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Synthesis of single- and double-chain fluorocarbon and hydrocarbon galactosyl amphiphiles and their anti-HIV-1 activity

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Abstract

Galactosylceramide (GalCer) is an alternative receptor allowing HIV-1 entry into CD4(−)/GalCer(+) cells. This glycosphingolipid recognizes the V3 loop of HIV gp120, which plays a key role in the fusion of the HIV envelope and cellular membrane. To inhibit HIV uptake and infection, we designed and synthesized analogs of GalCer. These amphiphiles and bolaamphiphiles consist of single and double hydrocarbon and/or fluorocarbon chain β-linked to galactose and galactosamine. They derive from serine (GalSer), cysteine (GalCys), and ethanolamine (GalAE). The anti-HIV activity and cytotoxicity of these galactolipids were evaluated in vitro on CEM-SS (a CD4(+) cell line), HT-29, a CD4(−) cell line expressing high levels of GalCer receptor, and/or HT29 genetically modified to express CD4. GalSer and GalAE derivatives, tested in aqueous medium or as part of liposome preparation, showed moderate anti-HIV-1 activities (IC_{50} in the 20–220 μM range), whereas none of the GalCys derivatives was found to be active. Moreover, only some of these anti-HIV active analogs inhibited the binding of [^{3}H]suramin (a polysulfonyl compound which displays a high affinity for the V3 loop) to SPC3, a synthetic peptide which contains the conserved GPGRAF region of the V3 loop. Our results most likely indicate that the neutralization of the virion through masking of this conserved V3 loop region is not the only mechanism involved in the HIV-1 antiviral activity of our GalCer analogs.

Keywords: Galactolipid; Galactosylceramide; Fluorocompound; Liposome; HIV; Gp120

1. Introduction

The human immunodeficiency virus (HIV), which causes AIDS, initiates the infection of the human CD4(+) macrophages, monocytes or lymphocytes T4, by the binding of its envelope glycoprotein gp120 to the cellular CD4 receptor and then to a coreceptor, such as mainly CCR5 or CXCR4 which are seven-transmembrane proteins of the chemokine receptor family (for recent reviews, see Refs. [1–4]). HIV can also infect, in vitro, CD4(−)

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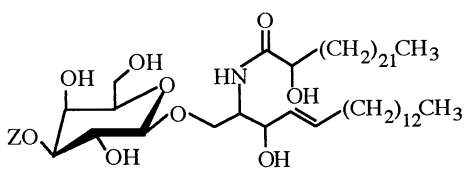
neural and colon epithelial cells. Galactosylceramide (GalCer) (Scheme 1) has been proposed as an alternative HIV receptor for the infection of these CD4(−) cells [5]. Their infection proceeds through gp120/GalCer interactions which involve the third variable domain (V3) of gp120 [6,7], although a region in the C2 domain has also been suggested to be important [8]. It has been further established that co-expression of CXCR4 on CD4(−) colon epithelial cells is necessary for their infection by HIV, raising the possibility that chemokine receptors may function as co-receptors for HIV entry into CD4(−)/GalCer(+) cells [5]. Galactosylceramide sulfate (or sulfatide), which is the natural sulfated derivative of GalCer, has in the past also been proposed as an alternative receptor for HIV [5]. However, the sulfatide has been recently shown to inhibit HIV-1 entry into CD4(−)/CXCR4(+) cells (the sulfatide mediates gp120 binding, but is not able to initiate the fusion event) [9].

The V3 loop also plays a fundamental role in infection after the virus has bound to CD4 and contains determinants for cell tropism (for a review, see Ref. [2]). The V3 domain is indeed involved in the association of the CD4/gp120 complex with the chemokine co-receptors. This association is required for conformational changes of gp41 (the transmembrane protein of the viral envelope) that mediate fusion of the viral membrane with the target cell membrane, hence virus entry into the cells. In addition, gp120 is also expressed at the surface of HIV-infected cells, indicating that the gp120 is a valuable target for the development of site-directed anti-HIV therapies. Ligands that are efficiently recognized by the viral or cellular gp120 could indeed be both (i) efficient competitors for the gp120/CD4(or GalCer)/co-receptor interactions, thus blocking acute and/or chronic HIV infection, and preventing the propagation of the virus, and (ii) targeting components for anti-HIV drug carrier systems directed either to HIV or to HIV-infected cells.

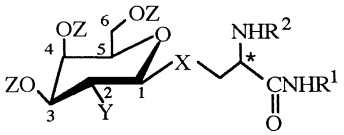


Galactosylceramide (GalCer) Z = H
Sulfated galactosylceramide (or sulfatide) Z = SO_3H

Scheme 1. Chemical structure of galactosylceramide (GalCer) and of its sulfated analog.

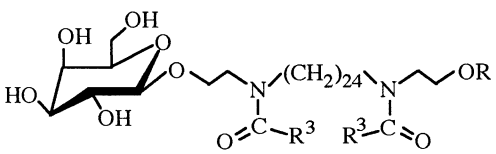
The potential of galactosphingolipids to inhibit HIV uptake and infection has recently instigated the syntheses of various galactolipids and the examination of their biological (anti-influenza virus and anti-HIV) activities [10–15]. As part of our contribution to this field, we reported in a preliminary communication the synthesis of some GalCer analogs deriving from β-galactosylated D,L- and L-serine and possessing fluorocarbon and/or hydrocarbon chains (compounds of the **GalSer** series in Scheme 2) [16]. We have now extended this series to: (i) new L- and (ii) D-serine derivatives; (iii) L-cysteine derivatives (compounds of **GalCys** series in Scheme 2); (iv) L-serine and L-cysteine galactosaminyl derivatives (compounds of **Gal(NHAc)Ser**, **Gal(NH_2)Cys** and **Gal(NHAc)Cys** series); (v) L-cysteine sulfated galactosaminyl analogs (**Gal(NHAc)(Sul)Cys** series), and (vi) ethanolamine-based galactosylated (bola)amphiphiles (compounds of the **GalAE** and **GalBAE** series). We describe here their synthesis together with the results of biological testings, including their anti-HIV-1 activity in various CD4(+), CD4(−) or GalCer(+) cell cultures and their ability to interact with SPC3, a synthetic peptide which is a mimic of the V3 loop (this assay proved useful to select analogs that recognize the V3 loop, a good correlation being generally found between the affinity for the V3 loop of a given analog and its anti-HIV activity) [14,17].

The highly fluorinated galactosylated (bola)amphiphiles were more particularly designed for their enhanced hydrophobic and lipophobic character resulting from the presence of the fluorocarbon chains [18]. These compounds should exhibit a stronger tendency to self-organize and to form galactosyl-rich clusters and domains or patches when incorporated within conventional membranes, thus increasing the interactions with gp120 [19,20]. They may further serve as components for the

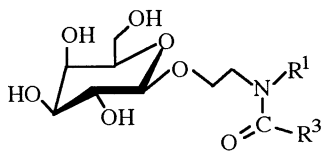


* = (D,L)-, (L)- or (D)-

CODE NAME	X	Y	Z	R^1	R^2
I-GalSer[C14](D,L), (D) or (L)	O	OH	H	$-(CH_2)_{13}CH_3$	H
I-GalSer[C16](L)	-	-	-	$-(CH_2)_{15}CH_3$	-
I-GalSer[F6C11](D,L)	-	-	-	$-(CH_2)_{11}C_6F_{13}$	-
II-GalSer[C14][F4C11](D,L)	-	-	-	$-(CH_2)_{13}CH_3$	$-(O)C(CH_2)_{10}C_4F_9$
II-GalSer[C16][F6C11](L)	-	-	-	$-(CH_2)_{15}CH_3$	$-(O)C(CH_2)_{10}C_6F_{13}$
II-GalSer[F4C11][F6C11](L)	-	-	-	$-(CH_2)_{11}C_4F_9$	$-(O)C(CH_2)_{10}C_6F_{13}$
I-Gal(NHAc)Ser[C14](L)	-	NHAc	-	$-(CH_2)_{13}CH_3$	H
I-GalCys[C14](L)	S	OH	-	-	-
II-GalCys[C14][C14](L)	-	-	-	-	$-(O)C(CH_2)_{12}CH_3$
I-Gal(NHAc)Cys[C14](L)	-	NHAc	-	-	H
II-Gal(NHAc)Cys[C14][C12](L)	-	-	-	-	$-(O)C(CH_2)_{10}CH_3$
II-Gal(NHAc)Cys[C14][C14](L)	-	-	-	-	$-(O)C(CH_2)_{12}CH_3$
II-Gal(NH_2)Cys[C14][C12](L)	-	NH_2	-	-	$-(O)C(CH_2)_{10}CH_3$
II-Gal(NH_2)Cys[C14][C14](L)	-	-	-	-	$-(O)C(CH_2)_{12}CH_3$
II-Gal(NHAc)(Sul)Cys[C14][C12](L)	-	NHAc	SO_3Na	-	$-(O)C(CH_2)_{10}CH_3$



CODE NAME	R^3	R
II-GalBAE[C24][C12](OH)	$-(CH_2)_{10}CH_3$	H
II-GalBAE[C24][F6C5](OH)	$-(CH_2)_4C_6F_{13}$	H
II-GalBAE[C24][F6C5]Gal	$-(CH_2)_4C_6F_{13}$	β-Gal



II-GalAE[C16][F8C7]

R^1 $-(CH_2)_{15}CH_3$

R^3 $-(CH_2)_6C_8F_{17}$

Scheme 2. Chemical structure and code name of the GalCer analogs reported in this study.

formulation of targeted fluorinated liposomes, which are attractive drug carrier and delivery systems [21], owing to their extended blood circulation times [22].

The anomeric β-configuration of galactosyl was selected for all galactosylated derivatives reported here, as this configuration seems to be essential for anti-HIV activity [23–25]. The hydrophobic chains have been connected to the sugar moiety through serine in order to mimic the ceramide part of GalCer. Although close analogs of the **GalSer** and **Gal(NHAc)Ser** compounds have been reported by other groups [12,13], they differ by the nature and length of the hydrophobic chains connected to serine. Supported by the anti-HIV activity found for *C*-glycosides (i.e., the carbohydrate moiety and the hydrophobic residue are linked together through a C–C bond) [10] and in order to avoid the in vivo enzymatic hydrolysis of glycosides, which is expected to reduce their potential anti-HIV activity, we designed the more stable *S*-glycosides deriving from cysteine. Modifications on the sugar moiety, which were found to increase anti-HIV activity in other series of glycolipids [10–15], were also performed. These consist of the replacement of the 2-OH

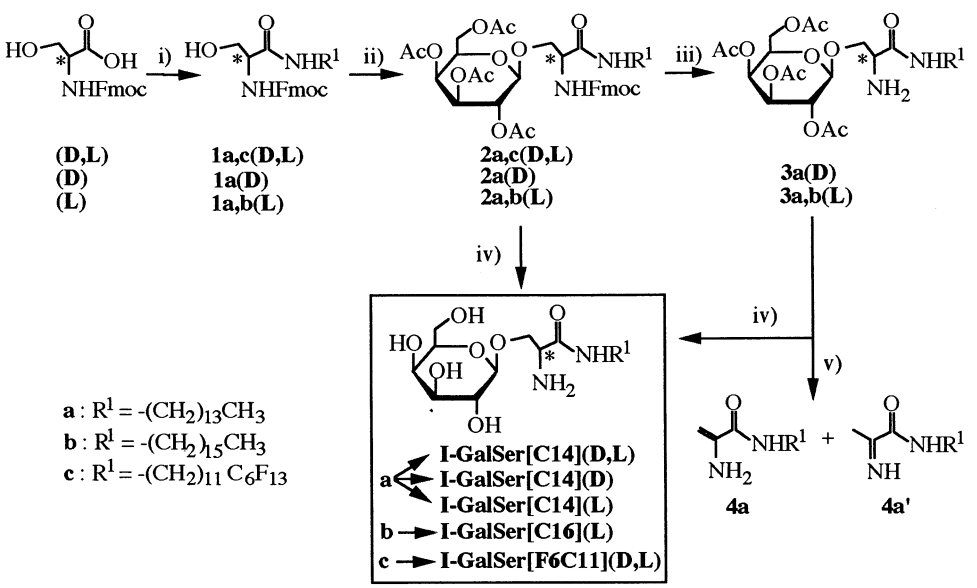
for an amino or acetamido group, and the sulfation of the OH of the galactosyl residue [9,13].

2. Results and discussion

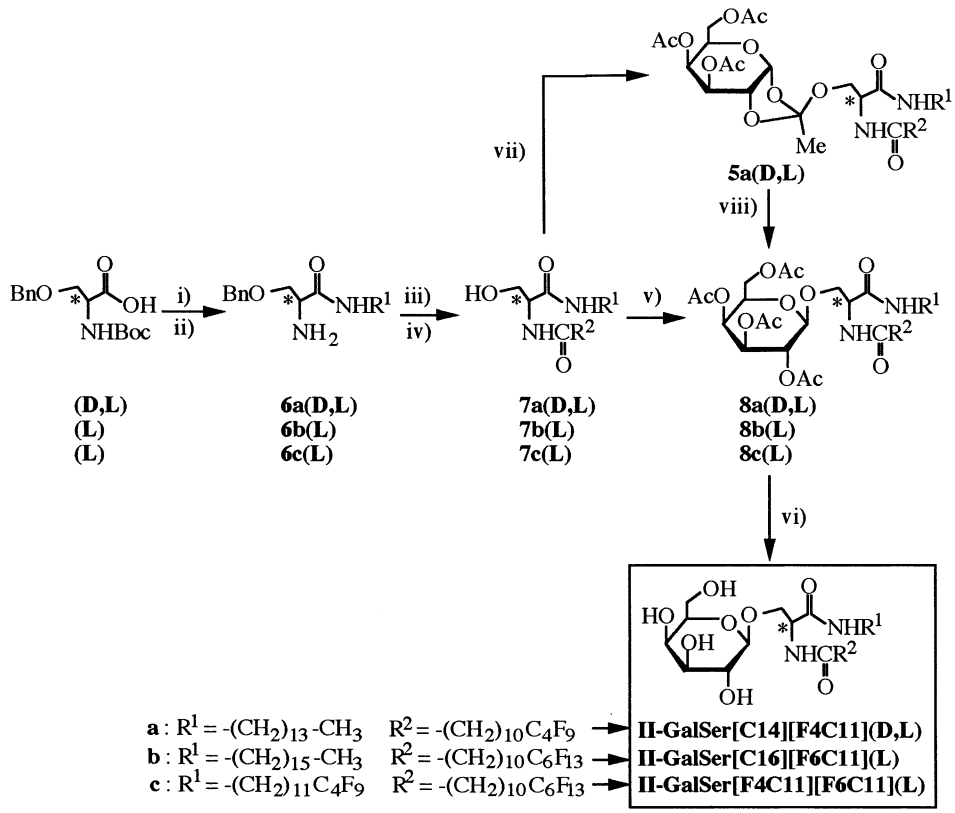
Synthesis.—For the preparation of the galactosylated derivatives shown in Scheme 2, three general synthetic approaches were considered:

1. O- or S-Glycosylation of serine, amidoethanol or cysteine building blocks containing one or two hydrophobic chains. This approach was applied for the synthesis of the monoamido **I-GalSer** (Scheme 3), **I-GalCys** (Scheme 5, path A) and **I-Gal(NHAc)Cys** (Scheme 9) series, of the diamido **II-GalSer** series (Scheme 4) and of the **II-GalAE** and **II-GalBAE** derivatives (Schemes 7 and 8);
2. Coupling of appropriately protected aminoacid and sugar, then successive conjugation of a hydrophobic chain on each of the aminoacid functions using conventional reactions in peptide chemistry. This approach, which has benefited from the recent development of efficient methods for the synthesis of glycosylated amino acids as building blocks [26,27], was applied for the synthesis of the single-chain **I-Gal(NHAc)Ser** derivatives (Scheme 9);
3. Alternatively, some double-chain cysteine galactolipids, e.g., compounds **II-GalCys** (Scheme 5, path B), **II-Gal(NH_2)Cys** and **II-Gal(NHAc)Cys** (Scheme 10), were prepared using a combination of strategy (i) and (ii), e.g., glycosylation of a single-chain amido cysteine derivative, the second lipophilic chain being introduced in a following step.

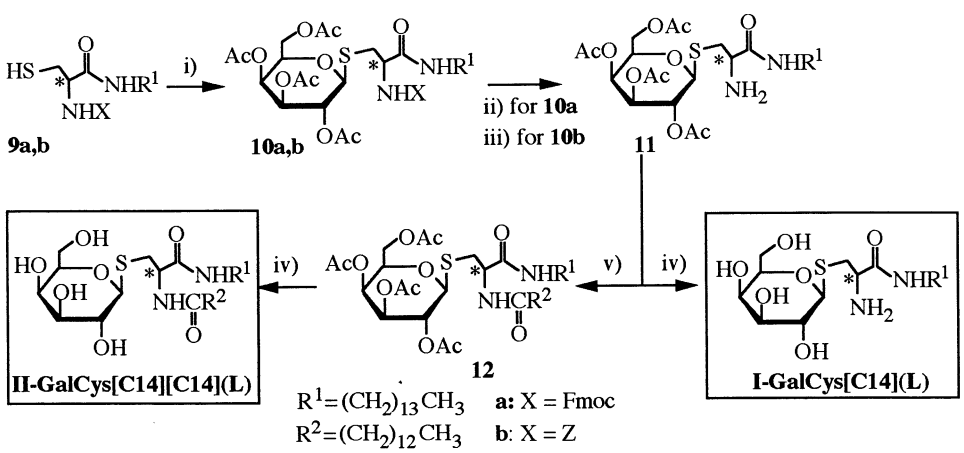
***GalSer** derivatives.* The synthesis of the monoamido and diamido long-chain serine β-galactosides **I-GalSer** and **II-GalSer** is presented in Schemes 3 and 4, respectively. It first requires the preparation of the aglycones **1** and **7**. The monoamido D,L-, L- and D-serine derivatives **1** were obtained in one step (50–80% yield range) starting from Fmoc-D,L-, Fmoc-L- or Fmoc-D-serine, respectively. The condensation of these Fmoc-derivatives with tetradecyl-, hexadecyl- or 11-(*F*-hexyl)-undecylamine was performed in the presence of DCC (or EDC)–HOBt and $NEt(iPr)_2$ (in order to avoid Fmoc-deprotection) [28]. The mixed hydrocarbon–fluorocarbon double-chain **7a** and **7b** aglycones and the fluorocarbon–fluorocarbon double-chain **7c** one were synthesized in four steps starting from *O*-benzyl-*N*-Boc-L- or D,L-serine (Scheme 4) [29].



Scheme 3. Synthetic route to the hydrocarbon and fluorocarbon single-chain **I-GalSer** derivatives. (i) R^1NH_2–DCC–HOBt–DMF; (ii) 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate [or $GalOC(=NH)CCl_3$]–TMSOTf–CH_2Cl_2; (iii) morpholine–$CHCl_3$; (iv) 2:1:1 MeOH–Et_3N–water; (v) MeONa–MeOH.



Scheme 4. Synthetic route to the hydrocarbon and fluorocarbon double-chain **II-GalSer** derivatives. (i) R^1NH_2–DCC–HOBt–DMF; (ii) CF_3CO_2H; (iii) R^2COCl–Et_3N–$CHCl_3$; (iv) H_2–Pd–C, AcOH–MeOH; (v) GalOC(=NH)CCl_3–TMSOTf–CH_2Cl_2; (vi) 2:1:1 MeOH–Et_3N–water; (vii) 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide, Ag_2CO_3–I_2–$CHCl_3$; (viii) $HgBr_2$–$MeNO_2$.



Scheme 5. Synthetic route to the hydrocarbon single-chain **I-GalCys(L)** and double-chain **II-GalCys(L)** derivatives. (i) 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose, $BF_3{\cdot}Et_2O$–CH_2Cl_2; (ii) morpholine–$CHCl_3$ for **10a**; (iii) H_2–Pd–C, MeOH for **10b**; (iv) 2:1:1 MeOH–Et_3N–water; (v) R^2CO_2H–DCC–HOBt–DMF.

Condensation of this protected aminoacid derivative with tetradecyl-, hexadecyl- or 11-(*F*-butyl)undecyl-amine in the presence of DCC–HOBt, then Boc-deprotection gave **6a**–**c**, respectively. Acylation of these latter compounds with the appropriate perfluoroalkylated acid chloride and further hydrogenolysis of the benzyl group afforded the diamido serines **7a**–**c** in almost 60% overall yields.

Galactosylation of aglycones **1** and **7** was best performed using the Schmidt method (Schemes 3 and 4, respectively) [30,31]. Thus, the β-galactosides **2** and **8** were obtained in yields ranging from 30 to 65% by reacting **1**

and **7** with GalOC(=NH)CCl_3 [32,33] and a catalytic amount of TMSOTf, respectively. This method was preferred to the Koenigs–Knorr glycosylation reaction [34]. Indeed, the condensation of D,L-serine derivative **7a** with 'GalBr' in the usual Koenigs–Knorr conditions (Scheme 4, step vii), gave mainly the 1,2-orthoester **5a** (anomeric ^{13}C-1 and quaternary ^{13}C resonance at 98 and 121 ppm, respectively) and the expected β-galactoside **8a**. The orthoester **5a**, when heated in nitromethane with a catalytic amount of $HgBr_2$ [34,35], was almost quantitatively converted into **8a** (64% overall yield for the glycosylation). Among the glycosylation methods tested, our attempts to prepare the β-galactosides by condensing the serine derivatives with 1,2,3,4,6-penta-*O*-acetyl-D-galactopyranose in the presence of a Lewis acid such as $SnCl_4$ or $BF_3{\cdot}Et_2O$ failed [36–38].

One drawback of the galactosylation which was encountered using the Schmidt method was the acetylation of the aglycones **1** and **7** (up to 20%), besides the formation of the expected galactosides **2** and **8**. Acetyl transfers from acetyl-protected sugars to the aglycone have often been reported to compete with glycosylation [34,39,40]. To account for this side-reaction, a reaction mechanism involving an orthoester intermediate has been postulated [40]. This is supported here by the formation of the orthoester when the galactosylation was performed in the conditions of the Koenigs–Knorr reaction (vide supra).

The low to moderate yields by which the β-galactosides **2** and **8** were obtained could also be due to the deactivation of the hydroxyl of serine resulting from the formation of an intramolecular hydrogen bond with the amide function. This accounts for the poor accepting properties of ceramides, *N*-acylsphingosines [41], serine and threonine derivatives [42] in the glycosidation reaction. In line with these results, a low reactivity has also been shown for the diamidoserine compounds **7** in the course of their phosphorylation [29].

The structure of the peracetylated galactosides was established by ^{1}H and ^{13}C NMR, the spectral assignments being ascertained by comparison with the data reported in literature for close analogs [43,44]: the anomeric H-1 proton resonance of compounds **2** and **8** is a doublet centered at $\sim$4.5 ppm ($J_{1,2}$ 7.8 Hz), in agreement with a β-configuration, unequivocally confirmed by the anomeric ^{13}C-1 resonance at $\sim$101.5 ppm (a α-configuration is characterized by a coupling of 2–3 Hz and an anomeric ^{13}C-1 resonance at 96–97 ppm).

The diamidoserine β-galactosides **II-GalSer** were obtained in 80–90% yields after acetyl deprotection of **8** in a 2:1:1 MeOH–NEt_3–water mixture [45]. By contrast, the deprotection of the serine amino group and of the galactose moiety of **2** to produce the monoamido-serine β-galactosides (**I-GalSer**) was most delicate. This deprotection, using a procedure similar to that described in literature [46,47], was best performed, in terms of efficiency and reproducibility, in two steps which consist first of the Fmoc cleavage using morpholine [28], then the deacetylation of **3** thus produced with 2:1:1 MeOH–NEt_3–water (overall yields $\sim$65%). We also found out that purification of **3** prior to deacetylation was essential for a clean reaction. Both the amino and galactose deprotection steps have to be carried out very rapidly (in about 1 h) in order to avoid degradation and β-elimination. This is related to the low stability of the *O*-glycosidic bond in basic media for glycopeptide with a serine residue [48] due to the acidity of the serine CH bond. By contrast, our attempts to deprotect **2a** in one step with 2:1:1 MeOH–NEt_3–water were not successful. Under these conditions, the deprotection took several hours for completion and led to a complex mixture consisting of the expected **I-GalSer[C14]** galactoside along with *N*-tetradecyl-2-amino-2-propenamide (**4a**) and *N*-tetradecyl-2-iminopropanamide (**4a′**), as a result of β-elimination. Compounds **4a** and **4a′** were further obtained almost quantitatively when the acetyldeprotection was performed with MeONa–MeOH on the Fmoc-deprotected **3a** (Scheme 3).

The ^{1}H and ^{13}C NMR spectra of the deprotected **I-GalSer** and **II-GalSer** are in full agreement with the proposed structures. Their anomeric carbon resonances are found in the 102.8–105.3 ppm range, consistent with the β-configuration. The D,L-serine galactosyl derivatives were obtained as their diastereoiso-

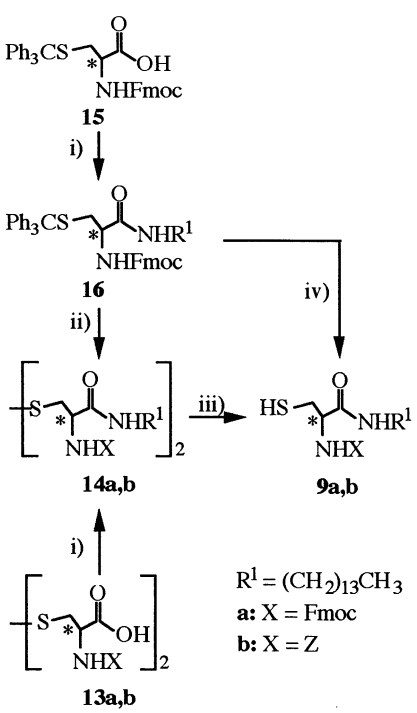
meric mixture. This is well reflected by their ^{13}C NMR spectrum which shows two ^{13}C resonances for several carbons (e.g., C-1β: 105.3 and 104.9 for **I-GalSer[C14](D,L)**). By contrast, the ^{13}C NMR spectra of the pure **I-GalSer[C14](D)** or **I-GalSer[C14](L)** diastereoisomers display a single resonance for each carbon, indicating that no racemization has occurred during the deprotection step of these derivatives.

GalCys *derivatives*. The synthesis of the monoamido and diamido long-chain L-cysteine galactosides (**I-GalCys(L)** and **II-GalCys(L)**, respectively) is presented in Scheme 5. The strategy used for the synthesis of the cysteine derivatives differs substantially from that described above for the preparation of the serine analogs. It also allows the preparation, from an unique single-chain derivative, of a larger range of double-chain compounds differing by the length and nature of the second hydrophobic chain.

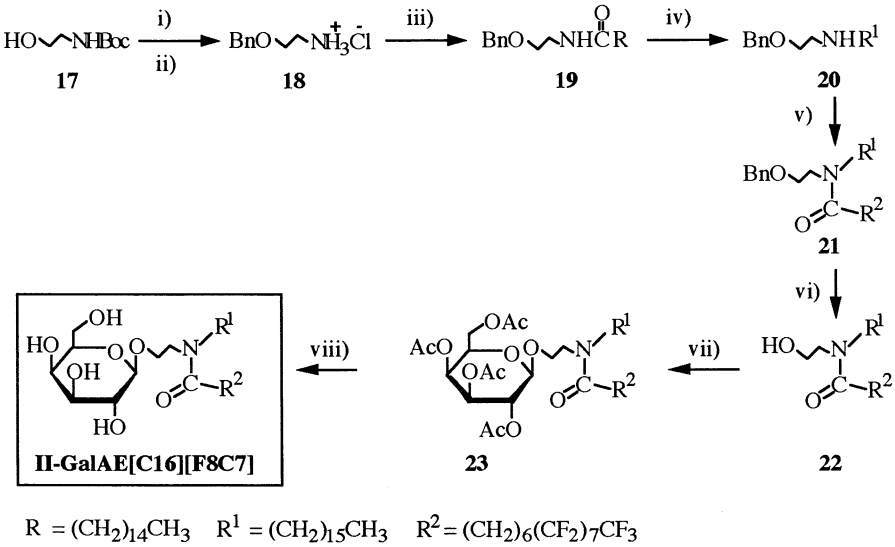
Both the monoamido **I-GalCys(L)** and diamido **II-GalCys(L)** derivatives have been prepared from the common single-chain galactosyl-protected cysteine intermediate **11**. The double-chain galactocysteine derivative **12** was obtained in 80% yield by condensing tetradecanoic acid with **11** using DCC–HOBt. Deacetylation of **11** and **12** was performed with 2:1:1 MeOH–NEt_3–water (from 60–75% yield), as described for the single- and double-chain **GalSer** derivatives.

The key single-chain galactosyl-protected cysteine synthon **11** was best obtained using a two-step procedure consisting into the galactosylation of the *N*-Fmoc-protected amido-cysteine **9a** followed by Fmoc-deprotection. The galactosylation of **9a** was performed in 93% yield with 1,2,3,4,6-penta-*O*-acetyl-D-galactopyranose in the presence of $BF_3{\cdot}Et_2O$, illustrating that thiols readily condense on peracetylated glycopyranoses in the presence of Lewis acids, in contrast to alcohols. The Fmoc deprotection of **10a** thus obtained was achieved with morpholine in ~80% yield. A second route for the synthesis of **11** starting from the benzyloxycarbonyl-(or *Z*)-protected derivative **9b** was also tested. Although the galactosylated *Z*-derivative **10b** could be readily obtained (78% yield), the Z-deprotection by catalytic hydrogenolysis failed however. Indeed, hydrogenolysis of **10b** led to a complex mixture in which we could identify the β-elimination products **4a** and **4a′**.

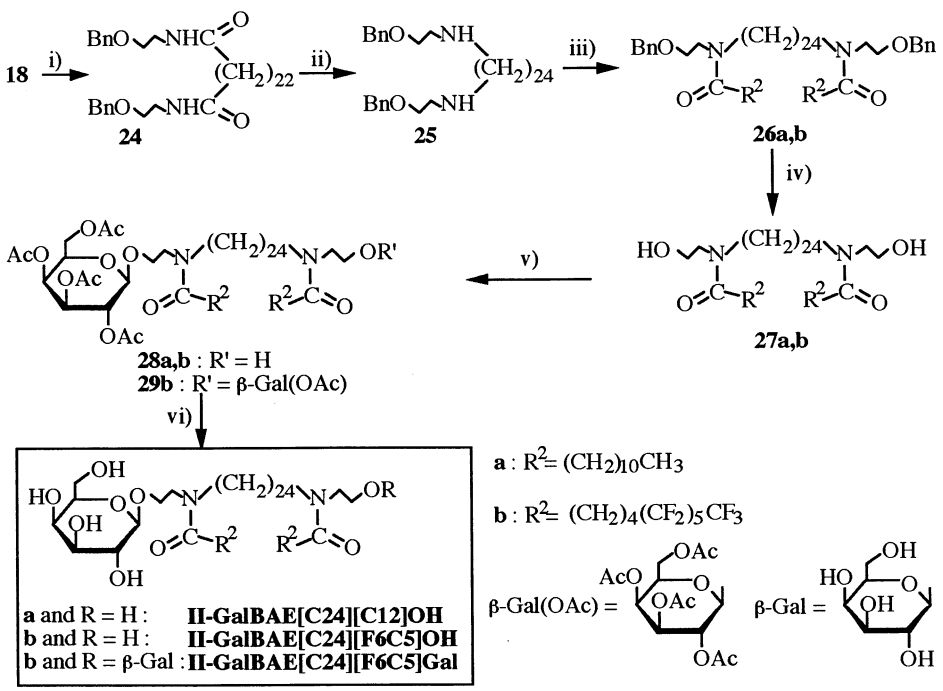
Concerning thiols **9a,b** (Scheme 6), they were prepared in two steps starting from Fmoc- or benzyloxycarbonyl-protected L-cystine **13a,b**, respectively. Coupling of tetradecylamine with **13a,b** using DCC–HOBt (90% yield), then reduction of the disulfide **14a,b** by action of Zn–AcOH (90% yield for **14a**) [49], or of dithiothreitol (70% yield for **14b**) [50] afforded thiol **9a,b**. Alternatively, compound **9a** was also prepared starting from *S*-trityl and *N*-Fmoc protected L-cysteine **15**, as shown in Scheme 6. Unfortunately, the trityl-deprotection of **16** with trifluoroacetic acid [51] was most difficult to achieve. Compound **9a** could however be obtained from **16** with acceptable yields (63%) by action of iodine [52], then reduction of the disulfide **14a** thus obtained. The ^1H NMR signal of the anomeric proton at 4.3 ppm ($J_{1,2}$ 9.3 Hz) and the ^{13}C NMR resonance of the anomeric car-



Scheme 6. Synthetic pathway for the monoamido-L-cysteine derivatives **9**. (i) R^1NH_2–DCC–HOBt–DMF; (ii) I_2, MeOH; (iii) Zn–AcOH for X = Fmoc; dithiothreitol for X = Z; (iv) CF_3CO_2H.



Scheme 7. Synthetic route to the hydrocarbon and fluorocarbon double-chain galactosyl-amidoethanol **II-GalAE** derivatives. (i) $PhCH_2Cl$–NaOH–*n*-$Bu_4N^+HSO_4^-$–water–CH_2Cl_2; (ii) CF_3CO_2H then HCl; (iii) RCOCl–Et_3N–CH_2Cl_2; (iv) $LiAlH_4$–THF; (v) R^2COCl–Et_3N–CH_2Cl_2; (vi) H_2–Pd–C, AcOH–MeOH; (vii) GalOC(=NH)CCl_3–TMSOTf–$CHCl_3$, −10 °C; (viii) 2:1:1 MeOH–Et_3N–water.



Scheme 8. Synthetic route to the bolaform hydrocarbon–hydrocarbon and hydrocarbon–fluorocarbon galactosyl-amidoethanol **II-GalBAE(OH)** and (bis)-galactosyl-amidoethanol **II-GalBAE(Gal)** derivatives. (i) Cl(O)C$(CH_2)_{22}$C(O)Cl–Et_3N–CH_2Cl_2; (ii) $LiAlH_4$–THF; (iii) R^2COCl–Et_3N–CH_2Cl_2; (iv) H_2–Pd–C–EtOH; (v) GalOC(=NH)CCl_3–TMSOTf–$CHCl_3$, −10 °C; (vi) MeOH–MeONa.

bon at 86–87 ppm confirmed the β-configuration of the *S*-glycosidic bond.

GalAE and GalBAE derivatives. The syntheses of the double-chain galactosyl amphiphiles **II-GalAE** and bolaamphiphiles **II-GalBAE(OH)** deriving from aminoethanol are presented in Schemes 7 and 8, respectively. They were obtained in 35 and 22% overall yields by galactosylation of aglycones **22** and **27** using the Schmidt method, then deacetylation of the resulting compounds **23** and **28** with MeONa–MeOH, respectively. The di-

galactosylated compounds **II-GalBAE(Gal)** were formed as by-products in the course of the bolaamphiphile **II-GalBAE(OH)** syntheses.

The presence of two equivalent hydroxyl functions in **27** and the very low solubility of the fluorocarbon–hydrocarbon **27b** made their mono-galactosylation difficult to control. We proceeded in a stochastic way, hence using stoichiometric quantities of the two reagents (1:1 molar ratio). A multi-step synthesis, including protection of one of the two hydroxyl functions, galactosylation, then deprotection, would probably not have given better results. It should be noted that the reaction mixtures after glycosylation were complex and difficult to purify. Formation of **28** was accompanied by that of the di-galactosylated compounds **29** (up to 8% in the case of **29b**), as expected, and by 2,3,4,6-tetra-*O*-acetyl-D-galactopyranose (which is also formed during the synthesis of **23**). It was most difficult to separate this latter compound from **23** and **28**. However, it was much easier to purify their respective **II-GalAE** and **II-GalBAE(OH)** derivatives after deacetylation.

^{1}H and ^{13}C NMR analyses of **23**, **28**, **29**, and of their respective deprotected **II-GalAE** and **II-GalBAE** analogs are in full agreement with the proposed structures. These analyses showed more particularly a β-configuration for the anomeric *O*-glycosidic bond (doublet at 4.39 ppm for the anomeric proton with a coupling constant $J_{1,2}$ of 7.8 Hz for **28**, a single resonance for the anomeric carbon in the 101.5–103.8 ppm range [43]).

The aglycone **22** and bolaform aglycones **27** were prepared in six steps from commercial *N*-(Boc)ethanolamine **17** with overall yields in the 20–30% range (Schemes 7 and 8, respectively). After *O*-benzyl-protection of **17** then *N*-Boc-deprotection, the *O*-benzyl-ethanolamine **18** thus obtained was condensed with palmitoyl chloride or α,ω-tetracosanoyl dichloride giving **19** and **24**, respectively. These amides were reduced into amine **20** and α,ω-tetracosanediamine **25**, respectively. The reduction was extremely slow for **24** owing to its low solubility in THF. The preparation of diamine **25** needed therefore two successive reductions for completion. Acylation of amine **20** with 7-(perfluorooctyl)heptanoyl chloride afforded **21** (83%). Acylation of diamine **25** with 5-(perfluorohexyl)pentanoyl chloride or dodecanoyl chloride afforded **26a** (90%) or **26b** (98%), respectively. *O*-Benzyl deprotection by hydrogenolysis of **21** and **26** afforded almost quantitatively building blocks **22** and **27**. High hydrogen pressure (40 atm) was necessary for the benzyl cleavage in the case of the bolaform compounds **26**. For some intermediates, both the ^{1}H and ^{13}C NMR spectra shows the doubling of some signals, which indicates the presence of two conformational isomers due to the amide bond [53].

*Single-chain **I-Gal(NHAc)Ser** and **I-Gal(NHAc)Cys** derivatives*. The preparation of the single-chain N-acetylated galactosamine compounds derived from serine and cysteine is depicted in Scheme 9. Their syntheses have been performed starting with the *N*-allyloxycarbonyl (Aloc)-protected galactosamine **32**. The *N*-Aloc approach for the synthesis of 2-amino-β-D-galactosides was chosen for its efficiency in glycosylation reactions, its β-selectivity [54], and the possibilities of chemoselective cleavage of the NH–Aloc function either into the acetamido group or into the free amino group under very smooth conditions [55,56] (possibilities which were used for the synthesis of the double-chain **II-Gal(NHAc)Cys** and **II-Gal(NH_2)Cys** compounds, vide infra). Stereocontrolled syntheses of 1,2-trans glycosaminides by electrophilic activation of an anomeric leaving group have indeed been achieved through participation of the C-2 amido substituent, such as NH–Aloc, -acetyl, -chloroacetyl, or -trichloroacetyl [34,54].

The key NH–Aloc–galactopyranose synthon **32** was prepared in four steps (40% overall yield) from D-galactosamine hydrochloride, applying the method used for the synthesis of its glucose analog [57]. The β-anomeric configuration of the resulting galactoside **32** was established by ^{1}H NMR (presence of a doublet for the anomeric proton at 5.69 ppm with $J_{1,2}$ 8.7 Hz), in agreement with the literature [58].

The β-galactosamine serine derivative **33** was obtained in ~40% yield by reacting **32** with *N*-Fmoc-L-serine in the presence of

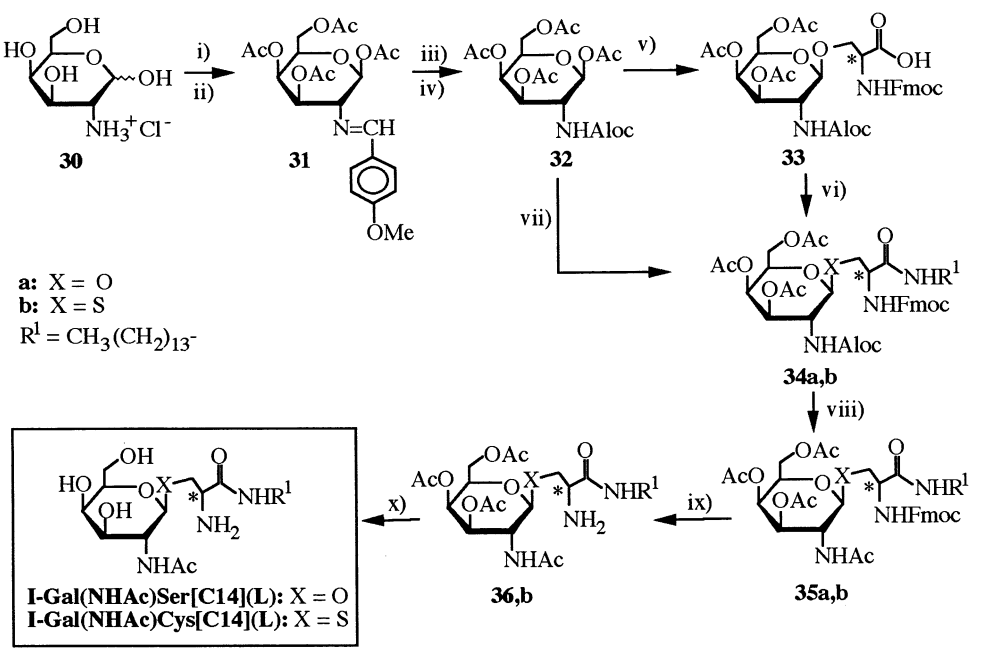
$BF_3{\cdot}Et_2O$. Conventional conjugation of tetradecylamine to the free acid function of **33** gave the single-chain derivative **34a**, which was converted in three steps into **I-Gal(NHAc)Ser[C14](L)** (53% overall yield). These steps consisted successively in the Aloc–Ac exchange with $Pd(PPh_3)_4$–Bu_3SnH–Ac_2O [55], Fmoc cleavage with morpholine, then deacetylation with 2:1:1 MeOH–NEt_3–water.

The single-chain **I-Gal(NHAc)Cys[C14](L)** analog was obtained using a very similar synthetic pathway (56% yield from **32**). The unique modification with the precedent strategy lies in the glycosylation step which was performed with **32** onto the pre-formed single-chain amido *N*-Fmoc-L-cysteine derivative **9a**.

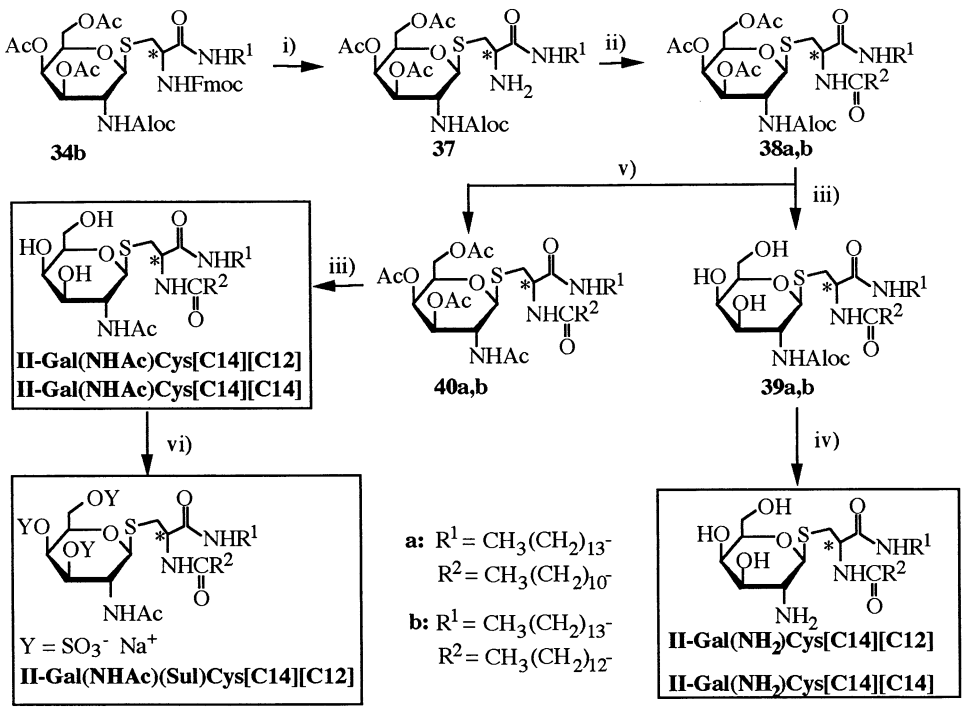
^1H and ^{13}C NMR analyses of **I-Gal(NHAc)Ser[C14](L)** and **I-Gal(NHAc)Cys[C14](L)** were consistent with the β-configuration of the *O*- and *S*-glycosidic bond. This is more particularly confirmed by the anomeric proton resonance which appears as a doublet at ~4.4 ppm with a large coupling constant ($J_{1,2}$ 8.1 and 10.3 Hz, respectively), and by the anomeric ^{13}C resonance which is located at 101.8 and 83.9 ppm for the serine and cysteine derivative, respectively.

Our attempts to obtain the fully deprotected **I-Gal(NH_2)Cys[C14](L)** analog of **I-Gal(NHAc)Cys[C14](L)** from compound **34b** were unsuccessful. Simultaneous deprotection of Aloc and Fmoc using $Pd(PPh_3)_4$–morpholine [55,59], then acetyl cleavage with 2:1:1 MeOH–NEt_3–water led to a complex mixture from which one could isolate **I-Gal(NHAc)Cys[C14](L)**, indicating that an acetyl transfer has occurred.

*Double-chain **II-Gal(NH_2)Cys** and **II-Gal(NHAc)Cys** derivatives*. The synthesis of the double-chain **II-Gal(NH_2)Cys** and **II-Gal(NHAc)Cys** derivatives is illustrated in Scheme 10. These derivatives were prepared in four steps starting from the same key single-chain compound **34b** in ~60% overall yields. After Fmoc-deprotection, the resulting **37** was acylated with lauric or myristic acid using EDC–HOBt. Deacetylation of **38** thus obtained by action of MeOH–NEt_3 (10:1 ratio), then Aloc cleavage in **39** with $Pd(PPh_3)_2Cl_2$ in the presence of Bu_3SnH–water [55,56] yielded derivatives **II-Gal(NH_2)Cys**. This sequence of deprotection has been applied in order to avoid any possibility of *O*- to *N*-acetyl migration (see preceding section). On the other hand, an Aloc–Ac exchange in **38** promoted



Scheme 9. Synthetic route to the single-chain L-serine **I-Gal(NHAc)Ser** and L-cysteine **I-Gal(NHAc)Cys** 2-acetamido-β-D-galactosyl derivatives. (i) *p*-MeOPhCHO, NaOH; (ii) Ac_2O, pyridine; (iii) 5 N HCl; (iv) allyl chloroformate; (v) *N*-Fmoc-L-serine, $BF_3{\cdot}Et_2O$–CH_2Cl_2; (vi) R^1NH_2–EDC–HOBt–DMF; (vii) **9a**, $BF_3{\cdot}Et_2O$; (viii) $Pd(Ph_3)_4$–Bu_3SnH–Ac_2O; (ix) morpholine; (x) 2:1:1 MeOH–Et_3N–water.



Scheme 10. Synthetic route to the double-chain cysteine galactosyl-amido **II-Gal(NH_2)Cys**, galactosyl-acetamido **II-Gal(NHAc)Cys** and sulfogalactosyl-acetamido **II-Gal(NHAc)(Sul)Cys** derivatives. (i) morpholine; (ii) R^2CO_2H–EDC–HOBt–DMF; (iii) 1:10 Et_3N–MeOH; (iv) $Pd(Ph_3)_2Cl_2$–Bu_3SnH–water; (v) $Pd(Ph_3)_2Cl_2$–Bu_3SnH–Ac_2O; (vi) SO_3·pyridine, DMF.

by the action of $Pd(PPh_3)_2Cl_2$–Bu_3SnH–Ac_2O [55], then deacetylation of **40** with MeOH–NEt_3 (10:1 ratio), afforded the **II-Gal(NHAc)Cys** derivatives.

The ^1H and ^{13}C NMR data collected on the galactosamine **II-Gal(NHAc)Cys** and **II-Gal(NH_2)Cys** derivatives, are also in full agreement with the proposed structures and, more particularly, with the anomeric β-configuration of the *S*-glycosidic bond.

*Double-chain **II-Gal(NHAc)(Sul)Cys[C14][C12]** derivative*. The O-sulfation of the N-acetylated **II-Gal(NHAc)Cys[C14][C12]** derivative was achieved with the sulfur trioxide–pyridine complex in DMF [60]. After treatment with Na_2CO_3, and chromatography on silica gel, **II-Gal(NHAc)(Sul)Cys[C14][C12]**, as its Na^+ salt, was isolated in 60% yield. Its structure was unambiguously attested to by ^1H and ^{13}C NMR, 2D (^1H–^1H) COSY, and ESIMS. The presence of the sulfate groups was confirmed by ESIMS (which indicated the presence three sulfate groups) and by TLC analysis (positive test with Azure A, which is a specific reagent for sulfated glycolipids [61]). It was noticed that the O-sulfation of **II-Gal(NHAc)Cys** was accompanied by a downfield shift of the signals corresponding to the CH–O protons and carbons by about 1–1.5 and 4–6 ppm, respectively.

Biological evaluation.—The aim of this study was to design and synthesize analogs of GalCer that were expected to bind to the V3 loop of HIV-1 gp120 and thus to block HIV-1 infection. The anti-HIV activity and cytotoxicity of the GalCer analogs was evaluated in vitro on CEM-SS, a CD4(+) cell line, HT-29, a CD4(−) cell line expressing high levels of GalCer receptor, and/or HT29 genetically modified to express CD4. Some of these analogs were also evaluated for their ability to inhibit the binding of [^{3}H]suramin (a polysulfonyl compound having high affinity for the V3 loop) to SPC3, a synthetic V3 peptide (eight GPGRAF motifs radially branched on uncharged poly-Lys core matrix). This peptide has been found to be an inhibitor of HIV-1 infection in both CD4(+) and CD4(−) cells [17,62]. Owing to their extremely low solubil-

ity in water, some single chain **I-GalSer** and **I-GalCys** and the double-chain **II-GalSer**, **II-GalCys** and **II-GalAE** derivatives were also tested as 2:1 PL–CH liposomal formulations. The results are collected in Table 1.

A specific anti-HIV activity, although moderate, was measured for some single-chain **I-GalSer** derivatives and some **II-GalSer** and **II-GalAE** liposomal formulations on CEM-SS cells (IC_{50} from 40 to 60 μM) and/or on GalCer(+)/CD4(−) or CD4(+) HT29 cells (IC_{50} from less than 20 to 216 μM). For comparison, an IC_{50} of 47 μM on HT29 CD4(−) cells has been reported for a soluble analog of GalCer which was also able to inhibit HIV-1 induced cell fusion as well as entry in CD4(+) cells [14], and suramin displays an IC_{50} of 38 μM on HT29 CD4(−) cells [63]. Most of the active compounds also proved to be well tolerated by the cells, at least over the concentration range investigated: the CC_{50} on CEM-SS were higher than 100 μM and inhibition on HT29 was not associated with any toxicity as evidenced by the XTT assay [14].

It should be noted that a similar antiviral activity of the galactolipid-based formulations

Table 1
Anti-HIV-1 activity and cytotoxicity of the GalCer analogs

Compound	Form [nm(SD)] [a]	CEM-SS		HT29 GalCer(+) CD4(−)	CD4(+)	[^{3}H]suramin/ SPC3 inhibition
		IC_{50} (μM)	CC_{50} (μM)	IC_{50} (μM)	IC_{50} (μM)	IC_{50} (μM)
I-GalSer[C14](D,L)	aq	60	>100	216		43
I-GalSer[C14](D)	aq	>100	>100	>1080		n.i. [e]
I-GalSer[C14](L)	aq	60	>100	216		n.i. [e]
I-GalSer[C16](L)	aq	50	75			
I-GalSer[F6C11](D,L)	aq	40	>100			
	F1 [110(30)]			>110 [b]	>110 [b]	n.i. [f]
I-Gal(NHAc)Ser[C14](L)	aq	>10 [b]	>10 [b]			
II-GalSer[C16][F6C11](L)	F1 [80(30)]			32		
II-GalSer[C14][F4C11](D,L)	F1 [75(25)]			36	36	
II-GalSer[F4C11][F6C11](L)	F1 [50(20)]			20 (IC_{70})	20 (IC_{40})	
I-GalCys[C14](L) [d]	F2 [90(25)]	[c]	27			
I-Gal(NHAc)Cys[C14](L) [d]	F2 [120(40)]	[c]	30			
II-GalCys[C14][C14](L) [d]	F2 [450(100)]	[c]	>25 [b]			
II-Gal(NHAc)Cys[C14][C12](L) [d]	F2 [500(100]	[c]	18			
II-Gal(NH_2)Cys[C14][C12](L) [d]	F2 [350(150)]	[c]	22			
II-Gal(NHAc)(Sul)Cys[C14][C12](L)	aq	[c]	>10 [b]			
II-GalBAE[C24][F6C5]OH [d]	F2 [160(40)]			26	28	[h]
II-GalBAE[C24][C12]OH [d]	F2 [160(50)]			[j]	[j]	[i]
II-GalAE[C16][F8C7] [d]	F2 [130(40)]			24	22	2
PL–CH (2:1 molar ratio)	[180(50)]	[c]	66 [k]	270 < IC_{50} < 540 [k]	270 < IC_{50} < 540 [k]	n.i. [g]

[a] aq: aqueous solution; F1 and F2: PL–CH–glycolipid liposome formulation 2:1:0.6 and 2:1:0.3 molar ratio, respectively. In brackets: the mean size in nm of the liposomes with the associated standard deviation (SD), as measured by light scattering.
[b] Not soluble or not dispersible as PL–CH-based liposomes at higher concentration.
[c] No specific anti-HIV activity was detected up to the corresponding CC_{50} concentration.
[d] When tested as aqueous solution, no activity was detected for a concentration of up to 10 μM.
[e] n.i.: no inhibition detected up to 170 μM.
[f] n.i.: no inhibition detected up to 110 μM.
[g] n.i.: no inhibition detected up to 550 μM of PL–CH 2:1.
[h] A maximum of 40% of inhibition is measured for a concentration ≥21 μM.
[i] A maximum of 20% of inhibition is measured for a concentration ≥30 μM.
[j] No specific activity could be detected for this galactolipid, its liposomal formulation being as active as the PL–CH controls.
[k] These values correspond to the PL concentration.

on the CD4(+) and CD4(−) HT29 cells is found. This result is not unexpected if the putative mechanism of action of the galactolipids consists indeed in the masking of the V3 loop, the V3 loop being also involved in the fusion process between the HIV-1 particle and the plasma membrane of the CD4(+) HT29 cells (through interaction with the CXCR4 chemokine receptor expressed by these cells [5,9]). Thus, our data suggest that the galactolipids may affect HIV-1 infection by two distinct mechanisms: (i) prevention of GalCer-mediated HIV-1 attachment to the surface of CD4(−)/GalCer(+) cells (see also discussion below) and (ii) post-binding inhibition of HIV-1 entry into CD4(+) cells.

It is also noticeable that the **II-GalSer** and **II-GalAE** liposomal formulations display a significantly higher anti-HIV activity on the HT-29 cells than the aqueous **I-GalSer** solutions. Furthermore, in a given series, the most active anti-HIV compounds or formulations were those containing fluorinated chains or components, respectively. This can be attributed to the formation of galactosyl-rich aggregates or domains which are more favored in the case of the highly fluorinated derivatives, owing to their higher hydrophobicity and lipophobicity. This in turn is expected to enhance their interaction with gp120, hence decreasing cellular infection [64,65]. Indeed, the nonlinear relationships found between the GalCer concentration and its binding to gp120 suggested a degree of cooperativity at higher GalCer concentration raising the possibility that gp120 preferentially or exclusively binds to glycolipid-rich domains in the liposomal bilayer [64].

Concerning the impact of the serine configuration (D or L isomer) on anti-HIV activity, our results, which show a comparable activity of the racemic mixture and L enantiomer of **I-GalSer[C14]** and no activity for the D enantiomer, suggest that the L enantiomer should be the active derivative in the racemic mixture (one should underscore that the L-configuration of serine is the same as that of sphingosine in the natural GalCer). Surprisingly, the L-stereoisomer did not show a higher activity than the D,L-mixture, and did not inhibited the binding of suramin to SPC3 (see below).

None of the β-*S*-galactosyl analogs (**GalCys** series) of GalCer, even when formulated as liposomes, was found to exhibit an anti-HIV activity, these formulations being rather cytotoxic. These derivatives were designed more particularly in view of the higher affinity of a β-c-galactosyl analog of GalCer for the HIV-1 gp120, as compared with its β-*O*-galactosyl homologue, which was attributed to improved resistance to both chemical and enzymatic deglycosylation [10]. The lack of antiviral activity of these β-*S*-galactosyl analogs shows that it is likely that steric factors and/or glycosyl conformation changes resulting from the O/C/S replacement [66] are almost as important for specific gp120 (V3) recognition, if this constitutes the mechanism of action of all of the GalCer analogs reported in this study (vide infra).

Concerning structure–activity relationships, our results obtained in the **GalSer** and **GalCys** series indicate that replacing one hydroxyl for a NHAc or a NH_2 group on the galactose did not significantly increase nor induce a specific anti-HIV activity. Neither could we obtain active **GalCys** compounds by substituting all the hydroxyles of **II-Gal(NHAc)Cys[C14][C12]** for a sulfate group as in **II-Gal(NHAc)(Sul)Cys[C14][C12]**. This result contrasts with that obtained in an analogous **Gal(NHAc)Ser** series for which sulfation substantially increased anti-HIV activity [13].

Attention was also paid to the demonstration that the anti-HIV activity found for some of the GalCer analogs described here was related to the masking of the V3 loop, as reported elsewhere for other GalCer analogs [14]. Among the anti-HIV active compounds, only **II-GalAE[C16][F8C7]** and **I-GalSer-[C14](D,L)** were able to prevent the binding of [^{3}H]suramin to SPC3 in a dose-dependent manner (IC_{50} of 2 and 43 μM, respectively). By contrast, **II-GalAE[C24][F6C5]OH** and **II-GalAE[C24][C12]OH**, which are structurally closely related to **II-GalAE[C16][F8C7]**, interfered with SPC3 recognition very weakly and in a dose-saturable manner. Moreover, and very surprisingly, the anti-HIV active **I-**

GalSer[C14](L), L-stereoisomer of **I-GalSer-[C14](D,L)**, and **I-GalSer[F6C11](D,L)** a fluorinated analog of **I-GalSer[C14](D,L)**, did not inhibit the binding of [^{3}H]suramin to SPC3.

These results most likely indicate that the neutralization of the virion through masking of the highly conserved GPGRAF region of the V3 loop is not the only mechanism involved in the HIV-1 antiviral activity of our GalCer analogs. The binding to a site of the V3 loop that does not involve the conserved motif as well as interference with other steps of viral replication are likely to occur. Further studies, including inhibition of binding to recombinant gp120 (in the presence or not of specific antibodies directed against V3 epitopes), inhibition of syncytia formation, etc., are necessary to fully understand our puzzling results and the mechanism of action of the different anti-HIV-1 active GalCer analogs described here.

3. Experimental

Chemical section

General. Unless indicated otherwise, the reactions were performed under anhyd N_2 using dry solvents and reagents. Anhydrous solvents were prepared by standard methods.

Dicyclohexylcarbodiimide (DCC), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), *N*-Boc-ethanolamine, tetracosanedioic acid, 4-pentenoic acid, 6-heptenoic acid, tetradecylamine, hexadecylamine, 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose, hydrazine acetate, 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU), trimethylsilyl trifluoromethanesulfonate (TMSOTf), 9-fluorenylmethyl chloroformate (Fmoc-Cl), 1-hydroxybenzotriazole (HOBt), *p*-anisaldehyde, allyl chloroformate, tributyltin hydride, sulfur trioxide pyridine complex, dithiothreitol, D-galactosamine hydrochloride, tetrakis-(triphenylphosphine)palladium(0) [$Pd(PPh_3)_4$], dichlorobis(triphenylphosphine)palladium(II) [$Pd(PPh_3)_2Cl_2$] and trichloroacetonitrile were purchased from Aldrich, *N*-Boc-*O*-benzyl-D,L-serine from Fluka, and boron trifluoride diethyl etherate from Sigma. *N*-Fmoc-3-*S*-trityl-L-cysteine was purchased from Novabiochem, *N*-Fmoc-L-cystine, *N*-benzyloxycarbonyl-L-cystine (*N*-*Z*-L-cystine) and *N*-Fmoc-D-serine from Bachem and *N*-Fmoc-L-serine from Propeptide.

N-Fmoc-D,L-serine was prepared by reaction between D,L-serine and Fmoc–Cl according to Ref. [67]. 11-(Perfluorobutyl)-undecylamine and 11-(perfluorohexyl)undecylamine were prepared according to Ref. [29], and 11-(perfluorobutyl)undecanoyl chloride and 11-(perfluorohexyl)undecanoyl chloride to Ref. [68]. 5-(Perfluorohexyl)pentanoic or 7-(perfluorooctyl)heptanoic acids were prepared from perfluorohexyl iodide (Elf-Atochem) and ethyl 4-pentenoate, or from perfluorooctyl iodide (Elf-Atochem) and 6-heptenoic acid, respectively [68]. Tetracosanedioyl dichloride, dodecanoyl chloride, 5-(perfluorohexyl)-pentanoyl chloride and 7-(perfluorooctyl)-heptanoyl chloride [68] were prepared from their respective acids following conventional procedure using $SOCl_2$. 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl bromide was prepared from 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose and 33% HBr–AcOH. The 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl trichloroacetimidate [GalOC(=NH)CCl_3] was obtained by 1-*O*-deacetylation of 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose by hydrazine acetate [69] followed by condensation with trichloroacetonitrile in the presence of DBU [32,33]. *N*-Fmoc-3-*O*-β-D-galactopyranosyl-L-serine was prepared according to Refs. [37,38].

Column chromatography purifications were carried out on Silica Gel 60 (E. Merck, 70-230 mesh). The purity of all new compounds was checked by thin-layer chromatography (TLC), NMR and elemental analysis. TLC analyses were performed on precoated Silica Gel F_{254} plates (E. Merck) with detection by UV and by charring with 50% methanol–sulfuric acid solution, $KMnO_4$, ninhydrin, Dragendorff's reagents (Sigma), or 5,5′-dithiobis(2-nitrobenzoic acid) (Aldrich). Optical rotations were measured with a Perkin–Elmer 141 polarimeter (1-dm cell) at 589 nm. Melting points, determined with a Reichert apparatus, are uncorrected. IR spectra were recorded on a Bruker FT-IFS 45 spectrometer as KBr discs

for the crystalline samples and as films for the neat liquids. ^{1}H, ^{13}C, and ^{19}F NMR spectra were recorded with a Bruker AC 200 spectrometer at 200, 50.3, and 188.3 MHz, respectively. Chemical shifts (δ) are given in ppm relative to the signal (i) for internal reference Me_4Si or indirectly to $CHCl_3$ (δ 7.27) for ^{1}H, (ii) for internal reference Me_4Si or indirectly to $CDCl_3$ (δ 76.9) for ^{13}C, (iii) to internal reference $CFCl_3$ for ^{19}F. Elemental analyses were performed by the Service Central de Microanalyse du CNRS. Electrospray mass analyses, positive or negative mode, were effected on a ADP220 Bellingham and Stanley apparatus.

Synthesis of the single-chain ***I-GalSer*** *derivatives*

3-O-*(β*-D-*Galactopyranosyl)*-D,L-*serine tetradecylamide* (**I-GalSer[C14](D,L)**). A solution of DCC (1.39 g, 6.7 mmol) in DMF (25 mL) was added dropwise at 0 °C to a solution of Fmoc-D,L-serine (2.0 g, 6.1 mmol), tetradecylamine (1.3 g, 6.1 mmol), and of HOBt (0.84 g, 6.2 mmol) in DMF (70 mL). The reaction mixture was stirred at 0 °C for 30 min, then at rt for 6 h. After evaporation of DMF under diminished pressure, the residue was chromatographed on silica gel ($CHCl_3$) yielding *N*-Fmoc-D,L-serine tetradecylamide (1.51 g, 48%), **1a(D,L)** as a white solid. R_f 0.42 (49:1 $CHCl_3$–MeOH). IR (ν cm^{-1}, KBr): 3440 (OH), 1700 (C=O carbamate), 1650 (C=O amide). ^{1}H NMR ($CDCl_3$–CD_3OD): δ 7.60 (d, *J* 8.0 Hz, 2 H, Fmoc), 7.50 (d, *J* 8.0 Hz, 2 H, Fmoc), 7.35–7.10 (m, 4 H, Fmoc), 4.30 (d, *J* 7.0 Hz, 2 H, C(O)OCH_2), 4.20–4.00 (m, 2 H, C(O)OCH_2CH and $HOCH_2CH$), 3.75 and 3.55 (AB part of an ABX system, J_{AB} 11.1, J_{AX} 5.6 Hz, 2 H, CH_2OH), 3.20 (t, *J* 7.0 Hz, 2 H, $NHCH_2$), 1.70–1.00 [m, 24 H, $(CH_2)_{12}$], 0.85 (t, *J* 7.0 Hz, 3 H, CH_3). ^{13}C NMR ($CDCl_3$–CD_3OD): δ 170.8 [C(O)NH], 157.0 [NHC(O)O], 141.3 and 143.7 (C, Fmoc), 127.8, 127.1, 125.0 and 120.0 (CH, Fmoc), 67.3 [C(O)OCH_2], 62.7 (CH_2OH), 55.5 ($HOCH_2CH$), 47.1 (C(O)OCH_2CH), 39.7 ($NHCH_2$), 33.9, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 26.9, 25.6, and 24.9 [$(CH_2)_{11}$], 22.7 (CH_2CH_3), 14.1 (CH_3).

Step 1 (galactosylation): TMSOTf (0.55 mL, 2.2 mmol) was added dropwise at rt to a solution of **1a(D,L)** (1.15 g, 2.2 mmol), Gal-OC(=NH)CCl_3 (1.41 g, 2.9 mmol) in CH_2Cl_2 (60 mL) containing 5 g of 4 Å molecular sieves. The mixture was stirred for 6 h, diluted with CH_2Cl_2, filtered through Celite, washed with saturated $NaHCO_3$ then with brine. The organic phase was dried with Na_2SO_4, filtered, and evaporated under diminished pressure. Purification by silica gel chromatography ($CHCl_3$) gave 3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-N^{α}-Fmoc-D,L-serine tetradecylamide (**2a(D,L)**, 0.54 g, 67%) as a white solid [^{13}C NMR ($CDCl_3$–CD_3OD): C-1β at 101.1 ppm].

Step 2 (one-step Fmoc- and acetyl-deprotection): a suspension of **2a(D,L)** (0.12 g, 0.14 mmol) in 5:1.5:1.5 MeOH–Et_3N–water was stirred at 35 °C for 15 h. The solvents were evaporated under diminished pressure and the residue was purified by chromatography ($CHCl_3$ to 1:3 $CHCl_3$–MeOH) giving a diastereoisomeric mixture (94%) of **I-GalSer[C14](D,L)**: R_f 0.09 (7:3 $CHCl_3$–MeOH). ^{1}H NMR (CD_3OD): δ 4.30 (d, *J* 7.0 Hz, 1 H, H-1 Gal), 4.20–2.90 (m, 9 H, H-2–6 Gal and OCH_2CH), 3.20 (t, *J* 7.0 Hz, 2 H, $NHCH_2$), 1.80–1.30 (m, 24 H, $(CH_2)_{12}$), 1.00 (t, *J* 7.0 Hz, 3 H, CH_3). ^{13}C NMR (CD_3OD): δ 172.6 and 172.3 (C(O)NH), 105.3 and 104.9 (C-1β Gal), 76.8 (C-5 Gal), 74.7 and 74.6 (C-3 Gal), 72.3 and 72.2 (C-2 Gal), 71.9 and 71.7 (OCH_2), 70.1 (C-4 Gal), 62.4 (C-6 Gal), 55.8 and 55.5 (OCH_2CH), 40.4 ($NHCH_2$), 32.9 ($CH_2CH_2CH_3$), 30.6, 30.5, 30.3, and 30.2 [$(CH_2)_9$], 27.9 ($NHCH_2CH_2$), 23.6 (CH_2CH_3), 14.3 (CH_3)

3-O-*(β*-D-*Galactopyranosyl)*-L-*serine tetradecylamide* (**I-GalSer[C14](L)**). *N*-Fmoc-L-serine tetradecylamide (**1a(L)**) was prepared in a similar way using Fmoc-L-serine with 77% yield. [R_f 0.42 (49:1 $CHCl_3$–MeOH); mp 122 °C: ^{1}H and ^{13}C NMR ($CDCl_3$–CD_3OD): identical to those of **1a(D,L)**, respectively]. Then, the galactosylation procedure as described above in Step 1, when applied to **1a(L)**, afforded **2a(L)** as a white solid (67% yield) and 3-*O*-acetyl-*N*-Fmoc-L-serine tetradecylamide as a by-product (25% yield). **2a(L)**: R_f 0.5 (4:1 CH_2Cl_2–Et_2O). $[\alpha]_D$ +5.7° (*c* 1.1; 4:1 $CHCl_3$–MeOH). IR (ν cm^{-1}, KBr): 1750 (C=O ester), 1655 (C=O amide). ^{1}H

NMR ($CDCl_3$–CD_3OD): δ 7.68 (d, J 8.0 Hz, 2 H, Fmoc), 7.55 (d, J 8.0 Hz, 2 H, Fmoc), 7.35–7.20 (m, 4 H, Fmoc), 5.24 (bd, $J_{3,4}$ 3.2 Hz, 1 H, H-4 Gal), 5.00 (dd, $J_{1,2}$ 7.4, $J_{2,3}$ 10.4 Hz, 1 H, H-2 Gal), 4.92 (dd, $J_{2,3}$ 10.4, $J_{3,4}$ 3.2 Hz, 1 H, H-3 Gal), 4.40 (d, J 7.4 Hz, 1 H, H-1 Gal), 4.30–3.60 [m, 9 H, H-5–6 Gal and OCH_2CH and $NHC(O)OCH_2CH$], 3.15 (m, 2 H, $NHCH_2$), 2.06, 1.97, 1.94, and 1.91 [all s, 12 H, $CH_3C(O)$], 1.50–1.10 [m, 24 H, $(CH_2)_{12}$], 0.80 (t, J 7.0 Hz, 3 H, CH_3). ^{13}C NMR ($CDCl_3$): δ 170.8 [C(O)NH], 170.4, 170.3, 169.9 and 169.4 [$CH_3C(O)$], 156.3 [NHC(O)O], 143.7 and 141.3 (C, Fmoc), 127.1, 127.8, 124.9 and 120.0 (CH, Fmoc), 101.5 (C-1β Gal), 70.9 and 70.8 (C-3-5 Gal), 69.6 (OCH_2CH), 68.7 (C-2 Gal), 67.1 (C-4 Gal), 66.9 [$C(O)OCH_2$], 61.3 (C-6 Gal), 54.2 (OCH_2CH), 47.1 [$C(O)OCH_2CH$], 39.7 ($NHCH_2$), 31.9 ($CH_2CH_2CH_3$), 29.7, 29.6, 29.5, 29.3, 29.2 and 29.1 [$(CH_2)_9$], 26.8 ($NHCH_2CH_2$), 22.6 (CH_2CH_3), 20.54, 20.45, 20.41, 20.38 [$CH_3C(O)$], 13.9 (CH_2CH_3).

3-*O*-Acetyl-*N*-Fmoc-L-serine tetradecylamide: R_f 0.6 (4:1 CH_2Cl_2–Et_2O). ^{13}C NMR ($CDCl_3$): δ 171.0 [C(O)NH], 168.6 [$CH_3C(O)$], 156.2 [NHC(O)O], 143.7 and 141.4 (C, Fmoc), 127.2, 127.9, 125.1, and 120.1 (CH, Fmoc), 64.3 (OCH_2CH), 67.5 [$C(O)OCH_2$], 49.5 (OCH_2CH), 47.2 [$C(O)OCH_2CH$], 39.9 ($NHCH_2$), 32.0 ($CH_2CH_2CH_3$), 29.7, 29.6, 29.5, 29.4, and 29.3 [$(CH_2)_9$], 26.9 ($NHCH_2CH_2$), 22.8 (CH_2CH_3), 20.9 [$CH_3C(O)$], 14.2 (CH_3).

2a(L) (150 mg, 0.17 mmol) and morpholine (1.15 mL) in $CHCl_3$ (2 mL) were stirred at rt for 7 h (Fmoc-deprotection). After evaporation of the solvent, the residue was purified by chromatography (CH_2Cl_2 to 1:1 CH_2Cl_2–Et_2O) giving 3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-L-serine tetradecylamide (**3a**(L), 76 mg, 76%) as a white solid. R_f 0.6 (23:2 $CHCl_3$–MeOH). ^1H NMR ($CDCl_3$): δ 7.34 (t, J 5.6 Hz, 1 H, $NHCH_2$), 5.33 (bd, $J_{3,4}$ 3.2 Hz, 1 H, H-4 Gal), 5.12 (dd, $J_{1,2}$ 7.8, $J_{2,3}$ 10.4 Hz, 1 H, H-2 Gal), 4.95 (dd, $J_{2,3}$ 10.4, $J_{3,4}$ 3.2 Hz, 1 H, H-3 Gal), 4.48 (d, J 7.8 Hz, 1 H, H-1 Gal), 4.07 (m, 2 H, H-6,6′ Gal), 3.86 (m, 2 H, H-5 Gal and OCH_aH_b), 3.77 (bt, J 8.2 Hz, 1 H, OCH_aH_b), 3.48 (dd, J 8.2, J 4.4 Hz, 1 H, OCH_2CH), 3.15 (q, J 6.6 Hz, 2 H, $NHCH_2$), 2.08, 2.00, 1.98, 1.91,[(all s, all 3 H, $CH_3C(O)$], 1.70 (bs, 2 H, NH_2), 1.50–1.10 [m, 24 H, $(CH_2)_{12}$], 0.81 (t, J 6.6 Hz, 3 H, CH_3). ^{13}C NMR ($CDCl_3$–CD_3OD): δ 171.0 [C(O)NH], 170.5, 170.2, 170.1, and 169.7 [$CH_3C(O)$], 101.3 (C-1β Gal), 71.9 (OCH_2CH), 71.0 (C-3 Gal), 70.8 (C-5 Gal), 68.9 (C-2 Gal), 67.1 (C-4 Gal), 61.3 (C-6 Gal), 55.0 (OCH_2CH), 39.4 ($NHCH_2$), 32.0 ($CH_2CH_2CH_3$), 29.9, 29.7, 29.7, 29.6, 29.5, 29.4, and 29.3 [$(CH_2)_9$], 27.0 ($NHCH_2CH_2$), 22.8 (CH_2CH_3), 20.9, 20.8, 20.7, and 20.6 [$CH_3C(O)$], 14.2 (CH_3).

The *O*-acetyl-deprotection, when performed on **3a**(L) as described above in Step 2, afforded, after chromatography ($CHCl_3$ to 1:1 $CHCl_3$–MeOH), **I-GalSer[C14]**(L) as a white solid (98% yield). R_f 0.5 (76:21:3 $CHCl_3$–MeOH–water). $[\alpha]_D$ 0° (c 0.77; MeOH). mp 180 °C. ^1H NMR ($CDCl_3$–CD_3OD): δ 4.05–3.40 (m, 10 H, H-1–6 Gal and OCH_2CH), 3.15 (t, J 6.8 Hz, 2 H, CH_2NH), 1.65–1.10 [m, 24 H, $(CH_2)_{12}$], 0.85 (t, J 6.6 Hz, 3 H, CH_3). ^{13}C NMR ($CDCl_3$–CD_3OD): δ 170.8 [C(O)NH], 102.8 (C-1β Gal), 74.8 (C-5 Gal), 73.0 (C-3 Gal), 70.7 (C-2 Gal), 70.1 (OCH_2), 68.7 (C-4 Gal), 61.1 (C-6 Gal), 54.0 (OCH_2CH), 39.4 ($NHCH_2$), 31.6 ($CH_2CH_2CH_3$), 29.4, 29.3, 29.0, and 28.9 [$(CH_2)_9$], 26.7 ($NHCH_2CH_2$), 22.3 (CH_2CH_3), 13.6 (CH_3). Anal. Calcd for $C_{23}H_{46}N_2O_7 \cdot 3/2H_2O$ (489.65): C, 56.42; H, 10.09; N, 5.72. Found: C, 56.19; H, 10.01; N, 5.88.

Note: a mixture of isomers **4a** and **4a′** (quantitative yields) was obtained when the deprotection was performed from **2a**(L) in a mixture of 2:1:1 MeOH–Et_3N–water at 35 °C for 12 h, or when reacting **2a**(L) with morpholine in DMF, then with a catalytic amount of NaOMe in MeOH. ^{13}C NMR ($CDCl_3$) of **4a** and **4a′**: δ 167.8 [C(O)NH, **4a′**], 161.0 [C(O)NH, **4a**], 132.6 (C=NH, **4a′**), 131.0 (CH_2=C, **4a**), 128.9 (CH_2=C, **4a**), 38.9 ($NHCH_2$), 32.0 ($CH_2CH_2CH_3$), 30.4, 29.9, 29.8, 29.5, and 29.1 [$(CH_2)_{10}$], 22.8 (CH_2CH_3), 14.2 (CH_3), 11.0 (CH_3, **4a′**).

3-O-*(β*-D-*Galactopyranosyl)*-D-*serine tetradecylamide* (**I-GalSer[C14](D)**). *N*-Fmoc-D-serine tetradecylamide, **1a**(D) was prepared in a similar way using Fmoc-D-serine with 50% yield [R_f 0.68 (9:1 $CHCl_3$–MeOH): ^1H and ^{13}C

NMR ($CDCl_3$–CD_3OD): identical to those of **1a**(D,L), respectively]. Then, the three-step procedure described for the preparation of **I-GalSer[C14]**(L), when applied to **1a**(D), gave successively **2a**(D) as an oil (purification by chromatography with CH_2Cl_2 to 99:1 CH_2Cl_2–Et_2O; 20%), **3a**(D) (80%) as a white solid, then **I-GalSer[C14]**(D) (85%) as a white solid.

2a(D) [R_f 0.40 (4:1 CH_2Cl_2–Et_2O)] and **3a**(D) [R_f 0.24 (24:1 $CHCl_3$–MeOH)]: ^1H and ^{13}C NMR ($CDCl_3$) spectra are identical to those of **2a**(L) and **3a**(L), respectively.

I-GalSer[C14](D): R_f 0.36 (76:21:3 $CHCl_3$–MeOH–water). $[\alpha]_D$ +3.6° (*c* 0.67; MeOH). ^1H NMR (CD_3OD): δ 4.20 and 4.02 (AB part of an ABX spectrum, J_{AB} 6.0, J_{AX} 9.0 Hz, 2 H, OCH_2CH), 3.80–3.30 (m, 8 H, H-1–6 Gal and OCH_2CH), 3.16 (t, *J* 7.0 Hz, 2 H, $NHCH_2$), 1.65–1.45 (m, 2 H, $NHCH_2CH_2$), 1.45–1.10 [m, 22 H, $(CH_2)_{11}$], 0.85 (t, *J* 6.0 Hz, 3 H, CH_3). ^{13}C NMR (CD_3OD): δ 170.0 [C(O)NH], 105.3 (C-1β Gal), 76.7 (C-5 Gal), 74.6 (C-3 Gal), 72.3 (C-2 Gal), 70.1 (C-4 Gal), 68.9 (OCH_2CH), 62.4 (C-6 Gal), 55.8 (OCH_2CH), 40.4 ($NHCH_2$), 32.9 ($CH_2CH_2CH_3$), 30.7, 30.6, 30.5, 30.3 and 30.2 [$(CH_2)_9$], 27.9 ($NHCH_2CH_2$), 23.5 (CH_2CH_3), 14.2 (CH_3). Anal. Calcd for $C_{23}H_{46}N_2O_7 \cdot 3/2H_2O$ (489.65): C, 56.42; H, 10.09; N, 5.72. Found: C, 56.54; H, 10.15; N, 5.85.

3-O-(β-D-Galactopyranosyl)-L-serine hexadecylamide (**I-GalSer[C16]**(L)). *N*-Fmoc-L-serine hexadecylamide (**1b**(L)) was prepared in a similar way as **1a**(D,L) using hexadecylamine. ^1H NMR ($CDCl_3$–CD_3OD) spectrum from δ 7.70–1.90 ppm identical to that of **1a**(D,L) then δ 1.50–1.10 [m, 28 H, $(CH_2)_{14}$], 0.80 (t, *J* 6.0 Hz, 3 H, CH_3). Then, the three-step procedure described for the preparation of **I-GalSer[C14]**(L), when applied to **1b**(L), gave **I-GalSer[C16]**(L) in 40% overall yield. ^1H NMR ($CDCl_3$–CD_3OD): spectrum from δ 4.10–3.15 identical to that of **I-GalSer[C14]**(L) then δ 1.65–1.10 [m, 28 H, $(CH_2)_{14}$], 0.85 (t, *J* 6.0 Hz, 3 H, CH_3). ^{13}C NMR ($CDCl_3$–CD_3OD), spectrum identical to that of **I-GalSer[C14]**(L). Anal. Calcd for $C_{25}H_{50}N_2O_7 \cdot 2H_2O$ (526.71): C, 57.01; H, 10.33; N, 5.32. Found: C, 57.12; H, 9.98; N, 5.21.

3-O-(β-D-Galactopyranosyl)-D,L-serine 11-(F-hexyl)undecylamide (**I-GalSer[F6C11]**(D,L)). The procedure described for the preparation of **1a**(D,L) when applied to a solution of DCC (0.56 g, 2.7 mmol) in $CHCl_3$ (10 mL) and a solution of Fmoc-D,L-serine (0.81 g, 2.5 mmol), 11-(F-hexyl)undecylamine hydrochloride (1.3 g, 2.5 mmol), $NEt(iPr)_2$ (0.86 mL, 5 mmol), and HOBt (0.34 g, 2.55 mmol) in $CHCl_3$ (80 mL) afforded *N*-Fmoc-D,L-serine 11-(*F*-hexyl)undecylamide (1.60 g, 83%) (**1c**(D,L)) as a white solid. R_f 0.23 (24:1 $CHCl_3$–MeOH). ^1H NMR ($CDCl_3$–CD_3OD): δ 7.70 (d, *J* 8.0 Hz, 2 H, Fmoc), 7.50 (d, *J* 8.0 Hz, 2 H, Fmoc), 7.45–7.15 (m, 4 H, Fmoc), 4.30 (d, *J* 6.5 Hz, 2 H, $C(O)OCH_2$), 4.15 (t, *J* 6.5 Hz, 1 H, $C(O)OCH_2CH$), 4.10 (m, 1 H, OCH_2CH), 3.85 and 3.60 (AB part of an ABX system, J_{AB} 11.2, J_{AX} 5.7, J_{BX} 4.5 Hz, 2 H, CH_2OH), 3.15 (t, *J* 7.0 Hz, 2 H, CH_2NH), 2.00 (t, *J* 8.0, J_{HF} 19.0 Hz, 2 H, CH_2CF_2), 1.70–1.10 [m, 18 H, $(CH_2)_9$]. ^{13}C NMR ($CDCl_3$–CD_3OD): δ 170.7 [C(O)NH], 156.7 [NHC(O)O], 143.6 and 141.2 (C, Fmoc), 127.6, 127.0, 124.8, and 119.8 (CH, Fmoc), 67.1 ($C(O)OCH_2$), 62.3 (CH_2OH), 55.9 (OCH_2CH), 47.0 [$C(O)OCH_2CH$], 39.5 ($NHCH_2$), 30.7 (t, J_{CF} 22 Hz, CH_2CF_2), 29.3, 29.2, 29.1, 29.0, and 28.9 [$(CH_2)_7$], 26.7 ($NHCH_2CH_2$), 19.9 (t, J_{CF} 4 Hz, $CH_2CH_2CF_2$). Then, the two-step procedure described for the preparation of **I-GalSer[C14]**(D,L), when applied to **1c**(D,L), gave successively 3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-*N*-Fmoc-L-serine 11-(*F*-hexyl)undecylamide, **2c**(D,L) (68% yield) [^{13}C NMR ($CDCl_3$–CD_3OD): C-1β at 100.1 and 100.3 ppm], and **I-GalSer[F6C11]**(D,L) as a white solid (40% yield).

Alternatively, the Fmoc- and acetyl-deprotection of **2c**(D,L) was also performed as follows: **2c**(D,L) (0.08 g, 0.07 mmol) in 2:1 DMF-morpholine (2.25 mL) was stirred until the total disappearance of **2c**(D,L). After evaporation to dryness, methanolic 1 M MeONa was added dropwise to the residue in anhyd MeOH and stirred at rt for 20 min. Purification by chromatography ($CHCl_3$ to 1:1 $CHCl_3$–MeOH) of the residue obtained after solvent evaporation afforded **I-GalSer-[F6C11]**(D,L) (45 mg, 85%): R_f 0.07 (7:3 $CHCl_3$–MeOH). ^1H NMR ($CDCl_3$–CD_3OD): δ 4.31 and 4.30 (d, *J* 7.0 Hz, 1 H, H-1 Gal),

4.20–3.40 (m, 9 H, H-2–6 Gal and OCH_2CH), 3.25 (t, *J* 7.0 Hz, 2 H, $NHCH_2$), 2.10 (tt, J_{HF} 18.0, *J* 8.0 Hz, 2 H, CH_2CF_2), 1.80–1.20 [m, 18 H, $(CH_2)_9$]. ^{13}C NMR ($CDCl_3$–CD_3OD): δ 172.2 and 172.3 [C(O)NH], 102.9 and 103.3 (C-1 Gal), 74.7 (C-5 Gal), 72.8 (C-3 Gal), 70.7 and 71.1 (OCH_2), 70.4 and 70.5 C-2 Gal), 68.3 (C-4 Gal), 60.6 (C-6 Gal), 53.9 and 54.3 (OCH_2CH), 38.7 ($NHCH_2$), 30.0 (t, J_{CF} 22.3 Hz, CH_2CF_2), 28.7, 28.5, 28.4, and 28.2 [$(CH_2)_7$], 26.1 ($NHCH_2CH_2$), 19.3 (t, J_{CF} 3.5 Hz, $CH_2CH_2CF_2$). ^{19}F NMR ($CDCl_3$–CD_3OD): δ −80.5 (3 F, CF_3), −113.8 (2 F, CF_2CH_2), −121.2, −122.2, and −122.8 [3 × 2 F $(CF_2)_3CF_2CH_2$], −125.5 (2 F, CF_3CF_2). Anal. Calcd for $C_{26}H_{39}F_{13}N_2O_7 \cdot 1H_2O$ (756.58): C, 41.28; H, 5.46; N, 3.70. Found: C, 41.33; H, 5.28; N, 3.05.

Synthesis of the double-chain ***II-GalSer*** *derivatives*

Synthesis of aglycones **7a**–**c**. The synthesis and characterization of compounds **6b**(L), **6c**(L), **7b**(L) and **7c**(L) starting from *N*-Boc-*O*-benzyl-L-serine have already been described in Ref. [29].

O-*Benzyl*-D,L-*serine tetradecylamide* (**6a**(D,L)): A solution of DCC (1.9 g, 9.3 mmol) in DMF (25 mL) was added dropwise at 0 °C to a solution of *N*-Boc-*O*-benzyl-D,L-serine (2.5 g, 8.5 mmol), Et_3N (1.2 mL, 8.5 mmol), tetradecylamine (1.8 g, 8.5 mmol), and HOBt (1.2 g, 8.6 mmol) in DMF (100 mL). The reaction mixture was stirred at 0 °C for 30 min, then at 50 °C for 20 h. After evaporation of DMF under diminished pressure, the residue was chromatographed on silica gel ($CHCl_3$) giving *N*-Boc-*O*-benzyl-D,L-serine tetradecylamide (3.0 g, 72%) of as a white solid [R_f 0.86 (49:1 $CHCl_3$–MeOH)]. IR (*ν* cm^{-1}, film): 1713 and 1659 cm^{-1} (C=O). ^1H NMR ($CDCl_3$): δ 7.35 (m, 5 H, Ph), 6.45 (t, 1 H, CH_2NH), 5.45 (m, 1 H, CHNH), 4.60 and 4.50 (AB system, *J* 11.7 Hz, 2 H, OCH_2Ph), 4.25 (m, 1 H, OCH_2CH), 3.90 and 3.55 (AB part of an ABX system, J_{AB} 9.2, J_{AX} 6.6, J_{BX} 3.9 Hz, 2 H, CH_2OBn), 3.25 (td, *J* 6.2, *J* 6.5 Hz, 2 H, $NHCH_2$), 1.50 [s, 9 H, $(CH_3)_3C$], 1.40–1.20 [m, 24 H, $(CH_2)_{12}$], 0.85 (t, *J* 6.8 Hz, 3 H, CH_3). ^{13}C NMR ($CDCl_3$): δ 169.9 [C(O)NH], 155.3 [OC(O)NH], 137.3 (C, Ph), 127.8 (CH, para Ph), 127.6 and 128.3 (CH, ortho and meta Ph), 80.1 [$C(CH_3)_3$], 73.3 ($PhCH_2$), 69.8 (CH_2O), 53.8 (OCH_2CH), 39.4 ($NHCH_2$), 31.8 ($CH_2CH_2CH_3$), 29.5, 29.4, 29.2, and 29.1 [$(CH_2)_9$], 28.1 [$(CH_3)_3C$], 26.7 (CH_2CH_2N), 22.5 (CH_2CH_3), 14.0 (CH_3).

A solution of *N*-Boc-*O*-benzyl-D,L-serine tetradecylamide (3.0 g, 6.1 mmol) in CF_3CO_2H (10 mL) was stirred at rt for 1 h. The reaction mixture was evaporated to dryness under diminished pressure. The residue was dissolved in $CHCl_3$, washed with 10% Na_2CO_3, dried over Na_2SO_4, filtered and evaporated to dryness under diminished pressure, affording **6a**(D,L) (2.4 g, 100%) as a white solid: R_f 0.32 (49:1 $CHCl_3$–MeOH). IR (*ν* cm^{-1}, film): 1639 cm^{-1} (C=O).

N-*[11-(*F*-Butyl)undecanoyl]*-D,L-*serine tetradecylamide* (**7a**(D,L)): Step 1: a solution of **6a**(D,L) (2.0 g, 5.1 mmol) and triethylamine (0.9 mL) in $CHCl_3$ (25 mL) was added dropwise at rt to a solution of 11-(*F*-butyl)-undecanoyl chloride (2.5 g, 6.1 mmol) in $CHCl_3$ (30 mL). The reaction mixture was stirred at rt for 24 h. After evaporation to dryness, the residue was chromatographed on silica gel ($CHCl_3$) giving *N*-[11-(*F*-butyl)-undecanoyl]-*O*-benzyl-D,L-serine tetradecylamide (3.2 g, 81%) as a white solid: R_f 0.71 (49:1 $CHCl_3$–MeOH). ^1H NMR ($CDCl_3$): δ 7.30 (m, 5 H, Ph), 6.85 (t, *J* 6.0 Hz, 1 H, CH_2NH), 6.50 (d, *J* 6.7 Hz, 1 H, CHNH), 4.60–4.50 (m, 3 H, OCH_2Ph and OCH_2CH), 3.75 and 3.50 (AB part of an ABX system, J_{AB} 9.2, J_{AX} 8.0, J_{BX} 4.3 Hz, 2 H, CH_2OBn), 3.20 (m, 2 H, $NHCH_2$), 2.20 [t, *J* 7.2 Hz, 2 H, $CH_2C(O)$], 2.00 (tt, *J* 7.5, J_{HF} 18.0 Hz, 2 H, CH_2CF_2), 1.70–1.10 [m, 40 H, $(CH_2)_8$ and $(CH_2)_{12}$], 0.85 (t, *J* 7.0 Hz, 3 H, CH_3). ^{13}C NMR ($CDCl_3$): δ 173.3 [$C(O)CH_2$], 170.0 [$CHC(O)$], 137.5 (C, Ph), 128.0 (CH para Ph), 128.6 and 127.8 (CH, ortho and meta Ph), 73.5 ($PhCH_2O$), 69.6 (CH_2OBn), 52.2 (OCH_2CH), 39.7 ($NHCH_2$), 36.6 (CH_2CO), 32.0 ($CH_2CH_2CH_3$), 30.4 (t, J_{CF} 22 Hz, CH_2CF_2), 29.7, 29.6, 29.5, 29.3, and 29.1 [$(CH_2)_9$ and $(CH_2)_6$], 26.9 (CH_2CH_2N), 25.6 (CH_2CH_2CO), 22.7 (CH_2CH_3), 20.0 (t, J_{CF} 4.0 Hz, $CH_2CH_2CF_2$), 14.1 (CH_3).

Step 2: H_2 (1 L/min) was bubbled through a suspension of *N*-[11-(*F*-butyl)undecanoyl]-*O*-

benzyl-D,L-serine tetradecylamide (3.1 g, 4.0 mmol) in MeOH (40 mL) and AcOH (10 mL) at 50 °C and containing Pd/C (0.31 g, 10% w/w). After cooling, the mixture was diluted with $CHCl_3$, filtered through Celite. The organic phase was washed with water, dried over Na_2SO_4, filtered and evaporated to dryness under diminished pressure affording compound **7a(D,L)** (2.8 g, 100%) as a white solid: R_f 0.25 (49:1 $CHCl_3$–MeOH). ^{1}H NMR ($CDCl_3$): δ 7.15 (m, 1 H, CH_2NH), 6.85 (d, J 7.1 Hz, 1 H, CHN*H*), 4.45 (m, 1 H, OCH_2CH), 4.05 and 3.50 (AB part of an ABX system, J_{AB} 11.5, J_{AX} 4.7, J_{BX} 3.0 Hz, 2 H, CH_2OH), 3.20 (td, J 6.7, J 6.3 Hz, 2 H, $NHCH_2$), 2.20 [t, J 7.2 Hz, 2 H, $CH_2C(O)$], 2.00 (tt, J 7.5, J_{HF} 18.0 Hz, 2 H, CH_2CF_2), 1.70–1.10 [m, 40 H, $(CH_2)_8$ and $(CH_2)_{12}$], 0.85 (t, J 7.0 Hz, 3 H, CH_3). ^{13}C NMR ($CDCl_3$): δ 174.4 [$NHC(O)CH_2$], 171.0 [$CHC(O)NH$], 63.1 (CH_2OH), 53.9 (OCH_2CH), 39.6 ($NHCH_2$), 36.5 (CH_2CO), 32.0 ($CH_2CH_2CH_3$), 30.8 (t, J_{CF} 22 Hz, CH_2CF_2), 29.7, 29.6, 29.4, 29.3, and 29.1 [$(CH_2)_9$ and $(CH_2)_6$], 27.0 (CH_2CH_2NH), 25.7 (CH_2CH_2CO), 22.7 (CH_2CH_3), 20.1 (t, J_{CF} 4.0 Hz, $CH_2CH_2CF_2$), 14.1 (CH_3). ^{19}F NMR ($CDCl_3$): δ −80.9 (3 F, CF_3), −114.1 (2 F, CF_2CH_2), −124.0 (2 F, $CF_2CF_2CH_2$), −125.6 (2 F, CF_2CF_3).

Synthesis of the ***II-GalSer*** *derivatives*

N-*[11-(*F-*Butyl)undecanoyl]*-O-*β*-D-*galacto*-*pyranosyl*-D,L-*serine tetradecylamide* (**II-GalSer[C14][F4C11](D,L)**): Step 1: galactosylation of **7a(D,L)**. A solution of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (0.7 g, 1.7 mmol) in anhyd $CHCl_3$ (15 mL) was added dropwise to **7a(D,L)** (1.2 g, 1.7 mmol), Ag_2CO_3 (0.66 g, 2.4 mmol), I_2 (43 mg) and of 4 Å molecular sieves (3 g) in $CHCl_3$ (40 mL). The suspension was shaken in the dark at rt for 7 days, then filtered through Celite which was washed with $CHCl_3$ (100 mL). The combined filtrates were then concentrated under diminished pressure. Column chromatography (3:2 CH_2Cl_2–hexane) of the residue led to a solid (1.2 g, 1.2 mmol) consisting in the β anomer **8a(D,L)** and the orthoester **5a(D,L)**. Conversion of the orthoester **5a(D,L)** into β-**8a(D,L)** was performed by refluxing the obtained solid with $HgBr_2$ (21 mg, 0.06 mmol) of in anhyd nitromethane for 8 h (^{1}H NMR monitoring shows the disappearance of the singlet of the orthoester methyl proton at 1.6 ppm). After concentration and chromatography (99:1 $CHCl_3$–MeOH), diastereoisomeric β-anomer **8a(D,L)** (0.8 g, 64%) was obtained as a white solid [^{13}C NMR ($CDCl_3$–CD_3OD): C-1β at 101.6 and 102.2 ppm].

Step 2: acetyl-deprotection of **8a(D,L)**. **8a(D,L)** (0.35 g, 0.35 mmol) was solubilized in 2:1:1 MeOH–Et_3N–water mixture (10 mL). The reaction mixture was stirred at 30 °C for 15 h until total disappearance of the starting compound (TLC monitoring). After evaporation and chromatography ($CHCl_3$ to 3:7 $CHCl_3$–MeOH) of the residue, **II-GalSer[C14][F4C11](D,L)** (130 mg, 45%) was obtained as a white solid: R_f 0.51 (17:3 $CHCl_3$–MeOH). ^{1}H NMR ($CDCl_3$–CD_3OD): δ 4.55 (t, J 5.1 Hz, 1 H, OCH_2CH), 4.50–3.40 (m, 9 H, H-1–6 Gal and OCH_2), 3.20 (t, J 7.2 Hz, 2 H, $NHCH_2$), 2.20 [t, J 7.1 Hz, 2 H, $CH_2C(O)$], 2.10 (tt, J_{HF} 17.9, J 8.1 Hz, 2 H, CH_2CF_2), 1.70–1.10 [m, 40 H, $(CH_2)_{12}$ and $(CH_2)_8$], 0.81 (t, J 7.3 Hz, 3 H, CH_3). ^{13}C NMR ($CDCl_3$–CD_3OD): δ 174.4 and 174.2 [$NHC(O)CH_2$], 170.1 and 170.0 [$C(O)NHCH_2$], 103.8 and 103.3 (C-1β Gal), 75.0 and 74.7 (C-5 Gal), 73.3 (C-3 Gal), 71.0 (C-2 Gal), 69.0 (C-4 Gal), 69.3 and 68.8 (OCH_2), 61.7 and 61.4 (C-6 Gal), 52.8 and 52.5 (OCH_2CH), 39.6 ($NHCH_2$), 36.1 and 36.0 [$CH_2C(O)$], 31.8 ($CH_2CH_2CH_3$), 30.6 (t, J_{CF} 22 Hz, CH_2CF_2), 28.9–30.1 $(CH_2)_6$ and $(CH_2)_9$, 26.8 (CH_2CH_2NH), 25.4 [$CH_2CH_2C(O)$], 22.5 (CH_2CH_3), 19.9 (t, J_{CF} 4.0 Hz, $CH_2CH_2CF_2$), 13.9 (CH_3). ^{19}F NMR ($CDCl_3$–CD_3OD): identical to that of **7a(D,L)**. Anal. Calcd for $C_{38}H_{65}F_9N_2O_8 \cdot 1/2H_2O$ (857.92): C, 53.20; H, 7.75; N, 3.27. Found: C, 53.15; H, 7.75; N, 3.38.

N-*[11-(*F-*Hexyl)undecanoyl]*-O-*β*-D-*galacto*-*pyranosyl*-L-*serine hexadecylamide* (**II-GalSer[C16][F6C11](L)**): When applied to **7b(L)** the galactosylation procedure described for the synthesis of **I-GalSer[C14](D)** afforded, after silica gel chromatography [$CHCl_3$ to 49:1 $CHCl_3$–MeOH)], pure β-anomer **8b(L)** as a white solid (60% yield) [^{13}C NMR ($CDCl_3$–CD_3OD): C-1β at 101.1 ppm]. Deacetylation of **8b(L)** in a 5:1.5:1.5 MeOH–Et_3N–water mixture at 30 °C for 15 h and chromatography ($CHCl_3$ to 3:7 $CHCl_3$–MeOH) gave **II-**

GalSer[C16][F6C11](L) as a white solid (90% yield): R_f 0.51 (17:3 $CHCl_3$–MeOH). $[\alpha]_D$ + 3.4° (*c* 0.97, 4:1 $CHCl_3$–MeOH). ^1H NMR ($CDCl_3$–CD_3OD): δ 4.60 (t, *J* 6.1 Hz, 1 H, OCH_2CH), 4.25 (d, *J* 6.5 Hz, 1 H, H-1 Gal), 4.05 (dd, *J* 10.2, *J* 5.1 Hz, 1 H, H-3 or 4 or 5 Gal), 4.00–3.40 (m, 7 H, H-2 Gal, H-6 Gal, two of H-3–5 Gal, OCH_2), 3.20 (t, *J* 7.0 Hz, 2 H, $NHCH_2$), 2.20 (t, *J* 8.3 Hz, 2 H, CH_2CO), 2.00 (tt, 2 H, J_{HF} 18.2, *J* 8.1 Hz, CH_2CF_2), 1.70–1.10 [m, 44 H, $(CH_2)_{14}$ and $(CH_2)_8$,], 0.85 (t, 3 H, CH_3). ^{13}C NMR ($CDCl_3$–CD_3OD): δ 174.2 [$CH_2C(O)NH$], 170.0 [$C(O)NHCH_2$], 103.8 (C-1β Gal), 74.8 (C-5 Gal), 73.3 (C-3 Gal), 71.1 (C-2 Gal), 69.6 (OCH_2), 69.3 (C-4 Gal), 62.0 (C-6 Gal), 52.8 (OCH_2CH), 39.7 ($NHCH_2$), 36.2 (CH_2CO), 31.8 (CH_2CH_2-CH_3), 30.8 (t, J_{CF} 22 Hz, CH_2CF_2), 29.6 to 29.0 [$(CH_2)_6$ and $(CH_2)_{11}$], 26.8 (CH_2CH_2NH), 25.5 (CH_2CH_2CO), 22.6 (CH_2CH_3), 20.0 (t, J_{CF} 4.0 Hz, $CH_2CH_2CF_2$), 13.9 (CH_3). ^{19}F NMR ($CDCl_3$–CD_3OD): identical to that of **I-GalSer[F6C11](D,L)**. Anal. Calcd for $C_{42}H_{69}F_{13}N_2O_8 \cdot 3/2H_2O$ (1004.01): C, 50.24; H, 7.23; N, 2.79. Found: C, 50.30; H, 7.16; N, 2.70.

N-*[11-(*F-*Hexyl)undecanoyl]*-O-*β*-D-*galacto*-*pyranosyl*-L-*serine 11-(*F-*butyl)-undecylamide* (**II-GalSer[F4C11][F6C11](L)**): When applied to **7c(L)** the galactosylation procedure described for the synthesis of **I-GalSer[C14](D)** afforded pure β anomer **8c(L)** as a white solid (30% yield) [^{13}C NMR (CD_3OD): C-1β at 101.1 ppm]. Deacetylation of **8c(L)** in a 5:1.5:1.5 MeOH–Et_3N–water mixture at 30 °C for 15 h and chromatography ($CHCl_3$ to 4:1 $CHCl_3$–MeOH) gave **II-GalSer-[F4C11][F6C11](L)** (60% yield): R_f 0.52 (17:3 $CHCl_3$–MeOH). $[\alpha]_D$ + 2.1° (*c*, 0.98; 4:1 $CHCl_3$–MeOH). ^1H NMR ($CDCl_3$–CD_3OD): from 4.60 to 2.20 ppm identical to that of **II-GalSer[C16][F6C11](L)**, then 2.00 (tt, J_{HF} 18.2, *J* 8.1 Hz, 4 H, CH_2CF_2), 1.70–1.10 [m, 34 H, $(CH_2)_9$ and $(CH_2)_8$]. ^{13}C NMR ($CDCl_3$–CD_3OD): from 174.2 to 36.2 ppm identical to that of **II-GalSer[C16][F6C11](L)**, then 30.7 and 30.8 (t, t, J_{CF} 22.3 Hz, CH_2CF_2), 29.6–29.0 [$(CH_2)_5$ and $(CH_2)_7$], 26.8 (CH_2CH_2NH), 25.5 (CH_2CH_2CO), 20.0 (t, J_{CF} 3.5 Hz, $CH_2CH_2CF_2$). ^{19}F NMR ($CDCl_3$–CD_3OD): δ −81.4 and −81.6 (2 × 3 F, CF_3), −115.1 (4 F, CF_2CH_2), −122.5, −123.4, −124.1, and −125.2 [4 × 2 F, $(CF_2)_3CF_2CH_2$ and $CF_2CF_2CH_2$], −126.6 (4 F, CF_3CF_2). Anal. Calcd for $C_{41}H_{58}F_{22}N_2O_8 \cdot 2H_2O$ (1160.91): C; 42.42; H, 5.38; N, 2.41. Found: C, 42.35; H, 5.29; N, 2.25.

Synthesis of the single-chain ***I-GalCys*** *and double-chain* ***II-GalCys*** *derivatives*

Synthesis of aglycones **9**

N-*Benzyloxycarbonyl*-L-*cysteine tetradecyl-amide* (**9b**): Tetradecylamine (1.68 g, 7.90 mmol) and HOBt (1.06 g, 7.85 mmol) was added at 0 °C to a solution of *N*-*Z*-L-cysteine **13b** (2.0 g, 3.93 mmol) in DMF (8 mL). After 15 min of stirring, DCC (1.63 g, 7.93 mmol) was added to the mixture and the solution was stirred at rt for 1 h. The solvent was removed under diminished pressure and the residue taken up in $CHCl_3$ and filtered. The solution was successively washed with 5% $KHSO_4$ (2 × 30 mL), water (2 × 30 mL), 10% $NaHCO_3$ (2 × 30 mL) and water (3 × 30 mL). After drying over Na_2SO_4 and filtration, the solvent was evaporated and the residue purified by chromatography (49:1 $CHCl_3$–MeOH) giving *N*,*N*′-bis(benzyloxycarbonyl)-L-cystine bis(tetradecylamide) (**14b**, 3.0 g, 85%) as a white solid: R_f 0.8 (49:1 $CHCl_3$–MeOH). mp 164 °C. IR (*v* cm^{-1}, KBr): 1690 (C=O carbamate), 1650 (C=O amide). ^1H NMR ($CDCl_3$–CD_3OD): δ 7.30 (bs, 10 H, Ph), 5.04 (s, 4 H, OCH_2), 4.67 (m, 2 H, SCH_2CH), 3.10 (m, 4 H, $NHCH_2$), 2.90 (m, 4 H, SCH_2), 1.50–1.04 [m, 48 H, $(CH_2)_{12}$], 0.80 (t, *J* 6.2 Hz, 6 H, CH_3). ^{13}C NMR ($CDCl_3$–CD_3OD): δ 169.7 [$C(O)NH$], 155.7 [NHC(O)O], 136.0 (C, Ph), 128.6 and 128.3 (CH, ortho and meta Ph), 127.6 (CH, para Ph), 67.3 (OCH_2), 55.4 (SCH_2CH), 39.9 ($NHCH_2$), 32.0 ($CH_2CH_2CH_3$), 29.8, 29.7, 29.6, 29.5 [$(CH_2)_9$ and SCH_2], 27.1 ($NHCH_2CH_2$), 22.8 (CH_2CH_3), 14.2 (CH_3).

Dithiothreitol (0.72 g, 6.93 mmol) was added to a solution of **14b** (2.0 g, 2.27 mmol) and Et_3N (1 mL, 7.2 mmol) in $CHCl_3$ (80 mL). After stirring at rt for 24 h, the solution was washed with 5% $KHSO_4$ (3 × 30 mL), then water (3 × 30 mL). After drying over Na_2SO_4 and filtration, the solvent was evaporated and the residue purified by chromatography ($CHCl_3$) giving **9b** (1.4 g, 70%) as a

white solid: R_f 0.7 (49:1 $CHCl_3$–MeOH). mp 113 °C. IR (ν cm^{-1}, KBr): 1665 (C=O carbamate), 1650 (C=O amide), 695 (C–S). ^1H NMR ($CDCl_3$): δ 7.23 (bs, 5 H, Ph), 6.37 (bs, 1 H, N*H*CH_2), 5.78 (d, *J* 8.3 Hz, 1 H, CHN*H*), 5.04 (s, 2 H, OCH_2), 4.30 (m, 1 H, SCH_2CH), 3.10 (td, *J* 8.3, *J* 6.8 Hz, 2 H, NHC*H*$_2$), 3.00 (m, 1 H, SC*H*$_a$H$_b$), 2.66 (m,1 H, SCH$_a$*H*$_b$), 1.60–1.10 [m, 24 H, $(CH_2)_{12}$], 0.80 (t, *J* 6.2 Hz, 3 H, CH_3). ^{13}C NMR ($CDCl_3$): δ 169.7 [*C*(O)NH], 156.0 [NHC(O)O], 136.1 (C, Ph), 128.7 and 128.4 (CH, ortho and meta Ph), 128.2 (CH, para Ph) 67.4 (OCH_2), 57.0 (SCH_2CH), 39.9 ($NHCH_2$), 32.0 ($CH_2CH_2CH_3$), 30.0, 29.9, 29.8, 29.7, 29.6, 29.5, 29.4, 27.2 [$(CH_2)_9$ and SCH_2], 27.0 ($NHCH_2CH_2$), 22.8 (CH_2CH_3), 14.2 (CH_3).

N-*(Fmoc)*-L-*cysteine tetradecylamide* (**9a**): Method 1: from *N*-Fmoc-*S*-trityl-L-cysteine (**15**). DCC (166 mg, 0.80 mmol) was added to a stirred solution of **15** (469 mg, 0.80 mmol), HOBt (108 mg, 0.80 mmol) and tetradecylamine (172 mg, 0.806 mmol) in anhyd DMF (6 mL). After 12 h, the mixture was filtered, the solvent removed under diminished pressure, and the residue taken up in $CHCl_3$ and filtered. The solution was washed with 8% $NaHCO_3$, water, 5% $KHSO_4$ then water. The organic phase was dried over $MgSO_4$, then filtered. After evaporation and chromatography of the residue (7:3 CH_2Cl_2–Et_2O), *N*-Fmoc-3-*S*-trityl-L-cysteine tetradecylamide (**16**) was obtained as a white solid (571 mg, 80%): R_f 0.13 (CH_2Cl_2); 0.45 (19:1 CH_2Cl_2–Et_2O). $[\alpha]_D$ 24 + 8.3° (*c* 1.2; $CHCl_3$). mp 47–49 °C. IR (ν cm^{-1}, KBr): 3415 and 3300 (NH), 1700 (C=O carbamate), 1660 (C=O amide). ^1H NMR ($CDCl_3$): δ 7.78 (dd, *J* 7.2, *J* 3.0 Hz, 2 H, Fmoc), 7.59 (d, *J* 7.2 Hz, 2 H, Fmoc), 7.45 (m, 7 H, Tr), 7.26 (m, 12 H, Tr), 5.88 (t, *J* 5.7 Hz, 1 H, N*H*CH_2), 5.21 (d, *J* 7.9 Hz, 1 H, N*H*C(O)O), 4.41 (d, *J* 6.7 Hz, 2 H, C(O)OCH_2), 4.21 (t, *J* 6.7 Hz, 1 H, C(O)OCH_2CH), 3.84 (ddd, *J* 7.9, *J* 7.4, *J* 5.7 Hz, 1 H, SCH_2CH), 3.18 (dt, *J* 6.6, *J* 5.7 Hz, 2 H, NHC*H*$_2$), 2.73 (dd, *J* 13.1, *J* 7.4 Hz, 1 H, SC*H*$_a$H$_b$), 2.63 (dd, *J* 13.1, *J* 5.7 Hz, 1 H, SCH$_a$*H*$_b$), 1.89 (m, 2 H, $NHCH_2CH_2$), 1.30 (m, 22 H, $(CH_2)_{11}CH_3$), 0.93 (t, *J* 6.7 Hz, 3 H, CH_3). ^{13}C NMR ($CDCl_3$): δ 169.9 [C(O)NH], 156.0 [NHC(O)O], 144.5 (C, Ph), 143.8, 143.7 and 141.4 (C, Fmoc), 129.7 (CH, ortho Ph), 128.1 (CH, meta Ph), 127.8 and 127.1 (CH, Fmoc), 127.0 (CH, para Ph), 125.1 and 120.1 (CH, Fmoc), 67.4 [C(O)O*C*H$_2$], 67.1 [C(Ph)$_3$], 54.2 (SCH_2CH), 47.2 [C(O)OCH_2CH], 39.7 ($NHCH_2$), 34.2 (CH_2S), 32.0 ($CH_2CH_2CH_3$), 29.8, 29.7, 29.6, 29.4, 29.3 and 26.9 [$(CH_2)_{10}$], 22.8 (CH_2CH_3), 14.2 (CH_3).

Compound **16** (163 mg, 0.20 mmol) was stirred in TFA (4 mL) for 5 h. After evaporation, ether was added and the organic phase was washed successively with water, aq $NaHCO_3$ (8%), and water until neutrality. After drying over $MgSO_4$ and filtration, the solvent was evaporated and the residue purified by chromatography (19:1 CH_2Cl_2–Et_2O) giving **9a** (33 mg, 30%) as a white solid: R_f 0.45 (19:1 CH_2Cl_2–Et_2O). mp 140 °C. ^1H NMR ($CDCl_3$): δ 7.78 (d, *J* 6.9 Hz, 2 H, Fmoc), 7.59 (d, *J* 7.2 Hz, 2 H, Fmoc), 7.33 (m, 4 H, Fmoc), 6.34 (bs, 1 H, N*H*CH_2), 5.80 [bd, *J* 7.9 Hz, 1 H, NHC(O)O], 4.47 [m, 2 H, C(O)O*C*H$_2$], 4.38 (m, 1 H, SCH_2CH), 4.22 [t, *J* 6.4 Hz, 1 H, C(O)OCH_2CH], 3.25 (dt, *J* 6.6, *J* 5.9 Hz, 2 H, NHC*H*$_2$), 3.12 (m, 1 H, SC*H*$_a$H$_b$), 2.70 (m, 1 H, SCH$_a$*H*$_b$), 1.82 (s, 1 H, SH), 1.50 (m, 2 H, $NHCH_2CH_2$), 1.26 [m, 22 H, $(CH_2)_{11}CH_3$], 0.90 (t, *J* 6.4 Hz, 3 H, CH_3). ^{13}C NMR ($CDCl_3$): δ 169.3 [*C*(O)NH], 156.1 [NHC(O)O], 143.7, 141.4 (C, Fmoc), 127.9, 127.1, 125.0 and 120.1 (CH, Fmoc), 67.2 [C(O)O*C*H$_2$], 56.3 (SCH_2CH), 47.2 [C(O)OCH_2CH], 39.8 ($NHCH_2$), 32.0 ($CH_2CH_2CH_3$), 29.7, 29.6, 29.5, 29.4, 29.3, 27.0, 26.9 [$(CH_2)_{10}$ and $HSCH_2$], 22.7 (CH_2CH_3), 14.2 (CH_3).

Method 2: from *N*-Fmoc-cystine **13a**. The procedure described for the synthesis of **9b** from **13b** when applied to **13a**, afforded *N*-Fmoc-L-cystine bis(tetradecylamide) (**14a**, 90%). Compound **14a** was also obtained in 63% yield by stirring, for 30 min at rt, **16a** and iodine in MeOH followed by addition of a 1 N aq sodium thiosulfate solution, extraction with $CHCl_3$, washing with water, then drying on $MgSO_4$, evaporation and chromatography: R_f 0.55 (19:1 CH_2Cl_2–Et_2O). mp 193–195 °C. IR (ν cm^{-1}, KBr): 1695 (CO carbamate), 1660 (CONH). ^1H NMR ($CDCl_3$): δ 7.68 (d, *J* 7.2

Hz, 2 H, Fmoc), 7.52 (d, *J* 7.0 Hz, 2 H, Fmoc), 7.33 (m, 4 H, Fmoc), 5.96 [d, *J* 7.9 Hz, 1 H, NHC(O)O], 4.87 (bs, 1 H, N*H*CH_2), 4.45–4.15 [m, 4 H, C(O)OC*H*$_2$CH and SCH_2C*H*], 3.15 (m, 2 H, NHC*H*$_2$), 2.95 (m, 2 H, SCH_2), 1.50–1.10 [m, 24 H, (C*H*$_2$)$_{12}CH_3$], 0.89 (t, *J* 6.3 Hz, 3 H, CH_3). ^{13}C NMR ($CDCl_3$): δ 169.7 [*C*(O)NH], 156.0 [NHC(O)O], 143.7, 141.4 (C, Fmoc), 127.9, 127.1, 125.0 and 120.1 (CH, Fmoc), 67.8 [C(O)O*C*H$_2$], 57.8 (SCH_2*C*H), 47.2 [C(O)-OCH_2*C*H], 39.2 ($NHCH_2$), 32.1 (*C*H_2CH_2-CH_3), 29.8, 29.7, 29.6, 29.5, 29.4, 29.2 [(*C*H$_2$)$_9$ and SCH_2], 27.1 ($NHCH_2$*C*H_2), 22.7 (*C*H_2-CH_3), 14.3 (CH_3).

Four portions of Zn (1.7 g) were added over 6 h to **14a** (2.7 g) in AcOH (30 mL) at 60 °C. The mixture was then diluted with $CHCl_3$ and filtered on Celite and washed with water until neutrality. The crude product was purified by chromatography ($CHCl_3$) giving **9a** (2.5 g, 90%).

Synthesis of **I-GalCys[C14](L)**

3-S-*(2,3,4,6-Tetra*-O-*acetyl-β*-D-*galacto-pyranosyl)*-N-*Fmoc*-L-*cysteine tetradecylamide* (**10a**): A solution of **9a** (0.21 g, 0.40 mmol), 1,2,3,4,6-penta-*O*-acetyl-D-galactopyranose (0.19 g, 0.48 mmol) and $BF_3{\cdot}Et_2O$ (250 μL, 2 mmol) in dry CH_2Cl_2 (5 mL) was stirred at rt for 24 h under anhyd N_2. The mixture was then diluted with CH_2Cl_2, washed with 10% $NaHCO_3$, then water until neutrality. After drying over Na_2SO_4 and filtration, the solvent was evaporated and the residue purified by chromatography (CH_2Cl_2) giving **10a** (0.32 g) as an oily compound (93%): R_f 0.40 (9:1 CH_2Cl_2–Et_2O). $[\alpha]_D$ 24 + 9.5° (*c* 0.67; $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.67 (d, *J* 7.2 Hz, 2 H, Fmoc), 7.51 (d, *J* 7.0 Hz, 2 H, Fmoc), 7.33 (m, 4 H, Fmoc), 6.53 (bs, 1 H, N*H*CH_2), 6.07 [d, *J* 7.3 Hz, 1 H, N*H*C(O)O], 5.34 (bd, 1 H, H-4 Gal), 5.18 (dd, $J_{2,3}$ 9.9 and $J_{1,2}$ 9.7 Hz, 1 H, H-2 Gal), 5.02 (dd, $J_{2,3}$ 9.9, $J_{3,4}$ 2.9 Hz, 1 H, H-3 Gal), 4.55 (d, *J* 9.7 Hz, 1 H, H-1 Gal), 4.30–3.95 [m, 7 H, H-5–6 Gal, C(O)OC*H*$_2$CH and SCH_2C*H*], 3.16 (m, 3 H, CH_2NH and SCH_aH_b), 2.82 (m, 1 H, SCH_aH_b), 2.02, 1.95, 1.89, and 1.87 [all s, all 3 H, $CH_3C(O)$], 1.40 (bs, 2 H, $NHCH_2CH_2$), 1.15 [m, 22 H, $(CH_2)_{11}$], 0.82 (t, *J* 6.1 Hz, 3 H, CH_3). ^{13}C NMR ($CDCl_3$): δ 170.4, 170.1, 169.9, 169.7, 169.6 [CH_3C(O) and C(O)NH], 156.0 [NHC(O)O], 143.7, 141.4, 141.3 (C, Fmoc), 127.9, 127.2, 125.1, 120.1 (CH, Fmoc), 85.6 (C-1β Gal) 75.1, 71.6, 67.0, 66.0 (C-2–5 Gal), 67.5 [C(O)OCH_2], 62.0 (C-6 Gal), 54.6 (SCH_2CH), 47.2 [C(O)OCH_2CH], 39.8 ($NHCH_2$), 34.2 (SCH_2), 32.0 (*C*$H_2CH_2CH_3$), 29.8, 29.7, 29.6, 29.5, 29.4, and 29.3 [(*C*H$_2$)$_9$], 26.9 (*C*H_2CH_2N), 22.7 (*C*H_2CH_3), 20.8 and 20.6 [*C*H_3C(O)], 14.2 (CH_2CH_3).

3-S-*(2,3,4,6-Tetra*-O-*acetyl-β*-D-*galacto-pyranosyl)*-N-*benzyloxycarbonyl*-L-*cysteine tetradecylamide* (**10b**): The above procedure described for the synthesis of **10a** when applied to **9b**, gave **10b** as an oily compound (78%): R_f 0.45 (9:1 CH_2Cl_2–Et_2O). ^{1}H NMR ($CDCl_3$): δ 7.27 (bs, 5 H, Ph), 6.52 (bs, 1 H, $NHCH_2$), 6.03 [d, *J* 7.1 Hz, 1 H, N*H*C(O)O], 5.36 (bs, 1 H, H-4 Gal), 5.22–4.96 (m, 2 H, H-2-3 Gal), 5.02 [s, 2 H, NHC(O)OCH_2], 4.55 (d, *J* 9.7 Hz, 1 H, H-1 Gal), 4.32–4.03 (m, 7 H, H-5–6 Gal, C(O)OC*H*$_2$CH and SCH_2C*H*), 3.17 (m, 3 H, CH_2NH and SCH_aH_b), 2.79 (m, 1 H, SCH_aH_b), 2.03, 1.98, 1.91, and 1.89 [all s, all 3 H, CH_3C(O)], 1.40 (bs, 2 H, $NHCH_2CH_2$), 1.15 [m, 22 H, $(CH_2)_{11}$], 0.80 (t, *J* 6.5 Hz, 3 H, CH_3). ^{13}C NMR ($CDCl_3$): δ 170.4, 170.1, 169.9, 169.7, 169.6 [CH_3C(O) and *C*(O)NH], 156.0 [NHC(O)O], 136.3 (C, Ph), 128.6 and 128.2 (CH, ortho and meta Ph), 128.2 (CH, para Ph) 85.3 (C-1β Gal), 75.1, 71.6, 67.5 and 66.8 (GalC-2–5), 67.1 [C(O)OCH_2], 62.0 (C-6 Gal), 54.5 (SCH_2*C*H), 39.7 ($NHCH_2$), 33.9 (SCH_2), 32.0 (*C*$H_2CH_2CH_3$), 29.8, 29.7, 29.6, 29.5, 29.4, and 29.3 [(*C*H$_2$)$_9$], 26.9 (*C*H_2CH_2N), 22.7 (*C*H_2CH_3), 20.8 and 20.6 [*C*H_3C(O)], 14.2 (CH_2CH_3).

3-S-*(2,3,4,6-Tetra*-O-*acetyl-β*-D-*galacto-pyranosyl)*-L-*cysteine tetradecylamide* (**11**): Compound **10a** (155 mg, 0.18 mmol) and morpholine (1.2 mL, 14 mmol) in $CHCl_3$ (2.4 mL) was stirred at rt for 8 h. After evaporation of the solvent, the crude product was purified by chromatography on silica gel (1:1 CH_2Cl_2–Et_2O) giving **11** (90 mg, 78%) as an oily compound: R_f 0.05 (4:1 CH_2Cl_2–Et_2O). $[\alpha]_D$ 24 − 11.3° (*c* 0.79; $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.38 (t, *J* 5.6 Hz, 1 H, N*H*CH_2), 5.37 (d, $J_{3,4}$ 3.2 Hz, 1 H, H-4 Gal), 5.16 (dd, $J_{2,3}$ 9.9, $J_{1,2}$ 9.8 Hz, 1 H, H-2 Gal), 5.00 (dd, $J_{2,3}$ 9.9 Hz, 1 H, H-3 Gal), 4.57 (d, *J* 9.8 Hz,

1 H, H-1 Gal), 4.20–3.90 (m, 3 H, H-5–6 Gal), 3.44 (ddd, *J* 3.9, *J* 8.0 Hz, 1 H, SCH_2CH), 3.27 (dd, *J* 13.9, *J* 3.8 Hz, 2 H, SCH_aCH_b), 3.22 (m, 2 H, CH_2NH), 2.88 (dd, *J* 13.9, *J* 8.0 Hz, 1 H, SCH_aH_b), 1.91, 2.00 and 2.10 [all s, 12 H, $CH_3C(O)$], 1.85 (bs, 2 H, NH_2), 1.10–1.50 [m, 24 H, $(CH_2)_{12}$], 0.80 (t, *J* 6.4 Hz, 3 H, CH_3). ^{13}C NMR ($CDCl_3$): δ 171.4 [*C*(O)NH], 170.6, 170.2, 170.0, and 169.7 [$CH_3C(O)$], 84.4 (C-1 Gal), 74.8, 71.4, 67.4, and 67.2 (C-2–5 Gal), 61.7 (C-6 Gal), 54.7 (SCH_2CH), 39.6 ($NHCH_2$), 35.5 (CH_2S), 32.0 ($CH_2CH_2CH_3$), 29.7, 29.6, and 29.4 [$(CH_2)_9$], 27.1 (CH_2CH_2NH), 22.8 (CH_2CH_3), 20.8, 20.7, and 20.6 [$CH_3C(O)$], 14.2 (CH_3).

3-S-*(β*-D-*Galactopyranosyl)*-L-*cysteine tetradecylamide* (**I-GalCys[C14](L)**): A solution of **11** (90 mg, 0.14 mmol) in 2:1:1 MeOH–Et_3N–water (2.5 mL) was stirred at rt for 1 h. After evaporation of the solvents, the crude product was purified by chromatography on silica gel (4:1 to 3:2 $CHCl_3$–MeOH) giving **I-GalCys[C14](L)** (40 mg, 60%) as a white powder: R_f 0.25 (76:21:3 $CHCl_3$–MeOH–water). $[\alpha]_D$ −7.3° (*c* 0.80; MeOH). ^1H NMR ($CDCl_3$–CD_3OD): δ 4.33 (d, *J* 9.3 Hz, 1 H, H-1 Gal), 3.89 (bd, *J* 2.8 Hz, 1 H, H-4 Gal), 3.86–3.56 (m, 5 H, SCH_2CH, H-2 Gal and H-5–6 Gal), 3.48 (dd, $J_{2,3}$ 9.1, $J_{3,4}$ 3.2 Hz, 1 H, H-3 Gal), 3.21 (t, *J* 6.8 Hz, 2 H, $NHCH_2$), 3.12 (dd, *J* 14.2, *J* 5.3 Hz, 1 H, SCH_aH_b), 2.83 (dd, *J* 14.2, *J* 8.2 Hz, 1 H, SCH_aH_b), 1.70–1.50 (m, 2 H, $NHCH_2CH_2$), 1.40–1.20 [m, 22 H, $(CH_2)_{11}$], 0.89 (t, *J* 6.6 Hz, 3 H, CH_3). ^{13}C NMR ($CDCl_3$–CD_3OD): δ 172.9 [C(O)NH], 86.0 (C-1β Gal), 79.2 (C-5 Gal), 74.6 (C-3 Gal), 69.5 (C-2 Gal), 69.2 (C-4 Gal), 61.7 (C-6 Gal), 54.7 (SCH_2CH), 39.4 ($NHCH_2$), 34.7 (SCH_2), 31.7 ($CH_2CH_2CH_3$), 29.4 and 29.1 [$(CH_2)_9CH_2CH_2CH_3$], 26.8 ($NHCH_2CH_2$), 22.4 (CH_2CH_3), 13.7 (CH_3). Anal. Calcd for $C_{23}H_{46}N_2O_6S{\cdot}1/2H_2O$ (487.69): C; 56.64; H, 9.71; N, 5.74; S 6.57. Found: C, 56.23; H, 9.44; N, 6.44.

Synthesis of **II-GalCys** *derivatives*

N-*Tetradecanoyl*-*3*-S-*(β*-D-*galactopyranosyl)*-L-*cysteine tetradecylamide* (**II-GalCys[C14][C14](L)**): DCC (40 mg, 0.19 mmol) was added at 0 °C to a mixture of **11** (115 mg, 0.178 mmol), tetradecanoic acid (45 mg, 0.19 mmol), and HOBt (27 mg, 0.19 mmol) in DMF (2 mL). The resulting mixture was stirred at rt overnight. After evaporation of the solvent, the residue was taken up in $CHCl_3$ and filtered. The solution was successively washed with 5% $KHSO_4$, water, 10% $NaHCO_3$, then water until neutrality. After drying over Na_2SO_4 and filtration, the solvent was evaporated and the residue purified by chromatography ($CHCl_3$) giving *N*-tetradecanoyl-3-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-L-cysteine tetradecylamide (**12**, 120 mg, 80%) as a white solid: R_f 0.40 (49:1 $CHCl_3$–MeOH). ^1H NMR ($CDCl_3$–CD_3OD): δ 5.39 (bs, H-4 Gal, 1 H), 5.08 (m, 2 H, H-2-3 Gal), 4.67 (d, *J* 8.6 Hz, 1 H, H-1 Gal), 4.08 (m, 4 H, SCH_2CH and H-5–6 Gal), 3.14 (m, 3 H, $NHCH_2$ and SCH_aCH_b), 2.87 (m, 1 H, SCH_aCH_b), 2.15 [t, *J* 7.1 Hz, 2 H, $NHC(O)CH_2$], 2.10, 1.99, 1.98, and 1.92 [all s, 3 H, $CH_3C(O)$], 1.50–1.30 [m, 4 H, $NHCH_2CH_2$ and $NHC(O)CH_2CH_2$], 1.30–1.10 [m, 42 H, $(CH_2)_{11}CH_3$ and $(CH_2)_{10}CH_3$], 0.80 (t, *J* 6.6 Hz, 6 H, CH_3CH_2). ^{13}C NMR ($CDCl_3$–CD_3OD): δ 174.1, 170.7, 170.4, 170.2, 170.1, and 169.9 [C(O)NH and $CH_3C(O)$], 84.6 (C-1 Gal), 74.5, 71.7, 67.5, and 67.2 (C-2–5 Gal), 61.7 (C-6 Gal), 52.7 (SCH_2CH), 39.4 ($NHCH_2$), 36.0 [$NHC(O)CH_2$], 32.8 (SCH_2), 31.7 ($CH_2CH_2CH_3$), 29.4, 29.1, and 29.0 [$(CH_2)_9CH_2CH_2CH_3$ and $(CH_2)_8CH_2CH_2CH_3$], 26.7 ($NHCH_2CH_2$), 25.5 [$NHC(O)CH_2CH_2$], 22.4 (CH_2CH_3), 20.2 and 20.1 [$CH_3C(O)$], 13.6 (CH_3).

A solution of **12** (120 mg, 0.14 mmol) in a 2:1:1 MeOH–Et_3N–water (2.5 mL) was then stirred at rt for 6 h. After evaporation to dryness under diminished pressure, the crude residue was purified by chromatography ($CHCl_3$ to 3:2 $CHCl_3$–MeOH) giving **II-GalCys[C14][C14](L)** (70 mg, 73%) of as a white solid: R_f 0.71 (76:21:3:$CHCl_3$–MeOH–water). $[\alpha]_D$ −7.1° (*c* 0.80; 4:1 $CHCl_3$–MeOH). ^1H NMR ($CDCl_3$–CD_3OD): δ 4.56 (dd, *J* 6.5 Hz, 1 H, SCH_2CH), 4.31 (d, *J* 9.1 Hz, 1 H, H-1 Gal), 3.85–3.46 (m, 5 H, H-2 Gal and H4-6 Gal), 3.41 (dd, $J_{2,3}$ 9.2, $J_{3,4}$ 2.9 Hz, 1 H, H-3 Gal), 3.15 (t, *J* 7.1 Hz, 2 H, $NHCH_2$), 2.82 (m, 2 H, SCH_2), 2.15 [t, *J* 7.5 Hz, 2 H, $C(O)CH_2$], 1.60–1.40 [m, 4 H, $NHCH_2CH_2$ and $C(O)CH_2CH_2$], 1.30–1.10 [m, 42 H, $(CH_2)_{11}$ and $(CH_2)_{10}$], 0.80 (t, *J* 6.0 Hz, 6 H, CH_3). ^{13}C

NMR ($CDCl_3$–CD_3OD): δ 174.3 and 170.5 [C(O)NH], 87.2 (C-1 Gal), 79.3, 74.8, 70.2, and 69.4 (C-2–5 Gal), 62.1 (C-6 Gal), 53.5 (SCH_2CH), 39.6 ($NHCH_2$), 36.2 [$C(O)CH_2$], 33.2 (SCH_2), 31.8 ($CH_2CH_2CH_3$), 29.6, 29.4, 29.2, and 29.1 [$(CH_2)_9CH_2CH_2CH_3$ and $(CH_2)_8CH_2CH_2CH_3$], 26.8 ($NHCH_2CH_2$), 25.6 [$C(O)CH_2CH_2$], 22.6 (CH_2CH_3), 13.8 (CH_3). Anal. Calcd for $C_{37}H_{72}N_2O_7S\cdot H_2O$ (707.07): C, 62.85; H, 10.55; N, 3.96; S, 4.54. Found: C, 62.65; H, 10.45; N, 3.97; S, 4.39.

Synthesis of the **II-GalAE** *and* **II-GalBAE** *derivatives*

N-*[2-(β-D-Galactopyranosyloxy)ethyl]*-N-*(hexadecyl)*-7-*(*F-*octyl)*-heptanamide (**II-GalAE[C16][F8C7]**). Step 1: *N*-Boc-ethanolamine (15.1 g, 94 mmol) and benzyl chloride (17.8 g, 1.5 eq, 141 mmol) in CH_2Cl_2 (40 mL) were added to an aq NaOH (10 eq, 37.8 g) solution (60 mL) and Bu_4NHSO_4 (0.07 eq, 2.26 g). The mixture was refluxed under vigorous stirring for 24 h. The organic phase was then washed until neutrality and dried over Na_2SO_4. After filtration, evaporation and chromatography (hexane), 2-benzyloxy-*N*-Boc-ethylamine (**17**, 23 g, 98%) as a white solid was obtained: R_f 0.32 (7:3 hexane–Et_2O). ^{1}H NMR ($CDCl_3$): δ 7.24, (m, 5 H, Ph), 4.98 (bs, 1 H, NH), 4.40 (s, 2 H, OCH_2Ph), 3.42 (t, *J* 5.1 Hz, 2 H, CH_2CH_2O), 3.21 (m, 2 H, CH_2N), 1.35 [s, 9 H, $(CH_3)_3$]. ^{13}C NMR ($CDCl_3$): δ 156.0 (CO), 138.2 (C, Ph), 128.6 and 127.9 (CH, ortho and meta Ph), 127.8 (CH, para Ph), 80.0 [$C(CH_3)_3$], 73.2 (OCH_2Ph), 69.4 (CH_2CH_2O), 41.0 (CH_2N), 28.6 (CH_3).

Step 2: compound **17** (23 g, 91.6 mmol) and TFA (20 mL) were stirred overnight at rt. After evaporation, the residue was dissolved in CH_2Cl_2, washed with 10% Na_2CO_3. The aq phase was extracted several times with CH_2Cl_2. The combined organic phases were dried over Na_2SO_4, filtered and evaporated to dryness under diminished pressure. The crude 2-(benzyloxy)ethylamine dissolved in Et_2O (200 mL) was precipitated as its HCl salt **18** (10.4 g 61%) with 2 mL of concentrated HCl (37% w/v): ^{1}H NMR (CD_3OD): δ 7.34 (m, 5 H, Ph), 4.59 (s, 2 H, OCH_2Ph), 3.68 (t, *J* 5.1 Hz, 2 H, CH_2CH_2O), 3.14 (t, *J* 5.1 Hz, 2 H CH_2N). ^{13}C NMR (CD_3OD): δ 138.8 (C, Ph), 129.4 and 128.9 (CH, ortho and meta Ph), 128.8 (CH, para Ph), 74.1 ($PhCH_2$), 66.7 (OCH_2), 40.6 (CH_2N).

Step 3: hexadecanoyl chloride (8.9 g, 32 mmol) in $CHCl_3$ (25 mL) was added dropwise at rt to a mixture of 2-(benzyloxy)-ethylamine·HCl (3.2 g, 17 mmol) and NEt_3 (7.3 mL, 52 mmol) in $CHCl_3$ (50 mL). The mixture was further stirred for 48 h, then evaporated to dryness. The crude residue was chromatographed on silica gel (9:1 $CHCl_3$–hexane) giving *N*-[2-(benzyloxy)ethyl]-hexadecylamide (**19**, 5.2 g, 78%) as a white powder: R_f 0.7 (49:1 $CHCl_3$–MeOH). IR (ν cm^{-1}, KBr): 3450 (NH), 1653 (C=O). ^{1}H NMR ($CDCl_3$): δ 7.26 (m, 5 H, Ph), 4.42 (s, 2 H, OCH_2Ph), 3.50–3.30 (m, 4 H, NCH_2CH_2O), 2.06 [t, *J* 7.2 Hz, 2 H, $CH_2C(O)$], 1.70–1.45 [m, 2 H, $CH_2CH_2C(O)$], 1.32–1.12 [m, 24 H, $(CH_2)_{12}$], 0.79 (t, *J* 6.1 Hz, 3 H, CH_3). ^{13}C NMR ($CDCl_3$): δ 173.2 (CO), 137.9 (C, Ph), 128.5 and 127.8 (CH, ortho and meta Ph), 127.8 (CH, para Ph), 73.1 (OCH_2Ph), 69.1 (CH_2OBn), 39.2 [$CH_2C(O)$], 36.8 (NCH_2), 31.9 ($CH_2CH_2CH_3$), 29.7, 29.6, 29.5, 29.4, and 29.3 [$(CH_2)_{10}$], 25.7 [$CH_2CH_2C(O)$], 22.7 (CH_2CH_3), 14.1 (CH_3).

Step 4: compound **19** (5.2 g, 13 mmol) in THF (25 mL) was added dropwise to $LiAlH_4$ (1.6 g, 43 mmol) suspended in 75 mL of Et_2O at 0 °C. After stirring at rt for 48 h and hydrolysis with a 1:1 THF–water solution, the organic phase was washed with water until neutrality, giving *N*-[2-(benzyloxy)ethyl]-hexadecylamine (**20**, 4.9 g, 100%) as a white solid: R_f 0.1 (49:1 $CHCl_3$–MeOH). ^{1}H NMR (CD_3OD): δ 7.36 (m, 5 H, Ph), 4.56 (s, 2 H, OCH_2Ph), 3.66 (t, *J* 5.1 Hz, 2 H, CH_2OBn), 2.90 (t, *J* 5.1 Hz, 2 H, OCH_2CH_2N), 2.68 [t, *J* 7.2 Hz, 2 H, $NCH_2(CH_2)_{14}$], 1.32 [m, 28 H $(CH_2)_{14}$], 0.93 (t, *J* 6.1 Hz, 3 H, CH_3). ^{13}C NMR (CD_3OD): δ 139.4 (C, Ph), 129.9 and 128.6 (CH, ortho and meta Ph), 128.8 (CH, para Ph), 74.0 (OCH_2Ph), 68.9 (CH_2OBn), 47.6 and 50.1 (NCH_2), 33.0 ($CH_2CH_2CH_3$), 30.7, 30.6, 30.5, 30.4, and 29.5 [$(CH_2)_{11}$], 28.1 ($CH_2CH_2CH_2N$), 23.6 (CH_2CH_3), 14.4 (CH_3).

Step 5: 7-(*F*-octyl)heptanoyl chloride (9.6 g, 17 mmol) in $CHCl_3$ (10 mL) was added dropwise at rt to a mixture of **20** (4.9 g, 13 mmol) and NEt_3 (5.5 mL, 39 mmol) in $CHCl_3$ (50

mL). The resulting mixture was stirred for 48 h, then evaporated to dryness. The crude residue was chromatographed on silica gel ($CHCl_3$) giving *N*-hexadecyl-*N*-[2-(benzyloxy)ethyl]-7-(F-octyl)heptylamide (**21**, 9.9 g, 83%) as a white solid: R_f 0.7 (49:1 $CHCl_3$–MeOH). IR (ν cm^{-1}, KBr): 1649 (C=O). ^1H NMR ($CDCl_3$): δ 7.23 (m, 5 H, Ph), 4.44 and 4.43 (s, s, 2 H, OCH_2Ph), 3.49 and 3.47 (2 t, *J* 4.4 Hz, 4 H, CH_2NCH_2), 3.25 (bt, *J* 5.1 Hz, 2 H, CH_2OBn), 2.24 and 2.25 [2 t, *J* 6.0 Hz, 2 H, $CH_2C(O)$], 2.16–1.76 (m, 2 H, CH_2CF_2), 1.58–1.16 [m, 36 H, $(CH_2)_4$ and $(CH_2)_{14}$], 0.80 (t, *J* 6.1 Hz, 3 H, CH_3). ^{13}C NMR ($CDCl_3$): δ 172.9 and 172.7 (CO), 138.3 (C, Ph), 128.4 and 127.6 (CH, ortho and meta Ph), 127.8 (CH, para Ph), 73.4 and 73.1 (OCH_2Ph), 68.8 and 68.3 (CH_2OBn), 49.4, 47.6, 46.3, and 46.1 (CH_2NCH_2), 32.9 and 32.8 [$CH_2C(O)$], 30.9 ($CH_2CH_2CH_3$), 29.7, 29.6, 29.4, 29.3, 29.1, 29.0, 27.7, 27.0, and 26.9 [$(CH_2)_4$ and $(CH_2)_{13}$], 25.1 [$CH_2CH_2C(O)$], 22.7 (CH_2CH_3), 20.1 (t, J_{CF} 5 Hz, $CH_2CH_2CF_2$), 14.1 (CH_3). ^{19}F NMR ($CDCl_3$): δ −81.3 (3 F, CF_3), −114.9 (2 F, CF_2CH_2), −122.4 (2 F, $CF_2CF_2CH_2$), −123.3 (2 F, $CF_2\gamma$ CH_2), −124.1 (2 F, $CF_2\delta$ CH_2), −126.7 (2 F, CF_2CF_3).

Step 6: hydrogen was bubbled through a suspension of Pd/C (0.9 g. 10% w/v) and **21** (9.9 g) in MeOH (30 mL) and AcOH (10 mL) for 4 h. After filtration, evaporation and chromatography (99:1 $CHCl_3$–MeOH), *N*-hexadecyl-*N*-[2-hydroxyethyl]-7-(F-octyl)heptylamide (**22**, 7.9 g, 88%), as a white solid was obtained: R_f 0.2 (49:1 $CHCl_3$–MeOH). IR (ν cm^{-1}, KBr): 1647 (C=O). ^1H NMR ($CDCl_3$): δ 3.71 (m, 2 H, CH_2OH), 3.48 (t, *J* 5.2 Hz, 2 H, NCH_2CH_2O), 3.22 (t, *J* 6.9 Hz, 2 H, $NCH_2CH_2CH_2$), 2.31 [t, *J* 7.2 Hz, 2 H, $CH_2C(O)$], 2.10 (2 t, *J* 8.2, J_{HF} 16.5 Hz, 2 H, CH_2CF_2), 1.65–1.48 (m, 4 H, CH_2CH_2CO and $CH_2CH_2CF_2$), 1.48–1.15 [m, 32 H, $(CH_2)_2$ and $(CH_2)_{14}$], 0.80 (t, *J* 6.1 Hz, 3 H, CH_3). ^{13}C NMR ($CDCl_3$): δ 62.9 (CH_2OH), 49.7 and 50.1 (CH_2NCH_2), 32.9 [$CH_2C(O)$], 31.9 ($CH_2CH_2CH_3$), 30.8 (t, J_{CF} 24 Hz, CH_2CF_2), 29.7, 29.6, 29 5, 29.3, 29.0, 28.9, 27.0, 26.8, and 25.0 [$(CH_2)_2$ and $(CH_2)_{13}$], 22.6 (CH_2CH_3), 20.0 (t, J_{CF} 5 Hz, $CH_2CH_2CF_2$), 14.0 (CH_3). ^{19}F ($CDCl_3$): identical to that of **21**. Anal. Calcd for $C_{33}H_{50}F_{17}NO_2$ (815.744): C, 48.58; H, 6.17; N, 1.71. Found: C, 48.56; H, 6.02; N, 1.97.

Step 7: the galactosylation procedure described for the preparation of **2a**(L), when applied to **22** (1.0 g, 1 eq, 1.23 mmol) of $GalOC(=NH)CCl_3$ (0.76 g, 1.25 eq, 1.54 mmol), and TMSOTf (0.06 mL, 0.33 mmol), afforded after workup and chromatography, *N*-[2-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyloxy)ethyl] - *N* - (hexadecyl) - 7 - (*F* - octyl)-heptylamide (**23**, 1.3 g). Deacetylation of **23** (1.3 g, 1.13 mmol) in 2:1:1 MeOH–Et_3N–water and workup as described for the preparation of **I-GalSer[C14]**(L) gave **II-GalAE[C16][F8C7]** (0.38 g, 34%): ^1H NMR ($CDCl_3$): δ 4.80–2.95 (m, 13 H, OCH_2CH_2N, CH_2N, H-1–6 Gal), 2.23 [t, *J* 7.2 Hz, 2 H, $CH_2C(O)$], 1.98 (m, 2H, CH_2CF_2), 1.51 [m, 4 H, $CH_2CH_2C(O)$, $CH_2CH_2CH_2N$], 1.30–1.17 [m, 32 H, $CH_3(CH_2)_{13}$, $(CH_2)_3CH_2CH_2C(O)$], 0.80 (t, *J* 6.4 Hz, 3 H, CH_3). ^{13}C NMR ($CDCl_3$): δ 174.0 (CO), 103.8 (C-1 Gal), 74.8 (C-5 Gal), 73.9 (C-3 Gal), 71.3 (C-2 Gal), 68.9 (C-4 Gal), 66.8 (OCH_2CH_2N), 61.5 (C-6 Gal), 48.8 (OCH_2CH_2N), 45.7 ($CH_2CH_2CH_2N$), 33.1 [$CH_2C(O)$], 32.0 ($CH_2CH_2CH_3$), 30.9 (t, J_{CF} 22.9 Hz, CH_2CF_2), 29.8–29.1 [$(CH_2)_{11}CH_2CH_2N$ and $(CH_2)_2CH_2CH_2CF_2$], 27.0 ($CH_2CH_2CH_2N$), 25.2 [$CH_2CH_2C(O)$], 22.8 (CH_3CH_2), 20.2 (bs, $CH_2CH_2CF_2$), 14.1 (CH_3). ^{19}F NMR ($CDCl_3$): identical to that of **21**. Anal. Calcd for $C_{39}H_{60}F_{17}NO_7$ (977.888): C, 47.90; H, 6.18; N, 1.43. Found: C, 47.45; H, 6.14; N, 1.58.

N^1-*[2-(β-D-Galactopyranosyloxy)ethyl]*-N^{24}-*(2 - hydroxyethyl)* - N^1,N^{24} - *bis[5 - (F - hexyl)-pentanoyl]-1,24-tetracosanediamide* (**II-GalBAE-[C24][F6C5](OH)**) *and* N^1,N^{24}-*bis[2-(β-D-galactopyranosyloxy)ethyl]* - N^1,N^{24} - *bis[5 - (F-hexyl)pentanoyl]-1,24-tetracosanediamide* (**II-GalBAE[C24][F6C5](Gal)**). Step 1: tetracosanedioyl dichloride (1.82 g, 4.2 mmol), 2-(benzyloxy)ethylammonium chloride (**18**, 1.9 g, 10.4 mmol) and Et_3N (5.2 mL, 37.6 mmol) in anhyd CH_2Cl_2 (100 mL) were stirred at 50 °C for 48 h. The organic phase was then washed with water until neutrality, dried over Na_2SO_4, filtered and evaporated to dryness under diminished pressure. After chromatog-

raphy (49:1 $CHCl_3$–MeOH) of the residue, N^1,N^{24} - bis[2 - (benzyloxy)ethyl] - 1,24 - tetracosanediamide (**24**, 1.96 g, 71%) was obtained as a white solid: R_f 0.56 (49:1 $CHCl_3$–MeOH). IR (ν cm^{-1}, KBr): 3320 (NH), 1640 (C=O). ^1H NMR ($CDCl_3$): δ 7.26 (m, 10 H, Ph), 5.70 (m, 2 H, NH), 4.44 (s, 4 H, OCH_2Ph), 3.41 and 3.49 (2m, 2 × 4 H, NCH_2CH_2), 2.08 [t, J 8.0 Hz, 4 H, $CH_2C(O)$], 1.52 [m, 4 H, $CH_2CH_2C(O)$], 1.18 [s, 36 H, $(CH_2)18$]. ^{13}C NMR ($CDCl_3$): δ 173.2 (CO), 138.0 (C, Ph), 128.5 and 127.9 (CH, ortho and meta Ph), 127.8 (CH, para Ph), 73.2 (OCH_2Ph), 69.1 (CH_2O), 39.3 (CH_2NH), 36.9 (CH_2CO), 29.73, 29.67, 29.5, 29.4, and 29.3 [$(CH_2)18$], 25.8 (CH_2CH_2CO).

Step 2: a mixture of **24** (3.24 g, 4.87 mmol) and $LiAlH_4$ (1.9 g) in anhyd THF (75 mL) was refluxed for 7 days. After hydrolysis with a 1:1 THF–water solution, filtration and extraction with $CHCl_3$, the solvents were evaporated. The crude product N^1,N^{24}-bis-[2 - (benzyloxy)ethyl] - 1,24 - tetracosanediamine (**25**) was then precipitated from $CHCl_3$ as its bis-(HCl) salt (2.35 g, 68%). Characterization was performed on a aliquot of **25** purified by chromatography (1:1 $CHCl_3$–CH_3OH): R_f 0.28 (1:1 $CHCl_3$–MeOH). ^1H ($CDCl_3$): 7.27 [m, 10 H, 2Ph], 4.46 [s, 4 H, 2(OCH_2Ph)], 3.53 [t, 4 H, 2(OCH_2CH_2NH)], 2.74 [t, J 5.2 Hz, 4 H, 2(OCH_2CH_2NH)], 2.52 [t, J 7.2 Hz, 4 H, 2(CH_2NH)], 1.64 [m, 2 H, 2(N*H*)], 1.41 [m, 4 H, 2($CH_2CH_2CH_2N$)], 1.18 [s, 40 H, $(CH_2)_{20}$]. ^{13}C ($CDCl_3$): 137.9 [2(C, Ph)], 128.4 and 127.8 [2(CH, ortho and meta Ph)], 127.7 [2(CH, ortho Ph)], 73.2 [2(OCH_2Ph)], 69.7 [2(OCH_2CH_2NH)], 50.0 [2(OCH_2CH_2NH)], 49.5 [2(CH_2N)], 30.2, 29.7, and 29.6 [$(CH_2)_{20}$], 27.4 [2(CH_2CH_2NH)].

Step 3: 5-(*F*-hexyl)pentanoyl chloride (1.3 g, 2.9 mmol) were added to a mixture of **25** (0.68 g, 0.96 mmol) as its HCl salt, and Et_3N (3 mL, 21.8 mmol) in anhyd CH_2Cl_2 (50 mL). The resulting solution was stirred at 60 °C for 3 days. The organic phase was then washed with water until neutrality, dried over Na_2SO_4, filtered and evaporated to dryness. Purification by chromatography ($CHCl_3$ to 1:1 $CHCl_3$–MeOH) of the residue afforded N^1,N^{24} - bis[2 - (benzyloxy)ethyl] - N^1,N^{24} - bis[5-(*F* - hexyl)pentanoyl] - 1,24 - tetracosanediamine (**26b**, 1.24 g, 90%) as a white powder: R_f 0.26 (49:1 $CHCl_3$–MeOH). ^1H NMR ($CDCl_3$): δ 7.23 (m, 10 H, Ph), 4.42 and 4.43 (2s, 2 H, 2 H, OCH_2Ph), 3.49 (m, 8 H, OCH_2CH_2N), 3.24 (t, J 7.3 Hz, 4 H, $CH_2CH_2CH_2N$), 2.29 (m, 4 H, CH_2CO), 2.00 (m, 4 H, CH_2CF_2), 1.59 (m, 12 H, CH_2CH_2CO, $CH_2CH_2CF_2$, CH_2CH_2-CH_2N), 1.18 [bs, 40 H, $(CH_2)_{20}$]. ^{13}C NMR ($CDCl_3$): δ 171.9 and 172.3 (CO), 137.8 and 138.3 (C, Ph), 128.3 and 128.5 (CH, meta Ph), 127.9 (CH, ortho Ph), 127.6 (CH, para Ph), 73.1 and 73.5 ($PhCH_2O$), 68.1 and 68.8 (CH_2O), 47.6 and 49.3 (OCH_2CH_2N), 46.1 and 46.3 (CH_2N), 32.5 and 32.6 (CH_2CO), 30.9 (t, J_{CF} 22 Hz, CH_2CF_2), 27.7 and 29.1–29.7 [$(CH_2)_{20}$], 26.9 and 27.0 (CH_2CH_2N), 24.7 (CH_2CH_2CO), 20.1 [t, J 3.7 Hz, $CH_2CH_2CF_2$). ^{19}F NMR ($CDCl_3$): δ −81.3 (6 F, CF_3), −114.8 (4 F, CF_2CH_2), −122.4, −123.4 and −124.0 [3 × 4 F, $(CF_2)_3$], −126.6 (4 F, CF_2CF_3).

Step 4: compound **26b** (6 g, 4.16 mmol) and Pd/C (0.6 g, 10% w/w) in EtOH (50 mL) were stirred under 40 atm H_2 for 21 days. After usual workup, N^1,N^{24}-bis[2-hydroxyethyl]-N^1, N^{24} - bis[5 - (*F* - hexyl)pentanoyl] - 1,24 - tetracosanediamine (**27b**, 4.19 g, 80%) was obtained: IR (ν cm^{-1}, KBr): 3400 (OH), 1610 (C=O). ^1H NMR ($CDCl_3$): δ 3.68 (t, 4 H, CH_2OH), 3.45 (t, J 4.9 Hz, 4 H, OCH_2CH_2N), 3.20 (t, J 7.7 Hz, 4 H, CH_2N), 2.32 (m, 4 H, CH_2CO), 2.03 (m, 4 H, CH_2CF_2), 1.57 (m, 12 H, $CH_2CH_2CF_2$, $CH_2CH_2CH_2N$, CH_2CH_2-CO), 1.18 [m, $(CH_2)_{20}$, 40 H]. ^{13}C NMR ($CDCl_3$): δ 174.2 (CO), 62.5 (CH_2OH), 50.0 (OCH_2CH_2N), 49.7 (CH_2N), 32.6 (CH_2CO), 30.9 (t, J_{CF} 22 Hz, CH_2CF_2), 29.1 to 29.7 [$(CH_2)_{20}$], 26.9 (CH_2CH_2N), 24.7 (CH_2CH_2-CO), 20.1 (t, J 3.5 Hz, $CH_2CH_2CF_2$). ^{19}F NMR ($CDCl_3$): identical to that of **26b**. Anal. Calcd for $C_{50}H_{74}F_{26}N_2O_4$ (1261.114): C, 47.62; H, 5.91; N, 2.22. Found: C, 47.34; H, 5.77; N, 2.08.

Step 5: the procedure described for the synthesis of **2a(D)** when applied to **27b**, afforded, after workup and chromatography ($CHCl_3$ to 49:1 $CHCl_3$–MeOH), N^1-[2-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyloxy)ethyl]-N^1,N^{24}-bis[5-(*F*-hexyl)pentanoyl]-N^{24}-(2-hydroxyethyl)-1,24-tetracosanediamide (**28b**, 34%), and a mixture of N^1,N^{24}-bis[2-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyoxyl)ethyl]-N^1,N^{24}-bis[5 - (*F* - hexyl)pentanoyl] - 1,24 - tetracosane-

diamide (**29b**) and 2,3,4,6-tetra-*O*-acetyl-D-galactopyranose, as white solids.

Compound **28b**: ^{1}H NMR ($CDCl_3$): δ 5.33 (d, *J* 3.2 Hz, 1 H, H-4 Gal), 5.17–4.90 (m, 2 H, H-2-3 Gal), 4.39 (d, *J* 7.8 Hz, 1 H, H-1 Gal), 4.20–3.85 (m, 5 H, GalOCH_2CH_2 and H-5–6 Gal), 3.68 (t, *J* 5 Hz, 2 H, CH_2OH), 3.42 (m, 4 H, OCH$_2$CH_2N), 3.20 (t, *J* 7.4 Hz, 4 H, CH_2N), 2.28 [m, 4 H, $CH_2C(O)$], 2.11–1.91 (m, 16 H, CH_3, CH_2CF_2), 1.63 [m, 12 H, $CH_2CH_2CH_2N$, $CH_2CH_2CF_2$, $CH_2CH_2C(O)$], 1.18 [bs, 40 H, $(CH_2)_{20}$]. ^{13}C NMR ($CDCl_3$): δ 174.3 and 172.1 [C(O)N], 170.4, 170.2, and 170.0 [*C*(O)CH$_3$], 101.5 (C-1 Gal), 68.5 (C-3 Gal), 68.2 (C-5 Gal), 67.3 (C-2 Gal), 66.1 (C-4 Gal), 62.6 (CH_2CH_2OH), 61.8 (C-6 Gal), 61.3 (GalO*C*H$_2$CH$_2$), 50.0 (N*C*H$_2$CH$_2$OH), 49.6 (*C*H$_2$NCH$_2$CH$_2$OH), 48.0 (GalOCH$_2$*C*H$_2$), 46.2 (*C*H$_2$NCH$_2$CH$_2$OGal), 32.6 (CH_2CO), 30.8 (t, J_{CF} 22 Hz, CH_2CF_2), 29.7 to 29.0 [$(CH_2)_{20}$], 26.8 (CH_2CH_2N), 24.7 (CH_2CH_2CO), 20.8, 20.6, and 20.5 (CH_3), 20.1 (t, J_{CF} 3.5 Hz, $CH_2CH_2CF_2$). ^{19}F NMR ($CDCl_3$): identical to that of **26b**.

Step 6: deacetylation of **28b** and of the mixture containing **29b** was performed with 1 M MeONa–MeOH at rt overnight. After evaporation, the crude residues were chromatographed twice on silica gel ($CHCl_3$ to 1:1 $CHCl_3$–MeOH) affording **II-GalBAE[C24]-[F6C5](OH)** (60%) and **II-GalBAE[C24]-[F6C5](Gal)** as white powders, respectively.

II-GalBAE[C24][F6C5](OH): ^{1}H NMR ($CDCl_3$): δ 4.42–3.16 (m, 24 H, CH_2N, GalOCH_2CH_2N, HOCH_2CH_2N, H-1–6 Gal and OH), 2.31 [m, 4 H, $CH_2C(O)$], 2.02 (m, 4 H, CH_2CF_2), 1.57 (m, 12 H, CH_2CH$_2$N, CH_2CH$_2$CF$_2$, CH_2CH$_2$CO), 1.18 [m, 40 H, $(CH_2)_{20}$]. ^{13}C NMR ($CDCl_3$): δ 174.2 and 174.3 (CO), 103.8 (C-1 Gal), 74.6 (C-5 Gal), 73.8 (C-3 Gal), 71.3 (C-2 Gal), 69.1 (GalO*C*H$_2$CH$_2$N), 66.8 (C-4 Gal), 62.2 (NCH$_2$*C*H$_2$OH), 61.8 (C-6 Gal), 49.9 (N*C*H$_2$CH$_2$OH), 49.6 (*C*H$_2$NCH$_2$CH$_2$OH), 48.6 (GalOCH$_2$*C*H$_2$N), 45.7 (*C*H$_2$NCH$_2$-CH$_2$OGal), 32.6 (*C*H$_2$CO), 30.9 (t, *J* CF 22 Hz, CH_2CF_2), 29.7–29.1 [$(CH_2)_{20}$], 26.8 (*C*H$_2$CH$_2$N), 24.7 (*C*H$_2$CH$_2$CO), 20.1 [t, J_{CF} 3.7 Hz, $CH_2CH_2CF_2$). ^{19}F NMR ($CDCl_3$): identical to that of **26b**. Anal. Calcd for $C_{56}H_{84}F_{26}N_2O_9$ (1423.258): C, 47.26; H, 5.95; F, 34.71; N, 1.97. Found: C, 47.59; H, 6.13; F, 32.58; N, 1.74.

II-GalBAE[C24][F6C5](Gal): ^{1}H NMR ($CDCl_3$–CD_3OD): δ 4.10 (d, $J_{1,2}$ 4 Hz, 1 H, H-1 Gal), 4.00–3.00 [m, 24 H, 2(OCH_2CH_2N, $CH_2CH_2CH_2N$, H-2–6 Gal)], 2.24 [m, 4 H, 2(CH_2CO)], 1.97 [m, 4 H, 2(CH_2CF_2)], 1.51 [m, 12 H, 2(CH_2CH$_2$N, CH_2CH$_2$CF$_2$, CH_2CH$_2$CO)], 1.11 [m, 40 H, $(CH_2)_{20}$]. ^{13}C ($CDCl_3$–CD_3OD): 173.3 (CO), 103.2 (C-1 Gal), 74.3 (C-5 Gal), 73.3 (C-3 Gal), 70.9 (C-2 Gal), 68.9 (C-4 Gal), 66.6 (O*C*H$_2$CH$_2$N), 61.2 (C-6 Gal), 48.5 (OCH$_2$*C*H$_2$N) partially hindered by CD_3OD], 45.5 (CH_2N), 32.4 (*C*H$_2$CO), 30.5 (t, J_{CF} 21 Hz, *C*H$_2$CF$_2$), 29.5–29.2 [$(CH_2)_{20}$], 26.6 (CH_2CH_2N), 24.5 (*C*H$_2$CH$_2$CO), 19.9 (t, J_{CF} 3.5 Hz, *C*H$_2$CH$_2$-CF$_2$). ^{19}F NMR ($CDCl_3$–CD_3OD): identical to that of **26b**.

N^1-*[2-(β-D-Galactopyranosyloxy)ethyl]*-N^{24}-*(2-hydroxyethyl)*-N^1,N^{24}-*bis(dodecanoyl)-1,24-tetracosanediamide* (**II-GalBAE[C24][C12]-(OH)**). Step 1: the procedure described for the synthesis of **26b** when applied to **25** (3.5 g, 1 eq, 4.93 mmol) as its HCl salt, dodecanoyl chloride (3.8 g, 3.4 eq, 17.4 mmol) and Et_3N (10 mL, 72 mmol) in anhyd $CHCl_3$ (150 mL) gave N^1,N^{24}-bis[2-(benzyloxy)ethyl]-N^1,N^{24}-bis(dodecanoyl)-1,24-tetracosanediamine (**26a**, 4.8 g, 98%) as a white powder: ^{1}H NMR ($CDCl_3$): δ 7.26 (m, 10 H, Ph), 4.43 and 4.44 (2s, 2 H, 2 H, OCH_2Ph), 3.49 (m, 4 H, OCH_2CH$_2$N), 3.47 (t, *J* 13.5 Hz, 4 H, OCH$_2$CH_2N), 3.24 (t, *J* 7.6 Hz, 4 H, CH_2N), 2.25 and 2.22 [2t, *J* ~8.0 Hz, 2 × 2 H, CH_2CO), 1.51 (m, 8 H, CH_2CH_2CO, $CH_2CH_2CH_2N$), 1.18 (bs, 72 H, $(CH_2)_{20}$ and $(CH_2)_8CH_3$), 0.81 (t, *J* 6.4 Hz, 6 H, CH_3). ^{13}C NMR ($CDCl_3$): δ 173.3 and 173.4 (CO), 137.9 and 138.5 (C, Ph), 128.5, 128.6 and 127.9 (CH, ortho and meta Ph), 127.7 (CH, para Ph), 73.3 and 73.6 (O*C*H$_2$Ph), 68.5 and 69.0 (O*C*H$_2$CH$_2$N), 47.7 and 49.6 (OCH$_2$*C*H$_2$N), 46.2 and 46.5 (CH_2N), 33.2 and 33.4 (*C*H$_2$CO), 32.1 (*C*H$_2$CH$_2$CH$_3$), 27.9 and 29.3–29.9 [$(CH_2)_{20}$ and $CH_3CH_2CH_2(CH_2)_6$], 27.0 and 27.2 (*C*H$_2$CH$_2$N), 25.6 (*C*H$_2$CH$_2$-CO), 22.8 (CH_3CH_2)], 14.3 (CH_3).

Step 2: the procedure described for the synthesis of **27b**, when applied to **26a** (4.83 g, 4.82 mmol), gave after 2 days of reaction and usual

workup, N^1,N^{24}-bis(2-hydroxyethyl)-N^1,N^{24}-bis(dodecanoyl)-1,24-tetracosanediamine (**27a**, 3.96 g, 100%): IR (ν cm^{-1}, KBr): 3390 (OH), 1610 (C=O). ^{1}H NMR ($CDCl_3$): δ 3.69 (t, 4 H, CH_2OH), 3.45 (t, J 4.0 Hz, 4 H, NCH_2CH$_2$OH), 3.20 (t, J 7.6 Hz, 4 H, CH$_2$N), 2.26 (t, J 7.5 Hz, 4 H, CH$_2$CO), 1.53 (m, 8 H, CH_2CH$_2$CO, CH$_2$CH_2CH$_2$N), 1.19 [m, 72 H, (CH$_2$)$_{20}$, (CH_2)$_8$CH$_3$], 0.81 (t, J 6.4 Hz, 6 H, CH$_3$). ^{13}C NMR ($CDCl_3$): δ 173.0 (CO), 63.1 (CH$_2$OH), 50.3 (HOCH$_2$$CH_2$N), 49.9 (CH$_2$N), 33.3 ($CH_2$CO), 32.0 ($CH_2CH_2CH_3$), 29.8–29.2 [(CH$_2$)$_{20}$ and (CH$_3$CH$_2$CH$_2$(CH$_2$)$_6$)], 26.9 (CH$_2$CH$_2$N), 25.5 (CH$_2$CH$_2$CO), 22.8 (CH$_3$$CH_2$)], 14.2 (CH$_3$). Anal. Calcd for $C_{52}H_{104}N_2O_4$ (821.42): C, 76.04; H, 12.76; N, 3.41. Found: C, 75.55; H, 12.85; N, 3.74.

Step 3: the galactosylation procedure described for the synthesis of **2a**(**D**) when applied to **27a**, afforded N^1-[2-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyloxy)ethyl]-N^{24}-(2-hydroxyethyl)-N^1,N^{24}-bis(dodecanoyl)-1,24-tetracosanediamide (**28a**) contaminated by 2,3,4,6-tetra-*O*-acetyl-D-galactopyranose. An aliquot was purified by chromatography for analysis. ^{1}H NMR ($CDCl_3$): δ 5.28 (m, 1 H, H-4 Gal), 5.10–4.80 (m, 2 H, H-2-3 Gal), 4.35 (d, J 7.9 Hz, ^{1}H, H-1 Gal), 4.10–3.75 (m, 5 H, GalOCH_2CH$_2$ and H-5–6 Gal), 3.64 (t, J 5.1 Hz, 2 H, CH_2OH), 3.38 (m, 4 H, NCH_2CH$_2$O), 3.16 (m, 4 H, CH$_2$N), 2.25 (t, J 7.7 Hz, 4 H, CH$_2$CO), 2.05–1.87 (m, 12 H, CH$_3$CO), 1.49 (m, 8 H, CH_2CH$_2$CO, CH$_2$CH_2CH$_2$N), 1.14 [m, 72 H, (CH$_2$)$_{20}$, (CH_2)$_8$CH$_3$)], 0.77 (t, J 6.7 Hz, 6 H, CH_3CH$_2$). ^{13}C NMR ($CDCl_3$): δ 175.6 (CO), 170.3 (COCH$_3$), 101.6 (C-1 Gal), 70.8 (C-3 Gal), 69.5 (C-5 Gal), 67.5 (C-2 Gal), 66.1 (C-4 Gal), 62.9 (CH$_2$OH), 61.8 (GalOCH$_2$CH$_2$), 61.3 (C-6 Gal), 50.2 (HOCH$_2$$CH_2$N), 49.9 ($CH_2NCH_2CH_2$OH), 49.6 (GalOCH$_2$$CH_2$N), 46.1 ($CH_2NCH_2CH_2$OGal), 33.2 ($CH_2$CO), 32.0 ($CH_2CH_2CH_3$), 29.2–29.8 and 27.1 [(CH$_2$)$_{20}$ and 2CH$_3$CH$_2$CH$_2$(CH_2)$_6$], 26.9 (CH$_2$CH$_2$N), 25.5 (CH$_2$CH$_2$CO), 22.8 (CH$_3$-CH$_2$), 20.9–20.6 (CH$_3$CO), 14.2 (CH$_3$CH$_2$).

Step 4: the deacetylation procedure used for the preparation of **II-GalBAE[C24]-[F6C5](OH)**, when applied to **28a**, gave, after chromatography ($CHCl_3$ to 1:1 $CHCl_3$–MeOH), **II-GalBAE[C24][C12](OH)** (22%) as a white powder: ^{1}H NMR ($CDCl_3$): δ 4.27–3.23 (m, 19 H, CH_2N, NCH$_2$CH_2OH, GalOCH$_2$CH_2N, HOCH$_2$CH_2 N, H-1–6 Gal), 2.29 (m, 4 H, CH$_2$CO), 1.60 (m, 8 H, CH_2CH$_2$CO, CH$_2$CH_2CH$_2$N), 1.26 (m, 72 H (CH$_2$)$_{20}$ and (CH_2)$_8$CH$_3$), 0.88 (t, J 6.4 Hz, 6 H, CH$_3$). ^{13}C NMR ($CDCl_3$): δ 174.5 and 175.7 (CO), 103.6 (C-1 Gal), 74.8 (C-5 Gal), 73.9 (C-3 Gal), 71.6 (C-2 Gal), 69.4 (C-4 Gal), 67.2 (GalOCH$_2$CH$_2$N), 62.8 (NCH$_2$$CH_2$OH), 62.5 (C-6 Gal), 50.2 (HOCH$_2$$CH_2$N), 49.9 ($CH_2NCH_2CH_2$OH), 48.6 (GalOCH$_2CH_2$N), 45.7 ($CH_2NCH_2CH_2$OGal), 33.3 [2($CH_2$CO)], 32.0 ($CH_2CH_2CH_3$), 29.8–29.2 [(CH$_2$)$_{20}$ and CH$_3$CH$_2$CH$_2$(CH$_2$)$_6$], 26.9 (CH$_2$$CH_2CH_2$N), 25.5 ($CH_2CH_2$CO), 22.8 (CH$_3$$CH_2$), 14.2 (CH$_3$). Anal. Calcd for $C_{58}H_{114}N_2O_9$ (983.563): C, 70.83; H, 11.68; N, 2.85. Found: C, 70.60; H, 11.64; N, 2.65.

Synthesis of the **Gal(NHAc)** *single-chain derivatives*

3-O-(2-Acetamido-2-deoxy-β-D-galactopyranosyl)-L-serine tetradecylamide (**I-Gal(NHAc)Ser[C14](L)**). Step 1: *p*-anisaldehyde (5.6 mL, 46 mmol) was added to a solution of D-galactosamine hydrochloride **30** (1.0 g, 4.6 mmol) in 1 N NaOH (4.7 mL). After stirring vigorously for 2 h at rt, the crystalline material was filtered off, washed with ice–water, EtOH and Et_2O, and dried, giving *N*-(*p*-methoxybenzylidene)-2-amino-2-deoxy-D-galactopyranose (1.0 g, 73%). This compound was then allowed to react with Ac_2O (2.0 mL, 21 mmol) in pyridine at 0 °C for 1 h, then at rt overnight. The reaction was poured in ice water and the precipitate was filtered and dried, affording *N*-(*p*-methoxybenzylidene)-1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-galactopyranose (**31**, 1.2 g, 75%) R_f 0.65 (24:1 $CHCl_3$–MeOH): ^{1}H NMR ($CDCl_3$): δ 8.14 (s, 1 H, N=CH), 7.59 (m, 2 H, Ph), 6.84 (m, 2 H, Ph), 5.85 (d, J 8.2 Hz, 1 H, H-1β Gal), 5.38 (d, J 3.2 Hz, 1 H, H-4 Gal), 5.18 (dd, $J_{2,3}$ 10.4, $J_{3,4}$ 3.2 Hz, 1 H, H-3 Gal), 4.11 (m, 3 H, H-5–6 Gal), 3.77 (s, 3 H, OCH$_3$), 3.53 (dd, $J_{2,3}$ 10.4, $J_{1,2}$ 8.2 Hz, 1 H, H-2 Gal), 2.10, 1.99, 1.96 and 1.82 (all s, 12 H, CH$_3$). ^{13}C NMR ($CDCl_3$): δ 170.5, 170.2, 169.8 and 168.9 [CH$_3$$C$(O)], 164.5 [$C$(O)Me], 162.4 ($C$CH=N), 130.3 (CH, Ph), 128.6 (CH=N), 114.2 (CH, Ph), 93.7 (C-1β Gal), 71.9, 71.7,

68.9 and 66.1 (C-2–5 Gal), 61.5 (C-6 Gal), 55.6 (OCH_3), 20.9, 20.8 and 20.6 [$CH_3C(O)$].

Step 2: the latter derivative **31** (1.1 g, 2.36 mmol) was dissolved in acetone (20 mL) and 5 N HCl (0.43 mL) was added. The reaction mixture was refluxed for 15 min, the precipitate was filtered and washed with Et_2O, yielding 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-β-D-galactopyranose hydrochloride (0.80 g, 85%). This compound (0.40 g, 1.0 mmol) was then reacted with $NaHCO_3$ (0.17 g) in water (10 mL). After 10 min of stirring, allyl chloroformate (0.15 mL, 1.4 mmol) in $CHCl_3$ (5 mL) was added and the reaction mixture was stirred for 1 h at rt. The aq layer was extracted twice with $CHCl_3$, the combined $CHCl_3$ solutions were washed with water. After drying with Na_2SO_4, the solution was filtered and evaporated to dryness under diminished pressure. The residue was recrystallized from Et_2O giving 1,3,4,6-tetra-*O*-acetyl-2-allyloxycarbonylamino-2-deoxy-β-D-galactopyranose (**32**, 0.40 g, 85%) as a white solid: R_f 0.64 (24:1 $CHCl_3$–MeOH). ^{1}H NMR ($CDCl_3$): δ 5.81 (m, 1 H, $CH{=}CH_2$), 5.69 (d, *J* 8.7 Hz, 1 H, H-1β Gal), 5.55 (d, *J* 9.6 Hz, 1 H, NH), 5.33 (d, *J* 3.0 Hz, 1 H, H-4 Gal), 5.13 (m, 3 H, $CH{=}CH_2$ and H-3 Gal), 4.50 (bd, 2 H, $OCH_2CH{=}$), 4.15-3.95 (m, 4 H, H-2 Gal, H-5 Gal, and H-6–6′ Gal), 2.10, 2.06, 1.97, and 1.94 (all s, 12 H, CH_3). ^{13}C NMR ($CDCl_3$): δ 170.5, 170.4, and 169.5 [$CH_3C(O)$], 156.1 [NHC(O)], 132.8 ($CH{=}CH_2$), 117.5 ($CH{=}CH_2$), 93.0 (C-1β Gal), 71.7 (C-3 Gal or C-5 Gal), 70.6 (C-3 Gal or C-5 Gal), 66.7 (C-4 Gal), 65.7 ($OCH_2CH{=}$), 61.6 (C-6 Gal), 51.4 (C-2 Gal), 20.9 and 20.7 [$CH_3C(O)$].

Step 3: **32** (150 mg, 0.348 mmol), N^{α}-Fmoc-L-serine (140 mg, 0.427 mmol), and $BF_3{\cdot}Et_2O$ (0.14 mL, 1.13 mmol) in CH_2Cl_2 (6 mL) were stirred overnight at rt. After adding CH_2Cl_2 (20 mL) and filtration over Celite, the organic phase was washed with 1 N HCl, then with water until neutrality. After drying over Na_2SO_4 and filtration, the solvent was evaporated and the residue purified by chromatography (99:1 to 19:1 $CHCl_3$–MeOH) giving 3-*O*-(3,4,6-tri-*O*-acetyl-2-allyloxycarbonylamino-2-deoxy-β-D-galactopyranosyl)-*N*-Fmoc-L-serine (**33**, 100 mg, 41%) as a white solid: R_f 0.30 (9:1 $CHCl_3$–MeOH). ^{1}H NMR (acetone-d_6): δ 7.84 (d, *J* 6.9 Hz, 2 H, Fmoc), 7.75 (d, *J* 7.2 Hz, 2 H, Fmoc), 7.50–7.25 (m, 4 H, Fmoc), 6.65 (m, 1 H, NH-Fmoc), 5.90 (m, 2 H, $OCH_2CH{=}CH_2$ and N*H*–Aloc), 5.36 (bs, 1 H, H-4 Gal), 5.30–5.10 (m, 3 H, $OCH_2CH{=}CH_2$, H-3 Gal), 4.80 (bs, 1 H, H-1 Gal), 4.70 (bs, 2 H, $OCH_2CH{=}CH_2$), 4.60–3.80 [m, 10 H, H-2 Gal, H-5–6 Gal, OCH_2CHNH and C(O)OCH_2CHFmoc], 2.11, 2.09, and 1.94 [all s, all 3 H, $CH_3C(O)$]. ^{13}C NMR (acetone-d_6): δ 170.6 and 170.3 [CH_3CO and COOH], 157.3 [NHC(O)O], 145.1, 145.0 and 142.0 (C, Fmoc), 134.0 ($OCH_2CH{=}CH_2$), 128.4, 128.0, 126.2 and 120.7 (CH, Fmoc), 117.2 ($OCH_2CH{=}CH_2$), 102.7 (C-1 Gal), 71.3 (C-5 Gal), 70.5 (C-3 Gal and OCH_2CHNH), 67.6 and 67.5 [C-4 Gal and C(O)OCH_2Fmoc], 66.1 ($OCH_2CH{=}CH_2$), 61.8 (-6 Gal), 56.0 [CHC(O)OH], 52.9 (C-2 Gal), 47.8 [C(O)OCH_2CH], 20.5 [$CH_3C(O)$].

Step 5: a solution containing **33** (90 mg, 0.128 mmol), tetradecylamine (27 mg, 0.126 mmol), EDC (27 mg, 0.14 mmol), and HOBt (17 mg, 0.126 mmol) in DMF (5 mL) was stirred at 0 °C for 1 h, then at rt overnight. After evaporation of DMF under diminished pressure, the residue was taken up in $CHCl_3$ and successively washed with 5% $KHSO_4$, 8% $NaHCO_3$, then water until neutrality. After drying over Na_2SO_4 and filtration, the solvent was evaporated and the residue purified by recrystallization in Et_2O giving 3-*O*-(3,4,6-tri-*O*-acetyl-2-allyloxycarbonylamino-2-deoxy-β-D-galactopyranosyl)-*N*-Fmoc-L-serine tetradecylamide (**34a**, 90 mg, 79%) as a white solid: R_f 0.40 (49:1 $CHCl_3$–MeOH). ^{1}H NMR ($CDCl_3$): δ 7.70 (d, *J* 7.2 Hz, 2 H, Fmoc), 7.52 (d, *J* 7.1 Hz, 2 H, Fmoc), 7.35–7.20 (m, 4 H, Fmoc), 6.40 (bs, 1 H, $NHCH_2$), 5.85–5.65 (m, 2 H, $OCH_2CH{=}CH_2$ and N*H*–Fmoc), 5.28 (bd, *J* 2.9 Hz, 1 H, H-4 Gal), 5.24-5.06 (m, 3 H, $NHC(O)OCH_2CH{=}CH_2$), 5.01 (dd, $J_{2,3}$ 11.2, $J_{3,4}$ 3.2 Hz, 1 H, H-3 Gal), 4.88 (d, *J* 8.6 Hz, 1 H, H-1 Gal), 4.60–3.60 [m, 12 H, H-2 Gal, H-5–6 Gal, OCH_2CH, $OCH_2CH{=}CH_2$, and C(O)OCH_2CH], 3.15 (m, 2 H, $NHCH_2$), 2.07, 1.96, and 1.94 [all s, all 3 H, $CH_3C(O)$], 1.55–1.35 (m, 2 H, $NHCH_2CH_2$), 1.35–1.10 [m, 22 H, $(CH_2)_{11}CH_3$], 0.81 (t, *J* 5.2 Hz, 3 H, CH_2CH_3]. ^{13}C NMR ($CDCl_3$): δ 170.6, 170.2

and 169.2 [CH_3C(O) and C(O)NH], 156.1 [NHC(O)O], 143.9 and 141.5 (C, Fmoc), 132.6 ($OCH_2CH{=}CH_2$), 127.9, 127.2, 125.2 and 120.2 (CH, Fmoc), 117.8 ($OCH_2CH{=}CH_2$), 102.5 (C-1 Gal), 71.2 (C-5 Gal), 70.2 (C-3 Gal), 69.9 (OCH_2CHNH), 67.2 [C(O)OCH_2-Fmoc], 66.8 (C-4 Gal), 66.0 ($OCH_2CH{=}CH_2$), 61.6 (C-6 Gal), 54.2 [*C*HC(O)NH], 52.5 (C-2 Gal), 47.3 [C(O)OCH_2CH], 39.9 ($NHCH_2$), 32.0 ($CH_2CH_2CH_3$), 29.8, 29.6, and 29.4 [$(CH_2)_9CH_2CH_2CH_3$], 27.0 ($NHCH_2CH_2$), 22.8 (CH_2CH_3), 20.7 [CH_3C(O)], 14.2 (CH_3).

Step 6: to a solution of **34a** (90 mg, 0.10 mmol) in CH_2Cl_2 (2 mL), Ac_2O (23 μL, 0.25 mmol), $Pd(PPh_3)_4$ (3 mg, 2.6×10^{-3} mmol), and Bu_3SnH (27 μL, 0.10 mmol) were added successively. The mixture was stirred for 1 h at rt. After filtration and evaporation of the solvent, the crude was recrystallized from Et_2O affording 3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-*N*-Fmoc-L-serine tetradecylamide (**35a**, 60 mg, 70%) as a white solid: R_f 0.25 (49:1 $CHCl_3$–MeOH): ^{1}H NMR ($CDCl_3$): δ 7.70 (d, *J* 7.1 Hz, 2 H, Fmoc), 7.53 (d, *J* 7.1 Hz, 2 H, Fmoc), 7.37–7.19 (m, 4 H, Fmoc), 6.47 (bs, 1 H, N*H*CH_2), 5.83 (bs, 2H, N*H*C(O)CH_3 and N*H*–Fmoc), 5.27 (bd, *J* 2.7 Hz, 1 H, H-4 Gal), 5.06 (dd, $J_{2,3}$ 11.2, $J_{3,4}$ 3.2 Hz, 1 H, H-3 Gal), 4.60–3.80 [m, 10 H, H-1-2 Gal, H-5–6 Gal, OCH_2, and C(O)OCH_2CH], 3.66 (m, 1 H, OCH_2CH), 3.16 (dt, *J* 6.2, *J* 5.7 Hz, 2H, $NHCH_2$), 2.07, 1.95, and 1.93 [all s, all 3 H, CH_3C(O)O], 1.82 [s, 3 H, CH_3C(O)NH], 1.60–1.10 [m, 24 H, $(CH_2)_{12}CH_3$], 0.82 (t, *J* 6.5 Hz, 3 H, CH_2CH_3]. ^{13}C NMR ($CDCl_3$): δ 170.9 and 170.8 [C(O)NH], 170.5, 170.2, and 169.3 [CH_3C(O)O], 156.2 [NHC(O)O], 143.8 and 141.4 (C, Fmoc), 127.9, 127.2, 125.1 and 120.1 (CH, Fmoc), 102.2 (C-1 Gal), 71.2 (C-5 Gal), 70.2 (C-3 Gal), 69.3 (OCH_2), 67.1 [C(O)OCH_2], 66.7 (C-4 Gal), 61.6 (C-6 Gal), 54.2 (OCH_2CH), 50.9 (C-2 Gal), 47.3 (C(O)OCH_2CH), 39.9 ($NHCH_2$), 32.0 ($CH_2CH_2CH_3$), 29.8, 29.7, 29.5, and 29.4 [$(CH_2)_9CH_2CH_2CH_3$], 27.0 ($NHCH_2CH_2$), 23.4 [CH_3C(O)NH], 22.8 (CH_2CH_3), 20.7 [CH_3C(O)], 14.2 (CH_3).

Step 7: compound **35a** (50 mg, 0.058 mmol) and morpholine (0.52 mL) in DMF (1 mL) were stirred at rt for 1 h 30. After evaporation of the solvent, the residue was purified by chromatography (24:1 to 9:1 $CHCl_3$–MeOH) giving 3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-L-serine tetradecylamide (**36a**, 35 mg, 90%) as a white solid: R_f 0.16 (24:1 $CHCl_3$–MeOH). ^{1}H NMR ($CDCl_3$): δ 7.38 (t, 1 H, N*H*CH_2), 6.13 [d, *J* 8.6 Hz, 1 H, N*H*C(O)CH_3], 5.28 (bd, *J* 2.9 Hz, 1 H, H-4 Gal), 5.08 (dd, $J_{2,3}$ 11.2, $J_{3,4}$ 3.3 Hz, 1 H, H-3 Gal), 4.56 (d, $J_{1,2}$ 8.4 Hz, 1 H, H-1 Gal), 4.15–4.01 (m, 3 H, H-2 Gal, H-6 Gal), 3.90-3.60 (m, 3 H, H-5 Gal and OCH_2), 3.50 (dd, *J* 7.6, *J* 4.7 Hz, 1 H, OCH_2CH), 3.16 (dt, *J* 6.3, *J* 5.8 Hz, 2 H, $NHCH_2$), 2.08 [s, 3 H, CH_3C(O)NH], 2.02 (s, 2 H, NH_2), 1.98, and 1.93 [all s, all 3 H, CH_3C(O)], 1.89 [s, 3 H, CH_3C(O)NH], 1.50–1.30 (m, 2 H, $NHCH_2CH_2$), 1.30–1.10 [m, 22 H, $(CH_2)_{11}CH_3$], 0.82 (t, *J* 6.5 Hz, 3 H, CH_2CH_3]. ^{13}C NMR ($CDCl_3$): δ 172.2 [C(O)NH], 170.7, 170.5, and 170.3 [CH_3C(O)], 101.7 (C-1 Gal), 72.0 (OCH_2), 71.0 (C-5 Gal), 70.4 (C-3 Gal), 66.8 (C-4 Gal), 61.6 (C-6 Gal), 55.3 (OCH_2CH), 50.9 (C-2 Gal), 39.4 ($NHCH_2$), 32.0 ($CH_2CH_2CH_3$), 29.7, 29.6, and 29.4 [$(CH_2)_9CH_2CH_2CH_3$], 27.0 ($NHCH_2CH_2$), 23.7 [CH_3C(O)NH], 22.7 (CH_2CH_3), 20.7 [CH_3C(O)], 14.2 (CH_3CH_2).

Step 8: the O-deacetylation of **36a** (34 mg, 0.054 mmol) was performed in a 2:1:1 MeOH–Et_3N–water mixture (0.72 mL) at rt for 1 h. After evaporation of the solvents, the crude residue was purified by chromatography (9:1 to 7:3 $CHCl_3$–MeOH) affording **I-Gal(N-HAc)Ser[C14](L)** (23 mg, 85%) as a white solid: R_f 0.40 (7:3 $CHCl_3$–MeOH). ESIMS: $m/z = 504.5$ $[M+H]^+$, 526.5 $[M+Na]^+$. ^{1}H NMR ($CDCl_3$–CD_3OD): δ 4.41 (d, *J* 8.1 Hz, 1 H, H-1 Gal), 4.00–3.50 (m, 9 H, OCH_2CH, H-2–6 Gal), 3.20 (t, *J* 6.8 Hz, 2 H, $NHCH_2$), 2.02 [s, 3 H, CH_3C(O)NH], 1.60–1.40 (m, 2 H, $NHCH_2CH_2$), 1.40–1.10 [m, 22 H, $(CH_2)_{11}CH_3$], 0.88 (t, *J* 6.5 Hz, 3 H, CH_2CH_3]. ^{13}C NMR ($CDCl_3$–CD_3OD): δ 172.0 [C(O)NH], 100.8 (C-1 Gal), 74.8 (C-5 Gal), 71.8 (C-3 Gal), 70.2 (OCH_2), 68.1 (C-4 Gal), 61.2 (C-6 Gal), 53.9 (OCH_2CH), 52.5 (C-2 Gal), 39.3 ($NHCH_2$), 31.5 ($CH_2CH_2CH_3$), 29.2, 29.1, 28.9, and 28.8 [$(CH_2)_9CH_2CH_2CH_3$], 26.5 ($NHCH_2CH_2$), 22.2 [CH_3C(O)NH], 22.1 [CH_2CH_3], 13.5 (CH_3).

Anal. Calcd for $C_{25}H_{49}N_3O_6S\cdot 3/2H_2O$ (546.77): C, 54.92; H, 9.59; N, 7.69; S, 5.86. Found: C, 54.78; H, 9.30; N, 7.58; S, 5.65.

3-S-*(2-Acetamido-2-deoxy-β-*D*-galactopyranosyl)*-L-*cysteine tetradecylamide* (**I-Gal(NHAc)Cys[C14](L)**). Step 1: compound **32** (0.36 g, 0.835 mmol), *N*-Fmoc-L-cysteine tetradecylamide (**9a**, 0.67 g, 1.24 mmol) and $BF_3\cdot Et_2O$ (0.4 mL, 3.25 mmol) in CH_2Cl_2 (15 mL) containing 4 Å molecular sieves in suspension were stirred at rt for 2 days. After filtration over Celite, the organic phase was washed with 8% $NaHCO_3$, then with water until neutrality. After drying over Na_2SO_4 and filtration, the solvent was evaporated and the residue purified by chromatography (19:1 to 7:3 CH_2Cl_2–Et_2O) giving 3-*S*-(3,4,6-tri-*O*-acetyl-2-allyloxycarbonylamino-2-deoxy-β-D-galactopyranosyl)-*N*-Fmoc-L-cysteine tetradecylamide (**34b**, 0.57 g, 75%) as a colorless oil: R_f 0.30 (7:3 CH_2Cl_2–Et_2O). $[\alpha]_D$ −0.5° (*c* 0.78; $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.70 (d, *J* 7.2 Hz, 2 H, Fmoc), 7.52 (d, *J* 7.1 Hz, 2 H, Fmoc), 7.35–7.20 (m, 4 H, Fmoc), 6.54 [bs, ^{1}H, C(O)N*H*CH_2], 6.03 (bs, 1 H, N*H*-Fmoc), 5.85–5.70 [m, 3H, N*H*C(O)OCH$_2$C*H*=CH$_2$], 5.30 (bs, 1 H, H-4 Gal), 5.25–4.95 (m, 3H, OCH$_2$CH=C*H*$_2$, H-3 Gal), 4.63 (d, $J_{1,2}$ 10.1 Hz, 1 H, H-1 Gal), 4.45 (m, 2 H, OC*H*$_2$CH=CH$_2$), 4.32 [m, 3 H, C(O)OC*H*$_2$CH, SCH$_2$CH], 4.12 [t, *J* 6.8 Hz, 1 H, C(O)OCH$_2$C*H*], 3.99 (m, 4 H, H-2 Gal, H-5 Gal, and H-6 Gal), 3.10-3.30 (m, 3 H, NHC*H*$_2$, SC*H*$_a$CH$_b$), 3.05–2.80 (m, 1 H, SCH$_a$C*H*$_b$), 2.04, 1.92, and 1.89 [all s, all 3 H, CH$_3$C(O)], 1.60–1.10 [m, 24 H, (C*H*$_2$)$_{12}$CH$_3$], 0.80 (t, *J* 6.5 Hz, 3 H, CH$_3$). ^{13}C NMR ($CDCl_3$): δ 170.5, 170.2, and 169.8 [*C*(O)NH and CH$_3$*C*(O)], 156.2 [NH*C*(O)O], 143.8, 141.4 (C, Fmoc), 132.7 (OCH$_2$*C*H=CH$_2$), 127.9, 127.2, 125.2, 125.0, 120.1 (CH, Fmoc), 117.6 (OCH$_2$CH=*C*H$_2$), 86.7 (C-1β Gal), 75.0 (C-5 Gal), 71.5 (C-3 Gal), 67.2 [C-4 Gal and C(O)O*C*H$_2$], 65.9 (O*C*H$_2$CH=CH$_2$), 62.2 (C-6 Gal), 54.4 (SCH$_2$*C*H), 50.9 (C-2 Gal), 47.2 [C(O)OCH$_2$*C*H], 39.9 (NHCH$_2$), 34.0 (CH$_2$S), 32.0 (*C*H$_2$CH$_2$CH$_3$), 29.8, 29.7, 29.5, and 29.4 [(*C*H$_2$)$_9$], 27.0 (NHCH$_2$*C*H$_2$), 22.8 (*C*H$_2$CH$_3$), 20.7 [*C*H$_3$C(O)], 14.2 (CH$_3$).

Step 2: the procedure described for the synthesis of **35a**, when applied to **34b**, afforded after recrystallization from Et_2O, 3-*S*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-*N*-Fmoc-L-cysteine tetradecylamide (**35b**) as a white solid (85%): R_f 0.38 (24:1 $CHCl_3$–MeOH). ^{1}H NMR ($CDCl_3$): δ 7.68 (d, *J* 7.2 Hz, 2 H, Fmoc), 7.51 (d, *J* 7.1 Hz, 2 H, Fmoc), 7.36–7.20 (m, 4 H, Fmoc), 6.56 (bs, 1 H, N*H*CH$_2$), 6.08 (d, *J* 7.2 Hz, 1 H, N*H*–Fmoc), 5.91 [d, *J* 9.1 Hz, 1 H, N*H*C(O)CH$_3$], 5.29 (bs, 1 H, H-4 Gal), 5.01 (dd, $J_{2,3}$ 10.5, $J_{3,4}$ 2.8 Hz, 1 H, H-3 Gal), 4.58 (d, $J_{1,2}$ 10.3 Hz, 1 H, H-1 Gal), 4.29–3.94 (m, 8 H, H-2 Gal, H-5–6 Gal, SCH$_2$C*H*, and C(O)OC*H*$_2$C*H*), 3.15–2.81 (m, 4 H, SCH$_2$, NHC*H*$_2$), 2.05, 1.92, 1.89, 1.85 [all s, all 3 H, CH$_3$C(O)O and C*H*$_3$C(O)NH], 1.50–1.10 [m, 24 H, (C*H*$_2$)$_{12}$CH$_3$], 0.80 (t, *J* 6.6 Hz, 3 H, CH$_2$C*H*$_3$). ^{13}C NMR ($CDCl_3$): δ 170.8, 170.7, 170.5, 170.3, and 169.7 [C(O)NH and CH$_3$*C*(O)O], 156.2 [NHC(O)O], 143.8 and 141.4 (C, Fmoc), 127.9, 127.2, 125.1 and 120.1 (CH, Fmoc), 86.5 (C-1 Gal), 75.1 (C-5 Gal), 71.3 (C-3 Gal), 67.1 [C-4 Gal and C(O)O*C*H$_2$], 62.1 (C-6 Gal), 54.4 (SCH$_2$*C*H), 49.2 (C-2 Gal), 47.3 [C(O)OCH$_2$*C*H], 39.9 (NHCH$_2$), 33.7 (SCH$_2$), 32.0 (*C*H$_2$CH$_2$CH$_3$), 29.8, 29.7, 29.5, and 29.4 [(*C*H$_2$)$_9$], 27.0 (NHCH$_2$*C*H$_2$), 23.3 [*C*H$_3$C(O)NH], 22.7 (*C*H$_2$CH$_3$), 20.7, 20.6 [*C*H$_3$C(O)], 14.2 (CH$_3$).

Step 3: the Fmoc deprotection as described for the preparation of **36a**, when applied to **35b**, gave after chromatography (24:1 to 9:1 $CHCl_3$–MeOH) 3-*S*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-L-cysteine tetradecylamide (**36b**) as a white solid (95%): R_f 0.11 (24:1 $CHCl_3$–MeOH). ^{1}H NMR ($CDCl_3$): δ 7.44 (t, *J* 5.5 Hz, 1 H, N*H*CH$_2$), 6.56 (d, *J* 9.2 Hz, 1 H, N*H*C(O)CH$_3$), 5.32 (bd, *J* 2.8 Hz, 1 H, H-4 Gal), 5.13 (dd, $J_{2,3}$ 10.7, $J_{3,4}$ 3.1 Hz, 1 H, H-3 Gal), 4.66 (d, $J_{1,2}$ 10.3 Hz, 1 H, H-1 Gal), 4.24–3.87 (m, 4 H, H-2 Gal, H-5–6 Gal), 3.43 (m, 1 H, SCH$_2$C*H*), 3.25–3.05 (m, 3 H, NHC*H*$_2$ and SC*H*$_a$CH$_b$), 2.71 (dd, *J* 8.7, *J* 13.6 Hz, 1 H, SCH$_a$C*H*$_b$), 2.09 [s, 3 H, CH$_3$C(O)], 1.99 [bs, 5 H, NH$_2$ and CH$_3$C(O)], 1.92 [s, 3 H, CH$_3$C(O)], 1.89 [s, 3 H, C*H*$_3$C(O)NH], 1.50–1.10 [m, 24 H, (C*H*$_2$)$_{12}$CH$_3$], 0.80 (t, *J* 6.6 Hz, 3 H, CH$_2$C*H*$_3$). ^{13}C NMR ($CDCl_3$): δ 173.2 [C(O)NH], 170.7, 170.5, and 170.3 [CH$_3$*C*(O)], 84.9 (C-1 Gal),

74.6 (C-5 Gal), 71.3 (C-3 Gal), 67.1 (C-4 Gal), 61.9 (C-6 Gal), 55.0 (SCH_2CH), 49.5 (C-2 Gal), 39.4 ($NHCH_2$), 36.0 (SCH_2), 32.0 ($CH_2CH_2CH_3$), 29.7, 29.6, and 29.4 [$(CH_2)_9$], 27.1 ($NHCH_2CH_2$), 23.7 [$CH_3C(O)NH$], 22.7 (CH_2CH_3), 20.7 [$CH_3C(O)$], 14.2 (CH_3CH_2).

Step 4: the O-deacetylation, as described for the synthesis of **I-Gal(NHAc)Ser[C14](L)**, when applied to **36b** gave after chromatography (9:1 to 7:3 $CHCl_3$–MeOH) **I-Gal(NHAc)Cys[C14](L)** as a white solid (85%): R_f 0.40 (7:3 $CHCl_3$–MeOH). ESIMS: m/z = 520.5 $[M+H]^+$, 542.5 $[M+Na]^+$. ^{1}H NMR ($CDCl_3$–CD_3OD): δ 4.38 (d, $J_{1,2}$ 10.3 Hz, 1 H, H-1 Gal), 4.10–3.50 [m, 7 H, H-2–6 Gal and SCH_2CH], 3.20–3.05 (m, 3 H, $NHCH_2$ and SCH_aCH_b), 2.70 (dd, J 8.7, J 13.6 Hz, 1 H, SCH_aCH_b), 1.94 [s, 3 H, $CH_3C(O)NH$], 1.50–1.10 [m, 24 H, $(CH_2)_{12}CH_3$], 0.83 (t, J 6.6 Hz, 3 H, CH_3). ^{13}C NMR ($CDCl_3$–CD_3OD): δ 172.8, 171.5 [C(O)NH], 83.9 (C-1 Gal), 79.2 (C-5 Gal), 72.8 (C-3 Gal), 68.8 (C-4 Gal), 62.0 (C-6 Gal), 53.9 (SCH_2CH), 51.1 (C-2 Gal), 39.6 ($NHCH_2$), 33.9 (SCH_2), 31.8 ($CH_2CH_2CH_3$), 29.5, 29.4, 29.1, 29.0, [$(CH_2)_9$], 26.8 ($NHCH_2CH_2$), 22.5 [$CH_3C(O)NH$], 22.1 [CH_2CH_3], 13.7 (CH_3). Anal. Calcd for $C_{25}H_{49}N_3O_6S\cdot1H_2O$ (537.76): C, 55.84; H, 9.56; N, 7.81; S, 5.96. Found: C, 55.55; H, 9.24; N, 7.58; S, 5.65.

Synthesis of the double-chain **Gal(NHAc)** *or* **Gal(NH_2)** *derivatives*

N-*Dodecanoyl*-*3*-S-*(amino*-*2*-*deoxy*-*β*-D-*galactopyranosyl)*-L-*cysteine tetradecylamide* (**II-Gal(NH_2)Cys[C14][C12](L)**). Step 1: compound **34b** (700 mg, 0.77 mmol) was stirred at rt for 1 h 30 with morpholine (7 mL) in DMF (14 mL). After evaporation of the solvent under diminished pressure, the residue was chromatographed on silica gel ($CHCl_3$ to 49:1 $CHCl_3$–MeOH) giving 3-*S*-(3,4,6-tri-*O*-acetyl-2-allyloxycarbonylamino-2-deoxy-β-D-galactopyranosyl)-L-cysteine tetradecylamide (**37**, 460 mg, 90%) as a white solid: R_f 0.27 (24:1 $CHCl_3$–MeOH). ^{1}H NMR ($CDCl_3$): δ 7.35 [t, J 5.5 Hz, 1 H, C(O)NH], 5.81 (m, 1 H, $OCH_2CH=CH_2$), 5.50 [d, J 8.8 Hz, 1 H, NHC(O)O], 5.32 (bd, J 3.1 Hz, 1 H, H-4 Gal), 5.27–5.00 (m, 3 H, $OCH_2CH=CH_2$ and H-3 Gal), 4.64 (d, J 10.2 Hz, 1 H, H-1β Gal), 4.50 (d, J 4.5 Hz, 2 H, $OCH_2CH=CH_2$), 4.10–3.80 (m, 4 H, H-2 Gal, H-5 Gal, and H-6 Gal), 3.45 (dd, J 8.7, J 3.8 Hz, 1 H, SCH_2CH), 3.22 (dd, J 14.2, J 8.6 Hz, 1 H, SCH_aCH_b), 3.15 (dt, J 6.6, J 5.8 Hz, 2 H, $NHCH_2$), 2.69 (dd, J 14.2, J 3.8 Hz, 1 H, SCH_aCH_b), 2.09, 1.99, and 1.93 [all s, all 3 H, $CH_3C(O)$], 1.86 (m, 2 H, NH_2), 1.60–1.40 (m, 2 H, $NHCH_2CH_2$), 1.30–1.10 [m, 22 H, $(CH_2)_{11}CH_3$], 0.81 (t, J 6.3 Hz, 3 H, CH_3). ^{13}C NMR ($CDCl_3$): δ 173.3, 173.2, 170.5, and 170.3 [s, $CH_3C(O)$, $C(O)NHCH_2$], 156.0 [NHC(O)O], 132.8 ($OCH_2CH=CH_2$), 117.7 ($OCH_2CH=CH_2$), 85.4 (C-1β Gal), 74.7 (C-5 Gal), 71.3 (C-3 Gal), 67.2 (C-4 Gal), 65.9 ($OCH_2CH=CH_2$), 61.9 (C-6 Gal), 55.1 (SCH_2CH), 51.2 (C-2 Gal), 39.5 ($NHCH_2$), 36.4 (SCH_2), 32.0 ($CH_2CH_2CH_3$), 29.8, 29.7, and 29.4 [$(CH_2)_9$], 27.1 ($NHCH_2CH_2$), 22.8 (CH_2CH_3), 20.8 [$CH_3C(O)$], 14.2 (CH_3).

Step 2: EDC (70 mg, 0.37 mmol) was added at 0 °C to a solution of **37** (214 mg, 0.31 mmol), dodecanoic acid (65 mg, 0.33 mmol), and HOBt (45 mg, 0.33 mmol) in DMF (10 mL). The reaction mixture was stirred at 0 °C for 1 h, then at rt for 3 days. DMF was removed under diminished pressure, and the residue taken up in $CHCl_3$ was successively washed with 5% $KHSO_4$, 8% $NaHCO_3$, then water until neutrality. After drying over Na_2SO_4 and filtration, the solvent was evaporated and the residue purified by successive recrystallization (Et_2O) giving *N*-dodecanoyl-3-*S*-(3,4,6-tri-*O*-acetyl-2-allyloxycarbonylamino-2-deoxy-β-D-galactopyranosyl)-L-cysteine tetradecylamide (**38a**, 245 mg, 90%) as a white solid: R_f 0.44 (24:1 $CHCl_3$–MeOH). ^{1}H NMR ($CDCl_3$): δ 6.74 [m, 2 H, NHC(O)], 5.80 (m, 1 H, $OCH_2CH=CH_2$), 5.65 [d, J 9.6 Hz, 1 H, NHC(O)O], 5.35 (bd, J 3.2 Hz, 1 H, H-4 Gal), 5.24–5.06 (m, 2 H, $OCH_2CH=CH_2$), 5.03 (dd, $J_{2,3}$ 10.7, $J_{3,4}$ 3.0 Hz, 1 H, H-3 Gal), 4.75 (d, $J_{1,2}$ 10.4 Hz, 1 H, H-1 Gal), 4.65–4.40 (m, 3 H, $OCH_2CH=CH_2$ and SCH_2CH), 4.15–3.90 (m, 4 H, H-2 Gal and H-5–6 Gal), 3.18 [m, 2 H, $C(O)NHCH_2$], 2.99 (dd, J 13.8, J 5.4 Hz, 1 H, SCH_aCH_b), 2.78 (dd, J 13.8, J 8.8 Hz, 1 H, SCH_aCH_b), 2.14 [t, J 7.1 Hz, 2 H, $NHC(O)CH_2$], 2.09 [s, 3 H, $CH_3C(O)$], 1.93 [s, 6 H, $CH_3C(O)$], 1.60–1.10 [m, 42 H, $(CH_2)_{12}CH_3$ and $(CH_2)_9CH_3$], 0.81 (t, J 6.3 Hz, 6 H, CH_3). ^{13}C NMR ($CDCl_3$): δ 173.6

[*C*(O)NHCH$_2$], 170.5, 170.3, and 169.9 [CH$_3$*C*(O)], 156.2 [NHC(O)O], 132.8 (OCH$_2$-CH=CH$_2$), 117.6 (OCH$_2$*C*H=CH$_2$), 87.3 (C-1 Gal), 74.9 (C-5 Gal), 71.6 (C-3 Gal), 67.3 (C-4 Gal), 65.9 (O*C*H$_2$CH=CH$_2$), 62.3 (C-6 Gal), 52.3 (SCH$_2$*C*H), 51.0 (C-2 Gal), 39.8 (NHCH$_2$), 36.5 [NHC(O)*C*H$_2$], 34.1 (SCH$_2$), 32.0 (*C*H$_2$CH$_2$CH$_3$), 29.7 and 29.4 [(C*H*$_2$)$_9$CH$_2$CH$_2$CH$_3$ and (C*H*$_2$)$_7$CH$_2$CH$_2$CH$_3$], 27.0 (NHCH$_2$C*H*$_2$), 25.7 [NHC(O)CH$_2$*C*H$_2$], 22.7 (*C*H$_2$CH$_3$), 20.7 and 20.6 [*C*H$_3$C(O)], 14.2 (CH$_3$).

Step 3: the deacetylation of **38a** (120 mg, 0.14 mmol) was performed in a 1:10 Et$_3$N–MeOH mixture (5 mL) at rt for 2 days. The solvents were evaporated to dryness under diminished pressure giving *N*-dodecanoyl-3-*S*-(2-allyloxycarbonylamino-2-deoxy-β-D-galacto-pyranosyl)-L-cysteine tetradecylamide (**39a**, 100 mg, 98%) as a white solid: R_f 0.12 (24:1 CHCl$_3$–MeOH). ^{1}H NMR (CDCl$_3$–CD$_3$OD): δ 5.70 (ddt, *J* 17.3, *J* 10.0, *J* 5.2 Hz, 1 H, OCH$_2$C*H*=CH$_2$), 5.10 (dd, *J* 17.3, *J* 1.4 Hz, 1 H, OCH$_2$CH=C*H*$_a$H$_b$), 5.00 (dd, *J* 10.0, *J* 1.4 Hz, 1 H, OCH$_2$CH=CH$_a$*H*$_b$), 4.44 (t, *J* 8.2, *J* 6.9 Hz, 1 H, SCH$_2$C*H*), 4.35 (d, 2 H, OC*H*$_2$CH=CH$_2$), 4.30 (bs, 1 H, H-1 Gal), 3.70–3.30 (m, 6 H, H-2–6 Gal), 3.01 (m, NHC*H*$_2$, 2 H), 2.73 (dd, *J* 14.1, *J* 8.2 Hz, 1 H, SC*H*$_a$CH$_b$), 2.58 (dd, *J* 14.1, *J* 6.9 Hz, 1 H, SCH$_a$C*H*$_b$), 2.03 [t, *J* 7.0 Hz, 2 H, NHC(O)C*H*$_2$], 1.50–1.00 [m, 42 H, (C*H*$_2$)$_{12}$CH$_3$ and (C*H*$_2$)$_9$CH$_3$], 0.67 (t, *J* 6.5 Hz, 6 H, CH$_3$). ^{13}C NMR (CDCl$_3$–CD$_3$OD): δ 175.1 and 171.3 [C(O)NH], 158.0 [NHC(O)O], 132.4 (OCH$_2$*C*H=CH$_2$), 117.4 (OCH$_2$CH=*C*H$_2$), 85.6 (C-1 Gal), 80.0 (C-5 Gal), 73.5 (C-3 Gal), 69.3 (C-4 Gal), 66.6 (O*C*H$_2$CH=CH$_2$), 62.6 (C-6 Gal), 53.7 (SCH$_2$*C*H), 53.5 (C-2 Gal), 40.0 (NHCH$_2$), 36.5 [NHC(O)-*C*H$_2$], 33.3 (SCH$_2$), 32.3 (*C*H$_2$CH$_2$CH$_3$), 30.0, 29.8, 29.7, and 29.6 [(C*H*$_2$)$_9$CH$_2$CH$_2$CH$_3$ and (C*H*$_2$)$_6$CH$_2$CH$_2$CH$_3$], 27.3 (NHCH$_2$C*H*$_2$), 26.1 [NHC(O)CH$_2$*C*H$_2$], 23.0 (*C*H$_2$CH$_3$), 14.2 (CH$_3$).

Step 4: deprotection of Aloc when applied to **39a** (100 mg, 0.13 mmol) in 1:1 DMF–CH$_2$Cl$_2$ (6 mL), water (50 μL), Pd(PPh$_3$)$_2$Cl$_2$ (5 mg, 7.12.10-3 mmol), and Bu$_3$SnH (60 μL, 0.22 mmol) afforded after 12 h at rt, filtration, evaporation of the solvent, chromatography (CHCl$_3$ to 49:1 CHCl$_3$–MeOH) and recrystallization (Et$_2$O), **II-Gal(NH$_2$)Cys[C14][C12](L)** (70 mg, 80%) as a white solid: R_f 0.20 (9:1 CHCl$_3$–MeOH); $[\alpha]_D$ −10.2° (*c* 0.53; 4:1 CHCl$_3$–MeOH). ^{1}H NMR (CDCl$_3$–CD$_3$OD): δ 4.58 (t, *J* 6.9 Hz, 1 H, SCH$_2$CH), 4.38 (d, *J* 10.0 Hz, 1 H, H-1 Gal), 3.80–3.50 (m, 5 H, H-2 Gal and H-4-6 Gal), 3.41 (dd, $J_{2,3}$ 9.8, $J_{3,4}$ 2.9 Hz, 1 H, H-3 Gal), 3.11 (t, *J* 7.1 Hz, 2 H, NHC*H*$_2$), 3.00–2.73 (m, 2 H, SCH$_2$), 2.15 [t, *J* 7.4 Hz, 2 H, NHC(O)C*H*$_2$], 1.60–1.30 (m, 4 H, NHCH$_2$C*H*$_2$ and NHC(O)CH$_2$C*H*$_2$), 1.30–1.00 [m, 38 H, (C*H*$_2$)$_{11}$CH$_3$ and (C*H*$_2$)$_8$CH$_3$], 0.80 (t, *J* 6.7 Hz, 6 H, CH$_3$). ^{13}C NMR (CDCl$_3$–CD$_3$OD): δ 174.4 and 170.4 [C(O)NH], 87.4 (C-1 Gal), 79.3 (C-5 Gal), 74.0 (C-3 Gal), 68.5 (C-4 Gal), 62.1 (C-6 Gal), 53.3 (SCH$_2$*C*H), 52.5 (C-2 Gal), 39.6 (NHCH$_2$), 36.1 [NHC(O)C*H*$_2$], 33.4 (SCH$_2$), 31.8 (*C*H$_2$CH$_2$CH$_3$), 29.5, 29.1, and 28.9 [(*C*H$_2$)$_9$CH$_2$CH$_2$CH$_3$ and (*C*H$_2$)$_6$CH$_2$CH$_2$-CH$_3$], 26.7 (NHCH$_2$C*H*$_2$), 25.5 [NHC(O)-CH$_2$*C*H$_2$], 22.5 (*C*H$_2$CH$_3$), 13.7 (CH$_3$). Anal. Calcd for C$_{35}$H$_{69}$N$_3$O$_6$S·3H$_2$O (714.07): C, 58.87; H, 10.59; N, 5.88; S, 4.49. Found: C, 59.09; H, 9.71; N, 5.75; S, 4.42.

N-*Tetradecanoyl-3*-S-*(amino-2-deoxy-β*-D-*galactopyranosyl)*-L-*cysteine tetradecylamide* (**II-Gal(NH$_2$)Cys[C14][C14](L)**). Step 1: the process used for the preparation of **38a** when applied to EDC (70 mg, 0.365 mmol), **37** (230 mg, 0.35 mmol), tetradecanoic acid (76 mg, 0.33 mmol) and HOBt (45 mg, 0.33 mmol), afforded *N*-tetradecanoyl-3-*S*-(3,4,6-tri-*O*-acetyl-2-allyloxycarbonylamino-2-deoxy-β-D-galactopyranosyl)-L-cysteine tetradecylamide (**38b**, 250 mg, 85%) as a white solid: R_f 0.44 (24:1 CHCl$_3$–MeOH). ^{1}H NMR identical to that of **38a** except at 1.60–1.10 [m, 46 H, (C*H*$_2$)$_{12}$CH$_3$ and (C*H*$_2$)$_{11}$CH$_3$]. ^{13}C NMR identical to that of **38a**.

Step 2: the deacetylation of **38b** (120 mg, 0.13 mmol) was performed in a 1:10 Et$_3$N–MeOH mixture (5 mL) at rt for 2 days. The solvents were evaporated to dryness under diminished pressure giving *N*-tetradecanoyl-3-*S*-(2-allyloxycarbonylamino-2-deoxy-β-D-galactopyranosyl)-L-cysteine tetradecylamide (**39b**, 87 mg, 85%) as a white solid: R_f 0.12 (24:1 CHCl$_3$–MeOH). ^{1}H NMR identical to that of **39a** except at 1.50–1.00 [m, 46 H,

(CH_2)$_{12}CH_3$ and (CH_2)$_{11}CH_3$]. ^{13}C NMR identical to that of **39a**.

Step 3: the Aloc-deprotection procedure, when applied to **39b**, as described for **39a**, afforded, after chromatography ($CHCl_3$ to 49:1 $CHCl_3$–MeOH) and recrystallization (Et_2O), **II-Gal(NH_2)Cys[C14][C14](L)** as a white solid (80%): R_f 0.18 (9:1 $CHCl_3$–MeOH); $[\alpha]_D$ −7.9° (*c* 0.80; 4:1 $CHCl_3$–MeOH). ^{1}H NMR identical to that **II-Gal(NH_2)Cys[C14][C12](L)** except at 1.60–1.00 [m, 46 H, (CH_2)$_{12}CH_3$ and (CH_2)$_{11}CH_3$]. ^{13}C NMR identical to that of **II-Gal(NH_2)Cys[C14][C12](L)**. Anal. Calcd for $C_{37}H_{73}N_3O_6S\cdot3H_2O$ (742.12): C, 59.88; H, 10.73; N, 5.66. Found: C, 60.24; H, 10.12; N, 5.67.

N-*Dodecanoyl-3*-S-*(2-acetamido-2-deoxy-β*-D-*galactopyranosyl)*-L-*cysteine tetradecylamide* (**II-Gal(NHAc)Cys[C14][C12](L)**). Step 1: the Aloc-deprotection procedure when applied to a CH_2Cl_2 solution (3 mL) of **38a** (124 mg, 0.14 mmol), Ac_2O (50 μL, 0.58 mmol), $Pd(PPh_3)_2Cl_2$ (5 mg, 7.12×10^{-3} mmol) and Bu_3SnH (40 μL, 0.15 mmol) gave, after stirring for 4 h at rt, evaporation of the solvent, and recrystallization from Et_2O, *N*-dodecanoyl-3-*S*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-L-cysteine tetradecylamide (**40a**, 110 mg, 91%) as a white solid: R_f 0.24 (24:1 $CHCl_3$–MeOH). ^{1}H NMR ($CDCl_3$): δ 6.77 [m, 2 H, NHC(O)], 6.31 [d, *J* 9.5 Hz, 1 H, NHC(O)CH_3], 5.34 (bd, *J* 2.9 Hz, 1 H, H-4 Gal), 5.03 (dd, $J_{2,3}$ 10.6, $J_{3,4}$ 3.0 Hz, 1 H, H-3 Gal), 4.71 (d, *J* 10.1 Hz, 1 H, H-1 Gal), 4.56 (m, 1 H, SCH_2CH), 4.33 (dd, $J_{1,2}$ 10.1, $J_{2,3}$ 10.6 Hz, 1 H, H-2 Gal), 4.10–4.00 (m, 3 H, H-5 Gal and H-6–6′ Gal), 3.18 (dt, *J* 6.4, *J* 5.6 Hz, 2 H, $NHCH_2$), 3.03 (dd, *J* 13.8, *J* 6.3 Hz, 1 H, SCH_aCH_b), 2.78 (dd, *J* 13.9, *J* 8.2 Hz, 1 H, SCH_aCH_b), 2.15 [t, *J* 7.3 Hz, 2 H, NHC(O)CH_2], 2.11, 1.93, 1.92 [all s, all 3 H, CH_3C(O)O], 1.89 [s, 3 H, CH_3C(O)NH], 1.60–1.10 [m, 42 H, (CH_2)$_{12}CH_3$ and (CH_2)$_9CH_3$], 0.82 (t, *J* 7 Hz, 6 H, CH_3CH_2). ^{13}C NMR ($CDCl_3$): δ 173.6, 170.7, 170.5, 170.3, and 169.9 [C(O)NH and CH_3C(O)], 86.7 (C-1 Gal), 75.0 (C-5 Gal), 71.5 (C-3 Gal), 67.2 (C-4 Gal), 62.3 (C-6 Gal), 52.2 (SCH_2CH), 49.0 (C-2 Gal), 39.9 ($NHCH_2$), 36.6 [NHC(O)CH_2], 33.6 (SCH_2), 32.0 ($CH_2CH_2CH_3$), 29.7, 29.6, and 29.4 [(CH_2)$_9$-$CH_2CH_2CH_3$ and (CH_2)$_6CH_2CH_2CH_3$], 27.0 ($NHCH_2CH_2$), 25.8 [NHC(O)CH_2CH_2], 23.4 [CH_3C(O)NH], 22.8 (CH_2CH_3), 20.8 and 20.6 [CH_3C(O)], 14.2 (CH_3CH_2).

Step 2: the deacetylation of **40a** (110 mg, 0.13 mmol) was performed in a mixture of 1:10 Et_3N–MeOH (5 mL) and $CHCl_3$ (2 mL) at rt for 2 days. After evaporation of the solvents, chromatography ($CHCl_3$ to 9:1 $CHCl_3$–MeOH) and recrystallization from Et_2O, **II-Gal(NHAc)Cys[C14][C12](L)** (76 mg, 80%) was obtained as a white solid: R_f 0.30 (9:1 $CHCl_3$–MeOH). $[\alpha]_D$ −6.3 ° (*c* 0.76; 4:1 $CHCl_3$–MeOH). ^{1}H NMR ($CDCl_3$–CD_3OD): δ 4.46 (dd, *J* 7.6, *J* 7.4 Hz, 1 H, SCH_2CH), 4.36 (d, *J* 10.1 Hz, 1 H, H-1 Gal), 3.83 (t, *J* 10.2 Hz, 1 H, H-2 Gal), 3.75–3.65 (m, 2 H, H-5 Gal, H-6 Gal), 3.56 (dd, $J_{6,6'}$ 12.2, $J_{5,6'}$ 3.3 Hz, 1 H, H-6′ Gal), 3.43 (dd, $J_{2,3}$ 10.3, $J_{3,4}$ 3.3 Hz, 1 H, H-3 Gal), 3.33 (m, 1 H, H-4 Gal), 3.01 (t, *J* 6.8 Hz, 2 H, $NHCH_2$), 2.81 (dd, *J* 14.2, *J* 7.6 Hz, 1 H, SCH_aCH_b), 2.58 (dd, *J* 14.2, *J* 7.4 Hz, 1 H, SCH_aCH_b), 2.06 [t, *J* 7.3 Hz, 2 H, NHC(O)CH_2], 1.82 [s, 3 H, CH_3C(O)NH], 1.50–1.25 [m, 4 H, C(O)NHCH_2CH_2 and NHC(O)CH_2CH_2], 1.25–1.10 [m, 38 H, (CH_2)$_{11}CH_3$ and (CH_2)$_8CH_3$], 0.70 (t, *J* 6.5 Hz, 6 H, CH_3CH_2). ^{13}C NMR ($CDCl_3$–CD_3OD): δ 174.4, 172.7, and 170.3 [C(O)NH], 84.3 (C-1 Gal), 79.2 (C-5 Gal), 73.3 (C-3 Gal), 68.6 (C-4 Gal), 62.0 (C-6 Gal), 52.6 (SCH_2CH), 51.5 (C-2 Gal), 39.4 ($NHCH_2$), 36.0 [NHC(O)CH_2], 32.9 (SCH_2), 31.6 ($CH_2CH_2CH_3$), 29.4, 29.2, 29.1, and 28.9 [(CH_2)$_9CH_2CH_2CH_3$ and (CH_2)$_6CH_2CH_2CH_3$], 26.7 ($NHCH_2CH_2$), 25.5 [NHC(O)CH_2CH_2], 22.4 [CH_3C(O)NH and CH_2CH_3), 13.7 (CH_3CH_2). Anal. Calcd for $C_{37}H_{71}N_3O_7S\cdot0.5H_2O$ (711.06): C, 62.50; H, 10.21; N, 5.91; S, 4.51. Found: C, 62.13; H, 10.06; N, 5.88; S, 4.43.

N-*Tetradecanoyl-3*-S-*(2-acetamido-2-deoxy-β*-D-*galactopyranosyl)*-L-*cysteine tetradecylamide* (**II-Gal(NHAc)Cys[C14][C14](L)**). Step 1: the same process as described for the synthesis of **40a**, when applied to **38b** (120 mg, 0.13 mmol) afforded *N*-tetradecanoyl-3-*S*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-L-cysteine tetradecylamide (**40b**, 110 mg, 95%) as a white solid: R_f 0.25

(24:1 $CHCl_3$–MeOH). ^1H NMR identical to that **40a** excepting at 1.60–1.00 [m, 46 H, $(CH_2)_{12}CH_3$ and $(CH_2)_{11}CH_3$]. ^{13}C NMR identical to that of **40a**.

Step 2: the deacetylation of **40b**, as described for **40a**, gave **II-Gal(NHAc)Cys[C14][C14](L)** as a white solid (90%): R_f 0.38 (9:1 $CHCl_3$–MeOH). $[\alpha]_D$ −5.0° (*c* 0.73; 4:1 $CHCl_3$–MeOH). ^1H NMR identical to that **II-Gal(NHAc)Cys[C14][C12](L)** except at 1.60–1.00 [m, 46 H, $(CH_2)_{12}CH_3$ and $(CH_2)_{11}CH_3$]. ^{13}C NMR identical to that of **II-Gal(NHAc)Cys[C14][C12](L)**.

N-*Dodecanoyl*-3-S-(2-*acetamido*-2-*deoxy*-3,4,6-*tri*-O-*sulfo*-β-D-*galacto*-*pyranosyl*)-L-*cysteine tetradecylamide* (**II-Gal(NHAc)-SulCys[C14][C12](L)**). **II-Gal(NHAc)Cys[C14]-[C12](L)** (35 mg, 0.048 mmol) and SO_3·pyridine (83 mg, 0.52 mmol) in anhyd DMF (3 mL) were stirred at rt for 4 h. The reaction mixture was evaporated to dryness under diminished pressure. The residue was poured in water (5 mL) containing Na_2CO_3 (55 mg, 0.52 mmol) then stirred at rt for 4 h. After evaporation to dryness, chromatography (49:1 to 9:1 $CHCl_3$–MeOH), and recrystallization from Et_2O, **II-Gal(NHAc)SulCys-[C14][C12](L)** (30 mg, 60%) as a white solid was obtained: R_f 0.27 (65:35:4 $CHCl_3$–MeOH–water). ESIMS: $m/z = 1012.5$ $[M - Na]^-$, 494.8 $[M - 2Na]^{2-}$, 322.1 $[M - 3Na]^{3-}$. ^1H NMR (CD_3OD): δ 5.03 (d, $J_{3,4}$ 3.1 Hz, 1 H, H-4 Gal), 4.87 (bs, 1 H, H-3 Gal), 4.71 (d, $J_{1,2}$ 10.1 Hz, 1 H, H-1β Gal), 4.56–4.23 (m, 3 H, H-5 Gal, H-6′ Gal and SCH_2CH), 4.18–4.02 (m, 2 H, H-2 Gal and H-6 Gal), 3.18 (m, 3 H, SCH_aCH_b and $NHCH_2$), 2.80 (dd, *J* 14.2, *J* 9.8 Hz, 1 H, SCH_aCH_b), 2.33 [t, *J* 7.3 Hz, 2 H, $NHC(O)CH_2$], 1.96 [s, 3 H, $CH_3C(O)NH$], 1.70–1.40 [m, 4 H, $C(O)NHCH_2CH_2$ and $NHC(O)CH_2CH_2$], 1.40–1.20 [m, 38 H, $(CH_2)_{11}CH_3$ and $(CH_2)_8CH_3$], 0.89 (t, *J* 6.5 Hz, 6 H, CH_3CH_2). ^{13}C NMR (CD_3OD): δ 176.4, 173.8, and 172.6 [C(O)NH], 85.4 (C-1β Gal), 77.9 (C-3 Gal), 77.0 (C-5 Gal), 74.6 (C-4 Gal), 68.6 (C-6 Gal), 54.7 (SCH_2CH), 50.6 (C-2 Gal), 40.4 ($NHCH_2$), 36.8 [$NHC(O)CH_2$], 33.0 (SCH_2), 32.8 ($CH_2CH_2CH_3$), 30.7, 30.6, 30.5, 30.4, 30.2, and 30.1 [$(CH_2)_9CH_2CH_2CH_3$ and $(CH_2)_6CH_2CH_2CH_3$], 27.8 ($NHCH_2CH_2$), 26.6 [$NHC(O)CH_2CH_2$], 23.5 (CH_2CH_3), 23.1 [$CH_3C(O)NH$], 14.4 (CH_3CH_2).

Biological section

Material. The soya phospholipon® 100 (PL), was a gift from Rhône Poulenc Rohrer, and contained phosphatidylcholines (> 95.0% w/w) and lysophosphatidylcholine (= 2.0%). Cholesterol (CH, grade 99%), Dubelcco modified phosphate buffered saline (DPBS) and penicillin–streptomycin solution were purchased from SIGMA. [^3H]-Suramin (49 Ci/mmol) was from Isotopchim (Ganagobie-Peyrus, France). SPC3 [$(GPGRAF)_8$–$(K)_4$–$(K)_2$–K–βA] was a generous gift from Eurithics (Paris, France).

Sample preparation. All the galactolipids were assayed in biological tests as solution in DPBS. Some of them (see Table 1) were also tested when formulated in the membrane of PL/CH (2:1 molar ratio) liposomes. In these preparations, the galactolipid/(PL + CH) molar ratio represented 0.3 or 0.6, i.e., 10 or 20% of total molar lipid amount (higher amounts of galactolipids led to liposomes that aggregate very rapidly).

Typically, dry lipid films were prepared by evaporation, under a dinitrogen stream, of an organic solution (MeOH–$CHCl_3$) containing PL (for a final concentration of 550–650 μM and 5–7 mM for the tests on CEM-SS and HT29 cells, respectively), CH (for a final concentration of 275–330 μM and 2.5–3.5 mM for the tests on CEM-SS and HT29 cells, respectively) and the galactolipid under investigation (10 or 20% of total molar lipid amount). After drying under reduced pressure for 30–60 min, the film was hydrated with 2 mL of a phosphate buffered saline (PBS, Sigma, pH 7.4) for 10 h at 50–55 °C, and about 10 cycles of freezing (liquid nitrogen) and heating (~50 °C or 90 °C) were performed. The raw dispersions were then allowed to stand for 24–48 h at 70 °C and then sonicated at 45 °C for 15–30 min (Branson Sonic Power Co. Sonifier Cell Disrupter B30). The preparations were centrifuged at 3000 rpm for 10 min. Alternatively, the liposomes were prepared by extrusion through polycarbonate membranes (Liposofast Milsch Equipment; size membrane pores 100 or 200 nm) of the raw lipid dispersions. The microbial proliferation in these preparations was prevented by

the addition (5 μL per mL of dispersion) of a penicillin (10,000 units) and streptomycin (10 mg/ml) in NaCl (0.9%) solution. Average particle sizes and size distributions were measured by laser light scattering on a Coulter N4MD sub-micron particle analyzer after preparation of the liposomes, then periodically during storage at rt (the particle sizes reported in Table 1 correspond to a storage period of 1 week). All the formulations were tested within 1 week of preparation.

Phospholipid and galactolipid concentrations were determined by the Stewart assay [70] and galactose assay [71], respectively. Galactose determination in the preparations made from the fluorinated galactosides was carried out by ^{19}F NMR using CF_3CH_2OH as internal standard. These determinations were consistent with the theoretical values.

Inhibition of HIV-1 infection

HT-29 cells: The human colonic adenocarcinoma cell line HT-29 was grown in 1:1 Dubelcco's modified Eagle's F12 medium (v/v) supplemented with 10% heat-inactivated fetal calf serum and 15 mM HEPES (pH 7.4). Cells were harvested from the culture flasks with trypsin-EDTA and subcultured every week. HT-29 were infected with a 1000 tissue culture infectious dose 50% ($TCID_{50}$) of HIV-1 (NDK) preincubated for 30 min at 37 °C in the presence or absence of the galactolipid under investigation. The cells were exposed to the mixture for 2 h. HIV-1 p24(gag) antigen, indicator of infection level, was measured in the culture supernatant from these cells 7 days post-infection, as described elsewhere [72]. The same procedure was used for the HT29 cells expressing CD4. These modified cells were obtained as described elsewhere [5]. Empty PL/CH liposomes as a control were also investigated.

CEM-SS cells: CEM-SS cells were infected with a dose of HIV-1 (LAI) infecting 50% of the cells, as described elsewhere [73]. 5 days later, the production of HIV-1 was evaluated by measuring the reverse transcriptase activity (RT) which expresses the presence of the virus in the culture supernatant. The tested compounds or liposomal formulations were added to the cell cultures after viral adsorption. RT inhibition% was measured in comparison with the non treated cells. AZT was used as a positive control ($IC_{50} = 4$ nM).

The galactoside IC_{50} values reported in Table 1 were determined from the curves of the RT inhibition% (CEM-SS), or of the p24 production% (HT29) against galactoside concentration.

Toxicity. The effects of the galactosides on CEM-SS or HT29 cell proliferation and viability were studied on non-infected cells in a colorimetric assay using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide] [74] or XTT (sodium 3′-[1-[(phenylamino) - carbonyl] - 3,4 - tetrazolium]-bis(4-methoxy-6-nitro)benzenesulfonic acid hydrate) [14] with various concentration of the tested product as described elsewhere, respectively.

Inhibition of [^{3}H]-suramin binding to SPC3. SPC3 (5 μM, 100 μL) was incubated in polyvinyl chloride 96-wells overnight at 4 °C. The wells were washed three times with 200 μL of DPBS and subsequently treated with DPBS 1% gelatin for 90 min at 37 °C to reduce non specific binding. The plates were then incubated for 1 h at 37 °C with 100 μL of 3H-suramin (106 counts/min/well) in either the absence or presence of increasing concentration of unlabelled suramine or GalCer analogs. The insoluble double-chain **GalAE** galactosides were tested formulated in liposomes and empty liposomes were used as negative controls. The plates were then washed five times with 200 μL of DPBS, each well was individualized, and the radioactivity associated to the wells was determined in a β scintillation counter (Beckman, Marseilles, France).

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References

[1] J.S. Cairns, M.P. D'Souza, *Nature Med.*, 4 (1998) 563–568.
[2] (a) E.A. Berger, *Nature Struct. Biol.*, 5 (1998) 671–674. (b) (a) E.A. Berger, *AIDS*, 11 (1997) S3–S16.
[3] D.C. Chan, P.S. Kim, *Cell*, 93 (1998) 681–684.
[4] D.R. Littman, *Cell*, 93 (1998) 677–680.

[5] O. Delézay, N. Koch, N. Yahi, D. Hammache, C. Tourres, C. Tamalet, J. Fantini, *AIDS*, 11 (1997) 1311–1318, and references therein.
[6] D.G. Cook, J. Fantini, S.L. Spitalnik, F. Gonzales-Scarano, *Virology*, 201 (1994) 206–214.
[7] N. Yahi, J.M. Sabatier, S. Baghdiguian, F. Gonzales-Scarano, J. Fantini, *J. Virol.*, 69 (1995) 320–325.
[8] S. Bhat, R.V. Mettus, E.P. Reddy, K.E. Ugen, V. Srikanthan, W.V. Williams, D.B. Weiner, *AIDS Res. Hum. Retrovirus*, 9 (1993) 175–181.
[9] J. Fantini, D. Hammache, O. Delezay, G. Pieroni, C. Tamalet, N. Yahi, *Virology*, 246 (1998) 211–220.
[10] C.R. Bertozzi, D.G. Cook, W.R. Kobertz, F. Gonzalez-Scarano, M.D. Bednarski, *J. Am. Chem. Soc.*, 114 (1992) 10639–10641.
[11] I. Rico-Lattes, J-C. Garrigues, E. Perez, C. André-Barrès, C. Madelaine-Dupuich, A. Lattes, M-D. Linas, A-M. Aubertin, *New J. Chem.*, 19 (1995) 341–344.
[12] H. Yoshida, K. Ikeda, K. Achiwa, H. Hoshino, *Chem. Pharm. Bull.*, 43 (1995) 594–602.
[13] K. Ikeda, T. Asahara, K. Achiwa, H. Hoshino, *Chem. Pharm. Bull.*, 45 (1997) 402–405.
[14] J. Fantini, D. Hammache, O. Delezay, N. Yahi, C. André-Barrès, I. Rico-Lattes, A. Lattes, *J. Biol. Chem.*, 272 (1997) 7245–7252.
[15] I. Rico-Lattes, M-F. Gouzy, C. André-Barrès, B. Guidetti, A. Lattes, *New J. Chem.*, 22 (1998) 451–457.
[16] L. Clary, J. Greiner, C. Santaella, P. Vierling, *Tetrahedron Lett.*, 36 (1995) 539–542.
[17] O. Delezay, D. Hammache, J. Fantini, N. Yahi, *Biochemistry*, 35 (1996) 15663–15671.
[18] J.G. Riess, F. Frézard, J. Greiner, MP Krafft, C. Santaella, P. Vierling, L. Zarif, in Y. Barenholz, D.D. Lasic (Eds.), *Handbook of Nonmedical Applications of Liposomes, from Design to Microreactors*, Vol. III, CRC Press, Boca Raton, 1996, pp. 95–139.
[19] D. Hammache, N. Yahi, G. Pieroni, F. Ariasi, C. Tamalet, J. Fantini, *Biochem. Biophys. Res. Commun.*, 246 (1998) 117–122.
[20] D. Hammache, G. Pieroni, N. Yahi, O. Delezay, N. Koch, H. Lafont, C. Tamalet, J. Fantini, *J. Biol. Chem.*, 273 (1998) 7967–7971.
[21] P. Vierling, C. Santaella, J.G. Riess, in F. Puisieux, P. Couvreur, J. Delattre, J.P. Devissaguet (Eds.), *Liposomes: New Systems and New Trends in their Applications*, Edition de Santé, Paris, 1995, pp. 293–318.
[22] C. Santaella, F. Frézard, P. Vierling, J.G. Riess, *FEBS Lett.*, 336 (1993) 481–483.
[23] N. Yahi, S. Baghdiguian, H. Moreau, J. Fantini, *J. Virol.*, 66 (1992) 4848–4854.
[24] J. Fantini, D.G. Cook, N. Nathanson, S.L. Spitalnik, F. Gonzalez-Scarano, *Proc. Natl. Acad. Sci. USA*, 90 (1993) 2700–2704.
[25] J. Fantini, N. Yahi, *Médecine/Sciences*, 9 (1993) 891–900.
[26] J. Kihlberg, M. Elofsson, L.A. Salvador, *Methods Enzymol.*, 289 (1997) 221–245.
[27] G. Arsequell, G. Valencia, *Tetrahedron Asymm.*, 8 (1997) 2839–2876.
[28] C.D. Chang, M. Waki, M. Ahmad, J. Meienhofer, E.O. Lundell, J.D. Haug, *Int. J. Peptide Protein*, 15 (1980) 59–66.
[29] L. Clary, C. Santaella, P. Vierling, *Tetrahedron*, 51 (1995) 13073–13088.
[30] R.R. Schmidt, *Angew. Chem., Int. Ed. Engl.*, 25 (1986) 212–235.
[31] R.R. Schmidt, in B.M. Trost, I. Fleming, E. Winterfieldt (Eds.), *Comprehensive Organic Chemistry*, Vol. 6, Pergamon, Oxford, 1991, pp. 33–66.
[32] R.R. Schmidt, R. Kläger, *Angew. Chem., Int. Ed. Engl.*, 24 (1985) 65–66.
[33] F.A.W. Koeman, J.P. Kamerling, J.F.G. Vliegenthart, *Tetrahedron*, 49 (1993) 5291–5304.
[34] A.F. Bochkov, G.E. Zaikov, *Chemistry of the O-Glycosidic Bond, Formation and Cleavage*, Pergamon, Oxford, 1979.
[35] J. Greiner, A. Manfredi, J.G. Riess, *New J. Chem.*, 13 (1989) 247–254.
[36] J. Banoub, D.R. Bundle, *Can. J. Chem.*, 57 (1979) 2085–2090.
[37] L.A. Salvador, M. Elofsson, J. Kihlberg, *Tetrahedron*, 51 (1995) 5643–5656.
[38] M. Elofsson, B. Walse, J. Kihlberg, *Tetrahedron Lett.*, 32 (1991) 7613–7616.
[39] D.M. Whitefield, S.P. Douglas, T.H. Tang, I.G. Csizmadia, H.Y.S. Pang, F.L. Moolten, J.J. Krepinski, *Can. J. Chem.*, 72 (1994) 2225–2238.
[40] H. Vegad, C.J. Gray, P.J. Somers, A.S. Dutta, *J. Chem. Soc., Perkin Trans. I*, (1997) 1429–1441.
[41] K.C. Nicolaou, T.J. Caufield, H. Kataoka, N.A. Stylianides, *Carbohydr. Res.*, 202 (1990) 177–182.
[42] R. Polt, L. Szabõ, J. Treiberg, Y. Li, V.J. Hruby, *J. Am. Chem. Soc.*, 114 (1992) 10249–10258.
[43] K. Bock, C. Pedersen, *Adv. Carbohydr. Chem. Biochem.*, 41 (1983) 27–66.
[44] K. Dill, E. Berman, A.A. Pavia, *Adv. Carbohydr. Chem. Biochem.*, 43 (1985) 1–49.
[45] P. Rosevear, T. VanAken, J. Baxter, S. Ferguson-Miller, *Biochemistry*, 19 (1980) 4108–4115.
[46] P. Schultheiss-Reimann, H. Kunz, *Angew. Chem., Int. Ed. Engl.*, 22 (1983) 62–63.
[47] H. Kunz, S. Birnbach, *Angew. Chem. Int. Ed. Engl.*, 25 (1986) 360362–1620.
[48] H.G. Garg, R.W. Jeanloz, *Adv. Carbohydr. Chem. Biochem.*, 43 (1985) 135–201.
[49] G.A. Roth, *J. Org. Chem.*, 60 (1995) 8105–8109.
[50] K.H. Wiesmuller, W. Bessler, G. Jung, *Hoppe-Seyler's Z. Physiol. Chem.*, 364 (1983) 593–606.
[51] I. Photaki, J. Taylor-Papadimitriou, C. Sakarellos, P. Mazarakis, L. Zervas, *J. Chem. Soc. (C)*, (1970) 2683–2687.
[52] B. Kamber, A. Hartmann, K. Eisler, B. Riniker, H. Rink, P. Sieber, W. Rittel, *Helv. Chim. Acta*, 63 (1980) 899–915.
[53] O. Locknoff, *Angew. Chem., Int. Ed. Engl.*, 30 (1991) 1611–1620.
[54] J. Banoub, P. Boullanger, D. Lafont, *Chem. Rev.*, 92 (1992) 1167–1195.
[55] E.C. Roos, P. Bernabé, H. Hiemstra, W.N. Speckamp, B. Kaptein, W.H.J. Boesten, *J. Org. Chem.*, 60 (1995) 1733–1740.
[56] O. Dangles, F. Guibé, G. Balavoine, S. Lavieille, A. Marquet, *J. Org. Chem.*, 52 (1987) 4984–4993.
[57] P. Boullanger, M. Jouineau, B. Bouammali, D. Lafont, G. Descotes, *Carbohydr. Res.*, 202 (1990) 151–164.
[58] K. Bock, H. Thogessen, *Annu. Rep. NMR Spectrosc.*, 13 (1982) 1–57.
[59] H. Kunz, H. Waldmann, *Angew. Chem., Int. Ed. Engl.*, 23 (1984) 71–72.
[60] T. Ikami, H. Hamajima, T. Usui, T. Mikani, H. Ishida, M. Kiso, A. Hasegawa, *J. Carbohydr. Chem.*, 16 (1997) 859–875.
[61] N. Iida, T. Toida, Y. Kushi, S. Handa, P. Fredman, L. Svennerholm, I. Ishiziha, *J. Biol. Chem.*, 264 (1988) 5974–5980.

[62] N. Yahi, J. Fantini, S. Baghdiguian, K. Mabrouk, C. Tamalet, J. Van Rietschoten, H. Rochat, J.M. Sabatier, *Proc. Natl. Acad. Sci. USA*, 92 (1995) 4867–4871.
[63] N. Yahi, J.-M. Sabatier, P. Nickel, K. Mabrouk, F. Gonzales-Scarano, J. Fantini, *J. Biol. Chem.*, 269 (1994) 24349–24353.
[64] D. Long, J.F. Berson, D.G. Cook, R.W. Doms, *J. Virol.*, 68 (1994) 5890–5897.
[65] D. Hammache, N. Yahi, M. Maresca, G. Pieroni, J. Fantini, *J Virol.*, 73 (1999) 5244–5248.
[66] P. Sears, C.H. Wong, *Angew. Chem., Int. Ed. Engl.*, 38 (1999) 2300–2324.
[67] M. Bodanszky, A. Bodanszky, *The Practice of Peptide Synthesis*, Springer, Berlin, 1984.
[68] A. Manfredi, S. Abouhilale, J. Greiner, J.G. Riess, *Bull. Soc. Chim. Fr.*, (1989) 872–878.
[69] G. Excoffier, D. Gagnaire, J.P. Utille, *Carbohydr. Res.*, 39 (1975) 368–373.
[70] J.C.M. Stewart, *Anal. Biochem.*, 104 (1980) 10–14.
[71] M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, F. Smith, *Anal. Chem.*, 28 (1956) 350–356.
[72] N. Yahi, S.L. Spitalnik, K.A. Stefano, P. De Micco, F. Gonzalez-Scarano, J. Fantini, *Virology*, 204 (1994) 550–557.
[73] C. Moog, A. Wick, P. Le Ber, A. Kirn, A.M. Aubertin, *Antiviral Res.*, 24 (1994) 275–288.
[74] R. Pauwels, J. Balzarini, M. Baba, D. Snoeck, P. Herdewijn, J. Desmyter, E. De Clercq, *J. Virol. Methods*, 20 (1988) 309–321.