Amphiphilic Anionic Analogues of Galactosylceramide: Synthesis, Anti-HIV-1 Activity, and gp120 Binding

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We describe the synthesis together with the results of anti-HIV-1 activity and gp120-monolayer binding experiments of new galactosyl amphiphiles, analogues of galactosylceramide, an alternative receptor used by HIV to infect CD4 negative cells. These compounds consist of single- and double-chain amphiphiles containing one or two galactose residues. To favor their clustering into galactosyl-rich microdomains, their molecular structure contains also an amino group or several hydroxyls or anionic groups, such as carboxylate, sulfate, sulfonate, and phosphate. Among the 12 new galactosylated compounds reported, a specific anti-HIV activity, although moderate (IC_{50} from 10 to 50 μM), was detected only for three of them, i.e., **I-GalSer-[CO2Na][C14], II-GalSer[C14][C7SO3Na]**, and **II-GalSer[C2SO4Na][C14]**, which contain an anionic group. The marked increase of surface pressure which was observed upon addition of gp120 into the aqueous subphase underneath the monolayers containing these galactolipids indicated gp120 insertion into the monolayers, suggesting that binding of these three derivatives to HIV-1 gp120 may be responsible for their anti-HIV activity.

Introduction

Compounds intended to inhibit the human immunodeficiency virus (HIV) at the early stages of its replication cycle, such as its adsorption on and entry into cells, are worthy candidates for combination therapy with the drugs presently used in clinics. HIV infects mainly CD4(+) lymphoid cells. Their infection is initiated by the binding of HIV (through its envelope glycoprotein gp120) to CD4 and then to a cellular coreceptor of the chemokine receptor family, such as mainly CCR5 or CXCR4,[1−6] according to the cell type infected and the virus tropism. The interactions of the gp120/CD4 complex with these coreceptors involve the V3 loop of gp120, which, thus, plays a key role in the complex fusion process of the HIV envelope with the cellular membrane. HIV can also infect in vitro many CD4(−) cells, including hepatocytes, natural killer cells, and neural and colon epithelial cells.[7] The infection of these two latter cell types has been proposed to occur through the binding of the V3 loop to the galactosylceramide (GalCer) receptor (see structure in Table 1) they express and more particularly to GalCer-rich microdomains. The nonlinear relationships found between GalCer concentration in a bilayer and its binding to gp120 suggests indeed the involvement of GalCer-rich microdomains,[8,9] thus providing an attachment platform for the virus onto the cell. It has been further established that coexpression of CXCR4 on CD4(−) colon epithelial cells was also necessary for their infection by HIV, raising the possibility that chemokine receptors may function as coreceptors for HIV entry into CD4(−)/GalCer(+) cells.[10] Analogues of GalCer which would strongly interact with the V3 loop are thus potential inhibitors of HIV uptake and infection.

These findings have urged the synthesis of various galactolipids and the examination of their anti-HIV activity.[11−21] As a part of our contribution into this field, we recently reported on GalCer analogues deriving from β-D-galactose and β-D-galactosamine (D,L)-, D- and L-serine (GalSer), L-cysteine (GalCys), and ethanolamine (GalAE), and possessing fluorocarbon and/or hydrocarbon hydrophobic chains (see examples in Table 1).[22,23] Several of the derivatives reported by others and our group were found to exhibit a moderate in vitro anti-HIV activity (IC_{50} in the 1−220 μM range[11−23]) on CD4(+) or on CD4(−)/GalCer(+) cell lines, whereas none of the GalCys derivatives was found to be active.[23] Moreover, only some of these anti-HIV active galactolipids 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.[15,23] These results most likely indicated that the neutralization of the virion through masking of this conserved V3 loop region was not the only mechanism involved in the HIV-1 antiviral activity of these GalCer analogues which might also affect HIV-1 infection by post-binding inhibition of HIV-1 entry into CD4(+) cells.

Unfortunately, neither the 3D structure of native gp120 nor that of the gp120/GalCer complex are known. Therefore, it is most difficult to design new GalCer analogues that would be more efficient anti-HIV com-

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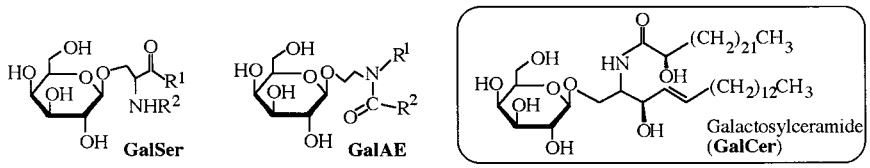
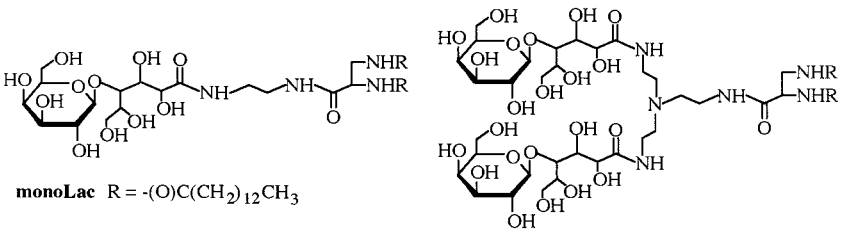
Table 1. Chemical Structure and Code Name of the Galactosylceramide (GalCer) Analogues



CODE NAME		R^1	R^2
First generation of GalCer analogs described in [23]			
I-GalSer[F6C11][NH2]	(IC_{50} = 40 μM*)	$-NH(CH_2)_{11}(CF_2)_5CF_3$	-H
I-GalSer[C14][NH2]	(IC_{50} = 60 μM*)	$-NH(CH_2)_{13}CH_3$	-H
II-GalAE[C16][F8C7]	(IC_{50} = 24 μM**)	$-NH(CH_2)_{15}CH_3$	$-(CH_2)_6(CF_2)_7CF_3$
Second generation of GalCer analogs described in this study			
I-GalSer[C11CO2Na][NH2]		$-NH(CH_2)_{11}CO_2Na$	-H
I-GalSer[CO2Na][C14]		-ONa	$-(O)C(CH_2)_{12}CH_3$
II-GalSer[C2OH][C14]		$-NH(CH_2)_2OH$	$-(O)C(CH_2)_{12}CH_3$
II-GalSer[C2SO4Na][C14]		$-NH(CH_2)_2OSO_3Na$	$-(O)C(CH_2)_{12}CH_3$
II-GalSer[C11CO2Na][C3CO2Na]		$-NH(CH_2)_{11}CO_2Na$	$-(O)C(CH_2)_2CO_2Na$
II-GalSer[C14][C11CO2Na]		$-NH(CH_2)_{13}CH_3$	$-(O)C(CH_2)_{10}CO_2Na$
II-GalSer[C14][C3CO2Na]		$-NH(CH_2)_{13}CH_3$	$-(O)C(CH_2)_2CO_2Na$
II-GalSer[C14][C7SO3Na]		$-NH(CH_2)_{13}CH_3$	$-(O)C(CH_2)_2C(O)NH(CH_2)_2SO_3Na$
II-GalSer[C14][P(OMe)(ONa)]		$-NH(CH_2)_{13}CH_3$	-P(O)(OMe)(ONa)
II-GalAE[F8C7][C11CO2Na]		$-(CH_2)_7(CF_2)_7CF_3$	$-(CH_2)_{10}CO_2Na$



* in CEM-SS cells

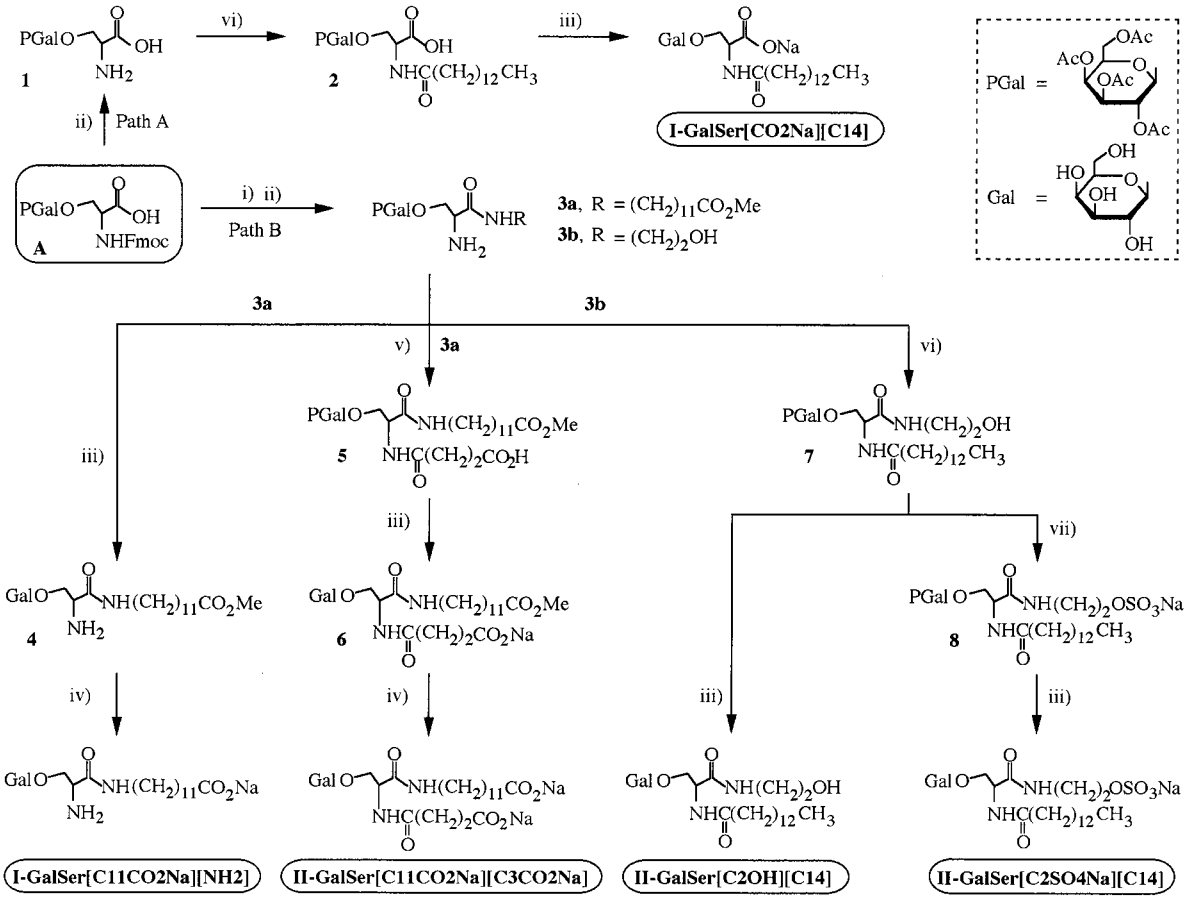
** in HT-29 cells

pounds than the former generation ones. Aiming at this goal and in order to have a better understanding of the role played by the structural elements of the GalCer analogues that influence their antiviral activity, we have performed a molecular modeling and dynamic study which included all structural analogues of GalCer known to display an anti-HIV activity[11−16,21] as well as those known to be inactive,[22,23] these molecules being inserted or not within a model membrane.[24] Most importantly, these molecular simulations (which will be reported elsewhere) indicated that the most active molecules were those that exhibited a stronger tendency to form galactosyl-rich microdomains within the membrane, in agreement with the above-mentioned experimental data from literature.[8,9]

We describe here the synthesis together with the results of biological testing, including the anti-HIV-1 activity in various cell cultures, and of gp120 binding experiments, of new series of amphiphilic galactosyl derivatives. These potential HIV inhibitors displayed in Table 1 were partly designed according to our molecular modeling and dynamic simulations[24] and to SAR data from literature.[11−16,21−23] Their molecular structure contains, in close proximity to or at variable distances from the galactosyl hydrophilic head, either an amino group or several hydroxyls or anionic groups, such as carboxylate, sulfate, sulfonate, and phosphate. One compound (i.e. **bisLac**, Table 1) with its ≪bouquet≫-like structure contains two galactosyl hydrophilic heads connected to a hydrophobic double-chain moiety. Several of these structural features were shown by our molecular modeling and dynamic study to favor the clustering of these derivatives within a model membrane, either through an intermolecular hydrogen bonding network or through complexation with divalent cations (Mg^{2+}, Ca^{2+}).[24]

Another feature from literature that was found to result in a substantial antiviral activity increase was the substitution of a carboxylate group for a terminal methyl in the alkyl chain of a GalCer analog.[15] Therefore, we have designed the highly fluorinated **II-GalAE-[F8C7][C11CO2Na]** in order to increase the anti-HIV activity of the previously prepared **II-GalAE[C16]-[F8C7]**.[23] The antiviral activity of **II-GalAE[C16]-[F8C7]** was attributed to the presence of the lipophobic fluorinated chain, which favors its self-association into galactosyl-rich patches when incorporated within conventional membranes. The anomeric β-configuration of

Scheme 1. Synthetic Route to the Hydrocarbon Single-Chain I-GalSer and Double-Chain II-GalSer Derivatives[a]



[a] (i) RNH_2/EDC/HOBt/CH_2Cl_2 with R = $-(CH_2)_{11}CO_2Me$ for **3a** and R = $-(CH_2)_2OH$ for **3b**; (ii) morpholine/DMF; (iii) MeOH/Et_3N/H_2O (2:1:1); (iv) 0.1 N NaOH in MeOH−H_2O (9:1); (v) succinic anhydride/Et_3N/CH_2Cl_2; (vi) tetradecanoic acid *N*-hydroxysuccinimide ester/CH_2Cl_2 or THF; (vii) SO_3·pyridine, pyridine.

galactosyl was selected for all galactosylated derivatives reported here, as this configuration seems to be essential for anti-HIV activity.[9,25] Finally, the GalSer series derive from L-serine because this configuration was found to led the more anti-HIV active compounds.[23]

Results and Discussion

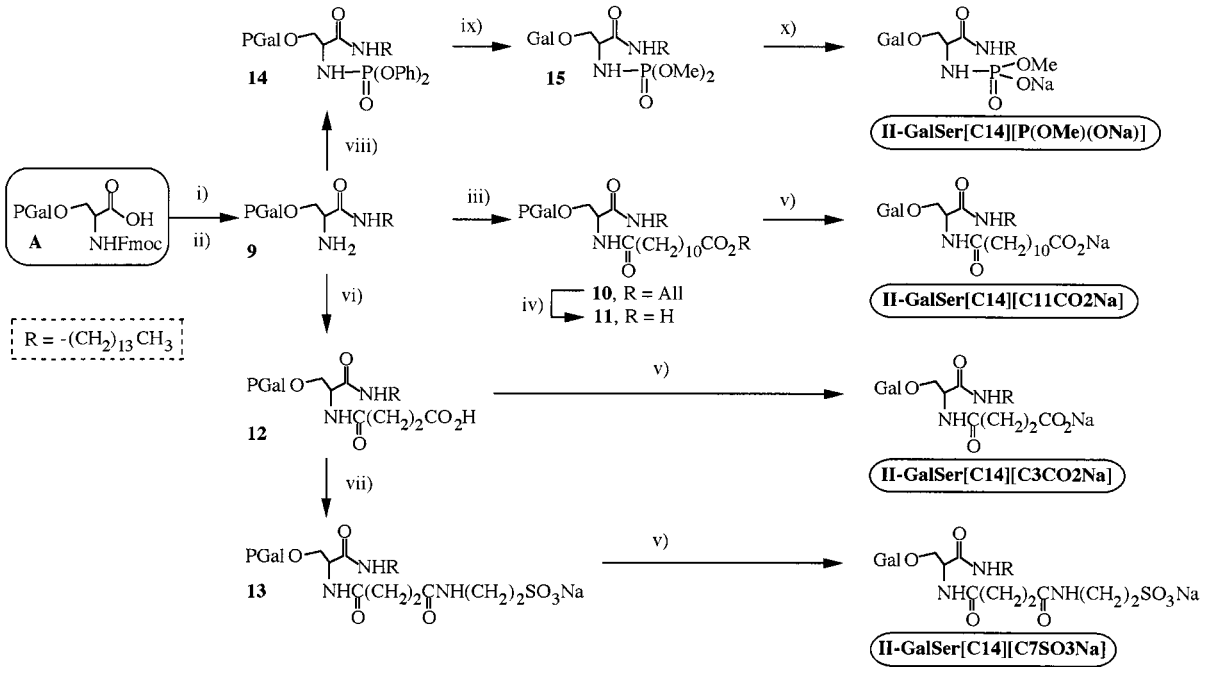
Chemistry. GalSer Series. All the galactosylated serine lipids described here and shown in Schemes 1 and 2 were prepared from the galactosylated L-serine synthon, **A**, using conventional coupling and deprotection reactions. Compounds of the **I-GalSer** series (Scheme 1) were obtained by condensing the selected functional group either onto its deprotected amino or onto its acid function. Compounds of the **II-GalSer** series (Schemes 1 and 2) were basically obtained by condensing successively two different functional groups, the first one onto the acid and the second one onto the amino function of **A**. These strategies which rely on the use of the glycosylated amino acid building block **A** benefited from the recent development of efficient methods for its synthesis.[23,26−28] These highly versatile strategies were further preferred to that consisting first in preparing the serine amino acid building blocks containing the suitable functional group(s) or chain(s) and then in their subsequent serine *O*-glycosylation, which is a most delicate step to perform in good yields. The structure of the key compound **A** was established by ^1H and ^{13}C NMR spectral assignments and further ascertained by comparison with the ^1H NMR data reported in the literature[28] and with ^{13}C NMR data for similar derivatives.[28−30] Its β-configuration is confirmed for instance by the anomeric C-1 resonance at 101.8 ppm.

The single chain compound presenting the serine carboxylate function, i.e., **I-GalSer[CO2Na][C14]**, was obtained from **A** in three steps with 57% overall yield (Scheme 1, path A). These steps consisted of the Fmoc-deprotection of **A** by action of morpholine, then acylation with tetradecanoic *N*-hydroxysuccinimide ester, and deacetylation with a MeOH:Et_3N:H_2O mixture followed by a cation-exchange.[23]

The two first steps of the preparation of **I-GalSer-[C11CO2Na][NH2]**, **II-GalSer[C11CO2Na]-[C3CO2Na]**, **II-GalSer[C2OH][C14]**, and **II-GalSer-[C2SO4Na][C14]** (Scheme 1, path B) and of the anionic **II-GalSer[C14]**-based series (Scheme 2) were very similar. They consisted of the condensation of **A** with the appropriate amine (e.g. methyl 12-aminododecanoate, ethanolamine, and tetradecylamine, respectively) in the presence of EDC/HOBt, followed by Fmoc-deprotection. Derivatives **3a**, **3b** (Scheme 1), and **9** (Scheme 2) were thus obtained with nearly 75, 60, and 60% yield, respectively.

Compound **3a**, by means of a two- and three-step process, was then converted into **I-GalSer[C11CO2Na]-[NH2]** and **II-GalSer[C11CO2Na][C3CO2Na]**, respectively (Scheme 1). **5** was obtained in fairly good yield

Scheme 2. Synthetic Route to the Hydrocarbon Double-Chain II-GalSer Derivatives (PGal and Gal as Defined in Scheme 1)[a]



[a] (i) $CH_3(CH_2)_{13}NH_2$/EDC/HOBt/CH_2Cl_2; (ii) morpholine/DMF; (iii) $CH_2{=}CHCH_2O(O)C(CH_2)_{10}CO_2H$/EDC/HOBt/$CH_2Cl_2$; (iv) $Pd(Ph_3)_4$/Et_2NH, CH_2Cl_2; (v) MeOH/Et_3N/H_2O (2:1:1); (vi) succinic anhydride/Et_3N/CH_2Cl_2; vii) a: *N*-hydroxysuccinimide/EDC/DMF, b: taurine/Et_3N; (viii) $(PhO)_2P(O)Cl$, pyridine; (ix) H_2/PtO_2/MeOH/Et_3N; (x) Et_3N/H_2O.

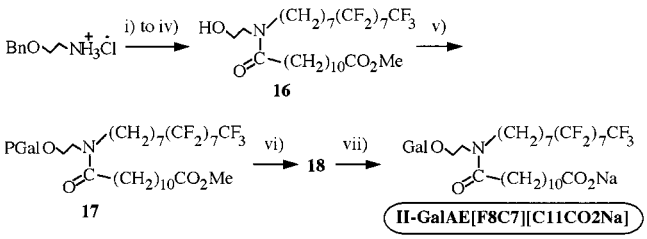
(95%) by addition of succinic anhydride on **3a** in the presence of Et_3N. Deacetylation of **3a** and **5**, with a MeOH:Et_3N:H_2O mixture, and methyl ester hydrolysis using 0.1 N NaOH afforded **I-GalSer[C11CO2Na]-[NH2]** and **II-GalSer[C11CO2Na][C3CO2Na]**, respectively. Owing to the low stability of the *O*-glycosyl-serine bond in basic media due to the acidity of the CH serine bond, the ester hydrolysis has to be carried out very carefully in order to avoid β-elimination.[23] While the hydrolysis of the methyl ester of the single-chain compound **4** was rapid and clean (**I-GalSer[C11CO2Na]-[NH2]** was obtained with 80% yield), hydrolysis of the double-chain compound **5a** needed 3 days for completion and was less selective (**II-GalSer[C11CO2Na]-[C3CO2Na]** was obtained with only 65% yield). This result confirmed that the stability toward hydrolysis of glycosyl-serine derivatives is higher for those having an unsubstituted amino-serine function.[31]

Compound **3b** by coupling with tetradecanoic *N*-hydroxysuccinimide ester gave **7** (70%), which, after conventional deacetylation, afforded **II-GalSer[C2OH]-[C14]** (35% yield from **A**). Alternatively, **7** when submitted to *O*-sulfation with the sulfur trioxide−pyridine complex in pyridine, followed by deacetylation and conversion into its Na^+ salt gave **II-GalSer[C2SO4Na]-[C14]** (25% yield from **A**).

The synthesis of the anionic **II-GalSer[C14]-[C11CO2Na]**, **II-GalSer[C14][C3CO2Na]**, **II-GalSer-[C14][C7SO3Na]**, and **II-GalSer[C14][P(OMe)(ONa)]** compounds was performed from the key amino synthon **9**, as outlined in Scheme 2. Thus, the coupling of **9** with dodecanedioic acid monoallyl ester in the presence of EDC/HOBt gave the double-chain compound **10** in 65% yield, which after allyl deprotection, deacetylation, and cation exchange afforded **II-GalSer[C14][C11CO2Na]**. The allyl cleavage was performed in 90% yield with catalytic $Pd(PPh_3)_4$ and diethylamine as transfer agent.[32] The allyl ester protection was preferred to the methyl ester one. Indeed, all our attempts to perform the hydrolysis in basic media of the methyl ester analogue of the allyl ester **10** were unsuccessful as mainly β-elimination products were formed. This further shows the low stability of the double-chain galactoserine derivatives under basic conditions. On the other hand, the *N*-succinic derivative **II-GalSer[C14][C3CO2Na]** was obtained by coupling **9** with succinic anhydride in the presence of Et_3N, followed by deacetylation of **12** thus obtained and a cation exchange (44% overall yield from **A**). Compound **12** was further used for the synthesis of the taurine derivative **II-GalSer[C14]-[C7SO3Na]** which was obtained by in situ activation of acid **12** with HOSu/EDC and then reaction with taurine in basic medium, followed by conventional acetyl-deprotection of **13** thus produced and cation exchange (30% overall yield from **A**).

Where the synthesis of the *N*-phosphoserine derivative **II-GalSer[C14][P(OMe)(ONa)]** is concerned (Scheme 2), it was prepared from **9** using a three-step procedure, which included (i) phosphorylation of **9** with diphenyl chlorophosphate in pyridine yielding **14** (80%), (ii) unexpected conversion (see below) of **14** into the dimethyl phosphate derivative **15** (87% yield) by bubbling hydrogen through a methanolic solution of **14**, catalytic PtO_2 and Et_3N,[33] and (iii) hydrolysis of one of the dimethyl ester phosphate bonds of **15** in aqueous Et_3N. When the diphenyl phosphate deprotection of **14** was performed using the conventional procedure (absence of Et_3N), we observed dephosphorylation and the quantitative formation of the starting material **9**. This is most probably due to the low stability of the *N*-phosphoric acid serine intermediate and autocatalytic hydrolysis. To neutralize the in situ generated *N*-

Scheme 3. Synthetic Route to the Hydrocarbon and Fluorocarbon Double-Chain Galactosyl-Amidoethanol II-GalAE Derivative (PGal and Gal as Defined in Scheme 1)[a]



[a] (i) $CF_3(CF_2)_7(CH_2)_6CO_2H$/EDC/HOBt/$Et_3N$/$CH_2Cl_2$; (ii) $LiAlH_4$/THF; (iii) $CH_3O(O)C(CH_2)_{10}CO_2H$/PyBrop/$Et_3N$/$CH_2Cl_2$; (iv) H_2/Pd/C, AcOH/MeOH; (v) $GalOC(=NH)CCl_3$/TMSOTf/$CHCl_3$, −10 °C; (vi) 2:1:1 MeOH/Et_3N/H_2O; (vii) 0.3 N NaOH/1:9 MeOH/H_2O.

phosphoric acid, we performed the deprotection step in the presence of Et_3N. Under these conditions, we obtained compound *N*-(dimethoxyphosphoryl)serine **15** as a result of a formal transesterification of **14** by methanol and of the expected deacetylation of the galactose residue. The structure of **15** was confirmed by ^1H, ^{13}C-DEPT, ^{31}P NMR, and mass spectrometry. More particularly, the presence of the $P(OMe)_2$ group is attested by a doublet ($J_{H,P}$ 11.2 Hz) at 3.66 ppm integrating for six protons and by two doublets at 54.3 and 54.4 ppm with characteristic $^2J_{C,P}$ coupling constants of ~6 Hz, attributable to two methoxy groups in the ^1H and ^{13}C-DEPT spectrum, respectively.

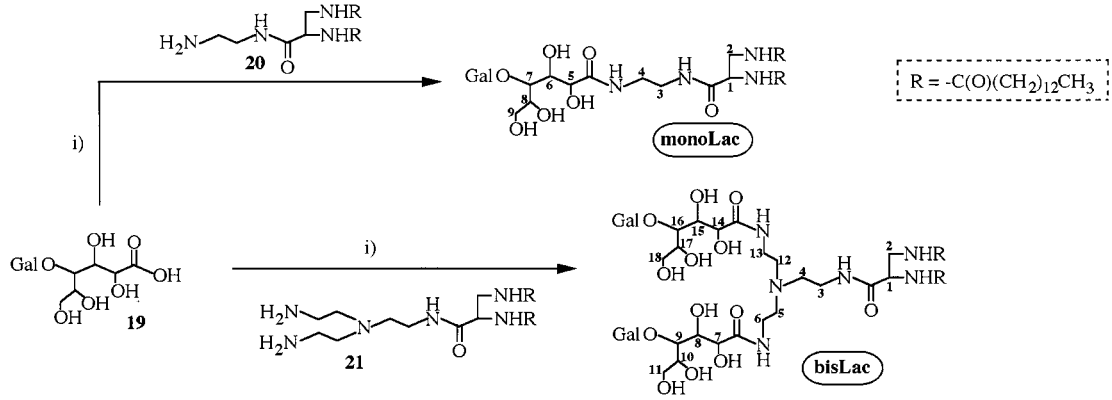
The hydrolysis of one of the dimethyl ester phosphate bonds of **15** was performed in aqueous Et_3N and afforded quantitatively **II-GalSer[C14][P(OMe)(ONa)]**, after cation-exchange and lyophilization. These conditions were expected to lead to the hydrolysis of both phosphate esters. However, a ^{31}P NMR monitoring of the hydrolysis of **15** showed its total consumption after 24 h of stirring at room temperature and its conversion into the monoester **II-GalSer[C14][P(OMe)-(O^-,Et_3NH^+)]** and no further evolution within the next 5 days. Indeed, the signal of the starting compound at 12.8 ppm was replaced by a resonance at 8.9 ppm, which is corresponding to that of the isolated material (as its sodium salt). The presence of one POMe group was unambiguously confirmed by ^{13}C-DEPT (doublet at 51.8 ppm with $^2J_{C,P}$ = 6 Hz, corresponding to a methoxy), ^{31}P NMR, and mass spectrometry. Our attempts to hydrolyze the second methyl ester phosphate bond without degradation were unsuccessful.

GalAE Series. The fluorinated galactolipid **II-GalAE-[F8C7][C11CO2Na]** which is derived from ethanolamine (Scheme 3) was obtained by galactosylation of **16** using the Schmidt procedure,[23,34] and then deacetylation of **17** thus was produced by a MeOH:Et_3N:H_2O mixture and hydrolysis of the methyl ester **18** in aqueous NaOH (25% overall yield from **16**). For the galactosylation of **16**, the Schmidt method, although it requires the preparation of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate as galactose donor, proved more efficient (35% yield) than the direct galactosylation with the commercially available 1,2,3,4,6-penta-*O*-acetyl-D-galactopyranose and $BF_3{\cdot}Et_2O$ as Lewis acid (25% yield). The aglycone **16** was prepared in four steps from 2-benzyloxyethylamine[23] in 50% overall yield using conventional procedures, including acylation with 7-(perfluorooctyl)heptanoic acid[23] in the presence of EDC/HOBt, $LiAlH_4$ reduction of the amide bond, then acylation with dodecanedioic acid monomethyl ester in the presence of PyBrop/Et_3N, and finally benzyl-deprotection by hydrogenolysis.

Lac Series. The synthesis of the lactobionamide derivatives, **monoLac** and **bisLac**, is presented in Scheme 4. Condensation of 1 or 2 equiv of the commercial lactobionic acid **19** with amine **20** or diamine **21** in methanol gave **monoLac** (60% yield) or **bisLac** (50% yield), respectively. This condensation step with lactobionic acid is very advantageous for introducing a galactosyl moiety; as in one step and without the need of any delicate glycosylation reaction, one obtains compounds with the required galactosyl β-configuration (which is already present in the starting material).[35]

Structural Characterization. All the new galactosylated compounds shown in Table 1 were characterized by ^1H and ^{13}C NMR (and also by ^{19}F NMR for **II-GalAE-[F8C7][C11CO2Na]**), and/or mass spectrometry, and elemental analyses. In addition to the specific NMR features already discussed for the different series of compounds, their respective ^1H and ^{13}C NMR spectra were all fully consistent with the β-configuration of the galactosyl residue. The ^{13}C shifts of the pyranosyl ring carbons were indeed in accordance with those reported in the literature, and more particularly the anomeric carbon resonances were found in an expected 102.9−

Scheme 4: Synthetic Route to the Lactobionamide Derivatives (Figures Refer to C and H Atom Numbering for ^1H and ^{13}C NMR; Gal as Defined in Scheme 1)[a]



[a] (i) MeOH, Δ.

Table 2. Anti-HIV-1 Activity and Cytotoxicity of the GalCer Analogues

compounds	IC_{50} (μM) CEM-SS	IC_{50} (μM) MT-4	CC_{50} (μM) CEM-SS	CC_{50} (μM) MT-4
I-GalSer[CO2Na][C14]	20	>100	>100	>100
I-GalSer[C11CO2Na][NH2]	>100	>100	>100	>100
II-GalSer[C14][C3CO2Na]	>100	>100	>100	>100
II-GalSer[C14][C11CO2Na]	>100	>100	>100	>100
II-GalSer[C11CO2Na][C3CO2Na]	>100	>100	>100	>100
II-GalSer[C2OH][C14]	>100	>100	>100	>100
II-GalSer[C2SO4Na][C14]	10	20	>100	>100
II-GalSer[C14][C7SO3Na]	50	>100	>100	>100
II-GalSer[C14][PO(OMe)(ONa)]	>100	>100	>100	>100
II-GalAE[F8C7][C11CO2Na][a]	>18	>18	>18	>18
monoLac	>100	>100	>100	>100
bisLac	32	33	44	38

[a] This compound was tested as PL/CH/glycolipid liposome formulation in a 2:1:0.15 molar ratio; the mean size of the liposomes with the associated standard deviation (SD) was 75 (25) nm, as measured by light scattering. In aqueous solution, no activity was detected for a concentration of up to 10 μM.

106.5 ppm range.[30] Furthermore, the anomeric proton signals could be seen in several cases, as a doublet in a 4.20−4.36 ppm range with a $^3J_{1,2}$ coupling constant of 7.20−7.36 Hz, characteristic of *trans*-1,2 one.[30]

Where the doubling of some ^{1}H and/or ^{13}C signals, which is seen for derivative **II-GalAE[F8C7]-[C11CO2Na]**, is concerned, it indicates rather the presence of conformational isomers in solution due to restricted rotation of the amide bond, as it has been already described.[23,36]

Biological Evaluation. The aim of this study was to synthesize new GalCer analogues that were designed according to molecular modeling and dynamic simulations[24] and to SAR data from literature[11−16,21−23] and expected to block HIV-1 cellular entry. The antiviral activity against HIV-1 (LAI and IIIB) and cytotoxicity of this second generation of GalCer analogues was evaluated in vitro on CEM-SS and MT4 cells according to published procedures.[37−39] The data are collected in Table 2.

Among the 12 new galactosylated compounds reported here, a specific anti-HIV activity, although moderate (IC_{50} from 10 μM to 50 μM), was detected only for three of them on the CEM-SS cell lines, i.e., **I-GalSer-[CO2Na][C14]**, **II-GalSer[C2SO4Na][C14]**, and **II-GalSer[C14][C7SO3Na]**, which belong to the GalSer series. The sulfate **II-GalSer[C2SO4Na][C14]**, which is the more active on CEM-SS cells, is the sole compound found to exhibit an anti-HIV activity on MT4 cells (IC_{50} = 20 μM). Furthermore, no cytotoxicity was detected for these derivatives for concentrations up to 100 μM on both cell lines.

Compounds **I-GalSer[CO2Na][C14]** and **II-GalSer-[C2SO4Na][C14]** were found to display higher anti-HIV activities on CEM-SS cells than the previously reported first generation of GalSer derivatives (Table 1) for which IC_{50} of 40−60 μM only were measured.[23] Furthermore, none of these first generation compounds was active on MT4 cells by contrast to the sulfate **II-GalSer[C2SO4Na][C14]** which protects to some extent these cells from HIV infection.

Concerning structure−activity relationships in the GalSer series, the most significant fact is that the more potent drugs were those possessing a hydrophobic anchor and, in close proximity to the galactose polar head, an ammonium/amine function (**I-GalSer[C14]-[NH2]**) or an anionic carboxylate (**I-GalSer[CO2Na]-[C14]**), sulfate (**II-GalSer[C2SO4Na][C14]**), or, and even further away from galactose, a sulfonated group as in **II-GalSer[C14][C7SO3Na]**. The anti-HIV activity in this GalSer series is decreasing on increasing the spacer length between the anionic sulfate/sulfonate and the serine core (**II-GalSer[C2SO4Na][C14]** vs **II-GalSer[C14][C7SO3Na]**). This anti-HIV activity is lost (i) on replacing the hydrophobic anchor by a hydrophilic chain (**I-GalSer[C14][NH2]** vs **I-GalSer[C11CO2Na]-[NH2]**, hence on introducing a carboxylate at the terminus of the hydrophobic spacer, see discussion below), (ii) on replacing the anionic sulfate by a neutral hydroxyl (**II-GalSer[C2SO4Na][C14] vs II-GalSer-[C2OH][C14]**), or (iii) on increasing the spacer length between the carboxylate and the serine core (**I-GalSer-[CO2Na][C14]** vs **II-GalSer[C14][C3CO2Na]** or **II-GalSer[C14][C11CO2Na]**).

Our results show also that introducing a sulfate or a sulfonate in place of a carboxylate or a phosphate is most beneficial for anti-HIV activity. Indeed, the sulfate derivative **II-GalSer[C2SO4Na][C14]** inhibits HIV infection of both CEM-SS and MT4 cell lines by contrast to its carboxylate analog **I-GalSer[CO2Na][C14]** which is active only on CEM-SS cells and at a lower level than the sulfate. Furthermore, if an anti-HIV activity was detected for the sulfonate **II-GalSer[C14][C7SO3Na]**, no such activity was found for its phosphate **II-GalSer-[C14][P(O)OMe)(ONa)]** or its carboxylate **II-GalSer-[C14][C3CO2Na]** homologues, while the opposite tendency was expected. Indeed, with the phosphate and carboxylate groups being located closer to the galactoserine core than the sulfonate, one expected a higher anti-HIV activity for these phosphate and carboxylate derivatives, as discussed above. These data underscore that both the nature of the anionic group and its close proximity to the galactose are of importance for anti-HIV activity. They are in perfect agreement with the recent observation that sulfatide, the natural sulfated congener of GalCer, has a potent anti-HIV activity in GalCer-expressing intestinal cells.[10] In addition, **II-GalSer[C2SO4Na][C14]** inhibits HIV-1 infection of T-cells, whereas the antiviral activity of sulfatide is limited to GalCer-expressing CD4-negative cells. To our knowledge, this is the first time that a synthetic sulfated analogue of GalCer with such a broad anti-HIV activity is described.

In the GalAE series, no anti-HIV activity was detected for **II-GalAE[F8C7][C11CO2Na]** neither as an aqueous solution nor as a liposomal formulation and for

concentration of up to about 20 μM (we were unable to prepare more concentrated and stable dispersions with this derivative). Thus, introducing a carboxylate group at the end of one of the lipophilic chains of **II-GalAE-[C16][F8C7]**, which was endowed with a substantial anti-HIV activity (IC_{50} = 24 μM on HT-29 cells[23]), has rather a detrimental effect on or, at least, does not improve anti-HIV activity. This is also the case, as discussed above when going from **I-GalSer[C14][NH2]** to **I-GalSer[C11CO2Na][NH2]**. These results contrast with the substantial increase of anti-HIV activity which was noticed in the literature for such a structural modification.[14] Therefore, the presence of a carboxylate group at the end of the hydrophobic chains of an analogue does not improve systematically its antiviral activity. This may be linked to the self-organizing properties of synthetic glycolipid analogues in aqueous phases, as recently discussed.[21]

Concerning the lactose derived compounds, neither **monoLac** nor **bisLac** displayed any specific anti-HIV activity, **bisLac** being rather cytotoxic. For **monoLac** and **bisLac**, we expected, at least, an HIV inhibition level in the same range as that reported for closely related *N*-alkyl-1-deoxylactitols[13,15] (IC_{50} = 1−10 μM; CEM-SS). More particularly, we expected a substantial HIV inhibition potency for **bisLac**: its ≪bouquet≫-like structure of the two galactosyl units connected to a hydrophobic anchor was aimed at enhancing its interaction with gp120, hence decreasing cellular infection.[8,9,40] The cytotoxicity of **bisLac** could be due to its potential detergent properties on cell membranes. Due to its pronounced cone-shaped geometry (polar head volume ≫ hydrophobic chain volume), **bisLac** when dispersed in water is expected to form rather micelles[41] (long-chain lactobionamide analogues were indeed reported to self-assemble into micelles[42]). Such aggregates are known to be more detergent on cell membranes than compounds organized into lamellar phases.

Where a correlation between the molecular modeling and dynamic simulations performed on some of the galactolipids reported here and their anti-HIV activity is concerned, it appears that clustering of these galactolipids within a membrane, as evidenced from our simulation study,[24] is not a sufficient condition for anti-HIV activity. This simulation study indicated indeed that the galactolipids reported here that were found to display an anti-HIV activity (i.e. **I-GalSer[CO2Na]-[C14]**, **II-GalSer[C2SO4Na][C14]**, and **II-GalSer-[C14][C7SO3Na]**), should cluster into galactosyl-rich microdomains. However, this study showed also formation of such clusters for **II-GalSer[C14][C3CO2Na]** which was found to be inactive.

Surface-Pressure Measurements − gp120 Binding to Glycolipid Monolayers at the Air−Water Interface. To provide further insight into the inhibition mechanism of the anti-HIV active GalCer analogues reported here, i.e., **I-GalSer[CO2Na][C14], II-GalSer-[C14][C7SO3Na]**, and **II-GalSer[C2SO4Na][C14]**, they were also tested for gp120 binding. This was done by analyzing the interaction of HIV-1 recombinant gp120 with the monolayer these lipids form when spread at the air−water interface, using a procedure reported elsewhere.[9,43,44] These experiments were performed by adding rgp120 (at a concentration of 10 nM corresponding to saturation) into the subphase of monolayers prepared at an initial pressure of 10 mN/m and then by recording the increase in surface pressure which is caused by the penetration of rgp120 into the monolayer. Among the three compounds tested, only **II-GalSer-[C14][C7SO3Na]** formed a stable monolayer. However, stable monolayers could be obtained when **I-GalSer-[CO2Na][C14]** and **II-GalSer[C2SO4Na][C14]** were incorporated into a glucosylceramide (GlcCer) monolayer, providing their relative content did not exceed 44 and 70%, respectively. The low stability of the pure **I-GalSer[CO2Na][C14]** or **II-GalSer[C2SO4Na][C14]** monolayers is most probably related to their substantial solubility in water. For these reasons, gp120 binding was evaluated onto these mixed monolayers, since the glycoprotein does not bind to GlcCer.[43]

Addition of rgp120 into the aqueous subphase underneath the **II-GalSer[C14][C7SO3Na]**, **I-GalSer-[CO2Na][C14]**/GlcCer, or **II-GalSer[C2SO4Na][C14]**/GlcCer monolayer induced a marked increase of surface pressure with a maximal $\Delta\pi$ increase of 8.5, 9.5, or 8.5 mN/m, respectively. Comparatively, a maximal $\Delta\pi$ increase of 2.5 and 12 mN/m was measured for a GlcCer (negative control) and GalCer (positive control) monolayer, respectively. This is a clear evidence of gp120 insertion into the galactolipid monolayers resulting from a primary interaction with the galactose residue. These data suggest that binding of these three derivatives to HIV-1 gp120 may be responsible for their anti-HIV activity in CD4-positive cells. This is in agreement with the current hypothesis that the assembly of the HIV-1 fusion complex requires, in addition to CD4 and a coreceptor, specific glycolipids which are present in restricted areas (microdomains) of the plasma membrane.[9,40,45,46]

Conclusion

The results of the present study underscore the interest of synthesizing carbohydrate-based therapeutic agents as alternative strategies for infectious diseases treatments.[21,47] Our future efforts will focus on demonstrating the clustering of the anti-HIV active GalSer derivatives into galactosyl-rich microdomains when incorporated into a membrane and the importance of the amino and anionic group in the formation of such microdomains, of their nature, and of their location with respect to the galactosyl residue, all factors that have an impact on anti-HIV activity, as evidenced in this study.

Experimental Section

Chemical Section. General. Unless otherwise indicated, reactions were conducted under an anhydrous nitrogen atmosphere using dry solvents and reagents. Anhydrous solvents were prepared by standard methods. β-D-Galactose pentaacetate, 12-aminododecanoic acid, 1-(3-*N*,*N*-dimethylaminopropyl)-3-ethylcarbodiimide (EDC), succinic anhydride, sulfur trioxide pyridine complex (SO_3·pyridine), tetradecylamine, dodecanedioic acid, allyl bromide, tetrakis(triphenylphosphine-)palladium(0) [$Pd(PPh_3)_4$], diethyl dodecanedioate, and *N*-hydroxysuccinimide (HOSu) were purchased from Aldrich, boron trifluoride diethyl etherate (BF_3/Et_2O) and 1-hydroxybenzotriazole (HOBt) from Sigma, and taurine and lactobionic acid from Fluka. Diphenyl chlorophosphate $(PhO)_2P(O)Cl$ and platinum oxide monohydrate were purchased from Accros, *N*-9-(fluorenylmethoxycarbonyl)-L-serine from Propeptide, and 1-bro-

motris(pyrrolidino)-phosphonium hexafluorophosphate (PyBrop) from Novabiochem. *N*-Hydroxysuccinimide ester of tetradecanoic acid was prepared by reaction of myristic acid and *N*-hydroxysuccinimide in the presence of EDC. Methyl 12-aminododecanoate was prepared by esterification of the corresponding acid with methanol. The 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl trichloroacetimidate ($GalOC(=NH)CCl_3$) and 7-(perfluorooctyl)heptanoic acid were prepared as described previously.[23] 2-Benzyloxyethylamine was prepared in two steps (60%) from commercial *N*-(*tert*-butoxycarbonyl)-ethanolamine.[23] Dodecanedioic acid monoallyl ester was obtained by reacting, in DMF, dodecanedioic acid with allyl bromide in the presence of K_2CO_3 [R_f = 0.51 (95:5 $CHCl_3$−MeOH). ^{1}H NMR ($CDCl_3$): δ 10.80 [bs, 1 H, C(O)OH], 5.85 (ddt, 1 H, J = 17.3, 11.6 and 5.7 Hz, 1H, $OCH_2CH{=}CH_2$), 5.24 (dd, J = 17.3 Hz, J = 1.5 Hz, 1H, $OCH_2CH{=}CH_aH_b$), 5.16 (dd, J = 11.6 Hz, J = 1.5 Hz, 1H, $OCH_2CH{=}CH_aH_b$), 4.50 (d, J = 5.7 Hz, 2H, $OCH_2CH{=}CH_2$), 2.30 [t, J = 7.3 Hz, 2H, $CH_2C(O)OH$], 2.26 [t, J = 7.4 Hz, 2H, $CH_2C(O)OAll$], 1.60−1.50 [m, 4H, $CH_2CH_2C(O)$], 1.30−1.15 [m, 12H, $(CH_2)_6$]. ^{13}C NMR ($CDCl_3$): δ 180.0 [C(O)OH], 173.6 [*C*(O)OAll], 132.4 (*C*H=CH_2), 118.1 (CH=*C*H_2), 65.0 ($OCH_2CH{=}$), 34.3 and 34.1 [$CH_2C(O)$], 29.4, 29.3, and 29.1 [$(CH_2)_6$], 25.0 and 24.7 [$CH_2CH_2C(O)$]]. Dodecanedioic acid monomethyl ester was obtained from diethyl dodecanedioate and $Ba(OH)_2$ in MeOH as an oil [R_f = 0.48 (95:5 $CHCl_3$−MeOH). ^{1}H NMR ($CDCl_3$): δ 3.61 (s, 3H, OCH_3), 2.28 [t, J = 7.3 Hz, 2H, $CH_2C(O)OH$], 2.25 [t, J = 7.5 Hz, 2H, $CH_2C(O)OMe$], 1.60−1.50 [m, 4H, $CH_2CH_2C(O)$], 1.40−1.15 [m, 12H, $(CH_2)_6$]. ^{13}C NMR ($CDCl_3$): δ 180.1 [C(O)OH], 174.5 [*C*(O)OMe], 51.5 (OCH_3), 34.2 [$CH_2C(O)$], 29.4, 29.3, 29.2, and 29.1 [$(CH_2)_6$], 25.0 and 24.8 [$CH_2CH_2C(O)$]]. 3-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-N^{α}-9-(fluorenylmethoxycarbonyl)-L-serine, **A**, was prepared according to published procedures and authentified by comparison of its NMR data with literature:[28] R_f = 0.3 (9:1 $CHCl_3$−MeOH). ^{1}H NMR ($CDCl_3$): δ 7.66 (d, J = 8.0 Hz, 2H, Fmoc), 7.50 (d, J = 8.0 Hz, 2H, Fmoc), 7.35−7.20 (m, 4H, Fmoc), 5.90 (m, 1H, NH), 5.27 (m, 1H, H-4 Gal), 5.10−4.95 (m, 2H, H-2,3 Gal), 4.40−3.60 [m, 10H, H-1β,5,6,6′ Gal, OCH_2CH and $C(O)OCH_2CH$], 2.10−1.90 [m, 12H, $CH_3C(O)$]. ^{13}C NMR ($CDCl_3$): δ 177.5 [C(O)OH], 170.6, 170.2 and 170.0 [$CH_3C(O)O$], 156.9 [NHC(O)O], 144.0, 143.8, and 141.3 (C, Fmoc), 127.8, 127.2, 125.2, and 120.1 (CH, Fmoc), 101.8 (C-1β Gal), 70.8 and 70.6 (C-3,5 Gal), 69.5 (OCH_2CH), 69.0 (C-2 Gal), 67.0 (C-4 Gal), 66.9 ($C(O)OCH_2$), 61.0 (C-6 Gal), 55.4 (OCH_2CH), 47.2 [$C(O)OCH_2CH$], 20.6, 20.5 and 20.4 [$CH_3C(O)$].

Column chromatography purifications were carried out using Merck silica gel 60 (mesh 70−230) and/or Sephadex LH 20 gel. The purity of all new compounds was checked by thin-layer chromatography (TLC), NMR, mass spectrometry, and/or elemental analysis. TLC analyses were performed on precoated silica gel F_{254} plates (Merck) and visualized by UV light (λ = 254 nm) and by charring with $KMnO_4$, 50% MeOH−H_2SO_4, or ninhydrine. Optical rotations were measured at 589 nm with a Perkin-Elmer 141 polarimeter (1-dm cell). ^{1}H, ^{19}F, ^{13}C, and ^{31}P NMR spectra were recorded on a Bruker AC 200 spectrometer at 200, 188.3, 50.3, and 81 MHz, respectively. Chemicals shifts are given in ppm (δ) relative to the solvent peak (i) $CHCl_3$ (δ 7.27) or CH_3OD (δ 3.35) for ^{1}H and (ii) $CDCl_3$ (δ 76.9) for ^{13}C and relative to external reference CCl_3F and 75% H_3PO_4 for ^{19}F and ^{31}P, respectively. Electrospray mass analyses, positive or negative mode, were run on a TSQ 7000 Finnigan Mat spectrometer. Elemental analyses were performed by the Service Central de Microanalyze of the CNRS.

General De-*O*-acetylation (Procedure A). β-D-Galactopyranoside **3a**, **5**, **2**, **7**, **8**, **11**, **12**, **13**, or **17** (0.10 mmol) in 1.8 mL of a 2:1:1 MeOH−Et_3N−H_2O mixture was stirred at room temperature for 2 h. After evaporation of the solvents, the crude residues were purified by chromatography ($CHCl_3$ to 7:3 or 3:2 $CHCl_3$−MeOH) affording the corresponding compound **4**, **6**, **I-GalSer[CO2Na][C14]**, **II-GalSer[C2OH][C14]**, **II-GalSer[C2SO4Na][C14]**, **II-GalSer[C14][C11CO2Na]**, **II-GalSer[C14][C3CO2Na], II-GalSer[C14][C7SO3Na]**, and **18** as white solids, respectively.

General Fmoc Cleavage (Procedure B). The *N*-Fmoc derivatives were deprotected in a 1:2 morpholine−DMF solution at room temperature for 4 h. After evaporation, the compounds were purified by chromatography.

General Amino Acid Coupling (Procedure C). EDC (1.1 equiv) was added to a CH_2Cl_2 solution of acid (1 equiv), amine (1 equiv), and HOBt (1.1 equiv). The reaction mixture was stirred at 0 °C for 1 h and then at room-temperature overnight. The reaction mixture diluted with $CHCl_3$ was successively washed with 5% $KHSO_4$, water, 8% $NaHCO_3$, and then water until neutrality. After drying with Na_2SO_4, filtration, evaporation, and purification, the compound was obtained.

Synthesis of I-GalSer[CO2Na][C14] (Scheme 1). 3-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-L-serine, 1. The procedure **B** when applied to 600 mg (0.93 mmol) of **A** gave, after chromatography (95:5 $CHCl_3$−MeOH), 335 mg (80%) of **1** as an oil. R_f = 0.25 (4:1 $CHCl_3$−MeOH). ^{1}H NMR ($CDCl_3$): δ 5.27 (m, 1H, H-4 Gal), 5.10−4.95 (m, 2H, H-2,3 Gal), 4.40−3.60 (m, 7H, H-1β,5,6,6′ Gal and OCH_2CH), 2.10−1.90 [m, 12H, $CH_3C(O)$], 1.80 (s, 2H, NH_2). ^{13}C NMR ($CDCl_3$): δ 177.5 [C(O)OH], 170.6, 170.2, and 170.0 [$CH_3C(O)$], 101.8 (C-1β Gal), 70.8 and 70.6 (C-3,5 Gal), 69.5 (OCH_2CH), 69.0 (C-2 Gal), 67.0 (C-4 Gal), 61.0 (C-6 Gal), 55.9 (OCH_2CH), 20.6, 20.5, and 20.4 [$CH_3C(O)$].

3-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-*N*-(tetradecanoyl)-L-serine, 2. Three hundred milligrams (0.69 mmol) of **1** and 235 mg (0.69 mmol) of *N*-hydroxysuccinimide ester of tetradecanoic acid in 10 mL of THF were stirred at room temperature for 24 h. After evaporation of the solvent, the crude residue was dissolved in $CHCl_3$ and washed with 1 N HCl and water until neutrality. After drying over Na_2SO_4 and filtration, the solvent was evaporated, and the residue was purified by chromatography (CH_2Cl_2 to 9:1 CH_2Cl_2−MeOH) giving 350 mg (80%) of **2** as a white solid. R_f = 0.30 (9:1 $CHCl_3$−MeOH). ^{1}H NMR ($CDCl_3$): δ 6.71 (m, 1H, NH), 5.33 (bs, 1H, H-4 Gal), 5.03 (m, 2H, H-2,3 Gal), 4.50 (m, 2H, H-1β,5 Gal), 4.02 (m, 5H, H-6,6′ Gal and OCH_2CH), 2.18 [m, 2H, $CH_2C(O)$], 2.08, 2.02, 1.99, and 1.91 [4s, each $3HCH_3C(O)$], 1.55 [m, 2H, $CH_2CH_2C(O)$], 1.19 [m, 20H, $[(CH_2)_{10}]$], 0.81 (t, J = 6.5 Hz, 3H, CH_2CH_3). ^{13}C NMR ($CDCl_3$): δ 174.9 [C(O)NH and C(O)OH], 170.5, 170.2, and 169.9 [$CH_3C(O)$], 101.4 (C-1β Gal),-70.8 (C-3,5 Gal), 69.0 (OCH_2CH and C-2 Gal), 67.1 (C-4 Gal), 61.0 (C-6 Gal), 54.1 (OCH_2CH), 36.5 [$CH_2C(O)$], 31.9 ($CH_2CH_2CH_3$), 29.8, 29.6, and 29.4 [$(CH_2)_8$], 25.6 [$CH_2CH_2C(O)$], 22.7 (CH_2CH_3), 20.8, 20.7, 20.6, and 20.5 [$CH_3C(O)$], 14.2 (CH_2CH_3).

3-*O*-(β-D-Galactopyranosyl)-*N*-(tetradecanoyl)-L-serine (Sodium Salt), I-GalSer[CO2Na][C14]. The procedure **A** when applied to 165 mg (0.25 mmol) of **2** gave, after treatment with Amberlite IRC-50 ion-exchange (Na^+), 115 mg (90%) of **I-GalSer[CO2Na][C14]**. R_f = 0.30 (35:15:2 $CHCl_3$−MeOH−H_2O). $[\alpha]_D$ = +8.0° (*c* 0.25; MeOH). ^{1}H NMR ($CDCl_3$−CD_3OD): δ 4.43 (sl, 1H, H-4 Gal), 4.26 (m, 2H, H-2,3 Gal), 3.85 (bs, 1H, H-1β Gal), 3.73 (m, 3H, H-5,6,6′ Gal), 3.51 (m, 3H, OCH_2CH), 2.23 [t, J = 7.4 Hz, 2H, $CH_2C(O)$], 1.59 [m, 2H, $CH_2CH_2C(O)$], 1.24 [m, 20H, $(CH_2)_{10}$], 0.85 (t, J = 6.5 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$−CD_3OD): δ 176.0 [C(O)ONa], 174.2 [C(O)NH], 103.8 (C-1β Gal), 74.9 (C-5 Gal), 73.0 (C-3 Gal), 70.7 (C-2 Gal), 70.5 (OCH_2CH), 68.9 (C-4 Gal), 61.1 (C-6 Gal), 54.5 (OCH_2CH), 35.9 [$CH_2C(O)$], 31.5 ($CH_2CH_2CH_3$), 29.4, 29.3, 29.2, 29.1, and 28.9 [$(CH_2)_8$], 25.4 [$CH_2CH_2C(O)$], 22.2 (CH_2CH_3), 13.2 (CH_3). This assignment was further confirmed by DEPT. Anal. Calc. for $C_{23}H_{42}NO_9Na\cdot 1/2H_2O$ (508.58): C, 54.32; H, 8.52; N, 2.75. Found: C, 54.21; H, 8.90; N, 2.80.

Synthesis of I-GalSer[C11CO2Na][NH2] (Scheme 1).3-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-L-serine-11-methoxycarbonylundecanamide, 3a. Procedure **C** when applied to 63 mg (0.33 mmol) of EDC and to 2 mL of CH_2Cl_2 containing 0.20 g (0.30 mmol) of **A**, 69 mg (0.30 mmol) of methyl 12-aminododecanoate, and 42 mg (0.31 mmol) of HOBt, afforded after chromatographic purification (98:2 $CHCl_3$−MeOH) 0.35 g (95%) of 3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-N^{α}-9-(fluorenylmethoxycarbonyl)-L-serine-11-methoxycarbonylundecanamide [R_f = 0.76 (95:5 $CHCl_3$−MeOH). ^{1}H NMR ($CDCl_3$): δ 7.73 (d, J = 7.2 Hz, 2H, Fmoc), 7.56 (d, J =

7.2 Hz, 2H, Fmoc), 7.40−7.25 (m, 4H, Fmoc), 6.37 (bs, 1H, N*H*CH_2), 5.75 [d, J = 6.7 Hz, 1H, NHC(O)O], 5.35 (bs, 1H, H-4 Gal), 5.12 (m, 1H, H-2 Gal), 4.99 (dd, J = 10.4, J = 3.2 Hz, 1H, H-3 Gal), 4.52 (d, J = 7.5 Hz, 1H, H-1β Gal), 4.45−3.95 [m, 9H, H-5,6,6′ Gal, OCH_2CH, and C(O)OCH_2CH], 3.62 [s, 3H, C(O)OCH_3], 3.21 (m, 2H, NHCH_2), 2.26 [t, J = 7.4 Hz, 2H, CH_2C(O)OCH_3], 2.10, 1.99 and 1.95 [s,s,s, 12H, CH_3C(O)], 1.65−1.45 [m, 4H, NHCH_2CH_2 and CH_2CH_2C(O)OCH_3], 1.22 [m, 14H, $(CH_2)_7$]. ^{13}C NMR ($CDCl_3$): δ 174.4 [C(O)NH], 170.5, 170.2, 170.1, 169.6 and 169.0 [CH_3C(O)O and CH_3OC(O)], 156.0 [NHC(O)O], 143.9, 143.7 and 141.4 (C, Fmoc), 127.9, 127.2, 125.1 and 120.1 (CH, Fmoc), 102.1 (C-1β Gal), 71.2 (C-3 Gal), 70.7 (C-5 Gal), 70.1 (OCH_2CHNH), 68.8 (C-2 Gal), 67.1 [C-4 Gal and C(O)OCH_2], 61.4 (C-6 Gal), 54.0 [OCH_2CHNH], 51.5 [C(O)OCH_3], 47.3 [C(O)OCH_2CH], 39.9 (NHCH_2), 34.2 [CH_2C(O)OCH_3], 29.6, 29.5, 29.3, and 29.2 [$(CH_2)_7$], 26.9 [NHCH_2CH_2], 25.0 [CH_2CH_2C(O)OCH_3], 20.8, 20.7, and 20.6 [CH_3C(O)]]. Procedure **B** when applied to 130 mg (0.15 mmol) of this methoxycarbonylundecanamide derivative afforded 80 mg (80%) of **3a** as a white solid after chromatography ($CHCl_3$ to 7:3 $CHCl_3$−MeOH). R_f = 0.79 (4:1 $CHCl_3$−MeOH). ^{1}H NMR ($CDCl_3$): δ 7.33 (d, J = 6.5 Hz, 1H, N*H*CH_2), 5.35 (bd, J = 3.3 Hz, 1H, H-4 Gal), 5.15 (dd, J = 7.8 Hz, J = 10.4 Hz, 1H, H-2 Gal), 4.97 (dd, J = 10.4 Hz, J = 3.3 Hz, 1H, H-3 Gal), 4.48 (d, J = 7.8 Hz, 1H, H-1β Gal), 4.15−3.35 [m, 9H, H-5,6,6′ Gal, OCH_2CH, and C(O)OCH_3], 3.18 (q, J = 6.6 Hz, 2H, NHCH_2), 2.27 [t, J = 7.4 Hz, 2H, CH_2C(O)OCH_3], 2.12, 2.04, 2.02, and 1.95 [4s, each 3HCH_3C(O)], 1.90 (s, 2H, NH_2), 1.65−1.45 [m, 4H, NHCH_2CH_2 and CH_2CH_2C(O)OCH_3], 1.23 [m, 14H, $(CH_2)_7$]. ^{13}C NMR ($CDCl_3$): δ 174.4 [C(O)NH], 171.8, 171.2, 170.2, 170.1, and 169.6 [CH_3C(O)O and CH_3OC(O)], 101.4 (C-1β Gal), 72.5 (C-3 Gal), 70.9 (C-5 Gal and OCH_2CHNH_2), 68.9 (C-2 Gal), 67.1 (C-4 Gal), 61.3 (C-6 Gal), 55.2 ($CHNH_2$), 51.5 [C(O)OCH_3], 39.3 (NHCH_2), 34.2 [CH_2C(O)OCH_3], 29.5, 29.4, 29.3, and 29.2 [$(CH_2)_7$], 26.9 [NHCH_2CH_2], 25.0 [CH_2CH_2C(O)OCH_3], 20.8, 20.7 ,and 20.6 [CH_3C(O)].

3-*O*-(*β*-D-Galactopyranosyl)-L-serine-11-methoxycarbonylundecanamide, 4. The procedure **A** when applied to 80 mg (0.12 mmol) of **3a** gave, after recrystallization in Et_2O, 45 mg (76%) of **4**. R_f = 0.27 (65:36:4 $CHCl_3$−MeOH−H_2O). ^{1}H NMR ($CDCl_3$−CD_3OD): δ 4.25 (d, J = 6.6 Hz, 1H, H-1β Gal), 4.00−3.45 (m, 12H, H-2−6 Gal, OCH_2CH and C(O)OCH_3), 3.18 (t, J = 6.9 Hz, 2H, NHCH_2), 2.30 [t, J = 7.2 Hz, 2H, CH_2C(O)OCH_3], 1.65−1.50 [m, 4H, NHCH_2CH_2 and CH_2CH_2C(O)OCH_3], 1.28 [m, 14H, $(CH_2)_7$]. ^{13}C NMR ($CDCl_3$−CD_3OD): δ 175.1 [C(O)NH], 171.8 [C(O)OCH_3], 103.9 (C-1β Gal), 75.8 (C-5 Gal), 73.8 (C-3 Gal), 71.4 (OCH_2CHNH_2), 70.9 (C-2 Gal), 69.2 (C-4 Gal), 61.6 (C-6 Gal), 54.6 ($CHNH_2$), 51.2 [C(O)OCH_3], 39.7 (NHCH_2), 34.0 [CH_2C(O)OCH_3], 29.7, 29.6, 29.5, 29.4, and 29.2 [$(CH_2)_7$], 27.0 [NHCH_2CH_2], 25.0 [CH_2CH_2C(O)OCH_3].

3-*O*-(*β*-D-Galactopyranosyl)-L-serine-11-carboxyundecanamide (Sodium Salt), I-GalSer[C11CO2Na][NH2]. Forty-five milligrams (0.094 mmol) of **4** were stirred with 3.3 mL of 0.1 N NaOH 9:1 MeOH−H_2O, at room temperature for 3 h. The reaction mixture was purified by chromatography (49:1 to 3:2 $CHCl_3$−MeOH) affording 37 mg (80%) of **I-GalSer[C11CO2Na][NH2]** as a white solid. R_f = 0.18 (65:36:4 $CHCl_3$−MeOH−H_2O). $[\alpha]_D$ = +7.3° (*c* 0.28; 4:1 MeOH−H_2O). ^{1}H NMR (CD_3OD): δ 4.36 (d, J = 7.0 Hz, 1H, H-1β Gal), 4.25−3.45 (m, 9H, H-2-6 Gal and OCH_2CH), 3.21 (t, J = 6.6 Hz, 2H, NHCH_2), 2.14 [t, J = 7.2 Hz, 2H, CH_2C(O)ONa], 1.65−1.45 [m, 4H, NHCH_2CH_2 and CH_2CH_2C(O)ONa], 1.27 [m, 14H, $(CH_2)_7$]. ^{13}C NMR (CD_3OD): δ 181.5 [C(O)O], 172.0 [C(O)NH], 104.8 (C-1β Gal), 76.9 (C-5 Gal), 74.7 (C-3 Gal), 72.3 (OCH_2CHNH), 71.3 (C-2 Gal), 70.2 (C-4 Gal), 62.5 (C-6 Gal), 55.5 ($CHNH_2$), 40.6 (NHCH_2), 38.0 [CH_2C(O)O], 30.7, 30.6, 30.5, 30.4, and 30.3 [$(CH_2)_7$], 27.9 [NHCH_2CH_2], 27.2 [CH_2CH_2C(O)O]. Anal. Calc.for $C_{21}H_{39}N_2O_9Na\cdot 3/2H_2O$ (513.56): C, 49.11; H, 8.24; N, 5.45. Found: C, 49.12; H, 8.25; N, 5.06.

Synthesis of II-GalSer[C11CO2Na][C3CO2Na] (Scheme 1). 3-*O*-(2,3,4,6-Tetra-*O*-acetyl-*β*-D-galactopyranosyl)-*N*-(3-carboxypropionyl)-L-serine-11-methoxycarbonylundecanamide, 5. Twenty-six milligrams (0.26 mmol) of succinic anhydride was added to a solution of 71 mg (0.12 mmol) of **3a** and 30 μL (0.21 mmol) of Et_3N in CH_2Cl_2 (3 mL). After having been stirred at room temperature for 24 h, the solution was washed with 5% $KHSO_4$ and then water. After having been dried with Na_2SO_4 and filtration, the solvent was evaporated, and the residue was purified by chromatography ($CHCl_3$ to 9:1 $CHCl_3$−MeOH) giving 80 mg (95%) of **5** as a white solid. R_f = 0.24 (95:5 $CHCl_3$−MeOH). ^{1}H NMR ($CDCl_3$): δ 7.04 (d, J = 7.3 Hz, 1H, CHN*H*), 6.71 [t, J = 5.1 Hz, 1H, C(O)N*H*CH_2), 5.36 (bd, J = 3.0 Hz, 1H, H-4 Gal), 5.12 (dd, J = 7.5, J = 10.4 Hz, 1H, H-2 Gal), 5.01 (dd, J = 10.4, J = 3.2 Hz, 1H, H-3 Gal), 4.60 (d, J = 7.4 Hz, 1H, H-1β Gal), 4.56 (m, 1H, H-5 Gal), 4.10 (m, 2H, H-6,6′ Gal), 3.93 (m, 2H, OCH_2CH), 3.72 (m, 1H, OCH_2CH), 3.63 (s, 3H, OCH_3), 3.18 (m, 2H, NHCH_2), 2.62 [m, 2H, CH_2C(O)OH], 2.50 [m, 2H, NHC(O)CH_2], 2.27 [t, J = 7.4 Hz, 2H, CH_2C(O)OCH_3], 2.12, 2.02, and 1.95 [s,s,s, 12H, CH_3C(O)], 1.60−1.40 [m, 4H, NHCH_2CH_2 and CH_2CH_2C(O)OCH_3], 1.40−1.20 [m, 14H, $(CH_2)_7$]. ^{13}C NMR ($CDCl_3$): δ 175.9 [C(O)OH], 174.4, 172.2, and 170.7 [C(O)NH and C(O)OCH_3], 170.2, 170.1, 169.7, and 169.4 [CH_3C(O)], 102.1 (C-1β Gal), 71.2, 70.8, 69.7, and 67.2 (C2−5 Gal and OCH_2CH), 61.4 (C-6 Gal), 52.6 (OCH_2CH), 51.5 (OCH_3), 40.0 (NHCH_2), 34.2 [CH_2C(O)OCH_3], 30.7 [NHC(O)CH_2CH_2C(O)OH], 29.6, 29.4, 29.3, and 29.2 [$(CH_2)_7$], 26.9 [NHCH_2CH_2], 25.0 [CH_2CH_2C(O)OCH_3], 20.8 and 20.6 [CH_3C(O)].

3-*O*-(*β*-D-Galactopyranosyl)-*N*-(3-carboxypropionyl)-L-serine-11-methoxycarbonylundecanamide, 6. The procedure **A** when applied to 75 mg (0.11 mmol) of **5** gave 50 mg (76%) of **6**. R_f = 0.30 (71:26:4 $CHCl_3$−MeOH−H_2O). ^{1}H NMR (CD_3OD−D_2O): δ 4.48 (t, J = 4.3 Hz, 1 H, H-4 Gal), 4.30−4.18 (m, 2H, H-1β,3 Gal), 3.83−3.70 (m, 4H, H-2,5,6,6′ Gal), 3.64 (s, 3H, OCH_3), 3.60−3.50 (m, 3H, OCH_2CH), 3.22−3.02 [m, 8H, $^+$HN(CH_2CH_3)$_3$ and NHCH_2], 2.58−2.42 (m, 4H, CH_2CH_2C(O)ONHEt$_3$), 2.30 [t, J = 7.3 Hz, 2H, CH_2C(O)OCH_3], 1.59−1.46 [m, 4H, NHCH_2CH_2 and CH_2CH_2C(O)OCH_3], 1.30−1.10 [m, 23H, $(CH_2)_7$ and $^+$HN(CH_2CH_3)$_3$]. ^{13}C NMR (CD_3OD): δ 180.3 [C(O)O], 177.0, 176.3, and 172.1 [C(O)NH and C(O)OCH_3], 104.6 (C-1β Gal), 76.6 (C-5 Gal), 74.4 (C-3 Gal), 72.2 (C-2 Gal), 70.1 (C-4 Gal), 70.0 (OCH_2CH), 62.3 (C-6 Gal), 55.0 (OCH_2CH), 52.5 (OCH_3), 47.7 [$^+$NH(CH_2CH_3)$_3$], 40.7 (NHCH_2), 34.8 [CH_2C(O)OCH_3], 33.1 and 32.9 [NHC(O)-CH_2CH_2C(O)O], 30.4, 30.3, 30.1, 30.0, 29.9, and 29.8 [$(CH_2)_7$], 27.7 (NHCH_2CH_2), 25.9 [CH_2CH_2C(O)OCH_3], 9.3 [$^+$NH-(CH_2CH_3)$_3$]. This assignment was further confirmed by DEPT.

3-*O*-(*β*-D-Galactopyranosyl)-*N*-(3-carboxypropionyl)-L-serine-11-carboxyundecanamide (Sodium Salt), II-GalSer-[C11CO2Na][C3CO2Na]. Fifty milligrams (0.074 mmol) of **6** were stirred with 2.6 mL of 0.1 N 9:1 NaOH MeOH−H_2O, at room temperature for 3 days. The reaction mixture was purified by gel filtration on Sephadex LH20 (MeOH) and then silica gel chromatography (49:1 to 1:1 $CHCl_3$−MeOH) affording 30 mg (66%) of **II-GalSer[C11CO2Na][C3CO2Na]** as a white solid. R_f = 0.50 (8:5:1 $CHCl_3$−MeOH−H_2O). $[\alpha]_D$ = +7.1° (*c* 0.26; H_2O). MS (ESI) *m*/*z* = 563.6 (M − 2Na + H)$^-$. ^{1}H NMR (D_2O): δ 4.46 (m, 1H, H-4 Gal), 4.31 (d, J = 7.4 Hz, 1H, H-1β Gal), 4.01 (dd, J = 10.6, J = 5.4 Hz, 1H, H-3 Gal), 3.84−3.41 (m, 7H, H-2,5,6,6′ Gal and OCH_2CH), 3.13 (m, 2H, NHCH_2), 2.47 [m, 4H, NHC(O)CH_2CH_2C(O)ONa], 2.18 [t, J = 6.9 Hz, 2H, CH_2C(O)ONa], 1.55−1.30 [m, 4H, NHCH_2CH_2 and CH_2-CH_2C(O)ONa], 1.30−1.10 [m, 14H, $(CH_2)_7$]. ^{13}C NMR (D_2O): δ 181.5 and 180.1 [C(O)ONa], 175.8 and 171.1 [C(O)NH], 102.9 (C-1β Gal), 75.2 (C-5 Gal), 72.7 (C-3 Gal), 70.7 (C-2 Gal), 68.7 (OCH_2CH), 68.6(C-4 Gal), 60.9 (C-6 Gal), 53.9 (OCH_2CH), 39.6 (NHCH_2), 36.0 [CH_2C(O)ONa], 32.0 and 31.6 [NHC(O)-CH_2CH_2C(O)O], 29.0, 28.8, 28.6, and 28.5 [$(CH_2)_7$], 26.2 (NHCH_2CH_2), 25.3 [CH_2CH_2C(O)ONa]. Anal. Calc. for $C_{25}H_{42}N_2O_{12}Na_2\cdot 2H_2O$ (644.62): C, 46.58; H, 7.19; N, 4.35. Found: C, 46.92; H, 7.22; N, 4.50.

Synthesis of II-GalSer[C2OH][C14] (Scheme 1).3-*O*-(2,3,4,6-Tetra-*O*-acetyl-*β*-D-galactopyranosyl)-L-serine-2-hydroxyethanamide, 3b. Procedure **C**, when applied to 95 mg (0.49 mmol) of EDC, 320 mg (0.48 mmol) of **A**, 35 μL (0.57 mmol) of ethanolamine, and 70 mg (0.51 mmol) of HOBt, afforded after chromatography (49:1 $CHCl_3$−MeOH) 310 mg (90%) of 3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-N^{α}-

9-(fluorenylmethoxycarbonyl)-L-serine-2-hydroxyethanamide as a white solid [R_f = 0.62 (9:1 $CHCl_3$−MeOH). ^1H NMR ($CDCl_3$): δ 7.71 (d, J = 7.2 Hz, 2H, Fmoc), 7.54 (d, J = 7.2 Hz, 2H, Fmoc), 7.40−7.23 (m, 4H, Fmoc), 6.91 (t, J = 6.5 Hz, 1H, N*H*CH_2), 5.89 [d, J = 6.9 Hz, 1H, NHC(O)O], 5.33 (bs, 1H, H-4 Gal), 5.11 (dd, J = 10.3 Hz, J = 7.7 Hz, 1H, H-2 Gal), 4.98 (dd, J = 10.5 Hz, J = 3.2 Hz, 1H, H-3 Gal), 4.47 (d, J = 7.6 Hz, 1H, H-1β Gal), 4.36 [m, 2H, C(O)OC*H*$_2$CH], 4.15−3.80 [m, 6H, H-5,6,6′ Gal, OC*H*$_2$CH and C(O)OCH$_2$C*H*], 3.72 (m, 1H, OCH$_2$C*H*), 3.63 (m, 2H, C*H*$_2$OH), 3.37 (m, 2H, NHC*H*$_2$), 2.10, 1.98, and 1.94 [s,s,s, 12H, CH_3C(O)]. ^{13}C NMR ($CDCl_3$): δ 170.7, 170.3, 170.1, 170.0, and 169.8 [CH_3*C*(O)O and C(O)NH], 156.0 [NHC(O)O], 143.8 and 141.4 (C, Fmoc), 127.9, 127.2, 125.1, and 120.1 (CH, Fmoc), 101.9 (C-1β Gal), 71.2 (C-3 Gal), 70.6 (C-5 Gal), 70.0 (O*C*H$_2$CH), 68.9 (C-2 Gal), 67.1 [C-4 Gal and C(O)O*C*H$_2$], 61.4 (CH_2OH and C-6 Gal), 54.4 (OCH$_2$*C*H), 47.2 [C(O)OCH$_2$*C*H], 42.5 (NHC*H*$_2$), 20.8, 20.7, and 20.6 [*C*H$_3$C(O)]]. The procedure **B** when applied to 300 mg (0.43 mmol) of this hydroxyethanamide derivative gave after chromatography (95:5 $CHCl_3$−MeOH) 130 mg (65%) of **3b** as a white solid. R_f = 0.20 (95:5 $CHCl_3$−MeOH). ^1H NMR ($CDCl_3$): δ 7.63 (t, J = 5.6 Hz, 1H, N*H*CH_2), 5.31 (bs, 1H, H-4 Gal), 5.10 (dd, J = 10.5 Hz, J = 7.7 Hz, 1H, H-2 Gal), 4.94 (dd, J = 10.5 Hz, J = 3.3 Hz, 1H, H-3 Gal), 4.44 (d, J = 7.7 Hz, 1H, H-1β Gal), 4.10 (m, 2H, H-6,6′ Gal), 3.90−3.75 (m, 3H, H-5 Gal and OC*H*$_2$CH), 3.60 (t, J = 5.0 Hz, 2H, C*H*$_2$OH), 3.51 (m, 1H, OCH$_2$C*H*), 3.34 (m, 2H, NHC*H*$_2$), 2.49 (bs, 3H, OH and NH_2), 2.10, 2.00, 1.98, and 1.91 [4s, each 3H, CH_3C(O)]. ^{13}C NMR ($CDCl_3$): δ 170.6 [*C*(O)NHCH$_2$], 170.2, 170.0, and 169.8 [CH_3*C*(O)], 102.1 (C-1β Gal), 71.4 (C-3 Gal), 70.6 (C-5 Gal), 69.9 (O*C*H$_2$CH), 68.9 (C-2 Gal), 67.1 (C-4 Gal), 61.4 (CH_2OH and C-6 Gal), 55.2 (OCH$_2$*C*H), 42.5 (NHCH$_2$), 20.8, 20.6, and 20.5 [CH_3C(O)].

3-O-(2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl)-*N*-(tetradecanoyl)-L-serine-2-hydroxyethanamide, 7. One hundred twenty milligrams (0.25 mmol) of **3b** and 93 mg (0.28 mmol) of *N*-hydroxysuccinimide ester of tetradecanoic acid were stirred in 5 mL of CH_2Cl_2 for 3 days. The reaction mixture diluted with CH_2Cl_2 was successively washed with 5% $KHSO_4$, water, 8% $NaHCO_3$, and then water until neutrality. After drying with Na_2SO_4 and filtration, the residue purified by chromatography (95:5 $CHCl_3$:MeOH) gave 120 mg (70%) of **7** as a white solid. R_f = 0.36 (9:1 $CHCl_3$−MeOH). ^1H NMR ($CDCl_3$): δ 6.98 (m, 1H, N*H*CH_2), 6.62 (m, 1H, CHN*H*), 5.32 (bs, 1H, H-4 Gal), 5.06 (dd, J = 7.7 Hz, J = 10.4 Hz, 1H, H-2 Gal), 4.96 (dd, J = 10.4 Hz, J = 3.3 Hz, 1H, H-3 Gal), 4.55 (m, 2H, H-1β,5 Gal), 4.25−3.85 (m, 4H, OC*H*$_2$CH and H-6,6′ Gal), 3.63 (m, 3H, C*H*$_2$OH and OCH$_2$C*H*), 3.36 (m, 2H, NHC*H*$_2$), 2.78 (s, 1H, OH), 2.18 [t, J = 7.5 Hz, 2H, C*H*$_2$C(O)], 2.10, 2.00, and 1.91 [s,s,s, 12H, CH_3C(O)], 1.53 [m, 2H, C*H*$_2$CH$_2$C(O)], 1.35−1.10 [m, 20H, $(CH_2)_{10}$], 0.80 (t, J = 6.5 Hz, 3 H, CH_2-CH_3). ^{13}C NMR ($CDCl_3$): δ 173.8 [NH*C*(O)CH$_2$], 170.6 [*C*(O)NHCH$_2$], 170.2, 170.0, and 169.8 [CH_3*C*(O)], 102.1 (C-1β Gal), 71.4 (C-3 Gal), 70.6 (C-5 Gal), 69.9 (O*C*H$_2$CH), 68.9 (C-2 Gal), 67.1 (C-4 Gal), 61.4 (CH_2OH and C-6 Gal), 52.5 (OCH$_2$*C*H), 42.5 (NHCH$_2$), 36.4 [*C*H$_2$C(O)], 32.0 (*C*H$_2$CH$_2$CH$_3$), 29.6, 29.5, 29.3, 29.1, and 28.8 [$(CH_2)_8$], 25.5 [*C*H$_2$CH$_2$C(O)], 22.7 (*C*H$_2$-CH_3), 20.8, 20.6, and 20.5 [*C*H$_3$C(O)], 14.1 (CH_2*C*H$_3$).

3-*O*-(β-D-Galactopyranosyl)-*N*-(tetradecanoyl)-L-serine-2-hydroxyethanamide, II-GalSer[C2OH][C14]. The procedure **A** when applied to 70 mg (0.10 mmol) of **7** gave 45 mg (85%) of **II-GalSer[C2OH][C14]**. R_f = 0.56 (70:30:4 $CHCl_3$−MeOH−H_2O). SM (ESI) m/z = 543.5 $(M + Na)^+$. ^1H NMR (1:1 $CDCl_3$−CD_3OD): δ 4.56 (dd, J = 4.5, J = 5.6 Hz, 1H, H-4 Gal), 4.20 (d, J = 7.2 Hz, 1H, H-1β Gal), 4.14 (dd, J = 10.3 Hz, J = 4.4 Hz, 1H, H-3 Gal), 3.85−3.40 (m, 9H, H-2,5,6,6′ Gal, C*H*$_2$-OH, and OC*H*$_2$C*H*), 3.30 (m, 2H, NHC*H*$_2$), 2.24 [t, J = 7.5 Hz, 2H, C*H*$_2$C(O)], 1.58 [m, 2H, C*H*$_2$CH$_2$C(O)], 1.24 [m, 20H, $(CH_2)_{10}$], 0.84 (t, J = 6.1 Hz, 3H, CH_3). ^{13}C NMR (1:1 $CDCl_3$−CD_3OD): δ 174.8 [NH*C*(O)CH$_2$], 170.8 [*C*(O)NHCH$_2$], 103.8 (C-1β Gal), 75.2 (C-5 Gal), 73.2 (C-3 Gal), 70.9 (C-2 Gal), 68.9 (O*C*H$_2$CH), 68.7 (C-4 Gal), 60.9 (C-6 Gal), 59.9 (CH_2OH), 53.2 (OCH$_2$*C*H), 41.5 (NHCH$_2$), 35.4 [*C*H$_2$C(O)], 31.4 (*C*H$_2$CH$_2$CH$_3$), 29.1, 29.0, 28.9, and 28.8 [$(CH_2)_8$], 25.2 [*C*H$_2$CH$_2$C(O)], 22.1 (CH_2*C*H$_3$), 13.1 (CH_3). This assignment was further confirmed by DEPT. Anal. Calc. for $C_{25}H_{48}N_2O_9$·3/2H_2O (547.69): C, 54.83; H, 9.39; N, 5.11. Found: C, 54.35; H, 8.99; N, 5.07.

Synthesis of II-GalSer[C2SO4Na][C14] (Scheme 1).3-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-*N*-(tetradecanoyl)-L-serine-2-sulfoxyethanamide (Sodium Salt), 8. Fifty-five milligrams (0.081 mmol) of **7** and 40 mg (0.25 mmol) of SO_3·pyridine in 3 mL of pyridine were stirred at room temperature for 1 h. Then, 20 mg of Na_2CO_3 in 5 mL of water was added to the reaction mixture at 0 °C. After evaporation of the solvents, the crude residue was extracted twice with 1:1 $CHCl_3$−MeOH mixture. The organic phases were evaporated and purified by chromatography (95:5 to 9:1 $CHCl_3$−MeOH) giving 50 mg (80%) of **8** as a white solid. R_f = 0.34 (4:1 $CHCl_3$−MeOH). ^1H NMR (CD_3OD−$CDCl_3$): δ 5.32 (bs, 1H, H-4 Gal), 5.00 (m, 2H, H-2,3 Gal), 4.54 (d, J = 7.0 Hz, 1H, H-1β Gal), 4.45 (m, 1H, H-5 Gal), 4.10−3.75 (m, 7H, OC*H*$_2$C*H*, CH_2OSO_3Na, and H-6,6′ Gal), 3.46 (m, 2H, NHC*H*$_2$), 2.19 [t, J = 7.5 Hz, 2H, C*H*$_2$C(O)], 2.08, 1.99, and 1.90 [s,s,s, 12H, CH_3C(O)], 1.53 [m, 2H, C*H*$_2$CH$_2$C(O)], 1.45−1.20 [m, 20H, $(CH_2)_{10}$], 0.80 (t, J = 6.6 Hz, 3H, CH_2C*H*$_3$). ^{13}C NMR (CD_3OD-$CDCl_3$): δ 176.4 [NH*C*(O)CH$_2$], 172.2 [*C*(O)NHCH$_2$], 171.8, 171.5, and 171.4 [CH_3*C*(O)], 102.1 (C-1β Gal), 72.1 and 72.0 (C-3,5 Gal), 70.1 (O*C*H$_2$CH), 69.7 (C-2 Gal), 68.4 (C-4 Gal), 67.7 (CH_2OSO_3Na), 62.5 (C-6 Gal), 54.4 (OCH$_2$*C*H), 40.4 (NHCH$_2$), 37.3 [*C*H$_2$C(O)], 33.0 (*C*H$_2$CH$_2$CH$_3$), 30.8, 30.7, 30.5, and 30.4 [$(CH_2)_8$], 26.7 [*C*H$_2$CH$_2$C(O)], 23.7 (*C*H$_2$CH$_3$), 21.7, 21.6, and 21.5 [*C*H$_3$C(O)], 15.0 (CH_2*C*H$_3$).

3-*O*-(β-D-Galactopyranosyl)-*N*-(tetradecanoyl)-L-serine-2-sulfoxyethanamide (Sodium Salt), II-GalSer[C2SO4Na]-[C14]. The procedure **A** when applied to 50 mg (0.063 mmol) of **8** gave, after treatment with Amberlite IRC-50 ion exchange (Na^+), 30 mg (80%) of **II-GalSer[C2SO4Na][C14]**. R_f = 0.18 (35:15:2 $CHCl_3$−MeOH−H_2O). MS (ESI) m/z = 599.5 $(M - Na)^-$. ^1H NMR (1:1 CD_3OD-$CDCl_3$): δ 4.62 (m, 1H, H-4 Gal), 4.30 (d, J = 7.2 Hz, 1H, H-1β Gal), 4.20 (dd, J = 10.2 Hz, J = 4.1 Hz, 1H, H-3 Gal), 4.13 (m, 2H, H-2,5 Gal), 3.90−3.50 (m, 9H, H-6,6′ Gal, C*H*$_2$C*H*$_2$OSO$_3$Na, and OC*H*$_2$C*H*), 2.32 [t, J = 7.0 Hz, 2H, CH$_2$C(O)], 1.65 [m, 2H, C*H*$_2$CH$_2$C(O)], 1.40−1.20 [m, 20H, $(CH_2)_{10}$], 0.92 (t, J = 6.8 Hz, 3H, CH_3). ^{13}C NMR (1:1 CD_3OD-$CDCl_3$): δ 174.8 [NH*C*(O)CH$_2$], 170.8 [*C*(O)NHCH$_2$], 103.6 (C-1β Gal), 75.0 (C-5 Gal), 73.1 (C-3 Gal), 70.8 (C-2 Gal), 68.9 (O*C*H$_2$CH and C-4 Gal), 65.8 (CH_2OSO_3Na), 61.0 (C-6 Gal), 53.1 (OCH$_2$*C*H), 38.9 (NHCH$_2$), 35.4 [*C*H$_2$C(O)], 31.5 (*C*H$_2$CH$_2$CH$_3$), 29.2, 29.1, 29.0, 28.9, and 28.8 [$(CH_2)_8$], 25.1 [*C*H$_2$CH$_2$C(O)], 22.1 (*C*H$_2$CH$_3$), 13.0 (CH_3). This assignment was further confirmed by DEPT. Anal. Calc. for $C_{25}H_{47}N_2O_{12}$-NaS·6H_2O (730.89): C, 41.09; H, 8.14; N, 3.83; S, 4.39. Found: C, 41.13; H, 7.90; N, 3.89; S, 4.16.

Synthesis of II-GalSer[C14][C11CO2Na] (Scheme 2). 3-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-L-serine Tetradecanamide, 9. Procedure **C**, when applied to 145 mg (0.75 mmol) of EDC, 450 mg (0.70 mmol) of **A**, 160 mg (0.73 mmol) of tetradecylamine, and 105 mg (0.77 mmol) of HOBt, gave after chromatography ($CHCl_3$) 480 mg (80%) of 3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-N^α-9-(fluorenylmethoxycarbonyl)-L-serine tetradecanamide as a white solid [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), 1700 (C=O carbamate), 1655 (C=O amide). ^1H NMR ($CDCl_3$−CD_3OD): δ 7.68 (d, J = 8.0 Hz, 2H, Fmoc), 7.55 (d, J = 8.0 Hz, 2H, Fmoc), 7.35−7.20 (m, 4H, Fmoc), 5.24 (bs, J = 3.2 Hz, 1H, H-4 Gal), 5.00 (dd, J = 7.4 Hz, J = 10.4 Hz, 1H, H-2 Gal), 4.92 (dd, ^3J = 10.4, Hz J = 3.2 Hz, 1H, H-3 Gal), 4.40 (d, J = 7.4 Hz, 1H, H-1β Gal), 4.30−3.60 [m, 9 H, H-5,6,6′ Gal, OC*H*$_2$C*H*, and C(O)OC*H*$_2$C*H*], 3.15 (m, 2H, NHC*H*$_2$), 2.06, 1.97, 1.94, and 1.91 [4s, each 3CH_3C(O)], 1.50−1.10 [m, 24H, $(CH_2)_{12}$], 0.80 (t, J = 7.0 Hz, 3H, CH_2C*H*$_3$). ^{13}C NMR ($CDCl_3$): δ 170.8 [C(O)NH], 170.4, 170.3, 169.9, and 169.4 [CH_3*C*(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 (O*C*H$_2$-CH), 68.7 (C-2 Gal), 67.1 (C-4 Gal), 66.9 [C(O)O*C*H$_2$], 61.3 (C-6 Gal), 54.2 (OCH$_2$*C*H), 47.1 [C(O)OCH$_2$*C*H], 39.7 (NHCH$_2$), 31.9 (*C*H$_2$CH$_2$CH$_3$), 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, and 20.38 [$CH_3C(O)$], 13.9 (CH_2CH_3)]]. The procedure **B** when applied to 150 mg (0.17 mmol) of this tetradecanamide derivative gave, after chromatography (CH_2Cl_2 to 1:1 CH_2Cl_2−Et_2O), 76 mg (76%) of **9** as a white solid. R_f = 0.6 (49:1 $CHCl_3$−MeOH). ^{1}H NMR ($CDCl_3$): δ 7.34 (t, J = 5.6 Hz, 1H, $NHCH_2$), 5.33 (bs, J = 3.2 Hz, 1H, H-4 Gal), 5.12 (dd, J = 7.8 Hz, J = 10.4 Hz, 1H, H-2 Gal), 4.95 (dd, J = 10.4 Hz, J = 3.2 Hz, 1H, H-3 Gal), 4.48 (d, J = 7.8 Hz, 1H, H-1β Gal), 4.07 (m, 2H, H-6,6′ Gal), 3.86 (m, 2H, H-5 Gal and OCH_aH_b), 3.77 (t, J = 8.2 Hz, 1H, OCH_aH_b), 3.48 (dd, J = 8.2 Hz, J = 4.4 Hz, 1H, OCH_2CH), 3.15 (q, J = 6.6 Hz, 2H, $NHCH_2$), 2.08, 2.00, 1.98, and 1.91 [4s, each $3HCH_3C(O)$], 1.70 (bs, 2H, NH_2), 1.50−1.10 [m, 24H, $(CH_2)_{12}$], 0.81 (t, J = 6.6 Hz, 3H, 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, 29.74, 29.70, 29.60, 29.55, 29.40, and 29.36 [$(CH_2)_9$], 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_2CH_3).

3-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-*N*-(11-allyloxycarbonyl-undecanoyl)-L-serine Tetradecanamide, 10. Procedure **C** when applied to 49 mg (0.26 mmol) of EDC, 160 mg (0.25 mmol) of **9**, 68 mg (0.25 mmol) of dodecanedioic acid monoallyl ester, and 38 mg (0.28 mmol) of HOBt afforded afetr chromatography ($CHCl_3$) 140 mg (65%) of **10** as a white solid. R_f = 0.54 (49:1 $CHCl_3$−MeOH). ^{1}H NMR ($CDCl_3$): δ 6.58 [m, 2H, C(O)N*H*], 5.83 (m, 1H, $OCH_2CH{=}CH_2$), 5.34 (bd, J = 2.5 Hz, 1H, H-4 Gal), 5.27−5.05 (m, 3H, H-2 Gal and $OCH_2CH{=}CH_2$), 4.97 (dd, J = 10.4 Hz, J = 3.3 Hz, 1H, H-3 Gal), 4.56 (d, J = 7.7 Hz, 1H, H-1β Gal), 4.50 (m, 3H, H-5 Gal and $OCH_2CH{=}CH_2$), 4.13 (m, 2H, H-6,6′ Gal), 4.05 (m, 2H, OCH_2CH), 3.66 (m, 1H, OCH_2CH), 3.16 (m, 2H, $NHCH_2$), 2.25 [t, J = 7.3 Hz, 2H, $CH_2C(O)OAll$], 2.15 [t, J = 7.2 Hz, 2H, $NHC(O)CH_2$], 2.08, 1.99, 1.98, and 1.91 [4s, each $3HCH_3C(O)$], 1.65−1.45 [m, 6H, $CH_2CH_2C(O)O$ and $NHCH_2CH_2$], 1.35−1.10 [m, 34H, $(CH_2)_6$ and $(CH_2)_{11}CH_3$], 0.80 (t, J = 6.6 Hz, 3H, CH_2CH_3]. ^{13}C NMR ($CDCl_3$): δ 173.4, 173.3, and 170.5 [$C(O)$-OAll and C(O)NH], 170.1, 169.9, 169.5, and 169.4 [$CH_3C(O)$], 132.5 ($OCH_2CH{=}CH_2$), 118.0 ($OCH_2CH{=}CH_2$), 102.2 (C-1β Gal), 71.3 (C-3 Gal), 70.8 (C-5 Gal), 69.9 (OCH_2CH), 68.9 (C-2 Gal), 67.2 (C-4 Gal), 64.9 ($OCH_2CH{=}CH_2$), 61.4 (C-6 Gal), 52.2 [OCH_2CH], 39.8 ($NHCH_2$), 36.4 [$NHC(O)CH_2$], 34.3 [$CH_2C(O)O$], 32.0 ($CH_2CH_2CH_3$), 29.7, 29.6, 29.4, and 29.1 [$(CH_2)_6$ and $(CH_2)_9CH_2CH_2CH_3$], 26.9 ($NHCH_2CH_2$), 25.6 [$CH_2CH_2C(O)NH$], 25.0 [$CH_2CH_2C(O)O$], 22.7 (CH_2CH_3), 20.8, 20.6, and 20.5 [$CH_3C(O)$], 14.1 (CH_2CH_3).

3-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-*N*-(11-carboxyundecanoyl)-L-serine Tetradecanamide, 11. Seventy-four milligrams of **10** (0.084 mmol), 130 μL of Et_2NH (1.25 mmol), and 5.0 mg (4.4×10^{-3} mmol) of $Pd[P(Ph)_3]_4$ in CH_2Cl_2 (3 mL) were stirred for 2 h at room temperature. After evaporation of the solvent, the solution was washed with 5% $KHSO_4$ and then water. After drying over Na_2SO_4 and filtration, the solvent was evaporated, and the residue was purified by chromatography ($CHCl_3$ to 1:1 $CHCl_3$−Et_2O) giving 65 mg (90%) of **11** as a white solid. R_f = 0.36 (95:5 $CHCl_3$−MeOH). ^{1}H NMR ($CDCl_3$): δ 7.02 [d, J = 7.1 Hz, 1H, CHN*H*], 6.66 [t, J = 6.5 Hz, 1H, $C(O)NHCH_2$], 5.36 (bd, J = 3.1 Hz, 1H, H-4 Gal), 5.13 (dd, J = 7.7 Hz, J = 10.4 Hz, 1H, H-2 Gal), 5.00 (dd, J = 10.4 Hz, J = 3.3 Hz, 1H, H-3 Gal), 4.64 (m, 1H, H-5 Gal), 4.57 (d, J = 7.7 Hz, 1H, H-1β Gal), 4.17 (m, 2H, H-6,6′ Gal), 3.96 (m, 2H, OCH_2CH), 3.69 (m, 1H, OCH_2CH), 3.20 (m, 2H, $NHCH_2$), 2.30 [t, J = 7.1 Hz, 2H, $CH_2C(O)OH$], 2.23 [t, J = 7.1 Hz, 2H, $NHC(O)CH_2$], 2.12, 2.03, 2.01, and 1.95 [4s, each $3HCH_3C(O)$], 1.70−1.45 [m, 6H, $NHCH_2CH_2$, $NHC(O)CH_2CH_2$ and $CH_2CH_2C(O)OH$], 1.35−1.10 [m, 34H, $(CH_2)_6$ and $(CH_2)_{11}CH_3$], 0.84 (t, J = 6.5 Hz, 3H, CH_2CH_3). ^{13}C NMR ($CDCl_3$): δ 177.8 [C(O)OH], 173.8 and 170.6 [C(O)NH], 170.2, 170.1, 169.9, and 169.6 [$CH_3C(O)$], 102.4 (C-1β Gal), 71.3 (C-3 Gal), 70.8 (C-5 Gal), 70.1 (OCH_2CH), 68.9 (C-2 Gal), 67.2 (C-4 Gal), 61.4 (C-6 Gal), 52.3 (OCH_2CH), 40.0 ($NHCH_2$), 36.3 [$NHC(O)CH_2$], 34.0 [$CH_2C(O)OH$], 32.0 ($CH_2CH_2CH_3$), 29.8, 29.6, 29.5, 29.3, 29.1, 28.9, and 28.8 [$(CH_2)_6$ and $(CH_2)_9$], 26.9 ($NHCH_2CH_2$), 25.5 [$NHC(O)CH_2CH_2$], 24.7 [$CH_2CH_2C(O)OH$], 22.7 (CH_2CH_3), 20.8, 20.7, and 20.4 [$CH_3C(O)$], 14.2 (CH_2CH_3).

3-*O*-(β-D-Galactopyranosyl)-*N*-(11-carboxyundecanoyl)-L-serine Tetradecanamide (Sodium Salt), II-GalSer[C14]-[C11CO2Na]. The procedure **A** when applied to 65 mg (0.077 mmol) of **11** gave, after treatment with Amberlite IRC-50 ion-exchange (Na^+), 45 mg (90%) of **II-GalSer[C14][C11CO2Na]**. R_f = 0. 69 (71:26:4 $CHCl_3$−MeOH−H_2O). IR (ν cm^{-1}, KBr): 1696 (C═O acide), 1645 (C═O amide). ^{1}H NMR ($CDCl_3$−CD_3-OD-D_2O): δ 4.51 (m, 1H, H-4 Gal), 4.23 (d, J = 7.4 Hz, 1H, H-1β Gal), 4.08 (dd, J = 10.4 Hz, J = 3.3 Hz, 1H, H-3 Gal), 3.80 (m, 1H, H-2 Gal), 3.69 (m, 3H, H-5,6,6′ Gal), 3.48 (m, 3H, OCH_2CH), 3.14 (m, 2H, $NHCH_2$), 2.24 [t, J = 7.8 Hz, 2H, $CH_2C(O)ONa$], 2.14 [t, J = 7.9 Hz, 2H, $NHC(O)CH_2$], 1.60−1.35 [m, 6H, $NHCH_2CH_2$, $NHC(O)CH_2CH_2$ and $CH_2CH_2C(O)$-OH], 1.35−1.10 [m, 34H, $(CH_2)_6$ and $(CH_2)_{11}CH_3$], 0.82 (t, J = 6.6 Hz, 3H, CH_3). ^{13}C NMR (DMSO-d_6): δ 172.2 and 169.3 [C(O)NH and C(O)ONa], 104.1 (C-1β Gal), 75.2 (C-5 Gal), 73.1 (C-3 Gal), 70.4 (C-2 Gal), 68.0 (C-4 Gal), 69.4 (OCH_2CH), 60.4 (C-6 Gal), 52.6 (OCH_2CH), 38.5 ($NHCH_2$), 35.1 [$NHC(O)CH_2$], 34.6 [$CH_2C(O)ONa$], 31.2 ($CH_2CH_2CH_3$), 29.0, 28.7, and 28.6 [$(CH_2)_6$ and $(CH_2)_9$], 26.2 ($NHCH_2CH_2$), 25.0 [$NHC(O)CH_2CH_2$], 24.8 [$CH_2CH_2C(O)OH$], 22.0 (CH_2CH_3), 13.9 (CH_3). This assignment was further confirmed by DEPT. Anal. Calc. for $C_{35}H_{65}N_2O_{10}Na\cdot 11/2H_2O$ (795.98): C, 52.81; H, 8.23; N, 3.52; Na, 2.89. Found: C, 53.00; H, 8.84; N, 3.17; Na, 2.33.

Synthesis of II-GalSer[C14][C3CO2Na] (Scheme 2).3-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)-*N*-(3-carboxypropionyl)-L-serine Tetradecanamide, 12. Twenty milligrams (0.20 mmol) of succinic anhydride were added to a solution of 120 mg of **9** (0.19 mmol) and 60 μL of Et_3N (0.43 mmol) in CH_2Cl_2 (3 mL). After stirring at room temperature for 24 h, the solution was washed with 5% $KHSO_4$ and then water. After drying over Na_2SO_4 and filtration, the solvent was evaporated, and the residue was purified by chromatography ($CHCl_3$ to 9:1 $CHCl_3$−MeOH) giving 130 mg (95%) of **12** as a white solid. R_f = 0.34 (95:5 $CHCl_3$−MeOH). ^{1}H NMR ($CDCl_3$): δ 7.10 [d, J = 6.9 Hz, 1H, CHN*H*], 6.84 [m, ^{1}H, $C(O)NHCH_2$], 5.32 (bd, J = 2.8 Hz, ^{1}H, H−4 Gal), 5.05 (dd, J = 7.4 Hz, J = 10.4 Hz, ^{1}H, H−2 Gal), 5.01 (dd, J = 10.4 Hz, J = 3.0 Hz, ^{1}H, H−3 Gal), 4.58 (d, J = 7.3 Hz, ^{1}H, H−1β Gal), 4.54 (m, ^{1}H, H−5 Gal), 4.08 (m, 2H, H−6,6¢ Gal), 3.94 (m, 2H, OCH_2CH), 3.71 (m, ^{1}H, OCH_2CH), 3.15 (dt, J = 6.6 Hz, J = 5.8 Hz, 2H, $NHCH_2$), 2.53 [m, 2H, $CH_2C(O)OH$], 2.45 [m, 2H, $NHC(O)$-CH_2], 2.08, 1.98, and 1.91 [s,s,s, 12H, $CH_3C(O)$], 1.42 (m, 2H, $NHCH_2CH_2$), 1.30−1.10 [m, 22H, $(CH_2)11CH_3$], 0.80 (t, J = 6.3 Hz, 3H, CH_2CH_3). ^{13}C NMR ($CDCl_3$): δ 176.4 [C(O)OH], 172.4 and 170.4 [C(O)NH], 169.9, 169.8, 169.5, and 169.3 [$CH_3C(O)$], 101.6 (C−1β Gal), 70.8 (C−3 Gal), 70.5 (C−5 Gal), 69.5 (C−2 Gal and OCH_2CH), 66.9 (C−4 Gal), 61.1 (C−6 Gal), 52.4 (OCH_2CH), 39.7 ($NHCH_2$), 31.7 ($CH_2CH_2CH_3$), 30.9 [NHC-$(O)CH_2$], 30.0 [$CH_2C(O)OH$], 29.5 and 29.1 [(CH_2)9], 26.7 ($NHCH_2CH_2$), 22.5 (CH_2CH_3), 20.6 and 20.4 [$CH_3C(O)$], 13.9 (CH_2CH_3).

3-*O*-(β-D-Galactopyranosyl)-*N*-(3-carboxypropionyl)-L-serine Tetradecanamide (Sodium Salt), II-GalSer[C14]-[C3CO2Na]. The procedure **A** when applied to 120 mg (0.16 mmol) of **12** gave, after treatment with Amberlite IRC-50 ion-exchange (Na^+), 45 mg (76%) of **II-GalSer[C14][C3CO2Na]**. R_f = 0.44 (71:26:4 $CHCl_3$−MeOH−H_2O). MS (ESI) m/z = 561.4 ($M - Na)^-$. ^{1}H NMR (CD_3OD-D_2O): δ 4.46 (m, 1H, H-4 Gal), 4.28 (d, J = 7.0 Hz, 1H, H-1β Gal), 4.17 (m, 1H, H-3 Gal), 3.80−3.35 (m, 7H, OCH_2CH and H-2,5,6,6′ Gal), 3.13 (m, 2H, $NHCH_2$), 2.46 [m, 4H, $CH_2CH_2C(O)ONa$], 1.43 (m, 2H, $NHCH_2CH_2$), 1.30−1.10 [m, 22H, $(CH_2)_{11}CH_3$], 0.80 (t, J = 6.7 Hz, 3H, CH_3). ^{13}C NMR (D_2O at 50 °C): δ 176.4 [C(O)ONa], 171.5 [C(O)NH], 104.0 (C-1β Gal), 76.0 (C-5 Gal), 73.8 (C-3 Gal), 71.7 (C-2 Gal), 69.9 (C-4 Gal), 69.6 (OCH_2CH), 61.8 (C-6 Gal), 54.6 (OCH_2CH), 40.5 ($NHCH_2$), 33.1 ($CH_2CH_2CH_3$), 31.3 and 31.4 [$NHC(O)CH_2$ and $CH_2C(O)OH$], 30.5, 30.1, and 29.7 [$(CH_2)_9$], 27.6 ($NHCH_2CH_2$), 23.3 (CH_2CH_3), 14.5 (CH_3). This assignment was confirmed by DEPT.

Synthesis of II-GalSer[C14][C7SO3Na] (Scheme 2). 3-*O*-(2,3,4,6-Tetra-*O*-acetyl-*β*-D-galactopyranosyl)-*N*-[(3-(2-sulfoethylcarbamoyl)propionyl]-L-serine Tetradecanamide, 13. Twenty milligrams (0.11 mmol) of EDC were added at 0 °C to a solution of 80 mg (0.11 mol) of **12** and 26 mg (0.23 mmol) of *N*-hydroxysuccinimide in 3 mL of DMF. The reaction mixture was stirred at 0 °C for 30 min and then at room temperature for 24 h. Eighteen milligrams (0.14 mmol) of taurine and 30 μL (0.22 mmol) of Et_3N were then added, and the mixture was stirred for 24 h. After evaporation of DMF under vacuo, the residue was chromatographed on silica gel ($CHCl_3$ to 4:1 $CHCl_3$−MeOH) giving 60 mg (65%) of **13** as a white solid. $R_f = 0.37$ (4:1 $CHCl_3$−MeOH). ^{1}H NMR (CD_3OD): δ 5.31 (bs, 1H, H-4 Gal), 5.03 (m, 2H, H-2,3 Gal), 4.56 (d, J = 6.9 Hz, 1H, H-1β Gal), 4.47 (m, 1H, H-5 Gal), 4.10 (m, 2H, H-6,6′ Gal), 4.00−3.70 (m, 3H, OCH_2CH), 3.54 (m, 2H, NHCH_2-CH_2SO_3), 3.15 (m, 2H, NHCH_2), 2.95 (m, 2H, NHCH_2CH_2SO_3), 2.60−2.40 [m, 4H, NHC(O)CH_2CH_2], 2.08, 1.99, 1.98, and 1.91 [4s, each 3H, CH_3C(O)], 1.52−1.40 (m, 2H, NHCH_2CH_2), 1.30−1.10 [m, 22H, (CH_2)$_{11}$$CH_3$], 0.80 (t, J = 6.3 Hz, 3H, CH_2CH_3). ^{13}C NMR ($CDCl_3$−CD_3OD): δ 173.4, 173.3, and 170.8 [C(O)NH], 170.4, 170.3, 170.1, and 169.7 [CH_3*C*(O)], 101.4 (C-1β Gal), 70.9 and 70.8 (C-3,5 Gal), 69.2 (OCH_2CH), 69.0 (C-2 Gal), 67.2 (C-4 Gal), 61.2 (C-6 Gal), 53.0 (OCH_2*C*H), 46.4 (NH*C*H_2-CH_2SO_3), 40.1 (NHCH_2), 35.4 (NHCH_2CH_2SO_3), 32.0 (*C*H_2CH_2-CH_3), 30.9 and 30.8 [NHC(O)*C*H_2CH_2], 29.8, 29.4, and 29.2 [(CH2)9], 27.1 (NHCH2CH2), 22.7 (CH2CH3), 20.7 and 20.6 [CH3C(O)], 14.1 (CH2CH3).

3-O−(*β*-D-Galactopyranosyl)-*N*-[(3-(2-sulfoethylcarbamoyl)propionyl]-L-serine Tetradecanamide, II-GalSer[C14][C7SO3Na]. The procedure **A** when applied to 57 mg (0.068 mmol) of **13** gave, after treatment with Amberlite IRC-50 ion-exchange (Na^+), 40 mg (80%) of **II-GalSer[C14][C7SO3Na]** as a white solid. $R_f = 0.17$ (71:26:4 $CHCl_3$−MeOH−H_2O). $[\alpha]_D$ = −11.7° (*c* 0.28; MeOH). IR (ν cm^{-1}, KBr): 1649 (C=O amide), 1210 (S=O). MS (ESI) *m*/*z* = 668.5 (M−Na)$^-$. ^{1}H NMR (CD_3-OD): δ 4.54 (m, 1H, H-4 Gal), 4.28 (d, J = 7.3 Hz, 1H, H-1β Gal), 4.23 (m, 2H, H-2,3 Gal), 3.84−3.70 (m, 3H, H-5,6,6′ Gal), 3.58 (m, 5H, OCH_2CH and NHCH_2CH_2SO_3), 3.17 (m, 2H, NHCH_2), 2.98 (t, J = 6.8 Hz, 2H, NHCH_2CH_2SO_3), 2.65−2.48 [m, 4H, NHC(O)CH_2CH_2], 1.51 (m, 2H, NHCH_2CH_2), 1.30−1.10 [m, 22H, (CH_2)$_{11}$$CH_3$], 0.80 (t, J = 6.6 Hz, 3H, CH_3). ^{13}C NMR (CD_3OD): 176.8, 176.1, and 173.4 [C(O)NH], 106.5 (C-1β Gal), 78.2 (C-5 Gal), 76.3 (C-3 Gal), 73.9 (C-2 Gal), 71.9 (C-4 Gal), 71.8 (OCH_2CH), 64.1 (C-6 Gal), 56.5 (OCH_2*C*H), 52.9 (NHCH_2CH_2SO_3), 42.3 (NHCH_2), 38.1 (NHCH_2CH_2SO_3), 34.5 (*C*H_2CH_2CH_3), 33.8 and 33.6 [NHC(O)CH_2CH_2], 32.3, 32.2, 31.9, and 31.8 [(CH_2)$_9$], 29.5 (NHCH_2*C*H_2), 25.2 (*C*H_2CH_3), 15.9 (CH_3). This assignment was confirmed by DEPT. Anal. Calc. for $C_{29}H_{54}N_3O_{12}NaS\cdot 2H_2O$ (727.84): C, 47.86; H, 8.03; N, 5.77; S, 3.16. Found: C, 47.85; H, 8.01; N, 5.47; S, 3.78.

Synthesis of II-GalSer[C14][OP(OMe)ONa] (Scheme 2).3-*O*-(2,3,4,6-Tetra-*O*-acetyl-*β*-D-galactopyranosyl)-*N*-(diphenoxyphosphoryl)-L-serine Tetradecanamide, 14. One hundred sixty microliters (0.76 mmol) of diphenyl chlorophosphate in 2 mL of pyridine were added to 95 mg (0.15 mmol) of **9** in 2 mL of pyridine at 0 °C. The mixture was stirred at 0 °C for 2 h. The reaction was poured into ice water, and the precipitate was filtered, washed twice with ice water, and dried. The residue was purified by chromatography ($CHCl_3$ to 49:1 $CHCl_3$−MeOH) giving 105 mg (80%) of **14** as a white solid. $R_f = 0.60$ (95:5 $CHCl_3$−MeOH). ^{1}H NMR ($CDCl_3$): δ 7.25 (m, 10H, Ph), 6.37 (t, J = 5.6 Hz, 1H, NHCH$_2$), 5.34 (bs, J = 3.3 Hz, 1H, H-4 Gal), 5.12 (dd, J = 7.8 Hz, J = 10.4 Hz, 1H, H-2 Gal), 4.95 (dd, J = 10.4 Hz, J = 3.3 Hz, 1H, H-3 Gal), 4.40 (d, J = 7.8 Hz, 1H, H-1β Gal), 4.25−4.00 (m, 4H, H-5,6,6′ Gal and OCH_aH$_b$), 3.87 (m, 1H, OCH$_a$$H_b$), 3.60 (dd, J = 6.8, J = 9.7 Hz, 1H, OCH$_2$CH), 3.11 (m, 2H, NHCH_2), 2.34 (m, 1H, NHP), 2.09, 2.02, 1.96, and 1.94 [4s, each 3HCH_3C(O)], 1.40−1.10 [m, 24H, (CH_2)$_{12}$], 0.84 (t, J = 6.6 Hz, 3H, CH_2CH_3). ^{13}C NMR ($CDCl_3$): δ 170.5, 170.2, 170.1, 169.9, and 169.4 [CH_3*C*(O) and C(O)NH], 150.6 (d, $^2J_{C,P}$ = 8.6 Hz, C Ph), 130.0 (CH, meta Ph), 125.4 (CH, para Ph), 120.4 (d, $^3J_{C,P}$ = 4.2 Hz, C ortho Ph), 101.8 (C-1β Gal), 71.1 and 70.7 (C-3,5 Gal and OCH_2CH), 68.8 (C-2 Gal), 67.1 (C-4 Gal), 61.3 (C-6 Gal), 54.7 (OCH_2*C*H), 39.9 (NHCH_2), 32.0 (*C*H_2CH_2CH_3), 29.8, 29.6, and 29.4 [(CH_2)$_8$], 26.9 (NHCH_2*C*H_2), 22.5 (*C*H_2CH_3), 20.8 and 20.6 [CH_3C(O)], 14.2 (*C*H_3). ^{31}P{^{1}H} NMR ($CDCl_3$): δ −1.52 (s).

3-*O*-(*β*-D-Galactopyranosyl)-*N*-(dimethoxyphosphoryl)-L-serine Tetradecanamide, 15. Hydrogen was bubbled through a suspension of 140 mg (0.17 mmol) of **14**, 40 mg of platinum(IV) oxide monohydrate, and 100 μL of Et_3N in 10 mL of MeOH at room temperature for 20 h. After filtration, evaporation, and chromatography (95:5 to 4:1 $CHCl_3$−MeOH), 80 mg (87%) of **15** as a white solid were obtained. $R_f = 0.55$ (4:1 $CHCl_3$−MeOH). MS (ESI) *m*/*z* = 593.5 (M + Na)$^+$. ^{1}H NMR ($CDCl_3$−CD_3OD): δ 4.18 (d, J = 7.2 Hz, 1H, H-1β Gal), 4.08 (dd, J = 10.0 Hz, J = 4.1 Hz, 1H, H-3 Gal), 3.66 (d, $J_{H,P}$ = 11.2 Hz, 6H, OCH_3), 3.85−3.40 (m, 8H, H-2,4-6 Gal and OCH_2CH), 3.14 (m, 2H, NHCH_2), 1.60−1.10 [m, 24H, (CH_2)$_{12}$], 0.82 (t, J = 6.6 Hz, 3H, CH_2CH_3). ^{13}C NMR ($CDCl_3$−CD_3OD): δ 172.6 [d, $^3J_{C,P}$ = 5 Hz, C(O)NH], 105.2 (C-1β Gal), 76.5 (C-5 Gal), 74.6 (C-3 Gal), 72.3 (C-2 Gal), 72.1 (d, $^3J_{C,P}$ = 5 Hz, OCH_2-CHNH), 70.1 (C-4 Gal), 62.6 (C-6 Gal), 56.5 (CHNHP), 54.4 and 54.3 [d,d, $^2J_{C,P}$ = 6 Hz, OCH_3], 40.7 (NHCH_2), 33.0 (*C*H_2-CH_2CH_3), 30.8, 30.7, 30.4, and 30.3 [(CH_2)$_8$], 27.9 (NHCH_2*C*H_2), 23.7 (*C*H_2CH$_3$), 14.7 (CH_2*C*H_3). ^{31}P{^{1}H} NMR ($CDCl_3$−CD_3-OD): δ 9.90 (s) [12.8 (s) in D_2O].

3-*O*-(*β*-D-Galactopyranosyl)-*N*-(hydroxymethoxyphosphoryl)-L-serine Tetradecanamide (Sodium Salt), II-GalSer[C14][OP(OMe)ONa]. Forty milligrams (0.070 mmol) of **15** and 100 μL of Et_3N in 5 mL of water were stirred at room temperature for 5 days. The crude residue was eluted on Amberlite IRC-50 ion-exchange (Na^+) column and then lyophilized giving 38 mg (95%) of **II-GalSer[C14][OP(OMe)-ONa]** as a white solid. $R_f = 0.67$ (71:26:4 $CHCl_3$−MeOH−H_2O). SM (ESI) *m*/*z* = 601.5 (M + Na)$^+$. ^{1}H NMR (D_2O): δ 4.33 (d, J = 7.0 Hz, 1H, H-1β Gal), 4.10−3.40 (m, 12H, OCH_3, OCH_2CH and H-2−6 Gal), 3.12 (m, 2H, NHCH_2), 1.60−1.10 [m, 24H, (CH_2)$_{12}$], 0.82 (t, J = 6.6 Hz, 3H, CH_2CH_3). ^{13}C NMR ($CDCl_3$−CD_3OD): δ 173.3 [d, $^3J_{C,P}$ = 5 Hz, C(O)NH], 103.4 (C-1β Gal), 75.0 (C-5 Gal), 72.7 (C-3 Gal), 71.5 and 71.4 (OCH_2CHNH), 70.6 (C-2 Gal), 68.5 (C-4 Gal), 60.8 (C-6 Gal), 55.6 (CHNHP), 51.8 (d, $^2J_{C,P}$ = 6 Hz, OCH_3), 39.5 (NHCH_2), 31.9 (*C*H_2CH_2-CH_3), 29.8, 29.7, 29.4, and 28.9 [(CH_2)$_8$], 26.9 (NHCH_2*C*H_2), 22.5 (*C*H_2CH$_3$), 13.8 (CH_2*C*H_3). ^{31}P {^{1}H} NMR (D_2O): δ 8.90 (s). Anal. Calc. for $C_{24}H_{48}N_2O_{10}NaP\cdot 5H_2O$ (668.69): C, 43.11; H, 8.74; N, 4.19; P, 4.63. Found: C, 42.95; H, 8.04; N, 4.17; P, 4.14.

Synthesis of II-GalAE[F8C7][C11CO2Na] (Scheme 3). Methyl *N*-2-Hydroxyethyl-*N*-[7-(*F*-octyl)heptyl]-11-carboxamidoundecanoate, 16. Procedure **C** when applied to 1.37 g (7.14 mmol) of EDC, 3.94 g (7.19 mmol) of 7-(perfluorooctyl)heptanoic acid, 1.34 g (7.16 mmol) of 2-benzyloxyethylamine hydrochloride, 1 mL of Et_3N (7.2 mmol), and 0.96 g (7.12 mmol) of HOBt afforded after chromatography (49:1 $CHCl_3$−MeOH) 4.8 g (95%) of *N*-(2-benzyloxy)-7-(*F*-octyl)-heptanamide as an oil [$R_f = 0.76$ (4:1 $CHCl_3$−MeOH). ^{1}H NMR ($CDCl_3$): δ 7.33 (m, 5H, Ph), 4.90 (bs, 1H, NH), 4.52 (s, 2H, OCH_2Ph), 3.54 (m, 2H, CH_2OBn), 3.49 (t, J = 5.4 Hz, 2H, NHCH_2), 2.17 [t, J = 7.1 Hz, 2H, CH_2C(O)], 2.04 (tt, $J_{H,F}$ = 18.8 Hz, J = 8.2 Hz, 2H, CH_2CF_2), 1.70−1.60 [m, 4H, CH_2-CH_2CF_2 and CH_2CH$_2$C(O)], 1.38−1.30 [m, 4H, (CH_2)$_2$]. ^{13}C NMR ($CDCl_3$): δ 173.0 [C(O)NH], 138.0 (C, Ph), 128.6 and 128.0 (CH, ortho and meta Ph), 127.9 (CH, para Ph), 73.3 (OCH_2Ph), 69.1 (*C*H_2OBn), 39.4 (CH$_2$NH), 36.6 [*C*H$_2$C(O)], 30.9 (t, $^2J_{C,F}$ = 22.2 Hz, *C*H_2CF_2), 28.9 [(CH_2)$_2$], 25.5 [CH_2*C*H$_2$C-(O)], 20.1 (*C*H_2CH$_2CF_2$). ^{19}F NMR ($CDCl_3$): identical to that of **16**, see below]. Four portions of 1.02 g of $LiAlH_4$ were added over 2 h to 4.15 g (6.1 mmol) of this amide in 50 mL of anhydrous THF; the suspension was then stirred at room temperature for 24 h. After hydrolysis with a 5:1 THF−H_2O solution, then 15% NaOH, and water, the mixture was filtered on Celite, and the solvents were evaporated. The crude product diluted with $CHCl_3$ was washed with water until neutrality and precipitated as its HCl salt. Filtration, washing with water and then ether, and drying afforded 3.4 g of *N*-[2-(benzyloxy)-ethyl]-7-(*F*-octyl)heptylamine (80%) [R_f = 0.72 (4:1 $CHCl_3$−

MeOH). ^{1}H NMR ($CDCl_3$−CD_3OD): δ 7.27 (m, 5H, Ph), 4.49 (s, 2H, OCH_2Ph), 3.74 (t, J = 5.2 Hz, 2H, CH_2OBn), 3.09 (m, 2H, CH_2CH$_2$OBn), 2.88 (t, J = 7.1 Hz, 2H, CH_2NH$_2^+$), 1.97 (tt, $J_{H,F}$ = 18.8, J = 8.2 Hz, 2H, CH$_2$CF$_2$), 1.69 (m, 2H, CH_2-CH$_2$NH$_2^+$), 1.51 (m, 2H, CH_2CH$_2$CF$_2$), 1.28 [m, 6H, (CH$_2$)$_3$]. ^{13}C NMR ($CDCl_3$−CD_3OD): δ 138.0 (C, Ph), 129.5 and 129.2 (CH, ortho and meta Ph), 129.0 (CH, para Ph), 74.5 (OCH$_2$-Ph), 65.8 (CH$_2$OBn), 48.7 (CH$_2$CH$_2$OBn), 48.0 (CH$_2$NH$_2^+$), 31.7 (t, $J_{C,F}$ = 22.2 Hz, CH$_2$CF$_2$), 29.8 and 29.6 [(CH$_2$)$_3$], 27.3 (CH$_2$-CH$_2$CH$_2$NH$_2^+$), 26.7 (CH$_2$CH$_2$NH$_2^+$), 21.0 (CH$_2$CH$_2$CF$_2$). ^{19}F NMR ($CDCl_3$): identical to that of **16**, see below]. PyBrop (1.4 g, 3.0 mmol) was added at 0 °C to a solution of 20 mL of CH$_2$-Cl$_2$ containing 0.73 g (3.0 mmol) of dodecanedioic acid monomethyl ester, 2.1 g (3.0 mmol) of this amine, and 1.4 mL (10 mmol) of Et$_3$N. The reaction mixture was stirred at 0 °C for 1 h and then at room temperature for 1 day. Workup, as described in procedure **C**, gave after chromatography ($CHCl_3$) 2.1 g (78%) of methyl *N*-[2-(benzyloxy)ethyl]-*N*-[7-(*F*-octyl)-heptyl]-11-carboxamidoundecanoate as a colorless oil [R_f = 0.30 ($CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.28 (m, 5H, Ph), 4.50 and 4.48 (2s, 2H, OCH$_2$Ph), 3.64 (s, 3H, CH$_3$O), 3.53 [m, 4H, CH$_2$-OBn and CH$_2$CH$_2$CH$_2$N), 3.31 (t, J = 7.6 Hz, 2H, CH$_2$CH$_2$-OBn), 2.31 [m, 4H, CH$_2$C(O)OMe and NC(O)CH$_2$], 1.98 (tt, $J_{H,F}$ = 18.8, J = 8.2 Hz, 2 H, CH$_2$CF$_2$), 1.59 [m, 8H, CH$_2$CH$_2$CF$_2$, NC(O)CH$_2$CH$_2$, CH$_2$CH$_2$C(O)OMe and CH$_2$CH$_2$CH$_2$N], 1.26 [m, 18H, (CH$_2$)$_3$ and (CH$_2$)$_6$]. ^{13}C NMR ($CDCl_3$): δ 173.4, 173.3, and 173.1 [C(O)OMe and C(O)N], 138.4 and 137.9 (C, Ph), 128.5 and 128.4 (CH, ortho and meta Ph), 127.8 and 127.6 (CH, para Ph), 73.5 and 73.2 (OCH$_2$Ph), 69.0 and 68.4 (CH$_2$OBn), 51.4 (OCH$_3$), 49.4 and 46.3 (CH$_2$CH$_2$OBn), 47.6 and 46.2 (CH$_2$-CH$_2$$CH_2$N), 34.1 [$CH_2$C(O)OMe], 33.2 and 33.1 [NC(O)CH$_2$], 30.9 (t, $J_{C,F}$ = 22.2 Hz, CH$_2$CF$_2$), 29.5, 29.3, 29.2, and 29.1 [(CH$_2$)$_2$ and (CH$_2$)$_6$], 27.6 (CH$_2$CH$_2$CH$_2$N), 26.8 and 26.7 (CH$_2$$CH_2CH_2$N), 25.5 [$CH_2CH_2$C(O)OMe], 25.0 [NC(O)CH$_2$$CH_2$], 20.1 ($CH_2CH_2CF_2$). The doubling of some signals indicates the presence of two conformational isomers due to the amide bond. ^{19}F NMR ($CDCl_3$): identical to that of **16**, see below]. Hydrogen was bubbled for 5 h at room temperature through a solution of 1.97 g (2.20 mmol) of this methyl ester derivative in MeOH (15 mL) and AcOH (2 mL) containing 0.2 g of Pd/C (10% w/w). The mixture was diluted with $CHCl_3$ and filtered through Celite. The organic phase was washed with water, dried over Na_2SO_4, filtered, and evaporated to dryness under vacuo affording 1.5 g (85%) of **16** as a white solid. R_f = 0.30 ($CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 3.74 (t, J = 4.6 Hz, 2H, CH_2OH), 3.64 (s, 3H, CH$_3$O), 3.50 (t, J = 4.7 Hz, 2H, CH_2CH$_2$OH), 3.26 (t, J = 7.5 Hz, 2H, CH$_2$CH$_2$CH_2N), 2.32 [m, 4H, CH_2C(O)OMe and NC(O)CH$_2$], 2.04 (tt, $J_{H,F}$ = 18.8 Hz, J = 8.2 Hz, 2 H, CH$_2$-CF$_2$), 1.59 [m, 8H, CH_2CH$_2$CF$_2$, NC(O)CH$_2$CH_2, CH_2CH$_2$C(O)-OMe, and CH$_2$CH_2CH$_2$N], 1.35−1.20 [m, 18H, (CH$_2$)$_3$ and (CH$_2$)$_6$]. ^{13}C NMR ($CDCl_3$): δ 175.5, 174.4, and 173.7 [C(O)-OMe and C(O)N], 62.9 and 60.6 (CH$_2$OH), 51.5 (OCH$_3$), 50.2 and 46.1 (CH$_2$CH$_2$OH), 49.8 and 46.1 (CH$_2$CH$_2$$CH_2$N), 34.2 [$CH_2$C(O)OMe], 33.4 and 33.2 [NC(O)CH$_2$], 30.9 (t, $^2J_{C,F}$ = 22.2 Hz, CH$_2$CF$_2$), 29.5, 29.3, 29.2, and 29.1 [(CH$_2$)$_2$ and (CH$_2$)$_6$], 27.6 (CH$_2$CH$_2$CH$_2$N), 26.8 and 26.7 (CH$_2$CH$_2$N), 25.4 [CH$_2$-CH$_2$C(O)OMe], 25.0 [NC(O)CH$_2$$CH_2$], 20.1 ($CH_2CH_2CF_2$). The doubling of some signals indicates the presence of two conformational isomers due to the amide bond. ^{19}F NMR ($CDCl_3$): δ −81.3 (3F, CF$_3$), −115.0 (2F, CF$_2$CH$_2$), −122.4 [6F, (CF$_2$)$_3$], −123.3 (2F, CF$_2$ γ CH$_2$), −124.1 (2F, CF$_2$ δ CH$_2$), −126.7 (2F, CF_2CF$_3$).

Methyl *N*-[2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyloxy)ethyl]-*N*-[7-(*F*-octyl)heptyl]-11-carboxamidoundecanoate, 17. Method D. A solution of 50 μL (0.26 mmol) of TMSOTf in 5 mL of $CHCl_3$ was added dropwise at −30 °C to a solution of 0.6 g (0.747 mmol) of **16** and 0.45 g (0.91 mmol) of GalOC(=NH)CCl$_3$ in $CHCl_3$ (20 mL) and containing 4 g of molecular sieves (4 Å). The resulting mixture was stirred at −30 °C for 6 h, then diluted with $CHCl_3$, filtered through Celite, and washed with a 8% $NaHCO_3$ and then with water until neutrality. The organic phase was dried over Na_2SO_4, filtered, and evaporated in vacuo. Purification by silica gel chromatography ($CHCl_3$) gave 0.24 g (35%) of **17** as an oil.

Method E. The procedure described for the preparation of the compound **A** when applied to 0.30 g (0.37 mmol) of **16**, 0.19 g (0.48 mmol) of 1,2,3,4,6-penta-*O*-acetyl-D-galactopyranose, and 150 μL of BF_3·Et$_2$O (1.22 mmol) gave after chromatography ($CHCl_3$) 90 mg (25%) of **17** as an oily compound. R_f = 0.54 (3:1 CH_2Cl_2−Et$_2$O). ^{1}H NMR ($CDCl_3$): δ 5.36 (bd, J = 2.5 Hz, 1H, H-4 Gal), 5.13 (dd, J = 7.7 Hz, J = 10.4 Hz, 1H, H-2 Gal), 4.99 (dd, J = 10.3 Hz, J = 3.4 Hz, 1H, H-3 Gal), 4.44 (d, J = 7.7 Hz, 1H, H-1β Gal), 4.11 (m, 2H, H-6,6′ Gal), 3.91 (m, 2H, CH_2OGal), 3.70 (m, 1H, H-5 Gal), 3.63 (s, 3H, OCH$_3$), 3.48 (m, 2H, NCH_2CH$_2$O), 3.28 (m, 2H, CH$_2$CH$_2$CH_2N), 2.28 [m, 4H, CH_2C(O)OMe and NC(O)CH$_2$], 2.11, 2.01, and 1.94 [s,s,s, 12H, $_{CH}$3C(O)], 1.92 (tt, JH,F = *1*8.8 Hz, J = 8.2 Hz, 2$_{H,\ C}$H2CF2), 1.$_{58}$ [m, 8H· CH2CH2CF2, NC(O)CH2CH2, CH2CH2C(O)OMe, and CH2CH2CH2N], 1.27 [m, 18H, (CH2)3 and (CH2)6]. 13C NMR (CDCl3): δ 174.1, 173.1, and 173.0 [C(O)OMe and C(O)N], 170.1, 170.0, 169.9, and 169.1 [CH$_3$$C$(O)], 101.5 and 101.3 (C-1β Gal), 70.9 and 70.8 (C-3,5 Gal), 69.0 and 68.6 (C-2 Gal), 68.5 and 67.7 (GalOCH$_2$), 67.2 and 67.1 (C-4 Gal), 61.2 (C-6 Gal), 51.2 (OCH$_3$), 49.3 and 47.1 (NCH$_2$CH$_2$O), 49.0 and 45.8 [CH$_2$CH$_2$$CH_2$N], 34.0 [$CH_2$C(O)OMe], 33.2 and 33.0 [NC-(O)CH$_2$], 30.9 (t, $^2J_{C,F}$ = 22.2 Hz, CH$_2$CF$_2$), 29.5, 29.4, 29.3, 29.2, 29.1, 29.0, and 28.9 [(CH$_2$)$_2$ and (CH$_2$)$_6$], 27.5 (CH$_2$CH$_2$-CH$_2$N), 26.7 and 26.6 (CH$_2$$CH_2CH_2$N), 25.4 [$CH_2CH_2$C(O)-OMe], 24.9 [NC(O)CH$_2$$CH_2$], 20.6, 20.5, 20.4, 20.3, and 20.0 [CH$_3$C(O)], 20.1 (CH$_2$CH$_2$CF$_2$). The doubling of some signals indicates the presence of conformational isomers due to the amide bond. Correlated ^{1}H/^{13}C COSY experience confirmed ^{1}H and ^{13}C signal attributions. ^{19}F NMR ($CDCl_3$): identical to that of **16**.

Methyl *N*-[2-(β-D-galactopyranosyloxy)ethyl]-*N*-[7-(*F*-octyl)heptyl]-11-carboxamidoundecanoate, 18. The procedure **A** when applied to 200 mg (0.17 mmol) of **17** gave 140 mg (80%) of **18**. R_f = 0.32 (9:1 $CHCl_3$−MeOH). ^{1}H NMR ($CDCl_3$): δ 5.10−3.00 (m, 16H, H-1−6 Gal, CH_2CH_2OGal, OCH$_3$, and CH$_2$CH$_2$CH_2N), 2.21 [m, 4H, CH_2C(O)OMe and NC-(O)CH$_2$], 1.97 (tt, $J_{H,F}$ = 18.8 Hz, J = 8.2 Hz, 2H, CH$_2$CF$_2$), 1.51 [m, 8H, CH_2CH$_2$CF$_2$, NC(O)CH$_2$CH_2, CH_2CH$_2$C(O)OMe, and CH$_2$CH_2CH$_2$N], 1.19 [m, 18H, (CH$_2$)$_3$ and (CH$_2$)$_6$]. ^{13}C NMR (CD_3OD-$CDCl_3$): δ 174.7 and 174.3 [C(O)OMe and C(O)N], 103.6 (C-1β Gal), 74.9 (C-5 Gal), 73.5 (C-3 Gal), 71.0 (C-2 Gal), 68.7 (C-4 Gal), 66.9 (GalOCH$_2$), 61.0 (C-6 Gal), 50.9 (OCH$_3$), 48.9 (NCH$_2$CH$_2$O), 45.7 and 45.6 (CH$_2$CH$_2$$CH_2$N), 33.6 [$CH_2$C-(O)OMe], 33.0 and 32.7 [NC(O)CH$_2$], 30.5 (t, $J_{C,F}$ = 22.2 Hz, CH$_2$CF$_2$), 29.1, 28.9, 28.8, and 28.6 [(CH$_2$)$_2$ and (CH$_2$)$_6$], 27.0 (CH$_2$CH$_2$CH$_2$N), 26.4 and 26.3 (CH$_2$$CH_2CH_2$N), 25.4 [CH$_2$-CH$_2$C(O)OMe], 25.2 and 24.6 [NC(O)CH$_2$$CH_2$], 19.8 ($CH_2CH_2$-CF$_2$). The doubling of some signals indicates the presence of two conformational isomers due to the amide bond. ^{19}F NMR ($CDCl_3$): identical to that of **16**.

***N*-[2-(β-D-Galactopyranosyloxy)ethyl]-*N*-[7-(*F*-octyl)-heptyl]-11-carboxamidoundecanoate (Sodium Salt), II-GalAE[F8C7][C11CO2Na].** One hundred milligrams (0.10 mmol) of **18** were stirred with 1 mL of 0.3 N NaOH 1:9 MeOH−H_2O, at room temperature for 5 days. The reaction mixture was purified by chromatography (49:1 to 4:1 $CHCl_3$−MeOH) affording 90 mg (90%) of **II-GalAE[F8C7][C11CO2Na]** as a white solid. R_f = 0.28 (9:1 $CHCl_3$−MeOH). [α]$_D$ = +2.9° (*c* 0.35; MeOH). SM (ESI) *m*/*z* = 950.5 (M−Na)$^-$. ^{1}H NMR (CD_3OD): δ 4.22−3.28 (m, 13 H, H-1−6 Gal, CH_2CH_2OGal and CH$_2$-CH$_2$CH_2N), 2.41 [t, ^3J = 7.2 Hz, 2H, CH$_2$C(O)ONa], 2.24 [t, J = 7.3 Hz, 2H, NC(O)CH$_2$], 2.10 (tt, $J_{H,F}$ = 18.8 Hz, J = 8.2 Hz, 2H, CH$_2$CF$_2$), 1.56 [m, 8H, CH_2CH$_2$CF$_2$, NC(O)CH$_2$CH_2, CH_2-CH$_2$C(O)OMe, and CH$_2$CH_2CH$_2$N], 1.29 [m, 18H, (CH$_2$)$_3$ and (CH$_2$)$_6$]. ^{13}C NMR (CD_3OD): δ 177.6 [C(O)O], 176.2 and 175.8 [C(O)N], 105.3 (C-1β Gal), 76.7 and 76.6 (C-5 Gal), 75.0 and 74.9 (C-3 Gal), 72.4 and 72.3 (C-2 Gal), 70.3 and 70.2 (C-4 Gal), 68.5 (GalOCH$_2$), 62.5 and 62.4 (C-6 Gal), 50.2 and 47,0 (NCH$_2$-CH$_2$O), 48.8 (CH$_2$CH$_2$$CH_2$N), 34.9 and 34.8 [$CH_2$C(O)O], 34.2 and 33.9 [NC(O)CH$_2$], 31.6 (t, $J_{C,F}$ = 22.2 Hz, CH$_2$CF$_2$), 30.5, 30.4, 30.3, 30.2, 30.1, 30.0, and 29.9 [(CH$_2$)$_2$ et (CH$_2$)$_6$], 28.3 (CH$_2$CH$_2$CH$_2$N), 27.7 and 27.5 (CH$_2$$CH_2CH_2$N), 26.7 and 26.6 [CH$_2CH_2$C(O)OMe], 26.0 and 25.9 [NC(O)CH$_2$CH$_2$], 21.2 (CH$_2$-CH$_2$CF$_2$). This assignment was confirmed by DEPT ^{13}C NMR.

The doubling of some signals indicates the presence of two conformational isomers due to the amide bond. ^{19}F NMR ($CDCl_3$): identical to that of **16**. Anal. Calc. for $C_{35}H_{49}F_{17}NO_9$Na (973.736): C, 43.10; H, 5.70; N, 1.44. Found: C, 43.16; H, 5.68; N, 1.54.

Synthesis of Monolac and BisLac (Scheme 4). Synthesis of *N*-{2-[2,3-(Ditetradecanamido)propionamido]ethyl}-lactobionamide, Monolac. One hundred milligrams (0.17 mmol) of **20**[48] and 65 mg (0.17 mmol) of lactobionic acid **19** in 6 mL of MeOH were refluxed for 24 h. After filtration, the precipate was washed twice with MeOH, dried, and purified by chromatography (90:10:1 to 70:30:1 $CHCl_3$−MeOH−H_2O). One hundred milligrams (60%) of **monoLac** as a white solid were obtained. R_f = 0.70 (70:30:3 $CHCl_3$−MeOH−H_2O). SM (ESI) m/z = 929.8 $(M + Na)^+$. ^{1}H NMR (CD_3OD-DMSO-d_6): δ 4.30 (m, 2H, H-2 and H-1β Gal), 3.80−3.10 [m, 14H, H-1,5−9 and H-2−6 Gal], 3.13 (m, 4H, H-3,4), 2.10 [m, 4H, $CH_2(CH_2)_{11}CH_3$], 1.60−1.45 [m, 4H, $CH_2CH_2(CH_2)_{10}CH_3$], 1.23 [m, 40H, $(CH_2)_{10}CH_3$], 0.85 (t, J = 6.2 Hz, 6H, CH_3). ^{13}C NMR (DMSO-d_6): δ 172.9, 172.6, 172.3, and 170.0 [C(O)NH], 104.5 (C-1β Gal), 82.8 (C-7), 75.6 (C-5 Gal), 73.2 (C-3 Gal), 71.1 (C-2 Gal), 70.3 (C-4 Gal), 72.1, 71.3, and 68.2 (C-5,6,8), 62.3 (C-9), 60.6 (C-6 Gal), 53.1 (C-2), 40.9, 38.1, and 37.7 (C-1,3,4), 35.3 [NHC(O)$CH_2(CH_2)_{11}CH_3$], 31.2 ($CH_2CH_2CH_3$), 29.0, 28.9, 28.8, and 28.7 [$(CH_2)_8$], 25.2 and 25.0 [NHC(O)$CH_2CH_2(CH_2)_{10}CH_3$], 22.0 (CH_2CH_3), 13.8 (CH_3). Anal. Calc. for $C_{45}H_{86}N_4O_{14}\cdot 3/2H_2O$ (934.22): C, 57.86; H, 9.44; N, 6.00. Found: C, 57.83; H, 9.52; N, 6.14.

Synthesis of *N*-{[2-(Lactobionamido)ethyl]-*N*-{2-[2,3-(ditetradecanamido)propionamido]ethyl}lactobionamide, Bislac. Fifty milligrams (0.076 mmol) of **21**[48] and 60 mg (0.16 mmol) of lactobionic acid in 6 mL of MeOH were refluxed for 24 h. After evaporation, the residue was chromatographed (40:25:4 $CHCl_3$−MeOH−H_2O), and the product obtained was washed with Et_2O and lyophilized giving 50 mg (50%) of **bisLac** as a white solid. R_f = 0.70 (8:5:1 $CHCl_3$−MeOH−H_2O). $[\alpha]_D$ = +7.6° (c 0.26; MeOH). SM (ESI) m/z = 1353.5 $(M + Na)^+$. ^{1}H NMR (CD_3OD): δ 4.60 (m, 1H, H-2), 4.50 (d, J = 7.0 Hz, 2H, H-1β Gal), 4,38 (m, 2H, H-4 Gal), 4.26 (m, 2H, H-3 Gal), 4.00−4.85 (m, 4H, H-2,5 Gal), 3.81−3.15 (m, 24 H, H-1,3,6−18 and H-5,6,6′ Gal), 2.61 (m, 6H, H-4,5,12), 2.19 [m, 4H, $CH_2(CH_2)_{11}CH_3$], 1.65−1.50 [m, 4H, $CH_2CH_2(CH_2)_{10}CH_3$], 1.28 [m, 40 H, $(CH_2)_{10}CH_3$], 0.89 (t, J = 6.2 Hz, 6 H, CH_3). ^{13}C NMR ($CDCl_3$−CD_3OD): δ 177.0, 176.2, 174.9, 174.8, and 172.0 [C(O)NH], 105.5 (C-1β Gal), 83.5 and 83.4 (C-7,14), 77.0 (C-5 Gal), 74.6 (C-3 Gal), 72.9 (C-2 Gal), 72.6 (C-4 Gal), 73.9, 72.3 and 70.2 (C-8−10,15−17), 63.6 (C-11,18), 62.6 (C-6 Gal), 54.8 (C-4,5,12), 54.2 (C-2), 42.0 (C-1), 39.1 and 38.6 (C-3,6,13), 37.1 [NHC(O)$CH_2(CH_2)_{11}CH_3$], 32.9 ($CH_2CH_2CH_3$), 30.8, 30.7, 30.6, 30.5, and 30.4 [$(CH_2)_8$], 26.9 and 26.7 [NHC(O)$CH_2CH_2(CH_2)_{10}CH_3$], 23.6 (CH_2CH_3), 14.4 (CH_3). Anal. Calc. for $C_{61}H_{114}N_6O_{25}\cdot 5H_2O$ (1421.67): C, 51.54; H, 8.79; N, 5.91. Found: C, 51.57; H, 8.76; N, 5.67.

Surface-Pressure Measurements: **gp120 Binding to Glycolipid Monolayers at the Air−Water Interface.** The surface pressure was measured with the μT trough S microtensiometer (Kibron Inc., Helsinki, Finland), and the data were collected and analyzed by the FilmWare 2.3 software. The apparatus was maintained at a temperature of 25 °C throughout the experiments. Pure lipids were prepared at a concentration of 1 mg/mL in 11:5:4 (v:v:v) hexane:chloroform:methanol, as previously described.[49] The lipid was carefully spread onto the surface of the trough filled with 800 μL of pure water obtained by filtration through a milli-Q water purification system (Millipore, Saint-Quentin, France). The amount of lipid was calculated in order to form a stable monomolecular film with a surface pressure of 10 mN/m, as previously determined by compression isotherms experiments. At least 5 min were allowed for the spreading solvent to evaporate and for the lipid molecules to distribute themselves throughout the film at the air−water interface. The stability of the monolayer was then checked by recording the pressure variations $\Delta\pi$ for 10 min (the monolayer can be considered as stable when $\Delta\pi$ is lower than 0.2 mN/m over this period), before addition of 10 nM recombinant gp120 (rgp120, HIV-1-IIIB isolate) into the subphase. The maximal increase in surface pressure ($\Delta\pi$max) was determined after the equilibrium was reached (60−120 min, depending on the extent of pressure variation). All experiments were carried out in triplicate, and each experiment was performed with a freshly prepared film.

Biological Section

Sample Preparation. All the galactolipids were assayed in biological tests as solution in Dubelcco modified phosphate buffered saline (DPBS). **II-GalAE-[F8C7][C11CO2Na]** was also tested formulated as PL/CH/**II-GalAE[F8C7][C11CO2Na]** (2:1:0.15 molar ratio; PL = soya phospholipon; CH = cholesterol) liposomes (higher amounts of galactolipid led to liposomes that aggregate very rapidly). These liposomes were prepared according to the procedure described in ref 23. Average particle sizes and size distributions were measured by laser light scattering on a Coulter N4MD submicron particle analyzer after preparation of the liposomes and then periodically during storage at room temperature (the particle sizes reported in Table 1 correspond to a storage period of one week). The formulation was tested within 1 week after preparation. Its phospholipid and galactolipid concentration was determined as described elsewhere.[23] Galactose determination in the preparation was also done by ^{19}F NMR using CF_3CH_2OH as internal standard. These determinations were consistent with the theoritical values.

Cells and Viruses. CEM-SS and MT4 cells were cultured in RPMI medium supplemented with 10% fetal calf serum. HIV-1 LAI and HIV IIIB were produced in CEM-SS cells as previously reported.[37]

Inhibition of HIV-1 Infection. CEM-SS Cells. CEM-SS cells were infected with HIV-1 LAI, as described elsewhere.[37] Five 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 formulation were added to the cell cultures after viral adsorption. RT inhibition percent was measured in comparison with the nontreated cells. The galactoside 50% inhibitory concentration of virus replication values (IC_{50}) reported in Table 1 were determined from the computer-generated median plot of the dose−effect data. AZT was used as a positive control (IC_{50} = 4 nM). The effects of the galactosides on CEM-SS cell proliferation and viability were measured on noninfected cells using a colorimetric assay (MTT test which is based on the capacity of living cells to reduce MTT to formazan.)[38] with various concentration of the tested product as described elsewhere. The 50% cytotoxicity concentration (CC_{50}) at which OD_{540} was reduced by one-half was calculated using the program mentioned above. Three measurements were carried out for each concentration of each compound on infected and noninfected cells. The entire experiment was repeated twice.

MT4 Cells. The anti-HIV-1 test based on MT4 infected cells was performed as described elsewhere.[38,39] Briefly, the replication of HIV-1 (IIIB) was followed by the cytopathogenic effect induced by the virus. MT4 cells were infected with a virus dose allowing 5 days later the death of 90% of the cells. The tested compounds were added in the cell culture medium after viral

adsorption. Cell viability was measured by the colorimetric MTT test. The drug IC50 values were determined from the curves of the protection percentage Δ against drug concentration. The effects of the galactosides on MT4 cell proliferation and viability were also measured on noninfected cells using the colorimetric MTT test after 5 days of incubation at 37 °C with various concentrations of the tested product. All the measurements were carried out in triplicate for each concentration of each compound, and the entire experiment was repeated twice.

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