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# Synthesis of C-5″ and C-6″-modified α-GalCer analogues as *i*NKT-cell agonists

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ABSTRACT

Alpha-galactosyl ceramide (α-GalCer) is a prototypical synthetic ligand of invariant natural killer T (*i*NKT) cells. Upon presentation by the MHC class I-like molecule CD1d, this glycolipid stimulates *i*NKT cells to secrete a vast amount of both pro-inflammatory Th1 and anti-inflammatory Th2 cytokines. Recently, we discovered that selected 6″-modified α-GalCer analogues may produce markedly Th1-biased responses due to the formation of either an additional anchor with CD1d or by establishing extra interactions with the T-cell receptor of *i*NKT cells. Here, we report a practical synthesis towards 6″-*O*-carbamate and galacturonamide analogues of α-GalCer and their evaluation as *i*NKT cell agonists in mice.

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## 1. Introduction

α-GalCer or KRN7000 (**1**, Fig. 1) is a synthetic glycolipid derived from a group of galactosylceramides found in marine sponge extracts showing promising anti-tumor activity in a B16-melanoma murine model and currently under investigation in different early phase human trials for patients with metastatic cancer.[1,2] α-GalCer is the prototypical antigen for *i*NKT-cells, a subset of T-lymphocytes, which show features of both innate and adaptive immunity. Whereas conventional T-cells are activated by recognition of peptide antigens presented by MHC class I or II molecules, *i*NKT-cells recognize lipid and glycolipid antigens in the context of CD1d expressed on antigen presenting cells.[3,4] CD1d shows striking structural analogy with the MHC class I protein,[5] however, it comprises a deeper and more lipophilic binding groove in line with the differences in antigen presentation. Indeed, crystal structures of both mouse and human CD1d bound to α-GalCer show that the lipid chains of α-GalCer are accommodated in two hydrophobic pockets. The F′ pocket binds the phytosphingosine chain while the fatty acyl chain fills the A′ pocket. As a result the galactose sugar is oriented towards the surface of CD1d, available for recognition by the TCR of *i*NKT cells.[6,7]

Engagement of the CD1d-α-GalCer complex by the TCR of *i*NKT cells results in a rapid production of Th1 and Th2 cytokines both by *i*NKT cells itself and by activation of bystander immune cells.[8] The robust cytokine production shows promise for a broad range of therapeutic applications, but despite its potent immune response α-GalCer showed limited therapeutic outcome in the clinic.[9,10] The concomitant release of both Th1 and Th2 cytokines, which have opposing effects in vivo, is believed to account for its poor efficacy. Indeed, α-GalCer analogues capable of skewing the cytokine profile have improved therapeutic potential.[11–13] OCH (**2**), characterized by a truncated phytosphingosine chain, improves disease in animal models of experimental autoimmune encephalitis and arthritis as a result of a Th2 biased cytokine secretion.[14,15] Stabilizing the anomeric bond by substituting a methylene unit for the glycosidic oxygen gave rise to α-*C*-GalCer (**3**), a compound with a marked Th1 response and superior malaria protection in mice.[16]

*Abbreviations:* *i*NKT cell, invariant natural killer T-cell; MHC, major histocompatibility complex; Th, T helper; CDI, 1,1′-carbonyldiimidazole; TEMPO, (2,2,6,6-tetramethylpiperidin-1-yl)oxyl; BAIB, bis(acetoxy)iodobenzene; HCTU, 2-(6-chloro-1-*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate; INF, interferon; IL, interleukin; APC, antigen presenting cell; BMDC, bone marrow dendritic cell; TCR, T cell receptor; NU-α-GalCer, 1-*O*-(6-naphtureido-6-deoxy-α-D-galactopyranosyl)-2-hexacosylamino-D-ribo-1,3,4-octadecanetriol.

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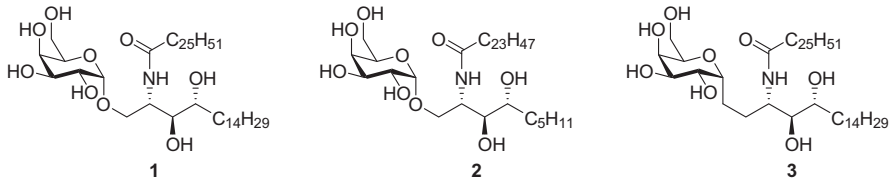



**Figure 1.** Structures of KRN7000 (**1**), OCH (**2**) and α-*C*-GalCer (**3**).

Modifications of the galactose moiety are mostly focusing on the 6″-position,[17,18] as other alterations generally result in poor antigenicity. Previously, we discovered that a C-6″-naphthylurea derivative (NU-α-GalCer, **4**, Fig. 2) induces a potent Th1 response both in a murine and a human setting resulting in a superior tumor protection in the B16 mouse melanoma model.[19] Crystallographic studies of the ternary CD1d-NU-α-GalCer–TCR complex revealed that the naphthyl moiety occupies a narrow binding pocket in CD1d above the A′ roof by induced fit, thereby enhancing the affinity for CD1d. The latter led us to investigate other modifications at the C-6″ position. Here we describe the synthesis of a series of C-6″-carbamate and C-5″-uronamide derivatives.

## 2. Results and discussion

### 2.1. Chemistry

Both the C-6″-carbamates **5a**–**5c** and the C-5″-amides **6a**–**6g** can be accessed from the common intermediate **8**, which is swiftly obtained upon treatment of the 4,6-benzylidene precursor with $Cu(OTf)_2$ and $BH_3$·THF. We previously started from the same intermediate for the synthesis of galacturonic acid and 6″-triazole analogues of α-GalCer.[20,21] The primary OH group of **8** was converted into carbamates **9a** and **9b** upon reaction with the appropriate isocyanate, while compound **9c** was obtained by treating **8** with 4-aminopyridine and 1,1′-carbonyldiimidazole (CDI).

The synthesis of the uronamides **11a**–**11g** commenced with the TEMPO/BAIB oxidation of the primary hydroxyl group of **8** to afford the galacturonic acid intermediate **10** (Scheme 1).[16] Successive coupling with the appropriate amine in the presence of 2-(6-chloro-1-*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HCTU) gave access to amides **11a**–**g**. Palladium-catalyzed hydrogenation furnished the desired carbamates **5a**–**5c** and amides **6a**–**6e**. However, this deprotection method failed to remove the benzyl protecting groups of the sulfur-containing compound **11f**, even under high pressure (50 bar), while overreduction of the naphthyl moiety was observed during hydrogenation of intermediates **9b** and **11g**. In case of naphthyl carbamate **9b**, careful monitoring of the reaction yet allowed obtaining the final product. For the naphthyl amide compound, however, undesired reduction of the naphthyl ring was observed before all benzyl groups were removed, thereby impeding isolation of the envisaged analogue. This led us to explore alternative deprotection conditions for compounds **11f** and **11g**. Treatment with aluminum chloride and dimethylaniline allowed to produce the desired products **6f** and **6g**, albeit in low yields.

### 2.2. Biological evaluation

The biological activity of all target glycolipids was assessed by measuring IFN-γ and IL-4 serum levels after intraperitoneal injection of 5 μg in mice (Fig. 3). Except for **6a** and **6f**, the C-5″-amides are generally weaker antigens than α-GalCer with a Th1 cytokine bias, mainly originating from significantly lower IL-4 levels compared to α-GalCer.

The C-6″-carbamates clearly exhibit stronger antigenic effects than the uronamides, but their cytokine profiles resemble that of α-GalCer.

It was shown that the remarkably high INF-γ release of the C-6″-carbamates (**5a**–**c**) stems from a higher APC-derived IL-12 production, compared with that of α-GalCer.[22] Notable, high IL-12 secretion was observed with **5c**, largely exceeding that induced by NU-α-GalCer. The crystal structure of the ternary mCD1d-5**b**-TCR complex indicates that the binding mode of **5b** in the parallels that of NU-α-GalCer. In particular, its naphthylcarbamate moiety also induces a hydrophobic pocket in CD1d by displacement of Met69 (Fig. 4),[23] indicating that the naphthyl moiety drives the observed induced fit rather than the nature of the linker, given that the length of the latter is appropriate. Probably, this extra interaction with CD1d compensates for the observed loss of the hydrogen bond between the 4″-OH group and Asn-30 of the TCR. Although the in vivo data indicate that **5b** is a potent antigen, at present we have no explanation for its distinct cytokine profile compared to NU-α-GalCer.

Previously, compound **5c** (PyrC-α-GalCer) was shown to be tilted towards the TCR thereby forming intimate contacts with the TCR instead of forming a third anchor inside CD1d.[18] The carbamate moiety of **5a**, on the other hand, was situated laterally above the CD1d binding group, suggesting that the smaller pyridine as well as the phenyl ring are not sufficient to induce the structural change within the A′ roof of CD1d, in contrast to both **5b** and NU-α-GalCer, which have the larger naphthyl ring.

## 3. Conclusions

In summary, we have synthesized a series of C-6″-carbamate and C-5″-galacturonamide derivatives of α-GalCer from a common intermediate **8**, further illustrating the highly convergent potential of the latter. Generally, the antigenic potency of the carbamates is

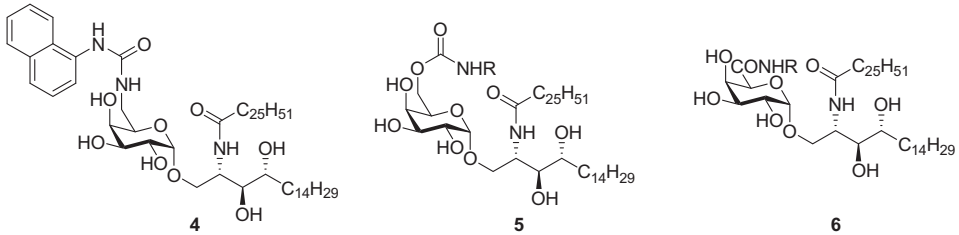
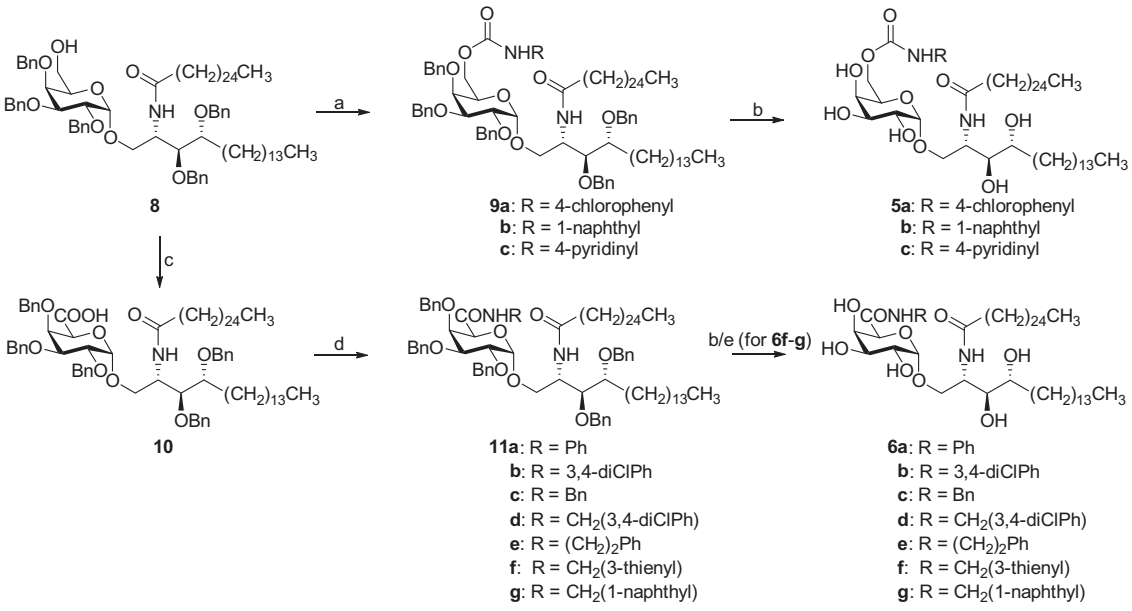



**Figure 2.** Structures of NU-α-GalCer (**4**), C-6″-carbamate (**5**) and C-5″-uronamide derivatives (**6**) of α-GalCer.




**Scheme 1.** Reagents and conditions: (a) (i) RNCO, DMF, 67–74% or (ii) $RNH_2$, CDI, DMF, 70 °C, 33%; (b) Pd black, $H_2$, EtOH/$CHCl_3$, 49–86%; (c) TEMPO, BAIB, $CH_2Cl_2/H_2O$, 87%; (d) $RNH_2$, HCTU, DIPEA, DMF, 53–87%; (e) $AlCl_3$, dimethylaniline, $CH_2Cl_2$, 11–15%.

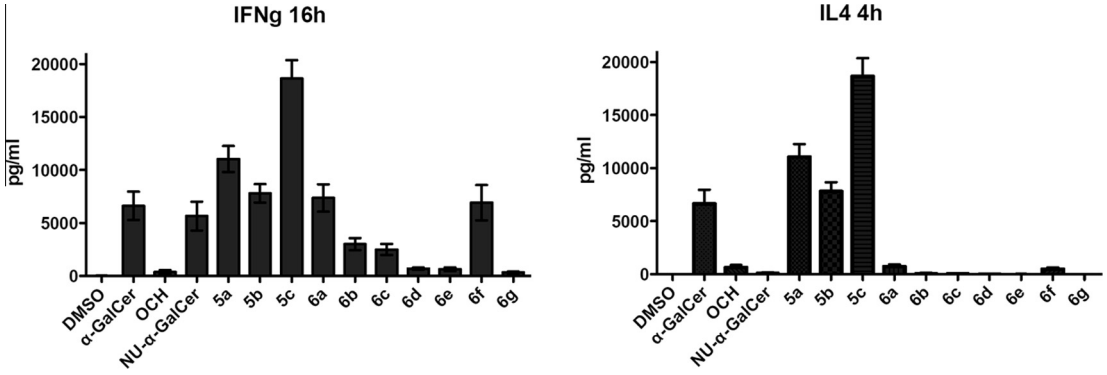



**Figure 3.** IFN-γ and IL-4 secretion, measured at respective 16 h and 4 h, after intraperitoneal injection of 5 μg of the glycolipids in mice (glycolipids were tested in at least 2 different experiments with 5–8 mice per group for each glycolipid).

superior to that of the uronamides, although within each series significant variations in cytokine profiles are observed depending on the substitution patterns.

The binding behavior of the naphthylcarbamate **5b** in the ternary complex is strikingly similar to that of NU-α-GalCer (**4**). The naphthyl moiety of both analogues occupies an extra binding pocket in CD1d. Determinants for the induction of this hydrophobic pocket possibly involve the size of the aromatic group and the correct positioning of a carbonyl that acts as H-bond acceptor. By varying the carbamate substituents it may be possible to increase the interaction with either the TCR or CD1d. A notable advantage of the carbamate derivatives over their urea analogues is that the synthesis of the former is significantly shorter.

## 4. Experimental section

### 4.1. Chemical synthesis

#### 4.1.1. General

Precoated Macherey-Nagel SIL G/UV254 plates were used for TLC, and spots were examined under UV light at 254 nm and further visualized by sulfuric acid-anisaldehyde spray. Column chromatography was performed on Biosolve silica gel (32–63 μm, 60 Å). NMR spectra were obtained with a Varian Mercury 300 Spectrometer. Chemical shifts are given in ppm ($\delta$) relative to the residual solvent signals, in the case of $CDCl_3$: $\delta$ = 7.26 ppm for $^1H$ and $\delta$ = 77.4 ppm for $^{13}C$ and in the case of pyridine-$d_5$: $\delta$ = 8.74, 7.58 and 7.22 ppm for $^1H$ and $\delta$ = 149.9, 135.5 and 123.5 ppm for $^{13}C$. Exact mass measurements were performed on a Waters LCT Premier XE TOF equipped with an electrospray ionization interface and coupled to a Waters Alliance HPLC system. Samples were infused in a $CH_3CN$/HCOOH (1000/1) mixture at 10 mL/min. All melting points were determined on a Büchi B-545 melting point apparatus and are uncorrected.

#### 4.1.2. Procedure for the synthesis of carbamates 9a and 9b

To a solution of compound **8** (0.07 mmol) in DMF (1 mL) was added the appropriate isocyanate (0.18 mmol). After stirring overnight, the reaction mixture was evaporated to dryness under reduced pressure. Purification by column chromatography (hexanes/EtOAc: 8/2) afforded carbamates **9a** (74%) and **9b** (67%).

**4.1.2.1. (2*S*,3*S*,4*R*)-3,4-Di-*O*-benzyl-1-O-(2,3,4-tri-O-benzyl-6-*O*-(4-chlorophenylcarbamoyl)-α-D-galactopyranosyl)-2-(hexacosanamido)octadecane-1,3,4-triol (9a).** $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 8.29 (s, 1H, NH), 7.31–7.10 (m, 29H, arom. H), 5.71 (d, *J* =

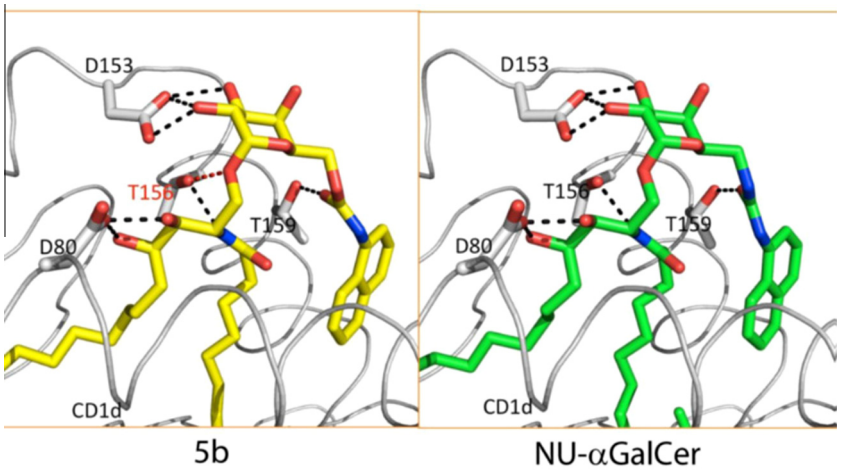



**Figure 4.** Crystal structure of **5b** bound to CD1d, compared with that of NU-α-GalCer.

6.6 Hz, 1H, NH), 4.88 (d, *J* = 11.6 Hz, 1H, $CH_2$-Ph), 4.79 (d, *J* = 3.9 Hz, 1H, H-1″), 4.75 (d, *J* = 11.6 Hz, 1H, $CH_2$-Ph), 4.67 (d, *J* = 12.0 Hz, 1H, $CH_2$-Ph), 4.62 (d, *J* = 11.9 Hz, 1H, $CH_2$-Ph), 4.61 (d, *J* = 11.8 Hz, 1H, $CH_2$-Ph), 4.57 (d, *J* = 11.6 Hz, 1H, $CH_2$-Ph), 4.52 (d, *J* = 11.4 Hz, 1H, $CH_2$-Ph), 4.51 (d, *J* = 11.8 Hz, 1H, $CH_2$-Ph), 4.42 (d, *J* = 11.6 Hz, 1H, $CH_2$-Ph), 4.41 (d, *J* = 11.6 Hz, 1H, $CH_2$-Ph), 4.33–4.26 (m, 2H, H-2, H-6″), 4.01–3.90 (m, 3H, H-2″, H-1), 3.86–3.75 (m, 4H, H-3, H-3″, H-4″, H-5″), 3.69 (dd, *J* = 1.6 Hz and 11.4 Hz, 1H, H-6″), 3.51–3.46 (m, 1H, H-4), 1.96–1.84 (m, 2H, $COCH_2$), 1.58–1.06 (m, 72H, $CH_2$), 0.77 (t, *J* = 6.9 Hz, 6H, $CH_3$).

$^{13}$C NMR (75 MHz, $CDCl_3$): δ 173.93, 153.51, 138.79, 138.73, 138.69, 138.47, 138.22, 137.63, 129.90, 128.96, 128.68, 128.64, 128.60, 128.57, 128.13, 128.04, 127.99, 127.90, 127.84, 127.73, 127.66, 119.69, 100.52, 80.54, 79.72, 79.48, 77.67, 77.45, 77.25, 76.83, 76.65, 75.20, 74.52, 73.88, 73.66, 73.62, 72.38, 70.26, 70.17, 65.87, 60.62, 52.34, 37.04, 32.17, 32.16, 30.88, 29.97, 29.96, 29.94, 29.92, 29.89, 29.79, 29.61, 29.60, 29.48, 26.17, 25.81, 22.93, 21.28, 14.43, 14.36.

Exact mass (ESI-MS) for $C_{92}H_{133}ClN_2O_{10}$ $[M+H]^+$ found, 1461.9786; calcd, 1461.9727.

**4.1.2.2. (2*S*,3*S*,4*R*)-3,4-Di-*O*-benzyl-1-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-(1-naphthylcarbamoyl)-α-D-galactopyranosyl)-2-(hexacosanamido) octadecane-1,3,4-triol (9b).** $^1$H NMR (300 MHz, pyridine-$d_5$): δ 10.66 (s, 1H, NH), 8.99 (d, *J* = 8.4 Hz, 1H, NH), 8.84 (d, *J* = 8.4 Hz, 1H, arom. H), 8.37 (d, *J* = 7 Hz, 1H, arom. H), 7.96 (d, *J* = 7.9 Hz, 1H, arom. H), 7.78 (d, *J* = 8.1 Hz, 1H, arom. H), 7.66–7.58 (m, 2H, arom. H), 7.55–7.29 (m, 26H, arom. H), 5.43 (d, *J* = 3.4 Hz, 1H, H-1″), 5.17 (d, *J* = 11.1 Hz, 1H, $CH_2$-Ph), 5.13 (d, *J* = 10.2 Hz, 1H, $CH_2$-Ph), 5.00–4.82 (m, 4H, $CH_2$-Ph, H-6″, H-2), 4.78–4.68 (m, 5H, H-6″, $CH_2$-Ph), 4.64–4.58 (m, 2H, H-5″, $CH_2$-Ph), 4.54 (dd, *J* = 2.0 and 8.4 Hz, 1H, H-3), 4.48–4.43 (m, 3H, H-1, H-2″, $CH_2$-Ph), 4.34 (dd, *J* = 2.7 Hz and 10.2 Hz, 1H, H-3″), 4.25–4.20 (m, 2H, H-1, H-4″), 3.97–3.93 (m, 1H, H-4), 2.66–2.56 (m, 2H, $COCH_2$), 2.15–1.18 (m, 72H, $CH_2$), 0.90 (t, *J* = 6.7 Hz, 3H, $CH_3$), 0.89 (t, *J* = 6.4 Hz, 3H, $CH_3$).

$^{13}$C NMR (75 MHz, pyridine-$d_5$): δ 172.21, 154.34, 138.55, 138.41, 138.21, 138.12, 127.65, 127.50, 127.29, 126.89, 126.80, 126.75, 126.58, 125.16, 125.11, 97.63, 80.00, 78.76, 77.89, 76.09, 74.88, 73.94, 73.24, 72.26, 71.67, 70.70, 68.77, 61.38, 50.13, 49.97, 48.02, 38.95, 35.66, 34.03, 30.97, 28.99, 28.87, 28.80, 28.74, 28.66, 28.43, 25.62, 25.27, 23.71, 21.78, 13.12.

Exact mass (ESI-MS) for $C_{96}H_{136}N_2O_{10}$ $[M+H]^+$ found, 1478.0220; calcd, 1478.0273.

**4.1.3. Procedure for the synthesis of carbamate 9c**

To a solution of compound **8** (50 mg, 0.04 mmol) in DMF (0.5 mL) was added CDI (31 mg, 0.18 mmol). After stirring overnight, the reaction mixture was heated until 70 °C and 4-aminopyridine was added. The reaction mixture was stirred at 70 °C during 48 h followed by evaporation to dryness under reduced pressure. Purification by column chromatography (hexanes/EtOAc: 7/3) afforded carbamate **9c** (26 mg, 33%).

**4.1.3.1. (2*S*,3*S*,4*R*)-3,4-Di-*O*-benzyl-1-*O*-(2,3,4-tri-*O*-benzyl-6-*O*-(4-pyridinylcarbamoyl)-α-D-galactopyranosyl)-2-(hexacosanamido)octadecane-1,3,4-triol (9c).** $^1$H NMR (300 MHz, $CDCl_3$): δ 7.87 (s, 1H, NH), 7.35–7.15 (m, 28H, arom. H), 6.93 (s, 1H, arom. H), 5.69 (d, *J* = 8.2 Hz, 1H, NH), 4.90 (d, *J* = 11.7 Hz, 1H, $CH_2$-Ph), 4.81 (d, *J* = 3.7 Hz, 1H, H-1″), 4.76 (d, *J* = 11.1 Hz, 1H, $CH_2$-Ph), 4.73 (d, *J* = 11.3 Hz, 1H, $CH_2$-Ph), 4.69 (d, *J* = 11.3 Hz, 1H, $CH_2$-Ph), 4.65 (d, *J* = 11.7 Hz, 1H, $CH_2$-Ph), 4.58 (d, *J* = 11.7 Hz, 1H, $CH_2$-Ph), 4.55 (d, *J* = 11.7 Hz, 1H, $CH_2$-Ph), 4.48 (d, *J* = 11.7 Hz, 1H, $CH_2$-Ph), 4.40 (d, *J* = 11.7 Hz, 1H, $CH_2$-Ph), 4.39 (d, *J* = 11.5 Hz, 1H, $CH_2$-Ph), 4.29–4.20 (m, 2H, H-2, H-6″), 4.12–4.06 (m, 1H, H-6″), 3.99 (dd, *J*= 3.3 Hz and 9.8 Hz, 1H, H-2″), 3.94 (m, 1H, H-5″), 3.85 (dd, *J* = 2.5 Hz and 10.1 Hz, 1H, H-3″), 3.81 (dd, *J* = 4.91 Hz and 11.0 Hz, 1H, H-1), 3.75 (app. s, 1H, H-4″), 3.68–3.64 (m, 1H, H-3), 3.62–3.56 (m, 1H, H-1), 3.48–3.43 (m, 1H, H-4), 1.86–1.74 (m, 2H, $COCH_2$), 1.54–1.09 (m, 72H, $CH_2$), 0.83–0.76 (m, 6H, $CH_3$).

$^{13}$C NMR (75 MHz, $CDCl_3$): δ 173.09, 148.33, 138.68, 138.62, 138.52, 138.01, 137.32, 130.68, 128.76, 128.73, 128.68, 128.66, 128.64, 128.60, 128.54, 128.16, 128.07, 128.05, 127.97, 127.82, 127.78, 127.68, 117.33, 99.09, 80.11, 79.67, 79.13, 74.56, 73.83, 73.48, 72.12, 68.33, 68.16, 66.68, 60.63, 56.66, 50.42, 36.97, 32.16, 31.82, 30.51, 30.04, 29.96, 29.93, 29.89, 29.84, 29.67, 29.64, 29.60, 29.59, 26.02, 25.94, 22.92, 22.88, 21.27, 14.22, 14.35.

Exact mass (ESI-MS) for $C_{91}H_{133}N_3O_{10}$ $[M+H]^+$ found, 1429.0229; calcd, 1429.0064.

**4.1.4. General procedure for the synthesis of amides (11a–11g)**

To a solution of **10** (150 mg, 0.11 mmol) in DMF (0.3 mL) and $CH_2Cl_2$ (0.7 mL) was added DIPEA (22 mg, 0.17 mmol). After stirring for 10 minutes at room temperature, HCTU (72 mg, 0.17 mmol) was added and the mixture was stirred for 30 min. Then the appropriate amine (0.17 mmol) was added and the solution was continued stirring overnight. After completion of the reaction, the mixture was evaporated to dryness. The residue was partitioned between $H_2O$ and EtOAc and the aqueous layer was

extracted with EtOAc. The organic layer was washed with brine and dried over $Na_2SO_4$. Purification by column chromatography (hexanes/EtOAc) and concentration under reduced pressure furnished the desired amides **11a** (87%), **11b** (84%), **11c** (53%), **11d** (82%), **11e** (82%), **11f** (85%) and **11g** (82%).

**4.1.4.1. (2*S*,3*S*,4*R*)-(2,3,4-Tri-*O*-benzyl-*N*-phenyl-α-D-galactopyranuronamidyl)-3,4-di-*O*-benzyl-2-(hexacosanamido)octadecane-3,4-diol (11a).** $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 8.20 (s, 1H, NH), 7.53 (d, $J$ = 1.0 Hz, 2H, arom. H), 7.39–7.08 (m, 28H, arom. H), 5.73 (d, $J$ = 8.2 Hz, 1H, NH), 4.98 (d, $J$ = 3.5 Hz, 1H, H-1″), 4.87 (d, $J$ = 10.8 Hz, 1H, $CH_2$-Ph), 4.84 (d, $J$ = 11.6 Hz, 1H, $CH_2$-Ph), 4.79 (d, $J$ = 12.7 Hz, 1H, $CH_2$- Ph), 4.71 (d, $J$ = 11.6 Hz, 1H, $CH_2$-Ph), 4.66 (d, $J$ = 11.6 Hz, 1H, $CH_2$-Ph), 4.62 (d, $J$ = 12.0 Hz, 1H, $CH_2$-Ph), 4.58 (d, $J$ = 10.8 Hz, 1H, $CH_2$-Ph), 4.55 (d, $J$ = 11.6 Hz, 1H, $CH_2$-Ph), 4.51–4.45 (m, 3H, $CH_2$-Ph and H-4″), 4.37 (app. d, $J$ = 1.1 Hz, 1H, H-5″), 4.26 (m, 1H, H-2), 4.07 (dd, $J$ = 3.5 Hz and 10.1 Hz, 1H, H-2″), 3.98 (dd, $J$ = 2.7 Hz and 10.0 Hz, 1H, H-3″), 3.90–3.76 (m, 3H, H-1, H-3), 3.55–3.49 (m, 1H, H-4), 1.92–1.78 (m, 2H, $COCH_2$), 1.68–1.22 (m, 72H, $CH_2$), 0.88 (t, $J$ = 6.7 Hz, 6H, $CH_3$).

$^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 173.07, 166.69, 138.73, 138.70, 138.55, 138.50, 138.40, 137.44, 129.16, 128.67, 128.65, 128.63, 128.61, 128.45, 128.35, 128.15, 128.07, 128.03, 128.01, 127.92, 127.90, 127.81, 127.70, 124.76, 120.19, 99.21, 79.97, 79.20, 78.55, 76.63, 76.10, 75.91, 74.10, 73.38, 72.72, 72.24, 72.14, 68.46, 50.19, 36.90, 32.16, 30.41, 30.05, 29.96, 29.94, 29.89, 29.82, 29.65, 29.61, 29.60, 26.11, 25.85, 22.93, 14.36.

Exact mass (ESI-MS) for $C_{91}H_{132}N_2O_9$ $[M+Na]^+$ found, 1419.9889; calcd, 1419.9831.

**4.1.4.2. (2*S*,3*S*,4*R*)-(2,3,4-Tri-*O*-benzyl-*N*-(3,4-dichlorophenyl)-α-D-galactopyranuronamidyl)-3,4-di-*O*-benzyl-2-(hexacosanamido) octadecane-3,4-diol (11b).** $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 8.17 (s, 1H, NH), 7.74 (d, $J$ = 2.2 Hz, 1H, arom. H), 7.40–7.14 (m, 27H, arom. H), 5.70 (d, $J$ = 8.2 Hz, 1H, NH), 4.95 (d, $J$ = 3.4 Hz, 1H, H-1″), 4.89–4.46 (m, 11H, $CH_2$-Ph and H-4″), 4.35 (d, $J$ = 1.2 Hz, 1H, H-5″), 4.31–4.25 (m, 1H, H-2), 4.04 (dd, $J$ = 3.4 Hz and 10.0 Hz, 1H, H-2″), 3.96 (dd, $J$ = 2.6 Hz and 10.2 Hz, 1H, H-3″), 3.91 (dd, $J$ = 4.9 Hz and 10.6 Hz, 1H, H-1), 3.80–3.74 (m, 2H, H-1 and H-3), 3.56–3.52 (m, 1H, H-4), 1.94–1.77 (m, 2H, $COCH_2$), 1.60–1.08 (m, 72H, $CH_2$), 0.88 (t, $J$ = 6.7 Hz, 6H, $CH_3$).

$^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 173.09, 167.17, 138.68, 138.66, 138.50, 138.43, 138.28, 136.81, 132.93, 130.62, 128.70, 128.68, 128.65, 128.64, 128.42, 128.40, 128.11, 128.09, 128.06, 128.01, 127.97, 127.94, 127.87, 127.70, 121.80, 119.37, 99.25, 79.92, 79.69, 78.49, 75.99, 74.11, 73.32, 72.94, 72.29, 68.67, 53.64, 50.20, 36.89, 32.16, 30.61, 30.05, 29.96, 29.94, 29.91, 29.89, 29.82, 29.66, 29.61, 29.59, 26.12, 25.85, 22.92, 14.35.

Exact mass (ESI-MS) for $C_{91}H_{130}Cl_2N_2O_9$ $[M+Na]^+$ found, 1487.9138; calcd, 1487.9046.

**4.1.4.3. (2*S*,3*S*,4*R*)-(2,3,4-Tri-*O*-benzyl-*N*-benzyl-α-D-galactopyranuronamidyl)-3,4-di-*O*-benzyl-2-(hexacosanamido)octadecane-3,4-diol (11c).** $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 7.37–7.17 (m, 30H, arom. H), 6.79 (app. t, $J$ = 5.9 Hz, 1H, NH), 5.74 (d, $J$ = 8.1 Hz, 1H, NH), 4.88 (d, $J$ = 3.7 Hz, 1H, H-1″), 4.85–4.42 (m, 12H, $CH_2$-Ph, H-4″, NH-$CH_2$), 4.35–4.28 (m, 2H, H-5″ and NH-$CH_2$), 4.26–4.18 (m, 1H, H-2), 4.04 (dd, $J$ = 3.4 Hz and 9.9 Hz, 1H, H-2″), 3.95 (dd, $J$ = 2.6 Hz and 10.0 Hz, 1H, H-3″), 3.82–3.71 (m, 3H, H-1 and H-3), 3.49 (m, 1H, H-4), 1.93–1.78 (m, 2H, $COCH_2$), 1.65–1.14 (m, 72H, $CH_2$), 0.87 (t, $J$ = 6.6 Hz, 6H, $CH_3$).

$^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 173.05, 168.75, 138.80, 138.76, 138.74, 138.51, 137.76, 128.87, 128.63, 128.59, 128.40, 128.25, 128.15, 128.06, 128.01, 127.96, 127.87, 127.84, 127.75, 127.67, 99.05, 80.00, 78.98, 78.61, 76.26, 76.03, 75.60, 74.04, 73.47, 72.59, 72.01, 71.92, 68.18, 60.61, 50.12, 43.37, 36.88, 32.16, 31.82, 31.14, 30.27, 30.06, 29.96, 29.94, 29.89, 29.84, 29.68, 29.61, 29.59, 26.16, 25.87, 22.92, 22.88, 14.43, 14.35.

Exact mass (ESI-MS) for $C_{92}H_{134}N_2O_9$ $[M+Na]^+$ found, 1433.9846; calcd, 1433.9982.

**4.1.4.4. (2*S*,3*S*,4*R*)-(2,3,4-Tri-*O*-benzyl-*N*-(3,4-dichlorobenzyl)-α-D-galactopyranuronamidyl)-3,4-di-*O*-benzyl-2-(hexacosanamido) octadecane-3,4-diol (11d).** $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 7.38–7.22 (m, 26H, arom. H), 7.14 (d, $J$ = 8.2 Hz, 1H, arom. H), 6.98 (dd, $J$ = 1.9 Hz and 8.2 Hz, 1H, arom. H), 6.82 (t, $J$ = 6.1 Hz, 1H, NH), 5.75 (d, $J$ = 8.0 Hz, 1H, NH), 4.92 (d, $J$ = 3.3 Hz, 1H, H-1″), 4.89 (d, $J$ = 11.0 Hz, 1H, $CH_2$-Ph), 4.82 (d, $J$ = 11.6 Hz, 1H, $CH_2$-Ph), 4.79 (d, $J$ = 12.1 Hz, 1H, $CH_2$-Ph), 4.74 (d, $J$ = 12.1 Hz, 1H, $CH_2$-Ph), 4.72 (d, $J$ = 11.8 Hz, 1H, $CH_2$-Ph), 4.65 (d, $J$ = 11.6 Hz, 1H, $CH_2$-Ph), 4.56 (d, $J$ = 11.6 Hz, 1H, $CH_2$-Ph), 4.55 (d, $J$ = 11.0 Hz, 1H, $CH_2$-Ph), 4.50–4.43 (m, 4H, $CH_2$-Ph, NH-$CH_2$ and H-4″), 4.30–4.26 (m, 2H, H-5″ and H-2), 4.20 (dd, $J$ = 5.4 Hz and 15.1 Hz, 1H, NH-$CH_2$), 4.05 (d, $J$ = 3.4 Hz and 10.1 Hz, 1H, H-2″), 3.96 (d, $J$ = 2.5 Hz and 10.0 Hz, 1H, H-3″), 3.88–3.73 (m, 3H, H-1 and H-3), 3.55–3.58 (m, 1H, H-4), 1.99–1.80 (m, 2H, $COCH_2$), 1.72–1.16 (m, 72H, $CH_2$), 0.89 (t, $J$ = 6.6 Hz, 6H, $CH_3$).

$^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 173.06, 168.95, 138.73, 138.69, 138.50, 138.47, 138.24, 132.72, 131.67, 130.87, 129.97, 128.66, 128.64, 128.62, 128.43, 128.13, 128.10, 128.03, 127.96, 127.91, 127.84, 127.68, 127.33, 99.14, 79.96, 79.33, 78.60, 77.69, 77.27, 76.84, 76.24, 75.98, 75.60, 74.06, 73.44, 72.73, 72.12, 71.97, 68.28, 50.18, 42.19, 36.91, 32.17, 30.42, 30.06, 29.97, 29.95, 29.90, 29.85, 29.69, 29.62, 29.60, 26.15, 25.88, 22.94, 14.37.

Exact mass (ESI-MS) for $C_{92}H_{132}Cl_2N_2O_9$ $[M+H]^+$ found, 1479.9365; calcd, 1479.9388.

**4.1.4.5. (2*S*,3*S*,4*R*)-(2,3,4-Tri-*O*-benzyl-*N*-phenethyl-α-D-galactopyranuronamidyl)-3,4-di-*O*-benzyl-2-(hexacosanamido)octadecane-3,4-diol (11e).** $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 7.38–7.12 (m, 30H, arom. H), 6.61 (t, $J$ = 6.0 Hz, 1H, NH), 5.78 (d, $J$ = 8.3 Hz, 1H, NH), 4.89 (d, $J$ = 3.3 Hz, 1H, H-1″), 4.87 (d, $J$ = 10.8 Hz, 1H, $CH_2$-Ph), 4.82 (d, $J$ = 11.7 Hz, 1H, $CH_2$-Ph), 4.77 (d, $J$ = 11.8 Hz, 1H, $CH_2$-Ph), 4.73 (d, $J$ = 10.5 Hz, 1H, $CH_2$-Ph), 4.72 (d, $J$ = 11.8 Hz, 1H, $CH_2$-Ph), 4.64 (d, $J$ = 11.7 Hz, 1H, $CH_2$-Ph), 4.59 (d, $J$ = 10.8 Hz, 1H, $CH_2$-Ph), 4.56 (d, $J$ = 11.5 Hz, 1H, $CH_2$-Ph), 4.51–4.49 (m, 1H, H-4″), 4.48 (d, $J$ = 11.8 Hz, 1H, $CH_2$-Ph), 4.46 (d, $J$ = 11.6 Hz, 1H, $CH_2$-Ph), 4.27–4.29 (m, 2H, H-2 and H-5″), 4.03 (dd, $J$ = 3.5 Hz and 10.0 Hz, 1H, H-2″), 3.94 (dd, $J$ = 2.7 Hz and 10.1 Hz, 1H, H-3″), 3.82 (dd, $J$ = 2.9 Hz and 6.5 Hz, 1H, H-3), 3.79–3.71 (m, 2H, H-1), 3.53–3.45 (m, 3H, NH-$CH_2$ and H-4), 2.73 (ddd, $J$ = 7.2 Hz, 13.5 Hz and 28.0 Hz, 2H, $CH_2$), 1.98–1.80 (m, 2H, $COCH_2$), 1.65–1.19 (m, 72H, $CH_2$), 0.88 (t, $J$ = 6.8 Hz, 6H, $CH_3$).

$^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 172.99, 168.65, 138.88, 138.80, 138.77, 138.75, 138.53, 138.51, 128.92, 128.78, 128.64, 128.62, 128.61, 128.39, 128.37,128.14, 128.06, 127.99, 127.95, 127.88, 127.85, 127.70, 126.70, 98.97, 80.11, 78.92, 78.61, 77.68, 77.45, 77.25, 76.83, 76.31, 76.03, 75.73, 74.06, 73.47, 72.53, 72.04, 71.81, 68.03, 50.11, 40.61, 36.91, 36.03, 32.16, 30.29, 30.07, 29.97, 29.94, 29.92, 29.89, 29.85, 29.69, 29.64, 29.61, 29.60, 26.21, 25.88, 22.93, 14.36.

Exact mass (ESI-MS) for $C_{93}H_{136}N_2O_9$ $[M+H]^+$ found, 1426.0337; calcd, 1426.0324.

**4.1.4.6. (2*S*,3*S*,4*R*)-(2,3,4-Tri-*O*-benzyl-*N*-(thiophen-3-ylmethyl)-α-D-galactopyranuronamidyl)-3,4-di-*O*-benzyl-2-(hexacosanamido) octadecane-3,4-diol (11f).** $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 7.30–7.13 (m, 25H, arom. H), 7.07 (dd, $J$ = 1.4 Hz and 5.0 Hz, 1H, arom. H), 6.81 (dd, $J$ = 1.3 Hz and 3.4 Hz, 1H, arom. H), 6.79–6.75 (m, 2H, arom. H and NH), 5.66 (d, $J$ = 8.3 Hz, 1H, NH), 4.81 (d, $J$ = 3.3 Hz, 1H, H-1″), 4.77 (d, $J$ = 11.0 Hz, 1H, $CH_2$-Ph), 4.73 (d, $J$ = 12.5 Hz, 1H, $CH_2$-Ph), 4.68 (d, $J$ = 12.0 Hz, 1H, $CH_2$-Ph), 4.64 (d,

$J$ = 11.6 Hz, 1H, $CH_2$-Ph), 4.58–4.42 (m, 7H, $CH_2$-Ph, NH-$CH_2$, H-4″), 4.40 (d, $J$ = 11.8 Hz, 1H, $CH_2$-Ph), 4.37 (d, $J$ = 11.6 Hz, 1H, $CH_2$- Ph), 4.20 (d, $J$ = 0.8 Hz, 1H, H-5″), 4.18–4.12 (m, 1H, H-2), 3.95 (dd, $J$ = 3.4 Hz and 10.0 Hz, 1H, H-2″), 3.87 (dd, $J$ = 2.6 Hz and 10.0 Hz, 1H, H-3″), 3.75–3.63 (m, 3H, H-1 and H-3), 3.48–3.40 (m, 1H, H-4), 1.88–1.69 (m, 2H, $COCH_2$), 1.58–1.07 (m, 72H, $CH_2$), 0.81 (t, $J$ = 6.7 Hz, 6H, $CH_3$).

$^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 173.02, 168.56, 140.27, 138.77, 138.76, 138.54, 138.52, 128.65, 128.63, 128.62, 128.39, 128.33, 128.19, 128.07, 128.01, 127.90, 127.88, 127.77, 127.69, 127.12, 126.52, 125.44, 99.06, 79.96, 79.01, 78.57, 77.70, 77.28, 76.86, 76.23, 76.03, 75.61, 74.05, 73.45, 72.58, 72.02, 71.89, 68.21, 50.10, 38.03, 36.90, 32.18, 32.17, 30.29, 30.08, 29.98, 29.96, 29.93, 29.91, 29.86, 29.70, 29.63, 29.61, 26.15, 25.88, 22.95, 14.38.

Exact mass (ESI-MS) for $C_{90}H_{132}N_2O_9S$ $[M+H]^+$ found, 1417.9757; calcd, 1417.9732.

#### 4.1.4.7. (2*S*,3*S*,4*R*)-(2,3,4-Tri-*O*-benzyl-*N*-(naphthalen-1-ylmethyl)-α-D-galactopyranuronamidyl)-3,4-di-*O*-benzyl-2-(hexacosanamido)octadecane-3,4-diol (11g).

$^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 7.98 (d, $J$ = 8.2 Hz, 1H, arom. H), 7.85 (d, $J$ = 7.8 Hz, 1H, arom. H), 7.77 (d, $J$ = 8.2 Hz, 1H, arom. H), 7.50–7.23 (m, 29H, arom. H), 6.85 (t, $J$ = 5.6 Hz, 1H, NH), 5.78 (d, $J$ = 8.4 Hz, 1H, NH), 4.92–4.36 (m, 15H, H-1″, $CH_2$-Ph, NH-$CH_2$, H-4″, H-5″), 4.28–4.20 (m, 1H, H-2), 4.04 (dd, $J$ = 3.2 Hz and 10.0 Hz, 1H, H-2″), 3.98 (dd, $J$ = 2.0 Hz and 10.0 Hz, 1H, H-3″), 3.82–3.69 (m, 3H, H-1 and H-3), 3.53–3.47 (m, 1H, H-4), 1.97–1.79 (m, 2H, $COCH_2$), 1.62–1.18 (m, 72H, $CH_2$), 0.90 (t, $J$ = 6.5 Hz, 6H, $CH_3$).

$^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 173.05, 168.73, 138.84, 138.75, 138.73, 138.53, 138.50, 134.02, 133.14, 131.56, 128.98, 128.95, 128.69, 128.65, 128.60, 128.43, 128.28, 128.18, 128.08, 127.98, 127.88, 127.84, 127.77, 127.71, 126.84, 126.51, 126.15, 125.67, 123.50, 99.02, 79.94, 78.89, 78.59, 77.71, 77.29, 76.86, 76.30, 76.06, 75.61, 74.06, 73.47, 72.59, 72.00, 71.95, 68.20, 50.07, 41.30, 36.88, 32.18, 30.22, 30.08, 29.99, 29.96, 29.91, 29.87, 29.71, 29.64, 29.62, 26.15, 25.89, 22.95, 14.39.

Exact mass (ESI-MS) for $C_{96}H_{136}N_2O_9$ $[M+H]^+$ found, 1462.0370; calcd, 1462.0324.

### 4.1.5. General procedure for debenzylation: (5a–5c and 6a–6e)

A solution of the protected amide/carbamate (0.03 mmol) in $CHCl_3$ (0.4 mL) and EtOH (1.2 mL) was hydrogenated under atmospheric pressure in the presence of palladium black (10 mg). Upon reaction completion, the mixture was diluted with pyridine and filtered through celite. The filter cake was rinsed with $CHCl_3$ and EtOH and the filtrate was evaporated to dryness. After purification by column chromatography (DCM/MeOH), final compounds **5a** (81%), **5b** (68%), **5c** (86%), **6a** (86%), **6b** (63%), **6c** (49%), **6d** (80%) and **6e** (79%) were afforded as white powders.

#### 4.1.5.1. (2*S*,3*S*,4*R*)-1-*O*-(6-*O*-(4-Chlorophenylcarbamoyl)-α-D-galactopyranosyl)-2-(hexacosanamido)octadecane-1,3,4-triol (5a).

$^1$H NMR (300 MHz, pyridine-$d_5$): $\delta$ 10.73 (s, 1H, NH), 8.58 (d, $J$ = 8.2 Hz, 1H, NH), 7.91 (d, $J$ = 8.7 Hz, 2H, arom. H), 7.40 (d, $J$ = 8.9 Hz, 2H, arom. H), 6.99 (br. s, 1H, OH), 6.75 (br. s, 1H, OH), 6.62 (d, $J$ = 3.7 Hz, 1H, OH), 6.45 (d, $J$ = 6.6 Hz, 1H, OH), 6.27 (d, $J$ = 6.8 Hz, 1H, OH), 5.51 (d, $J$ = 3.9 Hz, 1H, H-1″), 5.21–5.18 (m, 1H, H-2), 5.03 (dd, $J$ = 8.1 and 11.0 Hz, 1H, H-6″), 4.79 (dd, $J$ = 3.5 Hz and 11.0 Hz, 1H, H-6″), 4.66–4.57 (m, 3H, H-1, H-2″, H-5″), 4.39–4.24 (m, 5H, H-3″, H-4″, H-1, H-3, H-4), 2.46 (t, $J$ = 7.5 Hz, 2H, $COCH_2$), 1.98–1.18 (m, 72H, $CH_2$), 0.88 (t, $J$ = 6.3 Hz, 6H, $CH_3$).

$^{13}$C NMR (75 MHz, pyridine-$d_5$): $\delta$ 172.33, 153.39, 138.14, 128.14, 128.05, 126.14, 119.21, 100.24, 74.89, 71.53, 70.08, 69.72, 69.27, 68.86, 67.57, 64.78, 50.50, 35.64, 32.88, 30.96, 30.94, 29.31, 29.18, 28.98, 28.87, 28.84, 28.76, 28.74, 28.70, 28.63, 28.57, 28.45, 28.43, 25.37, 25.18, 21.77, 13.11.

Exact mass (ESI-MS) for $C_{57}H_{103}ClN_2O_{10}$ $[M+H]^+$ found, 1011.7393; calcd, 1011.7374; mp 162.0–164.0 °C.

#### 4.1.5.2. (2*S*,3*S*,4*R*)-1-*O*-(6-*O*-(1-Naphthylcarbamoyl)-α-D-galactopyranosyl)-2-(hexacosanamido)octadecane-1,3,4-triol (5b).

$^1$H NMR (300 MHz, pyridine-$d_5$): $\delta$ 10.68 (s, 1H, NH), 8.74–8.70 (m, 1H, arom H), 8.58 (d, $J$ = 8.6 Hz, 1H, NH), 8.26 (d, $J$ = 7.3 Hz, 1H, arom. H), 7.94 (d, $J$ = 7.5 Hz, 1H, arom. H), 7.74 (d, $J$ = 8.4 Hz, 1H, arom. H), 7.62–7.51 (m, 3H, arom. H), 5.58 (d, $J$ = 3.9 Hz, 1H, H- 1″), 5.28–5.22 (m, 1H, H-2), 5.17–5.06 (m, 1H, H-6″), 4.94 (dd, $J$ = 4.2 Hz and 11.2 Hz, 1H, H-6″), 4.84–4.65 (m, 3H, H-1, H-5″, H-2″), 4.48–4.37 (m, 4H, H-1, H-3, H-3″, H-4″), 4.33–4.28 (m, 1H, H-4), 2.51–2.46 (m, 2H, $COCH_2$), 1.93–1.23 (m, 72H, $CH_2$), 0.91–0.86 (m, 6H, $CH_3$).

$^{13}$C NMR (75 MHz, pyridine-$d_5$): $\delta$ 172.27, 154.65, 149.43, 135.00, 133.63, 127.56, 125.15, 125.11, 125.04, 123.71, 100.24, 75.11, 71.50, 70.10, 69.75, 69.29, 68.90, 64.83, 50.45, 35.66, 30.95, 30.94, 29.19, 28.95, 28.86, 28.83, 28.76, 28.74, 28.68, 28.62, 28.58, 28.45, 28.43, 25.32, 25.22, 21.76, 13.10.

Exact mass (ESI-MS) for $C_{61}H_{106}N_2O_{10}$ $[M+H]^+$ found, 1027.7919; calcd, 1027.7926; mp 159.0–161.0 °C.

#### 4.1.5.3. (2*S*,3*S*,4*R*)-1-*O*-(6-*O*-(4-Pyridinylcarbamoyl)-α-D-galactopyranosyl)-2-(hexacosanamido)octadecane-1,3,4-triol (5c).

$^1$H NMR (300 MHz, pyridine-$d_5$): $\delta$ 11.18 (s, 1H, NH), 8.83 (d, $J$ = 8.3 Hz, 1H, NH), 8.67 (dd, $J$ = 1.4 Hz and 4.8 Hz, 2H, arom. H), 7.93 (dd, $J$ = 1.4 Hz and 4.8 Hz, 2H, arom. H), 5.55 (d, $J$ = 3.8 Hz, 1H, H-1″), 5.26–5.20 (m, 1H, H-2), 5.08 (dd, $J$ = 8.2 Hz and 11.0 Hz, 1H, H- 6″), 4.77 (dd, $J$ = 3.4 Hz and 11.0 Hz, 1H, H-6″), 4.72–4.62 (m, 3H, H-1, H-2″, H-5″), 4.45–4.30 (m, 5H, H-3″, H-4″, H-1, H-3, H-4), 2.53 (t, $J$ = 7.5 Hz, 2H, $COCH_2$), 1.95–1.23 (m, 72H, $CH_2$), 0.88 (t, $J$ = 6.5 Hz, 6H, $CH_3$).

$^{13}$C NMR (75 MHz, pyridine-$d_5$): $\delta$ 173.94, 154.68, 151.19, 147.76, 113.56, 101.56, 76.57, 72.96, 71.66, 70.43, 53.40, 46.06, 37.19, 32.45, 30.72, 30.50, 30.38, 30.34, 30.27, 30.24, 30.17, 30.09, 29.94, 26.89, 26.75, 23.26, 14.61.

Exact mass (ESI-MS) for $C_{56}H_{103}N_3O_{10}$ $[M+H]^+$ found, 978.7662; calcd, 978.7722; mp decomposition.

#### 4.1.5.4. (2*S*,3*S*,4*R*)-(*N*-Phenyl-α-D-galactopyranuronamidyl)-2-(hexacosanamido)octadecane-3,4-diol (6a).

$^1$H NMR (300 MHz, pyridine-$d_5$): $\delta$ 9.88 (s, 1H, NH), 8.49 (d, $J$ = 8.8 Hz, 1H, NH), 8.12 (dd, $J$ = 1.1 Hz and 8.6 Hz, 2H, arom. H), 7.36–7.30 (m, 3H, arom. H and OH), 7.10 (t, $J$ = 7.3 Hz, 1H, arom. H), 6.79 (d, $J$ = 5.2 Hz, 1H, OH), 6.43 (d, $J$ = 5.9 Hz, 1H, OH), 6.12 (d, $J$ = 5.4 Hz, 1H, OH), 5.47 (d, $J$ = 3.8 Hz, 1H, H-1″), 5.27–5.24 (m, 1H, H-2), 5.02–4.99 (m, 3H, OH, H-4″ and H-5″), 4.66–4.55 (m, 2H, H-1 and H-2″), 4.44 (app. d, $J$ = 9.2 Hz, 1H, H-3″), 4.32–4.21 (m, 3H, H-1, H-3, H-4), 2.49–2.44 (m, 2H, $COCH_2$), 1.40–1.12 (m, 72H, $CH_2$), 0.88 (t, $J$ = 6.6 Hz, 6H, $CH_3$).

$^{13}$C NMR (75 MHz, pyridine-$d_5$): $\delta$ 173.77, 169.23, 139.70, 129.36, 120.95, 101.60, 76.53, 74.21, 72.76, 71.71, 71.36, 69.97, 69.18, 51.49, 37.07, 34.44, 32.44, 32.43, 30.67, 30.45, 30.35, 30.31, 30.24, 30.22, 30.19, 30.11, 30.07, 29.93, 29.91, 26.80, 26.71, 23.25, 14.60.

Exact mass (ESI-MS) for $C_{56}H_{102}N_2O_9$ $[M+H]^+$ found, 947.7612; calcd, 947.7658; mp 136.0–138.0 °C.

#### 4.1.5.5. (2*S*,3*S*,4*R*)-(*N*-(3,4-Dichlorophenyl)-α-D-galactopyranuronamidyl)-2-(hexacosanamido)octadecane-3,4-diol (6b).

$^1$H NMR (300 MHz, pyridine-$d_5$): $\delta$ 10.19 (s, 1H, NH), 8.52 (d, $J$ = 8.7 Hz, 1H, NH), 8.46 (d, $J$ = 2.4 Hz, 1H, arom. H), 7.92 (dd, $J$ = 2.4 Hz and 9.0 Hz, 1H, arom. H), 7.39 (d, $J$ = 9.0 Hz, 1H, arom. H), 7.28 (bs, 1H, OH), 6.85 (bs, 1H, OH), 6.45 (d, $J$ = 4.2 Hz, 1H, OH), 6.13 (bs, 1H, OH), 5.40 (d, $J$ = 3.6 Hz, 1H, H-1″), 5.27–5.23 (m, 1H, H-2), 5.03–4.96 (m, 3H, H-4″, H-5″ and OH), 4.62 (dd, $J$ = 3.6 Hz and 10.5 Hz, 1H, H-2″), 4.57 (dd, $J$ = 5.1 Hz and 10.8 Hz, 1H, H-3), 4.44 (dd,

$J$ = 1.8 Hz and 9.6 Hz, 1H, H-3″), 4.26–4.21 (m, 3H, H-1 and H-4), 2.49–2.44 (m, 2H, $COCH_2$), 2.29–1.18 (m, 72H, $CH_2$), 0.89 (t, $J$ = 6.6 Hz, 6H, $CH_3$).

$^{13}$C NMR (75 MHz, pyridine-$d_5$): $\delta$ 171.94, 168.21, 138.12, 131.03, 129.39, 125.19, 121.60, 121.10, 119.06, 100.22, 75.33, 72.80, 71.31, 70.27, 68.78, 68.42, 49.86, 35.56, 33.15, 30.95, 30.94, 29.17, 28.95, 28.86, 28.83, 28.76, 28.74, 28.70, 28.64, 28.58, 28.45, 28.43, 25.29, 25.21, 21.76, 13.10.

Exact mass (ESI-MS) for $C_{56}H_{100}Cl_2N_2O_9$ $[M+Na]^+$ found, 1015.6865; calcd, 1015.6884; mp 155.0–157.0 °C.

**4.1.5.6. (2*S*,3*S*,4*R*)-(*N*-Benzyl-α-D-galactopyranuronamidyl)-2-(hexacosanamido)octadecane-3,4-diol (6c).** $^1$H NMR (300 MHz, pyridine-$d_5$): $\delta$ 8.69 (dd, $J$ = 5.9 Hz and 6.8 Hz, 1H, NH), 8.47 (d, $J$ = 8.7 Hz, 1H, NH), 7.59 (d, $J$ = 5.9 Hz, 2H, arom. H), 7.30–7.17 (m, 4H, arom. H and OH), 6.40 (bs, 1H, OH), 5.58 (d, $J$ = 3.9 Hz, 1H, H-1″), 5.03–5.00 (m, 2H, H-2 and OH), 4.98–4.95 (m, 4H, H-4″, OH and NH-$CH_2$), 4.69 (dd, $J$ = 3.8 Hz and 9.9 Hz, 1H, H-2″), 4.63–4.56 (m, 2H, H-3 and H-5″), 4.44 (dd, $J$ = 3.1 Hz and 10.0 Hz, 1H, H-3″), 4.34–4.27 (m, 3H, H-1 and H-4), 2.48–2.43 (m, 2H, $COCH_2$), 1.93–1.09 (m, 72H, $CH_2$), 0.89 (t, $J$ = 6.7 Hz, 6H, $CH_3$).

$^{13}$C NMR (75 MHz, pyridine-$d_5$): $\delta$ 172.12, 169.18, 138.92, 127.50, 126.55, 125.87, 123.04, 121.66, 100.15, 75.12, 72.52, 71.26, 70.08, 69.96, 68.61, 67.55, 49.94, 41.71, 35.57, 32.98, 30.96, 30.94, 29.20, 28.98, 28.87, 28.83, 28.76, 28.74, 28.70, 28.63, 28.58, 28.45, 28.43, 25.32, 25.22, 21.77, 13.11.

Exact mass (ESI-MS) for $C_{57}H_{104}N_2O_9$ $[M+H]^+$ found, 961.7808; calcd, 961.7815; mp 128.0–130.0 °C.

**4.1.5.7. (2*S*,3*S*,4*R*)-(*N*-(3,4-Dichlorobenzyl)-α-D-galactopyranuronamidyl)-2-(hexacosanamido)octadecane-3,4-diol (6d).** $^1$H NMR (300 MHz, pyridine-$d_5$): $\delta$ 8.96 (dd, $J$ = 5.7 Hz and 7.1 Hz, 1H, NH), 8.48 (d, $J$ = 8.6 Hz, 1H, NH), 7.77 (d, $J$ = 1.9 Hz, 1H, arom. H), 7.41 (dd, $J$ = 2.0 Hz and 8.3 Hz, 1H, arom. H), 7.32 (d, $J$ = 8.2 Hz, 1H, arom. H), 6.75 (app. s, 1H, OH), 6.43 (d, $J$ = 5.7 Hz, 1H, OH), 6.13 (d, $J$ = 4.8 Hz, 1H, OH), 5.60 (d, $J$ = 3.8 Hz, 1H, H-1″), 5.26 (app. d, $J$ = 4.6 Hz, 1H, H-2), 5.01–4.92 (m, 3H, NH-$CH_2$, H-4″ and H-5″), 4.69–4.56 (m, H2, H-2″ and H-1), 4.49–4.42 (m, 2H, H-3″ and NH-$CH_2$), 4.33–4.29 (m, 3H, H-1, H-3 and H- 4), 2.46 (app. t, $J$ = 7.5 Hz, 2H, $COCH_2$), 1.97–1.16 (m, 72H, $CH_2$), 0.89 (t, $J$ = 6.7 Hz, 6H, $CH_3$).

$^{13}$C NMR (75 MHz, pyridine-$d_5$): $\delta$ 173.43, 170.90, 141.50, 132.57, 130.84, 130.65, 130.10, 127.77, 101.72, 76.86, 74.10, 72.75, 71.68, 71.44, 70.08, 51.32, 42.16, 37.07, 34.66, 32.45, 32.46, 30.71, 30.48, 30.38, 30.34, 30.27, 30.25, 30.20, 30.14, 30.10, 29.96, 29.94, 26.80, 26.72, 23.28, 14.61.

Exact mass (ESI-MS) for $C_{57}H_{102}Cl_2N_2O_9$ $[M+H]^+$ found, 1029.7021; calcd, 1029.7041; mp 144.0–146.0 °C.

**4.1.5.8. (2*S*,3*S*,4*R*)-(*N*-Phenethyl-α-D-galactopyranuronamidyl)-2-(hexacosanamido)octadecane-3,4-diol (6e).** $^1$H NMR (300 MHz, pyridine-$d_5$): $\delta$ 8.50 (d, $J$ = 8.7 Hz, 1H, NH), 8.13 (t, $J$ = 6.0 Hz, 1H, NH), 7.32–7.18 (m, 5H, arom. H), 7.03 (br. s, 1H, OH), 6.41 (br. s, 1H, OH), 6.13 (br. s, 1H, OH), 5.54 (d, $J$ = 3.9 Hz, 1H, H-1″), 5.27 (app. d, $J$ = 4.4 Hz, 1H, H-2), 4.99–4.91 (m, 2H, H-4″ and H-5″), 4.65 (dd, $J$ = 3.8 Hz and 10.0 Hz, 1H, H-2″), 4.60 (dd, $J$ = 5.4 Hz and 10.7 Hz, 1H, H-1), 4.41 (dd, $J$ = 3.1 Hz and 10.0 Hz, 1H, H-3″), 4.39–4.31 (m, 1H, H-4), 4.30 (dd, $J$ = 4.2 Hz and 10.7 Hz, 1H, H-1), 3.72 (dd, $J$ = 6.5 Hz and 14.4 Hz, 2H, NH-$CH_2$), 2.94 (app. t, $J$ = 7.5 Hz, 2H, $CH_2$), 2.48 (t, $J$ = 7.1 Hz, 2H, $COCH_2$), 1.97–1.18 (m, 72H, $CH_2$), 0.89 (t, $J$ = 6.6 Hz, 6H, $CH_3$).

$^{13}$C NMR (75 MHz, pyridine-$d_5$): $\delta$ 173.42, 170.26, 140.50, 136.52, 129.67, 129.16, 126.87, 123.50, 101.65, 76.91, 73.90, 72.78, 71.53, 71.49, 70.11, 69.17, 51.29, 41.44, 37.08, 36.72, 34.66, 32.46, 32.45, 30.71, 30.48, 30.37, 30.34, 30.27, 30.25, 30.21, 30.15, 30.11, 29.96, 29.94, 26.81, 26.73, 23.27, 14.61.

Exact mass (ESI-MS) for $C_{58}H_{106}N_2O_9$ $[M+H]^+$ found, 975.7974; calcd, 975.7977; mp 144.0–146.0 °C.

**4.1.6. Procedure for debenzylation of 6f and 6g**

To a solution of the protected amide (0.07 mmol) in $CH_2Cl_2$ (1 mL) was added aluminum chloride (109 mg, 0.82 mmol) and dimethylaniline (0.13 mL, 1.00 mmol). After stirring for 8 h, the reaction mixture was quenched with a 1 N solution of HCl (1.5 mL) followed by extraction with EtOAc. Next, the combined organic layers were washed with a saturated $NaHCO_3$ solution and brine, dried over $Na_2SO_4$ and evaporated to dryness. The resulting residue was submitted to column chromatography ($CH_2Cl_2$/MeOH: 28/2), yielding amides **6f** (10 mg, 15%) and **6g** (8.5 mg, 11%).

**4.1.6.1. (2*S*,3*S*,4*R*)-(*N*-(Thiophen-3-ylmethyl)-α-D-galactopyranuronamidyl)-2-(hexacosanamido)octadecane-3,4-diol (6f).** $^1$H NMR (300 MHz, pyridine-$d_5$): $\delta$ 8.86 (t, $J$ = 6.2 Hz, 1H, NH), 8.45 (d, $J$ = 8.5 Hz, 1H, NH), 7.25 (app. dd, $J$ = 1.2 Hz and 5.2 Hz, 2H, arom. H), 7.11 (br s, 1H, OH), 6.91 (dd, $J$ = 3.6 Hz and 5.1 Hz, 1H, arom. H), 6.68 (br. s, 1H, OH), 6.39 (br s, 1H OH), 6.12 (br. s, 1H, OH), 5.55 (d, $J$ = 3.8 Hz, 1H, H-1″), 5.28–5.21 (m, 1H, H-2), 5.0–4.82 (m, 4H, NH-$CH_2$, H-4″ and H-5″), 4.66 (dd, $J$ = 3.8 Hz and 9.9 Hz, 1H, H-2″), 4.56 (dd, $J$ = 5.4 Hz and 10.5 Hz, 1H, H-1), 4.41 (d, $J$ = 3.2 Hz and 9.9 Hz, 1H, H-3″), 4.35–4.19 (m, 2H, H-3 and H-4), 4.25 (dd, $J$ = 4.2 Hz and 10.6 Hz, 1H, H-1), 2.44 (app. t, $J$ = 7.4 Hz, 2H, $COCH_2$), 1.92–1.19 (m, 72H, $CH_2$), 0.89 (t, $J$ = 6.7 Hz, 6H, $CH_3$).

$^{13}$C NMR (75 MHz, pyridine-$d_5$): $\delta$ 171.87, 168.91, 141.99, 125.87, 124.66, 123.74, 100.23, 75.37, 72.51, 71.25, 70.01, 69.97, 68.60, 67.72, 53.86, 49.76, 36.91, 35.55, 33.13, 30.95, 30.94, 29.20, 28.97, 28.87, 28.83, 28.75, 28.74, 28.69, 28.63, 28.58, 28.45, 28.43, 25.29, 25.20, 21.76, 13.10.

Exact mass (ESI-MS) for $C_{55}H_{102}N_2O_9S$ $[M+H]^+$ found, 967.7431; calcd, 967.7379; mp 126.0–128.0 °C.

**4.1.6.2. (2*S*,3*S*,4*R*)-(*N*-(Naphtalen-1-ylmethyl)-α-D-galactopyranuronamidyl)-2-(hexacosanamido)octadecane-3,4-diol (6g).** $^1$H NMR (300 MHz, pyridine-$d_5$): $\delta$ 8.49 (d, $J$ = 8.7 Hz, 1H, NH), 8.29 (dd, $J$ = 2.7 Hz and 6.7 Hz, 1H, NH), 7.94 (d, $J$ = 7.2 Hz, 1H, arom. H), 7.87 (dd, $J$ = 3.1 Hz and 6.3 Hz, 1H, arom. H), 7.77 (d, $J$ = 8.3 Hz, 1H, arom. H), 7.59–7.38 (m, 4H, arom. H), 5.54 (d, $J$ = 3.7 Hz, 1H, H- 1″), 5.44 (dd, $J$ = 6.7 Hz and 15.6 Hz, 1H, NH-$CH_2$), 5.29–5.21 (m, 1H, H-2), 5.13–4.95 (m, 3H, NH-$CH_2$, H- 4″ and H-5″), 4.68 (dd, $J$ = 3.7 Hz and 9.8 Hz, 1H, H- 2″), 4.60 (dd, $J$ = 5.4 Hz and 10.6 Hz, 1H, H-1), 4.45 (dd, $J$ = 3.1 Hz and 10.0 Hz, H-3″), 4.43–4.29 (m, 2H, H-3 and H-4), 4.28 (dd, $J$ = 4.4 Hz and 10.7 Hz, 1H, H-1), 2.46 (app. t, $J$ = 7.1 Hz, 2H, $COCH_2$), 1.97–1.19 (m, 72H, $CH_2$), 0.89 (t, $J$ = 6.6 Hz, 6H, $CH_3$).

$^{13}$C NMR (75 MHz, pyridine-$d_5$): $\delta$ 173.42, 170.60, 134.50, 132.12, 129.31, 128.21, 126.85, 126.35, 126.27, 125.92, 101.77, 76.84, 74.18, 72.79, 71.63, 71.50, 70.12, 69.29, 55.37, 51.34, 41.33, 37.07, 34.62, 32.47, 30.82, 30.71, 30.48, 30.38, 30.34, 30.27, 30.25, 30.21, 30.15, 30.11, 29.96, 29.94, 26.81, 26.73, 23.28, 14.62.

Exact mass (ESI-MS) for $C_{61}H_{106}N_2O_9$ $[M+H]^+$ found, 1011.7974; calcd, 1011.7971; mp 121.0–123.0 °C.

### 4.2. Biological evaluation

#### 4.2.1. Mice

C57BL/6 and mice were in house bred (in accordance with the general recommendations for animal breeding and housing) or purchased from the Harlan Laboratory. Experiments were conducted according to the guidelines of the Ethical Committee of Laboratory Animals Welfare of Ghent University. Mice used for experiments were between 5 and 12 weeks old.

#### 4.2.2. In vivo activation of *i*NKT cells and serum analysis

For in vivo activation of *i*NKT cells C57BL/6 mice were intraperitoneally injected with 5 μg glycolipid (dissolved in PBS with DMSO). Subsequently mice were bled at 4 h and 16 h after injection. Serum was stored at −20 and analyzed for IL-4 and IFN-γ levels by ELISA.

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