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New Methods for the Synthesis of Novel Phenylpropanoid Glucose Esters

Dissertação para obtenção do Grau de Mestre em Química Bioorgânica

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Abstract

Phenylpropanoid glycosides are naturally occurring compounds, most commonly appearing in fruits and vegetables that possess biological activities such as antiviral, antimicrobial, antioxidant, antifungal and many others. They are composed of a sugar that is linked (with a glycoside linkage) to a hydroxycinnamoyl residue and/or another group. These types of compounds have been used in Asian traditional medicine for many years but only recently they began to be studied and synthetized for further uses. The reason behind this is the low percentage of these esters in the natural sources and the relatively high complexity of the carbohydrate molecules.

In this project we aimed to develop and optimize protocols for the synthesis of phenylpropanoid esters of glucose and methylglucopyranose substituted with a cinnamic acid moiety. In order to achieve that three different esterification techniques were tested; the Steglich esterification, the Mitsunobu esterification and transesterification through enzymatic catalysis. The first two being well known and established procedures and the last being a “greener” alternative that has an enormous potential in carbohydrate synthesis.

Keywords: glucose, phenylpropanoid esters, esterification, transesterification, Mitsunobu, Steglich, enzymatic catalysis

Resumo

Glicosídeos fenilpropanóicos são compostos naturais que se encontram mais comummente em frutas e vegetais e que possuem atividades biológicas, entre outras estão atividades antivíricas, antimicrobianas, antioxidantes e antifúngicas. Estes compostos são formados por um açúcar que está ligado através de uma ligação glicosídica a um ácido hidroxicinâmico e/ou a outro grupo. Durante muitos anos estes compostos foram usados na medicina asiática, mas apenas recentemente começaram a ser estudados e sintetizados para outros fins. Este facto deve-se à baixa percentagem destes compostos nas suas fontes naturais e à complexidade relativamente elevada da síntese envolvendo carboidratos.

Neste projeto o nosso objetivo passou por desenvolver e otimizar protocolos para a síntese de ésteres fenilpropanóicos da glucose e metilglucopiranose substituídos com ácido cinâmico. De modo a alcançar esses objetivos três técnicas de esterificação distintas foram testadas; a esterificação de Steglich, a esterificação de Mitsunobu e a transesterificação por catálise enzimática. Os dois primeiros tratam-se de procedimentos já bastante conhecidos e estabelecidos sendo o último uma alternativa mais "verde" que apresenta enorme potencial na síntese de carboidratos.

Palavras-chave: glucose, ésteres fenilpropanóicos, esterificação, transesterificação, Mitsunobu, Steglich, catálise enzimática

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List of Reagents

Reagent	Distributor	Empirical Formula	CAS number
Acetic acid	Sigma	$C_2H_4O_2$	64-19-7
Acetic anhydride	Panreac	$C_4H_6O_3$	108-24-7
Barium Oxide	Sigma	BaO	1304-28-5
Cinnamic acid	Aldrich	$C_9H_8O_2$	140-10-3
Cyclohexane	Riedel-de Häen	C_6H_{12}	110-82-7
Diethyl ether	Sigma	$C_4H_{10}O$	60-29-7
1,4-dioxane	Carlo Erba	$C_4H_8O_2$	123-91-1
DMAP	Alfa Aesar	$C_7H_{10}N_2$	1122-58-3
DMF	Carlo Erba	C_3H_7NO	68-12-2
Ethanol, 95%	Carlo Erba	C_2H_6O	64-17-5
Hydrochloric acid	Honeywell	HCl	7647-01-0
Hydrofluoric acid, 70% in pyridine	Sigma	HF	7664-39-3
Lipase acrylic resin from Candida antarctica	Sigma	-	9001-62-1
Piperidine	Sigma	$C_5H_{11}N$	110-89-4
Pyridine	Carlo Erba	C_5H_5N	110-86-1
Sodium sulphate	Honeywell	$NaSO_4$	7757-82-6
tert-butyl alcohol	Sigma	$C_4H_{10}O$	75-65-0
THF	Carlo Erba	C_4H_8O	109-99-9
Toluene	Carlo Erba	C_7H_8	108-88-3
Triethylamine	Carlo Erba	$C_6H_{15}N$	121-44-8

Abbreviations

Ac – Acetyl

Asp – Aspartic acid

ATP – Adenine triphosphate

ºC – Degrees Celsius

Cal-B - *Candida Antarctica*

COSY – Correlation Spectroscopy

d – doublet

DCC - *N*,*N*'-Dicyclohexylcarbodiimide

DIAD - Diisopropyl azodicarboxylate

DMAP – 4-*N*,*N*-dimethyl(amino)pyridine

DMF – *N*,*N*-dimethylformamide

DMSO – Dimethyl sulfoxide

DNA – Deoxyrribonucleic acid

EtOAc – Ethyl acetate

His - Histidine

HIV – Human Immunodeficiency Virus

HMBC – Heteronuclear Multiple Bond Correlation

HPLC - High-performance liquid chromatography

HSQC – Heteronuclear Single-Quantum Correlation

Hz – Hertz

m – multiplet

m.p. – melting point

mg – milligram

MPLC - Medium-performance liquid chromatography

ml – mililiters

NMR – Nuclear Magnetic Ressonance

PhGs - Phenylpropanoid glycosides

ppm – Parts per Million

Pyr – Pyridine

RNA – Ribonucleic acid

s – singlet

Ser - Serine

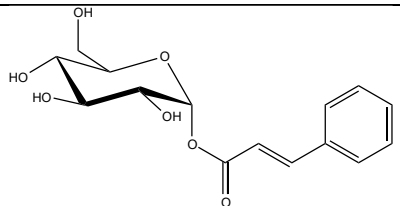
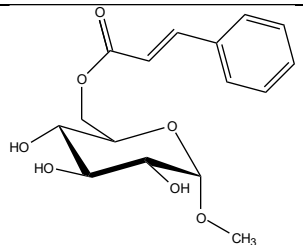
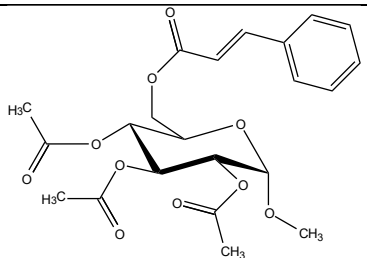
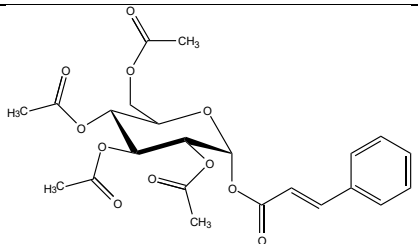
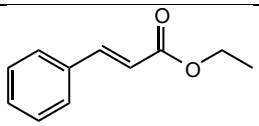
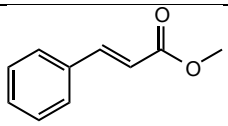
t – triplet

THF – Tetrahydrofuran

TLC – Thin Layer Chromatography

v/v – Volume per Volume

List of compounds

Compound Number	Structure	Name
1		**1-*O*-cinnamoyl-α-D-glucopyranose**
2		**6-*O*-cinnamoyl-α-D-methylglucopyranoside**
3		**2,3,4-*tri*-*O*-acetyl-6-*O*-cinnamoyl-α-D-methylglucopyranoside**
4		**2,3,4,6-*tetra*-*O*-acetyl-1-*O*-cinnamoyl-α-D-glucopyranose**
5		**Ethyl cinnamate**
6		**Methyl cinnamate**

1. Introduction

1.1. Sugars

1.1.1. What are they?

Carbohydrates, also known as saccharides or sugars, are one of the most important and common biological compounds found in most of the living organisms. These molecules are produced by photosynthetic processes (Fig. 1.1.) made by the plants, algae and certain bacteria in order to harness chemical energy from sunlight (in most cases) making them the major source of metabolic energy for both plants and animals that depend on plants as a food font.[1] Besides the nutritional role that sugars and starches play, saccharides also perform other tasks such as structure material in the form of cellulose, energy transport (ATP), cell surface carbohydrates as cell recognisers and are one of the most crucial compounds in DNA and RNA. Humans use carbohydrates not only as a food source but also in clothing e.g. cotton, construction materials, fuel and many other things with the likes of wood which are cellulose based, reinforcing the importance of all these different molecules.[2,3]

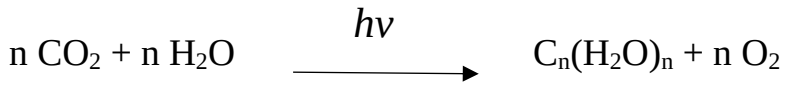


Figure 1.1. Chemical equation of photosynthesis.

1.1.2. Nomenclature

As shown in the figure above (Fig. 1.1.), the formula of many of the carbohydrates is written as $C_n(H_2O)_n$, therefore their name. Carbohydrate, as a generic term, illustrates a large group of organic substances namely aliphatic polyhydroxy aldehydes and ketones. These two carbonyl functionalized types of compound can be classified as aldoses and ketoses but there are more ways of characterizing sugars.[1] According to complexity, they can be simple carbohydrates, classified as monosaccharides that consist of just one monomer or complex carbohydrates, where are included disaccharides, molecules with two monomers, polysaccharides, composed by more than two aliquots and oligosaccharides, that are similar to a polysaccharide but with more than three and less than ten monosaccharides. The carbohydrate nomenclature can also be formed according to the monomer size, C_4 sugars are called tetroses, C_5 pentoses, C_6 hexoses and so on. Cyclic isomers are the favoured structural form of many of the monosaccharides with 5 or more carbon atoms in aqueous solution. These saccharides can resemble furan and pyran rings being then named furanoses and pyranoses (5 and 6 membered rings, respectively). With such a diverse possibility in the matter of nomenclature, carbohydrates have not been given systematic names, although the suffix -ose prevails.[4,5]

Table 1.1. Carbohydrate nomenclature.

Variants	Nomenclature			
Complexity	**Simple carbohydrates**		**Complex carbohydrates**	
	Monosaccharides		Di-, oligo- and polysaccharides	
Size	**Tetroses** (C_4)	**Pentoses** (C_5)	**Hexoses** (C_6)	**Heptoses** (C_7)
Carbonyl function	**Aldoses** (Aldehyde functionalized)		**Ketoses** (Ketone functionalized)	
Cyclic derivatives	**Furanoses** (5 membered rings)		**Pyranoses** (6 membered rings)	

1.1.3. Different types of sugars

1.1.3.1. Monosaccharides

Monosaccharides are carbohydrate molecules that cannot be hydrolysed into smaller pieces being the monomeric units of the oligosaccharides and polysaccharides referred above.

A monosaccharide is a polyhydroxy aldehyde or ketone comprising 3 to 7 carbon atoms and a series of hydrogen and oxygen atoms. The three most common monosaccharides are glucose, galactose and fructose which are all hexoses, the first two containing an aldehyde group and the last a ketone group, whose chemical formula is $C_6H_{12}O_6$.

These simple sugars can be classified by two different criteria: their carbonyl group and chirality. Despite aldoses and ketoses being isomers, aldoses have more asymmetric carbons than ketoses causing the first to have more possible configurations. Because each chiral carbon atom has a mirror image, there are 2^n (n being the number of chiral carbons in the molecule) possible arrangements of these atoms. For example, in the case of an aldohexose the 4 chiral carbon atoms allow the existence of 16 different atomic arrangements, in difference of the ketohexose that have 8 different arrangements due to the existence of just 3 chiral carbons.[1, 6, 7] For both cases half of the total conformations belong to what is known as the D series and the other half (the mirror image) belong to the L series.

Taking into account the Fischer projection, all sugars that have the hydroxyl group on the highest-numbered chiral carbon atom (C5 in the case 6 carboned molecules) positioned on the right-hand side are denominated D sugars and those in which the alcohol group is on the left side are designated L sugars (Fig. 1.2.).[6, 8]

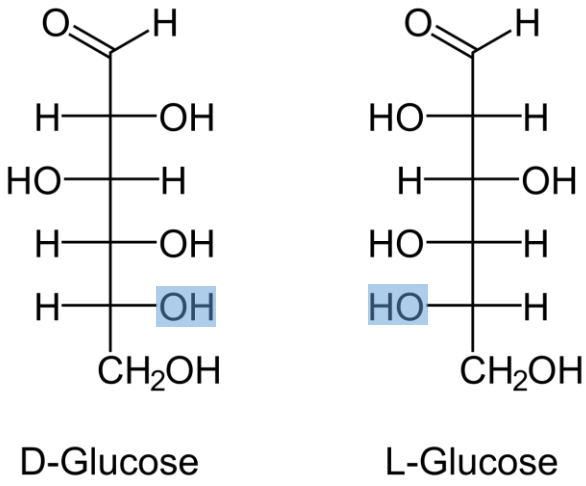


Figure 1.2. D- *vs* L- conformations of glucose according to the Fischer projection.

As was mentioned above, these compounds when in aqueous solutions are preferably in ring formations. This takes effect when the carbonyl groups of the aldehyde or ketone undergoes a nucleophilic attack by the unshared electrons of the oxygen from a hydroxyl group producing hemiacetals or hemiketals, respectively. In consequence of this cyclization a new chiral centre is formed. This new chiral position is called the anomeric position and it has a proper nomenclature. The name given to the configuration of the cyclic derivative where the hydroxyl group on the anomeric position (positioned on the right side of the ring oxygen) is on the opposite side (considering the equatorial symmetric plane) of the substituent on the left of the ring oxygen is α.

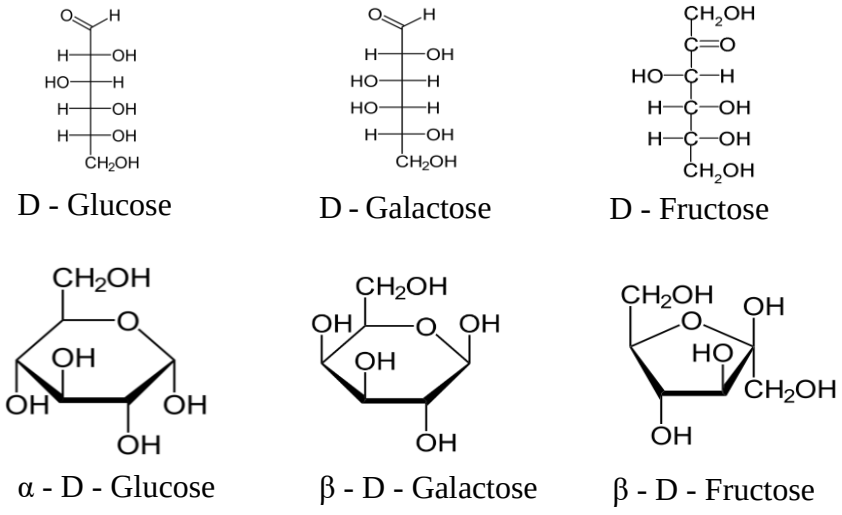


Figure 1.3. Cyclic and acyclic forms of glucose, galactose and fructose.

If both substituents are on the same side of the symmetric plane this configuration has the name β, as it is shown in figure 1.3.[1, 9, 10]

1.1.3.2. Di-, oligo- and polysaccharides

As the title shows, the compounds that will be addressed in this chapter are composed by two or more monosaccharides. In the case of the disaccharides the monomer linkage is called a glycosidic bond or acetal bridge (C - O - C) and it is formed between two carbons, where at least one of them must be an anomeric one, by elimination of one molecule of water. When both carbons entering in this interconnection are anomeric the sugars built are nonreducing disaccharides. This effect happens for the set of two possible ring formations (pyranose and furanose) and the anomeric configuration for both anomeric positions can also be α and β. Logically, the glycosidic bridge of the reducing disaccharides are comprised of one anomeric carbon from one of the units and a hydroxyl moiety from a non-anomeric position of the other unit.[11]

The three most common disaccharides are maltose, lactose and sucrose. Maltose is a reducing sugar, meaning that it can be broken down into two glucose monomers. The linkage occurs between C_1 (the anomeric carbon) from one monomer and the C_4 from the other. Maltose is formed most often from the partial hydrolysis of starch and glycogen and is used in the manufacture of beer. It is considered one of the sweetest sugars, above sucrose. Lactose, also known as milk sugar, unlike most carbohydrates is produced by mammals, in the milk, as opposed to the others that are mostly produced by plants. This compound, as well as maltose, is a reducing sugar with galactose and glucose moieties in its composition. The bond occurs between the anomeric carbon from galactose and the C_4 from glucose. Lastly, the most common and well-known carbohydrate, sucrose (also known as table sugar). Contrasting with the ones afore mentioned, sucrose is a nonreducing sugar making it a unique saccharide. The link between the two monomers is made with both anomeric carbons from α - D - glucose and β - D – fructose, so as long as this molecule stays intact, neither one of the monomers can uncyclize into an open-

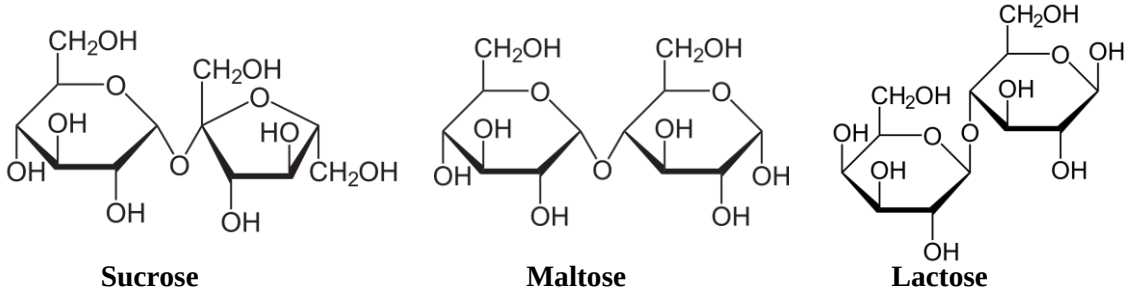


Figure 1.4. Haworth projection of sucrose, maltose and lactose.

chain arrangement. Therefore it can only happen in a single structure in the solid state as well as in solution, which causes it to exhibit some unusual properties. [11, 12]

Oligosaccharides, as well as polysaccharides, are composed by three or more monosaccharides by a glycosidic bond similar to the disaccharides. Although they are considered two different classes of sugars, there is no strict borderline between a high unit oligosaccharide and a polysaccharide being, the first, usually used to name defined structures. These two types of compounds can also be reducing or nonreducing depending on the terminal acetal linkage with the same conditions as for the disaccharides. Oligosaccharides may be found in nature with noncarbohydrate moieties (aglycons) at the reducing end and can also have branched structures.[11, 13]

1.1.4. Glucose

Starting with a brief historical context, glucose was first isolated from raisins in 1747 by the German chemist Andreas Marggraf. Its name was given in 1838 by Jean Dumas and later that century the structure was discovered by Emil Fischer.[14] This aldohexose is by far the most common saccharide there is; it appears in its basic form in fruits, plants, animal blood, as the skeleton in many glycosides, etc., and, as said before, in many di-, oligo- and polysaccharides. Glucose can occur in various forms being the most common the D - glucose form, which can also be called dextrose, originating by its dextrorotatory properties.[15, 16, 17] Naturally, a dextrorotatory molecule also has a laevorotatory form, in this case the laevorotatory form of D - glucose is D – fructose. The IUPAC name for glucose is 2,3,4,5,6 – pentahydroxyhexaldehyde and its molecular weight is 180.16 g/mol.

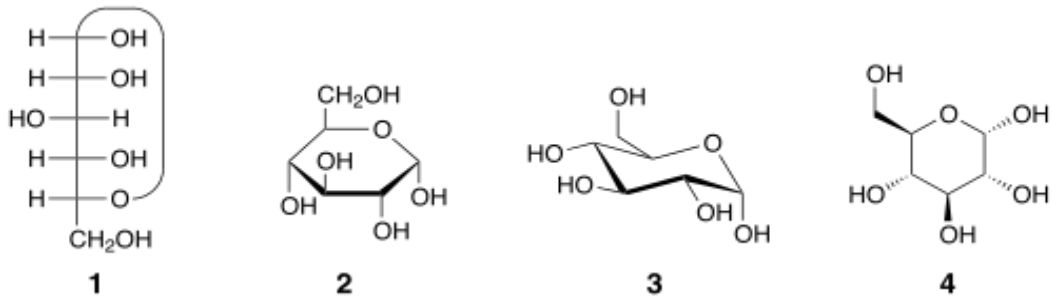


Figure 1.5. Different illustrations of α - D - glucopyranose. Fischer (1), Haworth (2), chair (3) and Reeves conformation (4).

When in ring form, this compound contains alcohol groups and an ether, differing to an aldehyde in the open-chain form. Those functional groups provide this molecule with unique properties (some of them presented in table 1.2.) and chemistry.

Table 1.2. Physical properties of glucose.

Physical properties	
Appearance	White crystals
Molecular weight	180.16 g/mol
Melting point	150 ºC
Density	1.562 g/cm^3 (18ºC)
Solubility	Polar solvents

D – Glucose when in aqueous solutions in equilibrium, as was before mentioned, has 2 anomeric conformations for each hemiacetalic forms (pyranose and furanose) as well as the acyclic form with prevalence of the β – anomeric isomer from pyranose (table 1.3.).[10] The ring closes when in the presence of water as it is described in the figure below (Fig. 1.6.).[18, 19]

Table 1.3. Configuration distribution of glucose, fructose and galactose in aqueous solution in equilibrium.[10]

	Pyranose		***Furanose***		
Carbohydrates	**α-anomer (%)**	**β-anomer (%)**	**α-anomer (%)**	**β-anomer (%)**	**Acyclic (%)**
Glucose	38.0	62.0	0.5	0.5	0.002
Fructose	2.5	65.0	6.5	25.0	0.8
Galactose	30.0	64.0	2.5	3.5	0.02

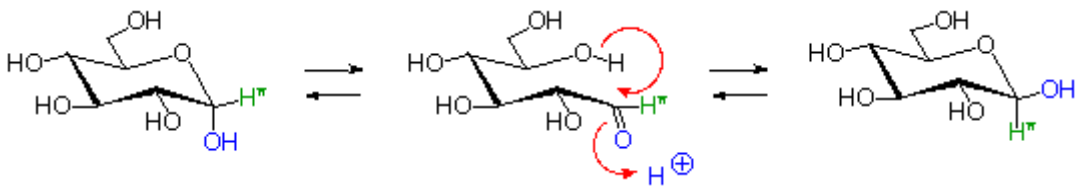


Figure 1.6. Hemiacetal formation mechanism.

This monosaccharide is a high factor to the maintenance of life since it is considered the universal energy transport molecule also being one of the main products of photosynthesis and is considered a starter in the process of respiration. In the mammals this *molecule of life* is stored in a compact way specially in the liver, in the form of glycogen, the "mirror" of starch in the plants that works as an energy storage unit. [16, 17]

1.2. Phenylpropanoid sugar esters

1.2.1. What are they? Where are they found?

Phenylpropanoid glycosides (PhGs) are naturally occurring compounds, most commonly appearing in fruits and vegetables that possess a wide variety of strong biological activities such as antiviral, antifungal, antibacterial, antitumoral, antioxidant, radical-scavenging properties and many others. These compounds have been unknowingly used for centuries in Asian traditional medicine, thus, these compounds are highly praised. [20, 21, 22] Although, the difficulty in extracting them from their natural source, due to the low percentage present (less than 5% w/w), has prevented our species from using them, until now.[23, 24] In the recent years, with the evolution of science, it is possible to study these molecules to their full potential and find new methods for their synthesis.

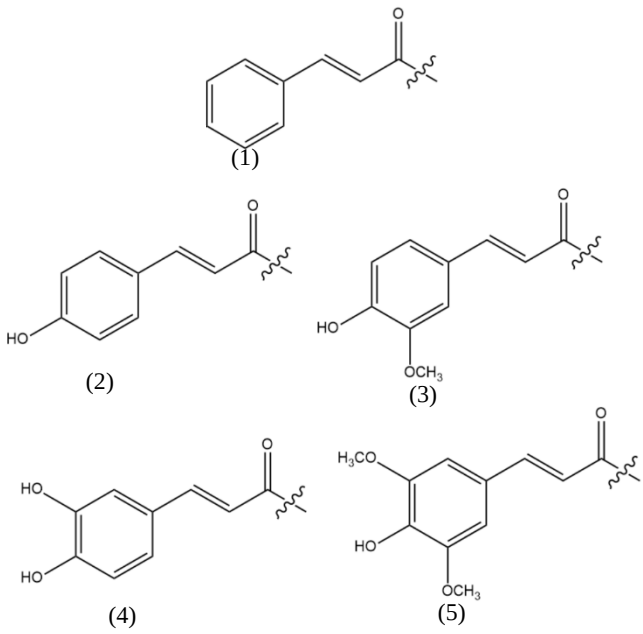


Figure 1.7. Cinnamic acid (1) and its four most common derivatives. (2) Coumaric acid; (3) Ferulic acid; (4) Caffeic acid and (5) Sinapic acid.

As said in chapter 1.1.3.2. these types of compounds are composed by two parts, the aglycon moiety and a glycone moiety (any mono-, di-, oligo- or polysaccharide with a reaction site where they can be substituted). The linkage between both parts happens through a glycosidic bond by elimination of a water molecule. In the case of the PhGs, the aglycon fraction is most commonly a cinnamoyl/cinnamic

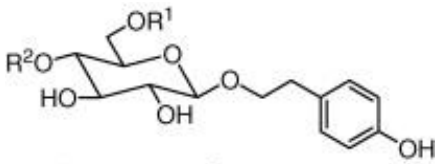
acid derivative – coumaric acid, ferulic acid, sinapic acid or caffeic acid (fig. 1.7.).

1.2.2. Examples of phenylpropanoid glycosides

1.2.2.1. PhGs from monosaccharides

Hundreds of phenylpropanoid glycosides have been isolated and characterized over the course of the last years, from simple PhGs, monosaccharides with one substituent to oligosaccharides with multiples substituents.[25]

Two of the widely known PhGs from monosaccharides are grayanoside A and syringalide B. As shown in the figure 1.8., these two compounds are considered isomers differing solely on the position of the feruloyl moiety, grayanoside A when the feruloyl moiety is on the position 4 and syringalide B when in the sixth site, both having a phenylethyl part in the anomeric carbon (C_1). These compounds are mostly found in the peel of *Prunus grayana* and in the leaves of *Syringa reticulata*, respectively, but can also be found in other plant species.[26]



1: R^1= feruloyl, R^2= H; grayanoside A
2: R^1= H, R^2= feruloyl; syringalide B

Figure 1.8. Structure of the isomers grayanoside A and syringalide B.[26]

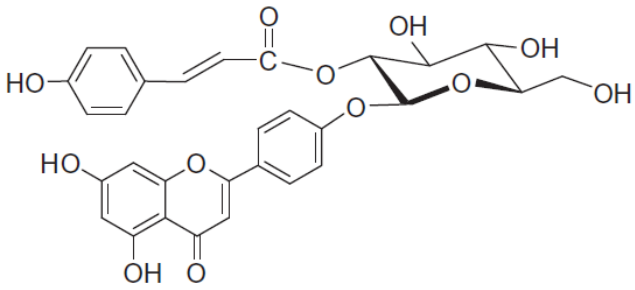


Figure 1.9. Structure of apigenin-4'-yl 2-O-(p-coumaroyl)-β-D-glucopyranoside.[21]

Another example of a compound that has an aglycon other than a hydroxycinnamic acid, we have apigenin-4'-yl 2-*O*-(p-coumaroyl)-β-D-glucopyranoside (fig. 1.9.). This glycoside happens naturally in club moss (*Palhinhaea cernua*) and it is one of its major bioactive components with the capability to inhibit the activity of xanthine oxidase that is a major problem in people with hyperuricemia, but can as well be used to treat rheumatism, hepatitis and nephrolithiasis (kidney stones) and, as many others, has been used in folk Chinese medicine, but just recently started to get pharmacologic interest.[21]

1.2.2.2. PhGs from disaccharides

PhGs from disaccharides, just like the ones covered before are compounds with strong biological activities that vary from antitumoral to immunomodulatory attributes.

Osmanthuside B6 (fig. 1.10.) is a disaccharide PhGs with a rhamnose (a natural deoxy sugar) molecule connected to glucose through a glycosidic bond between C_3 of glucose and the anomeric carbon of the first that is comprised of a coumaroyl residue and a phenylethyl group in positions C_6 and C_1 of the glucose moiety.[25] The majority of the known disaccharides PhGs also have in their composition a glucopyranosyl and a rhamnopyranosyl ring connected the same way as osmanthuside B6. Two of the best-known examples are acteoside and isoacteoside, also possessing cinnamoyl and phenylethyl aglycons.[27, 28] (fig. 1.11.)

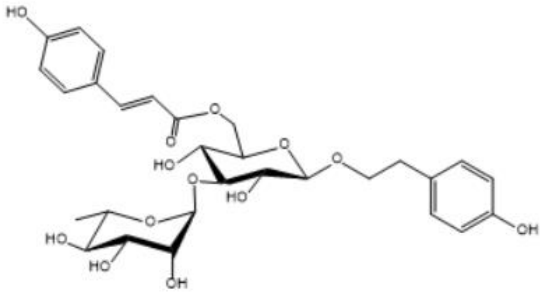
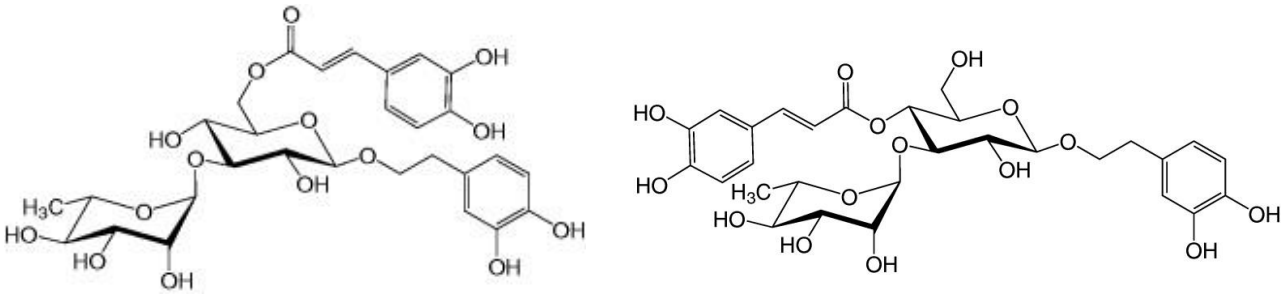


Figure 1.10. Structure of osmanthuside B6.



1 **2**

Figure 1.11. Structures of isoacteoside (1) and acteoside (2).

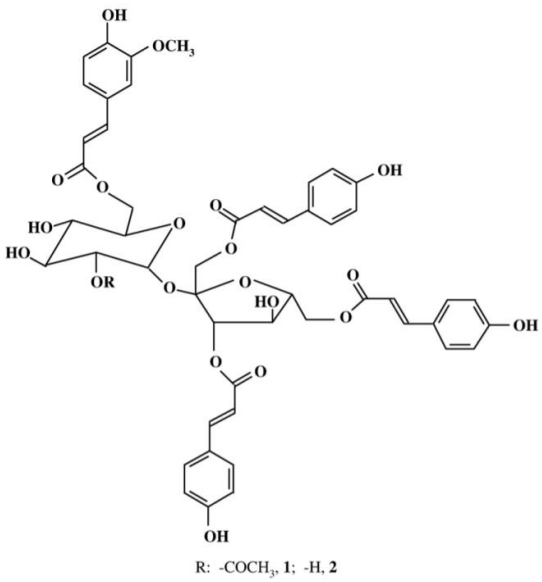
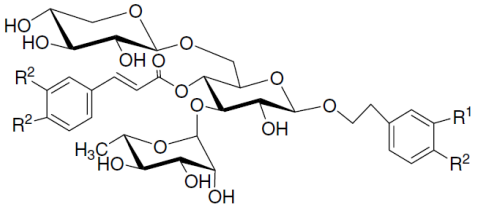


Figure 1.12. Structures of vanicoside A (1) and B (2).

Sucrose PhGs, alongside the compounds described above, are very common. The two compounds illustrated in the left figure, vanicoside A and vanicoside B, are both 2, 3, 6-tri-*p*-coumaroyl-6'-feruloyl diverging in the acetyl group present in position 2' of the glucopyranosyl moiety of vanicoside A. These are promising molecules due to their capability of enzymatic inhibition, i.e. protein kinase C and β-glucosidase, as reported in.[29]

1.2.2.3. PhGs from trisaccharides

Phenylpropanoid trisaccharides are not as common as the ones introduced above but are also provided with amazing properties and bioactivities. Arenarioside might be the most well-known compound of this class and can be found in various species of plants, e. g. *Orobanche arenaria* or *Jasminum nudiflorum*. In the illustration presented (fig. 1.13.) it is possible to observe the structures of arenarioside and one of its analogues, (4-hydroxylphenyl) ethyl α-L-rhamnopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→6)]-4-O-caffeoyl-β-D-glucopyranoside. As the name suggests, these molecules are composed by three sugar pyranoses - glucose, rhamnose and xylose as well as two aglycon fragments. For the first case, these two fractions are caffeic acid at the forth position of glucose and a dihydroxyphenyl ethyl at the anomeric position. For the analogue, the structure differs at the phenylethyl fragment, presenting merely a single hydroxyl group in the aromatic ring's fourth position.[23]



	R^1	R^2
1	OH	OH
2	H	OH

Figure 1.13. Structures of arenarioside (1) and one of its analogues (2).

1.3. Synthesis of PhGs

As mentioned before, PhGs just like many other bioactive compounds appear in nature in very low percentages. Therefore it is necessary to develop methods to synthetize them in order to access some of their pharmacologic value. Although these compounds are composed of sugars (molecules with abounding functional groups), the strategies to produce them do not vary much when compared with "normal" organic synthesis, giving more emphasis to protecting group approaches than direct synthetic procedures.

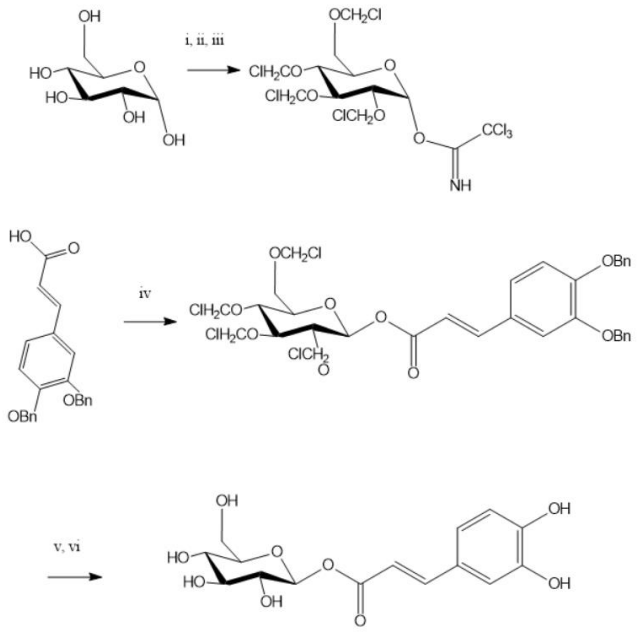
1.3.1. Protection strategies

Protecting group approaches are important in general organic chemistry but when working with carbohydrates this technique gains a whole new importance due to the high number of functional groups, mainly hydroxyl groups. Protecting group have more uses other than protecting, they can also grant other properties to the reacting molecules e.g. increase/decrease the reactivity of the compound in question or participating in reactions, providing different stereochemical outcomes. Due to the fact that there are a lot of different group to protect and unprotect between steps, in a single reaction these techniques can entail some unpredictable problems (mostly steric problems), most often requiring the need of changing to alternative methods.[30]

There are two major types of protecting groups, those that are permanent and those that are temporary. As the name suggests, the permanent are those that will remain through all the synthetic steps of the reaction, used to eliminate possible reactive sites for the entirety of the reaction being then eliminated at the end. The temporary groups are used when it is needed to protect some site(s) for a specific reaction step, being than removed to continue the sequence or when it is needed to have a specific group open to react, in the case of carbohydrates a free alcohol group. The removal of protecting groups (both types) has to be the least harmful possible to the integrity of the reaction, with that meaning that the conditions used in this process cannot interfere with the compound in the reaction medium. This also applies in the opposite case, the conditions used in the synthetic procedure should not interfere with the protective groups. [31, 32]

Despite permanent protection groups being one of the bases of organic chemistry, there are not a large number of possible groups that have all the criteria required. Acetates, benzoates, benzyl ethers and acetals are the only ones with the capability to perform this role well due to their high stability and yield. On the other hand, there are plenty of possibilities when it comes to temporary protection groups, although several of them have similar profiles between them or are not easy to apply in certain contexts. [31, 32]

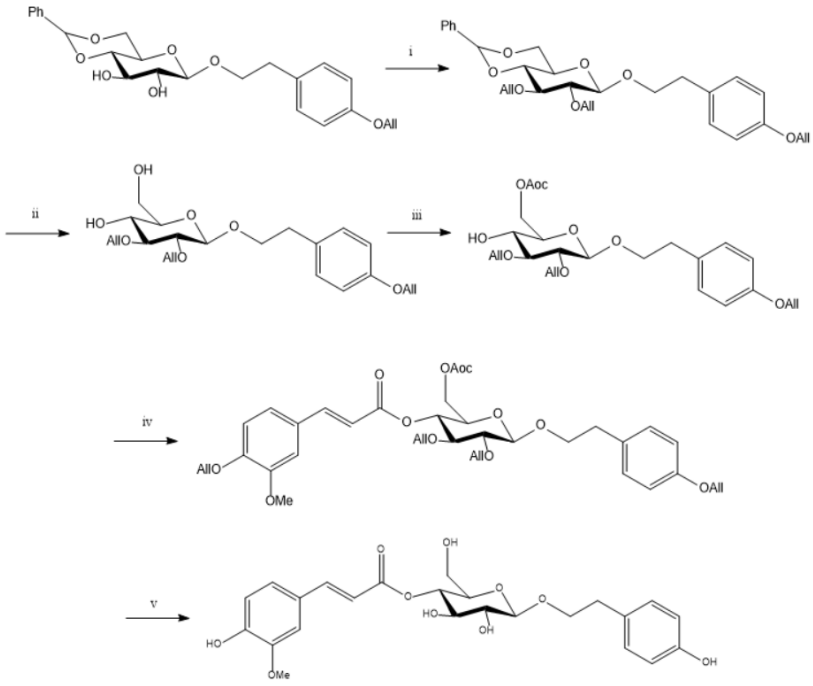
To demonstrate how these techniques can be used, two examples of PhGs synthesis are presented in the schemes below (scheme 1.1. and 1.2.).



Scheme 1.1. Synthesis of 1-*O*-β-Caffeoylglucose; i) $ClCH_2COCl$, CH_2Cl_2/Pyr (9:1), ii) AcO^-,$NH_2NH_3^+$ THF, iii) Cl_3CCN , DBU cat., CH_2Cl_2, iv) 2, AgOTf , CH_2Cl_2, 4Å sieves, v) Pyr/water (1:1), pH 6.7, vi) 1,4-cyclohexadiene 10% Pd/C, EtOH.[33]

In the first case the objective is the synthesis of 1-*O*-β-D-Caffeoylglucose. The first step into this synthesis is the protection of all the alcohols in the α-D-glucose with chloroacetyl groups. These protection groups were chosen because of the ease in their elimination since they could be removed under neutral conditions, preserving the integrity of the whole compound. Apart from the chloroacetyl group in the anomeric position, all the others will stay in their sites until the reaction is finished making them permanent protection groups. Considering that the final compound is a β-anomer and the initial reagent has a pure α configuration it is required to change it. This was achieved by anomeric deacylation (step 2) followed by activation with trichloroacetimidate which grants the molecule the β configuration in the fourth step. To prevent the functional groups from the phenolic moiety from taking part in any reaction, the caffeic acid was protected with benzyl groups.[33]

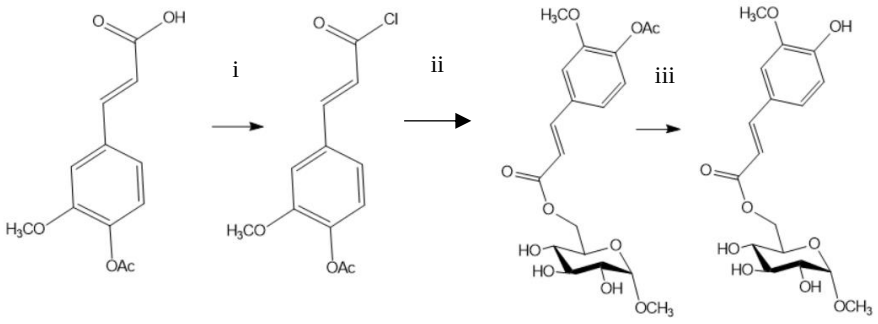
In this next case (scheme 1.2.) the objective is the synthesis of Syringalide B, the glucose phenylpropanoid compound referred in 1.2.2.1.[26] The initial intermediate of this reaction is 2-(4-Allyloxyphenyl)ethyl 4,6-*O*-benzylidene-β-D-glucopyranoside. This compound is protected in positions 4 and 6 of glucose by a benzylidene group and by an allyl group in the hydroxyl of the aromatic ring. This procedure starts by allylation of the first intermediate with allyl bromide, followed by the removal of the benzylidene group with acetic acid, steps 1 and 2, respectively. After these two stages, the molecule is left with both position 4 and 6 unprotected and free to react, but since the glycosylation must happen at the fourth position and the sixth is the most reactive of the two (primary alcohol), protection is needed. In this specific case, the allyl groups are considered permanent protection groups and both benzylidene and allyl 1-benzotriazoyl-carbonate are temporary.[26, 34]



Scheme 1.2. Synthesis of Syringalide B. i) Allyl bromide, NaH/DMF (dropwise); ii) 80% AcOH (80 °C); iii) AocOBt, Et_3N/anhydrous CH_2Cl_2; iv) 4-*O*-allylferulic acid, DCC/cat. DMAP, anhydrous CH_2Cl_2/ 4 Å sieves; v) Pd-C/*p*-TsOH, CH_3OH/H_2O 60-80 °C.[34]

1.3.2. Direct strategies

As aforementioned in the beginning of this chapter, protective strategies are important in the synthetic processes of PhGs but sometimes they are too complicated to use due to their flaws. Otherwise, direct synthesis is simplest and fastest to use, however is way more limited in terms of diversity because it singularly relies on the specificity of the reagents used.[30]



Scheme 1.3. Synthesis of Methyl 6-*O*-Feruloyl-glucopyranoside ester. i) $SOCl_2$, Benzene; ii) Methyl α-D-glucopyranose, Pyr. Cat. DMAP; iii) Pyrrolidine, EtOH.[20]

In the illustration above (scheme 1.3.) it is possible to observe a D-glucose molecule with a methoxy group in the anomeric position (methyl α-D-glucopyranose). Employing a direct synthesis method between the feruloyl moiety and the pyranose ring, the ester happens in the position 6 of glucose since it is the most reactive hydroxyl available.[20]

As it is possible to conclude, this method is extremely fast and simple as it needs just one step, but it limits the variety of possible products.

1.3.3. Enzymatic synthesis

The high complexity of carbohydrates can, in some cases make the called "normal" synthesis to falter due to its low capability of to fulfill stereo- regioselective as well as needing very demanding protecting and deprotecting strategies and there is where enzymatic catalysis gains a great importance. In comparison with chemical synthesis these methods are remarkably selective, do not require protecting/deprotecting strategies as well as working in aqueous solutions without the need of using harmful reactional conditions or toxic chemicals in addition to having the potential to reduce the formation of by-products. These characteristics make this method to have gained a rapid expansion in organic chemistry and considerable recognition in green chemistry

and that, nowadays is one very important aspect to consider due to all the environmental problems.[35–37]

One of the most used and established biocatalysts is *Candida Antarctica* also known as Lipase B or Cal-B. This enzyme is a serine hydrolase with an active site composed by Ser105, His224 and Asp187 that can work as a catalyst in multiple reactions, one of them being esterification.[37]

2. General methods

All the reactions were performed in round bottom flasks with volumes varying between 10 to 250 ml sealed with a serrated rubber. The inert atmosphere was accomplished by applying a rubber balloon filled with argon. The stirring was done with a magnetic bar and a magnetic heating plate.

Unless otherwise specified, all reagents were used directly from the laboratory stock without any type of further purification.

The most common organic solvents, obtained commercially, (dichloromethane, acetone, ethyl ether, hexane, chloroform, methanol) were freshly-distilled before use. Ethanol and pyridine were used directly from the laboratory stock (p.a.). DMF was distilled after being stirred with barium oxide for a day.

All the material/equipment used were already present in the laboratory.

All the TLC revelation was achieved by UV lighting followed by heating the layer after being submerged in a solution of sulphuric acid (5%) in methanol.

Unless otherwise mentioned, the TLC elution when the solution contained acetylated compounds was done in hexane and ethyl acetate (1:1, ratio). The eluent was ethyl acetate with acetone and water (10:10:1, respectively) for sugars with free hydroxyl groups.

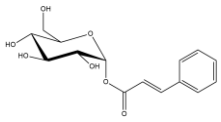
The reactions described are in the chronological order that they were made.

Both fusion point and optic rotation characterization test were preformed resourcing of equipment present in the laboratory.

The NMR tests were supplied by the department.

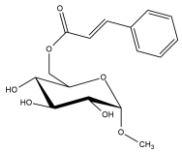
All the products, unless otherwise indicated, turned out to be white solids with a slight petrol/rubber odour.

2.1. Attempt to synthesize 1-*O*-cinnamoyl-α-D-glucopyranose (reaction 1.1/compound 1)



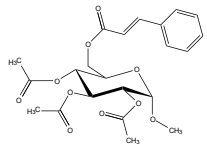
To a solution of 0.600 g of dehydrated glucose (0.0033 mol) in 10 ml of DMF were added 0.570 g of *trans*-cinnamic acid (0.0038 mol, 1.2 equivalents) and 0.720 g of DCC (0.0035 mol, 1.1 equivalents) as a catalyst. The reaction was stirred for 5 days at 60 ºC and was controlled by TLC analysis (ethyl acetate, acetone, water, 10:10:1 as the eluent). After two days a new stain appeared but did not changed for the rest of the reaction time. The reaction solvent was then evaporated under low pressure and purified by preparative chromatography, but it was not possible to isolate any product.

2.2. Attempt to synthesize 6-O-cinnamoyl-α-D-methylglucopyranoside (reaction 2.1/compound 2)



0.567 g of methyl α-D-glucose (0.0029 mol), along with 0.463 g of *trans*-cinnamic acid (0.0031 mol, 1.1 equivalents) and 0.655 g of DCC (0.0032 mol, 1.1 equivalents) plus catalytic amount of DMAP (about a spatula) were added to 12 ml of DMF in a round bottom flask. Throughout the 24 hours of reaction time, the temperature of the mixture was at 60 ºC and controlled by TLC analysis using EtOAc/Acetone/H_2O 10:10:1 as eluent. The work up was carried out by solvent evaporation and the separation was attempted by chromatographic column with a solvent gradient from AcOEt to 10:10:1 (AcOEt/Acetone/water), but no product was isolated.

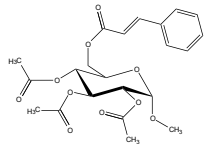
2.3. 2,3,4-*tri*-*O*-acetyl-6-*O*-cinnamoyl-α-D-methylglucopyranoside (reaction 3.1/compound 3)



In 12 ml of DMF, 0.595 g of methyl α-D-glucose (0.0030 mol) were dissolved together with 0.513 g of *trans*-cinnamic acid (0.0034 mol, 1.15 equivalents), 0.903 g of DCC (0.0044 mol, 1.5 equivalents) and cat. measure of DMAP. The reaction was stirred for 24 hours at 60 ºC, followed by solvent evaporation. The solid residue was dissolved in 12 ml of pyridine and 0.90 ml of acetic anhydride (0.0092 mol, 3 equivalents) were subsequently added. The mixture was left to react for 1 h at r.t. The mixture was worked up by mixing toluene with the pyridine solution to form an azeotrope and was than evaporated at reduced pressure. Separation was accomplished by chromatographic column using solvent gradient starting from hexane, ethyl acetate (5:1) to hexane, ethyl acetate (1:1) . The reaction yielded 0.191 g (13.3 %).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.79 – 7.66 (m, 2H), 7.60 – 7.45 (m, 4H), 7.45 – 7.36 (m, 6H), 6.50 (d, *J* = 15.9 Hz, 1H), 6.39 (d, *J* = 16.0 Hz, 1H), 5.70 – 5.56 (m, 1H), 5.53 (t, *J* = 9.8 Hz, 1H), 5.29 – 5.10 (m, 2H), 5.08 – 4.91 (m, 4H), 4.32 (dd, *J* = 24.5, 4.1 Hz, 2H), 4.27 – 4.12 (m, 2H), 4.08 (ddd, *J* = 14.0, 9.4, 3.7 Hz, 2H), 3.46 (d, *J* = 3.5 Hz, 6H), 2.16 – 2.09 (m, 7H), 2.07 (d, *J* = 1.8 Hz, 5H), 2.05 – 1.99 (m, 5H).

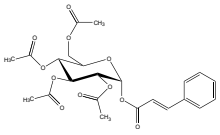
2.4. 2,3,4-*tri*-*O*-acetyl-6-*O*-cinnamoyl-α-D-methylglucopyranoside (reaction 3.2/compound 3)



To a solution of 0.636 g of methyl α-D-glucose (0.0032 mol) in 12 ml of DMF were added 0.503 g of *trans*-cinnamic acid (0.0033 mol, 1.1 equivalents) and 0.728 g of DCC (0.0035 mol, 1.1 equivalents) plus a spatula of DMAP. The reaction was carried on for 24 h at 40 ºC and was controlled by TLC analysis. The work up was accomplished by solvent evaporation. Acetylation was made with pyridine and acetic anhydride and lasted for 2 hours in this case. The mixture was dissolved in ethyl ether and washed with 3 portions (15 ml each) of the HCl solution followed by a saturated solution of potassium bicarbonate and 2 portions of brine. The organic phase was dried and evaporated. The residue was purified by a chromatography column (gradient ? to ?). Yield: 16.2 % (0.249 g).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.80 – 7.66 (m, 1H), 7.56 (dq, *J* = 8.9, 4.0, 3.6 Hz, 2H), 7.45 – 7.35 (m, 3H), 6.39 (d, *J* = 16.0 Hz, 1H), 5.71 – 5.62 (m, 1H), 5.18 – 5.11 (m, 1H), 5.09 – 5.01 (m, 1H), 5.01 – 4.92 (m, 1H), 4.35 (d, *J* = 3.6 Hz, 1H), 4.29 (td, *J* = 15.0, 13.6, 4.7 Hz, 1H), 4.24 – 4.15 (m, 1H), 4.08 (ddd, *J* = 14.0, 9.4, 3.7 Hz, 1H), 3.46 (d, *J* = 3.5 Hz, 3H), 2.17 – 2.10 (m, 4H), 2.07 (d, *J* = 1.7 Hz, 3H), 2.05 – 1.99 (m, 3H).

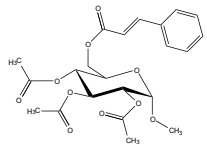
2.5. 2,3,4,6-*tetra*-*O*-acetyl-1-*O*-cinnamoyl-α-D-glucopyranose (reaction 4.1/compound 4)



In a round bottom flask 0.629 g of dehydrated glucose (0.0032 mol), 0.624 g of *trans*-cinnamic acid (0.0041 mol, 1.3 equivalents) and 0.788 g of DCC (0.0038 mol, 1.3 equivalents) plus catalytic amount of DMAP were mixed in 15 ml of DMF and stirred for 24 hours. After 20 hours reacting at 40 ºC the TLC (ethyl acetate, acetone and water; 10:10:1 as eluent) showed what appeared to be the product desired. 10 equivalents of acetic anhydride (≈ 3.03 ml) in 5 ml of pyridine and a small quantity of imidazole were added. The reaction was left for 2 days at r.t. and was controlled by TLC analysis (1:1, hexane and ethyl acetate). The work up followed was the liquid-liquid extraction with ethyl ether as the organic phase and HCl, potassium bicarbonate and brine solutions as washing phases. The separation was performed by double chromatographic column (eluents). The reaction yielded 0.167 g (11.4 %).

^{1}H NMR (400 MHz, Chloroform-d) δ 7.73 (d, J = 9.8 Hz, 1H), 7.69 (d, J = 9.7 Hz, 1H), 7.54 (p, J = 4.5 Hz, 4H), 7.39 (d, J = 2.6 Hz, 6H), 6.47 (dd, J = 16.2, 2.8 Hz, 1H), 6.39 – 6.32 (m, 1H), 5.88 – 5.78 (m, 1H), 5.76 (d, J = 7.6 Hz, 1H), 5.65 – 5.55 (m, 1H), 5.27 (d, J = 9.7 Hz, 1H), 5.20 (d, J = 3.9 Hz, 1H), 4.40 – 4.26 (m, 3H), 4.13 (t, J = 11.7 Hz, 2H), 3.98 – 3.86 (m, 1H), 2.20 (d, J = 6.3 Hz, 3H), 2.11 (dt, J = 7.4, 3.8 Hz, 7H), 2.08 – 2.04 (m, 7H).

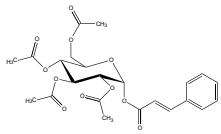
2.6. 2,3,4-*tri*-*O*-acetyl-6-*O*-cinnamoyl-α-D-methylglucopyranoside (reaction 3.3/compound 3)



The esterification process was accomplished after 24 hours using 0.644 g of the monosaccharide (0.0033 mol), 0.523 g cinnamic acid (0.0034 mol, 1.05 equivalents) plus 0.946 g of DCC (0.0046 mol, 1.4 equivalents) in 15 ml of DMF at room temperature. The reaction was controlled by TLC. Acetylation wise, the reaction using pyridine and acetic anhydride carried on for 20 hours. Due to a not so good separation in the first try, a second chromatography column had to be made with less polar eluents – ending in 3:1 (hexane:ethyl acetate). The final yield was 17.0%, corresponding to 0.250 g of product.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.81 – 7.69 (m, 1H), 7.56 (dq, *J* = 8.9, 4.0, 3.6 Hz, 2H), 7.44 – 7.34 (m, 3H), 6.46 – 6.34 (m, 1H), 5.74 – 5.58 (m, 1H), 5.23 (dd, *J* = 15.2, 9.9 Hz, 1H), 5.18 – 5.10 (m, 1H), 5.10 – 5.02 (m, 1H), 5.03 – 4.92 (m, 2H), 4.35 (d, *J* = 3.6 Hz, 1H), 4.32 – 4.25 (m, 1H), 4.25 – 4.12 (m, 1H), 4.08 (ddd, *J* = 14.0, 9.4, 3.7 Hz, 1H), 3.46 (d, *J* = 3.5 Hz, 3H), 2.12 (d, *J* = 10.9 Hz, 3H), 2.09 (d, *J* = 13.8 Hz, 3H), 2.06 – 1.99 (m, 3H).

2.7. 2,3,4,6-*tetra*-*O*-acetyl-1-*O*-cinnamoyl-α-D-glucopyranose (reaction 5.1/compound 4)



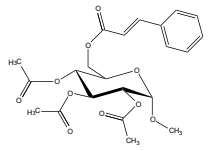
In 8 ml of DMF, 0.397 g of α-D-glucose (0.0022 mol) were dissolved alongside 0.809 g of PPh_3 (0.0031 mol, 1.4 equivalents), 0.441 g of *trans*-cinnamic acid (0.0030 mol, 1.35 equivalents) and ≈ 0.60 ml of DIAD (0.0029 mol, 1.3 equivalents) at 0 ºC when all the solid compounds are already dissolved. The reaction lasted for 24 hours at room temperature, followed by solvent evaporation. The solid from the previous reaction was dissolved in 8 ml of pyridine before being added 4.15 ml of acetic anhydride (0.044 mol, 20 equivalents) plus catalytic amount of imidazole. The mixture was left to react for 24 hours also at r.t. The work up was attained by liquid-liquid decantation with ethyl ether (organic phase) and aqueous solutions of HCl, potassium bicarbonate and brine as washing phases. Separation was accomplished by chromatographic column using solvent gradient starting with hexane and ending in hexane, ethyl acetate (9:2) . Due to lower separation accomplished one fraction was then put through preparative chromatography and the reaction yielded 0.122 g (11.6 %).

Melting point = 110 ºC -115 ºC (Lit. 151.6 ºC – 152.2 ºC)[38]

α[D] = 0.27

^{1}H NMR (400 MHz, Chloroform-*d*): δ 7.80 (dd, *J* = 16.0, 12.9 Hz, 1H), 7.61 (dd, *J* = 6.7, 3.0 Hz, 1H), 7.59 – 7.51 (m, 1H), 7.49 – 7.36 (m, 3H), 6.54 (d, *J* = 16.0 Hz, 1H), 6.49 (d, *J* = 3.7 Hz, 1H), 6.44 (d, *J* = 16.0 Hz, 0H), 5.88 (d, *J* = 7.9 Hz, 0H), 5.59 (t, *J* = 9.9 Hz, 1H), 5.31 (dt, *J* = 13.3, 8.8 Hz, 1H), 5.20 (td, *J* = 10.4, 3.4 Hz, 2H), 4.33 (td, *J* = 12.4, 11.6, 4.2 Hz, 1H), 4.21 (dt, *J* = 10.1, 3.1 Hz, 1H), 3.92 (ddd, *J* = 10.2, 4.5, 2.2 Hz, 0H), 2.11 (d, *J* = 2.7 Hz, 3H), 2.08 (s, 2H), 2.06 (s, 3H), 2.04 (s, 3H).

2.8. 2,3,4-*tri*-*O*-acetyl-6-*O*-cinnamoyl-α-D-methylglucopyranoside (reaction 6.1/compound 3)



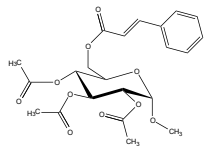
This time in the esterification process instead of using glucose the methylated derivative of glucose was chosen. This time the quantities were 0.218 g of the monosaccharide (0.0011 mol), 0.273 g of cinnamic acid (0.0018 mol, 1.5 equivalents), 0.409 g of PPh_3 (0.0016 mol, 1.3 equivalents) and ≈ 0.30 ml of DIAD (0.0016 mol, 1.3 equivalents). The reaction lasted for 24 hours at 40 ºC in 5 ml of DMF resulting in a yellow oil. The acetylation was achieved using pyridine along with acetic anhydride and imidazole followed by liquid-liquid separation with HCl, potassium bicarbonate, brine and ethyl ether as the organic phase. The reaction mixture was purified by chromatographic column with solvent gradient starting in hexane and finishing with 4:1, hexane, ethyl acetate. 0.135 g of product was obtained with a 26.5 % yield.

M. p. = 73 ºC – 81 º

α[D] = 0.37

^{1}H NMR (400 MHz, Chloroform-*d*): δ 7.73 (d, *J* = 16.1 Hz, 1H), 7.54 (t, *J* = 4.5 Hz, 2H), 7.44 – 7.35 (m, 3H), 6.48 (d, *J* = 16.0 Hz, 1H), 5.50 (t, *J* = 9.8 Hz, 1H), 5.12 (t, *J* = 9.9 Hz, 1H), 4.33 (d, *J* = 3.6 Hz, 2H), 4.08 (dd, *J* = 10.5, 3.6 Hz, 1H), 3.43 (s, 3H), 2.08 (s, 3H), 2.05 (s, 3H), 2.02 (s, 3H).

2.9. 2,3,4-*tri*-*O*-acetyl-6-*O*-cinnamoyl-α-D-methylglucopyranoside (reaction 6.2/compound 3)



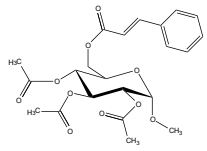
0.237 g of methyl α-D-glucose (0.0012 mol), 0.248 g of the cinnamoyl moiety (0.0017 mol. 1.4 equivalents) and 0.509 g of triphenylphosphine (0.0019 mol, 1.6 equivalents) were dissolved in 5 ml of DMF and then complemented with 0.35 ml of DIAD at 0 ºC. By TLC analysis it was possible to observe the appearance of a stain that could be the pretended product and the reaction was left reacting for 24 hours. To work up the reaction a liquid-liquid separation was done resourcing of methanol and hexane, the PPh_3 dissolved in the less polar, hexane and the remaining compounds went to the methanol phase (including DMF). The methanol was therefore evaporated, and 5 ml of pyridine were added together with 20 equivalents of acetic anhydride (≈ 2.25 ml) and cat. amount of imidazole. The mixture was reacting for 72 hours (over the weekend) and was worked up using a liquid-liquid separation. In order to achieve separation a chromatography column with solvent gradient (hexane to 9:2, hexane, ethyl acetate) was done. This reaction yielded 0.3726 g of product (68.9 %).

M. p. = 88 ºC – 90 ºC

α[D]= 0.41

^{1}H NMR (400 MHz, Chloroform-*d*): δ 7.73 (d, *J* = 16.0 Hz, 1H), 7.55 (dd, *J* = 6.5, 3.1 Hz, 2H), 7.43 – 7.36 (m, 3H), 6.59 – 6.35 (m, 3H), 5.51 (t, *J* = 9.7 Hz, 1H), 5.13 (t, *J* = 9.8 Hz, 1H), 4.92 (d, *J* = 3.6 Hz, 0H), 4.33 (d, *J* = 3.6 Hz, 2H), 4.07 (dt, *J* = 10.4, 3.7 Hz, 1H), 3.44 (s, 3H), 2.08 (s, 3H), 2.05 (s, 3H), 2.02 (s, 3H).

2.10. 2,3,4-*tri*-*O*-acetyl-6-*O*-cinnamoyl-α-D-methylglucopyranoside (reaction 6.3/compound 3)



In 20 ml of DMF, 2.216 g of methyl α-D-glucose (0.011 mol) were dissolved alongside 3.982 g of PPh3 (0.015 mol, 1.4 equivalents), 2.724 g of trans-cinnamic acid (0.018 mol, 1.7 equivalents) and ≈ 3.00 ml of DIAD (0.015 mol, 1.3 equivalents) at 0 ºC when all the solid compounds are already in solution. The reaction lasted for 24 hours at r.t., followed by the liquid-liquid separation methodology with methanol and hexane. The yellow oil from the previous reaction was dissolved in 20 ml of pyridine before being added about 14 ml of acetic anhydride (0.1480 mol, 10 equivalents) plus catalytic amount of imidazole. The mixture was left to react for 24 hours also at r.t. After 20 hours, by TLC analysis was possible to reckon the need to add more acetic anhydride

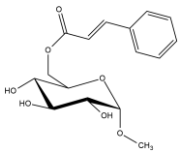
and imidazole, leaving the mixture reacting for an extra 24 hours. The work up was attained by liquid-liquid decantation. Separation was accomplished by chromatographic column using solvent gradient ending in hexane, ethyl acetate (4:1) starting with hexane, solvent used to pack the chromatographic column. Due to lower separation accomplished one fraction required another column and then was possible to detect the existence of one stain superimposing the stain correspondent to the product desired and that caused a yield of 14.0 % and a lower purification.

M. p.= 112 – 117 ºC

α[D]= 0.52

^{1}H NMR (400 MHz, Chloroform-*d*): δ 7.72 (d, *J* = 16.0 Hz, 1H), 7.53 (dt, *J* = 7.8, 3.9 Hz, 2H), 7.45 – 7.33 (m, 3H), 6.47 (d, *J* = 16.0 Hz, 1H), 5.50 (t, *J* = 9.8 Hz, 1H), 5.12 (t, *J* = 9.8 Hz, 1H), 4.33 (d, *J* = 3.6 Hz, 2H), 4.08 (dq, *J* = 10.3, 3.8 Hz, 1H), 3.43 (s, 3H), 2.08 (s, 3H), 2.04 (s, 3H), 2.01 (s, 3H).

2.11. 6-*O*-cinnamoyl-α-D-methylglucopyranoside (reaction 7.1/compound 2)



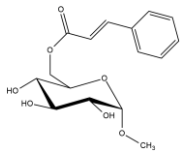
The esterification processes consisted of mixing 0.516 g of methyl α-D-glucose (0.0026 mol), 0.466 g of *trans*-cinnamic acid (0.0031 mol, 1.2 equivalents), 0.925 g of PPh_3 (0.0035 mol, 1.4 equivalents) and 0.60 ml DIAD (0.0031 mol, 1.2 equivalents), at 0 ºC, in 7 ml of DMF. The reaction lasted for 24 h at 40 ºC and then the DMF was evaporated at low pressure. To achieve a desired purification five chromatographic columns were done, although the first four were not done properly since the eluents used were not appropriate (ethyl acetate and acetone in different gradients). The eluent used in the fifth try was ethyl acetate:dichloromethane (1:1) which showed good results. This reaction provided 0.4752 g of product yielding (56.4 %).

M.p.= 73 – 75 ºC (lit. 73 – 75 ºC)[39]

α[D]= 0.53

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.73 (dd, *J* = 6.7, 3.0 Hz, 1H), 7.66 (d, *J* = 16.0 Hz, 1H), 7.47 – 7.40 (m, 1H), 6.66 (d, *J* = 16.0 Hz, 1H), 5.19 (d, *J* = 5.8 Hz, 0H), 4.90 (d, *J* = 4.8 Hz, 0H), 4.81 (d, *J* = 6.4 Hz, 1H), 4.57 (d, *J* = 3.6 Hz, 0H), 4.43 (dd, *J* = 11.9, 2.1 Hz, 0H), 4.20 (dd, *J* = 11.8, 6.6 Hz, 0H), 3.62 (ddd, *J* = 9.1, 6.6, 2.0 Hz, 0H), 3.29 (s, 1H), 3.21 – 3.10 (m, 1H), 2.51 (q, *J* = 2.0 Hz, 0H).

2.12. 6-*O*-cinnamoyl-α-D-methylglucopyranoside (reaction 7.2/compound 2)



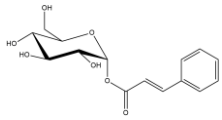
The synthesis of this molecule convoys the exact same protocol as the reaction aforementioned using 0.998 g of methyl glucose, 1.060 g of cinnamic acid, 1.762 g of PPh_3 and 1.3 ml of DIAD, differing solely in the purification step. Instead starting and finishing the chromatography with 1:1 (ethyl acetate:dichloromethane), a gradient was used. This time was also used ethyl acetate and 1:1 (AcOEt:acetone) from lower to higher in terms of solvent polarity. This reaction supplied 0.6170 g of product (37.3 % yield) from 0.0051 mol of limiting reagent (methyl α-D-glucose).

M.p.= 73 – 75 ºC (lit. 73 – 75 ºC)[39]

α[D]= 7.15

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.73 (dd, *J* = 6.4, 2.9 Hz, 1H), 7.66 (d, *J* = 16.0 Hz, 0H), 7.51 – 7.35 (m, 1H), 6.66 (d, *J* = 16.0 Hz, 0H), 5.19 (d, *J* = 5.8 Hz, 0H), 4.91 (d, *J* = 4.8 Hz, 1H), 4.82 (d, *J* = 6.4 Hz, 1H), 4.57 (d, *J* = 3.7 Hz, 0H), 4.49 – 4.36 (m, 0H), 4.20 (dd, *J* = 11.8, 6.6 Hz, 0H), 3.68 – 3.56 (m, 0H), 3.29 (s, 2H), 3.16 (td, *J* = 9.4, 5.3 Hz, 1H).

2.13. 1-*O*-cinnamoyl-α-D-glucopyranose (reaction 8.1/compound 1)



1.0915 g of α-D-glucose (0.0061 mol), along with 1.5005 g of cinnamic acid (0.010 mol, 1.7 equivalents), 2.0794 g of PPh_3 (0.0079 mol, 1.3 equivalents) and 1.6 ml of DIAD (0.0079 mol, 1.3 equivalents) were dissolved in 12 ml of DMF and reacted for 24 hours at 40 ºC. The reaction was controlled by TLC analysis using 10:10:1 as an eluent. The work up was attained by solvent evaporation under very low pressure. Since the starting material (α-D-glucose) is not soluble in AcOEt, recrystallization of this compound was underwent followed by filtration of the precipitated phase. The fraction that dissolved in ethyl acetate was then put through a silica column in which the eluents used were ethyl acetate and 1:1 (AcOEt:acetone) in a gradient fashion. This reaction gave 0.4518 g of final product (25.0% yield).

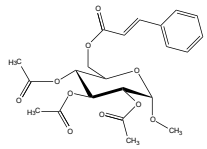
M.p.= 145 – 150 ºC (lit. 129 – 132 ºC)[40]

α[D]= 0.16

^{1}H NMR (400 MHz, DMSO-d_6): δ 7.74 (d, *J* = 4.1 Hz, 1H), 7.45 (d, *J* = 2.5 Hz, 1H), 7.45 – 7.43 (m, 1H), 6.68 (d, *J* = 5.8 Hz, 0H), 6.66 – 6.62 (m, 0H), 5.50 (d, *J* = 7.9 Hz, 0H), 5.34 (d, *J* = 5.2

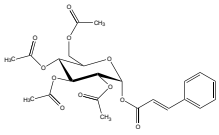
Hz, 0H), 5.21 (d, *J* = 5.8 Hz, 0H), 5.15 (d, *J* = 4.8 Hz, 0H), 5.12 – 5.07 (m, 0H), 5.04 (d, *J* = 5.4 Hz, 0H), 4.61 (t, *J* = 5.9 Hz, 0H), 3.50 – 3.46 (m, 0H), 3.26 (dddd, *J* = 12.3, 8.0, 4.9, 2.2 Hz, 0H), 2.51 (p, *J* = 1.9 Hz, 0H).

2.14. Deacylation of 3.3.



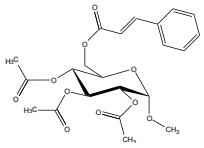
In a round bottom flask all the product that yielded from the reaction was dissolved in EtOH (95%) with addition of about 0.55 ml of piperidine (2 eq. for each acetyl group present in the molecule, total of 6 eq.). The flask was then put in a recipient with ice and salt at -20 ºC and left reacting for 60 hours. TLC analysis did not show any good results and reaction was discontinued.

2.15. Deacylation of 4.1.



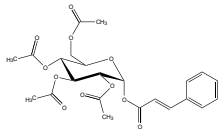
In order to cleave that protecting groups from this molecule a solution of $MeNH_2$ (33%) in EtOH – about 5.5 ml, corresponding to 40 equivalents for each acetyl group, 120 eq. total - was used in which was put to react 0.1644 g of product from reaction 4.1. in 4 ml of CH_2Cl_2. By TLC analysis (10:10:1 as eluent) was possible to conclude that every ester present in this compound were cleaved, including the cinnamoyl moiety. This method was also ceased.

2.16. Deacylation of a fraction from 3.3.



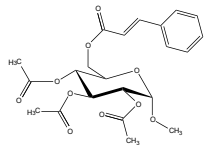
One of the fractions recovered from the chromatography column done in the purification process (0.3765 g of acetylated starting material) was divided in two parts with an objective of widen these studies. 0.1883 g of the compound was dissolved in 5 ml of EtOH and posterior added ≈ 0.50 ml of piperidine corresponding to a total of 4 equivalents. The half left was put to react with 4 eq. of $MeNH_2$ (33%) in EtOH (about 0.60 ml). The reactions were made at – 20 ºC during 24 h. Analysing the TLC layers (1:1, hexane:AcOEt) after 20 hours bring the conclusion that $MeNH_2$ is not able to selectively remove these protecting groups since the stain corresponding to the starting material (methyl α-D-glucose) is present in the application point of the layer. Although piperidine does not appear to remove the cinnamic moiety with such ease it is also evident that it does not remove the protection groups with the yield pretended even leaving the reaction for 60 hours.

2.17. Deacylation of a fraction from 4.1.



Following what happened in 2.16., a fraction from the purification of reaction 4.1. was divided in two halves to test two different possibilities. Both parts were dissolved in 5 ml of EtOH in whereat were added 1.58 ml of piperidine (10 eq. for each acetyl moiety, 40 eq. total) in one and 1.31 ml of pyrrolidine (also 10 eq. for each) in the other. Likewise, the reaction ensued at – 20 ºC over the weekend but at the end of the 60 hours both reactions turned out with a brown colour and by TLC analysis it was attainable to conclude that mixture degraded since the TLC layer did not show the presence of any sugar.

2.18. Deacylation of 3.2.



This reaction followed the same methodology as the one in 2.17. Both parts from a total of 0.1243 g of product were halved and put to react with 10 eq. of piperidine and pyrrolidine with 4 ml of EtOH. Unlike the deacylation described in 2.17. the reaction, this time, only lasted for 24 hours and in the case of pyrrolidine method it showed much greater results, by TLC study, than before. Although, the amount of possible final product was not substantial and could not be isolated.

2.19. Enzymatic synthesis (general method)

To achieve the esterification, more precisely a *trans*-esterification, the sugar moiety (α-D-glucose) was dissolved in a wide variety of solvents namely – dioxane, THF, DMF, pyridine, *t*-BuOH and a mixture of hexane and pyridine (1:1 ratio). To these solutions the solid phase was added, in this case it was a lipase B resin (about 50 to 200 mg) from *Candida Antarctica*, known by other names as CAL-B or Novozyme 435 together with methyl or ethyl cinnamate and, if necessary, a small batch or molecular sieves (3 Å). Depending on the solvent, the reaction times chosen were different although the temperature was the same (between 40 to 55 ºC).

2.20. Synthesis of methyl and ethyl cinnamates

Since none of the esters mentioned were available in stock their synthesis had be made. To achieve this objective, cinnamic acid chloride was dissolved in MeOH and EtOH, respectively with a few drops of sulphuric acid until all the cinnamic acid chloride disappeared. The reaction was controlled by TLC analysis (CCl_4 as the eluent) and then purified by resource of a silica column. Ethyl cinnamate turned out a colourless oil and the methyl cinnamate a white solid, both with a pleasant fruity odour.

3. Results and discussion

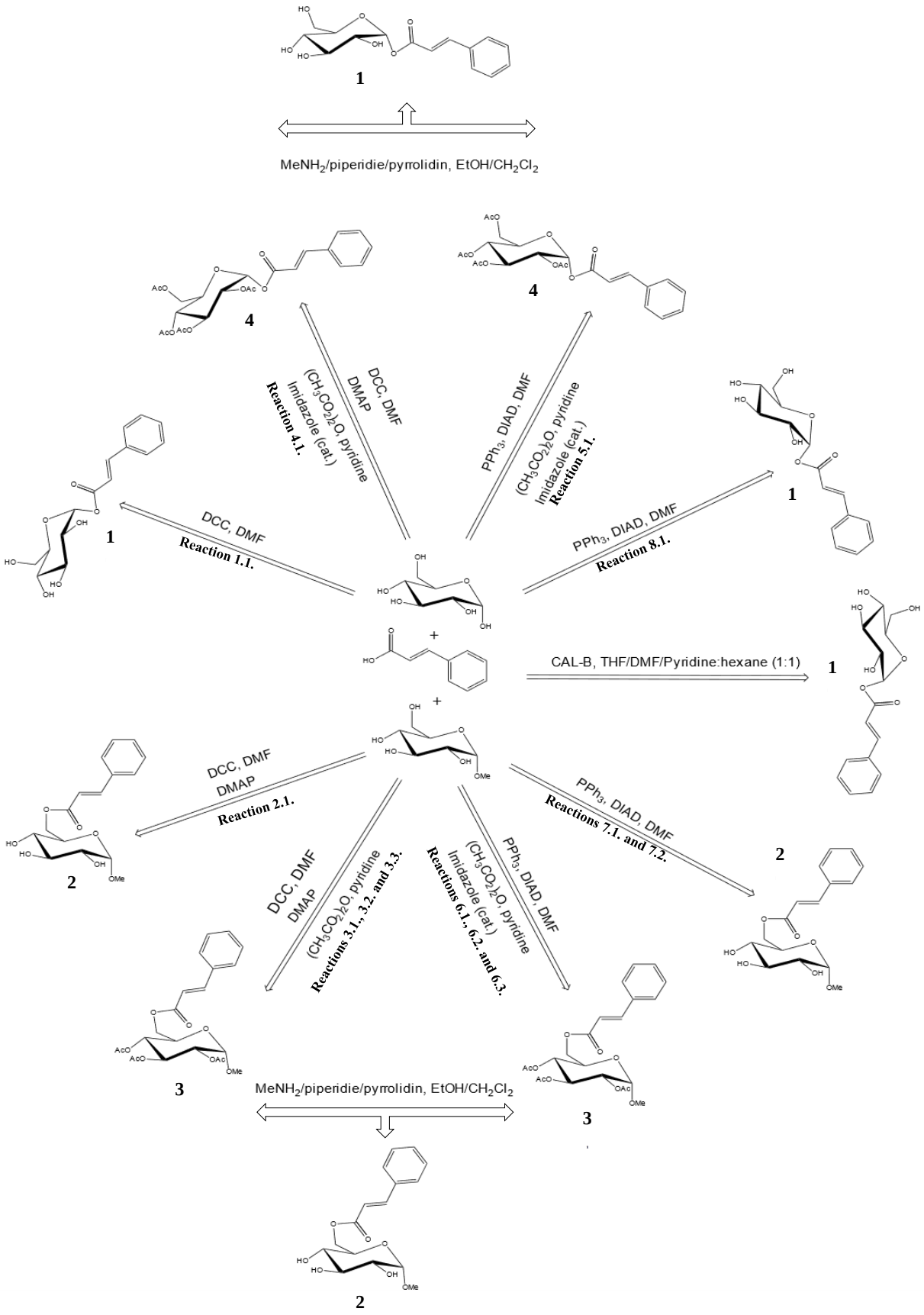
3.1. Objectives

As mentioned in the introduction chapter, PhGs are high bioactive compounds with multiple uses in nowadays concerns. One of the many lead-compounds in the synthesis of these molecules is glucose, the base molecule of our experimental labour.

This work is centred on the development of reproducible procedures towards the direct synthesis of four distinct phenylpropanoid glucose esters derivatized with cinnamic acid in the different hydroxyl groups. Although, the elevated complexity of carbohydrates, which is caused by its high functionality (large number of alcohol groups present) makes their chemical synthesis to be unique and unpredictable.

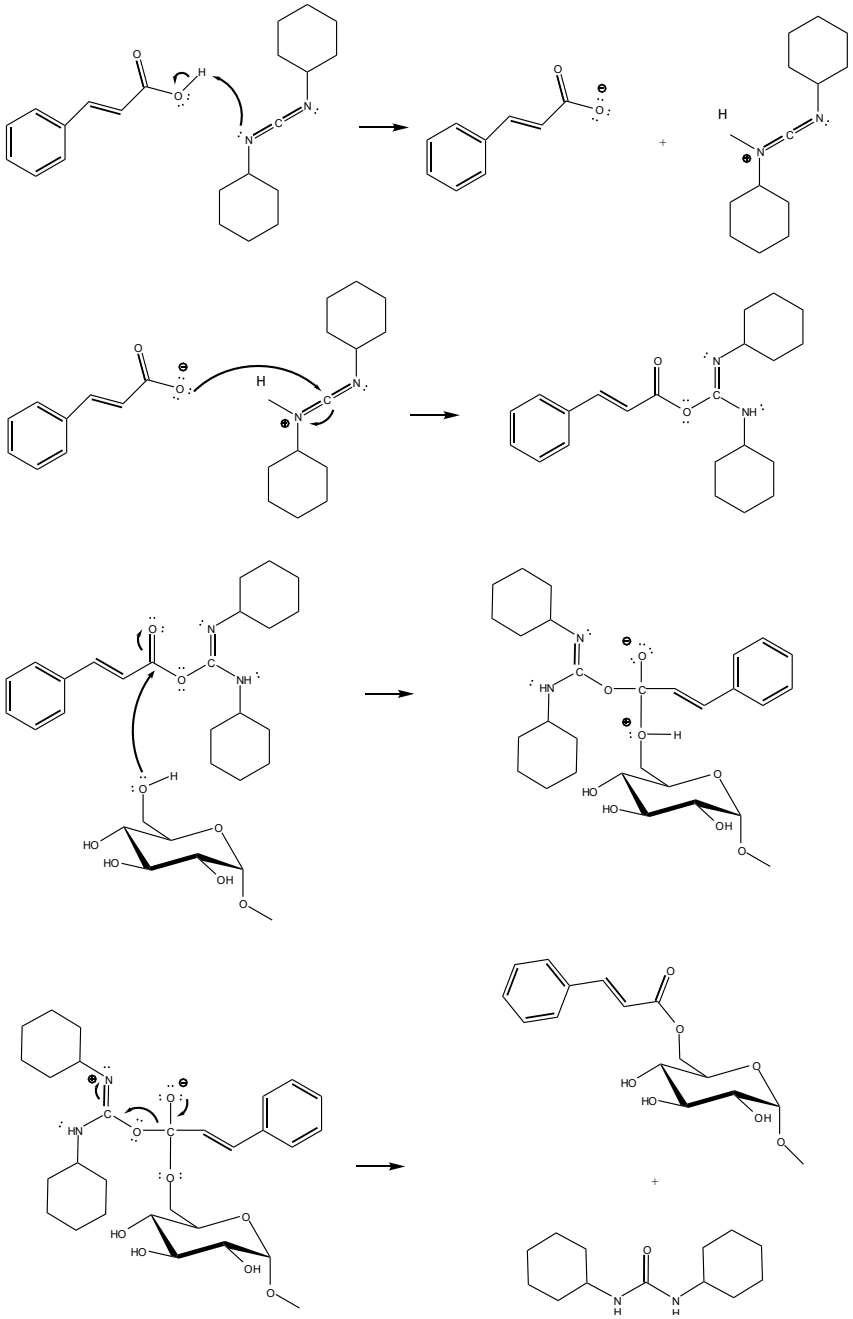
To antagonize these facts, three different synthetic methods were approached and optimized. The Steglich and Mitsunobu esterification with and without protection/deprotection techniques and enzymatic transesterification, the last one being a greener and more specific alternative to the previous.

3.2. General scheme



3.3. Steglich esterification

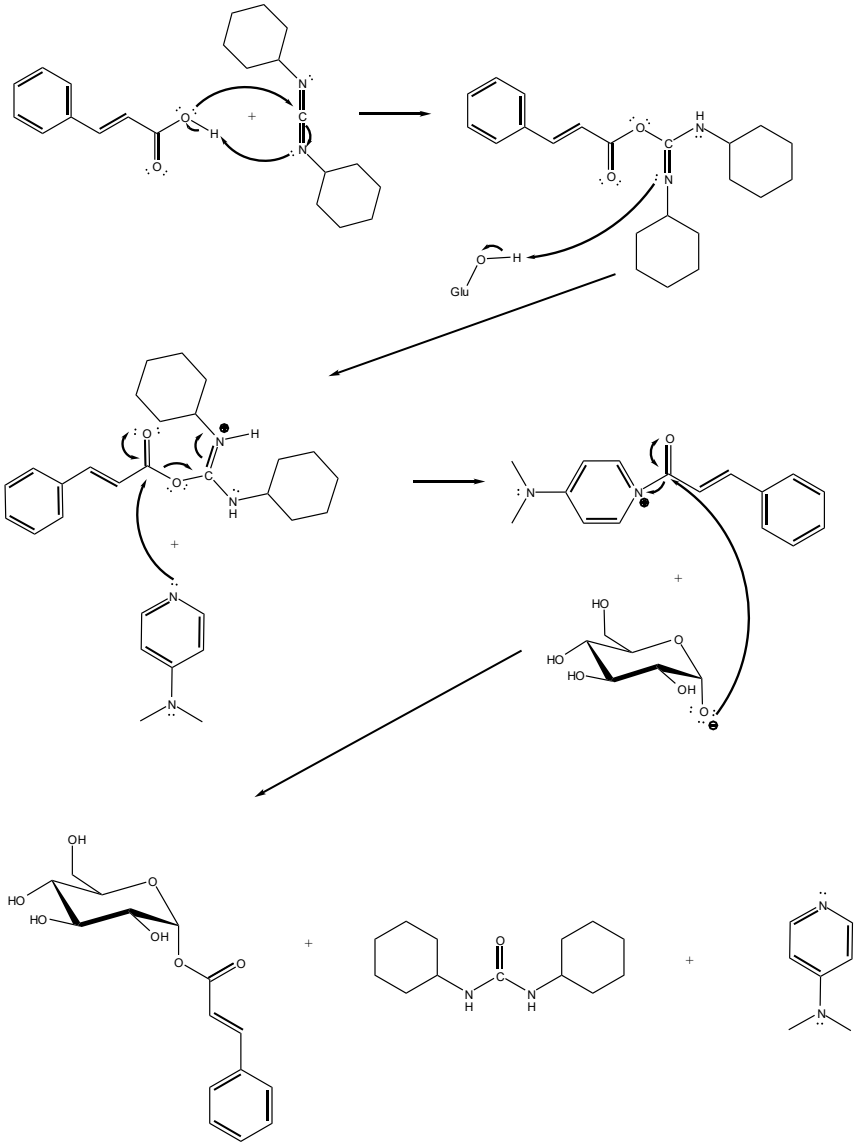
The Steglich procedure is one the most widely used and important methods to achieve esterification. This method works under mild conditions being optimal for carbohydrates, as well as for amino acid chemistry; and resources of dicyclohexylcarbodiimide (DCC) to activate the carboxylic acid as it can be seen in the mechanism bellow (Scheme 3.1.).[41]



Scheme 3.1. Steglich esterification. Mechanism of DCC-activation in the synthetic route of 6-O-cinnamoyl-α-D-methylglucopyranoside.

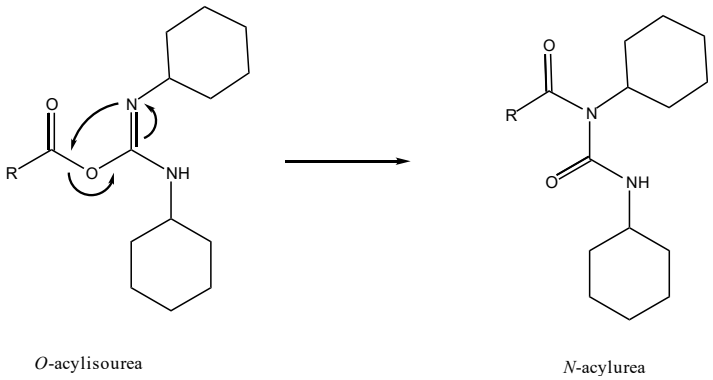
This mechanism is divided in five steps. The first step consists in the deprotonation of the carboxyl moiety via DCC with formation of cinnamate, followed by nucleophilic attack. At this point the carboxylic fraction is prone to be attacked by a nucleophile, in this case one of the hydroxyl groups from the methylglucopyranoside, causing, at last, the removal of the leaving group (dicyclohexylurea).[41, 42]

The method described above is not generally adopted due to the high variations in yields and sometimes undesirable products, more precisely *N*-acylureas caused by acyl migration (scheme 3.3.). Because of that, the most used procedure involves a catalytic reagent that is 4-(dimethylamino)pyridine (DMAP). This reagent, as referred in (add. Ref), accelerates the carboxylic acid activation and reduces the possible amount of side products causing, that way, an increment in the yield even when at room temperature. The mechanism of DMAP - catalysed reaction can be seen in the scheme bellow (scheme 3.2.).[41]



Scheme 3.2. Steglich esterification. Mechanism of DCC and DMAP in the synthetic route of 1-*O*-cinnamoyl-α-D-glucopyranose.

The catalytic effect accomplished by DMAP has to do with its stronger nucleophilicity when compared to an alcohol, in this case a D-glucose molecule.[43] In the Steglich esterification DMAP, as an excellent acyl transfer vehicle, reacts directly with the *O*-acylisourea, which causes the cleavage of the dicyclohexylurea and the formation of a reactive amide that reacts with the carbohydrate. This inhibit the possibility of the reaction leading to *N*-acylureas as side products.[41, 42, 44]

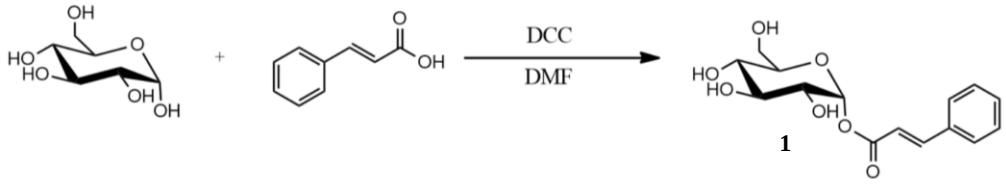


O-acylisourea *N*-acylurea

Scheme 3.3. Acyl migration mechanism. *O*-acylisourea to *N*-acylurea.[44]

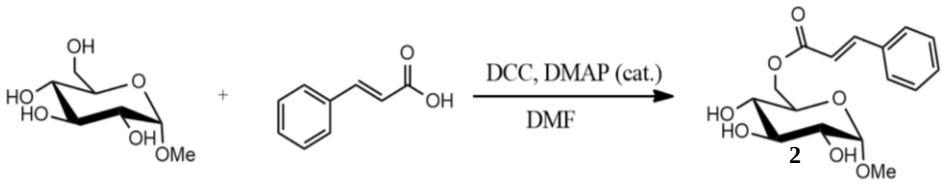
3.4. Direct Steglich esterification reactions

With the purpose of testing the two methods described in the introduction chapter at 1.3., different reactions were performed, as it is possible to see in the schemes 3.4. and 3.5. below (exemplifying the two general methods at different glucose positions). The first represents the reaction of glucose with cinnamic acid in DMF with solely DCC as the esterification agent. The second represents the same reaction but, instead, using methyl α-D-glucopyranoside as the sugar moiety and DMAP as well as DCC as reaction activators.



Scheme 3.4. General Steglich esterification method for the synthesis of 1-*O*-cinnamoyl-α-D-glucopyranose using DCC.

The reaction illustrated on scheme 3.4. was the first to be performed, which means it was far from being optimized. By TLC analysis it was possible to see the presence of a stain that could correspond to the desired product but interpreting the ^{1}H NMR spectrum it was easy to conclude the inexistence of neither the sugar nor the aromatic fraction of a possible PhG. This outcome could have something to do with the high probability of the reaction forming undesirable side products, but due to the low definition of the peaks this could not be established. The presence of water in the solution can represent other explanation for this result. Water, as a very strong nucleophile attacks the site where the alcohol groups from glucose should react, inhibiting it and making the reaction run in a cycle with the constant formation of the cinnamic acid.[43] Since DMF was distilled beforehand, the water can originate from bad dehydration of glucose or bad isolation of the reaction site. Due to the problems attained by this method, it was abandoned and further on we always used DCC/DMAP and the methyl derivative of glucose that does not need dehydration, unlike the D-glucose.



Scheme 3.5. General Steglich esterification method for the synthesis of 6-*O*-cinnamoyl-α-D-methylglucopyranoside using DCC and DMAP.

On account of the lack of success in the previous Steglich procedure, the DCC/DMAP method (scheme 3.5.) was adopted and optimized. In order to achieve maximum optimization, five reactions at different conditions and amounts of reagents were tested, one of them using glucose reaction 4.1.) and the four others using methyl α-D-glucose, as described in the following chapter, as they underwent protection of the remaining hydroxyl groups as well.

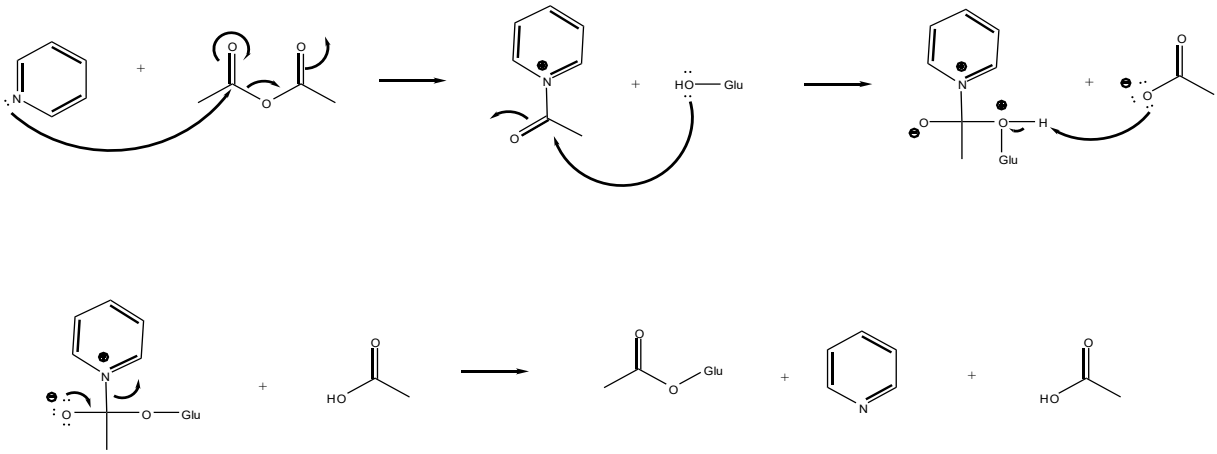
3.5. Direct Steglich esterification with acetylation reactions

Attending the acidic behaviour of the silica gel[45] we thought the bad yields and the absence of either the sugar or the cinnamoyl moieties in the ^{1}H NMR spectra could be a result of interaction between the product and the silicon dioxide (SiO_2). Thus, we tried a different method which consists of protecting the already esterified compound with acetyl groups, thus altering the polarity of the compound and its interaction with the silica gel.

Reaction 3.1. (presented in 2.3.) was the first endeavour to this method. The esterification part of this reaction followed the same path as the one described in scheme 3.5., differing in the conditions and quantity of reagents used (Table 3.1.). Just like the two previous reactions, the esterification did not go as planned, showing a very small stain corresponding to the desired product on the TLC plate. Despite the small quantity of compound obtained, the following acetylation was performed. Purification through silica gel column was not achieved to a great extent due to the presence of multiple compounds with very close polarities (the TLC plate showed four different stains closely together). The explanation for these results could be the reaction conditions. Comparing this reaction with the previous two, what they had in common was the temperature (60 ºC) that could influence the formation of unwanted reactions/products. The use of just 3 equivalents of acetic anhydride in the protection step (one for each hydroxyl group) was not ideal and could have led to the formation of molecules with different number of acetyl groups present, being the reason for the presence of various compound with close polarity indexes. Adding to that the reaction time - one hour, we concluded that to guarantee a higher yield it should have reacted longer.

Table 3.1. Conditions and quantities of reagents used in the esterification procedure of reactions 2.1. to 4.1.

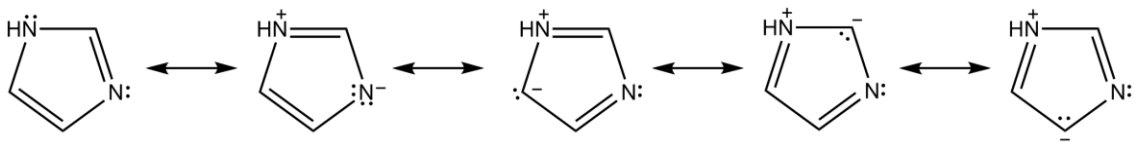
Sugars	*Reaction /compound*	*Reagents*				*Solvent*	*Temp. (ºC)*	*Reaction time*	*Yield (%)*
		Sugar (g)	***t*-cinnamic acid (g)**	**DCC (g)**	**DMAP**	**DMF (ml)**			
Methyl glucopyranose	2.1./2	0.567	0.463	0.655	Yes	12	60	24 h	-
	3.1./3	0.595	0.513	0.903	Yes	12	60	24 h	13.3
	3.2./3	0.636	0.503	0.728	Yes	12	40	24 h	16.2
	3.3./3	0.644	0.523	0.946	Yes	15	r.t.	24 h	17.0
Glucose	4.1./4	0.629	0.624	0.788	Yes	15	40	24 h	11.4



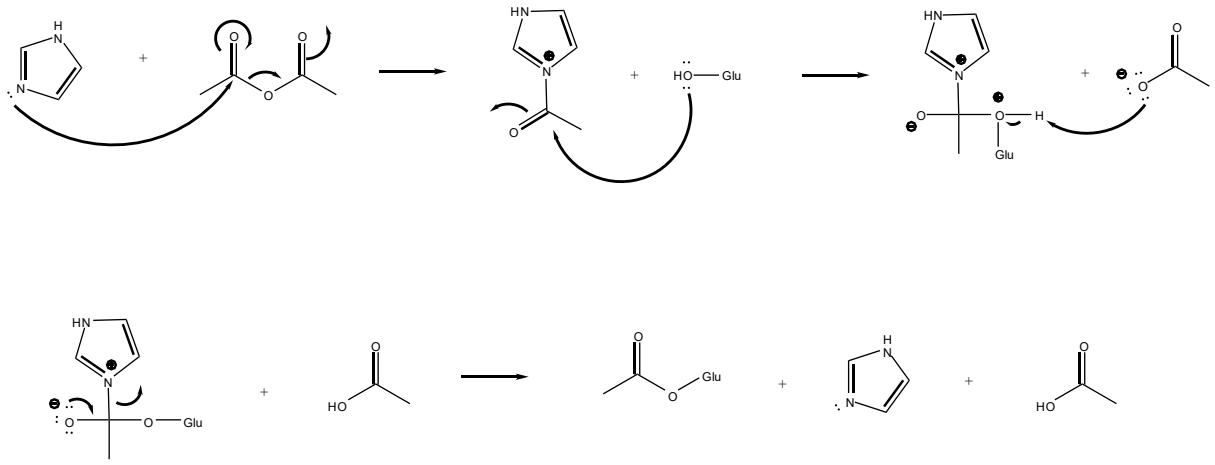
Scheme 3.6. Acetylation mechanism using pyridine as the base catalyst.

The second attempt (reaction 3.2.) on this method provided better results than the previous one. This time the esterification reaction was carried out at 40 ºC instead of 60 ºC which, appeared to have improved the yield of this step. Since we detected two possible problems in the acetylation reaction (the reaction time and the number of equivalents used) we decided to change just one parameter, the time. Thus, the acetylation reaction lasted for 24 hours and, as shown on the TLC plate, it had been enhanced. In the work up of this reaction, instead of the typical solvent evaporation we performed a liquid-liquid separation with HCl 1M to guarantee full removal of the pyridine, in case it had convoluted any problem in the purification task in the previous experiments. These changes caused the yield (16.2%) and the NMR spectrum purity to be improved, although the optimization still leaves space for further adjustments.

Due to the fact that the esterification process was apparently optimized, henceforth we did not change any of the parameters of this synthesis. On the contrary, the acetylation process still needed some development. In the third try (reaction 3.3.) of this method we changed two parameters relatively to the previous reaction, one of them was increasing the number of equivalents of acetic anhydride for each hydroxyl group present (about 10 equivalents total) and the addition of catalytic amount of imidazole. Although the acetyl transfer reaction occurs using just pyridine (scheme 3.6.), the catalytic mechanism of imidazole (scheme 3.8.) certainly grants a more complete reaction since it is more basic than pyridine (about 100 times more), because of the ability to stabilize charge through resonance between both nitrogen atoms (Scheme 3.7.).[46]



Scheme 3.7. Mechanism of resonance of imidazole.



Scheme 3.8. Acetylation mechanism using imidazole as the base catalyst.

The increment in yield after these changes in the acetylation step was visible, but once again the generated final product yield (17.0%) was far from the expected. The reason behind that was a poorly performed silica gel chromatography separation, where almost all of the desired compound coeluted with the starting material due to their highly close polarity even after two tries with different eluent gradients.

In the fourth and final attempt (reaction 4.1) with this method instead of using the methyl derivative of glucose we used glucose, not hydrated. Besides the optimization of both the esterification and the acetylation reactions, the outcome of this experiment was the same as the others. Just like what had happened in the other cases, the problem consisted in the lack of final yield caused by bad silica column separation.

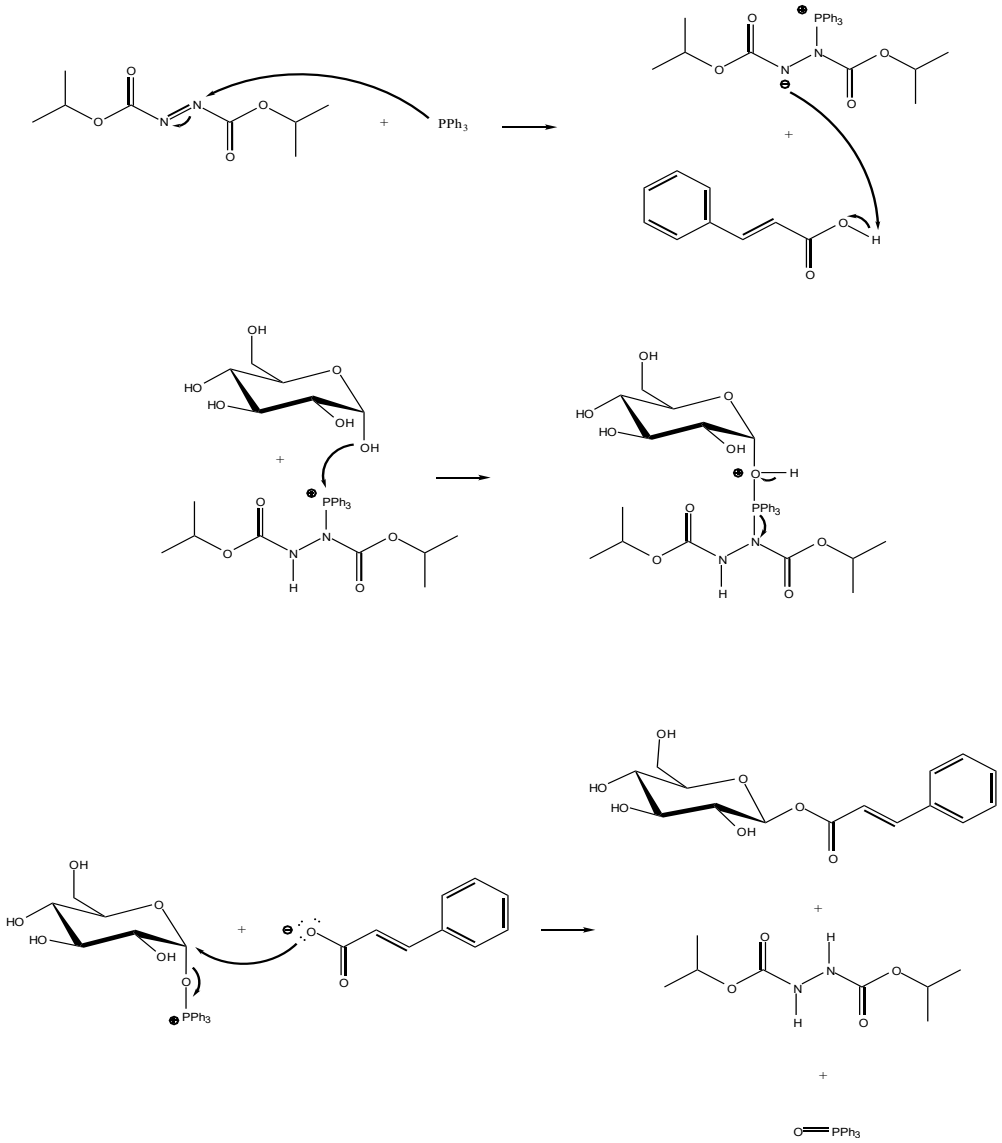
As for the ^{1}H NMR interpretation, by attending on the spectra from the products of reactions 3.1, 3.2, 3.3 and 4.1 (sections 6.1 to 6.4 in the appendix) it is clear to assume that we are in the presence of two different compound due to the fact that all the peaks are in duplicate. Since the only bidimensional spectra that we were able to obtain were those of reaction 3.3, conclusions could only be made for the compounds of reactions 3.1, 3.2 and 3.3. Using the COSY and HSQC spectra we were able to assign all the protons and carbons to their chemical shift along with their correlations, shown in the appendix (6.3.). By analysis of the HMBC spectrum we can assume that both the compounds present are conformers of each other because it is only visibly clear the correlation between the carbonyl carbon (165 ppm) and H2' (6.43 ppm), H3' (7.69 ppm) and H6 (4.33 ppm) being the last the only proton corresponding to the glucose backbone. Which means that the cinnamoyl moiety exclusively happened in the sixth position of glucose. As for reaction 4.1 it is not possible to conclude what is present in the final mixture because in that reaction, we used glucose and not the methylated derivative but, most likely we are in the presence of anomers or two isomers substituted by the cinnamoyl moiety in two different positions, presumably in positions 1 and 6.

Having that in mind we decided to try a whole new method, the Mitsunobu esterification.

3.6. Mitsunobu esterification

The Steglich method, although being one of the most common and important ways to synthetize esters, did not apply as well as expected when it comes to phenylpropanoid glucose esters. Because of that, we decided to address a new method, the Mitsunobu esterification. This reaction, as well as the previous has a significant role in organic and medicinal chemistry, because of its purview and stereospecificity adding to the mild conditions required. This method consists in the substitution of mainly primary and secondary alcohols with nucleophiles mediated by a redox combination between a dialkyl azodicarboxylate and a trialkyl/triarylphosphine.[47] A wide range of compounds that contain alcohol, thiol or amine groups are able to react under these conditions. In our case, the nucleophile is a carboxylic acid (cinnamic acid), the azodicarboxylate is DIAD (diisopropyl azodicarboxylate) and the phosphine is PPh_3 (triphenylphosphine). The reaction is not very solvent specific; THF, acetonitrile, ethyl acetate, DMF, etc. are possible candidates, being the last the one we chose since it met all the conditions needed to perform the task; dissolves all the compound used in the reactions, its boiling point is not too high or low, which is ideal since it allow us to use an higher temperature if needed and it is relatively undemanding in the work up assignments, and it is an aprotic solvent.

The mechanism of the Mitsunobu reaction is described in scheme 3.9. and it is divided in four steps. The first one is the addition of triphenylphosphine to DIAD, yielding a phosphonium salt/cation followed by protonation of the last with the proton originated from the carboxylic moiety. The third step is the attack from the hydroxyl group(s) from glucose with formation of an alkoxy phosphonium salt, followed by the fourth and final step that consists in a S_N2 reaction of which our product results, together with triphenylphosphine oxide and diisopropyl hydrazinedicarboxylate. Attending the last two compounds referred and the two catalysts used it



Scheme 3.9. Mitsunobu esterification mechanism with PPh_3 and DIAD in the synthetic route of 1-*O*-cinnamoyl-β-D-glucopyranose.

is noticeable that this catalytic system can be classified as a redox reaction, since the first one is the product of oxidation of triphenylphosphine and the second is the reduced product of DIAD.[47, 48, 49]

In regard to the conformation of the anomeric position of glucose, is noticeable an alteration in its conformation, changing from α to β. This event happens when the esterification is undergone

in a low sterically hindered secondary alcohol (sometimes tertiary alcohols), like in this case, making the reaction to have a unique aspect that can be very useful and exploitable.[47]

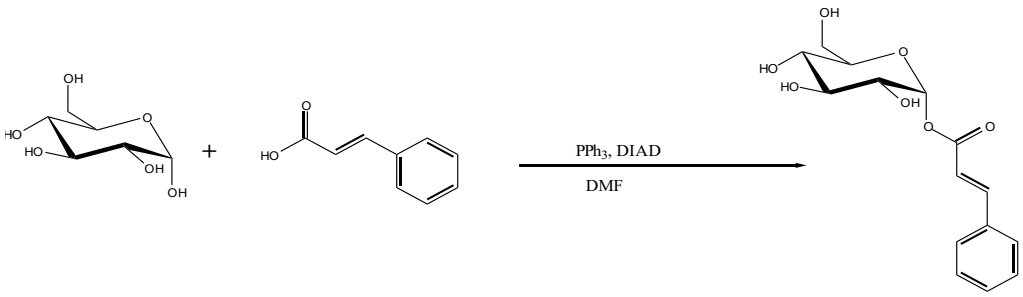
3.7. Direct Mitsunobu esterification reactions

Just like what was described in the previous chapter (the Steglich esterification), this part of our experimental work had as a base the development and optimization of a method to synthetize phenylpropanoid glucose esters, more precisely 6-*O*-cinnamoyl-α-D-methylglucopyranoside, 1-*O*-cinnamoyl-β-D-glucopyranose and their acetylated derivatives. In order to achieve this objective 6 different reactions were tried using different conditions and methods.

The first reaction tried (5.1) did not achieve the best results due to the lack of an optimized protocol. The TLC plates from the esterification process exhibited good results, the same was observed when the acetylation was carried out (as previously optimized). Having that in consideration, the problem could only come from the separation by silica column task. The close proximity in polarity of the components/products of the reaction, was the main problem. Analysing the ^{1}H NMR spectrum correspondent to what allegedly was the isolated product, revealed the lack of purification and a heavy presence of peaks in the aromatic region. The impurity present was most likely PPh_3 that was not separated properly. That is why, we decided to change the eluents of the silica gel column in order to separate the PPh_3 from the desired compound.

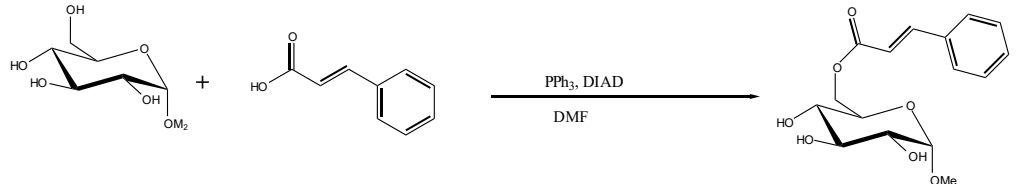
Table 3.2. Conditions and quantities of reagents used in the esterification procedure of reactions 5.1. to 6.3.

Sugars	*Reaction /compound*	*Reagents*				*Solvent*	*Temp. (ºC)*	*Reaction time*	*Yield (%)*
		Sugar (g)	***t*-cinnamic acid (g)**	**PPh_3 (g)**	**DIAD (ml)**	**DMF**			
Methyl glucopyranose	6.1./3	0.218	0.273	0.409	≈0.30	5	40	24 h	26.5
	6.2./3	0.237	0.248	0.509	≈0.35	5	r.t.	24 h	68.9
	6.3./3	2.216	2.724	3.982	≈3.00	20	r.t.	24 h	14.0
Glucose	5.1./4	0.397	0.441	0.809	≈0.60	8	r.t.	24 h	11.6



Scheme 3.10. Mitsunobu esterification scheme for the synthesis of 1-O-cinnamoyl-α-D-glucopyranose using PPh_3 and DIAD.

In the second try on this method (6.1), this time using the methylated derivative, the results were better than before. Although the Mitsunobu reaction supposedly works best at lower temperatures according to the literature[47, 49], we decided to try using mild heating (40 ºC) in this attempt. After 24 hours the TLC showed good results and the same was observed for the acetylation protocol. The changes to the eluent were mild, instead of ending in 9:2 (hexane, ethyl acetate) we decided to slightly increase the polarity (4:1; hexane, ethyl acetate). Although the results were apparently better, both in yield and in NMR purity, PPh_3 was still present in the mixture (to a lesser extent) and that caused a decrease in total yield (26.5%).



Scheme 3.11. General Mitsunobu esterification method for the synthesis of 6-O-cinnamoyl-α-D-methylglucopyranoside using PPh_3 and DIAD.

Even though the results in 6.1. were acceptable, the procedure still had room for improvement and so we decided to remove the heating from the reaction and change the work up method, in order to totally remove the PPh_3 from the mixture (in reaction 6.2.). The usage of methanol and hexane in a liquid-liquid separation is a good method to achieve that goal since methanol does not dissolve the sugar moiety but dissolves PPh_3 and hexane does the exact opposite as well as not being miscible with DMF (the reaction solvent). The work-up has to be done between the esterification and the acetylation steps since the acetyl protecting groups would make the polarity of the molecule to decrease and the method would not be viable. The absence of PPh_3 enhanced the ease in separation and that resulted in a very good final yield (68.9%) and purification.

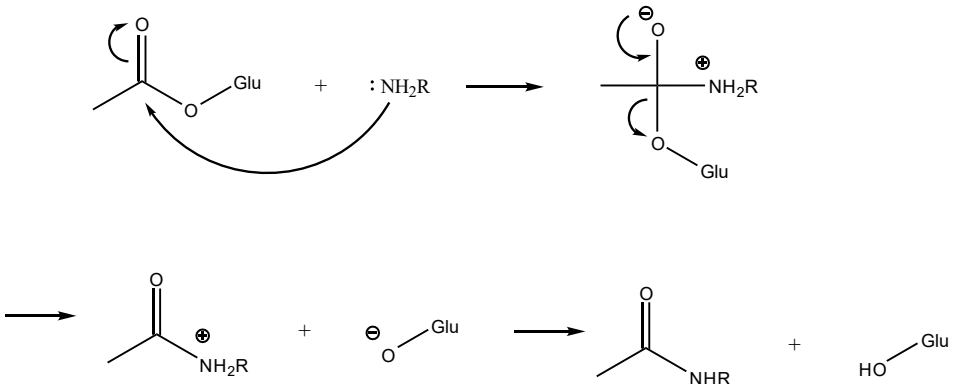
In the third attempt on this method we tried to reproduce the previous reaction. Initially, the process seemed to be working as well as it happened in the prior attempt, both the esterification and the acetylation reactions went as expected but the separation task was not successful. Probable explanation for this could lay on the long time that the mixture stayed in the silica column (over the weekend) and the formation of degradation products.

Unlike what happened in reactions 3.1 to 4.1, with exception of reaction 5.1, the ^{1}H NMR spectra of reactions 6.1, 6.2 and 6.3 (sections 6.5 to 6.8 in the appendix) do not exhibit any evidence of product mixture. This time, due to the clearer spectra we were able to easily identify and assign every proton and carbon to their respective chemical shifts along with their correlations using the COSY and HSQC spectra. By HMBC spectrum interpretation we were able to identify, just as it happened in the reactions 3.1 to 3.3 a correlation between the carbon from the carbonyl (166 ppm) and the proton in the sixth position (4.33 ppm) meaning that the cinnamoyl in present in that position.

3.8. Deacetylation reactions

In order to mimic the natural PhGs and their unique characteristics, the hydroxyl groups had to be deprotected. Thus, the compounds that were acetylated prior to separation via silica gel column have now to be deacetylated. To achieve this goal, several methods were tested using methyl amine, piperidine and pyrrolidine as cleavage reagents. Those reagents had to be selective to remove the acetyl groups from the molecule, without cleaving the cinnamoyl moiety since they are both esters.

Finding a method that could meet these requirements was by far the most challenging part of all our laboratorial work and the results were not satisfactory.



Scheme 3.12. Proposed mechanism for deacetylation with amines.

Piperidine was the initial experimental choice as it is used in other type of deprotections like the deprotection of Fmoc (fluorenylmethyloxycarbonyl) in peptide synthesis.[50, 51] Thus, this compound was a good candidate due to its low nucleophilicity that could help in the selective deprotection required to remove the acetyl groups and maintain the cinnamoyl fraction.[52] We decided to use 2 equivalents of piperidine for each acetyl group present. After 10 hours, by TLC analysis it was possible to observe multiple stains close to each other which meant that several different molecules with different numbers of acetyl groups were present. In the TLC plate of the reaction state at 35 hours, 2 different stains were present, and it was safe to assume that the stain with the higher Rf belonged to the cinnamoyl moiety, since it had fluorescence but did not reveal in the sugar stain. The second one had a very low Rf, did not reveal under UV light and revealed as a sugar, which meant we were in the presence of methyl glucose. With these facts it is evident that this method is not appropriate to complete the task.

The second attempt of deprotecting the compounds, the product of reaction 4.1 this time, we decided to try a method reported by T. Kawada et al.[27] in which they use 40% $MeNH_2$ in MeOH in a ratio of 40:1 in proportion to acetyl group, obtaining a yield of about 50%. After 1 and a half hours of reacting 33% $MeNH_2$ in EtOH (the most similar that we had in stock) with our compound, we were only in the presence of glucose and cinnamic acid.

In the last 3 experiments we used different amounts of amines ($MeNH_2$, piperidine and pyrrolidine) and the outcome was always the same. The majority of the initial compound was transformed in glucose and the rest was in too low amount to be possible to isolate and characterize.

Although the purification of the acetylated compounds by silica gel column is easier than the non-acetylated, we decided to abandon this method for obvious reasons.

3.9. Mitsunobu esterification reactions without acetylation

To bypass the problem aforementioned, we had to develop a method that could surpass the difficulties in isolating the non-acetylated products by chromatography column, objective that we were not able to achieve in previous experiments. Due to limited time we were only capable of experimenting 3 distinct procedures, 2 with the methyl derivative and one with glucose, in which the esterification method that was previously optimized was used.

The challenging part and the one that needed development and optimization was the purification exercise. This task had already been attempted in the earlier part of our experimental work without success. Those bad results could have been caused by multiple factors, but the lack of experience in working with these compounds might be the main one. In order to counteract that, we employed the practice gained throughout the time engaged learning how sugars behave in certain conditions to find a method that could resolve this problem. The first experiment was reaction 7.1. and in respect to the final yield obtained (superior to 55%) it is possible to state that it was a success. After 6 different efforts with distinct eluents gradients, in which the first 5 were not capable of isolating the desired product, we found one that accomplished the task – (1:1, AcOEt/CH_2Cl_2). Initially, this first attempt was supposed to be used as a lead to experiment various different eluents in order to find one that could work in further reactions but surprisingly, even after 6 tries putting our mixture through the silica column, we obtained one of the highest yields since the beginning of this project.

Table 3.3. Conditions and quantities of reagents used in the esterification procedure of reactions 7.1. to 8.1.

Sugars	*Reaction /compound*	*Reagents*				*Solvent*	*Temp. (ºC)*	*Reaction time*	*Yield (%)*
		Sugar (g)	***t*-cinnamic acid (g)**	**PPh_3 (g)**	**DIAD (ml)**	**DMF**			
Methyl glucopyranose	7.1./2	0.516	0.466	0.925	≈0.60	7	40	24 h	56.4
	7.2./2	0.998	1.060	1.762	≈1.30	12	r.t.	24 h	37.3
Glucose	8.1./1	1.092	1.501	2.080	≈1.60	12	40	24 h	25.0

The same did not happen in reaction 7.2. because of the excess of time that took us to finish the purification (our objective was trying to maximize the final yield by increasing the polarity at slower rate), causing the mixture to be interacting with the silica gel for a large amount of time and that led to product degradation and a lower yield (37.3%). Although the yield was not very high, it was an improvement when compared to most of the preceding assays.

The third and final Mitsunobu reaction was performed using glucose instead of methyl glucopyranoside. At first thought the two different initial reagents would not make a big difference when concerning the purification task, but when we tried to dissolve the reaction mixture in 1:1 (AcOEt/CH_2Cl_2), it would not dissolve. Despite the small differences between the both compounds the slight polarity dissimilarity alters the task completely. Since glucose does not dissolve in AcOEt, but the rest of the mixture components do, we decided to perform a recrystallization followed by filtration. Following that step we carried out a chromatography column like the two described atop using the same eluents. The final outcome of the reaction (25.0%) was relatively good, but presented two distinct stains superimposed, both corresponding to possible phenylpropanoid glucose esters that could be anomers or isomers due to their very approximate Rf's in TLC analysis.

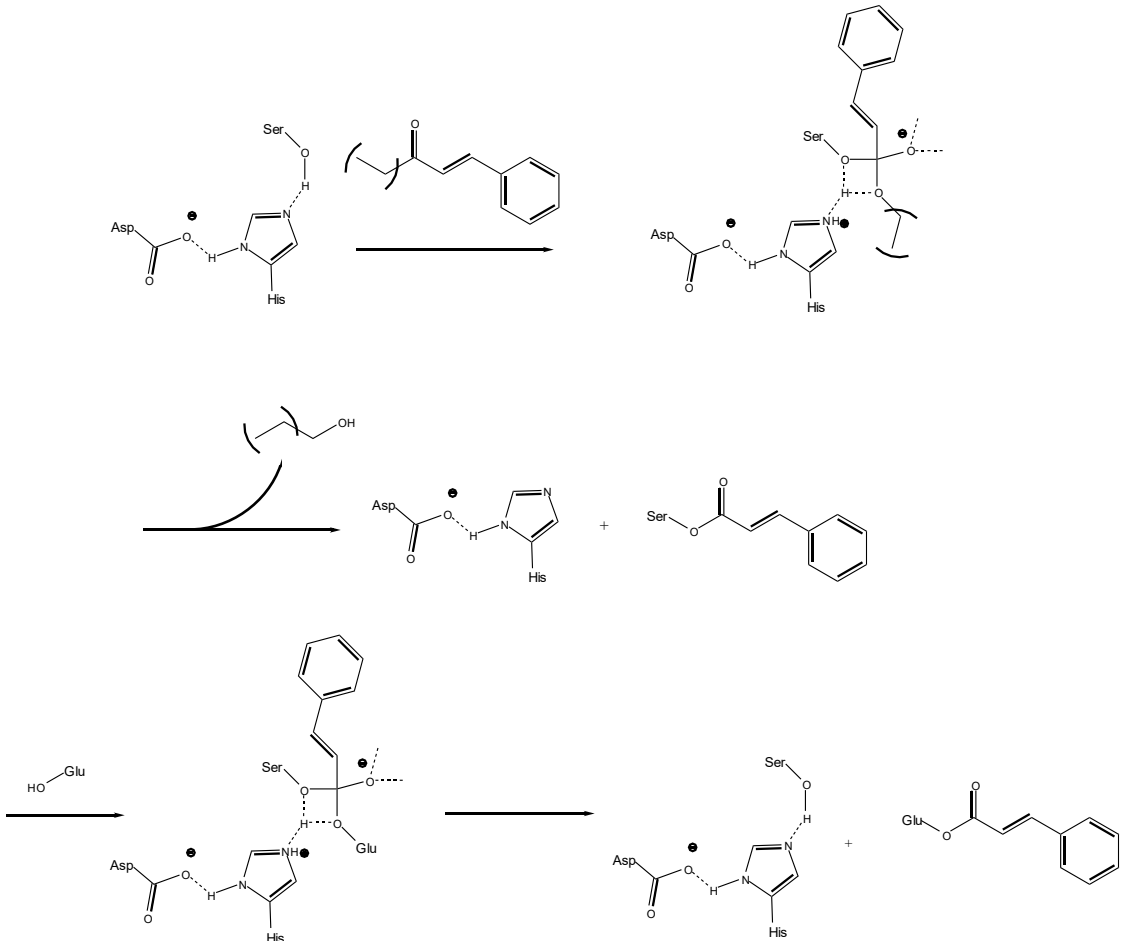
Considering all that was stated above, it is safe to assume that, considering the obvious difficulties of this task we achieved good results that could be easily reproduced, leaving still space for further optimization.

3.10. Enzymatic synthesis

In some cases, chemical synthesis of certain compounds entails very demanding and time-consuming protection and deprotection steps. Chemical synthesis of carbohydrates, in most of the occasions are included in that category and that is why novel methods to simplify those types of reactions are emerging, one of them being enzymatic synthesis. Enzymatic synthesis is considered a greener alternative to the "normal" chemical synthesis, since it does not use acrid or toxic chemicals/reagents, as well as being extremely specific in stereoselective synthetic procedures.[35-37]

For that reason, we decided to experiment this method since we are working with compounds where selectivity is what matters the most. To undertake this method, we realized 4 different enzymatic transesterifications in which we changed the solvent of the reactions (probably the most important factor for the reaction to happen) and the cinnamate. In the 3 initial reactions we used ethyl cinnamate with DMF, hexane:pyridine (1:1) and *t*-BuOH, respectively, but by TLC analysis it was possible to conclude that the first and last did not react at all unlike the one where we used hexane and pyridine. In that reaction, the TLC plate, after 3 days reacting, showed the appearance of a new stain correspondent to a PhG (it had the same rf as in the previous cases), but unfortunately we were not able to isolate and characterize this compound since it appeared to had degraded as we tried to retrieve it from the preparative chromatography silica after being in solution with MeOH for 48 hours (over the weekend).

Overall this method has not presented us with the best results apart from the reaction where hexane and pyridine were the solvents. Although the lack of optimization that lead to the impossibility of isolating and characterizing the product of that reaction it is acknowledgeable that this is a very promising method in carbohydrate synthesis that could work as an alternative to the lesser "green" procedures used nowadays.



Scheme 3.13. Proposed mechanism for the transesterification using Cal-B and methyl/ethyl cinnamates.[37]

The mechanism proposed for the transesterification using Cal-B as the enzymatic intermediate is illustrated above (scheme 3.13). This mechanism is divided in 4 steps, the initial one being the nucleophilic attack performed by the serine to the cinnamate with formation of a tetrahedral intermediate that evolves to the "acylated enzyme", as referred to by V. Ferrario et al.[37], with displacement of MeOH/EtOH. The second nucleophilic attack happens between the "acylated enzyme" and the secondary alcohols of glucose generating the second tetrahedral intermediate followed by the formation of the esterified alcohol and regeneration of the enzyme.

4. Conclusions and future perspectives

This project was based on three different approaches to achieve a common goal. That goal was the develop and optimize protocols for the synthesis of phenylpropanoid esters of glucose and one of its derivatives, the methyl glucopyranose. Those methods were the Steglich and Mitsunobu esterification and the transesterifications through enzymatic catalysis. The results attained for all the three methods are presented in the table below (table 4.1.).

Table 4.1. Final summary of all the reactions and respective yields.

Method	Sugar	Reaction	Solvent	Acetylation	Yield (%)
Steglich	Glucose	1.1.	-	No	-
		4.1.		Yes	11.4
		2.1.		No	-
	Methyl glucopyranose	3.1.		Yes	13.3
		3.2.		Yes	16.2
		3.3.		Yes	17.0
Mitsunobu	Glucose	5.1.		Yes	11.6
		8.1.		No	25.0
	Methyl glucopyranose	6.1.		Yes	26.5
		6.2.		Yes	68.9
		6.3.		Yes	14.0
		7.1.		No	56.4
		7.2.		No	37.3
Enzymatic catalysis	Glucose	-	THF	No	-
			Hexane:Pyridine (1:1)		
			t-ButOH		

Both the Steglich and the Mitsunobu esterification methods are already well established in the science community and for that reason the challenges in the esterification reactions involving these approaches were not very arduous to surpass. However, besides reactions 6.2. and 7.1., the yields were not that great, being the main cause for that the lack of success in the purification task that presented itself as the troublesome part of this experimental work. Initially we thought that the lack of good results were affected by the absence of experience concerning this exercise but in later reactions the results still were not the desired. The conclusions that could be extracted are that purifications of these compounds through silica gel chromatographic column may not be the method to use because of the complex nature of the carbohydrates and the close similarity in polarity that cause the outcomes to be inconsistent and so, the use of more refined methods such as HPLC or MPLC might be the ideal path to follow.

Although the acetylation processes undergone in some of the reactions described helped counteract some of the problems accrued in the purification tasks, the fact that specific deacetylation being impossible makes this exercise ineffective.

The reactions involving enzymatic catalysis did not presented the greatest results. Besides the reaction in which the solvent was pyridine:hexane (1:1) we were not able to withdraw any positive outcomes due to the time constrains that prevented us from creating a possible optimized protocol. Although it is possible to assume that optimal solvent selection is crucial in reaction involving these types of substrates.

In order to understand if these phenylpropanoid glucose esters have identical biologic activities to the other examples of PhGs reported, further biological activity studies, along with computational modelling is preeminent.

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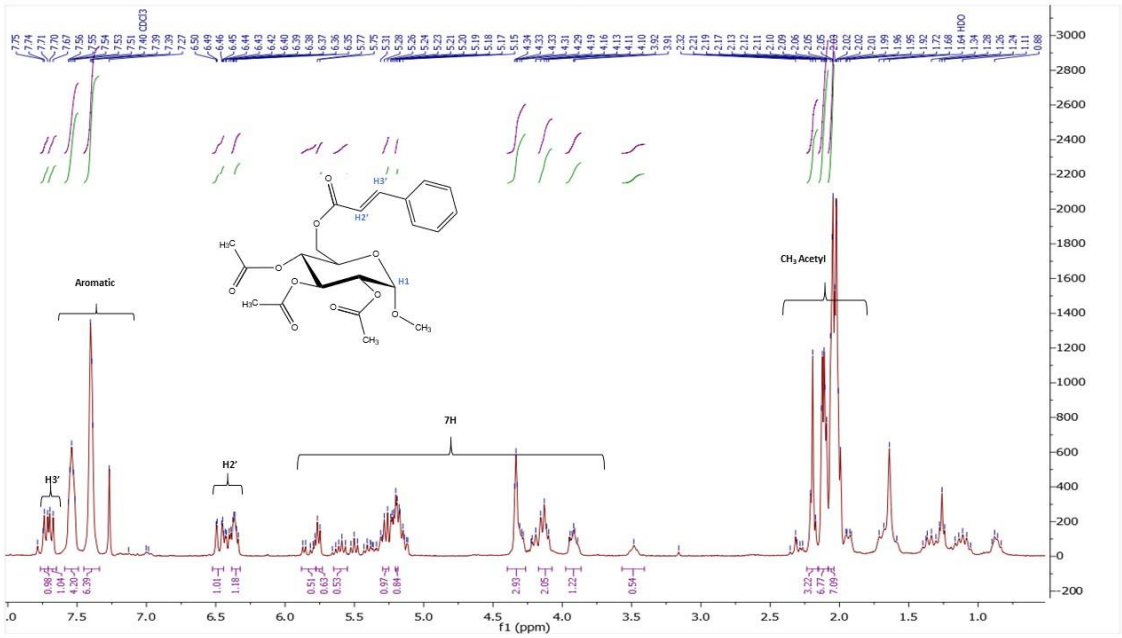
6. Appendix

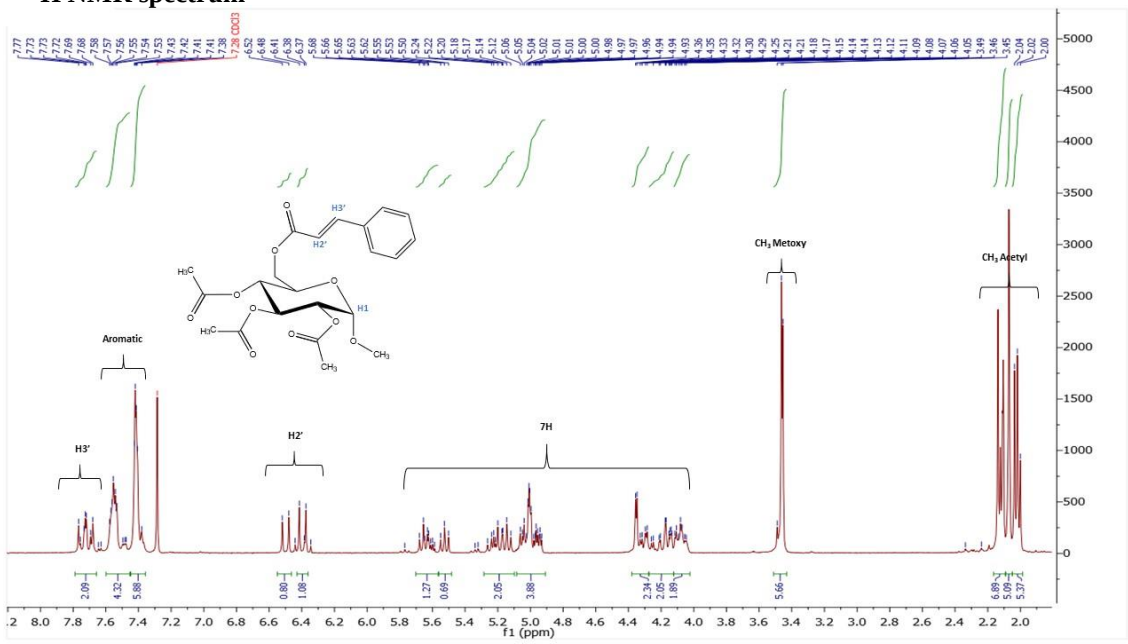
6.1. 2,3,4-*tri*-*O*-acetyl-6-*O*-cinnamoyl-α-D-methylglucopyranoside (3.1)

^{1}H NMR spectrum



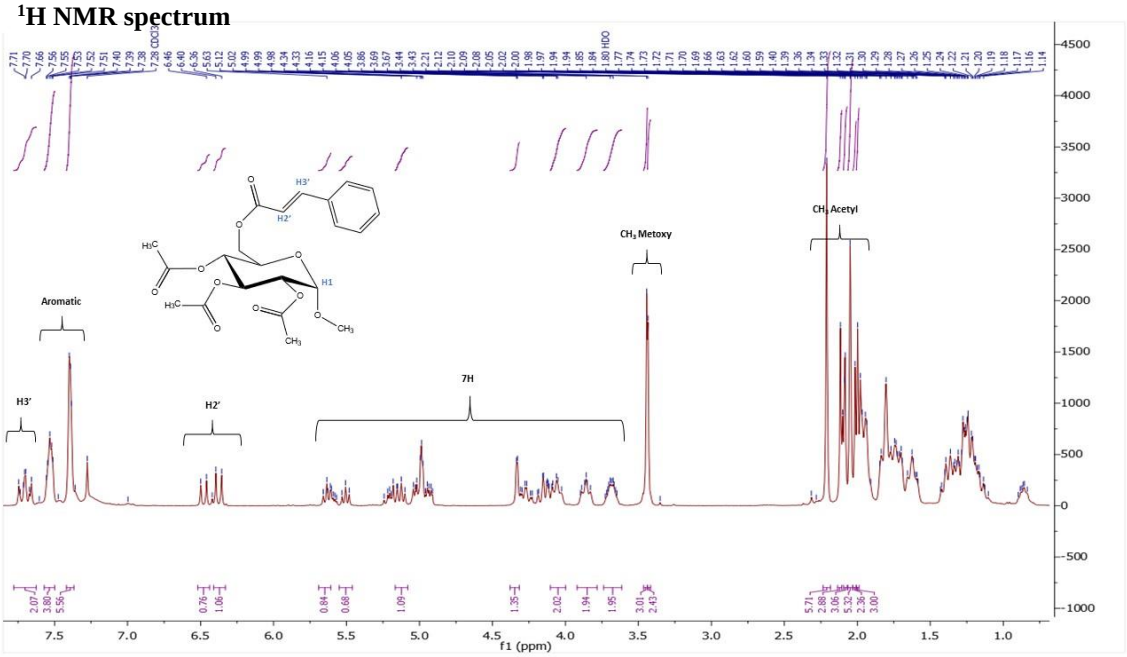
6.2. 2,3,4-*tri*-*O*-acetyl-6-*O*-cinnamoyl-α-D-methylglucopyranoside (3.2)

^{1}H NMR spectrum

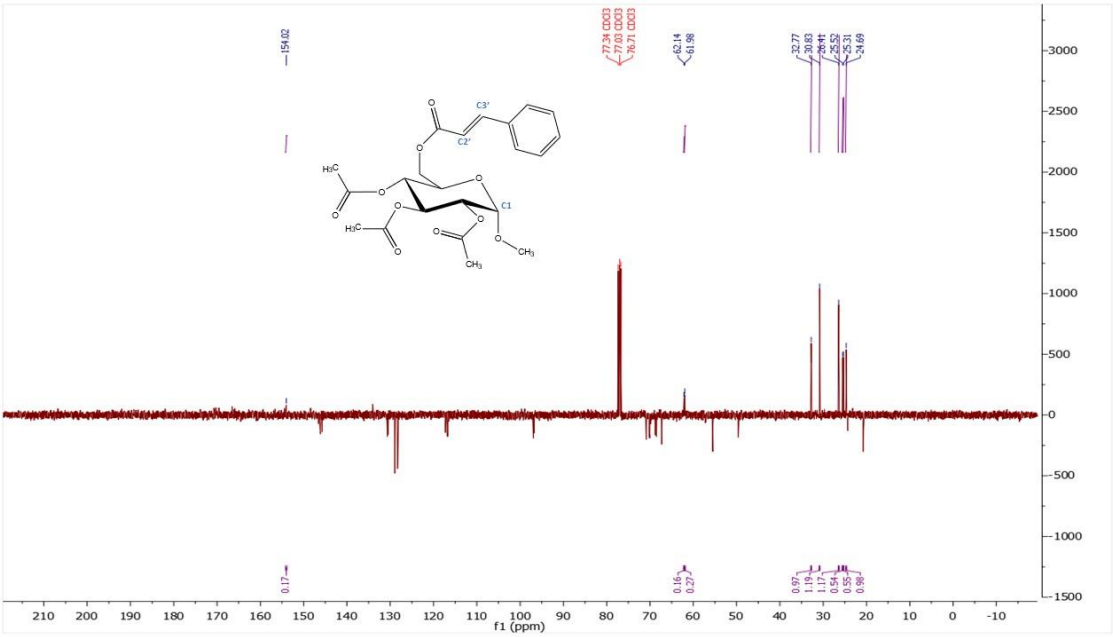


6.3. 2,3,4-*tri*-*O*-acetyl-6-*O*-cinnamoyl-α-D-methylglucopyranoside (3.3)

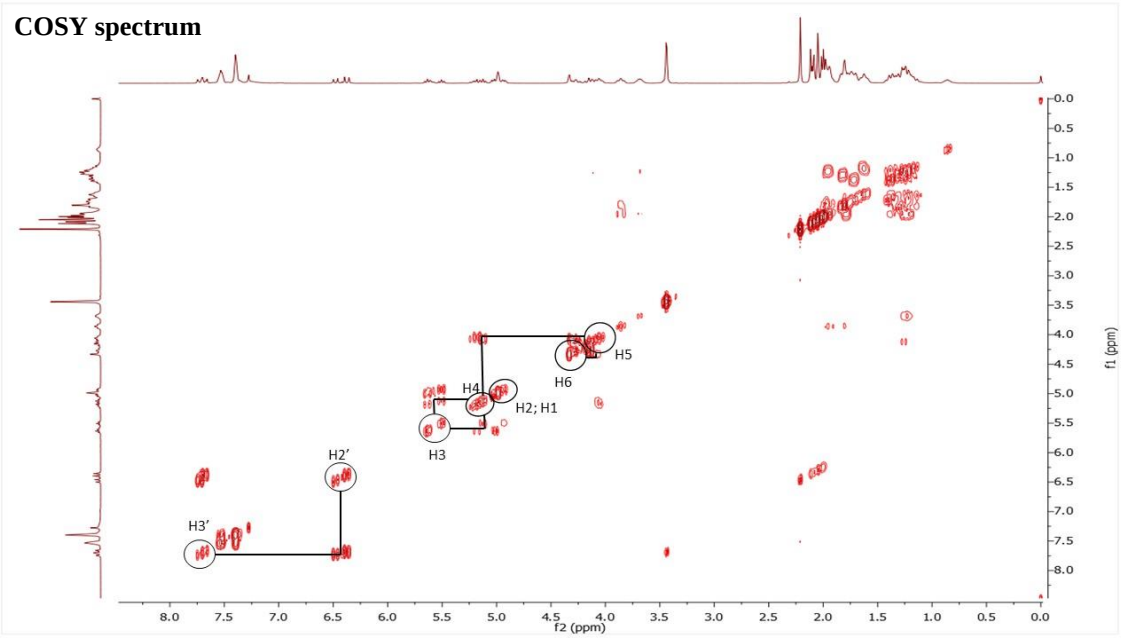
^{1}H NMR spectrum

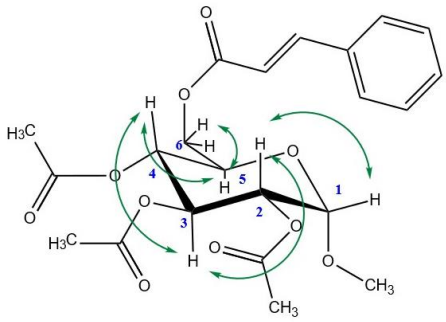


^{13}C NMR spectrum

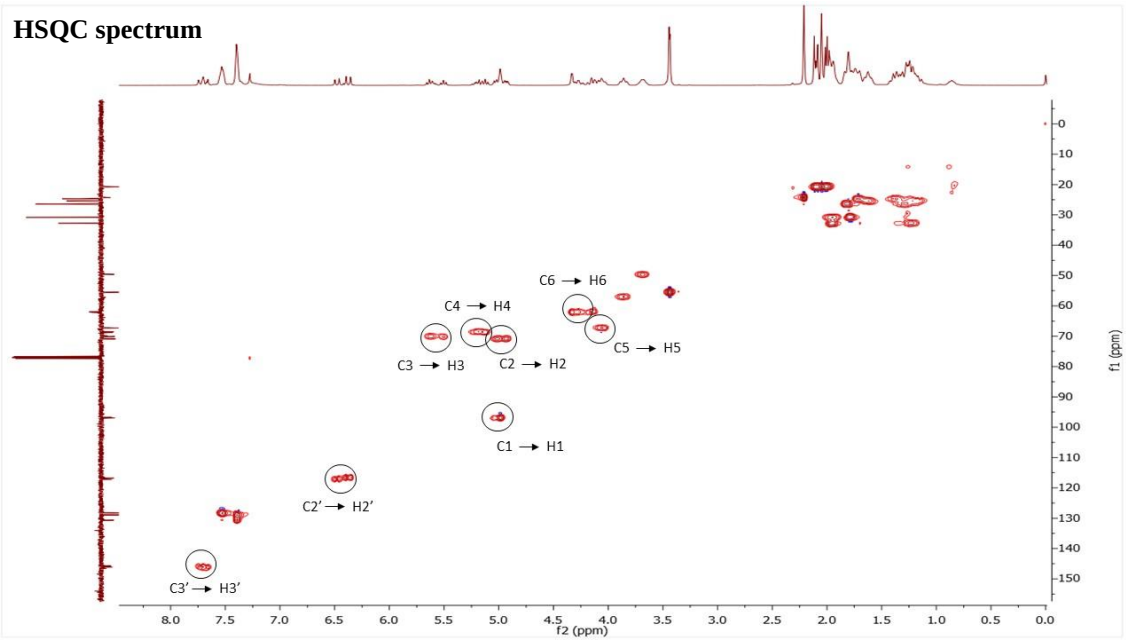


COSY spectrum





HSQC spectrum



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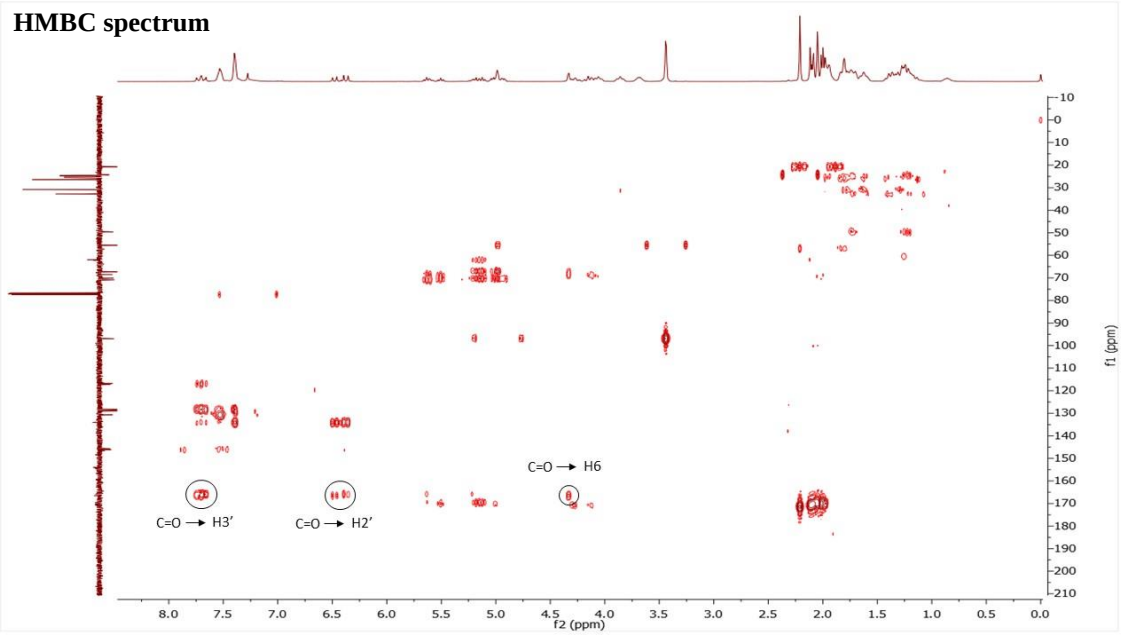
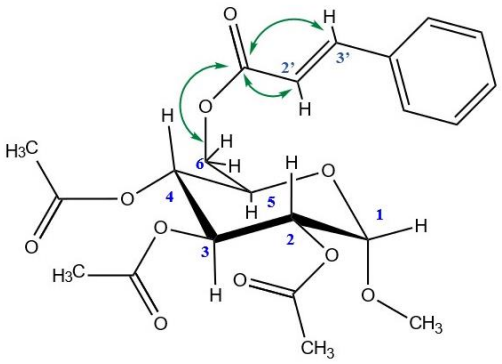
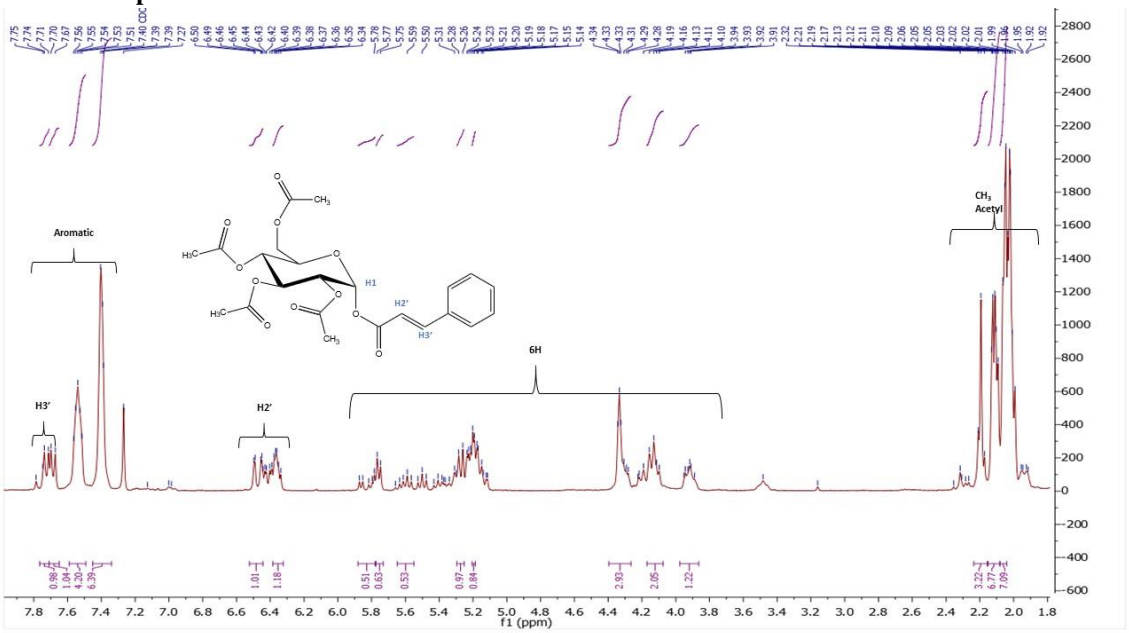




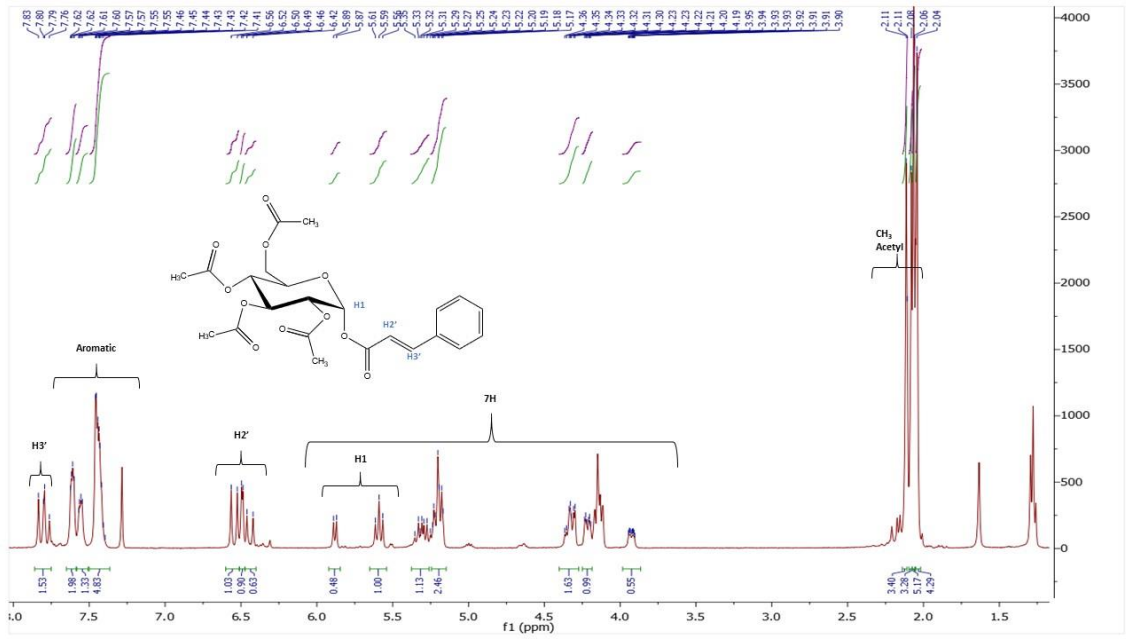
6.4. 2,3,4,6-*tetra*-*O*-acetyl-1-*O*-cinnamoyl-α-D-glucopyranose (4.1)

^{1}H NMR spectrum



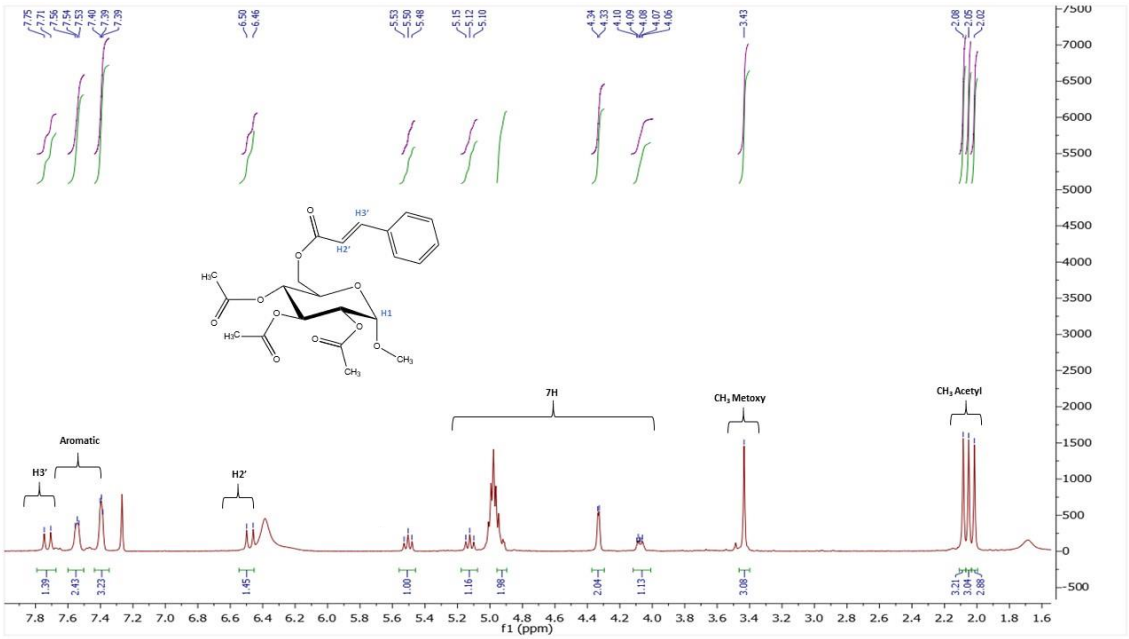
6.5. 2,3,4,6-*tetra*-*O*-acetyl-1-*O*-cinnamoyl-α-D-glucopyranose (5.1)

^{1}H NMR spectrum



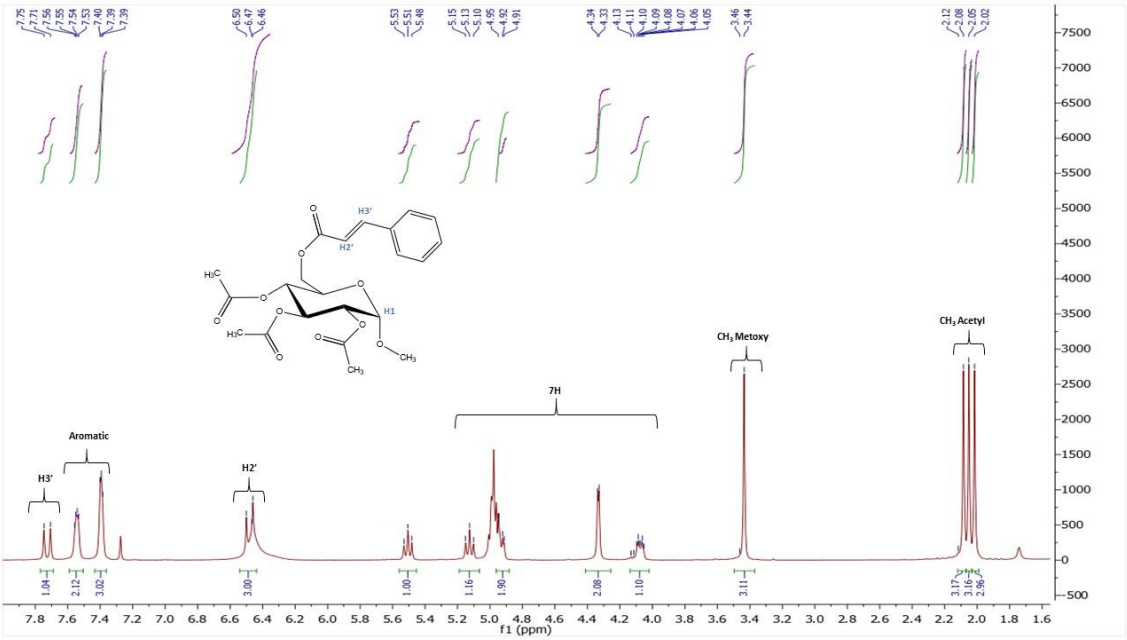
6.6. 2,3,4-*tri*-*O*-acetyl-6-*O*-cinnamoyl-α-D-methylglucopyranoside (6.1)

^{1}H NMR spectrum

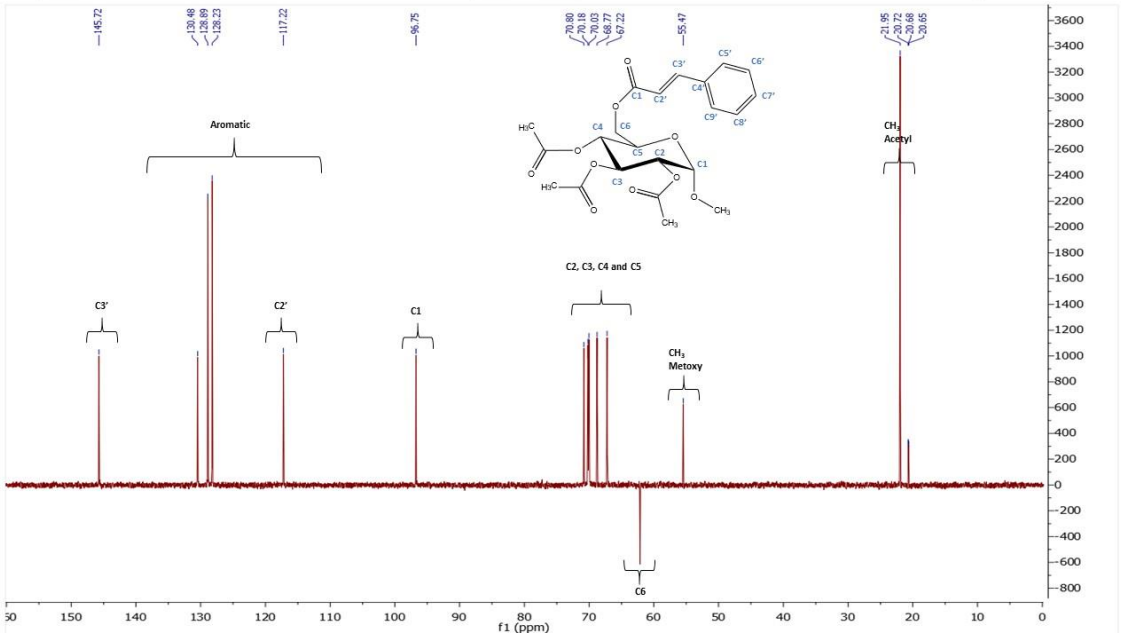


6.7. 2,3,4-*tri*-*O*-acetyl-6-*O*-cinnamoyl-α-D-methylglucopyranoside (6.2)

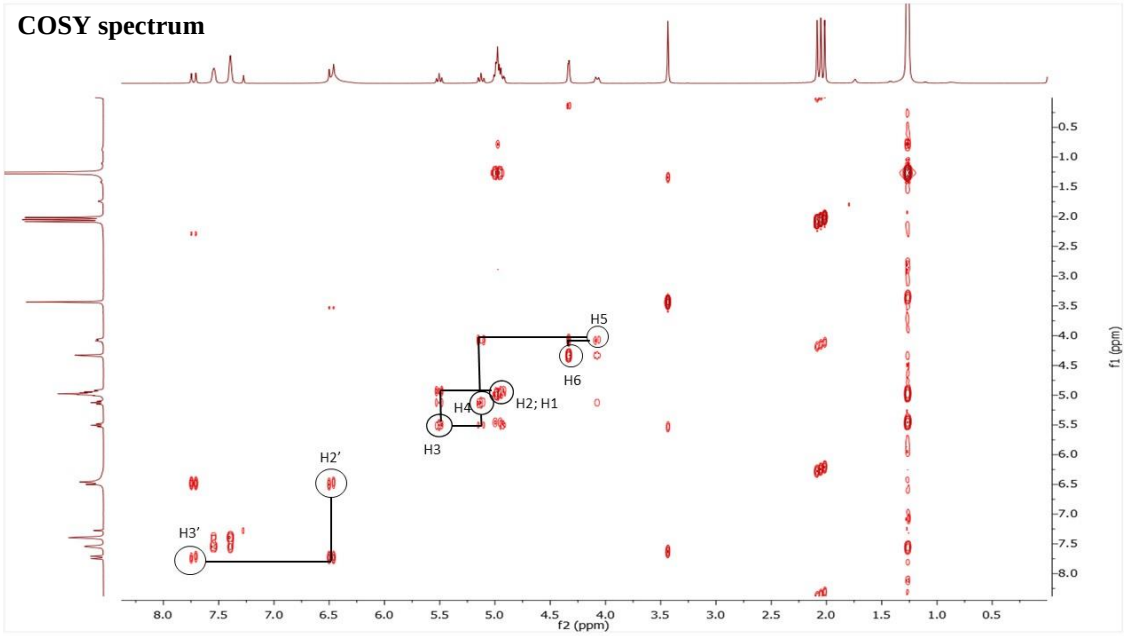
^{1}H NMR spectrum

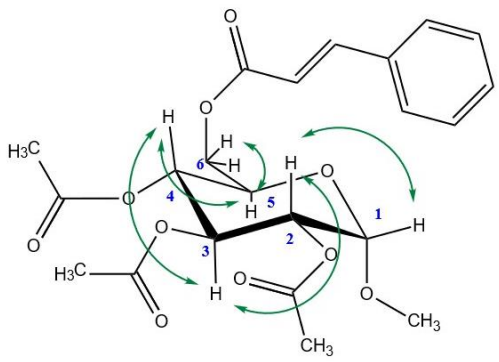


^{13}C NMR spectrum

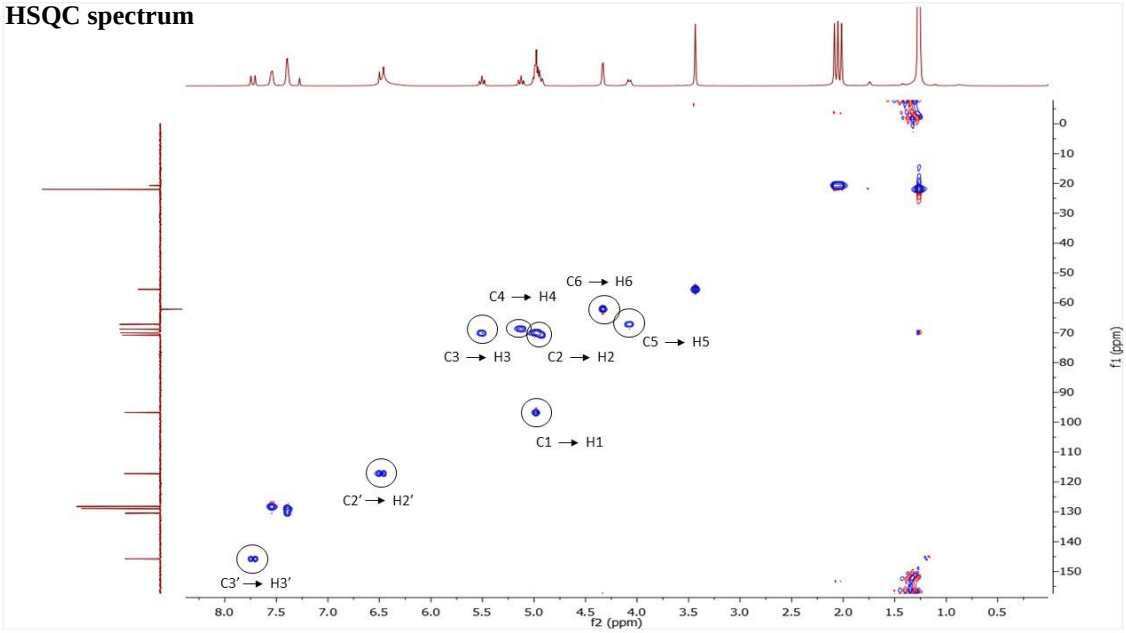


COSY spectrum

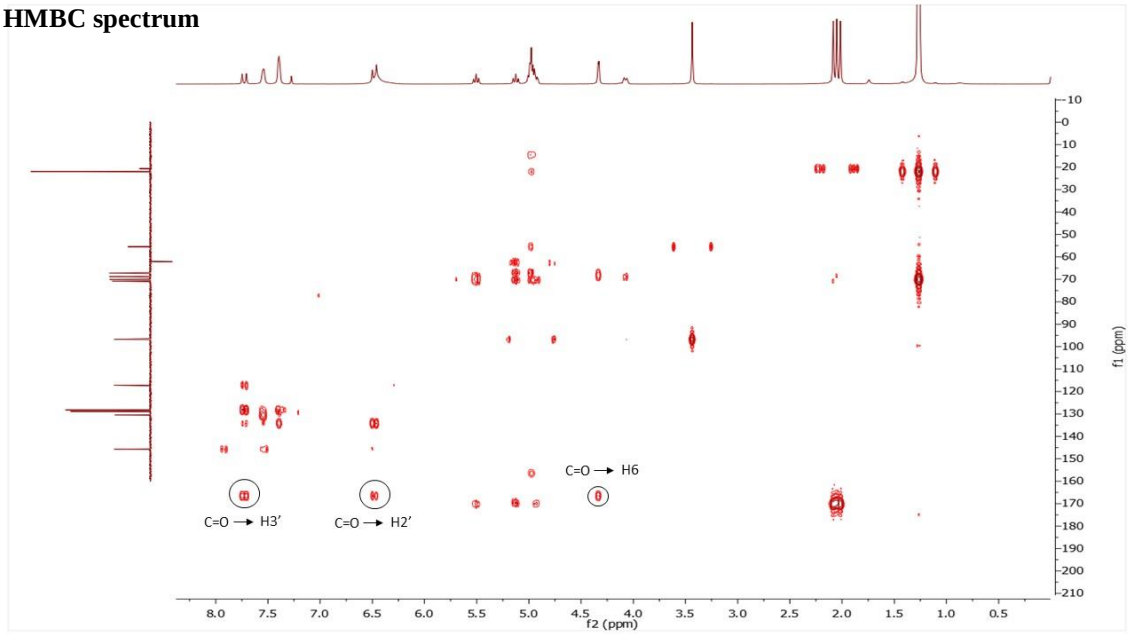


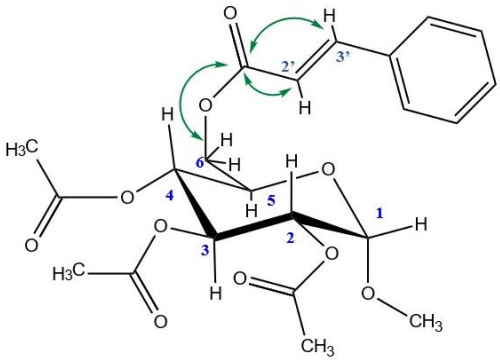


HSQC spectrum



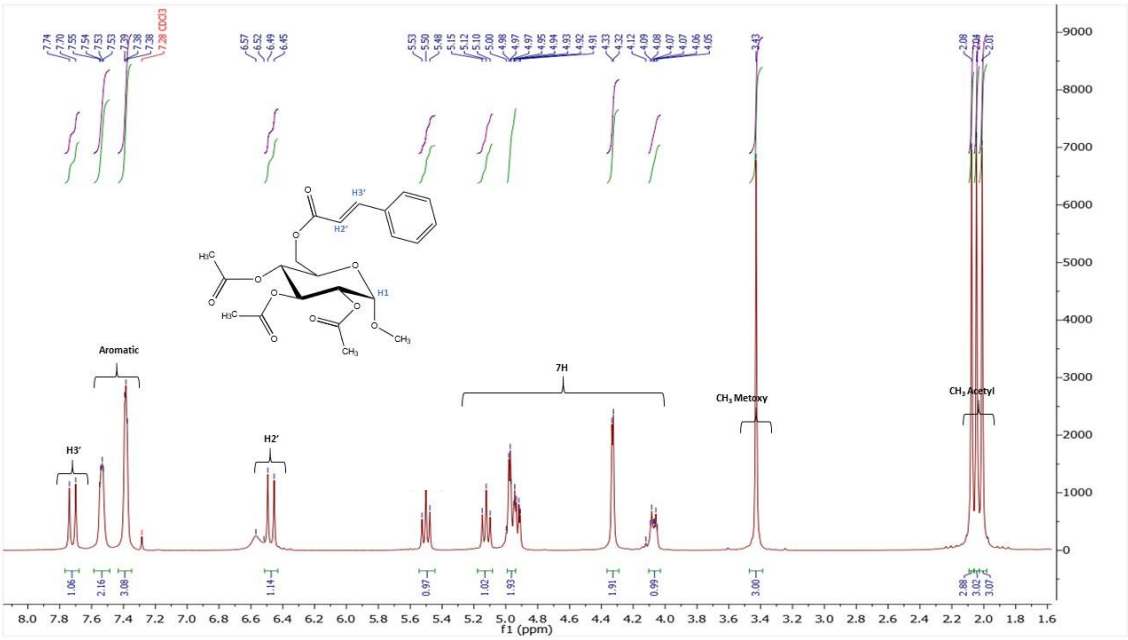
HMBC spectrum





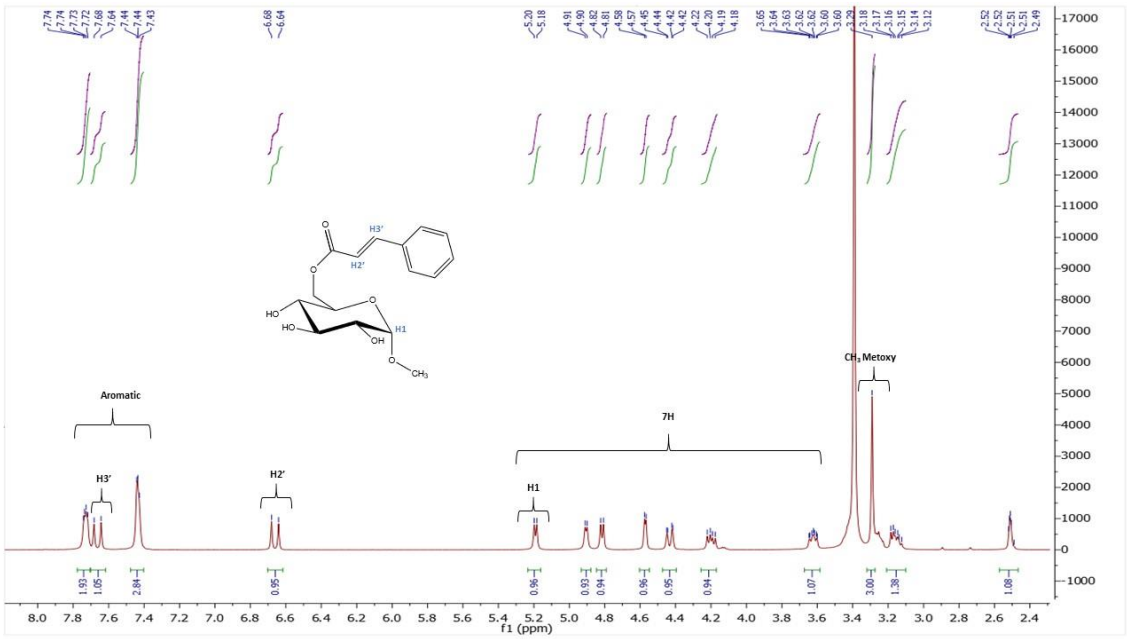
6.8. 2,3,4-*tri*-*O*-acetyl-6-*O*-cinnamoyl-α-D-methylglucopyranoside (6.3)

^{1}H NMR spectrum



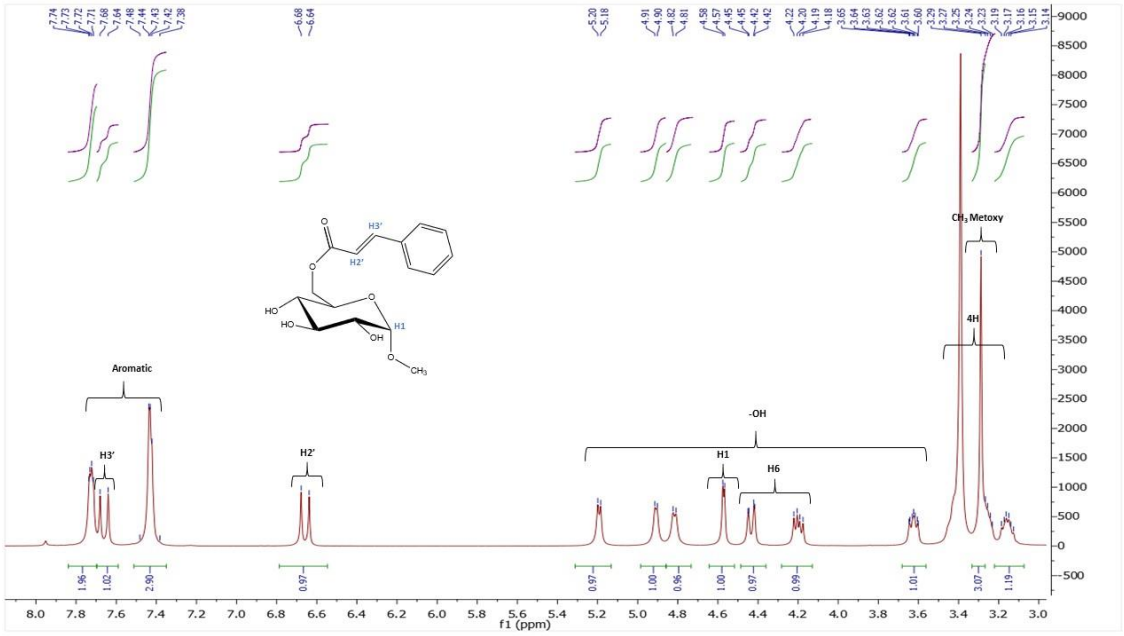
6.9. 6-*O*-cinnamoyl-α-D-methylglucopyranoside (7.1)

^{1}H NMR spectrum

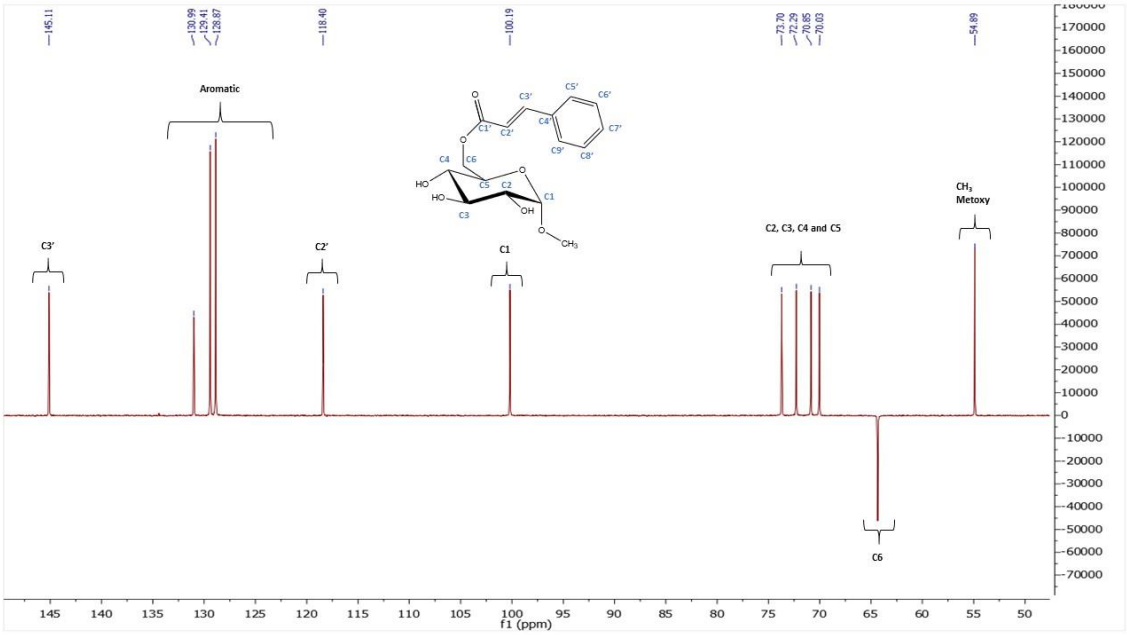


6.10. 6-*O*-cinnamoyl-α-D-methylglucopyranoside (7.2)

^{1}H NMR spectrum



^{13}C NMR spectrum



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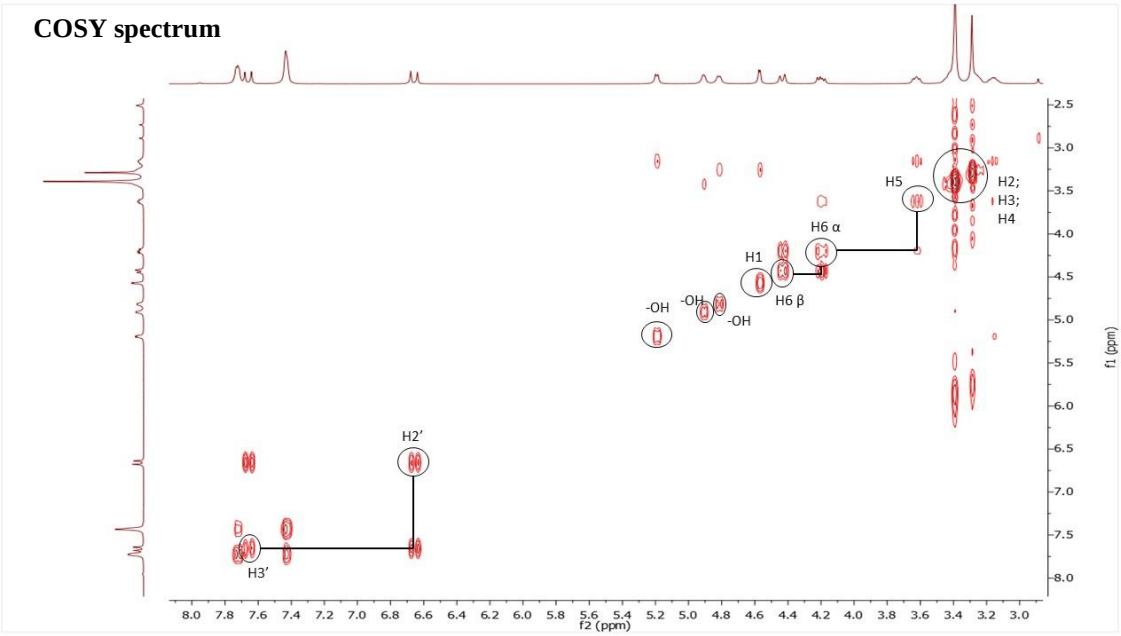
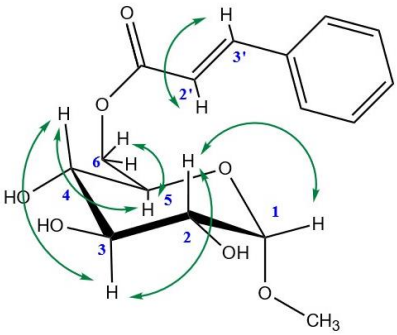
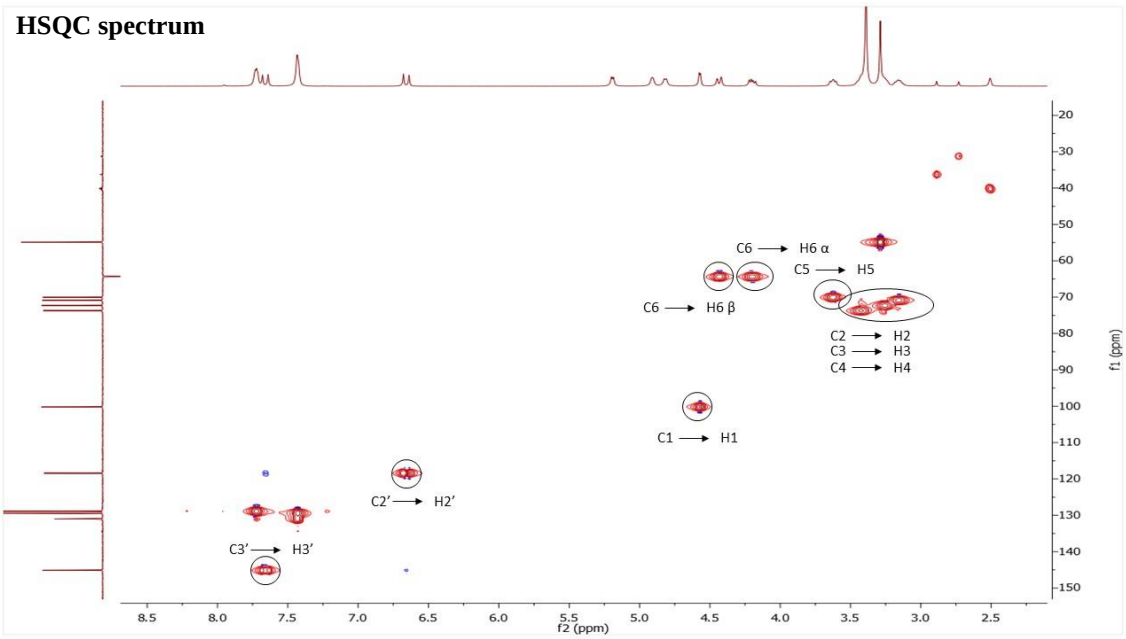




HSQC spectrum



6.11. 1-*O*-cinnamoyl-α-D-glucopyranose (8.1)

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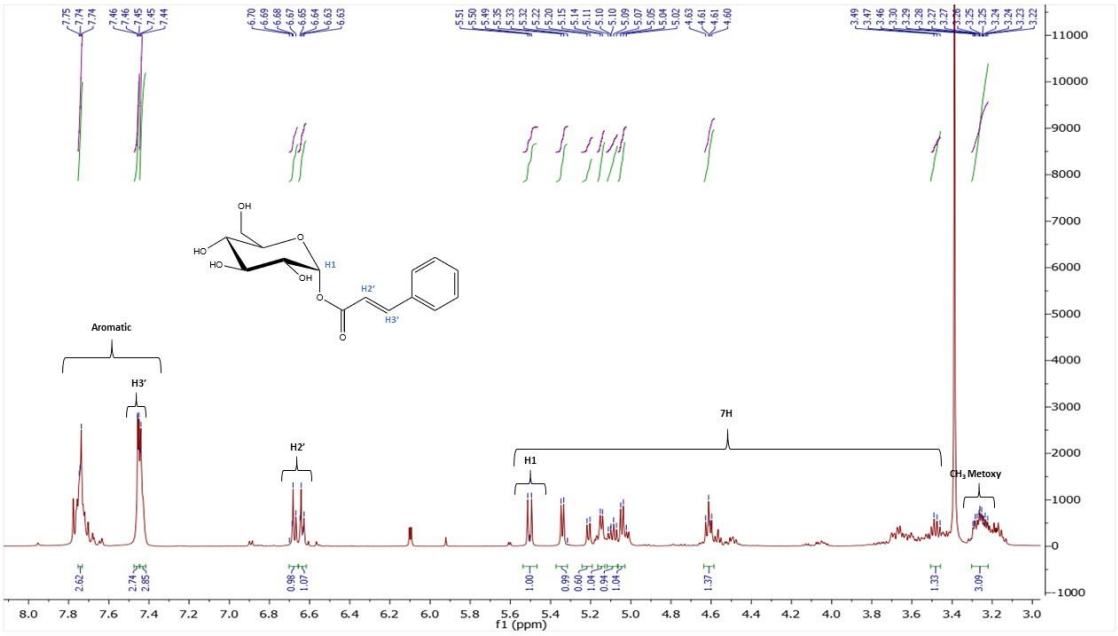

^{13}C NMR spectrum