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Linda Elizabeth Jewell
AUTEUR DE LA THÈSE / AUTHOR OF THESIS

M.Sc. (Chemistry)
GRADE / DEGREE

Department of Chemistry
FACULTÉ, ÉCOLE, DÉPARTEMENT / FACULTY, SCHOOL, DEPARTMENT

Synthesis of Glycolipids by *Fucus Distichus* and Natural Product Isolation and Characterization of *Euphorbia Iancifolia*

TITRE DE LA THÈSE / TITLE OF THESIS

T. Durst
DIRECTEUR (DIRECTRICE) DE LA THÈSE / THESIS SUPERVISOR

CO-DIRECTEUR (CO-DIRECTRICE) DE LA THÈSE / THESIS CO-SUPERVISOR

EXAMINATEURS (EXAMINATRICES) DE LA THÈSE / THESIS EXAMINERS

R. Ben

J.T. Arnason

Gary W. Slater

Synthesis of Glycolipids inspired by *Fucus distichus* and Natural Product Isolation and Characterization of *Euphorbia lancifolia*

Linda Elizabeth Jewell
B.Sc., University of Ottawa, Canada, 2007

Thesis submitted to the
School of Graduate Studies and Research
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In the

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Candidate	Supervisor
Linda Elizabeth Jewell	Professor Tony Durst

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Dedication

For my family and friends,

And for my fellow graduate students.

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Acknowledgements

I am extremely grateful towards a number of people who have helped me during my degree. Most importantly, I owe an enormous thank you to Dr. Tony Durst for giving me the opportunity to work in his lab and to tackle these engaging projects. Your kind, strong mentorship is an ideal to which all leaders should strive. You have made the last two years the most challenging, yet most rewarding, of my life.

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Lina, for putting up with me as I learned how to supervise, for always making me laugh, and for making me challenge myself; to David, for being so innocent, naive, and sweet; to Halima, for showing me the ropes and for making excellent reading suggestions for when I can't take any more chemistry; to Lynne, for her level-headedness and guidance when I was still fresh in the lab; to Devin, Margaret, Hooda, Emma, Ana Gargaun, Darija, Samira, Zimmer, Mélanie, Moe, and Eric, for being incredible (pre)-undergrads, for the excellent conversations, and for all the good times. I could fill the rest of my thesis by trying to express how much you have all meant to me. Thank you for your friendship.

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Last, and least, I wish to thank caffeine. I couldn't have done it without you!

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List of Abbreviations and Symbols

Ac	acetyl
Ac_2O	acetic anhydride
br	broad
brine	saturated NaCl solution
br s	broad singlet
°C	degrees Celsius
^{13}C NMR	carbon-13 nuclear magnetic resonance
$CDCl_3$	deuterated chloroform
COSY	correlation spectroscopy
δ	chemical shift
Δ	heat
d	doublet
DCC	dicyclohexylcarbodiimide
DCM	dichloromethane
dd	doublet of doublets
ddd	doublet of doublet of doublets
DEPT	distortionless enhancement polarization transfer
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
dt	doublet of triplets

EC_{50}	effective concentration for 50% mortality
EI	electronic ionization
equiv.	equivalents
Et	ethyl
Et_3N	triethylamine
Et_2O	diethyl ether
EtOAc	ethyl acetate
EtOH	ethanol
h	hours
HMQC	heteronuclear multiple quantum coherence
^1H NMR	proton nuclear magnetic resonance
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectroscopy
Hz	Hertz
IC_{50}	concentration to inhibit growth by 50%
IR	infrared
J	coupling constant
liq.	liquid
m	multiplet
M	molarity
$[M^+]$	molecular ion
Me	methyl
MeOH	methanol

MeOD-d_6	deuterated methanol
min	minutes
mL	millilitres
m.p.	melting point
MRSA	methicillin-resistant *Staphylococcus aureus*
MS	low resolution mass spectroscopy
MSSA	methicillin-susceptible *Staphylococcus aureus*
MW	molecular weight
m/z	mass to charge ratio
N	normality
NMR	nuclear magnetic resonance
NMO	N-methylmorpholine-N-oxide
OAc	acetoxy
P.C.	large-scale glass-backed plate chromatography
Ph	phenyl
ppm	parts per million
PTSA	p-toluenesulfonic acid
q	quartet
rt	room temperature
s	singlet
spp	species
t	triplet
TBAF	tetrabutyl ammonium fluoride

THF	tetrahydrofuran
TLC	thin layer chromatography
WHO	World Health Organization

Statement of Collaborator Contributions

Fucus distichus Project

This project was begun prior to my arrival in the Durst lab. Samples of *Fucus distichus* were collected by Virginie Treyvaud-Amiguet, a post-doctoral researcher in the Arnason lab. Purifications by column chromatography and by soxhlet were performed primarily by lab technician Halima Mao of the Durst lab, and the structural identification of the glycolipid was made by research assistant Muhammad Asim. Early synthetic routes were explored by Halima Mao, honours student Daria Klonowska, and co-op student Eric Teh. During my time on the project, I was assisted by co-op student Lina Chan.

Euphorbia lancifolia Project

Samples of *E. lancifolia* were collected by Tony Durst. Lina Chan provided assistance during the initial separation of the plant extracts. Some structural assignments were made with the assistance of Muhammad Asim.

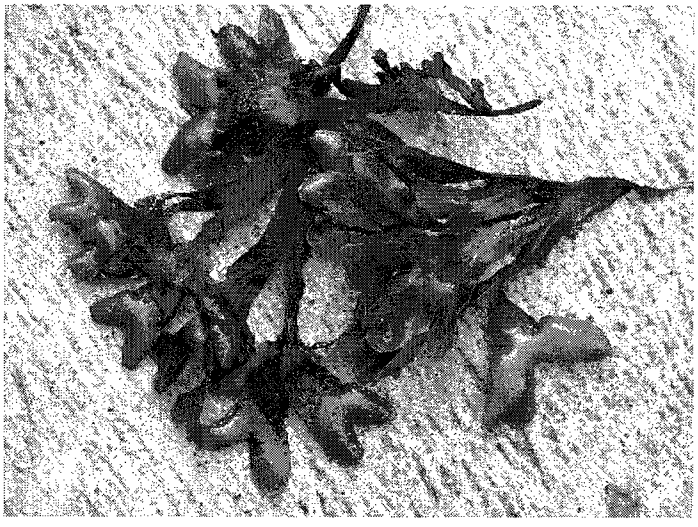
Abstract

The ethanolic extracts of *Fucus distichus*, a freshwater brown algae harvested from Ungava Bay in northern Québec, have demonstrated antiviral and antibiotic activity. The major component and potential active molecule from this seaweed was identified as a glycolipid consisting of β-*D*-galactose linked to two ω-linolenic acid chains via a glycerol unit. The synthesis of this glycolipid, as well as a number of derivatives, is described to investigate their antibiotic and antiviral activity relative to the natural product. One major family of analogues explored includes *C*-linked glycolipids, where a carbon-carbon linkage replaces the metabolically unstable C-O ester bond between the sugar and the glycerol in the natural product. The natural product, as well as eight other analogues were synthesized. Bioactivity data for some of the compounds is described.

In a second project, an investigation into the major constituents of *Euphorbia lancifolia* (Ixbut) was undertaken. Although there have been no in-depth reported chemical studies of *E. lancifolia* to date, traditional medicine in Latin America claims that the plant leaves can be used in herbal tea as a galactogogue, a drug which induces lactation. The goal of this study was to identify the major constituents of *E. lancifolia* as the first step in investigating the claims regarding the plant's biological activity. Nine compounds, including the novel triterpene derivative β-amyryl ferulate, were identified and isolated. Biological assays testing the ability of these compounds to interact with the metabolism of dopamine, an important hormone in lactation, will be undertaken shortly.

Chapter 1

Synthesis of Glycolipids Inspired by *Fucus distichus*



1.1 Introduction

1.1.1 Natural Product and Medicinal Chemistry

Throughout history, all cultures have discovered that various plants have interesting and desirable biological activity. Many societies carefully documented the species that were useful for various purposes; for example, as early as 1550 BC, the Ebers Papyrus was authored in Egypt and detailed hundreds of useful preparations made from local plants and animals. Unlike the Egyptians, most societies did not keep rigorous written records, but oral tradition and the accumulated knowledge of elders has preserved traditional medicine into the present day (1).

In contrast to the practice of traditional medicine, allopathic medicine describes "the broad category of medical practice that is sometimes called Western medicine, biomedicine, scientific medicine, or modern medicine" (2). The advent of drugs in allopathic medicine is linked to the first isolation of several important compounds, such as strychnine and morphine in the 1800s. In 1826, E. Merck released the first chemically pure pharmaceutical, morphine, to the general public (1).

Allopathic medicine remains inaccessible to one third of the world's population for geographic and economic reasons (2). Understanding the traditional remedies originating from around the world is beneficial for many reasons. Most importantly, investigations into traditional remedies elucidated the chemical entities present in these preparations; by identifying and studying these compounds, the safety and efficacy of the individual bioactive compounds of traditional remedies may be assessed in a rigorous, controlled setting. Such investigations suggest the side-effects and complications that are possible from a traditional

remedy that may make it unsafe for certain members of the population. Validating and standardizing these preparations also helps to increase consumer confidence in natural health products, providing an economical and increasingly safe alternative to allopathic medicine. Increasing Western interest in natural health products has provided a source of revenue for some remote communities. Finally, because many species which produce interesting natural products are native to parts of the world which are undergoing significant deforestation and development, highlighting the importance of natural product chemistry may help to drive conservation.

Using nature as a source of inspiration for pharmaceuticals is not a new idea. Many of the most historically important pharmaceuticals are directly isolated from organisms: morphine, penicillin, and taxol have inarguably saved and bettered millions of lives, and all three compounds are clinically useful without any chemical modification. The structures of all three examples are also complex, and their *de novo* syntheses require the creative application of modern synthetic techniques.

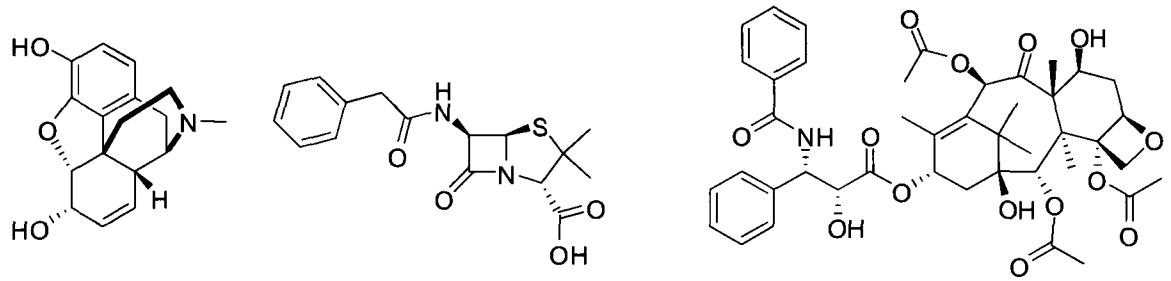


Figure 1.1.1 Historically important drugs used without chemical modification. **(a)** Morphine; **(b)** Penicillin G; **(c)** Taxol

Still more pharmaceuticals are inspired by natural products, but have undergone structural changes to enhance their biological activity and/or stability. Following the identification of a potentially useful compound, medicinal chemists alter the structure of a known compound in a systematic, rational manner. By evaluating the effect of each small change at numerous sites within a molecule, it is often possible to enhance the biological activity of a molecule of interest. Acetylsalicylic acid, known commonly as Aspirin, is the first example of a compound developed through medicinal chemistry.

Allopathic medicine has largely replaced traditional medicine in the Western world. Following the rapid rise in sophistication in synthetic organic chemistry and the increase in screening capabilities provided by biochemical advances, most pharmaceutical companies focused their drug development programs on synthetic molecules rather than natural products. Natural product study remains a challenging field for many reasons. Due to the time and effort required to secure pure compounds, many available samples are semi-pure extracts. If such an extract provides a "hit" in a biological screening, much effort is required to determine which component of the extract is active, whether the observed effect is due to a synergism between multiple compounds, or if some minor contaminant is causing a false-positive in the test (for example, inhibiting enzyme activity by behaving as a detergent). Although it is certainly possible to discover molecules with desirable activity through such a process, before a biological compound can be considered for further study, its structure needs to be unambiguously determined and a synthetic strategy must be developed. The development of a synthetic route and the structural analysis of synthetic compounds are obviously addressed before they are ever entered into a screening program; when a hit is generated, it is thus comparatively easy to generate larger quantities of the compound for

further testing. For these reasons, it is easy to appreciate why pharmaceutical companies are generally reluctant to rely upon natural product chemistry as a source for new lead structures (1).

Despite the obvious challenges inherent in natural product study, most pharmaceutical companies maintain an interest in learning from nature. Some developments in modern synthesis, such as combinatorial chemistry, have failed to deliver the libraries of useful compounds that they were initially expected to bring. As identification and separation techniques have improved in recent years, the ability of private or academic researchers to identify novel natural products and to begin to assess their biological activity has also progressed. By collaborating with these researchers, pharmaceutical companies are able to skip over the time-consuming screening and identification process which is necessary at the beginning of a research program (3). The enduring interest in natural products as useful pharmaceutical agents themselves, or as lead structures, is reflected by the number of chemical entities either inspired by or directly obtained from nature (**figure 1.1.2**). Only 30% of all new pharmaceuticals arise from purely synthetic studies: the remaining compounds are either directly biological in nature (such as peptides or proteins), are isolated from living organisms, are semi-synthetic products using a natural product as a scaffold, or take advantage of a pharmacophore found in nature (4). When the field is narrowed include only anticancer treatments, only 27% of the small molecules used since the 1940s are purely synthetic (4).

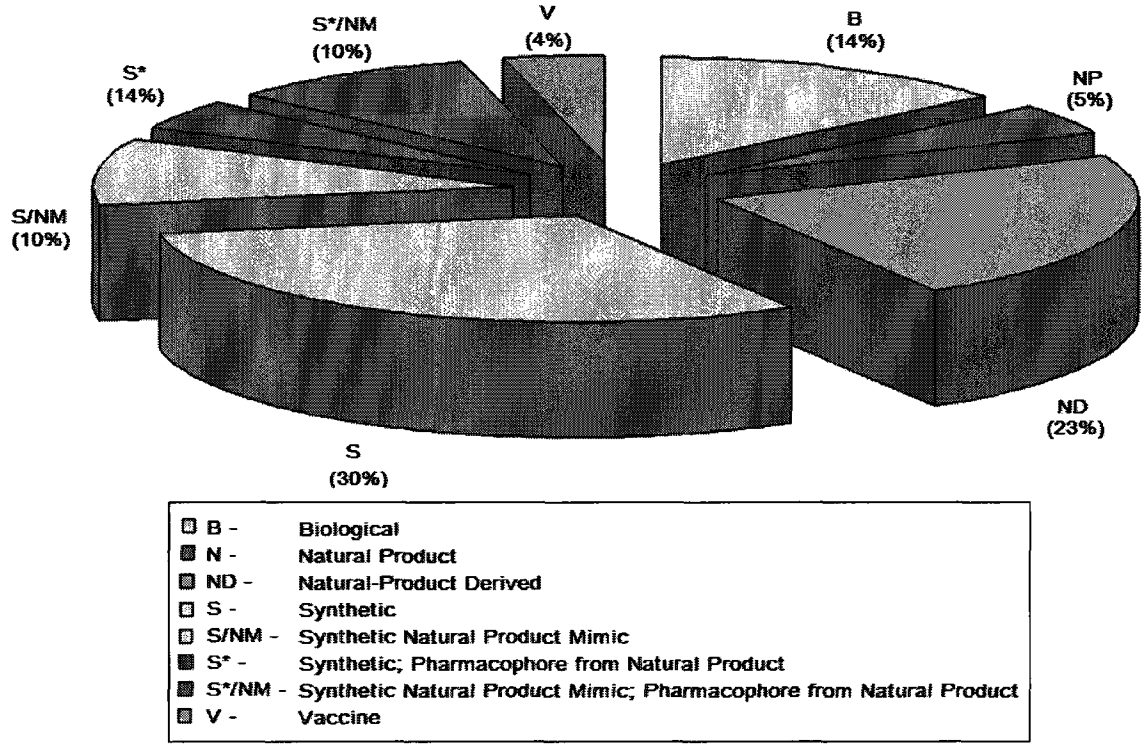


Figure 1.1.2 Sources of all new pharmaceutical chemical entities between January 1981 and June 2006 (N = 1184). Modified from (4).

This reliance upon nature to provide inspiration for pharmaceuticals has increased in recent years (**figure 1.1.3).** Beginning around 1990, purely synthetic compounds have represented a decreasing share of the pharmaceuticals on the market. Part of this change may be accounted for by the increased production of vaccines beginning in the mid-90's; however, even considering this change, synthetic compounds have given way to an increasing proportion of natural product-derived pharmaceuticals. These trends suggest that the screening of organisms may continue to yield economically important compounds in the years to come.

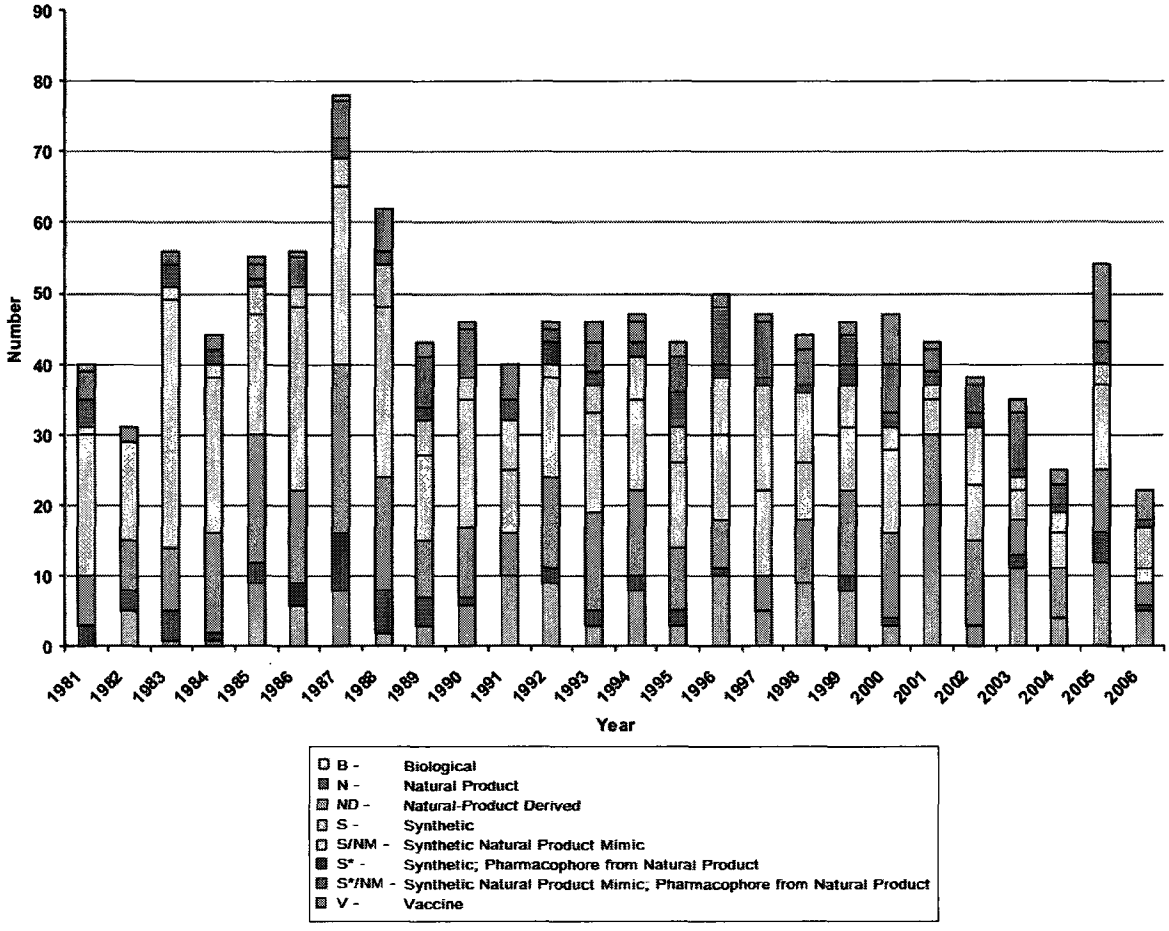


Figure 1.1.3 Sources of all new pharmaceutical chemical entities between January 1981 and June 2006 (N = 1184) by year. Modified from (4).

1.1.2 Marine Sources of Natural Products

Although compounds isolated from terrestrial species represent the vast majority of commercially important natural products, marine species offer a variety of highly potent, structurally complex molecules. The formal study of marine natural products has lagged behind terrestrial species for the simple reason that many aquatic species were inaccessible before modern developments in diving technology. Unlike the fungi or terrestrial plants responsible for producing or inspiring many of the compounds in **figure 1.1.2**, the marine species which produce interesting natural products are generally unculturable in a laboratory setting. This unfortunate feature makes the acquisition of large quantities of material for study a costly and difficult endeavour (5).

Although they may prove challenging to study formally, marine species, especially seaweeds, have played an important role in traditional medicine (6), (7), (8). Seaweeds are used for a variety of ailments including respiratory problems and dropsy. As goiters are caused by a lack of dietary iodine, many cultures have also discovered that seaweeds, which are generally very rich in iodine, are an effective treatment (9). One common observation regarding the biological activity of algae is their potency against both bacteria and viruses (10). Sulfated polysaccharides isolated from red algae inhibit the progression of retroviruses by apparently preventing replication and cell transformation (11). Some sulphated algal glycolipids have demonstrated activity against HIV (12). This history led to the discovery that *Fucus distichus*, an algae common to northern waters worldwide, possesses antibiotic and antiviral activity (13).

1.1.4 Previous Work on *Fucus distichus* and similar glycolipids

Studies into the proposed biological activity of *F. distichus* (**figure 1.1.4**) began at the University of Ottawa in 2005. In collaboration with Nunavik Biosciences, a company owned by the Inuit people of Nunavik (14), samples of *F. distichus* were collected from Ungava Bay (**figure 1.1.3**). Bioassay-guided fractionation via silica gel chromatography revealed that the polar extracts of the plant contained a component with antibiotic and antiviral properties.

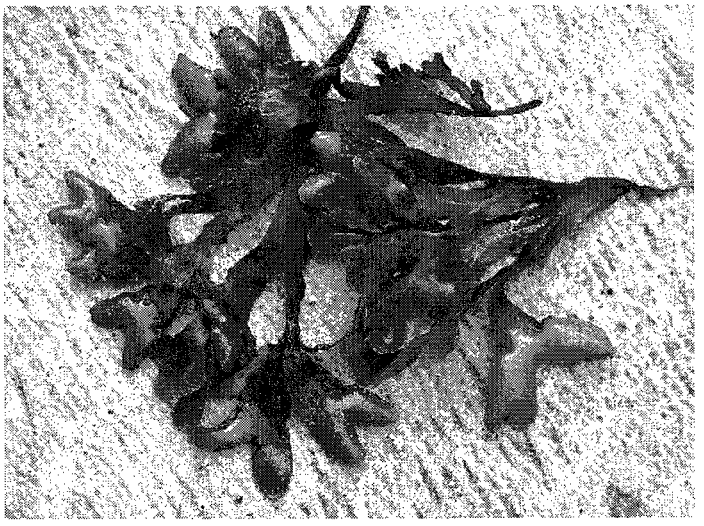


Figure 1.1.4 *F. distichus* harvested from British Columbia, Canada (24).

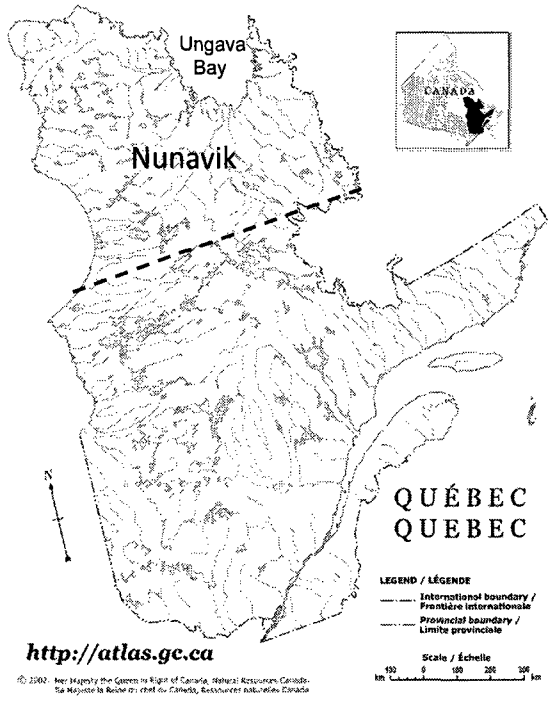


Figure 1.1.5 Map of Québec depicting the Nunavik region and Ungava Bay. Modified from (15).

The most active fraction from the column contained a complex mixture of sugars and sugar derivatives according to NMR. To facilitate the analysis of this mixture, the extract was polyacetylated before further chromatography. This modification allowed the isolation and purification of a single compound with biological activity more potent than any of the semi-pure extracts (**table 1.1.1**) (13). The proposed structure of this compound was a glycolipid consisting of *β-D*-galactose linked to glycerol, which was diesterified to linolenic acid (**figure 1.1.6**). These promising results revealed the need for more detailed study.

Table 1.1.1 Percent inhibition of *Herpes simplex* virus growth for crude ethyl acetate extract and increasingly polar fractions isolated by column chromatography compared to inhibition by isolated glycolipid

	Concentration (μg/mL)					
Fraction	200	20	2	0.2	0.02	0.002
Crude EtOAc	100	100	100	97	85	85
F1	41	0	-	-	-	-
F2	100	99	23	0	-	-
F3	100	100	92	0	-	-
F4	99	56	0	-	-	-
F5	100	100	100	56	-	-
F6	100	100	98	22	0	-
F7	100	100	100	76	31	0
F9	100	100	99	2	0	-
	Concentration (μg/mL)					
	100	10	1	0.1	0.01	0.001
Glycolipid	100	100	100	96	99	89

The stereochemistry at the C-2' position of the glycolipid was difficult to ascertain given the small amount of sample available from the algae. Literature precedent (ex. (16), (17)) suggests that the *S*-isomer (**1**) may be the natural product.

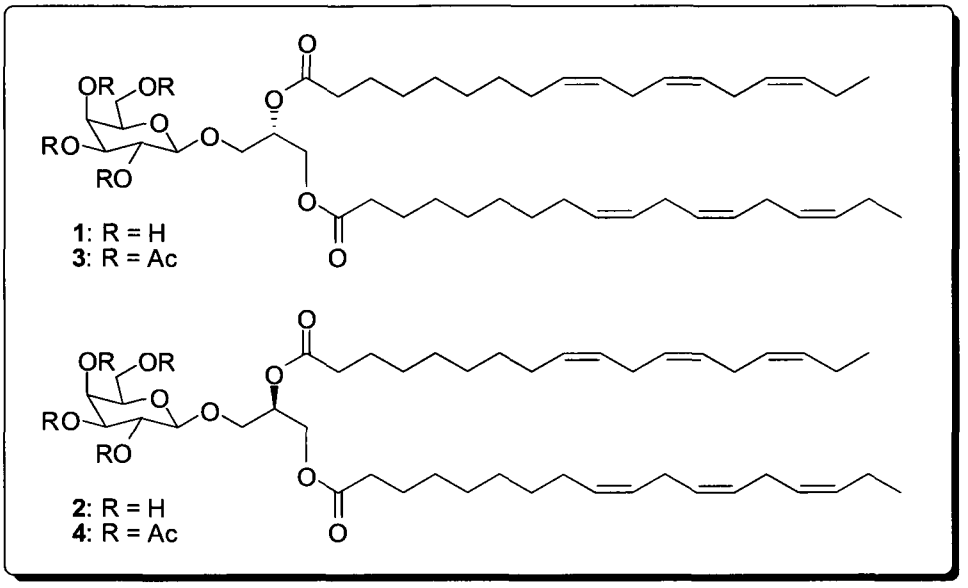


Figure 1.1.6 Glycolipid isolated from *F. distichus*. Although the natural product has free hydroxyl groups (**1,2**), to facilitate extraction, the natural product was tetra-acetylated (**3,4**) and the initial structural identification was made based upon this structure.

In 2003, researchers from Thailand reported the synthesis and anti-HSV activities of a series of glucose- and galactose-derived glycolipids with long-chain fatty acid tails of varying lengths and degrees of unsaturation. The results of their antiviral tests are presented in **table 1.1.3**. Examined as a whole, these results suggest a few key trends:

- Longer chains, with a higher degree of unsaturation, gave higher antiviral activity than shorter, more saturated chains. One notable exception to this trend is the lauroyl glycolipids (7b and 13a)
- Galactose-derived analogues gave higher activity than the equivalent glucose compounds (ex. entries 7m and 13d)
- The enantiopure glycolipid with *S*-stereochemistry at the C-2' position possessed only modestly improved activity over the racemic mixture (ex. entries 7g and 18b)

It is unknown whether the trends observed for antiviral activity will correlate with antibiotic activity as well. Despite the wide variety of glycolpids described in this work, one compound absent from their series is the di-linolenoyl galactose glycolipid **1** proposed as the active component of *F. distichus* responsible for its antiviral activity. Interestingly, these trends suggest that compound **1** may be the most active member of this series. Although this compound is known in the literature (ex. (18), (19)), it has never been investigated for antiviral or antibiotic activity.

Table 1.1.2 Inhibition of HSV strains 1 and 2 by synthetic glycolipids (50 μg/mL) (20)

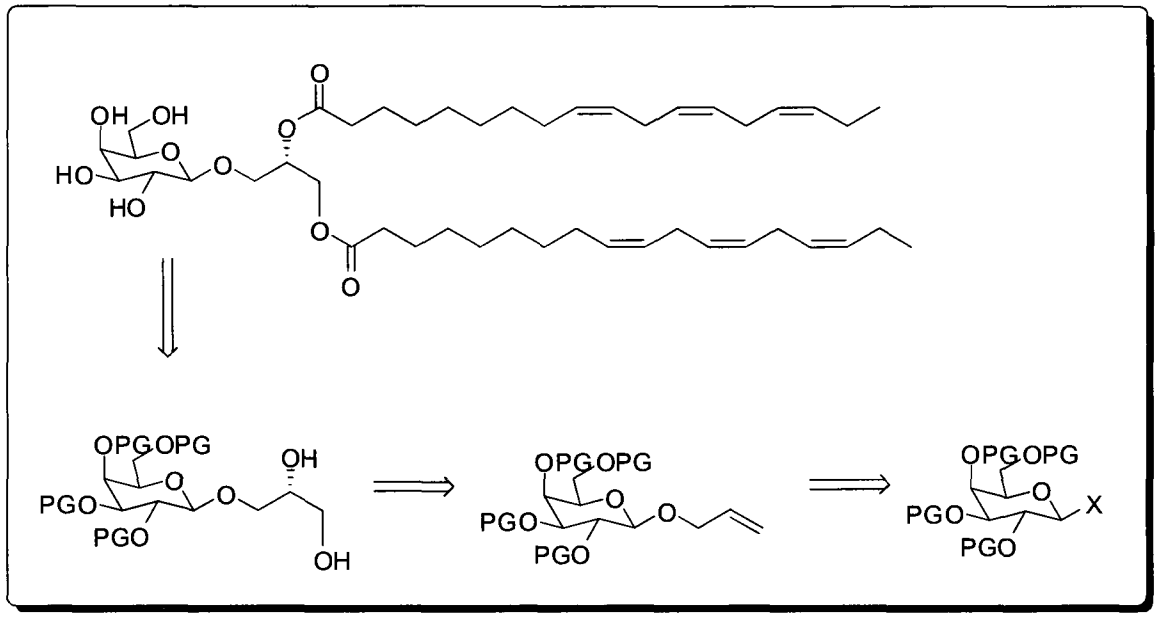
Compound	Sugar	Stereochemistry (C-2')	R_1	R_2	Inhibition (%)[a]	
					HSV-1	HSV-2
7a	glucose	rac.	decanoyl	decanoyl	0	0
7b	glucose	rac.	lauroyl	lauroyl	80±6	50±5
7c	glucose	rac.	steroyl	steroyl	0	0
7d	glucose	rac.	behenoyl	behenoyl	0	0
7e	glucose	rac.	oleoyl	oleoyl	0	25±3
7f	glucose	rac.	linoleoyl	linoleoyl	50±2	50±4
7g	glucose	rac.	linolenoyl	linolenoyl	94±4	97±4
7h	glucose	rac.	lauroyl	oleoyl	13±2	17±1
7i	glucose	rac.	steroyl	lauroyl	0	0
7j	glucose	rac.	steroyl	behenoyl	0	0
7k	glucose	rac.	behenoyl	lauroyl	0	20±1
7l	glucose	rac.	behenoyl	oleoyl	0	10±2
7m	glucose	rac.	linolenoyl	linoleoyl	90±5	70±2
13a	galactose	rac.	lauroyl	lauroyl	80±4	50±2
13b	galactose	rac.	linoleoyl	linoleoyl	55±2	50±2
13c	galactose	rac.	behenoyl	lauroyl	0	25±1
13d	galactose	rac.	linolenoyl	linleoyl	100±0	90±2
18a	glucose	S	lauroyl	lauroyl	80±8	50±4
18b	glucose	S	linolenoyl	linolenoyl	100±0	100±0

[b] Values are expressed as means ± S.D. of three replicates.

1.2 Results and Discussion

1.2.1 Proof-of-concept and natural product synthesis

The first goal of the glycolipid project was to validate the proposed structure of the active constituent of the algae extract through total synthesis. An adaptable synthetic strategy was designed to allow for the incorporation of different sugars, linkages, and fatty acid chains thereby enabling us to generate analogues.



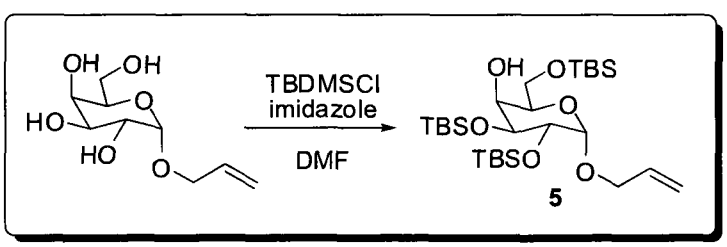
Scheme 1.2.1 Retrosynthetic analysis of glycolipid isolated from *F. distichus*

The retrosynthesis of this natural product is intuitive. The first disconnection is the linolenic acid chains to yield *β-D*-galactose linked to glycerol. This glycerol derivative may be synthesized either in racemic form or in either optical antipode by the dihydroxylation of the corresponding alkene. Introducing the alkene into suitably protected galactose in a

stereoselective fashion has been described in the literature on a number of occasions (ex. (21), (22)) and can generally be performed using allyl alcohol in the presence of a Lewis acid.

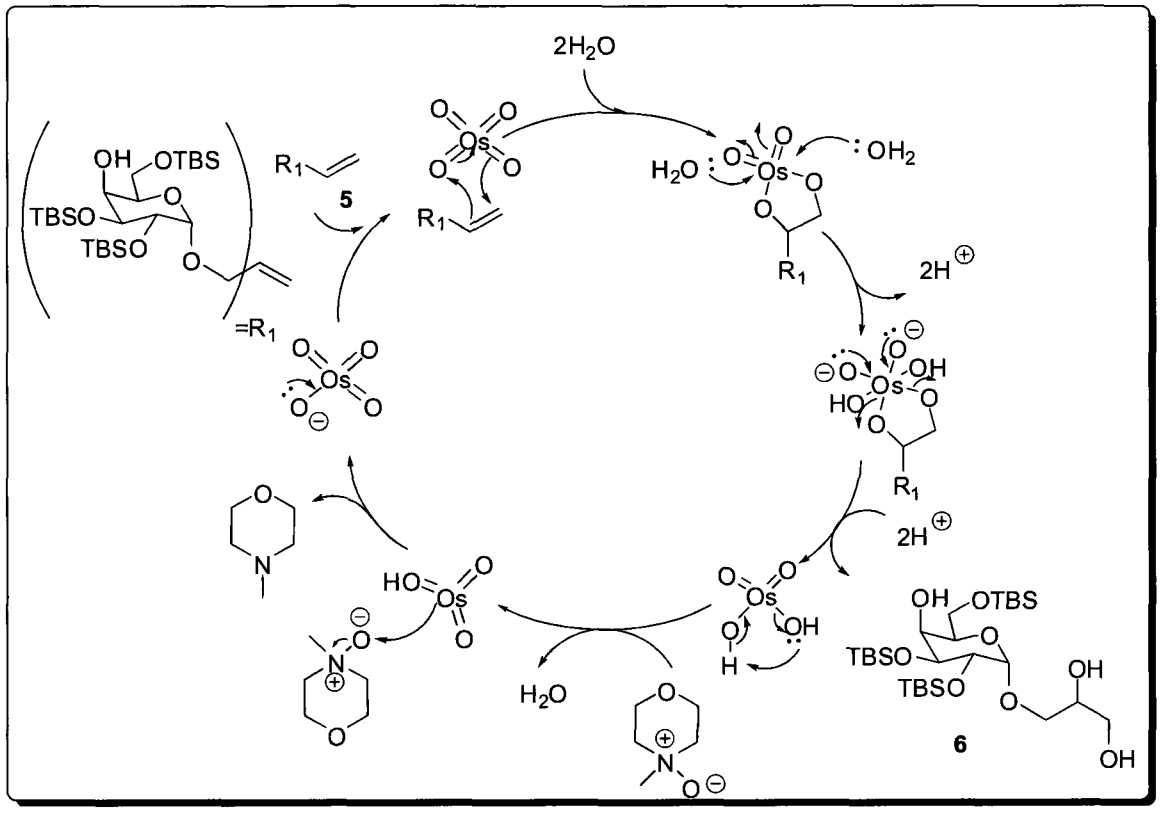
The first compound synthesized was the α-analogue of the natural product due to the ready availability of allyl-α-*D*-galactopyranoside. Initially, it was our plan to apply TBS groups to all four free hydroxyl groups of the sugar. However, the tetra-protection proved unsuccessful likely due to the hindered nature of the axial hydroxyl group at the 4 position on the sugar, and the triprotected derivative was instead obtained in 73% yield (**scheme 1.2.2**). The presence of only three TBS gropus, rather than four, was verified by the integration of the ^{1}H NMR. Fortunately, this hindrance also prevented the unintended esterification of this hydroxyl group in a later step in the synthesis, so the tri-TBS-protected sugar was carried forward without incident.

In this step, despite the use of an excess of TBDMS-Cl, a significant portion of the starting material remained di- or mono-protected. This material was re-protected in a second reaction and successfully utilized in the synthetic procedure.



Scheme 1.2.2 TBS-protection of *α*-O-allylgalactose. The triprotected sugar was the major product of this reaction rather than the tetraprotected sugar initially expected.

The next step of the synthesis necessitated the dihydroxylation of the protected alkene **5**. *Syn* dihydroxylation was achieved using a catalytic amount of OsO_4 and a slight excess of NMO as a re-oxidant (**scheme 1.2.3**). Because osmium is a highly toxic and expensive reagent, it is used in catalytic amounts; NMO is used as a sacrificial re-oxidant. To emulate the stereochemistry of the natural product, the Sharpless Asymmetric dihydroxylation was attempted. In this reaction, a chiral ligand creates a steric bias for one face of the molecule over the other; commercial reagents are available to selectively produce either diastereomer. The bulky chiral ligand is most effective at producing a high diastereomeric ratio when the alkene is highly substituted, especially with relatively large groups. The ratio tends to be poor when primary alkenes are used (23). Unfortunately, this poor selectivity was realized with alkene **5** and no obvious preference was detected. For this reason, dihydroxylation was performed with OsO_4 and NMO without the presence of a chiral catalyst.

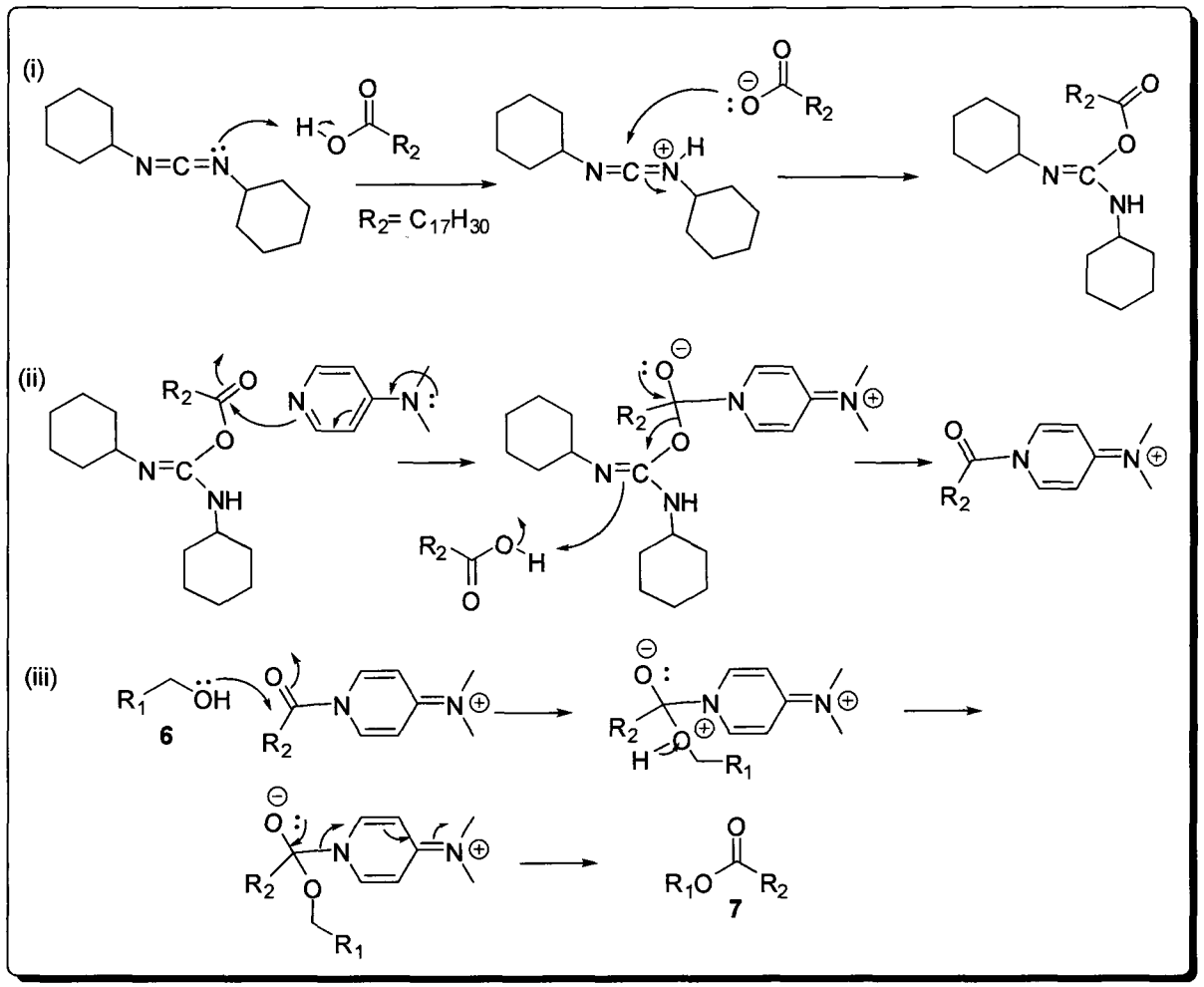


Scheme 1.2.3 Dihydroxylation of an alkene using OsO_4 with NMO as a reoxidant.

The successful dihydroxylation was confirmed by the disappearance of the alkene signals in both the proton [^{1}H δ: 5.87-5.97 (m, H-2'), 5.15-5.30 (m, H-3') for **5**;), 3.84-3.56 (m, H-2', H-3'a), 3.44 (dd, J = 10.5, 6.9 Hz, H-3'b) for **6**] and carbon [^{13}C δ: 134.2 (C-2'), 117.7 (C-3'), for **5**; ^{13}C δ: 69.9 (C-2'), 63.9 (C-3') for **6**] NMR spectra.

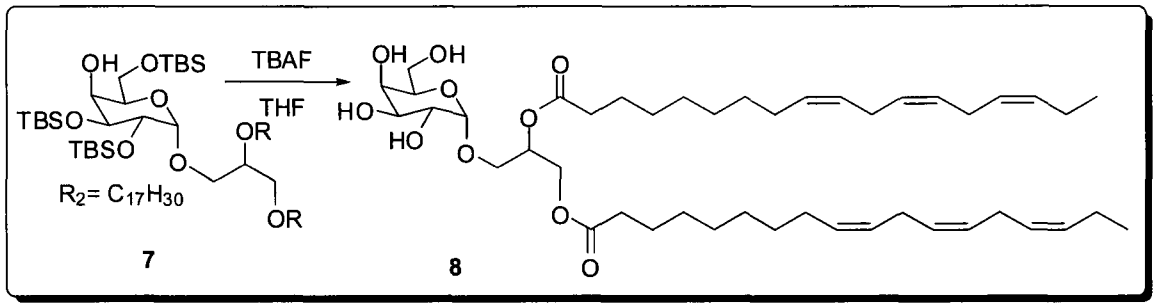
The esterification of the newly-installed hydroxyl groups of **6** was the next goal. The Steglich esterification (**scheme 1.2.4**), utilizing DCC and a catalytic amount of DMAP with an excess of linolenic acid, was expected to yield the diesterified product. The reaction was only modestly successful; although the desired product was obtained, the yield remained low

even after an extended reaction period (26% yield). This deficiency was addressed in a later synthesis, but optimization was not attempted with this substrate.



Scheme 1.2.4 Esterification of dihydroxylated sugar with linolenic acid. (i) DCC deprotonates the fatty acid, and the lone pair of the oxygen attacks the DCC's carbon. (ii) The acyl group is transferred to DMAP. (iii) The alcohol attacks the activated carbonyl, furnishing the ester.

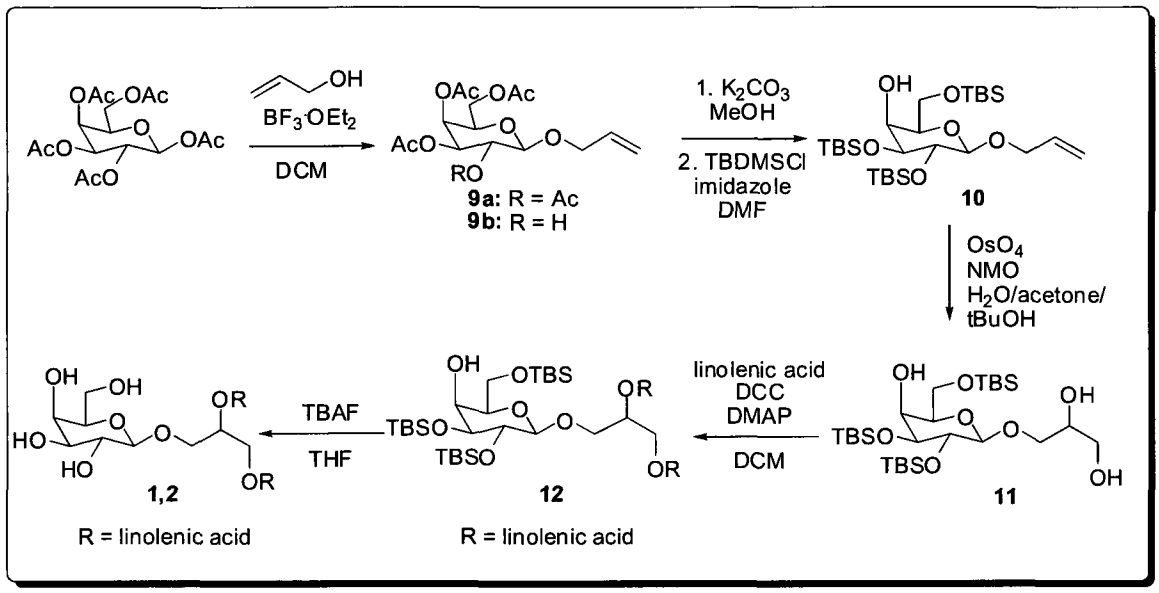
closely by TLC and was stopped when degradation of the starting material was observed. The desired glycolipid was isolated in 29% yield. The majority of the other material isolated was a mixture of semi-deprotected products which were collected and resubmitted to the reaction conditions.



Scheme 1.2.5 Deprotection of tri-*O*-TBS-*α*-*O*-glycolipid

This product was sent for evaluation in antibiotic and antiviral assays performed in the lab of Dr. J. Hudson at the University of British Columbia. The results of these tests are reported in **section 1.3**.

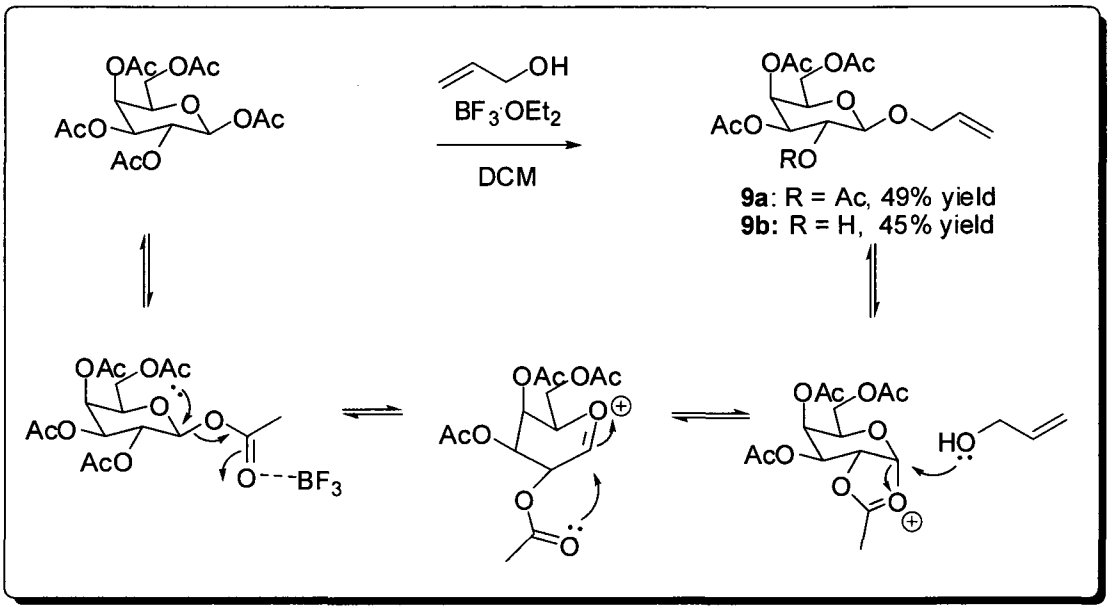
Following the proof-of-concept synthesis of the α-anomer of the natural glycolipid, the synthesis of the natural product was undertaken (**scheme 1.2.6**). The general procedures used in this synthesis are as described for the α-anomer.



Scheme 1.2.6 Synthesis of glycolipid isolated from *F. distichus*.

Penta-acetyl *β-D*-galactose (**scheme 1.2.7**) was treated with BF_3•OEt_2 and allyl alcohol in DCM. Boron trifluoride is a Lewis acid which coordinates to the OAc at the 1-position in the sugar. This coordination makes the OAc a good leaving group, and it is expelled by the internal oxygen's lone pair to form an oxonium ion. The allyl alcohol present in the reaction mixture may then attack from either the top or bottom face of the molecule: because the reaction is reversible under these conditions, the *β*-derivative where the allyl group is equatorial is formed preferentially due to the participation of the neighbouring acetyl group (**scheme 1.2.7**). This stereochemistry was confirmed by NMR. The relatively large coupling constant of H-1 to H-2 (J = 8.0 Hz) indicates an axial-axial relationship between the protons (compared to an equatorial-axial relationship, J ~ 2-4 Hz according to the Karplus relationship (24)). Both the 2,3,4,6-tetra-OAc and the 3,4,6-tri-OAc sugar derivatives were produced in this reaction. The formation of this second product may

be rationalized by the attack of either allyl alcohol or water onto the BF_3-activated carbonyl of the 3-OAc. The removal of this acetyl group did not prove to be problematic, as it was necessary to exchange the acetyl groups for TBS groups for the later stages of the synthesis.



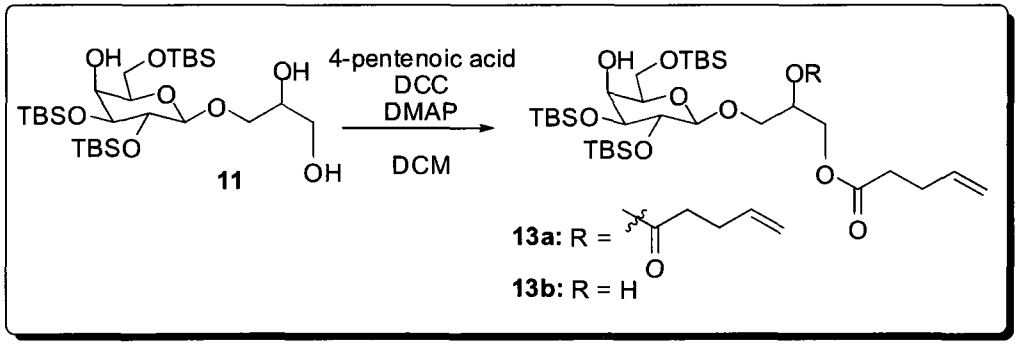
Scheme 1.2.7 Synthesis of allyl 2,3,4,6-tetra-O-acetyl-*β-D*-galactopyranoside

Deprotection of the allylated sugars **9a** and **9b** was achieved by treatment with methanol in the presence of a catalytic amount of K_2CO_3. This rapid transformation was followed by the reprotection of the 2,3, and 6-hydroxyl groups with TBS to produce **10.** Despite the obvious inefficiency in this deprotection-reprotection sequence, the removal of the acetyl groups was found to be necessary for the completion of the synthesis. Although earlier studies found that acetyl groups are effective at masking the hydroxyl groups for the duration of the synthesis and are stable under the reaction conditions utilized, the removal of these groups proved to be a serious challenge in the final step of the synthesis in the presence of the long-chain fatty esters. Despite literature precedence to the contrary (20), in our

hands, it was impossible to fully deprotect the sugar without also cleaving the fatty esters. A wide range of protecting groups were screened before the TBS groups were chosen (H. Mao, unpublished results). Under the relatively mild conditions of the synthesis, this protecting group remained in place. It was also possible to cleave all three protecting groups without perturbing the final product; TBS proved to be an ideal solution to this problem.

Following the protection of the carbohydrate's hydroxyl groups, the double bond of **10** was then oxidized to the corresponding diol, **11**, using OsO_4 as previously described. This transformation proceeded in 71% yield.

In the penultimate step, the newly-installed hydroxyl groups were esterified with linolenic acid. From the synthesis of the α-anomer, this esterification was anticipated to be troublesome. This problem was investigated by attempting the esterification with different carboxylic acids and a variety of different reaction conditions on both this substrate and upon some of the *C*-linked analogues, to be described later.



Scheme 1.2.8 Esterification of **11** with 4-pentenoic acid

Esterification of **11** using 4-pentenoic acid, DCC, and DMAP was more efficient than with linolenic acid, affording compound **15** in 54% yield (**scheme 1.2.8**). This improvement

suggested that the poor yields obtained in various esterifications with linolenic acid were likely caused by the physical properties of linolenic acid itself, rather than due to technique or the use of DCC. Because the synthesis of the natural product necessitated the use of linolenic acid, various solutions were explored.

The simplest solution seemed to be to enhance the reactivity of linolenic acid by synthesizing the corresponding acyl chloride. This transformation was attempted using both sulfuryl chloride and oxalyl chloride. Unfortunately the fatty acid appeared to degrade in both cases resulting in an unintelligible mixture of products, likely due to the attack of the chloride ion onto the double bonds of the linolenic acid. This route was abandoned.

The yield of the desired glycolipid **13** was improved to 46% by increasing the concentration of the starting material to 0.07 M. This concentration solubilised all of the reagents and allowed the stirring to continue for the duration of the reaction period despite the accumulation of the urea byproduct produced by DCC. In earlier studies, careful monitoring of the reaction by TLC revealed that the reaction's progress decreased as the solid precipitate inhibited stirring; more concentrated reactions were thus less successful.

The improvement of the esterification provided enough material to complete the synthesis. As in the preparation of the α-anomer, the final step in this sequence was the removal of the TBS groups by treatment with TBAF. This final step provided the proposed natural glycolipid as a racemic mixture of **1** and **2**.

As the glycolipid isolated from *F. distichus* was acetylated to facilitate its purification and structural identification, a small amount of the synthetic *β-O*-galactosyl glycolipid was acetylated to allow for comparison by NMR. The NMR spectrum of the acetylated natural product is very similar to that of the synthetic product (**figure 1.2.9**), in line with the initial

assignment. The minor discrepancies observed can be attributed to the fact that this NMR contains both the C-2'-*S* and C-2'-*R* isomers of the acetylated synthetic glycolipid **3** and **4**. We felt that this mixture was worth testing because previous study suggested that the racemic mixture was still highly active relative to the pure C-2'-*S* glycolipid (20). Both the acetylated glycolipids **3** and **4**, and the unacylated **1** and **2**, were sent for evaluation in antibiotic and antiviral assays.

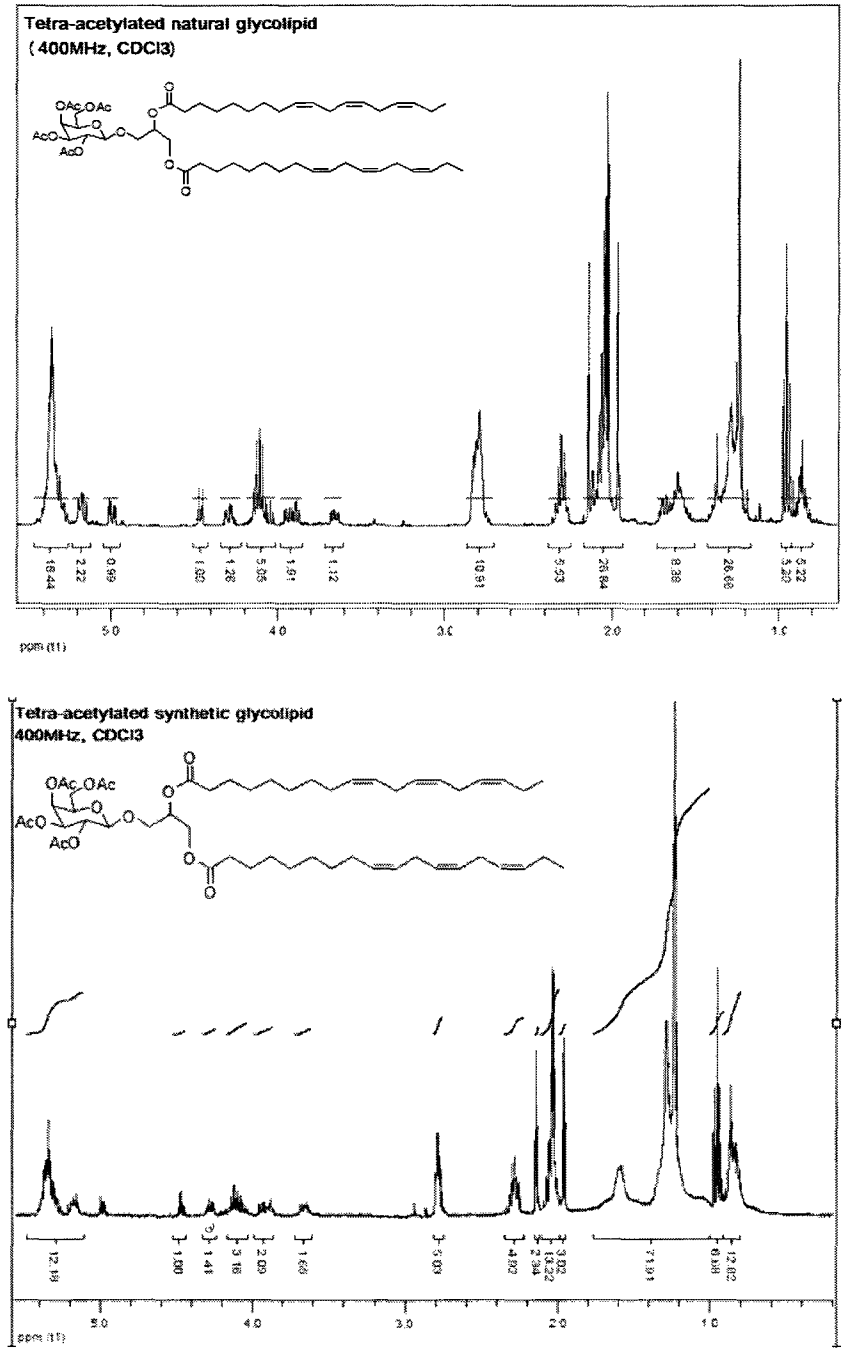


Figure 1.2.1 ^{1}H NMR of acetylated glycolipids

a) Natural product isolated from *F. distichus*

b) Synthetic *β-O*-galactosyl glycolipid

1.2.2 Synthetic analogues

Following the successful synthesis of the natural product, the preparation of a number of analogues was planned with the goal of improving their chemical and metabolic stability while maintaining or enhancing activity. In general, there are three main portions of the molecule: a sugar head, a short spacer (glycerol), and two chains (**figure 1.2.2**). Modification of these features provides a diverse set of related compounds which may be submitted for bioassay. Two major categories of analogues were planned: *O*-linked analogues, with an ether link to the sugar head group, and *C*-linked analogues, with a carbon-carbon bond between the sugar and the linker. The obvious advantage of the *C*-linked analogues is their improved chemical stability, especially in acidic environments.

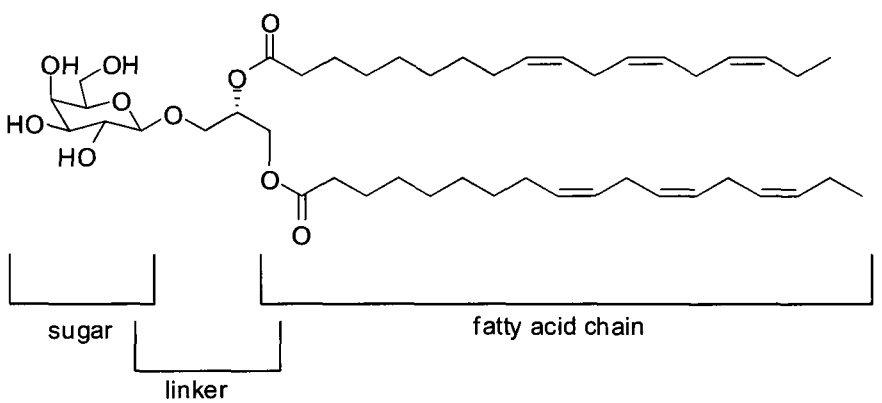
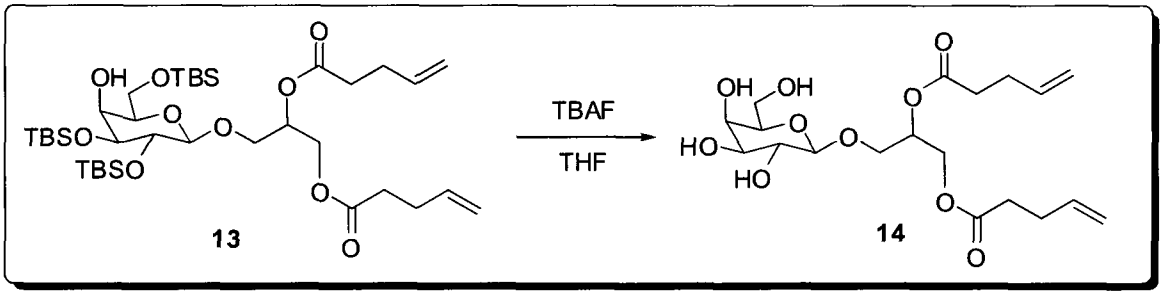


Figure 1.2.2 Schematic diagram of glycolipid. The structures of all analogues followed this basic pattern.

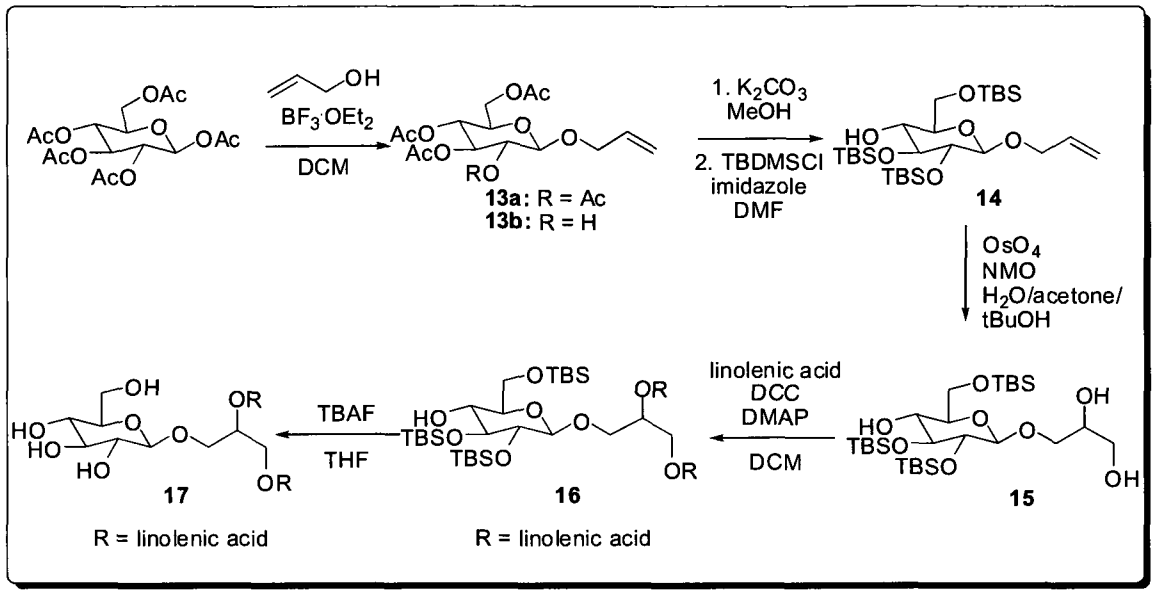
The first synthetic compound produced for bioassay was the pentenoyl glycolipid derived from protected compound **11** (**scheme 1.2.9**). This short-chain analogue was prepared for a number of reasons. First, and most simply, its protected precursor was

available from the optimization studies. The limited SAR study published by the Thai group (20) suggested that glycolipids with longer chains tended to possess higher antiviral activity. Because we already had the protected **13** in hand, we chose to complete the synthesis of **14** to probe this relationship further. The synthesis of **14** required only the removal of the TBDMS groups by treatment with TBAF. The desired compound was obtained in 44% yield after purification by column chromatography. The ^{1}H NMR of this compound displayed many obvious similarities to that of the mixture of **1** and **2**. The olefin protons, rather than appearing as a large multiplet as in **1** and **2**, ([^{1}H δ: 5.25-5.40 (12H, m, H-11', H-12', H-14', H-15', H-17', H-18')]) were instead apparent as two distinct sets of peaks ([^{1}H δ: 5.90-5.76 (2H, m, H-7'); 5.11-5.01 (4H, m, H-1, H-8')]).



Scheme 1.2.9 Synthesis of 4-pentenoyl *β-O*-galactosyl glycolipid **14**

The next analogue prepared was the *β-O*-linked glucosyl analogue of the natural product. This synthesis was analogous to the galactosyl glycolipid described previously (**scheme 1.2.10**). This glucose analogue was intended to provide information about the importance of the sugar head group in the glycolipid's antibiotic and antiviral activity.



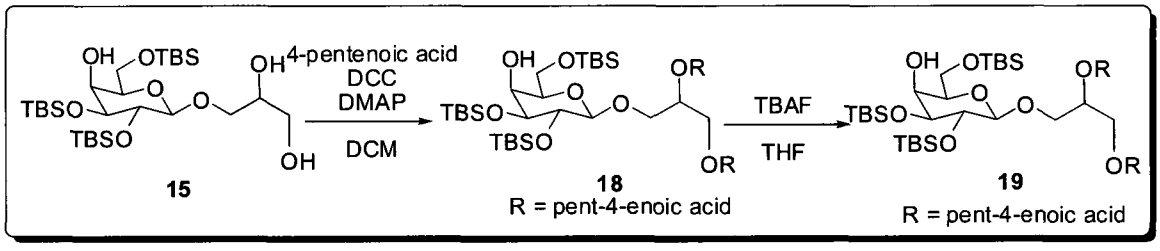
Scheme 1.2.10 Synthesis of glucose analogue of glycolipid isolated from *F. distichus*.

The *β-O*-allylation of penta-acetyl glucose was achieved with allyl alcohol in the presence of BF_3•OEt_2. A mixture of both the 2,3,4,6-tetra-acetylated (**13a**) and the 3,4,6-tri-acetylated glycosides (**13b**) were isolated in a 4:5 ratio with a combined yield of 92%. As described for the equivalent galactoside, both products were carried forward and the acetyl groups were removed in quantitative yield by treatment with a catalytic amount of K_2CO_3 in MeOH.

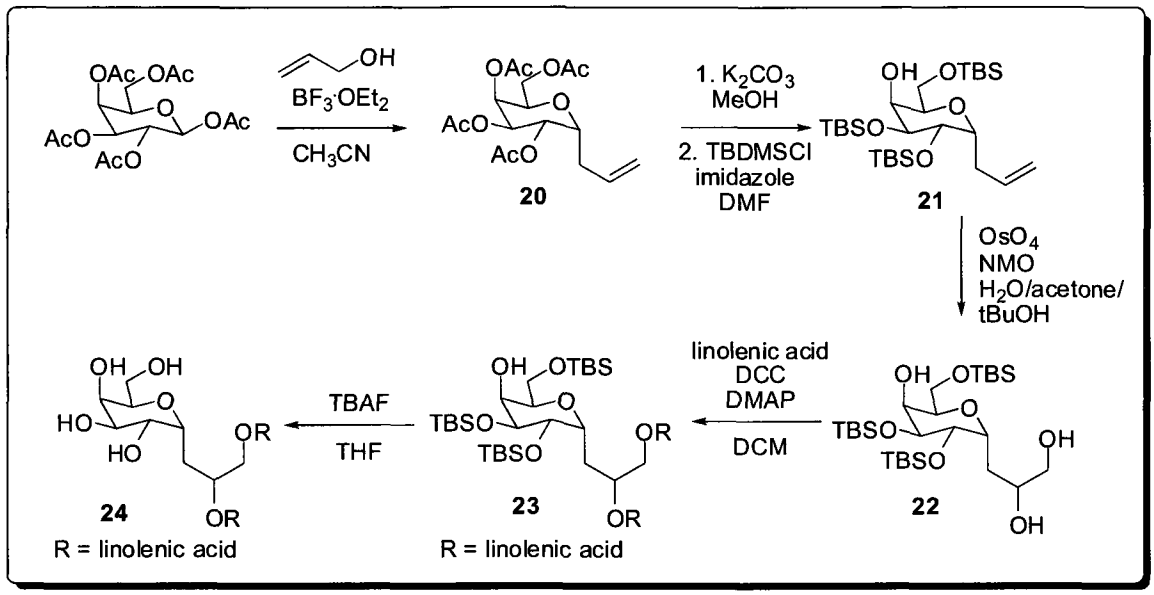
The reprotection of the glucoside with TBDMS-Cl again led primarily to the 2,3,6-tri-protected derivative **14**. Dihydroxylation proceeded without incident to provide the diol **15** in 80.3% yield. The optimization studies performed during the synthesis were applicable to the synthesis of this analogue. Performing this reaction at a high concentration as with the galactose glycolipid furnished the TBS-protected glucose analogue of the natural product **16**

in a satisfactory yield of 44%. Carefully-monitored deprotection of this compound led to the desired glycolipid **17**.

The pentenoyl glucose glycolipid was also synthesized to further the short-chain series containing galactose-derived glycolipid **12**. This simple analogue was prepared by esterifying the previously-described diol **15** with 4-pentenoic acid. As observed for the equivalent galactose compound **11**, this esterification proceeded in dramatically improved yield (100%) over the esterification with linolenic acid. The removal of the TBS protecting groups with TBAF afforded **19** in 73% yield.



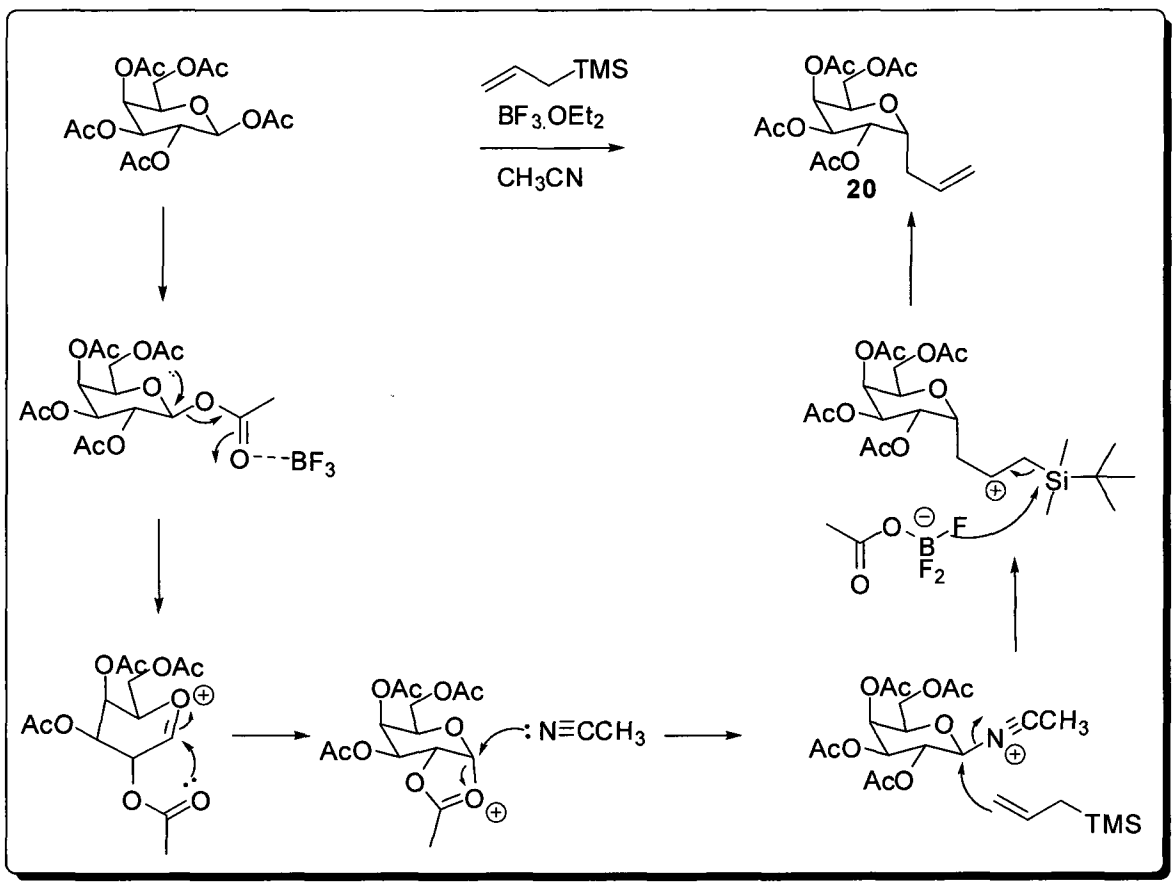
1.2.11 Synthesis of *β-O*-pentenoyl glucose glycolipid **19**



Scheme 1.2.12 Synthetic scheme towards *α-C*-linked galactosyl glycolipid **24**

As with the *O*-linked analogues, the first *C*-linked compounds synthesized were the *α-C*-linked sugars due to the relative simplicity of the synthetic route (**figure 1.2.12**). *α-C*-allylgalactose was produced by treating the penta-acetylated sugar with allyltrimethylsilane in the presence of BF_3•OEt_2. As in the *O*-allylation procedure, the role of the BF_3•OEt_2 is to behave as a Lewis acid, coordinating to the acetyl group at the 1 position to make it a more effective leaving group. The adjacent oxygen can donate its electrons, expelling the acetyl group and forming an oxonium ion in the process. The olefin of the allyltrimethylsilane then attacks the sugar; the resulting carbocation is stabilized by the β-silicon effect (25). Approach from the less-hindered bottom face of the sugar affords the *α*-anomer. The coordination of the BF_3 to the oxygen of the former acetyl group releases a fluoride ion, which attacks the silicon of the TMS, installing a double bond at the end of the carbon chain. The ^{1}H NMR of this product demonstrates only four acetyl groups [δ: 2.01 (s, 3H), 1.96 (s,

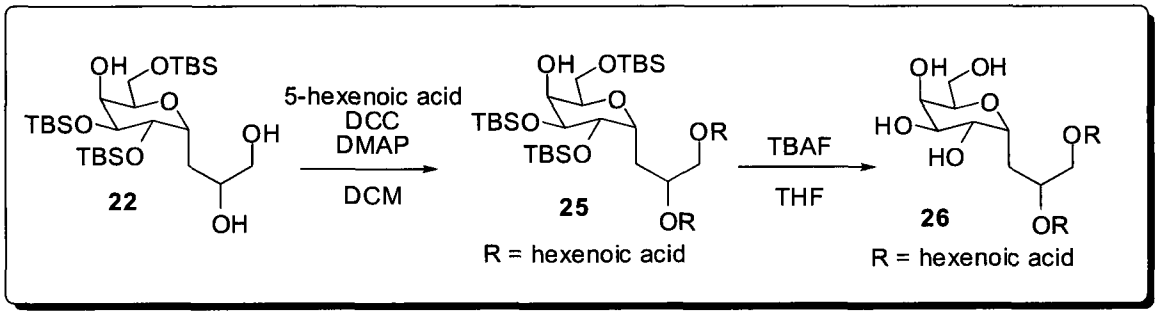
3H), 1.92 (s, 3H), 1.91 (s, 3H)] as well as the appearance of three olefin protons [δ: 5.71-5.60 (1H, m, H-9), 5.17-5.10 (2H, m, H-10)]. The stereochemistry of the anomeric centre was confirmed by comparison with the literature (26), as the direct observation of the H1-H2 coupling constant was difficult due to its complex multiplicity.



Scheme 1.2.13 Mechanism of allylation of penta-*O*-acetyl galactose

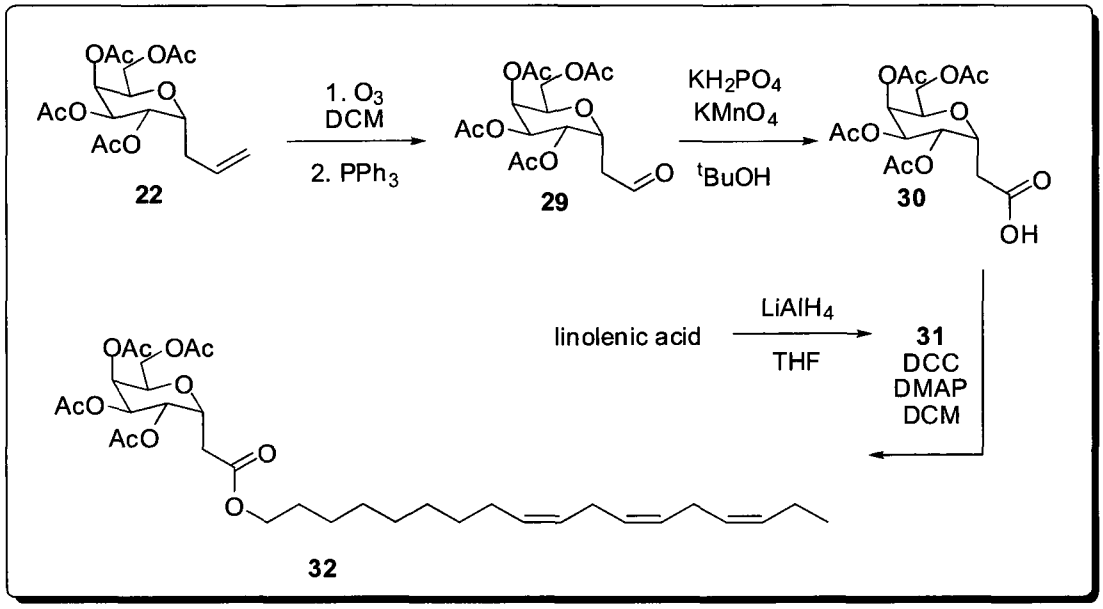
As with the *O*-linked analogues, it was necessary to remove the acetyl groups and replace them with the more suitable TBS groups before carrying forward in the synthesis. The deprotection-reprotection sequence was performed as described for the *β-O* analogues, furnishing protected glycoside **21** in 46% yield across 2 steps.

The desired glycolipid **24** was secured in 29% yield following its deprotection using TBAF. The low yield in this transformation may be explained by the very slow rate of deprotection; this yield accounts only for the fully deprotected product obtained after a single reaction. The remaining material was a mixture of semi-deprotected products, which were collected and re-submitted to the reaction conditions to provide more of the *C*-glycolipid **24**, providing a total yield of 41%.



Scheme 1.2.14 Synthesis of hexenoyl α-*C*-galactose-linked glycolipid **26**

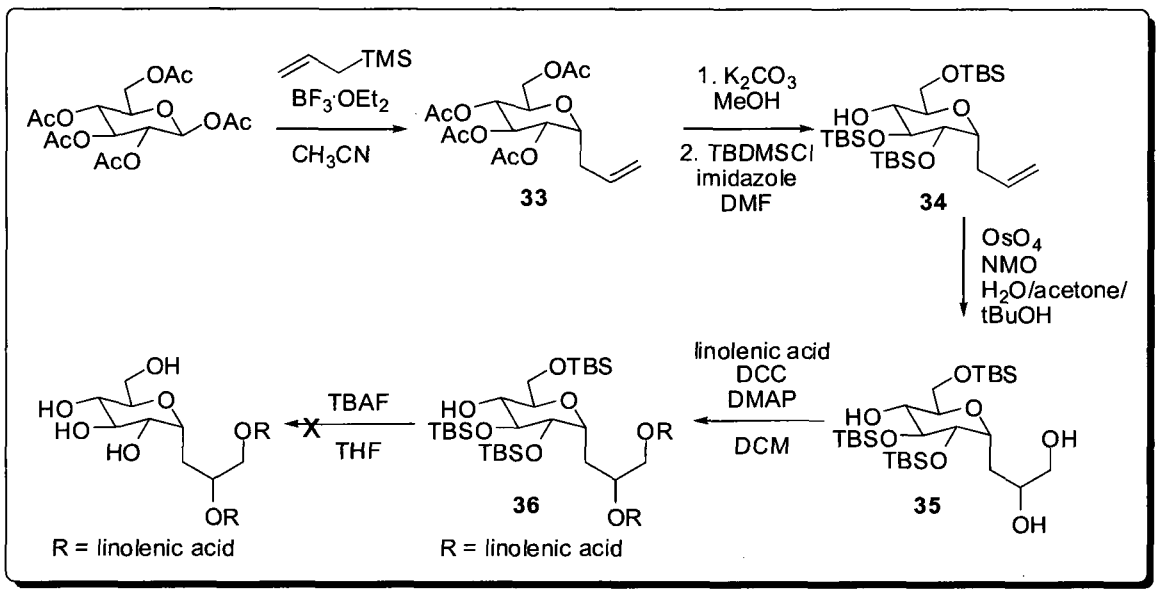
The short-chain glycolipid **28** was synthesized from the diol **22** in two steps. This small glycolipid was prepared to provide a further basis of comparison between the *C*- and *O*-linked glycolipids previously prepared.



Scheme 1.2.15 Synthesis of mono-linolenyl glycolipid from galactose-derived aldehyde **29**

A third *C*-α-galactose-linked glycolipid was planned as a mono-acylated derivative. With glycolipid **32**, we intended to probe the importance of having two linolenic acid chains. This mono-acylated compound was prepared quickly and in good yield beginning from the previously-synthesized α-*C*-allylgalactose. Allylgalactoside **22** was subjected to ozonolysis. This reaction proceeded smoothly, furnishing aldehyde **29** in 70% yield. The aldehyde was oxidized to the corresponding carboxylic acid **30** in 88% yield after treatment with the combined $KMnO_4$-KH_2PO_4 oxidant system (27). Separately, linolenic acid was reduced to its alcohol **31** by $LiAlH_4$. Coupling of the linolenol with the carboxylic acid **30** afforded the acetylated glycolipid **32** in 63% yield. The significantly improved yield of this esterification relative to the diesterification which produced glycolipid **25** is attributed to the fact that only a single coupling must be made. Furthermore, this single coupling is unhindered relative to the addition of linolenic acid to the 2'hydroxyl group of the diol **24**.

Glycolipid **32** was submitted for bioassay with its acetyl groups intact. We believed that deacetylation would be rapid under biological conditions, providing the deprotected glycolipid. Bioactivity results for this compound are given in **section 1.3**.

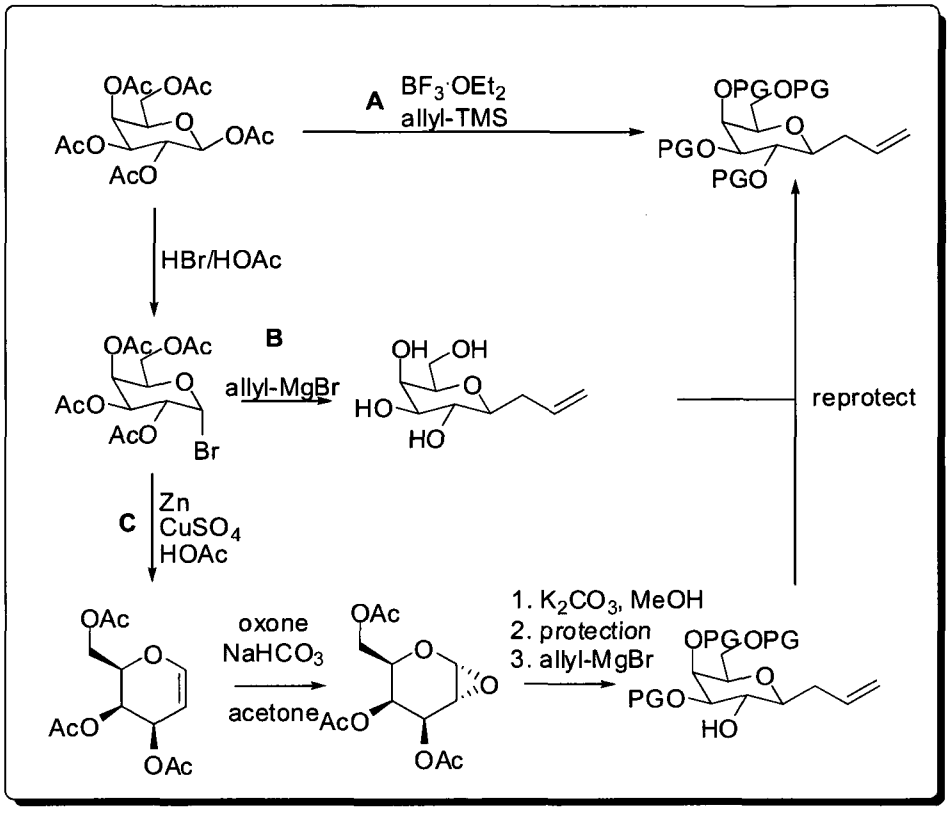


Scheme 1.2.16 Attempted synthesis of α-*C*-glucosyl glycolipid

The *C*-α-glucose-derived glycolipid analogous to the galactose-derived compound **24** was pursued to complete the compound series to be sent for bioassay.

Tetra-acetyl glucose was transformed into the α-*C*-allyl glucopyranoside **33** in 55% yield following purification and recrystallization in ether. A trace amount of the β-anomer was also detected, but was not pursued at this stage. The acetyl groups were exchanged for TBDMS protecting groups before the double bond was oxidized to furnish the diol **35.** The newly-installed hydroxyl groups were esterified to provide protected α-*C*-glucosyl ester **36** in 34% yield). Unfortunately, the deprotection of this ester was unsuccessful: none of the

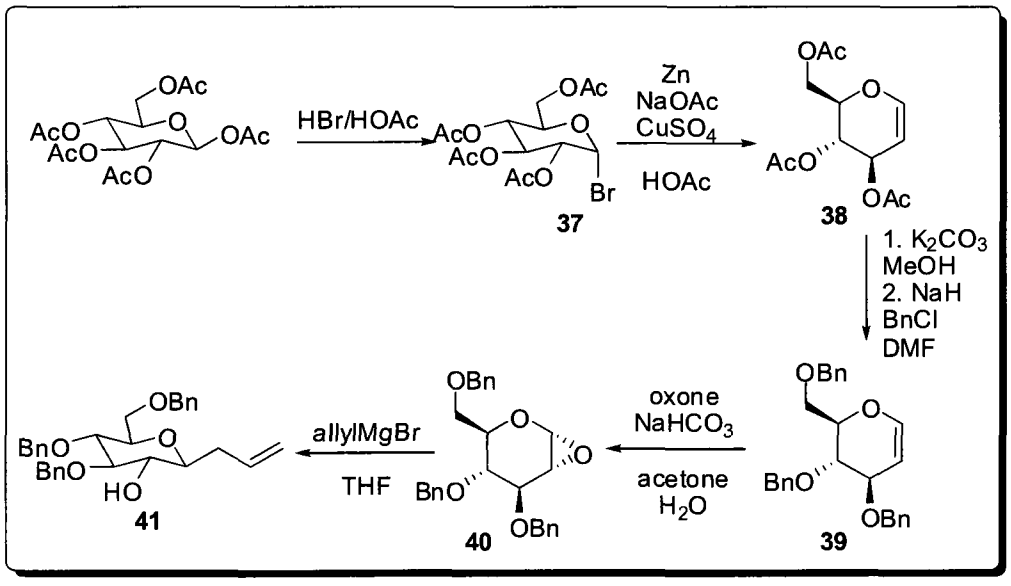
desired product was detected. NMR indicated no trace of the ester, implying that it had been destroyed in the deprotection experiment. It is possible that the particular TBAF used may have been improperly stored; the fluoride ion is basic (pka 10.8 in H_2O) (28), so any water present in the reaction mixture may have hydrolyzed the ester. Due to the inconsistent results obtained in the bioassay (**section 1.3**), this compound was not pursued further.



Scheme 1.2.17 Possible synthetic routes toward the *β-C*-allyl sugars.

Although the use of BF_3•OEt_2 and allyl trimethylsilane readily provided the α-anomers of both the glucose and galactose *C*-allyl compounds, the *β*-anomer was desired due to its similarity to the natural product. Although there are many possible routes to the desired

β-*C*-allyl sugars, each route considered (**scheme 1.2.12**) possessed a serious flaw: route **A**, while short, provides only minute quantities of the desired anomer; the reaction conditions strongly favour the α-anomer. Route **B** is also quite short, but the yield of the Grignard reaction is very poor, and because the acetyl groups are removed, hinders the isolation of the desired product. The third route, **C**, avoids the isolation problems inherent in **B**, and each step of the synthesis is high-yielding, unlike **A**; however, the route is so long that the yield is inevitably low after extensive handling. Route **B** was attempted on numerous occasions and with varying reaction conditions, but the anticipated problems with yield and handling did indeed limit the application of this procedure. For this reason, route **C** (**scheme 1.2.2**) was pursued as a means to obtain the desired allylated sugar.



Scheme 1.2.18 Synthesis of β-tri-benzyl *C*-allyl glucose **41**

Bromination of penta-acetyl glucose was achieved readily with a mixture of HBr/HOAc according to literature precedent (29). This reaction affords primarily the α-brominated sugar due to the anomeric effect (30). A slightly modified Fisher-Zach method (29) was used for the preparation of the galactal.

The epoxidation of the glucal proved to be a sensitive stage of the synthesis. On separate occasions, both the tri-benzylated and the tri-acetylated glucals were subjected to epoxidation conditions (31). Unfortunately, during the 6-hour reaction time suggested by the literature, both compounds were over-oxidized to the diol rather than to the desired epoxide. This problem was exacerbated by the identical Rf of the starting material and the desired epoxide, making the reaction difficult to monitor. Eventually, epoxidation of the acetylated glycal was abandoned as it was more prone to over-oxidation than the benzylated product; in any case, the acetyl groups would not survive the Grignard reaction planned for the next step, making the isolation of the product much more difficult. By decreasing the temperature from 0°C to -20°C and monitoring the reaction closely by TLC, it was possible to stop the reaction as soon as the undesired diol began to form. This also meant that the reaction was ended before the full conversion of the glucal to the epoxide. In a typical reaction, the ratio of starting material: product varied between 1.7:1 and 3:1 before the diol became visible by TLC (**figure 1.2.3**). Due to their similarity in polarity, the separation of these compounds was not attempted.

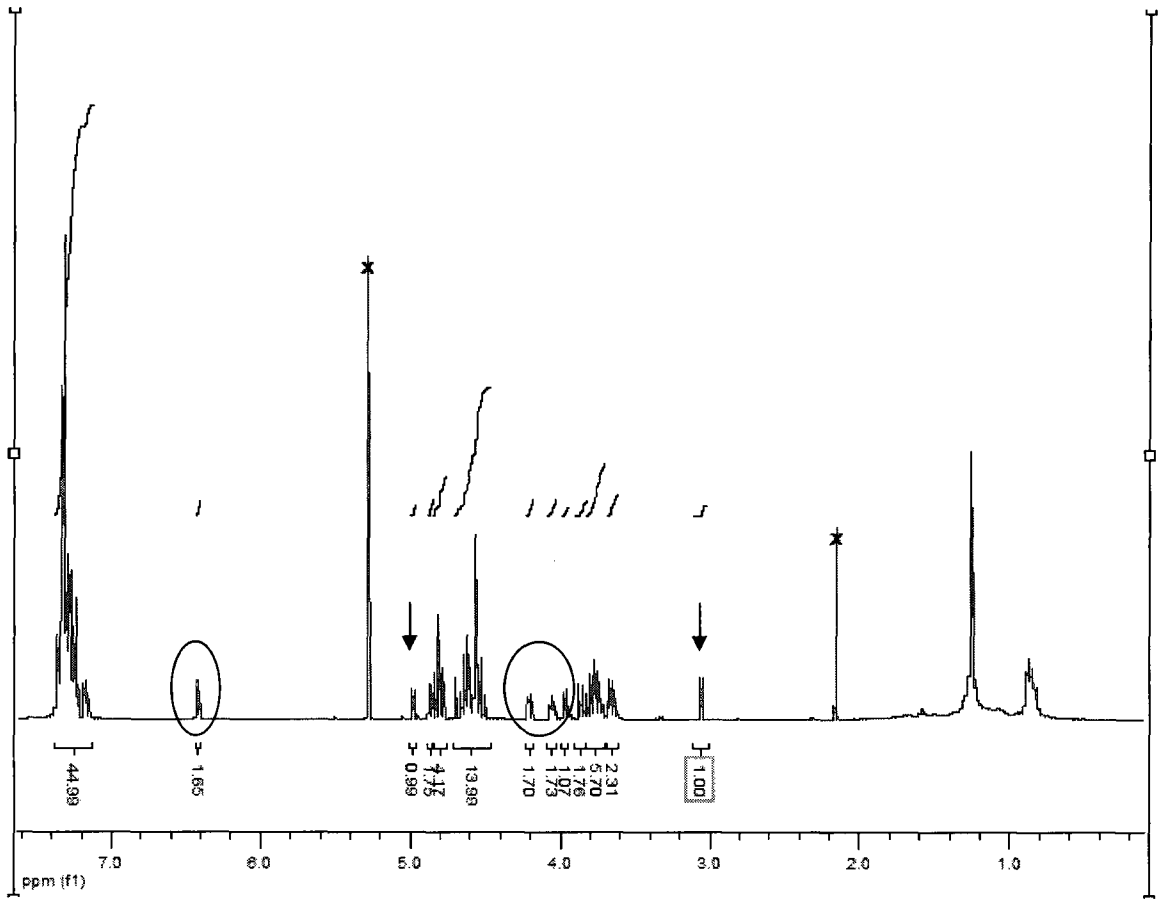
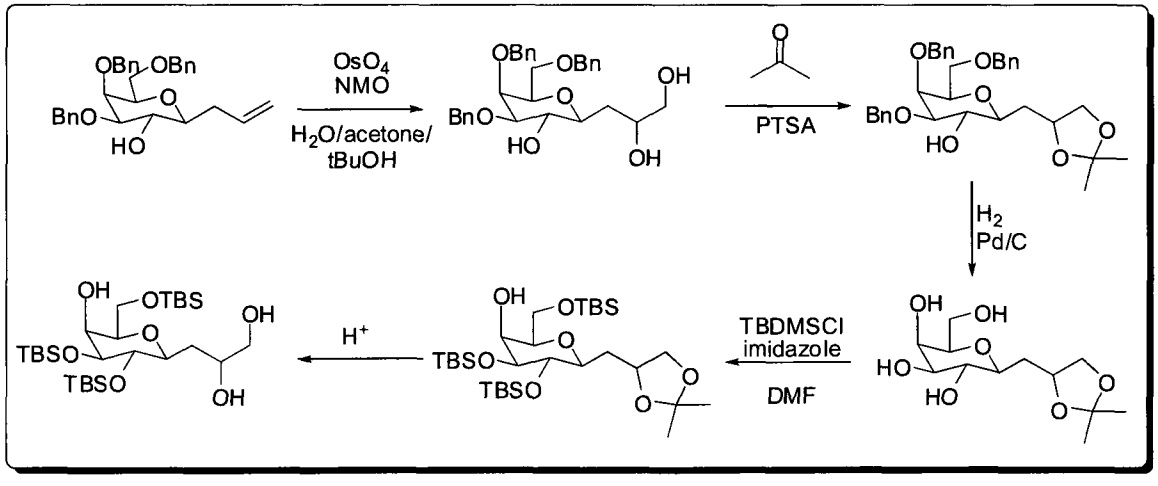


Figure 1.2.3 ^{1}H NMR depicting the mixture of epoxide and glucal obtained after the attempted oxidation of tri-*O*-benzyl glucal. Key signals for the glucal are indicated with a circle while peaks for the epoxide are indicated by an arrow.

Fortunately, the mixture of the epoxide **40** and the glucal **39** could be used directly in the alkylation step because the alkene is unreactive to the Grignard, and the Rf of the allylated product is significantly different from the glucal. As predicted, alkylation of the epoxide in the presence of excess allylmagnesium bromide proceeded smoothly and the desired *C*-glycoside **41** was readily separable from the glucal.

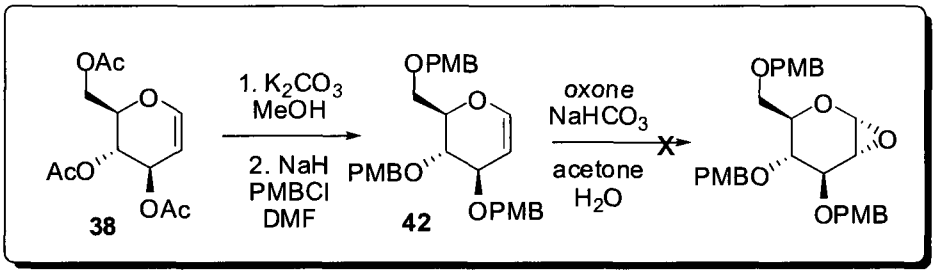
Before proceeding with the synthesis, the protecting groups needed to be changed into the TBDMS groups which had worked successfully in earlier studies. Because the removal of benzyl groups is generally achieved using hydrogenation or other conditions generally incompatible with alkenes (32), we felt that it was safer to change the protecting groups at this stage rather than proceeding to the end of the synthesis and risking the double bonds in the long-chain fatty acid.

To avoid the accidental reduction of the newly-installed olefin, a longer route was planned (**scheme 1.2.19**). First, the double bond could be dihydroxylated as originally planned in the sequence. The new hydroxyl groups could then be protected as an acetonide while the benzyl groups are removed and replaced with the desired TBS groups. Finally, removal of the acetonide will allow for the synthesis to proceed as described for the previous analogues.



Scheme 1.2.19 Alternative route towards 2,3,6-*O*-tri-TBDMS-*β*-*C*-galactopyranoside

As an alternative to this longer route, the PMB-protected glucal **42** was prepared. The deprotection of the PMB-protected *C*-glycoside was anticipated to proceed more easily as PMB groups can generally be removed under milder conditions relative to allowing more direct access to the useful TBDMS-protected glycoside. Surprisingly, the epoxidation of the PMB-protected glucal was unsuccessful despite repeated attempts.



Scheme 1.2.20 Attempted epoxidation of PMB-protected glucal **42** was unsuccessful.

With the desired *β-C*-allylglycosides secured on a small scale, the next goal was the scale-up of this reaction. Disappointingly, the scale-up proved extremely difficult. Despite the careful addition of oxone at a low temperature, this reaction could not be successfully replicated on a larger scale: rather than the desired epoxide, a variety of over-oxidized products including diols and aldehydes were identified when the reaction mixture was scaled up. This problem was not resolved during the timeframe of this project, but there are a number of plausible explanations. On a larger scale, the stirring of the biphasic reaction may have become less efficient. This poor mixing may over-expose the upper portion of the organic phase to the oxone generated in the aqueous layer. This would also explain why, in some trials, both starting material and overoxidized product were isolated with only trace amounts of epoxide detected. Similarly, allowing insufficient time for the reaction to cool, or

adding the oxone solution too quickly, may have also allowed the reaction to proceed too quickly.

The extreme variability in this step, coupled with the problems that were encountered in the bioassays (**section 1.3**), halted the progress of the glycolipid project.

1.3 Biological Studies

The glycolipids synthesized were subjected to the same antibacterial assays as the natural glycolipid. The bacterial species chosen for this analysis are clinically relevant organisms. The first species, *Propionibacterium acnes* is an anaerobic bacterium found among the flora on the facial skin of many humans. This bacteria is implicated in the common skin disease, acne (33), but may also play a role in serious inflammations and sepsis (34). The glycolipid was tested against both a clinical and a commercial (ATCC) strain of this bacteria.

Clostridium difficile is a common pathogen in the human gut. Overuse or misuse of antibiotics may allow colonies of antibiotic-resistant *C. difficile* to multiply rapidly when other gut flora are weakened. The resulting symptoms include bloating and diarrhoea, and death may occur in weakened patients (35).

Streptococcus pyogenes is among the bacteria that may be responsible for causing strep throat and more serious infections including scarlet fever (36).

MSSA and MRSA(methicillin-susceptible- and methicillin-resistant *Staphylococcus aureus,* respectively) are responsible for a variety of ailments including relatively minor skin infections, but also much more serious opportunistic infections including pneumonia (37).

The full procedure for the bacterial assays may be found in the 2008 report prepared for Nunavik Biosciences (38). In brief, pure bacterial cultures were made in suspension and were plated on blood agar plates or selective agar plates by serial dilution. Each bacterial preparation was treated with an equal volume of *F. distichus* dilution before being exposed to invisible light to one hour on a shaker and being plated in 2.5 μL aliquots at an appropriate

dilution. The aliquots were spread across the plate with sterile plastic loops. Control untreated samples were kept in the bio-safety cabinet during the treatment period. Duplicates for all samples were performed; duplicates were also performed on each plate. Controls were composed of duplicate + solvent, and a control was made for each bacterium + light + solvent.

The bacterial strains were grown and assayed on blood agar plates incubated at 35°C for 24 hours (*S. Pyogenes,* MRSA, and MSSA), 48 hours (*C. difficile* in an anaerobic chamber), or 72 hours (*P. acnes* in an anaerobic chamber). After the specified period, the plates were removed from the incubator and colonies were counted manually.

The first compound sent for antibiotic testing was **6**, the *α-D*-glucose derived glycolipid. Despite the inverted stereochemistry at the anomeric centre, this synthetic glycolipid displayed very high inhibition of all of the species it was tested against (**figure 1.3.1**). Even at a concentration of 12.5 μg/mL (16.1 μM), the glycolipid was still able to inhibit the growth of all six bacterial species by greater than 60%. Although these precise tests were not performed with the natural glycolipid, note that the activity of the natural product at 50 μg/mL is plotted on this graph as bullet points. For three out of the four bacterial species tested with both compounds, the activity is comparable; for *C. difficile*, the activity of the synthetic product is 20% higher than the natural product. These results reinforce earlier studies (20) suggesting that the stereochemistry at the anomeric centre of the sugar does not have a large impact on antibiotic activity.

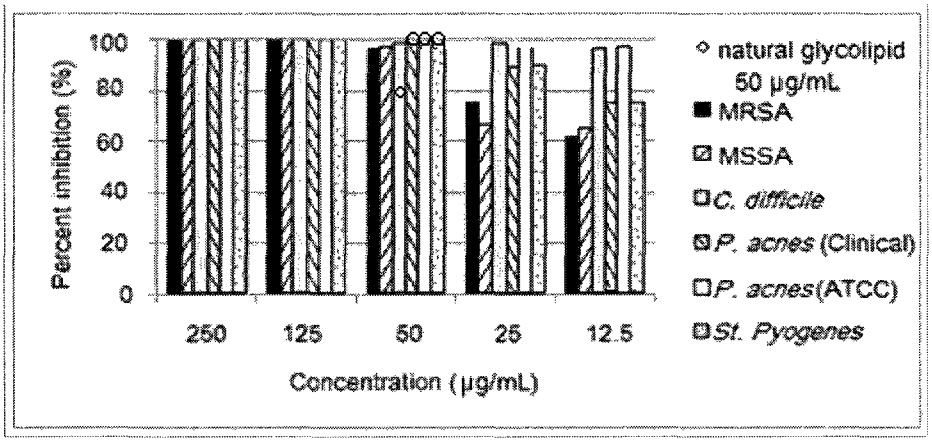


Figure 1.3.1 Percent inhibition of six bacterial species by *α-D*-galactosyl glycolipid **6** at concentrations ranging from 250 to 12.5 µg/mL and for natural glycolipid **1** at 50 µg/mL (38).

In addition to testing the synthetic glycolipid **6**, the crude algal extract and a number of other glycolipids isolated from the algae were also assayed against a range of bacteria at a concentration of 50 µg/ml (**table 1.3.1**). Interestingly, the crude algal extract displayed the highest activity against all four bacterial strains it was tested against. It outperformed both the glycolipid **1** as well as both of the isolated but unidentified glycerolipids, suggesting that its high activity may be the result of a synergistic effect between multiple active compounds, rather than solely due to the activity of the glycolipid **1**. The presence of multiple compounds with similar biological activity is a common strategy in nature known as phytochemical redundancy (39): requiring a pathogen or predator to acquire resistance to a large number of toxins provides more protection than possessing a single toxin.

Table 1.3.1 Ratios of treatments versus control at 50 μg/ml presented as percentages of inhibition of various bacterial strains (38)

	P. acnes Clinical	*P. acnes* ATCC	*C. difficile*	*St. pyogenes*	MSSA	MRSA
Crude algal extract	99.8%	100%	97.5%	100%	-	-
Algal glycerolipid a	20%	50%	50%	0%	-	-
Algal glycerolipid b	99.9%	99.3%	80%	100%	-	-
Natural glycolipid **1**	100%	99.9%	80%	100%	-	-
Acetylated glycolipid **2**	98.8%	70%	40%	100%	-	-
Synthetic **8**	100%	100%	99.7%	100%	99.4%	97.1%

Both the synthetic *β-O*-galactyl glycolipid **1** and the *β-O*-glucyl glycolipid **18** yielded promising results in preliminary antibiotic assays (**table 1.3.2**). Both compounds appeared to display similar biological activity, which is again in line with previously-published results for similar compounds (20). The activity of both analogues was comparable to that of the natural product against two of the three species tested: however, the natural glycolipid significantly outperformed both of the synthetic compounds against *C. difficile*. The most

likely explanation for this apparent discrepancy is that the natural product may have contained some trace contaminants which behaved as synergists in this assay.

Table 1.3.2 Antibacterial activities of synthetic *β-O*-glycolipids expressed as $\log_{10}$ cfu (colony forming units) inactivated by standard concentration of synthetic *β-D*-galactose- and *β-D*-glucose-derived glycolipids at 100 μg/mL (38), (13). The maximal limit of the assay was 4.

Bacterial strains	*β-D*-Gal GL	*β-D*-Glu GL	Natural glycolipid **1**
P. acnes ATCC	>3	>3	3
P. acnes Clinical	>3	>3	-
C. difficile	<1	<1	3
S. pyogenes	>3.2	>3.2	>3

The next set of compounds sent for biological assay included a number of short-chain glycolipids in addition to the *C-α*-galactose analogue of the glycolipid. Unfortunately, the results of this assay were disappointing. The most active compound tested at this time appeared to be unmodified linolenic acid. None of the glycolipids sent for assay possessed any significant activity (**table 1.3.3**).

Table 1.3.3 Antibacterial activities (log_{10} decrease) at a concentration of 100μg/mL for six compounds sent for assay in May 2008.

Compound	*Streptococcus pyogenes*	*Propionobacterium acnes*	*Clostridium difficile*
Linolenic acid	5.23	0.92	0.98
C-α-gal-pentenyl glycolipid	0.7	0.75	0.02
O-β-glu-pentenyl glycolipid	0.61	0.7	0.08
O-β-gal-pentenyl glycolipid	0.65	0.75	0.98
C-α-gal-linolenyl glycolipid	0.93	0.44	0.41
C-α-gal-mono-linolenyl glycolipid	1.71	0.66	0.89

The causes of these surprising results are unknown, but there are several variables to consider. First, and most seriously, it is possible that the compounds synthesized truly are poor antibiotics. The *O*-linked compounds which were evaluated at this time had only short fatty acid chains with a single unsaturation; previous studies suggest that higher degrees of unstaturation tend to enhance antibiotic activity (20). Additionally, the *C*-linked analoges tested were the first *C*-compounds to be tested in our assay, so there is no reference with which to compare them. Further testing is required to explore these results before any of the compounds, especially the *C*-analogues, should be discounted. Because there was no positive control included in this assay, it is also possible that there was simply a problem with the assay itself.

Significantly, we have observed that these glycolipids are generally unstable even when they are kept in a carefully controlled environment (ie. in a sealed vial flushed with N_2 and stored at -20°C). The antibiotic and antiviral tests for this project were performed in the laboratory of Dr. James Hudson at the University of British Columbia, so it was not possible to observe precisely how they were performed. If the synthetic glycolipids were exposed to warm temperatures during shipping, or if they were not properly stored after arriving in British Columbia, it is possible that the samples may have oxidized or otherwise degraded before the assays were performed.

Further antibiotic studies designed to address these important questions were planned to take place in the lab of Dr. J.T. Arnason at the University of Ottawa, but problems were encountered in securing the media and equipment required for these assays. Unfortunately, these delays coupled with the instability of the glycolipids themselves prevented the investigation of any other compounds.

1.4 Conclusion

A total of nine different glycolipids were synthesized in this project (**figure 1.4.1, 1.4.2**), including the natural products **1** and **3** and the acetylated derivatives **2** and **4**. Although early bioactivity results were promising, the extremely variable results obtained in later experiments questioned the validity of earlier studies. Further assays were planned at the University of Ottawa, but difficulties in obtaining the necessary resources prevented the investigation from taking place within the timeframe of this study. Because the linolenic acid chains of the glycolipid are very sensitive to oxidation, the half-life of these compounds, even in the freezer, is generally under two months (L. Jewell, unpublished results). This physical property precluded our ability to simply stockpile compounds for testing at a later date. Instead, a variety of intermediates have been made available should the problems with the bioassay be solved, and the syntheses of the analogues presented in this thesis may act as a guide in later studies.

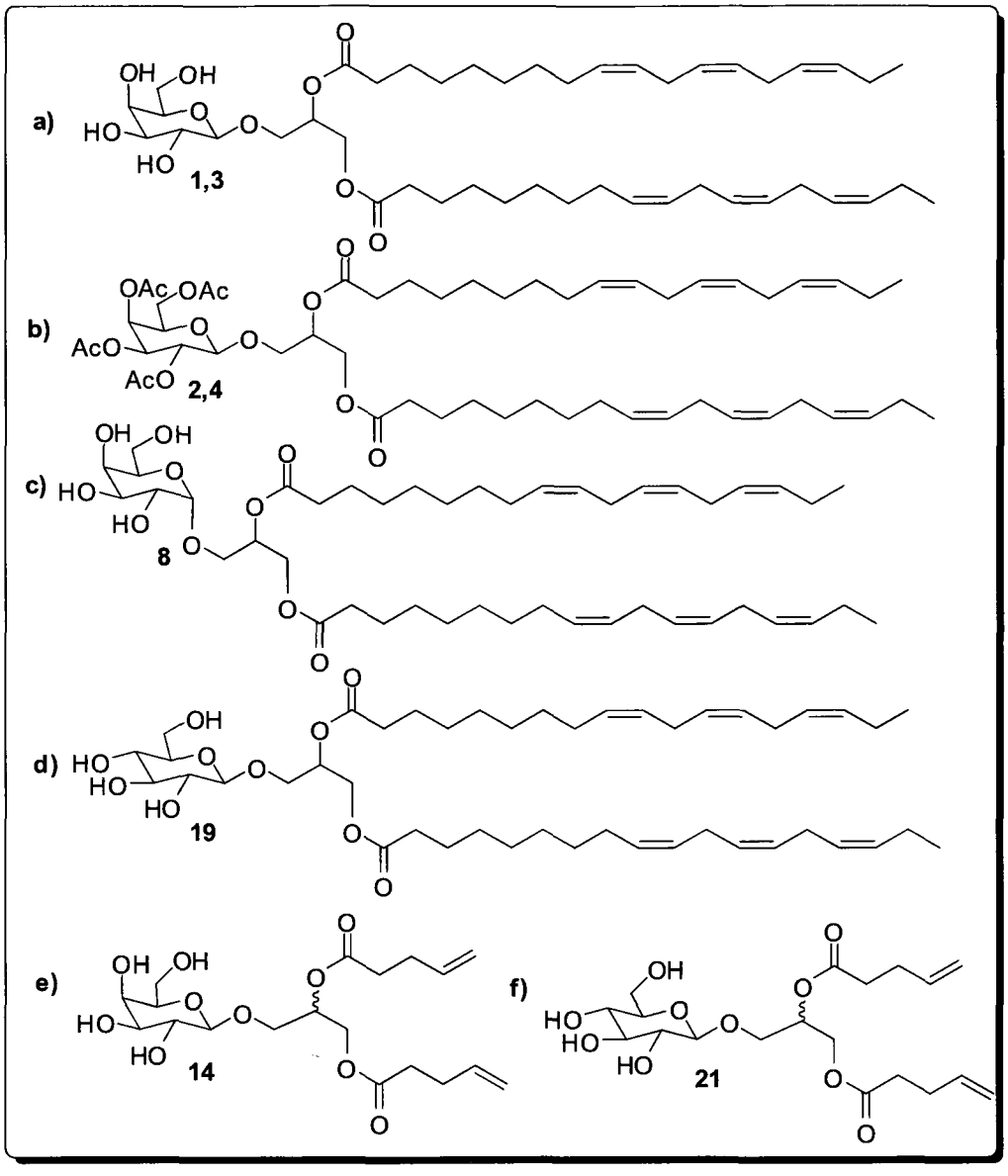


Figure 1.4.1 Summary of O-linked glycolipids synthesized and sent for bioactivity testing.

- **a)** 2',3'-propyl dilinolenate-*β* -*D*-Galactopyranoside **(1,2)**
- **b)** 2',3'-propyl dilinolenate-2,3,4,6-O-tetracetyl-*β*-*D*-Galactopyranoside **(2,4)**
- **c)** 2',3'-propyl di-linolenyl-α-*D*-galactopyranoside **(8)**
- **d)** 2',3'-propyl dilinolenate- *β*-*D*-Glucopyranoside **(19)**
- **e)** 2',3'-propyl dipent-4-enyl *β*-*D*-galactopyranoside **(18)**
- **f)** 2',3'-propyl dipent-4-enyl-*β* -*D*-Glucopyranoside **(21)**

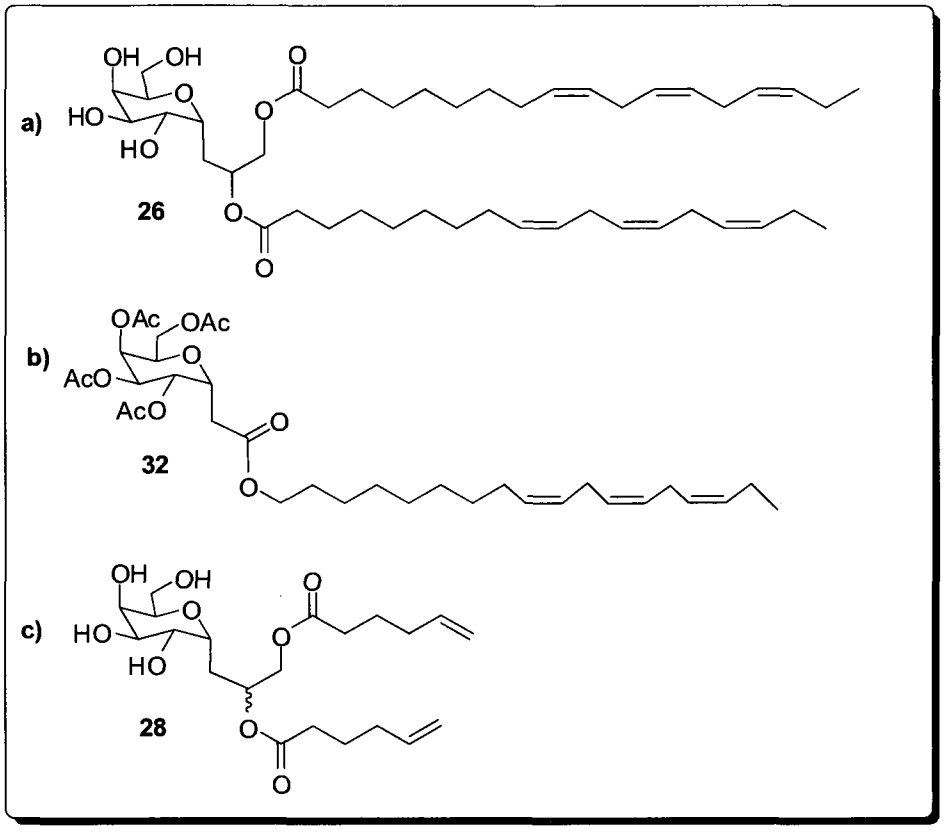


Figure 1.4.2 Summary of C-linked glycolipids synthesized

a) 2',3'-propyl dilinolenate-α-*C*-galactopyranoside (**26**)
b) 2'-ethyl linolenate-2,3,4,6-Tetra-*O*-acetyl-*C*-galactopyranoside (**32**)
c) 2',3'-propyl dihex-5-enyl-α-*C*-galactopyranoside (**28**)

The possibilities for future work on this project are myriad. Before more compounds are synthesized, the variability in the antibiotic tests must be addressed. First, realizing the goal of performing antibiotic assays at the University of Ottawa would allow compounds to be tested as soon as they are completed, resolving the question of whether the synthetic compounds truly possess poor activity, or if they had simply degraded before testing.

Once the bioassay has been standardized, there are many families of compounds which could be synthesized. A review of some of the compound classes, as well as a general retrosynthesis for each compound, is presented in **figure 1.4.3**.

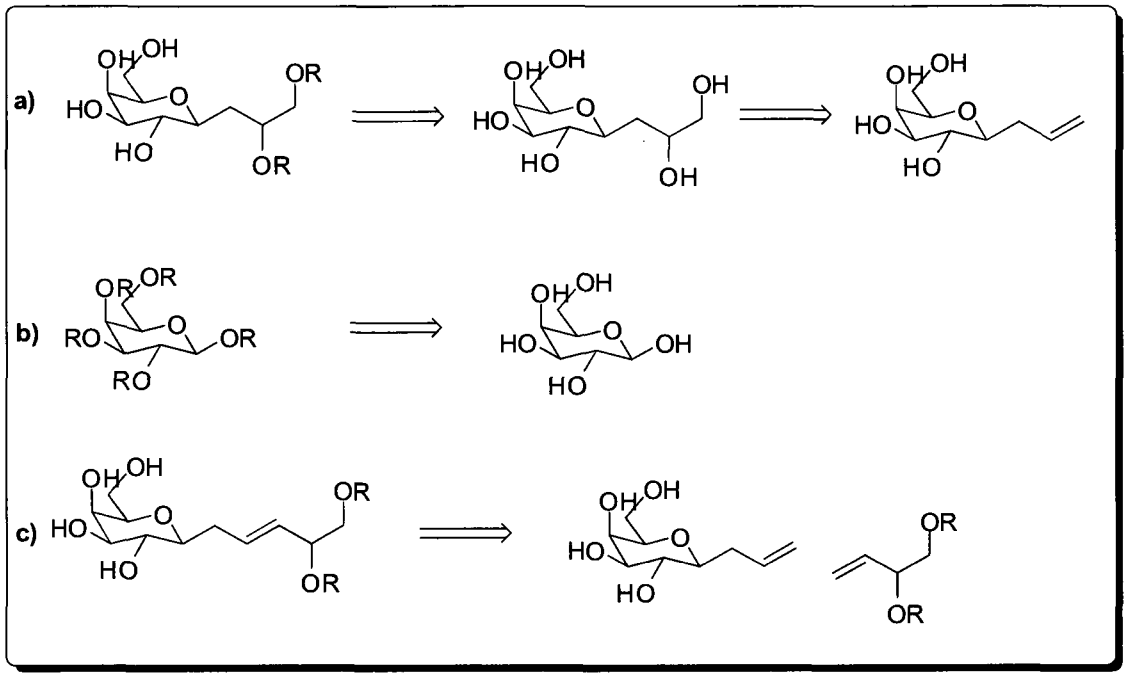


Figure 1.4.3 Retrosyntheses for further studies. **a)** *β-C*-glycolipids **b)** dendritic structures **c)** compounds with variable linker length generated by olefin metathesis.

First, the *β-C*-linked glycolipids (**a**) described in the discussion represent an important class of molecules due to their general similarity to the natural product. The partial synthesis provided in this thesis may be elaborated to provide the desired glycolipids following optimization.

Because linolenic acid is itself known to possess some antibiotic activity (40), a dendrite possessing many linolenic acid chains may provide interesting results in an *in vitro* study (**b**). One relatively easy dendrite-like structure would simply be a poly-linolenated

sugar. However, the large molar mass and acid sensitivity of such a molecule means that it will likely be poorly bioavailable and thus will not necessarily display the same activity *in vivo* as it may in early *in vitro* tests.

Olefin metathesis (**c**) using Grubbs' catalyst provides a rapid means of generating diversity from the *C*- and *O*-allyl-glycosides. These compounds may also be used to determine the optimal linker length.

Despite the problems encountered in this study, the methods presented in this thesis describe the synthesis of glycolipids with promising biological activity worthy of further study.

1.3 Experimental

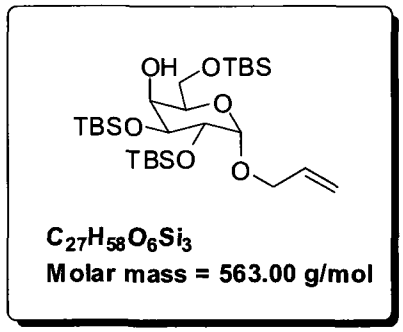
General:

Dichloromethane was distilled over calcium hydride before use. THF was distilled over sodium-benzophenone before use. Reactions were monitored by TLC using aluminum-backed TLC plates (EMD Chemicals, TLC Silica gel 60 F_{254}), which were visualized by UV light (254 nm) then permanently stained with Hannessian's stain. Purification by flash chromatography was performed using SiliCycle SiliFlash ® F60 silica gel of 230-400 mesh and glass columns fitted with a cotton plug and a sand base or a fritted glass filter. Ozonolysis was performed using a Yanco Industries ozonolysis apparatus. Reagents were purchased from Sigma Aldrich, Alfa Aesar, or Tokyo Chemical Corporation.

^{1}H and ^{13}C NMR spectra were acquired using a Bruker Avance 300, 400 or 500 MHz spectrometer. Samples were dissolved in deuterated chloroform, methanol, or acetone as indicated. Chemical shifts are reported in parts per million (ppm) and are referenced relative to the deuterated solvent used. ^{1}H integration is provided in parentheses and coupling constants are reported in Hz. ^{1}H splitting patterns are reported as singlets (s), broad singlets (br), doublets (d), triplets (t), quartets (q), multiplets (m), doublet of doublets (dd), or doublets of doublet of doublets (ddd). NMR data was assigned based upon supplemental NMR experiments including COSY, DEPT, and HETCOR, and by comparison with literature reports when available.

Mass spectral analyses were conducted with an Electrospray mass spectrometer. All spectral analyses were performed at facilities located at the University of Ottawa.

2,3,6-tri-O-TBS-α-O-allylgalactopyranoside (5)



$C_{27}H_{58}O_6Si_3$
Molar mass = 563.00 g/mol

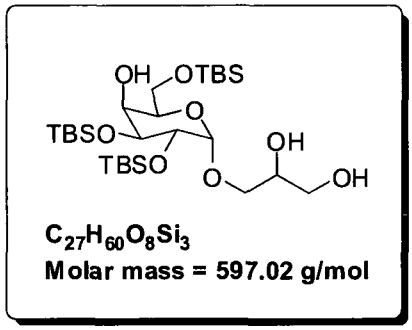
To commercially available α-O-allylgalactopyranoside (1. 0 g, 4.54 mmol) in 3 mL of DMF was added imidazole (1.85 g, 27.2 mmol). The resulting solution was cooled to 0°C and stirred for 10 minutes. *Tert*-butyl-chloro-dimethoxysilane (TBSCl) (4.11 g, 27.2 mmol) was added. The reaction mixture was stirred at 0°C for 30 minutes, then warmed to room temperature and monitored by TLC.

When complete, the reaction mixture was diluted with ether (5 mL) and transferred into a separatory funnel. Brine (15 mL) was added, and the resulting aqueous layer was drained. The remaining organic extracts were washed 3x with 5 mL of dH_2O, dried over $MgSO_4$, and evaporated to dryness. Following purification by flash chromatography (eluted with 5-10% ethyl acetate in hexanes), protected glycoside **5** was obtained as a viscous, colourless oil (1.878 g, 3.336 mmol, 73.4 % yield).

^{1}H NMR (400MHz, $CDCl_3$) δ: 5.87-5.97 (1H, m, H-2'), 5.15-5.30 (2H, m, H-3'), 4.78 (1H, d, J = 3.6Hz, H-1), 4.14-4.19 (1H, m, H-5), 3.73-3.99 (7H, m, H-2, H-3, H-4, H-6, H-7), 0.88-0.89 (33H, m, Si-$C(CH_3)_3$), 0.04-0.12 (20H, m, Si-$(CH_3)_2$)

^{13}C NMR (100MHz, $CDCl_3$) δ: 134.2 (C-2'), 117.7 (C-3'), 102.6 (C-1), 76.2 (C-5), 74.3 (C-3), 72.9 (C-4), 70.2 (C-2), 69.2 (C-1'), 61.7 (C-6), 26.1 (TBDMS), 18.2 (TBDMS), -4.0 (TBDMS)

2',3'-dihydroxypropyl 2,3,6-tri-O-TBS-α-*D*-galactopyranoside (6)



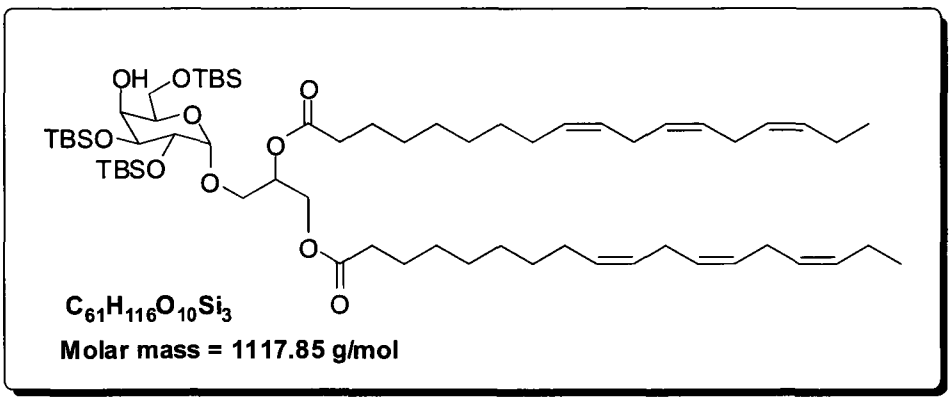
$C_{27}H_{60}O_8Si_3$
Molar mass = 597.02 g/mol

Alkene **5** (580 mg, 0.74 mmol) was dissolved in a mixture of 2:4:1 dH_2O: *tert*-butyl alcohol : acetone in a round-bottom flask equipped with a stir bar and wrapped with foil. NMO (130 mg, 1.10 mmol) was added, followed by a catalytic amount of OsO_4 (approximately 38 mg, 0.15 mmol). The reaction was stirred at room temperature and monitored by TLC. When complete after approximately 4 hours, the acetone was removed by evaporation. The resulting dark brown syrup was dissolved in 5 mL of saturated sodium thiosulfate solution and extracted 3x with 10 mL of ethyl acetate. The organic extracts were dried over $MgSO_4$, evaporated to dryness, and purified by flash chromatography (eluted with 30% ethyl acetate in hexanes). Diol **6** was obtained as a dark yellow oil (324 mg, 0.543 mmol, 73.3% yield).

^{1}H NMR (400MHz, $CDCl_3$) δ: 4.72 (1H, d, J = 1.8 Hz, H-1), 3.84-3.56 (10H, m, H-2, H-3, H-4, H-5, H-6, H-1', H-2', H-3'a), 3.44 (1H, dd, J = 10.5, 6.9 Hz, H-3'b)

^{13}C NMR (100MHz, $CDCl_3$) δ: 100.3 (C-1), 71.6 (C-5), 71.3 (C-2), 70.5 (C-3), 70.3 (C-4), 70.1 (C-1'), 69.9 (C-2'), 63.9 (C-3'), 62.4 (C-6), 25.0 (TBDMS), 18.2 – 18.0 (TBDMS), -4.1 - -5.5 (TBDMS)

2',3'-propyl dilinolenyl 2,3,6-O-TBS-α-*D*-galactopyranoside (7)



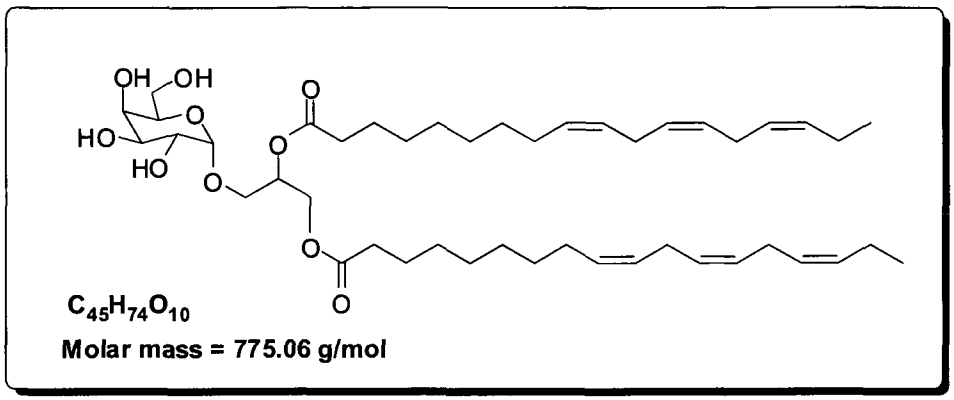
A mixture of diol **6** (200 mg, 0.336 mmol), DCC (206 mg, 1.01 mmol), and a trace amount of DMAP were dissolved in dry DCM (10 mL). Linolenic acid (278 mg, 1.01 mmol) was added and the reaction mixture was immediately sealed and flushed with N_2.

When the reaction was complete by TLC, it was quenched by the addition of diethyl ether. The mixture was filtered into a separatory funnel where it was washed with a saturated solution of ammonium chloride (10 mL). The organic extracts were dried over $MgSO_4$, evaporated to dryness, and purified by flash chromatography (eluted using a solvent gradient of 0-10% ethyl acetate in hexanes). Protected glycolipid **7** was obtained as a light yellow oil (110 mg, 0.089 mmol, 26.4% yield).

^{1}H NMR (400MHz, CDCl3) δ: 5.25-5.40 (12H, m, H-11', H-12', H-14', H-15', H-17', H-18'), 4.30-4.41 (1H, m, H-1), 3.12-3.40 (11H, m, H-2, H-3, H-4, H-5, H-6, H-1', H-2', H-3'), 2.78 (8H, dd, J = 5.6, 5.6 Hz, H-13', H-16'), 2.25-2.30 (4H, H-4'), 1.98-2.10 (8H, m, H-10', H-19'), 1.54-1.63 (4H, m, H-5'), 1.23-1.40 (16H, m, H-6', H-7', H-8', H-9'), 0.86-0.97 (37H, m, H-20', TBDMS), 0.04-0.12 (20H, m, TBDMS)

^{13}C NMR (100MHz, CDCl3) δ: 173.2 (C-4'), 131.9 (C-12'), 130.2 (C-91'), 128.2 (C-15'), 128.2 (C-16'), 127.7 (C-18'), 127.0 (C-13'), 99.6 (C-1), 71.2 (C-2'), 70.2 (C-5), 69.7 (C-4), 69.5 (C-2), 65.7 (C-3'), 65.6, 62.6 (C-1'), 62.0 (C-6), 34.2 (C-8'), 34.0 (C-10'), 29.3 (C-7'), 27.2 (C-11'), 26.0-25.5 (TBDMS; C-14', C-17'), 24.8 (C-16'), 20.5 (C-20'), 18.1-17.9 (TBDMS), 14.2 (C-21'), -4.1 - -5.5 (TBDMS)

2',3'-propyl di-linolenyl-α-*D*-galactopyranoside (8)



Compound **7** (160 mg, 0.139 mmol) was dissolved in tetrahydrofuran and tetra-N-butylammonium fluoride (0.56 mL of 1M solution) was added.

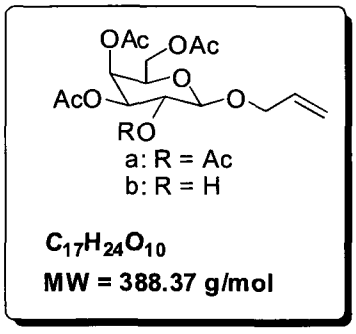
When complete, the reaction mixture was diluted with brine (10 mL) and extracted with ethyl acetate (3x, 10mL). The organic extracts were dried over $MgSO_4$, evaporated to dryness, and purified by flash chromatography (solvent gradient of 75-100% ethyl acetate in hexanes). Glycolipid **8** was obtained as a light yellow oil (31.5 mg, 40.6 mmol, 29.2% yield).

^{1}H NMR (400MHz, $CDCl_3$) δ: 5.19-5.41 (12H, m, H-12', H-13', H-15', H-16', H-18', H-19'), 4.92 (1H, d, J = 3.6 Hz, H-1), 4.35 (1H, dd, J = 4.0, 12.0 Hz, H-2), 4.06-4.19 (2H, m, H-3'), 3.70-3.93 (7H, m, H-4, H-5, H-6, H-1', H-2'), 3.61 (1H, dd, J = 4.0, 10.8 Hz, H-3), 2.71-2.81 (8H, m, H-14', H-17'), 2.27-2.32 (4H, m, H-5'), 1.99-2.09 (8H, m, H-11', H-20'), 1.54-1.62 (4H, m, H-6'), 1.16-1.39 (16H, m, H-7', H-8', H-9', H-10'), 0.95 (6H, t, J = 7.6 Hz, H-21')

^{13}C NMR (100MHz, CDCl3) δ: 173.3 (C-4'), 132.0 (C-12'), 130.2 (C-19'), 128.3 (C-15') , 128.2 (C-16'), 127.8 (C-18'), 127.2 (C-13'), 99.3 (C-1), 70.8 (C-2'), 70.3 (C-3), 70.1 (C-5),

69.4 (C-4), 69.3 (C-2), 66.7 (C-3'), 62.9 (C-1'), 62.2 (C-6), 34.3 (C-8'), 34.1 (C-10'), 29.2 (C-7'), 27.2 (C-11'), 25.6 (C-14', C-17'), 25.6 (C-6'), 20.6 (C-20'), 14.3 (C-21')

2,3,4,6-tetra-O-acetyl *β*-O-allylgalactopyranoside (9)



Penta-acetyl *β-D*-galactose (5.0 g, 12.8 mmol) and allyl alcohol (3.5 mL, 51.2 mmol) were dissolved in 100mL of dichloromethane (DCM). The reaction mixture was cooled to 0°C and flushed with N_2. $BF_3{\cdot}OEt_2$ (2.4 mL, 19.2 mmol) was added dropwise, and the resulting solution was stirred for 1 hour before being warmed to room temperature and stirred for a further 12 hours.

The reaction was quenched by the addition of ice water (50 mL). The organic layer was drained away and the remaining aqueous layer was extracted with ethyl acetate (3x 20 mL). The organic extracts were combined and dried over $MgSO_4$ before being concentrated. The resulting colourless syrup (crude mass 4.6 g) was purified by flash chromatography using 0-30% ethyl acetate in hexanes to yield both O-allylated glycolipid **9a** (2.14 g, 6.32 mmol, 49.4% yield) and **9b** (2.00 g, 5.77 mmol, 45.1% yield). Compounds **a** and **b** were combined before proceeding.

a:

^{1}H (400MHz, $CDCl_3$) δ: 5.64-5.74 (1H, m, H-2'), 5.22 (1H, dd, J = 0.8, 3.2 Hz, H-4), 5.08-5.13 (1H, m, H-1), 5.03 (2H, td, J = 2.0, 10.0 Hz, H-3'a, H-3'b), 4.87 (1H, dd, J = 3.2, 10.0 Hz, H-3), 4.39 (1H, d, J = 8.0 Hz, H-2), 4.18 (1H, ddt, J = 1.6, 4.8, 13.2 Hz, H-5), 3.96-4.00

(2H, m, H-6), 3.78 (1H, dt, J = 1.2, 8.0 Hz, H-1'), 1.88 (3H, s, OAc), 1.87 (3H, s, OAc), 1.86 (3H, s, OAc),1.80 (3H, s, OAc)

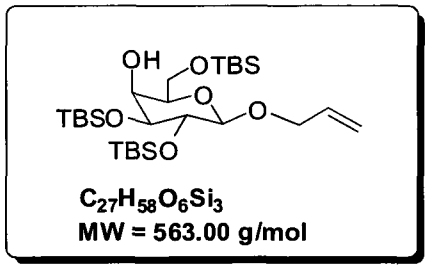
^{13}C (100MHz, CDCl3) δ: 170.1 (OAc), 170.0 (OAc), 169.8 (OAc), 169.1 (OAc), 133.3 (C-2'), 117.2 (C-3'), 99.5 (C-1), 70.8 (C-2), 70.5 (C-4), 69.8 (C-5), 68.7 (C-1'), 67.0 (C-3), 61.2 (C-6), 20.4 (OAc)

b:

^{1}H (400MHz, $CDCl_3$) δ: 5.77-5.87 (1H, m, H-2'), 5.36 (1H, dd, J = 1.2, 3.6 Hz, H-4), 5.21 (2H, m, H-3'a, H-3'b), 4.99 (1H, dd, J = 3.6, 10.4 Hz, H-3), 4.49 (1H, d, J = 8.0 Hz, H-1), 4.32 (1H, ddt, J = 1.6, 5.2, 13.2 Hz, H-5), 4.16 (1H, dd, J = 6.8, 11.2 Hz, H-2), 4.05-4.12 (2H, m, H-6), 3.87 (1H, dt, J =1.2, 8.0 Hz, H-1'), 2.03 (3H, s, OAc), 2.02 (3H, s, OAc), 1.95 (3H, s, OAc)

^{13}C (100MHz, CDCl3) δ: 170.1 (OAc), 170.0 (OAc), 169.8 (OAc), 169.1 (OAc), 133.3 (C-2'), 118.2 (C-3'), 99.5 (C-1), 70.8 (C-2), 70.5 (C-4), 69.8 (C-5), 68.7 (C-1'), 67.0 (C-3), 60.2 (C-6), 20.5 (OAc),

2,3,6-tri-O-TBS β-O-allylgalactopyranoside (10)



$C_{27}H_{58}O_6Si_3$
MW = 563.00 g/mol

Acetylated galactopyranoside **9** (5.0 g, 22.7 mmol) was dissolved in 10 mL of anhydrous methanol. Potassium carbonate (0.152 g, 1.1 mmol) was added and the heterogeneous solution was stirred overnight. Approximately 0.5 g of Amberlite H^+ resin was added to stop the reaction. After stirring for a further 10 minutes, the resin was filtered off and washed 2 x with 3 mL of methanol. The solvent was evaporated to yield the deprotected glycoside (4.89 g, 22.2 mmol, 98.5% yield) as a thick, orange syrup, which was used without further purification.

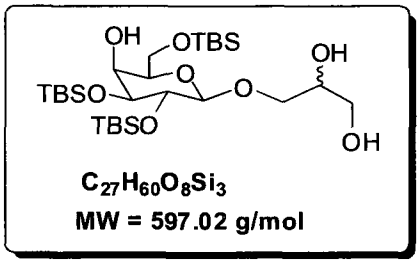
This syrup (1. 0 g, 4.54 mmol) was dissolved in 3 mL of DMF and imidazole (1.85 g, 27.2 mmol) was added. The resulting solution was cooled to 0°C and stirred for 10 minutes before TBDMS-Cl (4.11 g, 27.2 mmol) was added. The reaction mixture was stirred at 0°C for 30 minutes, then warmed to room temperature and monitored by TLC.

When complete, the reaction mixture was diluted with ether (5 mL) and transferred into a separatory funnel. Brine (15 mL) was added, and the resulting aqueous layer was drained. The remaining organic extracts were washed 3x with 5 mL of dH_2O, dried over $MgSO_4$, and evaporated to dryness. Following purification by flash chromatography (eluted with 5-10% ethyl acetate in hexanes), protected glycoside **10** was obtained as a viscous, colourless oil (1.878 g, 3.336 mmol, 73.4 % yield).

^{1}H NMR (400MHz, CDCl3) δ: 5.76-5.88 (1H, m, H-2'), 5.00-5.16 (2H, m, H-3'), 3.26-3.94 (8H, m, H-1, H-2, H-3, H-4, H-5, H-6, H-1'), 0.81-0.95 (33H, m, TBDMS), 0.04-0.12 (20H, m, TBDMS)

^{13}C NMR (100MHz, CDCl3) δ: 134.2 (C-2'), 117.7 (C-3'), 102.6 (C-1), 76.2 (C-5), 74.3 (C-3), 72.9 (C-4), 70.2 (C-2), 69.2 (C-1'), 61.7 (C-6), 26.1 (TBDMS), 18.2 (TBDMS), -4.0 (TBDMS)

2',3'-dihydroxypropyl 2,3,6-tri-O-TBS-*β*-*D*-galactopyranoside (11)

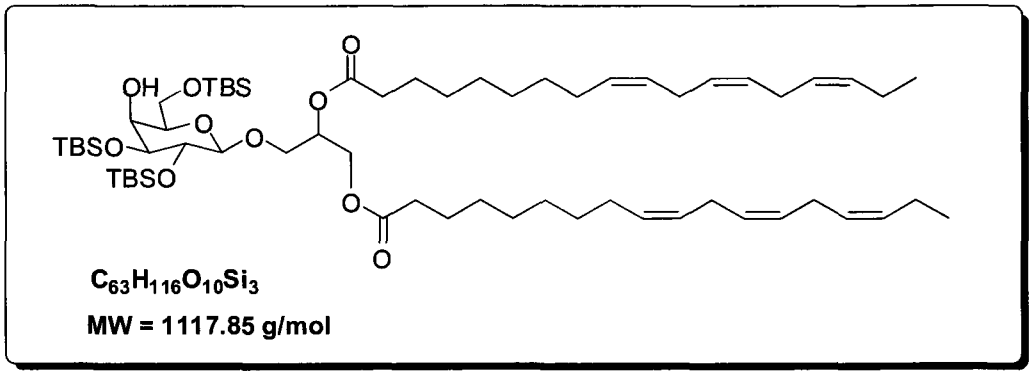


Protected glycoside **10** (300 mg, 0.532 mmol) was dissolved in a mixture of 2:4:1 dH_2O: *tert*-butyl alcohol: acetone in a round-bottom flask equipped with a stir bar and wrapped with foil. NMO (78 mg, 0.66 mmol) was added, followed by a catalytic amount of OsO_4 (approximately 25 mg, 0.09 mmol). The reaction was stirred at room temperature and monitored by TLC. When complete after approximately 4 hours, the acetone was removed by rotary evaporation. The resulting dark brown syrup was dissolved in 5 mL of saturated sodium thiosulfate solution and extracted 3x with 10 mL of ethyl acetate. The organic extracts were dried over $MgSO_4$, evaporated to dryness, and purified by flash chromatography (eluted with 30% ethyl acetate in hexanes). Dihydroxylated compound **11** was obtained as a dark yellow oil (225 mg, 0.377 mmol, 70.8 % yield).

^{1}H NMR (400MHz, $CDCl_3$) δ: 0.04-0.12 (20H, m, Si-CH_3), 0.81-0.95 (33H, m, Si-C(CH_3)-$_3$), 3.36-3.90 (11H, m, H-2, H-3, H-4, H-5, H-6, H-7, H-8, H-9), 4.11-4.17 (1H, m, H-1)

^{13}C NMR (100MHz, $CDCl_3$) δ: -4.0 (Si-C), 18.1 (Si-CH_3), 25.9 (Si-C(CH_3)$_3$), 26.0 (Si-C(CH_3)$_3$), 26.1 (Si-C(CH_3)$_3$), 62.0 (C-6), 63.3 (C-4), 64.0 (C-9), 69.3 (C-7), 71.1 (C-2), 72.7 (C-3), 74.6 (C-5), 75.9 (C-8), 104.9 (C-1)

2',3'-propyldilinolenyl 2,3,6-O-TBS-*β*-*D*-galactopyranoside (12)



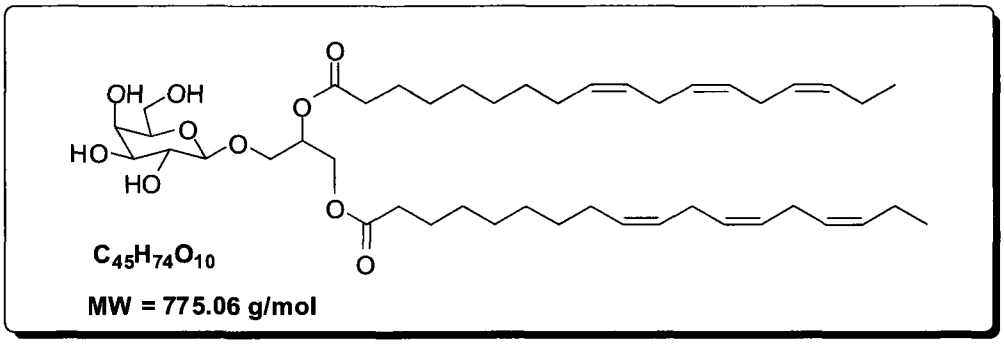
A mixture of diol **11** (200 mg, 0.336 mmol), DCC (206 mg, 1.01 mmol), and a trace amount of DMAP were dissolved in dry DCM (10 mL). Linolenic acid (278 mg, 1.01 mmol) was added and the reaction mixture was immediately sealed and flushed with N_2.

When the reaction was complete by TLC, it was quenched by the addition of diethyl ether. The mixture was filtered into a separatory funnel where it was washed with a saturated solution of ammonium chloride (10 mL). The organic extracts were dried over $MgSO_4$, evaporated to dryness, and purified by flash chromatography (eluted using a solvent gradient of 0-10% ethyl acetate in hexanes). Protected glycolipid **12** was obtained as a light yellow oil (179 mg, 160 mmol, 47.7% yield).

^{1}H NMR (400MHz, $CDCl_3$) δ: 5.25-5.40 (12H, m, H-12', H-13', H-15', H-16', H-18', H-19'), 4.30-4.37 (1H, m, H-1), 4.09-4.19 (2H, m, H-3'), 3.38-3.99 (9H, m, H-2, H-3, H-4, H-5, H-6, H-1', H-2'), 2.78 (8H, dd, J = 5.6, 5.6 Hz, H-14', H-17'), 2.25-2.30 (4H, H-4'), 1.98-2.10 (8H, m, H-11', H-20'), 1.54-1.63 (4H, m, H-5'), 1.23-1.40 (16H, m, H-6', H-7', H-8', H-9'), 0.86-0.97 (37H, m, H-21', TBDMS), 0.04-0.12 (20H, m, TBDMS)

^{13}C NMR (100MHz, $CDCl_3$) δ: 173.0 (C-4'), 132.0 (C-12'), 130.3 (C-19'), 128.3 (C-15'), 128.2 (C-16'), 127.8 (C-18'), 127.1 (C-13'), 103.7 (C-1), 74.4 (C-2'), 73.4 (C-3), 73.4 (C-5), 73.2 (C-4), 73.0 (C-2), 72.7 (C-3'), 69.0 (C1'), 62.9 (C-6), 34.3 (C-8'), 34.1 (C-5'), 29.6 (C-7'), 29.2 (C-9', C-10'), 27.2 (C-11'), 26.2-25.5 (C-6', C-14', C-17', TBDMS), 20.6 (C-20'), 18.2 (TBDMS), 14.3 (C-21'), -3.3 - -5.4 (TBDMS)

2',3'-propyl dilinolenate-*β*-*D*-Galactopyranoside (1,3)



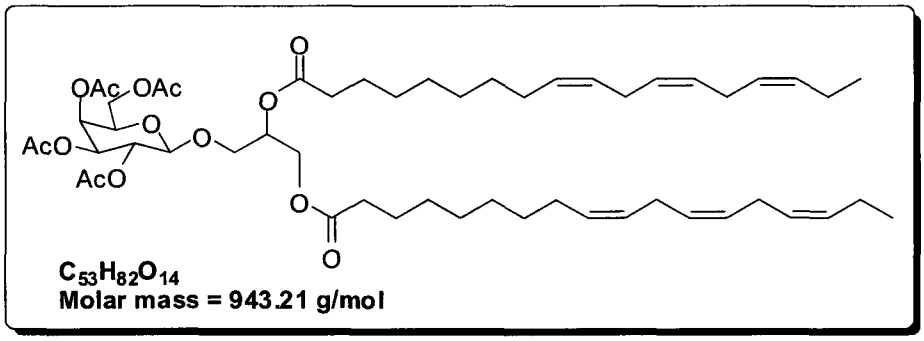
The TBS-protected glycolipid **12** (160 mg, 0.139 mmol) was dissolved in tetrahydrofuran and TBAF (0.56 mL of 1M solution) was added.

When complete, the reaction mixture was diluted with brine (10 mL) and extracted with ethyl acetate (3x, 10mL). The organic extracts were dried over $MgSO_4$, evaporated to dryness, and purified by flash chromatography (solvent gradient of 75-100% ethyl acetate in hexanes). The mixture of glycolipids **1** and **3** was obtained as a light yellow oil (31.5 mg, 40.6 mmol, 29.2% yield).

^{1}H NMR (400MHz, $CDCl_3$) δ: 5.25-5.40 (12H, m, H-11', H-12', H-14', H-15', H-17', H-18') 4.37 (1H, dd, J = 12.0, 3.6 Hz, H-3'a), 4.24 (1H, d, J = 7.6Hz, H-1), 4.15 (1H, dd, J = 12.0, 3.6 Hz, H-3'b), 3.49-4.07 (11H, m, H-2, H-3, H-4, H-5, H-6, H-1', H-2'), 2.78 (8H, dd, J = 5.6, 5.6 Hz, H-13', H-16'), 2.29 (4H, dd, J = 8.0, 14.8 Hz, H-4'), 2.00-2.07 (8H, m, H-10', H-19'), 1.56-1.66 (4H, m, H-5'), 1.32-1.37 (16H, m, H-6', H-7', H-8', H-9'), 0.95 (6H, t, J = 7.2 Hz, H-20')

^{13}C NMR (100MHz, $CDCl_3$) δ: 173.7 (C-4'), 132.0 (C-12'), 130.3 (C-19'), 128.4 (C-15'), 128.3 (C-16'), 127.8 (C-18'), 127.2 (C-13'), 103.9 (C-1), 74.6 (C-2'), 73.5 (C-3), 71.5 (C-5), 71.4 (C-4), 70.2 (C-2), 69.4 (C-3'), 68.3 (C-1'), 62.7 (C-6), 34.4 (C-8'), 34.2 (C-5'), 29.2 (C-7') 29.1 (C-9'), 29.1 (C-10'), 27.3 (C-11'), 25.7 (C-14', C-17'), 25.6 (C-6'), 20.6 (C-20'), 14.4 (C-21')

2',3'-propyl dilinolenate-2,3,4,6-O-tetracetyl-*β*-*D*-Galactopyranoside (2)



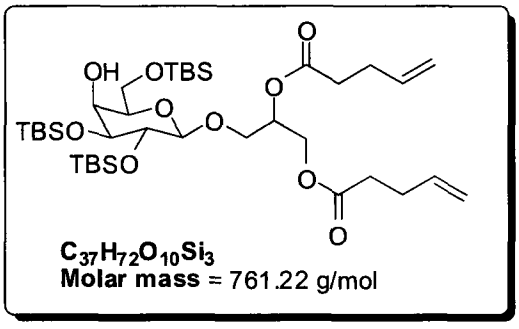
The glycolipids **1** and **2** (25 mg, $3.2x10^{-2}$ mmol) were dissolved in pyridine (1 mL) before Ac_2O (19 mg, 0.19 mmol) was added and the reaction flask was flushed with N_2. The reaction was monitored by TLC.

When complete, the solvent was removed on the rotovap and the compound was purified by flushing it through a Pasteur pipette plugged with cotton and filled with silica. The acetylated glycolipids **2** and **4** were obtained as a colourless oil (8.4 mg, $8.9x10^{-3}$ mmol, 28% yield).

^{1}H NMR (400MHz, $CDCl_3$) δ: 5.43-5.17 (12H, m, H-11', H-12', H-14', H-15', H-17', H-18'), 4.50-4.48 (1H, d, J = 7.6 Hz, H-1), 4.33-3.88 (10H, m, H-2, H-3, H-4, H-5, H-6, H-1', H-2'), 3.70-3.64 (2H, m, H-3'), 2.82 (8H, dd, J = 5.6, 5.6 Hz, H-13', H-16'), 2.33-2.28 (4H, m, H-4'), 2.15 (s, 3H, OAc), 2.16-1.98 (m, 14H, H-10', H-19', OAc), 1.98 (s, 3H, OAc), 1.65-1.55 (4H, m, H-5'), 1.30-1.25 1.32-1.37 (16H, m, H-6', H-7', H-8', H-9'), 0.98 (6H, t, J = 7.2 Hz, H-20')

^{13}C NMR (400MHz, $CDCl_3$) Unavailable due to computer crash

2',3'-propyl dipent-4-enyl 2,3,6-tri-TBS-*O*-*β*-*D*-galactopyranoside (13)



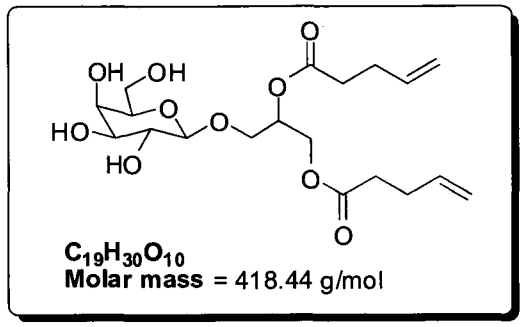
A mixture of compound **11** (100 mg, 0.168 mmol), DCC (104 mg, 0.503 mmol), and a trace amount of DMAP were dissolved in dry DCM (3 mL). 5-hexenoic acid (50.4 mg, 0.503 mmol) was added and the reaction mixture was immediately sealed and flushed with N_2. When the reaction was complete by TLC, it was quenched by the addition of diethyl ether. The mixture was filtered into a separatory funnel where it was washed with a saturated solution of ammonium chloride (10 mL). The organic extracts were dried over $MgSO_4$, evaporated to dryness, and purified by flash chromatography (eluted using a solvent gradient of 0-10% ethyl acetate in hexanes). Protected glycolipid **13** was obtained as a light yellow oil (69.1 mg, 0.0908 mmol, 54.0% yield).

^{1}H NMR (400MHz, $CDCl_3$) δ: 5.83-5.73 (2H, m, H-7'), 5.24-5.13 (1H, m, H-8'a), 5.02 (1H, dd, J = 17.2, 1.6 Hz, H-8'b), 4.97 (1H, dd, J = 10.8, 1.6 Hz, H-1), 4.37-4.31 (1H, m, H-4), 4.19-4.08 (2H, m, H-3, H-2), 3.93-3.40 (8H, m, H-5, H-6, H-1', H-2', H-3'), 2.42-2.31 (8H, m, H-5', H-6'), 0.90-0.85 (27H, m, TBDMS), 0.11-0.03 (18H, m, TBDMS)

^{13}C NMR (100MHz, $CDCl_3$) δ: 172.5 (C-4'), 172.1 (C-4'), 136.6 (C-7'), 115.6 (C-8'), 104.0 (C-1), 76.1 (C-4), 74.4 (C-3), 72.6 (C-2), 69.5 (C-1'), 69.1 (C-2'), 67.0 (C-3'), 63.0 (C-5),

61.5 (C-6), 33.5 (C-5'), 33.3 (C-6'), 28.8-25.8 (TBDMS), 18.2-18.1 (TBDMS), -3.3 - -5.4 (TBDMS)

2',3'-propyl dipent-4-enyl *β-D*-galactopyranoside (14)



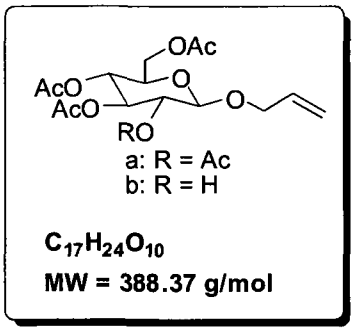
Protected glycolipid **13** (69.1 mg, 0.091 mmol) was dissolved in 3.0 mL of THF and TBAF (0.545 mL of 1M solution) was added.

When complete, the reaction mixture was diluted with brine (10 mL) and extracted with ethyl acetate (3x, 10mL). The organic extracts were dried over $MgSO_4$, evaporated to dryness, and purified by flash chromatography (solvent gradient of 75-100% ethyl acetate in hexanes). Glycolipid **14** was obtained as a light yellow oil (16.8 mg, 0.040 mmol, 44% yield).

^{1}H NMR (400MHz, $CDCl_3$) δ: 5.90-5.76 (2H, m, H-7'), 5.32-5.29 (1H, m, H-8'a), 5.11-5.01 (4H, m, H-1, H-8'), 4.44-3.56 (11H, m, H-2, H-3, H-4, H-5, H-6, H-1', H-2', H-3'), 2.49-2.37 (8H, m, H-5', H-6'), 2.03 (3H, br.s, OH)

^{13}C NMR (100MHz, $CDCl_3$) δ: **NMR data unavailable due to computer crash**

2,3,4,6-tetra-O-acetyl *β*-O-allylglucopyranoside (15)



Penta-acetyl *β-D*-glucose (5.0 g, 12.8 mmol) and allyl alcohol (3.5 mL, 51.2 mmol) were dissolved in 100mL of dichloromethane (DCM). The reaction mixture was cooled to 0°C and flushed with N_2. $BF_3 \cdot OEt_2$ (2.4 mL, 19.2 mmol) was added dropwise, and the resulting solution was stirred for 1 hour before being warmed to room temperature and stirred for a further 12 hours.

The reaction was quenched by the addition of ice water (100 mL). The organic layer was drained away and the remaining aqueous layer was extracted with ethyl acetate (3x 25 mL). The organic extracts were combined and dried over $MgSO_4$ before being concentrated. The resulting colourless syrup (crude mass 4.6 g) was purified by flash chromatography using 0-30% ethyl acetate in hexanes to yield both *O*-allylated glycolipid **15a** (1.98 g, 5.10 mmol, 39.8% yield) as a powdery white solid and **15b** (2.56 g, 6.59 mmol, 51.5% yield) as a colourless oil. Compounds **a** and **b** were combined before proceeding.

a: ^{1}H (400MHz, $CDCl_3$) δ: 5.86-5.77 (1H, m, H-2'), 5.23 (1H, dq, J = 17.2, 1.6 Hz, H-3'a), 5.17 (1H, dq, J = 10.4, 1.6 Hz, H-3'b), 5.16 (1H, d, J = 9.6 Hz, H-1), 5.06 (1H, t, J = 9.6 Hz, H-4), 4.99 (1H, dd, J = 9.6, 7.6 Hz, H-2), 4.53 (1H, d, J = 7.6 Hz, H-3), 4.30 (1H, ddt, J = 13.2, 3.6, 1.2 Hz, H-1'a), 4.18 (1H, dd, J = 4.8, 12.4 Hz, H-6a), 4.00-4.08 (2H, m, H-4, H-

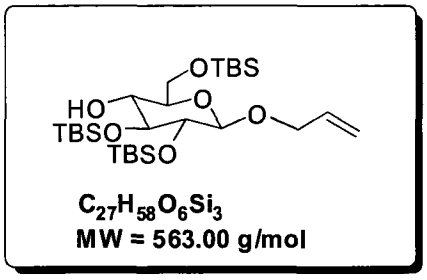
6b), 3.62 (1H, ddd, J =2.4, 4.8, 10.0 Hz, H-1'), 2.00 (3H, s, OAc), 1.96 (3H, s, OAc), 1.94 (3H, s, OAc), 1.92 (3H, s, OAc)

^{13}C (100MHz, $CDCl_3$) δ: 170.6 (OAc), 170.2 (OAc), 169.3 (OAc), 169.2 (OAc), 133.3 (C-2'), 117.6 (C-3'), 99.5 (C-1), 72.8 (C-2), 71.7 (C-4), 71.2 (C-5), 70.0 (C-1'), 68.4 (C-3), 61.9 (C-6), 20.7 (OAc), 20.6 (OAc), 20.5 (OAc), 20.5 (OAc)

b:

^{1}H (400MHz, $CDCl_3$) δ: 5.77-5.87 (1H, m, H-2'), 5.36 (1H, dd, J = 1.2, 3.6 Hz, H-3'a), 5.21 (3H, m, H-1 H-2, H-3'b), 4.99 (1H, dd, J = 3.6, 10.4 Hz, H-3), 4.49 (1H, d, J = 8.0 Hz, H-4), 4.32 (1H, ddt, J = 1.6, 5.2, 13.2 Hz, H-5), 4.16 (1H, dd, J = 6.8, 11.2 Hz, H-1'a), 4.05-4.12 (2H, m, H-6), 3.87 (1H, dt, J =1.2, 8.0 Hz, H-1'b), 2.03 (3H, s, OAc), 2.02 (3H, s, OAc), 1.95 (3H, s, OAc)

2,3,6-tri-O-TBS β-O-allylglucopyranoside (16)



$C_{27}H_{58}O_6Si_3$
MW = 563.00 g/mol

Acetylated glucopyranosides **15a** and **b** (5.0 g, 22.7 mmol) were dissolved in 10 mL of anhydrous methanol. Potassium carbonate (0.152 g, 1.1 mmol) was added and the heterogeneous solution was stirred overnight. Approximately 0.5 g of Amberlite H^+ resin was added to stop the reaction. After stirring for a further 10 minutes, the resin was filtered off and washed 2 x with 3 mL of methanol. The solvent was evaporated to yield the crude glycoside (4.89 g, 22.2 mmol, 98.5% yield) as a thick, orange syrup, which was used without further purification.

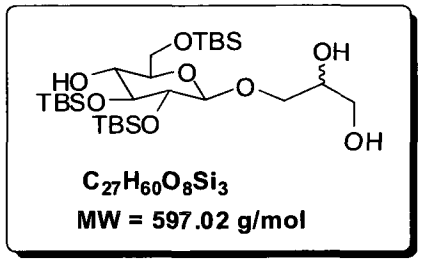
To glycoside (800 mg, 3.60 mmol) was dissolved in 3 mL of DMF and imidazole (1.5 g, 22 mmol) was added. The resulting solution was cooled to 0°C and stirred for 10 minutes before TBDMS-Cl (3.3 g, 22 mmol) was added. The reaction mixture was stirred at 0°C for 30 minutes, then warmed to room temperature and monitored by TLC.

When complete, the reaction mixture was diluted with ether (5 mL) and transferred into a separatory funnel. Brine (15 mL) was added, and the resulting aqueous layer was drained. The remaining organic extracts were washed 3x with 5 mL of dH_2O, dried over $MgSO_4$, and evaporated to dryness to yield 1.49 g of crude product. Following purification by flash chromatography (eluted with 5-10% ethyl acetate in hexanes), protected glycoside **16** was obtained as a viscous, colourless oil (1.22 g, 2.12 mmol, 58.9 % yield).

^{1}H NMR (400MHz, $CDCl_3$) δ: 5.88-5.98 (1H, m, H-2'), 5.23-5.28 (1H, m, H-3'b), 5.15-5.18 (1H, m, H-3'a), 4.30-4.34 (1H, m, H-1'b), 4.23 (1H, dd, J = 7.2, 10.8 Hz, H-1), 3.99-4.07 (1H, m, H-1'a), 3.70-3.90 (2H, m, H-6), 3.18-3.58 (4H, m, H-2, H-3, H-4, H-5), 0.88-0.92 (32H, m, TBDMS), 0.05-0.14 (20H, m, TBDMS)

^{13}C NMR (100MHz, $CDCl_3$) δ: 134.2 (C-2'), 117.5 (C-3'), 101.6 (C-1), 78.5 (C-5), 77.1 (C-3), 75.5 (C-4), 70.8 (C-2), 69.9 (C-1'), 62.6 (C-6), 26.1 (TBDMS), 18.3 (TBDMS), -4.0 (TBDMS)

2',3'-dihydroxypropyl 2,3,6-tri-O-TBS-*β*-*D*-glucopyranoside (17)

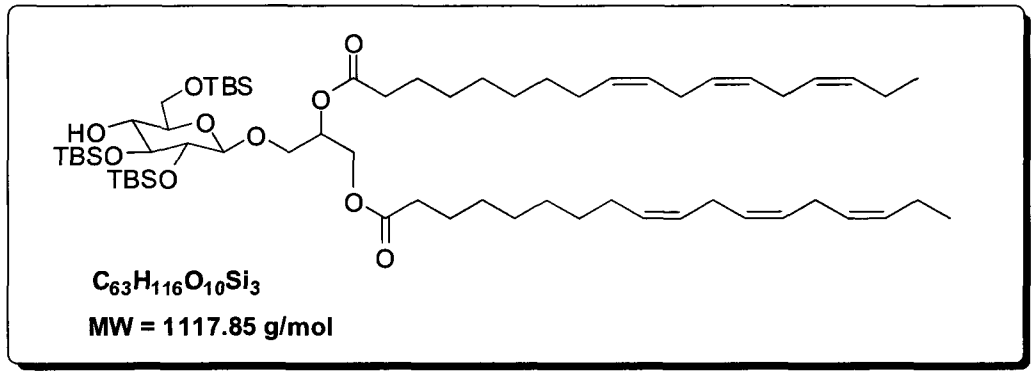


Glycoside **16** (150 mg, 0.267 mmol) was dissolved in 7 mL of a mixture of 2:4:1 dH_2O: *tert*-butyl alcohol : acetone in a round-bottom flask equipped with a stir bar and wrapped with foil. NMO (47 mg, 0.40 mmol) was added, followed by a catalytic amount of OsO_4 (approximately 17 mg, 0.05 mmol). The reaction was stirred at room temperature and monitored by TLC. When complete after approximately 4 hours, the acetone was removed by evaporation. The resulting dark brown syrup was dissolved in 5 mL of saturated sodium thiosulfate solution and extracted 3x with 10 mL of ethyl acetate. The organic extracts were dried over $MgSO_4$, evaporated to dryness, and purified by flash chromatography (eluted with 30% ethyl acetate in hexanes). Diol **17** was obtained as a dark yellow oil (128mg, 0.214 mmol, 80.3% yield).

^{1}H NMR (400MHz, $CDCl_3$) δ: 4.72 (1H, t, J = 3.2 Hz, H-1), 3.90-3.85 (1H, m, H-4), 3.81-3.45 (9H, m, H-2, H-3, H-5, H-6a, H-1', H-2', H-3'), 3.34 (1H, t, J = 8.4, H-6b), 2.52 (2H, br.s, OH), 0.90-0.87 (30H, m, TBDMS), 0.11-0.06 (18H, m, TBDMS)

^{13}C NMR (400MHz, $CDCl_3$) δ: 99.6 (C-1), 74.8 (C-4), 74.7 (C-5), 73.5 (C-3), 72.7 (C-1'), 72.0 (C-3'), 70.2 (C-2), 64.0 (C-2'), 63.9 (C-6), 26.1 (TBDMS), 25.9 (TBDMS), 18.4-18.3 (TBDMS), -3.31 - -5.36 (TBDMS)

2',3'-propyl dilinolenate 2,3,6-O-TBS-*β* -*D*-glucopyranoside (18)



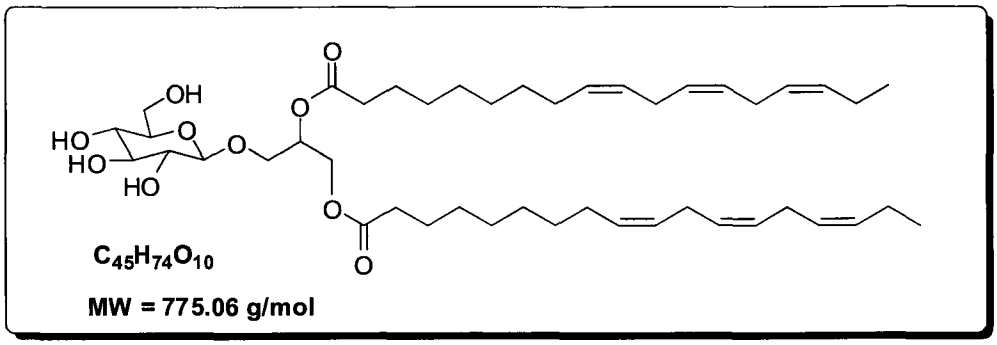
A mixture of diol **17** (28 mg, 0.047 mmol), DCC (29 mg, 0.141 mmol), and a trace amount of DMAP were dissolved in dry DCM (10 mL). Linolenic acid (39 mg, 0.141 mmol) was added and the reaction mixture was immediately sealed and flushed with N_2.

When the reaction was complete by TLC, it was quenched by the addition of diethyl ether. The mixture was filtered into a separatory funnel where it was washed with a saturated solution of ammonium chloride (10 mL). The organic extracts were dried over $MgSO_4$, evaporated to dryness, and purified by flash chromatography (eluted using a solvent gradient of 0-10% ethyl acetate in hexanes). Protected glycolipid **18** was obtained as a light yellow oil (23 mg, 0.021 mmol, 44% yield).

^{1}H NMR (400MHz, $CDCl_3$) δ: 5.25-5.40 (12H, m, H-12', H-13', H-15', H-16', H-18', H-19'), 4.30-4.37 (1H, m, H-1), 4.09-4.19 (2H, m, H-3'), 3.38-3.99 (9H, m, H-2, H-3, H-4, H-5, H-6, H-1', H-2'), 2.78 (8H, dd, J = 5.6, 5.6 Hz, H-14', H-17'), 2.25-2.30 (4H, H-4'), 1.98-2.10 (8H, m, H-11', H-20'), 1.54-1.63 (4H, m, H-5'), 1.23-1.40 (16H, m, H-6', H-7', H-8', H-9'), 0.86-0.97 (37H, m, H-21', TBDMS), 0.04-0.12 (20H, m, TBDMS)

^{13}C NMR (400MHz, $CDCl_3$) δ: 172.9 (C-4'), 131.8 (C-12'), 130.2 (C-19'), 128.3 (C-15'), 128.2 (C-16'), 127.8 (C-18'), 127.1 (C-13'), 102.5 (C-1), 78.4 (C'2), 75.3 (C-2'), 75.3 (C-3), 70.4 (C-5), 70.3 (C-4), 70.3 (C-2), 66.8 (C-3'), 62.9 (C-1'), 60.0 (C-6), 34.3 (C-8'), 31.5 (C-10'), 29.2 (C-7'), 27.2 (C-11'), 26.0-25.6 (C-14', C-17', TBDMS), 24.9 (C-6'), 20.6 (C-20'), 18.4 (TBDMS), 18.3 (TBDMS), 14.3 (C-21'), -3.9 - -5.3 (TBDMS)

2',3'-propyl dilinolenate-*β-D*-Glucopyranoside (18)



The TBS-protected glycolipid **17** (23 mg, 0.020 mmol) was dissolved in tetrahydrofuran and TBAF (0.12 mL of 1M solution) was added.

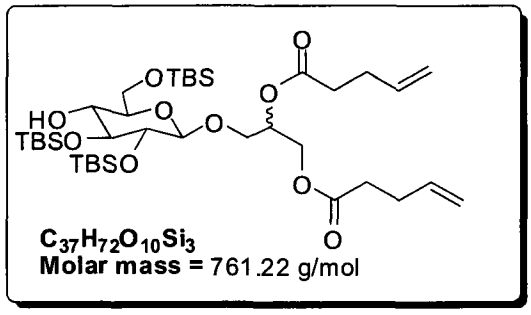
When complete, the reaction mixture was diluted with brine (10 mL) and extracted with ethyl acetate (3x, 10mL). The organic extracts were dried over $MgSO_4$, evaporated to dryness, and purified by flash chromatography (solvent gradient of 75-100% ethyl acetate in hexanes). The glycolipid **16** was obtained as a light yellow oil (8 mg, 0.010 mmol, 51.6% yield).

^{1}H NMR (400MHz, $CDCl_3$) δ: 5.20-5.41 (12H, m, H-12', H-13', H-15', H-16', H-18', H-19'), 4.36 (1H, dd, J = 12.0, 1.6 Hz, H-3'a), 4.29 (1H, d, J = 7.6Hz, H-1), 4.14 (1H, m, H-3'b), 3.28-3.92 (9H, m, H-2, H-3, H-4, H-5, H-6, H-1', H-2'), 2.78 (8H, dd, J = 5.6, 5.6 Hz, H-14', H-17'), 2.29 (4H, dd, J = 8.0, 14.8 Hz, H-5'), 2.00-2.07 (8H, m, H-11', H-20'), 1.56-1.66 (4H, m, H-6'), 1.32-1.37 (16H, m, H-7', H-8', H-9', H-10'), 0.95 (6H, t, J = 7.2 Hz, H-21')

^{13}C NMR (100MHz, $CDCl_3$) δ: 173.7 (C-4'), 132.0 (C-12'), 130.3 (C-19'), 128.4 (C-15'), 128.3 (C-16'), 127.8 (C-18'), 127.2 (C-13'), 103.5 (C-1), 76.3 (C-2'), 75.8 (C-3), 73.5 (C-5),

73.4 (C-4), 69.7 (C-2), 68.3 (C-3'), 62.6 (C-1'), 61.8 (C-6), 34.4 (C-8'), 34.2 (C-10'), 29.2 (C-7'), 27.3 (C-11'), 25.7 (C-14', C-17'), 25.6 (C-6'), 20.6 (C-20'), 14.4 (C-21')

2',3'-propyl dipent-5-enyl-2,3,6-O-TBS -*β* -*D*-Glucopyranoside (20)

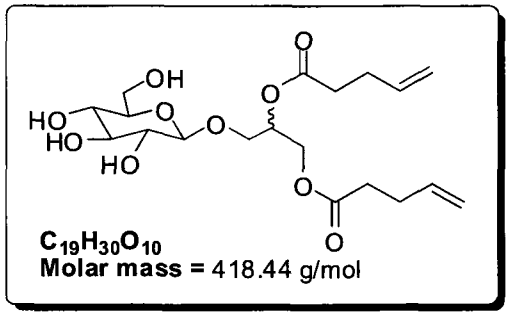


A mixture of diol **17** (90 mg, 0.13 mmol), DCC (93 mg, 0.45 mmol), and a trace amount of DMAP were dissolved in dry DCM (5 mL). Pent-4-enoic acid (45 mg, 0.45 mmol) was added and the reaction mixture was immediately sealed and flushed with N_2.

When the reaction was complete by TLC, it was quenched by the addition of diethyl ether. The mixture was filtered into a separatory funnel where it was washed with a saturated solution of ammonium chloride (10 mL). The organic extracts were dried over $MgSO_4$, evaporated to dryness, and purified by flash chromatography (eluted using a solvent gradient of 0-10% ethyl acetate in hexanes). Protected glycolipid **20** was obtained as a light yellow oil (100.4 mg, 0.131 mmol, 100% yield).

NO NMR data available due to computer crash

2',3'-propyl dipent-4-enyl-*β*-*D*-Glucopyranoside (21)



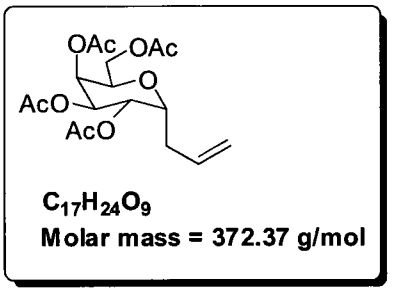
The TBS-protected glycolipid **20** (100 mg, 0.13 mmol) was dissolved in tetrahydrofuran (1 mL) and TBAF (0.23 mL of 1M solution) was added.

When complete, the reaction mixture was diluted with brine (10 mL) and extracted with ethyl acetate (3x, 10mL). The organic extracts were dried over $MgSO_4$, evaporated to dryness, and purified by flash chromatography (solvent gradient of 75-100% ethyl acetate in hexanes). The glycolipid **21** was obtained as a light yellow oil (40 mg, 0.096 mmol, 73% yield).

^{1}H NMR (400MHz, $CDCl_3$) δ: **Unavailable due to computer crash**

^{13}C NMR (400MHz, $CDCl_3$) δ: 173.1 (C-4'a), 172.8 (C-4'b), 136.5 (C-7'), 115.7 (C-8'), 99.3 (C-1), 73.9 (C-2'), 72.0 (C-3), 71.2 (C-5), 70.2 (C-4), 69.4 (C-2), 66.1 (C-3'), 62.8 (C-6'), 61.1 (C-1'), 33.4 (C-3'), 28.9 (C-4')

2,3,4,6-Tetra-*O*-acetyl-α-*C*-allyl galactopyranose (22)



$C_{17}H_{24}O_9$
Molar mass = 372.37 g/mol

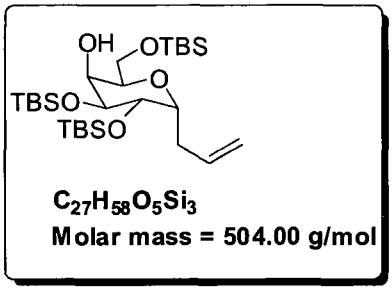
Penta-acetyl galactose (15.0 g, 38.5 mmol) was dissolved in acetonitrile (300 mL) and the reaction vessel was flushed with N_2 before being cooled to 0°C. Allyl-TMS (44 g, 385 mmol) was added slowly, followed by the dropwise addition of BF_3•OEt_2 (55 g, 385 mmol). The reaction mixture was warmed to RT before being heated to reflux and monitored by TLC.

When the reaction was complete after 5 hours, the solvent was removed by rotary evaporation. The residue was dissolved in chloroform (40 mL), then washed with water (50 mL), 10% $NaHCO_3$ (50 mL), and water (50 mL). The organic extract was dried over $MgSO_4$ and evaporated to dryness. The crude product (16.08 g) was recrystallized using ether to yield the desired *C*-glycoside **22** as an opaque white solid which was used without further purification (12.644 g, 88.2% yield).

^{1}H NMR (400MHz, $CDCl_3$) δ: 5.72-5.60 (1H, m, H-2'), 5.31-5.30 (1H, t, J = 2.8 Hz, H-3'a), 5.17-5.09 (2H, m, H-3, H-3'b), 5.05-4.89 (2H, m, H-2, H-4), 4.21-4.16 (1H, m, H-1), 4.09 (1H, dd, J = 12.8, 9.2 Hz, H-5), 4.01-3.96 (2H, m, H-6), 2.42-2.33 (1H, m, H-1'a), 2.25-2.17 (1H, m, H-1'b), 2.01 (3H, s, OAc), 1.96 (3H, s, OAc), 1.92 (3H, s, OAc), 1.91 (3H, s, OAc)

^{13}C NMR (100MHz, $CDCl_3$) δ: 170.2 (OAc), 169.7 (OAc), 169.5 (OAc), 169.4 (OAc), 133.1 (C-2'), 117.2 (C-3'), 71.1 (C-1), 67.9 (C-2), 67.9 (C-3), 67.6 (C-4), 67.3 (C-5), 61.1 (C-6), 30.6 (C-1'), 20.4 (OAc), 20.4 (OAc), 20.4 (OAc), 20.3 (OAc)

2,3,6-Tri-*O*-TBDMS-α-*C*-allyl galactopyranose (23)



Acetylated glycoside **22** (11.84 g, 31.83 mmol) was dissolved in 15 mL of anhydrous methanol. Potassium carbonate (0.880 g, 6.36 mmol) was added and the heterogeneous solution was stirred overnight. Approximately 0.5 g of Amberlite H^+ resin was added to stop the reaction. After stirring for a further 10 minutes, the resin was filtered off and washed 2 x with 5 mL of methanol. The solvent was evaporated to yield the desired product as a thick orange syrup (6.1 g, 30 mmol, 94.3% yield) which was used without further purification.

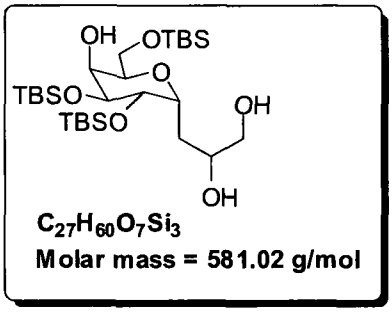
The deprotected glycoside (6.1 g, 30 mmol) was dissolved in 20 mL of DMF and imidazole (27 g, 180 mmol) was added. The resulting solution was cooled to 0°C and stirred for 10 minutes. TBDMS-Cl (12.3 g, 180 mmol) was added and the reaction mixture was stirred at 0°C for 30 minutes, then warmed to room temperature and monitored by TLC.

When complete, the reaction mixture was diluted with ether (40 mL) and transferred into a separatory funnel. Brine (30 mL) was added, and the resulting aqueous layer was drained. The remaining organic extracts were washed 3x with 30 mL of dH_2O, dried over $MgSO_4$, and evaporated to dryness. Following purification by flash chromatography (eluted with 5-10% ethyl acetate in hexanes), the desired tri-protected glycoside **23** was obtained as a viscous, colourless oil (7.62 g, 14.0 mmol, 46.5% yield).

^{1}H NMR (400MHz, $CDCl_3$) δ: 5.86-5.76 (1H, m, H-2'), 5.31-3.30 (1H, m, H-3'a), 5.17-5.09 (2H, m, H-3, H-3'b), 5.05-4.89 (2H, m, H-2, H-4), 4.21-4.16 (1H, m, H-1), 4.10 (1H, m, H-5), 4.01-3.96 (2H, m, H-6), 2.42-2.33 (1H, m, H-1'a), 2.20-2.15 (1H, m, H-1'b)

^{13}C NMR (100MHz, $CDCl_3$) δ: 170.4 (OAc), 170.0 (OAc), 169.8 (OAc), 169.7 (OAc), 133.4 (C-2'), 117.5 (C-3'), 71.6 (C-1), 68.2 (C-2), 68.2 (C-4), 67.8 (C-3), 67.6 (C-5), 61.4 (C-6), 30.8 (C-1'), 20.7 (OAc), 20.6 (OAc), 20.5 (OAc)

2',3'-dihydroxypropyl 2,3,6-Tri-*O*-TBDMS-α-*C*- galactopyranose (24)

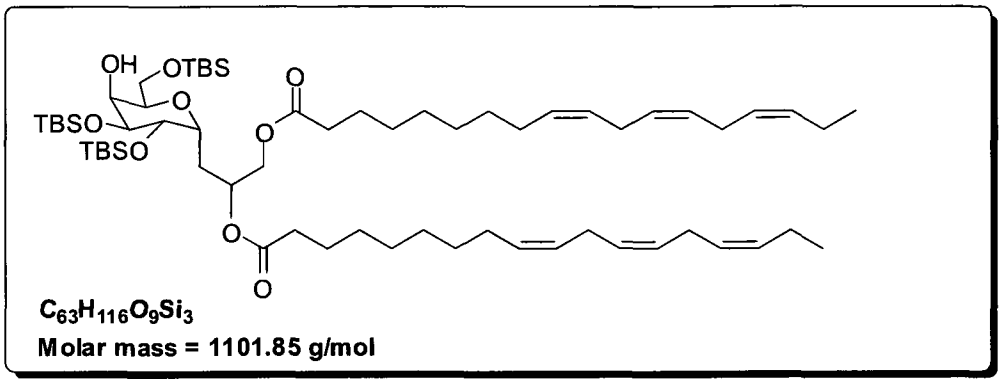


Protected glycoside **23** (330 mg, 0.60 mmol) was dissolved in a mixture of 2:4:1 dH_2O: *tert*-butyl alcohol: acetone (7 mL) in a round-bottom flask equipped with a stir bar and wrapped with foil. NMO (105 mg, 0.90 mmol) was added, followed by a catalytic amount of OsO_4 (approximately 30 mg, 0.12 mmol). The reaction was stirred at room temperature and monitored by TLC. When complete after approximately 2.5 hours, the acetone was removed by evaporation. The resulting dark brown syrup was dissolved in 5 mL of saturated sodium thiosulfate solution and extracted 3x with 10 mL of ethyl acetate. The organic extracts were dried over $MgSO_4$, evaporated to dryness, and purified by flash chromatography (eluted with 30% ethyl acetate in hexanes). Diol **24** product was obtained as a dark yellow oil (180 mg, 0.309 mmol, 51.6% yield).

^{1}H NMR (400MHz, $CDCl_3$) δ: 4.24-4.14 (2H, m, H-1, H-2), 3.88-3.37 (8H, m,), 2.63 (1H, br.d, OH), 2.45 (1H, br.s, OH), 1.86-1.75 (1H, m, H-1'a), 1.46-1.37 (1H, m, H-1'b), 0.87-0.86 (27H, TBDMS), 0.08-0.03 (18H, TBDMS)

^{13}C NMR (100MHz, $CDCl_3$) δ: 75.6 (C-2'), (C-3'), 71.9 (C-2'), 71.5 (C-1), 69.5 (C-4), 68.3 (C-3), 67.8 (C-2), 66.5 (C-5), 60.4 (C-6), 31.6 (C-1'), 25.9-25.8 (TBDMS), 18.3-18.0 (TBDMS), -4.6 - -5.5 (TBDMS)

2',3'-propyl dilinolenate-2,3,6-*O*-tri-TBDMS-α-*C*-galactopyranoside (25)



Diol **24** (2.0 g, 3.45 mmol), DCC (2.84 g, 13.8 mmol), and DMAP (trace) were combined in a dry RBF equipped with a stir bar. The flask was flushed with N_2 and 7 mL of dry, distilled DCM was added. Linolenic acid (2.40 g, 8.63 mmol) was solubilised in 1 mL of DCM and was added dropwise to the reaction mixture, and the reaction was stirred overnight.

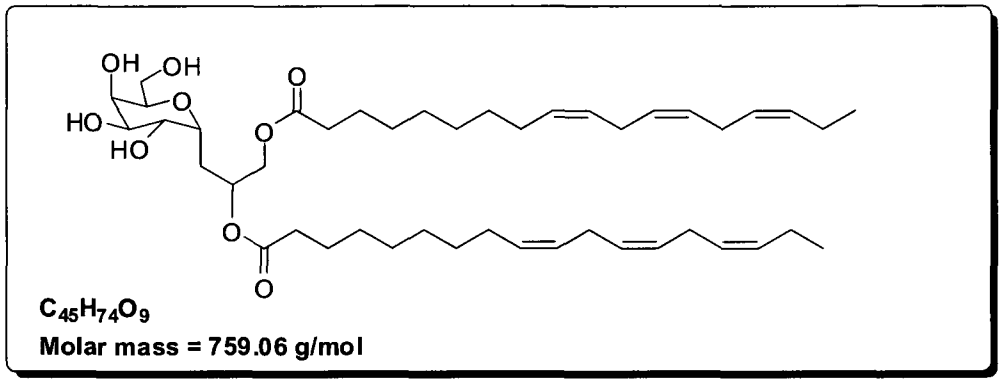
When the reaction was complete by TLC, it was quenched by the addition of diethyl ether. The solids were removed by filtration and washed well with ether. The organic extracts were washed with a saturated NH_4Cl solution then dried over $MgSO_4$, evaporated to dryness, and purified by flash chromatography (eluted using a solvent gradient of 0-10% ethyl acetate in hexanes). Protected *C*-glycolipid **25** was obtained as a light yellow oil (1.538 g, 1.39 mmol, 40.5% yield).

^{1}H NMR (400MHz, $CDCl_3$) δ: 5.40-5.26 (12H, m, H-12', H-13', H-15', H-16', H-18', H-19'), 4.34-3.45 (13H, m, H-1, H-2, H-3, H-4, H-5, H-6, H-2', H-3'), 3.20-3.14 (18H, m, H-), 2.78 (8H, dd, J = 5.6, 5.6 Hz, H-14', H-17'), 2.30-2.22 (4H, m, H-4'), 2.07-2.02 (8H, m, H-

11', H-20'), 1.57-1.53 (m, H-5'), 1.35-1.18 (16H, m, H-6', H-7', H-8', H-9', TBDMS), 0.95 (6H, t, J = 7.6 Hz, H-21'), 0.90-0.86 (36H, m, TBDMS), 0.10-0.03 (18H, m, TBDMS)

^{13}C NMR (100MHz, $CDCl_3$) δ: **Unavailable due to computer crash**

2',3'-propyl dilinolenate-α-*C*-galactopyranoside (26)

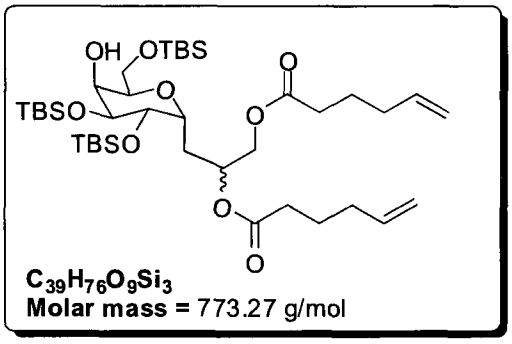


The protected *C*-glycolipid **25** (160 mg, 0.139 mmol) was dissolved in THF and TBAF (0.56 mL of 1M solution) was added.

When complete, the reaction mixture was diluted with brine (10 mL) and extracted with ethyl acetate (3x, 10mL). The organic extracts were dried over $MgSO_4$, evaporated to dryness, and purified by flash chromatography (solvent gradient of 75-100% ethyl acetate in hexanes). *C*-Glycolipid **26** was obtained as a light yellow oil (31.5 mg, 40.6 mmol, 29.2% yield).

NO NMR DATA AVAILABLE due to computer crash

2',3'-propyl dihex-5-enyl-2,3,6-*O*-tri-TBDMS-α-*C*-galactopyranoside (27)

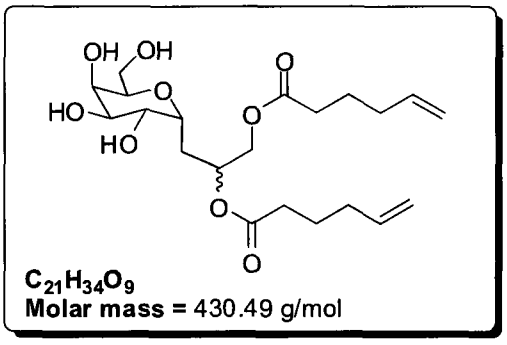


The diol **24** (140 mg, 0.241 mmol), DCC (174 mg, 0.845 mmol), and DMAP (trace) were combined in a dry RBF equipped with a stir bar. The flask was flushed with N_2 and1 mL of dry, distilled DCM was added. Pent-4-enoic acid (84 mg, 0.84 mmol) was solubilised in 1 mL of DCM and was added dropwise to the reaction mixture, and the reaction was stirred overnight. After 24 hours, another 2 equivalents of both the pentenoic acid (22 mg) and DCC (87 mg) were added.

When complete, the reaction mixture was diluted with diethyl ether (2 mL) and the solids formed during the reaction were removed by filtration. The filter cake was washed well with a further 2 mL of ether before the filtrate was washed with saturated NH_4Cl (5 mL). The organic extract was removed and dried over $MgSO_4$ before being concentrated to dryness. The crude product was purified by column chromatography to yield the protected glycolipid **27** as a yellow oil (60.7 mg, 7.85×10^{-2} mmol, 32.7% yield).

NO NMR DATA AVAILABLE due to computer crash

2',3'-propyl dihex-5-enyl-α-*C*-galactopyranoside (28)



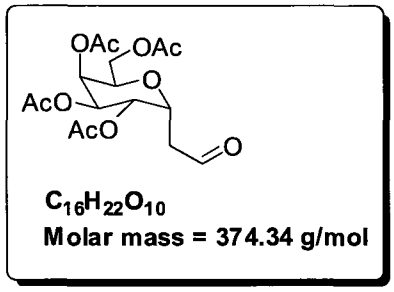
The protected glycolipid **27** (50 mg, $6.5x10^{-2}$ mmol) was dissolved in THF (2 mL) and a 1M solution of TBAF (0.39 mL, $3.9x10^{-1}$ mmol) was added dropwise. The reaction was monitored by TLC.

When complete, the reaction mixture was diluted with brine (5 mL) and extracted with ethyl acetate (3x 10mL). The organic extracts were dried over $MgSO_4$, evaporated to dryness, and purified by flash chromatography (solvent gradient of 75-100% ethyl acetate in hexanes). The *C*-glycolipid **28** was obtained as a light yellow oil (9.8 mg, $2.3x10^{-2}$ mmol, 35% yield).

^{1}H NMR (400MHz, $CDCl_3$) δ: 5.84-5.73 (2H, m, H-7'), 5.28-5.15 (1H, m, H-1), 5.06-4.97 (4H, m, H-8'), 4.44-3.62 (13H, m, H-2, H-3, H-4, H-5, H-6, H-2', H-3'), 2.44-2.30 (8H, m, H-5', H-6'), 1.91-1.87 (2H, m, H-1')

^{13}C NMR (100MHz, $CDCl_3$) δ: 173.2 (C-4'), 136.5 (C-7'), 115.7 (C-8'), 72.2 (C-1), 70.8 (C-2), 70.2 (C-3), 69.2 (C-4), 68.7 (C-5), 65.6 (C-2'), 64.3(C-3'), 61.7 (C-6), 33.6 (C-5'), 33.3 (C-6'), 28.7 (C-1')

2,3,4,6-Tetra-*O*-acetyl α-*D*-galactopyranosyl ethyl aldehyde (29)



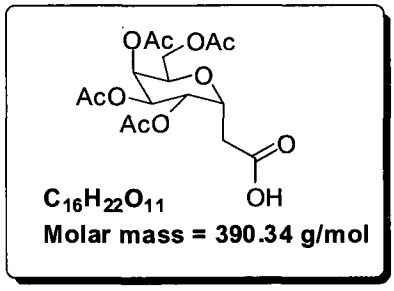
Glycoside **22** (0.500 g, 1.30 mmol) was dissolved in DCM (20 mL) in a dry RBF and the flask was flushed with N_2. The reaction flask was cooled to -78°C, then O_3 was bubbled into the flask until a dark blue colour persisted. The mixture was stirred at -78°C for 30 minutes before PPh_3 (0.852 g, 3.25 mmol) was added and the reaction was stirred for a further 30 minutes. Next, the reaction was warmed to 0°C, stirred at this temperature for 45 minutes, and then warmed to RT and stirred overnight.

The following day, the solvent was removed by rotary evaporation, and the residue was purified by flash chromatography. The desired aldehyde **29** was obtained as a colourless solid (0.341 g, 70.0% yield).

^{1}H NMR (400MHz, $CDCl_3$) δ: 9.71 (1H, t, J = 1.6 Hz, H-2'), 5.40 (1H, t, J = 3.2 Hz, H-4), 5.27 (1H, dd, J = 8.4, 4.8 Hz, H-3), 5.17 (1H, dd, J = 8.8, 3.6 Hz, H-2), 4.87-4.82 (1H, m, H-5), 4.34-4.28 (1H, m, H-1), 4.10-4.04 (2H, m, H-6), 2.71-2.61 (2H, m, H-1'), 2.10 (3H, s, OAc), 2.05 (3H, s, OAc), 2.04 (6H, s, OAc)

^{13}C NMR (100MHz, $CDCl_3$) δ: 198.3 (C-2'), 170.7 (OAc), 169.9 (OAc), 169.8 (OAc), 169.6 (OAc), 69.8 (C-1), 67.9 (C-2), 67.8 (C-3), 66.9 (C-4), 66.7 (C-5), 60.9 (C-6), 42.0 (C-1'), 20.8 (OAc), 20.8 (OAc), 20.7 (OAc), 20.6 (OAc)

2,3,4,6-Tetra-*O*-acetyl α-*D*-galactopyranosyl ethanoic acid (30)

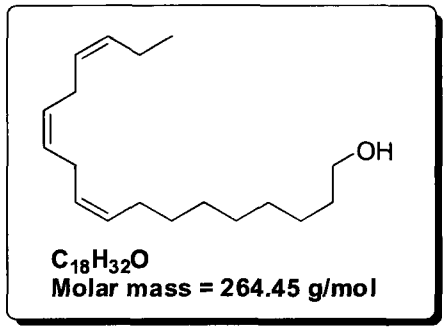


The aldehyde **29** (100 mg, 0.267 mmol) was dissolved in ^{t}BuOH (3 mL) and was slowly added to a 1.25 M solution of KH_2PO_4 (0.43 mL, 0.535 mmol). This solution was added to a 1.0 M solution of $KMnO_4$ (0.535 mL, 0.535 mmol), and the reaction mixture was stirred for 20 min. The reaction was quenched by the addition of saturated Na_2SO_3 until the $KMnO_4$ was neutralized (marked by the dissipation of the purple colour and the appearance of a dark brown precipitate). The reaction mixture was filtered and the filtrate was washed with EtOH. The EtOH was then removed by rotary evaporation and the resulting aqueous extract was acidified to a pH of 3 with HCl. This solution was extracted with DCM; the organic extracts were dried over $MgSO_4$, and rotovapped to dryness. The resulting carboxylic acid **30** was obtained as white foam (104 mg, 88.5% yield).

^{1}H NMR (300MHz, $CDCl_3$) δ: 5.42 (1H, t, J = 2.4 Hz, H-4), 5.33 (1H, dd, J = 9.0 Hz, 5.1 Hz, H-), 5.17 (1H, dd, J = 9.3 Hz, 3.3 Hz, H-), 4.74-4.68 (1H, m, H-), 4.24-4.06 (3H, m, H-), 2.75-2.58 (2H, m, H-), 2.12 (3H, s, OAc), 2.06 (3H, s, OAc), 2.04 (6H, s, OAc)

^{13}C NMR (100MHz, $CDCl_3$) δ: 174.9 (C-8), 171.1 (OAc), 170.5 (OAc), 170.4 (OAc), 170.1 (OAc), 70.1 (C-1), 69.7 (C-2), 68.3 (C-3), 67.9 (C-4), 67.6 (C-5), 61.6 (C-6), 31.5 (C-7), 21.1 (OAc)

Linolenol (31)



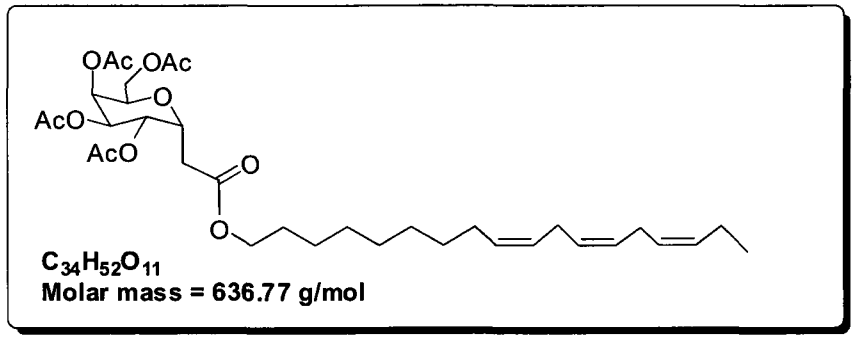
A dry rbf was charged with $LiAlH_4$ (273 mg, 7.18 mmol) and dry THF (5 mL), and the flask was sealed, flushed with N2, and cooled to 0°C. Separately, linolenic acid (1.0 g, 3.59 mmol) was dissolved in THF (2 mL) and this solution was added dropwise to the $LiAlH_4$ suspension. The reaction mixture was allowed to warm to RT and the reaction was monitored by TLC.

The reaction was quenched by the dropwise addition of H_2O until no further reaction was observed. A small amount of 10% HCl (2 mL) was added to complete the decomposition of the $LiAlH_4$. This solution was transferred to a separatory funnel and was extracted with ether (3x 10 mL). The combined organic layer was washed with brine, then dried over $MgSO_4$ and concentrated to dryness. The crude product (738 mg) was purified by column chromatography to yield the desired alcohol **31** (479 mg, 1.8 mmol, 50.5% yield).

^{1}H NMR (400MHz, $CDCl_3$) δ: 5.38-5.24 (6H, m, H-9, H-10, H-12, H-13, H-15, H-16), 4.25 (1H, br.s, OH), 3.57 (2H, t, J = 6.8 Hz, H-1), 2.77 (4H, dd, J = 6.0, 6.0 Hz, H-11, H-14), 2.07-1.99 (4H, m, H-8, H-17), 1.53-1.50 (2H, m, H-2), 1.33-1.27 (12H, m, H-3, H-4, H-5, H-6, H-7), 0.93 (3H, t, J = 7.6 Hz, H-18)

^{13}C NMR (400MHz, $CDCl_3$) δ: 131.9 (C-9), 130.3 (C-16), 130.2 (C-12), 128.2 (C-13), 127.7 (C-15), 127.1 (C-10), 62.8 (C-1), 32.6 (C-2), 29.6 (C-11), 29.5 (C-14), 29.4 (C-8), 29.2 (C-17), 27.2 (C-4), 25.8 (C-5) , 25.6 (C-6), 25.5, (C-7), 20.5 (C-3), 14.2 (C-18)

2'-ethyl linolenate-2,3,4,6-Tetra-*O*-acetyl-*C*-galactopyranoside (32)

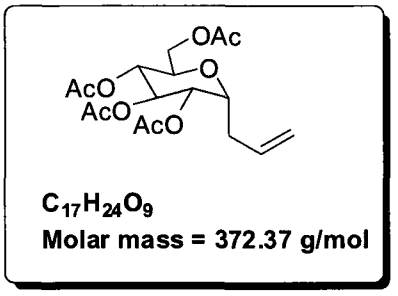


The carboxylic acid **30** (90 mg, 0.231 mmol), DCC (71.5 mg, 0.347 mmol), and DMAP (trace) were combined in a dry RBF equipped with a stir bar. The flask was flushed with N_2 and 0.5 mL of dry, distilled DCM was added. Linolenol **31** (91.5 mg, 0.347 mmol) was solubilised in 0.5 mL of DCM and was added dropwise to the reaction mixture, and the reaction was stirred overnight.

When complete, the reaction mixture was diluted with diethyl ether (2 mL) and the solids formed during the reaction were removed by filtration. The filter cake was washed well with a further 2 mL of ether before the filtrate was washed with saturated NH_4Cl (5 mL). The organic extract was removed and dried over $MgSO_4$ before being concentrated to dryness. The crude product was purified by column chromatography to yield the protected glycolipid **32** as a yellow oil (93.2 mg, 0.146 mmol, 63.3% yield).

NO NMR DATA AVAILABLE due to computer crash

2,3,4,6-Tetra-*O*-acetyl-α-*C*-allyl glucopyranose (33)



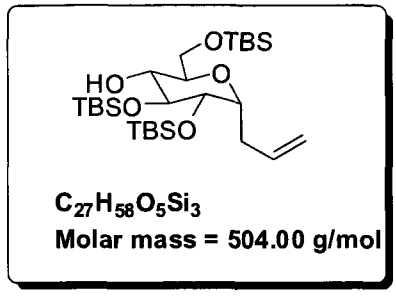
Penta-acetyl glucose (5.00 g, 12.8 mmol) was dissolved in acetonitrile (30 mL) and the reaction vessel was flushed with N_2 before being cooled to 0°C. Allyl-TMS (9.09 g, 64.1 mmol) was added slowly, followed by the dropwise addition of $BF_3{\bullet}OEt_2$ (7.32 g, 64.1 mmol). The reaction mixture was warmed to RT before being heated to reflux and monitored by TLC.

When the reaction was complete, the solvent was removed by rotary evaporation. The residue was dissolved in chloroform (20 mL), then washed with water (25 mL), 10% $NaHCO_3$ (25 mL), and water (25 mL). The organic extract was dried over $MgSO_4$ and evaporated to dryness. The crude product was purified by column chromatography to yield the desired glycoside **33** (2.609 g, 7.01 mmol, 54.7% yield).

^{1}H NMR (400MHz, $CDCl_3$) δ: 5.77-5.65 (1H, m, H-2'), 5.31 (1H, t, J = 12.0 Hz, H-), 4.94 (1H, t, J = 11.6 Hz, H-), 5.15-5.03 (3H, m, H-), 4.28-4.15 (2H, m, H-), 4.05 (1H, dd, J = 16.0, 3.6 Hz, H-), 3.86-3.80 (1H, m, H-), 2.58-2.47 (1H, m, H-6a), 2.37-2.26 (1H, m, H-6b), 2.04 (3H, s, OAc), 2.02 (3H, s, OAc), 2.01 (3H, s, OAc), 2.00 (3H, s, OAc)

^{13}C NMR (100MHz, $CDCl_3$) δ: 170.5 (OAc), 170.0 (OAc), 169.5 (OAc), 169.5 (OAc), 133.0 (C-2'), 117.7 (C-3'), 71.8 (C-1), 70.2 (C-3), 70.1 (C-2), 68.7 (C-4), 68.7 (C-5), 62.1 (C-6), 30.5 (C-1'), 20.6 (OAc), 20.6 (OAc), 20.6 (OAc)

2,3,6-Tri-*O*-TBDMS-α-*C*-allyl glucopyranose (34)



Glycoside **33** (2.609 g, 7.01 mmol) was dissolved in 5 mL of anhydrous methanol. Potassium carbonate (0.193 g, 1.40 mmol) was added and the heterogeneous solution was stirred overnight. Approximately 0.5 g of Amberlite H^+ resin was added to stop the reaction. After stirring for a further 10 minutes, the resin was filtered off and washed 2 x with 5 mL of methanol. The solvent was evaporated to yield the desired product as a thick orange syrup (1.37 g, 6.73 mmol, 96.1% yield) which was used without further purification.

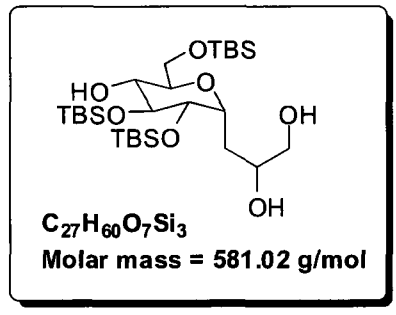
To the deprotected glycoside (290 g, 1.44 mmol) in 5 mL of DMF was added imidazole (392 mg, 5.76 mmol). The resulting solution was cooled to 0°C and stirred for 10 minutes before TBDMS-Cl (868 mg, 5.76 mmol) was added. The reaction mixture was stirred at 0°C for 30 minutes, then warmed to room temperature and monitored by TLC.

When complete, the reaction mixture was diluted with ether (10 mL) and transferred into a separatory funnel. Brine (10 mL) was added, and the resulting aqueous layer was drained. The remaining organic extracts were washed 3x with 30 mL of dH_2O, dried over $MgSO_4$, and evaporated to dryness. Following purification by flash chromatography (eluted with 5-10% ethyl acetate in hexanes), the protected glycoside **34** was obtained as a viscous, colourless oil (336 mg, 0.670 mmol, 46.5% yield).

^{1}H NMR (400MHz, $CDCl_3$) δ: 5.95-5.84 (1H, m, H-2'), 5.12 (1H, dq, J = 17.4, 1.2 Hz, H-3'$_t$), 5.05 (1H, ddd, J = 10.4, 1.2, 0.8 Hz, H-3'$_c$), 3.88 (1H, dd, J = 10.4, 4.4 Hz, H-2), 3.76 (1H, dd, J = 10.4, 6.0 Hz, H-3), 3.47-3.44 (2H, m, H-4, H-5), 3.30-3.19 (3H, m, H-1, H-6), 2.59-2.52 (1H, m, H-1'a), 2.28-2.21 (1H, m, H-1'b), 0.92-0.90 (27H, m, TBDMS), 0.16-0.08 (18H, m, TBDMS)

^{13}C NMR (100MHz, $CDCl_3$) δ: 134.6 (C-2'), 117.0 (C-3'), 80.1 (C-5), 78.2 (C-4), 77.9 (C-1), 74.1 (C-2), 73.5 (C-3), 65.0 (C-6), 36.3 (C-1'), 26.0 (TBDMS), 25.9 (TBDMS), 18.4 (TBDMS), -3.6 (TBDMS), -4.1 (TBDMS), -4.6 (TBDMS), -5.4 (TBDMS), -5.4 (TBDMS)

2',3'-dihydroxypropyl 2,3,6-Tri-*O*-TBDMS-*α*-*C*- glucopyranose (35)

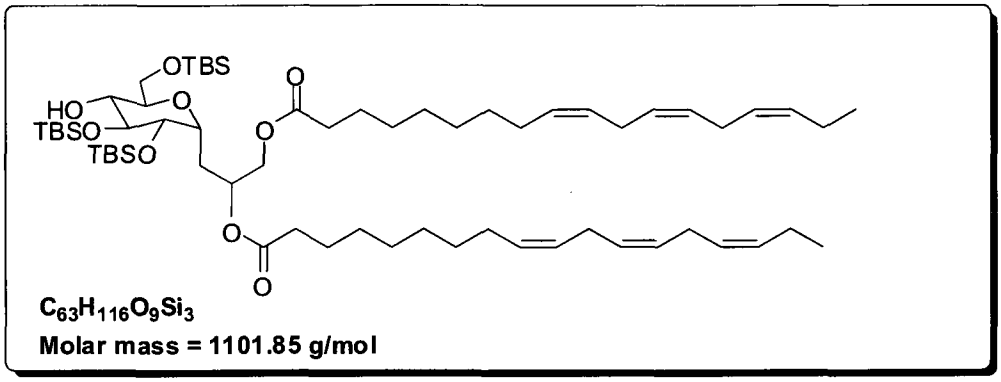


The protected glycoside **34** (220 mg, 0.401 mmol) was dissolved in a mixture of 2:4:1 dH_2O: *tert*-butyl alcohol: acetone (7 mL) in a round-bottom flask equipped with a stir bar and wrapped with foil. NMO (71 mg, 0.60 mmol) was added, followed by a catalytic amount of OsO_4 (approximately 6 mg, ~20 mmol). The reaction was stirred at room temperature and monitored by TLC. When complete after approximately 2.5 hours, the acetone was removed by evaporation. The resulting dark brown syrup was dissolved in 5 mL of saturated sodium thiosulfate solution and extracted 3x with 10 mL of ethyl acetate. The organic extracts were dried over $MgSO_4$, evaporated to dryness, and purified by flash chromatography (eluted with 30% ethyl acetate in hexanes). The diol **35** was obtained as a dark yellow oil (172 mg, 0.297 mmol, 74% yield).

^{1}H NMR (400MHz, $CDCl_3$) δ: 4.23-3.45 (9H, m, H-1, H-2, H-3, H-4, H-5, H-6a, H-2', H-3'), 3.29-3.22 (1H, m, H-6b), 1.99-1.79 (2H, m, H-1'), 0.90-0.87 (32H, m, TBDMS), 0.12-0.05 (18H, m, TBDMS)

^{13}C NMR (100MHz, $CDCl_3$) δ: 74.9 (C-4), 74.0 (C-2), 73.3 (C-3), 73.1 (C-3'), 72.3 (C-2'), 70.6 (C-5), 65.8 (C-1), 64.0 (C-6), 27.0 (C-1'), 26.0-25.8 (TBDMS), 18.5-18.0 (TBDMS), -3.7 - -5.5 (TBDMS)

2',3'-propyl dilinolenate-2,3,6-*O*-tri-TBDMS-α-*C*-glucopyranoside (36)



$C_{63}H_{116}O_9Si_3$
Molar mass = 1101.85 g/mol

Protected glycoside **35** (172 mg, 0.296 mmol), DCC (183 g, 0.889 mmol), and DMAP (trace) were combined in a dry RBF equipped with a stir bar. The flask was flushed with N_2 and 5 mL of dry, distilled DCM was added. Linolenic acid (247 g, 0.889 mmol) was solubilised in 1 mL of DCM and was added dropwise to the reaction mixture, and the reaction was stirred overnight.

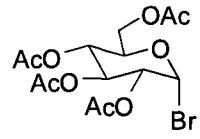
When the reaction was complete by TLC, it was quenched by the addition of diethyl ether. The solids were removed by filtration and washed well with ether. The organic extracts were washed with a saturated NH_4Cl solution then dried over $MgSO_4$, evaporated to dryness, and purified by flash chromatography (eluted using a solvent gradient of 0-10% ethyl acetate in hexanes). Protected *C*-glycolipid **36** was obtained as a light yellow oil (113 mg, 0.103 mmol, 34.7% yield).

^{1}H NMR (400MHz, $CDCl_3$) δ: 5.40-5.26 (12H, m, H-12', H-13', H-15', H-16', H-18', H-19'), 4.34 (1H, dd, J = 12.0, 3.2 Hz, H-3'a), 4.05 (1H, dd, J = 12.0, 5.2 Hz, H-3'b), 3.98-3.87 (1H, m, H-2'), 3.77-3.64 (2H, m, H-1, H-2), 3.60-3.54 (1H, m, H-3), 3.47-3.38 (2H, m, H-6), 3.27-3.24 (1H, m, H-5), 3.21-3.14 (3H, m, H-6, H-4), 2.78 (8H, dd, J = 6.0, 6.0 Hz, H-14',

H-17'), 2.29-2.22 (4H, m, H-4'), 2.07-1.17 (m, H-1', H-5', H-6', H-7', H-8', H-9', H-11', H-20'), 0.97-0.86 (m, H-21', TBDMS), 0.12-0.01 (20H, m, TBDMS)

^{13}C NMR (400MHz, $CDCl_3$) δ: 173.3 (C4'), 132.0 (C-12'), 130.2 (C-19'), 128.3 (C-15'), 128.2 (C-16'), 127.7 (C-18'), 127.1 (C-13'), 75.0 (C-1), 73.8 (C-2), 72.8 (C-3), 72.3 (C-5), 71.1 (C-4), 68.6 (C-2'), 65.0 (C-3'), 62.7 (C-6), 34.9 (C-8'), 34.1 (C-5'), 29.6 (C-7'), 29.2 (C-1'), 29.1 (C-9'), 29.1 (C-10'), 27.2 (C-11'), 26.0-25.5 (C-14', C-17', TBDMS), 24.7 (C-6'), 20.6 (C-20'), 18.3-18.0 (TBDMS), 14.3 (C-21'), -3.9 - -5.4 (TBDMS)

2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl bromide (37)



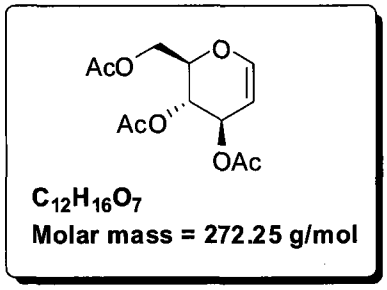
$C_{14}H_{19}BrO_9$
Molar mass = 411.20 g/mol

A flame-dried RBF equipped with a stir bar and charged with penta-acetyl glucose (5.0 g, 12.8 mmol) was flushed with N_2 and cooled to 0°C. A solution of 33% HBr in HOAc (35 mL) was added slowly, and the reaction mixture was stirred for 30 minutes before being warmed to room temperature and stirred for a further 60 minutes. The reaction was diluted with DCM (50 mL) and quenched by pouring it into ice-cold water (50 mL). The layers were separated, and the organic layer was washed repeatedly with water until the aqueous layer reached a pH of 7. The organic layer was then dried over $MgSO_4$ and rotovapped to dryness. The brominated sugar **37** was recrystallized from the crude product with Et_2O (4.4966 g, 10.94 mmol., 85.3% yield).

^{1}H NMR (400MHz, $CDCl_3$) δ: 6.59 (1H, d, J = 4.0 Hz, C-1), 5.54 (1H, t, J = 9.6 Hz, H-3), 5.14 (1H, t, J = 9.6 Hz, H-4), 4.82 (1H, dd, J = 10.0, 4.0, H-2), 4.33-4.27 (2H, m, H-6), 4.13-4.10 (1H, m, H-5)

^{13}C NMR (100MHz, $CDCl_3$) δ: 170.5 (OAc), 169.8 (OAc), 169.4 (OAc), 86.5 (C-1), 72.1 (C-3), 70.5 (C-2), 70.1 (C-4), 67.1 (C-5), 60.9 (C-6), 20.6 (OAc), 20.6 (OAc), 20.6 (OAc), 20.5 (OAc)

3,4,6-tri-*O*-acetyl-glucal (38)



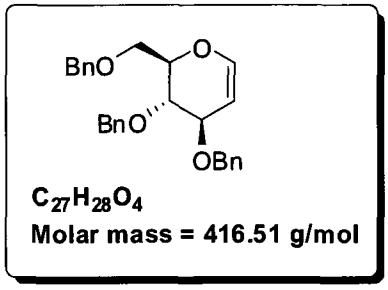
A round-bottom flask equipped with a magnetic stir bar was charged with the brominated sugar **37** (6.22 g, 15.1 mmol), zinc dust (5.4 g, 83 mmol), $CuSO_4$ (121 mg, 0.76 mmol), and NaOAc (6.0 g, 73 mmol). A solution of 60% HOAc in water (25 mL) was added and the reaction mixture was stirred at room temperature for one hour.

After the reaction was complete, the mixture was filtered through Celite. The Celite was washed well with EtOAc (3x 20 mL), and the resulting biphasic solution was separated. The organic extract was washed with saturated $NaHCO_3$ (20 mL), water (20 mL), and brine (20 mL), dried over $MgSO_4$, and rotovapped to dryness. The crude product (3.9819 g) was purified by column chromatography to yield the desired glucal **38** (1.6210 g, 5.95 mmol, 40% yield).

^{1}H NMR (400MHz, $CDCl_3$) δ: 6.31 (1H, dd, J = 8.4, 1.6 Hz, H-1), 5.19-5.16 (1H, m, H-2), 5.05 (1H, dd, J = 10.0, 7.6 Hz, H-4), 4.68 (1H, dd, J = 8.0, 4.4 Hz, H-3), 4.24 (1H, dd, J = 15.6, 7.2, H-6a), 4.13-4.07 (1H, m, H-5), 4.07 (1H, dd, J = 16.0, 4.0 Hz, H-6b), 1.92 (3H, s, OAc), 1.91 (3H, s, OAc), 1.88 (3H, s, OAc)

^{13}C NMR (100MHz, $CDCl_3$) δ: 170.3 (OAc), 170.1 (OAc), 169.3 (OAc), 145.5 (C-1), 98.8 (C-2), 73.4 (C-3), 67.2 (C-4), 67.0 (C-5), 61.2 (C-6), 20.7 (OAc), 20.6 (OAc), 20.5 (OAc)

3,4,6-tri-*O*-benzyl-*D*-glucal (39)



$C_{27}H_{28}O_4$
Molar mass = 416.51 g/mol

Tri-acetyl-glucal **38** (3.32 g, 12.1 mmol) was dissolved in 15 mL of anhydrous methanol. Potassium carbonate (334 mg, 2.42 mmol) was added and the heterogeneous solution was stirred overnight. Approximately 0.5 g of Amberlite H^+ resin was added to stop the reaction. After stirring for a further 10 minutes, the resin was filtered off and washed 2 x with 5 mL of methanol. The solvent was evaporated to yield the desired product as a thick orange syrup (1.73 g, 11.8 mmol, 96.9% yield) which was used without further purification.

A 60% dispersion of sodium hydride in mineral oil (2.62 g, 65.4 mmol) was washed 2x with hexanes before being suspended in DMF (5 mL) and cooled to 0°C. In a separate flask, the glucal (1.50 g, 10.9 mmol) was dissolved in DMF (5 mL) before being added to the sodium hydride suspension dropwise. This mixture was stirred for 10 minutes before the addition of benzyl chloride (8.28 g, 65.4 mmol). The reaction was warmed to room temperature and monitored by TLC. After 16 h, pyridine (2.6 g, 32.7 mmol) and a further portion of benzyl chloride (4.14 g, 32.7 mmol) was added.

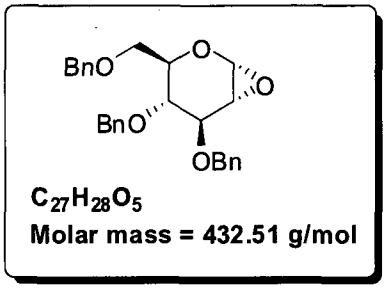
When complete, the reaction was quenched by the slow addition of a saturated solution of NH_4Cl (10 mL). This solution was extracted with DCM (3x 20 mL) and the combined

organic layers were dried over $MgSO_4$ and rotovapped to dryness. The benzylated glucal **39** was isolated as a white solid (3.80 g, 9.12 mmol, 83.7% yield)

^{1}H NMR (400MHz, $CDCl_3$) δ: 7.37-7.35 (15H, m, Bn), 6.47 (1H, dd, J = 6.4, 1.2 Hz, H-1), 4.91 (1H, dd, J = 6.0, 2.8 Hz, H-2), 4.87 (1H, d, J = 11.2 Hz, Bn), 4.70-4.57 (5H, Bn), 4.27-4.24 (1H, m, H-4), 4.13-4.09 (1H, m, H-3), 3.91 (1H, dd, J = 14.8, 6.0 Hz, H-6a), 3.87-3.78 (2H, m, H-5, H-6b)

^{13}C NMR (100MHz, $CDCl_3$) δ: 144.9 (C-1), 138.6 (Bn), 128.9 (Bn), 128.8 (Bn), 128.8 (Bn), 128.6 (Bn), 128.1 (Bn), 127.9 (Bn), 127.8 (Bn), 100.2 (C-2), 74.6 (C-3), 73.9 (Bn), 73.9 (C-4), 70.6 (C-5), 68.7 (C-6)

1,2-anhydro-3,4,6-tri-O-benzyl-*α*-*D*-Glucopyranose (40)



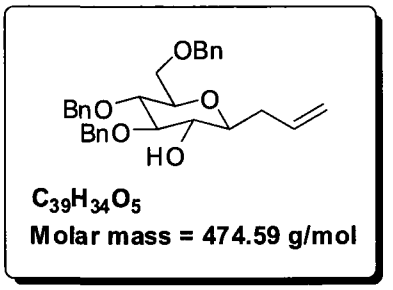
$C_{27}H_{28}O_5$
Molar mass = 432.51 g/mol

The glucal (335 mg, 0.805 mmol) was dissolved in a mixture of DCM (5 mL) and acetone (0.5 mL) before 10 mL of a saturated $NaHCO_3$ solution was added. The reaction mixture was stirred vigorously and cooled to -20°C in an ice-salt bath. Dropwise, over the course of 30 minutes, a solution of oxone in water (980 mg, 1.6 mmol in 3.5 mL) was added to the reaction mixture. After the addition was complete, the ice bath was removed and the reaction was stirred for 2.5 hours, during which the reaction was monitored closely by TLC. When overoxidation became apparent, the phases were separated, and the aqueous layer was re-extracted with DCM (3 x 5 mL). The combined organic phases were dried over $MgSO_4$ and rotovapped to dryness to yield 284 mg of crude product. NMR revealed a 3:1 ratio of product: starting material (calcd. 0.169 mmol, 21.1% yield). This product was carried forward without further purification.

^{1}H NMR (400MHz, $CDCl_3$) δ: 7.50-7.33 (15H, m, $Bn_{37,38}$), 6.56 (1H, d, J = 6.8 Hz, H-1_{37}), 5.10-4.63 (m, Bn, H-4_{38}), 4.36 (1H, d, J = 5.6 Hz, H-2_{38}), 4.34-4.20 (1H, m, H-4_{37}), 4.12 (1H, d, J = 7.6 Hz, H-3_{38}), 4.05 (1H, dd, J = 8.4, 6.0 Hz, H-3_{37}), 3.98-3.78 (3H, m, H-$5_{37,38}$, H-$6_{37,38}$), 3.15 (1H, d, J = 2.4 Hz, H-1_{38})

^{13}C NMR (100MHz, $CDCl_3$) δ: 144.9 (C_{37}-1), 138.6 (Bn), 138.3 (Bn), 138.2 (Bn), 137.8 (Bn), 128.7 (Bn), 128.5 (Bn), 128.1 (Bn), 128.1 (Bn), 128.0 (Bn), 127.9 (Bn), 127.8 (Bn), 100.2 (C_{37}-2), 79.2 (C-3), 77.7 (C-4), 77.0 (C-1), 76.0 (C-5), 74.6 (C_{37}-3), 73.9 (Bn), 73.7 (C_{37}-4), 72.3 (Bn), 70.6 (C_{37}-5), 69.6 (C-6), 68.7 (C_{37}-6), 68.4, 52.6 (C-2)

3, 4, 6-Tri-*O*-benzyl-*β*-*C*-allyl glucopyranose (41)



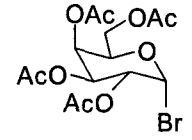
A 2-necked RBF was equipped with a stir bar and a rubber septum. The epoxide-glucal mixture (**38** and **39**) was dissolved in dry THF (20 mL) and the reaction flask was sealed, flushed with N_2, and cooled to 0°C. Dropwise, 1M allylmagnesium bromide (2.84 mL, 2.84 mmol) was added, and the reaction was monitored by TLC.

When the reaction was complete it was quenched by the addition of 10% HCl (10 mL). The sample was concentrated under reduced pressure, and the resulting syrup was dissolved in DCM (10 mL), transferred into a seperatory funnel, and extracted with water (2 x 10 mL). The organic extracts were dried over MgSO4 and rotovapped to dryness. The crude sample was purified by column chromatography to provide the glycoside **41** (293.3 mg, 0.6179 mmol, 43.5% yield).

^{1}H NMR (400MHz, $CDCl_3$) δ: 7.38-7.22 (15H, m, Bn), 6.03-5.89 (1H, m, H-2'), 5.19-5.09 (2H, m, H-3'), 4.98 (1H, d, J = 15.2 Hz, Bn), 4.84-4.74 (2H, m Bn), 4.69-4.57 (3H, m, Bn), 3.75-3.25 (7H, m, H-1, H-2, H-3, H-4, H-5, H-6,), 2.64-2.56 (1H, m, H-1'a), 2.38-2.29 (1H, m, H-1'b), 2.18 (1H, br.s, OH)

^{13}C NMR (100MHz, $CDCl_3$) δ: 138.2 (Bn), 135.7 (C-2'), 133.6 (Bn), 129.8-126.5 (Bn), 117.1 (C-3'), 87.7 (C-3), 85.8 (C-4), 80.1 (C-2), 78.3 (Bn), 75.2 (Bn), 74.5 (Bn), 73.5 (C-1), 72.5 (C-6), 68.9 (C-5), 36.3 (C-1')

2,3,4,6-tetra-*O*-acetyl-α-*D*-galactopyranosyl bromide (42)



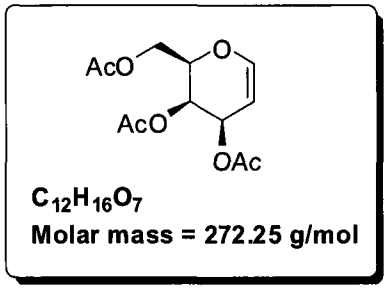
$C_{14}H_{19}BrO_9$
Molar mass = 411.20 g/mol

A flame-dried RBF equipped with a stir bar and charged with penta-acetyl galactose (10.0g, 25.1 mmol) was flushed with N_2 and cooled to 0°C. A solution of 33% HBr in HOAc (50 mL) was added slowly, and the reaction mixture was stirred for 30 minutes before being warmed to room temperature and stirred for a further 60 minutes. The reaction was diluted with DCM and quenched by pouring it into ice-cold water. The layers were separated, and the organic layer was washed repeatedly with water until the aqueous layer reached a pH of 7. The organic layer was then dried over $MgSO_4$ and rotovapped to dryness. The brominated sugar **41** was recrystallized from the crude product with Et_2O (9.5705 g, 23.37 mmol, 92.7% yield).

^{1}H NMR (400MHz, $CDCl_3$) δ: 6.67 (1H, d, J = 4.0 Hz, H-1), 5.50 (1H, dd, J = 3.2, 0.8 Hz, H-4), 5.38 (1H, dd, J = 10.4, 3.2 Hz, H-3), 5.03 (1H, dd, J = 10.8, 4.0 Hz, H-2), 4.47 (1H, td, J = 6.8, 0.8 Hz, H-5), 4.17 (1H, dd, J = 11.6, 6.4 Hz, H-6a), 4.09 (1H, dd, J = 11.2, 6.8 Hz, H-6b), 2.13 (3H, s, OAc-CH_3), 2.10 (3H, s, OAc-CH_3), 2.04 (3H, s, OAc-CH_3), 2.00 (3H, s, OAc-CH_3)

^{13}C NMR (100MHz, $CDCl_3$) δ: 107.1 (OAc), 169.8 (OAc), 169.6 (OAc), 169.5 (OAc), 87.9 (C-1), 70.8 (C-4), 67.7 (C-3), 67.5 (C-2), 66.7 (C-5), 60.6 (C-6), 20.5 (OAc), 20.4 (OAc), 20.4 (OAc), 20.3 (OAc)

3,4,6-tri-*O*-acetyl-galactal (42)



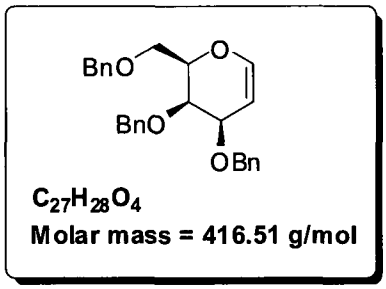
A round-bottom flask equipped with a magnetic stir bar was charged with the brominated sugar **41** (5.00 g, 12.2 mmol), zinc dust (4.4 g, 66.9 mmol), $CuSO_4$ (97 mg, 0.61 mmol), and NaOAc (4.80 g, 58.6 mmol). A solution of 60% HOAc in water (20 mL) was added and the reaction mixture was stirred at room temperature for one hour.

After the reaction was complete, the mixture was filtered through Celite. The Celite was washed well with EtOAc (3x 10 mL), and the resulting biphasic solution was separated. The organic extract was washed with saturated $NaHCO_3$ (10 mL), water (10 mL), and brine (10 mL), dried over $MgSO_4$, and rotovapped to dryness. The crude product was purified by column chromatography to yield the desired galactal **42** (667 mg, 2.45 mmol, 20.1% yield)

^{1}H NMR (400MHz, $CDCl_3$) δ: 6.31 (1H, dd, J = 8.4 Hz, 2.0 Hz, H-1), 5.41-5.39 (1H, m, H-2), 5.38 (1H, dt, J = 6.4 Hz, 2.8 Hz, H-3), 4.56 (1H, ddd, J = 8.4 Hz, 3.2 Hz, 2.0 Hz, H-), 4.20 (1H, t, J = 8.4 Hz, H-5), 4.08 (2H, dd, J = 8.8, 6.0 Hz, H-, H-), 1.98 (3H, s, OAc), 1.92 (3H, s, OAc), 1.86 (3H, s, OAc)

^{13}C NMR (100MHz, $CDCl_3$) δ: 170.3 (OAc), 170.0 (OAc), 169.9 (OAc), 145.3 (C-1), 98.7 (C-2), 72.6, 63.8, 63.6, 61.8, 20.6 (OAc), 20.5 (OAc), 20.4 (OAc)

3,4,6-tri-*O*-benzyl-*D*-galactal (44)



$C_{27}H_{28}O_4$
Molar mass = 416.51 g/mol

Tri-acetyl-galactal **42** (1.33 g, 4.90 mmol) was dissolved in 15 mL of anhydrous methanol. Potassium carbonate (0.880 g, 6.36 mmol) was added and the heterogeneous solution was stirred overnight. Approximately 0.5 g of Amberlite H^+ resin was added to stop the reaction. After stirring for a further 10 minutes, the resin was filtered off and washed 2 x with 5 mL of methanol. The solvent was evaporated to yield the desired product as a thick orange syrup (653 mg, 4.47 mmol, 91.3% yield) which was used without further purification.

A 60% dispersion of sodium hydride in mineral oil (984 mg, 24.6 mmol) was washed 2x with hexanes before being suspended in DMF (3 mL) and cooled to 0°C. In a separate flask, the glucal (600 mg, 4.10 mmol) was dissolved in DMF (2 mL) before being added to the sodium hydride suspension dropwise. This mixture was stirred for 10 minutes before the addition of benzyl chloride (3.11 g, 24.6 mmol). The reaction was warmed to room temperature and monitored by TLC.

When complete, the reaction was quenched by the slow addition of a saturated solution of NH_4Cl (10 mL). This solution was extracted with DCM (3x 10 mL) and the combined organic layers were dried over $MgSO_4$ and rotovapped to dryness to yield the benzylated galactal **44** (1.70 g, 4.10 mmol, 99% yield).

^{1}H NMR (400MHz, $CDCl_3$) δ: 7.33-7.24 (15H, m, Bn), 6.33 (1H, d, 7.2 Hz, H-1), 4.86-4.82 (2H, m, H-2, H-3), 4.63-4.36 (6H, m, Bn), 4.15-4.14 (2H, m, H-4, H-5), 3.75 (1H, dd, J = 13.6, 10.4 Hz, H-6'a), 3.62 (1H, dd, J = 13.6, 6.8 Hz, H-6'b)

^{13}C NMR (100MHz, $CDCl_3$) δ: 144.1 (C-1), 138.5 (Bn), 128.3 (Bn), 128.1 (Bn), 127.9 (Bn), 127.7 (Bn), 127.4 (Bn), 99.9 (C-2), 75.6 (C-3), 73.4 (C-4), 71.2 (C-5), 70.8 (Bn), 68.4 (C-6)

Chapter 2

Natural Product Isolation and Characterization of *Euphorbia lancifolia*

Only after the last tree has been cut down,
Only after the last river has been poisoned,
Only after the last fish has been caught,
Only then will you find that money cannot be eaten

(Native Cree Prophecy)

2.1 Introduction

2.1.1 Lactation

The production of milk, lactation, is primarily associated with the hormone prolactin. The secretion of prolactin is controlled by a number of factors, but the neurotransmitter dopamine is known to have a profound effect upon prolactin levels in the body. When dopamine binds to D_2-type dopamine receptors in milk-producing cells (lactotrophs) located in the pituitary gland, it inhibits the release of prolactin (**figure 2.1.1**) (41). Decreased levels of prolactin are associated with poor lactation success. During breastfeeding, low milk production is a common but complex problem and may be attributed to a number of psychological and biological factors including a disturbance in the levels of either prolactin or dopamine (41).

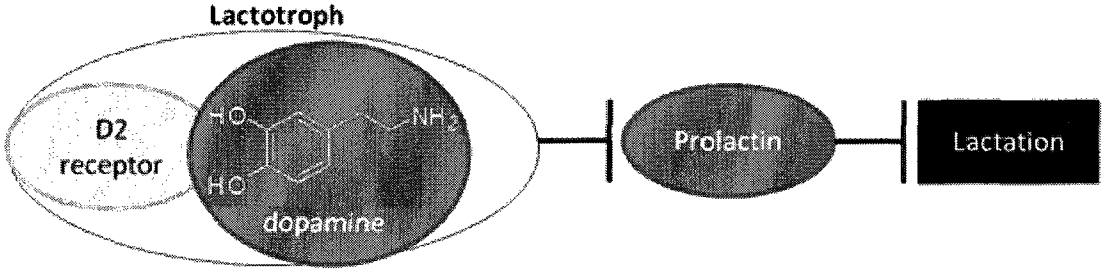


Figure 2.1.1 The effect of dopamine on lactation. When dopamine binds to D_2-type dopamine receptors in lactotroph cells in the pituitary, the secretion of prolactin is inhibited. This decrease in prolactin prevents lactation.

Historically, Western women who experienced difficulty producing enough breast-milk to feed their infants had little chance of improving their lactation output. Limited

lactation counselling was available, and after encountering difficulties, many women abandoned breastfeeding altogether in favour of commercially available formulas designed to meet the nutritional needs of their infants. However, studies revealing the importance of breast-milk in supporting the development of the immune system have lead to the current medical preference for breast-milk during early infancy (42). This change has also resulted in Western medicine's current standards for good lactation management: before and after giving birth, women are encouraged to breastfeed and are given advice by medical professionals, leading to an overall increased success rate in breastfeeding (43). Despite this improvement, some women are still unable to produce sufficient breast-milk to sustain their infants. Few pharmaceuticals exist to help improve lactation, and there are potentially dangerous risks associated with all available options (44).

2.1.2 Pharmaceutical galactogogues

Given the importance of dopamine in the regulation of prolactin, it is unsurprising that the three most commonly prescribed pharmaceuticals used to improve lactation are all dopamine antagonists. By interrupting the binding of dopamine to D_2 type receptors, the inhibitory effect of dopamine upon prolactin secretion may be avoided. Three of the most commonly prescribed synthetic galatagogues, all of which antagonize the binding of dopamine to D_2 receptors, are metoclopramide, sulpiride, and domperidone (**figure 2.1.2**) (44).

Metoclopramide is generally prescribed as an anti-emetic, to treat nausea, or as a prokinetic. In addition to behaving as a dopamine antagonist, it may act on seratonin receptors with agonist or antagonist effects depending upon their subtype. This complex activity is responsible for a number of side-effects, including dizziness and, rarely, extrapyramidal effects (inability to control bodily movements). Because of these side-effects, and because metoclopramide is known to cross the blood-brain barrier, its use in breastfeeding women is disfavoured relative to the other options (45).

Sulpiride is typically prescribed as an antipsychotic or an antidepressant. Although it is not likely to cause the same extrapyramidal side-effects as metoclopramide, sulpiride is transmitted via breast-milk to the suckling infant. It is commonly used in Africa as a galactogogue without apparent harm to infants, but its lack of demonstrated clinical safety retards its utility (46).

Domperidone, like metoclopramide, is used to treat emesis and nausea. Relative to the other two drugs described, domperidone is the least likely to cause side-effects, and its inability to cross the blood-brain barrier means that it is a safer choice for breastfeeding

women, although it is excreted in breast-milk. Although it is not approved by the FDA or other regulatory agencies for use as a galactogogue, it is commonly used "off-label" for this purpose (46).

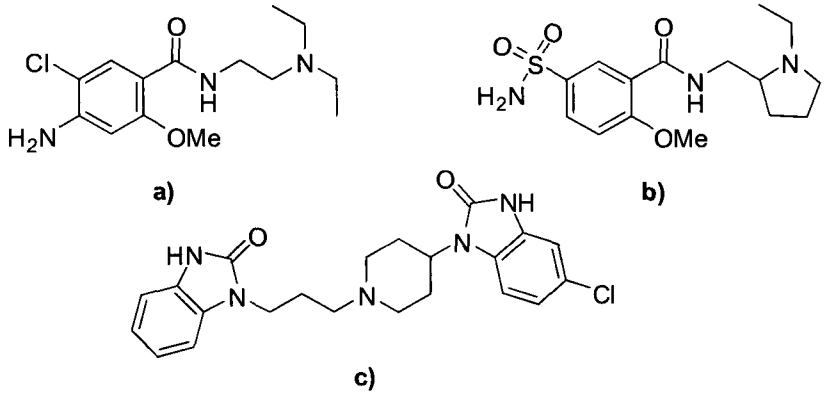


Figure 2.1.2 Pharmaceuticals which are used off-label to induce lactation.

a) Metoclopramide
b) Sulpiride
c) Domperidone

2.1.3 *Euphorbia lancifolia* (Ixbut)

Euphorbia lancifolia (Ixbut) is a medicinal plant in the Euphorbiaceae family native to Guatemala, Belize and Honduras. *E. lancifolia* is a perennial herb that is somewhat fleshy, has dark green leaves that span 5 to 9 centimetres long and grows as tall as 2 metres high. The plant is normally found growing in damp areas however it can also be found in pine forests or in open fields in south-eastern Mexico, Guatemala, Belize, El Salvador and Honduras (47). *E. lancifolia* is cultivated as a medicinal herb in villages and is used commonly as a galactogogue. Reports have also claimed that several villages in San Cristobal Verapaz, Guatemala prescribe the plant leaves as a medicine to cure sexual impotence (47).

The name ixbut originates from the early Mayan languages of the Pokom group where *ix* refers to women and *but* means "an increase in the flow or volume of water". It has been reported that for several centuries, postpartum Mayan women in Guatemala would take the plant leaves in an herbal tea to stimulate lactation and increase the flow of milk. The traditional Guatemalan preparation of *E. lancifolia* tea is to boil approximately 5 grams of the fresh leaves in 250 mL water, allow the liquid to cool, and add sugar as desired. The tea is then consumed three times a day. In other parts of Guatemala, instead of making an herbal tea, the fresh plant leaves are eaten raw, as in a salad (47).

Although few human studies of *E. lancifolia* exist, the plant has a rich history of folklore. Some reports claim that when the diet of cattle is supplemented with Ixbut, their milk output increases dramatically. Other tales state that women who have not given birth before, or aged grandmothers and great-grandmothers, are capable of lactating after drinking the herbal tea. One lofty story even claims that in the 1890s a doctor witnessed an elderly man who was able to nurse and ultimately sustain his great-grandchild after the infant's mother died in childbirth (47).

A series of controlled tests were conducted by Dr. Manuel Serrano in 1949 in collaboration with Merck & Co. In this study, women drank a cup of tea made from 5 leaves or 5 sections of the stem of *E. lancifolia* (approximately 5 grams of material) 6 times a day for 3 to 5 days. After drinking the herbal tea, 54 out of the 86 mothers showed an abundant increase in lactation, and some mothers who had been unable to nurse during a previous parturition were capable of effectively producing milk for several days. Chemical analysis of the milk produced before and after consumption of the herbal tea indicated that the quality of the milk did not have any significant changes (47). In the same year, Dr. Serrano conducted another study on *E. lancifolia* with 1800 women that were experiencing problems nursing their infants. The study found that 50% of the patients were able to produce milk only after consuming the plant, 35% of patients who were capable of nursing a little showed notable improvement after taking the plant, and only 15% of patients did not benefit from the plant (47).

Between 1949 and 1951, Dr. Efrén C. del Pozo conducted research on *E. lancifolia* in collaboration with Merck & Co. Several tests were carried out with nursing women in Mexico City and Guatemala City. The patients were given a dose of approximately 750 cc of an aqueous extract of *E. lancifolia* everyday for 25 days. Dr. Del Pozo concluded that the effects of *E. lancifolia* had been exaggerated; the more fantastic stories of non-post-partum women – or men – successfully nursing infants were most likely entirely untrue, and more research was necessary to determine whether a reproducible effect was present in nursing

women. Despite this conclusion, Merck & Co. has discontinued research on the galactogogue properties of *E. lancifolia* (47).

The utility of *E. lancifolia* in animals is also a matter of debate. For many years, *E. lancifolia* has been used in Guatemala as a supplement in cattle feed. Some dairy farmers claim that cows that are fed the plant as a supplement produce more milk compared to cows that are not. In 1911, a drug by the name of Galac-Latex was marketed as a galactogogue supplement for cattle feed in Guatemala where the main ingredient was *E. lancifolia*. The drug was well received but is now no longer available on the market (47). A 1927 study focused on the effect of this plant on dairy cows. Before the study one cow was only capable of producing on average 2 bottles (1.5 litres) of milk a day. After 11 days of receiving *E. lancifolia* as a dietary supplement, the cow's milk output had tripled to yield 6 bottles of milk a day (47). In 1949, the effects of *E. lancifolia* on goats in Guatemala City were studied. The experiment was conducted on 6 goats that were given a 5% aqueous infusion of the plant daily for 4 days. The results of the study showed no signs of toxicity and only a small increase in the production of milk (47).

In Cobán, Guatemala, there have been reports of cattle and horses that have died after consumption of the plant. Possible cause of death may be due to the inherent properties of the seeds, which are not utilized by the native population. It is probable that the species eaten by the animals may not have been *E. lancifolia*, but a different member of the Euphorbiciae family, many of which are toxic (47).

2.1.4 Current use of *Euphorbia lancifolia*

Presently, *E. lancifolia* is rarely used as a galactogogue in Guatemala City. This decline in interest is mainly due to the availability of many commercially prepared infant formulas, decreasing most women's dependence on breast-milk. In remote regions of Guatemala, where breast-milk is the major or sole source of food for infants, *E. lancifolia* is still used by some women. However, *E. lancifolia* is only taken when the nursing mothers are facing difficulties producing breast milk, rather than as a general way to supplement milk output (47).

E. lancifolia's history of medicinal use makes it of interest to some dairy farmers in Costa Rica. As there have not been any chemical or pharmacological studies performed on this plant, farmers are sceptical of using the plant in cattle feed. Before using *E. lancifolia* as a galactogogue, research is necessary to determine the compounds in the plant and any side effects that may be present when the plant is consumed by humans and cattle.

2.1.5 Other Natural Galactogogues

E. lancifolia is not the only plant with a history of use as a galactogogue. Many plants are traditionally used for this purpose, including *Trigonella foenum-graecum* (fenugreek), *Foeniculum vulgare* (fennel), and *Cnicus benedictus* (blessed thistle) (3). Interestingly, an African member of the *Euphorbiciae* family, *E. balsamifera*, also has a history of use as a galactogogue. However, many of these claims are based on anecdotal evidence and none have been sufficiently studied in a controlled clinical setting. This lack of evidence does not discredit the legitimacy of these claims, but indicates that scientific studies are required. Furthermore, as the active compound and mechanism of action of these plants is not understood, their safety for human consumption and a range of side effects or contraindications is still unknown (48).

The leaves and seeds of fenugreek are commonly used in Indian, Middle Eastern, and eastern European cooking. The seeds' strong, sweet taste is responsible for much of the flavour in cheaper maple syrup alternatives. Phytochemical investigations have detected a wide array of compounds, including lupeol, betulin, and betulinic acid. A mechanism of action for how fenugreek may benefit lactating women has not been proposed (49).

Like fenugreek, fennel is also culinary herb. It is known to contain a variety of compounds including xanthotoxin, α-amyrin, and β-sitosterol (50). Fennel also produces anethole (**figure 2.1.3**) which has demonstrated phytoestrogenic properties (51). This compound may contribute to the reported ability of fennel to improve lactation success.

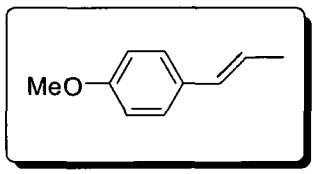


Figure 2.1.3 Anethole, a phytoestrogen present in fennel

Blessed thistle is a small, shrublike weed native to Europe. It produces a number of relatively common seconday metabolites including β-sitosterol, α-amyrin, and ursolic acid. Blessed thistle is also the source of the sesquiterpene cnicin, an uncommon and extremely bitter compound (52). Like fenugreek, no studies have investigated the cause of the proposed biological activity.

Euphorbia balsamifera is a tall African shrub. In Niger and Nigeria, it has a variety of uses including as a galactogogue in both cattle and women (53). A phytochemical investigation of this plant revealed a large number of triterpenes including lupeol, germanicol, and β-amyrin, among others (54).

2.2 Results and Discussion

2.2.1 Plant Extractions

There were two major goals in the *Euphorbia lancifolia* project:

1. To perform a general phytochemical investigation and identify the major constituents of this previously-unstudied plant, and
2. To examine the proposed biological activity of the plant and to identify the compound(s) responsible for this activity

Four batches of *E. lancifolia* leaves were collected in Heredia, Costa Rica, near the herbarium of the Universidad Nacional (**figure 2.2.1**) and stored in 1L Nalgene bottles. To preserve the leaves, they were stored in a mixture of water and ethanol. Prior to beginning isolation, the leaves of one bottle were filtered from the packing liquid and dried in the open air over the period of several days.

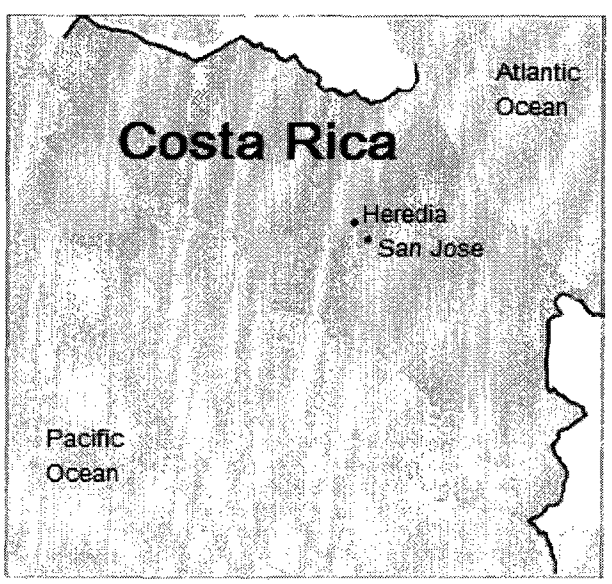


Figure 2.2.1 Map of Costa Rica with Heredia indicated. Modified from (55).

The packing liquid recovered from this manipulation was translucent and brown, and smelled strongly of sugar. This liquid was concentrated *in vacuo* to remove the ethanol and the aqueous remainder was extracted with EtOAc (3 x 25 mL). The combined organic layers were dried with magnesium sulfate ($MgSO_4$), filtered and concentrated *in vacuo*. ^1H NMR spectral data was taken, revealing a complex mixture of carbohydrates that were not analyzed further.

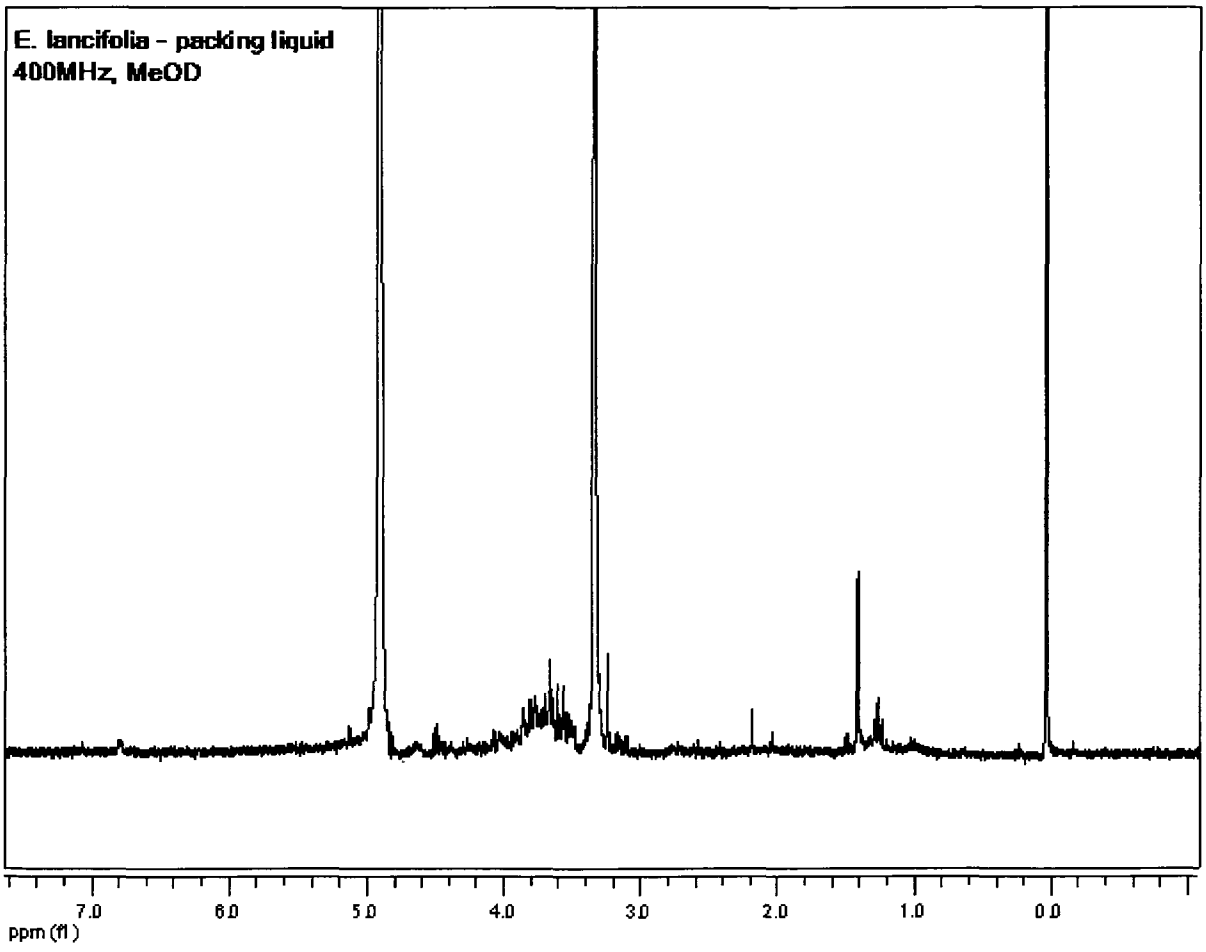


Figure 2.2.2 ^1H NMR spectrum of compounds extracted by packing liquid.

The aqueous extracts from this separation were transferred to a glass Petri dish and left out to dry for several days. This gave a crystalline solid that was brown and sticky with a strong, sweet odour. The mass of the sample was 3.71 g. ^{1}H NMR spectral data showed that the sample mainly consisted of carbohydrates. This mixture was further investigated by column chromatography.

The dried leaves recovered from the packing material were ground into a powder and extracted with hexanes, DCM, EtOAc, and finally MeOH (**table 2.2.1**). The major constituents of these fractions were determined after separation by column chromatography. Some compounds required further purification using either recrystalization or plate chromatography.

Table 2.2.1 Mass of material obtained during 1st extraction of *E. lancifolia*

Solvent	Mass of Material (g)
Hexane	1.11
DCM	0.5814
EtOAc	0.3858
MeOH	1.19

In total, four batches of leaves were investigated in this manner. Nine compounds were identified, including the novel compound β-amyryl ferulate (**49**). The compounds isolated were the pentacyclic triterpenes (**figure 2.2.3**) lupeol acetate (**46**), β-amyrin (**48**), germanicol pentanoate (**47**), β-amyryl ferulate (**51**), α-amyryl ferulate (**52**), β-amyryl p-hydroxycinnamate (**49**), and α-amyryl p-hydroxycinnamate (**50**), and a number of other plant metabolites (**figure 2.2.4**) including β-sitosterol (**54**), ethyl linolenate (**53**), phytol (**55**), and an unidentified porphyrin-type compound (**56**). Traces of plasticizer were also found to be

present in the extracts by ^{1}H NMR. The plasticizer is believed to have come from the plastic bags that were used to collect the plant samples.

The yield of these compounds based upon the dry weight of the leaves is summarized in **table 2.2.2**. The structural elucidation of these compounds was accomplished primarily using NMR and by comparison with data reported in the literature.

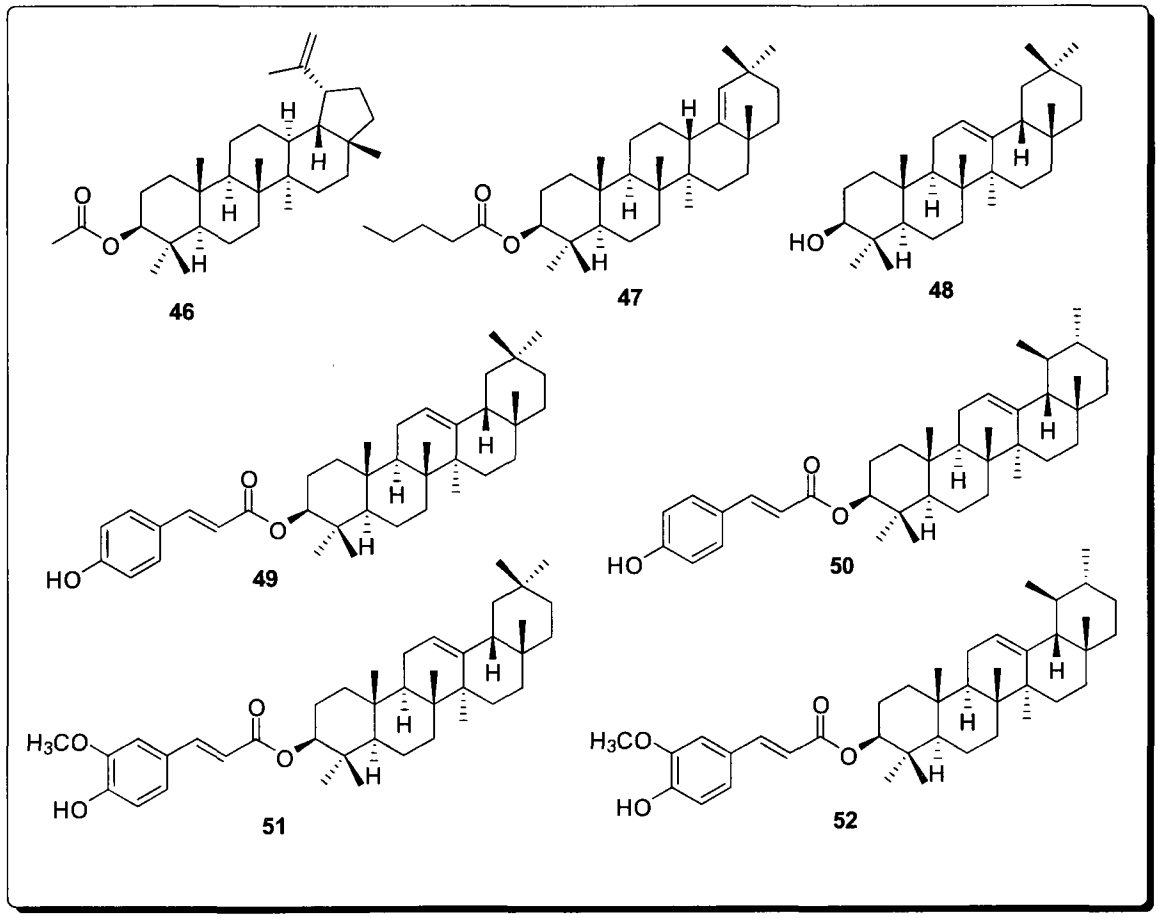


Figure 2.2.3 Triterpenes and triterpene derivatives isolated from *E. lancifolia*: lupeol acetate **(46)**; *β*-amyrin **(48)**; germanicol pentanoate **(47)**; *β*-amyryl p-hydroxycinnamate **(49)**; α-amyryl p-hydroxycinnamate **(50)**; *β*-amyryl ferulate **(51)**; α-amyryl ferulate **(52)**

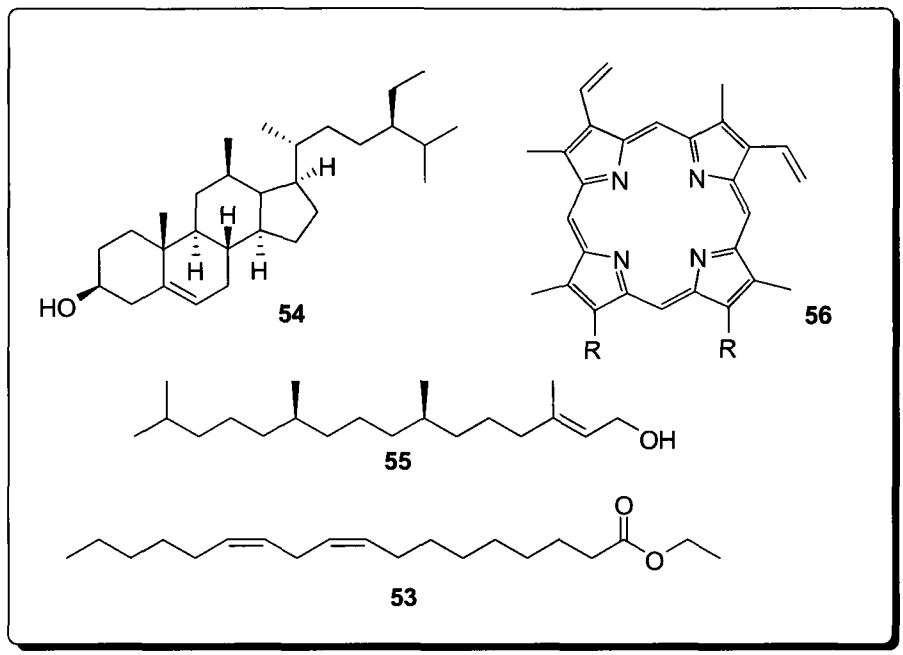


Figure 2.2.4 Other metabolites isolated from *E. lancifolia*: *β*-sitosterol **(54)**; phytol (**55**); ethyl linolenate (**53**); porphyrin derivative (**56**)

Table 2.2.2 Yield of compounds isolated from *E. lancifolia* based upon dry mass

Compound	Mass (mg)	% of dry mass of plant
β-sitosterol	2.8	$5.9x10^{-5}$
phytol	92.4	$1.23x10^{-3}$
ethyl linolenate	136.7	$1.825x10^{-3}$
germanicol pentanoate	98.1	$1.31x10^{-3}$
lupeol OAc	63.4	$8.46 x10^{-4}$
β-amyrin	25.1	$3.35 x10^{-4}$
α/*β*-amyryl ferulate	13.8	$1.84 x10^{-4}$
α/*β*-amyryl p-hydroxycinnamate	8.8	$2.7x10^{-4}$

2.2.2 Compounds Isolated from *Euphorbia lancifolia*

Pentacyclic triterpenoids

Triterpenes are an extremely common class of natural products derived from a 30-carbon precursor, generally squalene, which is itself composed of six 5-carbon isoprene units (56). There are eight major classes of triterpenoids: dammarane, euphane, hopane, isoeuphane, lanostane, lupane, oleanane, and ursane (57). The compounds isolated belong to the ursane, oleanane, and lupane triterpene families.

All of the triterpenes isolated from *E. lancifolia* are related biosynthetically (**figure 2.2.5**) (58). The biosynthesis of all triterpenes begins with squalene (**a**), which itself is assembled from two units of farnesyl pyrophosphate assembled head to tail. Squalene is selectively epoxidized into squalene oxide (**b**). When positioned correctly by enzymes, the cascade cyclization of **b** into the dammarenyl cation **c** may occur. A Wagner-Meerwein 1,2-hydride shift produces the baccharenyl cation **d**, which in turn cyclises to give the lupenyl cation **e**. This intermediate may follow two paths. Simple deprotonation of one of the neighbouring methyl groups affords lupeol. A ring expansion may also occur to produce the oleanyl cation **f**. Like the previous cation, a number of possible paths are available. Two 1,2-hydride shifts, followed by loss of H-12, affords β-amyrin. Germanicol is formed by the loss of H-19. Finally, the taraxasteryl cation **g** is formed by a 1,2-methyl shift. A series of hydride shifts, followed by the loss of H-12, produces α-amyrin.

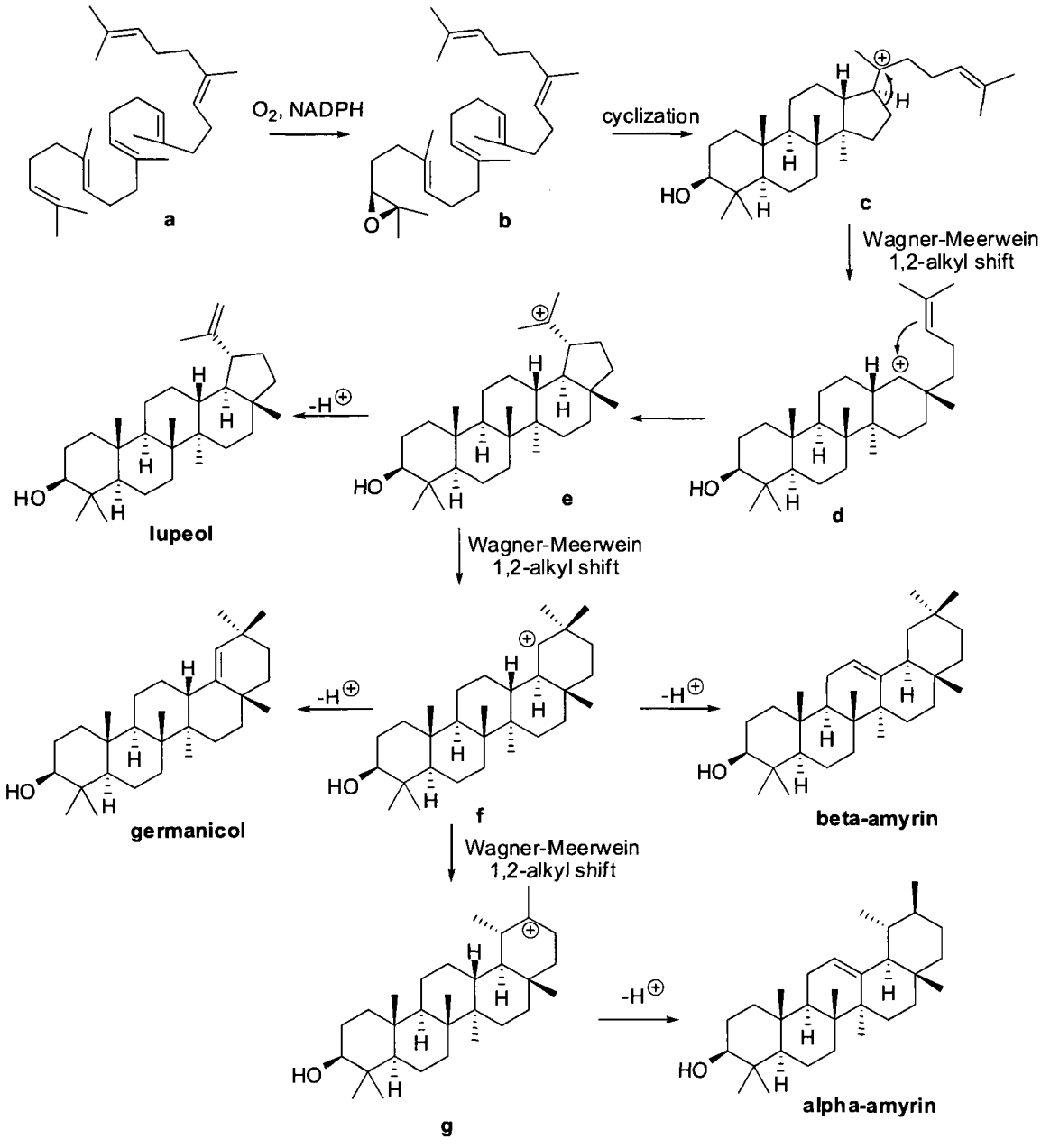


Figure 2.2.5 Biosynthetic pathway leading to lupeol, germanicol, and the amyrins (58).

Lupane triterpenes

Lupeol acetate (**46**) was isolated from the hexane extracts of *E. lancifolia* as a white solid after repeated purifications by flash chromatography and recrystalization from THF. The structural identification was made based upon key resonances in the ^{1}H and ^{13}C NMR spectra. The most distinctive signals were those of the C-29 olefinic protons [δ: 4.66 (1H, d, J = 2.4 Hz,); 4.54 (1H, dd, J = 2.4, 1.6 Hz)], the proton adjacent to the acetyl group at C-3 [δ: 4.46-4.42 (1H, m)], and the olefinic methyl group [δ: 1.66 (3H, d, J = 1.6 Hz)]. In addition, 6 methyl groups were visible in the upfield region of the spectrum [δ: 1.00 (s, 3H), 0.91 (s, 3H), 0.83 (s, 3H), 0.82 (s, 3H), 0.81 (s, 3H), 0.76 (s, 3H)]. The olefinic protons displayed a coupling constant (J = 2.4 Hz) consistent with a geminal relationship. In addition, one of the protons possessed long-range coupling to the C-30 methyl group as evidenced by their shared coupling constant (J = 1.6 Hz).

This natural product was isolated as its acetate ester. This assignment was based on the downfield shift of H-3 in the compound isolated [δ: 4.46-4.42 (1H, m)] relative to lupeol [δ: 3.19 (1H, br.m)] (59), as well as the presence of a methyl group [δ: 2.01 (3H, s)] consistent with an acetyl methyl. A single carbonyl carbon was also identified in the ^{13}C spectrum [δ: 171.3].

Table 2.2.3 compares the NMR data of the lupeol acetate isolated in this study with literature values.

Table 2.2.3 Comparison of ^{1}H and ^{13}C data observed for lupeol acetate with published data (60)

C	observed δ_H	literature δ_H	observed δ_C	literature δ_C
1			38.3	38.6
2			21.3	21.7
3	4.46-4.42 (m)	4.47	80.9	81.2
4			37.9	38.0
5			55.2	55.6
6			18.1	18.4
7			34.1	34.4
8			40.7	41.0
9			50.2	50.5
10			37.0	37.3
11			20.8	21.1
12			23.6	24.0
13			35.4	36.2
14			42.7	43.0
15			25.0	25.3
16			37.7	35.8
17			42.9	43.2
18			48.2	48.5
19			47.9	48.2
20			150.8	151.2
21			29.7	30.0
22			39.9	40.2
23	0.83 (s)	0.85	27.3	27.6
24	0.82 (s)	0.84	16.4	16.7
25	1.00 (s)	1.03	16.2	16.4
26	0.81 (s)	0.83	15.8	16.2
27	0.76 (s)	0.79	14.4	14.7
28	0.91 (s)	0.94	17.9	18.2
29	4.66 (d, J = 2.4 Hz); 4.54 (dd, J = 2.4, 1.6 Hz)	4.69 (s); 4.57 (s)	109.2	109.6
30	1.66 (d, J = 1.6 Hz)	1.69	19.2	19.5
1'			171.0	171.3
2'	2.01 (s)	2.05	27.8	28.2

Ursane and Oleanane Triterpenoids

The oleanane triterpene germanicol-3-pentanoate (**47**) was isolated as a white solid weighing 98.1 mg from the hexane extract of the plant. This compound was identified based upon a literature search, which revealed that germanicol 3-pentanoate has only been isolated from one other plant (61). Several key features of this compound's NMR spectrum were used to make this assignment. The identity of the triterpene as germanicol was initially made based on the singlet olefin proton on C-19 [δ: 4.84 (s)]. In an oleanane triterpene, the only position in the molecule where a double bond will result in one singlet rather than a doublet or triplet in the ^{1}H spectrum (**figure 2.2.6**) is the C-18 to C-19 bond (as in germanicol). This assignment is confirmed by the agreement of both the ^{1}H and ^{13}C NMR spectra with literature values (**table 2.2.4**). Rather than simple germanicol, the compound isolated was the pentyl ester of the simple triterpene. The presence of this unusual metabolite was deduced by key signals in the NMR spectra. The proton at C-3 was shifted downfield due to the adjacent oxygen's participation in an ester functionality rather than a simple hydroxyl group [^{1}H δ: 4.49-4.45 (m) and ^{13}C δ: 80.6 for the compound isolated; compare to ^{1}H δ: 3.20 and ^{13}C δ: 72.0 for germanicol] (62). Other distinctive signals in the ^{1}H spectrum include the methylene protons adjacent to the carbonyl [δ: 2.26 (d, J = 7.2 Hz)], and the methyl group at the end of the pentyl chain C-5' [δ: 0.93 (t, J = 7.6 Hz)]. The ^{13}C NMR spectrum confirmed the presence of 35 carbons. This structure is consistent with literature data (**table 2.2.4**).

Table 2.2.4 Comparison of germanicol pentanoate's ^{1}H and ^{13}C NMR data with published values (61)

C	observed δ_H	literature δ_H	observed δ_C	literature δ_C
1			38.6	38.47
2			23.8	23.61
3	4.49-4.45 (m)	4.45 (m)	80.6	80.47
4			37.9	37.74
5			55.6	55.44
6			18.2	18.03
7			34.5	34.22
8			40.8	40.63
9			51.1	50.99
10			36.8	37.02
11			21.1	21.00
12			26.2	26.07
13			38.4	38.27
14			43.3	43.20
15			27.5	27.40
16			37.7	37.58
17			34.4	34.40
18			142.7	142.56
19	4.84 (s)	2.26 [*sic*], 4.82	129.8	129.64
20			32.4	32.24
21			33.3	33.21
22			37.2	37.23
23	0.82	0.81	27.9	27.81
24	1.05	1.04	16.1	15.96
25	0.82	0.81	16.6	16.49
26	0.88	0.87	16.8	16.65
27	0.71	0.75	14.6	14.47
28	0.99	0.98	25.3	25.13
29	0.92	0.90	21.1	21.22
30	0.92	0.91	29.2	29.07

Table 2.2.4 Continued..

C	observed δ_H	literature δ_H	observed δ_C	literature δ_C
1'			173.5	173.60
2'	2.26 (d, J = 7.2 Hz)	2.26 (t, J = 7.5 Hz)	37.4	34.70
3'			25.3	24.73
4'			21.1	22.22
5'	0.93 (t, J = 7.6 Hz)	0.87 (t)	13.8	13.42

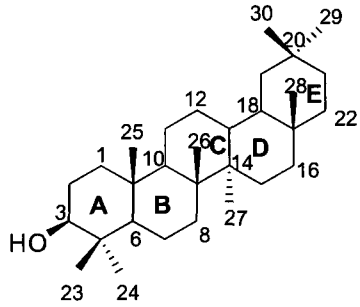


Figure 2.2.6 Numbered skeleton of a pentacyclic triterpene

Another oleanane triterpene, *β*-amyrin (**48**) was isolated from the hexane extract following plate chromatography. The structure of *β*-amyrin is similar to germanicol, described previously. The only difference is that the double bond is located between C-12 and C-13 rather than C-18 and C-19 (**figure 2.2.6**). The C-12 olefin proton of *β*-amyrin is an apparent triplet due to coupling with the protons of C-11 [δ: 5.16 (t, J = 3.6 Hz)]. Interestingly, *β*-amyrin was isolated separately from its structural isomer α-amyrin even though an ester of α-amyrin was also detected in this plant. No free α-amyrin was detected. The chemical shift of the C-3 proton adjacent to the hydroxyl group is also indicative of the fact that the free triterpene rather than the ester was isolated [^{1}H δ: 3.22-3.18 (m) and ^{13}C δ: 79.0 for the compound isolated; compare to ^{1}H δ: 4.69-4.60 (m) and ^{13}C δ: 83.0 for *β*-

amyryl p-hydroxycinnamate]. The spectroscopic properties of the compound isolated were identical with literature values (**table 2.2.5**).

Table 2.2.5 Comparison of β-amyrin's ^{1}H and ^{13}C NMR data with published values (63)

C	observed δ_H	literature δ_H	observed δ_C	literature δ_C
1			38.6	38.6
2			27.2	27.2
3	3.22-3.18 (m)	3.22 (dd, J = 10.9, 4.0 Hz)	79.0	79.0
4			38.8	38.8
5			55.2	55.1
6			18.4	18.4
7			32.5	32.6
8			39.8	39.8
9			47.6	47.6
10			37.0	36.9
11			23.5	23.5
12	5.16 (t, J = 3.6 Hz)	5.18 (t, J = 3.5 Hz)	121.7	121.7
13			145.2	145.2
14			41.7	41.7
15			26.2	26.1
16			26.9	26.9
17			32.6	32.5
18			47.2	47.2
19			47.2	47.2
20			31.1	31.1
21			34.7	34.7
22			37.2	37.1
23	1.00 (s)	1.00 (s)	28.1	28.1
24	0.79 (s)	(*sic*)	15.6	15.6
25	0.94 (s)	0.94 (s)	15.5	15.5
26	0.97 (s)	0.97 (s)	16.8	16.8
27	1.11 (s)	1.14 (s)	26.0	26.0
28	0.83 (s)	0.83 (s)	28.4	28.4
29	0.87 (s)	0.87 (s)	33.3	33.3
30	0.97 (s)	0.87 (s)	23.7	23.7

An inseperable isomeric mixture of α- and β-amyryl p-hydroxycinnamate (**49, 50**) was identified after repeated flash chromatography experiments followed by purification by plate chromatography. Unlike most of the other compounds described, the amyryl p-hydroxycinnamates were strongly UV active, which made them readily visible by TLC. Unfortunately, their isolation proved to be delicate due to their low abundance.

The identification of this mixture was aided by the aromatic portion of this molecule, which again marked it as unique relative to most of the other compounds isolated. The aromatic region of the ^{1}H NMR indicated the presence of an aromatic ring with a 1,4-substitution pattern and thus a plane of symmetry [^{1}H δ: 7.43 (2H, d, J = 8.4 Hz) and 6.83 (2H, d, J = 8.8 Hz)]. The large difference in the chemical shift of these protons also suggested that one of the substituents of the aromatic ring was electron-donating resulting in an upfield shift of two of the protons to 6.83 ppm) while the other was electron-withdrawing resulting in a downfield shift to 7.43 ppm. Additionally, the presence of a *trans*-double bond [^{1}H δ: 6.30 (d, J = 16.0 Hz) and 7.60 (d, J = 15.6 Hz)] makes the high-field region of the spectrum consistent with a p-hydroxycinnamate ester, possessing a hydroxyl group at the 4-position of the ring and an olefin conjugated to an ester.

The triterpene portion of this molecule was recognized as a 1.5:1 mixture of β- and α-amyrin primarily based upon the integration of the pairs of triplets at 5.19 and 5.13 ppm respectively which in either molecule corresponds to the H-12 olefin proton.

The ^{1}H and ^{13}C NMR data is identical to literature values for these compounds (table **2.2.6**).

Table 2.2.6 Comparison of β-amyryl p-hydroxycinnamate's ^{1}H and ^{13}C NMR data with published values (64).

C	observed δ_H	literature δ_H
1		
2		
3	4.69-4.60 (m)	4.64 (dd, J = 8.3, 7.8 Hz)
4		
5		
6		
7		
8		
9		
10		
11		
12	5.19 (dd, J = 3.6, 3.6 Hz)	5.19 (dd, J = 3.6, 3.6 Hz)
13		
14		
15		
16		
17		
18		
19		
20		
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		

Table 2.2.6 Continued...

C	observed δ_H	literature δ_H
1'		
2'	6.30 (d, J = 16.0 Hz)	6.30 (d, J = 16.0 Hz)
3'	7.60 (d, J = 15.6 Hz)	7.61 (d, J = 16.0 Hz)
4'		
5'	7.43 (d, J = 8.4 Hz)	7.43 (d, J = 8.8 Hz)
6'	6.83 (d, J = 8.8 Hz)	6.85 (d, J = 8.8 Hz)
7'		
8'	7.43 (d, J = 8.4 Hz)	7.43 (d, J = 8.8 Hz)
9'	6.83 (d, J = 8.8 Hz)	6.85 (d, J = 8.8 Hz)
OH	-	5.68

The final triterpene isolated from *E. lancifolia* was an inseperable mixture of α- and β-amyryl ferulate (**51, 52**). The small amount of this material present in the plant made its isolation a difficult process. The initial identification of this metabolite was made after several purifications of the hexane extracts of *E. lancifolia* followed by plate chromatography.

The novel compounds α- and β-amyryl ferulate were identified based upon key resonances in the aromatic region of the ^{1}H NMR spectrum. As previously described, the amyryl moiety of the molecule was identified primarily by the characteristic triplet of the alkene proton [^{1}H δ: 5.17 (dd, J = 3.6, 3.6 Hz)] and the multiplet of the C-3 proton adjacent to the hydroxyl group [^{1}H δ: 4.67-4.55 (m)].

The aromatic region of the spectrum revealed a phenyl ring containing three substituents, arranged in a 1, 3, 4-pattern as evidenced by the coupling of the three aromatic protons. The C-9' proton [^{1}H δ: 7.07 (dd, J = 8.0, 1.6 Hz)] has a meta relationship to the proton at C-5' [^{1}H δ: 7.04 (d, J = 1.6 Hz)] and an ortho relationship to the proton at C-8' [^{1}H

δ: 6.91 (d, J = 8.0 Hz)]. The chemical shifts of these protons also reveal that there is an electron-withdrawing group adjacent to C-9' and C-5'; this is consistent with a conjugated double bond, as in ferulic acid. The other two substituents appear to be electron donating; the sharp peak at 3.91 ppm is consistent with a methoxy group, and the broad singlet at 5.83 is characteristic of a phenol-OH proton.

Because amyryl ferulate is a novel compound, it is not possible to directly compare its NMR data with that from the literature. However, both the triterpene and ferulate portions of the molecule are consistent with similar compounds (**table 2.2.7, 2.2.8**).

Table 2.2.7 Comparison of amyryl portion of amyryl ferulate's ^{1}H and ^{13}C NMR data with that of amyryl p-hydroxycinnamate (64).

C	observed δ_H	literature δ_H	observed δ_C	literature δ_C
1			38.3	38.3
2			23.7	23.7
3	4.67-4.55 (m)	4.64 (dd, J = 8.3, 7.8 Hz)	80.8	81.0
4			38.0	38.0
5			55.3	55.3
6			18.3	18.3
7			32.7	32.6
8			41.7	41.7
9			47.6	47.6
10			36.9	36.9
11			23.7	23.7
12	5.17 (dd, J = 3.6, 3.6 Hz)	5.19 (dd, J = 3.6, 3.6 Hz)	121.7	121.7
13			145.2	145.2
14			39.9	39.8
15			26.9	26.2
16			26.2	26.9
17			32.5	32.5
18			47.2	47.3
19			46.8	46.8
20			31.1	31.1
21			34.7	34.8
22			37.3	37.2
23	0.97	0.99	28.0	28.1
24	0.86	0.88	16.2	16.8
25	0.92	0.94	15.6	15.6
26	0.97	0.98	16.8	16.9
27	1.12	1.14	26.0	26.0
28	0.82	0.83	28.1	28.4
29	0.90	0.90	33.3	33.4
30	0.84	0.86	23.6	23.6

Table 2.2.8 Comparison of ferulate portion of amyryl ferulate's ^{1}H and ^{13}C NMR data with that of 3α-*E*-feruloyltaraxerol (65)

C	observed δ_H	literature δ_H	observed δ_C	literature δ_C
1'			167.1	167.0
2'	6.29 (d, J = 16.0 Hz)	6.32 (d, J = 16.0 Hz)	116.2	116.8
3'	7.59 (d, J = 16.0 Hz)	7.59 (d, J = 16.0 Hz)	144.4	144.4
4'			127.2	127.1
5'	7.04 (d, J = 1.6 Hz)	7.06 (d, J = 1.5 Hz)	109.2	109.1
6'			146.7	146.7
7'			147.8	147.8
8'	6.91 (d, J = 8.0 Hz)	6.91 (d, J = 8 Hz)	114.7	114.6
9'	7.07 (dd, J = 8.0, 1.6 Hz)	7.08 (d, J = 8, 1.5 Hz)	123.1	123.2
10'	3.91 (s)	3.95	56.0	56.0
OH	5.83			

Other Metabolites

The phytosterol β-sitosterol **(54)** was the only compound identified from the EtOAc extract of *E. lancifolia*. An impure sample with a total mass of 2.8 mg was obtained (**table 2.2.9**).

The diterpene phytol **55** was isolated from the hexane and the DCM extracts of *E. lancifolia*. Phytol was identified by several unique peaks in the ^{1}H spectrum. The single olefin proton in this molecule appeared as a triplet of doublets due to its coupling to the adjacent methylene group and to H-4 via allylic coupling [^{1}H δ: 5.40 (td, J = 6.8, 1.2 Hz)]. The H-1 methylene protons appeared as a downfield signal due to the desheilding effects of the adjacent hydroxyl and olefin moieties [^{1}H δ: 4.14 (d, J = 6.8 Hz)]. The NMR data for this compound was consistent with literature values (**table 2.2.10**).

Table 2.2.9 Comparison of β-sitosterol's ^{1}H and NMR data with published values (66).

C	observed δ_H	literature δ_H
1		
2		
3	3.54-3.47 (m)	3.56 (m)
4		
5		
6	5.33 (dd, J = 3.2, 2.0 Hz)	5.39 (m)
7		
8		
9		
10		
11		
12		
13		
14		
15		
16		
17		
18	0.67 (s)	0.72 (s)
19	1.00 (s)	1.05 (s)
20		
21	0.91 (d, J = 6.4 Hz)	0.96 (d, J = 6.5 Hz)
22		
23		
24		
25		
26	0.82 (d, J = 6.7 Hz)	0.87 (d, J = 6.7 Hz)
27	0.81 (d, J = 6.8 Hz)	0.85 (d, J = 6.7 Hz)
28		
29	0.84 (t, J = 7.9 Hz)	0.89 (t, J = 7.4 Hz)

Table 2.2.10 Comparison of ^{1}H and ^{13}C data observed for phytol **(53)** with published data (67), (68).

C	observed δ_H	literature δ_H	observed δ_C	literature δ_C
1	4.14 (d, J = 6.8 Hz)	4.13 (d, J = 6.9 Hz)	59.4	59.4
2	5.40 (td, J = 6.8, 1.2 Hz)	5.40 (t, J = 6.9 Hz)	123.0	123.1
3			140.3	140.3
4	1.98 (d, J = 7.6)	1.96 (t, J = 7.6)	39.4	39.4
5			25.1	25.1
6			36.7	36.7
7			32.4	32.7
8			37.3	37.4
9			24.4	24.5
10			37.4	37.4
11			32.8	32.8
12			37.3	37.3
13			24.8	24.8
14			39.9	39.7
15			28.0	28.0
16			22.6	22.6
17			22.7	22.7
18			19.8	19.7
19			19.7	19.7
20	1.66 (s)	1.63 (s)	16.2	16.2

Ethyl linolenate **(53)** was identified in both the hexane and DCM extracts of the plant. This ester was a waxy, yellowish solid. It is important to note that this compound may not be a true natural product, but instead an artefact of the sample's storage in ethanol during the shipping process.

An unidentified porphyrin derivative was also isolated from the DCM extracts of *E. lancifolia*. The porphyrin structure was suggested by comparison of the ^{1}H NMR of the

unknown compound with that of a known porphyrin derivative. The low chemical shift of the methyl groups on the exterior of the porphyrin ring provided the initial clue as to the family of compounds to which this material belongs.

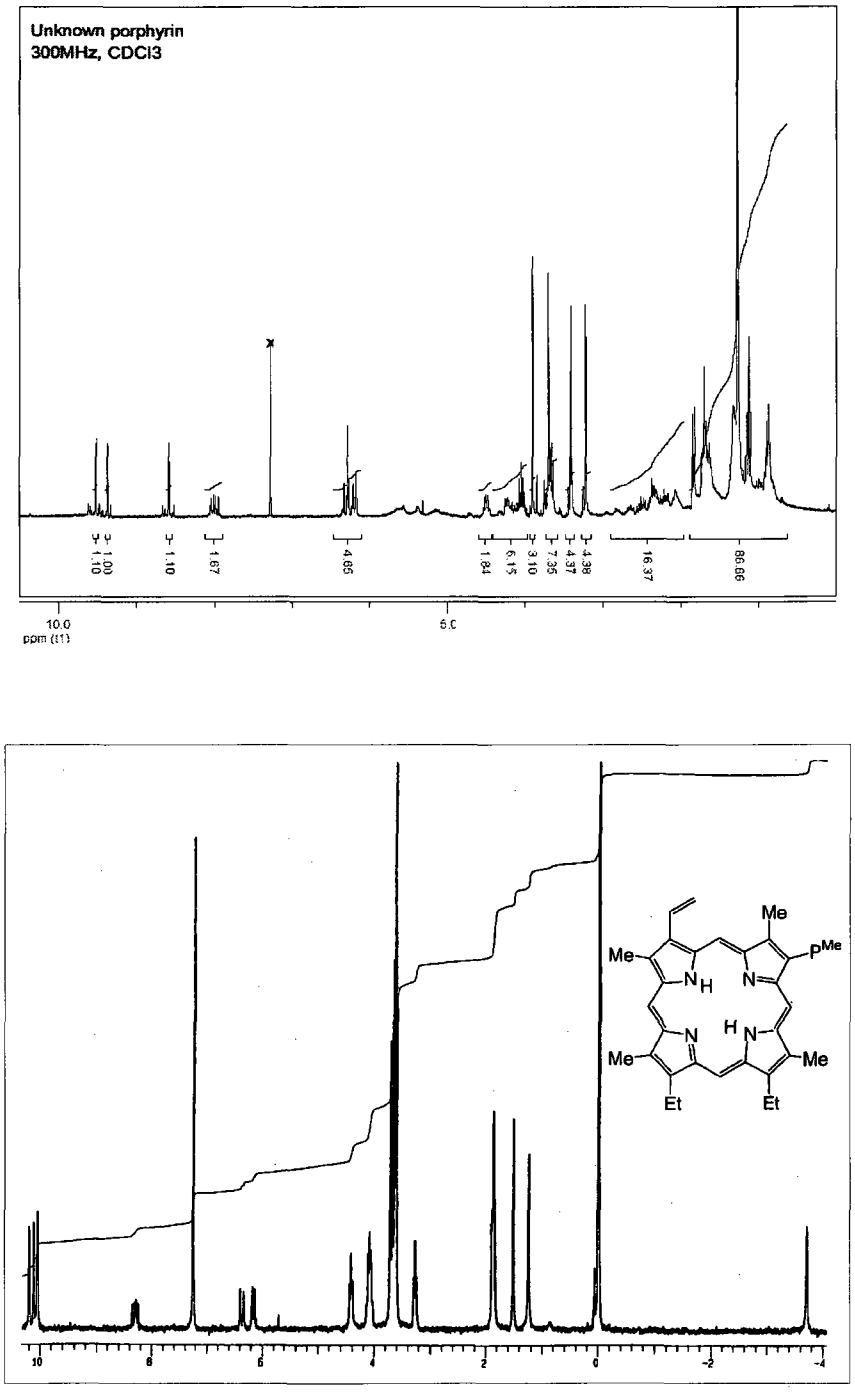


Figure 2.2.7 ^{1}H NMR spectrum of isolated porphyrin **56** (**a**) compared to ^{1}H NMR spectrum from the literature. (69) Both spectra were recorded in $CDCl_3$.

2.2.3 Literature Study of the Compounds Isolated from *Euphorbia lancifolia*

2.2.3.a Lupeol acetate

Lupeol acetate is a triterpene that has been previously isolated from many different *Euphorbia* species. The compound has also been isolated in the stem bark of *Alstonia scholaris* from the Apocynaceae family. *A. scholaris*, also called Indian devil tree, is a tropical evergreen tree native to India. Studies on the benzene extract of the stem bark of *A. scholaris* have found that the plant possesses anti-fertility activity. The main constituent in the benzene extract of the stem bark of *A. scholaris* was found to be lupeol acetate (70).

A 2005 study reported the effect of lupeol acetate on the reproductive system of male rats. The results of the study found that the compound caused a decrease in the weight of the reproductive organs (i.e. testes, epididymides, seminal vesicle and ventral prostate). Treatment with lupeol acetate resulted in reduction of sperm count and motility in male rats. This may have occurred as a result of a reduction in the testosterone output and the anti-androgenic effect of lupeol acetate; these effects are in line with reports of decreased fertility. Despite its reproductive effects, the study also found that lupeol acetate is non-toxic to the general body metabolism (70).

2.2.3.b Germanicol-3-pentanoate

In 2004, a study reported the isolation of germanicol-3-pentanoate from the methanol extract of *Sarcostemma clausum*, a laticiferous plant native to Florida. *S. clausum* has been used in Jamaica as a folk remedy for colds, and in Costa Rica and Guatemala as a larvicide against the botfly, *Dermatobia hominis*. Studies on the methanol extract of *S. clausum* have

found weak anti-microbial activity (71). Prior to this study, there have not been any other reports describing germanicol-3-pentanoate, although germanicol is a relatively common natural product.

Pentanoic acid in plants is generally derived from the α-oxidation of hexanoate, which is in turn derived from lipoxdiation (**figure 2.2.8**). Interestingly, pentanoic acid is a metabolite of linolenic acid, the ethyl ester of which was also detected in this plant (72).

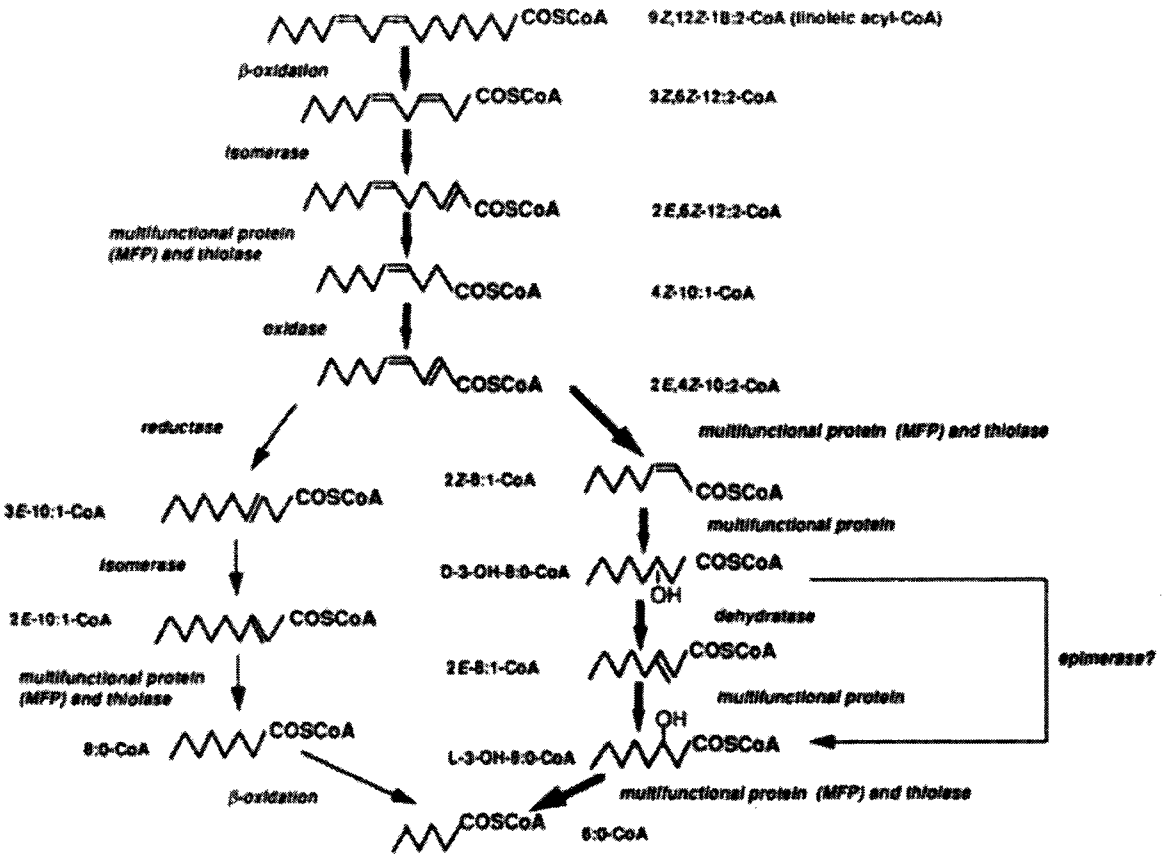


Figure 2.2.8 Oxidation of linoleic acid into pentanoic acid (modified from (72))

2.2.3.c β-Amyrin

Amyrins (**figure 2.2.9**) are naturally occurring pentacyclic triterpenes that exist in two isomeric forms, α and β, where the difference between these compounds is the position of one methyl group. Studies have shown that both α- and β-amyrin have many healing properties. In the Amazon and Northeast regions of Brazil, a resin collected from the trunk wood of the *Protium heptaphyllum* plant, also called almécega, is used in the treatment of wounds due to its anti-inflammatory and analgesic properties. The main constituent of this resin is an isomeric mixture of α- and β-amyrin. Previous studies have also found that α- and β-amyrins have anti-microbial, anti-depressant, anti-nociceptive, and gastroprotective activities (73).

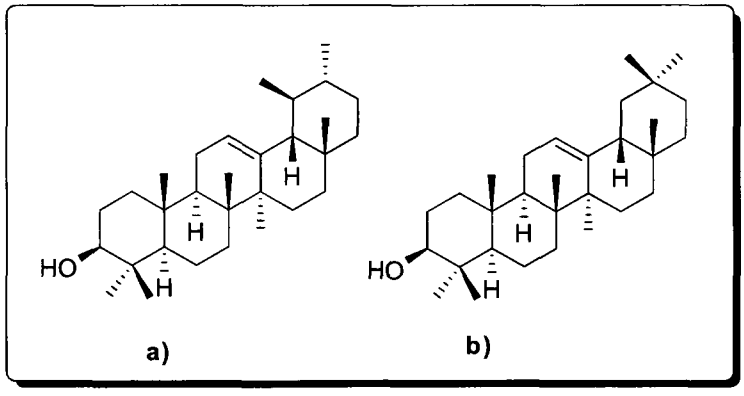


Figure 2.2.9 Comparison of the structures of **a)** α-amyrin and **b)** β-amyrin.

2.2.2.d α/β-amyryl p-hydroxycinnamate

There are only four previous reports in the literature describing the isolation of either α- or β-amyryl p-hydroxycinnamate. The coumaryl ester of β-amyrin was first isolated from *Acacia linarioides*, a shrub common to northern Australia, in 1997 (74). Although many members of the Acacia family are known to produce biologically active metabolites, no such claims have been made about *A. linariodes* specifically. However, the coumaryl ester of the amyrins has been found in many plants which do have a history of medicinal use.

β-amyryl p-hydroxycinnamate, along with a number of other triterpene-coumaryl esters, was isolated from *Casuarina equisetifolia*, a plant with demonstrated antioxidant properties, in 1999 (64). Similarly, α- and β-amyryl p-hydroxycinnamate were found in the EtOAc extract of *Anoectochilus formosanus* (75). The most recent study identified both isomers in the petroleum ether extract of a Taiwanese tree, *Barringtonia maunwongyathiae*. Topical application of an extract of this tree has been used to alleviate pain and inflammation caused by the resin of a more common tree, *Gluta usitata* (76). In these three studies, amyryl p-hydroxycinnamate was not responsible for any of the known or proposed biological properties of the plants it has been isolated from.

2.2.3e β-Amyryl ferulate

β-Amyryl ferulate consists of β-amyrin linked to ferulic acid. To date, the isolation of β-amyryl ferulate has not been reported in any literature.

Ferulic acid is a naturally occurring compound found in the leaves, seeds and cell walls of plants as a component of lignin. It has anti-oxidant and anti-radical activities and is often an ingredient in anti-aging supplements (77). As well, ferulic acid may protect against some cancers, bone degeneration, and menopausal symptoms (78). *In vitro* studies and animal testing have suggested that ferulic acid protects against breast and liver cancers by having direct anti-tumour activity or plays a role in apoptosis (79).

Sodium ferulate, a salt of ferulic acid, is used as an effective component in traditional Chinese medicine for the treatment of cardiovascular and cerebrovascular diseases. It is believed that sodium ferulate inhibits the production of inflammatory mediators, platelet aggregation and thrombus formation (80).

2.2.3.f β-Sitosterol

Phytosterols are analogous to cholesterol, which is only produced in animals. β-sitosterol is the most abundant phytosterol and it is present in a diverse array of plants. Consumption of phytosterols lowers cholesterol levels in humans by inhibiting the absorption of dietary cholesterol. This observation has lead to the use of β-sitosterol and other phytosterols as additives to foods which are naturally high in cholesterol, and may in part explain why some cultures which consume high-cholesterol diets in conjunction with relatively high levels of phytosterol-rich foods have high levels of cardiovascular health (81).

Although the mechanism is unknown, a number of studies suggest that β-sitosterol offers protection against breast, colon, and prostate cancer. One proposal is that β-sitosterol can be incorporated into the cell membrane, and that the resulting changes in the properties of the membrane inhibit the adhesion of cancerous cells onto the basement membrane, which lies at the surface between epithelial cells and connective tissue. This ultimately inhibits the growth of tumours (82).

2.2.3g Phytol

Phytol is a diterpene alcohol common in all plants usually found esterified to a chlorin ring forming chlorophyll. The compound is also used as a precursor for the synthesis of vitamin E and K_1. Studies have shown that phytol has various cellular and biological effects. Due to the hydrophobic nature of phytol, it may interact with cell membranes (83).

It has been known that isoprenoid compounds isolated from plants are useful immunologic adjuvants. Adjuvants are substances that modify the efficacy of vaccines by stimulating the immune system so that there is an increased response. A problem with these naturally occurring isoprenoids is that many of them are toxic. As a result, recent research has been focused on studying other isoprenoid compounds such as phytol and its derivatives to see if they possess any adjuvant activity (83).

A 2006 study found that phytol and phytanol (hydrogenated phytol) were better immunostimulants than other commonly used commercial isoprenoid adjuvants. These phytol-based adjuvants were found to have the ability to promote effective humoral and cell-

mediated immune responses at low doses. As well, phytol and phytanol were found to be non-toxic (83).

2.2.2g Ethyl linoleate

α-Linoleic acid is a long-chain fatty acid . In this study, the ester may be an artifact of the leaves' storage in ethanol.

Linoleic acid is very common in the plant kingdom, and is a common membrane component. Because it is so common, many studies have suggested its utility in an extremely wide variety of ailments including the proliferation of breast cancer (84), obesity reduction (85), cardiovascular health (86), and glucose tolerance (87).

2.2.2.h Porphyrin

Porphyrins are large aromatic molecules consisting of four pyrrole rings fused via methine bridges. These compounds are typically intensely coloured, and their extensive conjugation lends them interesting biological properties; porphyrin rings are present in and largely responsible for the biological activity of chlorophyll and heme (88).

The unidentified porphyrin identified in this study is likely derived from chlorophyll.

2.2.3 Biological Testing

To determine whether the crude extract or any of the purified compounds possessed biological activity similar to the pharmaceutical compounds previously described, two different *in vitro* biological assays were evaluated with the help of Andrew Waye, graduate student of J.T. Arnason and V. Trudeau at the University of Ottawa.

As explained previously, the three pharmaceuticals that are used most often to treat women who experience difficulty in producing breast-milk are metoclopramide, domperidone, and sulpiride. Because all three of these compounds are antagonists of the D_2 subtype of dopamine receptor, we were interested to know if any of the compounds isolated from *E. lancifolia* possessed the ability to bind to the dopamine receptor using a competitive binding assay developed in the lab of Dr. V. Trudeau. If they do bind, it is possible that they may be antagonists of the receptor. This may explain *E. lancifolia*'s reported activity as a galactogogue.

The first test contemplated is a competitive binding assay. In this assay, compounds with a strong affinity for D_2-type dopamine receptors displace tritium-labeled spiperone. The difference in radioactivity detected between control and sample wells indicates the relative number of receptors which are bound to a sample of interest rather than spiperone (22). Unfortunately, preliminary control experiments using this assay displayed unacceptably high variability and poor reproducability. For this reason, the competative binding assay was abandoned.

Instead, a second assay, assessing the compounds' ability to affect the activity of monoamine oxidase, was planned. Monoamine oxidase (MAO) is an enzyme which plays a critical role in the metabolism of a number of compounds including dopamine. In an *in vitro*

assay, the peroxide produced from the oxidation of dopamine to an aldehyde oxidizes the dye amplex red into the highly flourescent resorufin (**figure 2.2.10**). Resorufin may then be detected by spectrophotometric methods (89).

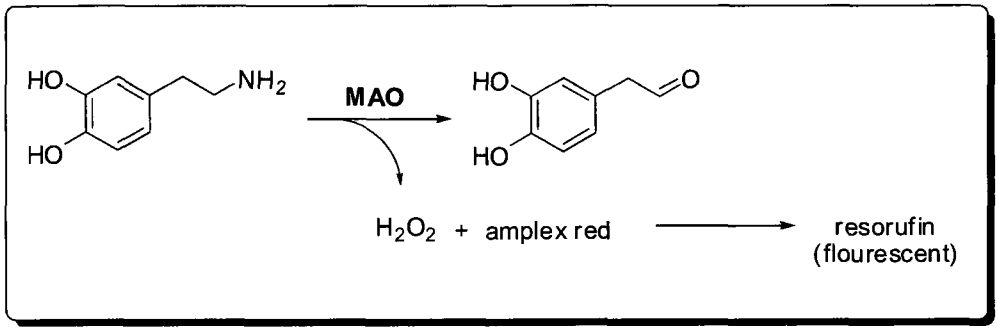


Figure 2.2.10 Metabolism of dopamine into dihydroxyphenaldehyde is achieved by the enzyme monoamine oxidase. This reaction produces peroxide, which oxidizes amplex red into the fluorescent resorufin.

Screening for galactogogues is not a well-established field. Most studies use feeding experiments on commercially relevant animals such as cows and goats, which are impractical on a small scale like the one used in this assay (44). Unfortunately, because lactation is an extremely complex biological event which can be significantly affected by various emotional and social factors, it can be very difficult to model in an *in vitro* assay. A demonstrated antagonism of the dopamine D_2 receptor would be only one possible mechanism through which *E. lancifolia* may improve lactation success.

2.2.4 Conclusion

It is unclear whether one of these compounds or another, unidentified constituent, is the active component responsible for the reported galactogogue effects. Further work to isolate all the main constituents present in the *E. lancifolia* leaves is necessary to come to a final conclusion. With the compounds that have been isolated, literature studies were conducted to summarize the effects of these compounds on humans and animals.

Interestingly, some of the compounds isolated from *E. lancifolia* are common to other plants used as galactogogues. Since there are no records in the literature of any studies investigating the proposed increase in lactation caused by these plants, it is not possible to know whether one or all of these compounds contribute to *E. lancifolia*'s utility as a galactogogue. It is also possible that a synergistic relationship exists between some of the triterpenes identified in this study.

Table 2.2.11 Comparison of triterpenes isolated from *E. lancifolia* and other plants used as galactogogues. The presence of a triterpene or its ester is indicated by a black box and its absence by a white box.

species	Metabolite				
	lupeol	germanicol	*β*-amyrin	α-amyrin	*β*-sitosterol
Euphorbia lancifolia	■	■	■	■	■
Fenugreek	■				
Fennel				■	■
Blessed thistle				■	■
Euphorbia balsamifera	■	■	■		

Fortunately, none of the compounds identified are known to be toxic or harmful within a biologically relevant dose range. Given *E. lancifolia*'s long history of use as a folk remedy, especially in a population as sensitive as nursing mothers, this result is unsurprising, but reassuring.

Investigating the medicinal plants found in remote regions of the world remains a fruitful area for research. Demonstrating the safety, and in many cases the utility, of plants with a history of medicinal use ensures that individuals who live without allopathic medicine for geographic or economic reasons retain confidence in the phytomedicines that may, at times, be the only source of health products available. Galactogogues are particularly important as breastmilk may be the only food source available to infants in remote communities. Although the research presented in this thesis does not fully demonstrate the safety or the mechanism of action of *E. lancifolia*, some phytochemical similarities with other suggested galactogogues and the absence of known toxins suggest that the consumption of *E. lancifolia* is harmless, if not truly beneficial.

2.4 Experimental

Flash chromatography was performed using SiliCycle SiliFlash ® F60 silica gel of 230-400 mesh and glass columns fitted with a cotton plug and a sand base or a fritted glass filter. Purifications were monitored by TLC (EMD Chemicals, TLC Silica gel 60 F_{254}), visualized by UV light (254 nm), then stained with Hannessian's stain. Glass-backed TLC plates (SiliCycle Glass Backed TLC Extra Hard Layer, 60Å, F-254) were used for the further purification of some UV-active compounds.

^{1}H and ^{13}C NMR spectra were acquired using a Bruker Avance 300, 400 or 500 MHz spectrometer. Samples were dissolved in deuterated chloroform, methanol, or acetone as indicated. Chemical shifts are reported in parts per million (ppm) and are referenced to the deuterated solvent used. ^{1}H integration is provided in parentheses and coupling constants are reported in Hz. ^{1}H splitting patterns are reported as singlets (s), broad singlets (br), doublets (d), triplets (t), quartets (q), multiplets (m), doublet of doublets (dd), or doublets of doublet of doublets (ddd). Mass spectral analyses were conducted with an Electrospray mass spectrometer. All spectral analyses were performed at facilities located at the University of Ottawa.

2.3.1 Extraction Procedure

The leaves of *Euphorbia lancifolia* were collected in the Tortuguero region of Costa Rica in April of 2008 and again in April 2009. The leaves were packed in Nalgene bottles in a mixture of 50% EtOH in H_2O, and were stored in this manner while they were shipped to Ottawa, Canada, and for a brief period in the lab. Two days before the extraction commenced, the leaves were removed from the packing liquid and dried in the fumehood. Once dried, the leaves were ground into a coarse powder.

For the first extraction, the leaf powder had a mass of 47.68 g. One half of this ground material was placed in an Erlenmeyer flask and soaked with hexanes (400 mL) overnight. The remaining half was reserved in a plastic container and put aside. By soaking the ground leaves with solvent, compounds in the material can be extracted out. The next day, the solvent was filtered off using vacuum filtration. The solvent extract was transferred into a round bottom flask where it was then concentrated *in vacuo*. The remaining material was weighed and transferred into a labelled vial. The filtered leaves were recovered and placed in an Erlenmeyer flask where they were then re-extracted with hexanes (400 mL) overnight. The following day, the solvent extract was filtered off, concentrated *in vacuo*, weighed and combined with the material obtained from the first extraction. The procedure was repeated using dichoromethane (DCM), EtOAc and methanol (MeOH) as the solvent (**table 2.3.1**), and a flow chart summarizing the leaf extraction procedure is presented in **figure 2.3.1**.

Table 2.3.1 Mass of material obtained during 1st extraction of *E. lancifolia*

Solvent	Mass of Material (g)
Hexane	1.11
DCM	0.5814
EtOAc	0.3858
MeOH	1.19

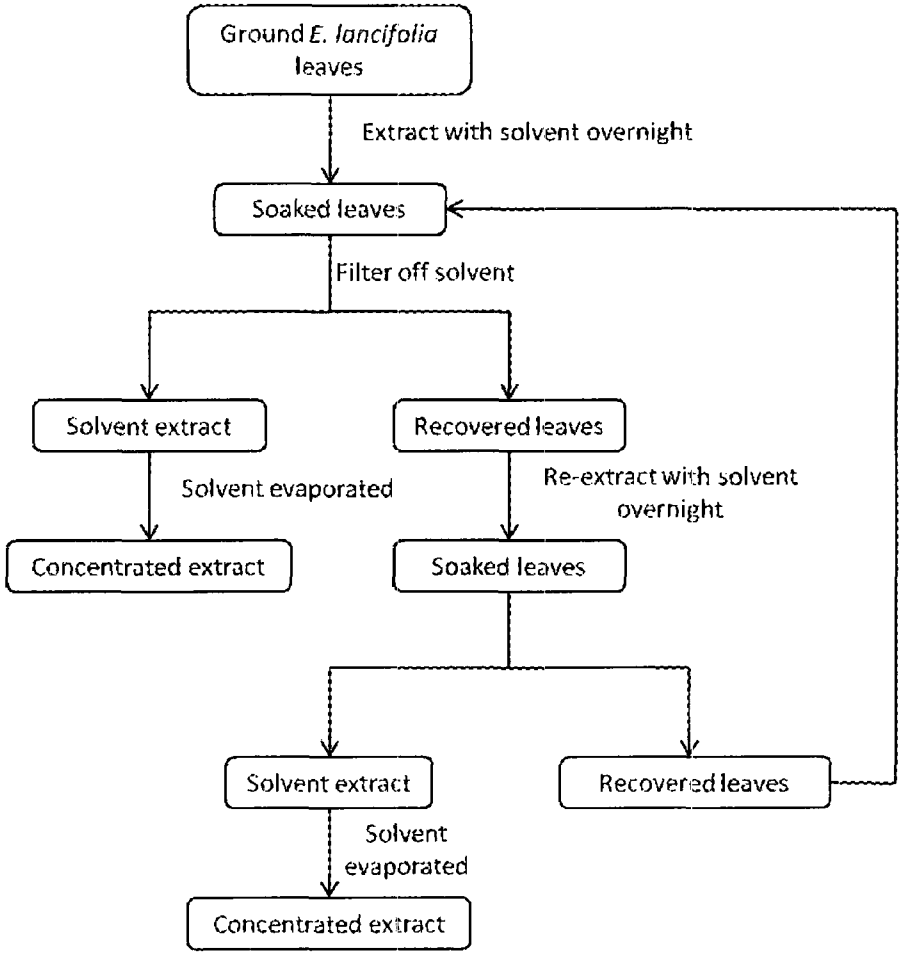


Figure 2.3.1 Flow chart of leaf extraction procedure

Throughout the course of the study, further extractions were necessary. In the third extraction, the ground leaves (74.89 g) were soaked in hexane (800 mL) overnight. The leaves were recovered by filtration, and the extract was evaporated to dryness (H1). The leaves were extracted with a second portion of hexane (800 mL) in the same manner to yield a second hexane extract (H2). This process was repeated to produce DCM (D1 and D2), and EtOAc (E1 and E2) extracts (**table 2.3.2**).

Table 2.3.2 Mass of material obtained during 3rd extraction of *E. lancifolia*

	Solvent		
	Hexane	DCM	EtOAc
Day 1 extracts (g)	2.7905	1.034	0.3155
Day 2 extracts (g)	0.7579	0.9543	0.3479
Total extracts (g)	3.5484	1.9883	0.6634

The contents of the packing liquid, in which the leaves were shipped, were also examined. The mixture was concentrated to approximately half to remove most of the EtOH before the sample was extracted with EtOAc (3x 20 mL). The organic extracts were dried over $MgSO_4$ and concentrated (PL-O). The remaining aqueous extracts were concentrated to dryness before being acetylated to facilitate their study (PL-A).

Before beginning column chromatography extractions on any of the extracts, thin layer chromatography (TLC) was performed. This preliminary investigation revealed their complexity and allowed the determination of an optimal solvent system required to separate them by column chromatography. The TLCs were viewed under ultraviolet (UV) light and

compounds that were UV active were noted. The TLCs were then stained with Hanessian's stain, which stains both UV active and non-UV active compounds a blue colour.

Hanessian's stain was made by dissolving ammonium molybdate (2.5 g) and cerium ammonium sulphate (1.0 g) in H_2O (90 mL) followed by the careful addition of concentrated sulphuric acid (10 mL).

Each sample was subjected to multiple purifications by column chromatography. The goal of the early columns was only to break each extract into more manageable fractions. Purified compounds were later isolated from these semi-pure fractions by column chromatography and recrystallization as necessary. Mixtures of UV-active compounds were further purified using plate chromatography. β-amyryl ferulate, β-amyryl p-hydroxycinnamate, phytol, and β-amyrin were all isolated in this manner.

Before running a column with the aqueous remainder from the packing liquid, the sample was acetylated. This was performed to aid in the separation and characterization of the sample. Converting the hydroxyl groups on the sugar moiety of the compounds to acetyl groups greatly decreases their polarity and prevents them from adhering too strongly to the stationary phase of the column.

Acetylation of the aqueous residue

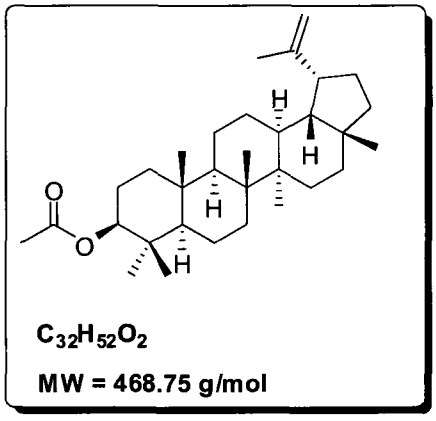
In a dry round bottom flask equipped with a magnetic stir bar, the aqueous residue was dissolved in pyridine (20 mL). Nitrogen was flushed through the flask and the reaction mixture was cooled to 0°C. Afterwards, acetic anhydride (10 mL) was added to the flask and the reaction mixture was left stirring for 48 h at room temperature. The reaction was then diluted with distilled water (40 mL) and left stirring at room temperature for 3 h. The reaction mixture was transferred into a separatory funnel and extracted with EtOAc, (3 x 30 mL). The combined organic extracts were further washed with sodium bicarbonate (30 mL) and brine (30 mL), dried with $MgSO_4$, filtered, and concentrated *in vacuo*.

Column chromatography was carried out on the acetylated organic residue using a hexane/EtOAc gradient. Even with acetylation, the compounds in the sample were not easily separable. Clear oils were obtained and ^1H NMR data indicated that the samples primarily consisted of sugars esterified to fatty acids of variable chain lengths. These compounds were not pursued further.

2.3.2 Spectroscopic Properties of the Compounds Identified

Lupeol Acetate (46)

Other names: Lupa-12,20(30)-dien-3-ol, acetate (7CI); 1H-Cyclopenta[a]chrysene, lupa-12,20(29)-dien-3-ol deriv.

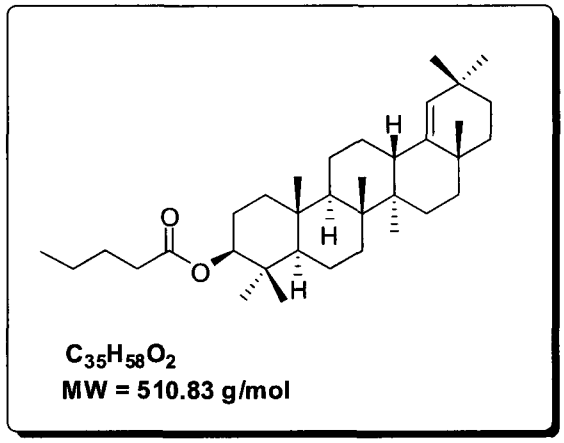


^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) 4.66 (1H, d, J = 2.4 Hz, H-29a), 4.54 (1H, dd, J = 2.4, 1.6 Hz, H-29b), 4.46-4.42 (1H, m, H-3), 2.01 (3H, s, H-2'), 1.66 (3H, d, J = 1.6 Hz, H-30), 1.00 (3H, s, H-25), 0.91 (3H, s, H-28), 0.83 (3H, s, H-23), 0.82 (3H, s, H-24), 0.81 (3H, s, H-26), 0.76 (3H, s, H-27)

^{13}C NMR (100MHz, $CDCl_3$): δ (ppm) 171.0 (C-1'), 150.8 (C-20), 109.2 (C-29), 80.9 (C-3), 55.2 (C-5), 50.2 (C-9), 48.2 (C-18), 47.9 (C-19), 42.9 (C-17), 42.7 (C-14), 40.7 (C-8), 39.9 (C-22), 38.3 (C-1), 37.9 (C-4), 37.7 (C-16), 37.0 (C-10), 35.4 (C-13), 34.1 (C-7), 29.7 (C-21), 27.8 (C-2'), 27.3 (C-23), 25.0 (C-15), 23.6 (C-12), 21.2 (C-2), 20.8 (C-11), 19.2 (C-30), 18.1 (C-6), 17.9 (C-28), 16.4 (C-24), 16.2 (C-25), 15.8 (C-26), 14.4 (C-27)

Germanicol Pentanoate (47)

Other names: Olean-18-en-3-ol, pentanoate, (3 β)- (9CI)

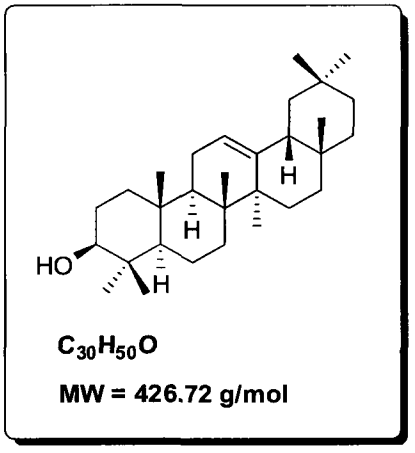


^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) 4.84 (1H, s, H-19), 4.49-4.45 (1H, m, H-3), 2.26 (2H, t, J = 7.2 Hz, H-2'), 1.05 (3H, s, H-24), 0.99 (3H, s, H-28), 0.93 (3H, t, J = 7.6 Hz, H-5'), 0.92 (3H, s, H-30), 0.92 (3H, s, H-29), 0.88 (3H, s, H-26), 0.82 (6H, s, H-23, H-25), 0.71 (3H, s, H-27)

^{13}C NMR (100MHz, $CDCl_3$): δ (ppm) 173.5 (C-1'), 142.7 (C-18), 129.8 (C-19), 80.6 (C-3), 55.6 (C-5), 51.1 (C-9), 43.3 (C-14), 40.8 (C-8), 38.6 (C-1), 38.4 (C-13), 37.9 (C-4), 37.7 (C-16), 37.4 (C-2'), 37.2 (C-22), 36.8 (C-10), 34.5 (C-7), 34.4 (C-17), 33.3 (C-21), 32.4 (C-20), 29.7 (C-3'), 29.2 (C-30), 27.9 (C-23), 27.5 (C-15), 26.2 (C-12), 25.3 (C-28), 23.8 (C-2), 21.1 (C-29), 21.1 (C-11), 18.2 (C-6), 16.8 (C-26), 16.6 (C-25), 16.1 (C-24), 14.6 (C-27), 13.8 (5')

β Amyrin (48)

Other names: Olean-12-en-3 β -ol (6CI,8CI); (+)-β -Amyrin; 3 β -Hydroxyolean-12-ene; NSC 527971; β -Amirin; β -Amyrenol; β -Amyrin; β -Amyrine

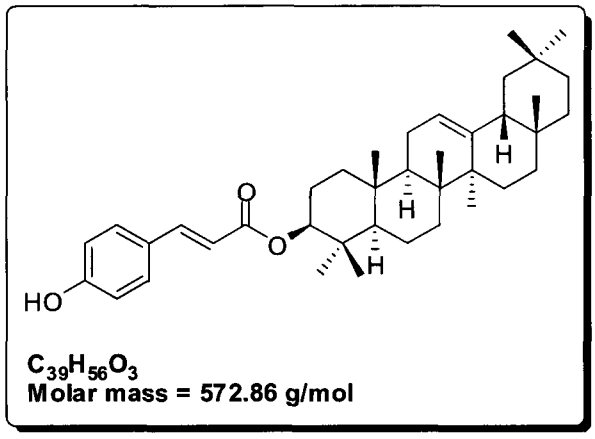


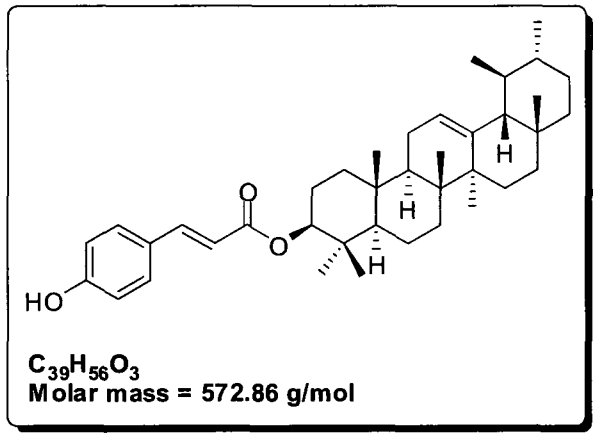
^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) 5.16 (1H, t, J = 3.6 Hz, H-12), 3.22-3.18 (1H, m, H-3), 1.11 (3H, s, H-27), 1.00 (3H, s, H-23), 0.97 (3H, s, H-26), 0.94 (3H, s, H-25), 0.87 (6H, s, H-29, H-30), 0.83 (3H, s, H-28), 0.79 (3H, s, H-24)

^{13}C NMR (100MHz, $CDCl_3$): δ (ppm) 145.2 (C-13), 121.7 (C-12), 79.0 (C-3), 55.2 (C-5), 47.6 (C-9), 47.2 (C-18), 46.8 (C-19), 41.7 (C-14), 39.8 (C-8), 38.8 (C-4), 38.6 (C-1), 37.2 (C-22), 37.0 (C-10), 34.7 (C-21), 33.4 (C-29), 32.6 (C-17), 32.5 (C-7), 31.1 (C-20), 28.4 (C-28), 28.1 (C-23), 27.2 (C-2), 26.9 (C-16), 26.2 (C-15), 26.0 (C-27), 23.7 (C-30), 23.5 (C-11), 18.4 (C-6), 16.8 (C-26), 15.6 (C-24), 15.5 (C-25)

β-Amyryl P-hydroxycinnamate (49), α-Amyryl P-hydroxycinnamate (50),

Other names: Olean-12-en-3-ol, (2E)-3-(4-hydroxyphenyl)-2-propenoate, (3β)- 3-(E)-Coumaroyl β-amyrin; 3-O-(E)-Coumaroyl- β -amyrin



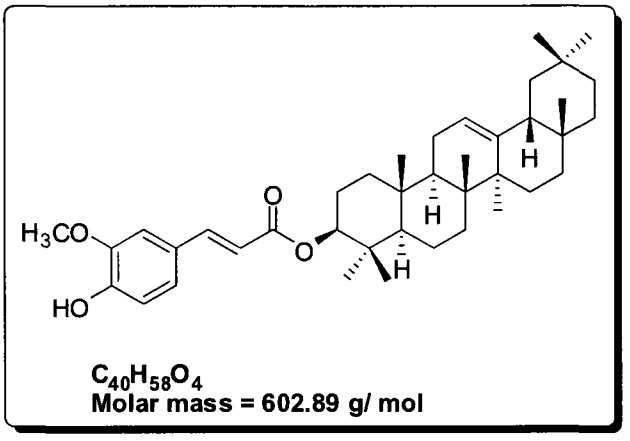


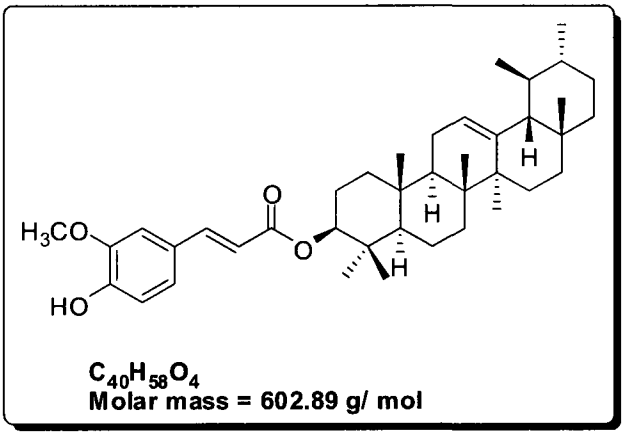
^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) 7.59 (1H, d, J = 16.0, H-3'), 7.41 (2H, d, J = 8.8Hz, H-5', H-9'), 6.82 (2H, d, J = 8.8Hz, H-6', H-8'), 6.28 (1H, d, J = 16.0 Hz, H-2'), 5.19 (1H, dd, J = 3.6, 3.6 Hz, H-12)

^{13}C NMR (100MHz, $CDCl_3$): δ (ppm) **Unavailable due to small amount of sample isolated**

β-Amyryl ferulate (51), α-Amyryl ferulate (52)

Other names: n/a



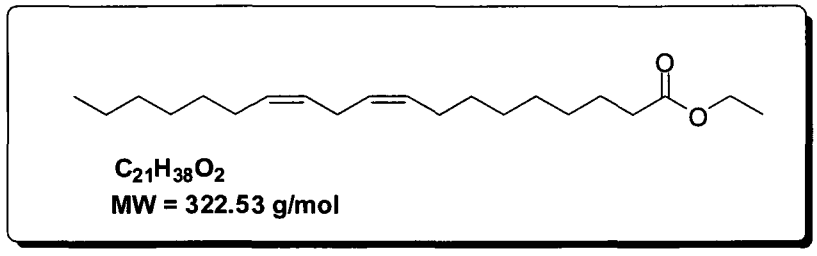


^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) 7.59 (1H, d, J = 16.0 Hz, H-3'), 7.07 (1H, dd, J = 8.0 Hz, 1.6 Hz, H-9'), 7.04 (1H, d, J = 1.6 Hz, H-5'), 6.91 (1H, d, J = 8.0 Hz, H-8'), 6.29 (1H, d, 16.0 Hz, H-2'), 5.83 (1H, s, OH), 5.17 (1H, dd, J = 3.2, 3.2 Hz, H-12β), 5.11 (1H, dd, J = 4.0, 4.0 Hz, H-12α), 4.67-4.55 (1H, m, H-3)

^{13}C NMR (100MHz, $CDCl_3$): δ (ppm) 167.1 (C-1'), 147.8 (C-7'), 146.7 (C-6'), 145.2 (C-13), 144.4 (C-3'), 127.2 (C-4'), 123.1 (C-9'), 121.7 (C-12), 116.2 (C-2'), 114.7 (C-8'), 109.2 (C-5'), 80.8 (C-3), 56.0 (C-10'), 55.3 (C-5), 47.6 (C-9), 47.2 (C-18), 46.8 (C-19), 41.7 (C-8), 39.9 (C-14), 38.3 (C-1), 38.0 (C-4), 37.3 (C-22), 36.9 (C-10), 34.7 (C-21), 33.3 (C-29), 32.7 (C-7), 32.5 (C-17), 31.1 (C-20), 28.1 (C-28), 28.0 (C-23), 26.9 (C-15), 26.2 (C-16), 26.0 (C-27), 23.7 (C-2), 23.7 (C-11), 23.6 (C-30), 18.3 (C-6), 16.8 (C-26), 16.2 (C-24), 15.6 (C-25)

Ethyl linoleate (53)

Other names: 9,12-Octadecadienoic acid (Z,Z)-, ethyl ester; Linoleic acid, ethyl ester (6CI,8CI); Ethyl (Z,Z)-9,12-octadecadienoate; Ethyl cis,cis-9,12-octadecadienoate; Ethyl linolate; Ethyl linoleate; Mandenol; Nikkol VF-E

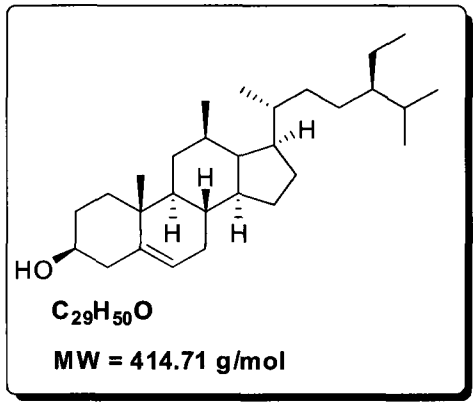


^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) 5.39-5.26 (4H, m, H-9, H-10, H-12, H-13), 4.08 (2H, q, J = 7.2 Hz, H-1'), 2.77 (2H, t, J = 6.4 Hz, H-11), 2.24 (2H, t, J = 7.6 Hz, H-2), 2.08-1.96 (4H, m, H-8, H-14), 1.64-1.54 (4H, m, H-7, H-15), 1.28-1.20 (17H, H-3, H-4, H-5, H-6, H-7, H-15, H-17, H-18)

^{13}C NMR (100MHz, $CDCl_3$): δ (ppm) 173.8 (C-1), 131.9, 130.2, 128.2, 128.2, 127.7, 127.1, 60.1 (C-1'), 34.4, 31.9, 29.7, 29.6, 29.6, 29.5, 29.4, 29.3, 29.2, 29.1, 29.1, 27.2, 25.6, 25.0, 22.7, 14.2, 14.1

β-sitosterol (54)

Other names: Nimbosterol (6CI); Stigmast-5-en-3 β -ol (8CI); (-)-β -Sitosterol; (24R)-Ethylcholest-5-en-3 β -ol; (24R)-Stigmast-5-en-3 β -ol; 22,23-Dihydrostigmasterol; 24 β -Ethylcholesterol; Angelicin; Angelicin (steroid); Azuprostat; Betaprost; Cinchol; Cupreol; Harzol; NSC 18173; NSC 49083; NSC 8096; Prostasal; Quebrachol; Rhammol; Rhamnol; SKF 14463; Sito-Lande; Sitosterol; Sobatum; Stigmasterol, 22,23-dihydro-; β 5-Stigmasten-3 β -ol; β -Dihydrofucosterol; β -Phytosterol; β -Sitosterin; β -Sitosterol

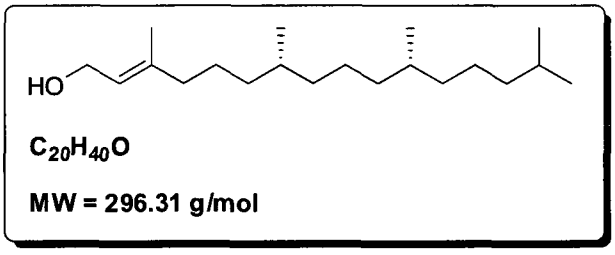


^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) 5.33 (1H, dd, J = 3.2, 2.0 Hz, H-6), 3.54-3.47 (1H, m, H-3), 2.30-0.74 (47H, m, H-1, H-2, H-4, H-7, H-8, H-9, H-11, H-12, H-14, H-15, H-16, H-17, H-18, H-19, H-20, H-21, H-22, H-23, H-24, H-25, H-26, H-27), 0.66 (3H, s, H-28)

^{13}C NMR (100MHz, $CDCl_3$): δ (ppm) **Unavailable due to small amount of sample isolated**

Phytol (55)

Other names: 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-; Phytol (6CI,8CI); (7R,11R,2E)-Phytol; (E)-Phytol; (E,R,R)-Phytol; 3,7,11,15-Tetramethylhexadec-2-en-1-ol; trans-Phytol



^{1}H NMR (400MHz, $CDCl_3$): δ (ppm) 5.40 (1H, td, J = 6.8, 1.2 Hz, H-2), 4.14 (2H, d, J = 6.8 Hz, H-1), 1.98 (2H, d, J = 7.6 Hz, H-4), 1.66 (3H, s, H-20),

^{13}C NMR (100MHz, $CDCl_3$): δ (ppm) 140.3 (C-3), 123.0 (C-2), 59.4 (C-1), 39.9 (C-14), 39.4 (C-4), 37.4 (C-10), 37.3 (C-8), 37.3 (C-12), 36.7 (C-6), 32.8 (C-11), 32.7 (C-7), 28.0 (C-15), 25.1 (C-5), 24.8 (C-13), 24.4 (C-9), 22.7 (C-17), 22.6 (C-16), 19.8 (C-18), 19.7 (C-19), 16.2 (C-20)

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Appendix 1 – NMR spectra for chapter 1

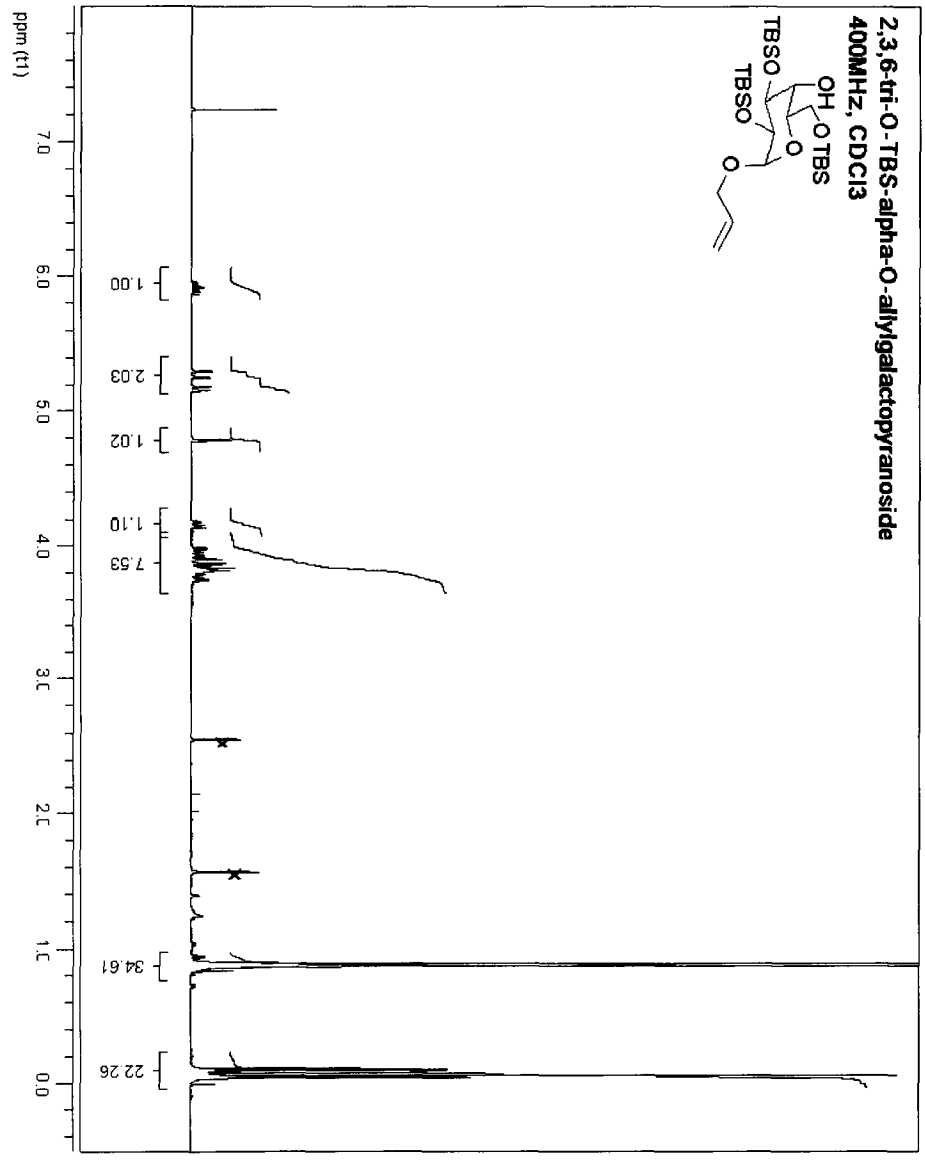

Figure a.1.1 ^{1}H NMR spectrum of 2,3,6-tri-O-TBS-α-O-allylgalactopyranoside (**5**) in $CDCl_3$

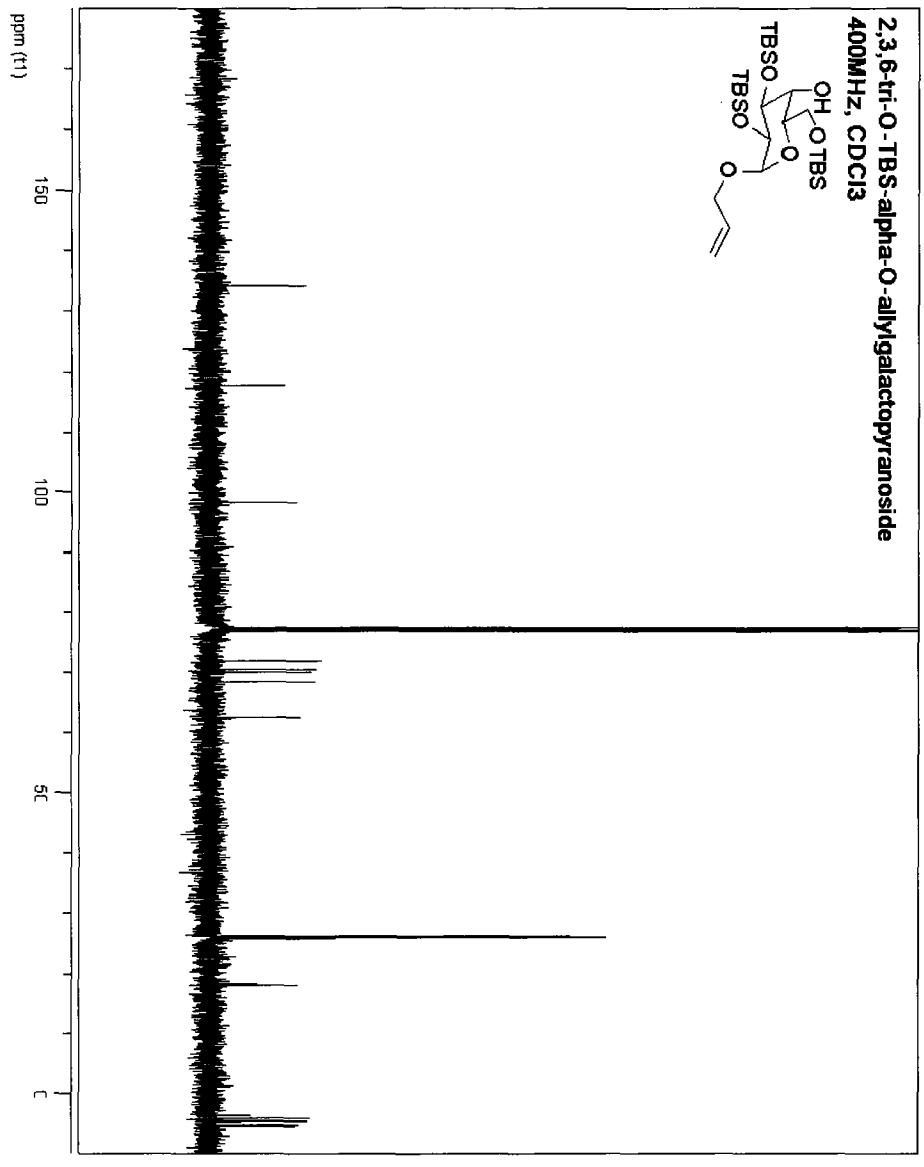

Figure a.1.2 ^{13}C NMR spectrum of 2,3,6-tri-O-TBS-α-O-allylgalactopyranoside (**5**) in $CDCl_3$

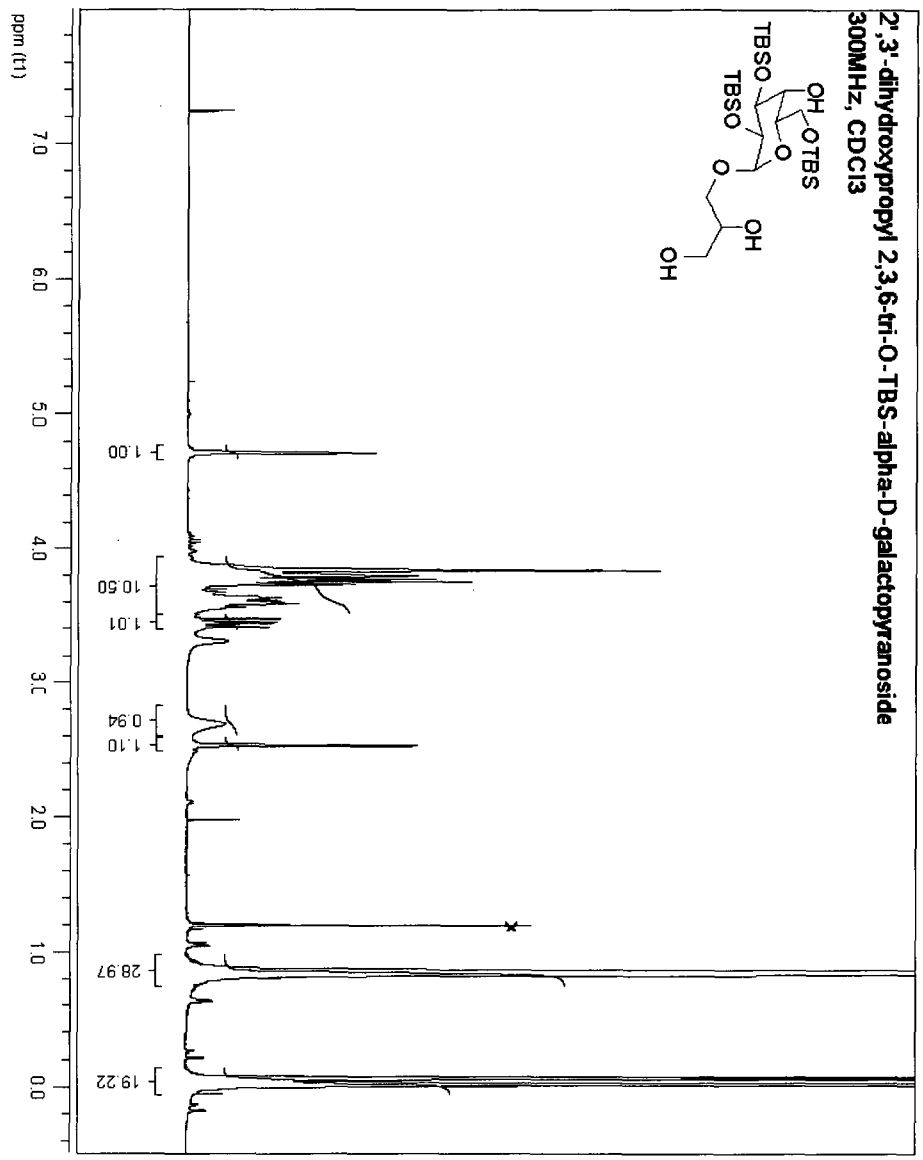

Figure a.1.3 ^{1}H NMR spectrum of 2',3'-dihydroxypropyl 2,3,6-tri-O-TBS-α-*D*-galactopyranoside (**6**) in $CDCl_3$

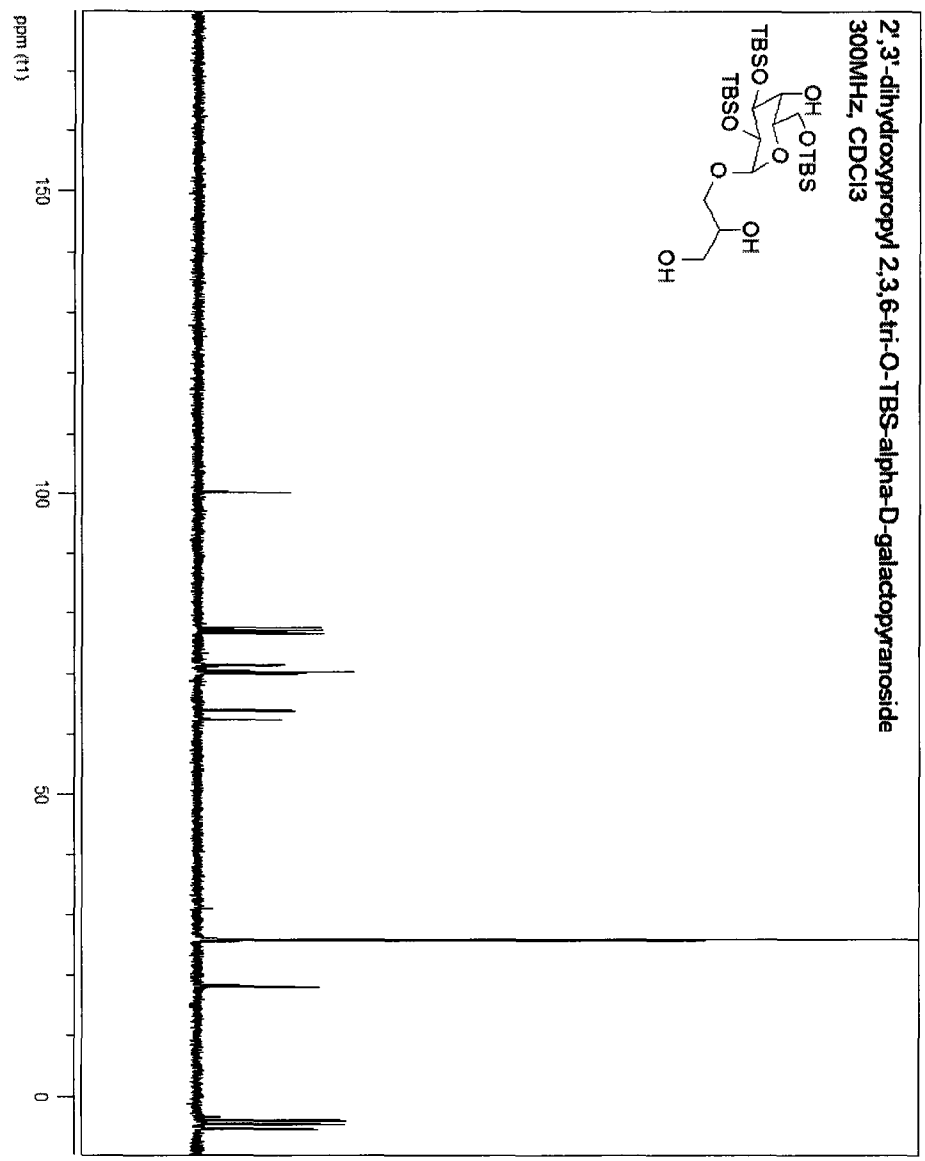

Figure a.1.4 ^{13}C NMR spectrum of 2',3'-dihydroxypropyl 2,3,6-tri-O-TBS-α-*D*-galactopyranoside (**6**) in $CDCl_3$

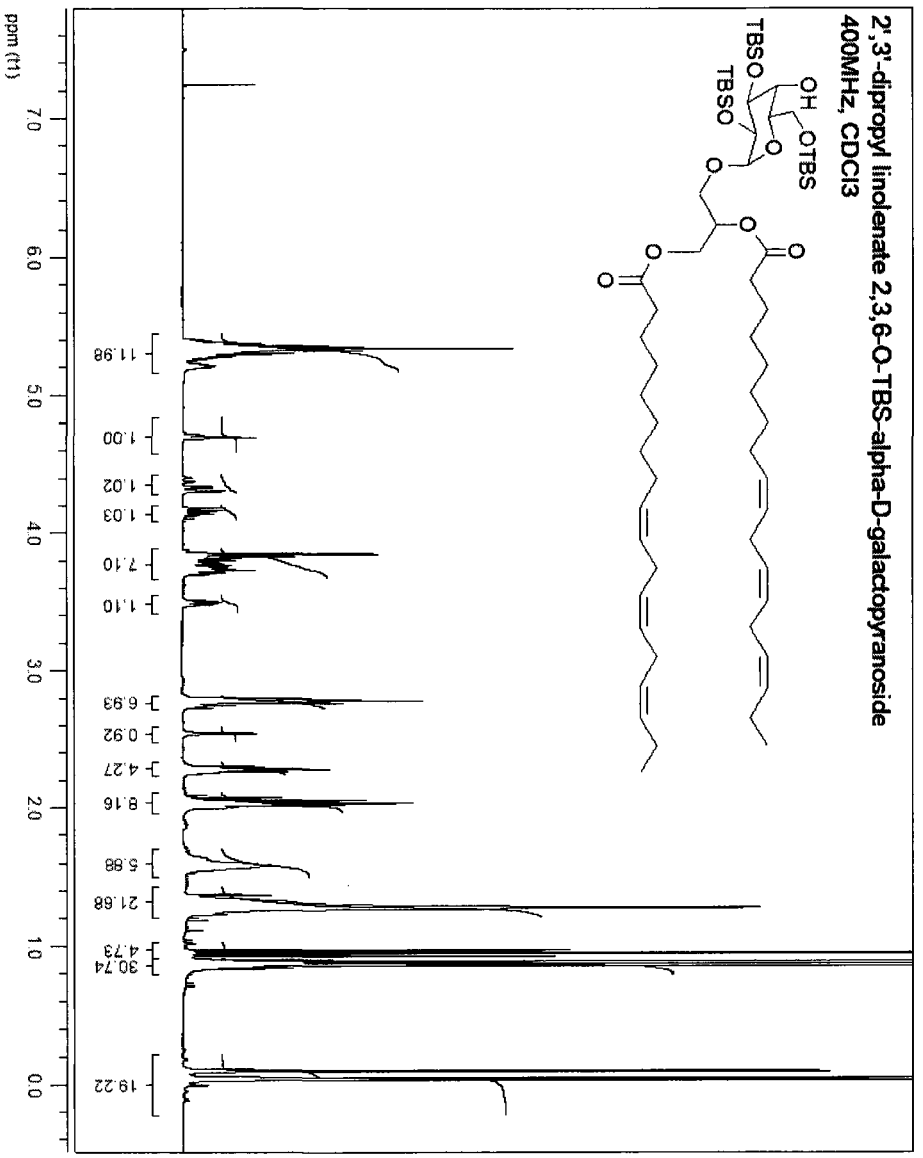

Figure a.1.5 ^{1}H NMR spectrum of 2',3'-dipropyl linolenate 2,3,6-O-TBS-α-*D*-galactopyranoside (**7**) in $CDCl_3$

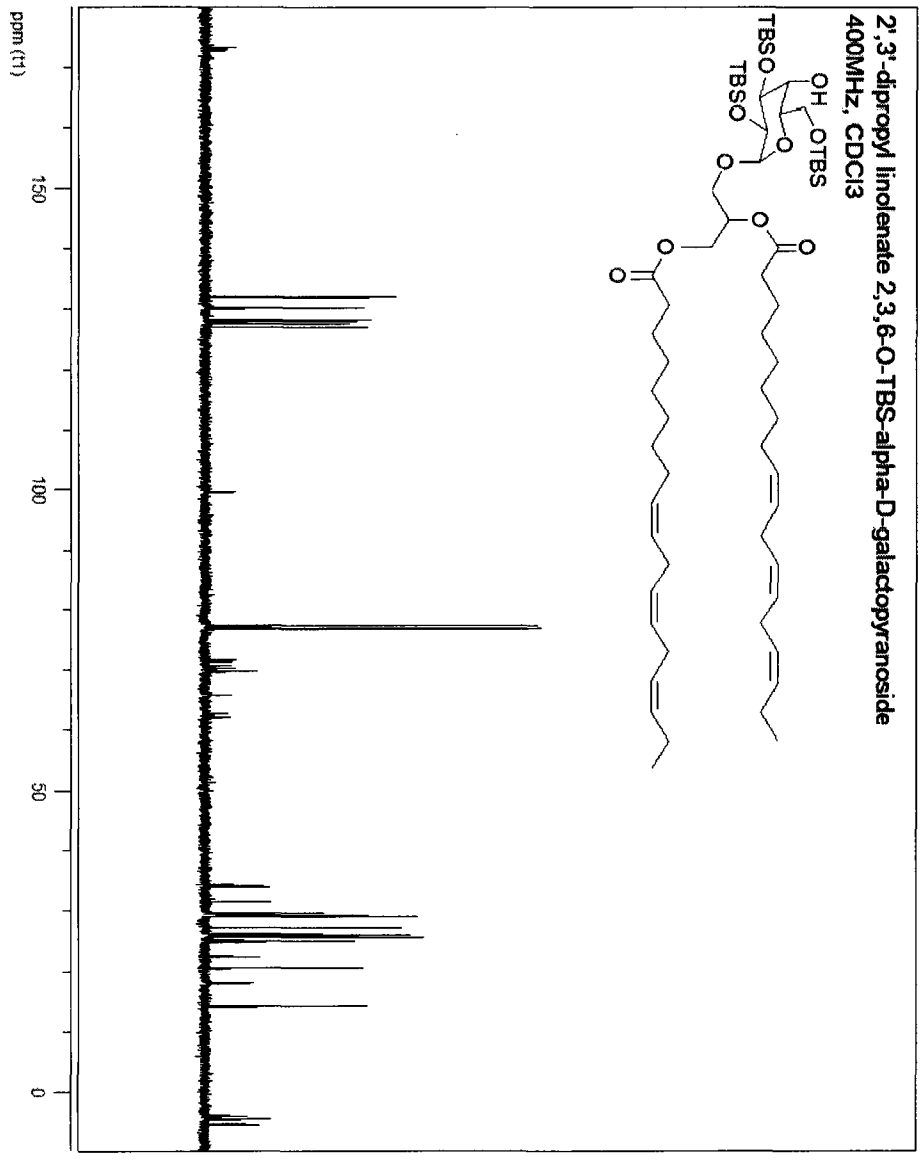

Figure a.1.6 ^{13}C NMR spectrum of 2',3'-dipropyl linolenate 2,3,6-O-TBS-α-*D*-galactopyranoside (**7**) in $CDCl_3$

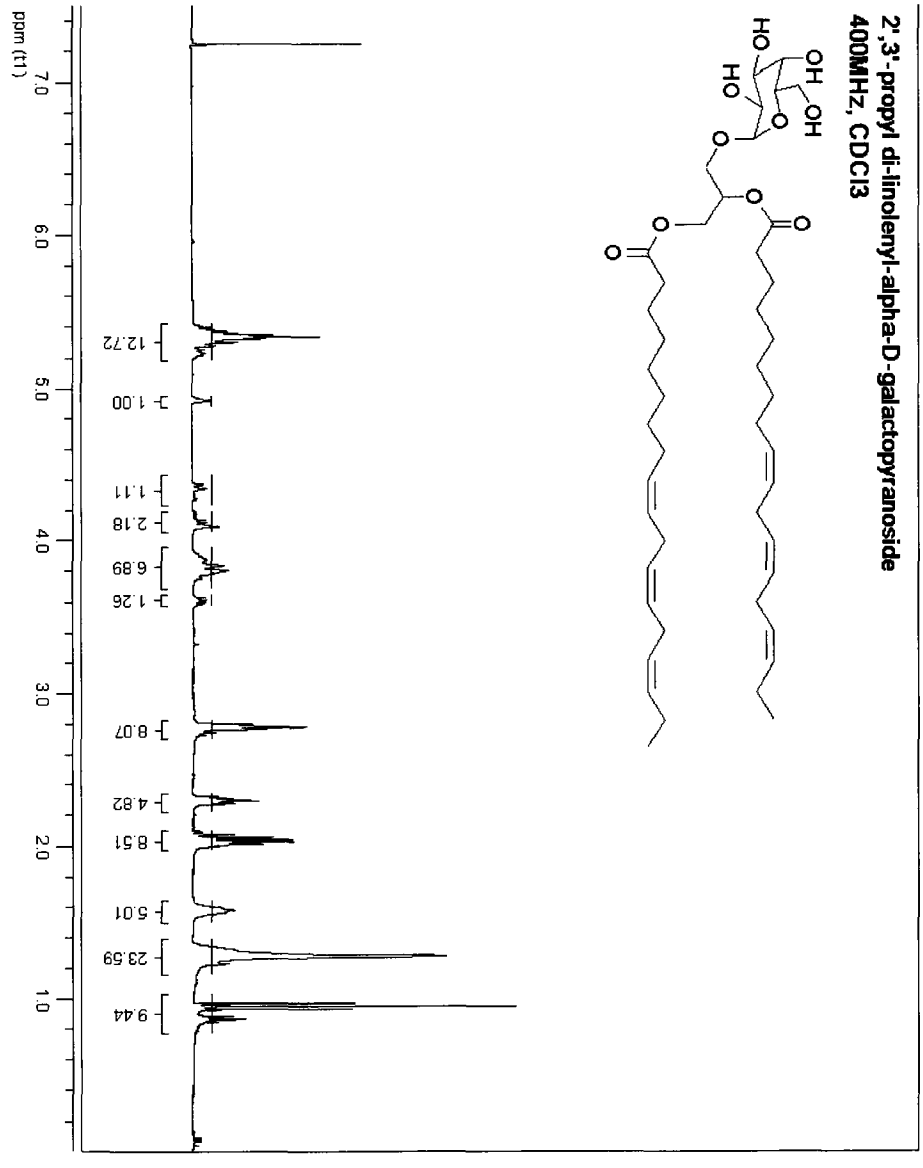

Figure a.1.7 ^{1}H NMR spectrum of 2',3'-propyl di-linolenyl-α-*D*-galactopyranoside (**8**) in $CDCl_3$

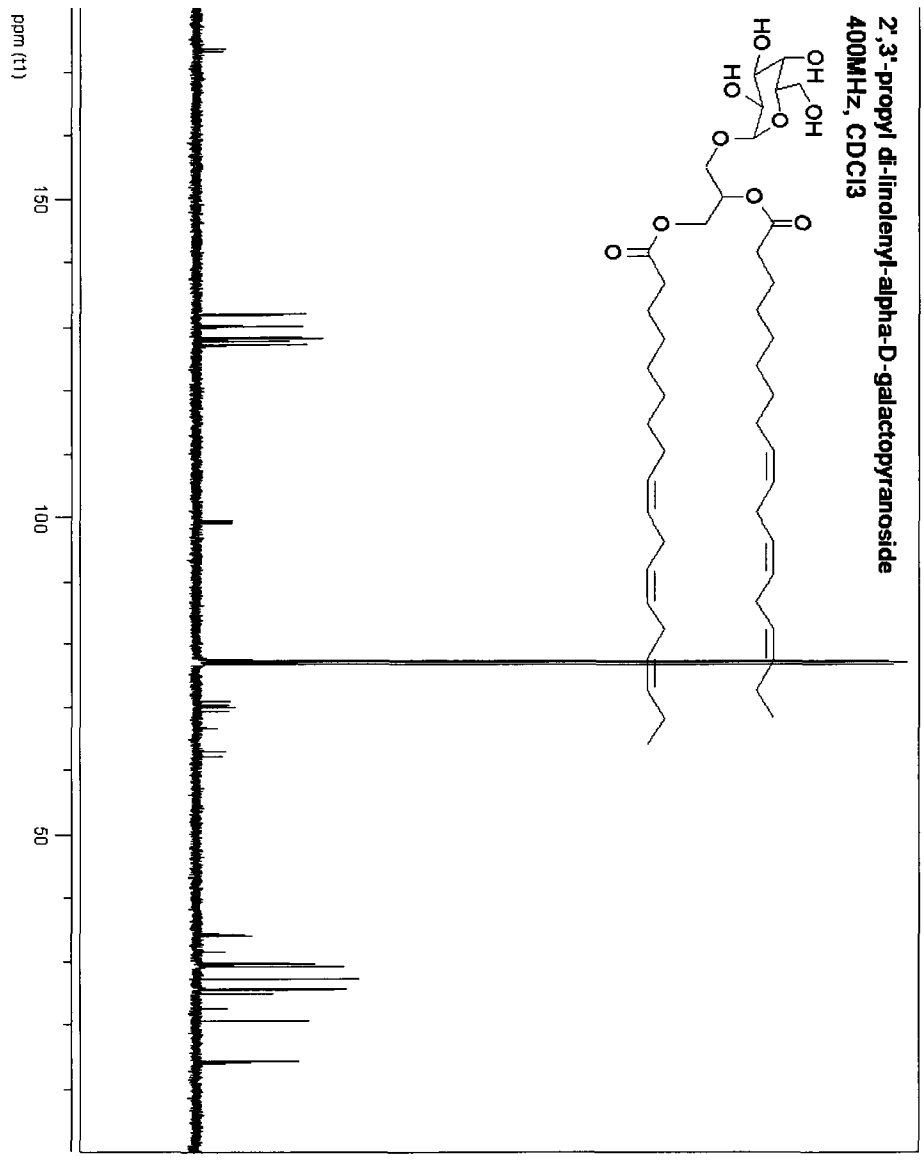

Figure a.1.8 ^{13}C NMR spectrum of 2',3'-propyl di-linolenyl-α-*D*-galactopyranoside (**8**) in $CDCl_3$

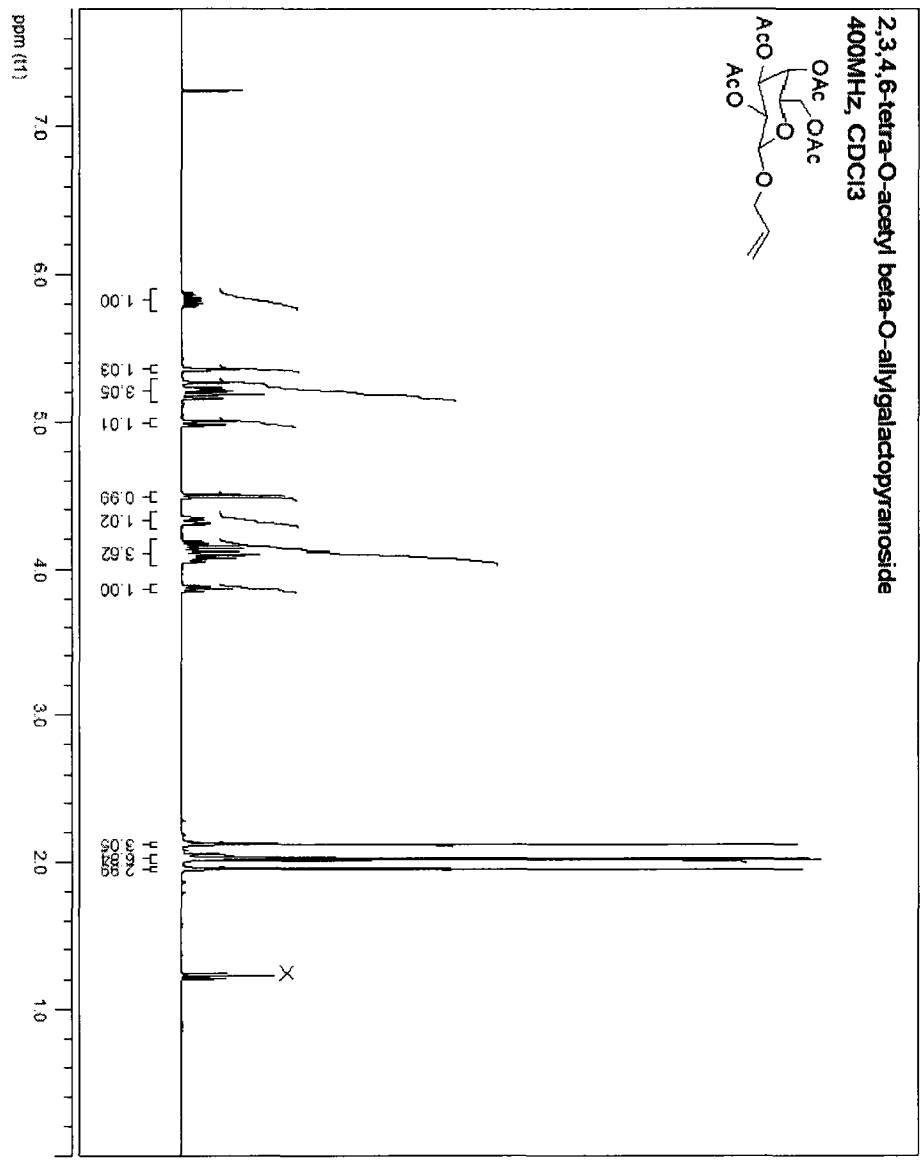

Figure a.1.9 ^{1}H NMR spectrum of 2,3,4,6-tetra-O-acetyl β-O-allylgalactopyranoside (**9**) in $CDCl_3$

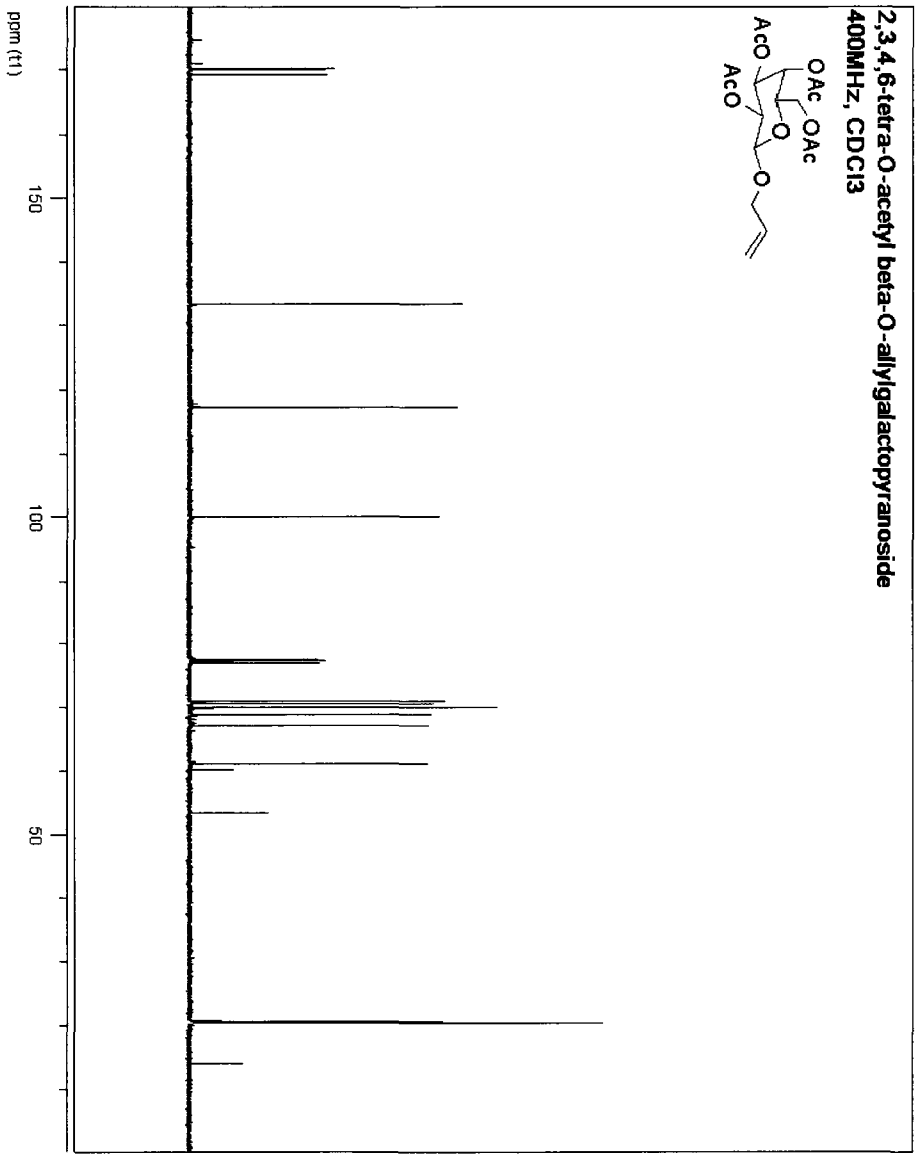

Figure a.1.10 ^{13}C NMR spectrum of 2,3,4,6-tetra-O-acetyl β-O-allylgalactopyranoside (**9**) in $CDCl_3$

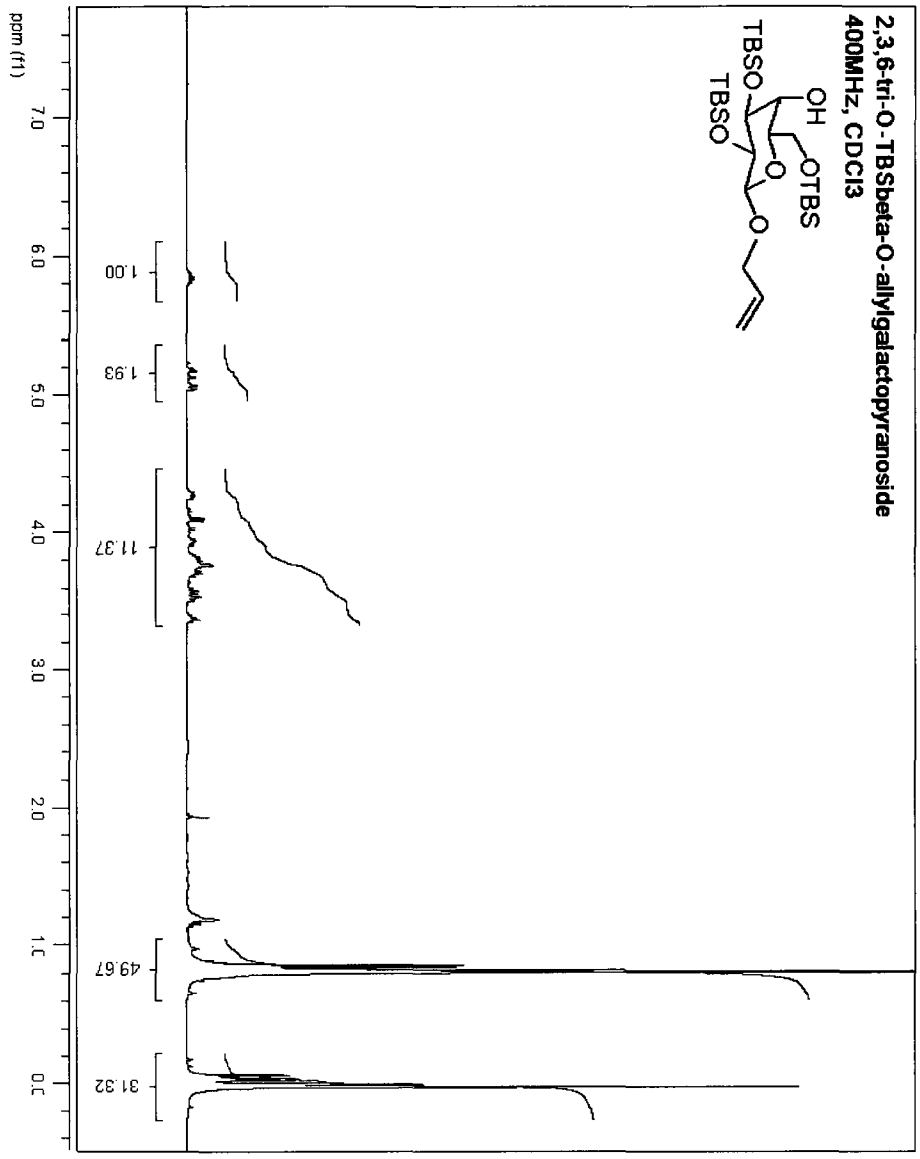

Figure a.1.11 ^{1}H NMR spectrum of 2,3,6-tri-O-TBS β-O-allylgalactopyranoside (**10**) in $CDCl_3$

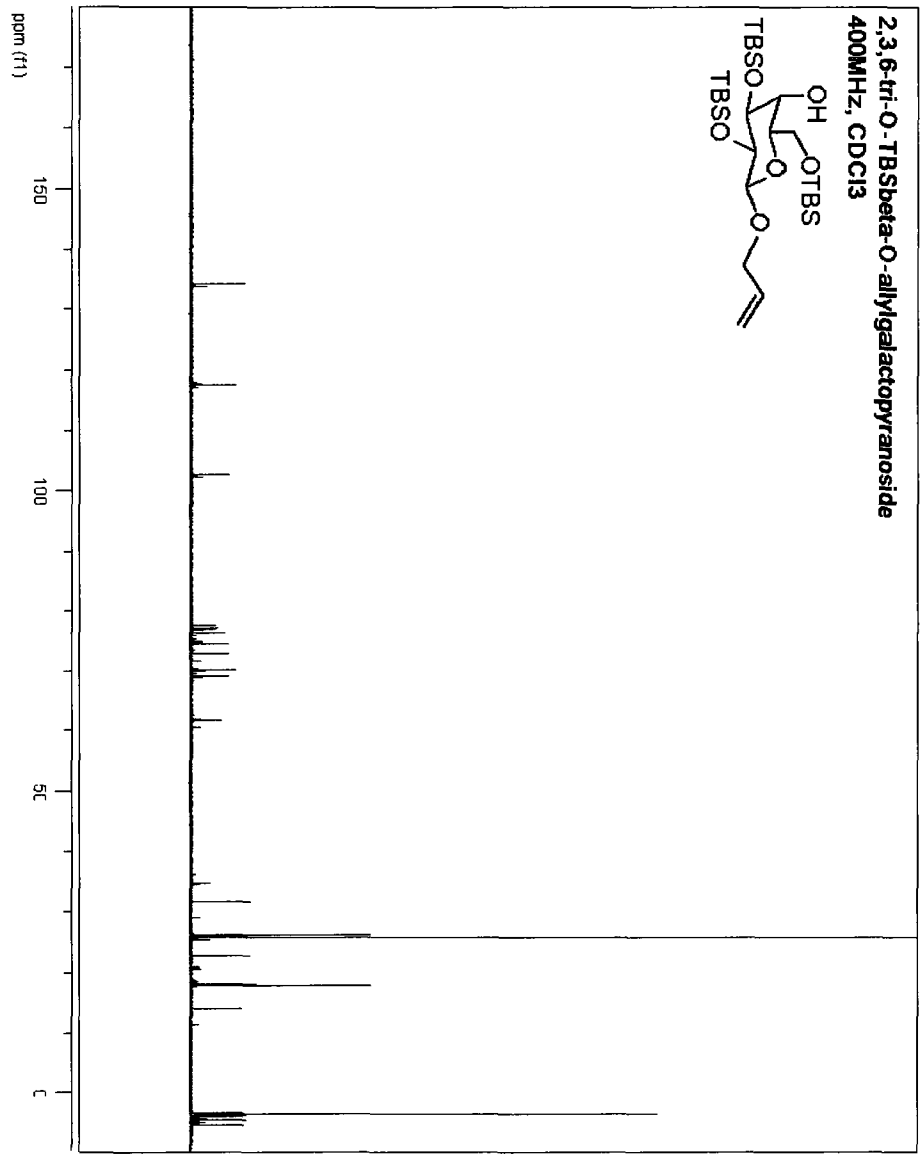

Figure a.1.12 ^{13}C NMR spectrum of 2,3,6-tri-O-TBS β-O-allylgalactopyranoside (**10**) in $CDCl_3$

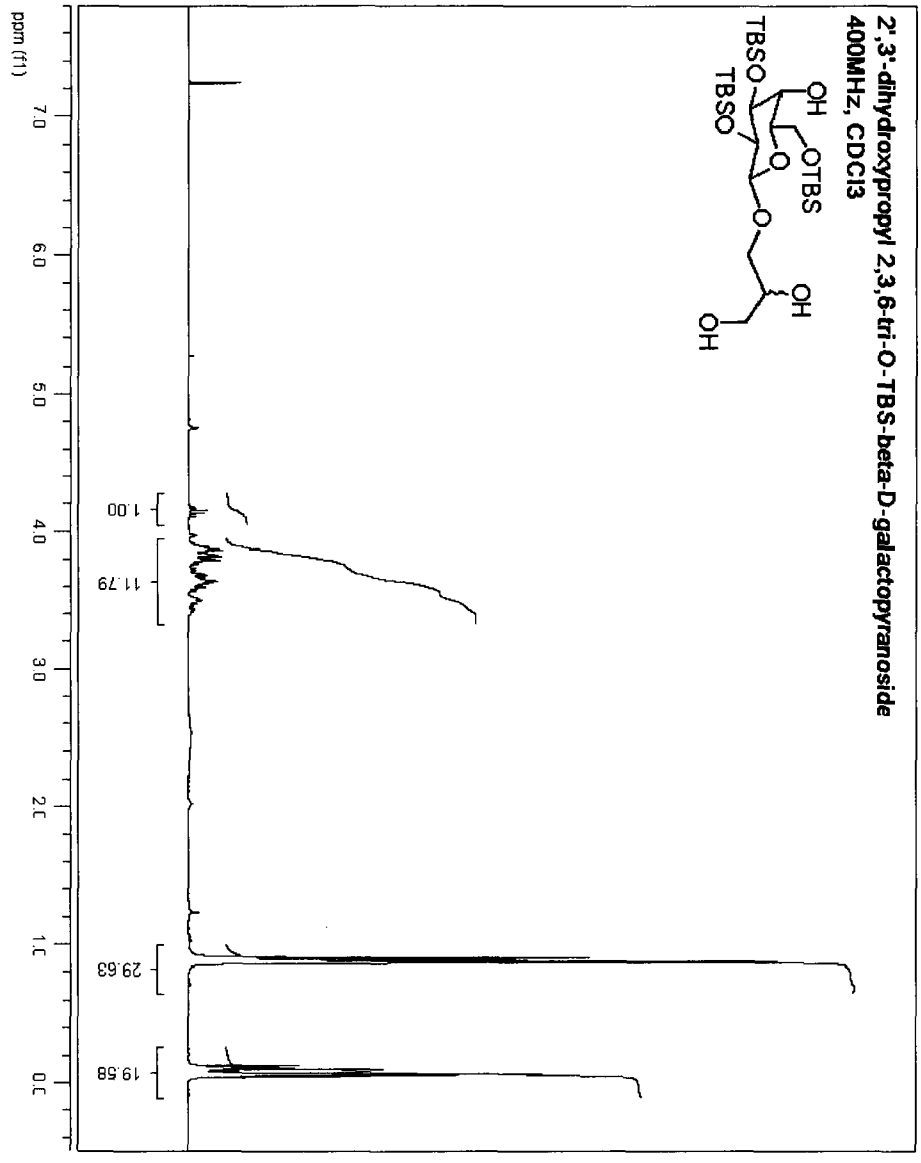

Figure a.1.13 ^{1}H NMR spectrum of 2',3'-dihydroxypropyl 2,3,6-tri-O-TBS β-O-allylgalactopyranoside (**11**) in $CDCl_3$

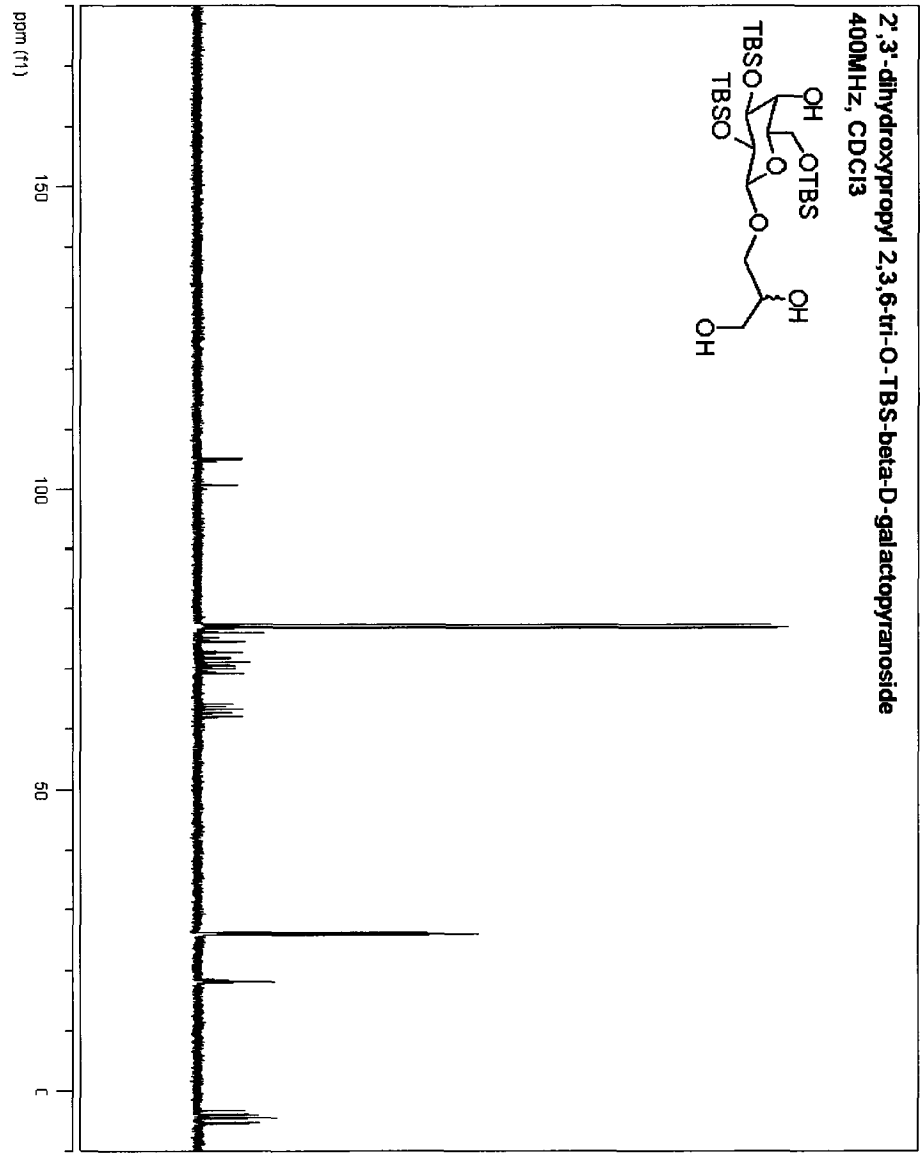

Figure a.1.14 ^{13}C NMR spectrum of 2',3'-dihydroxypropyl 2,3,6-tri-O-TBS β-O-allylgalactopyranoside (**11**) in $CDCl_3$

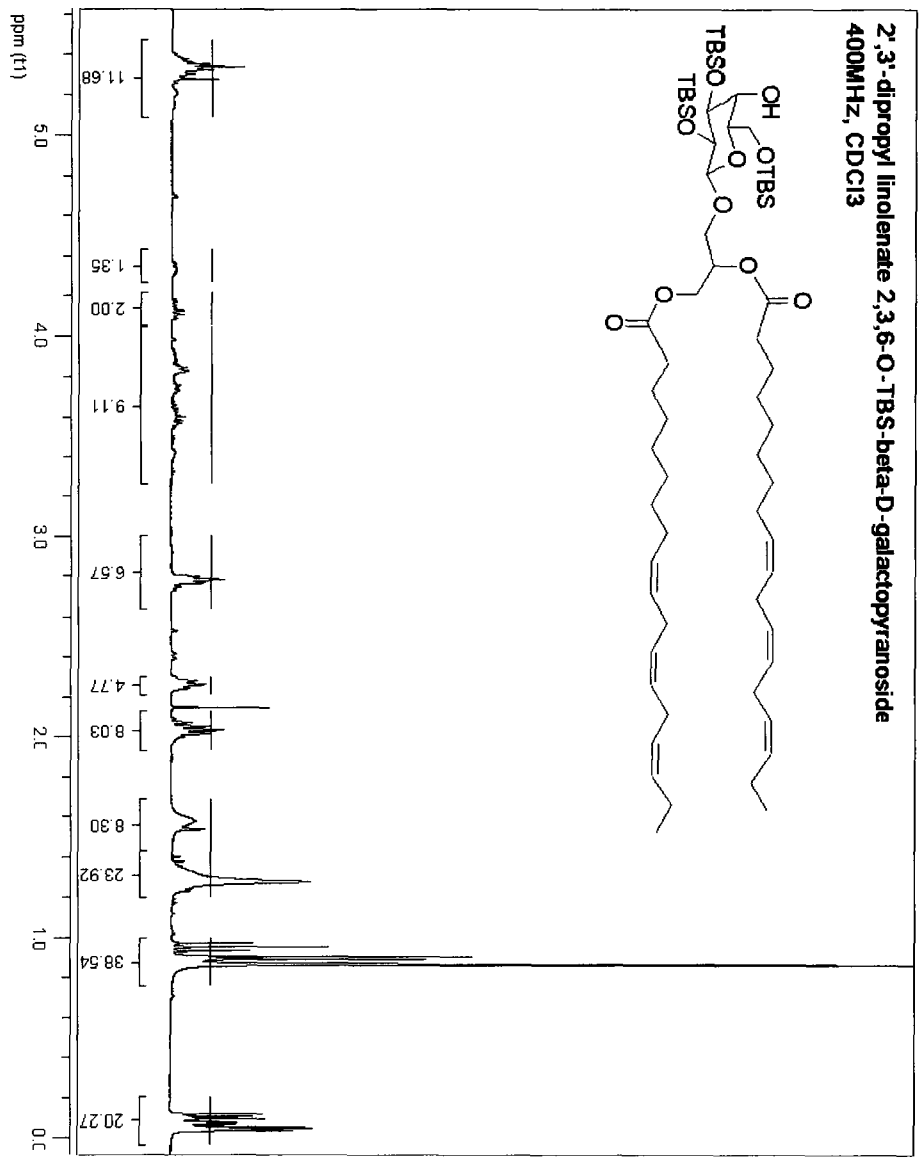

Figure a.1.15 ^{1}H NMR spectrum of 2',3'-dipropyl linolenate 2,3,6-tri-O-TBS β-O-allylgalactopyranoside (**12**) in $CDCl_3$

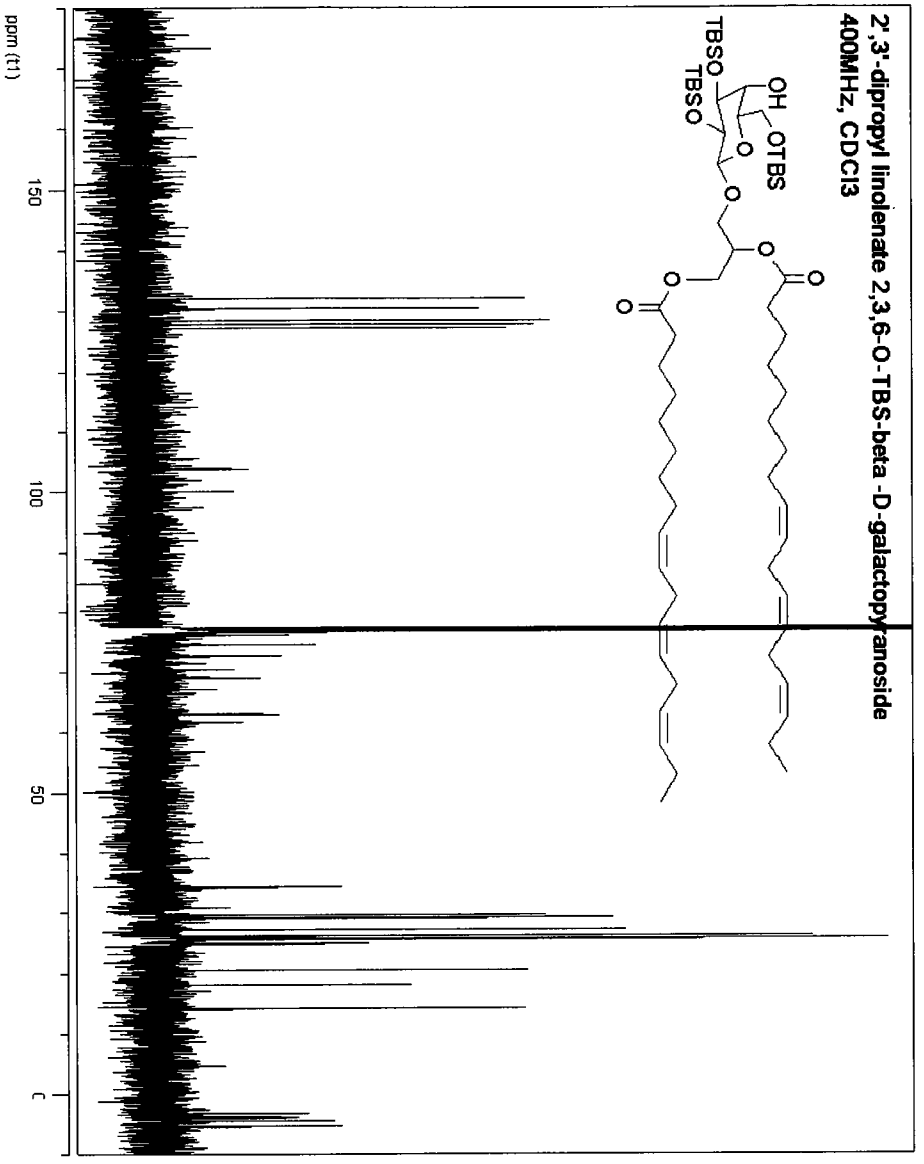

Figure a.1.16 ^{13}C NMR spectrum of 2',3'-dipropyl linolenate 2,3,6-tri-O-TBS β-O-allylgalactopyranoside (**12**) in $CDCl_3$

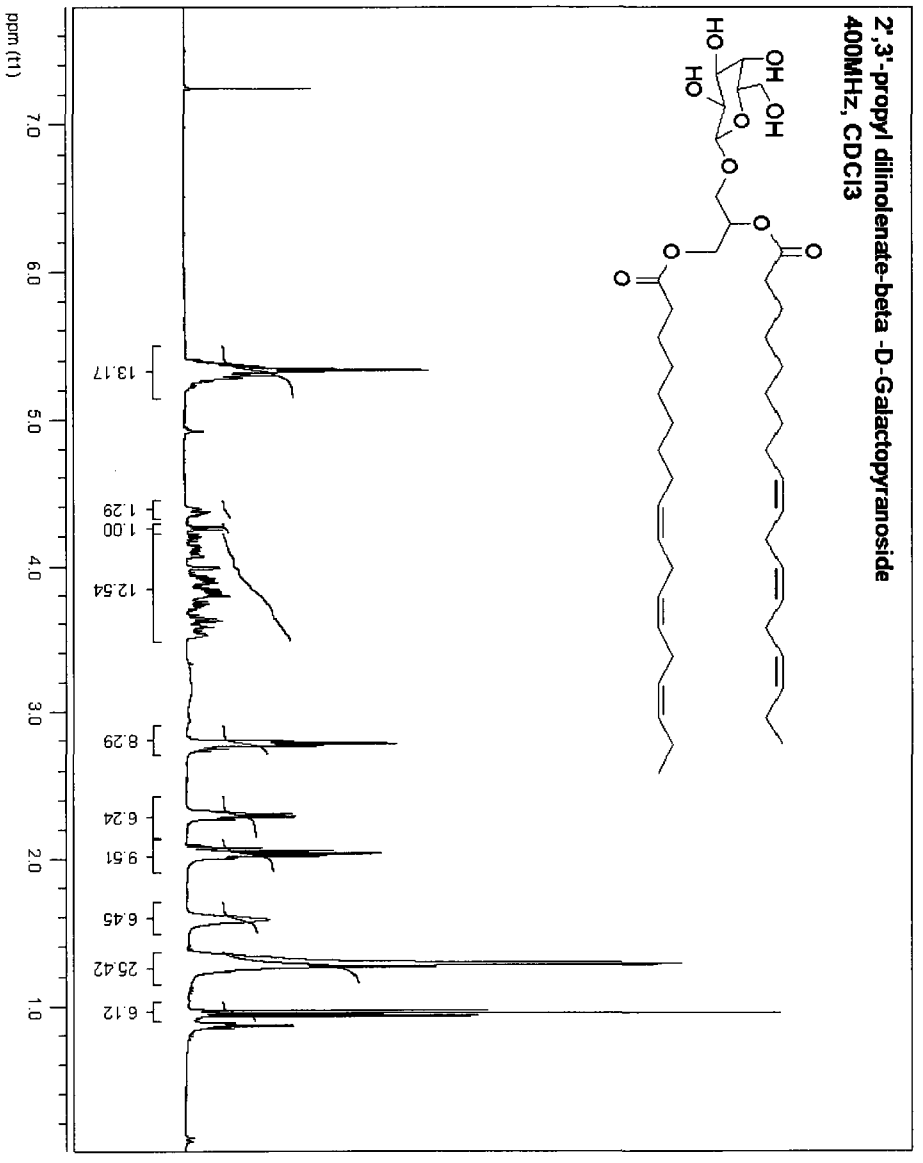

Figure a.1.17 ^{1}H NMR spectrum of 2',3'-dipropyl linolenate β-O-allylgalactopyranoside (**1**) in $CDCl_3$

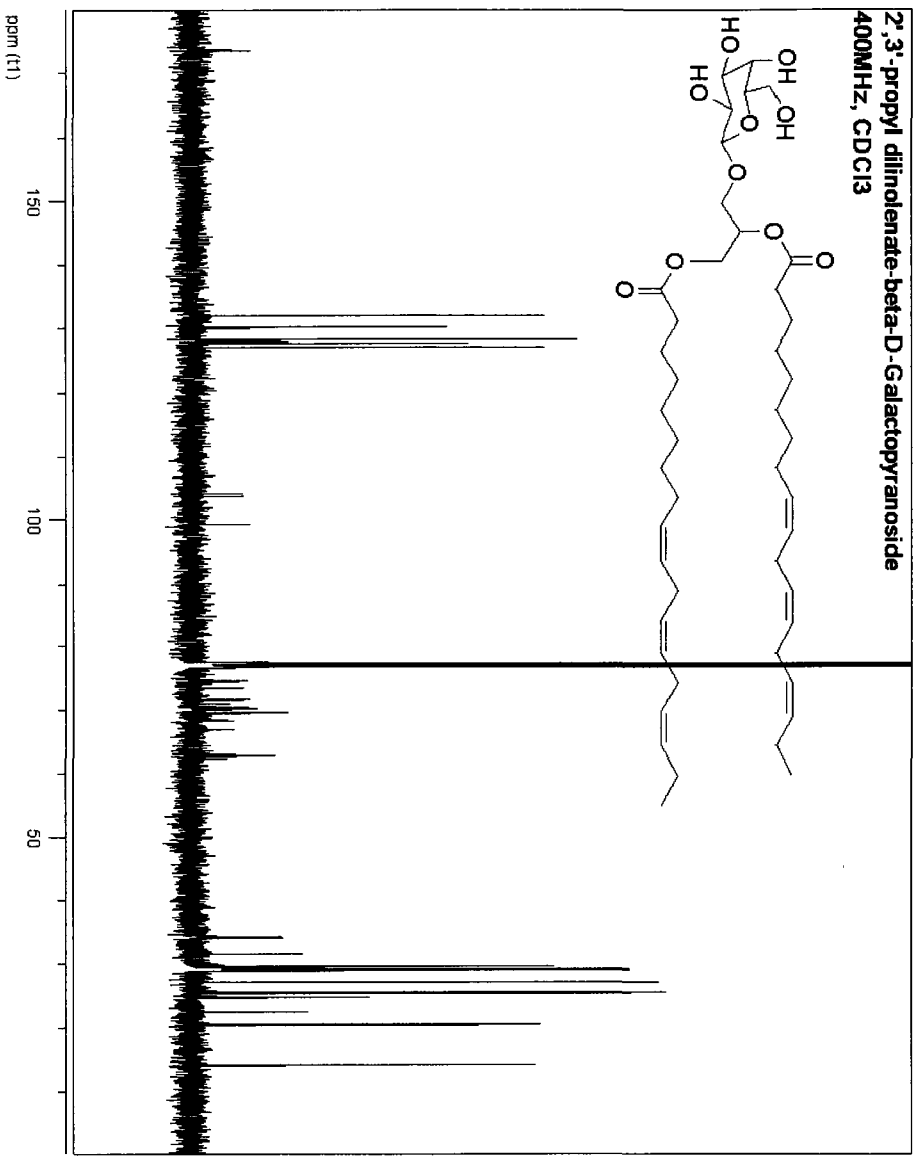

Figure a.1.18 ^{13}C NMR spectrum of 2',3'-dipropyl linolenate β-O-allylgalactopyranoside (**1**) in $CDCl_3$

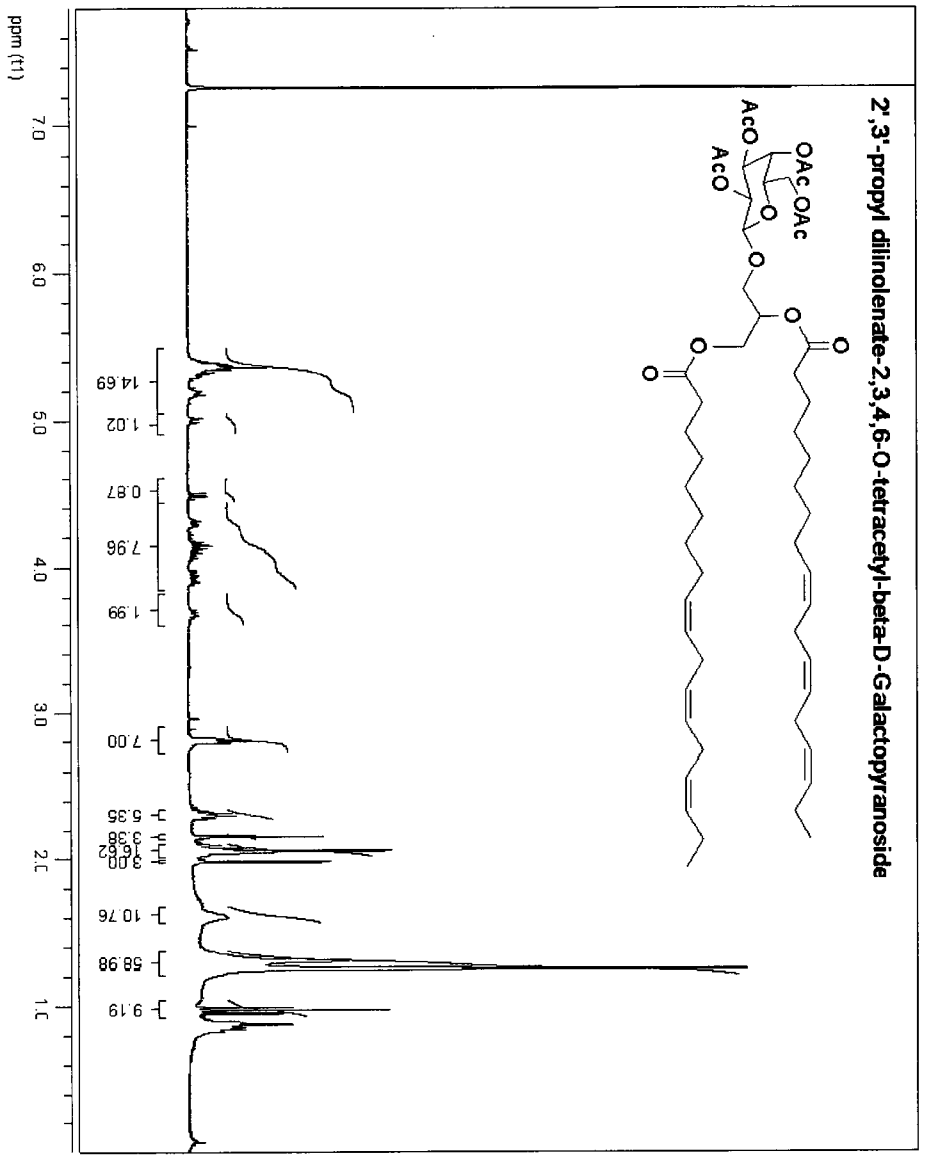

Figure a.1.19 ^{1}H NMR spectrum of 2',3'-dipropyl linolenate 2,3,4,6-*O*-tetraacetyl-*β*-O-allylgalactopyranoside (**2**) in $CDCl_3$

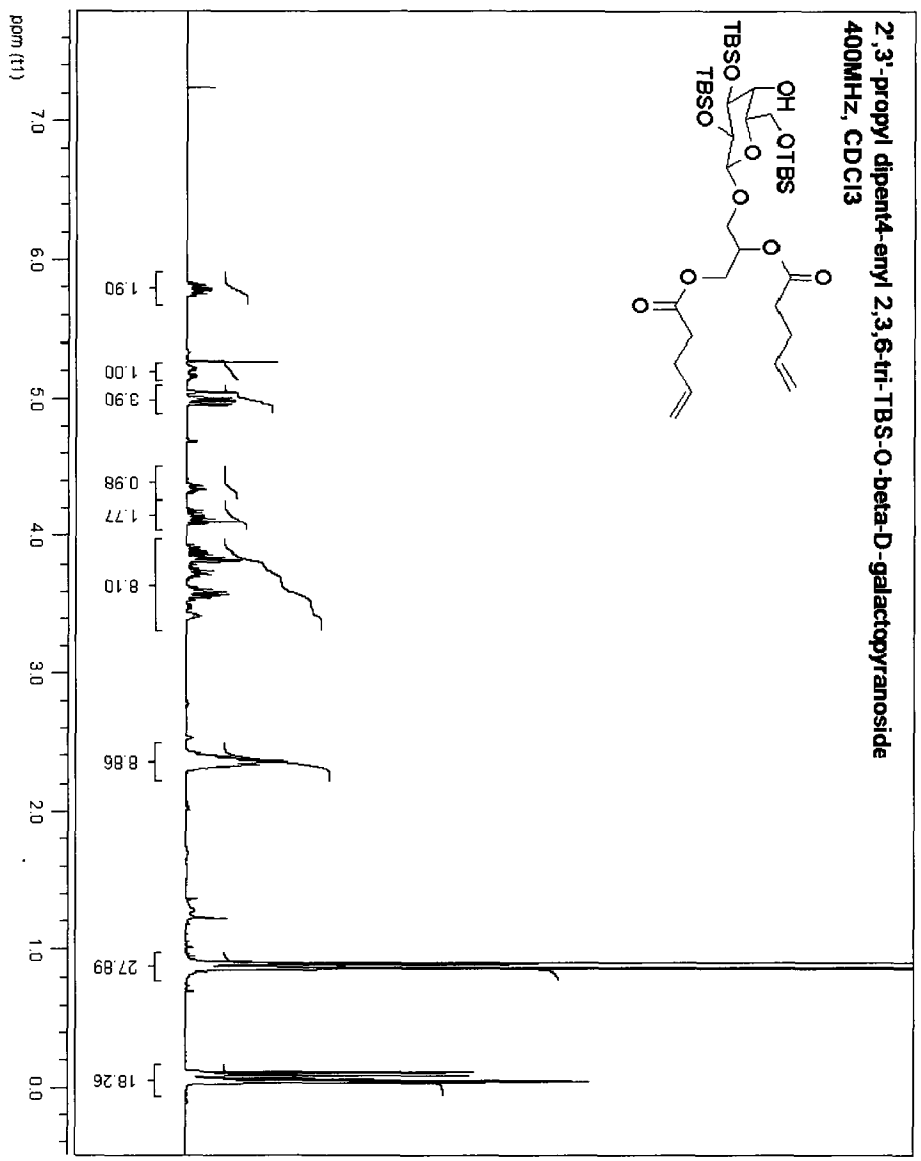

Figure a.1.20 ^{1}H NMR spectrum of 2',3'-propyl dipent-4-enyl 2,3,6-TBS-β-O-allylgalactopyranoside (**13**) in $CDCl_3$

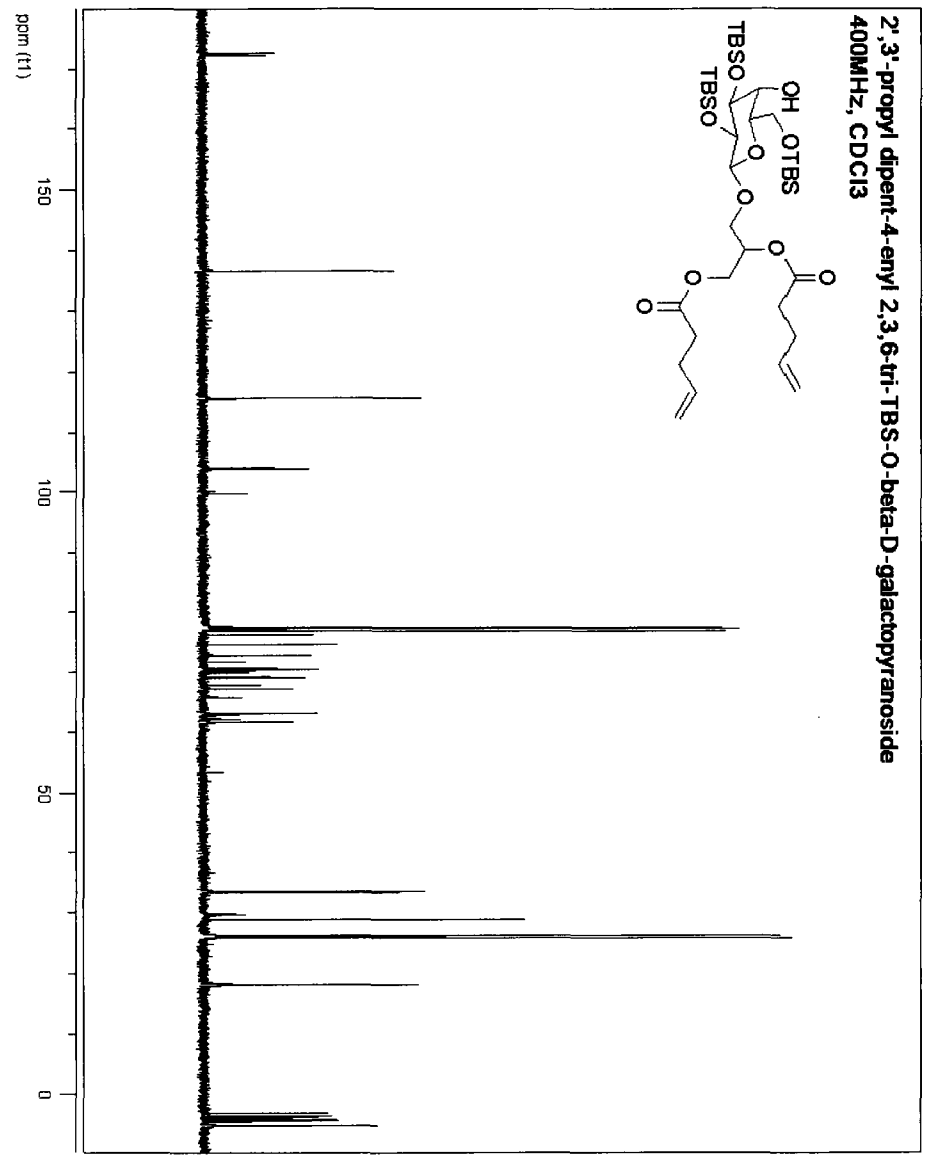

Figure a.1.21 ^{13}C NMR spectrum of 2',3'-propyl dipent-4-enyl 2,3,6-TBS-β-O-allylgalactopyranoside (**13**) in $CDCl_3$

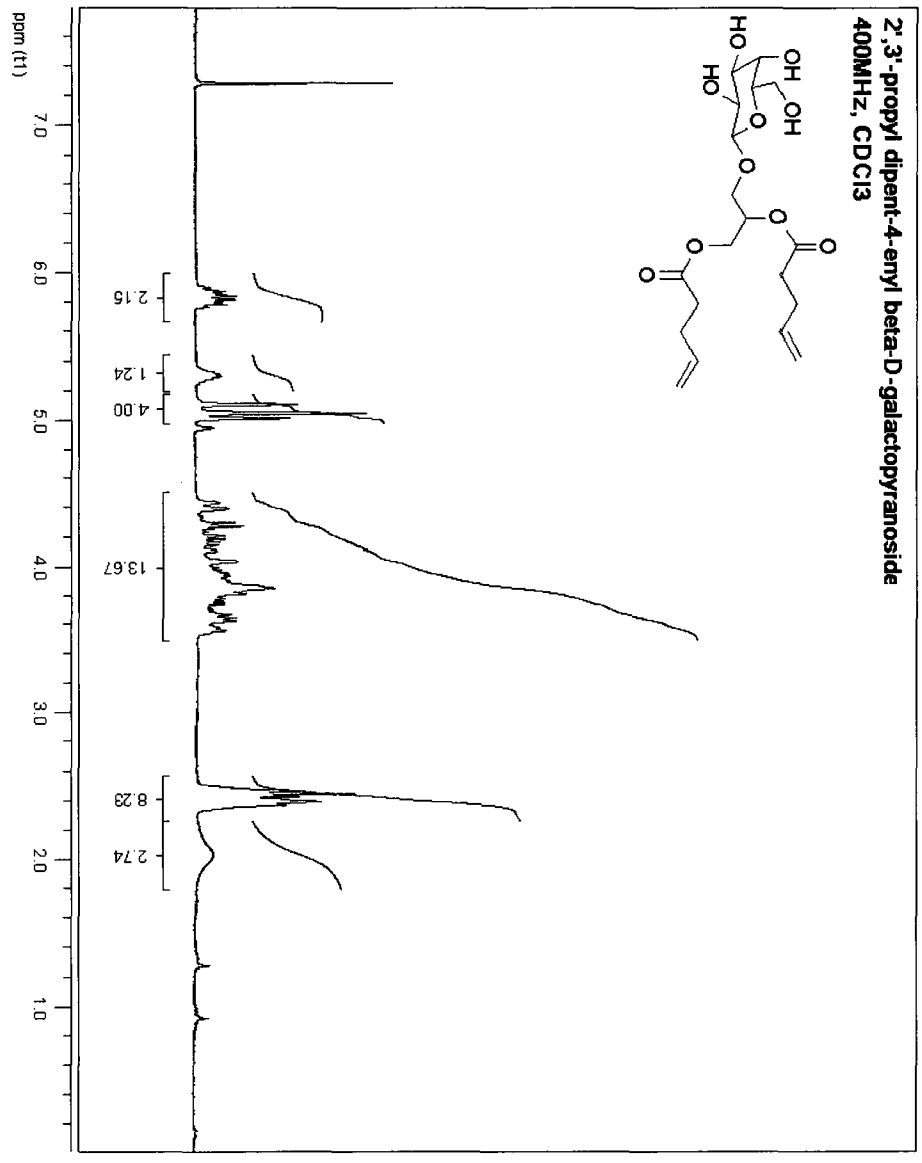

Figure a.1.22 ^{1}H NMR spectrum of 2',3'-propyl dipent-4-enyl -β-O-allylgalactopyranoside (**14**) in $CDCl_3$

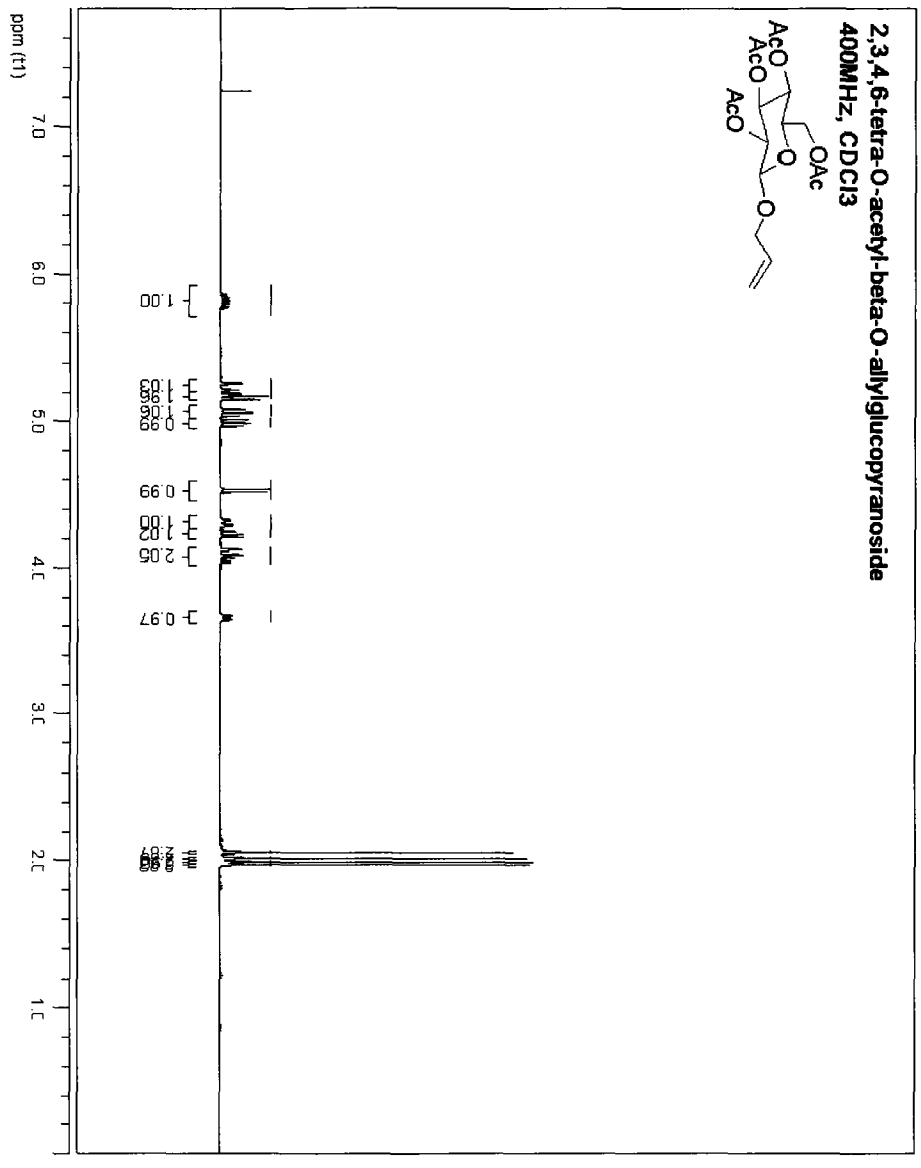

Figure a.1.23 ^{1}H NMR spectrum of 2,3,4,6-tetra-O-acetyl -β-O-allylglucopyranoside (**14**) in $CDCl_3$

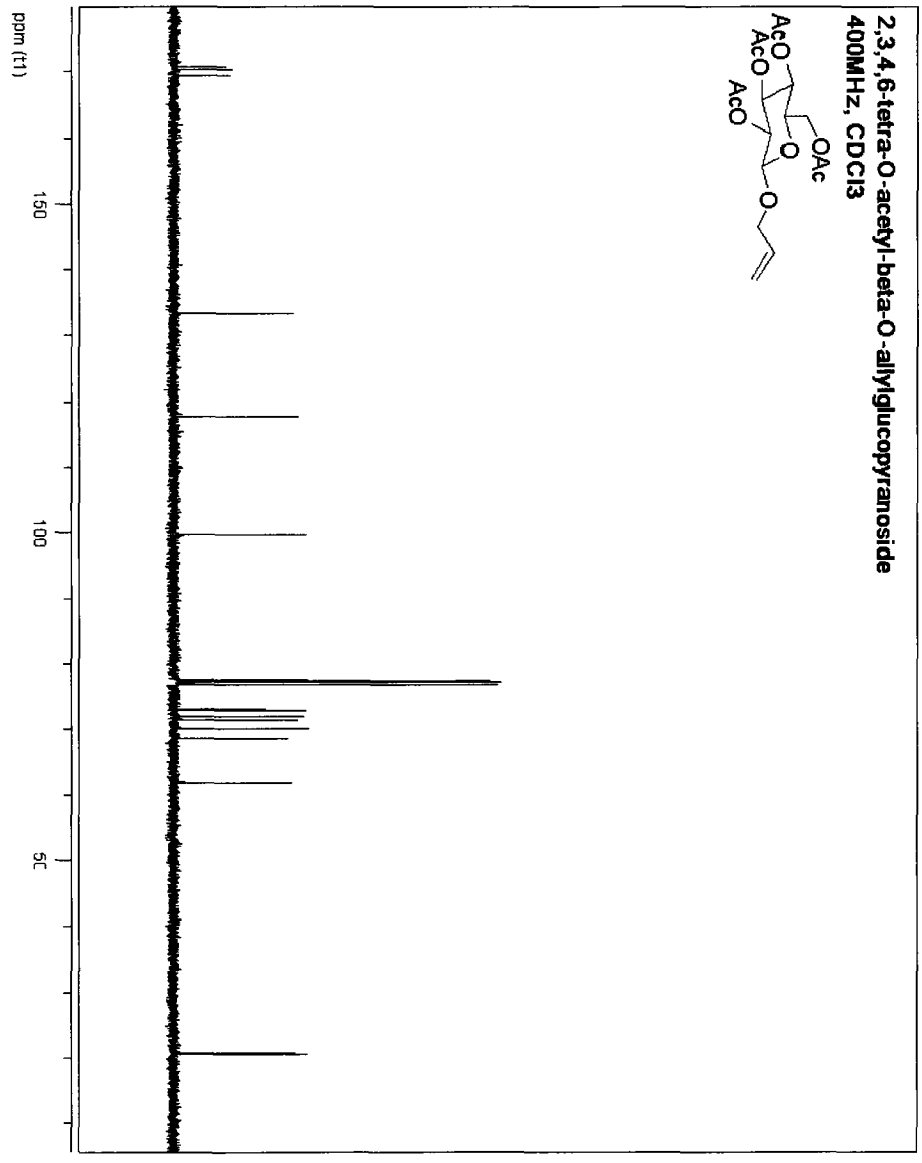

Figure a.1.24 ^{13}C NMR spectrum of 2,3,4,6-tetra-O-acetyl -β-O-allylglucopyranoside (**15**) in $CDCl_3$

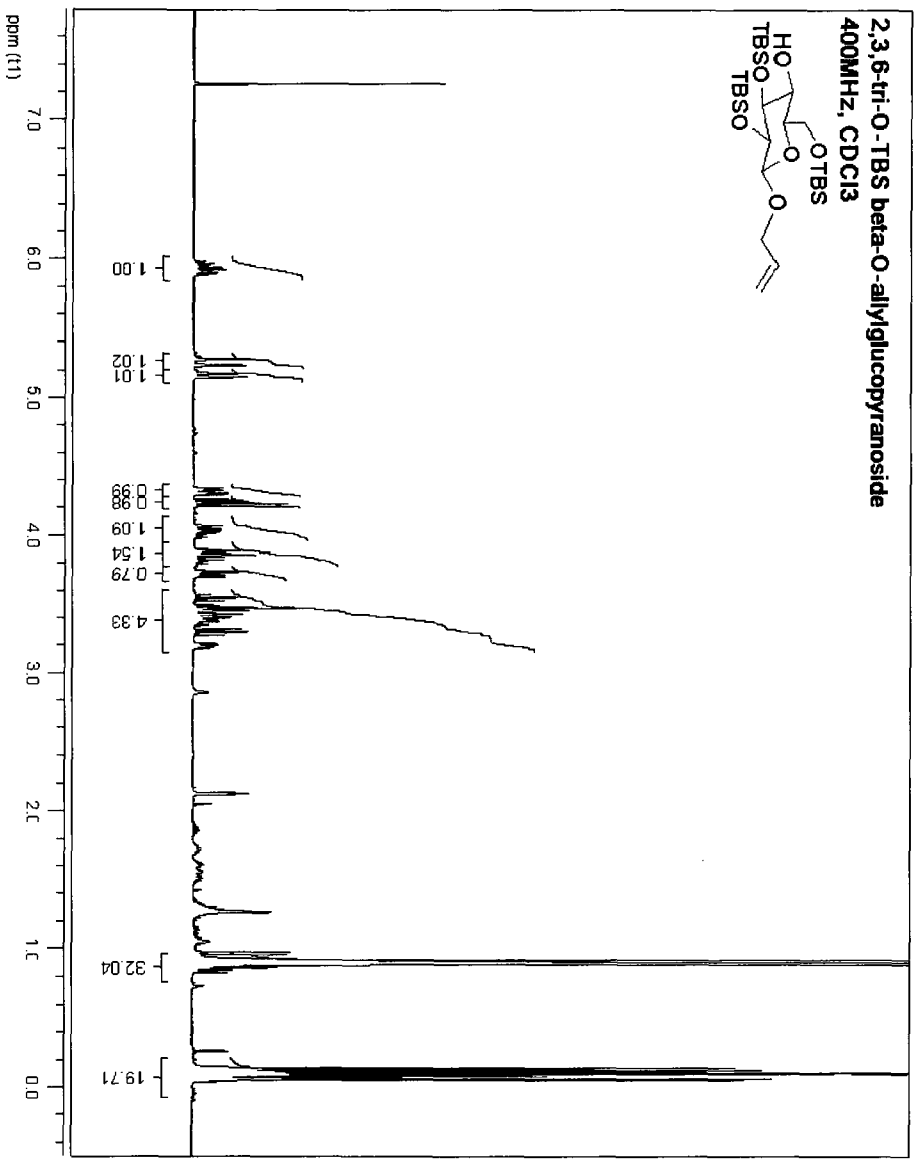

Figure a.1.25 ^{1}H NMR spectrum of 2,3,6-O-TBS -β-O-allylglucopyranoside (**16**) in $CDCl_3$

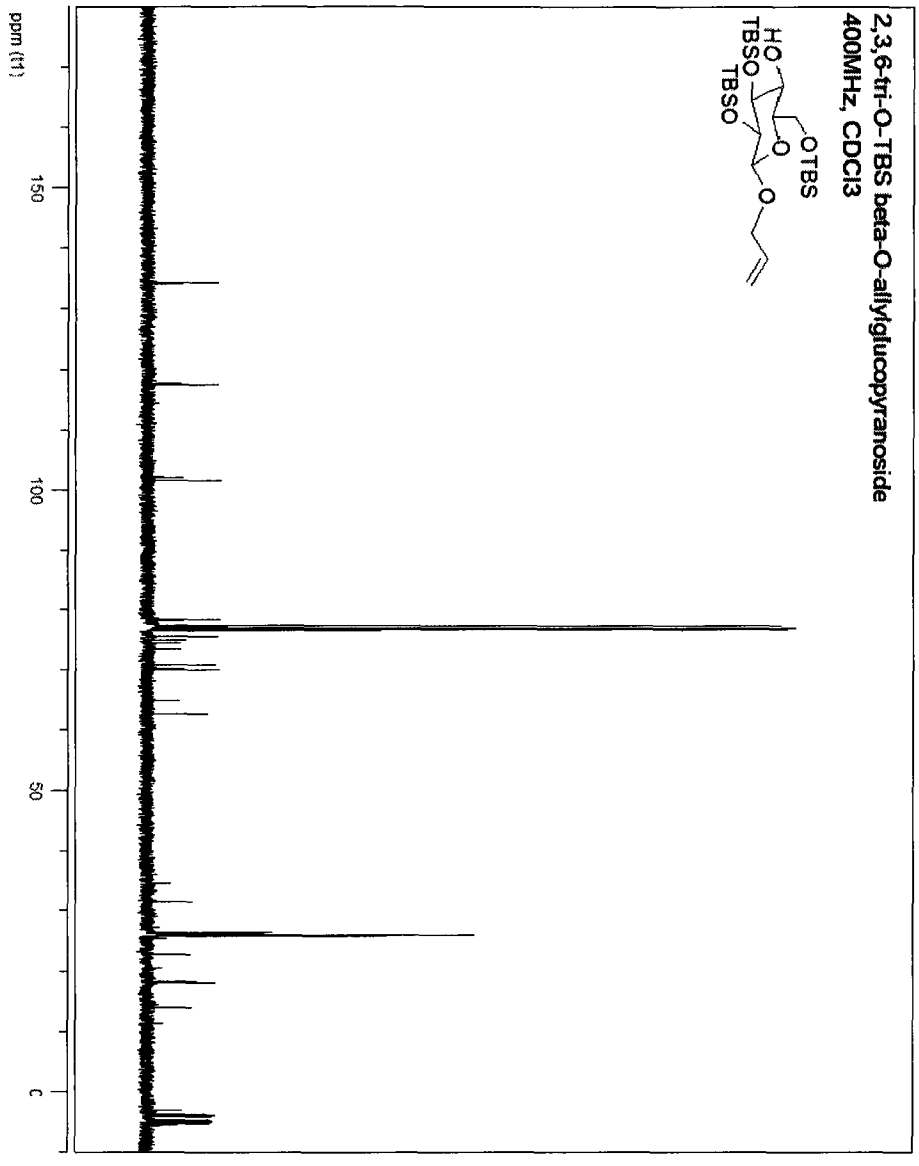

Figure a.1.26 ^{12}C NMR spectrum of 2,3,6-O-TBS -β-O-allylglucopyranoside (**16**) in $CDCl_3$

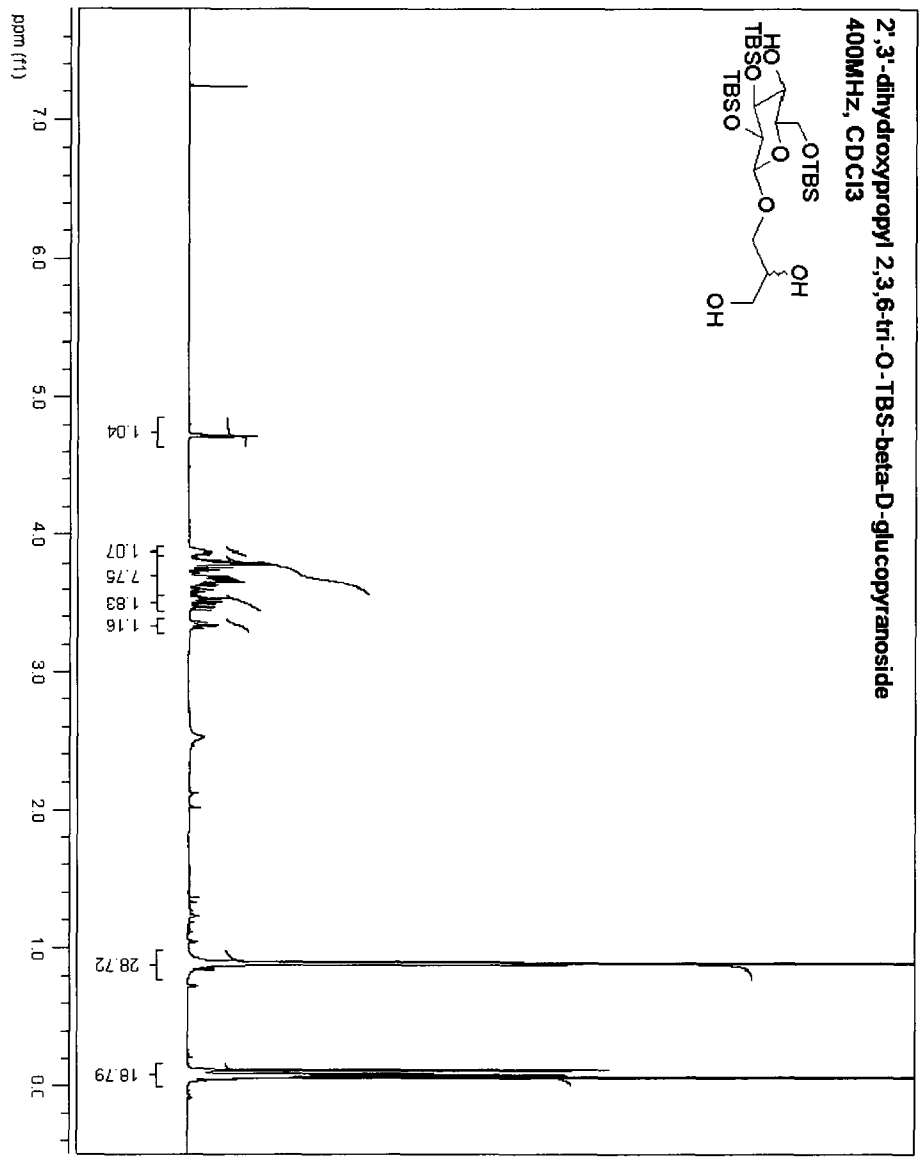

Figure a.1.27 ^{1}H NMR spectrum of 2',3'-dihydroxypropyl 2,3,6-O-TBS -β-O-allylglucopyranoside (**17**) in $CDCl_3$

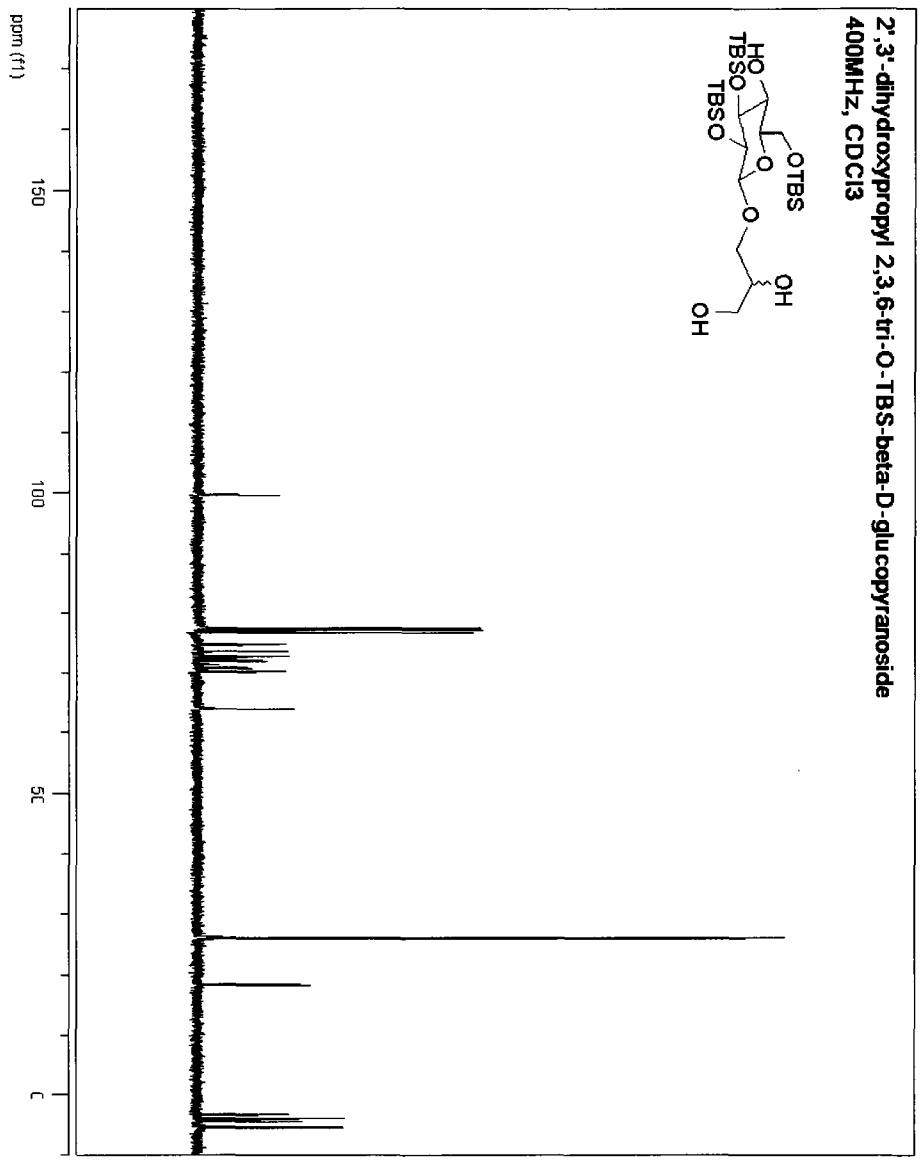

Figure a.1.28 ^{13}C NMR spectrum of 2',3'-dihydroxypropyl 2,3,6-O-TBS -β-O-allylglucopyranoside (**17**) in $CDCl_3$

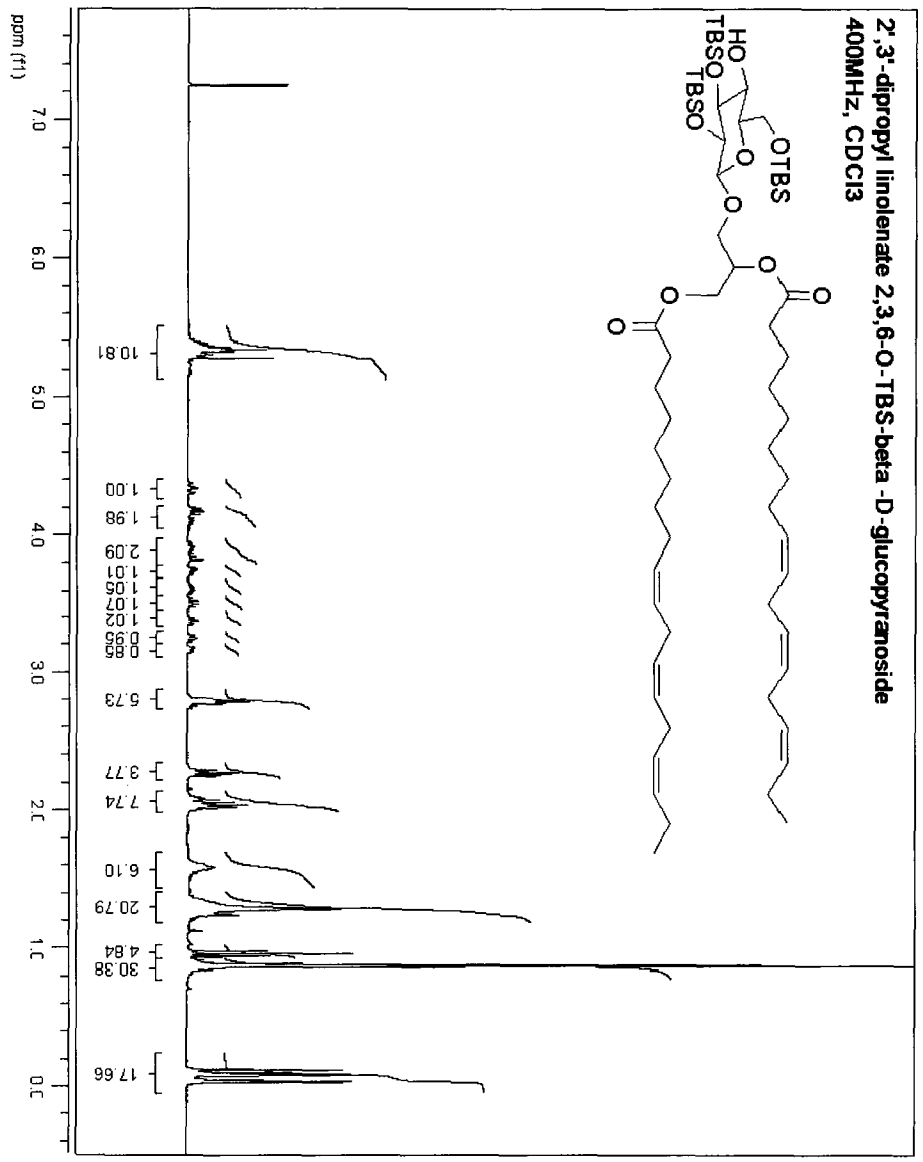

Figure a.1.29 ^{1}H NMR spectrum of 2',3'-dipropyl linolenatel 2,3,6-O-TBS -β-O-allylglucopyranoside (**18**) in $CDCl_3$

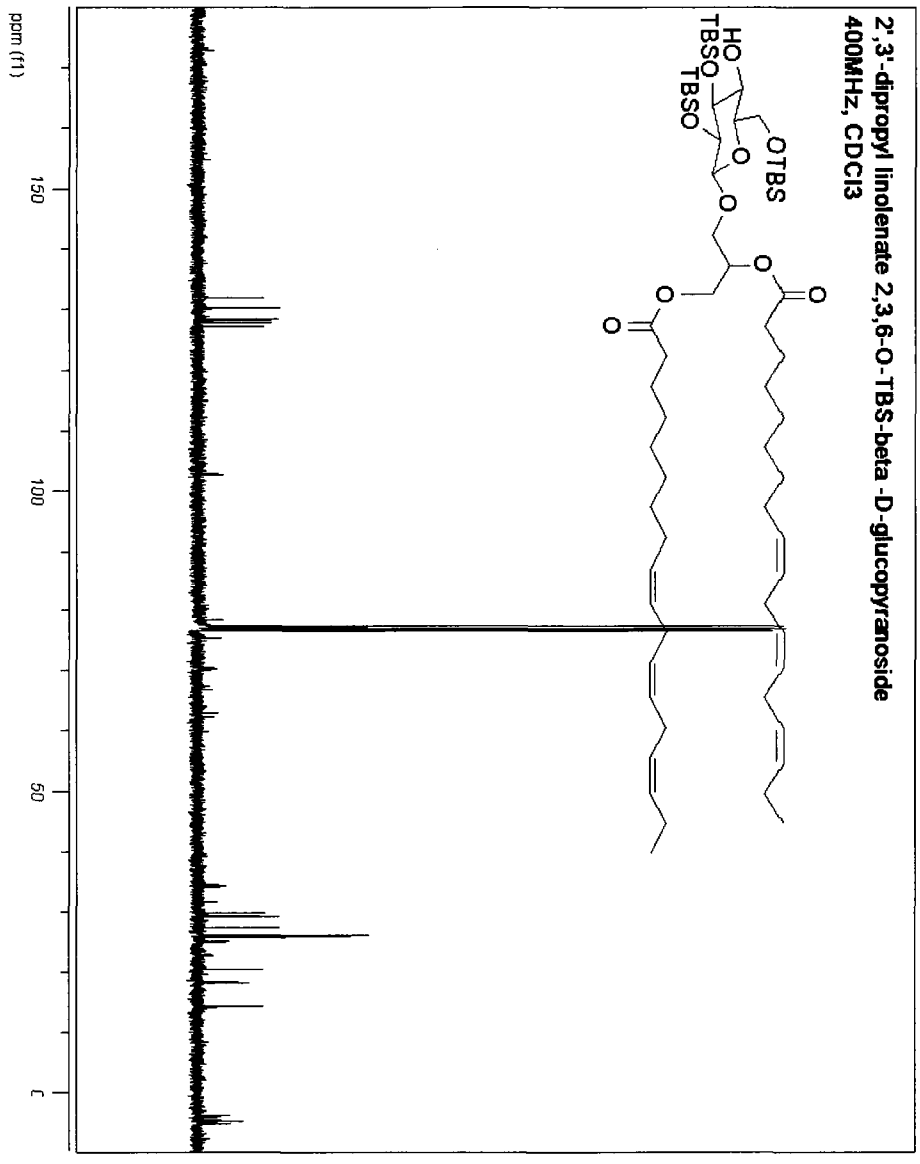

Figure a.1.30 ^{13}C NMR spectrum of 2',3'-dipropyl linolenatel 2,3,6-O-TBS -β-O-allylglucopyranoside (**18**) in $CDCl_3$

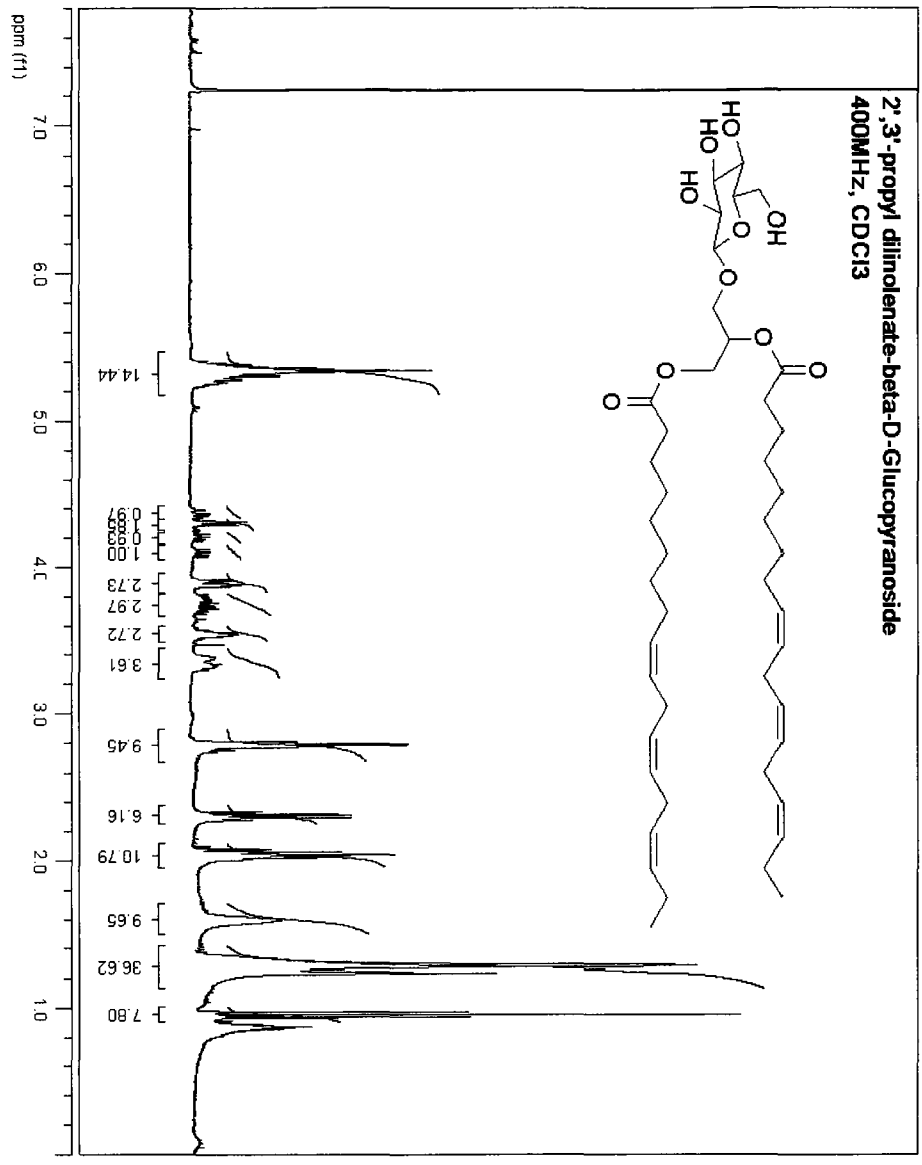

Figure a.1.31 ^{1}H NMR spectrum of 2',3'-dipropyl linoleate-β-O-allylglucopyranoside (**19**) in $CDCl_3$

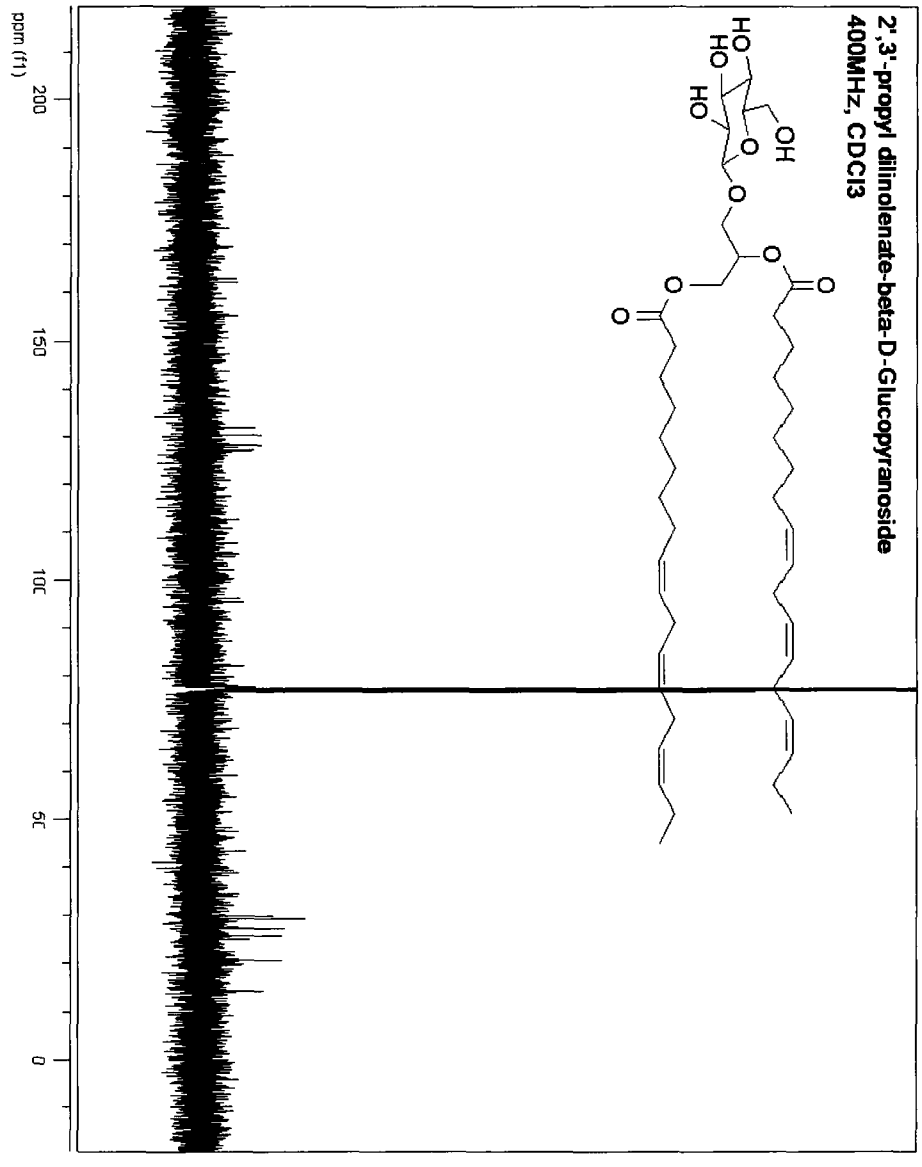

Figure a.1.32 ^{13}C NMR spectrum of 2',3'-dipropyl linoleate-β-O-allylglucopyranoside (**19**) in $CDCl_3$

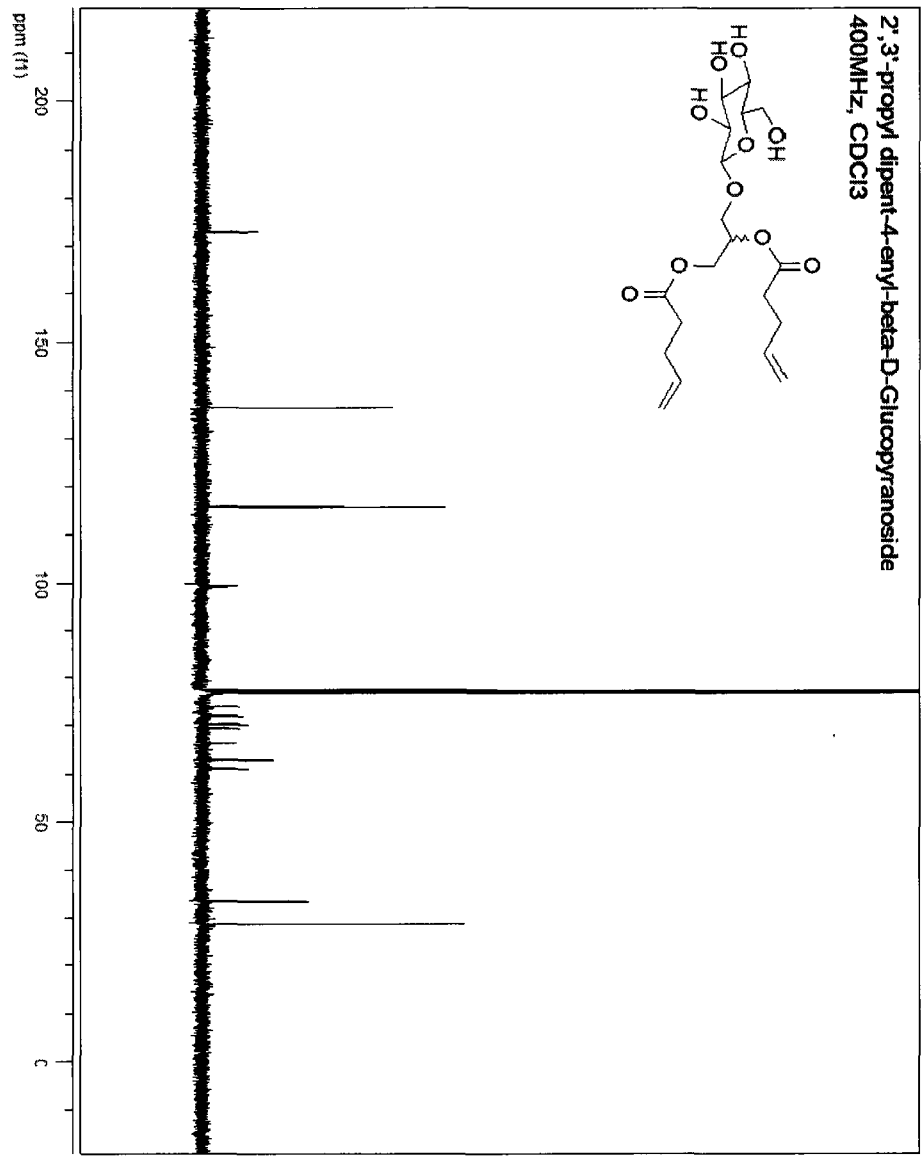

Figure a.1.33 ^{13}C NMR spectrum of 2',3'-dipropyl linoleate-β-O-allylglucopyranoside (**21**) in $CDCl_3$

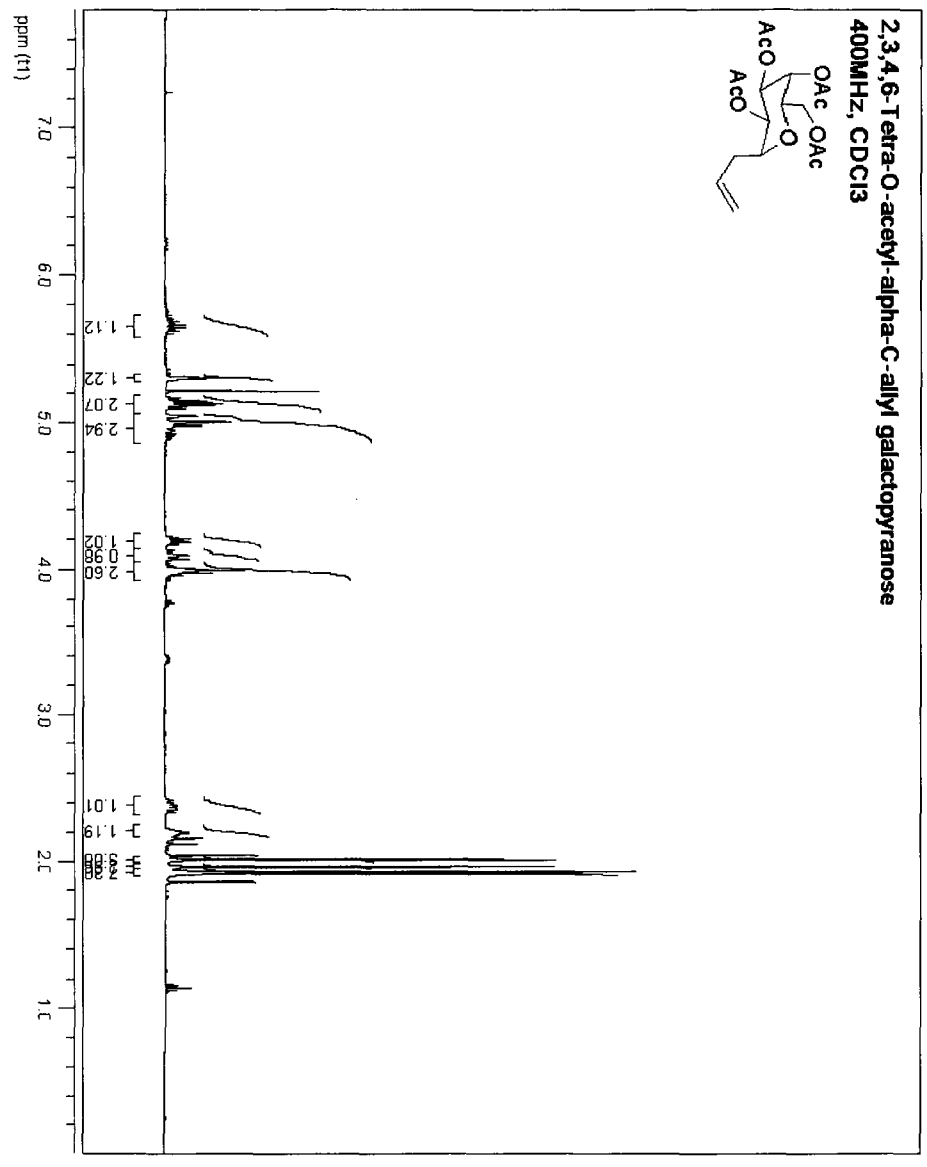

Figure a.1.34 ^{1}H NMR spectrum of 2,3,4,6-tetra-*O*-acetyl α-*C*-allyl galactopyranose (**22**) in $CDCl_3$

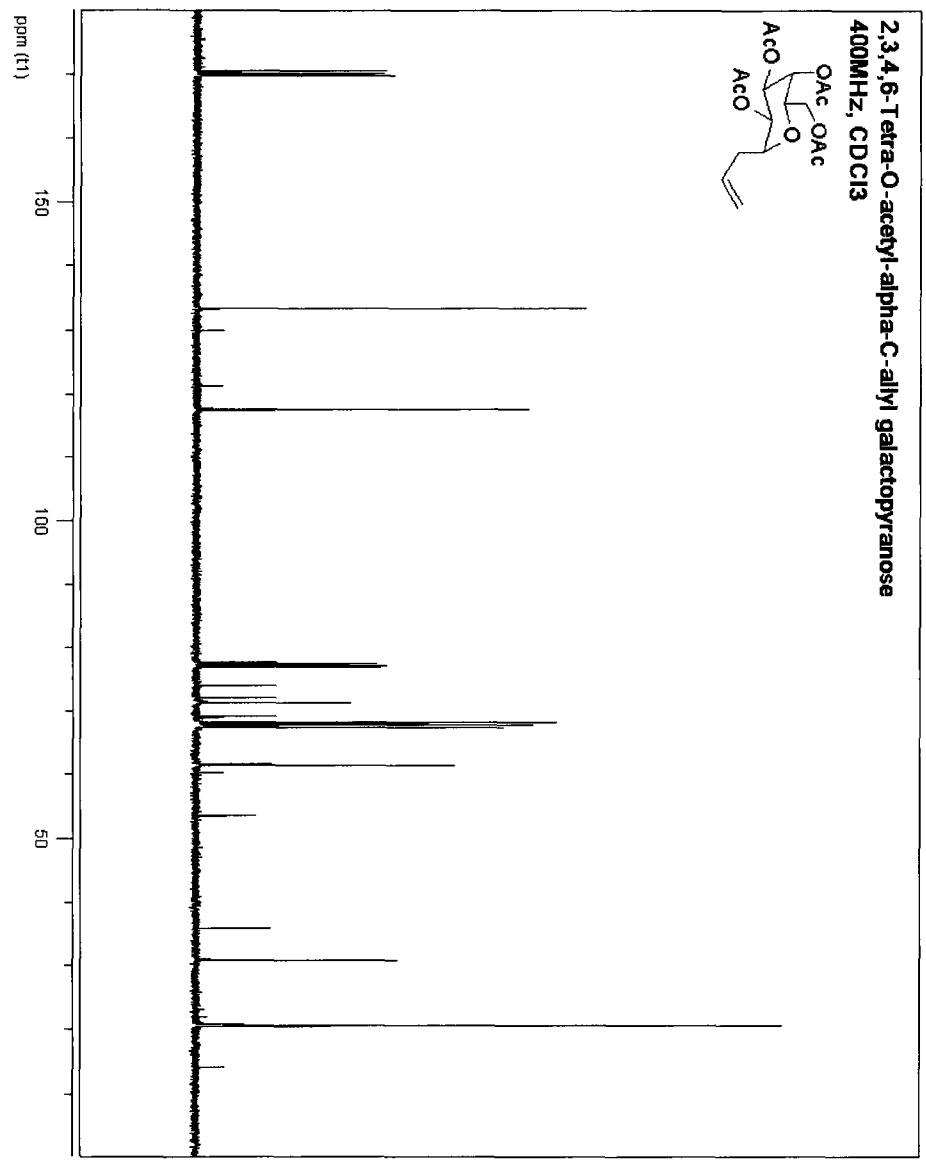

Figure a.1.35 ^{13}C NMR spectrum of 2,3,4,6-tetra-*O*-acetyl α-*C*-allyl galactopyranose (**22**) in $CDCl_3$

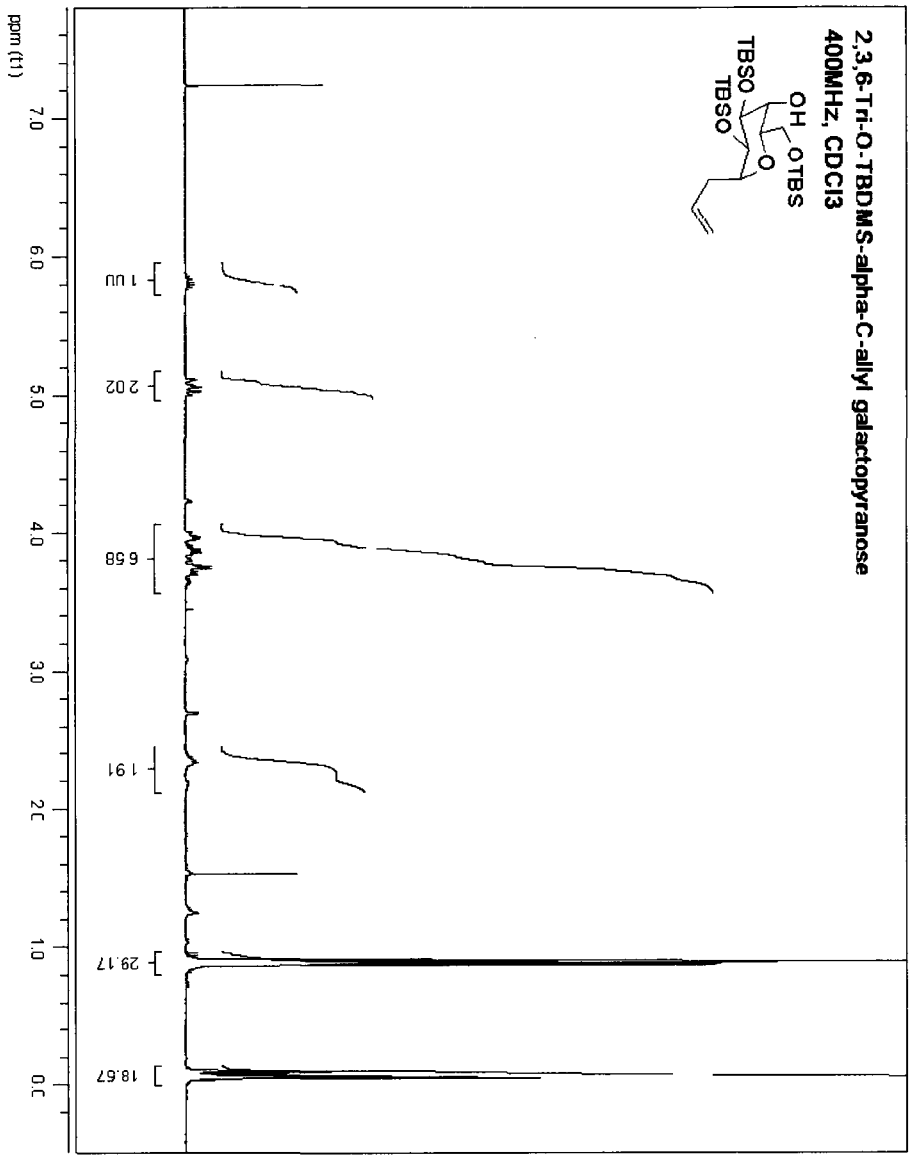

Figure a.1.36 ^{1}H NMR spectrum of 2,3,6-trio-*O*-TBDMS α-*C*-allyl galactopyranose (**23**) in $CDCl_3$

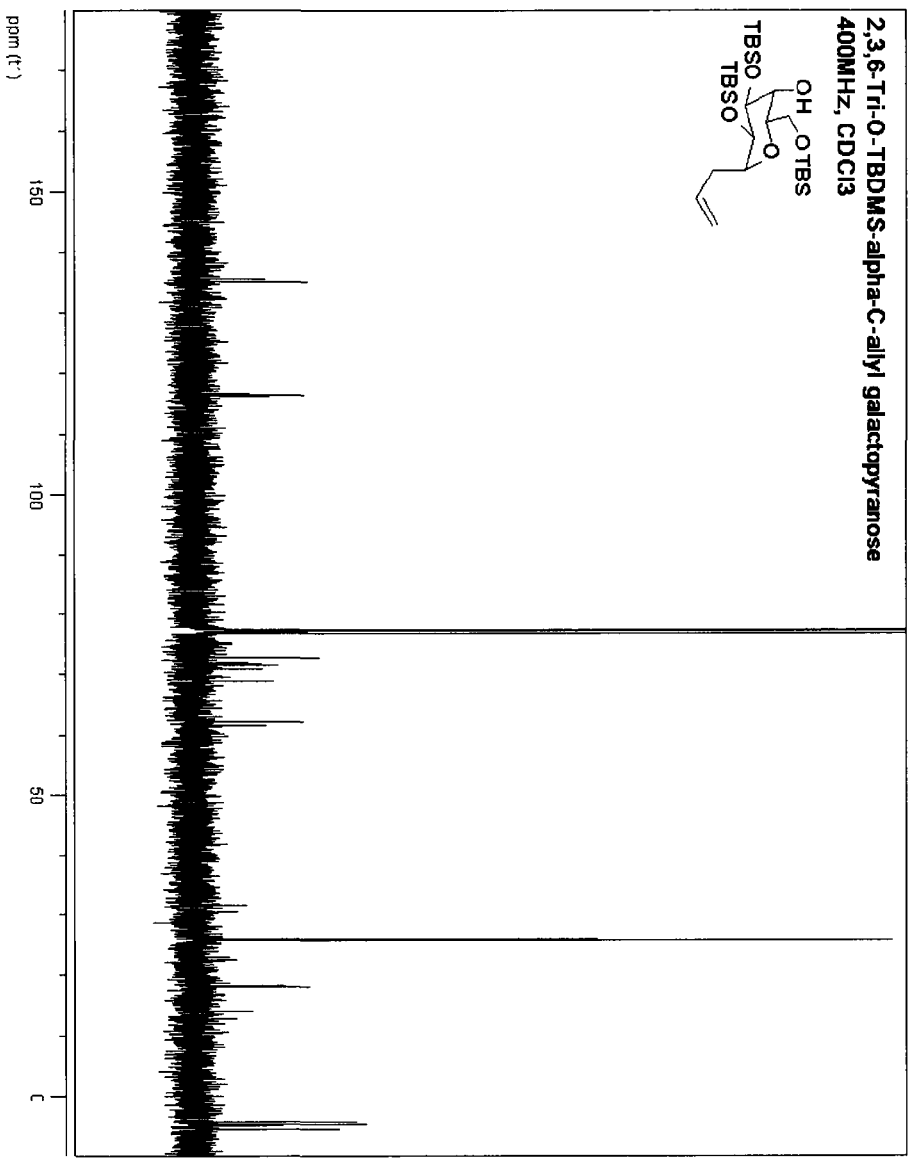

Figure a.1.37 ^{13}C NMR spectrum of 2,3,6-trio-*O*-TBDMS α-*C*-allyl galactopyranose (**23**) in $CDCl_3$

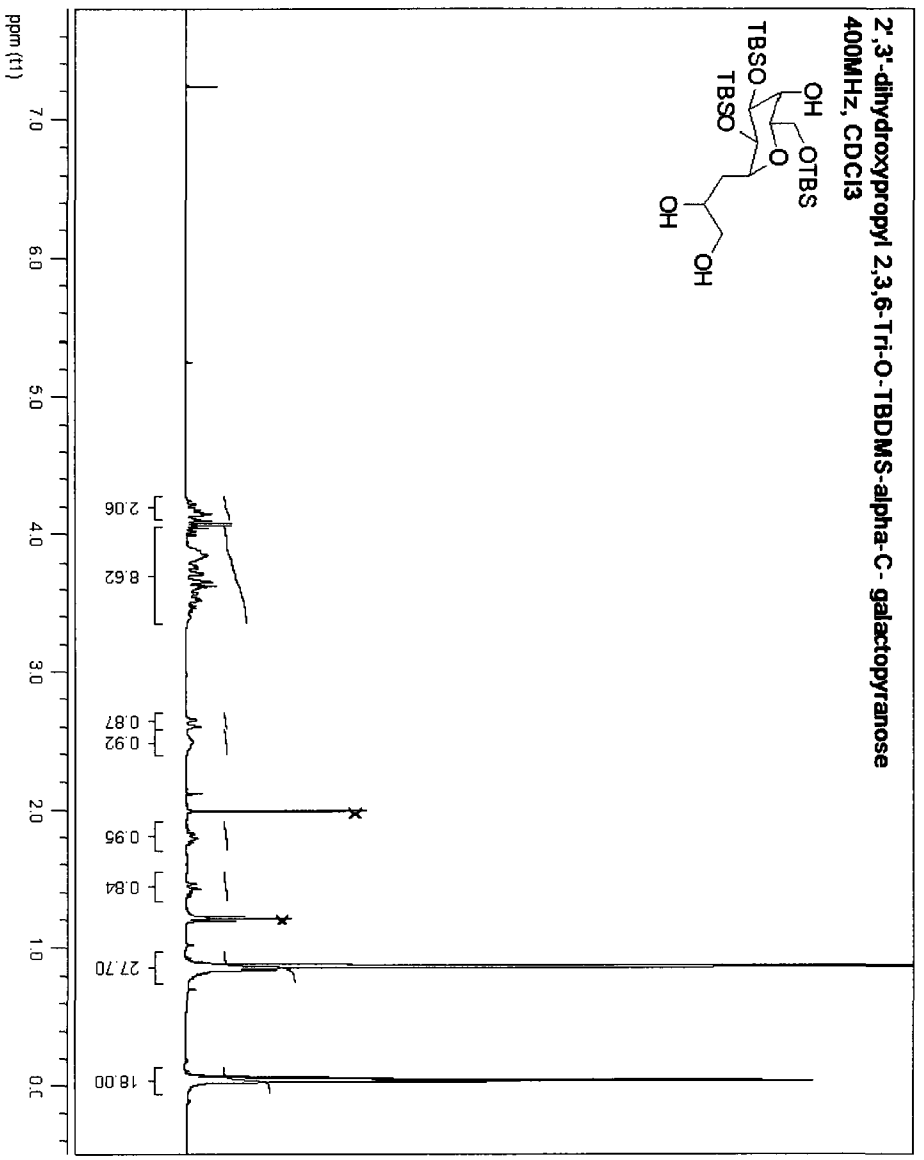

Figure a.1.38 ^{1}H NMR spectrum of 2',3'-dihydroxypropyl 2,3,6-trio-*O*-TBDMS *α*-*C*-allyl galactopyranose (**24**) in $CDCl_3$

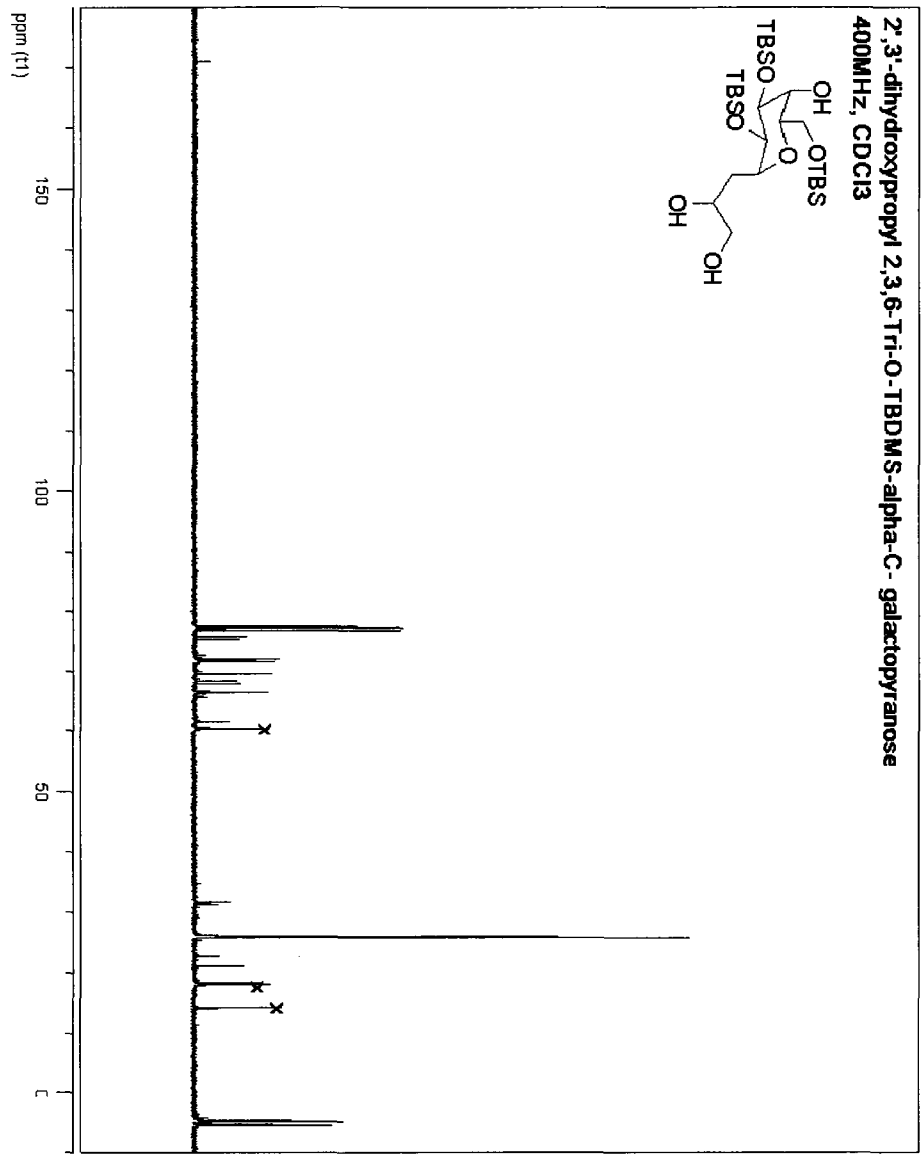

Figure a.1.39 ^{13}C NMR spectrum of 2',3'-dihydroxypropyl 2,3,6-trio-*O*-TBDMS *α*-*C*-allyl galactopyranose (**24**) in $CDCl_3$

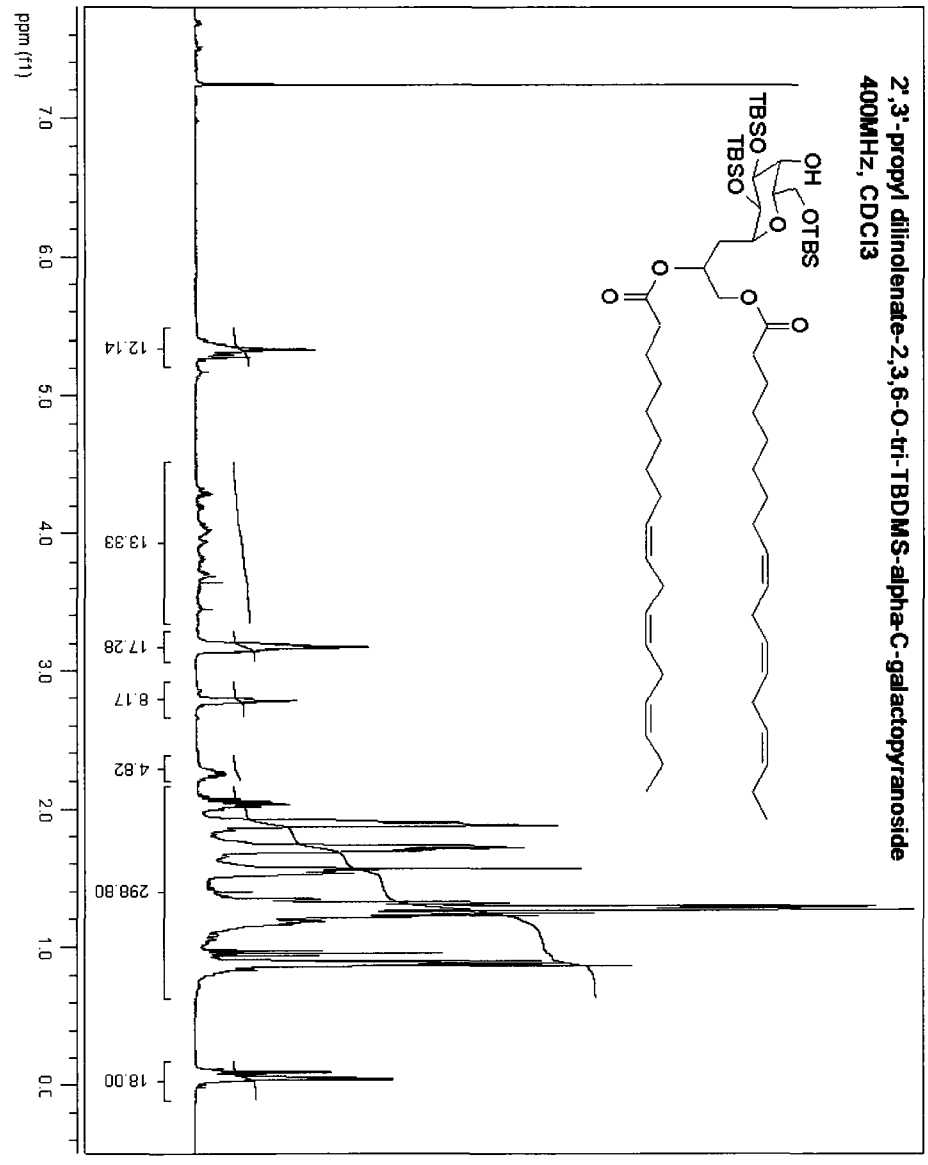

Figure a.1.40 ^{1}H NMR spectrum of 2',3'-propyl dilinolenyl-2,3,6-trio-*O*-TBDMS *α-C* galactopyranose (**25**) in $CDCl_3$

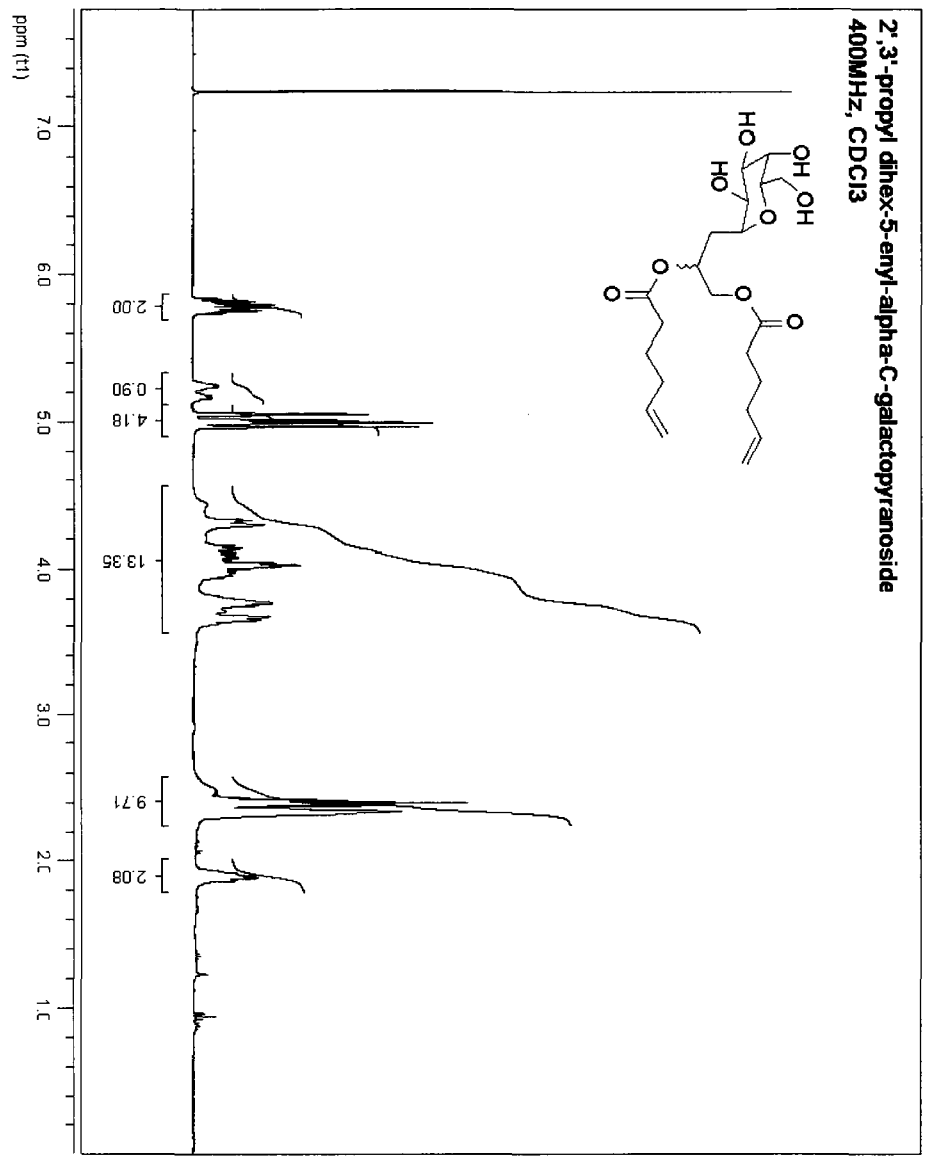

Figure a.1.41 ^{1}H NMR spectrum of 2',3'-propyl dihex-5-enyl-2 *α-C-* galactopyranose (**28**) in $CDCl_3$

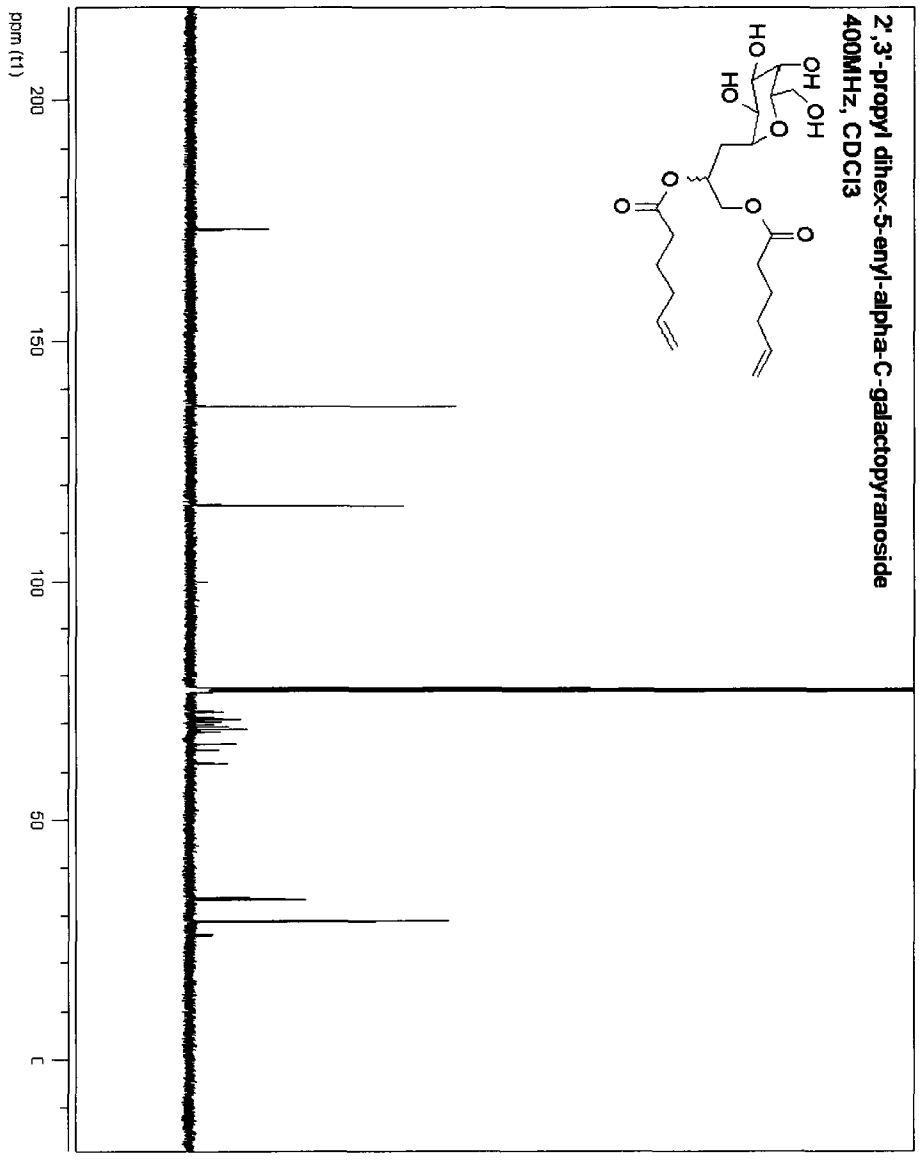

Figure a.1.42 ^{13}C NMR spectrum of 2',3'-propyl dihex-5-enyl-2 *α-C-* galactopyranose (**28**) in $CDCl_3$

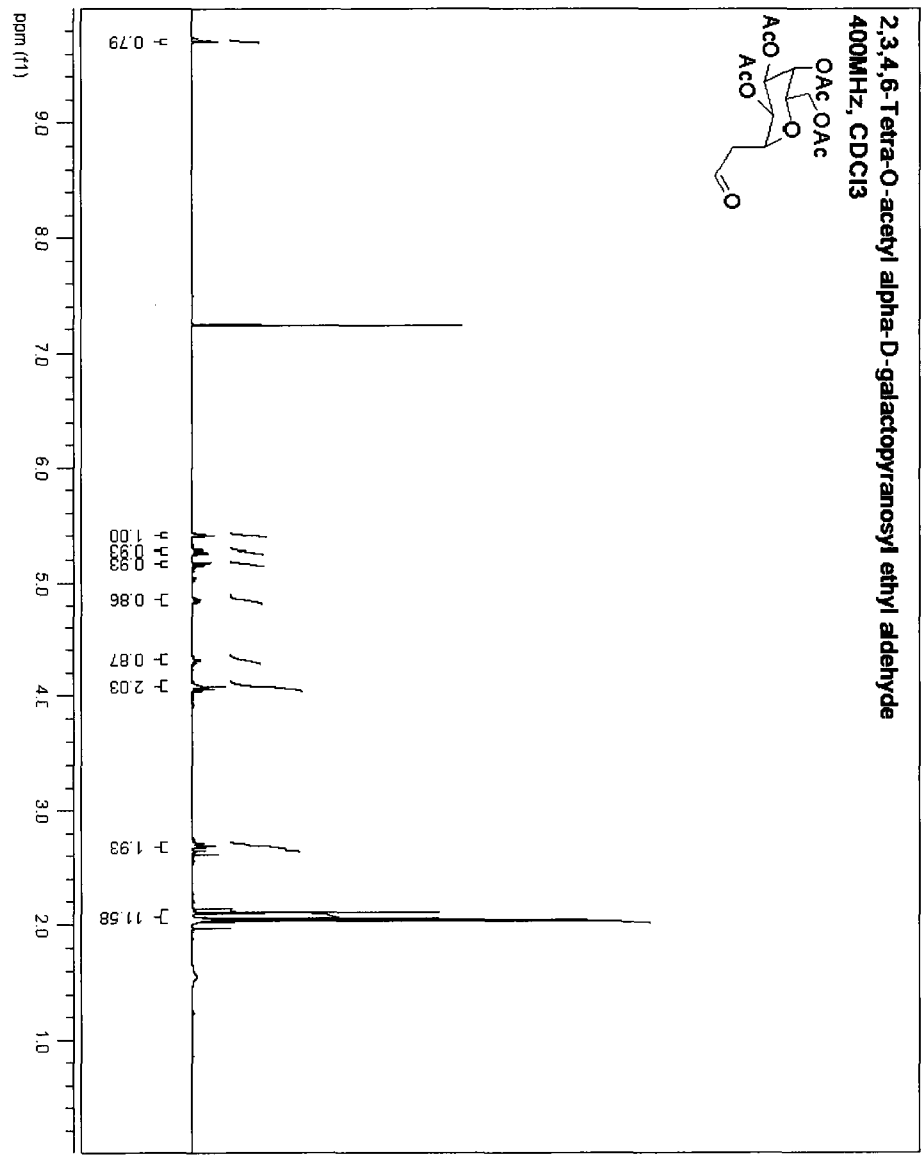

Figure a.1.43 ^{1}H NMR spectrum of 2,3,4,6-tetra-*O*=acetyl *α-D*-galactopyranosyl ethyl aldehyde (**29**) in $CDCl_3$

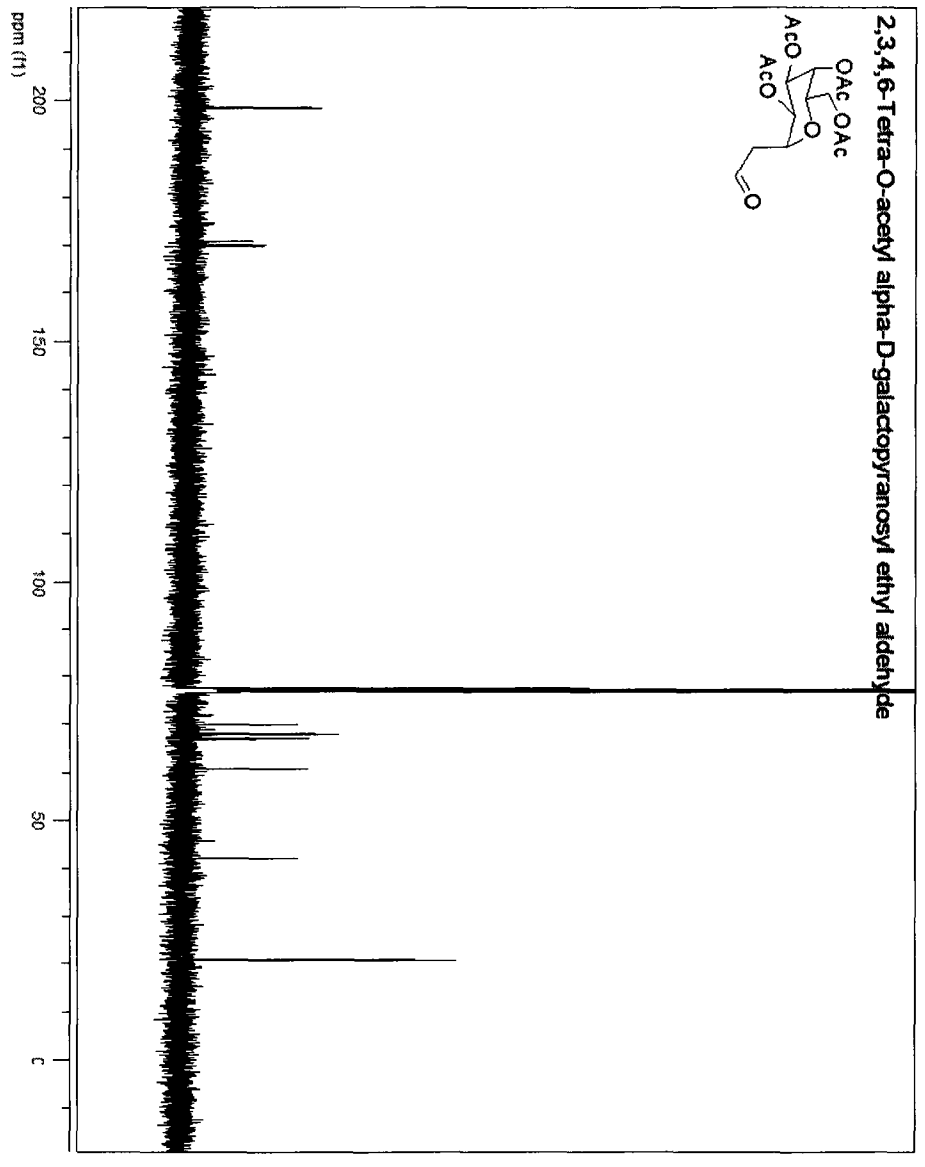

Figure a.1.44 ^{13}C NMR spectrum of 2,3,4,6-tetra-*O*=acetyl *α-D*-galactopyranosyl ethyl aldehyde (**29**) in $CDCl_3$

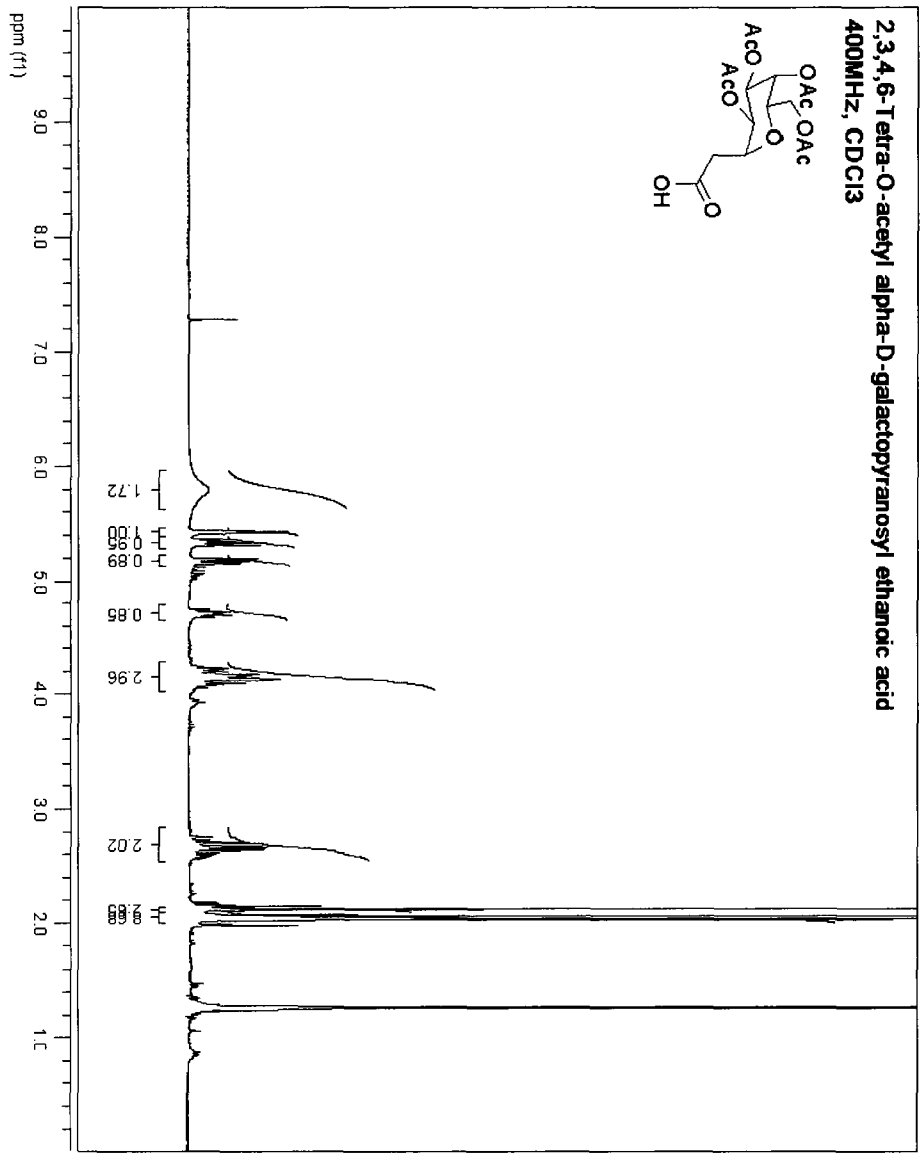

Figure a.1.45 ^{1}H NMR spectrum of 2,3,4,6-tetra-*O*=acetyl *α-D*-galactopyranosyl ethanoic acid (**30**) in $CDCl_3$

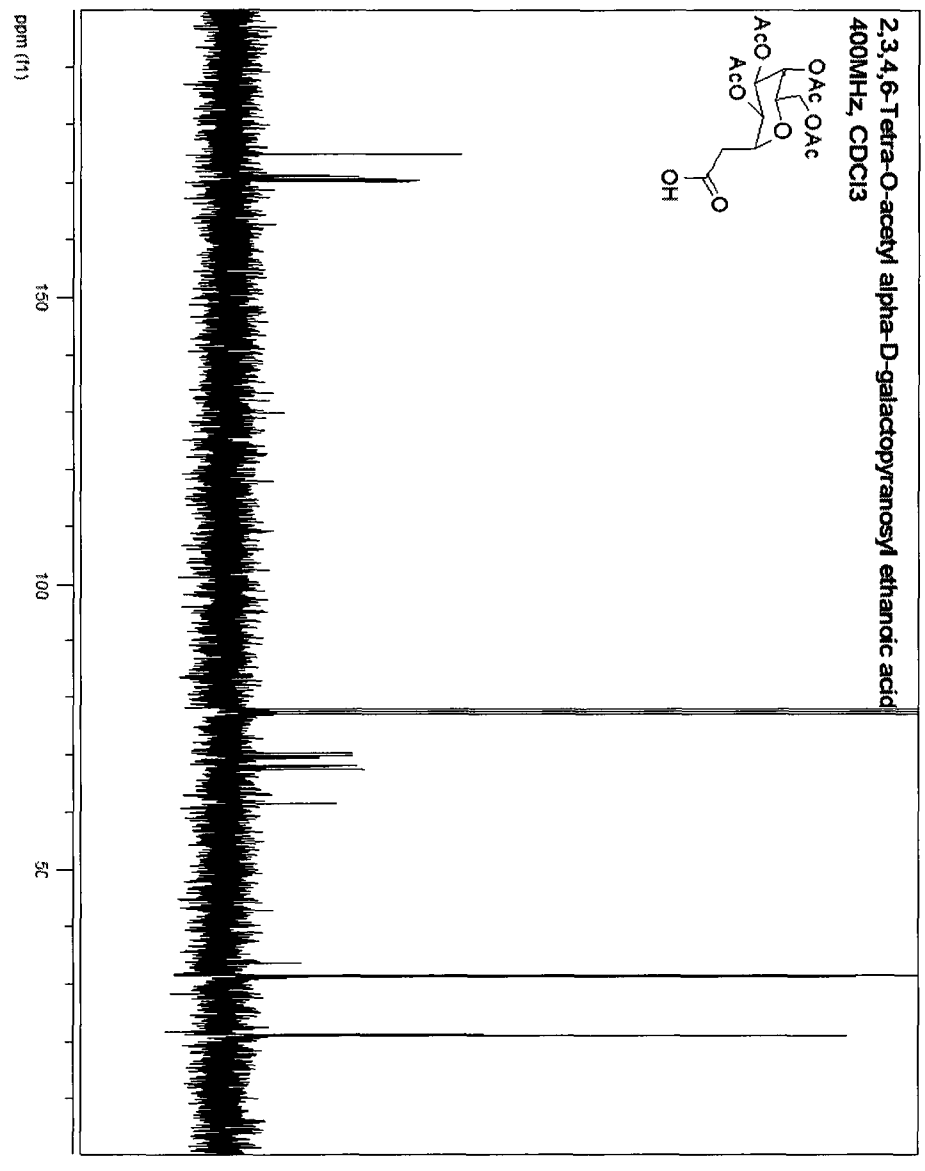

Figure a.1.46 ^{13}C NMR spectrum of 2,3,4,6-tetra-*O*=acetyl *α*-*D*-galactopyranosyl ethanoic acid (**30**) in $CDCl_3$

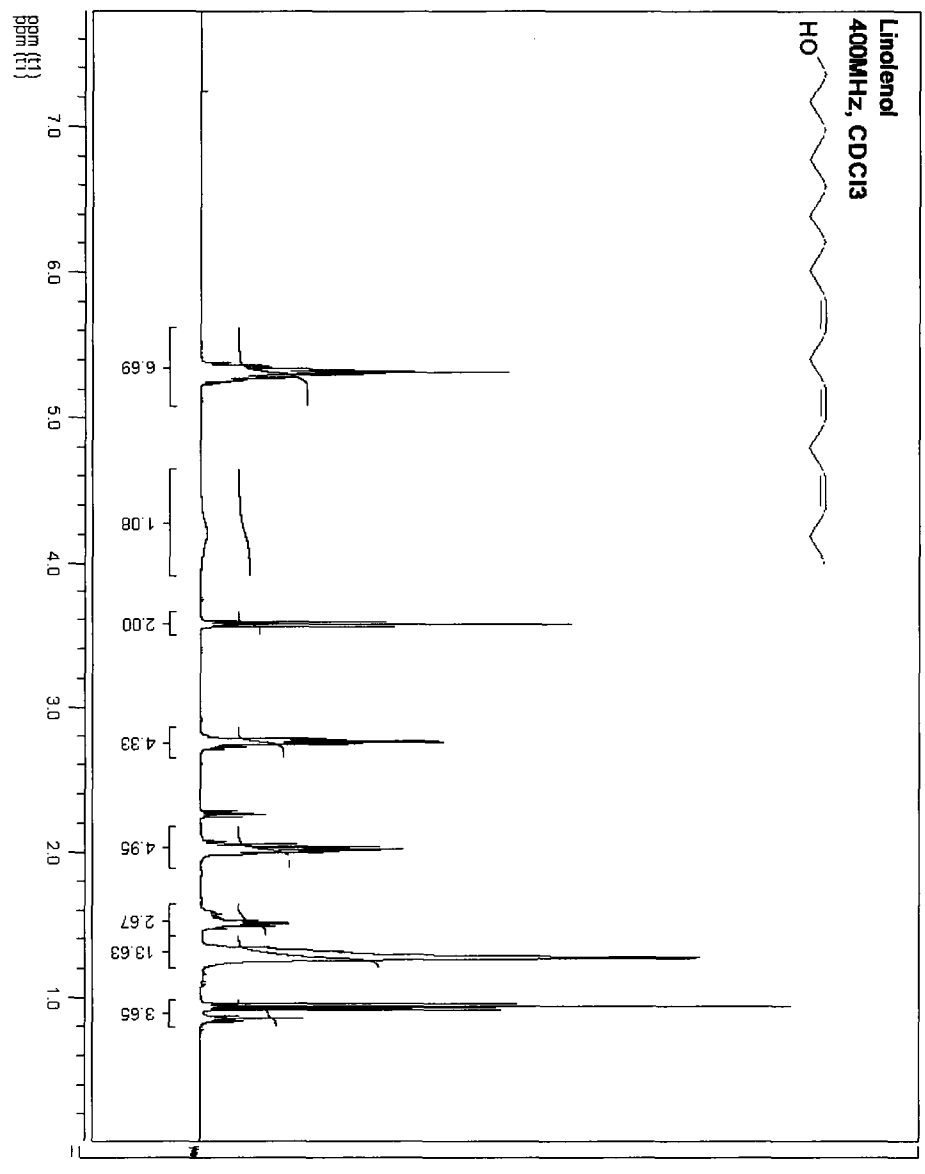

Figure a.1.47 ^{1}H NMR spectrum of linolenol (**31**) in $CDCl_3$

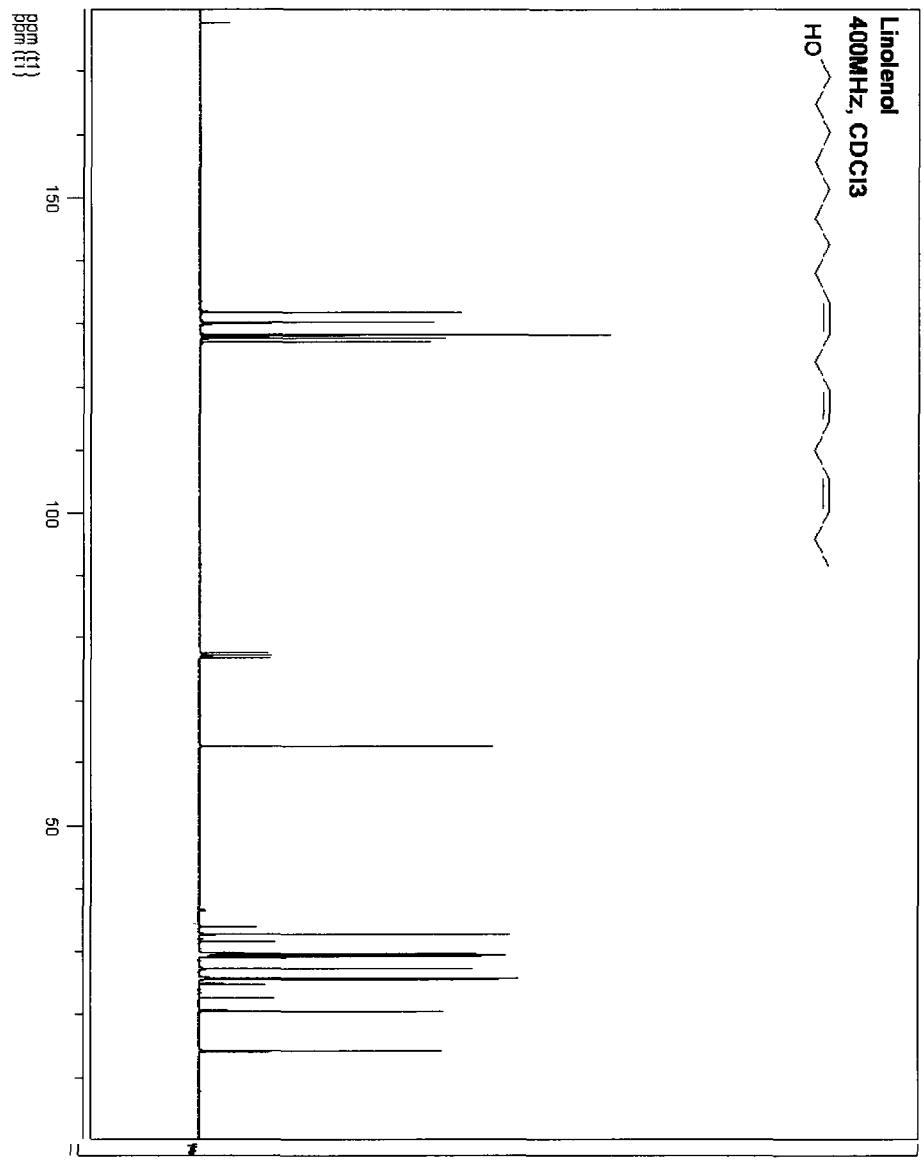

Figure a.1.48 ^{13}C NMR spectrum of linolenol (**31**) in $CDCl_3$

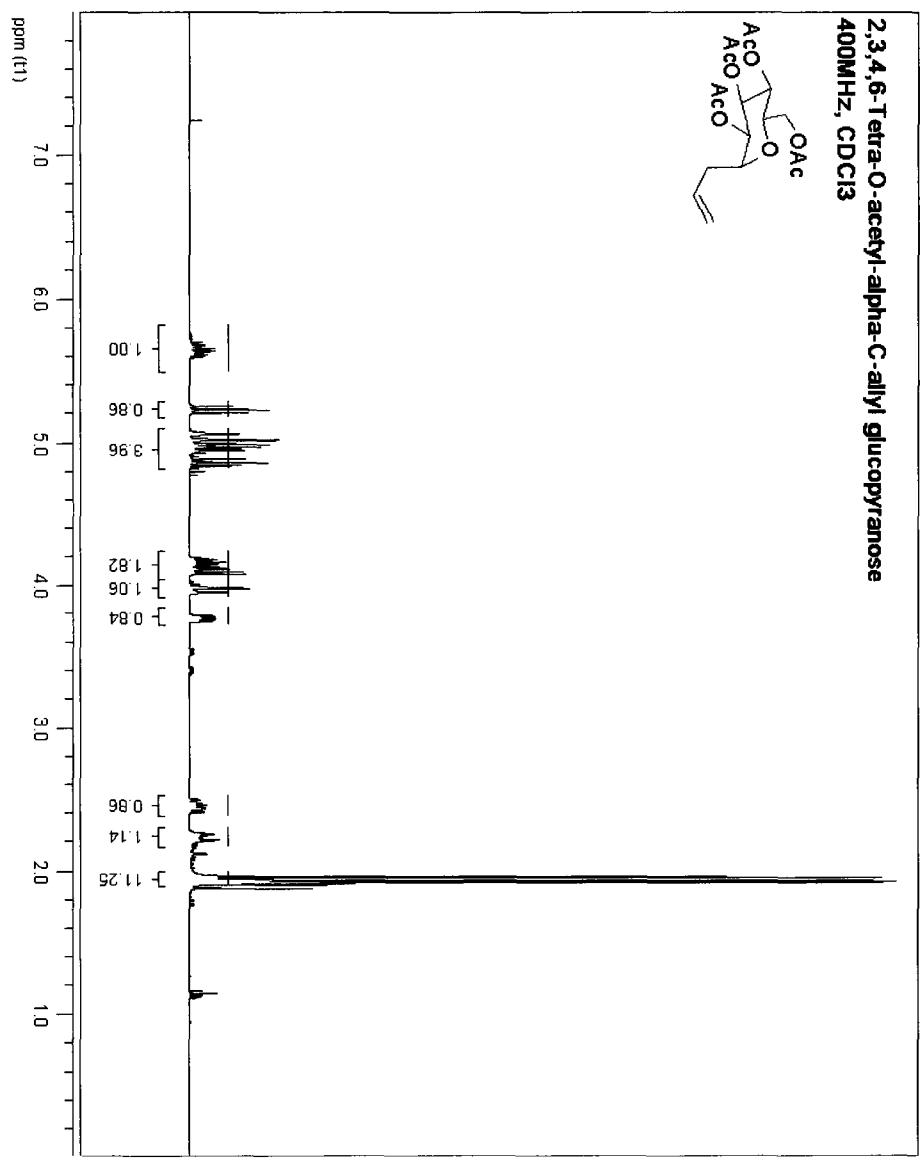

Figure a.1.49 ^{1}H NMR spectrum of 2,3,4,6-tetra-*O*-acetyl-α-*C*-allyl glucopyranose (**33**) in $CDCl_3$

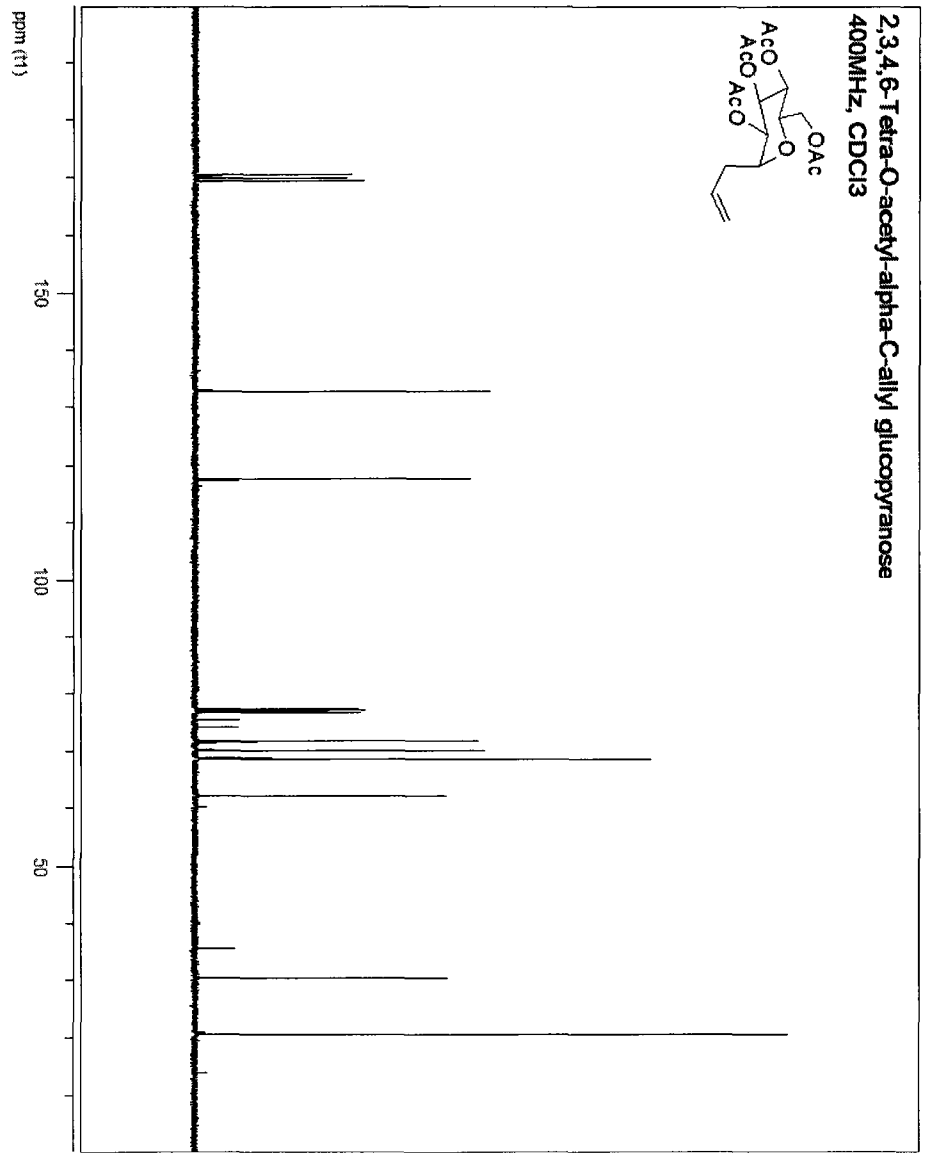

Figure a.1.50 ^{13}C NMR spectrum of 2,3,4,6-tetra-*O*-acetyl-α-*C*-allyl glucopyranose (**33**) in $CDCl_3$

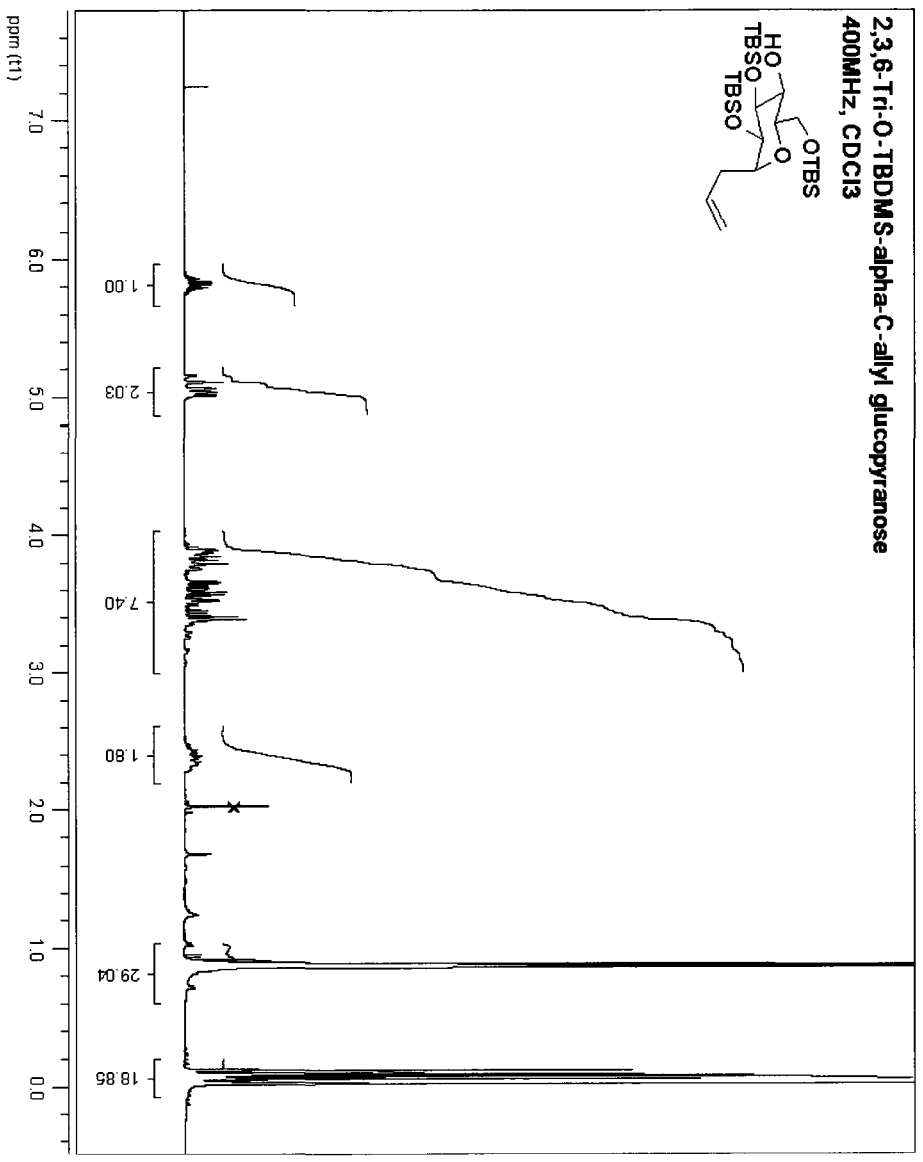

Figure a.1.51 ^{1}H NMR spectrum of 2,3,6-tri-*O*-TBDMS-*α*-*C*-allyl glucopyranose (**34**) in $CDCl_3$

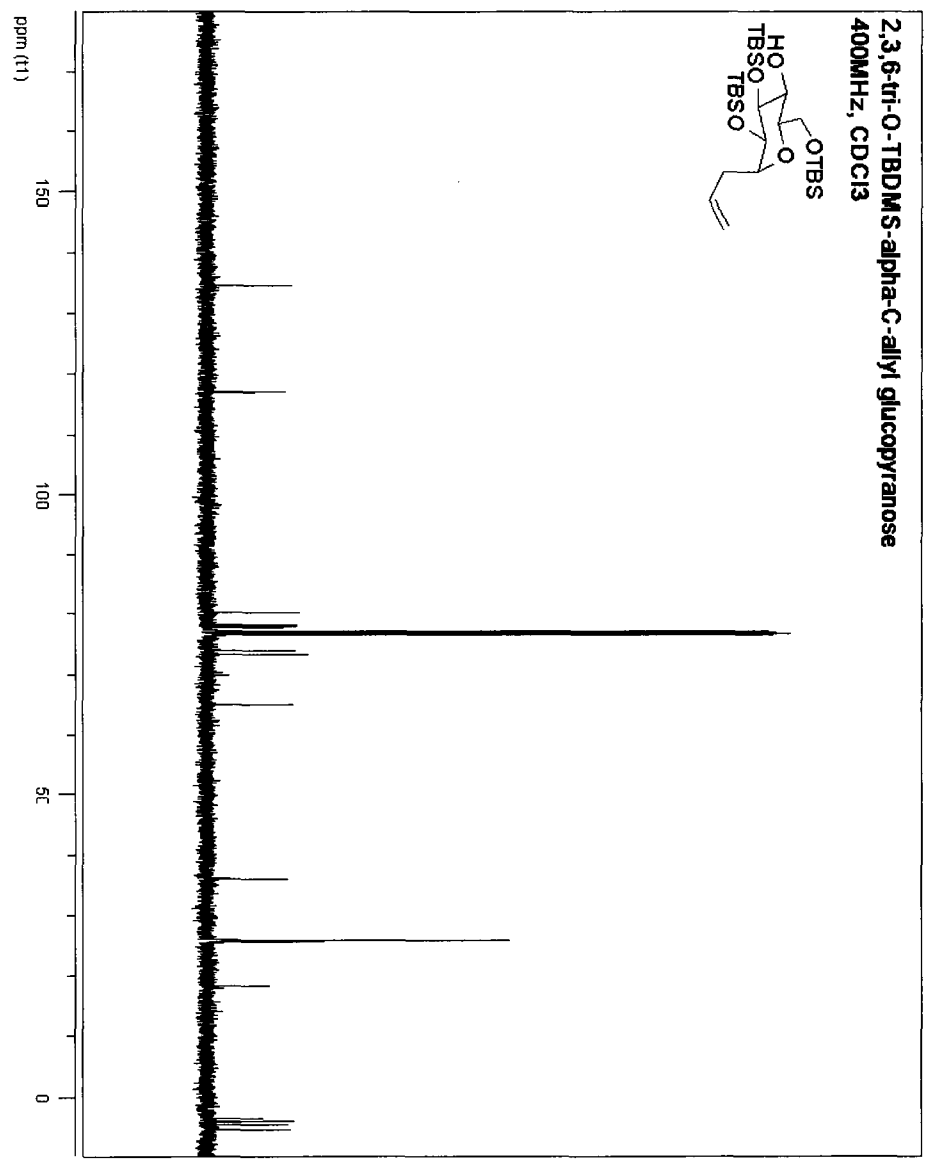

Figure a.1.53 ^{13}C NMR spectrum of 2,3,6-tri-*O*-TBDMS-α-*C*-allyl glucopyranose (**34**) in $CDCl_3$

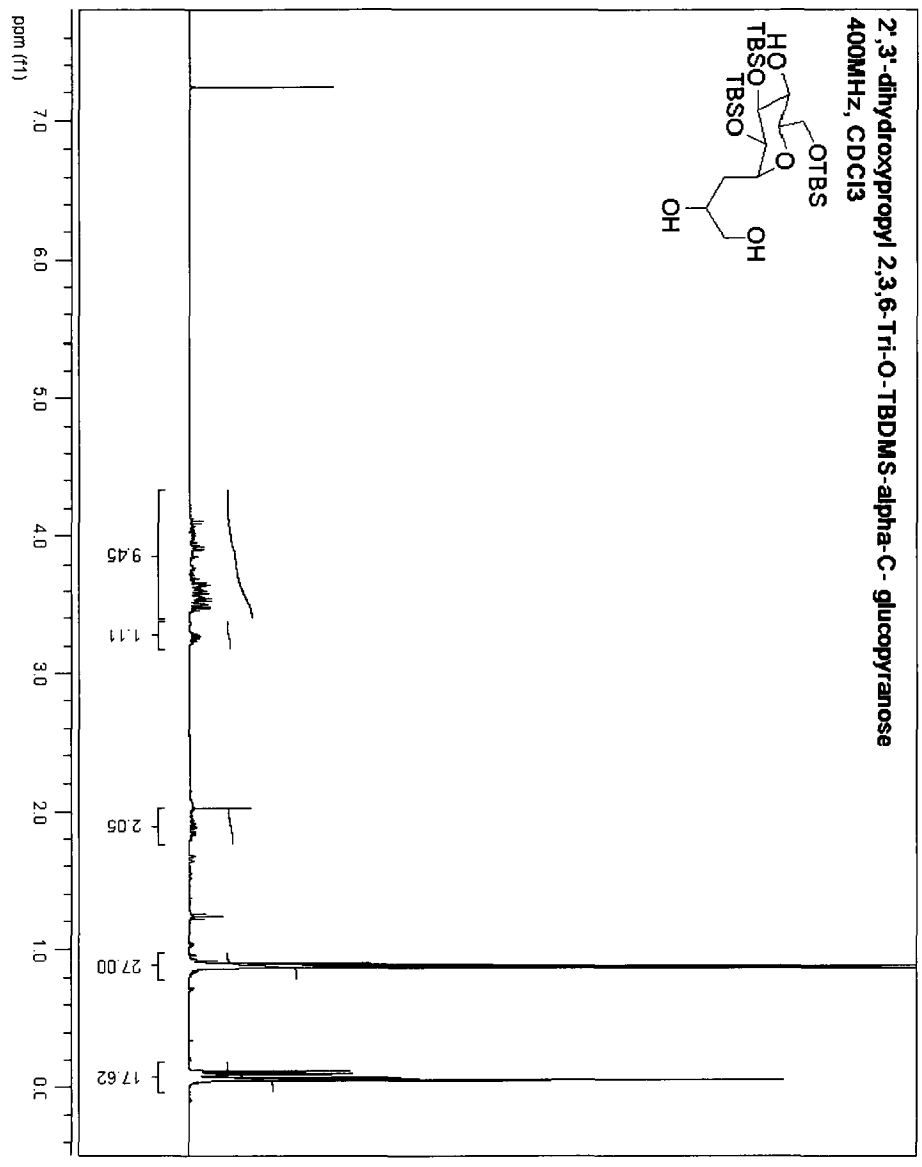

Figure a.1.54 ^{1}H NMR spectrum of 2',3'-dihydroxypropyl 2,3,6-tri-*O*-TBDMS-α-*C*-allyl glucopyranose (**35**) in $CDCl_3$

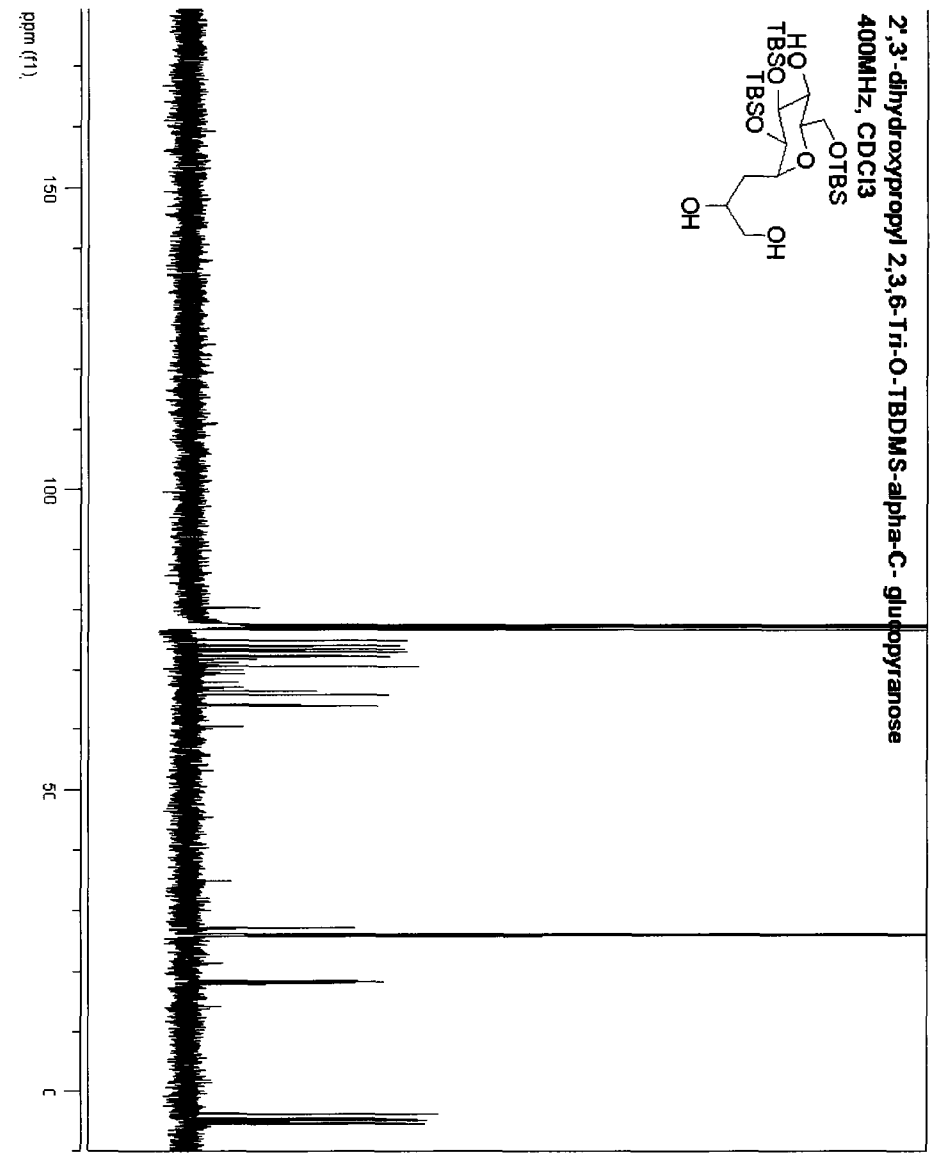

Figure a.1.55 ^{13}C NMR spectrum of 2',3'-dihydroxypropyl 2,3,6-tri-*O*-TBDMS-α-*C*-allyl glucopyranose (**35**) in $CDCl_3$

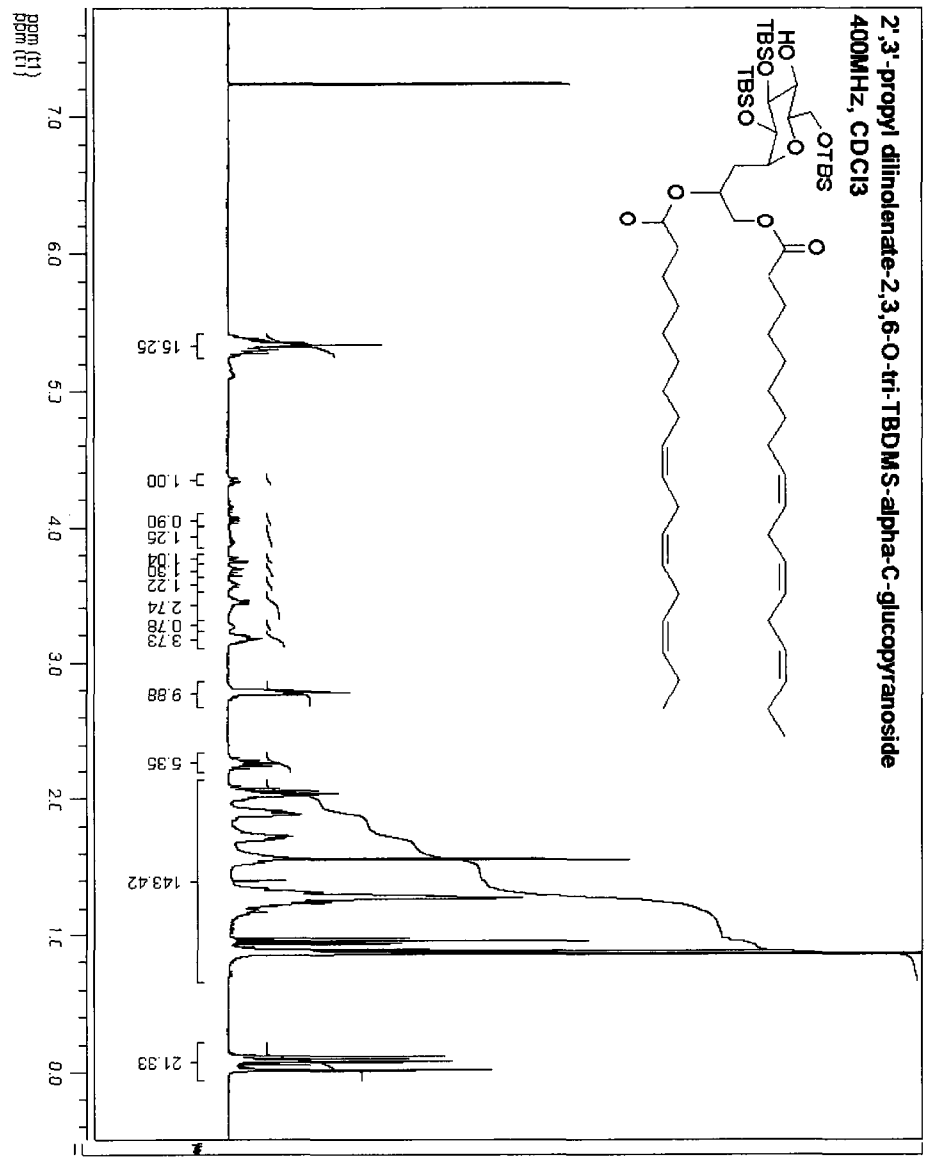

Figure a.1.56 ^{1}H NMR spectrum of 2',3'-propyl dilinolenyl 2,3,6-tri-*O*-TBDMS-α-*C*-allyl glucopyranose (**36**) in $CDCl_3$

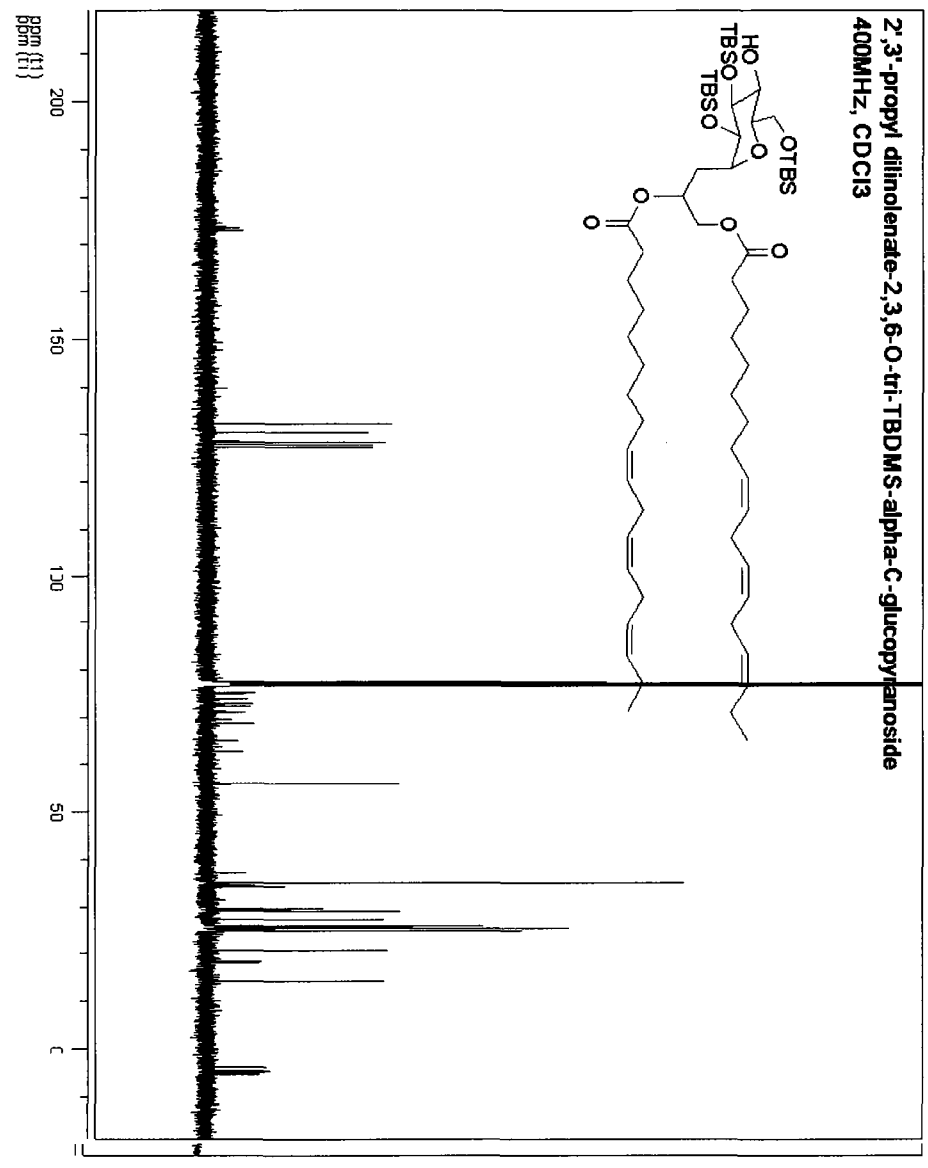

Figure a.1.57 ^{13}C NMR spectrum of 2',3'-propyl dilinolenyl 2,3,6-tri-*O*-TBDMS-α-*C*-allyl glucopyranose (**36**) in $CDCl_3$

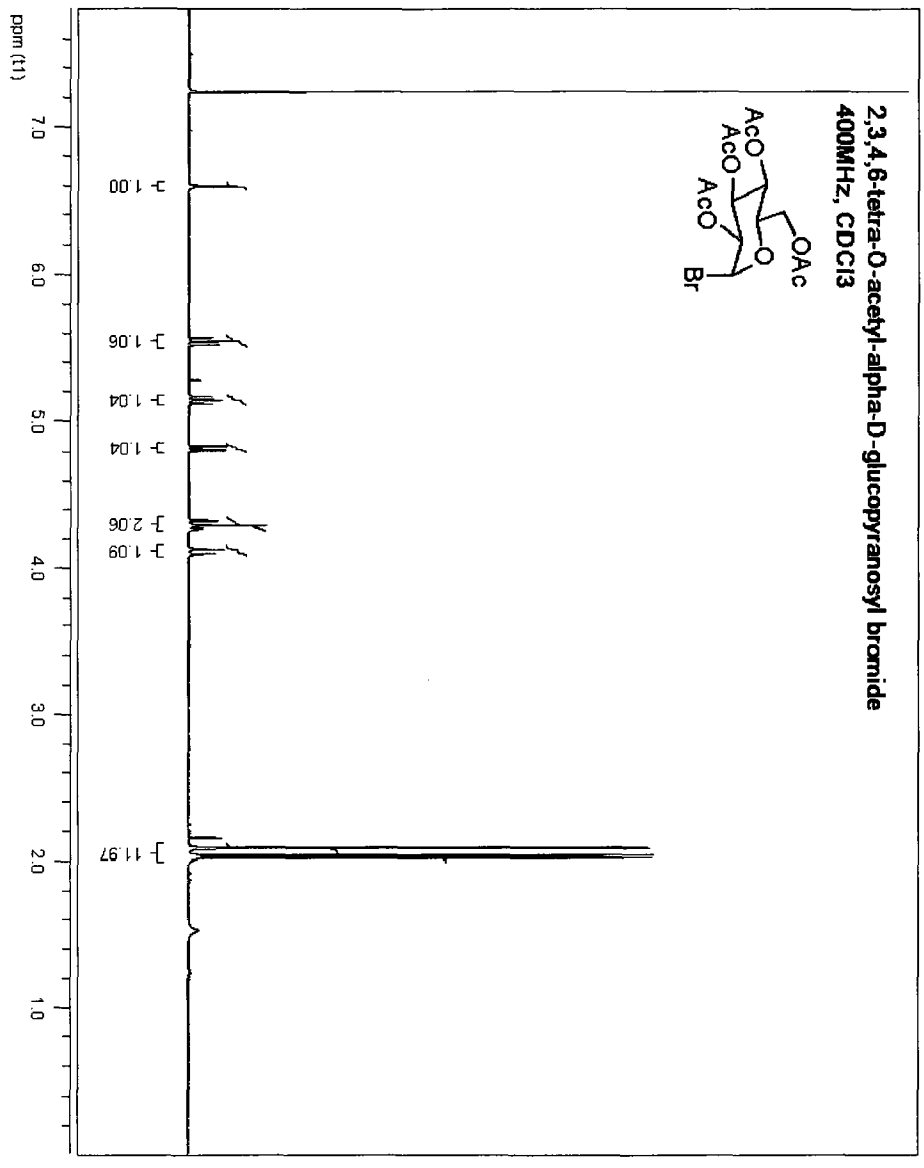

Figure a.1.58 ^{1}H NMR spectrum of 2,3,4,6-tetra-*O*-acetyl-*α*-*D*-glucopyranosyl bromide (**37**) in $CDCl_3$

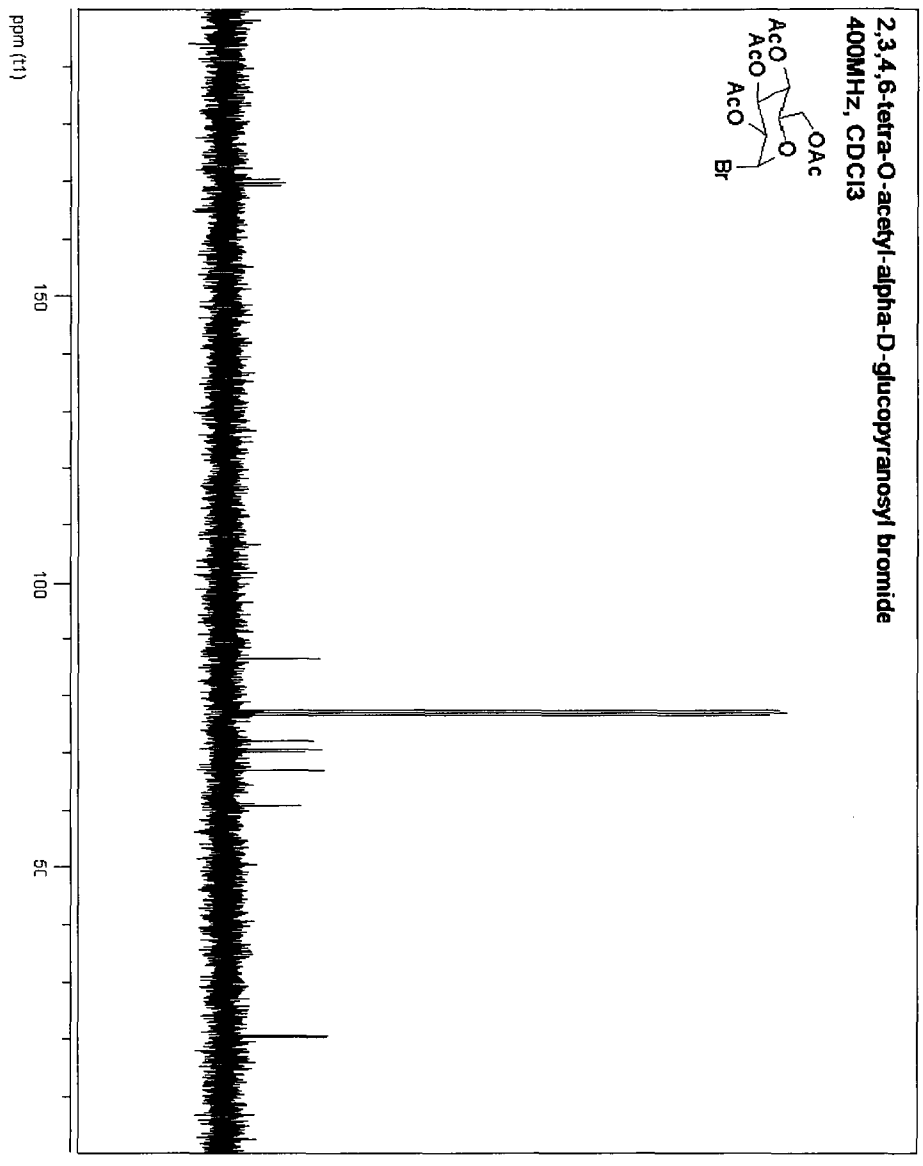

Figure a.1.59 ^{13}C NMR spectrum of 2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl bromide (**37**) in $CDCl_3$

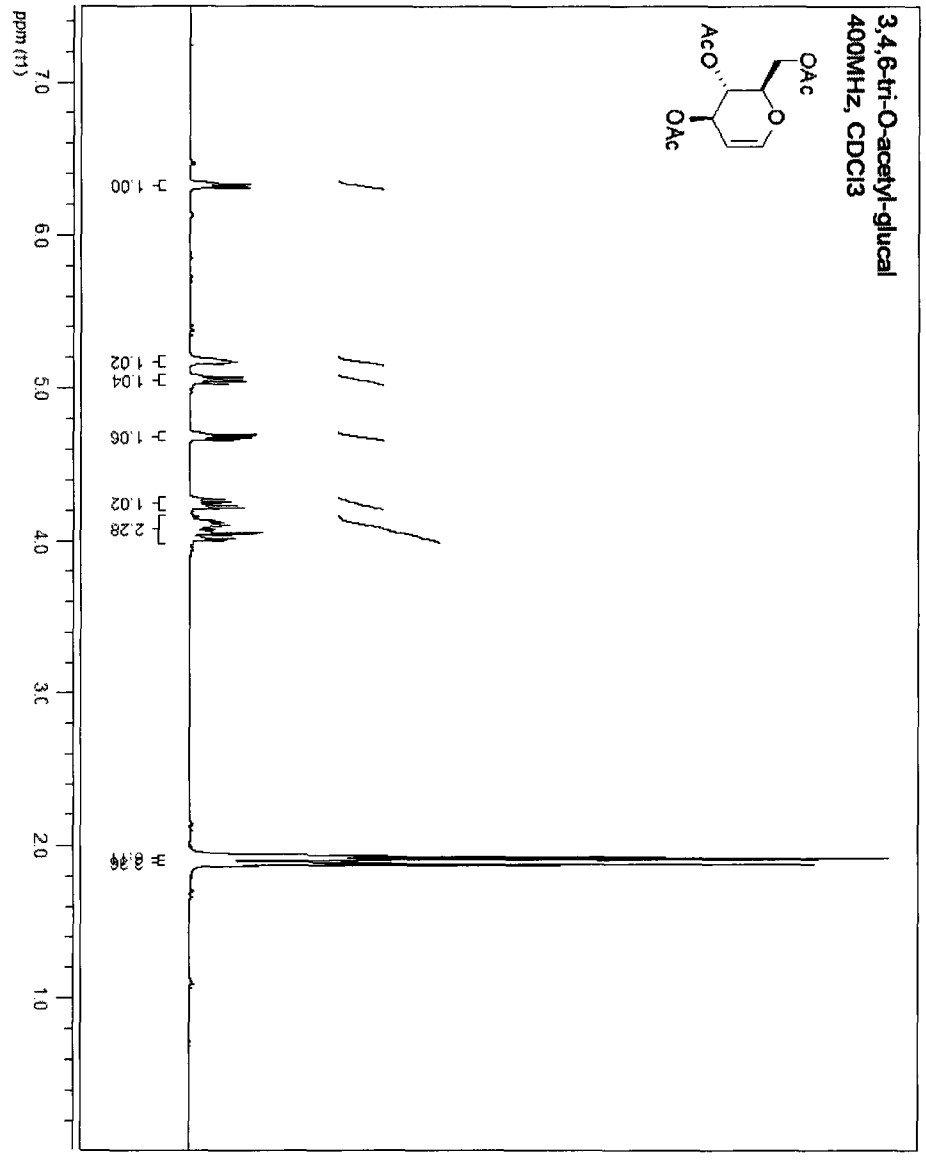

Figure a.1.60 ^{1}H NMR spectrum of 3,4,6-tri-*O*-acetyl-*D*-glucal (**38**) in $CDCl_3$

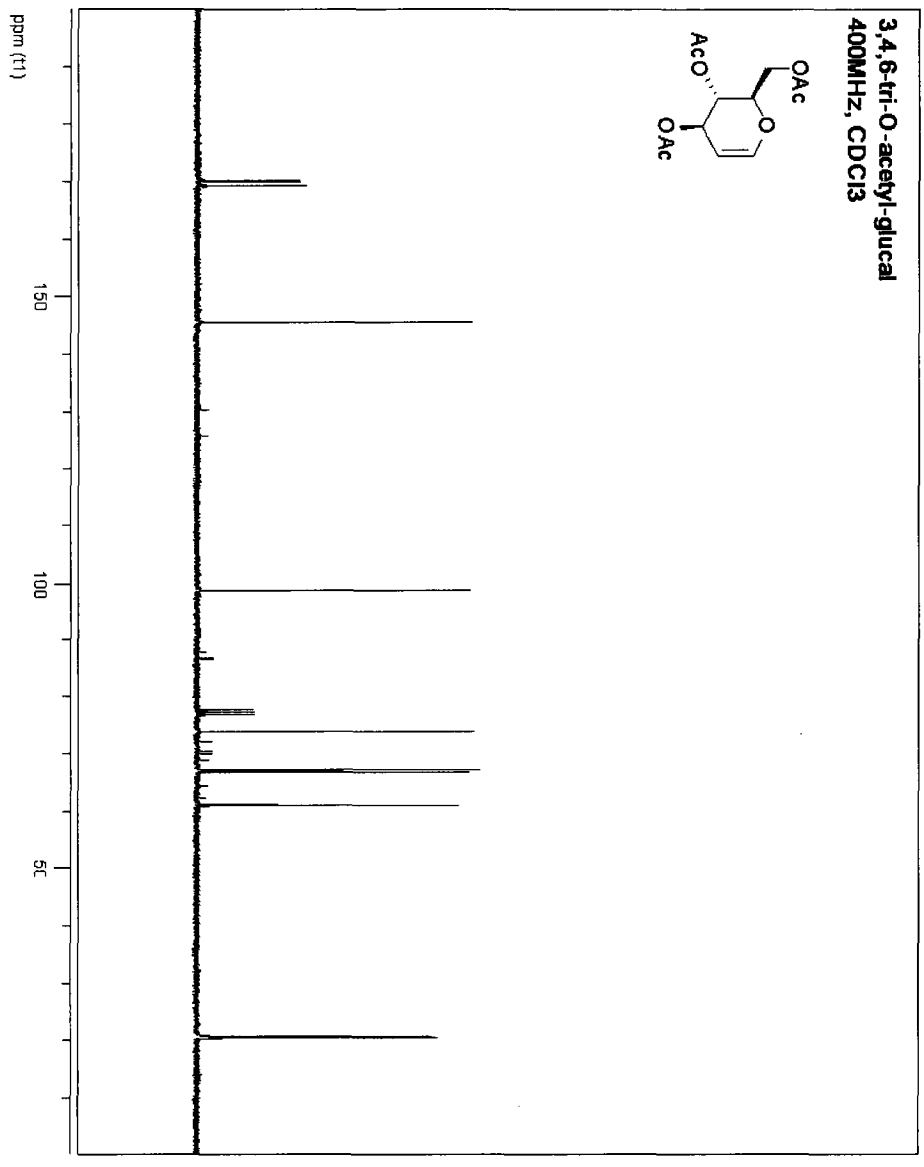

Figure a.1.61 ^{13}C NMR spectrum of 3,4,6-tri-*O*-acetyl-*D*-glucal (**38**) in $CDCl_3$

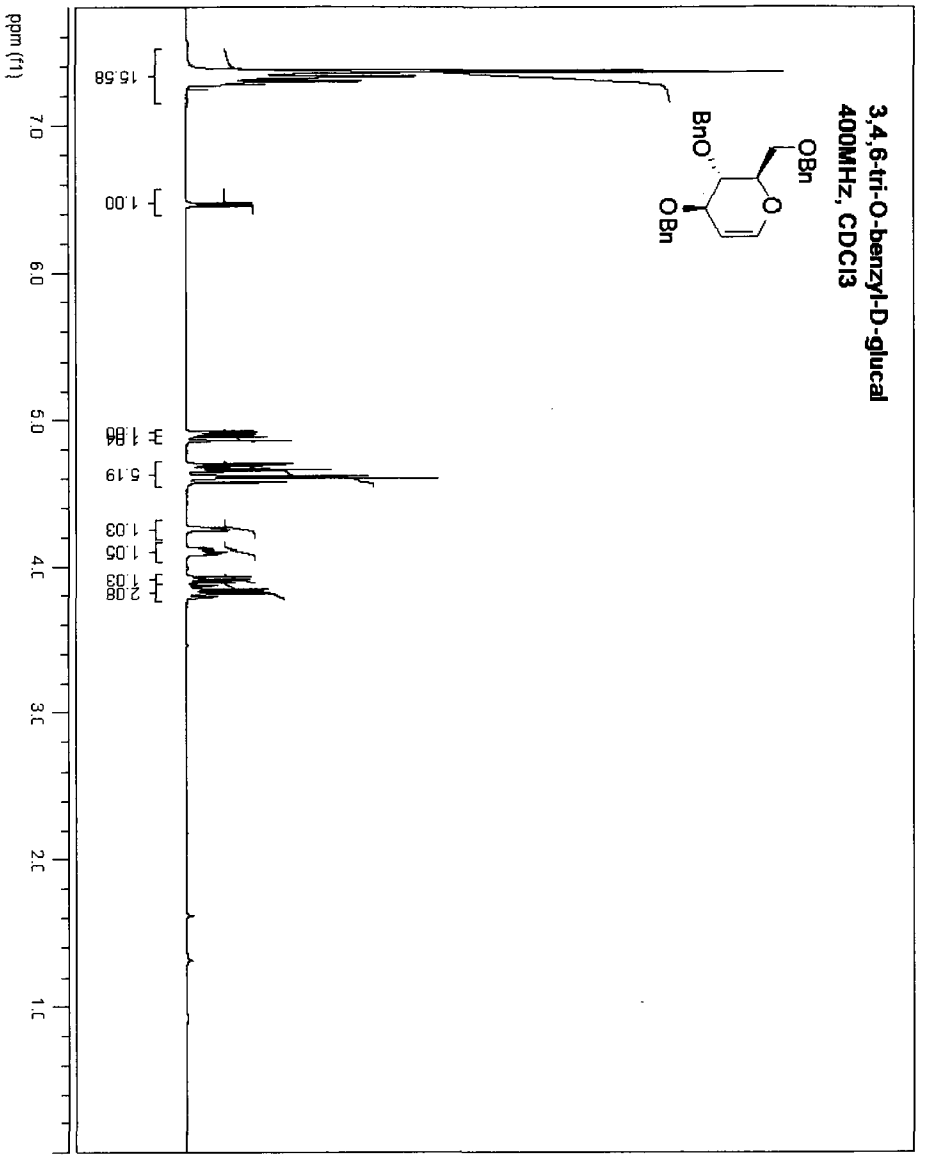

Figure a.1.62 ^{1}H NMR spectrum of 3,4,6-tri-*O*-benzyl-*D*-glucal (**39**) in $CDCl_3$

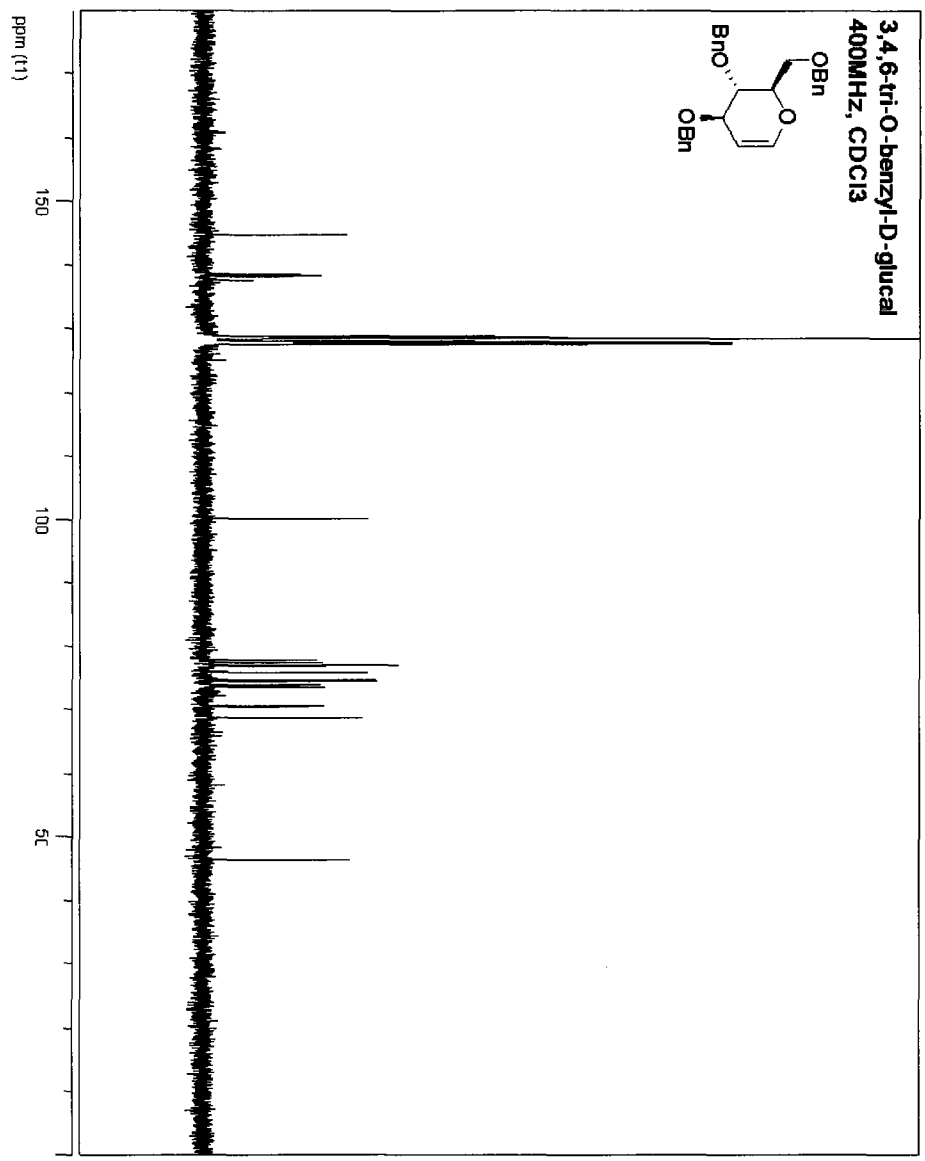

Figure a.1.63 ^{13}C NMR spectrum of 3,4,6-tri-*O*-benzyl-*D*-glucal (**39**) in $CDCl_3$

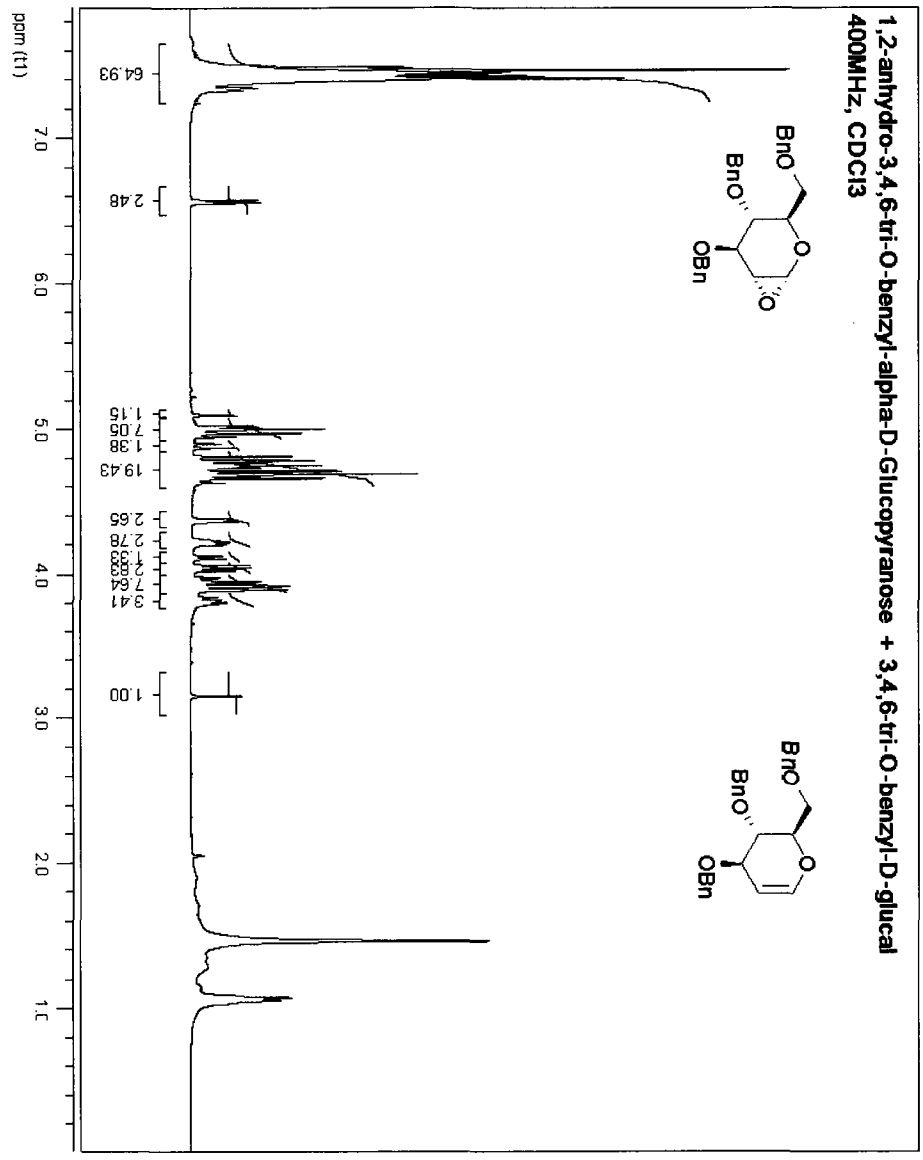

Figure a.1.64 ^{1}H NMR spectrum of 1,2-anhydro-3,4,6-tri-*O*-benzyl-*D*-glucopyranose (**40**) and 3,4,6-tri-*O*-benzyl-*D*-glucal (**39**) in $CDCl_3$

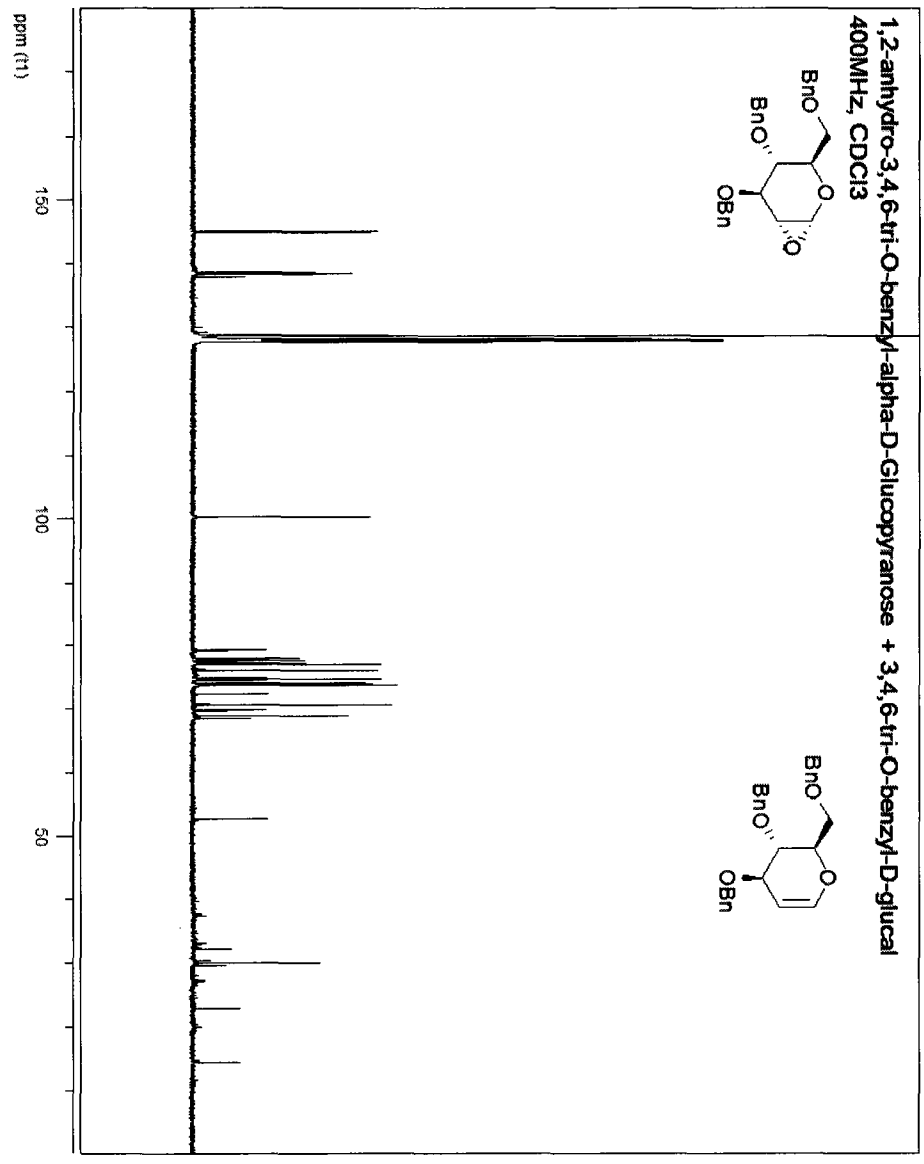

Figure a.1.65 ^{13}C NMR spectrum of 1,2-anhydro-3,4,6-tri-*O*-benzyl-*D*-glucopyranose (**40**) and 3,4,6-tri-*O*-benzyl-*D*-glucal (**39**) in $CDCl_3$

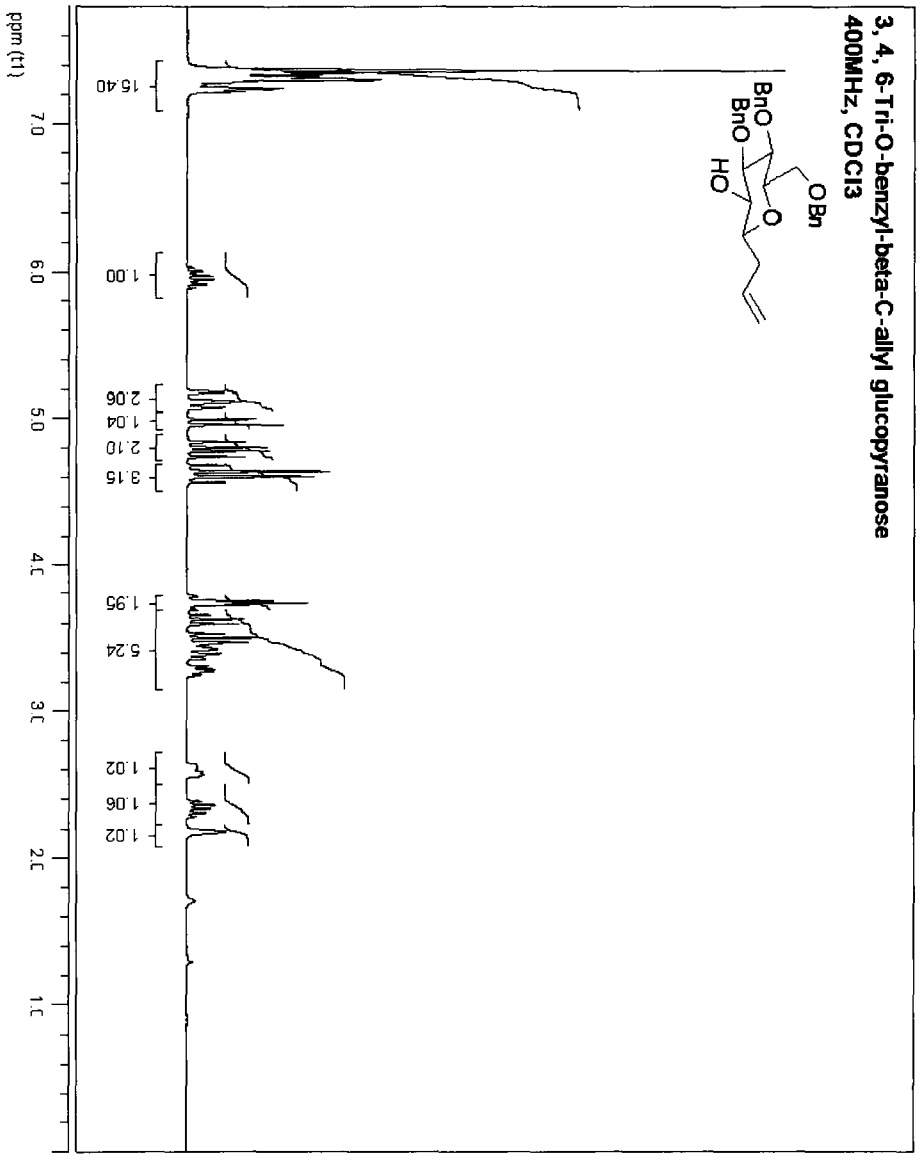

Figure a.1.66 ^{1}H NMR spectrum of 3,4,6-tri-*O*-benzyl- *β*-*C*-allyl glucopyranose (**41**)

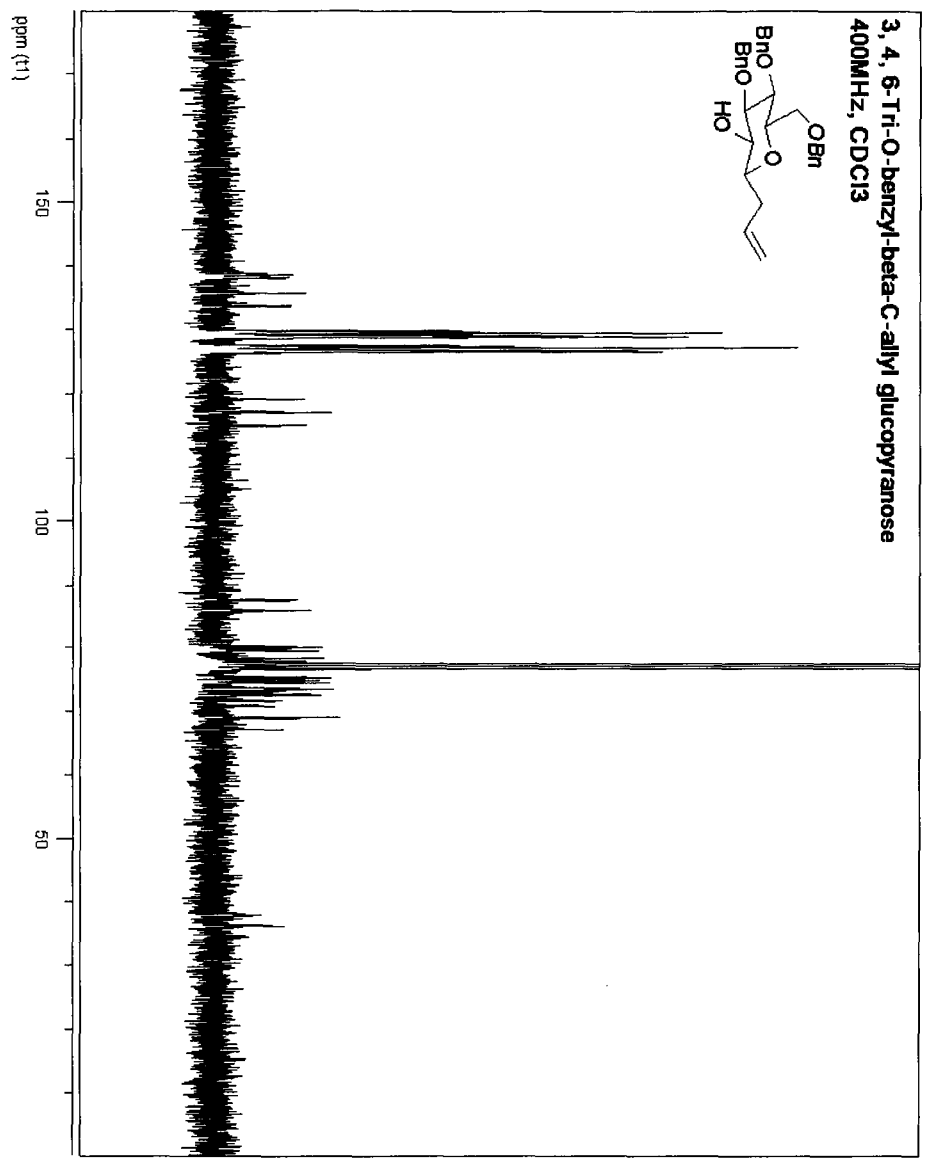

Figure a.1.67 ^{13}C NMR spectrum of 3,4,6-tri-*O*-benzyl-*β*-*C*-allyl glucopyranose (**41**)

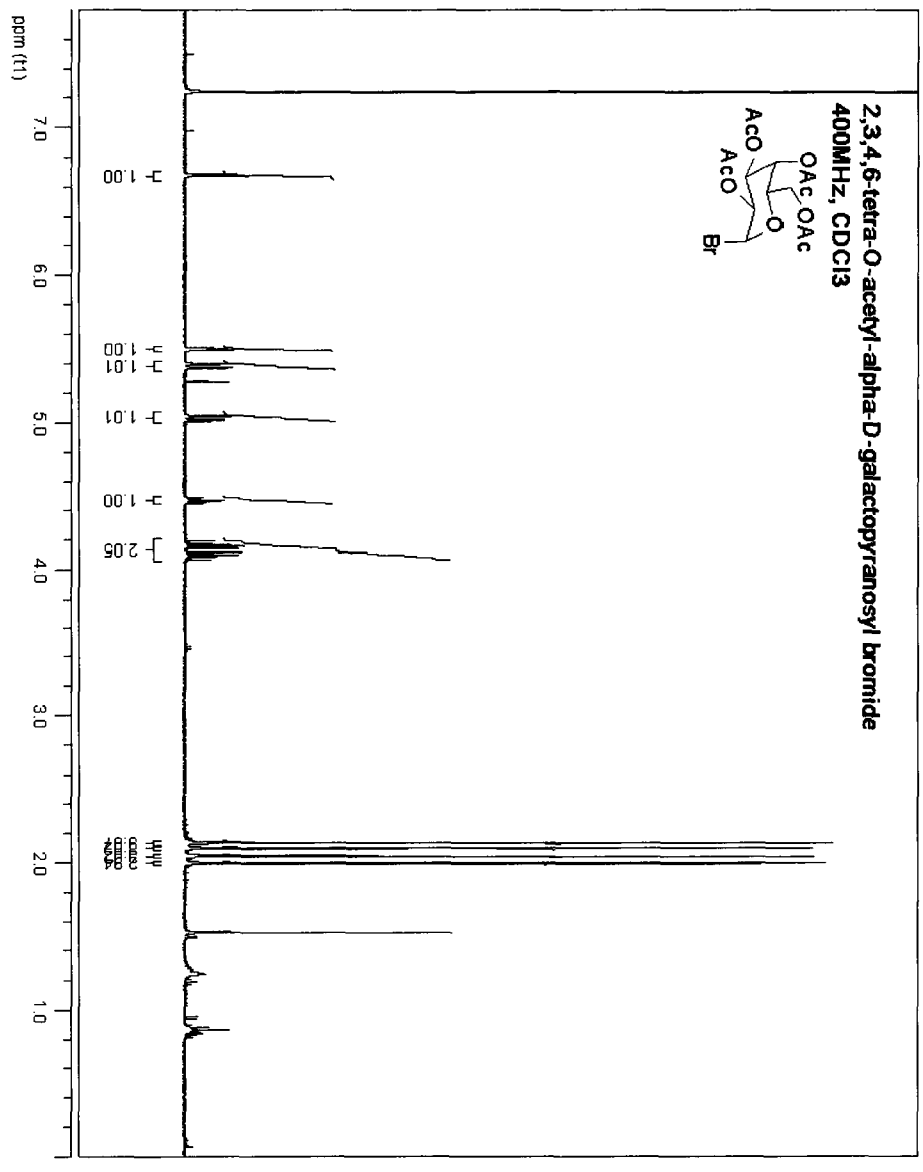

Figure a.1.68 ^{1}H NMR spectrum of 2,3,4,6-tetra-*O*-acetyl-*α*-*D*-galactopyranosyl bromide **(43)**

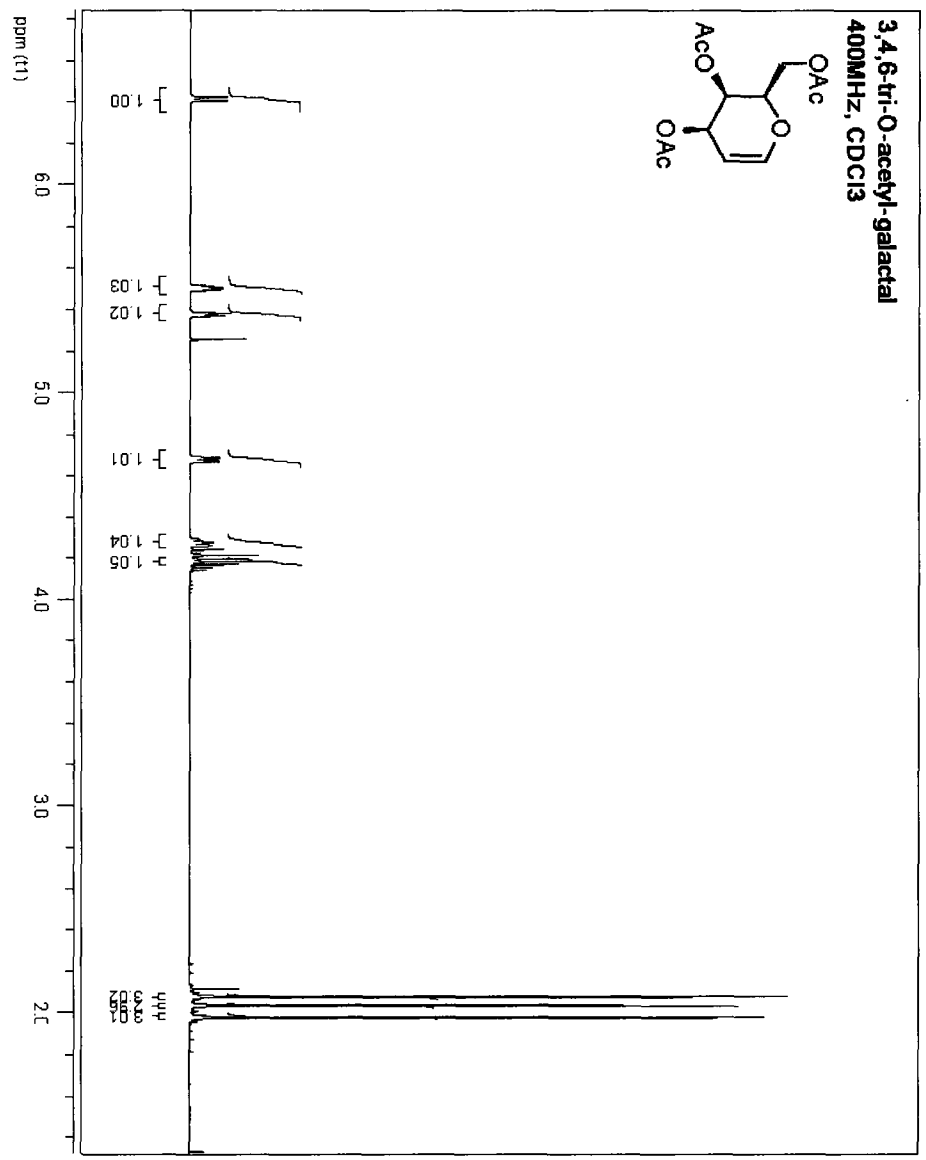

Figure a.1.69 ^{1}H NMR spectrum of 3,4,6-tri-*O*-acetyl-galactal (**44**)

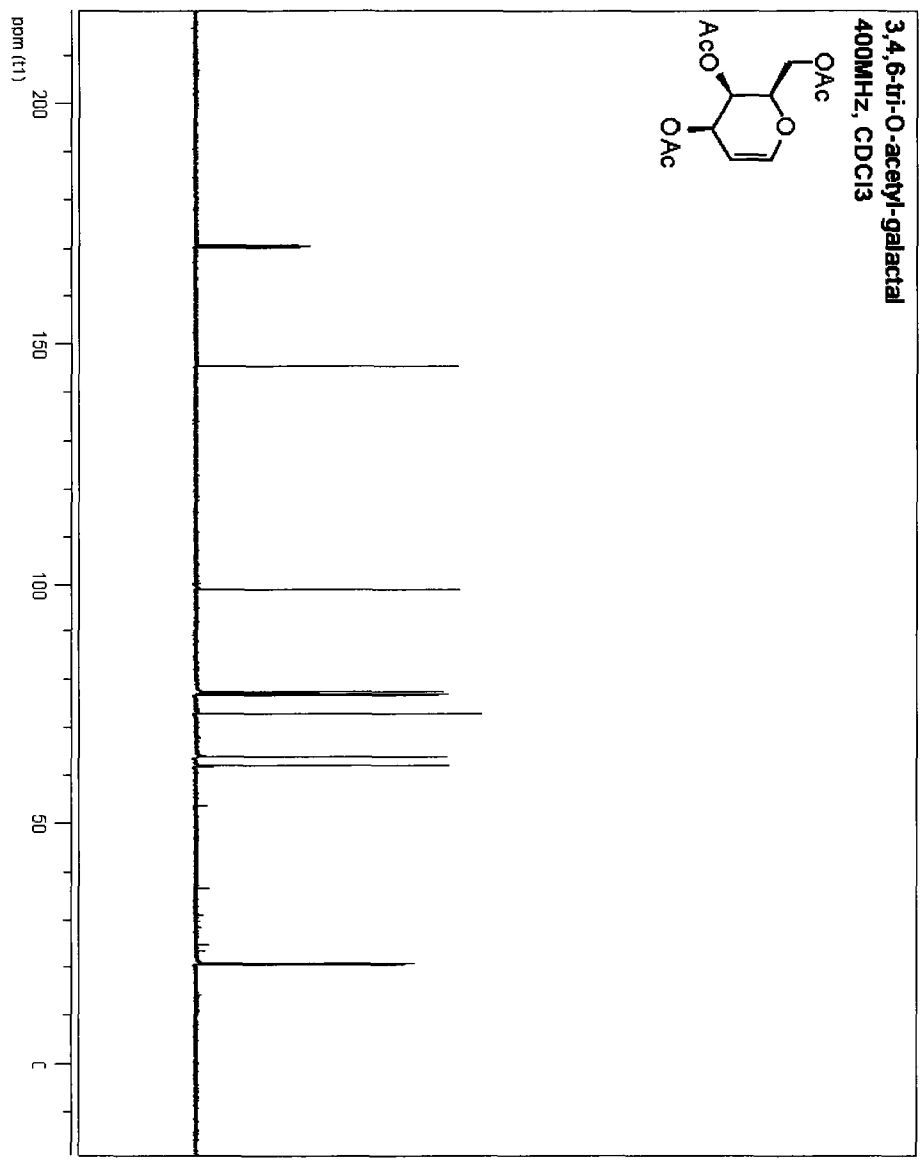

Figure a.1.70 ^{13}C NMR spectrum of 3,4,6-tri-*O*-acetyl-galactal (**44**)

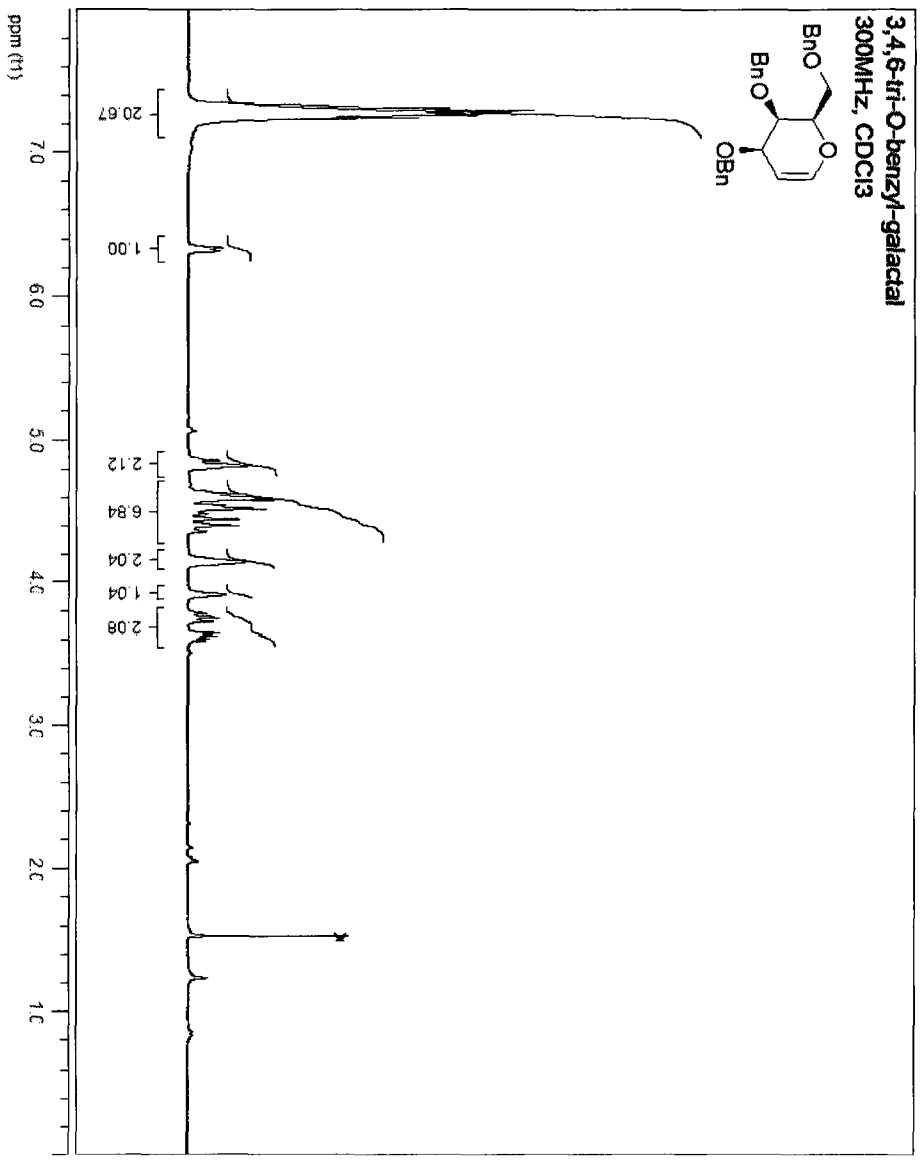

Figure a.1.71 ^{1}H NMR spectrum of 3,4,6-tri-*O*-benzyl-galactal (**45**)

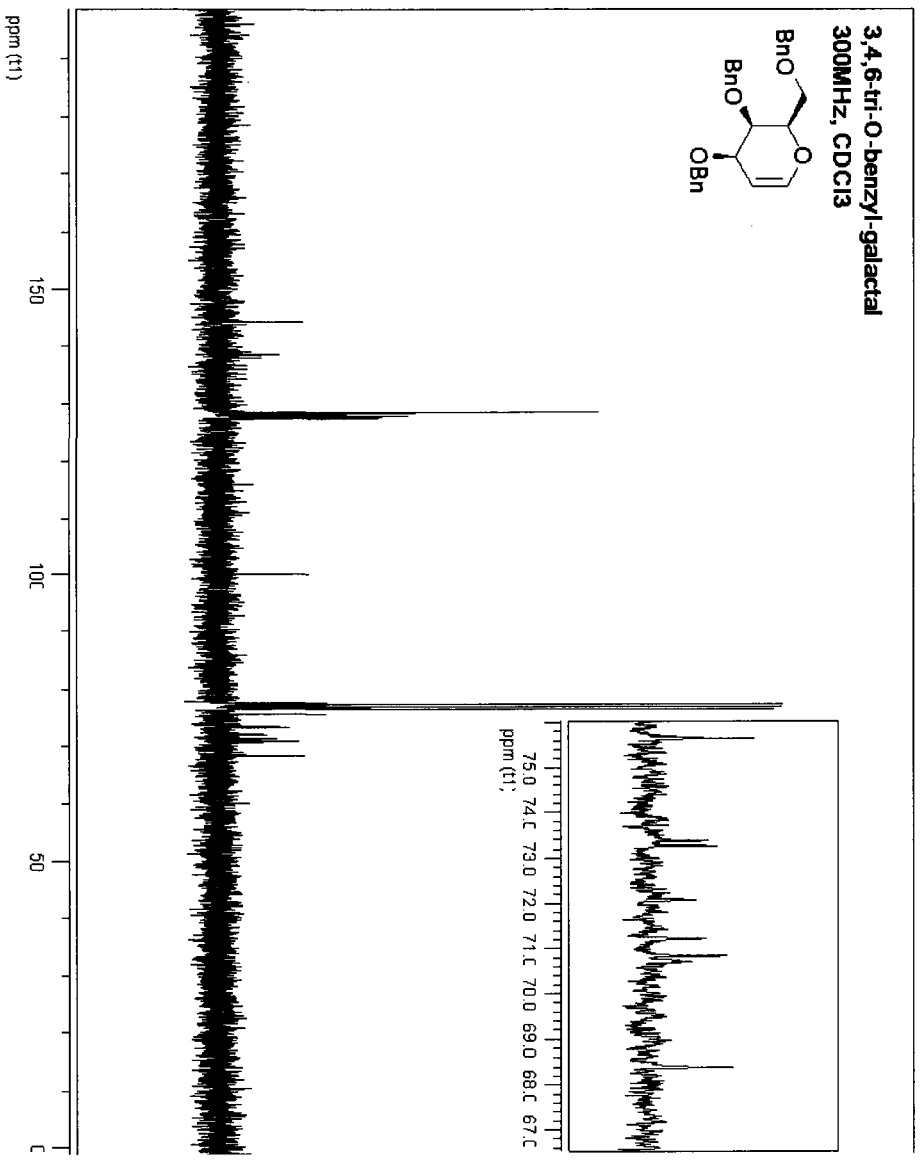

Figure a.1.72 ^{13}C NMR spectrum of 3,4,6-tri-*O*-benzyl-galactal (**45**)

Appendix 2 – NMR spectra for chapter 2

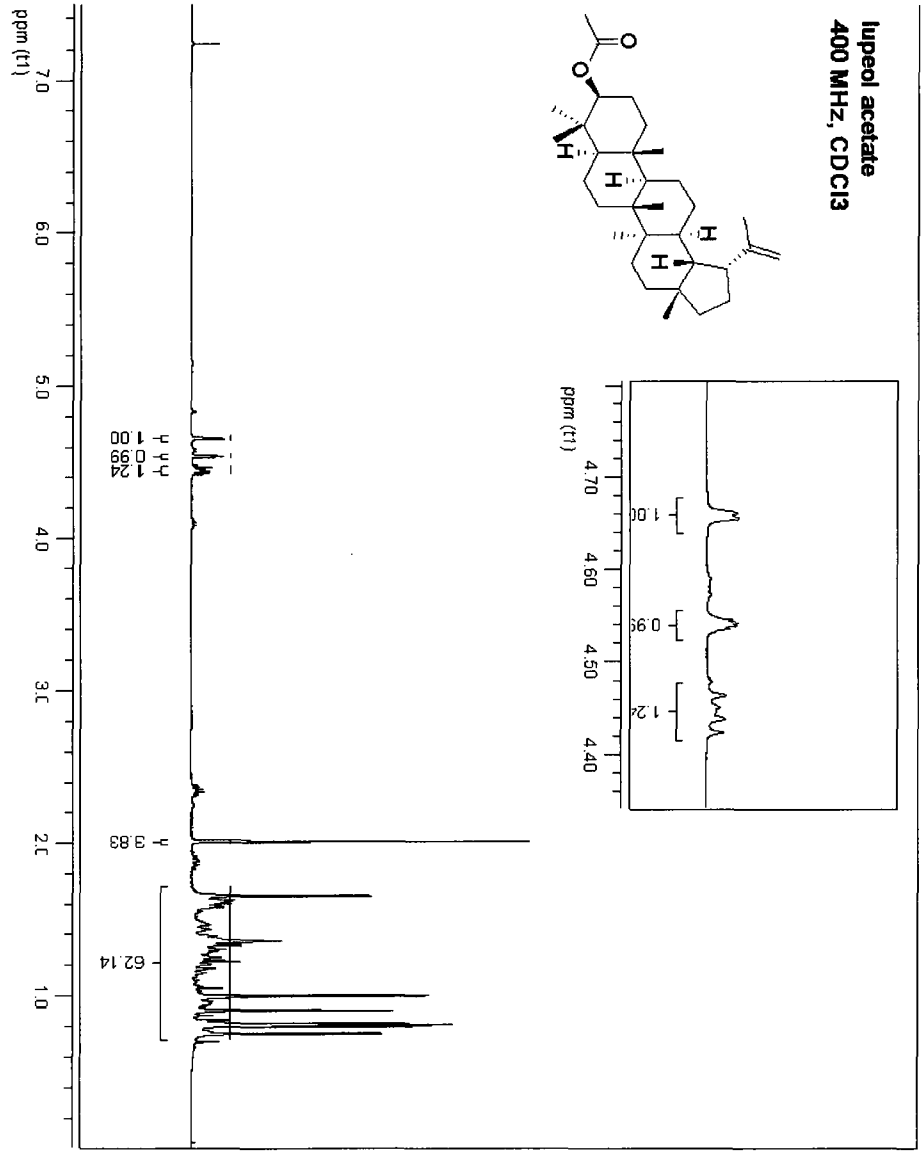

Figure a.2.1 ^{1}H NMR spectrum of lupeol acetate(**46**) in $CDCl_3$

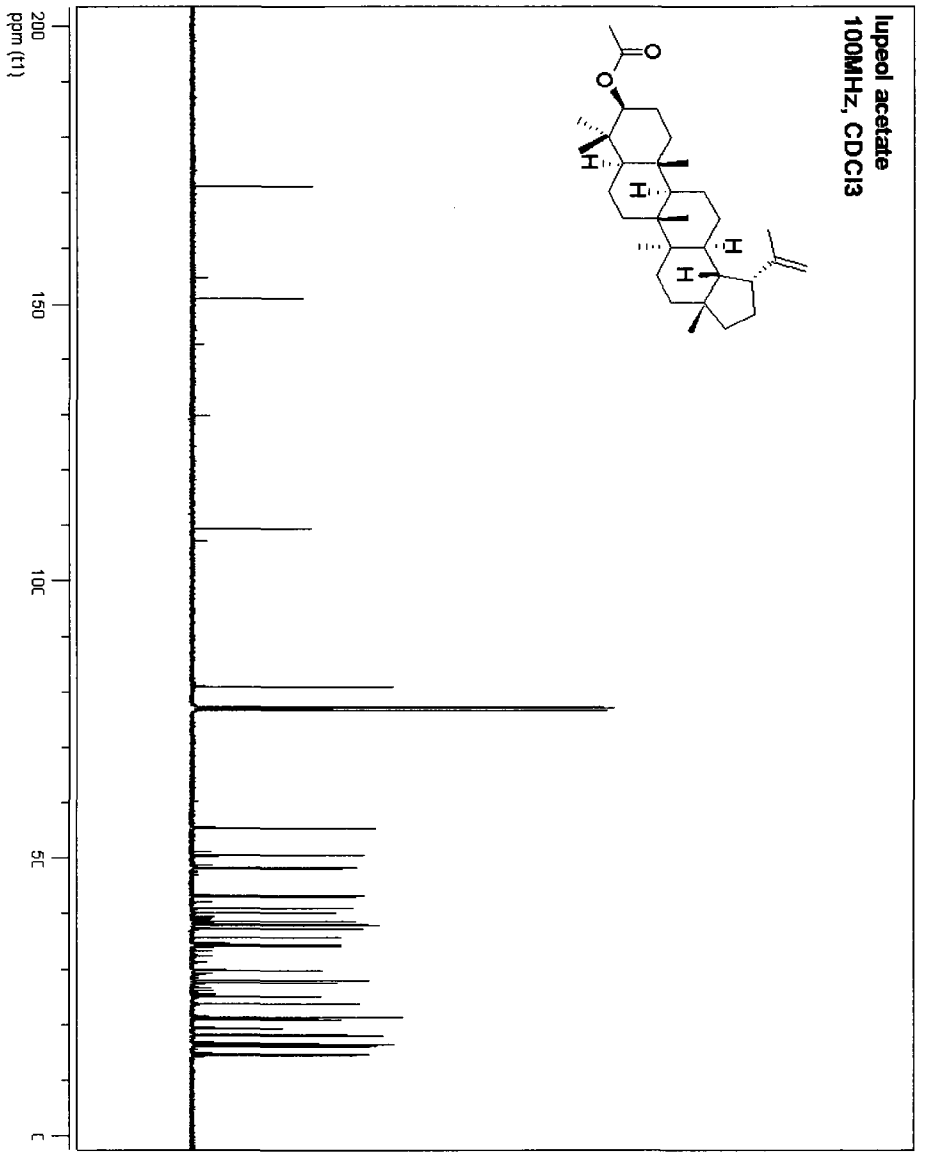

Figure a.2.2 ^{13}C NMR spectrum of lupeol acetate (**46**) in $CDCl_3$

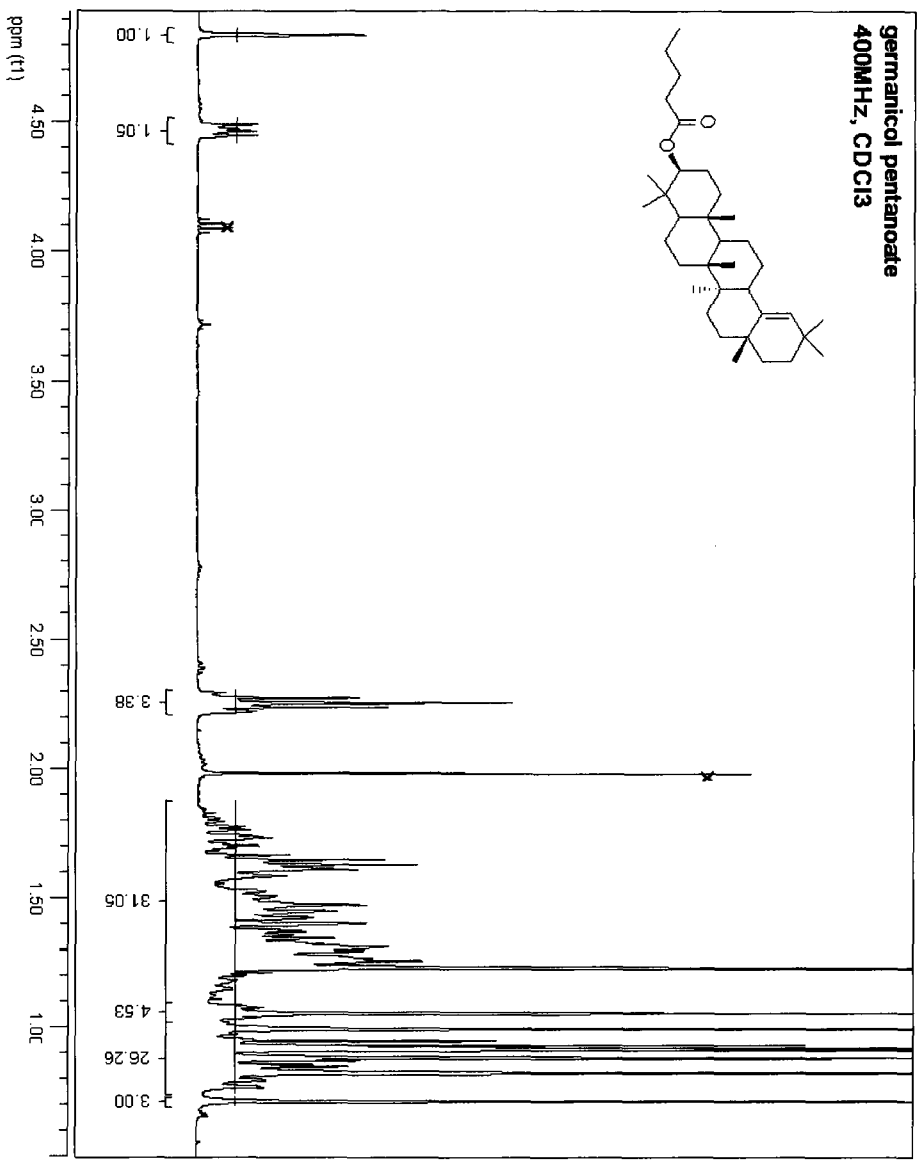

Figure a.2.3 ^{1}H NMR of germanicol pentanoate (**47**) in $CDCl_3$

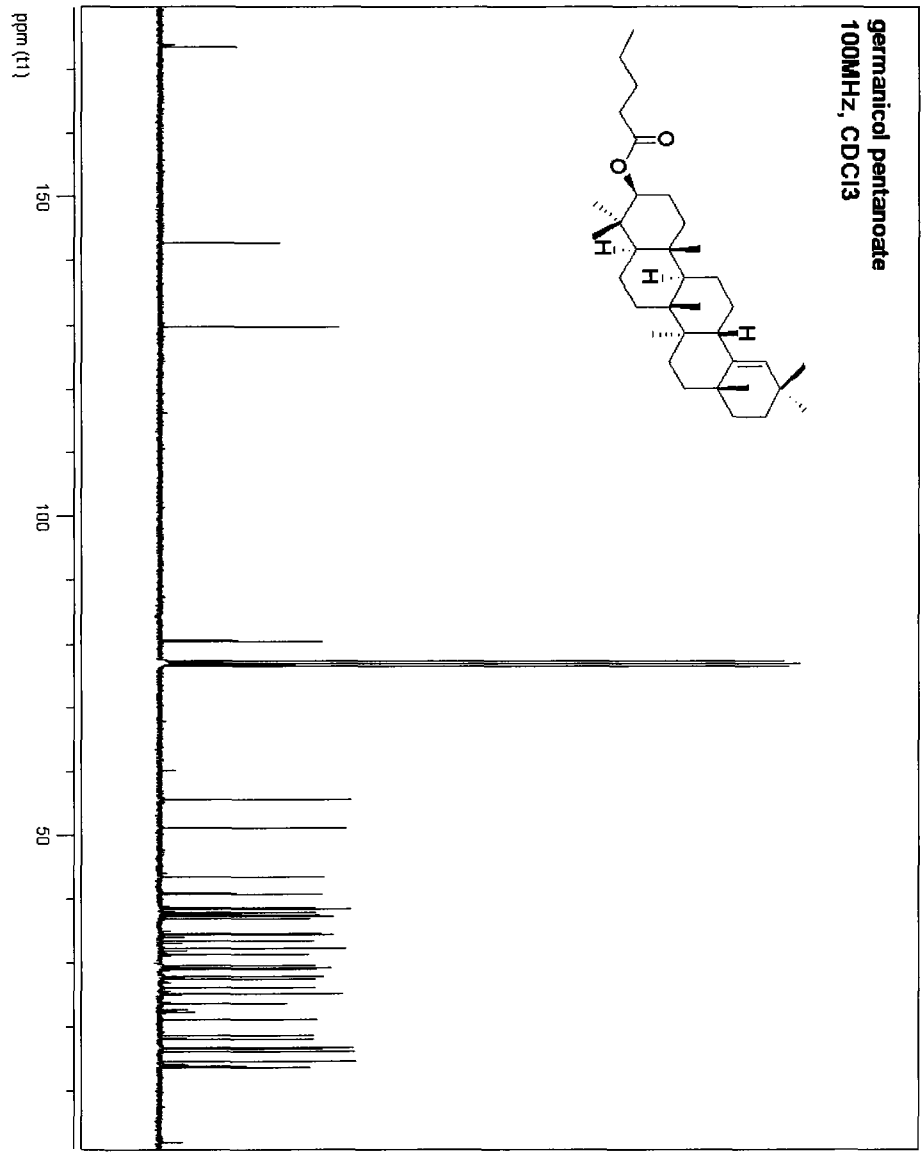

Figure a.2.4 ^{13}C NMR of germanicol pentanoate (**47**) in $CDCl_3$

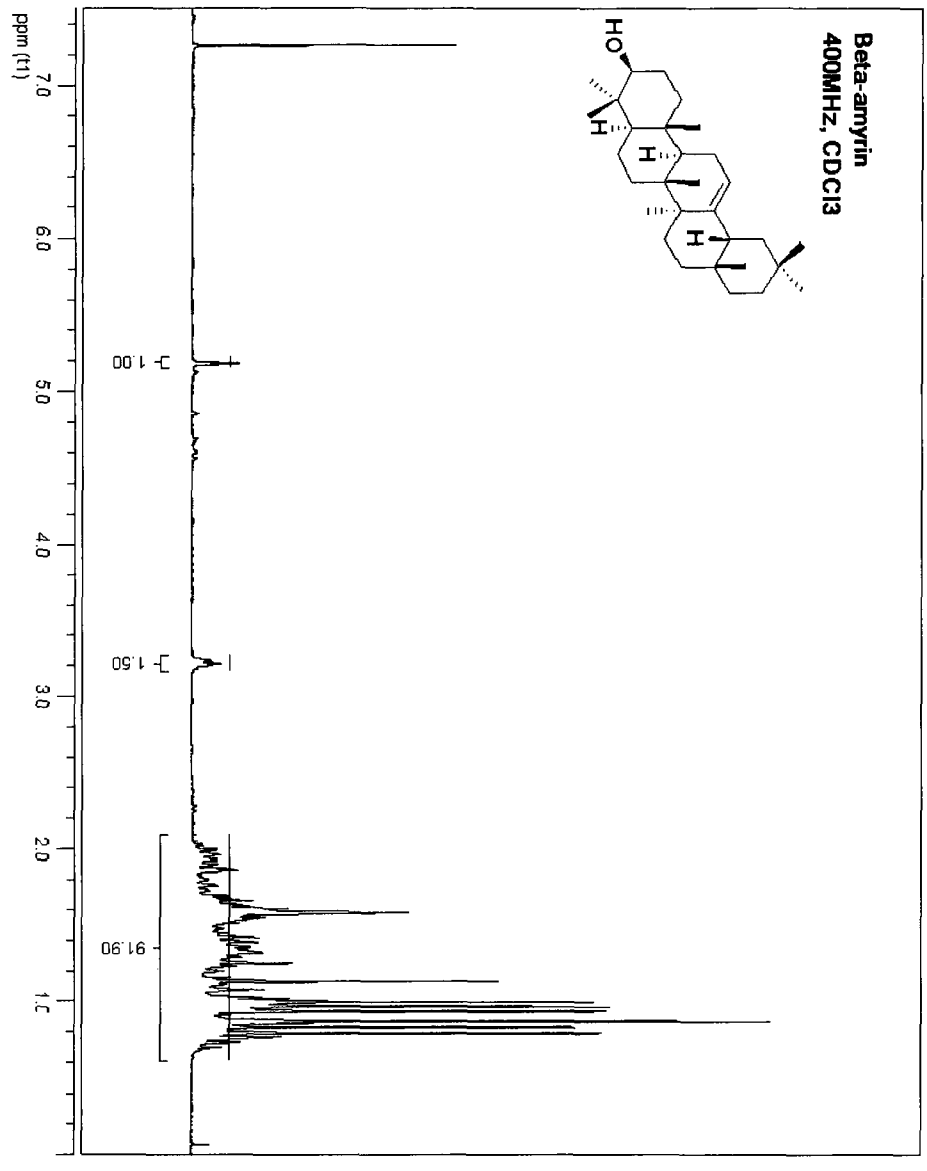

Figure a.2.5 ^{1}H NMR spectrum of β-amyrin (**48**) in $CDCl_3$

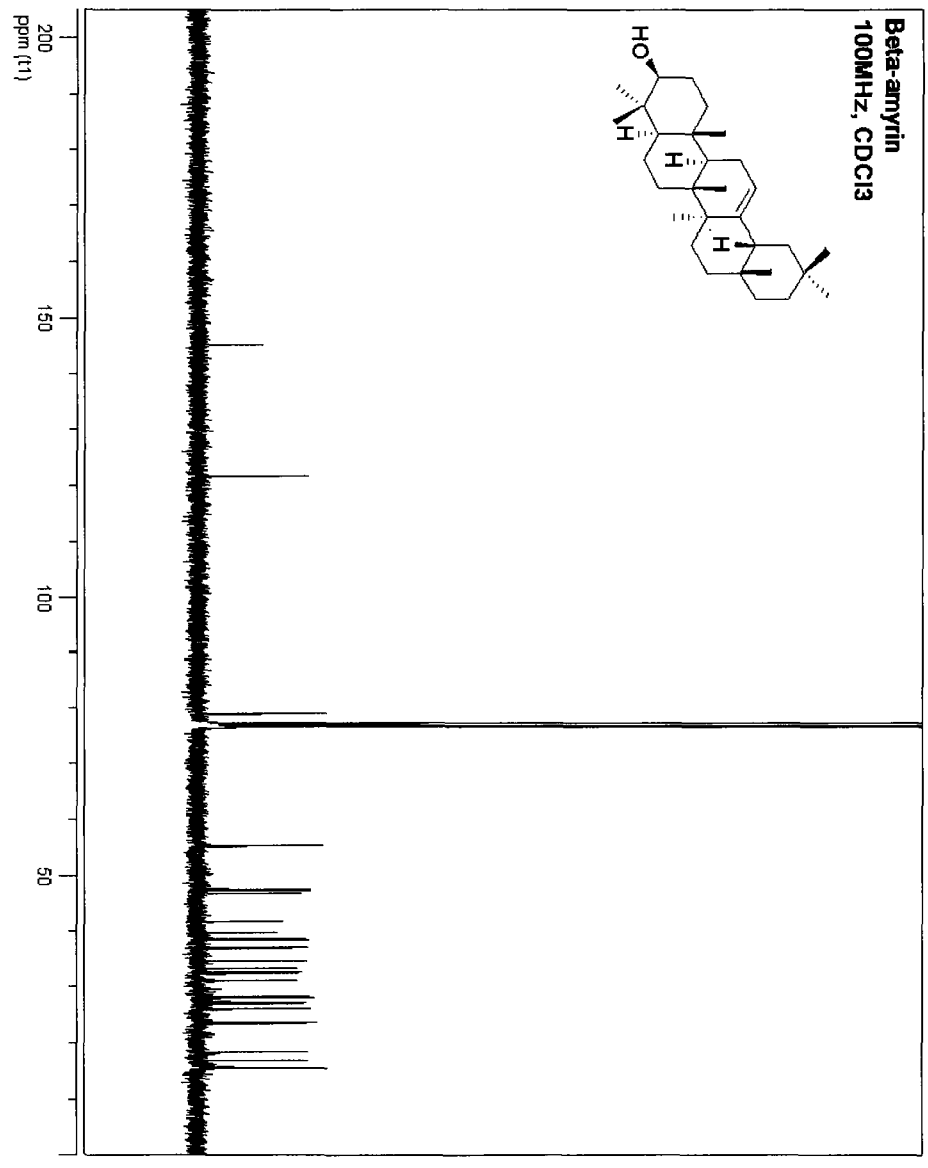

Figure a.2.6 ^{13}C NMR spectrum of β-amyrin (**48**) in $CDCl_3$

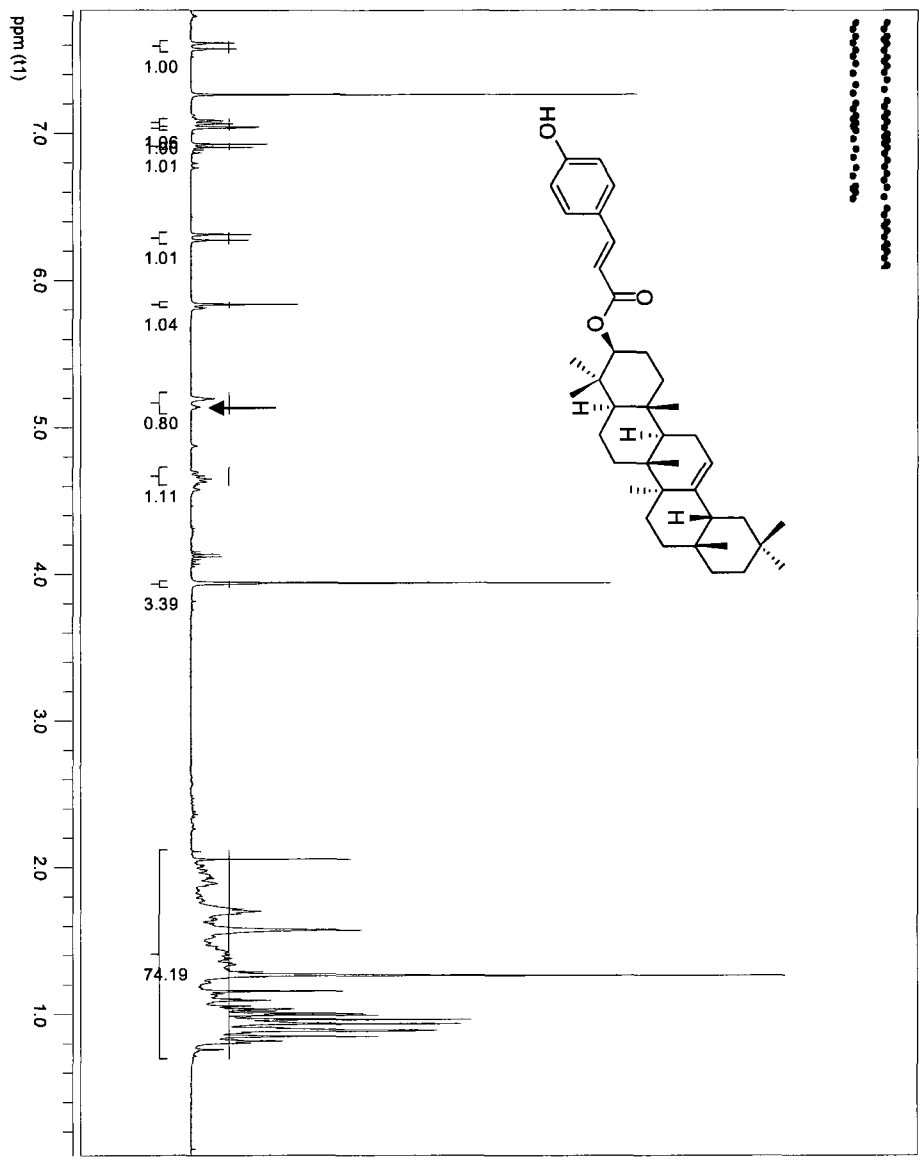

Figure a.2.7 ^{1}H NMR of isomeric mixture of α- (**50**) and *β*-amyryl p-hydroxycinnamate (**49**) in $CDCl_3$. Distinct signals belonging to the α- isomer are indicated by arrows.

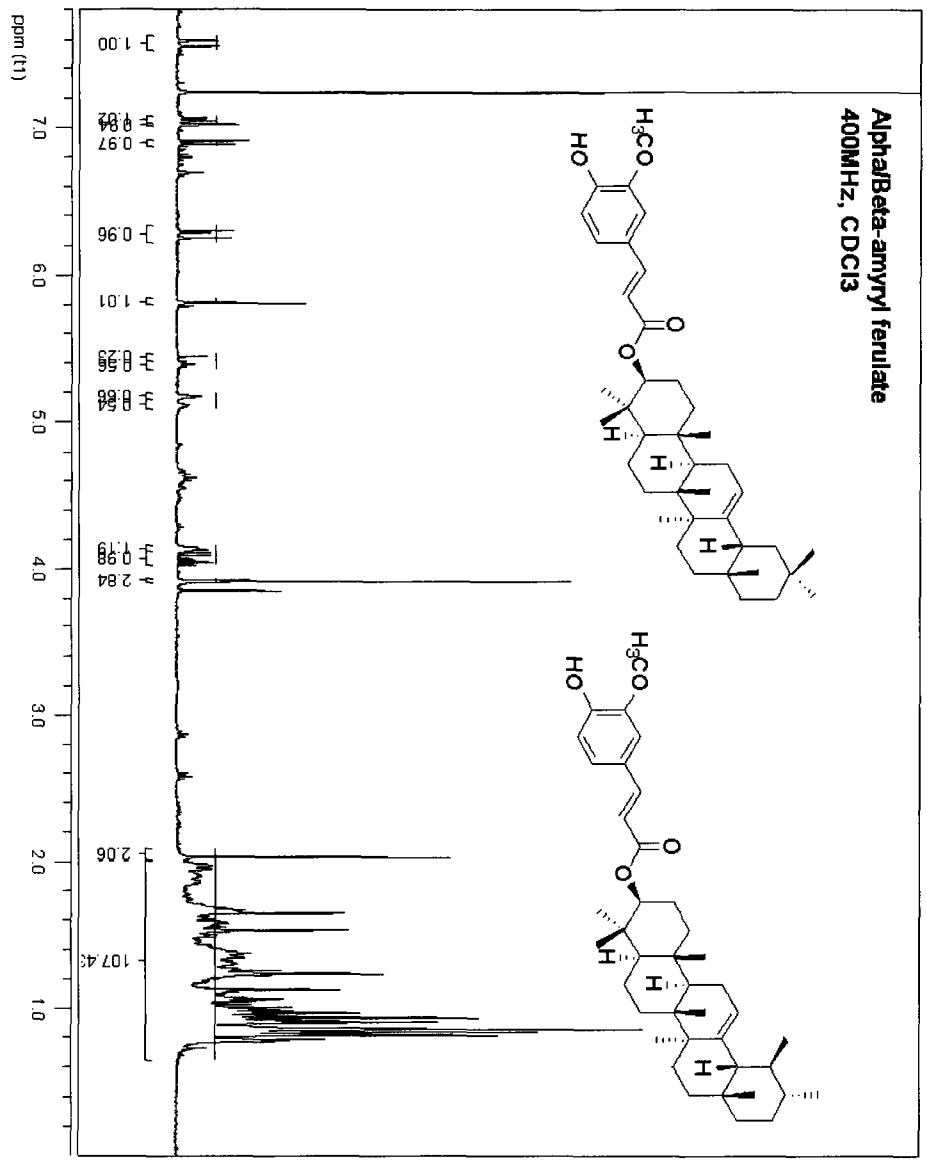

Figure a.2.8 ^{1}H NMR of isomeric mixture of α- (**52**) and *β*-amyryl ferulate (**51**) in $CDCl_3$. Distinct signals belonging to the α- isomer are indicated by arrows.

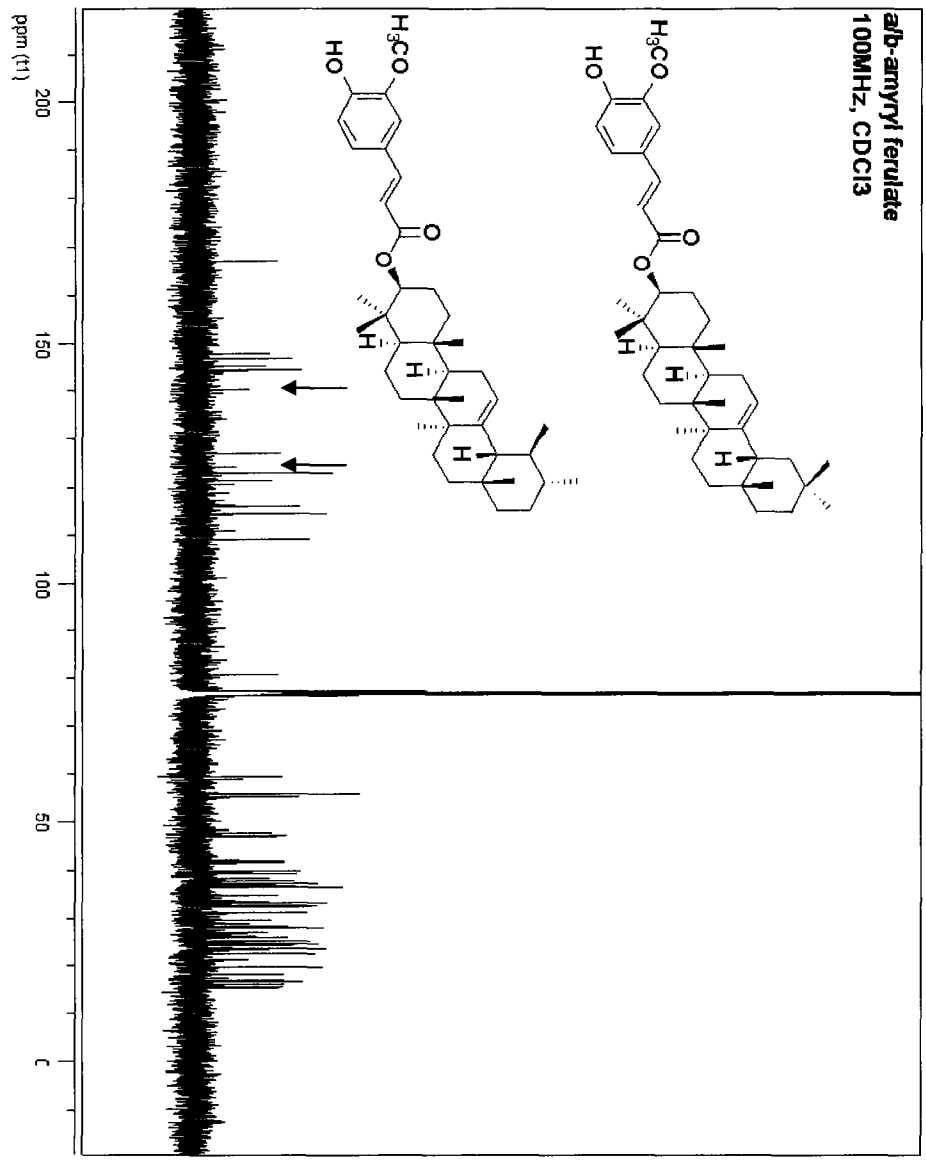

Figure a.2.9 ^{13}C NMR of isomeric mixture of α- (**52**) and *β*-amyryl ferulate(**51**) in $CDCl_3$. Distinct signals belonging to the α- isomer are indicated by arrows.

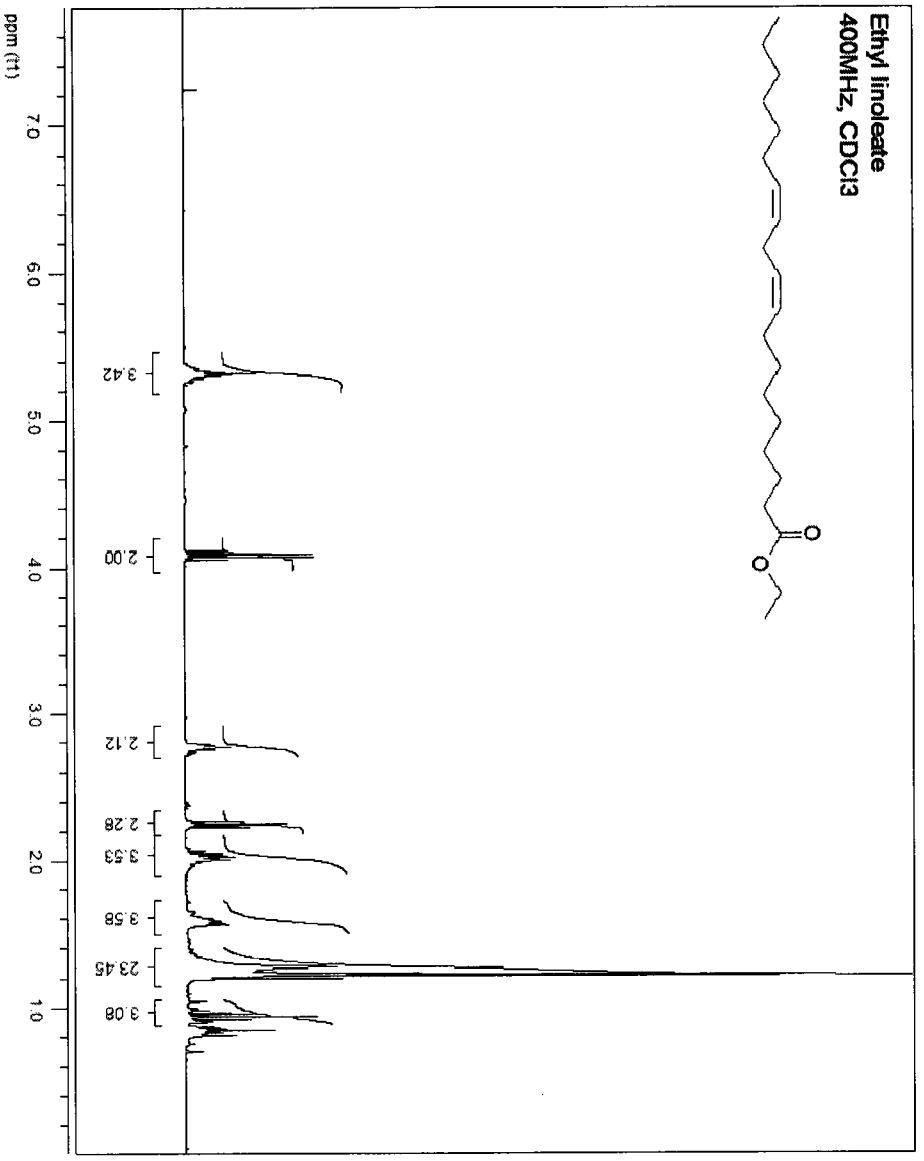

Figure a.2.10 ^{1}H NMR of ethyl linoleate in $CDCl_3$.

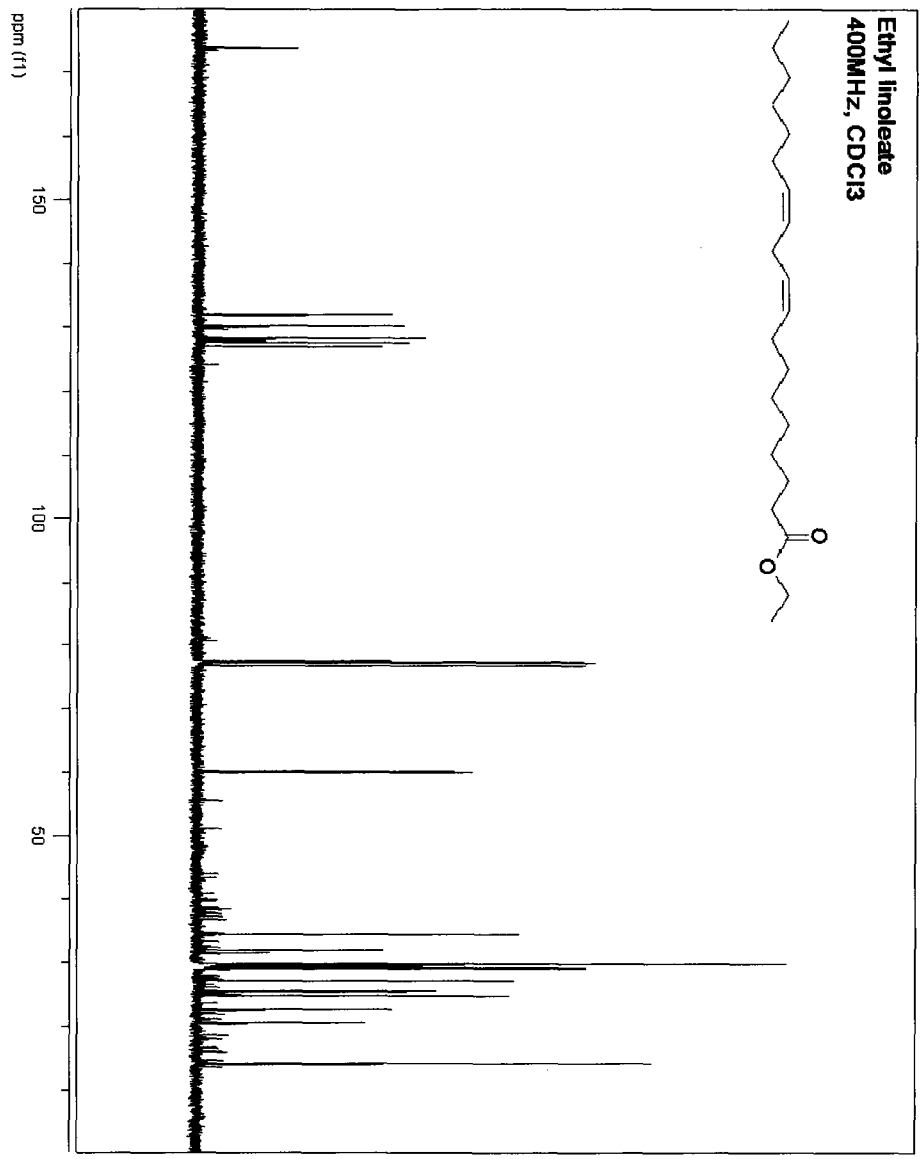

Figure a.2.11 ^{13}C NMR of ethyl linoleate in $CDCl_3$.

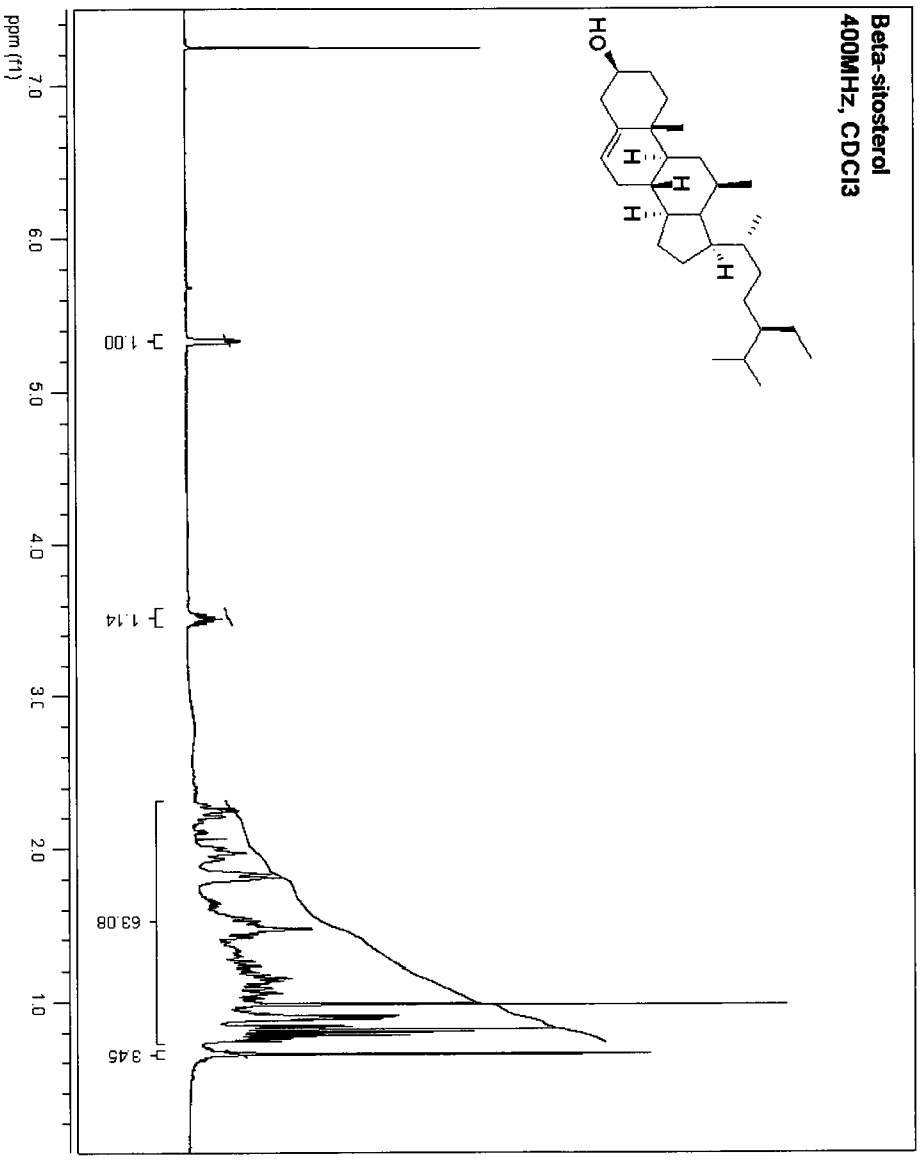

Figure a.2.12 ^{1}H NMR of β-sitosterol (**54**) in $CDCl_3$.

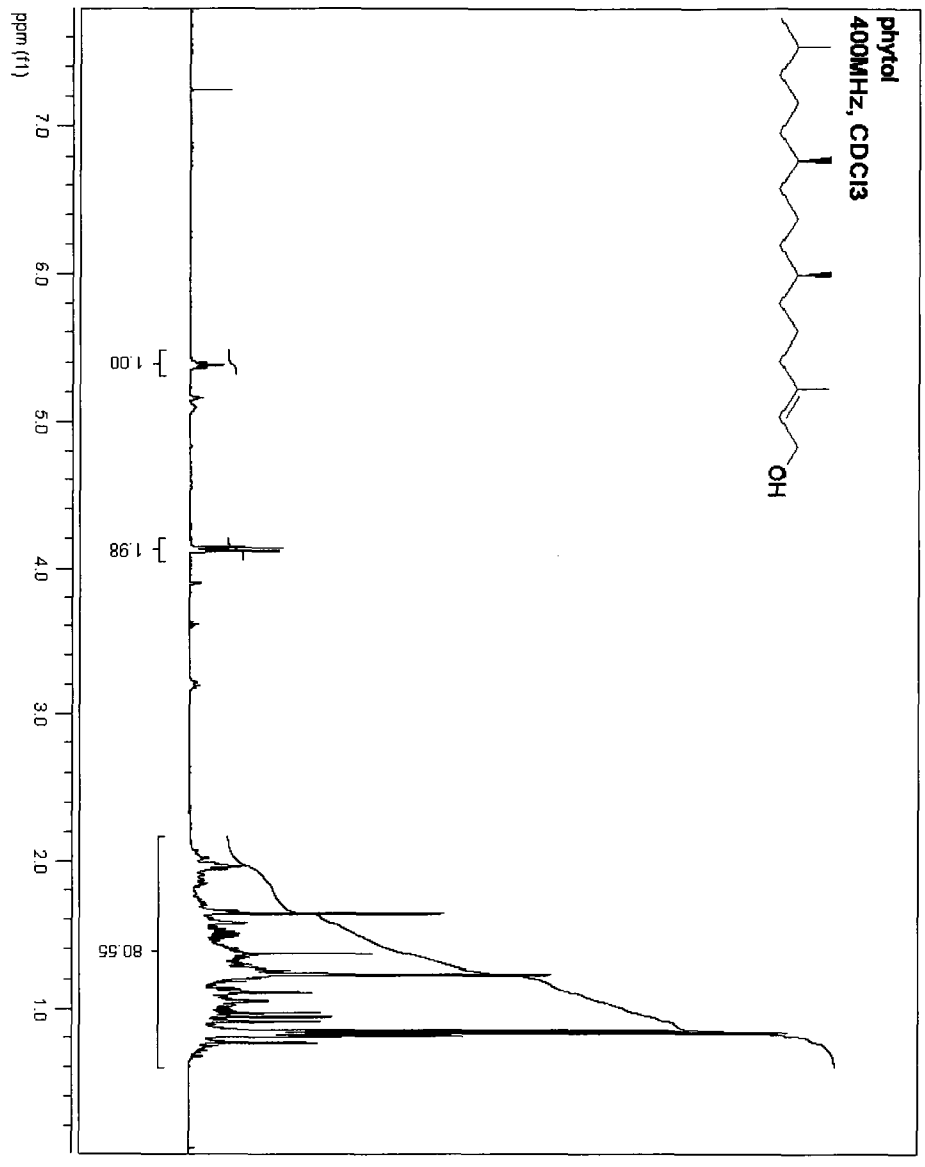

Figure a.2.13 ^{1}H NMR of phytol (**55**) in $CDCl_3$.

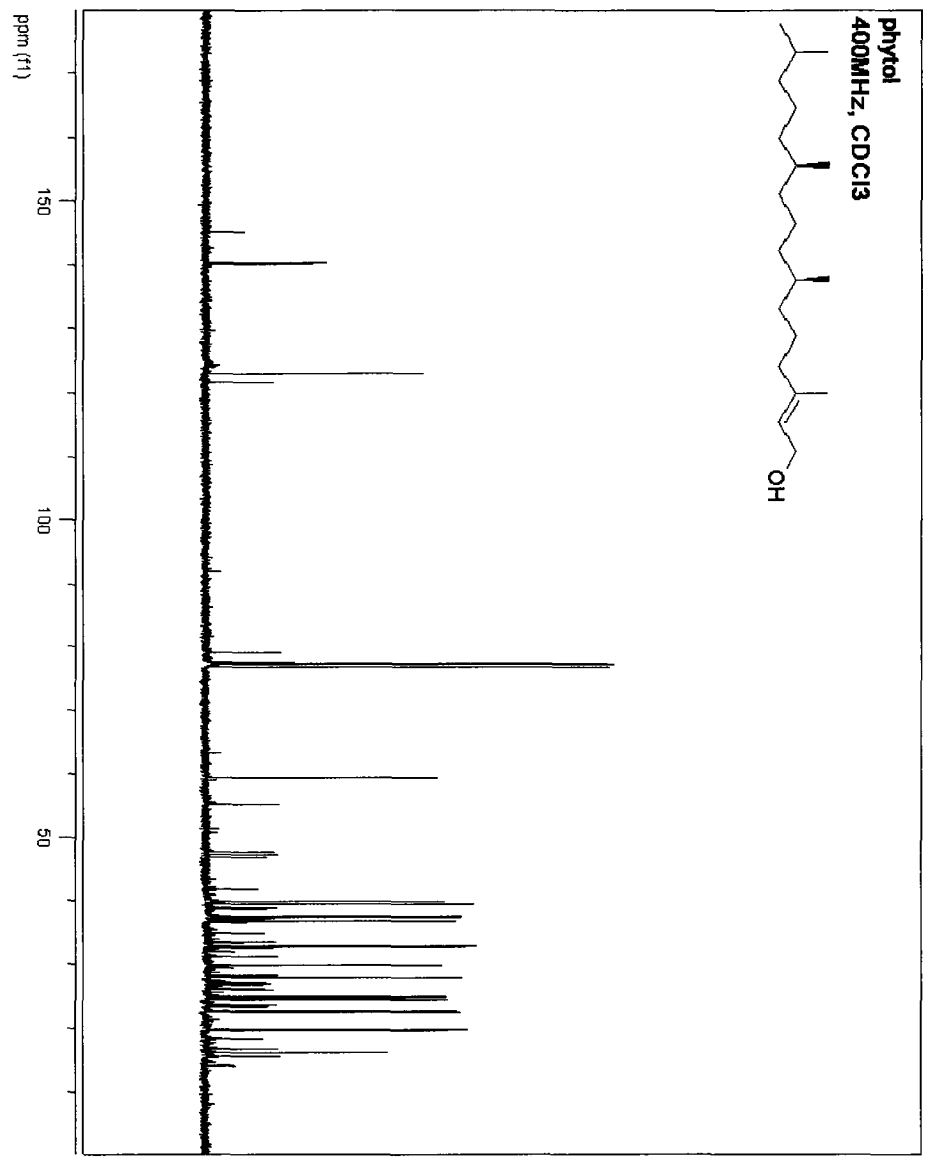

Figure a.2.14 ^{13}C NMR of phytol (**55**) in $CDCl_3$.

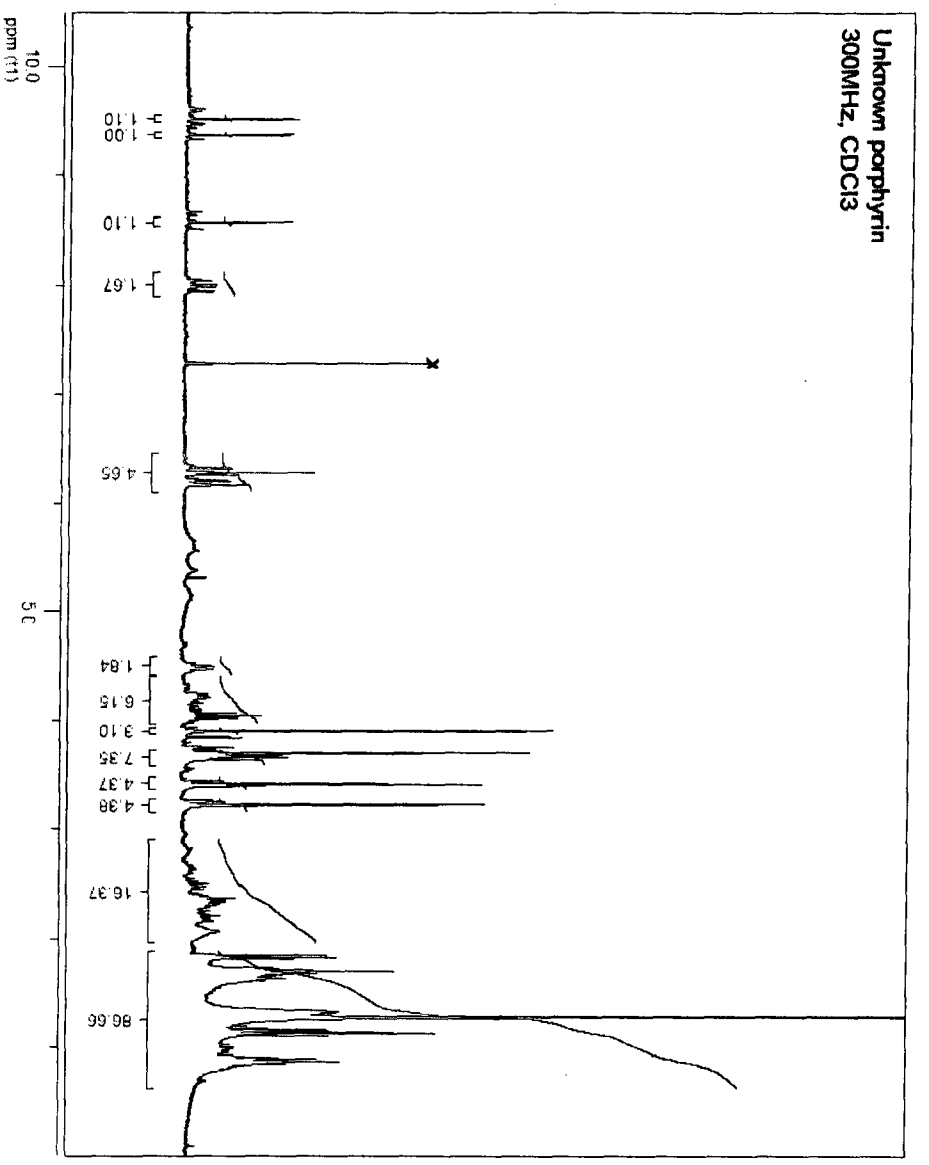

Figure a.2.15 ^{1}H NMR of porphyrin (**56**) in $CDCl_3$.