A versatile synthesis of αGalCer and its analogues exploiting a cyclic carbonate as phytosphingosine 3,4-diol protecting group

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Abstract: A convenient synthetic strategy to αGalCer and some relevant analogues by using a handily protected phytosphingosine is reported here. The conversion of the phytosphingosine amino group to azide and the protection of 3,4-diol as cyclic carbonate group, cleavable in mild basic conditions but resistant to acidic treatment, afforded quickly an excellent glycosyl acceptor. Its glycosylation with a proper galactosyl donor, gave a versatile intermediate in high yield and excellent stereoselectivity. To demonstrate the potentiality of the intermediate, three immunologically relevant compounds were chosen as model targets: αGalCer, dansyl alpha-galactosylceramide and 7DW8-5. These products were easily obtained in few steps and high yields to validate the synthetic route.

Keywords: α-Galactosylceramide analogues, glycosylation, glycosphingolipids, phytosphingosine acceptor

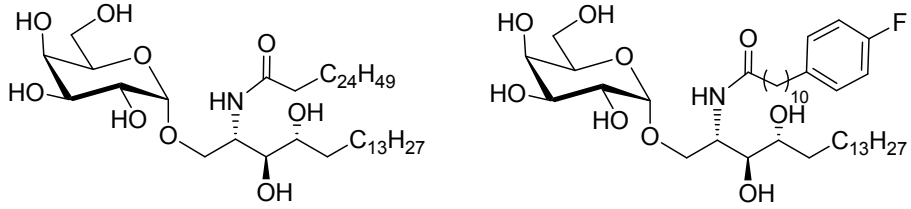
1. Introduction

Invariant natural killer T (iNKT) cells are a unique type of innate immune cells (T lymphocytes) that express an invariant α,β T-cell receptor. They recognize a range of foreign and endogenous lipids presented by CD1d glycoprotein (non-polymorphic MHC class I-like antigen presenting molecule). When activated they can influence the immune system with various effector and regulatory functions.[1]
α-Galactosylceramide (αGalCer or KRN7000 **1**, Fig 1), a synthetic glycolipid derived by the chemical simplification of Agelasphin, a natural product isolated from the marine sponge *Agelas Mauritianus*, is composed of a galactose α-linked to a phytosphingosine acylated with a long-chain fatty acid.[2] This glycolipid is the most extensively studied ligand for iNKT cells as it acts as a strong stimulator when bound to the CD1d glycoprotein. αGalCer is the reference compound for the evaluation of new CD1d ligands due to its potency and good biological characterisation. The complex between CD1d and αGalCer is recognised by the T-cell receptor of iNKT cells and induces the production of different cytokines that promote both an inflammatory response called T helper 1 (Th1), which governs the antitumor and antimicrobial activities, and an immunomodulatory response named T helper 2 (Th2), which can ameliorate various autoimmune diseases.[3]
For these reasons, iNKT cells represent a striking target for developing new therapeutics able to influence the human immune system in a broad range of settings.[1,4] Many analogues of αGalCer have been developed to date, by varying the structures of the sugar, of the acyl chain or of the phytosphingosine moiety allowing to understand the structural features essential or relevant for the immmunological activity.

In particular, some of the analogues showed a tendency in switching immune responses towards Th1 or Th2 dominance with respect to αGalCer, depending on the structural modifications

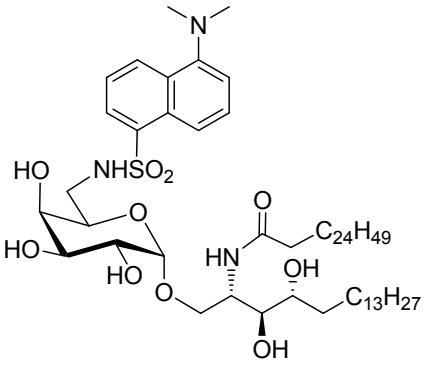
introduced. For example, an αGalCer derivative with a shortened sphingosine moiety (OCH analogue),[5] showed a Th2-biased NKT cell activation profile while the C-glycoside derivative of αGalCer showed Th1-polarizing action and an increased activity against malaria and B16-melanoma in mice.[6]

The biological interest towards modified αGalCer structures prompted the development of a huge number of synthetic approaches, in which researchers tried to optimize the synthetic schemes toward this class of compounds in term of yields, efficient glycosylation reactions and an overall reduced number of steps.[7] A first crucial aspect is the challenging stereoselective construction of 1,2-cis galactopyranosyl linkages. α-Gal-type glycosides can be formed by exploiting the anomeric effect and *in situ* anomerization,[8] by using appropriate solvents, and non-participating protecting groups at the C2-OH,[9] such as benzyl groups. Of particular interest are trimethylsilyl protecting groups[8b,c] which can give a rapid access to αGalCer structures. Often, galactosyl donors bearing a 4,6-*O*-benzylidene[10] (or 4,6-*O*-silylene[11]) protecting group are exploited as it can contribute to direct the attack of the acceptor from the α side, probably due to steric hindrance. [12] Moreover, a 4,6-*O*-arylidene protecting group allows an easy access to the C-6 position of galactose, for the development of αGalCer 6-OH modified analogues, which have been shown to have good or even enhanced biological activity.[13,14]



1 KRN7000

2 7DW8-5



3 Dansyl alpha-galactosylceramide

Figure 1 Structures of αGalCer and its analogues

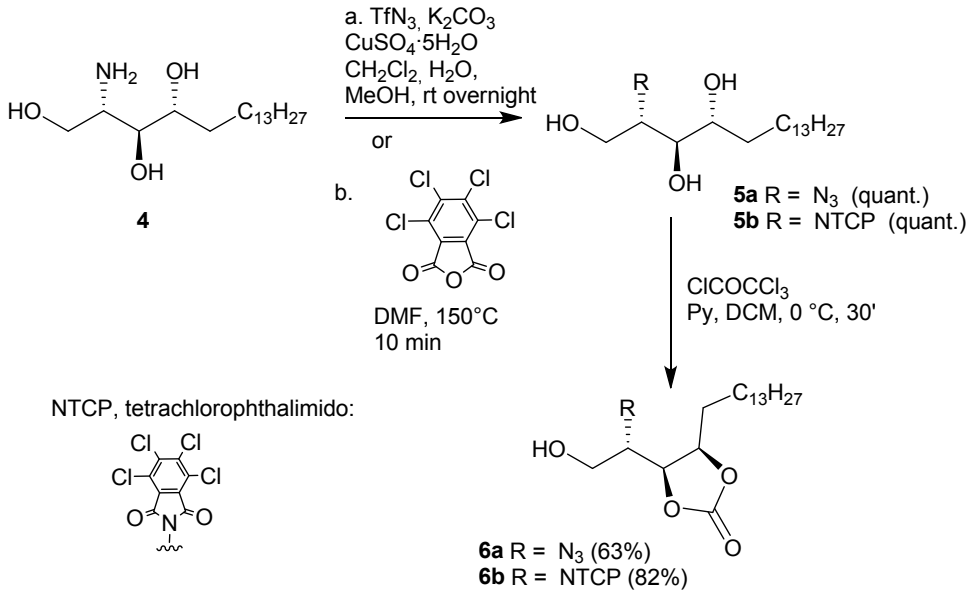
The other important aspect in the synthesis of αGalCer is represented by the choice of the sphingolipid acceptor for the glycosylation. For example, the use of ceramide allows quick access to αGalCer derivatives but usually with fair to low yields.[5] In fact, the presence of the amide on ceramide reduces the nucleophilicity of the primary hydroxyl group because of a hydrogen bond with the amide NH.[15] Alternative approaches use other types of phytosphingosine acceptors prepared either by protection of commercially available phytosphingosine or by synthesis from naturally occurring chiral precursors. In both cases, different steps of protection and deprotection are in general required. Commonly used protecting groups for the 2,3-OH groups of phytosphingosine are benzoyl,[16] benzyl,[17] isopropylidene,[18] silyl,[11,19] etc. In addition, *p*-methoxybenzyl ethers have found a convenient application in the synthesis of αGalCer derivatives.[20] One further different approach to αGalCer exploits the construction of the sphingoid backbone after the glycosylation of galactose with a proper phytosphingosine precursor,

such as a protected D-lyxose derivative. This strategy allows the access to different sphingosine analogues but does not permit to introduce modifications on the sugar part.[18,21]

In view of the above considerations, we developed a versatile synthesis for αGalCer and some analogues through a new versatile galactosyl sphingosine intermediate. To demonstrate the efficiency of the approach, we focused our efforts towards three representative structures (Figure 1) characterized by biological interest: αGalCer, 7DW8-5 **2**, a derivative characterized by a superior adjuvant activity on HIV and malaria vaccines in mice,[22] and the dansylated αGalCer **3** as a useful tool to monitor αGalCer uptake by cells.[23]

2. Results and discussion

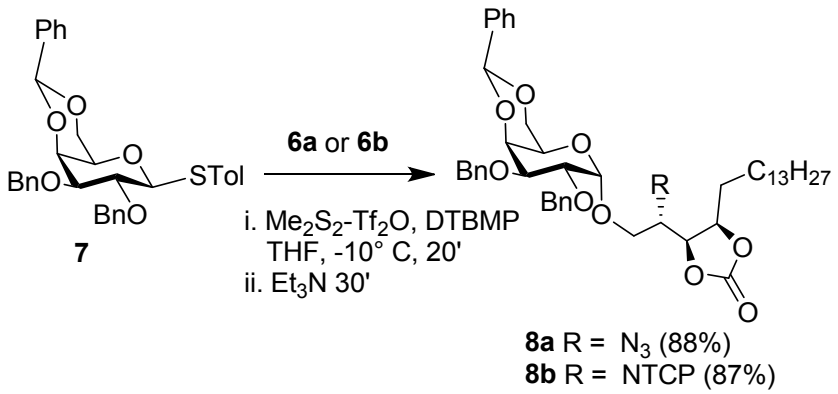
Our attention was initially focused on the sphingoid moiety. Key phytosphingosine acceptors were synthesized exploiting a cyclic carbonate as the protecting group for the 2,3-hydroxyl groups of phytosphingosine and masking the amino group as an azide or a tetrachlorophtalimide. The introduction of the carbonate 3,4-diol protecting group on phytosphingosine was reported only once in the literature[24] but never exploited to obtain phytosphingosine derivatives to be used as glycosyl acceptors in glycosylation reactions.



Scheme 1 Phytosphingosine acceptors synthesis.

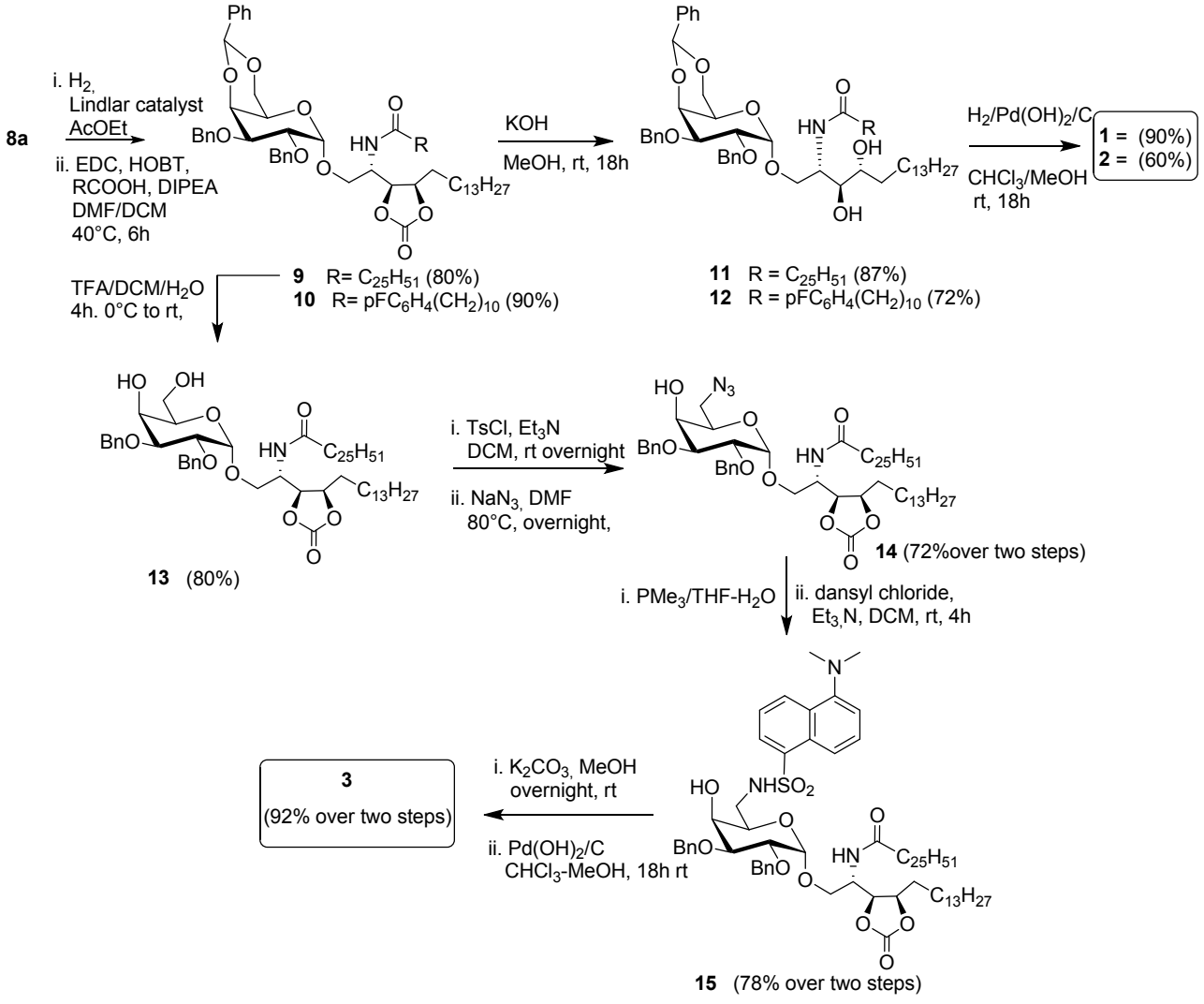
Commercial phytosphingosine **4** was converted into the azide **5a** by diazo-transfer[25] or into the tetrachlorophthalimido derivative **5b** according to our previously published procedure.[15] Differently from the approach described in ref. [24], 3,4-cyclic carbonate was introduced directly on triols **5a,b** as reported in Scheme 1. Each of the intermediates **5a** or **5b**, was dissolved in dry pyridine and added dropwise to a solution of an excess of trichloromethyl chloroformate in dichloromethane (DCM) at 0 °C. The reaction proceeded fast, and the mixture was quenched by pouring into ice-water after 30 min. After chromatographic purification, the cyclic carbonate **6a** was obtained in an acceptable 63% yield,[26] while **6b** was isolated in 82% yield by crystallisation.

4-Methylphenyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside **7**[27] was selected as glycosyl donor because of its stability, ease of preparation and for the α-directing effect of the benzylidene protecting group. The glycosylation reaction was performed on both acceptors **6a** and **6b** using Me_2S_2-Tf_2O as the promoter, according to the procedure reported by Fügedi.[28] (Scheme 2) and already exploited with the same donor but on a different acceptor in a previous synthesis of αGalCer analogues.[29]



Scheme 2: Glycosylation reaction

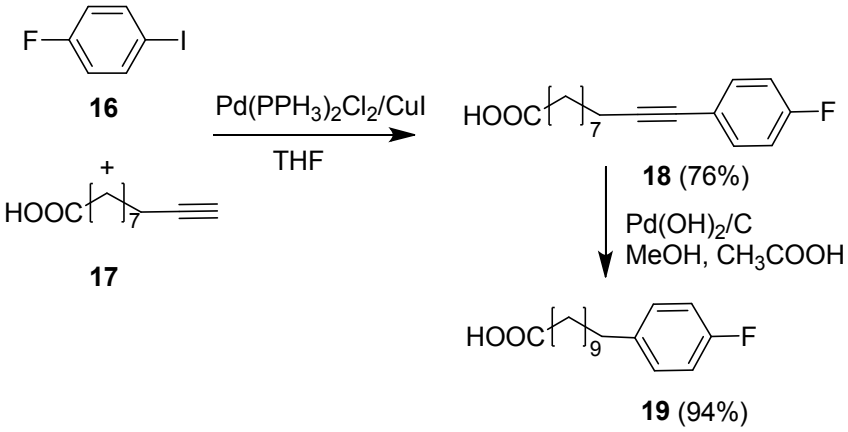
We were very pleased to obtain the expected products in high yield and complete stereoselectivity. Either a good match of donor-acceptor reactivity and the use of a buffered medium to perform the glycosylation contributed to our excellent results. We demonstrated that compounds **6a** and **6b** are feasible acceptors in the glycosylation with donor **7** that resulted more reactive than previously reported. [29] We then focused our attention on the introduction of the acyl chain on phytosphingosine.



Scheme 3 Synthesis of the final compounds

Disappointingly, when compound **8b** was submitted to standard tetrachlorophthalimide deprotection protocol (ethylendiamine in ethanol[30]), a complex mixture was obtained. Despite many attempts to deprotect either the NTCP or the cyclic carbonate or both in various conditions (ethylendiamine or hydrazine[31] in different conditions of solvent and temperature, sodium methoxide, potassium carbonate in methanol, sodium borohydride followed by treatment with acetic acid[32]), complex mixtures were always obtained. So intermediate **8b** was discarded. On the other hand, the azido intermediate **8a**, behaved as expected (Scheme 3). The azido group of **8a** was reduced by mild catalytic hydrogenation with Lindlar catalyst to the corresponding amine, which was condensed with hexacosanoic acid or with 11-(4-fluorophenyl)undecanoic acid **19** in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), *N,N*-diisopropylethylamine (DIPEA), and hydroxybenzotriazole (HOBt), to give compounds **9** and **10**.[17]

Acid **19** was prepared as shown in Scheme 4 in 71% yield over two steps, following a modified literature procedure.[33] Commercially available iodide **16** and alkyne **17** were coupled *via* Sonogashira reaction to give the alkyne **18** which afforded the carboxylic acid **19** by catalytic hydrogenation.



Scheme 4 Synthesis of the acid **19**

The hydrolysis of the cyclic carbonate on compounds **9** and **10** proceeded in mild conditions under Zémplen-like conditions with a catalytic amount of potassium hydroxide in methanol.[34] The so obtained diols **11** and **12** were directly subjected to catalytic hydrogenation to give the final compounds **1** and **2** in high yields.

The synthesis of compound **3** was more complex. Removal of the benzylidene protecting group from intermediate **9** in acidic conditions, using a mixture of TFA/DCM/H_2O, afforded diol **13**. The tosyl group was selectively introduced at the primary hydroxyl group of galactosyl moiety followed by nucleophilic substitution with sodium azide to obtain the 6-azido derivative **14**.[35] The azide was reduced to amine using the Staudinger reaction and directly submitted to sulphonylation by treatment with commercially available dansyl chloride in dichloromethane-triethylamine. Hydrolysis of the cyclic carbonate with potassium carbonate in methanol and final catalytic hydrogenation afforded in good yield the dansylated αGalCer **3**.

Conclusions

In conclusion, the synthetic route herein described allows an easy and convenient access to reported relevant αGalCer analogues. A new phytosphingosine acceptor was developed and obtained with a straightforward 2-steps synthesis. It performed satisfactorily in the glycosylation reaction to give the αGalCer backbone which was efficiently transformed into the target αGalCer derivatives **1**, **2**, and **3**. Indeed, the flexibility of our approach would permit the introduction of various acyl chains

on the ceramide moiety as well as modifications of the sugar part, so improving the access to αGalCer and analogues thereof.

Experimental section

Reagents were used as supplied without further purification unless otherwise stated. All reagents and solvents were purchased from TCI or Carlo Erba.

Thin layer chromatography was performed on silica gel plates with fluorescent indicator. Visualization was accomplished by UV light (254 and/or 365 nm) and/or by staining in ceric ammonium molybdate or sulfuric acid solution. Anhydrous solvents were obtained using activated molecular sieves (0.3 or 0.4 nm depending on the type of solvent). All reactions (if not specifically containing water as reactant, solvent or co-solvent) were performed under Argon atmosphere, in oven dried glassware. Flash column chromatography was performed following the procedure indicated on *J. Org. Chem*., 1978, **43**, 2923-2925, with 230-400 mesh silica gel. NMR spectra were recorded using a JEOL ECP 300 MHz spectrometer. Chemical shifts (δ) are quoted in parts per million referenced to the residual solvent peak. The multiplicity of each signal is designated using the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; quint, quintet; sext, sextet; hept, heptet; m, multiplet; br, broad. Coupling constants (*J*) are reported in Hertz (Hz). Mass spectra were recorded on a Thermo Finningan LCQ-deca XP-plus mass spectrometer equipped with an ESI source and an ion trap detector. High-resolution mass spectra were collected by electrospray ionization (ESI) spectroscopy on a QTof SYNAPT G2Si Mass Spectrometer Melting points were determined using a Stuart Scientific SMP3 apparatus and remain uncorrected. Optical rotations were measured on a JASCO P1010 polarimeter at 20°C.

(2*S*,3*S*,4*R*)-2-azidooctadecan-1-ol-cyclic 3,4-carbonate (6a)

Diphosgene (2.53 g, 12.80 mmol) was dissolved under Argon atmosphere in DCM (20 mL) and cooled at 0 °C. A solution formed by compound **5a** (0.88 g, 2.56 mmol) dissolved in Py (40 mL) was then added dropwise in 20'. After 30' the reaction mixture was slowly poured into ice (100 mL) and stirred for 1h. It was then transferred into a washing funnel, the layers separated and the aqueous was extracted with DCM (2x 100 mL), the organics combined and washed with HCl 1N (100 mL), brine, dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by flash chromatography (toluene/ethyl acetate 8:2) to obtain 0.60 g of a white solid (yield: 63%). Mp: 54-56 °C. $[\alpha]^{20}_D$: +48.5° (*c* 1.0, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.71 (m, 1H), 4.58 (dd, J_1 = 10.1 Hz, J_2 = 7.1 Hz, 1H), 4.12 (dd, J_1 = 11.6 Hz, J_2 = 2.8 Hz, 1H), 3.95 (dd, J_1 = 11.6 Hz, J_2 = 5.6 Hz, 1H), 3.71 (m, 1H), 2.10 (bs, 1H), 1.68 (m, 4H), 1.30 – 1.10 (m, 22H), 0.87 (t, J = 6.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.8, 79.7, 75.8, 62.7, 59.8, 32.0, 29.79, 29.77, 29.75, 29.69, 29.6, 29.5, 29.3, 29.0, 25.6, 22.8, 14.2. MS (ESI) *m/z* Calculated for $C_{19}H_{35}N_3O_4$: 369; Found: 392 $[M+Na]^+$. HRMS(ESI) calcd for $[C_{19}H_{35}N_3O_4 + Na]^+$: 392.2525, found: 392.2527.
IR (neat, cm^{-1}): 2918, 2850, 2144, 2103, 1789.

(2*S*,3*S*,4*R*)-2-tetrachlorophthalimidooctadecan-1-ol-cyclic 3,4-carbonate (6b)

Diphosgene (0.42 g, 2.12 mmol) was dissolved under Argon atmosphere in DCM (25 mL) and cooled at 0 °C. A solution formed by compound **5b** (0.50 g, 0.85 mmol) dissolved in Py (5 mL) was

then added dropwise in 20'. After 30' the reaction mixture was slowly poured into ice (100 mL) and stirred for 1h. It was then transferred into a washing funnel, the layers separated and the aqueous was extracted with DCM (2 x 100 mL), the organics combined and washed with HCl 1N (100 mL), brine, dried over Na_2SO_4 and concentrated under vacuum. The residue was crystallized by hot ethanol to obtain 0.43 g of a yellow solid (yield: 82%). Mp: 148-150 °C. $[\alpha]^{20}_D$: +9.5° (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.60 (dd, J_1 = 10.5 Hz, J_2 = 7.0 Hz, 1H), 4.73 – 4.55 (m, 2H), 4.10 (d, J = 6.1 Hz, 2H), 1.89 – 1.62 (m, 2H), 1.54 (d, J = 3.9 Hz, 1H), 1.40 – 1.10 (m, 24H), 0.87 (t, J = 6.7 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 163.6, 141.0, 130.3, 127.0, 79.4, 74.8, 60.4, 52.0, 32.0, 29.7, 29.5, 29.4, 29.0, 28.7, 25.3, 22.8, 14.3. MS (ESI) m/z Calculated for $C_{27}H_{35}Cl_4NO_6$: 611; Found: 610 $[M-1]^-$. HRMS(ESI) calcd for $[C_{27}H_{35}{}^{35}Cl_4NO_6 + Na]^+$: 632.1116, found: 632.1116.
IR (neat, cm^{-1}) 3433, 2918, 2051, 1754, 1718, 1470, 1389, 1364, 1350, 1095, 736.

(2*S*,3*S*,4*R*)-1-*O*-(2,3-di-O-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl)-2-azido-octadecan-1-ol-cyclic-3,4-carbonate (8a)

Preparation of Me_2S_2-Tf_2O (1.0 M): to a solution of dimethyl disulphide (1 mL, 11.3 mmol), in 7.5 mL of dry DCM cooled at -10 °C was added triflic anhydride (1.7 mL, 10 mmol). The mixture was stirred for 30' and the Me_2S_2-Tf_2O was obtained.

Compound **7** (0.83 g, 1.50 mmol), compound **6a** (0.83 g, 2.25 mmol) and 2,6-di tertbutyl-4-methyl pyridine (0.30 g, 1.50 mmol) were dissolved in dry THF (12.5 mL) under argon atmosphere and molecular sieves 4A° were added. The mixture was stirred at rt for 1h then cooled to -10 °C in a salt-ice bath before adding Me_2S_2-Tf_2O (1.0 M, 3 mL). After stirring for 20', the reaction was quenched with Et_3N (0.7 mL), diluted with DCM (20 mL). The organic layer was washed with water (2 x 40 mL), brine, dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (toluene/ethyl acetate 95:5) to obtain 1.05 g of a white waxy compound (yield: 88%). $[\alpha]^{20}_D$: +46.2° (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.52-7.25 (m, 15H), 5.49 (s, 1H), 5.01 (d, J = 3.7 Hz, 1H), 4.87 (d, J = 11.6 Hz, 1H), 4.79 (d, J = 11.6 Hz, 1H) 4.78 (m, 1H), 4.74 (d, J = 12.2 Hz, 1H), 4.64 (d, J = 11.3 Hz, 1H), 4.54 (ddd, J_1 = 10.2 Hz, J_2 = 7.3 Hz, J_3 = 2.8 Hz, 1H), 4.28-4.08 (m, 4H), 4.07-3.93 (m, 2H), 3.76 (dd, J_1 = 11.1 Hz, J_2 = 4.4 Hz, 1H), 3.67 (br s, 1H), 3.54 (ddd, J_1 = 10.1 Hz, J_2 = 4.2 Hz, J_3 = 2.4 Hz, 1H), 1.76-1.52 (m, 3H), 1.39-1.21 (m, 23H), 0.89 (t, J = 6.7 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.5, 138.8, 138.7, 137.9, 129.0, 128.6, 128.4, 128.3, 127.90, 127.85, 127.8, 127.7, 126.4, 101.2, 100.0, 79.4, 75.9, 75.7, 75.1, 74.7, 73.8, 72.2, 69.4, 68.4, 63.4, 57.9, 32.0, 29.8, 29.7, 29.6, 29.5, 29.3, 29.0, 25.6, 22.8, 14.2. MS (ESI) m/z Calculated for $C_{46}H_{61}N_3O_9$: 799; Found: 822 $[M+Na]^+$. HRMS(ESI) calcd for $[C_{46}H_{61}N_3O_9 + Na]^+$: 822.4306, found: 822.4309. IR (neat, cm^{-1}): 2923, 2853, 2104, 1807, 1454, 1099, 1050, 1028, 997, 740, 697.

(2*S*,3*S*,4*R*)-1-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl)-2-tetrachlorophthalimidooctadecan-1-ol-cyclic 3,4-carbonate (8b)

Preparation of Me_2S_2-Tf_2O (1.0 M): to a solution of dimethyl disulphide (1 mL, 11.3 mmol), in 7.5 mL of dry DCM cooled at -10 °C was added triflic anhydride (1.7 mL, 10 mmol). The mixture was stirred for 30' and the Me_2S_2-Tf_2O was obtained.

Compound **7** (1.00 g, 1.80 mmol), compound **6b** (1.43 g, 2.34 mmol) and 2,6-di tertbutyl-4-methyl pyridine (0.37 g, 1.80 mmol) were dissolved in dry THF (15 mL) under argon atmosphere and molecular sieves 4A° were added. The mixture was stirred at rt for 1h then cooled to -10 °C in a salt-ice bath before adding Me_2S_2-Tf_2O (1.0 M, 3.6 mL). After stirring for 20', the reaction was quenched with Et_3N (0.8 mL), diluted with DCM (20 mL). The organic layer was washed with water (2 x 40 mL), brine, dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (cyclohexane/ethyl acetate 9:1) to obtain 1.64 g of a white solid (yield: 87%). Mp: 103-105 °C. $[\alpha]^{20}_D$: +19.0° (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.58 – 6.90 (m, 15H), 5.59 (dd, J_1 = 10.7 Hz, J_2 =7.0 Hz, 1H), 5.44 (s, 1H), 4.85 (d, J = 3.2 Hz, 1H), 4.82 – 4.60 (m, 4H), 4.33 (s, 2H), 4.28 – 4.16 (m, 2H), 4.12 (d, J = 3.0 Hz, 1H), 4.01 (d, J = 12.6 Hz, 1H), 3.95 – 3.84 (m, 2H), 3.80 (dd, J_1 = 10.0 Hz, J_2 =3.2 Hz, 1H), 3.64 (s, 1H), 1.83 – 1.64 (m, 1H), 1.60 – 1.48 (m, 1H), 1.30 -1.10 (m, 24H), 0.88 (t, J = 6.6 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.7, 153.4, 140.7, 138.8, 138.2, 137. 9, 129.8, 128.9, 128.5, 128.20, 128.17, 127.8, 127.7, 127.5, 127.0, 126.4, 126.2, 101.0, 99.3, 79.4, 76.3, 76.0, 74.7, 74.6, 73.0, 72.6, 69.4, 65.8, 63.4, 50.1, 32.0, 29.76, 29.72, 29.54, 29.46, 29.4, 29.1, 28.7, 25.4, 22.8, 14.2. MS (ESI) m/z Calculated for $C_{51}H_{61}Cl_4NO_{11}$: 1042; Found: 1065 $[M+Na]^+$. HRMS(ESI) calcd for $[C_{51}H_{61}Cl_4{}^{35}NO_{11} + Na]^+$: 1062.2896, found 1062.2902. IR (neat, cm^{-1}): 2923, 2853, 1806, 1723, 1454, 1392, 1371, 1349, 1099, 1053, 739.

(2*S*,3*S*,4*R*)-1-*O*-(2,3-di-O-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl)-2-(hexacosanoyl)amino-octadecan-1-ol-cyclic-3,4-carbonate (9)

Under Ar atmosphere 0.63 g (0.79 mmol) of **8a** were dissolved in 60 mL of ethyl acetate and carefully degassed. Lindlar catalyst (0.63 g) was added and the reaction was stirred under H_2 atmosphere overnight. After restoring Ar atmosphere, solution was filtered through a plug of Celite and solvent was removed on vacuum. The amine was directly used without any further purification and split in two batch. Part of the crude, (0.46 g, 0.59 mmol) was then dissolved under Ar atmosphere in 16.7 mL of anhydrous DCM. The obtained solution was added to a solution previous prepared dissolving (under Ar atmosphere at 0 °C) the hexacosanoic acid (0.41 g, 1.04 mmol), DIPEA (0.34 g, 2.6 mmol), EDC (0.33 g, 1.73 mmol), HOBt (0.23 g, 1.73 mmol), in DMF (8.4 mL). The solution was stirred overnight at 40 °C and then it was allowed to cool-down to ambient. The solution was diluted with diethyl ether and washed with a 1N solution of HCl, a saturated solution of $NaHCO_3$, water and brine. After drying with $MgSO_4$, solvent was evaporated under vacuum and the crude was purified by flash chromatography, (cyclohexane/ethyl acetate 8:2) to give 0.53 g of pure **9** as waxy solid, in 80% yield. $[\alpha]^{20}_D$: +60.9° (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.52-7.25 (m, 15H), 6.00 (d, J = 8.9 Hz, 1H), 5.49 (s, 1H), 5.03 (d, J = 3.1 Hz, 1H), 4.90 (d, J = 11.0 Hz, 1H), 4.82-4.66 (m, 3H), 4.61 (d, J = 11.0 Hz, 1H), 4.44-4.18 (m, 4H), 4.11 (dd, J_1 = 10.1 Hz, J_2 = 3.0 Hz, 1H), 4.10 - 3.80 (m, 2H), 3.74 (m, 2H), 3.61 ($s_{app,}$ 1H), 2.03 (m, 2H), 1.70 -1.50 (m, 5H), 1.40 - 1.10 (m, 67H), 0.88 (t, J = 6.5 Hz, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 172.8, 154.0, 138.6, 138.2, 137.8, 129.1, 128.7, 128.5, 128.3, 128.2, 128.0, 127.9, 127.8, 126.4, 101.2, 100.0, 79.8, 76.10, 75.96, 74.6, 74.1, 71.5, 69.4, 67.8, 63.2, 47.6, 36.8, 32.0, 29.8, 29.6, 29.54, 29.48, 29.4, 29.2, 25.7, 25.6, 22.8, 14.2. MS (ESI) m/z Calculated for $C_{72}H_{113}NO_{10}$: 1152; Found: 1175 $[M+Na]^+$. HRMS(ESI) calcd for $[C_{72}H_{113}NO_{10} + Na]^+$: 1174.8262, found 1174.8270. IR (neat, cm^{-1}): 3274, 2917, 2850, 1829, 1541, 1469, 1101, 1053.

(2*S*,3*S*,4*R*)-1-*O*-(2,3-di-*O*-benzyl-α-D-galactopyranosyl)-2-(hexacosanoyl)amino-octadecan-1-ol-cyclic-3,4-carbonate (13)

Compound **9** (160 mg, 0.14 mmol) was dissolved in a mixture of DCM (11 mL) and water (0.5 mL). After cooling at 0°C, trifluoracetic acid (1 mL) was added and the mixture was left at 0°C for 2 h and at rt for 2h. The organic phase was washed with water (2 x 10 mL), brine, dried over $MgSO_4$ and concentrated under vacuum. The crude was purified by flash chromatography (cyclohexane/ethyl acetate 4:6) to give 117 mg of pure **13** as waxy solid, in 80% yield. $[\alpha]^{20}_D$: +52.3° (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.38-7.32 (m, 10H), 6.31 (d, J = 9.2 Hz, 1H), 4.87 (d, J = 3.0 Hz, 1H), 4.84-4.67 (m, 4H), 4.59 (d, J = 11.4 Hz, 1H), 4.55-4.45 (m, 1H), 4.38 (m, 1H), 4.11 (br s, 1H), 3.94-3.66 (m, 6H), 3.70 (dd, J_1 = 11.3 Hz, J_2 = 3.1 Hz, 1H), 2.49 (br s, 2H), 2.10 (m, 2H), 1.73 (m, 1H), 1.54 (m, 4H), 1.24 (m, 67H), 0.87 (t, J = 6.1 Hz, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.1, 154.2, 138.0, 137.97, 128.7, 128.6, 128.3, 128.1, 128.0, 99.6, 80.0, 77.4, 76.1, 74.0, 72.5, 70.2, 68.7, 68.4, 62.9, 57.8, 47.5, 36.6, 32.0, 29.8, 29.6, 29.5, 28.6, 25.7, 22.8, 14.2. MS (ESI) m/z Calculated for $C_{65}H_{109}NO_{10}$: 1064; Found: 1087 $[M+Na]^+$. HRMS(ESI) m/z calcd for $[C_{65}H_{109}NO_{10} + Na]^+$: 1086.7949, found 1086.7950. IR (neat, cm^{-1}): 3728, 2918, 2850, 1801, 1467, 1364, 1047.

(2*S*,3*S*,4*R*)-1-*O*-(6-azido-6-deoxy-2,3-di-*O*-benzyl-α-D-galactopyranosyl)-2-(hexacosanoyl)aminooctadecan-1-ol-cyclic-3,4-carbonate (14)

To a solution of **9** (117 mg, 0.11 mmol) in 2.5 mL of anhydrous DCM was added TsCl (63 mg, 0.33 mmol) followed by Et_3N (77 µL, 0.55 mmol) at 0 °C. The reaction mixture was raised up to rt and stirred for 8 h. Saturated aqueous NH_4Cl was slowly added to neutral. Two layers were separated, and the aqueous layer was extracted with DCM (3 x 20 mL). The organics were combined and dried over $MgSO_4$, and then concentrated *in vacuo*. The resulting residue was purified by flash chromatography (cyclohexane/ethyl acetate 8:2) to afford the intermediate 6-tosylate (117 mg, 87%, white solid). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.75 (d, J = 8.0, 2H), 7.34 (m, 12H), 6.26 (d, J = 8.8 Hz, 1H), 4.77 (d, J = 11.6 Hz, 1H), 4.71 (m, 5H), 4.57 (d, J = 11.5 Hz, 1H), 4.35 (m, 1H), 4.2 – 4.0 (m, 3H), 3.98 (m, 2H), 3.81 (m, 2H), 3.63 (d, J = 10.1 Hz, 1H), 2.4 (s, 3H), 2.13 (m, 2H), 1.62 (m, 8H), 1.24 (m, 65H), 0.87 (t, J = 6.4 Hz, 6H). To a solution of the intermediate (110 mg, 0.09 mmol) in 4.7 mL of anhydrous DMF was added NaN_3 (17 mg, 0.27 mmol). The reaction mixture was stirred at 80 °C overnight and cooled to rt. The reaction was diluted with 10 mL of water and diethyl ether (1:1), the phases separated. The aqueous was extracted with diethyl ether (2 x 10 mL). The organics combined were washed with water (2 x 10 mL), brine, dried over $MgSO_4$ and concentrated *in vacuo*. The crude was purified by flash chromatography (cyclohexane/ethyl acetate 8:2) to afford **14** (76 mg, 78%, yellow oil). $[\alpha]^{20}_D$: +47.9° (*c* 1.0, $CHCl_3$).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.35 (m, 10H), 6.16 (d, J = 9.3 Hz, 1H), 4.87 (d, J = 3 Hz, 1H), 4.83-4.67 (m, 4H), 4.59 (d, J = 11.3 Hz, 1H), 4.49 (t, J = 8.2 Hz, 1H), 4.39 (t, J = 9.2 Hz, 1H), 3.97 (br s, 1H), 3.83 (m, 4H), 3.73 (dd, J_1 = 11.3 Hz, J_2 = 2.9 Hz, 1H), 3.62 (m, 1H), 3.37 (dd, J_1 = 12.5 Hz, J_2 = 4.3 Hz, 1H), 2.4 (br s, 1H), 2.11 (m, 2H), 1.58 (m, 5H), 1.25 (m, 67H), 0.87 (t, J =5.8, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 172.8, 154.0, 137.9, 137.8, 128.8, 128.7, 128.3, 128.2, 128.1, 128.0, 99.4, 79.9, 77.1, 76.0, 74.1, 72.7, 69.5, 69.0, 67.7, 51.6, 47.3, 37.2, 32.0, 29.8, 29.5, 29.46, 29.4, 29.2, 28.6, 25.7, 22.8, 14.2. MS (ESI) m/z Calculated for $C_{65}H_{108}N_4O_9$: 1089; Found: 1112 $[M+Na]^+$. HRMS(ESI) calcd for $[C_{65}H_{108}N_4O_9 + Na]^+$: 1111.8014, found 1111.8022. IR (neat, cm^{-1}): 2918, 2848, 2100, 1802, 1655, 1540, 1467, 1264, 1055.

(2*S*,3*S*,4*R*)-1-*O*-(6-deoxy-6[*N*-(5-[dimethylamino]napth-1-ylsulfonyl)amino]2,3-di-*O*-benzyl-α-D-galactopyranosyl)-2-(hexacosanoyl)amino-octadecan-1-ol-cyclic-3,4-carbonate (15)

PMe_3 (10 µL, 0.096 mmol) was added to a solution of compound **14** (75 mg, 0.064 mmol) in THF (2 mL) at r.t. The resulting solution was stirred for 5 h. H_2O (10 µL) was then added and the reaction mixture was stirred for 1 h before being concentrated under reduced pressure. The residue was diluted in ethyl acetate and washed with water, brine, dried over $MgSO_4$ and evaporated to give the intermediate 6-amine. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.50 – 7.27 (m, 10H), 6.60 (d, *J* = 8.9 Hz, 1H), 4.89 (d, = 3.2 Hz, 1H), 4.85 – 4.70 (m, 4H), 4.60 (d, *J* = 11.3 Hz, 1H), 4.45 (m, 1H), 4.33 (m, 1H), 4.15 (br s, 1H), 3.96 (dd, J_1 = 9.8 Hz, J_2 = 3.3 Hz, 1H), 3.86 – 3.56 (m, 4H), 3.05 (br s, 2H), 2.86 (br s, 3H), 2.07 (m, 2H), 1.77 – 1.45 (m, 5H), 1.40 – 1.00 (m, 67H), 0.87 (t, *J* = 6.3 Hz, 6H).

The crude amine was dissolved in dry DCM, and dansyl chloride (17 mg, 0.064 mmol) and triethylamine (0.32 mmol) were sequentially added. The mixture was stirred at rt for 4h, the solvent was evaporated and the crude was directly purified by flash chromatography (cyclohexane/ethyl acetate 7:3) to afford **15** (65 mg, 78% over two steps, yellow wax). $[\alpha]^{20}_D$: +38.4° (*c* 0.5, MeOH). ^{1}H NMR ($CDCl_3$, 300 MHz) *δ* 8.56 (d, *J* = 7.8 Hz, 1H), 8.25 (d, *J* = 8.8 Hz, 1H), 8.23 (d, *J* = 7.4 Hz, 1H), 7.53 (m, 2H), 7.38 (m, 10H), 7.18 (d, *J* = 7.3 Hz, 1H), 6.29 (d, *J* = 8.6 Hz, 1H), 5.48 (br s, 1H), 4.72 (m, 5H), 4.55 (m, 2H), 4.39 (m, 1H), 3.97 (br s, 1H), 3.91 (m, 1H), 3.82-3.62 (m, 4H), 3.12 (m, 2H), 2.89 (s, 6H), 2.35 (br s, 1H), 2.14 (m, 2H), 1.65 – 1.45 (m, 5H), 1.25 (m, 67H), 0.87 (t, *J* = 6.5 Hz, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz) *δ* 173.1, 154.3, 151.5, 138.0, 137.9, 134.6, 130.6, 129.8, 129.7, 128.7, 128.6, 128.5, 128.2, 128.1, 128.0, 127.95, 123.5, 119.1, 115.5, 99.4, 80.1, 77.3, 75.9, 73.9, 72.5, 68.9, 67.6, 47.5, 45.6, 43.9, 36.7, 32.0, 29.8, 29.7, 29.6, 29.5, 29.2, 28.6, 25.73, 25.69, 22.8, 14.2. MS (ESI) m/z Calculated for $C_{77}H_{121}N_3O_{11}S$: 1296; Found: 1297 $[M+1]^+$. HRMS(ESI) calcd for $[C_{77}H_{121}N_3O_{11}S + Na]^+$: 1318.8620, found 1318.8610. IR (neat, cm^{-1}): 3352, 2922, 2852, 1804, 1658, 1531, 1457, 1320, 1143, 1049, 791.

(2*S*,3*S*,4*R*)-1-*O*-(6-deoxy-6[*N*-(5-[dimethylamino]napth-1-ylsulfonyl)amino]-α-D-galactopyranosyl)-2-(hexacosanoyl)amino-octadecan-1,3,4-triol (3)[23b]

To a solution of compound **15** (50 mg, 0.038 mmol) in MeOH (0.8 mL) was added K_2CO_3 (5 mg, 0.038 mmol, 1.0 equiv). After stirring at rt overnight, the mixture was diluted with ethyl acetate and organic layer washed with HCl 1N (10 mL), water, brine, dried over $MgSO_4$ and concentrated to provide the diol in quantitative yield. $Pd(OH)_2$ on carbon (10 mg) was added to a solution of the crude dissolved in a mixture of $CHCl_3$/EtOH (5 mL, 3/2, v/v) and the reaction stirred under H_2 for 17 h. at rt. The reaction mixture was then filtered through a Celite pad, the Celite was washed thoroughly with $CHCl_3$/EtOH (3/2, v/v), and the filtrate concentrated. The residue was purified by chromatography column (CH_2Cl_2/MeOH 9:1) obtaining a yellow wax (37 mg, 90% yield over two steps). ^{1}H NMR (Pyridine-*d*-5, 300 MHz) *δ* 9.73 (1H, t, *J* = 5.0 Hz), 9.02 (d, *J* = 8.6 Hz, 1H), 8.58 (d, *J* = 7.0 Hz, 1H), 8.53 (d, *J* = 8.3 Hz, 1H), 8.43 (d, *J* = 8.6 Hz, 1H), 7.50 (m, 2H), 7.11 (d, *J* = 7.6 Hz, 1H), 5.44 (d, *J* = 3.1 Hz, 1H,), 5.23 (m, 1H), 4.52 (m, 3H), 4.30-4.28 (m, 3H), 4.23 (dd, J_1 = 9.8 Hz, J_2 = 2.8 Hz, 1H), 4.16 (dd, J_1 = 11.3 Hz, J_2 = 5.5 Hz, 1H), 3.85 (t, *J* = 5.5 Hz, 2H), 2.72 (s, 6H), 2.44 (m, 3H), 1.82 (m, 5H), 1.31-1.23 (m, 66H), 0.86 (t, *J* = 6.7 Hz, 6H). ^{13}C NMR (Pyridine-*d*-5, 75 MHz) *δ* 173.0, 152.0, 137.4, 130.4, 130.3, 129.9, 128.9, 128.0, 120.3, 115.5, 101.1, 76.7, 72.3, 71.1, 71.0, 70.8, 69.7, 68.3, 50.9, 45.1, 44.8, 36.7, 34.4, 32.0, 30.3, 30.1, 29.9, 29.5, 26.4, 26.3, 22.8, 14.2. MS (ESI) m/z Calculated for $C_{62}H_{111}N_3O_{10}S$: 1090; Found: 1091 $[M+1]^+$.

11-(4-fluorophenyl)undec-9-ynoic acid (18)[32]

4-Fluoroiodobenzene (2.44 g, 11.00 mmol) was dissolved in dry THF under argon atmosphere (10 mL), $Pd(PPh_3)_2Cl_2$ (0.10 g, 0.14 mmol, 2.5 mol %), CuI (50 mg, 0.27 mmol, 5 mol %), $i Pr_2NH$ (1.67 g, 16.46 mmol) and the undec-10-ynoic acid (1.00 g, 5.49 mmol) were sequentially added. The mixture was stirred at rt for 3 h. The mixture was diluted with ethyl acetate and washed with HCl 1N (2 x 100 mL), water, brine, dried over $MgSO_4$ and concentrated under vacuum. The crude was purified by flash chromatography (toluene/ethyl acetate 6:4) to give 1.27 g of a pure yellow solid, in 76% of yield. MP: 66-68 °C

^1H NMR ($CDCl_3$, 300 MHz) δ 10.2 (br s, 1H), 7.35 (m, 2H), 6.97 (m, 2H), 2.37 (m, 4H), 1.62 (m, 4H), 1.34 (m, 8H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 180.5, 162.1 (d, J_{CF} = 246 Hz), 133.35 (d, J_{CF} = 7.9 Hz), 120.2 (d, J_{CF} = 2.9 Hz), 115.4 (d, J_{CF} = 21.8 Hz), 90.1, 79.6, 34.2, 29.2, 29.1, 29.0, 28.9, 28.8, 24.7, 19.4. ^{19}F ($CDCl_3$, 282 MHz) δ -112, MS (ESI) m/z Calculated for $C_{17}H_{21}FO_2$: 276; Found: 275 $[M-1]^-$.

11-(4-fluorophenyl)undecanoic acid (19)[32]

$Pd(OH)_2$ on carbon (0.30 g) was added to a solution of compound **18** (1.20 g, 4.3 mmol), dissolved in a mixture of MeOH (30 mL) and acetic acid (1.5 mL) and the reaction stirred under H_2 for 17 h. at rt. The mixture was then filtered through Celite, the Celite was washed thoroughly with methanol and the filtrate was concentrated. The residue was concentrated obtaining a white solid. (1.13 g, 94% yield). MP: 70-72 °C. ^1H NMR ($CDCl_3$, 300 MHz) δ 11.0 (br s, 1H), 7.11 (m, 2H), 6.95 (m, 2H), 2.57 (t, J = 7.6 Hz, 2H), 2.35 (t, J = 7.4 Hz, 2H), 1.62 (m, 4H), 1.34 (m, 12H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 180.5, 161.2 (d, J_{CF} = 241 Hz), 138.5 (d, J_{CF} = 2.3 Hz), 129.7 (d, J_{CF} = 7.6 Hz), 114.9 (d, J_{CF} = 20.8 Hz), 35.2, 34.2, 31.7, 29.6, 29.52, 29.48, 29.3, 29.2, 29.1, 24.8. ^{19}F ($CDCl_3$, 282 MHz) δ -118. MS (ESI) m/z Calculated for $C_{17}H_{25}FO_2$: 280; Found: 279 $[M-1]^-$.

(2*S*,3*S*,4*R*)-1-*O*-(2,3-di-O-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl)-2-[11-(4-fluorophenyl)undecanoyl]amino-octadecan-1-ol-cyclic 3,4-carbonate (10)

The second part of the crude amine produced after reduction of **8a**, (150 mg, 0.19 mmol) was then dissolved under Ar atmosphere in 5 mL of anhydrous DCM. The obtained solution was added to a solution previously prepared dissolving (under Ar atmosphere at 0 °C) the compound **19** (87 mg, 0.31 mmol), DIPEA (100 mg, 0.77 mmol), EDC (99 mg, 0.52 mmol), HOBt (70 mg, 0.52 mmol), in DMF (2.5 mL). The solution was stirred overnight at 40 °C and then it was allowed to cool-down to ambient. The solution was diluted with diethyl ether and washed with a 1N solution of HCl, a saturated solution of $NaHCO_3$, water and brine. After drying with $MgSO_4$, the solvent was evaporated under vacuum and the crude was purified by flash chromatography, (cyclohexane/ethyl acetate 8:2) to give 181 mg of pure **10** as yellow solid, in 90% yield. $[\alpha]^{20}_D$: +72.9° (*c* 1.0, $CHCl_3$). MP 70-72 °C. ^1H NMR ($CDCl_3$, 300 MHz) δ 7.53-7.33 (m, 15H), 7.10 (t, J = 8.0 Hz, 2H), 6.94 (t, J = 8.4 Hz, 2H), 6.03 (br d, J = 7.4 Hz, 1H), 5.49 (s, 1H), 5.02 (d, J = 2.5 Hz, 1H), 4.90 (d, J = 11.1 Hz, 1H), 4.77-4.69 (m, 3H), 4.61 (d, J = 11.3 Hz, 1H), 4.37 (m, 2H), 4.28-3.88 (m, 5H), 3.73 (br s, 2H), 3.67 (br s, 1H), 2.56 (t, J = 7.6 Hz, 2H), 2.04 (m, 2H), 1.54 (m, 7H), 1.20 (m, 35H), 0.88 (t, J = 6.3 Hz, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 172.8, 161.1 (d, J_{CF} = 249 Hz), 154.0, 138.6, 138.5, 138.47 (d, J_{CF} = 3 Hz), 138.2, 137.8, 129.72 (d, J_{CF} = 7.5 Hz), 129.1, 128.7, 128.5, 128.2, 127.8, 126.4, 115.0 (d, J_{CF} = 20.3 Hz), 101.2, 100.0, 79.9, 77.2, 76.1, 76.0, 74.6, 74.1, 71.6, 69.4, 67.7, 63.2, 47.6, 36.7, 35.2, 30.4, 30.1, 29.8, 29.6, 29.54, 29.48, 29.38, 29.30, 27.0, 25.7, 22.8, 14.2. ^{19}F ($CDCl_3$, 282 MHz) δ -118. MS (ESI) m/z Calculated for $C_{63}H_{86}FNO_{10}$: 1036; Found: 1059

$[M+Na]^+$. HRMS(ESI) calcd for $[C_{63}H_{86}FNO_{10} + Na]^+$: 1058.6133, found 1058.6143. IR (neat, cm^{-1}): 3527, 3374, 2918, 2850, 1800, 1686, 1509, 1095, 1073, 1040, 748.

(2*S*,3*S*,4*R*)-1-*O*-(α-D-galactopyranosyl)-2-[11-(4-fluorophenyl)undecanoyl]amino-octadecan-1,3,4-triol (2)[22b]

To a solution of compound **10** (158 mg, 0.15 mmol) in MeOH (6 mL) was added KOH (2 mg, 0.03 mmol). After stirring at rt overnight, 1 mL of water was added and the white solid **12** was filtered and washed with water and methanol and directly used for the next step. (110 mg, yield: 72%) ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.51-7.32 (m, 15H), 7.10 (t, J = 7.6 Hz, 2H), 6.94 (t, J = 8.6 Hz, 2H), 6.28 (br s, 1H), 5.47 (s, 1H). 4.97 (d, J = 2.7 Hz, 1H), 4.94 (d, J = 11.6 Hz, 1H), 4.76 (s, 2H), 4.67 (d, J = 11.2 Hz, 1H), 4.21 (m, 3H), 4.08 (m, 1H), 3.95 (m, 3H,), 3.82 (m, 1H), 3.47 (m, 3H), 2.56 (t, 1H, J = 7.4 Hz, 2H), 2.13 (m, 2H), 1.57 (m, 6H), 1.26 (m, 36H,), 0.88 (t, 1H, J = 5.5 Hz, 3H). MS (ESI) m/z Calculated for $C_{62}H_{88}FNO_9$: 1009; Found: 1010 $[M+1]^+$. $Pd(OH)_2$ on carbon (24 mg) was added to a solution of the crude dissolved in a mixture of $CHCl_3$/EtOH (8 mL, 1:1) and the reaction stirred under H_2 for 17 h. at rt. The reaction mixture was then filtered through Celite, which was thoroughly washed with $CHCl_3$/EtOH (1:1), and the filtrate was concentrated. The residue was purified by chromatography column (CH_2Cl_2/MeOH 9:1) to give **(2)** as a colourless wax. $[\alpha]^{20}_D$: +26.2° (*c* 0.5, CH_3OH:$CHCl_3$ 1:1). ^{1}H NMR (Pyridine-*d*-5, 300 MHz) δ 8.56 (d, J = 8.6 Hz, 1H), 7.16-7.07 (m, 4H), 5.55 (d, J = 3.7 Hz, 1H), 5.24 (m, 1H), 4.64 (m, 2H), 4.50 (m, 2H), 4.43-4.35 (m, 4H), 4.30 (br s, 2H), 2.51 (t, J = 7.4 Hz, 2H), 2.43 (t, J = 7.4 Hz, 2H), 2.27 (br s, 1H), 1.89-1.66 (m, 3H), 1.51 (m, 2H), 1.22 (m, 36H), 0.85 (t, J = 6.7 Hz, 3H). ^{13}C NMR (Pyridine-*d*-5, 75 MHz) δ 173.1, 161.4 (d, J_{CF} = 240 Hz), 138.95 (d, J_{CF} = 3.1 Hz), 130.2 (d, J_{CF} = 7.5 Hz), 115.2 (d, J_{CF} = 20.2 Hz), 115.0, 101.4, 76.6, 72.9, 72.4, 71.5, 70.9, 70.2, 68.5, 62.5, 51.3, 36.6, 35.1, 34.2, 32.0, 31.7, 30.2, 30.0, 29.9, 29.8, 29.7, 29.62, 29.58, 29.5, 29.3, 26.4, 26.2, 22.8, 14.2. ^{19}F (Pyridine-*d*-5, 282 MHz) δ -118. MS (ESI) m/z Calculated for $C_{41}H_{72}FNO_9$: 741; Found: 740 $[M-1]^-$.

(2S,3S,4R)-1-*O*-(α-D-galactopyranosyl)-2-(hexacosanoyl)aminooctadecan-1,3,4-triol (αGalCer) (1)

To a solution of compound **9** (83 mg, 0.07 mmol) in a mixture formed by MeOH (3 mL) and THF (1 mL) was added KOH (1 mg, 0.014 mmol). After stirring at rt overnight, the solvent was evaporated, the crude redissolved in DCM (10 mL), washed with water (2x10 mL), brine, dried over $MgSO_4$ and evaporated. The addition of methanol (3 mL), caused the precipitation of 70 mg of a white solid **(11)**, directly used for the last step (yield 87%). $[\alpha]^{20}_D$: +72.9° (*c* 1.0, $CHCl_3$). Mp 126-128 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.36 (m, 15H), 6.32 (d, J = 8.2 Hz, 1H), 5.47 (s, 1H), 4.98 (d, J = 3.4 Hz, 1H), 4.94 (d, J = 11.6 Hz, 1H), 4.76 (s, 2H), 4.68 (d, J = 11.3 Hz, 1H), 4.22 (m, 3H), 4.09 (dd, J_1 = 9.8 Hz, J_2 = 3.4 Hz, 1H), 3.95 (m, 3H), 3.82 (dd, J_1 = 10.1 Hz, J_2 = 2.4 Hz, 1H), 3.66 (br s, 1H), 3.52 (s, 1H), 3.45 (m, 2H), 2.27 (br s, 1H), 2.14 (t, J = 7.4 Hz, 2H), 1.58 (m, 4H), 1.26 (m, 68H), 0.88 (t, J = 6.1 Hz, 6H). ^{13}C NMR ($CDCl_3$, 75 MHz) 173.0, 138.4, 137.9, 137.8, 129.1, 128.6, 128.5, 128.3, 128.2, 127.85, 127.78, 126.4, 101.2, 99.6, 76.5, 76.3, 75.2, 74.7, 74.1, 73.4, 71.6, 69.9, 69.4, 63.1, 49.2 37.0, 33.5, 32.0, 29.8, 29.5, 25.9, 22.8, 14.2. MS (ESI) m/z Calculated for $C_{71}H_{115}NO_9$: 1126 Found: 1149 $[M+Na]^+$. IR (neat, cm^{-1}): 3415, 2919, 2850, 1738, 1620, 1544, 1497, 1101, 1054, 796. $Pd(OH)_2$ on carbon (13 mg) was added to a solution of the crude dissolved in a mixture of $CHCl_3$/MeOH (4 mL, 1/1, v/v) and the reaction stirred under H_2 for 17 h. at rt. The reaction mixture was then filtered through Celite, the Celite was washed thoroughly with hot

$CHCl_3$/MeOH (1/1, v/v), and the filtrate was concentrated under vacuum. The crude was purified by column chromatography (CH_2Cl_2/MeOH 9:1) obtaining a white solid **(1)**. (45 mg, yield 90%). Analytical data in agreement with literature report.[36]

Conflicts of interest

There are no conflicts to declare.

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Supplementary data

Supplementary data (^{1}H NMR and ^{13}C NMR spectra for all new compounds) associated with this article can be found, in the online version, at…….

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