

Marcas – Patentes
Modelos – Diseños
Nombres Comerciales – Enseñas
Propiedad Intelectual
Registros en el Exterior
Registros Sanitarios INVIMA – ICA
Acciones Judiciales – Administrativas
Derecho Comercial – Industrial
Asesoría – Contratos – Otros
Corresponsalia a Nivel Mundial



Mario Delgado Echeverry e Hijos SAS.

Abogados Consultores – Marcas y Patentes

Bogotá D.C. – Colombia
Avda. Kra. 24 Park Way No. 37 – 31 Of.
202
Tels. (571) 2444096 – 3690624
Fax (571) 2442496 A.A. No. 6207
E-mail: mde@mdelaw.com
mdelawof@cable.net.co
mdeabogados@cable.net.co

Miembros ACPI – ASIPI - AIPPI

Bogotá D.C., 1 de octubre de 2014

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 13-159964- -00005-0000

Fecha: 2014-10-01 16:34:34 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 378 FASENACIONALI
Act. 523 RESPUESTA Folios: 30

Señores
DIVISION DE NUEVAS CREACIONES
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
Ciudad

REFERENCIA: Expediente No. 13 – 159.964

TÍTULO: “MÉTODOS PARA PREPARAR GLICOESFINGOLÍPIDOS Y USOS DE LOS MISMOS”

SOLICITANTE: NATIONAL TAIWAN UNIVERSITY

TRÁMITE: Respuesta a Concepto Técnico notificado mediante Oficio No. 8.103 del 15 de julio de 2014.

JAIME EDUARDO DELGADO VILLEGAS, en mi condición de apoderado de la **NATIONAL TAIWAN UNIVERSITY**, dentro del término legal, presento respuesta a Concepto Técnico notificado mediante Oficio No. 8.103 del 15 de julio de 2014.

1. Excepción de Patentabilidad (Art 20 D 486 literal (d))

El Examinador Técnico de Patentes objeto la invención porque:

“El objeto de las reivindicaciones 22 a 26 no es patentable de acuerdo con el Art. Citado”.

R/: Nuestro poderdante de acuerdo a lo expresado por el Examinador Técnico de Patentes, eliminó las reivindicaciones 22 a 26.

Marcas – Patentes
Modelos – Diseños
Nombres Comerciales – Enseñas
Propiedad Intelectual
Registros en el Exterior
Registros Sanitarios INVIMA – ICA
Acciones Judiciales – Administrativas
Derecho Comercial – Industrial
Asesoría – Contratos – Otros
Corresponsalia a Nivel Mundial

MDE



Mario Delgado Echeverry e Hijos SAS.

Abogados Consultores – Marcas y Patentes

Bogotá D.C. - Colombia
Avda. Kra. 24 Park Way No. 37 – 31 Of.
202
Tels. (571) 2444096 – 3690624
Fax (571) 2442496 A.A. No. 6207
E-mail: mde@mdelaw.com
mdelawof@cablenet.co
mdeabogados@cablenet.co

Miembros ACPI – ASIPÍ - AIPPI

2. Unidad de invención (Art 25 D 486)

El Examinador objeta la **unidad de invención** de la Solicitud porque se refiere a 4, Grupos Inventivos:

“Los compuestos glicosfingolípidos en los cuales R1 es OH son conocidos (documento D2), por lo cual esta característica técnica común no es el “único concepto inventivo” de las invenciones, como se observa en la siguiente comparación:

“(…)”

“Por lo tanto, no hay unidad de invención entre los compuestos donde R1 tiene significados diferentes”.

“De otro lado, el compuesto intermediario 5 de la reivindicación 20, además de ser conocido (documento D2), sólo representa una parte de la estructura final del compuesto final; por lo cual no hay unidad de invención entre este tipo de compuestos intermediarios (que forman la cadena R del compuesto final) y los compuestos intermediarios que forman el anillo galactopiranosil del compuesto final; como se observa en la siguiente comparación:”

“(…)”

“Y el compuesto intermediario 3 de la reivindicación 20, además de estar sugerido (documento D1), sólo representa una parte de la estructura final del compuesto final; por lo cual no hay unidad de invención entre este tipo de compuestos intermediarios (que forman el anillo galactopiranosil del compuesto final) y los compuestos intermediarios que forman la cadena R del compuesto final; como se observa en la siguiente comparación:”

“Por lo tanto, los Grupos Inventivos están definidos por las siguientes características técnicas esenciales:

“1° Compuestos en los cuales R1 = OH y el segundo C después del grupo éter (-O-) está sustituido con N3, NH ó NHC(O)R2 (reivindicaciones 1, 2, 18, reivindicación 19 en parte, y reivindicación 20: compuestos 2, 8, 9, 10 y 11)”.

“2° Compuestos en los cuales R1 = N3, NH ó NHC(O)R2 y el segundo C después del grupo éter (-O-) está sustituido con N3, NH ó NHC(O)R2 (reivindicación 8, reivindicación 19 en parte, y reivindicación 20: compuestos 1, 12, 13, 14, 15 y 16)”.

“3° Compuestos intermedios que forman la cadena R del compuesto final donde R=alquilo terminado en arilo o heteroarilo (reivindicación 20: compuestos 5 y 7)”.

Marcas – Patentes
Modelos – Diseños
Nombres Comerciales – Enseñas
Propiedad Intelectual
Registros en el Exterior
Registros Sanitarios INVIMA – ICA
Acciones Judiciales – Administrativas
Derecho Comercial – Industrial
Asesoría – Contratos – Otros
Corresponsalía a Nivel Mundial

MDE



Mario Delgado Echeverry e Hijos SAS.

Abogados Consultores – Marcas y Patentes

Bogotá D.C. - Colombia
Avda. Kra. 24 Park Way No. 37 – 31 Of.
202
Tels. (571) 2444096 – 3690624
Fax (571) 2442496 A.A. No. 6207
E-mail: mde@mdelaw.com
mdelawof@cable.net.co
mdeabogados@cable.net.co

Miembros ACPI – ASIPI – AIPPI

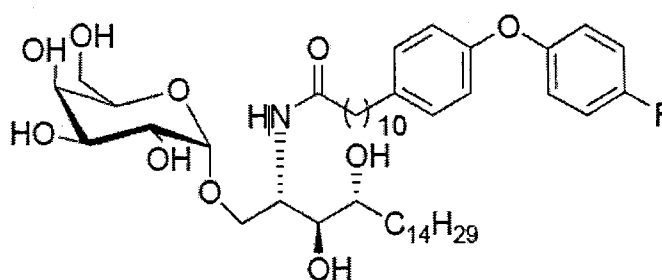
“4° Compuestos intermedios que forman el anillo galactopiranosil del compuesto final (reivindicación 20: compuestos 3, 4 y 6)”.

“Se sugiere presentar las solicitudes divisionales que considere necesarias, o limitar la solicitud inicial a una sola invención, eliminando de ella las otras invenciones”.

R/: Nuestro poderdante de acuerdo a lo expresado por el Examinador Técnico de Patentes, de la unidad de invención, eligió a los compuestos donde R1 = OH y los compuestos intermedios donde R = alquilo que terminan en anillo o heteroanillo (compuestos 5 y 7 de la reivindicación 20).

Se modificó la reivindicación 1, donde se incluyeron las diferentes etapas del proceso para preparar el compuesto quiral, teniendo en cuenta lo descrito en las reivindicaciones 2, 4 y 6 para los compuestos de fórmulas (6), (7), (5), (4) (3) y (2)

Se eliminaron las reivindicaciones 3, 4 y 6



Las reivindicaciones 18 a 21 son reivindicaciones de productos definidos por el proceso y se eliminaron con base a la presente elección del compuesto conocido, C34.

Se añadió la reivindicación 5: “El método de la reivindicación 1, en el que el grupo saliente que contiene tiol es thiotolueno (STOL)”.

Marcas – Patentes
Modelos – Diseños
Nombres Comerciales – Enseñas
Propiedad Intelectual
Registros en el Exterior
Registros Sanitarios INVIMA – ICA
Acciones Judiciales – Administrativas
Derecho Comercial – Industrial
Asesoría – Contratos – Otros
Corresponsalia a Nivel Mundial

MDE



Mario Delgado Echeverry e Hijos SAS.

Abogados Consultores – Marcas y Patentes

Bogotá D.C. - Colombia
Avda. Kra. 24 Park Way No. 37 – 31 Of.
202
Tels. (571) 2444096 – 3690624
Fax (571) 2442496 A.A. No. 6207
E-mail: mde@mdelaw.com
mdelawof@cable.net.co
mdeabogados@cable.net.co

Miembros ACPI – ASIPI - AIPPI

El soporte a las reivindicaciones, se encuentra en la Figura 1 y el apartado 0066 de la Especificación.

Por lo expresado anteriormente, el solicitante pone a consideración del Examinador Técnico de Patentes un **NUEVO CAPITULO REIVINDICATORIO**, el cual consta de cinco **(5) Nuevas Reivindicaciones** en tres folios.

1. DEL ANÁLISIS DE PATENTABILIDAD.

Novedad y Nivel Inventivo (art.16 y 18 D 486)

El Examinador objeta la **novedad** para las reivindicaciones 18 a 21 con los documentos **D1 (WO2008128062)**, **D2 (“In Vivo Protection Provided by a agents and chemotherapy Synthetic New Alpha-Galactosyl Ceramide Analog against Bacterial and Viral Infections in Murine Models”)** y el nivel inventivo para las reivindicaciones 1 – 17 y 22 porque:

“(…)”

“Las características esenciales del compuesto glicosfingolípido 1-O-(α-D-galactopiranosil)-2-(11-(4-(4-fluorofenoxi)fenil)undecanoil)amino-D-ribo-octadecan-1,3,4-triol (C34) de la reivindicación 18 habían sido divulgadas previamente en el documento D2, el cual enseña el compuesto glicosfingolípido 1-O-(α-Dgalactopiranosil)-2-(11-(4-(4-fluorofenoxi)fenil)undecanoil) amino-D-ribo-octadecan-1,3,4-triol (C34) (Pág. 4130, Col. 1, compuesto 5)”.

“En consecuencia, la reivindicación 18 no es nueva, según lo divulgado en el documento D2”.

“De otro lado, las reivindicaciones 18 a 21 no son nuevas, considerando que lo particular del estado de la técnica anula la novedad de lo particular que se reivindica, según lo divulgado en el documento D2:...”

Marcas – Patentes
Modelos – Diseños
Nombres Comerciales – Enseñas
Propiedad Intelectual
Registros en el Exterior
Registros Sanitarios INVIMA – ICA
Acciones Judiciales – Administrativas
Derecho Comercial – Industrial
Asesoría – Contratos – Otros
Corresponsalia a Nivel Mundial

MDE



Mario Delgado Echeverry e Hijos SAS.

Abogados Consultores – Marcas y Patentes

Bogotá D.C. - Colombia
Avda. Kra. 24 Park Way No. 37 – 31 Of.
202
Tels. (571) 2444096 – 3690624
Fax (571) 2442496 A.A. No. 6207
E-mail: mde@mdelaw.com
mdelawof@cablenet.co
mdeabogados@cablenet.co

Miembros ACPI – ASIPI - AIPPI

“(…)”

“Nivel inventivo.

“(…)”

“La diferencia entre la invención, definida en la reivindicación 1, y el proceso divulgado en el documento D2 consiste en que en los compuestos obtenidos R1 es OH, NH2 y NHCOR2 (donde R2 es H ó alquilo terminado en arilo), en lugar de los grupos OH y COOH en el anillo galactopiranosil”.

“No hay evidencia de que el proceso reivindicado, por el cual se obtienen diversos compuestos en los cuales R1 es OH, NH2 y NHCOR2 (donde R2 es H ó alquilo terminado en arilo), sea un proceso con algún efecto técnico diferente al que ya proporcionaba el del documento D2”.

“Por lo tanto, la persona normalmente conocedora de la materia, incluiría los grupos OH, NH2 y NHCOR2 (donde R2 es H ó alquilo terminado en arilo) en los compuestos del proceso divulgado en el documento D2, teniendo en cuenta que el documento D1 enseña el proceso para preparar compuestos que tienen los grupos OH, H, COOH, COO alquilo, CH2OH y CH2O alquilo (cuando el sustituyente R2 es alquilo) en el anillo galactopiranosil, que son grupos químicos que tienen la misma distribución estérica de las cargas, dado que son semejantes. Además, los compuestos del proceso alternativo reivindicado, de la misma manera que los compuestos del estado de la técnica, son también compuestos inmunogénicos que sirven como ligandos para las células NKT (“natural killer T”, que son linfocitos que tienen actividad antitumoral, antiviral y antibacterial). De manera que la reivindicación 1 de la solicitud se considera obvia y, en consecuencia, no tiene nivel inventivo”.

“Dado que las reivindicaciones dependientes 2 a 17 no presentan alguna característica adicional que conceda nivel inventivo al objeto de la reivindicación independiente, se considera que tampoco tienen nivel inventivo”.

“La composición de la reivindicación 22, que se caracteriza porque contiene un compuesto de las reivindicaciones precedentes, no tiene nivel inventivo, dado que aquellas no lo tienen”.

Marcas – Patentes
Modelos – Diseños
Nombres Comerciales – Enseñas
Propiedad Intelectual
Registros en el Exterior
Registros Sanitarios INVIMA – ICA
Acciones Judiciales – Administrativas
Derecho Comercial – Industrial
Asesoría – Contratos – Otros
Corresponsalia a Nivel Mundial

MDE



Mario Delgado Echeverry e Hijos SAS.

Abogados Consultores – Marcas y Patentes

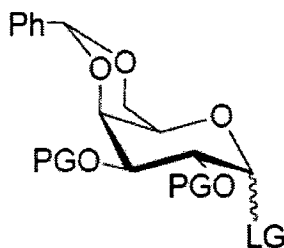
Bogotá D.C. - Colombia
Avda. Kra. 24 Park Way No. 37 – 31 Of.
202
Tels. (571) 2444096 – 3690624
Fax (571) 2442496 A.A. No. 6207
E-mail: mde@mdelaw.com
mdelawof@cablenet.co
mdeabogados@cablenet.co

Miembros ACPI – ASIPI – AIPPI

R/: Con relación a las objeciones del Examinador de Patentes con respecto a la falta de **Novedad y Nivel inventivo** de la invención en estudio, el solicitante desea resaltar lo siguiente:

En cuanto a la **novedad**, las reivindicaciones tienen por objeto cubrir un **nuevo método de fabricación de los compuestos**. El **compuesto C34 primario es conocido** y fue inventado originalmente por un subconjunto de los inventores (documento **D2**).

El **nivel inventivo** clave en el método del método es en la utilización del compuesto de fórmula (6):



(6)

en la que **LG es un grupo saliente que contiene tiol**.

El estado de la técnica anterior sólo describe el uso de un tricloroacetimidato ($\text{OC}(=\text{NH})-\text{CCl}_3$) grupo que el grupo saliente LG. (Ver en el documento **D1**. Figuras 7 y 7 (continuación) y los párrafos 0061, 000102, 000104, 000291 (compuesto 20), etc Como se describe en documento **D2**, el mismo glucósido tricloroacetimidato se utiliza en el mismo por Lin para la fabricación de C34.

De acuerdo con estos métodos, a fin de preparar glicósido-tricloro imidato (compuesto (6)), primero el grupo tio de 1-tio-glucósido se elimina por N-

Marcas – Patentes
Modelos – Diseños
Nombres Comerciales – Enseñas
Propiedad Intelectual
Registros en el Exterior
Registros Sanitarios INVIMA – ICA
Acciones Judiciales – Administrativas
Derecho Comercial – Industrial
Asesoría – Contratos – Otros
Corresponsalia a Nivel Mundial

MDE



Mario Delgado Echeverry e Hijos SAS.

Abogados Consultores – Marcas y Patentes

Bogotá D.C. - Colombia
Avda. Kra. 24 Park Way No. 37 – 31 Of.
202
Tels. (571) 2444096 – 3690624
Fax (571) 2442496 A.A. No. 6207
E-mail: mde@mdelaw.com
mdelawof@cablenet.co
mdeabogados@cablenet.co

Miembros ACPI – ASIPI - AIPPI

bromosuccinimida (NBS) para dar 1-OH-glucósido. A continuación, el 1-OH-glucósido se hace reaccionar con CCl_3CN y la base (normalmente K_2CO_3 , DBU u otros) para generar el compuesto donador de glycoside tricloroimidated (6). Este es el método descrito por Plettenberg O., J. Org. Chem. 67 (13): 4.559-4.564, en 4564 (2002), ver anexo.

Este ha sido el método aceptado para la síntesis de compuestos α -GalCer desde como se describe por Trappeniers (Chem Med Chem 2008, 3:.. 1061-1070) que fue citado durante la tramitación de Estados Unidos. (Ver Trappeniers en el Esquema 3, se adjunta copia)

El glicósido tricloro-imidated es un compuesto inestable. Para utilizar glicósido tricloro-imidated como donador de azúcar, que debe prepararse de nuevo inmediatamente antes de la etapa de glicosilación como se describe en el párrafo 0042 de nuestra especificación ("Debido a la imidato ser fácilmente hidrolizado y por lo general necesario para estar preparado fresco, el uso de este grupo saliente para la glicosilación en la síntesis a gran escala podría encontrarse con problemas de almacenamiento y purificación").

Este inconveniente de los métodos anteriores se supera mediante el uso de tioglicosido como un donante con catalizadores ácidos de Lewis y el uso de tamices moleculares para de-hidrato. (Véase el párrafo 0.042 de nuestra especificación). **El compuesto donador de tio-glucósido que se especifica en las reivindicaciones es muy estable y por lo general se puede conseguir por recristalización.**

Además, tenga en cuenta que los documento **D1** y **D2** describen métodos usando fosfina utilizado para reducir la azida. Se modificó el término

Marcas – Patentes
Modelos – Diseños
Nombres Comerciales – Enseñas
Propiedad Intelectual
Registros en el Exterior
Registros Sanitarios INVIMA – ICA
Acciones Judiciales – Administrativas
Derecho Comercial – Industrial
Asesoría – Contratos – Otros
Corresponsalia a Nivel Mundial

MDE



Mario Delgado Echeverry e Hijos SAS.

Abogados Consultores – Marcas y Patentes

Bogotá D.C. - Colombia
Avda. Kra. 24 Park Way No. 37 – 31 Of.
202
Tels. (571) 2444096 – 3690624
Fax (571) 2442496 A.A. No. 6207
E-mail: mde@mdelaw.com
mdelawof@cablenet.co
mdeabogados@cablenet.co

Miembros ACPI – ASIPI - AIPPI

"complejo de fosfina" se elimina de la reivindicación 5. El uso de agentes tales como hidruro de litio y aluminio, borohidruro de sodio o un complejo de borano reducción no se ha demostrado previamente para la preparación de compuestos C-34 relacionados.

Los inventores han observado que la **reducción de la azida por fosfina es bajo rendimiento, baja conversión, y su producto secundario fosfina-óxido es muy difícil de eliminar.** (Véase el apartado 0043 de nuestra especificación).

Por lo tanto, el objeto de la invención revelado en las nuevas reivindicaciones de la presente solicitud de patente de invención implica NOVEDAD Y NIVEL INVENTIVO y no son evidentes para una persona experta en la técnica en comparación con los documentos D1 y D2 del estado de la técnica mencionados por el Examinador de Patentes.

En conclusión, de acuerdo a lo expresado anteriormente, el solicitante pone a consideración del Despacho **UN NUEVO CAPÍTULO REIVINDICATORIO** el cual consta de cinco (5) **Nuevas Reivindicaciones** en tres folios, el cual cumple con los requisitos de **NOVEDAD, NIVEL INVENTIVO Y APLICACIÓN INDUSTRIAL** exigidos por la Decisión 486 del Acuerdo de la Comisión de la Comunidad Andina y se esta dando respuesta al Concepto Técnico notificado mediante Oficio No. 8.103 del 15 de julio de 2014.

Por lo tanto, solicito respetuosamente se proceda a **CONCEDER** la Patente de Invención titulada: **"MÉTODOS PARA PREPARAR GLICOESFINGOLÍPIDOS Y USOS DE LOS MISMOS"** a nombre de la sociedad **NATIONAL TAIWAN UNIVERSITY**, para las Nuevas Reivindicaciones 1 a 5 inclusive.

Marcas – Patentes
Modelos – Diseños
Nombres Comerciales – Enseñas
Propiedad Intelectual
Registros en el Exterior
Registros Sanitarios INVIMA – ICA
Acciones Judiciales – Administrativas
Derecho Comercial – Industrial
Asesoría – Contratos – Otros
Corresponsalia a Nivel Mundial

MDE

■ ■ ■ ■ ■
Mario Delgado Echeverry e Hijos SAS.

Abogados Consultores – Marcas y Patentes

Bogotá D.C. - Colombia
Avda. Kra. 24 Park Way No. 37 – 31 Of.
202
Tels. (571) 2444096 – 3690624
Fax (571) 2442496 A.A. No. 6207
E-mail: mde@mdelaw.com
mdelawof@cablenet.co
mdeabogados@cablenet.co

Miembros ACPI – ASIPI – AIPPI

Atentamente,


JAIME EDUARDO DELGADO VILLEGAS
C.C. No. 19.270.955 Bogotá
T.P. No. 43.308 C.S.J.

Anexo:

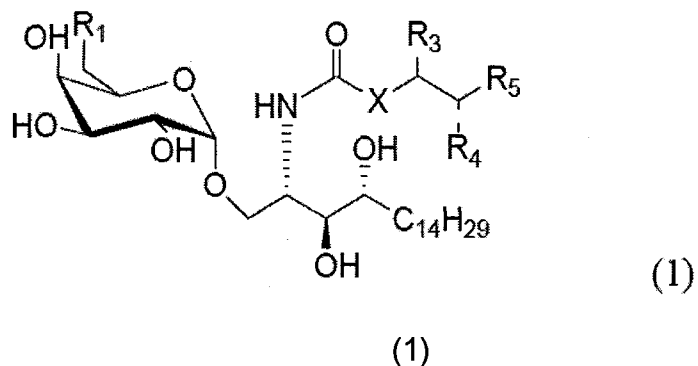
Nuevas Reivindicaciones (5) en tres folios.

Plettenberg O., J. Org. Chem en seis folios.

Trappeniers Chem Med Chem 2008 en el Esquema 3 en diez folios.

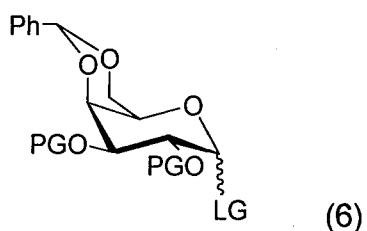
NUEVAS REIVINDICACIONES

1. Un proceso para preparar un compuesto quiral que comprende una forma R o S de un glicosfingolípido de la fórmula (1)

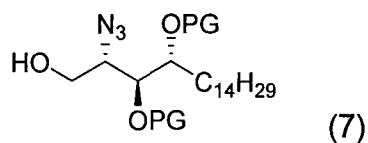


en donde $R_1=OH$, $R_2 = H$ o un alquilo, alquenilo, o alquilo terminando en arilo, arilo sustituido, heteroarilo, o heteroarilo sustituido, X =grupo alquilo, $R_3= OH$ o H , $R_4= OH$ o H , $R_5=$ arilo, arilo sustituido, heteroarilo, o heteroarilo sustituido, o una sal farmacéuticamente aceptable del mismo, en donde el compuesto de la fórmula 1 se prepara mediante

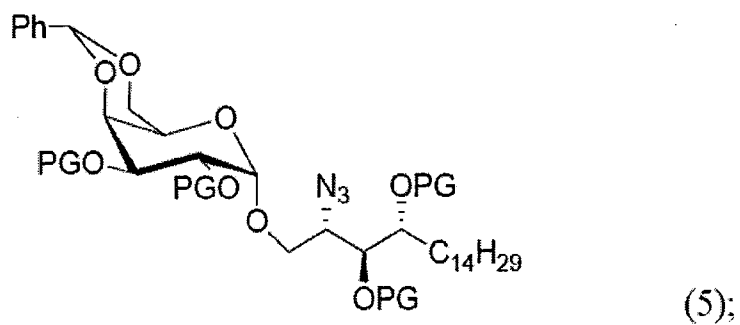
- (a) reacción de la alfa-glicosilación en presencia de un ácido de Lewis y, el uso de tamices moleculares para deshidratar, un compuesto de fórmula (6):



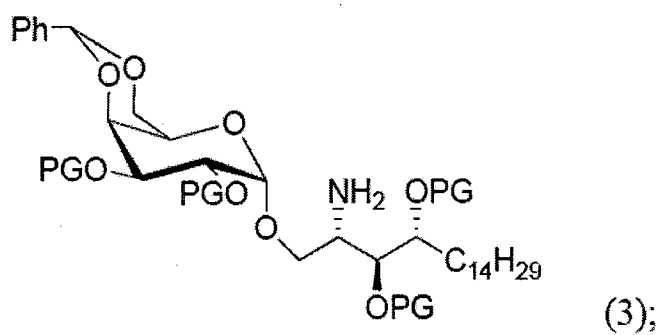
en donde PG es un grupo protector de hidroxilo y LG es un grupo saliente que contiene tiol, con un compuesto representado por la estructura de la fórmula (7):



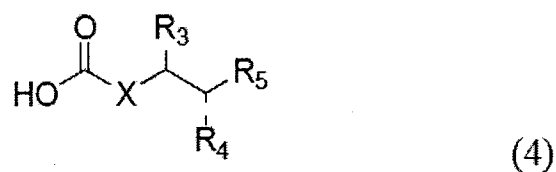
para obtener un compuesto de la fórmula (5)



- (b) reducir el compuesto de fórmula (5) para obtener un compuesto de fórmula (3)

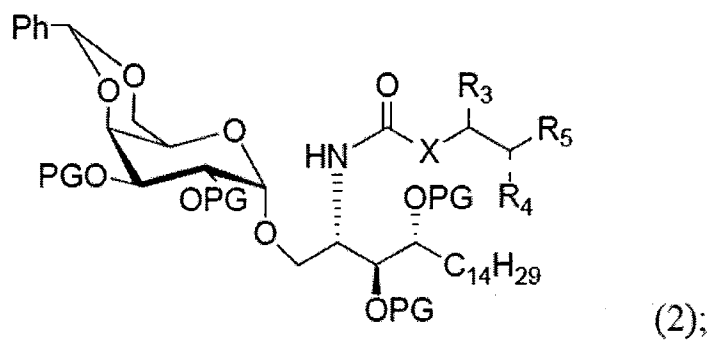


- (c) acoplamiento por medio de la etapa de formación del enlace amida con reactivos de acoplamiento, del compuesto de fórmula (3) con un compuesto de fórmula (4):



donde X=grupo alquilo, alquenilo, R3=H, OH, R4= H, OH, R5= arilo, arilo sustituido, heteroarilo, o heteroarilo sustituido, o una sal farmacéuticamente aceptable del mismo,

para obtener un compuesto de la fórmula (2):



- (d) desprotegiendo el compuesto de fórmula (2), con hidrógeno bajo catálisis de hidrogenación.
2. El método de la Reivindicación 1, en donde el catalizador de hidrogenación se selecciona a partir de Pd/C, Pd(OH₂), o Raney-Ni.
 3. El método de la Reivindicación 1, en donde la reducción en la etapa 1 (b) se consigue utilizando hidruro de litio y aluminio, borohidruro de sodio, complejo de borano, complejo de fosfina, reducción de enzima, hidrogenación, o hidrogenación por transferencia.
 4. El método de la Reivindicación 1, en donde el ácido de Lewis se selecciona de TMSOTf, Tf₂O, BF₃ · OEt₂, TfOH, Me₂S₂-Tf₂O.
 5. El método de la Reivindicación 1 en donde el grupo saliente que contiene tiol es tiotolueno (STol).

Synthesis of α -Galactosyl Ceramide, a Potent Immunostimulatory Agent

Oliver Plettenburg,[†] Vera Bodmer-Narkevitch, and Chi-Huey Wong*

Department of Chemistry and Skaggs Institute for Chemical Biology, The Scripps Research Institute,
10550 N. Torrey Pines Road, La Jolla, California 92067

wong@scripps.edu

Received March 7, 2002

α -Galactosyl ceramide has been identified to be a potent stimulatory agent for a novel immunological process, mediated by CD1 molecules. This paper describes a short and efficient synthesis of α -galactosyl ceramide. Starting from commercially available 2-deoxy galactose, a suitable sphingosine derivative was obtained in nine steps and 36% overall yield, which was subsequently glycosylated to give the target molecule. This flexible route will provide various glycolipids for further exploration of this interesting biological process.

Introduction

CD1 molecules represent a new class of highly conserved, antigen-presenting cell surface proteins.¹ They recognize and bind glycolipid antigens through lipid–protein interactions and present the sugar moiety of the antigen to a receptor on natural killer T-cells (NKT cells) to activate the immune system. In humans, five different isoforms of CD1 have been detected so far. Since the recognition event is highly specific for glycolipids and no carrier proteins are required, this novel defense mechanism has gained considerable interest in the past years, with the hope that a new type of vaccine may be developed in the future. Since the discovery of α -galactosyl ceramide **1** in 1993 from the marine sponge *Agelas mauritanus*,² it has been shown that **1** exhibits tumor growth inhibition activities.³ Further examination of the pharmaceutical potential of this class of compounds has led to the discovery that **1** is capable of initiating a CD1-mediated activation of NKT cells⁴ and preventing autoimmune diseases such as type I diabetes.⁵ Another derivative, whose sphingoside chain is truncated, seems to prevent autoimmune encephalomyelitis.⁶ It is worth noting that, although β -galactosyl ceramides are frequently found in mammalian tissues and do possess a

number of functions,^{7,8} α -linked galactosyl ceramides have not been detected in normal mammalian cells so far. They have been found in cancer⁹ or fetal¹⁰ cell lines, however. In addition to the unusual α -linkage between galactose and ceramide, the ceramide portion of **1** also differs from the normally found ceramide in which a *trans*-olefin instead of a dihydroxy geometry is in place.

A few syntheses of **1** have been published so far.^{11,12} As a part of our effort to understand the mechanism of CD1-mediated T-cell activation and to develop novel glycolipid vaccines, we are interested in developing a general and effective strategy for the synthesis of this compound and related structures.

Results and Discussion

Our synthesis starts from 2-deoxy galactose (Scheme 1), a relatively inexpensive starting material, which possesses all necessary stereochemical information found in compound **1**.

By Wittig reaction of a suitably protected 2-deoxy galactose derivative, it should be possible to introduce the long chain. The absence of an electron-withdrawing hydroxy group at C2 position should facilitate the opening of the pyranose ring. The single free hydroxy group generated in the Wittig reaction can be converted to an azide, serving as a protected form of the amide group.

* To whom correspondence should be addressed.

[†] Current address: Aventis Pharma Deutschland GmbH, DI&A Chemistry, D-65926 Frankfurt, Germany.

(1) (a) Burdin, M.; Kronenberg, M. *Curr. Opin. Immunol.* **1999**, *11*, 326–331. (b) Park, S.-H.; Bendelac, A. *Nature* **2000**, *406*, 788–792.

(2) (a) Natori, T.; Morita, M.; Akimoto, K.; Koezuka, Y. *Tetrahedron Lett.* **1993**, *34*, 5591–5592. (b) Natori, T.; Morita, M.; Akimoto, K.; Koezuka, Y. *Tetrahedron* **1994**, *50*, 2771–2784.

(3) Koezuka, Y.; Kazuhiro, M.; Sakai, T.; Takenori, N. *Recent Res. Dev. Cancer* **1999**, *1*, 341–354.

(4) Kawano, T.; Cui, J.; Koezuka, Y.; Taura, I.; Kaneko, Y.; Motoki, K.; Ueno, H.; Nakagawa, R.; Sato, H.; Kondo, E.; Koseki, H.; Taniguchi, M. *Science* **1997**, *278*, 1626–1629.

(5) (a) Sharif, S.; Arreaza, G. A.; Zucker, P.; Mi, Q.-S.; Sondhi, J.; Naidenko, O. V.; Kronenberg, M.; Koezuka, Y.; Delovitch, T. L. *Nature Med.* **2001**, *7*, 1057–1062. (b) Hong, S.; Wilson, M. T.; Serizawa, I.; Wu, L.; Nagendra, S.; Naidenko, O.; Miura, T.; Haba, T.; Scherer, D. C.; Wie, J.; Kronenberg, M.; Koezuka, Y.; van Kaer, L. *Nature Med.* **2001**, *7*, 1052–1056.

(6) Miyamoto, K.; Miyake, S.; Yamamura, T. *Nature* **2001**, *413*, 531–534.

(7) Harouse, J. M.; Bhat, S.; Spitalnik, S. L.; Laughlin, M.; Stefano, K.; Silberberg, D. H.; Gonzales-Scarano F. *Science* **1991**, *253*, 320–323.

(8) (a) Leeden, R. J. *Neurosci. Res.* **1984**, *12*, 147–159. (b) Hannun, Y. A.; Bell, R. M. *Science* **1989**, *243*, 500–507.

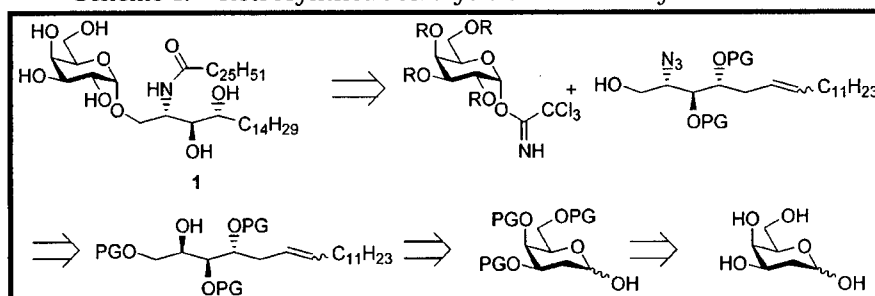
(9) Watanabe, K.; Matsubara, T.; Hakomori, S. *J. Biol. Chem.* **1976**, *251*, 2385–2387.

(10) Nishimura, K.; Nakamura, A. *J. Biochem.* **1985**, *98*, 1247–1254.

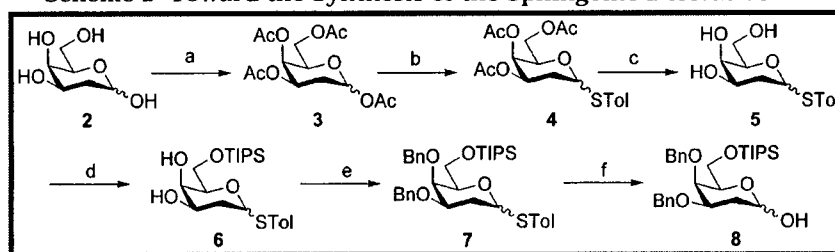
(11) (a) Morita, M.; Motoki, K.; Akimoto, K.; Natori, T.; Sakai, T.; Sawa, E.; Yamaji, K.; Koezuka, Y.; Kobayashi, E.; Fukushima, H. *J. Med. Chem.* **1995**, *38*, 2176–2187. (b) Morita, M.; Natori, T.; Akimoto, K.; Osawa, T.; Fukushima, H.; Koezuka, Y. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 699–704. (c) Figueroa-Pérez, S.; Schmidt, R. R. *Carbohydr. Res.* **2000**, *328*, 95–102. (d) Takikawa, H.; Muto, S.-e.; Mori, K. *Tetrahedron* **1998**, *54*, 3141–3150.

(12) Several derivatives have been reported: (a) NBD-derivative: Sakai, T.; Ehara, H.; Koezuka, Y. *Org. Lett.* **1999**, *1*, 359–361. (b) biotinylated derivative: Sakai, T.; Naidenko, O. V.; Iijima, H.; Kronenberg, M.; Koezuka, Y. *J. Med. Chem.* **1995**, *38*, 1836–1841.

Scheme 1. Retrosynthetic Analysis of α -Galactosyl Ceramide 1

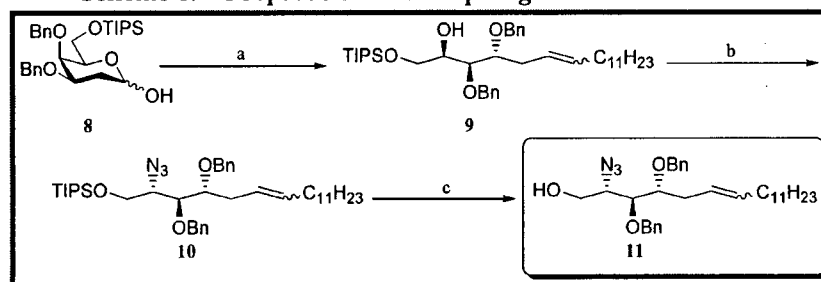


Scheme 2 Toward the Synthesis of the Sphingosine Derivative 11^a



^a (a) Ac₂O, py, quant; (b) TolSH, BF₃·OEt₂, 83%; (c) NaOMe in MeOH; 99.5%; (d) TIPSCl, imidazole, THF, 86%; (e) BnBr, NaH, DMF, 88%; (f) NBS, acetone, 77%.

Scheme 3. Preparation of the Sphingosine Derivative 11^a



^a (a) *n*-C₁₂H₂₅PPh₃Br, BuLi, THF, 92%; (b) HN₃, PPh₃, DEAD, THF, 93%; (c) TBAF, THF, 91%.

An ideal strategy is to have an easily and selectively cleavable protecting group at the primary hydroxy group and another type of mildly cleavable protecting group on the hydroxy groups at C3 and C4 positions. We decided to use benzyl ethers as the protection groups since this would allow simultaneous conversion of an azide to amine and reduction of the double bond while liberating the molecule in the final stage of the synthesis.

The route described in Scheme 2 involves the use of thiotoluene as a selective protecting group for the anomeric center. Free 2-deoxy-galactose was peracetylated and then reacted with thiocresol under BF₃ etherate catalysis to give thioglycoside **4**.

Subsequent cleavage of the acetates by sodium methoxide in methanol, followed by selective silylation of the 6-OH of **5** and benzylation of the hydroxy groups, gave **7**. The choice of the silyl protection group turned out to be important, since use of TBDMS or TBDPS led to cleavage or migration of the silyl group during benzylation.

Cleavage of the thiotoluene group was performed with NBS. The reaction is complete within minutes. The subsequent Wittig reaction was then carried out using 3 equiv of the Wittig reagent and 2.8 equiv of BuLi. The reaction proceeds very fast; after 2 h, little starting material can be detected by TLC. The *E/Z* ratio of the isolated olefinic product was determined to be 3.7:1. Our

attempt to introduce the azide group by mesylation of the free hydroxy group in **9** and substitution by azide gave either no conversion at low temperature, or decomposition at high temperature. This was surprising to some extent, since a successful conversion was described recently for a similar molecule.¹³ Changing the solvent from DMF to DMSO did not make any difference.¹⁴ Also, chloromesylation did not give the desired product.¹⁵

However, reaction with a freshly prepared HN₃ solution¹⁶ under Mitsunobu conditions led to the desired compound **10** in 93% yield in a very clean reaction. After desilylation with TBAF, the sphingosine derivative **11** was obtained in 91% yield (Scheme 3).

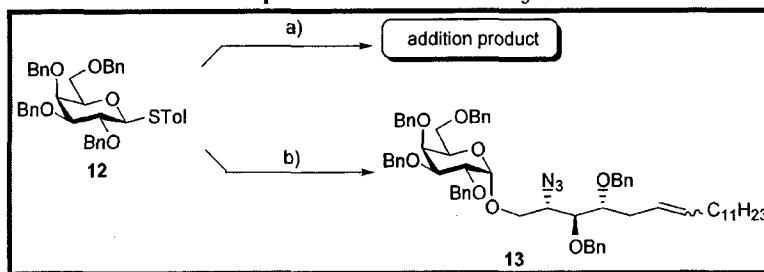
Overall, the sphingosine building block **11** was prepared in nine steps starting from commercially available 2-deoxy galactose in an overall yield of 36%. Our further strategy featured simultaneous deprotection after glycosylation by simple and mild hydrogenation, thereby cleaving all benzyl groups, removing the double bond, and reducing the azide to an amine. Amide formation using

(13) Ohlsson, J.; Magnusson, G. *Carbohydr. Res.* **2001**, *331*, 91–94.

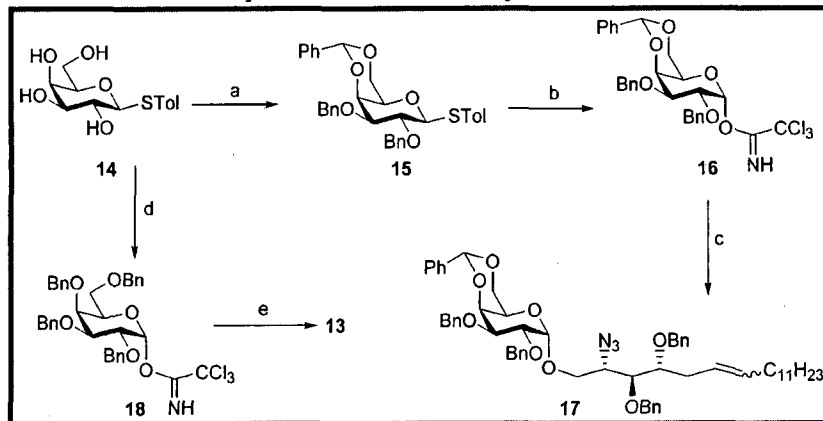
(14) Alvarez, S. G.; Alvarez, M. T. *Synthesis* **1997**, 413–414.

(15) Saotome, C.; Kanie, Y.; Kanie, O.; Wong, C.-H. *Bioorg. Med. Chem.* **2000**, *8*, 2249–2261.

(16) The preparation and handling of hydrazoic acid solutions are described: Wolff, H. *Org. React.* **1946**, *3*, 307–336.

Scheme 4. Preparation of α -Galactosyl Ceramide 13^a

^a (a) **11**, NIS, AgOTf; (b) **11**, DMTST, 39%.

Scheme 5. Preparation of α -Galactosyl Ceramides 13 and 17^a

^a (a) See ref 19; (b) (1) NBS, acetone, 95%; (2) CCl₃CN, DBU, 93%; (c) **11**, BF₃·OEt₂, 68%; (d) see ref 20; (e) **11**, BF₃·OEt₂, 50%.

standard techniques should yield the desired galactosyl lipid. This simple reaction sequence should facilitate the synthesis of glycolipids and related structures.

To simplify the deprotection of final products, a suitable glycosylation donor should be used so that the protected glycolipid product can be completely deprotected by hydrogenation. We first turned our attention to thioglycosides. In a first experiment, NIS and AgOTf were used as a promotor system and perbenzylated thiogalactose **12** as the donor, a system that was used successfully in preliminary experiments.¹⁷ Unfortunately in this case, the reaction led to the formation of several byproducts resulting from addition of electrophiles to the double bond (Scheme 4). Therefore, DMTST¹⁸ was used as another promotor to avoid the production of electrophilic iodine species. The reaction led to glycoside **13**, containing the desired α -linkage and whose exact mass corresponds to MNa - N₂. The observed isomerization of the double bond was deemed insignificant, since subsequent hydrogenation of either isomer would furnish the desired product. No β -anomer formation was observed. Further optimization allowed isolation of the galactosyl ceramide **13** in 39% yield.

On the other hand, Schmidt^{11c} had demonstrated in a similar case the benefits of using the 4,6-benzylidene-protected trichloroacetimidate **16**. **16** can be obtained easily from thiogalactose **14**¹⁹ (Scheme 5). BF₃ etherate was chosen as a mild promotor, delivering the galactosyl ceramide **17** in a clean reaction in 68% yield. A similar reaction on perbenzylated trichloroacetimidate **18**²⁰ yielded

the desired α -galactosyl ceramide **13** in 50% yield along with other products, the α/β ratio being 2.9:1. In both **13** and **17** *E/Z* ratio was found to be unchanged compared to the starting ceramide (3.7:1).

Our further investigations emphasized the hydrogenation of both **13** and **17** using Pearlmann's catalyst. The yields thus obtained were, however, relatively low, and we noticed that the reactions were accompanied by decomposition upon longer reaction times. An alternative synthetic strategy (Scheme 6) was therefore tried on galactosyl ceramide **17**. Staudinger reduction, followed by coupling of the free amine **19** with hexacosanoic acid gave the expected galactosyl ceramide **20** in 69% yield, which gave **1** upon hydrogenation with palladium black.

In summary, the new strategy described here for the synthesis of **1** and related glycolipids should provide access to glycolipid libraries for exploitation of their immunostimulating activities. The amide as well as the ceramide chain can be easily modified. In addition, the stereochemistry of the ceramide part can be altered by use of different starting sugars.

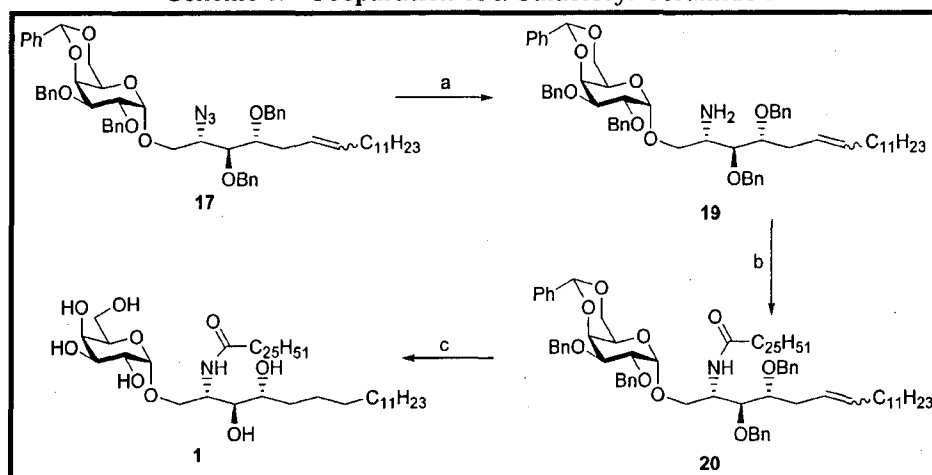
Experimental Section

General Methods. Tetrahydrofuran (THF) and diethyl ether (Et₂O) were distilled over sodium/benzophenone, and methylene chloride (CH₂Cl₂) over calcium chloride. Reagents of commercial quality were purchased and used without further purification. All reactions were carried out under an argon atmosphere with dry, freshly distilled solvents under anhydrous conditions, unless otherwise noted. Glycosylation experiments were performed by using molecular sieves, which were flame-dried right before the reaction under high vacuum. Analytical thin-layer chromatography was performed using silica gel 60 F₂₅₄ glass plates, compound spots were visualized

(17) Plettenburg, O.; Wong, C.-H. unpublished results.

(18) Ye, X.-S.; Wong, C.-H. *J. Org. Chem.* **2000**, *65*, 2410–2431.

(19) Zhang, Z.; Ollmann, I. R.; Ye, X.-S.; Wischnat, R.; Baasov, T.; Wong, C.-H. *J. Am. Chem. Soc.* **1999**, *121*, 734–753.

Scheme 6. Preparation of α -Galactosyl Ceramide 1^a

^a (a) PMe_3 , THF; then aq 1 M NaOH; (b) $\text{C}_{25}\text{H}_{51}\text{COOH}$, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC), 69%; (c) H_2 , Pd/EtOAc, quant.

by UV light (254 nm) and by staining with a yellow solution containing $\text{Ce}(\text{NH}_4)_2(\text{NO}_3)_6$ (0.5 g) and $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$ (24.0 g) in 6% H_2SO_4 (500 mL). Flash chromatography was performed on silica gel 60 Geduran (35–75 μm , EM Science).

1,3,4,6-Tetra-*O*-acetyl 2-Deoxy-D-galactopyranoside (3). 2-Deoxygalactose (10 g, 60.9 mmol) was dissolved in a mixture of 50 mL of pyridine and 50 mL of acetic anhydride. The mixture was stirred overnight. The solvent was removed in vacuo, and the resulting syrup was taken up in 200 mL of chloroform and extracted with sat. sodium bicarbonate solution (three to four times) and with brine (1 $\times$). The organic layer was dried over sodium sulfate, and the solvent was evaporated to give **3** (20.0 g, quant.) as a yellow oil. It was used for the next step without further purification. ^1H NMR (CDCl_3 , 500 MHz): δ = 5.75 (dd, 1H, J = 2.6, 10.1 Hz); 5.25 (d, 1H, J = 2.9 Hz); 5.07 (ddd, 1H, J = 12.5, 5.0, 3.1 Hz); 4.13 (dd, 1H, J = 11.0, 6.6 Hz); 4.07 (dd, 1H, J = 11.4, 6.6 Hz); 3.90 (dt, J = 6.6, 1.3 Hz); 2.11 (s, 3H); 2.09 (s, 3H); 2.00–2.07 (m, 2H); 2.00 (s, 3H); 1.96 (s, 3H). ^{13}C NMR (CDCl_3 , 101 MHz): δ = 170.37; 170.13; 169.80; 168.71; 91.59; 71.88; 67.93; 65.03; 61.51; 30.00; 20.91; 20.68; 20.62 (2C). HRMS: Calcd. ($\text{C}_{14}\text{H}_{20}\text{O}_9\text{Na}^+$): 355.0999, found 355.0996.

***p*-Methylphenyl 3,4,6-Tri-*O*-acetyl 2-deoxy-1-thio-D-galactopyranoside (4).** **3** (21.0 g, 53 mmol) was dissolved in 350 mL of dry dichloromethane. Thiocresol (13.22 g, 106 mmol, 2 equiv) was added. The reaction mixture was cooled to -40°C . $\text{BF}_3\cdot\text{OEt}_2$ (11.9 mL) was added slowly. The reaction mixture was allowed to warm to 0°C and stirred for 1 h at this temperature. The reaction was monitored closely by TLC (hexanes:ethyl acetate 1:1). After almost all starting material was consumed, the reaction was quenched by addition of saturated sodium bicarbonate solution. The organic layer was extracted with sat. NaHCO_3 (3 $\times$) and brine (1 $\times$), dried over sodium sulfate, and evaporated. The resulting oil was chromatographed on silica gel (hexanes:ethyl acetate 4:1) to give **4** as an oil (20.8 g, 83%). The α/β ratio was estimated from the ^1H NMR to be 2.5:1. ^1H NMR (CDCl_3 , 600 MHz): δ = 7.41 (d, 2H- β , J = 7.9 Hz); 7.35 (d, 2H- α , J = 7.9 Hz); 7.10 (d, 2H- β , J = 7.9 Hz); 7.09 (d, 2H- α , J = 7.9 Hz); 5.66 (d, 1H- α , J = 5.7 Hz); 5.36 (d, 1H- α , J = 2.6 Hz); 5.28–5.23 (m, 1H- α + 1H- β); 4.98 (ddd, 1H- β , J = 9.9, 7.2, 2.9 Hz); 4.75 (dd, 1H- β , J = 8.3, 5.7 Hz); 4.69 (t, 1H- β , J = 6.6 Hz); 4.16 (dd, 1H- β , J = 11.2, 7.2 Hz); 4.11–4.06 (m, 2H- α + 2H- β); 3.80 (dt, 1H- β , J = 0.9, 7.0 Hz); 2.44 (dt, 1H- α , J = 5.6, 12.8 Hz); 2.32 (s, 3H- β); 2.30 (s, 3H- α); 2.11 (s, 3H- α); 2.10 (s, 3H- β); 2.09–2.02 (m, 1H- α + 1H- β); 2.02 (s, 3H- β); 1.982 (s, 3H- α); 1.978 (s, 3H- α); 1.97 (s, 3H- β). ^{13}C NMR (CDCl_3 , 110 MHz): α -isomer: δ = 170.43; 170.20; 169.88; 137.75; 132.26; 130.04; 129.72; 84.01; 67.44; 66.68; 66.66; 62.42; 30.57; 21.09; 20.81; 20.64 (2C); β -isomer: δ = 170.27; 170.25; 169.96; 138.07; 132.55; 129.59; 129.48;

83.04; 74.61; 69.37; 65.35; 62.12; 31.46; 21.11; 20.77; 20.68. HRMS calcd ($\text{C}_{19}\text{H}_{24}\text{O}_7\text{SNa}^+$) 419.1135, found 419.1141.

***p*-Methylphenyl 2-Deoxy-1-thio-D-galactopyranoside (5).** **4** (1.4 g, 3.53 mmol) was dissolved in 75 mL of dry methanol. The suspension was cooled in an ice bath, and 3 mL of a solution of sodium methoxide in methanol was added. The reaction was allowed to warm to room temperature and stirred for 3 h. Amberlyst 15 (H^+ -form) was added to neutralize the sodium methoxide, the mixture was then diluted with methanol, and the exchange resin was filtered off. The resin was washed thoroughly and the filtrate was concentrated to give 950 mg (99.5%) of a white solid. ^1H NMR (MeOD, 600 MHz): δ = 7.29 (d, 2H- α + 2H- β , J = 8.3 Hz); 7.00 (d, 2H- α + 2H- β , J = 7.9 Hz); 5.46 (d, 1H- α , J = 5.5 Hz); 4.68 (dd, 1H- β , J = 11.0, 3.1 Hz); 4.18 (1, 1H- α , J = 6.1 Hz); 3.82 (ddd, 1H- α , J = 12.3, 4.8, 3.1 Hz); 3.74 (d, 1H- α , J = 2.6 Hz); 3.67–3.55 (m, 1H- α + 3H- β); 3.35 (dd, 1H- β , J = 11.2, 7.2 Hz); 3.21 (m, 1H, OH); 2.27 (dt, 1H- β + 1H- α , J = 2.0, 12.7 Hz); 2.20 (s, 3H- α , 3H- β); 1.86–1.81 (m, 1H- α + 1H- β). ^{13}C NMR (MeOD, 110 MHz): α -isomer δ = 138.55; 133.54; 132.66; 130.55; 86.22; 73.09; 69.54; 67.44; 62.74; 34.37; 21.10. β -isomer δ = 138.26; 132.47; 132.28; 130.52; 84.31; 80.69; 70.73; 68.44; 62.92; 35.52; 21.06. HRMS calcd ($\text{C}_{13}\text{H}_{18}\text{O}_4\text{SNa}^+$) 293.0818, found 293.0820.

***p*-Methylphenyl 2-Deoxy-1-thio-6-*O*-triisopropylsilyl-D-galactopyranoside (6).** **5** (553 mg, 2.05 mmol) was dissolved in 5 mL of dry THF. Imidazole (181 mg, 2.7 mmol) was added, and the reaction mixture was cooled to 0°C . TIPSCl (0.49 mL, 2.25 mmol, 1.1 equiv) was added dropwise. The reaction mixture was allowed to warm to room temperature and stirred for 2.5 h. The reaction was monitored by TLC and after completion quenched by addition of saturated NH_4Cl . The reaction mixture was diluted with ethyl acetate. The aqueous layer was separated and extracted with ethyl acetate. The combined organic layer was extracted with brine, dried over sodium sulfate, and evaporated. The residue was chromatographed on silica gel (hexanes:ethyl acetate 2:1) to give 750 mg (86%) of **6**. Since α/β assignment of the protons is difficult, integrals are given as absolute values, referring to the signal at 7.31 ppm as 2. Due to chromatography, α/β values changed to a ratio of about 1:1.4. ^1H NMR (CDCl_3 , 600 MHz): δ = 7.39 (d, 1.4H, J = 7.5 Hz); 7.32 (d, 2H, J = 7.9 Hz); 7.06 (2d, 3.4H); 5.61 (d, 1H, J = 5.3 Hz); 4.64 (dd, 0.7H, J = 11.8, 2.2 Hz); 4.22 (t, 1H, J = 4.8 Hz); 4.04 (s, 1H); 4.00–3.87 (m, 5.3H); 3.68 (b-s, 0.7H, OH); 3.62 (d, 1H, J = 3.1 Hz); 3.38 (t, 0.7H, J = 4.8 Hz); 3.24 (d, 0.7H, J = 5.3 Hz); 2.95 (bt, 3.0H, J = 10.1 Hz); 2.35–2.27 (m, 1.5H); 2.30 (s, 2.1H); 2.29 (s, 3H); 2.09–2.02 (m, 1.3H); 1.89–1.81 (m, 0.7H); 1.13–0.98 (m, 37.9H). ^{13}C NMR (CDCl_3 , 110 MHz): δ = 137.43; 136.91; 132.10; 131.40; 131.27; 130.05; 129.49; 129.45; 84.38; 82.96; 78.26; 70.18; 69.69; 69.33; 67.93; 66.46; 66.36; 63.76; 34.75; 33.59;

21.02; 20.97; 17.85; 17.83; 17.80; 17.77; 11.72; 11.69. calcd ($C_{22}H_{38}O_4SSiNa^+$) 449.2152, found 449.2158.

***p*-Methylphenyl 3,4-Di-*O*-benzyl-2-deoxy-1-thio-6-*O*-triisopropylsilyl- β -galactopyranoside (7).** **6** (700 mg, 1.58 mmol) was dissolved in 10 mL of DMF. The reaction mixture was cooled to -10°C (ice bath/ $NaCl$). Benzyl bromide (0.48 mL, 3.95 mmol, 2.5 equiv) was added, followed by 84 mg (3.32 mmol, 2.1 equiv) of sodium hydride (95%). The reaction was monitored by TLC (hexanes:ethyl acetate 2:1) and completed after 40 min at -10°C . The reaction was quenched by addition of solid NH_4Cl , diluted with water, and the reaction mixture was extracted with ethyl acetate (3 $\times$). The combined organic layer was extracted with water (3 $\times$) and brine, dried over sodium sulfate, and evaporated. The residue was subjected to column chromatography (hexanes:ethyl acetate 15:1) to give 880 mg (89%) of **7**. Ratio of the isomers after chromatography: approx. 1:0.6. ^1H NMR ($CDCl_3$, 600 MHz): δ = 7.45–7.25 (m, 24.6H); 7.09 (d, 2H, J = 8.3 Hz); 7.06 (d, 1.2H, J = 7.9 Hz); 5.68 (d, 1H, J = 5.7 Hz); 4.99 (d, 1.6H, J = 11.4 Hz); 4.72 (dd, 2.3H, J = 11.8, 3.1 Hz); 4.69–4.58 (m, 4.2H); 4.29 (t, 1H, J = 6.6 Hz); 4.05 (s, 1H); 3.97 (ddd, 1H, J = 12.2, 4.1, 2.5 Hz); 3.93 (s, 0.6 H); 3.89–3.84 (m, 2.1H); 3.74 (dd, 1H, J = 9.7, 5.7 Hz); 3.62 (ddd, 0.6H, J = 11.7, 4.3, 2.3 Hz); 3.44 (t, 0.6H, J = 6.6 Hz); 2.63 (dt, 1H, J = 12.7 Hz, 5.7 Hz); 2.33 (s, 3H); 2.325 (s, 2.1H); 2.20 (dd, 1H, J = 12.9, 4.6 Hz); 1.16–1.01 (m, 37H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 139.12; 138.94; 138.31; 138.20; 137.08; 136.94; 132.03; 131.57; 131.11; 130.55; 129.50; 129.39; 128.37; 128.32; 128.10; 127.98; 127.73; 127.69; 127.54; 127.33; 127.30; 127.24; 127.13; 84.88; 82.96; 79.71; 78.51; 75.43; 74.46; 74.03; 73.08; 72.35; 71.48; 70.40; 70.10; 62.36; 32.52; 31.86; 21.02; 17.96; 11.81. HRMS: calcd ($C_{36}H_{50}O_4SSiNa^+$) 629.3091, found 629.3075.

3,4-Di-*O*-benzyl-2-deoxy-6-*O*-triisopropylsilyl- β -galactopyranoside (8). **7** (820 mg, 1.35 mmol) was dissolved in 50 mL of acetone. The reaction mixture was cooled to 0°C , and NBS (281 mg, 1.59 mmol, 1.18 equiv) was added. The reaction mixture turned orange immediately. After 10 min, the reaction was quenched by addition of solid NH_4Cl . The mixture was diluted with water and ethyl acetate, and the aqueous layer was extracted with ethyl acetate (3 $\times$). The combined organic layer was extracted with brine, dried over sodium sulfate, and evaporated. The residue was subjected to column chromatography (hexanes:ethyl acetate 4:1) to give 520 mg (77%) of **8**. Ratio of α/β isomers: 1:0.4. ^1H NMR ($CDCl_3$, 600 MHz): δ = 7.40–7.22 (m, 14H); 5.42 (d, 1H, J = 2.2 Hz); 4.96 (d, 1.4H, J = 11.4 Hz); 4.70 (dd, 1.8H, J = 11.6, 5.5 Hz); 4.67–4.57 (m, 2.8H); 4.02–3.97 (m, 3H); 3.94 (s–b, 0.4 H); 3.90 (t, 0.4H, J = 9.2 Hz); 3.81 (dd, 1H, J = 9.7, 7.5 Hz); 3.80 (m, 0.4H); 3.73 (dd, 1H, J = 9.7, 5.7 Hz); 3.57 (ddd, 0.4H, J = 12.1, 4.2, 2.6 Hz); 3.52 (s–b, 0.4H); 3.40 (dd, 0.4H, J = 7.9, 5.7 Hz); 3.39 (s–b, 1H); 2.20 (dt, J = 12.5, 3.5 Hz); 2.15 (dt, 0.4 H, J = 12.3, 2.1 Hz); 2.00 (m, 0.4H); 1.98 (dd, 1H, J = 12.7, 4.0 Hz); 1.15–1.03 (m, 31H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 139.00; 138.89; 138.55; 138.21; 128.40; 128.34; 128.11; 128.04; 127.60; 127.36; 127.28; 94.82; 92.62; 77.14; 75.86; 74.53; 74.40; 74.26; 72.91; 71.76; 71.43; 70.36; 70.20; 62.38; 61.63; 34.90; 31.12; 17.98; 17.96; 14.14; 11.86; 11.84. HRMS calcd ($C_{29}H_{44}O_5SiNa^+$) 523.285, found 523.284.

3,4-Di-*O*-benzyl-1-*O*-triisopropylsilyl-octadec-6-ene-2-ol (9). Dodecyl triphenylphosphonium bromide (1.53 g, 3 mmol, 3 equiv) was suspended in 20 mL of dry THF. The mixture was cooled to -10°C . BuLi (1.75 mL, 2.8 mmol, 2.8 equiv) (1.6 M solution in hexanes) was added dropwise. The orange solution was stirred for 30 min, and then the starting material **8** (510 mg, 0.99 mmol in 15 mL of dry THF) was added slowly. The reaction was allowed to warm to room temperature and stirred overnight. The reaction was monitored by TLC using hexanes:ethyl acetate as a solvent system. For workup, the reaction mixture was quenched with methanol. Afterward 80% methanol in water was added and the reaction mixture was extracted with hexanes (4 $\times$). The combined hexanes layer was dried over sodium sulfate and evaporated, and the residue was subjected to column chromatography (hexanes:ethyl acetate 10:1) to yield 610 mg (92%) of **9**. ^1H NMR ($CDCl_3$, 600 MHz):

δ = 7.33–7.25 (m, 10H); 5.57–5.33 (m, 2H); 4.69 (d, 1H, J = 11.4 Hz); 4.60 (m, 3H); 3.94 (m, 1H); 3.72 (m, 4H); 3.07 (d, 1H, J = 4.8 Hz); 2.45 (dt, 1H, J = 15.4, 7.0 Hz); 2.39 (dt, 1H, J = 15.2, 5.6 Hz); 1.99 (q, 2H, J = 7.0 Hz); 1.33–1.20 (m, 19H); 1.12–0.99 (m, 21H); 0.86 (t, 3H, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 138.29; 133.64; 132.33; 128.34; 128.30; 128.00; 127.77; 127.70; 127.57; 124.99; 80.06; 78.14; 73.69; 72.71; 71.30; 63.86; 31.91; 29.67; 29.64; 29.57; 29.38; 29.35; 29.01; 27.54; 22.68; 17.99; 14.10; 14.10; 11.87. HRMS calcd ($C_{42}H_{70}O_4SiNa^+$) 675.4779, found 675.4780.

2-Azido-3,4-di-*O*-benzyl-1-*O*-triisopropylsilyl-octadec-6-ene (10). Sodium azide (6.5 g) was dispersed in 6.5 mL of water and 40 mL of toluene. The suspension was cooled to about 0°C . Concentrated sulfuric acid (1.35 mL) was added dropwise. While the internal temperature was kept below 10°C , the reaction mixture was stirred slowly for another 30 min. The toluene layer was decanted into a cooled conical flask (0°C), dried over sodium sulfate, and stored in a freezer. To determine the concentration, 0.3 mL of the solution and 0.3 mL of water were stirred in a vial and one drop of a phenolphthalein-solution (1% in MeOH) was added, and the solution was titrated with 0.25 N NaOH. Triphenylphosphine (482 mg, 1.84 mmol, 4 equiv) was dissolved in 15 mL of dry THF under argon. The solution was cooled to -78°C . DEAD (0.29 mL, 320 mg, 1.84 mmol, 4 equiv) and 1.3 mL (c = 1.4 M) of the previously prepared HN_3 solution were added. After stirring for 1 min, a solution of 300 mg (0.46 mmol) of **9** in 7 mL of THF was added rapidly. The reaction was stirred overnight. The reaction progress was checked by TLC (hexanes:ethyl acetate 9:1). The reaction was quenched by the addition of methanol (color changes from orange to light yellow), concentrated in vacuo, and chromatographed on silica gel (hexanes:ethyl acetate 20:1) to yield 263 mg (84%) of **10**. ^1H NMR ($CDCl_3$, 600 MHz): δ = 7.37–7.26 (m, 10H); 5.55–5.44 (m, 2H); 4.74–4.53 (m, 4H); 4.06 (dd, 1H, J = 10.5, 3.1 Hz); 3.84 (dd, 1H, J = 10.5, 7.9 Hz); 3.74–3.70 (m, 2H); 3.63 (dd, 1H, J = 6.1, 4.2 Hz); 2.48 (t, 1H, J = 5.5 Hz); 2.06 (m, 1H); 1.38–1.23 (m, 19H); 1.11–1.02 (m, 21H); 0.90 (t, 3H, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 138.32; 138.11; 132.34; 128.30; 128.27; 127.90; 127.67; 127.51; 125.14; 79.48; 78.89; 73.59; 72.05; 64.65; 64.62; 31.92; 29.68; 29.65; 29.60; 29.57; 29.40; 29.35; 27.90; 27.56; 22.68; 17.91; 14.11; 11.85. HRMS calcd ($C_{42}H_{70}N_3O_3Si^+$) 678.5024, found 678.5054.

2-Azido-3,4-di-*O*-benzyl-octadec-6-en-1-ol (11). **10** (250 mg, 0.37 mmol) was dissolved in 5 mL of dry THF. The reaction mixture was cooled to 0°C . 1 mL of a 1 M TBAF solution was added, and the reaction mixture was allowed to warm to room temperature. After 10 min, the reaction was controlled by TLC and found to be almost complete. After 20 min, the reaction was stopped by addition of 5 mL saturated $NaHCO_3$. The aqueous layer was extracted with ethyl acetate (4 $\times$), and the combined organic layer extracted with brine, dried, and evaporated. The residue was chromatographed on silica gel (hexanes:ethyl acetate 9:1) to yield 175 mg (91%) of **11**. ^1H NMR ($CDCl_3$, 600 MHz): δ = 7.39–7.26 (m, 10H); 5.51–5.48 (m, 1H); 5.48–5.38 (m, 1H); 4.70–4.61 (m, 3H); 4.56 (d, 1H, J = 11.4 Hz); 3.85 (dd, 1H, J = 11.4, 4.8 Hz); 3.80–3.68 (m, 4H); 2.46 (m, 2H); 2.03 (m, 2H); 1.37–1.22 (m, 18H); 0.88 (t, 3H, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$, 150 MHz): δ = 137.81; 137.58; 132.81; 128.44; 128.39; 127.97; 127.92; 127.88; 127.79; 124.40; 80.38; 78.57; 73.59; 72.26; 63.16; 62.24; 31.89; 29.65; 29.61; 29.54; 29.52; 29.36; 29.32; 28.11; 27.57; 22.66; 17.66; 14.09. HRMS calcd ($C_{33}H_{50}N_3O_3^+ - N_2$) 494.3629, found 494.3643.

2-Azido-3,4-di-*O*-benzyl-1-*O*-(2,3,4,5-tetra-*O*-benzyl- α -D-galactopyranosyl)- β -ribo-octadeca-6-en-1-ol (13). A solution of thioglycoside **12** (73 mg, 0.11 mmol) and sphingosine **11** (50 mg, 0.096 mmol) in 1.5 mL of anhyd CH_2Cl_2 was added over freshly dried powdered 4 Å molecular sieves and cooled to 0°C . Meanwhile, methyl disulfide (34.5 μL , 4 equiv) and methyl trifluoromethanesulfonate (43.9 μL , 4 equiv) were mixed in a separate vial to form solids. The solids in dry CH_2Cl_2 (0.5 mL) were added to the above solution, and the mixture was stirred at 0°C for 2.5 h. The reaction was quenched by the addition of 3 drops of saturated aqueous $Na_2S_2O_3$, diluted

with CH_2Cl_2 , and filtered through Celite. The organic layer was washed with saturated aqueous NaHCO_3 and brine, dried (MgSO_4), and concentrated. The residue was purified by column chromatography on silica gel (hexanes:EtOAc 7:1) to furnish **13** (39 mg, 39%) as an oil. ^1H NMR (CDCl_3 , 600 MHz): δ = 7.44–7.19 (m, 30H); 5.48–5.37 (m, 2H); 4.94 (d, 1H, J = 11.4 Hz); 4.89 (d, 1H, J = 3.2 Hz); 4.86–4.32 (m, 11H); 4.05 (dd, 1H, J = 10.0, 3.5 Hz); 3.96 (dd, 1H, J = 10.0, 2.0 Hz); 3.96–3.92 (m, 3H); 3.86–3.81 (m, 1H); 3.71–3.64 (m, 3H); 3.47 (m, 2H); 2.42 (m, 2H); 2.00 (m, 2H); 1.25 (m, 18H); 0.87 (t, 3H, J = 6.8 Hz). ^{13}C NMR (CDCl_3 , 150 MHz): δ = 138.82; 138.76; 138.65; 138.30; 138.09; 137.98; 132.42; 128.35; 128.34; 128.33; 128.31; 128.28; 128.22; 128.19; 127.79; 127.76; 127.75; 127.73; 127.72; 127.70; 127.64; 127.53; 127.51; 127.49; 127.43; 125.50; 98.60; 79.33; 78.89; 78.81; 76.41; 75.08; 73.68; 73.45; 73.14; 73.12; 73.08; 71.92; 69.02; 68.37; 62.24; 62.15; 31.92; 29.70; 29.68; 29.65; 29.60; 29.55; 29.48; 29.35; 27.95; 27.58; 22.68; 14.11. HRMS calcd ($\text{C}_{66}\text{H}_{81}\text{N}_3\text{O}_8\text{H}^+ - \text{N}_2$): 1016.6040, found 1016.6043.

O-(2,3-Di-O-benzyl-4,6-O-benzylidene-D-galactopyranosyl) Trichloroacetimidate (16). **15** (600 mg, 1.35 mmol) was dissolved in 45 mL of acetone. The reaction mixture was cooled to -20°C , and NBS (231 mg, 1.29 mmol, 1.2 equiv) was added. After 45 min, the reaction was quenched by addition of solid NH_4Cl . The mixture was diluted with water and ethyl acetate, and the aqueous layer was extracted with ethyl acetate (4 $\times$). The combined organic layer was extracted with brine (2 $\times$), dried over sodium sulfate, and evaporated. The residue was subjected to column chromatography (hexanes:ethyl acetate 1:1) to furnish 461 mg (95%) of intermediate deprotected product, which was subsequently solubilized in 10 mL of dry CH_2Cl_2 . CCl_3CN (1.028 mL, 10.28 mmol 10 equiv) and DBU (77 μL , 0.51 mmol) were added to the solution, and the mixture was stirred at room temperature for 2 h. The dark solution was then concentrated and purified by column chromatography on silica gel (hexanes:EtOAc 2:1 containing 1% NEt_3) to furnish **16** (567 mg, 93%) as a white foam. NMR and mass spectroscopic data correspond to the data given in the literature.²¹

2-Azido-3,4-di-O-benzyl-1-O-(2,3-di-O-benzyl-4,6-O-benzylidene- α -D-galactopyranosyl)-D-ribo-octadeca-6-ene-1-ol (17). A solution of trichloroacetimidate **16** (50 mg, 0.084 mmol, 1.1 equiv) and sphingosine derivative **11** (40 mg, 0.077 mmol) in 1.3 mL of anhyd Et_2O and 0.2 mL of anhyd THF was added over freshly dried powdered 4 Å molecular sieves and cooled to -20°C . $\text{BF}_3\cdot\text{OEt}_2$ (19.5 μL , 2 equiv) was added to the solution, and the mixture was stirred at -20°C for 1.5 h. Trichloroacetimidate **16** (22 mg, 0.036 mmol) was then added. Stirring was continued for 30 min followed by addition of another 12 mg (0.02 mmol) of donor. After 30 min, the mixture was diluted with EtOAc and filtered through Celite. The organic layer was washed with saturated aqueous NaHCO_3 and brine, dried (MgSO_4), and concentrated. The residue was purified by column chromatography on silica gel (hexanes:EtOAc 7:1) to furnish **17** (50 mg, 68%) as an oil. ^1H NMR (CDCl_3 , 500 MHz): δ = 7.52–7.25 (m, 25H); 5.45–5.41 (m, 3H); 4.92 (d, 1H, J = 3.5 Hz); 4.82 (d, 1H, J = 11.7 Hz); 4.79–4.49 (m, 7H); 4.11 (dm, 1H, J = 3.3 Hz); 4.05 (dd, 1H, J = 9.9, 3.3 Hz); 4.01–3.92 (m, 3H); 3.83–3.77 (m, 2H); 3.68–3.64 (m, 3H); 3.50 (m, 1H); 2.41 (m, 2H); 2.00 (m, 2H); 1.22 (m, 18H); 0.85 (t, 3H, J = 7.0 Hz). ^{13}C NMR (CDCl_3 , 125 MHz): δ = 138.72; 138.21; 137.95; 137.78; 137.77; 132.51; 128.35; 128.31; 128.24; 128.23; 128.21; 128.08; 127.80; 127.73; 127.68; 127.65; 127.64; 127.58; 127.50; 127.49; 127.44; 126.32; 101.02; 99.05; 79.20; 79.09; 75.71; 75.44; 74.65; 73.72; 73.44; 72.01; 71.96; 69.25; 68.29; 62.91; 61.91; 31.90; 29.67; 29.64; 29.58; 29.57; 29.39; 29.38; 29.33; 27.98; 27.57; 22.66; 14.11. HRMS calcd ($\text{C}_{59}\text{H}_{87}\text{N}_3\text{O}_8\text{Na}^+$): 974.5295, found 974.5306.

2-Amino-3,4-di-O-benzyl-1-O-(2,3-di-O-benzyl-4,6-O-benzylidene- α -D-galactopyranosyl)-D-ribo-octadeca-6-en-1-ol (19). The azide **17** (20 mg, 0.021 mmol) was dissolved in 0.5 mL of anhyd THF and cooled to 0°C . PMe_3 (0.11 mL of 1.0 M in THF, 0.11 mmol) was added to the solution, and the reaction was stirred for 45 min at 0°C and 2 h at room temperature. After complete disappearance of the starting material, 0.2 mL of aq 1 M NaOH was added to the mixture and stirred for 2 h. EtOAc was then added to the solution, and the mixture was washed with water (3 $\times$) and brine (1 $\times$), dried (MgSO_4), and concentrated. The residue was used for the next step without prior purification. ^1H NMR (CDCl_3 , 500 MHz): δ = 7.53–7.26 (m, 25H); 5.50 (m, 2H); 5.47 (s, 1H); 4.97 (d, 1H, J = 3.3 Hz); 4.85 (d, 1H, J = 11.7 Hz); 4.82–4.56 (m, 7H); 4.18 (dm, 1H, J = 2.3 Hz); 4.10–4.07 (m, 2H); 4.01–3.98 (m, 2H); 3.89 (m, 2H); 3.76 (m, 1H); 3.57 (m, 1H); 3.38 (m, 1H); 3.26 (m, 1H); 2.47 (m, 2H); 2.04 (m, 2H); 1.26 (m, 18H); 0.89 (t, 3H, J = 7.0 Hz). ^{13}C NMR (CDCl_3 , 125 MHz): δ = 138.73; 138.59; 138.48; 138.05; 137.81; 132.29; 128.84; 128.36; 128.37; 128.32; 128.28; 128.27; 128.09; 127.79; 127.67; 127.66; 127.62; 127.60; 127.54; 126.32; 101.04; 99.23; 79.61; 76.11; 75.72; 74.48; 74.47; 73.66; 73.61; 71.94; 71.79; 69.36; 69.37; 62.65; 62.77; 31.91; 29.69; 29.65; 29.64; 29.60; 29.43; 29.42; 29.35; 28.36; 27.61; 22.7; 14.12. HRMS calcd ($\text{C}_{59}\text{H}_{75}\text{NO}_8\text{Na}^+$): 948.5385, found 948.5379.

3,4-Di-O-benzyl-1-O-(2,3-di-O-benzyl-4,6-O-benzylidene- α -D-galactopyranosyl)-2-hexacosylamino-D-ribo-octadeca-6-en-1-ol (20). The crude amine **19** was dissolved in 0.5 mL of anhyd CH_2Cl_2 and hexacosanoic acid (26 mg, 0.06 mmol, 3 equiv) and 1-[3-(dimethylamino)-propyl]-3-ethylcarbodiimide hydrochloride (EDC) (11 mg, 0.063 mmol, 3 equiv) were added to the solution. The mixture was stirred at room temperature for 24 h and then diluted with CH_2Cl_2 and washed with water (3 $\times$) and brine (1 $\times$). The solution was dried (MgSO_4) and concentrated, and the residue was purified by column chromatography on silica gel (hexanes:EtOAc 4:1) to furnish **20** (11 mg, 69%) as an amorphous solid. ^1H NMR (CDCl_3 , 400 MHz): δ = 7.52–7.25 (m, 25H); 5.70 (d, 1H, J = 8.2 Hz); 5.47–5.45 (m, 3H); 4.93 (d, 1H, J = 3.5 Hz); 4.83 (d, 1H, J = 11.7 Hz); 4.78–4.48 (m, 7H); 4.38 (m, 1H); 4.16–4.04 (m, 3H); 4.07–4.04 (m, 3H); 3.94–3.87 (m, 1H); 3.77–3.73 (m, 1H); 3.59 (m, 1H); 2.45 (m, 1H); 2.09 (m, 1H); 1.87 (m, 2H); 1.73 (m, 2H); 1.50 (m, 2H); 1.35–1.20 (m, 62H); 0.90 (t, 6H, J = 7.0 Hz). ^{13}C NMR (CDCl_3 , 150 MHz): δ = 172.87; 138.67; 138.56; 138.37; 138.32; 137.81; 132.29; 128.84; 128.44; 128.37; 128.32; 128.31; 128.08; 127.87; 127.84; 127.76; 127.73; 127.66; 127.61; 127.57; 127.54; 126.31; 125.09; 101.00; 99.63; 79.93; 78.99; 76.13; 75.65; 74.40; 73.73; 73.34; 71.76; 71.59; 69.40; 68.24; 62.90; 50.20; 31.90; 29.72–29.61, 29.45; 29.42; 29.36; 27.99; 27.57; 25.71, 22.69; 14.13. HRMS calcd ($\text{C}_{85}\text{H}_{125}\text{NO}_9\text{Na}^+$): 1326.9246, found 1326.9248.

1-O-(α -D-galactopyranosyl)-2-hexacosylamino-D-ribo-1,3,4-octadecanetriol (1). A solution of **20** (11 mg, 0.008 mmol) in EtOAc (1 mL) was added to 3 mg of palladium black suspended in 1.5 mL of EtOAc and saturated with hydrogen. The reaction vessel was purged with hydrogen, and the mixture was stirred at room temperature for 10 h. The reaction mixture was diluted with pyridine, and the catalyst was removed by filtration over Celite. Evaporation of the solvent gave pure **1** (7 mg, 99.5%). The obtained spectroscopic data is identical to the one reported in the literature.^{11a} HRMS calcd ($\text{C}_{50}\text{H}_{99}\text{NO}_9\text{Na}^+$): 880.7212, found 880.7227.

Acknowledgment. O. P. acknowledges a fellowship of the Deutsche Forschungsgemeinschaft. V. B.-N. acknowledges the Swiss National Chemical Society fellowship. This research is supported by the Skaggs funds.

Supporting Information Available: ^1H and ^{13}C NMR spectral data of compounds **3–11**, **13**, **17**, **19**, **20**, and **1** is available free of charge via the Internet at <http://pubs.asc.org>.

JO0201530

(20) (a) Mottawia, M. S.; Olsen, C. E.; Denyer, K.; Smith, A. M.; Moller, B. L. *Carbohydr. Res.* **2001**, *330*, 309–318. (b) Wegmann, B.; Schmidt, R. R. *J. Carbohydr. Chem.* **1987**, *6*, 357–375.

(21) Koeman, F. A. W.; Kamerling, J. P.; Vliegthart, J. F. G. *Tetrahedron* **1993**, *49*, 5291–5309.

DOI: 10.1002/cmdc.200800021

Synthesis and in vitro Evaluation of α -GalCer Epimers

Matthias Trappeniers,^[a] Stijn Goormans,^[a] Katrien Van Beneden,^[b] Tine Decruy,^[b] Bruno Linclau,^[c] Aymen Al-Shamkhani,^[d] Tim Elliott,^[d] Christian Ottensmeier,^[d] Joern M. Werner,^[e] Dirk Elewaut,^[b] and Serge Van Calenbergh^{*[a]}

α -GalCer (also known as KRN7000) is an immunomodulatory glycolipid that is known to potently activate invariant natural killer T (NKT) cells upon CD1d-mediated stimulation. Because Th1 and Th2 cytokines, which are released after α -GalCer presentation, antagonize each other's effects, α -GalCer analogues that induce

a biased Th1/Th2 response are highly awaited. In this context, we report the synthesis and in vitro evaluation of α -Gal-D-xylo-Cer and two α -Gal-L-lyxo-Cer analogues, one with the natural acyl chain, the other with a truncated chain.

Introduction

Natural killer T (NKT) cells are a unique subset of T cells that express an invariant T cell antigen receptor (TCR). Unlike other T cells, NKT cells recognize glycolipid antigens when presented by the major histocompatibility complex (MHC) class I-like molecule CD1d.^[1] An extensively studied exogenous glycolipid activator of NKT cells is α -GalCer (also known as KRN7000, **1a**, Figure 1).^[2–4] α -GalCer was originally generated by the Kirin group from structure–activity studies of glycolipids that were isolated from the marine sponge *Agelas mauritanus*.

Stimulation of NKT cells by CD1d-mediated α -GalCer presentation leads to rapid release of T helper 1 (Th1) and T helper 2 (Th2) cytokines. Proinflammatory Th1 cytokines such as IFN- γ mediate antitumor, antiviral and antibacterial effects of α -GalCer, while immunomodulatory Th2 cytokines such as IL-4 delay or prevent the onset of autoimmune diseases like type 1 diabetes.^[5] Because Th1 and Th2 cytokines antagonize each other's effects, α -GalCer analogues that induce a biased Th1/Th2 response are highly awaited.

In 2005, the crystal structure of human CD1d in a complex with α -GalCer was elucidated, and it revealed the specific binding mode of α -GalCer to CD1d.^[6] The acyl chain of α -GalCer fits into the A' pocket by adopting a counterclockwise circular curve, and the sphingosine chain adopts an extended conformation to fit into the F' pocket and to reach the end of the binding groove. The galactose ring is well ordered and extends above the surface of the lipid-binding groove. The crystal structure revealed three hydrogen bonds between human CD1d and α -GalCer. The glycosidic linkage 1'-O is hydrogen bonded to Thr154, the 2'-OH group of the galactose ring forms a hydrogen bond to Asp151, and the 3-OH group on the sphingosine chain forms the third hydrogen bond to Asp80. These bonds are assumed to anchor α -GalCer in a proper orientation for recognition by the TCR of NKT cells.

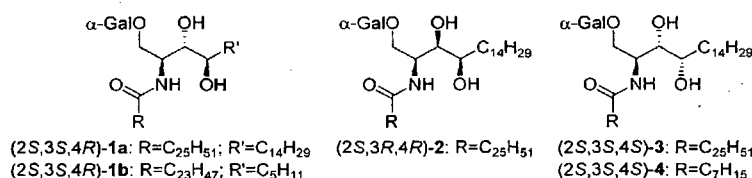


Figure 1. Structure of α -GalCer (**1a**, KRN7000), OCH (**1b**), α -Gal-D-xylo-Cer (**2**), and two α -Gal-L-lyxo-Cer analogues, one with the natural acyl chain (compound **3**) and the other with a truncated one (compound **4**).

In another crystallographic study of mouse CD1d in a complex with an OCH analogue, Asp80 was found to interact with both secondary hydroxy groups (i.e., 3-OH and 4-OH) of the sphingosine backbone.^[7] In addition, Arg79 is oriented in such a way that it can participate in hydrogen bonding with the same 3-OH. More recently, Wong and co-workers reported the crystal structure of mCD1d that was charged with the *Sphingomonas* glycolipid α -galacturonosyl ceramide (GalA-GSL), which is characterized by a shorter fatty acyl chain, the absence of a 4-OH on the sphingosine moiety, and the presence of a 6'-

[a] M. Trappeniers,* S. Goormans,* Prof. Dr. S. Van Calenbergh
Laboratory for Medicinal Chemistry
Faculty of Pharmaceutical Sciences, Ghent University
Harelbekestraat 72, 9000 Gent (Belgium)
Fax: (+32) 9-264-8146
E-mail: serge.vancalenbergh@ugent.be

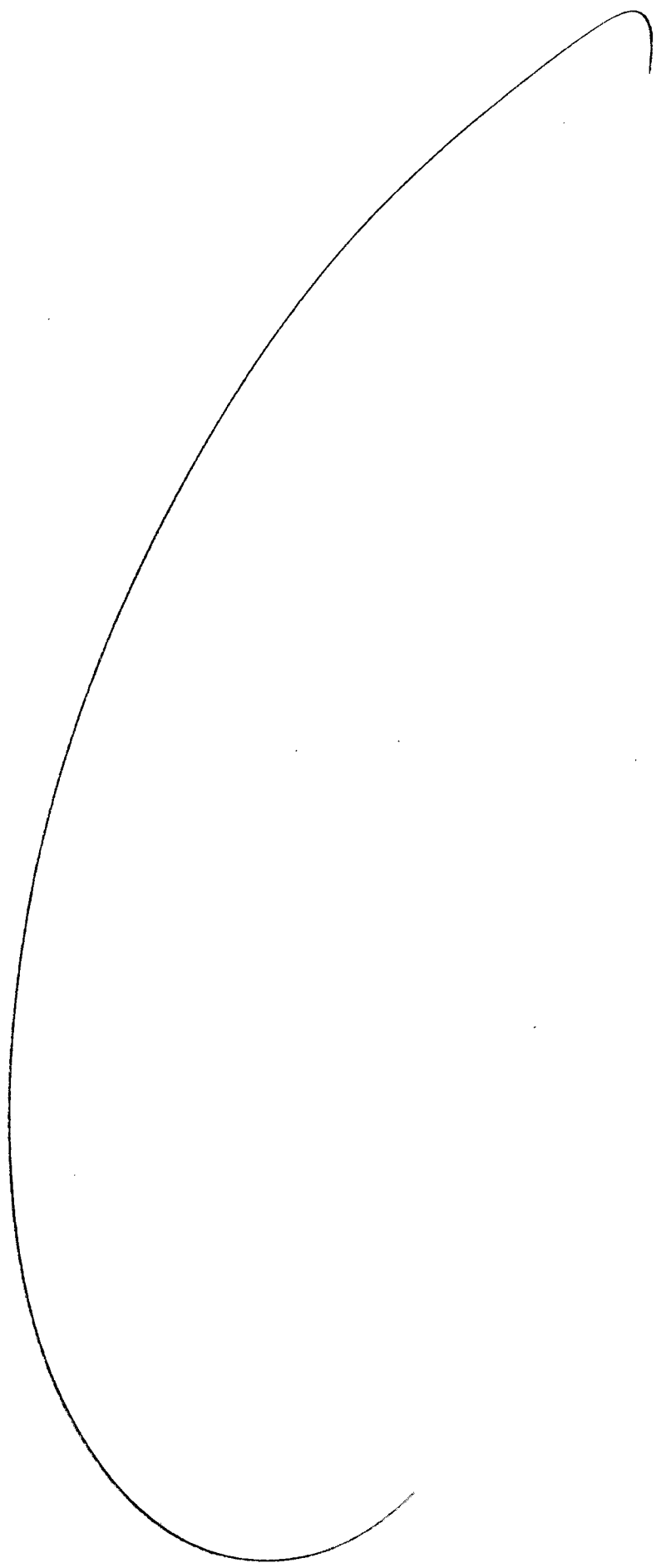
[b] Dr. K. Van Beneden, T. Decruy, Prof. Dr. D. Elewaut
Laboratory for Molecular Immunology and Inflammation
Department of Rheumatology, Ghent University Hospital, Ghent University
De Pintelaan 185, 9000 Ghent (Belgium)

[c] Dr. B. Linclau
School of Chemistry, University of Southampton
Highfield, Southampton SO17 1BJ (UK)

[d] Prof. Dr. A. Al-Shamkhani, Prof. Dr. T. Elliott, Prof. Dr. C. Ottensmeier
Cancer Sciences Division, School of Medicine
University of Southampton
Somers Cancer Research Building, MP824
Tremona Road, Southampton SO16 6YD (UK)

[e] Dr. J. M. Werner
School of Biological Sciences, University of Southampton
Bassett Crescent East, Southampton SO16 7PX (UK)

[*] These authors contributed equally to this work.



CO₂H group on the galactose unit.^[8] The absence of the 4-OH causes the sphinganine backbone to shift slightly deeper in the F' groove to establish an optimal hydrogen bond with the terminal oxygen atoms of Asp80. As a consequence, the hydrogen bond between Arg79 and the 3-OH of the sphinganine backbone is lost, and Arg79 is now oriented differently; this possibly affects the interaction of GalA-GSL with CD1d and the TCR.

Recently, Borg et al. reported the structure of a human NKT TCR in complex with CD1d that was bound to the potent NKT-cell agonist α -GalCer.^[9] Consistent with the previously determined structures,^[6,7] α -GalCer protrudes minimally from the CD1d cleft; only the galactosyl head group is exposed for recognition by the NKT TCR, and it interacts solely with the CDR1 α and CDR3 α loops. The galactose ring is sandwiched between Trp153 of CD1d and the aliphatic moiety of Arg95 α , the side-chain of which also hydrogen bonds to the 3-OH on the sphingosine chain. The 2'-OH, 3'-OH and 4'-OH of the galactose ring form hydrogen bonds to Gly96 α , Ser30 α , and Phe29 α , respectively, which are located on the invariant TCR α -chain. This mode of binding is consistent with the fine specificity that the NKT TCR exhibits for α -GalCer and its closely related analogues. Most reported analogues of α -GalCer result from modifications of the acyl chain,^[10,11] the glycosidic linkage,^[12,13] or the glycosyl residue,^[7,14–17] whereas relatively few alterations of the phytosphingosine part have been explored.^[11,18]

With the synthesis of OCH (1b, Figure 1) it was demonstrated that analogues with a truncated sphingosine base tend to predominantly trigger the production of Th2 cytokines.^[19,20] Other studies reported the synthesis of a 4-deoxy- α -GalCer congener,^[2,5,21,22] or analogues in which the phytoceramide moiety was replaced by a ceramide moiety, characterized by a double bond between C4 and C5.^[23,24] Interestingly, both types of analogues retained their ability to stimulate NKT cells; this suggests that the 4-OH is not critical for CD1d presentation.

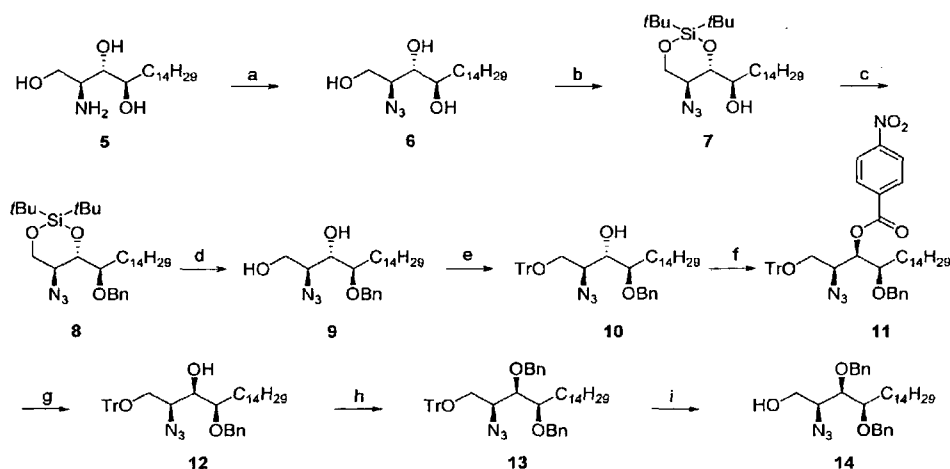
An overview of these modifications and the influence of the glycolipid structure on NKT cell response have been published by Savage et al.,^[25] and more recently by Wong and co-workers.^[26]

These studies illustrate the importance of exploring the stereochemistry at C3 and C4 of the phytosphingosine moiety. A few strategies are reported for the synthesis of D-xylo- and L-lyxo-phytosphingosine. The desired stereochemistry of the three chiral centers has been realized by using D-(-)-tartaric acid^[27–29] or a serine-derived 1,5-dioxaspiro[3.2]hexane^[30] as the chiral template, by an aldol condensation between an imino-glycinate that bears a chiral auxiliary and an appropriate aldehyde,^[31] or by asymmetric dihydroxylation of an (E)- α,β -unsaturated ester as the chiral induction stage.^[32] Remarkably, until now, no attempts to alter the stereochemistry of the secondary hydroxy groups (i.e., 3-OH and 4-OH) of the phytosphingosine moiety of α -GalCer have been published. Recent work by Kim et al.^[33] on the efficient synthesis of D-xylo-, L-lyxo-, and L-arabi-phytosphingosine from the natural D-ribo-phytosphingosine has prompted us to report the synthesis and in vitro evaluation of α -Gal-D-xylo-Cer (2) and two α -Gal-L-lyxo-Cer analogues, one with the natural acyl chain (compound 3), the other with a truncated one (compound 4) (Figure 1).

Results and Discussion

Chemistry

The synthesis of the D-xylo-phytosphingosine acceptor 14 was started from the commercially available D-ribo-phytosphingosine 5 (Scheme 1). Conversion of the primary amine to an azide, and subsequent protection of the hydroxy groups at C1 and C3 afforded the 1,3-di-*tert*-butylsilylene derivative 7.^[34] Benzyl protection of the remaining 4-OH group with freshly prepared benzyl 2,2,2-trichloroacetimidate, followed by silylene



Scheme 1. Reagents and conditions: a) N₃Tf, K₂CO₃, CuSO₄, CH₂Cl₂, CH₃OH, H₂O, RT, overnight, 98%; b) (tBu)₂Si(OTf)₂, DMF, pyridine, –20 °C, 4 h, 96%; c) benzyltrichloroacetimidate, TfOH (cat.), Et₂O, 0 °C → RT, 48 h, 58%; d) HF-pyridine, THF/pyridine (1:1), 0 °C, 0.5 h, 98%; e) TrCl, DMAP, pyridine, 70 °C, overnight, 87%; f) *p*-nitrobenzoic acid, DIAD, PPh₃, THF, RT, overnight, 71%; g) NaOMe, MeOH, RT, 1 h, 89%; h) BnBr, NaH, DMF, 0 °C → RT, overnight, 94%; i) ZnBr₂, CH₂Cl₂/iPrOH (85:15), RT, overnight, 73%.

deprotection gave diol **9**. The primary alcohol was then tritylated, and inversion of the 3-OH group under Mitsunobu conditions afforded the *p*-nitrobenzoic ester derivative **11**. Solvolysis of the activated ester and subsequent protection of the inverted 3-OH group as a benzyl ether gave the desired *D*-xylo-phytosphingosine acceptor **14** after final deprotection of the trityl group.

For the synthesis of the *L*-xylo-phytosphingosine acceptor **20**, we started from intermediate **7** (Scheme 2). Inversion of the 4-OH group under Mitsunobu conditions afforded the *p*-nitrobenzoic ester intermediate **15**. Solvolysis of the activated ester and subsequent deprotection of the silylene gave triol **17**. Selective tritylation of the primary alcohol, followed by a dibenzylization step, afforded the desired *L*-xylo-phytosphingosine acceptor **20** after final deprotection of the trityl group.

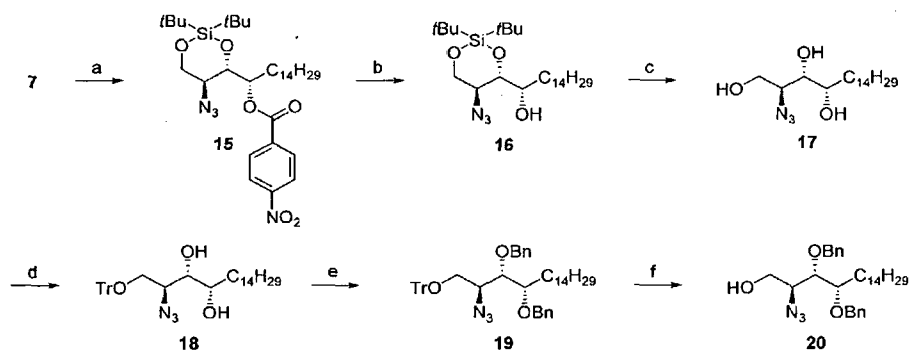
For the glycosidation step, the 4,6-benzylidene-protected trichloroacetimidate **21** was used as the galactosyl donor (Scheme 3).^[35] Reaction with the *L*-xylo-phytosphingosine ac-

ceptor **20** by using boron trifluoride diethyl etherate as the promoter afforded the desired α -glycoside **23** in 58% yield. The glycosidation of the *D*-xylo-phytosphingosine acceptor **14** was performed with trimethylsilyl triflate^[36] because it became clear during the course of this project that this promoter gave improved coupling yields. The desired α -glycoside **22** was isolated in 70% yield. Staudinger reduction, followed by acylation with the appropriate acid and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) as the coupling reagent gave the protected compounds **24–26**. Final hydrogenolysis afforded the desired analogues **2–4**.

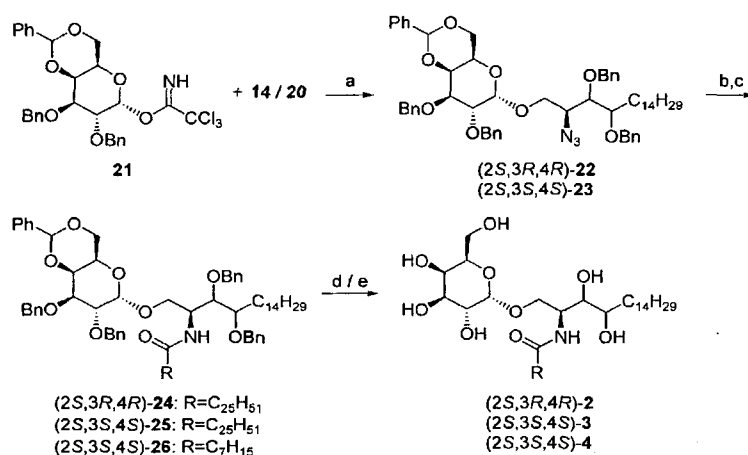
Confirmation of the inversion of the stereochemistry at C3 and C4 was established by preparing three bicyclic compounds **27**, **28** and **31**, and subjecting these to ¹H NMR spectroscopic selective decoupling experiments. The synthesis of the *trans*-fused bicyclic compounds **27** and **28** started from intermediates **7** and **16**, respectively (Scheme 4). Staudinger reduction of the azide, followed by cyclization with triphosgene afforded

the desired compounds **27** and **28**. For the synthesis of the *cis*-fused bicyclic compound **31**, intermediate **12** was used. Detritylation of the primary alcohol, followed by protection of the hydroxy groups at C1 and C3 afforded the 1,3-di-*tert*-butylsilylene derivative **30**. Reduction of the azide and simultaneous deprotection of the 4-OH group gave the desired *cis*-fused bicyclic compound **31** after cyclization with triphosgene.

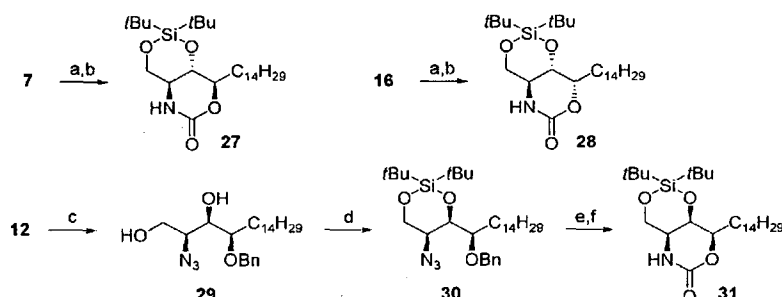
A ¹H NMR spectrum that was obtained by selective decoupling of H2 permitted us to determine the coupling constant between H3 and H4 (Figure 2). For the *trans*-fused derivative **27**, we found a ³*J*_{3,4} value of 9.4 Hz, which confirms the transdiaxial position of H3 and H4. Irradiation of H2 in compound **28** gave rise to a ³*J*_{3,4} value of 5.0 Hz, and compound **31** furnished a ³*J*_{3,4} value of 2.1 Hz. According to the Karplus equation, these values indicate a dihedral angle between 60° and 90°; this confirms the inversion of the stereochemistry at C3 (compound **31**) and C4 (compound **28**).



Scheme 2. Reagents and conditions: a) *p*-nitrobenzoic acid, DIAD, PPh₃, toluene, RT, overnight, 78%; b) NaOMe, MeOH, RT, 1 h, 90%; c) HF-pyridine, THF/pyridine (1:1), 0 °C, 0.5 h, 96%; d) TrCl, DMAP, pyridine, 70 °C, 4 h; e) BnBr, NaH, DMF, 0 °C → RT; f) ZnBr₂, CH₂Cl₂/iPrOH (85:15), RT, overnight, 63% over three steps.



Scheme 3. Reagents and conditions: a) **14**, TMSOTf, THF, 4 Å MS, –20 °C, 2 h, 70% or **20**, BF₃·Et₂O, Et₂O/THF, 4 Å MS, –20 °C, 3 h, 58%; b) 1) PMe₃, THF, RT, 2 h, 2) 1 M NaOH, RT, 2 h; c) C₂₅H₅₁COOH or C₇H₁₃COOH, EDC, CH₂Cl₂, RT, 18 h, 76% (**24**), 60% (**25**) and 64% (**26**); d) H₂, Pd black, CHCl₃/EtOH (1:3), 4 h, 68% (**2**); e) H₂, Pd/C, EtOAc, 43% (**3**) and 75% (**4**).



Scheme 4. Reagents and conditions: a) 1) PMe_3 , THF, RT, 2 h, 2) 1 M NaOH, RT, 2 h; b) triphosgene, $i\text{Pr}_2\text{EtN}$, CH_2Cl_2 , RT, 16 h, 64 % (27), 83 % (28) over two steps; c) ZnBr_2 , CH_2Cl_2 / $i\text{PrOH}$ (85:15), RT, overnight, 89 %; d) $(t\text{Bu})_2\text{Si}(\text{OTf})_2$, DMF, pyridine, -20°C , 4 h, 95 %; e) H_2 , Pd black, CHCl_3 / EtOH (1:3), 7 h; f) triphosgene, $i\text{Pr}_2\text{EtN}$, CH_2Cl_2 , RT, 16 h, 43 % (31) over two steps.

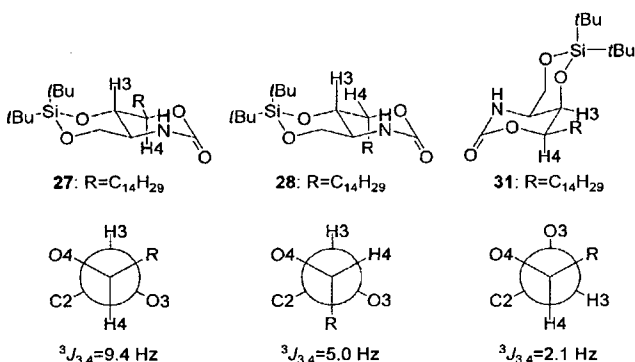


Figure 2. Determination of the coupling constant between H3 and H4 of bicyclic compounds 27, 28, and 31.

In vitro stimulation of NKT cells

To investigate whether these analogues could elicit NKT cell activation, splenocytes from B6 mice were cultured with different concentrations of glycolipids. Figure 3 demonstrates that all compounds induced significant IFN- γ and IL-4 production in a dose-dependent manner. Remarkably, epimer 4 activated NKT cells to induce similar levels of both IFN- γ and IL-4 compared with α -GalCer, while the level of IFN- γ was significantly lower when splenocytes were cultured at the lowest concentrations with either 2 or 3. A similar level of IFN- γ was observed after culturing the splenocytes with 1a, 2, or 3 at the highest concentration of 250 ng mL^{-1} . In addition, no cytokine production was observed when splenocytes from either $\text{Ja18}^{-/-}$ or $\text{CD1d}^{-/-}$ mice were cultured with α -GalCer or α -GalCer analogues; this indicates that these glycolipids induce CD1d-dependent TCR activation of NKT cells (Figure 3).

Conclusions

Herein we describe the synthesis and in vitro evaluation of α -Gal-*D*-xylo-Cer (2) and two α -Gal-*L*-xylo-Cer analogues, one with the natural acyl chain (compound 3), the other with a truncated one (compound 4). The in vitro data demonstrate

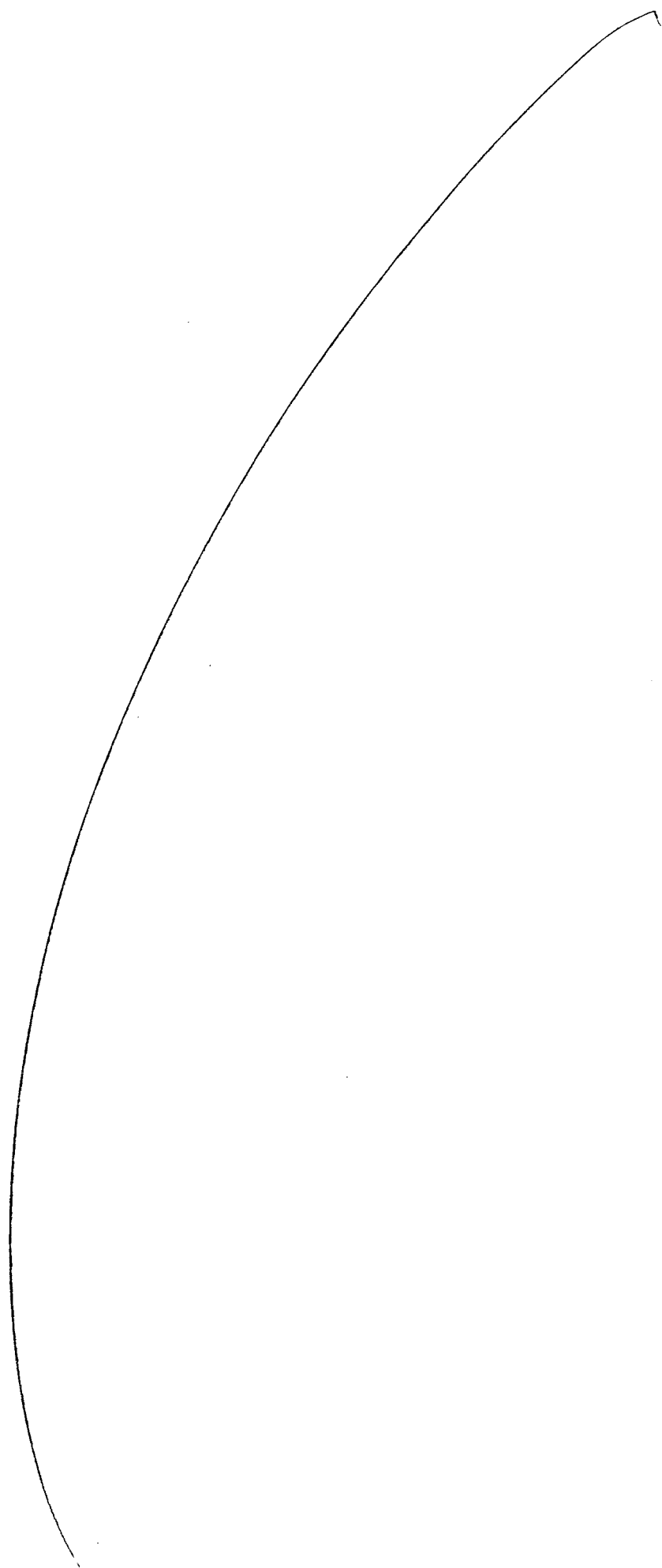
that these compounds showed significant biological activities through activation of NKT cells in both a TCR and CD1d-dependent manner. Furthermore, our data show evidence that a single modification by alteration of the stereochemistry of either the 3-OH or 4-OH in the phytosphingosine chain, causes the induction of differential cytokine levels. This observation can be probably explained by suggesting that these modifications might induce changes in the affinity for CD1d.

Experimental Section

General: NMR spectra were obtained with a Varian Mercury 300 spectrometer (Varian, Palo Alto, California, USA) or a Bruker Avance II 700 spectrometer. Chemical shifts are given in ppm (δ) relative to residual solvent peak, in the case of $[\text{D}_6]\text{DMSO}$: $\delta = 2.54 \text{ ppm}$ for ^1H and $\delta = 40.5 \text{ ppm}$ for ^{13}C , in the case of CDCl_3 : $\delta = 7.26 \text{ ppm}$ for ^1H and $\delta = 77.4 \text{ ppm}$ for ^{13}C , and in the case of $[\text{D}_3]\text{pyridine}$ $\delta = 7.18 \text{ ppm}$, 7.56 ppm and $\delta = 8.71 \text{ ppm}$ for ^1H and $\delta = 123.5$, 135.5 and 149.9 ppm for ^{13}C . All signals that were assigned to hydroxy groups were exchangeable with D_2O . Mass spectra and exact mass measurements were performed on a quadrupole/orthogonal-acceleration time-of-flight (Q/oaTOF) tandem mass spectrometer (qT of 2, Micromass, Manchester, UK) that was equipped with a standard electrospray ionization (ESI) interface. Samples were infused in a 2-propanol/water (1:1) mixture at $3 \mu\text{L min}^{-1}$. Precoated Merck silica gel F254 plates were used for TLC, and spots were examined under UV light at 254 nm and stained by sulfuric acid-anisaldehyde spray. Column chromatography was performed on ICN silica gel ($63\text{--}200 \mu\text{m}$, ICN, Asseldegem, Belgium).

(2S,3S,4R)-2-Azido-octadecane-1,3,4-triol (6): A mixture of NaN_3 (20.48 g , 315 mmol), CH_2Cl_2 (50 mL) and H_2O (50 mL) was cooled to 0°C , and Tf_2O (11.25 mL , 63 mmol) was added slowly. After 2 h stirring at 0°C , the mixture was separated. The aqueous layer was extracted with CH_2Cl_2 ($2 \times 25 \text{ mL}$) and the combined organic layers were washed with H_2O ($2 \times 100 \text{ mL}$). This freshly prepared TfN_3 solution (100 mL in CH_2Cl_2) was added to a mixture of phytosphingosine 5 (10 g , 31.5 mmol), K_2CO_3 (8.7 g , 62.9 mmol), CuSO_4 (50 mg , 0.32 mmol), H_2O (200 mL) and MeOH (600 mL). The mixture was stirred overnight and evaporated in vacuo to 200 mL of a white slurry. The precipitate was filtered, washed with H_2O ($5 \times 100 \text{ mL}$) and lyophilized to yield compound 6 (10.60 g , 98%) as a white solid. ^1H NMR (300 MHz , $[\text{D}_3]\text{pyridine}$): $\delta = 0.86$ (t, $J = 6.7$, 3H), $1.18\text{--}1.36$ (m, 22H), $1.60\text{--}1.74$ (m, 1H), $1.84\text{--}1.90$ (m, 2H), $2.12\text{--}2.24$ (m, 1H), $4.20\text{--}4.27$ (m, 1H), 4.32 (dd, $J = 4.4$ and 6.7 , 1H), 4.43 (ddd, $J = 3.5$, 4.1 and 7.6 , 1H), 4.57 (dd, $J = 7.5$ and 11.4 , 1H), 4.69 (dd, $J = 3.5$ and 11.4 , 1H), 6.38 (brs, 1H), 7.08 ppm (brs 2H); ^{13}C NMR (75 MHz , $[\text{D}_3]\text{pyridine}$): $\delta = 14.20$, 22.86 , 26.31 , 29.53 , 29.84 , 29.89 , 29.99 , 30.15 , 32.04 , 34.12 , 61.93 , 66.67 , 72.33 , 75.96 ppm ; HRMS (ESI-MS): m/z : for $\text{C}_{18}\text{H}_{37}\text{N}_3\text{O}_3$: calcd: 366.2733 [$M + \text{Na}$] $^+$, found: 366.2725 .

(2S,3S,4R)-2-Azido-1,3-O-di-(tert-butyl)silanedioctadecane-1,3,4-triol (7): A solution of 6 (10 g , 29.11 mmol) in DMF (200 mL) and pyridine (2.6 mL , 32.02 mmol) was cooled to -20°C and



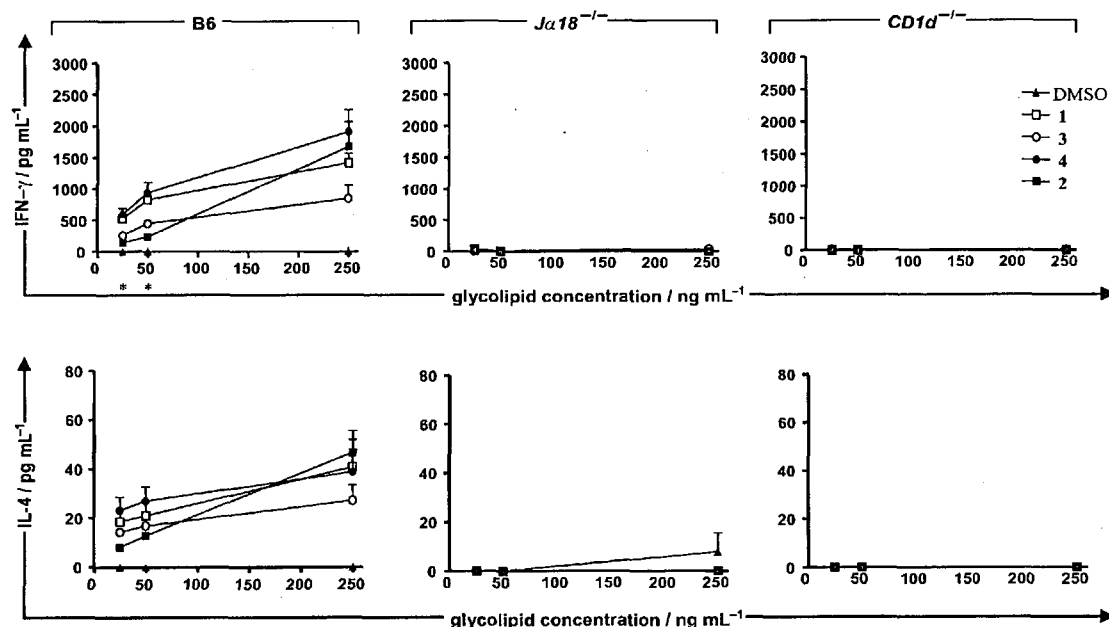


Figure 3. Properties of glycolipids to activate the TCR of NKT cells in a CD1d-dependent manner. Splenocytes of B6, $J\alpha 18^{-/-}$, and $CD1d^{-/-}$ mice were cultured with various concentrations of **1a** ($\square$), **2** ($\blacksquare$), **3** ($\circ$), **4** ($\bullet$), or DMSO ($\blacktriangle$). After 72 h, supernatants were harvested, and both IFN- γ (top row) and IL-4 (bottom row) levels were measured by ELISA. Data represent the mean $\pm$ SEM of 6–8 wells that were pooled from two experiments. (* $P < 0.05$ for both compounds **2** and **3** versus **1a** at glycolipid concentrations of 50 ng mL $^{-1}$ and 25 ng mL $^{-1}$; Mann–Whitney U test).

(*t*Bu) $_2$ Si(OTf) $_2$ (10.37 mL, 32.02 mmol) was added dropwise over 1 h. After additional stirring for 1 h at -20°C , the mixture was quenched with H $_2$ O (800 mL). The aqueous layer was extracted with EtOAc (3 $\times$ 250 mL) and the combined organic layers were washed with 1 M HCl (150 mL) and H $_2$ O (2 $\times$ 150 mL), dried over Na $_2$ SO $_4$, filtered and evaporated to dryness. The residue was purified by column chromatography with hexanes/EtOAc (95:5), to afford **7** (13.47 g, 96%) as a colorless oil. ^1H NMR (300 MHz, CDCl $_3$): δ = 0.87 (t, J = 6.7, 3H), 1.00 (s, 9H), 1.04 (s, 9H), 1.20–1.44 (m, 23H), 1.48–1.62 (m, 3H), 2.09 (d, J = 8.5, 1H), 3.51 (appdt, J = 4.7 and 10.0, 1H), 3.71–3.79 (m, 1H), 3.92 (dd, J = 6.0 and 10.0, 1H), 3.94 (dd, J = 10.0 and 10.1, 1H), 4.22 ppm (dd, J = 4.7 and 10.0, 1H); ^{13}C NMR (75 MHz, CDCl $_3$): δ = 14.34, 20.48, 22.92, 22.93, 25.88, 27.23, 27.71, 29.59, 29.83, 29.86, 29.89, 29.90, 29.92, 29.93, 31.10, 32.15, 58.99, 66.56, 73.15, 79.22 ppm; HRMS (ESI-MS): m/z : calcd for C $_{26}$ H $_{33}$ N $_3$ O $_3$ Si: 482.3783 [M –H] $^+$, found: 482.3780.

(2S,3S,4R)-2-Azido-4-O-benzyl-1,3-O-di-(*tert*-butyl)silanedioctadecane-1,3,4-triol (8): A solution of benzyl alcohol (12.3 mL, 118.85 mmol) in Et $_2$ O (45 mL) was cooled to 0°C and NaH (1.19 g, 29.71 mmol) was added. After 30 min stirring at 0°C , Cl $_3$ CCN (11.92 mL, 118.85 mmol) was added, and the solution was allowed to stir at room temperature for 1 h. The mixture was quenched with NaHCO $_3$ (50 mL) and the aqueous layer was extracted with Et $_2$ O (3 $\times$ 50 mL). The combined organic layers were dried over MgSO $_4$, filtered, and evaporated to dryness to afford benzyl 2,2,2-trichloroacetimidate as a brown oil. TfOH (208 μ L, 2.38 mmol) was added dropwise to a mixture of **7** (11.5 g, 23.77 mmol) and freshly prepared benzyl 2,2,2-trichloroacetimidate (118.85 mmol) in Et $_2$ O (55 mL). The brown mixture was stirred at room temperature for 48 h and quenched with NaHCO $_3$ (100 mL). The aqueous layer was extracted with Et $_2$ O (3 $\times$ 100 mL), and the combined organic layers were washed with H $_2$ O (50 mL), dried over MgSO $_4$, filtered, and

evaporated to dryness. The residue was purified by column chromatography with 1% Et $_3$ N (v/v) in hexanes/EtOAc (99:1) to afford compound **8** (7.9 g, 58%) as a colorless oil. ^1H NMR (300 MHz, CDCl $_3$): δ = 0.82 (t, J = 6.7, 3H), 0.92 (s, 9H), 0.98 (s, 9H), 1.20–1.44 (m, 23H), 1.48–1.62 (m, 3H), 3.45 (m, 1H), 3.52 (m, 1H), 3.82 (dd, J = 9.9 and 10.9, 1H), 3.98 (dd, J = 3.1 and 8.9, 1H), 4.12 (dd, J = 4.4 and 10.9, 1H), 4.52 (d, J = 12.0, 1H), 4.57 (d, J = 12.0, 1H), 7.20–7.30 ppm (m, 5H); ^{13}C NMR (75 MHz, CDCl $_3$): δ = 14.35, 20.57, 22.86, 22.92, 25.76, 27.24, 27.76, 29.59, 29.83, 29.86, 29.89, 29.90, 29.92, 29.94, 32.16, 59.37, 66.57, 72.18, 77.17, 81.15, 127.81, 128.19, 128.55, 138.73 ppm; HRMS (ESI-MS): m/z : calcd for C $_{33}$ H $_{39}$ N $_3$ O $_3$ Si: 596.4223 [M +Na] $^+$, found: 596.5014.

(2S,3S,4R)-2-Azido-4-O-benzyl-1,3,4-triol (9): A solution of HF in pyridine (3.99 mL, 28.78 mmol, 65–70%) was added dropwise to a solution of **8** (7.5 g, 13.08 mmol) in THF (65 mL) and pyridine (65 mL) at 0°C . After 30 min the mixture was diluted with EtOAc (150 mL), the organic layer was washed with 1 M HCl (3 $\times$ 50 mL) and H $_2$ O (3 $\times$ 50 mL), dried on Na $_2$ SO $_4$, filtered, and evaporated to dryness. The residue was purified by column chromatography with hexanes/EtOAc (7:3) to yield compound **9** (5.56 g, 98%) as colorless oil. ^1H NMR (300 MHz, CDCl $_3$): δ = 0.84 (t, J = 6.7, 3H), 1.20–1.38 (m, 23H), 1.42–1.56 (m, 2H), 1.62–1.68 (m, 1H), 2.40–2.58 (brs, 2H), 3.53 (m, 1H), 3.62 (m, 1H), 3.84 (dd, J = 5.0 and 11.7, 1H), 3.93 (m, 1H), 3.95 (dd, J = 4.4 and 11.7, 1H), 4.59 (s, 2H), 7.20–7.30 ppm (m, 5H); ^{13}C NMR (75 MHz, CDCl $_3$): δ = 14.35, 22.91, 25.53, 29.00, 29.59, 29.79, 29.83, 29.89, 29.93, 29.94, 29.95, 32.15, 62.97, 63.13, 72.23, 72.51, 79.76, 128.24, 128.26, 128.79, 138.02 ppm; HRMS (ESI-MS): m/z : calcd for C $_{25}$ H $_{43}$ N $_3$ O $_3$: 434.3377 [M +H] $^+$, found: 434.3372.

(2S,3S,4R)-2-Azido-4-O-benzyl-1-O-trityloctadecane-1,3,4-triol (10): A solution of **9** (5.4 g, 12.45 mmol), DMAP (384 mg, 3.11 mmol) and trityl chloride (3.90 g, 13.70 mmol) in pyridine

(125 mL) was stirred overnight at 70 °C. The mixture was quenched with NaHCO₃ and extracted with CH₂Cl₂ (3 × 150 mL). The combined organic layers were washed with brine, dried over Na₂SO₄, filtered, and evaporated to dryness. The residue was purified by column chromatography with hexanes/EtOAc (9:1) to yield compound **10** (7.33 g, 87%) as a yellow oil. ¹H NMR (300 MHz, CDCl₃): δ = 0.89 (t, *J* = 6.6, 3H), 1.20–1.38 (m, 23H), 1.42–1.52 (m, 2H), 1.54–1.60 (m, 1H), 2.32 (d, *J* = 4.1, 1H), 3.34–3.42 (m, 2H), 3.53–3.64 (m, 2H), 3.80 (ddd, *J* = 4.4, 4.7 and 6.9, 1H), 4.28 (d, *J* = 11.3, 1H), 4.43 (d, 1H, *J* = 11.3), 7.19–7.35 (m, 14H), 7.44–7.50 ppm (m, 6H); ¹³C NMR (75 MHz, CDCl₃): δ = 14.36, 22.93, 25.22, 28.78, 29.60, 29.81, 29.86, 29.90, 29.93, 29.94, 29.99, 32.16, 62.67, 64.32, 71.38, 71.99, 79.65, 87.71, 127.39, 127.99, 128.00, 128.17, 128.65, 128.90, 138.31, 143.77 ppm; HRMS (ESI-MS): *m/z*: calcd for C₄₄H₅₇N₃O₃: 698.4298 [M+Na]⁺, found: 698.4277.

(2S,3R,4R)-2-Azido-4-O-benzyl-3-O-(4-nitro)benzoyl-1-O-trityloctadecane-1,3,4-triol (11): DIAD (5.39 mL, 26.04 mmol) was added to a solution of triphenylphosphine (6.83 g, 26.04 mmol) and *p*-nitrobenzoic acid (4.34 g, 26.04 mmol) in THF (60 mL) at room temperature. After stirring for 1 h, compound **10** (7.0 g, 10.42 mmol) in THF (40 mL) was added, and the mixture was stirred overnight at room temperature. The next day, the THF was removed in vacuo, and the residue was purified by column chromatography with hexanes/EtOAc (97:3) to afford **11** (6.07 g, 71%) as a yellow oil. ¹H NMR (300 MHz, CDCl₃): δ = 0.84 (t, *J* = 6.7, 3H), 1.20–1.38 (m, 24H), 1.44–1.52 (m, 2H), 3.27 (dd, 1H, *J* = 6.1 and 10.1), 3.45–3.55 (m, 2H), 3.75 (ddd, *J* = 4.2, 5.9 and 5.9, 1H), 4.05 (d, *J* = 11.5, 1H), 4.43 (d, *J* = 11.3, 1H), 5.51 (dd, *J* = 4.7 and 5.8, 1H), 7.06–7.12 (m, 2H), 7.20–7.30 (m, 12H), 7.40–7.46 (m, 6H), 8.15 (d, *J* = 9.1, 2H), 8.27 ppm (d, *J* = 9.1, 2H); ¹³C NMR (75 MHz, CDCl₃): δ = 14.35, 22.92, 25.37, 29.60, 29.69, 29.75, 29.87, 29.89, 29.93, 29.94, 30.27, 32.16, 61.75, 63.15, 72.60, 74.76, 78.13, 87.58, 123.77, 127.47, 127.73, 127.87, 128.17, 128.51, 128.83, 131.22, 135.28, 138.10, 143.45, 150.86, 164.09 ppm; HRMS (ESI-MS): *m/z*: calcd for C₅₁H₆₀N₄O₆: 847.4411 [M+Na]⁺, found: 847.3873.

(2S,3R,4R)-2-Azido-4-O-benzyl-1-O-trityloctadecane-1,3,4-triol (12): NaOMe (400 mg, 7.02 mmol) was added to a solution of **11** (5.8 g, 7.02 mmol) in MeOH (70 mL) at room temperature. The white suspension was stirred for 1 h, quenched with a saturated NH₄Cl solution, and extracted with CH₂Cl₂ (3 × 100 mL). The combined organic layers were washed with brine (1 × 100 mL), dried over MgSO₄, filtered, and evaporated to dryness. The crude was purified by column chromatography with hexanes/EtOAc (9:1) to yield compound **12** (4.23 g, 89%) as a yellow solid. ¹H NMR (300 MHz, CDCl₃): δ = 0.82 (t, *J* = 6.7, 3H), 1.20–1.38 (m, 24H), 1.42–1.56 (m, 2H), 2.14 (d, *J* = 5.6, 1H), 3.24–3.42 (m, 4H), 3.62 (dd, *J* = 5.2 and 9.7, 1H), 4.14 (d, *J* = 11.1, 1H), 4.44 (d, *J* = 11.3, 1H), 7.10–7.26 (m, 18H), 7.35–7.40 ppm (m, 2H); ¹³C NMR (75 MHz, CDCl₃): δ = 14.35, 22.93, 25.30, 29.60, 29.82, 29.94, 30.09, 30.20, 31.92, 32.16, 63.53, 63.95, 72.42, 72.59, 79.55, 127.40, 127.49, 128.00, 128.03, 128.14, 128.68, 128.87, 138.23, 143.79 ppm; HRMS (ESI-MS): *m/z*: calcd for C₄₄H₅₇N₃O₃: 698.4298 [M+Na]⁺, found: 698.4279.

(2S,3R,4R)-2-Azido-3,4-di-O-benzyl-1-O-trityloctadecane-1,3,4-triol (13): NaH (266 mg, 6.65 mmol) was added to a solution of **12** (3.0 g, 4.43 mmol) in DMF (44 mL) at 0 °C. After 30 min stirring at 0 °C, benzyl bromide (806 μL, 6.65 mmol) was added, and the mixture was stirred overnight at room temperature. H₂O (200 mL) was added and the aqueous layer was extracted with CH₂Cl₂ (3 × 200 mL). The combined organic layers were washed with brine (3 × 100 mL), dried over MgSO₄, filtered, and evaporated to dryness. The residue was purified by column chromatography with hexanes/EtOAc (97:3) to yield compound **13** (3.19 g, 94%) as a color-

less oil. ¹H NMR (300 MHz, CDCl₃): δ = 0.82 (t, 3H, *J* = 6.7), 1.12–1.24 (m, 24H), 1.42–1.52 (m, 2H), 3.25–3.32 (m, 2H), 3.35–3.45 (m, 2H), 3.48–3.54 (m, 2H), 4.28 (d, *J* = 11.4, 1H), 4.36 (d, *J* = 11.4, 1H), 4.45 (d, *J* = 11.4, 1H), 4.58 (d, *J* = 11.5, 1H), 7.08–7.48 ppm (m, 25H); ¹³C NMR (75 MHz, CDCl₃): δ = 14.35, 22.93, 25.74, 29.60, 29.85, 29.94, 30.75, 30.77, 32.16, 62.40, 63.55, 73.27, 74.70, 79.83, 80.12, 127.17, 127.24, 127.37, 127.75, 127.83, 128.06, 128.12, 128.23, 128.48, 128.50, 128.86, 128.96, 138.33, 138.70, 143.83 ppm; HRMS (ESI-MS): *m/z*: calcd for C₅₁H₆₃N₃O₃: 788.4767 [M+Na]⁺, found: 788.4368.

(2S,3R,4R)-2-Azido-3,4-di-O-benzyl-1,3,4-triol (14): A solution of ZnBr₂ (7.7 g, 33.59 mmol, 1 M) in CH₂Cl₂/iPrOH 85:15 (33.6 mL) was added to compound **13** (1.59 g, 2.07 mmol), and the resulting yellow reaction mixture was stirred overnight at room temperature. H₂O (50 mL) was added, and the aqueous layer was extracted with CH₂Cl₂ (3 × 75 mL). The combined organic layers were washed with brine (1 × 100 mL), dried over MgSO₄, filtered and evaporated to dryness. The yellow crude was purified by column chromatography with hexanes/EtOAc (9:1) to afford compound **14** (792 mg, 73%) as a colorless oil. ¹H NMR (300 MHz, CDCl₃): δ = 0.82 (t, *J* = 6.7, 3H), 1.14–1.28 (m, 24H), 1.42–1.56 (m, 2H), 2.18 (brs, 1H), 3.47–3.55 (m, 3H), 3.57–3.64 (m, 2H), 4.46 (s, 2H), 4.57 (d, 1H, *J* = 11.5), 4.62 (d, 1H, *J* = 11.5), 7.18–7.32 ppm (m, 10H); ¹³C NMR (75 MHz, CDCl₃): δ = 14.35, 22.92, 26.24, 29.60, 29.78, 29.85, 29.90, 29.94, 30.18, 32.16, 62.69, 64.56, 73.22, 74.39, 79.44, 80.05, 128.20, 128.23, 128.33, 128.47, 128.72, 128.74, 138.02, 138.09 ppm. HRMS (ESI-MS): *m/z*: calcd for C₃₂H₄₉N₃O₃: 524.3846 [M+H]⁺, found: 524.3839.

(2S,3S,4S)-2-Azido-4-O-(4-nitro)benzoyl-1,3-O-di-(tert-butyl)silanedioctadecane-1,3,4-triol (15): DIAD (8.78 mL, 44.60 mmol) was added to a solution of triphenylphosphine (11.70 g, 44.60 mmol) and *p*-nitrobenzoic acid (7.45 g, 44.60 mmol) in toluene (60 mL) at room temperature. After stirring for 1 h, compound **7** (8.63 g, 17.84 mmol) in toluene (20 mL) was added, and the mixture was stirred overnight at room temperature. The next day, toluene was removed in vacuo, and the residue was purified by column chromatography with hexanes/EtOAc (97:3) to afford **15** (8.83 g, 78%) as a yellow oil. ¹H NMR (300 MHz, CDCl₃): δ = 0.86 (t, *J* = 6.6, 3H), 1.04 (s, 9H), 1.06 (s, 9H), 1.20–1.42 (m, 24H), 1.84–1.94 (m, 2H), 3.54 (apptd, *J* = 5.0 and 10.4, 1H), 3.91 (t, *J* = 10.8, 1H), 3.94 (dd, 1H, *J* = 1.8 and 10.0), 4.22 (dd, *J* = 5.0 and 10.8, 1H), 5.43 (apptd, *J* = 1.5 and 7.0, 1H), 8.23 (d, *J* = 9.1, 2H), 8.29 ppm (d, *J* = 9.1, 2H); ¹³C NMR (75 MHz, CDCl₃): δ = 14.35, 22.92, 25.37, 29.60, 29.69, 29.75, 29.87, 29.89, 29.93, 29.94, 30.27, 32.16, 61.75, 63.15, 72.60, 74.76, 78.13, 87.58, 123.77, 127.47, 127.73, 127.87, 128.17, 128.51, 128.83, 131.22, 135.28, 138.10, 143.45, 150.86, 164.09 ppm.

(2S,3S,4S)-2-Azido-1,3-O-di-(tert-butyl)silanedioctadecane-1,3,4-triol (16): NaOMe (680 mg, 12.64 mmol) was added to a solution of **15** (8.0 g, 12.64 mmol) in MeOH (100 mL) at room temperature. The white suspension was stirred for 1 h, quenched with a saturated NH₄Cl solution and extracted with CH₂Cl₂ (3 × 100 mL). The combined organic layers were washed with brine (1 × 100 mL), dried over MgSO₄, filtered and evaporated to dryness. The crude was purified by column chromatography (hexanes/EtOAc 9:1) to yield compound **16** (5.52 g, 90%) as a colorless oil. ¹H NMR (300 MHz, [D₅]pyridine): δ = 0.97 (t, *J* = 7.0, 3H), 1.11 (s, 9H), 1.13 (s, 9H), 1.22–1.42 (m, 22H), 1.50–1.62 (m, 2H), 1.86–2.10 (m, 2H), 3.97 (dd, *J* = 1.8 and 9.4, 1H), 4.05 (t, *J* = 12.3, 1H), 4.10–4.17 (m, 1H), 4.32–4.44 (m, 2H), 5.83 ppm (d, *J* = 7.0, 1H); ¹³C NMR (75 MHz, [D₅]pyridine): δ = 14.17, 20.39, 22.79, 22.84, 26.30, 27.11, 27.56, 29.51, 29.79, 29.82, 29.84, 29.87, 30.05, 32.03, 33.76, 58.77, 66.90,

70.44, 79.27 ppm; HRMS (ESI-MS): m/z : calcd for $C_{26}H_{53}N_3O_3$: 482.3778 $[M-H]^+$; found: 482.3781.

(25,35,45)-2-Azido-octadecane-1,3,4-triol (17): A solution of HF in pyridine (140 μ L, 1.01 mmol, 65–70%) was added dropwise to a solution of **16** (1.20 g, 2.48 mmol) in THF (10 mL) and pyridine (10 mL) at 0 °C. After 30 min the mixture was diluted with EtOAc (50 mL), the organic layer was washed with 1 M HCl (3 $\times$ 50 mL) and H₂O (3 $\times$ 50 mL), dried on Na₂SO₄, filtered, and evaporated to dryness. The residue was purified by column chromatography with hexanes/EtOAc (1:1) to yield compound **17** (818 mg, 96%) as a white powder. ¹H NMR (300 MHz, [D₅]pyridine): δ = 0.86 (t, J = 6.7, 3H), 1.21–1.40 (m, 22H), 1.48–1.62 (m, 1H), 1.66–1.78 (m, 1H), 1.81–1.92 (m, 1H), 1.98–2.12 (m, 1H), 4.02 (apptd, J = 1.8 and 7.9, 1H), 4.21–4.32 (m, 2H), 4.36–4.46 (m, 1H), 4.65 (ddd, J = 3.2, 5.0 and 11.4, 1H), 6.02 (d, J = 6.7, 1H), 6.44 (d, 1H, J = 7.9), 6.85 ppm (t, 1H, J = 5.6); ¹³C NMR (75 MHz, [D₅]pyridine): δ = 14.18, 22.85, 26.56, 29.52, 29.83, 29.86, 29.88, 29.95, 30.09, 32.04, 34.89, 63.14, 66.26, 70.92, 73.09 ppm; HRMS (ESI-MS): m/z : calcd for $C_{18}H_{37}N_3O_3$: 366.2733 $[M+Na]^+$, found: 366.2724.

(25,35,45)-2-Azido-3,4-di-O-benzyl-octadecane-1,3,4-triol (20): A solution of **17** (690 mg, 2.01 mmol), DMAP (61 mg, 0.50 mmol), and trityl chloride (842 mg, 1.56 mmol) in pyridine (5 mL) was stirred overnight at 70 °C. The mixture was diluted with EtOAc (20 mL) and washed with ice-cold HCl (0.1 M, 3 $\times$ 20 mL), H₂O (3 $\times$ 20 mL), and brine (1 $\times$ 20 mL). The organic layer was dried over Na₂SO₄, filtered and co-evaporated twice with toluene. NaH (643 mg, 16.08 mmol) was added to a solution of the crude compound **18** in DMF (10 mL) at 0 °C. After 30 min stirring at 0 °C, benzyl bromide (1.92 mL, 16.08 mmol) was added, and the mixture was stirred overnight at room temperature. H₂O (40 mL) was added, and the aqueous layer was extracted with CH₂Cl₂ (3 $\times$ 75 mL). The combined organic layers were washed with brine (3 $\times$ 50 mL), dried over MgSO₄, filtered, and evaporated to dryness. A solution of ZnBr₂ (13.8 g, 60.0 mmol, 1 M) in CH₂Cl₂/iPrOH (85:15, 60 mL) was added to the crude compound **19** and the resulting yellow mixture was stirred overnight at room temperature. The mixture was evaporated to dryness and dissolved in CH₂Cl₂ (20 mL). The solution was washed with H₂O (3 $\times$ 20 mL) and brine (1 $\times$ 20 mL), dried over Na₂SO₄, filtered, and evaporated to dryness. The crude was purified by column chromatography with hexanes/EtOAc (8:2) to afford compound **20** (665 mg, 63% over three steps) as a colorless oil. ¹H NMR (300 MHz, [D₅]pyridine): δ = 0.86 (t, J = 6.7, 3H), 1.20–1.34 (m, 22H), 1.42–1.61 (m, 2H), 1.82–1.92 (m, 2H), 3.92 (apptd, J = 3.8 and 6.2, 1H), 4.05 (dd, J = 3.8 and 7.0, 1H), 4.12 (apptd, J = 3.2 and 6.7, 1H), 4.26–4.35 (m, 1H), 4.42 (ddd, J = 2.9, 4.7 and 11.4, 1H), 7.01 (t, J = 5.28, 1H), 7.26–7.40 (m, 6H), 7.50–7.56 ppm (m, 4H); ¹³C NMR (75 MHz, [D₅]pyridine): δ = 14.35, 22.92, 26.24, 29.60, 29.78, 29.85, 29.90, 29.94, 30.18, 32.16, 62.69, 64.56, 73.22, 74.39, 79.44, 80.05, 128.20, 128.23, 128.33, 128.47, 128.72, 128.74, 138.02, 138.09 ppm; HRMS (ESI-MS): m/z : calcd for $C_{32}H_{49}N_3O_3$: 524.3846 $[M+H]^+$, found: 524.3870.

(25,3R,4R)-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 (22): A solution of **14** (401 mg, 0.77 mmol) in THF (5 mL) was added to a mixture of **21** (545 mg, 0.92 mmol) and powdered 4 Å molecular sieves in THF (5 mL). The mixture was cooled to –20 °C, and TMSOTf (21 μ L, 0.11 mmol) was added dropwise. After stirring for 1 h at –20 °C, the mixture was neutralized with Et₃N and filtered through celite. The filtrate was evaporated to dryness, and the resulting residue was purified by column chromatography (hexanes/EtOAc 5:1 + 1 V% Et₃N) to afford compound **22** (508 mg, 70%) as a white solid. ¹H NMR (300 MHz, CDCl₃): δ = 0.74 (t, J = 6.3, 3H),

1.14–1.20 (m, 22H), 1.30–1.46 (m, 4H), 3.37 (m, 1H), 3.40–3.50 (m, 4H), 3.63 (dd, J = 3.1 and 9.9, 1H), 3.74 (dd, J = 1.6 and 12.4, 1H), 3.80 (dd, J = 3.3 and 10.1, 1H), 3.88–4.00 (m, 2H), 4.38 (s, 2H), 4.46 (d, 1H, J = 11.7), 4.50 (d, J = 11.8, 1H), 4.52 (t, J = 2.3, 1H), 4.57 (d, J = 12.0, 1H), 4.60 (d, J = 12.4, 1H), 4.64 (d, J = 12.5, 1H), 4.68 (d, J = 12.0, 1H), 4.73 (d, J = 3.5, 1H), 5.30 (s, 1H), 7.10–7.25 (m, 23H), 7.34–7.38 ppm (m, 2H); ¹³C NMR (75 MHz, CDCl₃): δ = 14.28, 22.85, 25.80, 29.53, 29.77, 29.83, 29.87, 29.94, 30.50, 32.09, 61.83, 63.18, 68.51, 69.51, 72.26, 72.84, 73.15, 73.67, 74.53, 74.85, 75.64, 75.78, 77.37, 79.63, 79.95, 99.39, 101.21, 126.48, 127.73, 127.87, 127.91, 127.93, 128.15, 128.27, 128.39, 128.46, 128.47, 128.54, 128.65, 129.04, 137.95, 138.35, 138.54, 138.89 ppm; HRMS (ESI-MS): m/z : calcd for $C_{59}H_{75}N_3O_8$: 976.5452 $[M+Na]^+$; found: 976.5492.

(25,35,45)-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 (23): BF₃ $\cdot$ $\cdot$ Et₂O (280 μ L, 2.18 mmol) was added dropwise to a mixture of **21** (960 mg, 1.62 mmol), **20** (570 mg, 1.09 mmol) and powdered 4 Å molecular sieves in Et₂O/THF 13:1 (20 mL) at –20 °C. The mixture was stirred for 2 h at –20 °C, and an additional portion of compound **21** (960 mg, 1.62 mmol) was added. After 1 h, the mixture was diluted with EtOAc (50 mL) and filtered through celite. The filtrate was washed with a saturated NaHCO₃ solution (2 $\times$ 50 mL) and brine (1 $\times$ 50 mL), dried over Na₂SO₄, filtered, and evaporated to dryness. The residue was purified by column chromatography with hexanes/EtOAc (7:1) to afford compound **23** (578 mg, 56%) as a white powder. ¹H NMR (300 MHz, CDCl₃): δ = 0.81 (t, J = 6.7, 3H), 1.12–1.26 (m, 22H), 1.44–1.56 (m, 4H), 3.46 (apptd, J = 3.1 and 6.3, 1H), 3.53–3.58 (m, 2H), 3.62 (dd, J = 3.2 and 7.7, 1H), 3.71 (dd, J = 5.0 and 10.6, 1H), 3.90 (dd, J = 1.7 and 12.5, 1H), 3.92–3.99 (m, 2H), 4.04 (dd, J = 3.1 and 10.1, 1H), 4.08–4.13 (m, 2H), 4.42–4.47 (m, 3H), 4.58 (d, J = 11.6, 1H), 4.59 (d, J = 11.7, 1H), 4.67 (d, J = 12.3, 1H), 4.74 (d, J = 12.4, 1H), 4.79 (d, J = 11.7, 1H), 4.90 (d, J = 3.2, 1H), 5.40 (s, 1H), 7.13–7.35 (m, 23H), 7.43–7.47 ppm (m, 2H); ¹³C NMR (75 MHz, CDCl₃): δ = 14.36, 22.93, 26.09, 29.60, 29.82, 29.89, 29.90, 29.92, 29.95, 30.01, 30.32, 32.16, 61.16, 63.30, 68.21, 69.64, 72.20, 72.66, 73.93, 74.50, 74.82, 75.54, 76.14, 77.44, 78.18, 79.16, 99.26, 101.32, 126.58, 127.73, 127.78, 127.89, 127.94, 127.99, 128.27, 128.35, 128.35, 128.46, 128.52, 128.53, 128.56, 129.11, 138.07, 138.22, 138.64, 138.92, 138.95 ppm; HRMS (ESI-MS): m/z : calcd for $C_{59}H_{75}N_3O_8$: 976.5452 $[M+Na]^+$, found: 976.5516.

(25,3R,4R)-3,4-Di-O-benzyl-1-O-(2,3-di-O-benzyl-4,6-O-benzylidene- α -D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (24): A solution of trimethylphosphine in THF (2.66 mL, 2.66 mmol, 1 M) was added dropwise to a solution of **22** (508 mg, 0.53 mmol) in THF (5.4 mL) at room temperature. After stirring for 2 h at room temperature, a NaOH solution (10 mL, 1 M) was added and the mixture was allowed to stir for an additional 2 h. The solution was diluted with EtOAc (20 mL), washed with H₂O (3 $\times$ 20 mL) and brine (1 $\times$ 20 mL), dried over MgSO₄, filtered, and evaporated to dryness to afford the amine as a colorless oil. A mixture of the crude amine, EDC (204 mg, 1.06 mmol), hexacosanoic acid (333 mg, 0.80 mmol) in CH₂Cl₂ (8 mL) was stirred at room temperature for 18 h. The mixture was diluted with CH₂Cl₂ (20 mL), washed with H₂O (3 $\times$ 20 mL) and brine (1 $\times$ 20 mL), dried over MgSO₄, filtered, and evaporated to dryness. The residue was purified by column chromatography with hexanes/EtOAc (4:1) to afford compound **24** (532 mg, 76%) as a yellow oil. ¹H NMR (300 MHz, CDCl₃): δ = 0.88 (t, J = 6.9, 6H), 1.18–1.34 (m, 68H), 1.40–1.62 (m, 4H), 2.10 (t, J = 7.3, 2H), 3.42–3.52 (m, 3H), 3.57 (dd, J = 7.3 and 9.8, 1H), 3.72 (dd, J = 1.3 and 7.6, 1H), 3.83 (dd, J = 1.6 and 11.7, 1H), 3.87 (dd, J = 3.5 and 10.4, 1H), 4.01–4.10 (m, 3H), 4.38 (appq, J = 7.9,

1 H), 4.48 (d, $J=11.4$, 1 H), 4.55 (d, $J=11.4$, 1 H), 4.61 (d, $J=12.3$, 1 H), 4.64 (d, $J=12.0$, 1 H), 4.70 (d, $J=12.9$, 1 H), 4.76 (d, $J=12.3$, 1 H), 4.82 (d, $J=12.0$, 1 H), 4.83 (d, $J=11.7$, 1 H), 4.90 (d, $J=3.5$, 1 H), 5.42 (s, 1 H), 5.76 (d, $J=9.1$, 1 H), 7.18–7.40 (m, 23 H), 7.47–7.52 ppm (m, 2 H); ^{13}C NMR (75 MHz, CDCl_3): $\delta=14.36$, 22.93, 25.24, 26.07, 29.59, 29.61, 29.67, 29.83, 29.89, 29.94, 29.97, 30.11, 31.00, 32.16, 32.17, 37.12, 49.03, 63.16, 68.87, 69.56, 72.27, 73.37, 73.63, 74.99, 75.67, 75.82, 77.44, 79.82, 80.89, 99.49, 101.26, 126.58, 127.80, 127.84, 127.93, 127.95, 128.02, 128.06, 128.32, 128.55, 128.60, 128.64, 129.08, 138.05, 138.86, 138.91, 138.94, 172.89 ppm; HRMS (ESI-MS): m/z : calcd for $\text{C}_{85}\text{H}_{127}\text{N}_9\text{O}_9$: 1328.9408 $[\text{M}-\text{H}]^+$, found: 1329.0897.

(25,35,45)-3,4-Di-O-benzyl-1-O-(2,3-di-O-benzyl-4,6-O-benzylidene- α -D-galactopyranosyl)-2-hexacosylamino-octadecane-1,3,4-triol (25): A solution of trimethylphosphine in THF (1.90 mL, 1.90 mmol, 1 M) was added dropwise to a solution of **23** (360 mg, 0.38 mmol) in THF (4 mL) at room temperature. After stirring for 2 h at room temperature, a 1 M NaOH solution (1.9 mL) was added, and the mixture was allowed to stir for an additional 2 h. The solution was diluted with EtOAc (20 mL), washed with H_2O (3×20 mL) and brine (1×20 mL), dried over Na_2SO_4 , filtered and evaporated to dryness to afford the amine as a colorless oil. A mixture of the crude amine, EDC (225 mg, 1.14 mmol), and hexacosanoic acid (465 mg, 1.14 mmol) in CH_2Cl_2 (5 mL) was stirred at room temperature for 18 h. The mixture was diluted with CH_2Cl_2 (20 mL), washed with H_2O (3×20 mL) and brine (1×20 mL), dried over Na_2SO_4 , filtered, and evaporated to dryness. The residue was purified by column chromatography with hexanes/EtOAc (4:1) to afford compound **25** (298 mg, 60%) as a white solid. ^1H NMR (700 MHz, $[\text{D}_5]\text{pyridine}$): $\delta=0.84$ – 0.90 (m, 6 H), 1.20–1.34 (m, 65 H), 1.36–1.41 (m, 1 H), 1.43–1.49 (m, 1 H), 1.54–1.61 (m, 1 H), 1.78–1.89 (m, 2 H), 1.92–1.98 (m, 2 H), 2.41 (t, $J=7.4$, 2 H), 3.97 (dd, $J=5.8$ and 10.6, 1 H), 4.02 (s, 1 H), 4.10 (dd, $J=5.6$ and 10.2, 1 H), 4.18 (d, $J=12.2$, 1 H), 4.22 (t, $J=5.8$, 1 H), 4.35 (dd, $J=4.4$ and 10.2, 1 H), 4.39–4.44 (m, 3 H), 4.64 (d, $J=2.2$, 1 H), 4.73 (d, $J=10.9$, 1 H), 4.77 (d, $J=12.4$, 1 H), 4.80 (d, $J=11.2$, 1 H), 4.86–4.93 (m, 4 H), 4.97 (d, $J=11.4$, 1 H), 5.06 (m, 1 H), 5.42 (d, $J=2.1$, 1 H), 5.81 (s, 1 H), 7.26–7.40 (m, 16 H), 7.48 (d, $J=7.6$, 3 H), 7.53 (d, $J=7.5$, 2 H), 7.59 (d, $J=7.5$, 2 H), 7.78 (d, $J=7.3$, 2 H), 8.35 ppm (d, $J=7.9$, 1 H); ^{13}C NMR (75 MHz, $[\text{D}_5]\text{pyridine}$): $\delta=14.20$, 22.86, 22.88, 25.72, 26.30, 29.53, 29.57, 29.73, 29.76, 29.84, 29.88, 29.95, 29.97, 30.26, 30.85, 32.05, 32.06, 36.78, 50.64, 63.58, 67.86, 69.63, 71.48, 72.63, 73.62, 74.41, 74.49, 76.61, 77.10, 80.15, 80.51, 99.36, 100.97, 126.85, 127.74, 127.76, 127.80, 127.82, 127.85, 127.96, 128.10, 128.35, 128.40, 128.58, 128.64, 128.65, 129.00, 139.30, 139.44, 139.45, 139.53, 139.60, 172.80 ppm.

(25,35,45)-3,4-Di-O-benzyl-1-O-(2,3-di-O-benzyl-4,6-O-benzylidene- α -D-galactopyranosyl)-2-octanoylamino-octadecane-1,3,4-triol (26): A 1 M solution of trimethylphosphine in THF (1.10 mL, 1.10 mmol) was added dropwise to a solution of **23** (210 mg, 0.22 mmol) in THF (1 mL) at room temperature. After stirring for 2 h at room temperature, a 1 M NaOH solution (1.1 mL) was added, and the mixture was allowed to stir for an additional 2 h. The solution was diluted with EtOAc (20 mL), washed with H_2O (3×20 mL) and brine (1×20 mL), dried over Na_2SO_4 , filtered, and evaporated to dryness to afford the amine as a colorless oil. A mixture of the crude amine, EDC (127 mg, 0.66 mmol), octanoic acid (105 μL , 0.66 mmol) in CH_2Cl_2 (3 mL) was stirred at room temperature for 18 h. The mixture was diluted with CH_2Cl_2 (20 mL), washed with H_2O (3×20 mL) and brine (1×20 mL), dried over Na_2SO_4 , filtered, and evaporated to dryness. The residue was purified by column chromatography with hexanes/EtOAc (4:1) to afford compound **26**

(145 mg, 64%) as a white solid. ^1H NMR (700 MHz, $[\text{D}_5]\text{pyridine}$): $\delta=0.81$ (t, $J=6.9$, 3 H), 0.86 (t, $J=7.0$, 3 H), 1.15–1.35 (m, 30 H), 1.43–1.48 (m, 1 H), 1.54–1.59 (m, 1 H), 1.76–1.84 (m, 2 H), 1.92–1.97 (m, 2 H), 2.38 (t, $J=7.3$, 2 H), 3.97 (dd, $J=5.9$ and 10.6, 1 H), 4.02 (s, 1 H), 4.10 (dd, $J=5.6$ and 10.2, 1 H), 4.18 (dd, $J=1.4$ and 11.6, 1 H), 4.22 (dd, $J=4.7$ and 6.0, 1 H), 4.35 (dd, $J=4.4$ and 10.2, 1 H), 4.40–4.44 (m, 3 H), 4.64 (d, $J=2.2$, 1 H), 4.73 (d, $J=11.4$, 1 H), 4.77 (d, $J=12.3$, 1 H), 4.78 (d, $J=12.0$, 1 H), 4.80 (d, $J=11.4$, 1 H), 4.83 (1 H, d, $J=10.6$), 4.87 (1 H, d, $J=10.6$), 4.92 (1 H, d, $J=11.7$), 4.97 (1 H, d, $J=11.7$), 5.01 (m, 1 H), 5.42 (d, $J=2.6$, 1 H), 5.80 (s, 1 H), 7.26–7.39 (m, 15 H), 7.47 (dd, $J=1.4$ and 7.6, 4 H), 7.54 (d, $J=7.5$, 2 H), 7.59 (d, $J=7.5$, 2 H), 7.78 (d, $J=7.1$, 2 H), 8.33 ppm (d, $J=7.9$, 1 H); ^{13}C NMR (75 MHz, $[\text{D}_5]\text{pyridine}$): $\delta=14.15$, 14.21, 22.81, 22.87, 25.71, 26.26, 27.81, 28.37, 29.32, 29.55, 29.62, 29.87, 29.94, 29.50, 30.25, 30.84, 31.89, 32.05, 50.62, 63.59, 67.88, 69.62, 71.47, 72.62, 73.61, 74.41, 74.49, 76.60, 77.09, 80.17, 80.51, 99.36, 100.98, 127.75, 127.80, 127.82, 127.84, 127.96, 128.09, 128.35, 128.40, 128.58, 128.60, 128.64, 128.65, 129.00, 139.30, 139.44, 139.53, 139.59, 172.79 ppm; HRMS (ESI-MS): m/z : calcd for $\text{C}_{67}\text{H}_{91}\text{N}_9\text{O}_9$: 1054.6772 $[\text{M}+\text{H}]^+$, found: 1054.6716.

1-O-(α -D-Galactopyranosyl)-2-hexacosylamino-D-xylo-1,3,4-octadecanetriol (2): A solution of **24** (485 mg, 0.37 mmol) in $\text{CHCl}_3/\text{EtOH}$ (1:3, 12 mL) was hydrogenated under atmospheric pressure for 4 h in the presence of Pd black (50 mg). The solution was diluted with pyridine (20 mL), filtered through celite, and co-evaporated twice with toluene. The crude was purified by column chromatography with $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (9:1) to afford compound **2** (215 mg, 68%) as a white powder. ^1H NMR (300 MHz, $[\text{D}_5]\text{pyridine}$): $\delta=0.86$ (6 H, m), 1.18–1.42 (66 H, m), 1.62–1.90 (4 H, m), 2.00 (2 H, m), 2.44 (2 H, t, $J=7.5$), 4.08–4.18 (2 H, m), 4.25–4.32 (1 H, m), 4.34–4.50 (4 H, m), 4.50–4.58 (2 H, m), 4.60–4.68 (1 H, m), 4.98–5.08 (1 H, m), 5.48 (1 H, d, $J=3.5$), 5.93 (1 H, d, $J=5.0$), 6.26 (1 H, d, $J=4.4$), 6.30 (1 H, d, $J=3.9$), 6.37 (1 H, d, $J=7.2$), 6.46 (1 H, t, $J=5.5$), 6.53 (1 H, d, $J=5.74$), 8.25 ppm (1 H, d, $J=8.7$); ^{13}C NMR (75 MHz, $[\text{D}_5]\text{pyridine}$): $\delta=14.44$, 23.07, 23.09, 26.59, 26.76, 29.74, 29.78, 29.87, 29.98, 30.05, 30.12, 30.17, 30.23, 30.32, 32.25, 32.28, 34.02, 36.91, 51.09, 62.83, 69.32, 70.55, 71.11, 71.80, 72.55, 73.08, 74.32, 101.35, 173.95 ppm; HRMS (ESI-MS): m/z : calcd for (ESI-MS) for $\text{C}_{50}\text{H}_{99}\text{N}_9\text{O}_9$: 858.7392 $[\text{M}+\text{H}]^+$, found: 858.6768.

1-O-(α -D-Galactopyranosyl)-2-hexacosylamino-L-lyxo-1,3,4-octadecanetriol (3): A solution of **25** (275 mg, 0.21 mmol) in EtOAc (20 mL) was hydrogenated under atmospheric pressure for 16 h in the presence of 10% Pd/C (55 mg). The solution was diluted with pyridine (20 mL), filtered through celite, and co-evaporated twice with toluene. The crude was purified by column chromatography with $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (9:1) to afford compound **3** (77 mg, 43%) as a white powder. ^1H NMR (700 MHz, $[\text{D}_5]\text{pyridine}$): $\delta=0.88$ (m, 6 H), 1.18–1.43 (m, 66 H), 1.53–1.60 (m, 1 H), 1.72–1.79 (m, 2 H), 1.80–1.86 (m, 2 H), 2.07–2.13 (m, 1 H), 2.45 (t, $J=7.4$, 2 H), 4.12–4.17 (m, 2 H), 4.38–4.41 (dd, $J=5.6$ and 10.9, 1 H), 4.43–4.44 (dd, $J=6.0$ and 10.5, 1 H), 4.44–4.46 (dd, $J=3.1$ and 9.8, 1 H), 4.48–4.53 (m, 3 H), 4.55 (d, $J=3.0$, 1 H), 4.66 (dd, $J=3.8$ and 9.9, 1 H), 4.87–4.97 (m, 1 H), 5.49 (d, $J=3.8$, 1 H), 5.62 (brs, 1 H), 6.06 (brs, 1 H), 6.28 (brs, 1 H), 6.40 (brs, 3 H), 8.57 ppm (d, $J=8.8$, 1 H); ^{13}C NMR (75 MHz, $[\text{D}_5]\text{pyridine}$): $\delta=14.22$, 22.88, 26.30, 26.85, 29.55, 29.57, 29.65, 29.75, 29.85, 29.88, 29.94, 29.96, 30.07, 30.20, 52.88, 62.59, 69.91, 70.52, 70.80, 70.90, 71.49, 73.05, 73.59, 102.19, 174.62 ppm; HRMS (ESI-MS): m/z : calcd for $\text{C}_{50}\text{H}_{99}\text{N}_9\text{O}_9$: 858.7398 $[\text{M}+\text{H}]^+$, found: 858.7355.

1-O-(α -D-Galactopyranosyl)-2-octanoylamino-L-lyxo-1,3,4-octadecanetriol (4): A solution of **26** (120 mg, 0.11 mmol) in EtOAc (15 mL) was hydrogenated under atmospheric pressure for 16 h in the presence of 10% Pd/C (24 mg). The solution was diluted with

pyridine (20 mL), filtered through celite, and co-evaporated twice with toluene. The crude was purified by column chromatography with $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (95:5) to afford compound **4** (50 mg, 75%) as a white powder. ^1H NMR (700 MHz, $[\text{D}_5]\text{pyridine}$): δ = 0.79 (t, J = 7.0, 3H), 0.85 (t, J = 7.1, 3H), 1.10–1.18 (m, 5H), 1.20–1.38 (m, 25H), 1.52–1.59 (m, 1H), 1.71–1.78 (m, 3H), 1.80–1.85 (m, 1H), 2.06–2.12 (m, 1H), 2.42 (t, J = 7.5, 2H), 4.12–4.17 (m, 2H), 4.37–4.41 (dd, J = 5.6 and 10.9, 1H), 4.42–4.44 (dd, J = 6.5 and 11.1, 1H), 4.44–4.46 (dd, J = 3.4 and 10.1, 1H), 4.48–4.53 (m, 3H), 4.55 (d, J = 3.1, 1H), 4.66 (dd, J = 3.8 and 10.0, 1H), 4.86–4.91 (m, 1H), 5.48 (d, J = 3.8, 1H), 5.91 (brs, 6H), 8.56 ppm (d, J = 8.6, 1H); ^{13}C NMR (75 MHz, $[\text{D}_5]\text{pyridine}$): δ = 14.14, 14.21, 22.78, 22.87, 26.25, 26.84, 29.28, 29.52, 29.54, 29.85, 29.92, 29.94, 30.04, 30.20, 52.90, 62.59, 69.87, 70.54, 70.83, 70.90, 71.51, 73.06, 73.60, 102.21, 174.60 ppm; HRMS (ESI-MS): m/z : calcd for $\text{C}_{32}\text{H}_{53}\text{N}_1\text{O}_9$: 606.4581 $[M+H]^+$; found: 606.4557.

(2S,3S,4R)-1,3-O-Di-(tert-butyl)silanediyol-(2-N,4-O)-oxazinane-neoctadecane-1,3,4-triol (27): A solution of trimethylphosphine in THF (2.25 mL, 2.25 mmol, 1 M) was added dropwise to a solution of **7** (220 mg, 0.45 mmol) in THF (5 mL) at 0°C. After stirring for 15 min at room temperature, a 1 M NaOH solution (5 mL) was added, and the mixture was allowed to stir for an additional hour. The solution was diluted with EtOAc (20 mL), washed with H_2O (2 $\times$ 20 mL) and brine (2 $\times$ 20 mL), dried over Na_2SO_4 , filtered, and evaporated to dryness to afford the amine. $i\text{Pr}_2\text{EtN}$ (160 μL , 0.90 mmol) and triphosgene (270 mg, 0.90 mmol) were added to a solution of the crude amine in CH_2Cl_2 (2 mL), at 0°C. The mixture was stirred at room temperature overnight, quenched with MeOH (0.5 mL) and diluted with EtOAc (20 mL). The organic layer was washed with H_2O (2 $\times$ 20 mL) and brine (2 $\times$ 20 mL), dried over Na_2SO_4 , filtered, and evaporated to dryness. The crude was purified by column chromatography (hexanes/EtOAc 2:1) to afford compound **27** (140 mg, 64%) as a colorless wax. ^1H NMR (300 MHz, CDCl_3): δ = 0.86 (t, J = 6.7, 3H), 0.95 (s, 9H), 1.03 (s, 9H), 1.18–1.36 (m, 22H), 1.40–1.48 (m, 1H), 1.52–1.66 (m, 2H), 1.84–1.95 (m, 1H), 3.43 (ddd, J = 4.5, 9.2 and 10.5, 1H), 3.73 (t, J = 9.1, 1H), 3.79 (t, J = 10.4, 1H), 4.03–4.10 (m, 1H), 4.11 (dd, J = 4.7 and 10.2, 1H), 7.17 ppm (brs, 1H); ^{13}C NMR (75 MHz, CDCl_3): δ = 14.33, 20.08, 22.90, 23.02, 24.30, 27.11, 27.57, 29.57, 29.66, 29.69, 29.76, 28.87, 29.89, 29.90, 29.91, 30.94, 32.14, 53.70, 67.50, 72.18, 80.94, 154.52 ppm; HRMS (ESI-MS): m/z : calcd for $\text{C}_{27}\text{H}_{53}\text{N}_1\text{O}_4$: 484.3822 $[M+H]^+$, found: 484.3806.

(2S,3S,4S)-1,3-O-Di-(tert-butyl)silanediyol-(2-N,4-O)-oxazinane-neoctadecane-1,3,4-triol (28): A 1 M solution of trimethylphosphine in THF (1.16 mL, 1.16 mmol) was added dropwise to a solution of **16** (110 mg, 0.23 mmol) in THF (2.5 mL) at 0°C. After stirring for 1 h at room temperature, a 1 M NaOH solution (1.16 mL) was added, and the mixture was allowed to stir for an additional hour. The solution was diluted with EtOAc (20 mL), washed with H_2O (2 $\times$ 20 mL) and brine (2 $\times$ 20 mL), dried over Na_2SO_4 , filtered, and evaporated to dryness to afford the amine. $i\text{Pr}_2\text{EtN}$ (80 μL , 0.46 mmol) and triphosgene (137 mg, 0.46 mmol) were added to a solution of the crude amine in CH_2Cl_2 (2 mL), at 0°C. The mixture was stirred at room temperature overnight, quenched with MeOH (0.5 mL), and diluted with EtOAc (20 mL). The organic layer was washed with H_2O (2 $\times$ 20 mL) and brine (2 $\times$ 20 mL), dried over Na_2SO_4 , filtered, and evaporated to dryness. The crude was purified by column chromatography with hexanes/EtOAc (2:1) to afford compound **28** (91 mg, 83%) as a colorless oil. ^1H NMR (300 MHz, CDCl_3): δ = 0.87 (t, J = 6.7, 3H), 0.97 (s, 9H), 1.03 (s, 9H), 1.18–1.38 (m, 22H), 1.41–1.59 (m, 2H), 1.62–1.74 (m, 1H), 1.80–1.88 (m, 1H), 3.53 (apptd, J = 4.4 and 10.1, 1H), 3.80 (t, J = 10.2, 1H), 4.14 (dd,

J = 5.1 and 10.5, 1H), 4.19 (dd, J = 5.3 and 9.6, 1H), 4.33 (ddd, J = 1.9, 5.1 and 11.9, 1H), 7.10 ppm (brs, 1H); ^{13}C NMR (75 MHz, CDCl_3): δ = 14.34, 20.27, 22.91, 23.06, 25.87, 27.22, 27.58, 28.72, 29.58, 29.67, 29.69, 29.77, 29.86, 29.90, 29.92, 32.14, 49.53, 67.86, 70.84, 79.43, 153.82 ppm; HRMS (ESI-MS): m/z : calcd for $\text{C}_{27}\text{H}_{53}\text{N}_1\text{O}_4$: 484.3822 $[M+H]^+$, found: 484.3806.

(2S,3R,4R)-2-azido-4-O-benzyl-octadecane-1,3,4-triol (29): A 1 M solution of ZnBr_2 (4.14 g, 18.76 mmol) in $\text{CH}_2\text{Cl}_2/\text{PrOH}$ (85:15, 18.8 mL) was added to compound **12** (794 mg, 1.17 mmol), and the resulting yellow mixture was stirred overnight at room temperature. H_2O (25 mL) was added, and the aqueous layer was extracted with CH_2Cl_2 (3 $\times$ 50 mL). The combined organic layers were washed with brine (1 $\times$ 50 mL), dried over MgSO_4 , filtered, and evaporated to dryness. The yellow crude was purified by column chromatography with hexanes/EtOAc (3:1) to afford compound **29** (434 mg, 89%) as a colorless oil. ^1H NMR (300 MHz, CDCl_3): δ = 0.88 (t, J = 6.4, 3H), 1.23–1.34 (m, 22H), 1.34–1.44 (m, 2H), 1.52–1.70 (m, 2H), 2.33 (t, J = 5.9, 1H), 2.68 (d, J = 4.7, 1H), 3.42 (appq, J = 4.7, 1H), 3.58 (appq, J = 5.9, 1H), 3.74 (m, 1H), 3.83 (m, 2H), 4.50 (d, J = 11.1, 1H), 4.69 (d, J = 11.1, 1H), 7.31–7.41 ppm (m, 5H); ^{13}C NMR (75 MHz, CDCl_3): δ = 14.35, 22.92, 25.19, 29.59, 29.76, 29.81, 29.87, 29.89, 29.90, 29.92, 29.93, 30.08, 30.13, 32.15, 63.58, 63.77, 72.52, 73.81, 79.52, 128.24, 128.31, 128.84, 138.01 ppm; HRMS (ESI-MS): m/z : calcd for $\text{C}_{25}\text{H}_{43}\text{N}_3\text{O}_3$: 456.3202 $[M+Na]^+$, found: 456.3254.

(2S,3R,4R)-2-Azido-1,3-O-di-(tert-butyl)silanediyloctadecane-1,3,4-triol (30): A solution of **29** (430 mg, 0.99 mmol) in DMF (3 mL) and pyridine (97 μL , 1.19 mmol) was cooled at -20°C and $(t\text{Bu})_2\text{Si}(\text{OTf})_2$ (563 μL , 1.74 mmol) was added dropwise. After stirring for 1 h at -20°C , the mixture was quenched with H_2O (20 mL). The aqueous layer was extracted with EtOAc (3 $\times$ 25 mL), and the combined organic layers were washed with 1 M HCl (20 mL) and H_2O (2 $\times$ 20 mL), dried over Na_2SO_4 , filtered and evaporated to dryness. The residue was purified by column chromatography with hexanes/EtOAc (9:1) to afford **30** (540 mg, 95%) as a colorless oil. ^1H NMR (300 MHz, CDCl_3): δ = 0.88 (t, J = 6.4, 3H), 1.08 (s, 9H), 1.10 (s, 9H), 1.20–1.34 (m, 22H), 1.40–1.52 (m, 4H), 3.54 (m, 1H), 3.62 (appq, J = 1.8, 1H), 4.18 (dd, J = 1.8 and 7.6, 1H), 4.32 (dd, J = 1.8 and 12.9, 1H), 4.37 (dd, J = 2.4 and 12.9, 1H), 4.61 (d, J = 10.8, 1H), 4.92 (d, J = 10.8, 1H), 7.24–7.40 ppm (m, 5H); ^{13}C NMR (75 MHz, CDCl_3): δ = 14.36, 20.71, 22.93, 23.60, 25.67, 27.29, 28.02, 29.60, 29.83, 29.85, 29.90, 29.93, 29.94, 30.92, 32.16, 60.07, 65.50, 75.51, 79.61, 81.41, 127.74, 128.38, 128.52, 139.40 ppm; HRMS (ESI-MS): m/z : calcd for $\text{C}_{33}\text{H}_{59}\text{N}_3\text{O}_3\text{Si}$: 596.4223 $[M+Na]^+$; found: 596.4490.

(2S,3R,4R)-1,3-O-Di-(tert-butyl)silanediyol-(2-N,4-O)-oxazinane-neoctadecane-1,3,4-triol (31): A solution of **30** (200 mg, 0.35 mmol) in $\text{CHCl}_3/\text{EtOH}$ (1:3, 12 mL) was hydrogenated under atmospheric pressure for 7 h in the presence of Pd black (200 mg) and formic acid (13 μL , 0.35 mmol). The solution was diluted with pyridine (20 mL), filtered through celite, and co-evaporated twice with toluene to afford the crude amine as a colorless oil. $i\text{Pr}_2\text{EtN}$ (52 μL , 0.31 mmol) and triphosgene (92 mg, 0.31 mmol) were added to a solution of the crude amine in CH_2Cl_2 (2 mL), at 0°C. The mixture was stirred at room temperature overnight, quenched with MeOH (0.5 mL), and diluted with EtOAc (20 mL). The organic layer was washed with H_2O (2 $\times$ 20 mL) and brine (2 $\times$ 20 mL), dried over Na_2SO_4 , filtered, and evaporated to dryness. The crude was purified by column chromatography with $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (95:5) to afford compound **31** (73 mg, 43%) as a yellow oil. ^1H NMR (300 MHz, CDCl_3): δ = 0.86 (t, J = 6.6, 3H), 0.99 (s, 9H), 1.05 (s, 9H), 1.20–1.40 (m, 22H), 1.40–1.52 (m, 2H), 1.68–1.90 (m, 2H), 3.47 (dd, J = 2.1 and 4.6, 1H), 4.04 (dd, J = 1.5 and 12.8, 1H), 4.13 (appt, J =

6.7, 1H), 4.25 (m, 1H), 4.32 (dd, $J=1.8$ and 12.8, 1H), 6.54 ppm (brs 1H); ^{13}C NMR (75 MHz, CDCl_3): $\delta=14.33$, 20.66, 22.90, 23.65, 25.00, 27.06, 28.07, 29.57, 29.63, 29.68, 29.73, 29.85, 29.86, 29.88, 29.89, 29.91, 30.24, 32.14, 53.14, 65.91, 66.27, 80.47, 154.82 ppm; HRMS (ESI-MS): m/z : calcd for $\text{C}_{27}\text{H}_{53}\text{N}_4\text{O}_4\text{Si}$: 506.3642 $[M+\text{Na}]^+$, found: 506.3619.

In vivo experiments: C57BL/6J (B6) mice were originally purchased from The Jackson Laboratory (Bar Harbor, ME, USA), and $\text{CD1d}^{-/-}$ and $\text{J}\alpha 18^{-/-}$, both on B6 background, were kindly provided by Dr. François Trottein (Lille, France). Mice were bred in our breeding facility, and treated and used in agreement with the institutional guidelines. All animal procedures were approved by the Institutional Animal Care and Ethics Committee.

Dissolution of α -GalCer and α -GalCer-analogues: Stock solutions of **1**, **2**, **3** and **4** were prepared in 100% DMSO at a concentration of 1 mg mL $^{-1}$. Before use, the solutions were diluted with phosphate buffered saline (pH 7.4) to obtain a final concentration of 10 $\mu\text{g mL}^{-1}$.

In vitro stimulation with α -GalCer-analogues: Spleens from 8- to 12-week-old mice were removed and teased apart. After lysis of the erythrocytes with 0.17 M NH_4Cl , the remaining lymphocytes were washed three times with Dulbecco's phosphate-buffered saline (PBS). Cells were counted with trypan blue to exclude dead cells. Splenocytes were suspended in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, 100 U mL $^{-1}$ penicillin, 100 $\mu\text{g mL}^{-1}$ streptomycin, 0.03% glutamine, and 5×10^{-5} M 2-ME (all obtained from Life Technologies, Paisley, UK). Splenocytes were cultured in flat-bottomed 96-well plates at 2×10^6 cells mL $^{-1}$ per well in 200 μL , with a final concentration of glycolipids of 250 ng mL $^{-1}$, 50 ng mL $^{-1}$, or 25 ng mL $^{-1}$. After culture for 72 h, supernatants were harvested for determination of cytokine levels.

ELISA: The level of both IFN- γ and IL-4 in cell culture supernatants was measured by standard sandwich ELISA by using purified-capture and biotin-conjugated detection monoclonal antibodies and standards. After incubation with avidin peroxidase, ELISAs were developed with TMB substrate, followed by evaluation with a microplate reader.

Acknowledgements

M.T. is indebted to the Fund for Scientific Research-Flanders (Belgium; F.W.O.-Vlaanderen) for a position as Aspirant. The authors are indebted to the Fund for Scientific Research-Flanders (Belgium; F.W.O.-Vlaanderen) and Cancer Research Technology for financial support.

Keywords: α -galactosylceramide • cytokines • epimers • glycolipids • NKT cells

- [1] T. Kawano, J. Cui, Y. Koezuka, I. Toura, Y. Kaneko, K. Motoki, H. Ueno, R. Nakagawa, H. Sato, E. Kondo, H. Koseki, M. Taniguchi, *Science* **1997**, 278, 1626–1629.
- [2] M. Morita, K. Motoki, K. Akimoto, T. Natori, T. Sakai, E. Sawa, K. Yamaji, Y. Koezuka, E. Kobayashi, H. Fukushima, *J. Med. Chem.* **1995**, 38, 2176–2187.
- [3] T. Natori, Y. Koezuka, T. Higa, *Tetrahedron Lett.* **1993**, 34, 5591–5592.
- [4] T. Natori, M. Morita, K. Akimoto, Y. Koezuka, *Tetrahedron* **1994**, 50, 2771–2784.
- [5] K. O. Yu, S. A. Porcelli, *Immunol. Lett.* **2005**, 100, 42–55.
- [6] M. Koch, V. S. Stronge, D. Shepherd, S. D. Gadola, B. Mathew, G. Ritter, G. S. Fersht, R. R. Schmidt, E. Y. Jones, V. Cerundolo, *Nat. Immunol.* **2005**, 6, 819–826.
- [7] D. M. Zajonc, C. Cantu, J. Mattner, D. Zhou, P. B. Savage, A. Bendelac, I. A. Wilson, L. Teyton, *Nat. Immunol.* **2005**, 6, 810–818.
- [8] D. Wu, D. M. Zajonc, M. Fujio, B. A. Sullivan, Y. Kinjo, M. Kronenberg, I. A. Wilson, C. H. Wong, *Proc. Natl. Acad. Sci. USA* **2006**, 103, 3972–3977.
- [9] N. A. Borg, K. S. Wun, L. Kjer-Nielsen, M. C. J. Wilce, D. G. Pellicci, R. Koh, G. S. Besra, M. Bharadwaj, D. I. Godfrey, J. McCluskey, J. Rossjohn, *Nature* **2007**, 448, 44–49.
- [10] K. O. A. Yu, J. S. Im, A. Molano, Y. Dutronc, P. A. Illarionov, C. Forestier, N. Fujiwara, I. Arias, S. Miyake, T. Yamamura, Y. T. Chang, G. S. Besra, S. A. Porcelli, *Proc. Natl. Acad. Sci. USA* **2005**, 102, 3383–3388.
- [11] T. Toba, K. Murata, K. Nakanishi, B. Takahashi, N. Takemoto, M. Akabane, T. Nakatsuka, S. Imajo, T. Yamamura, S. Miyake, H. Annoura, *Bioorg. Med. Chem. Lett.* **2007**, 17, 2781–2784.
- [12] E. Kobayashi, K. Motoki, Y. Yamaguchi, T. Uchida, H. Fukushima, Y. Koezuka, *Bioorg. Med. Chem.* **1996**, 4, 615–619.
- [13] G. Yang, J. Schmieg, M. Tsuij, R. W. Franck, *Angew. Chem.* **2004**, 116, 3906–3910; *Angew. Chem. Int. Ed.* **2004**, 43, 3818–3822.
- [14] V. Costantino, E. Fattorusso, C. Imperatore, A. Mangoni, *Tetrahedron* **2002**, 58, 369–375.
- [15] L. Barbieri, V. Costantino, E. Fattorusso, A. Mangoni, N. Basilico, M. Mondani, D. Taramelli, *Eur. J. Org. Chem.* **2005**, 3279–3285.
- [16] L. Barbieri, V. Costantino, E. Fattorusso, A. Mangoni, E. Aru, S. Parapini, D. Taramelli, *Eur. J. Org. Chem.* **2004**, 468–473.
- [17] X. T. Zhou, C. Forestier, R. D. Goff, C. Li, L. Teyton, A. Bendelac, P. B. Savage, *Org. Lett.* **2002**, 4, 1267–1270.
- [18] K. Fuhshuku, N. Hongo, T. Tashiro, Y. Masudo, R. Nakagawa, K. Seino, M. Taniguchi, K. Mori, *Bioorg. Med. Chem.* **2008**, 16, 950–964.
- [19] K. Miyamoto, S. Miyake, T. Yamamura, *Nature* **2001**, 413, 531–534.
- [20] R. D. Goff, Y. Gao, J. Mattner, D. Zhou, N. Yin, C. Cantu, L. Teyton, A. Bendelac, P. B. Savage, *J. Am. Chem. Soc.* **2004**, 126, 13602–13603.
- [21] R. M. Ndonye, D. P. Izmirian, M. F. Dunn, K. O. A. Yu, S. A. Porcelli, A. Khurana, M. Kronenberg, S. K. Richardson, A. R. Howell, *J. Org. Chem.* **2005**, 70, 10260–10270.
- [22] D. Wu, D. M. Zajonc, M. Fujio, B. A. Sullivan, Y. Kinjo, M. Kronenberg, I. A. Wilson, C. H. Wong, *Proc. Natl. Acad. Sci. USA* **2006**, 103, 3972–3977.
- [23] Z. H. Jiang, S. Gandhi, R. R. Koganty, PCT Int. Appl. WO 2004028475.
- [24] W. Du, J. Gervay-Hague, *Org. Lett.* **2005**, 7, 2063–2065.
- [25] P. B. Savage, L. Teyton, A. Bendelac, *Chem. Soc. Rev.* **2006**, 35, 771–779.
- [26] D. Wu, M. Fujio, C. H. Wong, *Bioorg. Med. Chem.* **2008**, 16, 1073–1083.
- [27] X. Lu, H. S. Byun, R. Bittman, *J. Org. Chem.* **2004**, 69, 5433–5438.
- [28] X. Lu, R. Bittman, *Tetrahedron Lett.* **2005**, 46, 3165–3168.
- [29] T. Nakamura, M. Shiozaki, *Tetrahedron* **2001**, 57, 9087–9092.
- [30] A. J. Ndakala, M. Hashemzadeh, R. C. So, A. R. Howell, *Org. Lett.* **2002**, 4, 1719–1722.
- [31] Y. Cai, C. C. Ling, D. R. Bundle, *Org. Biomol. Chem.* **2006**, 4, 1140–1146.
- [32] L. He, H. S. Byun, R. Bittman, *J. Org. Chem.* **2000**, 65, 7618–7626.
- [33] S. Kim, N. Lee, S. Lee, T. Lee, Y. M. Ly, *J. Org. Chem.* **2008**, 73, 1379–1385; in this paper, *l*-lyxo-phytosphingosine was erroneously called *o*-lyxo-phytosphingosine, and the *l*-arabino analogue was incorrectly referred to as *o*-arabino-phytosphingosine.
- [34] R. J. B. H. N. van den Berg, C. G. N. Korevaar, G. A. van der Marel, H. S. Overkleef, J. H. van Boom, *Tetrahedron Lett.* **2002**, 43, 8409–8412.
- [35] a) O. Plettenburg, V. Bodmer-Narkevitch, C. H. Wong, *J. Org. Chem.* **2002**, 67, 4559–4564; b) F. A. W. Koeman, J. P. Kamerling, J. F. G. Vliegenthart, *Tetrahedron* **1993**, 49, 5291–5304.
- [36] S. Figueroa-Peréz, R. R. Schmidt, *Carbohydrate Res.* **2000**, 328, 95–102.

Received: January 29, 2008

Revised: March 6, 2008

Published online on April 16, 2008