Synthetic bolaamphiphilic analogs of galactosylceramide (GalCer) potentially binding to the V3 domain of HIV-1 gp 120: key role of their hydrophobicity

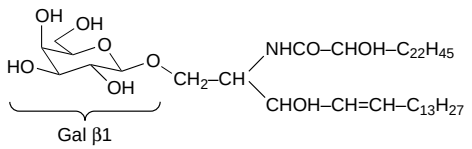
Isabelle Rico–Lattes,* Marie-Françoise Gouzy, Christiane André–Barrès, Brigitte Guidetti and Armand Lattes

Laboratoire des IMRCP (CNRS UMR 5623), Université Paul Sabatier, 118 route de Narbonne, 31062 Toulouse Cedex, France

New routes in three or four steps to synthetic bolaamphiphilic analogs of galactosylceramide (GalCer) are presented in this work, using unprotected lactose as the starting sugar. Some of these new anionic compounds obtained in good yields bind to the V3 domain of HIV-1 gp 120 and thus are the base of potential low-cost anti HIV-1 drugs. Moreover, structure-activity relationships show that the hydrophobic moiety of the molecule plays a key role in this activity.

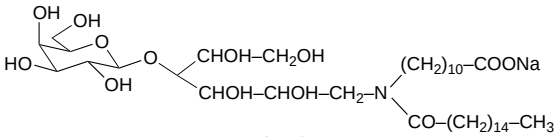
Synthèse de bolaamphiphiles analogues du galactosylceramide (GalCer) pouvant interagir avec la boucle V3 de la gp 120 du VIH-1: rôle clé de leur hydrophobie. De nouveaux composés bolaformes analogues du galactosylcéramide (GalCer) sont présentés dans ce travail. Leur synthèse en trois ou quatre étapes met en jeu le lactose non-protégé comme sucre de départ. Certains de ces nouveaux composés se lient à la boucle V3 de la gp 120 du VIH-1 et constituent donc une base pour obtenir de nouveaux agents anti VIH à faible coût. En outre, les relations structure-activité montrent que la partie hydrophobe de la molécule joue un rôle clé dans cette activité.

Galactosylceramide (GalCer), whose chemical structure is shown below, is an alternative receptor allowing HIV-1 entry into CD4 negative cells of neural and colonic origin.[1,2] Several lines of evidence suggest that this glycosphingolipid recognizes the V3 loop of the HIV-1 surface envelope glycoprotein gp 120.[3] This V3 domain is the major immunodominant epitope for the generation of neutralizing antibodies[4] and is essential for virus activity and tropism.[5,6]



Due to the high variability of the V3 loop sequence, the neutralizing activity of anti-V3 antibodies is generally restricted to one or, in the best cases, to a few related HIV-1 isolates, which renders vaccine development particularly difficult.[7] However, since the V3 domain of most HIV-1 strains contains several well-conserved basic amino acid residues,[8] it can bind to a wide variety of anionic compounds, such as Heparin and Suramin, which are efficient inhibitors of HIV-1 infection *in vitro*.[9–11] On the other hand, the V3 loop also plays a key role in the fusion process, probably by interacting with secondary receptors.[12,13]

Taking in account all these data, we decided to synthesize bolaamphiphilic analogs of GalCer with the aim to develop a new class of anti HIV-1 agents that could neutralize HIV-1 infection through masking of the V3 loop. Therefore, in a recent paper, we presented succinctly the synthesis of a new molecule of this type, which is a branched dissymmetric bolaamphiphile with one head derived from lactose and the other an anionic one: a carboxylate. This soluble analog of GalCer, previously named CA52[14] and whose structure is shown below, was shown strongly to inhibit the binding of ^{3}H-Suramin to SPC3, a V3-loop-derived synthetic peptide, with a 50% inhibitory concentration, IC50, of 0.7 μM. It also inhibits ^{3}H-Suramin binding to recombinant gp 120 in a similar manner (IC50 of 2.4 μM). Moreover, the mapping of the binding site on gp 120 showed that the region recognized by CA52 corresponds to the highly conserved consensus motif GPGRAF of the V3 loop. *This recent study also suggests that the hydrophobic moiety of the molecule plays an important role in its activity*. Indeed, the analog of CA52 with only seven methylene groups in the lateral alkyl chain was inactive and the analog with 10 methylene groups presented a weak activity (IC50 = 9.4 μM in the screening based on the interaction between SPC3 and ^{3}H-Suramin).[14]



CA52

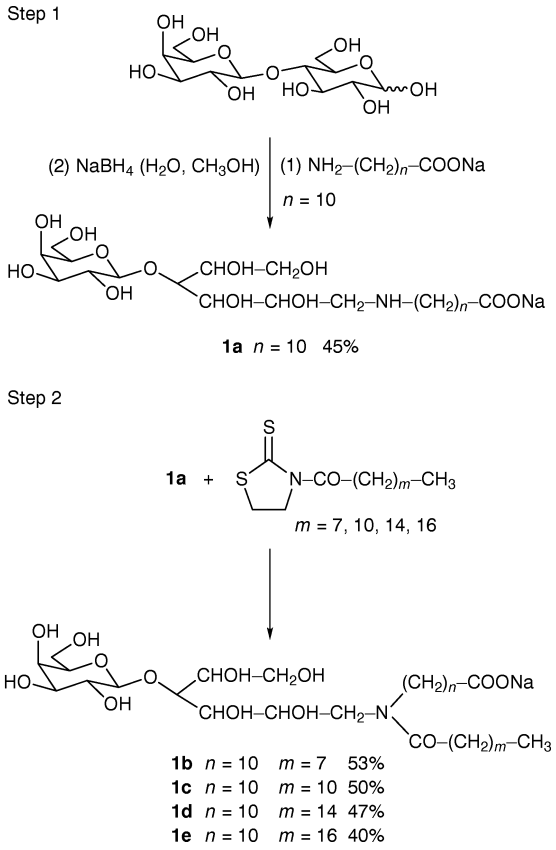
On the other hand, Wong and colleagues very recently developed new synthetic bolaamphiphilic molecules as analogs of Sialyl Lewis X tetrasaccharide, showing remarkable enhancement in selective inhibition by an additional negative charge and with long-chain hydrophobic groups.[15]

All these results prompted us to present in detail the synthesis of the bolaamphiphilic galactosylceramide analogs **1**, similar to CA52, to discuss their biological activity and therefore to propose a new type of bolaamphiphilic analog of GalCer, potentially more active than the first generation.

Results and Discussion

Synthesis of the bolaamphiphilic GalCer analogs 1, similar to CA52

The new analogs of GalCer developed in this study are obtained in only three steps from lactose. The synthetic scheme is illustrated in Scheme 1. The feature of note in this



Scheme 1 Synthetic scheme for *N*-[10-(sodium oxycarbonyl)decyl]-*N*-acyl-1-amino-1-deoxylactitols **1**

synthetic scheme is the use of unprotected lactose as the starting sugar, a low-cost disaccharide consisting of galactose and glucose units linked by a 1–4 junction in the β configuration. Thus, opening the glucose by reductive amination in the first two stages of the synthesis leads to the GalCer unit.[16] After chromatographic purification, the lateral alkyl chain is added *via* acylation of the secondary amine using *N*-acyl-thiazolidine-2-thiones.[16] A series of molecules of this type was synthesized with a varying number *m* of methylene groups in the lateral hydrophobic chain (Scheme 1).

Biological evaluation of GalCer analogs 1 in the ^{3}H-Suramin solid phase assay

As indicated for CA52 in the introduction, these analogs are screened in an assay based on the interaction between a V3-loop-derived synthetic peptide and ^{3}H-Suramin, which displays high affinity for the V3 loop. Therefore, according to precedent work on CA52, these analogs have been evaluated for their ability to inhibit the binding of ^{3}H-Suramin to SPC3, a synthetic peptide displaying eight V3 consensus motifs (GPGRAF), radially branched on an uncharged poly-lys core matrix.[14] This screening assay proved useful to select those analogs that recognized the V3 loop, and a good correlation was found between the anti HIV-1 activity of a given analog and its affinity for the V3 loop.

Table 1 summarizes the biological activity of the GalCer analogs **1**. The following comments can be made about these results: (*i*) Compounds with an insufficient hydrophobic lateral chain are inactive: **1a** without a lateral chain and **1b** with a too-short chain. (*ii*) In contrast, biological activity is optimum with a 14-methylene group lateral chain (IC50 = 0.7 μM). *The interaction between galactose and the V3 loop is specific but with a weak affinity. Thus, the hydrophobic long chain is necessary to interact with the hydrophobic portion of the V3 loop and therefore to enhance binding. In contrast with the sugar–protein interaction, these hydrophobic interactions are not specific but develop a high affinity.* (*iii*) Finally, when the hydrophobic chain is longer (16 methylene groups), the activity is slightly lower (IC50 = 1.2 μM), showing that an optimal hydrophobicity exists for this type of compound.

Table 1 Evaluation of *N*-[10-(sodium oxycarbonyl)decyl]-*N*-acyl-1-amino-1-deoxylactitols **1** in the ^{3}H-Suramin solid phase assay

Compound	*n*	*m*	IC50/μM
1a	10	—	>100
1b	10	7	>70
1c	10	10	9.4
1d (CA52)	10	14	0.7
1e	10	16	1.2

These results show that hydrophobic interactions play a key role in the recognition processes between proteins and ligand mimics. It is noteworthy that our results are in agreement with the recent and independent work of Wong *et al.* on mimics of Sialyl Lewis X tetrasaccharide.[15] Therefore, these data taken together show that hydrophobic interactions are of fundamental importance for the development of small molecule inhibitors of different types (antivirals, antibacterials, antiinflammatories, *etc.*).

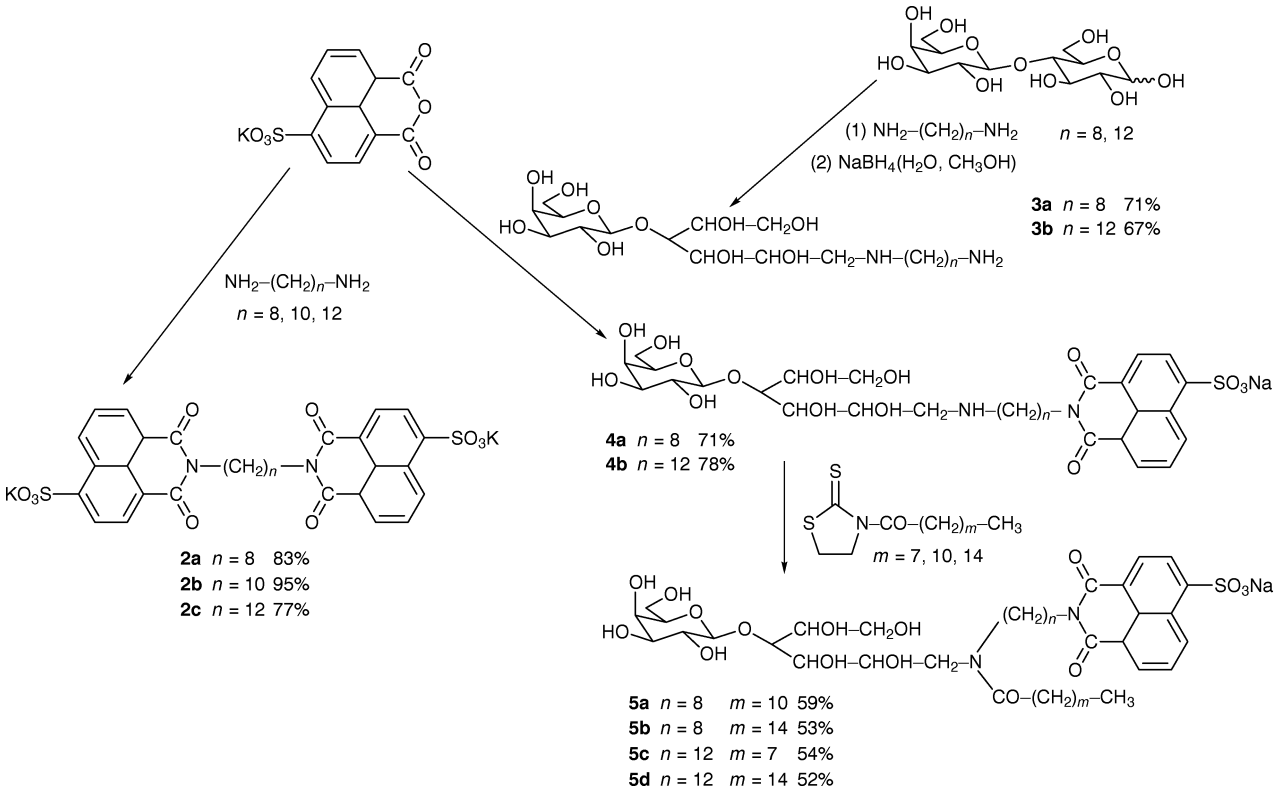
In a second phase, we decided tentatively to improve the biological activity of GalCer analogs by changing the carboxylate for an anionic motif, more closely related in structure to Suramin. Indeed, as we showed in a recent work, the region recognized by Suramin in the V3 loop only partially overlaps the region recognized by CA52.[14] So, it would be interesting to add a Suramin mimic to the structure of CA52, in place of the carboxylate, to improve binding between the compound and the V3 loop. In a previous work, Rideout and colleagues showed that sulfonated *N*-substituted naphthalimides are inhibitors of HIV *in vitro*.[17] Therefore, we decided to use this motif, both aromatic and anionic, as a structural analog of Suramin. Moreover, in a previous study, we demonstrated that bolaamphiphilic compounds of this type, derived from naphthalene disulfonic acid, are good mimics of Suramin.[18,19] Unfortunately, these previous compounds were only prepared in low yields. In the present study, we devised a new route to bis-sulfonated *N*-substituted naphthalimides.

Synthesis of *N*-substituted 1,8-naphthalimide derivatives toward a new series of bolaamphiphilic analogs of GalCer, 5

The synthetic routes to the synthesized compounds are summarized in Scheme 2. The synthesis of the bis-*N*-substituted 4-sulfo-1,8-naphthalimide derivatives **2** is performed by condensation of 4-sulfo-1,8-naphthalic anhydride on diamine in a molar ration of 2 : 1 in DMF at 90 °C. Easy access to these compounds is demonstrated by very good yields (Scheme 2), in contrast with those obtained with the previously used bis-*N*-substituted aminonaphthalenesulfonic acid.[18] The synthesis of the dissymmetric compounds **5** is performed in four steps: the lactose, solubilized in a small quantity of water, is added slowly to a methanolic solution of diamine and the products obtained are reduced by $NaBH_4$. After chromatographic purification, the compounds **3** are condensed with 4-sulfo-1,8-naphthalic anhydride to give the bolaforms **4** in good yields after purification. Then the lateral alkyl chain is added *via* acylation of the secondary amine using *N*-acyl thiazolidine-2-thiones, as for the first series of GalCer analogs **1**. After purification by chromatography, the new series of bolaamphiphilic analogs, **5**, of GalCer are obtained with 50–60% yields.

The bolaform compounds **2** have been synthesized to evaluate the fitness of the sulfononaphthalimide to mimic Suramin in V3 loop binding.

Biological studies are now underway and will be published in an upcoming paper. The preliminary results show a very



Scheme 2 Synthetic scheme for *N*-substituted 1,8-naphthalimide derivatives **5**

good similarity of the bolaform compounds **2** and Suramin, since the IC50 values for binding inhibition in ^{3}H-Suramin with SPC3 are respectively 0.9 μM for **2a**, 0.5 μM for **2b** and 0.4 μM for **2c**.

Conclusion

New anionic bolaamphiphilic analogs of galactosylceramide (GalCer) were synthesized in only three or four steps with good yields, starting from unprotected lactose. These compounds, depending on their hydrophobicity, could bind to the V3 domain of HIV-1 gp 120. They could be optimized to be the basis of potential low-cost anti HIV-1 drugs that neutralize HIV infection through masking of the V3 loop.

The key role of the hydrophobicity in the binding of the V3 loop shows the importance of the hydrophobic interactions in the conception of mimics of natural ligands to get inhibitors in various domains (for example in antiviral, antibacterial or antiinflammatory drug design). Work is now underway to investigate this new concept in anti-HIV drug design and therefore to prepare polyvalent inhibitors of the virus.

Experimental

Biological evaluation

Materials. SPC3 ($[GPGRAF]_8$—$[K]_4$—$[K]_2$—KbA) was generously provided by M. Mollard (Eurethics, Paris, France). The peptide was synthesized according to the Tam method and purified to homogeneity. The amino acid analysis of the purified peptide agreed with the deduced amino acid ratio. ^{3}H-Suramin (49 Ci mmol^{-1}) was purchased from Isotopchim (Ganagobie–Peyruis, France). Polyvinyl chloride multiwell plates (No 3911) were from Falcon–Becton Dickinson (Le Pont de Claix, France). Immulon 1 multiwell plates were from Poly Labo (Strasbourg, France).

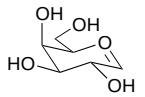
^{3}H-Suramin solid phase assay. SPC3 (100 μL) at the indicated concentration was incubated in polyvinyl chloride 96-well plates overnight at 4 °C. The wells were washed three times with 200 μL of phosphate buffered saline (PBS) and subsequently treated with PBS containing 1% gelatin for 90 min at 37 °C to reduce non-specific binding. The plates were then incubated with 100 μL of ^{3}H-Suramin (1 μCi mL^{-1}). After 1 h at 37 °C, the plates were washed five times with 200 μL of PBS, each well was individualized, and the radioactivity was determined in a β scintillation counter (Beckman, Marseille, France).

Inhibition of ^{3}H-Suramin binding to SPC3. The screening assay is based on the potential ability of synthetic GalCer analogs to compete with ^{3}H-Suramin for binding to SPC3. In this assay, 100 μL of SPC3 (5 μM) were deposited in polyvinyl chloride 96-well plates as described above. The indicated concentration of each drug was added in competition during ^{3}H-Suramin incubation. The plates were then washed and processed for radioactivity measurements exactly as for the ^{3}H-Suramin solid phase assay.

Syntheses

General. The ^{1}H and ^{13}C NMR spectra were recorded on Bruker AC200 and AC400 WB spectrometers at nominal frequencies of 200.1 and 400.1 MHz for the ^{1}H spectra and of 50.3 and 100.1 MHz for the ^{13}C spectra. The abbreviations d, t, m and dd indicate doublet, triplet, multiplet and split doublet, respectively. The chemical shifts are expressed in ppm with respect to tetramethylsilane (TMS). The mass spectra were recorded on a ZAB-HS spectrometer (WG-Analytical, Manchester, UK) using fast atom bombardment (FAB). The elemental analyses were carried out at the ENSCT (Toulouse, France). They agree with the proposed structures associated with small quantities of water and also ammonium carbonate, due to the ammonia used in the chromatographic purification.

***N*-[10-(Sodium oxycarbonyl)decyl]-1-amino-1-deoxylactitol, 1a.** To a solution of sodium 11-aminoundecanoate (22 mmol) in methanol (60 mL) is added lactose monohydrate (13.7 mmol) dissolved in water (30 mL). The mixture is stirred 12 h at room temperature, then heated at 55 °C for 6 h. The intermediate is not isolated. Sodium borohydride (15 mmol) is immediately added at room temperature in small portions to the reaction mixture and then stirred 12 h. The residual solution is evaporated *in vacuo*. The crude mixture is purified by chromatography on silica gel (230–400 mesh) by eluting with chloroform–methanol–30% ammonia solution: 5 : 3.5 : 1.5. After lyophilisation the pure product is obtained with 45% yield in two steps. $R_f = 0.11$. MS (FAB < 0, glycerol matrix/H_2O): 526 $(M - Na)^-$, 364 $(MH - Na - SugRes)^-$ where SugRes = sugar residue =



^1H NMR (250 MHz, D_2O) δ: 4.44 (d, $^3J = 7.6$ Hz, 1H, anomeric proton), 4.10–3.20 (m, 12H, C*H*OH and CH_2OH, saccharidic part), 3.00 (2t, 4H, twice CH_2NH), 2.10 (t, 2H, C$H_2CO_2^-$), 1.64 (m, 2H, CH_2CH$_2$NH), 1.48 (m, 2H, CH_2CH$_2$COO$^-$), 1.25 (m, 12H, 6 CH_2, aliphatic chain). ^{13}C NMR (100 MHz, D_2O–DMSO) δ: 186.3 (*C*OO$^-$), 105.5 (*C* anomeric), 80.9–70.5 (8 *C*HO—, disaccharidic part), 64.6 and 64.0 (twice *C*H$_2$OH), 53.8, 52.1 and 50.5 (*C*H$_2CO_2^-$ and twice *C*H$_2$NH), 31.5–28.1 (8 *C*H$_2$, aliphatic chain). Elemental analysis for $C_{23}H_{44}H_2O_{12}Na$: found C 43.62; H 8.23; N 4.78.

***N*-[10-(Sodium oxycarbonyl)decyl]-*N*-acyl-1-amino-1-deoxylactitols.** To a solution of compound **1a** (3.28 mmol) in 250 mL of DMF and 3.28 mmol of triethylamine is added the acylating agent, 3-acyl-thiazolidine-2-thione (6.40 mmol) prepared according to a procedure described elsewhere.[16] The mixture is stirred for 4 days at 60 °C. After evaporation to dryness, the residue is purified by chromatography on silica gel (eluent chloroform–methanol–30% ammonia solution: 6 : 3 : 1). Both the ^1H and ^{13}C NMR spectra show the doubling of some signals, which indicates the presence of two conformational isomers in solution due to the amide bond, as it has been already described.[20]

***N*-[10-(Sodium oxycarbonyl)decyl]-*N*-nonanoyl-1-amino-1-deoxylactitol, 1b.** Yield: 53%. $R_f = 0.27$ (eluent chloroform–methanol–30% ammonia solution: 5 : 3.5 : 1.5). MS (FAB < 0, glycerol matrix/DMSO): 666 $(M - Na)^-$, 504 $(MH - Na - SugRes)^-$. ^1H NMR (400 MHz, $CDCl_3$–CD_3OD 2 : 1) δ: 4.42 (d, $^3J = 7.6$ Hz, 1H, anomeric proton), 4.03–3.25 (m, 16H total: 12H, C*H*OH and CH_2OH, saccharidic part, and 4H, twice CH_2NCO). 2.38 and 2.30 (2t, 3 : 7, 2H, CH_2CON), 2.25 (t, 2H, C$H_2CO_2^-$), 1.56 (m, 6H, CH_2CH$_2CO_2^-$, CH_2CH$_2$CON and CH_2CH$_2$NCO), 1.26–1.22 (m, 22H, 11 CH_2, aliphatic chains), 0.84 (t, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$–CD_3OD 2 : 1) δ: 180.9 (*C*OO$^-$), 179.2 (*C*ON), 108.2 (*C* anomeric), 87.1–72.9 (8 *C*HO—, disaccharidic part), 66.4 and 65.5 (twice *C*H$_2$OH), 54.6, 53.8 and 48.7 (*C*H$_2CO_2^-$ and twice *C*H$_2$NCO), 38.2 (*C*H$_2$CON), 37.3–26.6 (14 *C*H$_2$, aliphatic chains), 17.8 (*C*H$_3$). Elemental analysis for $C_{32}H_{60}NO_{13}Na$: found C 53.93; H 9.32; N 2.34.

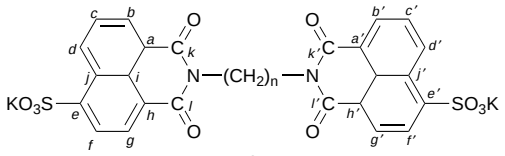
***N*-[10-(Sodium oxycarbonyl)decyl]-*N*-dodecanoyl-1-amino-1-deoxylactitol, 1c.** Yield: 50%. $R_f = 0.35$ (eluent chloroform–methanol–30% ammonia solution: 5 : 3.5 : 1.5). MS (FAB < 0, glycerol matrix/DMSO): 708 $(M - Na)^-$, 546 $(MH - Na - SugRes)^-$. ^1H NMR (400 MHz, $CDCl_3$–CD_3OD 2 : 1) δ: 4.42 (d, $^3J = 7.5$ Hz, 1H, anomeric proton), 4.02–3.12 (m, 16H total: 12H, C*H*OH and CH_2OH, saccharidic part, and 4H, twice CH_2NCO), 2.40 and 2.31 (2t, 3 : 7, 2H, CH_2CON), 2.24 (t, 2H, C$H_2CO_2^-$), 1.56 (m, 6H, CH_2CH$_2CO_2^-$, CH_2CH$_2$CON and CH_2CH$_2$NCO), 1.27–1.23 (m, 28H, 14 CH_2, aliphatic chains), 0.84 (t, $^3J = 7.1$ Hz, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$–CD_3OD 2 : 1) δ: 178.8 (*C*OO$^-$), 176.5 (*C*ON), 105.5 (*C* anomeric), 84.4–70.2 (8 *C*HO—, disaccharidic part), 63.7 and 62.7 (twice *C*H$_2$OH), 51.3, 51.1 and 47.4 (*C*H$_2CO_2^-$ and twice *C*H$_2$NCO), 35.9 (*C*H$_2$CON), 34.6–23.9 (17 *C*H$_2$, aliphatic chains), 15.1 (*C*H$_3$). Elemental analysis for $C_{35}H_{66}NO_{13}Na$: found C 55.40; H 9.55; N 2.33.

***N*-[10-(Sodium oxycarbonyl)decyl]-*N*-hexadecanoyl-1-amino-1-deoxylactitol, 1d.** Yield: 47%. $R_f = 0.45$ (eluent chloroform–methanol–30% ammonia solution: 5 : 3.5 : 1.5). MS (FAB < 0, glycerol matrix/DMSO): 764 $(M - Na)^-$, 602 $(MH - Na - SugRes)^-$. ^1H NMR (400 MHz, $CDCl_3$–CD_3OD 2 : 1) δ: 4.42 (d, $^3J = 7.4$ Hz, 1H, anomeric proton), 4.10–3.10 (m, 16H total: 12H, C*H*OH and CH_2OH, saccharidic part, and 4H, twice CH_2NCO), 2.40 and 2.31 (2t, 3 : 7, 2H, CH_2CON), 2.24 (t, 2H, C$H_2CO_2^-$), 1.56 (m, 6H, CH_2CH$_2CO_2^-$, CH_2CH$_2$CON and CH_2CH$_2$NCO), 1.26–1.22 (m, 36H, 18 CH_2, aliphatic chains), 0.83 (t, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$–CD_3OD 2 : 1) δ: 176.7 (*C*OO$^-$), 174.9 (*C*ON), 103.7 (*C* anomeric), 82.1–68.7 (8 *C*HO—, disaccharidic part), 62.0 and 60.2 (twice *C*H$_2$OH), 49.6, 49.3 and 48.7 (*C*H$_2CO_2^-$ and twice *C*H$_2$NCO), 33.9 (*C*H$_2$CON), 32.9–22.3 (21 *C*H$_2$, aliphatic chains), 14.1 (*C*H$_3$). Elemental analysis for $C_{39}H_{74}NO_{13}Na$: found C 57.33; H 9.86; N 2.28.

***N*-[10-(Sodium oxycarbonyl)decyl]-*N*-octadecanoyl-1-amino-1-deoxylactitol, 1e.** Yield: 40%. $R_f = 0.50$ (eluent chloroform–methanol–30% ammonia solution: 5 : 3.5 : 1.5). MS (FAB < 0, glycerol matrix/DMSO): 792 $(M - Na)^-$, 630 $(MH - Na - SugRes)^-$. ^1H NMR (400 MHz, $CDCl_3$–CD_3OD 2 : 1) δ: 4.44 (d, $^3J = 7.3$ Hz, 1H, anomeric proton), 4.04–3.40 (m, 16H total: 12H, C*H*OH and CH_2OH, saccharidic part, and 4H, twice CH_2NCO), 2.41 and 2.35 (2t, 3 : 7, 2H, CH_2CON), 2.25 (t, 2H, C$H_2CO_2^-$), 1.58 (m, 6H, CH_2CH$_2CO_2^-$, CH_2CH$_2$CON and CH_2CH$_2$NCO), 1.30–1.25 (m, 40H, 20 CH_2, aliphatic chains), 0.87 (t, $^3J = 6.9$ Hz, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$–CD_3OD 2 : 1) δ: 176.7 (*C*OO$^-$), 174.9 (*C*ON), 103.7 (*C* anomeric), 82.1–68.7 (8 *C*HO—, disaccharidic part), 62.0 and 60.2 (twice *C*H$_2$OH), 49.6, 49.3 and 48.7 (*C*H$_2CO_2^-$ and twice *C*H$_2$NCO), 33.9 (*C*H$_2$CON), 32.9–22.3 (23 *C*H$_2$, aliphatic chains), 14.1 (*C*H$_3$). Elemental analysis for $C_{41}H_{78}NO_{13}Na$: found C 54.45; H 9.87; N 4.25.

α,ω-Bis[4-(potassium sulfo)-1,8-naphthalimidyl]alkane, 2. A solution of 3 mmol of potassium 4-sulfo-1,8-naphthalic anhydride in 50 mL of DMF is added dropwise under argon to a solution of 1.5 mmol of 1,ω-diaminoalkane in 50 mL of DMF. The reaction mixture is heated to 90 °C for 3 days. The reaction is followed by thin layer chromatography on silica (eluent chloroform–methanol–30% ammonia solution: 6 : 3 : 1). The solvent is evaporated under vacuum (1 mm Hg) and the residue ground up in 20 mL of ethanol and then filtered. The white powder is dried under reduced pressure (10^{-1} mm Hg).

1,8-Bis[4-(potassium sulfo)-1,8-naphthalimidyl]octane, 2a. Yield: 83%. $R_f = 0.35$ (eluent chloroform–methanol–30% ammonia solution: 6 : 3 : 1). MS (FAB < 0, glycerol matrix/DMSO): 701 $(M - K)^-$, 685 $(M - 2K + Na)^-$, 637 (M − K



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$-SO_2)^-$. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 9.28 (dd, $^3J_{o'}$ = 8.6 Hz, ^4J_m = 1 Hz, 2H, $H_{e,e'}$), 8.49 (dd, ^3J_o = 7.3 Hz, ^4J_m = 1 Hz, 2H, $H_{g,g'}$), 8.47 (d, $^3J_{o''}$ = 7.6 Hz, 2H, $H_{b,b'}$), 8.23 (d, $^3J_{o''}$ = 7.6 Hz, 2H, $H_{c,c'}$), 7.88 (dd, ^3J_o = 7.3 Hz, $^3J_{o'}$ = 8.6 Hz, 2H, $H_{f,f'}$), 4.05 (t, ^3J = 7.4 Hz, 4H, CH_2N), 1.66 (m, 4H, CH_2CH$_2$N), 1.33 (m, 8H, 4 CH_2, aliphatic chain). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 163.4 and 163.0 ($C_{k,k'}$ and $C_{l,l'}$, twice C=O), 149.6 ($C_{d,d'}$), 134.0 ($C_{e,e'}$), 130.3 and 130.1 ($C_{b,b'}$ and $C_{g,g'}$), 128.0 and 127.4 ($C_{i,i'}$ and $C_{j,j'}$), 126.7 ($C_{f,f'}$), 124.9 ($C_{c,c'}$), 122.7 and 121.9 ($C_{h,h'}$ and $C_{a,a'}$), 39.6 (CH$_2$N), 28.6 to 26.4 (6 CH_2, aliphatic chain). Elemental analysis for $C_{32}H_{26}N_2S_2O_{10}K_2$: found C 50.97; H 3.62; N 3.79.

1,10-Bis[4-(potassium sulfo)-1,8-naphthalimidyl]decane, 2b. Yield: 95%. R_f = 0.37 (eluent chloroform–methanol–30% ammonia solution: 6 : 3 : 1). MS (FAB < 0, MNBA matrix/DMSO): 729 $(M-K)^-$, 691 $(M-2K+H)^-$. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 9.29 (dd, $^3J_{o'}$ = 8.5 Hz, ^4J_m = 1 Hz, 2H, $H_{e,e'}$), 8.50 (dd, ^3J_o = 7.3 Hz, ^4J_m = 1 Hz, 2H, $H_{g,g'}$), 8.48 (d, $^3J_{o''}$ = 7.5 Hz, 2H, $H_{b,b'}$), 8.25 (d, $^3J_{o''}$ = 7.5 Hz, 2H, $H_{c,c'}$), 7.89 (dd, ^3J_o = 7.3 Hz, $^3J_{o'}$ = 8.6 Hz, 2H, $H_{f,f'}$), 4.05 (t, ^3J = 7.3 Hz, 4H, CH_2N), 1.64 (m, 4H, CH_2CH$_2$N), 1.23 (m, 12H, 6 CH_2, aliphatic chain). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 163.4 and 163.0 ($C_{k,k'}$ and $C_{l,l'}$, twice C=O), 149.5 ($C_{d,d'}$), 134.0 ($C_{e,e'}$), 130.3 and 130.1 ($C_{b,b'}$ and $C_{g,g'}$), 128.0 and 127.4 ($C_{i,i'}$ and $C_{j,j'}$), 126.8 ($C_{f,f'}$), 124.9 ($C_{c,c'}$), 122.7 and 121.9 ($C_{h,h'}$ and $C_{a,a'}$), 39.6 (CH$_2$N), 28.6–26.4 (8 CH_2, aliphatic chain). Elemental analysis for $C_{34}H_{30}N_2S_2O_{10}K_2$: found C 50.75; H 4.20; N 3.51.

1,12-Bis[4-(potassium sulfo)-1,8-naphthalimidyl]dodecane, 2c. Yield: 77%. R_f = 0.37 (eluent chloroform–methanol–30% ammonia solution: 6 : 3 : 1). MS (FAB < 0, MNBA matrix/DMSO): 757 $(M-K)^-$, 719 $(M-2K+H)^-$, 637 $(M-2K-SO_2+H)^-$. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 9.30 (dd, $^3J_{o'}$ = 8.6 Hz, ^4J_m = 1 Hz, 2H, $H_{e,e'}$), 8.50 (dd, ^3J_o = 7.3 Hz, ^4J_m = 1 Hz, 2H, $H_{g,g'}$), 8.48 (d, $^3J_{o''}$ = 7.6 Hz, 2H, $H_{b,b'}$), 8.25 (d, $^3J_{o''}$ = 7.6 Hz, 2H, $H_{c,c'}$), 7.88 (dd, ^3J_o = 7.3 Hz, $^3J_{o'}$ = 8.6 Hz, 2H, $H_{f,f'}$), 4.05 (t, ^3J = 7.3 Hz, 4H, CH_2N), 1.68 (m, 4H, CH_2CH$_2$N), 1.25 (m, 16H, 8 CH_2, aliphatic chain). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 163.4 and 163.0 ($C_{k,k'}$ and $C_{l,l'}$, twice C=O), 149.5 ($C_{d,d'}$), 134.0 ($C_{e,e'}$), 130.3 and 130.1 ($C_{b,b'}$ and $C_{g,g'}$), 128.0 and 127.4 ($C_{i,i'}$ and $C_{j,j'}$), 126. 8 ($C_{f,f'}$), 124.9 ($C_{e,e'}$), 122.7 and 121.9 ($C_{h,h'}$ and $C_{a,a'}$), 39.6 (CH$_2$N), 28.6–26.4 (10 CH_2, aliphatic chain). Elemental analysis for $C_{36}H_{34}N_2S_2O_{10}K_2$: found C 52.33; H 4.42; N 3.51.

***N*-(ω-Aminoalkyl)-1-amino-1-deoxylactitol, 3.** A solution of 2 mmol of lactose monohydrate in 15 mL of water is added dropwise to a solution of 6 mmol of 1,ω-diaminoalkane in 15 mL of methanol. The reaction mixture is stirred for 12 h at room temperature; the intermediate product is not isolated. Sodium borohydride (3 mmol) is added, at room temperature, in small portions to the reaction mixture and then stirred for one day. The residual solution is evaporated under vacuum and the crude mixture is then purified by chromatography on silica gel eluting with a gradient of a chloroform–methanol–30% ammonia solution: 6 : 3 : 1 followed by 5 : 3.5 : 1.5.

***N*-(8-Aminooctyl)-1-amino-1-deoxylactitol, 3a.** Yield: 71%. R_f = 0.20 (eluent chloroform–methanol–30% ammonia solution: 2 : 2 : 1). MS (FAB > 0, glycerol matrix/DMSO): 471 $(MH)^+$, 309 $[(M+H-SugRes)H]^+$. ^{1}H NMR (200 MHz, D_2O) δ: 4.33 (d, ^3J = 7.8 Hz, 1H, anomeric proton), 4.09–3.39 (m, 12H, C*H*OH and CH_2OH, saccharidic part), 2.80 (m, 6H, CH_2NH$_2$ and twice CH_2NH), 1.49 (m, 4H, CH_2CH$_2$NH$_2$ and CH_2CH$_2$NH), 1.25 (m, 8H, 4 CH_2, aliphatic chain). ^{13}C NMR (50 MHz, D_2O) δ: 106.7 (*C* anomeric), 76.1–71.0 (8 *C*HO—, disaccharide part), 65.6 and 63.4 (twice *C*H$_2$OH), 54.3 and 50.8 (twice *C*H$_2$NH), 42.4 (*C*H$_2$NH$_2$), 32.9–22.3 (6 *C*H$_2$, aliphatic chain).

***N*-(12-Aminododecyl)-1-amino-1-deoxylactitol, 3b.** Yield: 67%. R_f = 0.39 (eluent chloroform–methanol–30% ammonia solution: 2 : 2 : 1). MS (FAB > 0, glycerol matrix/DMSO): 549 $(MNa)^+$, 527 $(MH)^+$, 365 $[(M+H-SugRes)H]^+$. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 4.25 (d, ^3J = 7.8 Hz, 1H, anomeric proton), 3.84–3.29 (m, 16H total: 12H, C*H*OH and CH_2OH, saccharidic part, and 4H, twice CH_2NH), 2.70 (m, 2H, CH_2NH$_2$), 1.25 (m, 20H, 10 CH_2, aliphatic chain). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 104.1 (*C* anomeric), 81.0–66.5 (8 *C*HO—, disaccharidic part), 62.2 and 60.8 (twice *C*H$_2$OH), 52.1 and 49.2 (twice *C*H$_2$NH), 42.4 (*C*H$_2$NH$_2$), 28.7–26.0 (10 *C*H$_2$, aliphatic chain).

***N*-{ω-[4-(sodium sulfo)-1,8-naphthalimidyl]alkyl}-1-amino-1-deoxylactitol, 4.** Product **3** (4.25 mmol) in methanol is added to a solution of potassium 4-sulfo-1,8-naphthalic anhydride (3.40 mmol) and 13 mmol of Et_3N in 50 mL of DMF. The mixture is stirred for 4 days at 60 °C. After evaporation of solvents, the crude product is purified by chromatography on silica gel eluting with a chloroform–methanol–30% ammonia solution: 7 : 2.5 : 0.5. The K cation is exchanged, after purification, for Na by an Amberlyst 15 resin (15-fold excess of Na sites) in DMF.

***N*-{8-[4-(Sodium sulfo)-1,8-naphthalimidyl]octyl}-1-amino-1-deoxylactitol, 4a.** Yield: 71%. R_f = 0.61 (eluent chloroform–methanol–30% ammonia solution: 2 : 2 : 1). MS (FAB < 0, glycerol matrix/DMSO): 729 $(M-Na)^-$, 665 $(M-Na-SO_2)^-$, 567 $(MH-Na-SugRes)^-$. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 9.28 (dd, $^3J_{o'}$ = 8.6 Hz, ^4J_m = 1 Hz, 1H, H_e), 8.49 (dd, ^3J_o = 7.4 Hz, ^4J_m = 1 Hz, 1H, H_g), 8.47 (d, $^3J_{o''}$ = 7.6 Hz, 1H, H_b), 8.24 (d, $^3J_{o''}$ = 7.6 Hz, 1H, H_c), 7.88 (dd, ^3J_o = 7.4 Hz, $^3J_{o'}$ = 8.6 Hz, 1H, H_f), 4.30 (d, ^3J = 7.1 Hz, 1H, anomeric proton), 4.05 (t, ^3J = 7.5 Hz, 2H, CH_2N), 3.73–2.88 (m, 16H total: 12H C*H*OH and CH_2OH, saccharidic part, and 4H, twice CH_2NH), 1.64 (m, 4H, CH_2CH$_2$NH and CH_2CH$_2$N), 1.31 (m, 8H, 4 CH_2, aliphatic chain). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 163.5 and 163.0 (C_k and C_l, twice C=O), 149.5 (C_d), 134.0 (C_e), 130.4 and 130.1 (C_b and C_g), 128.0 and 127.4 (C_i and C_j), 126.6 (C_f), 124.9 (C_c), 122.7 and 121.9 (C_h and C_a), 103.5 (*C* anomeric), 79.3–66.6 (8 *C*HO—, disaccharidic part), 61.9 and 60.7 (twice *C*H$_2$OH), 49.1 and 46.6 (twice *C*H$_2$NH), 39.6 (*C*H$_2$N), 28.4–25.2 (6 *C*H$_2$, aliphatic chain). Elemental analysis for $C_{32}H_{45}N_2SO_{15}Na$: found C 49.83; H 6.58; N 4.19.

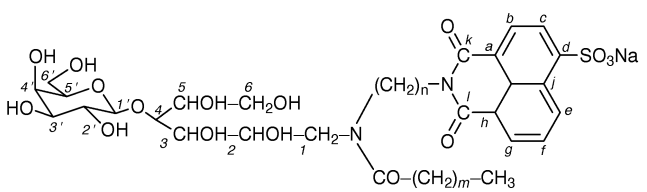
***N*-{12-[4-(Sodium sulfo)-1,8-naphthalimidyl]dodecyl}-1-amino-1-deoxylactitol, 4b.** Yield: 78%. R_f = 0.75 (eluent chloroform–methanol–30% ammonia solution: 2 : 2 : 1). MS (FAB < 0, glycerol matrix/DMSO): 785 $(M-Na)^-$, 623 $(MH-Na-SugRes)^-$. ^{1}H NMR (200 MHz, DMSO-d_6) δ: 9.26 (dd, $^3J_{o'}$ = 8.6 Hz, ^4J_m = 1 Hz, 1H, H_e), 8.49 (dd, ^3J_o =

7.3 Hz, ^4J_m = 1 Hz, 1H, H_g), 8.46 (d, $^3J_{o''}$ = 7.6 Hz, 1H, H_b), 8.22 (d, $^3J_{o''}$ = 7.6 Hz, 1H, H_c), 7.88 (dd, ^3J_o = 7.3 Hz, $^3J_{o'}$ = 8.6 Hz, 1H, H_f), 5.07–4.46 (m, 8H, O*H*, saccharidic part), 4.29 (d, ^3J = 7.1 Hz, 1H, anomeric proton), 4.03 (t, ^3J = 7.0 Hz, 2H, CH_2N), 3.72–2.86 (m, 16H total: 12H, C*H*OH and CH_2OH, saccharidic part, and 4H, twice CH_2NH), 1.63 (m, 4H, CH_2CH$_2$NH and CH_2CH$_2$N), 1.29–1.20 (m, 16H, 8 CH_2, aliphatic chain). ^{13}C NMR (50 MHz, DMSO-d$_6$) δ: 163.5 and 163.0 (C_k and C_l, twice C=O), 149.8 (C_d), 134.1 (C_e), 130.3 and 130.1 (C_b and C_g), 128.0 and 127.5 (C_i and C_j), 126.7 (C_f), 124.9 (C_c), 122.6 and 121.9 (C_h and C_a), 103.6 (*C* anomeric), 79.5–66.8 (8 *C*HO—, disaccharidic part), 62.0 and 60.7 (twice *C*H$_2$OH), 49.1 and 46.9 (twice *C*H$_2$NH), 39.6 (*C*H$_2$N), 28.7–25.2 (10 *C*H$_2$, aliphatic chain). Elemental analysis for $C_{36}H_{53}N_2SO_{15}Na$: found C 51.51; H 7.11; N 4.35.

***N*-{ω-[4-(Sodium sulfo)-1,8-naphthalimidyl]alkyl}-*N*-acyl-1-amino-1-deoxylactitol, 5.** To a solution of compound **4** (0.66 mmol) in 20 mL of DMF and 1.33 mmol of Et$_3$N is added to the acylating agent 3-acyl-thiazolidine-2-thione (0.99 mmol) prepared according to a procedure described elsewhere.[16] The mixture is stirred for 4–5 days at 60 °C. The solvent is evaporated under vacuum, then the residue is purified by chromatography on silica gel eluting with a chloroform–methanol–30% ammonia solution 7 : 2.5 : 0.5. Both the ^{1}H and ^{13}C NMR spectra show the doubling of some signals, which indicates the presence of two conformational isomers in solutions, due to the amide bond.[20]

***N*-{8-[4-(Sodium sulfo)-1,8-naphthalimidyl]octyl}-*N*-dodecanoyl-1-amino-1-deoxylactitol, 5a.** Yield: 59%. R_f = 0.33 (eluent chloroform–methanol–30% ammonia solution: 6 : 3 : 1). MS (FAB < 0, glycerol matrix/DMSO): 911 (M − Na)$^-$, 749 (MH − Na − SugRes)$^-$, ^{1}H NMR (400 MHz, DMSO-d$_6$) δ: 9.22 (dd, $^3J_{o'}$ = 8.6 Hz, ^4J_m = 1 Hz, 1H, H_e), 8.48 (dd, ^3J_o = 7.3 Hz, ^4J_m = 1 Hz, 1H, H_g), 8.46 (d, $^3J_{o''}$ = 7.6 Hz, 1H, H_b), 8.22 (d, $^3J_{o''}$ = 7.6 Hz, 1H, H_c), 7.87 (dd, ^3J_o = 7.3 Hz, $^3J_{o'}$ = 8.6 Hz, 1H, H_f), 4.29 (d, ^3J = 7.1 Hz, 1H, anomeric proton), 4.01 (t, ^3J = 7.1 Hz, 2H, CH_2N), 3.80–3.20 (m, 16H total: 12H, C*H*OH and CH_2OH, saccharidic part, and 4H, twice CH_2NCO), 2.28 and 2.22 (2t, 3 : 7, 2H, CH_2CON), 1.60 and 1.44 (m, 6H, CH_2CH$_2$CON, CH_2CH$_2$NCO and CH_2CH$_2$N), 1.29–1.14 (m, 24H, 12 CH_2, aliphatic chains), 0.77 (t, ^3J = 7.1 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ: 172.3 (*C*ON), 163.4 and 162.9 (C_k and C_l, twice C=O), 149.2 (C_d), 133.8 (C_e), 130.3 and 130.0 (C_b and C_g), 127.9 and 127.3 (C_i and C_j), 126.8 (C_f), 124.9 (C_c), 122.7 and 121.9 (C_h and C_a), 104.4 (*C* anomeric), 83.5–67.8 (8 *C*HO—, disaccharidic part), 61.9 and 60.1 (twice *C*H$_2$OH), 49.8 and 44.9 (twice *C*H$_2$NCO), 39.6 (*C*H$_2$N), 32.2–21.9 (16 *C*H$_2$, aliphatic chains), 13.8 (*C*H$_3$). Elemental analysis for $C_{44}H_{67}N_2SO_{16}Na$: found C 52.98; H 8.05; N 4.63.

***N*-{8-[4-(Sodium sulfo)-1,8-naphthalimidyl]octyl}-*N*-hexadecanoyl-1-amino-1-deoxylactitol, 5b.** Yield: 53%. R_f = 0.34 (eluent chloroform–methanol–30% ammonia solution: 6 : 3 : 1). MS (FAB < 0, glycerol matrix/DMSO): 967 (M − Na)$^-$, 805 (MH − Na − SugRes)$^-$. ^{1}H NMR (400 MHz, DMSO-d$_6$) δ: 9.16 (dd, $^3J_{o'}$ = 8.6 Hz, ^4J_m = 1 Hz, 1H, H_e), 8.49 (dd, ^3J_o = 7.3 Hz, ^4J_m = 1 Hz, 1H, H_g), 8.46 (d, $^3J_{o''}$ = 7.7 Hz, 1H, H_b), 8.19 (d, $^3J_{o''}$ = 7.7 Hz, 1H, H_c), 7.88 (dd, ^3J_o = 7.3 Hz, $^3J_{o'}$ = 8.6 Hz, 1H, H_f), 5.30–4.15 (m, 8H, O*H*, saccharidic part), 4.27 (d, ^3J = 7.1 Hz, 1H, anomeric proton), 4.04 (t, ^3J = 6.0 Hz, 2H, CH_2N), 3.96–3.29 (m, 16H total: 12H, C*H*OH and CH_2OH, saccharidic part, and 4H, twice CH_2NCO and CH_2N), 2.26 and 2.17 (2t, 3 : 7, 2H, CH_2COH), 1.56 and 1.40 (m, 6H, CH_2CH$_2$CON, CH_2CH$_2$NCO and CH_2CH$_2$N), 1.23–1.07 (m, 32H, 16 CH_2, aliphatic chains), 0.73 (t, ^3J = 7.1 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ: 172.8 (*C*ON), 163.3 and 162.9 (C_k and C_l, twice C=O), 148.5 (C_d), 133.6 (C_e), 130.4 and 130.0 (C_b and C_g), 127.9 and 127.2 (C_i and C_j), 126.9 (C_f), 125.0 (C_c), 122.8 and 121.8 (C_h and C_a) 104.1 (*C* anomeric), 83.0–65.8 (8 *C*HO—, disaccharidic part), 61.8 and 60.1 (twice *C*H$_2$OH), 49.8 and 44.8 (twice *C*H$_2$NCO), 39.6 (*C*H$_2$N), 32.2–21.9 (20 *C*H$_2$, aliphatic chains), 13.6 (*C*H$_3$). Elemental analysis for $C_{48}H_{75}N_2SO_{16}Na$: found C 54.31; H 8.39; N 4.50.



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***N*-{12-[4-(Sodium sulfo)-1,8-naphthalimidyl]dodecyl}-*N*-nonanoyl-1-amino-1-deoxylactitol, 5c.** Yield: 54%. R_f = 0.34 (eluent chloroform–methanol–30% ammonia solution: 6 : 3 : 1). MS (FAB < 0, glycerol matrix/DMSO): 925 (M − Na)$^-$, 763 (MH − Na − SugRes)$^-$. ^{1}H NMR (400 MHz, DMSO-d$_6$) δ: 9.26 (dd, $^3J_{o'}$ = 8.6 Hz, ^4J_m = 1 Hz, 1H, H_e), 8.49 (dd, ^3J_o = 7.3 Hz, ^4J_m = 1 Hz, 1H, H_g), 8.46 (d, $^3J_{o''}$ = 7.6 Hz, 1H, H_b), 8.22 (d, $^3J_{o''}$ = 7.6 Hz, 1H, H_c), 7.88 (dd, ^3J_o = 7.3 Hz, $^3J_{o'}$ = 8.6 Hz, 1H, H_f), 5.16–4.15 (m, 8H, O*H*, saccharidic part), 4.30 (d, ^3J = 7.1 Hz, 1H, anomeric proton), 4.04 (t, ^3J = 6.0 Hz, 2H, CH_2N), 3.80–3.30 (m, 16H total: 12H, C*H*OH and CH_2OH, saccharidic part, and 4H, twice CH_2NCO and CH_2N), 2.30 and 2.23 (2t, 3 : 7, 2H, CH_2CON), 1.65 and 1.46 (m, 6H, CH_2CH$_2$CON, CH_2CH$_2$NCO and CH_2CH$_2$N), 1.31–1.23 (m, 26H, 13 CH_2, aliphatic chains), 0.83 (t, ^3J = 7.1 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ: 172.4 (*C*ON), 163.4 and 163.0 (C_k and C_l, twice C=O), 149.2 (C_d), 133.8 (C_e), 130.4 and 130.0 (C_b and C_g), 127.9 and 127.3 (C_i and C_j), 126.8 (C_f), 124.9 (C_c), 122.7 and 121.9 (C_h and C_a), 104.4 (*C* anomeric), 83.5–67.8 (8 *C*HO—, disaccharidic part), 61.9 and 60.1 (twice *C*H$_2$OH), 49.7 and 44.9 (twice *C*H$_2$NCO), 39.6 (*C*H$_2$N), 32.2–21.9 (17 *C*H$_2$, aliphatic chain), 13.8 (*C*H$_3$). Elemental analysis for $C_{45}H_{69}N_2SO_{16}Na$: found C 51.83; H 8.15; N 5.06.

***N*-{12-[4-(Sodium sulfo)-1,8-naphthalimidyl]dodecyl}-*N*-hexadecanoyl-1-amino-1-deoxylactitol, 5d.** Yield: 52%. R_f = 0.35 (eluent chloroform–methanol–30% ammonia solution: 6 : 3 : 1). MS (FAB < 0, glycerol matrix/DMSO): 1023 (M − Na)$^-$, 861 (MH − Na − SugRes)$^-$. ^{1}H NMR (400 MHz, DMSO-d$_6$) δ: 9.21 (dd, $^3J_{o'}$ = 8.6 Hz, ^4J_m = 1.1 Hz, 1H, H_e), 8.45 (dd, ^3J_o = 7.3 Hz, ^4J_m = 1.1 Hz, 1H, H_g), 8.44 (d, $^3J_{o''}$ = 7.6 Hz, 1H, H_b), 8.21 (d, $^3J_{o''}$ = 7.6 Hz, 1H, H_c), 7.87 (dd, ^3J_o = 7.3 Hz, $^3J_{o'}$ = 8.6 Hz, 1H, H_f), 4.27 (d, ^3J = 7.0 Hz, 1H, anomeric proton), 4.00 (t, ^3J = 6.0 Hz, 2H, CH_2N), 3.69–3.29 (m, 16H total: 12H, C*H*OH and CH_2OH, saccharidic part, and 4H, twice CH_2NCO), 2.29 and 2.20 (2t, 3 : 7, 2H, CH_2CON), 1.60 and 1.42 (m, 6H, CH_2CH$_2$CON, CH_2CH$_2$NCO and CH_2CH$_2$N), 1.27–1.14 (m, 40H, 20 CH_2, aliphatic chains), 0.78 (t, ^3J = 7.1 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d$_6$) δ: 172.3 (*C*ON), 163.3 and 162.9 (C_k and C_l, twice C=O), 149.2 (C_d), 133.8 (C_e), 130.3 and 130.0 (C_b and C_g), 127.9 and 127.3 (C_i and C_j), 126.8 (C_f), 124.9 (C_c), 122.7 and 121.9 (C_h and C_a), 104.4 (*C* anomeric), 83.6–67.8 (8 *C*HO—, disaccharidic part), 61.9 and 60.1 (twice *C*H$_2$OH), 49.7 and 44.8 (twice *C*H$_2$NCO), 39.6 (*C*H$_2$N), 32.2–21.9 (24 *C*H$_2$, aliphatic chains), 13.7 (*C*H$_3$). Elemental analysis for

$C_{52}H_{83}N_2SO_{16}Na$: found C 53.92; H 8.68; N 4.39.

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