NOVA SCHOOL OF SCIENCE & TECHNOLOGY

CHEMISTRY DEPARTMENT

Catarina Isabel Baptista Cipriano

Bachelor in Chemistry

Synthesis of new phosphoglycoglycerol – evaluation as model to new putative therapeutic agents

Dissertation for obtaining the Master's degree in Bioorganic Chemistry

MASTER'S DEGREE BIOORGANIC CHEMISTRY

Universidade NOVA de Lisboa

November, 2021

NOVA SCHOOL OF SCIENCE & TECHNOLOGY

CHEMISTRY DEPARTMENT

Synthesis of new phosphoglycoglycerol – evaluation as model to new putative therapeutic agents

Dissertation for obtaining the Master's degree in Bioorganic Chemistry

Catarina Isabel Baptista Cipriano
Bachelor in Chemistry

Advisor: Paula Cristina de Sério Branco, Assistant Professor with Habilitation, FCT/UNL

Joint supervisor: Patrícia Isabel da Silveira Máximo, Researcher, LAQV, FCT/UNL

Júri:

President: António Jorge DiasParola. Full Professor, NOVA University Lisbon

Arguente: Amélia Pilar Grases dos Santos Silva Rauter, Full Professor, University of Lisbon

MASTER'S DEGREE BIOORGANIC CHEMISTRY

Universidade NOVA de Lisboa
November, 2021

Synthesis of new phosphoglycoglycerol – evaluation as model to new putative therapeutic agents

Acknowledgements

I would like to start by expressing my sincere thanks to all the people involved in making this work possible.

First of all, I would like to thank Professor Paula Branco for giving me the opportunity to participate in this project and for the guidance provided in my development as a chemist. A word of appreciation to teachers Luísa Ferreira and Ana Lourenço for their help whenever necessary.

A special thanks to Dr. Patrícia Máximo, who was always available to help me 7 days a week, at any time. It was a great pleasure to learn and absorb knowledge from a person who shows such passion for what she does every day.

I would like to thank all the members of laboratories 202 and 205 that were present throughout the last year. In special to Flávia Leitão and Beatriz Chicharo for staying till late with me in the lab and always be there when the work was not going so well and of course for my mascot form the lab my beautiful squeaky duck. I would also like to thank to, Rafael Rippel, for the orientation and teachings, to João Macara for always make jokes and try to make me laugh every day, to Sofia Santos for dealing with my dumb questions and to Inês Fino and Diana Gago, for the rides back to my hometown or my home in Serrado, for the early companion in the lab and deep conversation about the future. But a big tank to all of you to make me feel like I had a family away of mine, I will always miss our family lunches.

I would also like to tank to all my friends in my hometown that always supported me in this stressful stage of my life and always believed that I can do everything that I want.

Finally, a thank you to my family who gave me all possible conditions to succeed. To my family members who unfortunately are no longer present to witness the completion of this stage of my life.

Resumo

Os fosfoglicoglicerois são estruturas não tão abundantes na natureza, mas com um papel relevante a nível biológico, uma vez que são um dos constituintes da parede e membrana celular. Como tal, estas unidades estruturais são o objetivo de procura de novos alvos para intervenções terapêuticas.

Com este trabalho, visou-se a síntese de um novo fosfoglicoglicerol isolado de *Asparagopsis armata* com atividade antimicrobiana comprovada. A estrutura exata é ainda desconhecida; contudo, três unidades estruturais foram identificadas como, D-(+)-galactose, glicerol, e um grupo de fosfato de etanolamina estando a unidade de glicerol ligada em posição α no açúcar.

A síntese desta nova estrutura pode ser abordada em quatro etapas principais, a síntese da unidade de açúcar previamente protegida, a síntese do intermediário de glicerol; a reação de acoplamento entre o açúcar protegido e o intermediário de glicerol; e finalmente, uma reação de acoplamento entre o composto anterior e o grupo fosfato de etanolamina.

Para a preparação da fração de açúcar, foi utilizado o grupo de proteção benzilo, uma vez que um dos objetivos é evitar o efeito dos grupos participantes na configuração do produto de reação de acoplamento. A síntese da estrutura benzílica, consistiu numa substituição na posição anomérica de um acetobromo-α-D-galactose com um grupo tiol, seguida de uma hidrólise dos acetatos e proteção com brometo de benzilo. Foi também realizada uma halogenação da posição anomérica.

A síntese do intermediário de glicerol consistiu numa proteção seletiva dos grupos hidroxilo livres primários. Finalmente, a reação de acoplamento foi realizada entre o açúcar protegido e os dois intermediários de glicerol sintetizados. Para esta síntese foram desenvolvidas três vias diferentes; primeiro com o derivado de açúcar benzilado e com tiofenol na posição anomérica e o intermediário de glicerol protegido com um grupo benzilo e o grupo TBDPS em ambos os hidroxilos primários; uma segunda abordagem foi realizada entre o açúcar benzilado com flúor na posição anomérica com o mesmo intermediário de glicerol; e finalmente, a terceira abordagem foi realizada entre o açúcar benzilado e o intermediário de glicerol com benzilo e acetato como grupos protetores. Apenas na última abordagem foi possível obter o produto de acoplamento com ambos os grupos protetores no intermediário de glicerol e com a presença de dois anómeros. Nas outras reações nunca foi possível identificar o grupo TBDPS e o produto desililado identificado em quantidades vestigiais. Num trabalho futuro, tentar-se-á a desproteção do acetato e o acoplamento com o grupo fosfato de etanolamina.

Palavras-chave: fosfoglicoglicerol*; Asparagopsis Armata*; α-D-(+)-galactose; intermediário de glicerol; glicoglicerol.

Abstract

Phosphoglycerides are structures not so abundant in nature but with a relevant role at biological level since they are one of the constituents of the cell wall and membrane. As such, these structural units are the objective of the search for new targets for therapeutic interventions.

With this work, we aimed the synthesis of a new phosphogglycoglycerol isolated from *Asparagopsis armata* with proven antimicrobial activity. The exact structure is still unknown; however, three structural units were identified as, D-(+)-galactose, glycerol, and an ethanolamine phosphate group with the glycerol unit being α-linked in the sugar.

The synthesis of this new structure can be approached in four main steps, the synthesis of the previously protected sugar unit, the synthesis of the glycerol intermediate; the coupling reaction between the protected sugar and the glycerol intermediate; and finally, a coupling reaction between the previous compound and the ethanolamine phosphate group.

For the preparation of the sugar fraction, the benzyl protecting group was used, since one of the objectives is to avoid the effect of the participating groups on the configuration of the coupling reaction product. The synthesis of the benzyl structure, consisted of a substitution at the anomeric position of an acetobromo-α-D-galactose with a thiol group, followed by a hydrolysis of the acetates and protection with benzyl bromide. A halogenation of the anomeric position was also performed.

The synthesis of the glycerol intermediate consisted of a selective protection of the primary free hydroxy groups. Finally, the coupling reaction was performed between the protected sugar and the two synthesized glycerol intermediates. Three different routes were developed for this synthesis; first with the benzylated sugar derivative with thiophenol in the anomeric position and the protected glycerol intermediate with a benzyl group and the TBDPS group on both primary hydroxy; a second approach was performed between the benzylated sugar with fluorine in the anomeric position with the same glycerol intermediate; and finally, the third approach was performed between the benzylated sugar and the glycerol intermediate with benzyl and acetate as protecting groups. Only in the last approach was it possible to obtain the coupling product with both protecting groups on the glycerol intermediate and with the presence of two anomers. In the other reactions it was never possible to identify the TBDPS group and the desilylated product identified in trace amounts. In a future work, deprotection of the acetate and coupling with the ethanolamine phosphate group will be attempted.

Keywords: phosphoglycoglycerol; *Asparagopsis Armata*; α-D-(+)-galactose; glycerol intermediate; glycoglycerol.

INDEX

INDEX OF FIGURES

INDEX OF TABLES

INDEX OF SCHEMES

List of abbreviations

^{13}C NMR	Carbon Nuclear Magnetic Resonance
^{1}H NMR	Proton Nuclear Magnetic Resonance
NMR	Nuclear Magnetic Resonance
ATR	Attenuated Total Reflectance
ESI-MS	Electrospray Ionization – Mass Spectrometry
m/z	Mass-to-charge ratio
FTIR	Fourier Transform Infra-Red
IR	Infra-red
COSY	Correlation Spectroscopy
HMBC	Heteronuclear Multiple Bond Correlation
HSQC	Heteronuclear Single Quantum Coherence
s	Singlet
d	Duplet
t	Triplet
q	Quartet0
dd	Duplet Duplet
ddd,	Duplet Duplet Duplet
m	Multiplet
J	Multiplet coupling constant
ppm	Parts per million
r.t.	Room temperature
Eq.	Equivalents
Satd. aq.	Saturated aqueous
DCM	Dichloromethane
DMAP	4-dimethylaminopyrrolidine
TBDPSCl	*Tert*-butyl(cloro) diphenylsilane
TBDPS	*Tert*-butyl diphenylsilane
NBS	*N*-bromosuccinimide
NIS	*N*-iodosuccinimide
TMSOTf	Trimethylsilyl trifluoromethanesufonate
DMF	*N,N*-dimethylformamide
THF	Tetrahydrofuran
TBAB	Tetrabutylammonium bromide
DAST	Diethylaminosulfur trifluoride
PE	Phosphatidylethanolamine
CL	Cardiolipin
PG	Phosphatidylglycerol
S_N2	Bimolecular nucleophilic substitution
TLC	Thin Layer Chromatography

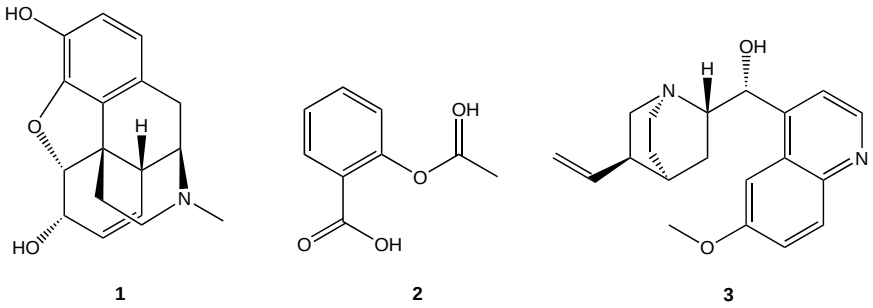
δ	Chemical shift
ν	Wavenumber (cm^{-1})
u	Atomic mass unit

1.
INTRODUCTION

1.1. Natural products used in medicine

Since ancient times, human beings have relied on plants and natural products derived from them for the treatment of diseases. With the development of science and the numerous examples of the innovative potential of natural extracts in medicine, it has been deduced that the effect of these extracts is in fact the effect of specific molecules with active biological properties. Therefore, it was necessary to begin the isolation of active principles from natural plants for their use in medicine.[1]

We have numerous examples of plant-derived extracts and compounds isolated from plants that have been widely used in the treatment of many significant diseases. The first natural product to be isolated and used for medicinal purposes was morphine **(1)** in the early 1800s, marketed by Merck in 1826, and in 1893 the first semisynthetic drug, aspirin **(2)**, was synthesized based in a natural product and marketed by Bayer in 1899[2]. The most revolutionary discovery in the medicine history was made in the 17th century with de discovery of quinine **(3)** for the treatment of malaria: this was the first chemical compound to be used for the treatment of an infectious disease[3]. The structures of these three compounds are shown in Figure 1.1.



1 2 3

Figure 1.1 – Examples of molecular structures of active compounds.

The 20th century was the most important for the discovery of new natural compounds with the development of new methods of analysis and the big advance of the chemical industry. These natural compounds can be found in a vast diversity of plants, animals, microorganisms, and marine organisms which are an abundant source of secondary metabo-

lites with different chemical structures and that play a very important role in the discovery and development of new drugs. The discovery of natural product-based structures is characterized by the discovery of an active compound that requires an individualized manipulation of the structure to reach the drug criterion.[4]

Generally, the natural products are directly extracted from their natural sources. However, the unlimited extraction of these compounds directly from their habitat is not possible, due to the ecosystem sustainability and profitability perspective. An alternative strategy is to use chemical synthesis to produce compounds with medicinal application. Unfortunately, the chemical synthesis of natural products is very difficult due to the high costs, difficulty in obtaining/collecting biological starting material in quantities suitable for the production of pharmaceutical preparations, the intrinsic variability of the biological material itself, the redundancy of the molecules discovered, low yields, impracticality of scale-up, difficulties in isolation and/or purification procedures, high toxicity of some active compounds, ecological and legal considerations. But despite of this problems is the best option to obtain natural products in large quantities.[5]

The global pharmaceutical market is valued at about $1.1 trillion annually. About 41% of these drugs originate, directly or indirectly, from natural products including plants (25%), microorganisms (13%) and animals (3%). Nowadays, natural products or derivatives continue to be one of the most important resources for the development of new drugs. Some examples of their application are: i) a direct source of therapeutic agents; ii) a source for the development of semi-synthetic drugs; iii) prototypes for the design of target molecules.[6],[7]

The last century of evolution in the syntheses of natural products for drug development has created the need for temporal studies and analyses of this evolution. One of the most important examples is the article review of Newman and Craig, were a detailed analysis made of the impact of the evolution of the use of natural products since 1981 to 2019. This analysis (Figure 1.2) shows a gradual increase in the use of natural products in the pharmaceutical industry until the year 1987 which marked the decline until today.[8]

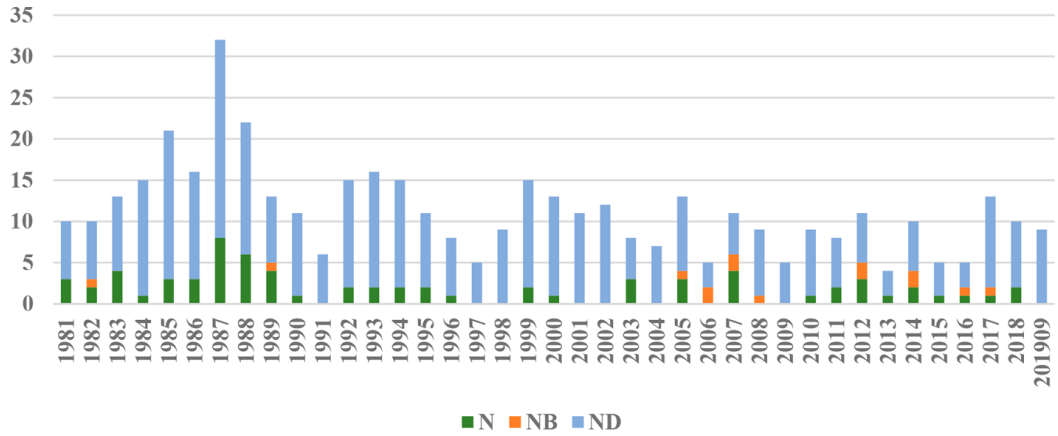


Figure 1.2 - Drugs that are directly or indirectly derived from natural products; N - unmodified natural products; NB - botanical products with the same mechanism of action as the natural extract; ND - derived from natural products with semi-synthetic modifications. Figure taken from the 2019 article by Newman and Cragg.

Despite the evolution of chemistry to produce new synthetic drugs, the natural products still hold out the best options for finding novel agents/active templates, which when worked on in conjunction with synthetic chemists and biologists, offer the potential to discover novel structures that can lead to effective agents in a variety of human diseases.

1.2. Marine organisms as a source of natural products

In recent years the recognition of the tremendous biodiversity of the marine sea life, made us realize the new chemistry and biomedical potential of marine organisms[9]. This was possible in part due to the drastic advances in both research techniques and strategies to address the key issues hampering marine natural product-bases drug discovery and development. For example, the application of microorganism-derived metabolites as chemotherapeutic agents in the treatment of infectious diseases and cancer. More recently, compounds obtained from marine organisms such as sponges, algae, and phytoplankton have been crucial in the discovery of new drugs.[10]

It is not known who was the first country to investigate the marine environment as a source of medicines, but it is known that both Japanese and Chinese herbs contained various mixtures of plants, including algae, as for example, the use of "Tyrian Purple", a dyestuff from a Mediterranean mollusk used in Roman times, which was known to show significant activity in some cancers and was used as part of traditional Chinese medicine for the treatment of leukemia.[11]

The focus on the marine natural products chemistry has essentially begun in the 1970s and has maturated in the decade of the 90s. By 1975 there were already three parallel tracks in marine natural products chemistry: marine toxins, marine drugs and marine chemical ecology[12]. It is the integration of the three fields of study that has given marine natural products chemistry its unique character and vigor. For a better estimate of the number of new compounds discovered each year, Faulkner wrote the first review article with natural compounds from marine organisms discovered between 1977-1987[13], where 2500 new metabolites with marine origin were identified. In the most recent review article, 1201 new compounds were discovered in publications in the year 2014[14]. These review articles give insight on the amount of natural compounds of interest to the pharmaceutical industry, which can be obtained from marine organisms only.

Medicinal chemistry has always focused on the discovery of new biological agents with anticancer properties derived from marine organisms. However, the first product from a marine organism to be approved by the Food and Drug Administration (FDA) was Ziconotide, a painkiller marketed as Prialt. This compound was originally extracted and purified from the venom of the marine snail *C. magus*, which is a fish-hunting species.[15],[16]

Besides the biological diversity from which these marine products can be extracted from, there has been a particular interest on algae species due to their exceptional abundance, ease of access and promising biological activity of the organic molecules they produce.

1.3. *Asparagopsis armata*

The alga *Aparagopisis armata* was described for the first time in 1855 by Harvey[17]. These algae were observed for the first time on the coasts of Western Australia and New Zealand. However, since 1925 it can be found on European coasts and on the coasts of the Northeast Atlantic, Mediterranean, South Africa, Middle East and Indo-Pacific region[18]. These algae are an invasive species with an impressive abundance. Therefore, it is of utmost importance to find strategies to mitigate the negative impacts resulting from this seaweed abundance, which can pass through the valorization of this marine resource, pointing out its biotechnological potential.[19]

This organism is very rich in biologically active molecules, particularly compounds with bromine, iodine, and ketones that cause toxic effects in other species[20]. Generally, red algae of the genus *Asparagopsis* are well known as sources of halogenated compounds with strong anti-fungal and antibacterial activity[21]. In McConnell and Fenical's paper[22], they were able to isolate and identify some extracts from *Asparagopsis armata*. They did several ethanol extractions and pentane washes and were able to isolate a few halomethanes including MeI, $CHCl_3$ and CCl_4. Figure 1.3 shows four ethyl esters, which are halogenated derivatives of acetic acid and acrylic acid.

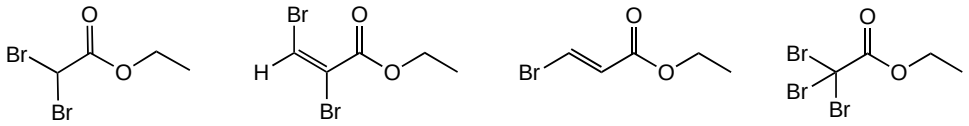


Figure 1.3 - Some ethyl esters isolated from an extract of *Asparagopsis armata.*

This seaweed is very abundant in the cost of Portugal and on the islands of the Azores and Madeira. It is therefore of national interest to explore the biologically active metabolites of this seaweed, as well as their applications in the medical field[23]. One example is described in this work, the development of a synthetic strategy for the preparation of a phosphoglycoglycerol isolated from an extract of *Asparagopsis armata*.

1.4. **Phosphoglycoglycerols**

Glycolipids represent a broad class of biologically active natural products with a diverse range of molecular structures and biological functions, many of which are essential for life[24]. They are mainly distributed in the kingdom of microorganisms and plants and can be found on the surface of cell membranes. These compounds can be classified by the nature of the lipid fragment such as glycoglycerolipids and glycosphingolipids, but glycoglycerolipids are in fact the main constituents of this class of compounds[25]. They can be found in natural products, seaweed, cyanobacteria, and higher plants.

The glycoglycerolipids are characterized by a mono- or oligosaccharide attached to the C3 position of the glycerol backbone. In Figure 1.4 the base structure of the glycoglycer-

olipids is represented. These compounds contain several biological activities such as anti-tumor[26], anti-viral[27], and anti-inflammatory activities[28]. However, the low natural abundance of these types of compounds coupled with the difficulty of isolation have created the need to develop new synthetic routes for the preparation of these molecules[29].

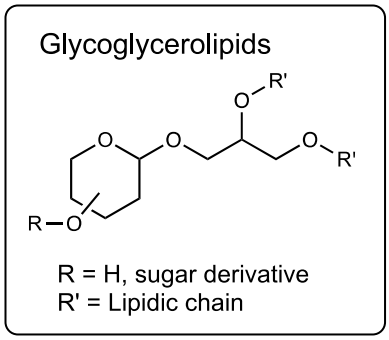


Figure 1.4 - Glycoglycerolipids base structure.

Phosphoglycerolipids are highly amphiphilic molecules and one of the most important constituents of cellular membranes. In addition to that they are a source of physiologically active compounds. These compounds are characterized from a glycerol backbone that is esterified to the carboxyl groups of the two fatty acid chains at the hydroxy groups at C-1 and C-2, which is represented in Figure 1.5. The hydroxy group in the C-3 is esterified by phosphoric acid which is further esterified by another alcohol such as ethanolamine, choline, serine, or myo-inositol[30].

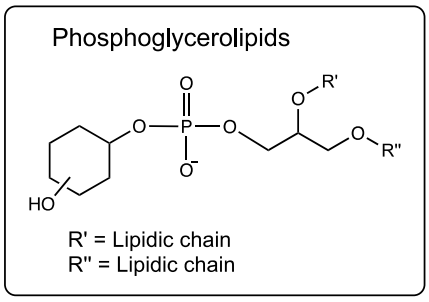
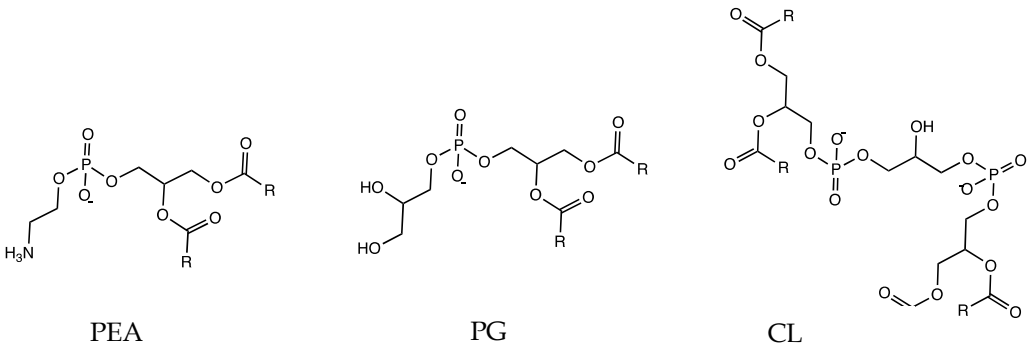


Figure 1.5 -Phosphoglycerolipids base structure.

One of the most common places were phosphoglycerolipids can be found is in bacterial membranes. The structure of the cell divides most bacteria in two families Gram-positive and Gram-negative. The Gram-positive bacteria generally contains lipoteichoic acids, phosphatidylglycerol (PG) and cardiolipin (CL). In contrast, the Gram-negative bacteria are primarily composed of phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and cardiolipin (CL)[31].



PEA PG CL

Figure 1.6 - Structure of phosphatidylethanolamine (PEA), phosphatidylglycerol (PG) and cardiolipin (CL).

This type of compounds can also act as signalization molecules. One of these examples, is the phosphatidylinositol, Figure 1.7 , one natural substrate specific for the phospholipase C [32]. This enzyme is part of a family of intracellular eukaryotic enzymes with important functions in the signal transduction. Several phospholipids have been synthetized with hydrophilic chains, followed by the study of the inhibition of the phospholipase C from the *Bacillus cereus*[33].

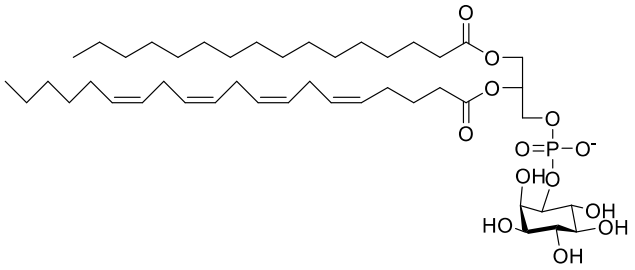


Figure 1.7 - Structure of phosphatidylinositol.

Phosphoglycoglycerolipids are structures derived from glycoglycerolipids, consisting of a sugar skeleton, mono- or disubstituted glycerol with lipid and phosphate chains. Although they are not very abundant in nature, these compounds play an important role at the biological level by being one of the constituents of the membrane and the cell wall[34]. The P-O bond can occur directly with the sugar unit, at positions C-1 or C-6, or with glycerol at one of the primary positions. When the oxygen atoms of the glycerol are not substituted with lipidic chains, we are in the presence of a new family, called phosphoglycocglycerols represented in the Figure 1.8.

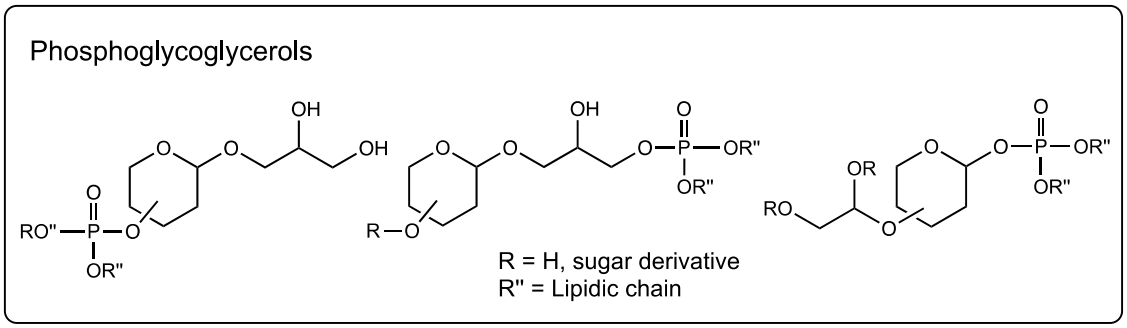


Figure 1.8 - Phosphoglycoglycerols base structures.

To survive in harsh environments, micoorganisms need to develop their membrane structures to adapt to the surroundings and ensure the constant stability and permeability of nutritional flow. One example of this type of microorganisms are the species *Thermus* and *Meiothermus.* In these two type of thermophilic bacterias the presence of phosphoglycolipids with biological roles related with to the thermal stability of the celular membrane have been reported. The bulky head groups would enhance the steric protection, possibly by stabilizing the membrane against enviromental stress through hydrogen bonding[35].

Another exemple, a phosphoglycoglycerol present in six species of the halophilic Gram-negative bacteria *Halomonas,* was identified and characterized. This was constituted by a unity of glucose, two glycerol's and one phosphate group as represented in the Figure 1.9. Halophilic organisms can resist to fluctuations of extracellular salinity using some strategies. One of those strategies consist of modifying the membrane structure, generally affecting phospholipids and their fatty acid composition[36].

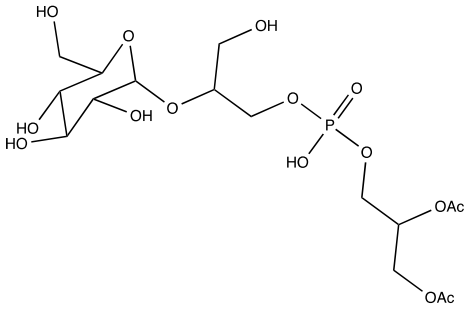


Figure 1.9 - Structure of PG1 phosphoglycerol isolated from *Halomonas* species.

There are also other less common structures of this type of compounds. For example, phosphoglycoglycerols containing a phosphocholine unit, such as GGPL-I and GGPL-III isolated from the *Mycoplasma fermentans,* Figure 1.10 [37].

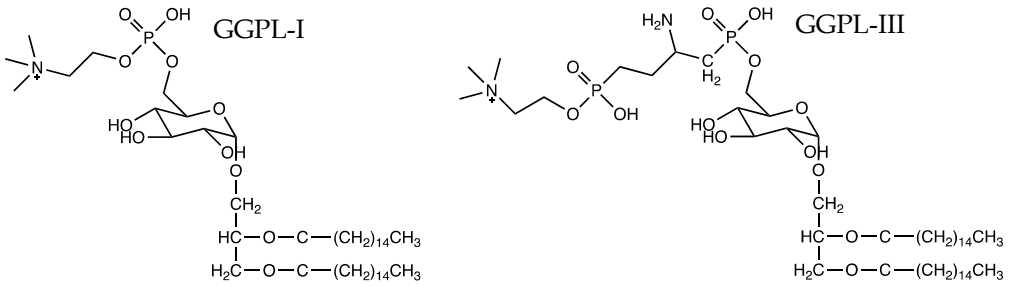


Figure 1.10 - Structures of phosphoglycerolipids containing phosphocholine (GGPL-I and GGPL-III) isolated from *Mycoplasma fermentans*.

Mycoplasma fermentans may probably play a role in human disease. In fact, much evidence has been accumulated in recent years suggesting that it may have an etiologic role as an opportunist in patients with human immunodeficiency virus infection and AIDS and may have a possible association with chronic arthritis[38],[39]. The GGPL-I and GGPL-III are the major components and the principal immunodeterminants of the *M. fermentans*[40].

Despite of all the examples given previously, there are few examples of phosphoglycoglycerols structures described in the literature. We can take the glycoglycerols and phosphoglycoglycerols as an example of two natural products that can be found in marine sources. Recently, the research group where this work was developed discovered a new phosphoglycoglyerol with anti-microbial properties from *Asparagopsis armata*.

The exact structure of this new compound is still unknown, but with resource to NMR spectroscopy they were able to identify different parts of the structure. The ^{13}C NMR analysis suggests the presence of a sugar unit, glycerol, and ethanolamine. Confirmation of the presence of the ethanolamine unit was done by ^{1}H NMR. The identification of the sugar and the position of the glycerol unit was made by X-ray spectroscopy in a derivative resulting from an acetylation reaction. The sugar identified was galactose and this method also allowed the determination of the α configuration at the anomeric position of the sugar. In all derivatization attempts, namely the acetylation reaction, the phosphorus fragment was not identified, due to possible hydrolysis.

The conjugation of spectroscopic data suggests the presence of a glycoglycerol unit derived from galactose attached to an ethanolamine phosphoamidate or phosphate group, Figure 1.11. Due to its biological activity and the absence of similar structures in the literature, the synthesis of a library of compounds with these fragments has awakened our interest.

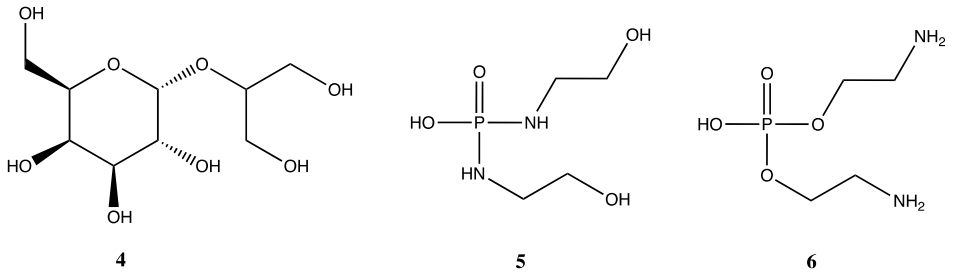


Figure 1.11 - Molecules present in the structure of a new phosphoglycerol isolated from *Asparagopsis armata*.

In the Figure 1.12 are some possible structures from the possible combination of the three fragments. Usually the phosphate-sugar bonds occur in the carbon C-6 of the sugar but it's still possible to occur a bond from phosphate to glycerol via carbon C-1. Then the ethanolamine fragment can be bond to the phosphate atom with the nitrogen atom, giving the possible structures **7** and **9**. The other bond possible occurs between the phosphate atom and the oxygen atom giving the structures **6** and **8**.

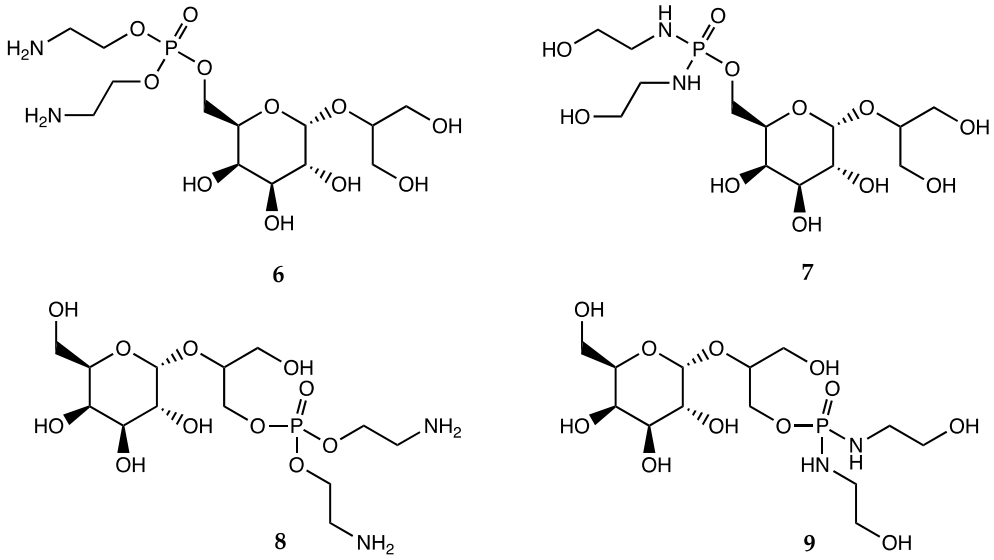


Figure 1.12 - Possible structures for a new phosphoglycerol isolated from *Asparagopsis armata*.

To prepare this library of compounds we will need several synthetic strategies. For all the compounds is necessary the preparation of the structure **4** as the first step. Then for the structures **8** and **9** we need the preparation of the phosphoamidate and phosphate groups. To obtain the final product we just need a coupling reaction. The binding of phosphorus atom to sugar via carbon C-6 is favorable because of the more accessible primary hydroxy group. For the structures **6** and **7** it is also necessary the preparation of the phosphoamidate and phosphate groups and then we just need a coupling reaction between these groups and the structure 4. This reaction needs to bind the phosphorus atom to the secondary hydroxy group of the glycerol group.

1.5. **Carbohydrates**

The abundance of carbohydrates in nature, their chemical properties and their diverse roles in biological systems make them attractive as a subject of study in chemistry and biology.

Glucose is the most abundant organic molecule on the planet and is a fundamental precursor unit of multiple biological macromolecules. Another very important monosaccharide, ribose, is present in its five-carbon cyclic form in our RNA and DNA[41].

Until recently, the role of sugars (low molecular weight monosaccharides and oligosaccharides) in the biological system was thought to be limited to energy production and as a structural component. However, they have been implicated in many cellular processes, including cell recognition and cellular transport. Because of their importance as fundamental units, they are synthetic targets and biological tools, and carbohydrates are useful and important models for total synthesis as well as for creating and developing new synthetic technologies[42].

For the most common monosaccharides - pentoses and hexoses - they have the structure of polyhydroxyaldehydes or polyhydroxyketones that exist in several isomeric forms, resulting from the existence of chirality centers. Structurally they occur naturally in the form of rings, which can have six members (pyranose form) or five members (furanose form)[43].

When the carbohydrates are in aqueous solutions, the pyranose and furanose forms tend to get to an equilibrium in solution, this phenomenon is called mutarotation represented in Figure 1.13. The first step towards this equilibrium occurs with the opening of one of the sugar's pyranoses anomers to give the open chain aldehyde. This reaction is a hemiacetal hydrolysis subjected to acid and base catalysis. The ring closing reactions are also catalyzed by acid or base. The first possibility is the regeneration of the 6-membered pyranose ring by attack of the 5-hydroxy group back onto C-1 aldehyde. However, the open chain hydroxy aldehyde, may also ring close by nucleophilic attack of the free 4-hydroxy group onto the aldehyde, thus forming a 5-membred ring. The hydroxy group may attack either face of the aldehyde, leading to either the α or β products in both cases[44].

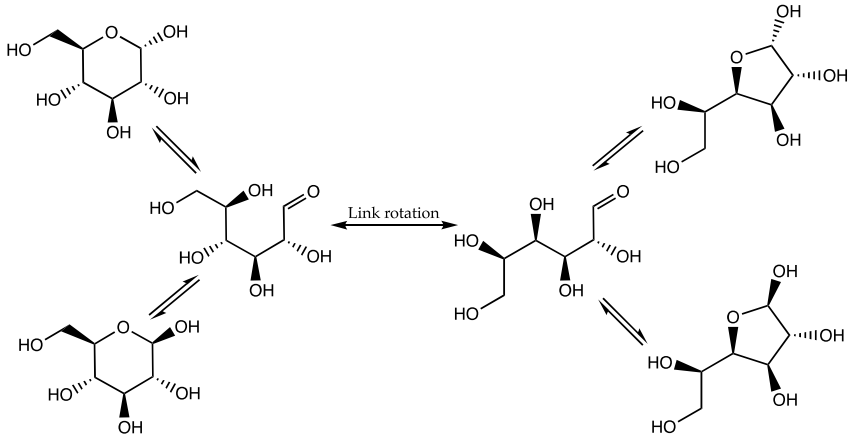


Figure 1.13 - Descriptive reaction scheme of the mutarotation phenomenon.

Due to the large number of hydroxy groups present in carbohydrates, it is important to use strategies for protecting these groups. There are two different types of protecting groups that require different conditions. It must be possible to introduce and remove groups permanently with great regiocontrol and great efficiency. Obviously, they must also be stable under glycosylation conditions and under conditions used for the removal of temporary groups.

The most efficient permanent protecting groups for these reactions are the acetates, benzoates, and benzyl ethers since they have the stability and at the same time the efficient introduction/deprotection properties needed to make them suitable for these protections.

The acetates and benzoates can be introduced and removed in high yields under mild conditions. Drawbacks as permanent protecting groups include their relatively limited base stability and their tendency to migrate, because they are participant groups. The benzyl ethers are usually performed under strongly basic conditions. They are stable towards almost any conditions, are easily removed under essentially neutral conditions and they are non-participant groups. One drawback is the rather harsh conditions usually employed for their introduction[45].

1.6. **Coupling Reactions**

The synthesis of phosphoglycoglycerols requires a previous preparation of the coupling materials. It requires a selective protection of the hydroxy groups of the sugar unit and of the primary alcohols from the glycerol unit. There are several procedures to do the protection of the two compounds used in this reaction. This is a stereoselective reaction and because of that the configuration of the anomeric position of the resultant product will depend on several factors. So, the protection groups used in the protection of the hydroxy groups of the sugar unit will play an important role in the obtention of the required final configuration.

Mannock et al[46] used the Koenigs-Knorr conditions to do the condensation reaction between the acetobromoglucose with the glycerol, Figure 1.14.

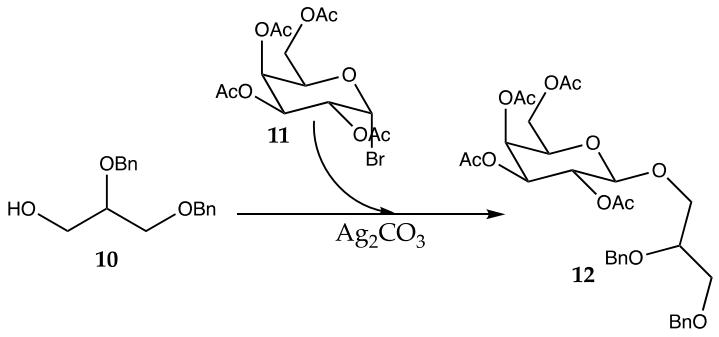


Figure 1.14 - Synthetic strategy used by Mannock et al. for the coupling reaction between a sugar unit and a glycerol unit.

As mentioned in the previous chapter the acetate protecting groups are participant groups. The use of participating neighboring protecting groups, in the C-2 position, promotes the 1,2-trans coupling with the acceptor, and consequently the preferential formation of the β-anomer, while non-participating groups such as the benzyl group promote the formation of a mixture of α and β products.

In Figure 1.15. the Koenigs-Knorr reaction one of the oldest glycosylation processes and a starting point in the glycoside syntheses is represented. This process involves coupling between acetoamide halides sugars as donors with reactive acceptor compounds having primary hydroxy groups, in the presence of Ag_2CO_3 or AgOTf. This type of reactions is valuable in the synthesis of simple glycosides such as β-(1-6) disaccharides. The presence of a halogen bonded to the anomeric center allows the formation of a five membered carbocation ring intermediate that blocks the α-face of the donor sugar favoring the formation of the β-structure[47],[48].

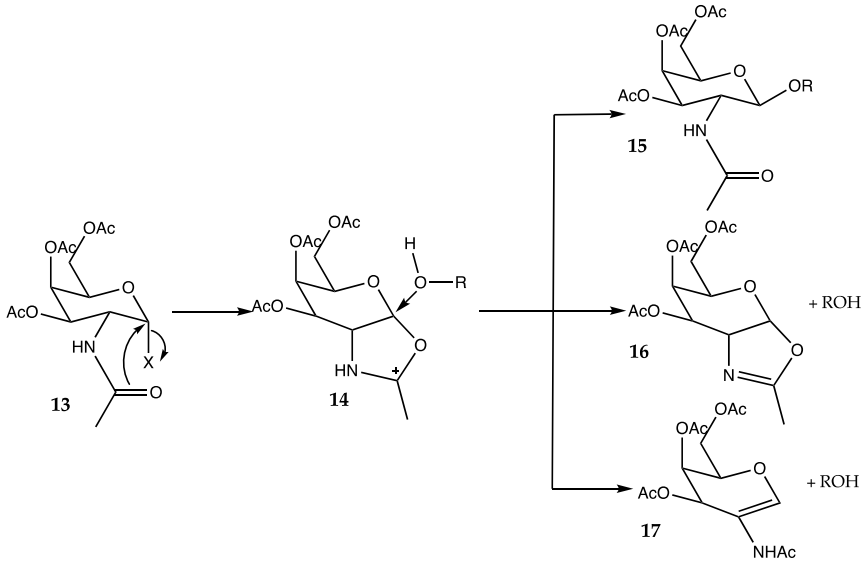


Figure 1.15 - Side reactions accompanying Koenigs–Knorr reactions.

Welzel et al[49] suggest a synthetic route for the preparation of the glycoglycerol using a benzylated sugar as the glycosidic donor and silver trifluoromethanesulfonate as the catalyst of the coupling reaction, Figure 1.16.

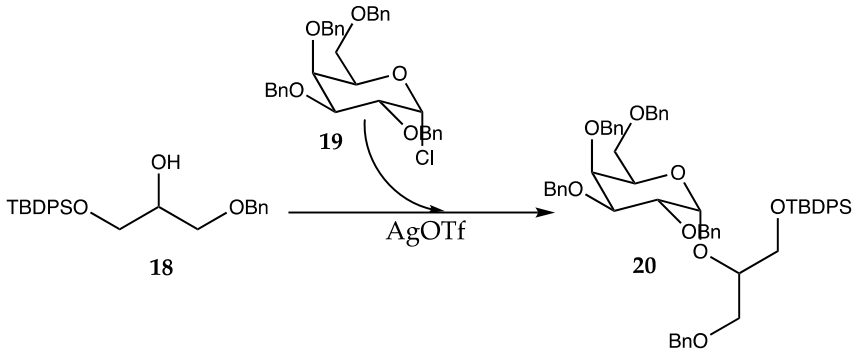


Figure 1.16 - Synthetic strategy used by Welzel et al. for the coupling reaction between a sugar unit and a glycerol unit sugar unit and a glycerol unit.

We can also find examples of other type of catalysts with applications in the condensation reaction between the sugar unit and the glycerol. The use of mercury salts as promoters, such as $Hg(CN)_2$, was introduced by Helferich and is also common in these reactions[50]. These types of reactions always require the use of a drying agent (the use of molecular sieves is a common procedure) to prevent hydrolysis from occurring in the sugar, which is more sensitive due to the presence of a good leaving group.

Sun et al[51] studied the effect of stereoselectivity of the reaction of Koenigs-Knorr glycosylation catalyzed by urea. The sugar donors used in these reactions are protected with non-participant benzyl groups, so the formation of the cation intermediary doesn't occur. The use of various donor and acceptor structures is described, as well as their effect on the configuration of the glycosylation product. In most cases, the formation of the α configuration is favored. The addition of phosphines to the reaction, however, had a steric effect on its stereoselectivity. The addition of tri-(2,4,6-trimethoxyphenyl)-phosphine (TTMPP), tripled the ratio of α/β (12.6:1) turning the previously poorly stereoselective glycosylation reactions into highly α-selective reactions. In contrast to traditional Koenigs-Knorr glycosylation methods, this described method is α-selective and the catalysis does not involve the use of heavy metals.

There is one more thing to have in mind when trying this type of reactions. The protecting groups of the hydroxy groups of the glycerol and the hydroxy group that is reacting also influence the reaction product. When protected with ether groups, the acceptors are much more reactive than when protected with ester groups, and the yield of the reactions is higher when the primary hydroxy group is free rather than the secondary hydroxy group[52].

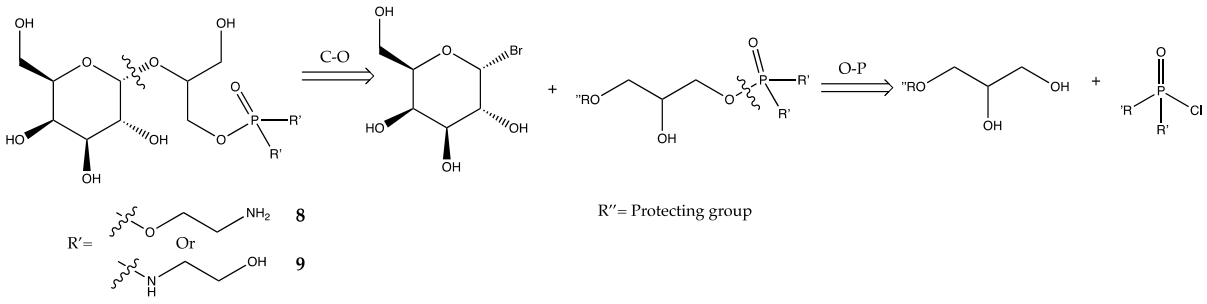
2.
Results and Discussion

2.1. Preambule

In this work, we attempted to synthesize two possible structures for a phosphoglycoglycerol previously isolated by our group from *Asparagopsis armata.* The isolated compound was characterized by ^{1}H and ^{13}C NMR, ATR-FTIR, and X-ray diffraction, although this analysis was not conclusive on the absolute structure of the compound. In this way, a research project was started aiming the synthesis of various phosphoglycoglycerol structures for comparison with the product of natural origin.

The structures outlined for this compound were similar to each other, presenting differences in the linkage position of the glycerol, phosphate, and ethanolamine groups to the galactose unit. To design a synthetic plan for the preparation of these structures, the retrosynthetic analysis represented in Scheme 2.1 was performed.

For structures **8** and **9**, in which phosphorus is bound to glycerol, starting with a C-O disconnection results in two synthons relating to an α-brominated galactose and a glycerophosphate or glycerophosphoamidate. Finally, an O-P disconnection is performed, thus obtaining the equivalent synthons of diamino phosphate chloride or diethanolamine chlorophosphate and glycerol protected on one of the primary hydroxy groups.

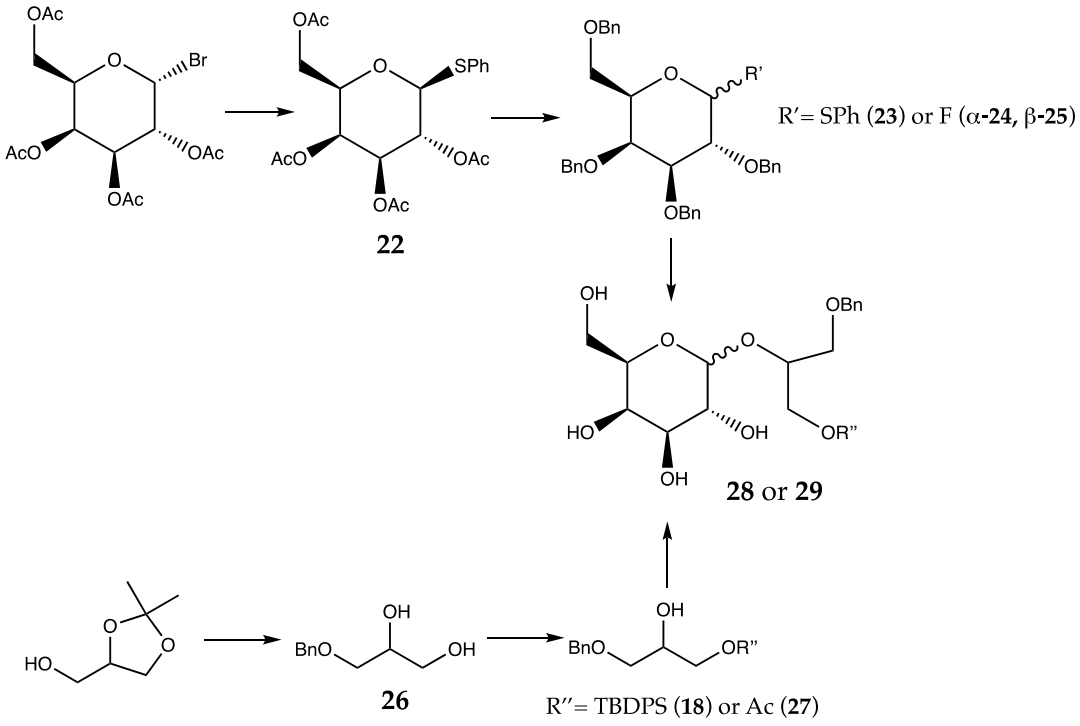


Scheme 2.1 - Retrosynthetic analysis of the structures to be synthesized.

The syntheses of this compounds can be divided in four synthetic milestones. First, the derivatization of the D-(+)-galactose (section 2.2). Then, the derivatization of the glycerol with the objective of further coupling to the sugar unit (section 2.3), followed, by the coupling reactions between the two groups previously synthesized (section 2.4). Finally, the

coupling reaction between the phosphoamidate or phosphate with the compound previously synthesized, will be performed in a future work carried out in this project.

For the derivatization of the D-(+)-galactose three different routes were proposed. Two of these routes begin with D-(+)-galactose but mixtures of isomeric compounds were obtained, and the other one started with acetobromo-α-D-galactose and gave with a yield of 65,52% the compound **23**. In this route a substitution reaction in the anomeric position with a fluor atom to see if the change in the reactivity of the anomeric position would influence the yields in the coupling reactions was also performed. Then a route for the protection of the primary alcohols of the glycerol using two different protecting groups was created; the goal was to test if the size of the protecting group had some effect in the coupling reactions. Finally, a coupling reaction was performed between the sugar and the glycerol previously synthesized as represented in the followed Scheme 2.2.



Scheme 2.2 - Synthetic strategies for the preparation of the compounds **28** or **29**.

2.2. Reaction of derivatization of the D-(+)-galactose

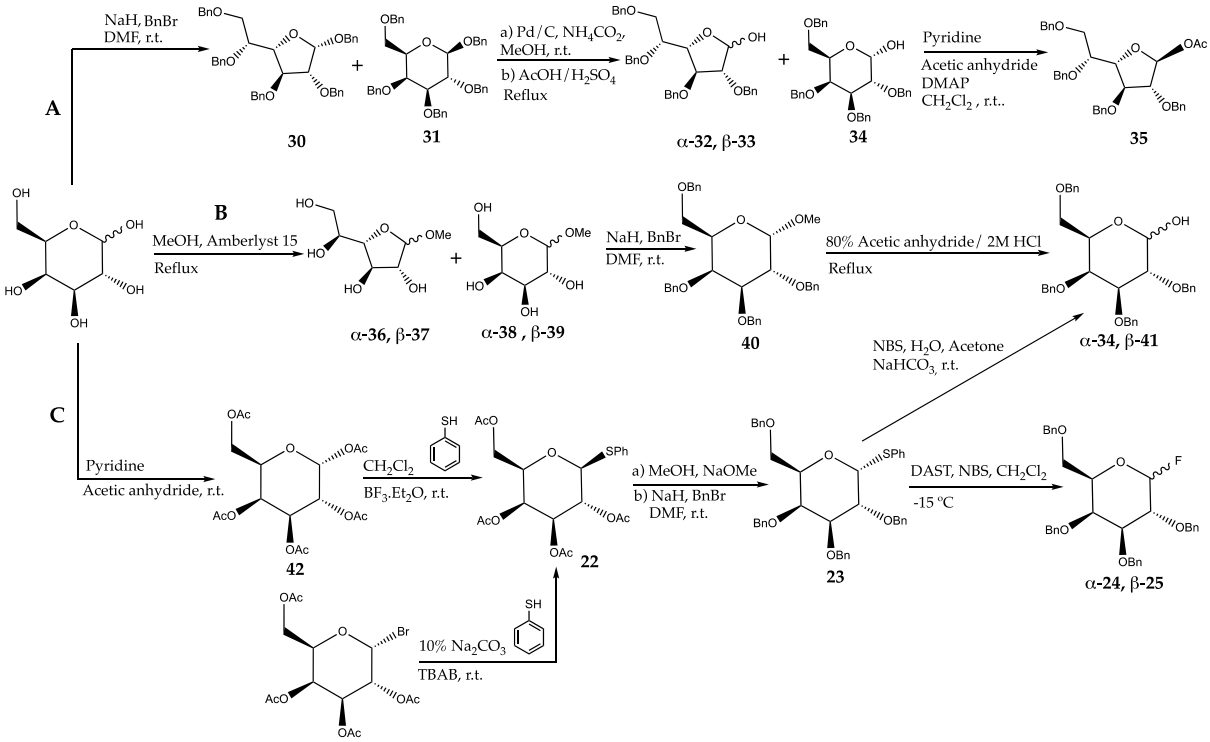
As mentioned further above in the introduction, the role of protecting functional groups is fundamental for the stereoselectivity of the coupling reactions. In the case of the phosphoglycoglycerol to be synthesized, whose configuration at the anomeric center was previously determined as α, a protection of the hydroxy groups of galactose with non-participating groups such as benzyl is necessary for obtaining majorly this configuration in the product of the coupling reaction.

In the section below, three different routes were tested for obtaining benzyl derivatized galactose and a thiophenol group or fluoride atom in the anomeric position as represented in the Scheme 2.3.

Path A started with the benzylation of all the positions of D-(+)-galactose, followed by a hydrolysis of the anomeric position and subsequent acetylation. This path didn´t work since a mixture of a furanose and pyranose were obtained in the first step and maintained in the next steps. As consequence of the formation of this mixture the major product obtained was a furanose and because of that the path B was envisaged.

Path B instead of starting with a benzylation of the sugar, we envisage a methylation of the anomeric position, then a benzylation of the remaining positions of galactose and finally a hydrolysis of the anomeric position. Although in this pathway the product was obtained with a very low yield.

So, because of the problems in the paths A and B, path C was developed. This path started with the acetylation of D-(+)-galactose, followed by a substitution reaction at the anomeric position to place the thiophenol group, then a hydrolysis and benzylation of the remaining position of the galactose, and finally another substitution reaction to place the fluoride atom at the anomeric position. In this path the reaction with commercial acetobromo-α-D-galactose was also tried, to eliminate one step of the route and increase the global yield of the reaction.



Scheme 2.3 - Synthetic approaches to the preparation of the derivatives of D-(+)-galactose.

2.2.1. Synthesis of penta-*O*-benzyl-D-galactopyranoside (31) - path A

The first pathway (path A) of this synthesis started with a simple benzylation reaction of D-(+)-galactose. This reaction was made with a simple treatment of the hydroxy groups of the sugar with benzyl bromide in the presence of a good base like sodium hydride. The mechanism of this reaction is a simple S_N2 displacement of the halide anion by the alkoxide which itself is formed by deprotonation of the alcohol.[44]

Despite de mechanism described, one pyranose (**31**) and one furanose (**30**) were obtained using the conditions of ambient temperature, 10 equivalents of sodium hydride and benzyl bromide, DMF as solvent and 5 to 7 days of stirring[53]. This can be due to the phenomenon of mutarotation that leads the sugars in solution to an equilibrium of the 4 possible forms. In a paper involving the preparation of various structures of benzylated sugars using excess of KOH in DMSO, Decoster *et al*.[54] demonstrate by ^{1}H NMR spectroscopy that the sugar was obtained in good yield in the form of α-furanose.

In this case the presence of the compound **30** and **31** in the mixture was verified resorting to ^{1}H and ^{13}C NMR spectra as presented in Table 2.1. The remaining signals were identified by comparation with the literature[53].

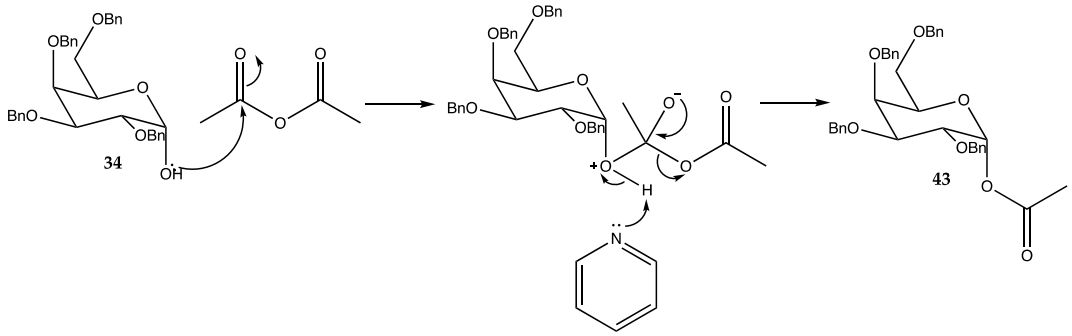
Table 2.1 - Proportion and chemical shift in the ^{1}H and ^{13}C spectrum of the obtained compounds.

	Proportion	^{1}H (ppm) – H1	^{13}C (ppm) – C1
α furanose (**30**)	1.01	4.96	98.3
β pyranose (**31**)	1.00	4.47	102.9

2.2.2. Synthesis of acetil-2,3,4,6-tetra-*O*-benzyl-D-galactopyranoside (43) - path A

Then after the hydrolyses described in the section 2.2.6 the mixture of the furanose (**31** and **32**) and pyranose (**33**) was submitted to an acetylation reaction at the anomeric position. This reaction is a simple addition/elimination mechanism of ester formation with an alcohol in a mixture of acetic anhydride and base. The base used not only serves to mop up the equivalent of acetic acid that is produced during the reaction, but it also catalyzes the reaction itself. Another method that can be used is to use Lewis's acid catalysis for the acetylation reaction.

In this case we used acetic anhydride and pyridine as base. The hydroxy group of the sugar attacks a carbonyl group of the acetic anhydride breaking the double bond and forming a tetrahedral intermediate which undergoes an elimination reaction, this time losing a carboxylate anion to give an ester (Scheme 2.4).[55]



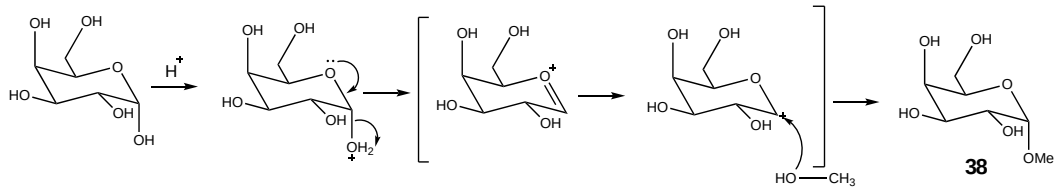
Scheme 2.4 - Mechanism of an acetylation reaction.

Since the starting material of this reaction was a mixture of compounds, we expected to obtain two different products from this reaction. Instead, just one was isolated, the acetil-2,3,4,6-tetra-*O*-benzyl-α-D-galactofuranoside (**35)**. The confirmation by ^{1}H NMR spectroscopy of the product was made with the presence of a singlet at 2.13 ppm with integration of 3 protons characteristic of the methyl group of the acetate and the position of the anomeric proton signal at 6.31 ppm. The remaining signals were verified by the integrals of the protons in the ^{1}H NMR spectrum, that were consistent with the literature[53]. Although the peaks at 4.83-4.32 ppm integrate to 11 protons instead of 8 this may be due to some impurity in the NMR tube.

2.2.3. Synthesis of Methyl 2,3,4,6-tetra-*O*-benzyl-D-galactopyranoside (38, 39) - path B

The preparation of this type of compounds is achieved through a Fischer glycosylation reaction. Usually, a source of anhydrous Brønsted acid ($SOCl_2$) or a Lewis acid ($ZnCl_2$) in the presence of excess of alcohol is used.

Lactol compounds are hemiacetals, and because of that they can react with alcohols to form acetals. This S_N1 reaction initially gives rise to the formation of a cyclic carbocation intermediate called a glycosyl cation. This glycosyl cation is then attacked by the alcohol to yield the acetal product.



Scheme 2.5 - Mechanism of a methoxylation of the anomeric position.

The mechanism in the Scheme 2.5 results in an equilibrium state. Acid treatment of any glycoside in these conditions can reverse this reaction to yield the initial carbohydrate. Since the reaction is thermodynamically controlled, the most frequent anomer that occurs is the most thermodynamically stable one (α). Considering steric effects, it would be logical to state that the most stable product of this reaction was predictably the β-anomer with the

anomeric substituent in the equatorial position, as opposed to the α-anomer with the substituent in the more impeded axial position.

The isolation of the thermodynamically preferred product indicates that there is some other factor in this reaction that stabilizes primarily the α-anomer. This stabilization is called the anomeric effect where electronegative substituents on a pyranose ring prefer to occupy the axial rather than the equatorial orientation. When an electronegative substituent in an axial position at the anomeric center exists, the oxygen atom in the ring has one of its lone pairs of electrons anti-periplanar to the C-OMe bond. This lone pair can participate in a stabilizing two electron interaction, which can be represented by the structures **38** and **44** in the Figure 2.1.[44]

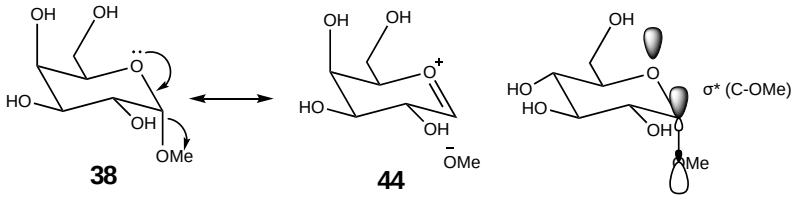


Figure 2.1 - Structures **38** and **44** and orbital representation.

These interactions between the electron pair of the oxygen atom and the antibonding orbital σ* of the C-OMe bond results in an electron delocalization and consequently the stabilization of the energy. The fact that these orbitals are in an anti-periplanar position optimizes their overlap. In general, this effect has implications for carbohydrate chemistry, as the formation of α-glycosides tends to be more favorable compared to β-anomers.

The anomeric effect can be explained as a destabilizing effect, which is a consequence of the dipole repulsion of an equatorial electronegative substituent on C1 and the resulting dipole of the free electron pair of the oxygen atom of the ring. This effect was initially considered to be an electrostatic repulsive effect that occurs only in the β conformation. In this reaction MeOH and Amberlyst 15 a powerful and selective acid catalyst were used under reflux[56]. A mixture of the α/β anomers for pyranose (**38, 39**) and furanose forms (**36, 37**) in a proportion showed in the Table 2.2 was obtained.

These compounds were identified in the ^{1}H and ^{13}C NMR spectra in the Appendix 4 - 1-methyl-α-D-galactopyranoside (38), 1-methyl-β-D-galactopyranoside (39), 1-methyl-α-D-galactfuranoside (36), 1-methyl-β-D-galactofuranoside and are represented with the integrals of the protons of the anomeric position; these peaks are consistent with the ones from the literature for both spectra [57], [58], [59], [60]. Although in the ^{13}C spectrum, the β pyranose and the α furanose have the same chemical shift.

Table 2.2 - Proportion and chemical shift in the ^{1}H and ^{13}C spectrum of the obtained compounds.

	Proportion	^{1}H (ppm) – H1	^{13}C (ppm) – C1
β pyranose (**39**)	3.37	4.31	108.1
α pyranose (**38**)	1.00	4.83	102.0

β furanose (**37**)	1.56	4.88	103.8
α furanose (**36**)	3.84	4.90	108.1

After obtaining a mixture of the α/β anomers for the pyranose (**38, 39**) and furanose forms (**36, 37**) the next step consisted in a selective protection of the hydroxy groups, followed by the hydrolysis of the anomeric position.

The protection was made with benzyl groups, with the same conditions described in the section 2.2.1 above. In this step it was possible to isolate the methyl-2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranoside (**40**). The presence of this compound was confirmed by the aromatic protons signal in the range of 7.43-7.27 ppm with integration to 27 protons (this excess can be due to the deuterated solvent or some residual benzyl bromide), and to the signal at 4.63 ppm, that refers to the H-1 proton in an α position due to the *J* coupling of 2.1 Hz. The rest of the peaks were verified by the integrals of the protons in the ^{1}H NMR spectrum compared with the ones in the literature[61].

In this step it was possible to isolate the α anomer of pyranose since is the preferred form of this product due to the anomeric effect characterized above in this section.

2.2.4. Synthesis of phenyl-2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopynanoside (22) - path C

Since the methyl α-D-galactopyranosyl benzylation reaction worked but with a very low yield in the obtention of the compound **40**, assays were performed using D-(+)-galactose as substrate again now using a different approach. In this route the protecting group chosen was the acetate since this protecting group has the stability and at the same time the efficient introduction/deprotection properties needed to make them suitable for the next reactions performed in this route.

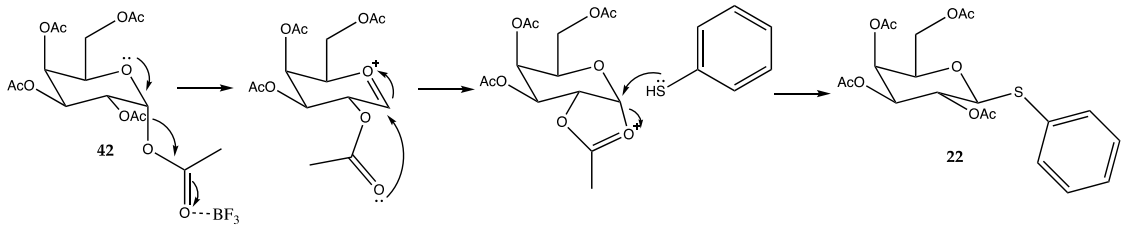
The acetylation of sugars is a relatively easy and quick reaction using acetic anhydride and pyridine as solvent and catalyst of the reaction. Usually, the acetylation of free sugars such as galactose tend to give a mixture of stereoisomers at the anomeric position, or anomers.[44]

It was possible to isolate one of the anomers penta-*O*-acetyl-α-D-galactopyranoside (**42**) with a yield of 45.7%, using the conditions described in the paragraph above[62]. The confirmation of the presence of this compound was made by ^{1}H NMR, in which the presence of the singlets at 2.18-1.95 ppm characteristic of the methyl groups of the acetate group are shown. The remaining signals were confirmed with the comparation of the integrals obtained and the ones from the ^{1}H NMR spectrum from the literature[62]. The β anomer appears in a mixture with both furanose forms, and isolation was not possible.

The next step in this route is the substitution reaction in the anomeric position with a thiophenol group. Thioglycosides are used in glycosylation reactions as glycosyl donors. Due to their thermal and chemical stability, they are used as stable intermediates for functional-group transformations as well as stereoselective glycosylation's. They can also be

transformed in various other glycosyl donors for example fluorides (section 2.2.7) and hence the thiol functionality is often used as a temporary anomeric protecting group[63].

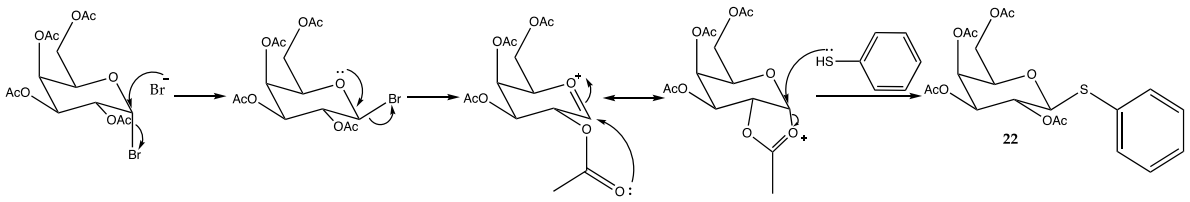
First this reaction was made with the compound **42** as starting material. In the mechanism of this reaction the anomeric acetate is efficiently displaced under the influence of an acidic catalyst. The procedure was to react the compound **42** dissolved in dichloromethane with a slight excess of thiol using a hard Lewis acid as promoter such as boron trifluoride etherate, which generally gives a high yield of mainly the β anomer due to the neighboring group participation, as represented in the Scheme 2.6[64].



Scheme 2.6 - Mechanism of an thioglycoside formation using D-(+)-galactose as starting material.

The product was isolated with a yield of 19.6%. This compound was characterized by ^{1}H NMR: it was possible to see the appearance of the signals in the aromatic zone at 7.55-7.30 ppm referring to the phenyl group of the thiophenol and as consequence the disappearance of one singlet in the zone of the methyl groups of the acetates at 2.14-2.00 ppm. In the ^{13}C NMR spectrum it was possible to see the disappearance of one signal at 170.4-169.5 ppm (corresponding to the signal of carbonyl group of the acetate) and the appearance of a signal at 132.5 ppm, referring to the quaternary carbon from the phenyl group of the thiophenol. The rest of the signals was confirmed, comparing the values obtained with the data described in the literature[65].

Then the starting material was changed for a commercial compound, acetobromo-α-D-galactose. This reaction was performed with the aim of reducing one step of the synthesis and increase the yield of the substitution reaction in the anomeric position. In this reaction Tetrabutylammonium bromide (TBAB) is used as catalyst to promote the interconversion of the α-glycosyl bromide to the reactive β-glycosyl bromide. This interconversion led to the formation of a 1,2-oxocarbonium ion by participation of the neighboring group, which finally furnished 1,2-*trans*-thioglycoside, as represented in the Scheme 2.7.[66]



Scheme 2.7 - Mechanism of a thioglycoside formation using acetobromo-α-D-galactose.

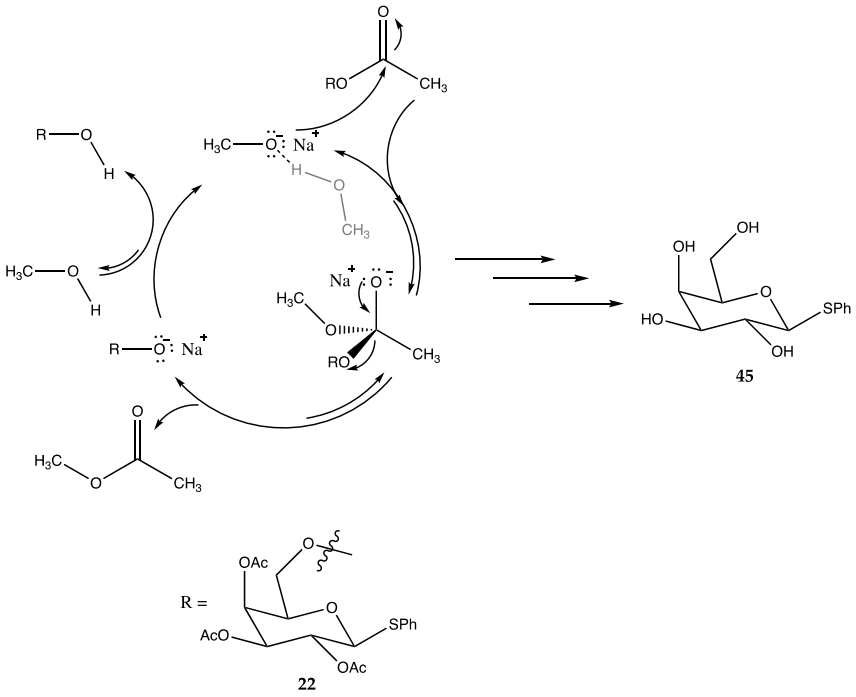
The product was isolated with a yield of 71% and was characterized by ^{1}H NMR in the same manner as described in the paragraph above, where the identity of the compound was confirmed, comparing the values obtained with the data described in the literature[65].

The first reaction made (Scheme 2.6) had very low yield this can be due to an incomplete reaction between the sugar and the thiophenol or due to a formation of a secondary product not identified. In the second reaction the yield was good, but in this reaction the group in the anomeric position is a bromide and this is a better leaving group than the acetate since this compound is stable enough to exist on its own when it leaves the molecule and it's a weaker base than the acetate. Due to this the reaction is easier to occur when the bromide is in the anomeric position. Besides the yields, in the second synthetic method the elimination of one step of the route is better for the obtention of the final product, and because of these two parameters this was the path chosen to obtain this compound.

2.2.5. Synthesis of phenyl-2,3,4,6-tetra-*O*-benzyl-1-thio-α-D-galactopynanoside (23) - path C

The last step of this route consisted in a hydrolysis of all de acetate protecting groups of the galactose and the protection of this positions with benzyl groups. This step is very important since the objective in the section 2.4 is to introduce the glycerol intermediate in the α position of the sugar as mentioned in the preamble. So, for this to occur the protecting groups in the sugar need to be non-participants and to accomplish this it is necessary to substitute the acetate groups by benzyl groups.

First a hydrolysis of the acetates is performed. This is a basic hydrolysis performed with methanol as a protic solvent and NaOMe as a base, usually called Zemplén transesterification (Scheme 2.8). This mechanism occurs with the transesterification of the acetate into methyl acetate via methanol-assisted methoxy anion $[Me–O–H\cdots O–Me]^-$ addition, which is the classical Zemplén mechanism with an added H-bond assistance.[64], [67]



Scheme 2.8 - Mechanism of an Zemplén hydrolysis.

The next step is the protection with benzyl groups. These two reactions are made *in situ* to avoid losses of yield during the reaction. This protection was performed in the same conditions described in section 2.2.1. In this final step the phenyl-2,3,4,6-tetra-*O*-benzyl-1-thio-α-D-galactopynanoside (**23**) was obtained with a yield of 60%. This compound was characterized by ^{1}H NMR where it was possible to see the disappearance of the singlets at 2.18-1.95 ppm characteristic of the methyl groups of the acetate group and the appearance of the signals in the aromatic zone at 7.45-7.38 ppm characteristics of the phenyl group of the benzyl groups, this zone integrates to 31 protons, but this can be due to the deuterated solvent or some excess of benzyl bromide. In the ^{13}C NMR it is possible to see that the quaternary carbons now appear at a lower chemical shift at 138.8-137.8 ppm for the benzyl groups compared with the acetates that appeared at 170.4-169.4 ppm. The rest of the peaks where confirmed, comparing the values obtained with the data described in the literature[63].

The obtention of the α anomer instead of the β anomer occurs due to an opening of the ring of galactose caused by the free pair of electrons from the thiophenol, forming a double bond between the sulfur atom and an oxonium atom. When the ring closes again it tends to close in the most stable configuration in this case is the α anomer.

2.2.6. Synthesis of 2,3,4,6-tetra-*O*-benzyl-α-D-galactopynanoside (34, 41)

It is known that the benzyl groups aren't a viable anomeric protecting group since it's controlled regioselective removal has not been achieved in diversely protected carbohydrates. The most popular hydrogenolysis reactions use $Pd/C/H_2$, although the removal of other benzyl groups is possible to occur; most unpopular methods use strong Lewis's acids

although it is probable that cleavage of the glycoside bonds and other protecting groups occurs[68].

So, for the synthesis of this compound several possible routes to obtain the same product but with different configurations were tried.

The first route made to obtain this product was route A, using a mixture of *per* benzylated galactoses in the pyranose and furanose forms described in section 2.2.1 as starting material. In this route two different methods to obtain this product were tried.

In the first method the conditions for the hydrogenolysis of the anomeric position were Pd/C, MeOH as solvent and ammonium formate. But as mentioned above these conditions can sometimes hydrolyze other positions on the carbohydrate. The analysis of the ^{1}H NMR spectrum present in the annexes was very difficult because it was not possible to calibrate the spectrum with the signal of the deuterated solvent and it's not possible to identify the anomer signal and conclude how many protons are in the anomeric zone. But all the information leads to the deprotection of more than one benzyl group. Due to the lack of conclusion with this reaction another method was tested which consisted in an acidic glycoside hydrolysis.

For this reaction was used a mixture of the compunds **30** and **31** as starting material. It is believed that this reaction occur by a mechanism that proceeds *via* an intermediate oxicarbenium ion, generated by protonation and elimination of the benzyl group at the anomeric center[69]. In this procedure acetic acid and a 1M solution of sulphuric acid was used and a mixture of three products, two furanoses (**32, 33**) and one pyranose (**34**) (in a proportion showed in the Table 2.3) were identified in the ^{1}H NMR spectrum presented in the Appendix 2 - 2,3,4,6-tetra-O-benzyl-α-D-galactopyranoside (34), 2,3,4,6-tetra-O-benzyl-α-D-galactofuranoside (32) and 2,3,4,6-tetra-O-benzyl-β-D-galactofuranoside . These signals are represented with the integrals of the protons of the anomeric position. All the signals were consistent with the ones from the literature for both spectra[53],[70].

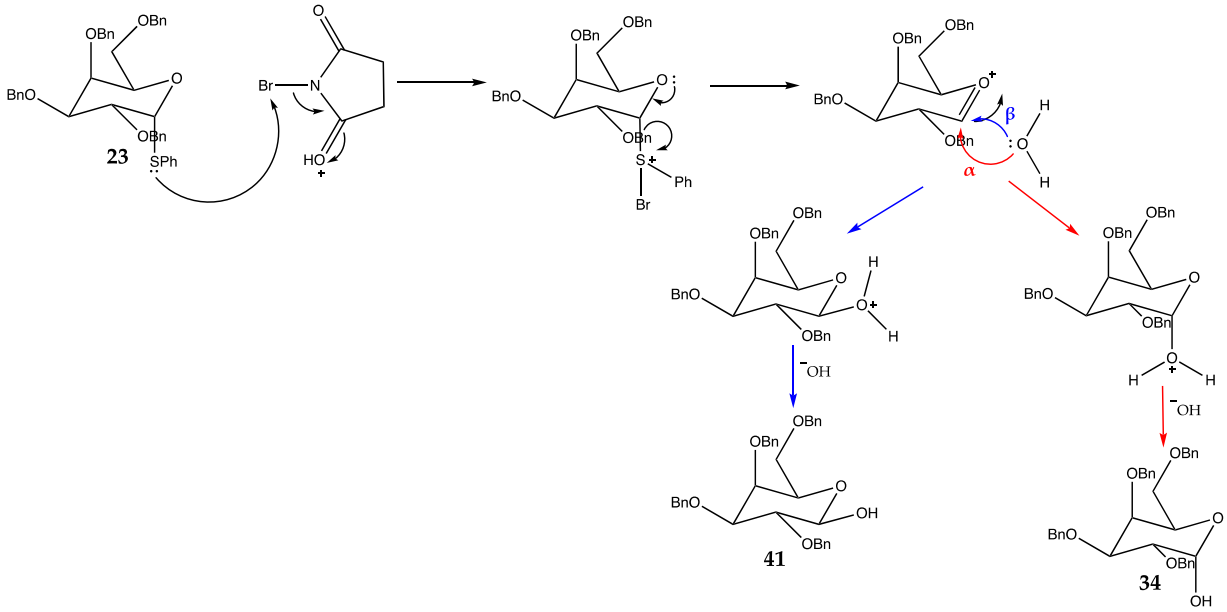
Table 2.3 - Proportion and chemical shift in the ^{1}H and ^{13}C spectrum of the obtained compounds.

	Proportion	^{1}H (ppm) – H1
β furanose (**33**)	1.00	5.47
β pyranose (**34**)	1.09	5.31
α furanose (**32**)	0.46	5.26

Path B was the second path tried and the starting material was the compound **40** obtained in the section 2.2.3. In this procedure again an acid glycoside hydrolysis was used although under different conditions. This time a mixture of 80% of acetic acid and 2M HCl in a ratio of 10:4 was used and the 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranoside (**34**) was obtained in 21.6 % yield[71]. The product was identified by comparation with the literature[53],[71].

Finally, in the route C the hydrolyses of the compound **23** obtained in the previous section was performed. The mechanism of this hydrolysis reaction is represented in the

Scheme 2.9. It starts with the activation of the anomeric sulfur by nucleophilic attack of sulfur on bromide with loss of succinimide. This converts the anomeric sulfide into a sulfonium leaving group since it is now positively charged. The loss of this anomeric sulfur produces a glycosyl cation, with can then be trapped by the water.[44]

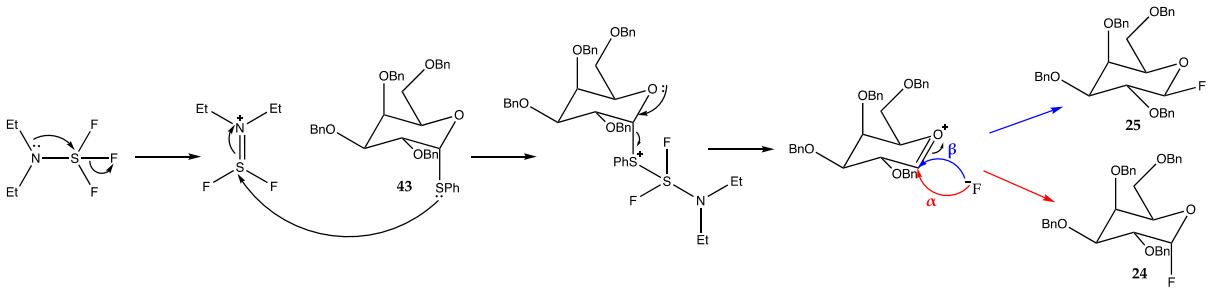


Scheme 2.9 - Mechanism of an hydrolysis of the anomeric position.

The isolated compound was 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranoside (**34**), with a yield of 61%. The presence of this compound was confirmed in the ^{1}H NMR spectrum due to the decrease of protons in the aromatic zone from 25 protons to 18, being consistent with the departure of the thiophenol group and to the singnal at 5.32 ppm referring to the H-1 proton in an β position due to the *J* coupling of 3.5 Hz. In the ^{13}C NMR spectrum the presence of the formed compound was confirmed with the lack of one signal in the quaternary carbon's zone, correspondent to the bond between the sulphur and the phenyl group. The rest of the signals were verified by the integrals of the protons in the ^{1}H NMR spectrum compared with the ones in the literature[53].

2.2.7. Synthesis of 2,3,4,6-tetra-*O*-benzyl-D-galactopynanoside fluoride (24, 25)

To finalize the sugar derivatization reactions, a fluorination of the anomeric position was performed. This reaction has the aim to test if the change of the reactivity of this position increases the yield of the reactions further described ahead in section 2.4. The first step of this mechanism is the activation of the thioglycoside group with the Vilsmeier-type electrophilic sulfinium cation species formed from diethylaminosulfur trifluoride (DAST). This change the anomeric sulfide into a sulfonium leaving group since it is now positively charged. The loss of this anomeric sulfur produces a glycosyl cation, with can then be trapped by the fluor (Scheme 2.10)[44].



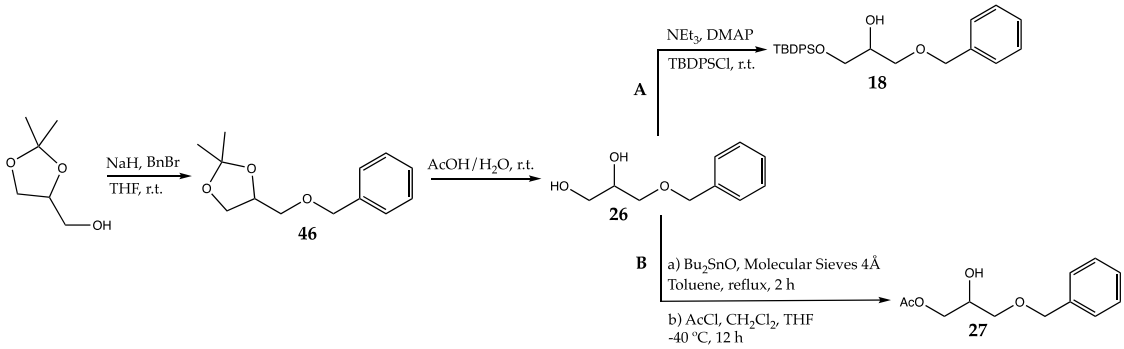
Scheme 2.10 - Mechanism of a fluorination reaction of the anomeric position.

In this reaction DAST and NBS are used simultaneously, and the yield of the reaction is 67%. This yield is good, but it could be better if instead of using NBS the reaction was performed in other conditions. When using NBS the generation of the unstable bromides leads to a troublesome purification of the desired fluorides because the bromogalactopyranosides are not converted to fluor-galactopyranosides *in situ* [72].In fact, glycosyl bromide was immediately generated in the absence of DAST as well and because of this the yield of the reaction is lower than the expected.

Nevertheless, it was possible to obtain and separate the two α (**24**) and β (**25**) anomers of this reaction, with a ratio of 1.00/1.00 and yield of 21% and 9% respectively. This can be verified by the integrals of the protons in the ^{1}H NMR spectrum, that are consistent with the ones from the literature. The final identification of these spectra are the anomeric protons and the coupling constants. For the 1-H α anomer the coupling constant is between an axial, equatorial position and because of that the *J* coupling is lower, in the 1-H β the coupling constant is between an axial, axial positions so the *J* is higher than the other one. The rest of the peaks were verified by the integrals of the protons in the ^{1}H NMR spectrum compared with the ones in the literature [73],[74].

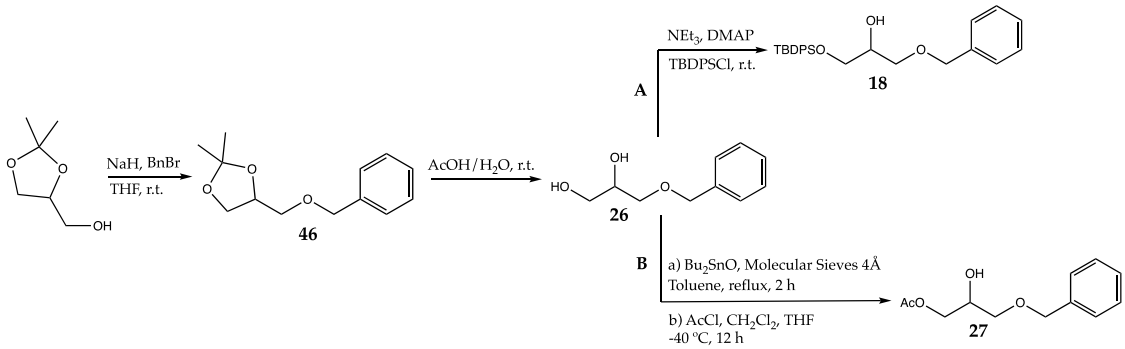
2.3. Reactions of derivatization of glycerol

As mentioned earlier the next step of this synthesis is the preparation of glycerol intermediates **18** and **26**. To accomplishes this the synthetic route presented in the



Scheme **2.11** was developed. These reactions were started with a commercial glycerol (*S*)-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol with the free primary hydroxy group being protected by reaction with benzyl bromide to obtain the product **51**. Then the opening of acetal occurs in acidic medium, followed by protection of the primary hydroxy group with

tert-butyldiphenylsilyl (route A) or acetate (route B). Route B was developed to increase the yields of the reaction and to reduce the steric hindrance in the secondary hydroxy group caused by the TBDPS group.



Scheme 2.11 - Synthetic approaches to the preparation of the glycerol derivatives.

2.3.1. Synthesis of 3-(benzyloxy)propane-1,2-diol (26)

The first two steps of this stage of the synthesis are the same for both routes. The first step consisted in the protection of the primary free hydroxy group with a benzyl group, the mechanism of this reaction is described in the section 2.2.1. In this step two different solvents were tried to reach to the desired product, presented in the Table 2.4.

Table 2.4 - Yields obtained for different reaction conditions.

Commercial glycerol	NaH (g)	BnBr (mL)	Solvent	η
500 mg	100	0.892	DMF (5 mL)	51%
500 mg	131	0.4	THF (3 mL)	91%

Usually in the etherification reactions with an S_N2 mechanism such as the Williamson reaction the solvents used are the aprotic and polar solvents that tend to increase the reaction rate as a result of raising the availability of the free nucleophile[55]. The two solvents used in this reaction (THF and DMF) are aprotic solvents and the big difference in the dielectric constant. Due do the higher dielectric constant of DMF it favours the reaction between the oxygen and the benzyl bromide.

The only justification for the results obtained in this reaction favoring the formation of the compound **46** in THF is the incompletion of the reaction with DMF caused by the difficulty to see de disappearance of the starting material when revealing the TLCs with potassium permanganate.

The formation of the product **46** was confirmed by ^{1}H NMR with the characteristic signals at 7.41-7.27 ppm in the aromatic zone referring to the phenyl group of the benzyl group and at 1.45 and 1.39 ppm in the aliphatic zone (two singlets referring to the methyl groups of the acetal group). In the ^{13}C NMR we were able to identify the characteristic signal of the quaternary carbon of the phenyl group at 138.1 ppm and the quaternary carbon of the

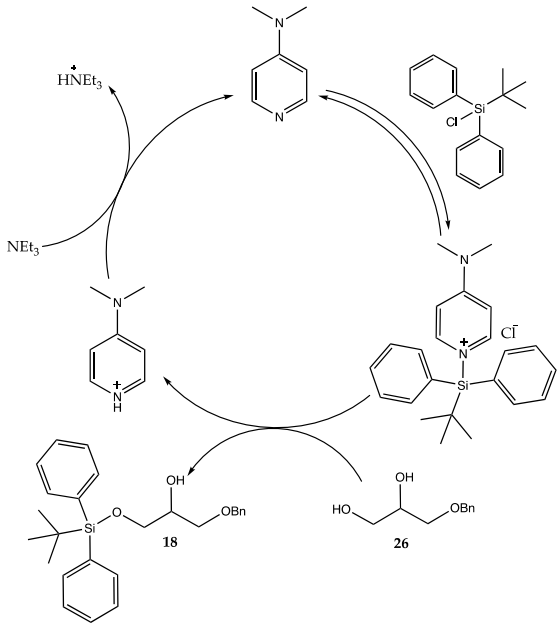
acetal group at 109.5 ppm. The identity of the compound was also confirmed, comparing the values obtained with the data described in the literature[75].

Then, a acidic hydrolysis reaction to open the acetal was performed. This hydrolysis occurred in an acidic medium of a mixture of AcOH/H_2O (4:1).

When the formation of the product **26** is seen by TLC and the starting material is consumed, the reaction in halted and the next reaction is performed without purification. This is made to increase the yields of the reactions.

2.3.2. Synthesis of 1-Benzyloxy-3-(*tert*-butyldiphenylsilyloxy)propan-2-ol (18)

Then, in path A the protection of the new primary free hydroxy group with a TBDPS group was performed. This protecting group is very good for the selective protection of primary alcohols due to their great stability under acidic and hydrogenolytic conditions. The protection reaction took place in basic medium, in the presence of triethylamine and 4-dimetilaminopiridina (DMAP), under catalytic conditions, and product was obtained with yield 33%[76]. In this type of reactions, the catalytic Lewis Bases such as DMAP are believed to react with silyl chlorides to form silyl pyridinium ion pairs, whose subsequent reaction with the alcohol substrate give the silyl ether products together with the protonated DMAP. To restore the catalyst the action of an auxiliary base such as triethylamine is required as represented in the Scheme 2.12[77].



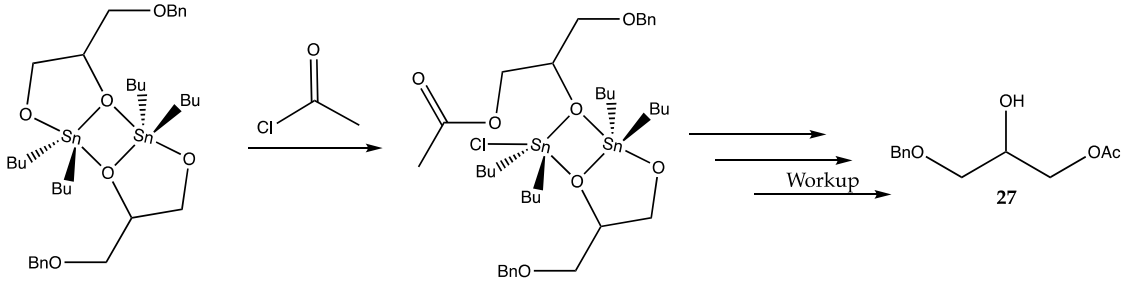
Scheme 2.12 - Mechanism of a silylation of the primary hydroxy from glycerol.

The presence of the product **18** was confirmed by ^{1}H NMR spectroscopy with the presence of the characteristic singlet at 1.04 ppm with integration to 9 protons characteristic of the methyl groups of the TBDPS group. The rest of the spectrum was confirmed by the comparation with literature.[78]

2.3.3. Synthesis of 3-(benzyloxy)-2-hydroxyprpyl acetate (27)

Due to the possible steric hindrance in the secondary hydroxy group caused by a large protecting group such as TBDPS used in the section above, an alternative route was created with the use of a smaller protecting group acetate.

This route B is divided in two steps made *in situ* without purification between them. The first step consists in the protection of the primary and secondary alcohols with dibutyltin oxide[79]. Dibutylstannylene acetals of terminal diols normally react preferentially on the primary oxygen atom. Technically one of the primary hydroxy is already protected with benzyl so, the other two hydroxy groups are free and there is a diol in the molecule. In the first step of this reaction the complex is form and then in the second step with the addition of the acetyl chloride occurs the reaction with the primary hydroxy since it is more accessible than the secondary hydroxy which is bound to two tin atoms (Scheme 2.13)[80],[81].

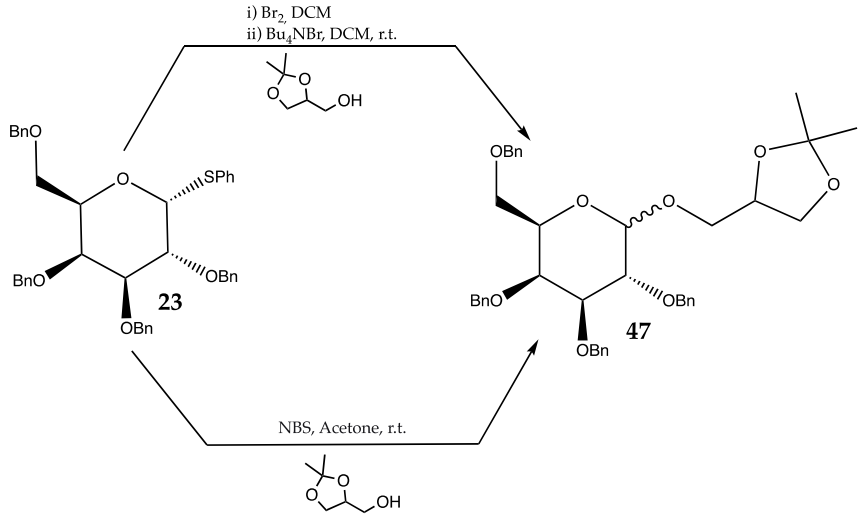


Scheme 2.13 - A proposed mechanism for the activation of tin intermediates by chloride.

The product 3-(benzyloxy)-2-hydroxyprpyl acetate (**27**) was obtained with a yield of 58%. The yield of this reaction is lower than the expected since the product with both hydroxy groups acetylated was formed as secondary product. The confirmation of the compound was made with ^{1}H NMR spectrum with the appearance of a singlet in at 2.1 ppm with integration to 3 protons characteristic of the methyl groups of the acetate. The rest of the spectrum was confirmed by the comparation with the spectrum from the literature.[79]

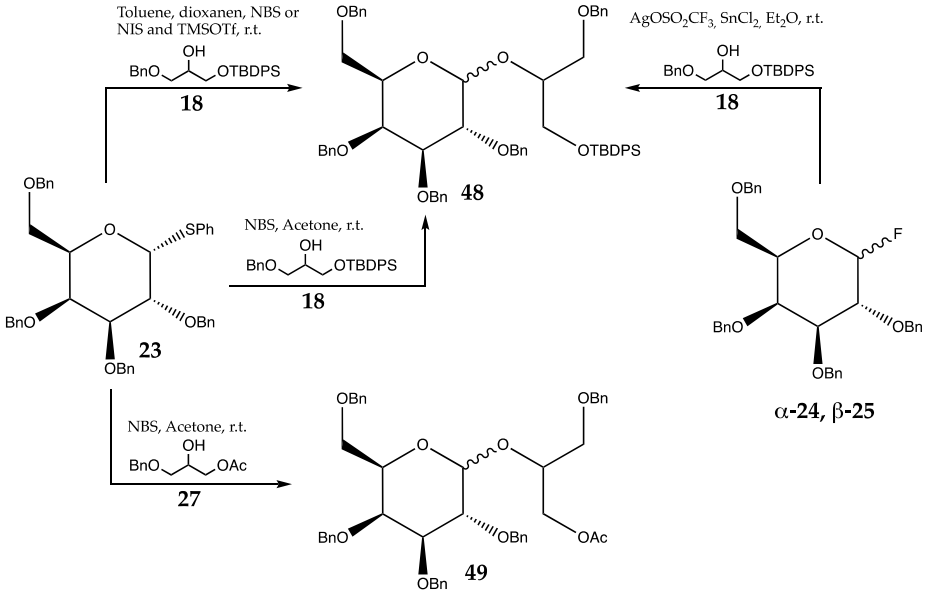
2.4. Coupling Reactions

In this section, several coupling reactions between galactose-derived sugar units and various glycerol derivatives are described. Firstly, to test various procedures for the coupling reaction between these derivatives, an assay was performed for the coupling between a commercial glycerol reagent, and a benzyl derivatized galactose with thiophenol in the anomeric position (Scheme 2.14).



Scheme 2.14 - Synthetic approaches to the preparation of the coupling reaction between the derivatized sugar and the commercial glycerol.

Then several reactions were made between the derivatized galactose, and the two different glycerol derivatives as represented in the Scheme 2.15. These reactions will be discussed in detail below.



Scheme 2.15 - Synthetic approaches to the preparation of the coupling reaction between the derivatized sugars and the derivatized glycerol's.

2.4.1. Synthesis of (2,2-dimethyl-1,3-dioxolan-4-yl)methyl-(2,3,4,6-tetra-*O*-benzyl)-galactopyranoside (47)

The first coupling reaction made in this project started with the phenyl-2,3,4,6-tetra-*O*-benzyl-1-thio-α-D-galactopynanoside (**23**) previously synthesized and the commercial (*S*)-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol. This first reaction had the objective to see if the coupling between the sugar with the thiophenol in the anomeric position and the free pri-

mary alcohol of the glycerol was able to occur. For the synthesis of this compound two different reactions were tried.

The first reaction was performed at room temperature and used NBS as the activator of the thioglycoside group and acetone as the solvent. The mechanism of this reaction is described in section 2.2.6. The obtained product was identified by mass spectroscopy, Appendix 17 - (2,2-dimethyl-1,3-dioxolan-4-yl)methyl-(2,3,4,6-tetra-O-benzyl)-galactopyranoside (47), with the molecular ion peak at 677.3 u. For the full characterization of this compound ^{1}H, ^{13}C, HMBC, HSQC and COSY spectra were made, although due to the low mass of this compound the spectra obtained were of poor quality preventing the characterization of the product. Thus, is still needed to optimize the reaction and to do a new characterization of compound **47**.

The second reaction for the synthesis of this compound was made in two steps without purification between them. In the first step of this reaction a substitution reaction in the anomeric position is supposed to occur, placing a bromine atom in this position. So, to accomplish this we used bromine and dichloromethane as solvent. This reaction starts with the activation of the anomeric sulfur by nucleophilic attack of sulfur on bromine with loss of bromide. This converts the anomeric sulfide into a sulfonium leaving group since it is now positively charged. The loss of this anomeric sulfur produces a glycosyl cation, with can then be trapped by the bromide.

The second step used tetrabutylammonium bromide and dichloromethane to place the commercial glycerol in the anomeric position[82]. ^{1}H NMR analysis of the several substances isolated (with very low mass) from this reaction showed that none of them corresponded to the desired product.

This can be due to the instability of the bromine atom in the anomeric position and the presence of water due to non-dried solvents or leaks in the systeam. Its possible that the presence of water combined with the instability of the bromine atom cause the hydrolysys producing a glycosyl cation, which can then be trapped by water forming a secondary product in this reaction.

2.4.2. Synthesis of 1-(benzyloxy)-3-((tert-butyldiphenylsilyl)oxy)propan-2-galactopyranoside (48, 49)

After confirming that the reaction between the sugar previously synthesized and the commercial glycerol was able to occur (although in low yield) the same reaction conditions were applied to the reaction between the sugars and the glycerol intermediate, 1-benzyloxy-3-(*tert*-butyldiphenyloxy) propan-2-ol (**18**), previously synthetized. This reaction is more difficult to perform since the secondary hydroxy group of the glycerol intermediate (**18**) is less reactive than the primary hydroxy group of commercial glycerol.

The first coupling reaction between these two compounds was made with phenyl-2,3,4,6-tetra-*O*-benzyl-1-thio-α-D-galactopynanoside (**23**) as the starting material. This reaction occurs according to the same mechanism as described in section 2.2.6. Two diferent activators for the thiphenol group were tried as presented in Table 2.5.

Table 2.5 - Yields obtained for different reaction conditions.

23 (mg)	Toluene (mL)	Dioxane (mL)	Activator (eq.)	**18** (mg)	η
54.00	2	6	NBS (2.11)	38.00	16.91%
74.60	2	5	NIS (1.04) and TMSOTf (1.74)	59.01	11.18%

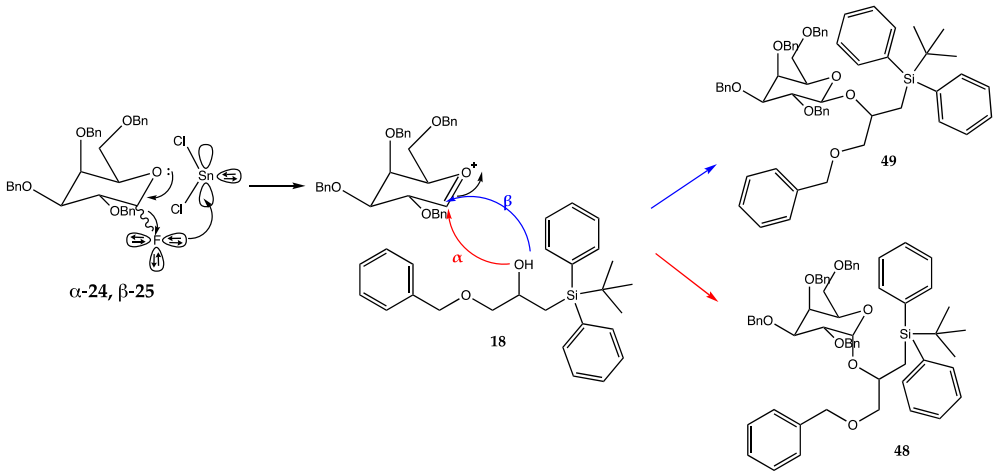
When using NBS as the activator of the thiophenol group several spots in the TLC were observed showing the formation of several products. The ^{1}H NMR spectra of the several substances isolated (with very low mass) from this reaction showed that none of them corresponded to the desired product.

When a mixure of NIS and TMSOTf several spots were also observerd in the TLC indicating the formation of several products but one of the spots was more intense than the others. In the ^{1}H NMR spectrum analysis it was possible to see that the characteristic singlet of the methyl groups of the TBDPS at 1.10 ppm was not present, and the aromatic zone just integrated to 25 protons instead of 35 protons. So, the mixture formed in this reaction was the compounds **50** and **51**.

Since the product with the TBDPS group in the glycerol was not isolated the second path was created. The procedure used in the hydrolysis of the thiophenol in the anomeric position described in section 2.2.6, was the same used for this reaction. In the first attempt of this reaction, we were able to see the formation of the expected product **48** or **49** by mass spectrometry, Appendix 18 - (1-(benzyloxy)-3-((tert-butyldiphenylsilyl)oxy)propan-2-galactopyranoside (48, 49). Although it was obtained in a mixture with the starting material (the peak at 650.2 u refers to the starting material and the one at 960.3 u to the product **48** or **49**).

Because of that, this reaction was made one more time. This time one product was isolated, but the TBDPS group was not present. In the analysis of the ^{1}H NMR spectrum it was possible to see again that the characteristic singlet of the methyl groups of the TBDPS at 1.10 ppm was not present, and the aromatic zone just integrate to 25 protons instead of 35 protons; comparing with the spectrum obtained from the previous reaction it was possible to conclude that they were in fact the same product (Appendix 19 - (1-(benzyloxy)-3-((tert-butyldiphenylsilyl)oxy)propan-2-galactopyranoside (52, 53)).

Then a third reaction was tried with another substrate, 2,3,4,6-tetra-*O*-benzyl-D-galactopynanoside fluoride (**24, 25**). The mechanism of this reaction is different of the previous reaction and is represented in the Scheme 2.16. In this glycosylation reaction, it is assumed that $SnCl_2$ behaves as a Lewis acid, where the vacant orbital accepts one of the three lone pairs in the fluorine atom of the glycosyl fluoride. As a result of this interaction, the C-F bond cleaves to give the glycosyl cation intermediate that is then attacked by an alcohol to give the glycoglycerol.



Scheme 2.16 - Mechanism of the coupling reaction between the compounds **24** and **25** and the compound **18**.

In this reaction the same mixture was isolated as before. When comparing the ^{1}H NMR spectrum of the two reactions with the different starting materials it was possible to see that the characteristic singlet of the methyl groups of the TBDPS at 1.10 ppm was not present, and the aromatic zone just integrate to 25 protons instead of 35 protons, as showed in the Appendix 19 - (1-(benzyloxy)-3-((tert-butyldiphenylsilyl)oxy)propan-2-galactopyranoside (52, 53) . Finally, and for this reaction it was possible to proceed with the identification of the product resorting to ^{13}C, HSQC, HMBC, and COSY spectrums.

When we started to analyze the spectrums obtained from this mixture was possible to see the formation of the two anomers α and β in the HSQC spectrum. In the Figure 2.1 are indicated the two anomers and due to the *J* we were able to conclude that the signal at 5.31 ppm was from the α anomer due the *J* of 3.7Hz and the signal at 4.69 ppm was from the β anomer due to the *J* of 7.4 Hz . The presence of the two anomers in the spectrum makes it impossible to identify the remaining protons of the molecules.

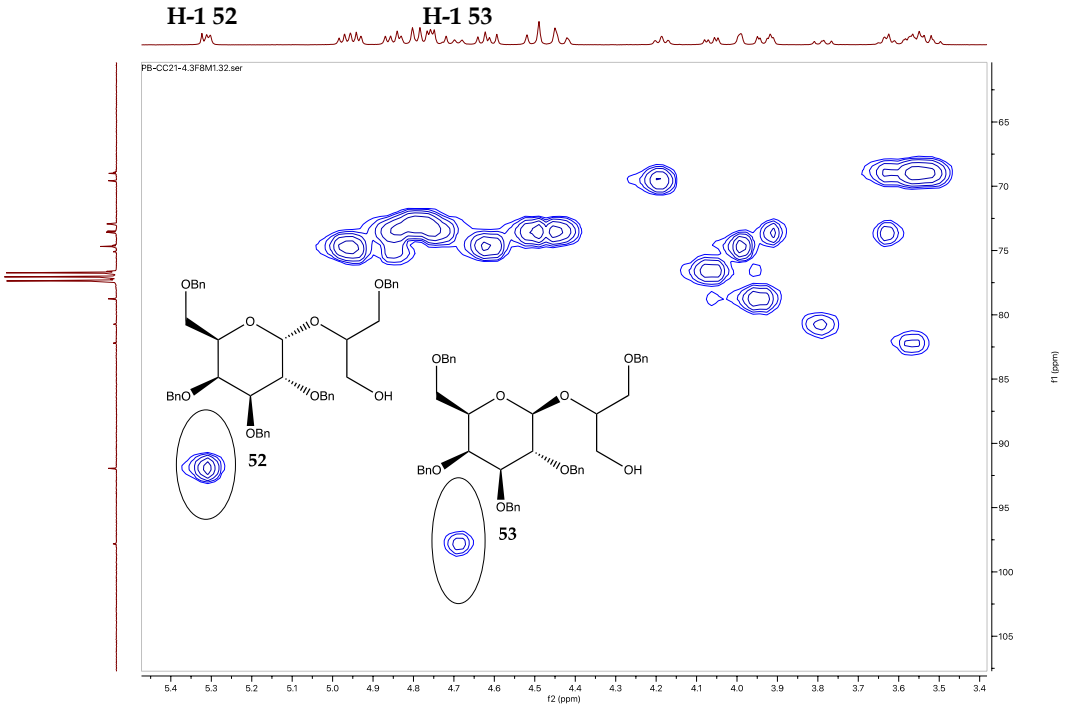


Figure 2.2 - HSQC spectrum with the identification of the α and β anomers of the compounds **52** and **53.**

It is possible that the use of too large protecting group, such as TBDPS, may cause a steric hindrance of the secondary alcohol, causing the formation of secondary products and lowering the yields of these type of reactions. Also, the conditions of the reactions are causing the deprotection of the TBDPS group. Due to the comparation between the yields of the reaction we can conclude that the galactose derivative with a thiophenol at the anomeric position is a better substract for these reactions.

2.4.3. Synthesis of (1-acetoxy-3-benzyloxy-2-propan)-2,3,4,6-tetra-*O*-benzylgalactopyranoside (50, 51)

As mentioned in the previous section the disappearance of the TBDPS group in the ^{1}H NMR of the obtained products led to the synthesis of a new glycerol intermediate with acetate as a protecting group instead of TBDPS. This new glycerol was used in a coupling reaction with the sugar derivative with thiophenol in the anomeric position. This reaction was carried out with in the same condition of hydrolysis of the anomeric position with NBS and acetone.

In this reaction it was possible to see again the formation of several secondary products in the TLC when the reaction was finished. The more intense spot in the TLC was analyzed with ^{1}H NMR spectroscopy and in a preliminary analysis it is possible to see the characteristic signal of the methyl group of the acetate at 1.99 ppm. This allowed us to conclude that the acetate was not hydrolyzed to a hydroxy group, like the TBDPS was in the previous reactions. But to verify that the product obtain was the [1-(acetyl)-2-(4-methoxy-benzyloxy)-

ethyl] [tetra-*O*-benzyl-D-galactopyranoside] (**50** or **51**), we performed the analyses of the ^{13}C, HSQC, HMBC, and COSY spectra from the obtained mass.

When we started to analyze the spectrums obtained from this mixture it was possible to conclude that the formation of the two anomers α and β had occurred. In HSQC spectrum present in the Figure 2.1 are indicated the two anomers and due to the *J* we were able to conclude that the signal at 4.57 ppm was from the β anomer due the *J* of 7.6Hz and the broad singlet at 5.20 ppm was from the α anomer . The presence of the two anomers in the spectrum makes it impossible to identify the remaining protons of the molecules.

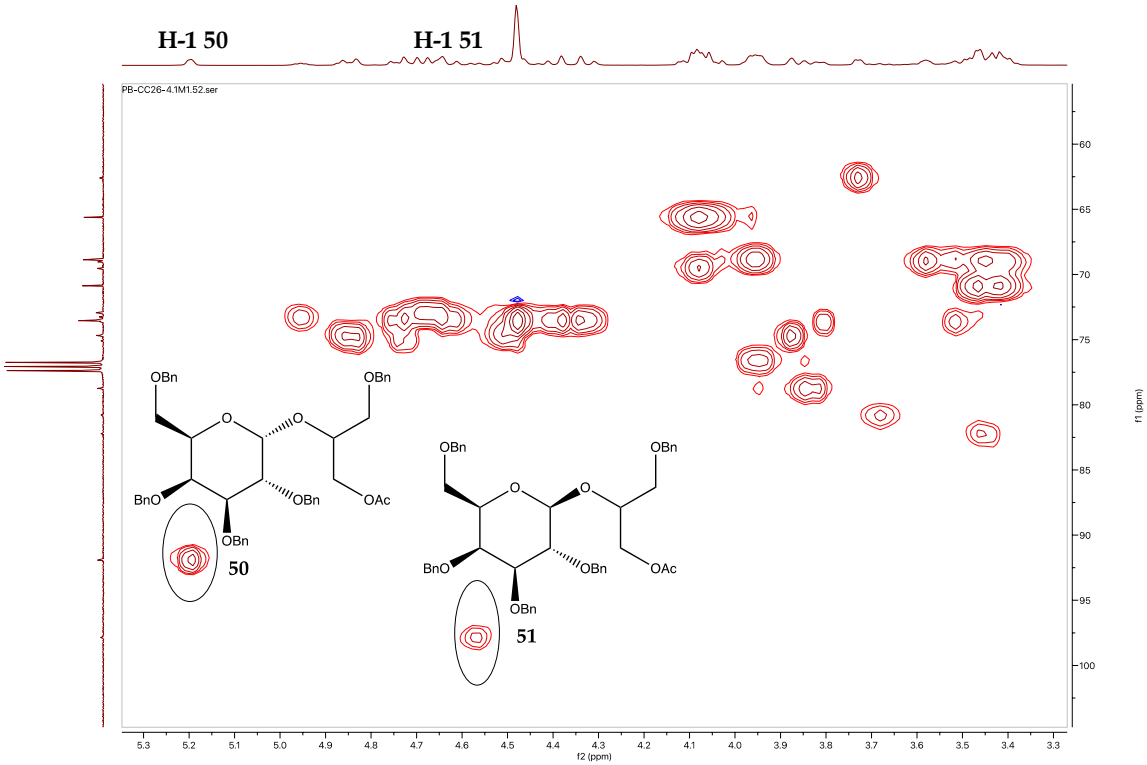


Figure 2.3 - HSQC spectrum with the identification of the α and β anomers of the compounds **50** and **51**.

The characterization of the product enabled us to conclude that this was in fact a mixture of the product **50** and **51**, with a yield of 29.21%. So, it's possible to conclude that the TBDPS group is not the best protecting group for the primary hydroxy of the glycerol since it's not stable during the reaction. Although this reaction was successful it still needs optimization to minimize the formation of secondary products.

3.
Conclusion

The work described in this thesis was aimed at the preparation of a phosphoglycoglycerol compound. This can be divided into the synthesis of three fragments, a D-(+)-galactose derivative, a glycerol derivative and a phosphonamidite or ethanolamine and then a subsequent coupling reaction between this three compounds.

For the synthesis of the D-(+)-galactose derivative were tried three different paths. In one case, benzylation of the D-(+)-galactose unite in basic medium followed by hydrolysis and acetylation of the anomeric position led to the furanose **34** as the major product. Due to this result an alternative route involving methylation of the anomeric position of the D-(+)-galactose followed by benzylation of the remaining positions and hydrolysis of the anomeric position was developed and compound **40**, was isolated with a very low yield. So, the best result for this synthesis was obtained starting with acetobromo-α-D-galactose and a substitution reaction at the anomeric position in order to place the thiophenol followed by the hydrolysis and benzylation of the remaining hydroxy groups of galactose. We have also tried a fluorination reaction in the anomeric position to see if a halogenated group in the anomeric position would be better in the coupling reaction, the bromide was not used due to it instability of this group in the anomeric position.

As expected the used of no participating groups in the protection of the D(+)-galactose lead to the formation of the α anomer as the prefer product.

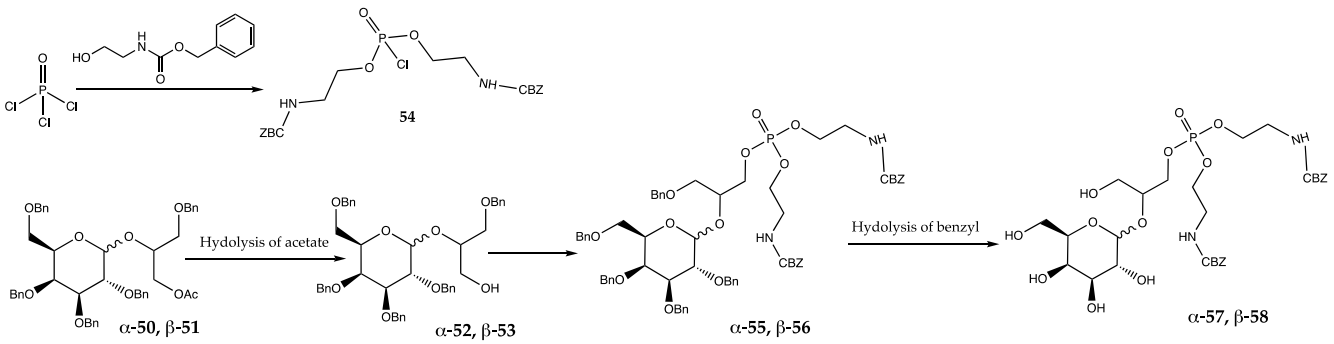
The glycerol intermediates synthesized were obtained by a benzylation reaction of the commercial glycerol ((*S*)-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol). Followed by the hydrolysis of the cetal group and protection of the remaining primary hydroxy group. In one case with an acetyl group or a large protecting group, TBDPS. This allowed us to teste the steric hindrance of the secondary hydroxy in the coupling reactions with the compound **23.**

Then the last step of this work concentrated in the coupling reactions between the sugar derivative and the glycerol intermediates. The reaction of compound **23** and a commercial glycerol allowed us to set the conditions to be applied in the coupling reactions. We were able to ensure that this reaction occurs, although the product was obtained with a low yield, which prevented us to proceed to a optimization of the reaction and a characterization of the compound. After this confirmation the same method was tried involving the com-

pound **18** and the sugar derivative **23, 24 and 25.** The low yield of the product **48** or **49** in this reaction wasn't a surprise since the reaction is more difficult to occur due to the secondary hydroxy group of the glycerol intermediate that is less reactive than the primary hydroxy group. In the several conditions used to try to perform this reaction again the hydrolysis of the TBDPS group occurred and only the products **52** and **53** were obtained in a mixture.

The best results were obtained with the coupling reaction preformed with the glycerol intermediate **26** and the sugar **23** with the obtention of the mixture of compounds **50** and **51** obtained in 29.21% yield**.** For that accounts the less lability and steric constrains of the acetate group on the glycerol in relation to the TBDPS protecting group. Despite of this in a future work is still needed an optimization of the reaction to minimize the formation of secondary products.

The next step in this project will be the optimization of the syntheses of the ethanolamine group and the coupling of these compounds and the compound **54**, followed by the hydrolysis of the benzyl groups and obtention of the final product represented in the Scheme 3.1.



Scheme 3.1 - Reaction for the obtention of the phosphoglycoglycerol.

4.
Experimental procedure

4.1. General information

The experimental part of this work involved the use of general laboratory procedures. All reagents, solvents and eluents used were purchased from Sigma-Aldrich, LaborSpirit, Alfa Aesar, Honeywell Riedel-de Häen, and were used without further purification, except when their dry use is mentioned. Solvent drying was performed using 3Å molecular sieves, previously activated in microwaves, for periods of 1 minute, interspersed with stirring, for a total of 10 minutes. After cooling the molecular sieves for 2 hours in vacuo and adding the respective solvent, the solvent was left to dry for at least 24 hours until its use. For weighing compounds, two scales, CP225D (Sartorious) and Analytical Plus, were used. The evolution of all reactions was followed by thin layer chromatography (TLC), using silica gel plates 60 F254 with 0.2 mm thickness on an aluminum support (Merck 5554). Preparative TLC was performed using Merk silica gel 60 (Merck 105744 and Merck 113895) on a glass surface with the described eluent for each case. TLC was revealed first using an ultraviolet lamp (λ = 254 nm), and subsequently using various chemical developers, such as potassium permanganate, sulfuric acid/ methanol (1:1) and phosphomolybdic acid reagent.[83]

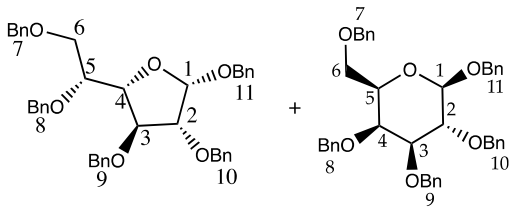
Some reaction steps involved a purification process on a normal phase flash chromatography column. For this purpose, 0.040-0.063 mm (230 - 400 mesh) silica gel (Merck 109385) and a compressed air stream was used on a column with a silica height between 15-20 cm. The column diameter was chosen according to the total sample mass to be separated. Characterization of the compounds was performed by Infrared spectroscopy, ^{1}H and ^{13}C *Nuclear magnetic resonance spectroscopy*, and in some cases mass spectroscopy (ESI-MS).

In nuclear magnetic resonance (NMR) analysis, proton (^{1}H) and carbon (^{13}C) spectra were acquired with a Bruker ARX400 spectrometer or Bruker Avance III 400 spectrometers at 400 MHz and 101 MHz for ^{1}H and ^{13}C respectively. The samples were prepared on 5 or 3 mm NMR tubes with the correspondent solvent. The values of the coupling constants, *J*, are given in Hz. The data are presented as follows, for ^{1}H-MRN spectra: deuterated solvent used, chemical shift (ppm), multiplicity, coupling constant, *J*, relative intensity, for ^{13}C-MRN spectra: solvent and chemical shift. The reference used is the solvent signal.

Infrared (IR) spectra were performed on a Perkin Elmer Spectrum 1000 FT-IR spectrophotometer acquired in the range 4000 cm $^{-1}$ to 400 cm $^{-1}$ with 4 scans. In the description only the most relevant frequencies are presented, and the data is given in the following order: sample holder, maximum absorption frequency (ν_{max} in cm^{-1}) and assignment of the functional group using ν for stretching and δ for bending.

4.2. Galactose derivatization reactions

4.2.1. Synthesis of penta-*O*-benzyl-D-galactopyranoside (31)



30 **31**

A solution of D-(+)-galactose (503.7 mg, 2.80 mmol) in dry dimethylformamide (10 mL) was cooled to 0°C, in an ice-water bath. Then, a base (15 eq.) was slowly added to the medium. After stirring for 15 min at room temperature under nitrogen, 4.78 mL (4.78 g, 28 mmol, 10 eq.) of benzyl bromide were added dropwise. The reaction was left stirring at room temperature for four days. Upon completion of the reaction verified by the observation of TLC (n-hexane/EtOAc - 9/1), water was added to the mixture, followed by extraction with (3x20mL) of ethyl acetate. The combined organic phases were washed with water, dried over Na_2SO_4, and after filtration and evaporation to dryness, the residue was purified by column chromatography on silica flash using a mixture of n-hexane/ ethyl acetate - 9/1 to give 0.801 mg (1.27 mmol, 45.5% yield) of a mixture containing penta-*O*-benzyl-β-D-galactopyranoside (**31**) and penta-*O*-benzyl-α-D-galactofuranoside (**30**) as a dark yellow oil.

Table 4.1 - Yields obtained for different reaction conditions.

Entry	Base	Temperature	Yield
1	NaH	no	45,5%
2	anhydrous pyridine	no	-[a]
3	anhydrous pyridine	80° C	-[a]

a – unidentified product

ν_{max} (cm^{-1}): 3087-3029 (ν C-H Ar); 2901-2870 (ν C-H aliphatic); 1498-1401 (ν C=C Ar); 1103-1026 (ν C-O); 744-693 (δ C-H Ar).

^{1}H NMR (500 MHz, Chloroform-*d*) δ: 7.43 – 7.26 (m, 47H), 4.99 (d, *J* = 12.2 Hz, 2H), 4.96 (d, *J* = 5.6 Hz, 1H), 4.83 – 4.50 (m, 15H), 4.49 – 4.44 (m, 1H), 4.40 (t, *J* = 7.3 Hz, 1H), 4.09 (dd, *J* = 7.7, 4.4 Hz, 1H), 4.05 (t, *J* = 6.5 Hz, 1H), 3.96 – 3.91 (m, 1H), 3.78 (q, *J* = 5.6 Hz, 1H), 3.74 – 3.62 (m, 3H), 3.60 – 3.54 (m, 1H).

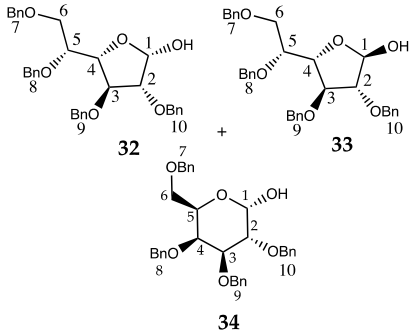
^{13}C NMR (101 MHz, Chloroform-*d*) δ: 138.8, 138.7, 138.6, 138.5, 138.2, 137.9, 137.6, 128.5 – 127.3, 102.8, 98.2, 84.1, 82.3, 80.9, 80.5, 79.6, 79.3, 75.2, 74.5, 73.6 – 73.5, 73.3, 73.1, 70.5, 68.9, 68.6.

4.2.2. Synthesis of 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranoside (34)

Method A:

A solution of 74.2 mg (0.118 mmol) of a mixture of penta-*O*-benzyl-β-D-galactopyranoside (**31**) and penta-*O*-benzyl-α-D-galactofuranoside (**30**) in dry methanol (4.68 mL) was stirred for 10 min, under nitrogen. Addition of 114 mg (1.07 mmol) of Pd/C was followed by the addition of 119 mg (1.92 mmol, 5 eq.) of ammonium formate and the mixture was stirred at room temperature for four days. Upon completion of the reaction, verified by TLC (n-hexane/EtOAc– 8/2), the mixture was filtered through a pad of celite, and the residue was washed with methanol (2x5 mL) and then dichloromethane (2x10 mL). The filtrate and washings were combined, and the solvents were evaporated to dryness, yielding 0.14 g of crude. The product wasn't obtained in this reaction.

Method B:



A solution of the mixture containing penta-*O*-benzyl-β-D-galactopyranoside (**31**) and penta-*O*-benzyl-α-D-galactofuranoside (**30**) (8.06 g) in acetic acid (98.06 mL, 1 eq.) was stirred for 5 min under nitrogen on a round-bottom flask. Then 24.31 ml (24.31 mmol, 1 eq.) of a 1M sulphuric acid solution were carefully added to the reaction medium. The reaction was refluxed for 5 h, until completion, verified by TLC (n-hexane/EtOAc - 8/2). The solution was cooled and a 1M NaOH solution was added dropwise until a value of pH 4 was observed. The acetic acid was evaporated under vacuum and the crude was extracted with ethyl acetate (3 x 20 mL). The combined organic phases were washed with satd. aq. $NaHCO_3$, dried over $MgSO_4$, and after filtration and evaporation to dryness, the residue was purified by column chromatography on silica flash using a mixture of n-hexane/ethyl acetate - 9/1 to give a mixture of 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranoside (**34**), 2,3,4,6-tetra-*O*-benzyl-α-D-galactofuranoside (**32**), 2,3,4,6-tetra-*O*-benzyl-β-D-galactofuranoside (**33**) as a colorless oil (0.2392 mg, 0.442 mmol,15.6% yield).

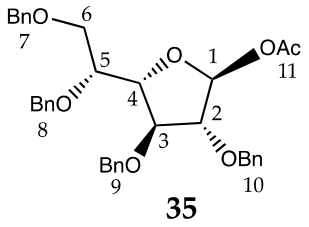
ν_{max} (cm^{-1}): 3420 (ν O-H); 3087-3033 (ν C-H Ar); 2913-2867 (ν C-H aliphatic); 1494-1451 (ν C=C Ar); 1092-945 (ν C-O); 732-693 (δ C-H Ar).

^{1}H NMR (500 MHz, Acetonitrile-d_3) δ: 7.42 – 7.27 (m, 33H), 5.47 (s, 1H), 5.31 (d, *J* = 3.9 Hz, 1H), 4.79 (d, *J* = 11.2 Hz, 0H), 4.72 (d, *J* = 11.5 Hz, 1H), 4.66 – 4.61 (m, 1H), 4.60 – 4.54 (m, 4H),

4.54 – 4.48 (m, 5H), 4.46 (d, *J* = 10.0 Hz, 1H), 4.42 (s, 0H), 4.14 – 4.06 (m, 1H), 4.03 – 3.98 (m, 2H), 3.93 (dd, *J* = 2.8, 1.4 Hz, 1H), 3.76 – 3.65 (m, 5H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ: 138.1, 137.9, 137.8, 137.6, 137.4, 133.7, 130.3, 129.0 – 127.6, 101.1, 96.4, 87.2, 84.7, 83.3, 82.3, 82.2, 81.7, 73.8, 73.7, 73.5, 73.4, 72.1, 72.0, 71.8, 70.9, 70.8.

4.2.3. Synthesis of acetil-2,3,4,6-tetra-*O*-benzyl-D-galactopyranoside (43)



35

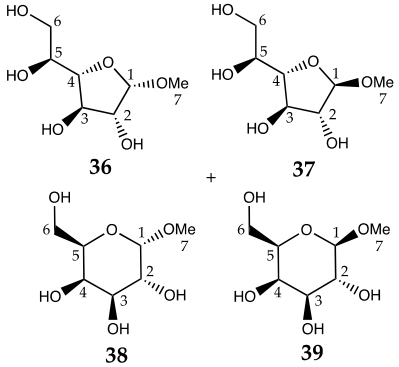
To a solution of a mixture of 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranoside (**34**), 2,3,4,6-tetra-*O*-benzyl-α-D-galactofuranoside (**32**), 2,3,4,6-tetra-*O*-benzyl-β-D-galactofuranoside (**33**) (238.5 mg) in dry DCM (2.75 ml), 0.1 ml of anhydrous pyridine (104.68 mg, 1.32 mmol, 3 eq.) were carefully added, followed by 0.15 ml (135.1o mg, 1.32 mmol, 3 eq.) of acetic anhydride. After stirring for 5 h at room temperature, the completion of the reaction was verified by TLC (n-hexane/EtOAc - 8/2) and satd. aq. $NaHCO_3$ was added. The crude was extracted with DCM and 1M HCl several times. The combined organic phases were washed with water. After drying over Na_2SO_4, and filtration and evaporation to dryness, the residue was purified by column chromatography on silica flash using a mixture of hexane/ethyl acetate - 9/1 to give 1-acetil-2,3,4,6-tetra-*O*-benzyl-β-D-galactofuranoside (**35**) as a colorless oil, yielding 0.2847 g of crude.

ν_{max} (cm^{-1}): 3087-3029 (ν C-H Ar); 2913-2867 (ν C-H aliphatic); 1498-1401 (ν C=C Ar); 1103-1026 (ν O-C); 732-697 (δ C-H Ar)

^{1}H NMR (400 MHz, Chloroform-*d*) δ: 7.44 – 7.28 (m, 20H), 6.31 (s, 1H), 4.83 – 4.32 (m, 10H), 4.14 – 4.06 (m, 2H), 3.85 (q, *J* = 5.2 Hz, 1H), 3.73 (d, *J* = 5.5 Hz, 2H), 2.13 (s, 3H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ: 170.1, 138.4-137.4, 128.8 – 127.5, 100.5, 87.2, 84.1, 83.1, 76.6, 73.4 -71.9, 70.6, 21.3.

4.2.4. Synthesis of Methyl D-galactopyranoside (38, 39)



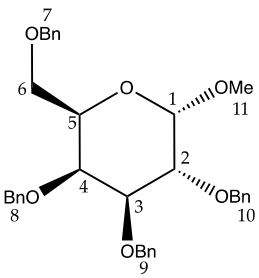
Amberlyst resin (118.8 mg, 0.377 mmol) was added, to a solution of D-(+)-galactose (510.1 mg, 2.83 mmol) in methanol (7.7 ml), This reaction mixture was kept at reflux for 20 h. TLC (DCM:MeOH: H_2O - 13:7:1 was used to ensure that the reaction was completed. The reaction was brought to room temperature, the resin was separated through filtration and the filtrate was concentrated to give a mixture of Methyl α-D-galactopyranoside (**38**), Methyl β-D-galactopyranoside (**39**), Methyl α-D-galactfuranoside (**36**), Methyl β-D-

galactofuranoside (**37**) as a yellow oil yielding 700 mg of crude, used without further purification.

^{1}H NMR (500 MHz, Deuterium Oxide): δ 4.90 (d, *J* = 1.9 Hz, 3H), 4.89 – 4.87 (m, 1H), 4.83 (d, *J* = 3.0 Hz, 3H), 4.31 (d, *J* = 7.9 Hz, 1H), 4.12 (dd, *J* = 4.6, 1.7 Hz, 2H), 4.09 – 4.02 (m, 8H), 3.98 – 3.93 (m, 6H), 3.93 – 3.87 (m, 4H), 3.85 – 3.68 (m, 20H), 3.67 – 3.62 (m, 4H), 3.52 – 3.47 (m, 1H), 3.42 (d, *J* = 9.8 Hz, 6H).

^{13}C NMR (101 MHz, Deuterium Oxide) δ: 108.1, 103.8, 102.0, 82.9, 80.7, 76.6, 70.9, 70.7, 62.7, 62.2, 61.0, 55.3, 54.9

4.2.5. Synthesis of Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranoside (40)



40

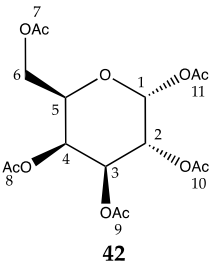
To a solution of 559.9 mg of a mixture of 1-methyl-α-D-galactopyranoside (**38**), 1-methyl-β-D-galactopyranoside (**39**), 1-methyl-α-D-galactfuranoside (**36**), 1-methyl-β-D-galactofuranoside (**37**) and 8 ml of dry dimethylformamide in an ice-water bath, sodium hydride (1.7456 g, 72.73 mmol, 15 eq.) was slowly added to the medium. After stirring for 15 min at room temperature under nitrogen, 3.42 ml (4.93 g, 28.82 mmol, 10 eq.) of benzyl bromide were added dropwise. The reaction was left stirring at room temperature for four days. Upon completion of the reaction verified by the observation of TLC (n-hexane/EtOAc - 9/1), the crude was dissolved in water and washed with (3x20mL) ethyl acetate. The combined organic phases were extracted with water, dried over Na_2SO_4 and, after filtration and evaporation to dryness, the residue was purified by column chromatography on silica flash using a mixture of n-hexane/EtOAc - 9/1) to give 162.9 mg (0.294 mmol, 10.2% yield) Methyl 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranoside (**40**) as a yellow oil.

ν_{max} (cm^{-1}): 3088-3030 (ν C-H Ar); 2906 (ν C-H aliphatic); 1497-1454 (ν C=C Ar); 1099-1049 (ν O-C); 736-701 (δ C-H Ar).

^{1}H NMR (500 MHz, Chloroform-*d*) δ: 7.43 – 7.25 (m, 20H), 4.97 (d, *J* = 11.4 Hz, 1H), 4.86 (dd, *J* = 11.9, 7.6 Hz, 2H), 4.78 – 4.69 (m, 3H), 4.60 (d, *J* = 11.5 Hz, 1H), 4.51 (d, *J* = 11.8 Hz, 1H), 4.42 (d, *J* = 11.8 Hz, 1H), 4.09 – 4.04 (m, 1H), 3.99 – 3.90 (m, 3H), 3.55 (d, *J* = 6.4 Hz, 2H), 3.40 (s, 3H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ : 138.5-137.7, 128.6 – 126.6, 107.0, 82.7, 72.1, 71.8, 70.9, 54.8.

4.2.6. Synthesis of penta-*O*-acetyl-α-D-galactopyranoside (42)



42

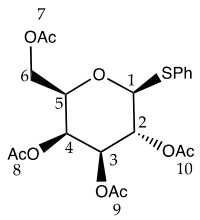
To a solution of D-(+)-galactose (0.5058 mg, 2.81 mmol, 1.00 eq.) with anhydrous pyridine (9.34 mL) in an ice-water bath, acetic anhydride (2.65 mL, 2.86 d, 28.04 mmol, 10.00 eq.) was added slowly with stirring. The reaction was allowed to warm to room temperature overnight. After the completion of the reaction was verified by TLC (n-hexane/EtOAc - 7/3), the crude was diluted in ethyl acetate and washed with 1M HCl (3x30 mL). The combined organic phases were washed with satd. aq. $NaHCO_3$ followed by water. The organic phase was dried over Na_2SO_4, and after filtration and evaporation to dryness, the residue was purified by column chromatography on silica flash using a mixture of n-hexane/EtOAc - 8/2 to give 499.2 mg (1.28 mmol, 45.7% yield) of penta-*O*-acetyl-α-D-galactopyranoside (**42**) as a colorless oil.

ν_{max} (cm^{-1}): 1746 (v C=O), 1369 (δ C-H aliphatic), 1206 (v C-O)

^{1}H NMR (400 MHz, Chloroform-*d*) δ: 6.37 (s, 1H), 5.49 (s, 1H), 5.32 (d, *J* = 2.4 Hz, 2H), 4.33 (t, *J* = 6.8 Hz, 1H), 4.14 – 4.03 (m, 3H), 2.15 (s, 6H), 2.03 (s, 3H), 2.01 (s, *J* = 1.6 Hz, 3H), 1.99 (s, *J* = 1.7 Hz, 3H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ: 170.4, 170.1, 169.9, 168.9, 89.7, 68.8, 67.4, 66.5, 61.3, 20.9, 20.6, 20.6.

4.2.7. Synthesis of phenyl-2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopynanoside (22)



22

To a solution of penta-*O*-acetyl-α-D-galactopyranoside (**42**) (102.8 mg, 1.28 mmol, 1 eq.) in DCM (0.3 M) and thiophenol (2.79 mL, 0.0281 mmol, 1.2 eq.), $BF_3{\cdot}Et_2O$ (2.71 mL, 0.0216 mmol, 1.5 eq.) were slowly added at 0° C under nitrogen. Then the reaction was left stirring at room temperature for 8 h. After completion of the reaction, verified by TLC (n-hexane/EtOAc - 7/3), the mixture was quenched with saturated aqueous NH_4Cl and washed with 0.3 N NaOH. The combined organic layers were dried with Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by column chromatography on silica flash using a mixture of n-hexane/EtOAc- 7/3 to give 87.5 mg (0.199 mmol, 19.6% yield) of 2,3,4,6-tetra-*O*-acetyl-1-thiophenol-β-D-galactopynanoside (**22**) as a colorless oil.

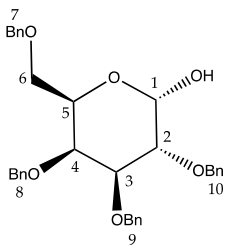
ν_{max} (cm^{-1}): 1746 (v C=O), 1368 (v C-H aliphatic), 1212 (δ O-C)

^{1}H NMR (500 MHz, Chloroform-*d*) δ: 7.54 - 7.30 (m, 5H), 5.44 (dd, *J* = 3.4, 1.1 Hz, 1H, H-4), 5.26 (t, *J* = 10.0 Hz, 1H, H-2), 5.07 (dd, *J* = 10.0, 3.3 Hz, 1H, H-3), 4.74 (d, *J* = 10.0 Hz, 1H, H-1

b), 4.25 – 4.12 (m, 2H, H6), 3.99 – 3.93 (m, 1H, H-5), 2.14 (s, 3H, $COCH_3$), 2.12 (s, 3H, $COCH_3$), 2.07 (s, 4H. $COCH_3$), 2.00 (s, 3H, $COCH_3$).

^{13}C NMR (101 MHz, Chloroform-*d*) δ: 170.4, 170.1, 169.9, 168.9, 89.7, 68.8, 67.4, 66.5, 61.3, 20.9, 20.6, 20.6.

4.2.8. Synthesis of 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranoside (34)



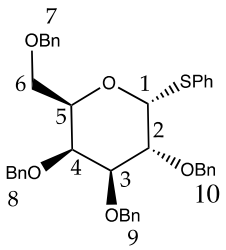
34

119 mg (0.215 mmol, 1.00 eq.) of 1-methyl-2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranoside (**40**) were dissolved in a solution of 80% acetic acid/ 2 M HCl (10/4, 5.35 mL per 119mg) and the reaction was kept at reflux for 11 h. The mixture was allowed to reach room temperature after the reaction was completed. Once again, that was assured by TLC (n-hexane/EtOAc- 7/3). Then the crude was dissolved in dichloromethane (2.98 mL, per 119 mg) and washed with satd. aq. $NaHCO_3$ (2x2.98 mL, per 119 mg). The combined organic layers were dried with Na_2SO_4, filtered, and evaporated to dryness to obtain 25 mg of compound **34** as a colorless oil.

ν_{max} (cm^{-1}): 3412 (ν O-H), 3087-3029 (ν C-H Ar); 2917-2867 (ν C-H aliphatic); 1498-1451 (ν C=C Ar); 1088-1026 (ν C-O); 736-693 (δ C-H Ar)

^{1}H NMR (500 MHz, Chloroform-*d*) δ: 7.41 – 7.26 (m, 50H), 5.30 (d, *J* = 3.6 Hz, 1H), 5.12 (s, 1H), 4.97 – 4.91 (m, 2H), 4.85 – 4.79 (m, 2H), 4.76 – 4.66 (m, 5H), 4.63 – 4.46 (m, 5H), 4.41 (dd, *J* = 11.9, 5.1 Hz, 2H), 4.19 (t, *J* = 6.3 Hz, 1H), 4.07 – 4.03 (m, 1H), 3.97 – 3.93 (m, 2H), 3.62 – 3.44 (m, 4H).

4.2.9. Synthesis of phenyl-2,3,4,6-tetra-*O*-benzyl-1-thio-α-D-galactopynanoside (23)



23

To a solution of 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopynanoside (**22**) (180.2 mg, 0,409 mmol, 1.0 eq.) in MeOH (0.25 M), 22.10 mg (0.409 mmol, 0.4 eq.) of NaOMe were added, at room temperature under nitrogen. After being stirred at room temperature for 2 h and upon completion of the reaction verified by the observation of TLC (n-hexane/EtOAc - 7/3), the mixture was concentrated under reduced pressure.

The crude of previous reaction was dissolved in DMF (0.15 M), and 110.8 mg (4.62 mmol, 6.0 eq.) of NaH were added in small portions at 0° C under N_2. After stirring for 15 min, 0.33 ml (2.77 mmol, 6.0 eq.) BnBr were added dropwise over 10 min. After being warmed to room temperature and stirred for 15 h and upon completion of the reaction verified by the observation of TLC (n-hexane/EtOAc - 7/3), the mixture was poured into water and extracted with ethyl acetate (20 mL x 3). The combined organic layers were dried with Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by column chroma-

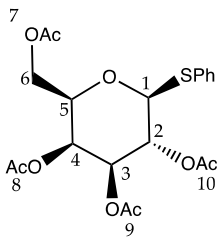
tography on silica flash using a mixture of hexane/AcOEt (7/3; v/v) to give 175 mg (0.277 mmol, 60% yield) of compound **23** as a yellow oil.

ν_{max} (cm^{-1}): 3088-3030 (ν C-H Ar); 2910-2867 (ν C-H aliphatic); 1451 (ν C=C Ar); 1094 (ν C-O); 737-694 (δ C-H Ar)

^{1}H NMR (400 MHz, Chloroform-*d*) δ: 7.44 – 7.28 (m, 25H), 5.00 (d, *J* = 11.5 Hz, 1H), 4.82 (d, *J* = 10.2 Hz, 1H), 4.76 (d, *J* = 2.2 Hz, 2H), 4.71 – 4.61 (m, 3H), 4.53 – 4.42 (m, 3H), 4.02 (d, *J* = 2.6 Hz, 1H), 3.97 (t, *J* = 9.5 Hz, 1H), 3.71 – 3.62 (m, 4H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ: 138.8, 138.4, 138.3, 137.9, 134.2, 131.5, 128.8, 128.5 – 127.4, 127.1, 87.7, 84.2, 75.7, 74.5, 73.6, 72.8, 72.1, 68.8.

4.2.10. Synthesis of phenyl-2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopynanoside (22)



22

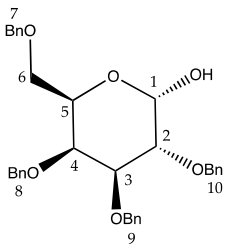
To a solution of acetobromo-α-D-(+)-galactose (84.5 mg, 0.205 mmol, 1.00 eq.) in 10% aq. Na_2CO_3 (5 mL), tiophenol (31.44 ml, 0.308 mmol, 1.5 eq.) and TBAB (3.79 mg, 0.0118mmol) were added. The reaction mixture was stirred for 4 h upon completion of the reaction verified by the observation of TLC (n-hexane/EtOAc - 7/3). The mixture was diluted with water and extracted with DCM (3x50mL). The organic layers were combined and washed with water, dried over Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by column chromatography on silica flash using a mixture of n-hexane/EtOAc (7/3; v/v) to give 64.30 mg (0.146 mmol, 67.44% yield) of compound **22** as a colorless oil.

ν_{max} (cm^{-1}): 2964 (ν C-H aliphatic); 1742 (ν C=O),1482-1366 (ν C-H), 1214-1044 (ν C-O); 741-690 (δ C-S)

^{1}H NMR (500 MHz, Chloroform-*d*) δ: 7.53 – 7.47 (m, 2H), 7.34 – 7.28 (m, 3H), 5.41 (dd, *J* = 3.4, 1.1 Hz, 1H), 5.23 (t, *J* = 10.0 Hz, 1H), 5.04 (dd, *J* = 10.0, 3.4 Hz, 1H), 4.71 (d, *J* = 10.0 Hz, 1H), 4.21 – 4.08 (m, 2H), 3.93 (ddd, *J* = 7.0, 6.1, 1.1 Hz, 1H), 2.11 (s, 3H), 2.09 (s, 3H), 2.03 (s, 3H), 1.96 (s, 3H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ: 138.8, 138.4 – 138.3, 137.9, 134.2, 131.5, 128.8, 128.5 – 127.4, 127.1, 87.7, 84.2, 75.7, 74.5, 73.6, 72.8, 72.1, 68.8.

4.2.11. Synthesis of 2,3,4,6-tetra-*O*-benzyl-α-D-galactopynanoside (34)



34

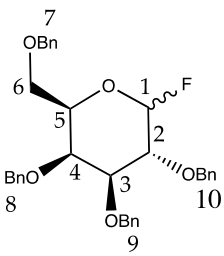
To a solution of 2,3,4,6-tetra-*O*-benzyl-1-thiophenol-α-D-galactopynanoside (**23**) (108.2 mg, 0,171 mmol, 9.00 eq.) in acetone (1.07 mL), *N*-bromosuccinimide (NBS) (64.25 mg, 0.361 mmol, 19.00 eq.) and water (0.114 mL) were added and the mixture was stirred for 15 min, time after which solid $NaHCO_3$ (0.2372 mg) was added. Upon completion of the reaction verified by the observation of TLC (n-hexane/EtOAc - 7/3) the solvent was removed by evaporation. The crude product was dissolved in ethylacetate and a saturated solution of satd. aq. $NaHCO_3$ was added. The organic phase was recovered, while the aqueous phase was extracted with EtOAc. The organic phases were combined, dried over Na_2SO_4 and filtered and evaporated to dryness. The residue was purified by column chromatography on silica flash using a mixture of n-hexane/EtOac (7/3; v/v) to give 51.30 mg (1.04 mmol, 61% yield) of compound **34** as a colorless oil.

ν_{max} (cm^{-1}): 3247 (ν O-H); 3088-3030 (ν C-H Ar); 2913-2867 (ν C-H aliphatic); 1098-1028 (ν C-O); 737-698 (δ C-H Ar).

^{1}H NMR (500 MHz, Chloroform-*d*) δ: 7.44 – 7.26 (m, 18H), 5.32 (d, *J* = 3.5 Hz, 1H), 5.00 – 4.41 (m, 8H), 4.20 (t, *J* = 6.5 Hz, 1H), 4.07 (dd, *J* = 9.8, 3.7 Hz, 1H), 3.99 (d, *J* = 2.8 Hz, 1H), 3.67 – 3.48 (m, 3H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ: 138.5, 138.4, 138.2, 137.7, 91.9, 76.6, 75.2 74.8, 74.7, 73.5, 73.0, 73.0, 69.5, 69.2.

4.2.12. Synthesis of 2,3,4,6-tetra-*O*-benzyl-D-galactopynanoside fluoride (24, 25)



α-24, β-25

The previous dried 2,3,4,6-tetra-*O*-benzyl-1-thiophenol-α-D-galactopynanoside (**23**) (108 mg, 0.17 mmol, 1.00 eq.) was dissolved in CH_2Cl_2 (0.63mL) under N_2 and cooled to -15 ºC. To the stirring solution was then added DAST (22.55 mL, 27.51 mg, 0.17 mmol, 1.50 eq.) and after 2 min NBS (30.38 mg, 0.170 mmol, 1.50 eq.) was added. Upon completion of the reaction verified by the observation of TLC (n-hexane/EtOAc - 8/2), the reaction mixture was diluted with DCM and washed with satd. aq. $NaHCO_3$ on and brine. The organic phases were combined, dried over Na_2SO_4, and filtered and evaporated to dryness. The residue was purified by column chromatography on silica flash using a mixture of hexane/EtOAc (9/1; v/v) to give 62.4 mg (0.115 mmol, 67.38% yield) of a mixture of the compounds **24** and **45** a colorless oil.

ν_{max} (cm^{-1}): 3087-3029 (ν C-H Ar); 2925-2870 (ν C-H aliphatic); 1455 (ν C=C Ar); 1142-1057 (ν C-F), 1026 (ν C-O); 798-597 (δ C-H Ar).

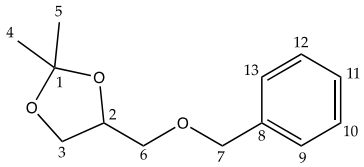
β anomer (25) - ^{1}H NMR (500 MHz, Chloroform-*d*) δ: 5.19 (dd, *J* = 53.2, 6.9 Hz, 1H), 4.96 (d, *J* = 11.6 Hz, 1H), 4.87 (d, *J* = 11.0 Hz, 1H), 4.81 – 4.78 (m, 1H), 4.75 (d, *J* = 7.4 Hz, 2H), 4.62 (d, *J* = 11.5 Hz, 1H), 4.52 – 4.42 (m, 2H), 4.15 (q, *J* = 7.1 Hz, 2H), 4.00 – 3.92 (m, 2H), 3.70 – 3.54 (m, 4H).

α anomer (24) - ^{1}H NMR (500 MHz, Chloroform-*d*) δ: 5.62 (dd, *J* = 53.7, 2.8 Hz, 1H), 4.97 (d, *J* = 11.3 Hz, 1H), 4.88 (d, *J* = 11.8 Hz, 1H), 4.84 (d, *J* = 11.8 Hz, 1H), 4.78 (d, *J* = 11.8 Hz, 1H), 4.75 (d, *J* = 11.8 Hz, 1H), 4.60 (d, *J* = 11.4 Hz, 1H), 4.53 – 4.42 (m, 2H), 4.18 – 3.94 (m, 4H), 3.57 (d, *J* = 6.5 Hz, 2H).

4.3. Synthesis of glycerol intermediates

4.3.1. Synthesis of ((S)-4-(benzyloxymethyl)-2,2-dimethyl-1,3-dioxolane) (46)

Method A:



46

A solution of (*S*)-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol (500 mg, 3.78 mmol, 1.00 eq.) in anhydrous DMF (2 mL) was cooled to 0°C, in an ice-water bath. Then sodium hydride (NaH; 60% in mineral oil, 100 mg, 1.00 eq.) was slowly added to the medium. After stirring for 15 min at room temperature under nitrogen, 0.892 ml (3.00 eq.) of benzyl bromide were added dropwise. Upon completion of the reaction verified by the observation of TLC (n-hexane/EtOAc – 7/3), NaH was quenched by slowly adding methanol to the reaction vessel at 0 ° C. After removal of solvent, the residue was dissolved in DCM and washed with water and the resulting water layer was extracted with DCM three times. The combined DCM layers were washed with brine and dried over Na_2SO_4. After removal of solvent, the residue was purified by flash column chromatography (n-hexane/EtOAc =7/3) to give 286.6 mg (0.552 mmol, 51.46% yield) of compound **46** as a colorless oil.

Method B:

A solution of (S)-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol (500 mg, 3.78 mmol, 1.00 eq.) in dry THF (3 mL) was cooled to 0°C, in an ice-water bath. Then sodium hydride (NaH; 60% in mineral oil, 131 mg 1.3. eq) was slowly added to the medium. After stirring for 15 min at room temperature under nitrogen, 0.4 mL (557.1 mg, 3.26 mmol, 1.3 eq.) of benzyl bromide were added dropwise. Upon completion of the reaction verified by the observation of TLC (n-hexane/EtOAc - 9/1), NaH was quenched by slowly adding H_2O to the reaction vessel at 0 °C. The crude was extracted with EtOAc (3 x 20 mL) and the organic phases were

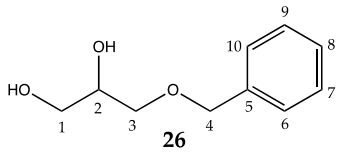
combined and concentrated at the rotary evaporator. The residue was purified by column chromatography on silica flash using a mixture of n-hexane/EtOAc (9/1; v/v) to give 509 mg (2.29 mmol; 91% yield) of compound **46** as a colorless oil.

ν_{max} (cm^{-1}): 3092-3034 (ν C-H Ar); 2987-2859 (ν C-H aliphatic); 1451-1373 (ν C-H aliphatic) 1253-1028 (ν C-O); 737-698 (δ C-H Ar).

^{1}H NMR (400 MHz, Chloroform-*d*) δ: 7.40 – 7.28 (m, 5H), 4.65 – 4.55 (m, 2H), 4.33 (p, *J* = 6.0 Hz, 1H), 4.08 (dd, *J* = 8.3, 6.5 Hz, 1H), 3.77 (dd, *J* = 8.2, 6.3 Hz, 1H), 3.55 (ddd, *J* = 33.6, 9.7, 5.6 Hz, 2H), 1.45 (s, 3H), 1.39 (s, 3H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ: 138.1, 128.5, 127.9, 109.5, 74.9, 73.6, 71.2, 67.0, 26.9, 25.5.

4.3.2. Synthesis of 3-(benzyloxy)propane-1,2-diol (26)

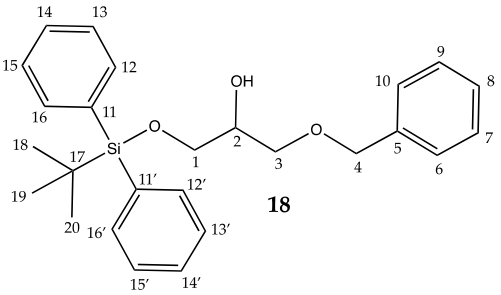


26

A solution of ((*S*)-4-(Benzyloxymethyl)-2,2-di-methyl-1,3-dioxolane) (536.7mg, 2,41 mmol) in 10 mL of an AcOH/H_2O solution (4:1). The reaction mixture was stirred at room temperature for 24h where the reaction was the reaction was verified by TLC (n-hexane/EtOAc; 6:4). The water was removed at the rotary evaporator. The product **25** was obtained in the form of oil (690.1 mg, 3.79 mmol) and proceeded to the next reaction without any purification step.

ν_{max} (cm^{-1}): 3391 (ν O-H); 3065-3030 (ν C-H Ar); 2925-2871 (ν C-H aliphatic); 1455 (δ C-H aliphatic) 1272-1024 (ν C-O); 737-694 (δ C-H Ar).

4.3.3. Synthesis of 1-Benzyloxy-3-(*tert*-butyldiphenylsilyloxy)propan-2-ol (18)



18

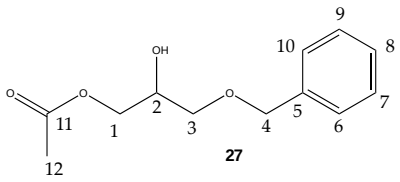
A solution of 3-(benzyloxy)propane-1,2-diol (**26**) (556 mg, 3.05 mmol, 1.00 eq.) in THF (3 mL) was cooled in an ice and water bath. To this solution was added NEt_3 (1,27 mL), 4-DMAP (35 mg 0.10 eq.) and BDPSCl (1.07 mL, 1.29 eq.) were added to this solution. Upon completion of the reaction verified by the observation of TLC (n-hexane/EtOAc – 8/2), it was terminated by adding 10 mL of an aqueous NH_4Cl solution. The product was extracted with AcOEt (3 x 20 mL). The organic phases were dried with Na_2SO_4 and concentrated at the rotary evaporator. The residue was purified by column chromatography on silica flash using a mixture of n-hexane/EtOAc (8/2; v/v) to give 428 mg (1.02 mmol; 33% yield) of compound **18** as a colorless oil.

ν_{max} **(cm^{-1}):** 3409 (ν OH); 3073 (ν C-H Ar); 2933-2859 (ν C-H aliphatic); 1428 (δ C-H aliphatic) 1277 (ν C-O); 1110 (ν Si-O); 741-702 (δ C-H Ar).

^{1}H NMR (500 MHz, Chloroform-*d*) δ: 7.72 – 7.26 (m, 15H), 4.56 (s, 2H), 3.96 (p, *J* = 5.4 Hz, 1H), 3.76 (d, *J* = 5.4 Hz, 2H), 3.60 (qd, *J* = 9.5, 5.3 Hz, 2H), 1.08 (s, 9H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ: 138.1, 135.6, 133.2, 129.8, 128.4, 127.8, 127.7, 73.4, 71.0, 70.8, 64.8, 26.9, 19.3.

4.3.4. Synthesis of 3-(benzyloxy)-2-hydroxypropyl acetate (27)



27

To a solution of 3-(benzyloxy)propane-1,2-diol (**26**) (4.90 g, 26.9 mmol, 1.00 eq.) in dry toluene (215 mL, 8.00 mL/mmol) was added dibutyltin oxide (6.70 g, 26.9 mmol, 1.00 eq.) and molecular sieves 4A˚ (2.0 g) at room temperature under nitrogen. After being heated under reflux and upon completion of the reaction verified by the observation of TLC (n-hexane/EtOAc – 8/2), the reaction mixture was concentrated in vacuo. The resulting residue was diluted with DCM (172 mL, 6.40 mL/mmol) and THF (43.0 mL, 1.60 mL/mmol), and acetyl chloride (1.92 mL, 26.9 mmol, 1.00 eq.) was added at –40 °C. After being stirred at the same temperature until the completion of the reaction verified by TLC, the reaction mixture was quenched with H_2O at –40 °C. The organic layer was separated, and the aqueous layer was extracted with DCM. The combined organic layers were washed with brine, dried over Na_2SO_4, and filtered. The filtrate was concentrated in vacuo and then the resulting residue was purified by column chromatography on silica gel (eluted with n-hexane/ETOAc = 4/1 to 3/1) to afford the compound **27** (3.50 g, 15.6 mmol, 58%) as a colorless oil.

ν_{max} **(cm^{-1}):** 3449 (ν OH); 3092-3030 (ν C-H Ar); 2952-2863 (ν C-H aliphatic); 1734 (ν C=O); 1451 (δ C-H aliphatic) 1234 (ν C-O); 198-1044 (ν Si-O); 741-698 (δ C-H Ar).

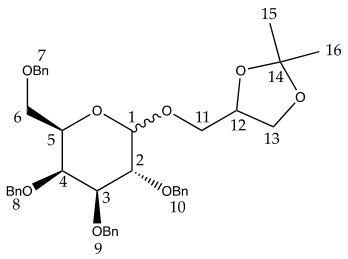
^{1}H NMR (400 MHz, Chloroform-*d*) δ: 7.42 – 7.29 (m, 5H), 4.58 (s, 2H), 4.31 – 4.01 (m, 4H), 3.62 – 3.47 (m, 2H), 2.64 (d, *J* = 4.8 Hz, 0H), 2.09 (s, 3H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ: 171.2, 137.6, 128.5, 127.9, 127.8, 73.5, 70.9, 68.6, 65.6, 20.8.

4.4. Coupling reactions

4.4.1. Synthesis of (2,2-dimethyl-1,3-dioxolan-4-yl)methyl-(2,3,4,6-tetra-O-benzyl)-galactopyranoside (47)

Method A:



47

To a solution of 2,3,4,6-tetra-*O*-benzyl-1-thiophenol-α-D-galactopynanoside (**23**) (40 mg, 0,063 mmol, 1.00 eq.) in acetone (0.39 mL), *N*-bromosuccinimide (0.032, 0.18 mmol, 1.00 eq.) and (*S*)-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol (0.2 mL, 3 eq.) were added and the mixure was left stiring. Upon completion of the reaction verified by the observation of TLC (n-hexane/EtOAc – 8/2) the solvent was removed by evaporation. The crude product was dissolved in EtOAc and a satd. aq. $NaHCO_3$ solution was added. The organic phase was recovered, while the aqueous phase was extracted with EtOAc. The organic phases were combined, dried over Na_2SO_4 and after filtration and evaporation to dryness the residue was purified by column chromatography on silica flash using a mixture of n-hexane/EtOAc (8/2; v/v) to give 7 mg (0.011 mmol, 17%) of compound **47** as a colorless oil. It was not possible to do the analysis of the spectrum due to the lack of resolution.

Method B:

To a solution 2,3,4,6-tetra-*O*-benzyl-1-thiophenol-α-D-galactopynanoside (**23**) (52 mg, 0.082 mmol, 1.0 eq.) in DCM (5 mL) at 0 °C, Br_2 (0.04 ml, 12.48mg, 0.078 mmol, 0.95 eq.) was added under stirring. After complete conversion of the 2,3,4,6-tetra-*O*-benzyl-1-thiophenol-α-D-galactopynanoside, molecular sieves 4Å were added to the mixture and stirred for another half hour at 0 °C. Then (*S*)-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol (7.60 mg, 0.058 mmol, 0.7 eq.) and Bu_4NBr (38.01, 0.123 mmol, 1.5 eq.) were added and the mixture was brought slowly to room temperature. Upon completion of the reaction verified by the observation of TLC (n-hexane/EtOAc – 8/2), the mixture was diluted with DCM and filtered through celite. The filtrate was washed with 5% $Na_2S_2O_3$, satd. solution of satd. aq. $NaHCO_3$ and then with water. The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography on silica flash n-hexane/EtOAc (8/2; v/v). There was no signal of the formation of the pretended product.

ν_{max} (cm^{-1}): 3065-3030 (ν C-H Ar); 2929-2878 (ν C-H aliphatic); 1458 (δ C-H aliphatic) 1277 (ν C-O); 1098-1028 (ν C-O); 741-706 (δ C-H Ar).

ESI-MS: 677 $[M+ NH_4]^+$

4.4.2. Synthesis of 1-(benzyloxy)-3-((*tert*-butyldiphenylsilyl)oxy)propan-2-galactopyranoside (48, 49)

Method A:

A mixture of phenyl-2,3,4,6-tetra-*O*-benzyl-1-thio-α-D-galactopynanoside (**23)** (54 mg, 0.085 mmol, 1.00 eq.) and 1-benzyloxy-3-(*tert*-butyldiphenylsilyloxy)propan-2-ol (**18**) (37.33 mg, 0.089 mmol, 1.04 eq.) was dissolved in toluene (5 mL) and evaporated under reduced pressure. This operation was repeated twice. Molecular sieves 4Å, dry toluene (2 mL), and dry dioxane (6 mL) were added and the mixture was stirred for 1 hr at room temperature. The mixture was cooled to 0 °C and NBS (4.85 mg, 0.085 mmol, 1.00 eq.) was added. Upon completion of the reaction verified by the observation of TLC (n-hexane/EtOAc – 8/2), the molecular sieves were filtered and washed with dichloromethane. The filtrate was then washed with 5% $Na_2S_2O_3$ (2 × 20 mL) and water (3 × 20 mL), dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography on silica flash n-hexane/EtOAc (8/2; v/v). The formation of the product was not identified.

Method B:

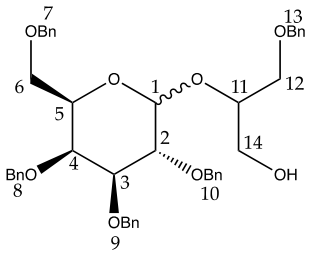
To a solution of phenyl-2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopynanoside (**23**) (54 mg, 0.0853 mmol, 1.00) in acetone (5 mL), *N*-bromosuccinimide (10.24 mg, 0.180 mmol, 2.11 eq.) and 1-Benzyloxy-3-(*tert*-butyldiphenylsilyloxy)propan-2-ol (**18**) (43.07 mg, 0.102 mmol, 1.20eq.) were added and the mixure was stirred under nitrogen. Upon completion of the reaction verified by the observation of TLC (n-hexane/EtOAc – 8/2) the solvent was removed by evaporation. The crude product was dissolved in EtOAc (100 mL) and a saturated solution of satd. aq. $NaHCO_3$ was added. The organic phase was recovered, while the aqueous phase was extracted by EtOAc (100 mL). The organic phases were combined, dried over Na_2SO_4 and after filtration and evaporation to dryness the residue was purified by column chromatography on silica flash using a mixture of hexane/AcOEt (8/2; v/v) to give 7 mg (0.0107 mmol, 16.91%) of a mixture of compounds **52** and **53** as a colorless oil.

Fisrt attempt - ESI-MS: 960 $[M+NH_4]^+$

Second attempt - was obtained the same product that is in method C.

Method C:

A mixture of phenyl-2,3,4,6-tetra-*O*-benzyl-1-thio-α-D-galactopynanoside (**23**) (74.60 mg, 0.117 mmol, 1.0 eq.) and 1-Benzyloxy-3-(*tert*-butyldiphenylsilyloxy)propan-2-ol (**18**) (59.01 mg, 0.140 mmol, 1.19 eq.) was dissolved in toluene (5 mL) and evaporated under reduced pressure. This operation was repeated



α-**52,** β-**53**

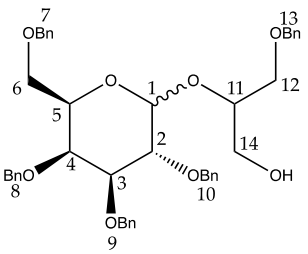
twice. Molecular sieves 4Å, dry toluene (2 mL), and dry dioxane (6 mL) were added and the mixture was stirred for 1 hr at room temperature. The mixture was cooled to 0 °C and NIS (27.58 mg, 0.123 mmol, 1.04 eq.) and TMSOTf (45.49 mg, 0.205 mmol, 1.74 eq.) were added. Upon completion of the reaction verified by the observation of TLC (n-hexane/EtOAc – 8/2), the molecular sieves were filtered and washed with dichloromethane. The filtrate was then washed with 5% $Na_2S_2O_3$ (2 × 20 mL) and water (3 × 20 mL), dried over Na_2SO_4, and evaporated under reduced pressure. The residue was purified by column chromatography on flash silica using a gradient of n-hexane/EtOAc eluents (9/1; 8/2; 7/3; 6/4; v/v) to give 9 mg (0.0199 Mmol, 11.18%) of the mixture of compounds **52** and **53** as a colorless oil.

ν_{max} (cm^{-1}): 3449 (ν OH); 3092-3026 (ν C-H Ar); 2921 (ν C-H aliphatic); 1494-1374 (δ C-H aliphatic); 1241 (ν C-O ether); 1098-1071 (ν C-O); 738-699 (δ C-H Ar)

^{1}H NMR (400 MHz, Chloroform-*d*) δ; 7.44 – 7.25 (m, 26H), 5.31 (d, *J* = 3.7 Hz, 1H), 4.96 (m, 2H), 4.85 (dd, *J* = 11.1, 4.7 Hz, 2H), 4.79 (d, *J* = 7.4 Hz, 2H), 4.78 – 4.74 (m, 1H), 4.72 (d, *J* = 3.8 Hz, 0H), 4.69 (d, *J* = 7.4 Hz, 1H), 4.62 (dd, *J* = 11.5, 7.3 Hz, 1H), 4.54 – 4.40 (m, 3H), 4.19 (t, *J* = 6.5 Hz, 1H), 4.06 (dd, *J* = 9.8, 3.7 Hz, 1H), 3.96 – 3.90 (m, 2H), 3.79 (dd, *J* = 9.7, 7.4 Hz, 1H), 3.66 – 3.48 (m, 4H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ: 138.6, 138.6, 138.6, 138.5, 138.4, 138.2, 137.9, 137.8, 128.5 – 127.4, 97.8, 91.9, 82.2, 80.7, 78.7, 77.2, 76.6, 75.1, 74.7, 74.6, 73.7 – 73.5, 73.5, 73.0, 72.9, 69.6, 69.0, 68.9.

4.4.3. Synthesis of 1-(benzyloxy)-3-((*tert*-butyldiphenylsilyl)oxy)propan-2-galactopyranoside (52, 53)



α-**52,** β-**53**

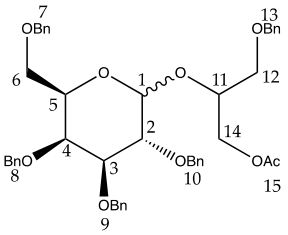
To a mixture of 2,3,4,6-tetra-*O*-benzyl-α/β-D-galactopynanoside fluoride (**24** and **25**) (107.00 mg, 0.197 mmol, 1.0 eq.) , silver trifluoromethanesulphonate (59.78 mg, 0.232 mmol, 1.18 eq.), tin chloride (44.12 mg, 0. 232 mmol, 1.18 eq.) and molecular sieves 4Å in ethyl ether (5mL), and a mixture of 1-Benzyloxy-3-(tert-butyldiphenyloxy)propan-2-ol (95.38 mg, 0.227 mmol, 1.15 eq.) in ethyl ether was added. The mixture was left under stirring at room temperature. After completion of the reaction verified by TLC (n-hexane/EtOAc - 8/2), the reaction mixture was filtered through celite. The filtrate was washed with a saturated aqueous solution of satd. aq. $NaHCO_3$ and water, dried and evaporated under vacuum. The residue was purified by column chromatography on flash silica using a gradient of n-hexane/EtOAc eluents (9/1; 8/2; 7/3; 6/4; v/v) to give 14 mg (0.0199 mmol, 11.75%) of the mixture of compounds **52** and **53** as a colorless oil.

ν_{max} (cm^{-1}): 3387 (ν OH); 3084-3030 (ν C-H Ar); 2925-2871 (ν C-H aliphatic); 1451 (δ C-H aliphatic); 1094-1028 (ν C-O); 733-702 (δ C-H Ar).

^{1}H NMR (400 MHz, Chloroform-*d*) δ; 7.44 – 7.25 (m, 26H), 5.31 (d, *J* = 3.7 Hz, 1H), 4.96 (m, 2H), 4.85 (dd, *J* = 11.1, 4.7 Hz, 2H), 4.79 (d, *J* = 7.4 Hz, 2H), 4.78 – 4.74 (m, 1H), 4.72 (d, *J* = 3.8 Hz, 0H), 4.69 (d, *J* = 7.4 Hz, 1H), 4.62 (dd, *J* = 11.5, 7.3 Hz, 1H), 4.54 – 4.40 (m, 3H), 4.19 (t, *J* = 6.5 Hz, 1H), 4.06 (dd, *J* = 9.8, 3.7 Hz, 1H), 3.96 – 3.90 (m, 2H), 3.79 (dd, *J* = 9.7, 7.4 Hz, 1H), 3.66 – 3.48 (m, 4H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ: 138.6, 138.6, 138.6, 138.5, 138.4, 138.2, 137.9, 137.8, 128.5 – 127.4, 97.8, 91.9, 82.2, 80.7, 78.7, 77.2, 76.6, 75.1, 74.7, 74.6, 73.7 – 73.5, 73.5, 73.0, 72.9, 69.6, 69.0, 68.9.

4.4.4. Synthesis of (1-acetoxy-3-benzyloxy-2-propan)-2,3,4,6-tetra-O-benzylgalactopyranoside (50, 51)



α-50, β-51

To a solution of phenyl-2,3,4,6-tetra-*O*-benzyl-1-thio-α-D-galactopynanoside (**23**) (62 mg, 0.097 mmol, 1.00 eq.) in acetone (5 mL), *N*-bromosuccinimide (20.93 mg, 0.118 mmol, 1.20 eq.) and 3-(benzyloxy)-2-hydroxypropyl acetate (46.3 6mg, 0,207 mmol, 2.11 eq.) were added and the mixure was stirred under nitrogen. Upon completion of the reaction verified by the observation of TLC (n-hexane/EtOAc – 8/2) the solvent was removed by evaporation. The crude product was dissolved in EtOAc and a saturated solution of satd. aq. $NaHCO_3$ was added. The organic phase was recovered, while the aqueous phase was extracted by EtOAc. The organic phases were combined, dried over Na_2SO_4 and after filtration and evaporation to dryness the residue was purified by column chromatography on silica flash using a mixture of n-hexane/EtOAc (8/2; v/v) to give 20 mg (0.0286 mmol, 29.21%) of a mixture of compounds **50** and **51** as a colorless oil.

ν_{max} (cm^{-1}): 3387 (ν OH); 3092-3034 (ν C-H Ar); 2917-2867 (ν C-H aliphatic); 1734 (ν C=O); 1497-1370 (δ C-H aliphatic); 1238 (ν C-O ether); 1098 (ν C-O); 738-699 (δ C-H Ar)

^{1}H NMR (400 MHz, Chloroform-*d*) δ: 7.24 (ddt, *J* = 21.0, 13.0, 7.4 Hz, 24H), 5.20 (d, *J* = 3.4 Hz, 1H), 4.96 (q, *J* = 5.0 Hz, 0H), 4.85 (dd, *J* = 11.5, 5.7 Hz, 1H), 4.69 (ddt, *J* = 25.6, 17.4, 8.7 Hz, 3H), 4.57 (d, *J* = 7.6 Hz, 0H), 4.52 (d, *J* = 7.0 Hz, 1H), 4.48 (s, 3H), 4.44 – 4.30 (m, 2H), 4.08 (qd, *J* = 11.5, 5.0 Hz, 3H), 3.95 (dd, *J* = 7.3, 4.0 Hz, 2H), 3.86 (d, *J* = 12.3 Hz, 1H), 3.75 – 3.66 (m, 1H), 3.59 (q, *J* = 5.5, 3.8 Hz, 1H), 3.45 (dtq, *J* = 22.0, 10.7, 5.7 Hz, 4H), 1.99 (s, 4H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ: 171.2, 170.9, 138.6, 138.6, 138.5, 138.4, 138.2, 137.9, 137.7, 128.7 – 127.2, 97.9, 91.9, 82.2, 80.8, 78.7, 76.6, 75.1, 74.78 – 74.6, 73.5, 73.25, 73.0, 70.9, 69.5, 69.01, 68.9, 65.6, 62.6, 21.1, 20.9.

BIBLIOGRAPHY

[1] S. Grabley and R. Thiericke, "Bioactive agents from natural sources: trends in discovery and application.," *Adv. Biochem. Eng. Biotechnol.*, vol. 64, pp. 101–154, 1999.

[2] D. J. Newman, G. M. Cragg, and K. M. Snader, "The influence of natural products upon drug discovery," *Nat. Prod. Rep.*, vol. 17, no. 3, pp. 215–234, 2000.

[3] J. Achan *et al.*, "Quinine, an old anti-malarial drug in a modern world: Role in the treatment of malaria," *Malar. J.*, vol. 10, pp. 1–12, 2011.

[4] Z. Guo, "The modification of natural products for medical use," *Acta Pharm. Sin. B*, vol. 7, no. 2, pp. 119–136, 2017.

[5] S. Bernardini, A. Tiezzi, V. Laghezza Masci, and E. Ovidi, "Natural products for human health: an historical overview of the drug discovery approaches," *Nat. Prod. Res.*, vol. 32, no. 16, pp. 1926–1950, 2018.

[6] J. B. Calixto, "The role of natural products in modern drug discovery," *An. Acad. Bras. Cienc.*, vol. 91, pp. 1–7, 2019.

[7] G. M. Cragg, D. J. Newman, and K. M. Snader, "Natural products in drug discovery and development," *J. Nat. Prod.*, vol. 60, no. 1, pp. 52–60, 1997.

[8] D. J. Newman and G. M. Cragg, "Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019," *J. Nat. Prod.*, vol. 83, no. 3, pp. 770–803, 2020.

[9] X. Liang, D. Luo, and H. Luesch, "Advances in exploring the therapeutic potential of marine natural products," *Pharmacol. Res.*, vol. 147, no. July, p. 104373, 2019.

[10] W. H. Gerwick and B. S. Moore, "Lessons from the past and charting the future of marine natural products drug discovery and chemical biology," *Chem. Biol.*, vol. 19, no. 1, pp. 85–98, 2012.

[11] D. J. Newman and G. M. Cragg, "Drugs and Drug Candidates from Marine Sources: An Assessment of the Current 'state of Play,'" *Planta Med.*, vol. 82, no. 9–10, pp. 775–789, 2016.

[12] D. J. Faulkner, "Highlights of marine natural products chemistry (1972-1999)," *Nat. Prod. Rep.*, vol. 17, no. 1, pp. 1–6, 2000.

[13] D. J. Faulkner, "Interesting aspects of marine natural products chemistry," *Tetrahedron*, vol. 33, no. 12, pp. 1421–1443, 1977.

[14] J. W. Blunt, B. R. Copp, R. A. Keyzers, M. H. G. Munro, and M. R. Prinsep, "Marine natural products," *Nat. Prod. Rep.*, vol. 32, no. 2, pp. 116–211, 2015.

[15] H. Malve, "Exploring the ocean for new drug developments: Marine pharmacology," *J. Pharm. Bioallied Sci.*, vol. 8, no. 2, pp. 83–91, 2016.

[16] M. S. Wallace, "Ziconotide: A new nonopioid intrathecal analgesic for the treatment of chronic pain," *Expert Rev. Neurother.*, vol. 6, no. 10, pp. 1423–1428, 2006.

[17] W. H. Harvey, "Some account of the marine botany of the Colony of Western Australia," *Trans. R. Irish Acad.*, vol. 22, no. 1849, pp. 525–566, 2016.

[18] S. Pinteus *et al.*, "Marine invasive macroalgae: Turning a real threat into a major opportunity - the biotechnological potential of Sargassum muticum and Asparagopsis armata," *Algal Res.*, vol. 34, no. June, pp. 217–234, 2018.

[19] S. Pinteus *et al.*, "Marine invasive species for high-value products' exploration – Unveiling the antimicrobial potential of Asparagopsis armata against human pathogens," *Algal Res.*, vol. 52, no. September, p. 102091, 2020.

[20] R. A. Marshall, D. B. Harper, W. C. McRoberts, and M. J. Dring, "Volatile bromocarbons produced by Falkenbergia stages of Asparagopsis spp. (Rhodophyta)," *Limnol. Oceanogr.*, vol. 44, no. 5, pp. 1348–1352, 1999.

[21] G. Genovese, L. Tedone, M. T. Hamann, and M. Morabito, "The Mediterranean red alga Asparagopsis: A source of compounds against Leishmania," *Mar. Drugs*, vol. 7, no. 3, pp. 361–366, 2009.

[22] O. J. McConnell, R. E. Longley, and F. E. Koehn, *The discovery of marine natural products with therapeutic potential.*, vol. 26. 1994.

[23] C. O. Silva, M. F. L. Lemos, R. Gaspar, C. Gonçalves, and J. M. Neto, "The effects of the invasive seaweed Asparagopsis armata on native rock pool communities: Evidences from experimental exclusion," *Ecol. Indic.*, vol. 125, no. October, 2021.

[24] I. Cheng-Sánchez and F. Sarabia, "Chemistry and biology of bioactive glycolipids of marine origin," *Mar. Drugs*, vol. 16, no. 9, 2018.

[25] G. Hölzl and P. Dörmann, "Structure and function of glycoglycerolipids in plants and bacteria," *Prog. Lipid Res.*, vol. 46, no. 5, pp. 225–243, 2007.

[26] C. Murakami *et al.*, "Effects of glycolipids from spinach on mammalian DNA polymerases," *Biochem. Pharmacol.*, vol. 65, no. 2, pp. 259–267, 2003.

[27] N. Chirasuwan *et al.*, "Anti-HSV-1 activity of sulpoquinovosyl diacylglycerol isolated from Spirulina platensis," *ScienceAsia*, vol. 35, no. 2, pp. 137–141, 2009.

[28] J. P. Bergé, E. Debiton, J. Dumay, P. Durand, and C. Barthomeuf, "In vitro anti-inflammatory and anti-proliferative activity of sulfolipids from the red alga Porphyridium cruentum," *J. Agric. Food Chem.*, vol. 50, no. 21, pp. 6227–6232, 2002.

[29] J. Zhang, C. Li, G. Yu, and H. Guan, "Total synthesis and structure-activity relationship of glycoglycerolipids from marine organisms," *Mar. Drugs*, vol. 12, no. 6, pp. 3634–3659, 2014.

[30] L. S. Jeremy M. Berg, John L. Tymoczko, *Biochemistry*, 5th ed. W. H. Freeman, 2002.

[31] T. Y. Lin and D. B. Weibel, "Organization and function of anionic phospholipids in bacteria," *Appl. Microbiol. Biotechnol.*, vol. 100, no. 10, pp. 4255–4267, 2016.

[32] E. Meldrum, P. J. Parker, and A. Carozzi, "The PtdIns-PLC superfamily and signal transduction," *BBA - Mol. Cell Res.*, vol. 1092, no. 1, pp. 49–71, 1991.

[33] P. W. Majerus, "Inositol phosphate biochemistry," *Annu. Rev. Biochem.*, vol. 61, no. 1, pp. 225–250, 1992.

[34] A. Al-Fadhli, S. Wahidulla, and L. D'Souza, "Glycolipids from the red alga Chondria armata (Kütz.) Okamura," *Glycobiology*, vol. 16, no. 10, pp. 902–915, 2006.

[35] Y. L. Yang *et al.*, "Structural elucidation of phosphoglycolipids from strains of the bacterial thermophiles Thermus and Meiothermus," *J. Lipid Res.*, vol. 47, no. 8, pp. 1823–1832, 2006.

[36] A. Giordano, F. M. Vella, I. Romano, and A. Gambacorta, "Structural elucidation of a novel phosphoglycolipid isolated from six species of Halomonas," *J. Lipid Res.*, vol. 48, no. 8, pp. 1825–1831, 2007.

[37] K. Matsuda, J. L. Li, and R. Harasawa, "Phosphocholine- C on t aining Glycoglycerolipids (G G PL- I and G G PL- III) Are Species-Speci fi c Major I mmunode t erminan t s o f Mycoplasma f ermen t ans," *Biochem. Biophys. Res. Commun.*, vol. 233, no. 2, pp. 644–649, 1997.

[38] K. Matsuda, T. Kasama, I. Ishizuka, S. Handa, N. Yamamoto, and T. Taki, "Structure of a novel phosphocholine-containing glycoglycerolipid from Mycoplasma fermentans," *J. Biol. Chem.*, vol. 269, no. 52, pp. 33123–33128, 1994.

[39] P. M. Furneri, A. Piperno, and G. Bisignano, *Antimycoplasmal Activity of Oleuropein*. Elsevier Inc., 2010.

[40] Y. Nishida, Y. Takamori, H. Ohrui, I. Ishizuka, K. Matsuda, and K. Kobayashi, "Synthesis and absolute configuration of a novel aminoglycoglycerolipid, species-specific major immunodeterminant of Mycoplasma fermentans," *Tetrahedron Lett.*, vol. 40, no. 12, pp. 2371–2374, 1999.

[41] F. Marcelo, "Total Synthesis and Stereochemical Assignment of Miharamycins," Universidade de Lisboa Faculdade de Ciências, Université Pierre et Marie Curie – Paris VI, 2008.

[42] K. A. Rosentrater and A. D. Evers, *Chemical components and nutrition*. 2018.

[43] K. C. Nicolaou and H. J. Mitchell, "Adventures in carbohydrate chemistry: New synthetic technologies, chemical synthesis, molecular design, and chemical biology," *Angew. Chemie - Int. Ed.*, vol. 40, no. 9, pp. 1576–1624, 2001.

[44] B. G. Davis and A. J. Fairbanks, *Carbohydrate Chemistry*, 1st ed. OXFORD, 2002.

[45] D. Rofifah, *The Organic Chemistry of Sugars*. Taylor & Francis, 2006.

[46] D. A. Mannock and R. N. Mcelhaney, "AN IMPROVED PROCEDURE FOR THE PREPARATION OF 1,2-DI-O-ACYL- 3.O-(~-D-GLUCOPYRANOSYL)-sn-GLYCEROLS," vol. 43, pp. 113–127, 1987.

[47] M. R. E. S. Aly and E. S. H. El Ashry, *Recent Advances Toward Robust N-Protecting Groups for Glucosamine as Required for Glycosylation Strategies*, 1st ed., vol. 73. Elsevier Inc., 2016.

[48] S. Co, "The Koenigs-Knorr reaction," vol. I, pp. 243–283.

[49] P. Welzel, "Synthesis of the repeating unit of the capsular antigen of neisseria meningitidis serogroup H," vol. 46, no. 15, pp. 5145–5154, 1990.

[50] G. Wulff and G. Rohle, "Results and Poblems of O-Glycoside Synthesis," vol. 13, no. 3, pp. 157–170, 1974.

[51] L. Sun, X. Wu, D. C. Xiong, and X. S. Ye, "Stereoselective Koenigs–Knorr Glycosylation Catalyzed by Urea," *Angew. Chemie - Int. Ed.*, vol. 55, no. 28, pp. 8041–8044, 2016.

[52] R. R. King and C. T. Bishop, " A Facile Route to 1 → 6 Linked Disaccharides: Syntheses of 6- O -β- D -Glucopyranosyl- D -glucose (Gentiobiose) and of 2-Acetamido-6- O -(2-Acetamido-2-deoxy-β- D -galactopyranosyl)-2-deoxy- D -galactose ," *Can. J. Chem.*, vol. 53, no. 13, pp. 1970–1972, 1975.

[53] J. S. Thomann *et al.*, "Novel glycolipid TLR2 ligands of the type Pam 2Cys-α-Gal: Synthesis and biological properties," *Eur. J. Med. Chem.*, vol. 51, pp. 174–183, 2012.

[54] E. Decoster, J. M. Lacombe, J. L. Strebler, B. Ferrari, and A. Pavia, "Une autre approche a la synthese de derives benzyles des monosaccharedes reducteurs. etude des equilibres pyrannose-furannose par r.m.n. du carbone-13," *J. Carbohydr. Chem.*, vol. 2, no. 3, pp. 329–341, 1983.

[55] J. Clayden, N. Greeves, and W. Stuart, *Organic Chemistry*, 2nd ed. OXFORD, 2012.

[56] M. Emmadi and S. S. Kulkarni, "Synthesis of Rare Deoxy Amino Sugar Building Blocks Enabled the Total Synthesis of a Polysaccharide Repeating Unit Analogue from the LPS of Psychrobacter cryohalolentis K5 T," *J. Org. Chem.*, vol. 83, no. 23, pp. 14323–14337, 2018.

[57] D. Takahashi, S. Hirono, C. Hayashi, M. Igarashi, Y. Nishimura, and K. Toshima, "Photodegradation of target oligosaccharides by light-activated small molecules," *Angew. Chemie - Int. Ed.*, vol. 49, no. 52, pp. 10096–10100, 2010.

[58] S. Vandenbussche *et al.*, "Aromatic-carbohydrate interactions: An NMR and computational study of model systems," *Chem. - A Eur. J.*, vol. 14, no. 25, pp. 7570–7578, 2008.

[59] R. G. S. Ritchie, N. Cyr, B. Korsch, H. J. Koch, and A. S. Perlin, "Carbon-13 Chemical Shifts of Furanosides and Cyclopentanols. Configurational and Conformational

Influences," *Can. J. Chem.*, vol. 53, no. 10, pp. 1424–1433, 1975.

[60] M. R. Richards, Y. Bai, and T. L. Lowary, "Comparison between DFT- and NMR-based conformational analysis of methyl galactofuranosides," *Carbohydr. Res.*, vol. 374, pp. 103–114, 2013.

[61] C. W. Chang *et al.*, "Automated Quantification of Hydroxyl Reactivities: Prediction of Glycosylation Reactions," *Angew. Chemie - Int. Ed.*, vol. 60, no. 22, pp. 12413–12423, 2021.

[62] B. Graham, A. E. R. Fayter, J. E. Houston, R. C. Evans, and M. I. Gibson, "Facially Amphipathic Glycopolymers Inhibit Ice Recrystallization," *J. Am. Chem. Soc.*, vol. 140, no. 17, pp. 5682–5685, 2018.

[63] S. R. Vidadala, S. A. Thadke, S. Hotha, and S. Kashyap, "Synthesis of thioglycosides from propargyl glycosides exploiting alkynophilic gold catalyst," *J. Carbohydr. Chem.*, vol. 31, no. 3, pp. 241–251, 2012.

[64] L. Gong *et al.*, "Ni-Catalyzed Suzuki-Miyaura Cross-Coupling of α-Oxo-vinylsulfones to Prepare C-Aryl Glycals and Acyclic Vinyl Ethers," *J. Am. Chem. Soc.*, vol. 141, no. 19, pp. 7680–7686, 2019.

[65] S. Escopy, Y. Singh, and A. V. Demchenko, "Triflic acid-mediated synthesis of thioglycosides," *Org. Biomol. Chem.*, vol. 17, no. 36, pp. 8379–8383, 2019.

[66] T. Ghosh, A. Santra, and A. K. Misra, "Appel-reagent-mediated transformation of glycosyl hemiacetal derivatives into thioglycosides and glycosyl thiols," *Beilstein J. Org. Chem.*, vol. 9, no. c, pp. 974–982, 2013.

[67] C. Pezzetta, S. Tshepelevitsh, I. Leito, and G. Poli, "Comment on 'zemplén transesterification: A name reaction that has misled us for 90 years' by B. Ren, M. Wang, J. Liu, J. Ge, X. Zhang and H. Dong,: Green Chemistry, 2015, 17, 1390-1394," *Green Chem.*, vol. 20, no. 10, pp. 2392–2394, 2018.

[68] N. K. Jalsa, "Regioselective removal of the anomeric O-benzyl from differentially protected carbohydrates," *Tetrahedron Lett.*, vol. 52, no. 49, pp. 6587–6590, 2011.

[69] W. W. Kallemeijn, M. D. Witte, T. Wennekes, and J. M. F. G. Aerts, *Mechanism-based inhibitors of glycosidases: Design and applications*, 1st ed., vol. 71. Elsevier Inc., 2014.

[70] V. S. Dorokhova *et al.*, "Synthesis and conformational analysis of vicinally branched trisaccharide β-d-Galf-(1 → 2)-[β-d-Galf-(1 → 3)-]-α-GalpfromCryptococcus

neoformansgalactoxylomannan," *Org. Biomol. Chem.*, vol. 19, no. 13, pp. 2923–2931, 2021.

[71] P. C. Bulman Page, Y. Chan, J. Liddle, and M. R. J. Elsegood, "Carbohydrate-derived iminium salt organocatalysts for the asymmetric epoxidation of alkenes," *Tetrahedron*, vol. 70, no. 40, pp. 7283–7305, 2014.

[72] K. Suzuki, Y. Ito, and O. Kanie, "An improved method for the synthesis of protected glycosyl fluorides from thioglycosides using N,N-diethylaminosulfur trifluoride (DAST)," *Carbohydr. Res.*, vol. 359, pp. 81–91, 2012.

[73] K. T. Huang and N. Winssinger, "IPy2BF4-mediated glycosylation and glycosyl fluoride formation," *European J. Org. Chem.*, no. 12, pp. 1887–1890, 2007.

[74] K. C. Nicolaou, R. E. Dolle, D. P. Papahatjis, and L. Randall, "Pratical Synthesis of Oligosaccharides. Partial Synthesis of Avermectin B1a," *J. Am. Chem. Soc.*, no. c, pp. 4189–4192, 1984.

[75] L. H. Urner *et al.*, "Dendritic Oligoglycerol Regioisomer Mixtures and Their Utility for Membrane," *Chem. Eur. J.*

[76] K. C. Nicolaou *et al.*, "Total synthesis and structural elucidation of azaspiracid-1. Construction of key building blocks for originally proposed structure," *J. Am. Chem. Soc.*, vol. 128, no. 7, pp. 2244–2257, 2006.

[77] P. Patschinski, C. Zhang, and H. Zipse, "The lewis base-catalyzed silylation of alcohols-a mechanistic analysis," *J. Org. Chem.*, vol. 79, no. 17, pp. 8348–8357, 2014.

[78] C. T. Liu, T. C. Tu, and L. Y. Hsu, "A new synthetic route towards the mono-O-protected anticonformationally constrained pyrimidine acyclic nucleoside," *Chem. Pharm. Bull.*, vol. 50, no. 8, pp. 1028–1030, 2002.

[79] M. Yoshida, K. Saito, H. Kato, S. Tsukamoto, and T. Doi, "Total Synthesis and Biological Evaluation of Siladenoserinol A and its Analogues," *Angew. Chemie - Int. Ed.*, vol. 57, no. 18, pp. 5147–5150, 2018.

[80] Y. Zhou, J. Li, Y. Zhan, Z. Pei, and H. Dong, "Halide promoted organotin-mediated carbohydrate benzylation: Mechanism and application," *Tetrahedron*, vol. 69, no. 13, pp. 2693–2700, 2013.

[81] T. B. Grindley, *Applications of tin-containing intermediates to carbohydrate chemistry*, vol. 53, no. 53. Elsevier Masson SAS, 1998.

[82] Z. B. Szabó *et al.*, "Synthesis of three regioisomers of the pentasaccharide part of the Skp1 glycoprotein of Dictyostelium discoideum," *Tetrahedron Asymmetry*, vol. 20, no. 6–8, pp. 808–820, 2009.

[83] I. J. Borowitz, *Thin-layer chromatography. A laboratory handbook*, vol. 21, no. 1. 1966.

.

A.

Appendix

Appendix 1 - penta-O-benzyl-β-D-galactopyranoside (29) and penta-O-benzyl-α-D-galactofuranoside (31)

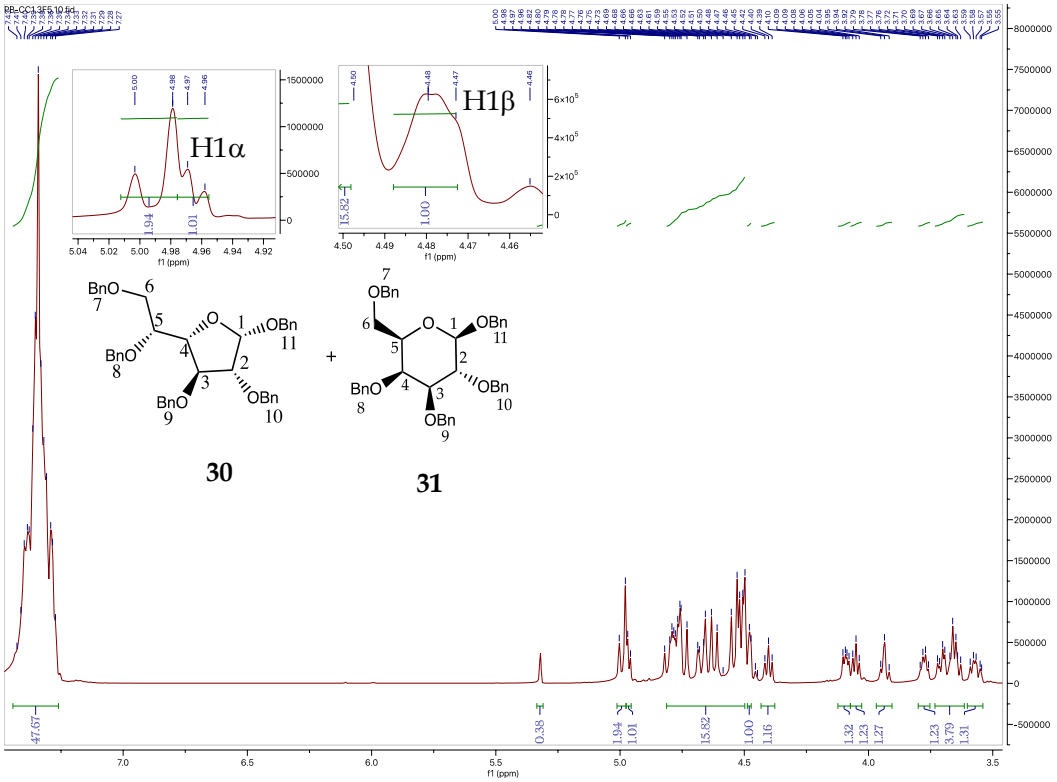


Figure 4.1 - ^{1}H NMR spectrum of compounds **30** and **31** (400 MHz, CDCl3).

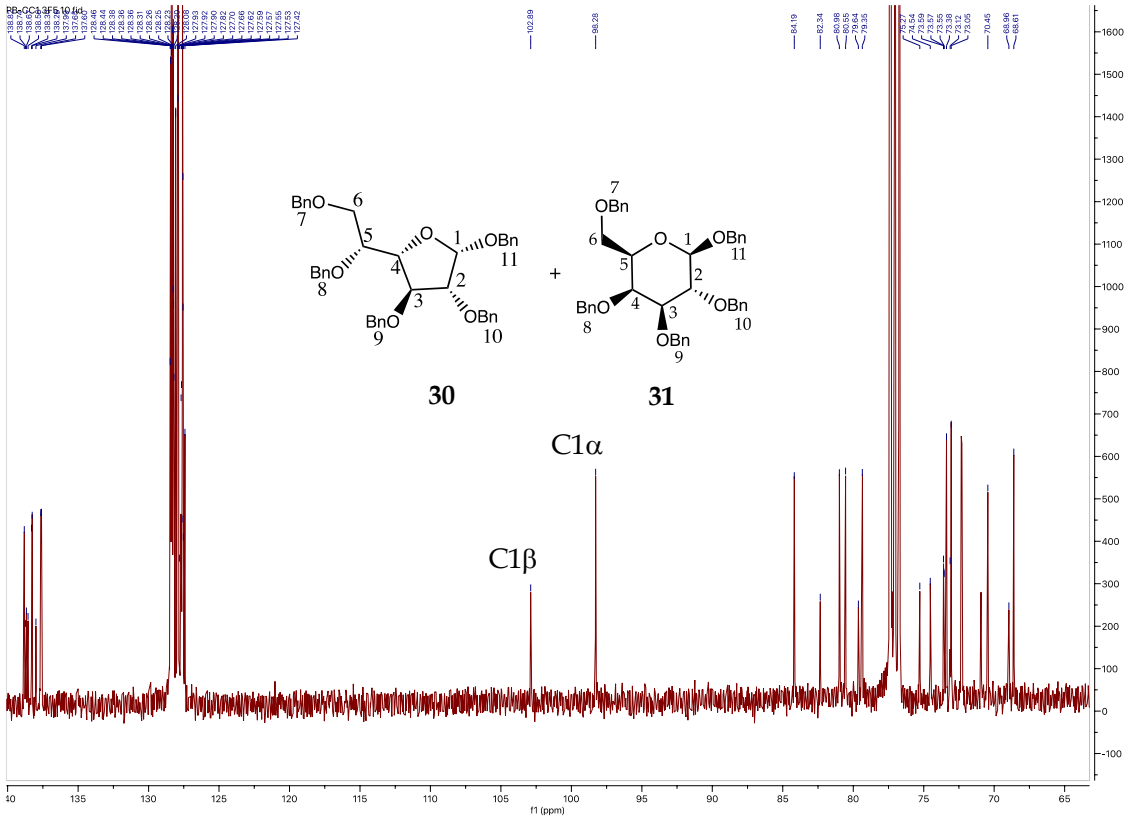


Figure 4.2 – ^{13}C NMR spectrum of compounds **30** and **31** (101 MHz, CDCl3).

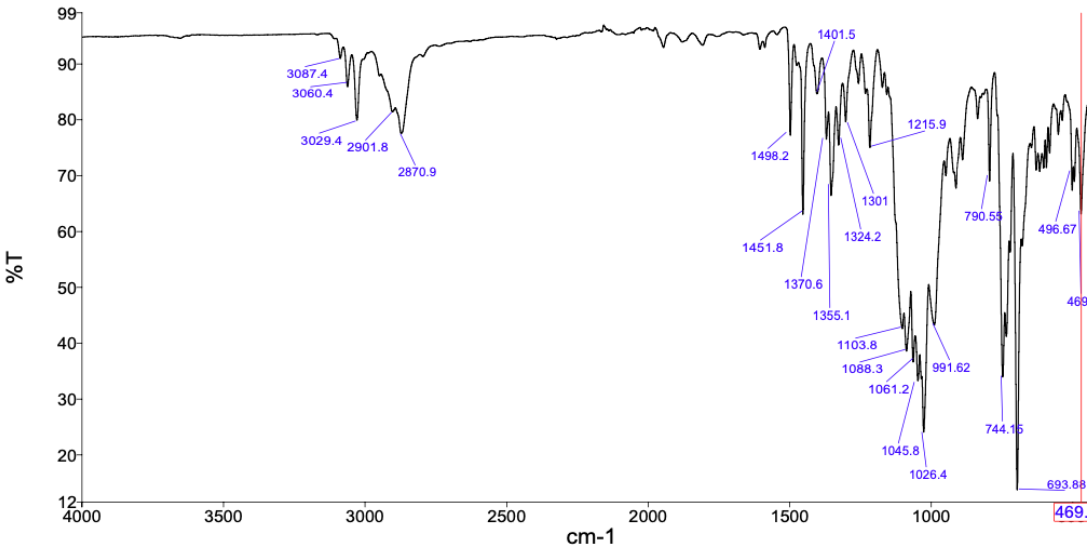


Figure 4.3 – IR spectrum of compounds **30** and **31**.

Appendix 2 - 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranoside (34), 2,3,4,6-tetra-*O*-benzyl-α-D-galactofuranoside (32) and 2,3,4,6-tetra-*O*-benzyl-β-D-galactofuranoside (33)

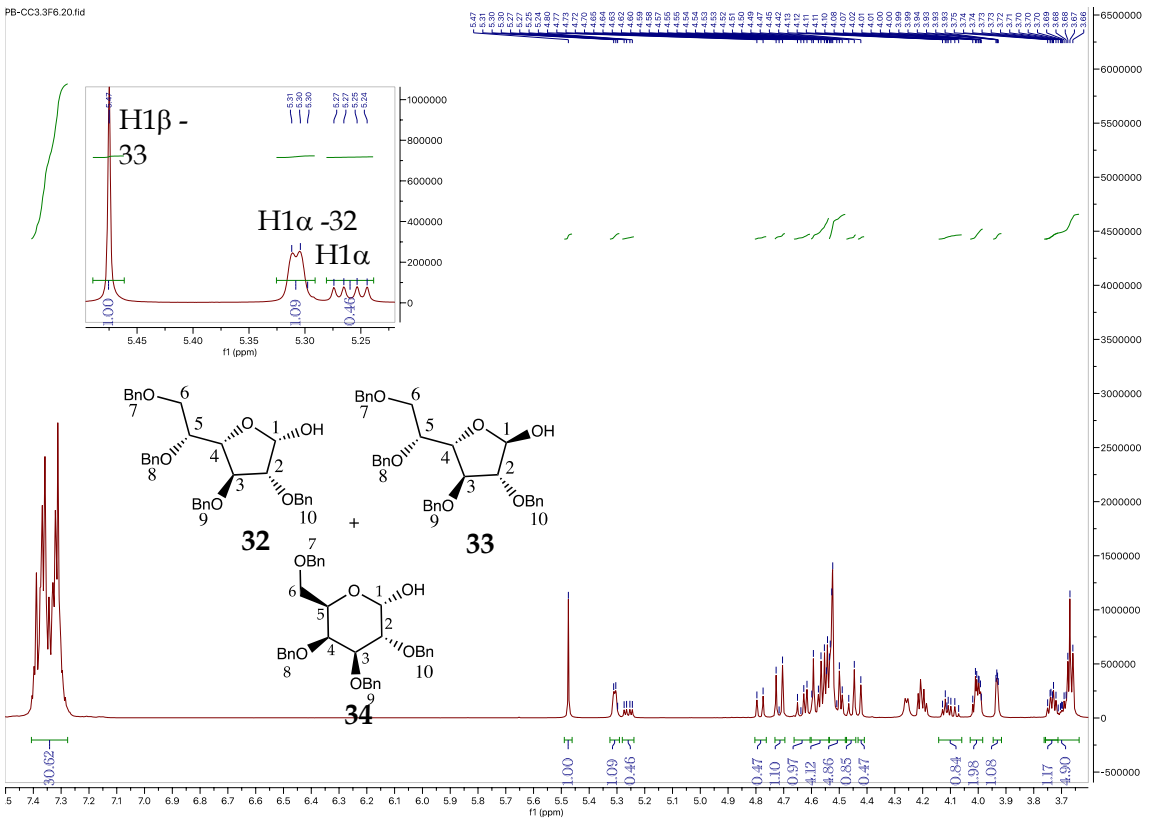


Figure 4.4 - ^{1}H NMR spectrum of compounds **32, 33** and **34** (400 MHz, CDCl3).

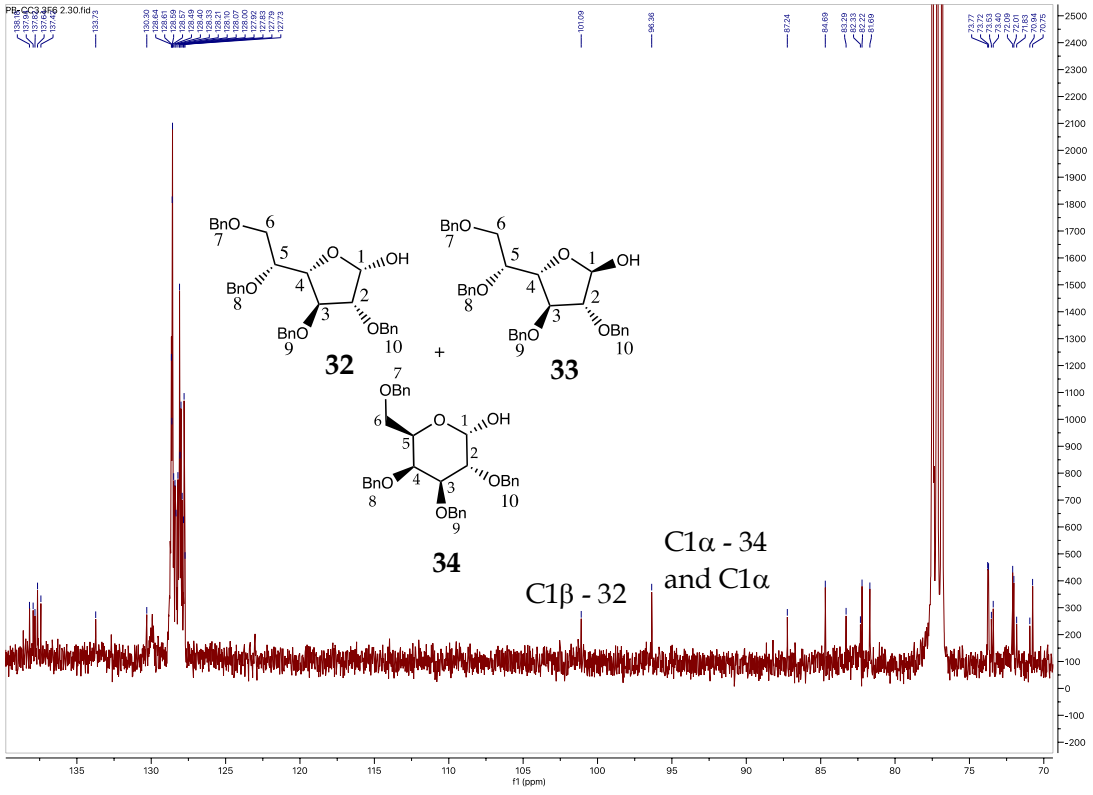


Figure 4.5 - ^{13}C NMR spectrum of compounds **32, 33** and **34** (101 MHz, CDCl3).

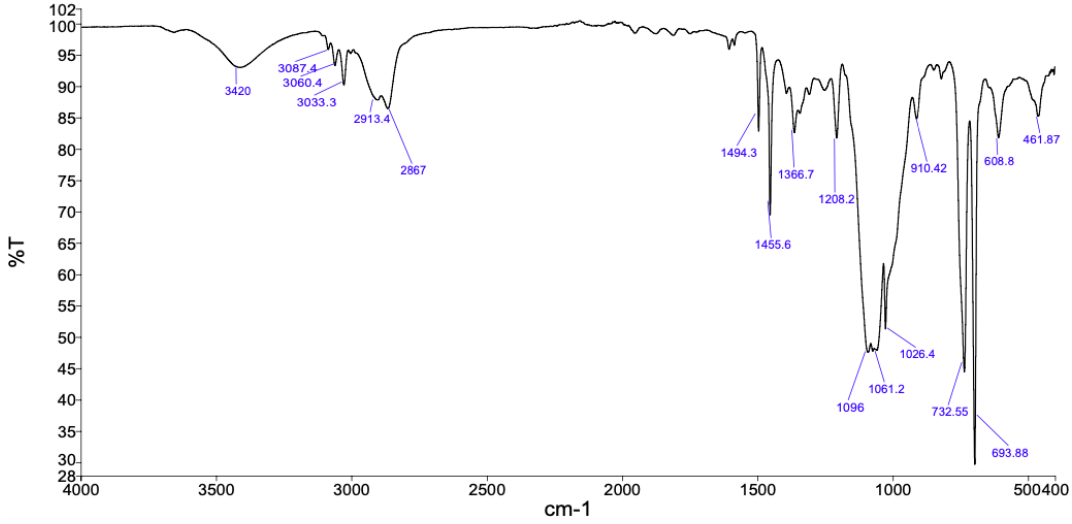


Figure 4.6 - IR spectrum of compounds **32, 33** and **34**

Appendix 3 - acetil-2,3,4,6-tetra-*O*-benzyl-β-D-galactofuranoside (35)

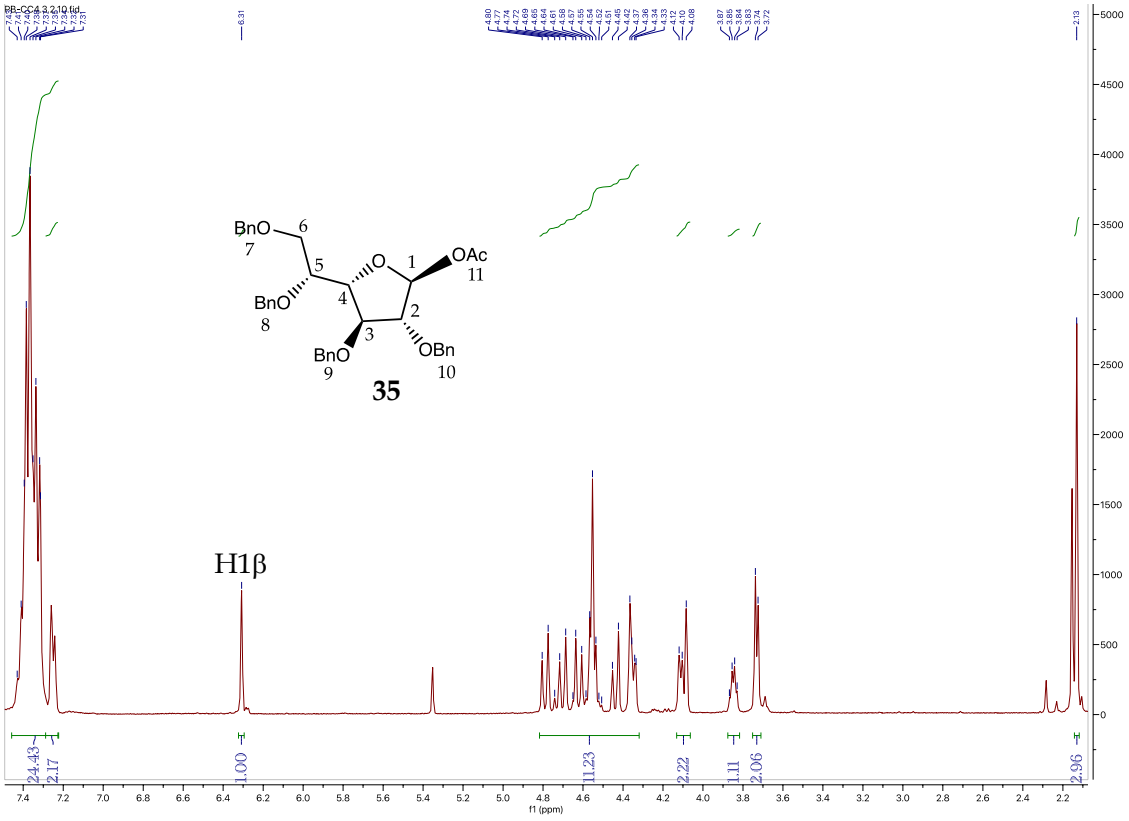


Figure 4.7 - ^{1}H NMR spectrum of compound **35** (400 MHz, CDCl3).

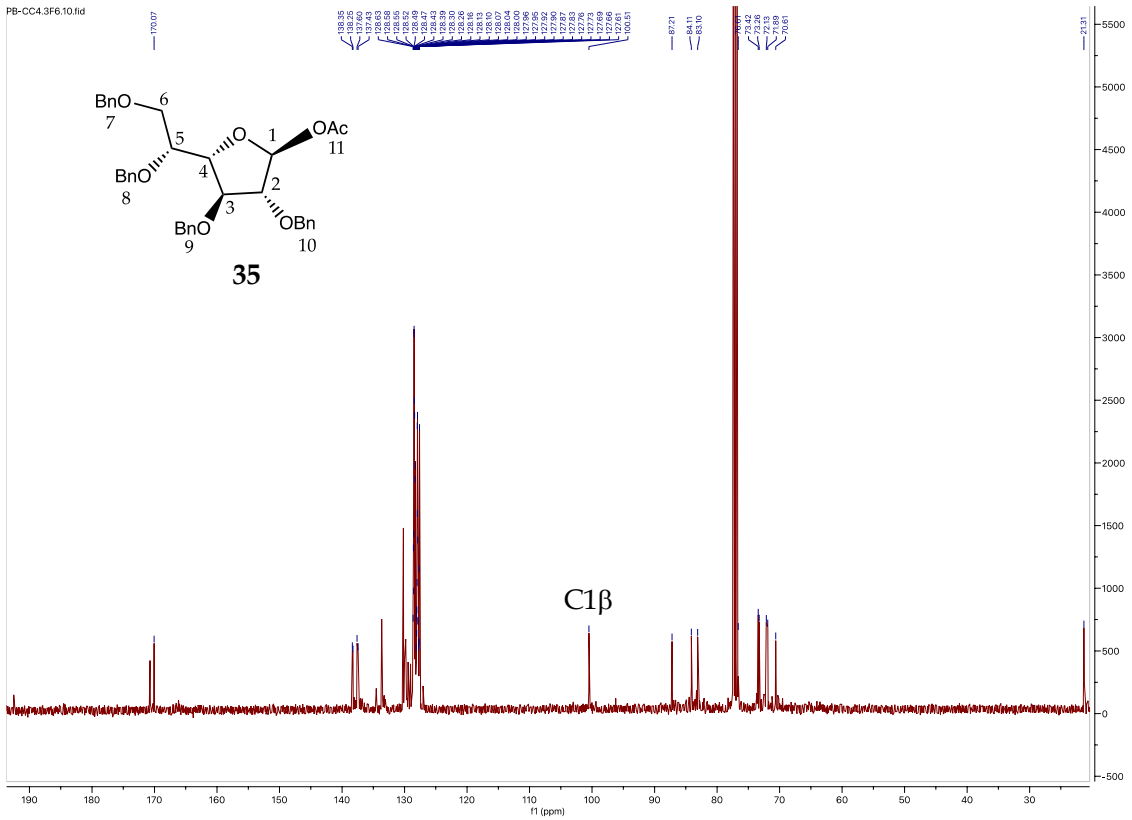


Figure 4.8 – ^{13}C NMR spectrum of compound **35** (101 MHz, CDCl3).

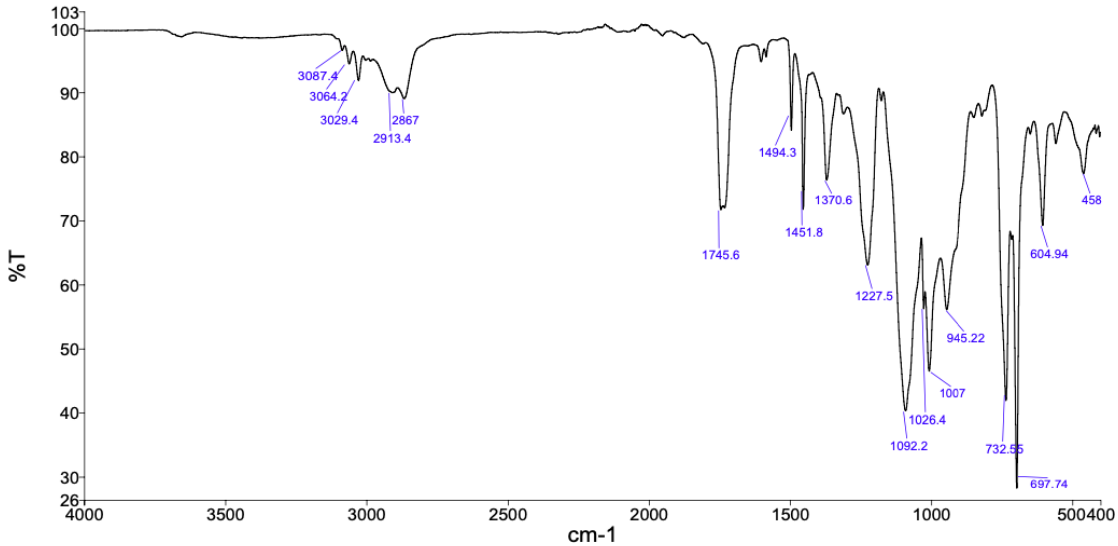


Figure 4.9 - IR spectrum of compound **35**.

Appendix 4 - 1-methyl-α-D-galactopyranoside (38), 1-methyl-β-D-galactopyranoside (39), 1-methyl-α-D-galactfuranoside (36), 1-methyl-β-D-galactofuranoside (37)

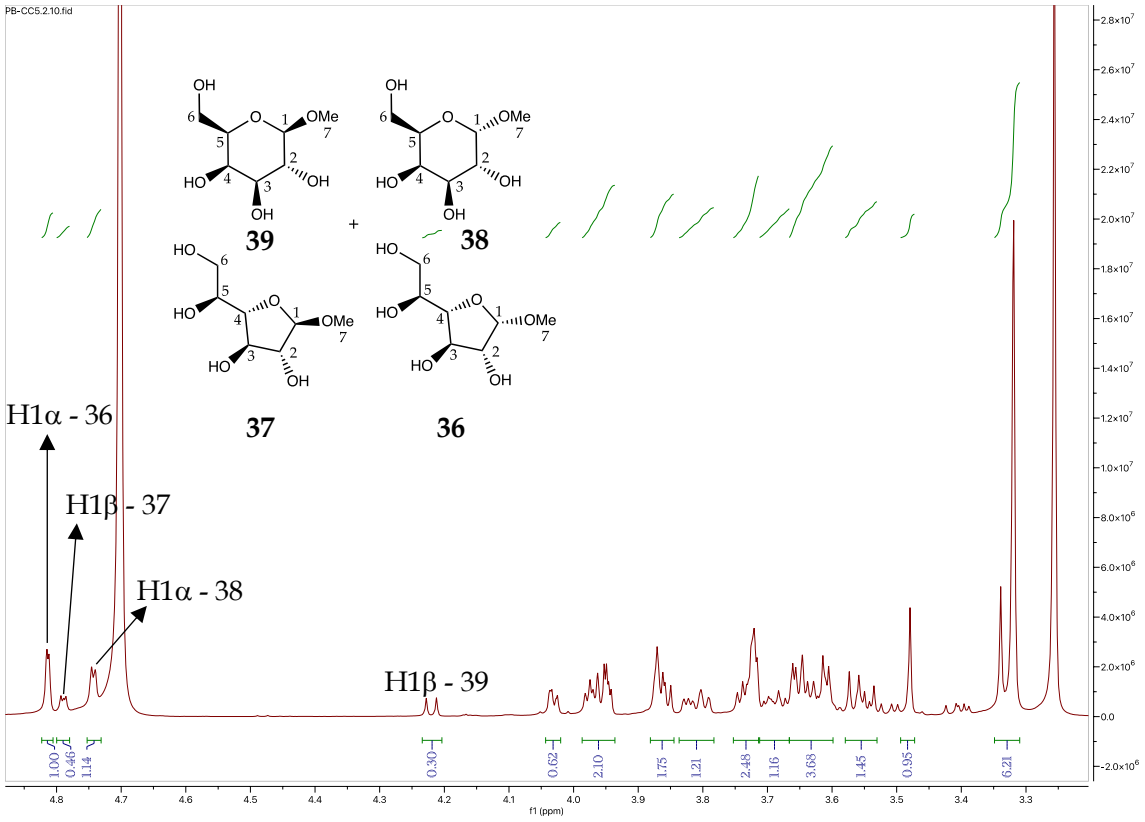


Figure 4.10 - ^{1}H NMR spectrum of compounds **36**, **37**, **38** and **39** (400 MHz, CDCl3).

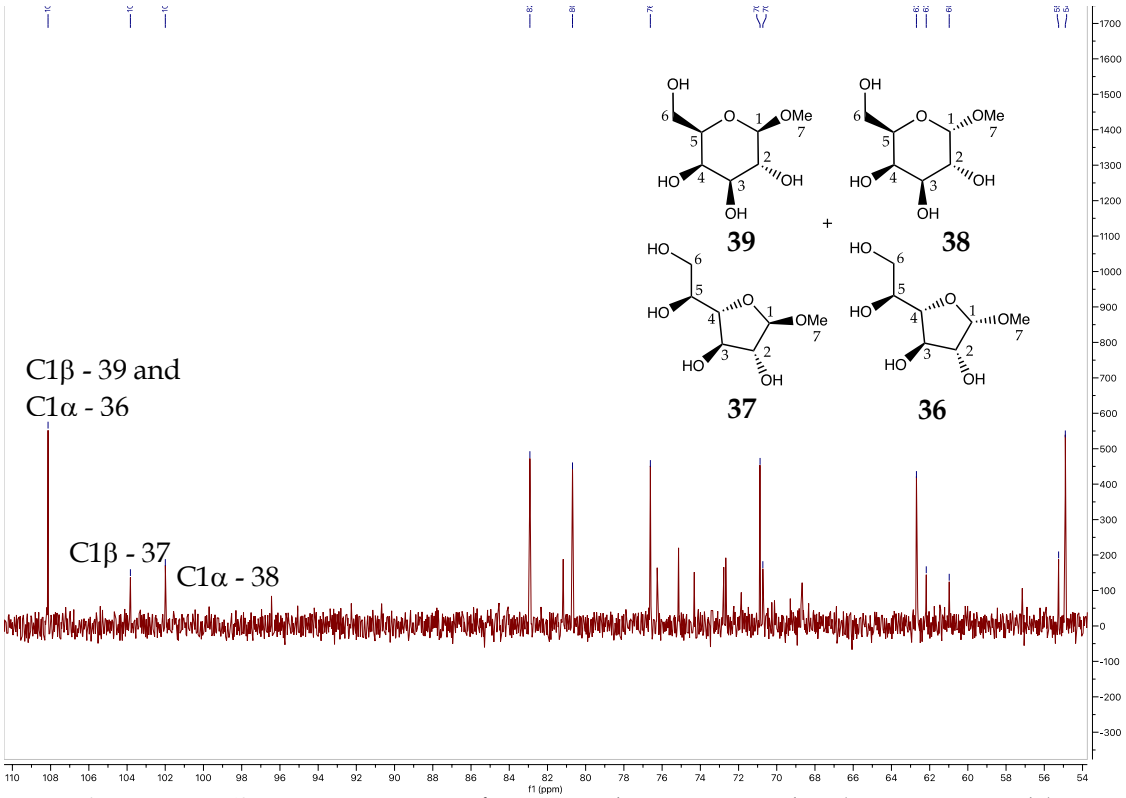


Figure 4.11 – ^{13}C NMR spectrum of compounds **36**, **37**, **38** and **39** (101 MHz, CDCl3).

Appendix 5 - 1-methyl-2,3,4,6-tetra-*O*-benzyl-b-D-galactopyranoside (40)

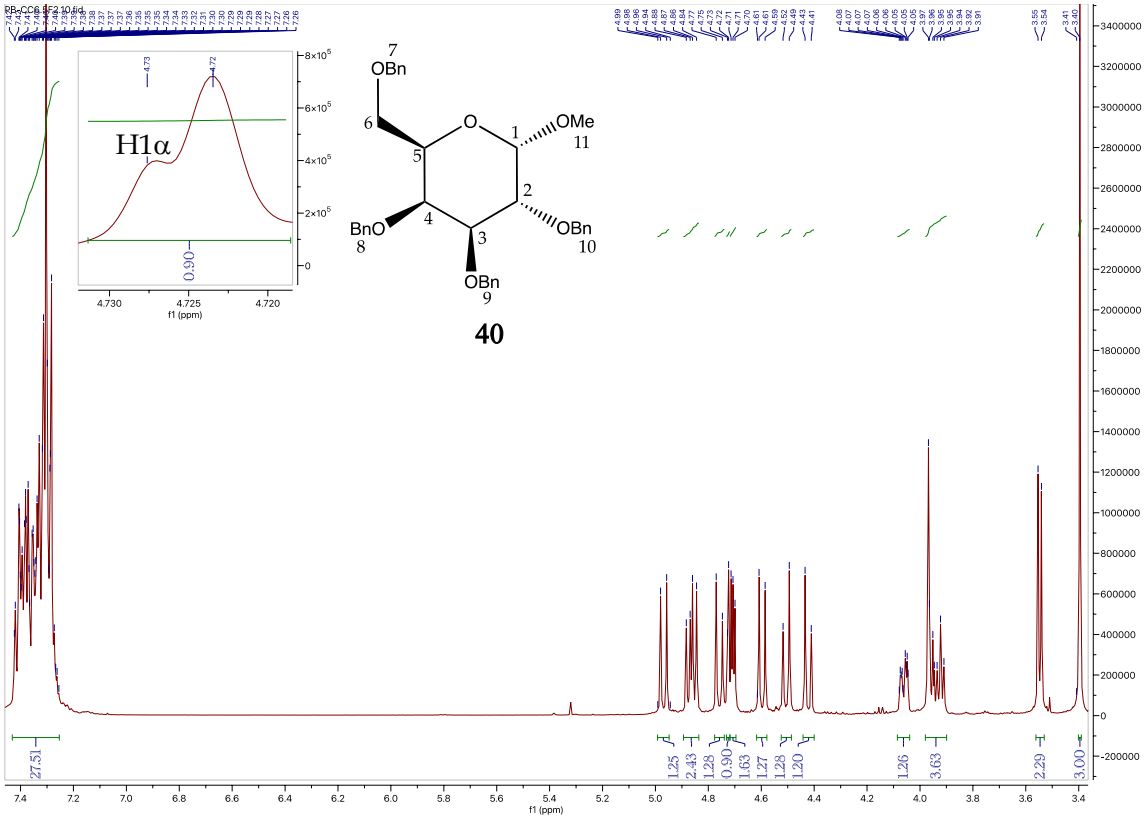


Figure 4.12 - ^{1}H NMR spectrum of compound **40** (400 MHz, CDCl3).

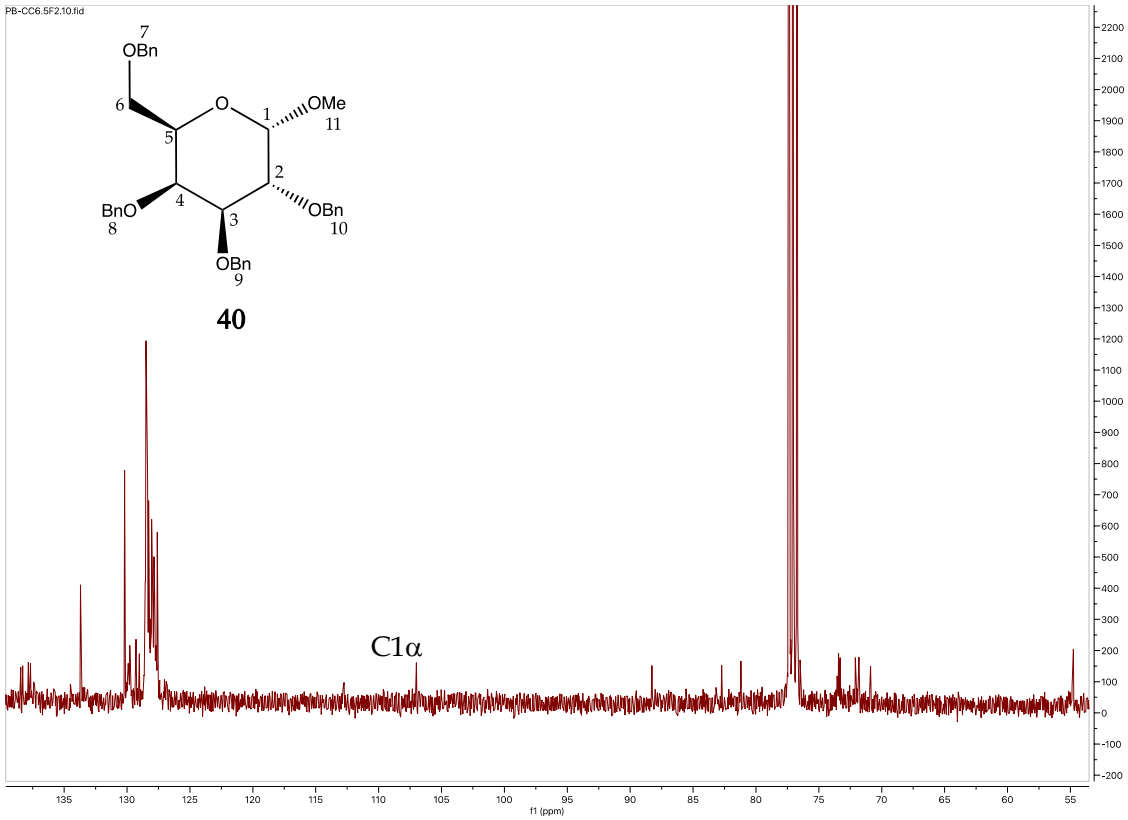


Figure 4.13 – ^{13}C NMR spectrum of compound **40** (101 MHz, CDCl3).

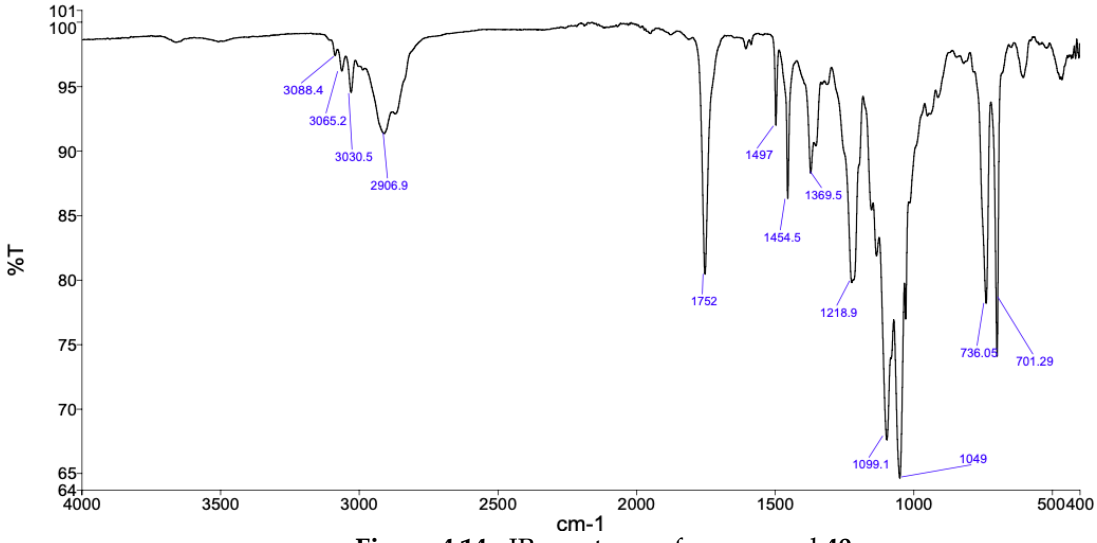


Figure 4.14 - IR spectrum of compound **40**.

Appendix 6 - penta-*O*-acetyl-a-D-galactopyranoside (42)

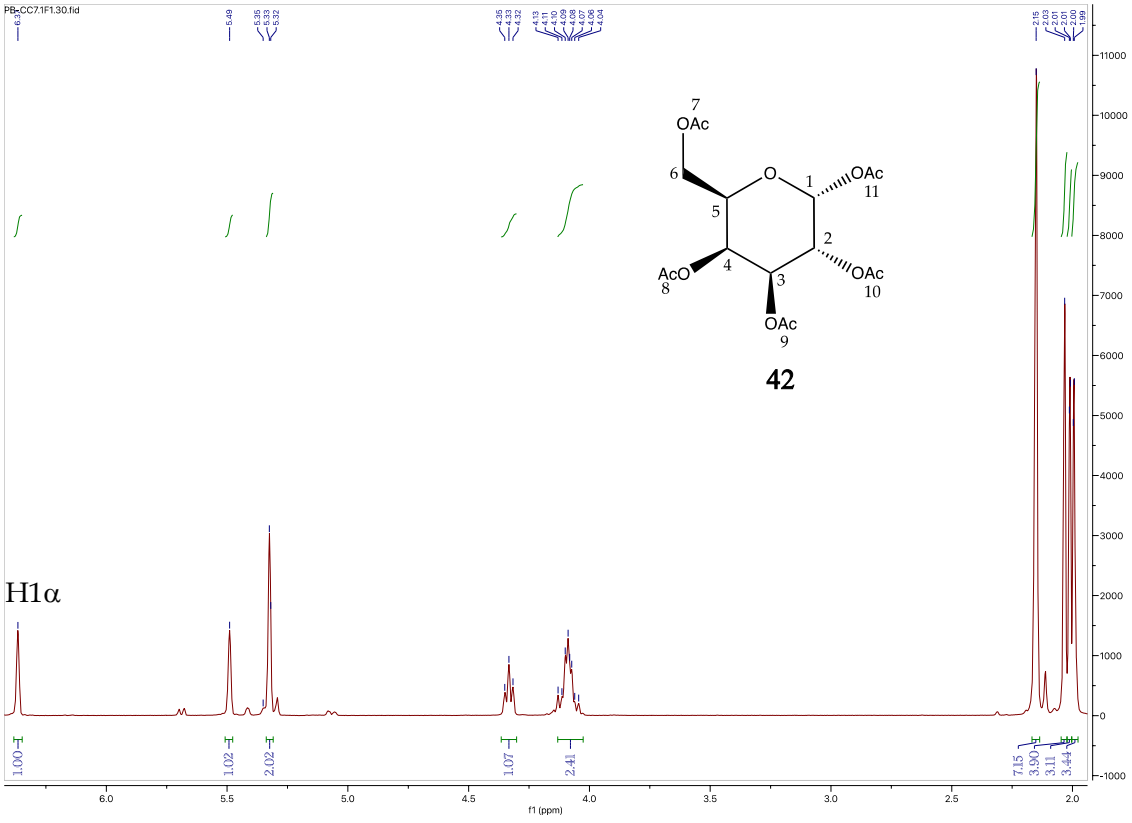


Figure 4.15 - ^{1}H NMR spectrum of compound **42** (400 MHz, CDCl3).

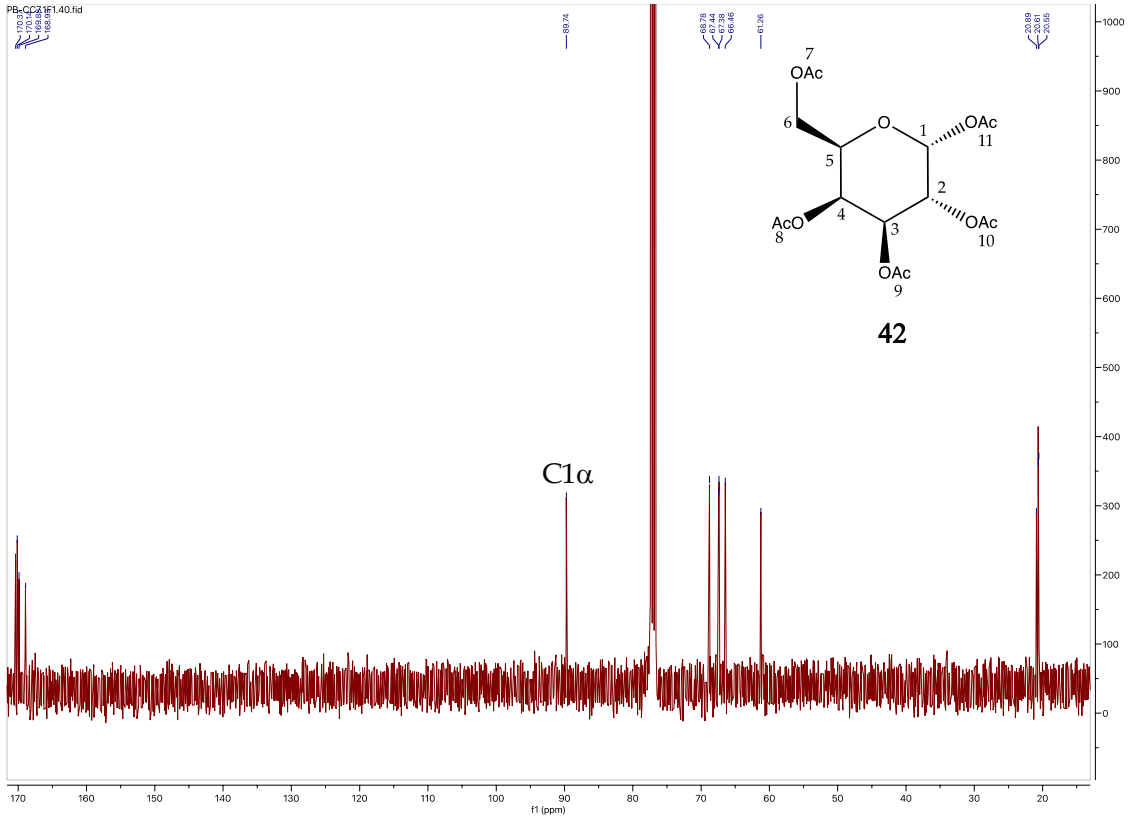


Figure 4.16 – ^{13}C NMR spectrum of compound **42** (101 MHz, CDCl3).

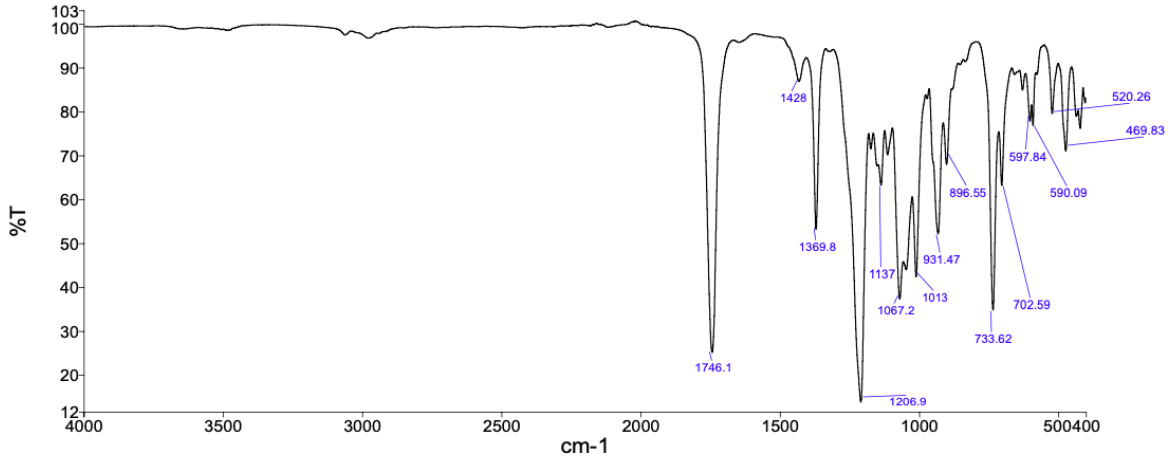


Figure 4.17 – IR spectrum of compound **42**.

Appendix 7 - phenyl-2,3,4,6-tetra-*O*-acetyl-1-thio-b-D-galactopynanoside (22)

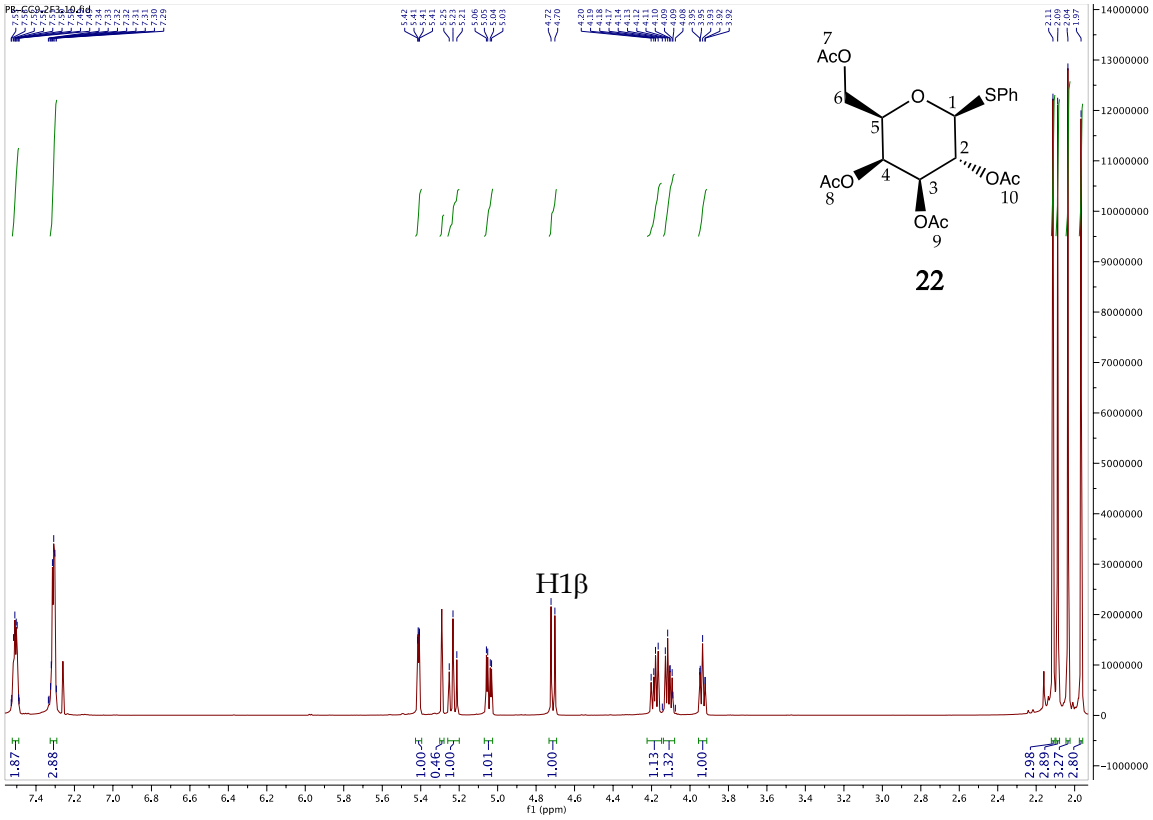


Figure 4.18 - ^{1}H NMR spectrum of compound **22** (400 MHz, CDCl3).

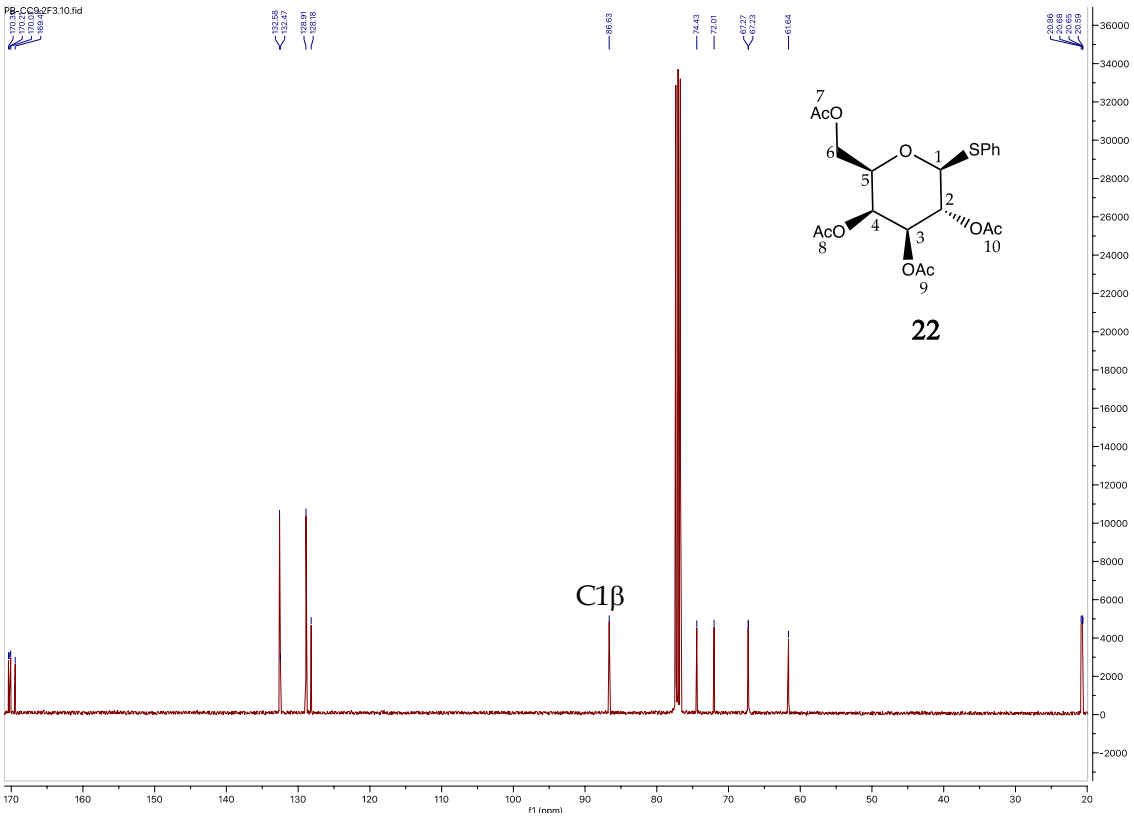


Figure 4.19 – ^{13}C NMR spectrum of compound **22** (101 MHz, CDCl3).

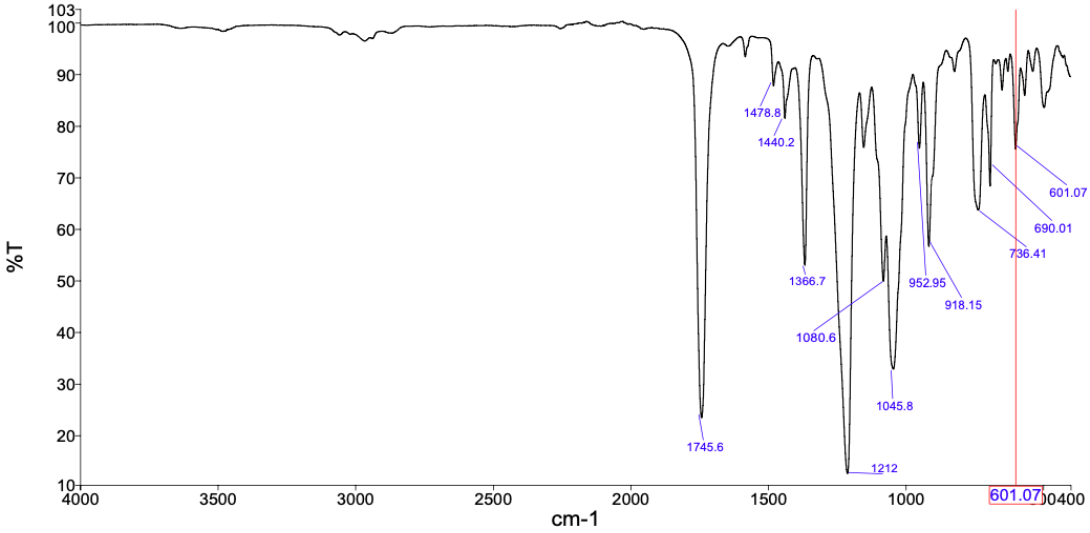


Figure 4.20 - IR spectrum of compound **22**.

Appendix 8 - 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranoside 34

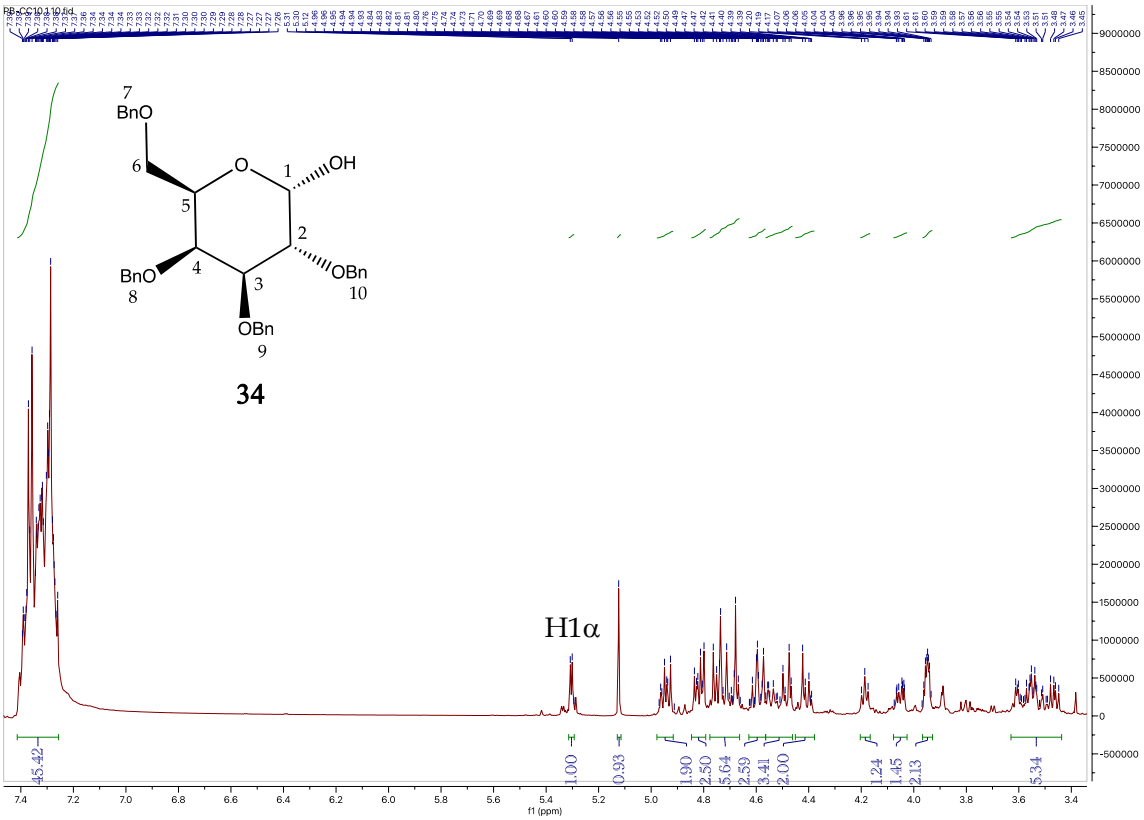


Figure 4.21 - ^{1}H NMR spectrum of compound **34** (400 MHz, CDCl3).

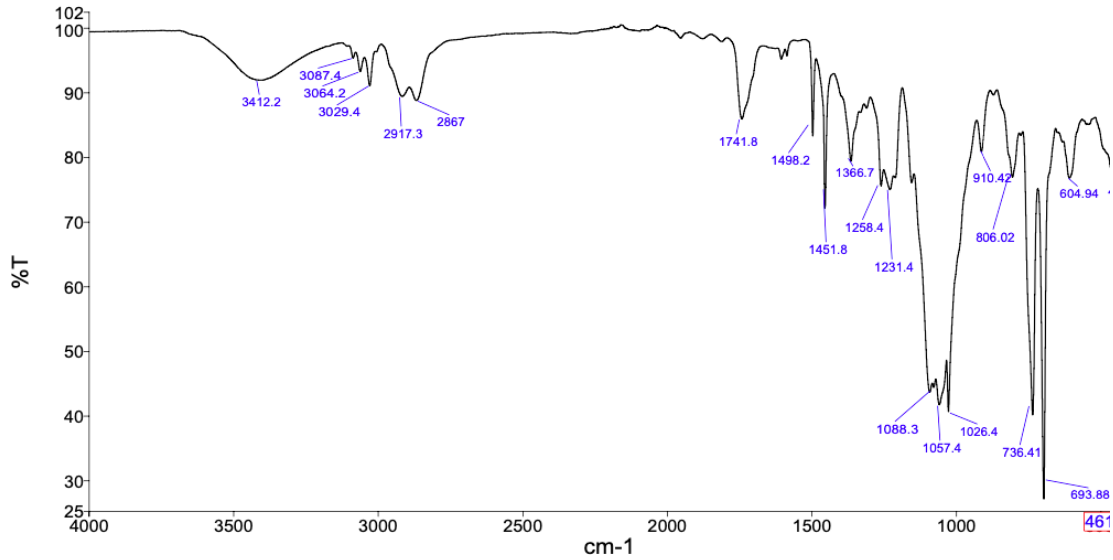


Figure 4.22 - IR spectrum of compound **34**.

Appendix 9 - phenyl-2,3,4,6-tetra-*O*-benzyl-1-thio-a-D-galactopynanoside (23)

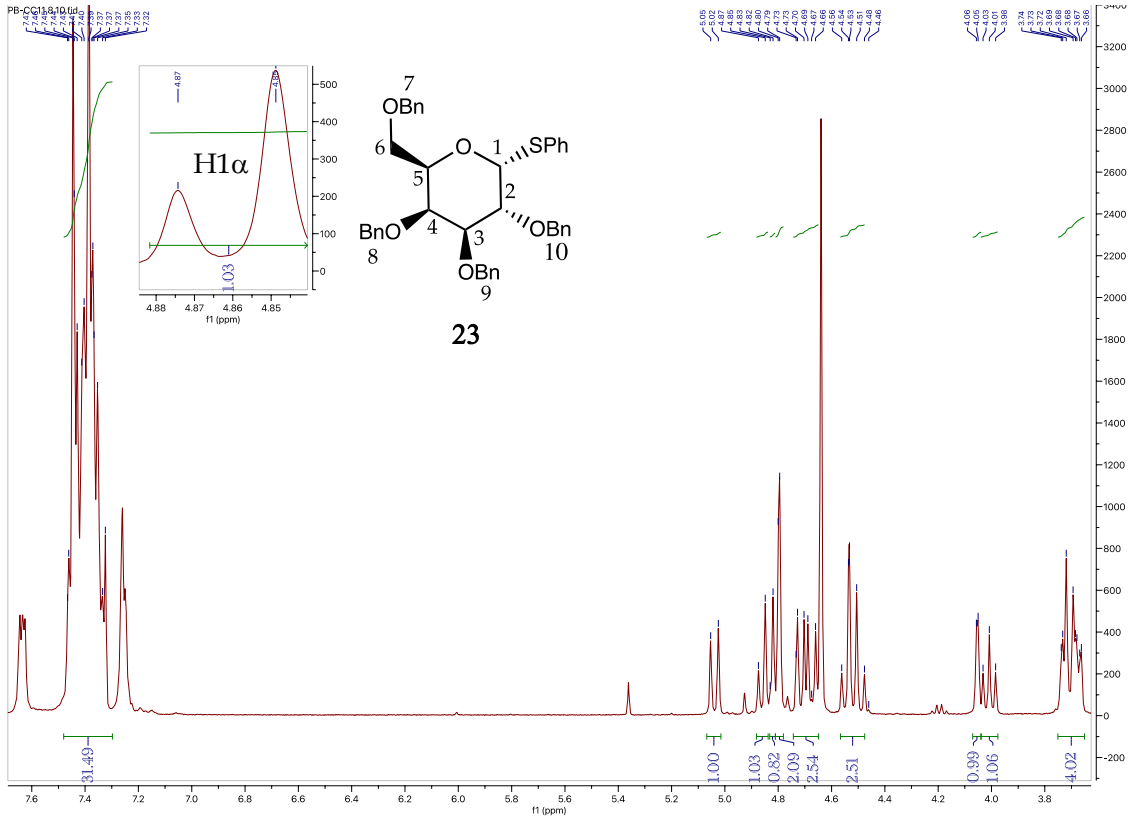


Figure 4.23 - ^{1}H NMR spectrum of compound **23** (400 MHz, CDCl3).

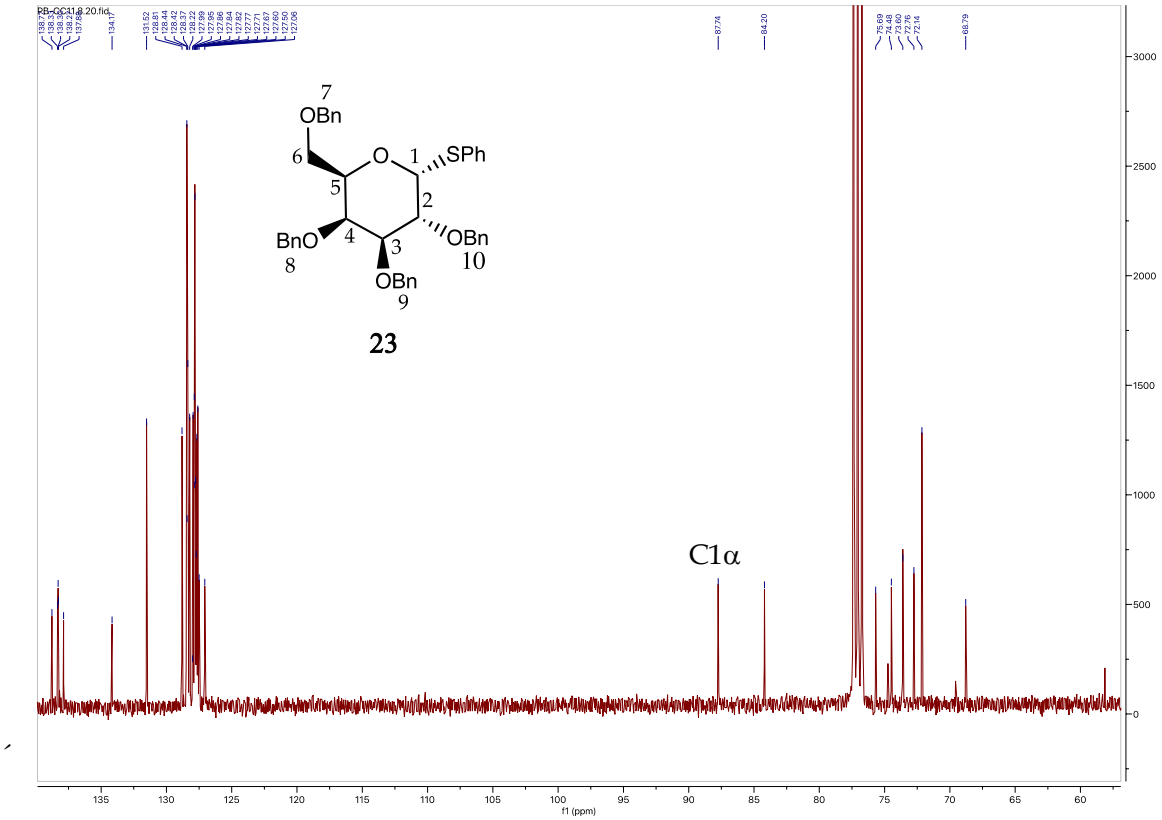


Figure 4.24 – ^{13}C NMR spectrum of compound **23** (101 MHz, CDCl3).

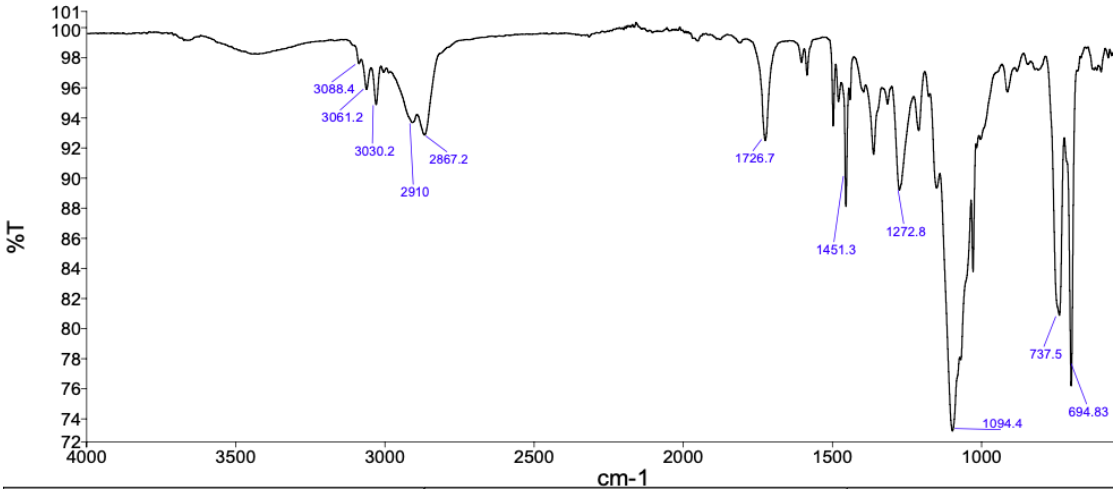


Figure 4.25 – IR spectrum of compound **23**.

Appendix 10 - phenyl-2,3,4,6-tetra-*O*-benzyl-1-thio-b-D-galactopynanoside (22)

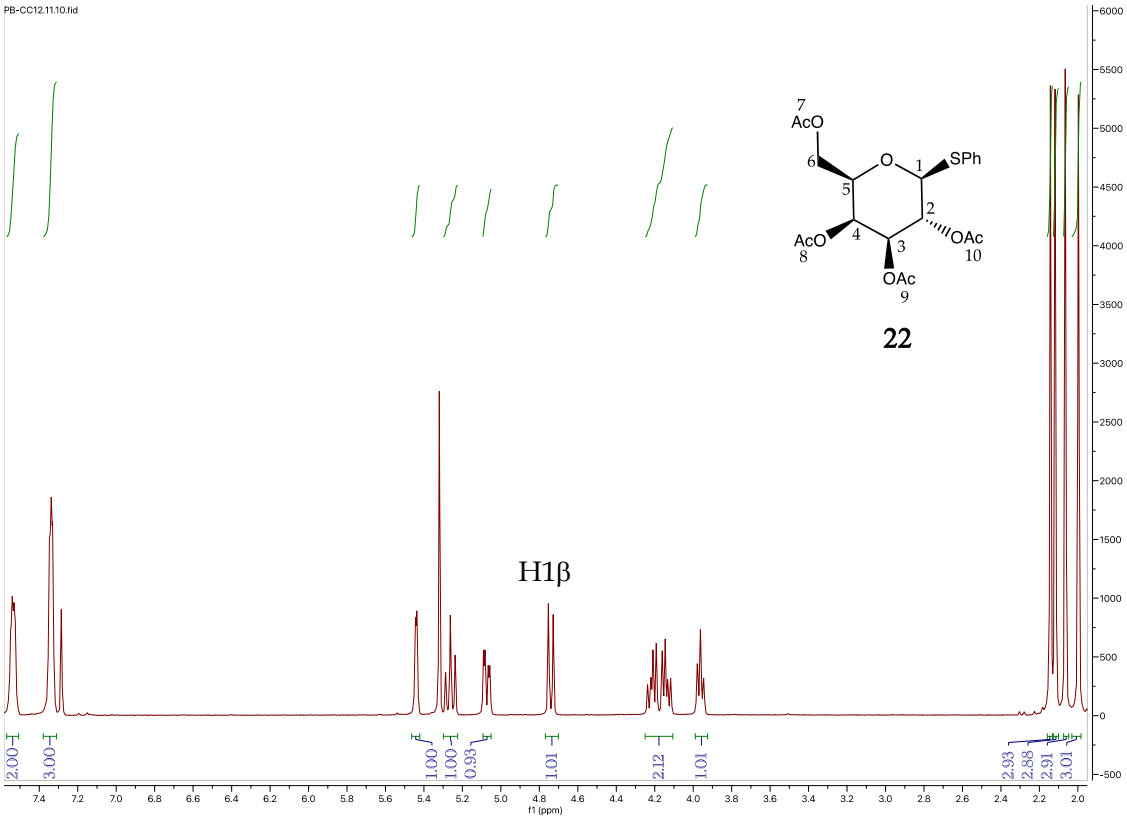


Figure 4.26 - ^{1}H NMR spectrum of compound **22** (400 MHz, CDCl3).

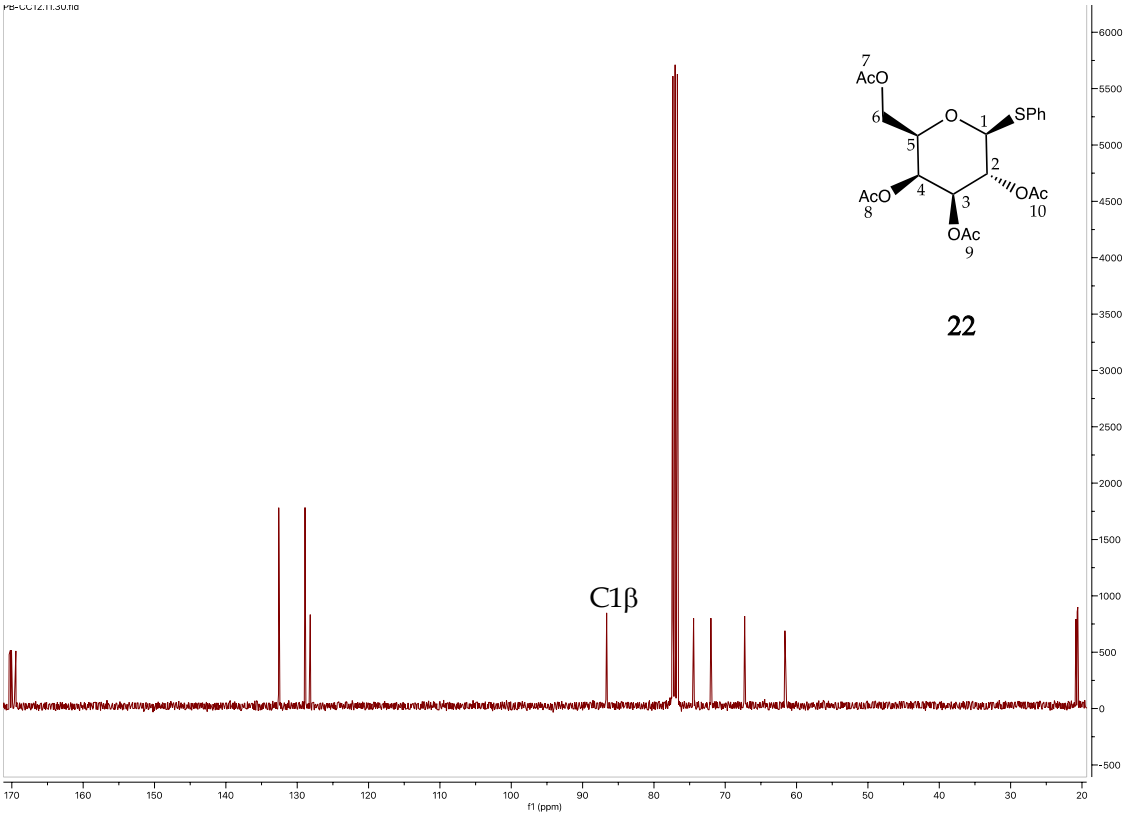


Figure 4.27 – ^{13}C NMR spectrum of compound **22** (101 MHz, CDCl3).

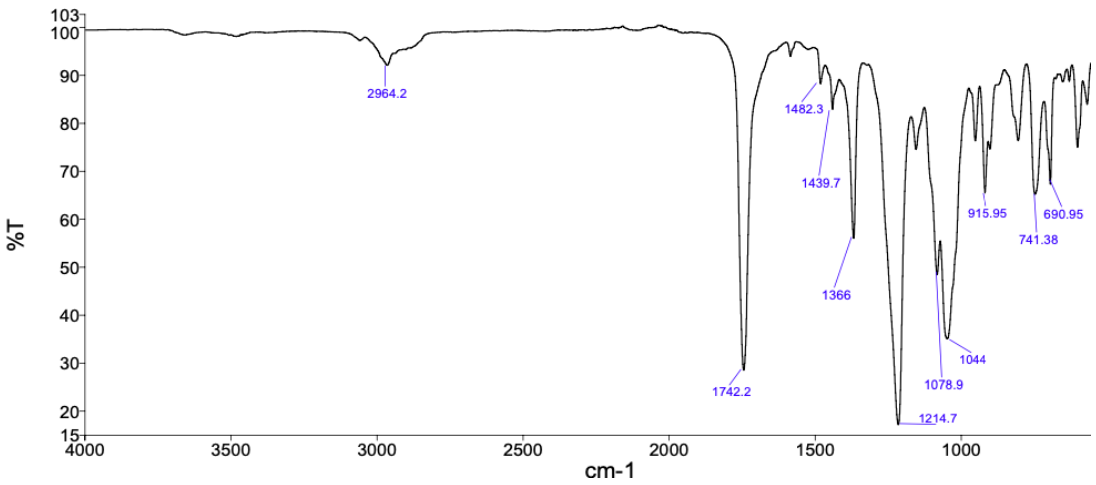


Figure 4.28 – IR spectrum of compound **22**.

Appendix 11 - 2,3,4,6-tetra-*O*-benzyl-a-D-galactopynanoside 34

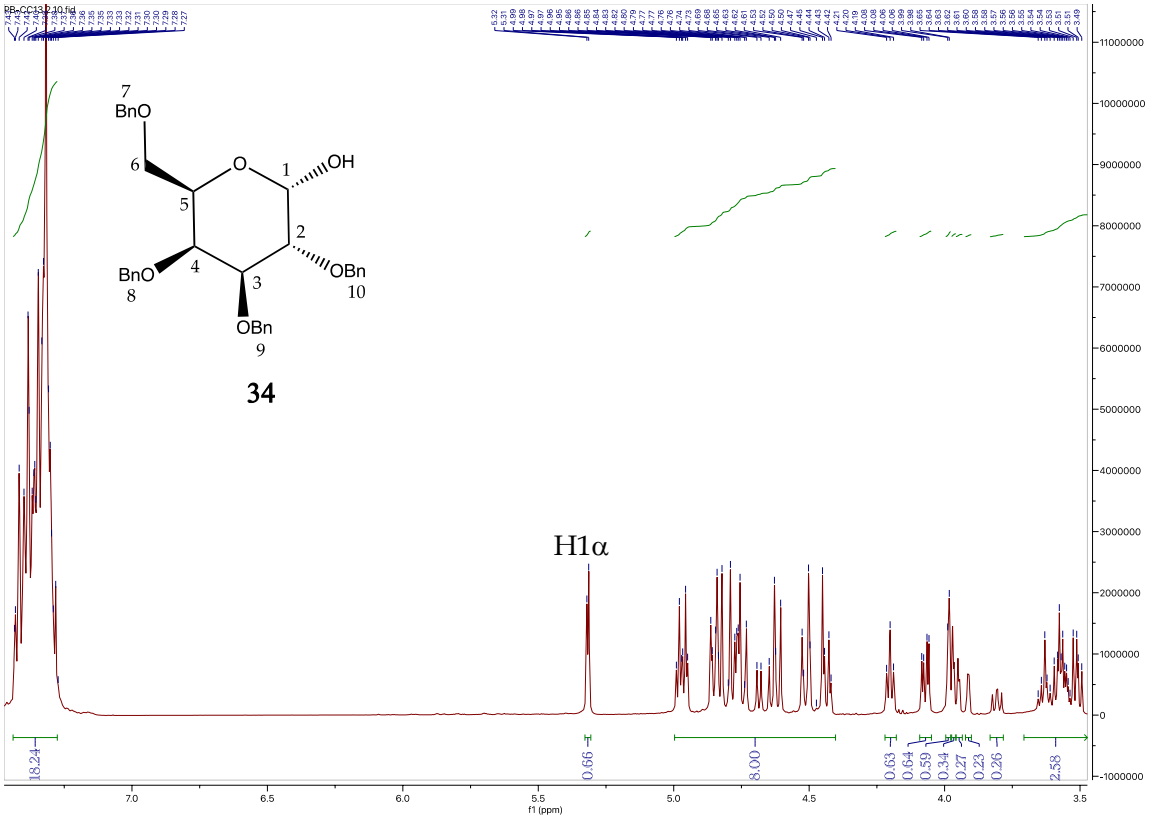


Figure 4.29 - ^{1}H NMR spectrum of compound **34** (400 MHz, CDCl3).

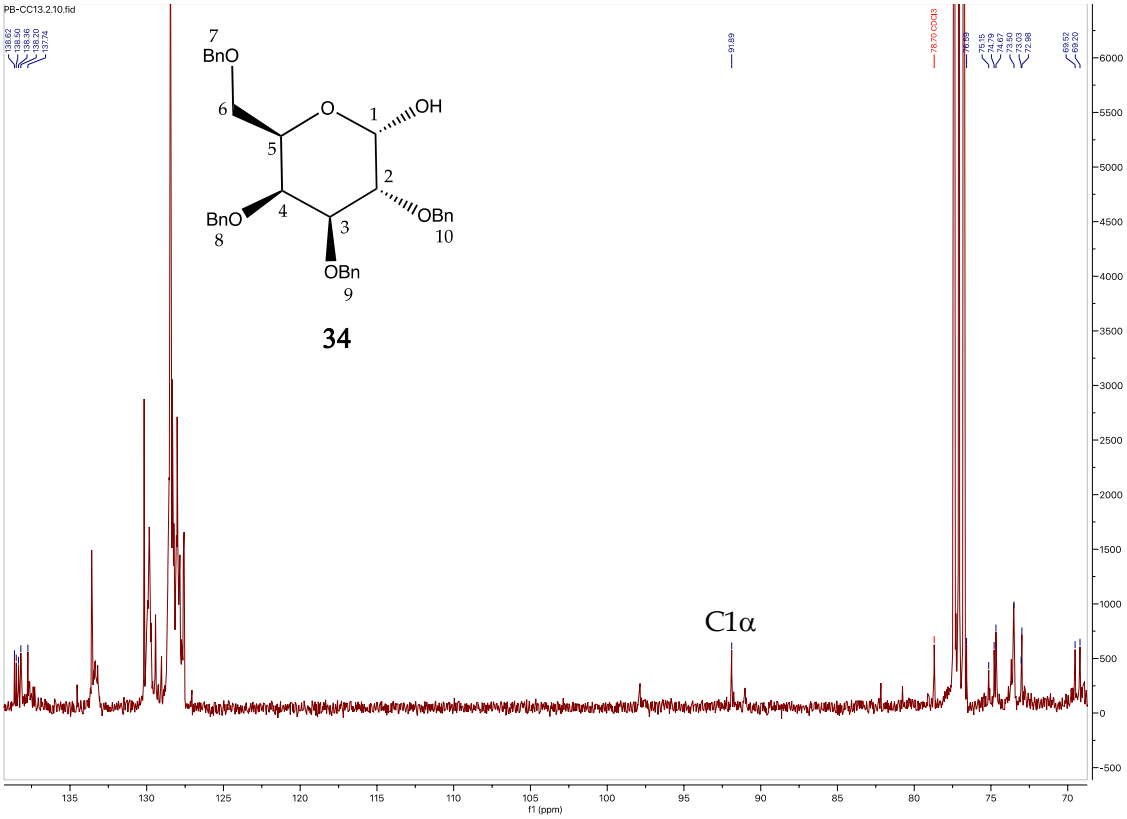


Figure 4.30 – ^{13}C NMR spectrum of compound **34** (101 MHz, CDCl3).

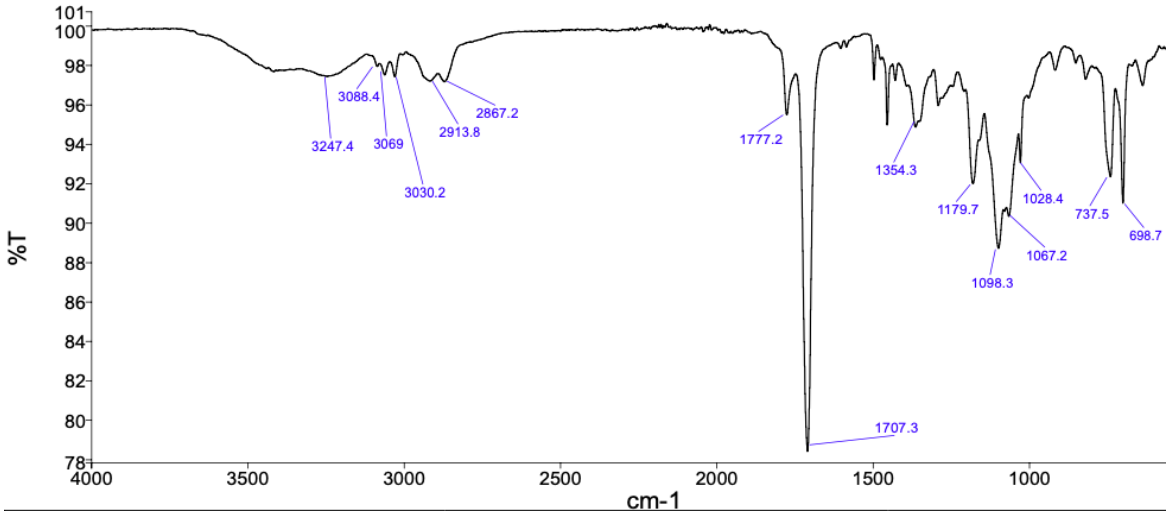


Figure 4.31 – IR spectrum of compound **34**.

Appendix 12 - 2,3,4,6-tetra-*O*-benzyl-D-galactopynanoside fluoride (24, 25)

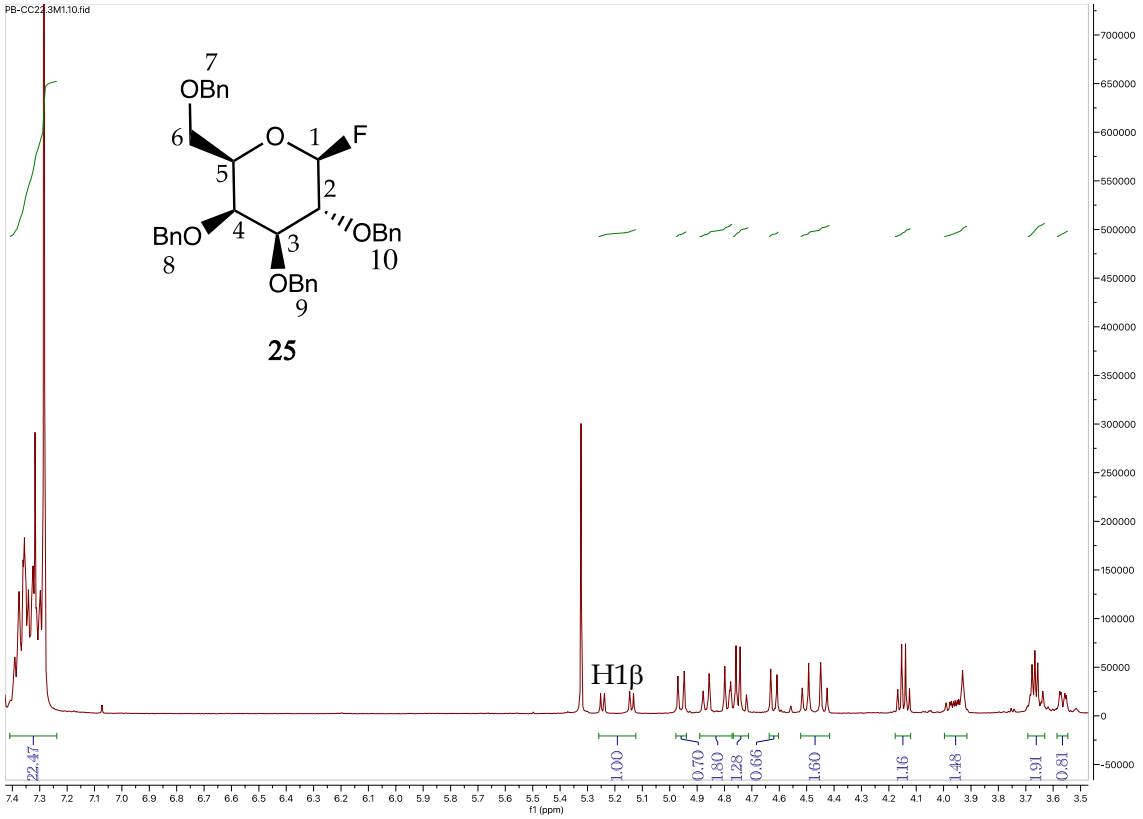


Figure 4.32 - 1H NMR spectrum of compound **25** (400 MHz, CDCl3).

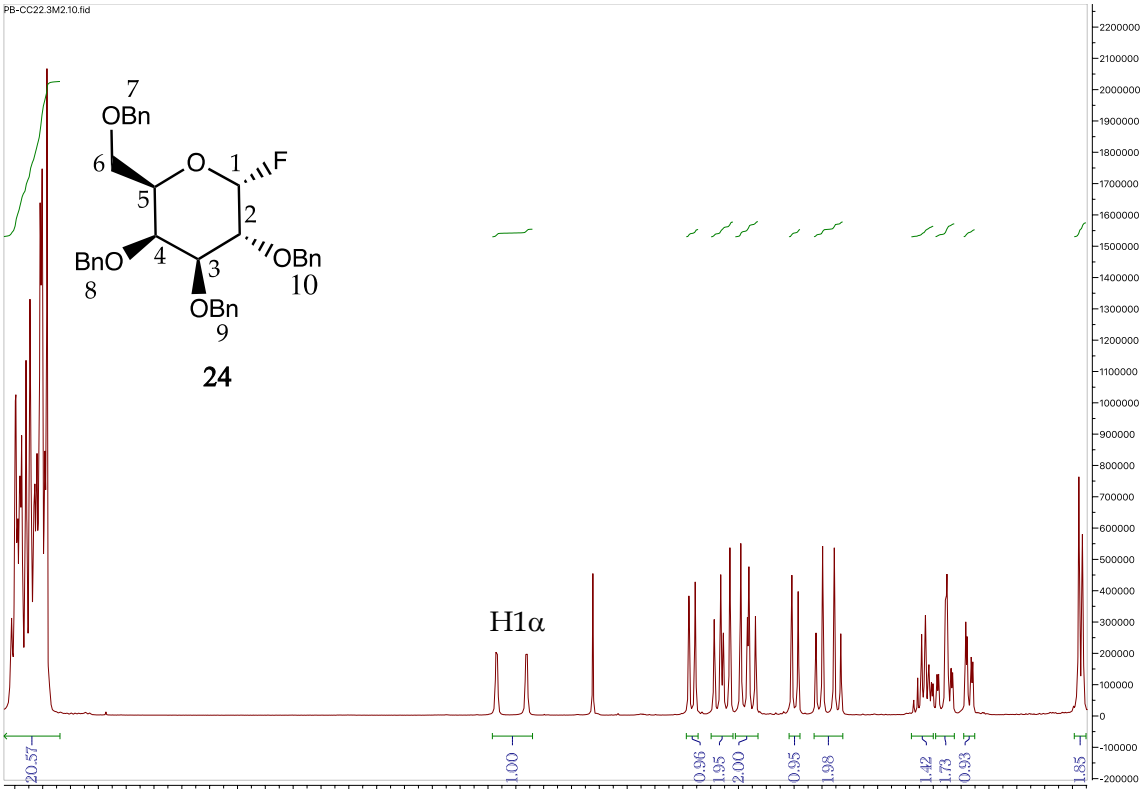


Figure 4.33 –^{13}C NMR spectrum of compound **24** (101 MHz, CDCl3).

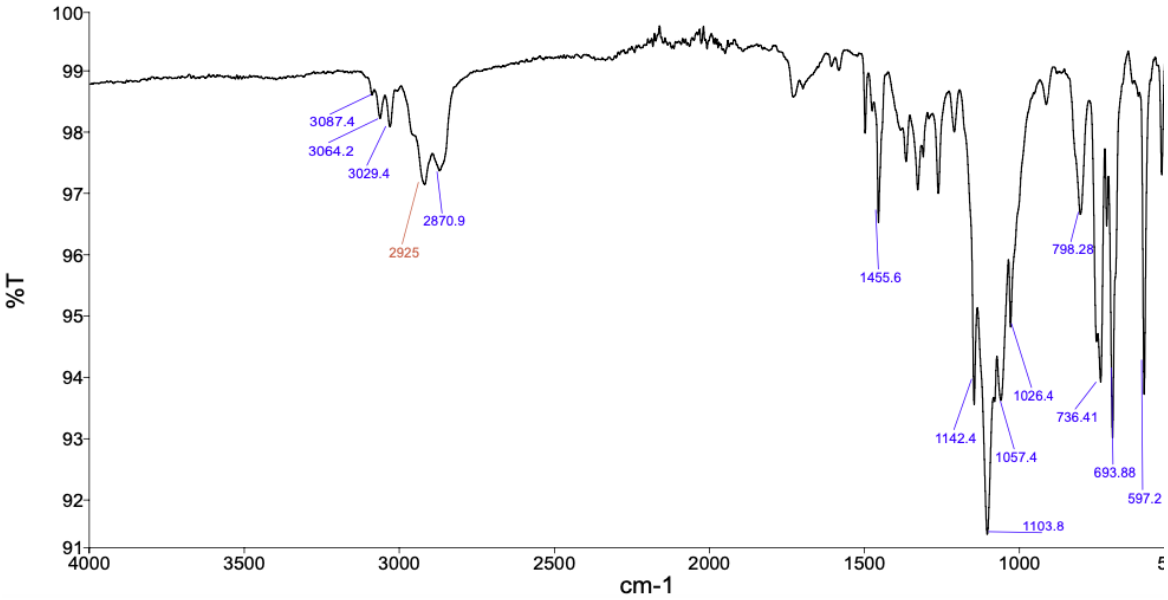


Figure 4.34 – IR spectrum of compounds **24** and **25**.

Appendix 13 - ((S)-4-(benzyloxymethyl)-2,2-dimethyl-1,3-dioxolane) (46)

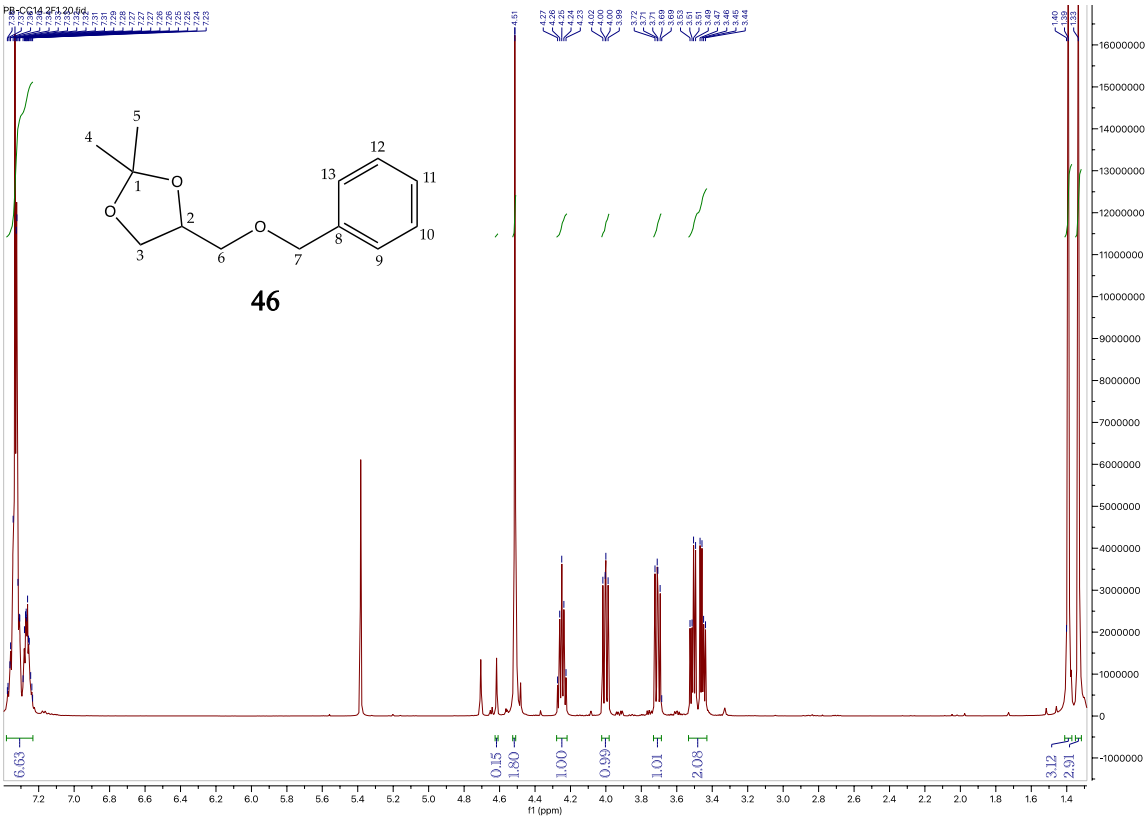


Figure 4.35 - ^{1}H NMR spectrum of compound **46** obtained with method A (400 MHz, CDCl3).

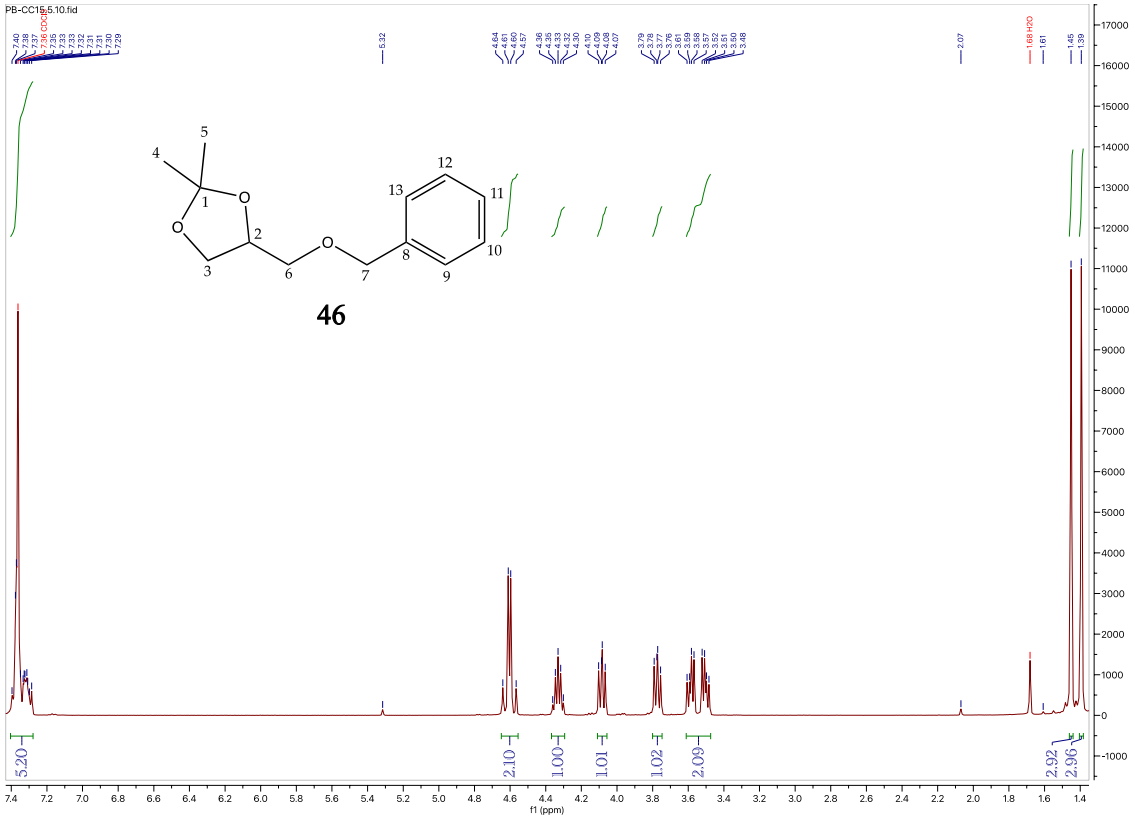


Figure 4.36 - ^{1}H NMR spectrum of compound **46** obtained with method B (400 MHz, CDCl3).

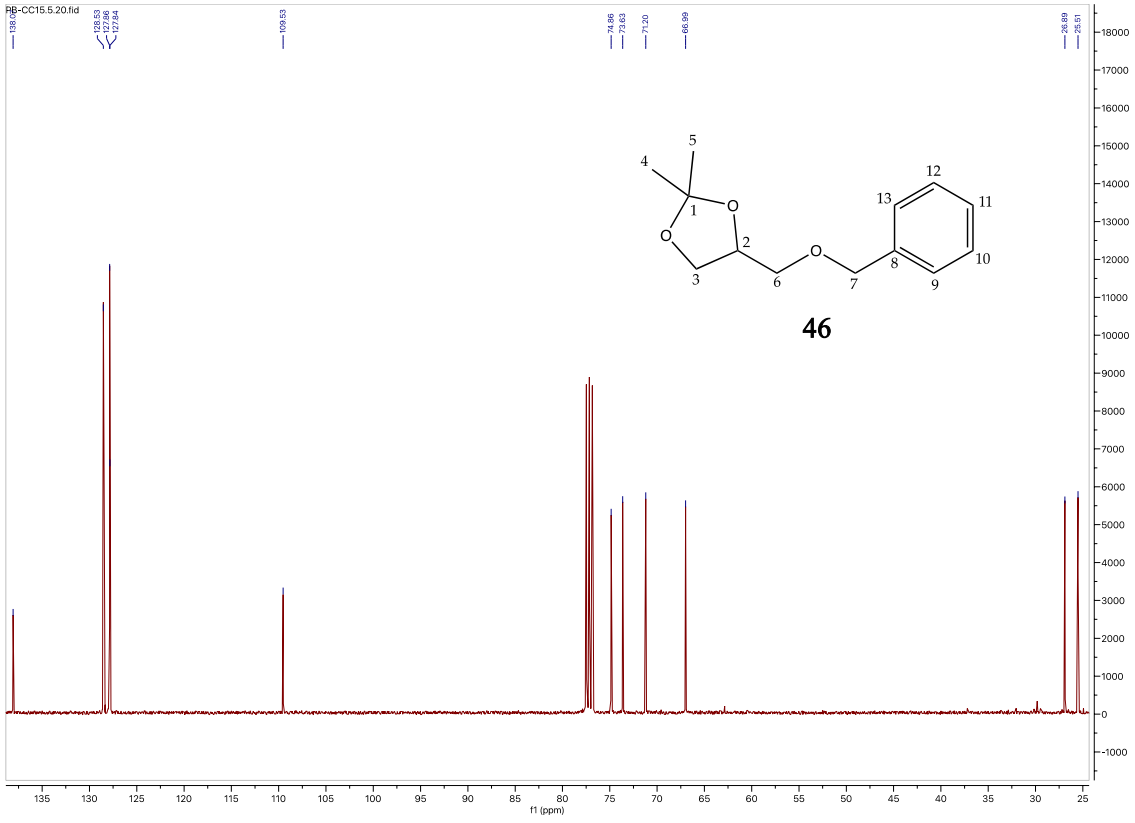


Figure 4.37 – ^{13}C NMR spectrum of compound **46** (101 MHz, CDCl3).

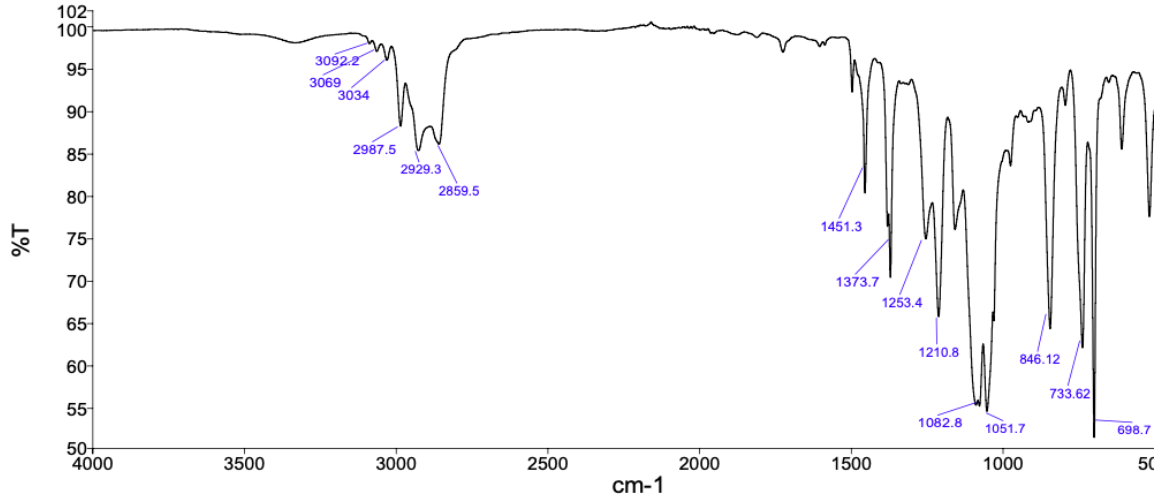


Figure 4.38 - IR spectrum of compound **46**.

Appendix 14 - 3-(benzyloxy)propane-1,2-diol (26)

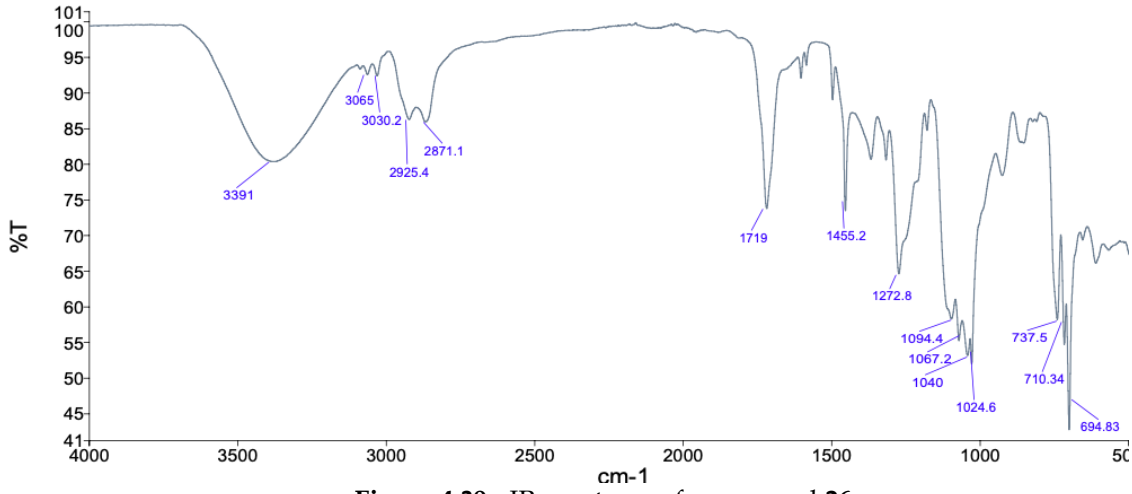


Figure 4.39 - IR spectrum of compound **26.**

Appendix 15 – 1-Benzyloxy-3-(*tert*-butyldiphenylsilyloxy)propan-2-ol (18)

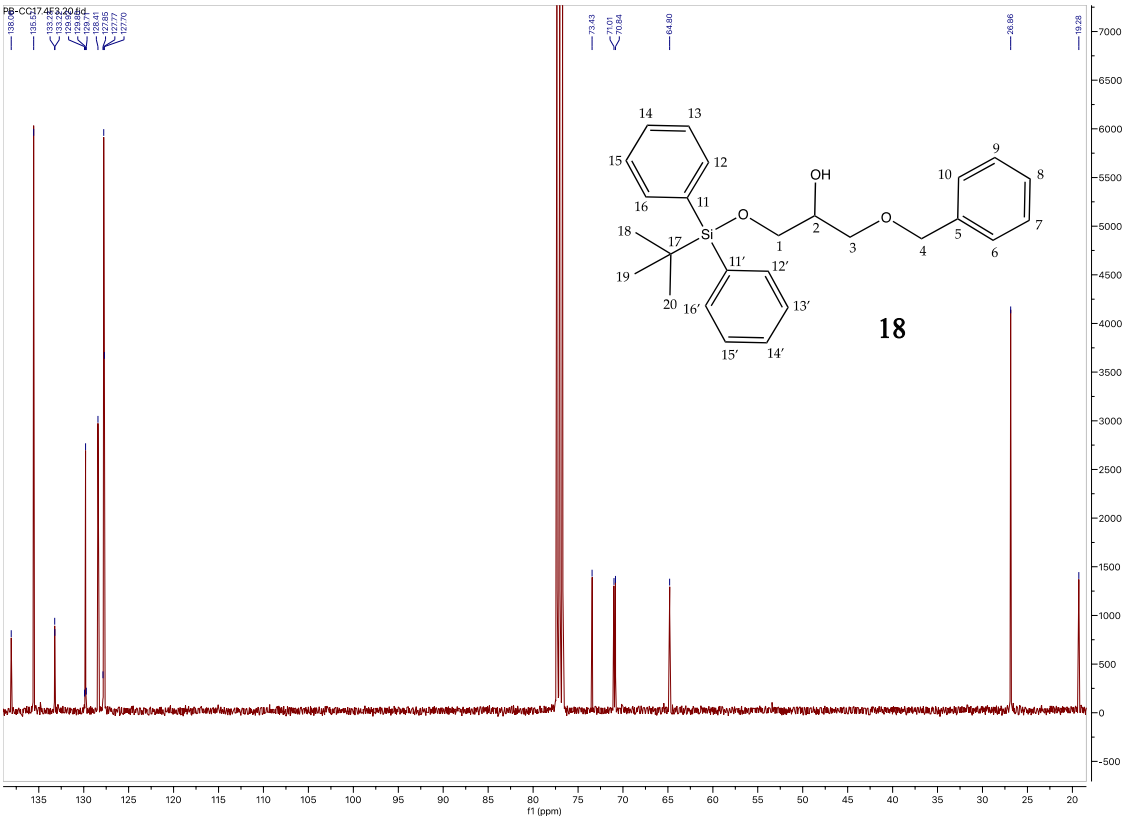


Figure 4.40 – ^{13}C NMR spectrum of compound **18** (400 MHz, CDCl3).

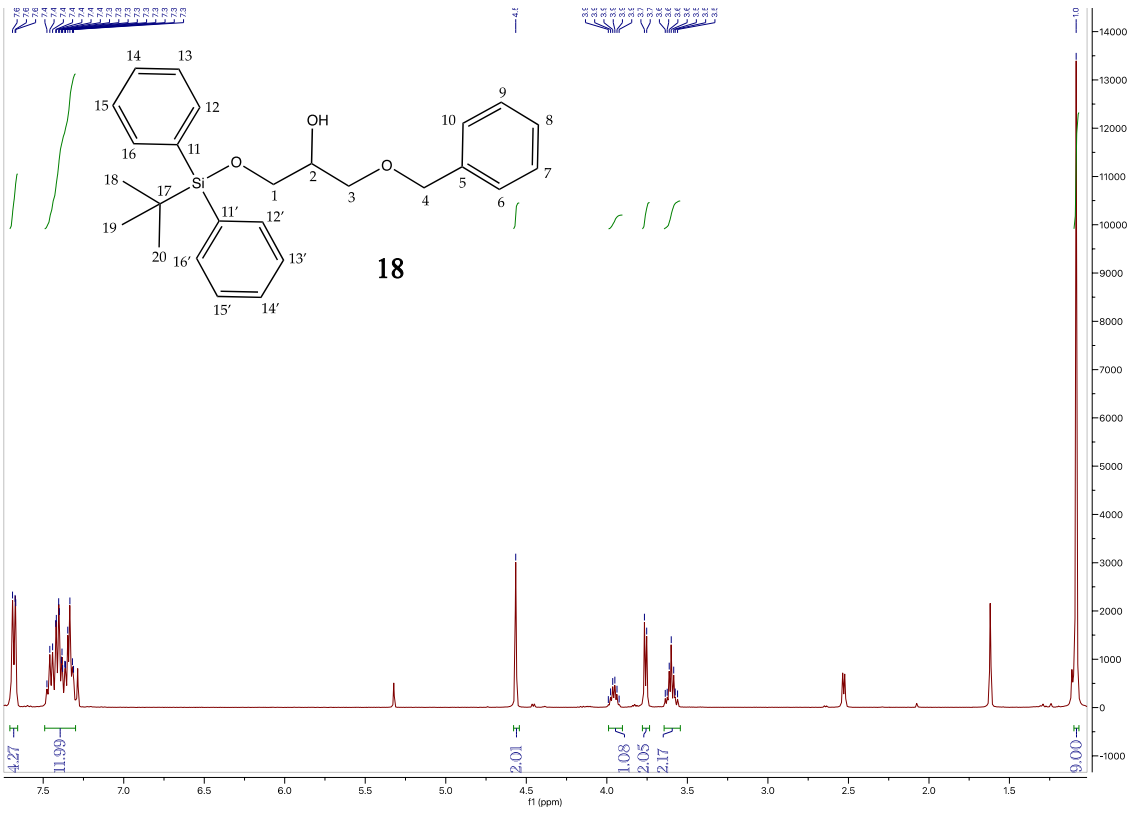


Figure 4.40 – ^{13}C NMR spectrum of compound **18** (101 MHz, CDCl3).

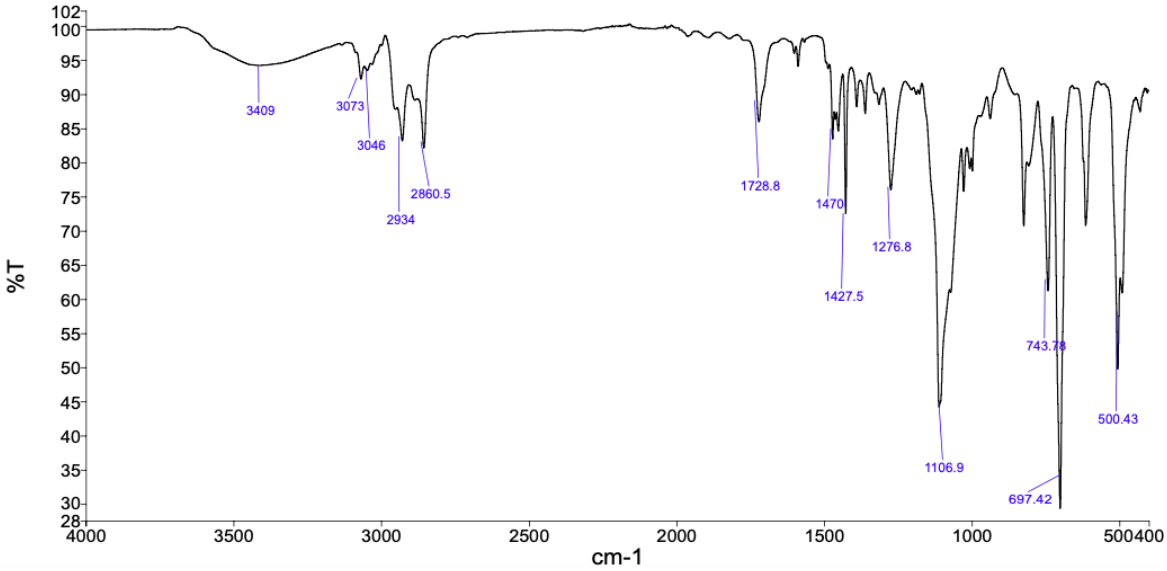


Figure 4.41 - IR spectrum of compound **18**.

Appendix 16 - 3-(benzyloxy)-2-hydroxypropyl acetate (27)

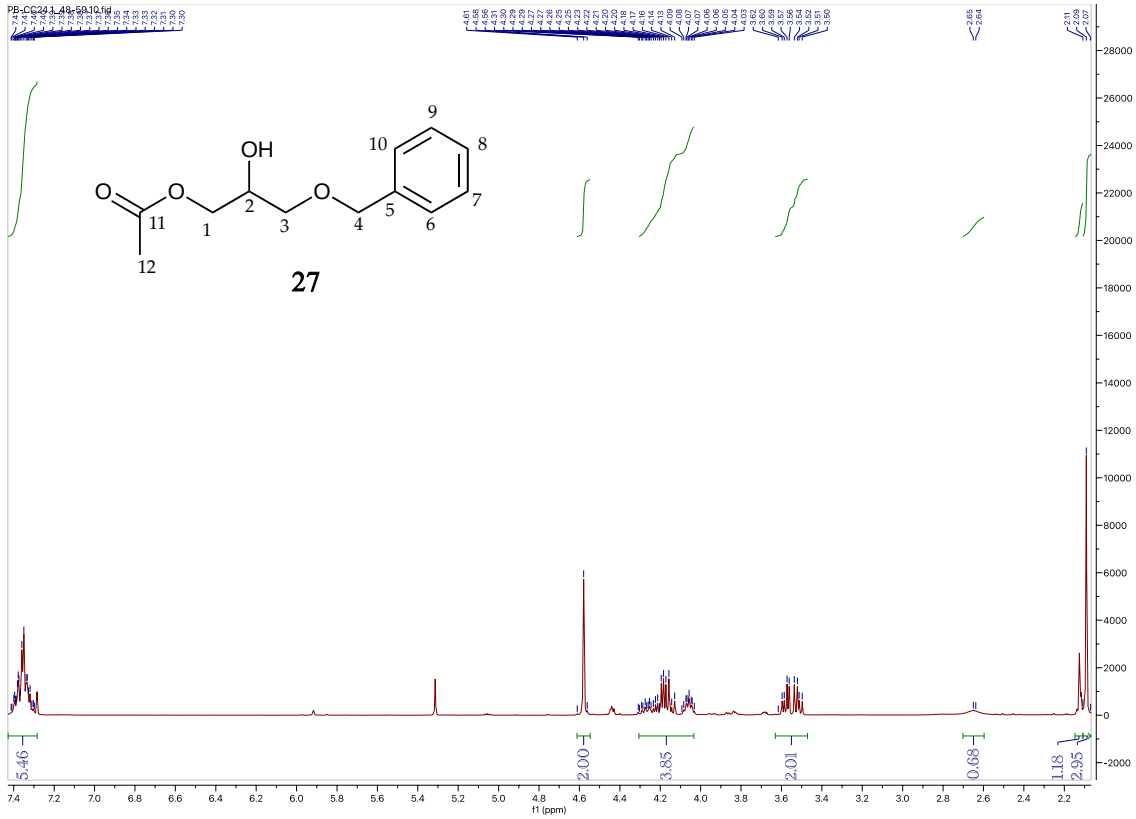


Figure 4.42 - ^{1}H NMR spectrum of compound **27** (400 MHz, CDCl3).

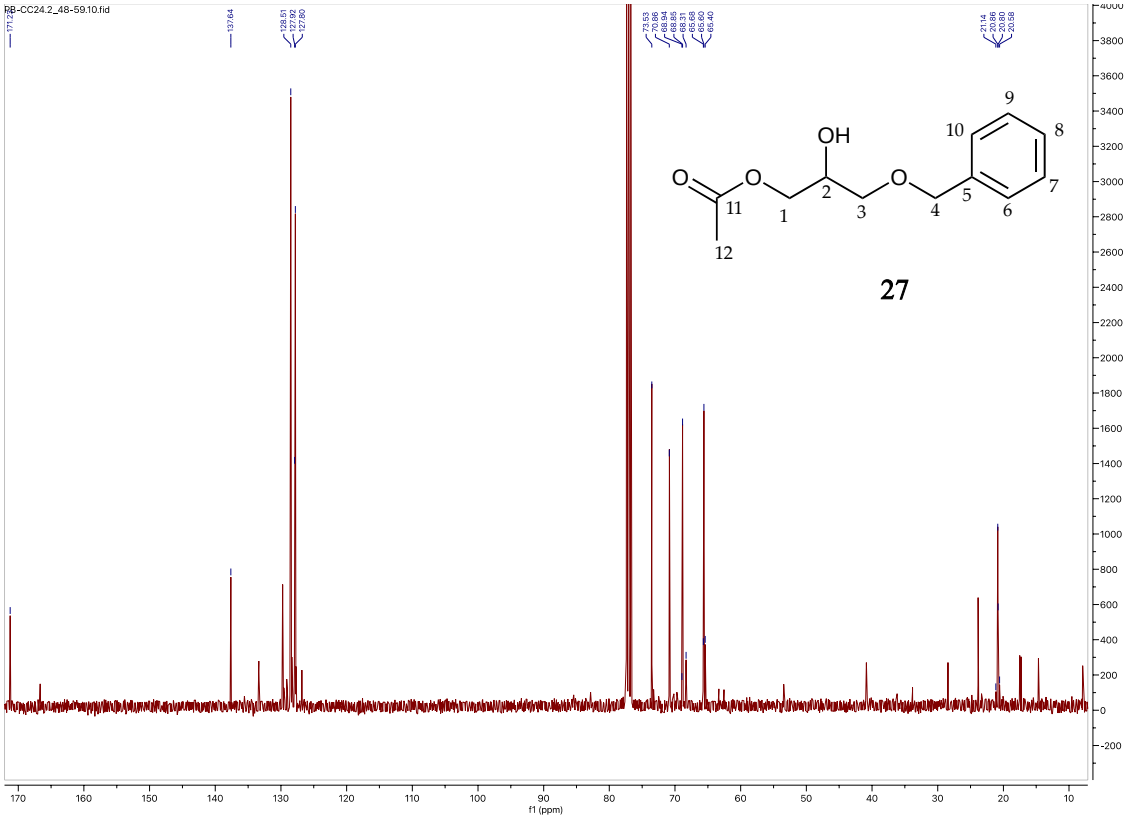


Figure 4.43 - ^{13}C NMR spectrum of compound **27** (101 MHz, CDCl3).

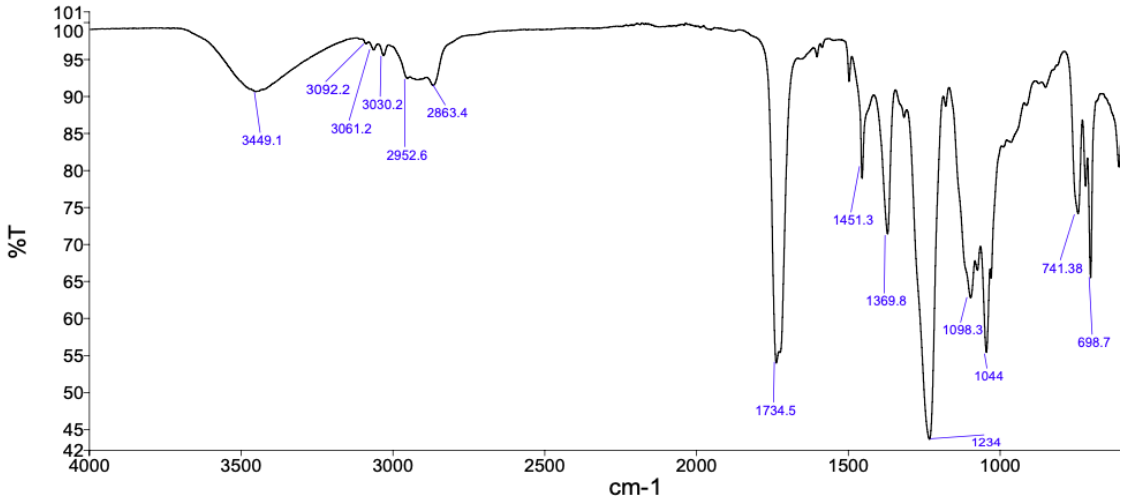


Figure 4.44 - IR spectrum of compound **27**.

Appendix 17 - (2,2-dimethyl-1,3-dioxolan-4-yl)methyl-(2,3,4,6-tetra-O-benzyl)-galactopyranoside (47)

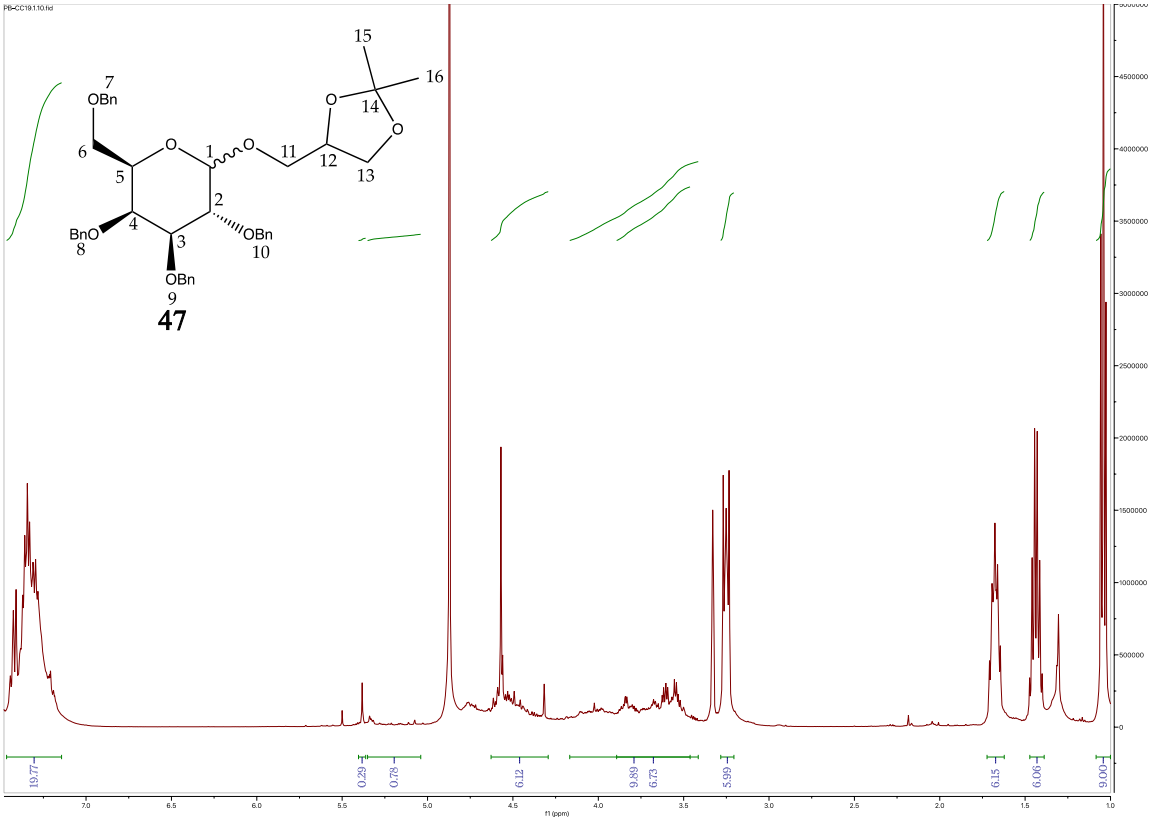


Figure 4.45 - ^{1}H NMR spectrum of the method A of the reaction 4.4.1 (400 MHz, CDCl3).

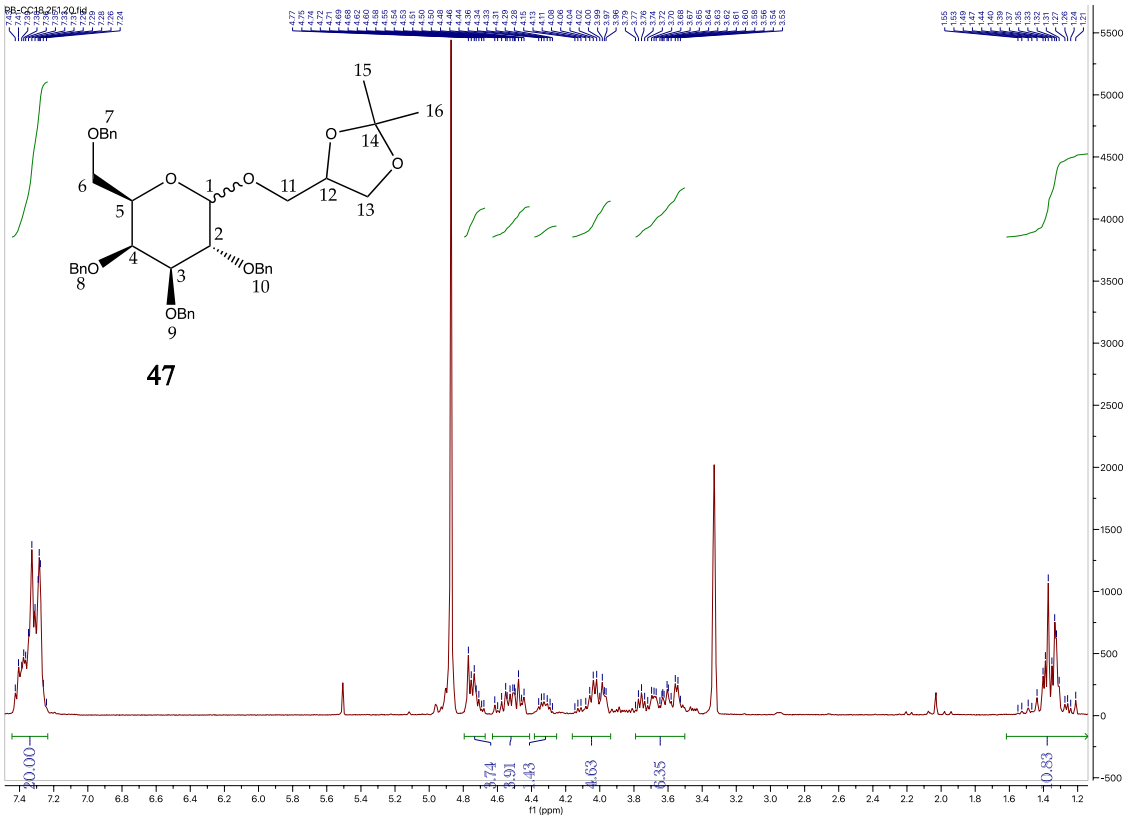


Figure 4.46 – ^{1}H NMR spectrum of compound **47** obtained in method B (400 MHz, CDCl3).

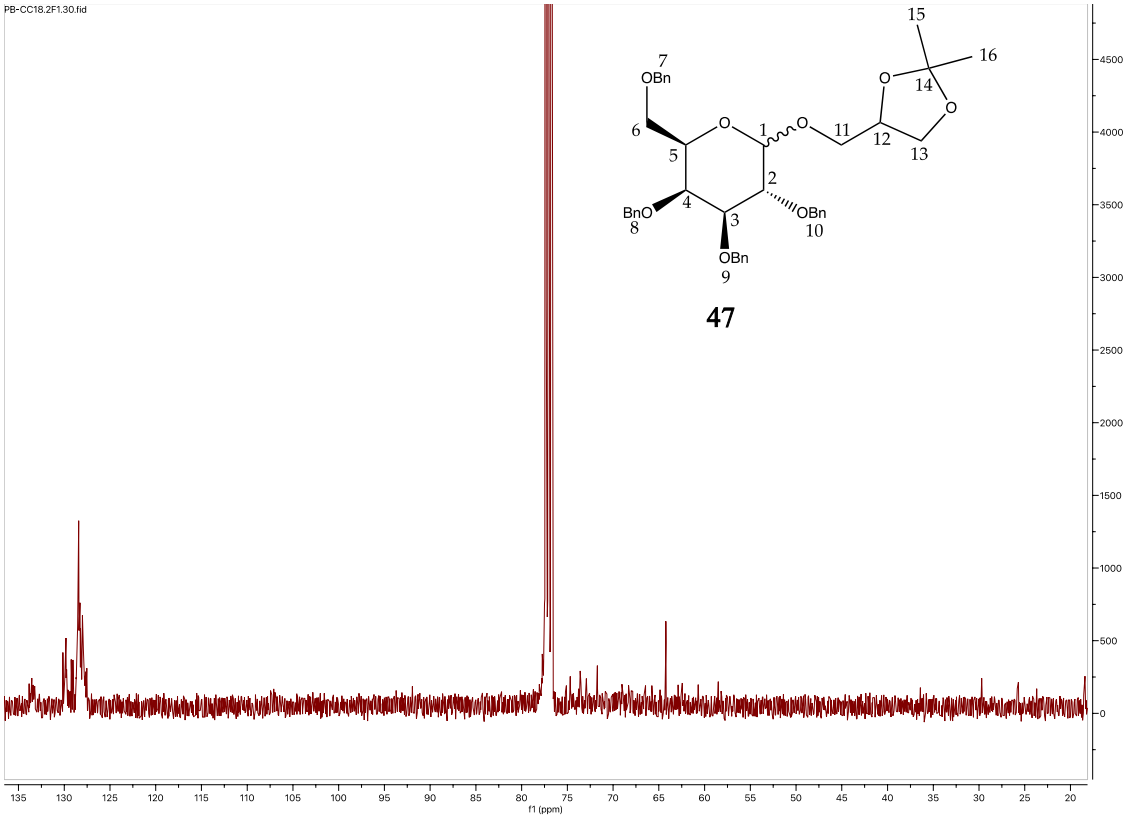


Figure 4.47 - ^{13}C NMR spectrum of compound **47** obtained in method B (101 MHz, CDCl3).

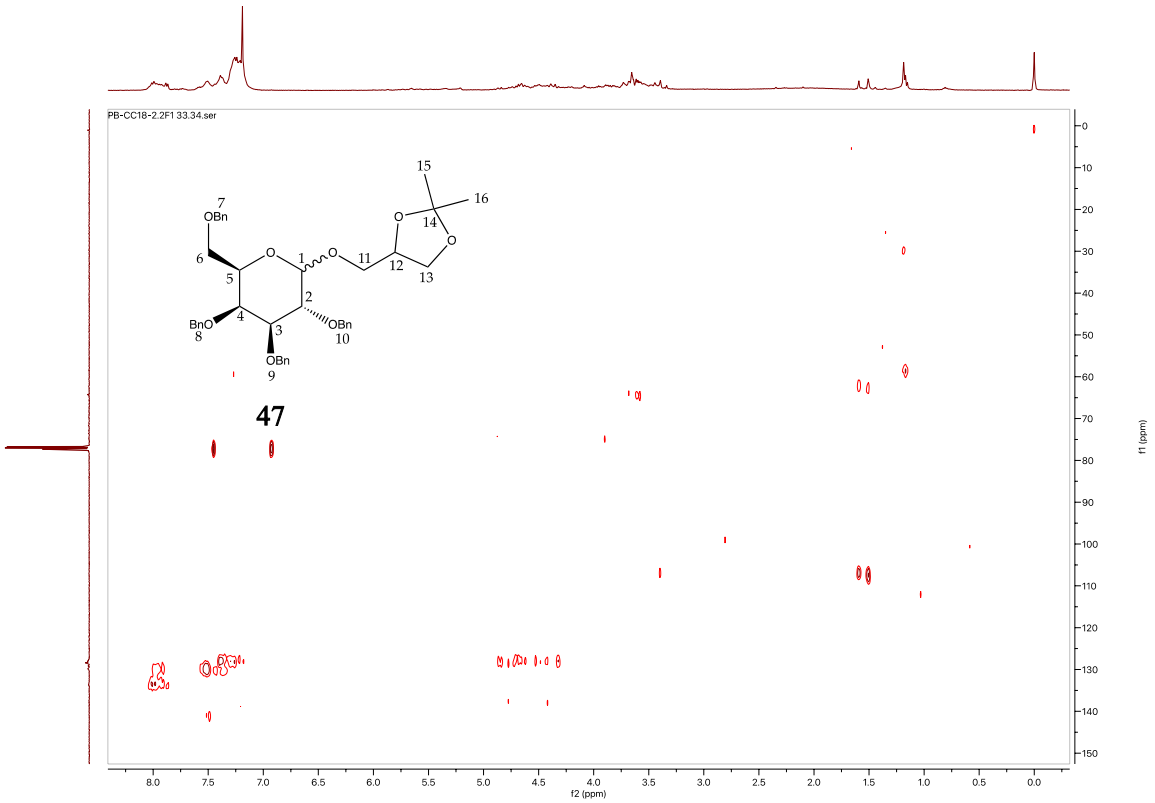


Figure 4.48 - HMBC spectrum of compound **47** (400 MHz, CDCl3).

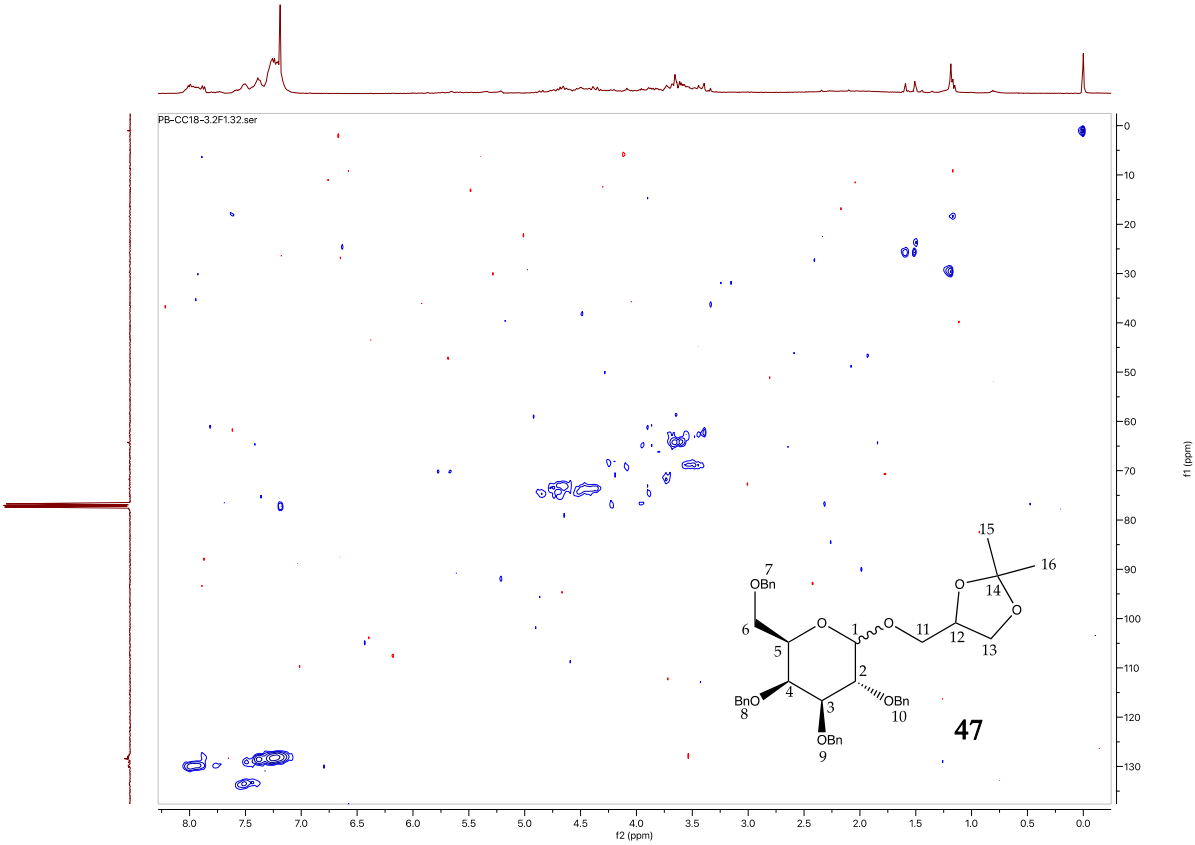


Figure 4.49 - HSQC spectrum of compound **47** (400 MHz, CDCl3).

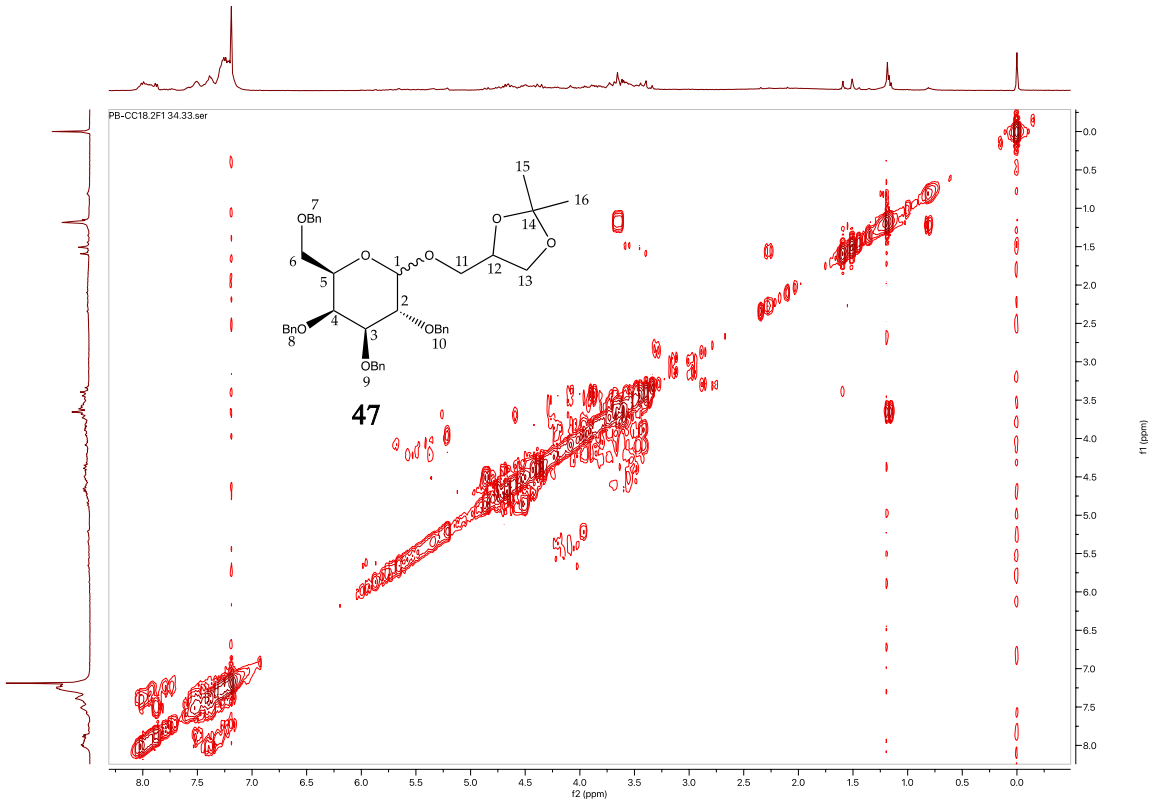


Figure 4.50 - COSY spectrum of compound **47** (400 MHz, CDCl3).

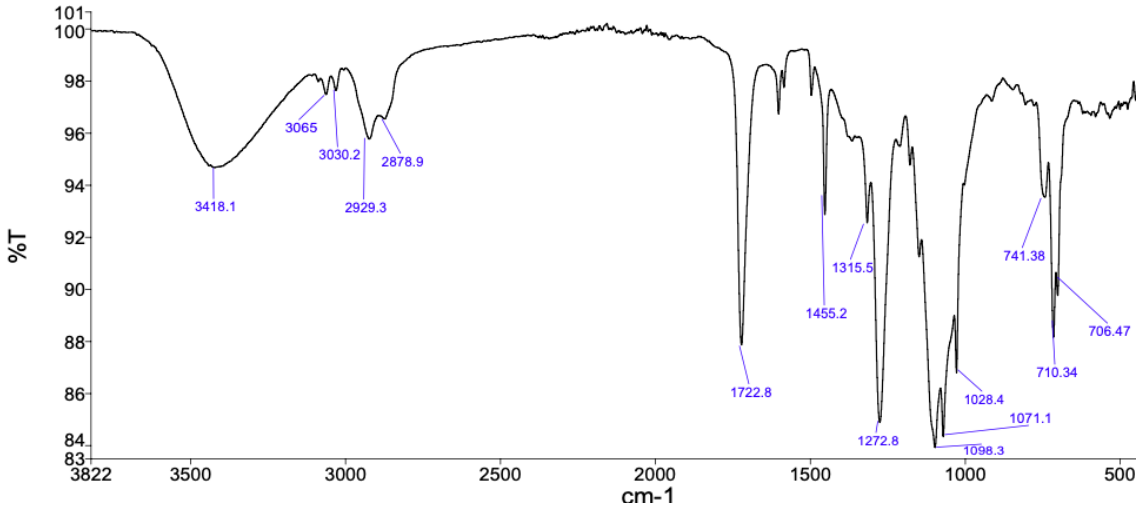


Figure 4.51 – IR spectrum of compound **47**.

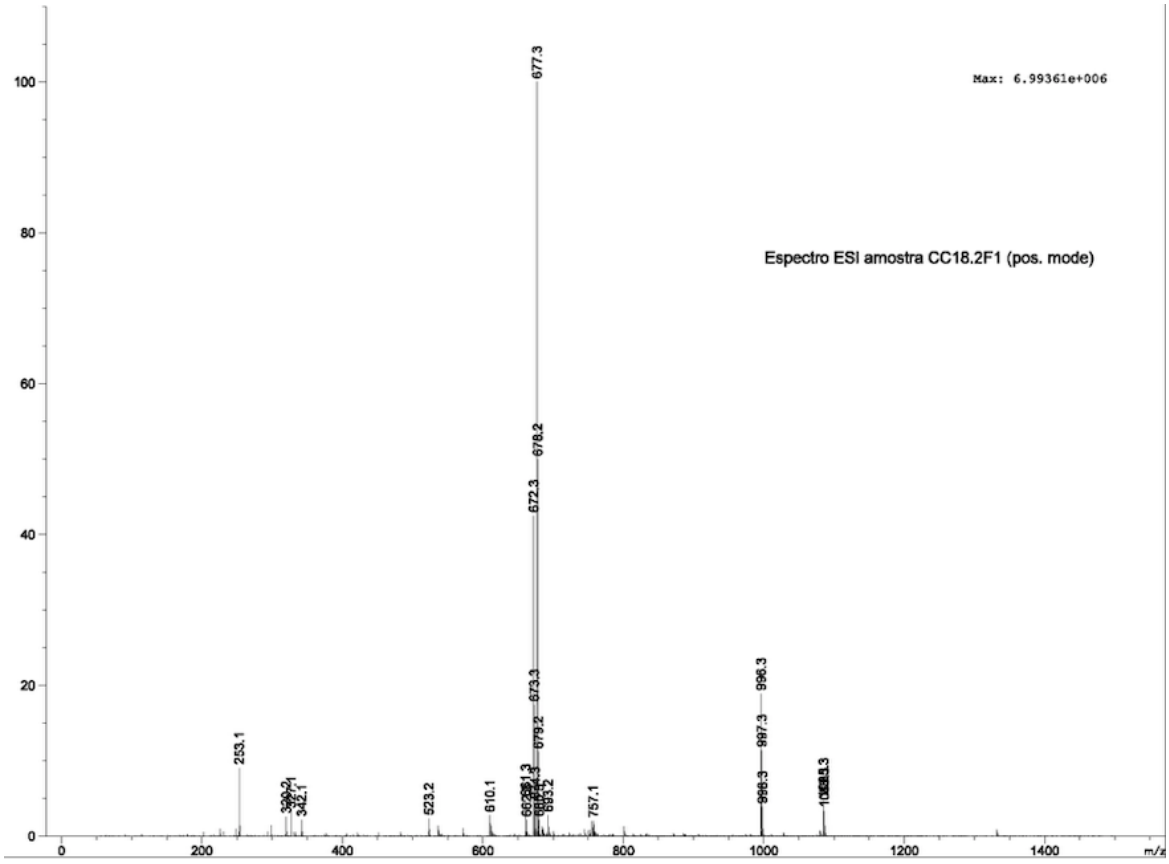


Figure 4.52 - Mass spectrum of compound **47**.

Appendix 18 - (1-(benzyloxy)-3-((tert-butyldiphenylsilyl)oxy)propan-2-galactopyranoside (48, 49)

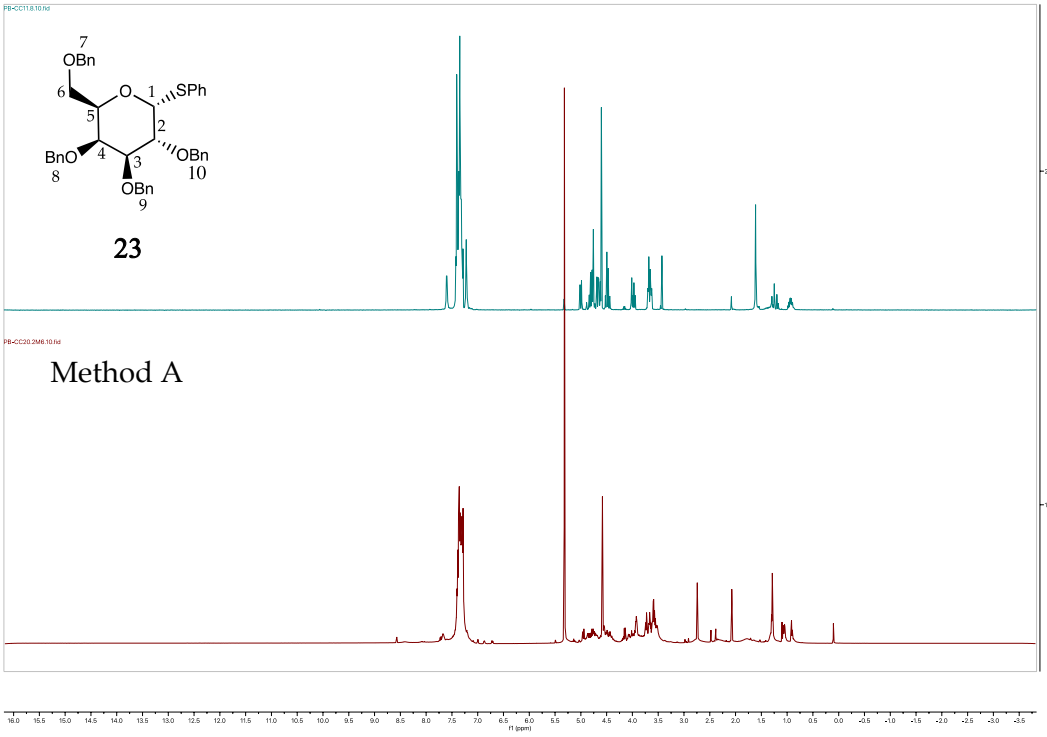


Figure 4.53 - Comparation between ^{1}H spectrums of the method A and the starting material (400 MHz, CDCl3).

Method B – First attempt

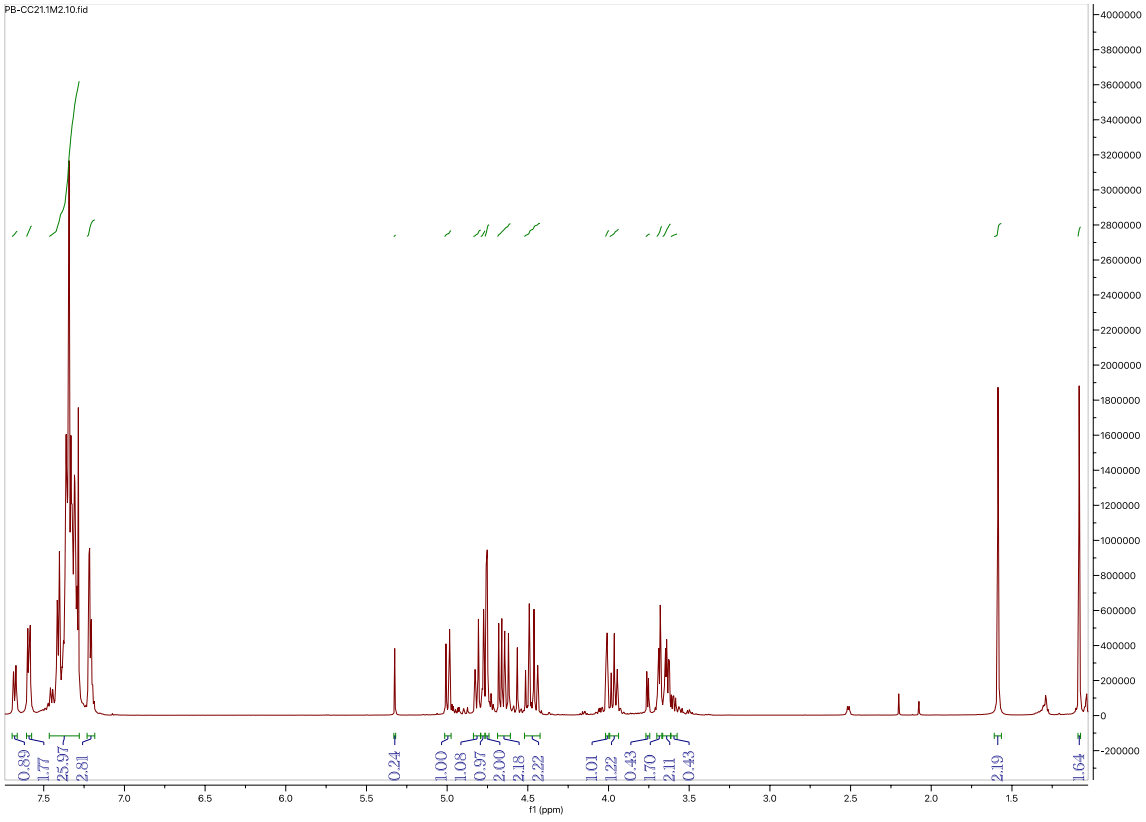


Figure 4.54 - ^{1}H NMR spectrum obtained in the first attempt with mixture of products (400 MHz, CDCl3).

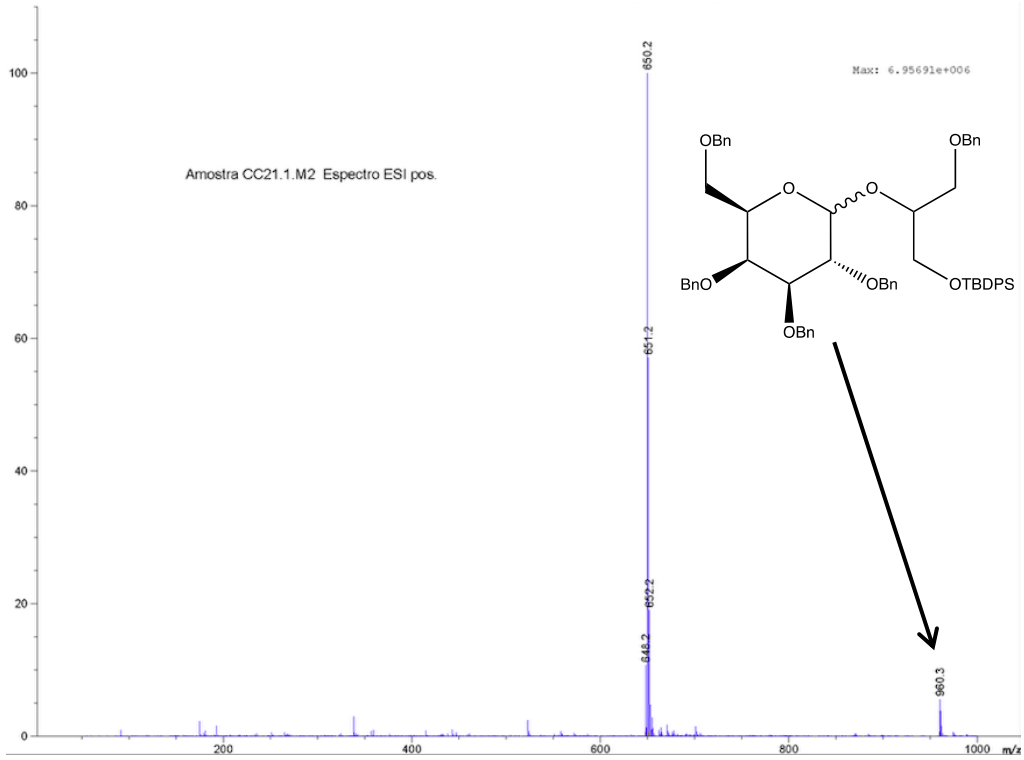


Figure 4.55 - Mass spectrum of compound **53**.

Method B – Second attempt

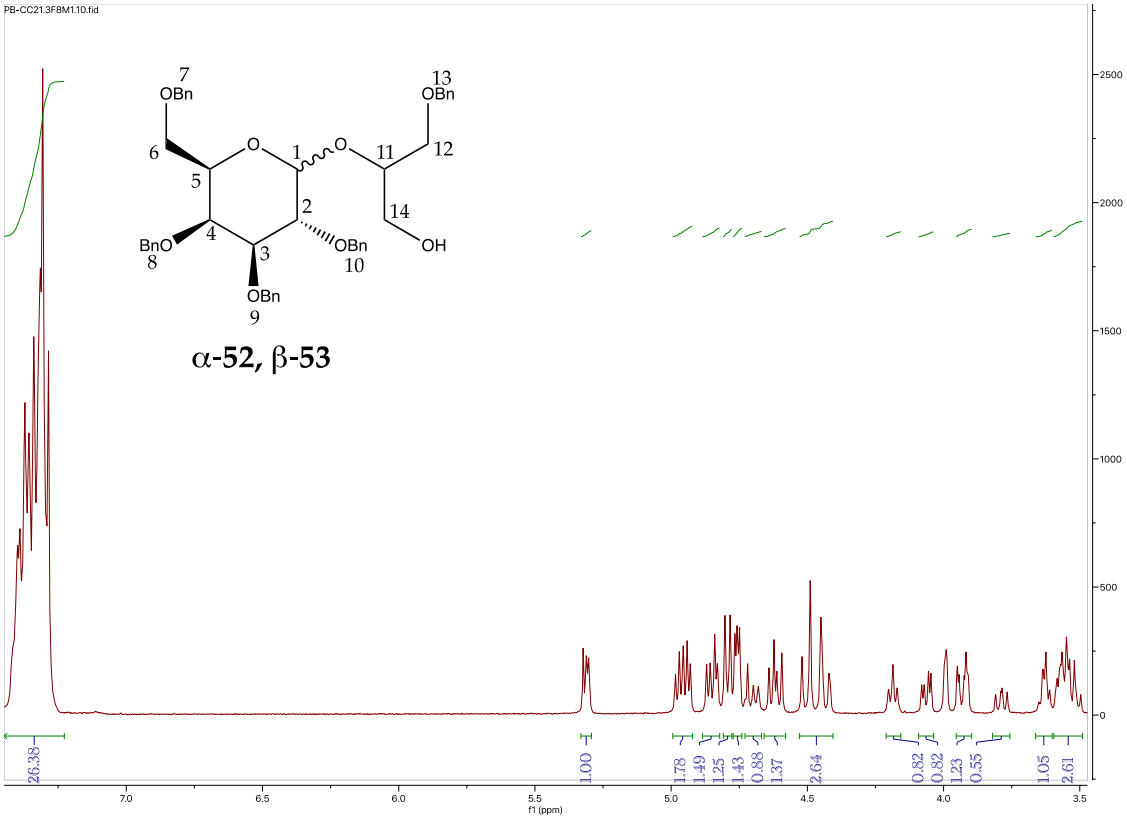


Figure 4.56 - ^{1}H NMR spectrum of compound **52** and **53** (400 MHz, CDCl3).

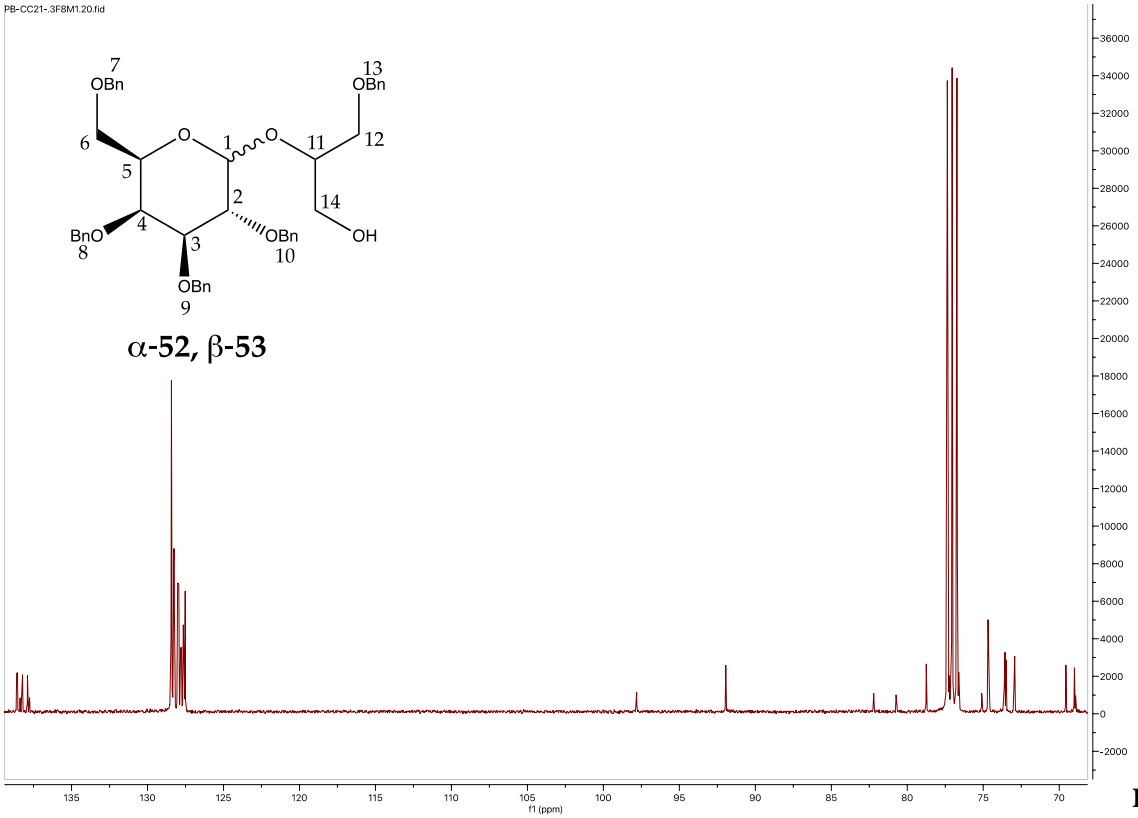


Figure 4.57 - ^{13}C NMR spectrum of compound **52** and **53** (101 MHz, CDCl3).

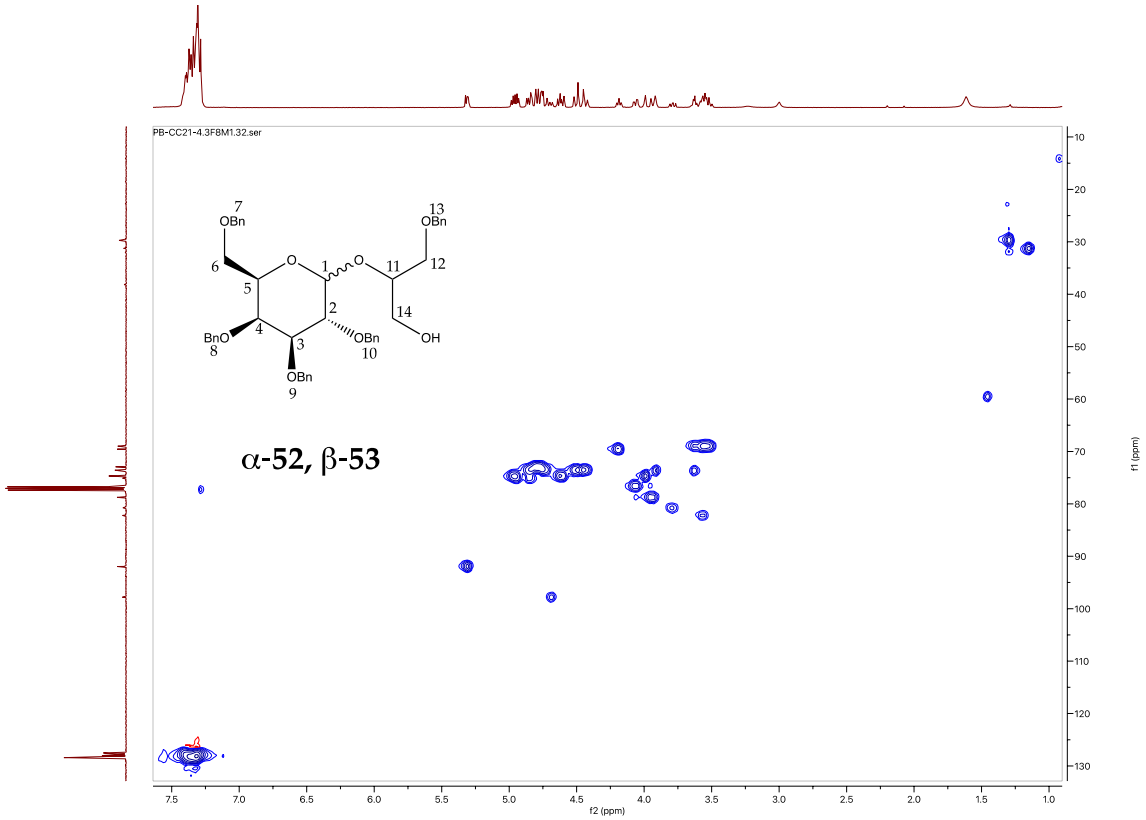


Figure 4.58 - HSQC spectrum of compound **52** and **53** (400 MHz, CDCl3).

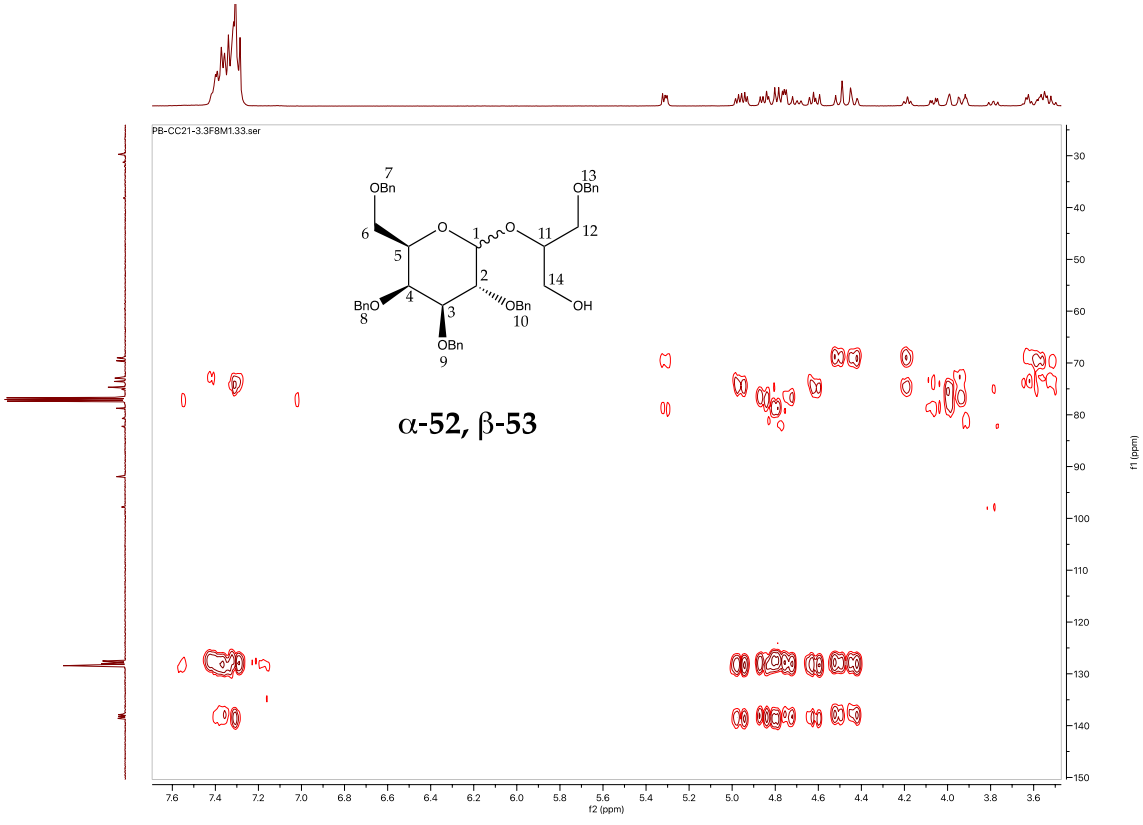


Figure 4.59 - HMBC spectrum of compound **52** and **53** (400 MHz, CDCl3).

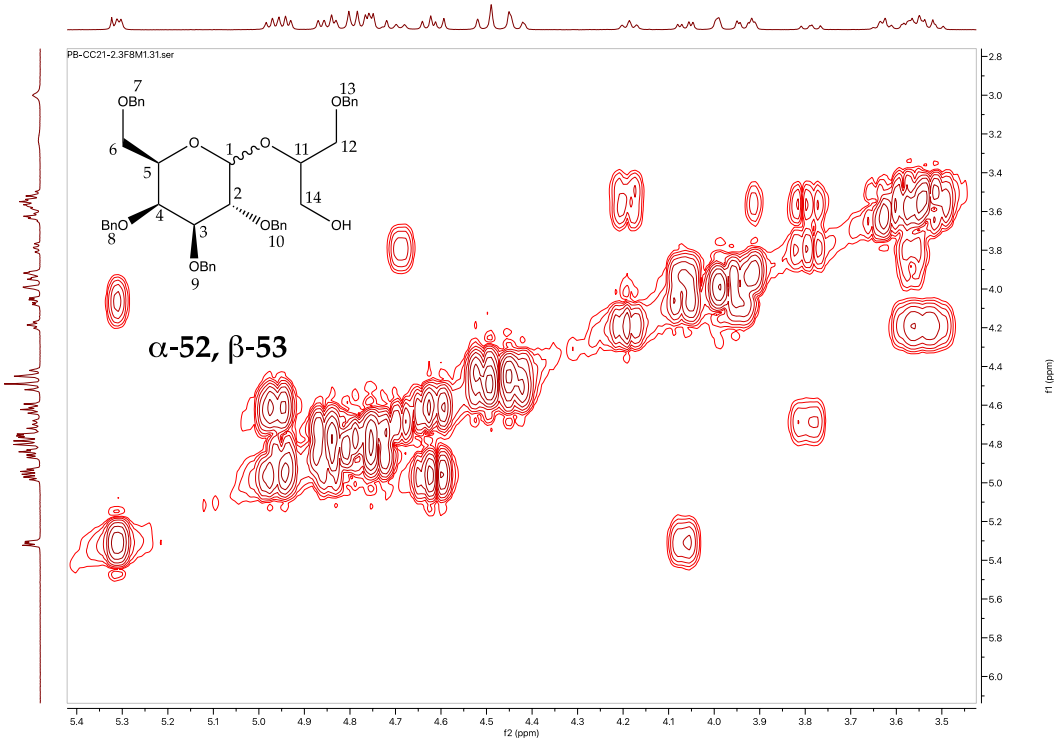


Figure 4.60 – COSY spectrum of compound **52** and **53** (400 MHz, CDCl3).

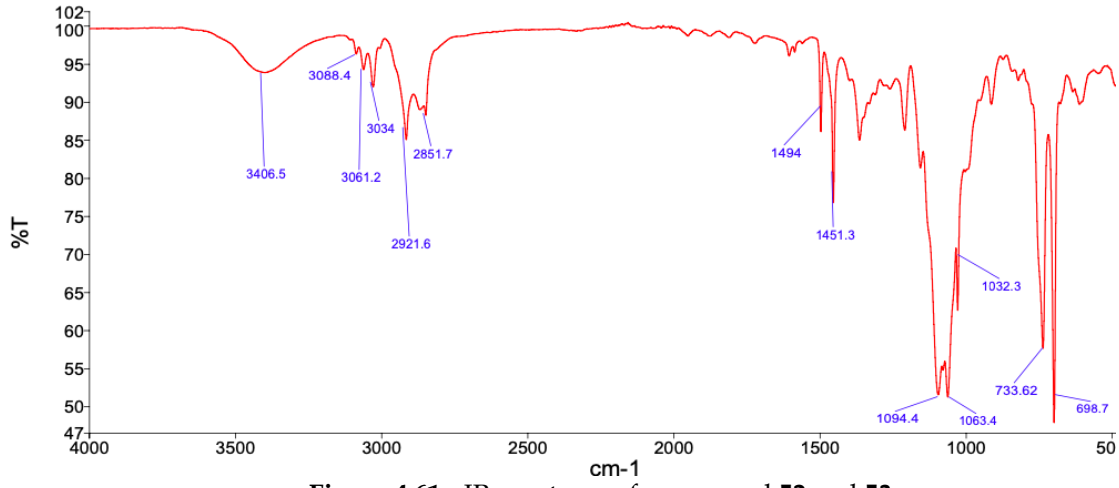


Figure 4.61 - IR spectrum of compound **52** and **53**.

Method C

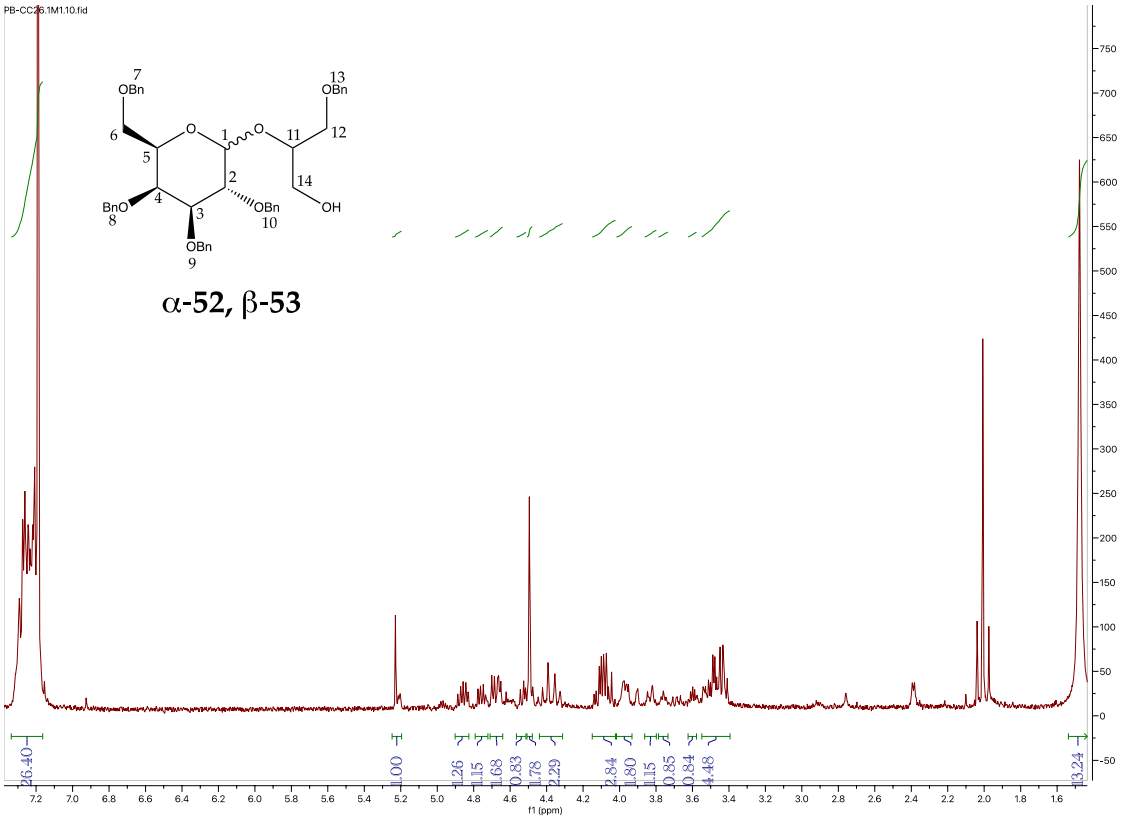


Figure 4.62 - ^{1}H NMR spectrum of compound **52** and **53** (400 MHz, CDCl3).

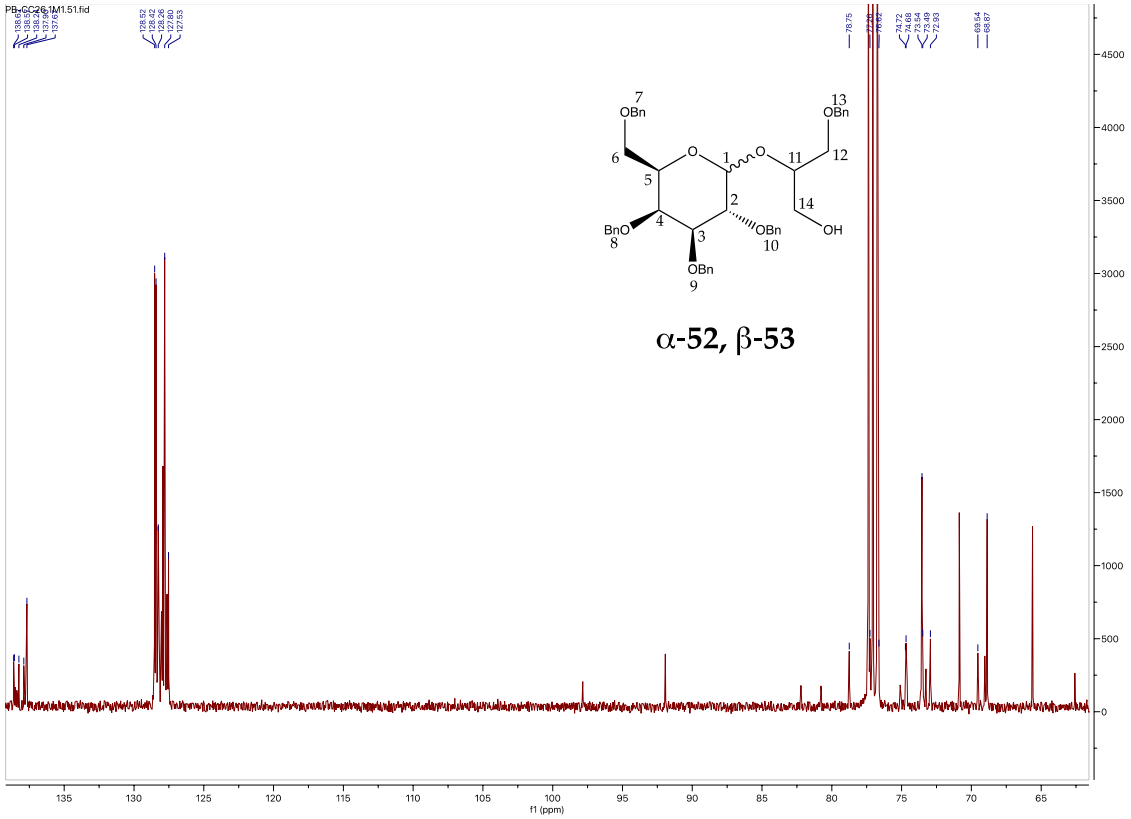


Figure 4.63 – ^{13}C NMR spectrum of compound **52** and **53** (101 MHz, CDCl3).

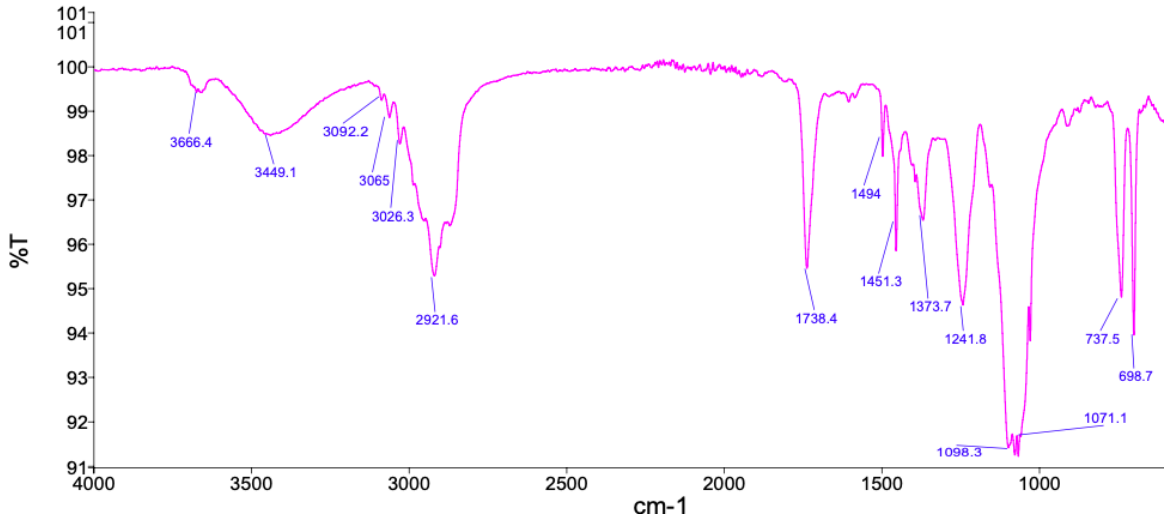


Figure 4.64 – IR spectrum of compound **52** and **53**.

Appendix 19 - (1-(benzyloxy)-3-((tert-butyldiphenylsilyl)oxy)propan-2-galactopyranoside (52, 53)

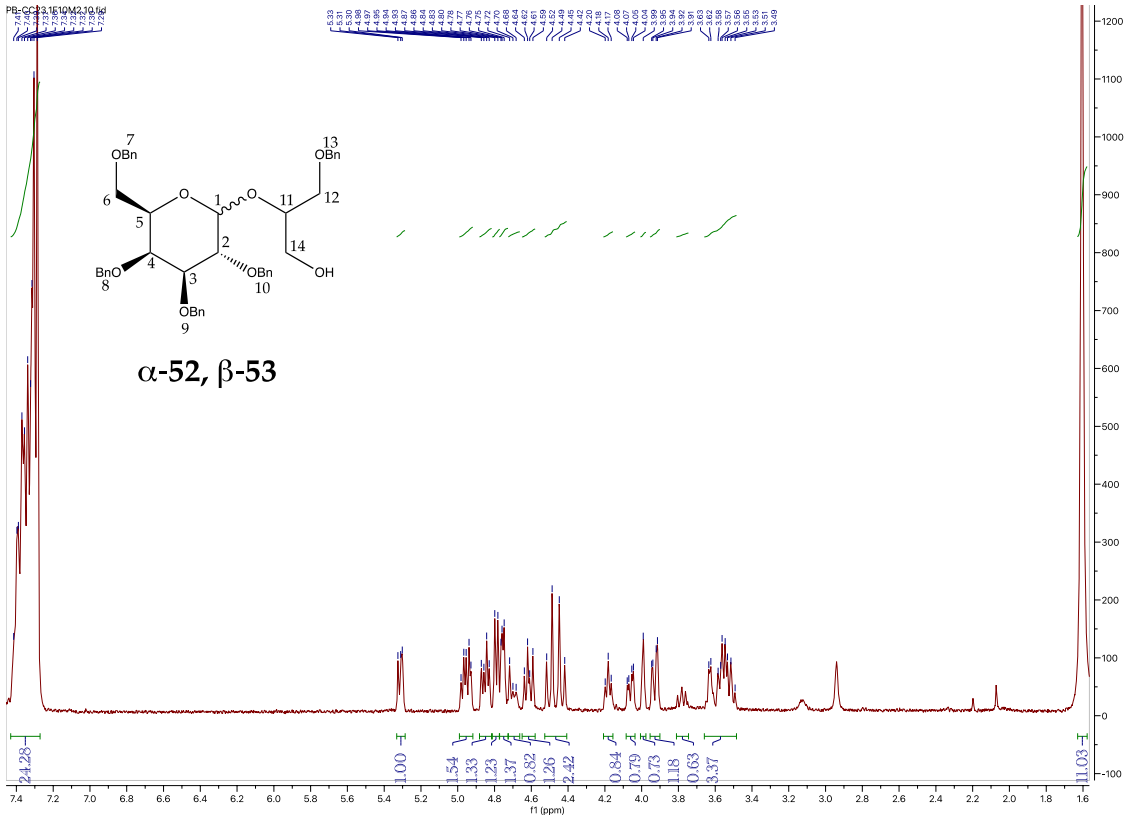


Figure 4.65 - ^{1}H NMR spectrum of compound **52** and **53** (400 MHz, CDCl3).

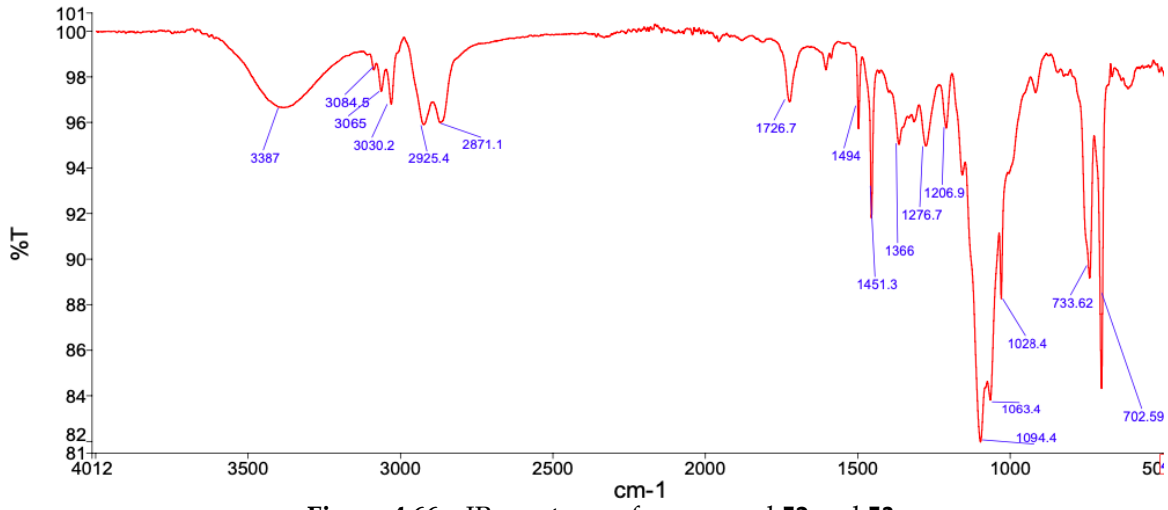


Figure 4.66 – IR spectrum of compound **52** and **53**.

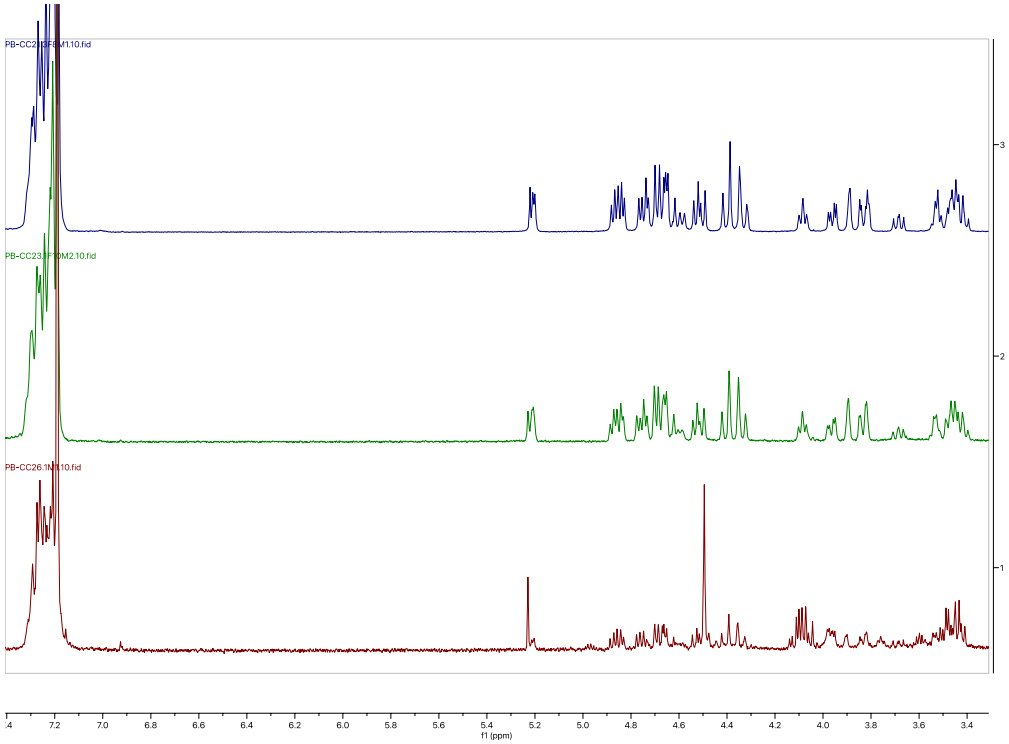


Figure 4.67 - Comparation between the 1H NMR spectrum for the three reactions were the compound **52** and **53** was found (400 MHz, CDCl3).

Appendix 20 - (1-acetoxy-3-benzyloxy-2-propan)-2,3,4,6-tetra-O-benzylgalactopyranoside (50, 51)

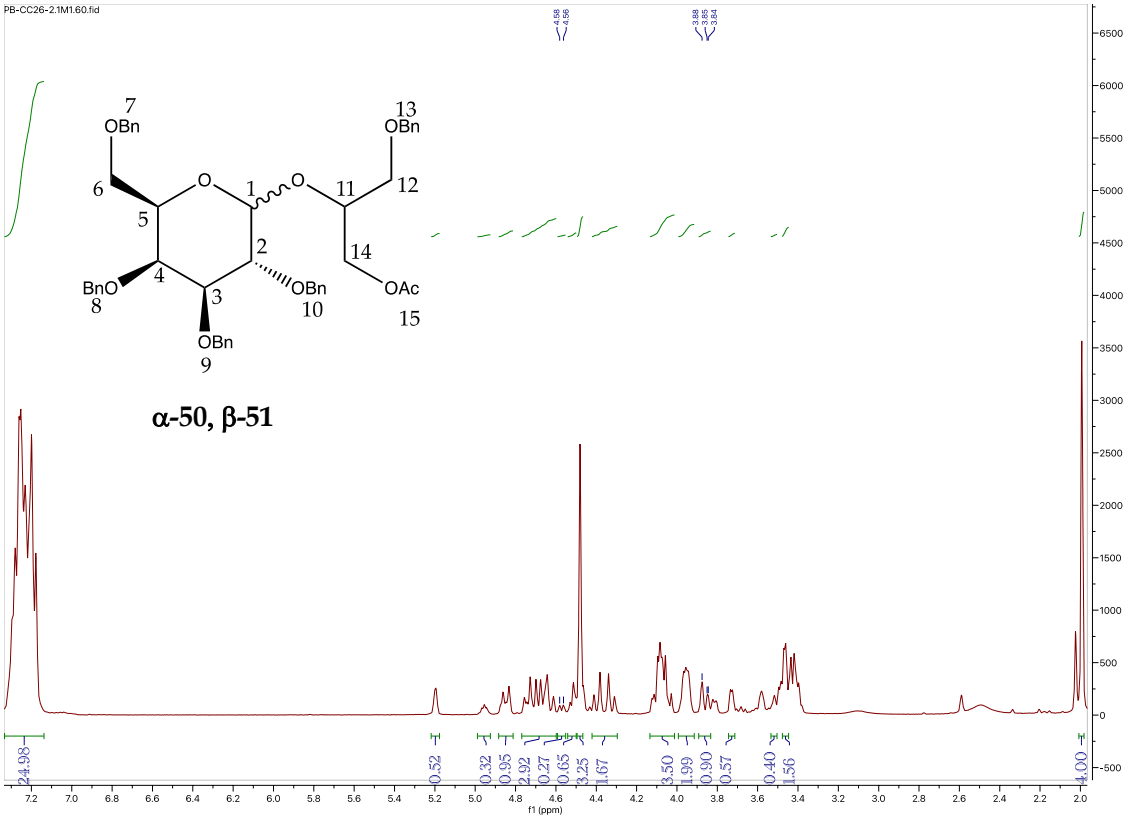


Figure 4.68 - ^{1}H NMR spectrum of compound **50** and **51** (400 MHz, CDCl3).

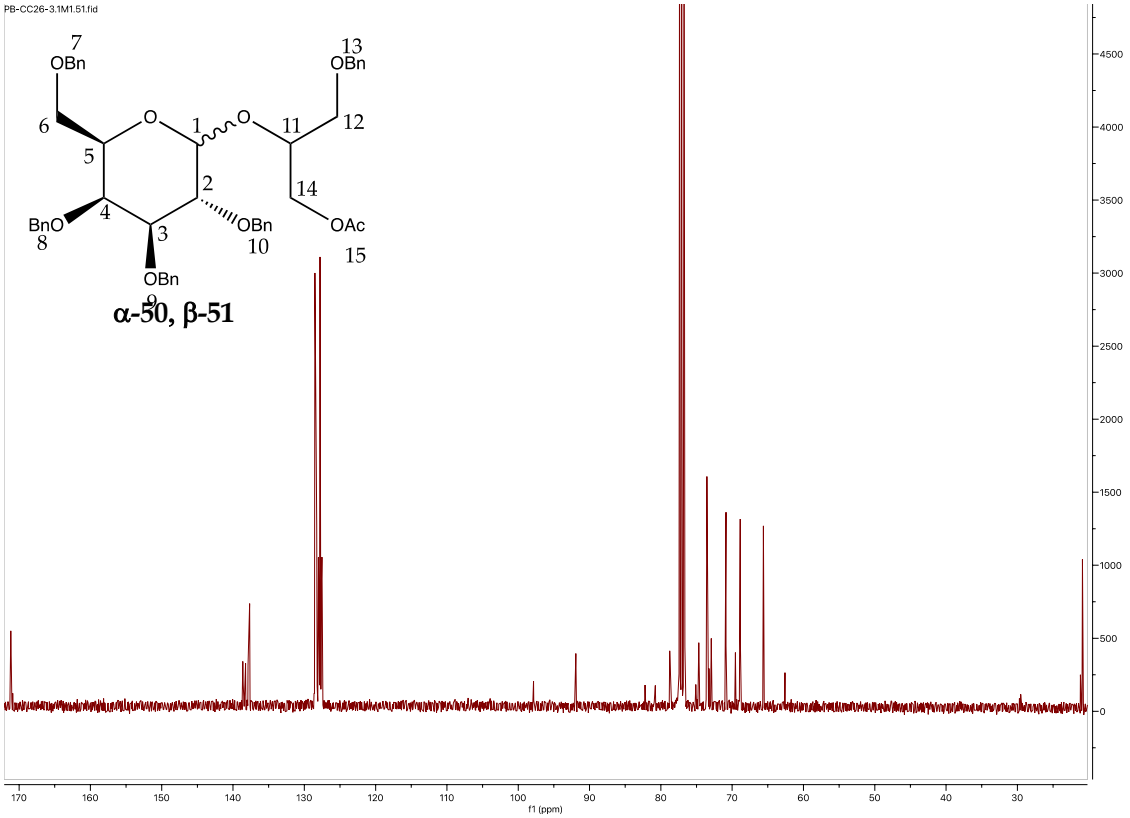


Figure 4.69 – ^{13}C NMR spectrum of compound **50** and **51** (101 MHz, CDCl3).

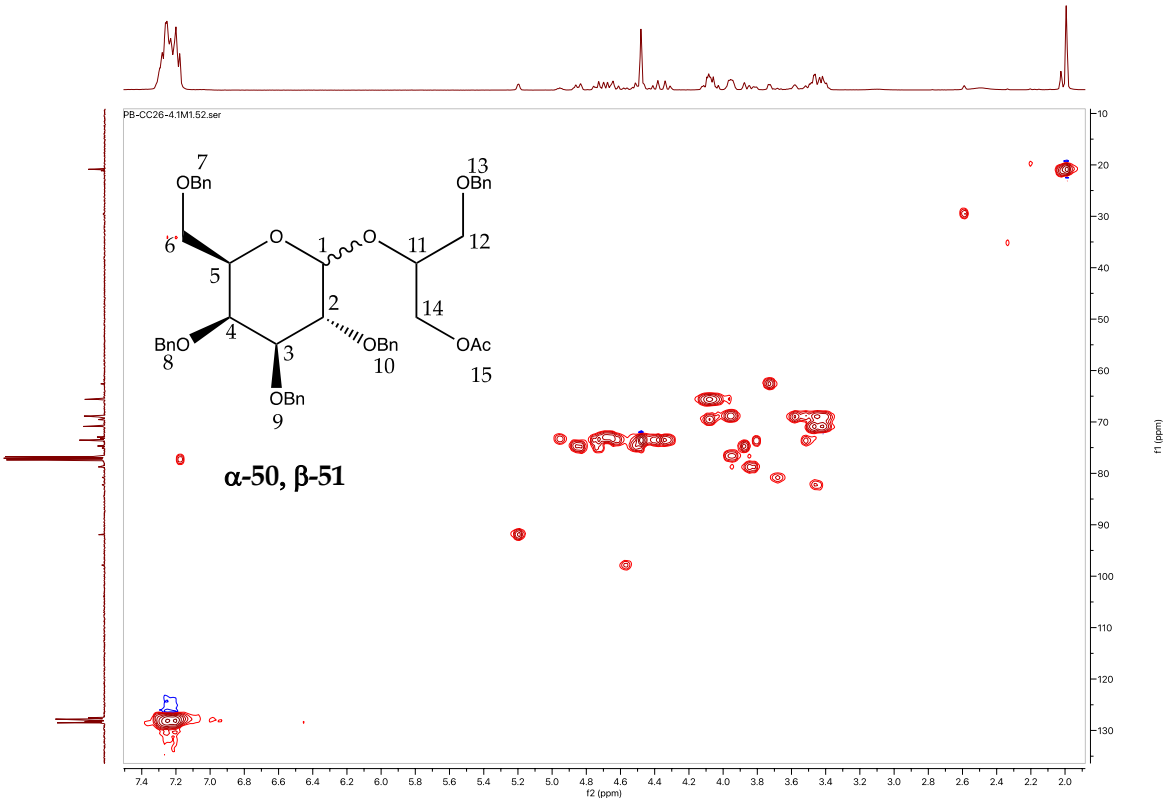


Figure 4.70 - HSQC spectrum of compound **50** and **51** (400 MHz, CDCl3).

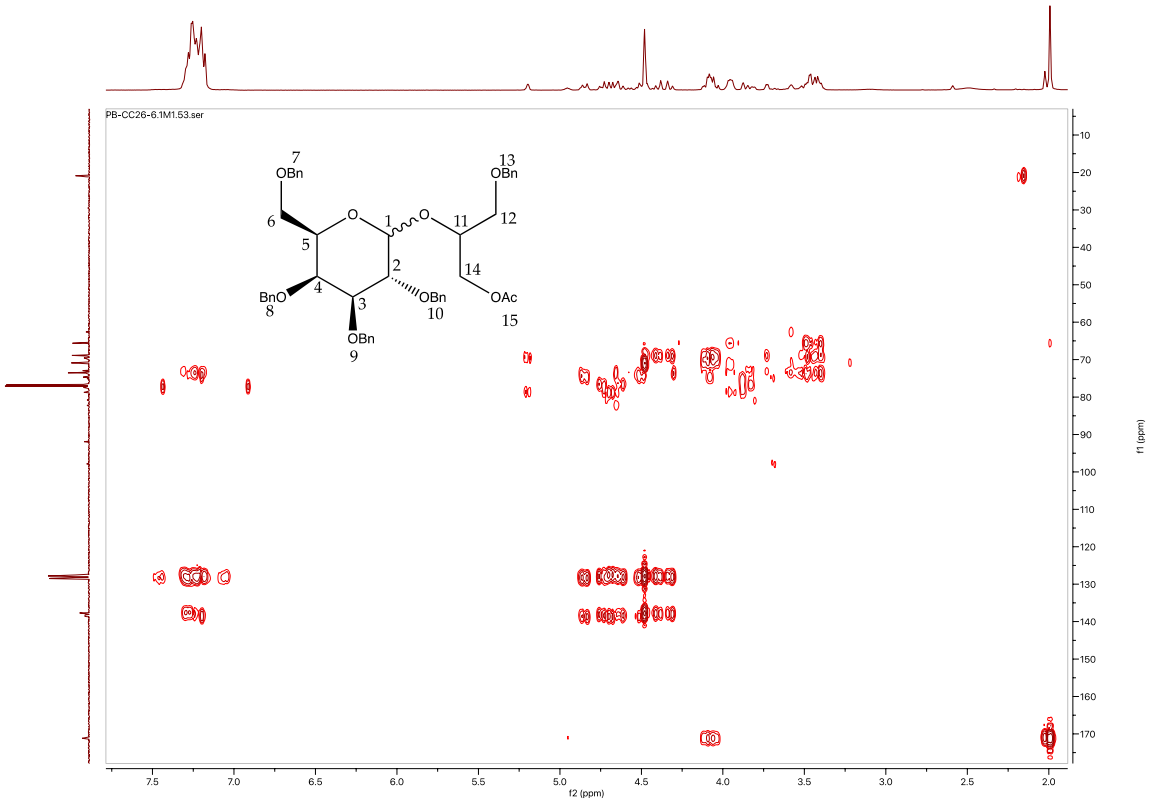


Figure 4.71 - HMBC spectrum of compound **50** and **51** (400 MHz, CDCl3).

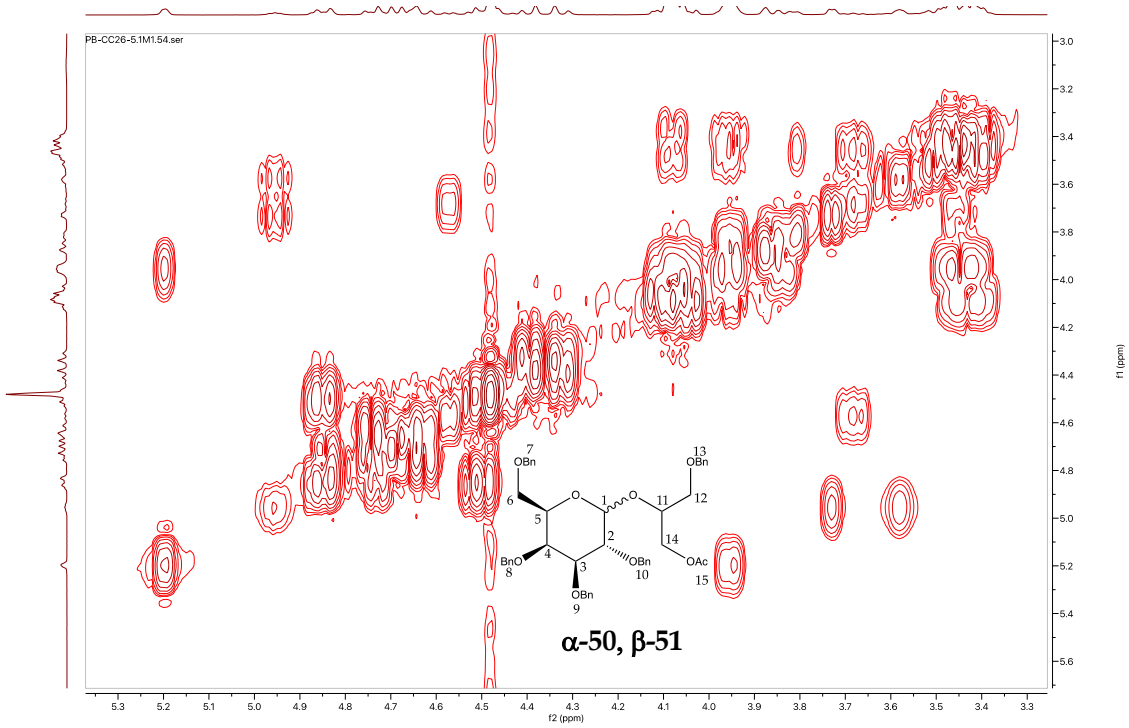


Figure 4.72 - COSY spectrum of compound **50** and **51** (400 MHz, CDCl3).

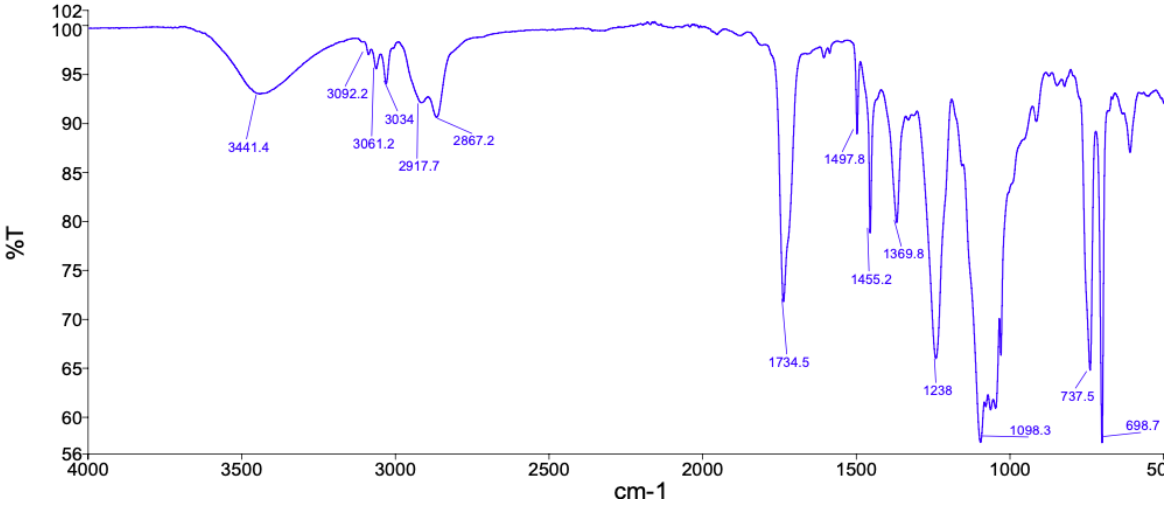


Figure 4.73 - IR spectrum of compound **50** and **51**.

2021

Catarina Isabel Baptista Cipriano

Synthesis of new phosphoglycoglycerol – evaluation as model to new putative therapeutic agents

NOVA SCHOOL OF SCIENCE & TECHNOLOGY