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Galactosylsphingamides: new α-GalCer analogues to probe the F′-pocket of CD1d

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Invariant Natural Killer T-cells (*i*NKT-cells) are an attractive target for immune response modulation, as upon CD1d-mediated stimulation with KRN7000, a synthetic α-galactosylceramide, they produce a vast amount of cytokines. Here we present a synthesis that allows swift modification of the phytosphingosine side chain by amidation of an advanced methyl ester precursor. The resulting KRN7000 derivatives, termed α-galactosylsphingamides, were evaluated for their capacity to stimulate *i*NKT-cells. While introduction of the amide-motif in the phytosphingosine chain is tolerated for CD1d binding and TCR recognition, the studied α-galactosylsphingamides showed compromised antigenic properties.

Invariant natural killer T-cells (*i*NKT-cells) are a small set of glycolipid reactive T-cells that bridge innate and adaptive immunity. They display characteristics of both the NK cell lineage (mouse NK1.1, human CD161) and conventional thymus-derived T-cells[1]. Their semi-invariant T-cell receptor (TCR) is composed of an invariant Vα14-Jα18 chain in mice (homologous Vα24-Jα18 chain in humans), preferentially paired with Vβ8.2, Vβ7 or Vβ2 (Vβ11 in humans). Whereas conventional T-cells are activated by peptide antigens presented by MHC proteins, *i*NKT-cells typically recognize glycolipid antigens presented by MHC class 1 like CD1d molecules on antigen presenting cells[2].

The prototypical antigen for *i*NKT-cell stimulation is KRN7000, also known as α-GalCer (**1**), a synthetic glycolipid derived from agelasphins found in marine sponge extracts (Fig. 1)[3,4]. Structural data illustrated that the lipid chains of α-GalCer are bound in two hydrophobic pockets deeply buried in CD1d[5,6]. The larger A′-pocket binds the fatty acid part while the linear F′-pocket accommodates the phytosphingosine chain. Consequently, the galactose sugar is positioned at the surface of the CD1d binding groove and is available for recognition by the TCR of *i*NKT-cells[7].

Upon stimulation, *i*NKT-cells rapidly secrete large amounts of pro-inflammatory Th1- (e.g., IFN-γ) and anti-inflammatory Th2-cytokines (e.g., IL-4). These cytokines mediate immune responses against tumors[8], microbial infections[1,9] and auto-immunity[10]. In addition, *i*NKT-cells transactivate other immune cells including dendritic cells, B- and T-cells, macrophages and NK-cells and are thus able to orchestrate complex immune functions[1]. Despite the potent immune response triggered by α-GalCer, this antigen showed limited therapeutic efficacy in several clinical studies[11]. The lack of therapeutic effect is attributed to the antagonizing activities of the secreted pro-inflammatory Th1- and anti-inflammatory Th2-cytokines. Accordingly, the search for improved α-GalCer analogues capable of inducing a biased cytokine release is ongoing.

To date, the growing library of α-GalCer analogues mainly consists of glycolipids with an altered fatty acid chain[12,13] and/or carbohydrate part[14–17]. In addition, a vast amount of non-glycosidic analogues, including *C*-glycosides[18–20] and cyclitol derivatives[21] exist. However, analogues with modifications in the phytosphingosine chain remain relatively scarce, due to the more challenging syntheses required to access these analogs.

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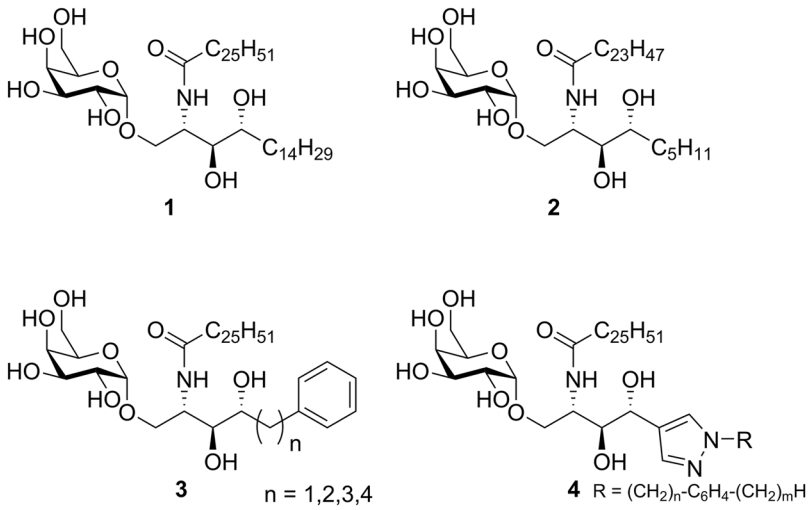


Figure 1. Structures of α-GalCer (**1**) and OCH (**2**) and known phytosphingosine-modified α-GalCer derivatives **3** and **4**.

A common strategy for the synthesis of α-GalCer analogues modified in the phytosphingosine moiety relies on the Wittig olefination on L-Garner's aldehyde, followed by stereoselective dihydroxylation of the Z-double bond furnishing the required D-ribo stereochemistry[14,22]. Although this approach may afford high stereoselectivity, it inevitably leads to minor unwanted isomers thereby lowering the overall yield and forcing demanding purification steps ahead in the synthesis.

Hitherto, a small number of phytosphingosine modified analogues have been reported, some of which exhibit interesting biological properties. Most notably, OCH (**2**), a glycolipid with a truncated phytosphingosine chain and a slightly trimmed fatty acid residue, became a prototypical Th2-polarizing *i*NKT-cell ligand[23,24]. Wong and coworkers reported derivatives with a terminal phenyl group in the sphingosine chain (**3**)[25]. Interestingly, the immunological properties of these analogues were strongly dependent on the relative position of the aromatic ring[12,22,26]. More recently, Park *et al.* demonstrated that selected derivatives bearing a pyrazole moiety in the phytosphingosine chain (**4**) give rise to a Th2-biased cytokine profile[27,28]. These examples demonstrate that alterations of the sphingosine chain are tolerated and may afford analogues with interesting cytokine profiles.

Up to now, the mechanism of cytokine polarization is poorly understood, although there is ample of evidence suggesting that the stability of the CD1d/glycolipid/TCR-complex is an important contributing factor[29]. The potent Th1-response observed for α-GalCer derivatives featuring aromatic moieties in the acyl chain, is attributed to the formation of additional Van der Waals interactions with amino acid residues lining the A'-pocket[30]. Consequently these glycolipids exhibit an enhanced affinity for CD1d resulting in the observed cytokine bias. In analogy, the potent Th1-polarizing sugar-modified galactosylceramides previously synthesized in our lab, also feature a greater CD1d-affinity due to the formation of an extra binding pocket in CD1d via induced-fit[31].

A study by McCarthy *et al.* indicates that although both the acyl chain and phytosphingosine contribute to the stability of the glycolipid/CD1d-complex, it is the phytosphingosine chain that controls TCR-binding and *i*NKT-cell activation, since ligand-induced conformational changes of the F'-pocket allosterically influence the CD1d/glycolipid footprint[32]. The aforementioned arguments excited us to explore a synthetic strategy for the preparation of phytosphingosine modified α-GalCer analogues, allowing us to introduce variations in the later stages of the synthesis via amide coupling. Molecular modeling studies indicate that the amide moiety, when placed at the right position, can potentially form a hydrogen bond with Tyr73 in the CD1d-binding groove and may consequently enhance the glycolipid/CD1d-complex stability (Fig. 2). The selected amide substituents are aimed at reinforcing the interactions (e.g., by π-π-stacking) with aromatic residues lining the F'-pocket.

To ensure acceptable solubility of the envisioned analogues, we opted to equip these initially with an octanoyl moiety, which was shown to retain most of the antigenic properties when replacing the *N*-hexacosanoyl group of α-GalCer[33].

Results and Discussion

Chemistry. An overview of the synthesized α-galactosylsphingamides is shown in Table 1.

As shown in the retrosynthetic analysis (Fig. 3), the α-galactosylsphingamides (**5a-h**, **6**–**9d** and **9f**) can be accessed from methyl ester **10**, which is obtained through Lewis acid catalyzed glycosylation of glycosyl acceptor **12**. The latter would be obtained by conversion of alcohol **13** to an azide with an inversion of stereochemistry at the carbon centre, followed by selective benzyl ether cleavage to afford the primary alcohol. The 2-deoxygalactose intermediate **14**, prepared from commercially available tri-*O*-acetyl-D-galactal (**16**), would serve as a substrate for a Wittig olefination with phosphonium ylid **15**.

The synthetic route towards glycosyl acceptor **12** is outlined in Fig. 4. It was decided to start from commercially available tri-*O*-acetyl-D-galactal (**16**), which was readily converted into the 3,4,6-tri-*O*-benzyl-protected derivative **17**[34]. Hydrolysis of the enol ether was achieved upon treatment with 4 m sulfuric acid, furnishing 2-deoxygalactose intermediate **14**[35]. Next, the Wittig reaction on **14** with methyl(triphenylphosphoranylidene)

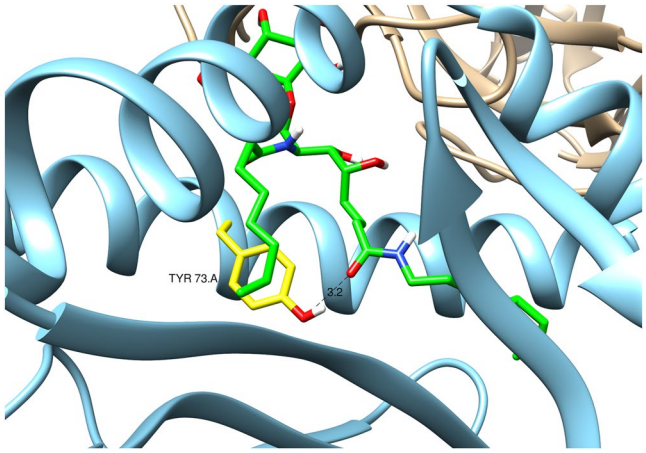


Figure 2. Model of the binding of **5a** to mouse CD1d. The **5a** carbons are depicted in green, while the Tyr73.A carbons are colored in yellow. The possible hydrogen bond with the amide oxygen atom is highlighted. Ribbons are colored as skyblue for the CD1d (chain A) and the mouse NKT TCR chains are colored in grey.

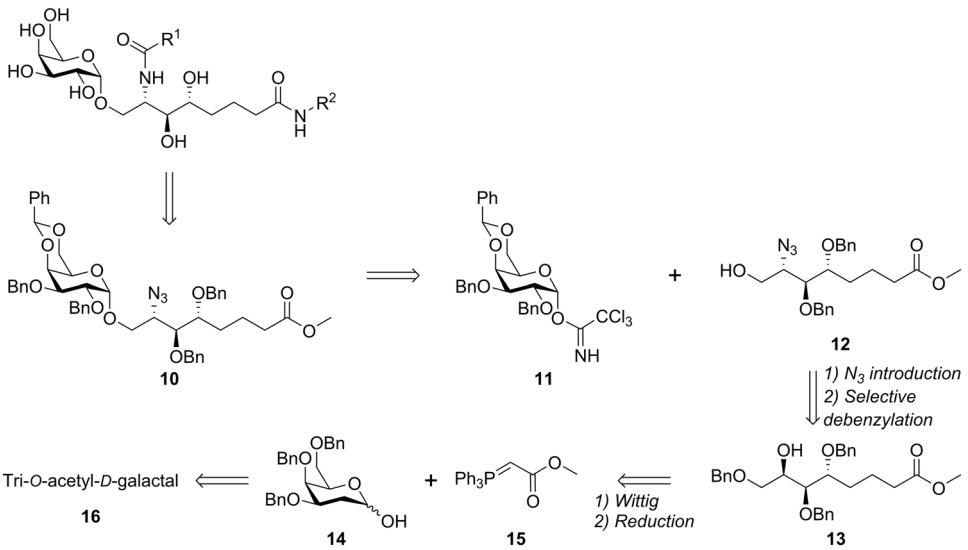


Figure 3. Retrosynthesis of the target α-galactosylsphingamides.

R^1 / R^2	$R^1 = C_7H_{15}$	$R^1 = C_{11}H_{23}$	$R^1 = C_{15}H_{31}$	$R^1 = C_{19}H_{39}$	$R^1 = C_{25}H_{51}$
C_9H_{19}	5a				
$(CH_2)_2Ph$	5b				
$(CH_2)_4Ph$	5c				
$(CH_2)_6Ph$	5d	6d	7d	8d	9d
$(CH_2)_8Ph$	5e				
Ph-*m*-C_5H_{11}	5f				9f
Ph-*p*-C_5H_{11}	5g				
$((CH_2)_2O)_2C_2H_5$	5h				

Table 1. Overview of the synthesized α-galactosylsphingamides.

acetate was investigated. When the reaction was carried out in THF, α,β-unsaturated ester **18** was obtained exclusively as the *E*-isomer in moderate yield (64%). Performing the reaction in refluxing toluene increased the yield to 79%, while also providing solely the *E*-isomer. To avoid Michael type side reactions and to eliminate the possibility for a 1,3-dipolar cycloaddition upon introduction of the azide, saturation of the double bond was

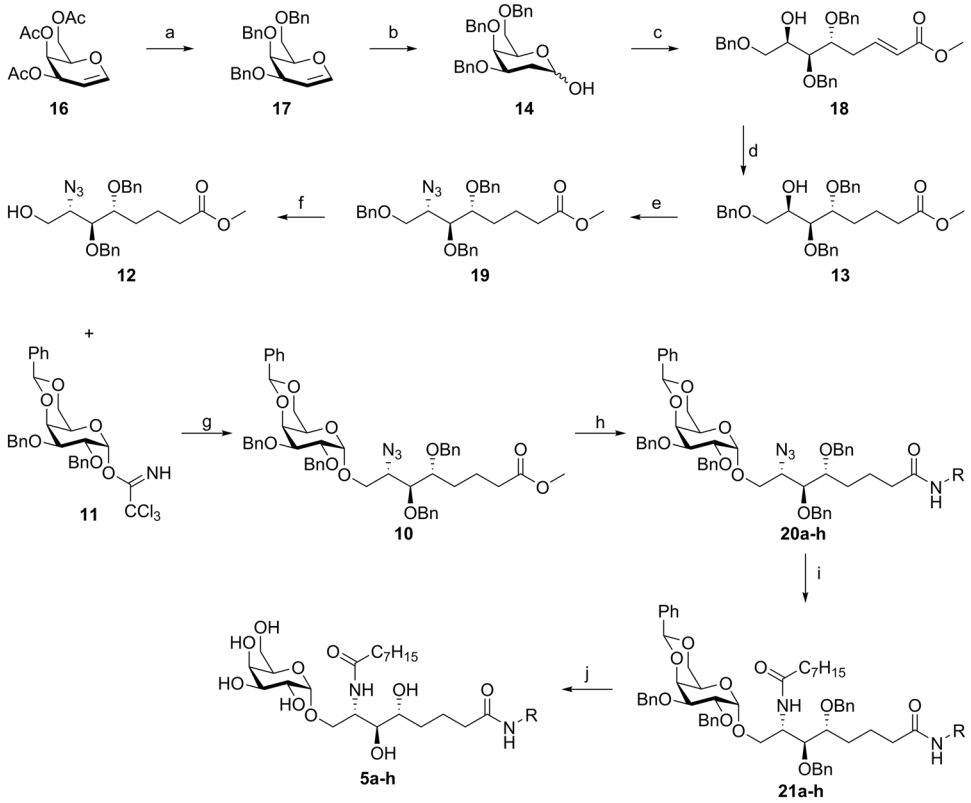


Figure 4. Reagents and conditions: (**a**) (i) Et_3N, H_2O, MeOH, RT, 4d; (ii) NaH, BnBr, DMF, RT, overnight, 94% (over 2 steps); (**b**) 4 M H_2SO_4, DMF, 0 °C → RT, overnight, 85%; (**c**) methyl (triphenylphosphoranylidene)acetate, toluene, 85 °C, 6 h, 79%; (**d**) $NiCl_2{\cdot}6\,H_2O$, $NaBH_4$, MeOH/THF, 0 °C, 1 h, 94%; (**e**) PPh_3, diethylazodicarboxylate, diphenylphosphorylazide, THF, −20 °C → RT, 7 h, 95%; (**f**) (i) $ZnCl_2$, AcOH/Ac_2O, 3.5 h, RT; (ii) MeOH, NaOMe, pH 10, RT, overnight, 67% over 2 steps; (**g**) TMSOTf, THF, −30 °C, 2 h, 67%; (**h**) $AlMe_3$, amine, CH_2Cl_2, reflux, overnight, 56%-85%; (**i**) (i) PMe_3, H_2O, THF, RT, 7 h; (ii) EDC, octanoic acid, CH_2Cl_2, RT, overnight, 51%–69% over 2 steps; (**j**) H_2, Pd black, MeOH, $CHCl_3$, RT, overnight, 37%–58%.

accomplished by conjugate reduction with nickel(II) chloride and sodium borohydride. Mitsunobu reaction with diphenyl phosphoryl azide (DPPA) converts the C2-OH to an azido group with the appropriate stereochemistry (**19**). Next, selective deprotection of the primary hydroxyl was achieved by treatment of **19** with zinc chloride in acetic anhydride followed by Zemplén deacetylation of the intermediate acetate to furnish acceptor **12**.

Next, TMSOTf-promoted glycosylation of acceptor **12** with galactosyl trichloroacetimidate **11**[36] afforded α-galactoside **10** in good yield and without notable formation of the β-glycoside. The ability to diversify the methyl ester after glycosylation is convenient as it reduces the number of linear steps towards the target α-GalCer analogues. A Lewis acid-catalyzed amidation with the appropriate amines gave intermediates **20a-h**. Next, the azido group was subjected to Staudinger reduction with trimethylphosphine and the resulting amine was coupled with octanoic acid using EDC. Finally, catalytic hydrogenolysis afforded the desired compounds **5a-h**. Analogues **6-9d** and **9f** with alternative acyl moieties were synthesized according to similar procedures.

Biological evaluation. To assess the biological activity of the α-galactosylsphingamides, we analyzed the IFN-γ- and IL-4-levels after intraperitoneal injection of 5 μg of the galactosylsphingamides **5a-h** in mice (Fig. 5).

The marginal cytokine release induced by this series indicates that the introduction of an amide moiety in the phytosphingosine chain compromises the antigenic potency.

In an effort to explain the poor antigenicity of **5a-h**, we next tested the binding of the Vα14Vβ8.2 TCR of the murine iNKT-cell hybridoma 2C12 to mCD1d-presenting ligands **5d,e** using surface plasmon resonance (SPR) and compared it with the binding of PBS-25[33], an α-GalCer analogue that also features an octanoic acid residue. It should be noted that in our hands, PBS-25 was inactive in the *in vivo* cytokine secretion assay (Supporting Figure S1). To further evaluate whether the low antigenicity of **5d** is resulting from the amide functionality in the phytosphingosine chain, we synthesized glycolipid **23** (Fig. 6), which is the amide-deleted counterpart of α-galactosphingamides **5d** and **5e**. Surprisingly, all ligands were bound with similarly high TCR affinities ($K_D = 38–50$ nM) (Fig. 7). Therefore, the TCR binding kinetics cannot explain the inability of the galactosylsphingamides to induce robust cytokine production *in vivo*. In addition, the maximal response (RU 120–220) observed for the individual sensorgrams of the α-galactosylsphingamides are comparable between the individual CD1d-ligand complexes, which were immobilized at similar levels, suggesting a similar CD1d ligand binding efficiency *in vitro*.

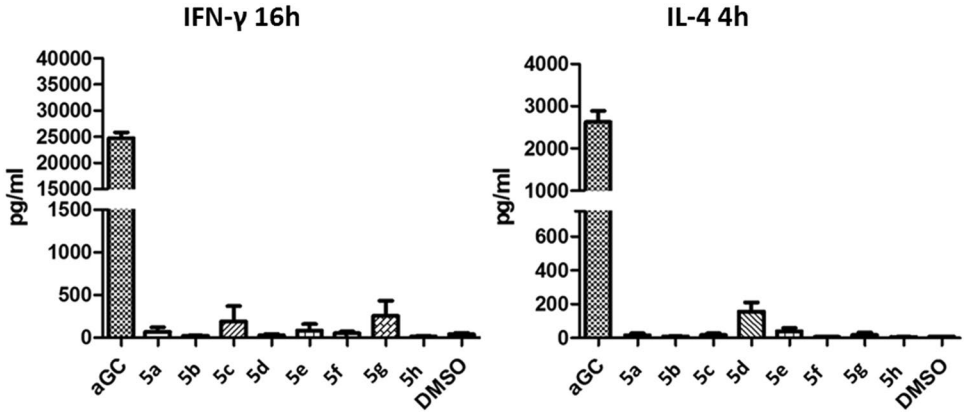


Figure 5. IL-4 and IFN-γ secretion, measured at respectively 4 h and 16 h, after intraperitoneal injection of 5 μg of the glycolipids in mice. Data for one individual experiment using 8 mice for each glycolipid.

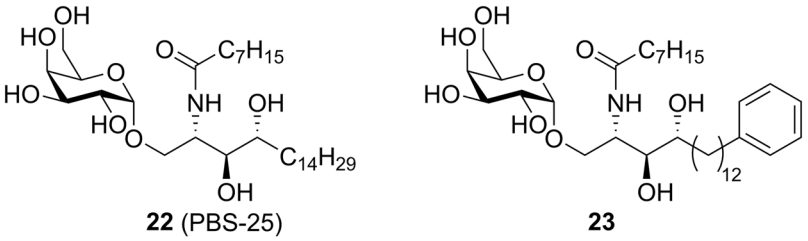
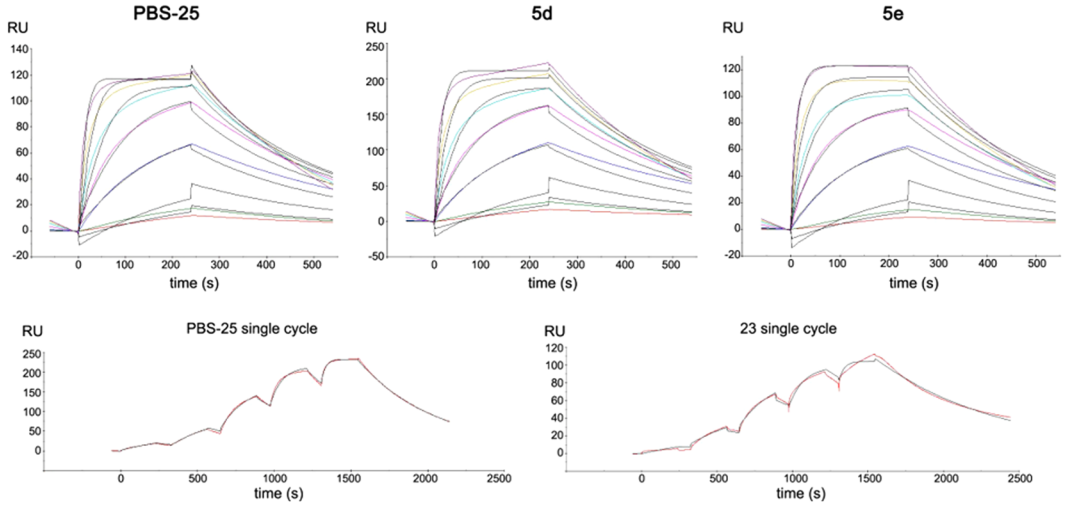


Figure 6. Structures of reference compounds PBS-25 (**22**) and **23**.

Since the current galactosylsphingamides are based on a C8 fatty acid, the short acyl chain will not fully occupy the A' pocket of CD1d. Likely similar to PBS-25[37], free fatty acid will be recruited to fill the remainder of the pocket. To assess whether these spacer lipids can potentially influence the ligand potency, we synthesized ligands based on **5d** that contained longer acyl chains of 12, 16, 20, and 26 carbon atoms (**6d**-**9d**). Since the acyl chain is introduced at the final stages of the synthesis, the elongated fatty acid chains could easily be introduced, demonstrating the versatility of our synthetic approach. We next analyzed antigenicity by measuring IFN-γ- and IL-4-levels after intraperitoneal injection of 5 μg of the acyl-elongated galactosylsphingamides **6**-**9d** in mice (Fig. 8). Surprisingly, cytokine levels are still inferior to those observed for α-GalCer but increase slightly upon elongating the acyl chain. Overall, the introduction of an amide functionality in the phytosphingosine chain appears to result in poor *i*NKT-cell stimulation. The recruitment of spacer lipids occupying the A' pocket was observed for **5a**-**d**, but the spacer lipid had no impact on the antigenic potency of the galactosylsphingamides since the antigenic potency of **8d** and **9d**, which have no space to fit a spacer lipid, also perform poorly.

Structural data. To gain insights into the poor antigenic potency of the glycolipids but high affinity TCR binding, we determined the crystal structure of the CD1d/**5d**-complex as well as that of the complex of CD1d with compound **23**, which resembles **5d** but lacks the amide group. As expected, **23** gives rise to well-defined electron density and is rigidly presented using the conserved H-bond network involving CD1d-residues Asp80 and the 3'- and 4'-OH of phytosphingosine, as well as CD1d Thr156 and the glycosidic oxygen, and most importantly, CD1d-residue Asp153 and the 2"-, and 3"-OH of the galactose (Fig. 9A–D). Surprisingly, galactosylsphingamide **5d** is presented less well ordered by CD1d, as judged by the less contoured electron density (Fig. 9A,B). This slight disorder of the galactose presentation correlates well with the increase in the H-bond distance between CD1d and **5d**. Especially the 2"-OH interaction with CD1d Asp153 is increased compared to **23** (3.0 vs. 2.6 Å, Fig. 9C,D), while the 3'-OH of phytosphingosine is also less intimately contacted by CD1d Asp80 (3.1 vs. 2.5 Å). Notewhorthy, the computationally modelled hydrogen bond between the amide oxygen and Tyr73 was not observed in the glycolipid/CD1d crystal structure. When looking down into the binding groove of CD1d (TCR view), we noticed lack of the F'-roof closure by **5d**, while **23** induced the F'-roof formation (Fig. 9E,F). This is likely the result of the amide group perturbing the hydrophobic nature of the F'-pocket in general. Based on our previous work on microbial glycolipids, we postulated that the TCR-dissociation rate is increased for glycolipids that do not close the F'-roof before TCR-binding[38]. However, since we have not observed any differences in the binding kinetics, especially the dissociation rate, we speculate that a compensatory mechanism will stabilize the TCR-interaction and this could likely be driven by the amide group and a novel interaction within the F'-pocket of CD1d. This will have to be investigated in the future using TCR-co-crystal structures.



Ligand	K_D (k_d/k_a) (nM)	k_a (1/Ms)	k_d (1/s)	Chi^2
PBS-25	43.8	9.98×10^4	43.3×10^{-4}	32.3
5d	49.7	7.85×10^4	39.0×10^{-4}	90
5e	49.5	7.36×10^4	36.4×10^{-4}	31
PBS-25 (sc)	49.2	3.92×10^4	19.3×10^{-4}	6.8
23 (sc)	38.1	3.09×10^4	11.8×10^{-4}	8.06

Figure 7. Real-time TCR-binding kinetics to mCD1d-presented ligands. Each curve represents the TCR binding sensorgram to CD1d-glycolipid complexes at a different TCR concentration after reference substraction (binding response to CD1d without added glycolipid). Sensorgrams (top) indicate similar lipid loading levels for **5d-e**. Single cycle TCR kinetics were measured for PBS-25 and 23. Here, TCR with increasing concentrations (3-fold) was sequentially added to CD1d-glycolipid with a final dissociation step of 10 min. Colored curves represent actual binding responses and black curves the fitted data. Kinetic values derived from the sensorgrams are listed below.

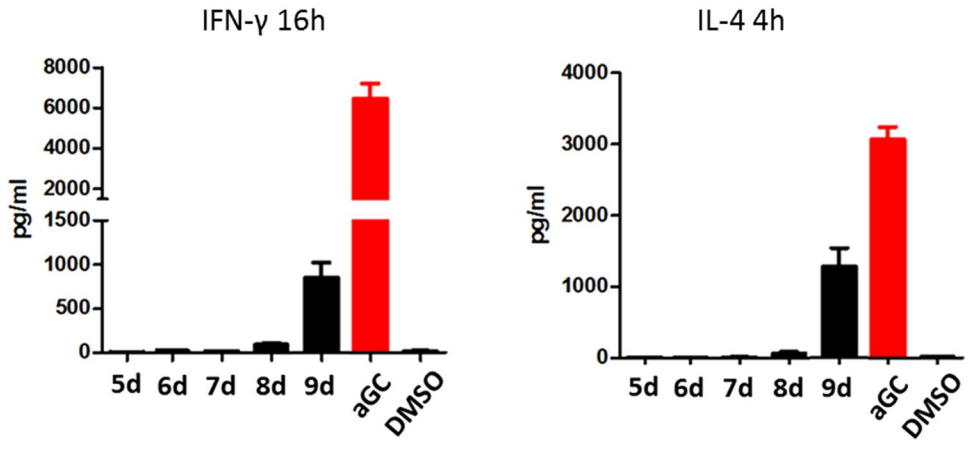


Figure 8. IFN-γ and IL-4 secretion, measured at 16 h and 4 h respectively, after intraperitoneal injection of 5 μg of the glycolipids in mice. Data for one individual experiment using 8 mice for each glycolipid.

We next compared the precise presentation of both ligand headgroups by CD1d. As suggested by the changes in hydrogen bond distances, the galactose of **5d** is slightly elevated compared to **23**, correlating with the increased distance in H-bonding interactions with CD1d (Fig. 10).

This slightly flexible presentation of **5d** is reminiscent to the presentation of the microbial glycosphingolipid GalA-GSL, which was also slightly shifted in the binding groove of CD1d compared to **22** but had comparable TCR association rates[39]. Therefore, the fine presentation of the galactose is likely not the reason for the biological inactivity of these galactosylsphingamides.

Since the only difference between both glycolipids is the presence (**5d**) or absence (**23**) of the amide group, this indicates that the amide is indeed responsible for the slight elevation of the galactose but more importantly for

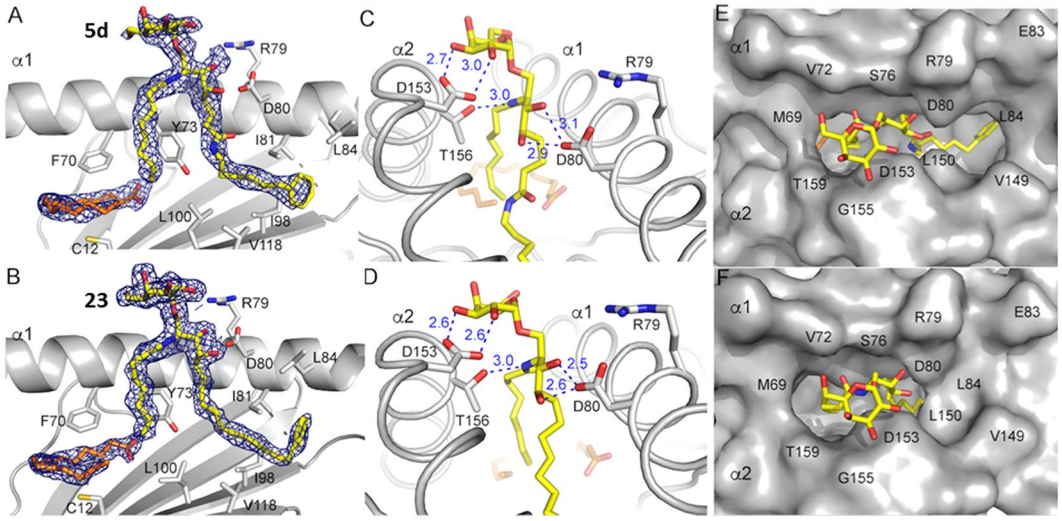


Figure 9. (**A**,**B**) Electron density map of **5d** and **23** with CD1d. Spacer lipid (palmitic acid) is depicted in orange. (**C**,**D**) H-bond interactions between **5d** and **23** (yellow) with CD1d (grey). (**E**,**F**) Glycolipid ligand presentation shown in "TCR view" from top with molecular surface of CD1d in grey and ligands as yellow sticks.

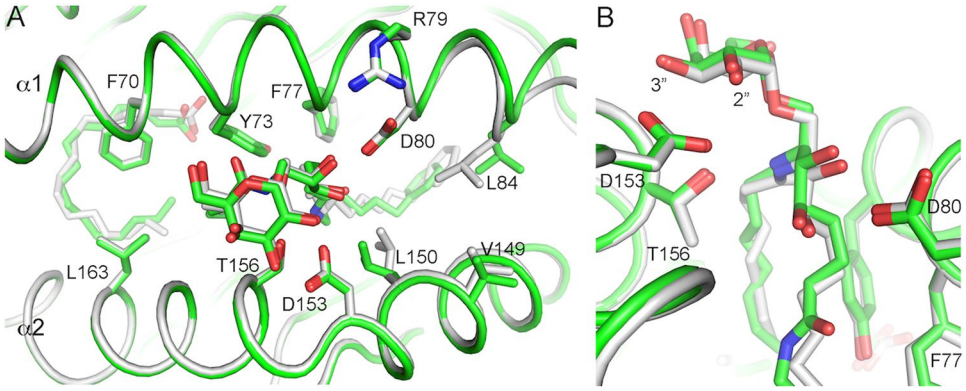


Figure 10. Comparison between **5d** (green) and **23** (grey)presented by CD1d.

the failure in closure of the F' roof in the binary complex, the latter of which is likely the main reason for the low antigenicity of the α-galactosylsphingamides (Fig. 10).

Conclusion

In summary, a series of α-GalCer analogues featuring an amide bond in the phytosphingosine were synthesized. The presented synthesis allows to alter an advanced methyl ester precursor after glycosylation, thereby reducing the number of linear steps towards the aimed α-GalCer analogues. Furthermore, the ability to diversify the acyl moiety in the penultimate step of the synthesis adds to the versatility of the presented synthesis route. Also, methyl ester **10** is an interesting intermediate for alternative derivatization of the phytosphingosine chain.

Biological evaluation revealed that the novel α-galactosylsphingamides are weak *i*NKT-cell antigens and that the main compromising factor is the implemented amide functionality. While extending the acyl chain offered partial restoration of the antigenic effect, overall *i*NKT-cell stimulation remained poor.

Crystal structure analysis revealed a slightly flexible presentation of the α-galactosylsphingamides to CD1d, where the sugar head group is slightly shifted in the CD1d binding groove. A similar binding has been observed for the microbial glycosphingolipid GalA-GSL, but never for known α-GalCer analogues[39]. These crystal structures support the hypothesis that although both the acyl chain and phytosphingosine contribute to the stability of the CD1d/glycolipid complex, it is the phytosphingosine chain that controls the CD1d/glycolipid footprint. How the amide group affects the biological activity of these compounds and how its presence compensates for the lack of the pre-formed F' roof formation upon TCR binding is currently unknown and will have to be characterized by determining CD1d-galactosylsphingamide-TCR structures.

Experimental part. *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 or by spraying with a solution of $(NH_4)_6Mo_7O_{24}{\cdot}4\ H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)_4{\cdot}2\ H_2O$ (10 g/L) in H_2SO_4 (10%) followed by charring. 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 (δ) relative to the residual solvent signals, in the case of $CDCl_3$: δ = 7.26 ppm for ^{1}H and δ = 77.4 ppm for ^{13}C and in the case of pyridine-d_5: δ = 8.74, 7.58 and 7.22 ppm for ^{1}H and δ = 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 in a CH_3CN/HCOOH (1000:1) mixture at 100 µL/min.

*3,4,6-Tri-O-benzyl-D-galactal (**17**).* 3,4,6-Tri-*O*-acetyl-D-galactal (22.05 g, 81 mmol) was dissolved in MeOH (250 mL), Et_3N (67 mL) and H_2O (8 mL) and the reaction mixture was stirred for 5 days at room temperature. After completion of the reaction the solvent was removed under reduced pressure. The crude product was dried by making azeotropic mixture with toluene to afford pure D-galactal in quantitative yield. This material was dissolved in dry DMF (250 mL) and cooled to 0 °C. Sodium hydride (60% dispersion, 13 g, 324 mmol) was added portionwise over a period of 15 min. After 30 minutes benzyl bromide (38.8 mL, 324 mmol) was added dropwise and the reaction mixture was stirred overnight, allowing the temperature to rise to room temperature. Upon completion of the reaction, the reaction mixture was quenched with MeOH at 0 °C. The mixture was extracted with EtOAc (4 × 200 mL) and the combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and the solvents were evaporated under reduced pressure. The crude was purified by silica gel chromatography (hexane/EtOAc: 9/1) furnishing 3,4,6-tri-*O*-benzyl-D-galactal (31.67 g, 94%) as a white solid.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 3.65 (dd, *J* = 10.1 and 5.1 Hz, 1 H, Ha-6) 3.78 (dd, *J* = 10.1 and 7.2 Hz, 1 H, Hb-6) 3.92–3.96 (m, 1 H, H-4) 4.15–4.21 (m, 2 H, H-3 and H-5) 4.41 (d, *J* = 12.2, 1 H, $\underline{CH_2}$Ph) 4.50 (d, *J* = 12.2, 1 H, $\underline{CH_2}$Ph) 4.57–4.68 (m, 3 H, H-2, $\underline{CH_2}$Ph) 4.82–4.90 (m, 2 H, H-2, $\underline{CH_2}$Ph) 6.36 (d, *J* = 6.4 Hz, 1 H, H-1) 7.24–7.36 (m, 15 H, arom. H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 68.38, 70.70, 70.82, 71.21, 73.26, 73.35, 75.63, 99.90, 127.37, 127.48, 127.62, 127.83, 128.08, 128.24, 128.32, 137.94, 138.30, 138.44, 144.12.

Exact mass (ESI-MS) for $C_{27}H_{28}NaO_4$ $[M + Na]^+$ found, 439.1878; calcd, 439.1880.

*2-Deoxy-3,4,6-tri-O-benzyl-D-galactose (**14**).* To a solution of 3,4,6-tri-*O*-benzyl-D-galactal (31.7 g, 76 mmol) in DMF (250 mL) maintained at 0 °C, H_2SO_4 (80 mL, 4.0 M) was added dropwise. The reaction mixture was allowed to rise to room temperature and was stirred for 16 h. Upon completion of the reaction, the mixture was neutralized with $NaHCO_3$ (400 mL, sat. aq.) and extracted with Et_2O (3 × 200 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, filtered and evaporated. The crude residue was purified by silica gel chromatography (hexane/EtOAc: 85/15) furnishing **14** (28.13 g, 85%) as a white solid.

^{1}H-NMR (α-anomer) (300 MHz, $CDCl_3$): δ 1.90–2.09 (m, 1 H, Ha-2) 2.23 (td, *J* = 12.4, 3.6 Hz, 1 H, Hb-2) 3.41–3.73 (m, 2 H, CH_2-6) 3.73–3.92 (m, 1 H, H-5) 4.00 (ddd, *J* = 12.0, 4.6 and 2.5 Hz, 1 H, H-3) 4.14 (t, *J* = 6.5 Hz, 1 H, H-4) 4.44 (d, *J* = 12.0 Hz, 1 H, $\underline{CH_2}$Ph) 4.52 (d, *J* = 12.0 Hz, 1 H, $\underline{CH_2}$Ph) 4.59–4.66 (m, 3 H, $\underline{CH_2}$Ph) 4.94 (d, *J* = 11.8 Hz, 1 H, $\underline{CH_2}$Ph) 5.47 (d, *J* = 3.1 Hz, 1 H, H-1) 7.20–7.43 (m, 15 H, arom. H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 30.92, 34.24, 69.0, 69.79, 70.08, 70.31, 71.65, 72.97, 73.29, 73.99, 74.19, 92.39, 94.54, 127.16, 127.40, 127.44, 127.53, 127.61, 128.02, 128.08, 128.22, 128.27, 137.67, 138.36, 138.45.

Exact mass (ESI-MS) for $C_{27}H_{30}NaO_5$ $[M + Na]^+$ found, 457.1987; calcd, 457.1985.

Data consistent with literature[40].

*(5R,6S,7R,E)-methyl 5,6,8- tri-O-benzyl-7-hydroxyoct-2-enoate (**18**).* 2-Deoxy-3,4,6-tri-*O*-benzyl-D-galactose **14** (3.7 g, 8.52 mmol) and methyl(triphenylphosphoranylidene)acetate (5.68 g, 17.04 mmol) were dissolved in anhydrous toluene (150 mL) and stirred for 6 h at 85 °C. The reaction mixture was evaporated under reduced pressure, and the residue was purified by silica gel chromatography (hexane/EtOAc: 8/2) to give **18** (3.29 g, 79%) as a slightly yellow oil.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.58 (ddd, *J* = 7.2, 5.7 and 1.3 Hz, 2 H, H-4) 3.48–3.60 (m, 2 H, H-8) 3.62–3.70 (m, 1 H, H-6) 3.73 (s, 3 H, OCH_3) 3.76–3.84 (m, 1 H, H-5) 3.96–4.05 (m, 1 H, H-7) 4.49–4.52 (m, 2 H, $\underline{CH_2}$Ph) 4.55–4.57 (m, 3 H, $\underline{CH_2}$Ph) 4.67 (d, *J* = 11.4 Hz, 1 H, $\underline{CH_2}$Ph) 5.88 (dt, *J* = 15.7 and 1.3 Hz, 1 H, H-2) 7.01 (dt, *J* = 15.7 and 7.4 Hz, 1 H, H-3) 7.23–7.40 (m, 15 H, arom. H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 33.81, 51.44, 69.66, 71.08, 72.56, 73.44, 74.06,78.48, 79.00, 123.21, 127.77, 127.86, 127.90, 127.94, 127.99, 128.07, 128.42, 128.45, 137.68, 137.75, 137.87, 145.57, 166.64.

Exact mass (ESI-MS) for $C_{30}H_{35}O_6$ $[M + H]^+$ found, 491.2427; Calcd., 491.2428.

*(5R,6S,7R)-methyl 5,6,8- tri-O-benzyl -7-hydroxyoctanoate (**13**).* $NiCl_2.6\ H_2O$ (800 mg, 3.36 mmol) and $NaBH_4$ (508 mg, 13.42 mmol) were added to a stirred solution of unsaturated ester **18** (3.29 g, 6.71 mmol) in MeOH (37 mL) and THF (3.7 mL) at 0 °C. After 1 h, the reaction mixture was filtered through Celite and the filter cake rinsed with Et_2O. Evaporation of the filtrate yielded **13** (3.09 g, 94% yield) as a yellow oil which could be used in the next step without further purification.

^{1}H-NMR (300 MHz $CDCl_3$) δ 1.52–1.88 (m, 4 H, H-2 and H-4) 2.16–2.50 (m, 2 H, H-3) 3.01 (br. s, 1 H, -OH) 3.55 (d, *J* = 5.8 Hz, 2 H, H-8) 3.60–3.73 (m, 5 H, OCH_3, H-5 and H-6) 3.98–4.05 (m, 1 H, H-7) 4.51–4.54 (m, 3 H, $\underline{CH_2}$Ph) 4.56–4.58 (m, 1 H, $\underline{CH_2}$Ph) 4.66 (d, *J* = 11.4 Hz, 1 H, $\underline{CH_2}$Ph) 4.72 (d, *J* = 11.4 Hz, 1 H, $\underline{CH_2}$Ph) 7.25–7.41 (m, 15 H, arom. H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 20.99, 30.27, 33.94, 51.48, 69.86, 71.06, 72.71, 73.42, 73.78, 78.97, 79.41, 127.71, 127.74, 127.85, 127.89, 127.96, 128.09, 128.38, 128.41, 138.03, 138.08, 173.83.

Exact mass (ESI-MS) for $C_{30}H_{36}KO_6$ $[M+K]^+$ found, 531.2151; Calcd., 531.2143.

*(5R,6S,7S)-methyl 7-azido-5,6,8- tri-O-benzyl-octanoate (**19**).* To a solution of compound **13** (3.09 g, 6.27 mmol) in anhydrous THF (100 mL) were added PPh_3 (3.29 g, 12.54 mmol), diethylazodicarboxylate (2.18 g, 5.7 mL, 12.54 mmol), and diphenylphosphorylazide (3.45 g, 2.71 mL, 12.54 mmol) at −20 °C. After addition, the reaction mixture was allowed to warm to room temperature and stirred for 7 h. The solvent was evaporated under reduced pressure to give an orange residue. Purification by silica gel chromatography (hexane/EtOAc: 9/1) afforded **19** (3.19 g, 95% yield) as a yellow oil.

^{1}H-NMR (300 MHz $CDCl_3$) δ 1.52–1.86 (m, 4 H, H-2 and H-4) 2.18–2.39 (m, 2 H, H-3) 3.55–3.83 (m, 8 H, OCH_3, H-5, H-6, H-7 and H-8) 4.57–4.62 (m, 3 H, $\underline{CH_2}$Ph) 4.65 (d, J = 11.5 Hz, 1 H, $\underline{CH_2}$Ph) 4.68 (d, J = 11.3 Hz, 1 H, $\underline{CH_2}$Ph) 4.78 (d, J = 11.3 Hz, 1 H, $\underline{CH_2}$Ph) 7.18–7.46 (m, 15 H, arom. H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 20.72, 29.07, 33.94, 51.47, 61.93, 70.08, 71.95, 73.34, 73.83, 78.65, 78.74, 120.18, 120.24, 126.09, 127.66, 127.69, 127.71, 127.77, 127.91, 128.00, 128.37, 128.38, 128.41, 130.03, 130.05, 137.81, 137.91, 138.09, 173.84.

Exact mass (ESI-MS) for $C_{30}H_{35}KN_3O_5$ $[M+K]^+$ found, 556.2210; Calcd., 556.2208.

*(5R,6S,7S)-methyl 7-azido-5,6-di-O-benzyl-8-hydroxyoctanoate (**12**).* Azide **19** (1.4 g, 2.7 mmol) and $ZnCl_2$ (7.38 g, 54 mmol) were dissolved in Ac_2O/AcOH (2:1, 21 mL). The reaction was allowed to stir for 3.5 h at room temperature. Next H_2O (10 mL) was added and the reaction mixture was extracted with CH_2Cl_2. The combined organic layers were washed with H_2O, $NaHCO_3$ (sat. aq.) and brine. Then the organic phase was dried over Na_2SO_4, filtered, and evaporated to afford 598 mg of crude acetate. The crude acetate was dissolved in MeOH (20 mL) and NaOMe (5.4 M solution in MeOH) was added till pH 10. After stirring overnight, Amberlite 120R (H^+ form) was added to neutralize the reaction mixture. The mixture was diluted with MeOH (20 mL) and the exchange resin was filtered off and rinsed with MeOH. Evaporation of the filtrate under reduced pressure yielded a solid residue that was purified by silica gel chromatography (hexane/EtOAc: 7/3) to afford **12** (771 mg, 67% yield) as a colorless oil.

^{1}H-NMR (300 MHz $CDCl_3$) δ 1.56–1.87 (m, 4 H, H-5 and H-7) 2.25–2.34 (m, 2 H, H-6) 2.38 (t, J = 6.3 Hz, 1 H, OH) 3.58–3.96 (m, 8 H, OCH_3, H-1, H-2, H-3 and H-4) 4.58 (dd, J = 20.1 and 11.4 Hz, 2 H, $\underline{CH_2}$Ph) 4.68 (dd, J = 17.8 and 11.2 Hz, 2 H, $\underline{CH_2}$Ph) 7.24–7.41 (m, 10 H, arom. H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 20.79, 29.38, 33.82, 51.51, 62.31, 63.11, 72.38, 73.71, 78.60, 79.84, 127.88, 128.00, 128.02, 128.11, 128.46, 128.52, 137.53, 137.75, 173.77.

Exact mass (ESI-MS) for $C_{23}H_{30}N_3O_5$ $[M+H]^+$ found, 428.2177; Calcd., 428.2180.

*(5R,6S,7S)-methyl-7-azido-5,6-di-O-benzyl-8-O-(2,3-di-O-benzyl-4,6-O-benzylidene-α-D-galactopyranosyl)-octanoate (**10**).* To a mixture of **11** (670 mg, 1.13 mmol) in THF (9 mL), a solution of **12** (323 mg, 0.76 mmol) in THF (6 mL) was added. The reaction mixture was cooled to −30 °C and TMSOTf (0.3 mL, 0.11 mmol) was added dropwise. After stirring for 2 h. at −30 °C, the reaction mixture was neutralized with Et_3N and evaporated to dryness. The residue was purified by silica gel chromatography (hexane/EtOAc: 8/2 + 1% v/v Et_3N) to afford **10** (438 mg, 67% yield) as colorless liquid which solidified upon storage.

^{1}H-NMR (300 MHz $CDCl_3$) δ 1.46–1.79 (m, 4 H, CH_2) 2.20 (t, J = 6.6 Hz, 2 H, CH_2) 3.52 (br s., 1 H, H-4") 3.56–3.71 (m, 7 H, H-2, H-2", H-4, Hb-6", OCH_3) 3.84 (dd, J = 12.5 and 1.5 Hz, 1 H, H-3") 3.93 (dd, J = 9.8 and 2.9 Hz, 1 H, Ha-1) 3.97–4.06 (m, 3 H, Hb-1, H-3 and Hb-6") 4.13 (dd, J = 3.2 and 1.0 Hz, 1 H, H-5") 4.43 (d, J = 11.5 Hz, 1 H, $\underline{CH_2}$Ph) 4.50–4.63 (m, 4 H, $\underline{CH_2}$Ph) 4.69 (d, J = 12.3 Hz, 1 H, $\underline{CH_2}$Ph) 4.76 (d, J = 12.5 Hz, 1 H, $\underline{CH_2}$Ph) 4.80 (d, J = 11.8 Hz, 1 H, $\underline{CH_2}$Ph) 4.92 (d, J = 3.2 Hz, 1 H, H-1") 5.41 (s, 1 H, H-8") 7.14–7.36 (m, 23 H, arom. H) 7.44–7.48 (m, 2 H, arom. H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 20.83, 29.23, 33.89, 51.47, 61.65, 63.00, 68.31, 69.31, 71.95, 72.00, 73.54, 73.84, 74.59, 75.41, 77.42, 78.42, 78.95, 99.14, 101.05, 126.33, 127.46, 127.50, 127.65, 127.69, 127.79, 127.83, 127.92, 128.09, 128.22, 128.26, 128.39, 128.85, 137.82, 137.91, 138.11, 138.73, 138.75, 173.80.

Exact mass (ESI-MS) for $C_{50}H_{56}N_3O_{10}$ $[M+H]^+$ found, 858.3948; Calcd., 858.3960.

General procedure for $AlMe_3$ assisted amide coupling. To a solution of glycoside **10** (500 mg, 0.58 mmol) in anhydrous CH_2Cl_2 (10 mL) was added the appropriate amine (5 eq.) and $AlMe_3$ (2 M in heptanes) (2.5 eq.). The reaction mixture was stirred overnight at reflux temperature. Next the reaction mixture was cooled to 0 °C and water was added. The mixture was extracted with CH_2Cl_2 and the combined organic phase was washed with H_2O and brine, dried over Na_2SO_4, filtered, and evaporated. Purification by column chromatography gave the desired amides **20a** (61%), **20b** (64%), **20c** (64%), **20d** (52%), **20e** (51%), **20 f** (68%), **20 g** (81%), **20 h** (60%).

*(5R,6S,7S)-7-azido-5,6-di-O-benzyl-8-O-(2,3-di-O-benzyl-4,6-O-benzylidene-α-D-galactopyranosyl)-N-nonyloctanamide (**20a**).* ^{1}H-NMR (300 MHz $CDCl_3$) δ 0.89 (t, J = 6.5 Hz, 3 H, terminal CH_3) 1.23–1.35 (m, 12 H, CH_2) 1.45 (dt, J = 13.4 and 7.8 Hz, 2 H, CH_2) 1.53–1.81 (m, 4 H, CH_2) 2.09 (t, J = 6.6 Hz, 2 H, CH_2) 3.14–3.24 (m, 2 H, NH$\underline{CH_2}$) 3.59–3.81 (m, 5 H, H-2, H-3, H-4, H-5" and Ha-6") 3.93 (dd, J = 12.6 and 1.5 Hz, 1 H, Ha-1) 3.98–4.04 (m, 1 H, Hb-6") 4.06 (d, J = 3.2 Hz, 1 H, H-3") 4.08–4.14 (m, 2 H, Hb-1 and H-2") 4.20 (dd, J = 3.5 and 1.2 Hz, 1 H, H-4") 4.49 (d, J = 11.5 Hz, 1 H, $\underline{CH_2}$Ph) 4.59–4.72 (m, 4 H, $\underline{CH_2}$Ph) 4.75 (d, J = 12.3 Hz, 1 H, $\underline{CH_2}$Ph) 4.82 (d, J = 12.3 Hz, 1 H, $\underline{CH_2}$Ph) 4.88 (d, J = 12.0 Hz, 1 H, $\underline{CH_2}$Ph) 4.98 (d, J = 3.3 Hz, 1 H, H-1") 5.32 (t, J = 5.1 Hz, 1 H, (CO)NH) 5.48 (s, 1 H, H-8") 7.22–7.43 (m, 23 H, arom. H) 7.51–7.56 (m, 2 H, arom. H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.10, 21.81, 22.65, 26.93, 29.25, 29.29, 29.50, 29.67, 31.84, 36.57, 39.50, 61.55, 63.03,

68.35, 69.33, 71.96, 72.02, 73.59, 73.87, 74.58, 75.38, 75.84, 78.35, 79.20, 99.19, 101.06, 126.34, 127.47, 127.52, 127.68, 127.71, 127.75, 127.78, 127.89, 127.98, 128.11, 128.23, 128.27, 128.40, 128.43, 128.49, 128.87, 137.83, 137.98, 138.14, 138.74, 172.41.

Exact mass (ESI-MS) for $C_{58}H_{76}N_5O_9$ $[M+NH_4]^+$ found, 986.5641; Calcd., 986.5638.

*(5R,6S,7S)-7-azido-5,6-di-O-benzyl-8-O-(2,3-di-O-benzyl-4,6-O-benzylidene-α-D-galactopyranosyl)-N-2-phenylethyl)octanamide (**20b**).* ^{1}H-NMR (300 MHz $CDCl_3$) δ 1.52–1.76 (m, 4 H, CH_2) 2.02–2.07 (m, 2 H, (CO)CH_2) 2.73 (t, J = 6.9 Hz, 2 H, H-11) 3.44 (q, J = 6.8 Hz, 2 H, NH$\underline{CH_2}$) 3.56 (app. s, 1 H, H-2) 3.57–3.63 (m, 2 H, H-4 and H-3) 3.66–3.75 (m, 2 H, H-5" and Ha-6") 3.88 (dd, J = 12.3 and 1.3 Hz, 1 H, H-3") 3.95 (d, J = 3.3 Hz, 1 H, Ha-1) 3.97–4.06 (m, 2 H, H-2" and Hb-6") 4.07–4.10 (m, 1 H, Hb-1) 4.15 (d, J = 2.9 Hz, 1 H, H-4") 4.43 (d, J = 11.6 Hz, 1 H, $\underline{CH_2}$Ph) 4.54–4.66 (m, 4 H, $\underline{CH_2}$Ph) 4.71 (d, J = 12.4 Hz, 1 H, $\underline{CH_2}$Ph) 4.78 (d, J = 12.4 Hz, 1 H, $\underline{CH_2}$Ph) 4.83 (d, J = 11.8 Hz, 1 H, $\underline{CH_2}$Ph) 4.93 (d, J = 3.2 Hz, 1 H, H-1") 5.29 (t, J = 5.6 Hz, 1 H, (CO)NH) 5.43 (s, 1 H, H-8") 7.12–7.38 (m, 28 H, arom. H) 7.48 (dd, J = 7.3 and 2.3 Hz, 2 H, arom. H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 21.71, 29.25, 35.70, 36.49, 40.49, 61.55, 63.03, 68.34, 69.32, 71.94, 72.01, 73.57, 73.85, 74.56, 75.37, 75.83, 77.86, 78.28, 78.35, 78.70, 79.08, 79.63, 99.17, 101.04, 126.32, 126.49, 127.46, 127.50, 127.66, 127.69, 127.76, 127.87, 127.95, 128.09, 128.21, 128.26, 128.38, 128.40, 128.61, 128.72, 128.85, 128.94, 128.97, 129.15, 129.23, 129.37, 129.49, 129.54, 129.68, 129.89, 130.00, 137.81, 137.96, 138.12, 138.72, 138.87, 140.00, 172.47.

Exact mass (ESI-MS) for $C_{57}H_{62}N_4NaO_9$ $[M+Na]^+$ found, 969.4413; Calcd., 969.4409.

*(5R,6S,7S)-7-azido-5,6-di-O-benzyl-8-O-(2,3-di-O-benzyl-4,6-O-benzylidene-α-D-galactopyranosyl)-N-(4-phenylbutyl)octanamide (**20c**).* ^{1}H-NMR (300 MHz $CDCl_3$) δ 1.43–1.78 (m, 8 H, CH_2) 2.07 (t, J = 6.8 Hz, 2 H, CH_2) 2.62 (t, J = 7.5 Hz, 2 H, CH_2) 3.21 (dd, J = 13.0 and 7.3 Hz, 2 H, NH$\underline{CH_2}$) 3.56 (app. s, 1 H, H-2) 3.60–3.67 (m, 2 H, H-4 and Ha-6") 3.69–3.79 (m, 2 H, H-5" and H-3) 3.91 (dd, J = 13.0 and 2.0 Hz, 1 H, Ha-1) 3.97–4.02 (m, 1 H, Hb-6") 4.04 (d, J = 2.9 Hz, 1 H, H-3") 4.07–4.10 (m, 2 H, H-2" and Hb-1) 4.18 (d, J = 2.9 Hz, 1 H, H-4") 4.47 (d, J = 11.5 Hz, 1 H, $\underline{CH_2}$Ph) 4.57–4.69 (m, 4 H, $\underline{CH_2}$Ph) 4.74 (d, J = 12.6 Hz, 1 H, $\underline{CH_2}$Ph) 4.81 (d, J = 12.6 Hz, 1 H, $\underline{CH_2}$Ph) 4.86 (d, J = 11.9 Hz, 1 H, $\underline{CH_2}$Ph) 4.98 (d, J = 3.2 Hz, 1 H, H-1") 5.29 (t, J = 5.2 Hz, 1 H, (CO)NH) 5.47 (s, 1 H, H-8") 7.14–7.41 (m, 28 H, arom. H) 7.49–7.53 (m, 2 H, arom. H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 21.79, 28.64, 29.22, 35.44, 36.54, 39.27, 61.55, 63.03, 68.35, 69.33, 71.95, 72.02, 73.59, 73.87, 74.57, 75.38, 75.84, 77.21, 78.32, 79.18, 99.18, 101.05, 125.82, 126.33, 127.47, 127.52, 127.67, 127.70, 127.75, 127.77, 127.87, 127.98, 128.10, 128.22, 128.27, 128.33, 128.37, 128.39, 128.42, 128.86, 137.81, 137.98, 138.11, 138.72, 142.05, 172.45.

Exact mass (ESI-MS) for $C_{59}H_{67}N_4O_9$ $[M+H]^+$ found, 975.4905; Calcd., 975.4903.

*(5R,6S,7S)-7-azido-5,6-di-O-benzyl-8-O-(2,3-di-O-benzyl-4,6-O-benzylidene-α-D-galactopyranosyl)-N-(6-phenylhexyl)octanamide (**20d**).* ^{1}H-NMR (300 MHz $CDCl_3$) δ 1.26–1.48 (m, 6 H, CH_2) 1.52–1.78 (m, 6 H, CH_2) 2.08 (t, J = 6.7 Hz, 2 H, CH_2) 2.59 (t, J = 7.5 Hz, 2 H, CH_2) 3.18 (dd, J = 13.4 and 7.2 Hz, 2 H, NH$\underline{CH_2}$) 3.59 (app. s, 1 H, H-2) 3.61–3.67 (m, 2 H, H-3 and H-4) 3.68–3.79 (m, 2 H, H-5" and Ha-6") 3.91 (dd, J = 12.7 and 1.1 Hz, 1 H, Ha-1) 3.98–4.02 (m, 1 H, Hb-6") 4.04 (d, J = 3.5 Hz, 1 H, H-3") 4.07–4.12 (m, 2 H, Hb-1 and H-2") 4.19 (d, J = 2.7 Hz, 1 H, H-4") 4.47 (d, J = 11.7 Hz, 1 H, $\underline{CH_2}$Ph) 4.57–4.72 (m, 4 H, $\underline{CH_2}$Ph) 4.75 (d, J = 12.4 Hz, 1 H, $\underline{CH_2}$Ph) 4.81 (d, J = 12.4 Hz, 1 H, $\underline{CH_2}$Ph) 4.86 (d, J = 12.1 Hz, 1 H, $\underline{CH_2}$Ph) 4.97 (d, J = 3.5 Hz, 1 H, H-1") 5.30 (t, J = 5.4 Hz, 1 H, (CO)NH) 5.47 (s, 1 H, H-8") 7.15–7.42 (m, 28 H, arom. H) 7.50–7.54 (m, 2 H, arom. H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 21.80, 26.77, 28.89, 29.23, 29.58, 31.31, 35.84, 36.55, 39.43, 61.54, 63.02, 68.34, 69.32, 71.95, 72.02, 73.57, 73.86, 74.56, 75.37, 75.83, 77.20, 78.33, 79.19, 99.17, 101.04, 125.62, 126.32, 127.46, 127.51, 127.67, 127.70, 127.74, 127.76, 127.87, 127.96, 128.09, 128.22, 128.24, 128.26, 128.35, 128.38, 128.42, 128.86, 137.81, 137.97, 138.11, 138.71, 142.59, 172.42.

Exact mass (ESI-MS) for $C_{61}H_{71}N_4O_9$ $[M+H]^+$ found, 1003.5232; Calcd., 1003.5216.

*(5R,6S,7S)-7-azido-5,6-di-O-benzyl-8-O-(2,3-di-O-benzyl-4,6-O-benzylidene-α-D-galactopyranosyl)-N-(8-phenyloctyl)octanamide (**20e**).* ^{1}H-NMR (300 MHz $CDCl_3$) δ 1.23–1.49 (m, 10 H, CH_2) 1.51–1.78 (m, 6 H, CH_2) 2.08 (t, J = 6.0 Hz, 2 H, CH_2) 2.59 (t, J = 7.5 Hz, 2 H, CH_2) 3.18 (dd, J = 13.0 and 6.5 Hz, 2 H, NH$\underline{CH_2}$) 3.59 (app. s, 1 H, H-2) 3.61–3.68 (m, 2 H, H-3 and H-4) 3.68–3.80 (m, 2 H, H-5" and Ha-6") 3.91 (dd, J = 12.4 and 1.4 Hz, 1 H, Ha-1) 4.98–4.03 (m, 1 H, Hb-6") 4.04 (d, J = 3.2 Hz, 1 H, H-3") 4.06–4.13 (m, 2 H, H-2" and Hb-1) 4.19 (dd, J = 3.1 and 0.9 Hz, 1 H, H-4") 4.47 (d, J = 11.4 Hz, 1 H, $\underline{CH_2}$Ph) 4.56–4.71 (m, 4 H, $\underline{CH_2}$Ph) 4.75 (d, J = 12.4 Hz, 1 H, $\underline{CH_2}$Ph) 4.82 (d, J = 12.4 Hz, 1 H, $\underline{CH_2}$Ph) 4.86 (d, J = 11.8 Hz, 1 H, $\underline{CH_2}$Ph) 4.98 (d, J = 3.2 Hz, 1 H, H-1") 5.31 (t, J = 5.1 Hz, 1 H, (CO)NH) 5.47 (s, 1 H, H-8") 7.14–7.43 (m, 28 H, arom. H) 7.49–7.54 (m, 2 H, arom. H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 21.82, 26.90, 29.22, 29.39, 29.66, 31.46, 35.95, 36.58, 39.48, 61.55, 63.03, 68.36, 69.34, 71.96, 72.04, 73.58, 73.87, 74.59, 75.38, 75.84, 77.21, 78.34, 79.21, 99.20, 101.06, 110.00, 125.57, 126.34, 127.48, 127.53, 127.68, 127.71, 127.76, 127.79, 127.89, 127.98, 128.11, 128.23, 128.27, 128.40, 128.43, 128.87, 137.83, 137.98, 138.14, 138.73, 142.82, 172.42.

Exact mass (ESI-MS) for $C_{63}H_{75}N_4O_9$ $[M+H]^+$ found, 1031.5575; Calcd., 1031.5529.

*(5R,6S,7S)-7-azido-5,6-di-O-benzyl-8-O-(2,3-di-O-benzyl-4,6-O-benzylidene-α-D-galactopyranosyl)-N-(3-pentylphenyl)octanamide (**20f**).* ^{1}H-NMR (300 MHz $CDCl_3$) δ 0.88 (t, J = 6.7 Hz, 3 H, terminal CH_3) 1.26–1.36 (m, 4 H, CH_2) 1.54–1.65 (m, 4 H, CH_2) 1.70–1.87 (m, 2 H, CH_2) 2.26 (t, J = 6.9 Hz, 2 H, CH_2) 2.56 (t, J = 8.0 Hz, 2 H, CH_2) 3.54–3.59 (m, 2 H, H-2 and H-3) 3.62–3.69 (m, 2 H, H-4 and Ha-6") 3.75 (dd, J = 6.7 and 3.6 Hz, 1 H, H-5") 3.85 (dd, J = 12.4 and 1.2 Hz, 1 H, Ha-1) 3.93–4.07 (m, 4 H, H-2", H-3", Hb-1 and Hb-6") 4.13 (d, J = 2.7 Hz, 1 H, H-4") 4.43 (d, J = 11.5 Hz, 1 H, $\underline{CH_2}$Ph) 4.64–4.52 (m, 4 H, $\underline{CH_2}$Ph) 4.68 (d, J = 12.5 Hz, 1 H,

CH_2Ph) 4.75 (d, J = 12.5 Hz, 1 H, CH_2Ph) 4.81 (d, J = 11.8 Hz, 1 H, CH_2Ph) 4.91 (d, J = 3.2 Hz, 1 H, H-1") 5.40 (s, 1 H, H-8") 6.85 (d, J = 7.2 Hz, 1 H, arom. H) 6.99 (s, 1 H, (CO)NH) 7.08–7.37 (m, 26 H, arom. H) 7.46 (dd, J = 7.3 and 2.2 Hz, 2 H, arom. H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 14.03, 21.78, 22.52, 28.95, 31.09, 31.52, 35.93, 37.45, 61.49, 63.04, 68.27, 69.32, 71.91, 72.06, 73.61, 73.95, 74.54, 75.38, 75.84, 77.21, 78.19, 79.37, 99.17, 101.05, 116.85, 116.95, 119.63, 124.29, 126.33, 127.48, 127.52, 127.67, 127.71, 127.79, 127.85, 127.94, 128.10, 128.23, 128.27, 128.39, 128.50, 128.75, 128.86, 137.78, 137.82, 137.91, 138.01, 138.72, 143.97, 170.72.

Exact mass (ESI-MS) for $C_{60}H_{68}N_4NaO_9$ $[M + Na]^+$ found, 1011.4886; Calcd., 1011.4879.

*(5R,6S,7S)-7-azido-5,6-di-O-benzyl-8-O-(2,3-di-O-benzyl-4,6-O-benzylidene-α-D-galactopyranosyl)-N-(4-pentylphenyl)octanamide (**20g**).* ^{1}H-NMR (300 MHz $CDCl_3$) δ 0.88 (t, J = 6.8 Hz, 3 H, terminal CH_3) 1.29–1.31 (m, 3 H, CH_2) 1.55–1.67 (m, 5 H, CH_2) 1.71–1.89 (m, 2 H, CH_2) 2.26 (t, J = 6.8 Hz, 2 H, CH_2) 2.57 (t, J = 7.6 Hz, 2 H, CH_2) 3.59–3.67 (m, 2 H, H-2 and H-5") 3.67–3.77 (m, 2 H, H-4 and Ha-6") 3.82 (dd, J = 6.8 and 3.6 Hz, 1 H, H-3) 3.92 (dd, J = 12.5 and 1.5 Hz, 1 H, Ha-1) 3.98–4.14 (m, 4 H, Hb-1, H-2", H-3" and Hb-6") 4.20 (d, J = 2.5 Hz, 1 H, H-4") 4.50 (d, J = 11.4 Hz, 1 H, CH_2Ph) 4.58–4.72 (m, 4 H, CH_2Ph) 4.75 (d, J = 12.3 Hz, 1 H, CH_2Ph) 4.82 (d, J = 12.3 Hz, 1 H, CH_2Ph) 4.88 (d, J = 11.7 Hz, 1 H, CH_2Ph) 4.98 (d, J = 3.2 Hz, 1 H, H-1") 5.47 (s, 1 H, H-8") 7.04–7.14 (m, 3 H, arom. H) 7.22–7.43 (m, 25 H, (CO)NH and arom. H) 7.51–7.56 (m, 2 H, arom. H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 14.02, 21.77, 22.52, 28.96, 31.18, 31.41, 35.30, 37.36, 61.50, 63.03, 68.26, 69.32, 71.91, 72.05, 73.60, 73.93, 74.54, 75.38, 75.83, 77.20, 78.21, 79.30, 99.16, 101.04, 119.77, 126.33, 127.48, 127.51, 127.67, 127.71, 127.78, 127.84, 127.94, 128.10, 128.22, 128.26, 128.39, 128.49, 128.81, 128.86, 135.43, 137.82, 137.91, 138.01, 138.71, 138.91, 170.66.

Exact mass (ESI-MS) for $C_{60}H_{68}N_4NaO_9$ $[M + Na]^+$ found, 1011.4874; Calcd., 1011.4879.

*(5R,6S,7S)-7-azido-5,6-di-O-benzyl-8-O-(2,3-di-O-benzyl-4,6-O-benzylidene-α-D-galactopyranosyl)-N-(2-(2-ethoxyethoxy)ethyl)octanamide (**20h**).* ^{1}H-NMR (300 MHz $CDCl_3$) δ 1.17 (t, J = 7.0 Hz, 3 H, terminal CH_3) 1.46–1.75 (m, 4 H, CH_2) 1.99–2.08 (m, 2 H, CH_2) 3.39 (q, 2 H, J = 5.2 Hz, NHCH_2) 3.43–3.62 (m, 11 H, CH_2, H-2, H-3 and H-4) 3.63–3.73 (m, 2 H, H-5" and Ha-6") 3.85 (dd, J = 12.6 and 1.8 Hz, 1 H, Ha-1) 3.91–3.97 (m, 1 H, Hb-6") 3.99 (d, J = 3.0 Hz, 1 H, H-3") 4.00–4.07 (m, 2 H, Hb-1 and H-2") 4.13 (d, J = 3.0 Hz, 1 H, H-4") 4.43 (d, J = 11.5 Hz, 1 H, CH_2Ph) 4.54 (d, J = 11.5 Hz, 1 H, CH_2Ph) 4.59–4.64 (m, 3 H, CH_2Ph) 4.69 (d, J = 12.6 Hz, 1 H, CH_2Ph) 4.76 (d, J = 12.6 Hz, 1 H, CH_2Ph) 4.81 (d, J = 11.8 Hz, 1 H, CH_2Ph) 4.92 (d, J = 3.2 Hz, 1 H, H-1") 5.41 (s, 1 H, H-8") 5.86 (t, J = 5.5 Hz, 1 H, (CO)NH) 7.16–7.37 (m, 23 H, arom. H) 7.44–7.48 (m, 2 H, arom. H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 15.60, 22.06, 29.82, 36.89, 39.50, 62.02, 63.43, 67.07, 68.78, 69.75, 70.14, 70.29, 70.71, 72.39, 72.45, 73.97, 74.26, 75.01, 75.82, 76.24, 77.63, 78.87, 79.50, 99.57, 101.47, 126.76, 127.88, 127.93, 128.10, 128.12, 128.19, 128.33, 128.52, 128.64, 128.69, 128.81, 128.82, 129.27, 138.25, 138.40, 138.58, 139.16, 172.98.

Exact mass (ESI-MS) for $C_{55}H_{67}N_4O_{11}$ $[M + H]^+$ found, 959.4799; Calcd., 959.4801.

Representative procedure for Staudinger reduction and acylation with EDC. To a solution of azide **20c** (350 mg, 0.36 mmol, 1eq.) in THF (5 mL) at room temperature, PMe_3 (1 M solution of in THF, 5.38 mL, 5.38 mmol, 15 eq.) was added dropwise. After stirring for 3 h at room temperature, H_2O (2 mL) was added and the reaction mixture was allowed to stir overnight at room temperature. Then the solvent was removed under reduced pressure. The crude product was dried by making azeotropic mixture with toluene to afford the crude amine. A mixture of the crude amine, EDC (112 mg, 0.72 mmol, 2 eq.) and octanoic acid (78 mg, 0.09 mL, 0.54 mmol, 1.5 eq.) in CH_2Cl_2 (10 mL) was stirred for 24 h at room temperature. The reaction mixture was diluted with CH_2Cl_2, washed with H_2O (2 × 10 mL) and brine (1 × 10 mL), dried over Na_2SO_4, filtered, and evaporated to dryness. Purification by silica gel chromatography gave the desired the desired amides. **21a** (74%), **21b** (76%), **21c** (82%), **21d** (80%), **21e** (81%), **21 f** (87%), **21 g** (87%), **21 h** (70%).

*(5R,6S,7S)-5,6-di-O-benzyl-8-O-(2,3-di-O-benzyl-4,6-O-benzylidene-α-D-galactopyranosyl)-7-octanamido-N-nonyloctanamide (**21a**).* ^{1}H-NMR (300 MHz $CDCl_3$) δ 0.85 (app. td, J = 6.7 and 2.8 Hz, 6 H, 2 x terminal CH_3) 1.19–1.35 (m, 18 H, CH_2) 1.39–1.54 (m, 4 H, CH_2) 1.60–1.83 (m, 6 H, CH_2) 1.90–1.97 (m, 2 H, CH_2) 2.02–2.10 (m, 2 H, CH_2) 3.17 (dd, J = 12.9 and 6.9 Hz, 2 H, NHCH_2)3.44 (m, 1 H, H-4) 3.52 (app. s, 1 H, H-5") 3.74 (dd, J = 7.0 and 2.0 Hz, 1 H, H-3) 3.82 (d, J = 4.8 Hz, 2 H, H-1) 3.89–3.96 (m, 2 H, H-4" and Ha-6") 4.04 (d, J = 3.6 Hz, 1 H, H-2") 4.07–4.14 (m, 2 H, H-2 and Hb-6") 4.18 (d, J = 3.2 Hz, 1 H, H-3") 4.39 (d, J = 11.6 Hz, 1 H, CH_2Ph) 4.47 (d, J = 11.6 Hz, 1 H, CH_2Ph) 4.55 (d, J = 11.8 Hz, 1 H, CH_2Ph) 4.62 (d, J = 11.8 Hz, 1 H, CH_2Ph) 4.72–4.77 (m, 3 H, CH_2Ph) 4.84 (d, J = 11.8 Hz, 1 H, CH_2Ph) 4.93 (d, J = 3.4 Hz, 1 H, H-1") 5.45 (s, 1 H, H-8") 5.98 (d, J = 8.4 Hz, 1 H, NH(CO)) 6.05 (t, J = 5.7 Hz, 1 H, (CO)NH) 7.19–7.40 (m, 23 H, arom. H) 7.47–7.52(m, 2 H, arom. H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 14.09, 21.84, 22.64, 25.65, 26.99, 28.82, 29.07, 29.26, 29.33, 29.52, 29.60, 31.76, 31.84, 36.03, 36.65, 39.48, 50.02, 63.00, 68.08, 69.36, 71.52, 71.72, 73.28, 73.98, 74.18, 75.61, 76.10, 78.88, 99.60, 101.01, 126.27, 127.57, 127.63, 127.70, 127.75, 127.86, 127.89, 127.92, 128.11, 128.31, 128.38, 128.41, 128.88, 137.73, 138.23, 138.26, 138.39, 138.55, 172.81, 173.01.

Exact mass (ESI-MS) for $C_{66}H_{89}N_2O_{10}$ $[M + H]^+$ found, 1069.6512; Calcd., 1069.6517.

*(5R,6S,7S)-5,6-di-O-benzyl-8-O-(2,3-di-O-benzyl-4,6-O-benzylidene-α-D-galactopyranosyl)-7-octanamido-N-(2-phenylethyl)octanamide (**21b**).* ^{1}H-NMR (300 MHz $CDCl_3$) δ 0.89 (t, J = 6.6 Hz, 3 H, terminal CH_3) 1.20–1.33 (m, 7 H, CH_2) 1.44–1.82 (m, 7 H, CH_2) 1.88–1.98 (m, 2 H, CH_2) 2.02–2.07 (m, 2 H, CH_2) 2.78 (t, J = 7.3 Hz, 2 H, CH_2) 3.40–3.54 (m, 4 H, H-4, H-5" and CH_2) 3.75 (dd, J = 7.0 and 2.4 Hz, 1 H, H-3) 3.79–3.94 (m, 3 H, H-1 and

Ha-6") 3.96 (d, $J = 3.3$ Hz, 1 H, H-4") 4.07 (d, $J = 3.3$ Hz, 1 H, H-2") 4.08–4.16 (m, 2 H, H-2 and Hb-6") 4.18 (d, $J = 3.2$ Hz, 1 H, H-3") 4.41 (d, $J = 11.7$ Hz, 1 H, $\underline{CH_2}$Ph) 4.50 (d, $J = 11.7$ Hz, 1 H, $\underline{CH_2}$Ph) 4.57 (d, $J = 11.7$ Hz, 1 H, $\underline{CH_2}$Ph) 4.64 (d, $J = 11.4$ Hz, 1 H, $\underline{CH_2}$Ph) 4.74–4.79 (m, 3 H, $\underline{CH_2}$Ph) 4.86 (d, $J = 11.3$ Hz, 1 H, $\underline{CH_2}$Ph) 4.96 (d, $J = 3.6$ Hz, 1 H, H-1") 5.46 (s, 1 H, H-8") 5.98 (d, $J = 8.5$ Hz, 1 H, NH(CO)) 6.14 (t, $J = 5.6$ Hz, 1 H, (CO)NH) 7.15–7.42 (m, 28 H, arom. H) 7.50–7.54 (m, 2 H, arom. H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 14.09, 21.77, 22.63, 25.67, 28.83, 29.08, 29.32, 31.77, 35.70, 36.00, 36.66, 40.60, 50.03, 62.99, 68.06, 69.35, 71.50, 71.73, 73.31, 74.02, 74.14, 75.64, 76.09, 76.57, 77.20, 77.43, 78.85, 78.88, 99.56, 101.01, 126.27, 126.33, 127.59, 127.64, 127.71, 127.79, 127.85, 127.91, 127.93, 128.11, 128.33, 128.36, 128.39, 128.42, 128.50, 128.71, 128.89, 137.73, 138.24, 138.27, 138.36, 138.54, 139.09, 172.92, 173.08.

Exact mass (ESI-MS) for $C_{65}H_{79}N_2O_{10}$ $[M + H]^+$ found, 1047.5768; Calcd., 1047.5729.

*(5R,6S,7S)-5,6-di-O-benzyl-8-O-(2,3-di-O-benzyl-4,6-O-benzylidene-α-D-galactopyranosyl)-7-octanamido-N-(4-phenylbutyl)octanamide (**21c**).* ^{1}H-NMR (300 MHz $CDCl_3$) δ 0.86 (t, $J = 6.6$ Hz, 3 H, terminal CH_3) 1.17–1.28 (m, 9 H, CH_2) 1.40–1.51 (m, 4 H, CH_2) 1.53–1.81 (m, 7 H, CH_2) 1.82–2.08 (m, 2 H, CH_2) 2.57 (t, $J = 7.5$ Hz, 2 H, CH_2) 3.19 (q, $J = 6.6$ Hz, 2 H, NH$\underline{CH_2}$) 3.43–3.51 (m, 2 H, H-4 and H-5") 3.72 (dd, $J = 6.8$ and 1.6 Hz, 1 H, H-3) 3.80 (d, $J = 4.8$ Hz, 2 H, H-1) 3.87–3.95 (m, 2 H, H-4" and Ha-6") 4.04 (d, $J = 3.2$ Hz, 1 H, H-2") 4.06–4.14 (m, 2 H, H-2 and Hb-6") 4.17 (d, $J = 3.0$ Hz, 1 H, H-3") 4.38 (d, $J = 11.5$ Hz, 1 H, $\underline{CH_2}$Ph) 4.46 (d, $J = 11.5$ Hz, 1 H, $\underline{CH_2}$Ph) 4.54 (d, $J = 11.7$ Hz, 1 H, $\underline{CH_2}$Ph) 4.61 (d, $J = 11.6$ Hz, 1 H, $\underline{CH_2}$Ph) 4.71–4.77 (m, 3 H, $\underline{CH_2}$Ph) 4.84 (d, $J = 11.6$ Hz, 1 H, $\underline{CH_2}$Ph) 4.92 (d, $J = 3.4$ Hz, 1 H, H-1") 5.44 (s, 1 H, H-8") 5.91 (d, $J = 8.5$ Hz, 1 H, NH(CO)) 6.08 (t, $J = 5.4$ Hz, 1 H, (CO)NH) 7.09–7.40 (m, 28 H, arom. H) 7.46–7.52 (m, 2 H, arom. H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 14.08, 21.80, 22.63, 25.65, 28.70, 28.77, 29.07, 29.18, 29.31, 31.76, 35.49, 35.96, 36.65, 39.24, 50.03, 63.00, 68.10, 69.36, 71.51, 71.74, 73.30, 73.99, 74.16, 75.61, 76.10, 77.20, 78.86, 99.64, 101.01, 125.72, 126.27, 127.56, 127.63, 127.72, 127.76, 127.88, 127.89, 127.91, 128.11, 128.27, 128.32, 128.34, 128.39, 128.42, 128.89, 129.96, 137.72, 138.22, 138.24, 138.39, 138.55, 142.18, 172.94, 173.06.

Exact mass (ESI-MS) for $C_{67}H_{83}N_2O_{10}$ $[M + H]^+$ found, 1075.6052; Calcd., 1075.6042.

*(5R,6S,7S)-5,6-di-O-benzyl-8-O-(2,3-di-O-benzyl-4,6-O-benzylidene-α-D-galactopyranosyl)-7-octanamido-N-(6-phenylhexyl)octanamide (**21d**).* ^{1}H-NMR (300 MHz $CDCl_3$) δ 0.86 (t, $J = 6.6$ Hz, 3 H, terminal CH_3) 1.16–1.37 (m, 12 H, CH_2) 1.38–1.83 (m, 10 H, CH_2) 1.87–2.11 (m, 4 H, CH_2) 2.56 (t, $J = 7.5$ Hz, 2 H, CH_2) 3.16 (q, $J = 7.5$ Hz, 2 H, NH$\underline{CH_2}$) 3.44–3.56 (m, 2 H, H-4 and H-5") 3.74 (dd, $J = 6.8$ and 2.4 Hz, 1 H, H-3) 3.82 (d, $J = 4.6$ Hz, 2 H, H-1) 3.88–3.99 (m, 2 H, H-4" and Ha-6") 4.05 (d, $J = 3.3$ Hz, 1 H, H-2") 4.07–4.15 (m, 2 H, H-2 and Hb-6") 4.18 (d, $J = 3.2$ Hz, 1 H, H-3") 4.39 (d, $J = 11.5$ Hz, 1 H, $\underline{CH_2}$Ph) 4.48 (d, $J = 11.4$ Hz, 1 H, $\underline{CH_2}$Ph) 4.55 (d, $J = 11.4$ Hz, 1 H, $\underline{CH_2}$Ph) 4.62 (d, $J = 11.6$ Hz, 1 H, $\underline{CH_2}$Ph) 4.72–4.79 (m, 3 H, $\underline{CH_2}$Ph) 4.84 (d, $J = 11.6$ Hz, 1 H, $\underline{CH_2}$Ph) 4.93 (d, $J = 3.5$ Hz, 1 H, H-1") 5.45 (s, 1 H, H-8") 5.93 (d, $J = 8.4$ Hz, 1 H, NH(CO)) 6.04 (t, $J = 5.7$ Hz, 1 H, (CO)NH) 7.11–7.43 (m, 28 H, arom. H) 7.48–7.52 (m, 2 H, arom. H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 14.08, 21.83, 22.63, 25.65, 26.82, 28.83, 28.94, 29.07, 29.31, 29.53, 31.35, 31.75, 35.85, 36.03, 36.66, 39.42, 50.04, 63.00, 68.14, 69.36, 71.53, 71.75, 73.28, 73.96, 74.19, 75.61, 76.11, 77.21, 78.90, 99.66, 101.01, 125.58, 126.27, 127.55, 127.62, 127.71, 127.74, 127.88, 127.91, 128.11, 128.21, 128.32, 128.34, 128.39, 128.42, 128.89, 137.73, 138.23, 138.25, 138.42, 138.56, 142.64, 172.82, 173.00.

Exact mass (ESI-MS) for $C_{69}H_{86}N_2NaO_{10}$ $[M + Na]^+$ found, 1125.6173; Calcd., 1125.6175.

*(5R,6S,7S)-5,6-di-O-benzyl-8-O-(2,3-di-O-benzyl-4,6-O-benzylidene-α-D-galactopyranosyl)-7-octanamido-N-(8-phenyloctyl)octanamide (**21e**).* ^{1}H-NMR (300 MHz $CDCl_3$) δ 0.87 (t, $J = 6.9$ Hz, 3 H, terminal CH_3) 1.17–1.35 (m, 14 H, CH_2) 1.37–1.84 (m, 12 H, CH_2) 1.87–2.00 (m, 2 H, CH_2) 2.02–2.11 (m, 2 H, CH_2) 2.58 (t, $J = 7.6$ Hz, 2 H, CH_2) 3.16 (dd, $J = 12.8$ and 7.5 Hz, 2 H, NH-CH_2) 3.45–3.51 (m, 1 H, H-4) 3.53 (br. s, 1 H, H-5") 3.75 (dd, $J = 6.8$ and 2.3 Hz, 1 H, H-3) 3.84 (d, $J = 4.9$ Hz, 2 H, H-1) 3.88–3.97 (m, 2 H, H-4" and Ha-6") 4.05 (d, $J = 3.4$ Hz, 1 H, H-2") 4.07–4.17 (m, 2 H, H-2 and Hb-6") 4.19 (d, $J = 2.9$ Hz, 1 H, H-3") 4.42 (d, $J = 11.6$ Hz, 1 H, $\underline{CH_2}$Ph) 4.54 (dd, $J = 22.3$ and 11.4 Hz, 2 H, $\underline{CH_2}$Ph) 4.65 (d, $J = 11.6$ Hz, 1 H, $\underline{CH_2}$Ph) 4.75–4.80 (m, 3 H, $\underline{CH_2}$Ph) 4.87 (d, $J = 11.6$ Hz, 1 H, $\underline{CH_2}$Ph) 4.96 (d, $J = 3.5$ Hz, 1 H, H-1") 5.48 (s, 1 H, H-8") 5.93 (d, $J = 8.5$ Hz, 1 H, NH(CO)) 6.04 (t, $J = 5.6$ Hz, 1 H, (CO)NH) 7.14–7.21 (m, 2 H, arom. H) 7.21–7.42 (m, 26 H, arom. H) 7.49–7.55 (m, 2 H, arom. H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 14.08, 21.85, 22.63, 25.65, 26.96, 28.82, 29.08, 29.25, 29.31, 29.42, 29.60, 31.46, 31.77, 35.94, 36.04, 36.65, 39.46, 50.03, 63.00, 68.07, 69.37, 71.52, 71.73, 73.31, 73.99, 74.18, 75.61, 76.10, 77.20, 78.88, 78.91, 99.61, 101.03, 125.54, 126.28, 127.59, 127.63, 127.71, 127.77, 127.86, 127.91, 128.12, 128.20, 128.32, 128.35, 128.40, 128.43, 128.90, 137.74, 138.24, 138.38, 138.56, 142.82, 172.82, 173.02.

Exact mass (ESI-MS) for $C_{71}H_{90}N_2NaO_{10}$ $[M + Na]^+$ found, 1153.6510; Calcd., 1153.6488.

*(5R,6S,7S)-5,6-di-O-benzyl-8-O-(2,3-di-O-benzyl-4,6-O-benzylidene-α-D-galactopyranosyl)-7-octanamido-N-(3-pentylphenyl)octanamide (**21f**).* ^{1}H-NMR (300 MHz $CDCl_3$) δ 0.83–0.88 (m, 6 H, 2 x terminal CH_3) 1.22–1.33 (m, 12 H, CH_2) 1.47–1.83 (m, 7 H, CH_2) 1.85–2.03 (m, 3 H, CH_2) 2.11–2.25 (m, 2 H, CH_2) 2.54 (t, $J = 7.4$ Hz, 2 H, CH_2) 3.46–3.53 (m, 2 H, H-4 and H-5") 3.73 (dd, $J = 7.7$ and 1.5 Hz, 1 H, H-3) 3.80 (d, $J = 4.9$ Hz, 2 H, H-1) 3.85–3.93 (m, 1 H, Ha-6") 3.95 (d, $J = 3.3$ Hz, 1 H, H-4") 4.05 (d, $J = 3.5$ Hz, 1 H, H-2") 4.07–4.14 (m, 2 H, H-2 and Hb-6") 4.17 (d, $J = 3.3$ Hz, 1 H, H-3") 4.38 (d, $J = 11.6$ Hz, 1 H, $\underline{CH_2}$Ph) 4.48 (d, $J = 11.6$ Hz, 1 H, $\underline{CH_2}$Ph) 4.57 (d, $J = 11.6$ Hz, 1 H, $\underline{CH_2}$Ph) 4.62 (d, $J = 11.5$ Hz, 1 H, $\underline{CH_2}$Ph) 4.74–4.80 (m, 3 H, $\underline{CH_2}$Ph) 4.86 (d, $J = 11.4$ Hz, 1 H, $\underline{CH_2}$Ph) 4.94 (d, $J = 3.6$ Hz, 1 H, H-1") 5.44 (s, 1 H, H-8") 6.00 (d, $J = 8.6$ Hz, 1 H, NH(CO)) 6.86 (d, $J = 7.6$ Hz, 1 H, arom. H) 7.12 (t, $J = 7.9$ Hz, 1 H, arom. H) 7.21–7.41 (m, 24 H, arom. H) 7.44–7.53 (m, 3 H, arom. H) 8.36 (s, 1 H, (CO)NH).

^{13}C NMR (75 MHz, $CDCl_3$) δ 14.03, 21.67, 22.53, 22.63, 25.70, 29.09, 29.33, 31.10, 31.54, 31.78, 36.01, 36.72, 50.00, 63.04, 67.99, 69.32, 71.49, 71.70, 73.26, 74.02, 74.13, 75.59, 76.10, 78.63, 99.55, 101.01, 116.86, 119.69, 123.80, 126.27, 127.56, 127.65, 127.73, 127.78, 127.82, 127.90, 127.93, 128.00, 128.12, 128.34, 128.37, 128.41, 128.45, 128.90, 137.74, 138.10, 138.14, 138.44, 138.56, 143.74, 171.59, 173.24.

Exact mass (ESI-MS) for $C_{68}H_{84}KN_2O_{10}$ $[M+K]^+$ found, 1127.5773; Calcd., 1127.5758.

*(5R,6S,7S)-5,6-di-O-benzyl-8-O-(2,3-di-O-benzyl-4,6-O-benzylidene-α-D-galactopyranosyl)-7-octanamido-N-(4-pentylphenyl)octanamide (**21g**).* ^{1}H-NMR (300 MHz $CDCl_3$) δ 0.86–0.93 (m, 6 H, 2 x terminal CH_3) 1.22–1.38 (m, 12 H, CH_2) 1.50–1.84 (m, 6 H, CH_2) 1.87–2.07 (m, 3 H, CH_2) 2.09–2.28 (m, 2 H, CH_2) 2.54 (t, *J* = 7.1 Hz, 2 H, CH_2) 3.46–3.54 (m, 2 H, H-4 and H-5") 3.75 (dd, *J* = 8.0, 1.2 Hz, 1 H, H-3) 3.79–3.83 (m, 2 H, H-1) 3.89 (dd, *J* = 12.5 and 1.3 Hz, 1 H, Ha-6") 3.95 (dd, *J* = 10.0 and 3.5 Hz, 1 H, H-4") 4.08 (d, *J* = 3.5 Hz, 1 H, H-2") 4.09–4.16 (m, 2 H, H-2 and Hb-6") 4.19 (d, *J* = 2.8 Hz, 1 H, H-3") 4.39 (d, *J* = 11.6 Hz, 1 H, $\underline{CH_2}$Ph) 4.49 (d, *J* = 11.6 Hz, 1 H, $\underline{CH_2}$Ph) 4.59 (d, *J* = 11.6 Hz, 1 H, $\underline{CH_2}$Ph) 4.64 (d, *J* = 11.3 Hz, 1 H, $\underline{CH_2}$Ph) 4.76–4.83 (m, 3 H, $\underline{CH_2}$Ph) 4.88 (d, *J* = 11.3 Hz, 1 H, $\underline{CH_2}$Ph) 4.95 (d, *J* = 3.5 Hz, 1 H, H-1") 5.46 (s, 1 H, H-8") 6.16 (d, *J* = 8.7 Hz, 1 H, NH(CO)) 7.06 (d, *J* = 8.5 Hz, 2 H, arom. H) 7.22–7.37(m, 22 H, arom. H) 7.38–7.46 (m, 3 H, arom. H) 7.49–7.5(m, 2 H, arom. H) 8.49 (s, 1 H, (CO)NH).

^{13}C NMR (75 MHz, $CDCl_3$) δ 14.02, 14.04, 14.08, 21.68, 22.52, 22.58, 22.63, 24.75, 25.69, 28.11, 28.90, 29.03, 29.09, 29.34, 31.23, 31.43, 31.62, 31.78, 33.64, 35.34, 36.51, 36.70, 49.97, 63.01, 67.87, 69.31, 71.50, 71.66, 73.27, 74.07, 74.12, 75.60, 76.07, 77.20, 78.55, 99.40, 101.01, 119.67, 126.27, 127.59, 127.68, 127.71, 127.82, 127.90, 127.94, 127.98, 128.12, 128.35, 128.37, 128.41, 128.44, 128.59, 128.90, 136.28, 137.72, 138.09, 138.14, 138.28, 138.35, 138.53, 171.57, 173.39.

Exact mass (ESI-MS) for $C_{68}H_{85}N_2O_{10}$ $[M+H]^+$ found, 1089.6204; Calcd., 1089.6199.

*(5R,6S,7S)-5,6-di-O-benzyl-8-O-(2,3-di-O-benzyl-4,6-O-benzylidene-α-D-galactopyranosyl)-7-octanamido-N-(2-(2-ethoxyethoxy)ethyl)octanamide (**21 h**).* ^{1}H-NMR (300 MHz $CDCl_3$) δ 0.89 (t, *J* = 6.8 Hz, 3 H, terminal CH_3) 1.18–1.35 (m, 12 H, CH_2 and terminal CH_3) 1.45–1.56 (m, 2 H, CH_2) 1.60–1.88 (m, 3 H, CH_2) 1.89–1.97 (m, 2 H, CH_2) 2.10 (t, *J* = 6.2 Hz, 2 H, CH_2) 3.40–3.48 (m, 2 H, CH_2) 3.50–3.62 (m, 10 H, H-4, H-5" and CH_2) 3.78 (dd, *J* = 6.1 and 2.4 Hz, 1 H, H-3) 3.80–3.99 (m, 4 H, CH_2-1, H-2 and Ha-6") 4.07 (d, *J* = 3.3 Hz, 1 H, H-4") 4.10–4.18 (m, 1 H, Hb-6") 4.19–4.25 (m, 2 H, H-2" and H-3") 4.46 (d, *J* = 11.6 Hz, 1 H, $\underline{CH_2}$Ph) 4.51 (d, *J* = 11.6 Hz, 1 H, $\underline{CH_2}$Ph) 4.59 (d, *J* = 11.6 Hz, 1 H, $\underline{CH_2}$Ph) 4.65 (d, *J* = 11.6 Hz, 1 H, $\underline{CH_2}$Ph) 4.73–4.78 (m, 3 H, $\underline{CH_2}$Ph) 4.87 (d, *J* = 11.4 Hz, 1 H, $\underline{CH_2}$Ph) 4.96 (d, *J* = 3.4 Hz, 1 H, H-1") 5.48 (s, 1 H, H-8") 5.89 (d, *J* = 8.4 Hz, 1 H, NH(CO)) 6.26 (t, *J* = 5.3 Hz, 1 H, (CO)NH) 7.22–7.44 (m, 23 H, arom. H) 7.51–7.55 (m, 2 H, arom. H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 14.08, 15.16, 21.82, 22.63, 25.67, 29.06, 29.16, 29.31, 31.75, 36.13, 36.68, 39.04, 50.12, 62.96, 66.61, 68.11, 69.38, 69.70, 69.81, 70.22, 71.57, 71.78, 73.30, 73.90, 74.23, 75.64, 76.12, 77.20, 78.94, 79.16, 99.58, 101.00, 126.28, 127.56, 127.60, 127.67, 127.70, 127.72, 127.88, 127.90, 128.09, 128.30, 128.33, 128.38, 128.41, 128.85, 137.77, 138.28, 138.30, 138.45, 138.60, 172.91, 173.00.

Exact mass (ESI-MS) for $C_{63}H_{83}N_2O_{12}$ $[M+H]^+$ found, 1059.5945; Calcd., 1059.5941.

General procedure for debenzylation. A solution of the protected amide (0.06 mmol) in $CHCl_3$ (3 mL) and EtOH (9 mL) was hydrogenated under atmospheric pressure in the presence of palladium black (35 mg). Upon reaction completion, the mixture was filtered through celite. The filter cake was rinsed with $CHCl_3$ and EtOH and the filtrate was evaporated to dryness. After purification by silica gel chromatography (10% → 18% MeOH in CH_2Cl_2), final compounds **5a** (43%), **5b** (30%), **5c** (42%), **5d** (56%), **5e** (56%), **5f** (78%), **5g** (71%), **5h** (53%) were obtained as white powders.

*(5R,6S,7S)-5,6-dihydroxy-8-O-(α-D-galactopyranosyl)-7-octanamido-N-nonyloctanamide (**5a**).* ^{1}H-NMR (300 MHz, pyridine-d_5) δ 0.77–0.91 (m, 6 H, 2 x terminal CH_3) 1.08–1.38 (m, 20 H, CH_2) 1.59 (dt, *J* = 14.8 and 7.6 Hz, 2 H, CH_2) 1.77 (dt, *J* = 14.8 and 7.6 Hz, 2 H, CH_2) 1.92–2.04 (m, 1 H, CH_2) 2.17–2.29 (m, 1 H, CH_2) 2.34–2.60 (m, 6 H, CH_2) 3.46 (dd, *J* = 13.0 and 6.8 Hz, 2 H, NH$\underline{CH_2}$) 4.26–4.37 (m, 3 H, Ha-1, H-3 and H-4) 4.37–4.45 (m, 3 H, H-4" and H-6") 4.48 (q, *J* = 6.2 Hz, 1 H, H-3") 4.55 (d, *J* = 2.9 Hz, 1 H, H-5") 4.60–4.69 (m, 2 H, Hb-1 and H-2") 5.19–5.28 (m, 1 H, H-2) 5.56 (d, *J* = 3.8 Hz, 1 H, H-1") 6.36 (br. s, 6 H, OH) 8.30 (t, *J* = 5.2 Hz, 1 H, NH(CO)) 8.46 (d, *J* = 8.6 Hz, 1 H, (CO)NH).

^{13}C NMR (75 MHz, pyridine-d_5) δ 14.60, 14.65, 23.23, 23.28, 23.47, 26.71, 27.78, 29.73, 29.88, 30.01, 30.18, 30.38, 30.66, 32.27, 32.43, 34.38, 37.14, 37.35, 40.11, 51.78, 63.03, 68.98, 70.68, 71.37, 71.96, 72.61, 73.42, 77.19, 101.92, 173.65.

Exact mass (ESI-MS) for $C_{31}H_{61}N_2O_{10}$ $[M+H]^+$ found, 621.4347; Calcd., 621.4326; m.p.: 95–97 °C.

*(5R,6 S,7S)-5,6-dihydroxy-8-O-(α-D-galactopyranosyl)-7-octanamido-N-(2-phenylethyl)octanamide (**5b**).* ^{1}H-NMR (300 MHz, pyridine-d_5) δ 0.80 (t, *J* = 6.2 Hz, 3 H, terminal CH_3) 1.11–1.35 (m, 8 H, CH_2) 1.77 (dt, *J* = 14.6 and 7.3 Hz, 2 H, CH_2) 1.89–2.04 (m, 1 H, CH_2) 2.11–2.26 (m, 1 H, CH_2) 2.31–2.55 (m, 6 H, CH_2) 2.93 (t, *J* = 7.2 Hz, 2 H, $\underline{CH_2}$Ph) 3.68 (q, *J* = 6.8 Hz, 2 H, NH$\underline{CH_2}$) 4.26–4.32 (m, 1 H, H-3) 4.32–4.54 (m, 6 H, H-4, Ha-1, H-4", H-5" and CH_2-6") 4.56 (d, *J* = 2.5 Hz, 1 H, H-3") 4.61–4.69 (m, 2 H, H-2" and Hb-1) 5.20–5.30 (m, 1 H, H-2) 5.57 (d, *J* = 3.4 Hz, 1 H, H-1") 7.24–7.35 (m, 3 H, arom. H) 8.43–8.54 (m, 2 H, arom. H).

^{13}C NMR (75 MHz, pyridine-d_5) δ 29.03, 37.66, 37.83, 41.15, 44.16, 44.44, 46.70, 48.82, 51.21, 51.58, 51.69, 56.18, 66.16, 77.46, 83.33, 85.09, 85.81, 86.38, 87.06, 87.83, 91.60, 116.30, 141.31, 143.62, 144.10, 155.13, 188.15, 188.27.

Exact mass (ESI-MS) for $C_{30}H_{51}N_2O_{10}$ $[M+H]^+$ found, 599.3541; Calcd., 599.3538; m.p. 56–58 °C.

(5R,6S,7S)-5,6-dihydroxy-8-O-(α-D-galactopyranosyl)-7-octanamido-N-(4-phenylbutyl)octanamide (***5c***). ^{1}H-NMR (300 MHz, pyridine-d_5) δ 0.80 (t, J = 6.6 Hz, 3 H, terminal CH_3) 1.07–1.42 (m, 10 H, CH_2) 1.54–1.67 (m, 4 H, CH_2) 1.76 (dt, J = 14.9 and 7.5 Hz, 2 H, CH_2) 1.90–2.04 (m, 1 H, CH_2) 2.12–2.27 (m, 1 H, CH_2) 2.30–2.58 (m, 6 H, CH_2) 3.44 (q, J = 5.8 Hz, 2 H, NH$\underline{CH_2}$) 4.25–4.44 (m, 6 H, Ha-1, H-3, H-4, H-4" and CH_2-6") 4.48 (q, J = 5.7 Hz, 1 H, H-3") 4.55 (d, J = 2.6 Hz, 1 H, H-5") 4.60–4.68 (m, 2 H, Hb-1 and H-2") 5.19–5.28 (m, 1 H, H-2) 5.56 (d, J = 3.8 Hz, 1 H, H-1") 6.47 (br. s, 6 H, OH) 7.17–7.25 (m, 3 H, arom. H) 7.28–7.35 (m, 2 H, arom. H) 8.28 (t, J = 5.3 Hz, 1 H, (CO)NH) 8.46 (d, J = 8.6 Hz, 1 H, NH(CO)).

^{13}C NMR (75 MHz, pyridine-d_5) δ 14.60, 23.23, 23.46, 26.72, 29.58, 29.72, 30.01, 30.21, 30.39, 32.27, 34.41, 36.07, 37.14, 37.33, 39.79, 51.78, 63.04, 68.98, 70.68, 71.38, 71.97, 72.62, 73.43, 77.21, 101.92, 126.46, 129.09, 129.23, 136.58, 143.22, 173.66, 173.67.

Exact mass (ESI-MS) for $C_{32}H_{55}N_2O_{10}$ $[M+H]^+$ found, 627.3860; Calcd., 627.3851; m.p. 57–59 °C.

(5R,6S,7S)-5,6-dihydroxy-8-O-(α-D-galactopyranosyl)-7-octanamido-N-(6-phenylhexyl)octanamide(***5d***). ^{1}H-NMR (300 MHz, pyridine-d_5) δ 0.77–0.87 (m, 3 H, terminal CH_3) 1.09–1.43 (m, 13 H, CH_2) 1.54 (dt, J = 12.6 and 6.3 Hz, 3 H, CH_2) 1.77 (dt, J = 14.4 and 7.1 Hz, 2 H, CH_2) 1.91–2.05 (m, 1 H, CH_2) 2.16–2.31 (m, 1 H, CH_2) 2.33–2.60 (m, 6 H, CH_2) 3.03 (q, J = 7.0 Hz, 2 H, CH_2) 3.43 (d, J = 5.8 Hz, 2 H, NH$\underline{CH_2}$) 4.27–4.46 (m, 6 H, Ha-1, H-3, H-4, H-4" and CH_2-6") 4.46–4.53 (m, 1 H, H-5") 4.56 (app. s, 1 H, H-3") 4.60–4.69 (m, 2 H, Hb-1 and H-2") 5.19–5.29 (m, 1 H, H-2) 5.56 (d, J = 2.4 Hz, 1 H, H-1") 6.40 (br. s, 6 H, OH) 7.19–7.29 (m, 3 H, arom. H) 7.30–7.39 (m, 2 H, arom. H) 8.29 (t, J = 5.7, 1 H, (CO)NH) 8.49 (d, J = 8.2 Hz, 1 H, NH(CO)).

^{13}C NMR (75 MHz, pyridine-d_5) δ 8.91, 14.59, 23.22, 23.45, 26.70, 27.58, 29.58, 29.71, 29.99, 30.35, 30.53, 32.08, 32.26, 34.37, 36.40, 37.13, 37.33, 40.05, 46.18, 51.78, 63.00, 68.92, 70.68, 71.34, 71.95, 72.56, 73.39, 77.17, 101.89, 126.41, 129.07, 129.21, 143.51, 173.66, 173.68.

Exact mass (ESI-MS) for $C_{34}H_{59}N_2O_{10}$ $[M+H]^+$ found, 655.4161; Calcd., 655.4164; m.p. 135–137 °C.

(5R,6S,7S)-5,6-dihydroxy-8-O-(α-D-galactopyranosyl)-7-octanamido-N-(8-phenyloctyl)octanamide (***5e***). ^{1}H-NMR (300 MHz, pyridine-d_5) δ 0.80 (t, J = 6.3 Hz, 3 H, terminal CH_3) 1.09–1.36 (m, 16 H, CH_2) 1.47–1.64 (m, 4 H, CH_2) 1.69–1.83 (m, 2 H, CH_2) 1.91–2.05 (m, 1 H, CH_2) 2.14–2.30 (m, 1 H, CH_2) 2.34–2.60 (m, 8 H, CH_2) 3.45 (dd, J = 13.1 and 6.8 Hz, 2 H, NH$\underline{CH_2}$) 4.26–4.45 (m, 6 H, Ha-1, H-3, H-4, H-4" and CH_2-6") 4.49 (q, J = 6.0 Hz, 1 H, H-5") 4.56 (d, J = 3.0 Hz, 1 H, H-3") 4.61–4.69 (m, 2 H, Hb-1 and H-2") 5.19–5.29 (m, 1 H, H-2) 5.57 (d, J = 3.5 Hz, 1 H, H-1") 6.56 (br. s, 6 H, OH) 7.21–7.29 (m, 3 H, arom. H) 7.33–7.40 (m, 2 H, arom. H) 8.30 (t, J = 5.4, 1 H, (CO)NH) 8.48 (d, J = 8.5 Hz, 1 H, NH(CO)).

^{13}C NMR (75 MHz, pyridine-d_5) δ 8.91, 14.58, 23.21, 23.45, 26.69, 27.73, 29.71, 29.80, 29.89, 29.99, 30.03, 30.61, 32.15, 32.26, 34.37, 36.47, 37.12, 37.33, 40.08, 46.18, 51.78, 63.00, 68.93, 70.67, 71.34, 71.95, 72.58, 73.40, 77.17, 101.88, 126.42, 129.09, 129.24, 143.59, 173.66.

Exact mass (ESI-MS) for $C_{36}H_{63}N_2O_{10}$ $[M+H]^+$ found, 683.4477; Calcd., 683.4477; m.p. 128–130 °C.

(5R,6S,7S)-5,6-dihydroxy-8-O-(α-D-galactopyranosyl)-7-octanamido-N-(3-pentylphenyl)octanamide (***5f***). ^{1}H-NMR (300 MHz, pyridine-d_5) δ 0.81 (t, J = 6.5 Hz, 6 H, 2 x terminal CH_3) 1.11–1.34 (m, 14 H, CH_2) 1.55 (dt, J = 14.8 and 7.4 Hz, 2 H, CH_2) 1.77 (dt, J = 14.8 and 7.4 Hz, 2 H, CH_2) 1.90–2.03 (m, 1 H, CH_2) 2.17–2.31 (m, 1 H, CH_2) 2.41 (t, J = 7.6 Hz, 2 H, CH_2) 2.55 (t, J = 7.6 Hz, 2 H, CH_2) 2.68 (dd, J = 14.2 and 7.0 Hz, 2 H, CH_2) 4.24–4.31 (m, 1 H, H-3) 4.31–4.45 (m, 5 H, H-4, Ha-1, H-4" and H-6") 4.48 (q, J = 5.4 Hz, 1 H, H-3") 4.55 (d, J = 2.4 Hz, 1 H, H-5") 4.60–4.69 (m, 2 H, H-2" and Hb-1) 5.20–5.29 (m, 1 H, H-2) 5.57 (d, J = 3.4 Hz, 1 H, H-1") 6.48 (br. s., 6 H, OH) 6.99 (d, J = 7.5 Hz, 1 H, arom. H) 7.32 (t, J = 7.7 Hz, 1 H, arom. H) 7.88 (d, J = 8.0 Hz, 1 H, arom. H) 7.98 (s, 1 H, arom. H) 8.47 (d, J = 8.7 Hz, 1 H, NH(CO)) 10.60 (s, 1 H, (CO)NH).

^{13}C NMR (75 MHz, pyridine-d_5) δ 14.51, 14.57, 23.10, 23.21, 23.41, 26.70, 29.70, 29.98, 31.76, 31.98, 32.25, 34.30, 36.57, 37.11, 38.21, 51.65, 63.02, 68.80, 70.63, 71.37, 71.93, 72.61, 73.40, 77.15, 101.82, 117.89, 120.54, 124.09, 129.41, 141.00, 144.22, 172.70, 173.69.

Exact mass (ESI-MS) for $C_{33}H_{57}N_2O_{10}$ $[M+H]^+$ found, 641.4001; Calcd., 641.4008; m.p. 165–167 °C.

(5R,6S,7S)-5,6-dihydroxy-8-O-(α-D-galactopyranosyl)-7-octanamido-N-(4-pentylphenyl)octanamide (***5g***). ^{1}H-NMR (300 MHz, pyridine-d_5) δ 0.81 (dt, J = 9.1 and 7.0 Hz, 6 H, 2 x terminal CH_3) 1.12–1.34 (m, 14 H, CH_2) 1.55 (dt, J = 14.8 and 7.4 Hz, 2 H, CH_2) 1.77 (dt, J = 14.9 and 7.5 Hz, 2 H, CH_2) 1.89–2.05 (m, 1 H, CH_2) 2.17–2.33 (m, 1 H, CH_2) 2.42 (t, J = 7.8 Hz, 2 H, CH_2) 2.53 (t, J = 7.8 Hz, 2 H, CH_2) 2.61–2.71 (m, 2 H, CH_2) 4.24–4.45 (m, 6 H, H-3, H-4, H-4", H-6" and Ha-1) 4.49 (q, J = 5.9 Hz, 1 H, H-3") 4.56 (d, J = 2.9 Hz, 1 H, H-5") 4.61–4.69 (m, 2 H, Hb-1 and H-2") 5.20–5.29 (m, 1 H, H-2) 5.57 (d, J = 3.7 Hz, 1 H, H-1") 6.41 (br. s, 6 H, OH) 7.23 (app. s, 2 H, arom. H) 8.01 (d, J = 8.4 Hz, 2 H, arom. H) 8.48 (d, J = 8.7 Hz, 1 H, NH(CO)) 10.63 (s, 1 H, (CO)NH).

^{13}C NMR (75 MHz, pyridine-d_5) δ 14.55, 14.58, 23.14, 23.22, 23.42, 26.70, 29.71, 29.99, 31.95, 32.26, 34.31, 35.86, 37.13, 38.14, 51.66, 63.04, 68.81, 70.66, 71.38, 71.95, 72.62, 73.42, 77.18, 101.85, 120.56, 129.43, 138.26, 138.75, 172.58, 173.68.

Exact mass (ESI-MS) for $C_{33}H_{57}N_2O_{10}$ $[M+H]^+$ found, 641.4011; Calcd., 641.4008; m.p. 147–149 °C.

(5R,6S,7S)-5,6-dihydroxy-8-O-(α-D-galactopyranosyl)-7-octanamido-N-(2-(2-ethoxyethoxy)ethyl)octanamide (***5h***). ^{1}H-NMR (300 MHz, pyridine-d_5) δ 0.80 (t, J = 6.5 Hz, 3 H, terminal CH_3) 1.08–1.22 (m, 8 H, terminal CH_3 and CH_2) 1.23–1.41 (m, 5 H, CH_2) 1.76 (dt, J = 14.8 and 7.4 Hz, 2 H, CH_2) 1.87–2.01 (m, 1 H, CH_2) 2.11–2.24 (m, 1 H, CH_2) 2.31–2.47 (m, 4 H, CH_2) 2.47–2.58 (m, 2 H, CH_2) 3.41 (q, J = 7.0 Hz, 2 H, CH_2) 3.49–3.54 (m, 2 H, CH_2) 3.56–3.61 (m, 2 H, CH_2) 3.62–3.70 (m, 2 H, CH_2) 4.22–4.44 (m, 6 H, Ha-1, H-3, H-4, H-4" and CH_2-6") 4.48 (q, J = 5.8 Hz, 1 H, H-5") 4.55 (d, J = 2.7 Hz, 1 H, H-3") 4.60–4.69 (m, 2 H, Hb-1 an H-2"d) 5.19–5.29 (m, 1 H, H-2) 5.56 (d, J = 3.7 Hz, 1 H, H-1") 6.43 (br. s, 6 H, OH) 8.43 (d, J = 8.4 Hz, 1 H, NH(CO)) 8.47–8.52 (m, 1 H, (CO)NH).

^{13}C NMR (75 MHz, pyridine-d_5) δ 14.59, 15.84, 23.23, 23.39, 26.71, 29.72, 30.00, 30.37, 32.27, 34.46, 37.14, 37.21, 40.14, 51.74, 63.03, 66.84, 68.93, 70.50, 70.67, 70.85, 71.01, 71.38, 71.96, 72.64, 73.42, 77.21, 101.90, 173.64, 173.86.

Exact mass (ESI-MS) for $C_{28}H_{55}N_2O_{12}$ $[M+H]^+$ found, 611.3750; Calcd., 611.3750; m.p. could not be determined due to hygroscopy.

(5R,6S,7S)-5,6-dihydroxy-8-O-(α-D-galactopyranosyl)-7-undecanamino-N-(6-phenylhexyl)octanamide (***6d***). ^{1}H-NMR (300 MHz, pyridine-d_5) δ 0.87 (t, J = 6.6 Hz, 3 H, terminal CH_3) 1.13–1.41 (m, 20 H, CH_2) 1.45–1.61 (m, 4 H, CH_2) 1.73–1.87 (m, 2 H, CH_2) 1.91–2.05 (m, 1 H, CH_2) 2.18–2.60 (m, 9 H, CH_2) 3.43 (q, J = 6.8 Hz, 2 H, NH$\underline{CH_2}$) 4.26–4.53 (m, 7 H, Ha-1, H-3, H-4, H-4", H-5" and CH_2-6") 4.56 (d, J = 3.1 Hz, 1 H, H-3") 4.61–4.71 (m, 2 H, Hb-1 and H-2") 5.21–5.31 (m, 1 H, H-2) 5.57 (d, J = 3.8 Hz, 1 H, H-1") 5.96 (br. s, 6 H, OH) 7.17–7.27 (m, 3 H, arom. H) 7.31–7.38 (m, 2 H, arom. H) 8.27 (t, J = 5.8 Hz, 1 H, (CO)NH) 8.48 (d, J = 8.7 Hz, 1 H, NH(CO)).

^{13}C NMR (75 MHz, pyridine-d_5) δ 14.69, 23.33, 23.47, 26.77, 27.59, 29.62, 29.99, 30.14, 30.20, 30.26, 30.32, 30.55, 32.11, 32.51, 34.41, 36.43, 37.18, 37.34, 40.08, 51.77, 63.05, 68.96, 70.70, 71.40, 71.98, 72.62, 73.45, 77.22, 101.93, 126.45, 129.10, 129.24, 143.53, 173.68, 173.72.

Exact mass (ESI-MS) for $C_{38}H_{67}N_2O_{10}$ $[M+H]^+$ found, 711.4781; Calcd., 711.4790; m.p. 91–93 °C.

(5R,6S,7S)-5,6-dihydroxy-8-O-(α-D-galactopyranosyl)-7-pentadecanamino-N-(6-phenylhexyl)octanamide (***7d***). ^{1}H-NMR (300 MHz, pyridine-d_5) δ 0.88 (t, J = 6.7 Hz, 3 H, terminal CH_3) 1.16–1.41 (m, 28 H, CH_2) 1.45–1.61 (m, 4 H, CH_2) 1.74–1.87 (m, 2 H, CH_2) 1.91–2.04 (m, 1 H, CH_2) 2.16–2.60 (m, 9 H, CH_2) 3.43 (q, J = 6.7 Hz, 2 H, NH$\underline{CH_2}$) 4.26–4.53 (m, 7 H, Ha-1, H-3, H-4, H-4", H-5" and CH_2-6") 4.56 (d, J = 3.2 Hz, 1 H, H-3") 4.61–4.71 (m, 2 H, Hb-1 and H-2") 5.21–5.30 (m, 1 H, H-2) 5.57 (d, J = 3.9 Hz, 1 H, H-1") 5.93 (br. s, 6 H, OH) 7.19–7.30 (m, 3 H, arom. H) 7.30–7.38 (m, 2 H, arom. H) 8.27 (t, J = 5.6 Hz, 1 H, (CO)NH) 8.47 (d, J = 8.6 Hz, 1 H, NH(CO)).

^{13}C NMR (75 MHz, pyridine-d_5) δ 14.69, 23.35, 23.47, 26.77, 27.59, 29.60, 30.02, 30.15, 30.18, 30.25, 30.34, 30.40, 30.57, 32.11, 32.52, 34.43, 36.43, 37.18, 37.34, 40.06, 51.77, 63.07, 68.99, 70.70, 71.40, 71.98, 72.64, 73.46, 77.25, 101.95, 126.43, 129.10, 129.24, 143.53, 173.61, 173.68.

Exact mass (ESI-MS) for $C_{42}H_{75}N_2O_{10}$ $[M+H]^+$ found, 767.5412; Calcd., 767.5416; m.p. 148–150 °C.

(5R,6S,7S)-5,6-dihydroxy-8-O-(α-D-galactopyranosyl)-7-nonadecanamino-N-(6-phenylhexyl)octanamide (***8d***). ^{1}H-NMR (300 MHz, pyridine-d_5) δ 0.88 (t, J = 6.6 Hz, 3 H, terminal CH_3) 1.14–1.40 (m, 36 H, CH_2) 1.45–1.61 (m, 4 H, CH_2) 1.70–1.88 (m, 2 H, CH_2) 1.90–2.05 (m, 1 H, CH_2) 2.18–2.58 (m, 9 H, CH_2) 3.44 (q, J = 6.7 Hz, 2 H, NH$\underline{CH_2}$) 4.23–4.54 (m, 7 H, Ha-1, H-3, H-4, H-4", H-5" and CH_2-6") 4.57 (d, J = 3.1 Hz, 1 H, H-3") 4.61–4.72 (m, 2 H, Hb-1 and H-2") 5.21–5.31 (m, 1 H, H-2) 5.57 (d, J = 3.8 Hz, 1 H, H-1") 5.83 (br. s, 6 H, OH) 7.20–7.27 (m, 3 H, arom. H) 7.31–7.38 (m, 2 H, arom. H) 8.28 (t, J = 5.6 Hz, 1 H, (CO)NH) 8.49 (d, J = 9.0 Hz, 1 H, NH(CO)).

^{13}C NMR (75 MHz, pyridine-d_5) δ 14.69, 23.35, 23.47, 26.78, 27.61, 29.62, 30.02, 30.20, 30.26, 30.32, 30.43, 30.55, 32.11, 32.52, 34.41, 36.43, 37.19, 37.34, 40.08, 51.77, 63.07, 68.96, 70.68, 71.40, 71.97, 72.64, 73.45, 77.22, 101.93, 126.43, 129.10, 129.24, 143.53, 173.66, 173.71.

Exact mass (ESI-MS) for $C_{46}H_{83}N_2O_{10}$ $[M+H]^+$ found, 823.6036; Calcd., 823.6042; m.p. 136–138 °C.

(5R,6S,7S)-5,6-dihydroxy-8-O-(α-D-galactopyranosyl)-7-hexacosylamino-N-(6-phenylhexyl)octanamide (***9d***). ^{1}H-NMR (300 MHz, pyridine-d_5) δ 0.88 (t, J = 6.4 Hz, 3 H, terminal CH_3) 1.11–1.43 (m, 42 H, CH_2) 1.43–1.62 (m, 6 H, CH_2) 1.81 (quint, J = 7.3 Hz, 2 H, CH_2) 1.91–2.07 (m, 2 H, CH_2) 2.12–2.30 (m, 2 H, CH_2) 2.34–2.61 (m, 8 H, CH_2) 3.03 (q, J = 7.4 Hz, 2 H, CH_2) 3.43 (q, J = 6.6 Hz, 2 H, NH$\underline{CH_2}$) 4.26–4.45 (m, 6 H, Ha-1, H-3, H-4, H-4" and CH_2-6") 4.49 (q, J = 5.9 Hz, 1 H, H-5") 4.56 (d, J = 2.9 Hz, 1 H, H-3") 4.60–4.70 (m, 2 H, Hb-1 and H-2") 5.19–5.30 (m, 1 H, H-2) 5.57 (d, J = 3.8 Hz, 1 H, H-1") 6.33 (br. s, 6 H, OH) 7.17–7.27 (m, 3 H, arom. H) 7.30–7.39 (m, 2 H, arom. H) 8.29 (t, J = 5.4 Hz, 1 H, (CO)NH) 8.49 (d, J = 8.5 Hz, 1 H, NH(CO)).

^{13}C NMR (75 MHz, pyridine-d_5) δ 8.89, 14.66, 23.32, 23.45, 26.75, 27.57, 29.59, 29.99, 30.14, 30.17, 30.25, 30.29, 30.37, 30.41, 30.52, 32.08, 32.49, 34.37, 36.40, 37.15, 37.31, 40.05, 46.15, 51.78, 63.00, 68.91, 70.67, 71.32, 71.95, 72.55, 73.40, 77.17, 101.87, 126.40, 129.07, 129.21, 143.50, 173.66, 173.69.

Exact mass (ESI-MS) for $C_{52}H_{95}N_2O_{10}$ $[M+H]^+$ found, 907.6976; Calcd., 907.6981; m.p. 116–118 °C.

(5R,6S,7S)-5,6-dihydroxy-8-O-(α-D-galactopyranosyl)-7-hexacosylamino-N-(3-pentylphenyl)octanamide (***9f***). ^{1}H-NMR (300 MHz, pyridine-d_5) δ 0.80 (t, J = 6.9 Hz, 3 H, terminal CH_3) 0.88 (t, J = 6.5 Hz, 3 H, terminal CH_3) 1.14–1.47 (m, 48 H, CH_2) 1.47–1.64 (m, 2 H, CH_2) 1.74–1.89 (m, 2 H, CH_2) 1.89–2.16 (m, 1 H, CH_2) 2.16–2.33 (m, 1 H, CH_2) 2.33–2.51 (m, 4 H, CH_2) 2.51–2.60 (m, 2 H, CH_2) 2.60–2.74 (m, 2 H, CH_2) 4.23–4.46 (m, 6 H, Ha-1, H-3", H-4, H-4" and CH_2-6") 4.46–4.53 (m, 1 H, H-3) 4.56 (br. s, 1 H, H-5") 4.60–4.71 (m, 2 H, Hb-1 and H-2") 5.20–5.31 (m, 1 H, H-2) 5.57 (d, J = 3.8 Hz, 1 H, H-1") 6.27 (d, J = 6.1 Hz, 1 H, OH) 6.32 (d, J = 3.7 Hz, 1 H, OH) 6.46–6.58 (m, 2 H, OH) 6.72 (br. s, 1 H, OH) 6.97–7.08 (m, 2 H, arom. H and OH) 7.33 (t, J = 7.8 Hz, 1 H, arom. H) 7.88 (d, J = 7.9 Hz, 1 H, arom. H) 7.97 (s, 1 H, arom. H) 8.46 (d, J = 8.5 Hz, 1 H, NH(CO)) 10.59 (s, 1 H, (CO)NH).

^{13}C NMR (75 MHz, pyridine-d_5) δ 14.54, 14.69, 23.13, 23.35, 23.44, 26.78, 30.02, 30.17, 30.20, 30.26, 30.32, 30.40, 30.44, 31.80, 32.02, 32.52, 34.35, 36.61, 37.18, 38.24, 51.68, 63.07, 68.87, 70.68, 71.42, 71.98, 72.65, 73.46, 77.22, 101.88, 117.91, 120.55, 124.11, 129.45, 141.05, 144.26, 172.71, 173.68.

Exact mass (ESI-MS) for $C_{51}H_{93}N_2O_{10}$ $[M+H]^+$ found, 893.6830; Calcd., 893.6825; m.p. 127–129 °C.

*N-((2S,3S,4R)-1-O-(α-D-galactopyranosyl)-3,4-dihydroxy-16-phenylhexadecan-2-yl)octanamide(**23**).* Compound **23** was synthesized in an analogous way as described in scheme 2 and 3. A Wittig olefination between **14** and triphenyl(10-decylphenyl)phosphonium bromide was followed by selective reduction of

Data collection statistics	CD1d-23	CD1d-5d
Space group	P212121	P212121
Cell dimension		
a, *b*, *c*, (Å)	42.2, 106.2, 106.9	42.2, 107.5, 109.6
a, b, g (°)	90, 90, 90	90, 90, 90
Resolution range (Å) [outer shell]	45–1.85 [1.92–1.85]	40–1.75 [1.81–1.75]
No. reflections	40,100	51,068
R_{meas} (%)	13.3 [97.9]	6.9 [58.1]
R_{pim} (%)	5.5 [41.3]	3.3 [27.5]
Multiplicity	5.4 [5.2]	4.3 [4.3]
Average I/sI	17.1 [2.5]	35.8 [3.1]
Completeness (%)	95.2 [98.6]	99.7 [100.0]
Refinement statistics		
No. atoms	3,435	3,385
Protein	2,992	2,929
Ligand (spacer/antigen)	18/46	18/46
Carbohydrate	56	52
Water	323	340
R/R_{free} (%)	21.4/24.3	20.3/22.1
Ramachandran plot (%)		
Favored	97.5	98.3
Allowed	100	100
R.m.s. deviations		
Bonds (Å)	0.008	0.008
Angles (°)	1.37	1.33
B-factors (Å^2)		
Protein	24.4	31.8
Spacer/Lipid	40.8/29.4	52.5/60.7
Carbohydrate	44.7	46.8
Water	31.0	39.7

Table 2. Data collection and refinement statistics of crystal structures of the CD1d-**23** and CD1d-**5d** complexes.

the double bond. The azide moiety on C2 was installed using a Mitsunobu inversion with hydrazoic acid (HN_3). Next, the primary hydroxyl group was selectively deprotected to give the corresponding acceptor, which was glycosylated with trichloroacetimidate donor **11**. Staudinger reduction of the azide, followed by amide formation with octanoic acid, yielded, after overall deprotection, compound **23**.

^{1}H-NMR (300 MHz, pyridine-d_5) δ 0.81 (t, J = 6.3 Hz, 3 H, terminal CH_3) 1.11–2.01 (m, 32 H, CH_2) 2.44 (t, J = 7.6 Hz, 2 H, CH_2) 2.60 (t, J = 7.6 Hz, 2 H, CH_2) 4.31–4.35 (m, 2 H, H-2" and H3) 4.38–4.46 (m, 3 H, Hb1 and CH_2-6") 4.47 (m, 1 H, H-4) 4.53 (m, 1 H, H-4") 4.57 (m, 1 H, H-5") 4.63–4.72 (m, 2 H, Ha1 and H-3") 5.24–5.34 (m, 1 H, H-2) 5.60 (d, J = 3.7 Hz, 1 H, H-1") 5.94 (br. s, 6 H, OH) 7.22–7.29 (m, 3 H, arom. H) 7.31–7.39 (m, 2 H, arom. H) 8.49 (d, J = 8.6 Hz, 1 H, NH).

^{13}C NMR (75 MHz, pyridine-d_5) δ 14.23, 22.89, 26.37, 26.50, 29.38, 29.56, 29.66, 29.82, 29.98, 30.01, 30.14, 30.38, 31.89, 34.42, 36.11, 36.77, 51.64, 62.68, 68.65, 70.30, 71.03, 71.67, 71.71, 72.50, 73.05, 76.72, 101.45, 128.73, 128.88.

Recombinant proteins, SPR studies and crystallography. Soluble mCD1-β2 M protein was expressed and purified from insect cells as described[41]. Glycolipid loading and SPR studies were performed as previously reported[27]. In brief, increasing concentrations of TCR (two-fold dilutions from 500 nM to 15.625 nM) were passed over streptavidin immobilized *CD1d* using a CAP chip on a Biacore T200. A reference substraction included the TCR binding response to CD1d to which no lipid has been added. In addition, single cycle kinetics were carried out with 3-fold dilutions of TCR (900nM-11nM), without reference substraction. Running buffer contained no detergent in an attempt to not extract these short-acyl chain ligands during the course of the experiment. CD1d-ligand-TCR complex formation, purification, crystallization and structure determination was performed as reported previously[19,25]. Data collection and refinement statistics are shown in Table 2. Structure of mCD1d-**5d** and mCD1d-**23** have been deposited in the Protein Data Bank (http://www.rcsb.org/) under accession codes 5TW2 and 5TW5, respectively.

In vivo cytokine secretion assay. Experiments were approved by and conducted according to the guidelines of the Ethical Committee of Laboratory Animal Welfare of Ghent University.

The different glycolipids were dissolved in DMSO at a concentration of 1 mg/mL, heated for 20 minutes at 80 °C and subsequently sonicated for 10 minutes at the same temperature. Next, 11 µL of the prepared solution

was diluted with PBS in order to obtain a 10 μg/mL solution and the resulting solution was again warmed to 80 °C for 20 minutes followed by sonication for 10 minutes at this temperature. Then, 500 μL (5 μg) was injected i.p. in 8 different C57BL/6 mice and blood was collected 4 h (IL-4) and 16 h (IFN-γ) post injection. The levels of IL-4 and INF-γ were determined by ELISA (eBioscience). KRN7000 was employed as control.

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Author Contributions

J.G., J.J., M.R. conceived and designed the synthetic experiments. J.G. and M.R. carried out the chemical synthesis. J.W. and D.M.Z. conceived and designed the structural chemistry experiments. S.G.R. carried out the crystallography experiments. T.D. carried out the *in vivo* cytokine determination under the supervision of D.E., M.F. performed the modeling studies. J.G., D.Z. and S.V.C wrote the manuscript.

Additional Information

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