

UNIVERSITY OF SPLIT
FACULTY OF CHEMISTRY AND TECHNOLOGY

**SYNTHESIS OF GLUCOSINOLATES:
GLUCONASTURTIIN (2-Phenylethyl Glucosinolate) AND
GLUCOMORINGIN ANALOGUE (4'-O-(α -D-Mannopyranosyl)
Glucosinalbin)**

DIPLOMA THESIS

JASNA BREKALO

Index number: 36

Split, October 2015.

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GRADUATE STUDY OF CHEMISTRY - ORIENTATION: ORGANIC
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SVEUČILIŠTE U SPLITU

KEMIJSKO-TEHNOLOŠKI FAKULTET

STUDIJ: KEMIJA - SMJER: ORGANSKA KEMIJA I BIOKEMIJA

SINTEZA GLUKOZINOLATA:

**GLUKONASTURCIN (2-Feniletil glukozinolat) I ANALOG
GLUKOMORINGINA (4'-O-(α -D-Manopiranozil)glukosinalbin)**

DIPLOMSKI RAD

JASNA BREKALO

Matični broj: 36

Split, Listopad 2015.

The work was done at the Institute of Organic and Analytical Chemistry (ICOA) in Orléans-France, under the supervision of Prof. Arnaud Tatibouët and supervisor Assist. Prof. Ivica Blažević at Faculty of Chemistry and Technology in Split-Croatia, in the time period from May to September 2015.

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Special thanks to my family, which has always been my extraordinary support.

Jasna Brekalo

OBJECTIVES OF THE THESIS

- To develop the synthesis of *gluconasturtiin* (2-phenylethyl isothiocyanates) and *glucomoringin* analogue (4-(α -D-mannosyloxy)benzyl glucosinolate) following the known procedure of Rollin *et. al.*, applying the hydroxamate disconnection approach.
- Gluconasturtiin is a natural glucosinolate, and it will be synthesized in order to prepare a large scale glucosinolate standard.
- The second one, an artificial glucosinolate which is closely related to glucomoringin, will be synthesized to explore the myrosinase action for preparation of the isothiocyanates.

SUMMARY

Using the known procedure of Rollin *et. al.*^{4,5} two different glucosinolates (GLs), *gluconasturtiin* (found in species belonging to Brassicaceae family), and *glucomoringin* analogue (not found in nature) were successfully synthesized. *Gluconasturtiin*, as one of the most widely distributed GLs in the cruciferous vegetables, has been synthesized using aldoxime pathway followed by hydroxamate disconnection. Chlorination of aldoxime by sodium hypochlorite solution afforded the corresponding hydroxyl chloride. Without further purification, this electrophilic acceptor reacted with 1-thio- β -D-glucopyranose tetraacetate in the presence of an organic base to produce the anomeric thiohydroxamate intermediate for further reactions of sulphation and deacetylation to gain an expected product *gluconasturtiin*.

A more striking application of the nitrovinyl pathway was the synthesis of the major GL of plant *Moringa oleifera*, known by its trivial name *glucomoringin*. This GL is an O-rhamnosylated form of *glucosinalbin*. Corresponding nitrovinyl derivate, under Lewis acid activation with triethylsilane as a source of the hydride ion, led to the formation of substituted acetohydroxymoil chloride. By *syn*-addition of 1-thio- β -D-glucopyranose thiohydroxamate intermediate was obtained for further reactions of sulphation and deacetylation to produce expected product *glucomoringin*.

Analysis of all the synthesized products and corresponding intermediates was performed using different spectroscopic techniques and methods. Characterization of molecule's mass was done by MSquadrupole - FIA method. IR spectroscopy was used to characterize various functional groups. NMR spectroscopy was used to record all C-C and H-H couplings between atoms in synthesized products. HPLC techniques as well as the inverse purification chromatography systems were used to purify all the resulting products.

Prema poznatim metodama za sintezu glukozinolata sintetizirana su dva različita spoja, glukonastrucin pronađen u vrstama iz porodice Brassicaceae i analog glukomoringina koji nije pronađen u prirodi.

Glukonasturcin kao jedan od u prirodi pronađenih GL među kupusnjačama je sintetiziran preko aldoksim puta koji omogućava formiranje tihidroksamatne skupine. Kloriranje aldoksima sa otopinom natrij-hipoklorita omogućava formiranje očekivanog hidroksimoil-klorida. Bez daljnjeg pročišćavanja ovaj elektrofilni akceptor u prisutnosti organske baze reagira s 1-tio- β -D-glukopiranoznim tetraacetatom. Ovom reakcijom dolazi do nastajanja anomernog tihidroksamata koji podliježe daljnjim reakcijama sulfatacije i deacetilacije u cilju formiranja očekivanog glukonasturcina.

Sinteza analoga glukomoringina (*4-(α -D-manoziloksi)benzil glukozinolat*), kao modificiranog oblika glukosinalbina sa O-manoziliranom formom, zahtijevnija je reakcija koja se izvodi preko nitrovinilnog puta. Odgovarajući nitrovinilni derivat, aktiviran korištenjem Lewisove kiseline, reagira sa trietilsilanom, kao izvorom vodikovog iona, te omogućava formiranje acetohidroksimoil-klorida. *Sin*-adicijom 1-tio- β -D-glukopiranoze dobiva se tihidroksimatni međuprodukt koji daljnim reakcijama sulfatacije i deactilizacije omogućava nastajanje očekivanog analoga glukomoringina.

Za analizu sintetiziranih spojeva korištene su različite spektroskopske tehnike. Za karakterizaciju molekulske mase je korištena MSkvadropol-FIA metoda. IR spektroskopijom su karakterizirane molekulske vibracije za karakteristične funkcijske skupine molekula, dok je NMR spektroskopija korištena za snimanje svih H-H i C-C interakcija između atoma u molekuli. HPLC tehnika kao i inverzna kromatografija su korištene prilikom dobivanja pročišćenih spojeva.

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LIST OF ABBREVIATIONS

Acet.	Acetone
OAc	Acetate
AcOH	Acetic acid
DMF	<i>N,N</i> -Dimethylformamide
DMSO	Dimethyl sulfoxide
Eq	Equivalent
ESP	Epithiospecifier protein
EtOAc	Ethyl acetate
GLs	Glucosinolates
HPLC	High performance liquid chromatography
IR	Infrared
MeOH	Methanol
NMR: s, d, t, m	Nuclear magnetic resonance: singlet, dublet, triplet, multiplet
ppm	Part per million
THF	Tetrahydrofuran
UDPG	Uridine-5'-diphosphate glucose
UV	Ultraviolet
PetEt	Petroleum ether

Glucosinolates are secondary metabolites which occur in all plant families of the Brassicales order. The major families are the Brassicaceae, Caricaceae, Euphorbiaceae, Moringaceae, Phytolaccaceae, Resedaceae and Tropealaceae. More than 130 different glucosinolates have been identified in these families as well as in our daily vegetables such as cabbage, broccoli, cauliflower and Brussels sprouts namely called crucifers.

The pungency of those plants is due to mustard oils produced from GLs when the plant material is chewed, cut, or otherwise damaged. Degradation of plant material through interaction with myrosinase enzyme results in GLs breakdown products: isothiocyanate, thiocyanate, epithionitrile, nitrile, and oxazolidinethione.

GLs hydrolytic and metabolic products act as chemoprotective agents for their fungicidal, bactericidal, nematocidal and allelopathic properties and have recently attracted intense research interest because of their chemoprevention attributes.

Researches about GLs are very active: contribution and species of many plants were detected. Amount of GLs in plant material is different in relation to the parts of plant from which they were isolated (seed, root, leaf, stem, flower). Dedicated extraction methods allow one to isolate a number of GLs from a plant material, but in many cases, organic synthesis brings crucial help for the production of natural GLs. In other respect, synthesis is the only way to elaborate a diversified range of artificial GL analogues. What is the most important, the synthesis can afford a higher amount of GLs.

In this work two different GLs were synthesized: *gluconasturtiin* (2-phenylethyl isothiocyanates) and *glucomoringin* analogue (4-(α -D-mannosyloxy)benzyl glucosinolate).

1. GENERAL SECTION

1. General section

Glucosinolates (GLs) are naturally occurring glycosides classified in the group of the cyanogenic glucosides. GLs basic skeleton consists of β -D-glucopyranose residue (glucone part), a O-sulphatated thiohydroximate moiety and a variable side chain (aglucone part) (Figure 1.).¹

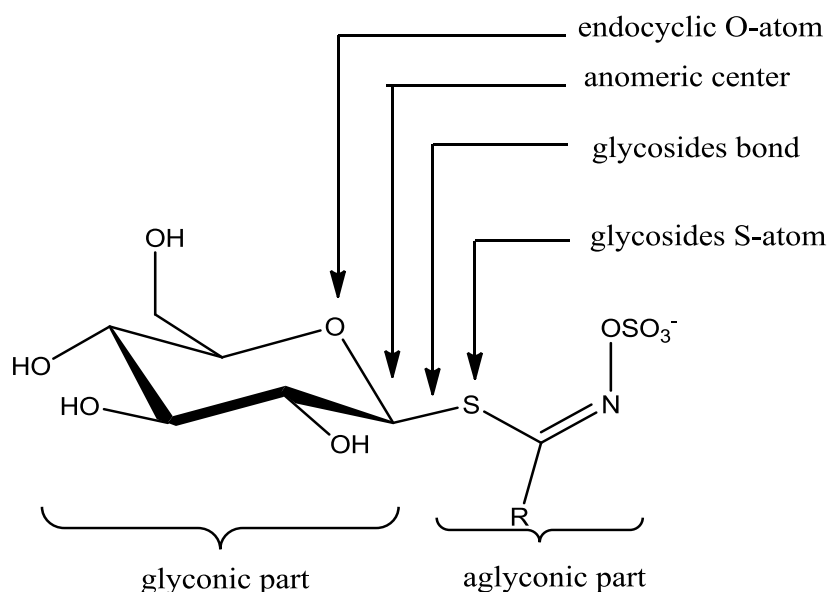
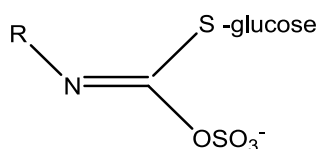


Figure 1. *The general structure of glucosinolates*

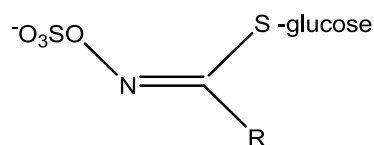
Different physical and chemical properties of GLs depend on the structures of glycone and aglycone part. Classification of the glycosides is based on an atom which is connecting glucone and aglucone part of the molecule. GLs are the S-glycosides connected to the anomeric atom of carbon in β -configuration like all naturally occurring glycosides.

The structure of GLs was generally assumed to be correct until 1957 when Ettlinger and Lundeen described their first chemical synthesis.² It was Gadamer in 1897, who proposed the first, but erroneous, isomeric structure, whereby the side chain would be linked to the nitrogen and the sulphate directly attached to the carbon of the thiohydroximate. However, this was revised by Ettlinger and Lundeen who gave the currently accepted structure (Figure 2.) where the β -

thioglucose unit is connected to a moiety and the sulphate is attached to the nitrogen with the Z-stereochemistry.



Gadamer, 1897



Ettlinger and Lundeen, 1957

Figure 2. Stereochemistry of GL molecule

One of the first isolated GLs were 2-propenyl or allyl GL from black (*Brassica nigra* L.) and 4-hydroxybenzyl GL from white (*Sinapis alba* L.) mustard seeds, also known by their trivial names: sinigrin and sinalbin, respectively. The remaining structural issue of the geometrical isomerism was based on the synthesis of sinigrin.¹

1.1. Biosynthesis of glucosinolates

It was assumed by Kjaer and Konti (1945) was assumed that natural amino acids are the precursors of the aglucone part of GLs. By proving the hypothesis it was found that GLs are derived from various amino acids. For example aliphatic GLs mainly originate from methionine but also from alanine, valine, leucine and isoleucine, while the aromatic GLs are derived from tyrosine and phenylalanine, and the indole GLs are derived from tryptophan (Figure 3.).²

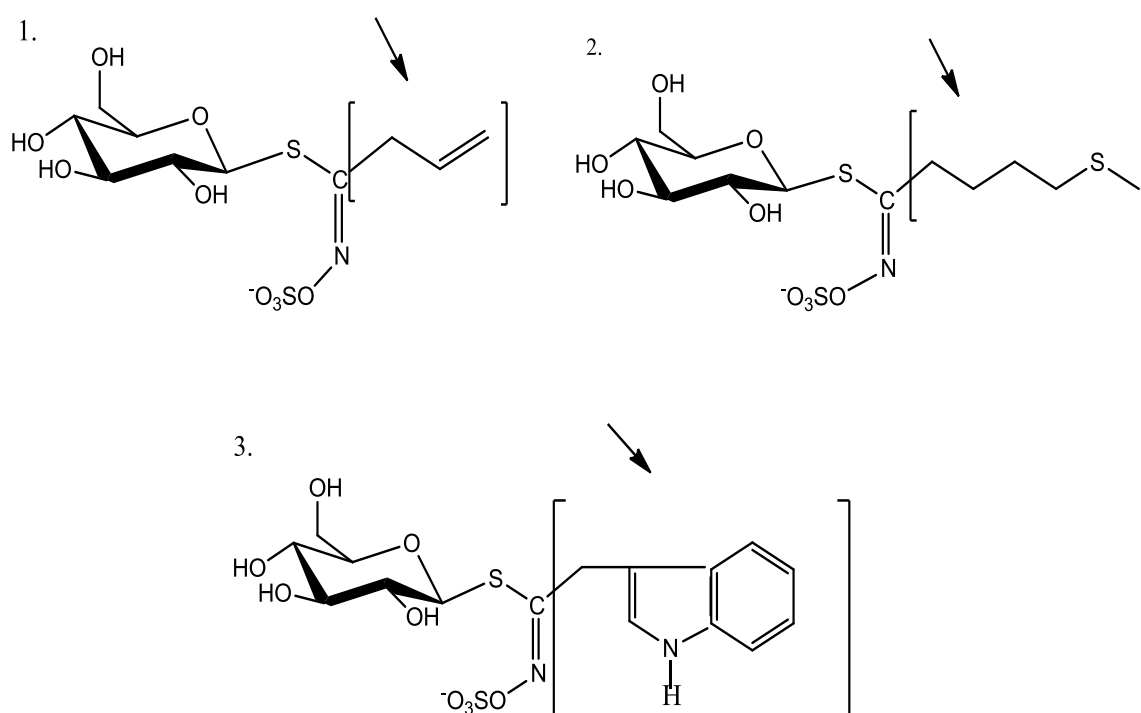


Figure 3. Presence of different aminoacid in GLs:

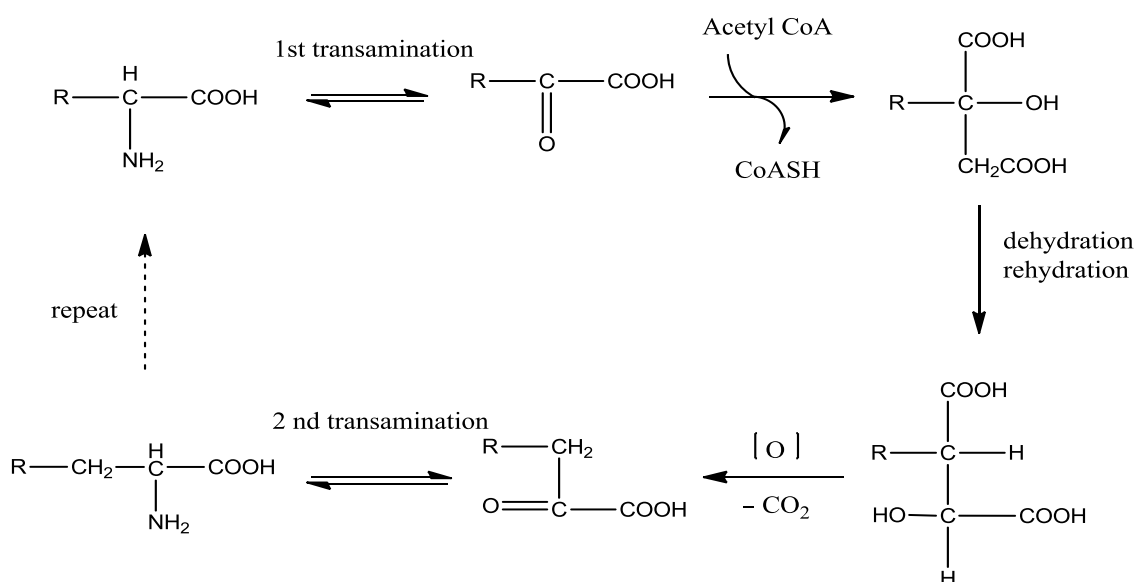
1. Allyl-glucosinolate (sinigrin),

2. 4-(Methylsulphanyl)butyl glucosinolate

3. Indole-3-yl-methyl glucosinolate

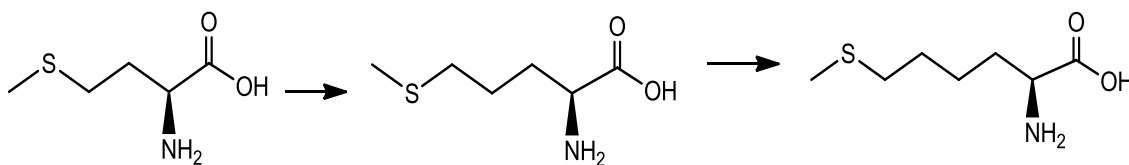
Glucosinolates can be biosynthesized in three distinct stages; side chain elongation, formation of the glycone moiety and then the secondary side chain modification.³

In the initial side chain elongation, the appropriate α -amino acid undergoes a transamination reaction to generate the corresponding α -keto acid (Scheme 1). This is followed by an aldol reaction with acetyl-CoA. A dehydration, rehydration sequence, followed by an oxidation and then decarboxylation occurs. Finally a second transamination takes place to recover the elongated amino acid functionality.³



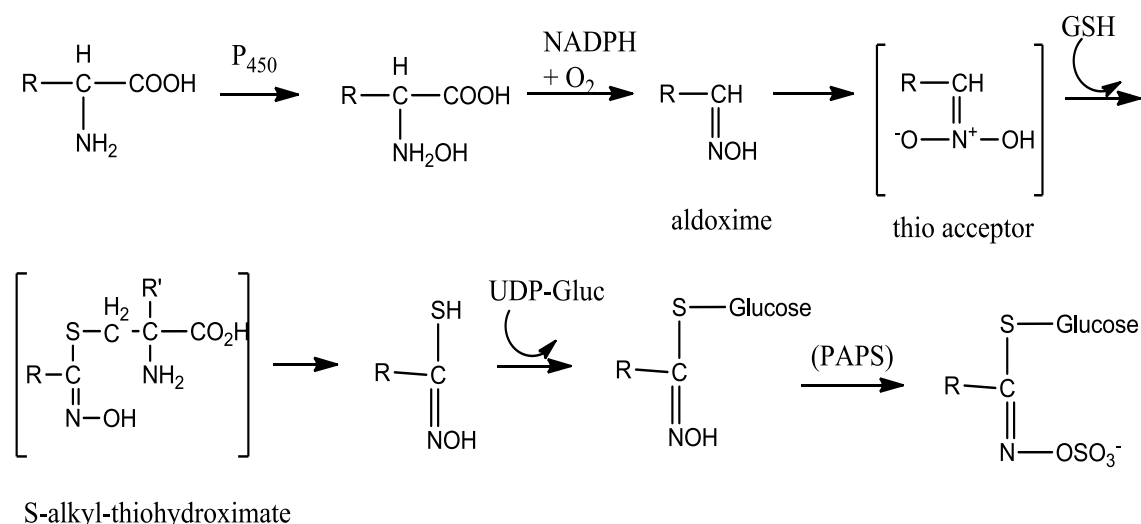
Scheme 1. Initial side chain elongation

The substrate can be subjected to this cycle a number of times, adding another carbon atom with each iteration. For example methionine, which is used as a precursor for aliphatic glucosinolates, can be converted to homomethionine and then to dihomomethionine and so forth using this biosynthetic cycle (Scheme 2).



Scheme 2. An example of chain extension.

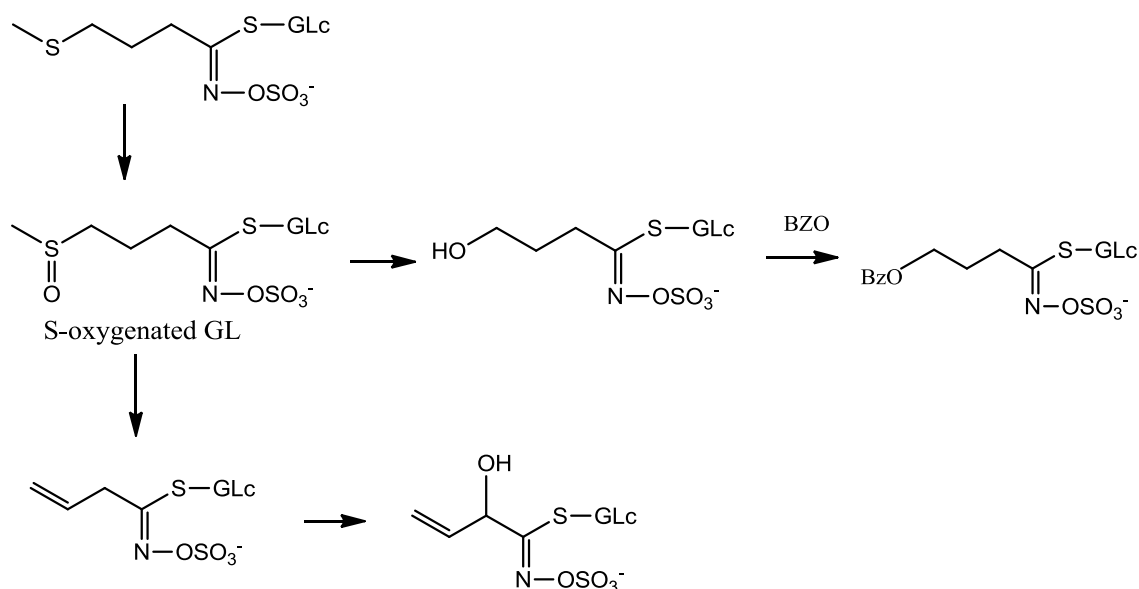
The second stage of the biosynthesis involves the formation of the aglucone moiety and it is initiated by cytochrome P450 oxidation of the amino acid precursor to give an *N*-hydroxyl amino acid. Little data is available regarding these steps due to the instability of the intermediates involved, although it is proposed that the oxime is initially converted to an acid-nitro compound, which acts as an acceptor for a thiol donor. The identity of the sulphur donor has recently been shown to be GSH, the reduced form of the tripeptide of glutamic acid, cysteine and glycine. A C-S lyase enzyme then cleaves the cysteine adduct to give the thiohydroximate, which undergoes S-thioglucoxylation by a soluble UDPG-thiohydroximate glucosyltransferase to yield the desulphoglucosinolate. The second stage of the biosynthesis concludes with sulphation by a 3'-phosphoadenosine-5'-phosphosulphate (PAPS) dependent enzyme to yield the complete glucosinolate.³



Scheme 3. Second stage of glucosinolate biosynthesis

In individual cases the biosynthesis continues with secondary side-chain modifications such as methylation, oxidation and hydrolysis. It is by these modifications that such a diverse range of glucosinolates is formed (Scheme 3.).³ For example the thiomethyl glucosinolate, can be oxidized to give the S-oxygenated glucosinolate, which in turn is converted to the hydroxylalkyl glucosinolate. Alternatively thiomethyl glucosinolate undergoes methylsulphide

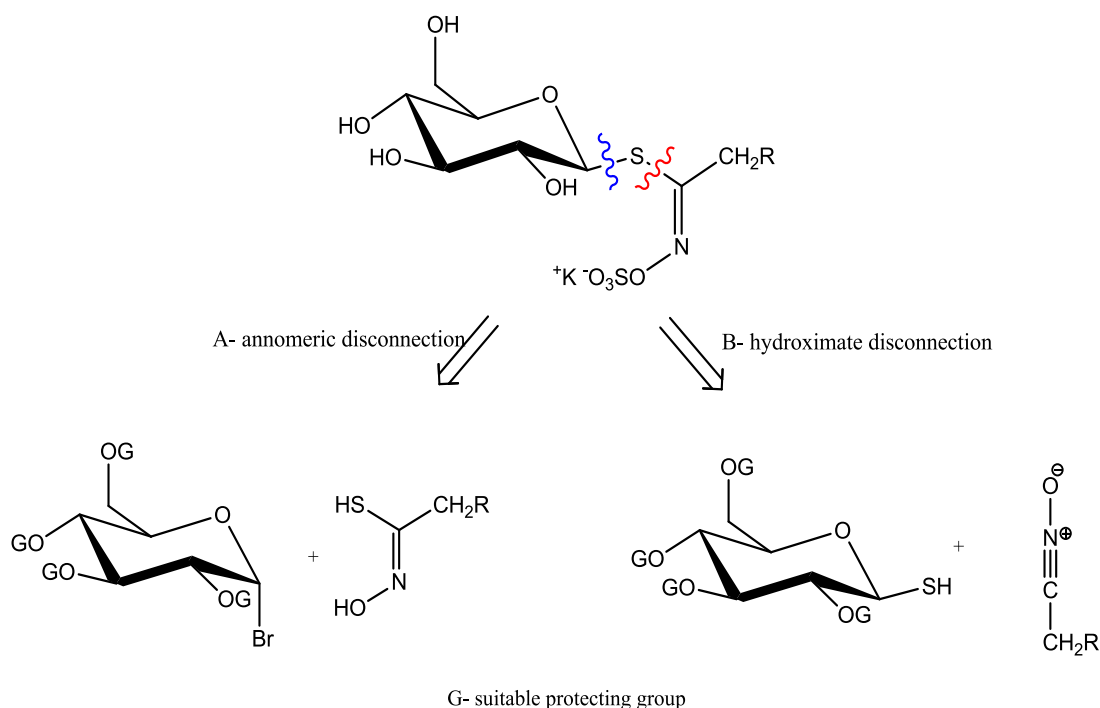
elimination to give the alkenyl glucosinolate, which can be further derivatized further to a hydroxyalkenyl glucosinolate. In further biosynthetic modifications the hydroxylalkyl side chain is esterified by the benzoate hydrolyzing enzyme (BZO) to afford benzoyloxy glucosinolate (Scheme 4.).



Scheme 4. Secondary side chain modifications

1.2. Synthesis of glucosinolates- the methods

From a chemical synthetic point of view, two major approaches – depicted in Scheme 5 – for the elaboration of GLs structures have been developed by a limited number of groups over the past 50 years: these are based on a retrosynthetic scheme where a single specific bond formation affords the GL skeleton and two types of disconnection have been considered, either on the anomeric center (A) or on to the hydroxymoi moiety (B).⁴

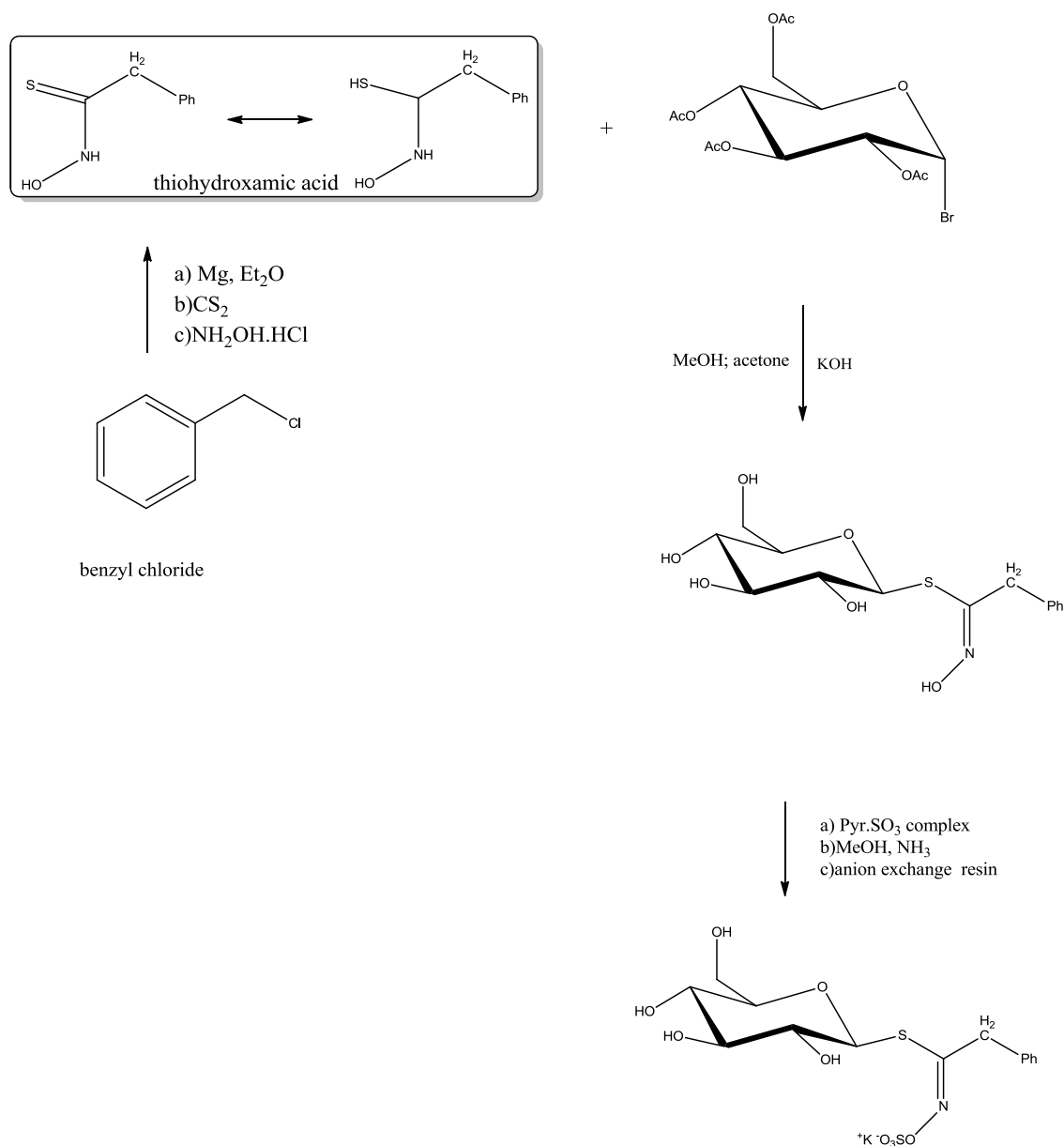


Scheme 5. Major approaches for the elaboration of GLs structures

Several synthetic routes to naturally occurring different GLs have been developed since the pioneering synthesis of glucotropaeolin (benzyl GL) by Ettlinger and Lundeen. During the 1960–1980 period, syntheses of simple aliphatic and arylaliphatic GLs were mainly performed by three groups: A. Kjaer, the major Brassicale chemistry expert in Denmark, M.H. Benn in Canada and A. MacLeod in Great-Britain. In the course of the 1990–2000 decade, indole-type GLs and their glyco-analogues and thiofunctionalized GLs were synthesized in group of Rollin and colleagues.³ A major part of this activity has been devoted to the synthetic elaboration of tailor-made artificial glucosinolate-like structures, with a view for exploring the recognition process of myrosinase, estimating the

relative importance of topical zones in the active site and searching for enzyme inhibitors.⁴

The anomeric disconnection scheme implies a “glycosidation-type” approach involving a standard electrophilic glucosyl donor and a thiohydroxamic acceptor. The first synthesis of glucotropaeolin by Ettlinger and Lundeen is based on that scheme: phenylacetothiohydroxamic acid (prepared in 33% yield from benzylmagnesium chloride, carbon disulphide and hydroxylamine) was reacted with acetobromoglucose under basic conditions to produce the glucosyl thiohydroximate (Scheme 6).²



Scheme 6. Synthesis of glucotropaeolin

Subsequent O-sulphation of the hydroxyamino group using sulphur trioxide pyridine complex gave the peracetylated glucotropaeolate anion, which could be isolated either as potassium or tetramethylammonium salt. Standard de-O-acetylation finally afforded glucotropaeolin after cation exchange purification. To date, this example remains unique as it has not been developed further.

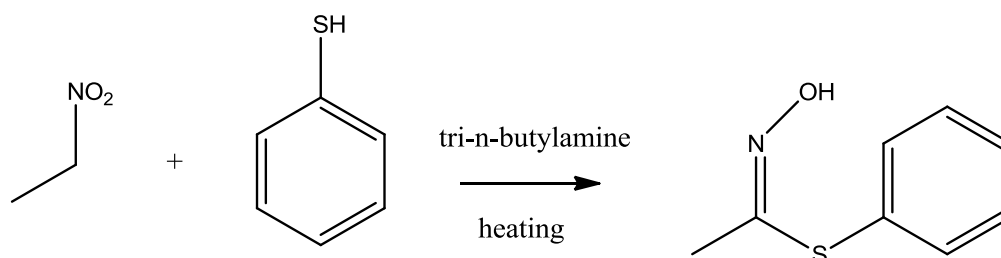
The thiohydroximate disconnection scheme is based on the 1,3-addition of a glycosyl mercaptan on a nitrile oxide. Because of their high lability, nitrile oxides have to be generated *in situ* from hydroxymoil chloride precursors through a 1,3-elimination under basic conditions (Scheme 17, given in chapter 3.). The key intermediate in the reaction is in fact the hydroxymoil chloride, which in turn also appears to be quite unstable in most cases. Indeed, the different approaches developed over the years for synthesizing GLs depend on three different ways to access hydroxymoil precursors – from aldoximes, from aliphatic nitronates or from nitrovinyl derivatives.⁴

1.2.1. The aldoxime pathway

Benn's pioneering work³ on the synthesis of many natural glucosinolates including; glucocapparin, gluconasturtiin, glucoputranjivin, glucosinalbin, glucoaubrietin, glucocochlearin used the aldoxime pathway. The key step for this method requires the chlorination of an oxime, synthesized from the corresponding aldehyde, using electrophilic chlorinating agents (Scheme 17, given in chapter 3.). One advantage of this route is that the starting alcohols, aldehydes or even oximes are commercially available. Those electrophilic acceptors were reacted in the presence of an organic base with 2,3,4,6-tetra-O-acetyl-1-thio- β -D-glucopyranose to produce in good yields the anomeric (Z)-thiohydroximate intermediates with complete stereocontrol in good yields. Subsequent O-sulphation with sulphur trioxide pyridine complex, followed by pyridine displacement with KHCO_3 and standard de-O-acetylation finally delivered the expected GLs in acceptable overall yields.⁴

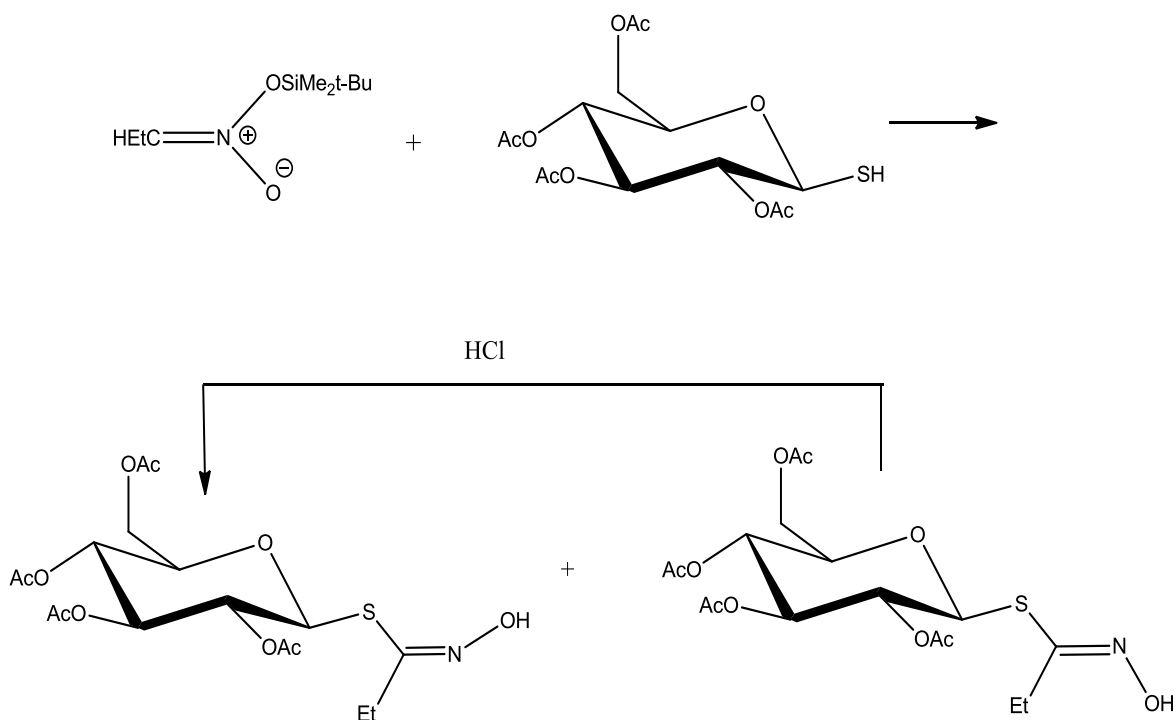
1.2.2. The nitronate pathway

Methodology emerged in 1954 by Copenhaver whereby a thiohydroximate is formed by using base induced condensation of primary nitroalkanes with thiols (Scheme 7).³



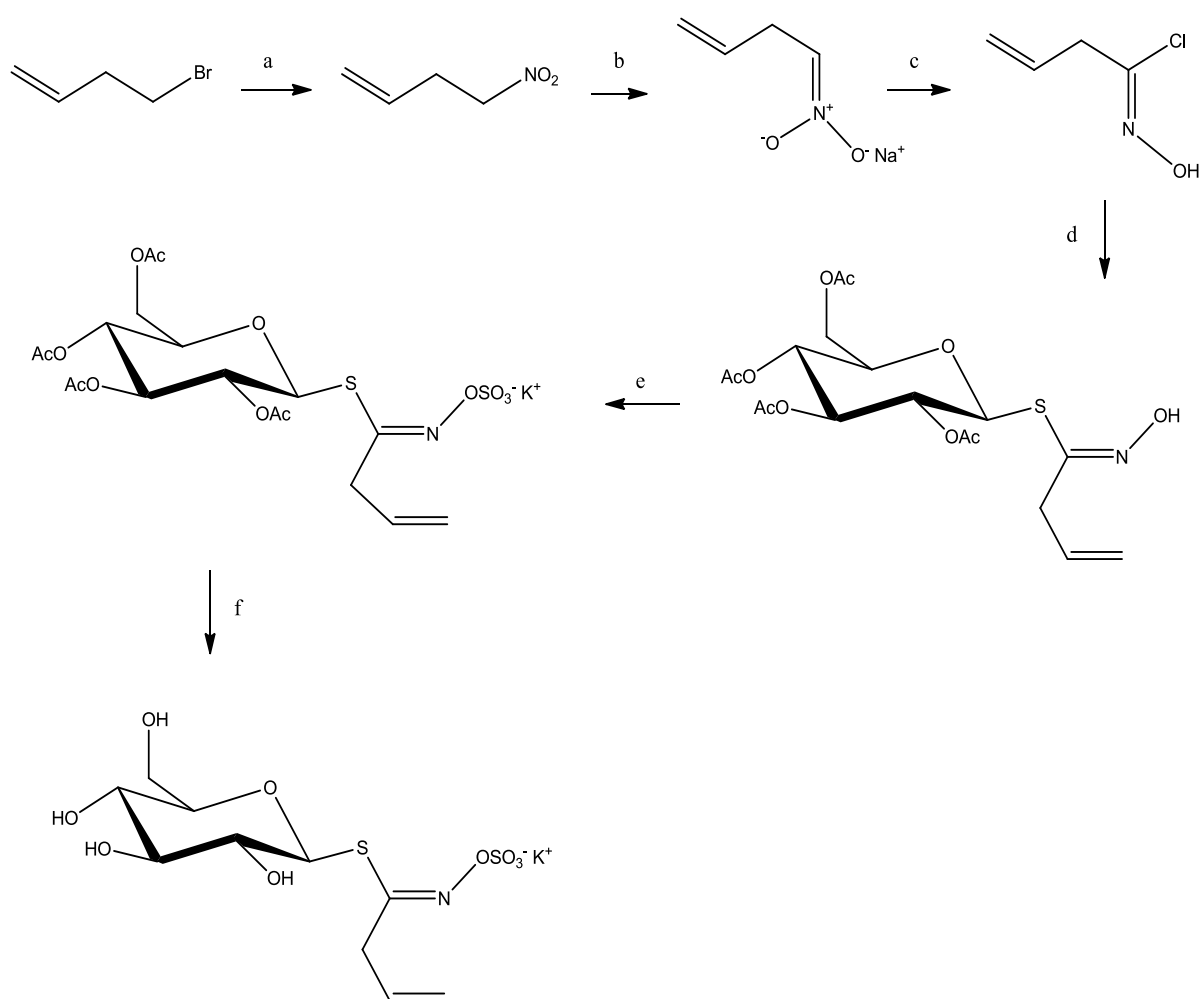
Scheme 7. The original Copenhaver conditions. Reagents and conditions: tri-n-butylamine, reflux

Benn later developed these conditions to explore their application towards glucosinolates.³ The original synthetic studies proved unsuccessful as they required harsh conditions, which were likely to deprotect the thioglucose moiety, and thus proved low yielding. However they observed that there was an intermediate nitronate anion, which could be formed under a range of milder conditions (Scheme 8). This anion has proven to be widely applicable in the area of GL synthesis.



Scheme 8. Benn's milder conditions. *Reagents and conditions:* NEt_3 , THF, 56% and 28%.

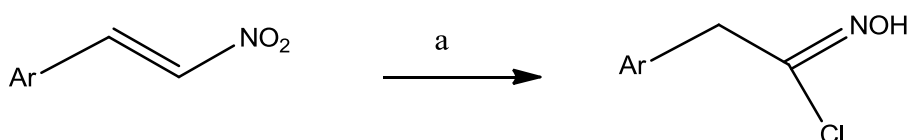
In more recent studies, as detailed in Scheme 9, the key stage of the reaction requires nucleophilic chlorination of the nitronate intermediate. Various techniques have been reported to achieve this for GLs including dry HCl and thionyl or lithium chloride. This pathway has been particularly favored in the synthesis of GLs, such as sinigrin which contains an alkene functionality in order to circumvent the possibility of alkene halogenation.³



Scheme 9. An example of the nitronate pathway in the synthesis of sinigrin.
Reagents and conditions: a) NaNO_2 , urea, DMSO; b) NaOEt ; c) LiCl-HCl ; d) acetylated thioglucose, NEt_3 ; e) $\text{py}\cdot\text{SO}_3$; f) NH_3/MeOH

1.2.3. The nitrovinyl pathway

In 1994 Kulkarni developed an alternative method for the formation of hydroxymoiil-chloride via nitroalkene precursors (Scheme 10).³



Scheme 10. Kulkarni methodology. Reagents and conditions a) TiCl_4 , Et_3SiH , CH_2Cl_2 .

Realizing the potential in this methodology Rollin *et. al.*⁴⁻⁵, applied it to the preparation of a range of GLs bearing aryl, alkyl and indolymethyl functionalities. They found that the approach had the advantage of a shorter reaction pathway than the nitronate method and that it was applicable to a wider range of substrates including indoles. A more striking application of the nitrovinyl method was reported for the first synthesis of the major GL of *Moringa* sp. (glucomoringin).⁴

In the order to explore the activity of enzyme myrosinase some of the modified structures of GLs were synthesized. An example of this method by nitrovinyl pathway is in (Scheme 20., given in chapter 3.).

1.3. Degradation of glucosinolates and their biological activity

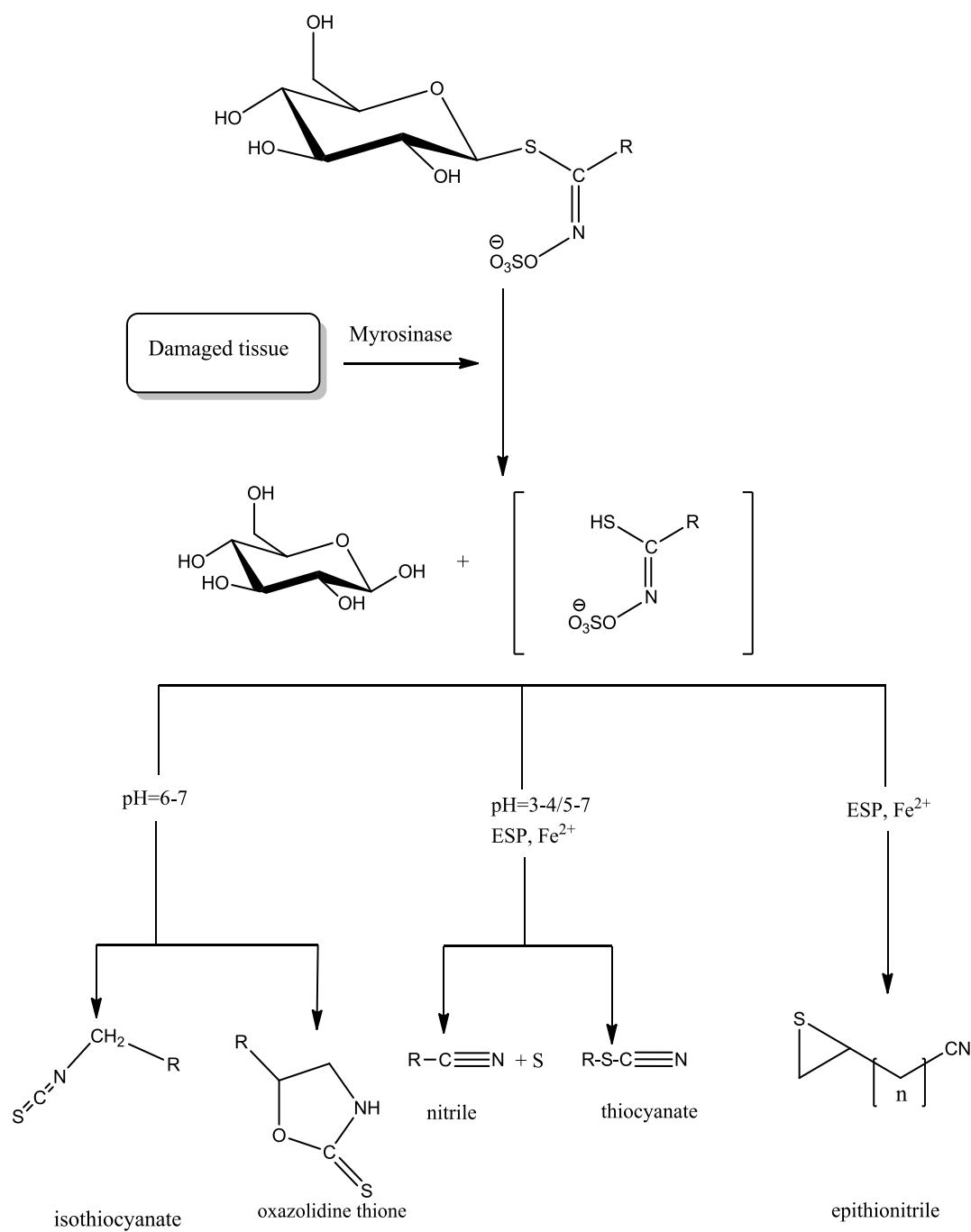
Damage to the cell structure of plants that contains GLs, by chopping, cooking, fermentation results in the enzymatic hydrolysis or a thermal decomposition. GLs are then transformed through degradation into a series of products which includes nitriles, isothiocyanates, thiocyanates, vinyl epithionitrile and oxazolidinethione. The degradation is catalyzed by the thioglucosidase enzyme (myrosinase).¹ The degradation of GLs could also occur with action of an acid or a base following a non-enzymatic pathway.

1.3.1. Enzymatic hydrolysis

The reaction of the myrosinase enzyme (thioglucoside glucohydrolase), initially located in a separate compartment of the cell in plants, produce GLs breakdown products - namely isothiocyanates, thiocyanates, nitriles, oxazolidin-2-thiones (Scheme 11.).¹

Attack of an herbivore, particularly by chewing, causes tissue disruption thereby bringing GLs into contact with myrosinase. Myrosinase is not identified as an enzyme, but as a family or a group of similarly acting enzymes. Myrosinases are the only known S-glucosidases, and show pronounced specificity for the GLs.⁵

Hydrolysis of the GLs in the presence of water gives glucose, sulphate, and an aglycone product. The final products are produced via an unstable intermediate, which is influenced by the reaction conditions. The conditions include the pH, nature of the aglucone and the presence of metal ions such as Fe^{2+} as well as coenzymes. The myrosinase catalysed glucosinolate hydrolysis can be modified also by the presence of proteins. For example, if specifier proteins are present then the formation of the isothiocyanates is impeded. Two such proteins have been identified to date; the epithiospecifer protein (ESP) from *Arabidopsis thaliana* L. Heynh. and the nitrile-specifer protein from *Pieris rapae* L. They do not share any sequence similarity, however both have been shown to encourage the formation of nitriles.⁶



Scheme 11. Enzymatic degradation of glucosinolates

1.3.2. Biological activity of glucosinolates

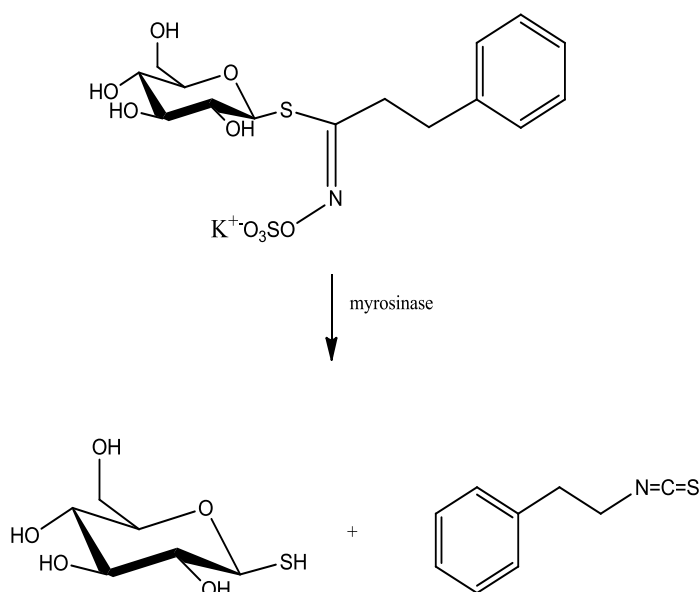
From the plant material, as a group of organic anions GLs are isolated in the form of a potassium or sodium salts. Chemicals produced by plants-namely called phytochemicals, are chemicals which may have an impact on health, or on flavor, texture, smell, or color of the plants, but are not required by humans as essential nutrients.

Phytochemicals secondary metabolites, GLs and/or their breakdown products have long been known for their fungicidal, bactericidal, nematocidal and allelopathic properties and have recently attracted intense research interest because of their anticancerogenic properties.⁶

Ingestion of about two servings per day of GLs vegetables rich in GLs may result in as much as a 50% reduction in the relative risk for cancer at certain sites. Some of the cancer chemoprotective activity of these vegetables is widely believed to be due to their content of major dietary components such as GLs.⁶ It has been shown that GLs vegetables are also rich in a number of vitamins and mineral as well as other more commonly recognize phytochemicals such as the carotenoids (including β -carotene or pro-vitamin A). For example *Moringa* leaves (Moringaceae family) contain more vitamin A than carrots, more calcium than milk, more iron than spinach, more vitamin C than oranges, and more potassium than bananas, and that the protein quality of *Moringa* leaves rivals that of milk or eggs.⁶

The major focus of much previous research has been on the negative aspects of GLs compounds because of the prevalence of certain-"antinutritional" or goitrogenic GLs in the protein-rich defatted meal from widely grown oilseed crops and in some domesticated vegetable crops. Efforts to avoid the goitrogenicity of rapeseed (*Brassica napus* L.), one of the most important oilseed crops in the world, led to the highly successful development of the oilseed crop "*Canola*". *Canola* seed contains about 40% oil and by regulation this oil must contain <2% erucid acid. The seed meal, which is fed to animals after oil is expressed, must have <30 μ mol of GLs per gram of the meal.⁵

GLs and their breakdown products have many important roles within nature. The metabolism of isothiocyanates in human volunteers has been examined after ingestion of a plant source of *sulphraphane* and *phenylethyl isothiocyanate*, and both studies strongly suggested a role of microflora in the digestive tract in the hydrolysis of the GLs to isothiocyanate. The antifungal and antimicrobial activity of glucosinolates and isothiocyanates are also widely known. For example phenethyl isothiocyanate and 4-methylsulphonylbutyl isothiocyanate have been reported to inhibit the growth of *Staphylococcus aureus* and *Penicillium glaucum*. 2-Phenylethyl isothiocyanate as breakdown product of *gluconasturiin* (Scheme 12.) has been shown to inhibit induction of lung and esophageal cancer in rat and mouse tumor models. This effect is well correlated with a reduction in carcinogen-DNA adduct formation and strongly suggested inhibition of cytochromes P450 as a mechanism of action. An analogous effect was observed in smokers who consumed watercress (a source of 2-phenylethyl glucosinolate) as well as an significant increase in the glucuronidation of nicotine metabolites, thus suggesting induction of the phase 2 detoxification enzyme UDP-glucuronosyl transferase activity in humans by 2-phenylethyl isothiocyanate.



Scheme 12. *2-Phenylethyl isothiocyanate, a breakdown product of gluconasturtiin*

There is, however, an opposite and positive side of this picture represented by the therapeutic and prophylactic properties of "nutritional" or - "functional" glucosinolates and their breakdown products. In many cultures throughout the tropics, differentiation between food and medicinal uses of plants (e.g. bark, fruit, leaves, nuts, seeds, tubers, roots, flowers), is very difficult since plants used span in both categories and this is deeply ingrained in the traditions and the fabric of the community.⁶

1.4. Analytical and spectroscopic methods

Many synthetic GLs as well as natural GLs have been analyzed by modern analytical and spectroscopic methods. Chromatography system, NMR, MS, IR and UV spectroscopy are the most used techniques for analysis.

1.4.1. Chromatography

The basic principle of chromatographic separation includes the mixture dissolved in the suitable solvent (mobile phase) passing over the stationary phase. Thereby, individual mixture components are kept at a different stage which leads to their separation at the outlet of the column. According to the physical-chemical processes chromatographic methods are divided into:

- Adsorption chromatography
- Partition chromatography
- Ion-exchange chromatography.

1.4.1.1. Thin-layer chromatography

A thin layer of some sorbent (*i. e.* silica gel) applied to the plate of glass, plastic, or aluminium foil of various dimensions is used as a stationary phase in thin-layer chromatography. Sorbent must have a large surface area and should be selective with respect to the separated substances. The sorbents can be divided into polar (aluminum oxide, silica, natural and artificial silicates) and non-polar (activated charcoal). The sorbents can also be organic substances mostly cellulose, polyamide etc.

The principle of thin-layer chromatography is simple. The sample is applied to a sorbents by point or line at a distance of 2.5 cm from the edge of the plate. The plate is placed in a sealed chamber saturated with vapors of the mobile phase. The different compounds will travel at different speeds, which will separate them from the mixture. The visualization of the compounds is carried out by the visulization reagent which causes coloration, or by UV-light. R_f (retardation factor) is defined as the ratio of the distance traveled by the center

of a spot to the distance traveled by the solvent. R_f is an important constant for a given substance under defined chromatographic conditions, and it can be used for identification of the components. It can have any value from 0 to 1. The R_f value depends on temperature, type of the solvent, the type of stationary phase and on the nature of the substance.

1.4.1.2. Column chromatography

Column chromatography is used for preparative purposes as for the isolation or purification of the compounds from a mixture. It is possible to have the presence of colored bands. This method is based on the different velocity of compounds migration through the stationary phase under the influence of the mobile phase and gravity. The mixture is distributed between the two phases according to the greater affinity for one or the other.

Column chromatography can be divided according to type of the stationary phase:

- Solid-liquid chromatography system (Fig. 4 and 5.)
- Liquid-liquid chromatography system,
- Ion exchange
- Gel chromatography



Figure 4. Solid-liquid chromatography system



Figure 5. GRACE Inverse Solid-liquid chromatography system

1.4.2. HPLC chromatography system

Systems used in chromatography are often categorized into one of four types based on the mechanism of action, adsorption, partition, ion-exchange and size exclusion. Adsorption chromatography arises from interactions between solutes and the surface of the solid stationary phase. Partition chromatography involves a liquid stationary phase that is immiscible with the eluent and is coated on an inert support. Ion exchange chromatography has a stationary phase with an ionically charged surface that is different from the charge of the sample. The technique is based on the ionization of the sample. The stronger the charge of the sample, the stronger the attraction to the stationary phase; therefore, it will take longer to elute off of the column. Size exclusion is as simple as screening samples by molecular size. The stationary phase consists of materials with precisely controlled pore size. Smaller particles are caught up in the column material and will elute later than larger particles.

HPLC instrumentation includes a pump, injector, column, detector and integrator or acquisition and display system. The heart of the system is the column where separation occurs. Detection of the eluting components is important, and the techniques used for the detection is dependent upon the detector used. The response of the detector to each component is displayed on a chart recorder or a computer screen and is known as a chromatogram. (Figure 6.)



Figure 6. HPLC system

1.4.3. Mass spectrometry

Mass spectrometry is a technique used for the analyzing of chemical species, based on translation of the sample in a gaseous state, its ionization and fragmentation and separations of obtained ions according to their mass or mass to charge ratio (relative m/e).

In a typical MS procedure, a sample, which may be solid, liquid, or gas, is ionized, for example by bombarding it with a current of electrons. This may cause some of the sample's molecules to break into charged fragments. An extraction system removes ions from the sample, which are then directed through the mass analyzer and onto the *detector*. The quadrupole mass analyzer (QMS) is one type of mass analyzer used in mass spectroscopy. The difference in masses of the fragments allows the mass analyzer to sort the ions by their mass-to-charge ratio. The detector measures the value of an indicator quantity and thus provides data for calculating the abundances of each ion present. Some detectors also give spatial information. (Figure 7.)

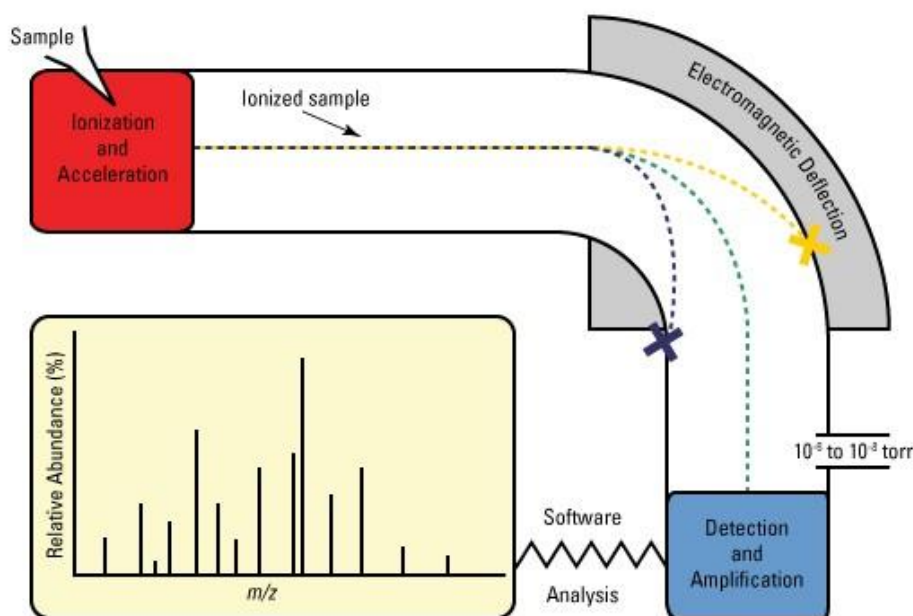


Figure 7. Scheme of the Mass spectrometer

1.4.4. IR spectroscopy

Infrared spectroscopy (IR spectroscopy) is the spectroscopy that deals with the infrared region of the electromagnetic spectrum, a radiation in range of longer wavelengths and lower frequencies than the visible light. It covers a range of techniques, mostly based on the absorption spectroscopy. For a given sample which may be solid, liquid, or gaseous, the method or technique of infrared spectroscopy uses instrument called infrared spectrometer (or spectrophotometer) to produce an infrared spectrum. Infrared spectroscopy exploits the fact that molecules absorb specific frequencies that are characteristic of their structure. These absorptions are resonant frequencies - the frequency of the absorbed radiation matches the transition energy of the bond or group that vibrates.

A basic IR spectrum is essentially a graph of infrared light absorbance (or transmittance) on the vertical axis vs. frequency or wavelength on the horizontal axis. Typical units of frequency used in IR spectra are reciprocal centimeters (sometimes called wave numbers), with the symbol cm^{-1} . Units of IR wavelength are commonly given in micrometers (formerly called "microns"), symbol μm , which are related to wave numbers in a reciprocal way. (Figure 8.)



Figure 8. IR spectrometer

1.4.5. NMR spectroscopy

NMR has become the preeminent technique for determining the structure of organic compound. Nuclear magnetic resonance is a technique that measures the energy (frequency) broadcast, by radio frequency waves, which is absorbed by a sample or an element. Radio frequency radiation acts to excite the rotation of the nuclei which has the electromagnetic field and acts as a magnet and as such these nuclei can be detected.

It should be noted that the nucleus absorbing energy of specific wavelengths required for the rotation of the nucleus whereby resulting the transmission of energy. It is said that the nuclei act as they are spinning. The energy which will produce spinning is namely called frequency of resonance. When the nuclei are spinning they possess a certain angle of rotation, which is called a spin. Spin is the quantum number or a slope that cannot attain value of zero.

Most pronounced isotopes of organic molecules have quantum number of 0. These isotopes cannot be recorded by the NMR technique. In the nature 1 of 100 C isotopes are ^{13}C , while the rest of them are ^{12}C . NMR spectrometer can only record ^{13}C nuclei. This technique is sensitive and requires a large magnetic field to detect the peaks. If the magnetic field is increased it is then possible to detect deuterium isotope which has the nuclear spin quantum number 1. (Figure 9. and 10.)

^{16}O and ^{14}N elements are also present in the organic molecules and they cannot be detected by NMR spectrometer, because only the nuclei with the odd atomic number or odd mass number and nuclear magnetic moment-spin, can be detected by the NMR spectrometer. However, for example, the nitrogen isotope ^{15}N , having odd atomic number, can be detected.

NMR spectroscopy (^{13}C , ^1H , DEPT, TOSCY, HSQC) is recorded for the structure confirmation of the synthetically obtained compounds.



Figure 9. NMR spectrometer 400 MHz



Figure 10. NMR spectrometer 250 MHz

2. EXPERIMENTAL SECTION

2.1. General methods

All reactions were carried out using oven-dried glassware under an atmosphere of dry argon. Solvent and reagents were obtained from Sigma-Aldrich, Acros, Alfa-Aesar or Carbosynth. All reagent-grade chemicals were obtained from commercial suppliers and were used as received.

Solvents were distilled following the procedures described by D.D. Perrin et. al.⁸ The quality of the used solvents is as follows:

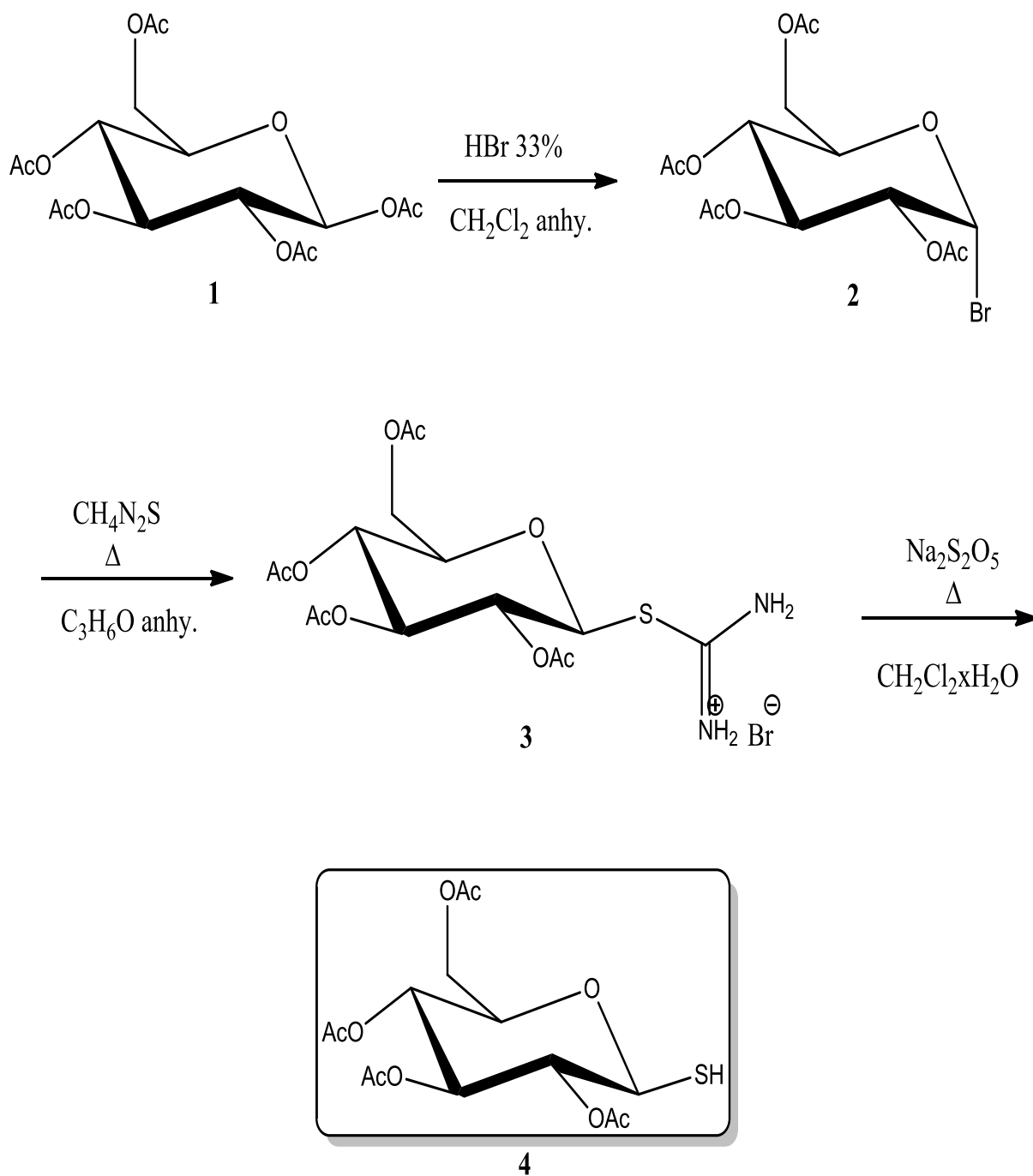
- ✓ THF was dried using Glass Technology Dry Solvent Station GTS100.
- ✓ Toluene was distilled and dried over CaH_2 .
- ✓ CH_2Cl_2 was distilled and dried over P_2O_5 .
- ✓ DMF (HPLC) were dried using activated 4Å molecular sieves.
- ✓ MeOH (HPLC) was dried using activated 3Å molecular sieves.
- ✓ Pyridine and triethylamine were dried over KOH.
- ✓ Chloroform (HPLC grade) was used without further purification.

Analytical thin-layer chromatography was performed using Silica Gel 60F254 precoated aluminium plates (Merck) with visualization by UV light and by charring with a 10% sulfuric acid solution in EtOH, phosphomolybdic acid or KMnO_4 . Flash chromatography was performed on silica gel 60N(spherical neutral, 40 – 63 μM) or using Reveleris® flash chromatography system. Some compounds which were not commonly isolated but were purified once in order to assess the structure of all the intermediates.

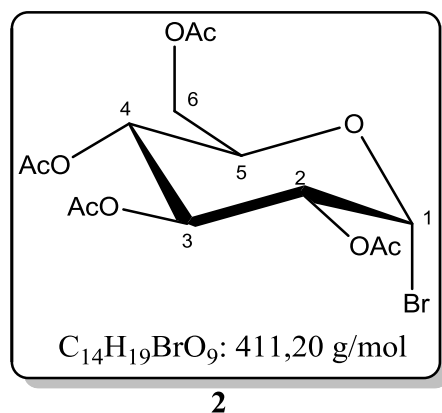
^1H and ^{13}C NMR spectra were recorded on Bruker Avance DPX 250 or Bruker Avance II 400 spectrometers. Chemical shifts were referenced to the residual solvent signal or to TMS as internal standard. Carbon signals were assigned by DEPT experiments. ^1H and ^{13}C NMR signals were attributed on the basis of H-H and H-C 2D correlations. Low-resolution mass spectra were recorded on a Perkin-Elmer Sciex API 300. High-resolution mass spectra were recorded on a Bruker Q-TOF MaXis spectrometer (precision 5 or 6 digits). The infrared spectra of compounds were recorded on a Thermo Scientific Nicolet iS10.

2.2. Compounds description

Typical procedure for the synthesis of the *1-thio-2,3,4,6-tetraacetate-β-D-glucose (4)*



Typical procedure for the synthesis of the *1-bromo-2,3,4,6-tetraacetate- α -D-glucose(2)*



In an oven dried 500 ml flask a solution of HBr in CH_3COOH 33%(45 mL, 25.4 mmol, 10 eq) was added dropwise to a solution of compound **1** (10 mL, 25.4 mmol) in anhydrous CH_2Cl_2 (20 mL) at -5°C under argon atmosphere. The reaction was stirred at room temperature for 2h then stopped by addition of CH_2Cl_2 (50 mL) and ice-cold water (60 mL).

Afterwards the aqueous phase was extracted with CH_2Cl_2 (2x100 mL and 150 mL) the organic phases were collected and washed successively with water (2x150 mL), and then with a saturated aqueous solution of NaHCO_3 (1x200 mL, until pH=8) and saturated solution of NaCl (1x150 mL).

The organic phases were dried over MgSO_4 , filtered and the solvent was removed under vacuum to give compound **2** (10.46 g, 99%) as a white solid. (figure 11.)

Rf: 0.38 (PetEt : EtOAc = 7:3)

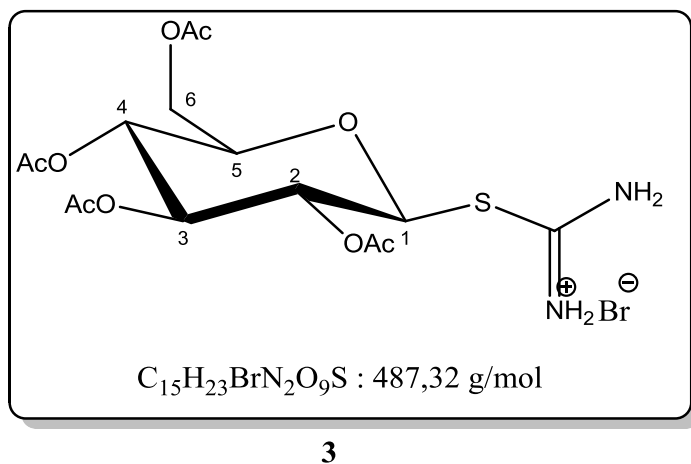
$^1\text{H-NMR}$ (CDCl_3 , 400MHz): δ (ppm)= 2.03, 2.05, 2.09, 2.10 (4s, 12H, CH_3CO), 4.08-4.16 (m, 1H, 5-H), 4.30 (m, 2H, 6-H), 4.83 (dd, $J_{2-1}=4.04\text{Hz}$, $J_{2-3}=9.98\text{Hz}$, 1H, 2-H), 5.16 (t, $J_4=9.49\text{Hz}$ 1H, 4-H), 5.57 (t, $J_3=9.86\text{Hz}$ 1H, 3-H), 6.60 (d, $J=4.03\text{Hz}$ 1H, 1-H).

$^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz): δ = 20.7, 20.75, 20.78, 20.8 (CH_3CO), 61.1 (C-6), 67.3 (C-3), 70.3 (C-2), 70.8 (C-4), 72.3 (C-5), 86.7 (C-1), 169.6, 169.9, 170.0, 170.6 (CH_3CO).



Figure 11. The synthesis of 1-bromo-2,3,4,6-tetraacetate- α -D-glucose(2)

Typical procedure for the synthesis of the 2,3,4,6-tetraacetate β -D-glucose-isothiuronium (3)



Thiourea (3.09 g, 40.6 mmol, 1.6 eq) was added to a solution of the compound 2 (10,239 g, 25.4 mmol, 1eq) in anhydrous acetone (dried over K_2CO_3), under inert atmosphere (argon). The reaction was stirred at 65° C during 30 min.

Slow dissolution of thiourea was observed after which a white precipitate was formed after additional 15-20 min. The reaction mixture was cooled at 0°C without stirring and the solvent was removed by filtration. The filtrate is evaporated to half-volume, cooled again at 0°C for another precipitation. All the collected precipitates gave compound **3** (10.43 g, 84%) as white solid (Figure 12.).

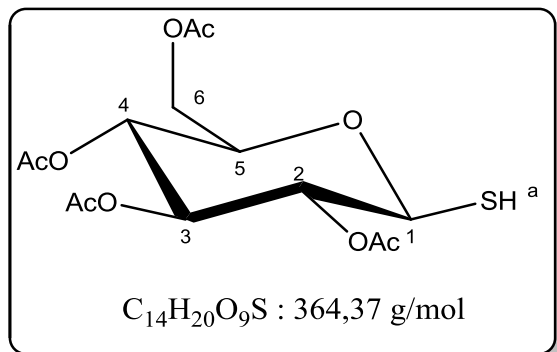
Rf: 0.15 (PetEt : EtOAc = 7:3)

1H -NMR (CD_3OD , 400MHz): δ (ppm)= 1.97, 2.01, 2.05, 2.15, (CH_3CO), 4.04-4.34 (m, 3H, 6-H, 5-H), 5.07-5.21 (dd, $J_{1-2}=9.93$ Hz, $J_{2-3}=20.85$ Hz, 2H, 4-H, 2-H), 5.36 (t, $J=9.47$ Hz, 1H, 3-H), 5.49 (d, $J_1=9.98$ Hz, 1H, 1-H).



Figure 12. The synthesis of 2,3,4,6-tetraacetate β -D-glucose-isothiuronium(**3**)

Typical procedure for the synthesis of **2,3,4,6-tetraacetate -1-thio- β -D-glucose (4)**



4

Sodium metabisulfite (6.99 g, 36.6 mmol, 2 eq) dissolved in water (70 mL) was added to a solution of the compound **3** (8.93 g, 18.3 mmol) dissolved in CH_2Cl_2 (70 mL). Reaction was heated for 1h with reflux (60°C).

After extraction of aqueous phase with CH_2Cl_2 (3x40 mL), organic phases were collected, washed with water (3x H_2O and 1xNaCl aq sat.) and dried over $MgSO_4$. The evaporation of the solvent under reduced pressure gave the expected compound **4** (5.69 g, 85%) as white solid. (Figure 13.)

Rf: 0.2 (PetEt : EtOAc = 7:3)

1H -NMR (CD_3OD , 400MHz): δ (ppm) = 1.97, 2.00, 2.04, 2.05 (4s, 12H, CH_3CO), 3.86-3.90 (m, 1H, 5-H), 4.12 (dd, $J_{6a,5}$ = 3.82 Hz, $J_{6a,6b}$ = 12.46 Hz, 1H, 6- H_a), 4.24 (dd, $J_{6b,5}$ = 2.56 Hz, $J_{6b,6a}$ = 12.76 Hz, 1H, 6- H_b), 4.76 (d, J = 9.80 Hz, 1H, 1-H), 4.90 (t, J = 9.37 Hz, 1H, 2-H), 5.05 (t, J = 9.74 Hz, 1H, 4-H), 5.24 (t, J = 9.37 Hz, 1H, 3-H).

^{13}C -NMR (CD_3OD , 100MHz): δ (ppm) = 20.48, 20.53, 20.6, 20.7 (CH_3CO), 63.3 (C-6), 69.7 (C-4), 75.0 (C-3), 75.1 (C-2), 77.1 (C-5), 79.3 (C-1), 171.19, 171.21, 171.5, 172.3 (CH_3CO). (Figure 19.)

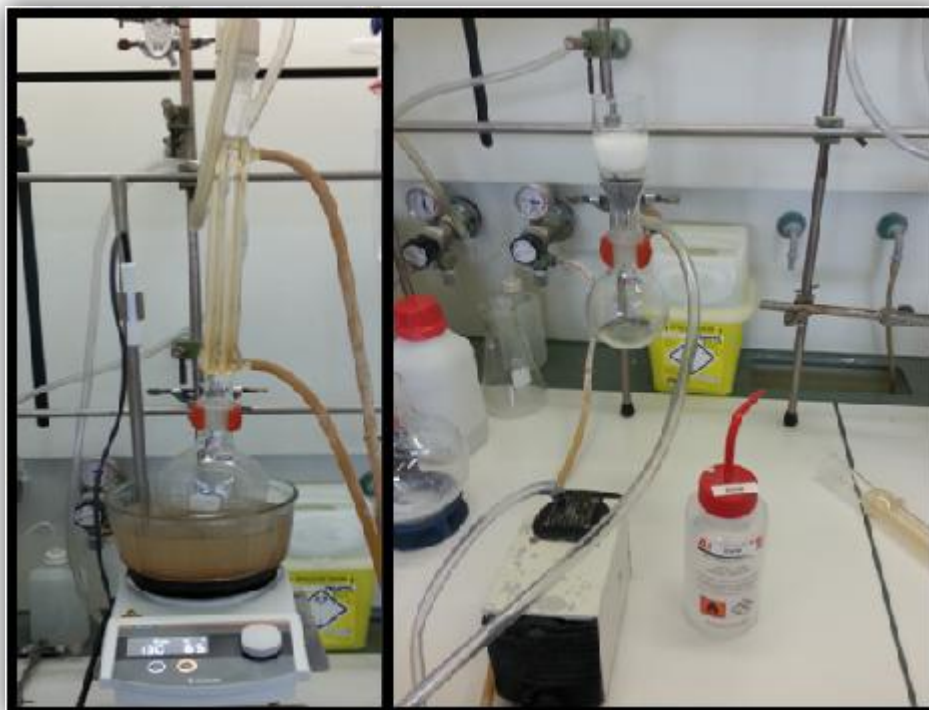
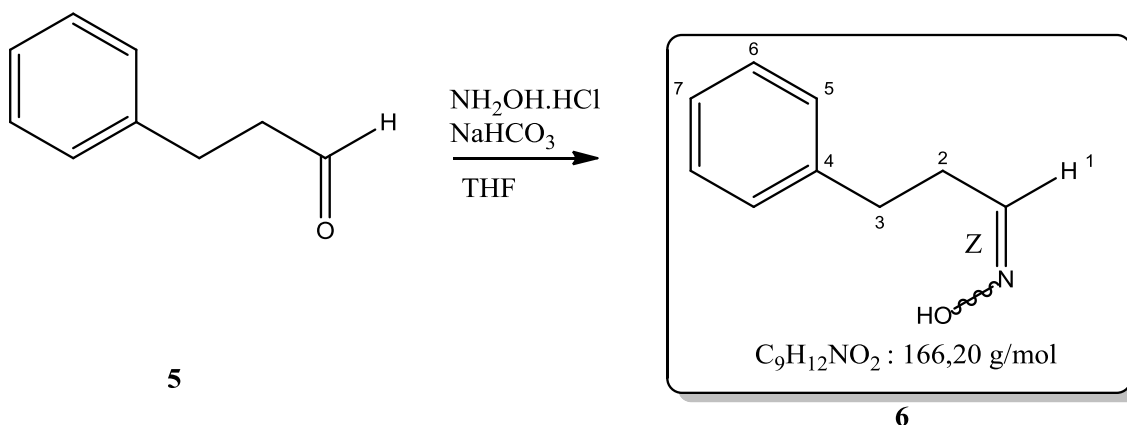


Figure 13 . The synthesis 2,3,4,6-tetraacetate -1-thio- β -D-glucose(4)

Typical procedure for the synthesis of the *3-phenylpropanal oxime*



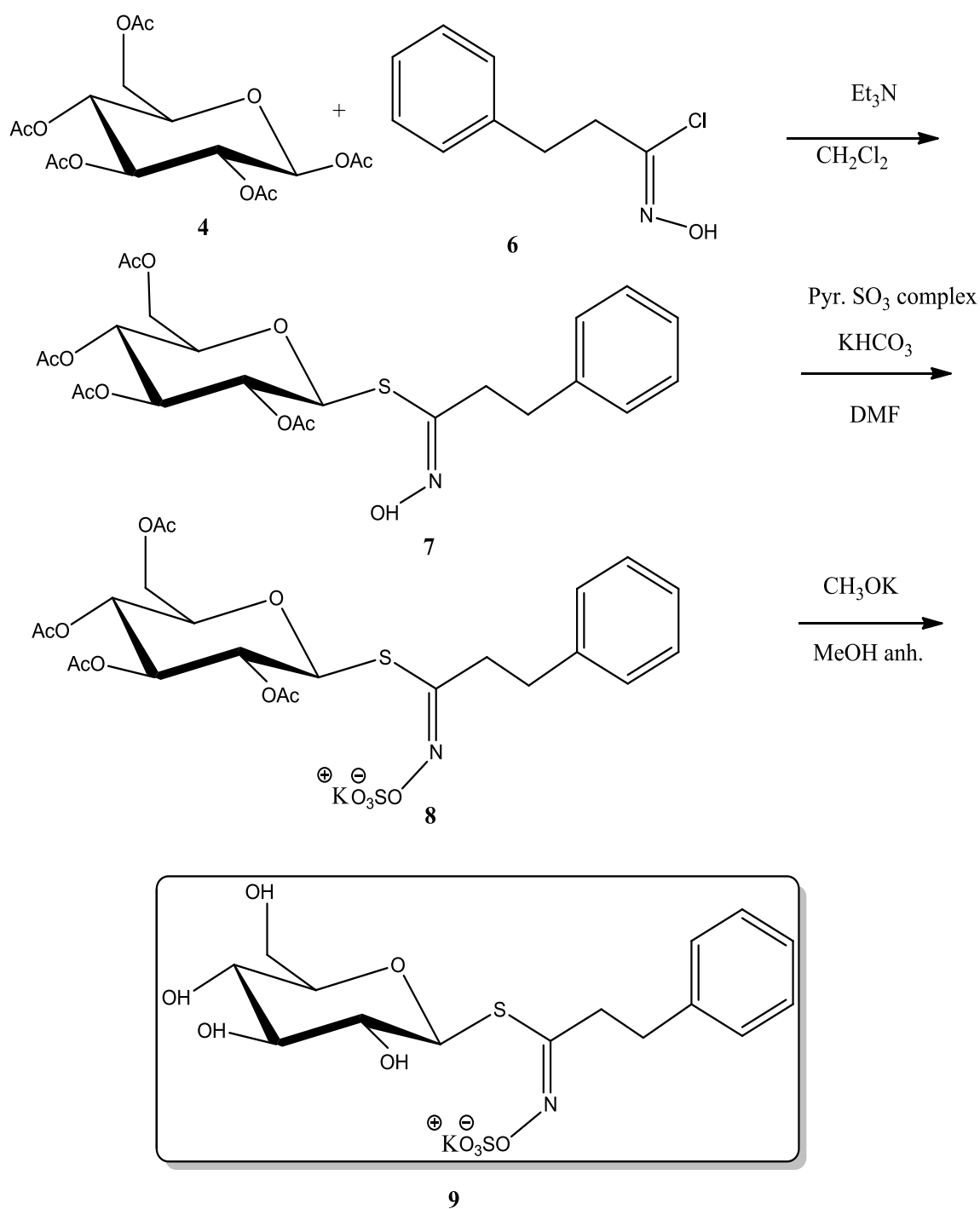
Tetrahydrofurane (30 mL), NaHCO_3 (3.1304 g, 37.2 mmol, 1 eq), and $\text{NH}_2\text{OH}/\text{HCl}$ (5.17 g, 74.4 mmol, 2 eq) were added to a solution of compound **5** (5.00 g, 37.2 mmol) in water (30 mL). Reaction was stirred for 3h at room temperature under an inert atmosphere. CH_2Cl_2 (30 mL) was added, and aqueous phase was extracted with CH_2Cl_2 (2x30 mL). The organic phases were collected, washed with H_2O (2x30 mL) and HCl (1M, 30 mL) and dried over MgSO_4 . The solvent was evaporated under reduced pressure to give **3-phenylpropanal oxime** (5.5391g, 99.8 %) as a white solid which was used without any further purification in the next step. The product is 88/12 E/Z mixture.

Rf 1= 0.46 (PetEt : EtOAc = 9:1)

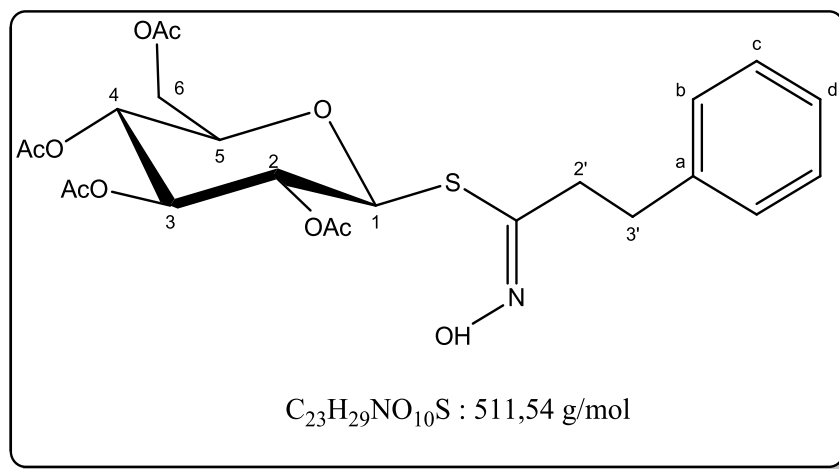
Rf 2= 0.67 (PetEt : EtOAc = 9:1)

$^1\text{H-NMR}$ (CDCl_3 , 400MHz): δ (ppm)= 2.71 (t, J = 7.30 Hz, 2H, 2-H) 2.82 (t, J =7.54 Hz, 2H, 3-H), 6.75 (t, J = 5.16 Hz, 1H, H^{-1}Z) 7.17-7.34 (m, 5H, 5- H_{ar} , 6- H_{ar} , 7- H_{ar}), 7.46 (t, J = 5.60 Hz, 1H, H^{-1}E), 8.48 (s, 1H, NOH).

$^{13}\text{C-NMR}$ (CDCl_3 , 100MHz): δ (ppm)= 26.4 (C-2), 32.0 (C-3), 126.23, 128.28, 128.3, 128.5 (aryl, C-4, C-5, C-6, C-7), 140.68 (C=N), 151.77 (C-1).(Figure 20.)

Typical procedure for the synthesis of the *gluconasturtiin* (9)

Typical procedure for the synthesis of the tetraacetylated *desulpho-gluconasturtiin* (**7**)



7

NaOCl (14%, 3.80 g, 75.00 mmol, 2.8 eq) was added to a solution of compound **6** (0.73 g, 27.00 mmol, 1 eq) in CH_2Cl_2 (50 mL), into a vigorously shaken separating funnel until the color changed from blue to yellow (10 min). The organic layer is separated and transformed into a flask where Et_3N (0.9835 g, 97.00 mmol, 3.6 eq) and a solution of compound **4** (1.00 g, 27.00 mmol) in CH_2Cl_2 (50 mL) were added successively. Reaction was stirred at $0^\circ C$ during 1:30h under argon. Afterwards, once the reaction was finished the organic phase was washed with NH_4Cl sat. (2x35 mL), and NaCl sat. (35 mL) then dried over $MgSO_4$ and concentrated under reduced pressure. The resulting crude compound was purified by silica gel column chromatography (PE/EA 7/3) to afford the desired product **7** (1.13 g, 95%) as a white solid. (figure 14.)

R_f : 0.57 (PetEt : EtOAc = 1:1):

1H -NMR ($CDCl_3$, 400 MHz): δ (ppm)= 1.91, 2.01, 2.03, 2.06 (4s, 12H, CH_3CO), 2.73-2.93 (m, 2H, 3'-H), 3.1 (t, J = 7.75 Hz, 2H, 2'-H), 3.65-3.73 (m, 1H, 5-H), 4.07-4.17 (m, 2H, 6-H), 4.95-5.12 (m, 3H, 2-H, 1-H, 4-H), 5.23 (t, J = 9.03 Hz, 1H, 3-H), 7.18-7.34 (m, 5H, b,c,d-H), 8.14 (s, 1H, N-OH).

^{13}C -NMR ($CDCl_3$, 100MHz): δ (ppm)= 20.49, 20.54, 20.55, 20.6 (CH_3CO), 33.2 (C-2'), 34.2 (C-3'), 62.11 (C-6), 70.1 (C-4), 73.7 (C-3), 76.1 (C-5), 79.9 (C-1),

80.4 (C-2), 126.5, 128.2, 128.7 (C-b,c,d) 140.5 (C-a), 169.2,169.3,170.2,170.6 (COO CH₃). (Figure 21.)

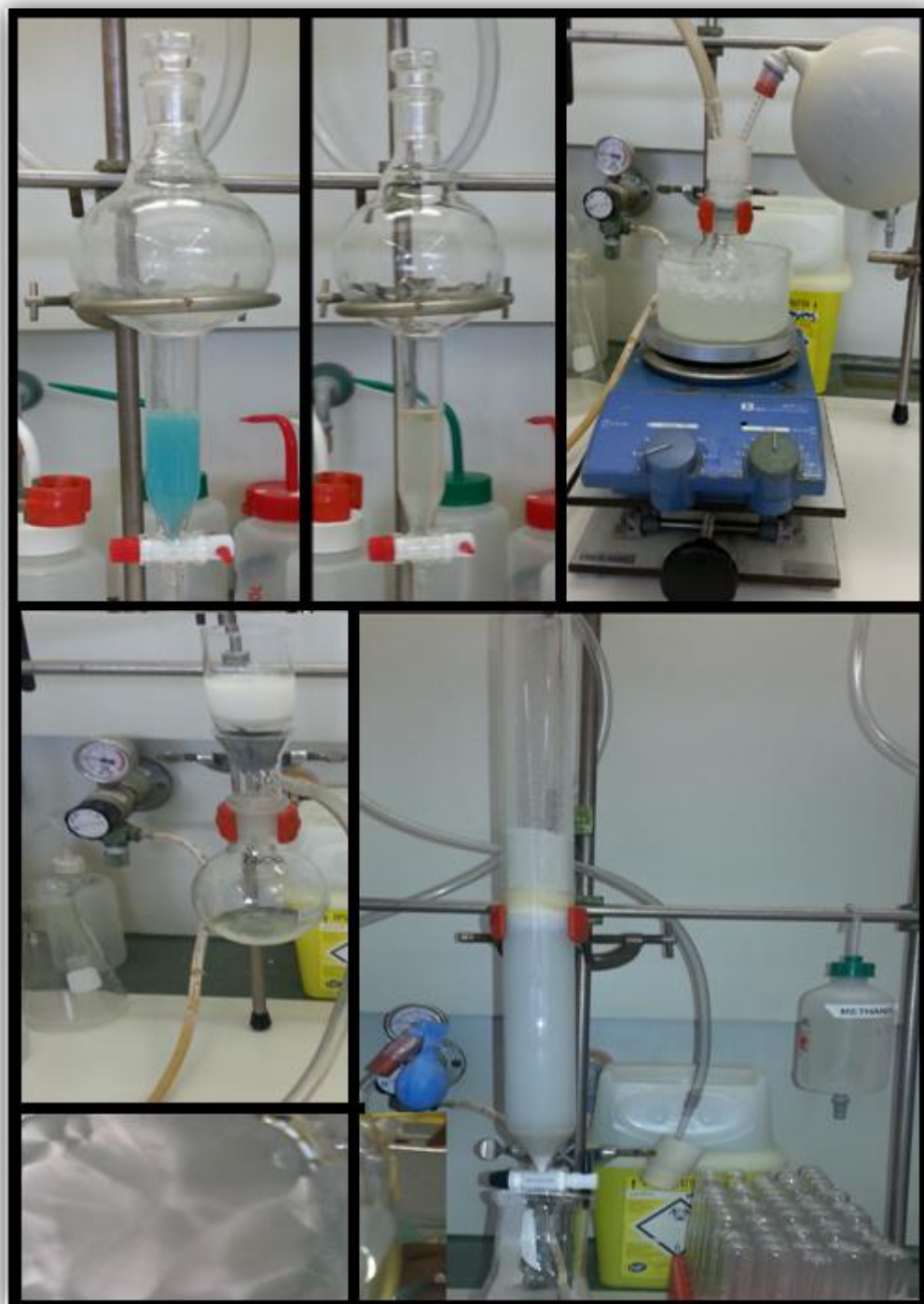
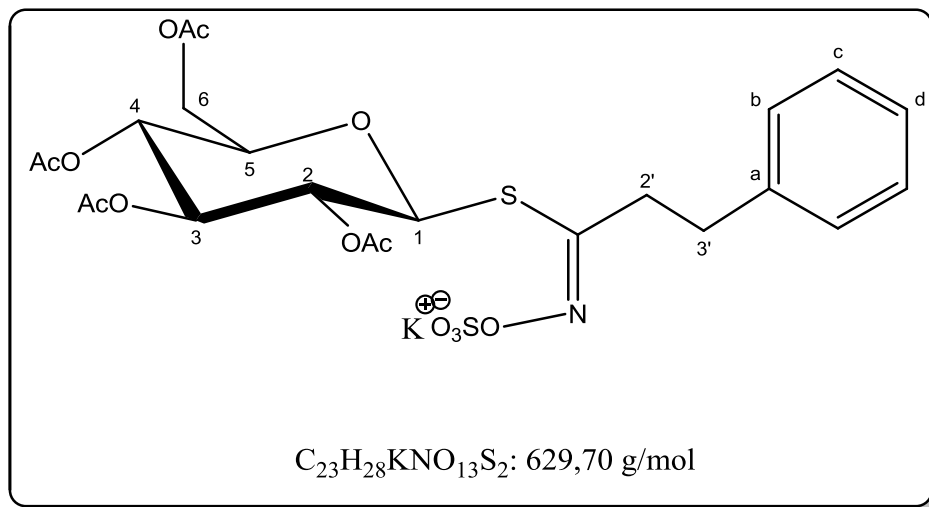


Figure 14. The synthesis of the desulpho-gluconasturtiin (**7**)

Typical procedure for the synthesis of the tetraacetylated-*gluconasturtiin*(**8**)



8

Sulfur trioxide pyridine complex (1.2045 g, 75.00mmol, 7eq) was added to a solution of **7** (0.75 g, 21.00mmol, 1eq) in anhydrous DMF (25mL), in to a flask (100 mL) under argon. Reaction was stirred at 65°C two days under argon. After that $KHCO_3$ (21 mL, 75.00mmol, 7eq, 0.5M) was added to the solution at room temperature. The solvent was evaporated under reduced pressure till dryness, and the crude product was coevaporated with toluene. After the evaporation the resulting crude compound was purified by silica gel column chromatography (EtOAc/MeOH 9/1) to afford the pure compound **8** (0.723 g, 76%) as a white solid. (figure 15.)

Rf : 0.325 (AcOEt : MeOH= 9:1)

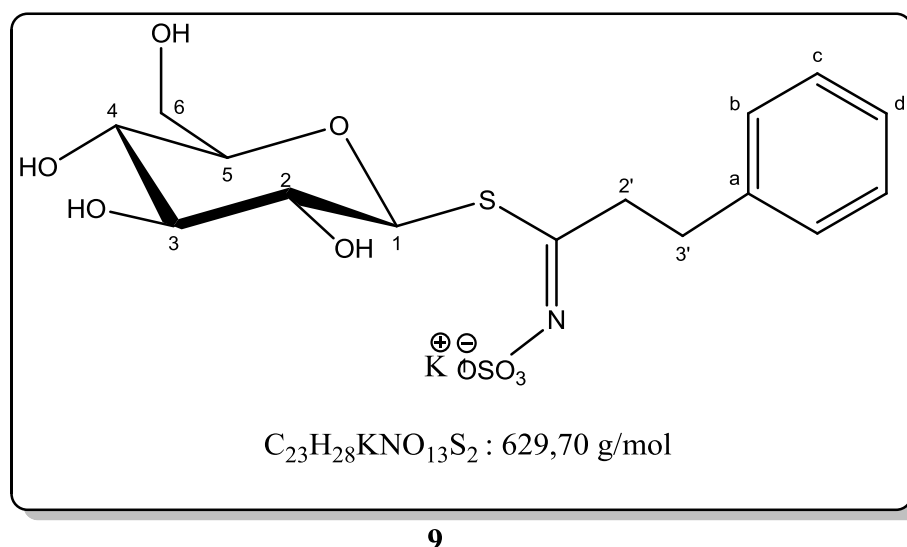
1H -NMR (CD_3OD , 400 MHz): δ (ppm)= 1.85, 1.99, 2.03, 2.04 (4s, 12H, CH_3CO), 2.90-3.15 (m, 4H, 2'-H, 3'-H), 3.91-3.98 (m, 1H, 5-H), 4.06-4.19 (m, 2H, 6-H), 4.95-5.08 (m, 2H, 4-H, 3-H), 5.28 (d, $J=10.10$ Hz, 1H, 1-H), 5.35 (t, $J=9.24$ Hz, 1H, 2-H), 7.16-7.36 (m, 5H, b,c,d-H) .

^{13}C -NMR (CD_3OD , 400 MHz): δ (ppm)=19.06, 19.10, 19.16, 19.21 (CH_3CO), 32.92 (C-3'), 34.07 (C-2'), 62.06 (C-6), 68.18 (C-4), 69.91 (C-3), 73.54 (C-2), 75.47 (C-5), 79.37 (C-1), 125.99,128.22 ,128.26 (C-b,c,d), 140.78 (C-a), 169.51, 169. 80, 170.08, 170.81 ($COOCH_3$). (Figure 22., given in chapter 6.)



Figure 15. The synthesis of the tetraacetylated-gluconasturtiin (**8**)

Typical procedure for the deacetylation of glycosylated *gluconasturtiin* (**9**)



After 30 min of stirring at room temperature, a solution of potassium methoxide (30.89 mg, 4.4 mmol, 0.4 eq) in anhydrous MeOH (20 mL) was added, under argon flux, to a solution of compound **8** (723.3 mg, 1.148 mmol) in anhydrous MeOH (20 mL).

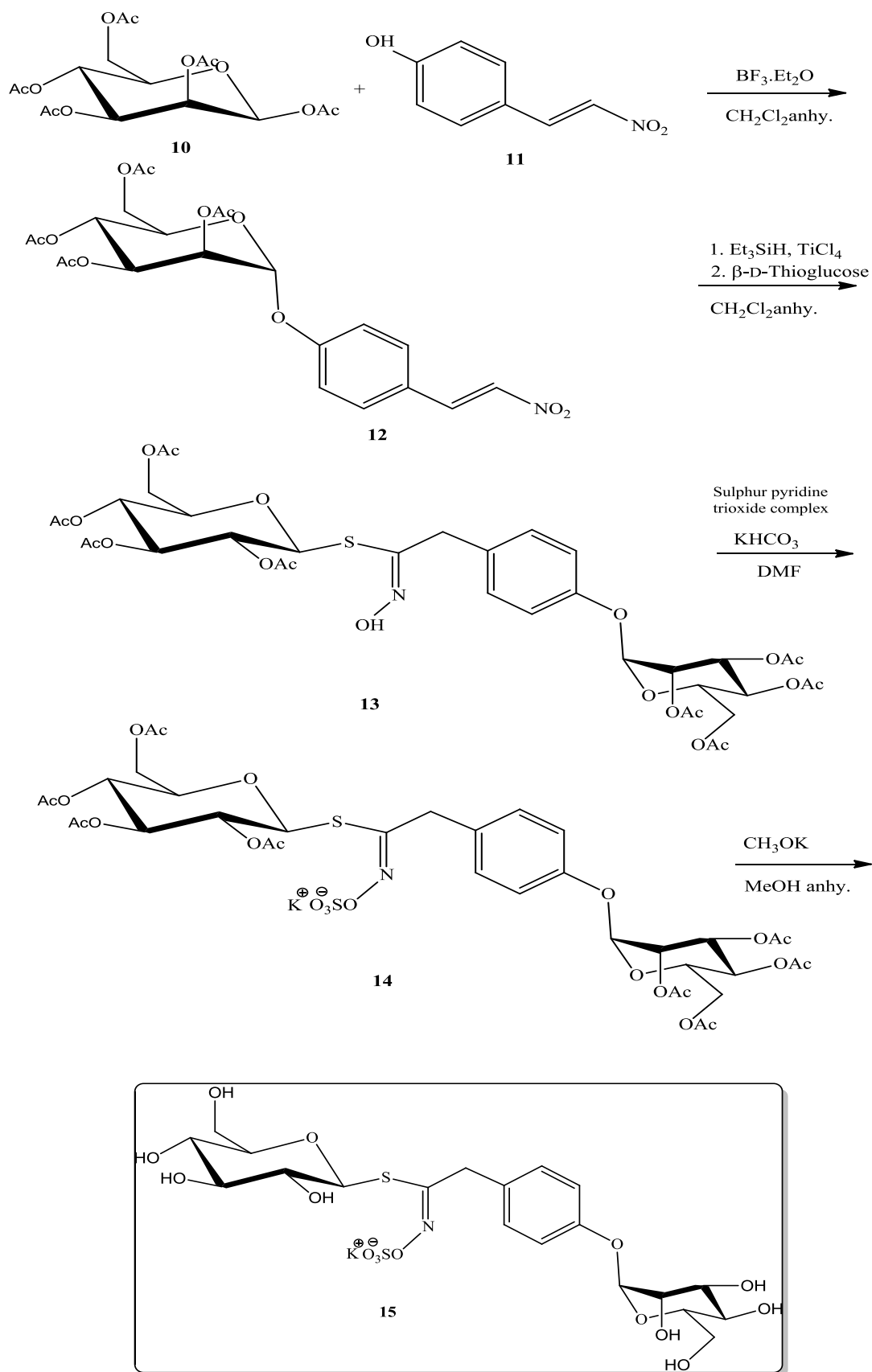
Afterwards, the mixture was stirred for 18h at the room temperature. Then the solvent was removed under high vacuum to give the crude residue (555.0 mg) which was purified by reverse flash chromatography system Grace (column: Reveleris C18 80g, Solvent A: H₂O/MeOH 99/1, Solvent D: MeOH, Flow Rate 40mL/min, UV1:227 nm, UV2:254 nm, ELSD Carrier: Iso-propanol), which in turn gave pure *gluconasturtiin* (270.1 mg, 51%) as white amorphous solid.

R_f: 0.8 (EtOAc:MeOH=9:1)

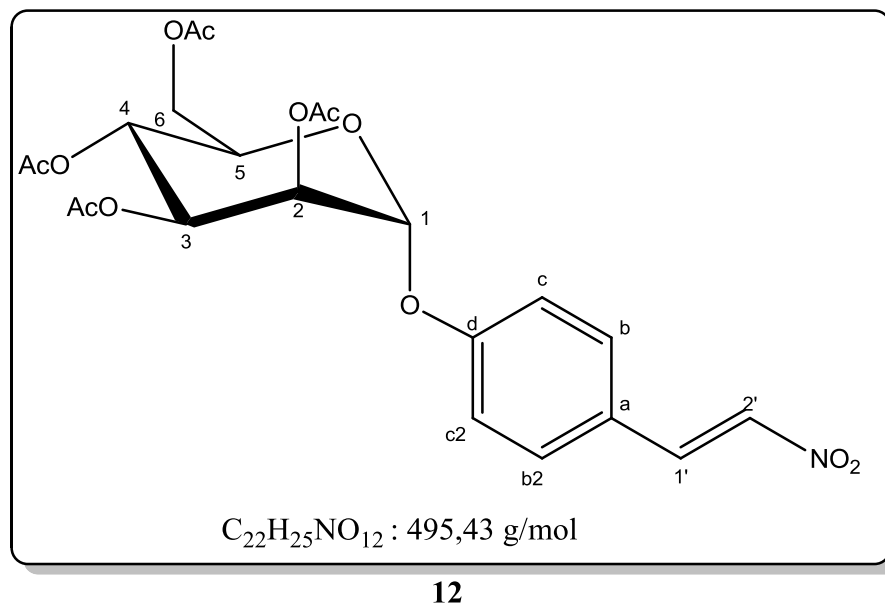
¹H-NMR (D₂O, 400 MHz): δ(ppm)= 2.93 (m, 2H, 3'-H), 3.00 (m, 2H, 2'-H), 3.32-3.47 (m, 4H, 2-H, 3-H, 4-H, 5-H), 3.608 (dd, J₁= 4.09 Hz, J₂= 12.32 Hz, 1H, 6-Ha), 3.77 (d, J= 12.62Hz 1H, 6-Hb) 4.82 (d J=9.52 Hz, 1H, 1-H), 7.16-7.36 (m, 5H, b,c,d-H_{ar}).

¹³C-NMR (D₂O, 400 MHz): δ(ppm)= 32.50 (C-2'), 33.90(C-3'), 60.60 (C-6), 69.00(C-5), 71.92(C-4), 77.02(C-3), 80.07(C-2), 81.64(C-1), 126.59, 128.71(C-b,c,d), 128.75 (C-a). (Figure 23)

IR: ν(cm⁻¹) = 3270.70 (-OH), 1733.06 (-C=N), 1496.31 (-C=C), 1042.65 (-C-O). (Figure 26.)

Typical procedure for the synthesis of the *glucomorignin analogue (15)*

Typical procedure for the synthesis of the *E-p*-(2,3,4,6-tetra-*O*-acetyl- α -D-mannopyranosyloxy)-2-nitrostyren (12**)**



Compound **11** (1.22 g, 74.00mmol, 1eq) was added to solution of compound **10** (2.90 g, 74.00mmol, 1eq) with anhydrous CH_2Cl_2 (29 mL) in a dry flask(250 mL) under inert atmosphere (argon). Afterwards a solution of boron trifluoride diethyl etherate (4.20g, 29.6 mmol, 4 eq) was successfully added. Reaction was stirred at room temperature for three days.

After hydrolysis (ice cold water - 40 mL) the aqueous phase was extracted with CH_2Cl_2 (3x40 mL). Organic phases were collected, dried over $MgSO_4$ and the solvent was evaporated under reduced pressure.

After the evaporation the resulting crude compound was purified by silica gel column chromatography (EtOAc/MeOH 9/1) to afford the pure compound **10** (1.62g, 44%) as an yellow solid. (Figure 16.)

Rf: 0.313 (PetEt:EtOAc = 7:3)

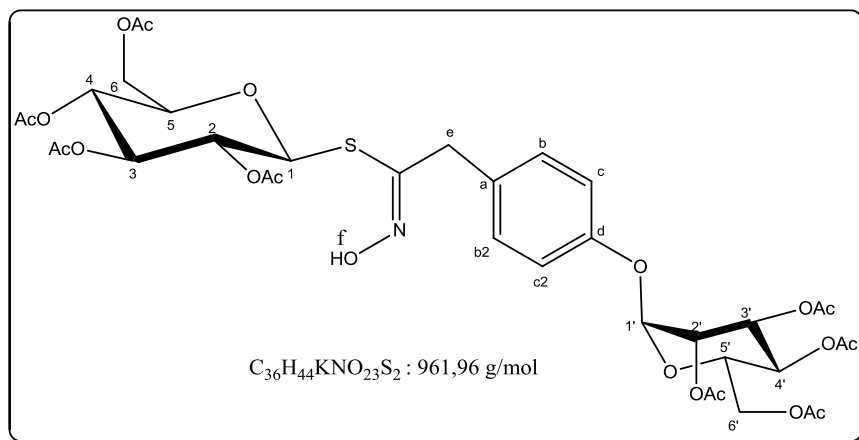
1H -NMR: ($CDCl_3$, 400MHz): δ (ppm)= 2.02, 2.04, 2.05, 2.25 (4s, 12H, CH_3CO), 4.00-4.14 (m, 2H, 5-H, 6-H), 4.27 (dd, $J_1=5.11$ Hz, $J_2=11.72$ Hz, 1H, 4-H), 5.37 (t, $J=9.828$ Hz, 1H, 3-H), 5.25-5.35 (m, 1H, 2-H), 5.82-5.93 (m, 1H, 1-H), 7.21 (d, $J=10.82$ Hz, 1H, 2'-H or 3'-H), 7.84 (d, $J=8.81$ Hz 1H, 2'-H or 3'-H), 8.02-8.17 (m, 1H, b,c-H).

^{13}C -NMR: (CDCl_3 , 100 MHz): $\delta(\text{ppm})$ = 20.61, 20.62, 20.64, 20.79 (CH_3CO), 61.9 (C-6), 65.6 (C-4), 68.6 (C-3), 69.0 (C-2), 69.5 (C-5), 95.3 (C-1), 117.2 (C-b, C-b2), 124.8 (C-d), 130.85 (C-c, C-c2) 136.14, 138.68 (C-1', C-2'), 158.42 (C-a), 169.6, 169.4, 170.4 (COOCH_3). (Figure 27. given in chapter 6.)



Figure 16. The synthesis of the *E*-*p*-(2,3,4,6-tetra-*O*-acetyl- α -*D*-mannopyranosyloxy)-2nitrostyren (**12**)

Typical procedure for the synthesis of the tetraacetylated-*glucomoringin* analogue (13)



13

Compound **12** (0.600 g, 1.2 mmol) was dissolved in an anhydrous CH_2Cl_2 (30 mL) in a flask (250 mL) under inert atmosphere (argon). Titanium tetrachloride (0.5060 g, 2.6 mmol, 2.2 eq, 0.29 mL) and triethylsilane (0.293 g, 2.5 mmol, 2.1 eq, 0.40 mL) were added to the solution, to give the expected hydroxymoil-chloride.

After 18h of stirring at room temperature, the reaction mixture was hydrolyzed (ice cold water-40 mL), and the aqueous phase was extracted with CH_2Cl_2 (2x40 mL). The organic phases were collected, and dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. The residue was dissolved in anhydrous CH_2Cl_2 (40 mL) along with *β -D-thioglucose* (0.525 g, 1.4 mmol, 1.2 eq) and trimethylamine (0.50 mL, 0.0036 mol, 3 eq) were successively added. After stirring for 1h at room temperature, the solvent was evaporated.

Chromatographic purification (PetEt : EtOAc = 1:1) of the residue provided pure compound **3** (0.636 g, 62%) as an yellow solid. (Figure 17.)

Rf: 0.543 (PetEt:EtOAc = 6:4)

1H -NMR ($CDCl_3$, 400 MHz): δ (ppm)= 1.98, 2.02, 2.04, 2.05, 2.08, 2.02 (6s, 24H, CH_3CO), 3.54-3.61 (m, 1H, 5-H), 3.90 (s, 2H, e-H), 4.00-4.34 (m, 5H, 5'-H, 6'-H), 4.81 (d, $J=10.10$ Hz, 1H, 1-H), 4.94-5.11 (m, 3H, 2-H, 3-H, 4-H), 5.39 (t, $J=10.37$ Hz, 1H, 4'-H), 5.45-5.48 (m, 1H, 2'-H), 5.55-5.59 (m, 2H, 1'-H, 3'-H), 7.08 (d, $J=8.08$ Hz, 2H, b,c- H_{ar}), 7.21 (d, $J=8.146$ Hz, 2H, b,c- H_{ar}), 8.14 (s, 1H, NOH).

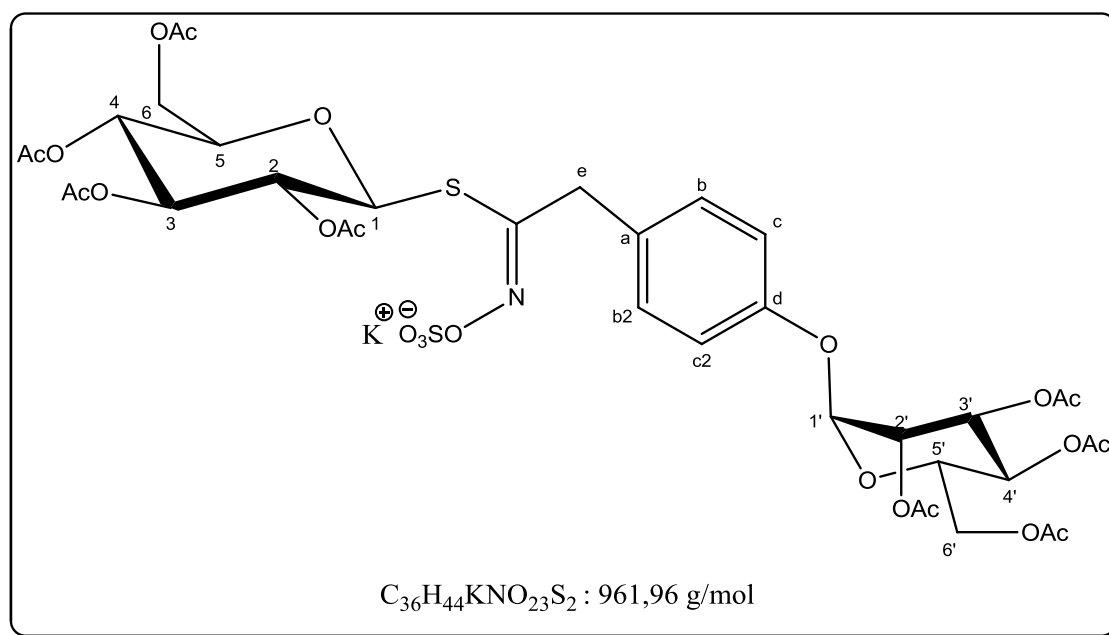
^{13}C -NMR (CDCl_3 , 100 MHz): $\delta(\text{ppm}) = 20.5, 20.54, 20.55, 20.7$ (CH_3CO), 38.0 (C-e), $62.0, 62.3$ (C-6', C-6), 65.9 (C-4'), 68.0 (C-4), 68.9 (C-3'), 69.2 (C-2'), 69.4 (C-5'), 70.1 (C-2), 75.8 (C-3), 76.1 (C-5), 79.4 (C-1), 96.2 (C-1'), 116.9 (C-b, C-b2), 129.4 (C-c, C-c2), 130.3 (C-d), 154.9 (C-a), 169.07 (C-N), $169.30, 169.73, 169.97, 169.99, 170.16, 170.43, 170.56$ (COOCH_3). (Figure 28, given in chapter 6.)

HRMS (ESI $^+$): m/z calculated for $\text{C}_{36}\text{H}_{44}\text{KNO}_{23}\text{S}_2$ ($[\text{M}+\text{H}]^+$): 843.80 , found 844.25 . (Figure 29., given in chapter 6.)



Figure 17. The synthesis of the tetraacetylated-glucomoringin analogue (**13**)

Typical procedure for the synthesis of the *p*-O-acetylated 4'-O-(α -D-mannopyranosyl)glucosinalbin (14**)**

**14**

Sulfur trioxide-pyridine complex (0.7907 g, 4.97 mmol, 7 eq) was added to a solution of compound **13** (0.6 g, 0.71 mmol) in anhydrous DMF (10.65 mL). Reaction was stirred at 55 °C for a 22h. Reaction mixture was then treated with an aqueous solution of $KHCO_3$ (4.97 mmol, 24.85 mL, 7 eq) and then the solvent was evaporated in vacuum.

Chromatographic purification (EtOAc: MeOH = 8:2) of the residue provided the desired compound **14** as white powdery solid (43.20 %). (Figure 18.)

Rf: 0.58 (EtOAc/ MeOH = 8:2)

1H -NMR (400 MHz, DMSO): δ (ppm)= 1.94, 1.95, 1.97, 1.98, 2.032.15 (6s, 24H, CH_3CO), 3.73 (d, $J=11.98$ Hz, 1H, 6- H_a), 3.85-3.98 (m, 4H, 5-H, 6'-H), 3.98-4.11 (m, 2H, 5'-H, 6'-H), 4.13-4.20 (m, 1H, 6- H_b), 4.82 (t, $J= 9.54$ Hz, 1H, 2-H), 4.91 (t, $J= 9.65$ Hz, 1H, 2'-H), 5.18(t, $J=9.88$ Hz, 1H, 4'-H,4-H), 5.26-5.36 (m, 4H, 1-H, 3-H,e-H,3'-H), 5.71(s, 1H, 1'-H), 7.133(d, $J=7.79$ Hz, 2H, b- H_{aryl} ,c- H_{aryl}), 7.31 (d, $J= 7.95$ Hz, 2H, 3-H, 5-H).

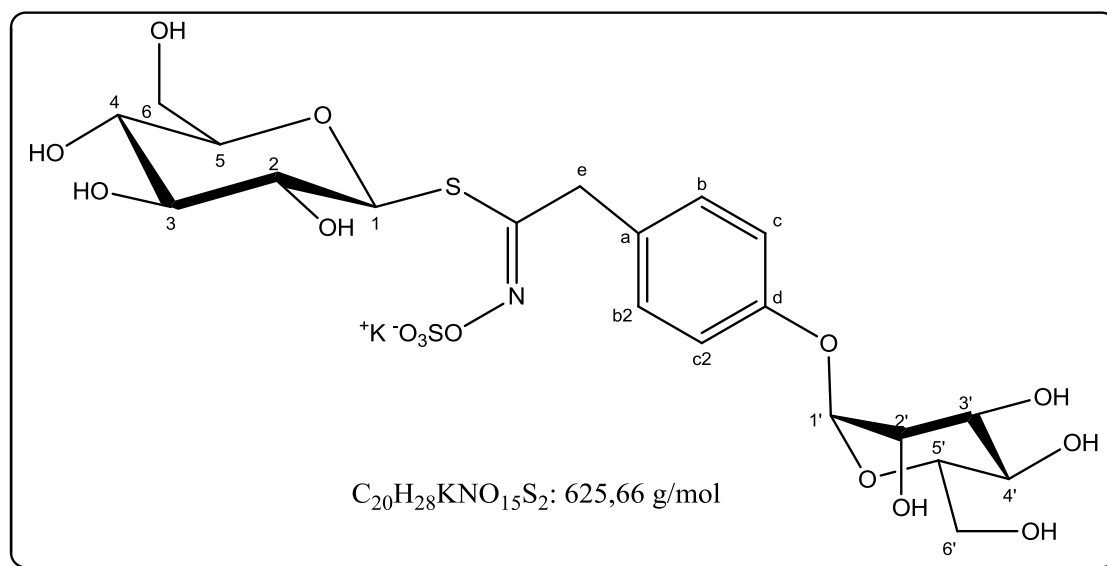
^{13}C -NMR (100 MHz, DMSO): δ (ppm)= 20.67, 20.74, 20.77, 20.88, 20.92, 21.08 (CH_3CO), 61.96(C-6), 62.09 (C-6'), 65.2 (C-4'), 68.12(C-4), 68.85 (C-3'), 68.97(C-2'), 69.09 (C-5'), 69.94 (C-2), 73.21 (C-3), 74.68 (C-5), 78.87 (C-1), 95.82 (C-1'), 117.39 (C-b2, C-b), 129.97 (C-c, C-c2), 130.97(C-d), 152.92 (C-

e), 154.50 (C-a), 169.61, 169.97, 170.01, 170.10, 170.39 (COOCH_3). (Figure 30., given in chapter 6.)



Figure 18. The synthesis of the sulpho-glucomoringin analogue (14)

Typical procedure for deacetylation of sulphate *p*-O-acetylated 4'-O-(α -D-mannopyranosyl)glucosinalbin (15**)**



15

Potassium methoxide (8.6mg, 0.122 mmol, 0.4 eq) was dissolved in MeOH anhyd..(5.2 mL) and added in to a solution of compound **14** (288.58 mg, 0,3mmol) in anhydrous MeOH (5.2mL) after 30 min of stirring at room temperature. The mixture was stirred 5h at room temperature and the solvent was removed under reduced pressure to give pure compound **15** (38.4 mg, 20%) as a white solid after inverse purification with Grace system (column: Reveleris C18 12g, Solvent A: H₂O, Solvent B:MeOH, Flow Rate 20mL/min, UV1:254 nm, UV2:280 nm, ELSD Carrier: Iso-propanol).

Rf: 0.4 (EtOAc : MeOH= 8:2)

¹H-NMR (400 MHz, D₂O): δ (ppm)= 3.15-3.22 (m, 1H, 5-H), 3.22-3.30 (m, 2H, 2-H, 3-H), 3.35 (t, J= 7.68 Hz, 1H, 4-H), 3.60 (s, 2H, 6-H), 3.65-3.77 (m, 4H, 4'-H, 5'-H, 6'-H), 3.96-4.01 (m, 1H, 3'-H), 4.04 (s, 2H, e-H), 4.12 (s, 1H, 2'-H), 4.65 (d, J=8.18 Hz, 1H, 1-H), 5.56 (s, 1H, 1'-H), 7.13 (d, J= 8.07 Hz, 2H, b,b2-H), 7.31 (d, J= 8.07 Hz, 2H, c,c2-H).

¹³C-NMR (100 MHz, D₂O): δ (ppm)= 37.49 (C-e), 60.29 (C-6), 60.68 (C-6'), 66.57 (C-4'), 69.73 (C-4), 69.90 (C-2'), 70.39 (C-3'), 71.78 (C-2), 73.38 (C-5'), 76.97 (C-3), 79.86 (C-5), 81.33 (C-1), 98.34 (C-1'), 117.61 (C-b2,C-b), 129.35

(C-c2, C-C), 129.40 (aryl C-d), 154.83 (aryl C-a), 162.71 (C-N). (Figure 31., given in chapter 6.)

IR: $\nu(\text{cm}^{-1})$ = 3332.16 (-OH), 2925.98 (-C-H), 1588.37 (-C=N), 1414.12 (-C=C), 1106.29 (-C-O). (Figure 32., given in chapter 6.)

3. DISCUSSION

3.1. Retro-synthetic approach of gluconasturtiin and analogue of glucomoringin

β -D-Glucose plays a central role in the biochemistry of the carbohydrates; it is stored in the form of dimers and polymers in much greater amounts than the other monosaccharide, and is thus the most readily available (Figure 19.)⁶.

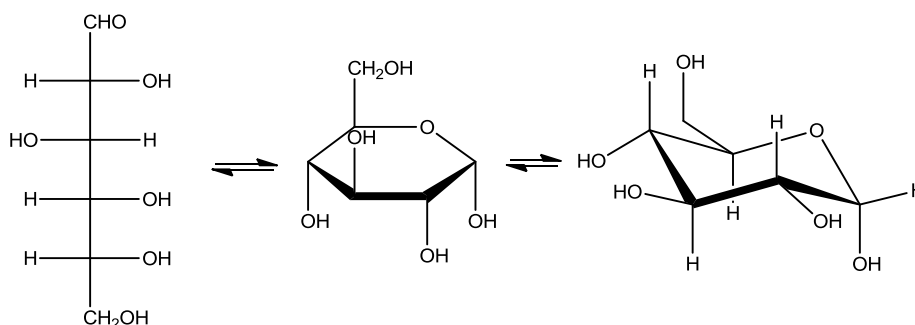
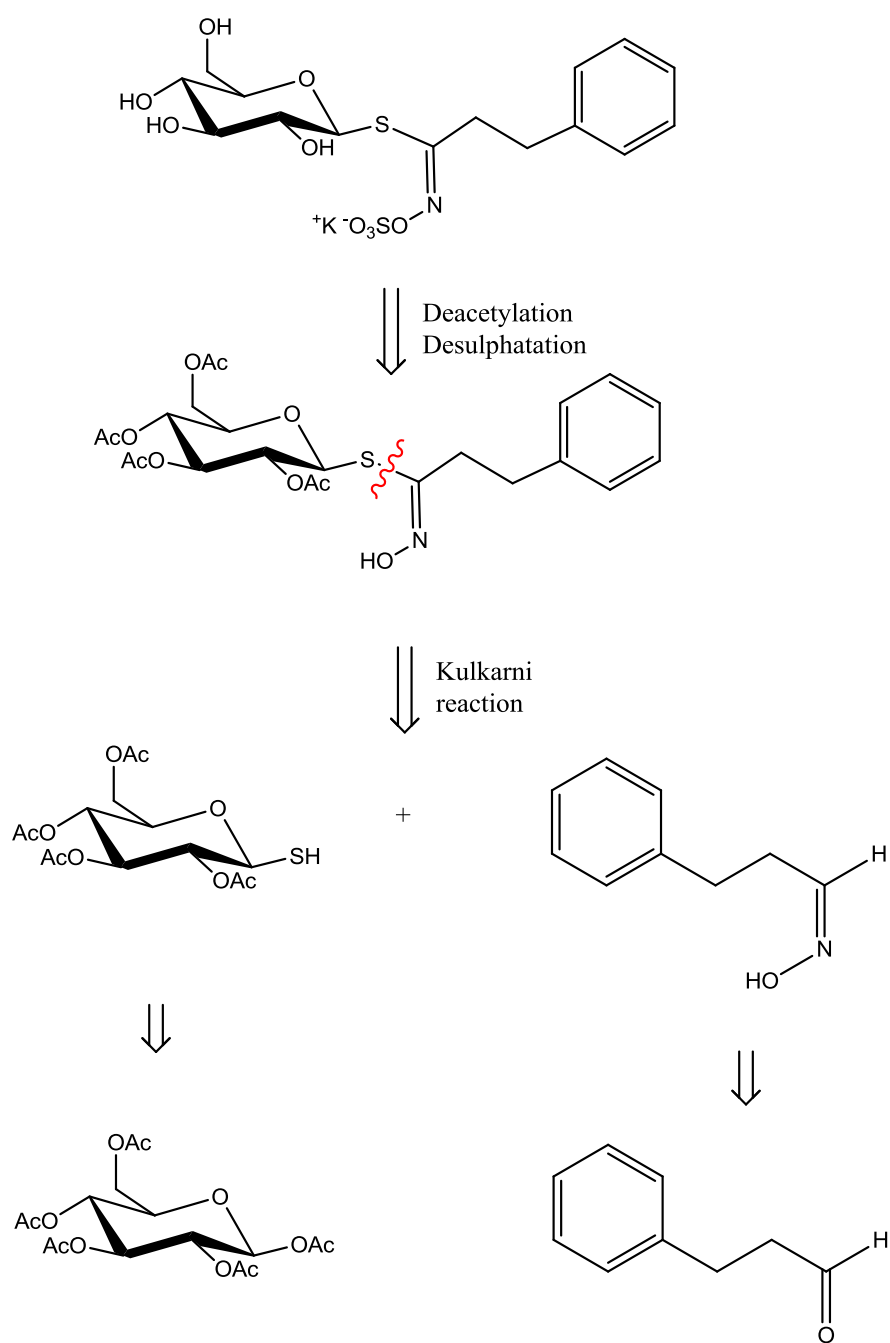


Figure 19. Forms of glucose

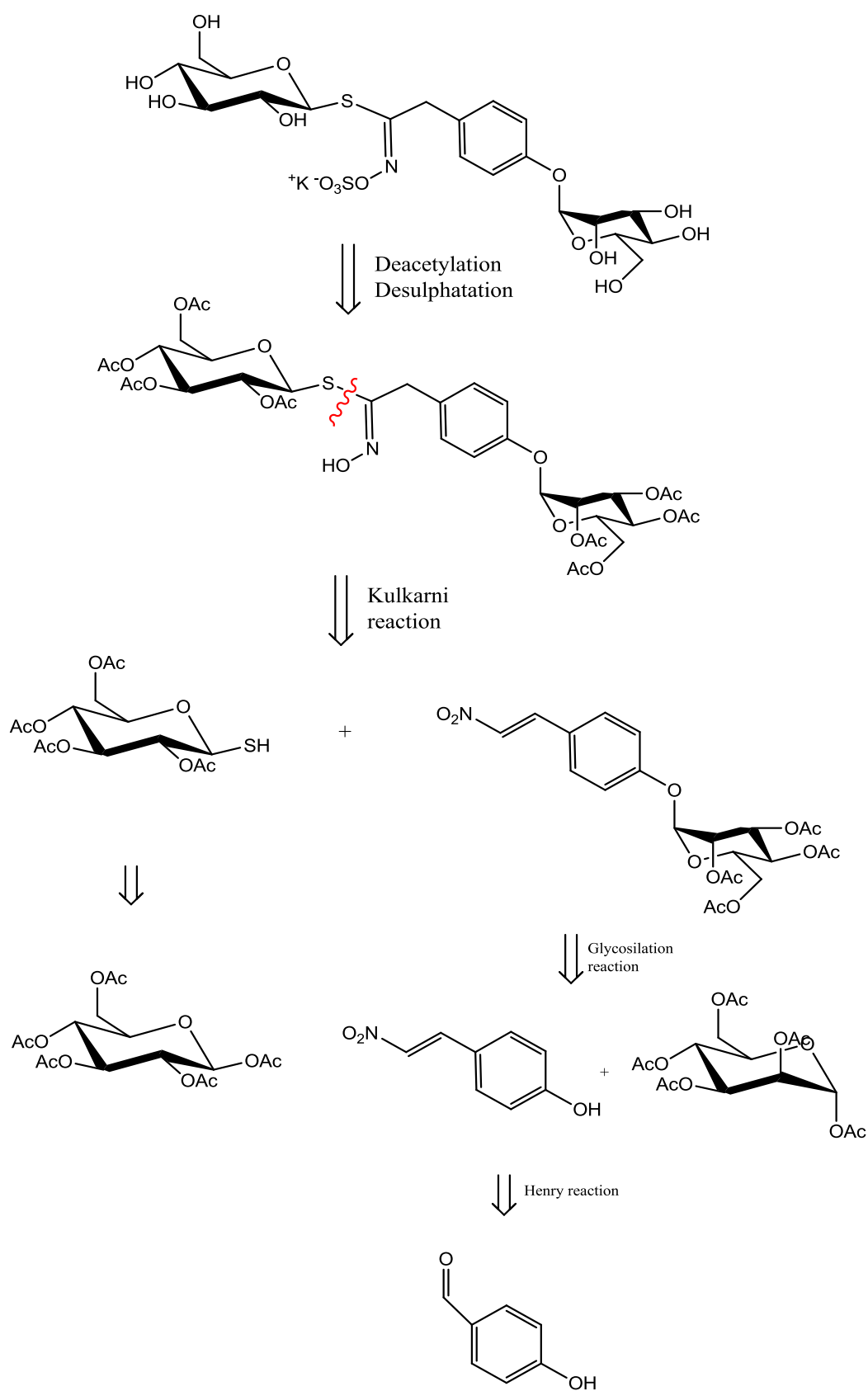
Thioglucose, modified form of β -D-glucose, in many cases was used as the sugar form in the synthesis of the GLs. In the synthesis of the GLs thioglucose produce an S-glucoside intermediate by interaction with oxime. Three major challenges of the glycosylation reaction remain:

- Regioselectivity -that is which particular hydroxyl group of the glycosyl acceptor reacts as the nucleophile
- Stereoselectivity- that is whether the newly formed interglycosidic linkage is specifically α or β
- Efficiency-that is the fact that alcohols are not particularly good nucleophiles, particularly hindered secondary hydroxyl groups of partially protected glycosyl acceptors, can result in often moderate overall yields.

This interaction will be explained by the reaction mechanism for the synthesis of GLs gluconasturtiin and glucomoringine analogue. Retro schemes of this two GL clearly show that thioglucose play a central role for the formation of GLs group (Schemes 13. and 14.).



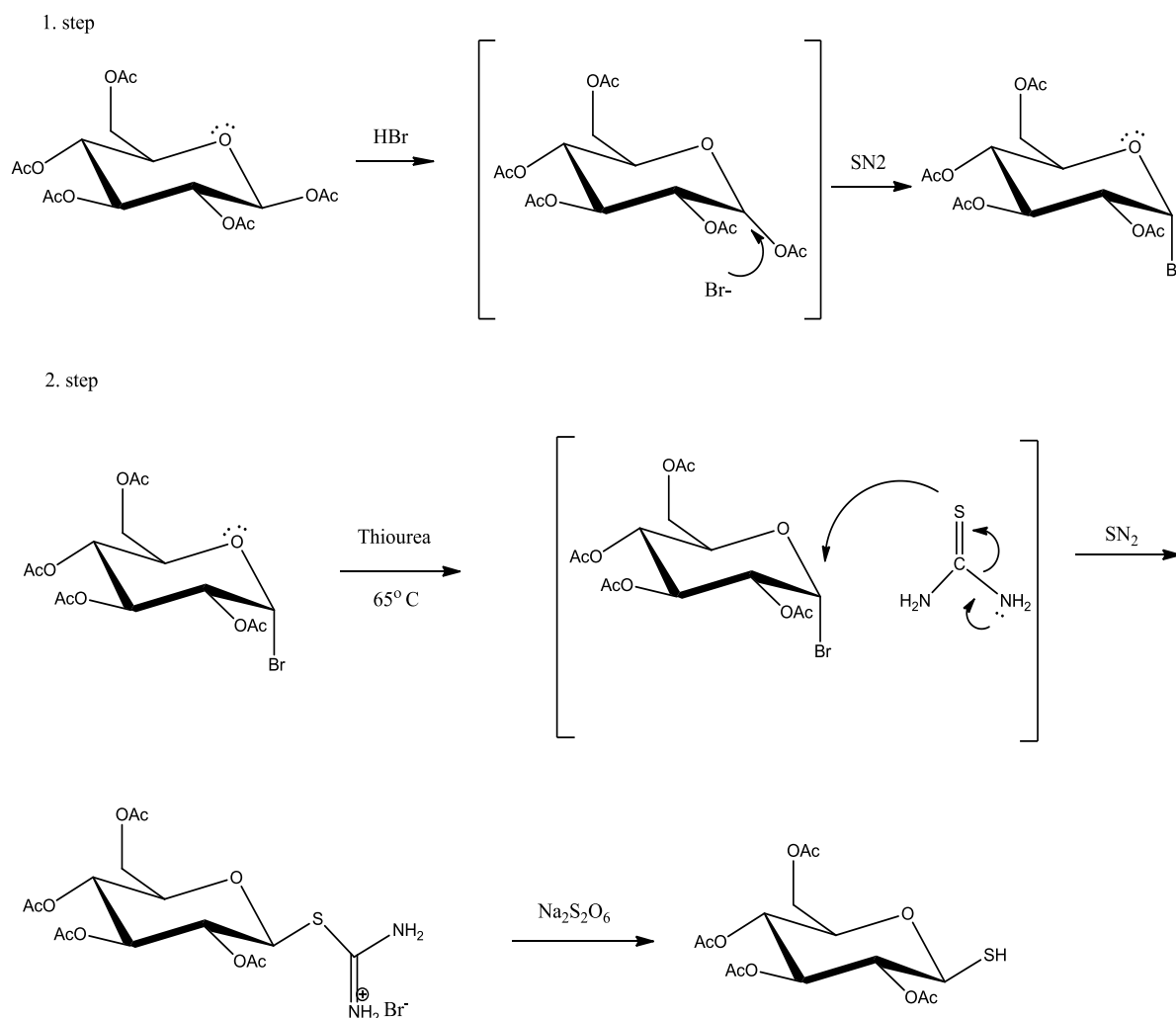
Scheme 13. Retro-synthetic approach of gluconasturtiin



Scheme 14. Retro-synthetic approach of glucomoringin analogue

3.2. The synthesis of tetracetylated β -D-thioglucose

β -D-Thioglucose, is required for both approaches as it represents the sugar unit for each of the desired GLs. The mechanism process for the synthesis of thioglucose was separated in two steps as detailed in Scheme 15.



Scheme 15. The synthesis of the β -D-thioglucose

In the first step a commercial compound of acetylated β -D-glucose was treated with HBr in acetic acid (33%). After aqueous extraction with CH_2Cl_2 solution of collected organic phase was evaporated to produce a crude residue. NMR spectrum shows very pure expected compound and thus it was not necessary to purify it. The yield of the compound synthesized was very good (99%). Under these conditions, bromides are formed exclusively as the α -anomers which are thermodynamically favored by the anomeric effect. ^1H -NMR spectroscopy indicated a coupling constant of 4.0 Hz at 6.62 ppm for H-1 indicating a *vicinal* relationship between the C-1 and C-2 hydrogens. Glycosyl bromides react readily with good nucleophiles. In second step thiolate anion of thiourea allows the β -formation of the thioglycosides, and gives tetraacetate thioglucose in a good yield (85%). Synthesis of thioglucose was performed twice in order to get a higher amount of the product which is necessary for further reactions. Reaction conditions contributed to the almost the same yield which is presented in Table 2.1.

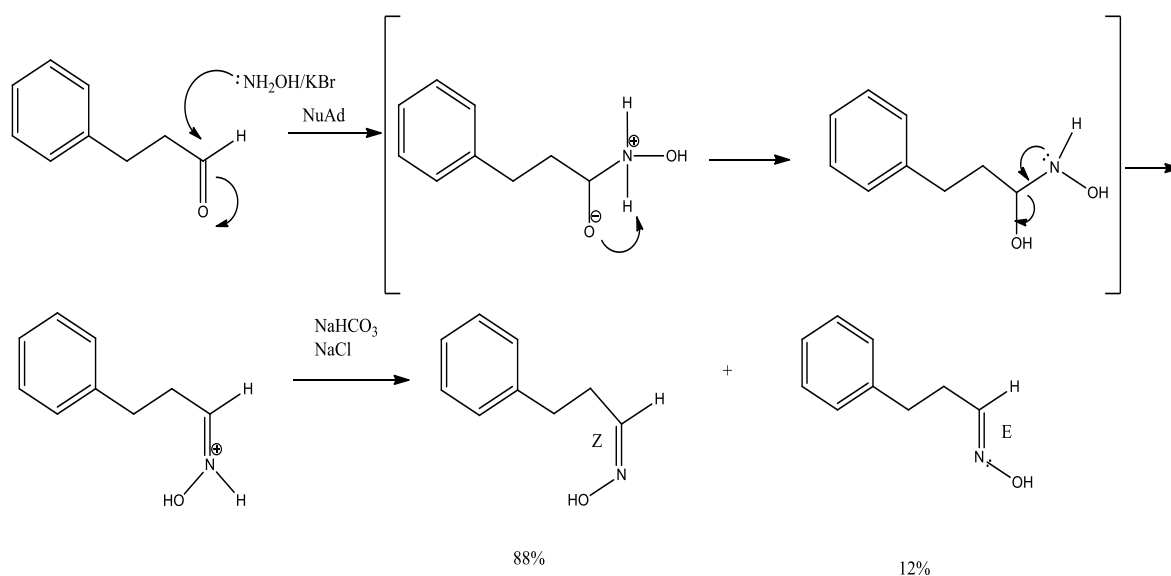
Table 2.1.

Reaction	mol of pentaacetate β -D-glucose	Eq of thiourea	Yield(%)
1.	0.0256	1.6	84
2.	0.0254	1.6	85

3.3. The synthesis of gluconasturtiin

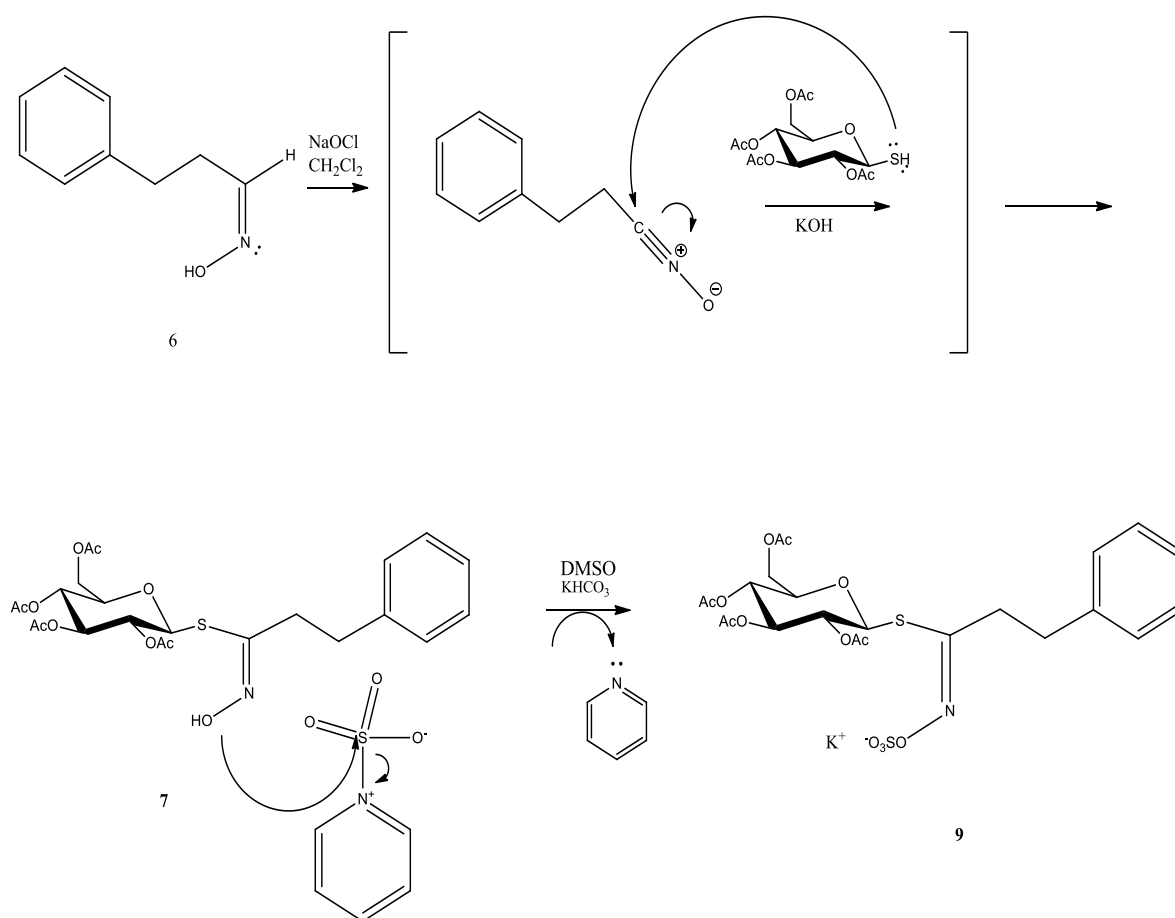
According to retro reactions ie. hydroxamate disconnection, synthesis of gluconasturtiin is based on the 1,3-addition of a nitrile oxide. Because of their high instability, nitrile oxides have to be generated *in situ* from hydroxymoi-chloride precursors through a 1,3-elimination under basic conditions.⁴ The key intermediate in the reaction is in fact the hydroxyl chloride, this halogenation represents an electrophilic substitution of the oxime (Scheme 17).

Under basic condition at room temperature, NH_2OH in hydrobromic acid by Nu addition on aldehyde group, produced an intermediate Schiff base, which produced the oxime. On the NMR spectrum a peak from *Z*- and *E*- configuration is visible. In this case about 88% of *Z*- configuration, and 12% *E*- configuration of molecule was produced (Scheme 16.).



Scheme 16. Mechanism for the synthesis of oxime: 3-phenylpropanal oxime

Without purification of oxime, hydroxymoiil-chloride can be isolated and subjected to dehydrohalogenation by slow addition of a base such as triethylamine. This addition allows a corresponding nitrile oxide as electrophilic acceptor to react with 1-thio- β -D-glucopyranose producing the anomeric (Z)-thiohydroximate intermediate in good yields. O- Sulphation with sulphur trioxide pyridine complex, followed by pyridine displacement with KHCO_3 produced expected tetraacetylated-gluconasturtiin (Scheme 18).



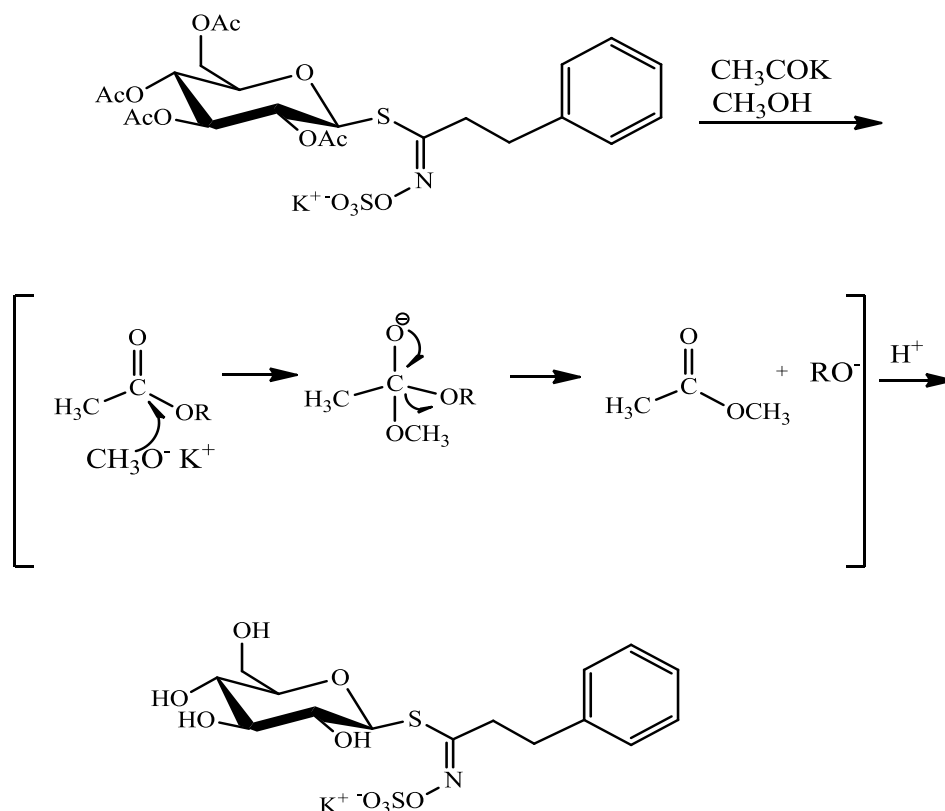
Scheme 17. Reaction mechanism for the synthesis if the tetraacetylated gluconasturtiin

The reaction of sulphation was one of the most problematic performing reactions, not due to the reaction conditions but because of the large lost yield of the obtained product. A Table 2.2 shows the intensive difference in the yield of the performed reactions, although the conditions of many reactions were the same. It was discovered that the solubility of sulphate tetraacetylated-gluconasturtiin (**9**) was unexpectedly poorly soluble in MeOH. This solvent is one of the most polar solvent used for the purification after addition of potassium in sulphate group which is presumed to affect the final yield. Reaction was repeated several times and it was possible to see the significant difference between the quantities of final sulphate-products. Further investigation showed that a molecule is completely soluble in a mixture of acetone and methanol (1mg in 0.1 ml of Acet:MeOH=1:1). Reaction 1, 5 and 6 have been performed using this mixture which resulted in much higher yield. These results have proven an assumption that the used solvent influenced the final yield. In addition the pyridine complex which was used was probably exposed to moisture what is not allowed for this reaction which is performed in inert atmosphere.

Table 2.2

Reaction	Eq of P.Complex	Time	Yield / %
1.	5	All night	78.29
2.	5	All night	23.82
3.	5	All night	27.17
4.	10	3days	36.22
5.	7	4days	76.50
6.	5	All night	41.00

Finally, standard de-O-acetylation produced the expected gluconasturtiin (Scheme 18).



Scheme 18. Standard de-O-acetylation process

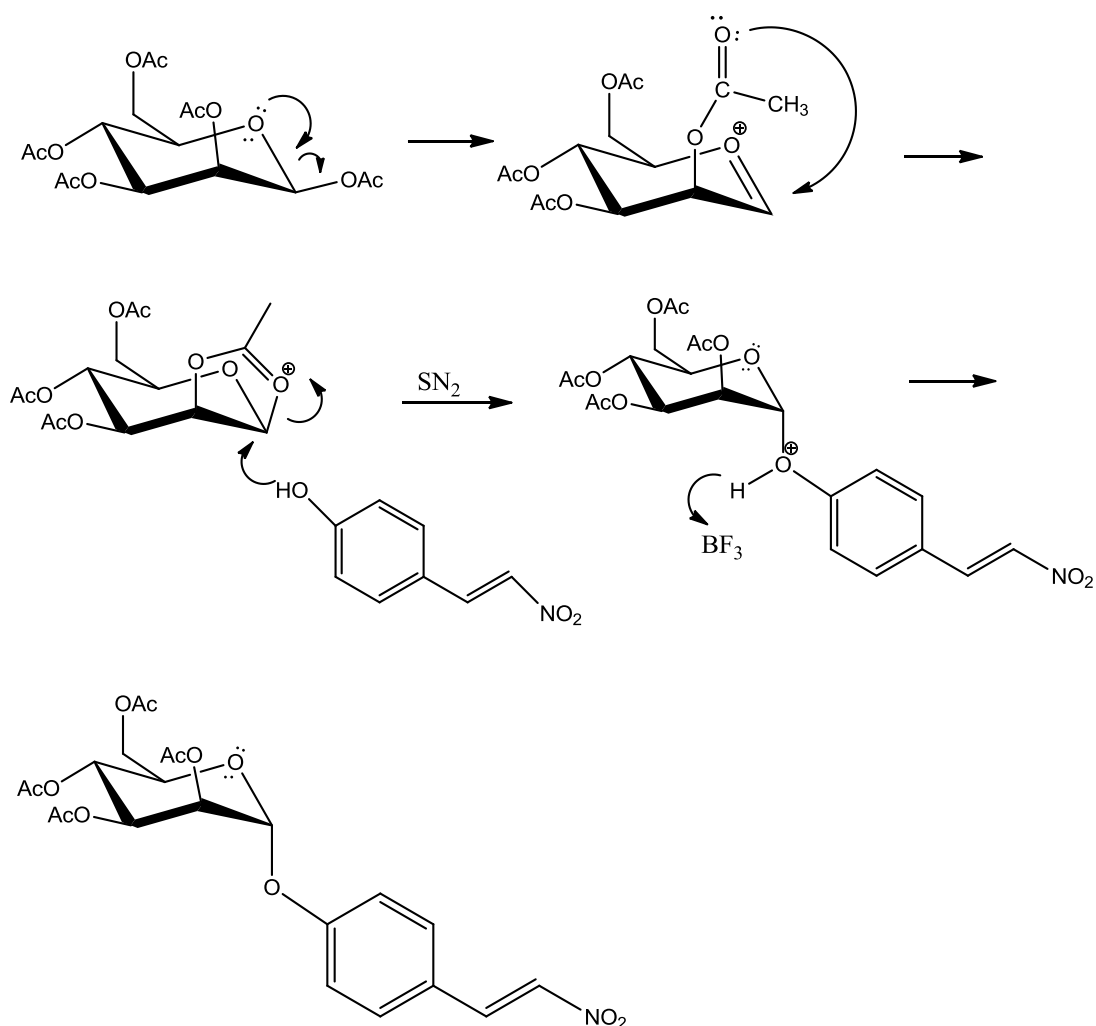
In HPLC analysis (Figure 26., given in chapter 6.) and NMR spectrum (Figure 24., given in chapter 6.) small impurities were noticed so the product was purified by inverse Grace system to allow very a pure compound. ^1H and ^{13}C spectrum were confirmed by correlation in COSY and HSQC spectrum (Figure 24. given in chapter 6.). Also product was recorded on an IR spectrum (Figure 27. given in chapter 6.). This spectrum corresponds to one found in literature with characteristic broad peak around 3270.70 cm^{-1} which is less pronounced then glucomoringin analogue which has more OH groups. Recording the angle of rotation by polarimeter unfortunately was not done because a solution of this compound was not clear.

Gluconasturtiin was used as a standard after isolation of the same GL from plant material. It was shown that both spectrum of inverse

chromatographic system correspond to the same GL (Figure 25. given in chapter 6.).

3.4. The synthesis of glucomoringin

One of the most useful ways to control the stereochemistry of the newly formed anomeric bond is by neighboring group participation of an ester protecting group, such as an acetate group. After the first step we can see that the participation of the carbonyl oxygen of the acetate protecting group on the 2 position of mannose may stabilize the intermediate glycosyl cation by cyclisation. Cyclic oxonium ion formed in this way is opened for S_N2 substitution of *p*-hydroxystyrene as nucleophile, which produces an α -anomer ie. *p*-nitrostyryl glycoside molecule (Scheme 19.).



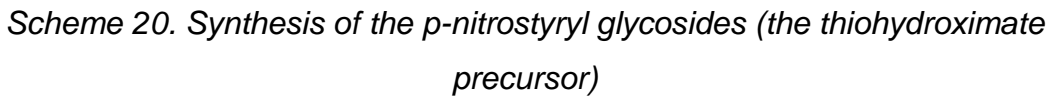
Scheme 19. Synthesis of the *p*-nitrostyryl glycoside

A method that avoids the conventional chlorination of *p*-nitrostyryl glycoside is the reaction with titanium tetrachloride, under Lewis acid activation with triethylsilane as the source of the hydride ion to produce hydroxymoyl chloride (Scheme 20.). Those electrophilic acceptors were reacted with 1-thio- β -D-glucopyranose to produce the anomeric thiohydroximate intermediate. Reaction was generated by *in situ* process. Forming of thiohydroximate intermediate was confirmed by TLC system. On TLC (using mobile phase MeOH:AcOEt= 8:2, and 9:1 with H₂SO₄/EtOH as a visualisation agent) four spots, with different R_f values were observed. Only spot which marks a thiohydroximate compound is UV visible which made purification easier, *i. e.* the tubes collected were only the ones that shows UV spot on TLC. The presence of 4 spots indicating different product explained the loss in yield during reaction 1 and 3 after purification system (Table 2.3.).

NMR spectrum showed an enough pure compound prepared for a next step. To confirm a molecule structure a MS spectrum by MS-FIA system was made where M⁺ spectrum showed a correct molecule mass (Figure 29. given in chapter 6.).

Table 2.3.

Reaction	Mol <i>p</i> -nitrostyryl glycoside	Eq of thioglucose	Yield after pu.
1.	0.0012	1.2	63%
2.	0.0018	1.2	57%
3.	0.0012	1.2	34 %

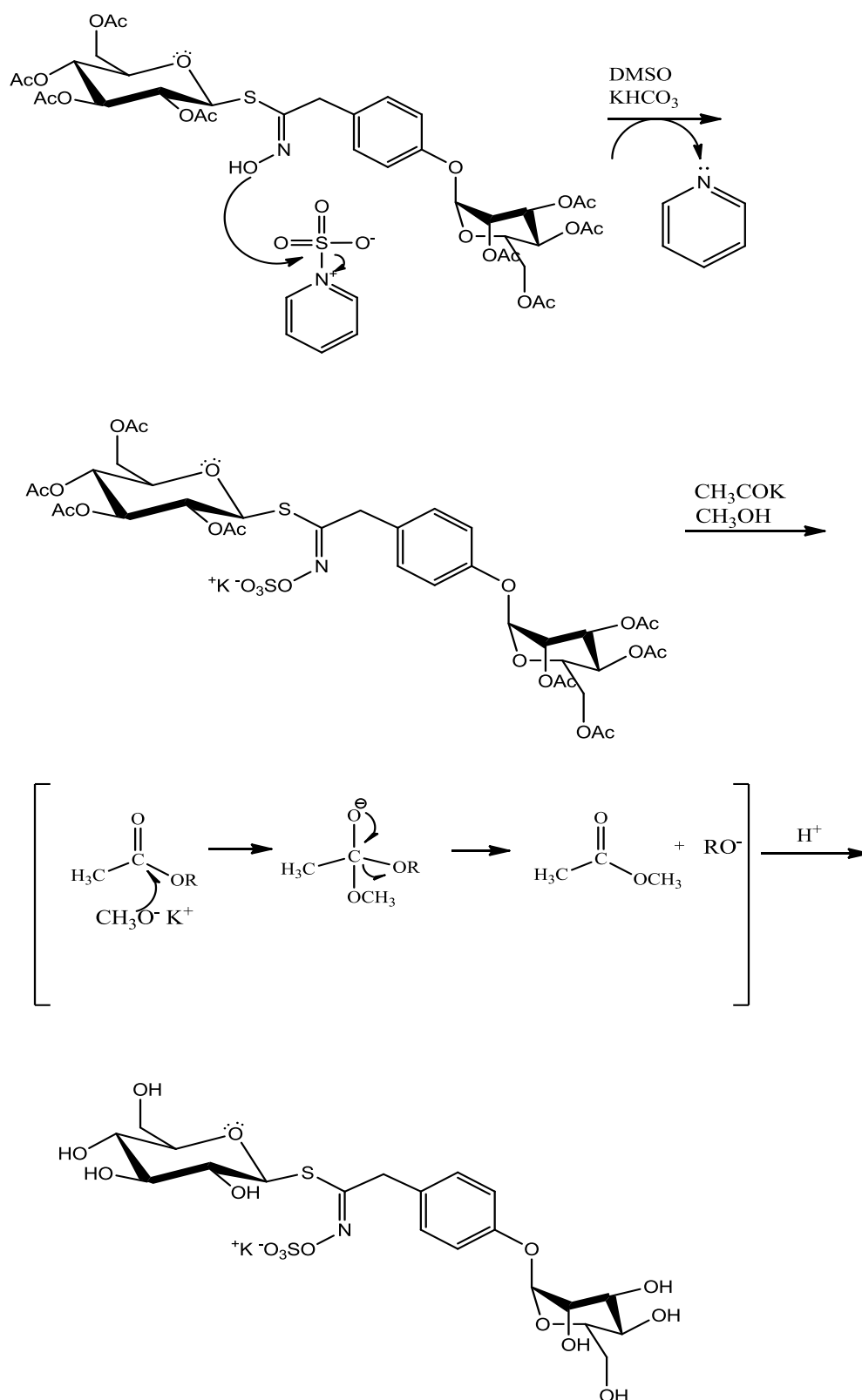


O-sulphation with sulphur trioxide pyridine complex, followed by pyridine displacement with KHCO_3 and standard de-O-acetylation delivered the expected analogue of glucomoringin (Scheme 21.). Reaction of sulphation, as in gluconasturtiin case presented a big challenge. As is to be expected acetylated glucomoringin analogue was formed, which was confirmed by NMR spectrum. ^1H and ^{13}C spectrum were confirmed by correlation in COSY and HSQC spectrum (Figure 32. given in chapter 6.). Even after purification was possible to see a huge peaks of DMF solvent on NMR spectrum. It was very hard to remove this solvent but by washing it with a large volume of toluene due to the azeotropic effect, the removal was successful.

The previous work on this analogue is scarce, and so, there are little information of this compound. In procedure by Rollin *et. al.*, a very high yield of the acetylated analogue of glucomoringin was formed, which did not happen in this case. Generally, it seems that reaction is favored to be performed in small amounts. If performed in higher scale the condition of chemicals stability are of huge importance and it could be a reason for lower yield of expected compound in our case (Table 2.4.).

Table 2.4.

Reaction	Eq of P.Complex	Time reaction	Yield
1.	7	22h	43 %
2.	5	3 days	32%
3.	5	4 days	0%



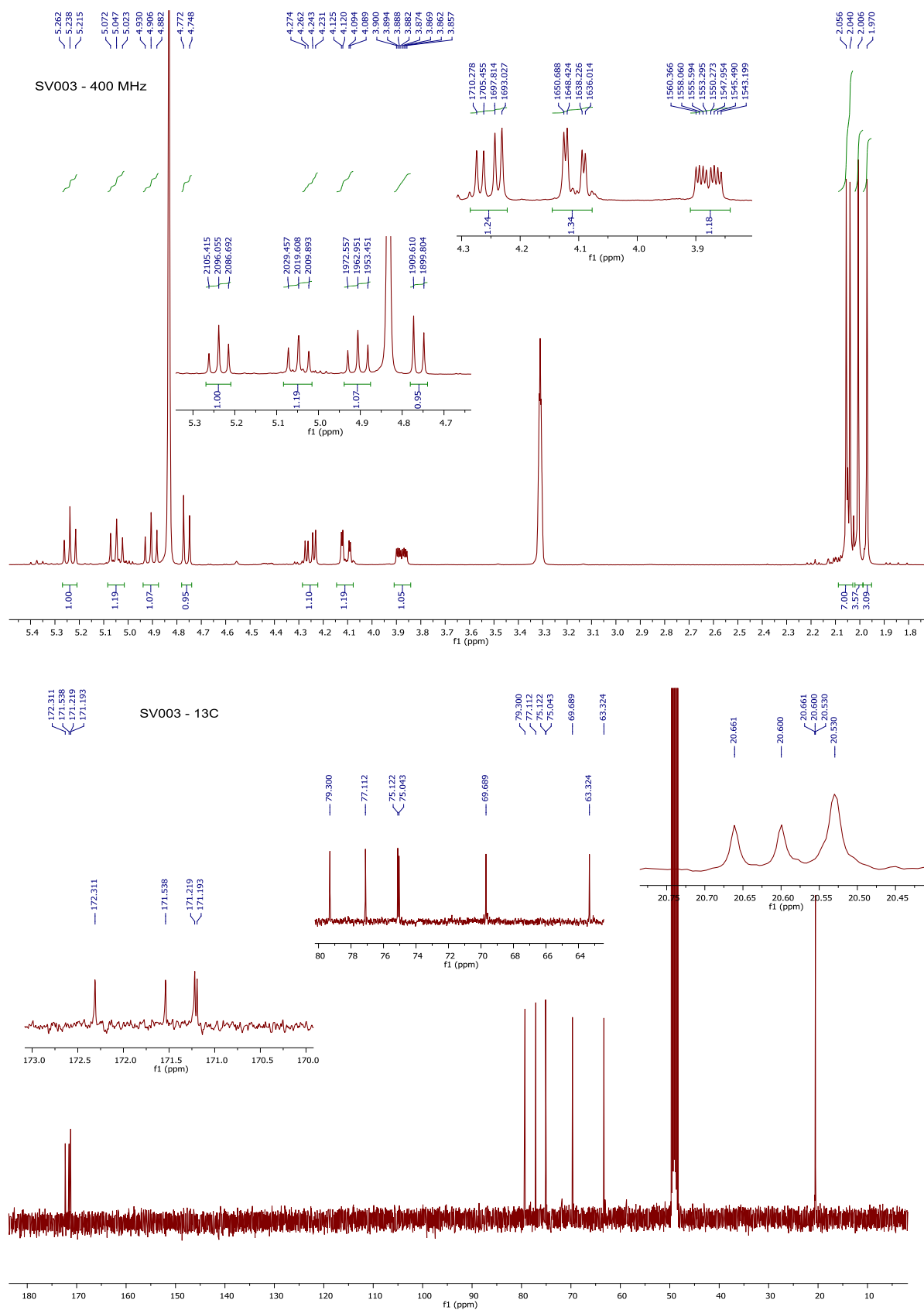
Scheme 21. Reaction mechanism of synthesis de-acetylated analogue of glucomoringin

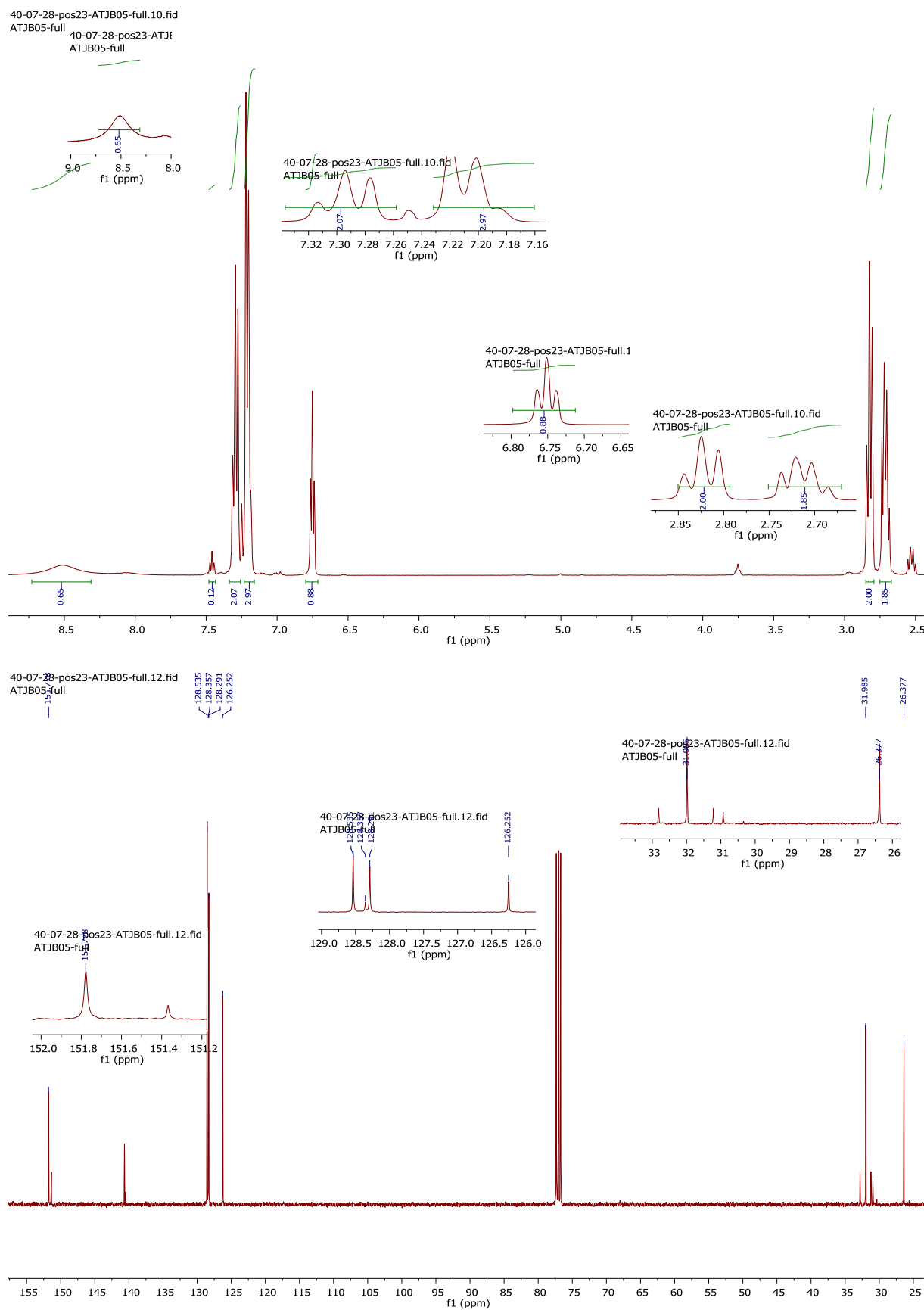
- Two different glucosinolates has been synthesized in acceptable yields: natural - gluconasturiin (**9**, 51 %), and another one unnatural - glucomoringine-4'-O-(α -D-manopyranosyl)glucosinalbin (**15**, 20%).
- The synthesis of thioglucose is the first step of obtaining the products **9**, and **15**. Although the reaction was not difficult to perform, it was noticed that when using a larger amounts of the reactants the yield decreased ca 15% (data not shown).
- In order to synthesize a gluconasturtiin aldoxime pathway was used followed by hydroxamate disconnection. Reaction of hydroxyl chloride with 1-thio- β -D-glucopyranose tetraacetate produced corresponding thiohydroxamate intermediar (**7**) in a high yield (95%).
- In synthesis of gluconasturtiin the purification conditions of sulphation reactions were optimized to obtain satisfactory yield. The compound (**8**) is produced in a low yield if only methanol was used as a solvent (from ca. 23 to 41%). Part of this compound was found in the crude precipitate, probably due to its weak solubility in MeOH. When a less polar solution of Acetone and MeOH (1:1) was used, the compound **8** was not lost in the precipitate, and this afforded a pure compound **8** without using a column chromatography (ca. 77%).
- Reactions for the synthesis of analogue of glucomoringin were followed by losing yields in almost every step. First problem was in the reaction for the synthesis of nitrovinyl derivate (**12**). The yield of the reaction product of hydroboration was decreasing depending of the time reaction: for 1 day (43%), 3 days (32%) and when the reaction was left to run 4 days no product was obtained (Table 2.4). It can be concluded that long time of the reaction does not benefit formation of the product. In table 2.2 it is also possible to notice the time of reaction influence the yield of the product, and thus it can be hypothesized that it is better to perform the reactions with shorter time period.
- This sulphation procedure due represented the most problematic step in both cases to obtain the desired sulphated compounds and should be optimized in the future.

- The spectroscopic data (^1H NMR, ^{13}C NMR, COSY, HSQC) confirmed the structure of all the synthesized products.

1. Ivica Blažević; *Free, glucosinolate degradation and glycosidically bound volatiles of plants from Brassicaceae family, doctoral thesis, University of Zagreb, Faculty of Science, (2009)*
2. Jasna Brekalo, *Composition of GLs of plants from Brassicaceae family: Dilpitaxis eruroides (L.) DC and Fibigia triquetra (DC), University of Split, Faculty of Chemistry and engineering, (2013)*
3. Susan Elizabeth Cobb; *The synthesis of natural and novel glucosinolates, doctoral thesis, University of St. Andrews, (2012)*
4. Patrick Rollin, Arnaud Tatibouët; *Glucosinolates: The syntetic approach, Comptes Rendus Chimie, 14 (2011) 194–210*
5. David Gueyrard, Renato Iori, Arnaud Tatibouët and Patrick Rollin; *Glucosinolate Chemistry: Synthesis of O-Glycosylated Derivates of Glucosinalbin, University of Orleans, (2010)*
6. Milan Dekić; *Phytochemical examination of selected species Brassicaceae and Geraniaceae family, doctoral thesis, University of Niš, Faculty of Science, (2011)*
7. Peter C. Collins, Robert J. Ferrier; *Monosaharides - Their Chemistry and their roles in natural products, Wiley, 1995*
8. D.D. Perrin, W. L. F. Armarego and D. R. Perrin, *Purification of Laboratory Chemicals, Pergamon, Oxford, 1986*

6. SUPPLEMENTARY MATERIAL

Figure 20. NMR spectrum of β -D-thioglucose



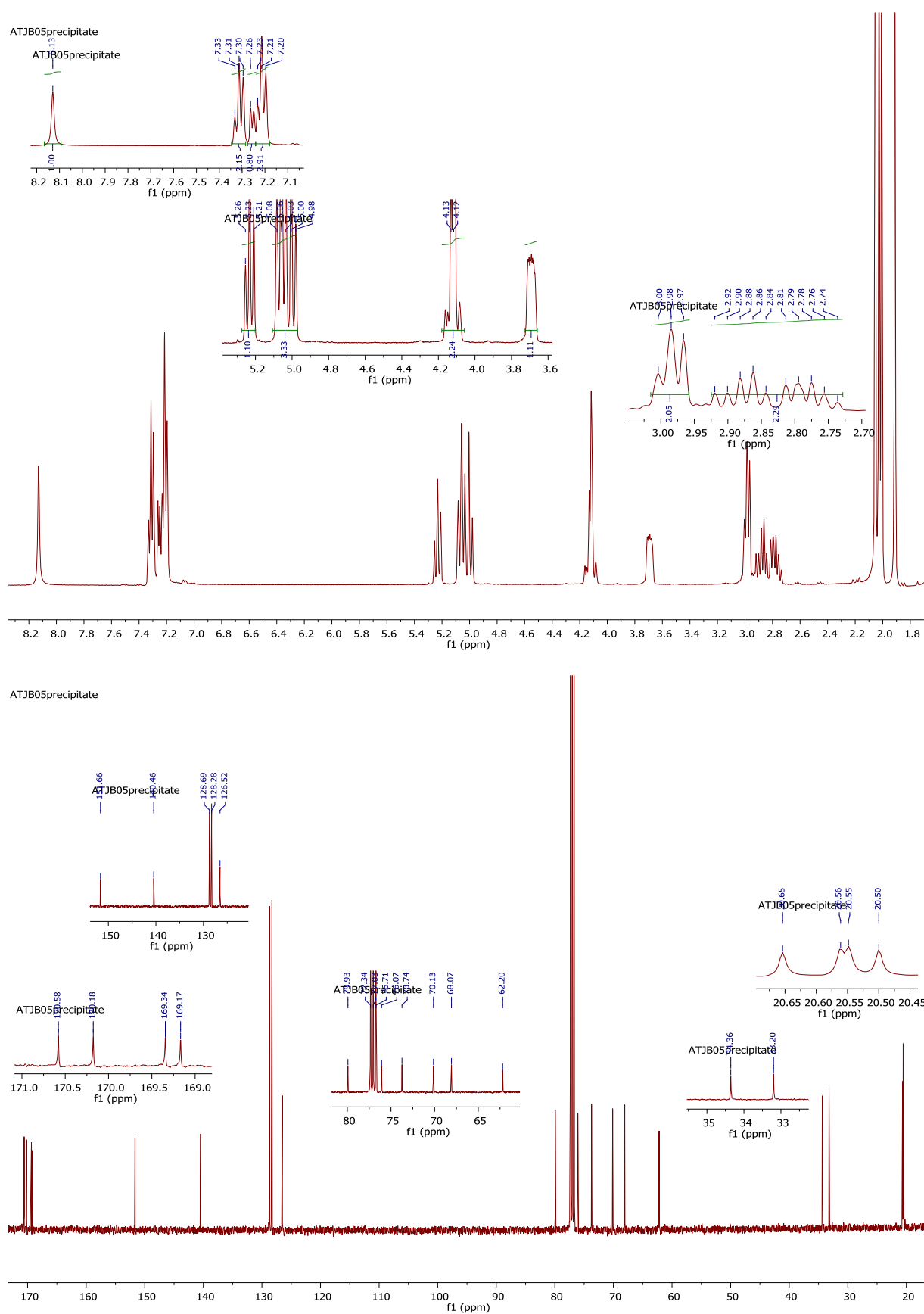


Figure 22. NMR spectrum of desulpho tetraacetylated-gluconaturtiin

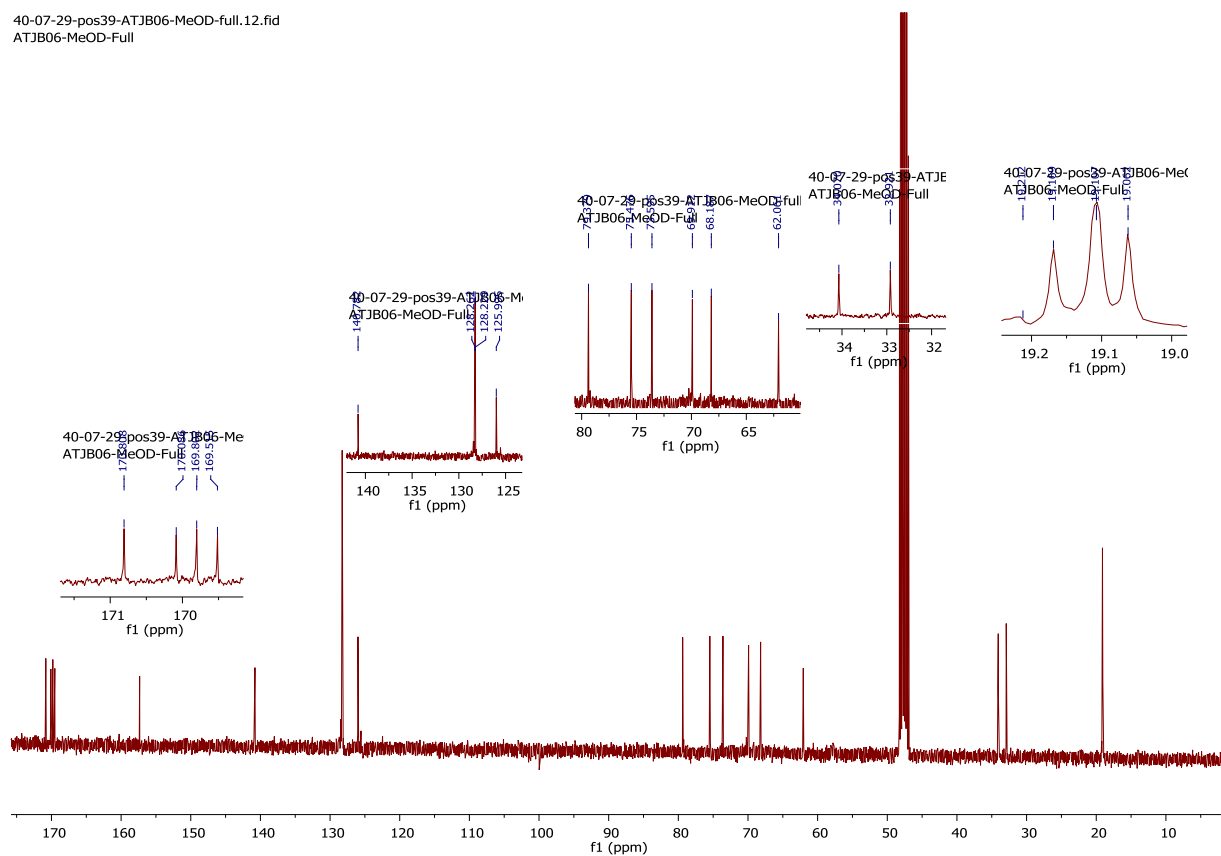


Figure 23. NMR spectrum of tetraacetylated gluconasturtiin

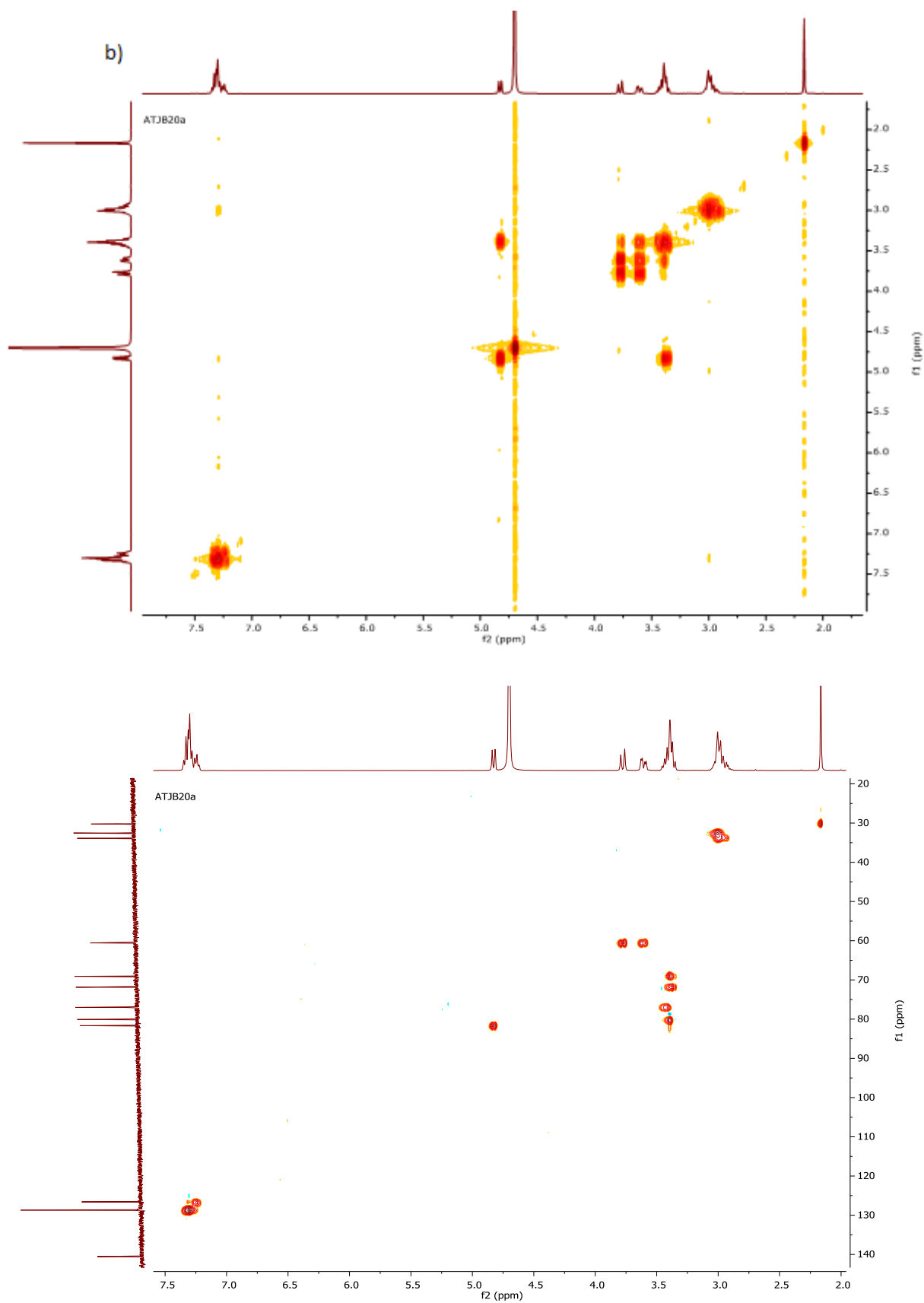


Figure 24. NMR spectrum of gluconasturtiin: a) 1D-NMR (^1H , ^{13}C)

b) 2D-NMR (COSY, HSQC)



Method Name: JB20 assay 2
Run Name: JB20 assay 400 mg
Run Date: 2015-07-22 09:44

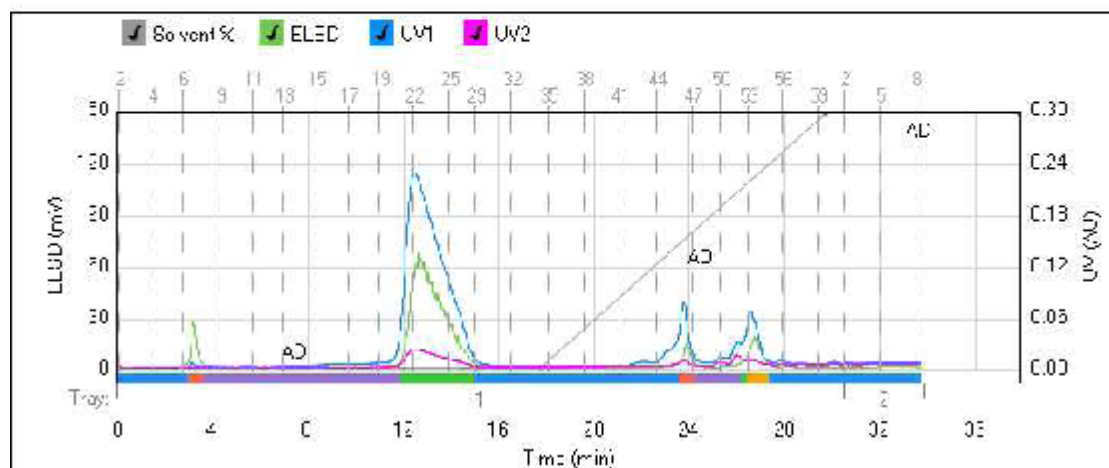
standard material

Column: Reveleris® C18 30g
Flow Rate: 40 mL/min
Equilibration: 3.7 min
Run Length: 38.0 min
Air Purge Time: 0 min

Slope Detection: Off
ELSD Threshold: 3 mV
UV Threshold: 0.05 AU
UV1 Wavelength: 227 nm
UV2 Wavelength: 324 nm

Collection Mode: Collect All
Pre-Vial Volume: 20 µL
Post-Vial Volume: 20 µL
Injection Type: Manual

ELSD Carrier: Iso-propanol
Solvent A: H₂O/MeOH 99/1
Solvent B: Water
Solvent C: i-Propanol
Solvent D: Methanol



Method Name: SFAT122 assay 3 C18
Run Name: SFAT122 assay 2 g
Run Date: 2015-07-22 10:52

plant material

Column: Reveleris® C18 30g
Flow Rate: 60 mL/min
Equilibration: 3.7 min
Run Length: 42.6 min
Air Purge Time: 0 min

Slope Detection: Off
ELSD Threshold: 3 mV
UV Threshold: 0.05 AU
UV1 Wavelength: 227 nm
UV2 Wavelength: 324 nm

Collection Mode: Collect None
Pre-Vial Volume: 20 µL
Post-Vial Volume: 20 µL
Injection Type: Manual

ELSD Carrier: Iso-propanol
Solvent A: H₂O/MeOH 99/1
Solvent B: Water
Solvent C: i-Propanol
Solvent D: Methanol

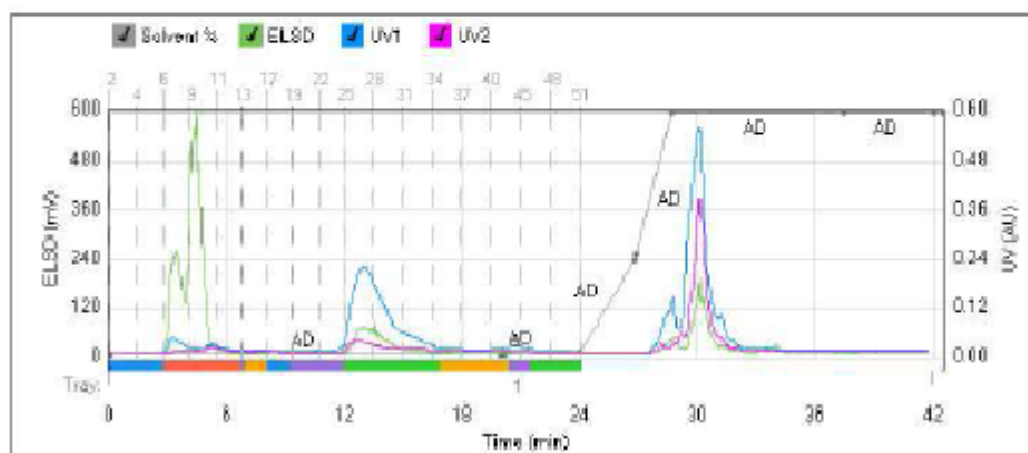


Figure 25. Comparison of the synthesized gluconasturtiin and GL from plant material

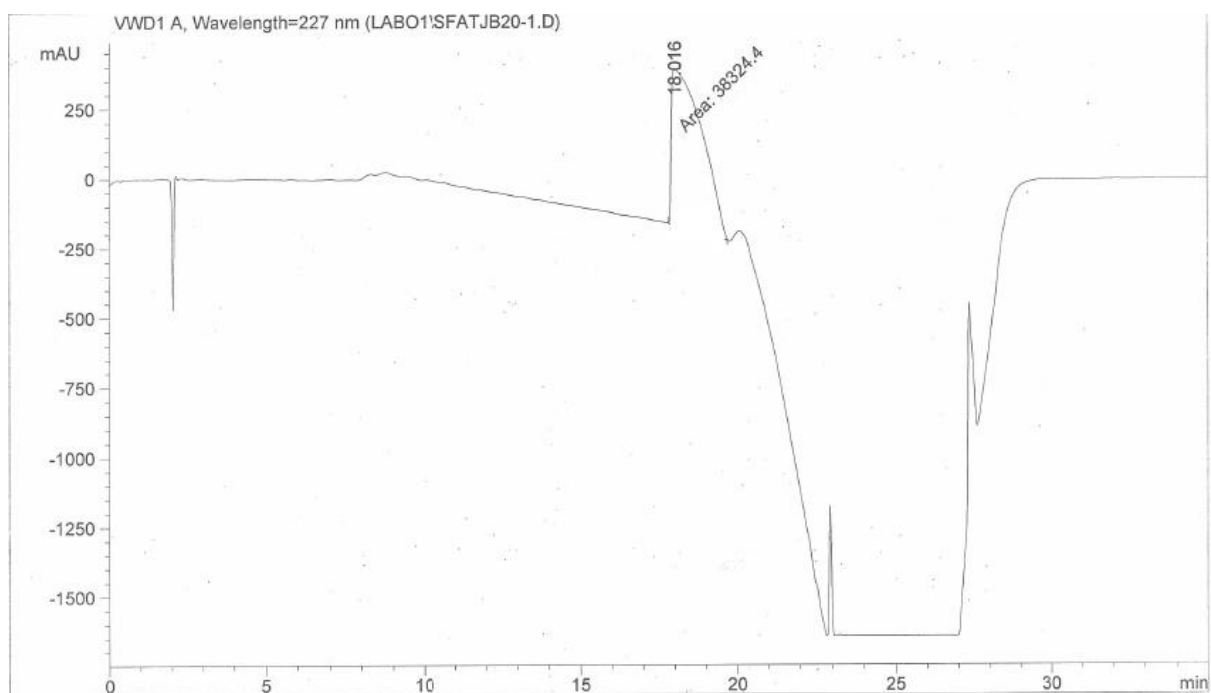


Figure 26. HPLC spectrum of gluconasturtiin

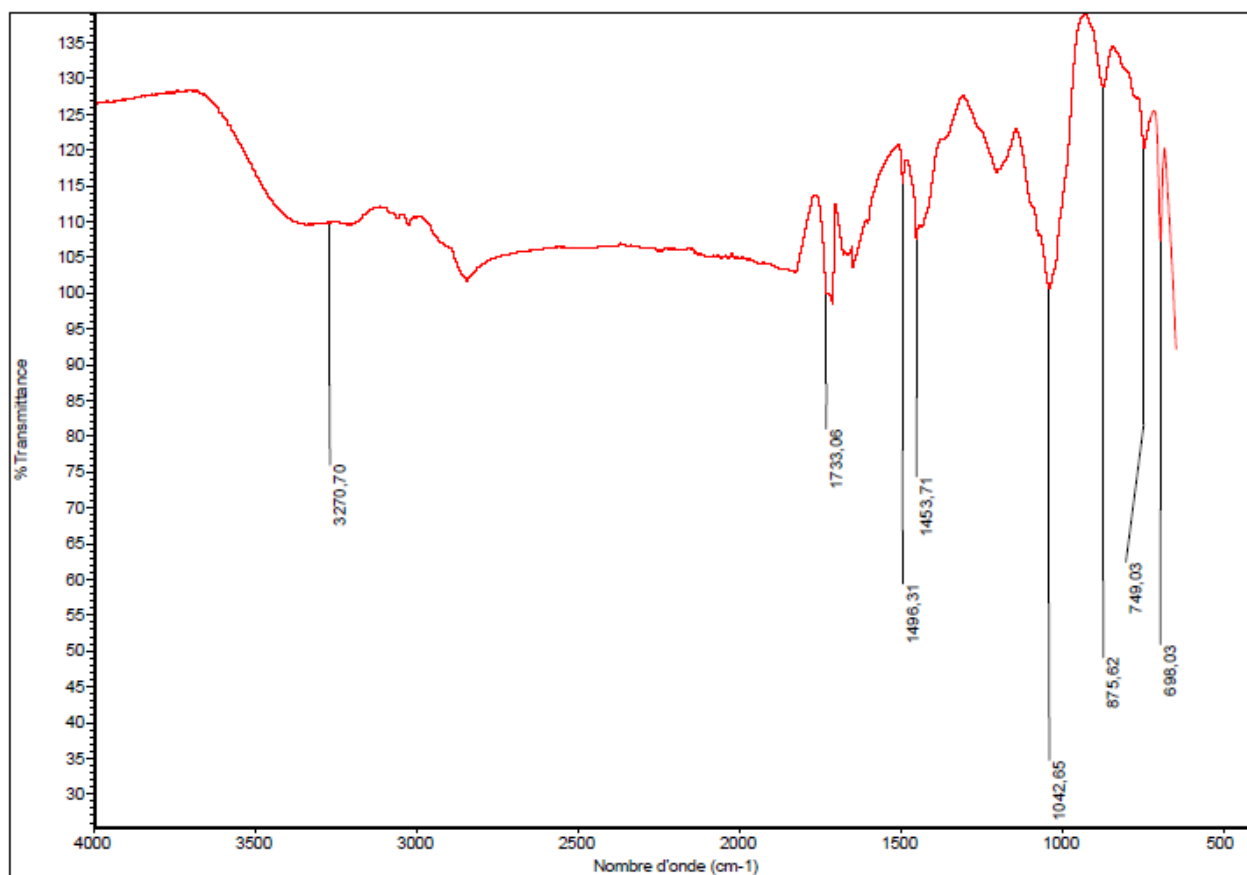
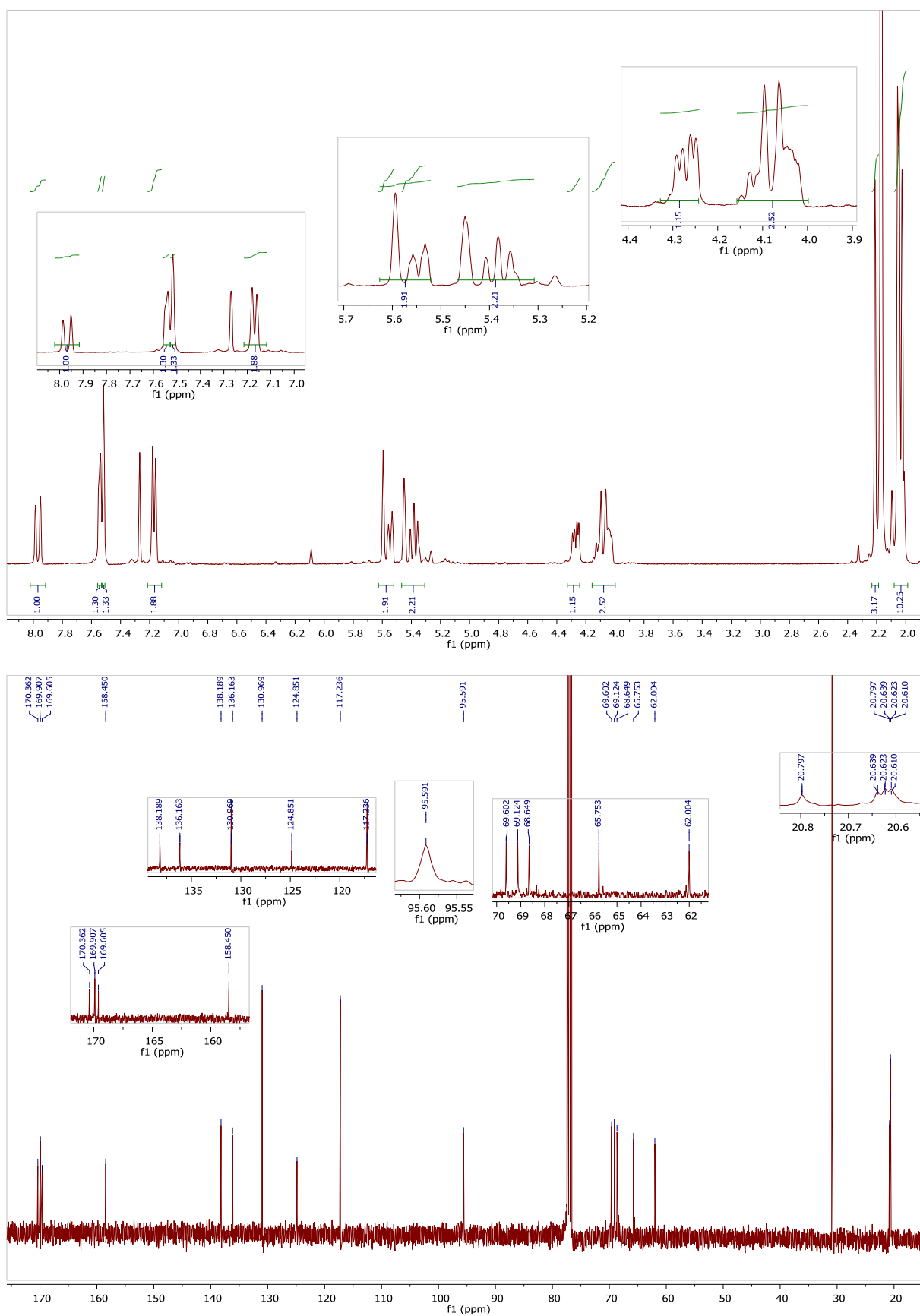


Figure 27. IR spectrum of gluconasturtiin

Figure 28. NMR spectrum of *p*-nitrostyryl glycosides

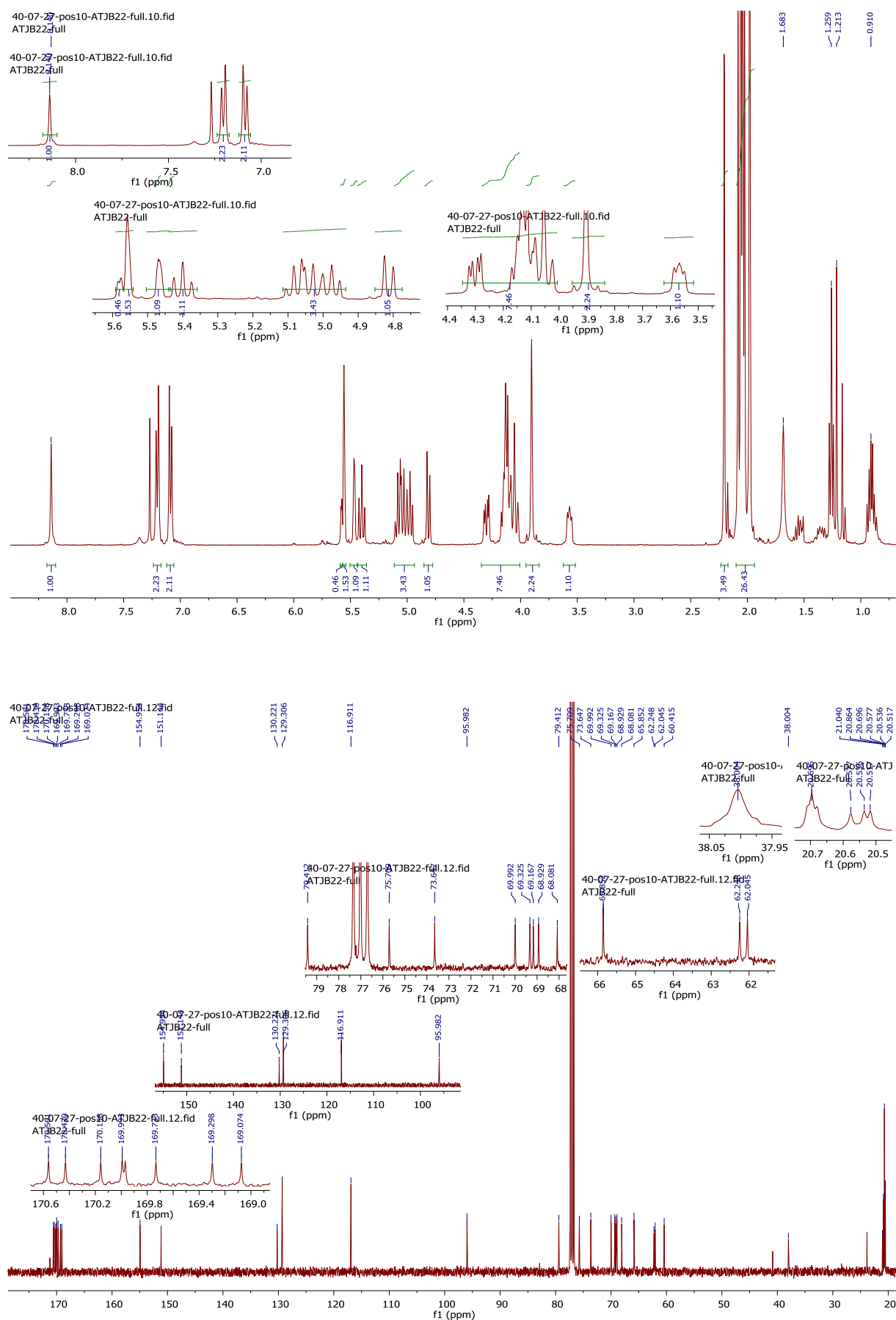


Figure 29. NMR spectrum desulpho tetracetylated analogue of glucomoringin

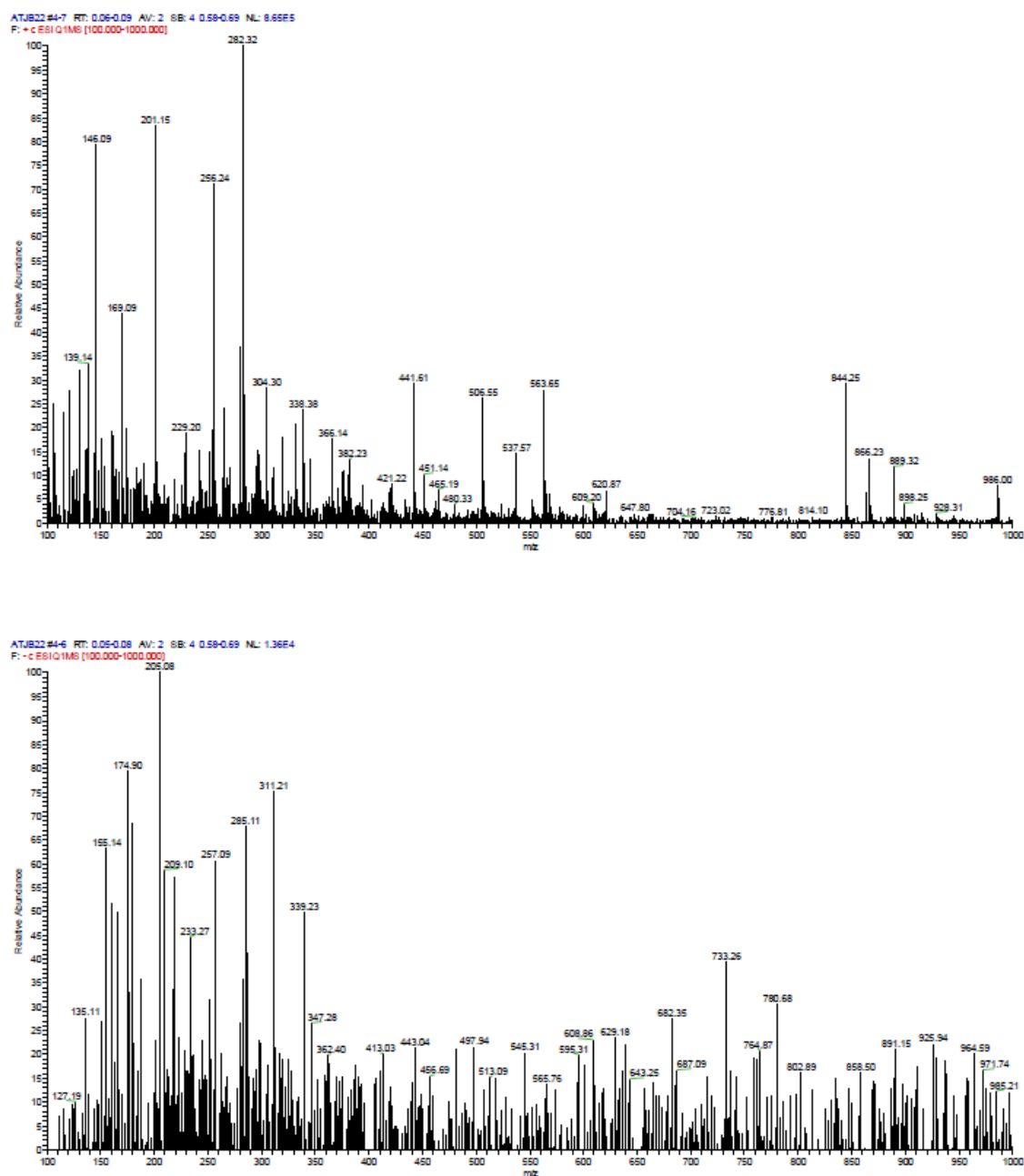


Figure 30. MS spectrum of desulpho tetracetylated analogue of glucomoringin

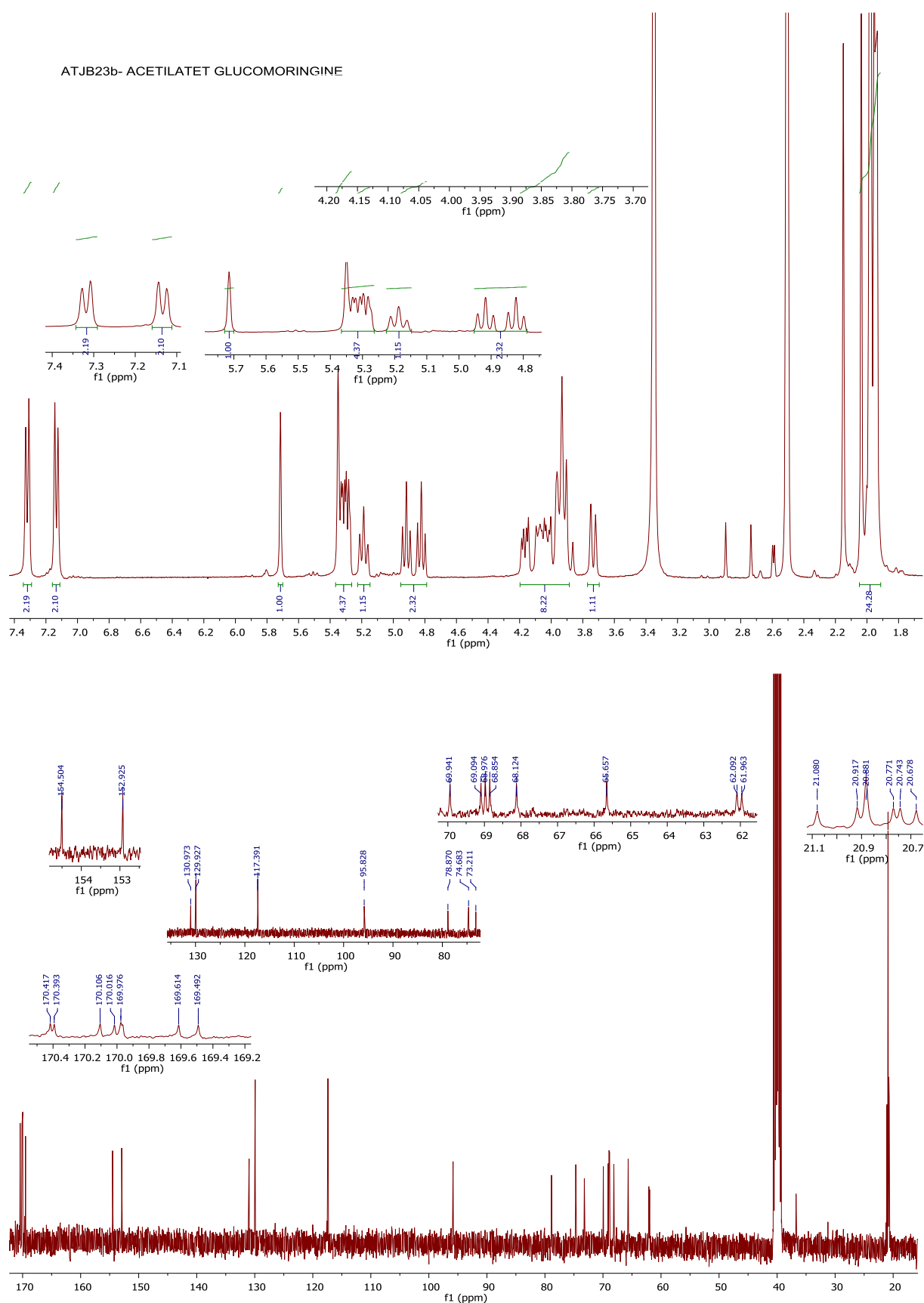
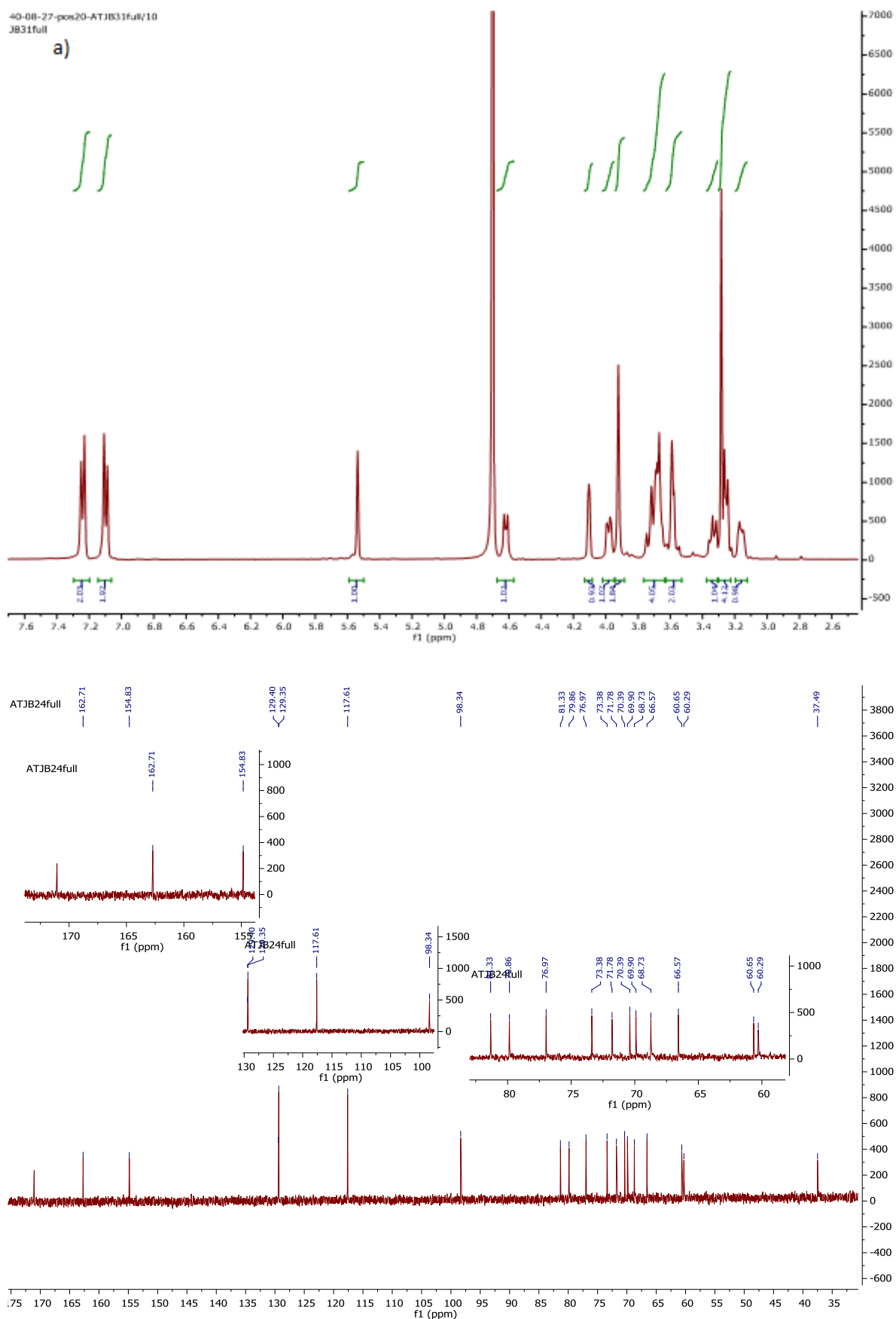


Figure 31. NMR spectrum of acetylated analogue of glucomoringine



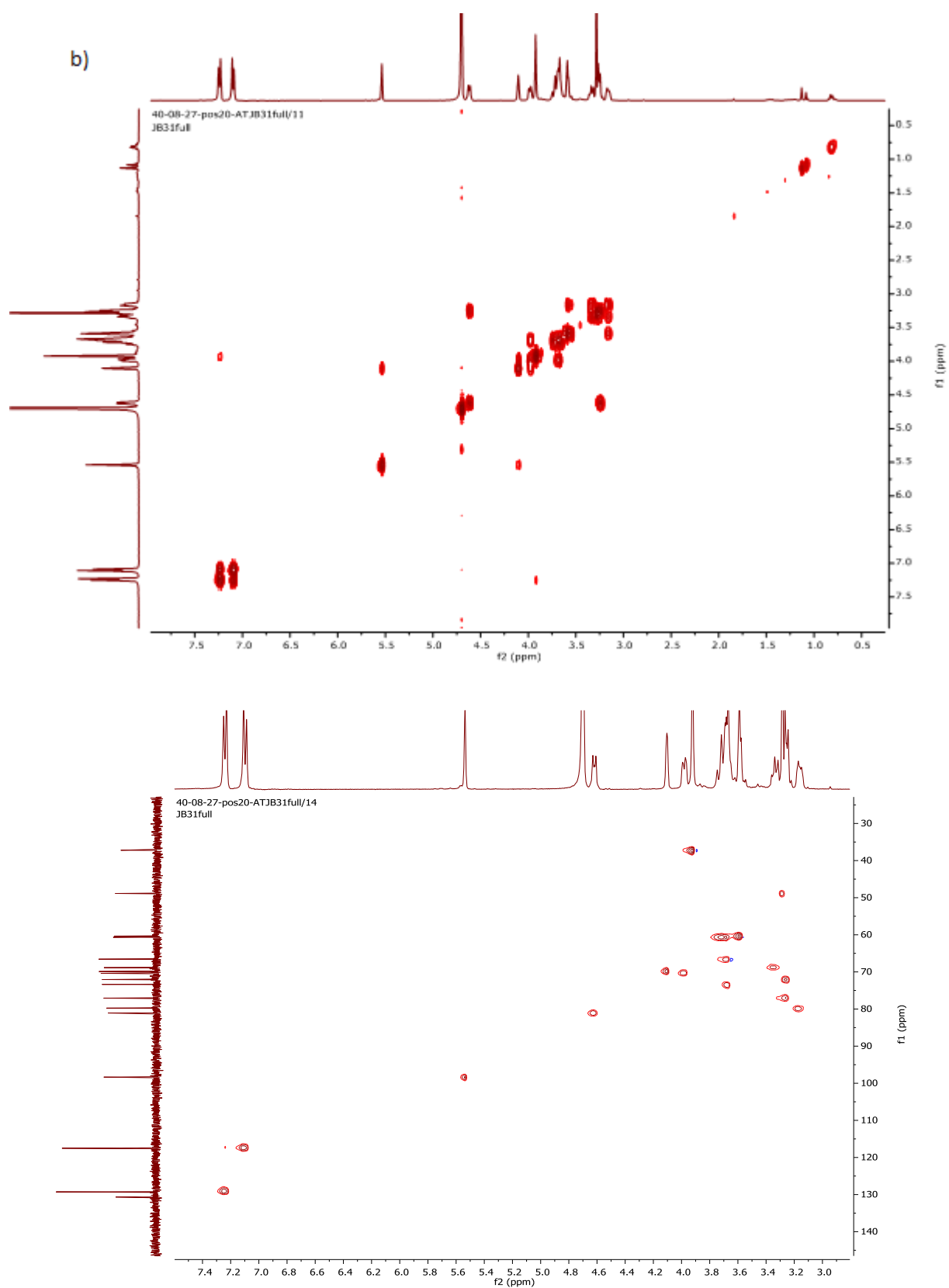


Figure 32. NMR spectrum of analogue of glucomoringin: a) 1D-NMR (^1H , ^{13}C)

b) 2D-NMR (COSY, HSQC)

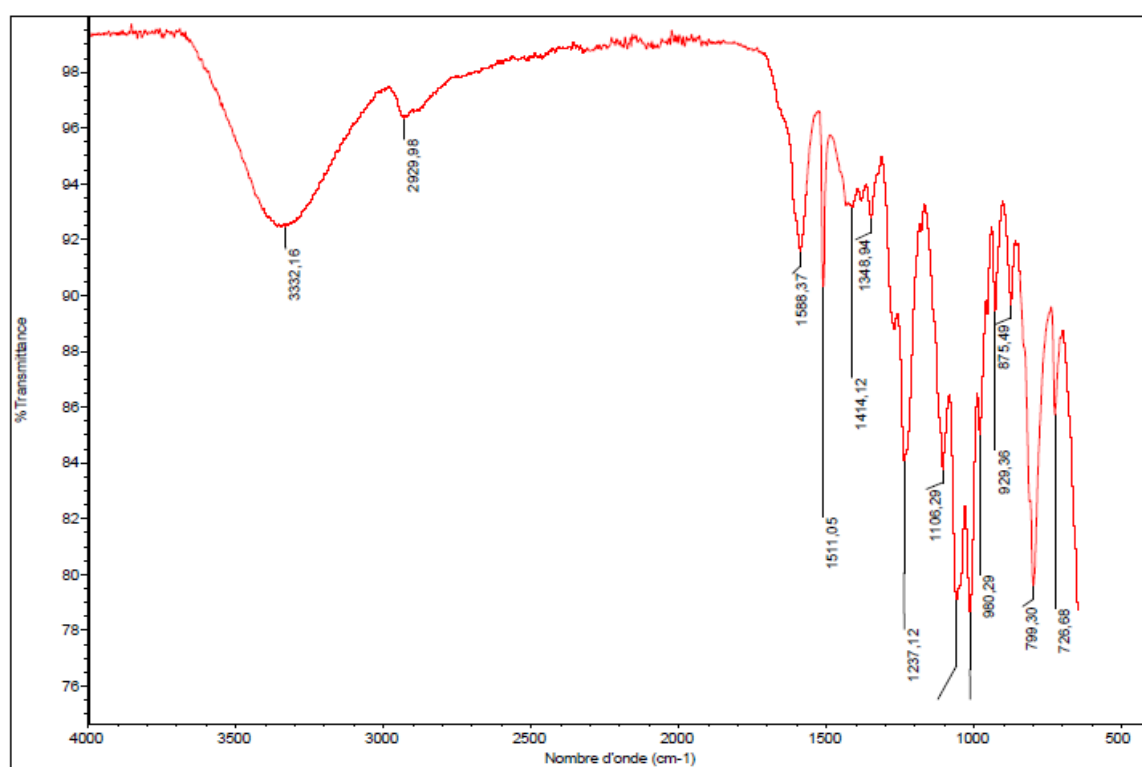


Figure 33. IR spectrum of analogue of glucomoringin