Mimetics of beta-Galactosylceramide with simple ceramide substitutes: Synthesis and binding to gp 120 of HIV-1

and

Enactment of chemistry knowledge by a high school student at a summer program

by

Line A. Augustin

A dissertation submitted to the Graduate Faculty in Chemistry in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

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Abstract

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Advisors: Professor David R. Mootoo, Professor Pamela Mills

This thesis is the account of two research works. The first part reports the synthesis of O- and C- and aza-C- glycosides of beta-Galactosylceramide (GalCer) that contain simple ceramide substitutes, and the initial results of their binding with gp120 of HIV-1. The O-glycosides were prepared via an established procedure. The C- and aza-C-glycosides originated from a central C1- substituted galactal precursor, and their synthesis is illustrative of a potentially general method for pairs of C- and aza-C- β-galactosides. They aza-C-glycoside with a simple C-17 hydrocarbon chain exhibited significant higher affinity than GalCer, whereas the corresponding C-glycoside was as active as GalCer.

The second part describes the ethnographic study of the enactment of the chemistry knowledge of a high school student at a summer program and the influence of a cultural practice, othermothering, on her ability to perform well on her chemistry Regents Exams. Kelly, an 11^{th} grade student exhibited very good understanding of the chemistry curriculum in the classroom, the laboratory period and the tutoring sessions where she plays a caring role for her peers. The same level of understanding was not reflected on the paper pencil exams taken during the summer program.

Acknowledgments

When I was clueless, you had answers; stubborn, you had patience; tearful, you had words of comfort and encouragement. I surely would not have made it without your support.

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The members of my thesis committee and Professor Grohmann. Special appreciation for your efforts, time and invaluable recommendations. I treasure your input.

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My mother, Bernadette Augustin and my family. Thank you for believing in me and for your tremendous care and support for so many years. Mom, thanks for seeds planted in a young mind.

My husband, Lesly. Next to God's grace, you're the best thing that happened to me. Thank you for your patience, your tolerance and your love. I love you very much!

My son, Miracles. This accomplishment is a testimony of God's endless grace for me. "Those who wait on the Lord shall renew their strength; they shall mount up with wings like eagles"

God, my Designer. Thank you for loving me, for writing the story on my life, in spite of my endeavors to write it myself. I thank you!

"For I know the plans I have for you, declares the Lord, plans to prosper you and not to harm you, plans to give you hope and a future."

Jeremiah 29:11

TO MY HUSBAND

LESLY SAINT-HILAIRE

Table of Contents

List of Illustrations and Tables

Symbols and Abbreviations

Ac	acetyl
Ac_2O	acetic anhydride
BMS	Borane Dimethyl Sulfide
Bn	benzyl
Brine	saturated aqueous sodium chloride solution
Br	broad
Bz	benzoyl
0°C	degree Celsius
ca.	approximately
calcd	calculated
^{13}C NMR	carbon-13 nuclear magnetic resonance spectrometry
COSY	correlation spectroscopy
CSA	camphorsulfonic acid
δ	chemical shift in ppm
d	doublet
DIB	iodobenzene diacetate
DCC	dicyclohexylcarbodiimide
DMAP	4-dimethylaminopyridine
DMF	N,N-dimethylformamide
ELISA	Enzyme-linked immunosorbent assays
Et_2O	diethyl ether
EtOAc	ethyl acetate

EtOH	ethanol
Eq	equivalent
FCC	flash column chromatography
FDA	Food and drugs Administration
g	gram
Gal	D-galactose
h	hour
H NMR	proton nuclear magnetic resonance spectrometry
HOAc	acetic acid
HRMS	high-resolution mass spectrometry
HPTLC	High performance thin-layer chromatography
Hz	hertz
IC_{50}	concentration of inhibitor resulting in the reduction of bonding to 50% of Maximum
J	coupling constant in Hertz
L	liter
M	molar
MeOH	methanol
mg	milligram
min	minute
mmol	millimole
MS	molecular sieves
Ph	phenyl

ppm	parts per million
q	quartet
qt	quantitative
rt	room temperature
s	singlet
t	triplet
TBDPS	tert-butyl diphenylsilyl
TIA	1-thio-1,2-O-isopropylidine acetal
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl
TMSOTF	trimethyl trifluoromethanesulfonate

Chapter 1

Introduction

1.1 AIDS - HIV virus

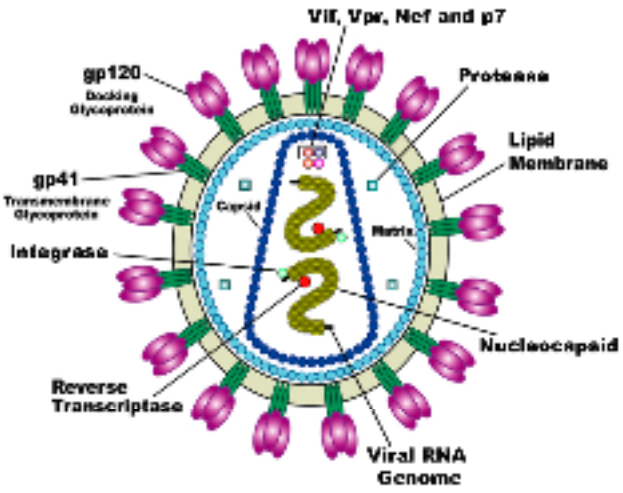
As of May 2006, the global AIDS (Acquired Immune Deficiency Syndrome) epidemic has killed more than 25 million people and new human immunodeficiency virus (HIV) infections have increased to an estimated 65 million people. People living with HIV worldwide are a total of 38.6 million[1]. AIDS is a global epidemic ravaging all people regardless of race, color, age, gender and religion.

HIV is mostly transmitted by infected blood, semen, vaginal fluids, and breast milk. HIV infects T-lymphocytes, thereby compromising the immune response. Infected individuals are unable to defend against infections and certain cancers, and develop opportunistic infections from viruses and bacteria that generally do not sicken the healthy.

The virion, an RNA virus, has a structure with numerous external spikes formed by the two major envelope proteins, the external gp120 and the transmembranous gp 41 (Figure 1.1). The protein gp120 lies on the outer envelope of the HIV virus, helps the virus to attach itself to a host cell, by binding to a receptor on the cell and mediates entry of the virus. The glycoprotein gp120 is anchored to the membrane of the virus along with gp 41, both coming from a cleaved protein, gp160. The structure of gp120 involves an outer domain, an inner domain and a bridging sheet. The protein is covered with a number of glycosylated sites, which are believed to prevent the human immune response from recognizing the virus[2]. Gp120 contains five highly variable regions, designated V1

through V5, the first four of which form loops through intramolecular disulfide bonds. The third variable loop, V3, is a major neutralizing determinant of HIV-1. The mechanism by which the V3 loop influences HIV-1 cell tropism remains unclear. The V3 loop or domain forms the binding site for the gp120 co-receptors and can bind to a variety of anionic compounds, such as sulfated polysaccharides, heparin and suramin[3]. Several studies[4] have confirmed the interactions of the V3 domain of gp120 with cell surface glycosphingolipids such as Galactosylceramide (GalCer) **1** and its sulfatide analog, 3'-sulfogalactosylceramide (SGC) **2**. Fantini et al have reported that masking of the V3 loop with synthetic soluble analogs of GalCer leads to the inhibition of HIV-1 entry in both CD4- and CD4+ cells[4b].

Figure 1.1 Structure of the HIV-1 virus[5]



When the glycoprotein binds to a receptor on the cell, it undergoes a series of structural changes that allows it to interact with co-receptors, which result the entry in the

membrane. To date, two receptors are known to facilitate the entry of the virus in the cell: CD4 receptor with co-receptors CXCR4 and CCR5, and GalCer with co-receptor GPR15/Bob. After initial contact and attachment to a cell, there is a cascade of intracellular events. First, the viral RNA and enzymes are released into the cytoplasm. The RNA undergoes reverse transcription where it is converted into viral DNA. Then the viral DNA enters the host cell nucleus where integrase enables integration of the viral DNA into cellular DNA. Once the viral DNA is integrated into the genetic material of the host cell, it is possible for HIV to exist in a latent state for many years. The next step of the infection process is the transcription of multiple messenger RNA from the viral DNA, which are then translated into viral proteins needed for viral assembly. Among the viral protein is HIV protease, which is required to process HIV proteins into their functional forms. Finally, all components assemble to form the virion, which is released from the cell and is ready to infect other cells. The end product is the production of massive numbers of new viral particles, death of the infected cells and ultimate devastation of the immune system.

The entry and fusion step and the three steps where an enzyme is involved in the process represent crucial steps where the infection can be stopped. Indeed, there are two types of drugs currently used in the treatment of HIV infection, acting on three of the four steps described above. They are entry and fusion inhibitors, reverse transcriptase and protease inhibitors. The enzyme inhibitors are usually used in a combination treatment known as Highly Active Anti-Retroviral Therapy (HAART), which aims to reduce the number of infected particles in the bloodstream (table 1.1).

Table 1.1 Examples of enzymes inhibitors used in HIV treatment

Transcriptase Inhibitors	**Protease Inhibitors**	**HAART combination**
Sustiva	Kaletra	Sustiva + Truvada
Retrovir	Norvir	Kaletra + Combivir
Combivir	Crixivan	Kaletra+Retrovir+ Emtriva

Because the HIV-virus lacks "proofreading enzymes" to correct errors made when it converts its RNA to DNA in reverse transcription, this step has a high error rate and often leaves mutations in the copied DNA. These mutations give variant forms of HIV very rapidly, allowing HIV to evolve quickly with a high genetic variability. This genetic variability produces growing resistance against the anti-retroviral drugs.

The only entry inhibitor that has been approved by the FDA is enfuvirtide, (also known as Fuzeon or T-20), a mimic of gp120[6]. Others drugs in this category are in

Table 1.2 Examples of entry and fusion inhibition drugs in clinical trials

Bind to receptors	**Mimic CD4**	**Mimic gp120**
AMD070 (CXCR4)	BMS-488043	TNX-355
PRO140 (CCR5)	Peptide T	Fuzeon
SCH-D (CCR5)	PRO 542	
UK-427, 857 (CCR5)		

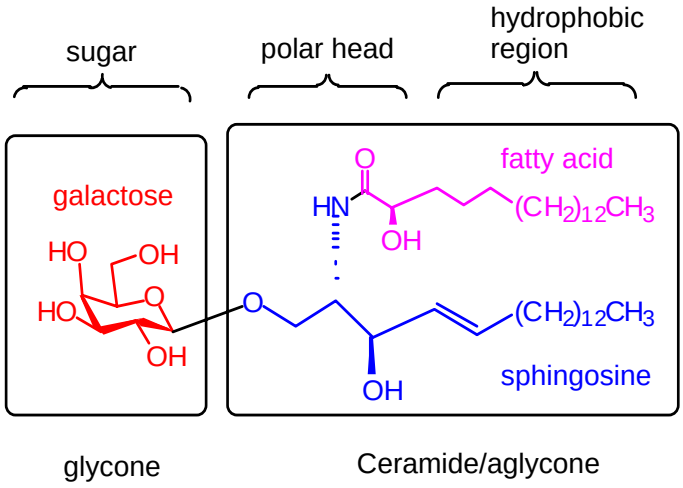
clinical trials[7]. These act as antagonists or agonists of gp120, CD4, CXCR4 or CCR5. (table 1.2).

Although, GalCer has been identified as a receptor of gp 120 and many research groups are involved in the synthesis and binding affinity of its analogs, no drug that targets GalCer and its binding with the virus has yet been developed.

1.2 Galactosylceramide (GalCer)

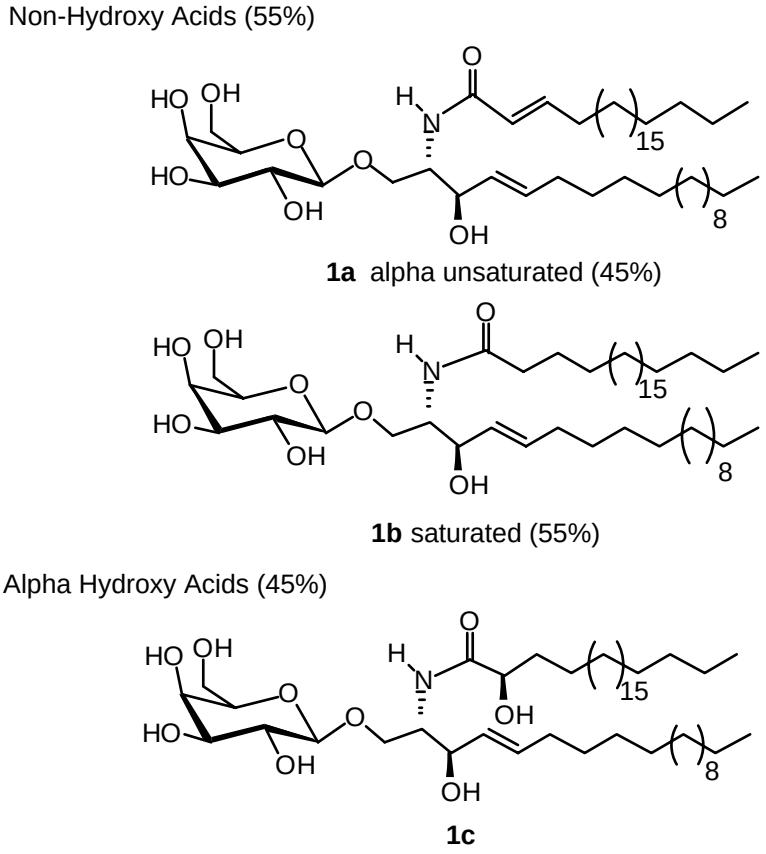
GalCer is the principal glycosphingolipid in brain tissue that was discovered in 1874[8] and was given the name cerebroside because of its three building blocks: a fatty acid, a long chain base and a hexose. Later, its structure was fully described to contain two major components: a sugar component (glycone) identified as galactose[9] and a ceramide (aglycone) composed of the long chain fatty acid and a sphingosine residues (figure 1.2). Its chemical structure was named N-Acylsphingosine-1-β-D-galactopyranoside.

Figure 1.2 Components of GalCer (1)



In the central nervous system, GalCer is a major lipid constituent of myelinating oligendrocytes, consistent with its high content in myelin. It is also present in neurons, particularly in axons. GalCer is also highly expressed in several epithelial tissues such as the mucosal intestinal epithelium, where it may promote the adhesion of various pathogens, including HIV and prions. GalCer is also one of the major glycosphingolipids expressed by intestinal epithelial cells, which are involved in the terminal hydrolysis and

Figure 1.3 Molecular structures and composition of GalCer



uptake of nutrients. It is isolated in three different molecular structures, two of them are structures of the non-hydroxy acid forms, **1a** and **1b**, and represent 55 percent of total extraction and the other structure is the alpha hydroxy acid structure **1c** which counts for

45 percent of total extraction[10a] (figure 1.3). Studies have revealed that the three structures behave differently when interacting with other species. For example, it has been observed that molecular interactions between the glycolipid and certain ligands only happen in structures with the α-hydroxy group and not in structures with a non-hydroxylated acyl chain[10b,10c]. So, depending on the molecular interactions being studied, analogs of one structural form are more suitable than the others.

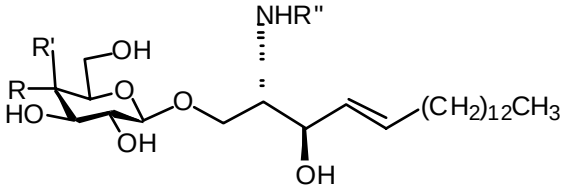
Within the ceramide, the two regions (the polar head and the hydrophobic tail) are believed to be significant in molecular interactions (figure 1.2). Indeed GalCer, like most sphingolipids, organized its molecules into supramolecular complexes in which the polar parts interact with water and is found in the external leaflet of the plasma membrane within lipid rafts[11,12]. This molecular arrangement allows GalCer to transport pathogens to different and respective cellular components and thus to be used as cellular binding sites for a wide variety of pathogens, including viruses, bacteria, fungi and parasites[13] such as, HIV-1, prions, and Borrelia burgdorferi. In HIV-1 attachment to the host cells, gp120 binds to GalCer on the enterocyte cell surface of CD4 negative cells. GalCer-containing lipid rafts allow the migration of HIV-1 virus on the cell surface until reaching a membrane protein called GPR15/Bob, a co-receptor, to which the complex is delivered[14].

Because analogs that demonstrate potent activity against gp120 – GalCer binding may serve as alternate HIV receptors in these CD4 negative cells, several structure-activity investigations with GalCer analogs have been undertaken in order to elucidate the molecular details of GalCer-gp 120 recognition[24,25,16]. Since GalCer is highly insoluble in

aqueous media, some strategies have been developed towards water-soluble analogs, with changes in both components[16,15]. The more systematic structure activity investigations have focused on variations of the sugar and the fatty acid chain. It is generally believed that specificity with respect to the galacto sugar sub-unit is high and that the presence or absence of the fatty acid residue, or changes in its length or level of hydroxylation can be

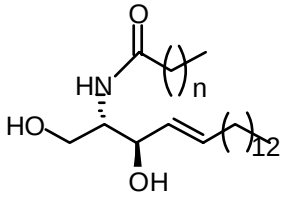
Figure 1.4 Structures activity: specificity of galactose

Harouse et al

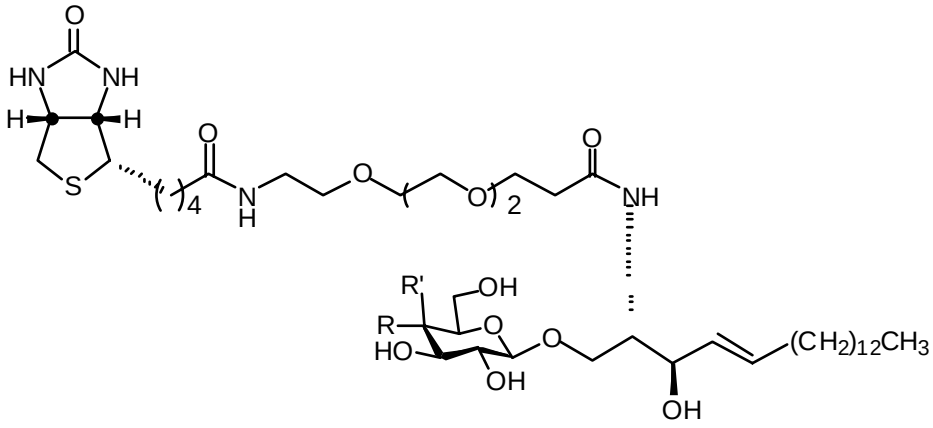


GalCer: R=H, R'=OH, R"=fatty chain
GlcCer: R=OH, R'=H, R"=fatty chain
LacCer: R=Gal, R'=H, R"=fatty chain
Psychosine: R=H, R'=OH, R"=H

Gervay-Hague et al



11 no binding affinity

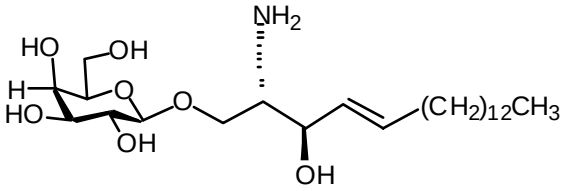


13 R=H, R'=OH, IC_{50} 96μM
14 R=OH, R'=H IC_{50} 18μM
15 R=Gal-β–O, R'=H, IC_{50} 19μM

tolerated[17,18,19]. However, anomalies to these trends have been reported. Harouse et al used HPTLC to determine the binding of several glycolipids to recombinant gp120 (figure 1.4). GalCer, glucosylceramide, lactosylceramide and psychosine were compared and only GalCer and psychosine were bound by gp120. Recent studies with water-soluble GalCer analogs suggest much lower specificity for the sugar[20] such as the Gervay-Hague

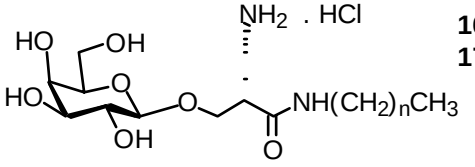
Figure 1.5 Structures activity: changes in the ceramide

Gervay-Hague et al



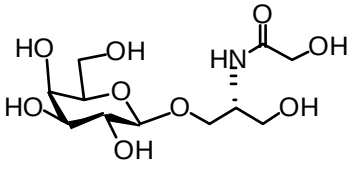
12 binds to rgp 120

Bednarski et al

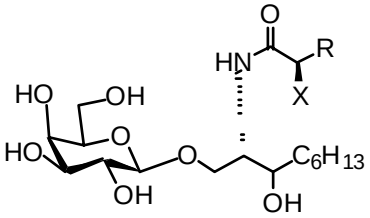


16 (n = 13): 86% inhibition at 2μM, IC_{50} 120μM
17 (n = 3): 34% inhibition at 3μM

Fantini et al



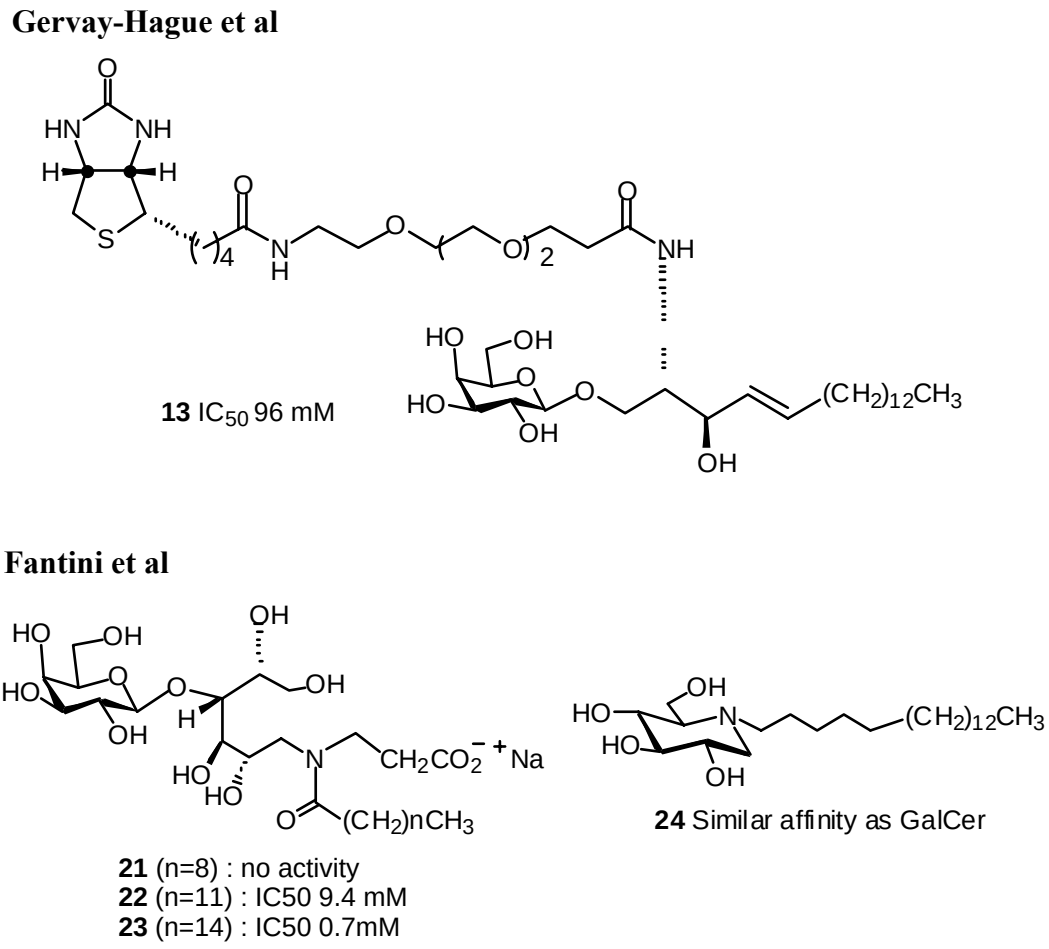
18 no binding affinity to gp120



19 R= C_8H_{17}, X=OH, active
20 R= C_4H_9, X= OH more active

study on a series of analogs with different sugar residues and ceramides (figure 1.4). In some analogs (**13-15**), the fatty acid was replaced with a polar triethyleneglycol-biotin and they were tested in an assay that measured the ability of the soluble ligand to inhibit the binding of gp120 to an immobilized biotionylated GalCer mimic. Different mono-

Figure 1.6 Structures activity: analogs with systematic variations



and di- saccharides showed similar activity as the analog with galactose residue **13**[20].

In another study, Bednarski tested analogs **16** and **17** (figure 1.5) by direct binding to rgp using an ELISA-type assay with HRP-gp120 and immobilized GalCer and obtained binding that was similar to GalCer-gp 120. But when he immobilized GalCer on HPTLC plates, there was a reduction in activity in regard to the length of the hydrocarbon chain[17b]. Analogs with shorter chain like **17** showed less inhibition ability than analogs with longer chain **16**. Other examples of analogs with ceramide substitutes of varying polarity have been reported to show good binding affinity[4b,22].

Studies have also shown a non-linear concentration dependence of gp 120 for GalCer, reinforcing the hypothesis of the involvement of GalCer-rich microdomains[23]. Jacques Fantini has reported monolayer-binding assay studies in which analogs showed different activity with gp 120, depending on the length of their ceramide chains[17]. In his studies analogs like **18** with only the sugar component and the polar head were found to have no binding affinity to gp 120 (figure 1.5). Analog **20** with a shorter fatty acid chain was more active than **19**, which contained a longer chain. However, with analogs **21-23**, with a very different substitute for the polar head region showed a reverse trend with respect to the length of the hydrocarbon chain (figure 1.6). These findings suggest that there may be an optimal hydrophobic surface area for binding. Furthermore, binding studies of N-Stearyl-1-deoxynojirimycin **24** and related derivatives with simple stearyl or stearoyl chains as ceramide substitutes with gp 120 reduced the yield of HIV infection by five orders of magnitude, showing comparable affinity to that of GalCer[15a]. The effects of variations in the sphingosine residue have not been studied as systematically.

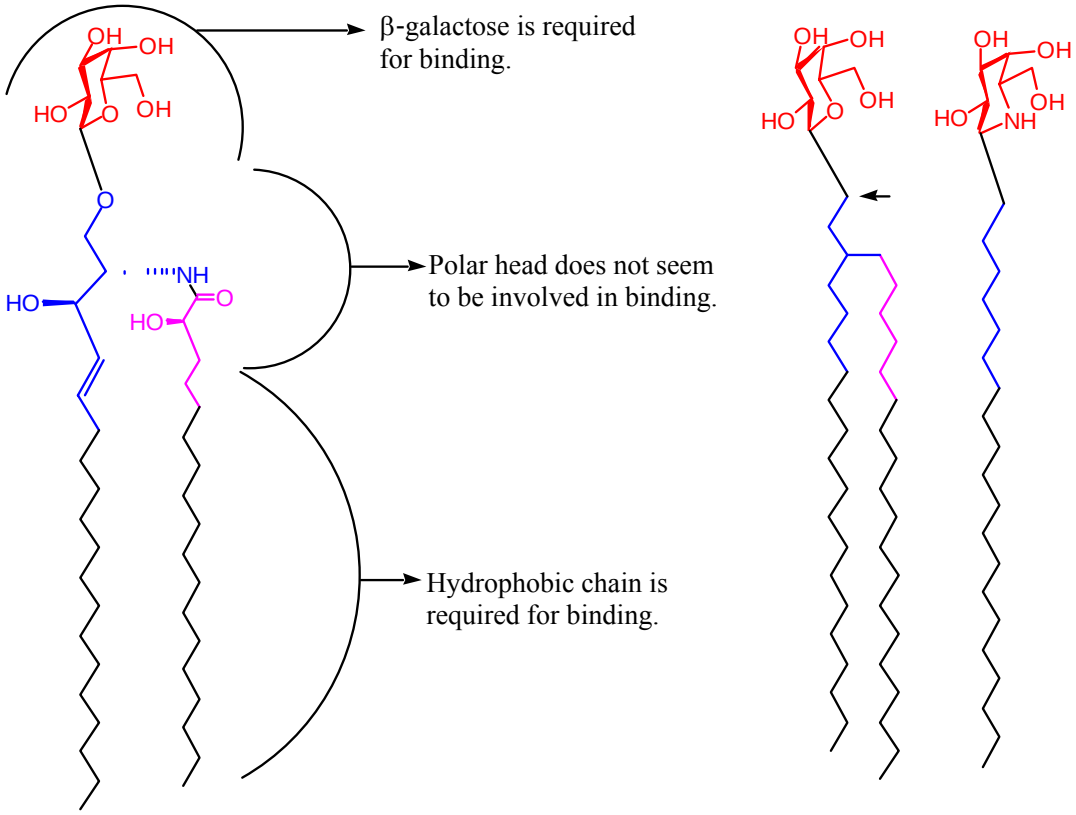
The molecular interactions of GalCer and gp 120 are still unclear. Understanding

such mechanism is crucial to develop fusion and entry inhibitors. Thus GalCer analogs remain of great interest, not only as potential new entry inhibitors, but also as mechanistic probes for the understanding of glycolipids-protein interactions.

1.3 Research goals

One of the research goals of our group is the elucidation of the molecular basis of the recognition process of the glycoprotein gp120 and the sphingolipid GalCer in order to develop potent inhibitors of gp120-GalCer binding which will prevent entry of the virus in the cell thus preventing infection and destruction of the immune system. Understanding of such molecular interactions should also bring clarification of protein-

Figure 1.7 Implications from previous studies

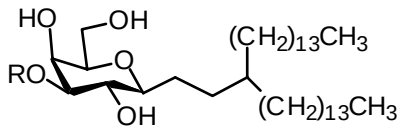


carbohydrate recognition involved in other disease mechanisms. Specifically, the aim of this dissertation is the synthesis of a series of GalCer analogs for biological testing and conformational studies, which should bring better understanding of the molecular recognition process and/or way to design other analogs for more efficient binding.

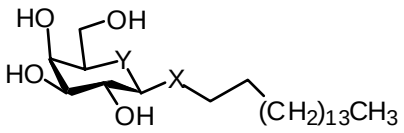
From previous studies on structures activity of GalCer analogs, we have three major trends. First, it has been observed than galactose and the hydrophobis region are required for binding. Second, modifications of the polar head from simple chains to highly hydroxylated structures are tolerated and did not prevent binding by rgp120. These results suggest that the polar head is not directly involved in the binding of gp 120 but may or may serve mainly as a scaffold to maintain an optimal orientation of the galactose residue relative to the hydrophobic region (figure 1.7). We targeted O- C- and aza-C-galactoside analogs with simple hydrocarbon residues as ceramide substitutes. These compounds are expected to have different conformational properties with respect to their glycosidic or pseudoglycosidic torsions, and based on the foregoing discussion, were expected to have bind gp 120 differently

Our laboratory has been involved in new methods for synthesis of C-glycosides.[26,27,28]. C-glycosides are appealing with regards to drug development because of their greater hydrolytic stability compared to O-glycosides[29,30]. However, the effect that replacement of the glycosidic oxygen with a methylene has on activity is less predictable. Indeed, examples of O-/C- glycoside partners of similar or opposing activities, depending on the biological system have been reported[31]. The synthesis of the series of C-glycosides, O-glycosides and aza-C-glycosides shown in figure 1.8, and the determination of their gp-120 binding[32] is the subject of this thesis.

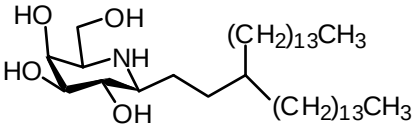
Figure 1.8 Targeted analogs



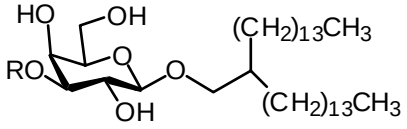
3 R=H
4 R=SO_3Na



5 Y=O, X=O
6 Y=O, X=CH_2
7 Y= NH, X=CH_2



8



9 R=H
10 R=SO_3Na

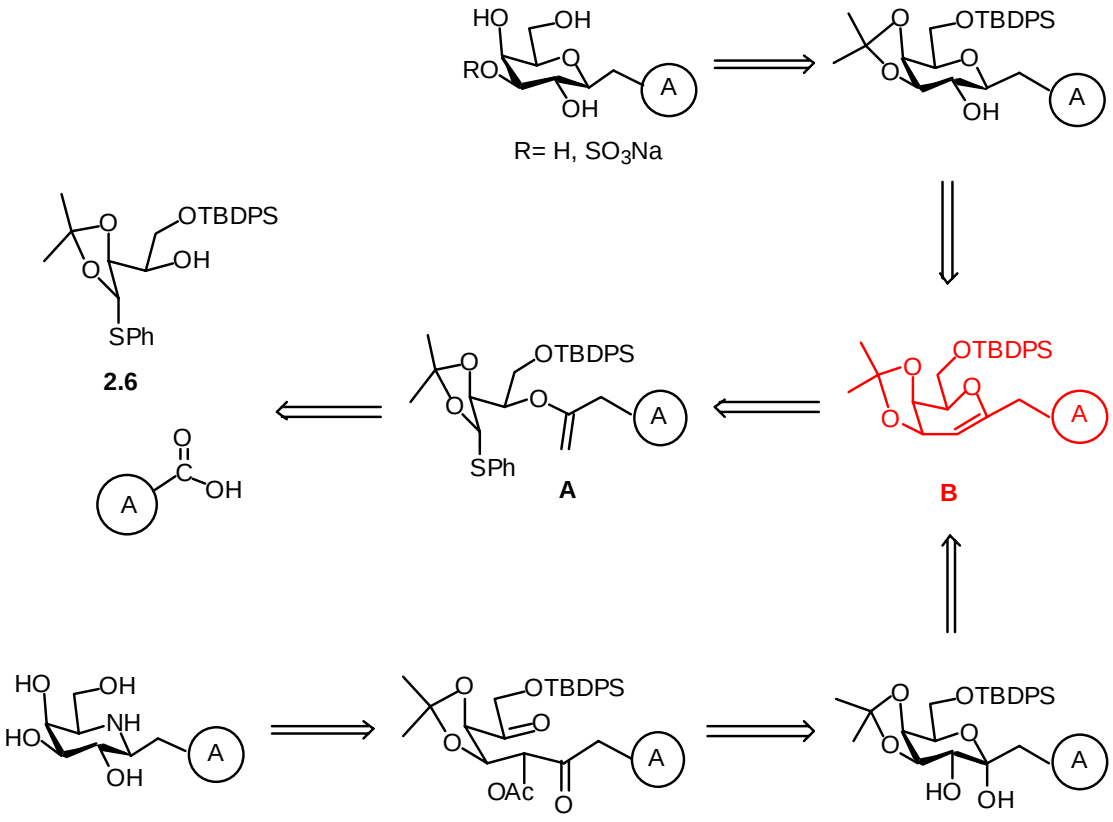
1.4 Methodology

The synthetic strategy follows previous work from our group[33] where a central C1-substituted galactal served as precursor to C- and aza-C- galactosides. The required galactal precursor **B** can be obtained from the oxocarbenium ion cyclization of an enol ether-thioacetal **A.** The enol ether will be produced by the Tebbe olefination of the ester which will be obtained from the coupling of 1-thio-1, 2-isopropylidene acetal, TIA **2.6**, the glycone component, and an acidic aglycone partner A-COOH. Stereoselective hydroboration of the galactal should afford the β-C-galactoside, which can be elaborated to the final targets (scheme 1.1).

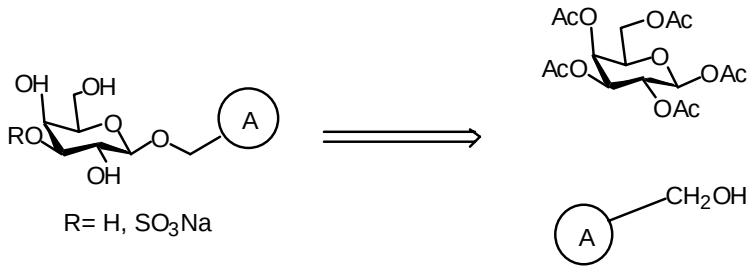
The aza analogs can also be obtained from glycal **B**. Dihydroxylation of the glycal is expected to provide the corresponding lactol with complete α-selectivity**.** Selective

Scheme 1.1 Retrosynthesis of targeted analogs

C- and Aza C- glycosides



O-glycosides



acetylation of the diol followed by oxidation will lead to the diketone, which will be subjected to a stereoselective double reductive amination to give desired β-aza-*C*-glycosides**.** Subsequent deprotection will provide the targeted β-aza-*C*-galatosides**.**

The synthesis of the O-analogs will be done using well-known glycosylation protocol. It will start with the coupling of the corresponding alcohol and the commercially available β-D-galactosepentaacetate followed by deprotection-protection manipulations to the desired products.

In Chapter 2, the synthesis of C-analogs is described followed by the synthesis of aza-analogs in chapter 3. The synthesis of the known O-analogs is repeated and reported in chapter 4. A summary of the synthesized targets and biological findings in two different assays are presented in Chapter 5.

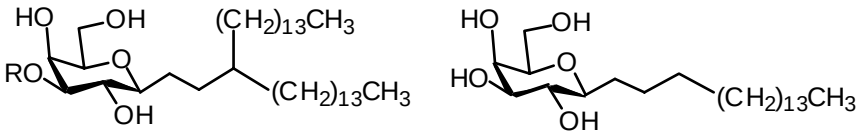
Chapter 2

Synthesis of the C-glycosides

2.1 Introduction

We started with the synthesis of the C-glycosides analogs in which the ceramide has been replaced either with a simple alkyl chain or a fatty chain, one of them being the sulfatide analog, SGC (Figure 2.1).

Figure 2.1 C-glycoside analogs



3 R=H
4 R=SO_3Na

6

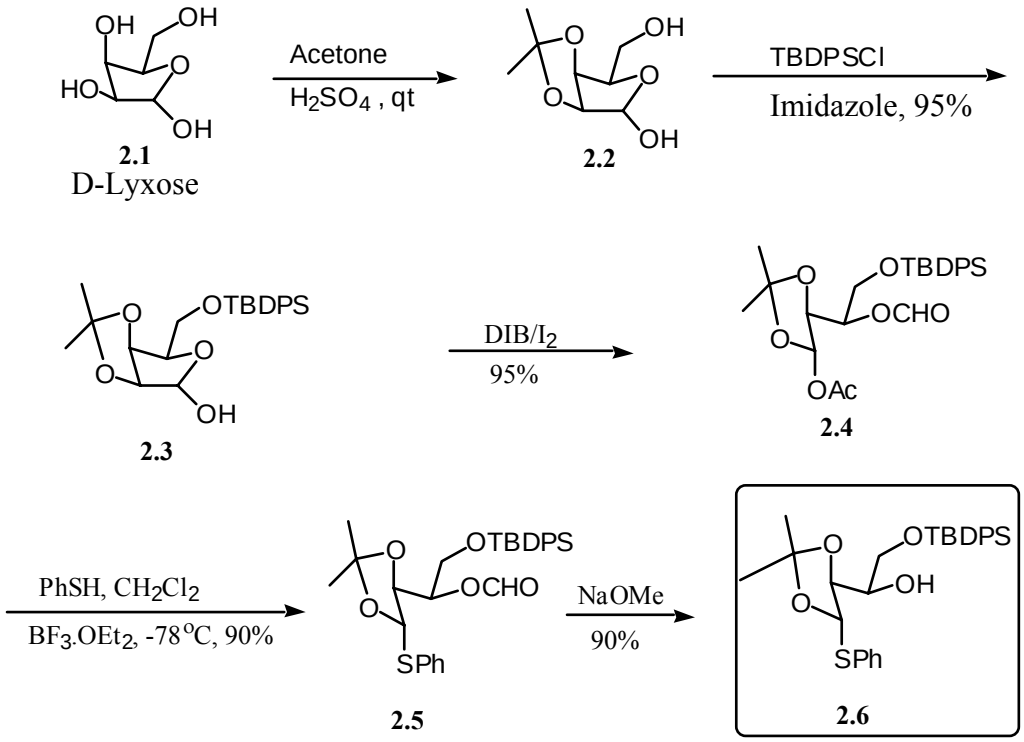
As described in chapter 1, the plan for the synthesis of **3**, **4** and **6** will follow previous work from our group on 1-thio-1, 2-isopropylidene acetal, TIA **2.6**. Esterification of TIA glycone component and an acidic aglycone partner **2.17** will give the ester. Tebbe olefination of the ester followed by thioacetal activation in enol ether will provide the C1-subtituted galactal **2.21**. Hydroboration-oxidation of **2.21** will afford the Galβ-C-saccharide, which will be elaborated to the desired **3** and **4** analogs.

2.2 Synthesis

2.2.1 Synthesis of the glycone component

The 1-thio-1, 2-isopropylidene acetal **2.6** was synthesized from D-lyxose, according to the procedure developed in our laboratory. D-lyxose was reacted with acetone in the presence of H_2SO_4 to obtain the acetonide **2.2**, which was converted to the silyl ether **2.3** using t-butyldiphenylsilylchloride in 90% yield. The reaction of **2.3** with (diacetoxy-iodo)benzene (DIB)/I_2 according to the Suarez[34] procedure, led to the acetate **2.4** in 95% yield. Treatment of **2.4** with thiophenol and boron trifluoride etherate at low temperature, followed by basic hydrolysis gave **2.6** in an overall yield of 81% (Scheme 2.1).

Scheme 2.1 Synthesis of Glycone component



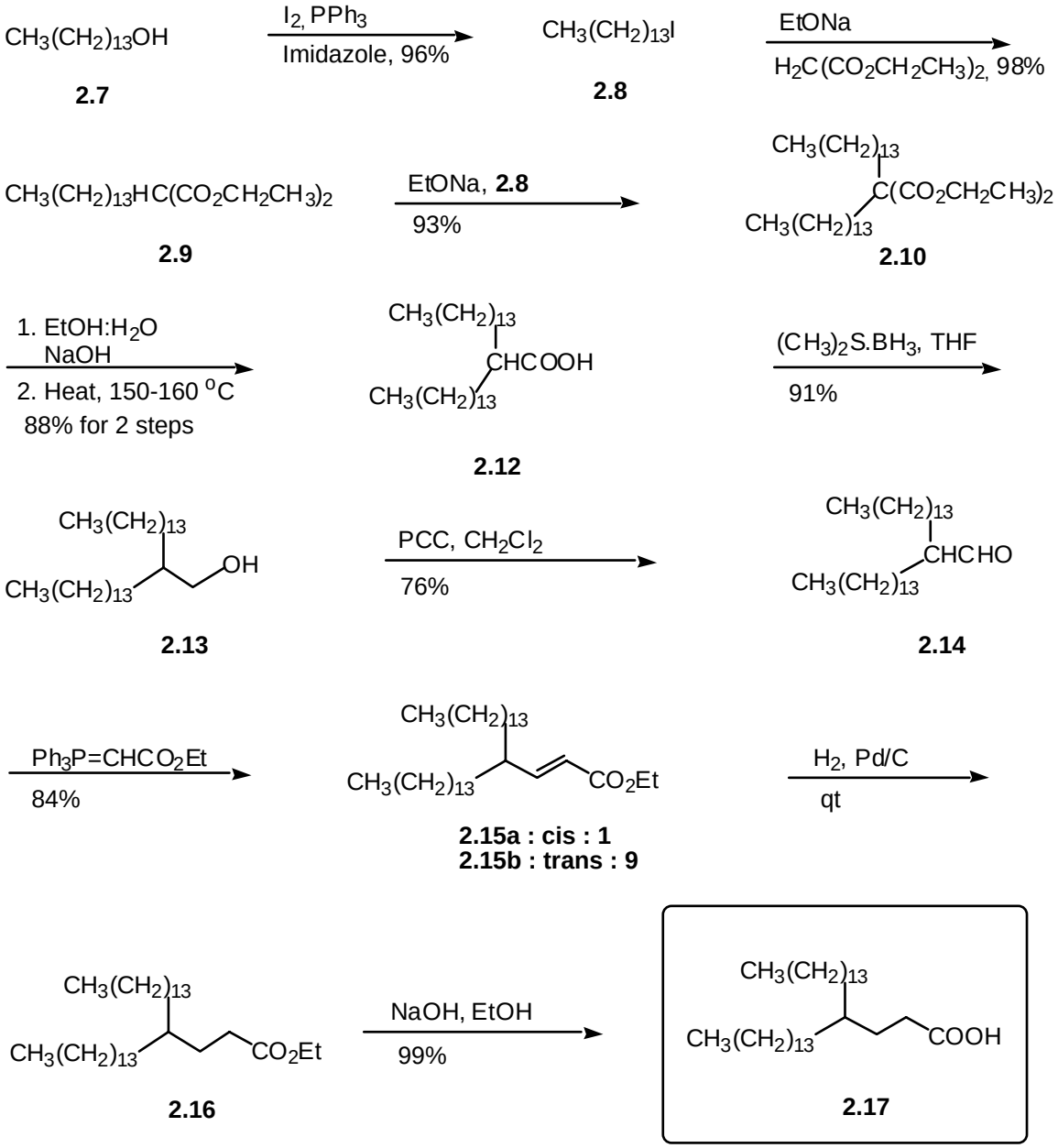
2.2.2 Synthesis of the aglycone component

The synthesis of the aglycone partner, 4-(tretradecyl)hexadecanoic acid **2.17**, started with the commercially available tetradecanol **2.7**. Iodination of the alcohol gave the iodide **2.8** in 96% yield. Double alkylation under basic conditions with dimethyl malonate gave the diethyl di-n-malonate **2.10**[35] in 92% yield. Hydrolysis with sodium hydroxide followed by decarboxylation produced the acid **1.12**. The alcohol **2.13** was obtained by reduction of **2.12** with Borane-Methyl Sulfide complex in THF in 91% yield. Oxidation of **2.13** with PCC, followed by Wittig reaction with methyl(triphenylphosphoranylidene)acetate and the aldehyde **2.14** yielded a mixture of unsaturated esters **2.15a** and **2.15b** in 84% yield. The diastereomers were found to be in a 1:9, cis:trans ratio. The known alcohol **2.13** will also be used as glycosyl donor in the glycosylation step of the synthesis of the O-glycoside analogs. The mixture of **2.15** was hydrogenated to give **2.16** in 89% yield. Hydrolysis of **2.16** with sodium hydroxide gave the aglycone **2.17** in an overall of 57% yield from the known alcohol **2.13** (Scheme 2.2).

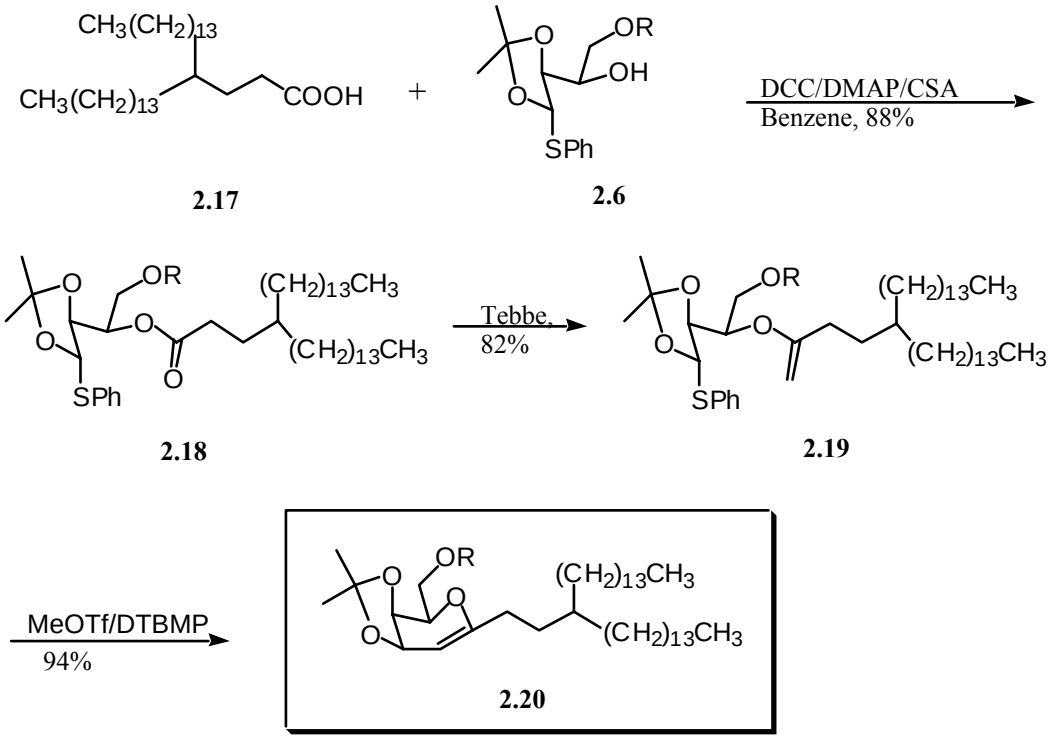
2.2.3 Synthesis of the C-glycosides

The synthesis of the C-glycosides started with the preparation of the glycal precursor. The glycone component **2.6** and the aglycone **2.17** were converted to ester **2.18** using dicyclohexylcarbodiimide and 4-dimethylaminopyridine in benzene in 88%

Scheme 2.2 Synthesis of Aglycone component



Scheme 2.3 Synthesis of the glycal precursor 2.20



2.17 **2.6** **2.18** **2.19** **2.20**

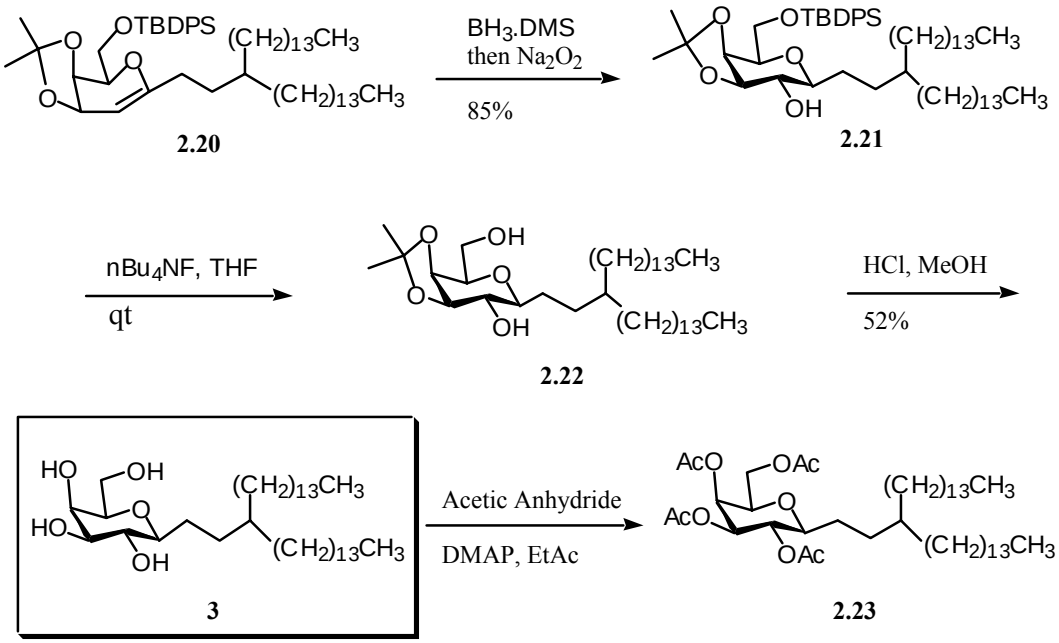
yield. The Tebbe methylenation of the ester **2.18** gave the enol ether **2.19** in 82% yield, which was treated with MeOTf/DTBMP/CH_2Cl_2 to give glycal **2.20** in 94% yield (Scheme 2.3).

Hydroboration-oxidation of **2.20** with BH_3·DMS then H_2O_2/NaOH yielded 85% of a single C-glycoside product **2.21** (scheme 2.4). Compound **2.21** was transformed to the desired C-glycoside **3** through the straightforward removal of the acetonide-protecting group with HCl in 52%. The stereochemistry of **3** was confirmed by HNMR analysis of derived tetraacetate **2.23** obtained from its acetylation with acetic anhydride in quantitative yield.

The coupling constants ($J_{1,2}$ = 9.5, $J_{2,3}$ = 9.5, $J_{3,4}$, $J_{4,5}$ < 3.0 Hz) were in agreement with the β-galactoside stereochemistry[36].

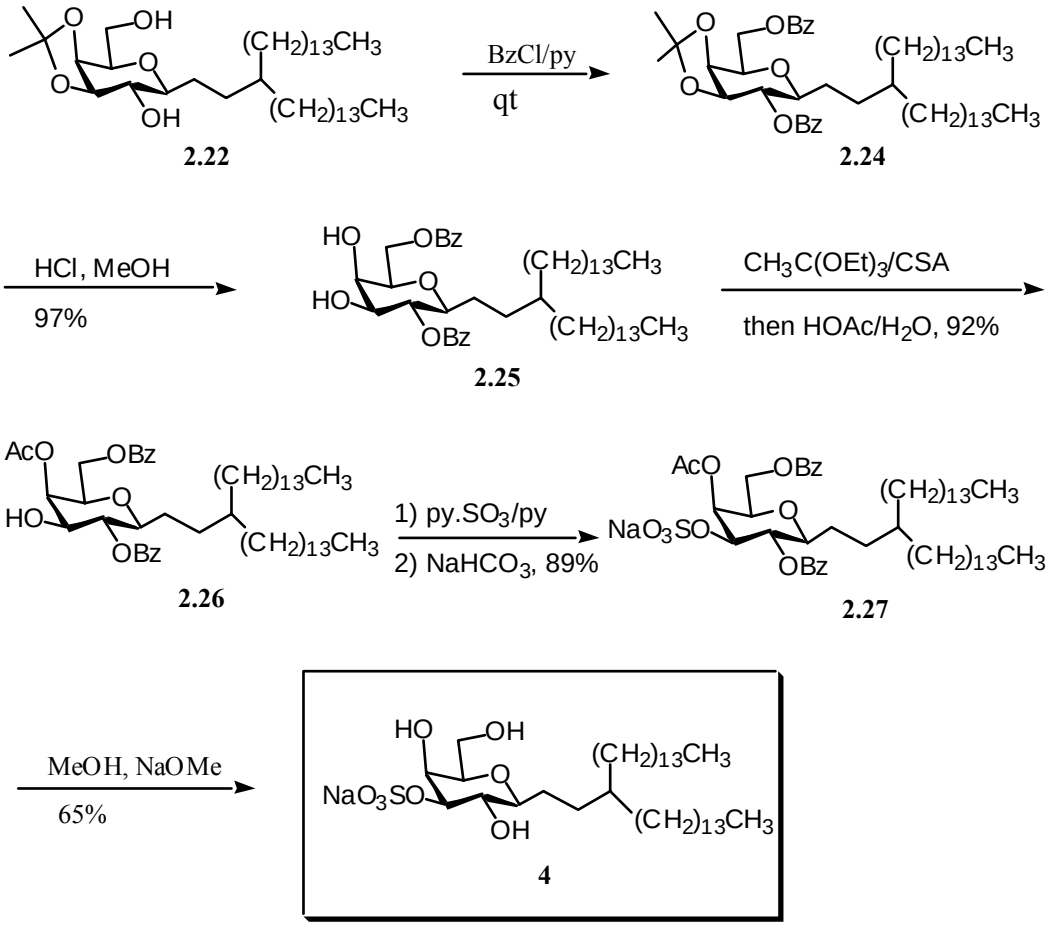
The synthesis of analog **4** continued with the desilyation of C-glycoside **2.21** with nBu_4NF in quantitative yield (scheme 2.5). The diol **2.22** was protected with benzoyl chloride in pyridine to give dibenzoate **2.24** in quantitative yield. Hydrolysis of the acetonide **2.24** under acidic conditions afforded **2.25** in yield. Selective acylation of the axial alcohol of **2.25** via regioselective cleavage of the orthoester with $CH_3C(OEt)_3$/CSA then HOAc/H_2O gave **2.26** in 92% yield. Sulfonation of the 3-OH according to the

Scheme 2.4 Synthesis of analog 3



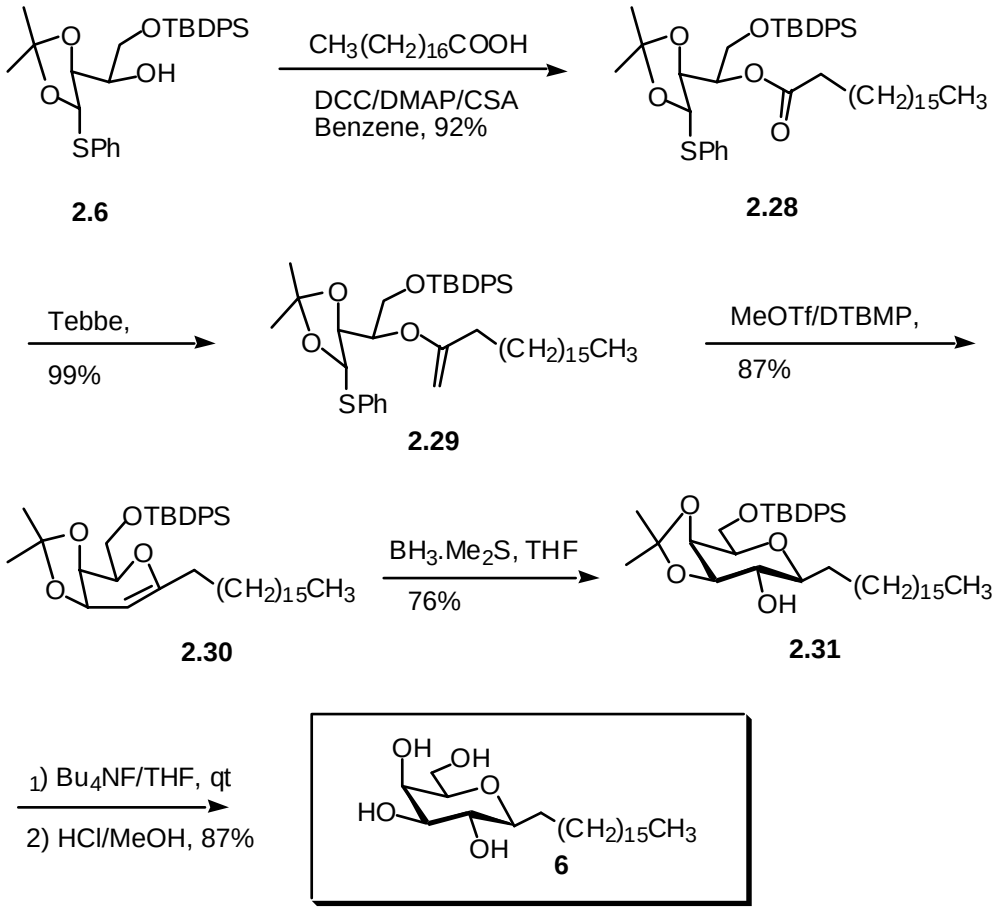
standard procedure py.SO_3/py yielded the desired product **2.27** in 89% yield. Basic hydrolysis-deprotection afforded the final product **4** in 65% yield.

Scheme 2.5 Synthesis of sulfatide 4



Analog **6** was previously prepared in this laboratory following the protocol that was described for **3,** using stearic acid as the acidic aglycone component (scheme 2.6). The synthesis produced glycal **2.30**, which was converted to C-glycoside **2.31** Deprotection of the silyl group and the acetonide provided the analog with the simple chain.

Scheme 2.6 Synthesis of C-glycoside 6



2.3 Summary

The synthesis of **3** and **4**, C-glycoside analogs of Galactosylceramide (GalCer) and 3'sulfogalactosylceramide (SGC) respectively, started from two commercially available materials, D-lyxose and tetradecanol. The 1-thio-1, 2-isopropylidene acetal, TIA **2.6**, obtained in five steps from D-lyxose, was the glycone component. The aglycone

2.17 partner was obtained in nine steps from tetradecanol in 43% yield. Following the coupling of TIA **2.6** and 4-Tetradecyloctadecanoic acid **2.17**, we obtained the key precursor, the glycal **2.20** in 68% yield from the esterification. Analog **3** was obtained in three steps from the glycal precursor in 44% yield and analog **4** was obtained seven steps from the glycal in 43% yield. Steric acid was used as aglycone to obtain analog **6** in 66% yield from the corresponding galactal **2.28**.

2.4 Experimental

General Synthesis

Unless otherwise stated, all reactions were carried out under a nitrogen atmosphere in oven-dried glassware using standard syringe and septa technique. Solvents were purified by standard procedures or used commercial sources as appropriate. Petroleum ether refers to the fraction of petroleum ether boiling between 40 and 60°C. Ether refers to diethyl ether. Unless otherwise stated thin-layer chromatography (TLC) was done on 0.25 mm thick precoated Silica Gel (HF-254, Whatman) aluminum sheets and flash column chromatography (FCC) was performed using Silica Gel 60 (32-63 mesh, Scientific Adsorbents). Elution for FCC usually employed a stepwise solvent polarity gradient, correlated with TLC mobility. The chromatograms were observed under UV (short and long wavelength) light and/or were visualized by heating plates that were dipped in ammonium molybdate solution. The spots were visualized by UV or charring with a solution of ammonium molybdate (VI) tetrahydrate (12.5g) and cerium (IV) sulfate tetrahydrate (5.0 g) in 10% aqueous sulfuric acid (500 mL). Optical rotations

($[\alpha]_D$) were recorded using a Rudolph Autopol III polarimeter at 589 nm (sodium D-line). NMR spectra were recorded using GE QE 300, JEOL 400 or Bruker Ultra Shield instruments. Unless otherwise stated spectra were recorded in $CDCl_3$ with residual $CHCl_3$ as the internal standard (δ_H 7.26, δ_C 77.0). Chemical shifts are quoted in ppm relative to tetramethylsilane (δ_H 0.00) and coupling constants (*J*) are given in Hertz. High-resolution mass spectrometry (HRMS) was performed on Micromass 70-SE-4F or Micromass Q-Tof ultima instruments at the Mass Spectrometry Laboratory of the University of Illinois, Urbana-Champaign.

1-Thio-1,2-O-isopropylidene Acetal 2.6

A solution of 2,3-*O*-isopropylidene-D-lyxofuranose **2.2** (2.7 g, 14 mmol), TBDPSCl (3.7 mL, 14 mmol), and imidazole (1.9 g, 28 mmol) in anhydrous DMF (25 mL) was stirred at 50°C for 1.5 h. The reaction mixture was then diluted with water and extracted with ether. The combined organic phase was washed with brine, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by FCC to give **2.3** (5.7 g, 95%): colorless oil; R_f = 0.20 (10% EtOAc/petroleum ether); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.06 (s, 9H), 1.26, 1.35 (both s, 3H ea), 3.94 (m, 2H), 4.33 (m, 1H), 4.58 (d, *J* = 7.0 Hz, 1H), 4.75 (m, 1H), 5.35 (s, 1H), 7.38, 7.70 (both m, 10H).

A solution of **2.3** (9.5 g, 21.6 mmol) in anhydrous cyclohexane (120 mL) containing diacetoxyiodobenzene (8.54 g, 26.5 mmol) and iodine (5.89 g, 23.2 mmol) was stirred under an atmosphere of argon at room temperature for 3 h. The reaction mixture was then diluted with water and extracted with ether. The organic phase was washed with aqueous $Na_2S_2O_3$ and brine, then dried (Na_2SO_4), filtered, and evaporated

under reduced pressure. The residue was purified by FCC to give **2.4** (10.0 g, 95%): clear oil; R_f = 0.45 (10% EtOAc/petroleum ether); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.05 (s, 9H), 1.46, 1.505 (both s, 3H ea), 2.10 (s, 3H), 3.82 (m, 2H), 4.51 (m, 1H), 4.58 (d, *J* = 7.0 Hz, 1H), 5.25 (m, 1H), 6.21 (d, *J* = 1.5 Hz, 1H), 7.40, 7.60 (both m, 10H), 8.05 (s, 1H); ESMS 509 (M + Na).

$BF_3{\cdot}OEt_2$ (1.3 mL, 12.4 mmol) was slowly added to a solution of **2.4** (5 g, 10.3 mmol) and thiophenol (2.12 mL, 20.6 mmol) in anhydrous CH_2Cl_2 (50 mL) at -78 C under an atmosphere of argon. The reaction mixture was warmed to -40°C and stirred at this temperature for 1 h or until TLC indicated complete disappearance of the starting material. Triethylamine (5 mL) was then added, and the reaction mixture was diluted with saturated aqueous $NaHCO_3$ and extracted with ether. The organic phase was washed with brine, then dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The crude material was dissolved in methanolic ammonia (100 mL) and stirred at room temperature for 30 min. Most of the solvent was removed under reduced pressure, and the residue was diluted with water and extracted with ether. The organic phase was washed with brine, then dried (Na_2SO_4), filtered, and evaporated under reduced pressure. FCC of the residue provided **2.6** (4.7 g, 90%): colorless oil; R_f = 0.50 (10% EtOAc/petroleum ether); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.07 (s, 9H), 1.47, 1.49 (both s, 6H), 2.32 (br s, 1H, D_2O ex), 3.80 (m, 3H), 4.18 (dd, *J* = 2.0, 7.0 Hz, 1H), 5.44 (d, *J* = 7.0 Hz), 7.20-7.80 (m, 15H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.5, 26.3, 27.1, 27.5, 65.3, 70.1, 80.4, 85.4, 111.5, 127.6, 127.9, 129.1, 129.9, 132.0, 133.3, 134.0, 135.7; ESMS 531 (M + Na). FABHRMS calcd for $C_{23}H_{31}O_4Si$ (M - SC_6H_5) 399.1992, found 399.1992.

Tetradecyl Iodide 2.8

Tetradecanol **2.2** (50g, 0.23 mol), Imidazole (16.1g, 0.23 mol), Triphenylphosphine (61.75g, 0.23 mol) were azeotroped with toluene (1200 mL). The mixture was cooled to rt and iodine (59.8g ,0.23 mol) was added. The reaction mixture was heated at reflux for 2 h, filtered through celite and washed with ether. FCC of the crude gave 72.9 g (96%) of **2.8 :** colorless syrup; R_f = 0.60 (2% ethyl acetate:petroleum ether); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.9 Hz, 3H), 1.26 (s, 22H), 1.82 (qt, *J* = 6.2 Hz, 2H), 3.17 (t, *J* = 6.9 Hz, 2H) . ^{13}C NMR (75 MHz, $CDCl_3$) δ 6.8,14.1, 22.8, 28.5, 29.4, 29.7, 30.4, 31.9, 33.6, 36.1.

Mono-alkyl-malonate 2.9

To a solution of sodium ethoxide (2.84g of sodium in 144 mL of ethanol) was added dimethyl malonate (18.7 mL, 0.12 mol). The mixture was heated at reflux at 70° C for 45 min and **2.8** were added dropwise. The reaction mixture was heated at reflux for 3 h. The cool mixture was then diluted with diethyl ether, washed with water and saturated NaCl. The organic phase was dried (Na_2SO_4), filtered and evaporated under reduced pressure. The residue was purified by FCC gave **2.9** (38.65 g, 98 %): Colorless oil; R_f = 0.60 (10% ethyl acetate:petroleum ether); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.9 Hz, 3H), 1.25(s, 22H), 1.87(q, *J* = 7.3 Hz, 2H), 3.30 (t, *J* = 7.69 Hz, 1H), 4.20 (q, *J* = 6.9 Hz, 4H).

Di-alkyl-malonate 2.10

To a solution of sodium ethoxide (2.45g of sodium in 140 mL of ethanol) was added **2.9** (38g, 0.11 mol). The mixture was heated at reflux at 70° C for 45 min and **2.8** (31.11g, 0.10 mol) was added dropwise. The reaction mixture was heated at refluxed for 3 h. The cool mixture was diluted with diethyl ether, washed with water and saturated NaCl. The organic phase was dried (Na_2SO_4), filtered and evaporated under reduced pressure. The residue was purified by FCC to give **2.10** (54.75 g, 93 %): Colorless oil; R_f = 0.60 (10% ethyl acetate:petroleum ether); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (t, *J* = 6.9 Hz 6H), 1.24 (s, 30H), 1.81 (m, 4H), 4.15(q, *J* = 6.9 Hz, 4H) . ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.1 , 23.6 , 25.2 , 31.3 , 33.6 , 58.4 , 62.2 , 173.2.

2-(Tetradecyl)hexanoic acid 2.12

To a solution of **2.10** (37.36g, 67.6 mmol) in 140 mL of ethanol, was added 140 mL of 6M sodium hydroxide. The mixture was refluxed overnight. After completion, the reaction mixture was neutralized with conc HCl, diluted with ether, washed with water, dried (Na_2SO_4), filtered and evaporated under reduced pressure to yield the crude **2.11**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98 (t, 6H, J = 6.96 Hz), 1.34 (m, 48H), 1.88 (m, 4H).The crude **2.11** (24.77g) was heated at 180˚C overnight. Recrystalization of the residue from ethyl acetate provided **2.12** (19.74 g, 88 %) as a white powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.96 Hz, 6H), 1.25 (s, 48H), 1.48 (m, 2H), 1.60 (m, 2H), 2.34 (m, 1H). $C_{30}H_{61}O_2$ HRMS calcd 453.4672, found 453.4672.

2-(Tetradecyl)hexadecyl-1-ol 2.13

To a solution of **2.12** (14.15g, 31.3 mmol) in dry THF (90 mL), cooled to 0 °C, was added borane-methyl sulfide complex (3.45 mL, 36 mmol) dropwise and the mixture was stirred for 1.5 h at room temperature. After completion of the reaction, the mixture was cooled again to 0 °C and dry methanol was carefully added. The solvent was removed by distillation at atmospheric pressure. The residue was washed with methanol and concentrated. FCC of the residue gave **2.13** (12.45g, 91 %): White powder; R_f = 0.41 (5% ethyl acetate:petroleum ether); ^{1} H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.6 Hz, 6H), 1.26 (s, 52H), 3,53 (d, *J* = 5.5 Hz, 2H) . ^{13} C NMR (75 MHz, $CDCl_3$) δ 14.7, 23.3, 27.7, 30.0, 30.4, 30.8, 31.8, 32.6, 41.4, 66.5. ESMS 473.5 (M+Cl).

2-Tetradecylhexadecanal 2.14

A powdered mixture of MS-4A sieves (10 g), Celite (8.54 g), florisil (0.85 g), sodium acetate (3.21 g, 39.2 mmol) and PCC (8.44 g, 39.2 mmol), in dichloromethane (200 mL) was stirred at rt for 0.5 h. A solution of 2-tetradecylhexadecan-1-ol **2.13** (3.43 g, 7.83 mmol) in dichloromethane (100 mL) was then introduced. The reaction mixture was stirred at rt for 4 h, then diluted with ether and filtered though Celite. The filtrate was concentrated under reduced pressure. FCC of the residue gave **2.14** (2.59 g, 76%): white powder; R_f = 0.88 (5% ethyl acetate:petroleum ether); ^{1}HNMR (300 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.6 Hz, 6H), 1.26 (bs, 48H), 1.44 (m, 2H), 1.57 (m, 2H), 2.21 (m, 1H), 9.54 (d, *J* = 2.9 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.1, 23.5, 27.9, 28.2, 29.8, 30.1, 30.2, 30.4, 32.7, 33.0, 52.8, 206.0. ESMS 487.4 (M+Cl).

Unsaturated ester 2.15

A solution of **2.14** (1.05g, 2.4 mmol) and dry acetonitrile (25 mL) was heated to 65 ˚C, then methyl(triphenylphosphoranylidene)-acetate (1.12g, 3.4 mol) was added. The reaction mixture was stirred for 3 h. The solvent was evaporated under reduced pressure. FCC provided cis/trans **2.15** (0.99g, 84%, cis/trans: 1/9) as a yellowish viscous liquid. R_f = 0.60 (10% ethyl acetate:petroleum ether);^{1} H NMR (300 MHz, $CDCl_3$) *Cis* isomer: δ 0.88 (t, J = 6.9 Hz, 6H), 1.25 (s, 52H), 3.69 (s, 3H, cis), 5.75 (d, *J* = 11.7 Hz, 1H), 5.89 (t, *J* = 10.6 Hz, 1H) *Trans* isomer: δ 0.88 (t, *J* = 6.9 Hz, 6H), 1.25 (s, 52H), 3.72 (s, 3H), 5.76 (d, *J* = 15.7 Hz, 1H), 6.74 (dd, *J* = 9.5 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.3, 22.9, 27.5, 29.6, 29.8, 29.9, 30.0, 32.2, 34.7, 42.9, 43.0, 51.4, 120.6, 154.1, 167.0.

Ester 2.16

To a solution of **2.15** (9.30g, 18.9 mmol) in ethyl acetate (180 mL) under nitrogen, was added palladium on carbon (1.86g, 10% of **2.15**). The mixture was stirred under balloon for 2 h, then filtered through celite. The filtrate was concentrated under reduced pressure to give **2.16** (9.34g, 89%) as a white powder; R_f = 0.52 (5% ethyl acetate:petroleum ether); ^{1} H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.9 Hz, 6H), 1.26 (s, 52H), (1.60 (m, 2H), 2.28 (t, *J* = 8.4 Hz, 2H), 3.66 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.3, 22.9, 26.8, 28.9, 29.6, 29.9, 30.3, 31.7, 32.2, 33.6, 37.3, 51.4, 174.3.

4-Tetradecyloctadecanoic acid 2.17

To a solution of **2.16** (8.33 g, 16.8 mmol) in ethanol (35 mL) was added 6M aqueous NaOH (35 mL). The reaction mixture was stirred at rt for 2 h, then neutralized with concentrated HCl and extracted with ether. The organic extract was dried (Na_2SO_4), filtered and evaporated under reduced pressure. FCC of the residue provided **2.17** (8.0 g, 99%) as a white powder; R_f = 0.26 (15% ethyl acetate:petroleum ether); ^{1}HNMR (300 MHz, $CDCl_3$) δ 0.89 (t, *J* = 6.9 Hz, 6H), 1.27 (s, 52H), 1.46 (m, 2H), 1.62 (m, 2H), 2.34 (t, *J* = 8.2 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.9, 23.5, 27.3, 30.1, 30.3, 30.5, 30.8, 30.9, 32.7, 34.1, 37.8, 180.1; ESMS (M^-) 479.3**.**

Thioacetal ester 2.18

DCC (96 mg, 0.47 mmol) was added at 0 ^oC to a solution of TIA-alcohol **2.6** (159 mg, 0.31 mmol), acid **2.17** (300 mg, 0.63 mmol), and DMAP (3.8 mg, 0.03 mmol) in anhydrous benzene (5 mL). The reaction was warmed to rt and stirred for 2 h. The mixture was diluted with ether and the resulting suspension filtered through Celite. The filtrate was washed with 0.1 N aqueous HCl and brine, dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The residue was purified by FCC to give ester **2.18** (265 mg, 87%): colorless oil; R_f = 0.79 (5% ethyl acetate:petroleum ether); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.89 (t, *J* = 6.0 Hz, 6H), 1.05 (s, 9H), 1.27 (bs, 55H), 1.44 (s, 3H), 1.49 (s, 3H), 2.28 (q, *J* = 6.6 Hz, 2H), 3.82 (m, 2H), 4.35 (dd, *J* = 4.6Hz, 1H), 5.24 (m, 1H), 5.28 (d, *J* = 6.6 Hz, 1H), 7.26-7.68 (m, 15H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.43, 20.5, 23.0, 26.9, 27.1, 27.54, 29.7, 30.0, 30.1, 30.4, 32.2, 33.6, 37.4, 62.5, 72.1, 80.4, 85.4, 127.8, 127.9, 129.1, 129.9, 132.5, 135.8, 184.2; ESMS (M+Cl) 1005.7.

Thioacetal-enol ether 2.19

Tebbe reagent in THF (3.1 mL, 0.5 M, 1.54 mmol), was added dropwise under an atmosphere of argon at -78^{o}C, to a solution of ester **2.18** (995 mg, 1.02 mmol) and pyridine (0.1 mL) in anhydrous THF (15 mL). The reaction was stirred at -78 ^{o}C , 0 ^{o}C and at rt for 20 min, then slowly poured into 1N aqueous NaOH at 0 ^{o}C. The resulting suspension was extracted with ether, and the organic phase was washed with brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by FCC on basic alumina (Brockmann I, 150 mesh) to give the enol ether **2.19** (816 mg, 82%): yellow oil; R_f = 0.89 (5% ethyl acetate:petroleum ether);^{1}H NMR (300 MHz, C_6D_6) δ 0.98 (t, *J* = 7.0 Hz, 6H), 1.25 (s, 9H), 1.39 (bs, 55H), 1.59 (s, 6H), 2.2 (t, *J* = 7.7 Hz, 2H), 4.0 (s, 2H), 4.19 (d, *J* = 5.86 Hz, 2H), 4.64 (m, 1H), 4.80 (dd, *J* = 4.6 Hz, 1H), 5.81 (d, *J* = 6.96 Hz, 1H), 7.20-7.80 (m, 15H); ^{13}C NMR (75 MHz, C_6D_6) δ 14.5, 20.5, 23.6, 27.2, 27.9, 28.4, 29.7, 30.4, 30.7, 31.2, 32.2, 33.6, 37.4, 62.5, 75.1, 80.4, 83.8, 86.6, 112.8, 129.9, 131.3, 133.5, 136.7, 163.2.

Glycal 2.20

A mixture of TIA-enol ether **2.19** (1.25 g, 1.29 mmol), 2,6-di-*tert*-butyl-4-methylpyridine (3.97 g, 19.34 mmol), and freshly activated, powdered 4A molecular sieves (2.00 g) in anhydrous CH_2Cl_2 (200 mL), was stirred for 30 min at rt under an argon atmosphere, then cooled to 0 ^{o}C. Methyl triflate (1.46 mL, 12.89 mmol) was then introduced and the mixture was warmed to rt and stirred for an additional 2 d, at which time triethylamine (2.60 mL) was added. The mixture was diluted with ether, washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), filtered and evaporated under

reduced pressure. The residue was purified by FCC over basic alumina (Brockmann I, 150 mesh) to give glycal **2.20** (1.04 g, 94%): clear oil; R_f = 0.40 (2% ethyl acetate:petroleum ether); ^{1}H NMR (300 MHz, C_6D_6) δ 0.98 (t, *J* = 6.9 Hz, 6H), 1.25 (s, 9H), 1.35 (bs, 55H), 1.60 (s, 6H), 2.2 (m, 2H), 4.08 (t, *J* = Hz, 1H), 4.22 (m, 2H), 4.30 (m, 2H), 4.62 (q, *J* = Hz, 1H), 4.8 (d, *J* = Hz, 1H), 7.20 (m, 5H), 7.85 (m, 5H); ^{13}C NMR (75 MHz, C_6D_6) δ 14.3, 21.7, 23.9, 27.8, 28.2, 28.8, 30.1, 30.4, 30.7, 31.2, 32.2, 33.6, 37.4, 63.5, 72.1, 80.4, 103.1, 127.8, 127.9, 129.1, 129.9, 135.5, 136.7; HRMS(ESI) calcd for $C_{56}H_{95}O_4Si$ (M-H) 859.7000, found 859.6969.

β-C-galactoside 2.21

$BH_3{\cdot}Me_2S$ (0.10 mL of 10.0-10.2 M solution) was added at 0 ^oC under an atmosphere of argon, to a solution of glycal **2.20** (246 mg, 0.254 mmol) in anhydrous THF (20 mL). The mixture was warmed to rt, stirred for an additional 1.5 h or until disappearance of the starting material at this temperature. The solution was cooled to 0 ^oC then treated with a mixture of 3N NaOH (1 mL) and 30% aqueous H_2O_2 (1 mL) for 30 min. The mixture was diluted with ether, washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), filtered and evaporated under reduced pressure. The residue was purified by FCC to give alcohol **2.21** (212 mg, 85%): colorless oil; R_f = 0.60 (10% ethyl acetate:petroleum ether);^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (t, *J* = 7.32 Hz, 6H), 1.10 (s, 9H), 1.30 (s, 52H), 1.55(s, 3H), 1.60 (s, 3H), 2.10 (m, 2H), 3.02 (t, *J* = 9.38 Hz, 1H), 3.5 (t, *J* = 7.42 Hz, 1H), 3.80 (t, *J* = 6.44 Hz, 1H), 3.95 (d, *J* = 6.68 Hz, 2H), 4.05 (t, *J* = 7.12 Hz, 1H), 4.25(d, *J* = 4.84 Hz 1H), 7.40 (m, 5H), 7.5 (m, 5H) ; ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.7, 19.8, 23.3, 26.9, 27.3, 29.4, 29.9, 30.3, 30.7, 32.5, 34.2, 38.1, 63.4, 74.5,

75.0, 7`9.4, 80.9, 110.2, 128.2, 129.5, 132.8, 134.2. LRMS ($M+NH_4^+$) 894.3.

(1S)-1,5-Anhydro-1-*C*-(3-tetradecylheptadec-1-yl)-D-galactitol 3

TBAF (5.56 mL of 1M solution) was added at rt to a solution of **2.21** (697 mg, 0.80 mmol) in anhydrous THF (3 mL) under nitrogen. The reaction mixture was stirred for 4 h, then concentrated under reduced pressure. FCC of the residue provided the diol **2.22** (500 mg, 99%): R_f = 0.13 (20% ethyl acetate:petroleum ether) ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.97 Hz, 6H), 1.25 (br s, 55H), 1.46 (s, 3H), 1.56 (s, 3H), 1.85 (m, 1H), 2.15 (d, *J* = 6.89 Hz, 1H), 2.31 (d, *J* = 3.06 Hz, 1H), 3.06 (m, 1H), 3.47 (t, *J* = 7.28 Hz, 1H), 3,78 (m, 2H), 3.93 (m, 1H), 4.03 (q, *J* = 5.57 Hz, 1H), 4.20 (dd, *J* = 2.02, 3.48 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.1, 22.6, 26.4, 26.7, 26.7, 28.2, 29.3, 29.6, 29.7, 30.1, 31.9, 33.5, 33.6, 37.5, 62.8, 70.5, 74.2, 74.4, 76.3, 76.7, 78.6, 80.4, 110.1.

A portion of the material from the previous step (105 mg, 0.18 mmol) in methanol (10 mL) was treated with a solution of HCl in ether (1mL) at for 20 min. The mixture was then neutralized with 1N NaOMe, diluted with brine and extracted with ether. The organic extract was dried (Na_2SO_4), filtered and evaporated *in vacuo*. FCC of the residue provided **3** (51 mg, 52%); R_f = 0.10 (60% ethyl acetate:petroleum ether). ^{1}H NMR (300 MHz, CD_3OD) δ 0.90 (t, *J* = 6.96 Hz, 6H), 1.30 (bs, 55H), 1.87 (m, 1H), 3.03 (t, *J* = 8.42 Hz, 1H), 3.40 (m, 3H), 3.70 (d, *J* = 5.86 Hz, 2H), 3.87 (bs, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 14.6, 23.9, 27.8, 30.3, 30.9, 31.3, 33.2, 34.7, 34.8, 38.8, 62.7, 70.9, 76.6, 79.6, 80.2, 82.2; FABHRMS: calcd for $C_{37}H_{74}O_5$ Na 621.5434, found 621.5434.

Tetraacetate 2.23

To a solution of **3** (5 mg, 0.0083 mmol) in ethyl acetate (1 mL) was added acetic anhydride (0.0013 mL, 0.0134 mmol) and DMAP (0.4 mg, .0033 mmol) at rt. After stirring for 1 hr, the reaction was quenched with methanol (0.3 mL), then concentrated under reduced pressure. FCC of the residue gave **2.23** (5.3 mg, 83%)**:** viscous liquid. R_f = 0.75 (20% ethyl acetate:petroleum ether) ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (t, *J* = 6.9 Hz, 6H), 1.26 (bs, 52H), 1.98 (s, 3H), 2.05 (s, 6H), 2.15 (s, 3H), 3.3 (t, *J* = 6.86 Hz, 1H), 3.83 (t, *J* = 6.68 Hz, 1H), 4. 10 (m, 2H), 5.02 (dd, *J* = 6.64 Hz, 1H), 5.10 (t, *J* = 9.73 Hz, 1H), 5.40 (d, *J* = 3.31 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.4, 21.1, 23.0, 27.0, 29.4, 29.7, 30.0, 30.4, 32.2, 34.0, 37.5, 61.9, 68.1, 72.7, 74.3, 76.6, 79.1, 169.8, 169.9, 170.2, 170.4

Compound 2.24

Benzoyl chloride (0.095 mL, 0.8150 mmol) was added at 5 ˚C to a solution of **2.22** (130 mg, 0.2037 mmol) in anhydrous pyridine (3 mL). The mixture was stirred for 1 h at 5 ˚C, then methanol (1mL) was added to the cold mixture. The mixture was stirred for an additional 1 h, then diluted with ethyl acetate (6 mL), washed with 1M aqueous sodium bicarbonate (3 mL), water (3 mL) and brine (3 mL). The organic phase was dried ($MgSO_4$), filtered and concentrated. FCC of the residue gave **2.24** (174 mg, qt) as a viscous liquid; R_f = 0.80 (10% ethyl acetate:petroleum ether). ^{1} H NMR (300 MHz, $CDCl_3$) δ 0.94 (t, *J* = 6.7 Hz, 6H), 3.4 (m, 1H), 4.16 (t, *J* = 5.5 Hz, 1H), 4.39 (m, 2H), 4.64 (dd, *J* = 7.7 Hz, 1H), 4.70 (dd, *J* = 4.8 Hz, 1H), 5.25 (q, *J* = 2.9 Hz, 1H), 7.20-7.8 (m, 10H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.8, 23.4, 27.1, 27.3, 28.4, 30.1, 30.4, 30.8,

32.7, 34.1, 34.4, 37.8, 64.8, 74.7, 74.8, 111.1, 128.3, 128.9, 130.3, 130.4, 130.6, 130.7, 133.6, 133.7, 135.4, 166.9.

Compound 2.25

Concentrated HCl in ether (0.1 mL) was added at rt to **2.24** (145 mg, 0.17 mmol) in methanol (3 mL). The reaction was followed by TLC and stopped after 20 min, neutralized with 0.1 NaOMe and concentrated under reduced pressure. FCC gave **2.25** (133 mg, 97%); R_f = 0.25 (20% ethyl acetate:petroleum ether). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.96 Hz, 6H), 1.26 (s, 52H), 1.50 (m, 2H), 1.67 (s, 2H), 2.91 (d, *J* = 3.8 Hz, 1H), 3.44 (m, 2H), 3.82 (t, *J* = 6.2 Hz, 2H), 4.05 (m, 1H), 4.54 (dd, *J* = 4.8 Hz, 1H), 4.65 (dd, *J* = 6.2 Hz, 1H), 5.09 (t, *J* = 9.2 Hz, 1H), 7.4-8.0 (m, 10H); HRMS (FAB) calcd for $C_{51}H_{82}O_7$ (M+H) 807.6139, found 807.6130.

Compound 2.26

A solution of **2.25** (57 mg, 0.071 mmol) in dry benzene (9 mL) and triethyl orthoacetate (9 mL) was treated with CSA (2.19 mg) at rt. The mixture was stirred for 1 h and then triethylamine (4.5 mL) was added followed by cold water (8 mL). The aqueous phase was extracted with ethyl acetate (3 x 10 mL), washed with aqueous sodium bicarbonate (20 mL) and water (20 mL) and brine (20 mL). The organic phase was dried ($MgSO_4$) and concentrated under reduced pressure. The residue was dissolved in dichloromethane (13 mL) and treated with 80% aqueous acetic acid (26 mL). The mixture was stirred at rt for 35 min. Toluene (3 x 13 mL) was added and the mixture was coevaporated under reduced pressure. FCC of the residue was purified and afforded **2.26**

(55 mg, 92%); R_f = 0.17 (20% ethyl acetate:petroleum ether) ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.87 (t, *J* = 6.59 Hz, 6H), 1.15-1.26 (bs, 52H), 1.51 (m, 2H), 2.24 (s, 3H), 3.50 (t, *J* = 7.69 Hz, 1H), 3.98 (m, 2H), 4.32 (q, *J* = 5.86 Hz, 1H), 4.49 (q, *J* = 4.03 Hz, 1H), 5.15 (t, *J* = 9.52 Hz, 1H), 5.52 (d, *J* = 3.66 Hz, 1H), 7.43-8.06 (m, 10H); HRMS (FAB) calcd for $C_{53}H_{84}O_8$ 848.6166, found 848.6166.

Compound 2.27

A solution of **2.26** (40 mg, 0.05 mmol) in dry dimethylformamide (2 mL) was treated with sulfur trioxide-trimethylamine complex (39 mg, 0.25 mmol) and the reaction mixture was stirred for 2 h, then sodium bicarbonate were added to saturation. The mixture was stirred overnight. The residue was diluted with dichloromethane and this mixture was filtered. The filtrate was evaporated and the residue was purified by silica gel chromatography to give **2.27** (40 mg, 89%); R_f = 0.75 (20% methanol:chloroform) ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.59 Hz, 6H), 1.10 (s, 2H), 1.25 (bs, 50H), 1.49 (m, 2H), 2.03 (s, 3H), 3.49 (t, *J* = 6.96Hz, 1H), 3.99 (peaks overlap, 2H), 4.30 (q, *J* = 5.13 Hz, 1H), 4.48 (d, *J* = 4.02 Hz, 1H), 5.17 (t, *J* = 9.52 Hz, 1H), 5.51 (d, *J* = 4.66 Hz, 1H), 7.33-8.01 (m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.9, 21.6, 23.5, 27.2, 27.3, 30.1, 30.5, 30.9, 32.7, 34.1, 34.3, 38.0, 70.2, 71.8, 75.2, 79.3, 128.8, 130.4, 133.5, 166.9. ESMS 927.5 (M−Na).

Sulfatide 4

To a solution of **2.27** (28 mg, 0.029 mmol) in 1.2 mL of methanol was added 0.5 mL (25% wt of Na in Methanol) of sodium methoxide. The reaction was stirred at rt for about

30 min. the reaction mixture was diluted with methanol, filtered and concentrated. FCC of the residue gave the desired sulfatide **4** (13 mg, 65%); R_f = 0.30 (20% methanol:chloroform). ^{1}H NMR (300 MHz, CD_3OD) δ 0.85 (t, *J* = 6.98 Hz, 6H), 1.23 (bs, 52H), 1.37 (m, 1H), 1.51 (m, 2H), 1.83 (m, 2H), 3.08 (m, 1H), 3.35 (t, *J* = 6.25 Hz, 1H), 3.49 (t, *J* = 9.38 Hz, 1H), 3.59 (d, *J* = 6.27 Hz, 2H), 4.09 (dd, *J* = 3.13, 6.27 Hz, 1H), 4.18 (d, *J* =3.10 Hz, 1H) ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.5, 23.9, 27.9, 27.9, 30.6, 30.9, 31.3, 33.2, 34.9, 35.0, 38.9, 62.9, 69.4, 71.1, 80.1, 82.2, 84.1; ESMS 677.4 (M-Na) HRMS (FAB) calcd for $C_{37}H_{73}O_8S$ (M-Na) 677.5026, found 677.5045.

Thioacetal ester 2.28

The coupling of TIA-alcohol **2.6** (1.00 g, 1.97 mmol) and stearic acid (840 mg, 2.95 mmol) was performed as described for **2.18**. The residue was purified by FCC to give ester **2.28** (1.40 g, 92%): colorless oil; R_f = 0.60 (10% ethyl acetate:petroleum ether); $[\alpha]_D$ –39.9 (c 5.00, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.9 Hz, 3H), 1.03 (s, 9H), 1.25 (m, 28H), 1.44, 1.48 (both s, 3H ea), 1.58 (m, 2H), 2.29 (m, 2H), 3.82 (m, 2H), 4.34 (dd, *J* = 3.6, 6.6 Hz, 1H), 5.25 (m, 2H), 7.20-7.70 (m, 15H).^{13}C NMR (125 MHz, $CDCl_3$) δ 14.7, 23.2, 25.5, 26.8, 27.2, 27.7, 29.8, 29.9, 30.0, 30.2, 30.2, 32.5, 34.8, 62.9, 72.0, 79.7, 85.8, 112.2, 128.2, 128.2, 129.5, 130.3, 130.3, 132.8, 133.5, 134.2, 136.1. FAB HRMS calcd for $C_{47}H_{70}O_5SSi$ (M+ Na) 797.4572, found 797.4611.

Thioacetal-enol ether 2.29

The Tebbe olefination of ester **2.28** (1.40 g, 1.80 mmol) was carried out following the same protocol as for **2.19**. The residue was purified by FCC on basic alumina

(Brockmann I, 150 mesh) to give the enol ether **2.29** (1.39 g, 99%): colorless oil; R_f = 0.65 (2% ethyl acetate:petroleum ether); $[\alpha]_D$ –57.0 (c = 1.4, $CHCl_3$); ^{1}H NMR (500 MHz, C_6D_6) δ 0.92 (t, *J* = 7.0 Hz, 3H), 1.18 (s, 9H), 1.32,1.35 (both s, 30H), 1.53, 1.52 (both s, 3H ea), 2.16 (t, *J* = 7.9 Hz, 2H), 3.95 (d, *J* = 3.43 Hz, 2H) , 4.15 (m, 2H), 4.52 (m, 1H), 4.77 (dd, *J* = 1.8, 7.0 Hz, 1H), 5.81 (d, *J* = 7.0 Hz, 1H), 7.10-7.80 (m, 15H). ^{13}C NMR (125 MHz, C_6D_6) δ 14.5, 23.3, 27.2, 30.0, 30.3, 30.8, 32.0, 32.5, 33.3, 33.9, 37.6, 62.2, 74.7, 80.6, 82.2, 85.5, 111.9, 128.7, 129.4, 130.2, 132.3, 133.9, 135.2, 135.2, 163.3. $C_{48}H_{72}O_4SSi$ LRMS (M+) 797.5

Glycal 2.30

The glycal was obtained as described for 2.30 with TIA-enol ether **2.29** (1.39 g, 1.80 mmol). The residue was purified by FCC over basic alumina (Brockmann I, 150 mesh) to give glycal **2.30** (1.19 mg, 87%): clear oil; R_f = 0.55 (2% ethyl acetate:petroleum ether); $[\alpha]_D$ +18 (c = 1.2, $CHCl_3$); ^{1}H NMR (500 MHz, C_6D_6) δ 0.92 (t, *J* = 7.0 Hz, 3H), 1.21 (s, 9H), 1.35 (m, 28H), 1.44, 1.46 (both s, partly buried under singlets at 1.35 6H), 2.11 (m, 2H), 4.00 (t, *J* = 6.7 Hz, 1H), 4.17 (qt, *J* = 6.5 Hz, 2H), 4.25 (m, 2H), 4.57 (dd, *J* = 3.0, 7.0 Hz, 1H), 4.72 (d, *J* = 8.3 Hz, 1H), 7.24, 7.81 (both m, 10H). ^{13}C NMR (125 MHz, C_6D_6): δ 14.9, 23.7, 27.6, 28.4, 29.2, 29.7, 30.7, 30.8, 31.2, 32.4, 32.9, 34.6, 38.1, 41.5, 64.7, 70.9, 72.7, 76.8, 98.7, 110.7, 127.8, 128.1, 128.9, 130.6, 136.6, 136.7, 157.3. FAB HRMS calcd for $C_{42}H_{65}O_4Si$ (M-H) 661.4652, found 661.4649.

β-C-galactoside 2.31

$BH_3.Me_2S$ (1.09 mL of a 1M solution in CH_2Cl_2, 1.09 mmol) was added at 0°C to a solution of glycal **2.30** (180 mg, 0.27 mmol) and in anhydrous THF, under an atmosphere of argon. The mixture was warmed to rt, and stirred for an additional 1h, or until tlc indicated complete disappearance of the starting material. At that time the solution was recooled to 0 °C, and a mixture of 3N NaOH (2 mL) and 30% aqueous H_2O_2 (2 mL) carefully added. After stirring at this temperature for an additional 30 min, the mixture was diluted with ether and washed with saturated aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), filtered and evaporated under reduced pressure. FCC of the residue provided **2.31** (185 mg, 76%): clear oil; R_f = 0.30 (15% ethyl acetate:petroleum ether); $[\alpha]_D$ +5.1 (c 1.7, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.89 (t, *J* = 7.0 Hz, 3H), 1.07 (s, 9H), 1.25 (m, 30H), 1.36 (s, 3H), 1.51 (s, 3H), 1.54 (m, partly buried under singlet at 1.51, 1H), 1.83 (m, 1H), 2.81 (m, 1H, D_2O ex.), 3.04 (br t, *J* = 7.0 Hz, 1H), 3.41 (dd, *J* = 9.9, 7.4 Hz, 1H), 3.80 (dt, *J* = 2.2, 8.1 Hz, 1H), 3.92 (m, 2H), 3.98 (dd, *J* = 5.5, 7.0 Hz, 1H), 4.30 (dd, *J* = 2.2, 5.5 Hz, 1H), 7.39, 7.72 (both m, 10H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.4, 19.5, 23.0, 25.6, 26.7, 27.1, 28.6, 29.6, 29.7, 30.0, 31.8, 32.2, 63.2, 74.0, 76.8, 78.4, 79.5, 109.8, 127.7, 127.8, 127.9, 129.7, 130.0, 133.6, 135.7, 135.8. FABHRMS calcd for $C_{38}H_{59}O_4Si$ (M-C_4H_9) 623.4132, found 623.4133.

(1S)-1,5-Anhydro-1-C-heptadecyl-D-galactitol 6.

TBAF (0.85 mL of 1M solution) was added at rt to a solution of **3.31** (190 mg, 0.279 mmol) in anhydrous THF (2 mL) under nitrogen. The reaction mixture was stirred for 4 h, then concentrated under reduced pressure. FCC of the residue provided the derived primary alcohol **2.32** (127 mg, qt): R_f = 0.13 (20% ethyl acetate:petroleum ether);

The alcohol (127 mg, 0.287 mmol) in methanol (2 mL) was treated with a solution of HCl in ether (8 drops) at for 10 min. The mixture was then neutralized with 1N NaOMe, diluted with brine and extracted with ether. The organic extract was dried (Na_2SO_4), filtered and evaporated *in vacuo*. FCC of the residue provided **6** (100 mg, 87 %). clear oil; R_f = 0.23 (10% methanol: chloroform); ^{1}H NMR (500 MHz, CD_3OD) δ 0.90 (t, *J* = 7.0 Hz, 3H), 1.29 (m, 33H), 1.59 (m, 1H), 1.84 (m, 1H), 3.06 (t, *J* = 7.9 Hz, 1H), 3.38 (m, 3H), 3.69 (m, 2H), 3.87 (m, 1H); FABHRMS calcd for $C_{23}H_{47}O_5$ (M+H) 403.3424, found 403.3423.

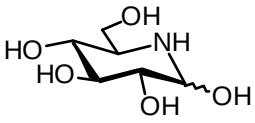
Chapter 3

Synthesis of Aza C-glycoside

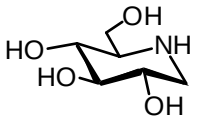
3.1 Introduction

Alkaloids mimicking the structures of monosaccharides are believed to be widespread in plants and microorganisms. These sugar mimics are classified into five structural classes: polyhydroxylated piperidines, pyrrolidines, indolizidines, pyrrolizidines and nortropanes. They are saccharides, in which the ring-oxygen is replaced with an imino-group; they are called azasugars. Nojirimycin, Deoxynojirimycin and galactostatin (figure 3.1) are examples of polyhydroxylated piperidines. The inhibitory activity of many of these azasugars toward carbohydrate-processing enzymes has suggested their use in a wide range of potential therapeutic strategies including the treatment of viral infections, cancer, diabetes, and tuberculosis. An example is N-Butyldeoxynojirimycin, which has been shown to exhibit anti-HIV activity in vitro and inhibition character of the replication of the virus[37].

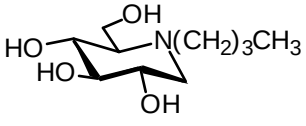
Figure 3.1 Examples of Azasugars



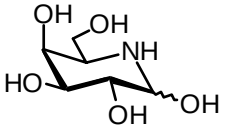
Nojirimycin, NJ



Deoxynojirimycin, DNJ



N-Butyldeoxynojirimycin

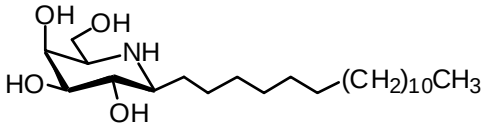


Galactostatin

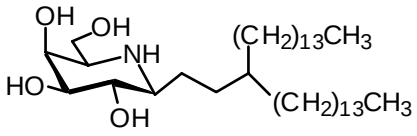
It is believed that the heterocyclic nitrogen mimics the oxonium ion transition state of the natural sugars when involved in glycoside hydrolysis and that their conformational resemblance to the natural sugars can explained their biological activity. Furthermore, as C-glycosides show better stability in chemical and enzyme hydrolysis, aza-C-glycosides show greater stability then their corresponding O-analogs.

As mentioned in the introductory chapter, N-stearyl-1-deoxynojirimycin **24** and related derivatives with simple stearyl or stearoyl chains as ceramide substitutes, exhibited gp-120 affinity that was comparable to GalCer[13,]. This chapter reports the synthesis aza-C-analogs **7** and **8**.

Figure 3.2 Aza-C-analogs of GalCer



7



8

A number of piperidine homoazasugars are known and described in the literature . As the homosugars have a close resemblance with five or six carbon sugars, most of the synthetic strategies make use of sugars as the starting material. The common methodologies used for the synthesis of piperidine azasugars are: 1) intramolecular

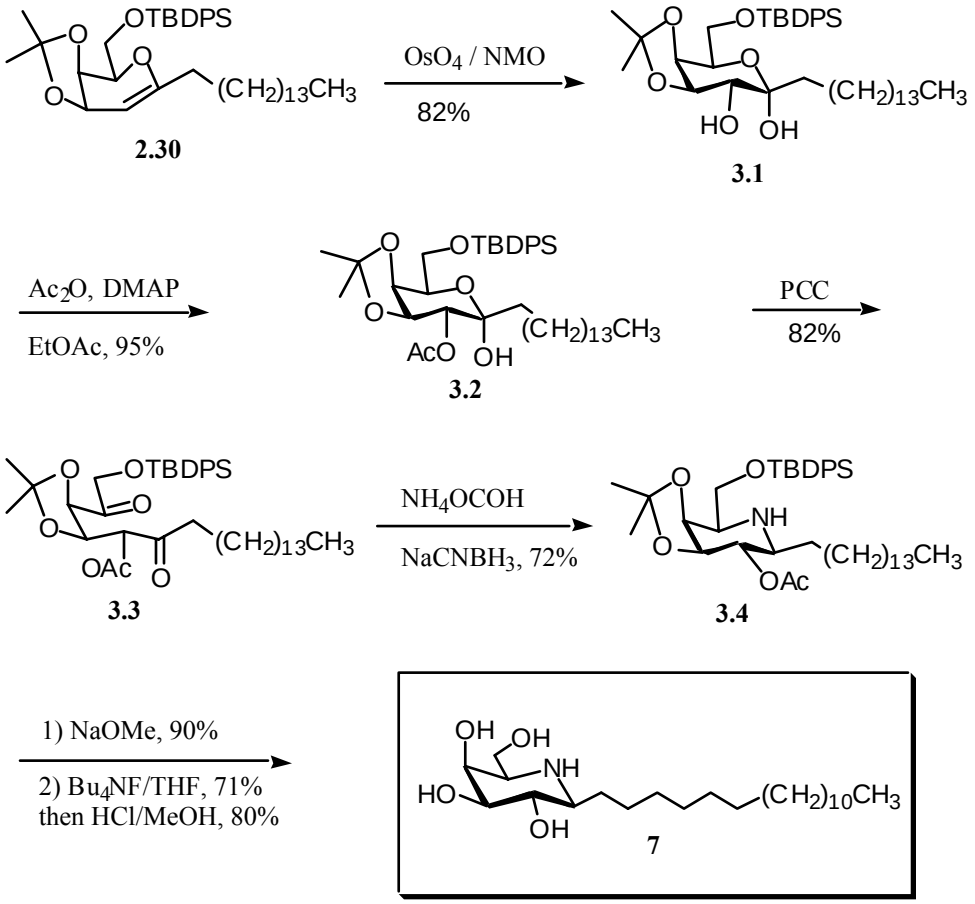
reductive amination, 2) intermolecular double reductive amination, 3) amino/amido mercuration, 4) intramolecular nucleophilic substitution, 5) synthesis from non-carbohydrate building block and aza-heterocycles and 6) enzyme catalyzed intramolecular reductive amination. In our approach, we started from non-carbohydrate components to obtain the corresponding glycal precursor and we applied intramolecular reductive amination to have the targeted Aza-C-analogs as described in the retrosynthesis. In previous work done in our laboratory, the synthesis of aza-α-D-gal (1'-1)-α-D-Mannopyranoside was completed via the reductive amination of a dicarbonyl sugar. So we envisaged that dihydroxylation of the glycal with osmium tetroxide-NMO would provide the corresponding diol**.** Selective acetylation of the diol followed by PCC oxidation and double reductive amination with ammonium formate and sodium cyanoborohydride will afford the desired azasugars**.** Subsequent deprotection will provide the targeted aza C-galatosides **7** and **8.**

3.2 Synthesis

3.2.1 Synthesis of Aza-analog 7

The azasugar **7** was previously prepared in this laboratory. This procedure was repeated. Thus, dihydroxylation of the **2.30**, the identical glycal in chapter 2, with osmium tetroxide-NMO provided diol **3.1** in 95% yield. Selective acetylation of **3.1** followed by PCC oxidation afforded the diketone **3.3** in 82% yield. Double reductive amination of the diketone **3.3** with ammonium formate and sodium cyanoborohydride with molecular sieves, gave the azasugar **3.4** as single diastereomer in 72% yield. Ester

Scheme 3.2 Synthesis of Aza analog 7

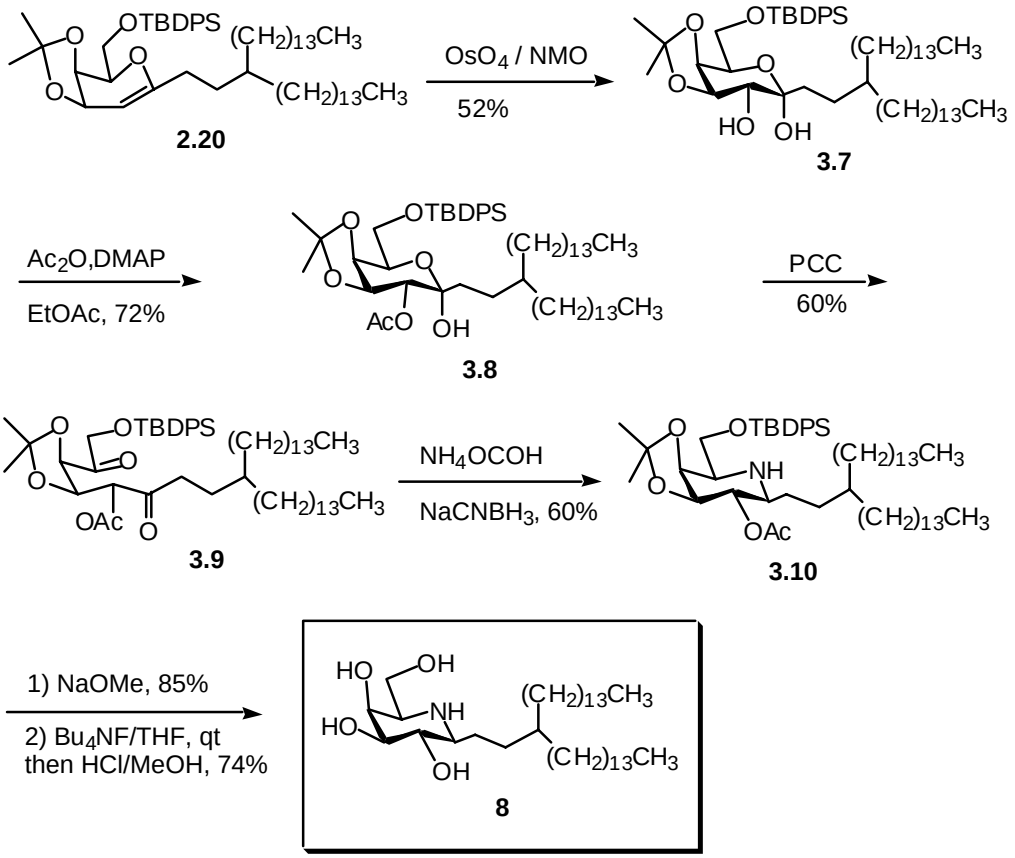


hydrolysis, and removal of the silyl and acetonide protecting groups provided the targeted aza C-galactoside **7** (Scheme 3.2).

3.2.2 Synthesis of Aza-analog 8.

The aza analog **8** was synthesized following the plan described for **7**. Dihydroxylation of glycal **2.21** from chapter 2, followed by selective acetylation of the dihydroxylation product and alcohol oxidation provided diketone **3.9**. Double reductive amination on **3.9** led to **3.10** in 60% yield. Finally alcohol afforded the desired product **8**.

Scheme 3.3 Synthesis of analog 8



The stereochemistry of both products **7** and **8** was assigned on the basis of *J* values, for **7** the values were $J_{1,2} = 9.9$, $J_{2,3} = 7.7$, $J_{3,4} = 5.1$, $J_{4,5} = 2.2$ Hz and for **8,** they were $J_{1,2} = 9.6$, $J_{2,3} = 7.8$, $J_{3,4} = 5.1$, $J_{4,5} = 2.3$ Hz.

3.6 Summary

Aza analogs **7** and **8** were obtained via the highly stereoselective double reductive animation of diketones precursors. These ketones were prepared from the glycals **2.21** and **2.28** which were the identical precursors for the corresponding C-glycosides in chapter 2. Analogs **8** and **9** were each prepared over seven steps, in 13 and 15% yield , from **2.21** and **2.28** respectively.

3.7 Experimental

Hemiacetal 3.1

N-methylmorpholine-N-oxide (0.57 mL, 60 wt% in H_2O, 3.32 mmol) and osmium tetroxide (2.08 mL 2.5 wt% in t-butanol, 0.17 mmol) were added to a solution of **2.30** (1.1 g, 1.66 mmol) in acetone (20 mL). The reaction mixture was stirred at rt for 0.5 h, at which time a solution of sodium bisulfite (0.57 mL, 1 N) was added and the mixture was stirred for additional 0.5 h. Most of the solvent was evaporated *in vacuo*, the residue was diluted with water and extracted with ethyl acetate. The combined organic phase was dried (Na_2SO_4), filtered, and evaporated *in vacuo*. FCC of the residue gave a mixture of anomers **3.1** (0.93 g, 80%): colorless oil; R_f = 0.10 (10% ethyl acetate:petroleum ether); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.4, 19.4, 22.39, 22.9, 25.2, 26.3, 27.7, 28.3, 29.6, 29.7, 32.1, 38.7, 63.2, 65.3, 69.0, 69.6, 72.7, 73.3, 76.3, 98.1, 109.4, 127.8, 128.0, 129.8, 130.1, 133.7, 135.7, 135.9. FAB HRMS calcd for $C_{42}H_{68}O_6Si$ (M + Na) 719.4684, found 719.4683**.**

Acetate 3.2

To a solution of **3.1** (0.9 g, 1.3 mmol) and DMAP (15.9 mg, 0.13 mmol) in ethyl acetate (10 mL) was added acetic anhydride (0.16 mL, 1.56 mmol) dropwise, the reaction mixture was stirred at rt for 5 min and was then diluted with methanol (0.5 mL). The mixture was evaporated under reduced pressure, and the residue was purified by FCC to give **3.2** (0.91 g, 95%): clear oil; R_f = 0.85 (20% ethyl acetate:petroleum ether); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (t, *J* = 7.0 Hz, 3H), 1.10 (s, 9H), 1.25 (s, 30H), 1.32, 1.53 (both s, 3H ea) 2.12 (s, 3H), 3.85, 3.96 (both m, 1H ea), 4.32 (m, 3H), 4.95 (d, *J* = 7.50 Hz, 1H), 7.40, 7.60 (both m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 19.6, 21.4, 22.1, 23.0, 26.9, 27.0, 27.1, 27.2, 28.0, 29.7, 29.8, 30.0, 30.2, 30.2, 32.2, 38.3, 63.2, 69.2, 73.4, 73.7, 75.3, 76.8, 97.9, 109.7, 127.7, 127.8, 127.9, 128.0, 128.1, 129.7, 129.8, 130.0, 133.1, 133.7, 133.8, 135.8, 170.3; FAB HRMS calcd for $C_{44}H_{71}O_5SiN$ 722.5180, found 722.5183.

Diketone 3.3

To a mixture of PCC (147 mg, 0.136 mmol), Celite (147 mg), florisil (15mg), sodium acetate (56 mg, 0.68 mmol) and freshly activated 4A molecular sieves (200 mg) and CH_2Cl_2 (3 mL) was added a solution of **3.2** (100 mg, 0.136 mmol) in CH_2Cl_2 (2 mL) The reaction mixture was stirred at rt for 3 hr under an argon atmosphere, and was then filtered through a bed of Celite. The filtrate was concentrated under reduced pressure, and the residue was purified by FCC to give **3.3** (82mg, 82%): colorless oil; R_f = 0.80 (10% ethyl acetate:petroleum ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (t, *J* = 7.0 Hz, 3H), 1.10 (s, 9H), 1.25 (br s, 30H), 1.27, 1.5 (both s, each 3H) 2.08 (s, 3H), 2.42 (m, 2H), 4.40

(ABq, J = 17.0 Hz, $\Delta\delta$ = 0.15 ppm, 1H), 4.66 (d, J = 2.2 Hz, 1H), 4.95 (dd, J = 2.2, 8.4 Hz, 1H), 5.17 (d, J = 8.4 Hz, 1H), 7.40, 7.60 (both m, 10H); FAB HRMS calcd for $C_{44}H_{68}O_7Si$ (M + Na) 759.4629, found 759.4632.

Azasugar 3.4

To a mixture of **3.3** (250 mg, 0.34 mmol), ammonium formate (38.0 mg, 0.6 mmol) and 4A powdered molecular sieves (100 mg) was added sodium cyanoborohydride (75 mg, 1.1 mmol) in one portion. The reaction mixture was stirred for 30 min at rt under an argon atmosphere. The solids were then removed by filtration through a bed of Celite, and washed by ether, and the filtrate was concentrated under reduced pressure. The residue was dissolved in ether (20 mL) and washed successively with saturated aqueous $NaHCO_3$, brine, dried (Na_2SO_4), filtered and concentrated under reduced pressure. FCC purification of the residue gave **3.4** (176.3 mg, 72%): clear oil; R_f = 0.45 (15% ethyl acetate:petroleum ether); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (br t, J = 7.0 Hz, 3H), 1.1 (s, 9H), 1.25 (s, 30H), 1.3, 1.6 (both s, each 3H), 1.2-1.4 (m, buried 2H), 2.45 (t, J = 9.3 Hz, 1H), 3.11 (m, 1H), 3.82 (m, 2H), 3.98 (m, 1H), 4.18 (m, 1H), 4.83 (dd, J = 7.8, 9.9 Hz, 1H), 7.40, 7.60 (both m, 10H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.4, 19.5, 21.4, 23.0, 25.9, 26.8, 26.9, 27.1, 28.1, 29.6, 29.9, 30.0, 31.6, 32.2, 57.3, 57.8, 64.4, 74.1, 76.5, 78.7, 86.9, 109.9, 127.8, 129.8, 133.5, 133.6, 134.9, 135.7, 170.3; FAB HRMS calcd for $C_{44}H_{71}O_5SiN$ (M + H) 722.5183, found 722.5179.

(1S)-1,5 dideoxy-1-C-heptadecyl-1,5 imino-D-galactitol 7

To a solution of **3.4** (150 mg, 0.21 mmol) in anhydrous methanol (5mL) was added sodium methoxide (21 mg, 0.21 mmol). The reaction mixture was stirred at rt for 0.5 h, then evaporated in *vacuo*. The crude material was dissolved in THF (5 mL) and treated with Bu_4NF (0.4 mL, 1M in THF) for 1h at rt. The mixture was then diluted with water and extracted with ether. The organic phase was washed with saturated aqueous $NaHCO_3$, brine, dried (Na_2SO_4), filtered and concentrated under reduced pressure. FCC of the residue the derivative **3.6** (64.1 mg, 72%): clear oil; R_f = 0.1 (40% ethyl acetate:petroleum ether); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (t, *J* = 7.0 Hz, 3H), 1.25 (br s, 32H), 1.32, 1.52 (both s, each 3H), 2.42 (m, 1H), 3.11 (m, 1H), 3.32 (dd, *J* = 7.8, 9.9 Hz, 1H), 3.82 (m, 2H), 3.98 (m, 1H), 4.18 (m, 1H).

To a solution of diol **3.6** (60mg, 0.14 mmol) in 5mL anhydrous methanol was added a solution of methanol-1M HCl (0.1mL, 0.1mmol), the reaction mixture was stirred at rt and monitored by TLC. When the starting material had completely disappeared, solid sodium methoxide was carefully added to a pH of 7. The solution was concentrated in vacuo and the residue purified by FCC to give **7** (43.5 mg, 80%): clear oil; R_f = 0.1 (20% methanol:ethyl acetate). ^{1}H NMR (500 MHz, CD_3OD) δ 0.90 (br t, *J* = 7.0 Hz, 3H), 1.30 (m, 28H), 1.50 (m, 2H), 1.62 (m, 1H), 1.94 (m, 1H), 2.00 (s, 1H, -NH), 2.78 (m, 1H), 3.15 (t, *J* = 6.5 Hz, 1H), 3.42 (dd, *J* = 3.0, 8.5 Hz, 1H), 3.62 (t, *J* = 10.0 Hz, 1H), 3.78 (m, 2H), 4.00 (br s, 1H); ^{13}C NMR (75 MHz, CD_3OD) δ 14.6, 23.9, 26.8, 30.6, 30.7, 30.9, 31.1, 32.0, 32.2, 60.9, 61.2, 61.5, 68.9, 71.7, 75.6. FAB HRMS calcd for $C_{23}H_{48}NO_4$ (M + H) 402.3583, found 402.3584.

Hemiacetal 3.7

The Hemiacetal was prepared from **2.20** (1.1 g, 1.66 mmol) following the same procedure described for **3.1**. FCC of the residue gave a mixture of anomers **3.7** (0.93 g, 80%): colorless oil; R_f = 0.10 (10% ethyl acetate:petroleum ether); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.1, 20.3, 22.3, 24.4, 26.1, 26.5, 26.7, 29.3, 29.6, 29.7, 30.1, 31.9, 33.4, 36.8, 68.5, 79.6, 110.5, 127.8, 127.9, 129.9, 130.0, 132.4, 132.7, 135.4, 135.7, 169.8, 205.0, 205.5; HRMS (ESI) calcd for $C_{58}H_{96}O_6$ NaSi 915.6874, found 915.6895.

Acetate 3.8

A solution of **3.7** (0.9 g, 1.3 mmol) was used to obtain the acetate as described for **3.2**. The residue was purified by FCC to give **3.8** (0.91 g, 95%): clear oil; R_f = 0.85 (20% ethyl acetate:petroleum ether); ^1H NMR (500 MHz, $CDCl_3$) δ 0.89 (t, *J* = 6.95 Hz, 6H), 1.06 (s, 9H), 1.26 (s, 52H), 1.55, 1.56 (both s, 3H ea) 2.15 (s, 3H), 3.87, 3.98 (both m, 1H ea), 4.32 (m, 2H), 4.37 (m, 1H), 5.07 (d, *J* = 6.40 Hz, 1H), 7.40, 7.60 (both m, 10H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 19.6, 21.4, 22.1, 23.0, 26.9, 27.0, 27.1, 27.2, 28.0, 29.7, 29.8, 30.0, 30.2, 30.2, 32.2, 38.3, 63.2, 69.2, 73.4, 73.7, 75.3, 76.8, 97.9, 109.7, 127.7, 127.8, 127.9, 128.0, 128.1, 129.7, 129.8, 130.0, 133.1, 133.7, 133.8, 135.8, 170.5; HRMS (ESI) calcd for $C_{58}H_{98}O_7$NaSi 957.6980, found 957.7031.

Diketone 3.9

The reaction was done following same protocol as described for **3.3** using **3.8** (100 mg, 0.136 mmol). The residue was purified by FCC to give **3.9** (82mg, 82%): colorless oil; R_f = 0.80 (10% ethyl acetate:petroleum ether); ^1H NMR (500 MHz, $CDCl_3$)

δ 0.87 (t, *J* = 6.8 Hz, 3H), 1.08 (s, 9H), 1.25 (br s, 52H), 1.47 (s, 6H) 2.06 (s, 3H), 2.39 (m, 2H), 4.44 (ABq, *J* = 17.0 Hz, Δδ = 0.16 ppm, 1H), 4.64 (d, *J* = 2.4 Hz, 1H), 4.92 (dd, *J* = 2.3, 8.5 Hz, 1H), 5.17 (d, *J* = 8.6 Hz, 1H), 7.40, 7.60 (both m, 10H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.1, 20.3, 22.6, 24.4, 26.1, 26.5, 26.6, 26.8, 29.3, 29.6, 29.7, 30.1, 31.9, 33.4, 36.8, 68.5, 79.6, 110.5, 127.8, 127.9, 129.9, 130.0, 132.4, 132.7, 135.4, 135.6, 169.8, 205.0, 205.5; HRMS (ESI) calcd for $C_{58}H_{96}O_7$ NaSi 955.6823, found 955.6864.

Azasugar 3.10

The reductive amination of **3.9** (250 mg, 0.34 mmol) was done according to the same procedure for **3.4**. FCC purification of the residue gave **3.10** (176 mg, 72%): clear oil; R_f = 0.45 (15% ethyl acetate:petroleum ether); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.89 (t, *J* = 6.95 Hz, 6H), 1.06 (s, 9H), 1.27 (s, 52H), 1.25-1.45 (m, 2H), 1.32, 1.56 (both s, each 3H), 2.10 (s, 3H) 2.45 (t, *J* = 9.7 Hz, 1H), 3.11 (m, 1H), 3.81 (m, 2H), 3.88 (m, 1H), 3.99 (m, H) 4.20 (q, *J* = 2.50 Hz, 1H), 4.85 (dd, *J* = 7.6, 9.7 Hz, 1H), 7.41, 7.71 (both m, 10H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.4, 19.5, 21.4, 23.0, 25.9, 26.8, 26.9, 27.1, 28.1, 29.6, 29.9, 30.0, 31.6, 32.2, 57.3, 57.8, 64.4, 74.1, 76.5, 78.7, 86.9, 109.9, 127.8, 129.8, 133.5, 133.6, 134.9, 135.7, 170.3.

(1S)-1,5 dideoxy-1-C-(3-tetradecylheptadec-1-yl)-1,5 imino-D-galactitol 8

The targeted aza-C-glycoside was obtained from **3.10** (150 mg, 0.21 mmol) as described for **7**. First we obtained **3.11** (64.1 mg, 72%): clear oil; R_f = 0.1 (40% ethyl acetate:petroleum ether); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (t, *J* = 6.8 Hz, 6H), 1.27 (br s, 52H), 1.38, 1.53 (both s, each 3H), 2.36 (m, 1H), 3.07 (m, 1H), 3.31 (dd, *J* = 7.32, 9.5

Hz, 1H), 3.82 (d, *J* = 5.12 Hz, 2H), 3.94 (dd, *J* = 5.12 Hz, 1H), 4.18 (dd, 1H, *J* = 3.96 Hz), then **8** (43.5 mg, 80%): clear oil; R_f = 0.1 (20% methanol:ethyl acetate). ^{1}H NMR (125 MHz, CD_3OD) δ 0.90 (br t, *J* = 7.0 Hz, 3H), 1.30 (m, 28H), 1.50 (m, 2H), 1.62 (m, 1H), 1.94 (m, 1H), 1.90 (s, 1H, -NH), 2.45 (t, *J* = 6.5 Hz, 1H), 2.82 (dd, *J* = 3.0, 8.5 Hz, 1H), 3.32 (m, 1H), 3.40 (t, *J* = 10.0 Hz, 1H), 3.65 (m, 2H), 3.90 (bs, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 13.9, 22.5, 26.5, 26.7, 29.0, 29.2, 29.5, 29.6, 29.6, 29.7, 30.1, 3.1, 31.8, 33.3, 33.6, 37.8, 48.7, 48.9, 49.1, 49.2, 49.4, 53.3, 58.9, 60.0, 62.7, 69.9, 73.0, 76.1; HRMS (ESI) calcd for $C_{37}H_{76}NO_4$ (M + H) 598.5774, found 598.5750 HRMS (ESI) calcd for $C_{58}H_{96}O_6$ NaSi 915.6874, found 915.6895.

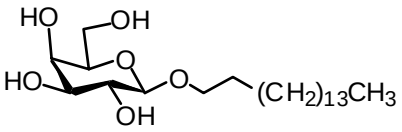
Chapter 4

Synthesis of O-glycosides

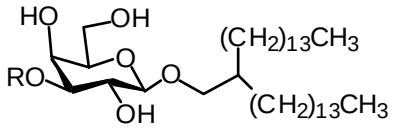
4.1 Introduction

Several methods have been reported for the stereoselective synthesis of glycosides with sensitive glycosyl donors and acceptors. However, the β-galactoside motif present in our targets **5** and **9** and **10** and the simplicity and stability of the aglycone segments, allowed for the use of a relatively straightforward procedure involving the Lewis acid mediated glycosidation of penta-O-acetylgalactopyranose and the requisite alcohol.

Figure 4.1 O-glycosides analogs of GalCer



5



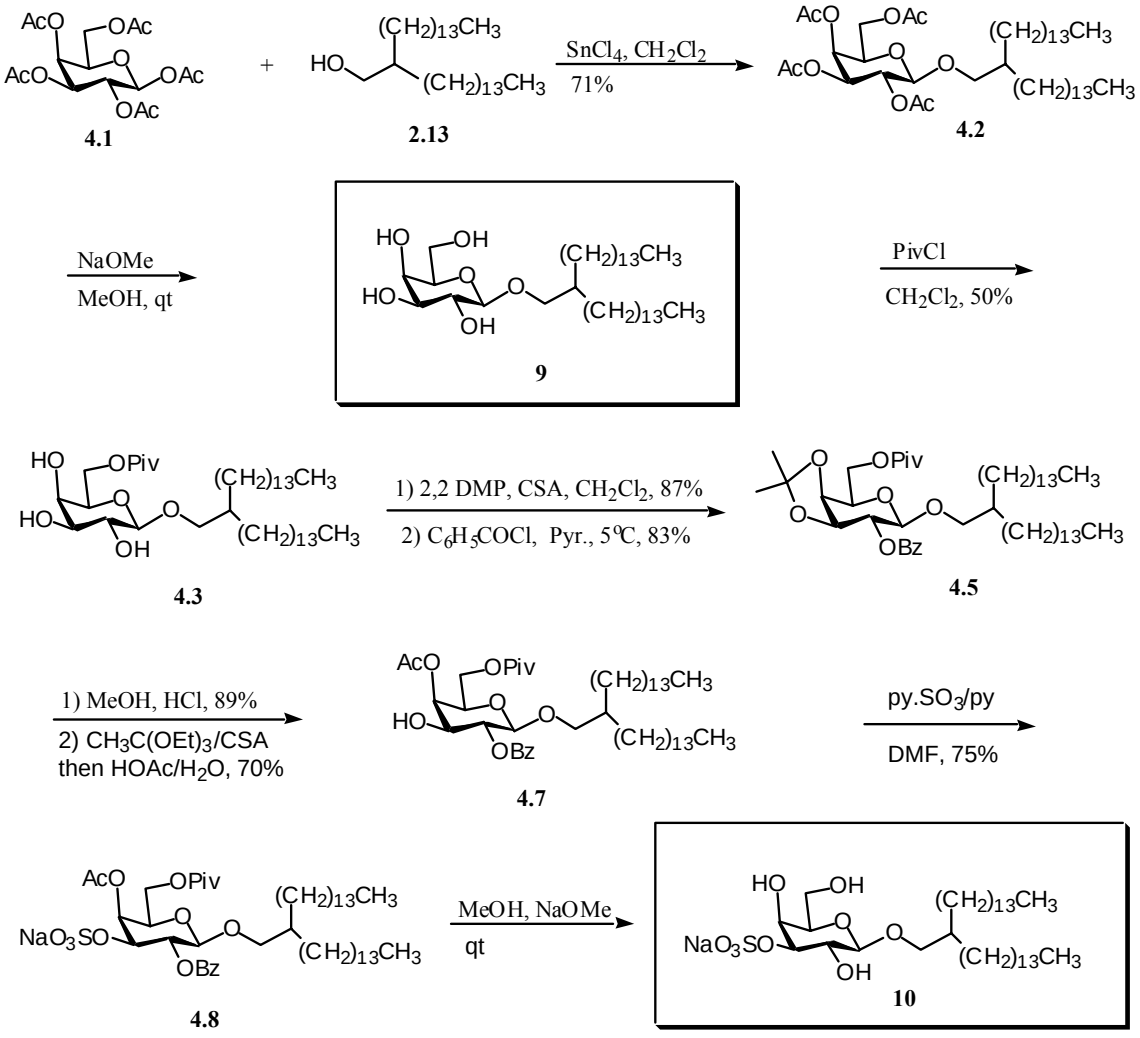
9 R=H
10 R=SO_3Na

4.2 Synthesis

4.2.1 Synthesis of analogs with a branched chain: 9 and 10

The synthesis started with the glycosylation of penta-O-acetyl-galactopyranose and alcohol **2.13** with tin (IV) chloride in dichloromethane to give the protected glycoside **4.2**. Basic hydrolysis of **4.2** with sodium methoxide produced the analog **9** in quantitative

Scheme 4.2 Synthesis of O-analogs with a branched chain

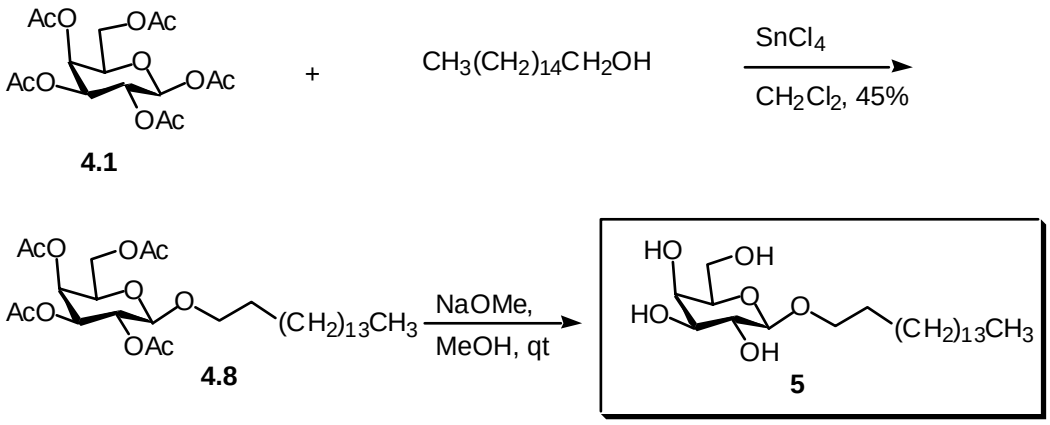


yield. The O-analog **9** was converted into the sulfated analog in seven steps. First the hydroxide group at C6 was protected with pivilate chloride in 50% yield. Protection of the hydroxide groups at C3 and C4 was done with 2,2 DMP to have the acetonide **4.4** with 85% yield, followed by benzoylation of the last hydroxide group at C2 to yield **4.5** in 83% yield. Selective acetylation of the axial hydroxide group at C4 with $CH_3C(OEt)_3$/CSA then $HOAc/H_2O$ in 70% yield followed the cleavage of the acetonide group with HCl (89%). Sulfonation of the 3-OH with the similar conditions as described in chapter 2, followed by basic hydrolysis provided the analog **10**.

4.2.2 Synthesis of an analog with a simple chain

The O-analog **5** was obtained in two steps following the same methodology described above. Glycosylation of the pentaacetategalactose and Cetyl alcohol provided the protected compound **4.8** in 45% yield. Basic hydrolysis gave the desired product **5** in quantitative yield.

Scheme 4.3 Synthesis of O-analog with a simple chain



4.3 Summary

The three known O-analogs **5**, **9** and **10** were synthesized using conventional methodology. Their synthesis started with glycosylation of tetraacetategalatose and the corresponding alcohol. Both tetra-ol **5** and **9** were obtained in two steps in 70% and 45% respectively. The sulfatide **10** was obtained in eight steps in 12% overall yield.

4.5 Experimental

Compound 4.2

Tin Chloride (0.471 mL, 2.56 mmol) was added under an atmosphere of nitrogen at rt to a solution of pentaacetategalactose (500 mg, 1.28 mmol) and dichloromethane (4 mL). The mixture was stirred and the alcohol **2.13** was added to the solution. The reaction mixture was stirred until completion for about 1 hr. The reaction mixture was then quenched with saturated $NaHCO_3$, the organic layer was extracted with dichloromethane, washed with brine, dried with Na_2SO_4 and concentrated under reduced pressure. The residue was purified by FCC to provide **4.2** (695 mg, 71%) as a syrup; R_f = 0.72 (30% ethyl acetate:hexanes); ^{1}H NMR (300 MHz, CD_3OD) δ 0.89 (t, *J* = 6.9 Hz, 6H), 1.49 (bs, 53H), 1.98 (s, 3H), 2.03 (s, 6H), 2.16 (s, 3H), 3.3 (dd, *J* = 6.61 Hz, 1H), 3.85 (dd, *J* = 5.1 Hz, 1H), 3.90 (m, 1H), 4.14 (dd, *J* = 7.0 Hz, 1H), 4.20 (dd, *J* = 6.8 Hz, 1H), 4.42 (d, *J* = 7.9 Hz, 1H), 5.02 (dd, *J* = 3.4 Hz, 1H), 5.20 (dd, *J* = 9.73 Hz, 1H), 5.40 (d, *J* = 3.31 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.4, 21.1, 23.0, 27.0, 29.5, 30.0, 30.5, 32.2, 34.0, 37.6, 61.9, 68.2, 72.7, 74.4, 76.6, 79.1, 169.9, 170.4.

O-glycoside 9

To a solution of **4.2** (840 mg, 1.09 mmol) in anhydrous methanol (20 mL) was added 2 mL of saturated sodium methoxide. The reaction mixture was stirred at rt for 10 min, then was neutralized with HCl and extracted with diethyl ether and concentrated under reduced pressure. FCC of the crude gave the desired compound **9** (652 mg, 99%); R_f = 0.13 (10% methanol:chloroform or 60% ethyl acetate:petroleum); ^{1}H NMR (500 MHz, CD_3OD) δ 0.90 (t, *J* = 6.96 Hz, 6H), 1.30 (bs, 53H), 1.87 (m, 1H), 3.03 (t, *J* = 8.42 Hz, 1H), 3.31 (bs, 2H), 3.40 (m, 2H), 3.70 (d, *J* = 5.86 Hz, 2H), 3.87 (bs, 1H); ^{13}C NMR (125 MHz, CD_3OD) δ 14.6, 23.9, 27.8, 30.3, 30.9, 31.3, 33.3, 34.7, 34.9, 38.8, 62.8, 70.9, 76.7, 79.7, 80.2, 82.2.

Compound 4.7

To a solution of compound **9** (1 g, 1.66 mmol) in dry dichloromethane (15 mL) and two small crystals of DMAP at 0 °C, was added 0.22 mL (1.75 mmol) of pivaloyl chloride. The reaction was stirred from 0 °C to rt for 1hr and diluted with methanol. The mixture was evaporated and the residue was purified to give **4.3** (600 mg, 53%) as a syrup; R_f = 0.70 (60% ethyl acetate:petroleum).

To a solution of **4.3** (36 mg, 0.053 mmol), dichloromethane (0.5 mL) and CSA (3.6 mg, 0.016 mmol) was added at rt 0.032 mL of 2,2-DMP. The reaction was stirred overnight then filtered and concentrated. FCC of the residue gave **4.4** (33 mg, 87%). Benzoyl chloride (0.04 mL, 0.343 mmol) was added at 5˚C to a solution of **4.4** (124 mg, 0.17 mmol) in anhydrous pyridine (1.5 mL). The mixture was stirred for 1 h at 5 ˚C, then methanol (1mL) was added to the cold mixture. The mixture was stirred for another hour,

then it was diluted with ethyl acetate (4 mL), washed with 1M aqueous sodium bicarbonate (2 mL), water (2 mL) and brine (2 mL). The organic phase was dried ($MgSO_4$), filtered and concentrated. FCC of the residue gave **4.5** (117 mg, 83%) as a viscous liquid; R_f = 0.50 (10% ethyl acetate:petroleum ether).

Concentrated HCl in ether (0.1 mL) was added at rt to **4.5** (117 mg, 0.24 mmol) in methanol (3 mL). The reaction mixture was followed and stopped after 20 min, neutralized with 0.1 NaOMe and concentrated under reduced pressure. FCC gave **4.6** (99 mg, 89%); R_f =0.25 (20% ethyl acetate:petroleum ether). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, *J* = 6.96Hz, 6H), 1.26 (s, 52H), 1.50 (m, 2H), 1.67 (s, 2H), 2.91 (d, *J* = 3.8 Hz, 1H), 3.44 (m, 2H), 3.82 (t, *J* = 6.2 Hz, 2H), 4.05 (m, 1H), 4.54 (dd, *J* = 4.8 Hz, 1H), 4.65 (dd, *J* = 6.2 Hz, 1H), 5.09 (t, *J* = 9.2 Hz, 1H), 7.4-8.0 (m, 10H)

A solution of **4.6** (99 mg, 0.05 mmol) in dry benzene (9 mL) and triethyl orthoacetate (9 mL) was treated with CSA (4 mg) at rt. The mixture was stirred for 1 h and then triethylamine (3.5 mL) was added followed by cold water (6 mL). The aqueous phase was extracted with ethyl acetate (3 x 10 mL), washed with aqueous sodium bicarbonate (15 mL) and water (15 mL) and brine (15 mL). The organic phase was dried ($MgSO_4$) and concentrated. The residue was dissolved in dichloromethane (10 mL) and treated with 80% aqueous acetic acid (20 mL). The mixture was stirred at rt for 35 min. Toluene (3 x 13 mL) was added and the mixture was coevaporated under vacuum. The residue was purified by silica gel chromatography and afforded **4.7** (114 mg, 92%); R_f = 0.20 (40% ethyl acetate:petroleum ether) ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, 6H), 1.45 (bs, 53H), 2.98 (bs, 1H), 2.20 (s, 3H) 3.33 (dd, *J* = 7.0 Hz, 1H), 3.87 (dd, *J* = 4.9 Hz,

1H), 4.1 (m, 2H), 4.41 (dd, *J* = 7.0 Hz, 1H), 4.59 (dd, *J* = 6.2 Hz, 1H), 4.65 (d, *J* = 8.0 Hz, 1H), 5.36 (dd, *J* = 10 Hz, 1H), 5.75 (d, *J* = 3.1 Hz, 1H), 7.2-7.55 (m, 5H).

Sulfatide 10

A solution of **4.7** (37 mg, 0.05 mmol) in dry dimethylformamide (2 mL) was treated with sulfur trioxide-trimethylamine complex (39 mg, 0.25 mmol) and the reaction mixture was stirred for 2 h, then sodium bicarbonate were added to saturation. The mixture was stirred overnight. The residue was diluted with dichloromethane and this mixture was filtered. The filtrate was evaporated and the residue was purified by silica gel chromatography to give **4.8** (30 mg, 79%); R_f = 0.70 (20% methanol:chloroform)**.**

To a solution of **4.8** (28 mg, 0.029 mmol) in 1.2 mL of methanol was added 0.5 mL (25% wt of Na in Methanol) of sodium methoxide. The reaction was stirred at rt for about 30 min. the reaction mixture was diluted with methanol, filtered and concentrated. FCC of the residue gave the desired sulfatide **10** (10 mg, 85%); R_f = 0.40 (20% methanol:chloroform). ^{1}H NMR (500 MHz, CD_3OD) δ 0.90 (t, *J* = 7.4 Hz, 3H), 1.29 (bs, 28H), 1.41 (m. 2H), 1.61 (m, 2H), 3.39-3.54 (overlapping peaks, 4H), 3.74 (d, *J* = 5.86 Hz, 2H), 3.79 (q, *J* = 6.35 Hz, 1H), 3.84 (d, *J* = 2.93 Hz, 1H), 4.18 (d, *J* = 7.81 Hz, 1H). ^{13}C NMR (125 MHz, CD_3OD) δ 14.6, 23.9, 27.8, 30.3, 30.9, 31.3, 33.2, 34.7, 34.9, 38.8, 62.8, 70.9, 76.7, 79.7, 80.2, 82.2.

Compound 4.8

The protected compound was obtained as described for **4.2**. The residue was purified by FCC to provide **4.2** (726 mg, 50%) as a syrup; R_f = 0.70 (30% ethyl

acetate:petroleum); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (t, *J* = 6.9 Hz, 6H), 1.49 (bs, 30H), 1.98 (s, 3H), 2.03 (s, 6H), 2.16 (s, 3H), 3.3 (dd, *J* = 6.61 Hz, 1H), 3.85 (dd, *J* = 5.1 Hz, 1H), 3.90 (m, 1H), 4.14 (dd, *J* = 7.0 Hz, 1H), 4.20 (dd, *J* = 6.8 Hz, 1H), 4.42 (d, *J* = 7.9 Hz, 1H), 5.02 (dd, *J* = 3.4 Hz, 1H), 5.20 (dd, *J* = 9.73 Hz, 1H), 5.40 (d, *J* = 3.31 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.4, 21.1, 23.0, 30.5, 32.2, 34.0, 37.6, 61.9, 68.2, 72.7, 74.4, 76.6, 79.1, 169.9, 170.4.

1-Hexadecyl-O-galactoside 5

The targeted tetraol compound was obtained with **4.8** (723 mg, 1.09 mmol) as described for **9**. FCC of the crude gave the desired compound **5** (645 mg, 99%); R_f = 0.20 (10% methanol:chloroform or 50% ethyl acetate:petroleum); ^{1}H NMR (500 MHz, CD_3OD) δ 0.90 (t, *J* = 7.02 Hz, 3H), 1.29 (bs, 28H), 1.38 (m. 2H), 1.62 (m, 2H), 3.44-3.55 (overlapping peaks, 4H), 3.73 (m, 2H), 3.83 (d, *J* = 2.75 Hz, 1H), 3.88 (q, *J* = 7.02 Hz, 1H), 4.20 (d, *J* = 7.32 Hz, 1H).^{13}C NMR (75 MHz, CD_3OD) δ 14.4, 21.1, 23.0, 30.5, 32.2, 34.0, 37.6, 61.9, 68.8, 72.7, 74.4, 76.6, 79.1.

Chapter 5

Biological findings and conclusion

5.1 Summary of synthesis and NMR data for synthetic analogs

Among glycolipids, Galactosylceramide (GalCer) **1** and its sulfated analog 3'sulfogalactosylceramide (SGC) **2** are of great interest due to their involvement in the fusion and entry process of HIV-virus in the cell. We have synthesized six analogs of GalCer and two analogs of SGC. The C- and aza-C-glycoside analogs originated from a C1-substituted galactal precursor, using the methodology developed in our research. The syntheses and NMR data for these synthetic analogs are summarized in Figures 5.1 and 5.2, and Tables 5.1 and 5.2.

Figure 5.1 Summary of the synthesis of the C-glycosides

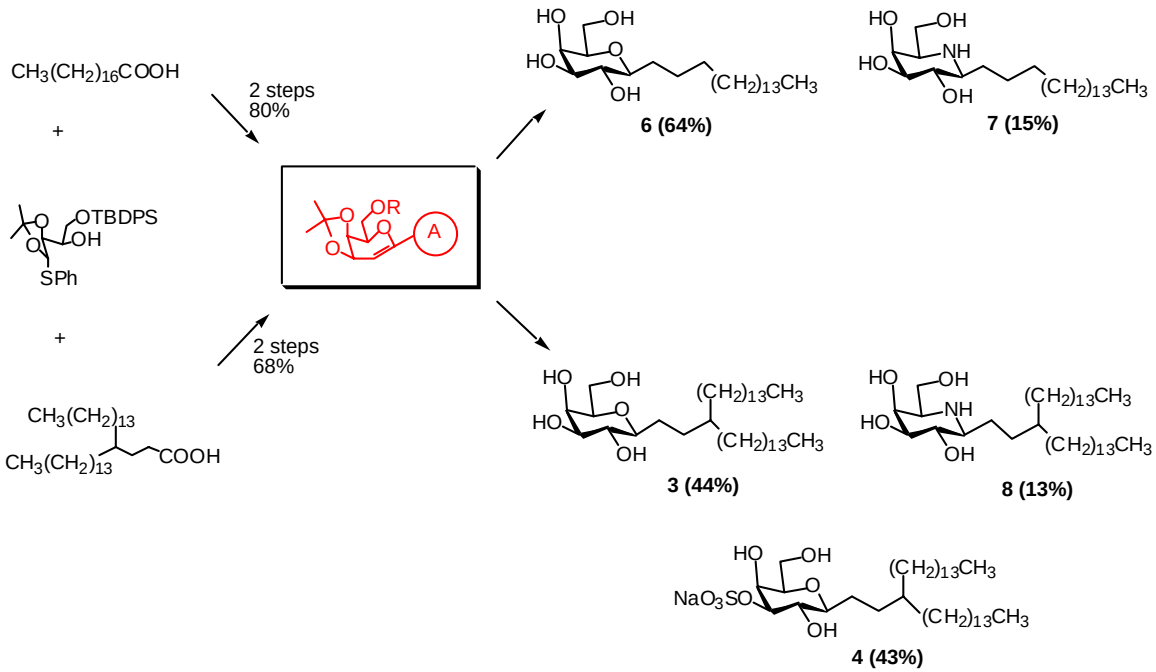


Table 1.5 1H NMR Data for the synthesized C-glycosides

	C-glycosides						**Aza-C-glycosides**			
	3		**4**		**6**		**7**		**8**	
H	ppm	m(*J*, Hz)	ppm	m(*J*, Hz)	ppm	m(*J*, Hz)	Ppm	m(*J*, Hz)	ppm	m(*J*, Hz)
1	3.03	t(8.42)	3.10	t(7.51)	3.06	t(7.9)	2.78	m	2.45	t(2.45)
2	3.42	*	3.56	t(9.38)	3.38	*	3.62	t(10.0)	3.40	*
3	3.42	*	4.23	d(3.10)	3.38	*	3.42	dd(3.0,8.5)	3.32	*
4	3.87	S	4.18	dd(3.13,6.27)	3.87	m	4.00	s	3.90	Bs
5	3.42	*	3.44	t(6.25)	3.38	*	3.15	t(6.5)	2.82	t(6.09)
6	3.70	d(5.86)	3.68	d(6.27)	3.69	m	3.78	m	3.65	d(6.23)

* peaks were overlapping or was unable to assign multiplicity.

Figure 5.2 Summary of the synthesis of the O-glycosides

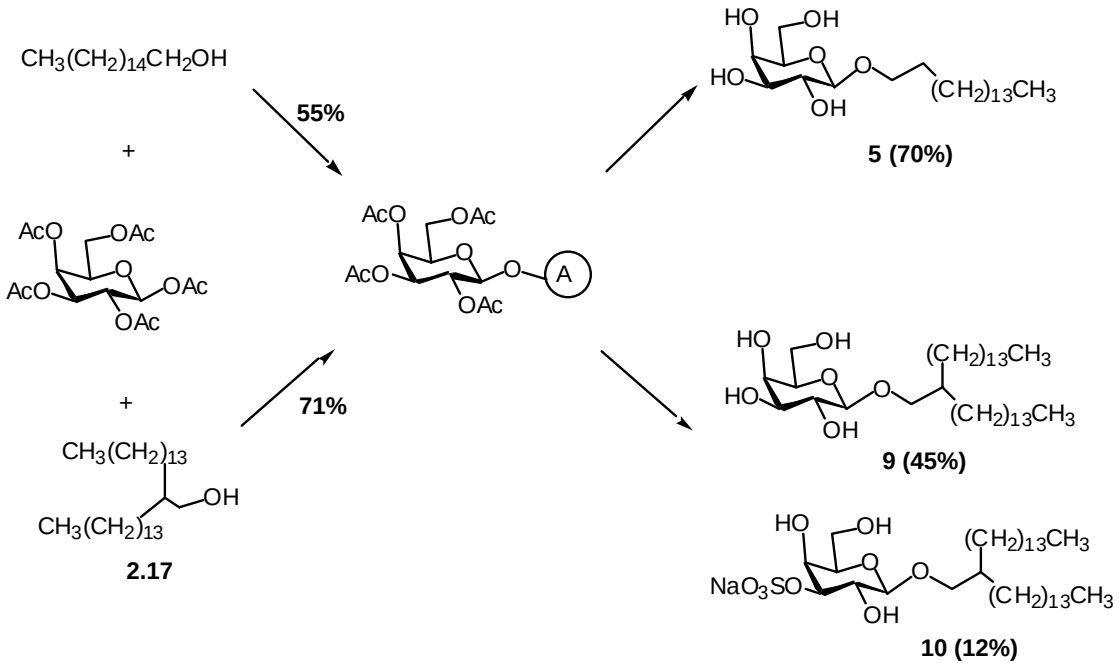


Table 5.2 1H NMR data for the O-glycosides

	O-glycosides					
	5		**9**		**10**	
H	ppm	m(*J*, Hz)	ppm	M(*J*, Hz)	ppm	m(*J*, Hz)
1	4.20	d(7.32)	3.87	bs	3.79	q(6.35)
2	3.44	*	3.31	*	3.39	*
3	3.88	q(7.02)	3.70	d(5.86)	4.18	d(7.81)
4	3.83	d(2.75)	4.19	m	3.84	d(2.93)
5	3.44	*	3.40	*	3.39	*
6	3.73	m	3.40	*	3.74	d(5.86)

5.2 Biological evaluation of GalCer analogs

The binding of the GalCer **1**, and analogs **6** and **7** to recombinant gp 120 was measured in a monolayer assay performed by Professor Jacques Fantini (Universite Paul Cezanne, Laboratoire de Biochimie et Physicochimie des Membranes Biologiques, INRA-UMR 1111, Faculte des Sciences et Techniques de Saint-Jerome, 13397, Marseille Cedex 20, France).

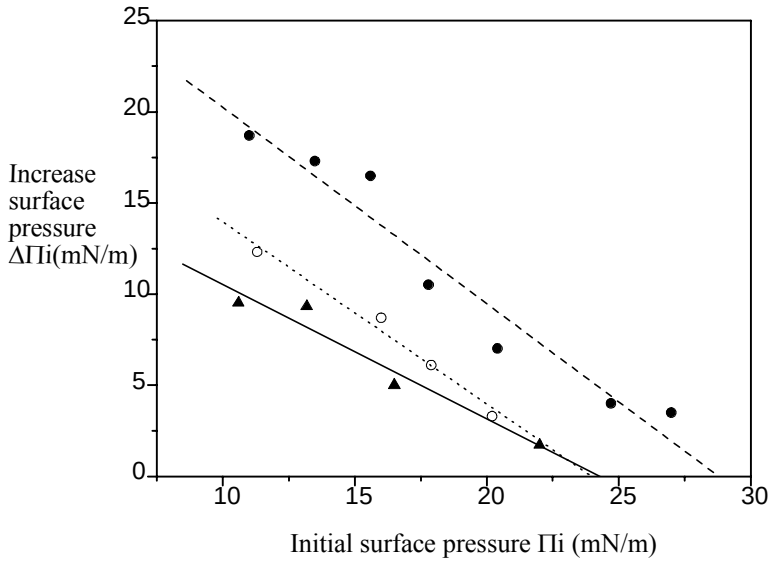
The change in surface pressure ($\Delta\pi$) at the air-water interface of the glycolipid monolayer, was determined, upon exposure to an aqueous solution of the recombinant gp 120 (10 nM)[39]. Measurements were obtained on a fully automated microtensiometer (μTROUGH SX; Kibron, Inc., Helsinki, Finland). The apparatus allowed the real-time recording of the kinetics of interaction of a soluble ligand with the monomolecular film, using a set of specially designed Teflon troughs. All experiments were carried out in a controlled atmosphere at 20 ± 1 °C. Monomolecular films of **1**, **6**, and **7** were spread on pure water subphases (volume of 800 μL) from hexane–chloroform–ethanol solution[44]. After spreading of the film, 5 min was allowed for solvent evaporation. To measure the interaction of gp120 with GalCer and its derivatives, recombinant gp120 (IIIB isolate, 10 nM) was injected in the subphase with a 10 μL Hamilton syringe, and pressure increases produced were recorded until reaching a stable value ($\Delta\pi$). The experiment was repeated at different values of the initial surface pressure (π_i) of the monolayer. The data were analyzed with the Filmware 2.5 program (Kibron, Inc.). The results are expressed as the variations of $\Delta\pi$ as a function of π_i for compounds **1**, **6**, and **7**. The accuracy of the system under our experimental conditions was ±0.25 mN/m for surface pressure.

Increase in surface pressure is associated with integration of gp120 into the glycolipid monolayer and is interpreted as a measure of gp120-glycolipid binding. Because the increase in surface pressure is a measure of binding of gp 120 to the glycolipid monolayer, these numbers suggest that **7** has a higher affinity for gp120 than **1** and **6**. Compound **6** showed comparable binding as GalCer **1**: the critical pressure of insertion, is in the range of 24 mN/m for both compounds. On the contrary, compound **7** showed a significantly higher value of the critical pressure of insertion (28.5 mN/m) compared to **1** and **6**.

This data concurs with earlier observations with related synthetic stearyl and stearoyl monosaccharide derivatives[11]. However, they contrast with other findings which indicate that fatty acid residue in the ceramide moiety is a critical requirement for binding. Thus, in the identical monolayer assay, the GalCer analog without the alpha hydroxy group in the acyl chain was completely inactive[13], and in a solid phase assay psychosine (without the acyl chain) is inactive[17]. The unusual behavior of the single chain GalCer analogues **6** and **7** may shed some light on the molecular mechanism of GalCer-gp120 interaction. It has been suggested that the polar head region of the ceramide is important for controlling a preferred orientation of the sugar for binding to gp 120. To the extent that the lipid monolayers used in this binding assay simulate GalCer molecules on the cell surface, and the simple hydrocarbon chains are not expected to favor a well defined conformation, the data for **6** and **7** might be an indication that the orientation of individual sugars is not critical as the geometry of a multivalent assembly of monomers. This speculation is consistent with the notion that the hydrophobic chains in GalCer facilitate clustering into microdomains, leading to multivalent binding to gp 120. That the

binding of the aza analog **7** is stronger than GalCer is in of itself noteworthy, and opens up new possibilities for mimetic design. This effect could be related to the degree of

Figure 5.3 Binding of gp 120 to glycolipid analogs 1, 6 and 7



1 (open circles, dashed line), **6** (full triangles, solid line), **7** (full circles, dotted line)

aggregation and geometry of the presumed microdomain, or to more intimate receptor contacts of the individual sugar residues[40]. Nevertheless, in the absence of a wider set of analogs additional speculation is premature. The gp120 binding of the remaining analogs and HIV fusion, infectivity and cytoxicity studies are in progress.

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Chapter 6

Enactment of chemistry knowledge by a high school student at a summer program

6.1 Introduction

This case study is to evaluate the acquisition of knowledge of one student participating in a five-week intensive chemistry program by analyzing her weekly performance on a standardized chemistry test and by looking at her interactions in different learning activities (fields). The analysis of her answers provided a pattern of incorrect responses on a specific topic and the analysis of her praxis in the different fields revealed a different understanding of what she knows about that specific topic. From these analyses, I recognized a behavioral characteristic of the student in three of the four fields studied. The microanalysis of the videotapes of the three fields has helped identify how Kelly demonstrated a caring attitude for her peers in the lecture, the laboratory periods and the tutoring sessions while showing a very good understanding of chemistry. But when she took the tests, the answers to the questions did not reflect the same level of understanding showed in the other fields. I attribute this difference to the caring role she was able to play in the first three fields. This role is known in the literature as "othermother". Contrary to what is known, the role of othermother of young girls in academic settings can be favorable in enacting their scientific knowledge. Specifically, in this study, I will show how a young Hispanic girl was able to use such a role to be efficient in a chemistry summer program.

An “othermother” can be defined as a woman other than a biological parent who fills the role of a mother; grandmothers (Gibson, 2002) and teenage girls often play this role. The role of othermother is traced by Collin (1990) back to slavery times when elderly women and young girls were expected to take care of young children and babies while their slave mothers were working on the plantation or accomplishing other tasks. It was used as a survival resource that served to educate the children about their origin.

This practice has become a practice among colored people and is reproduced in the community. It “can be associated with a universalized ethic of care, where othermothers within the community exhibit a sense of responsibility to the offspring of other mothers”, so “community othermothers work on behalf of the Black community by expressing ethics of caring and personal accountability, which embrace conceptions of transformative power and mutuality” (Case, 1997). Every female (grandmothers, sisters, aunts, cousins) share the responsibility of child rearing, the role of “othermother”. In a study conducted by Carol Gilligan and Amy Sullivan with twenty-six girls from poor and working-class Hispanic, Italian, Caribbean and African American, attending Boston public schools, 85% of them mentioned having an adult woman mentor, an “othermother” (Daigneault, 1998). As the girls started high school, their experiences with these “othermothers” were positive in strengthening their self-esteem.

In school settings, this practice is reproduced in the classroom when girls are expected to help boys who have been absent to catch up on the topics of the class. In contrast, boys are not usually expected or asked to carry out similar roles (Scantlebury, 2004). Also, in the case of the female adolescents, this role often keeps them away from school, depriving them from adequate educational opportunities. They often have to stay

home to take care of younger siblings. Scantlebury (2005) refers to this practice as a form of inequity between boys and girls.

6.2 The study

The Mathematics Sciences Partnership in New York City

The Mathematics Sciences Partnership in New York City (MSPinNYC) is a joint project of the Department of Education and the City University of New York funded by the National Science Foundation. The focus of the project is to improve high school students' performance in the critical areas of mathematics and the sciences through a combination of school reform and partnerships between college and high school faculty. Integral to the project is an intensive summer school. It is from this summer school that the case study is drawn.

Design of the study

This study was done while working on the qualitative evaluation of the MSPinNYC project. The evaluation team conducted ethnography and collected some quantitative data. A variety of data resources were used in an ethnographic analysis.

The observations of the students in the summer took place over a five-week period from July 12 to August 16, 2005. Two researchers observed the three fields (classrooms, the laboratory and the tutoring sessions) at various times throughout the study. The schedule was arranged so that each researcher observed all fields twice a week.

Through this case study, I explored the extent to which students understood the content of the activities in which they were engaged in the different fields and how they represented their understandings in the exam. Specifically I investigate how the structure provided by the summer program expanded or truncated the enactment of Kelly's chemistry knowledge and why.

Data sources and data collection

I observed the different fields, videotaped and audio taped them and interviewed selected participants. Most interviews were non-structured and the goal was to allow the interviewee to speak without interruption. A series of nominal group interviews were undertaken with different groups of participant (high school teachers, college faculty, undergraduate and high school students). Field notes were written and some were given to the teachers and other stakeholders.

A number of artifacts was collected, including copies of answer sheets from mock exams, copies of problems and questions solved in tutoring, copies of post-assessment of different class activities. Digital pictures of students' work were also taken. The data resources I used are the first four of the six Regents exams administered during the five weeks in which classes were held (the fifth one was given to the student before I could make a copy and I did not have access to the last one), vignettes from videotapes of the different activities and my field notes. The video vignettes allow me to analyze Kelly's behavior in each field, evaluate her learning, and describe her enactment of chemistry during the summer.

Data analysis and interpretation

Data were compiled into written field notes and/or video vignettes. Team meetings were scheduled twice a week; one meeting was with the team members observing the same subject group, the other was with all the members of the evaluation team. During the meetings, we discussed the data collected and decided on which aspects we would focus for the next week and the data needed to be collected. The findings were organized with illustrative vignettes of supporting evidence, exceptions and contradictions.

The Summer Program

In spring 2005, two high schools, namely Franklin High School and Orchard High School were chosen to participate in the MSPinNYC. Their first involvement in MSPinNYC was the summer program 2005 in which several mathematics, living environment, and chemistry teachers participated along with some of their students. It was held at Seeker College, which, for several years, was the host of a summer institute that serves as a model for the MSPinNYC.

During the five weeks, high school teachers (prospective, new and experienced), college faculty, and undergraduate students collaborated to improve their teaching practices and facilitate students' learning. There were four groups of students according to the subjects (1 Chemistry, 1 Living Environment and 2 Mathematics).

Twenty-seven students from the two schools were expected to participate in the chemistry session for the summer. Twenty-two students started the program, by the second week, the number increased to twenty-three students. Originally, three students

were designated as cadets and were expected to act as peer tutors in the classroom. However, after the first exam, because of their low performance, the cadets joined the class as students with the expectation that became these students would become tutors at their school during the academic year and during the next summer program. Although the students from Orchard High School did not take the chemistry Regents' exam at their school, these three students (upon recommendations of the school's principal and their teacher) were admitted to the program as cadets and were expected to take the official summer Regents exam at the end of the program.

During the five weeks, the teaching activities (fields) were scheduled from 10:00 AM to 5:00 PM as shown in the table below. The three activities were scheduled every day. During the first and the second week, the classroom and laboratory activities were done either in the morning or the afternoon. Tutoring sessions served as reinforcement for what was taught in the classroom and the laboratory. At the end of each week, the students were given a mock Regents exam as a measure of their learning and as a practice for the real Regents exam.

Table 6.1 Schedule for week 3 and week 4

	Monday	Tuesday	Wednesday		Thursday
10:00-12:00	Lecture	Lecture	Lecture	10:00-12:00	Lecture
12:00-1:00	Lunch	Brown Bag	Lunch	12:00-1:00	Brown Bag
1:00-3:00	Lab	Lab	Lab	1:00-4:00	Regents exam
3:00-5:00	Tutoring	Tutoring	Tutoring	4:00-5:00	Tutoring

The scores on the Regents exams were used as indicators of teaching and learning effectiveness and to arrange and rearrange the groups in the tutoring sessions. A total of six exams was administrated; five of them were previous Regents exams taken from the New York City Department of Education website[1], the last exam was the official summer Regents exam. Chemistry Regents exams for August 2004, January 2005, August 2003, June 2003, June 2004 and August 2005 were used for the mock Regents exams. The MSPinNYC administrators set the passing grade at 65 (except for chemistry, the official passing grade was 55 but will change to 65 for students entering high school from 2006). The reason for a higher passing grade was an attempt to motivate students to reach for higher passing grades. Answer sheets of each exam, except the final exam, were provided to the students to help them review. Some students spent time going over their incorrect answers. The teaching staff helped them during spontaneous breaks or before sessions. During the week before the official exam, previous Regents exams were reviewed in the classroom and the tutoring sessions. There were no lab sessions during the last week of the program.

The teaching staff schedule was planned to give everyone a chance to participate in the lectures either as a lead teacher or as a support teacher. During the lab periods, the teachers who were responsible for the lectures and the tutoring sessions were planning and getting ready for their teaching. This arrangement worked well due to the ratio of educators to students. In the chemistry TRT, there were twelve teaching staff and six to ten rotating others (students fulfilling field work requirements). Among the twelve, were four high school teachers, three college professors and five undergraduates.

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[1] www.nycenet.edu

Orchard High School

One of the reforms employed to get higher levels of performance from low achieving high schools has been the creation of either self-contained Small Learning Communities (SLCs) or Small Schools (SCs) from existing schools.

Small Learning Communities are "schools-within-a-school that would foster a greater sense of school community and belonging among the students and allow for closer and more comprehensive oversight of a student's academic and social progress."[2]

Small Schools may be in its own building or in a building with another school(s), but is organizationally, fiscally, and instructionally independent. Its teachers and students are self-selected. An ideally autonomous school is one that controls, not only its structure, budget, and learning program, but also (1) establishes its own transportation and school-day schedule; (2) has its own teachers and students; (3) has its own classroom space; and (4) once basic agreements are struck with others in the building about schedules and facilities, its use of space and time cannot be infringed upon.[3]

"The chief idea was that students would experience a small school feeling, go to classes in a confined part of the building, and be taught by a relatively small number of teachers who could personalize the experience" (Tobin, 2005). In 2002, aligning with this trend, Apple Valley High School was divided into three Small Schools. Orchard High School is one of them. From the school mission statement, the school was created to

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[2] From San Bernardino City School district website: http://www.sbcusd.k12.ca.us/new/

[3] This information is drawn from the work of Ancess & Ort, 1999; Gladden, 1998; Gregory, 2000; Kacan & Schipp, 2000; Lashway, 1998-99; Raywid, 1996; Small Schools Project, 2001a; and Wasley & Lear, 2001.

"provide a family-like atmosphere in which student needs receive immediate attention in order to foster student development and academic achievement". The school is located in the Bronx section of Region 9 of the New York City Department of Education in an urban, low income, working class, Latino and African American neighborhood. Orchard High School houses students from grade 9 to 12, with the academic 2005-2006 year's 12th grade being the first graduating class of the school. The average class size was 20 students. In 2004, 61 percent of the students were Hispanic, 38 percent were Black and less than one percent were Asian, with 51 percent female. Eighty two percent were eligible for free lunch. Only 8 percent of the students were enrolled as English Language Learners.

At the time of this study, the eleven teachers of the school were all licensed and had more than two years of teaching experience. Seven teachers hold at least a master's degree[4]. Four of these teachers participated in the summer program, each being the only teacher teaching his or her particular subject at the school. This is one of the reasons, besides not fulfilling the required laboratory activities, that the students at Orchard High School could not take the chemistry Regents' exam. Chemistry teacher Donovan is the only chemistry teacher at Orchard High School. During the school year, Donovan had to fulfill requirements for his masters degree and was not able to teach for several days. He missed so many classes that he was not able to cover several topics in the curriculum such as acids and bases, oxidation-reduction and organic chemistry. For this reason, all twenty students from Donovan's class were eligible for the summer program. However, seven signed up to come to the summer program and only four participated.

[4] The quantitative data were obtained from the school's Annual Report Card 2004.

Each Small school occupies a specific space in the building and interactions among the three schools' members are very limited. Orchard High School occupies the third floor and half of the fourth floor of the building that houses the Apple Valley community. The two other schools are accommodated within the remaining floors of the five-story building. A disadvantage of such structure is that each school inherited the classrooms on their allocated floors and, since there are no instructional interactions between the schools, specialized classrooms are not shared. This unbalanced distribution of resources left Orchard High School with no chemistry laboratory. The downside of the small school movement is not atypical of Apple Valley; similar problems were reported in Small Learning Communities opened in Philadelphia in the early 1970s (Tobin, 2005).

The student

Five students from Orchard High School started the MSPinNYC summer program. Three of them were girls and two were boys. One girl did not come because she was needed at home to take care of a younger sibling since her mother had to work. This study case focuses on one of the two remaining girls: Kelly. Kelly is an 11th grade student. She is 17 years old and was born in Ecuador. Her father is a jewelry polisher and her mother is a jewelry sample maker. She has three brothers and is the oldest child. She seems to be doing fairly well in school and had just passed her Living Environment Regents' exam with a scaled score of 70. Kelly was asked to write one paragraph describing her family and her neighborhood, she wrote the following:

> *Right now I live with my mom in Manhattan and my father lives in the Bronx. My parents have been divorced for 10 years and I am the oldest out of all my siblings.*

> *You could say that not only am I mother figure to them but also a role model to my three brothers. My neighborhood is an O.K. place to live at. I live in the projects, which I can consider a safe place. You would not get into problems if you just worry about yourself and don't get into any conflicts.*

Kelly is not just a student and a daughter but she has extended roles as an othermother and as a role model for her siblings, enacting a range of mother type responsibilities such as helping with cooking, laundry, etc. Kelly seems to be seriously aware of her influence to her siblings: "*You could say that not only am I mother figure to them but also a role model to my three brothers."*

Theoretical Framework

In fall 2004, when I got involved in the MSPinNYC project as a member of the evaluation team, I knew virtually nothing about educational research or education methodologies and pedagogies. I have training in organic chemistry and am interested in research in education. Although I had come to realize that content knowledge of a subject does not make someone a good teacher or educator, I did not learn much about pedagogical methods. I participated in the evaluation team with four students all candidates for a doctoral degree in urban education under the supervision of a distinguished professor of urban education. Often, I felt worthless and in despair. Fortunately for me, ethnography was the core part of the evaluation plan and none of the other students were familiar with qualitative evaluation. Together we learned how to employ fourth generation evaluation (Guba and Lincoln, 1989) in which numerous

quality criteria are applied to ensure that the data and associated analyses and interpretations are credible and that the evaluation makes a positive difference to the participants. The methodology requires participants, at all levels, to be identified and to be given a voice so that the study reflects a common shared experience. We learned how to observe, videotape, interview and interact with the different participants and how to obtain a variety of qualitative data resources such as field notes, interview transcripts, artifacts produced by the participants, email exchanges, vignettes, etc. A significant component of qualitative research is data analysis. In analyzing the data resources, two questions must be answered. The first one is: what is happening? And the second one is: why is it happening? Answering the first question was not much of a problem for me because it is describing what I see (although I later realized that description of what's happening relates strongly to the theoretical framework). But when I had to answer the second question I truly stumbled. Attempts to answer that question requires a theoretical background that I did not have.

As I was always interested in knowing more about how people learn, I became very interested in qualitative research because it seeks a wide understanding of a whole situation. Not only does it produce in-depth, comprehensive information, but also it uses a participant's background and observations to describe the context, the setting and all variables related to the subject under consideration, as well as the interactions salient to the study. I believe that the socio, cultural and economic context of a student's life is as significant as the way his or her brain works in mediating her abilities to learn and reflect knowledge. So when a science educator, Kenneth Tobin, professor of Urban Education of

the Graduate Center, introduced me to William Sewell's perspective[5] on culture, structure and agency, and after attending his classes, Theoretical and Empirical bases for Research in Urban Sciences, Mathematics and Technology Education, and Qualitative methods of Research in Urban Education, I decided that I would adopt Sewell's perspective along with Pierre Bourdieu's theory of power and practices and Randall Collin's sociology of emotions as theoretical background for my research. Also, I found that activity theory of Vygotsky could be a useful tool to collect information and guide in analysis. Activity theory allows for the study of different forms of praxis as developmental processes with both the individual and the social interconnected.

In this study, I predominantly used Sewell's approach in searching for patterns of coherence and contradictions, as they are dialectically interconnected and I tried to use them to understand the role of the structure of the summer program on Kelly's agency and enactment and production of her chemistry knowledge. Agency necessitates access to the resources of a field and the cultural capital needed to appropriate them; individuals use resources to meet their goals and for the production and reproduction of culture.

6.3 Results

6.3.1 Learning at the summer program

In the classroom

During the summer program, the teaching staff collaborated and cotaught in the three different fields. In the classroom, there were usually two lead teachers and the others served as support to the teaching process. As the TRT members became more

[5] See glossary for definition of: Sewell's perspective on culture; Pierre Bourdieu's theory of power and practices; Randall Collin's sociology of emotions and Activity theory.

aware of collaborative teaching, the teaching staff became more available to the students, sitting next to the students and assisting them to understand what was going on in the class and answering their questions. In this setting, the students could not only interact with the lead teachers but also with the teacher or teachers sitting next to or near to them and also with their peers.

From the videotapes of the three activities, I saw that Kelly was engaged in the class. She would discuss the questions asked with the other students usually the other cadets and with the teaching staff sitting next to her. She seemed to have a high level of awareness in class. She was attentive and very determined to pass the Regents exam with a good grade (the goal for the cadets was to pass with a scale score of 80 percent or more).

Kelly did not rely much on the teaching staff sitting with or near her. Once an assignment was given, she would try to do it on her own first. When needed, Kelly would look at the periodic table on the wall searching for answers. She would turn to her friends (who were also cadets and from the same school) for help first before asking the teaching staff nearby. And often she needed very little help. This excerpt below from field notes of a classroom activity shows how much Kelly tried to solve the problems herself first before asking for help and how able she was to continue working independently after the help was provided.

> (2:40) Kelly has her finger on her lips, she stares at her problem sheet, her eyebrows are raised up. She is trying to figure out the problems that the lead teacher just asked them to solve. She is focused. At her right is Hiris, a teaching staff and at her left is Donald. Donald is trying to solve the problems too. After a few seconds, Hiris starts to explain the problems with big hand gestures (2:43). Donald then focuses and tries to understand. For more than a minute, Kelly refuses help that is volunteered to her, purposely trying to solve the problem on

her own (still in the same posture and ignoring the conversation that is taking place between Hiris and Donald). Hiris continues to help Donald with (2:54).
Finally, Hiris addresses Kelly directly (3:02). Kelly opens her mouth, lift up her eyelashes to acknowledge her while focusing on the sheet. Kelly never raises her eyes to look at Hiris. Kelly rubs her lips with her finger, the finger that never leave her face, tight her teeth, turns to Donald, looks at his work, grimaces that she doesn't get it, takes the finger out of her face for a moment. Hiris points at something on her sheet; Kelly closes her lips and goes back to her initial posture while Hiris is trying to help. Now she gives full attention to Hiris. Donald is working on the problems.
Kelly seems to have returned to her own reflection when (3:13) she raises her hand in the direction of the blackboard, puzzled, points with her pen and asks Hiris about something on the blackboard. Immediately, Hiris gives her full attention to answering her question (3:15). At this point, Kelly seems to understand and starts to write on her sheet smiling as she does so. Hiris continues with her explanation, Donald is looking at what Kelly is writing. Kelly seems to have regained her confidence, putting herself down to write with a satisfied face. (3:20) Hiris points to something else on her sheet. She stops writing and pays attention, listening to her explanation. Hiris is leaning on her desk. Kelly gives her full attention. (3:25) Kelly shakes her head in approval and understanding, bites her lips and continues to write as soon as Hiris removes herself from the desk (3:27).
Hiris continues to interact with Donald while Kelly is back focusing on her work, solving the problems. She continues to work alone until (4:36) she requests help again from Hiris. Although Hiris is willing to help, Kelly only needs a little push and continues to work on her own until the end of the session.

Picture 1.- Passive engagement of Kelly in the classroom. She is looking at the board with a staring appearance. There are moments during the lecture; she seemed completely detached and not interested in what is being taught.

Picture 2. - At another lecture, Kelly yawned several times and kept playing with her hair. Was she not interested in the subject matter or was she just tired that day?

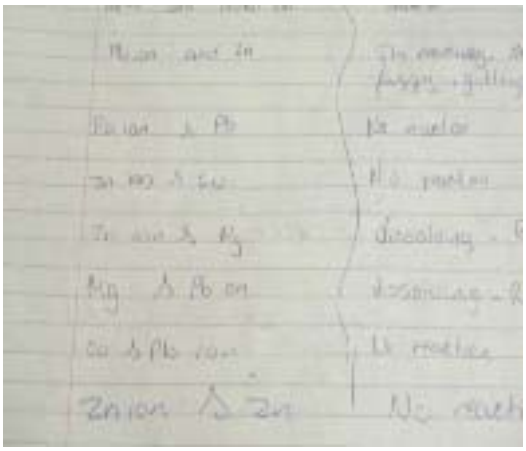
Although, she is usually active and well engaged in the classroom, at times, Kelly seemed bored or not challenged by the class materials. Pictures 1 and 2 show Kelly looking sleepy and bored in the lecture. This is a contradiction in Kelly's attitude for what was done in the classroom. Usually, she has a good engagement in the learning process but in these examples, Kelly has a passive engagement and was not in synchrony with the teaching staff. Maybe she was tired on that day or was hungry or did not understand what was being taught on that day. The reason of her passive engagement is not known and occurrences are sporadic and not consistent.

In the lab

Mostly the new and prospective teachers with the help of a more experienced high school teacher or college faculty taught the laboratory sessions. As in the classroom the teaching staff collaborated in the teaching and enacted different roles: there were two lead teachers and a minimum of two other teaching staff floating in the lab helping the students.

During one of the lab periods observed, Kelly seemed to be at ease. Although, she did not have any prior experiences with chemistry laboratory activities, she seemed very comfortable with what they were doing. The topic of the lab period was about the reactivity of metals and the students had to test the reactivity of different metals with different solutions.

worked well with two other cadets and
e different steps of the experiment. Here
ld, observing the reactivity of the metal in
te their observations. Earlier, Donald was
tion in one of the tubes with Fritz. Kelly
g him to continue his job.



Pictures 5. - and picture 6. - Kelly did the work for Donald in writing their observations from the reactions of the different metals and solutions. These pictures show the notes that Kelly wrote for the group.

More than proving how Kelly was involved in the lab, these pictures captured her enactment of her role of othermother in the laboratory activities. She was in control, getting things done and keeping the others in her group on tasks. She was not rude or authoritarian but caring and supporting. When the work assigned was not done, she

would do the job just to have the work done. It is interesting how she was able to use this role to be a caring peer, facilitating the understanding of chemistry and the work to be done. She initiated the division of labor within the group. She would double check on results given by other members of the group. Looking at her in the lab, one can see that she is a child that is used to having responsibilities. I can recognize in her behavior, the impact of her extended role as a mother figure for her siblings. In the lab, she is probably reproducing this role of taking care of things, coordinating activities (feeding time, study time and bed time) at home and helping with homework.

In the tutoring session

In the tutoring session, the size of the student groups varies from one to six (the six cadets were always together and the most challenged of these students had individual tutoring). Each group was assigned to one tutor. This tutor could be a college faculty, a high school teacher or an undergraduate. The students in a group were rearranged each week according to their grades from the mock Regents exam and their interactions with teaching staff. It was noticed that certain students developed better relationships with some of the teaching staff, so they were assigned in a group with that particular tutor. So, decisions on grouping depended in part on achievement and also on the rapport between the teacher and the student.

Whether she was tired at 3:00PM when the tutoring sessions started or she was not challenged by the tutoring assignments, Kelly would do the work and move on with not much enthusiasm, in contrast to her participation in the lecture and the lab. The assignments seemed to be easy for her. As soon as they were given and assigned she

would start working on the problems and would finish before the others. She participated in the discussion and was aware of what was happening and was in synchrony with the teaching staff and the group. Even when the others were discussing their assigned problem with the teaching staff, Kelly remained focused on what was being discussed, nodding her head or grimacing when the answers given by another student were incorrect but not answering the question unless the teaching staff asked someone from the group to help. The following is a vignette from field notes of a tutoring session held on July 14. In this vignette, there is evidence of synchrony between Kelly and Prof. Toddler. These synchronous interactions generated positive interactions that fostered not only a better environment for teaching and learning but also created mutual focus, which should bring entrainment.

> In this tutoring session, one of the college faculty was scheduled to work with the cadets. The lesson was on "phases and energy flow". Prof. Toddler discussed the problems with the group, solving each problem with a different student aloud, so that everyone could hear and benefit from the discussion. One after the other each cadet discussed the problem assigned trying to understand the principles related to the correct answer. Kelly answered her question correctly and engaged in the discussion on why the answer was right with Prof Toddler in a very calm and synchronous manner. She would shake her head in agreement; make gestures with her hands at about the same time as Prof Toddler (who used wide hands movements, like big waves). She kept eye contact with him, going rarely to her notes or the problem sheet for few seconds until they were done.

Analysis of the three teaching fields showed Kelly to be interacting well and participating in the learning process, enacting her chemistry in each of the fields. While in the classroom and the lab sessions, she was verbally engaged primarily with her peers, in the tutoring sessions, she interacted mostly with the teaching staff. In the lecture, she was engaged in the lessons, listening to the lead teacher, using the resources available when she needed them and discussing the problems with her friends.

Picture 7.- Synchronous hands gestures between Kelly and Prof. Toddler

During some lectures, the students were asked to go on the blackboard to solve problems, Kelly would be among the first to finish and would discuss with her friends about their answers. She usually solved them correctly. Kelly was also one of the students to review her Regents exam taken the week before and tried to solve the questions that she did not answer correctly. From observations of these fields, I can certainly deduce that Kelly is knowledgeable of the chemistry curriculum; she knows about the topics covered in these fields and enacted her knowledge solving the problems, discussing answers and results obtained, observing and drawing conclusion from her experiments.

Also, analysis of videotapes from these three fields revealed the enactment of Kelly's expanded role as an "othermother" in her relationship with the cadets specifically with Donald. The structure of these fields provided ways for her to enact her agency as a caregiver to Donald and in doing so; she was able to act as an othermother. The fields in the summer program had provided her with structure to enact her extended role and her knowledge. The structure of these fields had facilitated her agency. She used the structure provided by the activities of the program to play that role, using the resources available to

her. At home, she probably uses cooking, cleaning, laundry, babysitting to be an othermother; in the summer program, she used her knowledge of chemistry to enact othermother roles and as a result got to do a lot more chemistry in several fields. Her extended role allowed her to become proficient in chemistry. Kelly used the resources available to her as a student to act as an othermother, enacting positively her science and succeeding in her exams.

6.3.2 Kelly's Regents exam scores

Kelly was a quick learner, a focused and applied student. Although she was learning some of the concepts of the curriculum for the first time, often she did not seemed challenged by the assignments. From her participation in the lecture, the tutoring and the labs, Kelly showed understanding of the subjects being taught and because of that a high grade was expected in her Regents exam scores; a grade that would reflect the level of understanding that Kelly was expressing in these fields. Kelly's scores for the Regents exams went higher during the summer from a 21 on the first exam to 53 on the third and 66 on the last, with a higher score of 71 on the fifth exam. Kelly passed the official Regents examination with a scaled score of 78, two points less than the desired scaled score. Clearly, Kelly was able to answer more questions correctly after each week. The increase in her grades attests to her growing knowledge of chemistry. Just looking at the progression of the grades, one could hypothesize that this student was given instruction in chemistry, which was enabling her to make progress in her grades. She did learn chemistry during the summer program and was successful by reproducing this learning in her exams.

After observing Kelly and her interactions in the classroom, the laboratory and tutoring sessions, I expected her scores to be very high in the exams. This feeling was also shared by members of the chemistry TRT, who strongly believed in her capability to achieve high grades and expressed their confidence in her success. Although her grade was increasing each week, somehow there was some disappointment. When a student's participation in class is good, when one has seen her positive enactment in the laboratory and tutoring sessions, one can only be puzzled at her scores. So even though her attitude, participation and understanding of the materials were high during the different activities, her scores were not reflecting the same level of acquisition or understanding of the concepts as shown in the three fields.

Analysis of videotapes from the lectures and the tutoring sessions showed that there were times when Kelly looked bored. Maybe at these times, when she looked bored, sleepy or disinterested, she was actually confused and did not understand what was being taught. It is known that students who find it difficult to understand what is being done in class, sometimes express disinterest and indifference for the class. Could it be that Kelly was not bored at all but did not understand the materials and lost interest in what was happening? In this case there will be contradiction with her attitude in trying to solve problems on her own and trying to figure out some of them before she asked for help. Kelly always tried to understand what is being done with a positive and interactive attitude. Kelly was surely interested in learning chemistry during the summer.

Was she faking a level of understanding to look good in her boyfriend's eyes (although she always scored higher than him and often helped him with the assignments)? At times, especially in the laboratory, she seemed to work for both of

them. She would do a division of labor in the group and would do both her tasks and her boyfriend's (Donald). She would ask the other male member (there were mostly groups of three in the lab and the three cadets from Orchard High School always worked together) to get chemicals, pour solutions, or get glassware and would never insist that Donald do anything. In one of the labs, Donald was assigned to write the observations of the experiments, shortly after he stopped writing to discuss the experiment with the other student. Kelly started to write their observations. This situation did not seem to bother the other member of the group. In one tutoring session, where everyone was asked to work on a set of problems and was asked to give the answers and explanations on how the problems were solved, Kelly was helping Donald as much as she could. Obviously he was having difficulty answering and she seemed to have a good understanding of his assigned problems. At one point she was using her hands, gesturing, pulling up her eyelashes, as if to tell him: "Come on! Come on! You can do it, it's just …." Trying to motivate him and directing him in the right direction, still gesturing, she said: "whatever the list says" She did not want to answer for him but was encouraging him. This is one of the examples where Kelly was reproducing her role from her relationship with her siblings in her relationship with Donald. If Kelly is expanding her role of girlfriend to role model and othermother to Donald, why didn't she do her very best to score higher if she wanted to be a role model for him? Perhaps she did but was not successful.

Was Kelly among those whose exam scores do not reflect her actual knowledge? Is it possible that she really understood the topics taught during the year at her school and felt bored learning them over and was comfortable doing related questions in the Regents exams but was less comfortable in the new topics and did not get a full understanding of

them to obtain a higher score? If so, the program would have only reinforced her knowledge (learning time was actually review time for her) but did not quite make her more knowledgeable in chemistry. Her knowledge was not growing but was being refreshed. Having a control group during the summer could be a good experiment, where

Picture 8. - Here, Kelly is gesturing to help Donald answer questions about Arrhenius acids. This tutoring session happened the same day Prof Toddler and Donovan taught Arrhenius acids and bases. She was prompting him to use the list of acids and bases given in the reference table to identify which compound was an acid. The other students were listening: a couple looking at their sheet and reference tables. All were waiting patiently for Donald to understand the concept that High School teacher Daryl was explaining to him.

a group of students, who have never had chemistry in school, would participate in the summer program like the others. Comparison of their enactment of chemistry in the different fields could be a measure of the efficacy of the method adopted in the program.

Analysis of Four Regents exams

In an attempt to understand why Kelly's grades were not aligning with her enactment of chemistry in the other fields, I took a look at her answers to the first four Regents exams taken during the summer. The answers for Part A of the exams were analyzed. First I realized that Kelly's answers show a progression in her knowledge.

Most of the questions she missed about key ideas[6] of the chemistry core curriculum were answered correctly in the subsequent exams. Nevertheless, her answers for one key idea were not consistent with how chemical bonds are formed.

When questions corresponding to this key idea were definition questions, Kelly answered correctly:

Exam 2: question #9

> Covalent bonds are formed when electrons are:
> 1) transferred from one atom to another
> 2) captured by the nucleus
> 3) mobile within a metal
> 4) shared between two atoms (correct answer)

Exam 3: question #11

> Which type of bond is formed when electrons are transferred from one atom to another?
> 1) covalent
> 2) ionic (correct answer)
> 3) hydrogen
> 4) metallic

Her answers were also correct when the questions were repeated in a similar manner.

Exam 3: question #26

The bonds in the compound $MgSO_4$ can be described as
1) ionic only
2) covalent only
3) both ionic and covalent (correct answer)
4) neither ionic nor covalent

Exam 4: question #11

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[6] Principals and teachers have to design a curriculum from the core curriculum given by the Department of Education. The Regents exams are based on key ideas of the core curriculum. I used the key ideas of the core curriculum to analysis the answers.

Which compound contains both ionic and covalent bonds?

1) $CaCO_3$ (correct answer)
2) PCl_3
3) MgF_2
4) CH_2O

Since a similar question as the incorrectly answered question was not repeated in the other exam, I gave special attention to that question and noticed that Kelly was not able to identify covalent bonds between a set of compounds where three of them were bases and one an alcohol. At this point, the question was: is Kelly able to distinguish OH^- (hydroxide ion) in bases and RO^- (alkoxide group) from alcohol? Indeed I found that she did not answer any question correctly when she had to identify or recognize an Arrhenius base

Exam 1: question #5

Which compound contains only covalent bonds?

1) NaOH
2) $Ba(OH)_2$
3) $Ca(OH)_2$ (answer checked)
4) CH_3OH (correct answer)

Exam 2: question #30 (this question was not answered by Kelly)

Which compound could serve as a reactant in a neutralization reaction?

1) NaCl
2) KOH (correct answer)
3) CH_3OH
4) CH_3CHO

Exam 3: question #29 (this question was not answered)

A sample of $Ca(OH)_2$ is considered to be an Arrhenius base because it dissolves in water to yield

1) Ca^{2+} ions as the only positive ions in solution
2) H_3O^+ ions as the only positive ions in solution
3) OH^- ions as the only negative ions in solution (correct answer)

4) H^- ions as the only negative ions in solution

Exam 4: question #27 (question was not answered)

Given the reaction: $NH_3 + HCl \rightarrow NH_4Cl$
In this reaction, ammonia molecules (NH_3) act as a base because they
1) accept hydrogen ions (H^+) (correct answer)
2) accept hydroxide ions (OH^-)
3) donate Hydrogen ions (H^+)
4) donate hydroxide ions (OH^-)

I was prompted by these findings to go back to the videos of the classroom and the other fields to analyze her interactions while this subject (acids and bases) was taught. I found videos of the class activity and tutoring session where the subject was taught. Prof. Toddler and teacher Donovan taught the subject in the classroom. The text below is from the field notes on that lesson.

> Prof. Toddler and teacher Donovan teach the last 40 minutes of the lecture. The lesson is on the properties of acids and bases.
> Donovan starts by telling the students what the lesson will be about and what they are going to do in that period and leads the way for Toddler.
> Toddler starts the lesson by demonstrating the ability of acids and bases to conduct electricity. He uses a circuit with a lamp and different solutions, starting with distilled water, adding acid or base to the water to show the difference when the circuit is plunged in the solutions. The lamp does not light up with distilled water but does when either acid or base are added to distilled water. He concludes that acids and bases conduct electricity. In the meantime, on the board, Donovan is writing the definitions, observations from the demonstration and conclusion drawn from these observations as they are driven by Toddler. One part of the board has two columns, one for acids and one for bases. The students are very attentive and are writing the notes from the board without losing attention to Toddler's demonstration. Examples of what Donovan writes on the board are: Electrolyte – a solution that conducts electricity; Acids: - conduct electricity, - produce $H^{+;}$ Bases: - produce OH^-
>
> Kelly sits in the second row, between Donald and Fritz. She is engaged in synchronous interactions with the two teachers. She follows the demonstration done by Toddler and writes the notes put on the board at the same time Donovan writes them. From time to time, she addresses her friends.

Picture 9.- Toddler demonstrates the properties of acids and bases to the students while Donovan writes definitions, Toddler's observations and conclusions on the board. Students as well as teaching staff are in synchrony with them. Kelly is focused and pays attention to what both teachers are doing. The students as well as the other teachers present in the classroom are positively engaged with the teachers as coteaching is appropriately enacted in the classroom.

This class activity is a good example of coteaching that happened during the summer, where one teacher interacts with the students, facing them, demonstrating the lessons and never turning his back to them. In the meantime, the other teacher interacts with the class by writing all definitions, observations, and conclusions from the demonstration and everything that Toddler forgot to mention, on the board for the students. As the teachers are in synchrony, the students themselves and the other teachers in the room became in synchrony with them. Kelly is not an exception and was very focused on the demonstration and the writing on the board. As the lesson continues, she remains actively engaged. When a series of questions are given to the students to answer after watching segments of a movie about acids and bases, Kelly does well in answering them quickly and accurately and waits for the majority of the students to finish. Following is another excerpt from the field notes for this class activity.

> Kelly answers questions from the sheet, segment after segment. She looks comfortable and capable of doing them. As a matter of fact, no teachers are sitting near her. She interacts mostly with Donald while answering the questions. Enough time is given to the students to finish the work. Kelly is one of the students to finish and waits for the rest to be done before they can see another segment.

Picture 10.-In this picture, Kelly and Fritz are playing with Donald while waiting for the other students to finish the assignment. Kelly understands the lesson so well that answering the questions from the video movie is done quickly. As a matter of fact, it is the same for her friends (the other cadets); they are all done with the questions and are having fun playing with each other. The same day, the topic on acids and bases was reinforced in the tutoring session.

Later that day, Kelly shows a good understanding of the concept of the Arrhenius definition of acids and bases and properties. My earlier example of how Kelly was enacting her role of othermother in the tutoring session comes from the tutoring session of that day after the lesson taught by Toddler and Donovan. The pictures below show examples of problems that were solved that day in the tutoring about acids and bases. Kelly was helping Donald with question #4 seen in pictures 10 and 11. Question #4 is similar to the questions that Kelly missed in all the exams and yet, she was helping Donald answer the question in tutoring.

Question 4:

In an aqueous solution, which substance yields hydrogen ions as the only positive ion?
1)C_2H_5OH, 2) CH_3COOH, 3) KH, 4) KOH.

Kelly herself gave the correct answers for her assigned questions (#1 and 9):

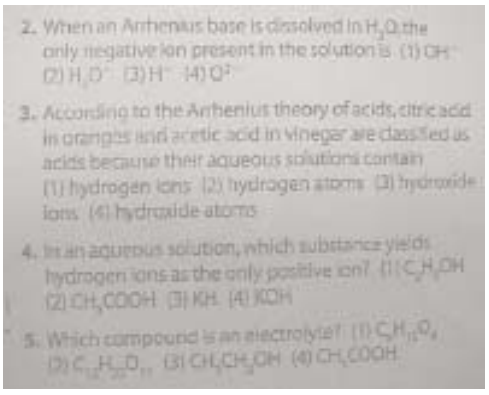
Question 1:
According to the Arrhenius theory, when an acidic substance is dissolved in water it will produce a solution containing only one kind of positive ion. To which ion does the theory refer?

1)acetate 2) hydrogen 3) chloride 4) sodium.

Question 9:

Which species is classified as an Arrhenius base?
1) CH_3OH 2) LiOH 3) PO_4^{3-} 4) CO_3^{2-}

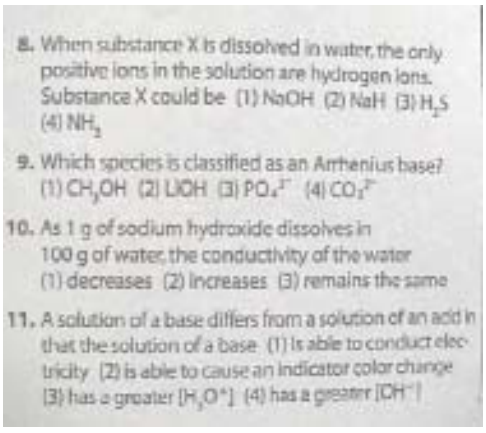
There is an obvious contradiction: from my field observations, Kelly exhibits a full and solid understanding of the concept of acid and base and yet did not reproduce that ability in her exams. Kelly mastered the subject well enough to be able to help Donald to find the correct answer to the question that she herself missed in the exams. Could it be that Kelly did understand what was taught during the program but did not retain the material? After the third week, when the lectures were divided into three 40 minutes sessions, after each session a post assessment was done. Artifacts collected from class activity revealed that Kelly understood what was done in class and answered the questions in a satisfactory manner. In the tutoring sessions, she was knowledgeable,


2. When an Arrhenius base is dissolved in H_2O, the only negative ion present in the solution is (1) OH^- (2) H_3O^+ (3) H^- (4) O^{2-}

3. According to the Arrhenius theory of acids, citric acid in oranges and acetic acid in vinegar are classified as acids because their aqueous solutions contain (1) hydrogen ions (2) hydrogen atoms (3) hydroxide ions (4) hydroxide atoms

4. In an aqueous solution, which substance yields hydrogen ions as the only positive ion? (1) C_2H_5OH (2) CH_3COOH (3) KH (4) KOH

5. Which compound is an electrolyte? (1) $C_6H_{12}O_6$ (2) $C_{12}H_{22}O_{11}$ (3) CH_3CH_2OH (4) CH_3COOH


8. When substance X is dissolved in water, the only positive ions in the solution are hydrogen ions. Substance X could be (1) NaOH (2) NaH (3) H_2S (4) NH_3

9. Which species is classified as an Arrhenius base? (1) CH_3OH (2) LiOH (3) PO_4^{3-} (4) CO_3^{2-}

10. As 1 g of sodium hydroxide dissolves in 100 g of water, the conductivity of the water (1) decreases (2) increases (3) remains the same

11. A solution of a base differs from a solution of an acid in that the solution of a base (1) is able to conduct electricity (2) is able to cause an indicator color change (3) has a greater $[H_3O^+]$ (4) has a greater $[OH^-]$

Pictures 11.- and 12.- are photos of problems solved in the tutoring session about acids and bases. Kelly answered questions 1 and 9 and helped Donald with question4.

did the assignments, discussed them with the teacher and, when permitted, she helped her friends. At the end of the week, when she took the Regents exams, she incorrectly answered some of the questions that previously she had shown a good understanding of during the lecture and the tutoring. During the summer program, no homework was given to the students; all learning activities were done at the college. If Kelly was not able to perform better on her exams because of a problem of retention, could it be that the positive progression of her grades is due to the Regents preparation?

Searching for a possible answer, I went to the answer sheets for the first three exams for four other cadets (two from Orchard and two from Franklin). Accordingly, I compared their answers to identify patterns and contradictions among the responses. Donald like Kelly answered all questions incorrectly and Fritz and Eugene only answered the question from the third exam correctly and Immanuel answered the questions on the first and third exams correctly. From my observations in the tutoring session, all the cadets but Donald, showed a sound understanding of Arrhenius acids and bases. What happened then in the exams? Again, this is a contradiction between the knowledge enacted in the lecture and the tutoring fields and the Regents exams.

Table 6.2 Answers from the cadets in Regents exams on questions on Arrhenius bases

	Kelly	Donald	Fritz	Immanuel	Eugene
1st Exam, #5	No	No	No	Yes	No
2nd Exam, #30	No	No	No	No	No
3rd Exam, #29	No	No	Yes	Yes	Yes

Correct answers (Yes), incorrect answers (No)

It is interesting to note that acids and bases were among the topics not covered during the academic year at Orchard High School. I considered the possibility that low performance on acids and bases was due to the topic not being taught at Orchard high school. This is possible since the three students from that school, who were cadets (the most advanced student in the summer class), failed to reproduce their understanding of the topic in the exams. The pattern for students to perform poorly on topics not taught at the High School did not hold up. I examined the answers of the students above for the two other topics not taught at Orchard high school: oxidation-reduction and organic chemistry and questions were answered satisfactorily.

6.4 Conclusion

Although, Kelly was actively involved and enacted her chemistry knowledge of the Arrhenius definition of acids and bases in the lecture and in the tutoring (the laboratory period where the subject was investigated was not observed), she was not able to reproduce that knowledge in the exams. Does that mean she does not know or understand the concept of Arrhenius bases or does it means she couldn't express her knowledge of Arrhenius base in her exam?

My observations of Kelly's enactment of chemistry in different fields confirm the fallibility of relying on one method of assessment, such as the Regents examination, to represent the knowledge of a student. Kelly enacted her knowledge of Arrhenius definitions of acids and bases very well in the classroom and in the tutoring session but yet was not able to enact that knowledge in her exams. In general, Kelly showed a very

good understanding of the topics of all the lessons, yet her scores on the exams did not reflect the knowledge she was enacting in the other fields.

I believe that Kelly was probably able to enact her knowledge in chemistry in the fields where she could extend her agency by being an othermother. In all three fields: classroom, laboratory and tutoring sessions, Kelly used her capital from being a helper, a guide and an othermother to her fellow students, especially her boyfriend Donald. Considering the Regents exams as another field, which is structured to emphasize an individual acting independently of others, Kelly could not act as an othermother and her capital that is used to promote the well being of others could not be enacted to show her knowledge of chemistry – as it can be in the other fields we explored in this study. In that field where she could not expand her extended role, she was less proficient. In this case, the role of othermother, contrary to Scantlebury's identification of this role as a source of inequality (Scantlebury, 2005), was favorable for the student. In a recent conversation with Kate Scantlebury, she communicated to me that recent studies showed corroboration with my findings of students using the role of othermother to their advantage in the classroom and in the learning process. Her research only revealed this trend when female students are grouped together; the inequality surfaces in a mixed group of boys and girls. In my case, Kelly worked mainly with boys and was able to use this role into her advantage. Kelly was able to enact her chemistry knowledge more when she was also able to be an othermother for the boys. Her agency was enacted in this role and allowed her to enact her knowledge. When her agency was truncated in the field of the exams, her enactment of chemistry was diminished.

As I am closing on this study case, I observed that Kelly only has male siblings and maybe acting as an othermother for the boys in the summer program was facilitated by the same sex of both groups of individuals (her siblings and the group of boys during the summer). It would be interesting to investigate the enactment of this role with respect to the sex and other patterns of the groups being cared for.

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Glossary

Enactment is the process of doing or performing something. It can be defined as a specific way in which teachers and/or students express their knowledge of a particular subject.

Entrainment implies that students should be able to repeat what teachers show them how to do. They should be able to carry a lesson along, repeat and apply what was taught. This is facilitated in the classroom by synchronous practices and positive interactions.

Ethnography (from the Greek *ethnos* = nation and *graphein* = writing) refers to the qualitative description of human social phenomena, based on fieldwork. Ethnography is a holistic research method founded in the idea that components/fields of a system cannot necessarily be accurately understood independently of each other.

Praxis is the process by which a theory or lesson becomes part of lived experience through a cycle of action-reflection-action. Rather than a theory being simply developed at the intellectual level, ideas are tested and experienced in the real world, followed by an opportunity for reflective contemplation and re-evaluation. In this way, abstract concepts are connected with lived reality.

Pierre Bourdieu's theory of power and practices: Bourdieu believed that society cannot be analyzed simply in terms of economic classes and ideologies. Much of his work concerns the independent role of educational and cultural factors. Instead of analyzing societies in terms of classes, Bourdieu uses the concept of *field*: a social arena in which people manoeuvre and struggle in pursuit of desirable resources. A field is a system of social positions structured internally in terms of power relationships. Different

fields can be either autonomous or interrelated and more complex societies have more fields.

Randall Collins' sociology of emotions: According to Collins, within a given group, interactions will be shaped by emotions. Positive emotions will generate positive or successful interactions while negative ones will produce unsuccessful interactions. In a learning environment, successful interactions are necessary to facilitate the teaching and learning experience. Nevertheless, there is no environment without both types of interactions. They co-exist in an equilibrium type of relationship and this equilibrium or relationship influences the structure of the classroom. This relationship can be identify by synchronous and asynchronous practices within the participants of a learning environment.

Stakeholder: Any person who is (or might be) affected by the project or/and can influence it. All participants are stakeholders. Examples are: principal investigator, students, Department of Board of Education staff, and High School teachers.

Theory of Vygotsky (activity theory): Activity theory is a Soviet psychological theory with roots in socio-cultural approach. It begins with the notion of activity. An activity is seen as a system of human "doing" whereby a subject works on an object in order to obtain a desired outcome. In order to do this, the subject employs tools. Which may be external or internal. Vygotsky stated that consciousness is constructed through a subject's interactions with the world and is an attribute of the relationship between subject and object. He supposed that higher mental processes are of a social origin.

William Sewell's cultural sociology perspective: For Sewell, a fundamental theorem of cultural sociology is the dialectical relationship between agency and structure. Agency

requires access to the resources of the field and the cultural capital needed to appropriate them; individuals use resources to meet their goals and, in so doing, change schema and practices which become part of the structure of the field and resources for the production and reproduction of culture.

Appendix

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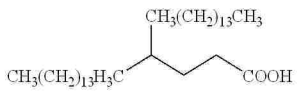

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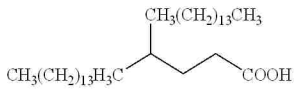
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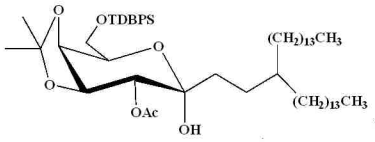
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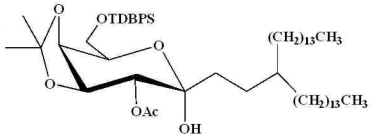
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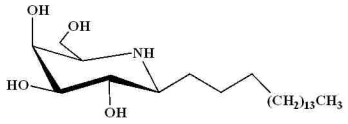

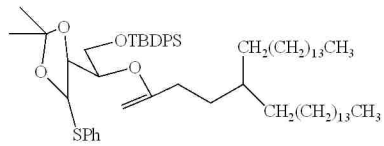
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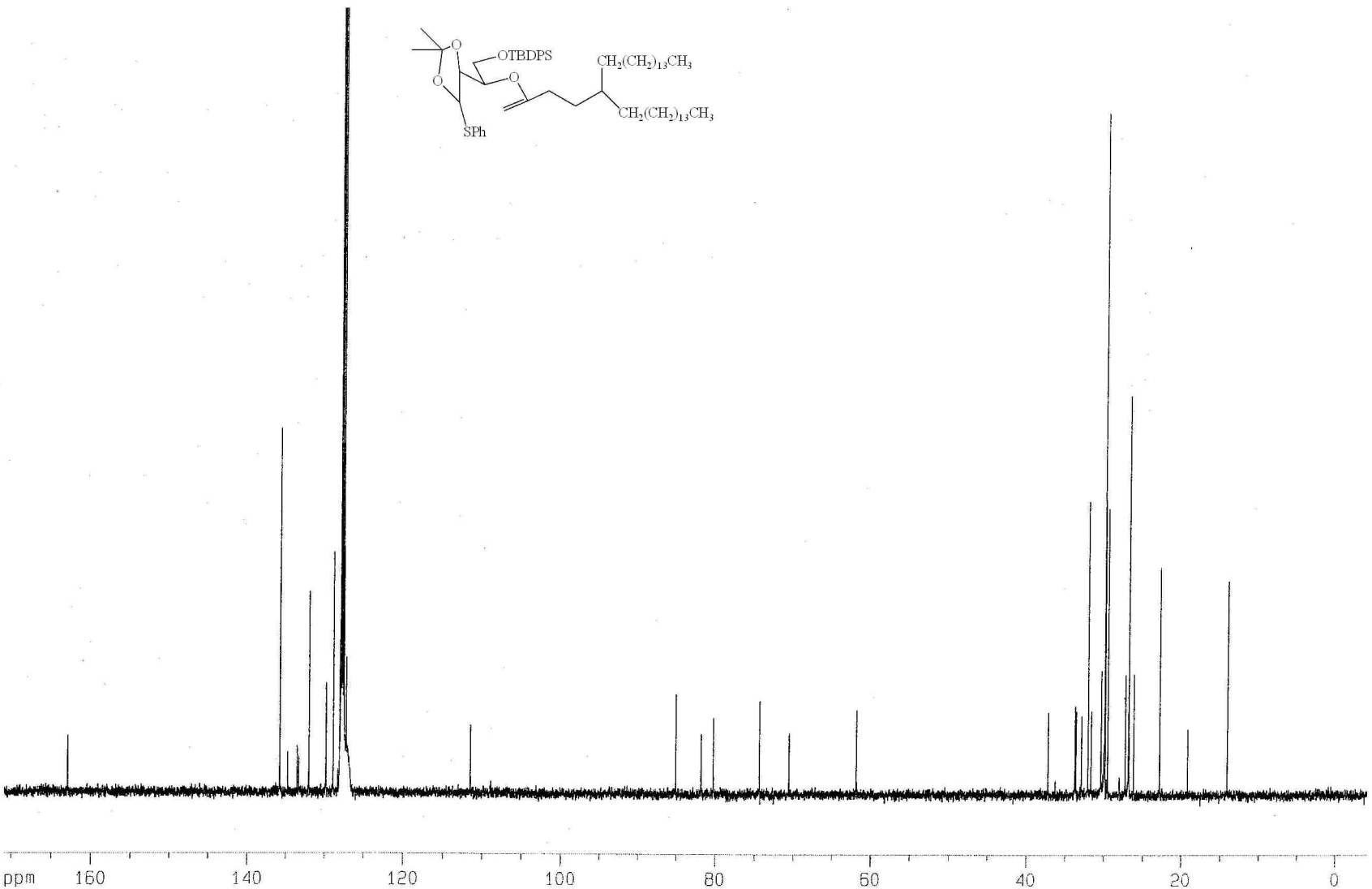
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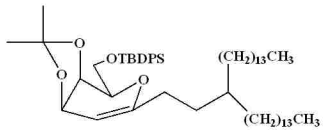
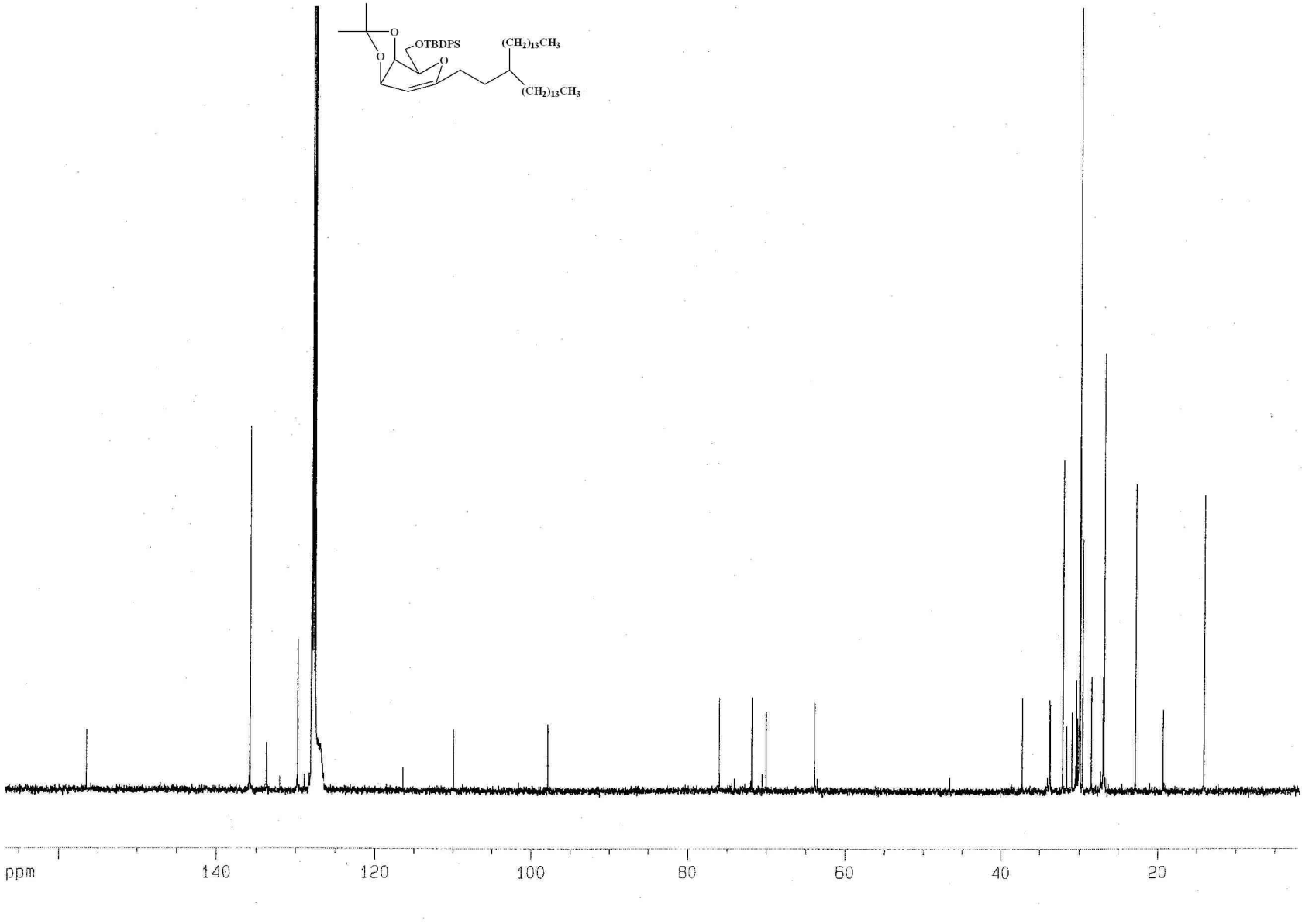
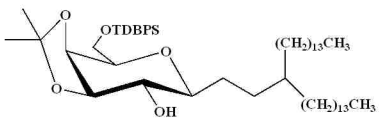


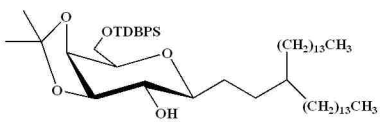

OTBDPS
(CH2)13CH3
(CH2)13CH3
ppm
140
120
100
80
60
40
20

132

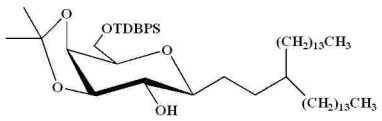

OTDBPS
O
O
O
O
OH
(CH2)13CH3
(CH2)13CH3

1.000
0.974
1.000
1.905
0.933
0.961
0.965
0.984
1.004
0.656
3.952
58.402
5.923
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

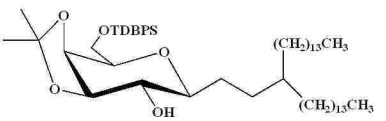
133

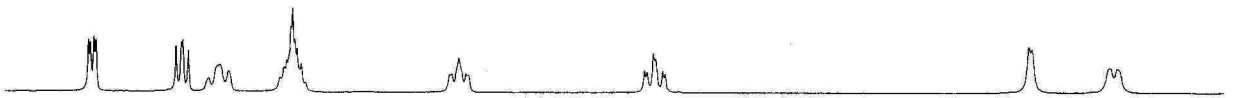


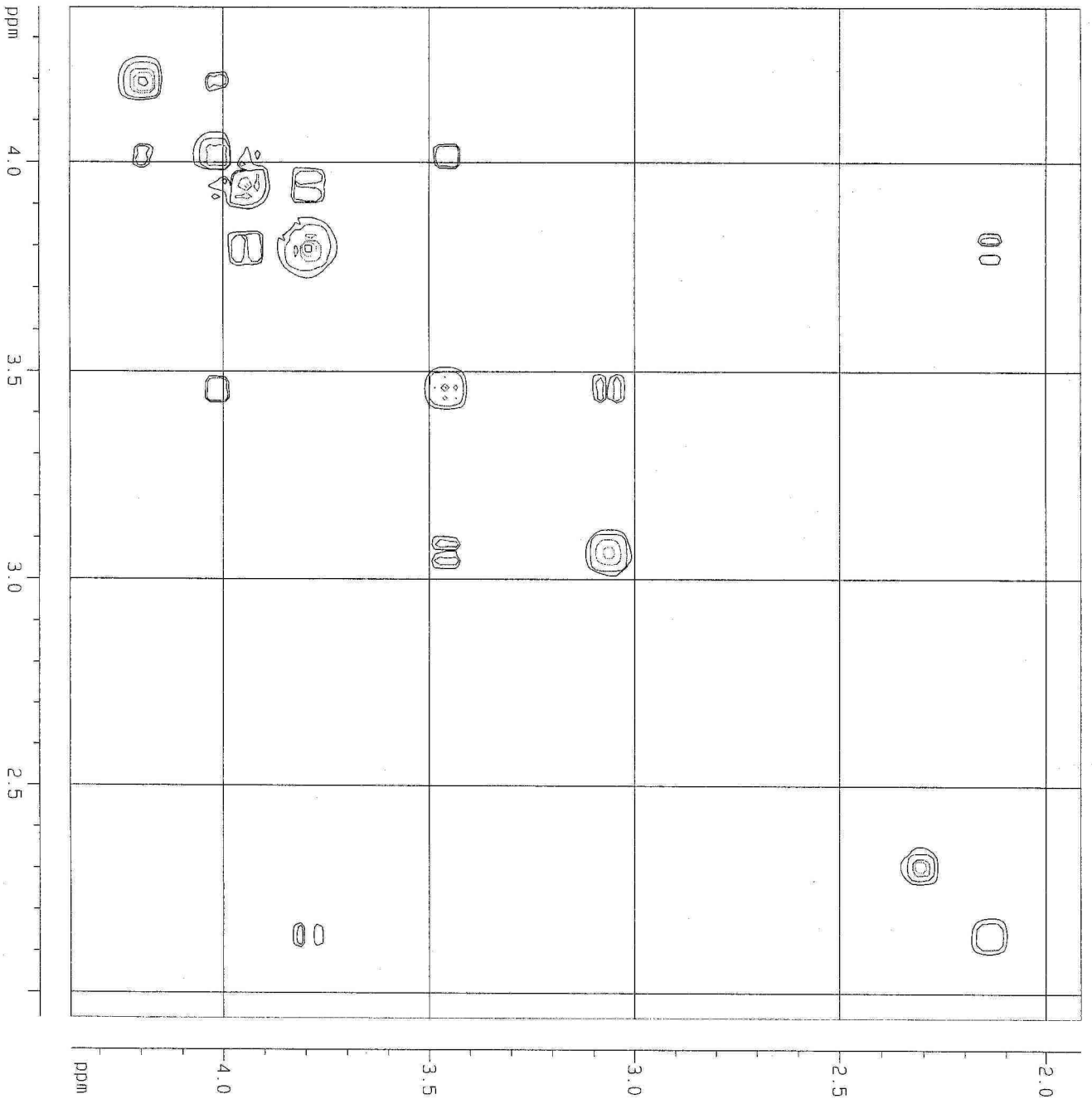
D_2O Exchange

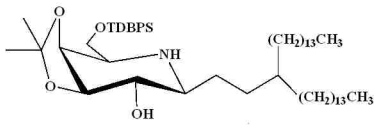

O
OTDBPS
O
O
OH
(CH2)13CH3
(CH2)13CH3

ppm
120
100
80
60
40
20

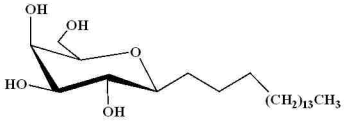

OTDBPS
O
O
O
OH
(CH2)13CH3
(CH2)13CH3



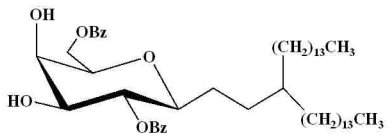

ppm
4.0
3.5
3.0
2.5
ppm
4.0
3.5
3.0
2.5
2.0


O
OTDBPS
NH
(CH2)13CH3
O
(CH2)13CH3
OH

Integral
3.930
5.814
1.000
2.911
0.944
0.917
1.852
2.198
4.313
58.355
9.481
6.228
pm
7
6
5
4
3
2
1

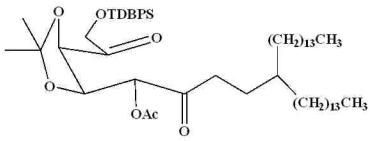

OH
OH
O
HO
OH
(CH2)13CH3

125

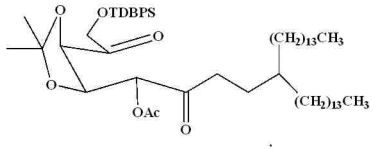

OH
OBz
O
HO
OBz
(CH2)13CH3
(CH2)13CH3

160
140
120
100
80
60
40
20
0
ppm

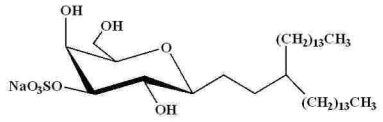
126


OTDBPS
O
O
O
OAc
O
(CH2)13CH3
(CH2)13CH3

Integral
10.255
4.208
4.992
80.545
ppm
7
6
5
4
3
2
1

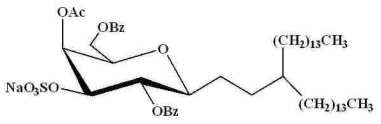

O
OTDBPS
O
(CH2)13CH3
O
OAc
O
(CH2)13CH3

ppm
200
180
160
140
120
100
80
60
40
20

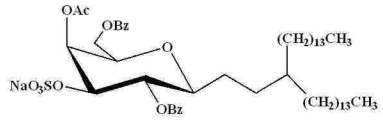

OH
OH
O
$(CH_2)_{13}CH_3$
NaO_3SO
OH
$(CH_2)_{13}CH_3$

5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5

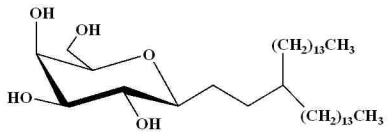
144



145

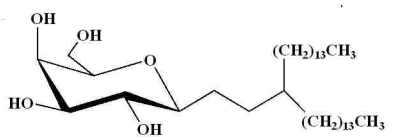

OAc
OBz
O
$(CH_2)_{13}CH_3$
NaO_3SO
OBz
$(CH_2)_{13}CH_3$

180
160
140
120
100
80
60
40
20
0
ppm

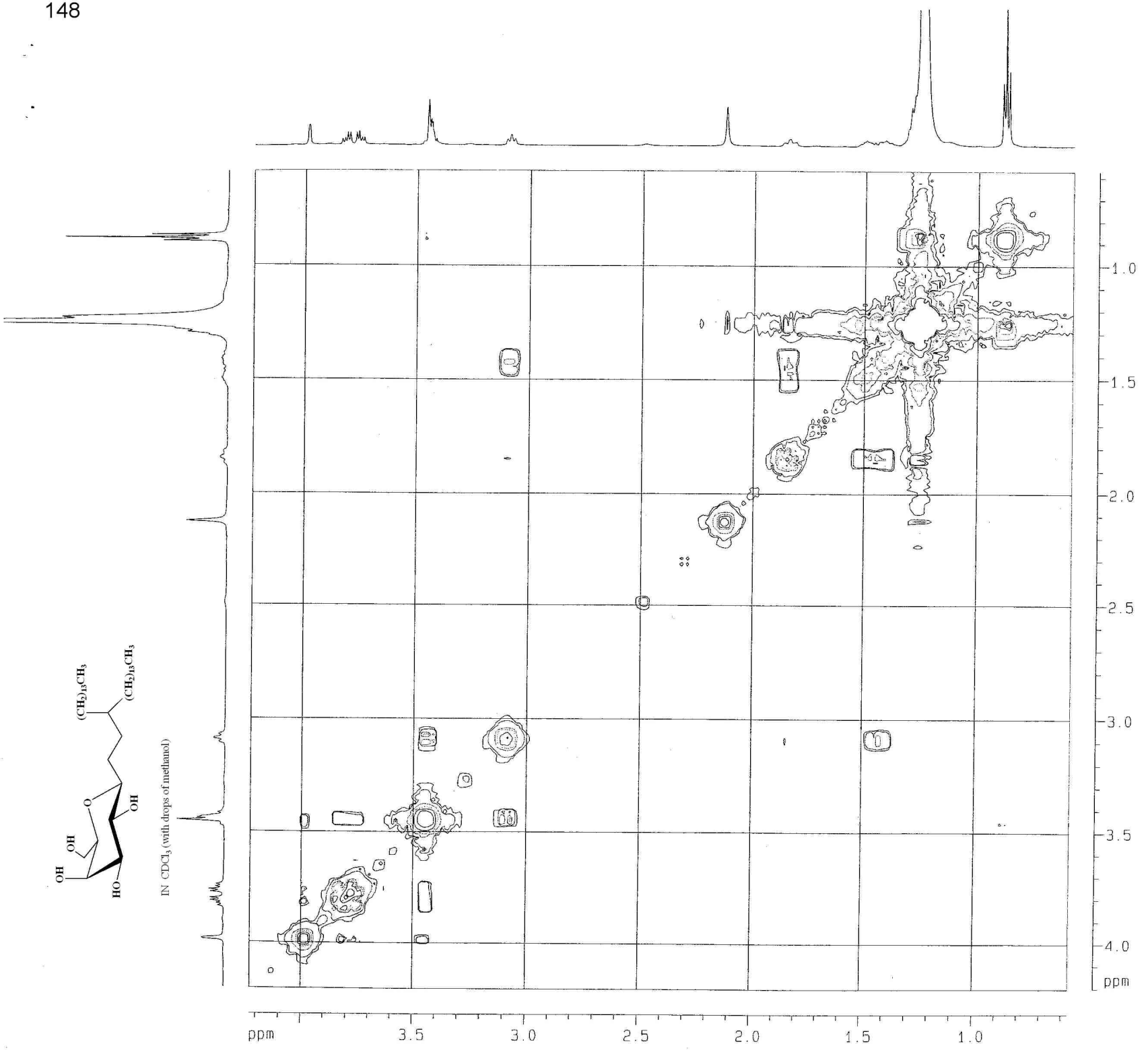


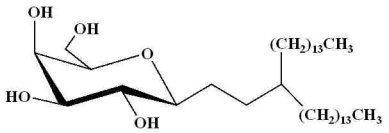
IN CD_3OD

147



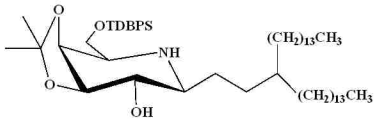
IN $CDCl_3$ (with drops of methanol)

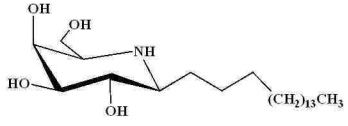

IN $CDCl_3$ (with drops of methanol)
ppm
3.5
3.0
2.5
2.0
1.5
1.0
1.0
1.5
2.0
2.5
3.0
3.5
4.0
ppm
$(CH_2)_{13}CH_3$
$(CH_2)_{13}CH_3$
OH
OH
OH
HO
O



IN $CDCl_3$ (with drops of methanol)

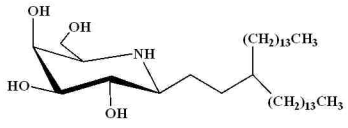
119



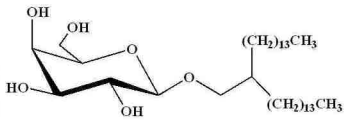

OH
OH
NH
HO
OH
(CH2)13CH3

80
60
40
20
0

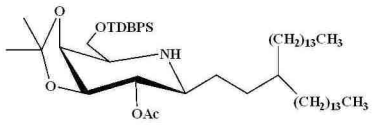
124



137

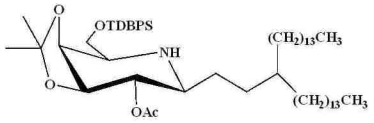


139


O
OTDBPS
NH
O
OAc
(CH2)13CH3
(CH2)13CH3

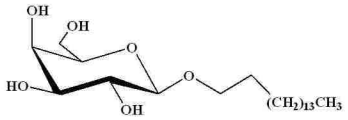
Integral
5.149
7.635
1.000
1.095
1.164
2.263
0.941
1.095
3.819
5.900
79.344
12.404
8.423
ppm
7
6
5
4
3
2
1

140

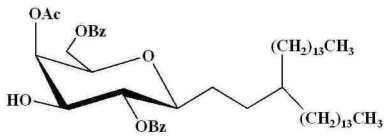

O
OTDBPS
NH
O
OAc
(CH2)13CH3
(CH2)13CH3

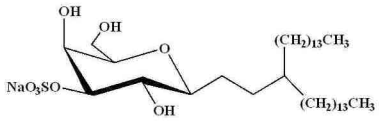
ppm
160
140
120
100
80
60
40
20

138



141




OH
OH
O
$(CH_2)_{13}CH_3$
NaO_3SO
OH
$(CH_2)_{13}CH_3$

40
120
100
80
60
40
20