LEWIS ACID CATALYZED STEREOSPECIFIC GLYCOSYLATION OF SECONDARY METABOLITES

A project report submitted in partial fulfilment
of the requirements for the degree of

MASTER OF SCIENCE

IN

CHEMISTRY (ORGANIC CHEMISTRY)

By

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CERTIFICATE

This is to certify that **Ms. ANJU AYYAPPAN** has worked on "**Lewis acid catalyzed stereospecific glycosylation of secondary metabolites**" under my supervision at the Organic Chemistry Section of CSIR - National Institute for Interdisciplinary Science and Technology, Thiruvananthapuram (during the period from January 2020 to July 2020) for the partial fulfilment of the Degree of Master of Science in Chemistry, School of chemical sciences, Mahatma Gandhi University, kottayam, Kerala-686560.

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CERTIFICATE

This is to certify that the work incorporated in this project entitled **"Lewis acid catalyzed stereospecific glycosylation of secondary metabolites"**, submitted by **Ms. ANJU AYYAPPAN** for the partial fulfilment of the requirement for the degree of Master of Science in Chemistry (Organic Chemistry), to the Director, School of Chemical Sciences, Mahatma Gandhi University, Kottayam, was carried out under the supervision of **Dr. K V RADHAKRISHNAN**, Principal Scientist, Organic Chemistry division of the CSIR-National Institute for Interdisciplinary Science and Technology, Thiruvananthapuram in the year 2020.

Dr. K. S. Devaky

Director
School of Chemical Sciences
MG University, Kottayam

Place: Priyadarsini Hills
Dated: 22.7.2020

DECLARATION

I hereby declare that the project work entitled **“Lewis acid catalyzed stereospecific glycosylation of secondary metabolites”** is carried out by me under the supervision of **Dr. K V RADHAKRISHNAN,** Organic Chemistry section of the CSIR- National Institute for Interdisciplinary Science and Technology, Trivandrum. The present work has not been submitted to any other Universities or Institute for the award of degree or diploma.

Anju

Thiruvananthapuram **ANJU AYYAPPAN**
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Chapter 1
Introduction

1.1 Introduction to Carbohydrates

Carbohydrate chemistry deals with the reactions and synthesis of often biologically important carbohydrate molecules. Living organisms are made up of different macromolecules like- carbohydrates, proteins, fats and lipids. Carbohydrates are one among the most common organic molecules that play a vital role in all biological systems; therefore, their chemistry and synthesis are of fundamental interest. Due to the easy access of many carbohydrates, they have been extensively investigated throughout the time of modern chemistry. In chemical view, carbohydrates are a class of simple organic compounds. It can be either aldehyde or a ketone with additional hydroxyl groups. The common chemical formula for carbohydrates is $C_n(H_2O)_n$, where the Carbon, Hydrogen and Oxygen are in the ratio of 1:2:1. They are the most abundant aldehyde compounds found in living organisms. They provide storage, transport and energy to bodies in the form of starch and glycogen, and also make up structural components such as cellulose in plants and chitin in animals (Figure 1.1). Additionally, they contribute to the immune system, fertilization, pathogenesis, blood clotting and development.[1]

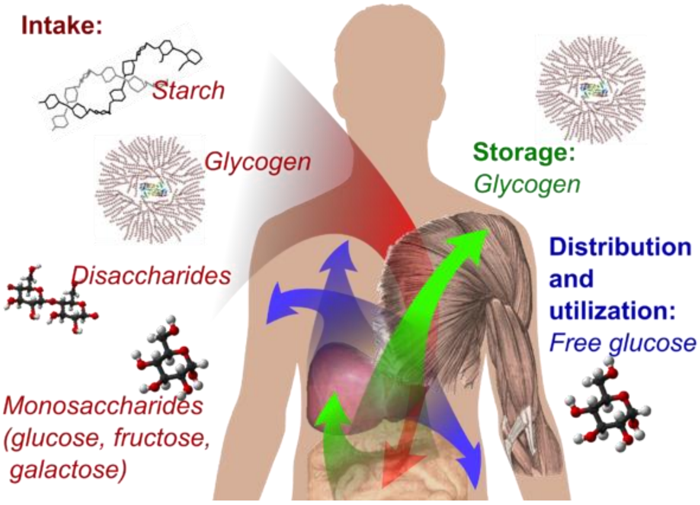


Figure 1.1: Glucose metabolism in human

1.1.1 Classification of carbohydrates

Carbohydrates are widely classified into simple and complex (polysaccharides). Simple sugars, based on the number of residue are further classified into monosaccharides and disaccharides; while polysaccharides based on the nature of repeating units are classified into homopolysaccharides and heteropolysaccharides.[2] A simple schematic representation of carbohydrate classification is given below (Figure 1.2).

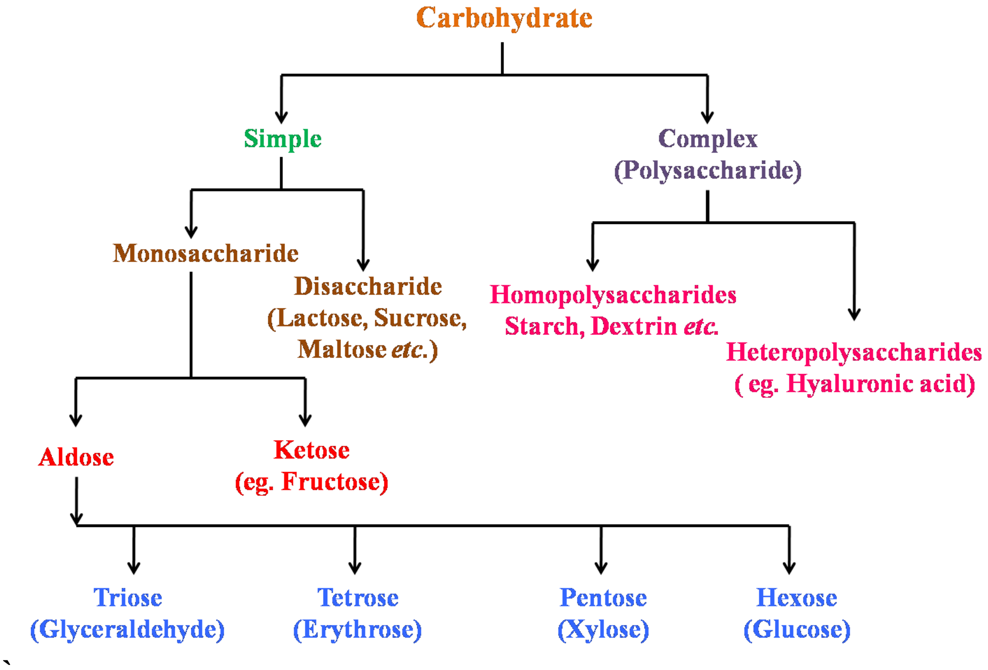


Figure 1.2: Classification of carbohydrates

Monosaccharides are the simple sugars which cannot be further hydrolysed. It consists of 3 carbons or more within the molecule. Monosaccharides may appear to be linear molecules, but when they come in contact with aqueous solutions, they tend to form 5-carbon ring structures, which make the molecules more stable. They are further classified by their placement of the carbonyl group into Aldoses and Ketoses. If Aldehyde functional group is present then it is called Aldoses (glucose and galactose) while if ketone is present then it is called Ketoses (fructose).

Combining two monosaccharides together would result in a disaccharide, linked via a glycosidic bond, and condensation reaction is the process that fuses two monosaccharides together. Such reactions form a disaccharide by removing a water molecule. Disaccharides are a class of simple sugar which on hydrolysis gives two monosaccharide residues. Maltose is a disaccharide mainly formed from two units of glucose joined with an α-(1→4) bond.

Polysaccharides are complex molecules composed of a large number of monosaccharide units joined together by glycosidic linkages. Homopolysaccharides are the class that have similar repeating units while in the case of heteropolysaccharides the repeating units are different. In starch the repeating unit is glucose, joined by glycosidic linkages. Dextrin is a group of low molecular weight carbohydrate_formed by the hydrolysis_of starch_or glycogen. They are mixtures of polymers of D-glucose units linked by α-(1→4) or α-(1→6) glycosidic bonds. While in Hyaluronic acid the repeating unit is Glucuronic acid and N-acetyl-D-Glucosamin (Figure 1.3).

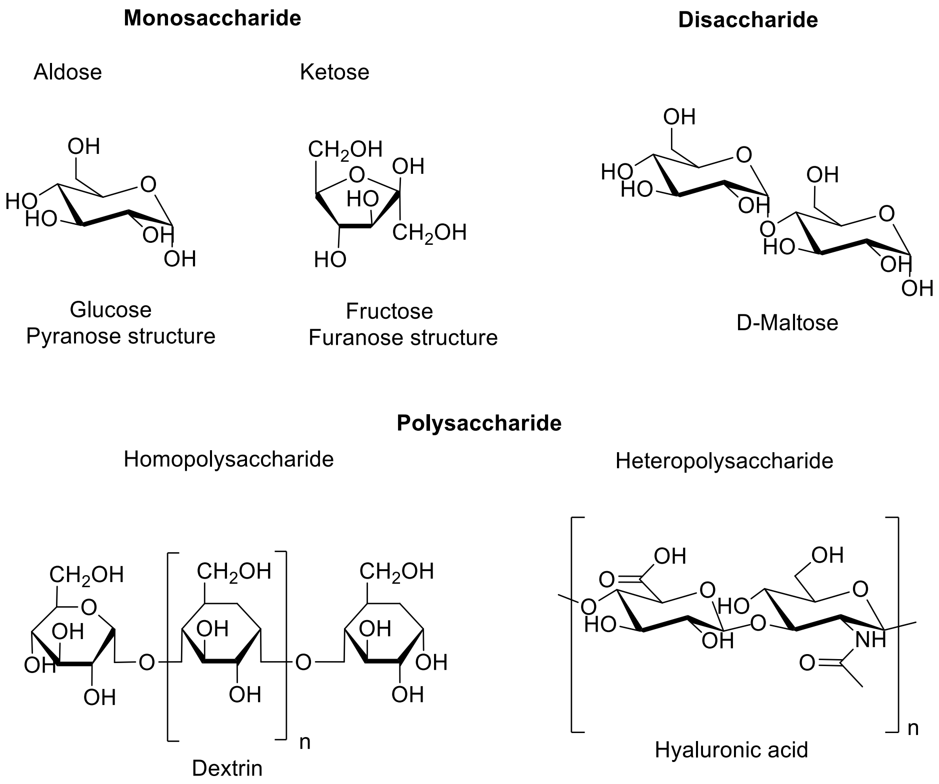
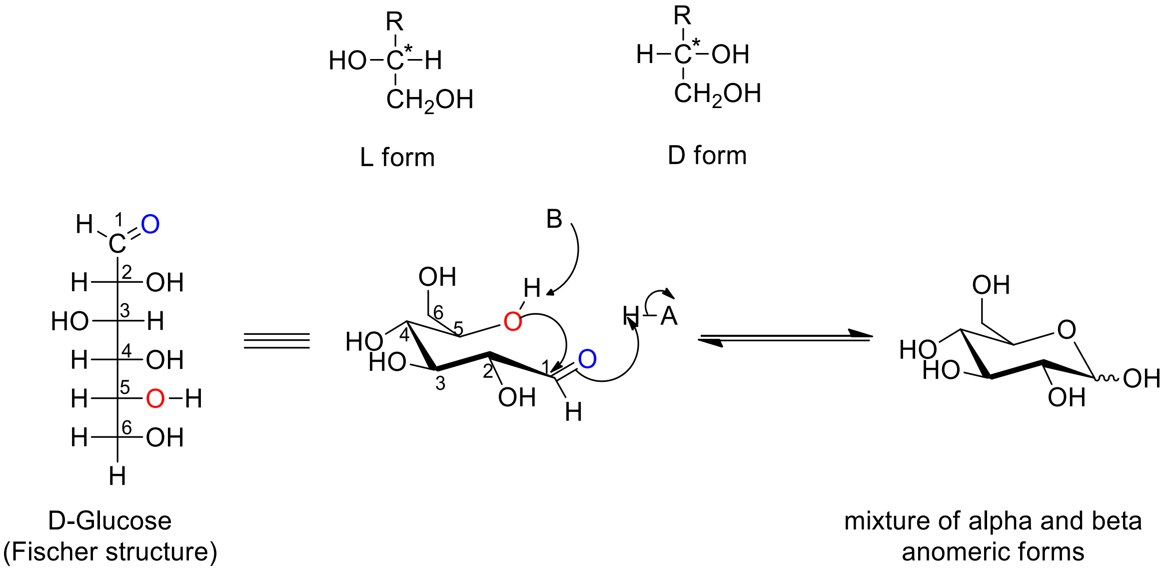


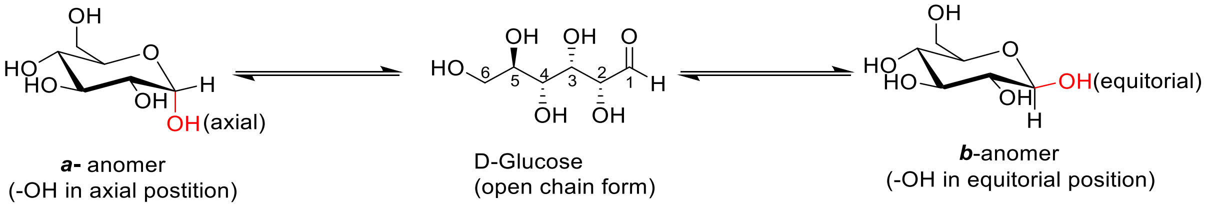
Figure 1.3: Structures of various types of carbohydrates

Due to the presence of more chiral carbons found in aldoses, there are more possible configurations in the aldose form than ketose. Carbons that have a hydroxyl group (-OH), disregarding both the first and last carbons are asymmetric. The asymmetric carbons lead to the two possible forms (R and S) which corresponds to the D- and L- configurations (Scheme 1.1). Most of the sugar molecules exist in their hemiacetal form. For example, in glucose the hydroxyl group from the 5th carbon will attack the carbonyl carbon, to form a hemiacetal intermediate. The carbon that will then change from a carbonyl carbon to a carbon with a hydroxyl will then be called an anomeric carbon. The new stereogenic centre that is created in the cyclic hemiacetal or hemiketal form of a carbohydrate is called the Anomeric carbon. It is hemiacetal or hemiketal that is bounded by an alcohol group and an ester bond. There are two different carbohydrate cyclic hemiacetal stereoisomers called anomers and are labelled as alpha or beta. If the hemiacetal -OH group is on the same side of the ring as the terminal $-CH_2OH$ substituent then it is said to be beta anomer and if it is on the opposite side then it is said to be alpha anomer.[3] The two anomers equilibrate in aqueous solution and this process is referred to as mutarotation. The process is catalyzed by acid, since hemiacetal formation is catalyzed by acid.



Scheme 1.1: Formation of hemiacetal form of D-Glucose

In the case of D-Glucose at equilibrium the **beta anomer predominates.**[4] This is because the -OH group of the anomeric carbon is more stable in equatorial position of the chair structure as shown in Scheme 4. In alpha-D-glucose, the -OH group on the anomeric carbon is axial as a result it experiences 1, 4 diaxial interactions leading to a reduction in its stability (Scheme 1.2). [5]



Scheme 1.2: Stability of anomers of D-glucose

1.1.1 Importance of Carbohydrates

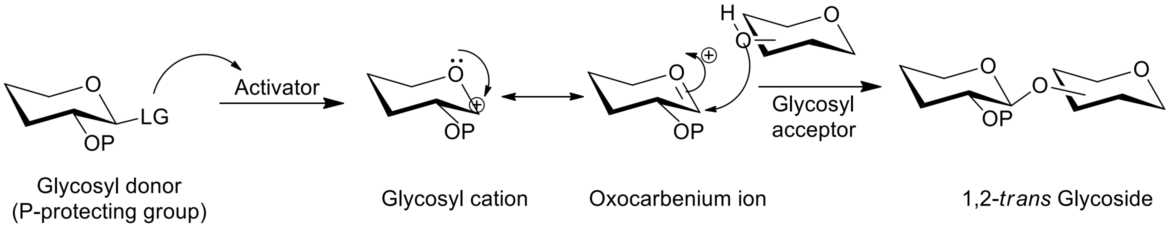
Glucose is the only sugar used by the body in various metabolic activities to provide energy for tissues. Therefore, all digestible polysaccharides, disaccharides, and monosaccharides are eventually to be converted into glucose or a metabolite of glucose by various liver enzymes. Because of its significant importance to proper cellular function, blood glucose levels must be kept relatively constant. Carbohydrates have several biochemical functions which are as follows;

- They are a fuel for cellular metabolism
- Used in several biosynthesis reactions
- Is converted into space-saving polysaccharides, such as glycogen and starch which provide stored energy for animal and plant cells respectively
- Used to form structural elements, such as chitin in animals and cellulose in plants
- The modified carbohydrates are important for an organism's fertilization, development, blood clotting and immune system function

Due to this structural diversity, carbohydrate chemistry can be applied to develop a broad range of complex therapeutic molecules and drugs, including pure carbohydrates as well as protein-linked carbohydrates, or glycoproteins. They also form the basis of revolutionary new vaccines and drugs to fight against malaria, HIV, and various other diseases. Carbohydrate-protein interactions play a crucial role in various critical intra- and intercellular events, such as cell differentiation, immune response, inflammation, cancer metastasis, and pathogen adhesion. As a result of these interactions, carbohydrates are of high biological and medicinal relevance. Glycobiology and carbohydrate chemistry act together to provide a guiding mechanism for sick cells *i.e.* enabling drugs to arrive with precision and act effectively without or with minimum side effects[6]. This can be used for developing targeted drug delivery systems thus increasing its bio-availability. In addition to this, it has been observed that drug-linked to sugar moieties participate in interactions between drugs and their biological targets by consequently increasing their pharmacokinetic properties (ADME), drug solubility and stability.

1.1.2 Glycosylation

Glycosylation is an important step in the carbohydrate chemistry. It involves the coupling of a glycosyl donor to glycosyl acceptor forming a glycoside using an activator under suitable reaction conditions. This results in the synthesis of complex polysaccharides which may play important roles in biological processes. Glycosyl donor consists of leaving group in its anomeric position while glycosyl acceptor can be monosaccharide, oligosaccharide that contains an available nucleophile such as unprotected hydroxyl. If the non-sugar compound acts as an acceptor then it known as aglycone.[7] The link between the glycon and aglycon is known as glycosidic bond. In the presence of activator the leaving group is abstracted resulting in the formation of a glycosyl cation which on further rearrangement forms oxocarbenium ion as the intermediate and finally resulting in the formation of 1,2 *trans* glycoside (Scheme 1.3).[8]



Scheme 1.3: General mechanism for glycosylation

Chemical glycosylation reaction is controlled by the principle of reactivity and selectivity. Glycosyl donor need to be sufficiently reactive to allow the nucleophile to react with it. If donor is much more reactive, then proper selectivity in glycosidic bond formation cannot be achieved. Here in our studies, glycosyl donor is a sugar group with a suitable leaving group at the anomeric position and glycosyl acceptor is a secondary metabolite with an unprotected hydroxyl group. The process of coupling of a glucose core and a secondary metabolite consist of various challenges including stereoselectivity, regioselectivity, chemoselectivity and enantioselectivity.

The environment and reaction conditions can improve, reduce or even overturn the inherent selectivities of a glycosyl donor and a glycosyl acceptor in the reaction. Temperature, stoichiometry, solvent, activating agent, water content, reaction time, anomeric leaving group of donor, and protecting groups are the main factors which have notable influence on the glycosylation reaction.[11] The outcome the reactions can be strongly determined by the above factors.

1.2 Natural Products (NPs)

Natural products are the compound or substance produced by a living organism. According to Kossel in 1891, natural products are broadly classified into primary metabolites and secondary metabolites. The former is involved normal growth, development, reproduction *etc*. of an organism (examples: sugars, nucleic acids, proteins *etc.*) while, the latter is involved in defense mechanism like antibiotics and pigments (example: flavonoids, alkaloids, terpenes, sterols *etc*.).

Natural products represent the richest source of novel molecular scaffolds in chemistry. The use of plants as medicines has a long history in the treatment of various diseases. Mankind has been continuously using plants for the treatment of various ailments[10] since the ancient period. According to WHO, about 80% of the world's population relies on traditional medicine for their primary health care. Obviously natural products will continue to be extremely important as sources of medicinal agents. They represent a significant source of drug compounds that are currently in the market for the treatment of a variety of diseases and some of them are used as dietary supplements, as dyes, flavouring agents, or ingredients in cosmetics industry for meeting demand for effective and safer use.

Many NPs have some historic importance. For example the alkaloid called *coniine*, the molecule in hemlock, killed *Socrates*. Some of them are essential for our survival but whose presence other than the optimum level can cause major problems. Biologically important NPs like cholesterol, a steroid molecule is the vital component of cell wall that causes heart attack when its level increases above optimal condition. Thromboxane is a polyketide whose excess level will clot all the blood in our body but its absence will create a deathly disease called *Haemophilia.* There are several classes of Secondary metabolites and they are said to show activities beneficial to humans, such as antimicrobial, anticancer, and radical-scavenging activities hence they are of more importance. Some of natural products along with mode of action and pharmaceutical use is given below (Table 1.1).[9]

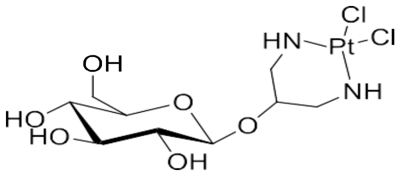
Table 1.1: Various glycosylated natural products and their pharmaceutical uses

Glycosylated natural product	**Mode of action**	**Pharmaceutical use**
Glycosylated natural products from microorganisms		
Anthracyclines	DNA intercalation Acute lymphoblastic/myeloblastic leukemias;	breast cancer, juvenile tumors, soft tissue, and aggressive sarcomas

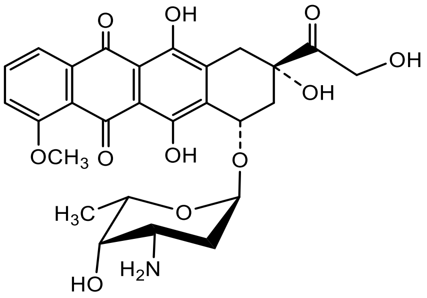
Macrolides	Binding to 50S rRNA	Respiratory tract and soft tissue infections caused by streptococci, pneumococci, staphylococci, and enterococci
Coumarins Gyrase Glycopeptides antibiotics	Gyrase inhibition , Binding to N-acyl-d-Ala-d-Ala termini of non-cross-linked lipid-PP-disaccharidepentapeptides	Infections caused by Staphylococcus epidermidis Infections by Gram-positive bacteria
Glycosylated natural products from plants		
Anthraquinones	Absorption, inhibition of Naþ/Kþ-ATPase	Laxative agents
Flavonoids	Radical-oxygen scavengers	Tone improvement in vein walls, treatment of congestive heart failure, and improvement in blood circulation
Etoposide	DNA-topoisomerase II	inhibition Anticancer therapy

1.3 Scope of the present work

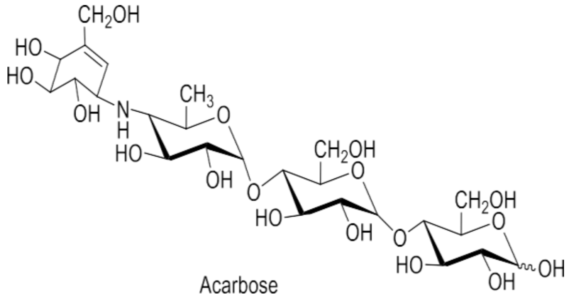
Many carbohydrate based medicines are available for the treatment of numerous diseases. Acarbose, Doxorubicin, Stavudine, Acadesine are some examples of currently used carbohydrate based drugs. Acarbose is a glycoside used for the treatment of type-2 diabetes mellitus and it was isolated from Actinoplanes microbial cultures. Doxorubicin is flavonoid glycoside used as an antibiotic. Cis-platin is an anti-cancer drug and by attachment of carbohydrate moiety to it, can reduce its side effects. Glycosides possess therapeutically beneficial properties and therefore they are widely used in the field of pharmaceutical science.



Cis platin with carbohydrate chain



Doxorubicin



Most of the bioactive secondary metabolites usually exist in plants, as their glycosides, but their bioavailability is lower. Generally metabolites derived from plants are insoluble in water and DMSO, which are the solvents used for the initial biological screening. So several studies are ongoing to enhance their solubility.

Biologically important carbohydrates can be extracted from natural sources. Majority of them exist in their heterogeneous form. Moreover effective separation still remains as a problem. The isolation glycosides from plants is a complex process. Usually glycosides are cleaved off during the isolation process, due to the acidic nature of silica in silica gel column chromatography and liberating pure aglycone part. However glycosides are considered to have more beneficial properties for humans than aglycone parts themselves. Therefore, recent investigations are focussing on the biological evaluation of secondary metabolites. Here in our studies, we are recombining the aglycone part with a glycone using glycosylation process. This can overcome the solubility issues and enhance their bioavailability. Hence, the process of glycosylation plays a major role in the development of new effective drug candidates. Carbohydrates are proving to be a valuable tool as drug leads due to its promising biological and therapeutic potentials.

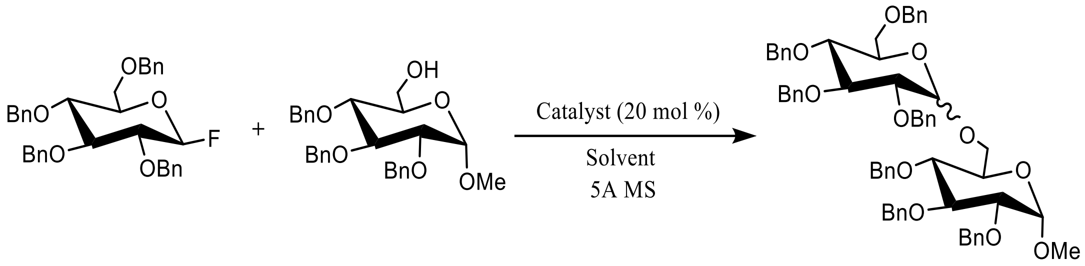
Chapter 2

Literature Review

Carbohydrate chemistry has emerged into an area of research by the pioneering discoveries of Emil Fisher, the great grandfather of carbohydrate chemistry. He was awarded Nobel Prize in 1902 for his ground breaking works in the field of carbohydrate chemistry. The major challenge in synthetic carbohydrate chemistry is more efficient, direct, and selective methods for the production of oligosaccharides, which is biologically and pharmacologically very active compounds.[18] Scientists experimented several ways to improve their preparation selectively by using different glycosyl donor acceptor pair, protecting groups, various catalysts, and reaction conditions. Carbohydrate chemistry is an area that has been undergoing many studies in the last few years.

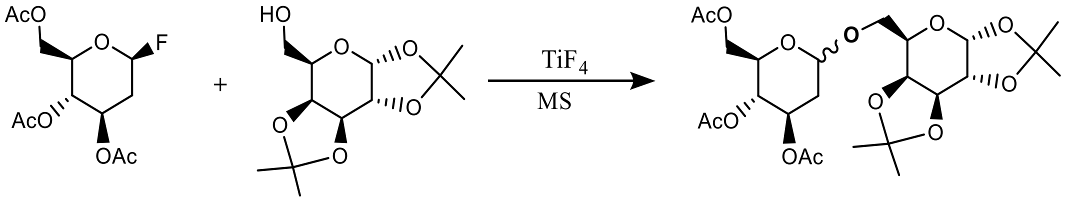
2.1. Glycosyl halides as glycosyl donor

Glycosyl halides including glycosyl fluorides, chlorides, bromides and iodides are used in chemical glycosylation reactions. Glycosyl fluorides are widely used in glycosylation reactions as glycosyl donors. The use of glycosyl fluorides as glycosyl donor were first introduced by Mukaiyama and his co-workers in 1981.[19] They were used stoichiometric mixture of stannous chloride and silver perchlorate ($SnCl_2$- $AgClO_4$) as a fluorophilic activating agent, which gives greater α- stereo selectivity starting from β-glycosyl fluoride donors. Glycosylation with a 4-OH acceptor produced a yield of 91% and α/β selectivity ratio of 80/20. This form of donor was incorporated into the complete synthesis of carbohydrates containing natural products and has received considerable attention as an attractive glycosyl donor.



Glycosylation Reaction Performed by the Mukaiyama and group

Moreover, Nicolaou, Vozny, and Kunz independently reported that glycosyl fluorides reacted effectively with a variety of free alcohols and silyl ethers using BF_3Et_2O as an activator to produce good yields of the corresponding *O*-glycosides.[20]



Hexane	Et_2O
0 ^{0}C, 55h	0 ^{0}C, 4.2%
65% (α/β= 2/1)	85% (α/β=1/4)

Noyori and group reported that SiF_4 and TMSOTf could be used as catalysts in the glycosylation reactions of glycosyl fluorides as donors and silyl ethers as acceptors in different solvents yielding O-glycosides.[21] For the selective glycosylation reactions, different lewis acids and bronsted acids are used as catalytic activators.

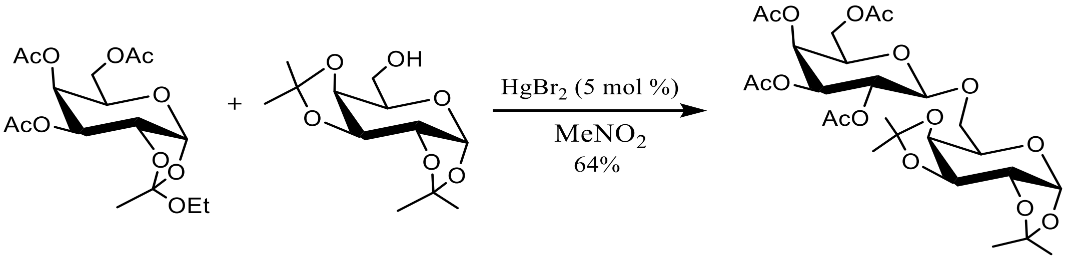
Glycosyl chlorides and glycosyl bromides are reported as glycosyl donors by Knoenigs and Knorr in 1901.[22] Their process used heavy metal salts (mainly salts of silver and mercury) as activating reagents. Nevertheless, other methods of glycosylation using glycosyl bromide or chloride in the lack of any metal were also commonly studied.

Among glycosyl halides, only few reports of the use of glycosyl iodide as an isolatable glycosyl donor are available due to their instability. Glycosyl iodides have been intensely investigated as glycosyl donors, since their discovery by Helferich and Gootz as glycosyl donors.[23] Glycosyl iodides can be activated by a catalytic amount of either a Lewis or Bronsted acid. Strong Bronsted acid, HI, is the by-product of the reaction with a protic nucleophile, and it became clear that equivalent amounts of an acid scavenger is needed in glycosylations. On the other hand, an anionic nucleophile can be used to improve the yield and reaction time and hence make the synthesis of oligosaccharide feasible. Therefore, glycosylation reactions using glycosyl iodide as donors are not feasible for oligosaccharide synthesis, in the absence of an acid scavenger.

Nowadays, chemists are extensively researching on various organo tin, boron, copper, caesium mediated glycosylation methods to perform regio and stereoselective glycosylation on partially protected or protection-less sugar moieties.[24]

2.2. Orthoesters as glycosyl donors

Over the past decades, the use of orthoesters as glycosyl donors has become extremely important. Kochetkov et al. played a significant role in the systematic study and application of orthoester methods. Kochetkov reported the first example for the use of orthoesters as glycosyl donor under catalytic conditions.[25] The use of catalytic amounts of $HgBr_2$ in CH_2Cl_2 mainly produce orthoester, whereas nitromethane as the solvent and more catalyst produce 1, 2-trans-glycoside.

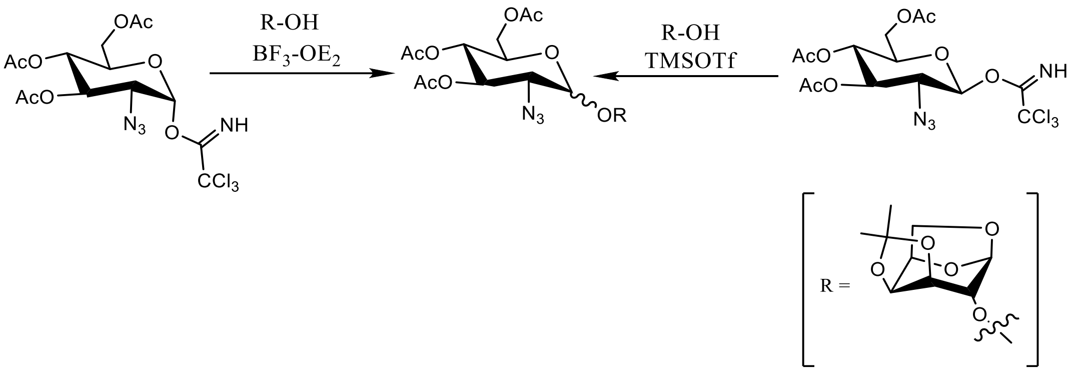


$HgBr_2$ catalyzed activation of an Orthoester

Generally, the use of simple orthoesters in the synthesis of oligosaccharide seems to be restricted by their low reactivity and the competing reaction with the alcohol produced by activating the donor. This has been resolved by the use of cyanoalkylidenes, but then it is important to tritylate the acceptor, which is a limitation. The most promising method is using orthoesters with a functional side group, which can be catalytically activated by creating a non-nucleophilic leaving group.

2.3. Triflates as Catalyst

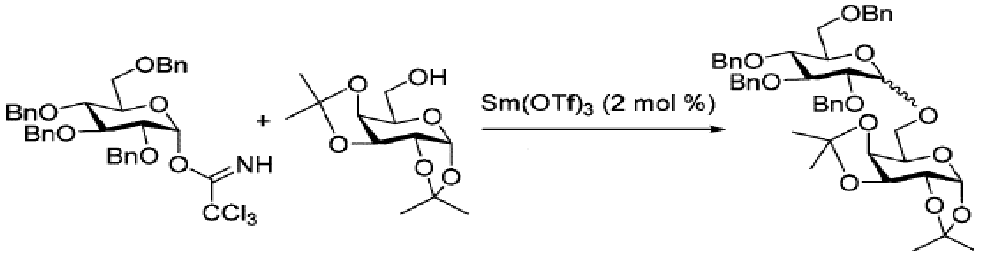
Grundler and Schmidt showed that the choice of catalyst could affect the stereoselectivity of a glycosylation reaction.[26] Also TMSOTf and TBDMSOTf were used by the Schmidt group as catalyst.



Lewis acid dependent selectivity introduced by Grundler and Schmidt

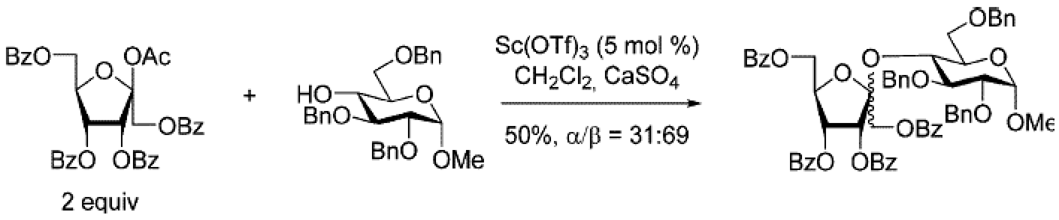
($BF_3.OEt_2$- α/β= 1:4, TMSOTf- α/β= 11:1)

In 1995, Schmidt and Castro-palomino introduced $Sn(OTf)_2$ as a catalytic activator for Trichloroacetimidate (TCA) donors. This catalyst resulted in high yields of 76-91% of disaccharides and trisaccharides on a disarmed glycosyl donor at room temperature within 15 minutes.[27] Then Adinolfi et al. reported in 2000, that $Sm(OTf)_3$ can be used as a catalytic activator for perbenzylated TCA donors.[28]



Activation of glycosyl donor by $Sm(OTf)_3$

Acetate groups are used as protecting groups in glycosylation reactions, but they were not frequently used for the synthesis of oligosaccharides because of the prolonged reaction time and harsh conditions. New approaches and methods are still emerging to make the donors more appealing. Yamanoi et al. reported that $Sc(OTf)_3$ could be used as a catalytic activator for the glycosyl acetate donors.[29]



$Sc(OTf)_3$ as a novel catalyst in Glycosyl acetate activation

Apart from above discussed donors and catalysts, various other type of donors and catalysts are used for glycosylation reactions. Glycosyl imidate donors, glycosyl esters, glycals, glycosyl carbonate and carbamate donors, thioglycosides, thiocyanates, phosphorous based, alkene or alkyne based glycosyl donors are some of the donors used for chemical glycosylation reactions.[30]

The field of carbohydrate research is developing gradually more towards catalytic glycosylation methods. The synthesis of glycosides via glycosylation is intrinsically challenging because every glycosylation leads to a pair of diasteromers. It not only decreases the yield of the desired product but also leads the reaction to the next step without further purification. Although so many attempts are established to overcome these issues in a limit, but not satisfactorily. New methods of controlling glycosidic bond formation have been developed in the field of carbohydrate chemistry. In recent years, many catalysts and leaving groups are introduced by carbohydrate chemists. Nowadays, glycoscience research is becoming an important area in synthetic carbohydrate chemistry.

2.4. Objectives of the present study

i. To reconnect various secondary metabolites including terpenoids, sterols *etc.* with different sugar moieties (*i.e.* primary metabolites) *via* glycosylation reactions using suitable glycosyl donors
ii. To study the difference in their bioavailability
iii. To examine how the nature of the sugar group affect their biological properties

Chapter 3

Results and Discussions

From the last few decades secondary metabolites have got great attention due to their potential role in human nutrition, cosmetics, drugs and their indispensable role in plant defence. This drastic change in interest of secondary metabolites is not purely academic but also of commercial nature. A large variety of these compounds have been isolated and identified from plants while only a few among them could be utilised as drugs, flavours, fragrances, insecticides, and dyes and thus have a great economic value. Further exploration of these compounds will yield valuable pharmaceutical, medicinal and other commercially important materials. Several secondary metabolites with very high bioactivity have been isolated so far, which prevent low density lipid peroxidation and thereby prevent atherosclerotic plaque formation, neurodegenerative diseases and ischemic injury. They also capture free radicals and chelate copper and iron ions, which can promote free radical generation. They can also stimulate enzymes which are involved in detoxification of cancer causing substances and inhibit inflammation and aging processes.[12] A large number of biologically active alkaloids also have been isolated from plants. At cellular level the action of alkaloids is quite variable, some effect nervous system, some effect protein synthesis, and other affect membrane transport and enzyme activities. Whereas the bioactive compound in question needs to be bioavailable before it can have an effect. It may be considered redundant to study the health effects of dietary bioactive compounds if their bioavailability is not also fully elucidated.

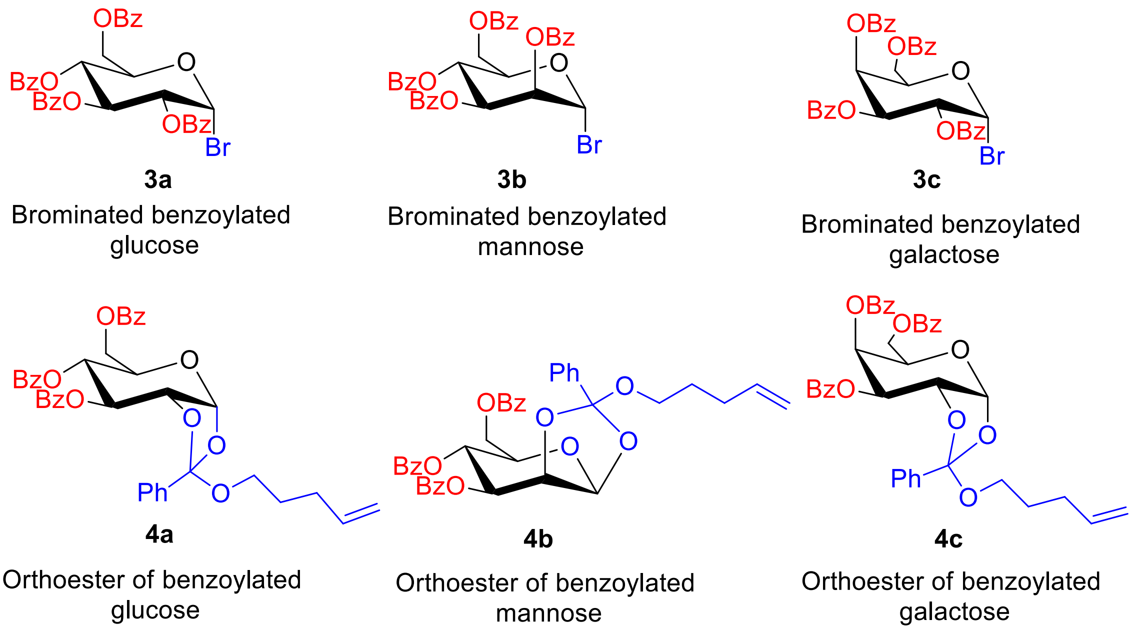
From a pharmacological perspective, bioavailability is the rate and extent to which the bioactive compound or a drug is absorbed and becomes available at the site of action. The molecular structure of a bioactive compound affects its absorption and hence their bioavailability considerably. The sugar moiety present in the glycosidic form of secondary metabolites has been suggested to be an important determinant for their absorption in humans.[13]

The design of small glycoconjugated organic molecules is one of the hot topics in current research due to their possible practical applications in biology as well as in material chemistry. Fluorescently labelled glycoconjugates provide ease of access toward biological events at the molecular level with high selectivity and sensitivity and are widely employed in diagnostic tool development. Moreover, sugar units are excellent building blocks in the supramolecular chemistry owing to their chirality and hydrogen bond forming ability. Moreover, monosaccharides form the constituent of significant biomolecules like NAD, FAD, RNA and also glucose represent the major energy source for live cells and stored as the metabolite. Because of these factors, carbohydrates play a crucial role in the development of efficacious drugs and diagnostic tools.

To accelerate the detection of positive hits and for improving the target selectivity and biocompatibility, carbohydrates can serve as a reliable detecting tool. The structural complexity of the carbohydrates makes the functionalization difficult compared to the presently used nucleic acids and proteins. Functionalization of carbohydrates may cause an alteration in the structure and their binding properties. Thus, carbohydrates have been less explored and remain as a challenging area of research. However, there have been extensive efforts on understanding interactions and functions of carbohydrate structures in the biological system.

3.1. Design strategy

Our strategy involved the design of monosugar appended glycoconjugates of secondary metabolites as shown in Scheme 3.1. First step involves the synthesis of starting materials (various glycosyl donors) for the *O*-glycosylation with different secondary metabolites.



3a Brominated benzoylated glucose

3b Brominated benzoylated mannose

3c Brominated benzoylated galactose

4a Orthoester of benzoylated glucose

4b Orthoester of benzoylated mannose

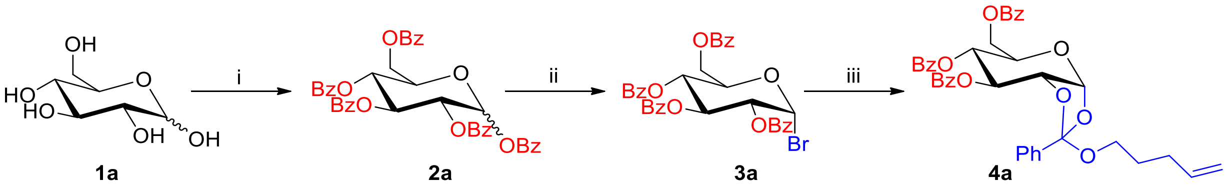
4c Orthoester of benzoylated galactose

Figure 3.1: Structure of various *O*-glycosylation donors

3.2. Synthesis strategy

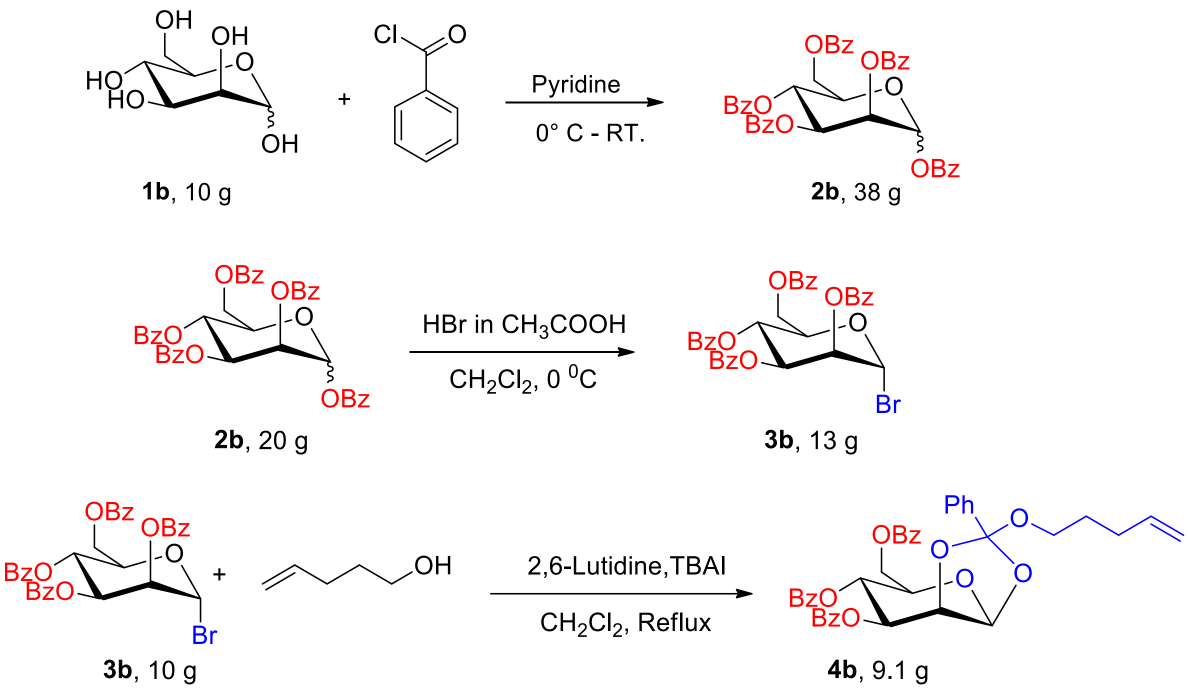
D-Glucose **1a** (20.5 g, 0.114 mol) and DMAP (0.1 g, 0.8 mmol) were dissolved in pyridine (250 ml) and, benzoyl chloride (100 ml, 0.862 mol) was slowly added with cooling and stirring. The resulting reaction mixture was stirred for 24 h at room temperature and then evaporated to dryness. The solid residue was dissolved in CH_2Cl_2, and water was carefully added with cooling and vigorous stirring to decompose the excess of benzoyl chloride.[14] The product (Rf 0.45 in Hex/EtOAc 2:1) was extracted with CH_2Cl_2, processed in the usual way, and dried to provide the crude perbenzoate **2a**, in approximately 85 % yield. The crude material (**3a**, 30 g, 0.042 mol) was dissolved in CH_2Cl_2 (200 ml) and was cooled to 0 ^oC, 30 % HBr/AcOH solution (115 ml) was slowly added and the reaction mixture was stirred at 0 ^oC for 1 h. The temperature of the reaction mixture was then raised up to, 10 ^oC, the flask was sealed and stored in the refrigerator (5 ^oC) overnight. The reaction mixture was then diluted with cold CH_2Cl_2 and, cold water (0 ^oC) was added. The organic layer was washed with cold water, cold saturated $NaHCO_3$ and dried. After evaporation of solvent the crude glycosyl bromide 2,3,4,6-tetra-*O*-benzoyl-*α*-D-glucopyranosyl bromide, **3a**, (Rf. 0.59 in Hex/EtOAc 2:1) was obtained in approximate yield 92 % (26.1 g).

The crude glycosyl bromide **3a** (20 g, 0.030 mol) was dissolved in dry CH_2Cl_2 (150 ml), and 2,6-lutidine (10 ml, 0.085 mmol), 4-pentenyl alcohol (7 ml, 0.07 mmol), tetra-butylammonium iodide (1.0 g, 2.7 mmol) were added. The resulting mixture was refluxed under argon for 24 h, when TLC (Rf 0.59 in Hex/EtOAc 3:1) showed complete disappearance of **3a**. After cooling to room temperature, water and diethyl ether were added, and the organic layer was washed with water, brine and dried. After evaporation the residue **4a** was filtered through silica gel using hexane/ethyl acetate (from 9:1 to 4:1) to effect partial purification from polar impurities (76 %). All the experimental procedures are well explained below.

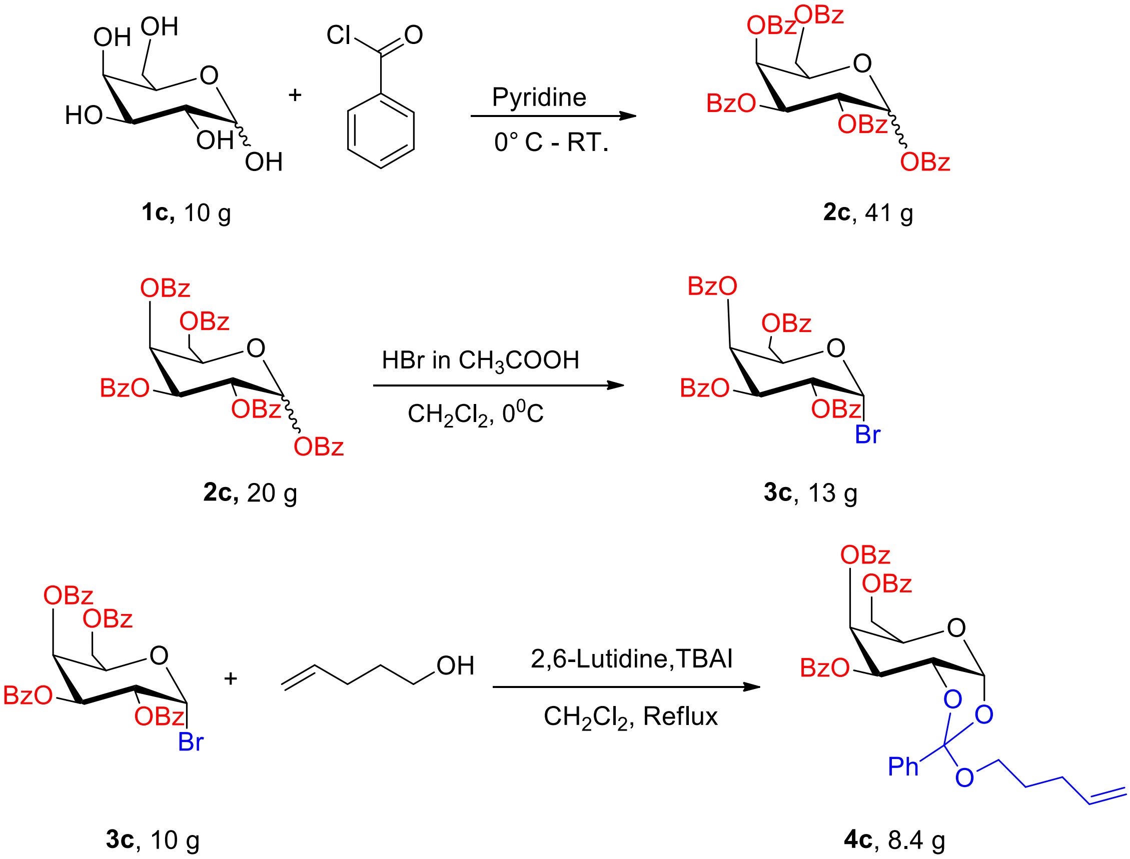


Scheme 3.1 : (i) PhCOCl, DMAP, pyridine, 24 h, RT, 85 % (ii) CH_2Cl_2, 0 ^oC, 30 % HBr/AcOH, 12 h, 92 % (iii) CH_2Cl_2, 2,6-lutidine, 4-pentenyl alcohol, nBu_4NI, 76 %.

The same protocol was also applied in the synthesis of mannose and galactose donors (Scheme 3.2 and 3.3). All the products were well characterised by usual spectroscopic analysis such as infrared, NMR and mass spectroscopic techniques.



Scheme 3.2: Synthesis of mannose glycosyl donors

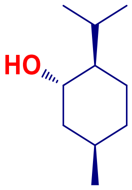


Scheme 3.3: Synthesis of galactose glycosyl donors

3.3. Selection of secondary metabolites

In the present study, we mainly chose those molecules having an alcoholic or phenolic hydroxyl group that are isolated from plant sources. Most of these molecules were isolated in our lab itself. They include isomenthol, kojic acid, stigmasterol and *β*-sitosterol.

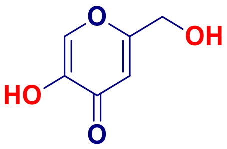
Isomenthol (**5b**) is the major component of the wild mint *Mentha arvensis*, a species of flowering plant in the mint family Lamiaceae (Figure 3.2A). In ayurveda, they are considered as appetizer and useful in gastric troubles. Traditionally, they are used to treat flatulence, digestive problems, gall bladder problems and coughs. The Aztecs used it for similar purposes and also to induce sweating and cure insomnia. The oil was extracted and rubbed into the skin for aches and pains. Mint extracts and menthol-related chemicals are used in food, drinks, cough medicines, creams and cigarettes. Menthol and isomenthol are widely used in dental care as a topical antibacterial agent, effective against streptococci and lactobacilli, and is commonly used in pharmaceutical and oral preparations like toothpastes, dental creams, beverages and tobacco[15]. In the present study, we chose the pure form of isomenthol that was purchased from the manufacturer Sigma-Aldrich. Kojic acid (**5c**) is a chelation agent produced by several species of fungi, especially *Aspergillus oryzae*. It is also a by-product in the fermentation process of malting rice, for use in the manufacturing of sake, the Japanese rice wine. It is a mild inhibitor of the formation of pigment in plant and animal tissues, and is used in food and cosmetics to preserve or change colours of substances. They are widely used in cosmetics to lighten skin and also used to treat skin diseases like melasma[16]. The co crystals of kojic acid with quercetin were found to have two times better cytotoxic activity to human cervical cancer cells (HeLa) and human colon cancer cells (Caco-2) in comparison with quercetin itself. In the present study, we used the kojic acid isolated from the fruits of *Dillenia indica* (elephant apple, Figure 3.2B) along with some compound purchased from the manufacturer Alfa-Aesar.



Isomenthol

5b

(A)



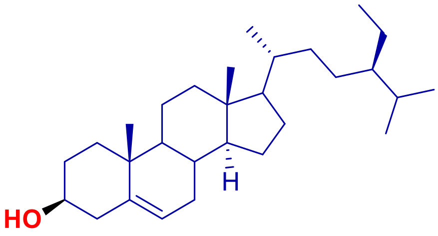
Kojic acid

5c

(B)

Figure 3.2: **(A)** Isomenthol, *Mentha arvensis* **(B)** Kojic acid, *Dillenia indica*

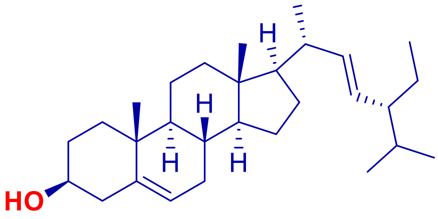
β-sitosterol (**5d**) and stigmasterol (**5e**) are phytosterols (most abundant plant sterols) with chemical structures similar to that of cholesterol (Figure 3.3).[17] Sitosterols are white, waxy powders with a characteristic odour and are hydrophobic and soluble in alcohols. Sterols have a major function to maintain the structure and physiology of cell membranes; they may be used in food manufacturing to increase the phytosterol content and potentially lowering the levels of LDL cholesterol. Stigmasterol is a constituent of various vegetables, legumes, nuts, seeds and unpasteurized milk. Pasteurization will inactivate stigmasterol. Edible oils contain higher amount than vegetables. Here we used these sterols isolated from different plant species, such as *Musa balbisiana*, *Pterocarpus marsupium*, *Anethum graveolens*, *Zingiber nimmonii etc.*



β-sitosterol

5d

Musa balbisiana *Zingiber nimmonii*



stigmasterol

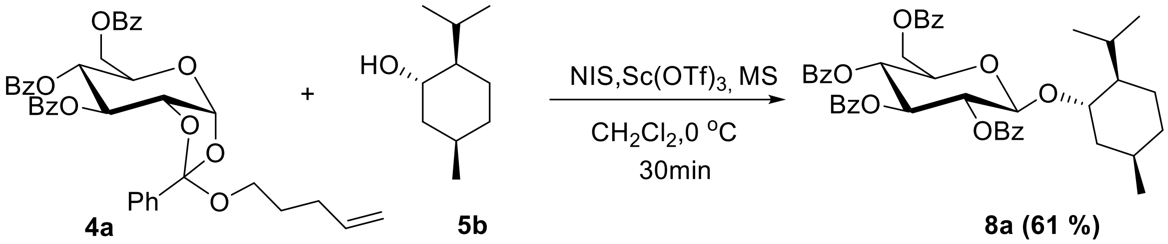
5e

Anethum graveolens

Figure 3.3: Structure of Sterols with some of the plants from which it is isolated

3.4. Glycosylation

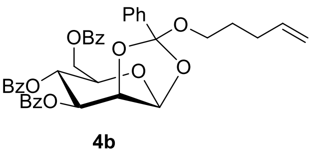
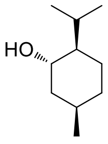
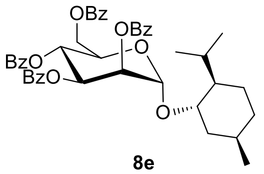
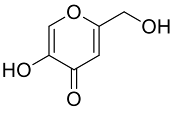
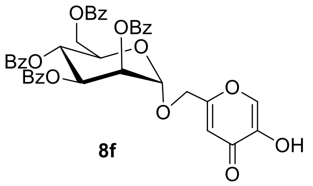
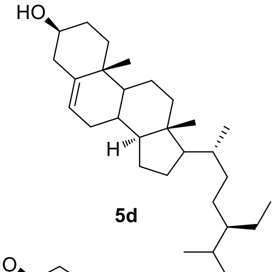
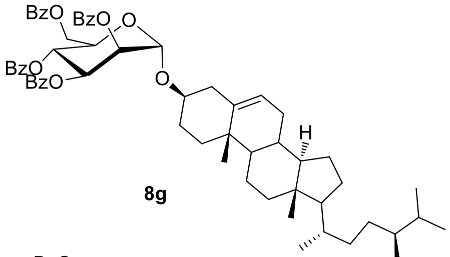
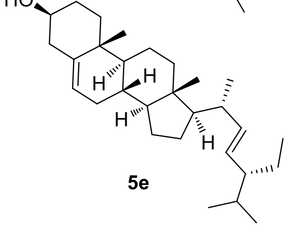
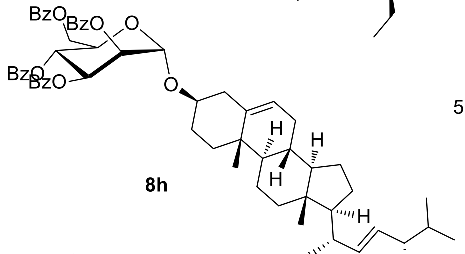
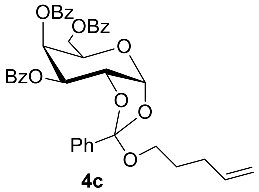
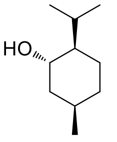
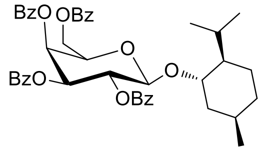
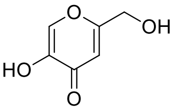
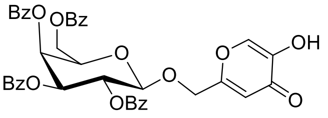
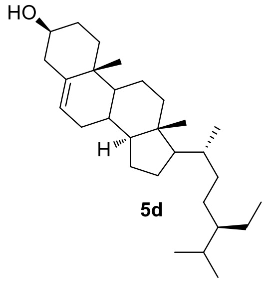
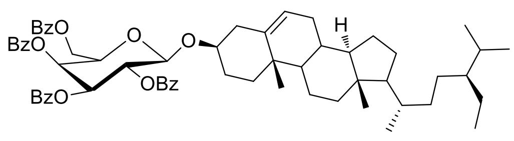
we carried out glycosylation reaction with n-pentenylorthoester glycosyl donors[14] (NPOE). Gluco NPOE **4a** (2 mmol) and acceptor **5b** (1 mmol) were azeotroped together with toluene. The resulting dry syrup was redissolved in dry CH_2Cl_2 (25 ml) at 0 °C. Molecular sieves and N-iodosuccinimide (NIS) (3.5 mmol) were stirred for 3 min and then $Sc(OTf)_3$ (0.2 mmol) was added. The reaction mixture was kept at room temperature for 30 min, and then the reaction was quenched with 10 % $Na_2S_2O_3$ and saturated $NaHCO_3$ aqueous solutions. The molecular sieves were filtered and washed with CH_2Cl_2 and the organic layer was washed with water, brine and dried. Column chromatography (Hex/EtOAc 4:1) provided the beta anomer compound **8a** in 61 % yield (Scheme 3.5).

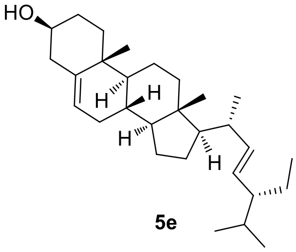
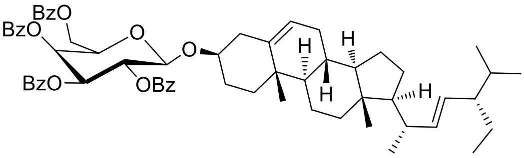


Scheme 3.5: Glycosylation with orthoester donors

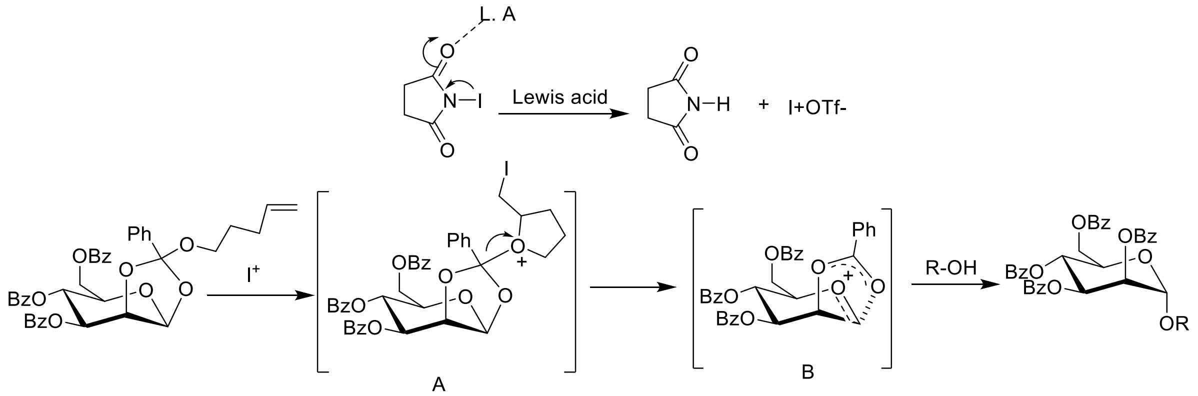
After the successful synthesis of the compound **8a**, we further optimised the reaction condition by changing the Lewis acids. When we employed $Yb(OTf)_3$ instead of $Sc(OTf)_3$, the yield was reduced to 33 %. Also, triflates like $In(OTf)_3$ and $Cu(OTf)_2$ did not furnish the product in more than 20 % yield. When we tried the reaction without forming the azeotrope, the product was obtained in 54 % yield. Hence we checked the generality of the reaction with various donors and acceptors under azeotrope free conditions. Results of our studies are tabulated below (Table 3.2). Interestingly, we also noticed that when we employed kojic acid as the donor, we obtained both the products; where the phenolic glycosylated products were obtained only in trace amounts.

Table 3.2: Glycosylation with various orthoester donors

No	Glycosyl donor	Acceptor	Product	Yield (%)
5	 **4b**	 **5b**	 **8e**	57
6	**4b**	 **5c**	 **8f**	26
7	**4b**	 **5d**	 **8g**	55
8	**4b**	 **5e**	 **8h**	57
9	 **4c**	 **5b**	 **8i**	48
10	**4c**	 **5c**	 **8j**	23
11	**4c**	 **5d**	 **8k**	56

No	Glycosyl donor	Acceptor	Product	Yield (%)
12	**4c**	 **5e**	 **8l**	58

From literature reports, a general mechanism for the gycosylation reaction with orthoester donors is given in scheme 1.3. Here, the triflate source is act as the promotor and the orthoester directing group preferably give *α*-anomer for mannose, while *β* for both glucose and galactose.[14] Initially, iodotriflate was formed from N-iodosuccinimide, that underwent a cyclisation with olefinic system of NPOE followed by ring expansion resulted in the formation of intermediate **A**. Further elimination of furanyl moiety (intermediate **B**) and subsequent addition of the hydroxy system resulted in the formation of the product (Scheme 3.6). The presence of orthoester as donor accelerates the glycosylation mechanism and preferentially leads to the formation of 1,2- *trans* Glycoside.



Scheme 3.6: General mechanism for glycosylation with orthoester donors

From these results, we can summarize as, in the presence of peracetylated donors, we are getting both the anomers, hence there is no specificity for the reaction. But, when we are employing the brominated donors, the glycosylation of phenolic/sp^2 system occurs predominantly, while in the presence of NPOE donors, the glycosylation of alcoholic hydroxyl group is the major product.

Chapter 4

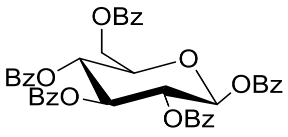
Experimental Section

All the chemicals were of the best grade commercially available and were used without further purification. All the solvents were purified according to standard procedures and dry solvents were obtained according to the literature methods and stored over molecular sieves. Analytical thin layer chromatography was performed with Merck TLC Silica gel F_{254} coated on aluminium sheets. Gravity column chromatography was performed using 230-400 or 100-200 mesh silica gel and mixtures of hexane-ethyl acetate were used for elution.

Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded on a Bruker AMX 500 spectrophotometer (CD_3CN, $CDCl_3$ and DMSO-d6 as solvents). Chemical shifts for ^{1}H NMR spectra are reported as δ in units of parts per million (ppm) downfield from $SiMe_4$ (δ 0.0). Multiplicities were given as: s (singlet); d (doublet); t (triplet); q (quartet); dd (double doublet); m (multiplet). Coupling constants are reported as J value in Hz. Carbon nuclear magnetic resonance spectra (^{13}C NMR) are reported as δ in units of parts per million (ppm) downfield from $SiMe_4$ (δ 0.0). For the structural assignment, 2-D NMR experiments were also conducted (^{1}H-^{1}H COSY, ^{1}H-^{13}C HMQC, ^{1}H-^{13}C HMBC). Mass spectra were recorded under EI/HRMS at 60,000 resolution using Thermo Scientific Exactive mass spectrometer.

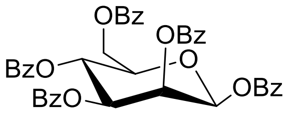
4.1. Spectral data

(2S,3R,4S,5R,6R)-6-((benzoyloxy)methyl)tetrahydro-2H-pyran-2,3,4,5-tetrayl tetrabenzoate (2a)

	**^{1}H NMR(500MHz, $CDCl_3$, TMS):** δ 8.18 (d, J = 5 Hz, 2H), 8.05 (d, J = 10 Hz, 2H), 7.97 (d, J = 10 Hz, 2H), 7.92-7.90 (m, 4H), 7.62 (t, J = 10 Hz, 1H), 7.36-7.31 (m, 5H), 7.27 (t, J = 5Hz, 4H), 6.93 (d, J = 3.5 Hz,1H), 6.40 (t, J = 10Hz, 1H), 5.94 (t, J=10 Hz, 1H), 5.75 (dd, J_1 = 10.5Hz, J_2 = 3.5 Hz, 1H), 4.71-4.67 (m, 2H), 4.54 (dd, J_1 = 12.5 Hz, J_2 = 4 Hz,1 H) ppm

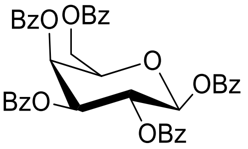
	^{13}C NMR(126MHz, $CDCl_3$, TMS): δ166.2, 166.0, 165.5, 165.3, 164.5, 148.0, 137.8, 134.0, 133.6, 133.5, 133.3, 133.2, 130.3, 130.1, 130.1, 129.9, 129.9, 129.8, 129.8, 129.5, 129.0, 128.9, 128.8, 128.7, 128.6, 128.5, 128.5, 128.4, 124.4, 90.1, 70.6, 68.9, 62.5 ppm **Mass Spectroscopic Analysis:** HRMS (ESI) for $C_{41}H_{32}NaO_{11}$ **Calcd $(M+Na)^+$:** 723.1842 **Found:** 723.1862 Colourless solid came in 20 % Ethyl acetate-Hexane mixture. **Yield:** 85 %

(2S,3S,4S,5R,6R)-6-((benzoyloxy)methyl)tetrahydro-2H-pyran-2,3,4,5-tetrayl tetrabenzoate (2b)

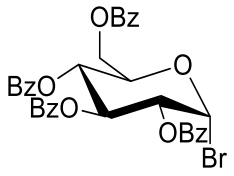
	**^{1}H NMR(500MHz, $CDCl_3$, TMS):** δ 8.24 (d, *J* = 10 Hz, 2H), 8.20 (d, *J* = 5 Hz, 1H), 8.13 (d, *J* = 7.5 Hz, 4H), 8.0-7.97 (m, 3H), 7.90 (d, *J* = 10 Hz, 2H), 7.65 (t, *J* = 7 Hz, 1H), 7.62 (m, 3H), 7.53 (t, *J* = 7Hz, 1H), 7.49-7.44 (m, 6H), 7.35-7.30 (m, 3H), 6.68 (brs, 1H), 6.34 (t, *J* = 10 Hz, 1H), 6.16-6.12 (m, 1H), 5.97 (s, 1H),), 4.75 (d, *J* = 12.5 Hz , 1H), 4.64-4.54 (m, 2H) ppm mixture of alpha and beta isomers. **^{13}C NMR(126MHz, $CDCl_3$,TMS):** δ166.1, 165.8, 165.3, 165.2, 163.9, 134.1, 133.7, 133.6, 133.4, 133.1, 130.2, 130.0, 130.0, 129.9, 129.8, 129.8, 129.0, 128.9, 128.8, 128.7, 128.5, 128.5, 128.5, 128.4, 91.4, 71.2, 70.1, 69.5, 66.2, 62.4 ppm **Mass Spectroscopic Analysis:** HRMS (ESI) for $C_{41}H_{32}NaO_{11}$ **Calcd $(M+Na)^+$:** 723.1842 **Found:** 723.1857 Colourless solid came in 20% Ethyl acetate-Hexane mixture. **Yiel**d: 98%

(2S,3S,4S,5R,6R)-6-((benzoyloxy)methyl)tetrahydro-2H-pyran-2,3,4,5-tetrayl tetrabenzoate (2c)

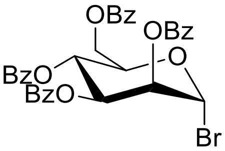
	^{1}H NMR(500MHz, $CDCl_3$, TMS): δ 8.11-8.00 (m, 7H), 7.97-7.80 (m, 6H),7.63-7.40 (m, 13H), 7.30-7.20(m, 10H), 6.30-6.10 (m,

	3H), 5.80-5.76 (m, 1H), 4.88-4.74 (m, 2H), 4.67-4.57 (m, 1H), 4.56-4.41 (m, 2H) ppm. (mixture of alpha and beta in 0.5:1 ratio) **^{13}C NMR(126MHz, $CDCl_3$,TMS):** δ 166.1, 166.0,165.9, 165.8, 165.5, 165.3, 164.5, 137.9, 133.8, 133.7, 133.5, 133.4, 133.3, 130.2, 130.0, 130.0, 130.0, 130.0,129.3, 129.1, 129.1, 129.1, 128.5,128.2, 125.3, 99.8, 93.10, 72.5, 71.6, 70.3, 69.5, 68.8, 68.6, 68.5, 68.0, 67.7, 63.6, 61.9, 61.8 ppm. **Mass Spectroscopic Analysis:** HRMS (ESI) for $C_{41}H_{32}NaO_{11}$ **Calcd $(M+Na)^+$:** 723.1842 **Found:** 723.1859 Colourless solid came in 20% Ethyl acetate-Hexane mixture. **Yield:** 98 %

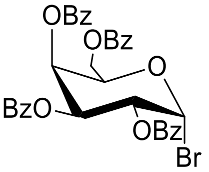
(2R,3R,4S,5R,6R)-2-((benzoyloxy)methyl)-6-bromotetrahydro-2H-pyran-3,4,5-triyl tribenzoate (3a)

	**^{1}H NMR (500MHz, $CDCl_3$,TMS):** δ 8.07 (d, J = 7.5 Hz, 2H), 8.00 (d, J = 7.5 Hz, 2H), 7.95 (d, J = 8 Hz, 2H), 7.88 (d, J = 8 Hz, 2H), 7.59-7.50 (m, 3H), 7.46-7.42 (m, 4H), 7.40-7.36 (m, 3H), 7.30 (t, J = 4.5 Hz, 2H), 6.87 (d, J = 2.5 Hz, 1H), 6.27 (t, J = 10Hz, 1H), 5.83 (t, J = 10 Hz, 1H), 5.33 (dd, J_1 = 10 Hz, J_2 = 3Hz, 1H), 4.74 (d, J = 10 Hz, 1H), 4.67 (d, J = 12.5 Hz, 1H), 4.52 (dd, J_1 = 12.5Hz, J_2 = 4 Hz, 1H) ppm **^{13}C NMR (125Hz, $CDCl_3$, TMS):** δ 166.0, 165.6, 165.3, 165.1, 133.8, 133.6, 133.3, 133.1, 129.9, 129.8, 129.7, 129.5, 129.0,128.8 ,128.6, 128.5, 128.5,128.4,128.4, 86.9, 72.8, 71.5, 70.7, 68.1, 62.0 ppm **Mass Spectroscopic Analysis:** HRMS (ESI) for $C_{34}H_{27}BrNaO_9$, **Calcd $(M+Na)^+$:** 681.0736 **Found:** 681.0733 and 683.0751 Pale Yellow solid. **Yield:** 92%

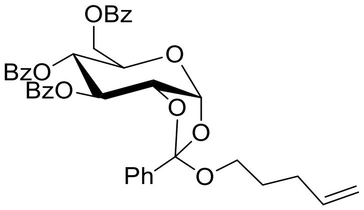
(2R,3R,4S,5S,6R)-2-((benzoyloxy)methyl)-6-bromotetrahydro-2H-pyran-3,4,5-triyl tribenzoate (3b)

	**^{1}H NMR (500MHz, CDCl$_3$, TMS):** δ 8.21 (d, J = 7Hz, 2H), 8.09 (d, J = 7.5 Hz, 4H), 7.96 (d, J = 7.5 Hz, 2H), 7.86 (d, J = 7.5 Hz, 2H), 7.69 (t, J = 7.5 Hz, 1H), 7.63 (t, J = 7.5 Hz, 1H), 7.59-7.57 (m, 2H), 7.44-7.37 (m, 4H), 7.30 (t, J = 8 Hz, 2H), 6.23 (brs, 1H), 6.29 (t, J = 10 Hz, 1H), 6.06 (dd, J_1= 10.5 Hz, J_2 = 3 Hz, 1H), 5.91 (brs, 1H), 4.70 (d, J = 12.5 Hz, 1H), 4.57 (d, J = 10 Hz, 1H), 4.50 (dd, J_1 = 12Hz, J_2 = 2.5Hz, 1H) ppm **^{13}C NMR (125Hz, CDCl$_3$, TMS):** δ 166.1, 165.7, 165.3, 163.9, 134.1, 133.7, 133.6, 133.4, 133.1, 130.2, 130.0, 129.8, 129.0, 128.8, 128.7, 128.5, 128.4, 91.4, 71.2, 70.0, 69.5, 66.2, 62.5 ppm **Mass Spectroscopic Analysis:** HRMS (ESI) for $C_{34}H_{27}BrNaO_9$ **Calcd (M+Na)$^+$:** 681.0736 **Found:** 681.0711 and 683.0742 Pale Yellow solid. **Yield:** 69 %

(2R,3R,4S,5S,6R)-2-((benzoyloxy)methyl)-6-bromotetrahydro-2H-pyran-3,4,5-triyl tribenzoate (3c)

	**^{1}H NMR(500MHz, CDCl$_3$, TMS):** δ 8.08-8.06 (m, 1H), 8.02-8.00 (m, 5H), 7.80(d, J = 7.5 Hz,2H), 7.55-7.45 (m, 3H), 7.44-7.39 (m, 6H), 7.28-7.26 (m, 3H), 6.13 (d, , J = 2 Hz, 1H), 6.08-6.06 (m, 1H), 5.73-5.61 (m, 2H),4.93 (t, J = 6 Hz, 1H), 4.66-4.63 (m, 1H), 4.49-4.46 (m,1H) ppm **^{13}C NMR(126MHz, CDCl$_3$,TMS):** δ 166.0, 165.6, 165.4, 165.3, 133.8, 133.6, 133.4, 133.4, 133.1, 130.0, 130.0, 130.0, 129.9, 129.9, 129.8, 129.8, 129.7, 129.2, 128.9, 128.8, 128.6, 128.5, 128.5, 128.4, 128.4, 88.3, 71.8, 68.9, 68.6, 68.1, 61.7 ppm **Mass Spectroscopic Analysis:** HRMS (ESI) for $C_{34}H_{28}BrO_9$ **Calcd (M+H)$^+$:** 659.0917 **Found:** 659.0911 and 661.0918 Pale Yellow solid. **Yield:** 69 %

(2R,3R,4S,5S,6R)-5-((benzoyloxy)methyl)-2-(pent-4-en-1-yloxy)-2-phenyltetrahydro-3aH-[1,3] dioxolo[4,5-b]pyran-6,7-diyl dibenzoate (4a)



^{1}H NMR(500MHz, CDCl$_3$, TMS): δ 8.09 (d, *J* = 7 Hz, 2H), 7.95 (t, *J* = 9 Hz, 4H), 7.78 (d, *J* = 6 Hz, 2H), 7.61-7.57 (m, 2H), 7.45-7.41 (m, 6H), 7.26 (t, *J* = 7.5 Hz, 2H), 6.94 (d, *J* = 7.5 Hz, 2H), 6.05 (d, *J* = 5Hz, 1H), 5.78 (s, 1H), 5.73-5.72 (m, 1H), 5.51 (d, *J* = 8.5 Hz, 1H), 4.98-4.90 (m, 2H), 4.78 (brs, 1H), 4.52 (dd, J_1 = 12 Hz, J_2 = 2 Hz, 1H), 4.38 (dd, J_1 = 12 Hz, J_2 = 4.5 Hz, 1H), 4.14 (t, *J* = 4Hz, 1H), 3.37-3.28 (m, 1H), 2.06-2.05 (m, 2H), 1.63-1.60 (m, 2H) ppm

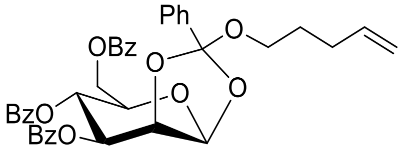
Mass Spectroscopic Analysis: HRMS (ESI) for $C_{39}H_{36}NaO_{10}$

Calcd (M+Na)$^+$: 687.2206 **Found:** 687.2233

Colourless solid came in 25 % Ethyl acetate-Hexane mixture.

Yield: 76 %

(3aS,5R,6R,7S,7aS)-5-((benzoyloxy)methyl)-2-(pent-4-en-1-yloxy)-2-phenyltetrahydro-3aH-[1,3]dioxolo[4,5-b]pyran-6,7-diyl dibenzoate (4b)



^{1}H NMR(500MHz,CDCl$_3$,TMS): δ 8.09 (d, *J* = 7.5 Hz, 2H), 8.06 (d, *J* = 7.5 Hz, 2H), 7.96 (d, *J* = 8 Hz, 2H), 7.86 (d, *J* = 7.5 Hz, 2H), 7.58- 7.54 (m, 2H), 7.50- 7.45 (m, 2H), 7.41-7.34 (m, 6H), 7.27-7.24 (m, 2H), 6.95 (d, *J* = 7.5 Hz, 2H), 6.18 (t, *J* = 10 Hz, 1H), 6.09-6.07 (m, 1H), 5.85-5.82 (m, 1H), 5.75 (s, 1H), 5.55 (s, 1H), 5.04 (d, *J* = 7.5 Hz, 1H), 4.96(d, *J* = 10.5 Hz, 1H), 4.75-4.70 (m, 2H), 4.49- 4.47 (m, 1H), 3.66 (t, *J* = 6 Hz, 2H), 2.16-2.11 (m, 2H), 1.68-1.65 (m, 2H) ppm

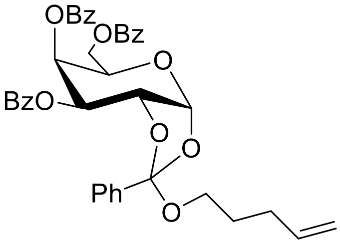
Mass Spectroscopic Analysis: HRMS (ESI) for $C_{39}H_{36}NaO_{10}$,

Calcd (M+Na)$^+$: 687.2206 **Found:** 687.2241

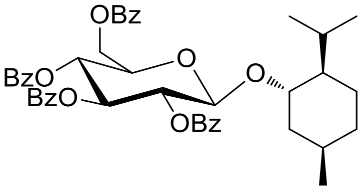
Colourless solid came in 25 % Ethyl acetate-Hexane mixture.

Yield: 90%

(2S,3aR,5R,6S,7S,7aR)-5-((benzoyloxy)methyl)-2-(pent-4-en-1-yloxy)-2-phenyl tetrahydro-5H-[1,3]dioxolo [4,5-b]pyran-6,7-diyl dibenzoate (4c)

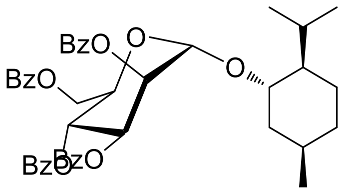
	**^{1}H NMR(500MHz,CDCl$_3$,TMS):** δ 8.09-8.06 (m, 2H), 8.03-7.98 (m, 3H), 7.81-7.79 (m, 2H), 7.56-7.51 (m,1H), 7.50-7.44 (m, 4H), 7.42-7.38 (m, 6H), 7.26-7.25 (m, 2H), 6.08-5.99 (m, 2H), 5.71-5.67 (m, 2H), 4.96-4.88 (m, 2H), 4.65-4.59 (m, 3H), 4.47-4.39 (m, 2H), 3.83-3.79 (m, 1H), 3.52-3.49 (m, 1H), 2.11-2.07 (m,2H), 1.73-1.70 (m, 2H) ppm **Mass Spectroscopic Analysis:** HRMS (ESI) for $C_{39}H_{36}NaO_{10}$ **Calcd (M+Na)$^+$:** 687.2206 **Found:** 687.2208 Colourless solid came in 25 % Ethyl acetate-Hexane mixture. **Yield:** 83%

(2R,3R,4S,5R,6R)-2-((benzoyloxy)methyl)-6-(((1S,2R,5R)-2-isopropyl-5-methyl cyclohexyl)oxy)tetrahydro-2H-pyran-3,4,5-triyl tribenzoate (8a)

	**^{1}H NMR(500MHz,CDCl$_3$,TMS):** δ 8.09 (d, *J* = 8 Hz, 2H), 7.97 (d, *J* = 8Hz ,2H), 7.93 (d, *J* = 8 Hz, 2H), 7.87 (d, *J* = 7.5 Hz, 2H), 7.6-7.52 (m, 3H), 7.45-7.38 (m, 8H), 7.34 (d, *J* = 11Hz, 1H), 5.94 (t, *J* = 9.5 Hz, 1H), 5.62 (t, *J* = 10 Hz ,1H), 5.57 (t, *J* = 9.5 Hz,1H), 4.88(d, *J* = 8 Hz ,1H), 4.63 (d, *J* = 12 Hz, 1H), 4.54-4.50 (m, 1H), 4.22-4.19 (m , 1H), 3.76 (s, 1H), 1.75-1.68 (m, 2H),1.44-1.30 (m, 2H),1.19-1.14 (m,3H), 0.96-0.86 (m, 2H), 0.80 (d, *J* = 6.5 Hz , 3H), 0.65 (d, *J* = 7 Hz, 3H), 0.59 (d, *J* = 6.5 Hz, 3H) **^{13}C NMR(126MHz, CDCl$_3$,TMS):** δ 166.3, 165.9, 165.8, 165.3, 133.5, 133.4, 133.2, 130.1, 130.0, 129.9, 129.9, 129.8, 129.8, 129.7, 129.7, 129.6, 128.5, 128.4, 128.4, 128.4, 128.3, 90.5, 74.2, 72.2, 70.1, 69.5, 67.8, 62.9, 20.6, 19.0,17.3, 14.7, 12.6 ppm **Mass Spectroscopic Analysis:** HRMS (ESI) for $C_{44}H_{46}NaO_{10}$

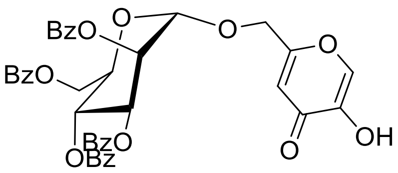
	Calcd (M+Na)$^+$: 757.2989 **Found:** 757.2985 Colourless viscous liquid came in 10 % Ethyl acetate-Hexane mixture. **Yield:** 61 %

(2R,3R,4S,5S,6S)-2-((benzoyloxy)methyl)-6-(((1S,2R,5R)-2-isopropyl-5-methyl cyclohexyl)oxy)tetrahydro-2H-pyran-3,4,5-triyl tribenzoate (8e)

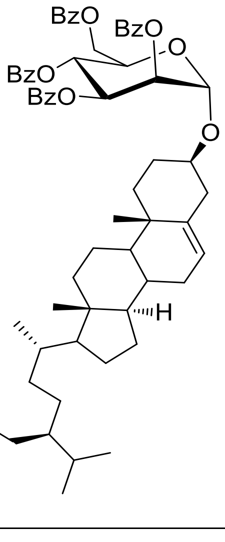
	**^{1}H NMR (500MHz, CDCl$_3$, TMS):** δ 8.08-8.07 (m, 1H), 8.03 (d, *J* = 7.5 Hz, 2H), 7.91 (d, *J* = 7.5 Hz, 2H), 7.87 (t, *J* = 7.5 Hz, 3H), 7.70 (d, *J* = 6 Hz, 2H), 7.58-7.47 (m, 4H), 7.39 (t, *J* = 7.5 Hz, 3H), 7.29 (d, *J* = 6 Hz, 3H), 5.89 (t, *J* = 10 Hz, 1H), 5.77 (s, 1H), 5.64 (dd, J_1 = 10 Hz, J_2 = 3.5 Hz, 1H), 5.07 (s, 1H), 4.45 (d, *J* = 12 Hz, 1H), 4.32 (dd, J_1 = 12Hz, J_2 = 4.5 Hz, 1H), 4.07 (t, *J* = 4.5 Hz, 1H), 3.68 (s, 1H), 1.74 (s, 1H), 1.60 (t, *J* = 7.5 Hz, 2H), 1.46-1.33 (m, 3H), 1.15 (d, *J* = 8.5 Hz, 2H), 1.00-0.92 (m, 1H) 0.69 (d, *J* = 6.5 Hz, 3H), 0.62 (d, *J* = 6.5 Hz, 3H), 0.58 (d, *J* = 6.5 Hz, 3H) ppm **^{13}C NMR (125Hz, CDCl$_3$, TMS):** δ 165.7, 165.3, 165.2, 163.8, 134.1, 133.7, 133.4, 133.1, 130.2, 130.0, 129.8, 129.8, 128.8, 128.8, 128.5, 128.4, 128.4, 91.4, 71.2, 70.0, 69.4, 66.2, 62.3, 30.9, 24.7, 19.0, 18.4, 16.8, 14.6, 12.4 ppm **Mass Spectroscopic Analysis:** HRMS (ESI) for C$_{44}$H$_{46}$NaO$_{10}$, **Calcd (M+Na)$^+$:** 757.2989 **Found:** 757.2991 Colourless solid came in 10 % Ethyl acetate-Hexane mixture. **Yield:** 57 %

(2R,3R,4S,5S,6S)-2-((benzoyloxy)methyl)-6-((5-hydroxy-4-oxo-4H-pyran-2-yl)methoxy)tetrahydro-2H-pyran-3,4,5-triyl tribenzoate (8f)

	^{1}H NMR(500MHz, CDCl$_3$, TMS): δ 8.12-8.10 (m, 2H), 8.09-8.05 (m, 2H), 8.02-7.95(m, 2H), 7.84-7.83 (m, 2H),

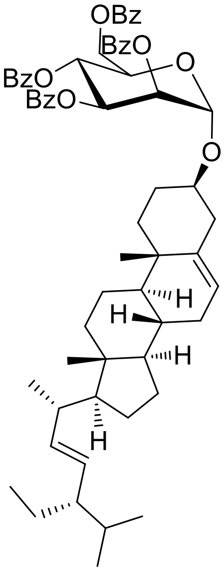
	7.61-7.51 (m, 2H), 7.50-7.45 (m, 1H), 7.41-7.37 (m, 6H), 7.29-7.26 (m, 3H), 6.28 (s, 1H), 6.23-6.19 (m, 1H), 6.10 (t, *J* = 10 Hz, 1H), 5.93- 5.84 (m, 2H), 5.70 (s, 1H), 5.09 (t, *J* = 9Hz, 1H), 4.76-4.68 (m, 1H), 4.49 (brs, 3H) ppm **^{13}C NMR(126MHz, CDCl$_3$, TMS):** δ 178.1, 166.4, 166.3, 166.2, 166.0, 133.9, 133.7, 133.5, 133.3, 133.2, 133.1, 129.9, 129.8, 128.5, 115.5, 100.1, 97.6, 70.6, 69.9, 69.0, 62.1, 55.5 ppm **Mass Spectroscopic Analysis:** HRMS (ESI) for $C_{40}H_{32}NaO_{13}$ **Calcd (M+Na)$^+$:** 743.1741 **Found:** 743.1738 Colourless solid came in 15 % Ethyl acetate-Hexane mixture. **Yield:** 26 %

(2R,3R,4S,5R,6S)-2-((benzoyloxy)methyl)-6-(((3R,10S,13S,14R)-17-((2S,5S)-5-ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl)oxy)tetrahydro-2H-pyran-3,4,5-triyl tribenzoate (8g)

	**^{1}H NMR(500MHz,CDCl$_3$, TMS):** δ 8.09-8.07 (m, 2H), 7.99-7.97 (m, 2H), 7.84-7.83 (m, 1H), 7.59-7.57 (m, 2H), 7.51-7.43 (m, 3H), 7.43-7.36 (m, 6H), 7.36-7.26 (m, 4H), 6.05-6.02 (m, 1H), 5.95-5.93(m, 1H), 5.65 (brs, 1H), 5.30-5.25 (m, 1H), 4.99-4.93 (m, 1H), 4.68-4.65 (m, 1H), 4.53-4.48 (m, 1H), 3.66-3.62 (m, 1H), 2.46-2.26 (m, 2H), 2.01-1.87 (m, 6H), 1.25-1.06(m, 32H), 0.89-0.67 (m, 8H) ppm **^{13}C NMR(126MHz, CDCl$_3$, TMS):** δ 163.1, 162.4, 162.3, 161.7, 137.5, 137.2, 133.2, 131.6, 130.7, 130.6, 130.1, 129.1, 127.1, 127.0, 126.7, 126.6, 126.2, 126.1, 125.6, 125.5, 125.3, 123.5, 121.7, 119.1, 118.5, 71.1, 69.0, 66.2, 64.4, 61.0, 53.8, 47.1, 42.8, 39.3, 37.0, 36.8, 34.1, 33.5, 31.0, 29.0, 28.8, 26.1, 25.3, 23.0, 21.3, 20.1, 18.0, 16.9, 16.3, 16.1, 15.8, 9.0, 8.9 ppm **Mass Spectroscopic Analysis:** HRMS (ESI) for $C_{62}H_{74}NaO_{10}$ **Calcd (M+Na)$^+$:** 1001.5180 **Found:** 1001.5184

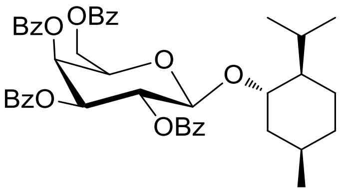
	Colourless solid came in 15 % Ethyl acetate-Hexane mixture. **Yield:** 55 %

(2R,3R,4S,5S,6S)-2-((benzoyloxy)methyl)-6-(((3R,8R,9R,10S,13S,14R,17S)-17-((2S,5S,E)-5-ethyl-6-methylhept-3-en-2-yl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13, 14,15, 16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl)oxy)tetrahydro-2H-pyran-3,4,5-triyl tribenzoate (8h)

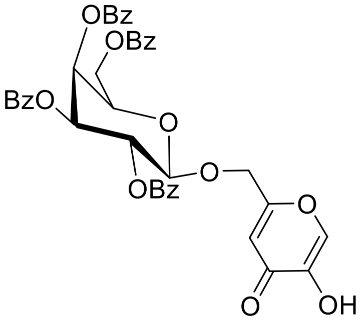
	**^{1}H NMR(500MHz,CDCl$_3$,TMS):** δ 8.09-8.08 (m, 2H), 8.07-8.06 (m, 1H), 7.99-7.98 (m, 2H), 7.98-7.97 (m, 2H), 7.60-7.59 (m, 2H), 7.58-7.56 (m, 3H), 7,52-7.50 (m, 4H), 7.43-7.36 (m, 4H), 7.36-7.27 (m, 3H), 6.07-5.93 (m, 1H), 5.65 (brs, 1H), 5.30-5.25 (m, 1H), 5.15-5.03 (m, 2H), 4.68-4.66 (m, 2H), 4.51-4.48 (m, 1H), 3.62 (brs, 1H), 2.46-1.87 (m, 16H), 1.66-1.25 (m, 15H), 1.13-0.84 (m, 10H), 0.81-0.70 (m, 5H) ppm **^{13}C NMR(126MHz, CDCl$_3$,TMS):** δ 165.1, 164.4, 164.3, 163.7, 139.5, 135.3, 133.6, 132.7, 132.6, 132.1, 131.2, 129.1, 129.0, 128.7, 128.7, 128.6, 128.2, 128.1, 127.6, 127.5, 127.3, 127.3, 125.5, 123.8, 121.1, 120.6 , 96.6, 73.1, 71.1, 68.2, 67.6, 66.4, 63.0, 55.8, 55.0, 49.1, 44.9, 41.3, 39.0, 38.8, 36.2, 35.5, 35.2, 33.0, 30.9, 30.8, 28.2, 27.3, 25.0, 23.3, 22.1, 20.0, 18.9, 18.3, 18.1, 17.8, 11.0, 10.9 ppm **Mass Spectroscopic Analysis:** HRMS (ESI) for $C_{63}H_{74}NaO_{10}$ **Calcd (M+Na)$^+$:** 1013.5180 **Found:** 1013.5183 Colourless solid came in 15 % Ethyl acetate-Hexane mixture. **Yield:** 57 %

(2R,3S,4S,5R,6R)-2-((benzoyloxy)methyl)-6-(((1S,2R,5R)-2-isopropyl-5-methylcyclo hexyl)oxy)tetrahydro-2H-pyran-3,4,5-triyl tribenzoate (8i)

	^{1}H NMR(500MHz,CDCl$_3$,TMS): δ 8.11 (d, *J*= 7.5 Hz,2H), 8.04 (d, *J*=8Hz ,2H), 7.95 (d, *J*=7.5 Hz, 2H), 7.79 (d, *J*=7.5Hz, 2H), 7.61(t J=7Hz,1H)7.56 (t, J=7.5Hz,1H) 7.52-7.47 (m,3H),7.44-7.41(m,3H),7.39-7.36(m,2H),7.26-7.22(m,2H), 5.98 (s, 1H), 5.82 (t, *J*=8.5Hz, 1H), 5.61 (

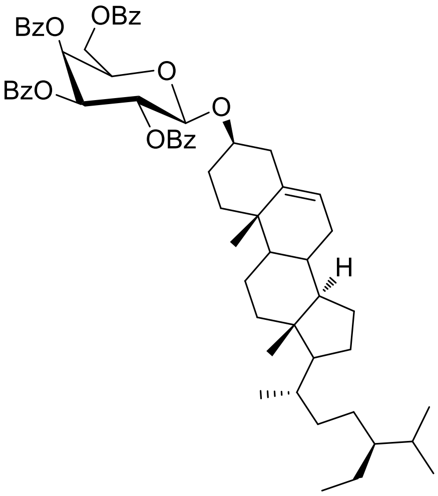
	dd , J_1=10.5Hz, J_2=7.5Hz, 1H), 4.83 (d, J=7.5 Hz, 1H), 4.64 (dd, J_1=11.5Hz, J_2=5.5Hz, 1H), 4.47-4.44(m,1H), 4.34 (t, J=6Hz ,1H), 3.76 (d, J=4.5Hz, 1H), 1.35-1.14 (m, 5H), 0.81 (d, J=7Hz, 3H), 0.63 (d, J=6.5Hz, 3H), 0.55 (d, J=6.5Hz, 3H) **^{13}C NMR(126MHz, CDCl$_3$, TMS):** δ 166.1, 165.8, 165.6, 165.2, 133.6, 133.3, 133.1, 130.1, 129.8, 129.8, 129.7, 128.6, 128.4, 128.3, 108.6, 101.9, 79.0, 78.8, 77.3, 77.0, 70.8, 71.9, 71.3, 68.3, 62.4, 57.8, 31.0, 30.0, 27.4, 25.3, 25.2, 2.8, 20.1, 18.0, 13.4 ppm **Mass Spectroscopic Analysis:** HRMS (ESI) for $C_{44}H_{46}NaO_{10}$ **Calcd (M+Na)$^+$:** 757.2989 **Found:** 757.2977 Colourless viscous liquid came in 10 % Ethyl acetate-Hexane mixture. **Yield**: 48 %

(2R,3S,4S,5R,6R)-2-((benzoyloxy)methyl)-6-((5-hydroxy-4-oxo-4H-pyran-2-yl)methoxy) tetrahydro-2H-pyran-3,4,5-triyl tribenzoate (8j)

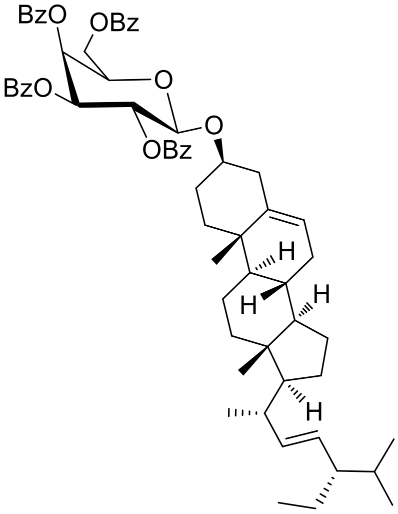
	**^{1}H NMR(500MHz,CDCl$_3$,TMS):** δ 8.02 (d, J = 7.5 Hz, 2H), 8.01 (d, J = 8 Hz, 2H), 7.97 (d, J = 8 Hz, 2H), 7.79 (d, J = 7.5 Hz, 2H), 7.62 (t, J = 7 Hz, 1H), 7.56 (t, J = 7.5 Hz, 1H), 7.51-7.36 (m, 9H), 7.25-7.23 (m, 3H), 6.0 (brs, 1H), 5.80 (t, J = 10 Hz, 1H), 5.67-5.60 (m, 2H), 4.84-4.67 (m, 3H), 4.71-4.67 (m, 1H), 4.44-4.40 (m, 1H), 4.33 (t, J = 6.5 Hz, 1H) ppm **^{13}C NMR(126MHz, CDCl$_3$,TMS):** δ 182.2, 166.1, 165.6, 156.6, 165.3, 130.1, 129.8, 129.7, 128.6, 128.5, 128.4, 128.3, 114.9, 101.7, 71.8, 71.3, 69.8, 69.7, 68.1, 62.0, 55.8 ppm **Mass Spectroscopic Analysis:** HRMS (ESI) for $C_{40}H_{32}NaO_{13}$ **Calcd (M+Na)$^+$:** 743.1741 **Found:** 743.1741

	Colourless solid came in 20 % Ethyl acetate-Hexane mixture. **Yield:** 58 %

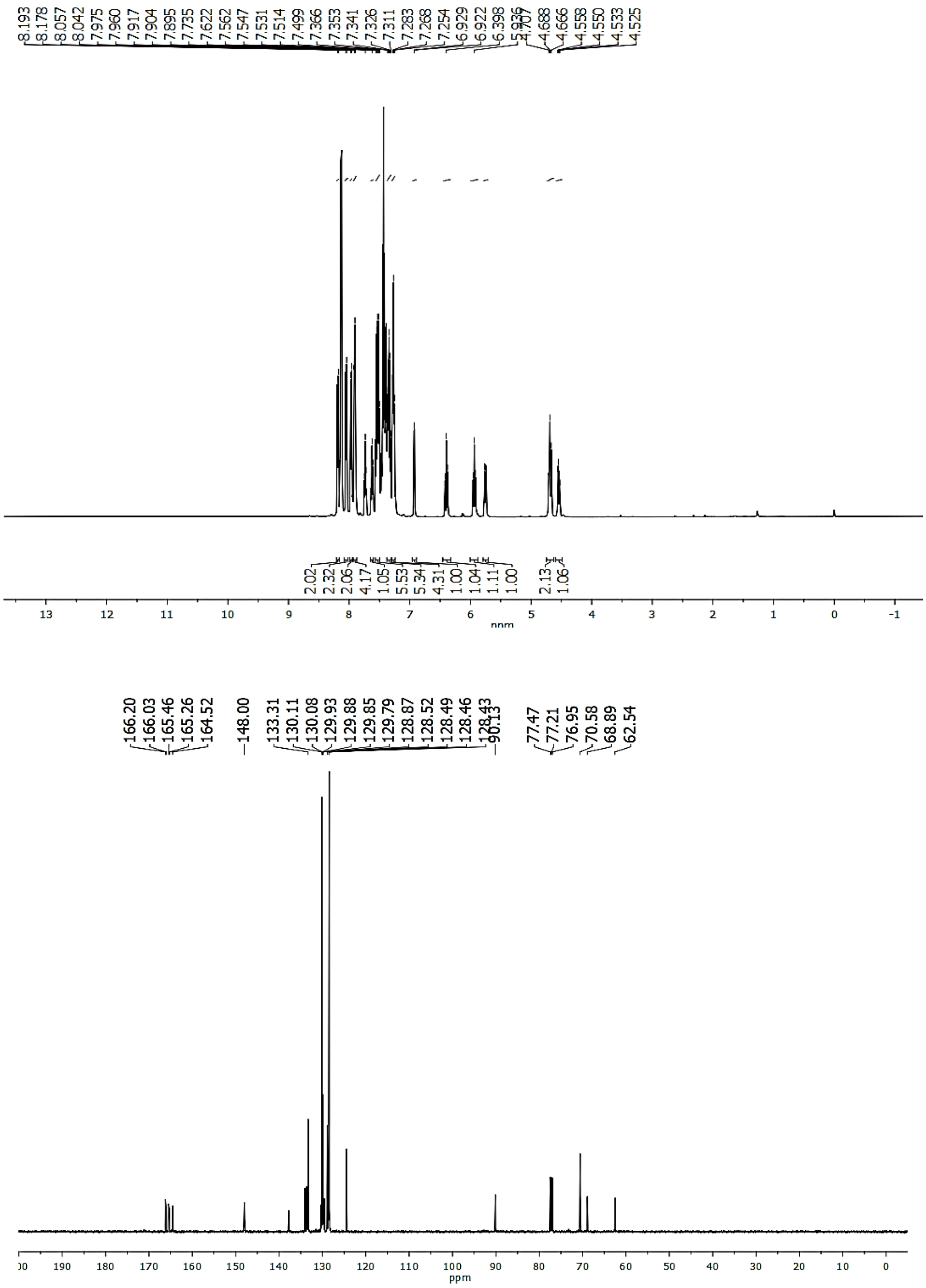
(2R,3S,4S,5R,6R)-2-((benzoyloxy)methyl)-6-(((3R,10S,13S,14R)-17-((2S,5S)-5-ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl)oxy)tetrahydro-2H-pyran-3,4,5-triyl tribenzoate (8k)

	**^{1}H NMR(500MHz,CDCl$_3$,TMS):** δ 8.11 (d, J = 7.5 Hz, 2H), 8.02 (d, J = 7.5 Hz, 2H), 7.96 (d, J = 7 Hz, 2H), 7.79 (d, J = 8.5 Hz, 2H), 7.56-7.37 (m, 5H), 7.27-7.23 (m, 5H), 7.18-7.14 (m, 2H), 5.97 (brs, 1H), 5.77 (t, J = 9Hz, 1H), 5.59-5.57 (m, 1H), 4.90 (d, J = 9 Hz, 1H), 4.69-4.65 (m, 1H), 4.43-4.40 (m, 1H), 4.32-4.31 (m, 1H), 3.56 (brs, 1H), 2.19-2.17 (m, 2H), 2.05-1.95 (m, 5H), 1.59-1.40 (m, 12H), 1.39-1.09 (m, 15 H), 1.05-0.62 (m, 15H) ppm **^{13}C NMR(126MHz, CDCl$_3$,TMS):** δ 166.1, 165.7, 165.6, 165.3, 140.3, 137.9, 133.6, 133.3, 133.2, 129.8, 129.7, 129.6, 129.1, 128.8, 128.6, 128.5, 128.4, 128.3, 128.2, 125.3, 122.0, 100.7, 80.9, 71.9, 71.3, 70.0, 68.2, 62.1, 56.7, 56.1, 50.1, 45.8, 42.3, 38.9, 37.1, 36.7, 36.1, 31.9, 31.8, 29.1, 26.0, 23.1, 21.5, 19.8, 19.3, 19.0, 18.8, 14.1, 12.0, 11.9 ppm **Mass Spectroscopic Analysis:** HRMS (ESI) for $C_{62}H_{74}NaO_{10}$ **Calcd (M+Na)$^+$:** 1001.5180 **Found:** 1001.5183 **Calcd (M+H)$^+$:** 979.5360 Colourless solid came in 15 % Ethyl acetate-Hexane mixture. **Yield:** 56 %

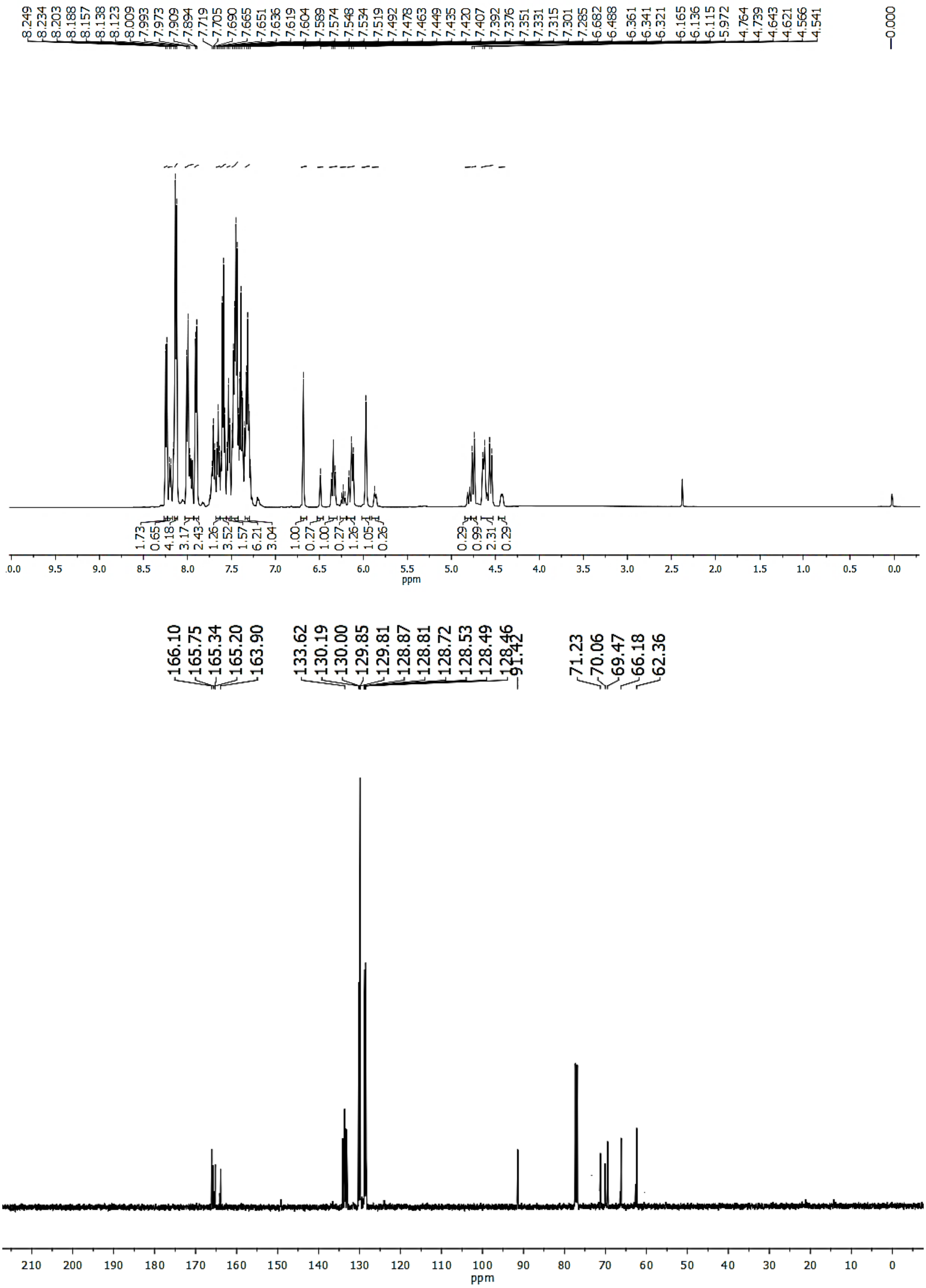
(2R,3S,4S,5R,6R)-2-((benzoyloxy)methyl)-6-(((3R,8R,9R,10S,13S,14R,17S)-17-((2S,5S,E)-5-ethyl-6-methylhept-3-en-2-yl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl)oxy)tetrahydro-2H-pyran-3,4,5-triyl tribenzoate (8l)

	**^{1}H NMR (500MHz, CDCl$_3$, TMS):** δ 8.11 (brs, 2H), 8.03 (brs, 2H), 7.97 (brs, 2H), 7.80 (brs, 2H), 7.62-7.60 (m, 1H), 7.57-7.39 (m, 9H), 7.26-7.24 (m, 2H), 5.98 (s, 1H), 5.80-5.76 (m, 1H), 5.60-5.59 (m, 1H), 5.17-5.14 (m, 1H), 5.03-5.02 (m, 1H), 4.93-4.92 (m, 1H), 4.68-4.67 (m, 1H), 4.44-4.41 (m, 1H), 4.33-4.32 (m, 1H), 3.57-3.56 (m, 1H), 2.19 (brs, 1H), 2.04-1.91 (m, 4H), 1.77-1.74 (m, 4H), 1.54-1.42 (m, 8H), 1.26-1.16 (m, 5H), 1.03-0.98 (m, 6H), 0.92 (s,3H), 0.85-0.80 (m, 12H), 0.68 (s, 3H) ppm **^{13}C NMR(126MHz, CDCl$_3$,TMS):** δ 166.0, 165.7, 165.6, 165.3, 140.3, 138.3, 133.6, 133.3, 133.2, 130.1, 129.8, 129.7, 129.5, 129.3, 129.1, 128.8, 128.6, 128.5, 128.4, 128.3, 128.3, 122.0, 100.7, 81.0, 71.9, 71.2, 70.0, 68.2, 62.1, 56.8, 56.0, 51.2, 50.1, 42.2, 40.5, 39.7, 38.9, 37.1, 36.7, 31.9, 31.8, 29.6, 25.4, 21.2, 21.1, 21.0, 19.3, 19.0, 12.3, 12.0 ppm **Mass Spectroscopic Analysis:** HRMS (ESI) for $C_{63}H_{74}NaO_{10}$ **Calcd (M+Na)$^+$:** 1013.5180 **Found:** 1013.5176 Colourless solid came in 15 % Ethyl acetate-Hexane mixture. **Yield:** 58 %

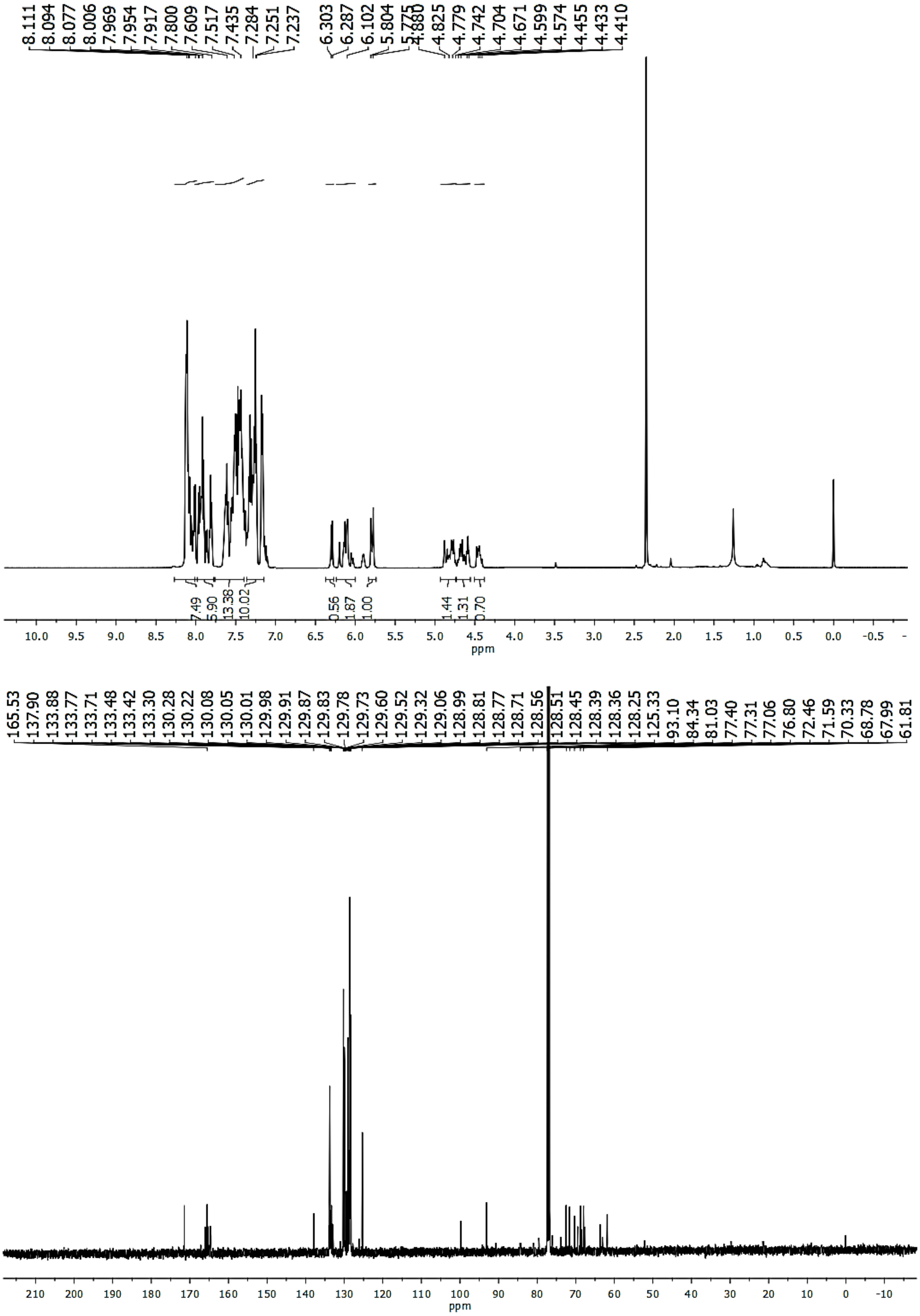
4.2. NMR Spectra



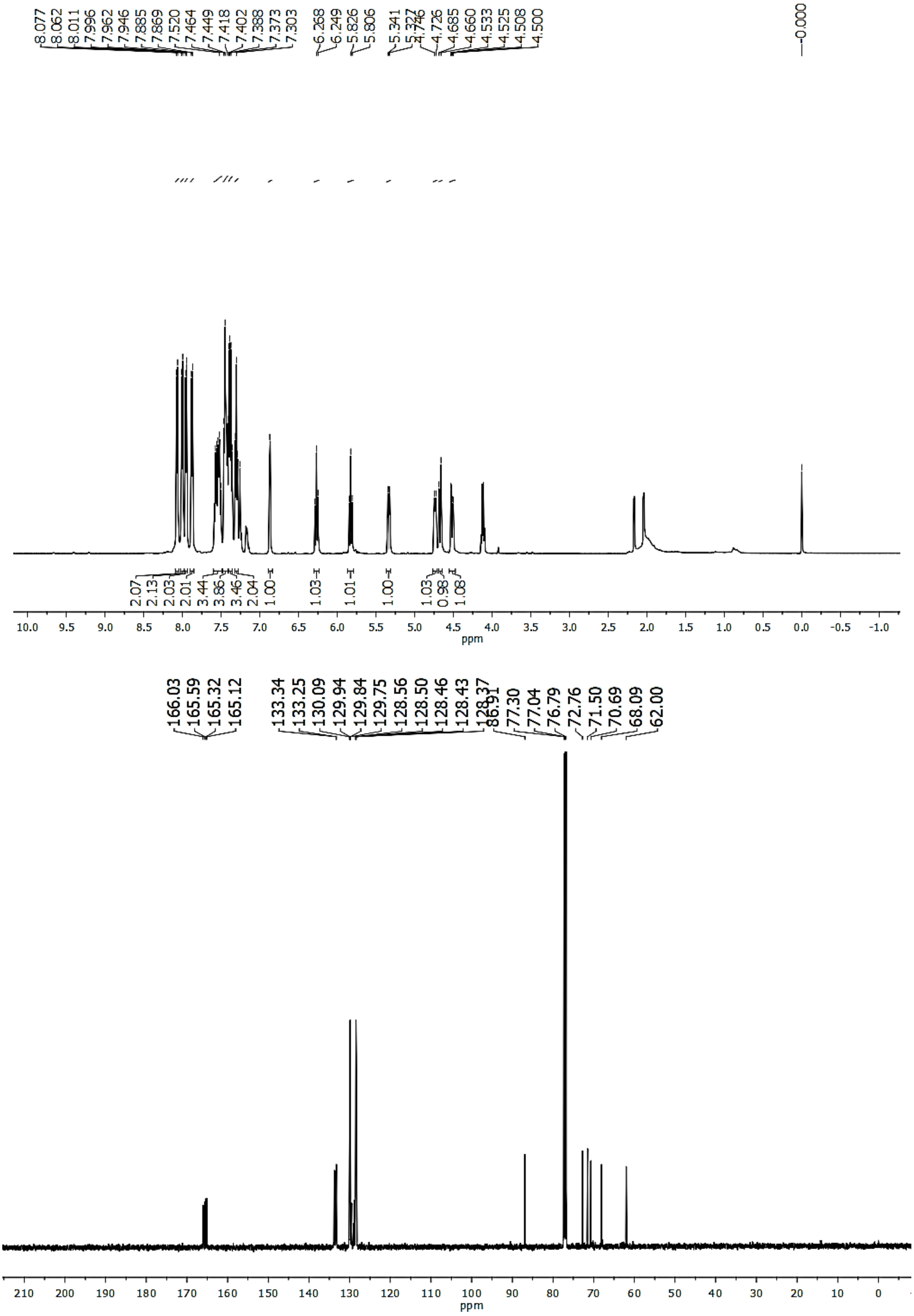
^{1}H and ^{13}C NMR of compound **2a**



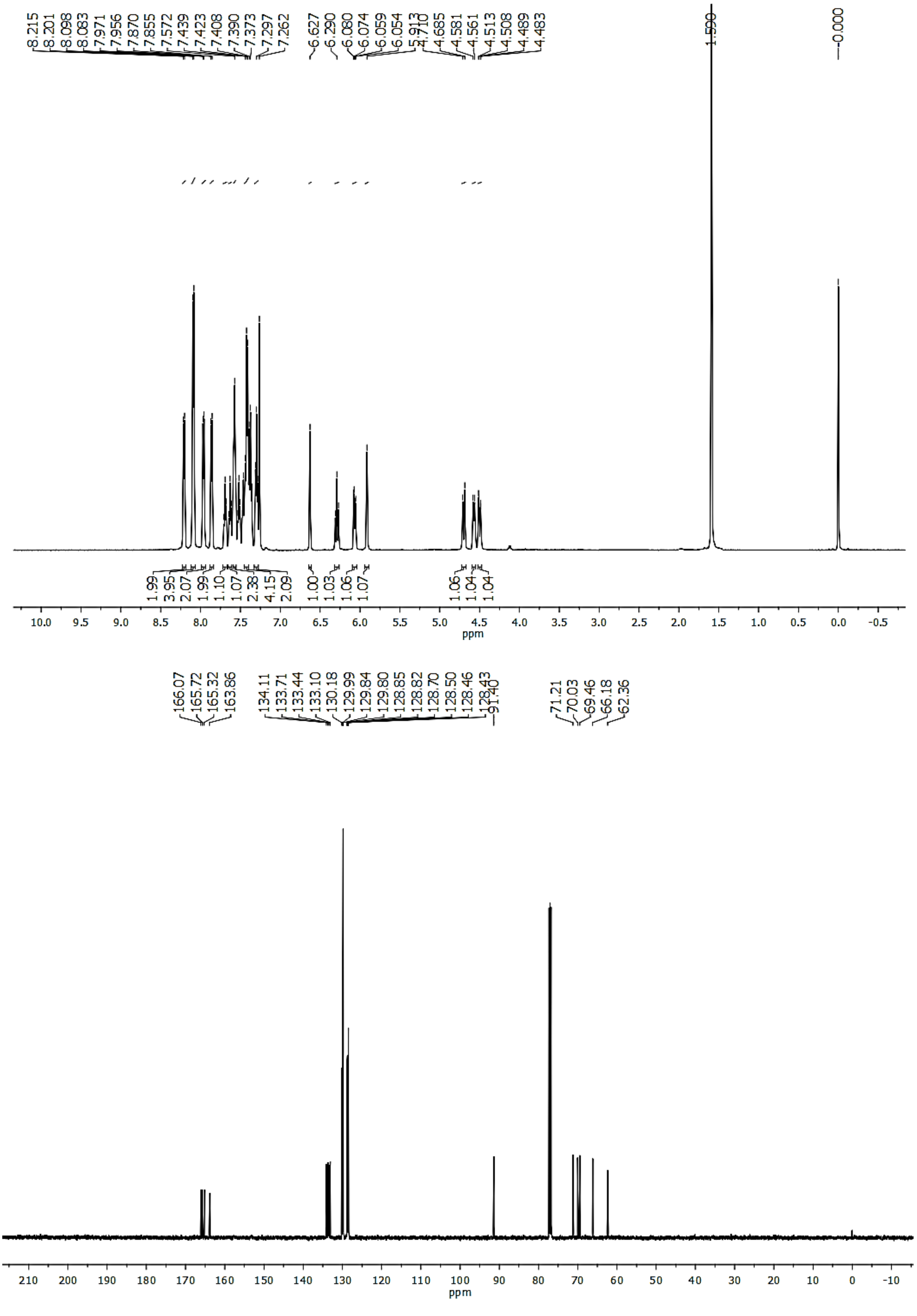
^{1}H and ^{13}C NMR of compound **2b**



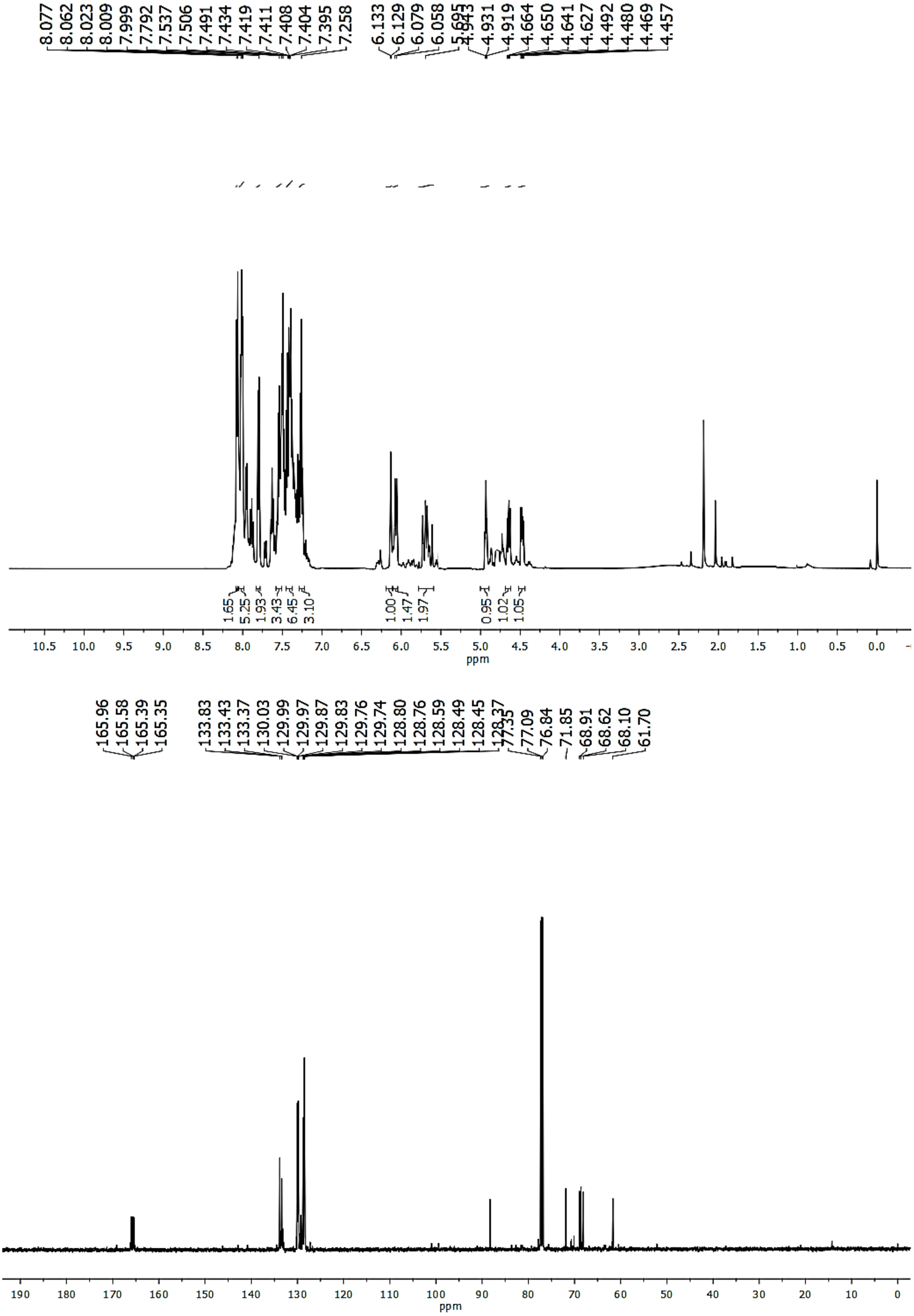
^{1}H and ^{13}C NMR of compound **2c**



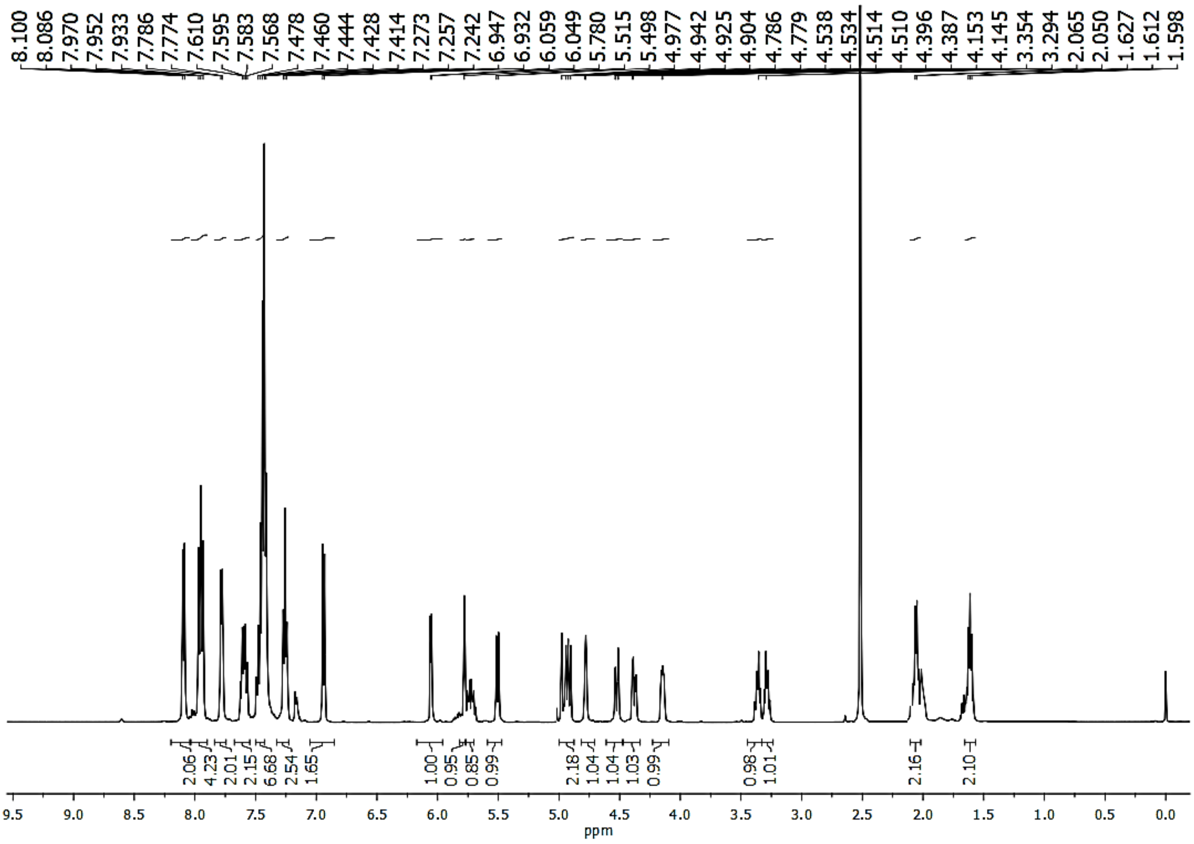
^{1}H and ^{13}C NMR of compound **3a**



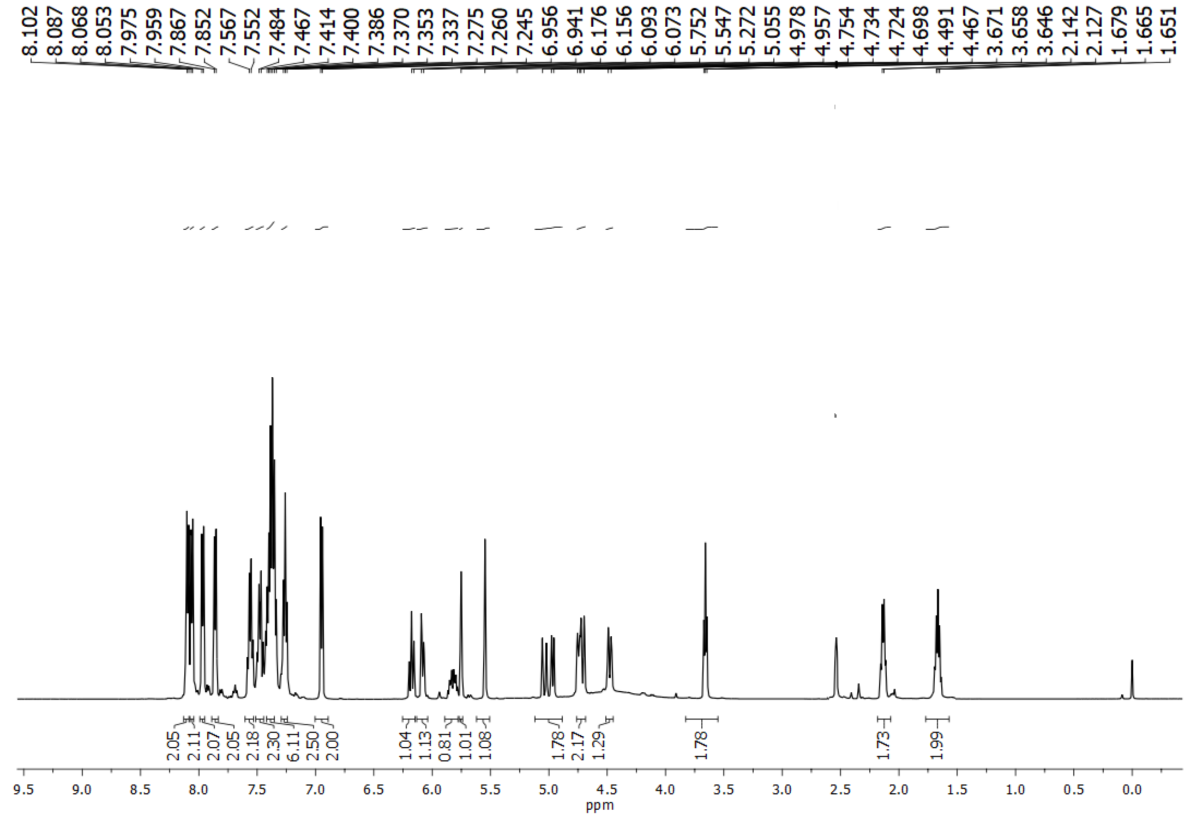
^{1}H and ^{13}C NMR of compound **3b**



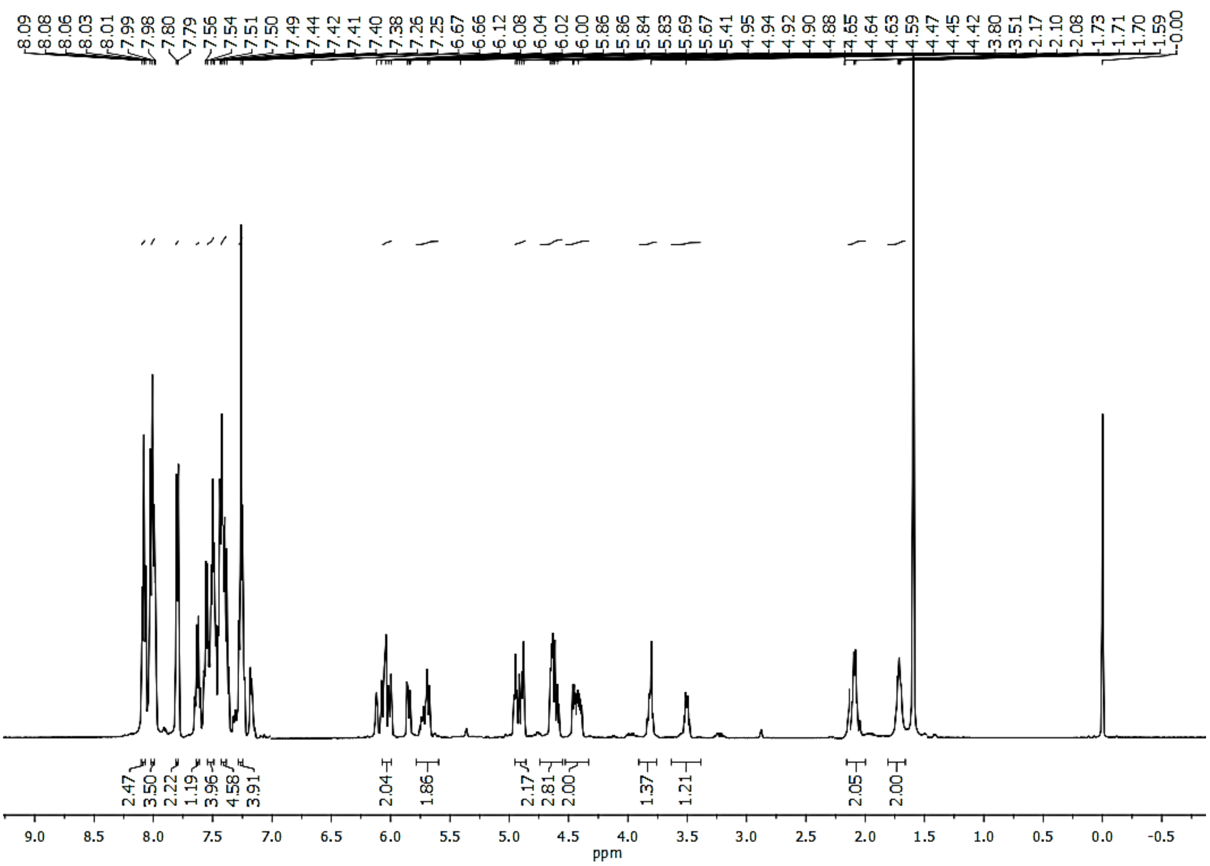
^{1}H and ^{13}C NMR of compound **3c**



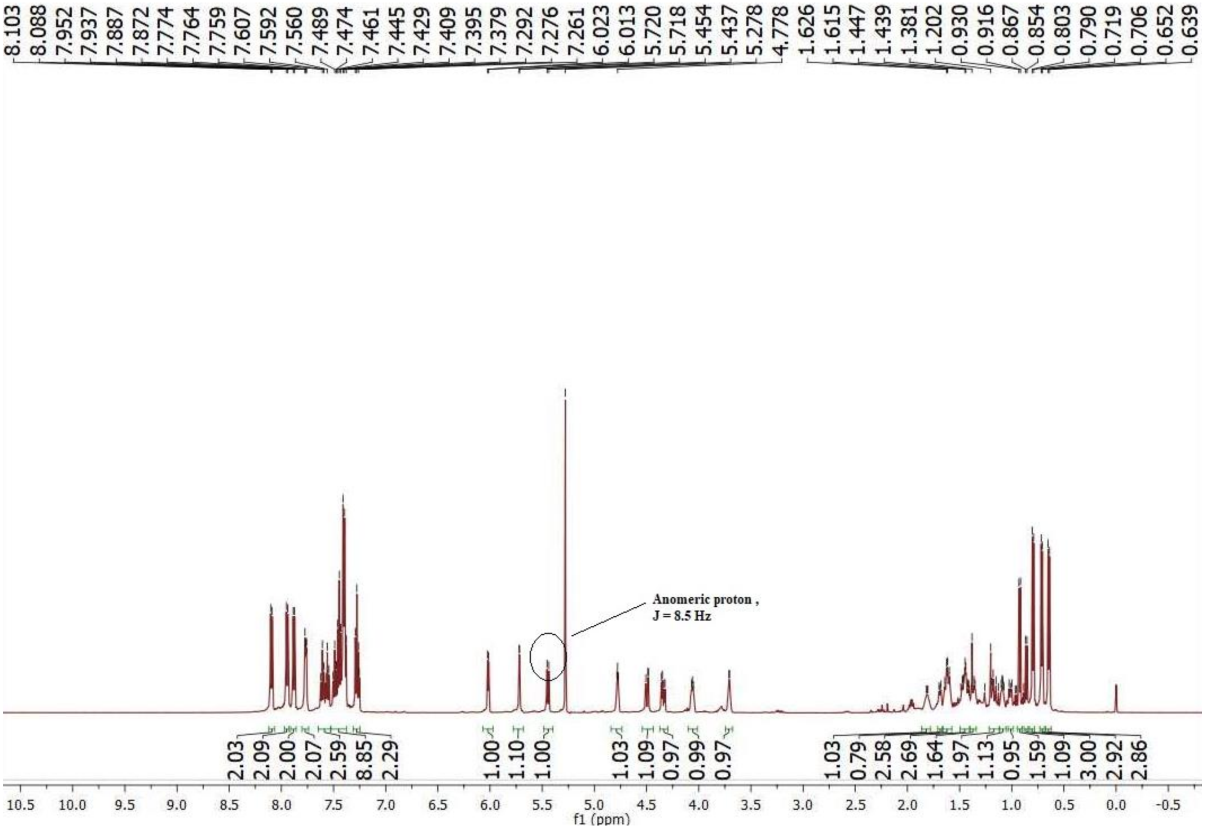
^{1}H NMR of compound **4a**

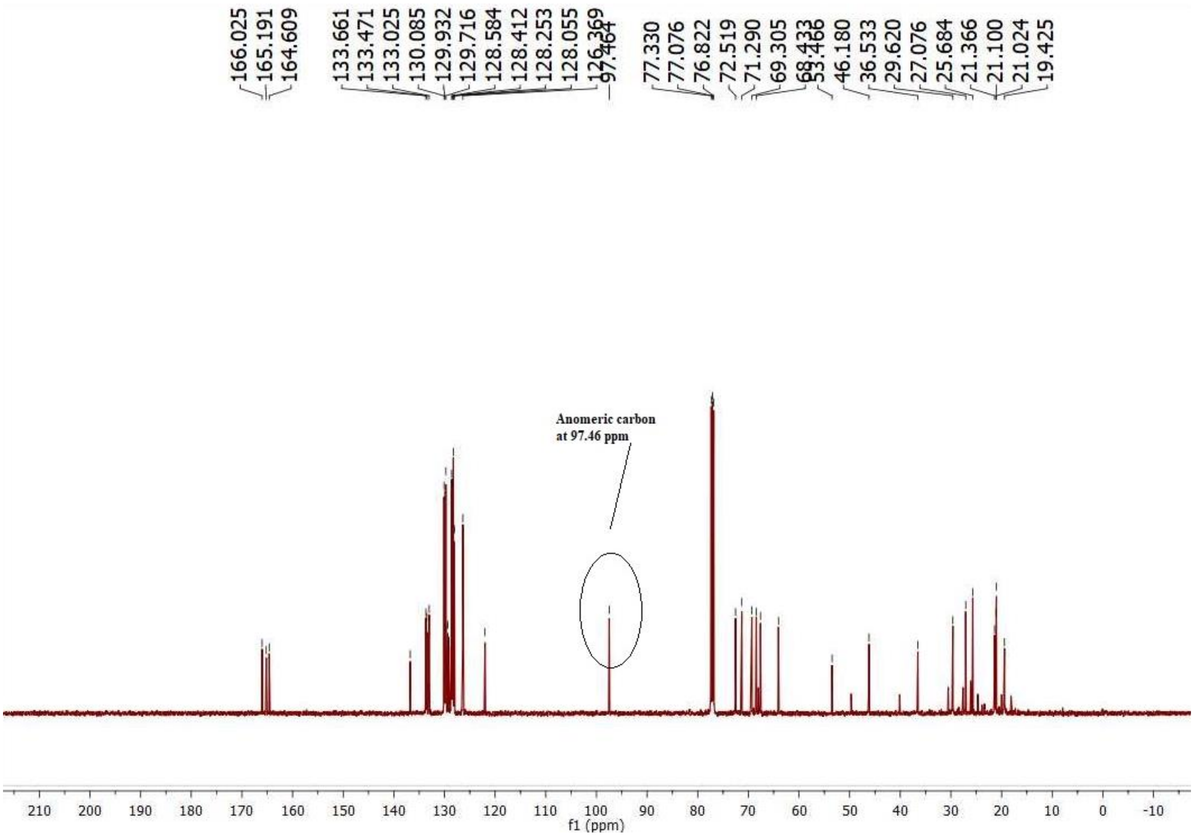


^{1}H NMR of compound **4b**

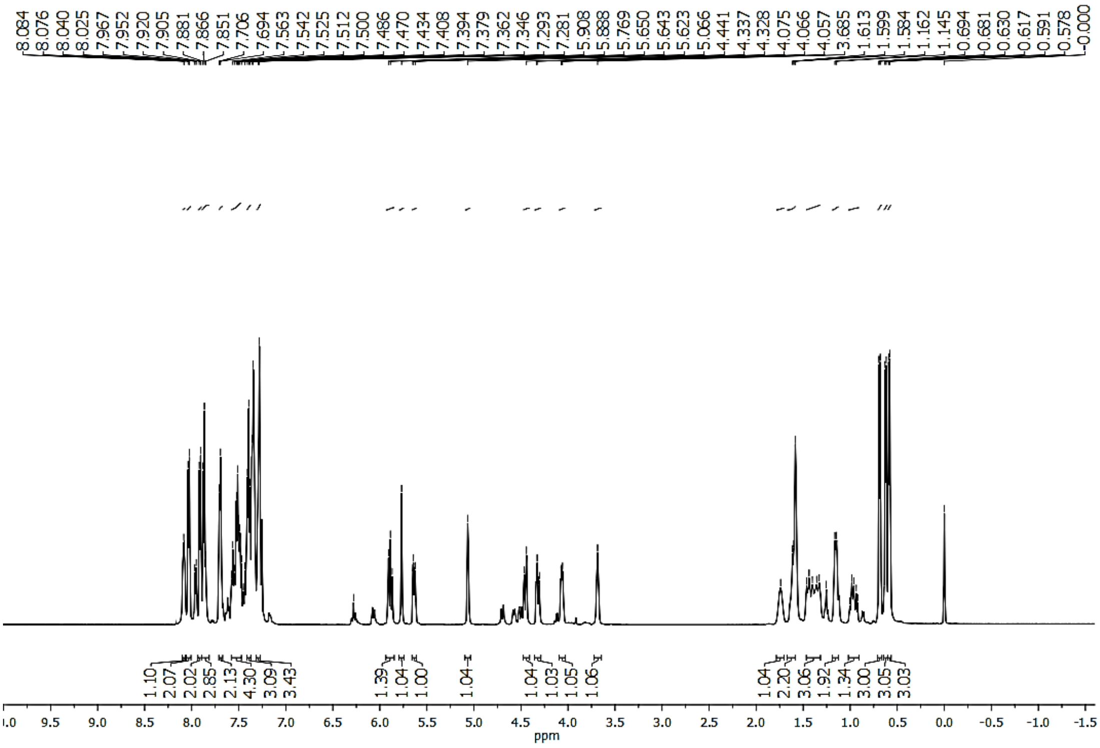


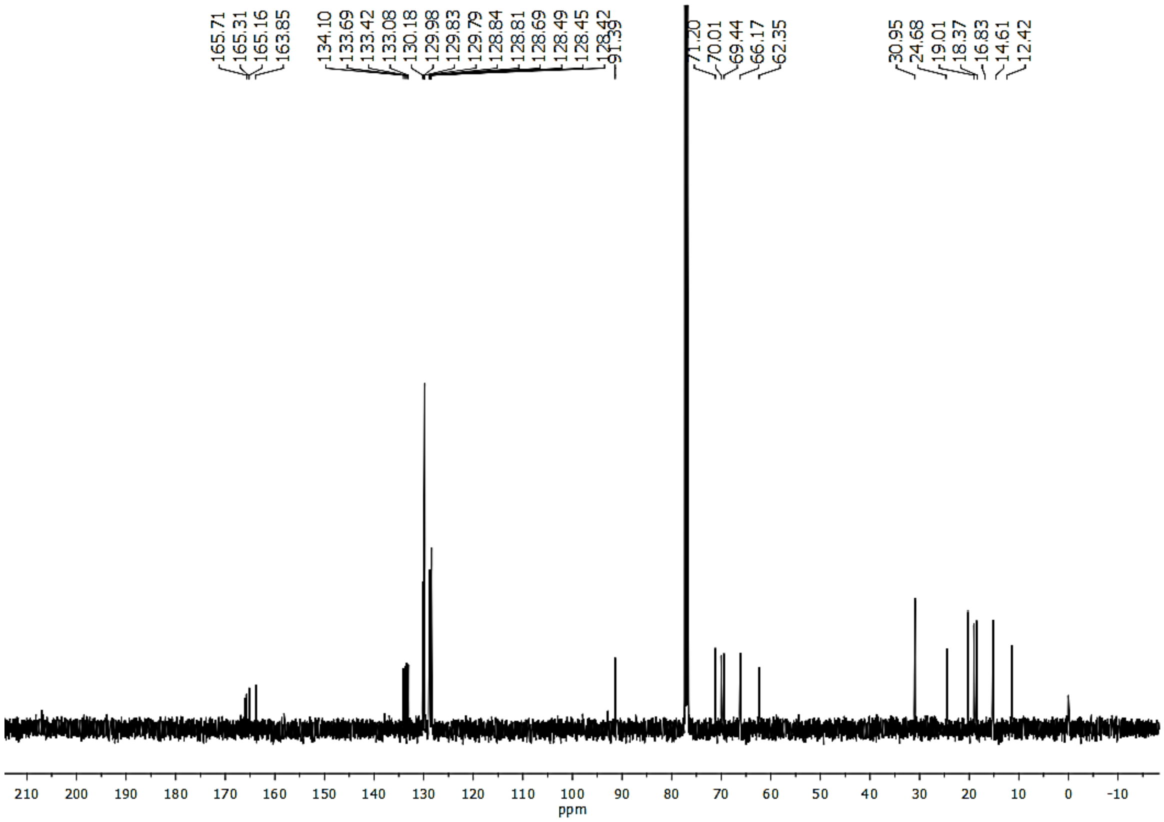
^{1}H NMR of compound **4c**



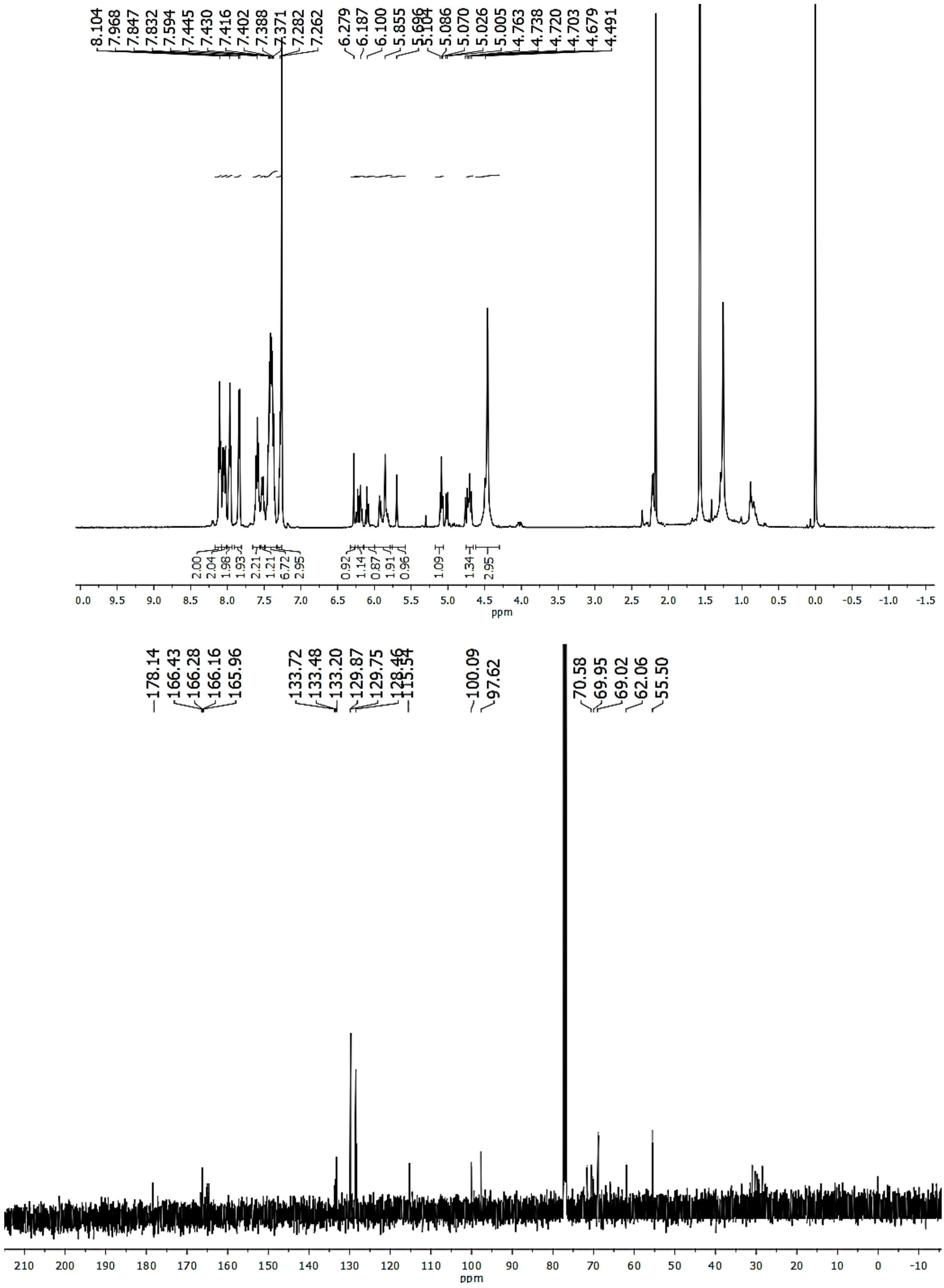


^{1}H and ^{13}C NMR of compound **8a**

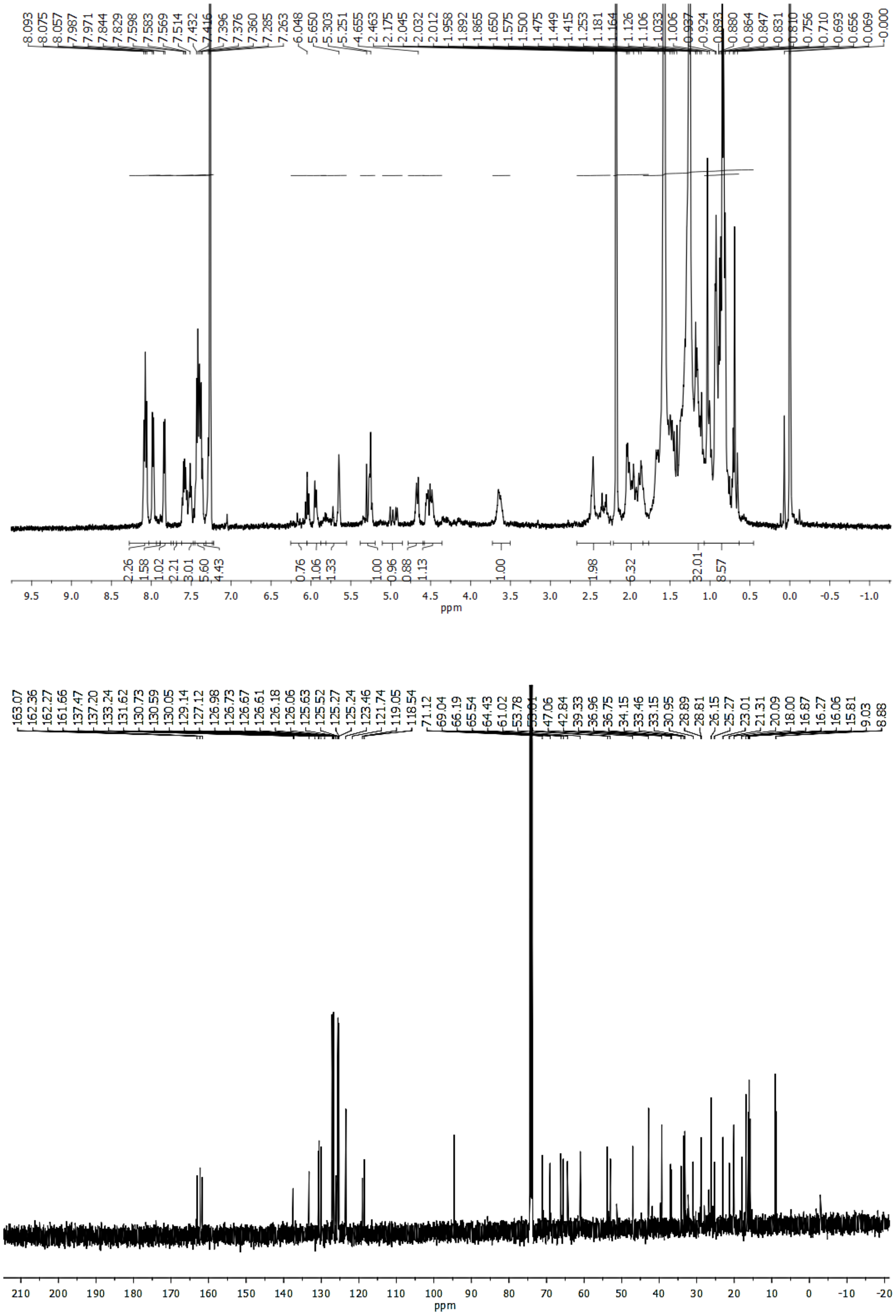




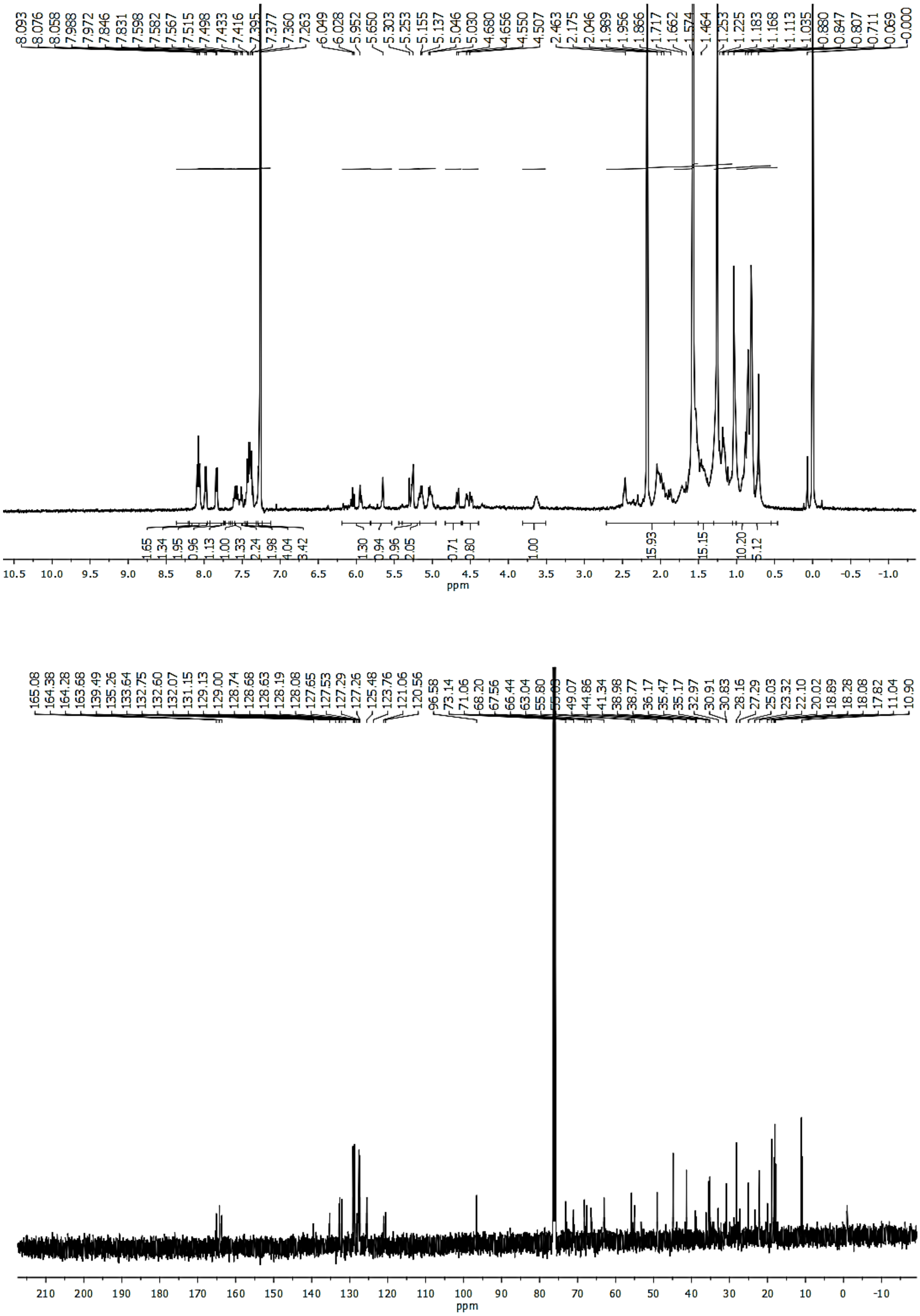
^{1}H and ^{13}C NMR of compound **8e**



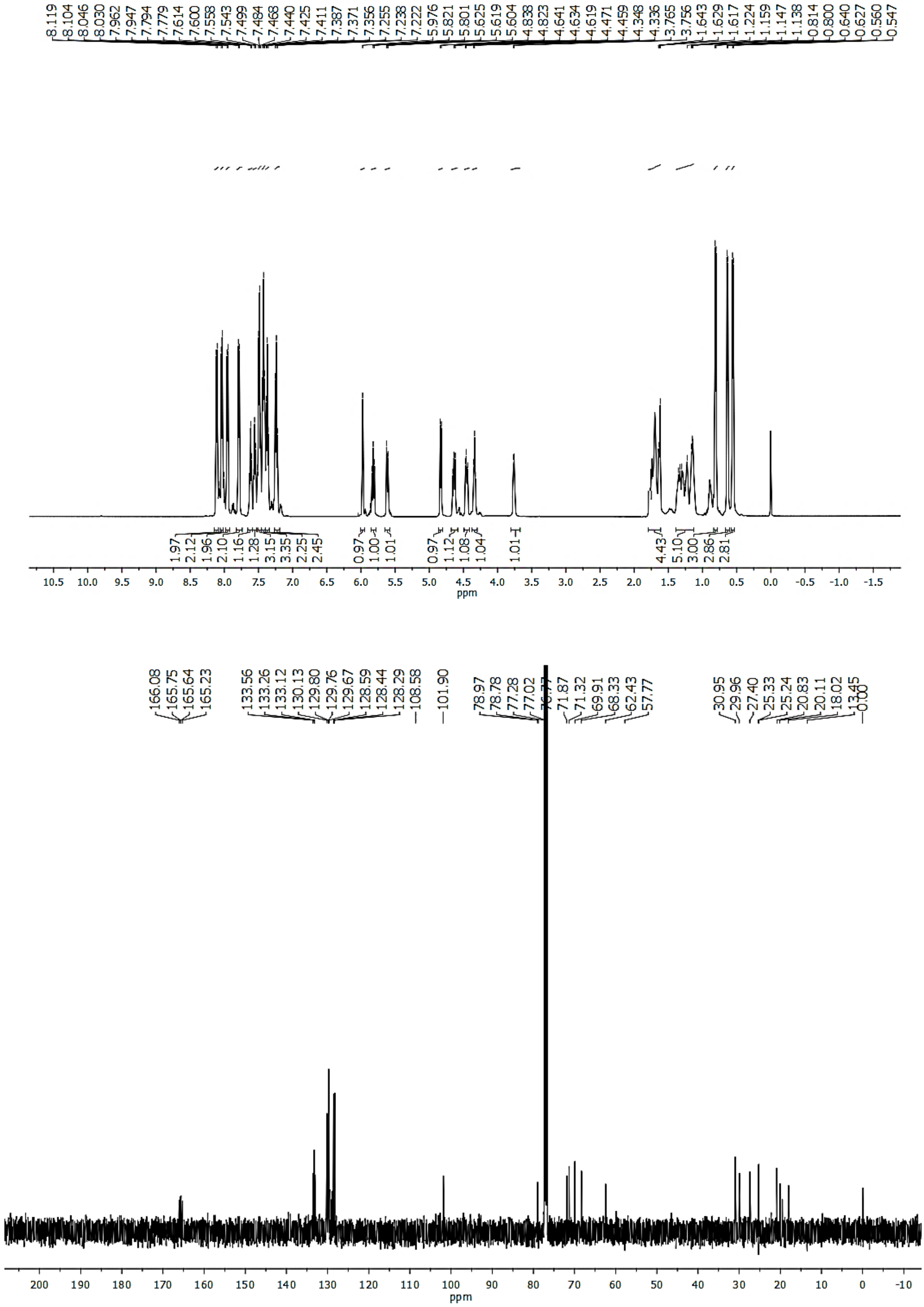
^{1}H and ^{13}C NMR of compound **8f**



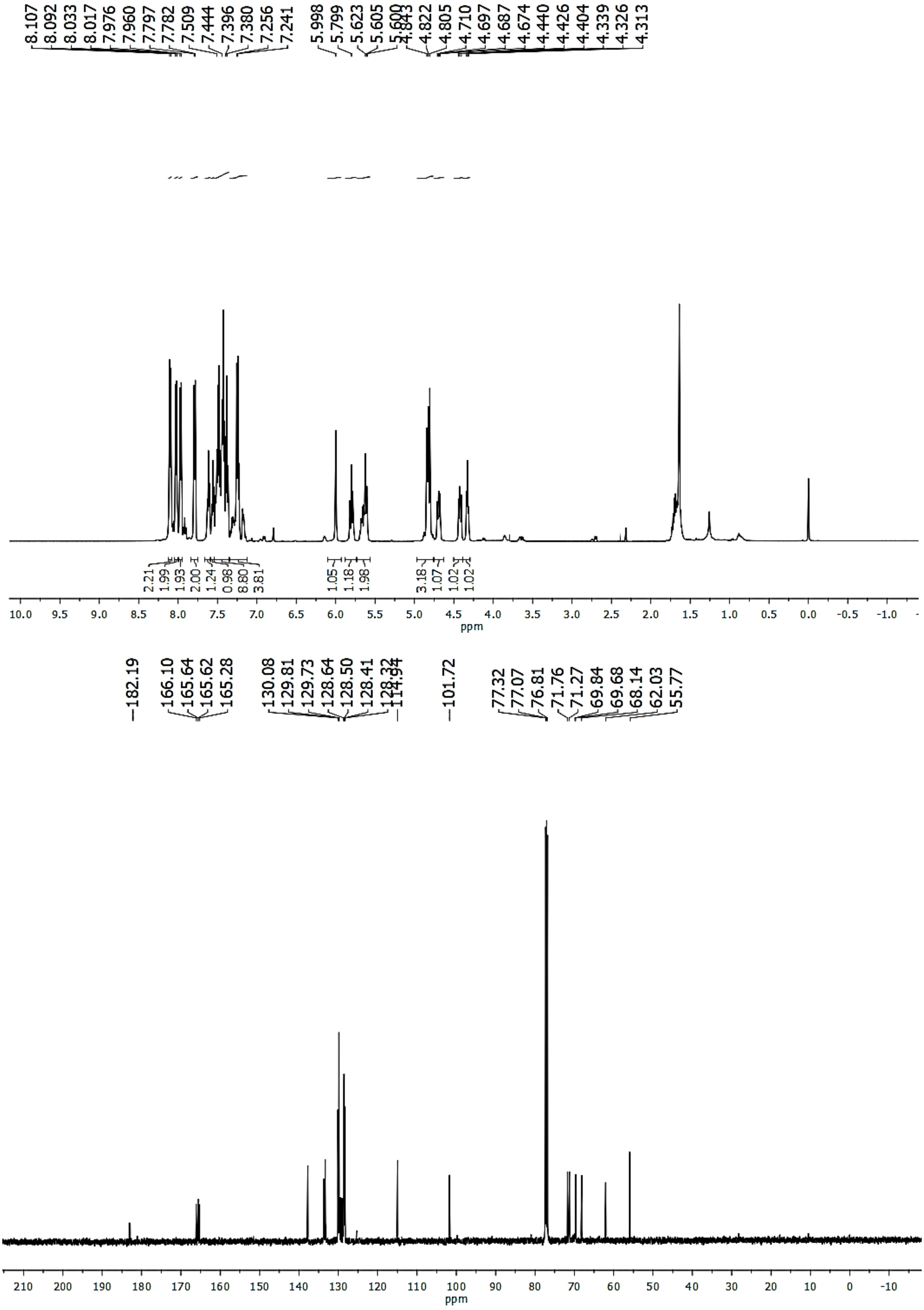
^{1}H and ^{13}C NMR of compound **8g**



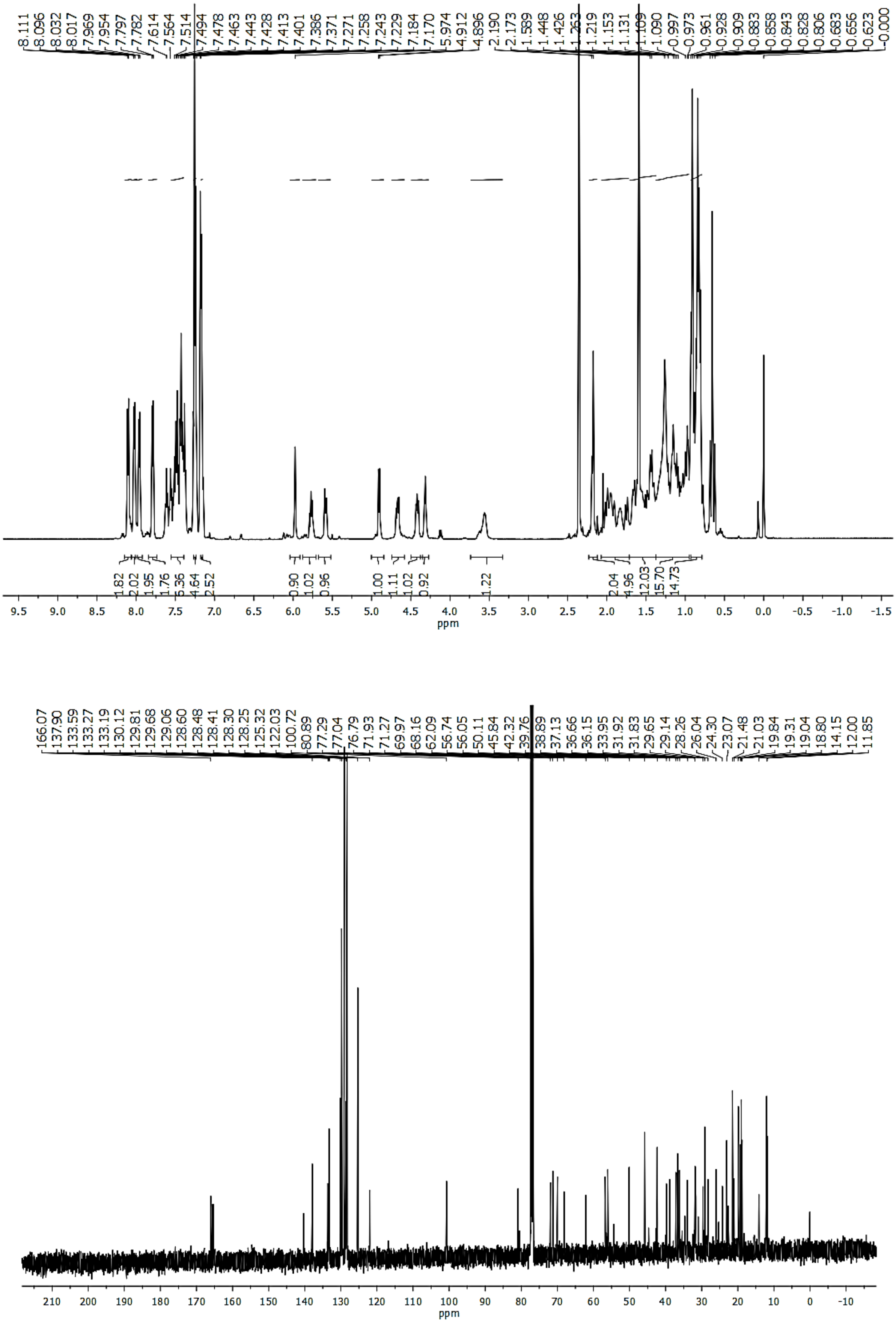
^{1}H and ^{13}C NMR of compound **8h**



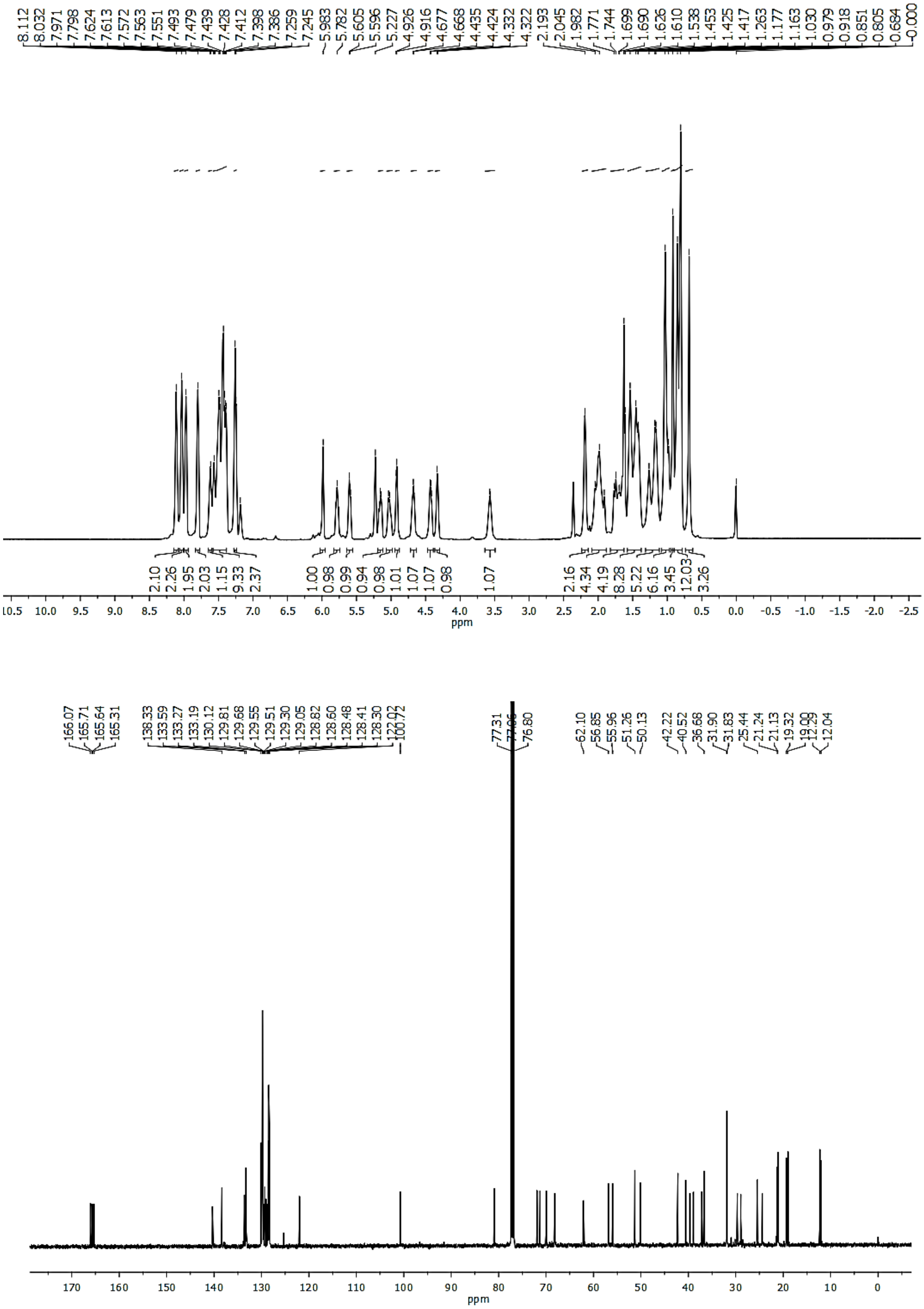
^{1}H and ^{13}C NMR of compound **8i**



^{1}H and ^{13}C NMR of compound **8j**



^{1}H and ^{13}C NMR of compound **8k**



^{1}H and ^{13}C NMR of compound **8l**

Conclusion

We could effectively carry out selective glycosylation of phenolic/sp^2 and alcoholic hydroxyl groups using different glycosyl donors. Brominated donors effectively coupled with phenolic system, while orthoester donors effectively coupled with alcoholic systems in the presence of a Lewis acid like $Sc(OTf)_3$,$Yb(Of)_3$. During this reactions the majority of the product obtained are the mixture of isomers .So we tried the same reactions in presence of sterically hindered organic Bronsted acid and Chiral bronsted acid as catalyst instead of the Lewis acid.The ultimate aim of this strategy is to enhance the stereospecificity of the reaction and also improves its yield enantioselectively.

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