UNIVERSITEIT GENT

FACULTY OF PHARMACEUTICAL SCIENCES

Synthesis of new α-GalCer analogues as iNKT cell targeting agents

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Thesis submitted in fulfilment of the requirements for the degree of Doctor in Pharmaceutical Sciences
Proefschrift voorgelegd tot het bekomen van de graad van Doctor in de Farmaceutische Wetenschappen

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Academic year 2013-2014

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Summary

α-Galactosylceramide (α-GalCer), a structurally optimized glycosphingolipid derived from extracts of the marine sponge *Agelas mauritianus*, is nowadays known as the prototypical ligand for invariant natural killer T (iNKT) cells. Following complexation with the antigen presenting glycoprotein CD1d, α-GalCer is recognized by the T cell receptor (TCR) of iNKT cells, resulting in the rapid release of both T helper 1 (Th1) and T helper 2 (Th2) cytokines. Th1 cytokines, such as IFN-γ, are involved in antitumor, antiviral and antibacterial responses, while Th2 cytokines, like IL-4, can ameliorate autoimmune diseases. Unfortunately, both types of cytokines tend to antagonize each other's biological effect, limiting the clinical effectiveness of α-GalCer. Hence, analogues capable of inducing a Th1/Th2 biased response are highly awaited. Towards this end, numerous modifications of all parts of α-GalCer, i.e. the galactose, the phytosphingosine and the acyl moieties, have already been investigated. An overview is given in Chapter 1.

S.A.R. studies revealed a wide variety of possible alterations in the ceramide part resulting in a shifted cytokine profile. By contrast, successful modifications of the galactose moiety are rather scarce and chiefly limited to the 6"-position. Encouraged by the Th1 biased response of certain C-6"-derivatised analogues, designed earlier in our laboratory, we decided to further explore modifications at this position. A divergent synthetic approach was developed that provides access to both 6"-*O*- and 6"-*N*-altered as well as 5"-modified α-GalCer analogues, and that allows late-stage introduction of alternative acyl chains. The elaboration of our methodology is described in Chapter 3, together with its application for the synthesis of two known Th1-featuring compounds, α-D-fucopyranosylceramide and α-D-galacturonosyl-ceramide, as well as two C6Ph acyl variants.

Chapter 4 describes the introduction of several new C-5" and C-6"-modifications, starting from a common key intermediate. A first group consists of seven aromatic-substituted uronamides and three C-6"-carbamates. With one exception, *in vivo* activation of mice iNKT cells by these compounds results in a Th1 polarization. The strongest Th1 antigens are found in the class of C-6"-carbamates, which also contains a Th2 polarizer. These C-6"-carbamates exhibit significantly enhanced antimetastatic effects in a B16 melanoma mouse model. Compared to α-GalCer, they were shown to interact much longer with CD1d. For two compounds this is reflected by the formation of more intimate contacts with CD1d. Strikingly, these additional contacts were not found for the strongest Th1 polarizer that, by contrast,

showed a novel interaction with the TCR. Hence, a clear relationship with the observed iNKT cell responses could not be established.

Secondly, a series of six α-GalCer analogues with differently 4-substituted 1,2,3-triazol-1-yl moieties connected to the 6”-position were synthesized. Both the phenyl-substituted derivatives as well as the butyl- and non-substituted compounds were able to stimulate iNKT cells better than α-GalCer in an *in vitro* mouse setting. We inferred that this increase in antigenic activity is mainly due to the triazole functionalization.

Additionally, a Th1-featuring C-5” and C-6”-modification was combined with an OCH ceramide moiety. Biological evaluation of these analogues demonstrated a reduced IFN-γ potential compared to their parent C-5”/C-6”-modified compounds.

The synthetic route was also utilized for the preparation of two C-6”-labeled α-GalCer derivatives, to be used in competition binding experiments.

Finally, Chapter 5 is dedicated to the synthesis of a potential iNKT cell antagonist that might be useful in the treatment of allergic inflammatory diseases, such as asthma, and other disorders characterized by inappropriate or excessive iNKT cell activation. Several attempts were needed to acquire the envisioned C-6”-naphthylurea α-mannosylceramide. The eventual succesful synthetic route also allowed late-stage conversion to the corresponding α-glucosyl derivative. Unfortunately, the preliminary biological experiments with NU-α-ManCer failed to show any antagonistic potential.

Samenvatting

α-Galactosylceramide (α-GalCer) is een structureel geoptimaliseerd glycosfingolipide, gebaseerd op extracten van de zeespons *Agelas mauritianus*, en wordt beschouwd als het prototype ligand voor de invariant natural killer T (iNKT) cellen. Na complexatie met het antigen-presenterend glycoproteïne CD1d wordt α-GalCer herkend door de T cel receptor (TCR) van deze iNKT cellen, die vervolgens gestimuleerd worden tot de productie van zowel T helper 1 (Th1) als T helper (Th2) cytokines. Th1 cytokines, zoals IFN-γ, spelen een rol in antitumorale, antivirale en antibacteriële responsen. Th2 cytokines daarentegen, zoals IL-4, kunnen auto-immuunziekten tegengaan. Helaas werken beide types cytokines elkaars biologisch effect tegen waardoor de klinische effectiviteit van α-GalCer beperkt is. Bijgevolg zijn analogen die de iNKT cel respons kunnen sturen naar een Th1 of Th2 richting dan ook sterk gewenst. Hiervoor werden reeds verschillende wijzigingen aangebracht aan de drie structurele onderdelen van α-GalCer, met name het galactosegedeelte, de fytosfingosineketen en de acylstaart. Een overzicht hiervan wordt weergegeven in Hoofdstuk 1.

Studies naar de structuur-activiteits-relatie (S.A.R.) hebben aangetoond dat het ceramidegedeelte op verschillende plaatsten kan veranderd worden teneinde een gepolariseerd cytokineprofiel te bekomen. Succesvolle wijzigingen aan het galactosegedeelte daarentegen zijn eerder schaars en voornamelijk beperkt tot de 6”-positie. Geïnspireerd door de Th1 polarizerende eigenschappen van bepaalde C-6”-derivaten die eerder in ons laboratorium gesynthetiseerd werden, hebben we besloten bijkomende modificaties aan deze positie te verkennen. Hiervoor werd een divergente syntheseroute ontwikkeld, die de mogelijkheid biedt om zowel 6”-*O*- als 6”-*N*-gemodifeerde α-GalCer analogen te bekomen, alsook analogen gewijzigd ter hoogte van C-5”. Bovendien kunnen in een laat stadium van de synthese wisselende acylketens ingevoerd worden. De uitwerking van deze methode staat beschreven in Hoofdstuk 3, samen met de toepassing ervan op de synthese van twee gekende Th1-polarizerende analogen, namelijk α-D-fucopyranosylceramide en α-D-galacturonosyl-ceramide. Daarnaast werden ook twee nieuwe varianten van deze verbindingen bereid, waarin de conventionele acylketen vervangen werd door een 6-phenylhexanoylketen (C6Ph).

Hoofdstuk 4 beschrijft de invoering van verscheidene nieuwe C-5” en C-6”-modificaties, vertrekkende vanuit een gemeenschappelijk sleutelintermediair. Een eerste groep bestaat uit zeven aromatisch gesubstitueerde uronamiden en drie C-6”-carbamaten. Op één uitzondering na induceren al deze verbindingen een Th1 gepolarizeerde respons *in vivo* in muizen. De

sterkste Th1 antigenen bevinden zich in de klasse van de C-6”-carbamaten, die daarnaast ook een Th2 polarizerende verbinding bevat. Deze C-6”-carbamaten oefenen significant betere antimetastatische effecten uit in een B16 melanoma muis model. In vergelijking met α-GalCer interageren deze analogen veel langer met CD1d. Voor twee componenten wordt dit weerspiegeld door de vorming van meer en betere contacten met CD1d. Dit werd echter niet teruggevonden voor de sterkste Th1 polarizer, die daarentegen een nieuwe interactie met de TCR vertoonde. Bijgevolg was het niet mogelijk om een algemeen verband te leggen met de waargenomen iNKT cel responsen.

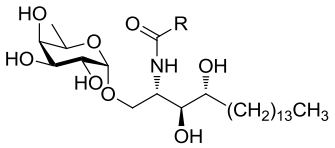
In tweede instantie werd een serie van zes α-GalCer analogen gesynthetiseerd met verschillende 4-gesubstitueerde 1,2,3-triazool-1-yl groepen ter hoogte van de 6”-positie. In een *in vitro* experiment met muis iNKT cellen vertoonden zowel de derivaten met een fenylsubstituent en een butylgroep, alsook de niet-gesubstitueerde variant, een betere stimulerende capaciteit dan α-GalCer. Hieruit hebben wij geconcludeerd dat de verhoogde antigene activiteit van deze analogen voornamelijk te wijten is aan de ingevoerde triazoolgroep.

Bijkomend werden twee analogen bereid die een C-5” en C-6”-modificatie combineren met een OCH-ceramide. Biologische evaluatie van deze verbindingen heeft aangetoond dat hun IFN-γ potentieel significant verlaagd is ten opzichte van de C-5”/C-6”-gemodificeerde referentieverbindingen.

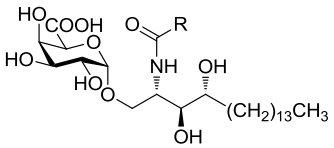
Verder werd de syntheseroute ook nog aangewend voor de bereiding van twee C-6”-gelabelde analogen. Deze verbindingen zullen toegepast worden in bindingscompetitie experimenten.

Tot slot wordt in Hoofdstuk 5 de synthese van een mogelijke iNKT cel antagonist beschreven. Een dergelijke antagonist zou nuttig kunnen zijn voor de behandeling van inflammatoire allergiën zoals astma, alsook van andere aandoeningen die gekenmerkt worden door een overmatige activatie van iNKT cellen. Om het beoogde C-6”-naftylureum-α-mannosylceramide derivaat te kunnen bekomen waren verschillende pogingen nodig. De uiteindelijk succesvolle syntheseroute liet toe om in een laat stadium het α-mannosylderivaat om te vormen naar het overkomstige α-glucosylderivaat. Helaas konden preliminaire biologische experimenten met NU-α-ManCer geen antagonistische eigenschappen aantonen.

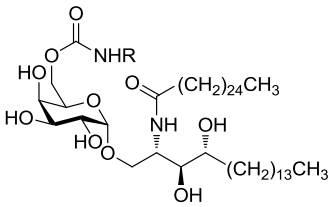
Overview of synthesized compounds



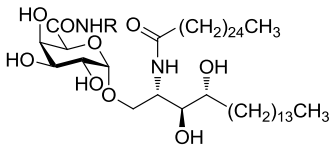
1: R = $(CH_2)_{24}CH_3$
2: R = $(CH_2)_5Ph$



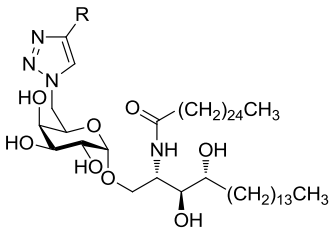
3: R = $(CH_2)_{24}CH_3$
4: R = $(CH_2)_5Ph$



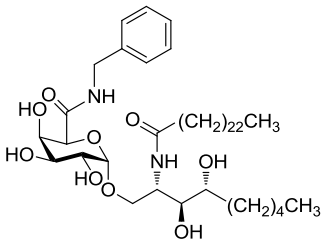
5a: R = 4-chlorophenyl
b: R = 1-naphthyl
c: R = 4-pyridyl



6a: R = Ph
b: R = 3,4-diClPh
c: R = Bn
d: R = CH_2(3,4-diClPh)
e: R = $(CH_2)_2Ph$
f: R = CH_2-(3-thienyl)
g: R = CH_2-(1-naphthyl)



8a: R = H
b: R = butyl
c: R = phenyl
d: R = benzyl
e: R = ethylphenyl
f: R = propylphenyl



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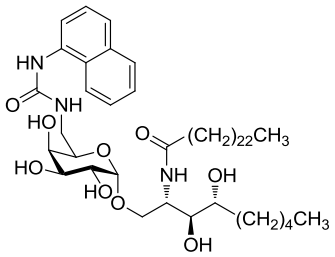

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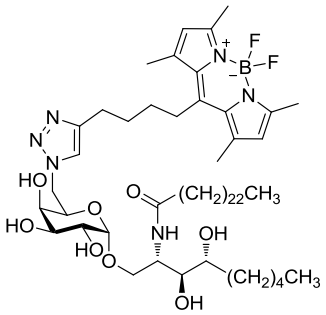

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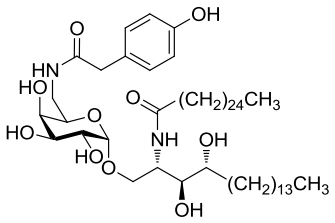

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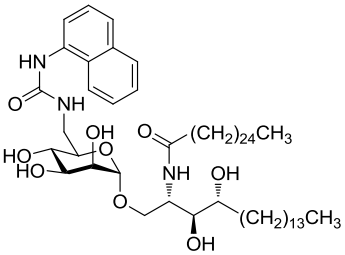
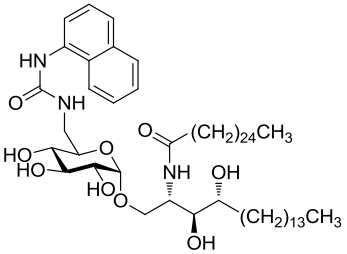


12
NU-α-ManCer



13
NU-α-GlcCer

List of abbreviations

AHR	Airway hyperreactivity
APC	Antigen-presenting cell
BAIB	(Diacetoxyiodo)benzene
BbGl	*Borrelia burgdorferi* glycolipid
BCR	B cell receptor
BMDC	Bone marrow dendritic cell
BODIPY	Boron dipyrromethene
C6Ph	6-Phenylhexanoyl
CD	Cluster of differentiation
CDI	1,1'-Carbonyldiimidazole
CDR	Complementarity determining regions
CuAAC	Copper-catalyzed azide-alkyne cycloaddition
DAMP	Damage-associated molecular pattern
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DC	Dendritic cell
DMF	Dimethylformamide
DIPEA	*N*,*N*-Diisopropylethylamine
DMAP	4-(Dimethylamino)-pyridine
DMSO	Dimethyl sulfoxide
DPPA	Diphenylphosphorazidate
EAE	Experimental allergic encephalomyelitis
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
ELISA	Enzyme-linked immunosorbent assay
ESI	Electrospray ionization
FucCer	Fucosylceramide
GalCer	Galactosylceramide
GD	Diasialoganglioside
GlcCer	Glucosylceramide
GSL	Glycosphingolipid
HCTU	2-(6-Chloro-1-H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate
HPLC	High pressure liquid chromatography

IFN	Interferon
iGb3	Isoglobotrihexosyl ceramide
IL	Interleukine
iNKT	invariant Natural killer T
ManCer	Mannosylceramide
MFI	Mean fluorescence intensity
MHC	Major histocompatibility complex
MS	Mass spectrometry
NHS	*N*-hydroxysuccinimide
NIS	*N*-iodosuccinimide
NK	Natural killer
NMR	Nuclear magnetic resonance
NU	Naphthylurea
PAMP	Pathogen-associated molecular pattern
PRR	Pattern recognition receptor
Rt	Room temperature
S.A.R.	Structure-activity relationship
SPR	Surface plasmon resonance
TBAF	Tetra(*n*-butyl)ammonium fluoride
TBDMSCl	*Tert*-butyldimethylsilyl chloride
TBSOTf	Trifluoromethanesulfonic acid *tert*-butyldimethylsilyl ester
TBAT	Tris-(benzyltriazolylmethyl)amine
TCR	T cell receptor
TEMPO	2,2,6,6-Tetramethyl-1-piperidinyloxy
Th	T helper
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TLR	Toll-like receptor
TMSOTf	Trimethylsilyl trifluoromethanesulfonate
TNF	Tumor necrosis factor
TSA	Toluenesulfonic acid
UV	Ultraviolet
VDW	Van der Waals
β_2m	β_2-microglobulin

CHAPTER 1

INTRODUCTION

1 Introduction

The immune system, classically divided into two arms, provides protection of the organism from invading pathogens and is responsible for the elimination of modified or altered cells. The innate immune system acts as a first line of defense associated with a rapid nonspecific response to a variety of microorganisms. By contrast, the acquired or adaptive immune system is rather slow-responding and reacts upon recognition of specific antigens. Furthermore it is characterized by the generation of immunological memory, ensuring a more rapid and effective response on subsequent exposure to the same pathogen.

Basically, microbial invasion is prevented by anatomical barriers such as the skin and the mucosal epithelia lining the respiratory, gastrointestinal and urogenital tracts. However, once infectious agents have penetrated tissues, both cellular as well as humoral components of the nonspecific system are brought into play causing an inflammatory response. Thereby, the first encountered immune cells are macrophages. Together with neutrophiles and dendritic cells (DCs) they belong to the family of phagocytic cells, capable of engulfing and digesting many pathogens. In addition, macrophages and DCs act as antigen-presenting cells (APC), necessary for initiating the adaptive immune response at a later stage. Other innate immune cells are eosinophils, basophils and mast cells, primarily exhibiting a secretory function. Finally, natural killer (NK) cells can recognize and kill modified cells through the release of lytic granules. The major humoral barrier of the innate arm is the complement system, composed of over twenty soluble proteins which induce a rapid killing response. This involves a catalytic cascade resulting in an increase of the vascular permeability, opsonization and recruitment of immune cells as well as direct killing of the microorganism through membrane lysis.

Detection of an altered homeostatic state by the innate immune system occurs through a limited repertoire of proteins, known as pattern recognition receptors (PRRs). These receptors are able to identify conserved components among a broad range of microorganisms in addition to alarm signals send out by stressed or damaged cells. Recognition of a so-called pathogen- or damage-associated molecular pattern (PAMP or DAMP) triggers the innate immune cells and proteins to eliminate the threat via their various effector mechanisms. Some activated cells including macrophages and DCs also release cytokines, which serve as

chemical mediators to attract other innate immune cells to the site of infection and contribute to the activation of the adaptive immune system.

An adaptive immune response is initiated when the innate system fails to eliminate the infectious agent and is achieved by specialized leucocytes, called lymphocytes. Among them, B cells are involved in humoral immune responses, while T cells mediate cellular immune responses. Both cell types express highly pathogen-specific receptors and are characterized by clonal expansion and differentiation into effector cells upon exposure to specific peptide antigens. A subset of the proliferating cells also differentiates into memory cells that persist after the infection, forming the basis of immunological memory.

Naive T lymphocytes constitute two major subtypes, $CD4^+$ and $CD8^+$ T cells. In contrast to B cells they cannot recognize soluble antigens but require association of the antigen with a major histocompatibility complex (MHC). In particular for $CD4^+$ T cells, antigens are displayed on MHC II molecules at the surface of antigen-presenting molecules (macrophages, DCs and B cells), while $CD8^+$ T cells recognize antigens presented by MHC I molecules, for example at the surface of virus-infected cells or tumor cells. As a result of their activation, the naive T cells differentiate into either Th1 and Th2 cells in case of $CD4^+$ T cells or cytotoxic T (Tc) cells in case of $CD8^+$ cells. In turn, Th1 cells release pro-inflammatory cytokines such as interferon-γ (IFN-γ) and tumor necrosis factor (TNF), thereby activating macrophages and NK cells. By contrast, Th2 cells produce anti-inflammatory cytokines including interleukine-4 (IL-4) leading to the activation of B cells. $CD8^+$ Tc cells on the other hand destroy viral-infected or tumor cells by means of perforine and granzyme secretion.

B cells are generally activated upon recognition of soluble proteins by their B cell receptor (BCR) in combination with stimulatory signals provided by Th2 cells. The BCR-bound antigen will be internalized followed by degradation to peptide fragments which can be presented by MHC II molecules at their cell surface. Furthermore, B-cell proliferation and differentiation generates plasma cells that secrete neutralizing antibodies.

In contrast to the conventional B and T cells described above, some B and T cells share innate characteristics, blurring the boundaries between both systems. These cells are referred to as innate-like B and T lymphocytes. Among them, natural killer T (NKT) cells are a prominent population in mouse and human shown to bridge the innate and acquired immune system. Whereas conventional T cells recognize peptide antigens displayed on MHC I or II molecules,

the majority of NKT cells recognize lipids or glycolipids presented by CD1d molecules. Other dissimilarities are their extremely rapid cytokine production following activation, and their lack of immunological memory. Because of their important functions in microbial and tumor immunity as well as autoimmune diseases, they have been the subject of extensive research in the past two decades. Hereby, α-galactosylceramide (α-GalCer) is often utilized as prototypical glycolipid for their activation.

1.1 NKT cells

NKT cells, first discovered in 1987, represent a unique thymus-derived lymphocyte subpopulation, originally characterized by the co-expression of the natural killer receptor NK1.1 (CD161) and a T-cell receptor (TCR).[1,2,3,4] In mice they localize preferentially to the liver (20-30% of the total lymphocytes), bone marrow (20-30%) and thymus (10-20%), whereas in human the frequency is much lower with a high degree of variability between individuals. Since the NK1.1 marker is not uniformly expressed by all NKT cells and additionally, some conventional T cells upregulate this marker upon activation, the elementary definition that NKT cells are $NK1.1^+$ T cells is inaccurate. Therefore, these heterogeneous cells are subdivided into at least three categories mainly based on their TCR phenotype and CD1d restriction.[2,3,5]

1.1.1 Classification

Type 1 NKT cells are defined as cells having an invariant TCR Vα chain rearrangement (Vα14-Jα18 in mice, Vα24-Jα18 in humans) conjugated with a limited Vβ chain repertoire (Vβ8.2, Vβ7 or Vβ2 in mice, Vβ11 in humans). As a consequence they are often referred to as 'invariant' NKT (iNKT) cells (Figure 1.1). They show high affinity for the glycosphingolipid α-GalCer presented by CD1d and are sometimes subdivided into a $CD4^+$ and a $CD4^-CD8^-$ or DN population, although human iNKT cells can be $CD8^+$ as well. Not all these cells express the NK1.1 marker and therefore, some authors prefer the term Vα14iT rather than iNKT cells. Type 2 NKT cells express more diverse TCR Vα chains and recognize different ligands, such as sulphatide and phospholipids, in the context of CD1d. Similar to type 1 NKT cells, they can be $CD4^+$ or DN and comprise both $NK1.1^+$ and $NK1.1^-$ subsets. Another population, classified as NKT-like cells, is also characterized by the expression of diverse TCRs but is CD1d-independent. Much less is known about these cells.

The analogues synthesized in this work are aimed at targeting iNKT cells. Hence only this type is considered hereafter.

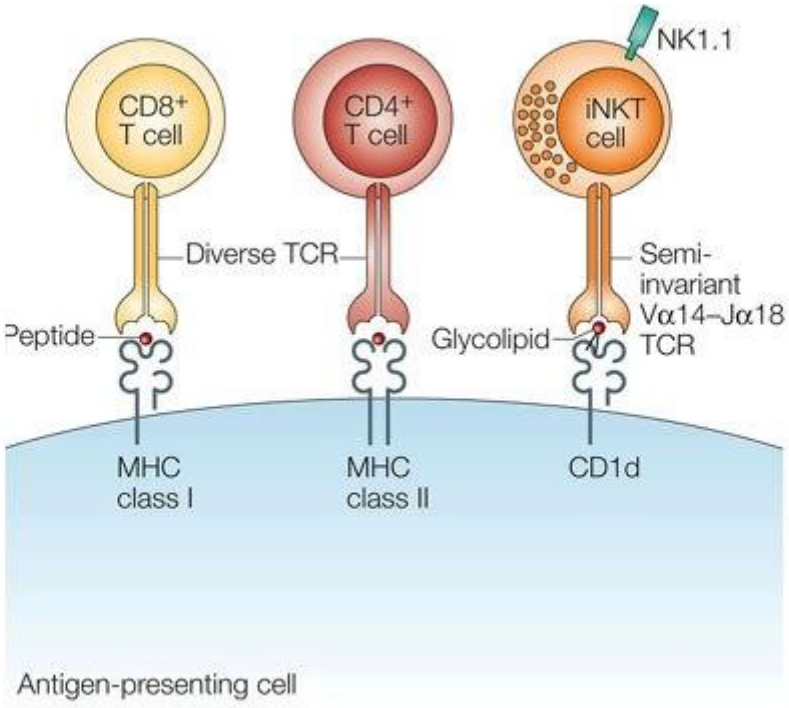


Figure 1.1 iNKT cells compared with conventional T cells.
(Van Kaer, 2005, Nat. Rev. Immunol.)

1.1.2 TCR structure

The structure of the iNKT TCR generally resembles that of other αβ TCRs. These heterodimeric receptors consist of an α and β chain, each subdivided into a constant (C) and variable (V) domain. Both chains have a transmembrane region by which the molecule is anchored in the cell membrane followed by a short cytoplasmic tail. The V segment is composed of three complementarity determining regions (CDRs). Together, these six hypervariable loops make up the antigen-recognition site of the TCR. Among them, CDR3β displays extensive junctional diversity resulting in slightly different conformations and variable loop lengths.[6,7]

1.2 Cluster of differentiation 1d (CD1d)

CD1d belongs to a third family of antigen-presenting molecules, the CD1 molecules.[8,9,10] These non-polymorphic glycoproteins are related to major histocompatibility complex (MHC) molecules and can be divided into two groups by sequence homology. Group I consists of CD1a, CD1b and CD1c isotypes, and group II comprises CD1d. CD1e on the other hand is

considered as an intermediate isotype thought to play an intracellular trafficking role. While humans express all isoforms, mice and rats contain only CD1d. They can be found at the surface of cortical thymocytes, macrophages, DCs and B cells where they present hydrophobic antigens, mostly glycolipids, for recognition by the TCR.

Analogous to MHC class I molecules, CD1 proteins are composed of a heavy chain with three extracellular domains (α1-α3) that are non-covalently associated with β_2-microglobulin (β_2m) (Figure 1.2). The α1 and α2 domains form the antigen-binding region that sit atop a six-stranded anti-parallel β-sheet platform. The α3 domain is attached to the cell membrane by a transmembrane segment, followed by a short cytoplasmic tail. The binding groove of CD1d is more narrow but substantially deeper compared to that of MHC-I and has two major pockets that connect directly to the surface, designated A' and F' in mice and A' and C' in humans. Their walls are made up of almost entirely hydrophobic residues, with the only polar residues situated at the bottom of the A' pocket and at the entrance of the groove. Hence, these molecules are highly suitable for the binding of lipid or glycolipid antigens.

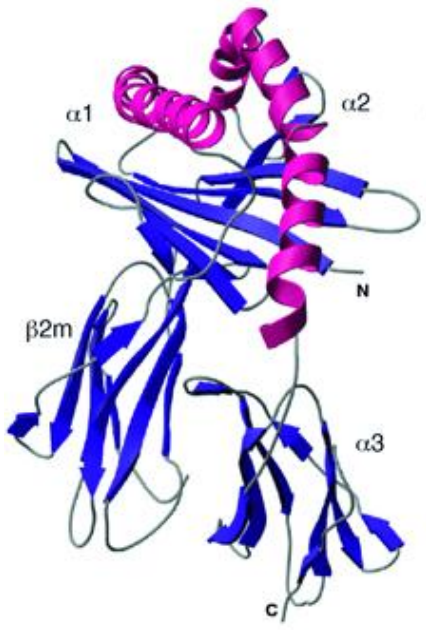


Figure 1.2 Crystal structure of mouse CD1d
(Zeng *et al*., 1997, Science).

1.3 The immunological role of iNKT cells

In response to antigen stimulation, iNKT cells are able to rapidly release massive amounts of cytokines, including pro-inflammatory Th1 cytokines such as IFN-γ and TNF-α, and anti-inflammatory Th2 cytokines such as IL-4, IL-10 and IL-13 (Figure 1.3).[11,12] This rapid

release, within 1-2 hours after TCR ligation, is thought to be due to the presence of high levels mRNAs encoding IL-4 and IFN-γ in resting iNKT cells.[13] This might also explain the initial Th0 cytokine profile induced by these cells in contrast to conventional T cells.[14] The produced cytokines in turn can activate several other cell types such as DCs, NK cells, macrophages and neutrophiles in the innate immune system and conventional B and T cells in the acquired immune system (Figure 1.3). Furthermore, iNKT cells can exhibit cytotoxic activities mediated by Fas-FasL interaction and the expression of granzyme B and perforin. However, it is still unclear whether all iNKT cells have this capability and trans-activated NK cells definitely play a predominant role herein. In tandem with their vigorous cytokine secretion, iNKT cells undergo surface receptor downregulation, followed by extensive cell expansion by days 2-3, before returning to homeostatic levels.[15,16]

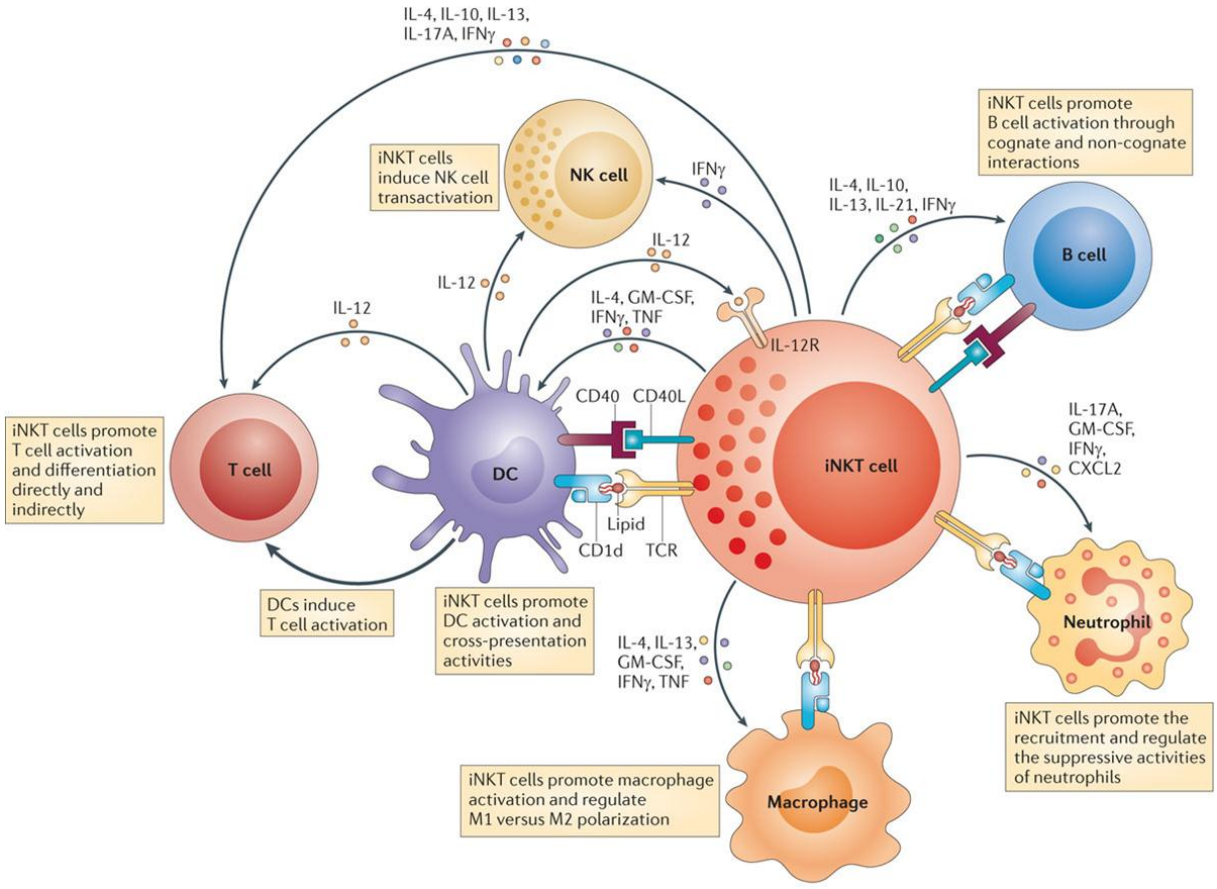


Figure 1.3 Cytokine release by activated iNKT cells inducing trans-activation of other leukocytes (Brennan *et al*., 2013, Nat. Rev. Immunol.).

Depending on the elicited cytokine profile, iNKT cells can mediate a wide range of protective and regulatory immune functions.[17] Th1 cytokines promote enhanced cell-mediated immunity, associated with tumor surveillance and host protection from a variety of infectious

agents including bacteria, viruses and parasites.[18,19] Th2-type cytokines on the other hand support humoral immunity through upregulation of antibody production and induce immunosuppressive responses essential for the prevention of autoimmune diseases as well as the maintenance of tolerance.[20,21] Homeostasis requires a carefully controlled balance between both types of cytokines and as a consequence any dysregulation can induce disease. Accordingly, a decreased number and/or impaired cytokine function of iNKT cells has been correlated with attenuated immune responses to pathogens and predisposition to the development of several autoimmune diseases as well as certain cancers.[22] However, whether the defect is a cause or consequence of the disease, or may in some cases simply reflect the accumulation of iNKT cells at sites of inflammation remains to be determined. Nevertheless, iNKT cells represent an important immunotherapeutic target with broad clinical potential.

The mechanisms controlling the outcome of iNKT cell activation are still poorly understood. Two independent studies showed that different human iNKT cell subsets are characterized by distinct cytokine profiles. Whereas $CD4^+$ iNKT cells are able to produce both Th1 and Th2 cytokines, $CD4^-$ subsets primarily produce Th1 cytokines.[23,24] In addition, the iNKT cell cytokine secretion is determined by the tissue of origin as well as by environmental aspects such as surrounding cytokines, chemokines and co-stimulatory molecules. Finally, distinct lipid antigens may also influence the resulting effector functions (see 1.7).

1.4 Distinct modes of iNKT cell activation

iNKT cells can respond to an array of different lipid-based antigens in complex with CD1d. The first discovered CD1d-binding iNKT cell ligand is the synthetic glycolipid α-GalCer. Since then, many more iNKT cell antigens have been described, classifiable into endogenous or self-antigens, microbial ligands and synthetic derivatives.

1.4.1 α-Galactosylceramide

During a screen for compounds that could prevent tumor metastases in mice, extracts derived from the marine sponge *Agelas mauritianus* elicited promising biological activities. Their antitumor properties were later attributed to a glycosphingolipid family called agelasphins, from which α-GalCer was structurally optimized (Figure 1.4).[25,26,27] Today this glycolipid, also known as KRN7000, is the most extensively studied antigen for iNKT cells.

α-GalCer contains a D-galactosyl head group attached to a ceramide, which in turn is composed of a C18 phytosphingosine base linked to a saturated C26 fatty acyl chain. A

striking structural feature is the α-anomeric linkage, as most mammalian glycolipids possess a β-glycosidic bond. Instead, the α-anomeric linkage is considered as a microbial signature.

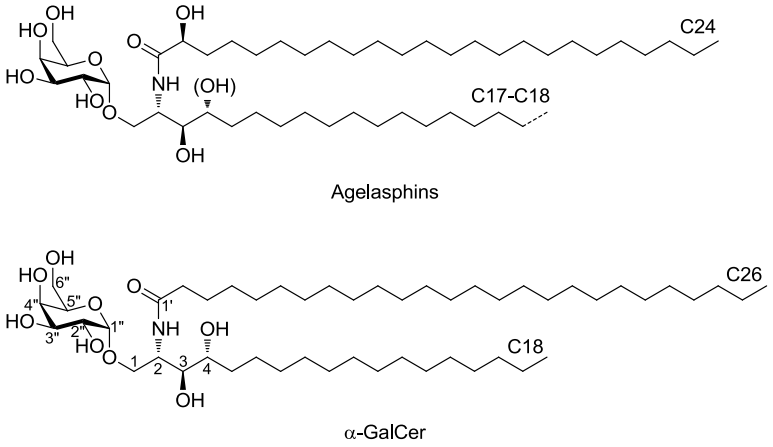


Figure 1.4 Structural comparison of agelasphins and α-GalCer (agelasphins with branched alkyl chains are not shown).

Activation of iNKT cells by strong agonists such as α-GalCer is suggested to occur in three distinct steps (Figure 1.5).[28] At first, interaction with the TCR leads to the secretion of primarily IL-4 and only a little IFN-γ. Secondly, upregulation of CD40 ligand (CD40L) by the activated iNKT cell followed by interaction with CD40 present on the DC cell induces DC maturation and secretion of IL-12. Subsequently, DC-derived IL-12 activates NK and iNKT cells for a second wave of IFN-γ production. This last step is required for maximal release of IFN-γ.

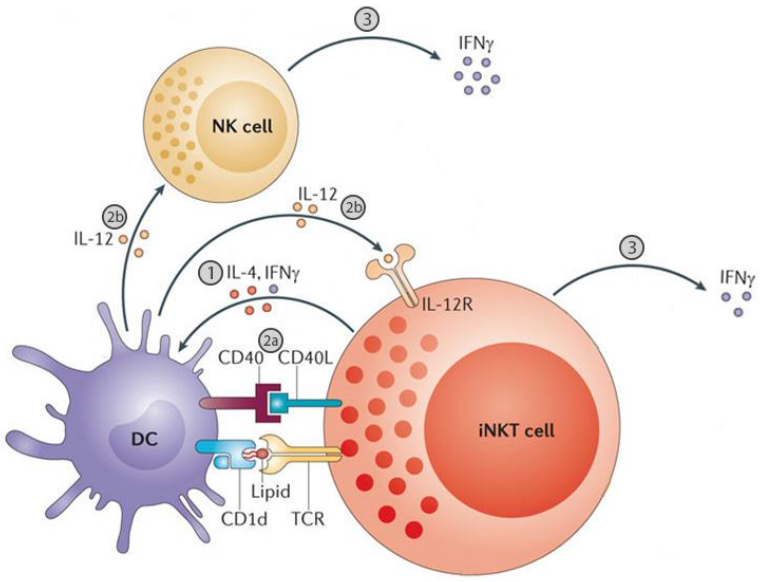


Figure 1.5 α-GalCer-mediated activation of iNKT cells (adapted from Brennan *et al.*, 2013, Nat. Rev. Immunol.).

1.4.2 Endogenous ligands

A remarkable feature of iNKT cells is their CD1d-restricted autoreactivity. Not only do self-lipids drive iNKT cell development in the thymus, they also contribute to peripheral iNKT cell activation implicated in many infectious and non-infectious diseases such as cancer or autoimmunity. However, the nature of these self-ligands remains a controversial issue. Initially, isoglobotrihexosyl ceramide (iGb3), composed of three serially linked glycosides β-linked to a ceramide, was proposed as the natural ligand essential for both thymic selection and peripheral activation of iNKT cells (Figure 1.6).[29] Meanwhile it is known that iGb3 cannot be the unique physiological antigen and although it is definitely an agonist for iNKT cells, its role as a natural selecting ligand has been questioned.[30,31] In the further search for specific self-lipids, other cellular glycosphingolipids have been identified as potential self-ligands such as the ganglioside GD3[32] and β-GlcCer (Figure 1.6).[33] Additionally, it has been demonstrated that self-antigens may not be restricted to GSLs but are likely more diverse in structure.[34] Consistent with this, several membrane phospholipids have been found associated with CD1d including phosphatidylinositol, phosphatidylcholine, sphingomyeline, phosphatidylglycerol and phosphatidylethanolamine.[35,36,] Recently, lysophosphatidylcholine has been suggested as a self-antigen for human iNKT cells (Figure 1.6).[37] Yet, this phospholipid does not appear to stimulate mouse iNKT cells indicating that self-antigens for mouse and human iNKT cells may be different.[34] Furthermore, the lipid self-antigens contributing to the thymic selection of iNKT cells may differ from those involved in iNKT cell activation in the periphery.

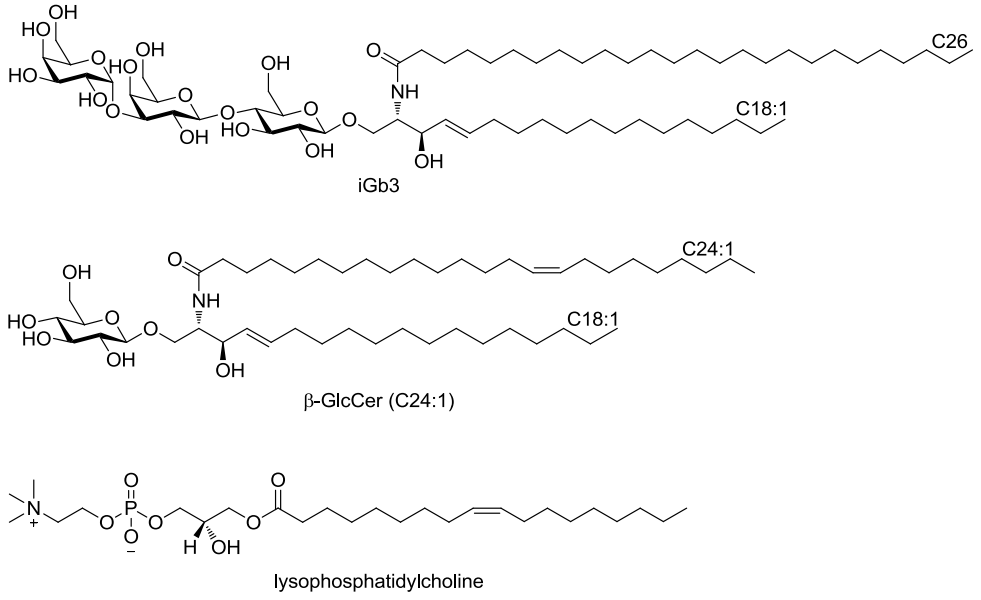


Figure 1.6 Structures of endogenous iNKT cell ligands.

1.4.3 Microbial activation

iNKT cells can become activated early in infection either through direct stimulation by microbe-specific CD1d-presented lipids or alternatively via an indirect pathway driven by TLR engagement of the APC.[38] Both mechanisms lead to Th1 type effector responses, including potent IFN-γ secretion.

1.4.3.1 Direct pathway

Microbial lipid antigens that activate iNKT cells have been identified in a broad range of microorganisms. For example *Sphingomonas* spp. bacteria express α-glucuronosylceramide (GSL-1) and α-galacturonosylceramide (GSL-1') shown to stimulate murine and human iNKT cells in a CD1d-dependent manner (Figure 1.7).[39,40,41] During infection with these bacteria in mice, iNKT cells appear to control both bacterial clearance and the septic shock response, providing evidence of a significant role for iNKT cells in antimicrobial defense. A second type of microbe containing cognate glycolipid antigens is *Borrelia burgdorferi*, the causative agent of Lyme disease.[42] The diacylglycerol-based lipids isolated from this bacteria are referred to as *B. burgdorferi* glycolipid-2 (BbGl-II), the fatty acids of which vary in length from C14 to C18 (Figure 1.7). Likewise, a direct role for iNKT cells in host defense and clearance of these microbes has been demonstrated.[43,44] Other iNKT cell stimulating lipids are likely present in *Plasmodium falciparum*,[45] *Trypanosoma* spp.,[45] *Leishmania* spp.,[46] *Ehrlichia* spp.,[39] *Streptococcus pneumoniae*,[47] *Helicobacter pylori*[48] and *Mycobacterium bovis* BCG.[49]

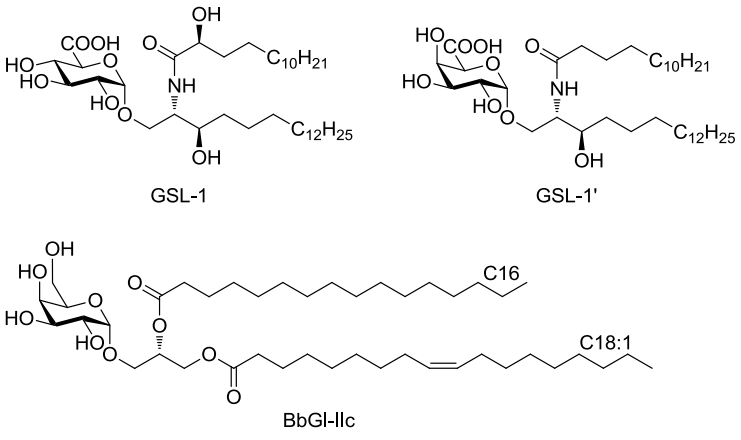


Figure 1.7 Structures of microbial iNKT cell ligands.

1.4.3.2 Indirect pathway

In a second pathway of iNKT cell activation during microbial infection, a unique mechanism is used that does not require TCR-mediated cognate recognition of bacterial lipid antigens.

Instead, IL-12 released by DCs in response to PAMPs or DAMPs such as Toll-like receptor (TLR) ligands, in concert with weak responses to CD1d-presented self-antigens is capable of stimulating iNKT cells (Figure 1.8). This has first been demonstrated in studies with *Salmonella typhimurium* and *Staphylococcus aureus* bacteria.[50] However, it remains to be determined which of the proposed self-lipids actually contribute to the activation of iNKT cells in this context. Furthermore, stimulation of iNKT cells independent of CD1d-mediated self-recognition has also been reported, for example in the case of *E. coli* where exposure to IL-12 and IL-18 produced by activated APCs is sufficient for iNKT cell activation.[51]

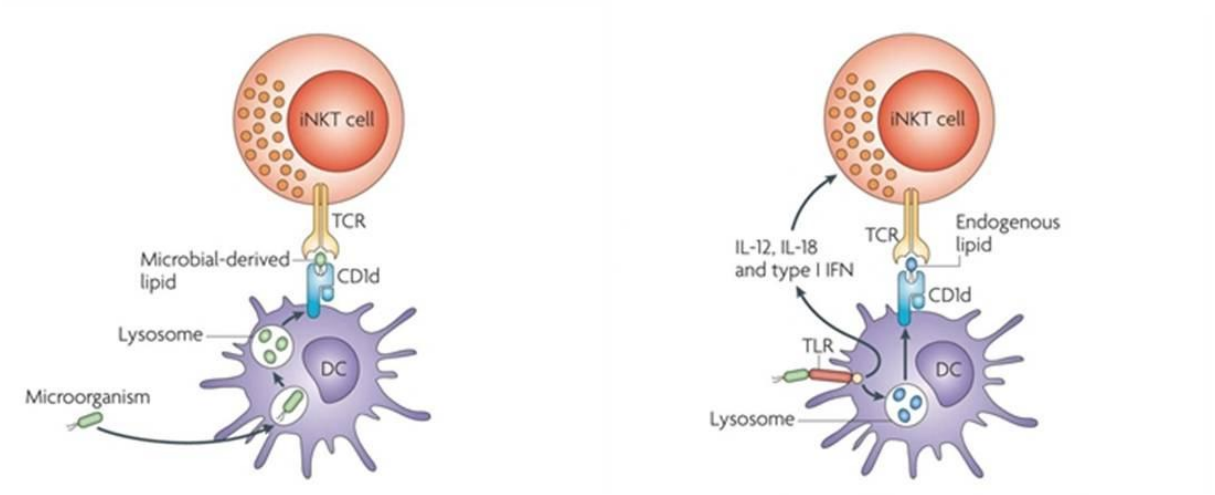


Figure 1.8 Microbial activation of iNKT cells: the left panel shows direct activation through iNKT TCR ligation by a microbial lipid ligand. By contrast, the right panel shows cytokine-dominated activation following TLR engagement whether or not combined with TCR stimulation by an endogenous lipid antigen (Cerundolo *et al*., 2009, Nat. Rev. Immunol.).

1.5 Structural basis for lipid antigen recognition

Binding of α-GalCer to CD1d is realized through accommodation of the acyl chain and sphingosine chain into the A' and F'/C' pockets respectively, giving rise to three hydrogen bonds with the glycolipid (Figure 1.9).[52,53] Specifically, the 3-OH on the sphingosine chain interacts with Asp80, the 2"-OH of the galactose ring with Asp151 (Asp153 in mice) and finally, the glycosidic oxygen is hydrogen-bonded to Thr154 (Thr156 in mice). These interactions are believed to contribute to the proper orientation of the galactose ring above the surface of CD1d for optimal recognition by the TCR.

Structural comparison of CD1d with and without α-GalCer reveals conformational changes in the F' pocket upon binding of α-GalCer, i.e. Leu84 and Leu150 are reoriented, leading to the formation of a closed 'roof' above the F' pocket.[53] This in turn affects the position of other

amino acids such as Arg79, known to be important for interaction with the TCR. As a result of the F' roof closure, the phytosphingosine chain is tightly tugged underneath by forming additional van der Waals interactions. Furthermore, the preformed F' roof allows binding of the TCR without significant accommodation of the CD1d/α-GalCer complex.

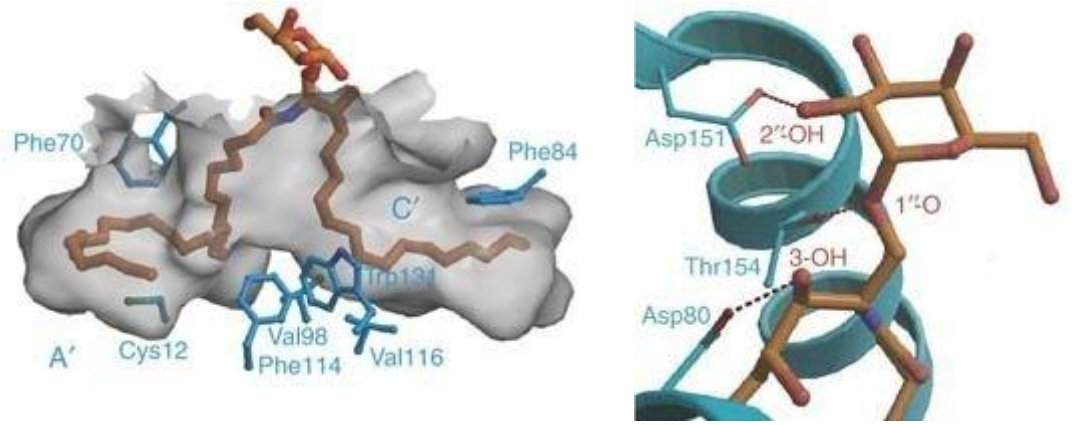


Figure 1.9 Presentation of α-GalCer by human CD1d: The left panel shows binding of the α-GalCer lipid chains in the A' and C' pocket of hCD1d. The right panel shows the hydrogen bonds formed between residues of hCD1d and α-GalCer (Koch *et al*., 2005, Nat. Immunol.).

Since mouse and human CD1d are highly homologous, the binding mode of α-GalCer in CD1d is very similar in both species. Nevertheless, a non-conservative polymorphism results in a different positioning of the galactose head group.[54] More specifically, a bulky tryptophane residue at position 153 in human CD1d, in contrast to a glycine residue at position 155 in mouse CD1d, pushes the galactose ring away causing a shift of up to 3Å (Figure 1.10).

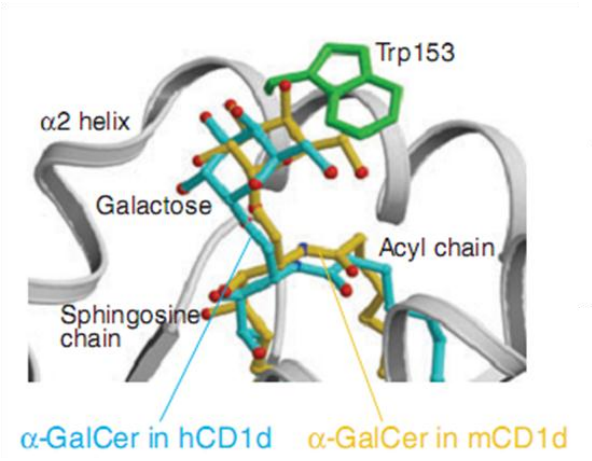


Figure 1.10 Species polymorphism in the presentation of α-GalCer by mouse and human CD1d leads to a small shift of the galactose ring. Orientation of α-GalCer when presented by human CD1d is depicted in blue and is superimposed on α-GalCer when complexed to mouse CD1, shown in yellow (Godfrey *et al*., 2005, Nat. Immunol.).

Despite the above mentioned difference in sugar head position, crystal structures of both mouse and human TCRs in complex with α-GalCer/CD1d show a highly conserved lock-and-key binding mode by the TCR (Figure 1.11).[55,56] In contrast to the diagonal footprint adopted by MHC-restricted TCRs, the iNKT TCR docks on a parallel manner to CD1d. Whereas the TCR-α chain sits above the opening of the CD1d binding groove, the TCR-β chain is situated above the closed end of the F' pocket. Consequently, recognition by the iNKT TCR is dominated by the invariant α-chain, contacting both CD1d and α-GalCer via several hydrogen bonds and van der Waals interactions. In particular for α-GalCer, these hydrogen bonds are formed between the 2", 3" and 4" hydroxyl groups of the galactose ring and Gly96 located in the CDR3α loop and Ser30 and Phe29 in the CDR1α loop respectively (in mice the 2"-OH and 3"-OH are both bound by Asn30 of CDR1α) (Figure 1.11). Additionally, the 3-OH group of the sphingosine base interacts with Arg95 in the CDR3α loop. The α1 and α2 helices of CD1d on the other hand interact with CDR3α and CDR2β.

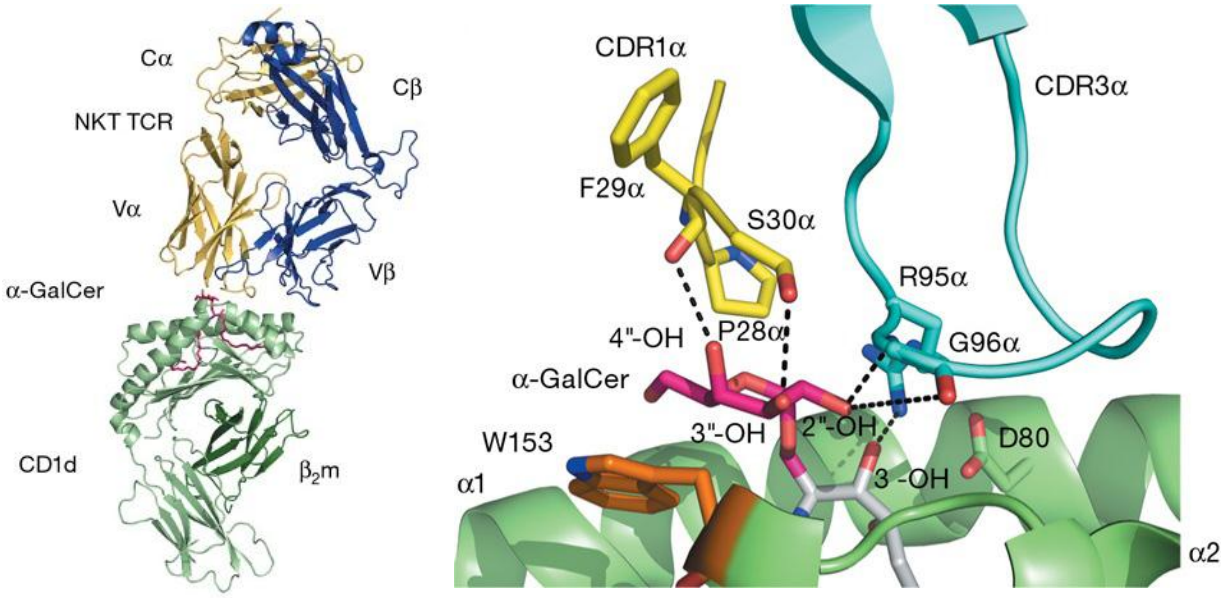


Figure 1.11 The left panel shows the ternary complex of α-GalCer with hCD1d and the TCR; the right panel shows the hydrogen bonds formed between residues of the TCR and α-GalCer (Borg *et al*., 2007, Nature).

The TCR footprint onto the CD1d/glycolipid complex is not only conserved between humans and mice, conferring reciprocal cross-species reactivity, but is also independent of the TCR-β composition and the lipid antigen associated with CD1d.[57, 58] An explanation for this conserved docking mode is provided by mutational analyses of the individual CDR residues, revealing a "hot spot" composed of germline-encoded amino acids within CDR1α, CDR3α and CDR2β.[59] Conversely, the hypervariable CDR3β loop can fine tune the interaction, thereby modulating the TCR affinity.[60]

Comparison of the ternary complex with α-GalCer to the one with GSL-1' and BbGl-IIc learns that the TCR can induce structural changes in both CD1d and the ligand orientation to maintain the conserved binding mode, probably causing the weaker potencies of these microbial antigens.[61] When GSL-1' and BbGl-IIc are bound by CD1d without the TCR, the roof over the F' pocket is not closed due to their different lipid chain structures. Closure does occur, however, upon binding by the TCR implicating structural changes above the F' pocket. This is associated with an energetic penalty, likely accounting for the reduced TCR affinities compared to α-GalCer. Furthermore, in case of BbGl-IIc, the sugar is rotated 60° counterclockwise from the optimal position when bound to CD1d without the TCR, attributed to a different binding orientation to CD1d. Hence, apart from conformational changes over the F' roof, binding of the TCR also involves a reorientation of the sugar head group by 60° in clockwise direction. This might explain the nearly tenfold weaker binding affinity compared to GSL-1'.[62] Together, these findings indicate that, even though the lipids are buried in the CD1d groove and do not directly contact the TCR, they are important in the formation of a TCR epitope and consequently can determine the antigenic potency.

1.6 Therapeutic potential and limitations

The therapeutic potential of harnessing iNKT cell responses has been demonstrated in various experimental models of human diseases. α-GalCer treatment showed antitumor effects against a broad range of experimental tumor lines including melanoma, sarcoma, colon carcinoma and lymphoma,[63] and can protect against spontaneous as well as carcinogen-induced primary tumor formation in mice.[64] Stimulation of iNKT cells via repeated administration of α-GalCer has also been reported to exhibit therapeutic effects in certain autoimmune diseases such as type 1 diabetes, experimental allergic encephalomyelitis (EAE), arthritis, lupus and experimental colitis.[65] Furthermore, α-GalCer-mediated activation of iNKT cells significantly increases immune responses to co-injected antigens providing opportunities for the generation of new adjuvant strategies to enhance the efficacy of various vaccines against infectious diseases and cancer.[66]

Despite these promising preclinical data, the clinical effectiveness of α-GalCer is rather limited. In a first phase I study consisting of IV administration of α-GalCer to patients with refractory solid tumors, only marginal therapeutic effects were observed.[67] Since administration of α-GalCer-pulsed DCs showed enhanced antitumor effects in murine models compared to free α-GalCer and additionally circumvent activation-induced anergy owing to

iNKT cell overstimulation, subsequent clinical trials were conducted following this approach.[68,69] Depending on the employed APC platform, modest to strong expansion of iNKT cells and secondary activation of NK and T cells following an initial drop in cell numbers were reported.[70] Moreover, increased levels of IFN-γ and IL-12 were seen. Nonetheless, no tumor regression could be observed except for one clinical trial utilizing intranasal administration of α-GalCer-pulsed APCs. These poor clinical responses are attributed to the opposing activities of simultaneous secreted Th1 and Th2 cytokines. Hence, α-GalCer analogues capable of inducing a biased Th1 or Th2 response are believed to afford superior clinical effectiveness.

1.7 Structure-activity relationship studies

In order to selectively induce Th1- or Th2-type cytokine production and to better understand the interaction mechanism of the CD1d/glycolipid/iNKT complex, many research groups have been generating new iNKT cell ligands by modification of the structure of α-GalCer. These alterations can be subdivided into four categories, i.e. variation of the ceramide part, modification of the configuration and the nature of the glycosidic bond, variation of the sugar moiety and finally polymodified compounds. Below, a brief overview of notable and/or relevant analogues is given.

1.7.1 Modifications of the ceramide moiety

An extensively studied modification in the ceramide moiety comprises truncation of the lipid chains, generally resulting in a Th2 bias. The best known analogue in this context is OCH containing a C9 phytosphingosine chain and a C24 acyl chain (Figure 1.12). Upon injection in mice, this compound induces a weak immune response with predominant IL-4 production, associated with a significant increased suppression of EAE and protection against diabetes and collagen-induced arthritis compared to α-GalCer.[71] Extended evidence of a correlation between acyl or phytosphingosine chain-shortened glycolipids and a Th2 cytokine-release profile has been provided by the group of Savage.[72] Yet, mere shortening of the acyl chain from C26 to C20 goes along with a significant decrease in overall potency. By introduction of insaturations into the latter, the stimulatory activity is augmented with retention of the Th2 skewing capacity.[73] The optimized compound among this class is C20:2 (Figure 1.12), a much stronger iNKT cell agonist compared to OCH.

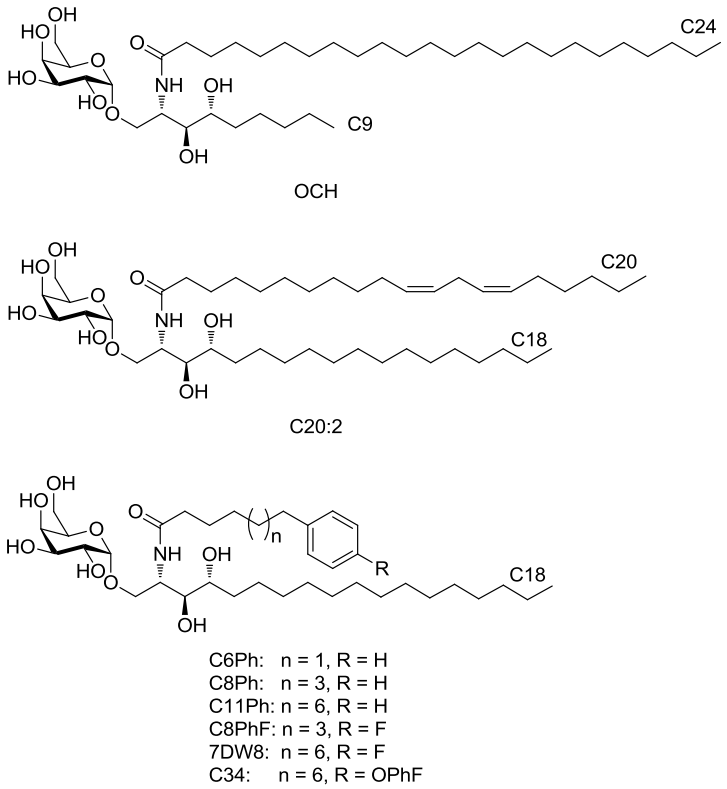


Figure 1.12 Structures of lipid chain-modified α-GalCer analogues.

With regard to the numerous aromatic side chains lining the binding groove of CD1d, the group of Wong introduced terminal aromatic rings into the fatty acyl chain of α-GalCer (Figure 1.12).[74] Interestingly, analogues C6Ph, C8Ph and C11Ph are four times more potent and biased for IFN-γ secretion, attributed to additional interactions with CD1d. For C8PhF, these effects are even more pronounced.[75] Recently, a more comprehensive screening of those and related analogues led to the identification of 7DW8 as a lead candidate with superior adjuvant activity on HIV and malaria vaccines in mice, and C34 as an adjuvant candidate for a breast cancer vaccine.[76,77]

In early structure-activity relationship (S.A.R.) studies of the polar part of the ceramide, comparison of the 4-deoxy and the 3,4-dideoxy analogues of α-GalCer indicated the importance of the 3-OH group, while the 4-OH group appeared to be less essential.[27,78,79] This is in accordance with the crystal structure of the ternary complex, where the 3-OH interacts with Asp80 of CD1d as well as with Arg95 of the TCR. However, more recently the 3-deoxy compound was found to be similarly effective at inducing iNKT cell responses as α-GalCer and 4-deoxy-α-GalCer.[80] A docking study suggested that the 4-OH group compensates for the role of the 3-OH group in making a hydrogen-bond network with CD1d, explaining the very

weak activity of the 3,4-dideoxy analogue. A 4,4-difluoro analogue showed moderate and slightly Th1 biased activity and thus confirms that the 4-OH group is not required for iNKT cell recognition.[81] Park *et al*. demonstrated via evaluation of all eight α-GalCer stereoisomers that the configuration of the C2-NH and the C3-OH group are crucial for the biological activity, while the stereochemistry of the C4-OH is less significant.[82] Strikingly, our group found earlier that the configuration of the C4-OH group appears to be more important than that of C3.[83] Finally, replacement of the 2-amide functionality with a 1,2,3-triazole moiety resulted in comparable stimulatory effects as α-GalCer and a pronounced Th2 response.[84]

1.7.2 Modifications of the glycosidic bond

The α-anomeric configuration of the glycosidic bond was previously assumed to be a crucial feature for iNKT cell recognition as β-GalCer (Figure 1.13) didn't show any TCR affinity in *in vitro* experimental settings and accordingly was unable to stimulate iNKT cell hybridomas.[78] However, other studies did reveal biological activities induced by β-anomeric GalCer albeit at much lower potency than α-GalCer.[85] Though low-level contamination with α-anomeric forms cannot be excluded, the different behavior of both type of analogues provides evidence that β-GalCer can be recognized by the TCR. Furthermore, recent crystal structure elucidation of the TCR/β-GalCer/CD1d complex showed that β-GalCer adopts a flat orientation after TCR ligation similar to α-anomeric compounds.[86] This allows the TCR to dock in an analogous manner onto CD1d/β-GalCer. Yet, flattening of the conformation presumably implicates a considerable energetic penalty, accounting for the much lower TCR affinity. A similar induced-fit recognition model has been demonstrated for iGb3.[87]

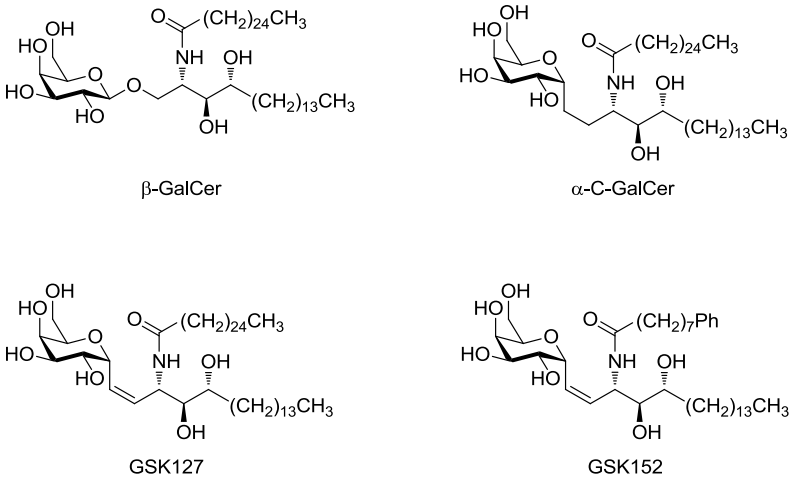


Figure 1.13 Structures of α-GalCer analogues modified at the glycosidic bond.

α-C-GalCer (Figure 1.13), characterized by a methylene group instead of the glycosidic oxygen, is definitely one of the best documented Th1 polarizers, shown to stimulate prolonged IL-12 secretion from DCs followed by long-lasting IFN-γ release from NK cells.[88] Due to the stable ether group, this compound displays higher resistance towards enzymatic and chemical degradation, resulting in a more stable presentation by CD1d despite its lower binding affinity. In *in vivo* mice experiments, the C-glycoside was 100 times more potent than α-GalCer against lung metastases and even 1000 times more effective against malaria. Unfortunately, it is not capable of significantly stimulating human iNKT cells, ruling out clinical benefits.[89] However, replacement of the glycosidic CH_2 by an E-olefin linker, e.g. in vinyl analogues GSK127 and GSK152 (Figure 1.13) leads to potent stimulatory activity of human iNKT cells biased towards a Th1 response.[89] Remarkably, these vinyl derivatives show a dichotomy in their potency of activating mouse versus human iNKT cells, i.e. GSK152 elicits a strong biological activity in human iNKT cells but is far less active in murine assays while GSK127 exhibits greater cytokine production in mice than in humans.

1.7.3 Modifications of the sugar moiety

Several studies have been performed to assess the S.A.R. of the galactose part of α-GalCer. Regarding the stabilizing hydrogen bonds with CD1d and the TCR, it is not surprising that the 2”-OH group is found critical for bioactivity. Upon substitution with a methoxy[90] or acetamide group,[91] the cytokine response is dramatically decreased. Likewise, removal of the hydroxyl group[92] or replacement by a fluoro group abolishes all activity.[93] Alteration of the 3”- and 4”-OH groups by contrast seems less destructive, yet weakens the bioactivity. This is exemplified in the 3”- or 4”-deoxy and -fluoro analogues of α-GalCer, which are still able to stimulate some iNKT cell hybridoma though less potent than α-GalCer.[94] Exceptions are 3”-*O*-sulfo-α-GalCer and some 4”-arylalkyl substituted derivatives (Figure 1.14), which afford activities comparable to or even better than the parent compound.[95,96] Inversion of the 4”-OH resulting in a glucosyl derivative (α-GlcCer) partially affects the activity,[97,98] while α-ManCer is unable to induce any activity, highlighting the importance of 2”-OH configuration.[78,98]

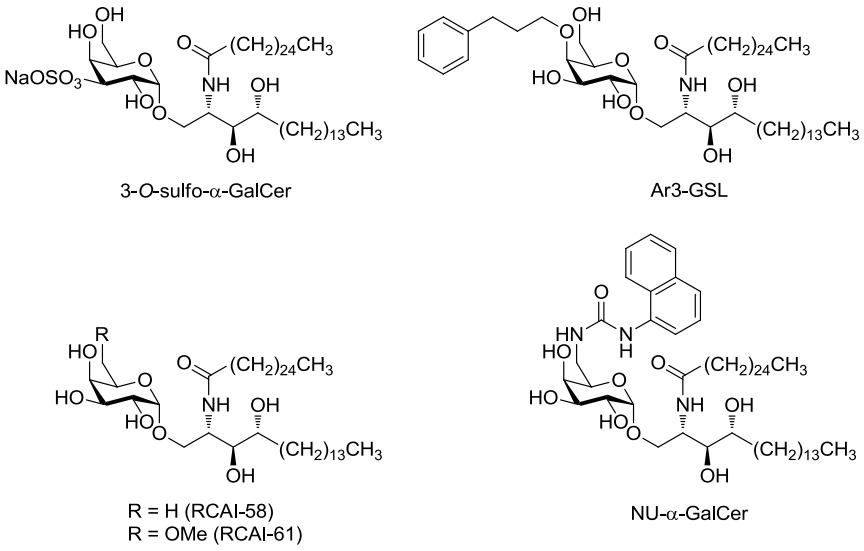


Figure 1.14 Structures of galactose-modified α-GalCer analogues.

In contrast to the secondary alcohol groups of the galactose, its primary 6”-OH group does not make any hydrogen bond with residues of CD1d nor the TCR, suggesting that modifications of this hydroxyl group may be well-tolerated. First evidence herefore was provided by a series diglycosylceramide analogues. Gal(α1→6)GalCer was able to stimulate iNKT cells without conversion to the parent monoglycosylceramide, as opposed to the corresponding diglycosylceramides at the other OH-groups (Gal(α1→2)GalCer, Gal(β1→3)GalCer and Gal(α1→4)GalCer), which lost their activity if the additional sugar cap was not enzymatically processed.[98,99] Later, Savage *et al*. demonstrated that attachment of small fluorophores or an acetamide group at the 6”-position resulted in modified α-GalCers that retained the capacity to stimulate iNKT cells.[100,101] These findings triggered the further exploration of this position and incited several research groups to synthesize 6”-modified analogues in the quest for polarizable iNKT cell ligands. For example Guzman and coworkers used a 6”-amide group to construct a water-soluble pegylated α-GalCer analogue which showed an increased biological activity skewed towards a Th2 response.[102] Mori and coworkers on the other hand reported a series of 6”-modified analogues of α-GalCer including a 6”-*O*-methylated (RCAI-61, Figure 1.14) and α-D-fucopyranosyl analogue (RCAI-58, Figure 1.14), both found superior to α-GalCer in inducing IFN-γ in mice.[103] Finally, our group recently reported a series of 6”-amide and 6”-urea derivatives that retained or even surpassed the antigenic potency of KRN7000 and, as an additional benefit, proved capable of producing a significant Th1-skewed cytokine response in vivo.[104] Among them, a urea compound referred to as NU-α-GalCer (Figure 1.14) showed marked *in vivo* antitumor effects and will be discussed in more detail below.

Apart from the numerous α-GalCer analogues modified at one of the glycosidic hydroxyl groups, also some nonglycosidic derivatives have been examined. Among them, threitolceramide (ThrCer, Figure 1.15), lacking the alcohol moieties at C-5" and C-6" of the galactose, is demonstrated to effectively activate iNKT cells.[105] Regarding the observed antigen-specific T and B cell response upon coinjection with OVA, ThrCer is considered a promising adjuvant for vaccination strategies, in particular because its weaker TCR binding affinity minimizes overstimulation of iNKT cells associated with the induction of anergy. A neoglycoside analogue, α-carba-GalCer (Figure 1.15), shares the stable ether structure of α-C-GalCer while retaining the H-bonding capacity of the glycosidic oxygen with the α2 helix of CD1d and is therefore believed to make a more stable complex with CD1d.[106] This compound results in a remarkably enhanced and prolonged IFN-γ production *in vivo* in mice and induces only half the amount of IL-4 compared to α-GalCer. Importantly, this potent Th1 polarizer can also stimulate human iNKT cells as opposed to α-C-GalCer.[107]

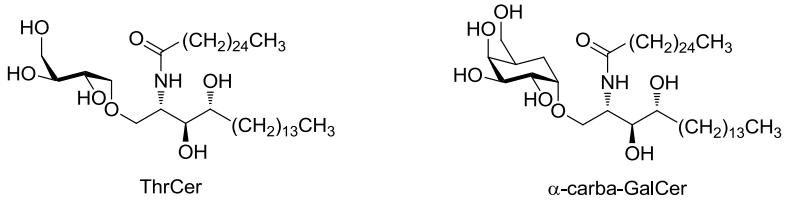


Figure 1.15 Structures of non-glycosidic α-GalCer analogues.

1.7.4 Polymodified α-GalCer analogues

With regard to the beneficial Th2 polarizing properties of OCH, Toba *et al*. prepared a series of phytosphingosine-altered OCH derivatives, including aliphatic, aromatic and ethereal compounds.[108] Compared to OCH most of these analogues induced a similar or reduced Th2 profile at the expense of antigenicity. In order to obtain a more potent Th2 immunomodulator, they also synthesized the C-glycoside variant of OCH (Figure 1.16). Remarkably, this compound was shown to have no cytokine production effect *in vitro* nor *in vivo*.

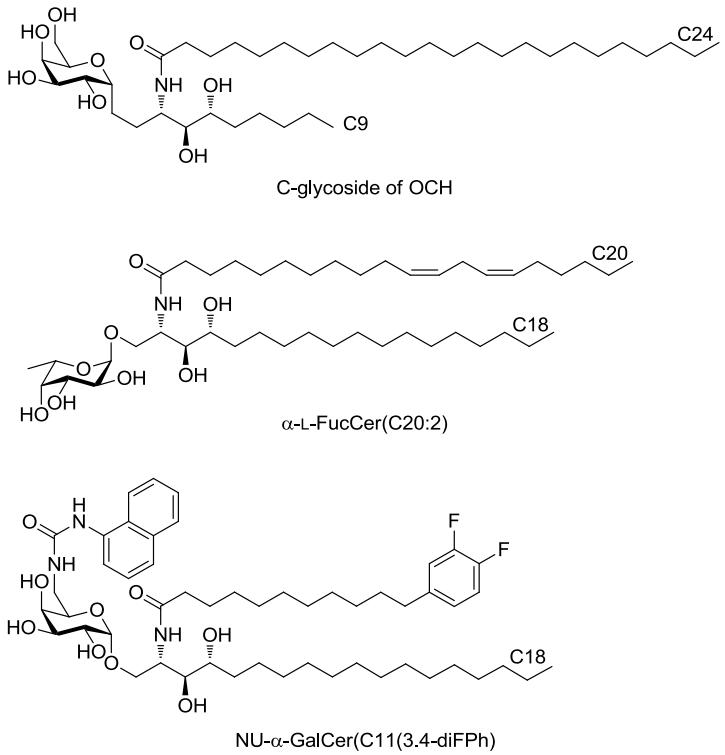


Figure 1.16 Structures of polymodified α-GalCer analogues.

Combination of a C20:2 acyl tail with an α-L-fucosyl head group also abrogates all activity (Figure 1.16).[109] Given the extremely potent biological activity exhibited by the α-GalCer variant of C20:2, this indicates that modification of the carbohydrate moiety can significantly alter the influence of the lipid moiety of the ligand on CD1d presentation and iNKT cell responses.

Finally, the group of Wong synthesized various α-GalCer analogues bearing differently substituted aromatic rings on both the acyl chain and the galactosyl 6"-position, including a C-6"-naphthylurea compound with an 11-(3,4-difluorophenyl)undecanoyl acyl chain (Figure 1.16), two Th1 featuring modifications.[110] This derivative retained or even surpassed the Th1 selectivity of the parent compounds although its potency was significantly decreased. This again illustrates that combination of two favorable modifications does not necessarily afford compounds with enhanced biological profiles.

1.8 Hypotheses concerning the polarization mechanism

As illustrated by the numerous synthetic α-GalCer analogues, the balance of Th1-Th2 cytokines can be affected by structurally different lipid antigens, representing a valuable tool for new immunotherapeutic strategies. However, the mechanisms by which alteration in

glycolipid structure controls the outcome of iNKT cell activation still remain unclear. Several explanations have been suggested including different binding affinities and pharmacokinetics, distinct cellular sites of antigen loading, and antigen targeting to different APCs.

1.8.1 Stability of the ternary complex

A well-established theory relates the induced cytokine pattern to the duration of TCR signaling, which in turn is influenced by the overall stability of the CD1d/glycolipid/TCR complex, i.e. the affinity of the TCR interaction, the half-life of the glycolipid/CD1d complex as well as the in vivo stability of the glycolipid. Oki *et al*. demonstrated that IFN-γ production by the iNKT cell requires longer TCR stimulation than for IL-4 production.[111] Furthermore, the mainstream IFN-γ following α-GalCer injection originates from trans-activated NK cells, also demanding a longer period of iNKT cell activation.[28] Consequently, ligands leading to long-lasting TCR signaling such as α-C-GalCer tend to induce a more pronounced Th1 polarization, while analogues with a short interaction time like OCH result in a Th2 bias (Figure 1.17).

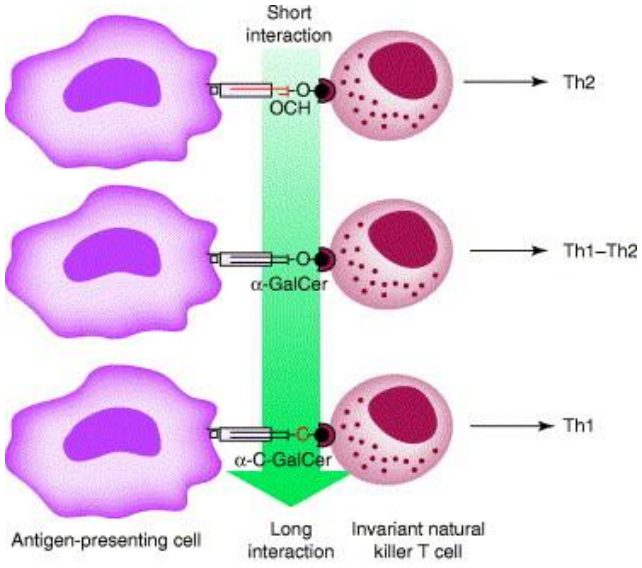


Figure 1.17 Correlation between TCR interaction time and the induced cytokine profile: Th2 polarizing analogues are characterized by a short interaction time while compounds with a Th1-skewed cytokine pattern induce long-lasting TCR signaling (Berkers and Ovaa, 2005, Trends in Pharmacol. Sci).

The reduced duration of TCR signaling by OCH is suggested to be the result of a decreased half-life of binding to CD1d as well as a reduced TCR affinity.[111,112] Indeed, McCarthy *et al*. demonstrated that shortening either the phytosphingosine or the acyl chain decreases the stability of the CD1d/glycolipid complex.[113] Interestingly, only for analogues with a truncated

phytosphingosine chain, but not for those with a shortened acyl chain, a reduced TCR affinity was observed. This is presumed to be a consequence of conformational changes within the incompletely filled F' pocket, altering the CD1d T cell recognition surface. On the other hand, CD1d molecules bound to analogues with a phenyl group in the acyl chain such as 7DW8 are characterized by a better binding avidity and stability for the TCR than α-GalCer, accounting for their Th1-skewed iNKT cell responses.[114] However, Sullivan *et al*. argued that differing CD1d/glycolipid half-lifes and TCR affinities do not provide a simple explanation for the altered cytokine profiles as α-C-GalCer binding to CD1d tends to be even shorter-lived compared to OCH and exhibits weaker binding to the TCR.[115] Conversely, C-glycoside forms long-lived functional complexes on the surfaces of APCs *in vivo* owing to its improved metabolic stability, yet allowing for a sustained activation of iNKT cells. Hence, they propose that in some cases the *in vivo* stability of the glycolipid may also be a critical determinant for the induction of polarized cytokine responses.

1.8.2 Cellular site of antigen loading

Another plausible explanation for the different cytokine profiles induced by altered glycolipid antigens involves their distinct uptake and presentation properties. It has been demonstrated that multiple analogues inducing Th2 cell-type cytokine responses are faster presented by CD1d and without requirement for intracellular loading than analogues with a mixed cytokine profile.[73,116] This might be attributed to the increased polarity and reduced hydrophobicity, which typically characterize Th2-polarizing analogues, as increased solubility facilitates access to the lipid binding site of cell surface expressed CD1d molecules. Furthermore, it has been shown that intracellular loading of a compound followed by lysosomal processing leads to accumulation of the glycolipid/CD1d complex into lipid rafts, whereas direct loading of CD1d molecules on the surface of the cell results in exclusion from such domains (Figure 1.18).[116] This might promote distinct TCR signaling, effectuating different iNKT cell responses.

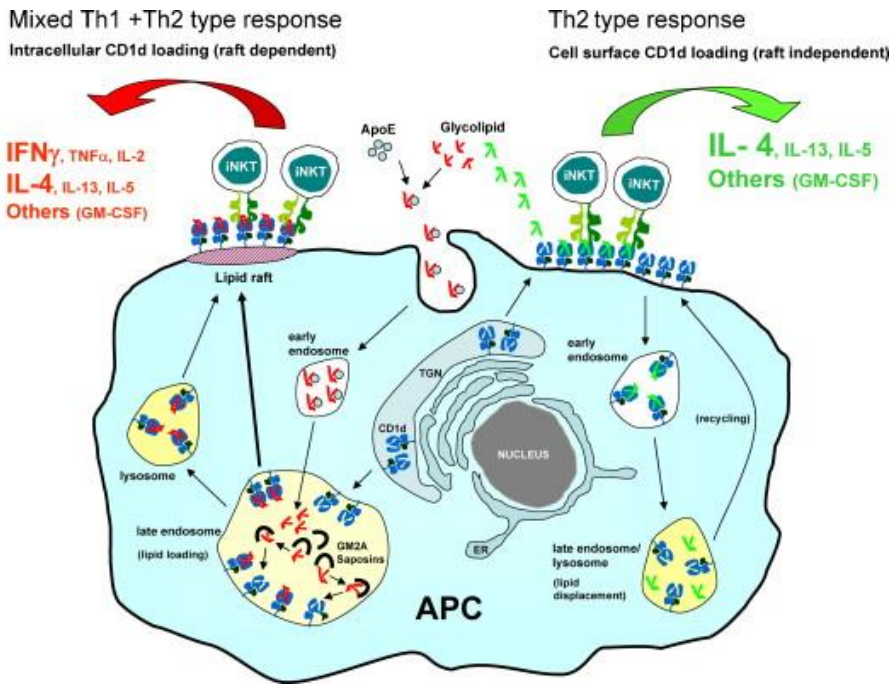


Figure 1.18 CD1d loading and intracellular processing affects the iNKT cell response: mixed Th1/Th2 inducers require internalization prior to CD1d loading, followed by lysosomal processing and preferential presentation in lipid rafts as opposed to Th2 polarizers, which are directly loaded onto CD1d molecules at the surface of APCs (Venkataswamy and Porcelli, 2010, Sem. Immunol.).

1.8.3 Presentation by different APCs

A third, alternative mechanism suggests that the different cytokine production of α-GalCer variants might be the consequence of presentation by different types of APCs. This assumption relies on a study in which iNKT cells stimulated by α-GalCer displayed by $CD1^+$ Schwann cells predominantly secrete Th2 cytokines.[117] Similarly, Besbradica *et al*. found that presentation of α-GalCer by B cells skews the iNKT cell outcome towards a Th2-type response.[118] Although other studies could not provide evidence for a correlation with the APC type used, a more recent study demonstrated that α-GalCer presentation requires CD1d expression by DCs, while presentation of Th2 variants is rather promiscuous.[119] Since the second and long-lasting wave of IFN-γ secretion is dependent on IL-12 derived from DCs, these analogues fail to induce a sustained IFN-γ production, accounting for the observed Th2 bias.

1.9 A closer look at NU-α-GalCer

NU-α-GalCer, one of the C-6”-modified compounds previously synthesized in our lab, is nowadays known as a strong Th1 polarizer, characterized by a markedly elevated IFN-γ and IL-12 production in mice compared to α-GalCer.[120] Additionally, in a B16 melanoma long

metastases model, NU-α-GalCer provided superior antitumor effects, in particular when loaded onto BMDCs. Interestingly, its Th1-skewing capacity was also observed in cultures of human peripheral blood mononuclear cells (PBMC) and purified human iNKT cells, in contrast to α-C-GalCer.

In order to gain more insight into the mechanism underlying the observed Th1 polarization, the crystal structure of NU-α-GalCer in complex with CD1d and the TCR was examined and compared to that of α-GalCer. This study demonstrated that all hydrogen bond interactions with the TCR and CD1d described for α-GalCer are conserved (Figure 1.19). Moreover, an additional H-bond is formed between the carbonyl oxygen of the urea linker and a threonine residue of CD1d. Strikingly, whereas the naphthyl group was expected to be exposed into the solvent, it was found facing down towards CD1d.

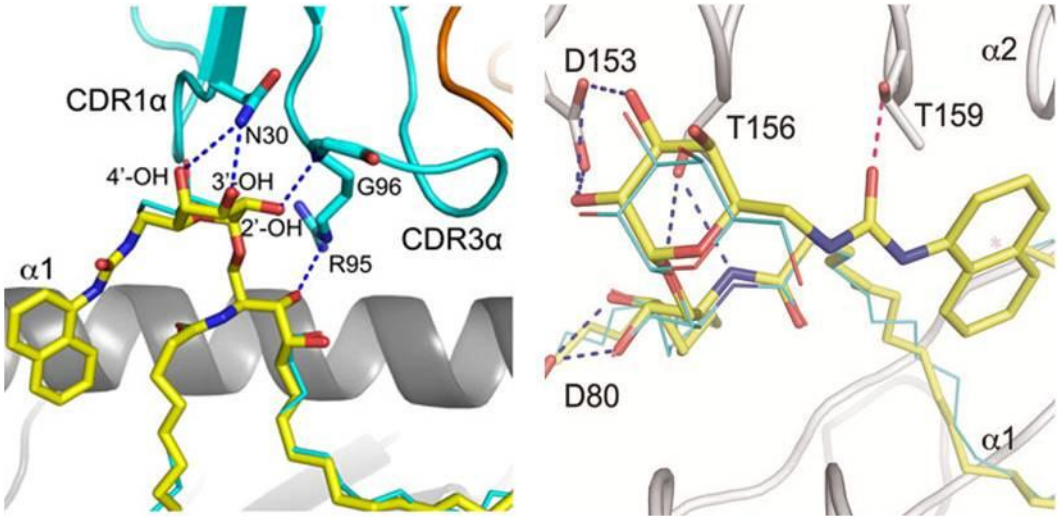


Figure 1.19 Crystal structure of the ternary complex with NU-α-GalCer: The left panel shows the binding of NU-α-GalCer (yellow) with CD1d and the TCR superimposed on α-GalCer (blue), highlighting the conserved H-bond interactions with the TCR and the downwards orientation of the naphthyl ureum group to CD1d. The right panel shows the conserved H-bond interactions with CD1d and the additional H-bond with Thr159.

A more detailed view of the binding mode of the galactose substituent learned that, in order to accommodate the naphthyl group, the α1 and α2 helices of CD1d are opened laterally, creating a small hydrophobic pocket on top of the A' roof (Figure 1.20). This binding pocket, also referred to as a third anchor, provides additional hydrophobic interactions with CD1d and, together with the additional hydrogen bond, enhances the stability of the complex with CD1d. This is clearly reflected by the higher affinity measured for CD1d and is proposed to account for the predominant Th1 cytokine secretion, in agreement with the first hypothesis.

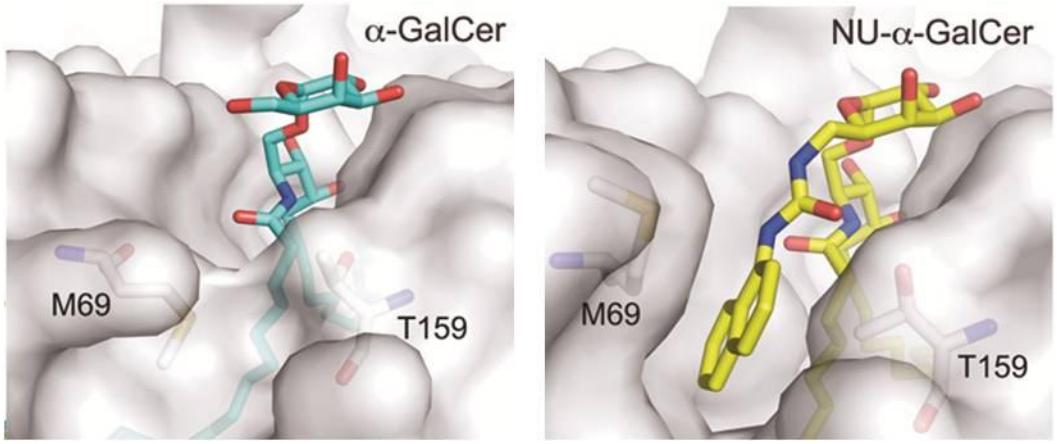
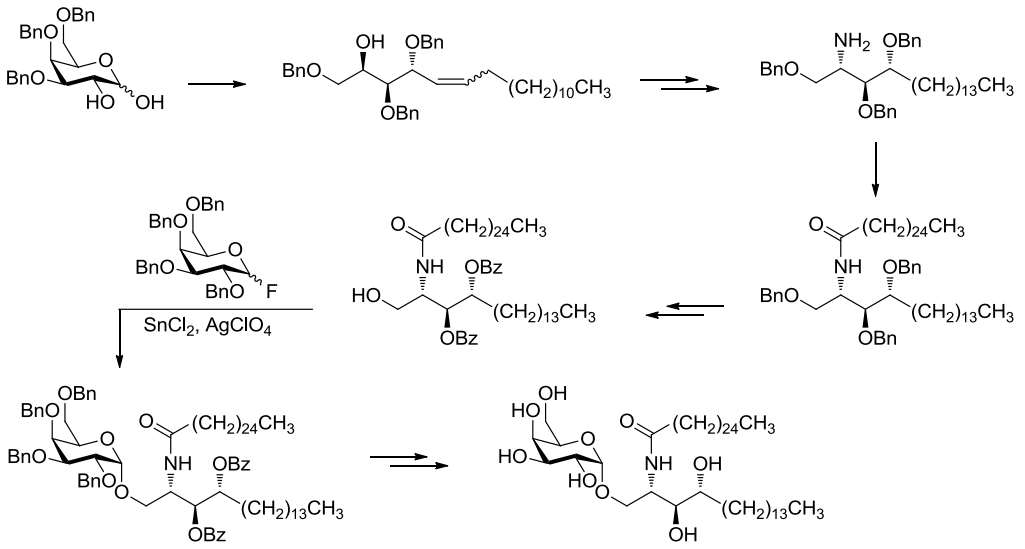


Figure 1.20 The left panel shows the binding of α-GalCer in CD1d with the galactosyl head group protruding at the surface of the antigen binding groove. The right panel shows the same for NU-α-GalCer illustrating the induction of the additional hydrophobic pocket by the naphthyl group.

1.10 Synthetic pathways towards α-GalCer

The small amount of agelasphins present in the marine sponge *Agelas mauritianus* prompted Morita and coworkers in 1993 to the initial synthesis of agelasphin 9b, the main compound among these active substances.[121] The difficult scale-up, however, incited the search for another candidate that could be synthesized more easily, and soon led to the first synthesis of α-GalCer by the same group (Scheme 1.1).[27] Since then, a wide variety of different synthetic strategies have been explored to optimize the synthesis of α-GalCer and related analogues.

Preparation of α-GalCer roughly involves the connection of three fragments, i.e. the galactose part, the D-*ribo*-phytosphingosine moiety and the acyl chain. Herefore, the two most convenient pathways are coupling of a properly protected sugar moiety with a totally functionalized ceramide part, or with a phytosphingosine building block followed by *N*-acylation.[122] Although less convergent, insertion of both fatty chains after the glycosidation step has also been described, allowing the development of α-GalCer analogues with various structural motifs in the lipid chain. Regardless of the chosen methodology, the major difficulty is the stereoselective formation of the glycosidic bond (vide infra), constituting the subject of many optimization attempts.

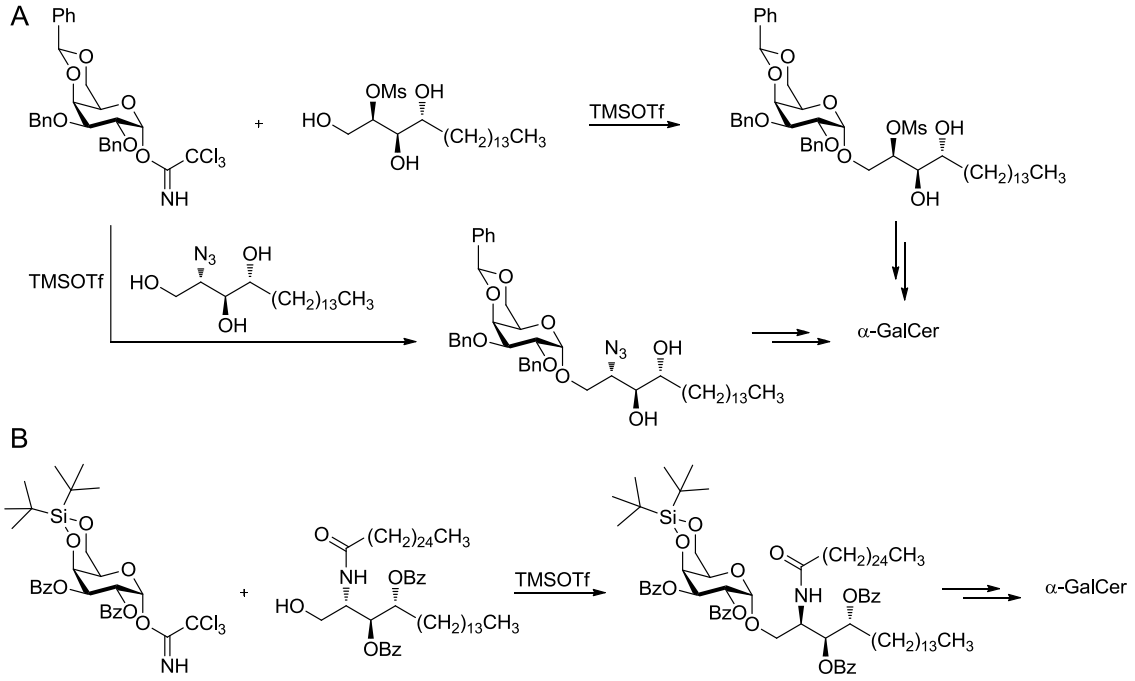


Scheme 1.1 First synthesis of α-GalCer by Morita *et al.*

The procedures found in the literature for the synthesis of D-*ribo*-phytosphingosine and its azido derivative are mainly based on two approaches, i.e. the chiral pool approach and the asymmetric induction approach.[123] In the former method, stereoselective synthesis of D-*ribo*-phytosphingosine starts from a naturally occurring chiral precursor, typically carbohydrates including D-galactose, D-lyxose and D-galactal. Other popular chiral starting materials comprise serine derivatives such as Garner's aldehyde. Asymmetric synthesis procedures by contrast use chiral auxiliaries and enantioselective catalytic reactions to introduce the desired chirality. Nowadays, however, D-*ribo*-phytosphingosine is commercially available as a derivative resulting from a yeast fermentation process, and therefore, these methods will not be discussed into more detail.

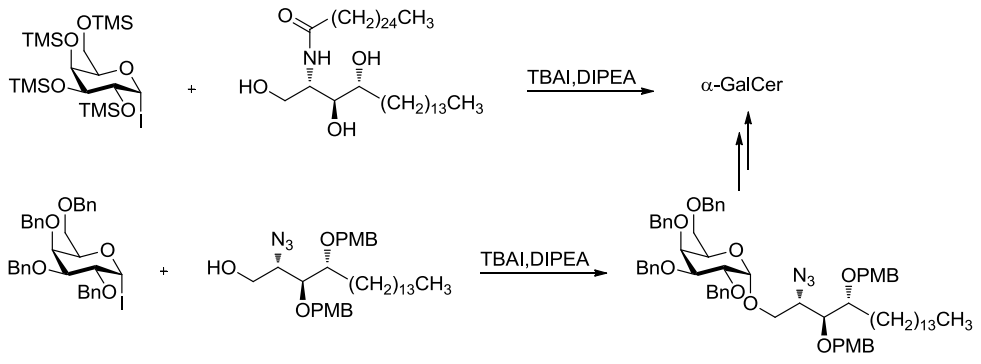
A drawback associated with the direct glycosylation of ceramide derivatives is the low product yield due to unfavorable hydrogen bond formation between the amide and the primary hydroxyl group, diminishing the nucleophilicity of the latter.[124] This can be ameliorated either by using a phytospingosine precursor as acceptor or by increasing the reactivity of the galactosyl donor. For example, Schmidt and coworkers elaborated a highly regio- and stereoselective glycosylation procedure with a 1,3,4-*O*-unprotected phytosphingosine derivative, taking advantage of the α-directing effect of a 4,6-*O*-benzylidene-protected trichloroacetimidate donor (Scheme 1.2, A).[125] Likewise, 4,6-di-*tert*-butylsilylene (DTBS)-protected galactosyl donors have been successfully applied in the synthesis of α-GalCer, for instance by the group of Kiso (Scheme 1.2, B).[126] This donor type

is reported as a powerful α-galactosylating agent, allowing the coupling of both ceramide and phytosphingosine derivatives.



Scheme 1.2 (A) Synthesis of α-GalCer by Schmidt *et al*. (B) Synthesis of α-GalCer by Kiso *et al*.

Another highly reactive glycosyl donor, recently introduced by Gervay-Hague in the convergent synthesis of α-GalCer, is per-*O*-silylated galactosyl iodide (Scheme 1.3, top).[127] α-Glycosylation with this donor involves *in situ* anomerization mediated by tetrabutylammonium iodide (TBAI). A similar methodology can be applied on a benzyl-protected galactosyl iodide in the glycosylation with azidophytosphingosine, affording the desired α-product in excellent yield and stereoselectivity (Scheme 1.3, bottom).[128] Inspired by the work of Gervay-Hague, Castillón and coworkers carried out a glycosylation reaction with the same galactosyl iodide donor, yet with a 1,3-*O*-stannyl ether-protected ceramide moiety.[129]



Scheme 1.3 Synthesis of α-GalCer by Gervay-Hague *et al*.

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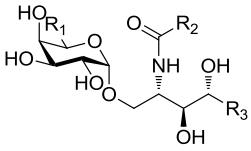
CHAPTER 2

OBJECTIVES

2 Objectives

In view of the interesting iNKT-cell mediated responses induced by the aforementioned C-6"-derivatised α-GalCer analogues (summarized in Table 2.1), in particular NU-α-GalCer, we wish to further explore modifications at this position. Via the synthesis of a wide range of C-5"- and C-6"-altered α-GalCer compounds, we aim to obtain new iNKT cell ligands with enhanced biological profiles. Furthermore, such compounds may contribute to our understanding of the cytokine polarization mechanism. Towards this end we want to develop a divergent synthetic approach that allows late-stage C-6"-functionalization as well as introduction of different acyl chains. As such, beneficial C-6" modifications can be combined with alternate acyl chains as in C6Ph (Table 2.1) to examine their mutual influence on the iNKT cell response.



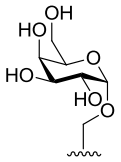
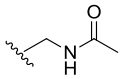
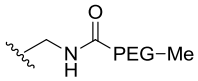
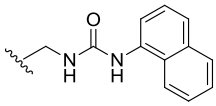
Glycolipid	R_1	R_2	R_3	Activity
Gal(α1→6)GalCer		$(CH_2)_{24}CH_3$	$(CH_2)_{13}CH_3$	iNKT cell activation similar to α-GalCer[a]
PBS-57		$(CH_2)_{13}CH=CH(CH_2)_7CH_3$	$(CH_2)_{13}CH_3$	Antigenicity similar to α-GalCer[b]
PEG-GalCer		$(CH_2)_{24}CH_3$	$(CH_2)_{13}CH_3$	Th2, stronger antigenicity than α-GalCer[c]
RCAI-58	CH_3	$(CH_2)_{24}CH_3$	$(CH_2)_{13}CH_3$	More potent IFN-γ inducer than α-GalCer[d]
RCAI-61	OCH_3	$(CH_2)_{24}CH_3$	$(CH_2)_{13}CH_3$	More potent IFN-γ inducer than α-GalCer[d]
NU-α-GalCer		$(CH_2)_{24}CH_3$	$(CH_2)_{13}CH_3$	Th1, antigenicity similar to α-GalCer[b]
C6Ph	CH_2-OH	$(CH_2)_5Ph$	$(CH_2)_{13}CH_3$	Th1, stronger antigenicity than α-GalCer[e]
OCH	CH_2-OH	$(CH_2)_{22}CH_3$	$(CH_2)_4CH_3$	Th2, weaker antigenicity than α-GalCer[b]

Table 2.1 Summary of the SAR relevant to this work.
[a] based on IL-2 measurement (mice, *in vitro*), [b] based on IL-4 and IFN-γ measurement (mice, *in vivo*), [c] based on IL-4 and IFN-γ measurement (mice, *in vitro*), [d] based on IFN- γ measurement (mice, *in vivo*), [e] based on IL-4 and IFN-γ measurement (human, *in vitro*).

At first, this method will be applied for the synthesis of α-D-fucopyranosyl analogues **1** and **2**, and galacturonic analogues **3** and **4** to prove its versatility (Figure 2.1). Compounds **1** and **3** are two known Th1 polarizers, while **2** and **4** are novel derivatives thereof, in which the conventional acyl moiety is replaced by a C6Ph moiety. The latter is known to considerably enhance the overall production of both Th1 and Th2 type cytokines and to skew the balance toward a Th1 type response.

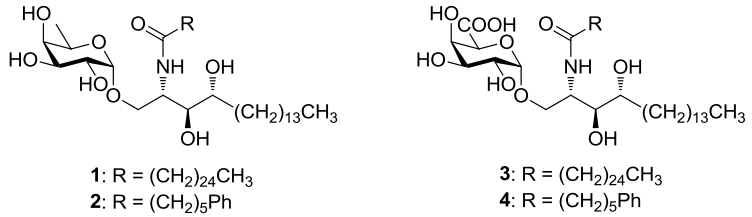


Figure 2.1 Structures of the α-D-fucopyranosyl and galacturonic analogues.

In order to gain more insight into the importance of the third anchor induced by NU-α-GalCer, and to determine the structural requirements to induce this anchor, we will explore closely related analogues, including C-6"-carbamates (**5**), uronamides (**6**) and C-5"-carbamate/urea derivatives (**7**) (Figure 2.2). Substitution of the urea functionality by a carbamate group provides more flexibility to the linker. Introducing an amide or carbamate/urea group at the C-5" position alters the distance of the carbonyl oxygen and the aryl substituent with respect to the carbohydrate moiety. Such modifications might change the interaction with CD1d and/or the TCR and, as a consequence, might influence the outcome of iNKT cell activation.

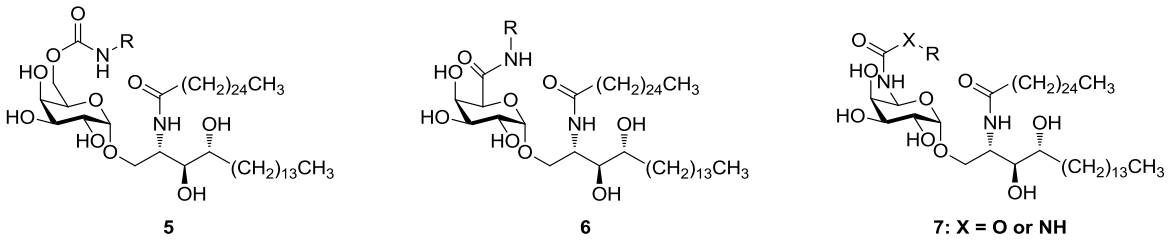
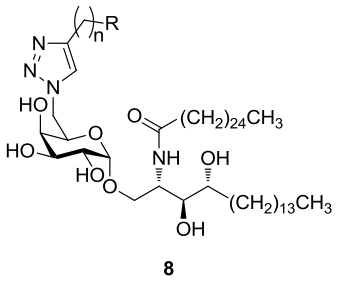


Figure 2.2 General structure of the envisioned C-6"-carbamates, uronamides and C5"-carbamate/urea derivatives.

Additionally, we intend to replace the Th1-featuring C-6"-amide functionality by a 1,2,3-triazole moiety (**8**, Figure 2.3). A 1,4-disubstituted triazole group, suggested as a bioisostere of a *trans*-amide bond, can be conveniently installed using a copper-catalyzed azide-alkyne cycloaddition reaction. Due to the commercial availability of a diverse array of aromatic-

substituted alkynes, the linker length can be easily varied, allowing to investigate its influence on the iNKT cell activity.



8

Figure 2.3 General structure of the C-6”-triazoles.

Crystal structure studies of α-GalCer complexed to CD1d and the TCR demonstrated that the lipid chains of the ceramide part are buried into the binding groove of CD1d and thus do not contact the TCR. Yet, truncation of the lipid chains appears to be sensed by the TCR, as illustrated by the lower TCR affinity for analogues with shorter phytosphingosine chains. This is attributed to a different CD1d T cell recognition surface and is likely to affect the bioactivities of sugar-modified α-GalCer analogues. Therefore we will combine interesting C-5” and C-6”-modifications with an OCH-like ceramide (**9**, Figure 2.4) and assess the effect on the iNKT cell response. Furthermore, glycolipids with shorter lipid chains are associated with an enhanced solubility in aqueous media, facilitating the biological experiments.

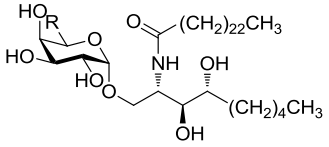


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Figure 2.4 General structure of C-5”- and C-6”-modified OCH derivatives.

Finally, the established methodology will be exploited for the synthesis of C-6”-labeled α-GalCer analogues **10** and **11** (Figure 2.5). Whereas the attached boron dipyrromethene (BODIPY) group in OCH derivative **10** is known for its excellent fluorescent properties, analogue **11** will serve as a reagent in the Chloramine-T method to obtain a radioiodine-labeled molecule. Both labeled compounds represent valuable biological tools and will prove useful in the implementation of competition binding assays by other research groups.

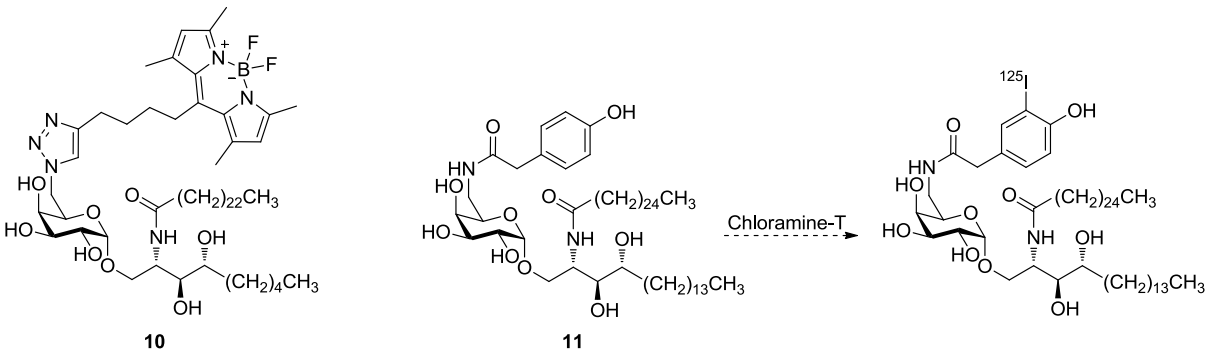


Figure 2.5 Structure of C-6”-labeled α-GalCer analogues.

In addition to the beneficial effects of activating iNKT cells, inappropriate or excessive activation can be detrimental for the host. For example, it can result in inflammatory or allergic disorders, such as bronchial asthma. In that regard, blockade of the iNKT cell response by a CD1d-dependent antagonist might be useful. Therefore we intend to synthesize a C-6”-naphthylureido-substituted α-mannosylceramide derivative (NU-α-ManCer, **12**, Figure 2.6), in which the naphthyl urea group is assumed to enhance the CD1d affinity, while the different orientation of the 2”-OH and 4”-OH groups of the mannosyl head group is expected to prevent interaction with the iNKT TCR.

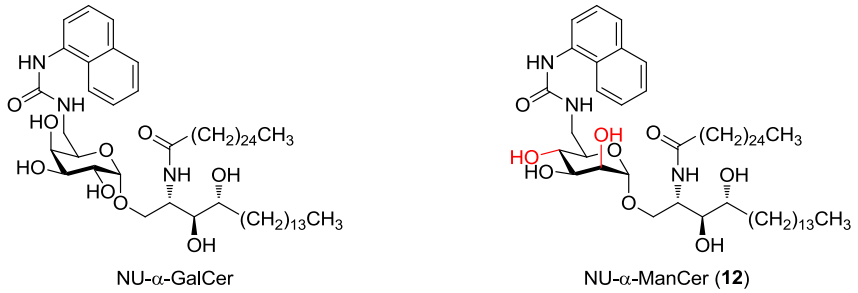


Figure 2.6 Structure of the known NU-α-GalCer and the envisaged NU-α-ManCer.

CHAPTER 3

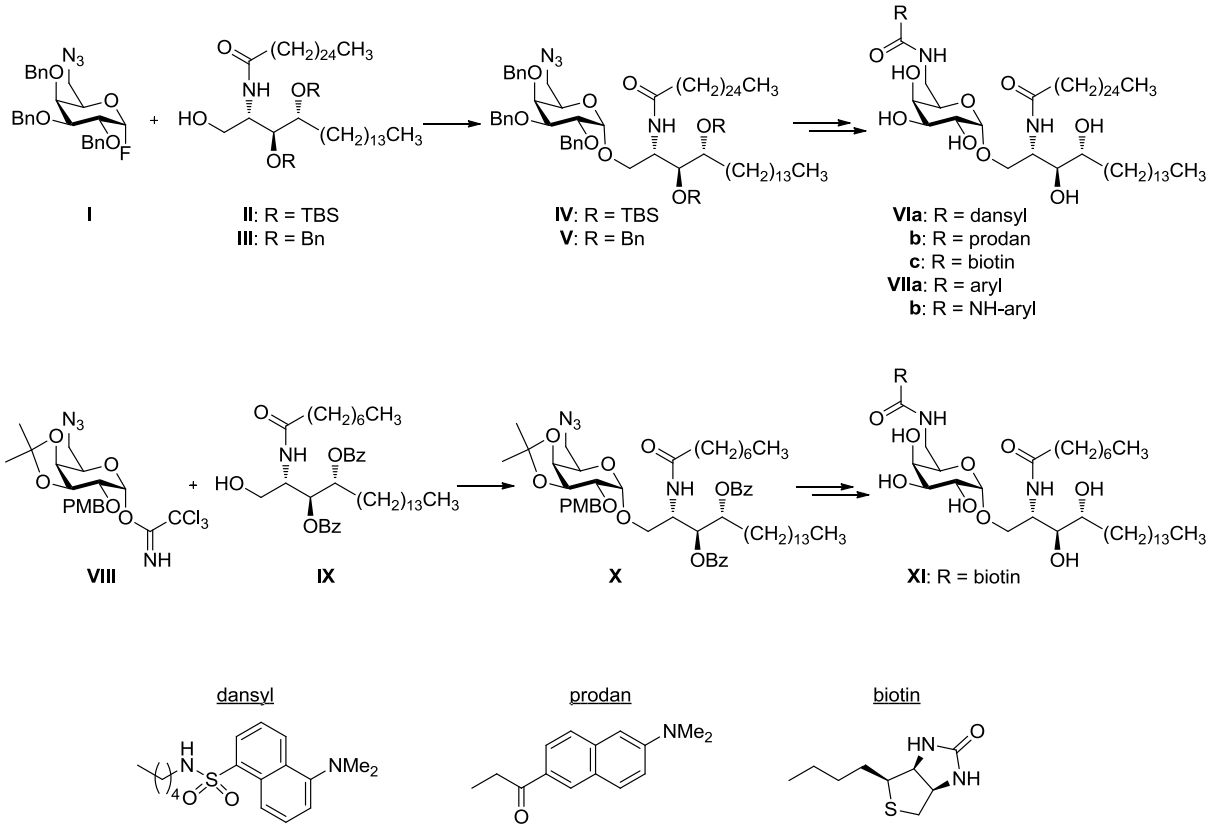
DEVELOPMENT OF A DIVERGENT APPROACH TOWARDS C-5”- AND C-6”-MODIFIED α-GALCER ANALOGUES

The content of this chapter was partially derived from:
Pauwels, N.; Aspeslagh, S.; Vanhoenacker, G.; Sandra, K.; Yu, E. D.; Zajonc, D. M.; Elewaut, D.; Linclau, B.; Van Calenbergh, S. *Org. Biomol. Chem.* **2011**, *9*, 8413-842.

3 Development of a divergent approach towards C-5”- and C-6”-modified α-GalCer analogues

3.1 Introduction

As outlined in the general introduction, several C-6”-modified α-GalCer analogues have already been synthesized. However, they concern mainly 6”-*N*-derivatized compounds, whereas we envisage both 6”-*O*- and 6”-*N*-alterations as well as 5”-modifications. This has crucial implications on the synthetic approach as the previously established methods are rather limited in their scope.



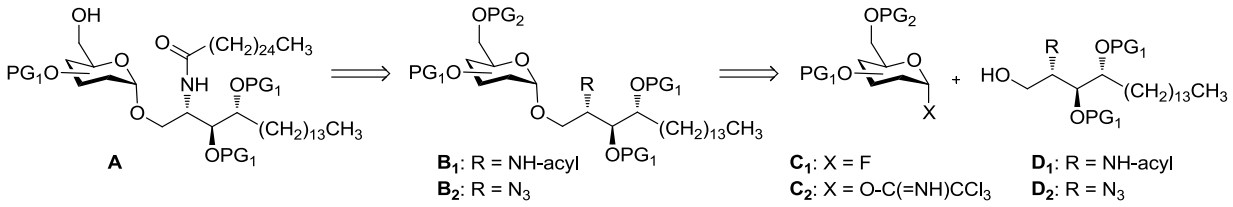
Scheme 3.1 Top: Synthetic strategy used by Zhou *et al*. for the preparation of C-6”-fluorophore- and biotin-labeled α-galactosylceramides **VIa-c** and adapted by Trappeniers *et al*. for the synthesis of C-6”-amides **VIIa** and urea derivatives **VIIb**. Middle: Synthetic strategy used by Wang *et al*. for the preparation of C-6”-biotin-labeled α-GalCer **XI**. Bottom: Structures of fluorophores and biotin.

For example, the strategy reported by Zhou *et al*. for the preparation of their C-6”-fluorophore- and -biotin-labeled α-galactosylceramides **VIa-c** consists of coupling benzyl-protected 6-azido-6-deoxy-galactosyl fluoride **I** with ceramide acceptor **II** followed by azide

reduction, deprotection and incorporation of the small fluorophores through amide bond formation (Scheme 3.1, top).[1] A slightly modified version of this methodology using ceramide acceptor **III** has been adapted by our group for the synthesis of aromatic substituted C-6"-amide and urea derivatives **VIIa** and **VIIb**.[2] Similarly, Wang and coworkers used 6-azido-galactosyl donor **VIII** in a glycosylation reaction with ceramide acceptor **IX** to produce C-6"-biotin-labeled α-GalCer **XI** (Scheme 3.1, middle).[3] Consequently, by incorporating the amine functionality early onto the carbohydrate masked as an azide, these strategies are restricted to 6"-*N*-derivatized compounds. Therefore, we want to explore a more divergent synthetic approach giving access to a wide variety of C-5" and C-6"-modified α-GalCer analogues.

An important aspect in our synthetic strategy is the stereoselective outcome in the glycosylation process, a major challenge in carbohydrate chemistry. In order to obtain optimal recognizable compounds for the iNKT TCR, an α-glycosidic bond is required. For galactose derivatives, this corresponds to the preparation of 1,2-*cis* glycosides which in general is more demanding than that of 1,2-*trans* glycosides. Yet, this linkage type can be formed by working in appropriate solvents under thermodynamic conditions.[4] Moreover, the stereoselectivity can be greatly enhanced by using a suitable galactosyl donor, preferably with a non-participating protecting group at the C-2 hydroxyl such as a benzyl group. Concerning the anomeric leaving group, three commonly employed donors in the synthesis of α-GalCer and related compounds are galactosyl fluoride,[1,5,6] galactosyl trichloroacetimidate,[7,8,9] and galactosyl iodide.[10,11] The latter has recently gained more interest and was shown to be an excellent α-directing donor in the glycosylation of unprotected ceramides by the group of Gervay-Hague.[12] However, previous elaboration of this methodology in our lab was found unsuccessful and therefore it is not considered in the current strategy. Finally, the desired late-stage introduction of diverse C-5"- and C-6"-functionalities implicates the use of a 6"-*O*-orthogonal protecting group, which is easily removable after the glycosylation process.

Taken together, we consider general structure **A** in Scheme 3.2 an ideal intermediate towards C-5"- and C-6"-modifications, compelling the synthesis of an appropriate C-6"-*O*-orthogonal protected α-galactoside **B**. Towards this end, two methods coupling galactosyl fluoride $\mathbf{C_1}$ or trichloroacetimidate $\mathbf{C_2}$ with ceramide moiety $\mathbf{D_1}$ or its azido precursor $\mathbf{D_2}$, respectively, will be attempted and evaluated for their α-selectivity.

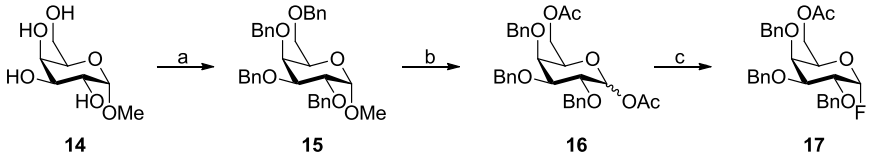


Scheme 3.2 General approach towards versatile intermediate **A**.

3.2 Strategy involving Mukaiyama glycosylation

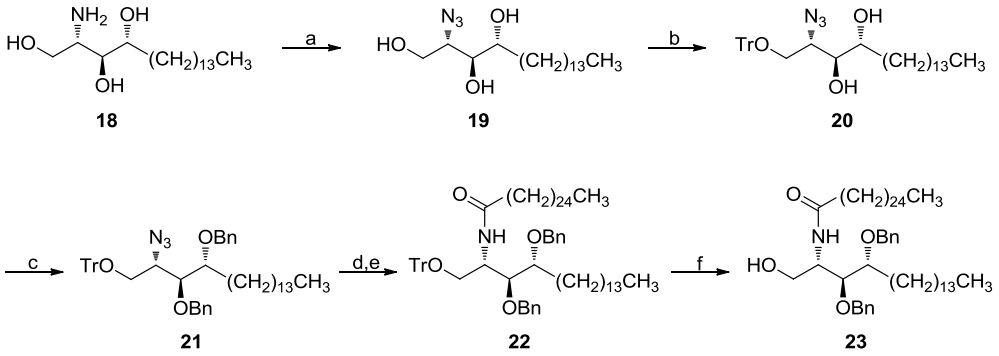
Since 1981 glycosyl fluorides have been utilized for effective glycosylation reactions because of their enhanced stability, ease of handling and higher stereoselectivity compared with other glycosyl halides. In a method developed by Mukaiyama and coworkers these practical glycosyl donors are activated by stannous(II) chloride, known to give predominantly 1,2-*cis* glycosides especially in the presence of silver perchlorate as a copromotor.[13]

Preparation of galactosyl fluoride donor **17** started by benzylation of the commercially available methyl-α-D-galactopyranoside **14** (Scheme 3.3). For the orthogonal C-6”-*O*-protection, we relied on the well-established acetolysis of benzyl ethers initialized by sulfuric acid and acetic anhydride.[14] Although Yang *et al*. reported that selective 6-*O*-debenzylation and simultaneous cleavage of the methyl glycosidic bond required a large excess of freshly fused $ZnCl_2$,[15] we similarly attained **16** by treatment with acetic anhydride, acetic acid and a catalytic amount of sulfuric acid. Subsequent introduction of the anomeric fluoride utilizing pyridinium polyhydrogen fluoride afforded exclusively the α-fluoride **17**.[16]



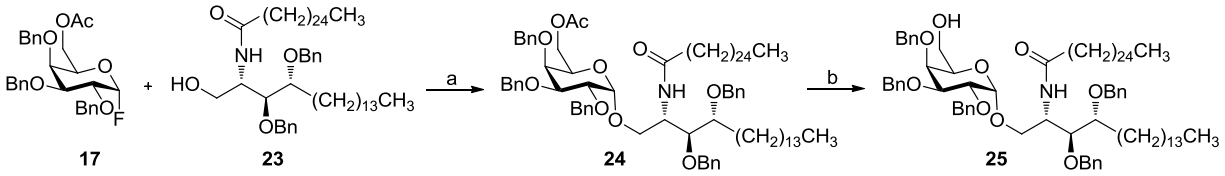
Scheme 3.3 Reagents and conditions: (a) NaH, BnBr, DMF, 0 °C-rt, overnight, 90%; (b) Ac_2O, AcOH, H_2SO_4, 0 °C-rt, overnight, 81%; (c) HF.pyridine, toluene, 0 °C-rt, 7 h, 74%.

The synthesis of ceramide **23** started from the readily available D-*ribo*-phytosphingosine **18** (Scheme 3.4). Metal-catalyzed diazotransfer afforded azidophytosphingosine **19**, which was submitted to a protocol analogous to Kratzer *et al*.[17] This consisted of selective tritylation of the primary OH followed by per-benzylation to give **21**. Staudinger reduction and acylation of the resulting amine with hexacosanoic acid in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) as a condensing agent afforded **22**. Final detritylation furnished the desired ceramide building block **23**.



Scheme 3.4 Reagents and conditions: (a) i. NaN_3, Tf_2O, CH_2Cl_2, H_2O, 0 °C, 2 h; ii. K_2CO_3, $CuSO_4$, H_2O, MeOH, rt, overnight, 76%; (b) TrCl, DMAP, pyridine, 70 °C, overnight, 85%; (c) NaH, BnBr, DMF, 0 °C-rt, overnight, 59%; (d) i. PMe_3, THF, rt, 2 h; ii. NaOH 1M, rt, 2 h; (e) $C_{25}H_{51}COOH$, EDC, CH_2Cl_2, rt, overnight, 83%; (f) $ZnBr_2$, CH_2Cl_2/iPrOH (8.5/1.5), rt, overnight, 85%.

With the galactosyl donor and ceramide acceptor in hand, Mukaiyama glycosylation could be performed resulting in the fully protected galactosylceramide **24** with an α/β ratio of 4/1 and a general yield of 69% (Scheme 3.5). Deacetylation under basic conditions then provided versatile intermediate **25**, which enables introduction of a variety of C-6”-modifications.



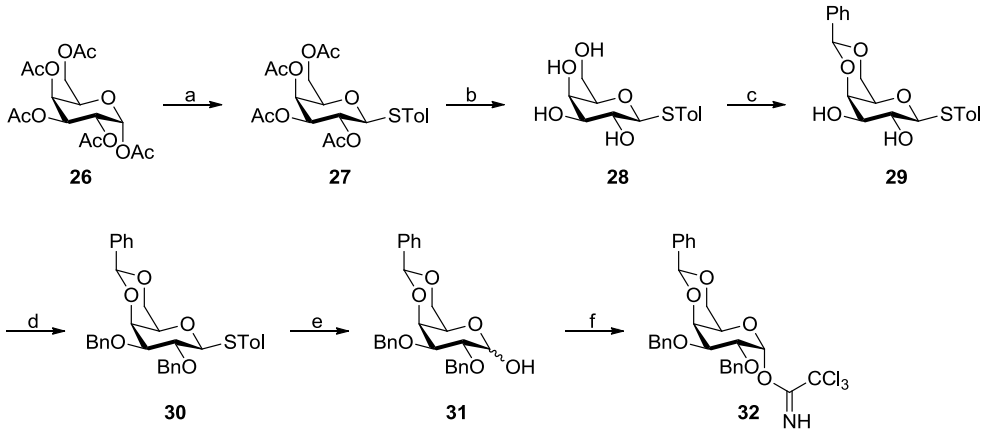
Scheme 3.5 Reagents and conditions: (a) $SnCl_2$, $AgClO_4$, THF, -10 °C, 1.5 h, 52%; (b) NH_3, MeOH, $CHCl_3$, rt, 48 h, 89%.

3.3 Strategy involving Schmidt glycosylation

Glycosyl trichloroacetimidates, introduced by Schmidt and Michel in 1980,[18] are currently among the most popular glycosyl donors for *O*-glycosylation.[19] Depending on the nature of the protecting group, solvent and the catalyst, α- or β- glycosides are usually formed with high stereoselectivity.[4,22] In particular for the synthesis of galactosyl cerebrosides, Schmidt demonstrated excellent α-selectivity by using a 4,6-*O*-benzylidene-protected galactosyl derivative.[7] This has been attributed to the *cis*-decalin ring system with the equatorial phenyl group preventing attack from the β-face.[20] Since the selective ring opening of 4,6-*O*-benzylidene-protected carbohydrates is a well-described method for obtaining protected saccharides having a free 6-OH group,[21] we decided to exploit a 4,6-*O*-benzylidene-protected galactosyl donor for the synthesis of our target intermediate. Contrary to the Mukaiyama procedure, this method is preferentially used for coupling azidosphingosines rather than

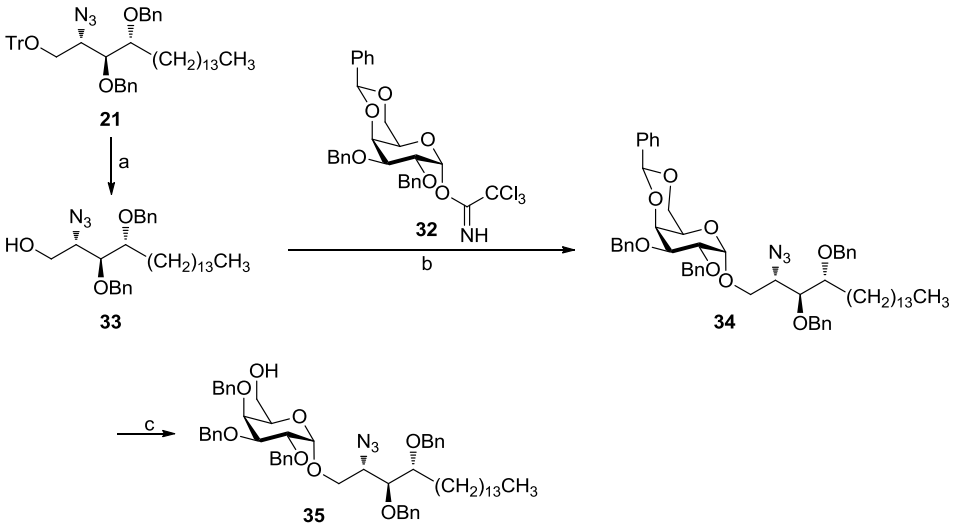
ceramides to circumvent the low nucleophilicity of ceramides associated with poor glycosylation yields.[22,23]

So, the synthesis of trichloroacetimidate intermediate **32** was started by treatment of penta-acetylated α-D-galactose **26** with *p*-thiocresol and BF_3.etherate yielding thiogalactoside **27**, followed by global Zemplen deacetylation to give **28** (Scheme 3.6). Next, introduction of a 4,6-*O*-benzylidene acetal was accomplished by treatment with benzaldehyde dimethylacetal and *p*-toluenesulfonic acid (*p*-TSA). Consecutive benzylation and removal of the anomeric thiotolyl group with *N*-iodosuccinimide (NIS) afforded **31**, which was then reacted with trichloroacetonitrile and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to selectively furnish the desired trichloroacetimidate **32** as the α-anomer.



Scheme 3.6 Reagents and conditions: (a) *p*-thiocresol, $BF_3.Et_2O$, CH_2Cl_2, 0 °C, 1 h, 80%; (b) NaOMe, MeOH, 0 °C-rt, 3 h; (c) benzaldehyde dimethylacetal, *p*-TSA, CH_3CN, rt, 5 h, 98% over 2 steps; (d) BnBr, NaH, DMF, 0 C-rt, 2 h, 79%; (e) NIS, acetone, H_2O, 0 °C-rt, 2.5 h, 95%; (f) Cl_3CCN, DBU, CH_2Cl_2, 0 °C, 5 h, 83%.

Azidophytosphingosine acceptor **33** could be easily obtained via detritylation of intermediate **21** and was subsequently used for trimethylsilyl trifluoromethanesulfonate (TMSOTf)-catalyzed coupling with galactosyl trichloroacetimidate **32** to acquire α-glycoside **34** in high yields (Scheme 3.7). Only a negligible amount of the β-anomer was observed, which could be easily removed by flash chromatography.



Scheme 3.7 Reagents and conditions: (a) $ZnBr_2$, CH_2Cl_2/iPrOH (8.5/1.5), rt, overnight, 51%; (b) TMSOTf, THF, -30 °C, 80%; (c) BH_3·THF, $Cu(OTf)_2$,CH_2Cl_2, 78%.

In order to address the 6”-position for further modifications, a regioselective opening of the benzylidene ring was required. This key step could be realized by treating **34** with BH_3.THF and $Cu(OTf)_2$, giving intermediate **35** (Scheme 3.7).[21] The regioselective opening was confirmed by combination of a COSY and HSQC experiment (Figure 3.1), which unambiguously proved that the carbon attached to the hydroxyl group possesses two protons, indicating that the free hydroxyl group was connected to C-6”.

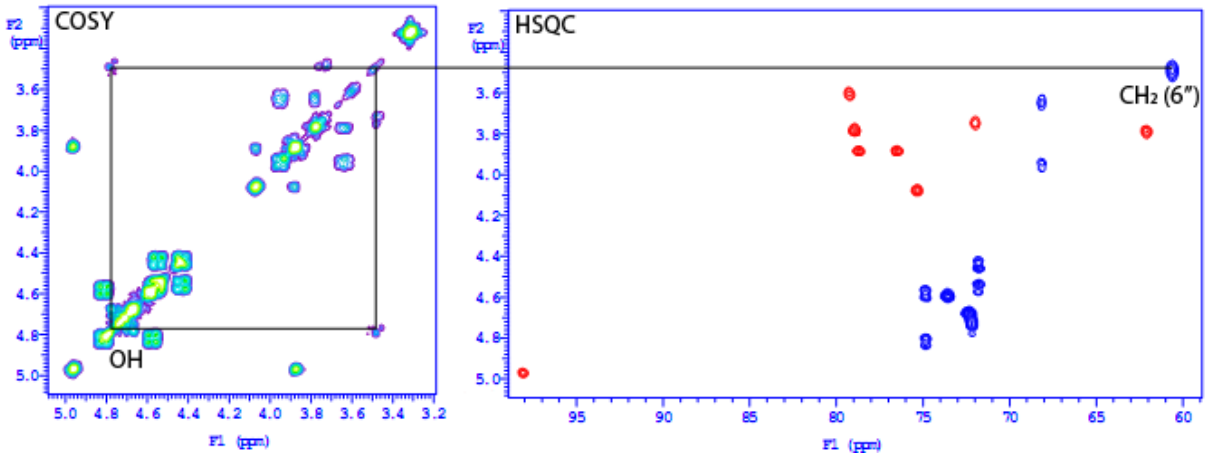


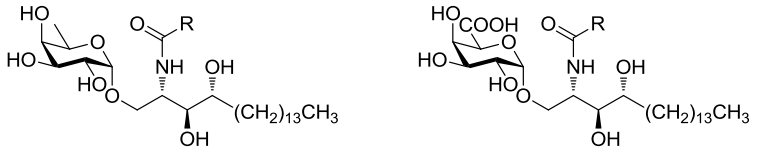
Figure 3.1 COSY and HSQC experiment of compound **35**.

Conclusively, both methods efficiently allow to synthesize a versatile intermediate towards C-5”- and C-6”-derivatized α-GalCer analogues through appropriate orthogonal C-6”-protection. However, the strategy involving Schmidt glycosylation affords more of the desired α-galactoside owing to a higher general yield and a better stereoselectivity. Moreover, this procedure offers the opportunity for late-stage introduction of alternative acyl chains in the

phytosphingosine moiety, thereby extending its potential scope. Hence this seems an exquisite approach for the assessment of S.A.R. studies, which was further applied for the preparation of our target compounds.

3.4 Proof-of-concept: synthesis of galacturonic acids and D-fucosyl analogues

To demonstrate the versatility of our method, it was first used to gain access to the known α-D-fucopyranosyl analogue **1**,[24] the galacturonic analogue **3**,[25] and two related derivatives **2** and **4** in which the conventional acyl moiety has been replaced by a 6-phenylhexanoyl (C6Ph) moiety, known to considerably enhance the overall production of both Th1 and Th2 type cytokines and to skew the balance toward a Th1 type response (Figure 3.2).[26] Compound **3** represents a synthetic analogue of GSL-1’ in which the ceramide part was replaced by that found in α-GalCer. GSL-1’ was shown to induce a more Th1-based immunity and to suppress tumor growth and prolong survival of mice bearing lung cancer more effectively than α-GalCer at equal doses. In contrast to GSL-1’, i.v. administration of compound **3** results in a drastically lower secretion of IFN-γ than that caused by α-GalCer administration. Despite this fact, it is more effective towards lung and breast cancer in mice compared to α-GalCer. Although **3** was reported for the first time in 2007 by Chang *et al*., its synthetic procedure has not been documented yet.



1: R = $(CH_2)_{24}CH_3$
2: R = $(CH_2)_5Ph$

3: R = $(CH_2)_{24}CH_3$
4: R = $(CH_2)_5Ph$

Figure 3.2 Structure of the envisaged α-D-fucopyranosyl analogues and galacturonic acid compounds.

In this context, an additional advantage of our strategy concerns the glycosylation of galacturonic acid donors, which surprisingly is not well-precedented.[27,28,29] This may be due to the deactivating effect of the electron-withdrawing C-5 carboxylic group, making the galacturonidation particularly challenging.[30] In addition, in their efforts to assess the influence of a pyranosyl C-5 carboxylate ester on the stereochemical outcome of glycosylation reactions, van der Marel and coworkers demonstrated that a C-5 ester is 1,5-*cis* or β-directing as opposed to C-5 methylene oxybenzyl, which induces little selectivity.[31] This selectivity arises from the $^3H_4$ half-chair conformer, indicating that in the intermediate pyranosyl

oxacarbenium ion intermediate, a C-5 carboxylate ester prefers to occupy a pseudoaxial position (Figure 3.3, top). In the presence of conflicting substituent preferences on electronic grounds (Figure 3.3, bottom; the C-2 and C-4 alkoxy groups are favorably oriented in a $^{4}H_{3}$ half-chair oxacarbenium ion conformer, while the C-3 alkoxy and C-5 ester substituents adapt a preferred position in the $^{3}H_{4}$ half-chair oxacarbenium ion conformer), the stereochemical outcome of per-benzyl-protected galacturonic ester donor remains speculative, but stereospecific α-glycosylation seems unattainable, despite the fact that destabilizing steric interactions predominate in the $^{3}H_{4}$ half-chair. Accordingly, a report of Seeberger *et al.*[29] on the synthesis of the *Sphingomonas* glycolipid GSL-1' illustrates that even after extensive optimization (including galactose protecting groups), glycosylation was achieved with a maximum 4.2/1 ratio of the α- and β-anomers, representing the most α-selective glycosylation reaction between a galacturonic acid building block and a ceramide reported so far.

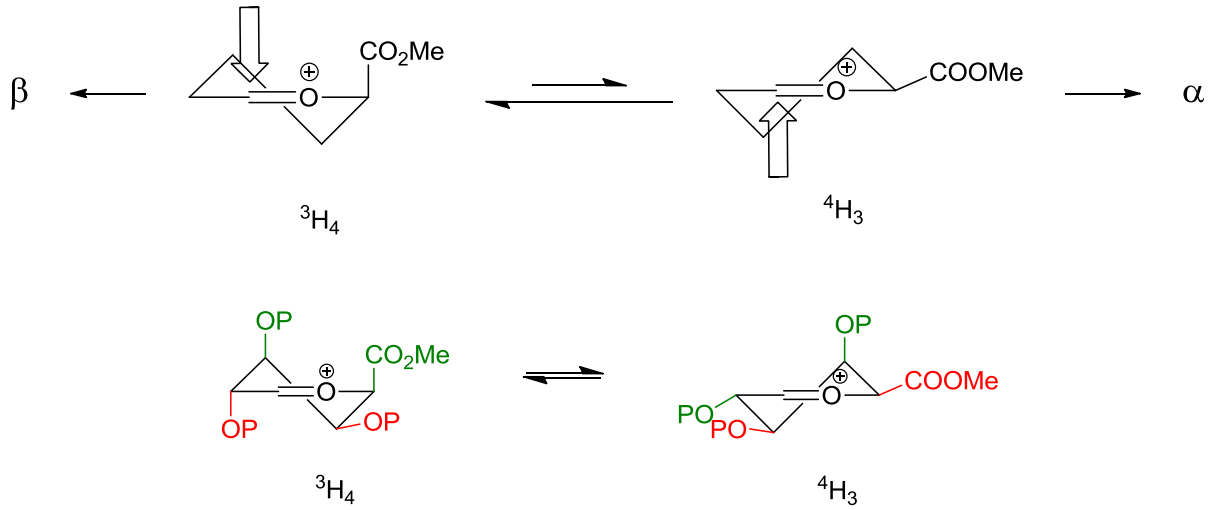


Figure 3.3 Top: Stereoselectivity of C-5-functionalized pyranosides. Bottom: Structures of the half-chair oxacarbenium ions involved in galactosidation. Substituents occupying a favorable position are depicted in green, while those occurring in an unfavorable position are colored red.

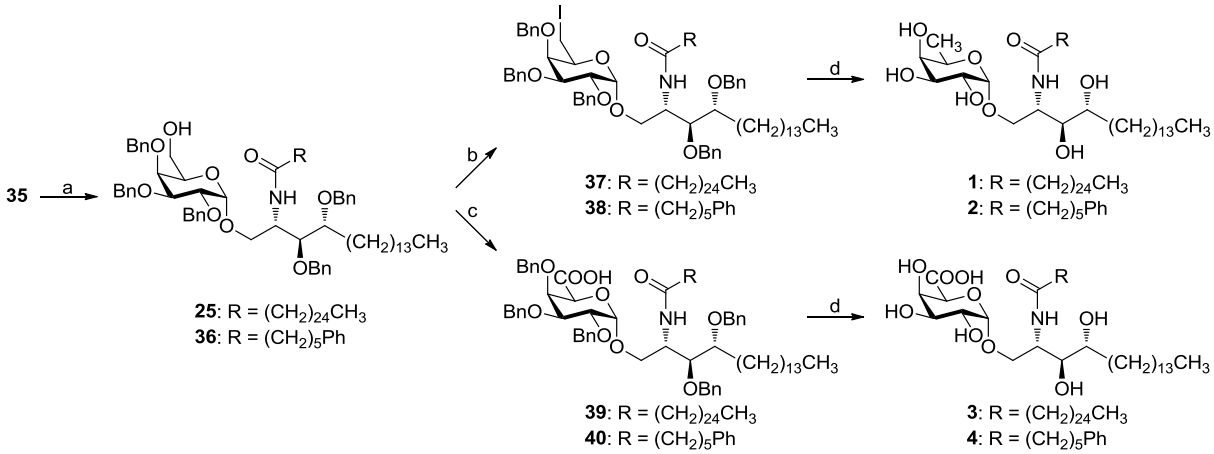
The synthesis of the α-D-fucosyl analogue RCAI-58 (**1**) by Mori and coworkers involved glycosylation under Mukaiyama conditions.[24] Unfortunately, neither yield nor anomeric ratio were mentioned. In contrast to the scarce reports about D-fucosylation, L-fucosylation is more prevalent, with reasonable α-selectivities.[32,33,34] Neglecting the potential influence on the α/β ratio due to the asymmetry of the sphingosine, glycosylation of this acceptor with D- and L-fucose is expected to proceed with the same stereoselectivity.

Scheme 3.8 summarizes the divergent route followed for the synthesis of both types of target analogues starting from versatile building block **35**. Staudinger reduction of the azide

followed by acylation of the resulting amino group with the appropriate acids gave compounds **25** and **36**. These in turn are useful intermediates primed for further C-5” and C-6”-modifications.

Towards the deoxygenation of the 6”-OH, **25** and **36** were first converted to the corresponding iodo analogues **37** and **38** by treatment with triphenylphosphine, imidazole and iodine. Our plan was to carry out the reductive dehalogenation and debenzylation in a single step. However, upon treatment with palladium black under hydrogen atmosphere, the reaction stopped at the stage of the deiodinated products still containing all benzyl groups, probably due to poisoning of the catalyst. After flash chromatography of the reaction mixture, the deiodinated intermediates were again subjected to catalytic hydrogenation (same conditions) to afford the target compounds **1** and **2**.

The galacturonic acid derivatives were prepared upon oxidation of the 6”-OH groups of **25** and **36** into the corresponding carboxylic acids **39** and **40** via a TEMPO/BAIB oxidation.[35] Final deprotection was accomplished by Pd-catalyzed hydrogenation to afford the desired compounds **3** and **4.**



Scheme 3.8 Reagents and conditions: (a) i. PMe_3, THF, NaOH, rt, 5 h; ii. EDC, RCOOH, CH_2Cl_2, rt, overnight, 71-81%; (b) PPh_3, I_2, imidazole, toluene, 110 °C, 30 min., 85%; (c) TEMPO, BAIB, CH_2Cl_2, H_2O, rt, overnight, 81-97%; (d) Pd black, H_2, rt, 49-86%.

3.5 Biological evaluation

To assess the biological activity of the galacturonic acid and α-D-fucopyranosyl analogues, serum levels of IFN-γ and IL-4 were measured after intraperitoneal injection of 5 μg of the corresponding glycolipids in mice (Collaboration with Prof. D. Elewaut). The cytokine secretion induced by these compounds is presented in Figure 3.4. Consistent with the results of Mori,[24] 6”-deoxy-analogue **1** is a superior IFN-γ inducer. However, where Mori and

coworkers only reported the IFN-γ secretion, we measured the IL-4 levels as well, revealing a strong Th2 response. Hence, we may infer that under these conditions **1** combines strong antigenicity with no net polarization. In combination with a C6Ph modified acyl chain, fucosyl analogue **2** induces only a weak cytokine secretion. So, combination of the two modifications known to enhance antigenic activity results in a remarkably decreased activity. In contrast to the *in vitro* response in human iNKT cells,[25] galacturonic acid **3** shows a minor cytokine secretion *in vivo* in mice. Combination with the C6Ph modified acyl chain in compound **4** abolishes all activity.

The low cytokine release of the C6Ph analogues **2** and **4** is surprising. Wong *et al.* demonstrated that this and related acyl moieties enhanced the stability for mCD1d, a finding that was further substantiated by cocrystal structures with that protein.[36] Furthermore, introduction of this acyl group in α-GalCer afforded a compound that produced more IFN-γ and less IL-4 from human iNKT cells compared to α-GalCer. It remains to be investigated if the very low antigenicity found for **2** and **4** is due to the fact that this acyl moiety negatively influences cytokine secretion in the mouse system (despite good affinity for mCD1d). Alternatively, our results may also indicate that modification of the carbohydrate moiety can significantly alter the influence of the lipid moiety of the ligand on CD1d presentation and iNKT cell responses. This is in accordance with observations made by Besra and coworkers.[34]

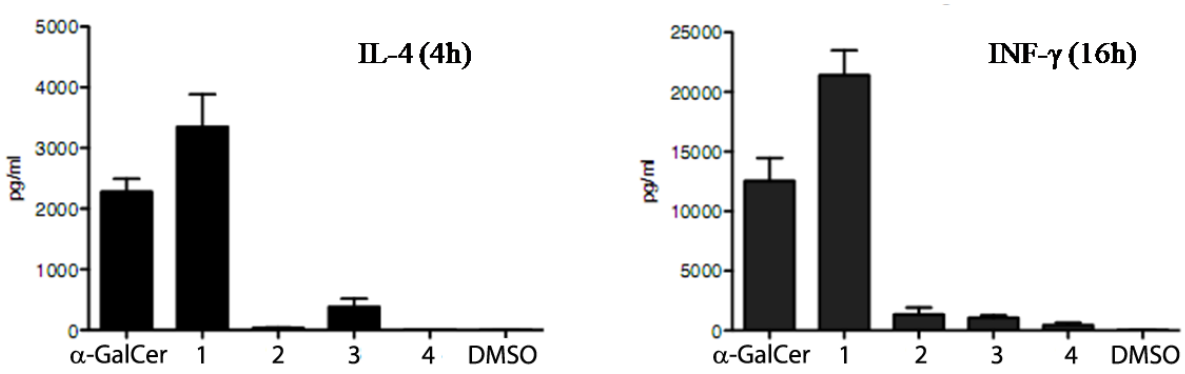


Figure 3.4 IL-4 and IFN-γ secretion, measured at respective 4 h and 16 h, after intraperitoneal injection of 5 µg of the glycolipids in mice.

We also determined the equilibrium binding affinities of the mouse Vα14Vβ8.2 NKT TCR toward the four CD1d-glycolipid complexes using surface plasmon resonance (SPR) (Table 3.1, Collaboration with Prof. D. Zajonc). Purified and biotinylated mouse CD1d was loaded with the four individual glycolipid ligands and coated on a Biacore sensor chip. Increasing concentrations of TCR were simultaneously passed over each flow channel of the

chip to measure real time binding kinetics. Interestingly, the binding phase (k_{ass}) of the TCR to each of the CD1d-presented glycolipids is similar, ranging from $0.8x10^5$-$1.3x10^5$ ($M^{-1}s^{-1}$) while the dissociation of the TCR (k_{diss}) can differ up to 10-fold between α-GalCer and **3** (compare $1.45x10^{-3}$ s^{-1} with $16.6x10^{-3}$ s^{-1}), resulting in over 10-fold different binding affinities (K_D=11.2nM-165nM).

Table 3.1 Equilibrium binding affinity of the TCR to the indicated glycolipid/CD1d complex, measured by SPR.

Glycolipid	$k_{ass}(M^{-1}s^{-1})$	$k_{diss}(s^{-1})$	$K_D(k_{diss}/k_{ass})$
α-GalCer	1.3E+05 ± 1E+03	1.5E-03 ± 4.5E-05	11.2 ± 0.2 nM
1	0.8E+05 ± 3E+03	5.3E-03 ± 2.9E-04	64.8 ± 6.0 nM
2	1.0E+05 ± 2E+04	6.5E-03 ± 1.8E-03	74.4 ± 25.0 nM
3	1.0E+05 ± 9E+03	16.6E-03 ± 5.5E-03	165.1 ± 38.3 nM
4	1.1E+05 ± 6E+03	8.1E-03 ± 1.2E-03	75.3 ± 15.2 nM

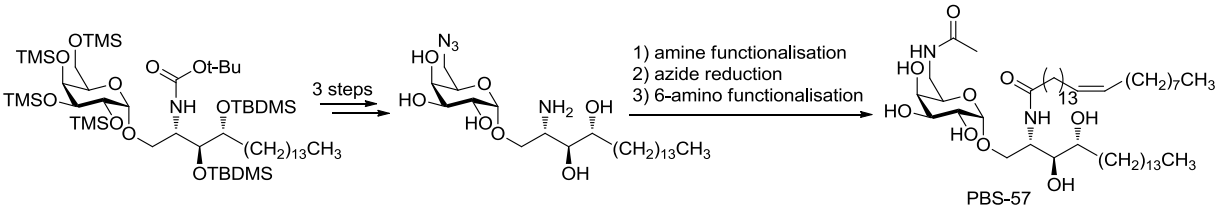
These data indicate that modifications of the galactose moiety and the acyl chain do affect iNKT TCR affinity but not to a degree that has been seen for weak microbial antigens, such as borrelial α-galactosyl diacylglycerolipids (K_D=6.2μM). As a result, the apparent discrepancy between relatively high affinity TCR interaction *in vitro* and weak iNKT cell activation *in vivo,* likely is a result of different pharmacokinetics of the lipids inside cells, rather than attributed by altered TCR binding kinetics.

3.6 Conclusion

We described a practical synthetic route to modify the 6”-position after the glycosidation, featuring the regioselective opening of a 4”,6”-*O*-benzylidene ring as the key step. As a proof-of-concept this method was employed to prepare the 6”-deoxy-analogue of α-GalCer (**1**), as well as the otherwise not so accessible galacturonic acid analogue **3**. The carboxylate group of the latter may be suitable for flexible substitution through an amide linkage and will be described in Chapter 4. An additional advantage of the reported procedure is that it allows to introduce alternative acyl moieties in the phytosphingosine moiety in the final stages of the synthesis, exploited for the preparation of compounds **2** and **4**.

During the course of this project, the group of Cox/Besra also reported a practical procedure to synthesize 6”-*N*-derivatized α-GalCer analogues relying on the orthogonal protection of the C-2 and C-6”-amino groups, and application of Gervay-Hague’s glycosylation methodology (Scheme 3.9).[37] Selective C-6”-TMS-ether cleavage nicely allowed introduction of the C-6”-

azido group and was followed by global deprotection prior to introduction of the acyl chain and further 6”-*N*-functionalization. Although not reported thus far, their synthetic route could also give access to 6”-*O*-derivatized compounds.



Scheme 3.9 The Cox/Besra method towards C-6”-modified α-GalCer analogues illustrated for C-6”-amino derivative PBS-57.

3.7 Experimental section

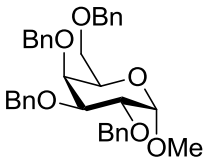
General

Precoated Macherey-Nagel SIL G/UV_{254} plates were used for TLC, and spots were examined under UV light at 254 nm and further visualized by sulfuric acid-anisaldehyde spray. Column chromatography was performed on Biosolve silica gel (63-200 μm, 60 Å). NMR spectra were obtained with a Varian Mercury 300 Spectrometer or a Bruker Avance II 500 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, in the case of DMSO-d_6: δ = 2.54 ppm for ^{1}H and δ = 40.5 ppm for ^{13}C, and in the case of pyridine-d_5: δ = 8.71, 7.56 and 7.18 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 CH_3CN/HCOOH (1000/1) mixture at 10 mL/min. The purity of the target compounds was assessed by HPLC. An Agilent Technologies 1100 HPLC system (Agilent Technologies, Waldbronn, Germany) was coupled to a Varian 385-LC Evaporative Light Scattering Detector (ELSD)(Agilent Technologies). The compounds were subjected to an Xbridge Shield C18 column (Waters, Millford, MA, USA) operated at 65°C. Mobile phases, delivered at a flow rate of 2 mL/min, consisted of 25 mM ammonium formate pH 5 (solvent A) and methanol (solvent B). A linear gradient from 70 % B to 100 % B was applied. The target compounds were dissolved in pyridine and further diluted a hundred fold in acetonitrile prior to injecting 2 µl.

2,3,4,6-tetra-*O*-benzyl-1-*O*-methyl-α-D-galactopyranoside (15)

To a solution of **14** (10.30 g, 53.02 mmol) in DMF (500 mL) at 0 °C was added NaH (12.72 g, 318.11 mmol). After stirring for 45 minutes, benzyl bromide (55.52 g, 318.11 mmol) was

added and the solution was stirred overnight at room temperature. Upon reaction completion, the mixture was quenched with brine and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by column chromatography (hexanes/EtOAc: 8.5/1.5) to afford compound **15** (26.37 g, 90 %) as a yellow oil.



^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.43-7.26 (m, 20H, arom. H), 4.95 (d, *J* = 11.5 Hz, 1H, CH_2-Ph), 4.86 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.84 (d, *J* = 12.1 Hz, 1H, CH_2-Ph), 4.74 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.69 (d, *J* = 3.7 Hz, 1H, H-1”), 4.69 (d, *J* = 12.7 Hz, 1H, CH_2-Ph), 4.58 (d, *J* = 11.5 Hz, 1H, CH_2-Ph), 4.49 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.40 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.05 (ddd, *J* = 0.9 Hz, 3.4 Hz and 10.8 Hz, 1H, H-2”), 3.97-3.89 (m, 3H, H-3”, H-4” and H-5”), 3.55 (d, *J* = 6.3 Hz, 2H, H-6), 3.40 (s, 3H, CH_3).

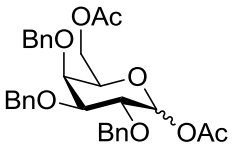
^{13}C-NMR (75 MHz, $CDCl_3$): δ 139.10, 138.91, 138.79, 138.25, 128.65, 128.60, 128.54, 128.49, 128.38, 128.03, 127.96, 127.85, 127.76, 99.06, 79.39, 76.71, 75.41, 75.00, 73.84, 73.75, 73.56, 69.49, 69.34, 55.63.

Exact mass (ESI-MS) for $C_{35}H_{38}O_6$ $[M+H]^+$ found, 555.2759; calcd, 555.2741.

Spectral data are consistent with the literature data.

1,6-di-*O*-acetyl-2,3,4-tri-*O*-benzyl-D-galactopyranoside (16)

A solution of **15** (23.59 g, 42.69 mmol) in AcOH (24 mL) and Ac_2O (18 mL) was cooled to 0 °C, followed by dropwise addition of H_2SO_4 (0.35 mL). The reaction mixture was allowed to warm to room temperature and was stirred overnight, then quenched with a saturated solution of $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was washed with a saturated solution of $NaHCO_3$ and brine, dried over $MgSO_4$ and evaporated. Purification by column chromatography (hexanes/EtOAc: 7/3) resulted in an anomeric mixture of compound **16** (18.49 g, 81 %) as a colorless oil.



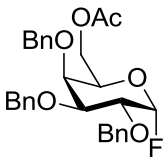
^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.41-7.16 (m, 15H, arom. H), 6.40 (d, *J* = 3.7 Hz, 0.8H, H-1_α”), 5.59 (d, *J* = 8.1 Hz, 0.2H, H-1_β”), 4.97 (dd, *J* = 11.4 Hz, 1H, CH_2-Ph), 4.88 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.80 (d, *J* = 12.3 Hz, 1H, CH_2-Ph), 4.80 (d, *J* = 12.3 Hz, 1H, CH_2-Ph), 4.72 (d, *J* = 11.4 Hz, 1H, CH_2-Ph), 4.62 (d, *J* = 11.2 Hz, 1H, CH_2-Ph), 4.19 (dd, *J* = 3.78 Hz and 9.74 Hz, 0.8H, H-2_α”), 4.13-3.87 (m, 5.2H, H-2_β”, H-3”, H-4”, H-5” and H-6”), 2.12 (s, 3H, OAc), 1.99 (s, 3H, OAc).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 170.81, 170.76, 169.65, 169.53, 138.72, 138.49, 138.28, 138.25, 139.19, 138.13, 128.79, 128.73, 128.70, 128.67, 128.61, 128.60, 128.26, 128.19, 128.09, 128.08, 128.02, 127.96, 127.89, 127.84, 127.66, 94.34, 90.89, 82.61, 78.82, 78.31, 75.60, 75.57, 74.93, 74.62, 74.45, 73.66, 73.64, 73.57, 73.39, 73.03, 71.06, 63.31, 63.14, 60.63, 21.37, 21.28, 21.24, 21.04, 21.02, 14.43.

Exact mass (ESI-MS) for $C_{31}H_{34}O_6$ $[M+Na]^+$ found, 557.2119; calcd, 557.2151.

6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl fluoride (17)

To HF in pyridine (24 mL) at 0 °C was added a solution of **16** (2.00 g, 3.74 mmol) in toluene (10 mL). The reaction mixture was gradually warmed to room temperature and stirred for 7 hours. The solution was poured into brine and extracted with CH_2Cl_2. The organic layer was washed with a saturated solution of $NaHCO_3$ and brine, followed by drying over Na_2SO_4. After evaporation of the organic solvent, the residue was purified by column chromatography (hexanes/EtOAc: 4/1) to furnish compound **17** (1.36 g, 74 %) as a white solid.



^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.42-7.27 (m, 15H, arom. H), 5.60 (dd, *J* = 2.6 Hz and 53.5 Hz, 1H, H-1''), 4.99 (d, *J* = 11.2 Hz, 1H, CH_2-Ph), 4.88 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.86 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.79 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.73 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.62 (d, *J* = 11.5 Hz, 1H, CH_2-Ph), 4.17-3.93 (m, 6H, H-2'', H-3'', H-4'', H-5'' and H-6''), 2.00 (s, 3H, OAc).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 170.77, 138.47, 138.17, 138.12, 128.75, 128.72, 128.66, 128.59, 128.53, 128.22, 128.16, 128.03, 127.81, 107.86, 104.86, 78.58, 76.10, 75.79, 74.89, 74.36, 74.05, 73.70, 71.35, 71.31, 63.39, 60.62, 21.28, 21.02, 14.44.

Exact mass (ESI-MS) for $C_{29}H_{31}FO_6$ $[M+Na]^+$ found, 517.1927; calcd, 517.1997.

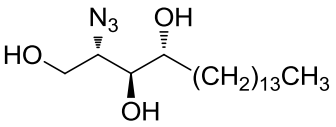
Spectral data are consistent with the literature data.

(2*S*,3*S*,4*R*)-2-azidooctadecane-1,3,4-triol (19)

To a suspension of NaN_3 (40.96 g, 630.00 mmol) in CH_2Cl_2 (100 mL) and H_2O (100 mL) at 0 °C was slowly added Tf_2O (22.5 mL). After stirring for 2 hours, the reaction mixture was separated. The aqueous layer was extracted with CH_2Cl_2 and the combined organic layer was washed with H_2O.

The freshly prepared TfN_3-solution (200 mL in CH_2Cl_2) was added to a mixture of D-*ribo*-phytosphingosine (20.00 g, 63.00 mmol), K_2CO_3 (17.40 g, 125.80 mmol) and $CuSO_4$ (100 mg, 0.64 mmol) in H_2O (400 mL) and MeOH (1.2 L). After stirring overnight, the solvent was

evaporated under reduced pressure to furnish 400 mL of a white slurry. The precipitate was filtered, washed with H_2O and lyophilized to yield **19** (16.37 g, 76 %) as a white solid.



^{1}H-NMR (300 MHz, pyridine-d_5): δ 7.03 (d, *J* = 5.5 Hz, 1H, OH), 6.90 (t, *J* = 6.1 Hz, 1H, OH), 6.35 (d, *J* = 6.1 Hz, 1H, OH), 4.70 (ddd, *J* = 3.5 Hz, 5.0 Hz and 11.4 Hz, 1H, H-1), 4.58 (ddd, *J* = 5.6 Hz, 7.6 Hz and 11.4 Hz, 1H, H-1), 4.43 (ddd, *J* = 3.5 Hz, 4.1 Hz and 11.4 Hz, 1H, H-2), 4.38-4.30 (m, 1H, H-3), 4.30-4.20 (m, 1H, H-4), 2.25-2.15 (m, 1H, CH_2), 2.00-1.86 (m, 2H, CH_2), 1.50-1.20 (m, 22H, CH_2), 0.88 (t, *J* = 6.7 Hz, 3H, CH_3).

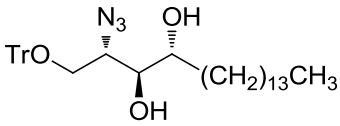
^{13}C-NMR (75 MHz, pyridine-d_5): δ 106.65, 76.50, 72.88, 67.47, 50.14, 34.66, 32.59, 30.70, 30.44, 30.39, 30.08, 26.86, 23.41, 14.75.

Exact mass (ESI-MS) for $C_{18}H_{37}N_3O_3$ $[M+Na]^+$ found, 366.2725; calcd, 366.2733.

Spectral data are consistent with the literature data.

(2*S*,3*S*,4*R*)-2-azido-1-*O*-trityloctadecane-1,3,4-triol (20)

To a solution of **19** (7.50 g, 21.83 mmol) in pyridine (218 mL) was added TrCl (6.83 g, 24.01 mmol) and DMAP (0.67 g, 5.46 mmol). After stirring overnight at 70 °C, the resulting orange reaction mixture was quenched with a saturated $NaHCO_3$ solution and extracted with CH_2Cl_2. The combined organic layer was washed with brine, dried over Na_2SO_4 and evaporated to dryness. Purification by column chromatography (hexanes/EtOAc: 9/1) afforded **20** (10.59 g, 85 %) as a yellow oil.



^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.49-7.25 (m, 5H, arom. H), 7.35-7.22 (m, 10H, arom. H), 3.67-3.62 (m, 2H, H-1 and H-3), 3.57-3.52 (m, 2H, H-2 and H-4), 3.45 (dd, *J* = 5.7 Hz and 10.1 Hz, 1H, H-1), 2.36 (d, *J* = 4.4 Hz, 1H, OH), 1.82 (t, *J* = 2.2 Hz, 1H, OH), 1.57-1.23 (m, 26H, CH_2), 0.88 (t, *J* = 6.6 Hz, 3H, CH_3).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 143.58, 128.78, 128.26, 128.14, 127.52, 127.48, 88.03, 74.44, 72.43, 63.71, 62.54, 32.16, 29.93, 29.89, 29.60, 25.84, 22.93, 14.36.

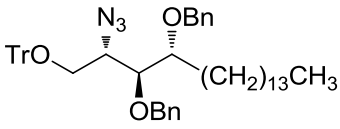
Exact mass (ESI-MS) for $C_{36}H_{51}N_3O_3$ $[M+Na]^+$ found, 608.3853; calcd, 608.3828.

Spectral data are consistent with the literature data.

(2*S*,3*S*,4*R*)-2-azido-3,4-di-*O*-benzyl-1-*O*-trityloctadecane-1,3,4-triol (21)

To a solution of **20** (10.58 g, 18.57 mmol) in DMF (190 mL) at 0 °C was added NaH (2.23 g, 55.70 mmol). After stirring for 45 minutes, benzyl bromide (6.66 mL, 55.70 mmol) was

added and the solution was stirred overnight at room temperature. Upon reaction completion, the mixture was quenched with H_2O and extracted with CH_2Cl_2. The combined organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by column chromatography (hexanes/EtOAc: 97/3) to afford compound **21** (8.43 g, 59 %) as a pale yellow oil.



^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.46-7.43 (m, 5H, arom. H), 7.38-7.34 (m, 2H, arom. H), 7.31-7.21 (m, 16H, arom. H), 7.10-7.07 (m, 2H, arom. H), 4.58 (d, *J* = 11.3 Hz, 1H, CH_2-Ph), 4.54 (d, *J* = 12.8 Hz, 1H, CH_2-Ph), 4.49 (d, *J* = 10.6 Hz, 1H, CH_2-Ph), 4.45 (d, *J* = 11.6 Hz, 1H, CH_2-Ph), 3.77 (ddd, *J* = 3.0 Hz, 5.6 Hz and 8.3 Hz, 1H, H-2), 3.63-3.44 (m, 3H, H-1, H-3 and H-4), 3.37 (dd, *J* = 8.1 and 10.0 Hz, 1H, H-1), 1.66-1.52 (m, 2H, CH_2), 1.35-1.22 (m, 24H, CH_2), 0.88 (t, *J* = 6.7 Hz, 3H, CH_3).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 144.27, 138.89, 138.46, 129.27, 128.94, 128.85, 128.78, 128.43, 128.37, 128.32, 128.22, 128.17, 128.13, 128.07, 127.57, 87.74, 79.97, 74.08, 72.66, 72.58, 63.77, 32.48, 30.39, 30.31, 30.26, 30.25, 30.23, 30.22, 30.18, 30.15, 29.92, 25.89, 23.24, 14.68.

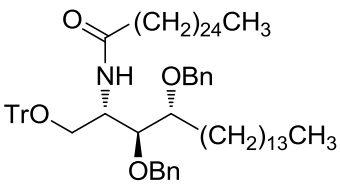
Exact mass (ESI-MS) for $C_{36}H_{51}N_3O_3$ $[M+Na]^+$ found, 788.4774; calcd, 788.4767.

Spectral data are consistent with the literature data.

(2*S*,3*S*,4*R*)-3,4-di-*O*-benzyl-2-hexacosylamino-1-*O*-trityloctadecane-1,3,4-triol (22)

To a solution of **21** (4.54 g, 5.92 mmol) in THF (5.3 mL) was added trimethylphosphine (29.62 mL, 29.62 mmol) dropwise. Upon stirring for 3.5 hours, NaOH was added and the reaction mixture was allowed to stir for an additional 2 hours. The aqueous layer then was extracted with EtOAc and the combined organic layers were washed with H_2O and brine, dried over $MgSO_4$ and evaporated to dryness to furnish the crude amine as a white slurry.

A suspension of hexacosanoic acid (3.53 g, 8.89 mmol) and EDC (2.27 g, 11.85 mmol) in CH_2Cl_2 (30 mL) was stirred for 30 minutes. Subsequently, a solution of the crude amine in CH_2Cl_2 (60 mL) was added. After stirring overnight, the mixture was extracted with CH_2Cl_2 and the organic layers were washed with H_2O and brine, dried over $MgSO_4$ and concentrated under reduced pressure. Purification by column chromatography (hexanes/EtOAc: 92/8) afforded **22** (5.48 g, 83 %) as a colorless oil.



^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.32-7.12 (m, 25H, arom. H), 5.50 (d, *J* = 9.1 Hz, 1H, NH), 4.73 (d, *J* = 11.4 Hz, 1H, CH_2-Ph), 4.41 (d, *J* = 10.8 Hz, 3H, CH_2-Ph), 4.26-4.17 (m, 1H, H-2), 3.76 (dd, *J* = 2.6 Hz and 6.7 Hz, 1H, H-3), 3.43-3.38 (m, 2H, H-1 and H-4), 3.23 (dd, *J* = 4.0 Hz and 9.5 Hz, 1H, H-1), 1.85 (t, *J* = 7.5 Hz, 2H, $COCH_2$), 1.51-1.18 (m, 72H, CH_2), 0.81 (t, *J* = 6.6 Hz, 6H, CH_3).

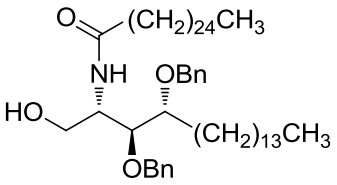
^{13}C-NMR (75 MHz, $CDCl_3$): δ 172.61, 144.07, 138.90, 138.81, 128.92, 128.58, 128.52, 128.05, 127.95, 127.79, 127.69, 127.28, 86.88, 80.63, 79.69, 73.51, 72.20, 62, 65, 60.62, 50.24, 37.14, 32.16, 30.16, 30.07, 29.96, 29.94, 29.89, 29.78, 29.67, 29.64, 29.61, 29.60, 26.22, 25.92, 22.93, 14.43, 14.35.

Exact mass (ESI-MS) for $C_{77}H_{115}NO_4$ $[M+Na]^+$ found, 1140.8667; calcd, 1140.8724; $[M+K]^+$ found, 1156.8420; calcd, 1156.8463.

Spectral data are consistent with the literature data.

(2*S*,3*S*,4*R*)-3,4-di-*O*-benzyl-2-hexacosylamino-octadecane-1,3,4-triol (23)

To a solution of **22** (5.48 g, 4.90 mmol) in CH_2Cl_2 (67 mL) and *i*PrOH (12 mL) was added $ZnBr_2$ (17.66 g, 78.42 mmol). The mixture was stirred overnight, then quenched with H_2O. After extraction with CH_2Cl_2, the combined organic layers were washed with H_2O and brine, dried over Na_2SO_4 and evaporated to dryness. The resulting residue was purified by column chromatography (hexanes/EtOAc: 7.5/2.5) furnishing **23** (3.66 g, 85 %) as a white solid.



^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.39-7.27 (m, 10H, arom. H), 6.02 (d, *J* = 8.1 Hz, 1H, NH), 4.71 (d, *J* = 11.5 Hz, 1H, CH_2-Ph), 4.66 (d, *J* = 11.2 Hz, 1H, CH_2-Ph), 4.60 (d, *J* = 11.5 Hz, 1H, CH_2-Ph), 4.45 (d, *J* = 11.5 Hz, 1H, CH_2-Ph), 4.16-4.11 (m, 1H, H-2), 3.99 (dt, *J* = 3.7 Hz and 11.5 Hz, 1H, ,H-1), 3.72-3.66 (m, 2H, H-3 and H-4), 3.60 (ddd, *J* = 3.9 Hz, 8.4 Hz and 11.8 Hz, 1H, H-1), 3.04 (dd, *J* = 4.3 Hz and 8.4 Hz, 1H, OH), 2.04-1.91 (m, 2H, $COCH_2$), 1.74-1.18 (m, 72H, CH_2), 0.88 (t, *J* = 6.7 Hz, 6H, CH_3).

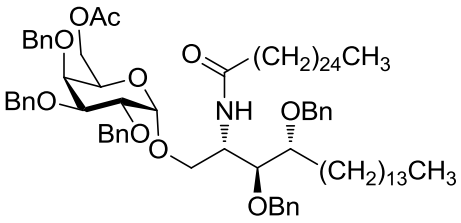
^{13}C-NMR (75 MHz, $CDCl_3$): δ 172.99, 138.29, 137.96, 128.84, 128.59, 128.30, 128.24, 128.10, 127.95, 82.39, 79.21, 73.19, 73.02, 63.10, 50.75, 36.85, 32.08, 30/96, 29.85, 29.82, 29.80, 29.74, 29.67, 29.63, 29.57, 29.52, 29.46, 29.43, 26.18, 25.79, 22.84, 14.26.

Exact mass (ESI-MS) for $C_{58}H_{101}NO_4$ $[M+Na]^+$ found, 898.7695; calcd, 898.7628.

Spectral data are consistent with the literature data.

(2*S*,3*S*,4*R*)-3,4-di-*O*-benzyl-1-*O*-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (24)

To a solution of ceramide **23** (225 mg, 0.26 mmol) in THF (2.6 mL) under argon atmosphere, $SnCl_2$ (115 mg, 0.60 mmol), $AgClO_4$ (127 mg, 0.60 mmol) and powdered 4 Å molecular sieves were added. A solution of **17** (100 mg, 0.20 mmol) in THF (2.6 mL) then was added at -10 °C and the mixture was stirred for 1.5 hours. After filtration over celite and rinsing of the filter cake with Et_2O, the organic solvent was evaporated under reduced pressure. Purification by column chromatography (hexanes/EtOAc: 8/2) yielded glycolipid **24** (143 mg, 52 %) as a white solid.



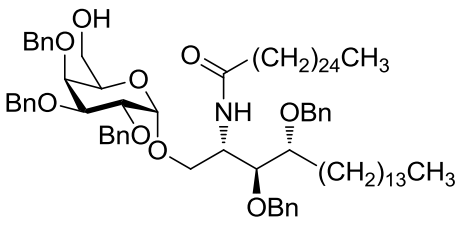
^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.40-7.21 (m, 25H, arom. H), 5.84 (d, *J* = 8.4 Hz, NH), 4.95 (d, *J* = 11.5 Hz, 1H, CH_2-Ph), 4.88 (d, *J* = 3.4 Hz, 1H, H-1"), 4.83 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.78 (d, *J* = 11.5 Hz, 1H, CH_2-Ph), 4.76 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.75 (d, *J* = 10.5 Hz, 1H, CH_2-Ph), 4.62 (d, *J* = 11.5 Hz, 1H, CH_2-Ph), 4.61 (d, *J* = 11.5 Hz, 1H, CH_2-Ph), 4.57 (d, *J* = 10.6 Hz, 1H, CH_2-Ph), 4.54 (d, *J* = 11.2 Hz, 1H, CH_2-Ph), 4.46 (d, *J* = 11.2 Hz, 1H, CH_2-Ph), 4.28-4.20 (m, 1H, H-2), 4.11-3.87 (m, 6H, H-2", H-3", H-4", H-5" and H-6"), 3.82-3.78 (m, 3H, H-1 and H-3), 3.52-3.50 (m, 1H, H-4), 1.98-1.88 (m, 5H, OAc and $COCH_2$), 1.63-1.23 (m, 72H, CH_2), 0.88 (t, *J* = 6.7 Hz, 6H, CH_3).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 172.97, 170.76, 138.79, 138.72, 138.59, 138.35, 128.59, 128.03, 127.99, 127.80, 127.65, 99.07, 80.07, 79.41, 79.22, 77.42, 74.78, 74.58, 73.76, 73.66, 73.37, 72.01, 69.06, 68.24, 63.64, 56.22, 50.35, 36.94, 32.15, 30.22, 29.93, 29.68, 29.59, 26.20, 25.93, 22.92, 20.97, 14.35.

Exact mass (ESI-MS) for $C_{87}H_{131}NO_{10}$ $[M+H]^+$ found, 1350.9780; calcd, 1350.9851; $[M+Na]^+$ found, 1372.9690; calcd, 1372.9671; $[M+K]^+$ found, 1388.9348; calcd, 1388.9410.

(2*S*,3*S*,4*R*)-3,4-di-*O*-benzyl-1-*O*-(2,3,4-tri-*O*-benzyl-6-hydroxy-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (25)

To a solution of **24** (1.12 g, 0.83 mmol) in $CHCl_3$ (8mL) was added NH_3 in MeOH (7 N, 25 mL). The mixture was stirred during 48 hours at room temperature followed by evaporation of the organic solvent. The resulting residue was suspended in H_2O and extracted with EtOAc. Subsequently, the organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. Purification by column chromatography (hexane/EtOAc: 7.2/2.5) provided compound **25** (967 mg, 89 %) as a colorless oil.



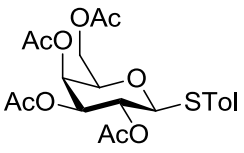
^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.39-7.25 (m, 25H, arom. H), 5.83 (d, *J* = 9.0 Hz, 1H, NH), 4.94 (d, *J* = 11.5 Hz, 1H, CH_2-Ph), 4.88 (d, *J* = 3.8 Hz, 1H, H-1”), 4.82 (d, *J* = 12.7 Hz, 1H, CH_2-Ph), 4.79 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.73 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.71 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.64 (d, *J* = 12.1 Hz, 1H, CH_2-Ph), 4.62 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.58 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.50 (d, *J* = 11.5 Hz, 1H, CH_2-Ph), 4.47 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.41-4.33 (m, 1H, H-2), 4.03 (dd, *J* = 3.7 Hz and 9.6 Hz, 1H, H-2”), 3.95 (dd, *J* = 7.6 Hz and 11.6 Hz, 1H, H-1), 3.85-3.82 (m, 3H, H-1, H-3”, H-4”), 3.70-3.62 (m, 3H, H-3, H-5”, H-6”), 3.55-3.50 (m, 1H, H-4), 3.48-3.42 (m, 1H, H-6”), 2.39 (t, *J* = 5.8 Hz, 1H, OH), 1.92-1.87 (m, 2H, $COCH_2$), 1.69-1.14 (m, 72H, CH_2), 0.88 (t, *J* = 6.8 Hz, 6H, CH_3).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 173.37, 138.86, 138.79, 138.67, 138.65, 138.48, 128.66, 128.64, 128.60, 128.17, 128.13, 128.06, 127.96, 127.94, 127.83, 127.67, 100.31, 80.68, 79.46, 77.66, 77.44, 77.24, 76.91, 76.81, 75.09, 74.77, 73.77, 73.43, 73.32, 72.08, 71.50, 70.04, 62.70, 50.90, 37.05, 32.16, 30.43, 29.95, 29.93, 29.89, 29.83, 29.66, 29.59, 26.01, 26.93, 22.92, 14.43, 14.35.

Exact mass (ESI-MS) for $C_{85}H_{129}NO_9$ $[M+H]^+$ found, 1308.9780; calcd, 1308.9746.

***p*-methylphenyl-2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (27)**

To a solution of galactose pentaacetate (10.00 g, 25.62 mmol) and *p*-thiocresol (3.5 g, 27.62 mmol) in CH_2Cl_2 (26 mL) at 0 °C was slowly added $BF_3.Et_2O$ (4 mL). After stirring for 1 hour at 0 °C, the reaction mixture was diluted with CH_2Cl_2 and quenched with a saturated solution of $NaHCO_3$. The organic layer was washed with H_2O and brine, dried over Na_2SO_4 and evaporated to dryness. The crude was purified by column chromatography (hexanes/EtOAc: 7/3) to afford compound **27** (9.29 g, 80 %) as a white solid.



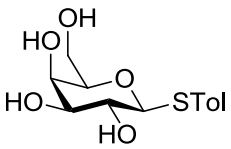
^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.39 (d, *J* = 8.1 Hz, 2H, arom. H), 7.11 (d, *J* = 8.1 Hz, 2H, arom. H), 5.39 (dd, *J* = 1.0 Hz and 3.2 Hz, 1H, H-4”), 5.20 (t, *J* = 10.0 Hz, 1H, H-2”), 5.02 (dd, *J* = 3.2 Hz and 10.0 Hz, 1H, H-3”), 4.63 (d, *J* = 10.0 Hz, 1H, H-1”), 4.17 (dd, *J* = 6.8 Hz and 11.3 Hz, 1H, H-6”), 4.09 (dd, *J* = 6.1 Hz and 11.3 Hz, 1H, H-6”), 3.89 (ddd, *J* = 0.8 Hz and 6.6 Hz, 1H, H-5”), 2.32 (s, 3H, CH_3), 2.09 (s, 3H, OAc), 2.08 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.95 (s, 3H, OAc).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 170.43, 170.26, 170.12, 169.49, 138.52, 133.23, 129.71, 128.71, 87.00, 74.44, 72.11, 67.38, 67.32, 61.67, 21.23, 20.93, 20.74, 20.70, 20.66.

Exact mass (ESI-MS) for $C_{21}H_{26}O_9S$ $[M+Na]^+$ found, 477.1032; calcd, 477.1195.
Spectral data are consistent with the literature data.

***p*-methylphenyl-1-thio-β-D-galactopyranoside (28)**

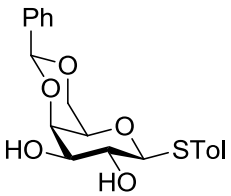
To a suspension of **27** (9.28 g, 20.41 mmol) in MeOH (90 mL) at 0 °C was added NaOMe (0.58 g, 10.21 mmol) in small portions. The reaction was allowed to stir for 3 hours at room temperature. Subsequently, the mixture was neutralized with Amberlite IR 120 (H^+ form) and diluted with MeOH. After filtration and rinsing with MeOH, the filtrate was concentrated under reduced pressure to yield compound **28** (6.06 g) as a white solid.



^{1}H-NMR (300 MHz, DMSO-d_6): δ 7.35 (d, *J* = 8.2 Hz, 2H, arom. H), 7.11 (d, *J* = 7.9 Hz, 2H, arom. H), 5.06 (d, *J* = 5.9 Hz, 1H, OH), 4.83 (d, *J* = 5.6 Hz, 1H, OH), 4.60 (t, *J* = 5.6 Hz, 1H, OH) 4.49 (d, *J* = 9.1 Hz, 1H, H-1”), 4.43 (d, *J* = 4.4 Hz, 1H, OH), 3.70 (t, *J* = 3.8 Hz, 1H, H-3”), 3.30-3.55 (m, 5H, H-2”, H-4”, H-5” and H-6”), 2.27 (s, 3H, CH_3).
^{13}C-NMR (75 MHz, DMSO-d_6): δ 136.46, 132.18, 130.83, 130.09, 88.84, 79.81, 75.39, 69.92, 60.00, 61.20, 21.27.
Exact mass (ESI-MS) for $C_{13}H_{18}O_5S$ $[M+Na]^+$ found, 309.0804; calcd, 309.0773.
Spectral data are consistent with the literature data.

***p*-methylphenyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (29)**

To a suspension of **28** (5.51 g, 19.25 mmol) in CH_3CN (74 mL), was added benzaldehyde dimethylacetal (3.52 g, 23.10 mmol). Upon adjustment of the pH until 3 by addition of *p*-TSA, a clear solution was obtained. After stirring for 5 hours, the resulting white suspension was neutralized with Et_3N and diluted with CH_2Cl_2. The organic layer was washed with H_2O and brine, dried over Na_2SO_4 and evaporated to dryness. The crude was recrystallised furnishing **29** (7.05 g, 98 %) as white crystals.



^{1}H-NMR (300 MHz, DMSO-d_6): δ 7.45 (d, *J* = 8.3 Hz, 2H, arom. H), 7.43-7.36 (m, 5H, arom. H), 7.09 (d, *J* = 7.9 Hz, 2H, arom. H), 5.54 (s, 1H, CH-Ph), 5.09 (d, *J* = 5.5 Hz, 1H, OH), 5.00 (, *J* = 5.6 Hz, 1H, OH), 4.58 (d, *J* =9.3 Hz, 1H, H-1”), 4.11 (app. d, *J* = 3.0 Hz, 1H, H-4”), 4.06-3.99 (m, 2H, H-6”), 3.60 (br. S, 1H, H-5”), 3.54-3.38 (m, 2H, H-2” and H-3”), 2.28 (s, 3H, CH_3).

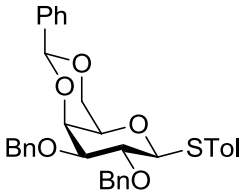
^{13}C-NMR (75 MHz, DMSO-d_6): δ 139.36, 136.96, 132, 28, 130.44, 130.02, 129.37, 128.54, 127.13, 100.54, 87.57, 76.77, 73.80, 69.91, 69.32, 68.55, 21.36.

Exact mass (ESI-MS) for $C_{20}H_{22}O_5S$ $[M+H]^+$ found, 375.1273; calcd, 375.1266; $[M+Na]^+$ found, 397.1109; calcd, 397.1086.

Spectral data are consistent with the literature data.

***p*-methylphenyl-2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (30)**

To a solution of **29** (7.00 g, 18.69 mmol) in DMF (94 mL) at 0 °C was added NaH (2.24 g, 56.08 mmol). After stirring for 30 minutes, benzyl bromide (6.7 mL, 56.08 mmol) was added and the solution was stirred for 2 hours at room temperature. Upon reaction completion, the mixture was quenched with H_2O and extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by column chromatography (hexanes/EtOAc: 6.5/3.5) to afford compound **30** (8.14 g, 79 %) as white crystals.



^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.61 (d, *J* = 7.9 Hz, 2H, arom. H), 7.54-7.26 (m, 15H, arom. H), 7.01 (d, *J* = 7.9 Hz, 2H, arom. H), 5.48 (s, 1H, CH-Ph), 4.72 (app. d, *J* = 3.5 Hz, 4H, CH_2-Ph), 4.57 (d, *J* = 9.4 Hz, 1H, H-1”), 4.37 (dd, *J* = 1.5 Hz and 12.3 Hz, 1H, H-6”), 4.14 (dd, *J* = 0.6 Hz and 3.5 Hz, 1H, H-4”), 3.98 (dd, *J* = 1.6 Hz and 12.5 Hz, 1H, H-6”), 3.84 (t, *J* = 9.4 Hz, 1H, H-2”), 3.62 (dd, *J* = 3.4 Hz and 9.2 Hz, 1H, H-3”), 3.41 (app. d, *J* = 0.9 Hz, 1H, H-5”), 2.31 (s, 3H, CH_3).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 138.83, 138.36, 138.17, 137.90, 133.71, 129.87, 129.27, 128.90, 128.63, 128.57, 128.42, 128.35, 128.06, 128.03, 127.91, 126.92, 101.59, 86.87, 81.70, 75.58, 73.90, 72.07, 70.01, 69.70, 21.40.

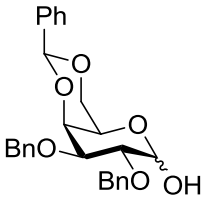
Exact mass (ESI-MS) for $C_{34}H_{34}O_5S$ $[M+H]^+$ found, 555.2230; calcd, 555.2205; $[M+Na]^+$ found, 577.2017; calcd, 577.2025; $[M+K]^+$ found, 593.1777; calcd, 593.1764.

Spectral data are consistent with the literature data.

2,3-di-*O*-benzyl-4,6-*O*-benzylidene-D-galactopyranoside (31)

To a suspension of **30** (8.10 g, 14.60 mmol) in acetone (162 mL) and H_2O (16.2 mL) at 0 °C was added *N*-iodosuccinimide (NIS) (3.60 g, 16.06 mmol). The reaction was allowed to stir for 2.5 hours at room temperature. Then a 0.5 M solution of $Na_2S_2O_3$ was added and the reaction mixture was allowed to stir for an additional hour. The aqueous layer was extracted with CH_2Cl_2 and the combined organic layers were washed with a 0.5 M solution of $Na_2S_2O_3$

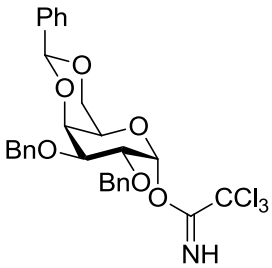
and brine. After drying over Na_2SO_4 and evaporation of the solvent, the residue was purified by column chromatography (hexanes/EtOAc: 1/1) to afford **31** (6.25 g, 95 %) as a white solid.



Exact mass (ESI-MS) for $C_{27}H_{28}O_6$ $[M+Na]^+$ found, 471.1788; calcd 471,1784; $[M+K]^+$ found, 487.1522; calcd, 487.1523.

2,3-di-*O*-benzyl-4,6-*O*-benzylidene-*α*-D-galactopyranosyl trichloroacetimidate (32)

To a solution of **31** (3.30 g, 7.36 mmol) in CH_2Cl_2 (80 mL) at 0 °C, DBU (0.5 mL, 3.68 mmol) and trichloroacetonitrile (7.38 mL, 73.6 mmol) were added. The reaction mixture was stirred for 5 hours, diluted with CH_2Cl_2 and celite was added. The solvent was removed under reduced pressure and the residue was purified by column chromatography (hexanes/EtOAc: 8.5/1.5 + 1 V% Et_3N) yielding **32** (3.62 g, 83 %) as white crystals.



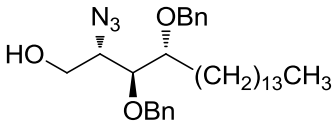
^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.57 (s, 1H, NH), 7.55-7.53 (m, 2H, arom. H), 7.40-7.26 (m, 13H, arom. H), 6.64 (d, *J* = 3.4 Hz, 1H, H-1”), 5.52 (s, 1H, CH-Ph), 4.83-4.75 (m, 4H, CH_2-Ph), 4.31-4.26 (m, 3H, H-2”, H-6” and H-4”), 4.08 (dd, *J* = 3.3 Hz and 10.0 Hz, 1H, H-3”), 4.01 (dd, *J* = 1.8 Hz and 12.6 Hz, 1H, H-6”), 3.84 (app. d, *J* = 0.9 Hz, 1H, H-5”).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 161.27, 138.63, 137.89, 129.21, 128.52, 128.47, 128.40, 128.21, 127.70, 127.63, 126.60, 101.34, 95.85, 75.31, 74.95, 74.71, 73.37, 72.45, 69.31, 65.50.

Exact mass (ESI-MS) for $C_{29}H_{28}Cl_3NO_6$ $[M+Na]^+$ found, 614.0919; calcd, 614.0880; $[M+K]^+$ found, 630.0668; calcd, 630.0619.

(2*S*,3*S*,4*R*)-2-azido-3,4-di-*O*-benzyl-octadecane-1,3,4-triol (33)

To a solution of **21** (8.40 g, 10.97 mmol) in CH_2Cl_2 (149 mL) and *i*PrOH (26.32 mL) was added $ZnBr_2$ (39.51 g, 175.44 mmol). The mixture was stirred overnight, then quenched with H_2O. After extraction with CH_2Cl_2, the combined organic layers were washed with H_2O and brine, dried over Na_2SO_4 and evaporated to dryness. The resulting residue was purified by column chromatography (hexanes/EtOAc: 9/1) furnishing **33** (2.92 g, 51 %) as a pale yellow oil.



^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.27-7.39 (m, 10H, arom.H), 4.72 (d, *J* = 11.1 Hz, 1H, CH_2-Ph), 4.67 (d, *J* = 10.2 Hz, 1H, CH_2-Ph), 4.63 (d, *J* = 10.5 Hz, 1H, CH_2-Ph), 4.57 (d, *J* = 11.4 Hz, 1H, CH_2-Ph), 3.89 (dd, *J* = 11.4 Hz and 5.9 Hz, 1H, H-1), 3.79 (ddd, *J* = 11.7 Hz, 6.7 Hz and 5.1 Hz, 1H, H-1), 3.60-3.73 (m, 3H, H-2, H-3 and H-4), 2.49 (dd, *J* = 6.2 Hz and 6.8 Hz, 1H, OH), 1.58-1.23 (m, 26H, CH_2), 0.88 (t, *J* = 6.8 Hz, 3H, CH_3).

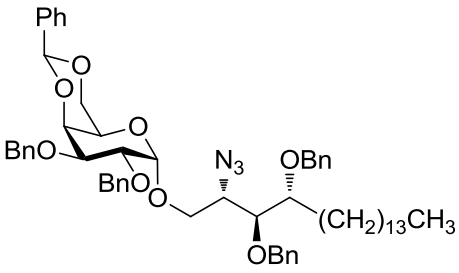
^{13}C-NMR (75 MHz, $CDCl_3$): δ 147.10, 138.20, 137.87, 128.76, 128.69, 128.34, 128.25, 128.23, 128.16, 128.08, 127.49, 80.75, 79.27, 77.45, 73.87, 72.79, 63.31, 62.50, 32.16, 30.47, 29.94, 29.92, 29.90, 29.84, 29.80, 29.60, 25.72, 22.93, 14.36.

Exact mass (ESI-MS) for $C_{32}H_{49}N_3O_3$ $[M+H]^+$ found, 524.3843; calcd, 524.3852.

Spectral data are consistent with the literature data.

(2*S*,3*S*,4*R*)-2-azido-3,4-di-*O*-benzyl-1-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl)-octadecane-1,3,4-triol (34)

To a mixture of galactosyl donor **32** (170 mg, 0.29 mmol) in THF (1 mL) under argon atmosphere was added a solution of **33** (100 mg, 0.19 mmol) in THF (2.8 mL). The solution was cooled to -30 °C and TMSOTf (5 µL, 0.03 mmol) was added. After stirring for 1 hour, the reaction mixture was neutralized with Et_3N and the solvent was evaporated under reduced pressure. The resulting residue was purified by column chromatography (hexanes/EtOAc: 9/1) affording glycolipid **34** (146 mg, 80 %) as a white solid.



^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.53-7.51 (m, 2H, arom. H), 7.41-7.21 (m, 23H, arom. H), 5.45 (s, 1H, CH-Ph), 4.97 (d, *J* = 3.2 Hz, 1H, H-1”), 4.86 (d, *J* = 12.0 Hz, 1H, CH_2-Ph), 4.81 (d, *J* = 13.2 Hz, 1H, CH_2-Ph), 4.77 (d, *J* = 10.4 Hz, 1H, CH_2-Ph), 4.74 (d, *J* = 12.3 Hz, 1H, CH_2-Ph), 4.67 (d, *J* = 11.7 Hz, 1H, CH_2-Ph), 4.60 (d, *J* = 11.4 Hz, 1H, CH_2-Ph), 4.58 (d, *J* = 11.7 Hz, 1H, CH_2-Ph), 4.50 (d, *J* = 11.7 Hz, 1H, CH_2-Ph), 4.16 (app. d, *J* = 3.5 Hz, 1H, H-4”), 4.14-4.07 (m, 2H, H-2” and H-6”), 4.04-3.99 (m, 2H, H-1 and H-3”), 3.88 (dd, *J* = 1.3 Hz and 12.6 Hz, 1H, H-6”), 3.73-3.69 (m, 3H, H-1, H-2 and H-3), 3.62 (ddd, *J* = 3.5 Hz and 7.1 Hz, 1H, H-4), 3.57 (app. s, 1H, H-5”), 1.65-1.26 (m, 26H, CH_2), 0.88 (t, *J* = 6.4 Hz, 3H, CH_3).

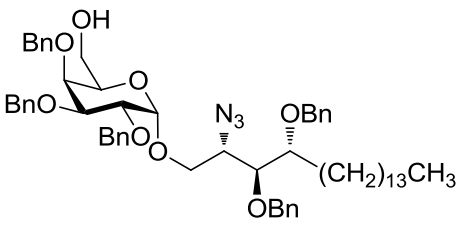
^{13}C-NMR (75 MHz, $CDCl_3$): δ 139.00, 138.60, 138.26, 138.07, 129.90, 129.10, 128.62, 128.59, 128.51, 128.47, 128.34, 128.15, 128.04, 128.00, 127.94, 127.91, 127.85, 127.75, 127.70, 126.58, 121.12, 101.30, 99.38, 79.65, 79.20, 77.90, 77.44, 76.04, 75.69, 74.91, 74.02,

73.74, 72.44, 72.31, 72.28, 69.56, 68.69, 63.22, 62.04, 32.16, 30.26, 30.01, 29.94, 29.92, 29.90, 29.88, 29.85, 29.72, 29.60, 29.47, 25.70, 22.92, 21.58, 14.35.

Exact mass (ESI-MS) for $C_{59}H_{75}N_3O_8$ $[M+H]^+$ found, 954.5652; calcd, 954.5627; $[M+Na]^+$ found, 976.5464; calcd, 976.5446; $[M+K]^+$ found, 992.5109; calcd, 992.5186.

(2*S*,3*S*,4*R*)-2-azido-3,4-di-*O*-benzyl-1-*O*-(2,3,4-tri-*O*-benzyl-6-hydroxy-α-D-galactopyranosyl)-octadecane-1,3,4-triol (35)

To a solution of **34** (50 mg, 0.05 mmol) in anhydrous CH_2Cl_2 (1.6 mL) under argon atmosphere was added copper(II) triflate (3 mg, 0.008 mmol) and BH_3.THF (0.26 mL, 0.26 mmol). After stirring for 1.5 hours at room temperature, the brown reaction mixture was quenched with methanol. Subsequently the mixture was diluted with EtOAc and washed with a saturated $NaHCO_3$ solution, water and brine. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by column chromatography (hexanes/EtOAc: 8.5/1.5) yielded **35** (37 mg, 73 %) as a colorless oil.



^{1}H NMR (300 MHz, DMSO-d_6): δ 7.38-7.7.22 (m, 25H, arom. H), 4.97 (d, *J* = 2.25 Hz, 1H, H-1”), 4.82 (d, *J* = 11.4 Hz, 1H, CH_2-Ph), 4.79-4.69 (m, 5H, CH_2-Ph, OH), 4.60-5.54 (m, 4H, CH_2-Ph), 4.44 (d, *J* = 11.6 Hz, 1H, CH_2-Ph), 4.08 (br. s, 1H, H-3”), 3.97-3.88 (m, 3H, H-1, H-3, H-2”), 3.79-3.73 (m, 3H, H-2, H-4”, H-5”), 3.67-3.62 (m, 2H, H-1, H-4), 3.52-3.46 (m, 2H, H-6”), 2.49-1.20 (m, 26H, CH_2) 0.85 (t, *J* = 6.6 Hz, 3H, CH_3).

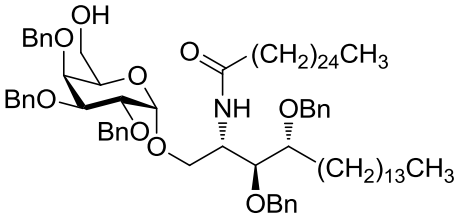
^{13}C NMR (75 MHz, $CDCl_3$): δ 138.96, 138.88, 138.63, 138.43, 138.30, 128.82, 128.72, 128.66, 128.63, 128.61, 128.54, 128.22, 128.13, 128.09, 128.00, 127.98, 127.86, 127.84, 127.78, 98.84, 79.56, 79.46, 79.06, 76.69, 75.43, 74.73, 73.96, 73.69, 73.48, 72.30, 71.12, 68.68, 62.70, 62.25, 32.19, 30.27, 30.02, 29.97, 29.93, 29.89, 29.87, 29.63, 25.61, 22.96, 14.39.

Exact mass (ESI-MS) for $C_{59}H_{77}N_3O_8$ $[M+Na]^+$ found, 978.5695; calcd, 978.5603.

(2*S*,3*S*,4*R*)-3,4-di-*O*-benzyl-1-*O*-(2,3,4-tri-*O*-benzyl-6-hydroxy-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (25)

To a solution of **35** (157 mg, 0.16 mmol) in THF (1.6 mL) was added dropwise trimethylphosphine (0.8 mL, 0.82 mmol). After stirring for 2.5 hours, a NaOH solution (3 mL, 1 M) was added and the mixture was allowed to stir for an additional 2.5 hours. The reaction mixture was extracted with EtOAc and the organic layer was washed with brine,

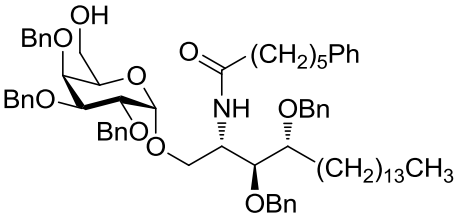
dried over Na_2SO_4 and concentrated under reduced pressure. The crude amine was dissolved in CH_2Cl_2 (2 mL) and added to a solution of EDC (63 mg, 0.33 mmol) and hexacosanoic acid (97 mg, 0.25 mmol) in CH_2Cl_2 (0.5 mL). This reaction mixture was stirred overnight at room temperature after which it was extracted with CH_2Cl_2. The organic layer was washed with brine and dried over Na_2SO_4. After evaporation of the organic solvent, the residue was purified by column chromatography (hexanes/EtOAc: 6/4) affording compound **25** (167 mg, 78 %) as a yellow oil.



Spectral data are consistent with those of **25** on page 62-63.

(2*S*,3*S*,4*R*)-3,4-di-*O*-benzyl-1-*O*-(2,3,4-tri-*O*-benzyl-6-hydroxy-α-D-galactopyranosyl)-2-(6-phenylhexanoyl)amino-octadecane-1,3,4-triol (36)

To a solution of **35** (120 mg, 0.13 mmol) in THF (1.3 mL) was added dropwise trimethylphosphine (0.6 mL, 0.63 mmol). After stirring for 2.5 hours, a NaOH solution (2.3 mL, 1 M) was added and the mixture was allowed to stir for an additional 2.5 hours. The reaction mixture was extracted with EtOAc and the organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude amine was dissolved in CH_2Cl_2 (1.4 mL) and added to a solution of EDC (48 mg, 0.25 mmol) and 6-phenylhexanoic acid (36 mg, 0.19 mmol) in CH_2Cl_2 (0.5 mL). This reaction mixture was stirred overnight at room temperature after which it was extracted with CH_2Cl_2. The organic layer was washed with brine and dried over Na_2SO_4. After evaporation of the organic solvent, the residue was purified by column chromatography (hexanes/EtOAc: 7/3) affording compound **36** (107 mg, 77 %) as a colorless oil.



^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.38-7.15 (m, 30H, arom. H), 5.84 (d, *J* = 8.6 Hz, 1H, NH), 4.94 (d, *J* = 11.4 Hz, 1H, CH_2-Ph), 4.86 (d, *J* = 3.7 Hz, 1H, H-1”), 4.83 (d, *J* = 11.7 Hz, 1H, CH_2-Ph), 4.80 (d, *J* = 11.7 Hz, 1H, CH_2-Ph), 4.74 (d, *J* = 11.7 Hz, 1H, CH_2-Ph), 4.69 (d, *J* = 11.7 Hz, 1H, CH_2-Ph), 4.66 (d, *J* = 11.7 Hz, 1H, CH_2-Ph), 4.60 (d, *J* = 11.7 Hz, 1H, CH_2-Ph), 4.55 (d, *J* = 11.4 Hz, 1H, CH_2-Ph), 4.52 (d, *J* = 11.7 Hz, 1H, CH_2-Ph), 4.48 (d, *J* =

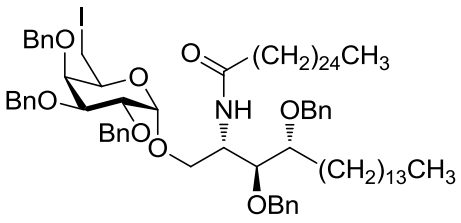
11.4 Hz, 1H, CH_2-Ph), 4.44 (d, *J* = 11.4 Hz, 1H, CH_2-Ph), 4.37 (m, 1H, H-2), 4.02 (dd, *J* = 9.9 Hz and 3.7 Hz, 1H, H-2"), 3.94 (dd, *J* = 11.4 Hz and 7.7 Hz, 1H, H-1), 3.85-3.82 (m, 3H, H-1, H-3"and H-4"), 3.70-3.62 (3H, H-3, H-5" and H-6"), 3.55-3.41 (m, 2H, H-4, H-6"), 2.57 (t, *J* = 7.1 Hz, 2H, CH_2-Ph), 1.97-1.80 (m, 2H, $COCH_2$), 1.69-1.26 (m, 34H, CH_2), 0.88 (t, *J* = 6.6 Hz, 3H, CH_3).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 173.26, 142.68, 138.84, 138.76, 138.66, 138.47, 128.68, 128.65, 128.62, 128.60, 128.51, 128.17, 128.14, 128.09, 127.98, 127, 95, 127.86, 127.84, 127.66, 125.93, 100.23, 80.57, 79.50, 79.43, 77.67, 77.45, 77.25, 76.82, 75.04, 74.76, 73.79, 73.40, 73.33, 72.06, 71.51, 69.98, 62.69, 51.08, 50.89, 36.87, 35.95, 32.16, 31.36, 31.16, 30.44, 30.02, 29.96, 29.92, 29.60, 29.12, 26.01, 25.73, 22.93, 14.36.

Exact mass (ESI-MS) for $C_{71}H_{93}NO_9$ $[M+H]^+$ found, 1104.7063; calcd, 1104.6923.

(2*S*,3*S*,4*R*)-3,4-di-*O*-benzyl-1-*O*-(2,3,4-tri-*O*-benzyl-6-iodo-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (37)

PPh_3 (18 mg, 0.07 mmol) was added to a solution of **25** (74 mg, 0.06 mmol) in toluene (0.4 mL) under argon followed by refluxing during 10 minutes. The mixture was cooled down to 80 °C and imidazole (11 mg, 0.17 mmol) and I_2 (19 mg, 0.07 mmol) were added. After refluxing for 20 minutes, the solution was concentrated under reduced pressure. The resulting residue was diluted with EtOAc and washed with a saturated $Na_2S_2O_3$ solution and water. The organic layer was dried on Na_2SO_4 and evaporated to dryness. Purification by column chromatography (hexanes/EtOAc: 9/1) yielded **37** (68 mg, 85 %) as a white solid.



^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.40-7.21 (m, 25H, arom. H), 5.84 (d, *J* = 8.6 Hz, 1H, NH), 5.03 (d, *J* = 11.2 Hz, 1H, CH_2-Ph), 4.84 (d, *J* = 11.7 Hz, 1H, CH_2-Ph), 4.83 (d, *J* = 3.4 Hz, 1H, H-1"), 4.79-4.72 (m, 3H, CH_2-Ph), 4.65-4.58 (m, 3H, CH_2-Ph), 4.52 (d, *J* = 11.7 Hz, 1H, CH_2-Ph), 4.49 (d, *J* = 11.7 Hz, 1H, CH_2-Ph), 4.30 (m, 1H, H-2), 4.08 (m, 1H, H-4"), 4.02 (dd, *J* = 10.0 Hz and 3.5 Hz, 1H, H-2"), 3.91-3.87 (m, 2H, H-1, H-3"), 3.84-3.77 (m, 3H, H-1, H-3, H-5"), 3.53 (ddd, *J* = 7.2 Hz and 3.6 Hz, 1H, H-4), 3.18 (dd, *J* = 9.9 Hz and 7.1 Hz, 1H, H-6"), 3.09 (dd, *J* = 9.9 Hz and 7.0 Hz, 1H, H-6"), 2.02-1.86 (m, 2H, $COCH_2$), 1.72-1.06 (m, 72H, CH_2), 0.88 (t, *J* = 6.7 Hz, 6H, CH_3).

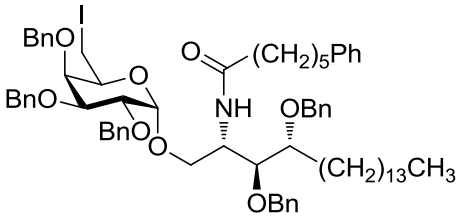
^{13}C-NMR (75 MHz, $CDCl_3$): δ 169.41, 135.12, 135.05, 135.00, 134.87, 134.74, 124.95, 124.90, 124.87, 124.39, 124.37, 124.30, 124.23, 124.16, 124.12, 124.08, 123.93, 101.28, 95.36, 76.43, 75.71, 75.61, 73.94, 73.94, 73.72, 73.52, 73.10, 72.70, 71.88, 71.63, 69.97,

69.80, 69.70, 68.44, 64.81, 46.66, 33.29, 28.44, 26.63, 26.37, 26.24, 26.21, 26.17, 26.12, 25.97, 25.91, 25.89, 25.87, 22.46, 22.26, 19.21, 10.63.

Exact mass (ESI-MS) for $C_{85}H_{128}INO_8$ $[M+H]^+$ found, 1418.8851; calcd, 1418.8757.

(2*S*,3*S*,4*R*)-3,4-di-*O*-benzyl-1-*O*-(2,3,4-tri-*O*-benzyl-6-iodo-α-D-galactopyranosyl)-2-(6-phenylhexanoyl)amino-octadecane-1,3,4-triol (38)

PPh_3 (20 mg, 0.08 mmol) was added to a solution of **36** (70 mg, 0.06 mmol) in toluene (0.4 mL) under argon followed by refluxing during 10 minutes. The mixture was cooled down to 80 °C and imidazole (13 mg, 0.19 mmol) and I_2 (21 mg, 0.08 mmol) were added. After refluxing for 20 minutes, the solution was concentrated under reduced pressure. The resulting residue was diluted with EtOAc and washed with a saturated $Na_2S_2O_3$ solution and water. The organic layer was dried on Na_2SO_4 and evaporated to dryness. Purification by column chromatography (hexanes/EtOAc: 8/2) yielded **38** (67 mg, 85 %) as a yellow oil.



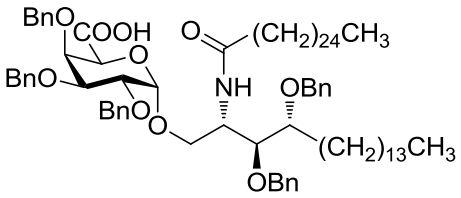
^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.42-7.16 (m, 30H, arom. H), 5.86 (d, *J* = 8.6 Hz, 1H, NH), 5.05 (d, *J* = 11.2 Hz, 1H, CH_2-Ph), 4.86 (d, *J* = 3.6 Hz, 1H, H-1”), 4.85 (d, *J* = 11.7 Hz, 1H, CH_2-Ph), 4.79 (d, *J* = 11.7 Hz, 1H, CH_2-Ph), 4.78 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.76 (d, *J* = 11.5 Hz, 1H, CH_2-Ph), 4.66 (d, *J* = 11.1 Hz, 1H, CH_2-Ph), 4.65 (d, *J* = 10.0 Hz, 1H, CH_2-Ph), 4.62 (d, *J* = 12.0 Hz, 1H, CH_2-Ph), 4.54 (d, *J* = 11.5 Hz, 1H, CH_2-Ph), 4.52 (d, *J* = 11.7 Hz, 1H, CH_2-Ph), 4.34 (m, 1H, H-2), 4.11-4.10 (m, 1H, H-4”), 4.05 (dd, *J* = 10.0 Hz and 3.5 Hz, 1H, H-2”), 3.95-3.89 (m, 2H, H-1, H-3”), 3.87-3.85 (m, 1H, H-5”), 3.83-3.79 (m, 2H, H-1, H-3), 3.59-3.54 (m, 1H, H-4), 3.21 (dd, *J* = 10.0 Hz and 7.2 Hz, 1H, H-6”), 3.12 (dd, *J* = 10.0 Hz and 6.9 Hz, 1H, H-6”), 2.60 (t, *J* = 7.6 Hz, 2H, CH_2-Ph), 2.03-1.86 (m, 2H, $COCH_2$), 1.73-1.26 (m, 32H, CH_2), 0.91 (t, *J* = 6.7 Hz, 3H, CH_3).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 169.14, 138.98, 135.09, 135.02, 134.97, 134.84, 134.72, 124.93, 124.88, 124.85, 124.76, 124.38, 124.36, 124.33, 124.29, 124.21, 124.14, 124.11, 124.07, 123.90, 122.17, 95.31, 76.35, 75.71, 75.57, 73.96, 73.54, 73.12, 72.69, 71.83, 71.60, 69.95, 69.75, 69.63, 68.39, 64.75, 56.87, 45.58, 33.11, 32.24, 28.42, 27.67, 26.62, 26.35, 26.21, 26.16, 25.86, 25.42, 22.42, 22.00, 19.19, 17.53, 10.69, 10.63.

Exact mass (ESI-MS) for $C_{71}H_{92}INO_8$ $[M+K]^+$ found, 1252.5511; calcd, 1252,5499.

(2*S*,3*S*,4*R*)-3,4-di-*O*-benzyl-1-*O*-(2,3,4-tri-*O*-benzyl-α-D-galactopyranosyluronate)-2-hexacosylamino-octadecane-1,3,4-triol (39)

TEMPO (22 mg, 0.14 mmol) and BAIB (578 mg, 1.80 mmol) were added to a mixture of **25** (940 mg, 0.72 mmol) in CH_2Cl_2 (4.7 mL) and H_2O (2.4 mL). The emulsion was vigorously stirred overnight at room temperature and the reaction was quenched with $Na_2S_2O_3$. After extraction of the aqueous layer with EtOAc, the organic layer was washed with a saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4 and evaporated. The residue was submitted to column chromatography (CH_2Cl_2/MeOH: 24/1 with 1% formic acid), affording compound **39** (823 mg, 87 %) as a yellow oil.



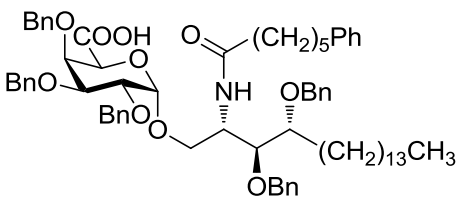
^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.32-7.19 (m, 25H, arom. H), 5.79 (br. s, 1H, NH), 4.97 (br. s, 1H, H-1"), 4.86-4.28 (m, 13H, CH_2-Ph, H-2, H-4", H-5"), 4.02 (dd, *J* = 3.4 Hz and 9.9 Hz, 1H, H-2"), 3.90 (dd, *J* = 2.6 Hz and 10.0 Hz, 1H, H-3"), 3.88-3.83 (m, 1H, H-1), 3.76-3.69 (m, 2H, H-1 and H-3), 3.53-3.50 (m, 1H, H-4), 1.93-1.74 (m, 2H, $COCH_2$), 1.59-1.08 (m, 72H, CH_2), 0.86 (t, *J* = 6.8 Hz, 6H, CH_3).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 173.30, 169.69, 138.59, 138.55, 138.38, 138.16, 128.70, 128.66, 128.47, 128.38, 128.21, 128.14, 128.13, 128.09, 128.01, 127.98, 127.66, 99.32, 79.65, 79.57, 78.14, 77.67, 77.45, 77.25, 76.82, 76.47, 75.84, 75.55, 74.07, 73.23, 73.10, 72.16, 71.21, 68.97, 50.15, 36.90, 32.17, 30.47, 30.04, 29.97, 29.94, 29.91, 29.89, 29.87, 29.84, 29.81, 29.67, 29.61, 29.60, 29.54, 25.99, 25.90, 22.93, 14.36.

Exact mass (ESI-MS) for $C_{85}H_{127}NO_{10}$ $[M+Na]^+$ found, 1344.9396; calcd, 1344.9352.

(2*S*,3*S*,4*R*)-3,4-di-*O*-benzyl-1-*O*-(2,3,4-tri-*O*-benzyl-α-D-galactopyranosyluronate)-2-(6-phenylhexanoyl)amino-octadecane-1,3,4-triol (40)

TEMPO (3 mg, 0.02 mmol) and BAIB (77 mg, 0.2 mmol) were added to a mixture of **36** (106 mg, 0.10 mmol) in CH_2Cl_2 (0.6 mL) and H_2O (0.3 mL). The emulsion was vigorously stirred overnight at room temperature and the reaction was quenched with $Na_2S_2O_3$. After extraction of the aqueous layer with EtOAc, the organic layer was washed with a saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4 and evaporated. The residue was submitted to column chromatography (CH_2Cl_2/MeOH: 29/1 with 1% formic acid), affording compound **40** (104 mg, 97 %) as a yellow oil.



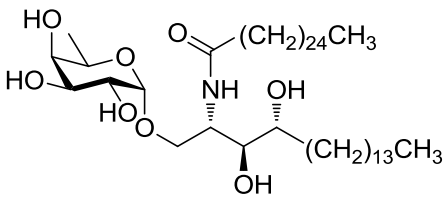
^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.34-7.14 (m, 30H, arom. H), 5.80 (d, *J* = 8.2 Hz, 1H, NH), 4.95 (d, *J* = 3.4 Hz, 1H, H-1”), 4.91-4.42 (m, 12H, CH_2-Ph, H-4”, H-5”), 4.42-4.31 (m, 1H, H-2), 4.06 (dd, *J* = 10.0 and 3.5 Hz, 1H, H-2”), 3.93 (dd, *J* = 10.1 and 2.6 Hz, 1H, H-3”), 3.89 (dd, *J* = 10.5 and 4.9 Hz, 1H, H-1), 3.80 (m, 2H, H-1, H-3), 3.54 (m, 1H, H-4), 2.67 (t, *J* = 7.7 Hz, 2H, CH_2-Ph), 1.92-1.77 (m, 2H, $COCH_2$), 1.67-1.19 (m, 34H, CH_2), 0.90 (t, *J* = 6.7 Hz, CH_3).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 173.33, 170.50, 142.73, 138.57, 138.55, 138.44, 138.42, 138.25, 128.71, 128.70, 128.68, 128.66, 128.62, 128.52, 128.46, 128.32, 128.25, 128.18, 128.16, 128.10, 128.02, 128.00, 127.96, 127.92, 127.64, 125.93, 99.93, 79.64, 79.45, 78.23, 77.72, 77.29, 76.87, 76.18, 75.95, 75.43, 74.07, 73.22, 73.07, 72.12, 71.17, 69.03, 50.23, 36.74, 35.98, 32.18, 31.38, 30.47, 30.06, 29.98, 29.93, 29.63, 29.12, 25.98, 25.72, 22.95, 14.39.

Exact mass (ESI-MS) for $C_{71}H_{97}NO_{10}$ $[M+H]^+$ found, 1118.6841; calcd, 1118.6716.

(2*S*,3*S*,4*R*)-1-*O*-(6-deoxy-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (1)

A solution of compound **37** (65 mg, 0.05 mmol) in MeOH (2.5 mL) was hydrogenated under atmospheric pressure in the presence of palladium black (20 mg). After consumption of the starting material, 1 spot was visible on TLC, corresponding with the deiodinated product. After a quick purification by column chromatography (hexanes/EtOAc: 7/3), the product was dissolved in MeOH (3 mL) and hydrogenated under atmospheric pressure in the presence of palladium black (15 mg). Upon completion of the reaction, the mixture was diluted with MeOH and filtered through celite. The filter cake was rinsed with MeOH and the filtrate was evaporated to dryness. After purification by column chromatography (CH_2Cl_2/MeOH:8/2), compound **1** (18 mg, 47 %) was afforded as a white powder.



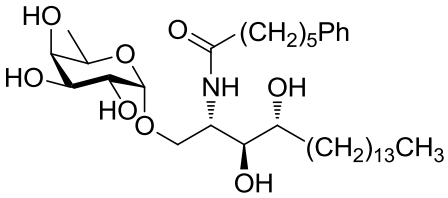
^{1}H-NMR (300 MHz, pyridine-d_5): δ 8.43 (d, *J* = 8.7 Hz, 1H, NH), 6.94 (d, *J* = 4.8 Hz, 1H, OH), 6.53 (d, *J* = 5.8 Hz, 1H, OH), 6.42 (d, *J* = 6.5 Hz, 1H, OH), 6.16 (d, *J* = 4.5 Hz, 1H, OH), 6.11 (d, *J* = 5.8 Hz, 1H, OH), 5.48 (d, *J* = 3.7 Hz, 1H, H-1”), 5.31 (m, 1H, H-2), 4.67 (dd, *J* = 10.5 Hz and 5.3 Hz, 1H, H-1), 4.59-4.56 (m, 1H, H-2”), 4.42-4.29 (m, 5H, H-1, H-3, H-4, H-3”, H-5”), 4.09-4.04 (m, 1H, H-4”), 2.47 (t, *J* = 7.4 Hz, $COCH_2$), 2.31-1.27 (m, 72H, CH_2), 1.11 (t, *J* = 7.1 Hz, 3H, CH_3), 0.89 (t, *J* = 6.7 Hz, 6H, CH_3).

^{13}C-NMR (75 MHz, pyridine-d_5): δ 171.88, 100.25, 75.62, 72.08, 71.39, 70.44, 68.72, 67.34, 66.32, 59.09, 50.02, 35.63, 33.30, 30.94, 29.20, 28.96, 28.85, 28.83, 28.75, 28.74, 28.69, 28.64, 28.57, 28.44, 28.43, 25.31, 25.23, 21.76, 19.63, 16.03, 13.10.

Exact mass (ESI-MS) for $C_{50}H_{99}NO_8$ $[M+H]^+$ found, 842.7474; calcd, 842,7444.

(2*S*,3*S*,4*R*)-1-*O*-(6-deoxy-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (2)

A solution of compound **38** (66 mg, 0.05 mmol) in MeOH (2.5 mL) was hydrogenated under atmospheric pressure in the presence of palladium black (20 mg). After consumption of the starting material, 1 spot was visible on TLC, corresponding with the deiodinated product. After a quick purification by column chromatography (hexanes/EtOAc: 7/3), the product was dissolved in MeOH (2.5 mL) and hydrogenated under atmospheric pressure in the presence of palladium black (15 mg). Upon completion of the reaction, the mixture was diluted with MeOH and filtered through celite. The filter cake was rinsed with MeOH and the filtrate was evaporated to dryness. After purification by column chromatography (CH_2Cl_2/MeOH: 9.2/0.8), compound **2** (22 mg, 70 %) was afforded as a white powder.



^{1}H-NMR (300 MHz, CD_3OD): δ 7.88 (d, *J* = 8.9 Hz, 1H, NH), 7.26-7.10 (m, 5H, arom. H), 4.79 (app. s, 1H, H-1"), 4.22-4.17 (m, 1H, H-2), 3.95 (dd, *J* = 13.3 Hz and 6.7 Hz, 1H, H-5"), 3.84 (dd, *J* = 10.5 Hz and 4.4 Hz, 1H, H-1), 3.77-3.70 (m, 2H, H-2", H-3"), 3.65-3.52 (m, 4H, H-1, H-3, H-4, H-4"), 2.61 (t, *J* = 7.7 Hz, 2H, CH_2-Ph), 2.21 (t, *J* = 7.5 Hz, 2H, $COCH_2$), 1.69-1.24 (m, 32H, CH_2), 1.20 (t, *J* = 6.7 Hz, 3H, CH_3), 0.89 (t, *J* = 6.7 Hz, 3H, CH_3).

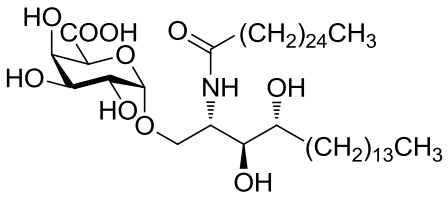
^{13}C-NMR (75 MHz, CD_3OD): δ 174.47, 142.55, 128.21, 128.11, 125.52, 99.86, 74.20, 72.42, 71.77, 70.50, 68.78, 66.81, 66.60, 50.59, 35.98, 35.57, 31.90, 31.85, 31.31, 29.64, 29.59, 29.31, 28.77, 25.82, 25.77, 22.56, 15.52, 13.28.

Exact mass (ESI-MS) for $C_{36}H_{63}NO_8$ $[M+H]^+$ found, 638.4659; calcd, 638.4626.

(2*S*,3*S*,4*R*)-1-*O*-(α-D-galactopyranosyluronate)-2-hexacosylamino-octadecane-1,3,4-triol (3)

A solution of compound **39** (105 mg, 0.08 mmol) in $CHCl_3$ (1.3 mL) and EtOH (3.8 mL) was hydrogenated under atmospheric pressure in the presence of palladium black (15 mg). Upon reaction completion, the mixture was diluted with pyridine and filtered through celite. The filter cake was rinsed with $CHCl_3$ and EtOH and the filtrate was evaporated to dryness. After

purification by column chromatography (CH_2Cl_2/MeOH:8/2), compound **3** (54 mg, 78 %) was afforded as a white powder.



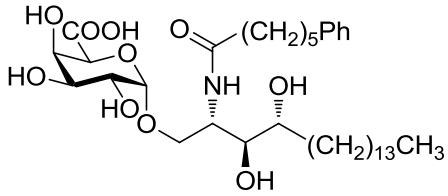
^{1}H-NMR (500 MHz, pyridine-d_5): δ 8.46 (d, *J* = 8.7 Hz, 1H, NH), 5.64 (d, *J* = 3.4 Hz, 1H, H-1”), 5.27-5.26 (m, 1H, H-2), 5.15 (app. s, 1H, H-5”), 5.02 (app.s, 1H, H-4”), 4.69-4.66 (m, 2H, H-2”, H-1), 4.50 (dd, *J* = 9.9 Hz and 3.1 Hz, 1H, H-3”), 4.37 (dd, *J* = 10.6 Hz and 4.5 Hz, 1H, H-3), 4.30 (app. s, 2H, H-1, H-4), 2.46-2.41 (m, 2H, $COCH_2$), 2.26-1.23 (m, 72H, CH_2), 0.85 (t, *J* = 6.7 Hz, CH_3).

^{13}C-NMR (75 MHz, pyridine-d_5): δ 171.93, 171.07, 152.04, 149.40, 105.00, 100.53, 75.48, 71.51, 71.34, 70.99, 70.05, 68.64, 68.08, 49.98, 35.61, 33.18, 30.94, 29.48, 29.20, 28.97, 28.86, 28.83, 28.76, 28.74, 28.69, 28.64, 28.60, 28.45, 28.43, 25.31, 25.22, 21.76, 13.10.

Exact mass (ESI-MS) for $C_{50}H_{97}NO_{10}$ $[M-H]^-$ found, 870.7089; calcd, 870.7039.

(2*S*,3*S*,4*R*)-1-*O*-(α-D-galactopyranosyluronate)-2-(6-phenylhexanoyl)amino-octadecane-1,3,4-triol (4)

A solution of compound **40** (35 mg, 0.03 mmol) in MeOH (2 mL) was hydrogenated under atmospheric pressure in the presence of palladium black (5 mg). Upon reaction completion, the mixture was diluted with MeOH and filtered through celite. The filter cake was rinsed with MeOH and the filtrate was evaporated to dryness. After purification by column chromatography (CH_2Cl_2/MeOH:8/2), compound **4** (14 mg, 68 %) was afforded as a white powder.



^{1}H-NMR (500 MHz, DMSO-d_6): δ 7.78 (d, *J* = 6.3 Hz, 1H, NH), 7.28-7.14 (m, 5H, arom. H), 4.78 (d, *J* = 3.4 Hz, 1H, H-1”), 4.07-3.93 (m, 3H, H-2, H-4”, H-5”), 3.74 (app. s, 1H, H-1), 3.62-3.38 (m, 5H, H-2”, H-3”, H-1, H-3, H-4), 2.57-2.52 (m, 4H, $COCH_2$, CH_2-Ph), 1.59-1.23 (m, 32H, CH_2), 0.86 (t, *J* = 6.9 Hz, CH_3).

^{13}C-NMR (75 MHz, DMSO-d_6): δ 172.65, 142.97, 128.90, 128.86, 126.22, 100.10, 75.03, 73.65, 71.45, 71.31, 70.51, 68.55, 67.90, 50.44, 36.09, 35.78, 32.86, 31.98, 31.59, 30.05, 29.95, 29.86, 29.82, 29.79, 29.77, 29.71, 29.40, 29.12, 28.91, 26.09, 25.90, 22.78, 14.64.

Exact mass (ESI-MS) for $C_{36}H_{61}NO_{10}$ $[M-H]^-$ found, 666.4213; calcd, 666.4223.

3.8 References

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CHAPTER 4

SYNTHESIS OF DIVERSE C-5”- AND C-6”-MODIFIED α-GALCER ANALOGUES

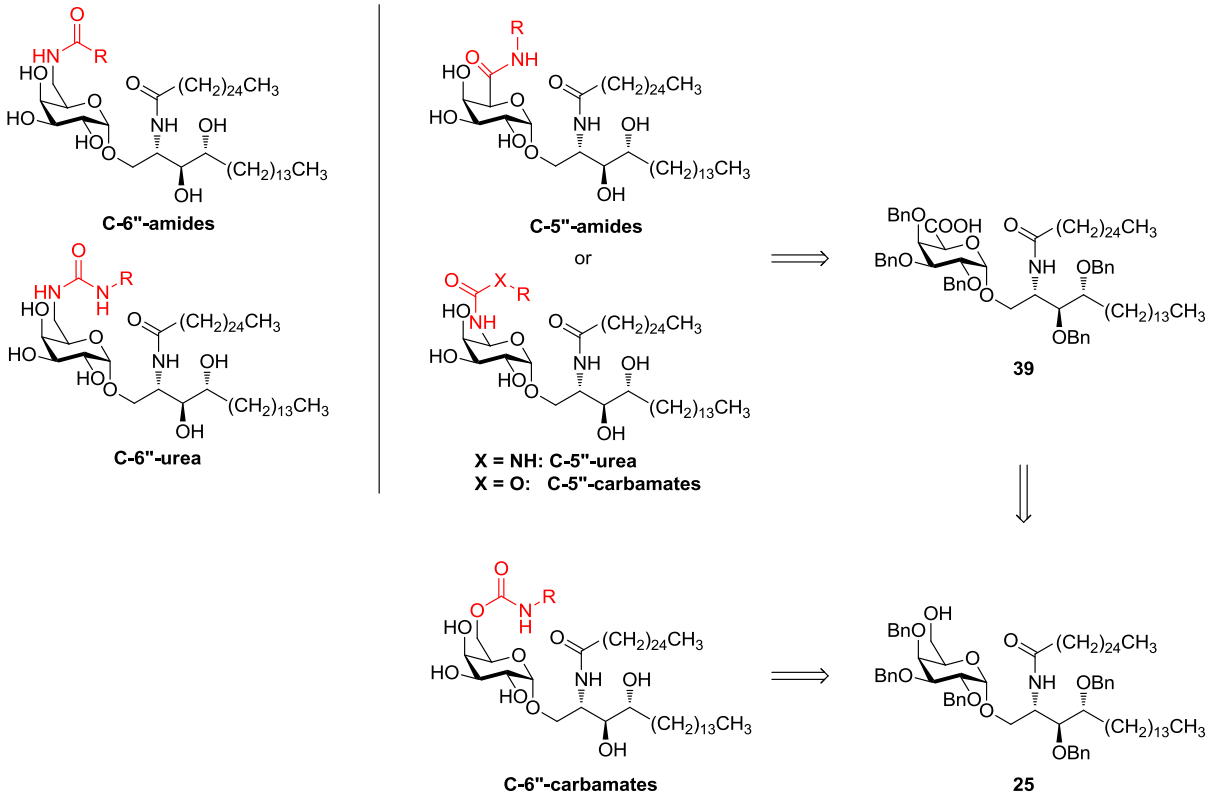
The content of this chapter was partially derived from:

- Aspeslagh, S.; Li, Y., Yu, E. D.; Pauwels, N.; Trappeniers, M.; Girardie, E.; Decruy, T.; Van Beneden, K.; Venken, K.; Drennan, M.; Leybaert, L.; Wang, J.; Franck, R. W.; Van Calenbergh, S.; Zajonc, D.; Elewaut, D. *EMBO J.* **2011**, *30*, 2294-2305.
- Pauwels, N.; Aspeslagh, S.; Elewaut, D.; Van Calenbergh, S. *Bioorg. Med. Chem.* **2012**, *20*, 7149-7154.

4 Synthesis of diverse C-5”- and C-6”-modified α-GalCer analogues

4.1 C-5”-amides, C-5”-urea/carbamate derivatives and C-6”-carbamates

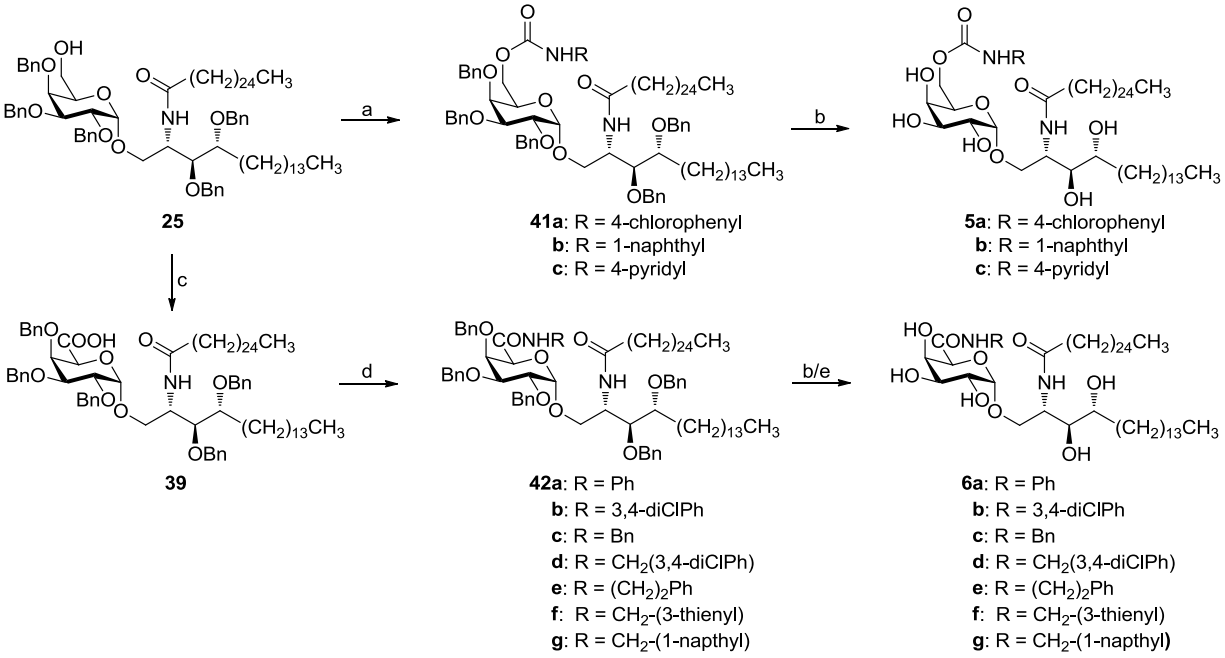
As suggested at the end of the previous chapter, galacturonic acid **39**, acquired upon TEMPO/BAIB-mediated oxidation of compound **25**, is an excellent precursor for the synthesis of C-5”-amide-substituted α-GalCer analogues (Scheme 4.1). These compounds differ from the previously synthesized C-6”-amides in that the amide functionality is inversed and located one position closer to the galactose moiety. In analogy, C-5”-modified compounds related to the known C-6”-urea derivatives can be prepared by submitting the same carboxylate **39** to a Curtius rearrangement reaction with relevant amines. A similar reaction with primary alcohols gives access to C-5”-carbamates. The corresponding C-6”-carbamates on the other hand can be obtained via treatment of **25** with the appropriate isocyanates. Together, biological evaluation of these compounds allows to investigate the influence of the linker type and length on the iNKT cell response.



Scheme 4.1 Retrosynthetic scheme for the synthesis of C-5”-amides, C-5”-urea/carbamate derivatives and C-6”-carbamates.

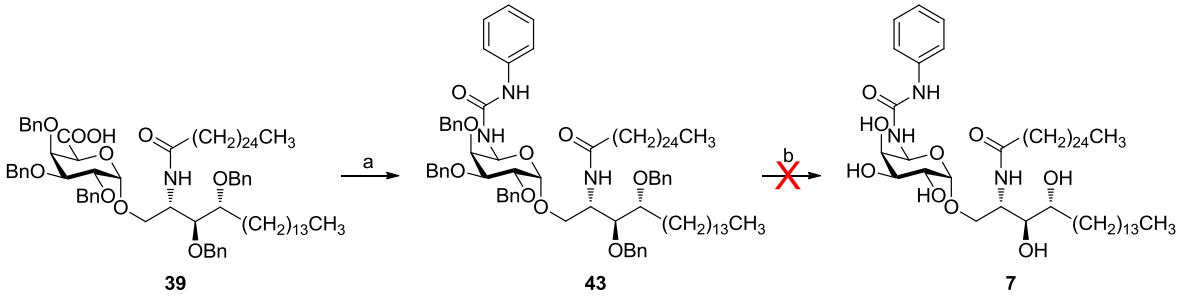
4.1.1 Synthesis

Scheme 4.2 illustrates the synthesis of C-6”-carbamates **5a**-**5c** and C-5”-amides **6a**-**6g** starting from the common intermediate **25**. Reaction of the primary hydroxyl group with the appropriate isocyanate allowed introduction of the C-6”-carbamate group, affording **41a** and **41b**. Since pyridin-4-yl isocyanate was not commercially available, compound **41c** was obtained by treating **25** with 4-aminopyridine and 1,1’-carbonyldiimidazole (CDI). On the other hand, oxidation of the primary hydroxyl group of **25** via a TEMPO/BAIB reaction, followed by coupling with the appropriate amine using 2-(6-Chloro-1-H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HCTU) afforded C-5”-amides **42a**-**42g**. Finally, the desired carbamates **5a**-**5c** and amides **6a**-**6e** were obtained after global deprotection involving a palladium-catalyzed hydrogenation. Unfortunately, this deprotection method was inconvenient for sulfur-containing compound **42f**, even under high pressure (50 bar). In addition, during hydrogenation of derivatives **41b** and **42g**, overreduction of the naphthyl group occurred. In case of naphthyl carbamate **41b**, carefully monitoring of the reaction yet allowed obtaining the final product. For the naphthyl amide compound, however, this undesired reduction was observed in the early stage of the reaction, impeding isolation of the envisaged analogue. Consequently, compounds **42f** and **42g** were alternatively deprotected in the presence of aluminium chloride and dimethylaniline, rendering the desired products **6f** and **6g** in low yields.



Scheme 4.2 Reagents and conditions: (a) i. RNCO, DMF, 67%-74% or ii. RNH_2, CDI, DMF, 70 °C, 33%; (b) Pd black, H_2, EtOH/$CHCl_3$, 49%-86%; (c) TEMPO, BAIB, CH_2Cl_2/H_2O, 87%; (d) RNH_2, HCTU, DIPEA, DMF, 53%-87 %; (e) $AlCl_3$, dimethylaniline, CH_2Cl_2, 11%-15%.

For the preparation of C-5”-urea derivative **7**, a Curtius rearrangement reaction was carried out involving refluxing of galacturonic acid **39** in toluene in the presence of Et_3N, diphenylphosphorazidate (DPPA), Ag_2CO_3 and aniline (Scheme 4.3). Regrettably, palladium-catalyzed hydrogenation of **43** to remove the benzyl groups appeared to be destructive for this compound, as no product formation could be observed and only low-molecular mass fragments were detected.



Scheme 4.3 Reagents and conditions: (a) Et_3N, DPPA, aniline, Ag_2CO_3, toluene, 110 °C, 3.5 h, 50%; (b) H_2, Pd black, EtOH, $CHCl_3$.

4.1.2 Biological evaluation

Similar to the galacturonic acid and α-D-fucopyranosyl analogues, the biological activity of the C-6”-carbamates and C-5”-amides was assessed by measurement of IFN-γ and IL-4 serum levels after intraperitoneal injection of 5 μg of the corresponding glycolipids in mice (Collaboration with Prof. D. Elewaut). The cytokine secretion induced by these compounds is presented in Figure 4.1. These data demonstrate that the C-5”-amides are weaker agonists than α-GalCer, except **6a** and **6f** which are equally potent IFN-γ inducers. The C-5”-amides generally induce a Th1 polarization, although less pronounced compared to NU-α-GalCer. The C-6”-carbamates on the other hand result in a much stronger iNKT cell response, with **5a** and **5c** skewing the balance towards a Th1 response, while **5b** has rather a Th2 polarizing effect.

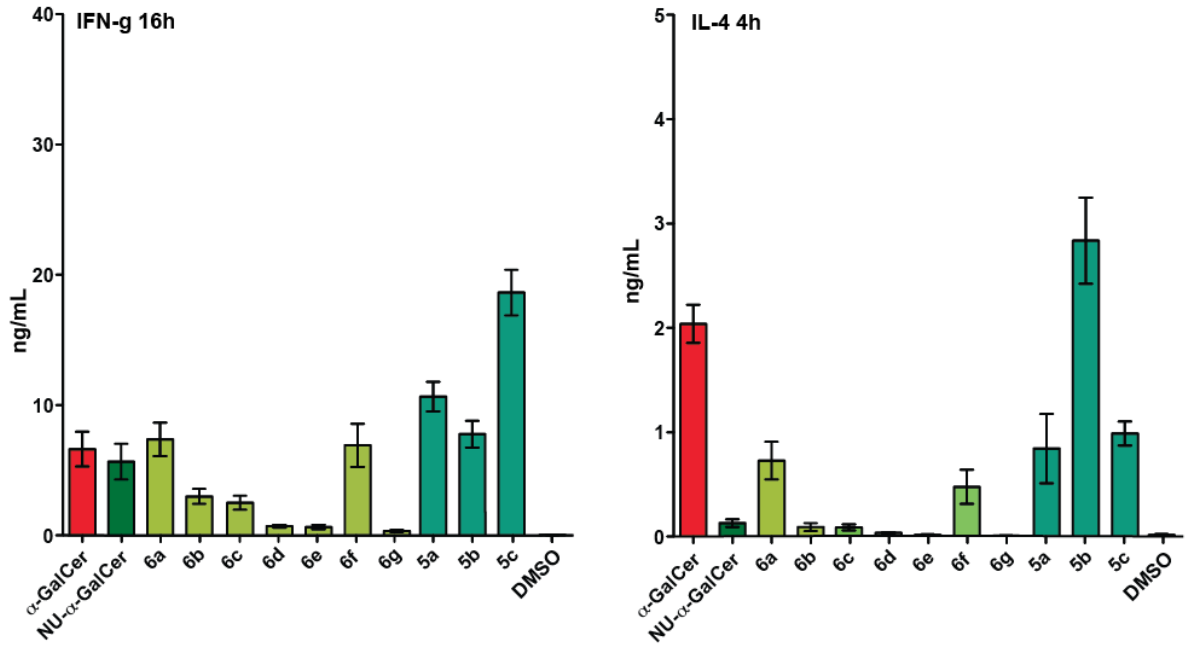


Figure 4.1 IFN-γ and IL-4 secretion, measured at respective 16 h and 4 h, after intraperitoneal injection of 5 µg of the glycolipids in mice.

In order to reveal a contingent relation between the nature of the linker and the observed differences in iNKT cell response, C-5"- amide **6c**, further referred to as BnNH-GSL-1', was studied in more detail and compared to NU-α-GalCer. SPR experiments demonstrated that the TCR binding affinity toward BnNH-GSL-1' is much weaker than to NU-α-GalCer (Table 4.1, Collaboration with Prof. D. Zajonc). Whereas the association rate of TCR binding is comparable, the dissociation of BnNH-GSL-1' is much faster than NU-α-GalCer. This indicates that the chemical differences between both glycolipids mostly affect the dissociation rate and thus the stability of the CD1d/glycolipid/TCR complex.

Table 4.1 Equilibrium binding affinity of the TCR to the indicated glycolipid/CD1d complex, measured by SPR.

Glycolipid	**$k_{ass}(M^{-1}s^{-1})$**	**$k_{diss}(s^{-1})$**	**$K_D(k_{diss}/k_{ass})$**
α-GalCer	1.3E+05 ± 1.2E+03	1.5E-03 ± 4.5E-05	11.2 ± 0.2 nM
NU-α-GalCer	9.9E+04 ± 2.0E+04	3.8E-03 ± 8.7E-04	39.6 ± 13.5 nM
BnNH-GSL-1'	1.2E+05 ± 9.2E+03	2.2E-02 ± 2.8E-04	187.3 ± 17.0 nM

To clarify the decreased stability of the ternary complex with BnNH-GSL-1', its crystal structure was elucidated and compared to the one of NU-α-GalCer and α-GalCer (Figure 4.2). Analogous to NU-α-GalCer, an additional hydrogen bond is formed between the carbonyl oxygen of the amide linker and Thr159 of CD1d. However, as the carbonyl oxygen of BnNH-

GSL-1’ is located closer to the galactose part, the galactose is tilted slightly down toward CD1d, moving the 4”-OH away from the TCR. This results in a loss of the hydrogen bond between this hydroxyl group and Asn30 of the TCR, accounting for weaker TCR binding. Moreover, the C-5”-aromatic substituent of BnNH-GSL-1’ is exposed into the solvent, in contrast to NU-α-GalCer where the naphthyl ring induces an additional hydrophobic pocket in CD1d. This suggests a lower binding affinity toward CD1d and, together with the reduced TCR affinity, explains the decreased stability of the ternary complex.

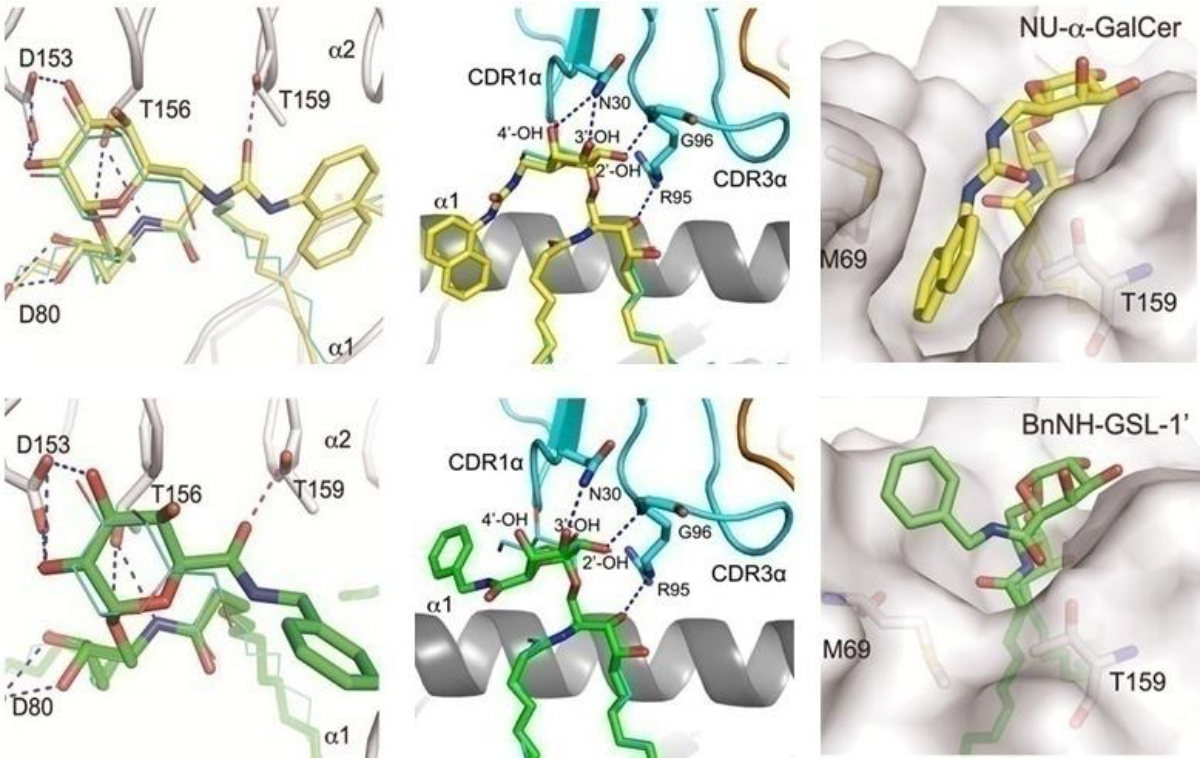


Figure 4.2 Crystal structure comparison of the ternary complexes with NU-α-GalCer (top, yellow) and BnNH-GSL-1’ (bottom, green), both superimposed on α-GalCer (blue).

Subsequently, we turned our attention to the C-6”-carbamate class, characterized by a remarkably high IFN-γ release. It is well-established that optimal IFN-γ production by iNKT cells requires APC-derived IL-12. For example, the IFN-γ level of the prototypical Th1 polarizer α-C-GalCer was shown to be greatly reduced in IL-12-deficient mice.[1] Therefore, we also examined the IL-12 production induced by these new glycolipids. The results, represented in Figure 4.3, show significantly higher IL-12 production for all three analogues compared to α-GalCer, with **5c** markedly exceeding the IL-12 amount induced by NU-α-GalCer. A similar trend was seen for the IFN-γ and IL-12 secretion when bone marrow dendritic cells (BMDCs) pulsed with these glycolipids were injected in mice (Figure 4.3, right) and for **5c** these cytokine levels were even increased up to 24 hours. Importantly, **5c**

was also able to induce a Th1-biased cytokine secretion in cultures of purified human iNKT cells.

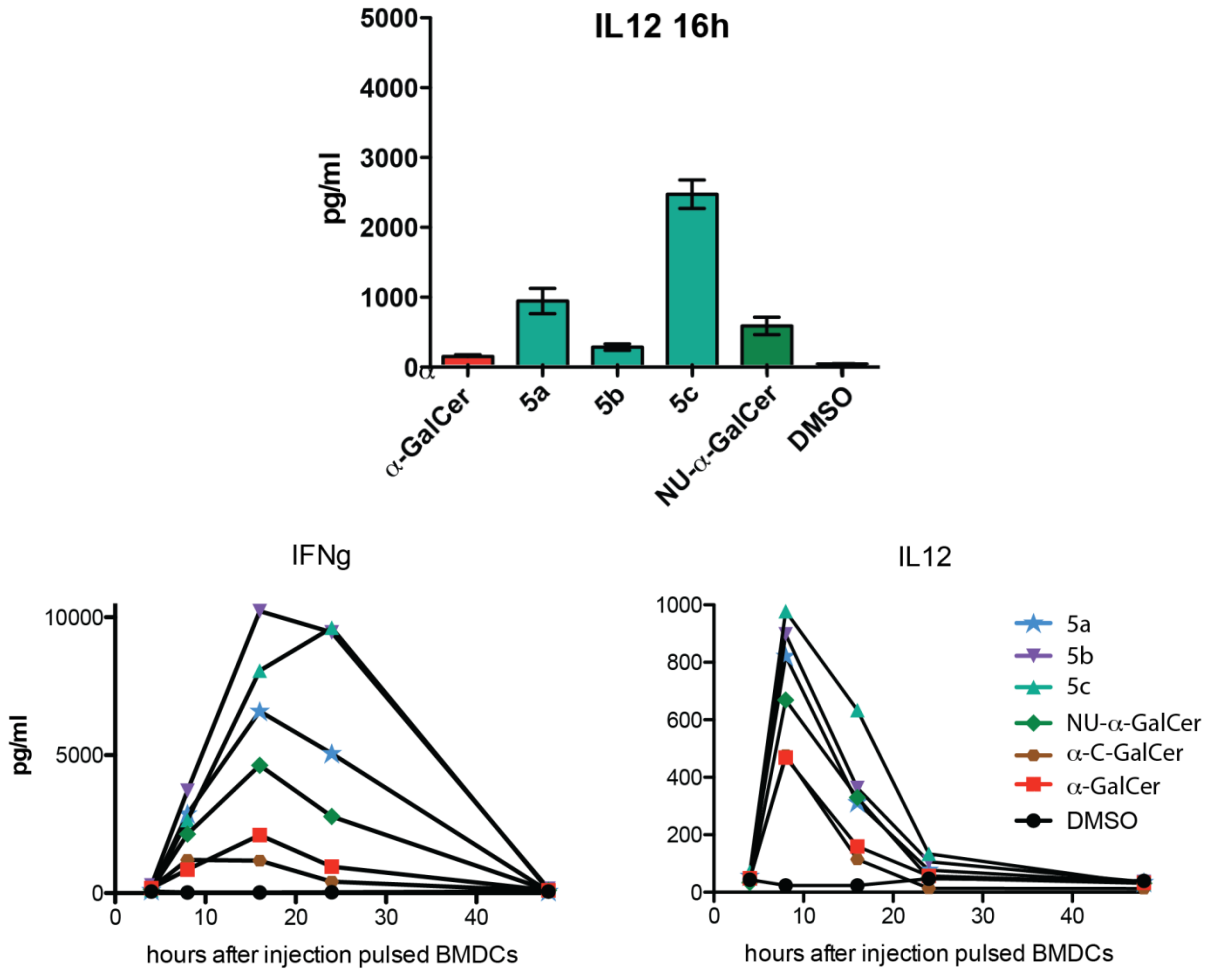


Figure 4.3 Top: IL-12 secretion, measured at 16 h after intraperitoneal injection of 5 μg of the glycolipids in mice. Bottom: IFN-γ and IL-12 levels at different time points after injection of glycolipid-pulsed BMDCs.

The equilibrium dissociation constant (K_D) of the TCR towards the different carbamate-based glycolipids presented by CD1d was assessed using SPR. For all three compounds, similar TCR affinities were observed, with **5c** equaling α-GalCer (Table 4.2). This indicates that they may follow an analogous binding mode with the TCR as α-GalCer.

Table 4.2 Equilibrium binding affinity of the TCR to the indicated glycolipid/CD1d complex, measured by SPR.

Glycolipid	**k_{ass}($M^{-1}s^{-1}$)**	**k_{diss}(s^{-1})**	**K_D(k_{diss}/k_{ass})**
α-GalCer	5.06E+04 ± 6.60E+03	1.34E-03 ± 9.00E-05	26.45 ± 4.55 nM
5a	5.27E+04 ± 1.47E+04	2.62E-03 ± 7.20E-04	49.30 ± 0.30 nM
5b	3.83E+04 ± 5.30E+03	1.50E-03 ± 7.35E-04	37.10 ± 14.10 nM
5c	6.61E+04 ± 1.21E+04	1.61E-03 ± 1.10E-04	25.00 ± 3.00 nM

The CD1d/glycolipid stability on the other hand was investigated via a lipid decay experiment, measuring the IL-2 production as a surrogate marker for the remaining binary complexes. Parallel to C-GalCer and NU-α-GalCer, the IL-2 levels of the C-6”-carbamate antigens were longer-lasting than that of α-GalCer and additionally were very similar to each other (Figure 4.4). Hence, we can conclude that the CD1d/glycolipid stability is comparable for all tested Th1 polarizing analogues and that it is significantly higher than that of CD1d/α-GalCer.

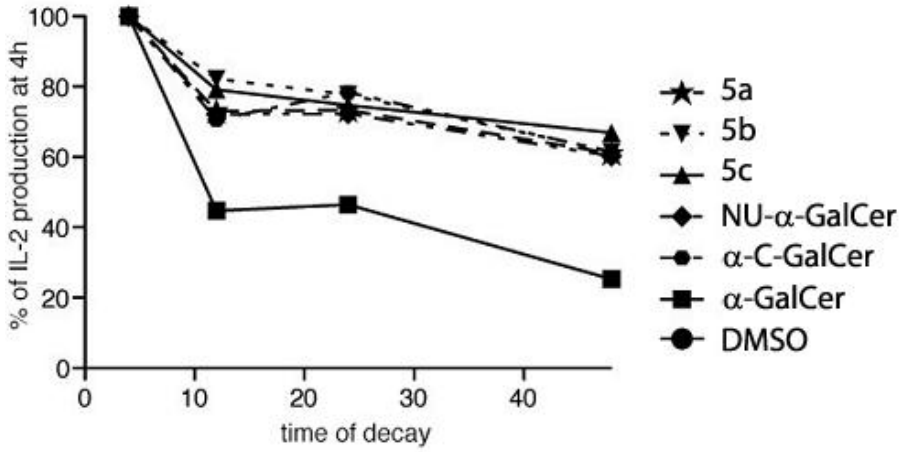


Figure 4.4 Stability analysis of the glycolipid/CD1d complexes.

To rationalize the observed binding kinetics, crystal structures of the ternary complexes with **5a** and **5c** were elucidated (Figure 4.5, Collaboration with Prof. D. Zajonc). Concerning the interactions with the TCR, the hydrogen bonds with the 2”-OH and 3”-OH groups are maintained, while for the 4”-OH group this contact is lost. The pyridine ring of **5c** makes intimate van der Waals (VDW) contact with Gln52 of the TCR (3.5-3.8 Å), an interaction previously unseen in any other structure. This might compensate for the lost hydrogen bond with the 4”-OH group and, as a consequence, explains the comparable TCR affinity to α-GalCer. By contrast, the C-6”-aryl groups of **5a** and NU-α-GalCer are more distant from the Gln52 residue, at 6.4-6.9 Å and 8.0-12.9 Å respectively, which is in agreement with the observed TCR affinity hierarchy.

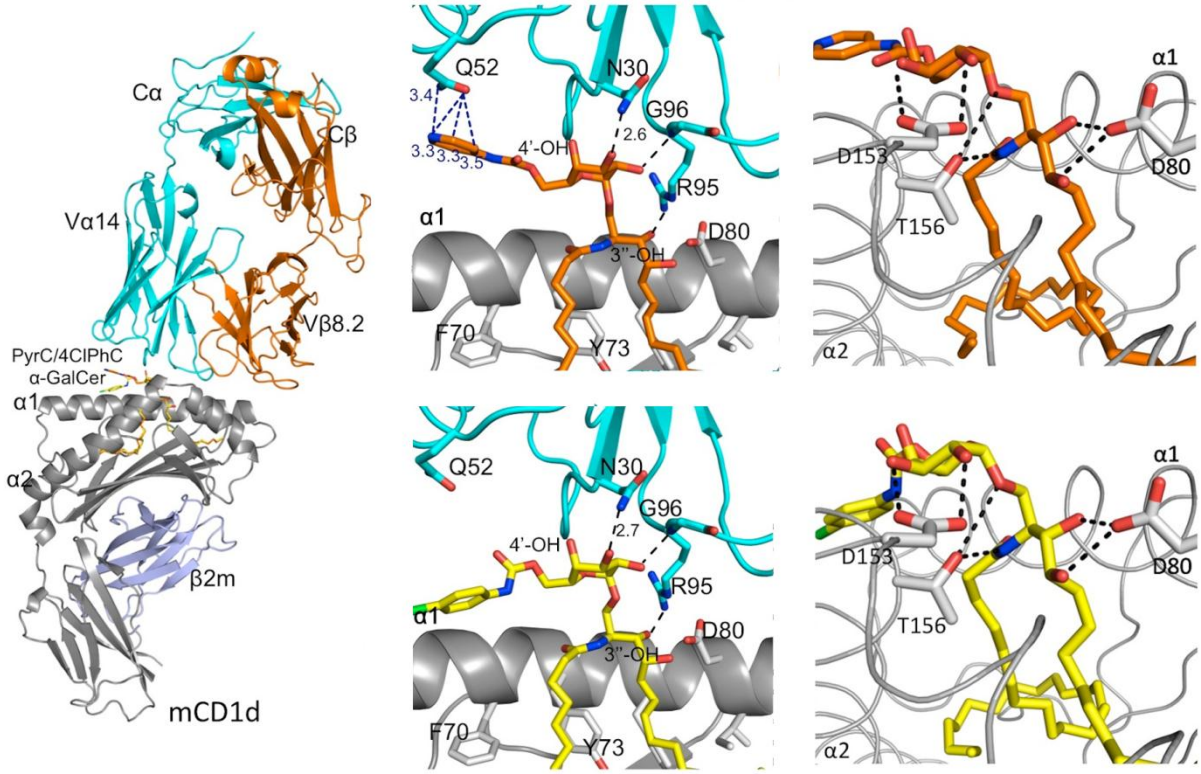


Figure 4.5 Crystal structures of the ternary complexes with **5c** (top) and **5a** (bottom). The left panel shows the overall binding by mCD1d and the TCR. The middle panel illustrates the interactions with the TCR, while the right panel illustrates those with CD1d.

Regarding the binding with CD1d, all hydrogen bonds found for α-GalCer are conserved. In contrast to NU-α-GalCer, neither the aromatic substituents of **5a** and **5c**, nor their carbonyl oxygen were found to induce additional interactions with CD1d. Nevertheless, analysis of the buried surface areas (BSA) between the glycolipids and CD1d indicates that **5a** binds as extensively to CD1d as NU-α-GalCer (1.124 vs 1.145 Å²), mostly attributed to novel VDW interactions with CD1d, in particular involving Met69, Met162 and Thr159. This is in accordance with its enhanced CD1d stability observed in the lipid decay experiment. Although **5c** showed a similar CD1d stability, its crystal structure indicates that it interacts less extensively with CD1d (1.045 Å²), comparable to α-GalCer (1.027 Å²). This suggests that in this case, factors other than the binding kinetics might play a role in the overall stability of the CD1d/glycolipid complex. Concerning the binding mode of **5b** in the ternary complex, we know from personal communication that it parallels that of NU-α-GalCer (Figure 4.6). In particular, it also induces an additional hydrophobic pocket in CD1d.

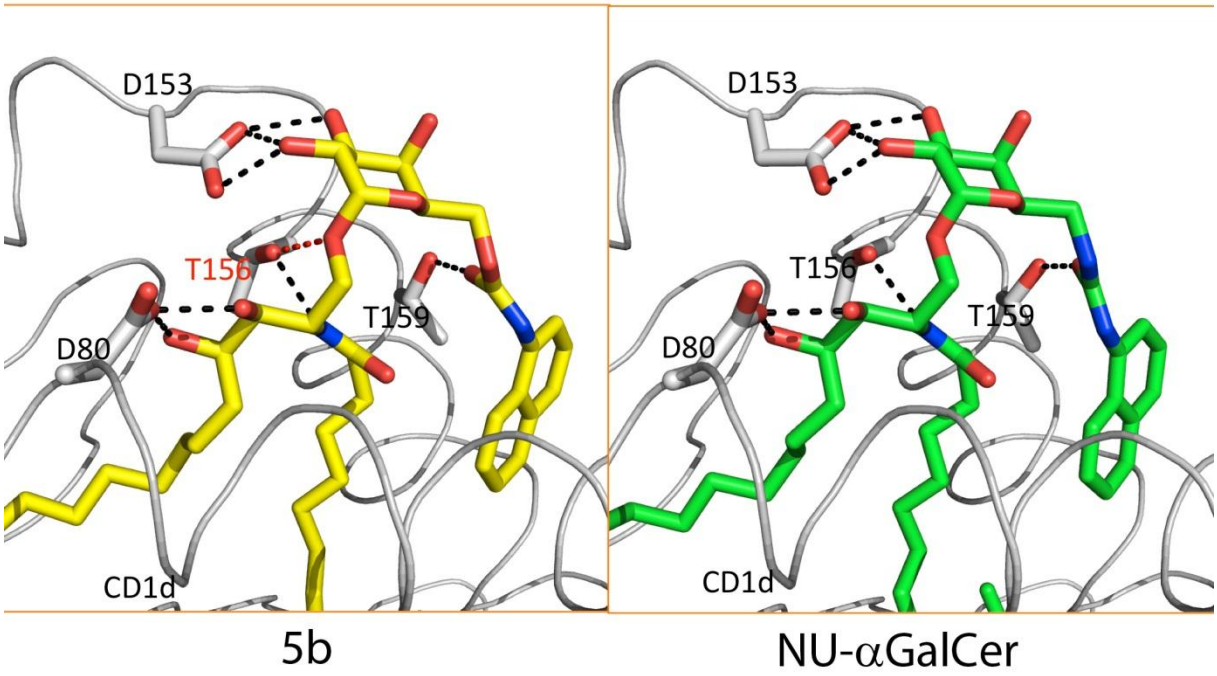


Figure 4.6 Crystal structure of **5b** bound to CD1, compared with that of NU-α-GalCer.

Finally, the high IFN-γ and IL-12 levels of the C-6”-carbamate analogues led us to investigate their anti-metastatic potential in a B16 lung melanoma model (Collaboration with Prof. D. Elewaut). All three compounds were able to reduce the quantity of lung nodules significantly more than α-GalCer (Figure 4.7). In analogy with its ability to induce high levels of IFN-γ and IL-12 secretion, **5c** was superior in preventing tumor metastases, even in comparison with NU-α-GalCer.

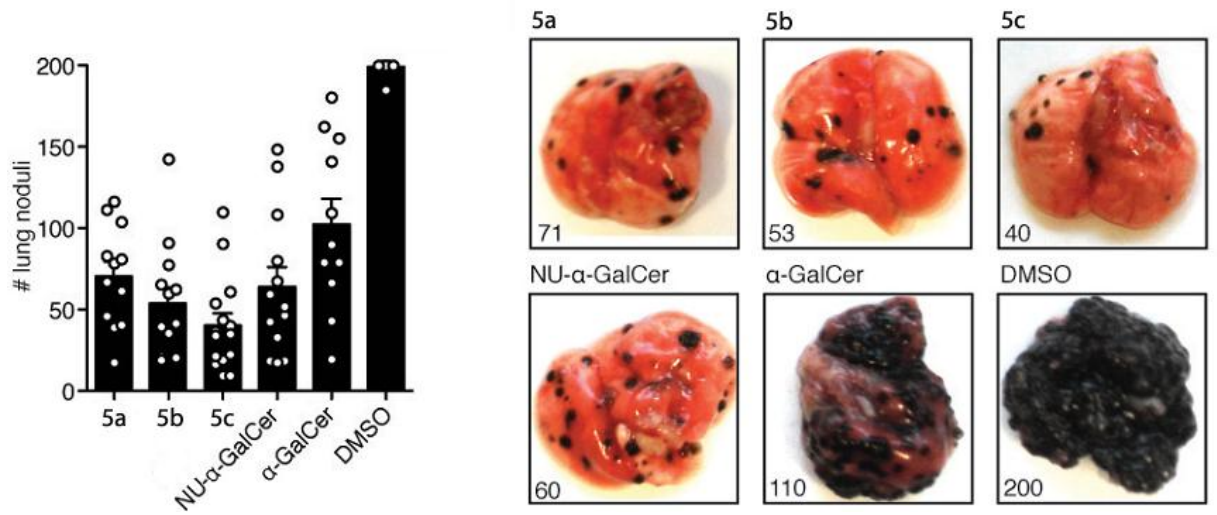


Figure 4.7 Left: number of long noduli in a B16 mouse melanoma model upon injection of 10 000 BMDCs loaded with the corresponding glycolipid (each dot represents an individual mouse). Right: pictures of the most representative lung for each glycolipid.

4.1.3 Conclusion

Starting from intermediate **25**, we could furnish a series of C-5”-amides and C-6”-carbamates in only a few steps, highlighting the versatility of our established synthetic route. Although the preparation of C-5”-carbamates and C-5”-urea derivatives could not be realized, data comparison of the succeeded C-5”-amide and C-6”-carbamate compounds with that of NU-α-GalCer does provide some insights concerning the influence of the linker and the aromatic substituent on the iNKT cell response. The possible effect of the C-5” or C-6” substituents is twofold. At first, C-6”-substitution with certain aromatic groups can result in novel interactions with the TCR, as illustrated by the VDW contacts between Gln52 and the pyridine ring of **5c**. However, this analogue also loses an H-bond between the 4”-OH and Asn30, resulting in no net TCR affinity enhancement. Secondly, increased interactions with CD1d may arise, such as the induction of an additional hydrophobic pocket and the formation of an extra hydrogen bond with Thr159, both increasing the stability of the binary complex. For the induction of the additional hydrophobic pocket, the size of the aromatic group seems to be the key determinant rather than the linker type or length. Indeed, this so-called third anchor is thus far only observed with NU-α-GalCer and **5b**, two naphthyl-containing derivatives. On the other hand, the linker length might affect the orientation of the carbohydrate moiety and thus the recognition of the CD1d/glycolipid epitope by the TCR, as shown for BnNH-GSL-1’. However, whether all these different interactions are related with the Th1 polarization induced by these compounds, and if so, what underlies their different potencies, is not yet clear and definitively needs further research. Furthermore, the apparently contradicting cytokine secretion induced by **5b** raises a lot of questions.

4.2 C-6”-triazoles

The copper-catalyzed azide–alkyne cycloaddition (CuAAC) variant of Huisgen’s 1,3-dipolar cycloaddition provides an easy and reliable method to link two building blocks via a 1,2,3-triazole moiety. Besides being a rigid linking unit, the 1,4-disubstituted triazole also possesses desirable pharmacological properties.[2] It is metabolically stable and, due to its high aromatic stabilization, resistant towards acid and basic hydrolysis and towards reductive and oxidation conditions. It has a large dipole moment (about 5 D) and is able to participate in hydrogen bond formation, dipole-dipole and π-stacking interactions. Since the aforementioned crystal structure of NU-α-GalCer and BnNH-GSL-1’ revealed an extra hydrogen bond between the carbonyl oxygen of the substituent and Thr159 of CD1d, the capacity of a triazole moiety to

act as H-bonding acceptor is most appealing. Moreover, a 1,4-disubstituted triazole group is suggested as a bioisostere of a *trans*-amide bond, which is interesting in view of the distinct Th1 profile of α-GalCer analogues with aromatic amides on C-6", including a phenyl-substituted amide (PhA-α-GalCer).[3] Hence, its corresponding triazole compound **9c** is synthesized and evaluated for comparative reasons. Finally, a docking experiment of C-6"-(1,2,3-triazol-1-yl)-substituted α-GalCer (**8a**) in the structure of the ternary complex indicates that introduction of aromatic substituents in position 4 of the triazole ring might favorably occupy the cleft that accommodates the naphthyl group of NU-α-GalCer (Figure 4.8, Performed by dr. H. De Winter). Hence, such aromatic substituents may induce a similar hydrophobic pocket in CD1d. This led us to select a homologous series of aromatic alkynes, allowing us to investigate the influence of the linker length between the triazole ring and the aromatic group on the iNKT cell activity.

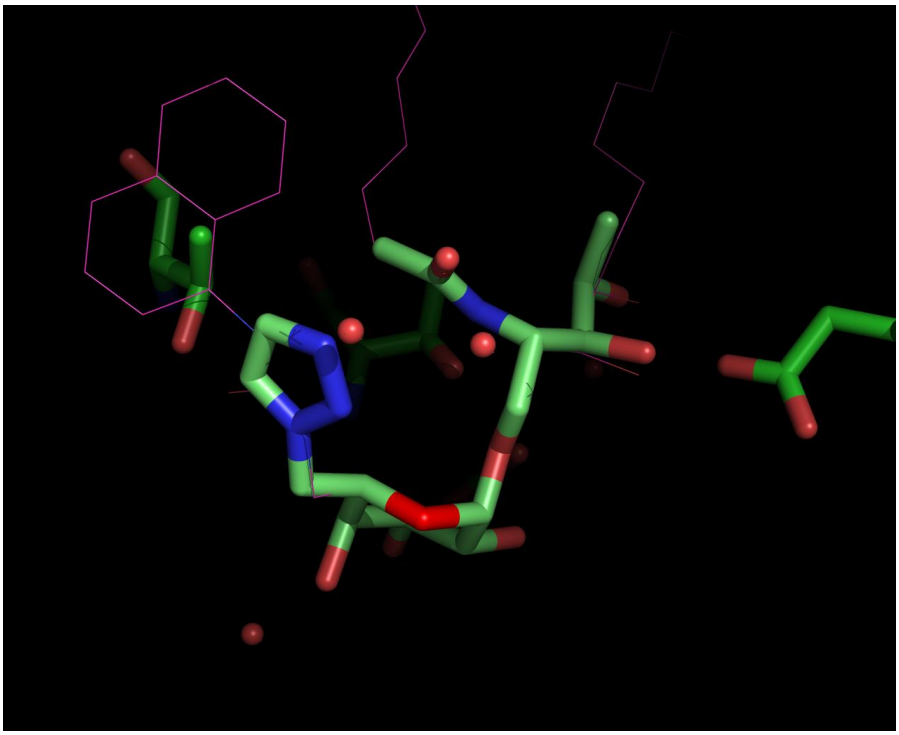


Figure 4.8 Docking of a 6"-triazole-substituted α-GalCer analogue (green: triazole compound; purple: NU-α-GalCer).

Incorporation of a triazole unit at the C-6" position has very recently been described by the group of Besra for the synthesis of homodimeric α-GalCer analogues.[4] Pegylated as well as alkylene spacers of varying lengths were used to link the two α-GalCer units (Figure 4.9). Depending on the linker length, the pegylated dimers showed similar or lower iNKT cell stimulation as α-GalCer. By contrast, the alkylene derivatives were only marginally active, regardless the length of the linker.

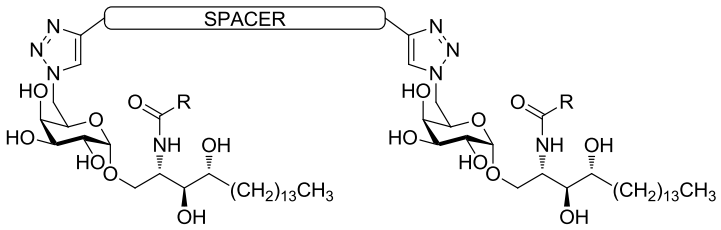
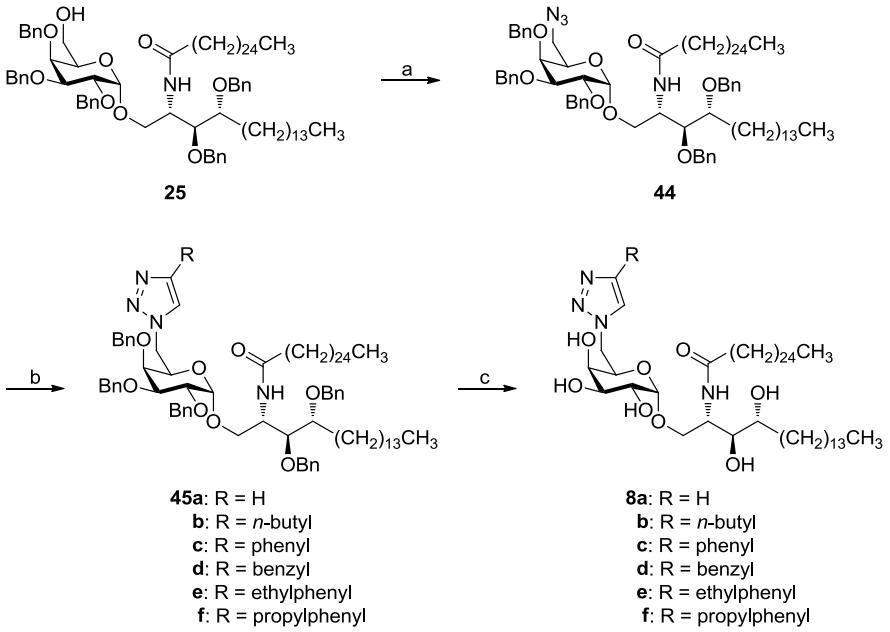


Figure 4.9 General structure of homodimeric α-GalCer analogues synthesized by the group of Besra.

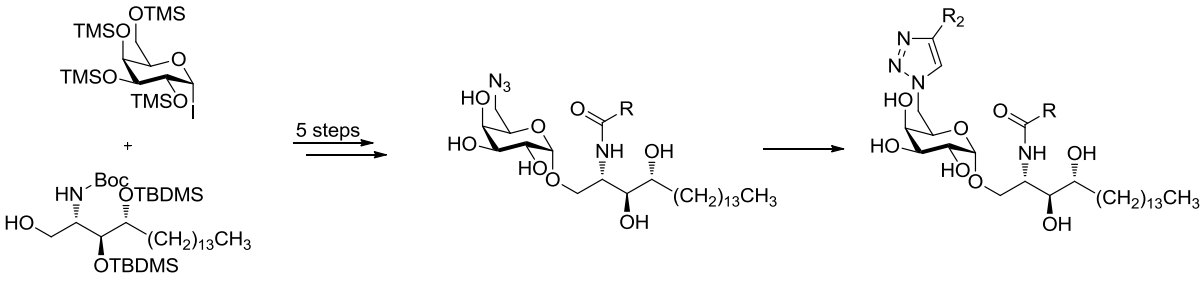
4.2.1 Synthesis

The synthesis of the desired triazoles started from intermediate **25** (Scheme 4.4). Conversion of the primary hydroxyl group to an azide group via a Mitsunobu reaction with DPPA afforded compound **44** in excellent yield. Initial attempts to convert **44** to triazole **45b** using a CuAAC reaction at ambient temperature did not result in triazole formation, even after 48 hours. Based on a report by Carvalho and co-workers[5] and our own lab experiences, we then performed the CuAAC reaction under microwave conditions (70 °C) in the presence of the appropriate alkyne, successfully obtaining compounds **45b-45f** in good yields. Due to solubility reasons, DMF was chosen as a solvent, rather than common mixtures such as H_2O/tBuOH or H_2O/THF. Aberrantly, the unsubstituted triazole **45a** was acquired by treatment with neat vinylacetate (120 °C). This procedure provides an improved and simple method compared to other reported alternatives such as the use of TMS-acetylene or acetylene gas.[6] Final debenzylation by catalytic hydrogenolysis afforded the desired analogues **8a-8f** in moderate yields.



Scheme 4.4 Reagents and conditions: (a) Ph_3P, DEAD, DPPA, THF, -20 °C, 92%; (b) appropriate alkyne, sodium ascorbate, $CuSO_4$, DMF, μW (70-120 °C, 250 W), 60%-97%; (c) Pd black, H_2, EtOH/$CHCl_3$, rt, 22%-53%.

During the course of this work, Besra and co-workers reported the synthesis of structurally related triazoles including phenyl-substituted triazole **8c**. In contrast to our method, they performed the CuAAC reaction on an unprotected 6’’-azido-6’’-deoxy-α-GalCer derivative, applying conventional heating (Scheme 4.5).[7]



Scheme 4.5 Synthetic overview of the related 1,4-disubstituted triazoles prepared by Besra and coworkers.

4.2.2 Biological evaluation

To assess the antigenic activity of the final compounds, BMDCs were loaded during 20 hours with 100 ng/mL glycolipid. After 16 hours of coculture with 2C12 cells (iNKT cell hybridoma), IL-2 secretion was determined by ELISA (Collaboration with Prof. D. Elewaut). **8a-8f** showed an efficacy to release IL-2 that was higher or comparable to α-GalCer (Figure 4.10). The analogues in which an *n*-butyl (**8b**) or phenyl (**8c**) substituent is directly attached to the triazole ring tend to induce the highest IL-2 secretion. Strikingly, the response of phenyl-substituted triazole **8c** contrasts with the *in vitro* results obtained by Besra and co-workers, where a remarkably reduced iNKT cell stimulatory activity is observed for this compound.[7] A possible explanation for this discrepancy is the direct incubation of glycolipids, BMDCs and iNKT cell hybridomas in their assay, while we used glycolipid-loaded BMDCs. Introduction of varying linker lengths between the triazole and the phenyl moieties doesn’t seem to significantly influence the antigenic activity. Hence we speculated that the mere presence of a triazol-1-yl ring at position 6” suffices to cause a superior antigenic activity than α-GalCer. Affirmatively, DCs loaded with the unsubstituted triazole analogue **8a** were even better in stimulating iNKT cells to release IL-2. This is consistent with the potent iNKT cell activity of the precursor azide reported in the publication of Besra and co-workers.[7] The IL-2 secretion induced by *n*-butyl containing analogue **8b** is in line with the alkyl-substituted triazoles of Besra, where the one with an octyl chain is far more active than the one with a longer undecyl chain. Contradictory to the docking experiment, however, these data indicate that 1,4-substitution of the triazole moiety doesn’t significantly impact on the interaction with CD1d and the TCR, provided that the attached group doesn’t exceed a certain size.

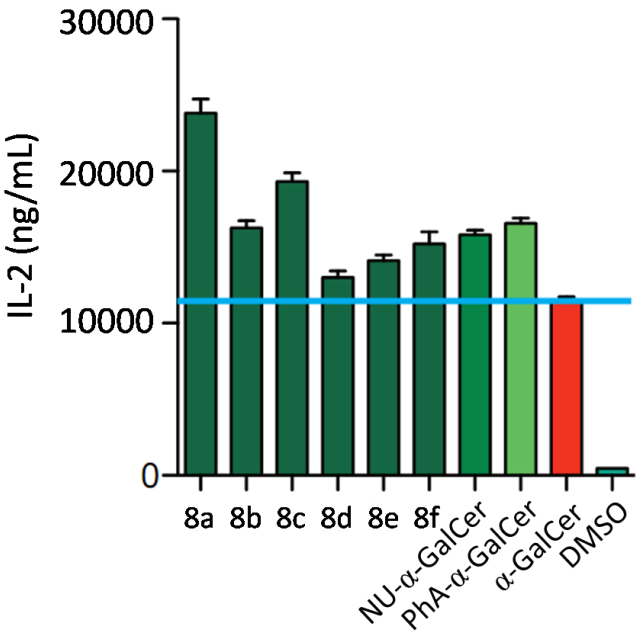


Figure 4.10 IL-2 secretion after coculture of 2C12 cells (iNKT cell hybridoma) with glycolipid (100 ng/mL) loaded BMDCs.

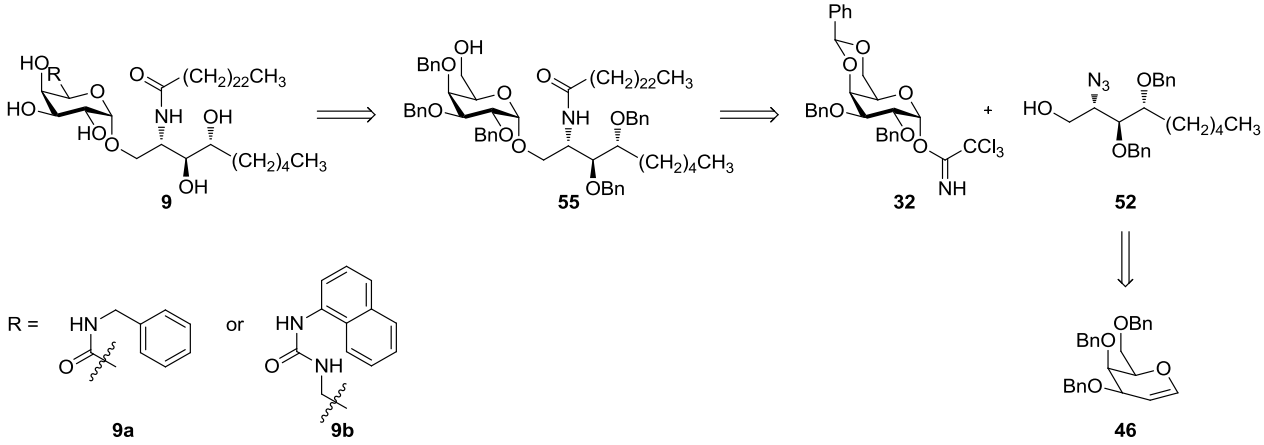
4.2.3 Conclusion

In summary, a series of phenyl-substituted C-6”-triazolyl α-GalCer analogues as well as a *n*-butyl- and non-substituted triazole analogue have been synthesized using a copper-catalyzed azide-alkyne cycloaddition reaction. Compared to our approach, the almost simultaneously published method by Besra and coworkers, using an unprotected 6”-azido precursor, is attractive in that it allows diversification at the very end of the synthesis. Evaluation of the *in vitro* IL-2 secretion induced by these compounds reveals them to act as potent iNKT cell stimulating ligands. We suggest that the observed increase in antigenic activity is mainly due to the triazole functionalization whereby further substitution at C-4 of this heterocycle with small groups is tolerated.

4.3 OCH-variants of NU-α-GalCer and BnNH-GSL-1’

As evidenced by several polymodified α-GalCer analogues, including the herein reported C6Ph derivative of α-D-FucCer (**2**), combination of two favorable modifications does not necessarily afford compounds with enhanced biological profiles. Furthermore, it is unlikely that differing CD1d/glycolipid complex stabilities and TCR affinities alone determine the outcome of iNKT cell activation. Therefore we decided to combine two contradictory modifications, i.e. a C-6”-naphthyl urea substituent known to enhance the CD1d affinity, and an OCH-like ceramide moiety associated with a decreased half-life of binding to CD1d and a

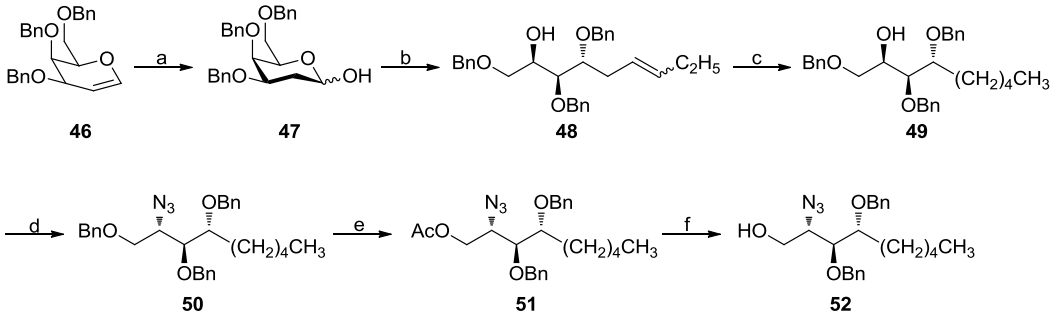
reduced TCR affinity. Introduction of such an OCH ceramide is also expected to improve the solubility of the glycolipid in aqueous mixtures, facilitating biological experiments. As a comparison, the OCH variant of BnNH-GSL-1’ is also included. Towards this end, a similar strategy as for the previous C-6”- and C-5”-modified α-GalCer compounds can be applied, using the same galactosyl donor **32** in a Schmidt glycosylation procedure (Scheme 4.6). Yet, a shorter phytosphingosine base is required, which, in contrast to the C18-phytosphinsosine base, is not commercially available and is therefore prepared following a chiral pool approach.



Scheme 4.6 Retrosynthesis of NU-OCH (**9a**) and BnNH-GSL-1’-OCH (**9b**).

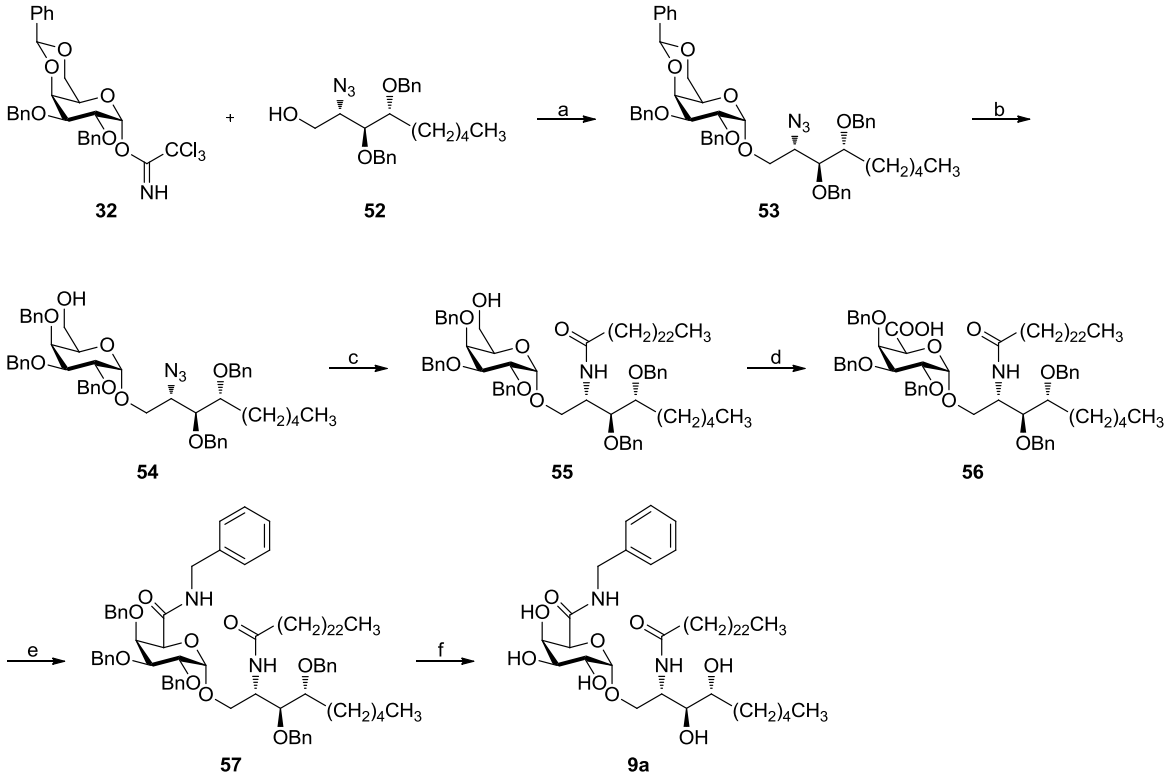
4.3.1 Synthesis

The C9-phytosphingosine building block was prepared relying on a method reported by Niu *et al.*, starting from tri-*O*-benzyl-D-galactal **46** (Scheme 4.7).[8] Hydrolysis of the galactal building block in the presence of HBr and triphenylphosphine resulted in the corresponding 2-deoxysugar **47**. The latter was treated with the appropriate Wittig reagent, derived from reacting *n*-propyl triphenylphosphonium bromide in the presence of *n*-BuLi at -12 °C, leading to alkene **48** as a mixture of two isomers. In order to facilitate the NMR spectral analysis of the following compounds, we have decided to reduce the double bond at this stage, in contrast to Niu *et al.* who combined this with the global deprotection at the end of the synthesis. The selective reduction was accomplished quantitatively by a palladium-catalyzed hydrogenation in the presence of small amounts ammonia, affording alkane **49**.[9] Next, the hydroxyl group was converted to an azido group under Mitsunobu conditions, thereby generating the desired configuration. Primary debenzylation of the obtained azide **50** by treatment with $ZnCl_2$ in a mixture of acetic anhydride and acetic acid, followed by deacetylation under Zemplen conditions furnished the desired azidophytosphingosine precursor **52**.



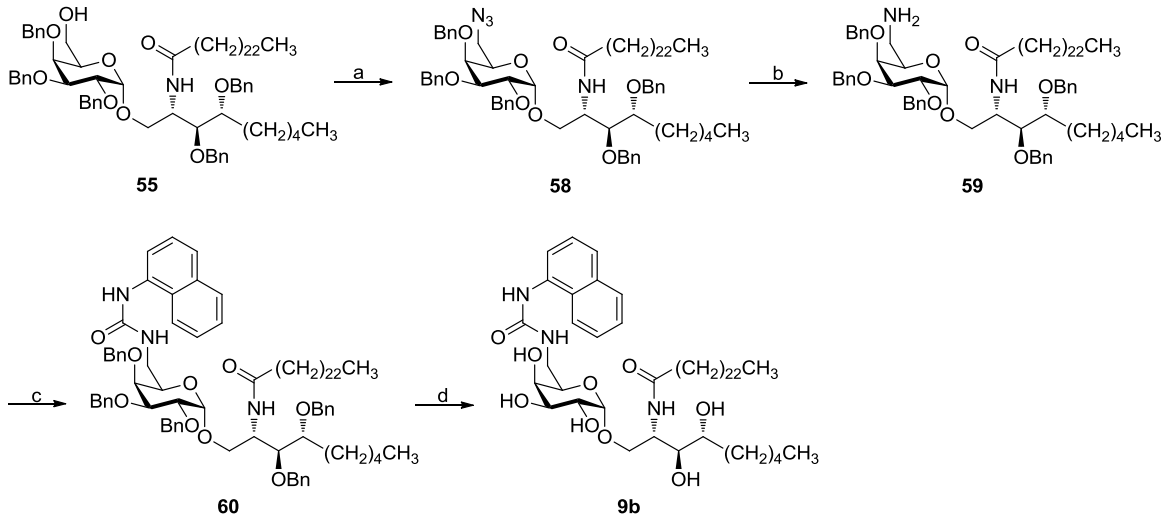
Scheme 4.7 Reagents and conditions: (a) HBr, PPh_3, CH_2Cl_2, rt, 24 h; (b) [*n*-$PrPPh_3$]$^+Br^-$, BuLi, THF, -12 °C, 3 h, 55% over 2 steps; (c) Pd/C, H_2, NH_3, MeOH, rt, 16 h, 99%; (d) Ph_3P, DEAD, DPPA, THF, -20 °C-rt, overnight, 89%; (e) $ZnCl_2$, Ac_2O, AcOH, rt, 5 h; (f) NaOMe, MeOH, rt, overnight, 69% over 2 steps.

Having obtained the appropriate acceptor **52**, Schmidt glycosylation with galactosyl donor **32** was successfully realized (Scheme 4.8). Regioselective opening of the benzylidene ring, as described earlier for intermediate **35**, allowed to introduce the proposed C-5”- and C-6”-modifications. For the envisaged benzylamide derivative, this implicated conversion to carboxylic acid **56** by a TEMPO/BAIB oxidation reaction, followed by condensation with benzylamine in the presence of DIPEA and HCTU. Debenzylation by a catalytic hydrogenation then afforded the final C-5”-amide-substituted OCH derivative **9a**.



Scheme 4.8 Reagents and conditions: (a) TMSOTf, THF, -30 °C, 2 h, 61%; (b) $Cu(OTf)_2$, BH_3.THF, CH_2Cl_2, rt, 5 h, 68%; (c) i. PMe_3, NaOH, THF, 6.5 h; ii. EDC, $C_{23}H_{47}COOH$, CH_2Cl_2, rt, overnight, 79%; (d) TEMPO, BAIB, CH_2Cl_2, H_2O, rt, overnight, 98%; (e) DIPEA, HCTU, benzylamine, DMF, CH_2Cl_2, rt, overnight, 88%; (f) H_2, Pd Black, $CHCl_3$, EtOH, rt, 74%.

The C-6”-urea-substituted OCH derivative on the other hand could be easily accessed starting from intermediate **55** (Scheme 4.9). Mitsunobu reaction converted the primary hydroxyl group to an azido group, giving compound **58**. Staudinger reduction followed by reaction of the obtained amine **59** with 1-naphthylisocyanate resulted in urea compound **60**. Final deprotection under the usual hydrogenation conditions furnished the desired C-6”-naphthylurea OCH analogue **9b**.



Scheme 4.9 Reagents and conditions: (a) PPh_3, DEAD, DPPA, THF, -20 °C-rt, overnight, 98%; (b) PMe_3, NaOH, THF, rt, 6 h; (c) naphthylisocyanate, DMF, 0 °C-rt, overnight, 60% over 2 steps; (d), H_2, Pd black, $CHCl_3$, EtOH, 26%.

4.3.2 Biological evaluation

The *in vivo* cytokine secretion measured upon intraperitoneal injection of 5 µg of the truncated glycolipids in mice is shown in Figure 4.11. Despite the combination with a Th2-featuring OCH-ceramide, the C-5”- and C-6”-altered compounds still induce a Th1 polarization. However, their capacity to release IFN-γ is clearly lower compared to the parent compounds, rendering a nice trend of increasing Th1 profiles. Neglecting other possible influencing factors, this might suggest that the additional hydrogen bond between the carbonyl oxygen of both C-6”-linkers and CD1d compensates for the decreased VDW interactions due to the shorter lipid chains. Further investigation is required to evaluate this hypothesis, including crystal structure elucidation of the corresponding ternary complexes and affinity measurements.

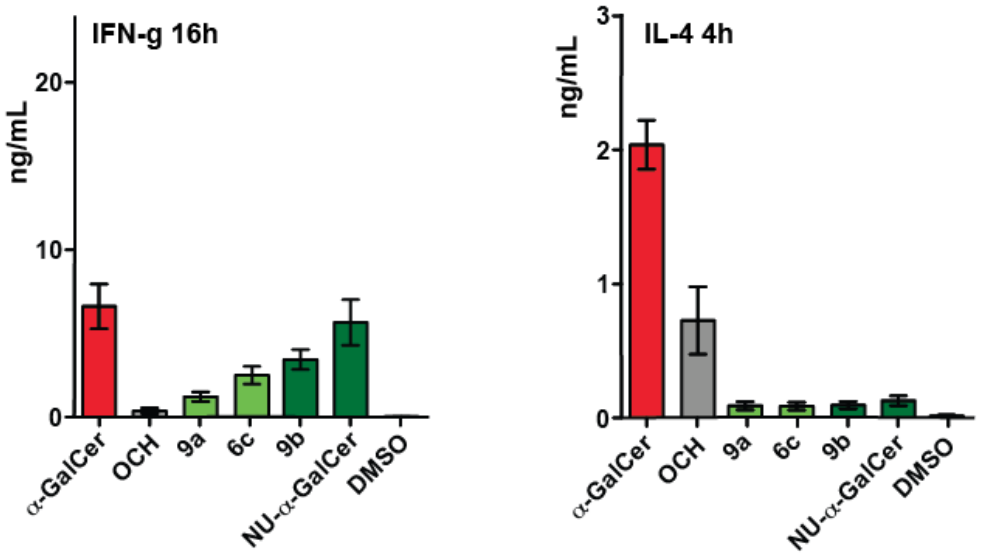


Figure 4.11 INF-γ and IL-4 secretion, measured at respective 16 h and 4 h, after intraperitoneal injection of 5 µg of the glycolipids in mice.

4.4 Binding assay in a human setting

The aforementioned *in vivo* and *in vitro* experiments were mainly accomplished in a mouse setting. However, mouse and human CD1d have been proven to be slightly different due to species polymorphisms. In particular, a glycine-tryptophane mutation leads to a small shift of the galactose ring (see Chapter 1). Therefore, in a final experiment, human CD1d-expressing cells were pulsed for 3 hours at 37 °C with the different C-5" and C-6"-modified α-GalCer analogues followed by staining with fluorescently-conjugated human iNKT TCR tetramers for 20 minutes at room temperature (Collaboration with Prof. S. Gadola). The TCR-staining is dependent on different factors, i.e. the "bioavailability" of the compound or how well it gets loaded onto CD1d, how stable it is kept in the CD1d binding groove, and the headgroup structure of the compound. The latter may help stabilizing the CD1d-binding of the glycolipid and may influence binding of the TCR to the combined CD1d/glycolipid epitope. However, with α-galactosylceramides the key factors are the binding to CD1d and the stability of the CD1d/glycolipid complex. Hence, the observed mean fluorescence intensity (MFI), normalized to α-GalCer, mainly gives an indication of the degree of CD1d/glycolipid complex formation (Figure 4.12). Two compounds that bind markedly better to CD1d than α-GalCer are **2** and **4**, both containing a CD1d affinity-enhancing C6Ph acyl chain. In contrast to the herein reported cytokine secretions in mice, these date do follow the human findings with the corresponding C6Ph compound reported by Wong *et al.* Furthermore, galacturonic acid **3** binds hCD1d at least equivalent to α-GalCer, whereas in mice only minor cytokine

release was observed. Surprisingly, the C-6”-naphthyl-substituted analogues NU-α-GalCer and **5b**, characterized by induction of an additional pocket in mouse CD1d, appear to bind less efficient to human CD1d compared to α-GalCer. Nevertheless, both compounds were previously found to induce a Th1 polarization in a human setting. On the other hand, **5c** results in a higher fluorescence intensity, while in mCD1d less intimate contacts were observed for this compound.

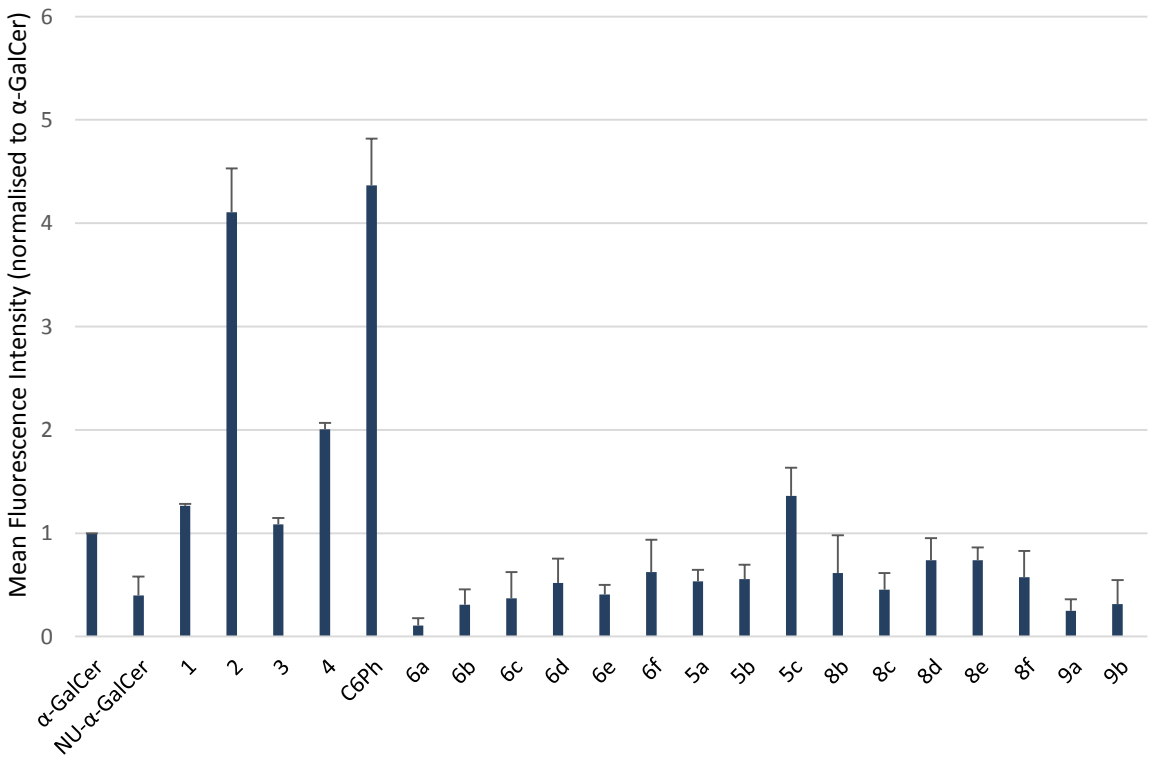


Figure 4.12 iNKT TCR tetramer staining of glycolipid-pulsed CD1d molecules.

Together, these data suggest that the minor structural differences between human and mouse CD1d can result in a different degree of glycolipid binding to CD1d, in turn influencing complex formation with the TCR. This may explain some of the discrepancies between human and mouse iNKT cell responses observed for certain glycolipids. Furthermore, the decreased hCD1d binding by NU-α-GalCer is in conflict with the observed Th1 polarization induced by this compound. This indicates that, at least for this compound, other predominating factors are probably involved in the outcome of human iNKT cell activation.

4.5 C-6”-labeled analogues

In the context of collaborations with other research groups, synthesis of two labeled α-GalCer analogues was desired. On the one hand, a BODIPY (boron-dipyrromethene)-labeled OCH

derivative was envisaged for the implementation of a fluorescence polarization assay, allowing real-time measurement of ligand binding and competition binding experiments. A convenient way to append the fluorescent group was via a C-6”-triazole linker (**10**, Figure 4.13). On the other hand, a radioactive glycolipid that strongly binds to CD1d was needful for another competition binding assay. Towards this end, we provided a tyrosine-like precursor with the *p*-hydroxyphenyl group connected through a C-6”-amide bond (**11**, Figure 4.13). This derivative offers easy access to a iodine-labeled molecule via the Chloramine-T method.

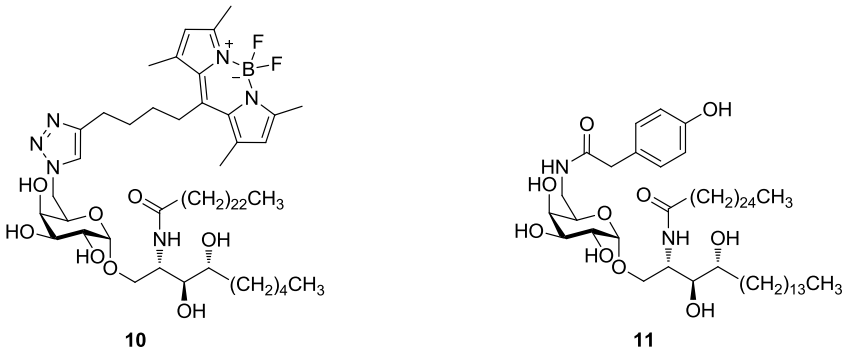
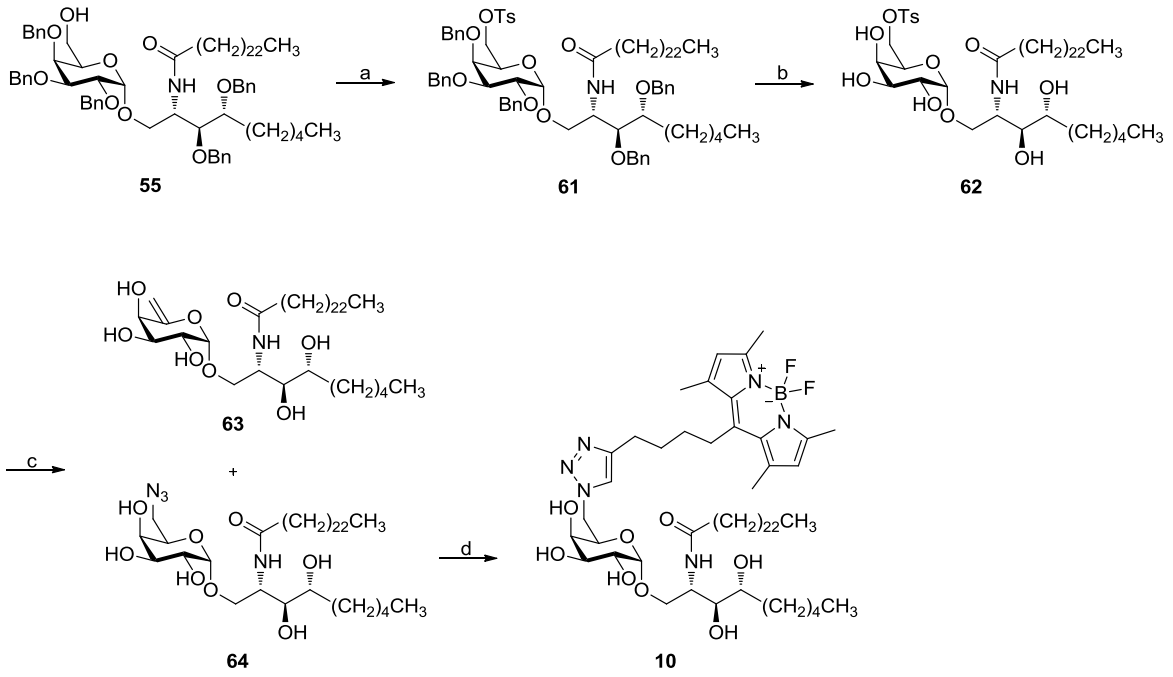


Figure 4.13 Structure of the C-6”-labeled α-GalCer derivatives.

4.5.1 Synthesis of a C-6”-BODIPY-triazole OCH analogue

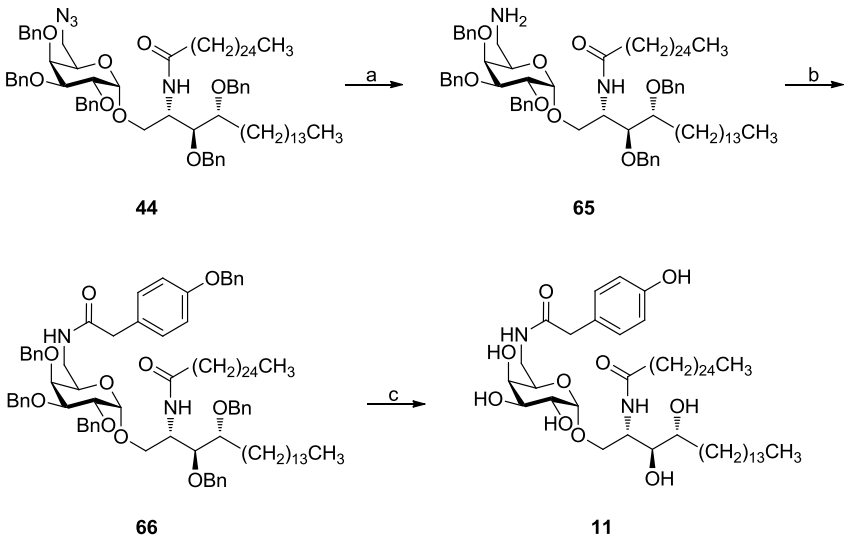
The synthetic pathway of C-6”-BODIPY analogue **10** is outlined in scheme 4.10. Intermediate **55** served as starting material. A slightly different approach compared to the previously synthesized C-6”-triazole derivatives was applied since the BODIPY core is not compatible with catalytic hydrogenation conditions. Therefore, the primary hydroxyl group was first tosylated followed by global deprotection of the secondary hydroxyl groups. Treatment of **62** with sodium azide then afforded azide **64,** in addition to a considerable amount of the undesired elimination product **63**. Finally, a CuACC click reaction with the alkyne-functionalized BODIPY furnished the envisaged BODIPY derivative **10**.[10] As microwave conditions could be harmfull for the BODIPY core, this reaction was performed at room temperature in the presence of tris-(benzyltriazolylmethyl)amine (TBTA).



Scheme 4.10 Reagents and conditions: (a) TsCl, pyridine, 0 °C-rt, overnight, 98%; (b) H_2, Pd black, $CHCl_3$, EtOH, 79%; (c) NaN_3, DMF, 60 °C, 2 days, 38%; (d) CuI, TEA, TBTA, alkyne, DMF, H_2O, 3 h, 91%.

4.5.2 Synthesis of a C-6”-radioactive precursor

Intermediate **44,** obtained in the synthesis of the C-6”-triazole derivatives, was submitted to Staudinger reduction (Scheme 4.11). Next, the resulting amine **65** was reacted with (benzyloxy)phenylacetic acid in the presence of DIPEA and HCTU to yield amide **66**. Global debenzylation gave the final compound **11**.



Scheme 4.11 Reagents and conditions: (a) PMe_3, NaOH, THF, 5.5 h; (b) (benzyloxy)phenylacetic acid, DIPEA, HCTU, DMF, CH_2Cl_2, overnight, 93% over 2 steps; (c) H_2, Pd black, $CHCl_3$, EtOH, 48%.

4.6 Experimental part

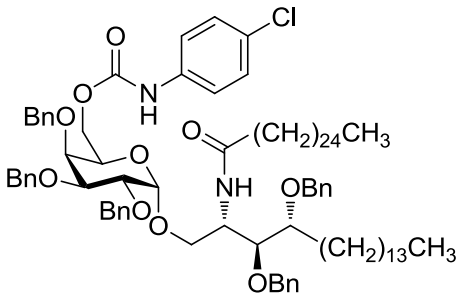
General

Precoated Macherey-Nagel SIL G/UV_{254} plates were used for TLC, and spots were examined under UV light at 254 nm and further visualized by sulfuric acid-anisaldehyde spray. Column chromatography was performed on Biosolve silica gel (63-200 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 ^1H 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 ^1H and δ = 149.9, 135.5 and 123.5 ppm for ^{13}C. IR spectra were recorded on a Varian Scimitar 800 FT-IR spectrometer as KBr pellets. The absorption peaks are reported in cm^{-1}. Exact mass measurements were performed on a Waters LCT Premier XE TOF equipped with an electrospray ionization interface and coupled to a Waters Alliance HPLC system. Samples were infused in a CH_3CN/HCOOH (1000/1) mixture at 10 mL/min.

Procedure for the synthesis of carbamates 41a and 41b

To a solution of compound **25** (0.07 mmol) in DMF (1 mL) was added the appropriate isocyanate (0.18 mmol). After stirring overnight, the reaction mixture was evaporated to dryness under reduced pressure. Purification by column chromatography (hexanes/EtOAc: 8/2) afforded carbamates **41a** (74 %) and **41b** (67 %).

*(2S,3S,4R)-3,4-di-O-benzyl-1-O-(2,3,4-tri-O-benzyl-6-O-(4-chlorophenylcarbamoyl)-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (**41a**)*

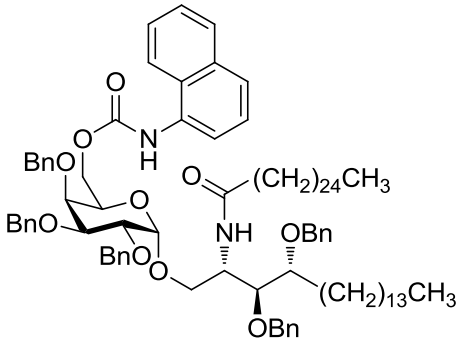


^1H NMR (300 MHz, $CDCl_3$): δ 8.29 (s, 1H, NH), 7.31-7.10 (m, 29H, arom. H), 5.71 (d, *J* = 6.6 Hz, 1H, NH), 4.88 (d, *J* = 11.6 Hz, 1H, CH_2-Ph), 4.79 (d, *J* = 3.9 Hz, 1H, H-1"), 4.75 (d, *J* = 11.6 Hz, 1H, CH_2-Ph), 4.67 (d, *J* = 12.0 Hz, 1H, CH_2-Ph), 4.61 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.62 (d, *J* = 11.9 Hz, 1H, CH_2-Ph), 4.57 (d, *J* = 11.6 Hz, 1H, CH_2-Ph), 4.52 (d, *J* = 11.4 Hz, 1H, CH_2-Ph), 4.51 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.42 (d, *J* = 11.6 Hz, 1H, CH_2-Ph), 4.41 (d, *J* = 11.6 Hz, 1H, CH_2-Ph), 4.33-4.26 (m, 2H, H-2, H-6"), 4.01-3.90 (m, 3H, H-2", H-1), 3.86-3.75 (m, 4H, H-3, H-3", H-4", H-5"), 3.69 (dd, *J* = 1.6 Hz and 11.4 Hz, 1H, H-6"), 3.51-3.46 (m, 1H, H-4), 1.96-1.84 (m, 2H, $COCH_2$), 1.58-1.06 (m, 72H, CH_2), 0.77 (t, *J* = 6.9 Hz, 6H, CH_3).

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

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

*(2S,3S,4R)-3,4-di-O-benzyl-1-O-(2,3,4-tri-O-benzyl-6-O-(1-naphthylcarbamoyl)-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (**41b**)*



^{1}H NMR (300 MHz, pyridine-d_5): δ 10.66 (s, 1H, NH), 8.99 (d, *J* = 8.4 Hz, 1H, NH), 8.84 (d, *J* = 8.4 Hz, 1H, arom. H), 8.37 (d, *J* = 7 Hz, 1H, arom. H), 7.96 (d, *J* = 7.9 Hz, 1H, arom. H), 7.78 (d, *J* = 8.1 Hz, 1H, arom. H), 7.66-7.58 (m, 2H, arom. H), 7.55-7.29 (m, 26H, arom. H), 5.43 (d, *J* = 3.4 Hz, 1H, H-1"), 5.17 (d, *J* = 11.1 Hz, 1H, CH_2-Ph), 5.13 (d, *J* = 10.2 Hz, 1H, CH_2-Ph), 5.00-4.82 (m, 4H, CH_2-Ph, H-6", H-2), 4.78-4.68 (m, 5H, H-6", CH_2-Ph), 4.64-4.58 (m, 2H, H-5", CH_2-Ph), 4.54 (dd, *J* = 2.0 and 8.4 Hz, 1H, H-3), 4.48-4.43 (m, 3H, H-1, H-2", CH_2-Ph), 4.34 (dd, *J* = 2.7 Hz and 10.2 Hz, 1H, H-3"), 4.25-4.20 (m, 2H, H-1, H-4"), 3.97-3.93 (m, 1H, H-4), 2.66-2.56 (m, 2H, $COCH_2$), 2.15-1.18 (m, 72H, CH_2), 0.90 (t, *J* = 6.7 Hz, 3H, CH_3), 0.89 (t, *J* = 6.4 Hz, 3H, CH_3).

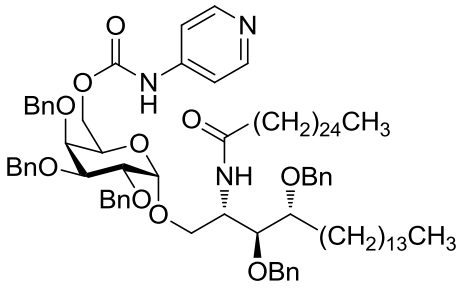
^{13}C NMR (75 MHz, pyridine-d_5): δ 172.21, 154.34, 138.55, 138.41, 138.21, 138.12, 127.65, 127.50, 127.29, 126.89, 126.80, 126.75, 126.58, 125.16, 125.11, 97.63, 80.00, 78.76, 77.89, 76.09, 74.88, 73.94, 73.24, 72.26, 71.67, 70.70, 68.77, 61.38, 50.13, 49.97, 48.02, 38.95, 35.66, 34.03, 30.97, 28.99,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

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

Procedure for the synthesis of carbamate 41c

To a solution of compound **25** (50 mg, 0.04 mmol) in DMF (0.5 mL) was added CDI (31 mg, 0.18 mmol). After stirring overnight, the reaction mixture was heated until 70 °C and 4-aminopyridine was added. The reaction mixture was stirred at 70 °C during 48 hours followed by evaporation to dryness under reduced pressure. Purification by column chromatography (hexanes/EtOAc: 7/3) afforded carbamate **41c** (26 mg, 33 %).

*(2S,3S,4R)-3,4-di-O-benzyl-1-O-(2,3,4-tri-O-benzyl-6-O-(4-pyridinylcarbamoyl)-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (**41c**)*



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

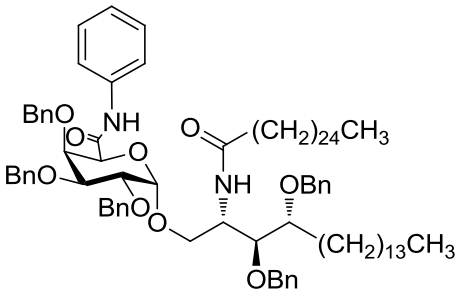
^{13}C NMR (75 MHz, $CDCl_3$): δ 173.09, 148.33, 138.68, 138.62, 138.52, 138.01, 137.32, 130.68, 128.76, 128.73, 128.68, 128.66, 128.64, 128.60, 128.54, 128.16, 128.07, 128.05, 127.97, 127.82, 127.78, 127.68, 117.33, 99.09, 80.11, 79.67, 79.13, 74.56, 73.83, 73.48, 72.12, 68.33, 68.16, 66.68, 60.63,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

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

General procedure for the synthesis of amides (42a-42g)

To a solution of **39** (150 mg, 0.11 mmol) in DMF (0.3 mL) and CH_2Cl_2 (0.7 mL) was added DIPEA (22 mg, 0.17 mmol). After stirring for 10 minutes at room temperature, HCTU (72 mg, 0.17 mmol) was added and the mixture was stirred for 30 minutes. Then the appropriate amine (0.17 mmol) was added and the solution was continued stirring overnight. After completion of the reaction, the mixture was evaporated to dryness. The residue was partitioned between H_2O and EtOAc and the aqueous layer was extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. Purification by column chromatography and concentration under reduced pressure furnished the desired amides **42a** (87 %), **42b** (84 %), **42c** (53 %), **42d** (82 %), **42e** (82 %), **42f** (85 %) and **42g** (82 %).

*(2S,3S,4R)-3,4-di-O-benzyl-1-O-(2,3,4-tri-O-benzyl-6-deoxy-5-phenylamide-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (**42a**)*

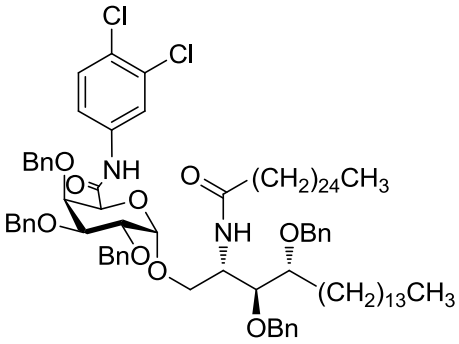


^{1}H NMR (300 MHz, $CDCl_3$): δ 8.20 (s, 1H, NH), 7.53 (d, *J* = 1.0 Hz, 2H, arom. H), 7.39-7.08 (m, 28H, arom. H), 5.73 (d, *J* = 8.2 Hz, 1H, NH), 4.98 (d, *J* = 3.5 Hz, 1H, H-1”), 4.87 (d, *J* = 10.8 Hz, 1H, CH_2-Ph), 4.84 (d, *J* = 11.6 Hz, 1H, CH_2-Ph), 4.79 (d, *J* = 12.7 Hz, 1H, CH_2-Ph), 4.71 (d, *J* = 11.6 Hz, 1H, CH_2-Ph), 4.66 (d, *J* = 11.6 Hz, 1H, CH_2-Ph), 4.62 (d, *J* = 12.0 Hz, 1H, CH_2-Ph), 4.58 (d, *J* = 10.8 Hz, 1H, CH_2-Ph), 4.55 (d, *J* = 11.6 Hz, 1H, CH_2-Ph), 4.51-4.45 (m, 3H, CH_2-Ph and H-4”), 4.37 (app. d, *J* = 1.1 Hz, 1H, H-5”), 4.26 (m, 1H, H-2), 4.07 (dd, *J* = 3.5 Hz and 10.1 Hz, 1H, H-2”), 3.98 (dd, *J* = 2.7 Hz and 10.0 Hz, 1H, H-3”), 3.90-3.76 (m, 3H, H-1, H-3), 3.55-3.49 (m, 1H, H-4), 1.92-1.78 (m, 2H, $COCH_2$), 1.68-1.22 (m, 72H, CH_2), 0.88 (t, *J* = 6.7 Hz, 6H, CH_3).

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

Exact mass (ESI-MS) for $C_{91}H_{132}N_2O_9$ $[M+Na]^+$ found, 1419.9889; calcd, 1419,9831.

*(2S,3S,4R)-3,4-di-O-benzyl-1-O-(2,3,4-tri-O-benzyl-6-deoxy-5-(3,4-dichlorophenylamide)-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (**42b**)*



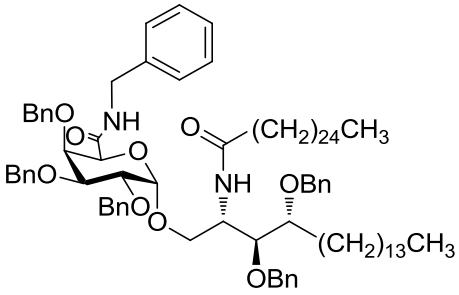
^{1}H NMR (300 MHz, $CDCl_3$): δ 8.17 (s, 1H, NH), 7.74 (d, *J* = 2.2 Hz, 1H, arom. H), 7.40-7.14 (m, 27H, arom. H), 5.70 (d, *J* = 8.2 Hz, 1H, NH), 4.95 (d, *J* = 3.4 Hz, 1H, H-1”), 4.89-4.46 (m, 11H, CH_2-Ph and H-4”), 4.35 (d, *J* = 1.2 Hz, 1H, H-5”), 4.31-4.25 (m, 1H, H-2), 4.04 (dd, *J* = 3.4 Hz and 10.0 Hz, 1H, H-2”), 3.96 (dd, *J* = 2.6 Hz and 10.2 Hz, 1H, H-3”), 3.91 (dd, *J* = 4.9 Hz and 10.6 Hz, 1H, H-1), 3.80-3.74 (m, 2H, H-1 and H-3), 3.56-3.52 (m, 1H, H-4), 1.94-1.77 (m, 2H, $COCH_2$), 1.60-1.08 (m, 72H, CH_2), 0.88 (t, *J* = 6.7 Hz, 6H, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ 173.09, 167.17, 138.68, 138.66, 138.50, 138.43, 138.28, 136.81, 132.93, 130.62, 128.70, 128.68, 128.65, 128.64, 128.42, 128.40, 128.11, 128.09, 128.06, 128.01, 127.97, 127.94, 127.87, 127.70, 121.80, 119.37, 99.25, 79.92, 79.69, 78.49,

75.99, 74.11, 73.32, 72.94, 72.29, 68.67, 53.64, 50.20, 36.89, 32.16, 30.61, 30.05, 29.96, 29.94, 29.91, 29.89, 29.82, 29.66, 29.61, 29.59, 26.12, 25.85, 22.92, 14.35.

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

*(2S,3S,4R)-3,4-di-O-benzyl-1-O-(2,3,4-tri-O-benzyl-6-deoxy-5-benzylamide-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (**42c**)*

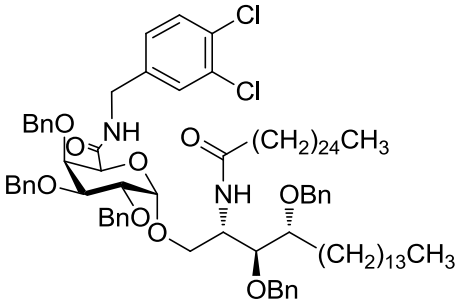


^{1}H NMR (300 MHz, $CDCl_3$): δ 7.37-7.17 (m, 30H, arom. H), 6.79 (app. t, *J* = 5.9 Hz, 1H, NH), 5.74 (d, *J* = 8.1 Hz, 1H, NH), 4.88 (d, *J* = 3.7 Hz, 1H, H-1"), 4.85-4.42 (m, 12H, CH_2-Ph, H-4", NH-C*H*$_2$), 4.35-4.28 (m, 2H, H-5" and NH-C*H*$_2$), 4.26-4.18 (m, 1H, H-2), 4.04 (dd, *J* = 3.4 Hz and 9.9 Hz, 1H, H-2"), 3.95 (dd, *J* = 2.6 Hz and 10.0 Hz, 1H, H-3"), 3.82-3.71 (m, 3H, H-1 and H-3), 3.49 (m, 1H, H-4), 1.93-1.78 (m, 2H, $COCH_2$), 1.65-1.14 (m, 72H, CH_2), 0.87 (t, *J* = 6.6 Hz, 6H, CH_3).

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

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

*(2S,3S,4R)-3,4-di-O-benzyl-1-O-(2,3,4-tri-O-benzyl-6-deoxy-5-(3,4-dichlorophenmethylamide)-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (**42d**)*



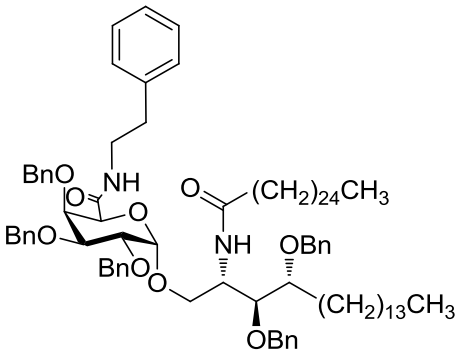
^{1}H NMR (300 MHz, $CDCl_3$): δ 7.38-7.22 (m, 26H, arom. H), 7.14 (d, *J* = 8.2 Hz, 1H, arom. H), 6.98 (dd, *J* = 1.9 Hz and 8.2 Hz, 1H, arom. H), 6.82 (t, *J* = 6.1 Hz, 1H, NH), 5.75 (d, *J* = 8.0 Hz, 1H, NH), 4.92 (d, *J* = 3.3 Hz, 1H, H-1"), 4.89 (d, *J* = 11.0 Hz, 1H, CH_2-Ph), 4.82 (d, *J* = 11.6 Hz, 1H, CH_2-Ph), 4.79 (d, *J* = 12.1 Hz, 1H, CH_2-Ph), 4.74 (d, *J* = 12.1 Hz, 1H, CH_2-Ph), 4.72 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.65 (d, *J* = 11.6 Hz, 1H, CH_2-Ph), 4.56 (d, *J* = 11.6 Hz, 1H, CH_2-Ph), 4.55 (d, *J* = 11.0 Hz, 1H, CH_2-Ph), 4.50-4.43 (m, 4H, CH_2-Ph, NH-CH_2 and H-4"), 4.30-4.26 (m, 2H, H-5" and H-2), 4.20 (dd, *J* = 5.4 Hz and 15.1 Hz, 1H, NH-CH_2), 4.05 (d, *J* = 3.4 Hz and 10.1 Hz, 1H, H-2"), 3.96 (d, *J* =

2.5 Hz and 10.0 Hz, 1H, H-3”), 3.88-3.73 (m, 3H, H-1 and H-3), 3.55-3.58 (m, 1H, H-4), 1.99-1.80 (m, 2H, $COCH_2$), 1.72-1.16 (m, 72H, CH_2), 0.89 (t, *J* = 6.6 Hz, 6H, CH_3).

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

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

*(2S,3S,4R)-3,4-di-O-benzyl-1-O-(2,3,4-tri-O-benzyl-6-deoxy-5-phenethylamide-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (**42e**)*

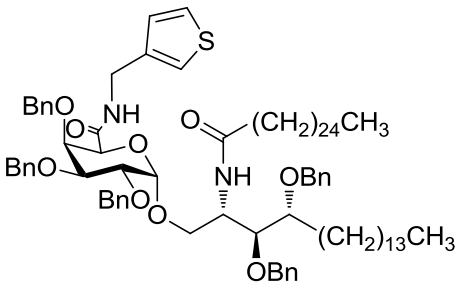


^{1}H NMR (300 MHz, $CDCl_3$): δ 7.38-7.12 (m, 30H, arom. H), 6.61 (t, *J* = 6.0 Hz, 1H, NH), 5.78 (d, *J* = 8.3 Hz, 1H, NH), 4.89 (d, *J* = 3.3 Hz, 1H, H-1”), 4.87 (d, *J* = 10.8 Hz, 1H, CH_2-Ph), 4.82 (d, *J* = 11.7 Hz, 1H, CH_2-Ph), 4.77 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.73 (d, *J* = 10.5 Hz, 1H, CH_2-Ph), 4.72 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.64 (d, *J* = 11.7 Hz, 1H, CH_2-Ph), 4.59 (d, *J* = 10.8 Hz, 1H, CH_2-Ph), 4.56 (d, *J* = 11.5 Hz, 1H, CH_2-Ph), 4.51-4.49 (m, 1H, H-4”), 4.48 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.46 (d, *J* = 11.6 Hz, 1H, CH_2-Ph), 4.27-4.29 (m, 2H, H-2 and H-5”), 4.03 (dd, *J* = 3.5 Hz and 10.0 Hz, 1H, H-2”), 3.94 (dd, *J* = 2.7 Hz and 10.1 Hz, 1H, H-3”), 3.82 (dd, *J* = 2.9 Hz and 6.5 Hz, 1H, H-3), 3.79-3.71 (m, 2H, H-1), 3.53-3.45 (m, 3H, NH-CH_2 and H-4), 2.73 (ddd, *J* = 7.2 Hz, 13.5 Hz and 28.0 Hz, 2H, CH_2), 1.98-1.80 (m, 2H, $COCH_2$), 1.65-1.19 (m, 72H, CH_2), 0.88 (t, *J* = 6.8 Hz, 6H, CH_3).

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

Exact mass (ESI-MS) for $C_{93}H_{136}N_2O_9$ $[M+H]^+$ found, 1426.0337; calcd, 1426.0324; $[M+Na]^+$ found, 1448.0142; calcd, 1448.0144; $[M+K]^+$ found, 1463.9927; calcd, 1463.9882.

*(2S,3S,4R)-3,4-di-O-benzyl-1-O-(2,3,4-tri-O-benzyl-6-deoxy-5-thiofuranmethylamide-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (**42f**)*

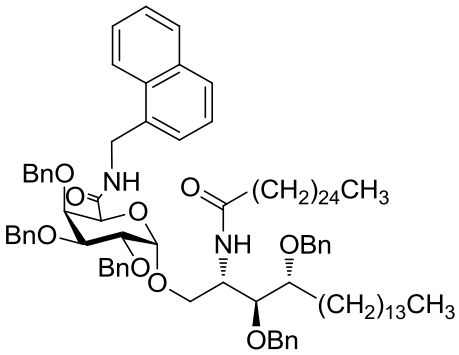


^{1}H NMR (300 MHz, $CDCl_3$): δ 7.30-7.13 (m, 25H, arom. H), 7.07 (dd, *J* = 1.4 Hz and 5.0 Hz, 1H, arom. H), 6.81 (dd, *J* = 1.3 Hz and 3.4 Hz, 1H, arom. H), 6.79-6.75 (m, 2H, arom. H and NH), 5.66 (d, *J* = 8.3 Hz, 1H, NH), 4.81 (d, *J* = 3.3 Hz, 1H, H-1"), 4.77 (d, *J* = 11.0 Hz, 1H, CH_2-Ph), 4.73 (d, *J* = 12.5 Hz, 1H, CH_2-Ph), 4.68 (d, *J* = 12.0 Hz, 1H, CH_2-Ph), 4.64 (d, *J* = 11.6 Hz, 1H, CH_2-Ph), 4.58-4.42 (m, 7H, CH_2-Ph, NH-CH_2, H-4"), 4.40 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.37 (d, *J* = 11.6 Hz, 1H, CH_2-Ph), 4.20 (d, *J* = 0.8 Hz, 1H, H-5"), 4.18-4.12 (m, 1H, H-2), 3.95 (dd, *J* = 3.4 Hz and 10.0 Hz, 1H, H-2"), 3.87 (dd, *J* = 2.6 Hz and 10.0 Hz, 1H, H-3"), 3.75-3.63 (m, 3H, H-1 and H-3), 3.48-3.40 (m, 1H, H-4), 1.88-1.69 (m, 2H, $COCH_2$), 1.58-1.07 (m, 72H, CH_2), 0.81 (t, *J* = 6.7 Hz, 6H, CH_3).

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

Exact mass (ESI-MS) for $C_{90}H_{132}N_2O_9S$ $[M+H]^+$ found, 1417.9757; calcd, 1417,9732; $[M+Na]^+$ found, 1439.9514; calcd, 1439,9551; $[M+K]^+$ found, 1455.9220; calcd, 1455,9291.

*(2S,3S,4R)-3,4-di-O-benzyl-1-O-(2,3,4-tri-O-benzyl-6-deoxy-5-(1-naphthalenmethylamide)-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (**42g**)*



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

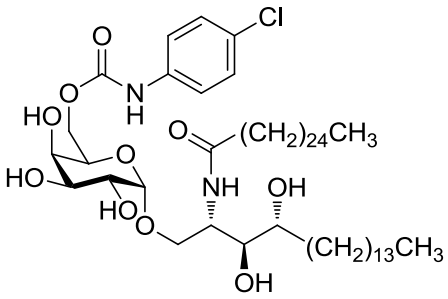
^{13}C NMR (75 MHz, $CDCl_3$): δ 173.05, 168.73, 138.84, 138.75, 138.73, 138.53, 138.50, 134.02, 133.14, 131.56, 128.98, 128.95, 128.69, 128.65, 128.60, 128.43, 128.28, 128.18, 128.08, 127.98, 127.88, 127.84, 127.77, 127.71, 126.84, 126.51, 126.15, 125.67, 123.50, 99.02, 79.94, 78.89, 78.59, 77.71, 77.29, 76.86, 76.30, 79.06, 75.61, 74.06, 73.47, 72.59, 72.00, 71.95, 68.20, 50.07, 41.30, 36.88, 32.18, 30.22, 30.08, 29.99, 29.96, 29.91, 29.87, 29.71, 29.64, 29.62, 26.15, 25.89, 22.95, 14.39.

Exact mass (ESI-MS) for $C_{96}H_{136}N_2O_9$ $[M+H]^+$ found, 1462.0370; calcd, 1462,0324; $[M+Na]^+$ found, 1484.0066; calcd, 1484,0144; $[M+K]^+$ found, 1499.9858; calcd, 1499,9883.

General procedure for debenzylation: method A (5a-5c and 6a-6e)

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

*(2S,3S,4R)-1-O-(6-O-(4-chlorophenylcarbamoyl)-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (**5a**)*

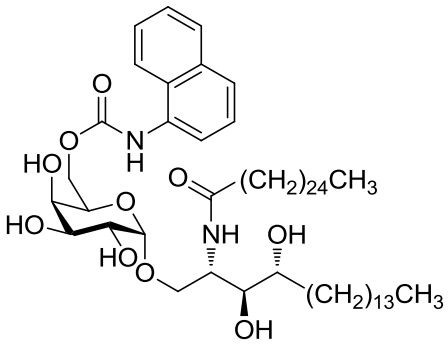


^{1}H NMR (300 MHz, pyridine-d_5): δ 10.73 (s, 1H, NH), 8.58 (d, *J* = 8.2 Hz, 1H, NH), 7.91 (d, *J* = 8.7 Hz, 2H, arom. H), 7.40 (d, *J* = 8.9 Hz, 2H, arom. H), 6.99 (br. s, 1H, OH), 6.75 (br. s, 1H, OH), 6.62 (d, *J* = 3.7 Hz, 1H, OH), 6.45 (d, *J* = 6.6 Hz, 1H, OH), 6.27 (d, *J* = 6.8 Hz, 1H, OH),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

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

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

*(2S,3S,4R)-1-O-(6-O-(1-naphthylcarbamoyl)-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (**5b**)*

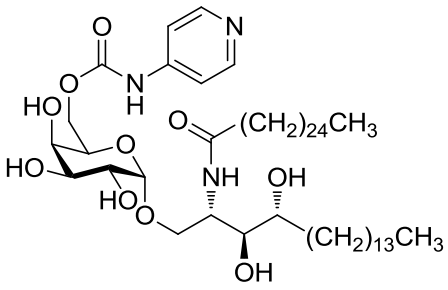


^{1}H NMR (300 MHz, pyridine-d_5): δ 10.68 (s, 1H, NH), 8.74-8.70 (m, 1H, arom H), 8.58 (d, *J* = 8.6 Hz, 1H, NH), 8.26 (d, *J* = 7.3 Hz, 1H, arom. H), 7.94 (d, *J* = 7.5 Hz, 1H, arom. H), 7.74 (d, *J* = 8.4 Hz, 1H, arom. H), 7.62-7.51 (m, 3H, arom. H), 5.58 (d, *J* = 3.9 Hz, 1H, H-1”), 5.28-5.22 (m,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

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

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

*(2S,3S,4R)-1-O-(6-O-(4-pyridinylcarbamoyl)-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (**5c**)*

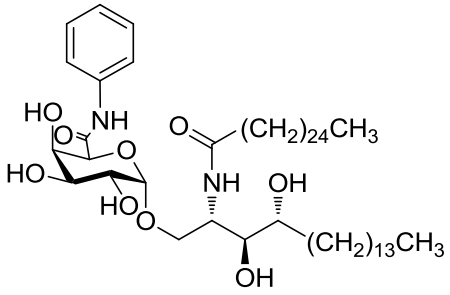


^{1}H NMR (300 MHz, pyridine-d_5): δ 11.18 (s, 1H, NH), 8.83 (d, *J* = 8.3 Hz, 1H, NH), 8.67 (dd, *J* = 1.4 Hz and 4.8 Hz, 2H, arom. H), 7.93 (dd, *J* = 1.4 Hz and 4.8 Hz, 2H, arom. H), 5.55 (d, *J* = 3.8 Hz, 1H, H-1”), 5.26-5.20 (m, 1H, H-2), 5.08 (dd, *J* = 8.2 Hz and 11.0 Hz, 1H, H-6”), 4.77 (dd,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

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

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

*(2S,3S,4R)-1-O-(6-deoxy-5-phenylamide-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (**6a**)*

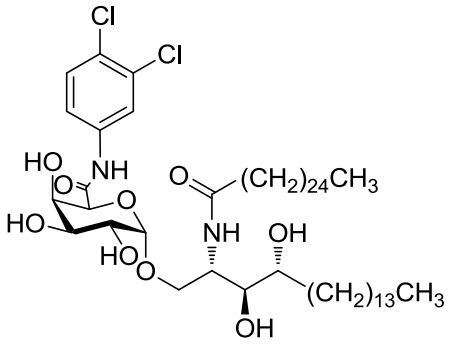


^{1}H NMR (300 MHz, pyridine-d_5): δ 9.88 (s, 1H, NH), 8.49 (d, *J* = 8.8 Hz, 1H, NH), 8.12 (dd, *J* = 1.1 Hz and 8.6 Hz, 2H, arom. H), 7.36-7.30 (m, 3H, arom. H and OH), 7.10 (t, *J* = 7.3 Hz, 1H, arom. H), 6.79 (d, *J* = 5.2 Hz, 1H, OH), 6.43 (d, *J* = 5.9 Hz, 1H, OH), 6.12 (d, *J* = 5.4 Hz, 1H, OH),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

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

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

*(2S,3S,4R)-1-O-(6-deoxy-5-(3,4-dichlorophenylamide)-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (**6b**)*

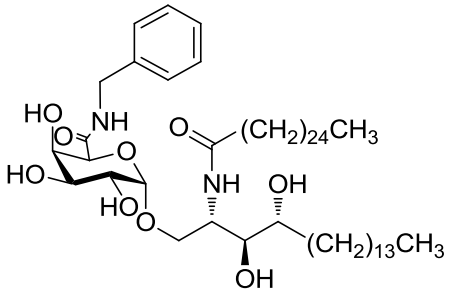


^{1}H NMR (300 MHz, pyridine-d_5): δ 10.19 (s, 1H, NH), 8.52 (d, *J* = 8.7 Hz, 1H, NH), 8.46 (d, *J* = 2.4 Hz, 1H, arom. H), 7.92 (dd, *J* = 2.4 Hz and 9.0 Hz, 1H, arom. H), 7.39 (d, *J* = 9.0 Hz, 1H, arom. H), 7.28 (bs, 1H, OH), 6.85 (bs, 1H, OH), 6.45 (d, *J* = 4.2 Hz, 1H, OH), 6.13 (bs, 1H, OH), 5.40 (d, *J* = 3.6 Hz, 1H, H-1"), 5.27-5.23 (m, 1H, H-2), 5.03-4.96 (m, 3H, H-4", H-5" and OH), 4.62 (dd, *J* = 3.6 Hz and 10.5 Hz, 1H, H-2"), 4.57 (dd, *J* = 5.1 Hz and 10.8 Hz, 1H, H-3), 4.44 (dd, *J* = 1.8 Hz and 9.6 Hz, 1H, H-3"), 4.26-4.21 (m, 3H, H-1 and H-4), 2.49-2.44 (m, 2H, $COCH_2$), 2.29-1.18 (m, 72H, CH_2), 0.89 (t, *J* = 6.6 Hz, 6H, CH_3).

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

Exact mass (ESI-MS) for $C_{56}H_{100}Cl_2N_2O_9$ $[M+Na]^+$ found, 1015.6865; calcd, 1015,6884.

*(2S,3S,4R)-1-O-(6-deoxy-5-benzylamide-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (**6c**)*

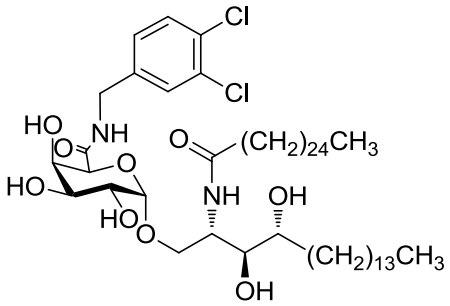


^{1}H NMR (300 MHz, pyridine-d_5): δ 8.69 (dd, *J* = 5.9 Hz and 6.8 Hz, 1H, NH), 8.47 (d, *J* = 8.7 Hz, 1H, NH), 7.59 (d, *J* = 5.9 Hz, 2H, arom. H), 7.30-7.17 (m, 4H, arom. H and OH), 6.40 (bs, 1H, OH), 5.58 (d, *J* = 3.9 Hz, 1H, H-1”), 5.03-5.00 (m, 2H, H-2 and OH), 4.98-4.95 (m, 4H, H-4”, OH and NH-C*H*$_2$), 4.69 (dd, *J* = 3.8 Hz and 9.9 Hz, 1H, H-2”), 4.63-4.56 (m, 2H, H-3 and H-5”), 4.44 (dd, *J* = 3.1 Hz and 10.0 Hz, 1H, H-3”), 4.34-4.27 (m, 3H, H-1 and H-4), 2.48-2.43 (m, 2H, $COCH_2$), 1.93-1.09 (m, 72H, CH_2), 0.89 (t, *J* = 6.7 Hz, 6H, CH_3).

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

Exact mass (ESI-MS) for $C_{57}H_{104}N_2O_9$ $[M+H]^+$ found, 961.7808; calcd, 961.7815; $[M+Na]^+$ found, 983.7621; calcd, 983.7634; $[M+H]^+$ found, 999.7262; calcd, 999.7373.

*(2S,3S,4R)-1-O-(6-deoxy-5-(3,4-dichlorophenmethylamide)-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (**6d**)*

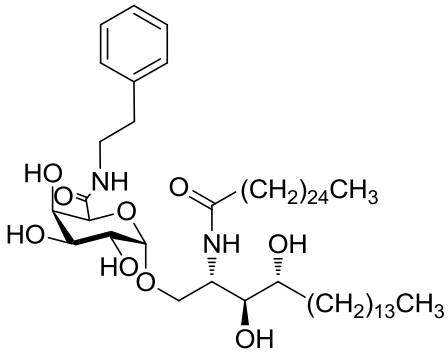


^{1}H NMR (300 MHz, pyridine-d_5): δ 8.96 (dd, *J* = 5.7 Hz and 7.1 Hz, 1H, NH), 8.48 (d, *J* = 8.6 Hz, 1H, NH), 7.77 (d, *J* = 1.9 Hz, 1H, arom. H), 7.41 (dd, *J* = 2.0 Hz and 8.3 Hz, 1H, arom. H), 7.32 (d, *J* = 8.2 Hz, 1H, arom. H), 6.75 (app. s, 1H, OH), 6.43 (d, *J* = 5.7 Hz, 1H, OH), 6.13 (d, *J* = 4.8 Hz, 1H, OH), 5.60 (d, *J* = 3.8 Hz, 1H, H-1”), 5.26 (app. d, *J* = 4.6 Hz, 1H, H-2), 5.01-4.92 (m, 3H, NH-CH_2, H-4” and H-5”), 4.69-4.56 (m, H2, H-2” and H-1), 4.49-4.42 (m, 2H, H-3” and NH-CH_2), 4.33-4.29 (m, 3H, H-1, H-3 and H-4), 2.46 (app. t, *J* = 7.5 Hz, 2H, $COCH_2$), 1.97-1.16 (m, 72H, CH_2), 0.89 (t, *J* = 6.7 Hz, 6H, CH_3).

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

Exact mass (ESI-MS) for $C_{57}H_{102}Cl_2N_2O_9$ $[M+H]^+$ found, 1029,7021; calcd, 1029.7041.

*(2S,3S,4R)-1-O-(6-deoxy-5-phenethylamide-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (**6e**)*



^{1}H NMR (300 MHz, pyridine-d_5): δ 8.50 (d, *J* = 8.7 Hz, 1H, NH), 8.13 (t, *J* = 6.0 Hz, 1H, NH), 7.32-7.18 (m, 5H, arom. H), 7.03 (br. s, 1H, OH), 6.41 (br. s, 1H, OH), 6.13 (br. s, 1H, OH), 5.54 (d, *J* = 3.9 Hz, 1H, H-1"), 5.27 (app. d, *J* = 4.4 Hz, 1H, H-2), 4.99-4.91 (m, 2H, H-4" and H-5"), 4.65 (dd, *J* = 3.8 Hz and 10.0 Hz, 1H, H-2"), 4.60 (dd, *J* = 5.4 Hz and 10.7 Hz, 1H, H-1), 4.41 (dd, *J* = 3.1 Hz and 10.0 Hz, 1H, H-3"), 4.39-4.31 (m, 1H, H-4), 4.30 (dd, *J* = 4.2 Hz and 10.7 Hz, 1H, H-1), 3.72 (dd, *J* = 6.5 Hz and 14.4 Hz, 2H, NH-CH_2), 2.94 (app. t, *J* = 7.5 Hz, 2H, CH_2), 2.48 (t, *J* = 7.1 Hz, 2H, $COCH_2$), 1.97-1.18 (m, 72H, CH_2), 0.89 (t, *J* = 6.6 Hz, 6H, CH_3).

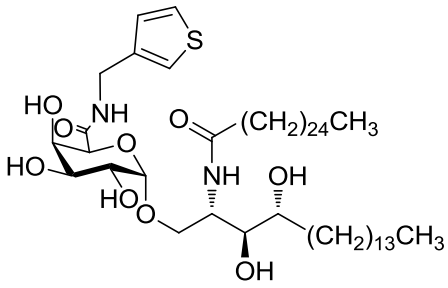
^{13}C NMR (75 MHz, pyridine-d_5): δ 173.42, 170.26, 140.50, 136.52, 129.67, 129.16, 126.87, 123.50, 101.65, 76.91, 73.90, 72.78, 71.53, 71.49, 70.11, 69.17, 51.29, 41.44, 37.08, 36.72, 34.66, 32.46, 32.45, 30.71, 30.48, 30.37, 30.34, 30.27, 30.25, 30.21, 30.15, 30.11, 29.96, 29.94, 26.81, 26.73, 23.27,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

Exact mass (ESI-MS) for $C_{58}H_{106}N_2O_9$ $[M+H]^+$ found, 975.7974; calcd, 975.7977; $[M+Na]^+$ found, 997.7779; calcd, 997.7796.

General procedure for debenzylation: method B (6f and 6g)

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

*(2S,3S,4R)-1-O-(6-deoxy-5-thiofuranmethylamide-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (**6f**)*

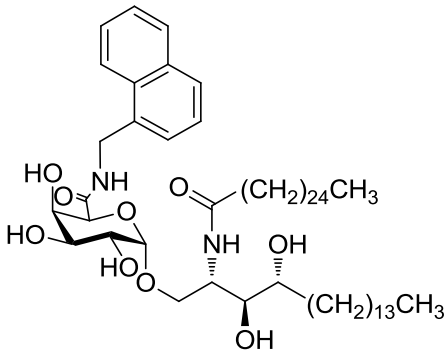


^{1}H NMR (300 MHz, pyridine-d_5): δ 8.86 (t, *J* = 6.2 Hz, 1H, NH), 8.45 (d, *J* = 8.5 Hz, 1H, NH), 7.25 (app. dd, *J* = 1.2 Hz and 5.2 Hz, 2H, arom. H), 7.11 (br. s, 1H, OH), 6.91 (dd, *J* = 3.6 Hz and 5.1 Hz, 1H, arom. H), 6.68 (br. s, 1H, OH), 6.39 (br. s, 1H OH), 6.12 (br. s, 1H, OH), 5.55 (d, *J* = 3.8 Hz, 1H, H-1”), 5.28-5.21 (m, 1H, H-2), 5.0-4.82 (m, 4H, NH-CH_2, H-4” and H-5”), 4.66 (dd, *J* = 3.8 Hz and 9.9 Hz, 1H, H-2”), 4.56 (dd, *J* = 5.4 Hz and 10.5 Hz, 1H, H-1), 4.41 (d, *J* = 3.2 Hz and 9.9 Hz, 1H, H-3”), 4.35-4.19 (m, 2H, H-3 and H-4), 4.25 (dd, *J* = 4.2 Hz and 10.6 Hz, 1H, H-1), 2.44 (app. t, *J* = 7.4 Hz, 2H, $COCH_2$), 1.92-1.19 (m, 72H, CH_2), 0.89 (t, *J* = 6.7 Hz, 6H, CH_3).

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

Exact mass (ESI-MS) for $C_{55}H_{102}N_2O_9S$ $[M+H]^+$ found, 967.7431; calcd, 967.7379; $[M+Na]^+$ found, 989.7236; calcd, 967.7198; $[M+K]^+$ found, 1005.7026; calcd, 1005.6938.

*(2S,3S,4R)-1-O-(1-naphthaleneamide-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (**6g**)*



^{1}H NMR (300 MHz, pyridine-d_5): δ 8.49 (d, *J* = 8.7 Hz, 1H, NH), 8.29 (dd, *J* = 2.7 Hz and 6.7 Hz, 1H, NH), 7.94 (d, *J* = 7.2 Hz, 1H, arom. H), 7.87 (dd, *J* = 3.1 Hz and 6.3 Hz, 1H, arom. H), 7.77 (d, *J* = 8.3 Hz, 1H, arom. H), 7.59-7.38 (m, 4H, arom. H), 5.54 (d, *J* = 3.7 Hz, 1H, H-1”), 5.44 (dd, *J* = 6.7 Hz and 15.6 Hz, 1H, NH-CH_2), 5.29-5.21 (m, 1H, H-2), 5.13-4.95 (m, 3H, NH-CH_2, H-4” and H-5”), 4.68 (dd, *J* = 3.7 Hz and 9.8 Hz, 1H, H-2”), 4.60 (dd, *J* = 5.4 Hz and 10.6 Hz, 1H, H-1), 4.45 (dd, *J* = 3.1 Hz and 10.0 Hz, H-3”), 4.43-4.29 (m, 2H, H-3 and H-4), 4.28 (dd, *J* = 4.4 Hz and 10.7 Hz, 1H, H-1), 2.46 (app. t, *J* = 7.1 Hz, 2H, $COCH_2$), 1.97-1.19 (m, 72H, CH_2), 0.89 (t, *J* = 6.6 Hz, 6H, CH_3).

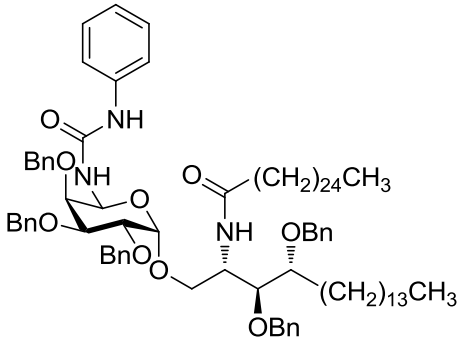
^{13}C NMR (75 MHz, pyridine-d_5): δ 173.42, 170.60, 134.50, 132.12, 129.31, 128.21, 126.85, 126.35, 126.27, 125.92, 101.77, 76.84, 74.18, 72.79, 71.63, 71.50, 70.12, 69.29, 55.37, 51.34,

41.33, 37.07, 34.62, 32.47, 30.82, 30.71, 30.48, 30.38, 30.34, 30.27, 30.25, 30.21, 30.15, 30.11, 29.96, 29.94, 26.81, 26.73, 23.28, 14.62.

Exact mass (ESI-MS) for $C_{61}H_{106}N_2O_9$ $[M+H]^+$ found, 1011.7974; calcd, 1011.7971; $[M+Na]^+$ found, 1033.7747; calcd, 1033,7796; $[M+K]^+$ found, 1049.7509; calcd, 1049,7535.

(2*S*,3*S*,4*R*)-3,4-di-*O*-benzyl-1-*O*-(2,3,4-tri-*O*-benzyl-6-deoxy-5-phenylureido-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (43)

To a solution of **39** (96 mg, 0.07 mmol) in toluene (1 mL) was added Et_3N (0.02 mL, 0.15 mmol) and DPPA (0.03 mL, 0.15 mmol). The mixture was stirred while refluxing for 1.5 hours followed by addition of Ag_2CO_3 (2 mg, 0.01 mmol) and aniline (7 mg, 0.07 mmol). Upon additional refluxing during 3 hours, the reaction mixture was cooled to 0 °C, quenched with NH_4Cl and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated to dryness. Purification by column chromatography (hexanes/EtOAc: 8.2/1.8) yielded derivative **43** (52 mg, 50 %) as a pale yellow oil.

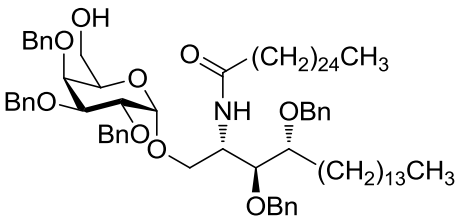


^{1}H NMR (300 MHz, $CDCl_3$): δ 7.43-7.05 (m, 31H, arom. H, NH), 5.98 (s, 1H, NH), 5.47 (app. d, *J* = 10.0 Hz, 1H, H-5"), 5.33 (d, *J* = 9.7 Hz, 1H, NH), 5.01 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.88 (d, *J* = 10.9 Hz, 1H, CH_2-Ph), 4.87 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.77 (d, *J* = 11.5 Hz, 1H, CH_2-Ph), 4.75-4.70 (m, 3H, H-1", CH_2-Ph), 4.67 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.65 (d, *J* = 11.5 Hz, 1H, CH_2-Ph), 4.56-4.47 (m, 4H, CH_2-Ph, H-1), 3.99-3.92 (m, 3H, H-2, H-2", H-3"), 3.78 (app. s, 1H, H-4"), 3.61-3.55 (m, 2H, H-3, H-4), 2.09-2.03 (m, 2H, $COCH_2$), 1.70-1.16 (m, 72H, CH_2), 0.88 (t, *J* = 6.7 Hz, 6H, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ 172.51, 153.26, 138.12, 137.98, 137.39, 137.34, 137.32, 136.79, 128.12, 127.81, 127.78, 127.42, 127.26, 127.23, 127.17, 127.15, 126.99, 126.75, 126.66, 126.43, 126.26, 123.06, 119.42, 99.38, 79.38, 77.41, 76.61, 75.71, 73.75, 73.61, 72.85, 72.75, 72.39, 72.14, 70.55, 49.78, 35.42, 30.93, 30.91, 28.73, 28.69, 28.64, 28.51, 28.40, 28.37, 28.35, 28.23, 25.23, 24.82, 21.68, 13.11.

Exact mass (ESI-MS) for $C_{91}H_{133}N_3O_9$ $[M+H]^+$ found, 1413.0187; calcd, 1413.0120.

(2*S*,3*S*,4*R*)-3,4-di-*O*-benzyl-1-*O*-(2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (25).

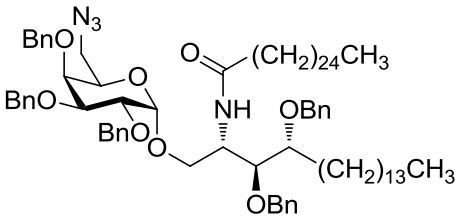


IR: 3485, 3319, 3036, 2922, 2851, 1647

(Other spectral data and preparation: see Chapter 3, p62-63)

(2*S*,3*S*,4*R*)-3,4-di-*O*-benzyl-1-*O*-(6-azido-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (44).

To a solution of intermediate **25** (500 mg, 0.38 mmol) and triphenylphosphine (400 mg, 1.53 mmol) in THF (25 mL) at -20 °C were added DEAD (0.7 mL, 2.2 M) and DPPA (0.33 mL, 1.53 mmol). The reaction mixture was stirred overnight and after completion of the reaction, the solvent was removed under reduced pressure. Purification by column chromatography (hexanes/EtOAc: 9/1) resulted in azide **44** (471 mg, 92 %) as a white solid.



^{1}H NMR (300 MHz, $CDCl_3$): δ 7.42-7.26 (m, 25H, arom. H), 5.87 (d, *J* = 8,6 Hz, 1H, NH), 4.98 (d, *J* = 11.4 Hz, 1H, CH_2-Ph), 4.84 (d, *J* = 3.8 Hz, 1H, H-1"), 4.82-4.72 (m, 4H, CH_2-Ph), 4.64 (d, *J* = 11.9 Hz, 1H, CH_2-Ph), 4.61-4.57 (m, 2H, CH_2-Ph), 4.51 (d, *J* = 11.5 Hz, 1H, CH_2-Ph), 4.49 (d, *J* = 11.7 Hz, 1H, CH_2-Ph), 4.32-4.24 (m, 1H, H-2), 4.02 (dd, *J* = 3.5 Hz and 10.1 Hz, 1H, H-2"), 3.91-3.83 (m, 3H, H-3", H-1, H-4"), 3.81-3.74 (m, 3H, H-3, H-1, H-5"), 3.55-3.51 (m, 1H, H-4), 3.47 (dd, *J* = 8.1 Hz and 12.4 Hz, 1H, H-6"), 2.99 (dd, *J* = 6.2 Hz and 12.4 Hz, 1H, H-6"), 2.01-1.85 (m, 2H, $COCH_2$), 1.64-0.98 (m, 72H, CH_2), 0.88 (t, *J* = 6.6 Hz, 6H, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ 173.01, 150.14, 150.03, 138.89, 138.78, 138.73, 138.60, 138.32, 130.30, 130.28, 128.68, 128.66, 128.63, 128.61, 128.57, 128.15, 128.06, 128.04, 127.97, 127.91, 127.84, 127.77, 127.69, 126.36, 126.34, 120.49, 120.43, 99.34, 80.09, 79.56, 79.04, 77.66, 77.44, 77.24, 76.81, 75.07, 74.87, 73.68, 73.51, 72.06, 70.25, 69.04, 51.61, 50.37, 36.98, 32.16, 30.06, 29.96, 29.94, 29.89, 29.84, 29.69, 29.64, 29.61, 29.59, 26.16, 25.95, 22.92, 14.35.

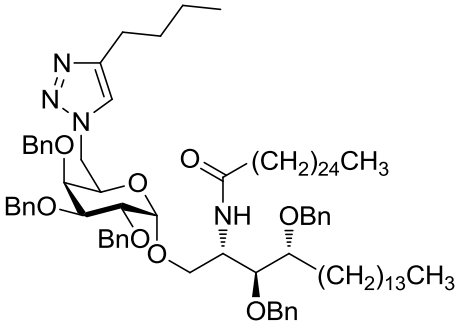
IR: 3312, 2978, 2918, 2849, 2097, 1645.

Exact mass (ESI-MS) for $C_{85}H_{128}N_4O_8$ $[M+H]^+$ found, 1333.9846; calcd, 1333.9805.

General procedure for CuAAC click reaction

To a solution of azide **44** (80 mg, 0.06 mmol) in DMF (2 mL) was added the appropriate alkyne, sodium ascorbate and copper(II) sulfate. The reaction mixture was stirred for 20 minutes at 70 °C in the microwave. After extraction with EtOAc, the organic phase was washed with brine and dried over Na_2SO_4, followed by evaporation of the solvent. Purification by column chromatography (hexanes/EtOAc: 7.5/2.5) afforded the desired triazoles **45b** (74 %), **45c** (60 %), **45d** (95 %), **45e** (80 %) and **45f** (97 %).

*(2S,3S,4R)-3,4-di-O-benzyl-1-O-(2,3,4-tri-O-benzyl-6-deoxy-6-(4-butyltriazol-1-yl)-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (**45b**).*

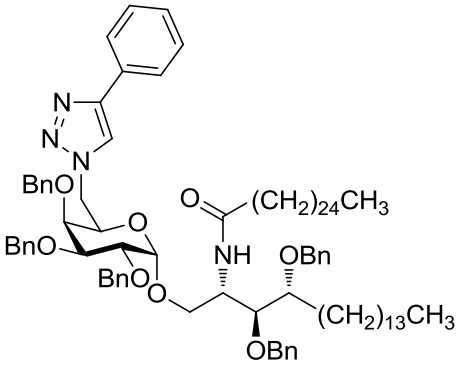


^{1}H NMR (300 MHz, $CDCl_3$): δ 7.27-7.03 (m, 26H, arom. H), 5.63 (d, *J* = 8.5 Hz, 1H, NH), 4.94 (d, *J* = 11.1 Hz, 1H, CH_2-Ph), 4.78 (d, *J* = 3.5 Hz, 1H, H-1”), 4.73 (d, *J* = 11.7 Hz, 1H, CH_2-Ph), 4.71 (d, *J* = 11.7 Hz, 1H, CH_2-Ph), 4.64 (d, *J* = 11.7 Hz, 2H, CH_2-Ph), 4.57 (d, *J* = 11.7 Hz, 1H, CH_2-Ph), 4.56 (d, *J* = 11.1 Hz, 1H, CH_2-Ph), 4.48 (d, *J* = 11.7 Hz, 1H, CH_2-Ph), 4.40 (d, *J* = 11.7 Hz, 1H, CH_2-Ph), 4.39 (d, *J* = 11.7 Hz, 1H, CH_2-Ph), 4.31 (dd, *J* = 5.9 Hz and 13.7 Hz, 1H, H-6”), 4.21-4.15 (m, 1H, H-2), 4.12 (dd, *J* = 5.9 Hz and 13.1 Hz, 1H, H-6”), 4.00-3.96 (m, 2H, H-2”, H-5”), 3.80 (dd, *J* = 2.6 Hz and 10.2 Hz, 1H, H-3”), 3.68-3.59 (m, 3H, H-4”, H-3, H-1), 3.52-3.41 (m, 2H, H-1, H-4), 2.57 (app. t, *J* = 7.7 Hz, 2H, CH_2), 1.88-1.71 (m, 2H, $COCH_2$), 1.56-1.05 (m, 76H, CH_2), 0.84 (t, *J* = 7.3 Hz, 3H, CH_3), 0.81 (t, *J* = 6.8 Hz, 6H, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ 173.01, 148.40, 138.82, 138.77, 138.56, 138.49, 138.44, 128.76, 128.68, 128.65, 128.62, 128.57, 128.16, 128.11, 128.09, 127.99, 127.92, 127.81, 127.66, 122.29, 99.41, 79.87, 79.78, 79.25, 77.67, 77.45, 77.25, 76.82, 76.47, 74.95, 74.76, 73.81, 73.51, 73.41, 72.08, 70.17, 50.28, 36.87, 32.16, 31.75, 30.43, 30.08, 29.96, 29.94, 29.91, 29.89, 29.85, 29.70, 29.61, 29.59, 26.06, 25.88, 25.52, 22.92, 22.55, 14.35, 14.05.

Exact mass (ESI-MS) for $C_{91}H_{138}N_4O_8$ $[M+H]^+$ found, 1416.0526; calcd, 1416.0593.

*(2S,3S,4R)-3,4-di-O-benzyl-1-O-(2,3,4-tri-O-benzyl-6-deoxy-6-(4-phenyltriazol-1-yl)-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (**45c**).*

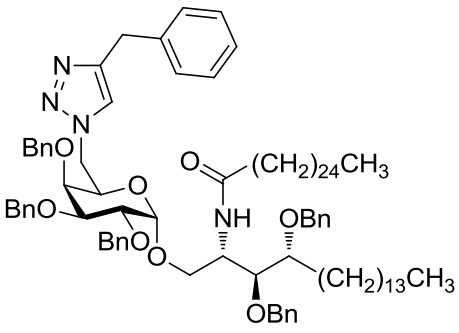


^{1}H NMR (300 MHz, $CDCl_3$): δ 7.93-7.72 (m, 3H, arom. H), 7.31-7.18 (m, 28H), 5.55 (d, *J* = 8.2 Hz, 1H, NH), 4.95 (d, *J* = 10.9 Hz, 1H, CH_2-Ph), 4.78 (app. s, 1H, H-1"), 4.73-4.55 (m, 6H, CH_2-Ph), 4.47-4.34 (m, 4H, CH_2-Ph, H-6"), 4.25-4.18 (m, 2H, H-2, H-6"), 4.06-3.97 (m, 2H, H-5", H-2"), 3.79 (app. d, *J* = 10.0 Hz, 1H, H-3"), 3.69-3.52 (m, 4H, H-4", H-3, H-1), 3.42-3.41 (m, 1H, H-4), 1.82-1.66 (m, 2H, $COCH_2$), 1.49-0.99 (m, 72H, CH_2), 0.80 (t, *J* = 6.5 Hz, 6H, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ 173.19, 162.87, 138.76, 138.68, 138.58, 138.51, 138.48, 130.85, 129.03, 128.82, 128.69, 128.68, 128.63, 128.58, 128.28, 128.21, 128.13, 128.01, 127.98, 127.91, 127.81, 127.68, 125.88, 121.47, 99.69, 80.15, 79.45, 79.27, 77.70, 77.49, 77.28, 76.86, 76.45, 75.06, 74.75, 73.80, 73.60, 73.40, 72.00, 70.10, 68.90, 50.41, 36.86, 36.73, 32.17, 31.69, 30.50, 30.08, 29.95, 29.90, 29.84, 29.65, 29.60, 25.86, 22.93, 14.37.

Exact mass (ESI-MS) for $C_{93}H_{134}N_4O_8$ $[M+H]^+$ found, 1436.0295; calcd, 1436.0274, $[M+Na]^+$ found 1458.0104; calcd, 1458.0094, $[M+K]^+$ found 1473.9843, calcd, 1473.9833.

*(2S,3S,4R)-3,4-di-O-benzyl-1-O-(2,3,4-tri-O-benzyl-6-deoxy-6-(4-benzyltriazol-1-yl)-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (**45d**)*



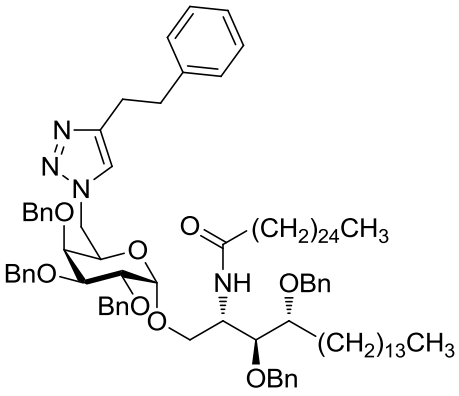
^{1}H NMR (300 MHz, $CDCl_3$): δ 7.30-7.09 (m, 31H, arom. H), 5.63 (d, *J* =8.6 Hz, 1H, NH), 4.92 (d, *J* = 11.1 Hz, 1H, CH_2-Ph), 4.74 (d, *J* = 2.9 Hz, 1H, H-1"), 4.70 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.69 (d, *J* = 13.0 Hz, 1H, CH_2-Ph), 4.66 (d, *J* = 12.1 Hz, 1H, CH_2-Ph), 4.64 (d, *J* = 11.6 Hz, 1H, CH_2-Ph), 4.56 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.50 (d, *J* = 11.1 Hz, 1H, CH_2-Ph), 4.48 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.39 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.38 (d, *J* = 11.6 Hz, 1H, CH_2-Ph), 4.25 (dd, *J* = 5.6 Hz and 13.8 Hz, 1H, H-6"), 4.16-4.09 (m, 2H, H-2, H-6"), 3.98-3.93 (m, 4H, CH_2-Ph, H-2", H-5"), 3.79 (dd, *J* = 2.4 Hz and 10.1 Hz, 1H, H-3"), 3.68 (app. s, 1H, H-4"), 3.64 (dd, *J* = 3.5 Hz and 5.7 Hz, 1H, H-3), 3.55 (dd, *J* = 6.1 Hz and 10.7 Hz, 1H, H-1), 3.45-

3.38 (m, 2H, H-4, H-1), 1.89-1.70 (m, 2H, $COCH_2$), 1.54-1.18 (m, 72H, CH_2), 0.80 (t, *J* = 6.7 Hz, 6H, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ 172.98, 147.54, 139.32, 138.82, 138.53, 138.46, 138.34, 128.85, 128.83, 128.71, 128.69, 128.64, 128.62, 128.58, 128.17, 128.11, 128.06, 128.04, 128.00, 127.95, 127.88, 127.81, 127.68, 126.97, 123.19, 99.27, 79.97, 79.58, 79.21, 77.67, 77.45, 77.25, 76.83, 76.42, 74.90, 74.71, 73.82, 73.49, 73.39, 72.04, 70.06, 68.41, 50.52, 50.17, 36.89, 32.38, 32.16, 30.34, 30.10, 29.97, 29.94, 29.89, 29.86, 29.71, 29.61, 29.60, 26.11, 25.90, 22.93, 14.36.

Exact mass (ESI-MS) for $C_{94}H_{136}N_4O_8$ $[M+H]^+$ found, 1450.0453; calcd, 1450.0431, $[M+Na]^+$ found 1472.0304; calcd, 1472.0250.

*(2S,3S,4R)-3,4-di-O-benzyl-1-O-(2,3,4-tri-O-benzyl-6-deoxy-6-(4-ethylphenyltriazol-1-yl)-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (**45e**).*

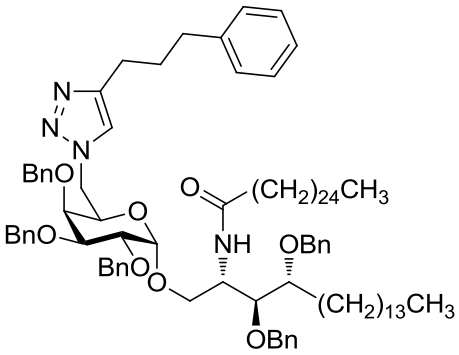


^{1}H NMR (300 MHz, $CDCl_3$): δ 7.30-7.06 (m, 31H, arom. H), 5.64 (d, *J* = 8.5 Hz, 1H, NH), 4.93 (d, *J* = 11.3 Hz, 1H, CH_2-Ph), 4.77 (d, *J* = 3.7 Hz, 1H, H-1”), 4.73-4.62 (m, 3H, CH_2-Ph), 4.60 (d, *J* = 10.9 Hz, 1H, CH_2-Ph), 4.57 (d, *J* = 11.7 Hz, 1H, CH_2-Ph), 4.52 (d, *J* = 9.2 Hz, 1H, CH_2-Ph), 4.48 (d, *J* = 9.6 Hz, 1H, CH_2-Ph), 4.40 (d, *J* = 11.5 Hz, 1H, CH_2-Ph), 4.39 (d, *J* = 11.5 Hz, 1H, CH_2-Ph), 4.29 (dd, *J* = 5.92 Hz and 13.7 Hz, 1H, H-6”), 4.22-4.15 (1H, H-2), 4.14 (dd, *J* = 6.7 Hz and 13.9 Hz, 1H, H-6”), 4.01-3.95 (m, 2H, H-2”, H-5”), 3.79 (dd, *J* = 2.6 Hz and 10.2 Hz, 1H, H-3”), 3.65-3.57 (m, 3H, H-4”, H-3, H-1), 3.51-3.41 (m, 2H, H-1, H-4), 2.96-2.82 (m, 4H, CH_2-CH_2-Ph), 1.88-1.70 (m, 2H, $COCH_2$), 1.53-1.04 (m, 72H, CH_2), 0.81 (t, *J* = 6.7 Hz, 6H, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ 173.04, 147.29, 141.38, 138.80, 138.76, 138.56, 138.48, 138.41, 128.76, 128.69, 128.66, 128.61, 128.58, 128.17, 128.11, 128.09, 128.01, 127.93, 127.82, 127.65, 126.31, 122.61, 99.37, 79.84, 79.25, 77.67, 77.45, 77.25, 76.83, 76.48, 74.93, 74.71, 73.82, 73.54, 73.42, 72.08, 70.09, 68.50, 50.36, 50.28, 36.88, 35.71, 32.16, 30.45, 30.08, 29.96, 29.89, 29.86, 29.70, 29.61, 29.59, 27.61, 26.05, 25.89, 22.93, 21.28, 14.43, 14.36.

Exact mass (ESI-MS) for $C_{95}H_{138}N_4O_8$ $[M+H]^+$ found, 1464.0540; calcd, 1464.0593.

*(2S,3S,4R)-3,4-di-O-benzyl-1-O-(2,3,4-tri-O-benzyl-6-deoxy-6-(4-propylphenyltriazol-1-yl)-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (**45f**).*



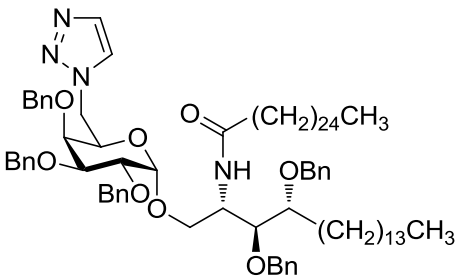
^{1}H NMR (300 MHz, $CDCl_3$): δ 7.36-7.17 (m, 31H, arom. H), 5.73 (d, *J* = 8.5 Hz, 1H, NH), 5.03 (d, *J* = 11.9 Hz, 1H, CH_2-Ph), 4.87 (d, *J* = 3.5 Hz, 1H, H-1"), 4.82 (d, *J* =11.8 Hz, 1H, CH_2-Ph), 4.80 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.73 (d, *J* = 12.0 Hz, 2H, CH_2-Ph), 4.66 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.65 (d, *J* = 11.27 Hz, 1H, CH_2-Ph), 4.57 (d, *J* = 11.6 Hz, 1H, CH_2-Ph), 4.49 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.47 (d, *J* = 11.6 Hz, 1H, CH_2-Ph), 4.40 (dd, *J* = 6.0 Hz and 13.8 Hz, 1H, H-6"), 4.32-4.20 (m, 2H, H-2, H-6"), 4.11-4.05 (m, 2H, H-5", H-2"), 3.89 (dd, *J* = 2.6 Hz and 10.0 Hz, 1H, H-3"), 3.76 (app. d, *J* = 1.5 Hz, 1H, H-4"), 3.74-3.66 (m, 2H, H-3, H-1), 3.59 (dd, *J* = 4.6 Hz and 10.7 Hz, 1H, H-1), 3.54-3.50 (m, 1H, H-4), 2.77-2.64 (m, 4H, CH_2-CH_2-Ph), 2.05-1.81 (m, 4H, CH_2, CH_2, $COCH_2$), 1.61-1.28 (m, 72H, CH_2), 0.90 (t, *J* = 6.7 Hz, 6H, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ 173.04, 147.93, 142.09, 138.84, 138.79, 138.57, 138.51, 138.44, 128.79, 128.76, 128.72, 128.70, 128.66, 128.64, 128.61, 128.58, 128.18, 128.13, 128.10, 128.02, 127.94, 127.83, 127.68, 126.17, 126.07, 122.41, 99.43, 79.80, 79.26, 77.73, 77.30, 76.88, 76.50, 74.96, 74.75, 73.84, 73.54, 73.44, 72.08, 70.15, 68.94, 50.31, 36.88, 35.65, 34.87, 32.18, 31.29, 30.42, 30.30, 30.09, 29.98, 29.96, 29.94, 29.91, 29.87, 29.72, 29.63, 26.06, 25.91, 25.39, 22.95, 18.06, 14.38.

Exact mass (ESI-MS) for $C_{96}H_{140}N_4O_8$ $[M+H]^+$ found, 1478.0772; calcd, 1478,0749.

(2*S*,3*S*,4*R*)-3,4-di-*O*-benzyl-1-*O*-(2,3,4-tri-*O*-benzyl-6-deoxy-6-(triazol-1-yl)-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (45a).

Azide **44** was dissolved in vinyl acetate and stirred at 120 °C in the microwave. After 6 hours, the reaction mixture was evaporated to dryness. Purification by column chromatography (hexanes/EtOAc: 7.5/2.5) afforded the desired triazole **45a** (93 mg, 71 %).



^{1}H NMR (300 MHz, $CDCl_3$): δ 7.53 (dd, *J* = 0.9 and 7.3 Hz, 2H, arom. H), 7.36-7.22 (m, 25H, arom. H), 5.68 (d, *J* = 8.5 Hz, 1H, NH), 5.01 (d, *J* = 11.2 Hz, 1H, CH_2-Ph), 4.84 (d, *J* = 3.6 Hz, 1H, H-1"), 4.80 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.78 (d, *J* = 11.8 Hz, 1H, CH_2-

Ph), 4.71 (d, *J* = 11.7 Hz, 1H, CH_2-Ph), 4.70 (d, *J* = 11.7 Hz, 1H, CH_2-Ph), 4.64 (d, *J* = 11.7 Hz, 1H, CH_2-Ph), 4.63 (d, *J* = 11.2 Hz, 1H, CH_2-Ph), 4.55 (d, *J* = 11.6 Hz, 1H, CH_2-Ph), 4.47 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.45 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.39 (dd, *J* = 5.2 Hz and 13.9 Hz, 1H, H-6"), 4.29 (dd, *J* = 7.7 Hz and 13.9 Hz, 1H, H-6"), 4.23-4.17 (m, 1H, H-2), 4.07-4.01 (m, 2H, H-2", H-5"), 3.87 (dd, *J* = 2.6 Hz and 10.1 Hz, 1H, H-3"), 3.77 (app. d, *J* = 1.3 Hz, 1H, H-4"), 3.68 (dd, *J* = 3.6 Hz and 5.2 Hz, 1H, H-3), 3.62 (dd, *J* = 6.9 Hz and 10.6 Hz, 1H, H-1), 3.55-3.47 (m, 2H, H-1, H-4), 1.95-1.77 (m, 2H, $COCH_2$), 1.61-1.13 (m, 72H, CH_2), 0.88 (t, *J* = 6.7 Hz, 6H, CH_3).

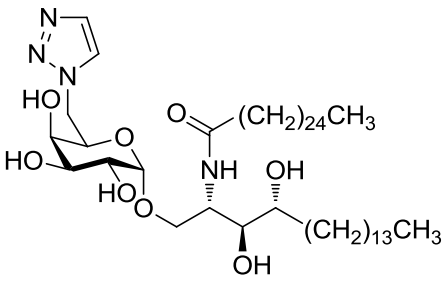
^{13}C NMR (75 MHz, $CDCl_3$): δ 172.95, 149.61, 138.59, 138.49, 138.33, 138.27, 138.08, 133.65, 128.57, 128.51, 128.48, 128.45, 128.41, 128.37, 128.03, 127.89, 127.88, 127.85, 127.79, 127.73, 127.62, 127.47, 124.77, 116.19, 99.01, 79.69, 79.61, 78.96, 76.25, 74.73, 74.63, 73.58, 73.38, 73.11, 71.96, 69.94, 50.40, 50.04, 36.69, 31.94, 30.28, 29.83, 29.74, 29.69, 29.67, 29.62, 29.46, 29.39, 29.37, 25.86, 25.67, 22.70, 14.13.

Exact mass (ESI-MS) for $C_{87}H_{130}N_4O_8$ $[M+H]^+$ found, 1359.9907; calcd, 1359.9967, $[M+Na]^+$ found, 1381.9797; calcd, 1381.9786.

General procedure for debenzylation of the C-6"-triazoles

A solution of the protected triazole (0.06 mmol) in $CHCl_3$ (0.4 mL) and EtOH (1.2 mL) was hydrogenated under atmospheric pressure in the presence of palladium black (10 mg). Upon reaction completion, the mixture was diluted with pyridine and filtered through celite. The filter cake was rinsed with $CHCl_3$ and EtOH and the filtrate was evaporated to dryness. After purification by column chromatography (CH_2Cl_2/MeOH: 8/2), final compounds **8a** (31 %), **8b** (50 %), **8c** (40 %), **8d** (22 %), **8e** (31 %) and **8f** (53 %) were obtained as white powders.

*(2S,3S,4R)-1-O-(6-deoxy-6-(triazol-1-yl)-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (**8a**).*



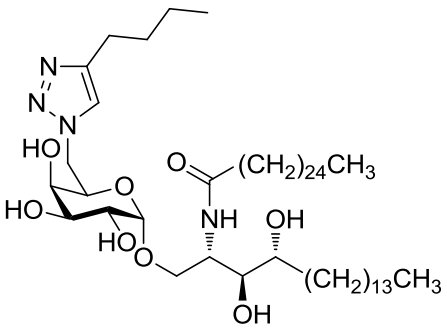
^{1}H NMR (300 MHz, pyridine-d_5): δ 8.42 (d, *J* = 8.9 Hz, 1H, NH), 8.21 (d, *J* = 0.9 Hz, 1H, arom. H), 7.92 (d, *J* = 0.9 Hz, 1H, arom. H), 5.5 (d, *J* = 3.7 Hz, 1H, H-1"), 5.26-5.19 (m, 1H, H-2), 5.14-4.99 (m, 2H, H-6"), 4.67-4.61 (m, 2H, H-2", H-5"), 4.38-4.30 (3H, H-1, H-3", H-3), 4.26-4.21 (m, 2H, H-4, H-4"), 4.09 (dd, *J* = 4.9 Hz and 10.6 Hz, 1H, H-1), 2.50-2.36 (m, 2H, $COCH_2$), 2.01-1.09 (m, 72H, CH_2), 0.88 (t, *J* = 6.7 Hz, 6H, CH_3).

^{13}C NMR (75 MHz, pyridine-d_5): δ 171.92, 149.07, 148.72, 148.36, 147.99, 135.00, 134.66, 134.33, 134.00, 133.64, 132.60, 124.24, 122.98, 122.65, 122.32, 121.99, 121.61, 99.98, 75.54, 71.23, 69.78, 69.67, 69.47, 68.57, 67.06, 50.30, 49.77, 35.57, 33.34, 30.94, 29.21, 28.97, 28.86, 28.83, 28.75, 28.69, 28.63, 28.58, 28.43, 25.27, 25.20, 21.76, 13.10.

IR: 3462, 3451, 3335, 2922, 2850, 1653.

Exact mass (ESI-MS) for $C_{52}H_{100}N_4O_8[M+H]^+$ found, 909.7681; calcd, 909.7619, $[M+Na]^+$ found, 931.7450; calcd, 931.7439.

*(2S,3S,4R)-1-O-(6-deoxy-6-(4-butyltriazol-1-yl)-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (**8b**).*

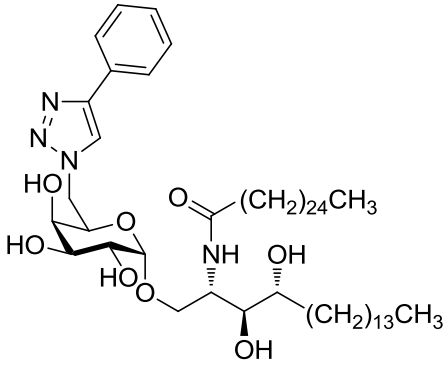


^{1}H NMR (300 MHz, pyridine-d_5): δ 8.42 (d, *J* = 8.7 Hz, 1H, NH), 7.93 (s, 1H, arom. H), 6.83 (br. s, 1H, OH), 6.37 (br. s, 1H, OH), 6.05 (br. s, 1H, OH), 5.52 (d, *J* = 3.9 Hz, 1H, H-1"), 5.26-5.19 (m, 1H, H-2), 5.03 (app. d, *J* = 6.5 Hz, 2H, H-6"), 4.70-4.62 (m, 2H, H-5", H-2"), 4.41 (dd, *J* = 5.4 Hz and 10.7 Hz, 1H, H-1), 4.32 (dd, *J* = 3.2 Hz and 9.9 Hz, 1H, H-3"), 4.27-4.22 (m, 3H, H-3, H-4", H-4), 4.16 (dd, *J* = 4.9 Hz and 10.6 Hz, 1H, H-1), 2.83 (t, *J* = 7.6 Hz, 2H, CH_2), 2.48-2.39 (m, 2H, $COCH_2$), 1.94-1.11 (m, 76H, CH_2), 0.93-0.86 (m, 9H, CH_3).

^{13}C NMR (75 MHz, pyridine-d_5): δ 171.94, 100.11, 75.56, 71.24, 69.83, 69.72, 69.41, 68.62, 67.25, 50.21, 49.90, 35.58, 33.32, 30.94, 30.81, 29.24, 28.98, 28.86, 28.83, 28.75, 28.74, 28.71, 28.64, 28.61, 28.44, 28.43, 25.29, 25.21, 24.59, 21.76, 21.39, 13.10, 12.82.

Exact mass (ESI-MS) for $C_{56}H_{108}N_4O_8$ $[M+H]^+$ found, 965.8295; calcd, 965.8240, $[M+Na]^+$ found, 987.8087; calcd, 987.8059, $[M+K]^+$ found, 1003.7825; calcd, 1003.7799.

*(2S,3S,4R)-1-O-(6-deoxy-6-(4-phenyltriazol-1-yl)-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (**8c**).*



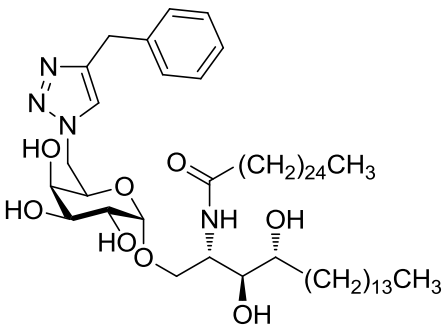
^{1}H NMR (300 MHz, pyridine-d_5): δ 8.66 (s, 1H, arom. H), 8.40 (d, *J* = 8.7 Hz, 1H, NH), 8.21-8.18 (m, 2H, arom. H), 7.50-7.45 (m, 2H, arom. H), 7.38-7.33 (m, 1H, arom. H), 5.55 (d, *J* = 3.7 Hz, 1H, H-1"), 5.28-5.19 (m, 1H, H-2), 5.10 (app. d, *J* = 5.5 Hz, 2H, H-6"), 4.75 (t, *J* = 6.6 Hz, 1H, H-5"), 4.66 (dd, *J* = 3.7 Hz and 9.8 Hz, 1H,

H-2”), 4.45 (dd, *J* = 5.6 Hz and 10.6 Hz, 1H, H-1), 4.35 (dd, *J* = 3.2 Hz and 9.9 Hz, 1H, H-3”), 4.27-4.22 (m, 3H, H-3, H-4, H-4”), 4.15 (dd, *J* = 4.9 Hz and 10.8 Hz, 1H, H-1), 2.45-2.22 (m, 2H, $COCH_2$), 1.90-1.17 (m, 72H, CH_2), 0.88 (t, *J* = 6.7 Hz, 6H, CH_3).

^{13}C NMR (75 MHz, pyridine-d_5): δ 171.95, 149.40, 149.07, 148.71, 148.35, 146.54, 134.66, 134.33, 134.00, 130.91, 128.07, 126.94, 124.95, 122.99, 122.66, 122.33, 122.00, 121.60, 121.01, 100.05, 75.56, 71.22, 69.79, 69.54, 69.25, 68.60, 67.07, 50.34, 49.83, 35.54, 33.32, 30.94, 29.23, 28.97, 28.87, 28.83, 28.75, 28.74, 28.70, 28.61, 28.58, 28.44, 28.43, 25.26, 25.18, 21.76, 13.10.

Exact mass (ESI-MS) for $C_{58}H_{104}N_4O_8$ $[M+H]^+$ found, 985.7953; calcd, 985.7927, $[M+Na]^+$ found, 1007.7729; calcd, 1007.7746.

(2S,3S,4R)-1-O-(6-deoxy-6-(4-benzyltriazol-1-yl)-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol ***(8d)***

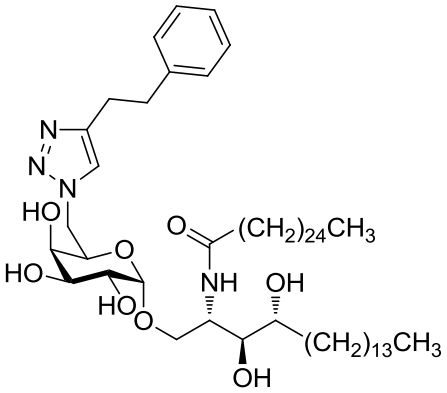


^{1}H NMR (300 MHz, pyridine-d_5): δ 8.41 (d, *J* = 8.7 Hz, 1H, NH), 7.91 (s, 1H, arom. H), 7.47-7.45 (m, 2H, arom. H), 7.41-7.36 (m, 2H, arom. H), 7.28-7.26 (m, 1H, arom. H), 5.48 (d, *J* = 3.7 Hz, 1H, H-1”), 5.23-5.19 (m, 1H, H-2), 5.01-4.99 (m, 2H, H-6”), 4.64-4.59 (m, 2H, H-2”, H-5”), 4.36 (dd, *J* = 5.2 Hz and 10.7 Hz, 1H, H-1), 4.32-4.25 (m, 3H, H-4, H-4”, H-3, CH_2-Ph), 4.21 (app. d, *J* = 2.4 Hz, 1H, H-3”), 4.14 (dd, *J* = 4.6 Hz and 10.7 Hz, 1H, H-1), 2.48-2.41 (m, 2H, $COCH_2$), 1.93-1.26 (m, 72H, CH_2), 0.88 (t, *J* = 6.6 Hz, 6H, CH_3).

^{13}C NMR (75 MHz, pyridine-d_5): δ 171.92, 149.06, 148.70, 148.35, 147.98, 146.06, 139.26, 135.00, 134.66, 134.33, 133.99, 133.64, 128.02, 127.78, 125.45, 122.98, 122.65, 122.32, 122.20, 121.99, 100.13, 75.55, 71.30, 69.79, 69.71, 69.47, 68.56, 67.44, 50.37, 49.83, 35.58, 33.29, 31.33, 30.95, 29.24, 28.98, 28.87, 28.83, 28.75, 28.74, 28.71, 28.64, 28.61, 28.55, 28.44, 28.43, 28.37, 25.31, 25.22, 21.76, 13.10.

Exact mass (ESI-MS) for $C_{59}H_{106}N_4O_8$ $[M+H]^+$ found, 999.8134; calcd, 999.8089, $[M+Na]^+$ found, 1021.7906; calcd, 1021.7908, $[M+K]^+$ found, 1037.7676; calcd, 1037.7648.

*(2S,3S,4R)-1-O-(6-deoxy-6-(4-ethylphenyltriazol-1-yl)-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (**8e**).*

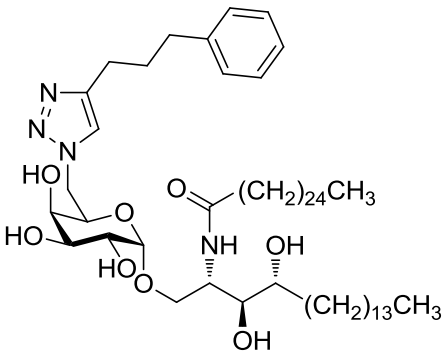


^{1}H NMR (300 MHz, pyridine-d_5): δ 8.43 (d, *J* = 8.7 Hz, 1H, NH), 7.88 (s, 1H, arom. H), 7.36-7.31 (m, 3H, arom. H), 7.27-7.25 (m, 2H, arom. H), 5.50 (d, *J* = 3.9 Hz, 1H, H-1”), 5.26-5.19 (m, 1H, H-2), 5.01-4.96 (m, 2H, H-2”, H-5”), 4.67-4.61 (m, 2H, H-6”), 4.39 (dd, *J* = 5.3 Hz and 10.6 Hz, 1H, H-1), 4.61 (dd, *J* = 3.2 Hz and 9.9 Hz, 1H, H-3” or H-4”) 4.56 (m, 2H, H-3, H-4), 4.49 (app. d, *J* = 2.4 Hz, H-3” or H-4”), 4.15 (dd, *J* = 5.0 Hz and 10.7 Hz, 1H, H-1), 3.22-3.11 (m, 4H, CH_2), 2.47-2.40 (m, 2H, $COCH_2$), 1.94-1.24 (m, 72H, CH_2), 0.89 (t, *J* = 6.7 Hz, 6H, CH_3).

^{13}C NMR (75 MHz, pyridine-d_5): δ 173.49, 150.58, 150.21, 149.86, 147.64, 142.41, 136.17, 135.84, 135.51, 129.24, 129.12, 126.72, 124.49, 124.16, 123.83, 123.50, 123.18, 123.12, 101.57, 77.11, 72.76, 71.34, 71.22, 70.91, 70.14, 51.76, 51.42, 37.10, 36.35, 34.85, 32.46, 32.45, 30.75, 30.49, 30.38, 30.34, 30.27, 30.25, 30.21, 30.14, 30.11, 29.96, 29.94, 28.44, 26.80, 26.72, 23.27, 14.61.

Exact mass (ESI-MS) for $C_{60}H_{108}N_4O_8$ $[M+H]^+$ found, 1013.8259; calcd, 1013.8240.

*(2S,3S,4R)-1-O-(6-deoxy-6-(4-propylphenyltriazol-1-yl)-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (**8f**).*



^{1}H NMR (300 MHz, pyridine-d_5): δ 8.38 (d, *J* = 8.7 Hz, 1H, NH), 7.93 (s, 1H, arom. H), 7.37-7.24 (m, 5H, arom. H), 7.11 (br. s, 1H, OH), 6.94 (br. s, 1H, OH), 6.82 (d, *J* = 2.9 Hz, 1H, OH), 6.36 (d, *J* = 5.0 Hz, 1H, OH), 6.04 (d, *J* = 3.9 Hz, 1H, OH), 5.51 (d, *J* = 3.7 Hz, 1H, H-1”), 5.26-5.18 (m, 1H, H-2), 5.03 (d, *J* = 5.03 Hz, 2H, H-6”), 4.71-4.62 (m, H-5”, H-2”), 4.41 (dd, *J* = 5.4 Hz and 10.4 Hz, 1H, H-1), 4.33-4.20 (m, 5H, H-3”, H-3, H-4, H-4”), 4.15 (dd, *J* = 5.0 Hz and 10.7 Hz, 1H, H-1), 2.86 (t, *J* = 7.6 Hz, 2H, CH_2), 2.72 (t, *J* = 7.6 Hz, 2H, CH_2), 2.41 (ddd, *J* = 3.0 Hz and 7.5 Hz, 2H, $COCH_2$), 2.10 (m, 2H, CH_2), 1.90-1.18 (m, 72H, CH_2), 0.87 (t, *J* = 6.6 Hz, 6H, CH_3).

^{13}C NMR (75 MHz, pyridine-d_5): δ 173.43, 150.58, 150.22, 149.87, 149.49, 148.06, 142.91, 136.51, 136.17, 135.84, 135.51, 135.14, 129.33, 129.10, 126.51, 124.49, 124.16, 123.83, 123.50, 123.10, 101.63, 77.07, 72.75, 71.34, 71.21, 70.88, 70.13, 68.79, 51.70, 51.40, 37.08, 35.92, 34.83, 32.46, 32.45, 31.95, 30.75, 30.50, 30.38, 30.34, 30.27, 30.25, 30.22, 30.15, 30.12, 29.96, 29.94, 26.79, 26.72, 25.92, 23.28, 14.62.

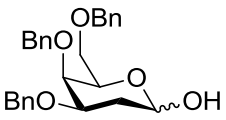
Exact mass (ESI-MS) for $C_{61}H_{110}N_4O_8$ $[M+H]^+$ found, 1027.8478; calcd, 1027.8402.

Computational modeling of C-6"-triazole 8a (performed by Dr. H. De Winter)

The starting conformation of the triazole was generated using OpenBabel version 2.3.1[11] and graphical visualisation of the complex was done with PyMol.[12] The structure was subsequently positioned into the binding pocket of Nu-α-GalCer (crystal structure was taken from the PDB, code 3QUZ) by superimposing the sugar ring of the triazole compound onto the corresponding ring of Nu-α-GalCer. Torsion angles were rotated to adopt a low energy conformation.

3,4,6-tri-*O*-benzyl-2-deoxy-D-galactopyranose (47)

To a solution of 3,4,6-tri-*O*-benzyl-D-galactal (2 g, 4.80 mmol) in CH_2Cl_2 (30 mL), a mixture of 48 % HBr (8.0 mL) and PPh_3 (17.6 g, 67.22 mmol) in CH_2Cl_2 (80 mL) was slowly added. The solution was vigorously stirred for 24 hours and the reaction mixture then was extracted with EtOAc. The combined organic layers were washed with a saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4 and evaporated to dryness to afford the crude product **47**.

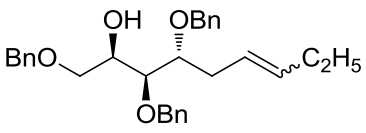


Exact mass (ESI-MS) for $C_{27}H_{30}O_5$ $[M+H]^+$ found, 435.2198; calcd, 435.2166, $[M+Na]^+$ found, 457.1977; calcd, 457.1986, $[M+K]^+$ found, 473.1725; calcd, 473.1725.

(2*R*,3*S*,4*R*,6E/Z)-1,3,4-tri-*O*-benzyl-non-6-en-2-ol (48)

n-Propyl triphenylphosphonium bromide (9.3 g, 24.01 mmol) was dissolved in THF (60 mL). The mixture was cooled to -12 °C, followed by dropwise addition of *n*-BuLi (12 mL, 1.6 M in hexane). After stirring for 1 hour at room temperature, the reaction mixture was again cooled to -12 °C and a solution of the crude galactopyranose **47** in anhydrous THF (90 mL) was added. After stirring for 2 hours at -12 °C, the reaction was quenched by addition of a saturated NH_4Cl solution. The mixture was extracted with CH_2Cl_2 and the organic layer was washed with brine, dried over Na_2SO_4 and evaporated. Column chromatography

(hexanes/EtOAc: 9/1) resulted in an isomeric mixture of the desired product **48** (1.22 g, 55 % over 2 steps) as a colorless oil.



^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.28-7.18 (m, 15H, arom. H), 5.44-5.33 (m, 2H, H-6, H-7), 4.57-4.37 (m, 6H, CH_2-Ph), 4.03-3.99 (m, 1H, H-2), 3.70-3.65 (m, 1H, H-4), 3.55-3.51 (m, 1H, H-3), 3.47-3.45 (m, 2H, H-1), 3.04-3.02 (m, 1H, OH), 2.39-2.30 (m, 2H, H-5), 2.04-1.91 (m, 2H, H-8), 0.91-0.83 (m, 3H, CH_3).

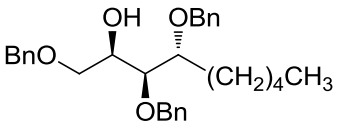
^{13}C-NMR (75 MHz, $CDCl_3$): δ 138.38, 138.34, 138.30, 135.47, 134.26, 132.41, 132.27, 132.15, 128.81, 128.62, 128.59, 128.31, 128.17, 128.12, 128.08, 128.04, 127.92, 128.89, 124.85, 124.61, 79.93, 79.82, 79.02, 78.76, 73.75, 73.66, 73.58, 73.56, 72.86, 72.71, 71.35, 69.96, 69,90, 34.24, 29.94, 28.94, 25.94, 14.34.

Exact mass (ESI-MS) for $C_{30}H_{36}O_4$ $[M+H]^+$ found, 461.2701; calcd, 461.2686, $[M+Na]^+$ found, 483.2522; calcd, 483.2506, $[M+K]^+$ found, 499.2265; calcd, 499.2245.

Spectral data are consistent with the literature data.

(2*R*,3*S*,4*R*)-1,3,4-tri-*O*-benzyl-non-2-ol (49)

Palladium on carbon (41 mg) was added to a solution of compound **48** (822 mg, 1.79 mmol) in 0.1 M NH_3 in MeOH (8.22 mL). The suspension was stirred for 16 hours under hydrogen atmosphere followed by filtration through celite and rinsing of the filter cake with MeOH. The product was concentrated under reduced pressure to obtain **49** (821 mg, 99 %) as a colorless oil.



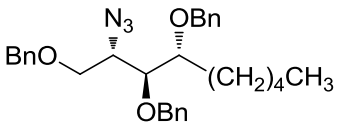
^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.28-7.18 (m, 15H, arom. H), 4.62 (d, *J* = 11.3 Hz, 1H, CH_2-Ph), 4.58 (d, *J* = 11.1 Hz, 1H, CH_2-Ph), 4.49-4.38 (m, 4H, CH_2-Ph), 3.96 (m, 1H, H-2), 3.64-3.58 (m, 1H, H-4), 3.54 (dd, *J* = 3.2 Hz and 4.1 Hz, 1H, H-3), 3.48-3.45 (m, 2H, H-1), 3.09 (d, *J* = 4.6 Hz, 1H, OH), 1.61-1.12 (m, 8H, CH_2), 0.80 (t, *J* = 6.8 Hz, CH_3).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 138.51, 138.37, 138.33, 128.64, 128.62, 128.60, 128.34, 128.18, 128.08, 128.05, 127.92, 127.91, 80.17, 79.27, 73.78, 73.61, 73.05, 71.32, 70.10, 32.14, 31.22, 25.52, 22.83, 14.27.

Exact mass (ESI-MS) for $C_{30}H_{38}O_4$ $[M+H]^+$ found, 463.2861; calcd, 463.2843, $[M+Na]^+$ found, 485.2627; calcd, 485.1662.

(2*S*,3*S*,4*R*)-2-azido-1,3,4-tri-*O*-benzyl-nonane-1,3,4-triol (50)

To a solution of compound **49** (874 mg, 1.89 mmol) in THF (160 mL) were added Ph_3P (1.98 g, 7.56 mmol), DEAD (3.45 mL (2.2 M), 7.56 mmol) and DPPA (1.7 mL, 7.56 mmol) at -20 °C. The mixture was stirred for 6 hours at this temperature followed by stirring at room temperature overnight. The solvent was evaporated under reduced pressure and the residue was directly submitted to column chromatography (hexanes/EtOAc: 9.5/0.5) to give compound **50** (821 mg, 89 %) as a clear oil.



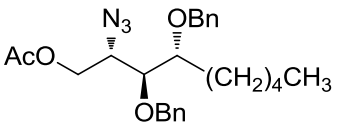
^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.36-7.26 (m, 15H, arom. H), 4.71 (d, *J* = 11.1 Hz, 1H, CH_2-Ph), 4.62 (d, *J* = 11.5 Hz, 1H, CH_2-Ph), 4.58 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.56 (d, *J* = 12.4 Hz, 1H, CH_2-Ph), 4.53 (d, *J* = 11.5 Hz, 1H, CH_2-Ph), 4.52 (d, *J* = 12.1 Hz, 1H, CH_2-Ph), 3.83-3.76 (m, 2H, H-1 and H-2), 3.73-3.67 (m, 2H, H-1 and H-3), 3.62 (ddd, *J* = 3.8 Hz and 7.6 Hz, 1H, H-4), 1.83-1.27 (m, 8H, CH_2), 0.89 (t, *J* = 6.8 Hz, 3H, CH_3).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 128.66, 128.61, 128.25, 128.16, 127.99, 127.94, 127.91, 127.88, 79.47, 79.38, 74.05, 73.59, 72.27, 70.43, 62.32, 32.21, 30.06, 25.24, 22.86, 14.30.

Exact mass (ESI-MS) for $C_{30}H_{37}N_3O_3$ $[M+H]^+$ found, 488.29907; calcd, 488.2908, $[M+Na]^+$ found, 510.2726; calcd, 510.2727, $[M+K]^+$ found, 526.2469; calcd, 526.2467.

(2*S*,3*S*,4*R*)-1-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-nonane-1,3,4-triol (51)

Compound **50** (820 mg, 1.68 mmol) was dissolved in 27 mL Ac_2O/AcOH (2:1 v/v) followed by addition of $ZnCl_2$ (4.6 g, 33.62 mmol). After stirring for 5 hours at room temperature, the reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was washed with water and a saturated Na_2CO_3 solution, dried over Na_2SO_4 and evaporated under reduced pressure to afford the crude product **51** (849 mg) as an orange-brown oil.

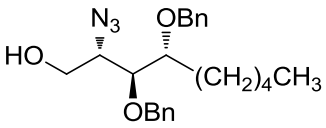


Exact mass (ESI-MS) for $C_{25}H_{33}N_3O_4$ $[M+H]^+$ found, 440.2539; calcd, 440.2544, $[M+Na]^+$ found, 462.2356; calcd, 462.2363, $[M+K]^+$ found, 478.2153; calcd, 478.2103.

(2*S*,3*S*,4*R*)-2-azido-3,4-di-*O*-benzyl-nonane-1,3,4-triol (52)

The crude compound **51** (848 mg) was dissolved in MeOH (45 mL) and the pH was adjusted to 10 by addition of NaOMe. After stirring overnight, the pH of the reaction mixture was neutralized by addition of Amberlite IR 120 (H^+ form) resin. The mixture was filtered and the solvent was evaporated under reduced pressure. Co-evaporation with water and toluene,

followed by column chromatography (hexanes/EtOAc: 8/2) yielded compound **52** (443 mg, 69 % over 2 steps) as a yellow oil.



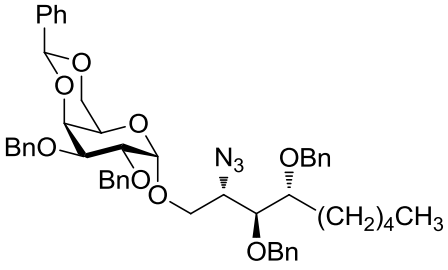
^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.39-7.26 (m, 10H, arom. H), 4.72 (d, *J* = 11.1 Hz, 1H, CH_2-Ph), 4.67 (d, *J* = 9.3 Hz, 1H, CH_2-Ph), 4.63 (d, *J* = 9.6 Hz, 1H, CH_2-Ph), 4.57 (d, *J* = 11.5 Hz, 1H, CH_2-Ph), 3.90 (dd, *J* = 5.3 Hz and 11.8 Hz, 1H, H-1), 3.79 (dd, *J* = 5.1 Hz and 11.6 Hz, 1H, H-1), 3.73-3.61 (m, 3H, H-2, H-3 and H-4), 1.71-1.23 (m, 8H, CH_2), 0.89 (t, *J* = 7.0 Hz, CH_3).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 138.20, 137.87, 128.76, 128.69, 128.34, 128.25, 128.23, 128.08, 80.74, 79.27, 73.87, 72.79, 63.30, 62.49, 32.12, 30.44, 25.40, 22.82, 14.26.

Exact mass (ESI-MS) for $C_{23}H_{31}N_3O_3$ $[M+H]^+$ found, 398.2436; calcd, 398.2438, $[M+Na]^+$ found, 420.2256; calcd, 420.2258, $[M+K]^+$ found, 436.2001; calcd, 436.1997.

(2*S*,3*S*,4*R*)-2-azido-3,4-di-*O*-benzyl-1-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl)-nonane-1,3,4-triol (53)

To a solution of compound **32** (1.03 g, 1.74 mmol) in THF (9 mL) under argon atmosphere was added a solution of **52** (506 mg, 1.33 mmol) in THF (10 mL). The mixture was cooled to -30 °C and TMSOTf (0.04 mL, 0.20 mmol) was added. After stirring for 2 hours, the reaction mixture was neutralized with Et_3N followed by concentration under reduced pressure. Purification by column chromatography (hexanes/EtOAc: 8.5/1.5) resulted in compound **53** (677 mg, 61 %) as a white solid.



^{1}H NMR (300 MHz, $CDCl_3$): δ 7.38-7.35 (m, 2H, arom. H), 7.26-7.06 (m, 23H, arom. H), 5.30 (s, 1H, CH-Ph), 4.81 (d, *J* = 3.3 Hz, 1H, H-1"), 4.70 (d, *J* = 12.0 Hz, 1H, CH_2-Ph), 4.62 (d, *J* = 10.1 Hz, 1H, CH_2-Ph), 4.58 (d, *J* = 12.4 Hz, 1H, CH_2-Ph), 4.56 (d, *J* = 11.3 Hz, 1H, CH_2-Ph), 4.51 (d, *J* = 11.7 Hz, 1H, CH_2-Ph), 4.45 (d, *J* = 11.1 Hz, 1H, CH_2-Ph), 4.43 (d, *J* = 11.5 Hz, 1H, CH_2-Ph), 4.34 (d, *J* = 11.5 Hz, CH_2-Ph), 4.00 (dd, *J* = 0.7 Hz and 3.13 Hz, 1H, H-4"), 3.98-3.94 (m, 1H, H-2"), 3.93-3.84 (m, 3H, H-6", H-3", H-1), 3.73 (dd, *J* = 1.6 Hz and 12.6 Hz, 1H, H-6"), 3.60-3.53 (m, 3H, H-3, H-1, H-2), 3.51-3.45 (m, 1H, H-4), 3.42 (app. d, *J* = 0.7 Hz, 1H, H-5"), 1.51-1.05 (m, 8H, CH_2), 0.73 (t, *J* = 7.0 Hz, 3H, CH_3).

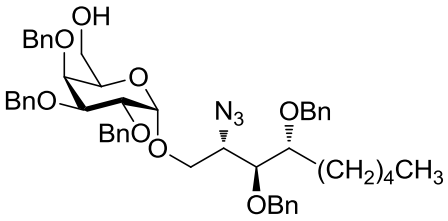
^{13}C NMR (75 MHz, $CDCl_3$): δ 139.01, 139.00, 138.60, 138.26, 138.07, 129.11, 128.63, 128.60, 128.52, 128.47, 128.35, 128.16, 128.05, 128.01, 127.95, 127.92, 127.87, 127.76,

127.71, 126.59, 101.31, 99.39, 79.66, 79.18, 77.68, 76.04, 75.69, 74.91, 74.02, 73.75, 72.31, 72.29, 69.56, 68.70, 63.23, 62.05, 32.18, 30.23, 25.38, 22.86, 14.30.

Exact mass (ESI-MS) for $C_{50}H_{57}N_3O_8$ $[M+H]^+$ found, 828.4247; calcd, 828.4218, $[M+Na]^+$ found, 850.4062; calcd, 850.4038, $[M+K]^+$ found, 66.3802; calcd, 866.3777.

(2*S*,3*S*,4*R*)-2-azido-3,4-di-*O*-benzyl-1-*O*-(2,3,4-tri-*O*-benzyl-6-hydroxy-α-D-galactopyranosyl)-nonane-1,3,4-triol (54)

To a solution of compound **53** (677 mg, 0.82 mmol) in anhydrous CH_2Cl_2 (25 mL) under argon atmosphere was added copper(II) triflate (45 mg, 0.12 mmol) and BH_3.THF (1 M, 4.1 mL). After stirring for 5 hours at room temperature, the reaction was quenched with MeOH. The mixture was extracted with EtOAc and the organic layer was washed with a saturated $NaHCO_3$ solution, water and brine followed by drying over Na_2SO_4 and evaporation until dryness. The residue was submitted to column chromatography (hexanes/EtOAc: 8/2) to afford the desired compound **54** (464 mg, 68 %) as a clear colorless oil.



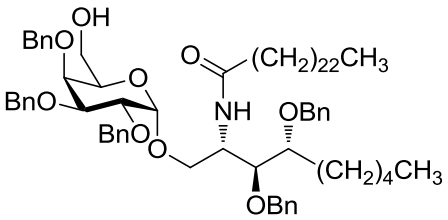
^{1}H NMR (300 MHz, $CDCl_3$): δ 7.41-7.21 (m, 25H, arom. H), 4.97 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.92 (d, *J* = 3.4 Hz, 1H, H-1"), 4.88 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.81 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.75 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.70 (d, *J* = 12.1 Hz, 1H, CH_2-Ph), 4.67 (d, *J* = 10.9 Hz, 1H, CH_2-Ph), 4.63 (d, *J* = 11.5 Hz, 1H, CH_2-Ph), 4.62 (d, *J* = 11.2 Hz, 1H, CH_2-Ph), 4.59 (d, *J* = 11.8 Hz, CH_2-Ph), 4.51 (d, *J* = 11.5 Hz, 1H, CH_2-Ph), 4.08 (dd, *J* = 3.6 Hz and 10.1 Hz, 1H, H-2"), 4.00-3.95 (m, 2H, H-3", H-1), 3.86 (app. d, *J* = 1.6 Hz, 1H, H-4"), 3.73-3.67 (m, 4H, H-5", H-1, H-2, H-3), 3.67-3.61 (m, 2H, H-4, H-6"), 3.42 (dd, *J* = 4.4 Hz and 11.2 Hz, 1H, H-6"), 1.67-1.21 (m, 8H, CH_2), 0.87 (t, *J* = 7.0 Hz, 3H, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ 138.94, 138.87, 138.61, 138.40, 138.28, 128.81, 128.71, 128.64, 128.61, 128.59, 128.52, 128.21, 128.10, 128.07, 127.98, 127.95, 127.85, 127.83, 127.82, 127.76, 98.83, 79.55, 79.44, 79.05, 76.66, 75.40, 74.70, 73.94, 73.67, 73.45, 72.28, 71.09, 68.68, 62.69, 62.23, 60.63, 32.17, 30.22, 25.28, 22.85, 21.29, 14.44, 14.29.

Exact mass (ESI-MS) for $C_{50}H_{57}N_3O_8$ $[M+H]^+$ found, 828.4247; calcd, 828.4218, $[M+Na]^+$ found, 850.4062; calcd, 850.4038, $[M+K]^+$ found, 66.3802; calcd, 866.3777.

(2*S*,3*S*,4*R*)-3,4-di-*O*-benzyl-1-*O*-(2,3,4-tri-*O*-benzyl-6-hydroxy-α-D-galactopyranosyl)-2-tetracosylamino-nonane-1,3,4-triol (55)

To a solution of **54** (463 mg, 0.56 mmol) in THF (5.6 mL) was added dropwise trimethylphosphine (2.8 mL, 2.79 mmol). After stirring for 3.5 hours, a NaOH solution (10.3 mL, 1 M) was added and the mixture was allowed to stir for an additional 3 hours. The reaction mixture was extracted with EtOAc and the organic layer was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The crude amine was dissolved in CH_2Cl_2 (7 mL) and added to a solution of EDC (214 mg, 1.12 mmol) and tetracosanoic acid (308 mg, 0.84 mmol) in CH_2Cl_2 (1.5 mL). This reaction mixture was stirred overnight at room temperature after which it was extracted with CH_2Cl_2. The organic layer was washed with brine and dried over Na_2SO_4. After evaporation of the organic solvent, the residue was purified by column chromatography (hexanes/EtOAc: 7/3) affording compound **55** (509 mg, 79 %) as a yellow oil.



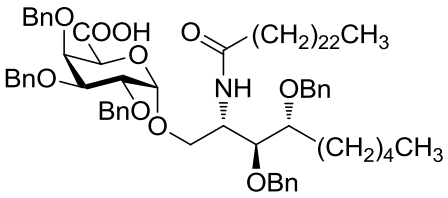
^{1}H NMR (300 MHz, $CDCl_3$): δ 7.39-7.26 (m, 25H, arom. H), 5.85 (d, *J* = 9.0 Hz, 1H, NH), 4.95 (d, *J* = 11.5 Hz, 1H, CH_2-Ph), 4.85 (d, *J* = 4.0 Hz, 1H, H-1”), 4.83 (d, *J* = 12.7 Hz, 1H, CH_2-Ph), 4.80 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.73 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.72 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.64 (d, *J* = 11.7 Hz, 1H, CH_2-Ph), 4.63 (d, *J* = 11.5 Hz, 1H, CH_2-Ph), 4.59 (d, *J* = 12.1 Hz, 1H, CH_2-Ph), 4.55 (d, *J* = 12.1 Hz, 1H, CH_2-Ph), 4.47 (d, *J* = 11.5 Hz, 1H, CH_2-Ph), 4.42-4.33 (m, 1H, H-2), 4.03 (dd, *J* = 3.7 Hz and 9.6 Hz, 1H, H-2”), 3.95 (dd, *J* = 7.8 Hz and 11.5 Hz, 1H, H-1), 3.87-3.82 (m, 3H, H-1, H-3”, H-4”), 3.70-3.63 (m, 3H, H-3, H-5”, H-6”), 3.56-3.51 (m, 1H, H-4), 3.47 (dd, *J* = 7.8 Hz and 13.4 Hz, 1H, H-6”), 1.93-1.87 (m, 2H, $COCH_2$), 1.69-1.24 (m, 50H, CH_2), 0.90-0.84 (m, 6H, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ 173.42, 138.86, 138.80, 138.68, 138.65, 138.48, 128.67, 128.65, 128.18, 128.13, 128.07, 127.97, 127.95, 127.85, 127.68, 100.31, 80.70, 79.49, 79.45, 77.45, 76.82, 75.08, 74.78, 73.78, 73.42, 73.33, 72.10, 71.51, 70.02, 62.69, 60.63, 50.90, 37.05, 32.16, 30.38, 29.94, 29.89, 29.83, 29.66, 29.59, 25.94, 25.68, 22.92, 22.87, 21.28, 14.43, 14.35, 14.30.

Exact mass (ESI-MS) for $C_{74}H_{107}NO_9$ $[M+H]^+$ found, 1154.8068; calcd, 1154,8024.

(2*S*,3*S*,4*R*)-3,4-di-*O*-benzyl-1-*O*-(2,3,4-tri-*O*-benzyl-α-D-galactopyranosyluronate)-2-tetracosylamino-nonane-1,3,4-triol (56)

TEMPO (2.7 mg, 0.02 mmol) and BAIB (70 mg, 0.22 mmol) were added to a mixture of **55** (100 mg, 0.09 mmol) in CH_2Cl_2 (0.6 mL) and H_2O (0.3 mL). The emulsion was vigorously stirred overnight at room temperature and the reaction was quenched with $Na_2S_2O_3$. After extraction of the aqueous layer with EtOAc, the organic layer was washed with a saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4 and evaporated. The residue was submitted to column chromatography (CH_2Cl_2/MeOH: 29/1 with 1% formic acid), affording compound **56** (99 mg, 98 %) as a yellow oil.



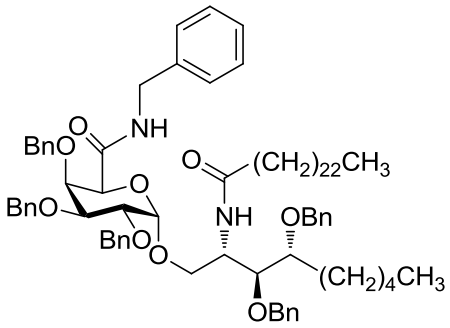
^{1}H NMR (300 MHz, $CDCl_3$): δ 7.34-7.26 (m, 25H, arom. H), 5.91 (d, *J* = 8.0 Hz, 1H, NH), 5.00 (d, *J* = 3.7 Hz, 1H, H-1”), 4.88 (d, *J* = 10.9 Hz, 1H, CH_2-Ph), 4.79 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.75 (d, *J* = 9.4 Hz, 1H, CH_2-Ph), 4.72 (d, *J* = 11.2 Hz, 1H, CH_2-Ph), 4.65 (d, *J* = 10.9 Hz, 1H, CH_2-Ph), 4.63 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.61 (d, *J* = 10.9 Hz, 1H, CH_2-Ph), 4.54 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.49 (d, *J* = 11.5 Hz, 1H, CH_2-Ph), 4.48 (d, *J* = 11.5 Hz, 1H, CH_2-Ph), 4.40-4.33 (m, 3H, H-4”, H-5”, H-2), 4.04 (dd, *J* = 3.3 Hz and 10.1 Hz, 1H, H-2”), 3.93 (dd, *J* = 2.6 Hz and 10.1 Hz, 1H, H-3”), 3.88 (dd, *J* = 5.0 Hz and 10.7 Hz, 1H, H-1), 3.79 (dd, *J* = 6.7 Hz and 10.7 Hz, 1H, H-1), 3.72 (dd, *J* = 3.7 Hz and 5.0 Hz, 1H, H-3), 3.55-3.50 (m, 1H, H-4), 1.94-1.82 (m, 2H, $COCH_2$), 1.66-1.24 (m, 50H, CH2), 0.91-0.84 (m, 6H, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ 173.59, 171.47, 170.89, 138.61, 138.59, 138.48, 138.42, 138.22, 128.66, 128.64, 128.43, 128.35, 128.21, 128.14, 128.06, 127.96, 127.92, 127.65, 99.21, 79.77, 79.35, 78.23, 77.48, 76.31, 76.00, 75.42, 74.02, 73.21, 73.02, 72.11, 69.06, 60.66, 53.66, 50.18, 36.89, 32.16, 30.34, 29.94, 29.90, 29.85, 29.67, 29.60, 25.92, 25.65, 22.93, 22.89, 21.27, 14.42, 14.36, 14.21.

(2*S*,3*S*,4*R*)-3,4-di-*O*-benzyl-1-*O*-(2,3,4-tri-*O*-benzyl-5-benzylamide-6-deoxy-α-D-galactopyranosyl)-2-tetracosylamino-nonane-1,3,4-triol (57)

To a solution of **56** (99 mg, 0.09 mmol) in DMF (0.3 mL) and CH_2Cl_2 (0.7 mL) was added DIPEA (0.04 mL, 0.22 mmol). The solution was stirred for 10 minutes followed by addition of HCTU (90 mg, 0.22 mmol). After stirring for another 30 minutes, benzylamine (0.02 mL, 0.22 mmol) was added and the reaction mixture was allowed to stir overnight. Upon reaction completion, the organic solvent was removed by evaporation and the resulting residue was

submitted to column chromatography (hexanes/EtOAc: 6/4) to yield compound **57** (94 mg, 88 %) as a yellow solid.



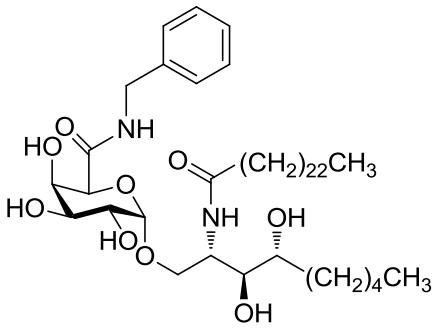
^{1}H NMR (300 MHz, $CDCl_3$): δ 7.39-7.16 (m, 30H, arom. H), 6.80 (t, *J* = 5.8 Hz, 1H, NH), 5.78 (d, *J* = 8.3 Hz, 1H, NH), 4.89 (d, *J* = 3.7 Hz, 1H, H-1''), 4.88 (d, *J* = 10.7 Hz, 1H, CH_2-Ph), 4.81 (d, *J* = 11.6 Hz, 1H, CH_2-Ph), 4.78 (d, *J* = 10.3 Hz, 1H, CH_2-Ph), 4.73 (d, *J* = 11.9 Hz, 1H, CH_2-Ph), 4.72 (d, *J* = 11.6 Hz, 1H, CH_2-Ph), 4.63 (d, *J* = 11.6 Hz, 1H, CH_2-Ph), 4.59 (d, *J* = 11.3 Hz, 1H, CH_2-Ph), 4.55 (d, *J* = 12.0 Hz, 1H, CH_2-Ph), 4.54-4.51 (m, 2H, H-4'', CH_2-Ph), 4.51 (d, *J* = 10.7 Hz, 1H, CH_2-Ph), 4.47 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.45 (d, *J* = 11.6 Hz, 1H, CH_2-Ph), 4.35-4.29 (m, 2H, CH_2-Ph, H-5''), 4.27-4.19 (m, 1H, H-2), 4.04 (dd, *J* = 3.4 Hz and 10.1 Hz, 1H, H-2''), 3.96 (dd, *J* = 2.7 Hz and 10.1 Hz, 1H, H-3''), 3.83-3.77 (m, 2H, H-1, H-3), 3.74 (dd, *J* = 5.6 Hz and 10.3 Hz, 1H, H-1), 3.52-3.48 (m, 1H, H-4), 1.97-1.79 (m, 2H, $COCH_2$), 1.68-1.13 (m, 50H, CH_2), 0.88 (t, *J* = 6.6 Hz, 3H, CH_3), 0.86 (t, *J* = 6.7 Hz, 3H, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ 172.81, 168.48, 138.56, 138.52, 138.50, 138.28, 137.53, 128.64, 128.40, 128.36, 128.17, 128.02, 127.91, 127.84, 127.77, 127.76, 127.72, 127.64, 127.61, 127.52, 127.45, 127.42, 98.81, 79.77, 78.75, 78.36, 77.21, 76.00, 75.80, 75.37, 73.82, 73.22, 72.33, 71.78, 71.69, 67.93, 49.87, 43.14, 36.65, 31.94, 31.92, 29.97, 29.70, 29.65, 29.60, 29.43, 29.38, 29.35, 25.63, 25.56, 22.68, 22.66, 14.11, 14.08.

Exact mass (ESI-MS) for $C_{81}H_{112}N_2O_9$ $[M+H]^+$ found, 1257.8498; calcd, 1257.8441.

(2*S*,3*S*,4*R*)-1-*O*-(5-benzylamide-6-deoxy-α-D-galactopyranosyl)-2-tetracosylamino-nonane-1,3,4-triol (9a)

A solution of **57** (92 mg, 0.07 mmol) in $CHCl_3$ (1 mL) and EtOH (3 mL) was hydrogenated under atmospheric pressure in the presence of palladium black (10 mg). Upon reaction completion, the mixture was diluted with pyridine and filtered through celite. The filter cake was rinsed with $CHCl_3$ and EtOH and the filtrate was evaporated to dryness. After purification by column chromatography (CH_2Cl_2/MeOH: 28/2), final compound **9a** (44 mg, 74 %) was obtained as a white powder.



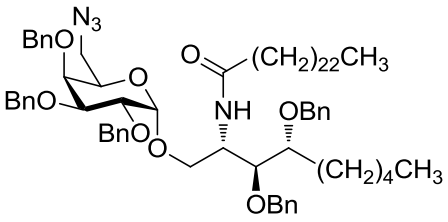
^{1}H NMR (300 MHz, pyridine-d_5): δ 8.71-8.67 (m, 1H, NH), 8.46 (d, *J* = 8.7 Hz, 1H, NH), 7.31-7.19 (m, 5H, arom. H), 6.71 (br. s, 1H, OH), 6.39 (d, *J* = 5.6 Hz, 1H, OH), 6.10 (br. s, 1H, OH), 5.59 (d, *J* = 3.9 Hz, 1H, H-1”), 5.29-5.21 (m, 1H, H-2), 5.03-4.89 (m, 3H, H-4”, H-5”, CH_2-Ph), 4.68 (dd, *J* = 3.5 Hz and 9.8 Hz, 1H, H-2”), 4.63-4.56 (m, 2H, H-1, CH_2-Ph), 4.44 (dd, *J* = 2.6 Hz and 9.5 Hz, 1H, H-3”), 4.31-4.26 (m, 3H, H-1, H-3, H-4), 2.47-2.42 (m, 2H, $COCH_2$), 2.30-1.19 (m, 50H, CH_2), 0.88 (t, *J* = 6.8 Hz, 3H, CH_3), 0.82 (t, *J* = 7.1 Hz, 3H, CH_3).

^{13}C NMR (75 MHz, pyridine-d_5): δ 173.40, 170.58, 140.50, 135.14, 128.99, 128.04, 127.35, 124.49, 123.11, 101.74, 76.92, 74.10, 72.73, 71.61, 71.49, 70.11, 69.17, 51.26, 43.20, 37.06, 34.62, 32.77, 32.45, 30.36, 30.33, 30.24, 30.18, 30.12, 30.08, 29.94, 26.71, 26.38, 23.34, 23.27, 14.61, 14.59.

Exact mass (ESI-MS) for $C_{46}H_{82}N_2O_9$ $[M+H]^+$ found, 807.6121; calcd, 807.6093.

(2*S*,3*S*,4*R*)-3,4-di-*O*-benzyl-1-*O*-(6-azido-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-galactopyranosyl)-2-tetracosylamino-nonane-1,3,4-triol (58)

Compound **55** (78 mg, 0.07 mmol) was dissolved in THF (6 mL) and the solution was cooled to -20 °C. PPh_3 (71 mg, 0.27 mmol), DEAD (0.12 mL, 0.27 mmol) and DPPA (0.06 mL, 0.27 mmol) were added and the mixture was stirred for 6 hours, after which it was allowed to warm up to room temperature. After stirring overnight, the reaction mixture was evaporated and the residue was submitted to column chromatography (hexanes/EtOAc: 8.5/1.5) resulting in compound **58** (78 mg, 98 %) as a clear yellow oil.



^{1}H NMR (300 MHz, $CDCl_3$): δ 7.42-7.17 (m, 25H, arom. H), 5.91 (d, *J* = 8.8 Hz, 1H, NH), 4.99 (d, *J* = 11.6 Hz, 1H, CH_2-Ph), 4.86 (d, *J* = 3.8 Hz, 1H, H-1”), 4.84 (d, *J* = 11.6 Hz, 1H, CH_2-Ph), 4.82 (d, *J* = 11.6 Hz, 1H, CH_2-Ph), 4.78 (d, *J* = 11.6 Hz, 1H, CH_2-Ph), 4.75 (d, *J* = 11.3 Hz, 1H, CH_2-Ph), 4.65 (d, *J* = 11.6 Hz, 1H, CH_2-Ph), 4.60 (d, *J* = 11.9 Hz, 1H, CH_2-Ph), 4.59 (d, *J* = 11.6 Hz, 1H, CH_2-Ph), 4.52 (d, *J* = 11.6 Hz, 1H, CH_2-Ph), 4.50 (d, *J* = 11.6 Hz, 1H, CH_2-Ph), 4.32-4.25 (m, 1H, H-2), 4.03 (dd, *J* = 3.4 Hz and 10.0 Hz, 1H, H-2”), 3.92-3.84 (m, 3H, H-3”, H-1), 3.82-3.76 (m, 3H, H-4”, H-5”, H-3), 3.55 (dd, *J* = 3.8 Hz and 7.2 Hz, 1H, H-4), 3.48 (dd, *J* = 8.0 Hz and 12.4 Hz, 1H,H-6”), 2.32 (dd, *J* = 5.0 Hz and 12.5 Hz, 1H, H-

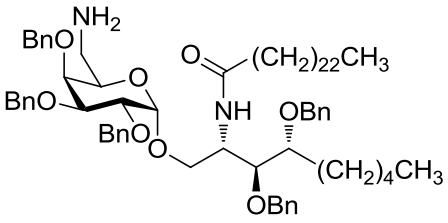
6”), 2.00-1.89 (m, 2H, $COCH_2$), 1.70-1.24 (m, 50H, CH_2), 0.89 (t, J = 6.6 Hz, 3H, CH_3), 0.87 (t, J = 6.9 Hz, 3H, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ 173.10, 150.14, 150.03, 138.89, 138.78, 138.73, 138.58, 138.32, 130.30, 130.29, 128.69, 128.68, 128.64, 128.61, 128.58, 128.17, 128.15, 128.07, 127.99, 127.92, 127.85, 127.79, 127.71, 126.37, 126.35, 120.50, 120.43, 99.31, 80.10, 79.54, 79.04, 77.69, 76.72, 75.06, 74.88, 73.72, 73.52, 73.49, 72.07, 70.27, 68.98, 51.61, 50.38, 36.99, 32.19, 32.16, 30.23, 29.95, 29.90, 29.83, 29.69, 29.64, 29.60, 25.97, 25.81, 22.93, 22.90, 14.36, 14.32.

Exact mass (ESI-MS) for $C_{74}H_{106}N_4O_8$ $[M+H]^+$ found, 1179.8011; calcd, 1179.8083.

(2*S*,3*S*,4*R*)-3,4-di-*O*-benzyl-1-*O*-(6-amino-2,3,4-tri-*O*-benzyl-α-D-galactopyranosyl)-2-tetracosylamino-nonane-1,3,4-triol (59)

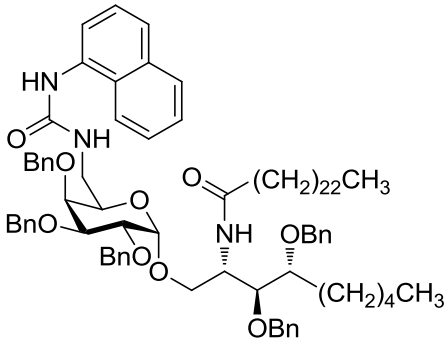
To a solution of **58** (77 mg, 0.07 mmol) in THF (1 mL) was added PMe_3 (0.33 mL, 0.33 mmol) dropwise. After stirring for 4 hours, NaOH (2 mL, 1 M) was added, followed by an additional stirring for 2 hours. Upon reaction completion, the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated to dryness to give the crude amine **59** (85 mg) as a yellow oil.



Exact mass (ESI-MS) for $C_{74}H_{108}N_2O_8$ $[M+H]^+$ found, 1153.8164; calcd, 1153,8184.

(2*S*,3*S*,4*R*)-3,4-di-*O*-benzyl-1-*O*-(2,3,4-tri-*O*-benzyl-6-deoxy-6-(3-(1-naphthyl)ureido-α-D-galactopyranosyl)-2-tetracosylamino-nonane-1,3,4-triol (60)

To a solution of the crude amine **59** in DMF (1 mL) at 0 °C was added naphthylisocyanate (0.01 mL, 0.08 mmol). The reaction mixture was allowed to stir at room temperature overnight followed by evaporation to dryness. Purification by column chromatography (hexanes/EtOAc: 7/3) resulted in compound **60** (52 mg, 60 %) as a yellow oil.

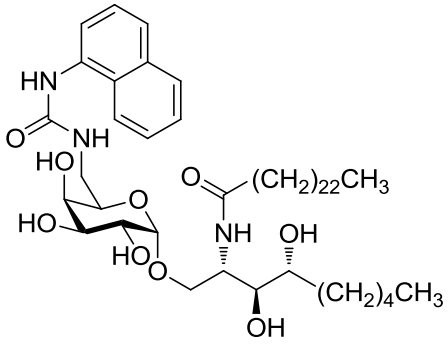


^{1}H NMR (300 MHz, pyridine-d_5): δ 9.42 (s, 1H, NH), 9.04 (d, *J* = 8.9 Hz, 1H, NH), 8.64 (d, *J* = 6.6 Hz, 1H, arom. H), 8.51 (d, *J* = 8.3 Hz, 1H, arom. H), 8.29 (d, *J* = 8.1 Hz, 1H, 1H, arom. H), 7.94-7.91 (m, 1H, arom. H), 7.72-7.27 (m, 28H, arom. H), 7.01 (dd, *J* = 3.6 Hz and 6.3 Hz, 1H, NH), 5.34 (d, *J* = 3.3 Hz, 1H, H-1”), 5.15 (d, *J* = 11.3 Hz, 2H, CH_2-Ph), 4.97-4.85 (m, 3H, CH_2-Ph, H-2), 4.81-4.71 (m, 5H, CH_2-Ph), 4.53 (d, *J* = 11.6 Hz, 1H, CH_2-Ph), 4.47-4.42 (m, 2H, H-3, H-5”), 4.41-4.37 (m, 2H, H-2”, H-1), 4.31 (dd, *J* = 2.5 Hz and 10.1 Hz, 1H, H-3”), 4.24 (d, *J* = 5.3 Hz and 10.6 Hz, 1H, H-1), 4.19 (app. d, *J* = 1.7 Hz, 1H, H-4”), 4.16-4.12 (m, 1H, H-6”), 3.97-3.91 (m, 2H, H-4, H-6”), 2.69-2.54 (m, 2H, $COCH_2$), 2.06-1.09 (m, 50H, CH_2), 0.89 (t, *J* = 6.5 Hz, 3H, CH_3), 0.79 (t, *J* = 6.8 Hz, 3H, CH_3).

^{13}C NMR (75 MHz, pyridine-d_5): δ 174.14, 157.54, 151.05, 140.04, 139.29, 139.80, 139.74, 136.56, 135.23, 129.25, 129.13, 129.05, 128.97, 128.79, 128.32, 128.22, 128.08, 128.01, 126.95, 126.79, 126.56, 126.44, 126.37, 126.25, 124.74, 122.76, 122.64, 120.48, 119.50, 99.81, 81.31, 80.24, 79.89, 77.55, 77.21, 75.58, 74.58, 73.77, 73.05, 72.30, 71.58, 51.54, 37.12, 32.56, 32.46, 30.45, 30.37, 30.26, 30.17, 29.94, 26.80, 26.59, 23.28, 14.59.

(2*S*,3*S*,4*R*)-1-*O*-(6-deoxy-6-(3-(1-naphthyl)ureido-α-D-galactopyranosyl)-2-tetracosylamino-nonane-1,3,4-triol (9b)

A solution of **60** (51 mg, 0.04 mmol) in $CHCl_3$ (0.4 mL) and EtOH (1.2 mL) was hydrogenated under atmospheric pressure in the presence of palladium black (5 mg). Upon reaction completion, the mixture was diluted with pyridine and filtered through celite. The filter cake was rinsed with $CHCl_3$ and EtOH and the filtrate was evaporated to dryness. After purification by column chromatography (CH_2Cl_2/MeOH: 28/2), final compound **9b** (9 mg, 26 %) was obtained as a white powder.



^{1}H NMR (300 MHz, pyridine-d_5): δ 9.52 (s, 1H, NH), 8.71 (d, *J* = 8.7 Hz, 1H, NH), 8.50 (d, *J* = 7.6 Hz, 1H, arom. H), 8.46 (dd, *J* = 3.4 Hz and 6.2 Hz, 1H, arom. H), 7.90 (dd, *J* = 3.3 Hz and 6.1 Hz, 1H, arom. H), 7.65 (d, *J* = 8.2 Hz, 1H, arom. H), 7.54 (d, *J* = 7.8 Hz, 1H, arom. H), 7.50-7.45 (m, 2H, arom. H), 7.26-7.15 (m, 1H, NH), 5.55 (d, *J* = 3.9 Hz, 1H, H-1”), 5.36-5.28 (m, 1H, H-2),

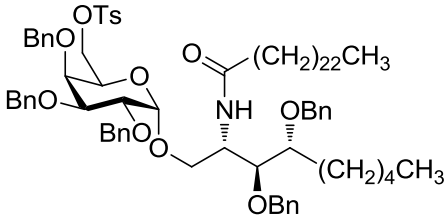
4.67 (dd, *J* = 4.7 Hz and 10.9 Hz, 1H, H-2”), 4.61 (dd, *J* = 3.7 Hz and 9.5 Hz, 1H, H-1), 4.53 (dd, *J* = 5.2 Hz and 7.6 Hz, 1H, H-5”), 4.43-4.35 (m, 3H, H-1, H-3, H-3”), 4.33-4.20 (m, 3H, H-4, H-4”, H-6”), 4.15-4.07 (m, 1H, H-6”), 2.49-2.45 (m, 2H, $COCH_2$), 2.25-1.19 (m, 50H, CH_2), 0.88 (t, *J* = 6.7 Hz, 3H, CH_3), 0.79 (t, *J* = 7.0 Hz, 3H, CH_3).

^{13}C NMR (75 MHz, pyridine-d_5): δ 172.57, 156.45, 147.97, 135.03, 133.68, 127.66, 126.68, 125.34, 124.91, 124.70, 122.98, 121.61, 121.33, 118.26, 100.68, 75.57, 71.50, 70.12, 70.05, 68.93, 67.99, 50.61, 41.02, 35.68, 33.31, 31.24, 30.94, 28.86, 28.82, 28.80, 28.73, 28.66, 28.57, 28.43, 25.26, 24.90, 21.81, 21.75, 13.10, 13.06.

Exact mass (ESI-MS) for $C_{50}H_{85}N_3O_9$ $[M+H]^+$ found, 872.6391; calcd, 872,6364.

(2*S*,3*S*,4*R*)-3,4-di-*O*-benzyl-1-*O*-(2,3,4-tri-*O*-benzyl-6-deoxy-6-*O*-tosyl-α-D-galactopyranosyl)-2-tetracosylamino-nonane-1,3,4-triol (61)

To a solution of **55** (407 mg, 0.35 mmol) in pyridine at 0 °C was added tosyl chloride (82 mg, 0.43 mmol). The reaction mixture was allowed to stir overnight at room temperature. Upon reaction completion, the solvent was removed by evaporation. Purification by column chromatography (hexanes/EtOAc: 8.5/1.5) afforded **61** (452 mg, 98 %) as a clear colorless oil.



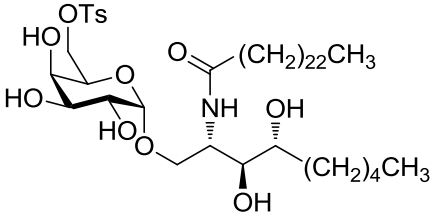
^{1}H NMR (300 MHz, $CDCl_3$): δ 7.69 (d, *J* = 8.1 Hz, 2H, arom. H), 7.36-7.20 (m, 27H, arom. H), 5.85 (d, *J* = 8.5 Hz, 1H, NH), 4.91 (d, *J* = 11.3 Hz, 1H, CH_2-Ph), 4.81 (d, *J* = 11.3 Hz, 1H, CH_2-Ph), 4.79 (d, *J* = 3.2 Hz, 1H, H-1”), 4.76 (d, *J* = 12.1 Hz, 1H, CH_2-Ph), 4.75 (d, *J* = 11.5 Hz, 1H, CH_2-Ph), 4.74 (d, *J* = 11.7 Hz, 1H, CH_2-Ph), 4.62 (d, *J* = 11.7 Hz, 1H, CH_2-Ph), 4.59 (d, *J* = 11.9 Hz, 1H, CH_2-Ph), 4.50 (d, *J* = 11.5 Hz, 1H, CH_2-Ph), 4.49 (d, *J* = 11.5 Hz, 2H, CH_2-Ph), 4.28-4.19 (m, 1H, H-2), 4.02-3.83 (m, 6H, H-2”, H-3”, H-4”, H-5”, H-6”), 3.82-3.77 (m, 2H, H-1, H-3), 3.71 (dd, *J* = 4.6 Hz and 10.5 Hz, 1H, H-1), 3.56-3.51 (m, 1H, H-4), 2.41 (s, 3H, CH_3), 2.00-1.86 (m, 2H, $COCH_2$), 1.69-1.18 (m, 50H, CH_2), 0.88 (t, *J* = 5.8 Hz, 3H, CH_3), 0.86 (t, *J* = 6.1 Hz, 3H, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ 172.84, 144.96, 138.70, 138.61, 138.38, 138.28, 138.03, 132.49, 129.87, 128.44, 128.36, 128.32, 128.26, 127.96, 127.92, 127.78, 127.73, 127.67, 127.57, 127.51, 127.41, 104.76, 99.07, 79.90, 78.85, 78.54, 77.21, 76.36, 74.63, 74.13, 73.51, 73.36, 73.12, 71.75, 68.76, 78.65, 49.94, 36.67, 31.97, 31.92, 29.86, 29.70, 29.65, 29.61, 29.46, 29.39, 29.35, 25.67, 25.56, 22.68, 21.63, 14.11, 14.10.

Exact mass (ESI-MS) for $C_{81}H_{113}NO_{11}S$ $[M+H]^+$ found, 1308.8115; calcd, 1308.8113; $[M+Na]^+$ found, 1330.8131; calcd, 1330.8188.

(2*S*,3*S*,4*R*)-1-*O*-(6-deoxy-6-*O*-tosyl-α-D-galactopyranosyl)-2-tetracosylamino-nonane-1,3,4-triol (62)

A solution of **61** (451 mg, 0.34 mmol) in $CHCl_3$ (1.6 mL) and EtOH (4.8 mL) was hydrogenated under atmospheric pressure in the presence of palladium black (45 mg). Upon reaction completion, the mixture was diluted with pyridine and filtered through celite. The filter cake was rinsed with $CHCl_3$ and EtOH and the filtrate was evaporated to dryness. After purification by column chromatography (CH_2Cl_2/MeOH: 28/2), compound **62** (234 mg, 79 %) was obtained as a white powder.



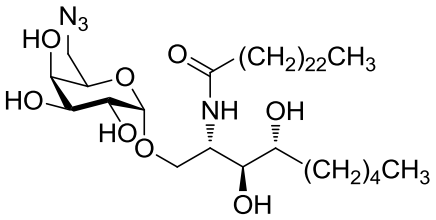
^{1}H NMR (300 MHz, CD_3OD): δ 7.79 (d, *J* = 8.4 Hz, 2H, arom. H), 7.43 (d, *J* = 8.1 Hz, 2H, arom. H), 4.77 (app. s, 1H, H-1"), 4.20-4.14 (m, 3H, H-1, H-5"), 4.01 (t, *J* = 6.1 Hz, 1H, H-2), 3.79-3.71 (m, 4H, H-2", H-3", H-6", H-3), 3.62-3.57 (m, 2H, H-4, H6"), 3.53 (dd, *J* = 2.3 Hz and 6.0 Hz, 1H, H-4"), 2.45 (s, 3H, CH_3), 2.22 (ddd, *J* = 2.6 Hz and 7.5 Hz, 2H, $COCH_2$), 1.64-1.21 (m, 50H, CH_2), 0.91 (t, *J* = 6.7 Hz, 3H, CH_3), 0.89 (t, *J* = 6.6 Hz, 3H, CH_3).

^{13}C NMR (75 MHz, CD_3OD): δ 174.60, 145.32, 133.18, 129.87, 127.95, 99.62, 74.11, 71.75, 69.89, 69.81, 69.48, 68.90, 68.67, 67.06, 50.36, 36.08, 31.95, 31.89, 31.68, 29.61, 29.57, 29.49, 29.37, 29.29, 29.24, 25.92, 25.53, 22.61, 22.55, 20.44, 13.31, 13.26.

Exact mass (ESI-MS) for $C_{46}H_{83}NO_{11}S$ $[M+H]^+$ found, 858.5725; calcd, 858.5765; $[M+Na]^+$ found, 880.5571; calcd, 880.5585.

(2*S*,3*S*,4*R*)-1-*O*-(6-azido-6-deoxy-α-D-galactopyranosyl)-2-tetracosylamino-nonane-1,3,4-triol (64)

To a solution of **62** (233 mg, 0.27 mmol) in DMF (1 mL) was added NaN_3. The reaction mixture was heated to 60 °C and was stirred for 2 days. The resulting white suspension was filtered, followed by evaporation of the solvent under reduced pressure. The residue was purified by column chromatography (CH_2Cl_2/MeOH: 96/4), giving **64** (75 g, 38 %) as a white solid.



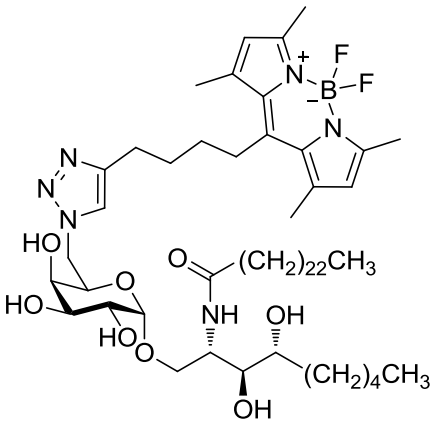
^{1}H NMR (300 MHz, CD_3OD): δ 4.89 (d, *J* = 3.1 Hz, 1H, H-1”), 4.23-4.17 (m, 1H, H-2), 3.96 (dd, *J* = 4.8 Hz and 8.8 Hz, 1H, H-5”), 3.90 (dd, *J* = 4.4 Hz and 10.5 Hz, 1H, H-1), 3.81-3.76 (m, 3H, H-2”, H-3”, H-4”), 3.70 (dd, *J* = 4.2 Hz and 10.3 Hz, 1H, H-1), 3.64-3.52 (m, 3H, H-3, H-4, H-6”), 3.26 (dd, *J* = 4.4 Hz and 12.7 Hz, 1H, H-6”), 2.22 (app. t, *J* = 7.2 Hz, 2H, $COCH_2$), 1.64-1.22 (m, 50H, CH_2), 0.91 (t, *J* = 6.7 Hz, 3H, CH_3), 0.90 (t, *J* = 6.7 Hz, 3H, CH_3).

^{13}C NMR (75 MHz, CD_3OD): δ 174.59, 99.72, 74.08, 71.77, 70.42, 70.16, 70.03, 68.78, 67.04, 51.41, 50.44, 36.06, 31.93, 31.90, 31.67, 29.61, 29.57, 29.48, 29.38, 29.29, 29.22, 25.92, 25.53, 22.60, 22.56, 13.30, 13.26.

Exact mass (ESI-MS) for $C_{39}H_{76}N_4O_8$ $[M+H]^+$ found, 729.5774; calcd, 729.5741; $[M+Na]^+$ found, 751.5574; calcd, 751.5561.

(2*S*,3*S*,4*R*)-1-*O*-(6-deoxy-6-(4-BODIPY-triazol-1-yl))-α-D-galactopyranosyl)-2-tetracosylamino-nonane-1,3,4-triol (10)

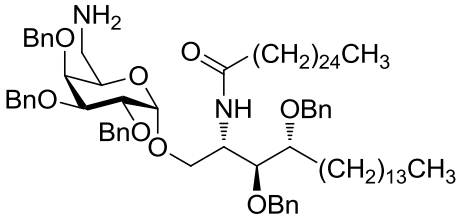
To a solution of **64** (22 mg, 0.03 mmol) in DMF (0.8 mL) and H_2O (0.2 mL) was added TEA (50 µL), CuI (4 mg, 0.02 mmol), TBTA (2 mg, 0.004 mmol) and the alkyne (17 mg, 0.06 mmol). After stirring for 3 hours, the solvent was removed by evaporation. The resulting residue was purified by preparative TLC (CH_2Cl_2/MeOH: 27/3) to furnish final compound **10** (29 mg, 91 %) as an orange powder.



^{1}H NMR (300 MHz, CD_3OD): δ 7.75 (s, 1H, arom. H), 6.12 (s, 2H, arom. H), 4.81 (d, *J* = 3.4 Hz, 1H, H-1”), 4.62-4.55 (m, 2H, H-6”), 4.19 (app. t, *J* = 6.3 Hz, 1H, H-5”), 4.10 (dd, *J* = 4.6 Hz and 10.6 Hz, 1H, H-2), 3.82 (dd, *J* = 1.3 Hz and 2.7 Hz, 1H, H-4”), 3.77 (dd, *J* = 3.4 Hz and 8.5 Hz, 1H, H-2”), 3.74 (dd, *J* = 3.2 Hz and 9.9 Hz, 1H, H-3”), 3.54-3.45 (m, 3H, H-1, H-3, H-4), 3.38 (dd, *J* = 4.5 Hz and 10.3 Hz, 1H, H-1), 3.07-3.02 (m, 2H, CH_2), 2.78 (t, *J* = 7.4 Hz, 2H, CH_2), 2.44 (s, 6H, CH_3), 2.42 (s, 6H, CH_3), 2.15 (t, *J* = 7.5 Hz, 2H, CH_2), 1.90 (t, *J* = 7.4 Hz, 2H, CH_2), 1.75-1.22 (m, 52H, CH_2), 0.91-0.87 (m, 6H, CH_3).

Exact mass (ESI-MS) for $C_{58}H_{99}BF_2N_6O_8$ $[M+H]^+$ found, 1057.7662; calcd, 1057.7664; $[M+Na]^+$ found, 1079.7499; calcd, 1079.7483.

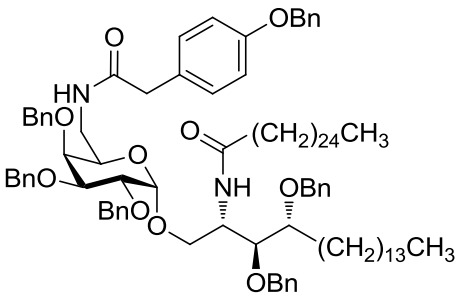
(2*S*,3*S*,4*R*)-3,4-di-*O*-benzyl-1-*O*-(6-amino-2,3,4-tri-*O*-benzyl-6-deoxy-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (65).



To a solution of **44** (126 mg, 0.09 mmol) in THF (1.4 mL) was added trimethylphosphine (0.47 mL, 1M) dropwise. After stirring for 3 hours, NaOH (2.8 mL, 1M) was added and the reaction mixture was allowed to stir for an additional 2.5 hours. The solution was extracted with EtOAc, followed by washing of the organic layer with brine, drying over Na_2SO_4 and evaporation of the solvent to afford amine **65** as a yellow oil.

(2*S*,3*S*,4*R*)-3,4-di-*O*-benzyl-1-*O*-(2,3,4-tri-*O*-benzyl-6-(4-*O*-benzyl)-benzylamido-6-deoxy-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (66).

To a solution of (benzyloxy)phenylacetic acid (60 mg, 0.24 mmol) in DMF (0.3 mL) and CH_2Cl_2 (0.7 mL) was added DIPEA (0.04 mL, 0.24 mmol). After stirring for 10 minutes, HCTU (99 mg, 0.24 mmol) was added and the solution was allowed to stir for an additional 30 minutes. The mixture was then added to a solution of the crude amine **65** in DMF (0.3 mL) and CH_2Cl_2 (0.7 mL). Upon stirring overnight, the solvent was removed by evaporation and the resulting residue was submitted to column chromatography (hexanes/EtOAc: 7/3) giving **66** (134 mg, 93%) as a white solid.



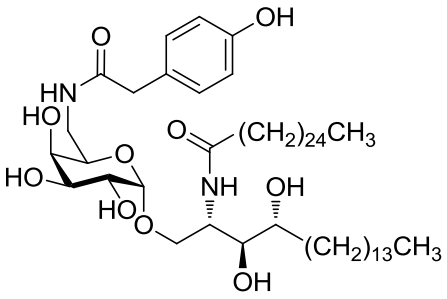
^{1}H NMR (300 MHz, $CDCl_3$): δ 7.41-7.19 (m, 30H, arom. H), 7.08-7.04 (m, 2H, arom. H), 6.90-6.86 (m, 2H, arom. H), 5.96 (d, *J* = 8.8 Hz, 1H, NH), 5.67 (dd, *J* = 4.2 Hz and 7.4 Hz, 1H, NH), 5.01 (d, *J* = 16.0 Hz, 2H, $COCH_2$-Ph), 4.91 (d, *J* = 11.4 Hz, 1H, CH_2-Ph), 4.80 (d, *J* = 11.6 Hz, 1H, CH_2-Ph), 4.79 (d, *J* = 3.7 Hz, 1H, H-1”), 4.77 (d, *J* = 12.9 Hz, 1H, CH_2-Ph), 4.76 (d, *J* = 10.8 Hz, 1H, CH_2-Ph), 4.72 (d, *J* = 11.7 Hz, 1H, CH_2-Ph), 4.62 (d, *J* = 11.4 Hz, 2H, CH_2-Ph), 4.58 (d, *J* = 11.2 Hz, 1H, CH_2-Ph), 4.57 (d, *J* = 11.4 Hz, 1H, CH_2-Ph), 4.48 (d, *J* = 11.6 Hz, 1H, CH_2-Ph), 4.46 (d, *J* = 11.7 Hz, 1H, CH_2-Ph), 4.25-4.17 (m, 1H, H-2), 3.99 (dd, *J* = 3.7 Hz and 10.1 Hz, 1H, H-2”), 3.86-3.74 (m, 4H, H-3”, H-4”, H-1, H-3), 3.69 (app. t, *J* = 6.2 Hz, 1H, H-5”), 3.59 (dd, *J* = 4.3 Hz and 10.3 Hz, 1H, H-1), 3.53-3.32 (m, 2H, H-4, H-6”), 3.10 (ddd, *J* = 4.1 Hz, 7.5 Hz and 13.6 Hz, 1H, H-6”), 2.04-1.87 (m, 2H, $COCH_2$), 1.68-1.08 (m, 72H, CH_2), 0.88 (t, *J* = 6.7 Hz, 6H, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ 173.11, 171.75, 158.21, 138.87, 138.84, 138.69, 138.56, 138.50, 137.13, 130.63, 130.60, 128.81, 128.77, 128.66, 128.62, 128.58, 128.16, 128.10, 128.06, 127.98, 127.95, 127.89, 127.83, 127.68, 127.65, 127.40, 115.40, 115.23, 99.32, 80.08, 79.40, 79.17, 77.44, 76.59, 75.07, 74.78, 73.80, 73.75, 73.24, 71.96, 70.20, 69.26, 68.64, 50.49, 42.86, 36.92, 32.16, 30.09, 30.04, 29.97, 29.94, 29.89, 29.74, 29.66, 29.61, 29.59, 26.25, 25.97, 22.92, 14.35.

Exact mass (ESI-MS) for $C_{100}H_{142}N_2O_{10}$ $[M+H]^+$ found, 1532.0707; calcd, 1532.0743; $[M+Na]^+$ found, 1554.0514; calcd, 1554.0562; $[M+K]^+$ found, 1570.0314; calcd, 1570.0302.

(2*S*,3*S*,4*R*)-1-*O*-(6-benzylamido-6-deoxy-α-D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (11).

A solution of **11** (120 mg, 0.0.8 mmol) in $CHCl_3$ (1 mL) and EtOH (3 mL) was hydrogenated under atmospheric pressure in the presence of palladium black (12 mg). Upon reaction completion, the mixture was diluted with pyridine and filtered through celite. The filter cake was rinsed with $CHCl_3$ and EtOH and the filtrate was evaporated to dryness. After purification by column chromatography (CH_2Cl_2/MeOH: 27/3), compound **11** (39 mg, 48 %) was obtained as a white powder.



^{1}H NMR (300 MHz, pyridine-d_5): δ 8.68 (t, *J* = 5.7 Hz, 1H, NH), 8.50 (d, *J* = 8.7 Hz, 1H, NH), 7.50 (d, *J* = 8.5 Hz, 2H, arom. H), 7.18 (d, *J* = 8.5 Hz, 2H, arom. H), 5.50 (d, *J* = 3.9 Hz, 1H, H-1”), 5.28-5.26 (m, 1H, H-2), 4.61-4.56 (m, 2H, H-2”, H-1), 4.48-4.44 (m, 1H, H-5”), 4.36-4.26 (m, 5H, H-1, H-3, H-4, H-3”, H-4”), 3.95-3.89 (m, 1H, H-6”), 3.87-3.81 (m, 3H, H-6”, CH_2-Ph), 2.55-2.41 (m, 2H, $COCH_2$), 1.94-1.14 (m, 72H, CH_2), 0.88 (t, *J* = 6.7 Hz, 6H, CH_3).

^{13}C NMR (75 MHz, pyridine-d_5): δ 173.36, 172.48, 157.89, 131.09, 127.34, 116.51, 101.42, 76.92, 72.60, 71.25, 70.70, 70.10, 68.60, 51.32, 43.17, 41.49, 36.91, 32.22, 30.51, 30.27, 30.14, 30.11, 30.03, 30.01, 29.98, 29.93, 29.88, 29.72, 29.70, 26.61, 26.53, 23.03, 14.37.

Exact mass (ESI-MS) for $C_{58}H_{106}N_2O_{10}$ $[M+H]^+$ found, 991.7939; calcd, 991.7926; $[M+Na]^+$ found, 1013.7784; calcd, 1013.7745.

4.7 References

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[12] http://www.pymol.org

CHAPTER 5

SYNTHESIS OF A POTENTIAL iNKT CELL ANTAGONIST

5 Synthesis of a potential iNKT cell antagonist

5.1 Introduction

There is emerging evidence that iNKT cells play an important pathogenic role in allergic diseases, such as contact hypersensitivity[1] and asthma.[2,3] Particularly in the latter, their function has been extensively studied over the past few years. This has provided new insights into the disease mechanism, opening the door to novel therapeutic strategies.

The most elementary explanation of asthma, characterized by airway inflammation and airway hyperreactivity (AHR), involves an interaction between mast cells, eosinophils and $CD4^+$ Th2 cells.[2,4] The first phase, allergen sensitization, results from an adaptive immune response to common aeroallergens, leading to the development of allergen-specific $CD4^+$ Th2 cells and IgE. Subsequent challenge with allergen activates these $CD4^+$ Th2 cells, in turn enhancing the growth, differentiation and recruitment of eosinophils, basophils, B cells and mast cells via the secretion of IL-4, IL-5, IL-9 and IL-13 (Figure 5.1). B cell-derived IgE stimulates mast cells to rapidly release chemical mediators such as leukotrienes and histamine, increasing the airway resistance. The produced IL-4 and IL-13 on the other hand give rise to AHR.

So clearly, MHC class II-restricted $CD4^+$ Th2 cells play an important role in the pathogenesis of bronchial asthma. However, a number of clinical observations cannot be explained by this paradigm, suggesting that several additional processes and pathways must be involved.[2] Indeed, different murine models of asthma indicate the requirement of iNKT cells producing IL-4 and IL-13 in the development of AHR.[5,6] Moreover, respiratory administration of α-GalCer has been demonstrated to rapidly induce AHR and airway inflammation in naive mice, even in MHC class II-deficient types.[7] Although in human asthma more functional studies are required, the currently available findings also support the clinical relevance of iNKT cells. For example, an increased number of pulmonary $CD4^+$ iNKT cells is observed in many but not all asthmatic patients.[2,8] These iNKT cells might be activated by substances naturally occurring in our environment such as pathogen- or pollen-derived glycolipids and ozone, thereby inducing AHR either directly or by licensing Th2 cells (Figure 5.1).[3,4,5,7] In

addition, it has been shown that pro-Th2 cytokines like IL-25, IL-33 and TSLP (thymic stromal lymphopoietin) act on iNKT cells to exacerbate the allergic symptoms.

In accordance with these findings, the CD1d-dependent iNKT cell antagonist DPPE-PEG (di-palmitoyl-phosphatidyl-ethanolamine covalently attached to polyethyleneglycol) completely abrogates the development of AHR in OVA- and α-GalCer-induced murine models.[9] Hence, blockade of iNKT cells with an iNKT cell-specific antagonist might be highly effective in the treatment of asthma.

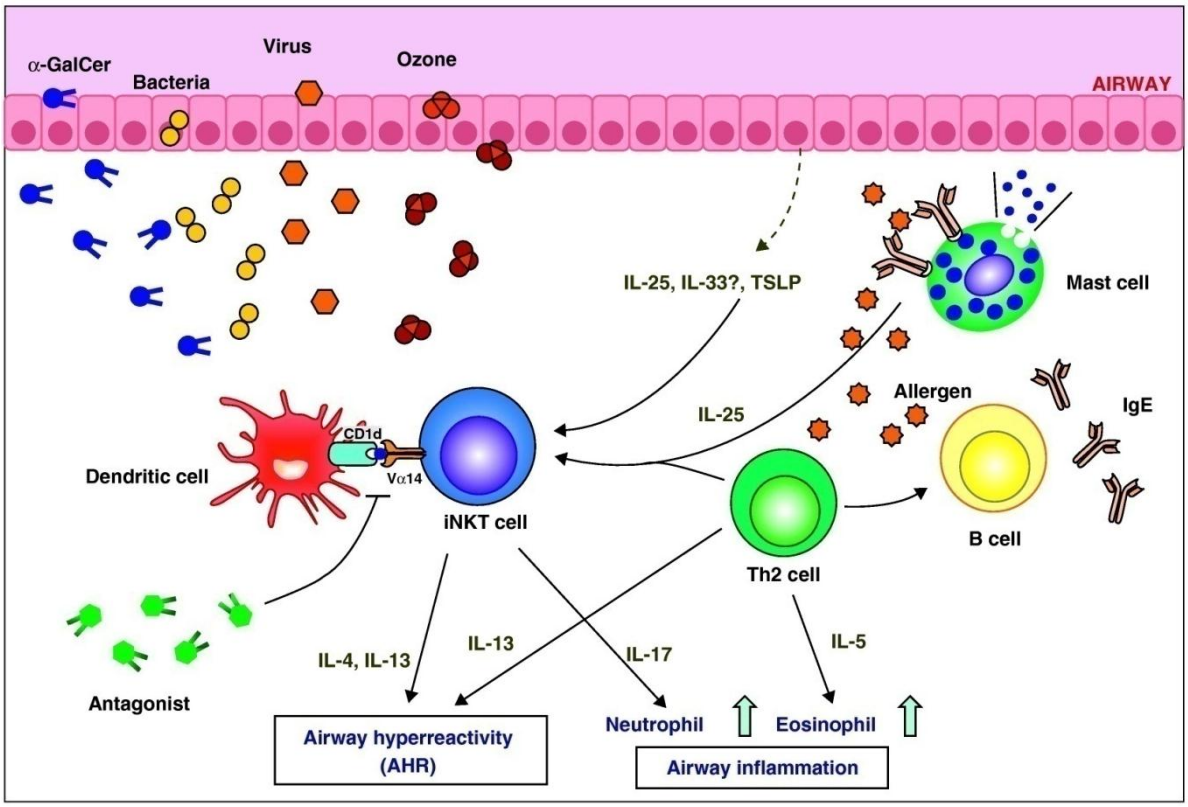


Figure 5.1 Activated $CD4^+$ Th2 cells and iNKT cells in the development of AHR and airway inflammation.

Apart from DPPE-PEG, only two other CD1d-dependent antagonists have been described thus far. In 2009, the group of Vasella synthesized a thioamide-substituted 1,4-triazole derivative of 7-oxaceramide (Figure 5.2).[10] This compound was able to compete with α-GalCer for binding to plate-bound human CD1d, thereby reducing the α-GalCer-mediated iNKT cell activation. However, this competition was only achieved at a 15-fold molar excess over α-GalCer and could not be observed in living cells. The latter is in all probability due to the instability of the thioamide moiety inside living cells. More recently, the natural ganglioside GD3 (Figure 5.2) was identified as a high-affinity, competitive CD1d-ligand that inhibits the activation of iNKT cells both *in vitro* and *in vivo*.[11] Taking into account the

abundant presence of GD3 in ovarian cancer-associated ascites fluid, this suggests that ovarian cancer tumors may use GD3 to inhibit the antitumor iNKT cell response as an early mechanism of tumor immune evasion. Contrary to this observation, another group reported earlier that mice immunized with $GD3^{+}$ human melanoma or with GD3-loaded DCs developed a GD3-mediated CD1d-restricted iNKT cell response, characterized by IL-4, IL-10 and IFN-γ secretion and polarized toward Th2 upon further immunization.[12] As the information currently available concerning iNKT cell inhibition is thus rather scarce, the synthesis of new iNKT cell antagonists is highly desirable.

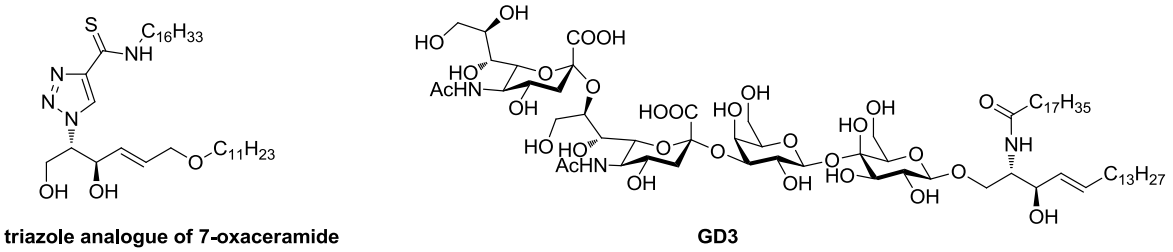


Figure 5.2 Structures of CD1d-dependent iNKT cell antagonists.

As outlined in the introduction, the 2”-, 3”- and 4”-OH groups of the α-GalCer carbohydrate moiety are involved in the interaction with the TCR. Consequently, α-ManCer is unable to significantly activate iNKT cells due to the loss of hydrogen bonds with the 2”-OH and 4”-OH group. On the other hand, NU-α-GalCer, designed earlier in our laboratory, induces a potent Th1 polarization, which has been related to increased interactions with CD1d, namely the induction of an additional binding pocket by the naphthylurea substituent and an extra hydrogen bond. Via the synthesis of NU-α-ManCer derivative **12** (Figure 5.3) we envisage to obtain an iNKT cell antagonist which may be useful in the treatment of allergic inflammatory responses such as asthma and other disorders characterized by inappropriate iNKT cell activation.

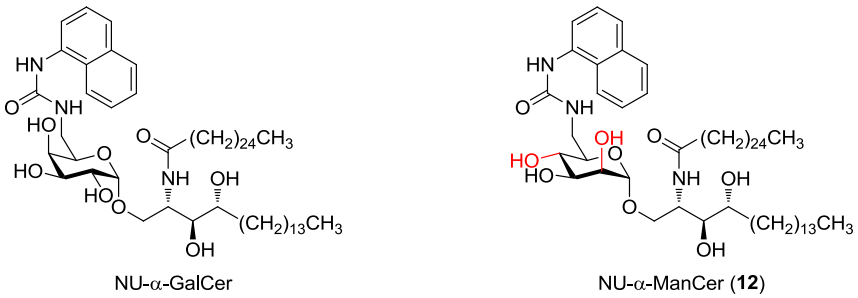
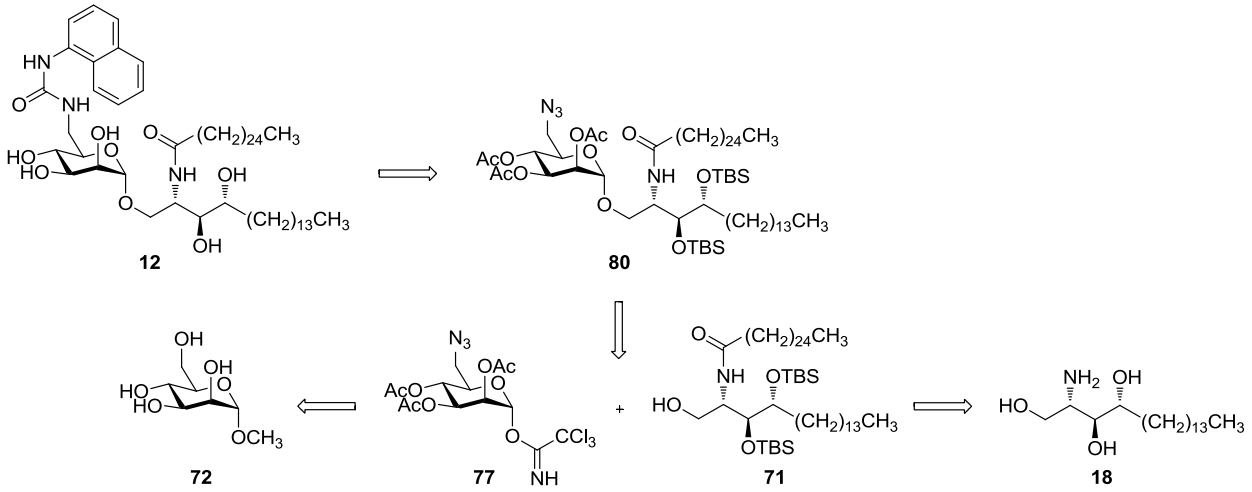


Figure 5.3 Structure of NU-α-GalCer and NU-α-ManCer.

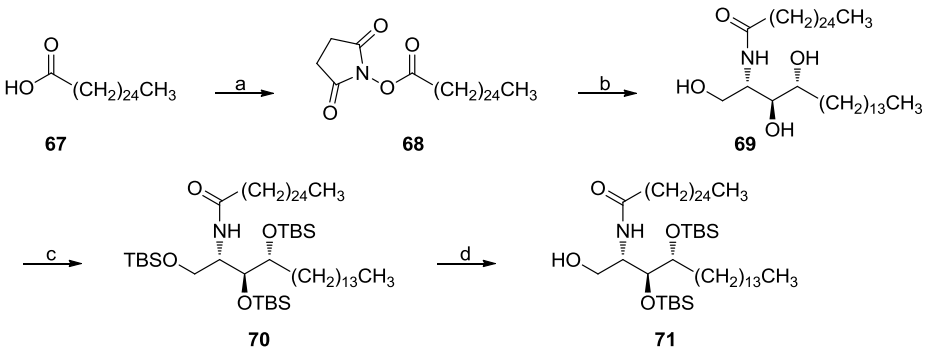
5.2 Synthesis of NU-α-ManCer

The retrosynthesis of our first attempt towards the preparation of NU-α-ManCer **12** is shown in Scheme 5.1. We intended to couple glycosyl donor **77** and acceptor **71** under Schmidt's conditions, whereby the 2"-acetyl group favors α-selectivity through neighbouring group participation. Staudinger reduction of the obtained C-6"-azido-mannosylceramide **80** followed by reaction with 1-naphthylisocyanate permits to introduce the desired naphthylurea substituent. Final deprotection of the acetyl and silyl groups then gives the envisaged NU-α-ManCer **12**.



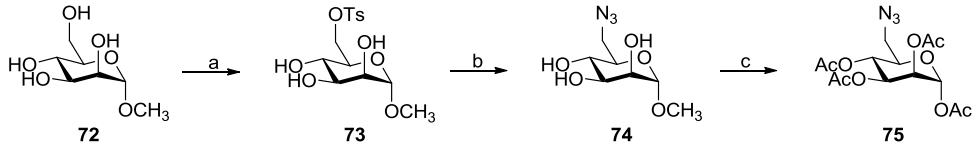
Scheme 5.1 Retrosynthesis of the first strategy towards NU-α-ManCer **12**.

For the synthesis of ceramide building block **71**, a procedure similar to that of Kim *et al*. was employed.[13] First an *N*-hydroxysuccinimide ester of hexacosanoic acid **67** was prepared as acyl donor for condensation with phytosphingosine to afford the unprotected ceramide **69** (Scheme 5.2). Next, the free hydroxyl groups were silylated with TBSOTf in the presence of 2,6-lutidine giving intermediate **70**. Upon regioselective removal of the primary silyl group with HF.pyridine, ceramide acceptor **71** was obtained.



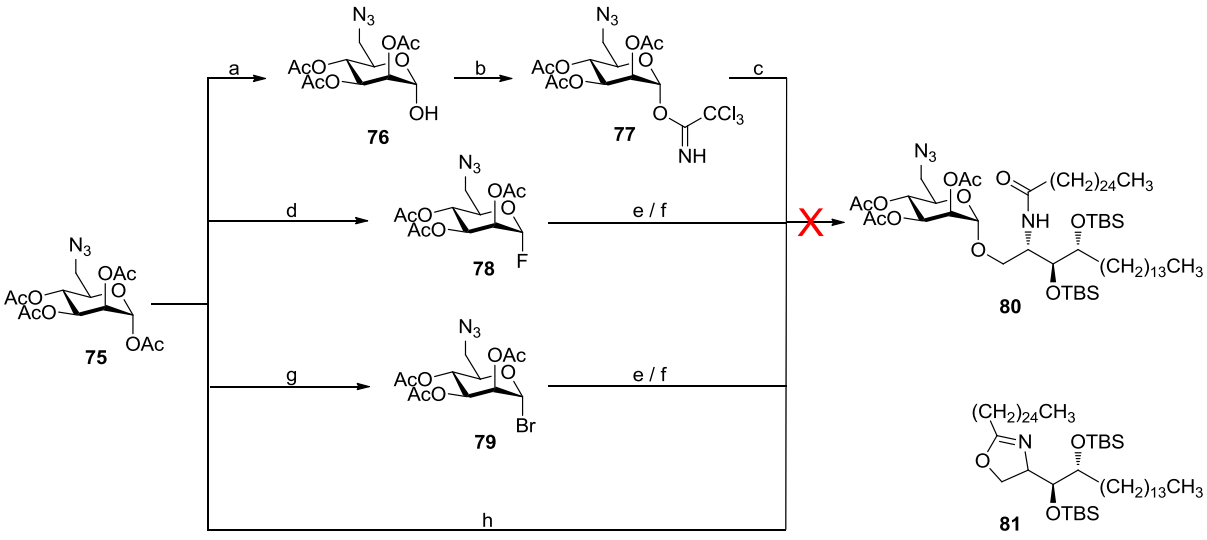
Scheme 5.2 Reagents and conditions: (a) NHS, CH_2Cl_2, 40 °C, overnight; (b) phytosphingosine, Et_3N, THF, 50 °C, overnight; (c) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C-rt, 6 h, 37% over 3 steps; (d) HF.pyridine, THF, pyridine, 0 °C, 1.5 h, 70%.

Preparation of the mannosyl donor started from the commercially available methyl-α-D-mannopyranoside **72** (Scheme 5.3). After selective tosylation of the primary alcohol giving **73**, an azide group at the 6”-position was introduced to furnish **74**. Subsequent acetylation of the remaining hydroxyl groups and the anomeric position under acidic conditions produced intermediate **75**.



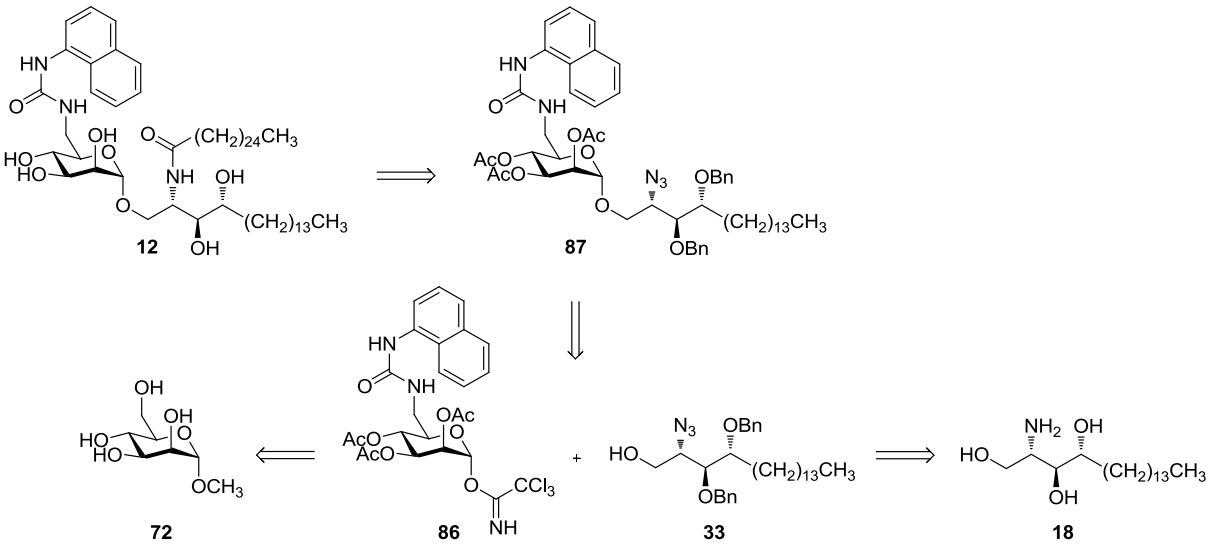
Scheme 5.3 Reagents and conditions: (a) TsCl, pyridine, 0 °C, 20 min, 71%; (b) NaN_3, DMF, 60 °C, 3 days, 93%; (c) Ac_2O, AcOH, H_2SO_4, 0 °C-rt, 2 days, 83%.

Removal of the anomeric acetyl group in **75** using benzylamine, followed by substitution with trichloroacetonitrile in the presence of DBU afforded trichloroacetimidate **77** (Scheme 5.4). Unfortunately, glycosylation of **71** with **77** could not be realized, probably due to the low nucleophilicity of the ceramide. To compensate for this, the more reactive mannosyl fluoride donor **78** was used for glycosylation under Mukaiyama conditions. This didn’t result in the expected mannosylceramide derivative either. Instead, conversion of ceramide **71** to an undesired oxazoline derivative was observed (**81**). Subsequently, several other mannosyl donors and activator systems were applied, again without success (Scheme 5.4).



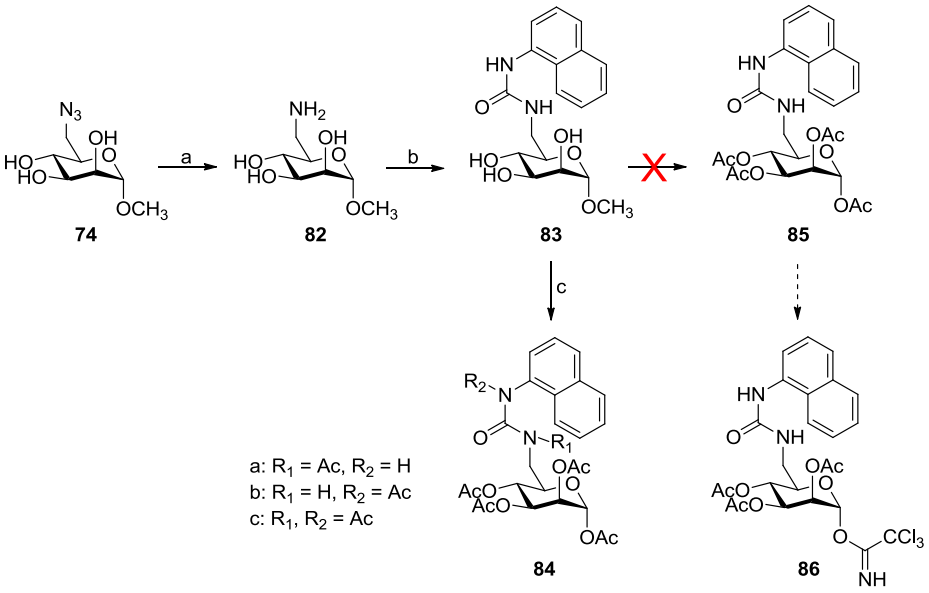
Scheme 5.4 Reagents and conditions: (a) $BnNH_2$, THF, 50 °C, 24 h, 94%; (b) CCl_3CN, DBU, CH_2Cl_2, 0 °C, 5 h, 49%; (c) **71**, TMSOTf, THF, -30 °C, 24 h; (d) HF.pyridine, pyridine, toluene, -20 °C-rt, overnight, 73%; (e) **71**, $SnCl_2$, $AgClO_4$, THF, -10 °C for 1 h-rt for 27 h; (f) **71**, $Hg(CN)_2$, nitromethane, 90 °C, 3-20 h; (g) AcOH, AcBr, MeOH, rt, overnight; (h) **71**, $BF_3.Et_2O$, CH_2Cl_2, 0 °C-rt, 5 h.

Therefore, we decided to follow a new strategy which allows coupling of the more reactive azidosphingosine **33** with glycosyl donor **86** (Scheme 5.5). This implicates introduction of the acyl chain after Schmidt's glycosylation as opposed to the first attempt, followed by global deprotection to yield **12**.



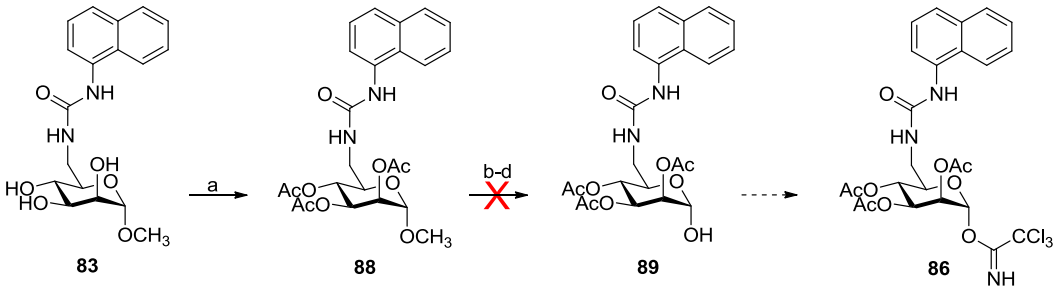
Scheme 5.5 Retrosynthesis of the second strategy towards NU-α-ManCer **12**.

The preparation of mannosyl donor **86** started with the Staudinger reduction of azide intermediate **74** and subsequent reaction of the resulting amine **82** with 1-naphthylisocyanate to produce the corresponding naphthylurea derivative **83** (Scheme 5.6). Yet, during the following acetylation of the free hydroxyl groups under acidic conditions, the NH groups of the urea functionality were acetylated as well, resulting in compounds **84a-c** instead of the desired product **85**.



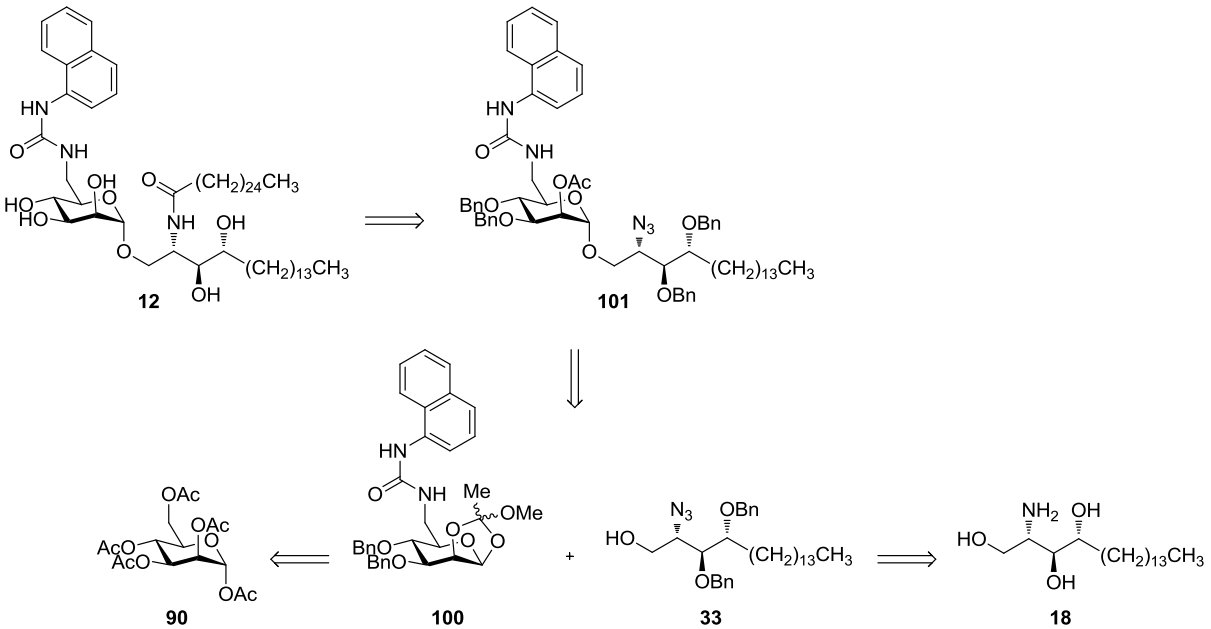
Scheme 5.6 Reagents and conditions: (a) i. PMe_3, THF, 2 h; ii. H_2O, 3 h; (b) 1-naphthylisocyanate, DMF, 0 °C-rt, 1 h, 89% over 2 steps; (c) Ac_2O, AcOH, H_2SO_4, 0 °C-rt, overnight.

In order to circumvent the unwanted *N*-acetylation of **83**, the free OH groups were acetylated under mild basic conditions, furnishing **88** (Scheme 5.7). Then, anomeric demethylation prior to incorporation of the trichloroacetimidate group was attempted using several methods (b-d).[14,15,16] Regrettably, none of these efforts were successful.



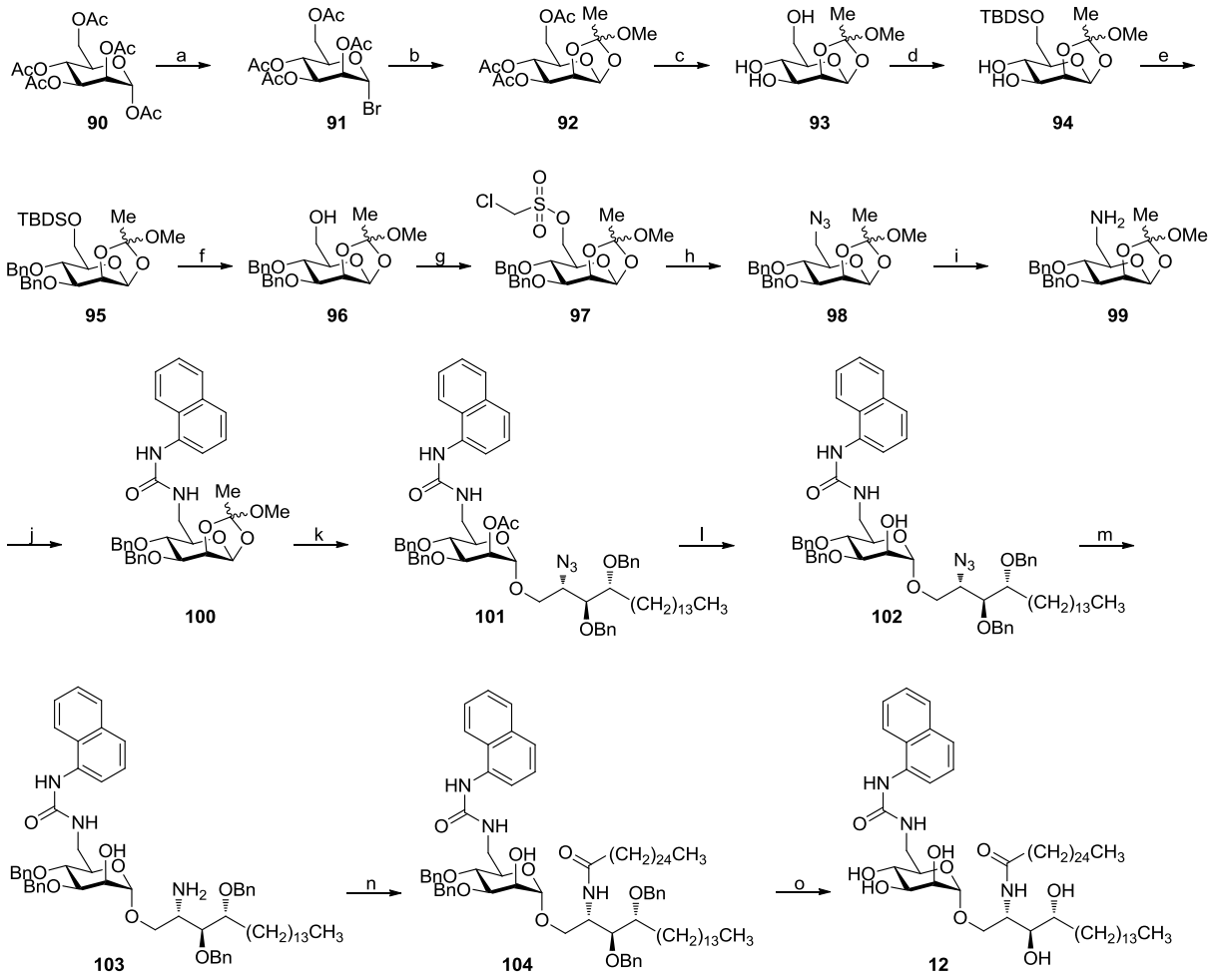
Scheme 5.7 Reagents and conditions: (a) Ac_2O, pyridine, 0 °C-rt, 1 h, 50%; (b) H_2SO_4, AcOH, 0 °C-rt (c) i. BCl_3, CH_2Cl_2, -78 °C; ii. Ag_2CO_3, CH_3CN, H_2O; (d) $TrBF_4$, CH_2Cl_2, rt.

To overcome the aforementioned difficulties, a third alternative synthetic route was developed exploiting a glycosyl donor with a 1,2-orthoester functionality (Scheme 5.8). Sugar 1,2-orthoesters are often considered undesired side products in the Koenigs-Knorr glycosylation.[17] However, by slightly changing the experimental conditions they may become the predominant product, which may subsequently be used for glycosylation as pioneered by Kochetkov and co-workers, leading stereospecifically to 1,2-*trans*-glycosides.[18,19] Similar to the previous attempt, introduction of the acyl chain takes place after the glycosylation process followed by removal of the benzyl groups to acquire NU-α-ManCer **12**.



Scheme 5.8 Retrosynthesis of the third strategy towards NU-α-ManCer **12**.

Generally, the synthesis of sugar 1,2-orthoesters starts from an acylated glycosyl halide, an alcohol and a base. Here, peracetylated α-D-mannopyranosyl bromide **91**, prepared from the commercially available penta-*O*-acetyl-α-D-mannose **90** was used in the presence of methanol and 2,6-lutidine (Scheme 5.9). This resulted in the formation of two inseparable diastereoisomers of orthoester **92** in a ratio 9:1 (*exo*:*endo*), consistent with data found in literature.[20]



Scheme 5.9 Reagents and conditions: (a) HBr, Ac_2O, AcOH, 0 °C-rt, overnight, (b) 2,6-lutidine, MeOH, CH_2Cl_2, rt, overnight, 67% over 2 steps; (c) NH_3, MeOH, rt, overnight; (d) TBDMSCl, imidazole, THF, rt, 30 h, 76% over 2 steps; (e) NaH, BnBr, DMF, 0 °C-rt, overnight, 76%; (f) TBAF, THF, -78 °C-rt, 3 h; (g) McCl, pyridine, 0 °C, 30 min.; (h) NaN_3, DMF, 80 °C, overnight, 39% over 3 steps; (i) i. PMe_3, THF, 2 h; ii. H_2O, 2.5 h; (j) 1-naphthylisocyanate, DMF, 0 °C-rt, 1 h, 68% over 2 steps; (k) **33**, TMSOTf, CH_2Cl_2, 0 °C, 1 h, 17-34 %; (l) NaOMe, MeOH, 0 °C-rt, 3 days, 50%; (m) PMe_3, THF, 2h; ii. H_2O, 2.5 h; (n); EDC, CH_2Cl_2, rt, overnight, 64% over 2 steps; (o) Pd black, H_2, EtOH/$CHCl_3$, 5 h, 34%.

Once the 1,2-orthoester functionality was introduced, the remaining acetyl groups were removed with a mixture of ammonia in methanol. Selective silylation at the primary position using *tert*-butyldimethylsilyl chloride (TBDMSCl) followed by benzylation of the secondary hydroxyl groups afforded compound **95**. Next, orthogonal deprotection of the silyl group with

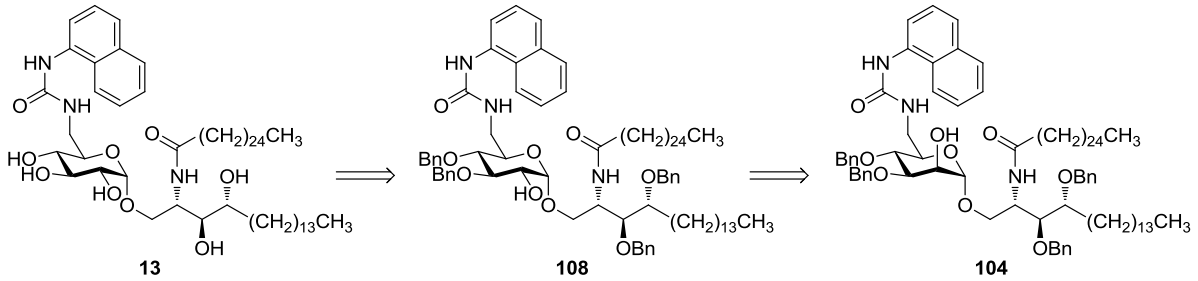
tetra(*n*-butyl)ammonium fluoride (TBAF) provided product **96,** allowing subsequent selective manipulation of the 6”-position. After nucleophilic displacement of the corresponding monochloromesylate **97** with sodium azide, the resulting 6-azido derivative **98** was submitted to Staudinger reduction, giving amine **99**. Reaction with 1-naphthylisocyanate then yielded the desired naphthylurea intermediate **100** to be used as glycosyl donor.

The orthoester glycosylation consisted of reacting compound **100** with acceptor **33** in the presence of catalytic amounts TMSOTf. Unfortunately, the desired glycoside **101** could only be obtained in very low yield (17%), owing to competing glycosylation of the extruded methanol from the initial orthoester. Since the undesired methyl glycoside and the isomeric starting compound **100** have the same Rf, distinction was only possible by NMR analysis, impeding easy monitoring of the reaction. In order to reduce the formation of this side product, molecular sieves were employed, leading to a doubling of the reaction yield (34%).

In the following step, the 2”-*O*-acetyl group regenerated during the glycosylation reaction was removed under basic conditions to give intermediate **102**. Next, introduction of the acyl chain was achieved by Staudinger reduction and subsequent coupling with cerotic acid in the presence of EDC. Lastly, palladium-catalyzed hydrogenolysis of compound **104** provided the desired NU-α-ManCer derivative **12**.

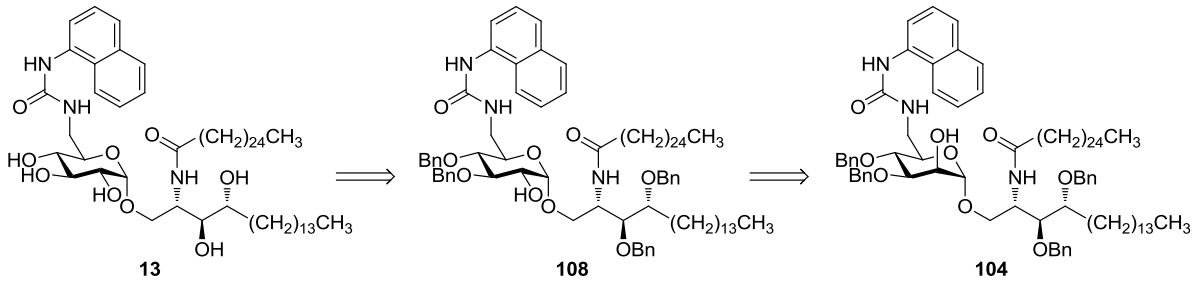
5.3 Synthesis of NU-α-GlcCer

Having obtained mannose intermediate **104** possessing a C-2” free hydroxyl group, we turned our attention to invert the stereochemistry of this group to acquire the corresponding glucose derivative **13** (Scheme 5.10). Compared to α-GalCer, α-GlcCer shows an activity level that is only slightly reduced. Similarly, α-GlcCer compounds containing a C24:0 or C20:2 acyl tail are equally potent in inducing iNKT cell expansion than the corresponding α-GalCer compounds.[21] By comparing the biological activity of NU-α-GlcCer **13** with that of NU-α-GalCer and NU-α-ManCer, additional information could be gained concerning the importance of the C-4” hydroxyl group.



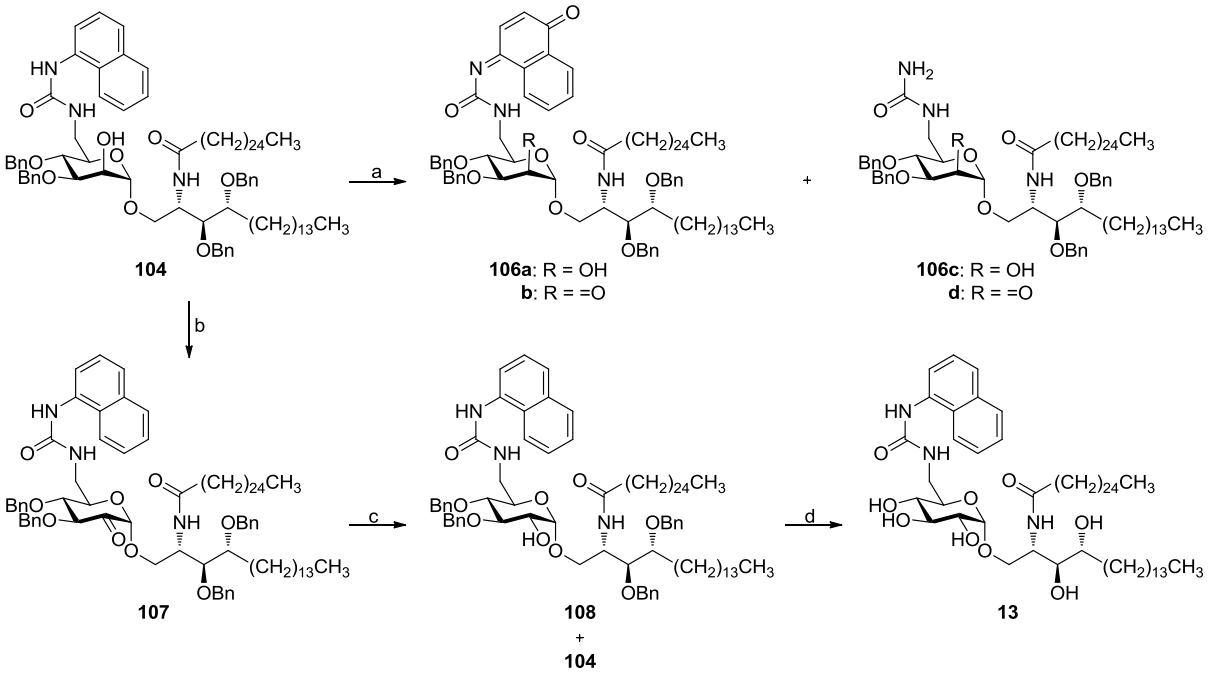
Scheme 5.10 Retrosynthesis of NU-α-GlcCer **13**.

Towards this end, a Mitsunobu reaction with benzoic acid was carried out, known as a synthetic tool for alcohol inversion (Scheme 5.11). Subsequent removal of benzoyl and benzyl groups of **105** would then deliver the envisioned NU-α-GlcCer **13**. However, the stated Mitsunobu conditions didn't result in any product formation, neither when the more reactive *p*-nitrobenzoic acid was used.[22] Difficulties associated with nucleophilic displacement reactions at the 2-position of hexoses have been reported earlier.[23,24,25]



Scheme 5.11 Reagents and conditions: (a) PPh_3, DEAD, benzoic acid or *p*-nitrobenzoic acid, THF, 0 °C-rt, 24 h.

In a second attempt, epimerization of the C-2" center via an oxidation-reduction sequence was explored. This strategy is most commonly used for the synthesis of β-mannosides but there are also some reports on the construction of α-glucosides.[26,27,28] Oxidation of the C-2" hydroxyl group first was performed using Dess Martin reagent (Scheme 5.12). However, under these conditions oxidation of the naphthyl system was observed, leading to a mixture of **106a-d**. Therefore, oxidation according to Swern was tried, successfully resulting in 2"-keto-derivative **107,** which was then reduced with sodium borohydride to furnish a mixture of glucoside **108** and the initial mannoside **104**. Both epimers could be separated by column chromatography and the manno-derivative was recycled. Finally, debenzylation afforded the proposed NU-α-GlcCer **13**. ^{1}H-NMR analysis of this compound showed an anomeric coupling constant of 3.8 Hz, in line with other related α-GlcCer analogues and thus confirming the nature of the carbohydrate moiety.[21]



Scheme 5.12 Reagents and conditions: (a) DMP, CH_2Cl_2, 2 h, rt; (b) oxalyl chloride, DMSO, CH_2Cl_2, Et_3N, -78 °C, 2 h; (c) $NaBH_4$, THF, 0 °C-rt, 5 h, 16% over 2 steps; (d) Pd black, H_2, EtOH/$CHCl_3$, rt, 33%.

5.4 Biological evaluation

So far, only NU-α-ManCer has been evaluated for its antagonistic potential, providing some preliminary results. A first experiment aimed at determining whether NU-α-ManCer acts as an inhibitor of iNKT TCR binding. Towards this end, human CD1d-expressing T2 lymphoblast cells were pulsed with vehicle, α-GalCer, OCH, GD1a or NU-α-ManCer (**12**), prior to staining with iNKT TCR tetramers (Collaboration with Prof. S. Gadola). As shown in Figure 5.4, the intensity of the tetramer stains between CD1d-expressing T2 lymphoblasts pulsed with vehicle alone or NU-α-ManCer were comparable, suggesting that NU-α-ManCer does allow some iNKT TCR binding to CD1d.

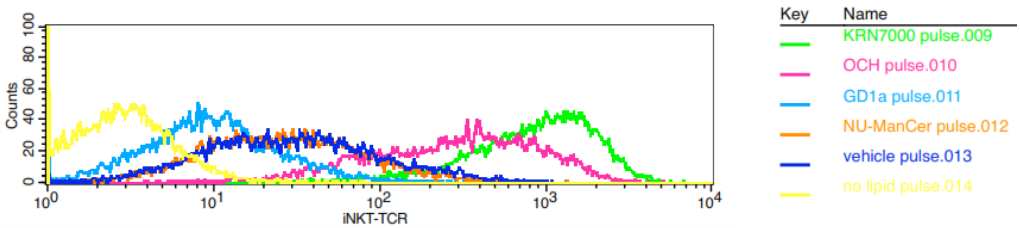


Figure 5.4 Inhibition of iNKT TCR binding.

To investigate whether NU-α-ManCer acts as a competitor of lipid binding, human CD1d-expressing T2 lymphoblasts were pulsed for 1 hour with varying concentrations of OCH, followed by pulsing for 2 hours with vehicle, disialoganglioside 1a (GD1a) or NU-α-ManCer

as competitor ligands. Staining with iNKT TCR tetramers allowed to determine OCH lipid displacement. The results are presented in Figure 5.5 and demonstrate that, while GD1a could displace OCH, NU-α-ManCer was comparable to the vehicle background control.

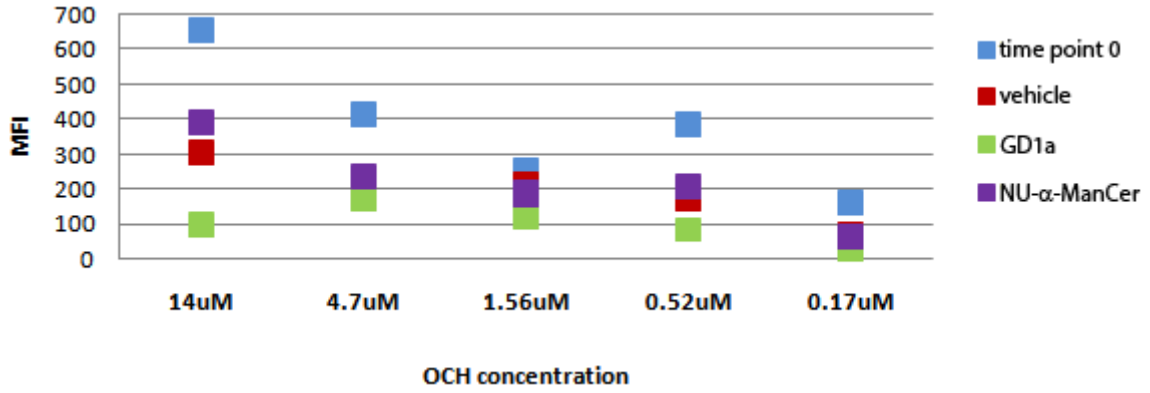


Figure 5.5 Lipid binding competition experiment.

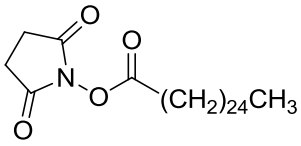
5.5 Conclusion

The synthesis of NU-α-ManCer proved to be less straightforward than initially expected due to several difficulties encountered during, or in anticipation of the glycosylation reaction. Through application of a 1,2-orthoester-based glycosylation strategy, the intended mannosyl derivative could eventually be attained. This approach also allowed late-stage conversion to the corresponding glucosyl derivative via an oxidation-reduction sequence. Based on the preliminary results of the competition assays with NU-α-ManCer, we assume that this compound does not act as an iNKT cell antagonist. Additional experiments to extend and rationalize these findings as well as evaluation of NU-α-GlcCer are in progress.

5.6 Experimental section

***N*-succinimidyl hexacosanoate (68)**

To a solution of hexacosanoic acid (4.50 g, 11.34 mmol) in CH_2Cl_2 was added *N*-hydroxysuccinimide (NHS) (1.57 g, 13.61 mmol). The mixture was heated to 40 °C and stirred overnight. After being poured in water and extraction with Et_2O, the combined organic layers were washed with brine, dried over $MgSO_4$ and evaporated to dryness to furnish **69** (3.61 g) as a white powder.



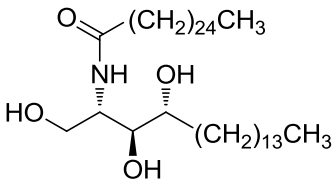
^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.83 (br. s, 2H, $COCH_2$), 2.59 (t, *J* = 7.4 Hz, 2H, $COCH_2$), 1.74 (ddd, *J* = 7.5 Hz and 15.2 Hz, 2H, CH_2), 1.43-1.18 (m, 44H, CH_2), 0.88 (t, *J* = 6.7 Hz, 3H, CH_3).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 169.14, 168.65, 31.91, 30.92, 29.68, 29.61, 29.54, 29.34, 29.07, 28.78, 25.57, 24.56, 22.67, 14.09.

Spectral data are consistent with the literature data.

(2*S*,3*S*,4*R*)-2-hexacosylamino-octadecane-1,3,4-triol (69)

To a solution of **68** (3.61 g) in THF (100 mL) was added phytosphingosine (1.93 g, 6.10 mmol) and Et_3N (3 mL, 21.96 mmol). The resulting suspension was heated to 50 °C. After stirring overnight, a clear solution was obtained, which was allowed to cool down to room temperature. The solution was then diluted with EtOAc (50 mL), resulting in a white suspension. After centrifugation for 30 minutes, the supernatant was removed and the white residue was evaporated to dryness to afford **69** (3.99 g) as a white powder.



^{1}H-NMR (300 MHz, pyridine-d_5): δ 8.28 (d, *J* = 8.5 Hz, 1H, NH), 5.06 (dt, *J* = 4.8 Hz and 9.6 Hz, 1H, H-2), 4.53-4.43 (m, 2H, H-1 and H-3), 4.37 (dd, *J* = 4.8 Hz and 6.4 Hz, 1H, H-1), 4.30-4.26 (m, 1H, H-4), 2.47 (t, *J* = 7.4 Hz, 2H, $COCH_2$), 2.31-1.19 (m, 72H, CH_3).

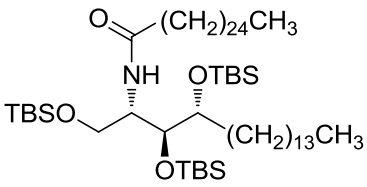
^{13}C-NMR (75 MHz, pyridine-d_5): δ 176.96, 75.65, 71.96, 63.95, 52.63, 35.70, 32.85, 30.93, 29.11, 28.93, 28.81, 28.79, 28.71, 28.66, 28.60, 28.55, 28.39, 25.43, 25.19, 21.72, 13.04.

Exact mass (ESI-MS) for $C_{44}H_{89}NO_4$ $[M+H]^+$ found, 696.6869; calcd, 696,6870; $[M+Na]^+$ found, 718.6682; calcd 718,6689.

Spectral data are consistent with the literature data.

(2*S*,3*S*,4*R*)-1,3,4-tri-*O*-*tert*-butyldimethylsilyl-2-hexacosylamino-octadecane-1,3,4-triol (70)

To a solution of **69** (1.50 g) in CH_2Cl_2 (12 mL), 2,6-lutidine (2.3 mL, 19.41 mmol) was added. The mixture was cooled to 0 °C and TBSOTf (3.0 mL, 12.94 mmol) was added. The reaction mixture was allowed to stir for 6 hours at room temperature. After quenching with a saturated $NaHCO_3$ solution, the aqueous layer was extracted with CH_2Cl_2. Then, the combined organic layers were washed with a 1M HCl solution, a saturated $NaHCO_3$ solution and brine, followed by drying over Na_2SO_4 and removal of the solvent under reduced pressure. Purification by column chromatography (hexanes/EtOAc: 97/3) yielded compound **70** (1.65 g, 37 % over 3 steps) as a clear colorless oil.



^{1}H-NMR (300 MHz, $CDCl_3$): δ 5.78 (d, *J* = 8.5 Hz, 1H, NH), 3.94-3.87 (m, 1H, H-2), 3.83 (dd, *J* = 4.2 Hz and 9.9 Hz, 1H, H-1), 3.78 (dd, *J* = 1.4 Hz and 7.3 Hz, 1H, H-3), 3.66-3.57 (m, 2H, H-1 and H-4), 2.01 (t, *J* = 7.5 Hz, 2H, $COCH_2$), 1.60-1.15 (m, 72H, CH_2), 0.93-0.80 (m, 33H, CH_3), 0.10-0.08 (m, 3H, CH_3), 0.05-0.01 (m, 15H, CH_3).

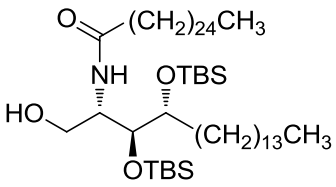
^{13}C-NMR (75 MHz, $CDCl_3$): δ 177.81, 80.96, 80.92, 66.94, 58.10, 42.71, 37.75, 37.49, 35.55, 35.26, 35.23, 35.22, 35.18, 35.02, 34.98, 34.95, 34.93, 34.92, 31.91, 31.66, 31.63, 31.43, 31.34, 31.25, 28.25, 23.91, 23.80, 23.70, 19.67, 2.61, 2.05, 1.74, 0.93, 0.37, 0.34.

Exact mass (ESI-MS) for $C_{62}H_{131}NO_4Si_3$ $[M+H]^+$ found, 1038.9584; calcd, 1038.9459; $[M+Na]^+$ found, 1060.9448; calcd 1060.9278; $[M+K]^+$ found, 1076.9200; calcd, 1076.9017.

Spectral data are consistent with the literature data.

(2*S*,3*S*,4*R*)-3,4-di-*O*-*tert*-butyldimethylsilyl-2-hexacosylamino-octadecane-1,3,4-triol (71)

Compound **70** (411 mg, 0.40 mmol) was dissolved in THF (4.1 mL) in a polyethylene vial and cooled to 0 °C. Then, 2.4 mL of a stock solution consisting of pyridine (3.0 mL) and HF.pyridine (2.7 mL) in THF (7.5 mL) was added. After stirring for 1.5 hours, the reaction mixture was quenched with a saturated $NaHCO_3$ solution and extracted with EtOAc. The combined organic layers were washed with a saturated $NaHCO_3$ solution, dried over $MgSO_4$ and evaporated to dryness. The resulting residue was purified by column chromatography (hexanes/EtOAc: 9/1) to afford **71** (237 mg, 70 %) as a clear colorless oil.



^{1}H-NMR (300 MHz, $CDCl_3$): δ 6.17 (d, *J* = 7.8 Hz, 1H, NH), 4.12 (app. d, *J* = 11.2 Hz, 1H, H-1), 4.01-3.95 (m, 1H, H-2), 3.83 (t, *J* = 2.8, 1H, H-3), 3.68 (ddd, *J* = 2.6 Hz and 6.4 Hz, 1H, H-4), 3.51 (app. t, *J* = 7.7 Hz, 1H, H-1), 3.09 (d, *J* = 6.7 Hz, 1H, OH), 2.12-2.04 (m, 2H, $COCH_2$), 1.58-1.08 (m, 72H, CH_2), 0.88-0.77 (m, 24H, CH_3), 0.02-0.00 (m, 12H, CH_3).

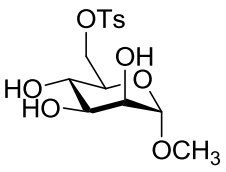
^{13}C-NMR (75 MHz, $CDCl_3$): δ 177.58, 68.52, 56.16, 41.85, 39.33, 36.84, 34.71, 34.61, 34.59, 34.57, 34.54, 34.45, 34.39, 34.28, 30.91, 30.87, 30.74, 30.52, 27.60, 23.06, 23.03, 19.03, 1.15, 0.84, 0.39, 0.00.

Exact mass (ESI-MS) for $C_{56}H_{117}NO_4Si_2$ $[M+H]^+$ found, 924.8608; calcd, 924.8594.

Spectral data are consistent with the literature data.

6-*O*-toluenesulfonyl-1-*O*-methyl-α-D-mannopyranoside (73)

Tosylchloride (5.98 g, 31.44 mmol) was added in small portions to a solution of methyl-α-D-mannopyranoside **72** (5.00 g, 25.77 mmol) in pyridine (50 mL) at 0 °C. The mixture was stirred for 20 minutes, followed by evaporation of the solvent. Purification by column chromatography (CH_2Cl_2/MeOH: 96/4) afforded **73** (6.34 g, 71 %) as a white foam.



^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.80 (d, *J* = 8.4 Hz, 2H, arom. H), 7.34 (d, *J* = 8.1 Hz, 2H, arom. H), 4.67 (d, *J* = 1.3 Hz, 1H, H-1), 4.35 (dd, *J* = 4.2 Hz and 10.9 Hz, 1H, H-6), 4.28 (dd, *J* = 1.6 Hz and 11.0 Hz 1H, H-6), 3.91 (app. s, 1H, H-2), 3.75-3.71 (m, 3H, H-3, H-4 and H-5), 3.30 (s, 3H, OCH_3), 2.43 (s, 3H, CH_3).

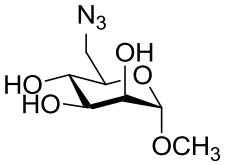
^{13}C-NMR (75 MHz, $CDCl_3$): δ 145.23, 132.90, 130.12, 128.25, 101.17, 71.86, 70.74, 70.18, 69.71, 67.33, 55.31, 21.87.

Exact mass (ESI-MS) for $C_{14}H_{20}O_8S$ $[M+H]^+$ found, 349.0992; calcd, 349.0952; $[M+Na]^+$ found, 371.0795; calcd 371.0771; $[M+K]^+$ found, 387.0534; calcd, 387.0510.

Spectral data are consistent with the literature data.

6-azido-6-deoxy-1-*O*-methyl-α-D-mannopyranoside (74)

To a solution of **73** (6.33 g, 18.18 mmol) in DMF (55 mL) at 60 °C was added NaN_3 (13.12 g, 201.80 mmol) in several portions. After stirring for 3 days, the resulting white suspension was filtered, followed by evaporation of the solvent under reduced pressure. The residue was purified by column chromatography (CH_2Cl_2/MeOH: 96/4), giving **74** (3.70 g, 93 %) as a colorless syrup.



^{1}H-NMR (300 MHz, $CDCl_3$): δ 4.68 (d, *J* = 1.5 Hz, 1H, H-1), 3.88 (dd, *J* = 1.5 Hz and 3.2 Hz, 1H, H-2), 3.74-3.62 (m, 3H, H-3, H-4 and H-5), 3.49-3.48 (m, 2H, H-6), 3.35 (s, 3H, OCH_3).

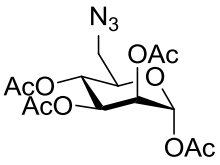
^{13}C-NMR (75 MHz, $CDCl_3$): δ 101.23, 71.86, 71.60, 70.93, 68.41, 55.41, 51.60.

Exact mass (ESI-MS) for $C_7H_{13}N_3O_5$ $[M+Na]^+$ found, 242.0767; calcd 242.0747; $[M+K]^+$ found, 258.0511; calcd, 258.0487.

Spectral data are consistent with the literature data.

1,2,3,4-tetra-*O*-acetyl-6-azido-6-deoxy-1-*O*-methyl-α-D-mannopyranoside (75)

To a solution of **74** (4.42 g, 20.17 mmol) in AcOH (210 mL) and Ac_2O (210 mL) at 0 °C was added concentrated H_2SO_4 (4.5 mL) dropwise. The reaction mixture was allowed to stir at room temperature for 2 days and then quenched with iced water. After extraction with CH_2Cl_2, the combined organic layers were washed with iced water and a saturated solution of $NaHCO_3$, dried over $MgSO_4$ and co-evaporated with toluene twice. Purification by column chromatography (hexanes/EtOAc: 6/4) yielded compound **75** (6.22 g, 83 %) as a colorless syrup.



^{1}H-NMR (300 MHz, $CDCl_3$): δ 6.09 (d, *J* = 1.7 Hz, 1H, H-1), 5.35-5.33 (m, 2H, H-3 and H-4), 5.2-5.24 (m, 1H, H-2), 4.02-3.98 (m, 1H, H-5), 3.39 (dd, *J* = 3.0 Hz and 13.5 Hz, 1H, H-6), 3.31 (dd, *J* = 5.5 Hz and 13.5 Hz, 1H, H-6), 2.18 (s, 3H, CH_3), 2.17 (s, 3H, CH_3), 2.06 (s, 3H, CH_3), 2.01 (s, 3H, CH_3).

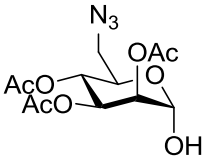
^{13}C-NMR (75 MHz, $CDCl_3$): δ 170.24, 170.00, 169.81, 168.25, 90.57, 72.08, 68.78, 68.50, 66.71, 50.94, 21.27, 21.06, 20.97, 20.90, 20.86.

Exact mass (ESI-MS) for $C_{14}H_{19}N_3O_9$ $[M+Na]^+$ found, 396.1037; calcd 396.1014; $[M+K]^+$ found, 412.0786; calcd, 412.0753.

Spectral data are consistent with the literature data.

2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-methyl-α-D-mannopyranoside (76)

To a solution of compound **75** (262 mg, 0.70 mmol) in dry THF (3.6 mL) was added benzylamine (0.1 mL). The reaction mixture was allowed to stir at 50 °C for 24 hours under argon atmosphere. Since both starting material and desired product had the same Rf (hexanes/EtOAc: 6/4), mass spectrometry was needed to determine reaction completion. Subsequently, the solvent was evaporated under reduced pressure followed by purification by column chromatography (hexanes/EtOAc: 6/4) to give compound **76** (219 mg, 94 %) as a pale yellow syrup.



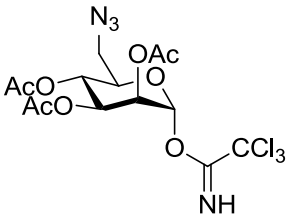
^{1}H-NMR (300 MHz, $CDCl_3$): δ 5.42 (dd, *J* = 3.1 Hz and 10.1 Hz, 1H, H-3), 5.29-5.26 (m, 3H, H-1, H-2 and H-4), 4.19 (ddd, *J* = 4.8 Hz and 9.5 Hz, 1H, H-5), 3.36-3.34 (m, 2H, H-6), 2.16 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 2.00 (s, 3H, CH_3).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 170.43, 170.24, 170.14, 92.28, 70.24, 70.12, 68.73, 67.43, 51.37, 20.96, 20.92, 20.79.

Exact mass (ESI-MS) for $C_{12}H_{17}N_3O_8$ $[M+Na]^+$ found, 354.0923; calcd 354.0908; $[M+K]^+$ found, 370.0663; calcd, 370.0647.

2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-α-D-mannopyranosyl trichloroacetimidate (77)

To a solution of **76** (194 mg, 0.59 mmol) in CH_2Cl_2 (6 mL) at 0 °C, DBU (0.05 mL, 0.29 mmol) and trichloroacetonitrile (0.5 mL, 5.87 mmol) were added. The reaction mixture was stirred for 5 hours, diluted with CH_2Cl_2 and celite was added. After filtration, the solvent was removed under reduced pressure and the residue was purified by column chromatography (hexanes/EtOAc: 7.5/2.5 + 1 V% Et_3N) yielding **77** (136 mg, 49 %) as a pale yellow syrup.



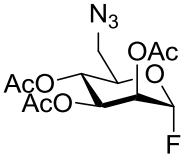
^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.81 (s, 1H, NH), 6.39 (d, *J* = 2.0 Hz, 1H, H-1), 5.47 (d, *J* = 1.9 Hz and 3.2 Hz, 1H, H-2), 5.41-5.38 (m, 2H, H-3 and H-4), 4.18-4.11 (m, 1H, H-5), 3.38-3.36 (m, 2H, H-6), 2.20 (s, 3H, CH_3), 2.07 (s, 3H, CH_3), 2.01 (s, 3H, CH_3).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 170.03, 169.97, 169.88, 159.93, 94.44, 72.70, 68.82, 68.04, 66.55, 50.94, 20.98, 20.93, 20.85.

Exact mass (ESI-MS) for $C_{14}H_{17}Cl_3N_4O_8$ $[M+Na]^+$ found, 497.0020; calcd 497.0004; $[M+K]^+$ found, 512.9760; calcd, 512.9744.

2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-α-D-mannopyranosyl fluoride (78)

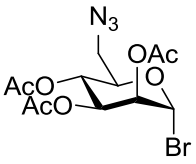
Anhydrous pyridine (1.0 mL) and a HF solution in pyridine (12.3 mL) were placed in a polyethylene vessel. The solution was cooled to -20 °C and a solution of **75** (256 mg, 0.69 mmol) in toluene (2.6 mL) was added. The reaction mixture was gradually warmed to room temperature and stirred overnight, then quenched with a saturated $NaHCO_3$ solution. The aqueous layer was extracted with CH_2Cl_2 followed by washing of the combined organic layer with a saturated $NaHCO_3$ solution and brine. After drying over Na_2SO_4 and removal of the solvent under reduced pressure, the residue was purified by column chromatography (hexane/EtOAc: 7/3) to furnish compound **78** (167 mg, 73%) as a colorless syrup.



^{1}H-NMR (300 MHz, $CDCl_3$): δ 5.60 (dd, *J* = 1.9 Hz and 48.6 Hz, 1H, H-1), 5.41-5.39 (m, 1H, H-2), 5.38-5.33 (m, 2H, H-3 and H-4), 4.16-4.09 (m, 1H, H-5), 3.46 (dd, *J* = 2.8 Hz and 13.6 Hz, 1H, H-6), 3.34 (dd, *J* = 5.4 Hz and 13.6 Hz, 1H, H-6), 2.19 (s, 3H, CH_3), 2.07 (s, 3H, CH_3), 2.02 (s, 3H, CH_3).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 169.76, 169.71, 169.59, 106.04, 103.07, 72.05, 72.02, 68.00, 67.98, 67.87, 67.35, 65.94, 60.40, 50.68, 21.06, 20.70, 20.69, 20.67, 20.59, 14.21.

2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-α-D-mannopyranosyl bromide (79)



To a solution of **75** (200 mg, 0.53 mmol) in AcOH (1.9 mL), AcBr (0.32 mL, 4.29 mmol) and MeOH (0.07 mL, 1.72 mmol) were added. After stirring overnight, the reaction mixture was co-evaporated twice with toluene, affording compound **79** as an orange syrup.

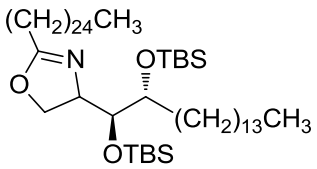
(2*S*,3*S*,4*R*)-3,4-di-*O*-*tert*-butyldimethylsilyl-1-*O*-(2,3,4-tri-*O*-acetyl-6-azido-6-deoxy-α-D-mannopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (80)

Procedure 1

To a mixture of mannosyl donor **77** (126 mg, 0.20 mmol) in THF (0.8 mL) was added a solution of ceramide **71** (187 mg, 0.26 mmol) in THF (2.1 mL). The solution was cooled to -30 °C and TMSOTf (5 µL, 0.03 mmol) was added. The reaction mixture was stirred under argon atmosphere but even after 24 hours, formation of the desired product could not be observed.

Procedure 2

To a solution of ceramide **71** (284 mg, 0.31 mmol) in THF (0.94 mL) under argon atmosphere, $SnCl_2$ (135 mg, 0.71 mmol), $AgClO_4$ (148 mg, 0.71 mmol) and powdered 4 Å molecular sieves were added. A solution of **78** (80 mg, 0.24 mmol) in THF (0.94 mL) was then added at -10 °C and the mixture was stirred for 1 hour. Since the majority of both starting materials remained unchanged, the solution was gradually warmed to room temperature. After stirring for 27 hours, complete conversion of ceramide **71** to a more apolar product was observed, while mannosyl donor **78** was still intact. The mixture was filtered over celite and the filter cake was washed with Et_2O. The combined filtrates were evaporated to dryness and the resulting residue was purified by column chromatography (hexanes/EtOAc: 95/5), affording oxazoline **81** (15 mg, 6%) as a colorless oil. However, no desired product could be obtained.



^{1}H-NMR (300 MHz, $CDCl_3$): δ 4.35 (app. t, *J* = 7.5 Hz, 1H, H-1), 4.22-4.16 (m, 1H, H-2), 4.09 (dd, *J* = 7.5 Hz and 9.9 Hz, 1H, H-1), 3.92 (app. s, 1H, H-3), 3.63-3.59 (m, 1H, H-4), 2.23-2.08 (m, 2H, $COCH_2$), 1.66-0.93 (m, 72H, CH_2), 0.91-0.62 (m, 24H, CH_3), 0.07-0.01 (m, 12H, CH_3).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 39.92, 36.70, 36.00, 35.08, 34.62, 34.47, 34.44, 34.43, 34.39, 34.35, 34.32, 34.22, 34.17, 34.13, 34.03, 32.98, 30.75, 30.68, 30.38, 27.46, 22.88, 22.83, 18.88, 4.75, 1.01, 0.85, 0.71, 0.23.

Exact mass (ESI-MS) for $C_{56}H_{115}NO_3Si_2$ $[M+H]^+$ found, 906.8552; calcd 906.8494.

Procedure 3

To a solution of ceramide **71** (411 mg, 0.44 mmol) in THF (1.37 mL) under argon atmosphere, $SnCl_2$ (197 mg, 1.04 mmol), $AgClO_4$ (216 mg, 1.04 mmol) and powdered 4 Å molecular sieves were added. A solution of **79** (139 mg, 0.35 mmol) in THF (0.94 mL) was then added at -10 °C. After stirring for 2 hours, mass spectrometry revealed formation of oxazoline **81**. Unfortunately, no desired product could be detected.

Procedure 4

A solution of **79** (188 mg, 0.48 mmol) in nitromethane (15 mL) was added dropwise to a solution of ceramide **71** (220 mg, 0.24 mmol) in nitromethane (5 mL). $Hg(CN)_2$ (121 mg, 0.48 mmol) was added. After stirring for 3 hours at 90 °C under argon atmosphere, TLC showed several spots including the one corresponding to oxazoline **81**. The mixture was cooled to room temperature and diluted with EtOAc, followed by washing with water, a saturated $NaHCO_3$ solution and brine. Next, the solution was dried over $MgSO_4$ and evaporated to dryness. Unfortunately, mass spectrometry of the crude mixture could not detect the envisaged product.

Procedure 5

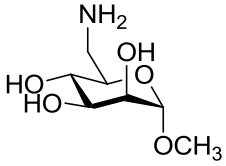
A solution of **78** (153 mg, 0.46 mmol) in nitromethane (7.5 mL) was added dropwise to a solution of ceramide **71** (212 mg, 0.23 mmol) in nitromethane (2.5 mL). $Hg(CN)_2$ (116 mg, 0.46 mmol) was added. After stirring for 20 hours at 90 °C under argon atmosphere, again no formation of the desired product could be observed and the reaction was stopped.

Procedure 6

A solution of mannosyl derivative **76** (40 mg, 0.11 mmol) in CH_2Cl_2 (4 mL) was added to a solution of ceramide **71** (100 mg, 0.11 mmol) in CH_2Cl_2 (12 mL). The solution was cooled to 0 °C followed by dropwise addition of $BF_3.Et_2O$. The mixture was allowed to stir for 5 hours at room temperature under argon atmosphere. TLC revealed several spots, however mass spectrometric analysis could not identify the desired product.

6-amino-6-deoxy-1-*O*-methyl-α-D-mannopyranoside (82)

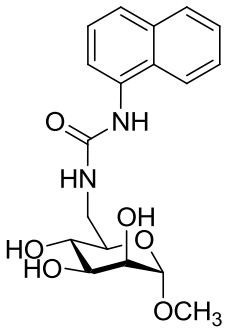
To a solution of azide **74** (500 mg, 2.28 mmol) in THF (20 mL) was added a 1 M solution of trimethylphosphine in THF (22 mL). After stirring for 2 hours, H_2O (20 mL) was added and the reaction mixture was allowed to stir for an additional 3 hours. The solvent was then removed by co-evaporation with toluene, yielding amine **82,** which was used without further purification.



Exact mass (ESI-MS) for $C_7H_{15}NO_5$ $[M+H]^+$ found, 194.1024; calcd, 194.1029; $[M+Na]^+$ found, 216.0849; calcd, 216.0848; $[M+K]^+$ found, 232.0589; calcd, 232.0587.

6-deoxy-6-(1-naphthyl)ureido-1-*O*-methyl-α-D-mannopyranoside (83)

To a solution of the crude amine **82** (100 mg) in DMF (5 mL) at 0 °C was added 1-naphthylisocyanate (1.18 mL, 0.40 mmol). The mixture was allowed to stir for 1 hour at room temperature, followed by removal of the organic solvent by evaporation. Purification by column chromatography (CH_2Cl_2/MeOH: 95/5) afforded **83** (109 mg, 89 % over 2 steps).



^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.81-7.77 (m, 1H, arom. H), 7.63-7.60 (m, 1H, arom. H), 7.49 (dd, *J* = 0.9 Hz and 7.5 Hz, 1H, arom. H), 7.42 (d, *J* = 8.3 Hz, 1H, arom. H), 7.30-7.18 (m, 3H, arom. H), 6.10 (t, *J* = 5.6 Hz, 1H, NH),4.42 (d, *J* = 1.4 Hz, 1H, H-1), 3.64 (dd, *J* = 1.8 Hz and 3.3 Hz, 1H, H-2), 3.56-3.47 (m, 2H, H-6 and H-3), 3.41 (app. t, *J* = 9.3 Hz, 1H, H-4), 3.35-3.29 (m, 1H, H-5), 3.21 (ddd, *J* = 3.4 Hz, 6.1 Hz and 14.3 Hz, 1H, H-6), 3.09 (s, 3H, OCH_3).

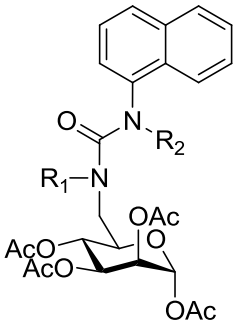
^{13}C-NMR (75 MHz, $CDCl_3$): δ 158.83, 134.69, 133.99, 128.78, 128.38, 126.38, 126.29, 126.05, 125.24, 121.78, 120.86, 101.68, 71.94, 71.07, 70.88, 68.01, 55.01, 40.80.

Exact mass (ESI-MS) for $C_{18}H_{22}N_2O_6$ $[M+H]^+$ found, 363.1549; calcd, 363.1556; $[M+Na]^+$ found, 385.1360; calcd, 385.1376; $[M+K]^+$ found, 401.1115; calcd, 401.1115.

1,2,3,4-tetra-*O*-acetyl-6-deoxy-6-(1-naphthyl)ureido-α-D-mannopyranose (85)

A solution of **83** (98 mg, 0.27 mmol) in Ac_2O (2.8 mL) and AcOH (2.8 mL) was cooled to 0 °C, followed by addition of concentrated H_2SO_4 (0.06 mL). After stirring overnight at room temperature, one major polar spot was observed on TLC. The reaction mixture was poured into iced water and extracted with CH_2Cl_2. The combined organic layers were washed with a

saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4 and evaporated to dryness. Mass spectrometric analysis identified **84a-c**, yet no desired product could be detected. Repeating the reaction under more careful monitoring of the reaction temperature and time didn't change the result.



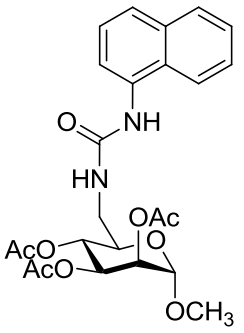
a: R_1 = Ac, R_2 = H
b: R_1 = H, R_2 = Ac
c: R_1, R_2 = Ac

Exact mass (ESI-MS) for $C_{27}H_{30}N_2O_{11}$ (a-b) $[M+Na]^+$ found, 581.1769; calcd, 581.1747; $[M+K]^+$ found, 597.1496; calcd, 597.1487.

Exact mass (ESI-MS) for $C_{29}H_{32}N_2O_{12}$ (c) $[M+Na]^+$ found, 623.1876; calcd, 623.1853; $[M+K]^+$ found, 639.1507; calcd, 389.1592.

2,3,4-tri-*O*-acetyl-6-deoxy-6-(1-naphthyl)ureido-1-*O*-methyl-α-D-mannopyranoside (88)

83 (66 mg, 0.18 mmol) was dissolved in pyridine (4 mL), the mixture was cooled to 0 °C and Ac_2O (2 mL) was added. The reaction was allowed to stir at room temperature for 1 hour. After removal of the organic solvent, the crude was purified by column chromatography (hexanes/EtOAc: 1/1) to afford **88** (44 mg, 50 %).

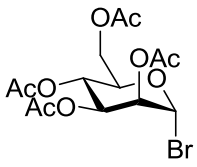


^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.09-8.06 (m, 1H, arom. H), 7.89-7.86 (m, 1H, arom. H), 7.78 (d, *J* = 8.2 Hz, 1H, arom. H), 7.59-7.44 (m, 4H, arom. H), 5.24 (dd, *J* = 3.4 Hz and 10.1 Hz, 1H, H-3), 5.14 (dd, *J* = 1.7 Hz and 3.4 Hz, 1H, H-2), 5.13 (app. s, 1H, NH), 5.08 (app. t, *J* = 10.0 Hz, 1H, H-4), 4.46 (d, *J* = 1.7 Hz, 1H, H-1), 3.80-3.73 (m, 1H, H-5), 3.69 (ddd, *J* = 2.8 Hz, 7.3 Hz and 14.2 Hz, 1H, H-6), 3.21 (ddd, *J* = 4.6 Hz, 7.3 Hz and 14.2 Hz, 1H, H-6), 3.07 (s, 3H, OCH_3), 2.03 (s, 3H, OAc), 2.01 (s, 3H, OAc), 1.97 (s, 3H, OAc).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 170.35, 170.10, 170.08, 157.03, 134.74, 133.24, 130.04, 128.71, 127.44, 127.10, 126.85, 125.96, 124.05, 122.30, 98.53, 69.75, 69.69, 69.12, 67.34, 55.11, 41.04, 20.98, 20.96, 20.90.

Exact mass (ESI-MS) for $C_{24}H_{28}N_2O_9$ $[M+H]^+$ found, 489.1875; calcd, 489.1868; $[M+Na]^+$ found, 511.1697; calcd, 511.1687; $[M+K]^+$ found, 527.1445; calcd, 527.1426.

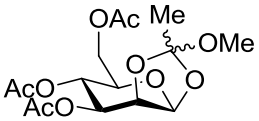
2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide (91)



To a solution of **90** (20.00 g, 51.24 mmol) in Ac_2O (15 mL) at 0 °C was added a solution of HBr in AcOH (45 mL). After stirring overnight, the reaction was quenched with ice, followed by extraction with CH_2Cl_2. The combined organic layers were washed with a saturated $NaHCO_3$ solution, dried over Na_2SO_4 and co-evaporated twice with toluene to afford compound **91** as a pale yellow syrup.

3,4,6-tri-*O*-acetyl-1,2-*O*-(1-methoxyethylidene)-β-D-mannopyranose (92)

To a solution of intermediate **91** in CH_2Cl_2 (110 mL) was added a mixture of 2,6-lutidine (16 mL) in MeOH (110 mL). After stirring overnight, the reaction mixture was extracted with CH_2Cl_2. The combined organic layers were washed with H_2O and a saturated $NaHCO_3$ solution, followed by drying over Na_2SO_4 and evaporation to dryness. The resulting residue was purified by column chromatography (hexanes/EtOAc: 6.5/3.5 + 1 V% Et_3N) to yield compound **92** (11.83 g, 67 % over 2 steps) as a yellow powder.



^{1}H-NMR (300 MHz, $CDCl_3$): δ 5.50 (d, *J* = 2.6 Hz, 1H, H-1), 5.30 (t, *J* = 9.8 Hz, 1H, H-4), 5.15 (dd, *J* = 4.0 Hz and 9.9 Hz, 1H, H-3), 4.62 (dd, *J* = 2.6 Hz and 4.0 Hz, 1H, H-2), 4.25 (dd, *J* = 4.9 Hz and 12.2 Hz, 1H, H-6), 4.14 (dd, *J* = 2.7 Hz and 12.1 Hz, 1H, H-6), 3.68 (ddd, *J* = 2.8 Hz, 4.8 Hz and 9.5 Hz, 1H, H-5), 3.28 (s, 3H, OCH_3), 2.12 (s, 3H, OAc), 2.08 (s, 3H, OAc), 2.06 (s, 3H, OAc), 1.52 (s, 3H, CH_3).

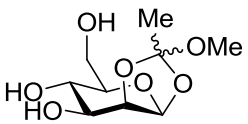
^{13}C-NMR (75 MHz, $CDCl_3$): δ 170.66, 170.39, 169.43, 124.52, 97.40, 71.36, 70.64, 65.51, 62.34, 49.94, 24.36, 20.77, 20.73, 20.68.

Exact mass (ESI-MS) for $C_{15}H_{22}O_{10}$ $[M+Na]^+$ found, 385.1121; calcd, 385.1111; $[M+K]^+$ found, 401.0873; calcd, 401.0850.

Spectral data are consistent with the literature data.

1,2-*O*-(1-methoxyethylidene)-β-D-mannopyranose (93)

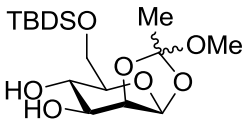
To a suspension of compound **92** (11.77 g, 34.98 mmol) in MeOH (120 mL) was added a solution of NH_3 in MeOH (7 N, 60 mL). After stirring overnight, the reaction mixture was evaporated to dryness, giving the crude **93** as a pale yellow syrup.



Exact mass (ESI-MS) for $C_9H_{16}O_7$ $[M+Na]^+$ found, 259.0790; calcd, 259.0794; $[M+K]^+$ found, 275.0532; calcd, 275.0533.

6-*O*-*tert*-butyldimethylsilyl-1,2-*O*-(1-methoxyethylidene)-β-D-mannopyranose (94)

To a suspension of **93** in THF (164 mL) was added imidazole (4.64 g, 68.16 mmol), giving a clear solution. Adding TBDMSCl (5.65 g, 37.49 mmol) again led to a white suspension. After stirring for 30 hours, the reaction mixture was filtered over celite, followed by rinsing of the filter cake with Et_2O. Evaporation of the solvent and purification of the residue by column chromatography (CH_2Cl_2/MeOH: 9.5/0.5 + 1 V% Et_3N) gave compound **94** (8.70 g, 76 % over 2 steps) as off-white crystals.



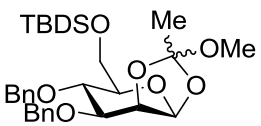
^{1}H-NMR 5.45 (d, *J* = 2.4 Hz, 1H, H-1), 4.50 (dd, *J* = 2.6 Hz and 3.8 Hz, 1H, H-2), 3.96 (dd, *J* = 4.4 Hz en 10.6 Hz, 1H, H-6), 3.85 (app. t, *J* = 9.2 Hz, 1H, H-4), 3.81 (dd, *J* = 5.9 Hz and 10.5 Hz, 1H, H-6), 3.78-3.75 (m, 1H, H-3), 3.31 (s, 3H, OMe), 3.28 (ddd, *J* = 1.3 Hz, 4.4 Hz and 5.8 Hz, 1H, H-5), 1.68 (s, 3H, CH_3), 0.90 (s, 9H, CH_3), 0.10 (s, 6H, CH_3).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 124.22, 97.73, 78.54, 73.06, 72.73, 70.35, 64.52, 49.80, 25.95, 25, 91, 24.90, 18.35, -5.37, -5.41.

Exact mass (ESI-MS) for $C_{15}H_{30}O_7Si$ $[M+Na]^+$ found, 373.1664; calcd, 373.1658; $[M+K]^+$ found, 389.1405; calcd, 389.1398.

3,4-di-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-1,2-*O*-(1-methoxyethylidene)-β-D-mannopyranose (95)

To a solution of **94** (8.70 g, 26.01 mmol) in DMF (262 mL) at 0 °C was added NaH (3.12 g, 78.03 mmol). After stirring for 30 minutes, benzyl bromide (9 mL, 78.03 mmol) was added and the solution was stirred overnight at room temperature. Upon reaction completion, the mixture was quenched with H_2O and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by column chromatography (hexanes/EtOAc: 8/2 + 1 V% Et_3N) to afford compound **95** (10.54 g, 76 %) as a pale yellow syrup.



^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.41-7.26 (m, 10H, arom. H), 5.30 (d, *J* = 2.3 Hz, 1H, H-1), 4.93 (d, *J* = 10.7 Hz, 1H, CH_2-Ph), 4.80-4.75 (m, 2H, CH_2-Ph), 4.73 (d, *J* = 10.7 Hz, 1H, CH_2-Ph), 4.36 (dd, *J* = 2.5 Hz and 3.9 Hz, 1H, H-2), 4.00 (app. d, *J* = 9.3 Hz, 1H, H-4) 3.94 (dd, *J* = 3.0 and 10.9 Hz, 1H, H-

6), 3.81 (dd, *J* = 1.9 Hz and 11.1 Hz, 1H, H-6), 3.72 (dd, *J* = 4.0 Hz and 9.3 Hz, 1H, H-3), 3.28 (s, 3H, OCH_3), 3.22 (dt, *J* = 2.5 Hz en 9.2 Hz, 1H, 5), 1.74 (s, 3H, CH_3), 0.90 (s, 9H, CH_3), 0.07 (s, 6H, CH_3).

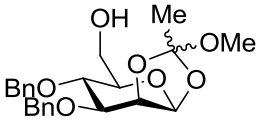
^{13}C-NMR (75 MHz, $CDCl_3$): δ 138.6, 138.0, 128.5, 128.4, 128.0, 127.9, 127.7, 124.0, 97.6, 79.2, 77.3, 75.2, 74.8, 73.8, 72.5, 62.0, 49.6, 25.9, 24.5, 18.2, -5.3, -5.4.

Exact mass (ESI-MS) for $C_{29}H_{42}O_7Si$ $[M+Na]^+$ found, 553.2608; calcd, 553.2597; $[M+K]^+$ found, 569.2346; calcd, 569.2337.

Spectral data are consistent with the literature data.

3,4-di-*O*-benzyl-1,2-*O*-(1-methoxyethylidene)-β-D-mannopyranose (96)

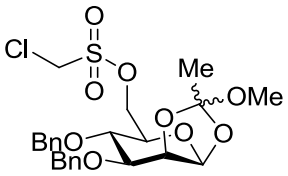
To a solution of **95** (10.54 g, 19.86 mmol) in THF (108 mL) at -78 °C was added a 1M solution of TBAF in THF (24 mL). After stirring for 1 hour, the solution was allowed to stir at room temperature. 2 hours later, the reaction mixture was diluted with EtOAc, washed with brine and dried over Na_2SO_4. Evaporation to dryness afforded compound **96** as a syrup.



Exact mass (ESI-MS) for $C_{23}H_{28}O_7$ $[M+Na]^+$ found, 439.1737; calcd, 439.1733; $[M+K]^+$ found, 455.1493; calcd, 455.1472.

3,4-di-*O*-benzyl-1,2-*O*-(1-methoxyethylidene)-6-monochloromethylsulfonyl-β-D-mannopyranose (97)

To a solution of the crude **96** in pyridine (79 mL) at 0 °C was added chloromethanesulfonylchloride (2 mL, 19.86 mmol). After stirring for 30 minutes, the red-brown reaction mixture was quenched with iced water and extracted with CH_2Cl_2. The combined organic layers were washed with a 1M solution of HCl, a saturated solution of $NaHCO_3$ and H_2O. Drying over Na_2SO_4 and concentration under reduced pressure resulted in **97** as a brown syrup.

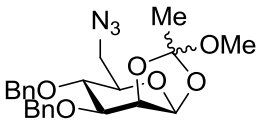


Exact mass (ESI-MS) for $C_{24}H_{30}ClO_9S$ $[M+Na]^+$ found, 551.1120; calcd, 551.1119; $[M+K]^+$ found, 567.0857; calcd, 567.0858.

6-azido-3,4-di-*O*-benzyl-6-deoxy-1,2-*O*-(1-methoxyethylidene)-β-D-mannopyranose (98)

A solution of the crude **97** in DMF (53 mL) was heated to 80 °C followed by addition of NaN_3 (2.58 g, 39.72 mmol). Upon reaction completion, the mixture was quenched with H_2O

and extracted with Et_2O. The combined organic layers were washed with H_2O, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by column chromatography (hexanes/EtOAc: 8/2 + 1 V% Et_3N), yielding compound **98** (3.45 g, 39 % over 3 steps) as a yellow syrup.



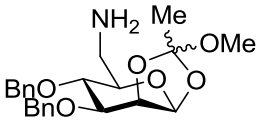
^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.42-7.26 (m, 10 H, arom. H), 5.34 (d, *J* = 2.5 Hz, 1H, H-1), 4.97 (d, *J* = 10.9 Hz, 1H, CH_2-Ph), 4.78 (app. s, 2H, CH_2-Ph), 4.66 (d, *J* = 10.9 Hz, 1H, CH_2-Ph), 4.42 (dd, *J* = 2.4 Hz en 4.0 Hz, 1H, H-2), 3.88 (app. t, *J* = 8.9 Hz, 1H, H-4), 3.73 (dd, *J* = 4.0 Hz en 9.3 Hz, 1H, H-3), 3.51 (dd, *J* = 3.4 Hz en 8.2 Hz, 1H, H-6), 3.42 (dd, *J* = 2.6 Hz en 4.4 Hz, 1H, H-6), 3.38 (m, 1H, H-5), 3.29 (s, 3H, OCH_3), 1.76 (s, 3H, CH_3).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 137.98, 137.60, 128.59, 128.56, 128.52, 128.12, 128.10, 128.06, 127.98, 124.11, 97.54, 78.88, 76.90, 75.43, 74.34, 73.40, 72.24, 51.19, 49.98, 24.12.

Exact mass (ESI-MS) for $C_{23}H_{27}N_3O_6$ $[M+Na]^+$ found, 464.1796; calcd, 464.1798; $[M+K]^+$ found, 480.1539; calcd, 480.1537.

6-amino-3,4-di-*O*-benzyl-6-deoxy-1,2-*O*-(1-methoxyethylidene)-β-D-mannopyranose (99)

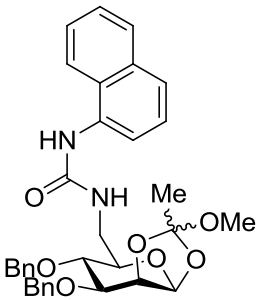
To a solution of **98** (3.45 g, 7.81 mmol) in THF (64 mL), a 1M solution of trimethylphosphine in THF (39 mL) was added dropwise. After stirring for 2 hours, H_2O (64 mL) was added and the reaction mixture was allowed to stir for an additional 2.5 hours. The solution was extracted with EtOAc, followed by washing of the organic layer with brine, drying over Na_2SO_4 and evaporation of the solvent to afford amine **99** as a yellow oil.



Exact mass (ESI-MS) for $C_{23}H_{29}NO_6$ $[M+H]^+$ found, 416.2086; calcd, 416.2073; $[M+Na]^+$ found, 438.1905; calcd, 438.1893.

3,4-di-*O*-benzyl-6-deoxy-6-(3-(1-naphthyl)ureido-1,2-*O*-(1-methoxyethylidene)-β-D-mannopyranose (100)

To a solution of **99** in DMF (110 mL) at 0 °C, 1-naphthylisocyanate was added. After stirring for 1 hour at room temperature, the solvent was evaporated under reduced pressure. Purification of the residue by column chromatography (hexanes/EtOAc: 6/4 + 1 V% Et_3N) resulted in compound **100** (3.04 g, 68 % over 2 steps) as a white powder.



^{1}H-NMR (300 MHz, CD_3OD): δ 8.02-7.99 (m, 1H, arom. H), 7.87-7.84 (m, 1H, arom. H), 7.69-7.63 (m, 2H, arom. H), 7.52-7.25 (m, 13H, arom. H), 5.43 (d, *J* = 2.1 Hz, 1H, H-1), 4.87 (d, *J* = 12.6 Hz, 1H, CH_2-Ph), 4.77 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.72 (d, *J* = 10.5 Hz, 1H, CH_2-Ph), 4.69 (d, *J* = 11.4 Hz, 1H, CH_2-Ph), 4.57 (app. t, *J* = 3.0 Hz, 1H, H-2), 3.88 (dd, *J* = 4.0 Hz and 9.1 Hz, 1H, H-3), 3.80 (app. d, *J* = 11.6 Hz, 1H, H-6), 3.60 (app. t, *J* = 8.9 Hz, 1H, H-4) 3.48-3.43 (m, 1H, H-5), 3.38 (app. d, *J* = 12.9 Hz, 1H, H-6), 3.25 (s, 3H, OCH_3), 1.60 (s, 3H, CH_3).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 162.85, 157.17, 138.18, 137.90, 134.67, 133.49, 129.35, 128.77, 128.69, 128.31, 128.14, 126.80, 126.60, 126.07, 123.96, 122.86, 122.19, 97.69, 79.08, 75.44, 74.68, 73.59, 72.66, 50.05, 41.09, 36.72, 31.69, 29.92, 24.37, 14.43.

Exact mass (ESI-MS) for $C_{34}H_{36}N_2O_7$ $[M+H]^+$ found, 585.2607; calcd, 585.2601; $[M+Na]^+$ found, 607.2421; calcd, 607.2420; $[M+K]^+$ found, 623.2146; calcd, 623.2160.

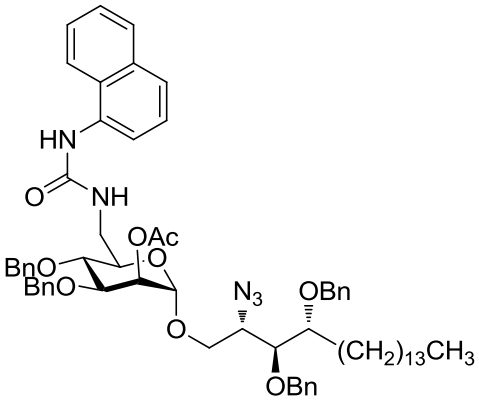
(2*S*,3*S*,4*R*)-2-azido-3,4-di-*O*-benzyl-1-*O*-(2-*O*-acetyl-3,4-di-*O*-benzyl-6-deoxy-6-(3-(1-naphthyl)ureido-α-D-mannopyranosyl)-octadecane-1,3,4-triol (101)

Procedure 1

A solution of (2*S*,3*S*,4*R*)-2-azido-3,4-di-benzyl-octadecane-1,3,4-triol (0.45g, 0.86 mmol) in CH_2Cl_2 (0.5 mL) was added to a suspension of mannosyldonor **100** (0.10g, 0.17 mmol) in CH_2Cl_2 (1.6 mL) at 0 °C. After stirring for 15 minutes under argon atmosphere, TMSOTf (0.01 mL, 0.05 mmol) was added, followed by stirring for an additional hour. The reaction mixture then was diluted with CH_2Cl_2, washed with a saturated solution of $NaHCO_3$ and H_2O, dried over Na_2SO_4 and evaporated to dryness. Purification by column chromatography (hexanes/EtOAc: 7.5/2.5) afforded compound **101** (0.03 g, 17 %) as a white powder.

Procedure 2

To a mixture of mannosyl donor **100** (0.10 g, 0.17 mmol) and powdered 4 Å molecular sieves in CH_2Cl_2 (1.6 mL) at 0 °C was added a solution of (2*S*,3*S*,4*R*)-2-azido-3,4-di-benzyl-octadecane-1,3,4-triol (0.45g, 0.86 mmol). After stirring for 15 minutes under argon atmosphere, TMSOTf (0.01 mL, 0.05 mmol) was added, followed by stirring for an additional hour. The reaction mixture then was diluted with CH_2Cl_2, washed with a saturated solution of $NaHCO_3$ and H_2O, dried over Na_2SO_4 and evaporated to dryness. Purification by column chromatography (hexanes/EtOAc: 7.5/2.5) afforded compound **101** (0.06 g, 34 %) as a white powder.



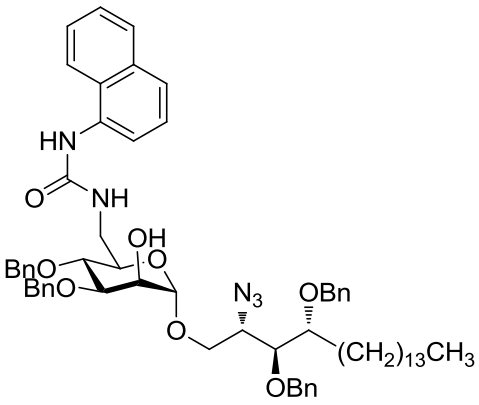
^{1}H-NMR (300 MHz, CD_3Cl_3): δ 7.98-7.86 (m, 1H, arom. H), 7.85-7.81 (m, 1H, arom. H), 7.69-7.66 (m, 1H, arom. H), 7.55-7.42 (m, 4H, arom. H), 7.41-7.20 (m, 20H, arom. H), 6.62 (s, 1H, NH), 5.32 (dd, *J* = 1.9 Hz and 3.4 Hz, 1H, H-2"), 4.87-4.82 (m, 1H, NH), 4.66 (d, *J* = 11.0 Hz, 1H, CH_2-Ph), 4.64 (d, *J* = 11.2 Hz, 1H, CH_2-Ph), 4.64 (*J* = 3.2 Hz, 1H, H-1"), 4.63-4.58 (m, 3H, CH_2-Ph), 4.56 (d, *J* = 11.4 Hz, 1H, CH_2-Ph), 4.51-4.48 (m, 2H, CH_2-Ph), 3.94 (dd, *J* = 3.2 Hz and 8.3 Hz, 1H, H-3"), 3.83 (dd, *J* = 2.4 Hz and 10.3 Hz, 1H, H-1), 3.67-3.52 (m, 6H, H-2, H-3, H-4, H-4", H-5" and H-6"), 3.44 (dd, *J* = 8.1 Hz and 10.3 Hz, H-1), 3.38 (dd, *J* = 4.8 Hz and 11.5 Hz, 1H, H-6"), 1.99 (s, 3H, CH_3), 1.63-1.17 (m, 26H, CH_2), 0.88 (t, *J* = 6.8 Hz, 3H, CH_3).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 173.77, 172.53, 157.00, 155.76, 138.48, 137.95, 137.25, 134.54, 133.31, 128.73, 128.06, 127.50, 126.83, 126.21, 124.92, 123.70, 122.05, 120.74, 119.51, 99.65, 98.43, 80.24, 79.86, 79.38, 79.01, 78.64, 78.16, 76.46, 76.04, 75.61, 74.24, 73.12, 72.25, 71.88, 71.55, 71.02, 70.32, 68.53, 67.67, 67.30, 66.46, 50.00, 48.77, 41.52, 40.28, 37.12, 35.88, 32.17, 30.94, 30.57, 29.94, 28.74, 28.38, 26.02, 24.79, 22.93, 21.70, 14.37, 13.14.

Exact mass (ESI-MS) for $C_{65}H_{81}N_5O_9$ $[M+H]^+$ found, 1076.6069; calcd, 1076.6113; $[M+Na]^+$ found, 1098.5884; calcd, 1098.5932; $[M+K]^+$ found, 1114.5629; calcd, 1114.5671.

(2*S*,3*S*,4*R*)-2-azido-3,4-di-*O*-benzyl-1-*O*-(3,4-di-*O*-benzyl-6-deoxy-6-(3-(1-naphthyl)ureido-α-D-mannopyranosyl)-octadecane-1,3,4-triol (102)

A 30 % solution of NaOMe in MeOH (0.4 mL, 2.10 mmol) was added to a solution of **101** (0.45 g, 0.42 mmol) in MeOH (1.7 mL) at 0 °C. The mixture was allowed to stir at room temperature but after 2 days, a lot of starting material remained and an additional amount of NaOMe in MeOH was added (0.4 mL, 2.10 mmol). After stirring overnight, the reaction mixture was neutralized with Amberlite IR 120 (H^+ form). Filtration and rinsing with MeOH and CH_2Cl_2 was followed by evaporation of the solvent under reduced pressure. Purification by column chromatography (hexanes/EtOAc: 7/3) furnished **102** (0.22 g, 50 %) as a yellow syrup.



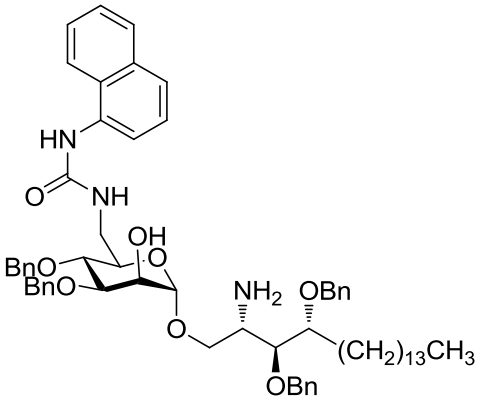
^{1}H-NMR (300 MHz, CD_3Cl_3): δ 7.99-7.97 (m, 1H, arom. H), 7.84-7.80 (m, 1H, arom. H), 7.66-7.59 (m, 2H, arom. H), 7.45-7.23 (m, 23H, arom. H), 6.84 (br. s, 1H, NH), 5.10 (br. s, 1H, NH), 4.82 (d, *J* = 10.6 Hz, 1H, CH_2-Ph), 4.69 (d, *J* = 1.5 Hz, 1H, H-1"), 4.67 (d, *J* = 10.4 Hz, 1H, CH_2-Ph), 4.63 (d, *J* = 11.1 Hz, 2H, CH_2-Ph), 4.59 (d, *J* = 11.8 Hz, 1H, CH_2-Ph), 4.57-4.50 (m, 3H, CH_2-Ph), 3.94 (dd, *J* = 1.7 Hz and 3.0 Hz, 1H, H-2"), 3.86-3.83 (m, 2H, H-1 and H-3"), 3.68-3.59 (m, 4H, H-2, H-3, H-5" and H-6"), 3.57-3.48 (m, 2H, H-4 and H-4"), 3.49 (dd, *J* = 8.3 Hz and 10.1 Hz, 1H, H-1), 3.39-3.34 (m, 1H, H-6"), 1.63-1.23 (m, 26 H, CH_2), 0.88 (t, *J* = 6.8 Hz, 3H, CH_3).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 171.40, 157.16, 138.39, 138.36, 138.02, 137.96, 134.63, 133.74, 129.17, 128.80, 128.69, 128.66, 128.63, 128.55, 128.27, 128.23, 128.21, 128.11, 128.07, 128.01, 126.63, 126.40, 126.22, 126.12, 122.58, 122.17, 99.81, 79.99, 79.42, 79.22, 76.84, 75.46, 74.56, 73.90, 72.34, 72.31, 70.96, 68.58, 68.17, 62.18, 60.63, 40.92, 32.16, 30.14, 30.01, 29.94, 29.91, 29.87, 29.82, 29.60, 25.37, 22.93, 21.28, 14.43, 14.36.

Exact mass (ESI-MS) for $C_{63}H_{79}N_5O_8$ $[M+H]^+$ found, 1034.6049; calcd, 1034.6007; $[M+Na]^+$ found, 1056.5845; calcd, 1056.5826; $[M+K]^+$ found, 1072.5543; calcd, 1072.5566.

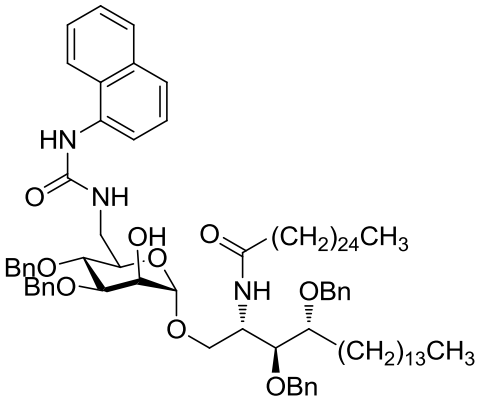
(2*S*,3*S*,4*R*)-2-amino-3,4-di-*O*-benzyl-1-*O*-(3,4-di-*O*-benzyl-6-deoxy-6-(3-(1-naphthyl)ureido-α-D-mannopyranosyl)-octadecane-1,3,4-triol (103)

To a suspension of **102** (0.37 g, 0.36 mmol) in THF (2.9 mL), a 1M solution of trimethylphosphine in THF (1.8 mL) was added dropwise. After stirring for 2 hours, H_2O (2.9 mL) was added and the reaction mixture was allowed to stir for an additional 2.5 hours. The solution was extracted with EtOAc, followed by washing of the organic layer with brine, drying over Na_2SO_4 and evaporation of the solvent to afford amine **103**.



Exact mass (ESI-MS) for $C_{63}H_{81}N_3O_8$ $[M+H]^+$ found, 1008.6169; calcd, 1008.6102; $[M+Na]^+$ found, 1030.5984 calcd, 1030.5921; $[M+K]^+$ found, 1046.5747; calcd, 1046.5661.

(2*S*,3*S*,4*R*)-3,4-di-*O*-benzyl-1-*O*-(3,4-di-*O*-benzyl-6-deoxy-6-(3-(1-naphthyl)ureido-α-D-mannopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (104)



Hexacosanoic acid (0.28 g, 0.72 mmol) was added to a suspension of EDC (0.17 g, 0.90 mmol) in CH_2Cl_2 (1.5 mL). After stirring for 30 minutes, a suspension of **103** was added and the solution was allowed to stir overnight. The reaction mixture was diluted with CH_2Cl_2, washed with H_2O and brine, dried over Na_2SO_4 and evaporated to dryness. The resulting residue was purified by column chromatography (hexanes/EtOAc: 6.5/3.5) to yield compound **104** (0.32 g, 64 % over 2 steps) as a yellow syrup.

^{1}H-NMR (300 MHz, CD_3Cl_3): δ 8.08-8.04 (m, 1H, arom. H), 7.82-7.78 (m, 2H, arom. H), 7.64-7.58 (m, 2H, arom. H), 7.45-7.24 (m, 22H, arom. H), 5.81 (d, *J* = 8.5 Hz, 1H, NH), 5.56 (br. s, 1H, NH), 4.79 (d, *J* = 10.6 Hz, 1H, CH_2-Ph), 4.71 (d, *J* = 1.9 Hz, 1H, H-1”), 4.71-4.54 (m, 6H, CH_2-Ph), 4.45 (d, *J* = 11.7 Hz, 1H, CH_2-Ph), 4.40-4.36 (m, 1H, H-2), 3.82 (app. d, *J* = 1.5 Hz, 1H, H-2”), 3.79-3.70 (m, 2H, H-3” and H-1), 3.65-3.57 (m, 6H, H-1, H-6”, H-3, H-4, H-4” and H-5”), 3.50-3.44 (m, 1H, H-6”), 2.53 (br. s, 1H, OH), 1.99-1.83 (m, 2H, $COCH_2$), 1.64-1.11 (m, 72H, CH_2), 0.89 (t, *J* = 6.5 Hz, 6H, CH_3).

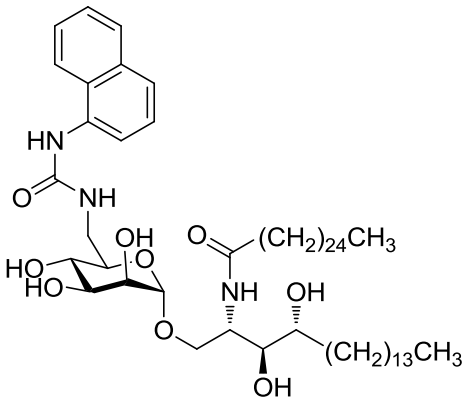
^{13}C-NMR (75 MHz, $CDCl_3$): δ 173.77, 157.04, 138.48, 138.40, 138.23, 137.96, 134.55, 134.30, 128.80, 128.74, 128.73, 128.66, 128.49, 128.27, 128.19, 128.16, 128.10, 128.07, 126.22, 126.16, 126.08, 124.94, 122.07, 120.77, 99.69, 80.24, 79.87, 79.37, 77.49, 75.48, 75.39, 73.13, 72.35, 72.25, 71.56, 68.53, 67.67, 50.00, 41.50, 37.12, 32.18, 30.57, 29.98, 29.96, 29.91, 29.77, 29.63, 29.61, 29.54, 29.52, 26.12, 26.03, 22.95, 14.38.

Exact mass (ESI-MS) for $C_{89}H_{131}N_3O_9$ $[M+H]^+$ found, 1386.9740; calcd, 1386.9964; $[M+Na]^+$ found, 1408.9675; calcd, 1408.9783; $[M+K]^+$ found, 1424.9347; calcd, 1424.9522.

(2*S*,3*S*,4*R*)-1-*O*-(6-deoxy-6-(3-(1-naphthyl)ureido-α-D-mannopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (12)

A solution of compound **104** (0.15 g, 0.11 mmol) in EtOH/$CHCl_3$ (3.6 mL/1.2 mL) was hydrogenated under atmospheric pressure for 5 hours in the presence of palladium black (15 mg). The solution then was filtered through celite, followed by rinsing of the filter cake with MeOH and evaporation of the solvent under reduced pressure. Purification by column

chromatography (CH_2Cl_2/MeOH: 28/2) afforded compound **12** (0.04 g, 34 %) as a white powder.



^{1}H-NMR (300 MHz, pyridine-d_5): δ 9.48 (s, 1H, NH), 8.94 (d, *J* = 8.3 Hz, 1H, NH), 8.53 (dd, *J* = 7.4 Hz, 1H, arom. H), 8.45-8.42 (m, 1H, arom. H), 7.89 (m, 1H, arom. H), 7.64 (d, *J* = 8.2 Hz, 1H, arom. H), 7.53-7.44 (m, 3H, arom. H), 7.22 (s, 1H, NH (under pyridine)), 5.45 (app. s, 1H, H-1''), 5.24-5.14 (m, 1H, H-2), 4.63 (dd, *J* = 3.1 Hz en 10.4 Hz, 1H, H-1), 4.54-4.53 (m, 3H, H-2'', H-3'' and H-4''), 4.45-4.37 (m, 2H, H-3 and H-5''), 4.35-4.28 (m, 3H, H-1, H-4 and H-6''), 4.20-4.14 (m, 1H, H-6''), 2.48 (dd, *J* = 5.0 Hz and 7.3 Hz, 2H, $COCH_2$), 1.97-1.22 (m, 72H, CH_2), 0.88 (t, *J* = 6.6 Hz, 6H, CH_3).

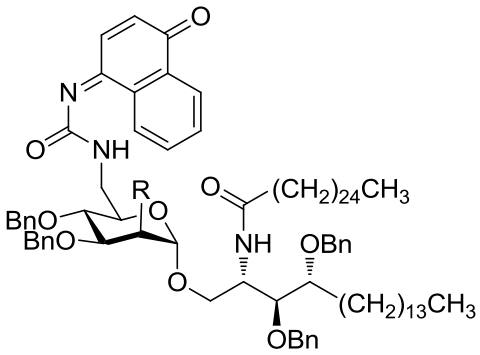
^{13}C-NMR (75 MHz, $CDCl_3$): δ 172.93, 156.50, 135.10, 133.62, 127.63, 126.28, 125.32, 124.81, 124.60, 121.61, 121.28, 117.61, 101.57, 74.58, 72.78, 71.66, 71.23, 70.94, 68.53, 66.72, 51.65, 41.27, 35.73, 32.60, 30.96, 30.94, 29.15, 28.96, 28.89, 28.84, 28.77, 28.74, 28.68, 28.58, 28.54, 28.46, 28.43, 25.41, 25.33, 21.77, 21.76, 13.11.

Exact mass (ESI-MS) for $C_{61}H_{107}N_3O_9$ $[M+H]^+$ found, 1026.8130; calcd, 1026.8086; $[M+Na]^+$ found, 1048.7919; calcd, 1048.7905; $[M+K]^+$ found, 1064.7678; calcd, 1064.7644.

(2*S*,3*S*,4*R*)-3,4-di-*O*-benzyl-1-*O*-(3,4-di-*O*-benzyl-6-deoxy-6-(3-(1-naphthyl)ureido-α-D-mannopyran-2-on)-2-hexacosylamino-octadecane-1,3,4-triol (107)

Dess-Martin oxidation

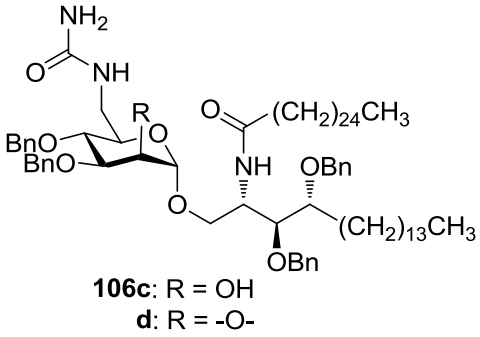
To a solution of **104** in CH_2Cl_2 was added Dess Martin periodinane. After stirring for 2 hours at room temperature, the reaction mixture was diluted with CH_2Cl_2 and sequentially washed with $Na_2S_2O_3$ and brine. The organic layer then was dried over Na_2SO_4 followed by concentration under reduced pressure to give a mixture containing mainly the undesired **106a-d**.



106 a: R = OH
b: R = -O-

Exact mass (ESI-MS) for $C_{89}H_{129}N_3O_{10}$ (a) $[M+H]^+$ found, 1400.9719; calcd, 1400.9756; $[M+Na]^+$ found, 1422.9591; calcd, 1422.9576; $[M+K]^+$ found, 1438.9343; calcd, 1438.9315.

Exact mass (ESI-MS) for $C_{89}H_{127}N_3O_{10}$ (b) $[M+H]^+$ found, 1398.9630; calcd, 1398.9600; $[M+Na]^+$ found, 1420.9406; calcd, 1420.9419; $[M+K]^+$ found, 1436.9088; calcd, 1436.9159.



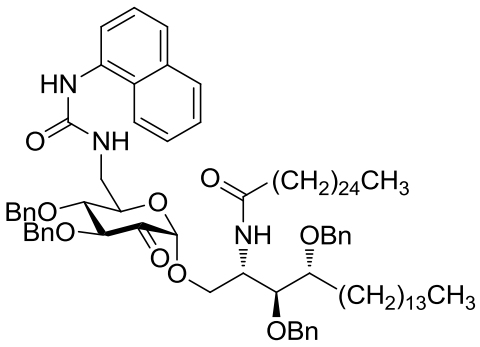
106c: R = OH
d: R = -O-

Exact mass (ESI-MS) for $C_{79}H_{125}N_3O_9$ (c) $[M+H]^+$ found, 1260.9498; calcd, 1260.9494; $[M+Na]^+$ found, 1282.9339; calcd, 1282.9314; $[M+K]^+$ found, 1298.9089; calcd, 1298.9053.

Exact mass (ESI-MS) for $C_{79}H_{123}N_3O_9$ (d) $[M+H]^+$ found, 1258.9302; calcd, 1258.9338; $[M+Na]^+$ found, 1280.9169; calcd, 1280.9157; $[M+K]^+$ found, 1296.8853; calcd, 1296.8896.

Swern oxidation

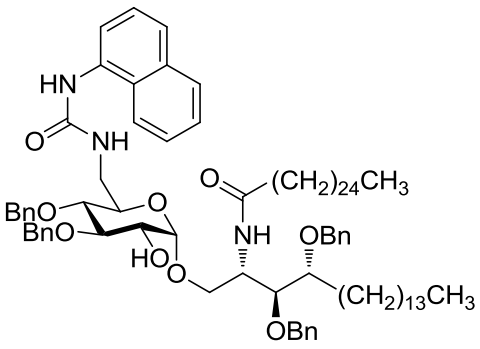
Dimethylsulfoxide (0.05 mL, 0.67 mmol) was added to a solution of oxalyl chloride (0.03 mL; 0.32 mmol) in CH_2Cl_2 (3 mL) at -78 °C. After stirring for 30 minutes, a solution of **104** (320 mg, 0.23 mmol) in CH_2Cl_2 (3 mL) was added to the reaction and the stirring was continued for another 45 minutes. Et_3N (0.14 mL, 0.99 mmol) was then added to the reaction and the cooling bath was removed. After another hour of stirring, the reaction was diluted with CH_2Cl_2 and the organic layer was sequentially washed with 5 % HCl, saturated $NaHCO_3$ and brine. Subsequent drying over Na_2SO_4 and evaporation to dryness provided the crude ketone **107** (303 mg), which was used without any further purification.



Exact mass (ESI-MS) for $C_{89}H_{129}N_3O_9$ $[M+H]^+$ found, 1384.9815; calcd, 1384.9807; $[M+Na]^+$ found, 1406.9630; calcd, 1406.9627; $[M+K]^+$ found, 1422.9333; calcd, 1422.9366.

(2*S*,3*S*,4*R*)-3,4-di-*O*-benzyl-1-*O*-(3,4-di-*O*-benzyl-6-deoxy-6-(3-(1-naphthyl)ureido-α-D-glucopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (108)

To a solution of **107** in THF (6 mL) at 0 °C was added $NaBH_4$ (21 mg, 0.25 mmol). After stirring for 5 hours at room temperature, the reaction mixture was concentrated under reduced pressure. Purification by column chromatography (CH_2Cl_2/EtOAc: 8.7/1.3) furnished **108** (56 mg, 16 % over 2 steps) as an orange solid.



^{1}H-NMR (300 MHz, CD_3Cl_3): δ 7.98-7.95 (m, 1H, arom. H), 7.76-7.71 (m, 1H, arom. H), 7.68 (d, *J* = 7.3 Hz, 1H, arom. H), 7.55 (d, *J* = 8.2 Hz, 1H, arom. H), 7.42-7.14 (m, 24H, arom. H and NH), 5.76 (d, *J* = 8.8 Hz, 1H, NH), 5.14 (br. s, 1H, NH), 4.79 (d, *J* = 11.1 Hz, 1H, CH_2-Ph), 4.74 (d, *J* = 10.5 Hz, 1H, CH_2-Ph), 4.66 (d, *J* = 11.1 Hz, 1H, CH_2-Ph), 4.62 (d, *J* = 11.7 Hz, 1H, CH_2-Ph), 4.57 (d, *J* = 10.8 Hz, 1H, CH_2-Ph), 4.50 (d, *J* = 3.8 Hz, 1H, H-1''), 4.47 (br. s, 2H, CH_2-Ph), 4.33 (d, *J* = 11.7 Hz, 1H, CH_2-Ph), 4.35-4.31 (m, 1H, H-2), 3.71 (dd, *J* = 3.5 Hz and 11.4 Hz, 1H, H-1), 3.59-3.50 (m, 5H, H-1, H-3, H-4, H-3'' and H-5''), 3.48-3.44 (m, 1H, H-6''), 3.42-3.32 (m, 2H, H-2'' and H-6''), 3.16 (app. t, *J* = 9.2 Hz, 1H, H-4''), 2.39 (d, *J* = 8.5 Hz, 1H, OH), 1.98-1.78 (m, 2H, $COCH_2$), 1.66-1.10 (m, 72H, CH_2), 0.81 (t, *J* = 6.7 Hz, 6H, CH_3).

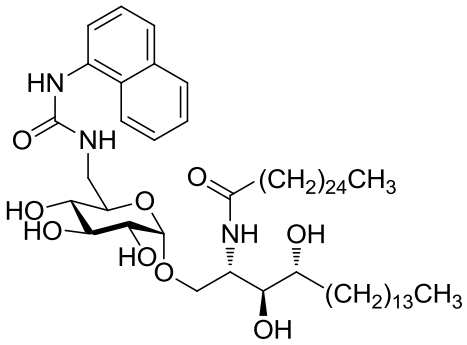
^{13}C-NMR (75 MHz, $CDCl_3$): δ 173.85, 156.93, 138.88, 138.33, 138.15, 138.07, 134.57, 134.10, 128.89, 128.71, 128.69, 128.64, 128.62, 128.58, 128.53, 128.41, 128.37, 128.34, 128.29128.14, 128.12, 128.09, 127.90, 126.35, 126.25, 126.18, 125.34, 121.98, 121.04, 99.95, 83.40, 81.11, 79.06, 78.70, 77.47, 75.53, 75.46, 73.53, 73.19, 72.25, 71.00, 69.79, 50.13, 41.45, 37.09, 32.18, 30.69, 29.97, 29.95, 29.92, 29.90, 29.88, 29.78, 29.62, 29.60, 29.57, 25.96, 25.78, 22.94, 14.37.

Exact mass (ESI-MS) for $C_{63}H_{79}N_5O_8$ $[M+H]^+$ found, 1387.0018; calcd, 1386.9964; $[M+Na]^+$ found, 1408.9834; calcd, 1408.9783; $[M+K]^+$ found, 1424.9579; calcd, 1424.9522.

(2*S*,3*S*,4*R*)-1-*O*-(6-deoxy-6-(3-(1-naphthyl)ureido-α-D-glucopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (13)

A solution of compound **108** (56 mg, 0.038 mmol) in EtOH/$CHCl_3$ (1.2 mL/0.4 mL) was hydrogenated under atmospheric pressure for 16 hours in the presence of palladium black (5 mg). The solution then was filtered through celite, followed by rinsing of the filter cake with MeOH and evaporation of the solvent under reduced pressure. Purification by column

chromatography (CH_2Cl_2/MeOH: 28/2) afforded compound **13** (13 mg, 33 %) as a white powder.



^{1}H-NMR (300 MHz, pyridine-d_5): δ 9.51 (s, 1H, NH), 8.67 (d, *J* = 8.5 Hz, 1H, NH), 8.56 (d, *J* = 6.7 Hz, 1H, arom. H), 8.43 (dd, *J* = 2.6 Hz and 9.4 Hz, 1H, arom. H), 7.90 (dd, *J* = 3.2 Hz and 6.2 Hz, 1H, arom. H), 7.65 (d, *J* = 8.2 Hz, 1H, arom. H), 7.54 (d, *J* = 7.9 Hz, 1H, arom. H), 7.50-7.42 (m, 2H, arom. H), 7.13 (t, *J* = 5.3 Hz, 1H, NH), 6.55 (br. s, 1H, OH), 6.23 (br. s, 1H, OH), 5.48 (d, *J* = 3.8 Hz, 1H, H-1”), 5.32-5.31 (m, 1H, H-2), 4.67 (dd, *J* = 5.1 Hz and 11.3 Hz, 1H, H-1), 4.52 (app. t, *J* = 9.1 Hz, 1H, H-3”), 4.43-4.38 (m, 1H, H-5”), 4.36-4.28 (m, 4H, H-3, H-4, H-1 and H-6”), 4.11 (dd, *J* = 5.9 Hz and 13.5 Hz, 1H, H-6”), 4.04 (dd, *J* = 3.8 Hz and 9.7 Hz, 1H, H-2”), 3.97 (app. t, *J* = 9.2 Hz, 1H, H-4”) 2.44 (t, *J* = 7.6 Hz, 2H, $COCH_2$), 1.90-1.21 (m, 72H, CH_2), 0.88 (t, *J* = 6.6 Hz, 6H, CH_3).

^{13}C-NMR (75 MHz, pyridine-d_5): δ 172.62, 156.41, 135.11, 133.66, 127.68, 126.32, 125.35, 124.86, 124.63, 121.99, 121.59, 117.65, 100.09, 75.42, 73.59, 72.31, 71.83, 71.69, 67.64, 50.46, 41.31, 35.69, 33.35, 30.95, 30.94, 29.18, 28.94, 28.87, 28.82, 28.75, 28.74, 28.66, 28.57, 28.44, 28.43, 25.27, 21.76, 13.10.

Exact mass (ESI-MS) for $C_{61}H_{107}N_3O_9$ $[M+H]^+$ found, 1026.8115; calcd, 1026.8086.

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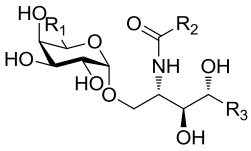
CHAPTHER 6

GENERAL CONCLUSION

6 General conclusion

We developed an efficient route towards the synthesis of a wide variety of C-5" and C-6"-modified α-GalCer analogues. This method also allows the late-stage incorporation of alternative acyl chains. A summary of the acquired data of the synthesized compounds is represented in Table 6.1. Consistent with earlier reports in literature we showed via the synthesis of deoxy compound **2** and galacturonic acid **4** that combination of two favorable modifications does not necessarily lead to compounds with an enhanced biological profile. Instead, we observed a remarkably decreased iNKT cell activity for these compounds.



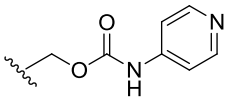
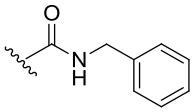
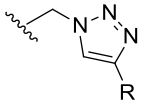
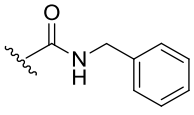
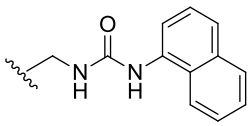
Glycolipid	R_1	R_2	R_3	Activity
1	CH_3	$(CH_2)_{24}CH_3$	$(CH_2)_{13}CH_3$	No net polarization, strong antigenicity[a]
2	CH_3	$(CH_2)_5Ph$	$(CH_2)_{13}CH_3$	Very weak antigenicity[a]
3	COOH	$(CH_2)_{24}CH_3$	$(CH_2)_{13}CH_3$	Very weak antigenicity[a]
4	COOH	$(CH_2)_5Ph$	$(CH_2)_{13}CH_3$	Very weak antigenicity[a]
5c		$(CH_2)_{24}CH_3$	$(CH_2)_{13}CH_3$	Th1, strong antigenicity[a]
6c		$(CH_2)_{24}CH_3$	$(CH_2)_{13}CH_3$	Weak Th1, weaker antigenicity than α-GalCer[a]
8		$(CH_2)_{24}CH_3$	$(CH_2)_{13}CH_3$	Similar iNKT cell activation than α-GalCer[b]
9a		$(CH_2)_{22}CH_3$	$(CH_2)_4CH_3$	Th1, weaker antigenicity than **6c**[a]
9b		$(CH_2)_{22}CH_3$	$(CH_2)_4CH_3$	Th1, weaker antigenicity than NU-α-GalCer[a]

Table 6.1 Summary of the acquired data.
[a] based on IL-4 and IFN-γ measurement (mice, *in vivo*), [b] based on IL-2 measurement (mice, *in vitro*).

The introduction of several aromatic groups via different linker types at C-5” or C-6” showed that the effect can be twofold. Certain aromatic groups can result in novel interactions with the TCR as illustrated by 4-pyridyl substituted carbamate **5c**. On the other hand, increased interactions with CD1d might arise, for example by the formation of an extra hydrogen bond and/or by the induction of an additional hydrophobic pocket. As the latter has only been observed for the two naphthyl-containing compounds (NU-α-GalCer and **5b**), it is likely that the size of the aromatic group is the key determinant rather than the linker type or length. However, the linker length might affect the orientation of the carbohydrate moiety as has been shown for BnNH-GSL-1’ (**6c**).

Regarding the therapeutic potential, we identified the carbamate-based glycolipids **5** as a new class of iNKT cell ligands with superior anti-tumor effects.
Based on the preliminary results of the competition assays with NU-α-ManCer (**12**) we assume that this compound does not act as an iNKT cell antagonist, hence will not be useful in the treatment of allergic inflammatory disorders.

The promising results of the C-6”-carbamates encourage further exploration of this class of compounds by substituting the C-6”-linker with other aromatic and non-aromatic groups. On the other hand, combination with other modifications, for example with C-type glycosides, might also lead to interesting findings. Finally, more research with regard to the human system is urgently awaited.

APPENDIX

CURRICULUM VITAE

Curriculum Vitae

Personalia

Name	Nora Pauwels
Date of birth	August 21, 1985
Place of birth	Ghent
E-mail:	nora.pauwels@ugent.be

Education

2008-present	PhD Pharmaceutical Sciences Title: Synthesis of new α-GalCer analogues as iNKT cell targeting agents Laboratory for Medicinal Chemistry, Ugent Promotor: Prof. Serge Van Calenbergh
2003-2008	Master in Pharmaceutical Sciences, *magna cum laude*, Ugent Thesis: Synthèse de nouveaux pseudo-nucléotides susceptibles de moduler la multi-chimiorésistance des traitements des cancers cérébraux (Laboratoire de Chimie Thérapeutique, Université Claude Bernard Lyon I)
1997-2003	Sciences-Maths, St-Bavo Humaniora, Ghent

Teaching experience

2008-2012	Tutor "Seminars Medicinal Chemistry", 3rd bachelor Pharmaceutical Sciences Prof. Serge Van Calenbergh
2009-2010	Tutor "Problem-based Pharmaceutical Education", 3rd bachelor Pharmaceutical Sciences Prof. Niek Sanders

Supervision of master students

2008-2009	Eline Van Hecke Thesis: Curtiusomlegging van een galacturonzuurderivaat als model voor nieuwe α-GalCer analogen
2010-2011	Annelies Comeyne

	Thesis: Synthese van een 6”-deoxy-6”-naftylureumderivaat van α-mannosylceramide
2011-2012	Sabah Kasmi
	Thesis: Synthese van een 6”-deoxy-6”-naftylureumderivaat van α-mannosylceramide

Scientific publications

Synthesis of a sugar-modified α-GalCer analogue as a potential iNKT cell antagonist.

Pauwels N., Comeyne A., Guillaume J., Risseeuw M., Tocheva A., Gadola S., Van Calenbergh S.

In preparation

Enhanced TCR footprint by a novel glycolipid increases NKT dependent tumor protection.

Aspeslagh S., Nemčovič M., **Pauwels N.**, Venken K., Van Calenbergh S., Zajonc D. M., Elewaut D.

Journal of Immunology 191(6): 2916-2925 (2013).

Synthesis of 6”-triazole-substituted α-GalCer analogues as potent iNKT cell stimulating ligands.

Pauwels N., Aspeslagh S., Elewaut D., Van Calenbergh S.

Bioorganic and Medicinal Chemistry. 20(24): 7149-7154 (2012).

Crystal structures of bovine CD1d reveal altered αGalCer presentation and a restricted A' pocket unable to bind long-chain glycolipids.

Wang J., Guillaume J., **Pauwels N.**, Van Calenbergh S., Van Rhijn I., Zajonc D. M.

PLoS One 7(10): e47989 (2012).

Galactose-modified iNKT cell agonists stabilized by an induced fit of CD1d prevent tumour metastasis.

Aspeslagh S., Li Y., Yu E. D., **Pauwels N.,** Trappeniers M., Girardie E., Decruy T., Van Beneden K., Venken K., Drennan M., Leybaert L., Wang J., Franck R. W., Van Calenbergh S., Zajonc D., Elewaut D.

The EMBO Journal. 30(11): 2294-2305 (2011).

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Pauwels N., Aspeslagh S., Vanhoenacker G., Sandra K., Yu E. D., Zajonc D. M., Elewaut D., Linclau B., Van Calenbergh S.

Organic and Biomolecular Chemistry. 9(24): 8413-8421 (2011).

Conferences

Annual One-Day Meeting on Medicinal Chemistry of SRC & KVCV

Liège, Belgium, November 30, 2012

EFMC-ISMC. 22nd International Symposium on Medicinal Chemistry

Berlin, Germany, September 2-6, 2012

Poster presentation: Versatile Synthesis of C-5” and C-6”-modified α-GalCer analogues as new iNKT cell ligands (N. Pauwels, S. Aspeslagh, D. Elewaut and S. Van Calenbergh)

ChemCYS. 11th Chemistry Conference for Young Scientists

Blankenberge, Belgium, March 1-2, 2012

Oral Communication: Versatile Synthesis of C-5”- and C-6”-modified α-GalCer analogues as new iNKT cell ligands (N. Pauwels, S. Aspeslagh, D. Elewaut and S. Van Calenbergh)

Annual One-Day Meeting on Medicinal Chemistry of SRC & KVCV

Ghent, Belgium, November 25, 2011

6th International Symposium on CD1 and NKT cells

Chicago, USA, September 23-27, 2011

Poster presentation: Versatile synthesis and biological evaluation of C-5”- and C-6”-modified α-GalCer analogues as new NKT cell ligands (N. Pauwels, S. Aspeslagh, D. Zajonc, D. Elewaut and S. Van Calenbergh)

Glen. 1st Glycolipid European Network Congress

Reims Champagne-Ardenne University, France, November 16-17, 2010

Oral Communication: Versatile Synthesis and biological evaluation of C-6”-modified α-GalCer analogues as new NKT-cell ligands (N. Pauwels and S. Van Calenbergh)

EFMC-ISMC. 21st International Symposium on Medicinal Chemistry

Brussels, Belgium, September 5-9, 2010

Poster presentation: Versatile Synthesis of C-5”-modified α-GalCer analogues as new NKT-cell ligands (N. Pauwels and S. Van Calenbergh)

10th Flemish Youth Conference of Chemistry (VJC 10)

Blankenberge, March 1-2, 2010.

Poster presentation: Synthesis of C-5”-modified α-galactosylceramide analogues.

(N. Pauwels and S. Van Calenbergh)

Prize for best poster presentation Medicinal Chemistry

13th Sigma-Aldrich Organic Synthesis Meeting

Sol Cress, Spa, Belgium, December 3-4, 2009.

Poster presentation: Synthesis of C-5’-modified α-galactosylceramide analogues (N. Pauwels and S. Van Calenbergh)

Annual One-Day Meeting on Medicinal Chemistry of SRC & KVCV

Brussels, Belgium, November 6, 2009

12th Sigma-Aldrich Organic Synthesis Meeting

Sol Cress, Spa, Belgium, December 4-5, 2008

Annual One-Day Meeting on Medicinal Chemistry of SRC & KVCV

Leuven, Belgium, November 7, 2008

Dankwoord

Na 5 jaar onderzoek is het hoog tijd om een aantal mensen in de bloemetjes te zetten die rechtstreeks of onrechtstreeks tot dit werk hebben bijgedragen.

In de eerste plaats wil ik een woord van dank richten aan mijn promotor, prof. Serge Van Calenbergh. Niet alleen voor de kans die ik gekregen heb om in jouw labo te starten, maar des te meer voor de vele kansen en onvergetelijke ervaringen die nadien volgden. Al op de eerste dag zei je dat je deur altijd zou openstaan en dat was zeker niet gelogen. Serge, bedankt voor je geduld en vertrouwen, ook als de resultaten soms wat op zich lieten wachten. Ook voor het nauwgezet en kritisch nalezen van mijn artikels en doctoraat.

Dit werk had niet gerealiseerd kunnen worden zonder de biologische evaluatie van de gesynthetiseerde analogen. Ik ben dan ook veel dank verschuldigd aan de verschillende biologen die deze experimenten hebben uitgevoerd. Bedankt prof. Dirk Elewaut en Sandrine Aspeslagh voor de bepalingen van de cytokinesecreties. Thanks a lot prof. Dirk Zajonc for the crystallographic studies. I'm also very grateful to prof. Stephan Gadola and Anna Tocheva for the realization of the TCR tetramer staining experiments.

Uiteraard had mijn periode in het labo nooit zo aangenaam geweest zonder de hulp en aanwezigheid van mijn goede collega's. Martijn and Kiran, you were always ready to answer my questions and to advise me when I encountered a problem. Thanks a lot! Joren en Annelies, bedankt voor de boeiende α-GalCer discussies en de gezellige babbels in de bureau en het labo. René, ray of sunshine, I'll miss your funny statements, your sometimes strange questions and your ever-present smile. Dries en Arno, ook bij jullie kon ik steeds terecht voor een goed gesprek en niet te vergeten, onze loopmiddagen/avonden samen met Joren, Izet en Annelies waardoor we eens letterlijk en figuurlijk konden uitwaaien. Lijun, although we only shared a short period in the lab, you were a very nice person to be around. Good luck to all of you with the continuation of your projects! Ik mis nu al onze koffiemomentjes en de frietjes op vrijdag. Izet en Annelies, ontzettend bedankt voor de dagelijkse administratieve en technische ondersteuning. Zonder jullie zou het met momenten toch serieus in de soep gedraaid zijn. Ook de oud-collega's mogen hier zeker niet vergeten worden: Ulrik, Radim, Matthias, Thomas, Sara, Shari en Jolien. Speciale dank aan Sara voor het uitwerken van mijn voorkaft.

In het leven buiten het labo kon ik steeds rekenen op een fantastisch thuisfront. Mama en papa, jullie hebben sowieso al enorm veel gedaan voor mij tijdens mijn studiejaren farmacie, maar ook in de afgelopen 5 jaar kon ik niets te veel aan jullie vragen. Ontzettend bedankt daarvoor! Steven, we lopen elkaars deur dan wel niet plat, je immer aanwezige humor op elke bijeenkomst voorzag me telkens van de nodige ontspanning. Lieve Oma en Bompa, bedankt voor jullie luisterend oor en de deugddoende aanmoedigingen.

Tot slot kom ik bij de belangrijkste persoon in mijn leven. Davy, ik denk niet dat ik met woorden kan beschrijven hoezeer ik je dankbaar ben voor je steun en hulp tijdens de voorbije jaren. In gelijk welke gemoedstoestand ik van het labo thuiskwam - enthousiast, ongeduldig, zenuwachtig of teleurgesteld - steeds was je er om naar mij te luisteren, samen een vreugdedansje te doen of om mij op te peppen. Bedankt voor alles!