Towards the Synthesis of alpha-Aminogalactosylceramides

Shaun C. Christian

University of Connecticut - Storrs, shaun.christian@uconn.edu

Recommended Citation
https://opencommons.uconn.edu/gs_theses/71
Towards the Synthesis of α-Aminogalactosylceramides

Shaun C. Christian, B.S.

B.S., University of Maine at Orono, 2008

A Thesis
Submitted in Partial Fulfillment of the
Requirements for the Degree of
Master of Science
at the
University of Connecticut
2011
Towards the Synthesis of α-Aminogalactosylceramides

Presented by
Shaun C. Christian, B.S.

Major Advisor__________________________________________________________
Amy R. Howell

Associate Advisor______________________________________________________
Mark Peczuh

Associate Advisor______________________________________________________
Dennis Wright

University of Connecticut
2011
Acknowledgements

First and foremost I would like to thank God for blessing my family, friends, and I every day. Without him absolutely none of this would be possible.

I would like to thank my mother Selina Pernell Christian and my father Charles Melvin Christian Jr. for their love and support throughout my entire academic career. If it wasn’t for their guidance I may not have chosen to further my academic endeavors after my undergraduate experience. I would also like to thank my Aunt Carol for consistently showing me love and support. She has been a perpetual force motivating me towards finishing both my undergraduate and graduate work. I thank them for continually teaching me the important morals and values of life. I also appreciate them showing me the tools to success and trusting in my ability to use them.

I want to thank Dr. Amy R. Howell my graduate advisor and friend for her all of her guidance and wisdom. Her hospitality and kindness knows no bounds. She was always there to encourage and motivate me whenever I felt discouraged or distracted. She not only taught me the solutions to problems but gave me the independence to seek out the answers myself which I truly appreciate.

I would like to thank all of my friends in Massachusetts that have supported me through my entire academic career. They have always been understanding and encouraging when I didn’t have time spend with them or if I needed to cancel prior engagements to fulfill requirements for school. To be completely honest I wouldn’t do them justice unless I named them specifically because they have been that influential in my life. I would like to thank Norman Widamen for his encouraging words, constant support, and the invites to the Wednesday lunches. To him I say, “Your Bankai is derived from a Zanpakuto of friendship”. I would like to say thank you to Jesus Figueroa for his one on one advice, support, and encouragement. He consistently keeps me on my toes with his unfathomable wit. I would like to thank Daniel Kee for his heart to heart talks, fun spirit, encouragement, advice, and Friday night antics. He was among the firsts to level up in friendship without even knowing it. I would also like to thank Richard Franklin for his sense of humor, sense of adventure, and hospitality. Only the legends were in attendance at Mattoon St.

Finally I would like to thank all of my friends at the University of Connecticut. They have taught me so many things about life and how that applies to the Chemistry department. Only the people present during my experience know the stories that occurred. But I would like to send a special thanks to one Michael Hyland who taught me things from controlled throws to levels above Pitbull status. His friendship, hospitality, and encouragement had inspired me to refer to myself in a simple phrase, “When all everyone knew about were goons, Shaun Christian was a goblin!”
List of Abbreviations

Ac    acetyl
Ac$_2$O    acetic anhydride
AcOH    acetic acid
BAIB    [bis(acetoxy)iodo]benzene
BINAP    2,2'-bis(diphenylphosphino)-1,1'-binaphthyl
BnBr    benzyl bromide
Boc    tert-butyloxy carbonyl
Boc$_2$O    di-tert-butyl dicarbonate
br    broad
Bu$_4$NF    tetrabutylammonium fluoride
CBr$_4$    tetrabromomethane
CH$_2$Cl$_2$    methylene chloride
CHCl$_3$    chloroform
cm    centimeters
d    doublet
DCC    $N,N'$-dicyclohexyl carbodiimide
dd    doublet of doublets
ddd    doublet of doublet of doublets
dddd    doublet of doublet of doublet of doublets
DIAD    diisopropyl azodicarboxylate
DMAP    4-dimethylaminopyridine
DMF    dimethyl formamide
EDC    1-ethyl-3-(3-dimethylamino propyl) carbodiimide
Et$_2$O    diethyl ether
EtOAc    ethyl acetate
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>EtOH</td>
<td>ethanol</td>
</tr>
<tr>
<td>Fmoc</td>
<td>fluorenylmethyloxycarbonyl</td>
</tr>
<tr>
<td>g</td>
<td>gram</td>
</tr>
<tr>
<td>GalCer</td>
<td>galactosylceramide</td>
</tr>
<tr>
<td>h</td>
<td>hour</td>
</tr>
<tr>
<td>H</td>
<td>hydrogen</td>
</tr>
<tr>
<td>H$_2$</td>
<td>dihydrogen</td>
</tr>
<tr>
<td>H$_2$NNH$_2$</td>
<td>hydrazine</td>
</tr>
<tr>
<td>H$_2$O</td>
<td>hydrogen oxide</td>
</tr>
<tr>
<td>HBr</td>
<td>hydrobromic acid</td>
</tr>
<tr>
<td>HCl</td>
<td>hydrochloric acid</td>
</tr>
<tr>
<td>HN(OMe)Me</td>
<td>methoxy(methyl)amine</td>
</tr>
<tr>
<td>HRMS</td>
<td>high resolution mass spectroscopy</td>
</tr>
<tr>
<td>Hz</td>
<td>hertz</td>
</tr>
<tr>
<td>IR</td>
<td>infrared</td>
</tr>
<tr>
<td>J</td>
<td>J-coupling</td>
</tr>
<tr>
<td>KBr</td>
<td>potassium bromide</td>
</tr>
<tr>
<td>LiAl(Ot-Bu)$_3$H</td>
<td>tri-$t$-butoxyaluminum hydride</td>
</tr>
<tr>
<td>M</td>
<td>molecular or molarity</td>
</tr>
<tr>
<td>m</td>
<td>multiplet</td>
</tr>
<tr>
<td>MeOH</td>
<td>methanol</td>
</tr>
<tr>
<td>MeSO$_3$H</td>
<td>methanesulfonic acid</td>
</tr>
<tr>
<td>MgCl</td>
<td>magnesium chloride</td>
</tr>
<tr>
<td>MgSO$_4$</td>
<td>magnesium sulfate</td>
</tr>
<tr>
<td>MHz</td>
<td>megahertz</td>
</tr>
<tr>
<td>min</td>
<td>minutes</td>
</tr>
<tr>
<td>Symbol</td>
<td>Chemical Name</td>
</tr>
<tr>
<td>-------------</td>
<td>--------------------------------------</td>
</tr>
<tr>
<td>mL</td>
<td>milliliter</td>
</tr>
<tr>
<td>mmol</td>
<td>millimole</td>
</tr>
<tr>
<td>N$_2$</td>
<td>dinitrogen</td>
</tr>
<tr>
<td>Na$_2$S$_2$O$_3$</td>
<td>sodium thiosulfate</td>
</tr>
<tr>
<td>NaH</td>
<td>sodium hydride</td>
</tr>
<tr>
<td>NaHCO$_3$</td>
<td>sodium bicarbonate</td>
</tr>
<tr>
<td>NaOH</td>
<td>sodium hydroxide</td>
</tr>
<tr>
<td>NaOMe</td>
<td>sodium methoxide</td>
</tr>
<tr>
<td>NaOt-Bu</td>
<td>sodium tert-butyl alcohol</td>
</tr>
<tr>
<td>NH$_4$Cl</td>
<td>ammonium chloride</td>
</tr>
<tr>
<td>NHS</td>
<td>N-hydroxysuccinimide</td>
</tr>
<tr>
<td>NK T</td>
<td>natural killer T-cell</td>
</tr>
<tr>
<td>NMM</td>
<td>N-methylmorpholine</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>NOD</td>
<td>non-obese diabetic mice</td>
</tr>
<tr>
<td>OC(NH)CCl$_3$</td>
<td>trichloroacetimidate</td>
</tr>
<tr>
<td>P$_2$S$_5$</td>
<td>phosphorus pentasulfide</td>
</tr>
<tr>
<td>Pd$_2$(dba)$_3$</td>
<td>tris(dibenzylideneacetone)dipalladium(0)</td>
</tr>
<tr>
<td>PhSH</td>
<td>thiophenol</td>
</tr>
<tr>
<td>PNP</td>
<td>p-nitrophenyl</td>
</tr>
<tr>
<td>PPh$_3$</td>
<td>triphenyl phosphine</td>
</tr>
<tr>
<td>ppm</td>
<td>parts per million</td>
</tr>
<tr>
<td>Pyr</td>
<td>pyridine</td>
</tr>
<tr>
<td>q</td>
<td>quartet</td>
</tr>
<tr>
<td>rt</td>
<td>room temperature</td>
</tr>
<tr>
<td>s</td>
<td>singlet</td>
</tr>
</tbody>
</table>
SEM      [2-(trimethylsilyl)ethoxy]methyl

**t**      triplet

**TBAF**    tetrabutylammonium fluoride

**TBAHS**   tetra-\(n\)-butylammonium hydrogensulfate

**TBAI**    tetrabutylammonium iodide

**TBDMSOTf (TBSOTf)** tert-butyldimethylsilyl trifluoromethanesulfonate

**TBDPSCl** tert-butyldiphenylsilyl chloride

**t-BuOH**  tert-butyl alcohol

**TEMPO**   (2,2,6,6-Tetramethylpiperidin-1-yl)oxyl

**TFA**     trifluoroacetic acid

**THAI**    tetrahexylammonium iodide

**THF**     tetrahydrofuran

**TLC**     thin layer chromatography

**TMSN\(_3\)** trimethylsilyl azide

**TOF**     time of flight

**TsOH**    \(p\)-toluenesulfonic acid
Table of Contents

Introduction 1
   I. Galactosylceramide Background 1
      A. Composition Of A Galactosylceramide 1
      B. Immunomodulation 2
   II. Discovery Of KRN7000 3
   III. Research On KRN7000 Analogs 3
      A. OCH – A Th2 Biasing Glycolipid 3
      B. Anomeric Replacement: α-C-GalCer 6
      C. α-S-GalCer 7
   IV. Target Of Research (α-N-GalCer) 8
      A. Approach To α-N-GalCer’s 9
      B. Approach To α-N-formyl-N-GalCer 10
   V. Summary 12

Results and Discussion 12
   I. Research Objective 12
   II. Synthesis Of α-N-galactosylceramide 12
   III. Synthesis Of α-N-formyl-N-galactosylceramide 16

Conclusion 19

Experimental 21
   Hexacosanoic acid 2,5-Dioxo-pyrrolidin-1-yl ester (14a) 21

   p-Nitrophenyl hexacosanoate (14b) 22

   Hexacosanoic acid (2,3-Dihyроxy-1-hydroxymethyl-heptadecyl) amide (15) 22

   (2S,3S,4R)-1,3,4-Tri-t-butyldimethylsilyloxy-2-hexanosoylaminooctadecane (16) 23
(2S,3S,4R)-3,4-Bis-tert-butyldimethylsilyloxy-2-hexacosanoylamino-1-octadecanol (17)

(2S,3S,4R)-2-(N-Pentacosanoylamino)-3,4-di-tert-butyldimethylsilyloxy-1-octadecaphalimide (18)

[2-Hydroxy-1-(methoxymethylcarbamoyl)ethyl]carbamic acid tert-butyl ester (20)

(2S)-2-(N-tert-Butoxycarbonyl)amino-1-hydroxyoctadecan-3-one (21)

(2S)-2-(N-tert-Butoxycarbonyl)amino-1-tert-butyldiphenylsilanyloxyoctadecan-3-one (22)

(2S,3R)-2-(N-tert-Butoxycarbonyl)amino-1-tert-butyldiphenylsilanyloxyoctadecan-3-ol (23)

(2S,3R)-2-(N-tert-Butoxycarbonyl)amino-1-tert-butyldiphenylsilanyloxy-3-benzyloxyoctadecane (24)

(2S,3R)-2-(N-tert-Butoxycarbonyl)amino-1-tert-butyldiphenylsilanyloxy-3-benzyloxyoctadecane (24)

(2S,3R)-2-(N-tert-Butoxycarbonyl)amino-3-benzyloxyoctadecan-1-ol (25)

(2S,3R)-3-Benzylxoxy-2-(N-t-butoxycarbonyl)amino-1-octadecaphalimide (26)

(2S,3R)-3-Benzylxoxy-2-(N-t-butoxycarbonyl)amino-1-octadecamine (27)

Methyl 2,3,4,6-tetra-O-benzyl-α-D-galactopyranoside (28)

2,3,4,6-Tetra-O-benzyl-D-galactopyranose (29)

1-O-Acetyl, 2,3,4,6-tetra-O-benzyl-β-D-galactopyranose (30)

2,3,4,6-Tetra-O-benzyl-α-D-galactopyranosyl bromide (31)
$N^\alpha$(Fluoren-9-ylmethyloxycarbonyl)$-N^\gamma$(2,3,4,6-tetra-$O$-benzyl-$\beta$-$D$-galactopyranosyl)$-N^\gamma$-formyl-$L$-asparagine-$t$-butyl-ester (35) 32

(2S,3S,4R)-2-($N$-Hexacosanoylamino)-3,4-di-$t$-butyldimethylsilanyloxy-1-octadecanoic acid (36) 32

(2S,3R)-3-Benzylxy-2-($N$-$t$-butoxycarbonyl)amino-1-octadecanoic acid (38) 33

References 34
Introduction

I. Galactosylceramide Background

In 1993, Koezuka and coworkers were researching marine natural products with anti-tumor activities at the Kirin Brewery, which led to the discovery of agelasphin (e.g. agelasphin 9b). Research on these natural products led to the creation of the KRN7000 (Figure 1).

![Figure 1: Structures of Agelasphin 9b and KRN7000](image)

KRN7000 has been reported to stimulate NKT cells through its presentation by the CD1d protein. This stimulatory pathway may have potential for the treatment of tumors, infectious diseases, and autoimmune conditions. Further studies involving alterations to this molecule are being investigated to observe how differences in glycolipid functionality alter immune responses. In the following paragraphs the structure and bioactivity of some of the glycolipids related to the goals of this thesis will be discussed.

A. Composition Of A Galactosylceramide

Generally, a galactosyl ceramide (a type of glycolipid) (Figure 2) is made up of a galactosyl moiety connected to a ceramide by what is known as a glycosidic bond. The ceramide consists of two hydrophobic lipid chains, one an amide acyl chain and the other a sphinganine, either of which can vary based on chain length, branching, or functional groups attached. In the next section the influence of some glycolipids on the immune system will be presented.
Glycolipids have been shown to increase immunoactivity by interacting with the immune systems lymphocytes. Lymphocytes are white blood cells that serve various functions in the immune system. They are also related to adaptive immunity (immunity that develops as we age), as opposed to innate immunity (basic immunity that responds immediately but non-specifically to infections and pathogens). The two major classes of lymphocytes are distinguished by cells which grow independently of the thymus (B cells) and that grow inside the thymus (T cells). Natural Killer T-lymphocytes (NKT cells) are a subset of lymphocytes that have T-cell receptors located on their surface. The glycolipid’s alkyl chains are utilized by binding to the CD1d protein of an antigen presenting cell through crevice-like tunnels. Once bound, the exposed sugar head of the glycolipid interacts with the T-cell receptor of the NKT cell. Interaction between the T-cell receptor and glycolipid causes the release of cytokines from the NKT cells. There are two groups of cytokines, T helper 1 (Th1) and T helper 2 (Th2), categorized based on their response upon release. Cytokines like interferon-γ (IFN-γ) and interleukin-2 (IL-2) exhibit an inflammatory response (Th1) associated with controlling bacterial, parasitic and viral infections as well as tumors. Interleukin-4 (IL-4) and IL-10 are examples of two cytokines responsible for an immunomodulatory response (Th2), which has been known to ameliorate autoimmune diseases, such as multiple sclerosis, lupus, rheumatoid arthritis, and type I diabetes. In the next section the research leading to the discovery of KRN7000 and its biological activity will be discussed.
II. Discovery Of KRN7000

In 1993, Koezuka and coworkers were investigating marine natural products for bioactivity and came across the agelasphins, in particular, agelasphin 9b (extracted from the sponge *Agelas Mauritianus*) (Figure 1). The interestingly rare feature about these agelasphins was their α-glycosidic bonds, as opposed to the β-glycosidic bonds found in more advanced organisms. Around this time it was also discovered that glycolipids cause lymphocyte cell proliferation. Further studies\(^\text{11}\) of the bioactivity led to structural determination that indicated the C4 hydroxyl group as nonessential for bioactivity and the C3 hydroxyl group as necessary for T-cell stimulation. Alteration of the lipid chains led to the creation of the galactosylceramide KRN7000.

KRN7000 is now widely known for its interaction with NKT cells leading to the release of both Th1 and Th2 cytokines. It has been shown\(^\text{10}\) that Th2 cytokines can antagonize the Th1 response. Ideally, controlling which cytokines are released would prove beneficial to those suffering from infectious or autoimmune diseases. Interest surrounding the medicinal properties of the glycolipid led researchers to investigate methods to manipulate the molecule resulting in the controlled release of Th1 and Th2 cytokines. In the next section previous research involving key KRN7000 derivatives that lead to a biased cytokine response will be discussed, including background information leading to the target structures of this thesis.

III. Research On KRN7000 Analogs

A. OCH – A Th2 Biasing Glycolipid

Among the analogs of KRN7000 is OCH (Figure 3), a compound which showed bias in the release of Th2 cytokines, IL-4 and IL-10. Further studies showed that OCH inhibited experimental autoimmune encephalitis\(^\text{12}\) and collagen-induced arthritis\(^\text{13}\). Inspired by the ameliorative implications,
Miyake and coworkers researched the glycolipid’s (Figure 3) effect on type 1 diabetes. Tests were conducted on non-obese diabetic mice (NOD), a model for type I diabetes in humans. KRN7000 was used as a control to test the activity of OCH.

![Figure 3: Structure of OCH](image)

While observing the incidence of diabetes in 30 week old NOD mice that were given multiple doses of OCH at 5 weeks of age, the glycolipid showed comparable inhibition results (reduction from 75% to 27%) to KRN7000. In an experiment observing insulitis (inflammation of islets of the pancreas, which can lead to diabetes), the inflammations were categorized by the amount of inflamed areas observed (Figure 4).

To observe levels of insulitis pancreatic sections were removed and examined. Data showed lower grade 3 levels and higher grade 0 levels in mice dosed with OCH compared to mice dosed with KRN7000. Although OCH was not as potent in the stimulation of NKT cells (as KRN7000), the glycolipid showed more inhibitory effect of insulitis in the microscopic appearance of pancreatic samples. Overall, OCH has provided evidence indicating its inhibition of insulitis and diabetes.\(^\text{14}\)
OCH test results have been debated between researchers trying to determine plausible reasons for its superiority over KRN7000 in models for diabetes. Variables, such as defects in NKT cells, might have led to an increase of NKT cells in transgenic mice, which would give off more Th2 cytokines, contaminating the accuracy of the results for cytokine release. The results for the spontaneous development of diabetes could have been shifted in favor of the OCH glycolipid if there were any bacterial infections in the mice, because a combination of mycobacterium extract with KRN7000 would cause the NKT cells to predominately produce Th1 cytokines, nullifying the Th2 effect. Although these are not definitively the reasons behind the results, the fact remains that OCH has shown a biased release of Th2 cytokines, IL-4 and IL-10, and has assisted validation of the pursuit of altering the glycolipid structure to provide controlled responses. Another area of interest concerning the alteration of the glycolipid structure and controlled cytokine release is the glycosidic bond between sugar and ceramide.

**Figure 4:** Levels of insulitis inflammation

Grade 4 (severe intra-insulitis: cell infiltration in an area more than 50% of an islet)

Grade 3 (moderate intra-insulitis: cell infiltration in an area more than 25% but less than 50% of an islet)

Grade 2 (mild intra-insulitis: cell infiltration in the area less than 25% of an islet)

Grade 1 peri-insulitis but no intra-insulitis

Grade 0 no inflammation
Discussion below will focus more on research concerning the glycosidic bond between sugar and ceramide.

B. Anomeric Replacement: α-C-GalCer

It is known that KRN7000 releases high levels of both Th1 and Th2 cytokines (i.e. IFN-γ and IL-4, respectively, are generally monitored). Moreover, the unbiased release antagonizes the health benefits related to both types of cytokines. Research\(^{16}\) on encephalomyelitis in mice has shown that a synthetic analogue possessing a truncated sphingosine chain resulted in stimulating the release of only IL-4, which better protected mice from encephalomyelitis. This alteration to the glycoside led researchers\(^{16-17}\) to synthesize a KRN7000 derivative with a hydrophobic methylene (CH2) as the glycosidic link between the sugar and ceramide instead of the hydrophilic oxygen (O) (Figure 5). The substitution was meant to deter \(α\)-galatosidase catabolism in vivo\(^{18-19}\) and allow observation of any difference in NKT cell stimulation.

![Figure 5: Franck and Tsuji’s methylene galactosylceramide\(^{17}\)](image)

In multiple comparisons between KRN7000 and \(α\)-C-GalCer, test results consistently provided data\(^{17,20-21}\) which led to the conclusion that \(α\)-C-GalCer is a better candidate for the treatment of infectious and autoimmune diseases than KRN7000. Comparison tests\(^{17}\) regarding malaria have shown that \(α\)-C-GalCer’s antimalaria effect lasted 3 days longer than KRN7000. In regards to tumor studies\(^{20}\) \(α\)-C-GalCer has been shown to exhibit similar T cell stimulation to KRN7000 when using a dosage 1000 times less than KRN7000. A binding stability experiment\(^{20}\) showed that \(α\)-C-GalCer required less time to bind to NKT cells and stimulated the cells more significantly than KRN7000. In more recent studies,\(^{21}\) \(α\)-C-
GalCer was used as an adjuvant to a live attenuated influenza virus vaccine and was shown to increase the immunogenicity and enhance protection provided by the vaccine in wild type mice. The next section will discuss previous research conducted in the Howell group focusing particularly on the glycosidic bond.

C. α-$S$-GalCer

The emerging understanding and medicinal potential of the α-glycosylceramides had attracted the attention of the Howell group. Previous research in our group related to glycolipids was inspired by the KRN7000 derivative α-$C$-GalCer. The comparison of the CH2 glycolipid to KRN7000 has previously been discussed and has led to the conclusion that alterations to the glycosidic bond can have biased and beneficial effects. Following that approach an investigation was conducted exploring the link between the sugar and ceramide. One previous glycolipid target in the group was α-$S$-GalCer 3.

This approach took advantage of treating a nucleophilic ceramide with an electrophilic sugar donor. Ceramide 1 (Scheme 1) was treated with phosphorus pentasulfide forming 2. Glycosylation was attempted using a trichloroacetimidate activated by a Lewis acid but unfortunately no desired product 3 was isolated. Thiol glycosylation following the above method had been previously reported successfully, but researchers used simple alkyl acceptor groups in comparison to the ceramide.

![Scheme 1: Glycosylation of a protected α-$S$-GalCer](image)

Another approach was attempted, targeting α-$S$-GalCer (Scheme 2). Beginning with phytosphingosine 4, the molecule was Boc-protected, fully silyl protected, selectively deprotected, and then brominated following Yamamoto’s procedure forming the electrophilic alkyl chain, 5. Following precedent literature, α-$S$-GalCer 7 was synthesized as an inseparable anomeric mixture. The thiol sugar was then
alkylated using Schimdt’s reported approach, resulting in the protected glycolipid 6. The thiol glycoside was fully deprotected and acylated to form the targeted \(\alpha-S\)-GalCer 7.

![Synthesis of \(\alpha-S\)-GalCer](image)

### Scheme 2: Synthesis of \(\alpha-S\)-GalCer

The successfully synthesized \(\alpha-S\)-GalCer was tested for biological activity, and although it shared a similar structure to KRN7000, it did not show any biological activity. One speculation was that oxidation of the thiol glycoside could have occurred, interfering with interaction between the \(S\)-GalCer and NKT cell. Although the \(S\)-GalCer may not have shown beneficial effects in regards to NKT cell stimulation, it provided information regarding substitution of oxygen with a closely related atom. Continuation of the glycosidic link investigation by the Howell group led to the goals of this thesis.

IV. Target Of Research (\(\alpha-N\)-GalCer)

The goals of this thesis were to synthesize \(\alpha-N\)-GalCer 8 and \(\alpha-N\)-formyl-\(N\)-GalCer 9 (Figure 6). These targets were chosen because we were inspired by \(\alpha-C\)-GalCer’s bioactivity and wanted to synthesize glycosylceramides that carried similarities but had not been explored previously. The next two sections will discuss literature methods used to approach \(N\)-glycosides.
A. Approach To α-N-GalCer’s

As with S-GalCer’s there are two main approaches to synthesizing N-glycosides. The first couples an electrophilic sugar donor with an amine. The next method utilizes a nucleophilic sugar coupled with an electrophile.

Some researchers have reported creating alkylglycosylamines by coupling simple aminoalkyl groups with unprotected sugar donors (Scheme 3). In relation to the sugar, mannose commonly results in alpha glycosylations, while glucose and galactose yield alpha and beta glycosylated products (beta predominating). There have been no reports of the synthesis of N-glycosylceramides. Marisa Blauvelt, in the Howell group did try to glycosylate an aminoceramide using an unprotected sugar donor, but the approach was unsuccessful.

An alternative approach would be to use a halide donor sugar. Our group has experience coupling galactosyl iodides or bromides with ceramides or related sphingoid bases. Consequently we will examine the reaction of these glycosyl halides with aminoceramides. Glycosylations reported by Jacquelyn Gervay-Hague treated an alpha sugar donor with a sphinganine (acceptor) resulting in an alpha

**Figure 6:** Target α-N-GalCer molecules

**Scheme 3:** Formation of an alkylglycosylamine using mannose.
glycosylated product. To synthesize the first target (8) a similar approach to Jacquelyn Gervay-Hague’s will be attempted (Figure 7).

**Figure 7: Approach to α-N-GalCer**

Regarding the second approach to forming α-N-GalCer’s (mentioned above) there have been reports of successfully synthesizing α-N-GalCer’s using nucleophilic sugar donors (Scheme 4). For example, the synthesis of spicamycin involves a nucleophilic sugar donor reacting with an electrophilic chloropurine. The sugar 10 underwent azide formation using trimethylsilyl azide. Azide 11 was reduced using hydrogen with Lindlar’s catalyst, forming 12. The heterocyclic alkylation via amide coupling formed 13.

**Scheme 4: Formation of alkylglycosylamine using galactose.**

B. Approach To α-N-formyl-N-GalCer

A proposed method to the synthesis of α-N-formyl glycolipids was found in recent chemistry performed by Danishefsky’s group. Their investigation of complex chemistry was inspired by the Passerini reaction (Figure 8). The reaction (also referred to as an acyl transfer) called for a carboxylic acid and an isonitrile resulting in an amide product.
Danishefsky proposed a mechanism between the isonitrile and carboxylic acid (Figure 9), as illustrated in a reported reaction using a fully protected isonitrile and aspartic acid (Scheme 5). This reaction's outcome has been shown to be influenced anomerically by the isonitrile used for the acyl transfer; an alpha isonitrile would result in an alpha formylated product and a beta isonitrile would result in a beta formylated product.

![Figure 9: Danishefsky’s proposed acyl transformation.](image)

**Scheme 5:** Acyl transfer using tetrabenzylated isonitrile and aspartic acid.

Our initial approach to $\alpha$-N-formyl-$N$-GalCer 9 will take advantage of Danishefsky’s method (discussed above) to form $N$-formyl glycolipids. An alpha nucleophilic sugar donor will be treated with an electrophilic ceramide acceptor (Figure 10) to synthesize our desired formyl product.
V. Summary

Past research has shown that KRN7000 anomeric replacements (e.g. α-C-GalCer) have produced interesting results. Further investigations on these replacements would lead to a better understanding of the glycolipid structure and potentially biased T cell stimulation. The Howell group’s research has reported that it is possible to synthesize α-S-galactosylceramides. The investigations in this thesis have been focused on anomeric replacement with nitrogen.

Results and Discussion

I. Research Objective

The goals of these studies were:

1. To synthesize an α-N-galactosylceramide analog of KRN7000
2. To synthesize an α-N-formyl-N-galactosylceramide analog of KRN7000
3. To compare NKT cell stimulatory abilities of α-N-galactosylceramide and α-N-formyl-N-galactosylceramide to their parent compound KRN7000 in collaboration with the lab of Professor Brian Wilson of the University of Florida

II. Synthesis Of α-N-galactosylceramide

A simple and straightforward method was required for the synthesis of the complex structure of α-N-galactosylceramide. Since the Howell group had success with coupling galactosyl halides with ceramides or related sphingoid bases this method was examined first. As mentioned before (Figure 7, pg.

Figure 10: Approach to α-N-formyl-N-GalCer
Following Sanghee’s reported\textsuperscript{30} \(\alpha\)-GalCer approach (Scheme 6), the synthesis of \(\alpha\)-\(\text{N}\)-GalCer began by coupling commercially available cerotic acid with \(N\)-hydroxysuccinimide, providing ester \(14a\) in 43\% yield. Due to the esters less than moderate yields an alternate ester formation was examined.\textsuperscript{31} Cerotic acid was treated with \(p\)-nitrophenol to afford ester \(14b\) in 60\% yield. Ester \(14b\) could be isolated using column chromatography, but it was also found that purification of \(14b\) could be accomplished by recrystallization using ethyl acetate giving 75\% yield. The ester was then coupled to phytosphingosine, forming ceramide \(15\), isolated in 99\% yield. Previously this had been purified via column chromatography, but it was discovered that \(15\) could also be purified by recrystallization using ethyl acetate. Ceramide \(15\) was then fully silyl protected, providing \(16\) in 93\% yield. Selective deprotection gave the free primary alcohol \(17\) in 62\% yield. The original procedure\textsuperscript{30} called for 10\% trifluoroacetic acid (TFA), but the reaction did not reach completion until the concentration of TFA was increased to 50\%. An amination similar to the Gabriel synthesis\textsuperscript{32} was then performed on alcohol \(17\). The selectively deprotected ceramide was treated with phthalimide under Mitsunobu conditions, forming \(18\) in 76\% yield. Selective deprotection of \(18\) using hydrazine was attempted. Although the starting material was consumed, based on TLC, we were unable to isolate clean aminoceramide \(19\). As proof of aminoceramide formation, it was acetylated. Although pure acetylated product was not isolated, \(^{13}\)C NMR data from the acetylation showed two carbonyl peaks, which led us to believe that aminoceramide \(19\) was successfully synthesized. The glycosylation of \(19\) was attempted but no desired product was isolated. Consequently, the approach was revised.
The Howell group has found that working with sphinganines (aminodiols) rather than phytosphingosines (aminotriols), is sometimes more straightforward. We decided to proceed with the synthesis using a protected sphinganine (Figure 11). Prior to the ceramide formation the aminodiol will be coupled with the halosugar donor. The sphingoid base will be formed using a simple and selective route.\textsuperscript{33}

\textbf{Figure 11: Approach to revised α-N-GalCer}

Following a procedure developed in the group\textsuperscript{33} (Scheme 7) the synthesis of the selectively protected sphingoid base began by treating Boc-protected serine with methoxy(methyl)amine hydrochloride, forming Weinreb amide 20. This was treated with two equivalents of a sacrificial base to deprotonate the primary alcohol of the carbonate. Then, pentadecylmagnesium bromide was added, forming ketone 21 in 62% yield over two steps. The alcohol was silyl protected, providing 22 in 85% yield. The protected sphinganine was then reduced, giving the secondary alcohol 23 in 50% yield. Benzylolation gave the fully protected sphingoid base 24 in 54% yield. Selective deprotection provided 25 in 25% yield. The low yield in the deprotection was due to loss of material during work-up. The primary
amine was formed following the same procedure described in the attempted synthesis of the aminoceramide 19. Reaction with phthalimide gave 26 in 61% yield. A deprotection was attempted using sodium borohydride with acetic acid in isopropyl alcohol, but no desired product was isolated. An alternate deprotection with hydrazine produced 27 in 25% yield. During purification of the phthalimide deprotection via TLC plate a second spot was observed close to the desired product. This had also been observed with the attempted aminoceramide purification. The $^1$H and $^{13}$C NMRs showed only the desired aminodiol in the isolated product.

Scheme 7: Synthesis of sphingoid base

The synthesis of the glycosyl donor began with commercially available methyl-$\alpha$-D-galactopyranoside(28) (Scheme 8). Following the approach of Jacquelyn Gervay-Hague’s reported halosugar synthesis, global benzyl protection of methoxygalactoside, followed by anomeric hydrolysis gave 29 in 9% yield over two steps. Acetylation of the lactol formed 30. Purification of 30 did not afford a sufficient yield so the product was obtained by a labmate. Treatment of the acetylated sugar with hydrobromic acid provided bromosugar 31. Due to its sensitivity to silica gel, the sugar was directly used in the glycosylation without purification.
Glycosylation of the protected aminosphingoid base 27 with bromosugar 31 was performed following a method reported by Jacquelyn Gervy-Hague (Scheme 9). A compound that displayed similar spectral characteristics to the desired product was observed but, unfortunately, could not be isolated.

Scheme 8: Synthesis of bromosugar

Scheme 9: Attempted synthesis of revised α-N-GalCer

III. Synthesis Of α-N-formyl-N-galactosylceramide

Our synthesis of α-N-formyl-N-galactosylceramide (9) will require treatment of an electrophilic sugar with a nucleophilic ceramide (Figure 12). Based on the study reported by Danishefsky (Scheme 5, pg. 12) we will treat an isonitrile with a carboxylic acid derived from the corresponding ceramide. The beta isonitrile will be used to test the methodology of the acyl transfer reaction. Once the methodology proves successful, the alpha isonitrile will be used to create the desired N-formyl glycolipid. The isonitrile will be synthesized using Danishefsky’s methods, and the acid will be synthesized by oxidation of the ceramide’s primary alcohol.
A model study was conducted prior to the α-N-formyl-N-GalCer synthesis, to test Danishefsky’s reported acyl transfer (Scheme 5, pg. 12). Commercially available aspartic acid 34 was treated with isonitrile 33, prepared by labmate Dr. Stewart Richardson, giving 35 in 52% yield (Scheme 10). The results of this study gave us enough cause to pursue the acyl transfer with ceramide.

The approach towards 9 began with previously synthesized ceramide alcohol 17 (Scheme 6, pg. 14). Following a procedure presented by Danishefsky, compound 17 was oxidized to carboxylic acid 36 in 37% yield (Scheme 11). Excess TEMPO and BAIB were necessary to complete the conversion to carboxylic acid. The acid was then treated with the protected isonitrile 33 to form product 37. Unfortunately, the desired product from the acyl transfer reaction was not observed.
Scheme 11: Attempted synthesis of α-N-formyl-N-GalCer

Messy TLC and NMR data led to concerns about the stability of the ceramide under the high temperature reaction conditions. Byproducts observed via TLC showed potential signs of ceramide decomposition (e.g. deprotections). An alternate approach will be taken to address steric complications with the ceramide and take advantage of our previously synthesized sphingoid base 25.

The reaction conditions for the acyl transfer reaction reported by Danishefsky appeared to be too harsh for the ceramide-derived carboxylic acid. It was decided that a simpler structure and different protecting groups may tolerate the reaction conditions better. The alternative route will use the protected isonitrile 33 and an oxidized form of the sphingoid base (creating the acid) for the acyl transfer (Figure 13). As stated previously, a beta isonitrile will be used to test the methodology of the acyl transfer with the sphingoid base.

Figure 13: Revised approach to α-N-formyl-N-GalCer
Sphingoid base 25 (Scheme 7, pg. 16) was oxidized to carboxylic acid 37 in 81% yield (Scheme 12). Acid 38 was reacted with 32 to form the revised 6-N-formyl-N-GalCer product 39. Unfortunately no desired product was isolated, and NMR data showed evidence of byproducts. It was concluded that high temperatures caused the decomposition of the protected sphingoid base and ceramide which led to the unsuccessful acyl transfer with the protected isonitrile.

\[
\begin{align*}
25 &\xrightarrow{\text{TEMPO, PAIB}} \text{CH}_2\text{Cl}_2/\text{Water, 81%} \quad &\text{O} &\text{O} &\text{Bn} \\
\text{N} &\text{HIBoc} \\
 38 &\xrightarrow{150^\circ\text{C, Microwave}} \text{CHCl}_3 \\
32 + 38 &\xrightarrow{150^\circ\text{C, Microwave}} 39
\end{align*}
\]

Scheme 12: Attempted synthesis of revised 6-N-formyl-N-GalCer

Conclusion

The syntheses of complex amino glycosides have been shown to hold a few obstacles. The synthesis of the aminoceramide was not completed due to complications during purification. The acetylation of an isolated aminotriol did suggest that the aminoceramide was synthesized, but in the next reaction no glycosylated product was isolated. The alternative 6-N-GalCer glycosylation using a sphingoid base, resulted in a compound with similar spectral characteristics to the desired product, but the glycosylated sphinganine was not isolated. The 6-N-formyl-N-GalCer synthesis was also not successful. Based on 1H NMR and TLC, it appeared that decomposition of the ceramide and sphinganine carboxylic acids occurred under the reaction conditions.

Future attempts to synthesize amino galactosylceramides will continue to focus on using aminosphingoid base derivative 35. The 6-N-formyl-N-GalCer approach will focus on identifying less extreme conditions. One approach would use a fully deprotected isonitrile 40 (Figure 14). The formation
of the N-formyl group is driven by hydrogen bonding of the free C2′′ hydroxyl group on the sugar providing compound 41, followed by deprotection of the silyl groups to obtain target 9.

![Reaction Scheme](image)

**Figure 14:** Formation of N-formyl-N-GalCer using unprotected isonitrile

Another approach uses an acylated thiolphenol as opposed to a carboxylic acid in the acyl transfer reaction. The product will be a di-thiophenol substituted carbon 43 (Scheme 13), but this can be hydrolyzed to the formyl group using p-toluene sulfonic acid in water to afford the α-N-formyl-N-galactosylceramide product 44, followed by full deprotection to provide desired target 9.

![Scheme 13](image)

**Scheme 13:** Formation of N-formyl-N-GalCer using thiophenol
Experimental

General. Melting points were determined in open Pyrex capillary tubes and are uncorrected. Infrared spectra were recorded on a Nicolet 750 FT-IR spectrometer. $^1$H NMR spectra were recorded at 300 MHz on a Bruker Avance Ultrashield 300-NMR spectrometer, at 400 MHz on a Bruker Avance DRX-400 NMR spectrometer, and at 500 MHz on a Bruker Avance 500-NMR spectrometer. Chemical shifts (δ) are reported in ppm and coupling constants (J) are reported in Hertz. Abbreviations used are as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, dd = doublet of doublets, ddd = doublet of double of doublets, dt = doublet of triplets. $^{13}$C NMR spectra were recorded on a Bruker Avance Ultrashield 300-NMR spectrometer at 75 MHz, on a Bruker Avance DRX-400 NMR spectrometer at 100 MHz, or on a Bruker Avance 500-NMR spectrometer at 125 MHz. High-resolution mass spectra were determined by the Mass Spectroscopy Facility in the Department of Chemistry at the University of Connecticut in Storrs, Connecticut and the Mass Spectroscopy Facility in the Department of Chemistry and Biochemistry at the University of Notre Dame in Notre Dame, Indiana. Column chromatography was performed with flash silica, 40 microns. Thin-layer chromatography was carried out on silica gel (Silica Gel 60 F$_{254}$) glass plates. Spots were visualized by UV and/or 10% molybdic acid in ethanol. Tetrahydrofuran was obtained from a distillation dispensing unit under neutral alumina (CH$_2$Cl$_2$, pyridine, and toluene were obtained in the Chemistry Department stock room located at University of Connecticut in Storrs, Connecticut and dried over 4Å MS).

![Hexacosanoic acid 2,5-Dioxo-pyrrolidin-1-yl ester (14a)](image)

Hexacosanoic acid 2,5-Dioxo-pyrrolidin-1-yl ester (14a)

Hexacosanoic acid (0.50 g, 1.3 mmol) was dissolved in CH$_2$Cl$_2$ (15 mL) under N$_2$ at rt. EDC (0.27 g, 1.4 mmol) and N-hydroxysuccinimide (0.18 g, 1.6 mmol) were added to the solution. The reaction mixture
was heated to 40 °C for 6 h. The reaction was diluted with H₂O (10 mL), and the organic layer was extracted with Et₂O (30 mL). The organic layer was then washed with brine (10 mL), dried (MgSO₄), and concentrated under reduced pressure. Purification by flash chromatography on silica gel (PetEt/EtOAc 85:15) afforded 14a as a white solid (0.28 g, 43%):³³ ¹H NMR (300 MHz, CDCl₃) δ 2.83 (s, 4H), 2.59 (t, J = 7.5 Hz, 2H), 1.74 (m, 2H), 1.39 (m, 2H), 1.25 (m, 42H), 0.87 (t, J = 6.8 Hz, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 169.1, 168.6, 31.9, 30.9, 29.7, 29.6, 29.5, 29.3, 29.1, 28.8, 25.6, 24.5, 22.7, 14.1

**p-Nitrophenyl hexacosanoate (14b)**

Hexacosanoic acid (1.0 g, 2.5 mmol) was dissolved in CH₂Cl₂ (260 mL) under N₂ at rt. p-Nitrophenol (0.39 g, 2.8 mmol), DMAP (0.060 g, 0.51 mmol), and DCC (0.55 g, 2.7 mmol) were added to the reaction flask, and the mixture was stirred for 8 h. The solution was filtered, and the filtrate was concentrated under reduced pressure. Purification by recrystallization (EtOAc) afforded 14b as a yellow solid (0.98 g, 75%):³¹ ¹H NMR (400 MHz, CDCl₃) δ 8.29 (d, J = 9.1 Hz, 2H), 7.30 (d, J = 9.3 Hz, 2H), 2.62 (t, J = 7.5 Hz, 2H), 1.78 (m, 2H), 1.28 (m, 44H), 0.90 (t, J = 6.8 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 171.3, 155.5, 145.2, 125.2, 122.4, 34.3, 31.9, 29.7, 29.7, 29.6, 29.6, 29.4, 29.4, 29.4, 29.2, 29.0, 24.7, 22.7, 14.1

**Hexacosanoic acid (2,3-Dihyroxy-1-hydroxymethylheptadecyl) amide (15)**

p-Nitrophenyl hexacosanoate (14b) (0.46 g, 0.89 mmol) was dissolved in pyridine (17 mL) under N₂ at rt. Phytosphingosine (0.23 g, 0.74 mmol) was then added, and the solution was allowed to stir for 65 h. The solution was then concentrated under reduced pressure. Purification by recrystallization (EtOAc) afforded 15 as a yellow solid (0.51 g, 99%):³⁰ ¹H NMR (500 MHz, pyr) δ 8.34 (d, J = 8.5 Hz, 1H), 5.10-5.05 (m,
1H), 4.49 (m, 1H), 4.45 (m, 1H), 4.33 (m, 1H), 4.23 (m, 1H), 2.46 (t, J = 7.3 Hz, 2H), 2.18-1.21 (m, 72H), 0.88 (t, J = 6.7 Hz, 6H).

(2S,3S,4R)-1,3,4-Tri-<sup>t</sup>-butyldimethylsilyloxy-2-hexacosanoylamino-1-octadecanol (17)

(2S,3S,4R)-1,3,4-Tri-<sup>t</sup>-butyldimethylsilyloxy-2-hexacosanoylamino-1-octadecane (16)

Hexacosanoic acid (2,3-dihygroxy-1-hydroxymethylheptadecyl) amide (15) (1.5 g, 2.2 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (50 mL) at 0 °C. TBSOTf (4.4 g, 17 mmol) and 2,6-lutidine (3.5 g, 33 mmol) were added, and the solution was stirred at rt for 10 h. The reaction was quenched with MeOH (12 mL). The mixture was then diluted with Et<sub>2</sub>O (100 mL) and washed with H<sub>2</sub>O (55 mL), saturated aqueous NaHCO<sub>3</sub> (55 mL), and brine (55 mL). The organic layer was dried (MgSO<sub>4</sub>), filtered, and concentrated under reduced pressure. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 98:2) afforded 16 as a colorless oil (2.13 g, 93%):<sup>30</sup> <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 5.82 (d, J = 8.7 Hz, 1H) 3.94 (m, 1H), 3.87 (dd, J = 9.9, 4.2 Hz, 1H), 3.82 (dd, J = 7.2, 1.2 Hz, 1H), 3.67 (m, 1H), 3.63 (dd, J = 9.9, 4.5 Hz, 1H), 2.14 (t, J = 7.7 Hz, 2H), 1.64-1.46 (m, 6H), 1.25 (m, 66H), 0.93-0.86 (m, 33H), 0.13-0.04 (m, 18H); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 172.2, 75.4, 75.4, 61.4, 52.5, 37.2, 32.2, 31.9, 30.0, 29.7, 29.5, 29.4, 29.4, 29.4, 26.4, 26.1, 26.1, 25.9, 25.8, 22.7, 18.4, 18.2, 18.2, 14.1, -3.5, -3.8, -4.6, -5.2, -5.2, -5.6.
aqueous NaOH (22 mL) and diluted with Et₂O (64 mL). The two layers were separated, and the organic layer was washed with H₂O (31 mL), saturated aqueous NaHCO₃ (31 mL), and brine (31 mL), dried (MgSO₄), and concentrated under reduced pressure. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 80:20) afforded 17 as a colorless oil (1.18 g, 62%):³⁰ ¹H NMR (300 MHz, CDCl₃) δ 6.24 (d, J = 7.8 Hz, 1H), 4.21 (m, 1H), 4.06 (m, 1H) 3.90 (m, 1H), 3.76 (m, 1H), 3.59 (m, 1H), 3.15 (m, 1H) 2.18 (t, J = 7.5 Hz, 2H), 1.48-1.62 (m, 6H), 1.25 (m, 66H), 0.93 (s, 9H), 0.91 (s, 9H), 0.85-0.94 (m, 6H), 0.11 (s, 6H), 0.08 (s, 6H); ¹³C NMR (75 MHz, CDCl₃) δ 172.6, 77.5, 76.4, 63.6, 51.3, 37.0, 34.4, 31.9, 29.8, 29.7, 29.6, 29.5, 29.4, 26.0, 26.0, 25.8, 25.6, 22.7, 18.2, 16.9, 14.1, -3.8, -4.1, -4.5, -4.9.

(2S,3S,4R)-2-(N-Pentacosanoylamino)-3,4-di-tert-butyldimethylsilanyloxy-1-octadecaphthalimide (18)

(2S,3S,4R)-3,4-Bis-tert-butyldimethylsilyloxy-2-hexacosanoylamino-1-octadecanol (17) (0.16 g, 0.18 mmol) was dissolved in dry THF (26 mL) under N₂ at rt. Triphenylphosphine (0.23 g, 0.88 mmol), DIAD (0.039 g, 0.19 mmol), and phthalimide (0.031 g, 0.21 mmol) were added, and the solution was stirred for 3.5 h. The mixture was concentrated under reduced pressure. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 95:5) afforded 18 as a clear oil (0.14 g, 76%): [α]²⁵ Dü -5.19 (c 1.4, CHCl₃); IR (KBr) 2924, 2854, 1717, 1683 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.83 (dd, J = 2.4, 5.4 Hz, 2H), 7.72 (dd, J = 2.4, 5.4 Hz, 2H), 5.98 (d, J = 8.1 Hz, 1H), 4.42 (m, 2H), 3.81 (m, 3H), 2.12 (ddd, J = 7.6, 7.6, 7.6 Hz, 1H), 2.01 (ddd, J = 7.6, 7.6, 7.6 Hz, 1H), 1.70 (m, 4H), 1.60 (m, 4H), 1.41 (m, 4H), 1.28 (br, s, 60H), 1.02 (s, 9H), 0.95 (s, 9H), 0.90 (m, 6H), 0.13 (m, 12H) ¹³C NMR (100 MHz, CDCl₃) δ 172.8, 169.1, 134.2, 132.2, 123.4, 51.1, 37.8, 37.1, 34.6, 32.1, 30.0, 29.9, 29.9, 29.8, 29.8, 29.6, 29.5, 29.4, 26.3,
26.3, 26.1, 25.6, 22.9, 18.6, 18.4, 14.3, -3.3, -3.8, -4.3, -4.7; HRMS (TOF) calcd for C_{64}H_{121}N_{2}O_{5}Si_{2} (M^+ + H) 1053.8814. Found 1053.8859.

[2-Hydroxy-1-(methoxymethylcarbamoyl)ethyl]carbamic acid tert-butyl ester (20)

Boc-L-serine (6.00 g, 29 mmol) was dissolved in dry CH_{2}Cl_{2} (115 mL) and the solution cooled to -15 °C under N_{2}. N,O-Dimethylhyroxylamine hydrochloride (3.0 g, 30 mmol) was added, followed by N-methylmorpholine (3.1 g, 30 mmol). After 5 min 1-(3-methylaminopropyl-3-ethylcarbodoiimide hydrochloride (5.8 g, 30 mmol) was added in five portions over 30 min. After being stirred for 1 h at -15 °C, the reaction was quenched with HCl (1 M, 17 mL), and the layers were separated. The aqueous layer was extracted with CH_{2}Cl_{2} (3 x 34 mL). The organic extracts were combined, washed with saturated NaHCO_{3} (17 mL) and H_{2}O (17 mL), dried (MgSO_{4}), and concentrated to provide 20 as a white solid (5.55 g, carried to the next step without purification):^{33} \text{^1H NMR (400MHz, CDCl}_3\text{)} \delta 5.71 (br, s, 1H), 4.95 (br, s, 1H), 3.82-3.78 (m, 5H), 3.23 (s, 3H), 2.90 (br s, 1H), 1.43 (s, 9H).

(2S)-2-(N-tert-Butoxycarbonyl)amino-1-hydroxyoctadecan-3-one (21)

[2-Hydroxy-1-(methoxymethylcarbamoyl)ethyl]carbamic acid tert-butyl ester (20) (15 g, 59 mmol) was dissolved in dry THF (117 mL) under N_{2}. The solution was cooled to -15 °C, and isopropylmagnesium chloride (60 mL, 2 M) was added dropwise, affording a clear solution. After 5 min, pentadecylmagnesium bromide (0.26 M in THF, 212 mL, 76 mmol) was added at -15 °C. The resulting solution was allowed to warm to rt overnight. The mixture was cooled to -15 °C, and HCl (1 M, 180 mL) was added, followed by EtOAc (135 mL). The two layers were separated, and the aqueous layer was extracted with CH_{2}Cl_{2} (3 x
30 mL). The organic extracts were combined, washed with H₂O (270 mL), dried (MgSO₄), and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 85:15) afforded 21 as a white solid (14 g, 62% over two steps): ³¹H NMR (400 MHz, CDCl₃) δ 5.48 (br s, 1H), 4.26 (m, 1H), 3.94 (m, 2H), 2.63 (br s, 1H), 2.55 (m, 2H), 1.57 (m, 2H), 1.45 (s, 9H), 1.27-1.22 (m, 24H), 0.88 (t, J = 6.8 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 208.1, 164.0, 80.3, 70.1, 68.9, 63.3, 61.6, 52.3, 49.8, 39.9, 31.9, 29.7, 29.6, 29.4, 29.3, 29.2, 28.3, 23.5, 22.7, 14.1.

(2S)-2-(N-tert-Butoxycarbonyl)amino-1-tert-butyldiphenylsilanyloxyoctadecan-3-one (22)

A catalytic amount of DMAP was added to a stirred solution under N₂ of (2S)-2-(N-tert-butoxycarbonyl)amino-1-hydroxyoctadecan-3-one (21) (7.4 g, 19 mmol) and imidazole (3.8 g, 56 mmol) in dry DMF (21 mL). After 20 min TBDPSCl (6.1 g, 22 mmol) was added dropwise, and the reaction mixture was stirred overnight. In the morning the reaction was diluted with saturated aqueous NH₄Cl (50 mL), and the aqueous layer was extracted with CH₂Cl₂ (37 mL). The organic layer was washed with H₂O (37 mL) and brine (37 mL), dried (MgSO₄), and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 98:2) afforded 22 as a clear oil (10 g, 85%): ³¹H NMR (400 MHz, CDCl₃) δ 7.60 (m, 4H), 7.39 (m, 6H), 5.53 (d, J = 7.7 Hz, 1H), 4.33 (m, 1H), 4.04 (dd, J = 10.6, 3.1 Hz, 1H), 3.90 (dd, J = 10.9, 3.8 Hz, 1H), 2.51 (m, 2H), 1.58 (m, 2H), 1.44 (s, 9H), 1.26 (m, 24H), 1.03 (s, 9H), 0.89 (t, J = 7.0 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 207.4, 155.3, 135.5, 128.4, 129.9, 127.8, 79.8, 64.2, 61.3, 60.3, 41.3, 40.0, 32.1, 29.7, 29.6, 29.4, 29.0, 28.3, 27.6, 26.7, 26.2, 23.3, 22.7, 22.6, 20.9, 20.4, 19.4, 19.2, 18.7, 14.3.

(2S,3R)-2-(N-tert-Butoxycarbonyl)amino-1-tert-butyldiphenylsilanyloxyoctadecan-3-ol (23)
LiAl(O-t-Bu)₃H (1.1 g, 4.3 mmol) was added to dry EtOH (7.6 mL) at -78 °C under N₂. (2S,3R)-2-(N-tert-Butoxycarbonyl)amino-1-tert-butylphenylsilyloxyoctadecan-3-one (22) (0.46 g, 0.72 mmol) in dry EtOH (7.6 mL) was added to the reaction flask dropwise. After stirring for 6 h at -78 °C the reaction mixture was diluted with CH₂Cl₂ (2.2 mL) and neutralized with 10% citric acid (22 mL). The solution was allowed to stir and reach rt over 1.5 h. The cloudy suspension was extracted with CH₂Cl₂ (3 x 7 mL). The organic extracts were combined and washed with H₂O (4 x 7 mL). The organic layer was then washed with brine (7 mL), dried (MgSO₄), and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 95:5) afforded 23 as a clear oil (0.23 g, 50%):³³ ¹H NMR (300 MHz, CDCl₃) δ 7.62 (m, 4H), 7.41 (m, 6H), 5.30 (d, J = 8.1 Hz, 1H), 3.93 (m, 1H), 3.90 (m, 1H), 3.67 (m, 1H), 3.57 (br s, 1H), 2.88 (m, 2H), 1.44-1.06 (m, 45H), 0.87 (t, J = 7.0 Hz, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 155.9, 135.8, 132.8, 132.7, 130.2, 130.1, 128.1, 128.0, 79.6, 74.0, 64.4, 54.7, 34.7, 32.1, 31.1, 29.8, 29.6, 28.6, 27.1, 26.1, 22.9, 19.4, 14.3.

(2S,3R)-2-(N-tert-Butoxycarbonyl)amino-1-tert-butylphenylsilyloxy-3-benzyloxyoctadecane (24)

(2S,3R)-2-(N-tert-Butoxycarbonyl)amino-1-tert-butylphenylsilyloxyoctadecan-3-ol (23) (0.14 g, 0.21 mmol) was dissolved in dry DMF (1 mL) under N₂ and cooled to 0 °C. Tetrabutylammonium iodide (0.12 g, 0.32 mmol) and sodium hydride (60% in mineral oil, 0.0070 g, 0.30 mmol) were then added. After 15 min benzylobromide (0.055 g, 0.32 mmol) was added dropwise via syringe. The solution was allowed to stir at 0 °C for an additional 15 min before it was removed from the cooling bath and allowed to stir at rt for 45 min. The reaction was then quenched with saturated aqueous NH₄Cl (2 mL), and the solution was extracted with EtOAc (4 x 3 mL). The combined organic extracts were washed with H₂O (3 mL) and brine (3 mL), dried (MgSO₄), and concentrated. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 97:3) afforded 24 as a clear oil (0.085 g, 54%):³³ ¹H NMR (300 MHz, CDCl₃) δ
7.65 (m, 4H), 7.63-7.27 (m, 11H), 4.71 (d, \(J = 8.0\) Hz, 1H), 4.54 (d, \(J = 11.3\) Hz, 1H), 4.49 (d, \(J = 11.3\) Hz, 1H), 3.86 (m, 2H), 3.72 (dd, \(J = 9.0, 4.1\) Hz, 1H), 3.58 (m, 1H), 1.42 (s, 9H), 1.42 (s, 2H), 1.26 (br s, 26H), 1.05 (s, 9H), 0.88 (t, \(J = 7.0\) Hz, 3H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta 155.8, 138.8, 135.9, 133.5, 130.0, 128.5, 128.0, 127.7, 79.3, 79.1, 72.3, 63.1, 32.2, 30.7, 30.1, 29.9, 29.9, 29.8, 29.6, 28.6, 27.1, 25.6, 22.9, 19.5, 14.4.

(2S,3R)-2-\((N\text{-}tert\text{-}Butoxycarbonyl)amino\)-3-benzyloxyoctadecan-1-ol (25)

(2S,3R)-2-\((N\text{-}tert\text{-}Butoxycarbonyl)amino\)-1-\(\text{tert}\text{-}butyldiphenylsilyl\)oxy-3-benzyloxyoctadecane (24) (0.15 g, 0.20 mmol) was dissolved in THF (0.8 mL) under N\(_2\), and the solution was cooled to 0 °C. TBAF (0.11 mL, 1.0 M in THF) was added, and the solution was stirred for 4 h at rt. The reaction mixture was concentrated, and the residue was dissolved in CH\(_2\)Cl\(_2\) (2.4 mL). The solution was washed with brine (5 mL), dried (MgSO\(_4\)) and concentrated again. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 80:20) afforded 25 as a white solid (0.025 g, 25%): \(^{31}\)H NMR (400 MHz, CDCl\(_3\)) \(\delta 7.32 (m, 5H), 5.27 (d, \(J = 7.4\) Hz, 1H), 4.63 (d, \(J = 11.4\) Hz, 1H), 4.48 (d, \(J = 11.4\) Hz, 1H), 3.95 (m, 1H), 3.65 (m, 3H), 2.95 (s, 1H), 1.67 (m, 1H), 1.44 (s, 9H), 1.26 (br s, 27H), 0.88 (t, \(J = 6.8\) Hz, 3H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta 156.2, 138.2, 128.7, 128.1, 128.0, 82.0, 79.6, 73.0, 62.5, 53.5, 32.1, 31.5, 30.0, 29.9, 29.8, 29.7, 29.6, 28.6, 25.8, 22.9, 14.3.

(2S,3R)-3-Benzylxy-2-\((N\text{-}t\text{-}butoxycarbonyl)amino\)-1-octadecaphthalimide (26)

(2S,3R)-2-\((N\text{-}tert\text{-}Butoxycarbonyl)amino\)-3-benzyloxyoctadecan-1-ol (25) (1.0 g, 2.0 mmol) was dissolved in dry THF (160 mL) at rt under N\(_2\). Triphenylphosphine (2.7 g, 10 mmol), DIAD (0.45 g, 2.2
mmol), and phthalimide (0.36 g, 2.4 mmol) were added, and the solution was stirred for 3.5 h. The mixture was concentrated under reduced pressure. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 90:10) afforded 26 as a white solid (0.77 g, 61%): mp 84-88 °C; [α]_D^{25} -27.3 (c 3.4, CHCl_3); IR (KBr) 2924, 2854, 1717 cm⁻¹; ^1^H NMR (300 MHz, CDCl_3) δ 7.75 (br s, 2H), 7.61 (br s, 2H), 7.24 (m, 5H), 4.87 (d, J = 9.3 Hz, 1H), 4.61 (d, J = 11.7 Hz, 1H), 4.41 (d, J = 11.7 Hz, 1H), 4.06 (br s, 1H), 3.75 (m, 2H), 3.51 (br s, 1H), 1.69 (br s, 1H), 1.40 (br s, 3H), 1.20 (s, 23H), 1.11 (s, 9H), 0.81 (t, J = 6.3 Hz, 3H); ^13^C NMR (75 MHz, CDCl_3) δ 168.7, 155.9, 138.6, 133.9, 128.6, 127.2, 123.3, 80.7, 79.3, 77.6, 77.2, 76.8, 72.3, 51.8, 38.0, 32.1, 31.2, 30.0, 29.9, 29.9, 29.8, 29.7, 29.5, 28.2, 25.7, 23.1, 22.9, 14.3; HRMS (TOF) calcd for C_{38}H_{57}N_2O_5 (M⁺ + H) 621.4267. Found 621.4289.

(2S,3R)-3-Benzoyloxy-2-(N-t-butoxycarbonyl)amino-1-octadecamine (27)

(2S,3R)-3-Benzoyloxy-2-(N-t-butoxycarbonyl)amino-1-octadecapthalimide (26) (1.5 g, 2.5 mmol) was dissolved in MeOH (15 mL) at rt under N_2. The solution was treated with hydrazine monohydrate (0.18 g, 3.7 mmol) and allowed to stir for 3 h at 65 °C. After 2 h a TLC (CH_2Cl_2/MeOH 98:2) showed starting material; so hydrazine (0.060 mL, 1.2 mmol) was added. A white precipitate resulted. After 1 h the mixture was passed through Celite and concentrated. Purification by flash chromatography on silica gel (CH_2Cl_2/MeOH 98:2) afforded 27 as a yellow oil (0.29 g, 25%): [α]_D^{25} -11.0 (c 1.7, CHCl_3); IR (KBr) 3447, 2924, 2854, 1717 cm⁻¹; ^1^H NMR (300 MHz, CDCl_3) δ 7.21 (m, 5H), 5.01 (d, J = 7.8 Hz, 1H), 4.57 (d, J = 11.4 Hz, 1H), 4.43 (d, J = 11.4 Hz, 1H), 3.59 (m, 1H), 3.48 (m, 1H), 2.80 (br, s, 2H), 1.55 (m, 2H), 1.38 (s, 9H), 1.20 (s, 26H), 0.83 (t, J = 6.3 Hz, 3H); ^13^C NMR (75 MHz, CDCl_3) δ 156.2, 138.8, 128.0, 127.9, 81.0, 79.4, 72.5, 54.5, 41.7, 32.1, 31.1, 30.1, 29.9, 29.9, 29.8, 29.7, 29.6, 28.6, 25.8, 22.9, 14.3; HRMS (TOF) calcd for C_{30}H_{55}N_2O_3 (M⁺ + H) 491.4213. Found 491.4196.
Methyl 2,3,4,6-tetra-\textit{O}-benzyl-\textit{\textgreek{a}}-D-galactopyranoside (28)

Sodium hydride (60% in mineral oil, 2.5 g, 103 mmol) in DMF (50 mL) was added to methyl-\textit{\textgreek{a}}-D-galactopyranoside (28) (2.0 g, 10 mmol) in two fractions. The flask was then placed in a cold water bath for 30 min. BnBr (11 g, 62 mmol) in DMF (10 mL) was then added to the reaction flask. The reaction was stirred at rt for 12 h under N\textsubscript{2} flow. The solution was neutralized with brine (25 mL), and the organic layer was extracted with Et\textsubscript{2}O (50 mL x 3). The combined organic layers were washed with water (50 mL x 3) and brine (50 mL), dried (MgSO\textsubscript{4}), and concentrated under reduced pressure. The product was carried to the next step without further purification.\textsuperscript{35}

\[\text{Methyl 2,3,4,6-tetra-\textit{O}-benzyl-D-galactopyranose (29)}\]

Methanesulfonic acid (2.1 g, 21 mmol) was dissolved in H\textsubscript{2}O (10 mL). Methyl 2,3,4,6-tetra-\textit{O}-benzyl-\textit{\textgreek{a}}-D-galactopyranoside (28) (7.9 g, 14 mmol) was dissolved in acetic acid (60 mL). The methanic sulfonic acid solution was then added dropwise to the galactopyranoside solution. The resultant solution was heated to 80 °C for 6.5 h. The mixture was then cooled to rt and concentrated under reduced pressure. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 7:3) afforded 29 as a colorless oil (0.72 g, 9% over two steps).\textsuperscript{35} \textsuperscript{1}H NMR (200 MHz, CDCl\textsubscript{3}) \(\delta\) 7.40-7.18 (m, 20H), 5.97-4.36 (m, 8H), 5.27 (d, \(J = 3.6\) Hz, 1H), 4.19-3.45 (m, 6H).
1-\textit{O}-Acetyl, 2,3,4,6-tetra-\textit{O}-benzyl-\textbeta-D-galactopyranose (30)

2,3,4,6-Tetra-\textit{O}-benzyl-D-galactopyranose (29) (0.72 g, 1.3 mmol) was dissolved in pyridine (3 mL) and was cooled to 0 °C. Acetic anhydride (0.27 g, 2.6 mmol) was then added to the reaction flask dropwise. The reaction was stirred overnight. The mixture was diluted with EtOAc (3 mL) and washed with ice cold water (3 mL). The organic layer was then separated and washed with water (3 mL) and brine (3 mL), dried (MgSO$_4$), and concentrated under reduced pressure. 1-\textit{O}-Acetyl, 2,3,4,6-tetra-\textit{O}-benzyl-\textbeta-D-galactopyranose (30) was not purified.$^{35}$

2,3,4,6-Tetra-\textit{O}-benzyl-\textalpha-D-galactopyranosyl bromide (31)

1-\textit{O}-Acetyl, 2,3,4,6-tetra-\textit{O}-benzyl-\textbeta-D-galactopyranose (30) (0.20 g, 0.34 mmol) was dissolved in CH$_2$Cl$_2$ (9 mL) and cooled to 0 °C. HBr (33% in AcOH) (0.17 mL) was then added to the reaction flask dropwise. The solution was allowed to stir for 1.5 h. The reaction mixture was then poured over ice cold saturated aqueous NaHCO$_3$ (10 mL) and stirred slowly. The organic and aqueous layers were separated. The aqueous layer was extracted with cold EtOAc (10 mL x 3). The combined organic layers were washed with water (10 mL) and brine (10 mL), dried (MgSO$_4$), and concentrated under reduced pressure. The product (31) was used without further purification.$^{37}$
Aspartic acid (34) (0.027 g, 0.066 mmol) was added to 2,3,4,6-Tetra-O-benzyl-β-D-galactopyranosyl isonitrile (33) (0.025 g, 0.046 mmol) in anhydrous CHCl₃ under N₂. The reaction mixture was sealed and heated to 150 °C in a microwave for 45 minutes. Once the reaction was complete the solvent was remove via reduced pressure. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 3:1) afforded 35 as a yellow oil (0.023 g, 52%).

**N≡-(Fluoren-9-ylmethyloxycarbonyl)-N²-(2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl)-N²-formyl-L-asparagine-t-butyl-ester (35)**

Aspartic acid (34) (0.027 g, 0.066 mmol) was added to 2,3,4,6-Tetra-O-benzyl-β-D-galactopyranosyl isonitrile (33) (0.025 g, 0.046 mmol) in anhydrous CHCl₃ under N₂. The reaction mixture was sealed and heated to 150 °C in a microwave for 45 minutes. Once the reaction was complete the solvent was remove via reduced pressure. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 3:1) afforded 35 as a yellow oil (0.023 g, 52%).

**Aspartic acid (34)** (0.027 g, 0.066 mmol) was added to 2,3,4,6-Tetra-O-benzyl-β-D-galactopyranosyl isonitrile (33) (0.025 g, 0.046 mmol) in anhydrous CHCl₃ under N₂. The reaction mixture was sealed and heated to 150 °C in a microwave for 45 minutes. Once the reaction was complete the solvent was remove via reduced pressure. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 3:1) afforded 35 as a yellow oil (0.023 g, 52%).

**N≡-(Fluoren-9-ylmethyloxycarbonyl)-N²-(2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl)-N²-formyl-L-asparagine-t-butyl-ester (35)**

Aspartic acid (34) (0.027 g, 0.066 mmol) was added to 2,3,4,6-Tetra-O-benzyl-β-D-galactopyranosyl isonitrile (33) (0.025 g, 0.046 mmol) in anhydrous CHCl₃ under N₂. The reaction mixture was sealed and heated to 150 °C in a microwave for 45 minutes. Once the reaction was complete the solvent was remove via reduced pressure. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 3:1) afforded 35 as a yellow oil (0.023 g, 52%).

**Aspartic acid (34)** (0.027 g, 0.066 mmol) was added to 2,3,4,6-Tetra-O-benzyl-β-D-galactopyranosyl isonitrile (33) (0.025 g, 0.046 mmol) in anhydrous CHCl₃ under N₂. The reaction mixture was sealed and heated to 150 °C in a microwave for 45 minutes. Once the reaction was complete the solvent was remove via reduced pressure. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 3:1) afforded 35 as a yellow oil (0.023 g, 52%).

**Aspartic acid (34)** (0.027 g, 0.066 mmol) was added to 2,3,4,6-Tetra-O-benzyl-β-D-galactopyranosyl isonitrile (33) (0.025 g, 0.046 mmol) in anhydrous CHCl₃ under N₂. The reaction mixture was sealed and heated to 150 °C in a microwave for 45 minutes. Once the reaction was complete the solvent was remove via reduced pressure. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 3:1) afforded 35 as a yellow oil (0.023 g, 52%).
vigorously until starting material was no longer visible via TLC (petroleum ether/EtOAc 7:3). The reaction was diluted with CH₂Cl₂ (20 mL) and washed with saturated aqueous Na₂S₂O₃ (11 mL), H₂O (11 mL), and brine (11 mL). The organic layer was combined, dried (MgSO₄) and concentrated under reduced pressure. Purification by flash chromatography on silica gel (petroleum ether/EtOAc 3:2) afforded 36 as a white solid (0.038 g, 37%): mp 75-77 °C; [α]²⁵_D -9.6 (c 0.7, CHCl₃); IR (KBr) 3446, 2918, 2850, 1792, 1652 cm⁻¹: ¹H NMR (300 MHz, CDCl₃) δ 6.73 (d, J = 4.9 Hz, 1H), 4.69 (d, J = 5.1 Hz, 1H), 4.01 (s, 1H), 3.80 (d, J = 7.8 Hz, 1H), 2.20 (t, J = 7.8 Hz, 2H), 1.50 (m, 8H), 1.27 (s, 65H), 0.91 (s, 6H), 0.85 (s, 18H), 0.22 (s, 3H), 0.07 (s, 3H), 0.06 (s, 3H) ¹³C NMR (100 MHz, CDCl₃) δ 173.0, 169.5, 80.3, 79.7, 55.4, 51.7, 36.9, 34.8, 32.1, 32.0, 29.9, 29.8, 29.7, 29.7, 29.6, 29.5, 29.4, 26.5, 26.0, 25.9, 25.8, 25.8, 25.6, 22.7, 18.2, 18.0, 14.1, -3.9, -4.1, -4.5, -4.7; HRMS (TOF) calcd for C₆H₁₁₆NO₅Si₂ (M⁺ + H) 938.84. Found 938.8387.

(2S,3R)-3-Benzzyloxy-2-(N-tert-butoxycarbonyl)amino-1-octadecanoic acid (38)

(2S,3R)-2-(N-tert-Butoxycarbonyl)amino-3-benzyloxyoctadecan-1-ol (25) (0.10 g, 0.20 mmol) was dissolved in CH₂Cl₂ (4.4 mL)/H₂O (2.2 mL) at rt under N₂. TEMPO (0.0060 g, 0.041 mmol) followed by BAIB (0.16 g, 0.51 mmol), was added to the reaction flask. The mixture was stirred vigorously until starting material was no longer visible via TLC (petroleum ether/EtOAc 7:3). The reaction was diluted with CH₂Cl₂ (20 mL) and washed with saturated aqueous Na₂S₂O₃ (11 mL), H₂O (11 mL), and brine (11 mL). The organic layer was, dried (MgSO₄), and concentrated under reduced pressure. Purification by flash chromatography on silica gel (CH₂Cl₂/MeOH 99:1) afforded 38 as a light brown oil (0.083 g, 81%): [α]²⁵_D -0.3 (c 1.8, CHCl₃); IR (KBr) 3432, 2924, 2853, 1717, 1652 cm⁻¹: ¹H NMR (300 MHz, CDCl₃) δ 7.22 (m, 5H), 5.15 (s, 1H), 4.62 (d, J = 11.1 Hz, 1H), 4.53 (d, J = 11.1 Hz, 1H), 3.71 (m, 1H), 1.60 (m, 2H), 1.40 (s, 9H), 1.22 (s, 26H), 0.84 (t, J = 6.9 Hz, 3H) ¹³C NMR (100 MHz, CDCl₃) δ 175.3, 155.9,
HRMS (TOF) calcd for C\textsubscript{30}H\textsubscript{52}NO\textsubscript{5} (M\textsuperscript{+} + H) 506.3845. Found 506.3887.

References


