Title:

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α-GalCer analogues were synthesized that combine known Th1 polarizing C6”-modifications with a *C*-glycosidic linkage. We employed a protecting group strategy that allowed the preparation of both saturated and unsaturated derivatives with variable C6”-substituents. Selected analogues demontrate promising activity in mice. Interestingly, introduction of a 6”-O-pyridinylcarbamaoyl substituent to α-*C*-GalCer restores its antigenicity in human *i*NKT cells.

Introduction

In the past two decades immunogenic glycolipids have gained increasing interest due to their potential as vaccine adjuvants to fight microbial infections and cancer, as well as for the treatment for autoimmune disorders.[1] In this context, the most extensively studied glycolipid is α-galactosylceramide (α-GalCer, **1**, Figure 1), which consist of a ceramide moiety that is α-anomerically coupled to a galactopyranosyl moiety. α-GalCer is known to bind with high specificity to CD1d, a protein present on antigen presenting cells.[2] Upon recognition of the CD1d-α-GalCer complex by the T cell receptor (TCR) of invariant natural killer T cells (*i*NKT cells) large amounts of both pro-inflammatory T-helper 1 (Th1) and anti-inflammatory Th2 cytokines are secreted. The concomitant release of both cytokine types, which have opposing roles *in vivo*, is believed to limit the clinical outcome of α-GalCer therapy.[3] Consequently, potent analogues able to skew the cytokine response towards either Th1 or Th2 are of great interest.

Glycolipid synthesis groups furnished a plethora of α-GalCer analogues[1] and to date, the best known Th1-polarizing glycolipid is the *C*-glycoside analogue of α-GalCer, α-*C*-GalCer (**2**),[4] which shows increased activity against malaria and B16-melanoma in mice.[5] The prolonged secretion of Th1-cytokines like IFN-γ and IL-12 forms the basis of the observed Th1-profile[6] and is likely due to the increased metabolic stability of α-*C*-GalCer. Unfortunately, in contrast to α-GalCer, α-*C*-GalCer is a weak antigen for human *i*NKT cells. However *C*-glycosides having an *E*-alkene linker between the galactose sugar and the ceramide do stimulate human *i*NKT cells.[7]

We have previously shown that introducing aromatic urea or carbamate substituents at the C6”-position of the galactopyranose unit, can result in powerful Th1-biasing *i*NKT-cell antigens. The Th1-bias observed for α-NU-GalCer (**3**) arises from an increased stability of the CD1d-glycolipid complex as the naphthyl moiety induces and occupies an additional hydrophobic pocket in CD1d, thereby enhancing the affinity.[8] The pyridine ring of PyrC-α-GalCer (**4**) on the other hand makes extra contacts with the TCR, thereby enhancing the stability of the ternary complex.[9]

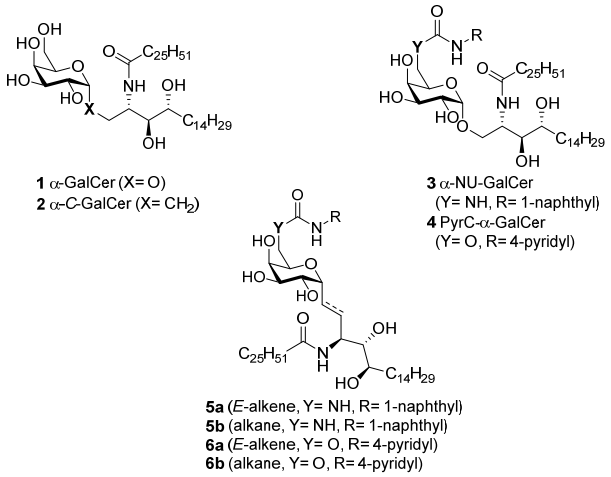

Figure 1. Structures of glycolipids **1** to **6**

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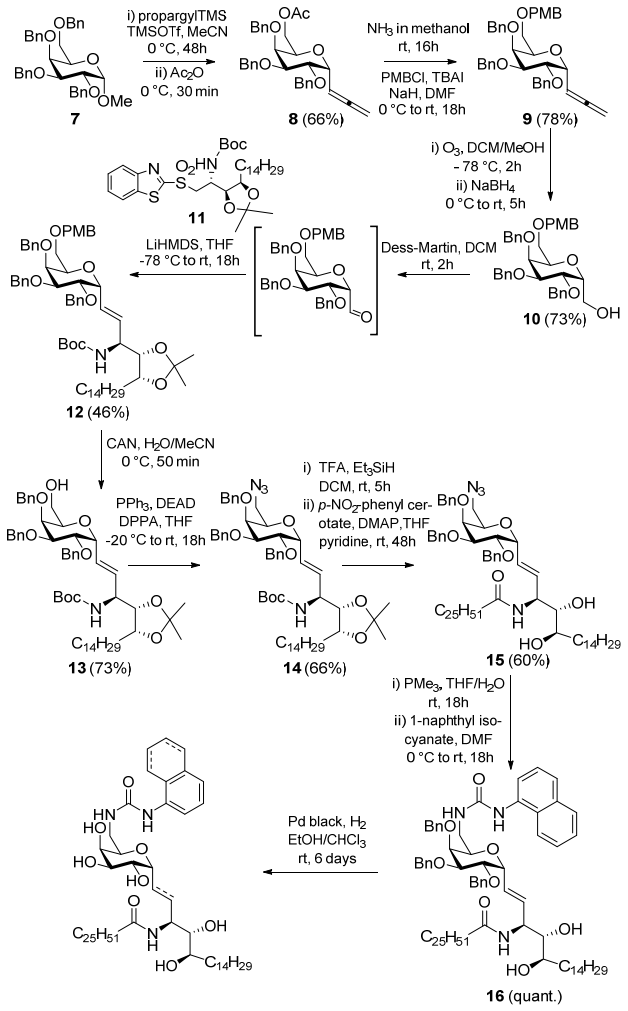
Electronic Supplementary Information (ESI) available: ^{1}H- and ^{13}C-NMR spectra of new compounds + additional figures. See DOI: 10.1039/x0xx00000x

The idea of combining the above mentioned C6”-modifications with a *C*-glycosidic linkage seems obvious, yet no synthetic strategy has been developed to date with the exception of a C6”-methoxy analogue featuring an *E*-alkene connection together with a modified phytosphingosine lacking the 4-hydroxy.[10] Here, we report a divergent synthetic route towards C6”-modified α-*C*-GalCer derivatives **5a**,**b** and **6a**,**b** as new metabolically stable *i*NKT cell agonists.

Results and discussion

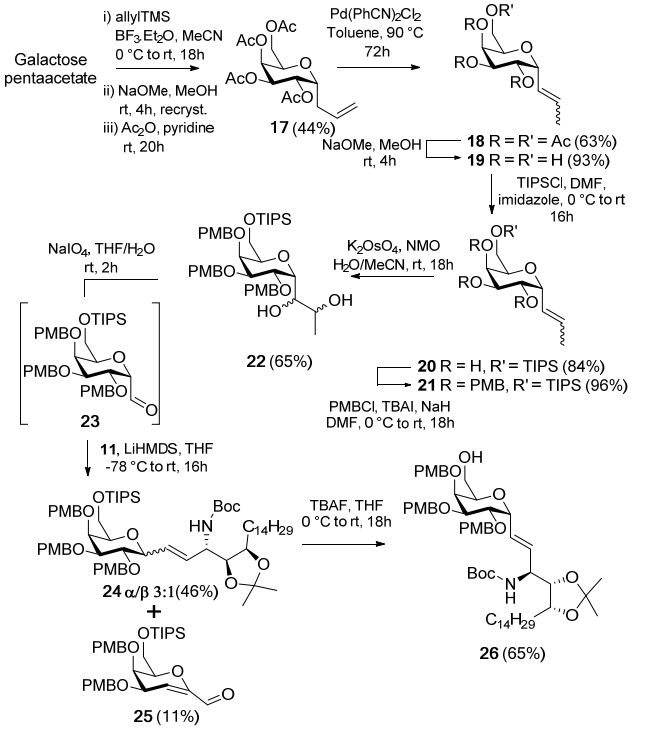
Chemical synthesis

Initially we modified the procedure of Chen et al.[11] utilizing the Julia-Kocienski olefination method to join the ceramide part to the sugar, as it allows the formation of both saturated and unsaturated derivatives. To allow swift manipulation of the C6”-position an orthogonally protected galactose formaldehyde was required. Treatment of perbenzylated α-methyl galactoside **7** with propargyltrimethylsilane and trimethylsilyl triflate, followed by the addition of acetic anhydride, furnished allene **8** (Scheme 1).



Scheme 1.

Next, the acetate group was removed with ammonia in methanol and the alcohol was protected as PMB-ether. Ozonolysis of **9** and *in situ* reduction of the product with sodium borohydride gave primary alcohol **10**, which was reoxidized to the desired galactose formaldehyde enabling Julia-Kocienski olefination with the known benzothiazole **11**.[11] Unfortunately the ozonolysis/reduction sequence was poorly reproducible as only trace amounts of desired alcohol were obtained in most attempts. Olefin **12** was obtained without epimerization at the anomeric center and deprotection of the PMB-ether was accomplished with ceric ammonium nitrate at low temperature and short reaction time to avoid unwanted acetonide cleavage. The primary alcohol was converted to an azide by means of a Mitsunobu reaction with DPPA, followed by deprotection of the acid labile protecting groups with TFA and acylation of the resulting amine with the *p*-nitrophenyl ester of cerotic acid. A troublesome side reaction occurring during TFA treatment is the conversion of the released amine to the trifluoroacetamide, which significantly lowered the yield. Addition of triethylsilane to suppress this side reaction did not particularly improve the reaction outcome.[6] Staudinger reduction followed by addition of 1-naphthyl isocyanate provided protected urea **16**. Catalytic hydrogenolysis and alkene saturation using Pd-black under hydrogen atmosphere proceeded sluggishly resulting in an inseparable mixture of products, including saturation of the naphthyl to the tetraline ring and incomplete double bond saturation. As Birch reduction is incompatible with the naphthyl moiety we were forced to reconsider our strategy.



Scheme 2.

To circumvent the unwanted saturation of the naphthyl ring, protecting group cleavage without hydrogenation was desirable. Considering the protecting group strategy of the sulfone we opted for PMB-groups on the sugar aldehyde, as a single acid hydrolysis step could remove all protecting groups. Rather than employing the allene approach, which suffers from low yields due to the unreliable ozonolysis/reduction step and the requirement of expensive reagents such as propargyltrimethylsilane, we synthesized propene **18** by standard *C*-glycosylation of galactosyl pentaacetate followed by palladium-catalyzed isomerization of the double bond (Scheme 2). Zemplén deacetylation and protection of the primary alcohol as triisopropylsilyl ether gave propenylgalactoside **20**, which was treated with PMBCl to mask the remaining hydroxyl functions. Propene **21** was subjected to an osmium-catalyzed dihydroxylation to furnish vicinal diol **22**.

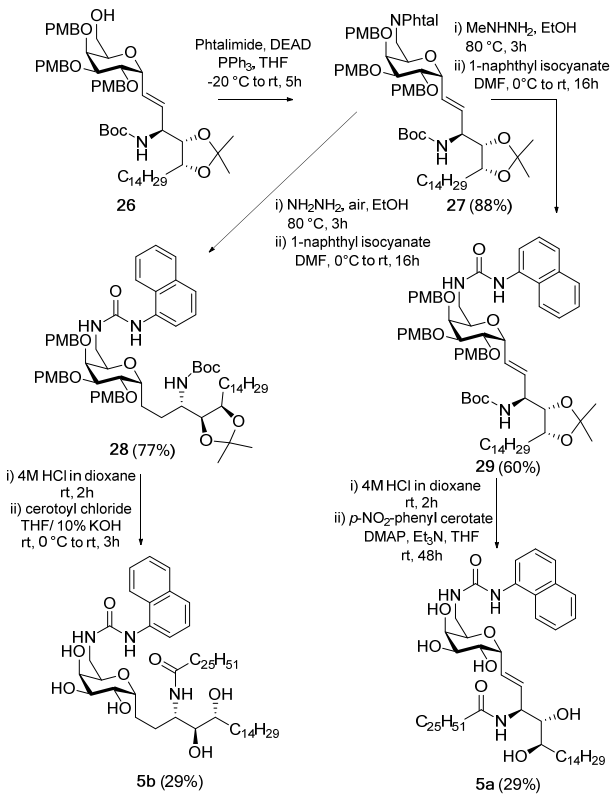
Oxidation of **22** with sodium periodate afforded *C*-formyl galactoside **23** amenable for Julia-Kocienski olefination with the previously used sulfone. Using the same coupling protocol as for **12**, an inseparable mixture of α- and β-anomers was obtained together with side product **25** due to β-elimination. The α/β-ratio could be increased by slow addition of the aldehyde to the deprotonated sulfone in dilute conditions, which also reduced the formation of elimination product. After deprotection of the silyl ether with tetrabutylammonium fluoride both anomers could be easily purified by column chromatography to furnish alcohol **26** as the single α-anomer.

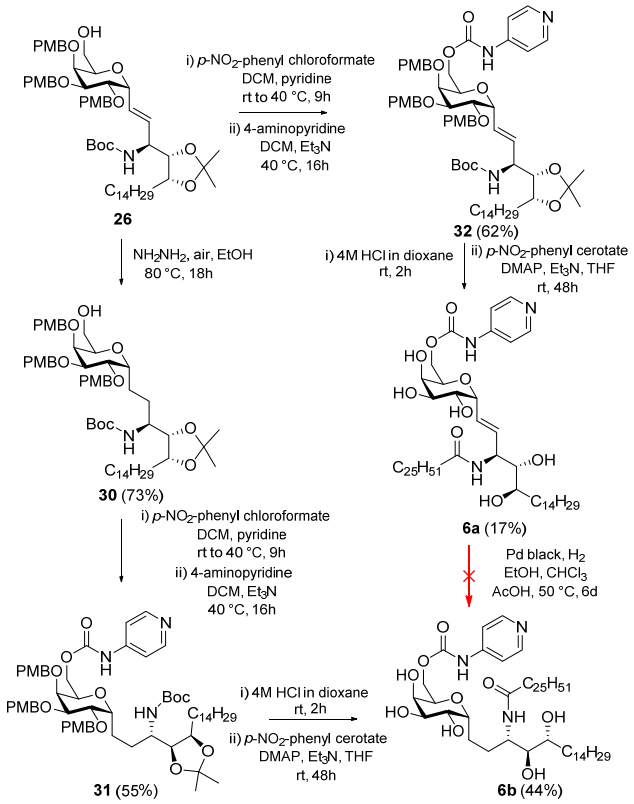
Mitsunobu reaction of **26** with hydrazoic acid to introduce an azide functionality at C6” did not furnish the desired product.

Scheme 3

The use of DPPA as azide source gave no reaction, while mesylation followed by sodium azide treatment gave the undesired elimination product. To our delight Mitsunobu reaction with phthalimide swiftly yielded **27** in good yield (Scheme 3). Treatment with hydrazine monohydrate not only liberated the free amine but conveniently also reduced the alkene via diimide reduction. Switching hydrazine for methylhydrazine allowed to remain the double bond intact. Next, treatment of the crude amines with 1-naphthyl isocyanate furnished the corresponding urea derivatives. To avoid trifluoroacetamide formation the acid-labile protecting groups were removed by dissolving the intermediates **28** and **29** in a 4 M HCl in dioxane solution. An attempt to acylate the resulting amines by treatment with cerotoyl chloride in a biphasic mixture of THF and saturated sodium acetate solution[12] resulted in a mixture of the desired compound and the corresponding acetamide, probably due to mixed anhydride formation. Addition of the *p*-nitrophenyl ester of cerotic acid in DCM gave **5a**, while acylation with cerotoyl chloride in a biphasic mixture of THF and 1 M KOH solution gave **5b**. The low yields associated with the final acylation are likely due to the low solubility of the final compounds and the cerotic acid derivative.

Since 4-pyridylisocyanate is not commercially available the primary alcohol of **26** was first treated with *p*-nitrophenyl chloroformate and the mixed anhydride was subsequently turned into the 4-pyridylcarbamate by addition of 4-aminopyridine (Scheme 4).





Scheme 4.

Deprotection with HCl and acylation gave **6a**. Obtaining the saturated carbamate through catalytic hydrogenation proved arduous since the pyridine nitrogen poisons the palladium catalyst. Addition of acetic acid to protonate the pyridine improved the conversion rate, although full saturation of the double bond could not be attained. Instead, we used the diimide reduction with hydrazine to saturate intermediate **26**. Further conversion following previously used reactions furnished saturated derivative **6a**.

Immunological activity

The biological activity of the C-glycosides **5**-**6** was assessed by measuring the IFN-γ and IL-4 levels after intraperitoneal injection of 1 µg of the corresponding glycolipids in Balb/c mice (Figure 2).

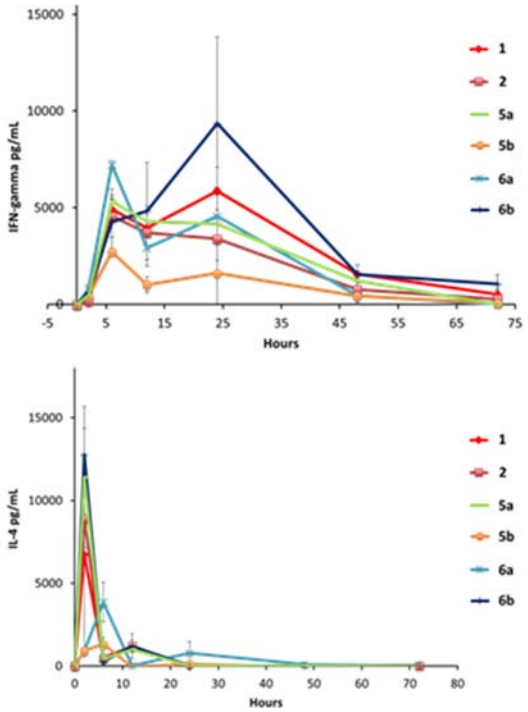


Figure 2: IFN-γ and IL-4 secretion, measured over the course of 72h, after intraperitoneal injection of 1 µg of the final compounds in Balb/c mice.

With the exception of **5b**, all glycolipids show superior antigenic properties as compared to α-*C*-GalCer. Moreover, pyridine carbamate **6b** is, to the best of our knowledge, the first *C*-glycoside that shows superior antigenic properties as compared to α-GalCer. In general the pyridine carbamate modified analogues are more potent than the naphthylurea derivatives. A similar trend is observed when the cytokine profile was investigated in C57BL/6 mice (Figure S1). However, while in Balb/c mice the unsaturated derivatives are less potent than the saturated *C*-glycosides, the opposite is true in C57BL/6 mice.

In addition, the *C*-glycosides were evaluated for their ability to stimulate human *i*NKT cells (Figure 3). We were delighted to observe that all final compounds activated human *i*NKT cells, , although this is most prominent for the pyridinylcarbamate analogues **6a**-**b**. This indicates that the appended C6"-substituents have the ability to restore the antigenic potency of α-*C*-GalCer. Furthermore, in line with previous findings, the *E*-alkene unsaturated derivatives display superior antigenic properties as compared to the saturated *C*-glycosides. In accordance with the results in Balb/c mice, the pyridine carbamates **6** surpass the antigenic potency of the naphthylurea compounds **5**. Although pyridine carbamate **6a** is less antigenic than its parent *O*-glycoside **4** (Figure S2), it shows more powerful human *i*NKT cell activation than α-GalCer.

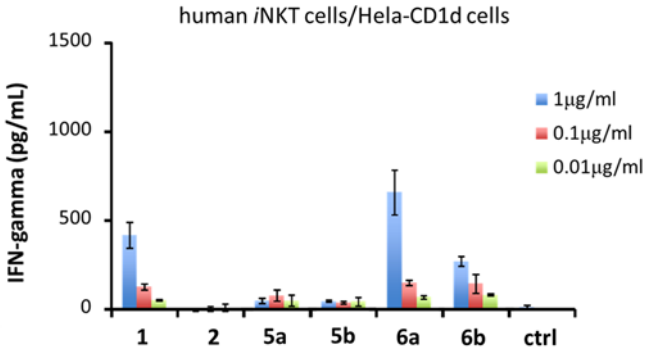


Figure 3: IFN-γ secretion after coculture of human *i*NKT cells and Hela CD1d cells with the corresponding *C*-glycosides

Conclusions

In summary, we developed a versatile synthetic strategy allowing the preparation of both saturated and unsaturated α-*C*-GalCer derivatives with variable C6"-substituents. All prepared *C*-glycosides were able to stimulate human *i*NKT cells, denoting that the appended C6"-substituents have the ability to restore the antigenic potency of α-*C*-GalCer, which lacks activity on human *i*NKT cells.

Experimental section

(3S,4S,5R)-1-(2',3',4'-tri-*O*-(4-methoxybenzyl)-6'-*O*-tri-isopropylsilyl-α-*C*-D-galactopyranosyl)-3-tert-butyloxy-carbonylamino-4,5-Di-*O*-isopropylidene-1-nonadecene-4,5-diol (24)

To a solution of diol **22** (930 mg, 1.23 mmol) in THF (16 mL) and H_2O (8 mL) was added $NaIO_4$ (1.32 g, 6.15 mmol) portionwise over a period of 1h. The reaction mixture was stirred at room temperature for 2.5h. Upon disappearance of the starting material (as judged by TLC), the mixture was diluted with Et_2O (30 mL) and the aqueous layer was extracted with Et_2O (3 x 30 mL). The combined organic phases were washed with saturated $NaHCO_3$ solution (100 mL), dried over Na_2SO_4, filtered and concentrated at 30 °C to afford crude

aldehyde which was used without further purification in the next step.

Lithium bis(trimethylsilyl)amide (1 M in THF, 2.46 mL, 2.46 mmol) was slowly added to a solution of sulfone **11** (786 mg, 1.23 mmol) in anhydrous THF (40 mL) at -78 °C. After 1.5h, the above prepared crude aldehyde in THF (40 mL) was added dropwise over a period of 2h. The mixture was allowed to warm to room temperature and stirred overnight. The reaction was quenched with water (50 mL) and extracted with Et_2O (3x 50 mL). The combined organic phases were washed with H_2O and brine, dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash chromatography (5 → 25% EtOAc in hexanes) to afford a mixture of α and β alkenes **24** (640 mg, 46%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) (mixture of anomers): δ 0.89 (t, J = 6.3 Hz, 3 H, terminal CH_3) 0.99 – 1.11 (m, 21 H, i-prop) 1.20 – 1.35 (m, 25 H, CH_2 and CH_3) 1.36 – 1.46 (m, 12 H, tBu and CH_3) 1.49 – 1.70 (m, 4 H, CH_2) 3.56 (dd, J = 9.2 and 2.5 Hz, 1 H, H-3) 3.71 – 3.78 (m, 3 H, H-3' and CH_2-6) 3.78 – 3.83 (m, 10 H, 3 x OCH_3 and H-5') 3.84 – 4.02 (m, 2 H, H-4 and H-5) 4.02 – 4.20 (m, 2 H, H-4' and H-2) 4.25 – 4.75 (m, 7 H, $\underline{CH_2}$PhOMe, H-1 and NH) 4.82 (d, J = 10.8 Hz, 1 H, $\underline{CH_2}$PhOMe) 5.87 – 6.01 (m, 2 H, CH=CH) 6.77 – 6.90 (m, 6 H, arom. H) 7.15 – 7.30 (m, 6 H, arom. H); ^{13} C NMR (75 MHz, $CDCl_3$): δ 11.91, 12.49, 14.11, 18.06, 18.12, 22.69, 25.53, 26.79, 27.53, 28.35, 29.02, 29.36, 29.60, 29.65, 29.69, 31.92, 52.21, 55.23, 61.81, 72.62, 72.80, 72.95, 73.98, 74.82, 79.47, 80.56, 107.97, 113.54, 113.65, 113.68, 125.76, 128.92, 129.02, 129.36, 129.66, 129.71, 130.85, 131.05, 131.18, 154.90, 159.00, 159.08; Exact mass (ESI-MS) for $C_{66}H_{106}NO_{12}Si$ $[M+H]^+$ found, 1132.7462; calcd, 1132.7479.

(3S,4S,5R)-1-(6'-hydroxy-2',3',4'-tri-*O*-(4-methoxybenzyl)-α-*C*-D-galactopyranosyl)-3-tert-butyloxycarbonylamino-4,5-Di-*O*-isopropylidene-1-nonadecene-4,5-diol (26)

A solution of **24** (2.3 g, 2.03 mmol) in THF (20 mL) was cooled to 0 °C and tetrabutylammonium fluoride (1 M in THF, 4.06 mL, 4.06 mmol) was added dropwise. The reaction mixture was allowed to reach room temperature and was stirred overnight. The reaction mixture was diluted with DCM (150 mL) and washed with saturated aqueous NH_4Cl solution (75 mL) and H_2O (75 mL). The aqueous layer was back extracted with DCM (2 x 50 mL) and the combined organic layers were washed with brine (100 mL). The organics were dried over Na_2SO_4, filtered and concentrated under reduced pressure and the residue was purified by flash column chromatography (0 → 30% EtOAc in hexanes) to provide α-glycoside **26** (1.28 g, 65 %) as a colourless oil together with the corresponding β-glycoside (362 mg, 18%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89 (t, J = 6.6 Hz, 3 H, terminal CH_3) 1.31 (s, 3 H, CH_3) 1.22 – 1.34 (m, 22 H, CH_2) 1.40 (s, 3 H, CH_3) 1.44 (s, 9 H, tBu) 1.48 – 1.71 (m, 4 H, CH_2) 2.02 (br. s, 1 H, OH) 3.51 – 3.62 (m, 2 H, H-5 and Ha-6') 3.81 (s, 3 H, OCH_3) 3.82 (s, 3 H, OCH_3) 3.82 (s, 3 H, OCH_3) 3.79 – 4.01 (m, 5 H, H-2, H-4, H-4', H-5' and Hb-6') 4.10 – 4.19 (m, 1 H, H-3) 4.25 – 4.36 (m, 1 H, H-3') 4.49 – 4.70 (m, 7 H, $\underline{CH_2}$PhOMe, H-1 and NH) 4.74 (d, J = 11.6 Hz, 1 H, $\underline{CH_2}$PhOMe) 5.84 (dd, J = 16.3 and 3.7 Hz, 1 H, $\underline{CH\text{-}1}$=CH) 5.93 (dd, J = 16.3 and 4.4 Hz, 1 H, CH=$\underline{CH\text{-}2}$) 6.83 – 6.90 (m, 6 H, arom. H) 7.19 – 7.27 (m, 6 H, arom. H); ^{13} C NMR (75 MHz, $CDCl_3$): δ 14.11, 22.69, 25.40, 26.85, 27.21, 28.37, 28.81, 29.36, 29.56, 29.62, 29.65, 29.69, 31.91, 52.15, 55.26, 61.86, 72.07, 72.88, 73.00, 73.03, 74.25, 77.21, 77.73, 78.01, 79.50, 79.61, 108.02, 113.71, 113.77, 113.82, 126.38, 129.13, 129.53, 129.88, 130.26, 130.50, 130.61, 131.43, 154.90, 159.17, 159.23, 159.35; Exact mass (ESI-MS) for $C_{57}H_{86}NO_{12}$ $[M+H]^+$ found, 976.6146; calcd, 976.6145.

(3S,4S,5R)-1-(2',3',4'-tri-*O*-(4-methoxybenzyl)-6'-phtalimidoyl-6'-deoxy-α-*C*-D-galactopyranosyl)-3-tert-butyloxycarbonylamino-4,5-Di-*O*-isopropylidene-1-nonadecene-4,5-diol (27)

To a solution of alcohol **26** (100 mg, 0.1 mmol) in anhydrous THF (2 mL) at -20 °C was added PPh_3 (79 mg, 0.3 mmol), DEAD (40% DEAD in toluene, 0.09 mL, 0.3 mmol) and phtalimide (44 mg, 0.3 mmol) in this order. The resulting mixture was allowed to reach room temperature and was stirred for 5h. Upon complete consumption of the starting material the solvents were removed under reduced pressure and the resulting residue was purified by flash column chromatography (10 → 30% EtOAc in hexanes) giving phtalimide **27** (100 mg, 88%) as a white waxy solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89 (t, J = 6.6 Hz, 3 H, terminal CH_3) 1.20 – 1.35 (m, 26 H, CH_3 and CH_2) 1.35 (s, 3 H, CH_3) 1.42 (s, 9 H, tBu) 1.46 – 1.66 (m, 3 H, CH_2) 3.50 – 3.60 (m, 1 H, H-5') 3.69 – 3.74 (m, 1 H, H-4') 3.80 – 3.81 (m, 2 H, CH_2-6') 3.82 (s, 4 H, H-5 and OCH_3) 3.82 (s, 3 H, OCH_3) 3.83 (s, 3 H, OCH_3) 3.98 (dd, J = 5.0 and 2.9 Hz, 1H, H-4) 4.04 – 4.13 (m, 1 H, H-3') 4.19 – 4.77 (m, 10 H, H-1', H-2', H-3, $\underline{CH_2}$PhOMe and NH) 5.67 (dd, J = 15.8 and 6.2 Hz, 1 H, $\underline{CH\text{-}1}$=CH) 5.91 (dd, J = 15.7 and 4.0 Hz, 1 H, CH=$\underline{CH\text{-}2}$) 6.82 – 6.93 (m, 6 H, arom. H) 7.10 – 7.17 (m, 2 H, arom. H) 7.22 – 7.27 (m, 2 H, arom. H) 7.32 – 7.38 (m, 2 H, arom. H) 7.66 – 7.70 (m, 2 H, Phtal) 7.79 – 7.83 (m, 2 H, Phtal); ^{13} C NMR (75 MHz, $CDCl_3$): δ 14.12, 22.69, 25.47, 26.99, 27.22, 28.40, 29.36, 29.59, 29.69, 31.92, 51.72, 55.28, 71.52, 72.63, 72.85, 76.79, 77.21, 77.82, 79.30, 79.72, 107.85, 113.73, 113.76, 123.10, 123.25, 123.59, 127.01, 129.19, 122.22, 129.50, 129.59, 129.80, 130.21, 130.38, 130.50, 130.64, 131.91, 132.08, 132.34, 133.60, 133.92, 134.30, 154.81, 159.13, 159.32, 168.30, 168.73; Exact mass (ESI-MS) for $C_{65}H_{88}N_2NaO_{13}$ $[M+Na]^+$ found, 1127.6172; calcd, 1127.6179.

(3S,4S,5R)-1-(2',3',4'-tri-*O*-(4-methoxybenzyl)-6'-naphtureido-6'-deoxy-α-*C*-D-galactopyranosyl)-3-tert-butyloxycarbonyl-amino-4,5-Di-*O*-isopropylidene-nonadecane-4,5-diol (28)

A solution of alkene **27** (100 mg, 0.09 mmol) in EtOH (4 mL) and hydrazine monohydrate (0.4 mL) was heated till reflux and stirred for 18h. Upon completion of the reaction, DCM (20 mL) was added and the organic layer was washed with 1 M NaOH solution (20 mL). The aqueous layer was back extracted with DCM (2 x 20 mL) and the combined organic phase was washed with H_2O (50 mL). The organics were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude amine was used in the next step without further purification. The amine was dissolved in DMF (2 mL) and cooled to 0 °C. Next 1-naphthyl isocyanate (34 µL, 0.23 mmol) was added and

the mixture was stirred overnight at room temperature. The solvents were removed under reduced pressure and the residue was purified by flash column chromatography (10 → 30% EtOAc in hexanes) to furnish urea **28** (80 mg, 77% over 2 steps) as a light brown solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89 (t, J = 6.6 Hz, 3 H, terminal CH_3) 1.15 – 1.32 (m, 27 H, CH_3 and CH_2) 1.38 (s, 3 H, CH_3) 1.42 (s, 9 H, tBu) 1.46 – 1.78 (m, 6 H, CH_2) 3.43 – 3.56 (m, 1 H, H-4) 3.64 – 3.75 (m, 3 H, H-5, CH_2-6') 3.79 (s, 3 H, OCH_3) 3.82 (s, 3 H, OCH_3) 3.82 (s, 3 H, OCH_3) 3.86 – 3.99 (m, 4 H, H-3, H-2', H-4' and H-5') 4.02 – 4.10 (m, 1 H, H-3') 4.37 (d, J = 12.4 Hz, 1 H, $\underline{CH_2}$PhOMe) 4.41 – 4.68 (m, 6 H, H-1' and $\underline{CH_2}$PhOMe) 5.69 (br. s, 1 H, NH) 6.78 – 6.91 (m, 6 H, arom. H) 7.11 – 7.26 (m, 6 H, arom. H) 7.41 – 7.53 (m, 4 H, naphthyl and NH) 7.60 – 7.73 (m, 1 H, naphthyl) 7.82 – 7.88 (m, 2 H, naphthyl) 7.98 – 8.06 (m, 1 H, naphthyl); ^{13} C NMR (75 MHz, $CDCl_3$): δ 14.12, 22.69, 25.39, 25.77, 26.76, 27.11, 28.43, 28.92, 29.37, 29.43, 29.57, 29.66, 29.71, 30.92, 31.92, 50.21, 55.25, 72.02, 72.53, 72.83, 73.53, 73.76, 77.20, 77.75, 79.82, 107.85, 113.74, 120.37, 121.62, 124.50, 125.80, 126.02, 128.46, 129.31, 129.47, 129.63, 129.73, 130.20, 130.37, 130.64, 134.11, 134.24, 155.87, 156.44, 156.96, 159.19, 159.34; Exact mass (ESI-MS) for $C_{68}H_{96}N_3O_{12}$ $[M+H]^+$ found, 1146.6990; calcd, 1146.6989.

(3S,4S,5R)-1-(2',3',4'-tri-*O*-(4-methoxybenzyl)-6'-naphtureido-6'-deoxy-α-*C*-D-galactopyranosyl)-3-tert-butyloxycarbonyl-amino-4,5-Di-*O*-isopropylidene-1-nonadecene-4,5-diol (29)

To a solution of alkene **27** (243 mg, 0.22 mmol) in degassed EtOH (3 mL) was added methylhydrazine (46 µL, 0.88 mmol). The resulting mixture was heated till reflux and stirred for 18h under nitrogen atmosphere. Upon completion of the reaction, DCM (20 mL) was added and the organic layer was washed with 1 M NaOH solution (20 mL). The aqueous layer was back extracted with DCM (2 x 20 mL) and the combined organic phase was washed with H_2O (50 mL). The organics were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude amine was used in the next step without further purification. The amine was dissolved in DMF (5 mL) and cooled to 0 °C. Next 1-naphthyl isocyanate (82 µL, 0.57 mmol) was added and the mixture was stirred overnight at room temperature. The solvents were removed under reduced pressure and the residue was purified by flash column chromatography (10 → 30% EtOAc in hexanes) to furnish urea **29** (152 mg, 60% over 2 steps) as a light brown solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89 (t, J = 6.6 Hz, 3 H, terminal CH_3) 1.19 – 1.32 (m, 27 H, CH_3 and CH_2) 1.39 (s, 3 H, CH_3) 1.44 (s, 9 H, tBu) 1.48 – 1.71 (m, 2 H, CH_2) 3.40 – 3.59 (m, 3 H, H-3'and CH_2-6') 3.77 (s, 3 H, OCH_3) 3.78 – 3.81 (m, 1 H, H-5) 3.81 (s, 3 H, OCH_3) 3.82 (s, 3 H, OCH_3) 3.84 – 3.98 (m, 3 H, H-4, H-4' and H-5') 4.09 – 4.19 (m, 1 H, H-2') 4.24 – 4.34 (m, 1 H, H-3) 4.44 – 4.67 (m, 7 H, H-1', $\underline{CH_2}$PhOMe and NH) 4.70 (d, J = 10.9 Hz, 1 H, $\underline{CH_2}$PhOMe)5.07 (br. s, 1 H, NH) 5.78 (dd, J = 16.4 and 3.9 Hz, 1 H, $\underline{CH\text{-}1}$=CH) 5.88 (dd, J = 16.4 and 4.0 Hz, 1 H, CH=$\underline{CH\text{-}2}$) 6.78 – 6.91 (m, 6 H, arom. H) 7.15 – 7.27 (m, 6 H, arom. H) 7.41 – 7.53 (m, 3 H, naphthyl) 7.69 (d, J = 7.9 Hz, 2 H, naphthyl) 7.83 – 7.88 (m, 1 H, naphthyl) 7.95 – 8.00 (m, 1 H, naphthyl); ^{13} C NMR (75 MHz, $CDCl_3$): δ 14.11, 22.67, 25.51, 25.69, 26.76, 28.37, 28.41, 28.67, 29.34, 29.53, 29.60, 29.65, 29.69, 30.90, 31.91, 40.87, 55.23, 72.91, 72.97, 73.43, 74.13, 74.88, 76.22, 77.20, 77.85, 79.50, 108.13, 113.68, 113.71, 113.76, 113.79, 121.80, 125.80, 125.91, 126.12, 126.50, 126.76, 126.96, 128.47, 128.55, 129.10, 129.15, 129.39, 129.50, 129.79, 130.06, 130.32, 130.43, 130.47, 130.53, 130.64, 130.75, 133.56, 134.30, 134.43, 154.88, 155.43, 159.16, 159.23; Exact mass (ESI-MS) for $C_{68}H_{94}N_3O_{12}$ $[M+H]^+$ found, 1144.6827; calcd, 1144.6832.

(3S,4S,5R)-1-(6'-hydroxy-2',3',4'-tri-*O*-(4-methoxybenzyl)-α-*C*-D-galactopyranosyl)-3-tert-butyloxycarbonylamino-4,5-Di-*O*-isopropylidene-nonadecane-4,5-diol (30)

A solution of alkene **26** (121 mg, 0.12 mmol) in EtOH (5 mL) and hydrazine monohydrate (0.5 mL) was heated till reflux and stirred for 18h. Upon completion of the reaction, DCM (20 mL) was added and the organic layer was washed with 1 M NaOH solution (20 mL). The aqueous layer was back extracted with DCM (2 x 20 mL) and the combined organic phase was washed with H_2O (50 mL). The organics were dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (0 → 50% EtOAc in hexanes) to provide saturated glycoside **30** (88 mg, 73 %) as a yellowish oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, J = 6.6 Hz, 3 H, terminal CH_3) 1.31 (s, 3 H, CH_3) 1.20 – 1.33 (m, 24 H, CH_2) 1.39 (s, 3 H, CH_3) 1.42 (s, 9 H, tBu) 1.47 – 1.78 (m, 6 H, CH_2) 2.56 (br. s, 1 H, OH) 3.53 – 3.73 (m, 4 H, H-2', H-3', H-4' and Ha-6') 3.81 (s, 3 H, OCH_3) 3.81 (s, 3 H, OCH_3) 3.81 (s, 3 H, OCH_3) 3.86 – 4.00 (m, 4 H, H-2, H-3, H-4 and H-5) 4.03 – 4.14 (m, 2 H, H-1 and Hb-6') 4.41 (d, J = 11.7 Hz, 1H, $\underline{CH_2}$PhOMe) 4.45 – 4.67 (m, 6 H, $\underline{CH_2}$PhOMe and NH) 6.82 – 6.91 (m, 6 H, arom. H) 7.13 – 7.28 (m, 6 H, arom. H); ^{13} C NMR (75 MHz, $CDCl_3$): δ 14.08, 22.64, 23.88, 25.50, 26.70, 27.37, 28.32, 28.63, 28.90, 29.31, 29.51, 29.57, 29.65, 31.88, 50.21, 55.20, 60.32, 72.12, 72.50, 72.83, 73.73, 73.93, 75.02, 76.24, 77.82, 79.47, 79.81, 107.74, 113.71, 113.74, 129.22, 129.31, 129.48, 129.60, 130.29, 130.52, 155.26, 159.16, 159.25; Exact mass (ESI-MS) for $C_{57}H_{88}NO_{12}$ $[M+H]^+$ found, 978.6314; calcd, 978.6301.

(3S,4S,5R)-1-(2',3',4'-tri-*O*-(4-methoxybenzyl)-6'-*O*-(4-pyridinylcarbamoyl)-α-*C*-D-galactopyranosyl)-3-tert-butyloxy-carbonylamino-4,5-Di-*O*-isopropylidene-nonadecane-4,5-diol (31)

To a solution of alcohol **30** (324 mg, 0.33 mmol) in anhydrous DCM (12 mL) was added p-nitrophenyl chloroformate (100 mg, 0.5 mmol) and pyridine (53 µL, 0.66 mmol). The mixture was heated to 40 °C for 8h. After complete disappearance of the starting material the mixture was diluted with DCM (30 mL) and washed successively with saturated $NaHCO_3$ solution (20 mL), H_2O (20 mL) and brine (20 mL). Next the aqueous layer was extracted with DCM (2 x 50 mL) and the combined organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude p-nitrophenyl carbonate was used without further purification in the next reaction.

The crude carbonate was dissolved in anhydrous DCM (8 mL) followed by addition of 4-aminopyridine (155 mg, 1.65 mmol) and Et_3N (91 µL, 0.66 mmol). The reaction mixture was heated to reflux temperature and was stirred overnight. The mixture

was diluted with DCM (30 mL) and washed successively with saturated $NaHCO_3$ solution (20 mL), H_2O (20 mL) and brine (20 mL). Next the aqueous layer was extracted with DCM (2 x 50 mL) and the combined organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (0 → 50% EtOAc in hexanes) to provide carbamate **31** (200 mg, 55 %) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, J = 6.5 Hz, 3 H, terminal CH_3) 1.14 – 1.36 (m, 24 H, CH_3 and CH_2) 1.36 – 1.51 (m, 16 H, tBu, CH_2 and CH_3) 1.57 – 1.71 (m, 4 H, CH_2) 1.74 – 1.87 (m, 1 H, CH_2) 3.56 – 3.73 (m, 2 H, H-3' and H-5') 3.81 (s, 3 H, OCH_3) 3.82 (s, 3 H, OCH_3) 3.83 (s, 3 H, OCH_3) 3.84 – 3.97 (m, 4 H, H-3, H-4, H-5 and H-4') 4.04 – 4.15 (m, 1 H, Ha-6') 4.15 (dd, J = 6.6 and 3.3 Hz, 1H, H-2) 4.27 (q, J = 6.3 Hz, 1 H, H-1) 4.44 – 4.67 (m, 7 H, Hb-6', $\underline{CH_2}$PhOMe and NH) 4.72 (d, J = 11.4 Hz, 1H, $\underline{CH_2}$PhOMe) 6.82 – 6.92 (m, 6 H, arom. H) 7.16 – 7.30 (m, 6 H, arom. H) 7.55 (d, J = 6.2 Hz, 2 H, pyridine) 8.44 (d, J = 6.4 Hz, 2 H, pyridine) 8.66 (s, 1 H, NHC(O)O); ^{13} C NMR (75 MHz, $CDCl_3$): δ 14.12, 22.69, 25.54, 26.35, 27.18, 27.92, 28.43, 28.73, 29.36, 29.43, 29.51, 29.66, 29.69, 31.92, 51.17, 55.29, 70.34, 72.88, 73.11, 73.84, 77.95, 79.56, 79.87, 108.14, 112.46, 113.74, 113.80, 129.24, 129.48, 129.74, 130.11, 130.41, 130.53, 147.91, 152.67, 155.66, 159.25, 159.37; Exact mass (ESI-MS) for $C_{63}H_{92}N_3O_{13}$ $[M+H]^+$ found, 1098.6619; calcd, 1098.6625.

(3S,4S,5R)-1-(2',3',4'-tri-*O*-(4-methoxybenzyl)-6'-*O*-(4-pyridinylcarbamoyl)-α-*C*-D-galactopyranosyl)-3-tert-butyloxy-carbonylamino-4,5-Di-*O*-isopropylidene-1-nonadecene-4,5-diol (32)

To a solution of alcohol **26** (187 mg, 0.19 mmol) in anhydrous DCM (8 mL) was added p-nitrophenyl chloroformate (57 mg, 0.29 mmol) and pyridine (31 µL, 0.38 mmol). The mixture was heated to 40 °C for 8h. After complete disappearance of the starting material the mixture was diluted with DCM (30 mL) and washed successively with saturated $NaHCO_3$ solution (20 mL), H_2O (20 mL) and brine (20 mL). Next the aqueous layer was extracted with DCM (2 x 50 mL) and the combined organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude p-nitrophenyl carbonate was used without further purification in the next reaction.

The crude carbonate was dissolved in anhydrous DCM (5 mL) followed by addition of 4-aminopyridine (89 mg, 0.95 mmol) and Et_3N (53 µL, 0.38 mmol). The reaction mixture was heated to reflux temperature and was stirred overnight. The mixture was diluted with DCM (30 mL) and washed successively with saturated $NaHCO_3$ solution (20 mL), H_2O (20 mL) and brine (20 mL). Next the aqueous layer was extracted with DCM (2 x 50 mL) and the combined organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (0 → 50% EtOAc in hexanes) to provide carbamate **32** (129 mg, 62 %) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, J = 6.6 Hz, 3 H, terminal CH_3) 1.13 – 1.34 (m, 24 H, CH_3 and CH_2) 1.34 – 1.50 (m, 14 H, tBu, CH_2 and CH_3) 1.59 – 1.75 (m, 2 H, CH_2) 1.74 – 1.87 (m, 1 H, CH_2) 3.54 (dd, J = 9.0 and 2.2 Hz, 1 H, H-3") 3.71 – 3.79 (m, 1 H, H-4") 3.81 (s, 3 H, OCH_3) 3.81 (s, 3 H, OCH_3) 3.83 (s, 3 H, OCH_3) 3.90 (d, J = 11.2 Hz, 1 H, Ha-6") 3.99 (d, J = 9.0 Hz, 1 H, H-5") 4.04 – 4.21 (m, 2 H, H-5, H-2") 4.31 – 4.42 (m, 2 H, H-3, H-4) 4.48 – 4.73 (m, 7 H, H-1", Hb-6, $\underline{CH_2}$PhOMe and NH) 4.77 – 4.88 (m, 2 H, $\underline{CH_2}$PhOMe) 5.89 (dt, J = 16.5 and 2.0 Hz, 1 H, $\underline{CH\text{-}2}$=CH) 6.03 (dt, J = 16.5 and 2.2 Hz, 1 H, CH=$\underline{CH\text{-}1}$) 6.82 – 6.94 (m, 6 H, arom. H) 7.20 – 7.31 (m, 6 H, arom. H) 7.53 (d, J = 5.6 Hz, 2 H, pyridine) 8.44 (d, J = 6.2 Hz, 2 H, pyridine) 8.55 (s, 1 H, NHC(O)O); ^{13} C NMR (75 MHz, $CDCl_3$): δ 14.11, 22.67, 25.60, 26.32, 27.19, 28.37, 28.88, 29.34, 29.48, 29.56, 29.63, 31.91, 51.60, 55.26, 66.19, 70.45, 72.74, 73.03, 73.18, 74.08, 76.28, 77.20, 77.98, 79.76, 80.43, 108.61, 112.43, 113.74, 113.80, 115.74, 125.36, 126.15, 129.10, 129.44, 129.88, 130.12, 130.47, 130.60, 147.98, 152.61, 155.46, 159.20, 159.35, 163.58; Exact mass (ESI-MS) for $C_{63}H_{90}N_3O_{13}$ $[M+H]^+$ found, 1096.6450; calcd, 1096.6468.

(3S,4S,5R)-1-(6"-naphtureido-6"-deoxy-α-*C*-D-galacto-pyranosyl)-3-hexacosylamino-1-nonadecene-4,5-diol (5a)

A solution of **29** (125 mg, 0.11 mmol) in 4 M HCl in dioxane (5 mL) was stirred 3h at room temperature. The reaction was monitored with mass spectrometry and upon completion the solvents were evaporated under reduced pressure. The crude amine thus obtained was used without further purification in the next step. The amine was suspended in THF (2 mL) and a catalytic amount of 4-DMAP was added. Next p-nitrophenyl hexacosanoate (85 mg, 0.17 mmol) was added followed by addition of Et_3N (1 mL). The resulting mixture was stirred for 3 days at room temperature. Evaporation of the solvents and purification of the resulting residue by means of column chromatography (2 → 12% MeOH in DCM) yielded **5a** (32 mg, 29%) as a white solid. ^{1}H NMR (300 MHz, pyridine-d_5): δ 0.89 (t, J = 6.6 Hz, 6 H, terminal CH_3) 1.00 – 1.49 (m, 64 H, CH_2) 1.49 – 1.96 (m, 5 H, CH_2) 2.20 – 2.38 (m, 2 H, CH_2) 2.47 (t, J = 7.4 Hz, 2H, CH_2) 3.22 (app t, J = 5.6 Hz, 1 H, CH_2) 4.16 (t, J = 5.5 Hz, 2 H, CH_2-6") 4.19 – 4.34 (m, 3 H, H-4, H-5 and H-3") 4.34 – 4.38 (m, 1 H, H-5") 4.59 (t, J = 6.2 Hz, 1 H, H-4") 4.79 (dd, J = 9.7 and 6.2 Hz, 1 H, H-2") 5.03 – 5.09 (m, 1 H, H-1") 5.85 – 5.93 (m, 1 H, H-3) 6.08 (br. s, 5 H, OH) 6.84 (dd, J = 16.2 and 3.8 Hz, 1 H, $\underline{CH\text{-}1}$=CH) 6.94 (dd, J = 16.2 and 6.0 Hz, 1 H, CH=$\underline{CH\text{-}2}$) 7.43 – 7.54 (m, 2 H, naphthyl) 7.61 – 7.72 (m, 2 H, naphthyl) 7.84 – 7.91 (m, 1 H, naphthyl) 8.52 (dd, J = 7.6 and 1.1 Hz, 1 H, naphthyl) 8.57 – 8.62 (m, 1 H, naphthyl) 8.68 (d, J = 9.0 Hz, 1 H, NH) 9.69 (s, 1 H, NHC(O)NH); ^{13} C NMR (75 MHz, pyridine-d_5): δ 14.69, 23.35, 26.72, 26.87, 30.02, 30.20, 30.23, 30.29, 30.32, 30.41, 30.46, 30.55, 30.80, 32.54, 35.05, 37.37, 42.35, 44.63, 46.24, 54.46, 70.27, 71.54, 72.71, 73.00, 76.93, 78.91, 119.65, 123.26, 124.45, 126.25, 126.43, 126.87, 127.73, 128.19, 129.13, 132.28, 135.24, 136.70, 158.30, 173.08; Exact mass (ESI-MS) for $C_{62}H_{108}N_3O_8$ $[M+H]^+$ found, 1022.8133; calcd, 1022.8131; m.p. 173 – 175 °C.

(3S,4S,5R)-1-(6"-naphtureido-6"-deoxy-α-C-D-galacto-pyranosyl)-3-hexacosylamino-nonadecane-4,5-diol (5b)

A solution of **28** (150 mg, 0.13 mmol) in 4 M HCl in dioxane (6 mL) was stirred 2.5h at room temperature. The reaction was monitored with mass spectrometry and upon completion the solvents were evaporated under reduced pressure. The crude

amine was used without further purification in the next step and was dissolved in THF (5 mL) and 10% KOH solution (2.5 mL) and cooled to 0 °C. In a separate flask cerotic acid (199 mg, 0.5 mmol) was refluxed for 2h in oxalylchloride (5 mL) and the crude acid chloride was obtained after evaporation of the solvent by a stream of nitrogen and subsequent drying on high-vacuum. The crude acid chloride was dissolved in THF (5 mL) to obtain a 0.1 M solution of acid chloride in THF and dropwise added (2 mL, 0.2 mmol) to the biphasic mixture. The reaction mixture was stirred for 3h at 0 °C and TLC showed complete conversion of the starting material. Next the aqueous layer was extracted with THF (3 x 15 mL) and the combined organic layer was dried over Na_2SO_4, filtered and evaporated.

The resulting residue was purified by column chromatography (2 → 12% MeOH in DCM) followed by trituration with EtOAc and yielded **5b** (38 mg, 29%) as a white solid. ^{1}H NMR (500 MHz, pyridine-d_5): δ 0.92 (t, J = 6.1 Hz, 6 H, 2 x terminal CH_3) 1.18 - 1.51 (m, 67 H, CH_2) 1.62 - 1.98 (m, 4 H, CH_2) 2.21 – 2.32 (m, 3 H, CH_2) 2.42 - 2.63 (m, 4 H, CH_2) 4.20 - 4.39 (m, 6 H, H-4, H-5, H-3”, H-5” and CH_2-6”) 4.48 (br. s, 1 H, H-4”) 4.60 – 4.66 (m, 2 H, H-1” and H-2”) 5.09 – 5.16 (m, 1 H, H-3) 7.48 - 7.57 (m, 4 H, naphthyl) 7.65 (d, J = 7.9 Hz, 1 H, naphthyl) 7.92 (d, J = 7.3 Hz, 1 H, naphthyl) 8.43 (d, J = 8.6 Hz, 1 H, NH) 8.59 (t, J = 7.3 Hz, 2 H, naphthyl and NH) 9.38 (s, 1 H, NH); ^{13} C NMR (125 MHz, pyridine-d_5): δ 14.68, 23.38, 23.58, 26.15, 27.02, 27.10, 27.76, 30.05, 30.06, 30.18, 30.23, 30.29, 30.36, 30.38, 30.44, 30.46, 30.49, 30.56, 30.83, 32.59, 32.60, 35.11, 37.68, 41.67, 52.90, 70.43, 71.31, 72.79, 73.75, 73.86, 75.86, 78.23, 119.32, 123.13, 123.64, 124.22, 126.17, 126.41, 127.00, 128.14, 129.30, 135.40, 136.20, 137.04, 150.72, 157.90, 174.67; Exact mass (ESI-MS) for $C_{62}H_{110}N_3O_8$ $[M+H]^+$ found, 1024.8292; calcd, 1024.8287; m.p. 187 – 189 °C.

(3S,4S,5R)-1-(6”-O-(4-pyridinylcarbamoyl)-α-*C*-D-galacto-pyranosyl)-3-hexacosylamino-nonadecane-4,5-diol (6a)

A solution of **31** (180 mg, 0.16 mmol) in 4 M HCl in dioxane (6 mL) was stirred 3h at room temperature. The reaction was monitored with mass spectrometry and upon completion the solvents were evaporated under reduced pressure. The crude amine thus obtained was used without further purification in the next step. The amine was suspended in THF (3 mL) and a catalytic amount of 4-DMAP was added. Next p-nitrophenyl hexacosanoate (125 mg, 0.24 mmol) was added followed by addition of Et_3N (0.5 mL). The resulting mixture was stirred for 2 days at room temperature. Evaporation of the solvents and purification of the resulting residue by means of column chromatography (4 → 18% MeOH in DCM) yielded **6a** (27 mg, 17%) as a white solid. ^{1}H NMR (300 MHz, pyridine-d_5): δ 0.89 (t, J = 5.6 Hz, 6 H, terminal CH_3) 1.01 – 1.60 (m, 60 H, CH_2) 1.60 – 2.05 (m, 8 H, CH_2) 2.05 – 2.40 (m, 6 H, CH_2) 2.40 – 2.84 (m, 4 H, CH_2) 4.20 – 4.43 (m, 4 H, H-4, H-5, H-2” and H-5”) 4.43 – 4.62 (m, 2 H, H-3”, H-4”) 4.63 – 4.76 (m, 1 H, H-1”) 4.85 (d, J = 10.2 Hz, 1H, Ha-6”) 5.00 – 5.17 (m, 1 H, H-3) 5.24 (dd, J = 10.2 and 8.9 Hz, 1 H, Hb-6”) 7.92 (d, J = 5.3 Hz, 2 H, pyridine) 8.53 (d, J = 8.5 Hz, 1 H, NH) 8.68 (d, J = 5.6 Hz, 2 H, pyridine) 10.89 (s, 1 H, NHC(O)O); ^{13} C NMR (75 MHz, pyridine-d_5): δ 14.69, 23.35, 26.77, 26.93, 27.06, 27.91, 30.02, 30.20, 30.23, 30.29, 30.32, 30.43, 30.58, 30.78, 32.54, 34.49, 37.41, 52.67, 66.26, 70.36, 70.82, 72.39, 72.61, 73.45, 75.93, 77.87, 113.61, 115.68, 131.30, 144.52, 147.73, 151.36, 154.84, 173.87; Exact mass (ESI-MS) for $C_{57}H_{106}N_3O_9$ $[M+H]^+$ found, 976.7925; calcd, 976.7924; m.p. 177-179 °C.

(3S,4S,5R)-1-(6”-*O*-(4-pyridinylcarbamoyl)-α-*C*-D-galacto-pyranosyl)-3-hexacosylamino-1-nonadecene-4,5-diol (6b)

A solution of **32** (53 mg, 0.05 mmol) in 4 M HCl in dioxane (1 mL) was stirred 4h at room temperature. The reaction was monitored with mass spectrometry and upon completion the solvents were evaporated under reduced pressure. The crude amine thus obtained was used without further purification in the next step. The amine was suspended in THF (1 mL) and a catalytic amount of DMAP was added. Next p-nitrophenyl hexacosanoate (29 mg, 0.055 mmol) was added followed by addition of Et_3N (0.1 mL). The resulting mixture was stirred for 2 days at room temperature. Evaporation of the solvents and purification of the resulting residue by means of column chromatography (4 → 14% MeOH in DCM) yielded **6b** (20 mg, 44%) as a white solid. ^{1}H NMR (300 MHz, pyridine-d_5): δ 0.89 (t, J = 6.6 Hz, 6 H, terminal CH_3) 1.09 – 1.58 (m, 63 H, CH_2) 1.58 – 2.38 (m, 11 H, CH_2) 4.20 – 4.32 (m, 3 H, H-4, H-5 and H-4”) 4.41 (br. s, 1 H, H-3”) 4.61 – 4.69 (m, 1 H, H-5”) 4.76 (dd, J = 8.1 and 5.8 Hz, 1H, H-2”) 4.84 – 5.05 (m, 1 H, Ha-6”) 5.06 – 5.23 (m, 2 H, H-1” and Hb-6”) 5.82 – 5.93 (m, 1 H, H-3) 6.33 (br.s, 1 H, OH) 6.63 (br. s, 1 H, OH) 6.83 (dd, J = 16.5 and 3.5 Hz, 1 H, CH-1=CH) 6.98 (dd, J = 16.5 and 5.9 Hz, 1 H, CH=CH-2) 7.84 (d, J = 5.6 Hz, 2 H, pyridine) 8.60 - 8.72 (m, 3 H, pyridine and NH) 10.98 (s, 1 H, NHC(O)O); ^{13} C NMR (75 MHz, pyridine-d_5): δ 14.68, 23.33, 26.80, 26.84, 30.00, 30.17, 30.20, 30.26, 30.32, 30.40, 30.52, 30.72, 32.52, 35.02, 37.31, 54.17, 66.06, 70.50, 70.74, 72.79, 72.97, 73.16, 75.75, 78.54, 113.56, 127.83, 131.79, 147.62, 151.36, 154.67, 173.13; Exact mass (ESI-MS) for $C_{57}H_{104}N_3O_9$ $[M+H]^+$ found, 974.7764; calcd, 974.7767; m.p. 173-175 °C.

Determination of Serum Cytokine Concentrations.

The serum concentrations of IFN-γ and IL-4 were assessed 2, 6, 12, 24, 48, and 72 h after i.v. administration of 1 μg of α-GalCer, α-*C*-GalCer, or the indicated *C*-glycoside analogues in Balb/c-mice. Cytokine levels were measured by way of a sandwich ELISA (eBioscience).

Quantification of the level of cytokines produced by human iNKT cell lines by ELISA

$1x10^4$ human iNKT cells were cocultured with $4x10^4$ Hela cells transfected with a CD1d gene, in the presence of an indicated concentration of each glycolipid. After 24-h incubation at 37 °C, the culture supernatants were collected and the concentrations of human IFN-γ and IL-4 in the supernatants were determined by ELISA (eBioscience).

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