Chemistry of Glucoisosaccharinic Acids

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Abstract

The pulp and paper industries generate very large amounts of organic waste throughout the various processes of papermaking. Alpha and beta-glucoisosaccharinic acids ((2*S*,4*S*)-2,4,5-trihydroxy-2-(hydroxymethyl) pentanoic acid and (2*R*,4*S*)-2,4,5-trihydroxy-2-(hydroxymethyl)pentanoic acid) are the major components of pulp waste. They are produced in any process in which cellulosic materials are treated with aqueous alkali and constitute 30-40% of the total organic waste generated. Thus, they are potentially valuable platform chemicals with a highly functionalised carbon skeleton, with fixed chirality at C-2 and C-4, which makes them ideal starting materials for use in synthesis, instead of the current practice of burning such a valuable resource as a source of energy.

Due to the difficulties involved in isolating the 2*R*-epimer from the mixture and the lack of a simple and easily repeatable procedure to prepare it, to date only the chemistry of the 2*S*-epimer has been widely studied and only the 2S-epimer has been used in synthesis. In order to assess the potential of these saccharinic acids as platform chemicals, the protecting group chemistry of the lactone form of the alpha-glucoisosaccharinic acid was explored. The use of single and multiple step reaction pathways leading to the regioselective protection of the three different hydroxyl groups and strategies for protecting the three different hydroxyl groups individually or in pairs was explored and is reported in this thesis. These various strategies were subsequently applied to a mixture of the two epimers leading to the preparation, in a three step procedure, of 6-benzoyl-5-*tert*-butyldimethylsilyl-β-glucoisosaccharino-1,4-lactone, a derivative of the 2*R*-epimer.

An attempt to convert the 2*S*-epimer into the 2*R*-epimer through the inversion of their stereochemistry via Sharpless asymmetric dihydroxylation reactions is also reported. A maximum conversion of 52% was obtained after optimization of the procedure.

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Abbreviations

Protecting groups

Ac	Acetyl
Bn	Benzyl
Bz	Benzoyl
Cbz	Benzyloxycarbonyl
Fmoc	9-Fluorenymethoxycarbonyl
TBDMS	*t*-Butyldimethylsilyl
TIPDS	1,3-(1,1,3,3-Tetraisopropyldisiloxanylidene)
TIPS	Triisopropylsilyl

Reagents

DIPEA	Diisopropylethylamine
DMAP	4-*N*,*N*-Dimethylaminopyridine
DMSO	Dimethylsulfoxide
TBAB	Tetrabutylammonium bromide
TBAF	Tetrabutylammonium fluoride
PTSA	*p*-Toulenesulfonic acid
Pyr	Pyridine

Others

1D	One dimensional NMR spectroscopy
2D	Two dimensional NMR spectroscopy
COSY	Correlation spectroscopy
d	Doublet
dd	Double doublet
DEPT	Distortionless enhancement by polarization transfer
ESI	Electrospray ionization
GC-MS	Gas chromatography-mass spectrometry
h	Hour
HMBC	Heteronuclear multiple bond correlation

HPAEC-PAD	High performance anion exchange chromatography-pulsed amperometric detection
HRMS	High resolution mass spectrometry
HSQC	Heteronuclear single quantum coherence
Hz	Hertz
J	Coupling constant
L/ILW	Low and intermediate radioactive waste
m	Multiplet
M	Molar
MP	Meting point
NMR	Nuclear magnetic resonance
NOESY	Nuclear overhauser effect spectroscopy
PPI	Pulp and paper industries
ppm	Part per million
Rf	Retardation factor
RT	Room temperature
s	Singlet
t	Triplet

1.0 Introduction

1.1 General introduction

This chapter provides a general introduction to the research that has been carried out and details of the aims and objectives of this work.

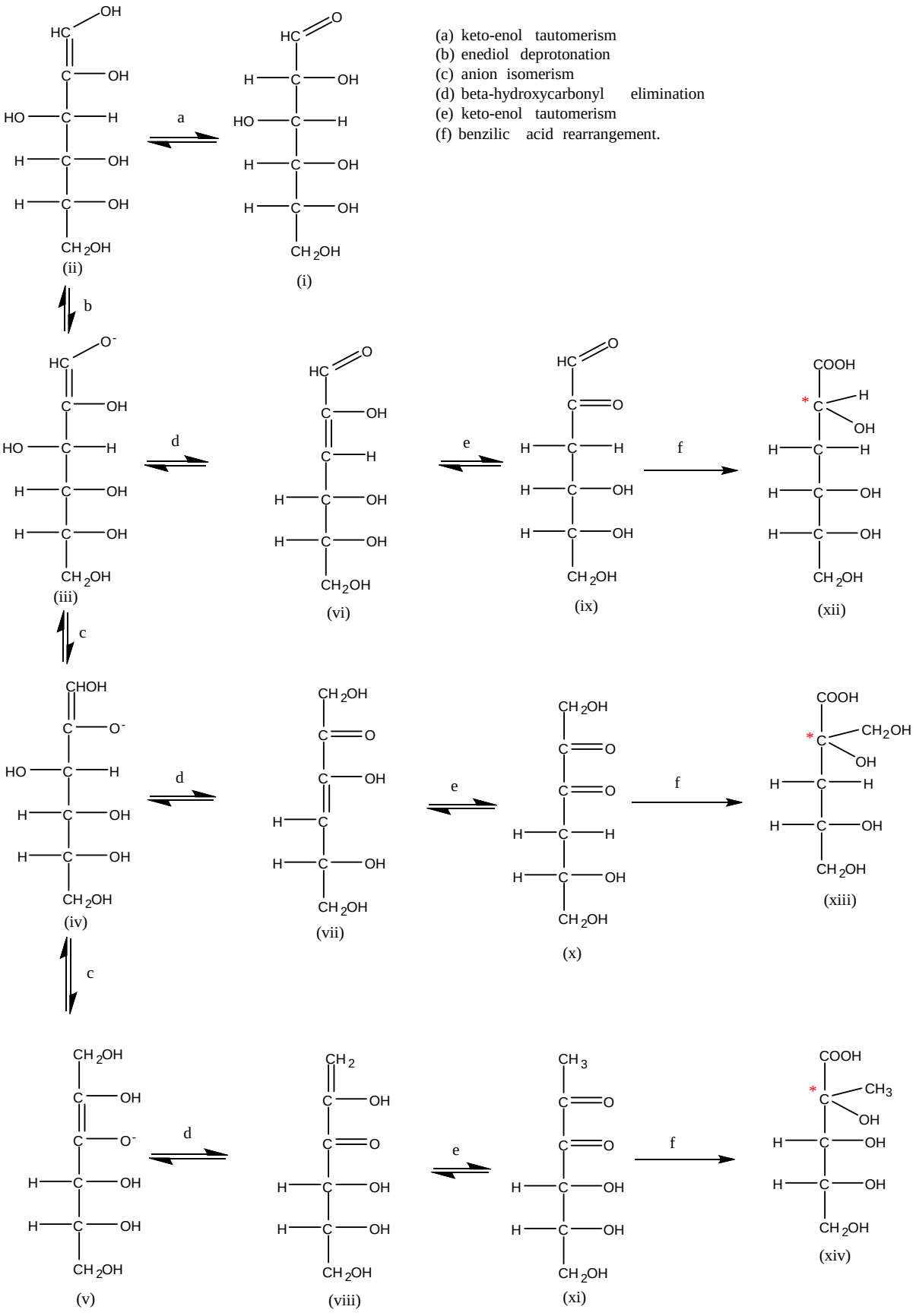
Glucoisosaccharinic acids are produced by any process in which cellulosic materials are treated with aqueous alkali, which generates a large quantity of degradation products with glucoisosaccharinic acids being one of the most abundant of such products. The production of glucoisosaccharinic acids as a primary waste product during the processing of cellulosic materials has raised a number of concerns and is of interest for a number of reasons:

- Glucoisosaccharinic acids (GISA) are potentially valuable platform chemicals with highly functionalised carbon skeletons which could substitute petroleum-based materials as starting materials in the synthesis of value added products.
- Glucoisosaccharinic acids have metal chelating properties which reduce the presence of radioactive and transition metals within various waste disposal streams which poses a threat to the intregrity of their storage facilities. This is particularly related to nuclear waste storage facilities.
- They are a potential carbon feed for microbial activities that can generate methane as a fuel.

The literature on the use of glucoisosaccharinic acids as starting materials in synthesis and study of their metal chelating properties is limited and there is a need to explore a better understanding of their chemistry.

1.2 Occurrence of glucoisosaccharinic acids

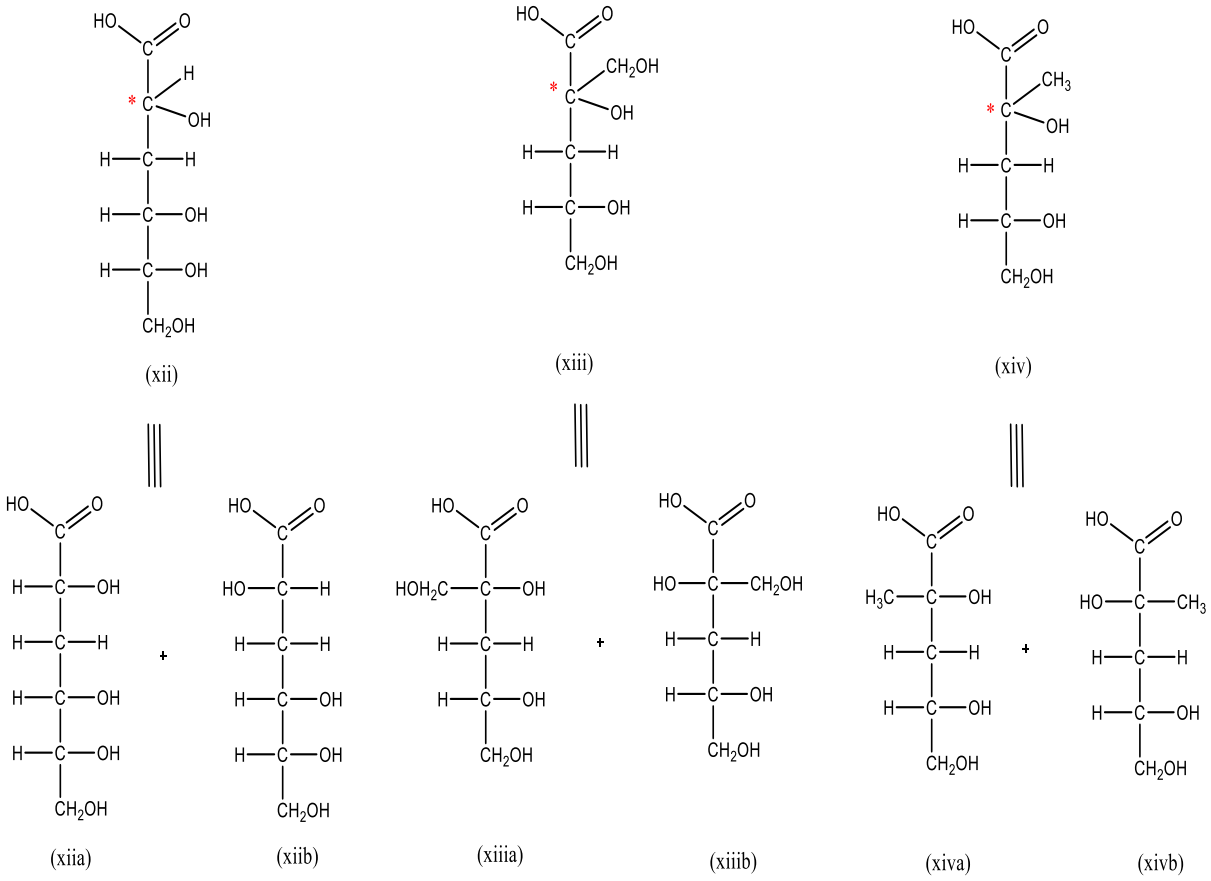
Glucoisosaccharinic acids are a group of branched-chain poly-hydroxyl acids that are amongst the acids generated when carbohydrates are treated with aqueous alkali. They were first reported in 1838 by Eugene Peligot [1] when he treated glucose with calcium hydroxide, and the analysis of the first crystalline lactone (α-D-glucosaccharin) produced from the hexose-alkali reaction led Peligot to the conclusion that it was an isomer of sucrose and as such it was named as saccharin and the corresponding free acid as glucosaccharinic acid [2, 3]. The basic principles of glucose degradation by alkali, involving the tautomerism of aldoses and ketoses with enediols, were proposed by Nef in 1907. Later, Isbell suggested a reaction mechanism that was a modified version of the earlier suggested mechanism by Nef (1907), to explain the production of glucosaccharinic acids when carbohydrates are treated with alkali [4 5]. The Nef-Isbell mechanism, as it has come to be known, for the alkaline degradation of D-glucose is shown below (Scheme 1.1):



Scheme 1.1: Nef-Isbell mechanism for the alkaline degradation of D-glucose (i) and the production of saccharinic acids [6].

The first stage of the reaction sequence involves a tautomerism reaction with the abstraction of the acidic proton at C2 and the development of the double bond between C1 and C2 with the simultaneous protonation of the oxyanion leading to the production of an enediol (**ii**) from glucose. The loss of a proton gives the enediol anion (**iii**). Anion isomerisation (c) then takes place resulting in a mixture of equilibrium intermediate anions (**iii**-**v**) through the Lobry de Bruyn/Alberda-van Ekenstein transformation that generates a mixture of D-glucose, D-mannose and D-fructose depending on the site and face where reprotonation takes place. This is considered as the first stage of the alkaline degradation of D-glucose [7]. The second stage involves the β-hydroxycarbonyl elimination that generates key intermediates diketodeoxyglycitol (**vi**-**viii**) and their corresponding dicarbonyl compounds via keto-enol tautomerism. In the final step of the Nef-Isbell mechanism, the dicarbonyl compounds (**ix**-**xi**) undergo a benzilic acid rearrangement to produce their corresponding glucosaccharinic acids (**xii**-**xiv**).

There are three structurally isomeric forms of saccharinic acids produced from the alkaline degradation of D-glucose and all three occur as mixtures of anomers having different configuration at C2. These are a mixture of 3-deoxy-D-ribo-hexonic and 3-deoxy-D-arabino-hexonic acids (**xii**) also known as α- and β-D-glucometasaccharinic acids (**xiia** & **xiib**), a mixture of 3-deoxy-2-C-(hydroxymethyl)-D-erythro-pentonic and 3-deoxy-2-C-(hydroxymethyl)-D-threo-pentonic acids (**xiii**) also known as α- and β-D-glucoisosaccharinic acids (GISA) (**xiiia** & **xiiib**). Also, a mixture of 2-C-methyl-D-erythro-pentonic and 2-C-methyl-D-threo-pentonic acids (**xiv**) also known as α- and β-D-glucosaccharinic acids (**xiva** & **xivb**) [8-10]



Scheme 1.2. Structural isomeric form of glucosaccharinic acids [11].

It is worth noting that the gluco-prefix identifies GISA as being derived from a glucose unit or from a monomer or a 1, 4-glucan such as in cellulose, maltose and amylose. In the early and some current literature describing GISAs [2-4, 8-10, 12-17], these molecules are widely referred to as metasaccharinic acids, isosaccharinic acids (ISA) and saccharinic acids.

1.3 Pulp and paper industry (PPI): A major source of glucoisosaccharinic acids

The pulp and paper industry (PPI) is one of the largest industrial sectors and is present in a broad range of geographic regions. The industry produces a large amount of waste including wastewater, the latter is due to the large volume of water used per ton of product and in some places, there is water reuse [18]. The industry uses wood from plants as its primary raw material for the production of pulp and paper products and the

chemical composition for a type of wood, or even a tree cannot be defined precisely [6]. However, the major constituents can be divided into two categories: carbohydrates, which constitute (70 ± 5%), and lignin (20 ± 5%). The carbohydrate materials consist of cellulose and hemicellulose, the average proportion inhardwood are: cellulose (45%), hemicelluloses (34%) and lignin (21%). In softwood the average proportions are: cellulose (42%), hemicelluloses (28%) and lignin (29%) [7].

The continuous activities of the industry in sourcing their raw material, wood, to meet the increasing demand of paper products, has led to deforestation of a lot of forest reserves around the world even though most countries around the world have laws governing various activities within the forest reserves. In Nigeria, for example, the forest reserves of the country are under the Ministry of Environment (at the national level) and Ministries of Forestry (at the state levels). Forest Research Institute of Nigeria (FRIN) is a federal agency saddled with the responsibility of the various forestry research activities and was recently mandated to coordinate the various reforestation programmes in the country under the FRIN (establishment) Act 2018, after losing almost ninety percent of country's forest resources in the last six decades [19]. PPI contributes less to deforestation in Nigeria compared to fuelwood and wood charcoal for domestic and industrial activities as a source of energy [20, 21] which could be due to the lack of softwood species that has longer fibres. Hence the industry heavily depends on the importation of such raw material while utilising the abundantly available species of hardwood unchecked. Reforestation on the other hand, is not a quick process and is much slower than deforestation-it takes a lot of time for trees to grow. This has led to an increase in the use of recovered fibres (RCFs) from recycled papers, paperboard, cardboard printing and publishing papers, packaging paper and board [22, 23], and lignocellulosic biomass as a source of raw material for the industry.

The PPI generates significant amounts of organic waste products during the various processes of pulping and papermaking which includes acetic, formic, glucoisosaccharinic, glycolic and lactic acids and likewise inorganic materials [24, 25]. Hence, it is desirable to develop alternative uses that could employ this waste as feedstock to generate fine and bulk chemicals, and advance waste treatment processes to serve as essential tools for compliance with the stringent environmental regulations. At the same time there is the opportunity to increase the profitability of the PPI which is current declining with their being a reduction in pulp and paper markets. The latter is driven by societal initiatives and awaness of deforestation preventive policies and programmes such as paperless, online newspaper and technological advancements. Nevertheless, the economic value of waste generated from PPI is very low [18].

- Cellulose

As was briefly mentioned above, the primary raw material used in the manufacture of paper is cellulose. Cellulose is a linear polysaccharide and a polymer of anhydroglucose units linked through β-(1,4)-glycosidic bonds. The pyranose rings are in the $^4C_1$ conformation, and the average number of monomeric units is known as the degree of polymerisation. The insolubility of cellulose in either water or organic solvents also constitutes a difficulty in the transformation of cellulose. A cellulose molecule has a non-reducing end and a reducing end (Figure 1). The reducing end responds to both oxidation and reduction processes and plays a vital in the alkaline degradation of

cellulose.

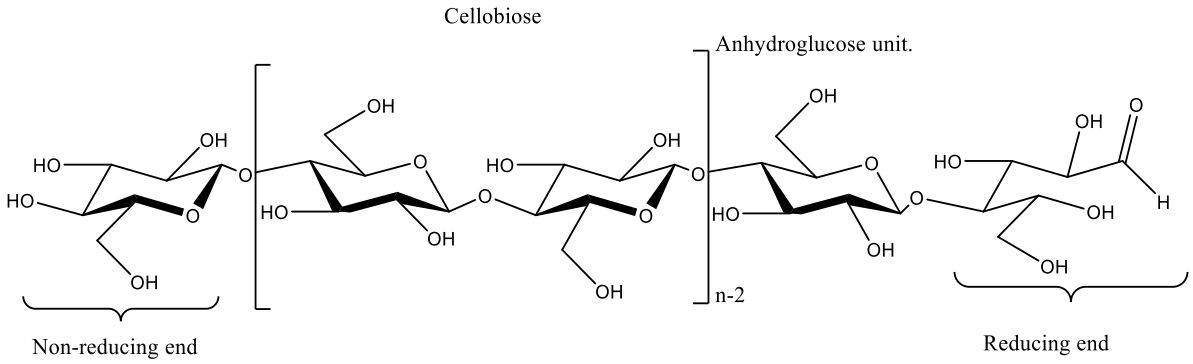


Figure 1 Chemical structure of cellulose [26]

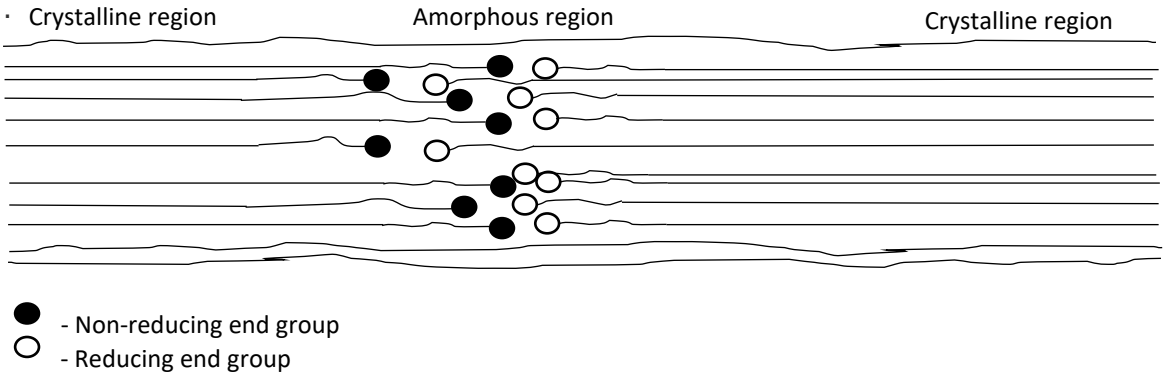


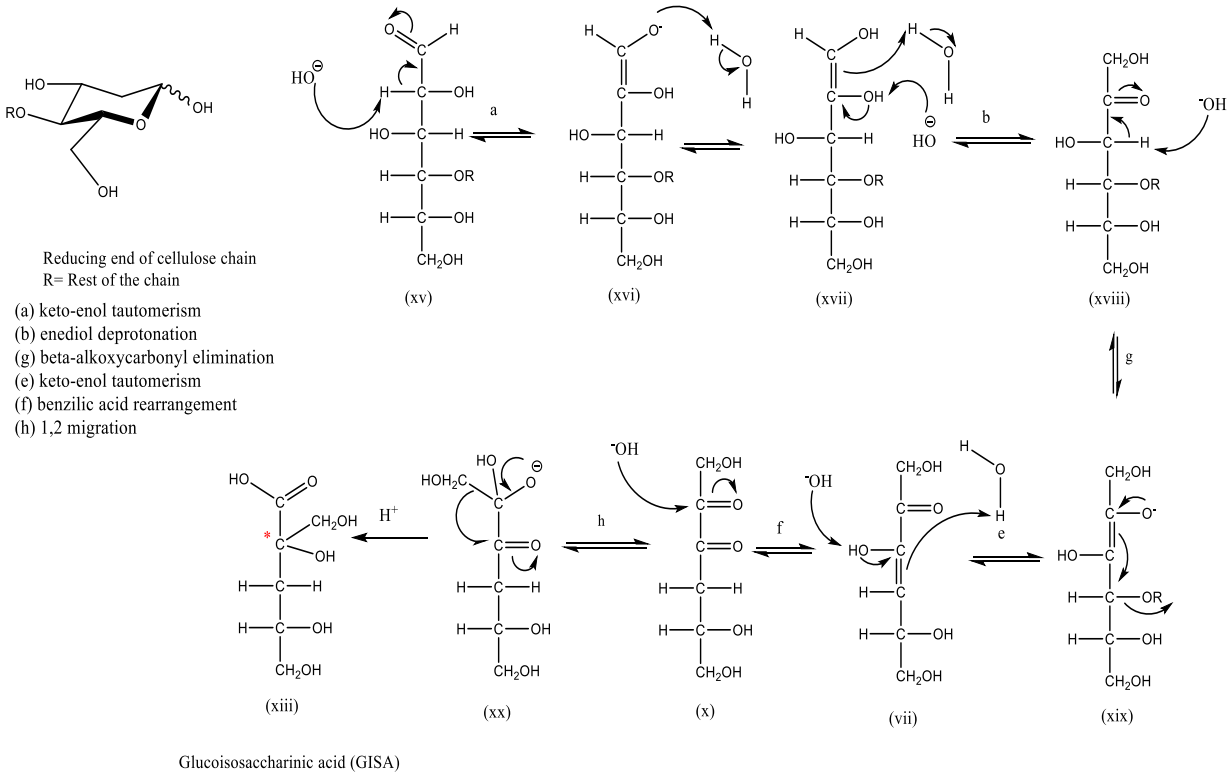
Figure 2 Physical structure of cellulose [27].

The cellulose molecules are arranged into strands as cellulose micro fibrils through intensive intra- and intermolecular hydrogen bonding. The micro fibrils have amorphous and crystalline regions with the majority of alkaline degradation occurring in the amorphous regions (Figure 2). [27]

1.3.1 Application of cellulose in pulp and paper industry.

The most commonly used process for the production of cellulose pulp is the Kraft process that uses sodium hydroxide and sodium sulphite as primary pulping chemicals, also known as cooking liquor or white liquor, at elevated temperatures. The Kraft process begins with the alkaline hydrolysis of the wood fibre that causes swelling of the

fibre and dissolves lignin in to the cooking liquor that leads to the breaking of the structural linkage between lignin and carbohydrates, which also decreases the crystallinity and ultimately the degree of polymerisation DP via the peeling reaction at the reducing end of the structure. Both cellulose and hemicellulose are vulnerable to the peeling reaction and it is this process that leads to the production of saccharinic acids. The cellulose alkaline degradation pathway is shown below and the first steps are the same as those involved in the alkaline degradation of glucose:

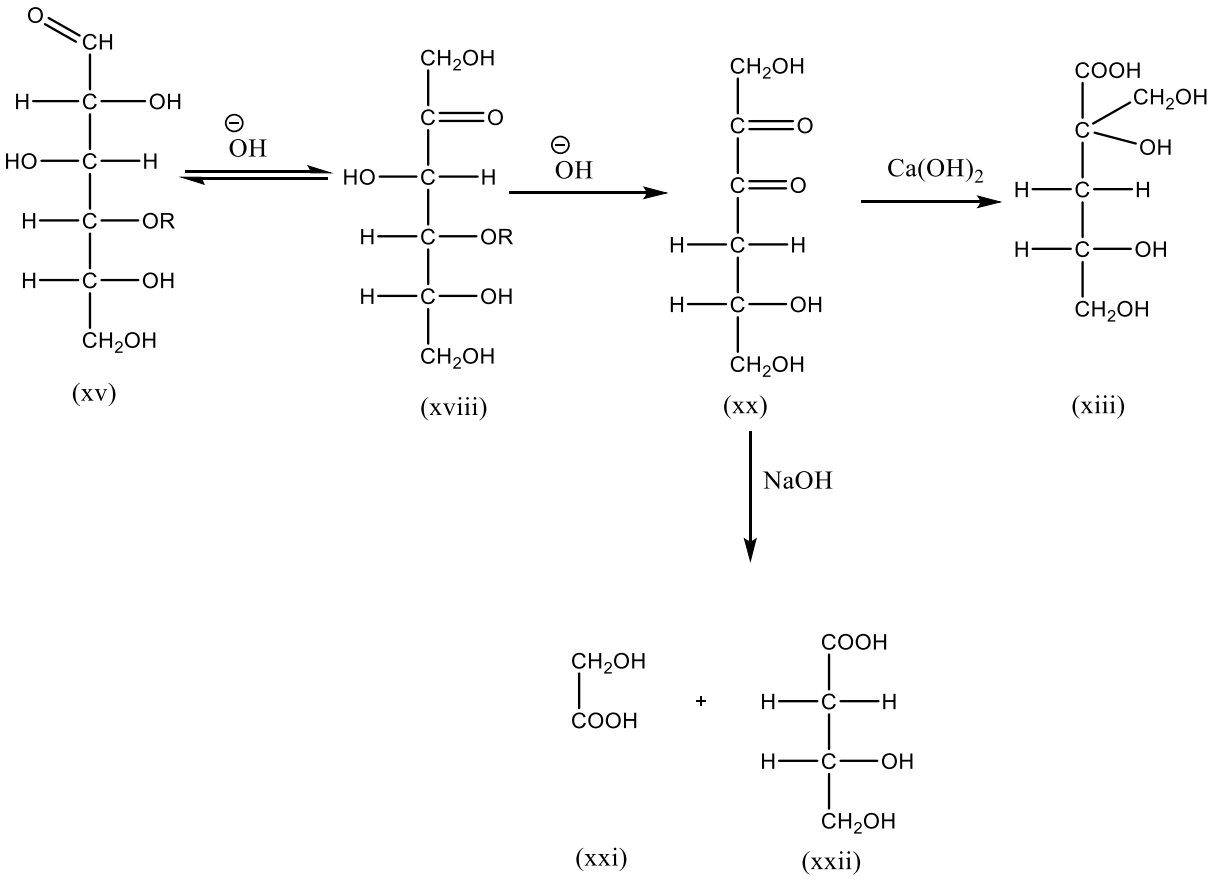


Scheme 1.3: Alkaline degradation of cellulose (peeling reaction) [4, 7, 8, 10, 28]

In the first part of the peeling reaction, the reducing monosaccharide undergoes a series of enolisation and tautomerisation steps leading to its transformation from 4-*O*-substituted D-glucose (**xv**) to 4-*O*-substituted D-fructose (**xviii**) in which the original carbonyl carbon migrates from C1 to C2. The peeling step is the β-elimination of the rest of cellulose chain to generate a key intermediate 4-deoxy-D-glycero-2, 3-

hexodilulose (x), which then undergoes a benzilic acid rearrangement to give a mixture of α-GISA and β-GISA.

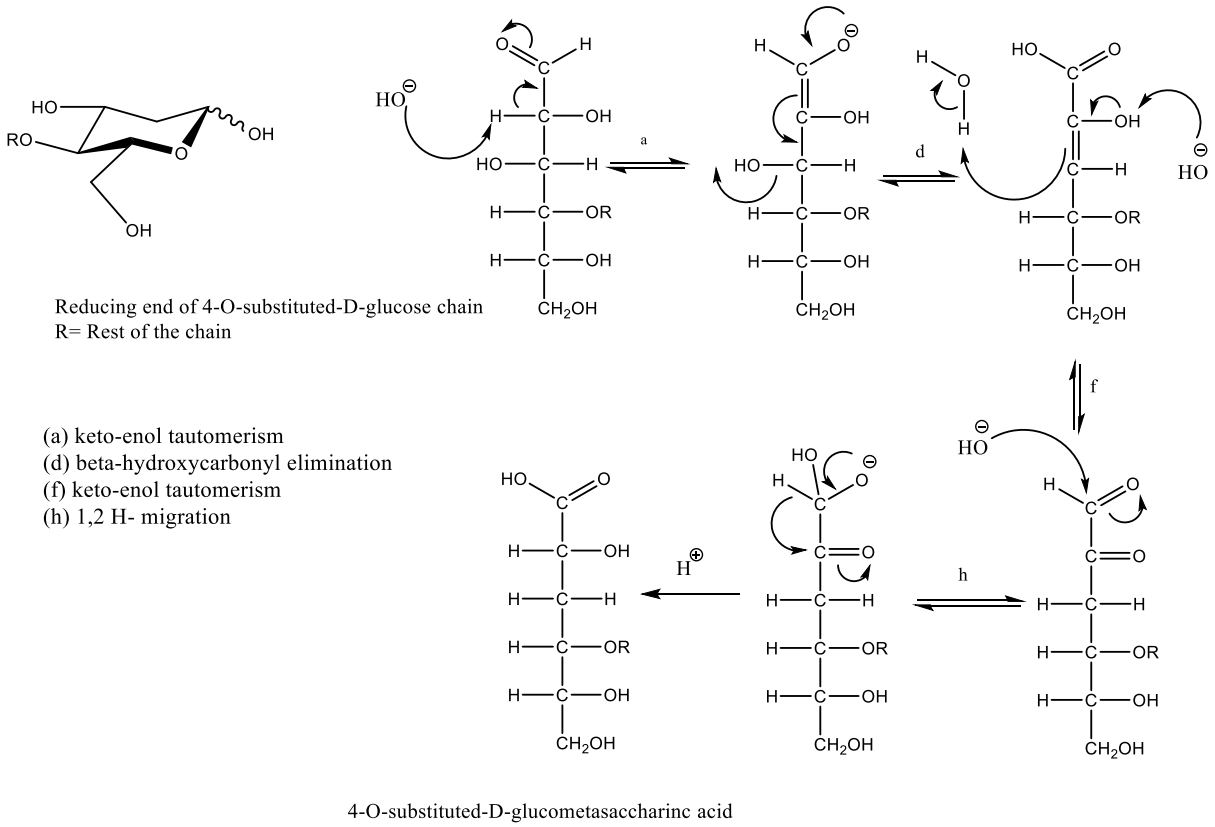
The formation of glucoisosaccharinic acids is favoured due to the presence of calcium ion in the alkaline degradation of cellulose, with calcium hydroxide as a base by catalysing its production from 4-deoxy-D-glycero-2, 3-hexodilulose (x) (Scheme 1.4), whereas in the case of sodium hydroxide, formation of large amount of fragmentation products such as glycolic acid (xxi) and D-3,4-diydroxybutyric acid (xxii) dominates [7, 11].



Scheme 1.4. Fragmentation products of peeling reaction [11].

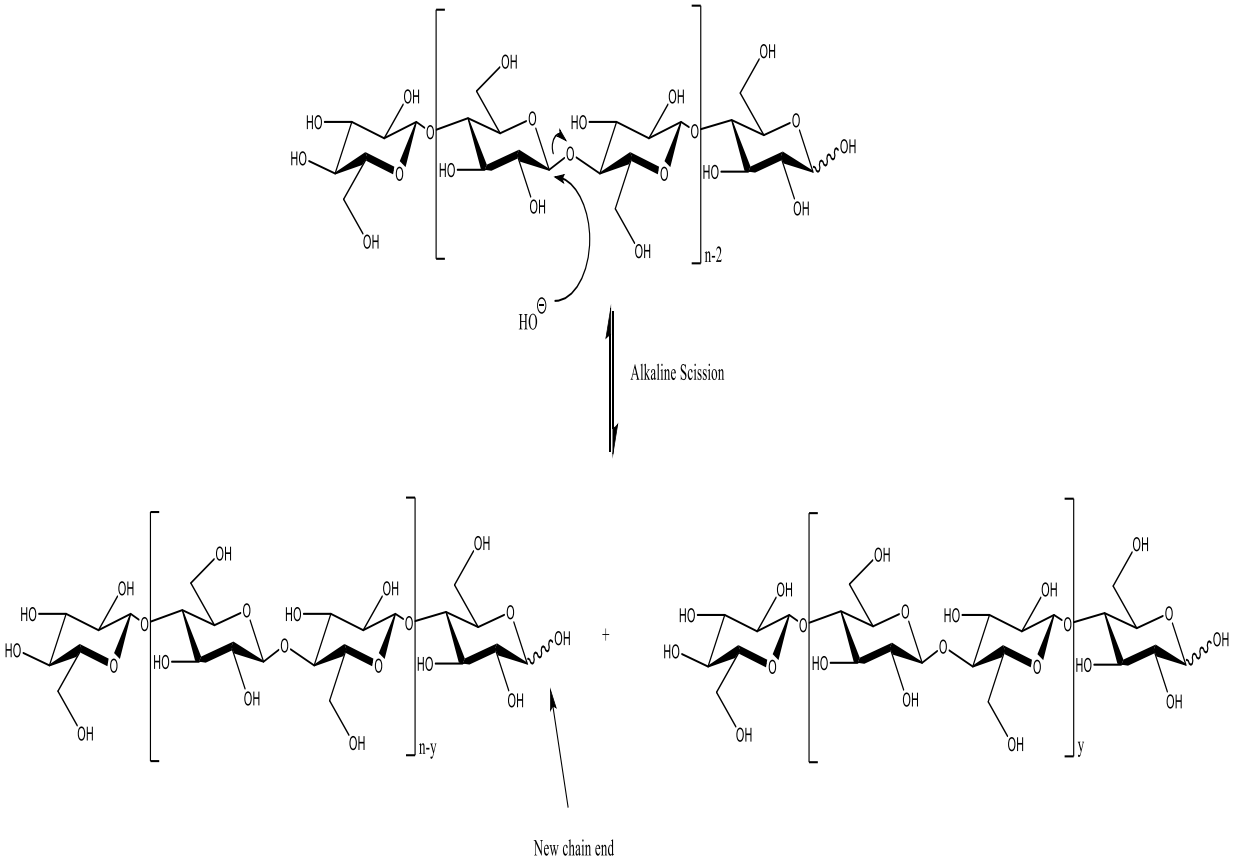
1.3.1.1 Stopping reaction and mid-chain scission of cellulose.

If the peeling of the cellulose structure from the reducing ends were to continue indefinitely, the whole of the cellulosic material would degrade eventually, which is usually not the case, in most cases, either a rearrangement which converts the reducing end to an alkali-stable unit (stopping) takes place or when the peeling gets to the crystalline non-accessable region it (physically) stops. The stopping reaction in cellulose pulping arises from the pathway which gives glucometasaccharinic acid from glucose (Scheme 1.5); the reducing end is converted to an alkali-stable carboxylic acid [29], which is resistant to further alkaline degradation, and results from elimination of hydroxy group at C3 (xvi) that competes with the removal of the rest of the cellulose chain from C4 (xvi).



Scheme 1.5: Alkaline degradation of cellulose (stopping reaction) [7, 14]

As the temperature continues to elevate, random alkaline scission (hydrolysis) of the glycosidic linkage occurs resulting in a decrease in DP (Scheme 1.6) and is followed by peeling from the new reducing end produced by the chain scission [30], which seems not to depend on the presence of the molecular oxygen [31]. However, stopping reaction has a larger effect at such higher temperatures, it results in more significant weight losses than the alkaline degradation at a lower temperature.



Scheme 1.6. Alkaline scission of cellulose [30].

1.4 Glucoisosaccharinic acids: A potential raw material for the production of chemicals.

The alkaline degradation of cellulose and hemicellulose generates a range of volatile and non-volatile aliphatic acids. Glucoisosaccharinic acids are one of the major degradation products (Table 1), and then isolation from the black liquor after the Kraft pulping processes and subsequent use as the potential chemical has attracted substantial interest in recent studies [32-34]. Due to the presence of lignin and other wood degradation products, the isolation of the aliphatic acids from the black liquor presents a complex separation problem, which to date has not been solved satisfactorily. Presently the only use that this waste stream has been put to is the heat produced when the aliphatic acids are burnt with lignin which is utilised to generate steam power electricity for the Kraft mill.

Black liquor is a complex mixture of water, various organic materials and inorganic cooking chemical residuals (see Table 1). The fraction of aliphatic carboxylic acids contains volatile formic and acetic acids and a wide range of non-volatile aliphatic acids. The non-volatile acids are the low-molecular-weight hydroxy acids with 2-4 carbon atoms (glycolic, lactic and 2-hydroxybutanioc acids) and high-molecular-weight hydroxy acids with 5 or 6 carbon atom (2-hydroxybutanoic, 3-deoxy-pentanoic, xyloisosaccharinic and glucoisosaccharinic acids). The high-molecular-weight hydroxy acids can undergo intramolecular self-esterification to form cyclic esters (lactones) [35].

Table 1 Main aliphatic carboxylic acids and their composition in black liquor of both hard- and softwood [35, 36]

Acids	Hardwood (Birch) 53% yield	Softwood (Pine) 47% yield
Acetic Acid	120	50
Formic Acid	50	70
Hydroxy acids;		
Glycolic	15	10
Lactic	45	45
2-Hydroxybutanoic	65	15
2,5-Dihydroxypentanoic	10	10
3-Deoxypentanoic	5	5
Xyloisosaccharinic	45	45
Glucoisosaccharinic	35	160

The approximate values are in Kg/ton of pulp produced in the kraft process

A significant amount of work has been undertaken in an attempt to fractionate components of this mixture [32, 37]. After the removal of the lignin from the black liquor it is possible to recover the volatile formic and acetic acids (around 50% of the total acids) by direct distillation and the low-molecular-weight hydroxy acids (about 15% of the total acids) by catalytic esterification followed by distillation. After the distillations, the high-molecular-weight hydroxyl-acids (mainly, glucoisosaccharinic, xyloisosaccharinic, 2, 5-hydropentanoic and 3-deoxypentanoic acids), which represent approximately 35% of the total acids, are left as residual material. A schematic of the procedures used to fractionate the various acids is shown below (Figure 3).

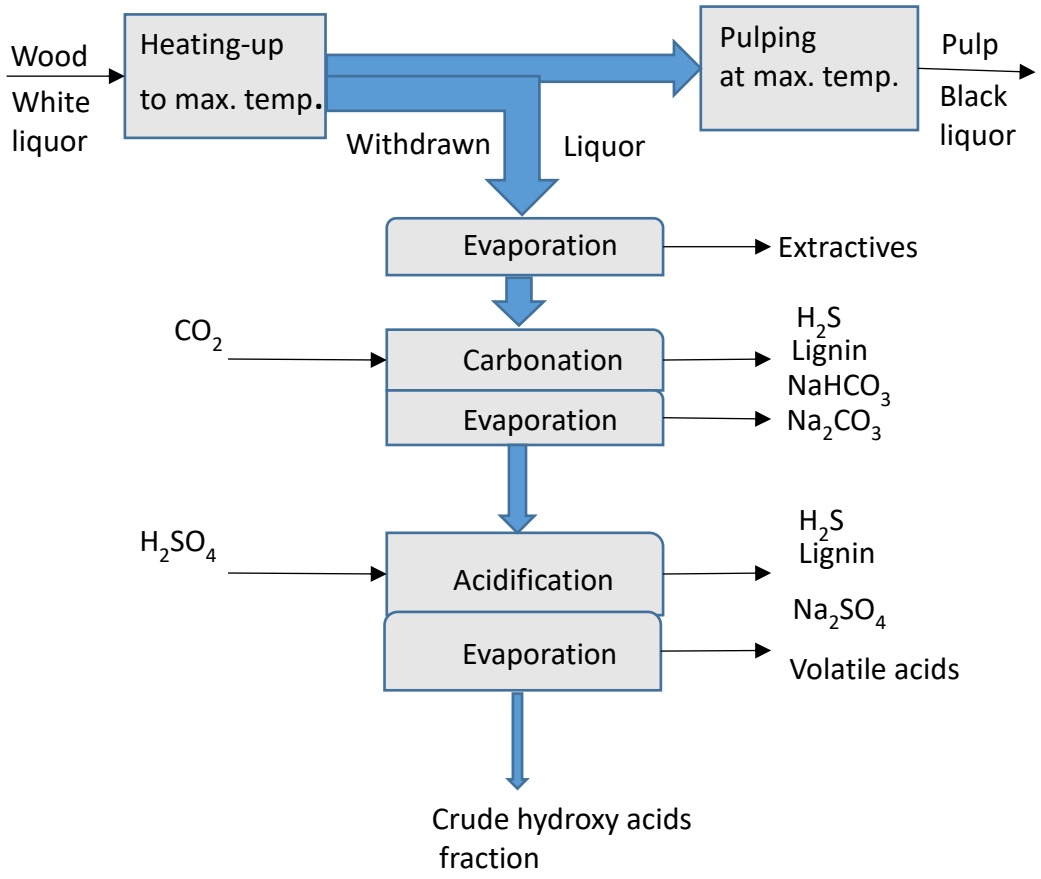


Figure 3. Schematic process for the isolation of hydroxy acids [33].

In general, low-molecular-weight hydroxy acids such as glycolic and lactic acids can also be produced by alternative methods, however, they are valuable materials and can be used in many applications such as the polylactic acid products that are used as biodegradable plastics [38, 39], for medical services [40] and glycolic acids for cosmetic products [41]. So far, the utilisation of the main non-volatile hydroxy acids such as glucoisosaccharinic acid, and its derivatives, has only been studied to a limited extent [33]. The residual organic materials can be converted to useful products using the most suitable of the following methods rather than the current energy generation option through steam power turbines.

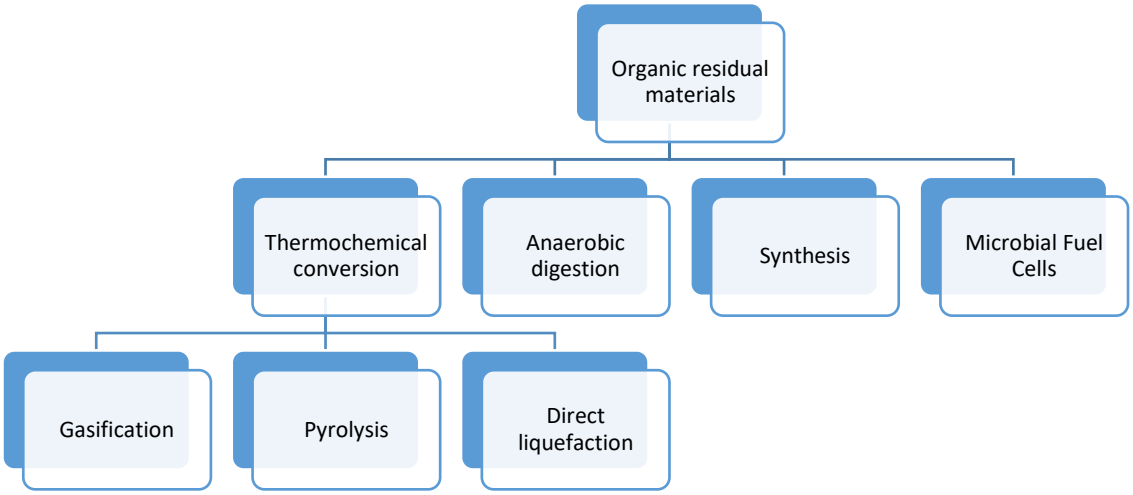


Figure 4. Alternative methods for the conversion of non-volatile organic residual materials for PPI to useful products.

1.4.1 Anaerobic microbial digestion (AD) of organic residual materials from PPI.

Anaeorbic digestion (AD) is the microbial degradation of organic compounds into different useful products, including methane, carbon dioxide and hydrogen by a microbial consortium in an oxygen-free environment, which is employed widely in the treatment of various industrial wastes [42] [43]. The use of AD for treatment of PPI waste has advantages over the existing combustion methods of disposal including the production of a higher calorific fuel via the derived of biogas (Figure 5), which is mainly composed of methane, which can itself be used as fuel to generate heat for the production plants [43].

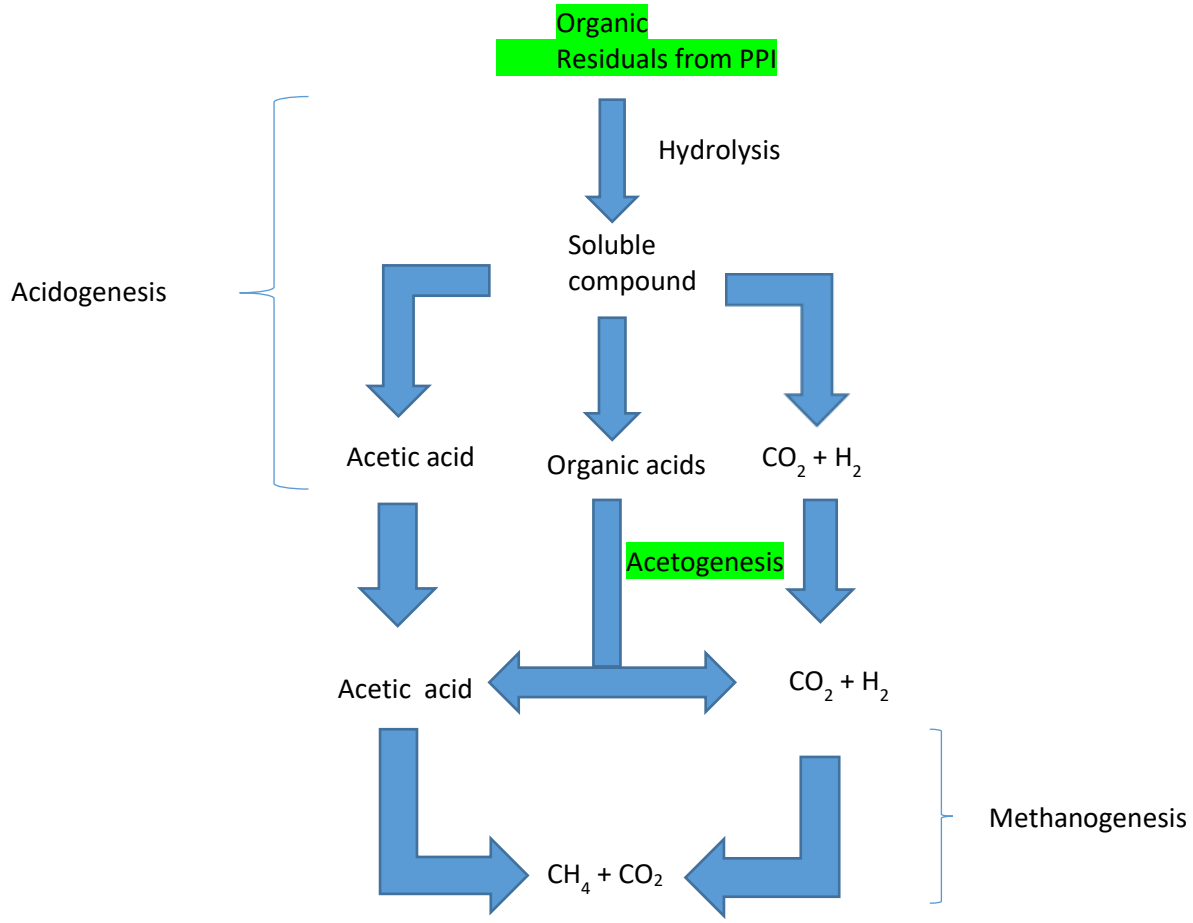


Figure 5. Schematic presentation of anaerobic microbial digestion of organic residual materials [43, 44]

AD generally involves the transformation of insoluble organic materials into soluble compounds through enzymatic hydrolysis, conversion of the soluble compounds into organic acids, then to acetic acid by the bacteria through acidogenesis and acetogenesis. Finally, the methanogenesis to generate methane (50-75%), carbon dioxide (25-50%) [44]. In the case of the non-volatile organic residuals produced by PPI, those that are already organic acids require a simplified anaerobic conversion to methane [45]. In spite of these advantages, the method needs some improvement in the stability of the process at an industrial scale to accommodate the large quantities of hot heterogeneous by-products generated by PPI daily and the removal of lignin, the

presence of which tends to inhibit to the AD performance [46, 47]. The adoption of appropriate operating conditions can potentially enhanced methane production.

1.4.2 Use of saccharinic acids within microbial fuel cells

The development of microbial fuel cells (MFC) has a long history, MFCs are sometimes referred to as bio-electrochemical systems that produce an electric through the metabolic activity of microorganisms [48]. From the chemical perspective of the process, MFC is an electrochemical cell where bacteria catalyse the oxidation and reduction in the electrochemical reaction. It consists of an anode and cathode compartment separated by a cation-specific membrane. In the anode compartment the organic residues are oxidized by the bacteria, which releases protons and electrons [49] (Figure 6). Electron are transferred to the anode due to the electrogenic activities of the bacteria and further to the cathode through an external circuit and the protons are transferred to the cathode through the proton exchange membrane. Electrons and protons are consumed in the aerobic chamber, combining with oxygen to form water. In the case of glucoisosaccharinic acid, the MFC equations are;

$$C_6H_{12}O_6 + 6H_2O \longrightarrow 6CO_2 + 12e^- + 12H^+ \quad \text{Anaerobic chamber}$$

$$3O_2 + 12H^+ + 12e^- \longrightarrow 6H_2O \quad \text{Aerobic chamber}$$

The electricity in the system is generated as a result of a separation of the cathode in the aerobic (electron acceptor) chamber from the anode in the anaerobic bacteria chamber (electron donor), and the movement of the electron from anode to cathode electrode is a spontaneous process that generates electricity in the MFC system

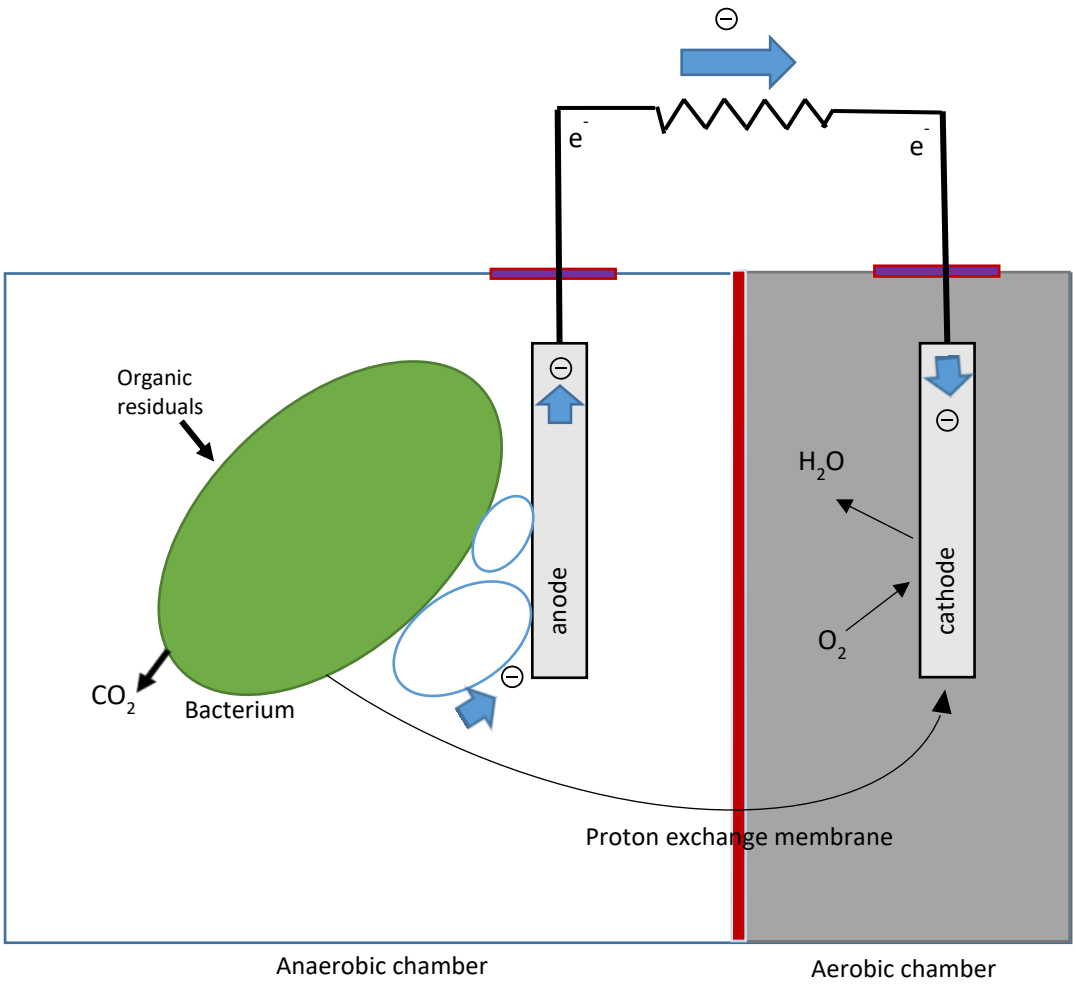


Figure 6. Schematic representation of the electric current production from the organic residual materials present in the waste treatment process in an MFC system.

The organic residual materials from the PPI are difficult to process within MFCs due the heterogeneous nature of their specific compositions. The possibility of the presence of lignin or its degradation products such as phenolic compounds, which have inhibitory effect on the activities of the bacteria in the MFC system and their presence at high concentration, hinders the metabolism of the microorganisms [50]. However, some studies have shown that the bacteria can develop various mechanisms that allow them adapt to such harmful environments [51]. The isolation of bacteria that adapt to such environments is a crucial factor for building efficient MFCs for the treatment of the organic by-products generated from the PPI [52]. The production of bioenergy through

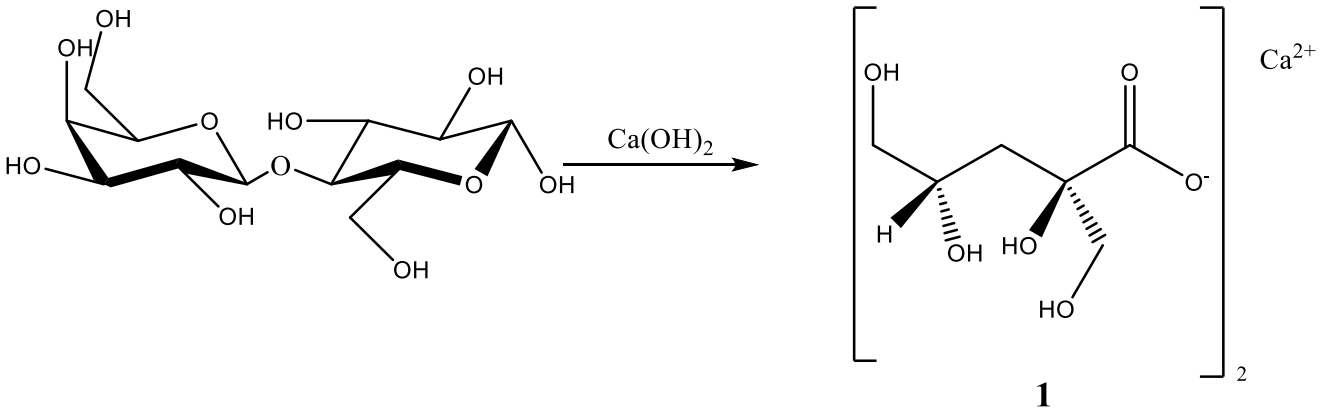
microbial activities is a current area of research at the University of Huddersfield and, despite the fact that the power produced by the MFC system is relatively low, it has great potential [53].

1.4.3 Thermochemical conversion of waste streams

The thermal degradation of organic materials is carried out by a straight-forward method, the chemical reactions that take place result in products which include gases, condensable liquids and solid residues [54]. Of the two primary methods, gasification is performed under oxidative conditions at a relatively high temperature (1000 ^{o}C), whereas pyrolysis proceeds in the absence of oxygen at lower temperatures (200-500 ^{o}C) [55]. Gasification has in recent years received considerable attention due to its potential for electricity generation if the gas produced can be burned in a gas turbine for combined-cycle power generation. Gasifiers with this capability are still at the development stage and now attention is shifting towards production of synthetic gas by gasification of the residual black liquor as a raw material for making higher value chemicals [56]. In contrast, pyrolysis results primarily in char and liquids. Liquefaction is a specific type of pyrolysis that produces highly heterogeneous solids, liquids and gaseous products, whereas many targeted applications of such products, such as combustion in turbines to generate power, require optimized and highly standardised liquid fuels with low levels of impurities (i.e. char, ash and nitrogen) [57]. The refining of the heterogeneous mixture of organic waste generated from PPI after dehydration to meet these requirements is an energy demanding process, is not considered to be an easy route [58]. However, extensive efforts have been made to optimise the conversion of organic residual materials into synthetic gas and liquid products [59].

1.4.4a Synthesis of α-glucoisosaccharinic acid

The most frequently employed method for producing α-glucoisosaccharinic acid was developed by Whistler and BeMiller [16, 42]



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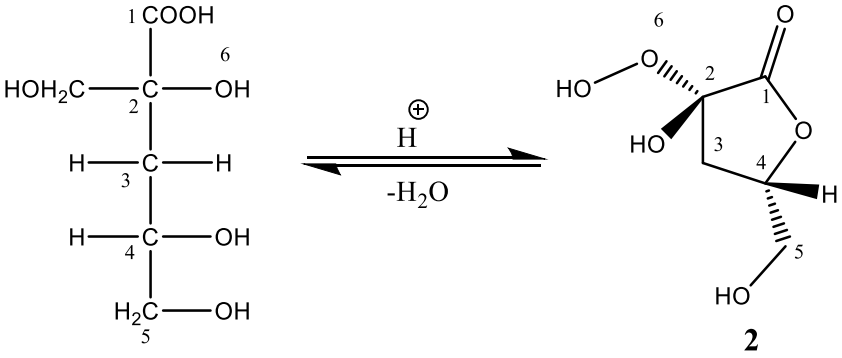
Equation 1. Alkaline degradation of lactose [11]

The procedure involves treating lactose with an aqueous solution of calcium hydroxide which produces a mixture of α-and β-isomers of glucoisosaccharinic acid along with a number of other small molecules. If the solution is reduced in volume and cooled to 4 ^{o}C a relatively clean sample of α-glucoisosaccharinic acid can be recovered (Eq. 1). Unfortunately, due to the difficulty in separating the β-isomer in its enantiomeric pure form from their mixture, only the α-GISA can be obtained directly from this procedure. The calcium salt of α-GISA is only sparingly soluble in organic solvents and, as such, it is normal to convert the acid to its lactone. This is achieved by adding oxalic acid (Scheme 7a) to a solution of the calcium salt and separating the lactone from the calcium oxalate that is formed through the application of a cation exchange column which provides H^{+} and removes Ca^{2+}.



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Scheme 1.7a. Acid-catalysed lactonisation of α-GISA to form α-D-glucoisosaccharino-1,4-lactone α-GISAL.

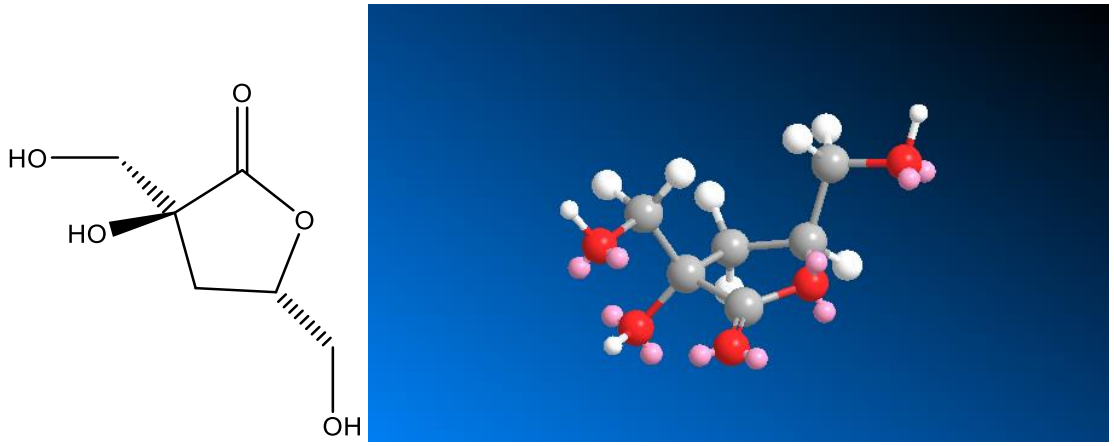
1.4.4a.1 Use of α-GISAL as a starting material in synthesis

The use of α-GISAL as a starting material in synthesis is rare, despite the ease of its preparation. Bennis *et al.* have reported incorporation α-GISAL into the synthesis of a small number of heterocycles including protected piperidine [60] and pyrrolidine [61] and Hanessian and Roy have incorporated it in the synthesis of the antibiotic spectinomycin [62]. Forent *et al.* [17] and Bertounesque *et al.* [63] have used α-GISAL in the synthesis of a range of anthracycline analogues and Wolf *et al.* utilised it in the synthesis of nucleoside analogues with antitumor or antiviral activity [64]. Bock *et al.* utilised α-GISAL in the synthesis of an optically-active methylene lactone [65]. Almond prepared a number of halogenated derivative of α-GISAL [66, 67].

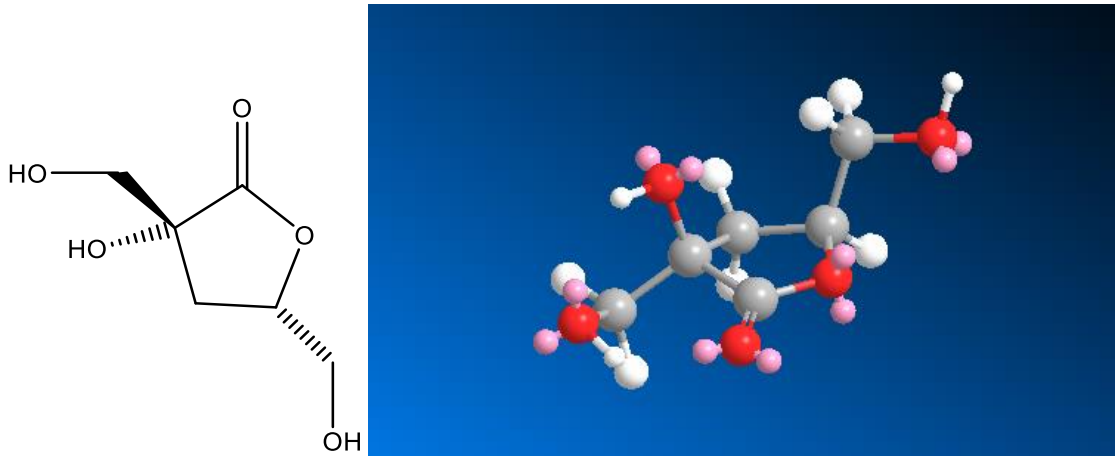
One of the main objectives of this research was to explore the protecting group chemistry of α-GISAL and to develop potentially valuable molecules for use as starting materials to prepare a number of useful platform chemicals which could be used in synthesis.

1.4.4a.2 Studies of the properties of α-GISA and its lactone

α-GISA has been widely used in studies of its metal chelating properties [68], especially the complexation with actinides [12, 69-75]. It has been demonstrated that the salts of α-GISA are able to complex metal ions including radioisotopes, which increases their solubility in water. As a consequence, another industry that undertakes work with glucoisosaccharinic acids is the nuclear waste storage, which is interested in the metal chelating properties of glucoisosaccharinic acids with radioactive elements that can form complexes within its storage repositories (see discussion below, section 1.5). GISAs are also of interest to the domestic and municipal sewage storage facilities because GISAs can form complexes with transition metals [15] that are present in such facilities and aid their leaching in to neighbouring environment [76]. In contrast to the significant work done to determine the physical properties of α-GISA very little research has been reported on the properties of β-GISA [77, 78].



A: α-GISAL



B: β-GISAL

Figure 7 Energy minimisation of α- and β-GISAL

The only difference in the structure of the two epimers is the configuration of the substituents at C2 and this gives the two molecules very different physical properties. Comparison of the 3D-structures (see figure 7) determined by MM2 energy minimisation studies) of the two epimers clearly demonstrates the *cis* arrangement of the hydroxymethylene groups in the α-GISA (Fig. 7A) and the *trans* arrangement in β-GISA (Fig. 7B). The different physical properties are most clearly demonstrated by comparing their aqueous solubilities: the α-anomer is only sparingly soluble in water whilst the β-

anomer is very soluble in aqueous solution. The arrangement of substituents will also influence the relative reactivity of the hydroxyl-groups and this is likely to be most clearly the case in their reactions with bulky protecting groups.

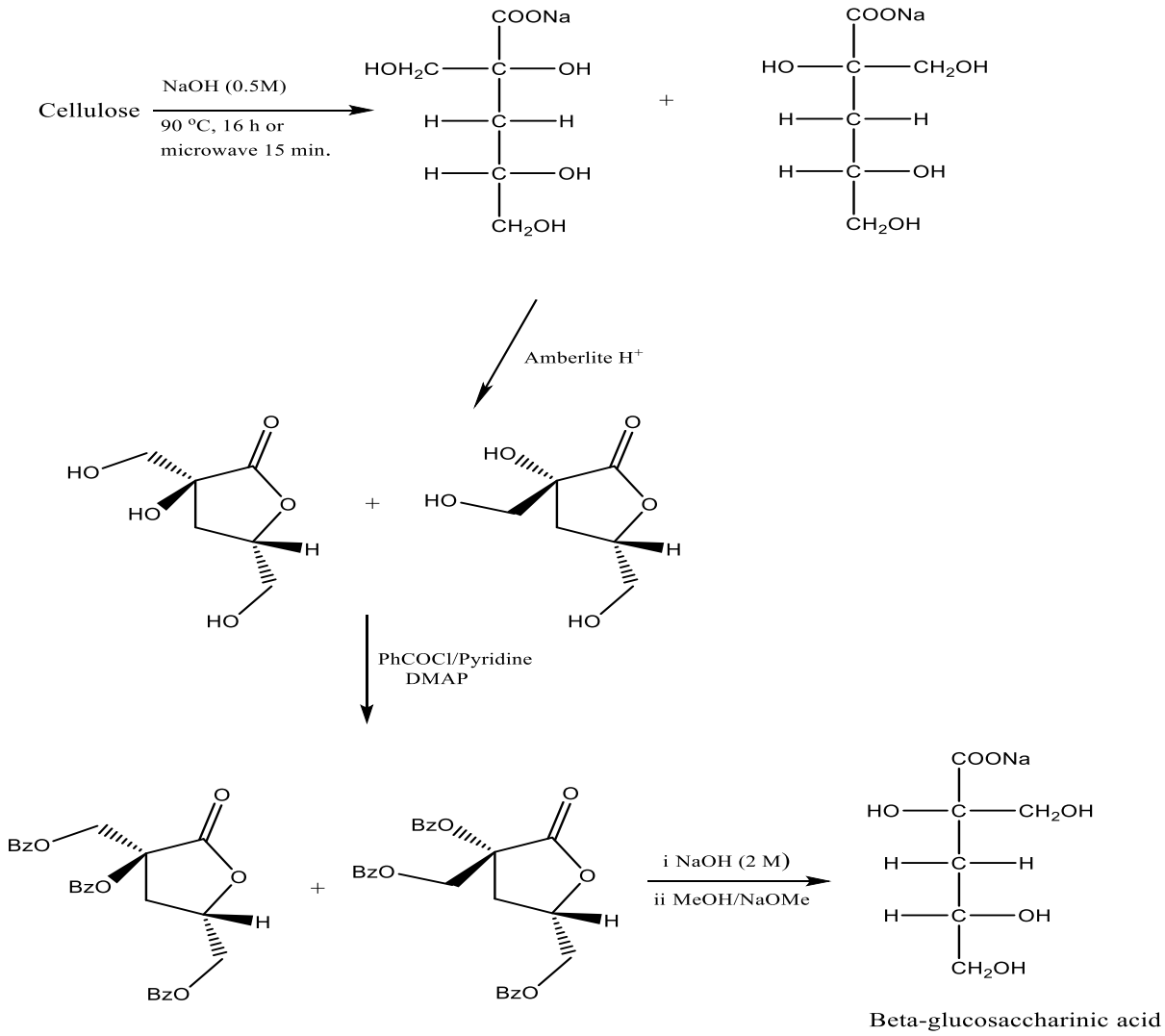
1.4.4b Synthesis of β-glucoisosaccharinic acid

In contrast to what was the case for α-GISA, there is only a very limited number of methods for the synthesis of β-GISA and these methods all require complex purification steps. The reactions involve the alkaline treatment of lactose, guaran and 4-O-substituted D-glucose analogues [78-82]. The original method reported for the preparation and isolation of β-GISA was published more than five decades ago by Whistler and BeMiller [79] who degraded guaran, a polymer of D-galacto-D-mannoglycan or lactose under alkaline and anaerobic conditions using combinations of sodium hydroxide and calcium hydroxide as the base at various temperatures from ambient to autoclaving at 120 °C. The β-GISA was isolated from reactions that were carried out at room temperature for four months, however, at higher temperatures more complex mixtures of products were obtained.

Four years after Whistler and BeMiller's publication in 1965, Feast *et al.* [81] also reported the synthesis of analytical quantities of β-GISA which were generated from the alkaline degradation of lactose in an aqueous calcium hydroxide solution. They characterised the product saccharinic acids as their trimethyl silyl derivatives using gas-liquid chromatography and reported that both α- and β-GISA were produced in approximately equal amounts, whereas, Whistler and BeMiller [79] and Whistler and Medcalf [80], in procedures where lactose was degraded in liquid ammonia, reported the production of a majority of β-GISA. The fact that Feast *et al.* only used calcium hydroxide, whereas Whistler and BeMiller used a combination of sodium hydroxide and calcium hydroxide

in their reaction could be the reason for the different product ratios. In 1968, Alfredsson and Samuelson [83], reported that a mixture β-GISA and α-GISA in approximately 3:1 ratio (188.2 *vs.* 63 mmol per 100 g) was produced, when cellulose was degraded in sodium hydroxide under anaerobic conditions. This issue of proportion has since been settled by Greenfield *et al.*[84] when they reported that, when sodium hydroxide is used as the alkaline species a higher proportion of β-GISA is produced than α-GISA, and, approximately equal proportions of both epimers are produced when only calcium hydroxide is used. In their work on the identification of chemical degradation products they reported the isolation of calcium salts of β-GISA (0.3 g) from the alkaline degradation of microcrystalline cellulose (50 g) using sodium hydroxide as base, under anaerobic condition at room temperature for thirteen days. After fractionation using preparative anion exchange column and evaporation of appropriate fractions to produce a syrup that was precipitated with calcium hydroxide to give the calcium salts.

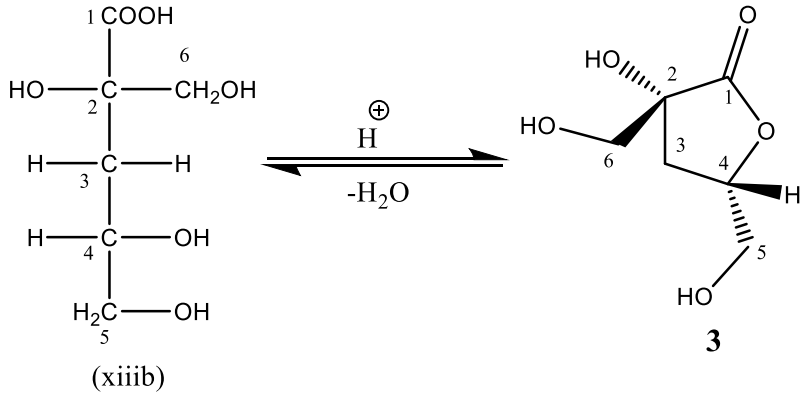
More recently, Glaus *et al.* [12] reported a method for the preparation of β-GISA by treating microcrystalline cellulose with lime water at room temperature under anaerobic conditions for periods of up to six months, and isolated pure β-GISA (220 mg) by repeatedly passing the crude mixture through an analytical high-performance anion-exchange chromatography HPAEC system and the product was characterised by MS/MS, GC-MS and NMR. Shaw *et al.* [77] recently optimised Whistler and BeMiller's method by incorporating it with the approach of Greenfield *et al.* (Scheme 1.8), to produce pure β-GISA (0.8 g) from the alkaline degradation of cellulose (200 g) with sodium hydroxide (0.5 M) under anaerobic condition at 90 ^{o}C for sixteen hours, or using microwave heating for five minutes, to generate a mixture of two epimers of GISA in a 3:1 ratio (Figure 8)



Scheme 1.8. Shaw's method for the synthesis of Na(β-GISA)

The isomers were separated as their tribenzoate derivatives using very careful normal phase column chromatography and collecting 400 fractions. The final product was recovered after removal of the esters using the Zemplen procedure. The purified β-GISA material was used to determine its aqueous pK_a which was determined to be 3.61. Almond *et al*. [85] adapted Shaw's method for the synthesis β-GISA and reported the study of its complexation properties with nickel ions.

The lack of a simple, straightforward and easily repeated method for the synthesis of β-GISA and its derivatives has led to a very limited amount of research being undertaken on β-GISA and this is an issue which will be addressed in the work reported here.



Scheme 7b. Acid-catalysed lactonisation of β-GISA to form β-GISAL.

It is clear from the literature that the original proportion of the two epimers that is present plays a key role in attempts to recover β-GISA. Furthermore, the purity of the mixture also influences the purification process: other impurities that are present in black liquor bind strongly to anion exchange columns and prevent the isolation of clean β–GISA. One of the aims of the current work was to determine if a synthetic route could be produced which would allow gram scale quantities of β-GISA to be produced. The idea was to design a robust method for the production of β-GISA. One potential starting material would be α-GISA, employing a route involving the inversion of the stereochemistry at C2, and this is the starting point chosen in the second part of the research programme described here

1.5 Roles of GISA on the storage of low and intermediate-level nuclear waste

In the nuclear industry, the main constituent of the organic matter in low and intermediate level radioactive waste is cellulose, and the degradation of cellulosic material in the underground concrete storage facilities used to contain nuclear waste is known to create strong alkaline conditions (pH 12-13) which result in the formation of saccharinic acids[12]. The most significant degradation products are (2S, 4S)-2, 4, 5-trihydroxy-2-(hydroxymethyl) pentanoic acid (α-D-glucoisosaccharinic, α-GISA) acid and (2R, 4S)-2, 4, 5-trihydroxy-2-(hydroxymethyl) pentanoic acid (β-D-glucoisosaccharinic acid, β-GISA), and as was discussed above, these are strong complexants of many cations of lanthanide and actinides [70]. Cellulose degradation products may have a damaging effect on the barrier function of concrete and promote the migration of heavy metal ions or radionuclides [86] because they enhance the solubility and decrease the sorption of these radionuclides by complexation [71]. The long-term equilibrium concentration of α and β-GISA in cement pore water is an important parameter in the assessment of the role of cellulose in the safety of a low and intermediate level waste (L/ILW) repositories [13, 71, 87].

The exact nature of the degradation products that are formed strongly depends on the nature of the solution the cellulosic materials are exposed to, and the length of time the cellulose is exposed [87]. The presence of calcium ions in cement catalyses the benzilic acid rearrangement to favour the production of glucoisosaccharinic acids and the relatively low temperature also favours the formation of glucoisosaccharinic acids making them the main degradation products in the alkaline repository environment. It has previously been shown that GISAs can form stable complexes with the tri-and tetravalent radionuclide ions of americium (Am^{3+}) and plutonium (Pu^{4+})[70], which influences their sorption on the cement phase. This could lead to an enhanced release

of these radionuclides in the neighbouring environment, which makes GISAs a threat to the integrity of such facilities [68, 72-75].

1.6 Protecting group chemistry of glucoisosaccharinic acids.

Exploring the protecting group chemistry of glucoisosaccharinic acids is a way of generating derivatives of glucoisosaccharinic acids which would be potential starting materials for use in synthetic chemistry. The readily available α-GISAL **2** (Scheme 7a) has two primary hydroxy groups at positions 5 and 6, that should in theory have similar reactivities, except that the hydroxy group 6 is in close proximity (beta) to the carbonyl carbon at position 1, which will withdraw electron density. The molecule also contains a tertiary hydroxy group bonded at C2 that is immediately adjacent (alpha) to the carbonyl carbon, which will also withdraw electron density by inductive effects, and this hydroxyl group is sterically hindered. Finally, the remaining functionality in GISA is the lactone ring. These features of **2** are the same in β-GISAL **3**, which has the opposite configuration at C2 and the same configuration at C4 which makes them epimers (2*S*, 4*S*-α–GISA and 2*R*, 4*S*-β-GISA).

Protecting groups are chemical entities that are temporarily employed during the synthesis of compounds and they are especially useful when manipulating the functionality of multifunctional molecules such as carbohydrates. Frequently, syntheses involve many steps, each employing different reaction conditions that could affect both target and non-target sites within a molecule in a planned reaction. Protecting groups are employed in order to block reaction at the non-target site and must be stable to both the reagents and conditions of such reactions, and then must be amenable to removal at the end of the reaction.

In this work attempts will be described to attain full regiochemical control over the selective protection and deprotection of the three hydroxyl groups of glucoisosaccharinic acid (at position 2,5 and 6) individually or in pairs, in single or multiple step reaction procedures. This thesis will also describe how the various protection strategies can be used to develop molecules with different hydrophobicities that can be employed in the separation of glucosaccharinic acids from black liquor and for the production of their derivatives as platform compounds for further synthesis.

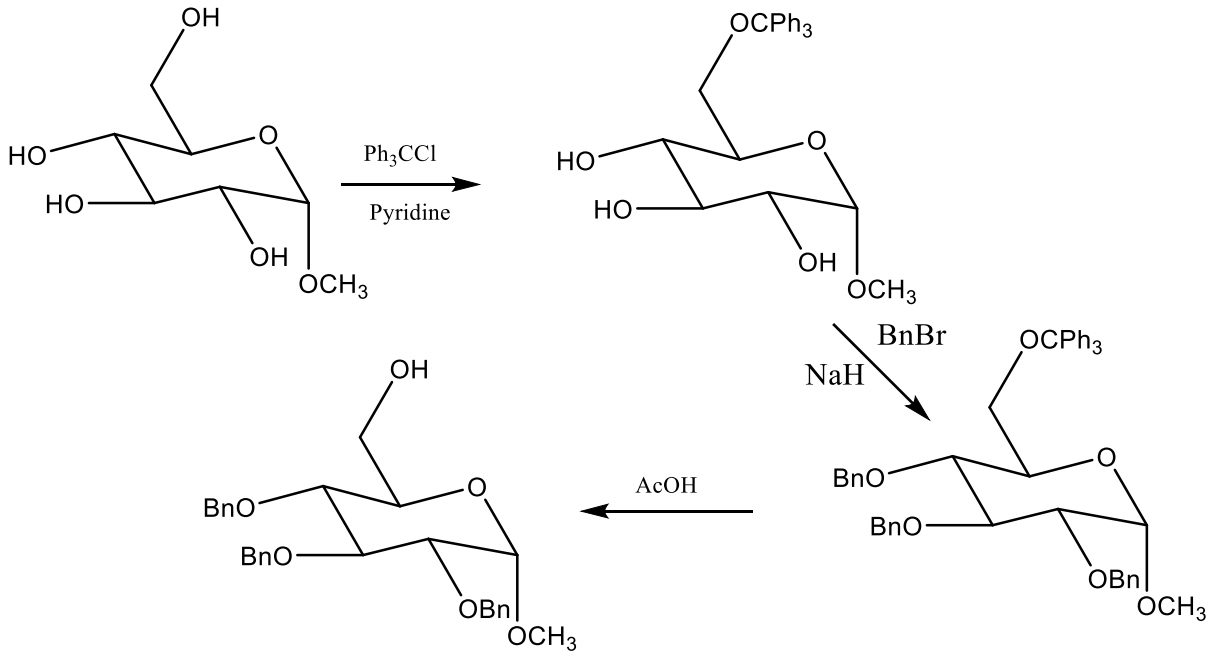
The following strategies were developed for the regioselective protection of the hydroxyl groups of α-GISAL **2** (See Table 5 in Chapter 3), a number of which were subsequently applied to a mixture of α-GISAL **2** and β-GISAL **3**:

1. The preparation of 2,5,6-tri-O-protected-α-GISAL in a single step, using the same protecting group or in two step procedures using orthogonal protection and different protecting groups;
2. The preparation of di-O-protected-α-GISAL (5,6-di, 2,6-di and 2,5) in single or multiple step one-pot sequential procedures;
3. The preparation of mono-O-protected-α-GISAL.

These strategic approaches were carried out using the protecting groups that are commonly applied to protect hydroxyls in monosaccharides (Table 2) [67], each protecting group was chosen for a specific purpose for the realisation of the set objectives.

1.6.1 Protecting group chemistry applied to carbohydrates

A range of different protecting groups is routinely employed in carbohydrate chemistry [88-90] with acetals (isopropylidenes and benzylidenes) esters (acetates and benzoates) and ethers (alkyl, aryl and silyl) being some of the most frequently employed. A list of protecting groups and the reaction conditions employed to add the different protecting groups is provided in the table below. The scientific literature includes a number of different protecting group strategy which allow for the regioselective protection of different hydroxyl groups in monosaccharides with the most notable including the use of the trityl-group to protect primary hydroxyl-groups of hexoses and the use of an orthogonal protecting group, such as benzyl for the protection of the rest of the secondary hydroxyl group to produce a fully protected compound which is followed by deprotection of the trityl group.

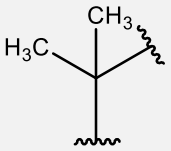
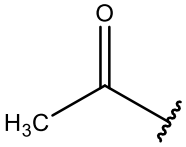
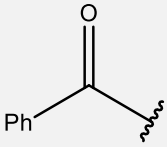
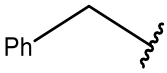
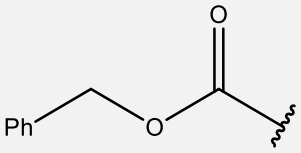
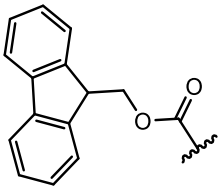


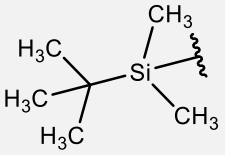
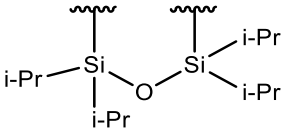
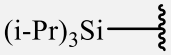
Scheme 1.9: protection of different hydroxyl groups in monosaccharides.

One of the issues with the choice of protecting groups for use with polar molecules is the solubility of the substrate in organic solvents. With carbohydrates it is frequently

necessary to use polar solvents and, as can be seen from the examples included in the table below, pyridine is often a solvent of choice as it can dissolve the polyhydroxylated aldehydes and ketones and, at the same time, it serves at a base catalyst in a wide range of reaction conditions. It is frequently the case that the addition of a protecting group increases the hydrophobicity of molecules and their solubility in organic solvents, as such, the derivatization can support the further manipulation of the molecules in synthetic procedures.

Table 2: A list of protecting group and reaction condition commonly used in carbohydrate synthesis.

Protecting groups	*Structures*	*Reaction conditions*
Acetal		Acetone, Con. H_2SO_4, $CuSO_4$, 3 h, RT, 62% [61]
Acetyl (Ac)		Ac_2O or AcCl, DMAP, Pyr; 80 ^oC, 95 % [91]
Benzoyl (Bz)		BzCl, Pyr, DCM, 3 h, 96% [92]
Benzyl (Bn)		BnBr, TBAI, NaH, THF, 20 ^oC, 3 h, 99% [91, 93]
Benzyloxycarbonyl (Cbz)		CbzCl, Pyr; DCM, RT, 24 h 87% [94]
Fluorenylmethoxycarbonyl (Fmoc)		FmocCl, Pyr; 20 ^oC, 40 min, 81-96% [95]

tert-Butyldimethylsilyl (TBDMS)		TBDMSCl, Et_3N, DMAP, DCM, RT, 3 h, 77-80% [96]
1,1,3,3-Tetraisopropyldisiloxane-1,3-diyl (TIPDS)		TIPDS, Pyr, RT, 24 h, 50-83% [97, 98]
Triisopropylsilyl (TIPS)		TIPSCl, imidazole, DMF, 12 h, 98% [99]

- Acetal protection

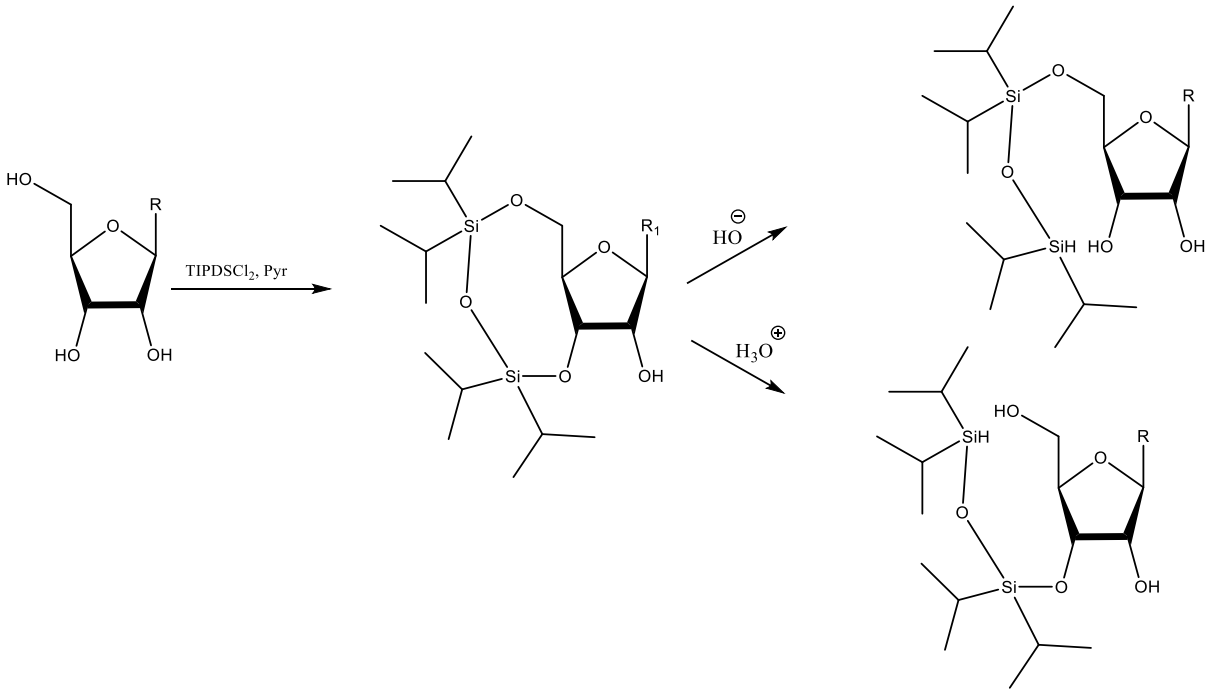
The acetal group (isopropylidene group) is very useful for the protection of vicinal hydroxy groups in carbohydrates synthesis such as oligosaccharides and in the preparation of rare monosaccharide and related compounds such as L-ascorbic acid (vitamin C) [100]. It was employed to protect the hydroxyls at position 2 and 6, where it gives a five-membered cyclic ring. It is formed by the reaction of vicinal hydroxyls with the ketone in the presence of acid. It is generally stable under basic and catalytic hydrogenolysis conditions, but acetals are acid labile [89]. The acetal protecting group has previously been used to protect α-GISA by Florent *et al.* [17]

- Ether protecting group.

It is sometimes necessary to use a protecting group for a carbohydrate hydroxyl group that is stable to both acidic and basic conditions and ethers fall into this category. Benzyl ethers are very useful protecting groups in synthetic carbohydrate chemistry [101, 102], they are stable to both acidic and basic conditions, but, they can be removed by hydrogenolysis over a palladium catalyst under neutral conditions [103]. Benzyl ethers are

moderate in size and in the work to be described in the following chapter, our attempts to protect all the three hydroxyl groups of α-GISAL in a single or in a two-step strategy will be discussed as will work centering on using benzyl ethers as an orthogonal protecting group.

The silyl ether groups; TBDMS, TIPS and TIPDS, have been widely used for the regioselective protection of primary hydroxyl groups of carbohydrates [88]. The ability of silyl protecting groups to be converted to another functional group directly without going through a deprotection step is a valuable transformation in the synthesis of glucoisosaccharinic acids as platform chemicals. Silyl-protected alcohols have been directly converted to acetates [104, 105] aldehydes [106], bromides [107, 108] and ketones [109] without a prior deprotection step to liberate the alcohol. The greater bulkiness of TIPS makes it more stable than TBDMS to acid hydrolysis. However, the TIPS group is more stable than TBDMS to basic condition [99], to the extent that a TBDMS protection can be removed in the presence of a TIPS on the same compound under basic conditions [88]. TIPDS was selected for its particular value in ribonucleoside chemistry for the selective substitution of the 3',5'-hydroxyl groups pairs and for the release of these groups specifically under acidic and basic conditions (Scheme 10).



Scheme 1.10: TIPDS protection in ribonucleoside chemistry.

The cyclic TIPDS ether can be removed like TBDMS and TIPS in the presence of fluoride ions [110].

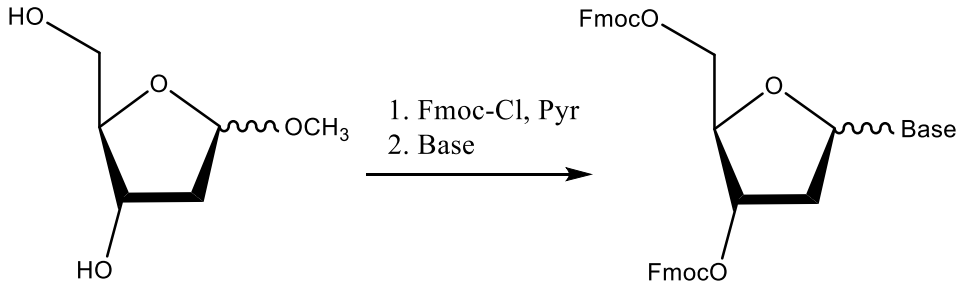
TBDMS was selected for the regioselective protection of the primary hydroxyl groups of α-GISAL at positions 5 and 6 in a single step, and then an orthogonal protection of the tertiary hydroxyl at position 2. TIPS was selected to protect the most sterically unhindered and reactive hydroxyl group between the two primary hydroxyl groups, so as to understand their various reactivity that could potentially be beneficial in our pursuit of generating molecules which allow the two epimers to be separated from a mixture. Whereas the TIPDS was particularly selected to form a cyclic ether across two hydroxyl groups of the molecule based on their relative configuration, which could possibly lead to the separation of the β-GISA from a mixture due to their different configurations where either it reacts or remains unreacted.

- Ester protecting groups.

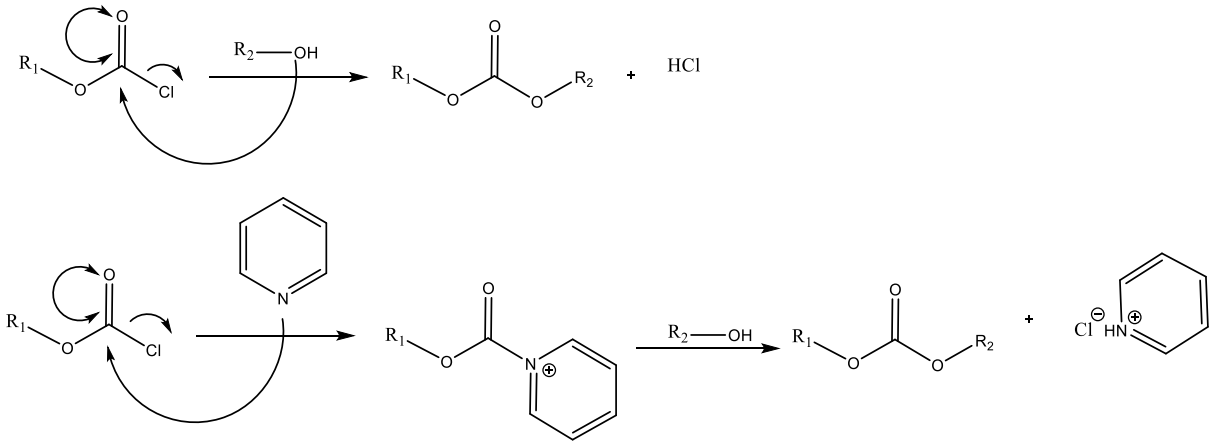
Acetate and benzoate are base-labile protecting groups that are useful for protection under conditions where other protecting groups are not stable i.e. in acidic conditions where acetals and most silyl ethers cannot be employed. Acetates are commonly used within the analysis of carbohydrates where they are employed as a derivatisation step in their GC-MS analysis. They are small groups and, as such, are sterically undemanding, but can have a tendency to migrate [111, 112]. Benzoyl esters have chromophores and have been used in the protection of β-GISA by Whistler [79], Feast [81] and Shaw [77]. They are introduced by the reactions of the acid chlorides in pyridine, which both catalyses the reactions and neutralises the liberated acid. Both the acetyl and benzoyl groups were employed as protecting groups in the current work.

- Carbonates

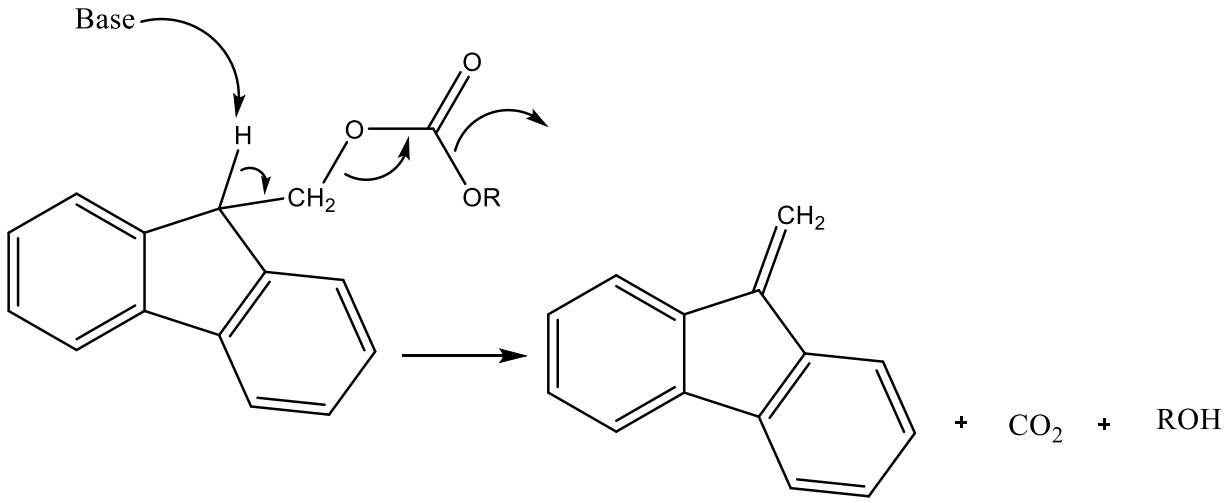
Fluorenylmethoxycarbonyl (Fmoc) and benzyloxycarbonyl (Cbz) are carbonate protecting groups, usually employed for regioselective protection of accessible alcohols [113, 114] . Protection of alcohols using Cbz to afford the corresponding benzyl carbonate is typically achieved in the presence of an organic base such as pyridine, and for a more selective protection in the presence of alcohols, the protection is achieved using DMAP [115] . It can even be used for the efficient protection of hindered electron-deficient hydroxyls if NaH is used as the base and working with either an ether or DCM as solvent. The Cbz protecting group is stable under both basic and acidic conditions but, like benzyl ethers, it is susceptible to catalytic hydrogenolysis [116]. Fmoc is base-labile and can be easily removed under mild basic conditions. Ben-Hattar and Jiricny [113] used Fmoc for the protection of the 3’ and 5’ hydroxyls of deoxyribose for the introduction of a modified base.



Scheme 1.11. Fmoc protection reported by Ben-Hattar and Jiricny



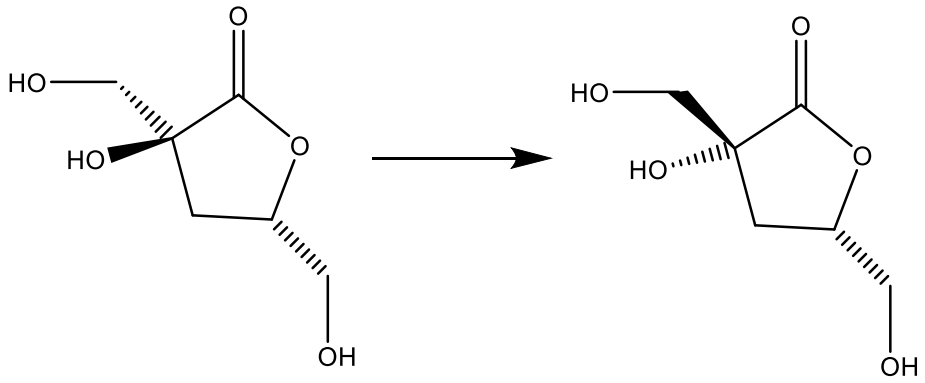
Scheme 1.12: Pyridine-catalysed and uncatalysed acyl transfer reactions of the chloroformate groups.



Scheme 1.13: Removal of an Fmoc protecting group under mild basic conditions [95].

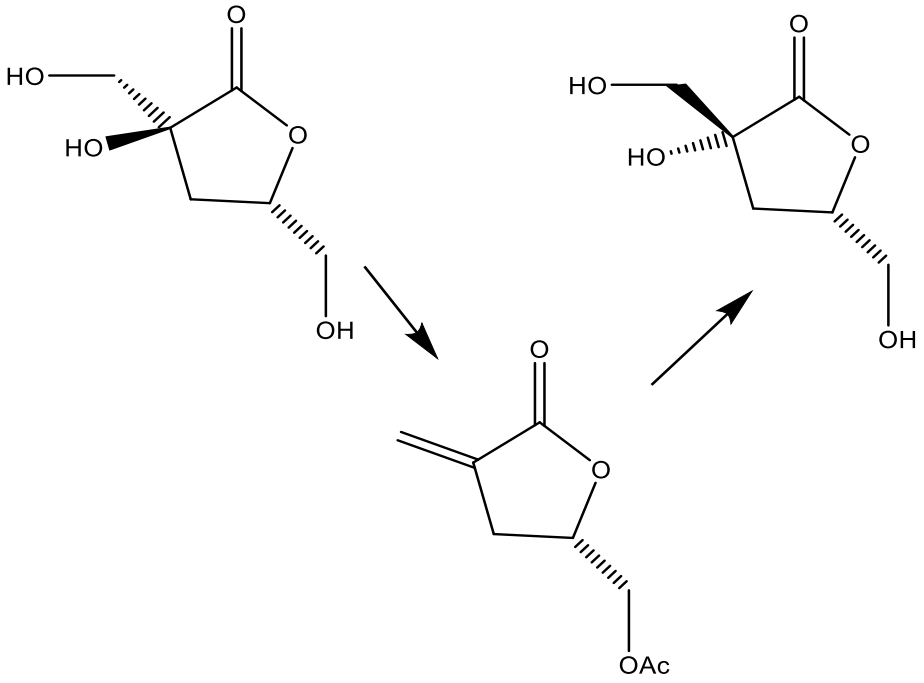
1.7 Inversion of stereochemistry of chiral alcohols.

In a number of preliminary studies performed by researchers at the University of Huddersfield, attempts were made to convert α-GISAL into β-GISAL by inversion of the stereochemistry at C2 (Scheme 1.14).



Scheme 1.14

Work by Almond described attempts to use the Mitsunobu reaction to directly invert the stereochemistry at C2, but without success. In an alternative approach, Almond activated the hydroxyl group at C2, as either a halogen or as a sulphonate ester, in order to promote inversion by substitution, but again, without any success. One method which gave partial success was the conversion of the α-GISAL into the methylene lactone using procedures that were originally reported by Bock *et al.* [65] (Scheme 1.15) and its subsequent dihydroxylation using Sharpless conditions, which gave a very low yield of both α and β-GISA.



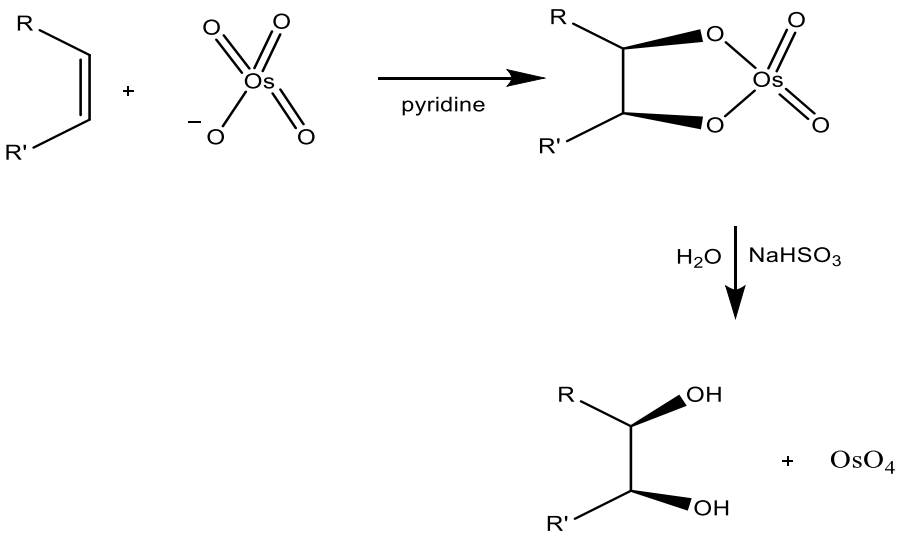
Scheme 1.15

Unfortunately, Almond [66] reported problems with the reaction sequence and in particular with poor yields in the production of the α-methylene lactone and thus, this avenue of work was abandoned. As part of the current work, an investigation was undertaken to understand the chemistry of the synthesis of the α-methylene lactone and its subsequent transformations, including its polymerisation and dihydroxylation.

The dihydroxylation of alkenes is a very common reaction and for which a range of catalysts has been developed including catalysts which can promote the stereospecific *syn*-addition of two hydroxyl-groups. The success of this work, which was developed by Barry Sharpless culminated in his being jointly awarded the Nobel Prize for Chemistry in 2001 [117]. The metal-catalysed asymmetric dihydroxylation of olefins using the Sharpless reaction has been extensively reviewed [118]. The Sharpless dihydroxylation is an example of a set of reactions known as ligand-accelerated reactions in which an increased rate of reaction occurs when the substrate coordinates as a ligand to a metal centre along with a second chiral ligand. The second ligand enables the complex to act

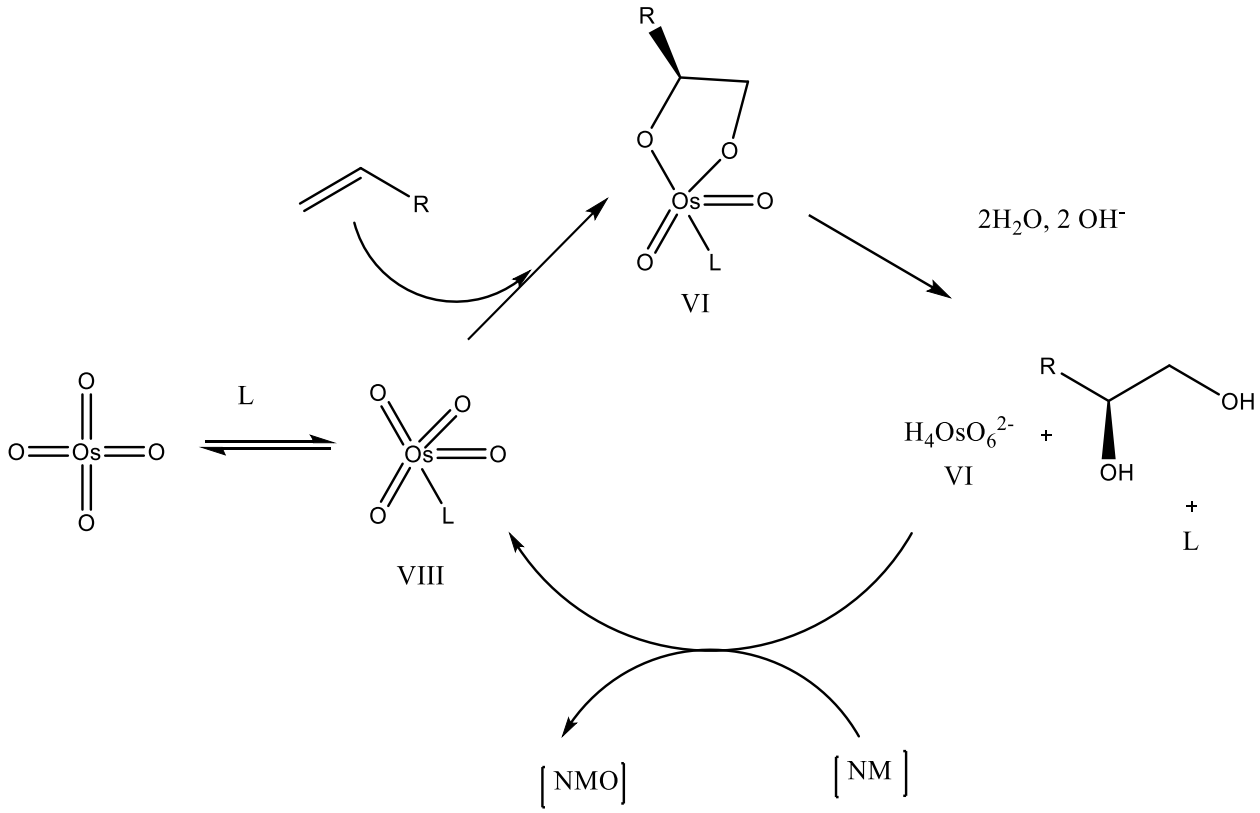
as a chiral catalyst. In the Sharpless dihydroxylation reaction this can favour the production of a particular stereochemically-defined dihydroxylated product.

Historically, the *syn*-dihydroxylation of alkenes was carried out by reacting an alkene with a stoichiometric equivalent of osmium tetroxide. The reaction is thought to proceed via a 1,3-dipolar addition (scheme 1.16) to give an osmate ester (osmylate).



Scheme 1.16: The stoichiometric osmylation of an alkene [118]

The osmylate is then hydrolysed and this is normally performed by adding a reducing agent which reduces the Os(IV) that is generated in the reaction to osmium metal and this prevents the reverse reaction from occurring. Osmium is a very expensive metal and the reagent osmium tetroxide is extremely toxic and it was not very long before catalysts were added, along with a stoichiometric amount of a cooxidant, to reduce the amount of osmium tetroxide needed to achieve complete reaction under catalytic conditions. One of the first procedures introduced by the Upjohn company [119-121] used N-methylmorpholine N-oxide (NMO) as an oxidant regenerate Os(VIII), in the Upjohn procedure only 1 mol % of OsO_4 was required to effect complete oxidation of an alkene (scheme 1.17).



Scheme 1.17: Catalytic cycles for the asymmetric dihydroxylation using NMO.

One of the draw-backs of the basic reaction is that addition can occur on both faces of the alkene and, as a consequence, racemic mixtures of products is obtained. Sharpless introduced his asymmetric dihydroxylation in order to avoid the production of racemic products. In the asymmetric dihydroxylation a single enantiomer of a chiral ligand is added which coordinates to the osmium centre. The chiral ligand of choice depends on what the preferred stereochemical outcome of the reaction is, but the most frequently employed ligands are derivatives of the alkaloid dihydroquinidine (DHQD) [122]. The chiral ligand coordinates to the osmium and one face of the alkene and this directs attack from the opposite enantioface.

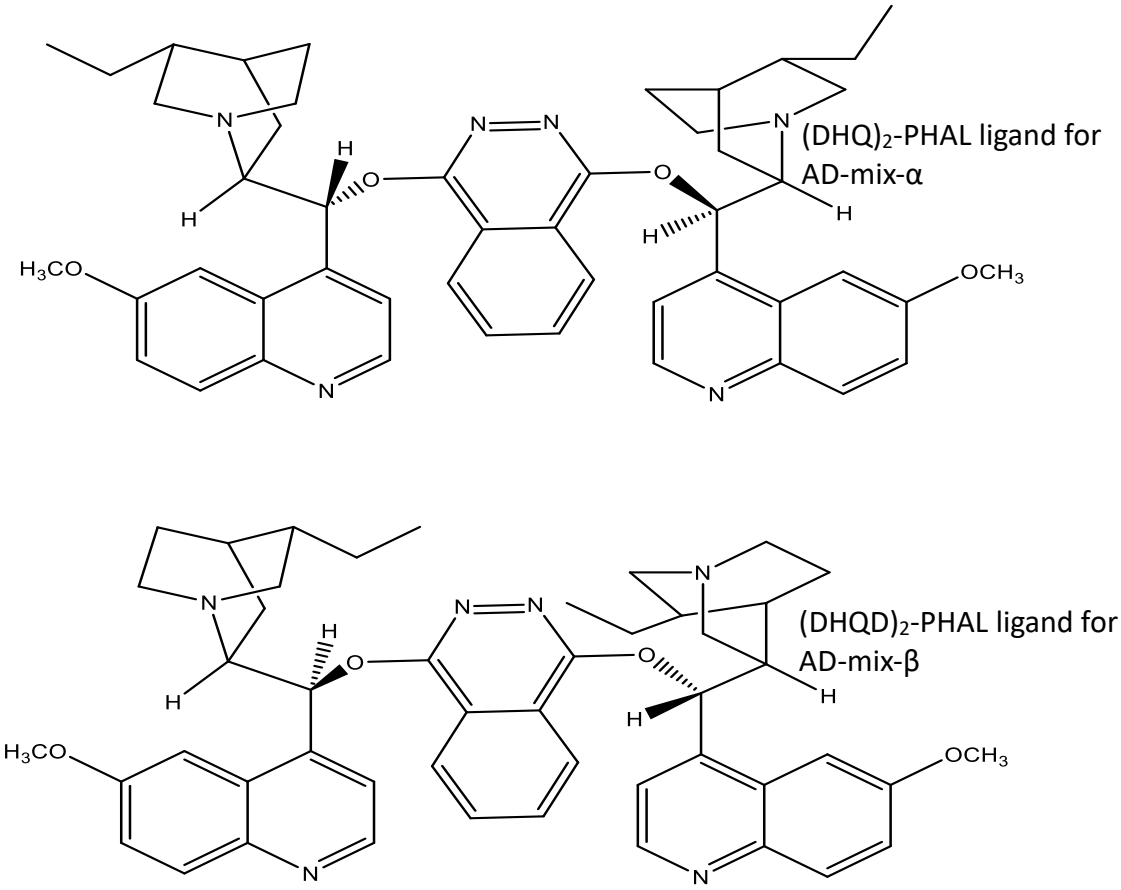
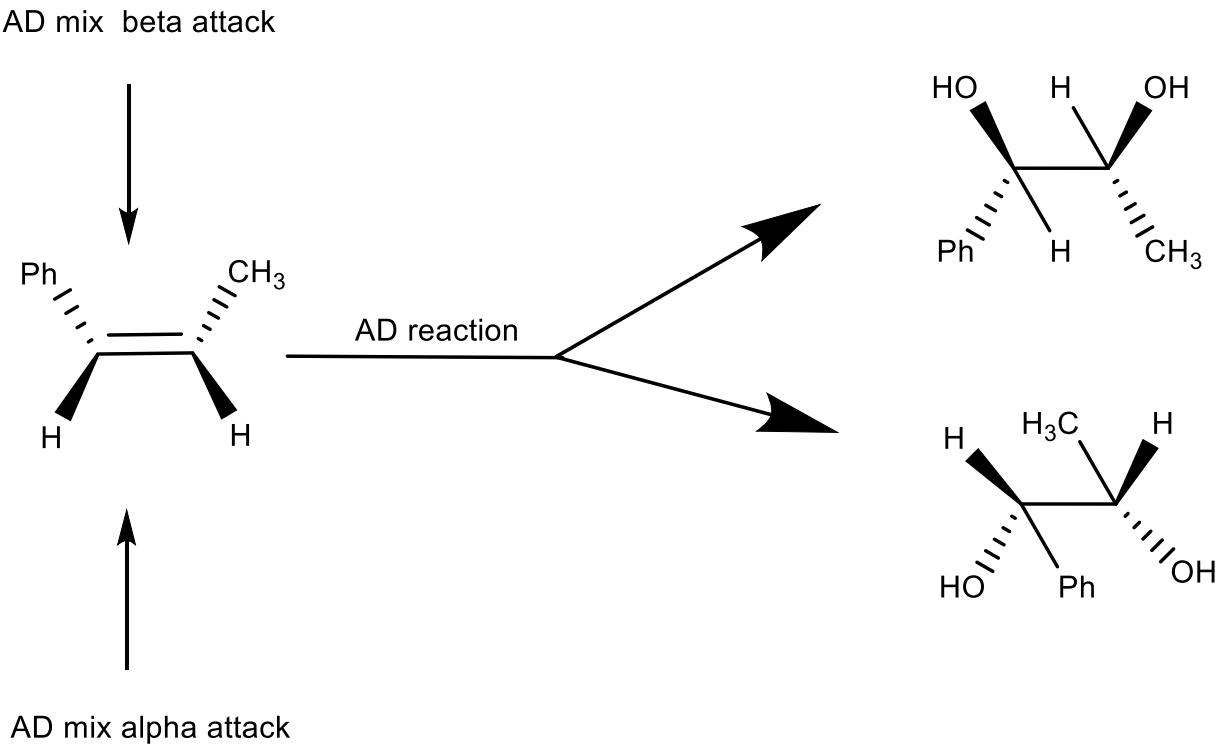


Figure 8 AD-mix α and AD-mix β [123]

The cinchona alkaloid backbone structure of the ligand is key in providing high enantioselectivity, and this could be due to the presence of the binding pocket formed by the aromatic systems acting as a floor with the additional substituted quinoline serving as a wall [124]. The aromatic rings provide stacking stabilization: the natural ligand DHQD gives rise to reaction on one face while dihydroquinine (DHQ) favours reaction at the other face. This enhances the rate and thus the enantioselectivity of the desired reaction significantly due to the stabilisation of the transition state caused by the aromatic stacking interactions which are especially significant with aromatic containing molecules. Therefore, the enantioselectivity of the reaction depends on the AD-Mix used (alpha contains DHQ and beta-DHQD) [125, 126] and the nature of the olefin used for the reaction. The *cis*-disubstituted olefin experiences steric hindrance that results in a low enantiomeric excess [126] with AD-mix β (Scheme 1.18). This implies that the attack on the alkene is favoured by the steric configuration and faces as below;



Scheme 1.18: The mnemonic device for predicting the enantioselectivity in AD reactions [126]

These commercially available mixtures, which contain the chiral auxiliary and the oxidant $K_3Fe(CN)_6$ [127] give organic chemists the ability to choose which diol they synthesise. In the context of the research being described here, the aim was to explore if the asymmetric dihydroxylation of the α-methylene lactone could be used to provide a robust synthetic methods to β-GISA.

1.8 Statement of Aims

The main aim of the current work is to develop glucoisosaccharinic acids as potential platform chemicals. To achieve this target two different programmes of work were attempted. The first was to explore methods for selectively protecting the different hydroxyl-groups in glucoisosaccharinic acid. The objective of this first section of work was to identify synthetic methods that could be used to regioselectively protect either one, two or all three of the hydroxyl groups using one-step procedures. In the first

instance, the protecting groups that were employed were those that have been used in classical carbohydrate chemistry (and in protection of the sugar components of nucleic acids). The majority of the initial exploratory reactions were carried out using α-GISA, which is readily available from the reaction of lactose with aqueous alkali. In extending these one-pot reactions, the project attempted to establish if it was possible to add orthogonal protecting groups regioselectivity to produce initially di-protected α-GISA derivatives. If successful then the lessons learnt in this section were to be used to direct attempts to generate tri-protected species containing different protecting groups.

The second aim of the project was to provide a robust and simplified method for producing or isolating β-GISA. A number of different approaches had been identified to achieve this aim. The first was to transform pure α-GISA into β-GISA. Previous attempts to invert the stereochemistry at C2 using stereospecific substitution reactions have failed (Michael Almond). In the current work attempts were made to invert the stereochemistry at C2 through performing an elimination, to generate an sp^2 centre at C2, followed by performing a stereoselective oxidative addition at C2 resulting in inversion of configuration.

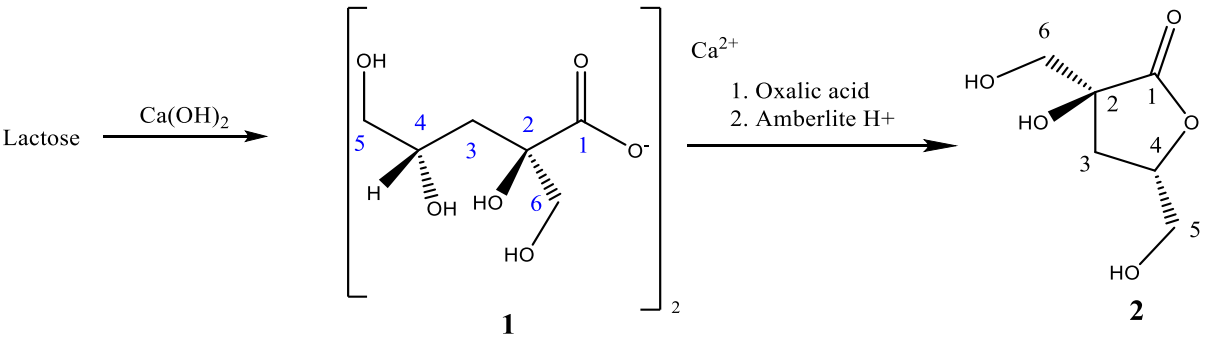
The final section of work focused on producing mixtures of α and β-GISA that are rich in the β-GISA e.g. from reactions of cellulose with $Ca(OH)_2$ and then to use the protecting group chemistry, developed in the early part of the project, to develop mixtures of molecules which could easily be separated. In this section of work, particular emphasis was made on trying to develop bifunctional protecting groups, which could preferentially react with one of the epimers. It was hoped that the cis-arrangement of the primary hydroxyls in α-GISA could be protected by a hydrophobic bifunctional

protecting group, the product of which would be extracted from an aqueous phase whilst leaving the unreacted β-GISA in solution.

2.0 Results and Discussion.

2.1 Synthesis of glucoisosaccharinic acids

The main objective of this research was to identify methods for utilising cellulosic waste streams in the production of platform chemicals, with the focus being on developing strategies for isolating derivatives of α-glucoisosaccharinic acid, which can easily be employed as a reagent in synthesis. A second aim was to provide a robust route for the production of β-glucoisosaccharinic acid. As mentioned in the introduction, during paper production, the processing of wood pulp with aqueous alkali generates large quantities of black liquor that contains an approximately equal amount of alpha and beta-glucoisosaccharinic acids, which are present amongst small amounts of a large number of other hydroxylated aliphatic carboxylic acids. The first part of the work undertaken in this research was to generate stocks of α-glucoisosaccharinic acid and to generate mixtures of α- and β-glucoisosaccharinic acid that are of a similar consistency to that of black liquor. The procedure that was employed for this synthesis was developed by Whistler and is routinely used to generate pure alpha-glucoisosaccharinic acid [16].



Scheme 2.1: Whistler method for the preparation of pure α-glucoisosaccharinic acid.

In this work several modifications were made in an attempt to improve the overall yield of α–glucosaccharinic acids and to reduce the reaction time. In the original procedure, calcium hydroxide (13.5 g) was added to a solution of lactose (50 g in 500 mL of water)

which was left stirring at room temperature for 3 days before being heated for 10 h at 90 ^{o}C. It was not clear why the reaction was stirred at room temperature before adding the calcium hydroxide and it was suspected that this may have been a legacy of when the procedure was used with polymeric substrates which normally be allowed to swell before being reacted with a base. In the procedure developed in the current research, the volume of water reduced (300 mL) and the reaction mixture, once prepared, was immediately heated for 18 h at 90 ^{o}C. Upon cooling, a slightly improved yield of the desired Ca(α-GISA)$_2$ **1** was obtained (+5%), but the reaction was completed in a much shorter time. After the solid α-GISA had been recovered by filtration a dark brown solution was left. It was recognised that this solution was a valuable source of saccharinic acids and analysis by HPAEC-PAD indicated that it contained a mixture of Ca(β-GISA)$_2$ and Ca(α-GISA)$_2$ in a 3:1 ratio, along with small amounts of other impurities.

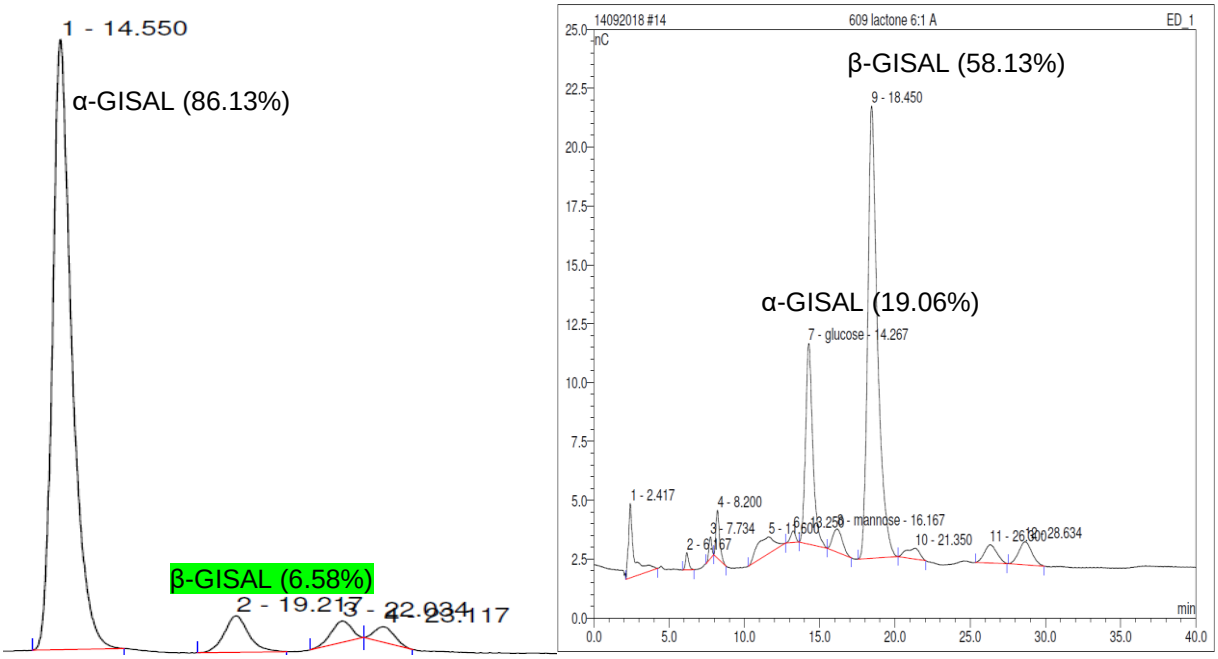


Figure 9 HPAEC-PAD chromatogram of mixture of β-GISAL & α-GISAL with some impurities in the filtrate and that of precipitated pure α-GISAL with traces of β-GISAL.

It is the difference in aqueous solubility of the calcium salts of the two diastereoisomers that causes Ca(α-GISA)$_2$ to preferentially precipitate from a concentrated aqueous solution. Ca(β-GISA)$_2$ has a much higher solubility in aqueous solution and pure samples cannot be recovered directly from the reaction. A crude mixture of solid Ca(β-GISA)$_2$ and Ca(α-GISA)$_2$ was obtained from the liquid after using a combination of rotary evaporation and freeze-drying to remove the remaining liquid. Freeze drying was necessary to remove trace levels of water, which would otherwise have interfered in reactions involving activated carbonyl compounds. The purity of the Ca(α-GISA)$_2$ **1** was determined using ^{1}H-NMR spectroscopy (Figure 10),where the chemical shift data matched those given in the literature [12, 128, 129]. The NMR analysis confirms that the desired product was produced in high purity (>95%) with only trace levels of other glucosaccharinic acids being present.

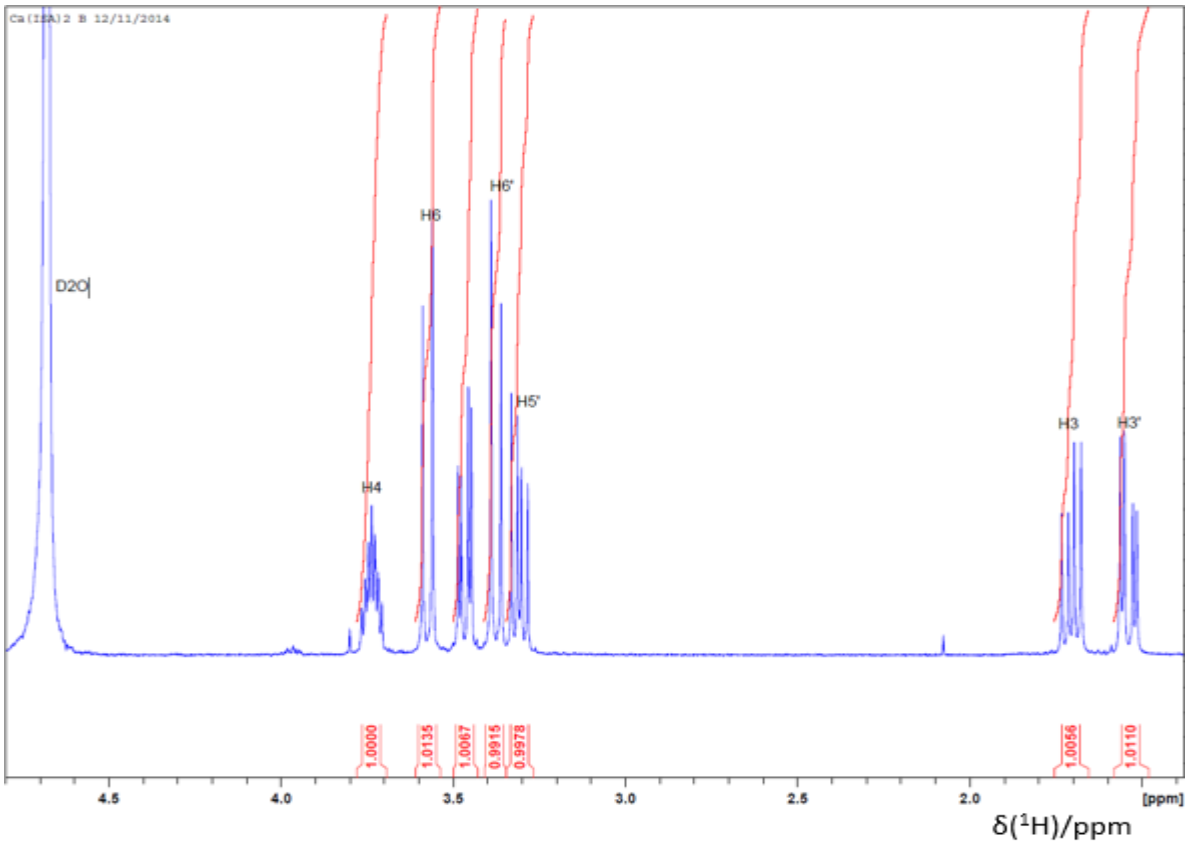
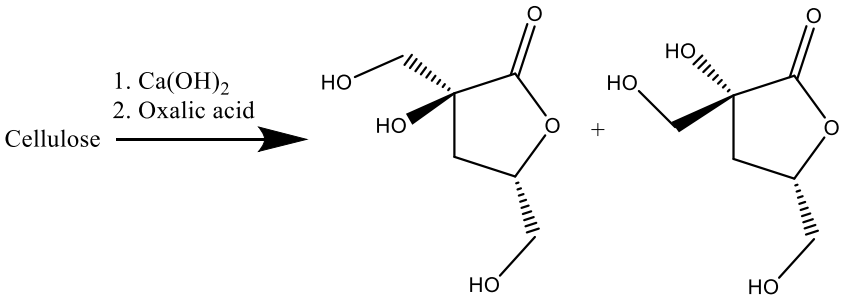


Figure 10 ^{1}H NMR of calcium glucoisosaccharinate recorded in D_2O.

In a separate experiment, the modified Whistler method which was described above, was repeated but replacing lactose with cellulose which generated an aqueous solution containing approximately equal amounts of Ca(α-GISA)$_2$ and Ca(β-GISA)$_2$ as reported by Shaw *et al.* [77]. All other variables were kept the same.



2 **3**

Unfortunately, the calcium salts of GISAs are very hydrophilic and only have limited solubility in organic solvents. Before they could be used in synthesis it was necessary to improve their solubilities in organic solvents. In the next step, the GISAs were converted to their corresponding lactones *via* an acid-catalysed intramolecular self-esterification to form α-glucoisosaccharino-1,4-lactone (α-GISAL **2**) when starting from Ca(α-GISA)$_2$, or a mixture of **2** and β-glucoiosaccharino-1,4-lactone (β-GISAL **3**) when starting from a mixture of the calcium salts of α- and β-GISA.

It is worth noting that the NMR spectra can easily distinguish between the two epimers. In α-GISAL the two H3 protons occur at similar chemical shifts whereas in the β-GISAL the two H3 protons occur at significantly different chemical shifts. In an attempt to understand why this is the case, energy minimised structures were calculated using molecular mechanics (MM2-level of theory, Figure 11). The first thing to note is that in the minimised structures the two epimers are predicted to have a small, but significant, difference in stability: the α-GISA total energy is calculated to be 10.08 kcal/mol whilst

the β-isomer is calculated to have a total energy of 4.34 kcal/mol and this may reflect the greater steric clashes present in the α-GISA.

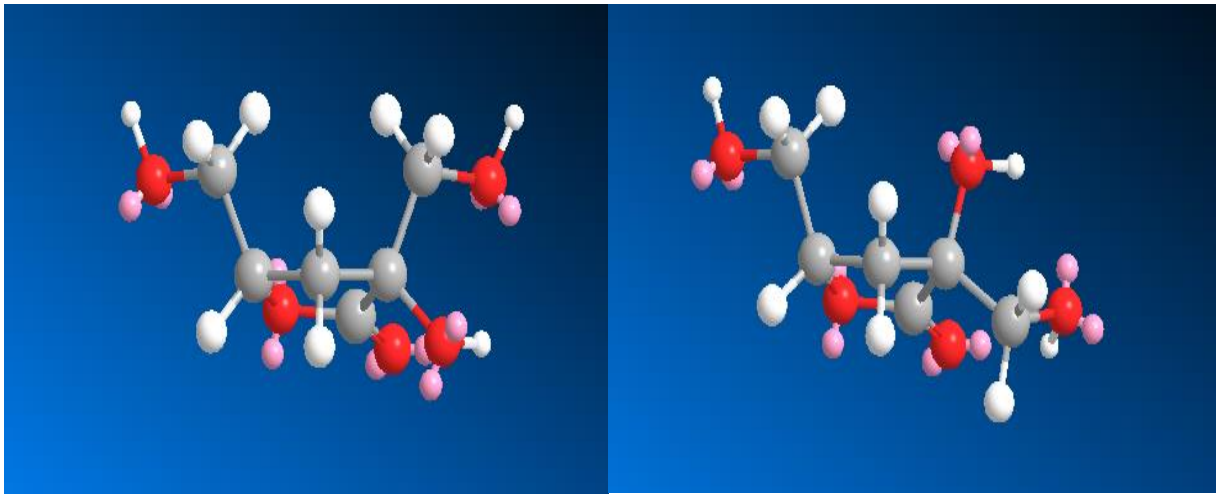


*Figure 11. The energy minimisation structures of α-GISAL **2** and β-GISAL **3***

In the α-GISAL minimised structure, when viewed with the C3-methylene group pointing forward, C2-C3-C4 are approximately co-planar and the two hydroxy methylene groups are on the same face of the lactone ring with one of the C3-methylene hydrogens located in a hydrophobic pocket aligned with the methylene groups of C5 and C6, whereas the other hydrogen points into space where it will be exposed to solvent. In β-GISAL the two hydroxy methylenes are on opposite faces of the ring. Now, in the energy minimised structure, when viewed with the C3-methylene group pointing forward, again C2-C3-C4 are located approximately co-planar and the top H3 is sandwiched in an eclipsed conformation between a hydroxymethylene and the electron ring oxygen of the C4-hydroxyl. The unsymmetrical arrangement of neighbouring groups must shield one of the H3s whilst at the same time deshield the other.

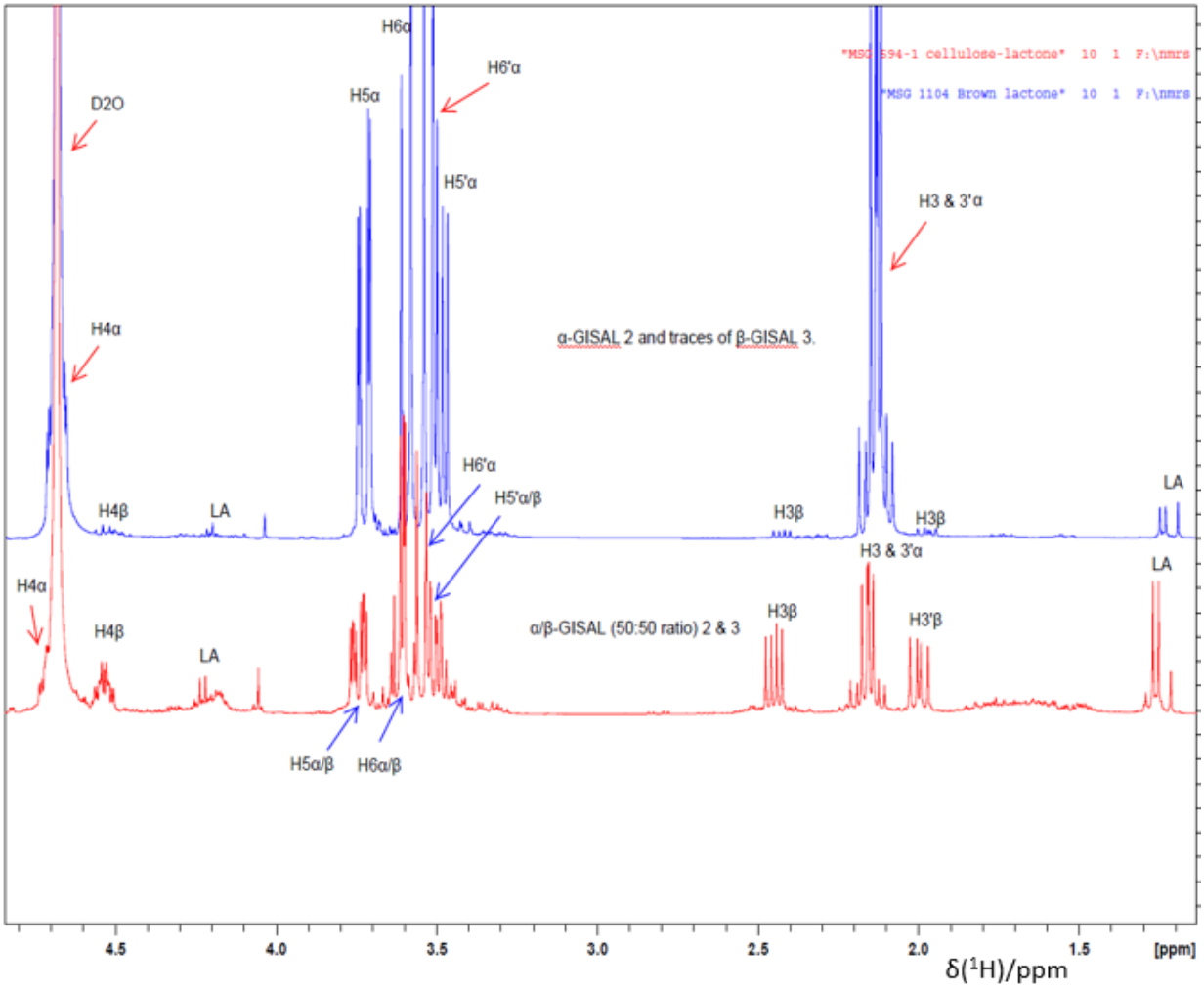


*Figure 12 ^{1}H NMR spectra of α-GISAL **2** and a mixture of α/β-GISAL **2** & **3** in a 50/50 ratio*

The proton NMR spectra confirm the lactonisation of the α-GISA **1** and the 50/50 α/β-GISA mixture to generate α-GISAL **2** (in blue) and α/β-GISAL **2** and **3** (in red). In the α-GISA the downfield shift of the H3s from 1.71/1.55 (dd/dd) to 2.13 (2 x dd) in the spectra **1** and **2**, are due to the lactone being formed and the chemical shifts are in agreement with the literature for α-GISAL **2**[128, 12] and for β-GISAL **3**[77, 82]**.** At the same time, evidence for acid and lactone forms was also obtained by inspection of the compounds' IR spectra which showed a strong absorbance at 1585 cm^{-1}, characteristic of a carboxylate salt in **1** and 1755 cm^{-1} in **2**, which is characteristic of a lactone.

2.2 Protecting group chemistry.

Having prepared the lactones, the next experiments were designed to protect the hydroxyl groups at positions 2, 5 and 6, either individually, in pairs or all together. It was also of interest to develop orthogonal protecting group strategies using a combination of acid and base-labile protecting groups. The protecting groups employed are those that have been routinely used to protect hydroxyl functionalities in sugars including silyl ethers, alkyl ethers, esters, carbonates and acetals.

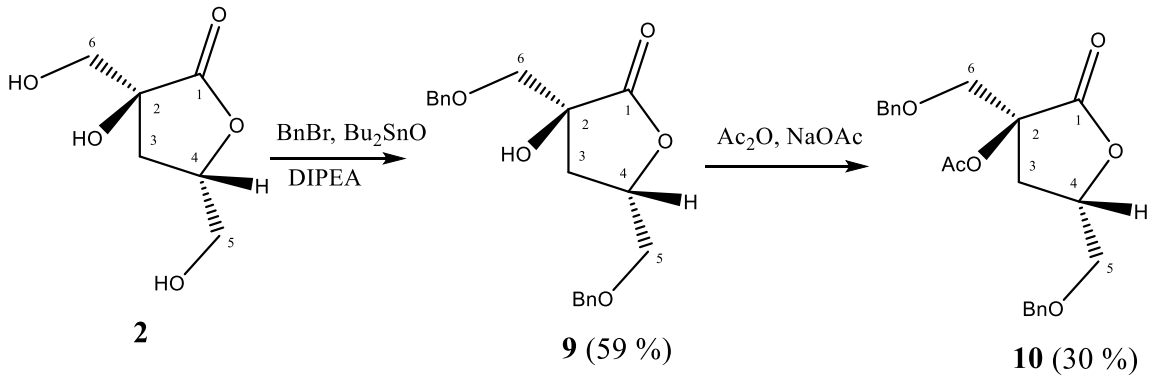
2.2.1 Ethers

In this section, derivatives of glucoisosaccharinic acids produced by direct substitution of hydroxy groups to form ethers is discussed. Ethers are among the most used protecting groups in carbohydrate synthesis for the protection of hydroxyl groups in carbohydrate-based polyols. Selective protection during acylation, acetylation or halogenation are issues that have been addressed by the developments of a number of methods. Ethers are formed and removed under a wide variety of conditions [88].

2.2.1.1 Benzyl Ether

The most frequently used derivatives as protecting groups are benzyl ethers, and these are usually prepared by reaction of a monosaccharide derivative with excess benzyl bromide in the presence of a stoichiometric amount of base in a polar aprotic solvent (NaH in DMF) and they can be cleaved to give an unblocked alcohol and toluene by hydrogenolysis, usually over a palladium catalyst [130]. Several attempts were made to prepare the 5,6-di-O-benzyl-α-GISAL **9** using benzyl bromide and sodium hydride as a base in DMF and these failed, with only ring-opened lactone products being obtained. An alternative benzylation procedure has recently been described by Giordano and Ladonisi [131] using a tin-mediated solvent-free method which they applied to the regioselective benzylation of primary alcohols in carbohydrate-based polyols. In the

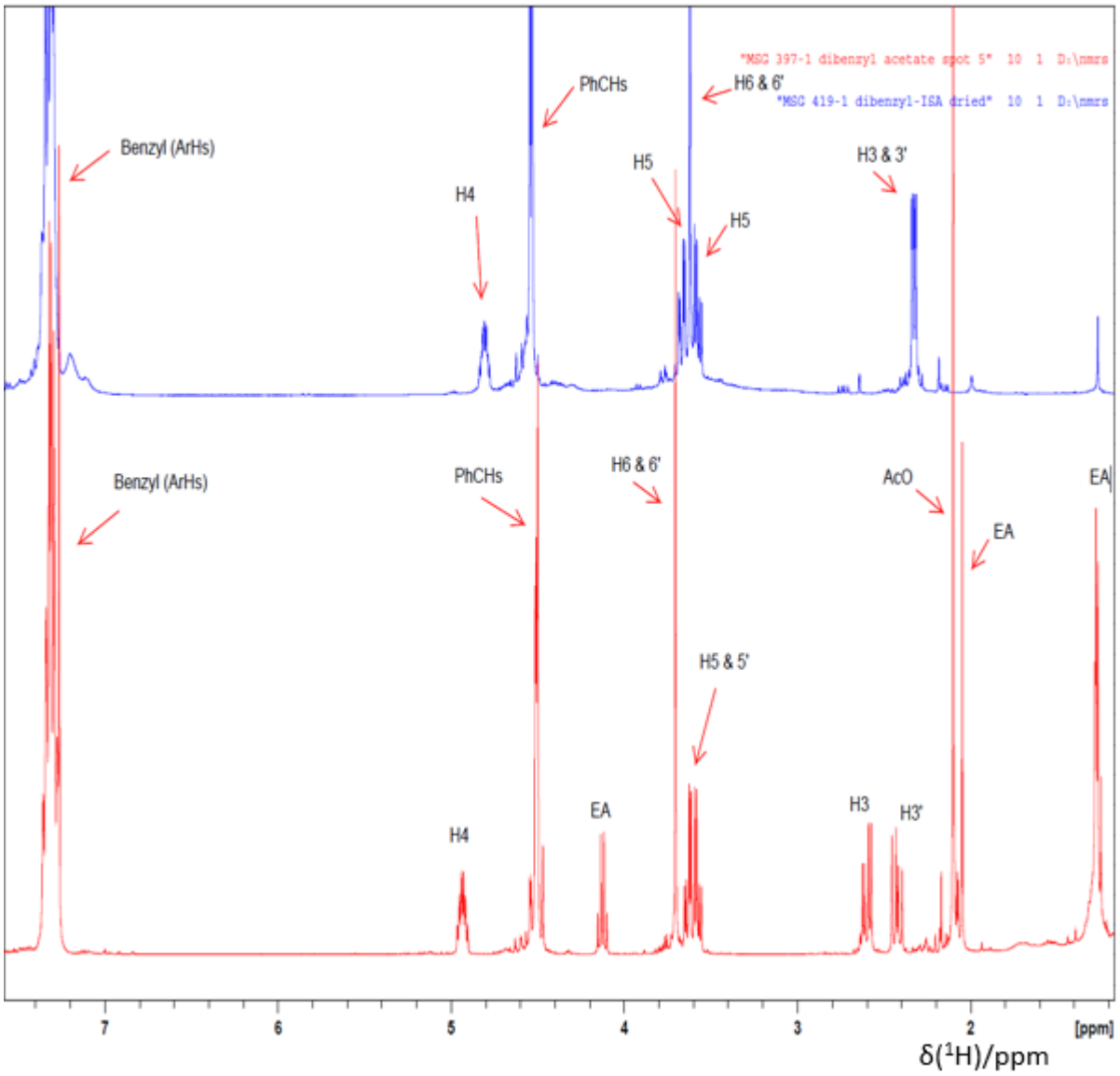
procedure a combination of a stoichiometric amounts of benzyl bromide and DIPEA as a base was reacted with the polyol in the presence of a tin reagent as a catalyst. When this procedure was attempted using α-GISAL **2** as the starting material, the desired product **9** (59% yield) was prepared.



2 **9** (59 %) **10** (30 %)

Scheme 2.3

To confirm that the reaction was with the primary alcohols at positions 5 and 6, the product was subsequently acetylated using acetic anhydride and sodium acetate to form **10**, (see scheme 2.3) and the locations of the benzyl and acetyl groups were confirmed through inspection of a combination of the ^{1}H-spectrum (Figure 13) and a HMBC spectrum (Figure 14). In the HMBC spectrum, long range scalar coupling is visible between the methylene carbons of the two benzyl groups and the methylene protons attached to C5 and C6.



*EA stands for ethyl acetate impurities

*Figure 13. ^{1}H NMR spectra of 5,6-di-O-benzyl-α-GISAL **9** and 2-O-acetyl-5,6-di-O-benzyl-α-GISAL **10**.*

The ^{1}H NMR spectra of **9** and **10** are compared in figure 13, and individual protons have been assigned on the two spectra, including those for the acetyl group which appears at 2.10 ppm in **10**. Interestingly, adding the acetyl group causes the splitting of the H3 (2 x dd) at 2.33 ppm in **9** to give individual resonances at 2.60 and 2.42 ppm respectively in **10**. In contrast, the splitting pattern for the H6 protons simplifies and moves downfield to 3.70 ppm from 3.62 ppm in **9.**

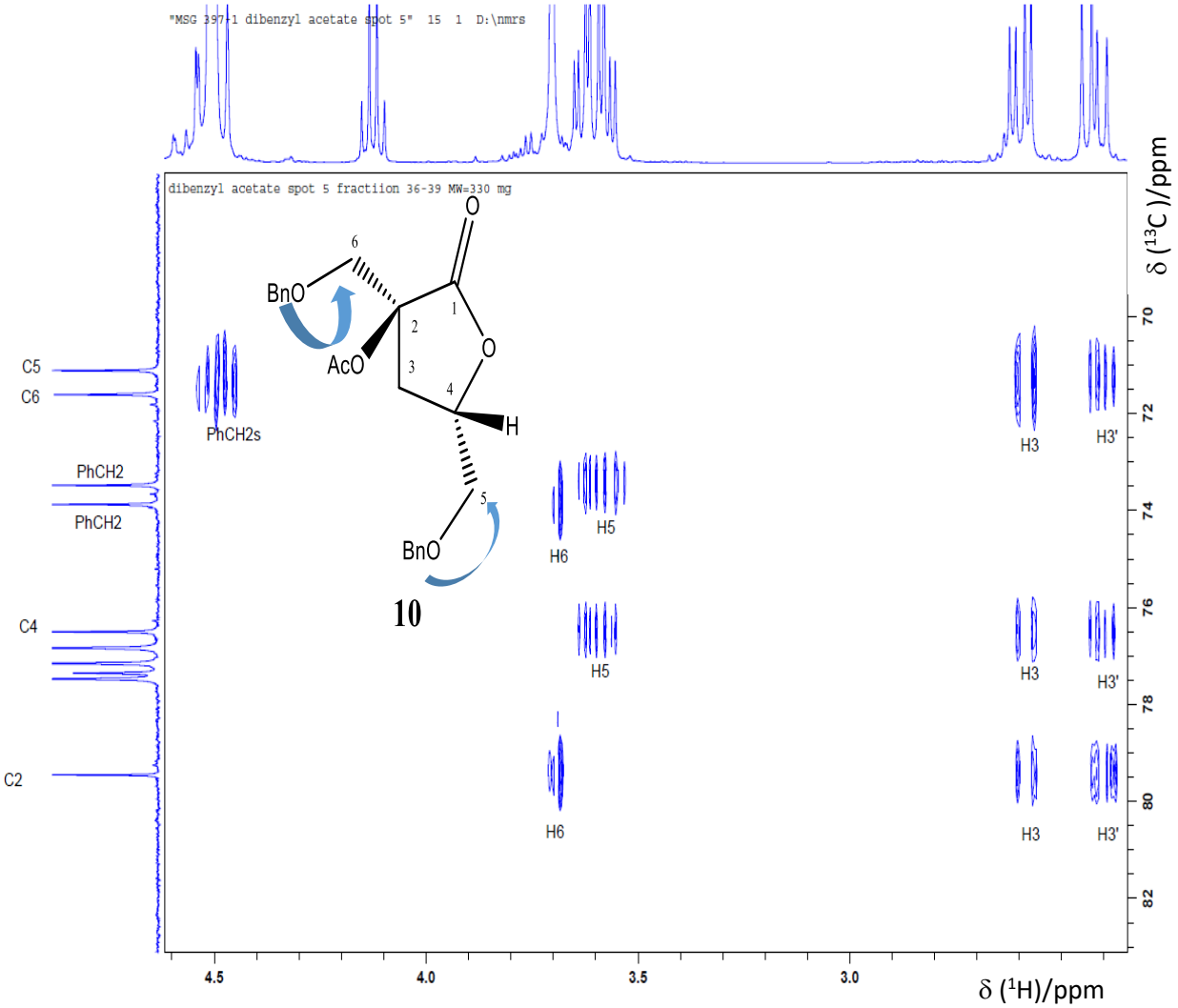


*Figure 14 HMBC of 2-O-acetyl-5,6-di-O-benzyl-α-GISAL **10** (expanded region).*

The expanded carbonyl region of the HMBC spectra of **10** (Figure 14) includes coupling between H3s, H6 and H4 and the GISAL carbonyl (C1) at 173.66 ppm through multiple bonds. In contrast, for the second carbonyl at 170.00 ppm, coupling is only observed with the methyl of the acetyl group, which is consistent with the addition of the acetyl group at position 2.

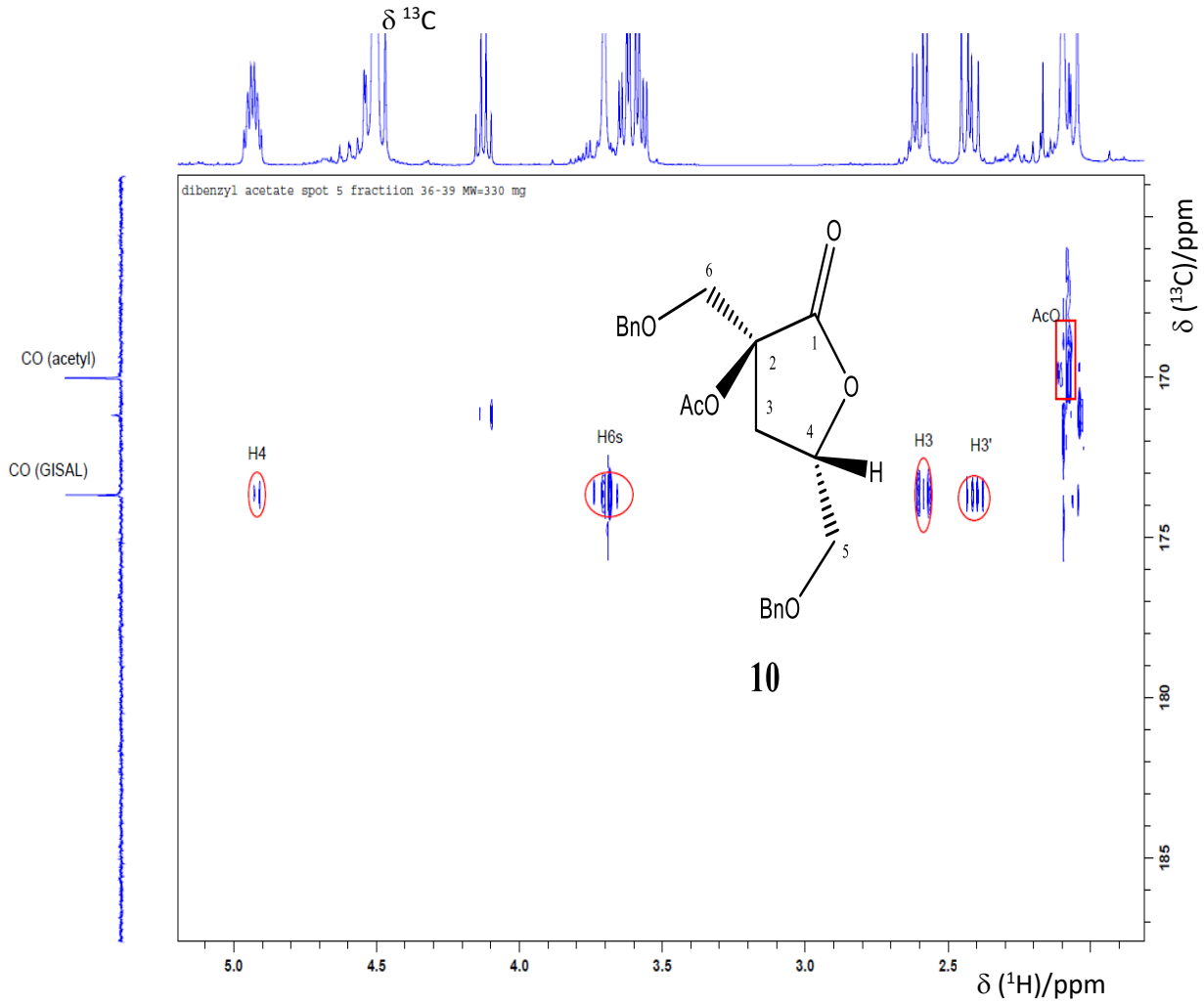


Figure 15. HMBC of 2-O-acetyl-5,6-di-O-benzyl-α-GISAL ***10****.*

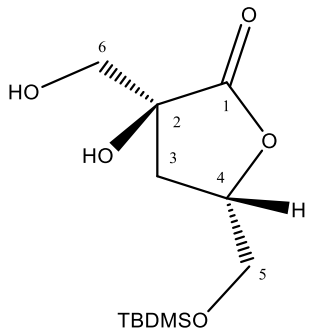
2.2.1.2 Silyl Ethers.

A wide variety of silicon protecting groups is available. At first, we considered whether the trimethylsilyl ether group, being less bulky, could be used to protect all three hydroxyl groups in a single step, but, because of its hydrolytic sensitivity, it was considered that other groups would be better if products were to be used in subsequent reaction sequences. The use of the *t*-butyldimethylsilyl (TBDMS) protecting group was explored first. TBDMS was chosen because it is approximately 1000 times more stable to acid hydrolysis than the trimethylsilyl ether counterpart [132, 133]. The TBDMS group is stable

under basic conditions and is usually removed by reaction with tetra-butylammonium fluoride in THF [132].

i. Preparation of *t*-butyldimethylsilyl derivatives of α-GISAL

The α-GISAL **2** was reacted with an increasing number of equivalents (up to ten equivalents) of TBDMSCl in pyridine. Reaction with 1.1 equivalent gave the mono-protected TBDMS derivative of GISAL **30** with the silyl group adding at position 5; this represents one of the only reactions that gave a single regioisomer and highlights the greater nucleophilicity of the 5-hydroxy group compared to that of the 6-hydroxyl group in reaction with a sterically hindered electrophilic centre. Calculation of the negative charge associated with the hydroxyl oxygens suggests that the C5 –OH has marginally more electron density than the C6-OH (-0.371 Vs -0.366) and the difference in reactivity is more likely to reflect steric clashes that arise in forming the transitions state leading to the respective products.



30 (46% Yield)

The successful preparation of **30** was an important step that subsequently led to the successful separation of β-GISAL derivatives using a one-pot sequential reaction (see later discussion 3.4).

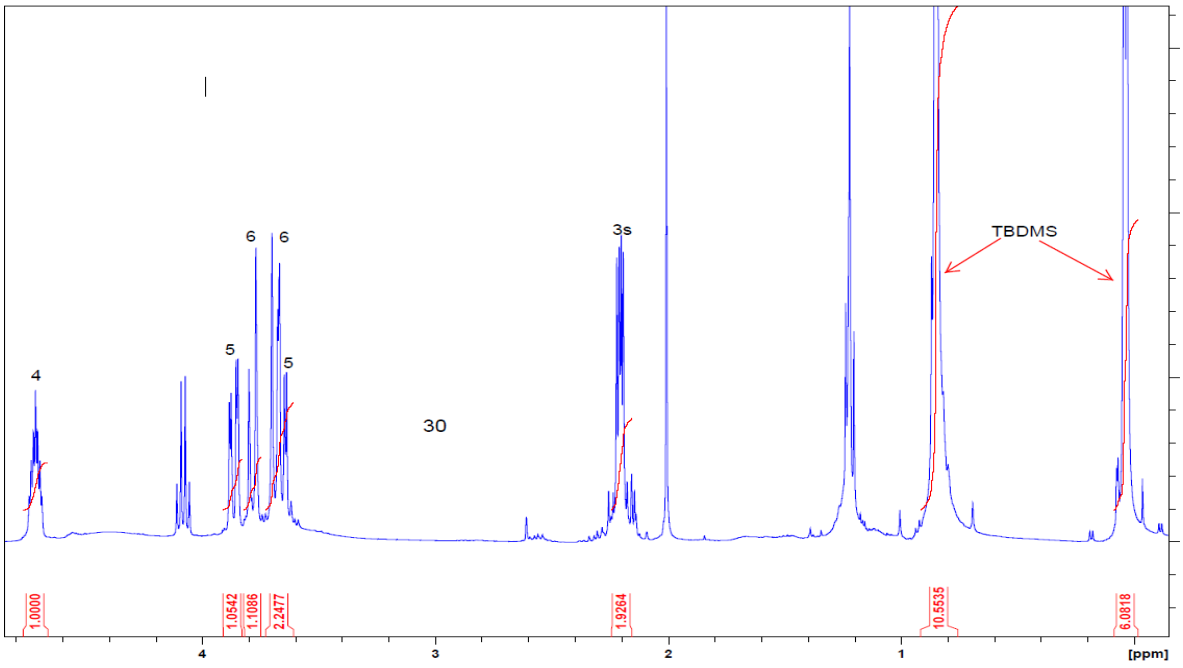


*Figure 16. ^{1}H NMR spectrum of **30***

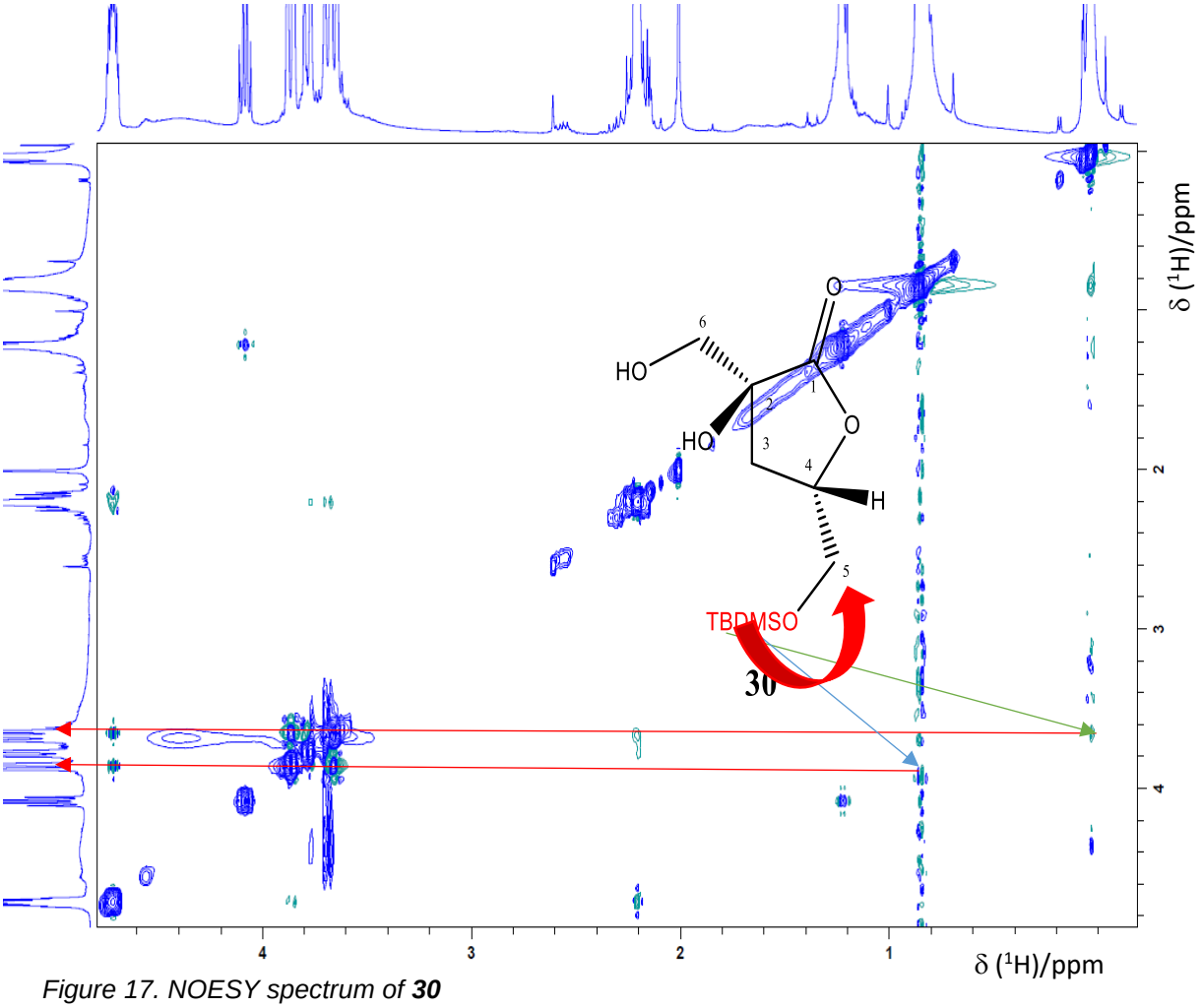
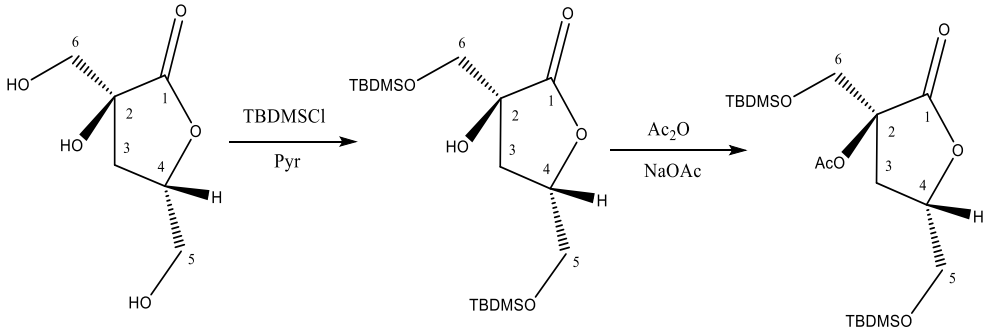


*Figure 17. NOESY spectrum of **30***

The NOESY spectrum of **30** above, shows an NOE between the TBDMS to the two methylene protons on C5 (red arrows), which confirms the presence of TBDMS protection on the hydroxyl group at position 5.

When the number of equivalents of TBDMSCl was raised above two, the only product that was obtained was the disubstituted derivative 5,6-di-O-TBDMS-α-GISAL **27**.

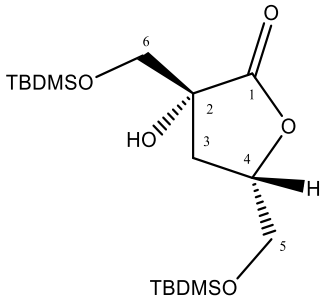


2 **27** (69%) **28** (81%)

Scheme 2.4

The disubstituted derivative 5,6-di-O-TBDMS-α-GISAL **27** was the only product when ten equivalents of the TBDMSCl was used in the reaction; none of the 2,5,6-trisubstituted produced was visible, and this is likely to be the result of the large size of the protecting group which prevents reaction at the tertiary centre. The ground state stabilities of the mono-, di- and tri-substituted derivatives were calculated using MM. The mono-silyl derivative had a total ground state energy of 11.68 kcal/mol, this increased to 13.90 kcal/mol in the disubstituted derivative and was calculated to be 20.61 kcal/mol in the trisubstituted. It was, however, possible to acetylate the 2-position of **27**: reaction of the 5,6-di-O-TBDMS-α-GISAL **27** with acetic anhydride and sodium acetate gave product **28** in a high yield.

As one of the objectives of the work was to develop a procedure which would make it possible to separate β-GISAL from a mixture of α- and β-GISAL, the same reaction was attempted on a mixture of the two lactones (in a 3:1 ratio), and after chromatography, a low yield (5%) of the desired 5,6-di-O-TBDMS-β-GISAL **29** was obtained. However, when the same reaction was attempted on a mixture of β-GISAL and α-GISAL (in a 50:50 ratio), a crude mixture of **27** and **29** was obtained, and all efforts to separate them were unsuccessful.



29 (5%)

Again, a series of NMR spectra was recorded in order to confirm the location of the protecting groups.

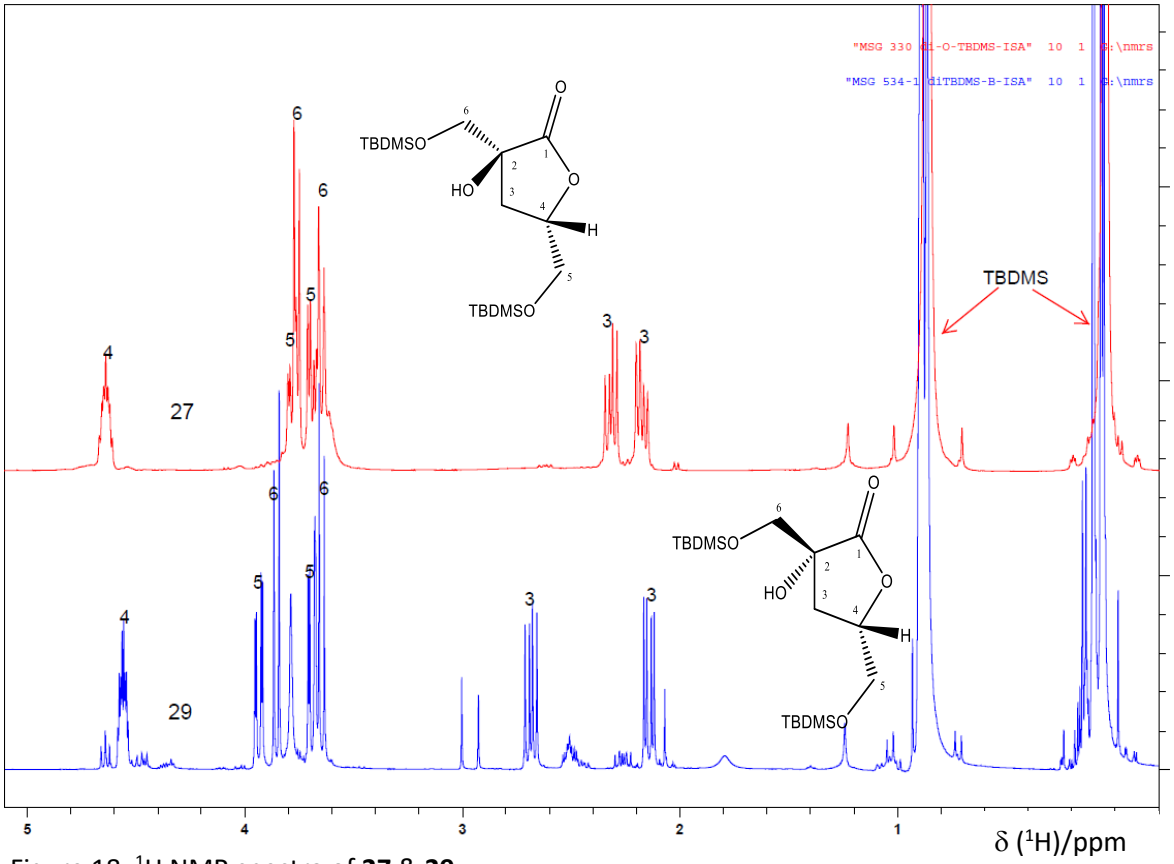


Figure 18. ^{1}H NMR spectra of **27** & **29**.

Inspection of the ^{1}H-NMR spectra of the two disubstituted products identified the locations of the different protons (Figure 18). The chemical shifts of TBDMS (butyl and methyl protons) in **27** and **29** are very similar. However, the TBDMS at position 6 is closer to the carbonyl of the lactone and its signals are shifted very slightly downfield compared to the corresponding signals for the TBDMS group at position 5, due to its inductive effect. The signals at 0.89 & 0.88 ppm are for the TBDMS on position 6 in **29** & **27** and 0.86 & 0.85 are for the position 5 in **29**, **27** & **30** (Figure 16) respectively.

A significant difference was observed in the positions of TBDMS signals between the two epimers; (2S, 4S)-5,6-di-O-TBDMS-α-GISAL **27** and (2R, 4S)-5,6-di-O-TBDMS-β-GISAL **29,** which are at -3.67, -5.44, -5.55 & -5.61 ppm and -5.49, -5.53, -5.56 & -5.63 ppm respectively, which could be due to the difference in configuration at carbon 2 and the steric repulsion.

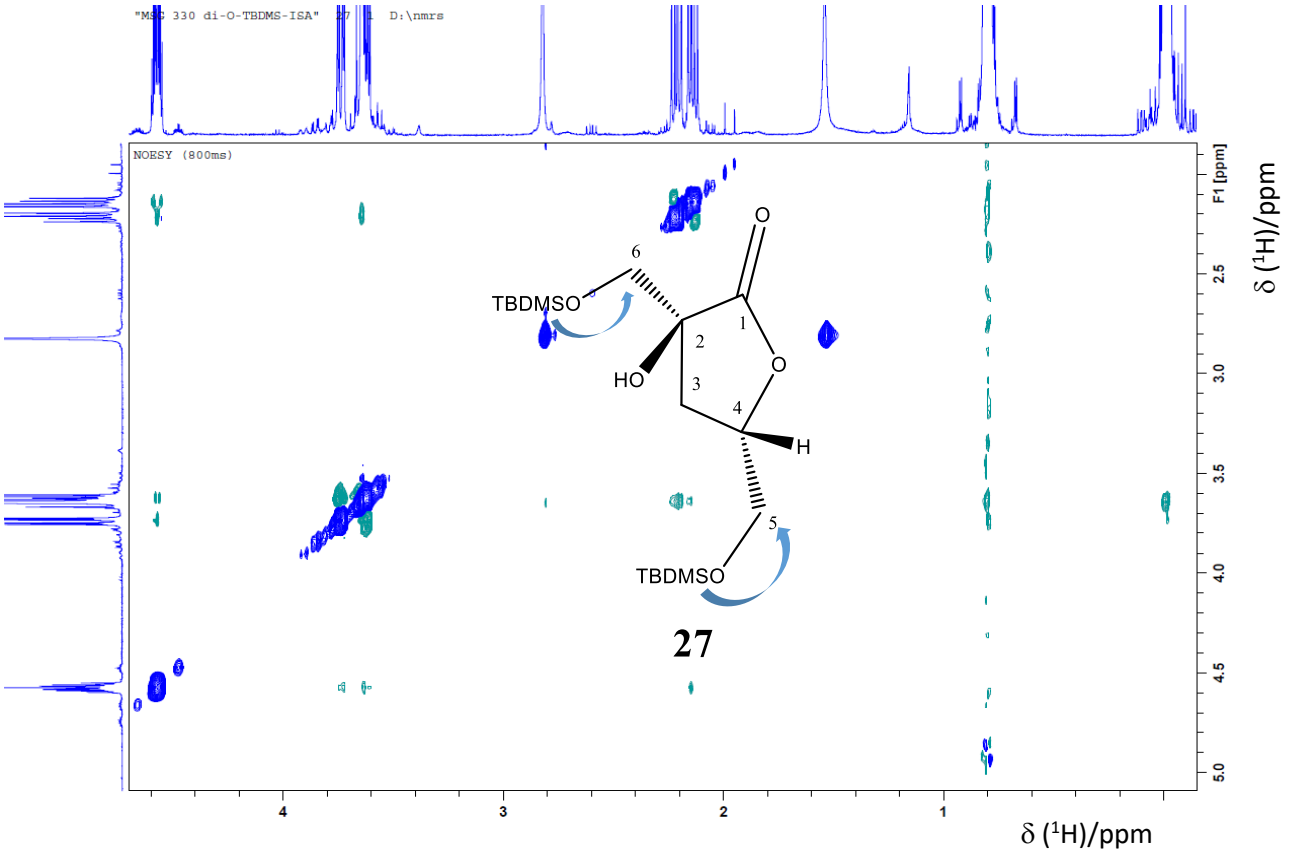


*Figure 19. NOESY spectrum for (2S, 4S)-5,6-di-O-TBDMS-α-GISAL **27***

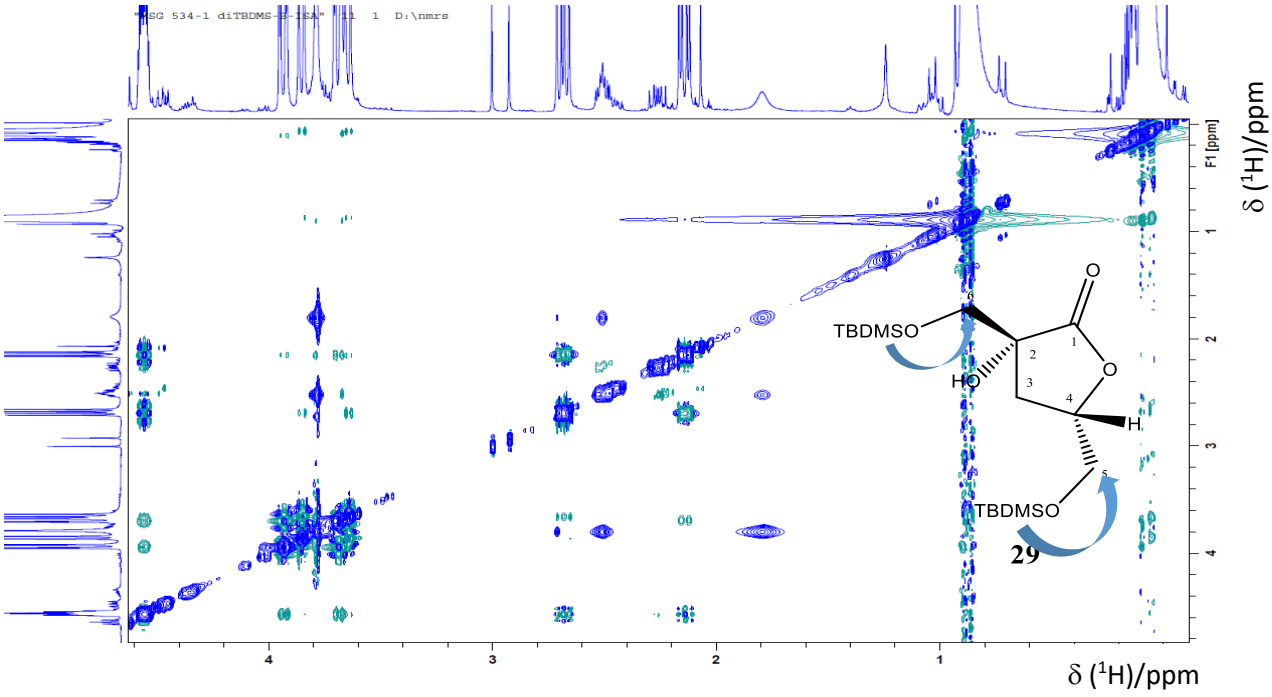


Figure 20. NOESY spectrum (2R, 4S)-5,6-di-O-TBDMS-β-GISAL **29**

The NOESY spectra of (2S,4S)-5,6-di-O-TBDMS-α-GISAL **27** (Figure 19) and (2R,4S)-5,6-di-O-TBDMS-β-GISAL **29** (Figure 20) both show reasonable NOEs between the methyl protons of the TBDMS and the methylenes at positions 5 and 6 which confirms their presence at these two positions.

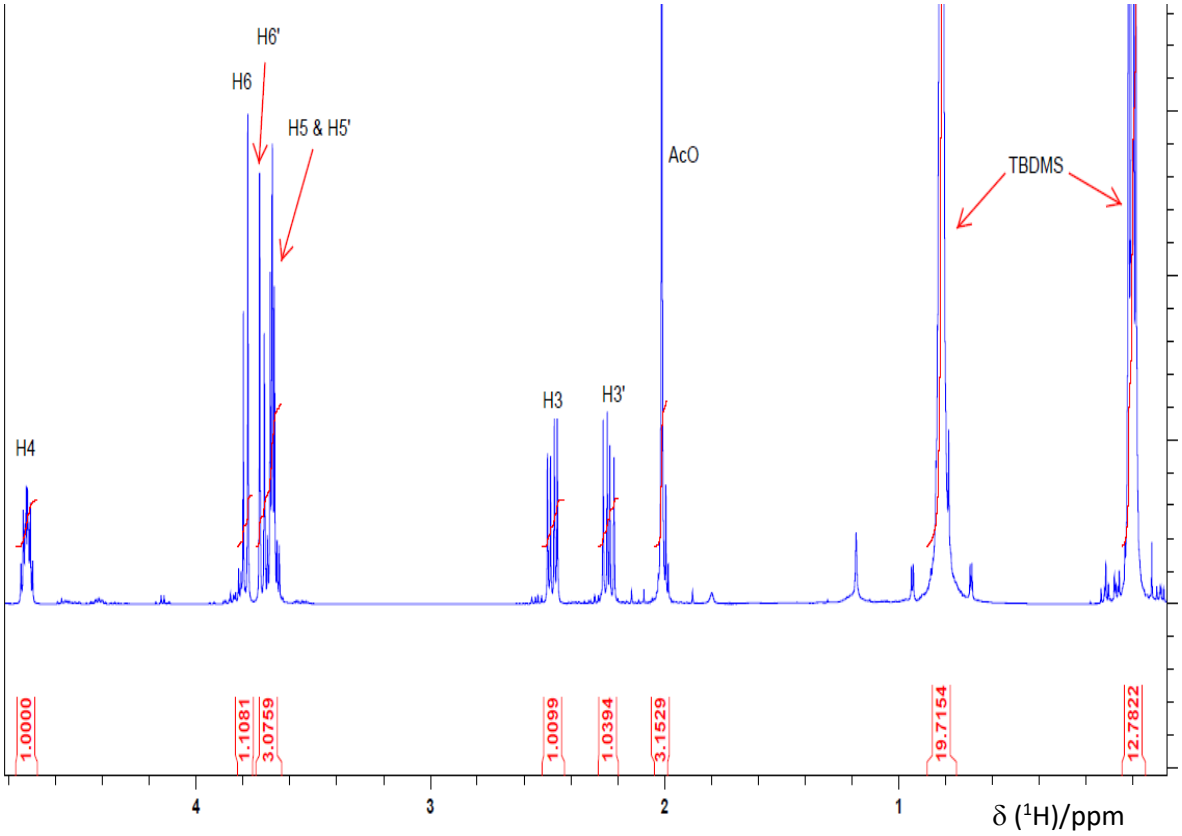


*Figure 21.¹H NMR of 2-O-acetyl-5,6-di-O-TBDMS-α-GISAL **28***

Further confirmation of the locations of the silyl groups was obtained from inspection of ^{1}H and HMBC spectra of the product obtained after reaction of the remaining hydroxyl group with acetic anhydride, which identify that the acetyl group had added at position 2 and that there was no migration of the protecting groups. The addition of an acetyl to generate **28** led to a downfield shift of all the protons adjacent to carbon 2 (Figure 21),

compared to their chemical shift In the starting material **27**: the H3s moved from 2.32 & 2.17 to 2.48 & 2.24 and H6s moved from 3.76 & 3.65 to 3.79 & 3.72 ppm.

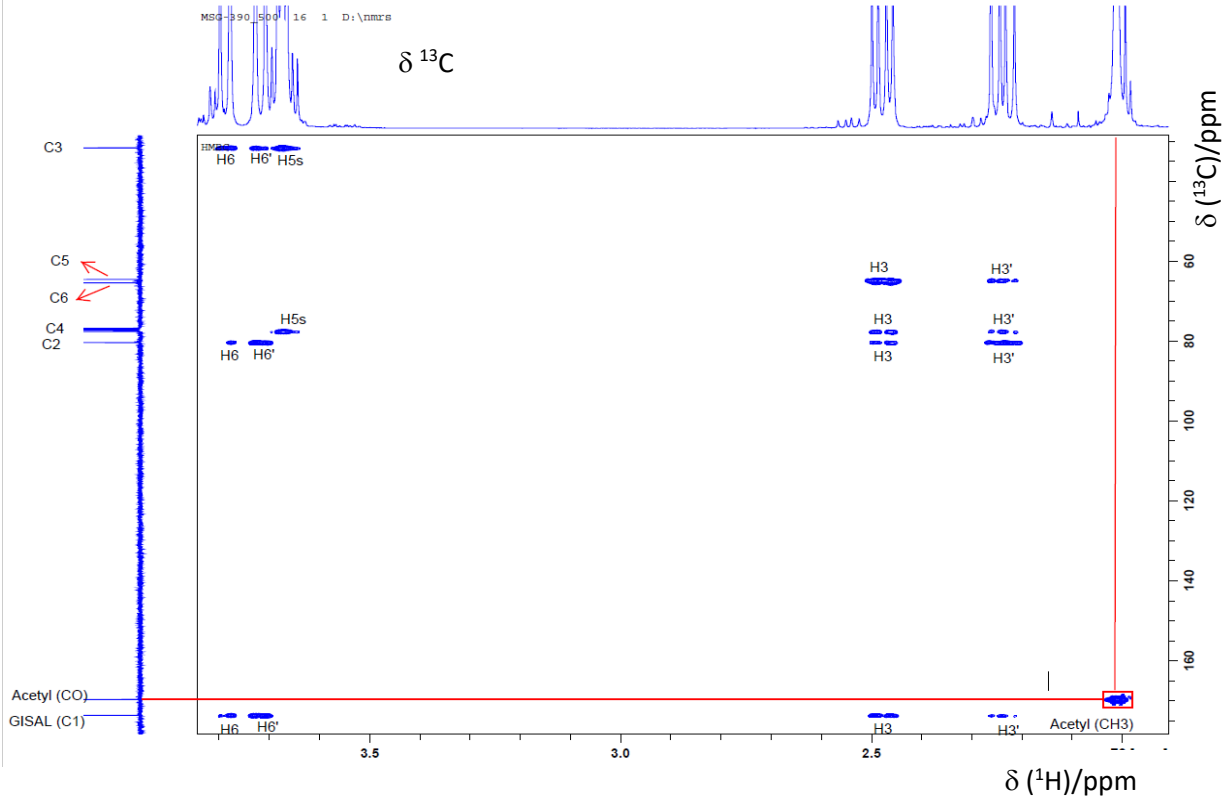


*Figure 22. HMBC of 2-O-acetyl-5,6-di-O-TBDMS-α-GISAL **28***

The expanded carbonyl region of the HMBC spectrum of **28** (Figure 22) shows the coupling between H3s and H6s with GISAL's carbonyl (C1) at 173.70 ppm through multiple bonds and the coupling between the methyl of the acetyl group to its corresponding carbonyl at 169.74 ppm and no coupling to any of the CH_2 groups

ii. TIPS and TIPDS derivatives of α-GISAL

In an attempt to enhance the regioselectivity of the reaction, the triisopropylsilyl protecting group, a bulkier reagent, was employed. It was hoped that this would add only to the most sterically accessible hydroxyl group. When five equivalents of the reagent were reacted with GISAL **2** in the presence of DMAP as a catalyst, a single product was obtained and relative integrations of the isopropyl and H3 protons on the ^{1}H-NMR (Figure 23) suggested that a mono-protected triisopropylsilyl ether had been

obtained. The NOESY spectrum (Figure 24) of the product **33** contained an NOE between the methyl protons of TIPS and those of the methylene at position 5. This result confirmed the higher reactivity of the hydroxyl group attached at carbon 5 over that of 6 towards bulky protecting groups

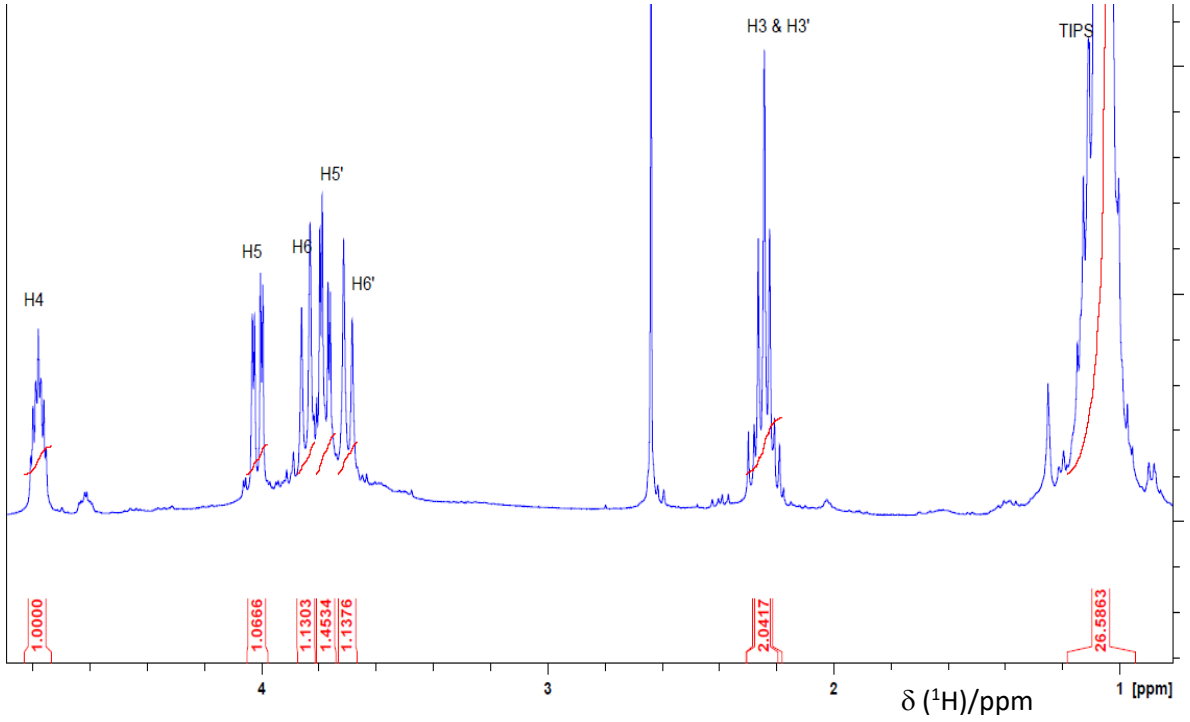


*Figure 23. ^{1}H NMR spectrum of 5-O-TIPS-α-GISAL **33***

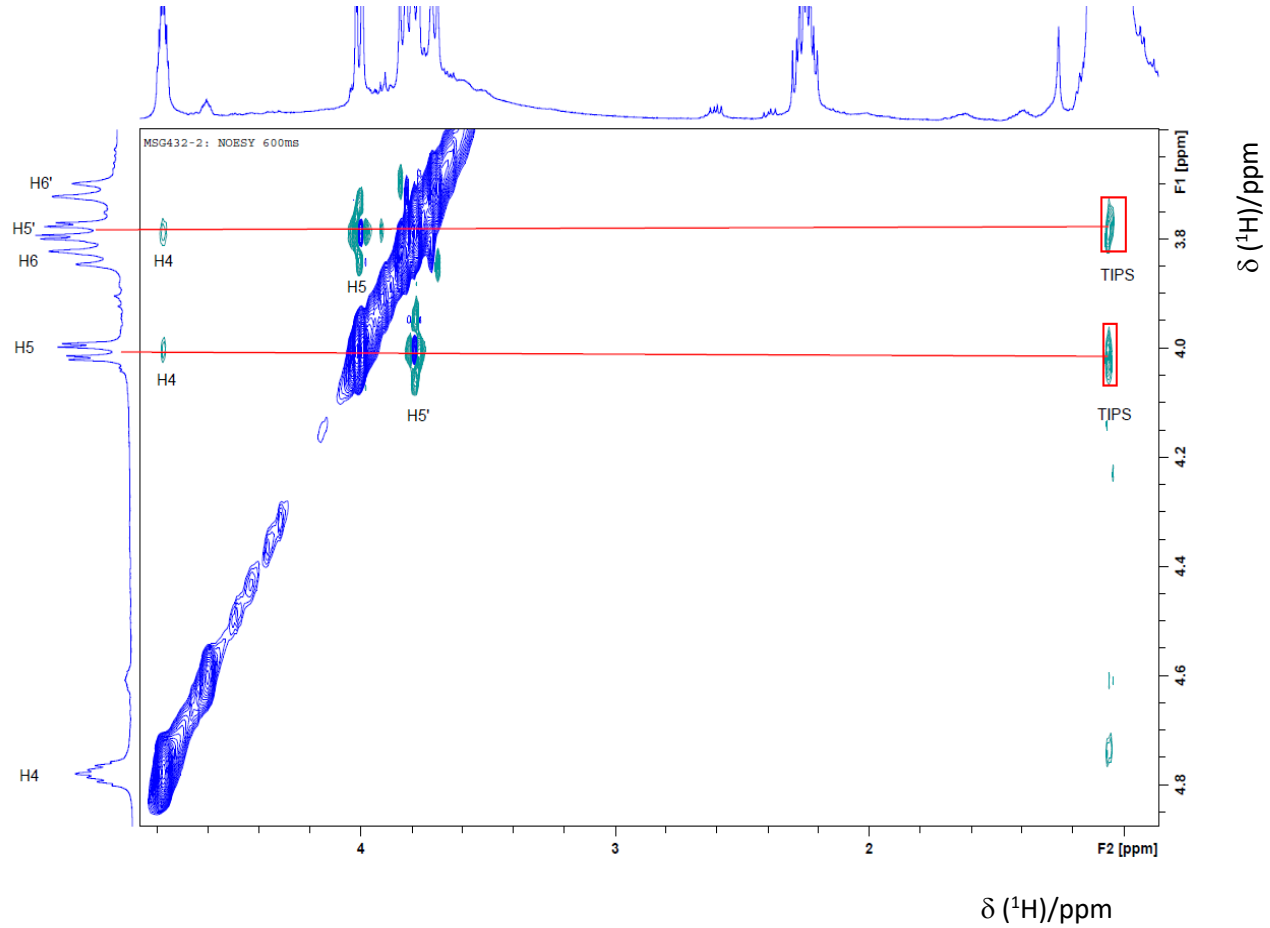
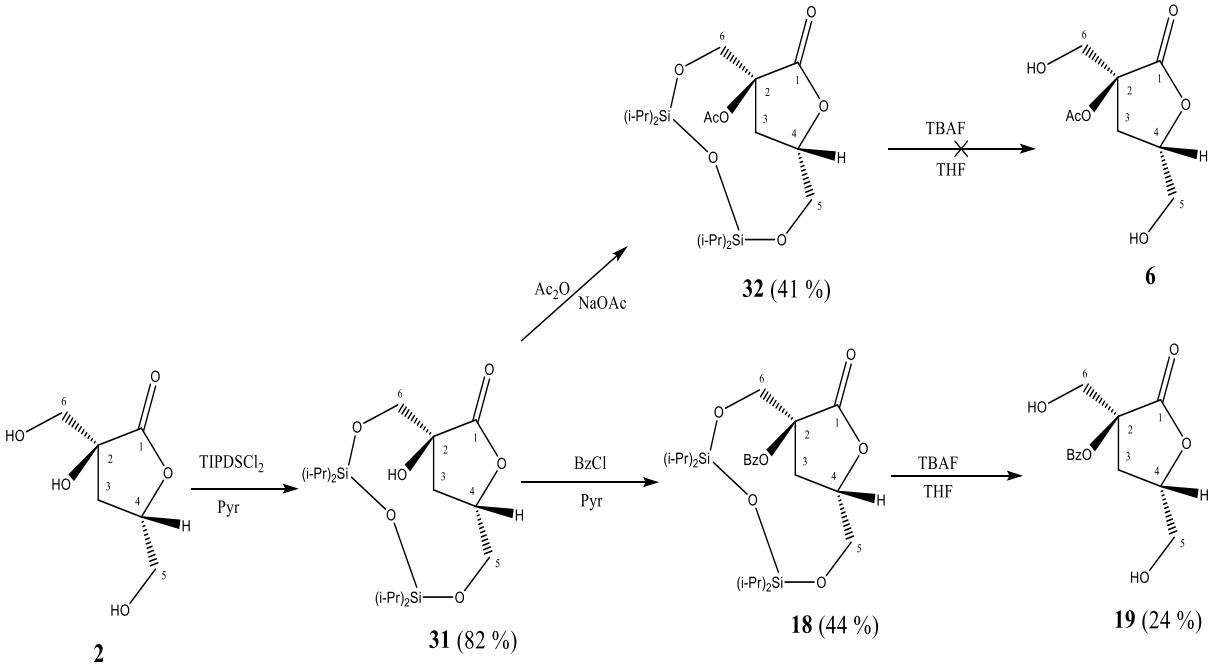


Figure 24. NOESY spectrum for 5-O-TIPS-α-GISAL ***33***

In the next experiments an attempt was made to protect both the 5 and 6-hydroxyl groups using a single protecting group; 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (TIPDS) has proved to be of particular value in ribonucleoside chemistry for the selective protection of the 3'- & 5'-hydroxyl groups and then for the specific release of each of these groups [98]. This work aimed to see if it was possible to observe a similar reaction with α-GISAL where the two primary hydroxyls are on the same face of the ring system and if this reaction was possible with the β-GISAL where the two primary hydroxyls are located on opposite faces of the lactone ring.



Scheme 2.5: The use of TIPDS protecting group to generate platform derivatives of α-GISAL.

In the initial experiment α-GISAL **2** was treated with TIPDSCl in pyridine which gave, after chromatography, the target product **31**; in a high yield (82%), in which the protecting group had added to both the primary hydroxyl groups. The resulting compound was reacted with acetic anhydride to form the 2-acetyl derivative **32** and with benzoyl chloride in pyridine to form the 2-benzoyl derivative **18** using the method reported by Shaw [128]. Mixed results were obtained when attempts were made to remove the protecting groups: the TIPDS protecting group of **18** was cleaved cleanly to form **19** using TBAF in THF whereas the acetyl group of **32** was also removed when it was treated with TBAF in THF**.**

Again, the identities of the compounds and locations of the protecting groups were confirmed using a combination of a ^{1}H spectrum (Figure 25), a HMBC spectrum (Figure 26) and a NOESY NMR spectrum (Figure 27).

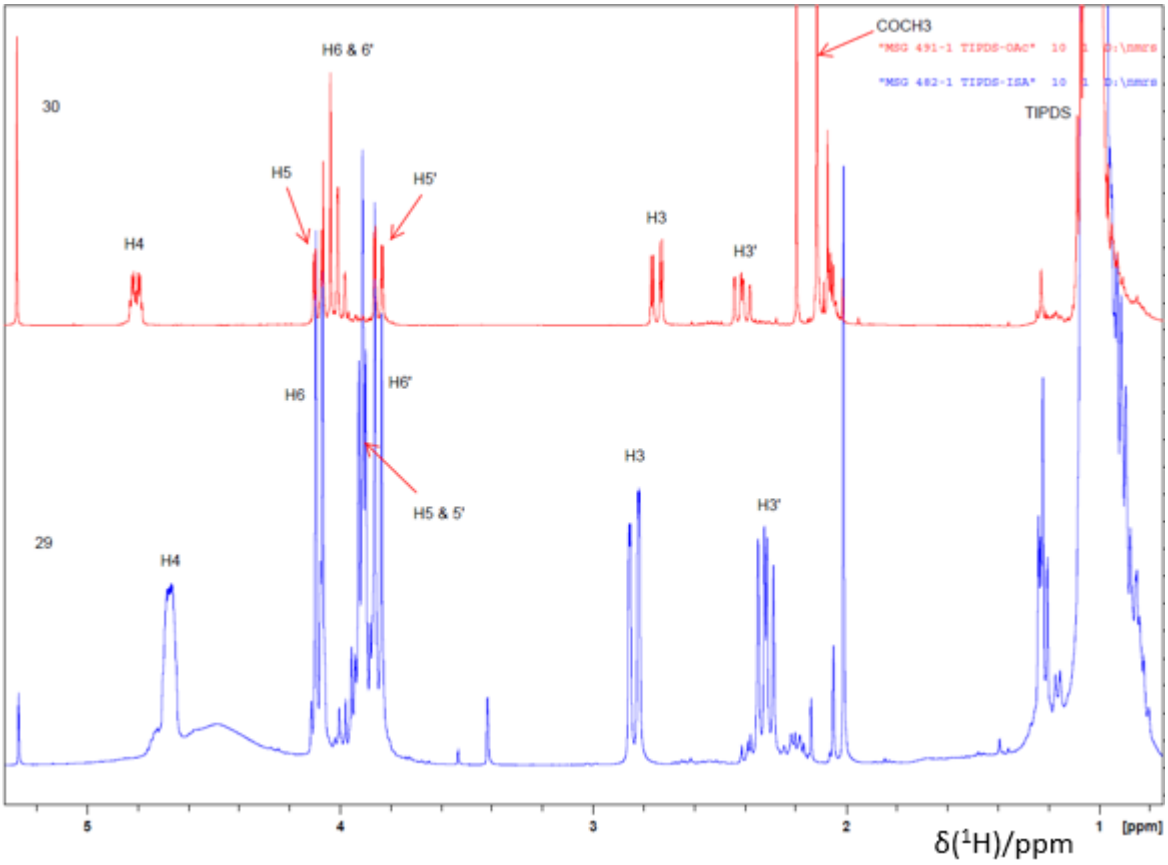


*Figure 25. ^{1}H NMR spectra of 5,6-O-TIPDS-α-GISAL **31** and 2-O-acetyl-5,6-O-TIPDS-α-GISAL **32***

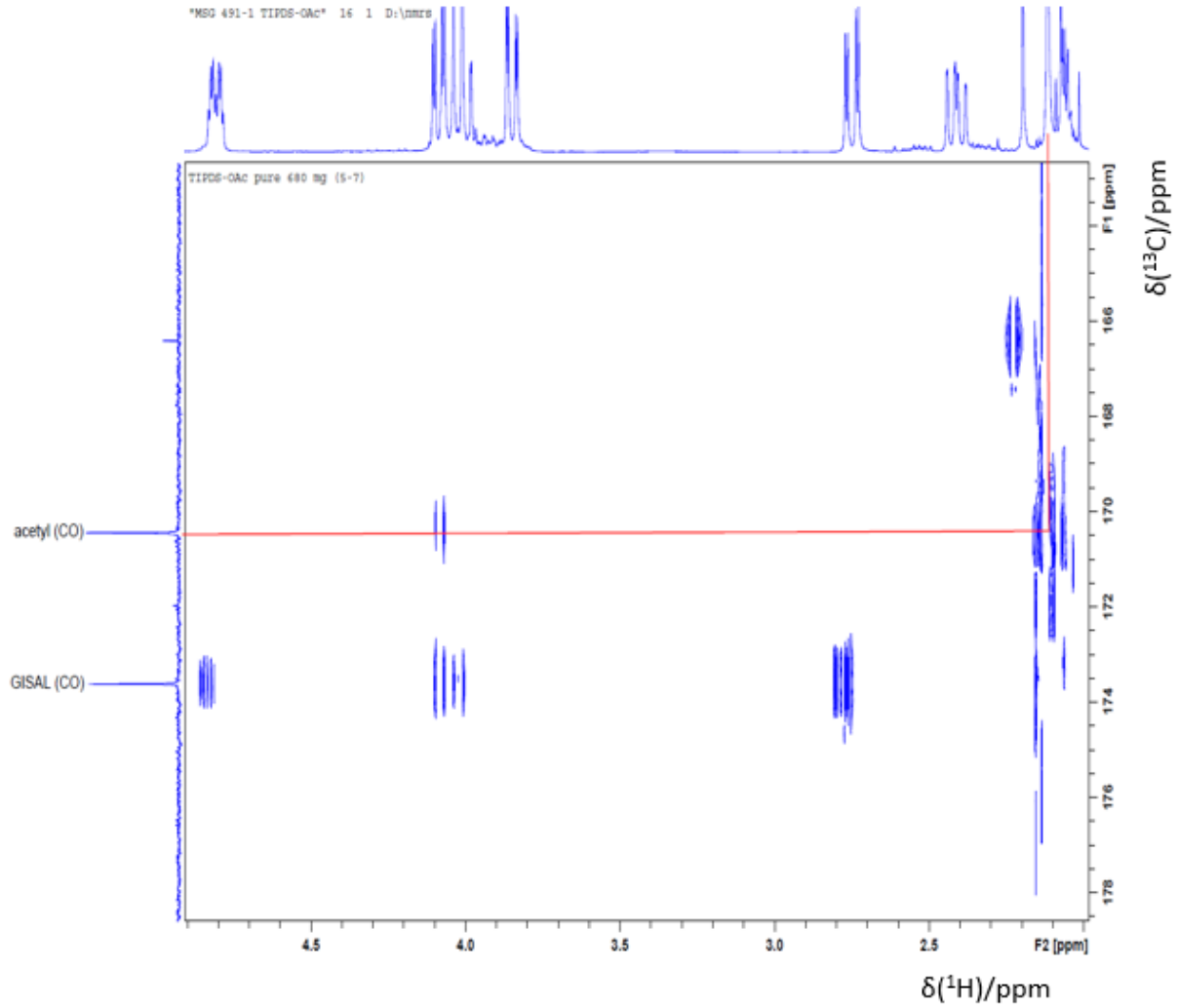


*Figure 26. HMBC spectrum of 2-O-acetyl-5,6-TIPDS-α-GISAL **32***

The expanded carbonyl region of the HMBC spectra of **32** shows the coupling of H3s and H6s to GISAL's carbonyl (C1) at 173.60 ppm through multiple bonds. The coupling between the methyl of the acetyl group to its corresponding carbonyl at 170.40 ppm and the absence of any coupling to the methylene group confirms the presence of the acetyl at position 2.

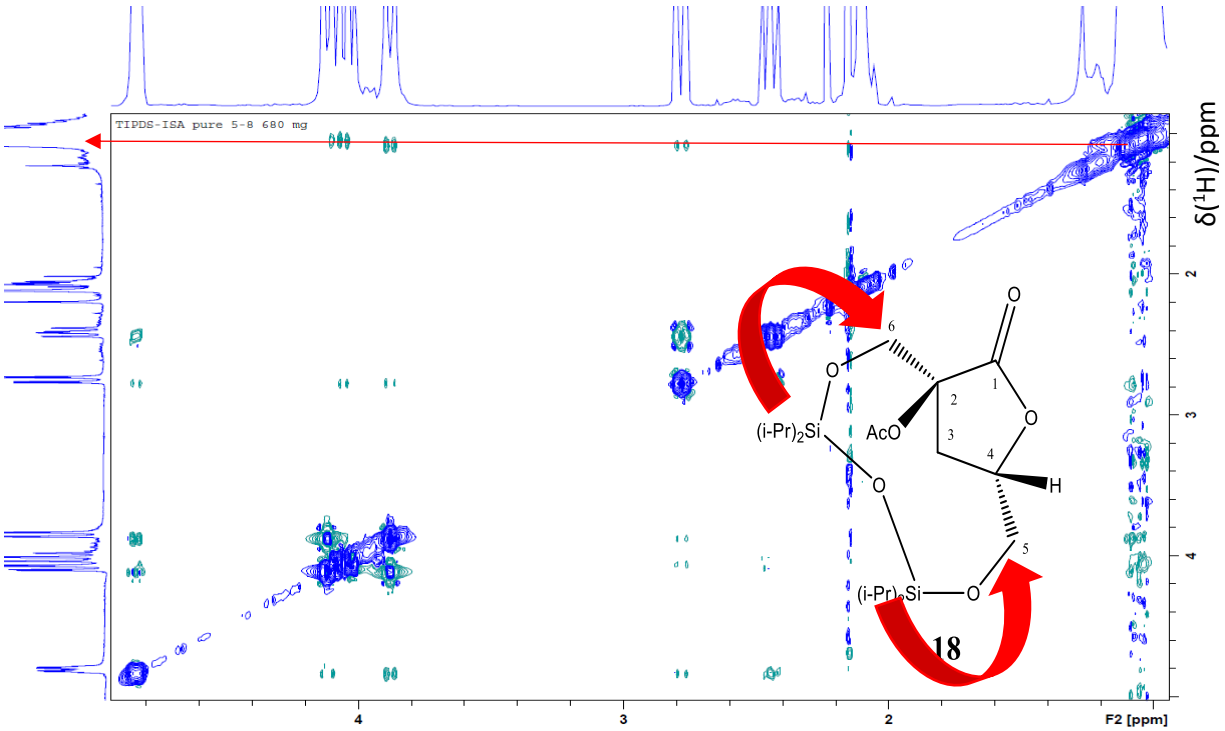
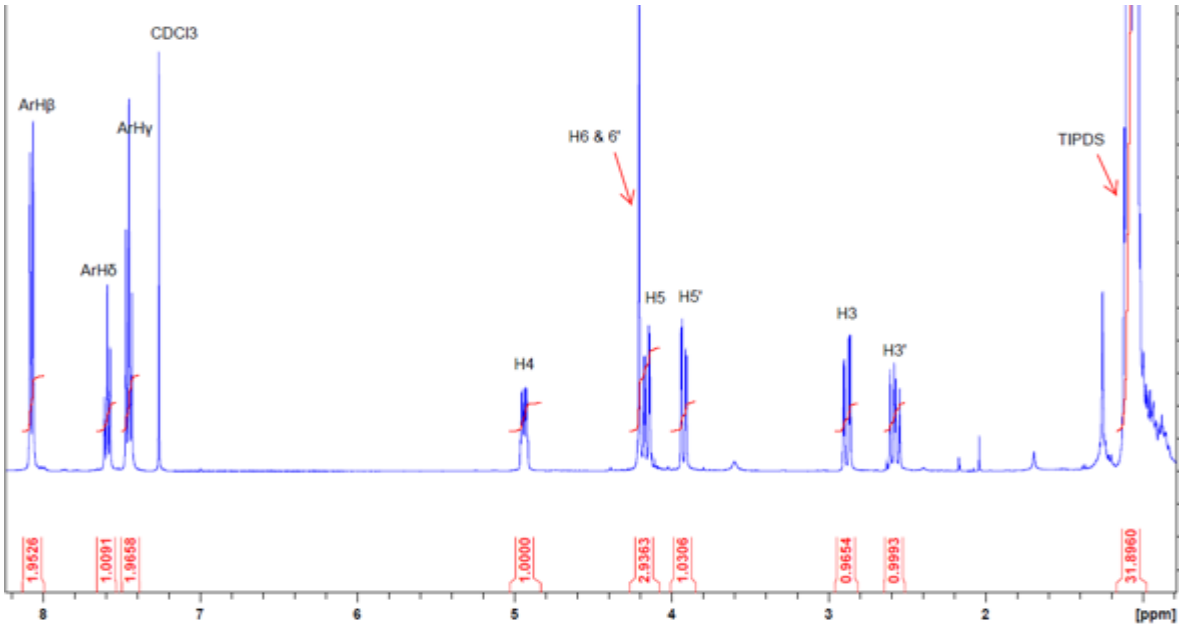


*Figure 27. NOESY spectrum of **32***

δ(¹H)/ppm

The NOESY spectrum of **32** (Figure 27) shows a coupling through space (NOE) between the methyl protons of TIPDS at both positions 5 and 6 to the methylene protons on carbons 5 and 6, which confirms the simultaneous protection of the primary hydroxyl groups at position 5 and 6 was successful.



δ(¹H)/ppm

Figure 28. ¹H NMR spectrum of 2-O-benzoyl-5,6-O-TIPDS-α-GISAL **18**

The ^{1}H NMR spectrum of the benzoyl derivative **18** (Figure 28) shows the TIPDS signals at 1.10-1.03 ppm and the presence of the aromatic peaks at 8.07, 7.59 & 7.45 ppm, which integrate to 2, 1 & 2 respectively, confirming the presence of benzoyl group in **18**.

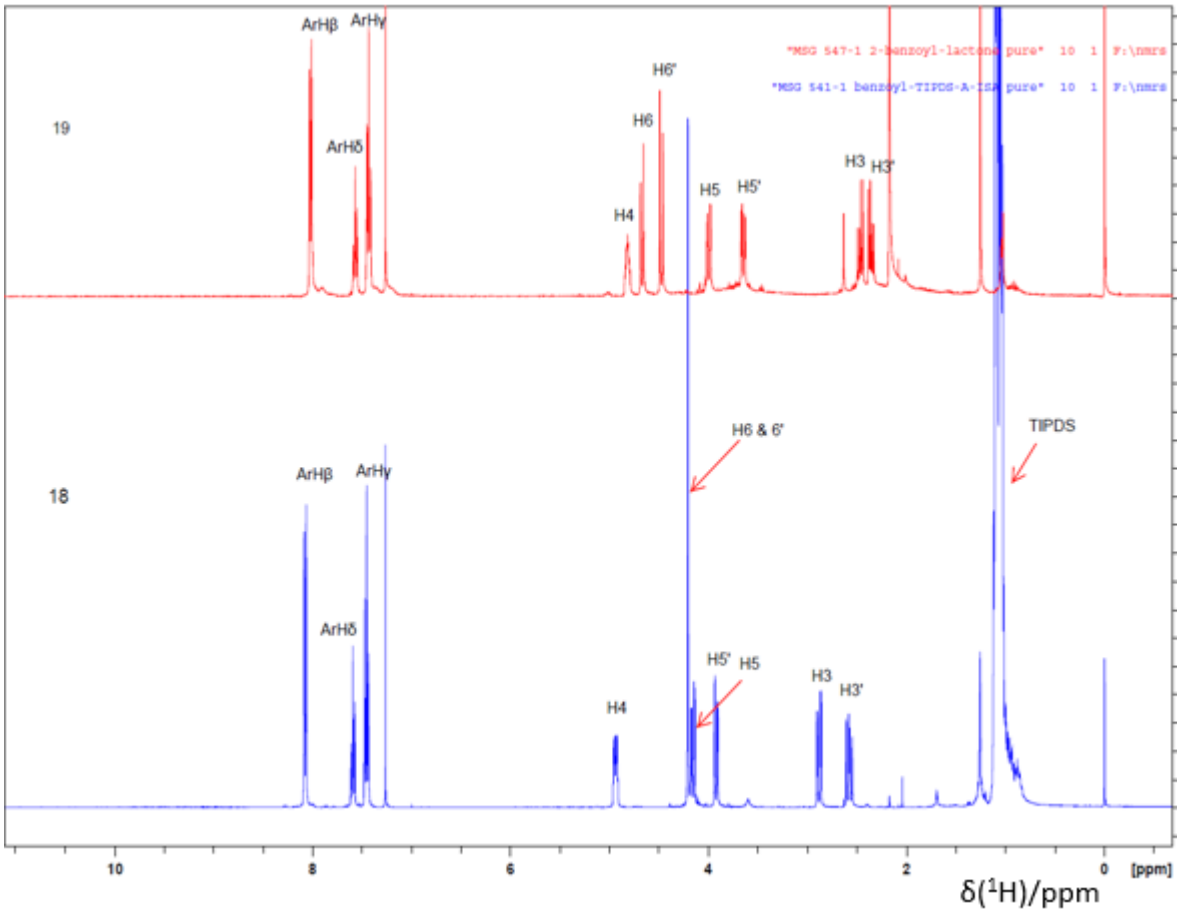


Figure 28. ^{1}H NMR of 2-O-benzoyl-5,6-TIPDS-α-GISAL (**18**) and 2-O-benzoyl-α-GISAL (**19**).

On the HMBC spectrum for **18** (Figure 30) there is coupling through multiple bonds between; H3, H6s & H4 to the GISAL carbonyl (pink line) at 173.61 ppm and H3s, H6s & H4 to C2 (green line), while that between H6s through ArHγ & ArHβ to PhCO (red line) at 165.8 ppm confirms the presence of the benzoyl group at position 2.

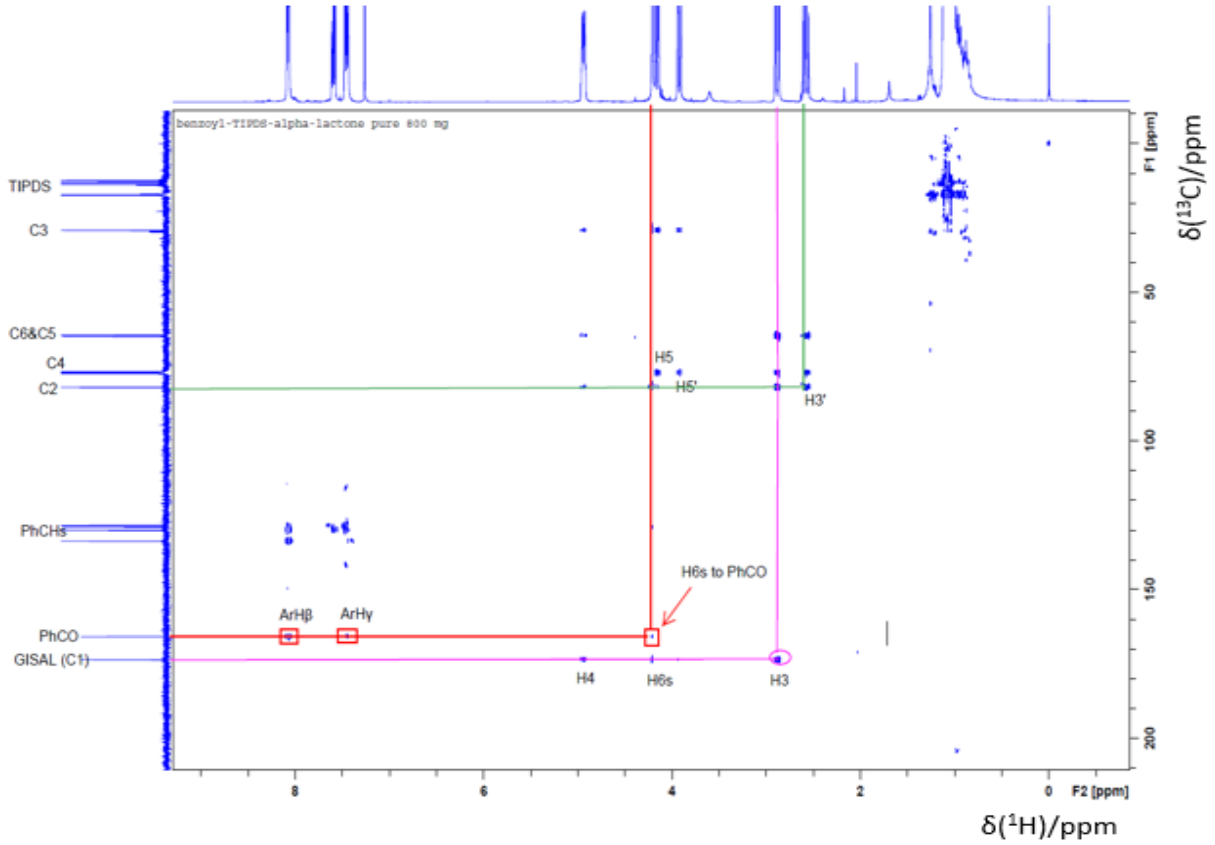


Figure 29. HMBC of 2-O-benzoyl-5,6-O-TIPDS-α-GISAL ***18***

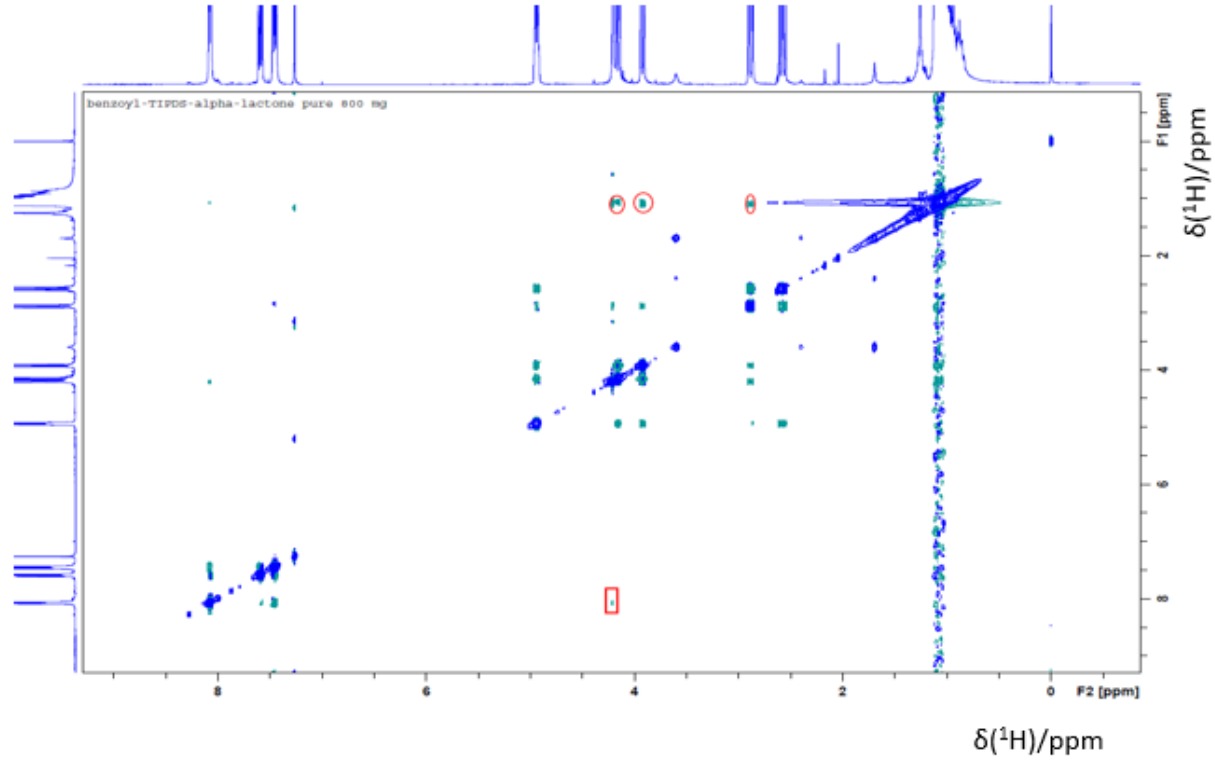


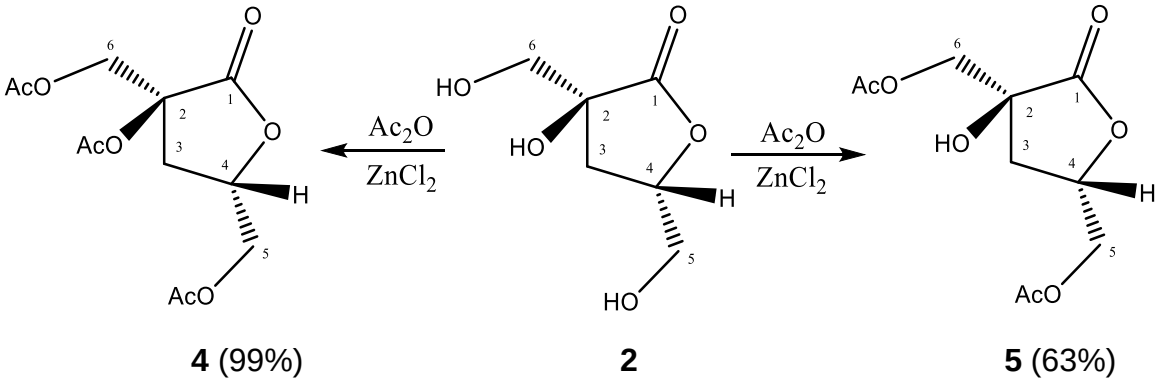
Figure 30. NOESY spectrum of 2-O-benzoyl-5,6-O-TIPDS-α-GISAL ***18***

The NOESY spectrum, (Figure 31) shows an NOE between H6s, H5s & H3 and TIPDS which undoubtedly settles the presence of the TIPDS ether at positions 5 and 6 while the NOE between H6s and ArHβ unequivocally confirms the presence of the benzoyl group at position 2.

Given that in previous experiments, it was not possible to add a bulky protecting group at 2-position it was surprising to find that reaction of **31** with benzoyl chloride in pyridine had led to the production of the tri-protected **18**. The NMR spectrum of **18** contains the TIPDS signals and signals of the benzoyl at 8.01, 7.57 & 7.3 ppm, the absence of the TIPDS signals in **19** confirms the cleavage of the TIPDS ethers at positions 5 and 6, and that the benzoyl group is retained.

2.2.2 Use of esters as protecting groups for α-GISA

2.2.2.1 Synthesis of acetates



4 (99%) **2** **5** (63%)

Scheme 2.6: Acetylation of α-GISAL.

The acetate derivatives of α-GISAL **2** were prepared by reacting **2** with acetic anhydride. Reaction with a large excess of acetic anhydride employing either zinc chloride or sodium acetate as catalyst generated the 2,5,6-triacetyl-α-GISAL **4** in high yield. When the same reaction was attempted with 2.2 equivalents of acetic anhydride the primary product was the 5,6-diacetyl-α-GISAL **5**. All attempts to form a mono-acetyl derivative in a direct reaction failed to give the desired product and mixtures of mono and diacetyl products were recovered. It is clear that there is very little difference in the activation

energies of the reaction of the two hydroxyl groups with small nucleophiles and this suggests that the regioselectivity observed in the formation of the silyl ether is driven by the steric demand of the bulky silyl groups. It was, however, possible to form the 2-acetyl-α-GISAL **6** indirectly, i.e. through orthogonal protection of the 5,6-hydroxyl groups as carbonates (see discussions below), followed by acetylation of the 2-hydroxyl group using acetic anhydride and zinc chloride and then removal of the carbonate groups.

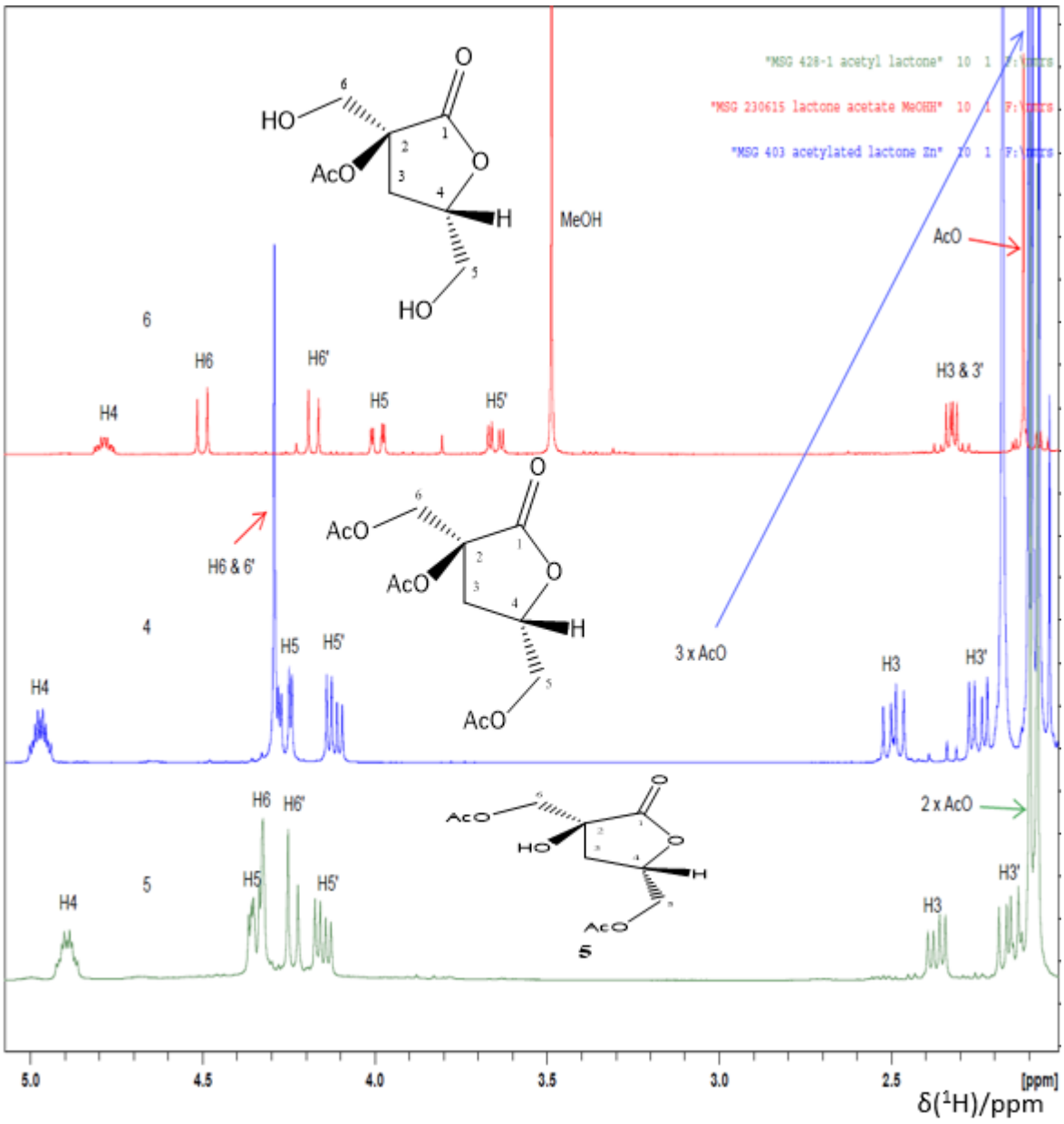
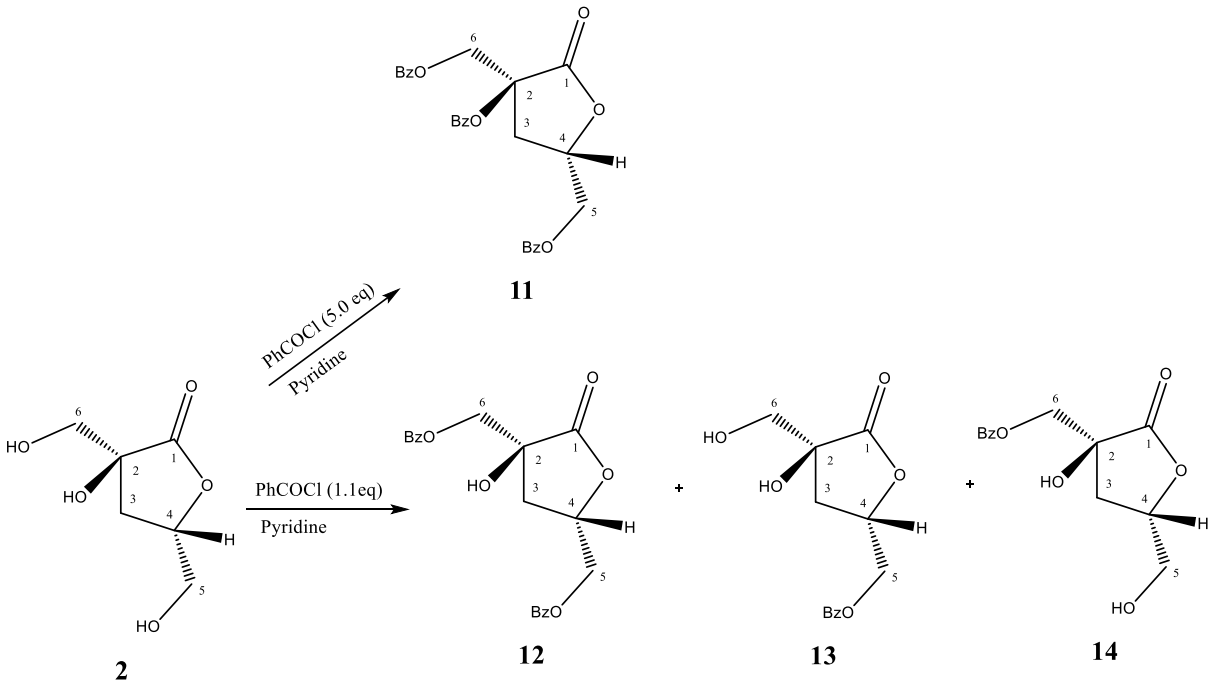


Figure 31. ^{1}H NMR spectra of 2-O-acetyl-α-GISAL **6**, 2,5,6-tri-O-acetyl-α-GISAL **4** and 5,6-di-O-acetyl-α-GISAL **5**.

The ^{1}H NMR spectra of the different acetyl derivatives are provided in Figure 32; the acetyl peaks are at 2.12, 2.10 & 2.08 ppm for **4**, 2.10 & 2.08 ppm for **5** and 2.12 ppm for **6**, and these signals confirm the presence of the acetyl groups in all three compounds. Addition of the electron withdrawing acetate group at position 5 causes a very significant downfield shift of the two H5s, whilst the addition at position 6 causes one of the H6s to move upfield whilst the other moves a small distance downfield.

2.2.2.2 Benzoates esters

To get a pure sample of β-GISA for use as a reference compound, the tribenzoate esters of α-GISAL **2** were prepared. The procedure used here to protect α-GISAL **2** as its benzoates esters was that reported by Shaw *et al*. and were used with only minor adjustments. The tribenzoyl-α-GISAL **11** was prepared using **2** as starting material and reaction with excess benzoyl chloride (five equivalents) in pyridine, which, after chromatography gave **11** in high yield. ^{1}H & ^{13}C NMR chemical shifts for **11** are in agreement with the values reported in the literature [134].



Scheme 2.7: Benzoylation of α-GISAL

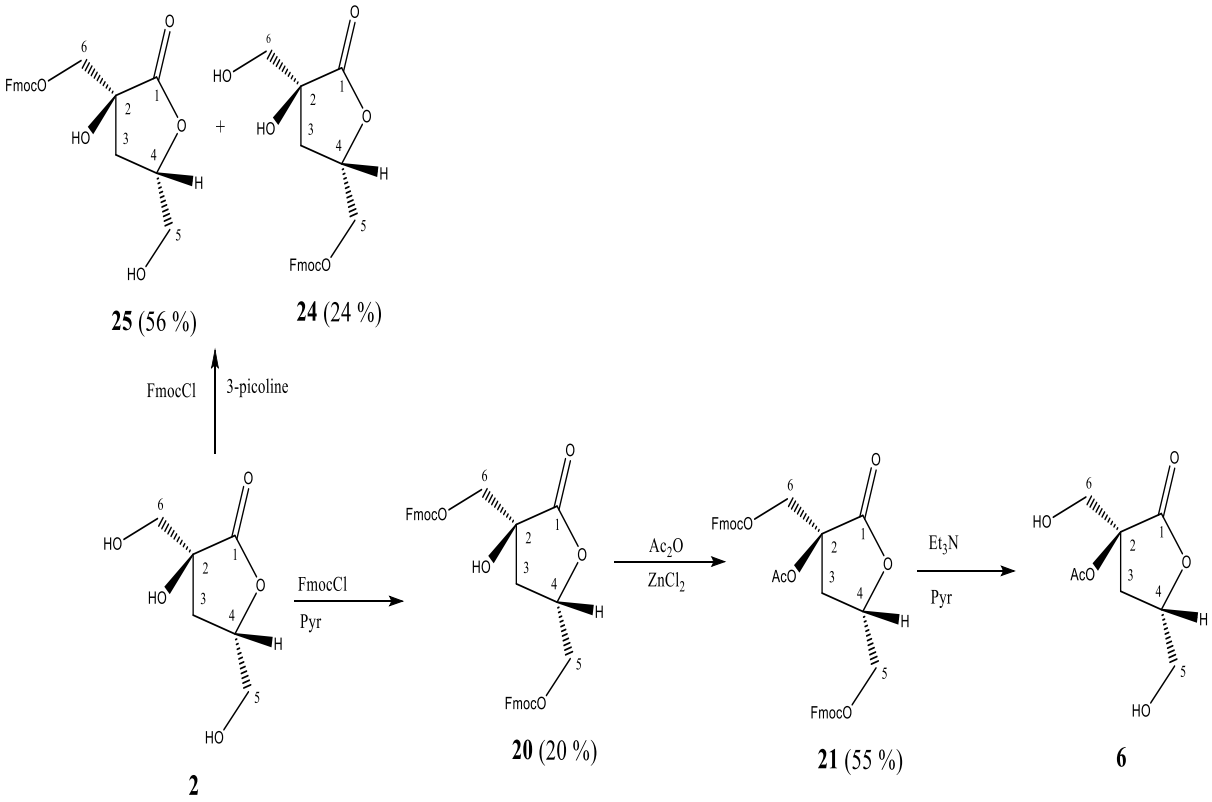
As was the case in the attempts to prepare mono-acetates, attempts to prepare the mono-protected benzoate derivatives of the glucoisosaccharinic acids by reacting a stoichiometric amount of benzoyl chloride (1.1 eq) with α-GISAL **2** gave a mixture containing 5,6-di-O-benzoyl-α-GISAL **12** and two mono-substituted derivatives 5-O-benzoyl-α-GISAL **13** & 6-O-benzoyl-α-GISAL **14** in a 60/40 ratio. The mixture of **13** & **14** was subsequently used as starting material in the production of the β-GISAL derivative *via* dihydroxylation reactions. (See later discussion, section 3.5)

2.2.3 Carbonates

Like esters, carbonates can be removed by basic hydrolysis, but carbonates are generally much less vulnerable to hydrolysis due to the resonance effect of the second oxygen[135]

2.2.3.1 Flourenymethylcarbonyl (Fmoc) derivatives of α-GISAL **2**

As was stated above, it was possible to form the mono-acetyl derivative **6** in a three-step procedure using a reaction sequence involving the incorporation of orthogonal protecting groups. It involved the initial preparation of the 5,6-di-O-Fmoc-α-GISAL **20**; which was prepared by reaction **2** with Fmoc chloride in pyridine with DMAP as catalyst [136]. The next step involved the acetylation of the hydroxyl group at position 2 to form 2-O-acetyl-5,6-di-O-Fmoc-α-GISAL **21** then the deprotection of the Fmoc groups using trimethylamine in dry pyridine [136-138] to form **6** (scheme 2.8).



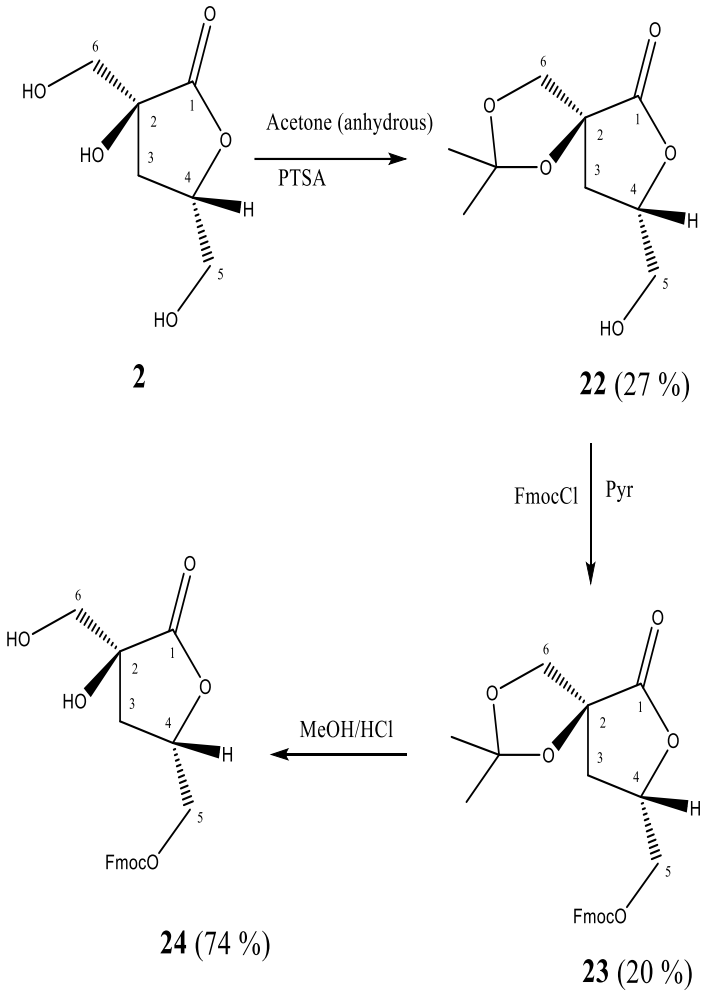
Scheme 2.8: The use Fmoc protection to form 2-*O*-acetyl-α-glucoisosaccharino-1,4-lactone **6.**

It is worth mentioning at this point that the reaction of α-GISAL **2** with the large and bulky Fmoc gave a mixture of products. In addition to the di-O-Fmoc-α-GISAL derivative **20**, the reaction also gave small amounts of the mono-Fmoc derivative of GISA, i.e. both 5-O-Fmoc-α-GIAL **24** and 6-O-Fmoc-α-GISAL **25** (scheme 2.8). A significant effort and a number of reaction attempts were made to optimise the yield for this reaction, but without a great deal of success (see table 3). The highest yields of the protected α-GISAL were obtained when 3-picoline was used as a solvent instead of pyridine, after chromatography **24** (24%), **25** (56%) and **20** (<5%) were obtained.

Table 3: Actual yields obtained in attempts to optimise Fmoc protection.

FmocCl molar equiv.	Catalyst	Reaction time (h)	Yield of **20** (%)	Yield **24** (%)	Yield **25** (%)	Unreacted FmocCl (approx.%)
2.2	none	3	5.2	22	<1	26
2.2	DMAP (non-stoichiometric)	3	20	unknown	unknown	14
2.2	ETT (non-stoichiometric)	3	10	12	<1	24
2.2	none	170	5.4	20	9.6	25
2.2	DMAP (stoichiometric)	0.5	<1	13	<1	N/A
2.2	ETT (stoichiometric)	0.5	11	<1	<1	N/A
1.1	none	170	9.0	<5	<5	N/A
10	none	0.5	<1	2.8	<1	N/A
2.2	none	3	15	23	<1	22

The mono-substituted derivative **24** was also synthesised using a three-step procedure which involved the preparation of 2,6-O-isopropylidene-α-GISAL **22** [17] followed by reaction of the hydroxyl at position 5 with an Fmoc group to form 2,6-O-isopropylidene-5-O-Fmoc-α-GISAL **23**. The subsequent hydrolysis of the acetal gave the desired product **24** in high yield (scheme 2.9). The 5-O-Fmoc-α-GISAL **24** was also used as starting material for the preparation of β-GISAL *via* a dihydroxylation reaction (see later discussion, section 3.5), while 6-O-Fmoc-α- GISAL **25** was treated with hydrogen bromide in acetic acid (33% v/v) to give 2,5-di-O-acetyl-6-O-Fmoc-α-GISAL **26** [139].



Scheme 2.9 Preparation of 5-O-Fmoc-α-GISAL

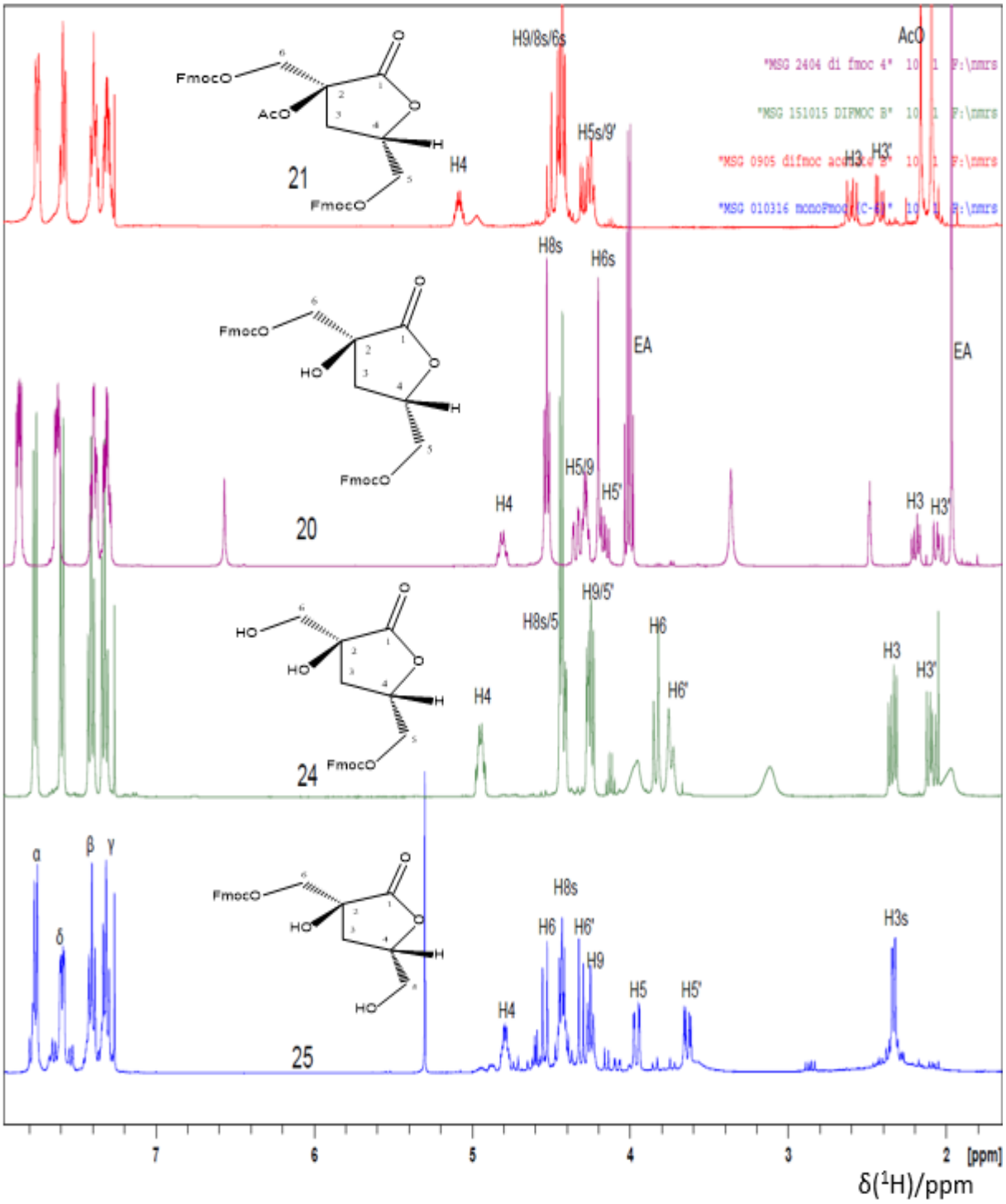


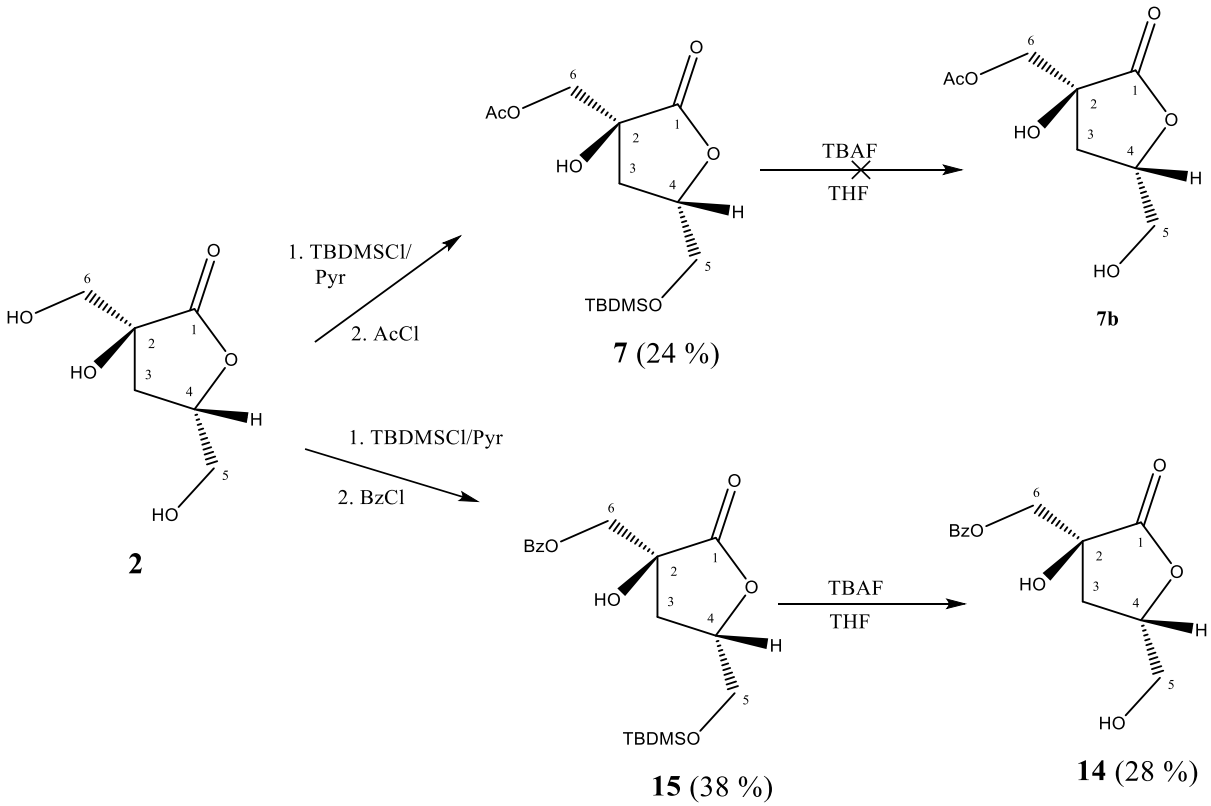
*Figure 32: ^{1}H NMR spectra of **21**, **20**, **24** and **25**.*

In the NMR spectra (Figure 33) the locations of the protecting groups were identified from observing the downfield shifts of the neighbouring methylene signals. In **25** the H6s are shifted downfield relative to the H5s and the opposite is true for **24**. In **20**, both

H5s and H6s are shifted downfield and unsurprisingly in **21** the H6s are shifted even further downfield.

2.3 Regioselective placement of orthogonal protecting groups on α-GISAL

The next approach to be attempted was to use a one-pot sequential reaction to add different protecting groups at different positions. In the first step, TBDMSCl (1:1 eq) was reacted with α-GISAL **2** with the silylation reaction taking place exclusively at the 5-OH, after the first reaction, the conditions for adding an acetyl group as an orthogonal protecting group on 6-OH were applied to successfully produce 6-O-acetyl-5-O-TBDMS-α-GISAL **7**. Unfortunately, when an attempt to remove the silyl protecting group to give the 6-mono-O-acetyl-α-GISAL **7b** was made, the silyl-group was successfully removed with TBAF, however, all efforts to extract the desired product from the aqueous layer were unsuccessful.



Scheme 2.10: One-pot sequence reaction for the regioselective protection of α-GISAL.

To get around this problem, the one-pot sequential reaction was repeated using a larger ester group at the position 6 in order to increase the hydrophobicity of the product. Using a benzoyl group as protection for the 6-OH instead of acetyl successfully generated the 6-O-benzoyl-5-O-TBDMS-α-GISAL **15**, which after deprotection of the TBDMS at position 5, gave 6-O-benzoyl-α-GISAL **14**.

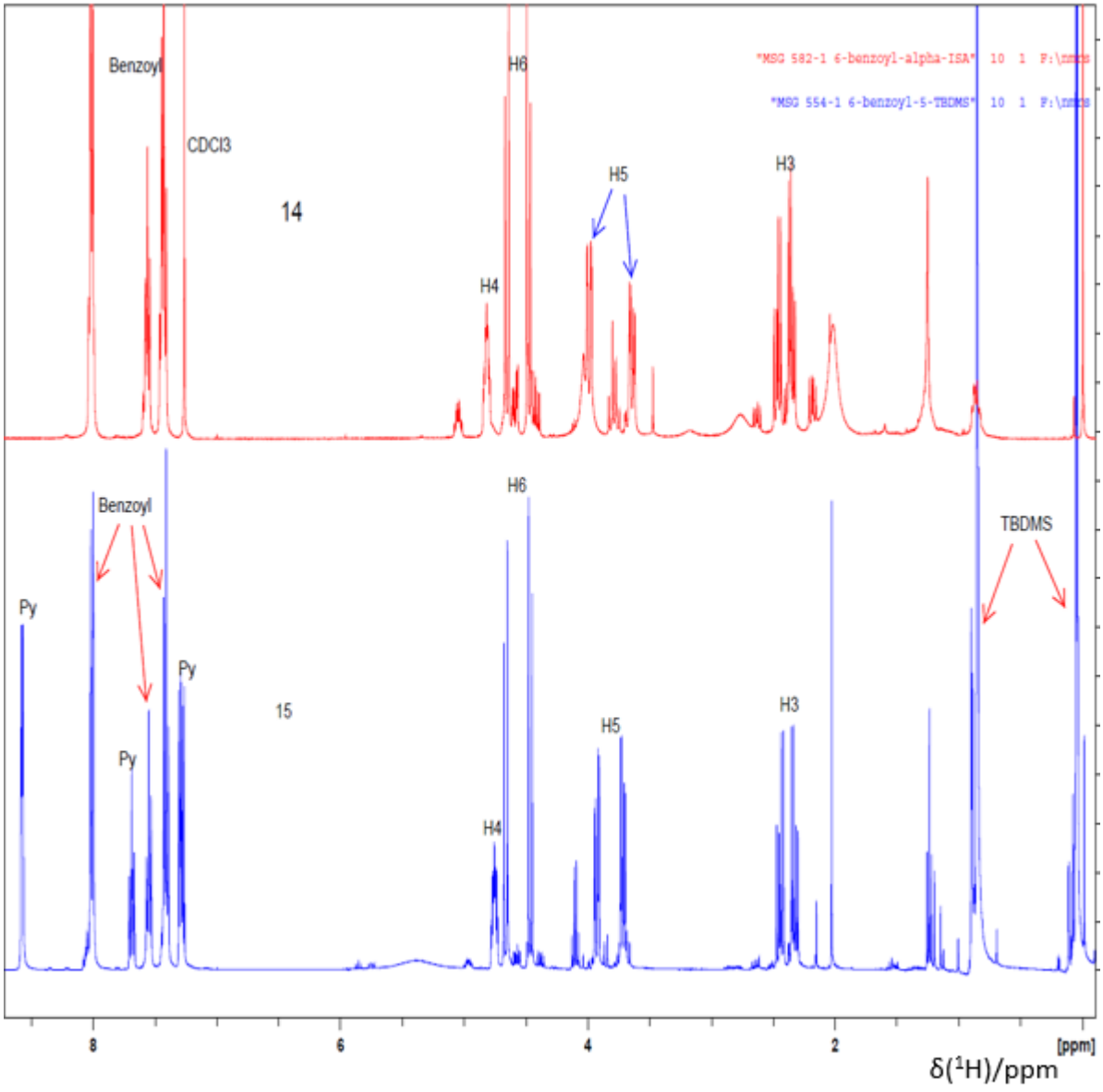


*Figure 33. ^{1}H NMR spectra of 6-O-benzoyl-5-O-TBDMS-α-GISAL **15** and 6-O-benzoyl-α-GISAL **14.***

The ^{1}H NMR spectrum of **15** shows the TBDMS signals at 0.85, 0.05 & 0.04 ppm and the benzoyl (aromatic peaks) at 8.01, 7.55 & 7.41 ppm, which integrated to 2, 1 & 2 respectively and the absence of the TBDMS signal in the spectrum of **14** with benzoyl signals retained, suggests the selective cleavage of the TBDMS in **14.**

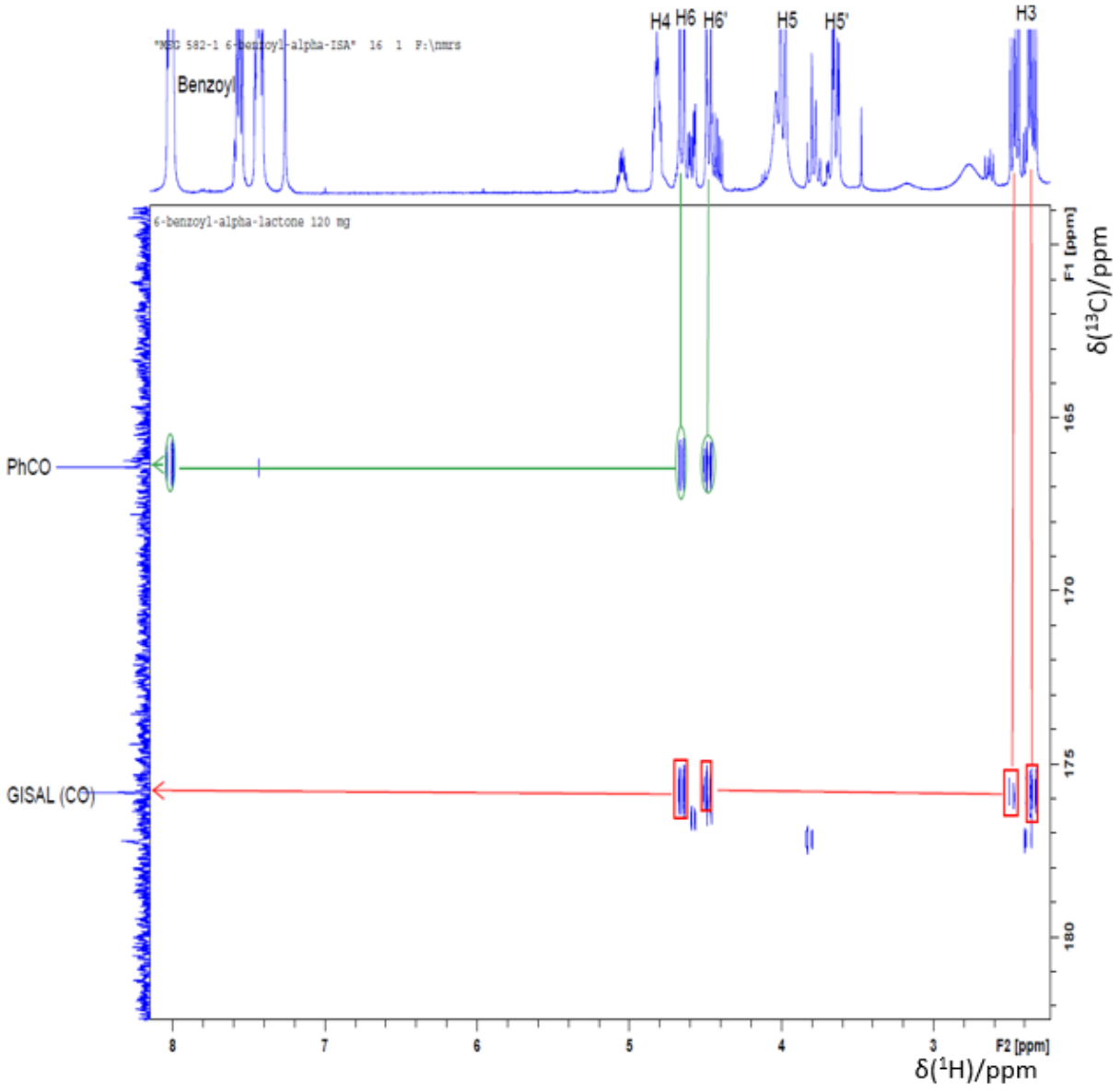


*Figure 34.]HMBC of 6-O-benzoyl-α-GISAL **14***

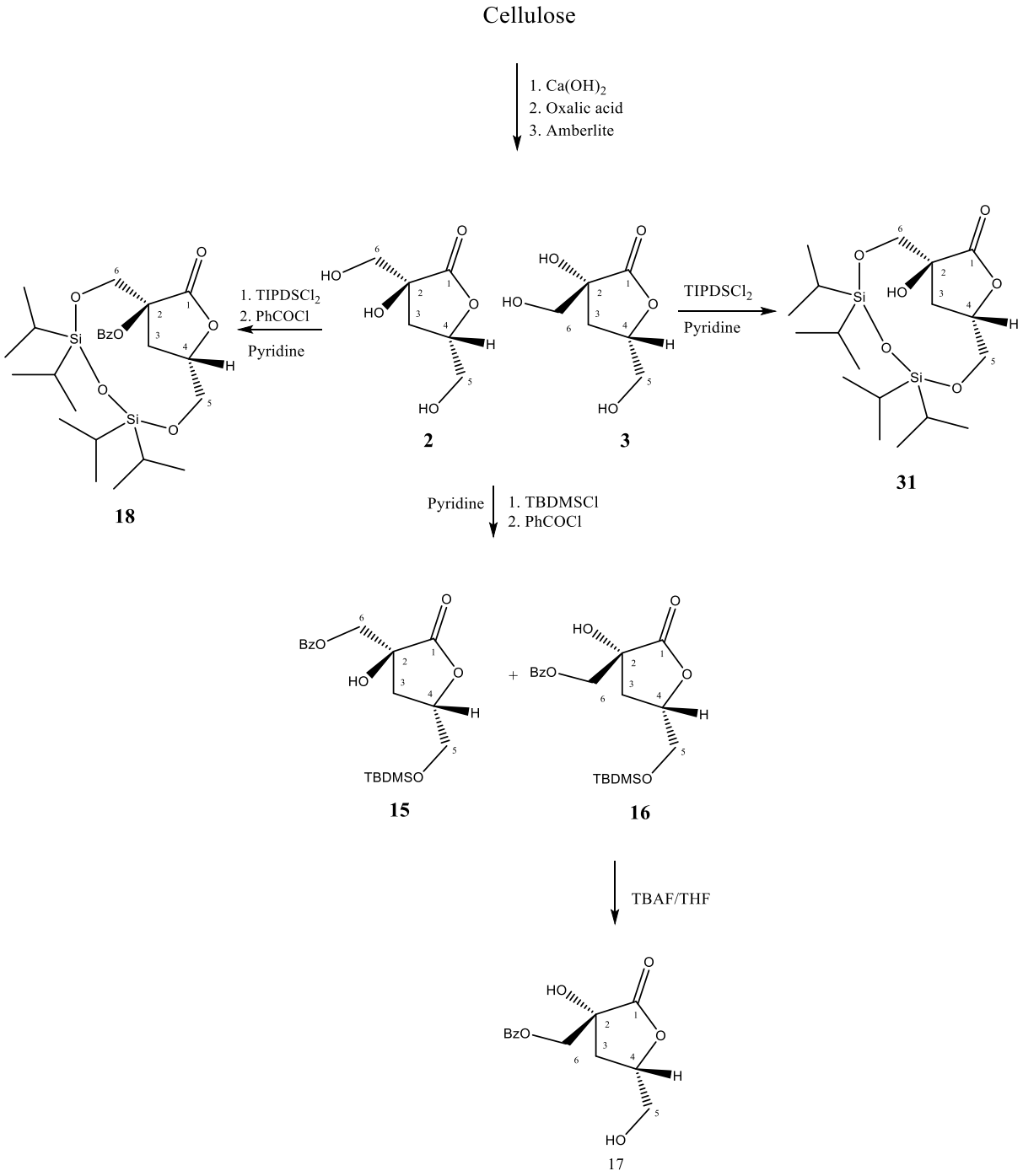
The HMBC spectrum, (Figure 35) shows coupling through multiple bonds between H3s, and H6s to the GISAL carbonyl at 175.8 ppm (red line) and that between H6s and ArHβ

to the PhCO at 166.4 ppm (green line), confirms the presences of the benzoyl at position 6 in **14.**

2.3.3 Use of protecting groups to aid the separation of α-GISAL and β-GISAL

As was stated in the introduction, there is a developed procedure where lactose is reacted with aqueous alkali to give ready access to α-GISAL **2**. In contrast, there are no simple methods for preparing β-GISAL. The most efficient method for preparing β-GISAL involves preparation of a mixture of the tribenzoate esters of both α-GISAL 2 and β-GISAL, which it is possible to separate using very careful column chromatography. While the main focus of the protecting group chemistry was to develop strategies giving access to regioselectively protected α-GISAL **2**, derivatives for use as platform chemicals; may provide an opportunity to apply the chemistry to mixtures of α-GISAL **2** and β-GISAL **3,** the products of which may be easier to separate.

The first experiment to be attempted on the 50:50 mixture of α-& β-GISAL, which had been prepared from cellulose, was reaction with the bifunctional TIPDS group, which, when applied to α-GISAL was shown to bridge between the hydroxyl groups at positions 5 & 6. When the reaction was repeated with a mixture of α-& β-GISAL (**2** & **3**) in a 50/50 ratio as starting material in pyridine, a sample of 5,6-O-TIPDS-α-GISAL **31** was obtained, which implies that the α-GISAL **2** component of the mixture has reacted with the TIPDS group as expected leaving behind the desired β-GISAL **3** in the aqueous phase. However, all efforts to isolate the unreacted β-GISAL **3** from the aqueous layer including freeze-drying were not successful and it is likely that other impurities, derived from the cellulose starting material which were present in the reaction mixture, gave intractable tars which could not be separated.



Scheme 2.11: A robust method for the separation of the β-GISAL derivative **16** from a mixture of α- and β-GISAL **2** & **3**.

In the next approach, the one-pot sequential reaction (discussed above, section 2.3) was repeated on the mixture of α- and β-GISA (**2** & **3**) using a benzoyl group to protect the 6-OH to form 6-O-benzoyl-5-O-TBDMS-α-GISAL **15**, followed by the deprotection

of the TBDMS at position 5 to form 6-O-benzoyl-α-GISAL **14** (Scheme 2.11) . The first two steps in the procedure successfully led to the preparation of a mixture of 6-O-benzoyl-5-O-TBDMS-α-GISAL **15** and 6-O-benzoyl-5-O-TBDMS-β-GISAL **16** that were subsequently separated in to their pure epimeric forms using column chromatography. (See appendix for the full characterisation of **16**).

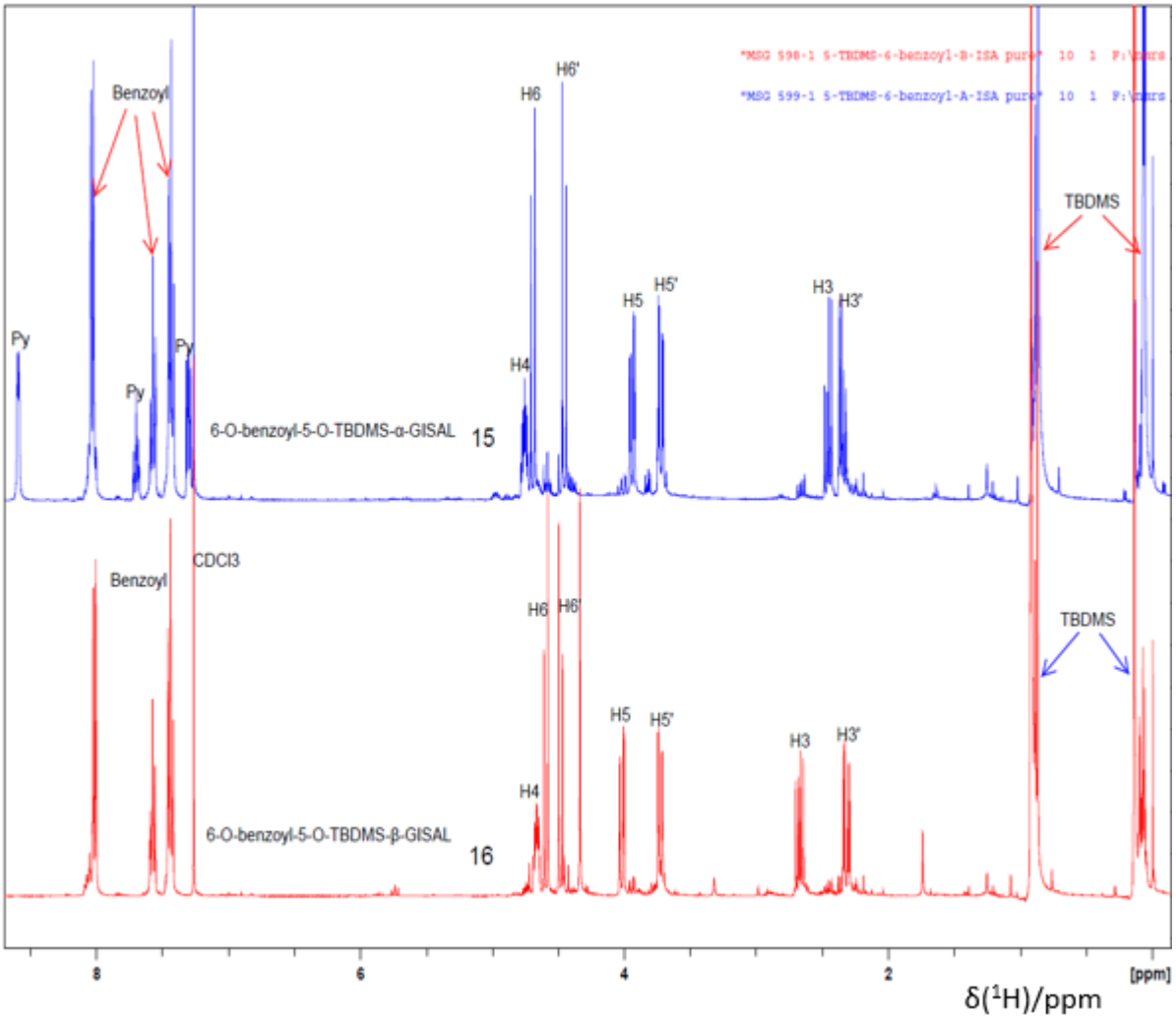


*Figure 35. ^{1}H NMR of **15** and **16**.*

As was the case for the corresponding lactones, the two epimers gave NMRs that are very similar (Figure 36) with the most significant difference between the two being the chemical shifts of the protons of the C3 methylene groups, in the α-epimer the two

protons have similar chemical shifts whilst in the β-epimer the two have different chemical shifts.

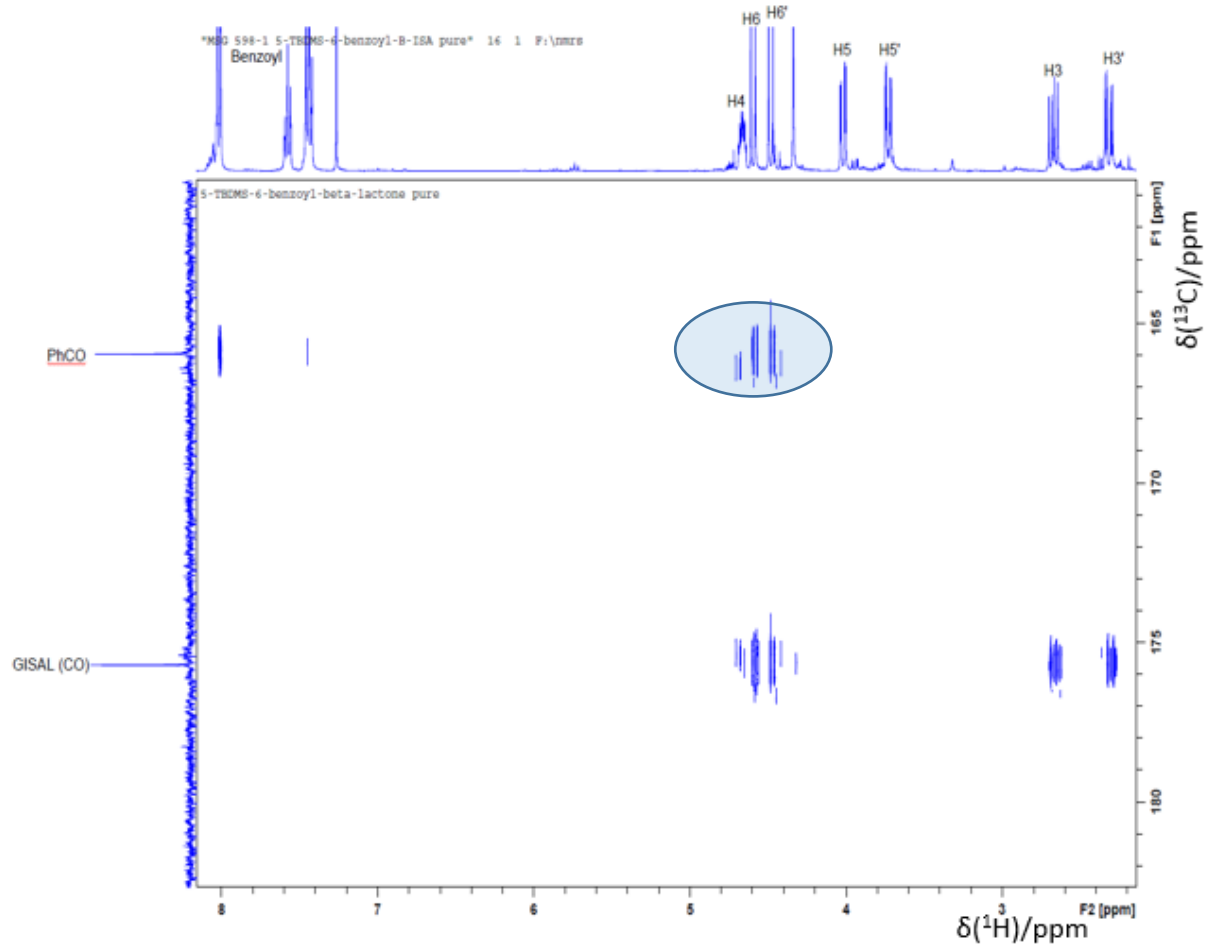


*Figure 36. HMBC spectrum of 6-O-benzoyl-5-O-TBDMS-β-GISAL **16**.*

The location of the benzoyl at C6 was confirmed by inspection of the HMBC spectrum of **16**, which has a long range coupling between the benzoyl carbonyl and the C6-methylene hydrogens (Figure 37). In a similar way, the location of the TBDMS group was confirmed through the observation of a NOE between the methyls of the TBDMS and the protons of the C5 methylene group on the NOE spectrum for **16** (Figure 38).

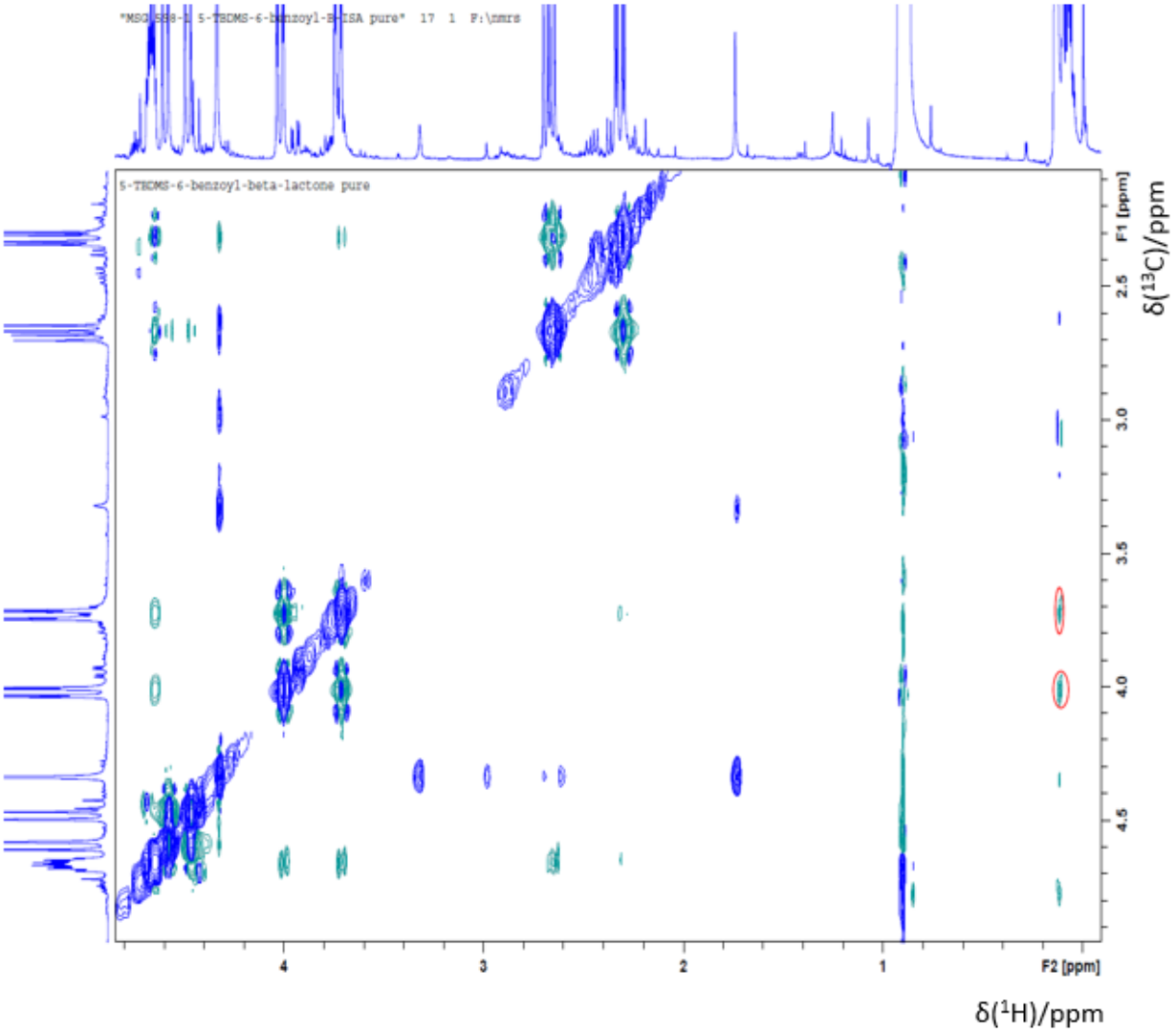


Figure 37. NOESY spectra for 6-O-benzoyl-5-O-TBDMS-β-GISAL ***16.***

In the next step, the method which has previously been reported to remove the silyl protecting group from **15** was then applied to 6-O-benzoyl-5-O-TBDMS-β-GISAL **16** to remove the TBDMS group at position 5 and to give 6-O-benzoyl-β-GISAL **17** and this was successful. Analysis of the NMR spectra (Figure 39) again confirmed that there was no loss or migration of the benzoyl group during the removal of the TBDMS; a long range scalar coupling between the benzoyl carbonyl and the C6-methylene is still visible on the HMBC spectrum (Figure 40).

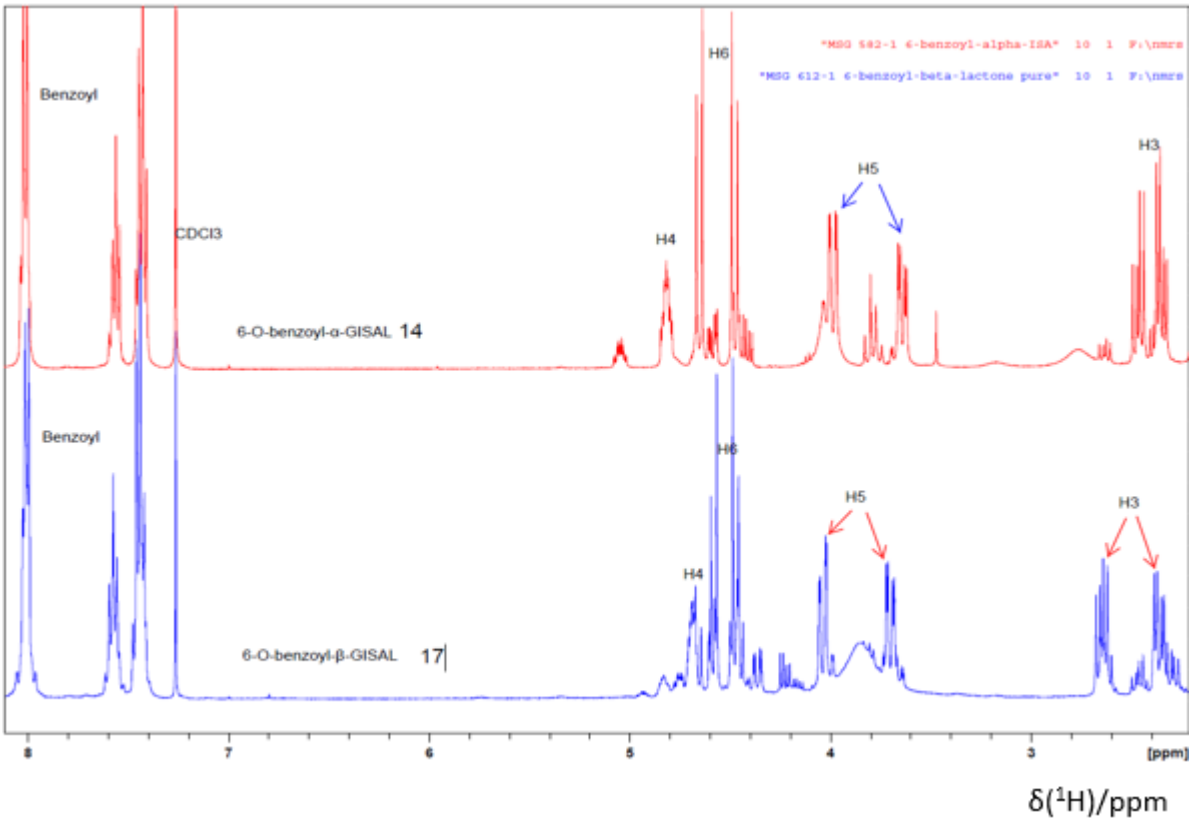


*Figure 38. ^{1}H NMR spectra of **14** and **17**.*

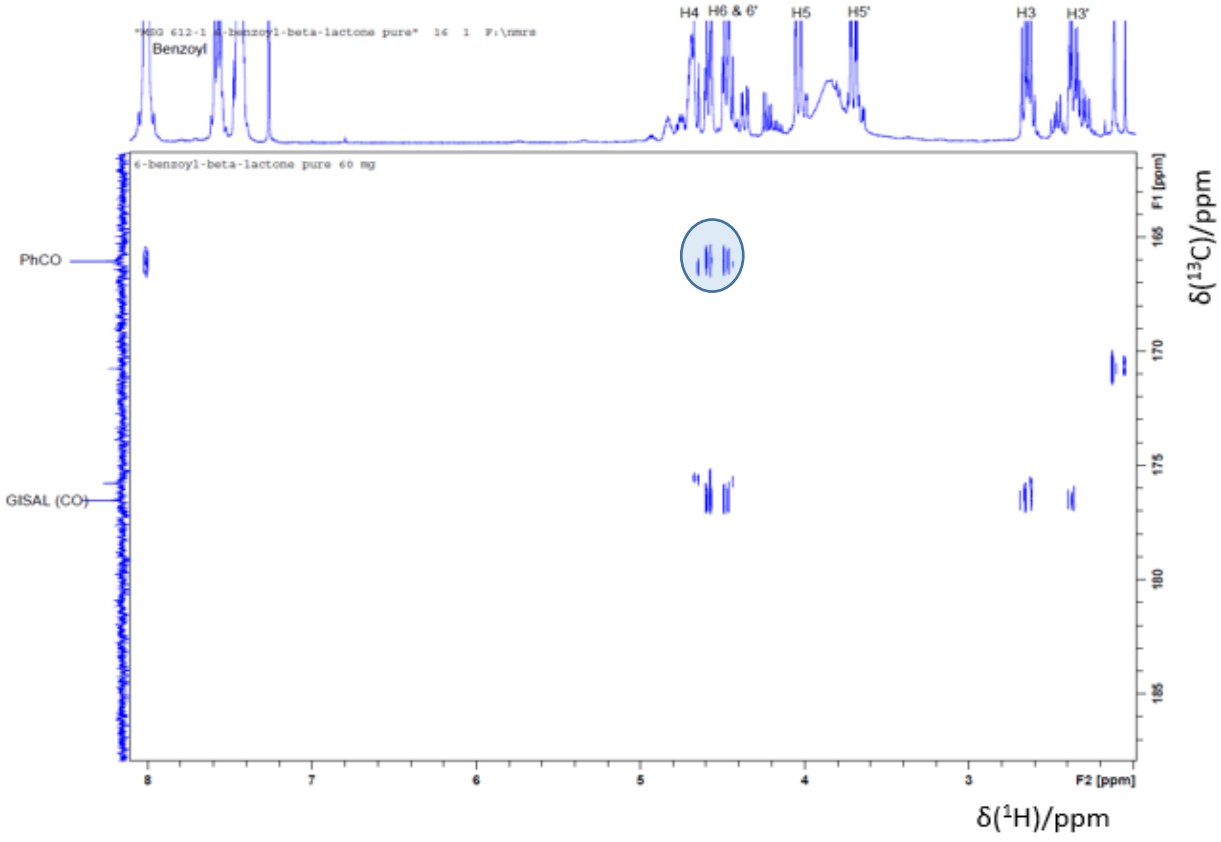


*Figure 39. HMBC spectrum of 6-O-benzoyl-β-GISAL **17**.*

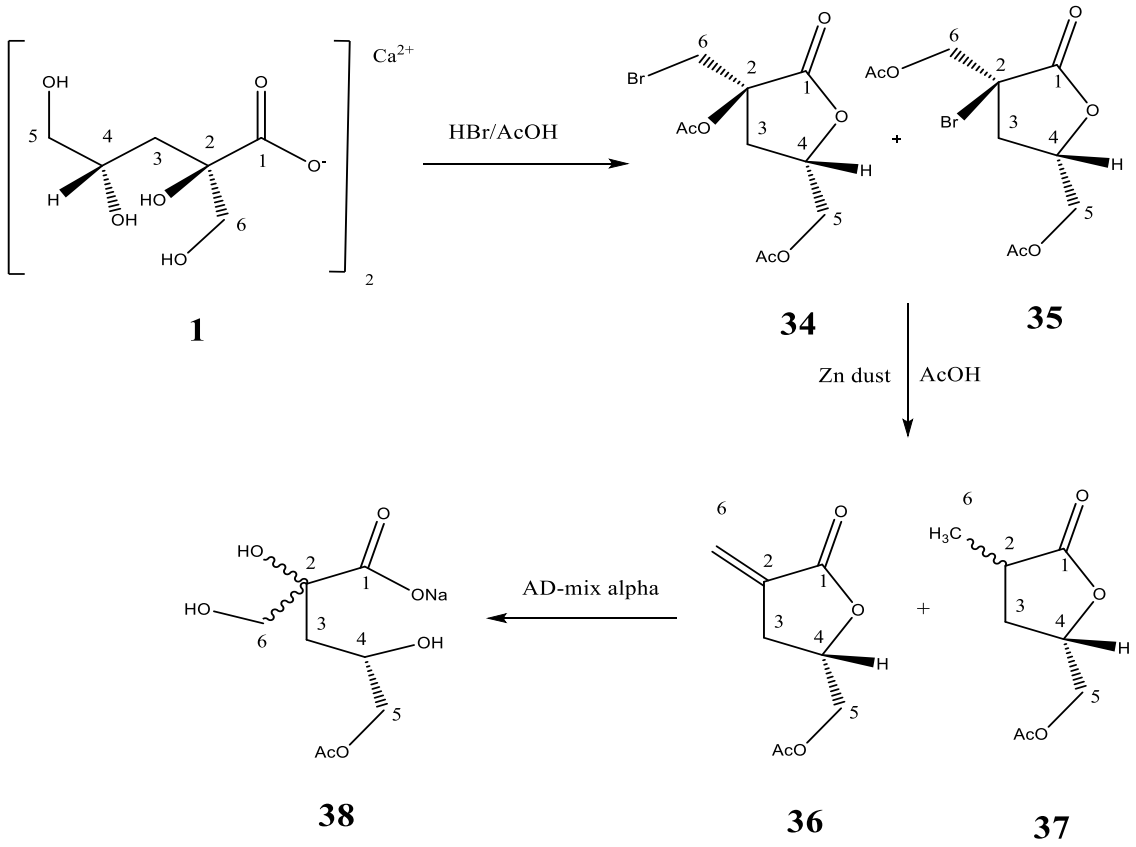
2.4 Attempted conversion of a α-GISAL to a β-GISAL derivative *via* a Sharpless dihydroxylation reaction.

As stated earlier, the other potential option for making β-GISA accessible is to covert the readily available and easily accessible α-GISA to a β-GISA derivative. One possible route was *via* the α-methylene lactone **36** whose synthesis had previously been reported by Bock *et al.* [65]. As was discussed in the introduction, the dihydroxylation of an olefin to a diol by an asymmetric dihydroxylation reaction is well known and involves the use of a chiral ligand associated with a metal catalyst (osmium) and can be achieved using commercially available premixes: AD Mix α or AD Mix β [118] depending on which epimer is required. The first step in this reaction sequence required the preparation of the α–methylene lactone.

2.4.1 Preparation of the α-methylene lactone using Herbert and Rolland's method [140]

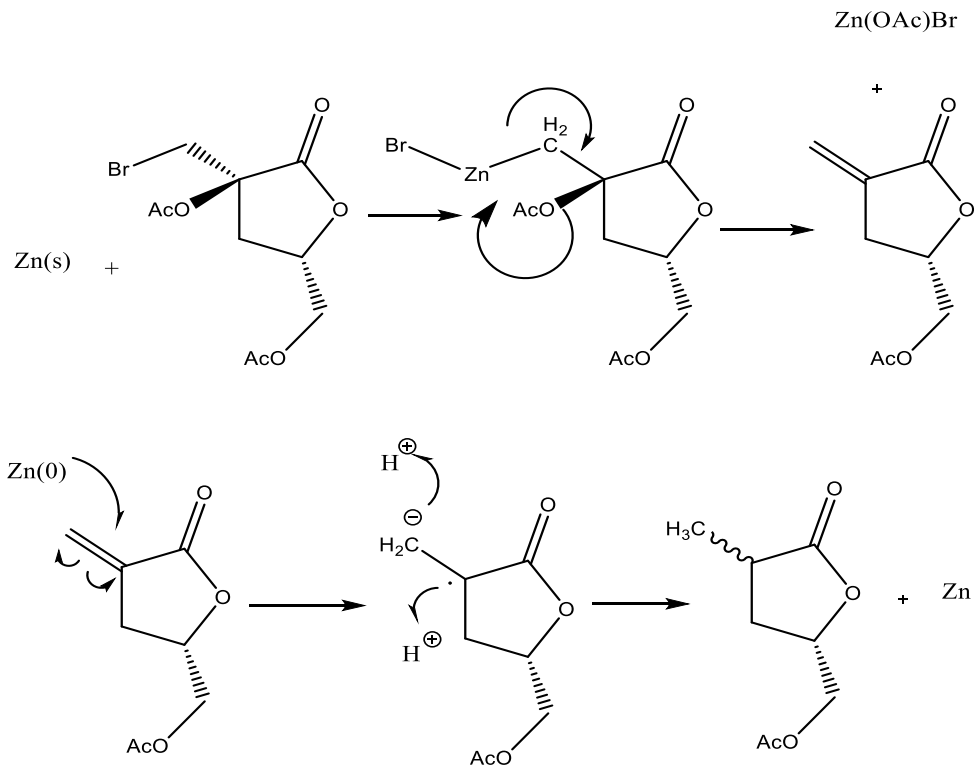
The method reported by Bock *et al*. [65] for the synthesis of the optically active α-methylene lactone, starting from Ca(α-GISA) **1**, was adopted in later experiments, and a number of significant modifications were made to improve the outcome of the reaction.

The first step involved the preparation of a mixture of the acetoxybromo-lactone isomers (**34** and **35**). Calcium GISA was treated with hydrogen bromide in acetic acid to form a 9:2 mixture of the acetoxybromo-lactones **34** & **35**. The reaction is believed to proceed through the formation of an acetoxonium ion intermediate that is then attacked by the bromide anion either at carbon 6 or 2. In the next step the mixture of **34** and **35** was treated with zinc dust in acetic acid to give two main products: the methylene lactone **36** and a further reduced product the methyl lactone **37** as a mixture of epimers. In a subsequent experiment, the two epimers of **37** were isolated in the organic phase recovered from the dihydroxylation of the mixture containing **36 & 37** (see later discussion).



Scheme 2.12: Sharpless dihydroxylation of Ca(α-GISA)$_2$ using AD-mix α.

Unfortunately, in our hands, the zinc metal-catalysed elimination of bromoacetate across the C2-C6 *via* Boord elimination [141] to form the desired lactone was not very high yielding. No matter how this reaction was carried out, the elimination was followed by a rapid hydrogenation of a large percentage of the resulting alkene to give significant quantities of the methyl lactone **37** as a significant product. The reaction is thought to occur by an oxidative insertion of the zinc metal into the C-Br bond followed by thermally induced elimination of the BrZnOAc (see scheme 2.13). It is likely that the metal, which is present in large excess in the reaction system, in suspension with acetic acid is also able to reduce the product alkene.



Scheme 2.13 : Boord elimination and hydrogenation reactions [141]

When the Bock procedure was repeated exactly as reported in the literatue [65] the product contained 5-O-acetyl-2-methylene-α-D-glucoisosaccharino-1,4-lactone **36** and 5-O-acetyl-2-methyl-α-D-glucoisosaccharino-1,4-lactone **37** (58% **36** and 16% **37**) along with 26% of the starting material **34** & **35** (Figure 41). This mixture was used as the starting material in the first exploratory dihydroxylation reaction.

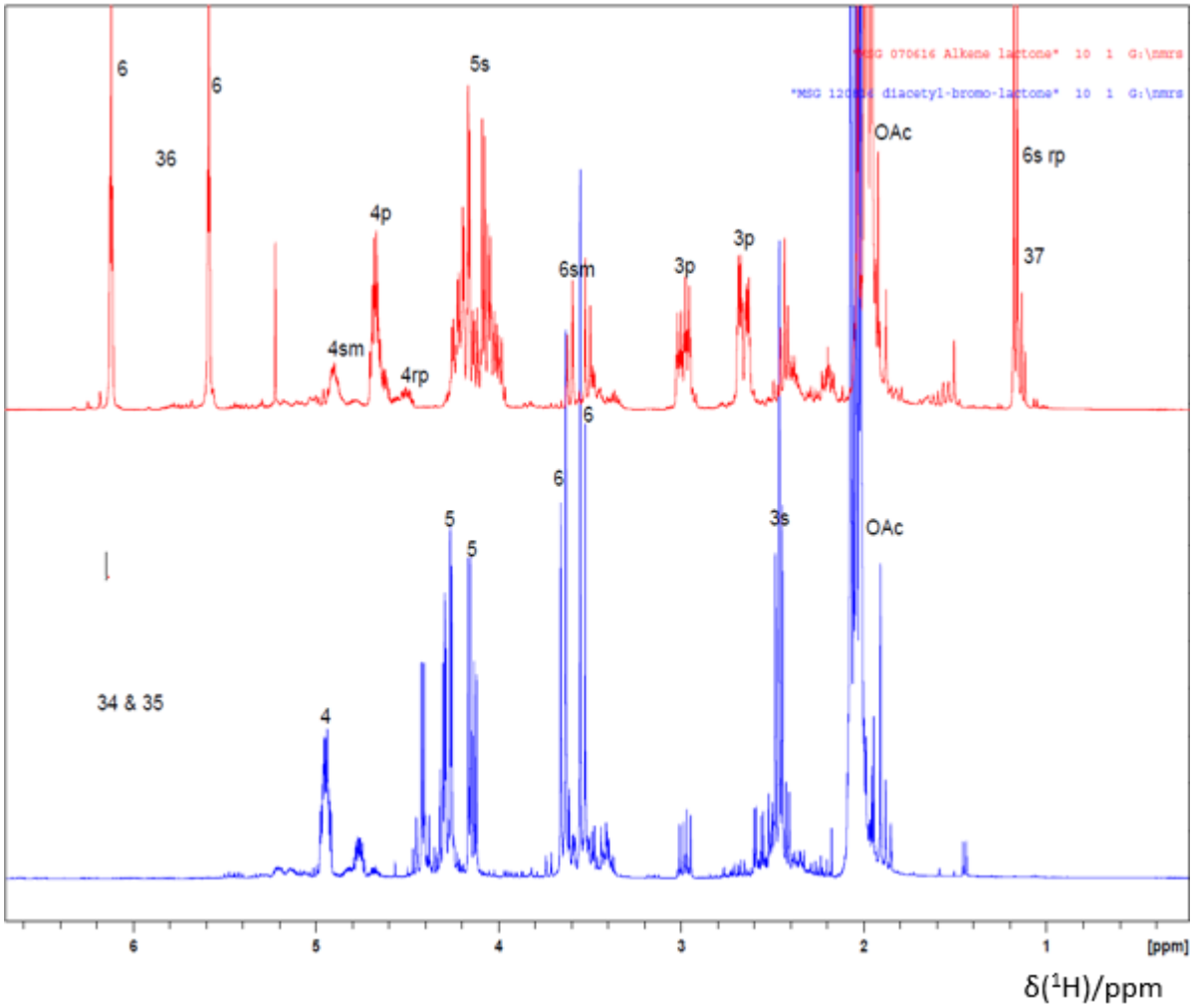
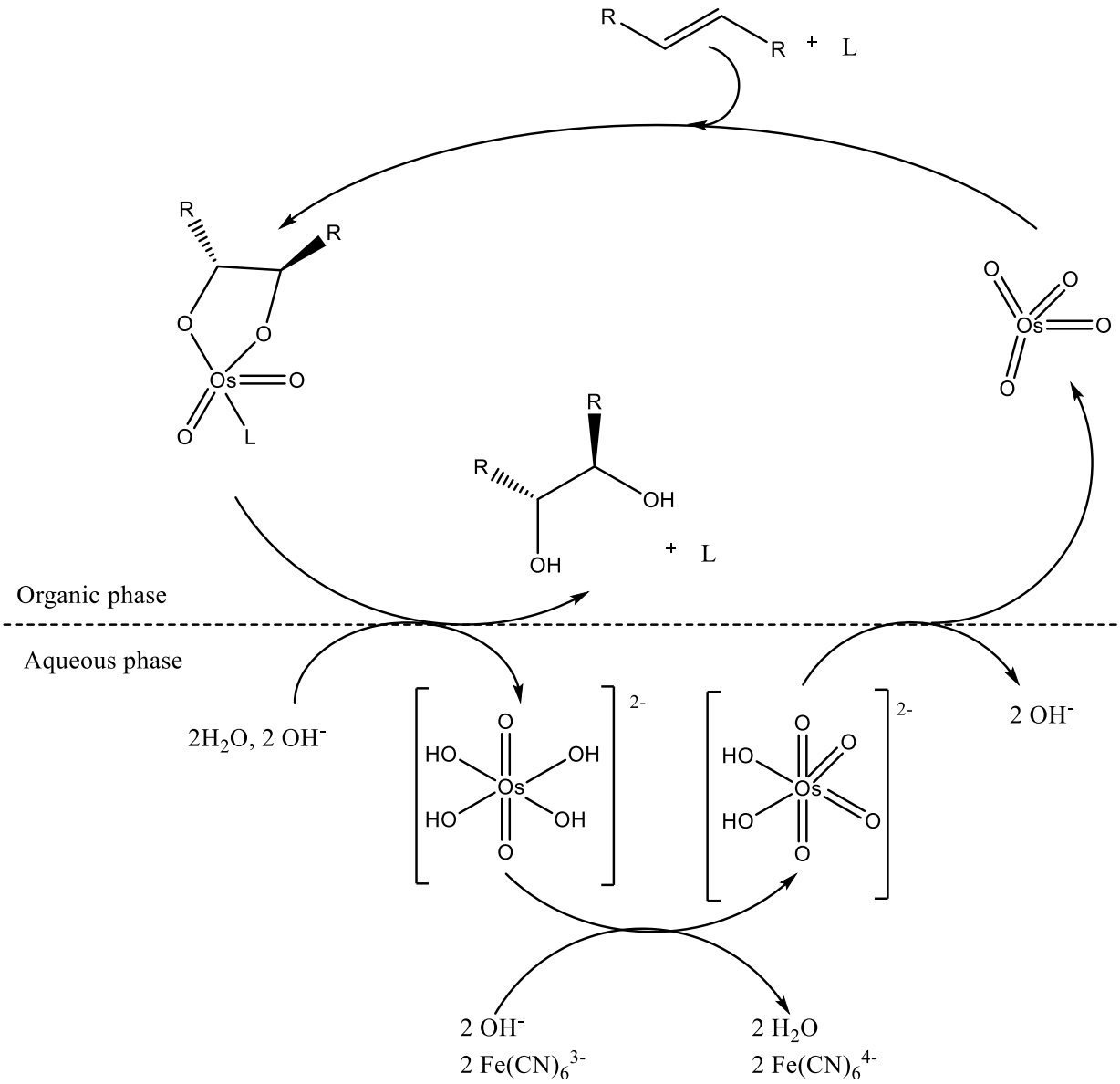


Figure 40.¹H NMR spectra of a mixture of 36 & 37 and diastereoisomers 34 & 35.

2.4.2. The Sharpless Asymmetric Dihydroxylation (AD)

The dihydroxylation reaction was carried out using the Sharpless method and in the first instance the AD mix α was employed as the catalyst as this had previously been shown to give *syn*-addition to the correct face of the alkene [118]. In the standard method that is employed to undertake Sharpless dihydroxylations for terminal alkenes the AD mix, which contains $K_2OsO_2(OH)_4$ as the catalytic oxidant, the chiral ligand $(DHQD)_2PHAL$ in AD mix β or $(DHQ)_2PHAL$ in AD mix α, the base K_2CO_3 and the sacrificial oxidant $K_3Fe(CN)_6$, are dissolved in 50:50 mixture of *t*-butanol and water to form a bright orange biphasic reaction mixture. The mixture is stirred at room temperature until the reagents

dissolve and then is cooled to 0 °C before the alkene is added in one portion and the resulting slurry is stirred vigorously until the alkene has all reacted (this typically takes between 6 and 24 h depending on the reactivity of the alkene).



Scheme 2.14: Osmium catalytic cycle of an AD reaction with $K_3Fe(CN)_6$ as the cooxidant

During this period the alkene adds to the osmium-ligand complex which is in the organic phase to form an osmylate. Base present at the interface of the two solvents then hydrolyses the osmylate with the reduced osmium (VI) species transferring to the aqueous phase where it is reoxidized using the ferric cyanide to give osmium tetroxide (VIII) which transfers back to the organic phase and recombines with alkene and ligand for a second catalytic cycle. Once all the alkene has reacted the remaining oxidant was reduced and in the first set of experiments reported here this was done by adding sodium sulphite and allowing the reaction mixture to warm up to room temperature over approximately 30 minutes. After the reaction, the reaction mixture was extracted with ethyl acetate and the desired product and the ligand were expected to be in the organic phase and the inorganic components including the hydrolysed osmium salts remain in the aqueous phase.

Using the α-methylene lactone as an alkene and employing the standard reaction procedures described above, at the end of the reaction the aqueous phase was extracted with ethyl acetate and the only products that were present in the organic layer were the two epimers of **37** and their NMR chemical shifts were the same as previously reported [65]. There was no evidence for the starting material in the organic extract which suggested that the α-methylene lactone had reacted. Unfortunately, the product could not be extracted and it was suspected that the high polarity of the product meant that it was being retained in the aqueous layer along with the ligand and other residual inorganic materials. An equal volume of ethanol was added to the aqueous layer in an attempt to lower the polarity of the solution to see if anything would precipitate. To our surprise, a phase separation was observed and quite by chance the combination of ethanol, water and a high ionic strength generated from the mixture of sodium salts led to the phase separation of the water and ethanol mixture. When the ethanol layer was

evaporated a crude solid was obtained and the ^{1}H NMR spectrum suggested that a complex mixture was present which potentially contained the ring- opened product **38**. The presence of the ring-opened lactone was confirmed from inspection of the ^{1}H NMR spectrum which contained a significant number of signals between 2 and 3 ppm that correspond to a very significant upfield shift of the C3-methylene group which is frequently observed in ring-opened products. In an attempt to effect a ring closure, the crude product was re-dissolved and left stirring in an acidified aqueous solution (pH 3-4) to encourage lactone formation, it was hoped that the lactone could be extracted from the aqueous phase and be purified, but this was not successful. This reaction was repeated several times, but with the same outcome on each occasion. One possible reason for obtaining ring-opened products is that the initial product formed after dihydroxylation of the α-methylene lactone would be mixture containing α-GISA & β-GISA bearing a C5-acetoxy group which would have a reasonable solubility in aqueous solution and which would partition into the basic aqueous layer during the dihydroxylation reaction. Once in the aqueous phase the lactone ring would undergo hydrolysis to give ring opened products.

In an attempt to overcome the ring opening problem, it was decided to use a starting lactone that had a large protecting group at position 5; that could improve the hydrophobicity of the products and which contained a chromophore that would help in locating products and simplify the product separation using chromatography. The reaction was repeated using 5-O-Fmoc-α-D-glucoisosaccharino-1,4-lactone **24** as a starting material and the first step was to react **24** with HBr in acetic acid and this gave a mixture of the acetoxybromo-lactones (**39** & **40**). This reaction worked well and gave a 9:2 ratio of the two isomers (determined from the integral ratios of the H4-protons, Fig 2.34) as was observed for the parent lactone α-GISAL (Scheme 2.15).

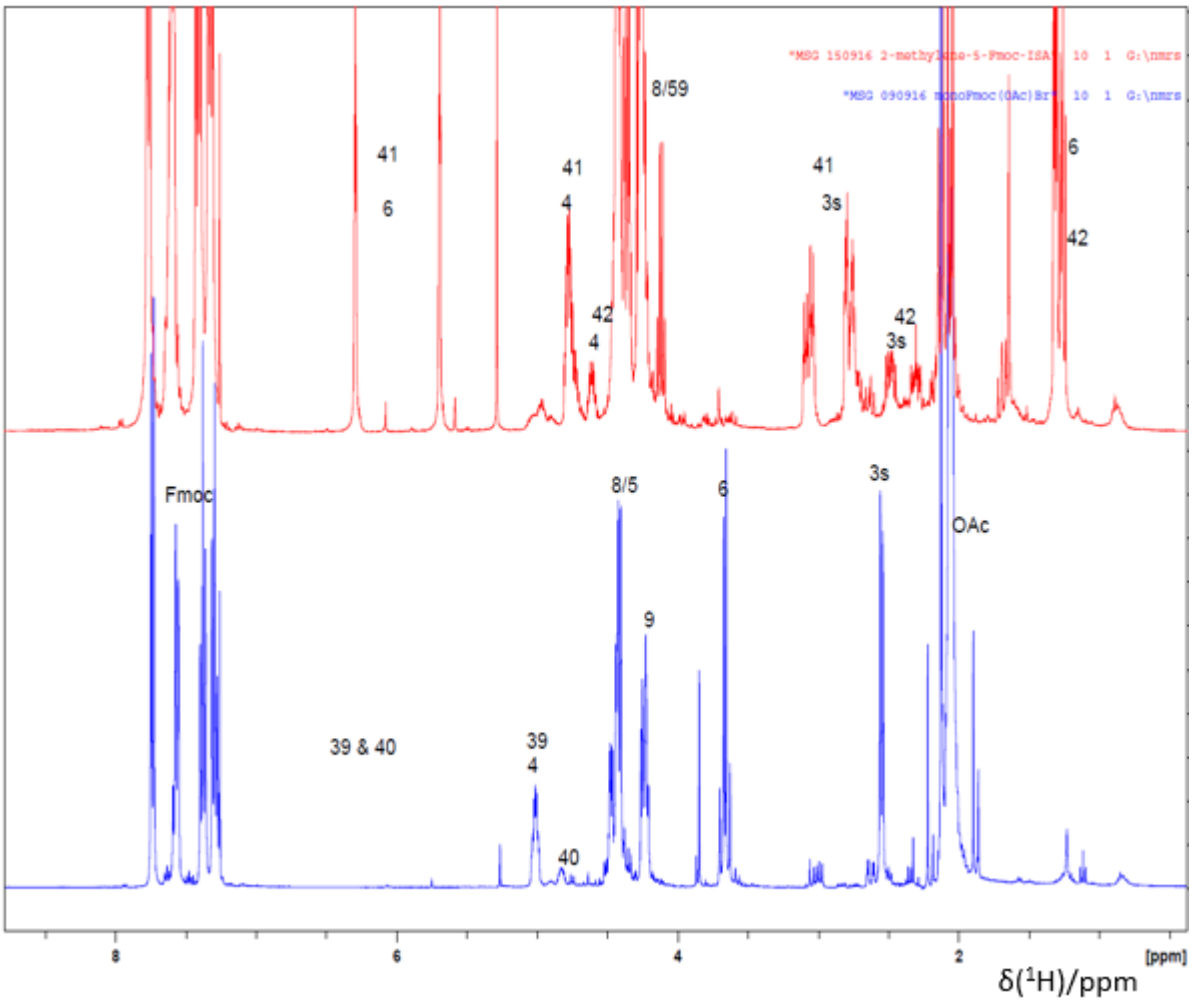
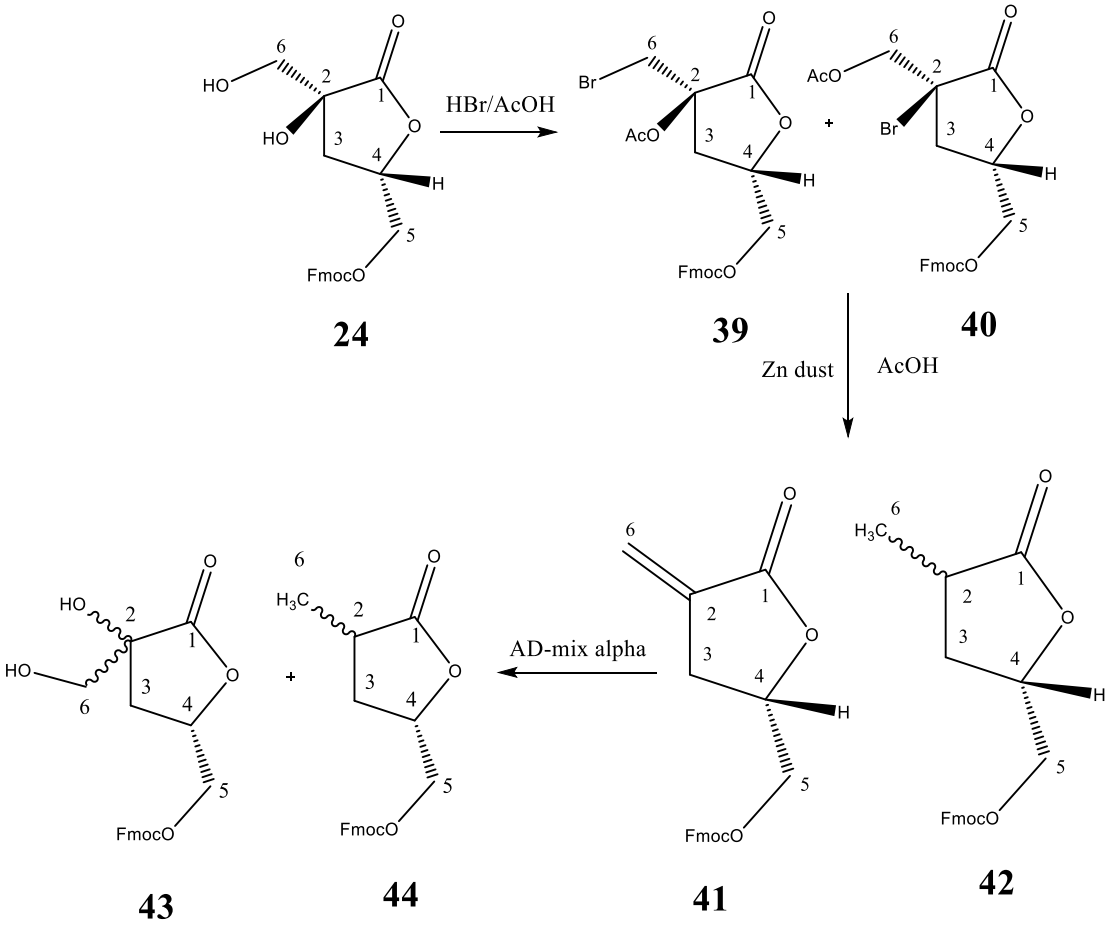


*Figure 41. ^{1}H NMR spectra of a mixture of **41** & **42** and **39** & **40**.*

The acetoxybromo lactone isomers, **39** and **40,** were subsequently transformed into the C5-Fmoc protected methylene lactone **41** (55%) using the same methods as discussed above, and again as well as getting the desired product, this was accompanied by the reduced methyl-lactone (31%) **42** (Figure 42).

On this occasion, after the asymmetric dihydroxylation, a small amount of the desired diol **41** was extracted in the organic layer.



Scheme 2.15: Sharpless dihydroxylation of **24** using AD-mix α.

On closer inspection of the mixture of products from the dihydroxylation of **41** (80 % yield) together with a small amount of unreacted **42** were all extracted in the organic layer with their lactone ring intact, but inspection of their NMR spectrum (Fig. 2.35) shows that the reactant had not reacted completely, only 70% of **41** was utilised in this reaction and also a signal of an acetyl group was observed. The latter may indicate that a degree of transesterification had taken place in the reaction leading to the formation of **39**.

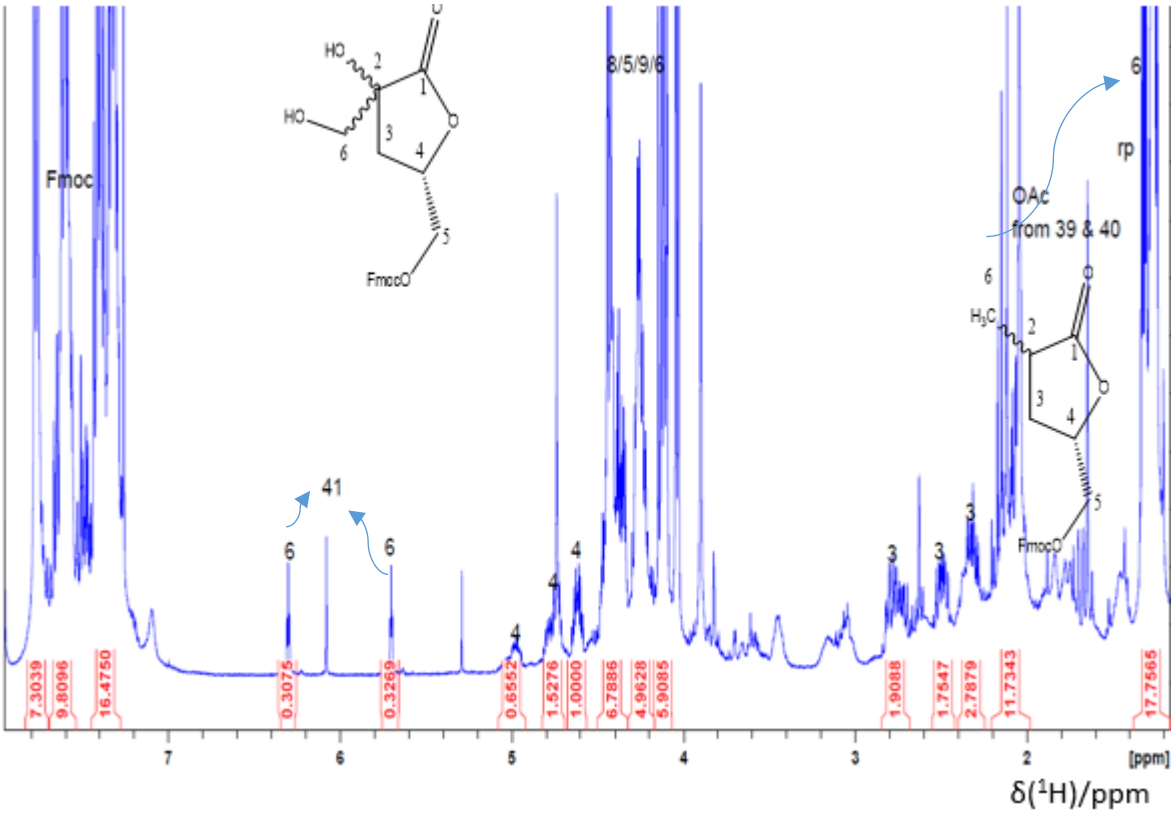
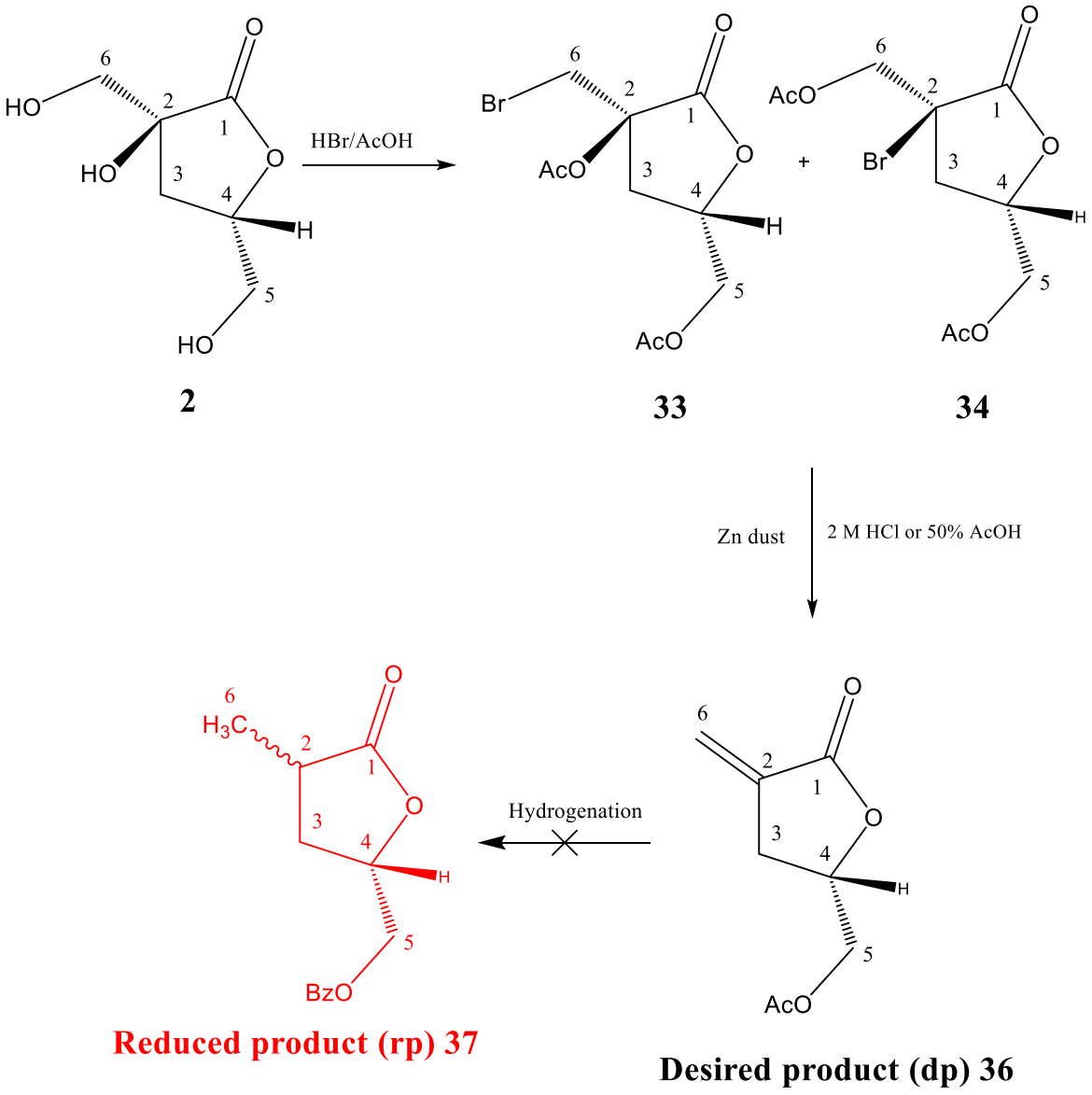


Figure 42.^{1}H NMR spectrum of 43 & 44.

At this stage, whilst it was clear that the alkene dihydroxylation was taking place, because of the complexity of the product mixture that was being obtained, it was decided to adopt a new strategy and to look more closely at the alkene formation reaction: to see if it was possible to prevent the reduction of the alkene in order that a clean alkene could be taken into the dihydroxylation reaction.

2.4.3 Optimization of the synthetic step leading to the production of the α-methylene lactone.

The objective of this section of work was to develop a method that could be used to prepare only the required olefin for the dihydroxylation reaction without the reduced product (Scheme 2.16) and to employ an α-GISAL derivative that has a less bulky protecting group at position 5 that could serve the vital role played by the Fmoc group on **24** in the last set of reactions.



Scheme 2.16: Optimizaation of alkene production.

2.4.3.1 Preparation of methylene lactone *via* Bock's method.

The method for the preparation of **36** using zinc dust and HCl (4 M) in ethanol was reported by Bock *et al*. [65] and this was the starting point for this work, the method was employed as reported and gave **36** (77%) and the further reduced products (23%) when performed on a large scale. All efforts to separate **36** and **37** using column chromatography and vacuum distillation failed and it was reported in the original publication that the alkene is unstable at high temperatures. The presence of the further reduced product required an optimisation of the synthesis method in order to prevent its formation in the reaction.

In the original paper the elimination step was performed in two parts. In the first step the bromoacetate (1 g) derivatives were dissolved in ethanol (22 mL), the zinc powder (8 g) was added and then the reaction mixture was stirred at room temperature over a period of 30 minutes. After 30 minutes, a solution of 4 M HCl was added and, after stirring for an additional 30 minutes, the reaction was stopped. Excess zinc was filtered off and the filtrate was evaporated to dryness. It was suspected that the reduction was taking place during the second phase of the reaction and this was confirmed by monitoring the production of the reduced product by GC-MS, the amount of the alkene dropped and this was accompanied by the increase in the amount of the reduced product.

As a consequence, several attempts were made to optimise the method in order to practically understand the role of the reagents used, particular attention was paid to the use of solvents and acid type, along with various acid concentrations and the zinc mixing periods being employed (Table 4).

The most successful reactions (highlighted in blue) were those in which the excess zinc was filtered from the reaction before adding the acid and at that stage the issue of further

reduced products was resolved (experiments 459-579). This was confirmed by inspection of the ^{1}H NMR spectra obtained from experiments 459-579.

Table 4. Optimization of the preparation of α-methylene lactone (dp) and suppression of the production methyl lactone (rp); the further reduced product

Reaction No. (MSG)	Acid/mixing time (min) (A = AcOH)	Part 2 mixing time (min)	Solvent	Result (%)	
				dp	rp
327	50 % A /30	30	EtOAc	66	34
328	50 % A /10	10	EtOAc	0	0
329	50 % A /25	25	EtOAc	57	43
334	Nil	10	EtOH	0	0
336	Nil	30	EtOH	0	0
340	50 % A /10	2.5 eq/10	EtOAc	44	10
341	Nil	60	EtOAc	0	0
342	Nil	240	EtOAc	0	0
354	4M HCl /30	30	EtOH	71	29
357	4M HCl (4 mL)/30	30	EtOH	0	0
377	HCl/10	10	EtOH	52	47
459	HCl/10	30	EtOH	40	0
475	HCl/5	30	EtOH	79	0
550	HCl/1	30	EtOH	71	0
573	2M HCl/5	30	EtOH	80	0
579	2M HCl/1	30	EtOH	51	0

At this stage, the prepared alkene with no reduced product was used for the dihydroxylation reaction using AD-mix-β. At the end of the reaction, sodium salts of a mixture of ring-opened products including Na(α-GISA) (41%) and Na(β-GISA) (38%) was obtained (Figure 44). The identity of the products was confirmed by comparison of the retention of the signals wit those of a standard (Figure 45). This result clearly shows a significant production of the β-epimer and a decrease of the α-epimer compared to the mixtures generated directly from either cellulose or lactose.

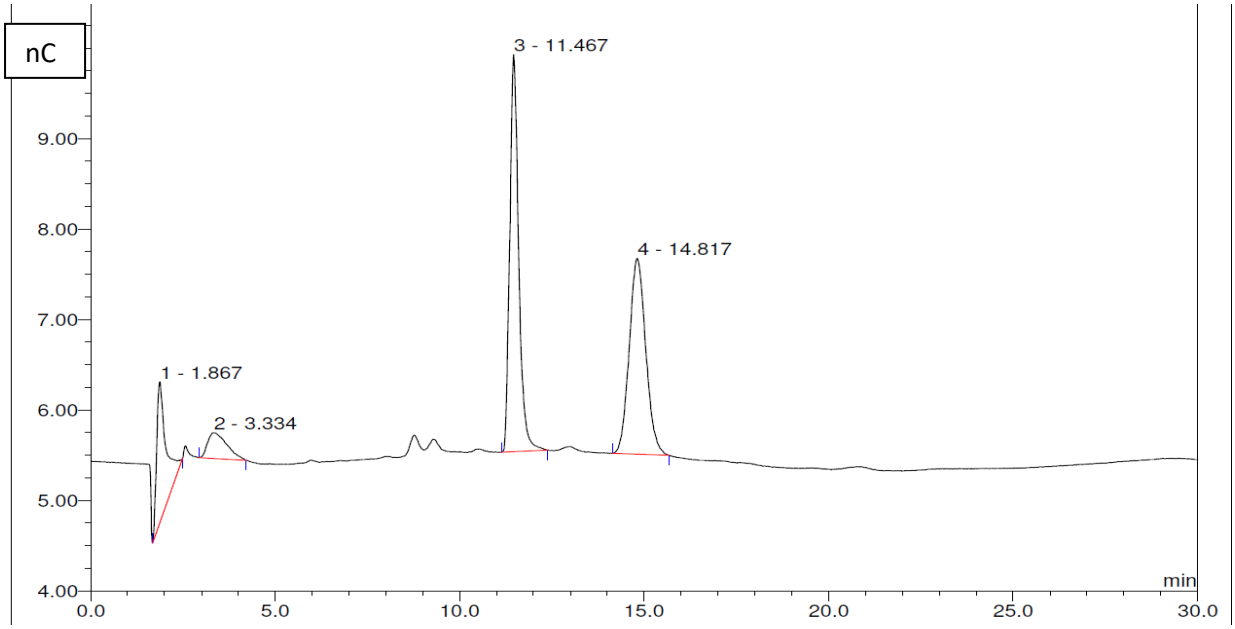


Figure 43. HPAEC chromatogram of opened ring α-and β-NaGISA using a pulsed ampeometic detector.

The search for solutions to the problem continued and, in the first instance, efforts were made to prevent ring opening during the dihydroxylation by using a buffered system as has previously described for terminal olefins [142-144] where under basic conditions epoxide formations had been observed. This method was applied directly on methylene lactone **36** produced using the optimised elimination procedure, and at the end of the reaction our products β-GISAL(OAc) (52%) and α-GISAL(OAc) (48%) (Figure 46) were obtained with their lactone rings intact.

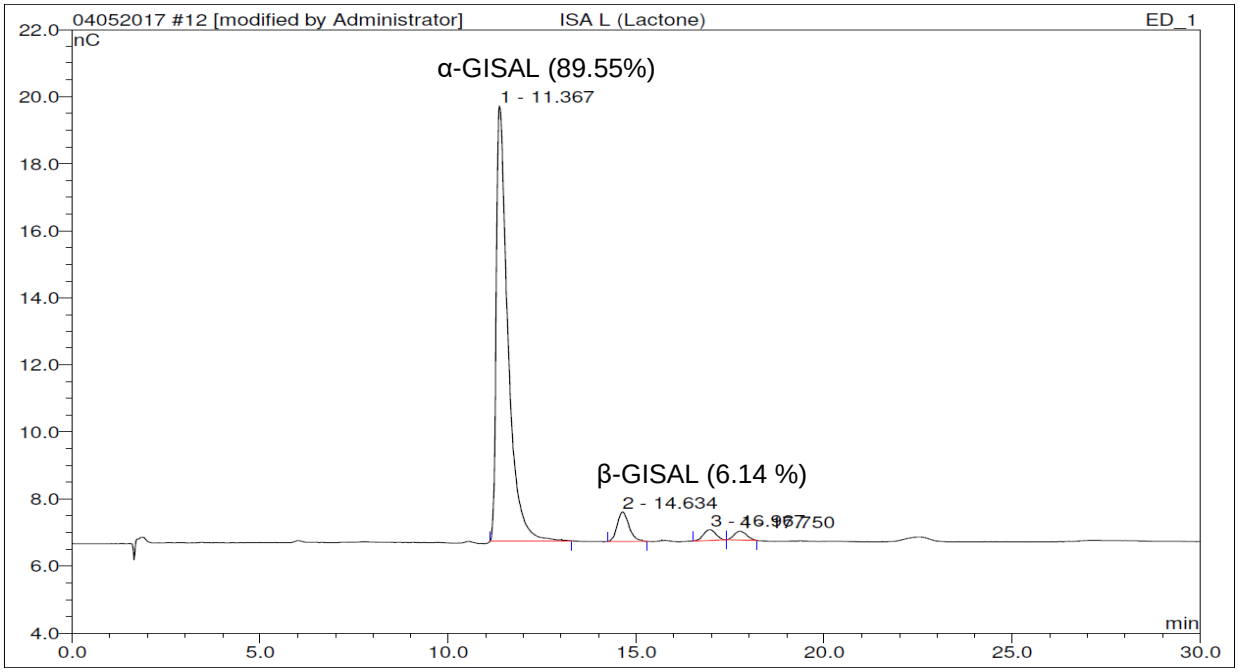


Figure 44. HPAEC chromatogram of the control (α-GISAL and β-GISAL).

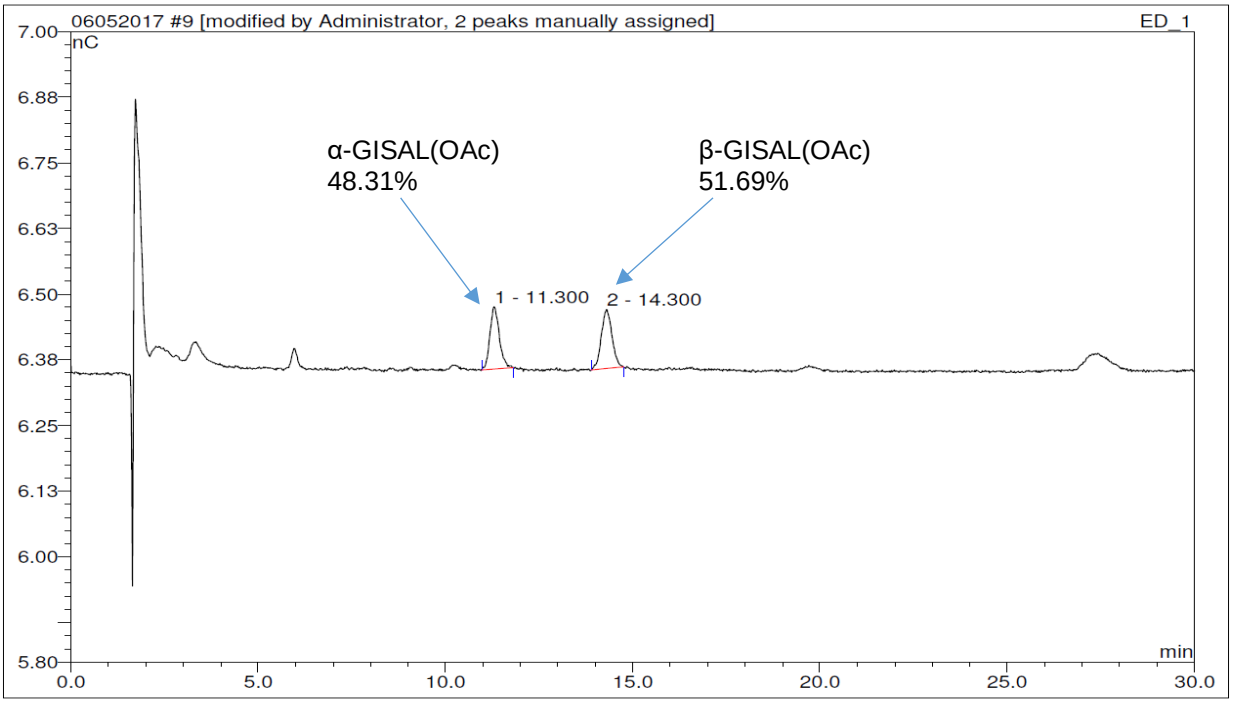
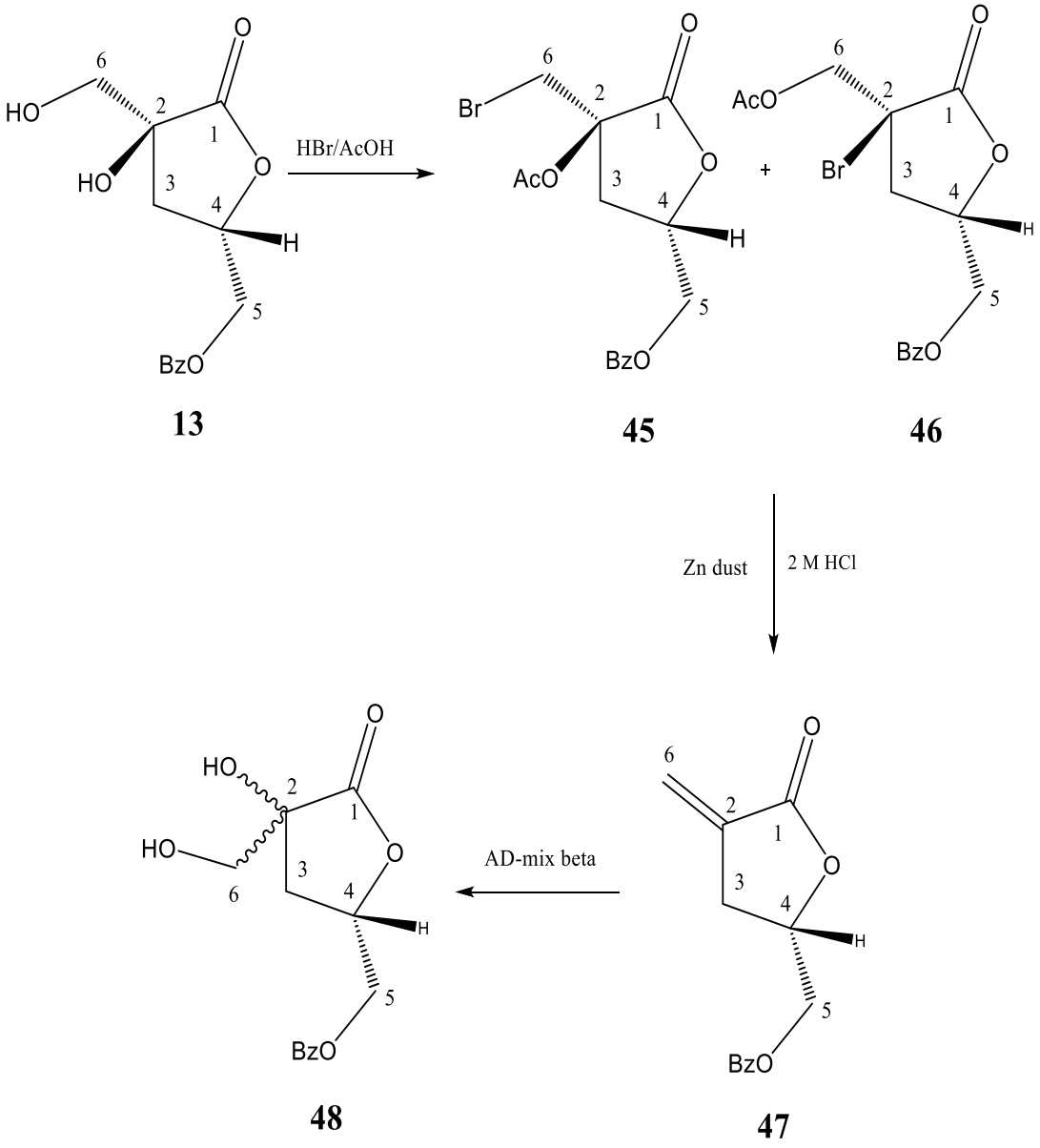


Figure 45. HPAEC chromatogram of the products obtained from the AD reactions using a buffered system

It is clear from the isolation of a mixture of epimers that the reaction is not stereoselective and the obtained result is the best we could possibly have, which was the same as those reported on the AD reaction of other terminal alkyl-substituted olefins which have low selectivity [145]. In the next set of experiments, we focused our attention on the use of a starting material that has a protecting group at position 5 of the α-GISAL **2** that has similar chemical properties to **24**, but which is not as bulky as Fmoc.



13 **45** **46** **48** **47**

Scheme 2.17: Sharpless dihydroxylation of **13** using AD-mix β.

5-Benzoyl-α-GISAL **13** was prepared for this purpose. Again, the isomeric bromoacetates **45** & **46** were formed by treatment of the lactone **13** with HBr in acetic acid. The bromoacetate was then converted into the modified methylene lactone using the optimised elimination procedure to give **47** (Figure 47). Finally, α-methylene lactone (**47**) was subjected to the dihydroxylation reaction in a buffered solution and at the end of the reaction, a mixture of four products (780 mg) was extracted in the organic phase. After chromatography, a mixture of two products (170 mg; 5-benzoyl-α-GISAL (19%), 5-benzoyl-β-GISAL (44%) & a mixture of their opened ring species (37%)) was isolated (Figure 48) and their separation was not possible because the two compounds had similar polarities.

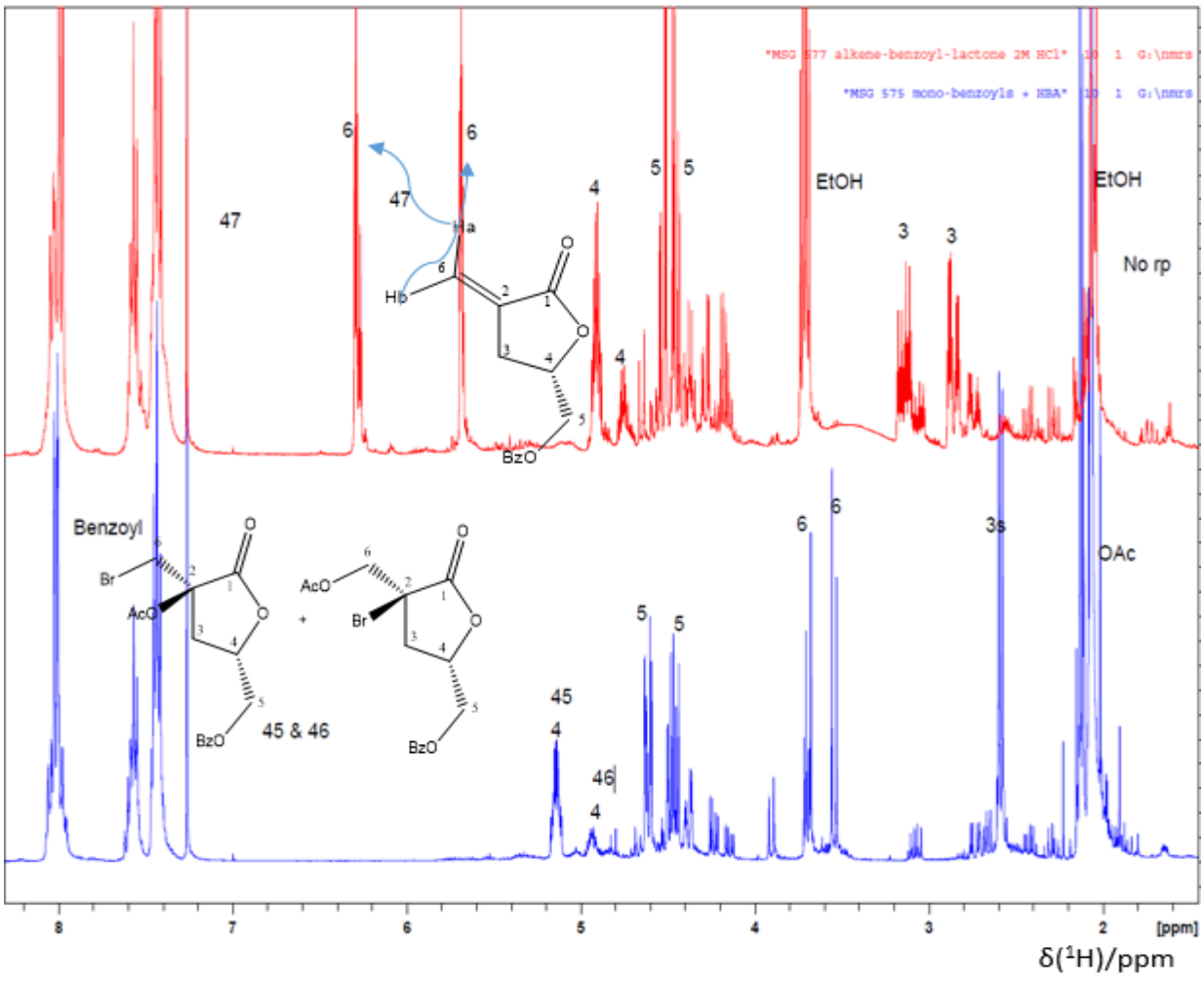


*Figure 46. ^{1}H NMR spectra of a mixture of **45** & **46** and **47** with no reduced product.*

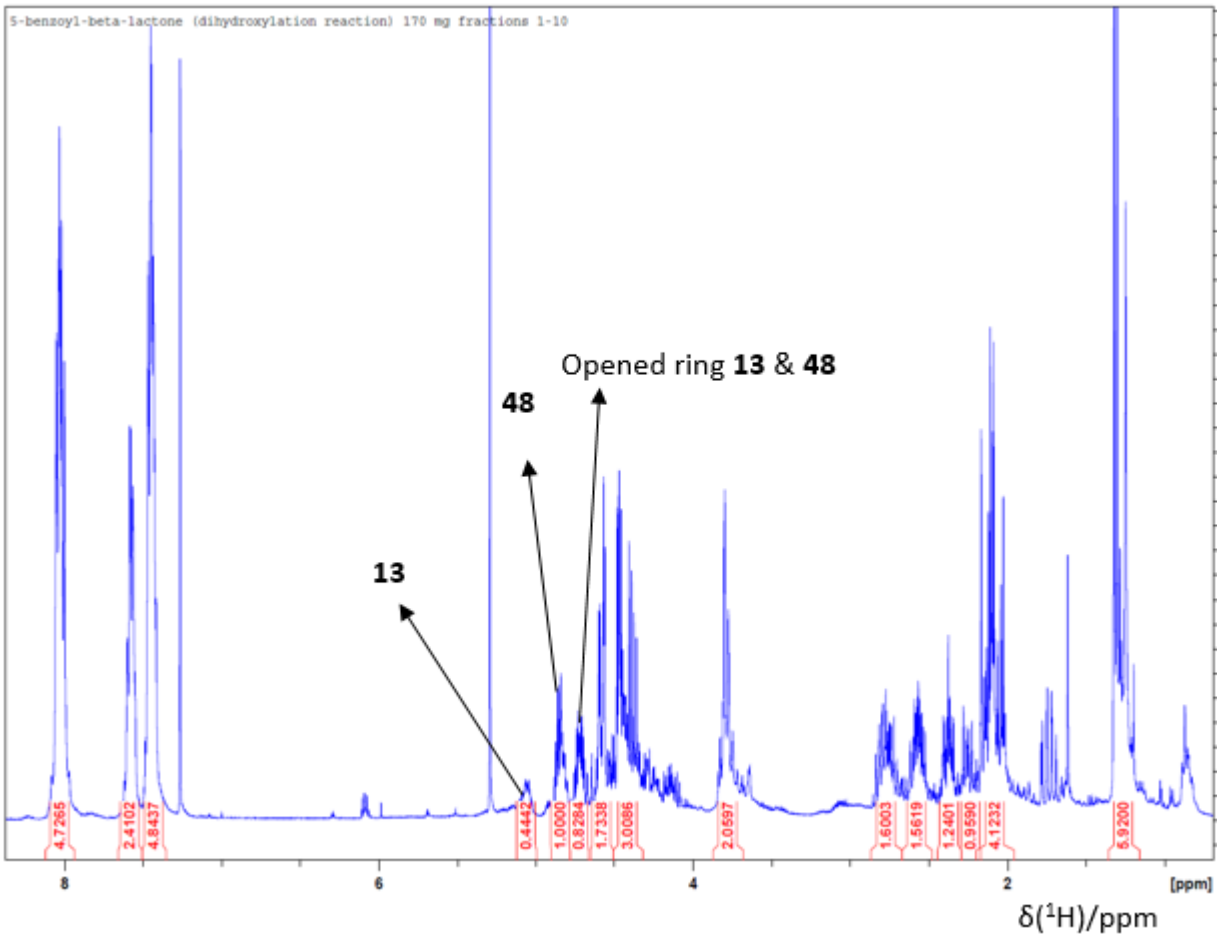


*Figure 47. ^{1}H NMR spectrum of a complex mixture of 5-benzoyl-β-GISAL **48**, 5-benzoyl-α-GISAL **13** and their opened ring species.*

3.0 Conclusion and future work

3.1 Conclusion

Most of the reactions used in this research to generate the protected glucoisosaccharinic acids derivatives are similar as those applied for the protection hydroxyls in carbohydrates. The main differences in their outcomes are related to the steric demands and attempts to put bulky protecting groups on to a tertiary hydroxyl group which is alpha to a carbonyl carbon. In order to get reaction at the tertiary hydroxyl group, the use of small, sterically undemanding protecting groups or forcing conditions were required. The attempted synthesis of the mono-protected glucoisosaccharinic acids unsurprisingly led to the isolation of mixtures of products. However, the higher reactivity of the primary hydroxyl group at position 5 makes it the preferred initial point of reaction and this was the case when reaction was with a bulky-silylating agent. This was a turning point in the strategic method for the separation of the derivatives of α- and β-GISAL from a mixture which contained a similar mixture to that present in the black liquor that is generated in Kraft pulping process.

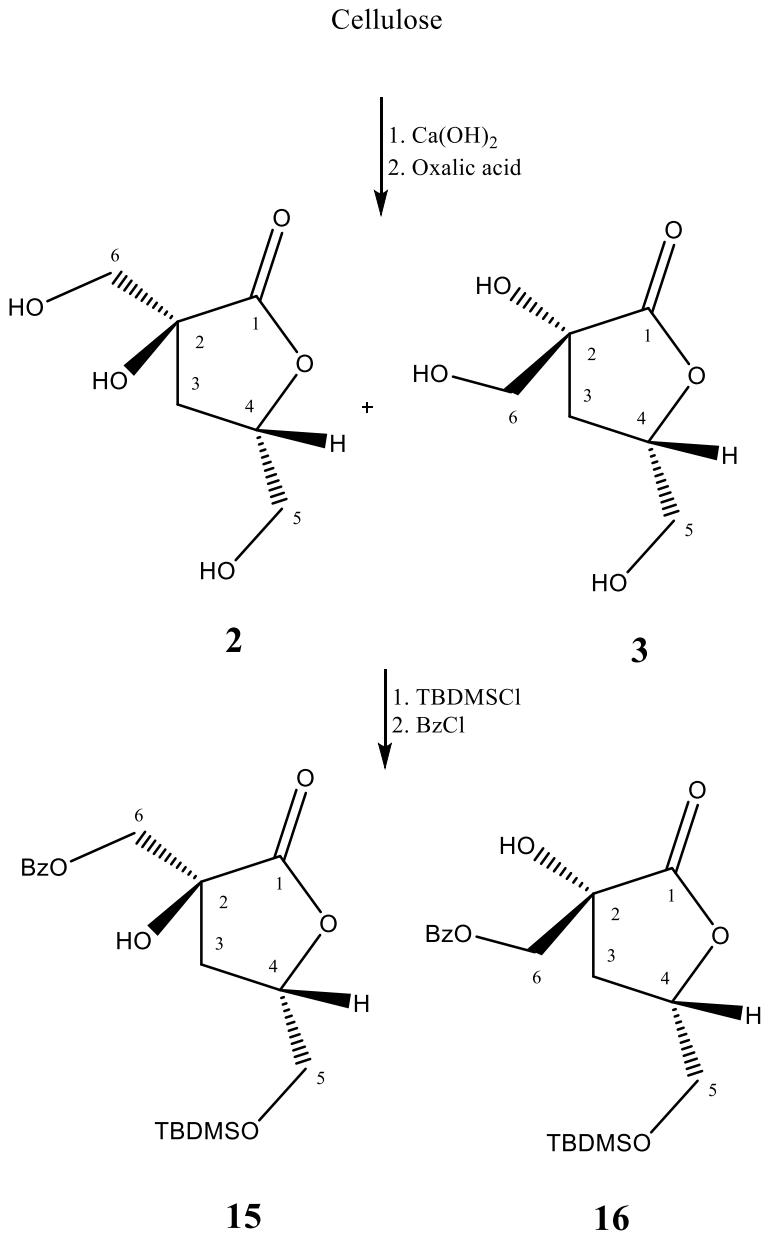
Despite various difficulties, the use of multiple steps and the employment of orthogonally protected hydroxyls gave access to a wide range of novel α- and β-glucoisosaccharinio-1,4-lactone derivatives (Table 5) which could hopefully be utilised in the synthesis of value-added products.

Table 5. List of all the novel compounds prepared as part of this work.

Cpd/N	Novel compounds	Yield (%)
4	2,5.6-tri-*O*-acetyl-α-glucoisosaccharino-1,4-lactone	99
5	5,6-di-*O*-acetyl-α-glucoisosaccharino-1,4-lactone	63
6	2-*O*-acetyl-α-glucoisosaccharino-1,4-lactone	-
7	6-*O*-acetyl-5-*O*-TBDMS-α-glucoisosaccharino-1,4-lactone	24
9	5,6-di-*O*-benzyl-α-glucoisosaccharino-1,4-lactone	59
10	2-*O*-acetyl-5,6-di-*O*-benzyl-α-glucoisosaccharino-1,4-lactone	30
12	5,6-di-*O*-benzoyl-α-glucoisosaccharino-1,4-lactone	23
13	5-*O*-benzoyl-α-glucoisosaccharino-1,4-lactone	60
14	6-*O*-benzoyl-α-glucoisosaccharino-1,4-lactone	28
15	6-*O*-benzoyl-5-*O*-TBDMS-α-glucoisosaccharino-1,4-lactone	38
16	6-*O*-benzoyl-5-*O*-TBDMS-β-glucoisosaccharino-1,4-lactone	25
17	6-*O*-benzoyl-β-glucoisosaccharino-1,4-lactone	37
18	2-*O*-benzoyl-5,6-*O*-TIPDS-α-glucoisosaccharino-1,4-lactone	44
19	2-*O*-benzoyl-α-glucoisosaccharino-1,4-lactone	24
20	5,6-di-*O*-Fmoc-α-glucoisosaccharino-1,4-lactone	20
21	2-*O*-acetyl-5,6-di-*O*-Fmoc-α-glucoisosaccharino-1,4-lactone	55
23	5-*O*-Fmoc-2,6-*O*-isopropylidene-α-glucoisosaccharino-1,4-lactone	20
24	5-*O*-Fmoc-α-glucoisosaccharino-1,4-lactone	73
25	6-*O*-Fmoc-α-glucoisosaccharino-1,4-lactone	56
26	2,5-di-*O*-acetyl-6-*O*-Fmoc-α-glucoisosaccharino-1,4-lactone	80
27	5,6-di-*O*-TBDMS-α-glucoisosaccharino-1,4-lactone	69
28	2-*O*-acetyl-5,6-di-*O*-TBDMS-α-glucoisosaccharino-1,4-lactone	81
29	5,6-di-*O*-TBDMS-β-glucoisosaccharino-1,4-lactone	5
30	5-*O*-TBDMS-α-glucoisosaccharino-1,4-lactone	46

31	5,6-*O*-TIPDS-α-glucoisosaccharino-1,4-lactone	82
32	2-*O*-acetyl-5,6-*O*-TIPDS-α-glucoisosaccharino-1,4-lactone	41
33	5-*O*-TIPS-α-glucoisosaccharino-1,4-lactone	59
45	5-*O*-benzoyl-2-methylene-α-glucoisosaccharino-1,4-lactone	89

The protecting group strategies designed and implemented on α-GISAL as starting material used a combination of an acid and base labile protecting groups and led to the generation of a number of novel derivatives of α-glucoisosaccharinio-1,4-lactone. The work also led to the identification of the most suitable combinations of protecting groups for the successful separation of the mixture of α-and β-glucoisosaccharinic acids prepared using a modified Feast method [81]. The modified method involves the treatment of cellulose with calcium hydroxide as a base to generate a mixture of the two isomers in approximately equal amounts. The identified suitable protecting groups' combination was applied on the mixture which led to the isolation of a β-GISAL derivative in a robust three step procedure that is simple and easily repeatable.



Scheme 4.1: The final robust method for the isolation of a β-GISAL derivative **16.**

In the second part of the work, attempts to convert α-GISA to β-GISA were successfully carried out using Sharpless dihydroxylation reactions, but the stereoselectivity of the final dihydroxylation reaction was not high enough to generate a single enantiomer and routinely a mixture of both epimers, the 2*R*, 4*S* and 2*S*,4*S,* was recovered. The initial Sharpless dihydroxylations, when performed under basic conditions, generated complex mixtures of products with ring-opened lactone products also being present.

However, after suppression of the formation of the further reduced product, and by carrying out the dihydroxylation reaction in a buffered system, an improved yield of the desired β-GISA derivative 5-acetyl-β-glucoisosaccharino-1,4-lactone was generated: increase to 52% from an original 20%.

The use of a starting material with a bulky protecting group (benzoyl) at position 5, again in the absence of the further-reduced product, and using the buffered system for the AD reaction, gave 5-benzoyl-α-glucoisosaccharino-1,4-lactone **13** (19%) and 5-benzoyl-β-glucoisosaccharino-1,4-lactone **48** (44%). The chemical shifts for the H-4 protons (5.11-5.01 ppm and 4.89-4.80 ppm) are in agreement with the 5.42-5.34 ppm and 5.00-4.94 ppm of the α- and β-tribenzoate derivatives of GISA reported by Shaw *et al.* [77]

The separation of **13** and **48** was not successful due to their similar polarities. This could be overcome with the protection of the primary hydroxyl group at position 6, prior to their separation using column chromatography.

The development of a robust method for the isolation β-GISA derivatives from a mixture will hopefully make the β-isomer of GISA more accessible for synthesis of value added products, for the study of its metal chelating properties within nuclear waste storage facilities and as a source of energy for microbial activities for methane generation. It is also hoped that the wide range of novel derivatives of the α-glucoisosaccharino-1,4-lactone prepared in this work will serve as platform chemicals for various synthetic industries.

3.2 Future work

- Optimisation of products with low yield.

Most of the reactions undertaken during this work employed either bulky protecting groups, used forcing reaction conditions or multi-step procedures and many of the reactions give, at least before purification steps were employed, mixtures of products only provided moderate yields of the desired products. The optimisation of the yield of these products would be a necessary part of future research work. At the completion of this project a stage has been reached where procedures have been identified which promote the reactions with the desired regioselectivity. However, for only a limited number of these were conditions optimised with respect to generating high yielding reactions. For the rest of the routes yields need to be optimised prior to their incorporation into synthetic schemes.

Direct isolation of β-GISA using HPAEC-PAD

The method for the isolation of the β-GISA using analytical HPAEC from its mixture was reported by Glaus *et al*. [12] but using starting materials containing a diverse range of products many of which fouled the columns used for the separation procedures. This approach could now be repeated to isolate β-GISA but employing the cleaner mixtures generated from the Sharpless dihydroxylation reactions. The difficulties experienced by Glaus as reported, are due to the presence large quantity of by-products in the mixture, a similar challenge that made Shaw's [128] attempt to isolate the β-GISA using analytical HPAEC unsuccessful.

Optimization of the Sharpless dihydroxylation of the a-methylene lactone

The use of α-methylene lactone as starting material successful provided a 52% conversion of the α-GISA derivative to β, via Sharpless dihydroxylation reaction. The

conversion was carried out using the commercially available premix- AD-mix. A better conversion could hopefully be obtained if, the require components of AD-mix including the cinchona alkaloid ligand are added to the reaction separately and applied to a more clean olefin and employing either NMO or $K_3Fe(CN)_6$ as the reoxidant.

Further work could also focus on developing other reactions of the α-methylene lactone. Attempted stereoselective epoxidation of methylene lactone should be carried out. The incorporation of the α-methylene lactone into polymers on its own and in combination with methyl acrylate could also be explored.

4.0 Materials and Experimental Methods

4.1 General Reagents

The general reagents used in this research were purchased from either Sigma-Aldrich UK (Gillingham, UK) or Fisher Scientific UK (Loughborough, UK) unless otherwise specified.

4.2 General Methods

4.2.1 Nuclear Magnetic Resonance (NMR)

All NMR spectra in this report were recorded using a Bruker Ascend 400 MHz spectrometer using Bruker pulse sequences at room temperature unless otherwise specified. Samples were dissolved in D_2O or $CDCl_3$ (Goss Scientific Ltd, Crewe, UK) unless otherwise stated and chemical shifts are given in parts per million (ppm) referenced to TMS (0 ppm) for $CDCl_3$ (7.27 ppm) and TPS for D_2O or residual HOD (4.79 ppm at RT). Both 1D (^{1}H, ^{13}C, ^{13}C DEPT 135) and 2D (COSY, HSQC, HMBC, NOESY) NMR spectra were obtained for most of the products.

4.2.2 High Resolution Mass Spectrometry (HRMS)

High resolution mass spectrometry (HRMS) of the synthesised compounds was performed using an Agilent 6210 TOF-MS spectrometer fitted with a dual ESI interface and operated in either positive or negative ion mode using Agilent Mass Hunter quantitative analysis software version B.06.00. 2012 by Dr Jack Blackburn at the University of Huddersfield.

4.2.3 Fourier-Transform Infrared (FT-IR)

All FT-IR spectra were recorded using a Thermo Nicolet 380 spectrophotometer (Thermo Fisher, Hemel Hempstead, Hertfordshire) using ATR.

4.2.4 High-Performance Anion-Exchange Chromatography Coupled with Pulsed Amperometery Detector (HPAEC-PAD).

All HPAEC-PAD analyses were performed using a Thermofisher ICS3000 Ion Chromatography system, Sunnyvale CA, USA, equipped with CarboPac PA20 column (150 mm x 3 mm). The samples were eluted using NaOH (50 mM) at a flow rate of 0.3 mL min^{-1}.

4.2.5 Gas Chromatography-Mass Spectrometry (GC-MS).

GC-MS analyses were performed on an Agilent 7890A GC system (Agilent Technologies, Edinburgh, UK) coupled to an Agilent 7683B Injector with an Agilent 5975B MSD quadrupole MS. The samples were eluted from a HP-5 column (30 m x 0.25 mm x 0.25 µm) using helium as a carrier (9 psi, FR 1 mL min^{-1}) and using the following temperature program: start temperature 140 °C, hold time 1 min and a final column temperature of 220 °C reached *via* a rising gradient of 2.5 °C min^{-1}.

4.2.6 Column chromatography and analytical thin layer chromatography (TLC)

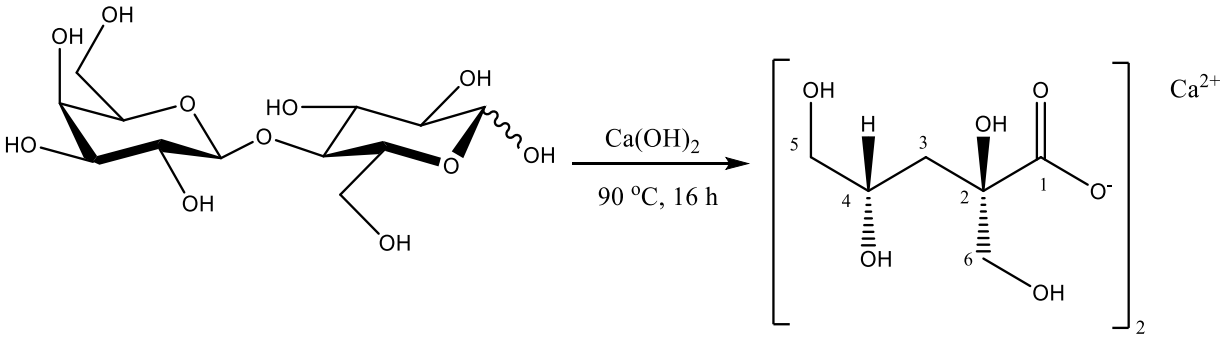
TLC was conducted using Alugram® SIL G/UV_{254} plates (Machery-Nagel, Düren, Germany). Chromopore-bearing fractions were identified using a Spectroline CM-10 Fluorescence Analysis Cabinet at 254 nm. All column chromatography was carried out using silica gel 63-200 µm as the stationary phase and a column with (dimensions 60 x 2 cm) unless otherwise specified.

4.2.7 Melting points (MP)

Melting points were determined using a Bibby Scientific Stuart SMP10 micro-furnace.

4.3 Production of glucoisosaccharinic acid derivatives.

4.3.1 Preparation of calcium (2*S*, 4*S*)-2,4,5-trihydroxy-2-(hydroxymethyl) pentanoic acid ($Ca(\alpha\text{-GISA})_2$). (**1**) [146]



1

Alpha-lactose monohydrate (50 g, 0.14 mol) was dissolved in ultra-pure water (300 mL), and calcium hydroxide (13.5 g, 0.18 mol) was added to the solution. The heterogeneous mixture was sonicated for five min and flushed with oxygen-free nitrogen. While stirring, the mixture was heated under reflux for 16 h at 90 ° C, and then filtered while hot. The brown filtrate was reduced in volume using rotary evaporation leaving (100 mL), it was then stored in a fridge for several days over which time a white precipitate formed. The white solid in the brown solution provided the desired $\alpha\text{-Ca(GISA)}_2$, which was filtered under vacuum, and the resulting solid was washed with cold water (10 mL), ethanol (5 mL) and acetone (5 mL). The filtrate was concentrated to dryness to give a dark brown sticky syrup of $\beta\text{-Ca(GISA)}_2/\alpha\text{-Ca(GISA)}_2$ in a 3:1 ratio which was used later (see section 2.4.4.3 after going through 2.3.2). The $\alpha\text{-Ca(GISA)}_2$ was dried in an oven overnight at 120 ° C to give a crude light brown solid (10.29 g, 25.9 mmol, 18% yield).

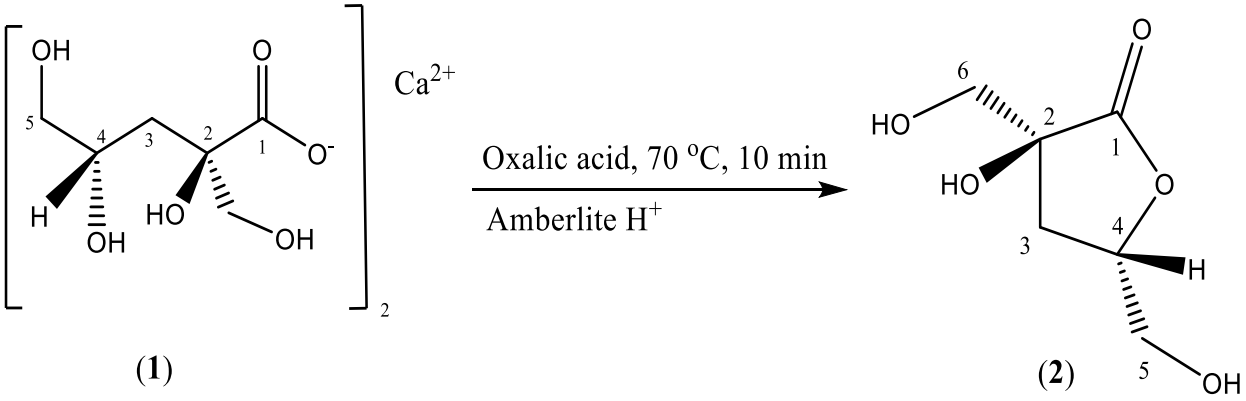
^{1}H **NMR** (400 MHz, D_2O) 3.77-3.74 (m, 1H, H-4). 3.58 (d, 1H, $J_{6,6'}$= 11.40 Hz, H-6), 3.47 (dd, 1H, $J_{5,4}$=3.36 Hz, $J_{5,5'}$= 11.50 Hz, H-5), 3.38 (d, 1H, $J_{6',6}$= 11.44 Hz, H-6'), 3.32 (dd, 1H, $J_{5',4}$=3.36 Hz, $J_{5',5}$= 11.50 Hz, H-5'), 1.71 (dd, 1H, $J_{3,4}$=8.04 Hz, $J_{3,3'}$= 14.45 Hz, H-3), 1.55 (dd, 1H, $J_{3',4}$= 4.20 Hz, $J_{3',3}$= 14.55 Hz, H-3')

^{13}C **NMR** (100 MHz, D_2O) 178.44 (C1), 79.41 (C2), 67.7 (C6), 65.9 (C5), 68.3 (C4), 37.6 (C3).

HRMS (m/z) Calculated mass for $C_6H_{10}O_6$ [M-H^-] 180.0634 found 180.0636.

FT-IR (cm^{-1}): 3307.1 (O-H), 2965.9, 2881.0 (C-H), 1585.3 (C=O), 1453.5, 1385.2 (C-H).

4.3.2 Preparation of α-D-glucoisosaccharino-1,4-lactone (α-GISAL) (2). [146]



(**1**) (**2**)

Ca(α-GISA)$_2$ (25 g, 62.8 mmol) was dissolved in water (100 mL) and the solution was heated at 70 °C for 10 min, then a solution of oxalic acid dihydrate (6.0 g) in water (60 mL) was added slowly. The mixture was then heated for 10 min while stirring at the same temperature. The mixture was filtered while hot, and the filtrate was passed through a column (3.4 × 45 cm) of Amberlite (H^+) cation-exchange resin. The column was preconditioned with HCl (1 M, 200 mL) its effluent and washings (water 100 mL) were concentrated under reduced pressure to give a thick brown partly crystalline syrup (8.48 g, 52.3 mmol, 42 % yield).

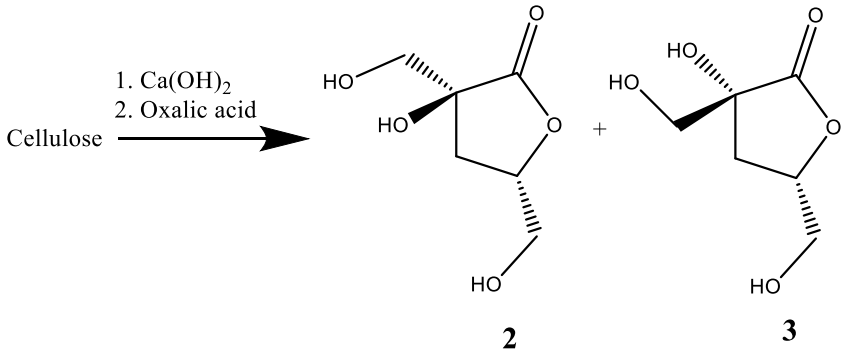
^1H **NMR** (400 MHz, D_2O) 4.72-4.65 (m, 1H, H-4), 3.73 (dd, 1H, $J_{5,4}$= 2.76 Hz, $J_{5,5'}$=12.40 Hz, H-5) 3.59 (d, 1H, $J_{6,6'}$ =11.60 Hz, H-6), 3.52 (d, 1H, $J_{6',6}$ =11.60 Hz, H-6'), 3.49 (dd, 1H, $J_{5',4}$= 4.96 Hz, $J_{5',5}$=12.40 Hz, H-5') 2.13, 2.14 (2 x dd, 2H, $J_{3,4}$= 6.0 Hz, $J_{3,3'}$=7.76 Hz, H-3 & 3').

^{13}C **NMR** (100 MHz, D_2O) 178.4 (C1), 79.4 (C4), 76.4 (C2), 63.2 (C6), 62.4 (C5), 32.8 (C3).

FT-IR (cm^{-1}): 3326.8 (O-H), 2933.7 (C-H), 1755.3 (C=O), 1437, 1370 (C-H), 1197.8, 1087.3 & 1042.8 (C-O).

MP: 88-89 ^oC lit. 91-92 ^oC [61]

4.3.3 Preparation of an intimate mixture of α-D-glucoisosaccharino-1,4-lactone (α-GISAL) (2) and β-D-glucoisosaccharino-1,4- lactone (β-GISAL) (3) via a cellulose degradation reaction



2 **3**

Avicel (200 g) was suspended in ultra-pure water UPW (1.6 L) in a large round bottom flask (2 L) and calcium hydroxide $Ca(OH)_2$ (54 g) was added slowly, while stirring at elevated temperature (40-50 ^oC) under an atmosphere of nitrogen; then the reaction was allowed to stir for 72 h at 90 ^oC. After 72 h, the reaction mixture was filtered while hot, the filtrate was concentrated to (300 mL) using a rotary evaporator, and the resulting solution was stored in a fridge for 7 days. After this time, the mixture was filtered and the filtrate containing the calcium salt of α-and β-GISA was passed through a preconditioned (1M HCl, 200 mL) cation exchange column (Amberlite, 6 x 40 cm). The eluted material was concentrated and freeze-dried to give a sticky brown crystalline syrup (7.9 g) containing a 50:50 mixture of α-GISAL and β-GISAL with small traces of lactic acid.

^{1}H **NMR** (400 MHz, D_2O **2**) 4.74-4.70 (m, 1H, H-4), 3.73 (dd, 1H, $J_{5,4}$= 2.80 Hz, $J_{5,5'}$= 5.84 Hz, H-5) 3.62 (d, 1H, $J_{6,6'}$ = 11.76 Hz, H-6). 3.55 (d, 1H, $J_{6',6}$ =11.76 Hz, H-6'), 3.53 (dd, 1H, $J_{5',4}$= 1.24 Hz, $J_{5',5}$=5.84 Hz, H-5') 2.16 (2 x dd, 2H, $J_{3,4}$= 6.80 Hz, $J_{3,3'}$=7.74 Hz, H-3 & 3').

^{13}C **NMR** (100 MHz, D_2O) 178.44 (C1), 79.40 (C4), 76.42 (C2), 63.18 (C6), 62.37 (C5), 32.76 (C3).

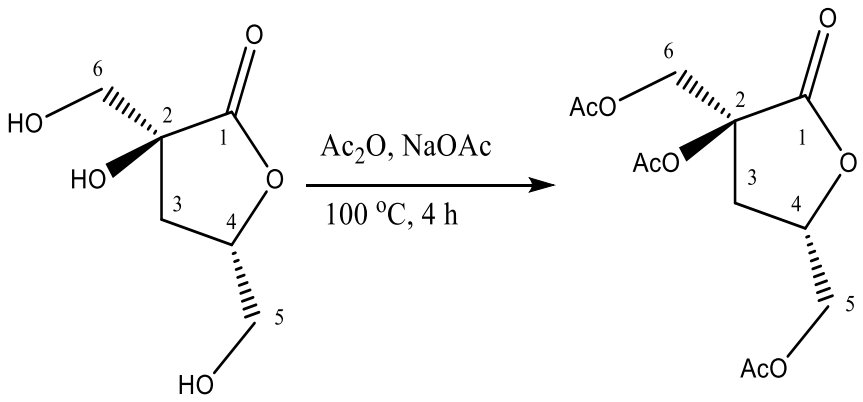
^{1}H **NMR** (400 MHz, D_2O **3**) 4.56-4.50 (m, 1H, H-4), 3.76 (dd, 1H, $J_{5,4}$= 6.84 Hz, $J_{5,5'}$= 5.76 Hz, H-5) 3.60, 3.61 (2 x d, 1H, $J_{6,6'}$ =1.92 Hz, H-6 & 6'), 3.49 (dd, 1H, $J_{5',4}$= 1.6 Hz, $J_{5',5}$= 5.76 Hz, H-5') 2.45 (dd, 1H, $J_{3,4}$= 6.76 Hz, $J_{3,3'}$= 13.57 Hz, H-3), 2.00 (dd, 1H, $J_{3,4}$= 9.16 Hz, $J_{3,3'}$= 13.57 Hz, H-3').

^{13}C **NMR** (100 MHz, D_2O) 179.42 (C1), 78.65 (C4), 76.64 (C2), 64.48 (C6), 62.41 (C5), 33.63 (C3).

4.4 Protecting Group Chemistry using GISAL as starting material

4.4.1 Synthesis of acetyl derivatives of α-glucoisosaccharino-1,4-lactone.

4.4.1.1 Preparation of 2,5,6-tri-*O*-acetyl-α-D-glucoisosaccharino-1,4-lactone (4)



2 **4**

Dried α-glucoisosaccharino-1,4-lactone **2** (1.0 g; 6.17 mmol) was added whilst stirring to an ice cooled solution of acetic anhydride (10 mL). Once the lactone had dissolved a catalytic amount of sodium acetate (0.5 g) was added and the reaction was heated to 100 °C for 4 h. The reaction was halted by addition of the contents of the round bottom flask to ice cold water (100 mL) and the solution was stirred at room temperature for a further 1 h. The organic products were then extracted into chloroform (3 × 60 mL) and the combined organic extracts were dried over anhydrous magnesium sulphate and concentrated at reduced pressure to give a golden crystalline syrup (1.77 g; 6.14 mmol; yield: 99%).

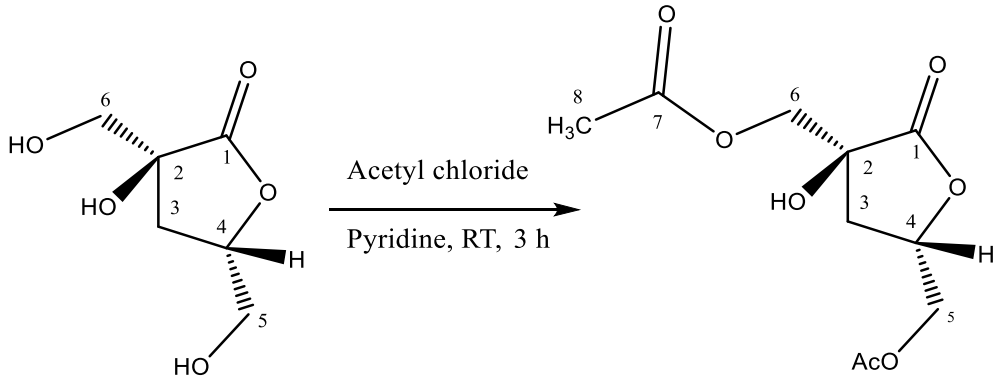
^{1}H **NMR** (400 MHz, $CDCl_3$): 5.01-4.95 (m, 1H, H-4), 4.30 (s, 2H, H-6s), 4.27 (dd, 1H, $J_{5',4}$= 3.4 Hz, $J_{5',5}$ = 12.3 Hz, H-5'), 4.13 (dd, 1H, $J_{5,4}$ = 6.7 Hz, $J_{5,5'}$ = 12.3 Hz, H-5), 2.50 (dd, 1H, H-3, $J_{3',4}$= 9. 0 Hz, $J_{3',3}$ =14.7 Hz), 2.25 (dd, 1H, H3', $J_{3',4}$ = 6.3 Hz, $J_{3',3}$ = 14.7 Hz), 2.11, 2.10, 2.08 (3s, 9H, 3 × C$\underline{H_3}$CO)

^{13}C **NMR** (100 MHz, $CDCl_3$): 172.0 (C1), 170.6, 170.0, 169.9 (3 x $CH_3\underline{C}O$), 77.9 (C2), 74.7 (C4), 65.3 (C6), 64.8 (C5), 32.1 (C3), 20.6, 20.6, 20.5 (3 x CH_3CO).

HRMS (m/z) Calculated mass for $C_{12}H_{16}O_8$ $[M+NH_4]^+$ 306.1183, found 306.1187.

FT-IR (cm^{-1}): 2959.0 (C-H), 1780.7, 1736.7 (C=O), 1437.0, 1369.7 (C-H), 1202.3, 1045.4 (C-O).

4.4.1.2 Preparation of 5,6-di-*O*-acetyl-α-D-glucoisosaccharino-1,4-lactone (5)



2 **5**

Dried α-G1SAL **2** (500 mg, 3.09 mmol) was dissolved in pyridine (5 mL) while stirring at room temperature for 10 mins. Acetyl chloride (470 µL; 6.48 mmol, 2.1 eq) was added cautiously at room temperature. The reaction was allowed to proceed uninterrupted for 3 h at room temperature. The reaction was halted by adding dichloromethane (30 mL) followed by ultra-pure water (30 mL). The organic layer was separated and the aqueous layer was further extracted with dichloromethane (2 × 30 mL). The combined organic layer was washed with 1% copper sulphate solution (2 × 50 mL) and dried over magnesium sulphate, then concentrated to give **5** (1.20 g; 5.61 mmol; 63% yield).

^{1}H **NMR** (400 MHz, $CDCl_3$) δ 4.89-4.86 (m, 1H, H-4), 4.36 (dd, 1H, $J_{5,4}$ = 3.08 Hz, $J_{5,5'}$ = 2.44 Hz, H-5), 4.33 (d, 1H, $J_{6,6'}$=4.16 Hz, H-6) 4.24 (d, 1H, $J_{6',6}$=11.44 Hz, H-6'), 4.15 (dd, 1H, $J_{5',4}$ = 6.08 Hz, $J_{5',5}$ = 6.32 Hz, H-5'), 2.37 (dd, 1H, $J_{3,4}$ = 6.56 Hz, $J_{3,3'}$ = 13.60

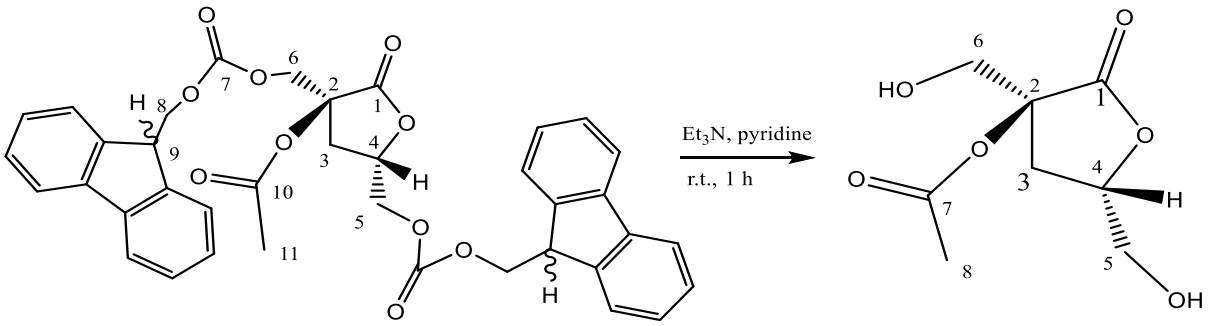
Hz, H-3), 2.16 (dd, 1H, $J_{3',4}$ = 8.84 Hz, $J_{3',3}$ = 13.60 Hz, H-3') 2.10 & 2.08 (2s, 6H, 2 x CH_3CO).

^{13}C **NMR** (100 MHz, $CDCl_3$): 175.1 (C1), 170.6 & 170.5 (2 x CH_3CO), 75.0 (C4), 74.4 (C2), 65.3 (C6), 64.6 (C5), 34.8 (C3), 20.7 & 20.6 (2 x CH_3CO)

HRMS (m/z): Calculated mass for $C_{10}H_{14}O_7$ $[M+Na]^+$ 269.0748, found 269.0740.

FT-IR (cm^{-1}): 3079.3 (O-H), 1781.0, 1742.6 (C=O), 1482, 1372.5 (C-H), 1232.6, 1195.6 (C-O).

4.4.1.3 Preparation of 2-*O*-acetyl-α-D-glucoisosaccharino-1, 4-lactone (6) via deprotection of 2-*O*-acetyl-5,6-di-*O*-Fmoc-α-D-glucoisosaccharino-1, 4-lactone. (21) [147, 148]



21 **6**

Triethylamine (4 mL 28.57 mmol) was added to a solution of 2-*O*-acetyl-5,6-di-*O*-Fmoc-α-GISAL **21** (1.17g, 1.81 mmol) in anhydrous pyridine (20 mL). After stirring for 1 h at room temperature the solvents were removed under reduced pressure to give a crude brown residue. The crude product was flushed through a bed of silica (column, 6 x 40 cm) using ethyl acetate to remove the Fmoc degradation products. The desired product **6** (470 mg) was obtained from the column by eluting with methanol.

^1H **NMR** (400 MHz, $CDCl_3$): δ 4.81-4.76 (m, 1H, H-4), 4.50 (d, 1H, $J_{6,6'}$=11.64 Hz, H-6), 4.18 (d, 1H, $J_{6'6}$=11.68 Hz, H-6'), 3.99 (dd, 1H, $J_{5,4}$= 2.68 Hz, $J_{5,5'}$=12.72 Hz, H-5), 3.65

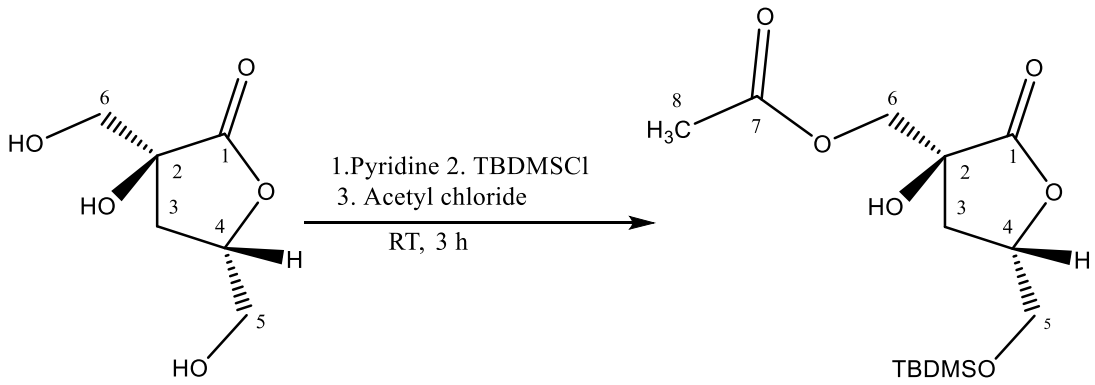
(dd, 1H, $J_{5,4}$= 4.08 Hz, $J_{5'5}$=12.80 Hz, H-5') 2.34, 2.35 (2 × dd, 2H, $J_{3,4}$= 5.16 Hz, $J_{3,3'}$=7.54 Hz, H-3 & H3'), 2.12 (s, 3H, CH_3CO)

^{13}C **NMR** (100 MHz, $CDCl_3$): δ 175.5 (C1), 170.8 (C7), 78.2 (C2), 74.9 (C4), 65.8 (C6), 63.1 (C5), 33.7 (C3), 20.7 (C8).

HRMS (m/z) Calculated mass for $C_8H_{12}O_6$ $[M+Na]^+$ 227.0526 found 227.0527

FT-IR: 3380.7 (O-H), 2944.2, 2833.2 (C-H), 1726.2 (C=O), 1447.7, 1374.9 (C-H), 1242.5, 1025.3 (C-O).

4.4.1.4 Preparation of 5-*O*-tert-butyldimethylsilyl-6-*O*-acetyl-α-D-glucoisosaccharino-1,4-lactone (7). [149]



2 **7**

Dried α-GISAL **2** (500 mg, 3.09 mmol) was dissolved in pyridine (6 mL) whilst stirring for 10 min at room temperature. It was then added cautiously to *tert*-butyldimethylsilyl chloride TBDMSCl (520 mg; 3.45 mmol; 1.1 eq) while stirring at room temperature. The reaction was allowed to proceed for 1 h, then acetyl chloride (250 µL; 3.40 mmol; 1.1 eq) was added cautiously. The reaction was allowed to continue for 2 h at room temperature. After 2h, the reaction was halted by adding DCM (50 mL), followed by water (50 mL). The aqueous layer was further extracted with DCM (2 × 30 mL) and the combined organic layer was dried over sodium sulphate and concentrated to give crude **7** (3.30 g) as a brown syrup, which was purified using chromatography to give the

desired product as a white semi-crystalline syrup (300 mg; 0.754 mmol; 24 % yield); (Rf: 0.42; Hexane/EtOAc 5:1 v/v).

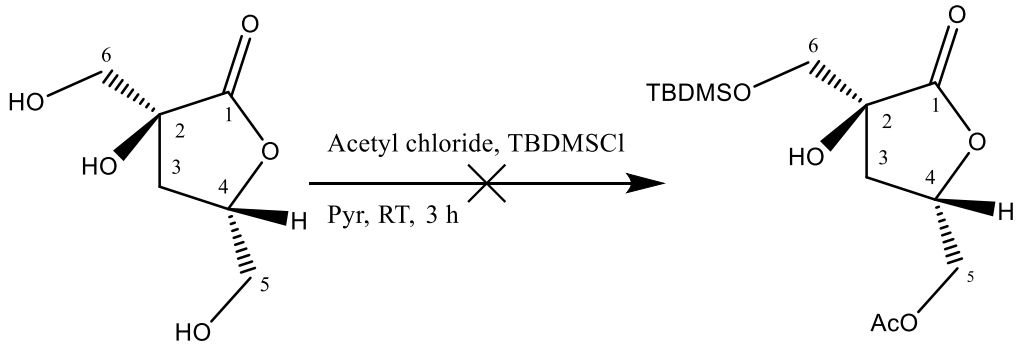
^{1}H **NMR** (400 MHz, CDCl3) 4.72-4.68 (m, 1H, H-4), 4.37 (d, 1H, $J_{6,6'}$ = 11.56 Hz, H-6), 4.19 (d, 1H, $J_{6',6}$ = 11.56 Hz, H-6'), 3.92 (dd, 1H, $J_{5,4}$ = 3.12 Hz, $J_{5,5'}$ = 11.72, H-5), 3.66 (dd, 1H, $J_{5',4}$ = 3.36 Hz, $J_{5',5}$ = 11.72, H-5'), 2.38 (dd, 1H, $J_{3,4}$ = 8.08 Hz, $J_{3,3'}$ = 13.83 Hz, H-3), 2.23 (dd, 1H, $J_{3',4}$ = 6.88 Hz, $J_{3',3}$ = 13.83 Hz, H-3'), 2.08 (CH_3CO), 0.87 (s, 9H, TBDMS), 0.06 & 0.05 (2 × s, 6H TBDMS).

^{13}C **NMR** (100 MHz, $CDCl_3$) 175.5 (C1), 170.8 (C7), 77.97(C4), 74.9 (C2), 65.6 (C6), 63.3 (C5), 33.7 (C3), 25.8 & 18.35 (TBDMS), 20.7 (C8), -5.39, -5.48 (TBDMS).

HRMS (m/z): Calculated mass for $C_{14}H_{26}O_6Si$ $[M+Na]^+$ 341.1391, found: 341.1390.

FT-IR (cm^{-1}): 3419.8 (O-H), 2953.8, 2929.6, 2857.1 (C-H), 1750.2 (C=O), 1462.9, 1376.5 (C-H), 1202.5, 1129.0 (C-O), 1044.3 (Si-OR), 1010.8 (Si-CH), 832.8, 776.6 ($SiMe_2$).

4.4.1.5. Attempted preparation of 5-*O*-acetyl-6-*O*-tert-butyldimethylsilyl-α-D-glucoisosaccharino-1,4-lactone (8) in a one-pot sequence reaction [149].



2 **8**

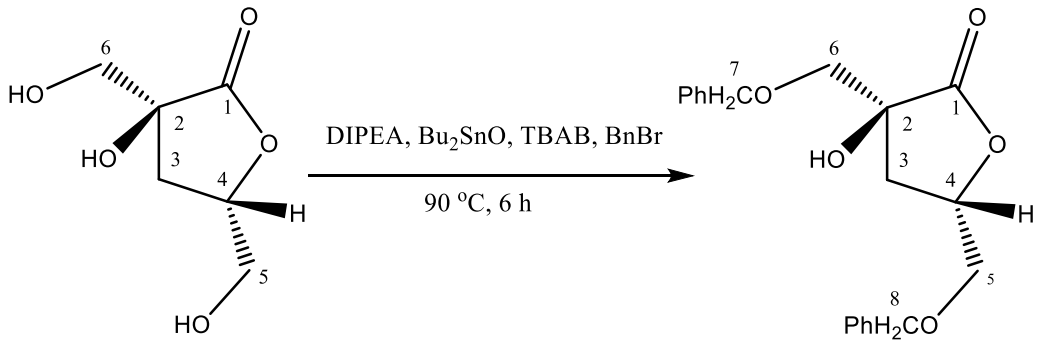
Dried α-glucoisosaccharino-1,4-lactone **2** (600 mg, 3.70 mmol) was dissolved in pyridine (6 mL) whilst stirring for 20 min at room temperature. It was then added cautiously to acetyl chloride (300 µL; 4.07 mmol; 1.1 eq) while stirring at room

temperature. The reaction was allowed to proceed for 1 h, then tert-butyldimethylsilyl chloride TBDMSCl (620 mg; 4.11 mmol; 1.1 eq) was added cautiously. The reaction was allowed to continue for 2 h at room temperature. After 2 h, the reaction was halted by adding of DCM (50 mL), followed by water (50 mL). The aqueous layer was further extracted with DCM (2 × 30 mL) and the combined organic layer was dried over sodium sulphate and concentrated to give a crude **8** sample (1.34 g) as a brown semi-crystalline syrup.

NMR analysis showed that none of the desired product was obtained but the major product was the 5,6-di-O-acetyl-glucoiosaccharino-1,4-lactone **5** which had already been isolated (see section 3.4.1.2).

4.4.2 Synthesis of benzyl and benzoyl derivatives of α and β-glucoisosaccharino-1,4-lactone.

4.4.2.1 Preparation of 5,6-di-*O*-benzyl-α-D-glucoisosaccharino-1,4-lactone (9) [150]



2 **9**

Dried α-GISAL **2** (1.0 g, 6.17 mmol) was dissolved in *N,N*-diisopropylethylamine (DIPEA) (2.3mL, 4 eq), and catalytic amounts of dibutyltin oxide (154 mg, 0.1 eq) and tetrabutylammonium bromide (597 mg, 0.3 eq) were added while stirring. Benzyl bromide (BnBr) (6 mL, 4 eq), was added slowly and the reaction was allowed to proceed for 4 h at 90 °C. Second portions of BnBr and DIPEA (2 eqs each) were added and the

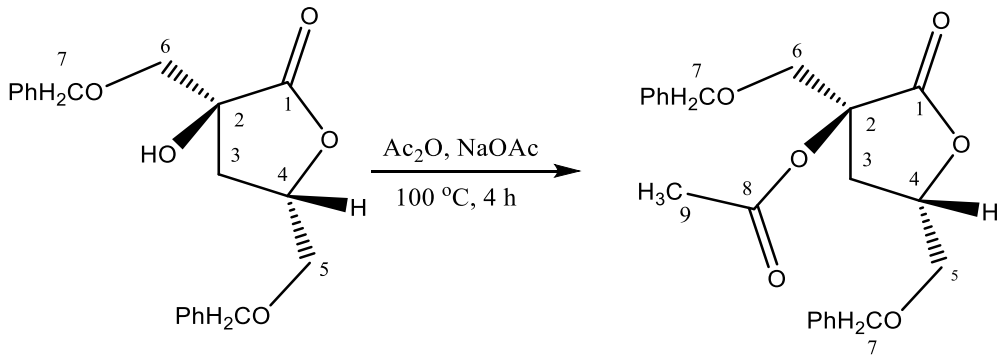
reaction continued for further 2 h at 90 ºC. The reaction was halted by pouring the reaction solution into a mixture of DCM (50 mL) and water (50 mL). The organic layer was separated, and the aqueous phase was extracted with DCM (2 × 50 mL). The combined organic extracts were dried over anhydrous sodium sulphate and concentrated to dryness to give a crude product (8.19 g) as a golden syrup which was purified by column chromatography to give the product as an oil (1.24 g, 3.62 mmol; yield: 59 %; EtOAc/Hexane 1/1 v/v).

^{1}H **NMR** (400 MHz, $CDCl_3$) 7.34-7.29 (m, 10H, ArH), 4.83-4.77 (m, 1H, H-4), 4.54 (m, 4H, 2 x H-7), 3.67 (dd, 1H, $J_{5,4}$ = 3.48 Hz, $J_{5,5'}$ = 10.97 Hz, H-5), 3.62 (m, 2H, H-6 & H-6'), 3.57 (dd, 1H, $J_{5',4}$ = 5.20 Hz, $J_{5',5}$ = 10.98 Hz, H-5'), 2.33 (dd, 2H, $J_{3,4}$ = 2.12 Hz, $J_{3,3'}$ = 7.50 Hz, H-3 & H-3')

^{13}C **NMR** (100 MHz, $CDCl_3$) 176.79 (C1), 137.59, 137.39 (ArCq), 128.50, 127.89, 127.84, 127.79 (ArC), 76.78 (C4), 75.34 (C2), 73.73, 73.56 (C7), 72.05 (C6), 70.88 (C5), 34.61 (C3).

HRMS (m/z): Calculated mass for $C_{20}H_{22}O_5$ $[M+Na]^+$ 365.1359, found 365.1358.

4.4.2.2 Preparation of 2-*O*-acetyl-5,6-di-*O*-benzyl-α-D-glucoisosaccharino-1,4-lactone (10)



9 **10**

5,6-Dibenzyl-α-GISAL **9** (1.0 g, 2.92 mmol) was dissolved in acetic anhydride (10 mL) and sodium acetate (0.5 g) was added as a catalyst. The reaction was allowed to proceed for 4 h whilst stirring at 100 °C. After 4 h, the whole content of the reaction vessel was transferred onto ice cold water (100 mL) and stirred for 1h to convert excess acetic anhydride to acetic acid. The organic product was extracted in DCM (2 x 100 mL), dried over anhydrous sodium sulphate and concentrated to give a brown semi-crystalline crude syrup (1.97 g) which was purified by column chromatography to give **10** as a colourless oil (330 mg; 0.859 mmol; 30 %); (Rf: 0.21; EtOAc/Hexane 1/1 v/v).

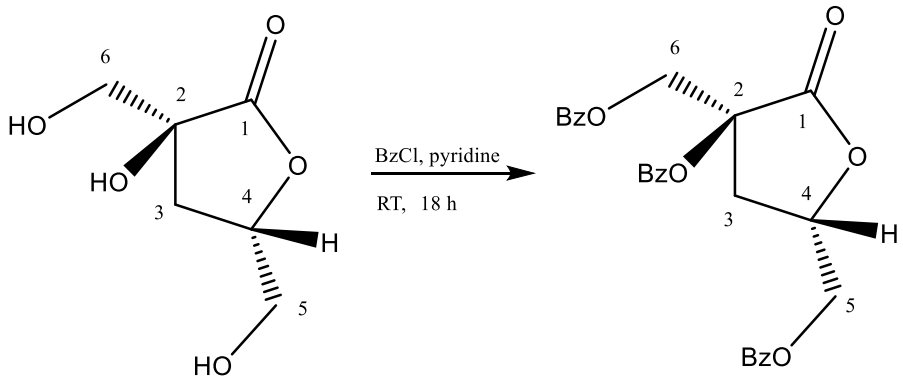
^{1}H **NMR** (400 MHz, $CDCl_3$) 7.33-7.26 (m, 10H, Ar<u>H</u>), 4.96-4.90 (m, 1H, H-4), 4.52-4.49 (t, 4H, J = 4.72 Hz, H-7s), 3.70 (s, 2H, H-6), 3.63 (dd, 1H, $J_{5,4}$ = 3.96 Hz, $J_{5,5'}$ = 10.7 Hz, H-5), 3.57 (dd, 1H, $J_{5',4}$ = 5.04, $J_{5',5}$ = 10.7 Hz, H-5'), 2.60 (dd, 1H, $J_{3,4}$ = 5.84 Hz, $J_{3,3'}$ = 14.3 Hz, H-3), 2.42 (dd, 1H, $J_{3',4}$ = 5.12 Hz, $J_{3',3}$ = 14.3 Hz, H-3'), 2.10 (s, 3H, C<u>H</u>$_3$CO).

^{13}C **NMR** (100 MHz, $CDCl_3$): 173.66 (C1), 170.00 (C8), 137.72 & 137.20 (ArCq), 128.48, 128.46, 127.89, & 127.78 (ArC), 79.44 (C2), 76.51 (C4), 73.86 & 73.46 (2 x Ph<u>C</u>H$_2$), 71.59 (C6), 71.10 (C5), 31.96 (C3), 20.63 (<u>C</u>H$_3$CO).

HRMS (m/z) Calculated mass for $C_{22}H_{24}O_6$ $[M+NH_4]^+$ 402.1911, found 402.1910.

FT-IR (cm^{-1}): 2866 (C-H), 1775 & 1740 (C=O), 1453 (Ar C-C), 1369 (C-H) 1205 & 1096 (C-O), 736 & 697 (Ar C-H).

4.4.2.3 Preparation of 2,5,6-tri-O-benzoyl-α-D-glucoisosaccharino-1,4-lactone (11) [134]



2 **11**

Dried α-GISAL **2** (1.0 g, 6.17 mmol) was dissolved in pyridine (10 mL) for 10 min at room temperature then cooled to 0 °C, before benzoyl chloride BzCl (4 mL, 5.0 eq) was added slowly while stirring. The reaction was allowed to proceed for 2 h at 0 °C and 16 h at room temperature. After 16 h, water (50 mL) was added, and the reaction was stirred for 1 h to hydrolyse any excess benzoyl chloride after which time the reaction was quenched with the addition of DCM (50 mL). The organic layer was separated, and the aqueous layer was extracted with further DCM (2 × 50 mL). The combined organic layer was washed with saturated $NaHCO_3$ (2 × 50 mL) and dried over sodium sulphate then concentrated to dryness to give a brown semi-crystalline crude syrup (4.64 g) which was purified using column chromatography to give a white solid **11** (2.01 g, 4.24 mmol, 69% yield); (Rf: 0.45, Hexane/EtOAc 4/1 v/v.)

^{1}H **NMR** (400 MHz, $CDCl_3$): 8.08-8.04 (m, 6H, 6 x ArHβ) 7.62-7.59 (m, 3H, 3 x ArHδ), 7.50-7.44 (m, 6H, 6 x ArHγ), 5.37-5.33 (m, 1H, H-4), 4.90 (d, 1H, $J_{6,6'}$ = 11.20 Hz, H-6),

4.67 (d, 1H, $J_{6',6}$ = 11.20 Hz, H-6'), 4.62 (dd, 1H, $J_{5,4}$ = 3.36 Hz, $J_{5,5'}$ = 12.29 Hz, H-5), 4.49 (dd, 1H, $J_{5',4}$ = 6.44 Hz, $J_{5',5}$ = 12.26 Hz, H-5'), 2.80 (dd, 1H, $J_{3,4}$ = 8.64 Hz, $J_{3,3'}$ = 14.99 Hz, H-3), 2.60 (dd, $J_{3',4}$ = 7.04 Hz, $J_{3',3}$ = 14.97 Hz, H-3')

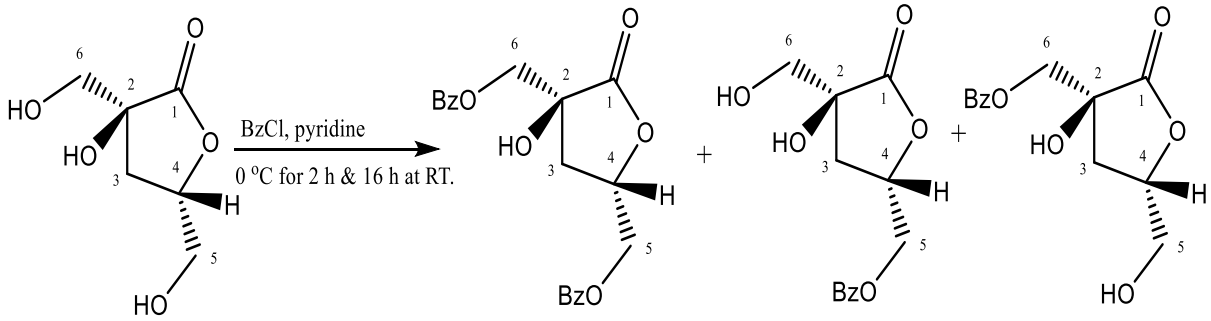
^{13}C **NMR** (400 MHz, $CDCl_3$): 171.9 (C1), 166.2, 165.7, 165.5 (CO), 134.2, 133.8, 133.5 (3 x ArCδ), 130.2-130.1 (ArCβ), 129.84-129.77 (ArCα), 128.7, 128.6, 128.5 (3 x ArCγ)), 78.4 (C2), 75.4 (C4), 66.1 (C6), 65.3 (C5), 32.7 (C3).

HRMS (m/z) Calculated mass for $C_{27}H_{22}O_8$ $[M+H]^+$ 475.1387, found 475.1386.

FT-IR (cm^{-1}): 1771 & 1722 (C=O), 1451 (Ar C-C), 1262, 1233, 1092 & 1062 (C-O), 701 & 684 (Ar C-H).

MP: 95-96 °C.

4.4.2.4 Preparation of 5,6-di-*O*-benzoyl-α-D-glucoisosaccharino-1,4-lactone (12), 5-*O*-benzoyl-α-D-glucoisosaccharino-1,4-lactone (**13**) and 6-*O*-benzoyl-α-D-glucoisosaccharino-1,4-lactone (**14**). [134]



2 **12** **13** **14**

Dried α-GISAL **2** (5.0 g, 30.86 mmol) was dissolved in pyridine (10 mL) for 10 min at room temperature then cooled to 0 °C, before benzoyl chloride BzCl (4 mL, 1.1 eq) was added slowly while stirring. The reaction was allowed to proceed for 2 h at 0 °C and 16 h at room temperature. After 16 h, water (50 mL) was added and the reaction was stirred for 1 h to hydrolyse any excess benzoyl chloride, after which time the reaction was

quenched with the addition of DCM (50 mL). The organic layer was separated and the aqueous layer was extracted with DCM (2 × 50 mL). The combined organic layer was washed with saturated $NaHCO_3$ (2 × 50 mL) and dried over sodium sulphate then concentrated to dryness to a give brown semi-crystalline crude syrup (10.41 g) which was purified using column chromatography to give as semi-crystalline syrups **12** (2.6 g, 7.00 mmol, 23% yield); (Rf: 0.28, Hexane/EtOAc 2/1 v/v) and a mixture of **13** & **14** (3.79 g; **13** (2.27 g. 13.70 mmol, 44% yield) & **14** (1.52 g, 9.2 mmol, 30% yield)) in a methanol wash.

^{1}H **NMR** (400 MHz, $CDCl_3$, **12**): 8.01 (d, 4H, *J* = 17.81 Hz, ArHβ), 7.57-7.67 (m, 2H, ArHδ), 7.42-7.37 (m, 4H, ArHγ), 5.11-5.05 (m, 1H, H-4), 4.68 (d, 1H, $J_{6,6'}$ = 11.64 Hz, H-6), 4.66 (dd, 1H, $J_{5,4}$ = 3.08 Hz, $J_{5,5'}$ = 12.54 Hz, H-5), 4.47 (d, 1H, $J_{6',6}$ = 11.56 Hz, H-6'), 4.45 (dd, 1H, $J_{5',4}$ = 5.96 Hz, $J_{5',5}$ = 12.54 Hz, H-5'), 2.53 (dd, 1H, $J_{3,4}$ = 6.32 Hz, $J_{3,3'}$ = 13.85 Hz, H-3), 2.36 (dd, 1H, $J_{3',4}$ = 9.20 Hz, $J_{3',3}$ = 13.87 Hz, H-3').

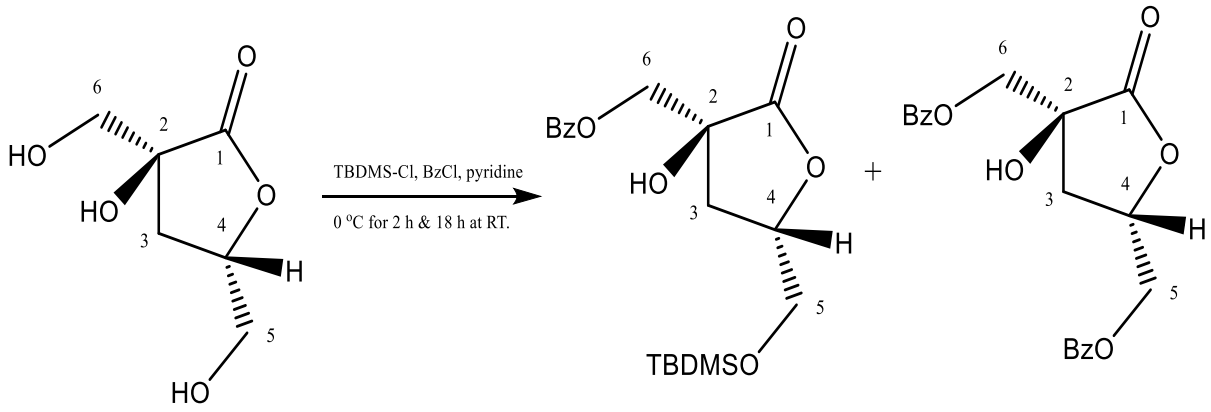
^{13}C **NMR** (100 MHz, $CDCl_3$):174.78 (C1), 166.21 & 166.16 (PhCO), 133.61 & 133.49 (ArCδ), 129.78 & 129.75 (ArCβ), 129.18 & 129.01 (ArCα), 128.60 & 128.54 (ArCγ), 75.57 (C4), 75.01 (C2), 65.85 (C6), 64.77 (C5), 34.95 (C3).

HRMS (m/z) Calculated mass for $C_{20}H_{18}O_7$ $[M+Na]^+$ 393.0945, found 393.0938.

FT-IR (cm^{-1}): 3514.6 (O-H), 2951.0 & 2857.1 (C-H), 1783.7, 1715.5 & 1714.8 (C=O), 1448.9 (Ar C-C), 1267.5 & 1197.9 (C-O), 729.6 & 704.7 (Ar C-H).

13 & **14** were in a 60/40 mixture that was used as starting material (see section 3.5.4) without any further characterisation.

4.4.2.4 Preparation of 5-*O*-tert-butyldimethylsilyl-6-*O*-benzoyl-α-D-glucoisosaaharino-1,4-lactone (15) and 5,6-di-*O*-benzoyl-α-glucoisosaccharino-1,4-lactone (12) [134, 151]



2 **15** **12**

Dried α-GISAL **2** (1.0 g; 6.17 mmol) was dissolved in pyridine (6 mL) whilst stirring for 10 min at room temperature. It was then added cautiously to *tert*-butyldimethylsilyl chloride (TBDMSCl 1.0 g; 6.79 mmol; 1.1 eq) while stirring at room temperature. The reaction was allowed to proceed for 2 h at room temperature. After 2 h, the reaction was cooled to 0 °C and benzoyl chloride (800 µL; 5.69 mmol; 0.9 eq) was added cautiously and the reaction was allowed to continue for a further 2 h at 0 °C, then for 16 h at room temperature. After 16 h, the reaction was halted by adding DCM (50 mL), followed by water (50 mL) and the layers were separated. The aqueous layer was extracted with DCM (2 x 30 mL) and the combined organic layer was dried over sodium sulphate and concentrated to give a brown crude syrup (3.59 g) which was purified using column chromatography to give **15** (750 mg;1.97 mmol; 32% yield); (Rf: 0.66; Hexane/EtOAc 4:1 v/v) and **12** (590 mg;1.59 mmol; 26% yield); (Rf: 0.26; Hexane/EtOAc 2:1 v/v).

^{1}H **NMR** (400 MHz, $CDCl_3$ **15**): 8.01 (d, 2H, J = 8.18 Hz, ArHβ), 7.55-7.50 (m, 1H, ArHδ), 7.41-7.35 (m, 2H, ArHγ), 4.78-4.73 (m, 1H, H-4), 4.66 (d, 1H, $J_{6,6'}$ = 11.48 Hz, H-6), 4.46 (d, 1H, $J_{6',6}$ = 11.48 Hz, H-6'), 3.93 (dd, 1H, $J_{5,4}$ = 3.32 Hz, $J_{5,5'}$ = 11.64 Hz, H-5), 3.72 (dd, 1H, $J_{5',4}$ = 3.72 Hz, $J_{5',5}$ = 11.66 Hz, H-5'), 2.45 (dd, 1H, $J_{3,4}$ = 8.32 Hz, $J_{3,3'}$ = 13.75

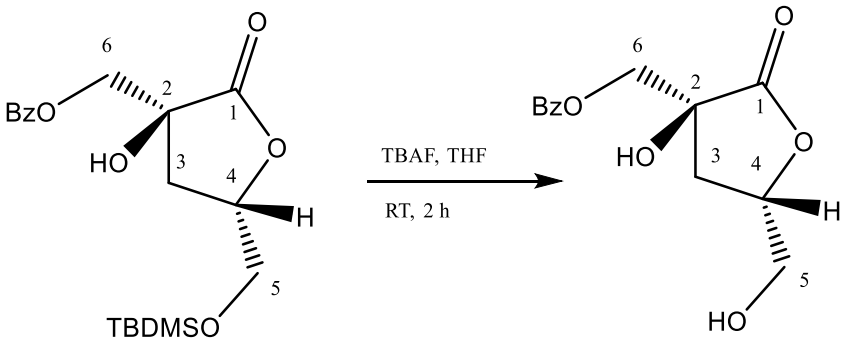
Hz, H-3), 2.33 (dd, 1H, $J_{3',4}$ = 6.72 Hz, $J_{3',3}$ = 13.73 Hz, H-3'), 0.85 (s, 9H, TBDMS), 0.05 & 0.04 (2s, 6H, TBDMS).

^{13}C **NMR** (100 MHz, $CDCl_3$):175.67 (C1), 166.25 (PhCO), 133.36 (ArCδ), 129.80 (ArCβ), 129.39 (ArCα), 128.46 (ArCγ), 78.00 (C4), 75.03 (C2), 66.09 (C6), 63.63 (C5), 34.26 (C3), 25.82 & 18.40 (TBDMS), -5.38 & -5.47 (TBDMS).

HRMS (m/z) Calculated mass for $C_{19}H_{28}O_6Si$ $[M+H]^+$ 381.1728, found 381.1733.

FT-IR (cm^{-1}): 3434.8 (O-H), 1777.4 & 1723.6 (C=O), 1451.7 (Ar C-C), 1268.6 & 1107.9 (C-O), 833.3 & 708.0 (Ar C-H).

4.4.2.5 Preparation of 6-*O*-benzoyl-α-D-glucoisosaccharino-1,4-lactone (**14**) *via* deprotection of 5-*O*-tert-butyldimethylsilyl-6-*O*-benzoyl-α-D-glucoisosaaharino-1,4-lactone (**15**)



15 **14**

6-Benzoyl-5-TBDMS-α-GISAL **15** (610 mg, 1.61 mmol) was dissolved in THF (3 mL) then tetrabutylammonium fluoride (460 mg, 1.76 mmol, 1.1 eq) was added cautiously. The reaction was stirred for 2 h at room temperature, after which, the reaction was quenched by the addition of DCM (30 mL) followed by water (30 mL). The layers were separated and the aqueous layer further extracted with DCM (2 x 30 mL). The combined organic layer was dried over sodium sulphate and concentrated to give the crude product as a yellow oil (480 mg) which was purified via column chromatography to give

14 as a white semi-solid (120 mg, 0.45 mmol, 28% yield) (Rf = 0.42, Hexane/EtOAc 1/2).

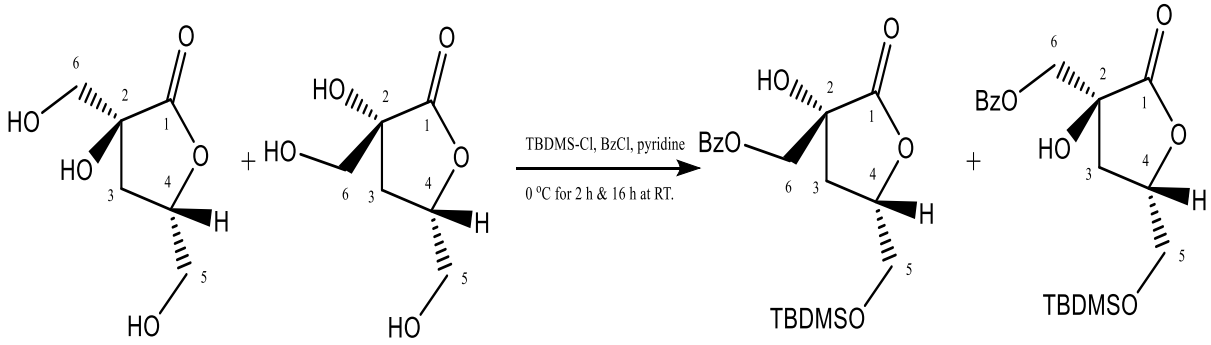
^{1}H **NMR** (400 MHz, $CDCl_3$): 8.01 (d, 2H, *J* = 7.1 Hz, ArHβ), 7.56 (t, 1H, *J* = 7.1 Hz, ArHδ), 7.43 (t, 2H, *J* = 7.56 Hz, ArHγ), 4.85-4.79 (m, 1H, H-4), 4.65 (d, 1H, $J_{6,6'}$ = 11.56 Hz, H-6) 4.48 (d, 1H, $J_{6',6}$ = 11.60 Hz, H-6'), 3.99 (dd, 1H, $J_{5,4}$ = 2.48 Hz, $J_{5,5'}$ = 12.76 Hz, H-5), 3.64 (dd, 1H, $J_{5',4}$ = 4.16 Hz, $J_{5',5}$ = 12.80 Hz, H-5'), 2.47 (dd, 1H, $J_{3,4}$ = 8.36 Hz, $J_{3,3'}$ = 13.90 Hz, H-3), 2.35 (dd, 1H, $J_{3',4}$ = 6.92 Hz, $J_{3',3}$ = 13.83 Hz, H-3').

^{13}C **NMR** (100 MHz, $CDCl_3$): 175.8 (C1), 166.4 (PhCO), 133.6 (ArCδ), 129.8 (ArCβ), 129.1 (ArCα), 128.6 (ArCγ), 78.5 (C4), 75.3 (C2), 66.0 (C6), 63.0 (C5), 33.7 (C3).

HRMS (m/z) Calculated mass for $C_{13}H_{14}O_6$ $[M+Na]^+$ 289.0683, found 289.0690.

FT-IR (cm^{-1}): 3353.4 (O-H), 1774.0 & 1720.0 (C=O), 1451.1 (Ar C-C), 1266 & 1202 (C-O), 726.4 & 707.6 (Ar C-H).

4.4.2.6 Preparation of 5-*O*-tert-butyldimethylsilyl-6-*O*-benzoyl-β-D-glucoisosaaharino-1,4-lactone (16) and 5-*O*-tert-butyldimethylsilyl-6-*O*-benzoyl-α-D-glucoisosaaharino-1,4-lactone (15) [134, 151]



2 **3** **16** **15**

A dried mixture of α & β-GISAL 1:1 ratio (2.0 g; 12.35 mmol, 1:1) was dissolved in pyridine (10 mL) whilst stirring for 10 min at room temperature. It was then added cautiously to TBDMSCl (2.0 g; 13.58 mmol; 1.1 eq) while stirring at room temperature.

The reaction was allowed to proceed for 2 h at room temperature, and was then cooled to 0 ^{o}C before benzoyl chloride (1.6 mL; 11.38 mmol; 0.9 eq) was added cautiously and allowed to continue reacting for 2 h at 0 ^{o}C, then 16 h at room temperature. After 16 h, the reaction was halted by addition of the reaction solution to DCM (50 mL) and water (50 mL). The aqueous layer was separated and was further extracted with DCM (2 x 50 mL). The combined organic layer was washed with brine (2 x 100 mL), dried over anhydrous sodium sulphate and concentrated to give a mixture of **15** & **16** (5.66 g) as a brown syrup which was separated by column chromatography to give **16** (590 mg; 1.55 mmol; 25% yield); (Rf: 0.56; Hexane/EtOAc 7:1 v/v) and **15** (880 mg; 2.32 mmol; 38% yield); (Rf: 0.50; Hexane/EtOAc 6:1 v/v).

^{1}H **NMR** (400 MHz, $CDCl_3$ **15,** α): 8.02 (d, 2H, *J* = 8.18 Hz, ArHβ), 7.55 (t, 1H, *J* = 3.73 Hz, ArHδ), 7.43 (t, 2H, *J* = 3.87 Hz, ArHγ), 4.77-4.73 (m, 1H, H-4), 4.69 (d, 1H, $J_{6,6'}$ = 11.48 Hz, H-6), 4.46 (d, 1H, $J_{6',6}$ = 11.48 Hz, H-6'), 3.93 (dd, 1H, $J_{5,4}$ = 3.32 Hz, $J_{5,5'}$ = 11.64 Hz, H-5), 3.72 (dd, 1H, $J_{5',4}$ = 3.72 Hz, $J_{5',5}$ = 11.66 Hz, H-5'), 2.46 (dd, 1H, $J_{3,4}$ = 8.32 Hz, $J_{3,3'}$ = 13.75 Hz, H-3), 2.35 (dd, 1H, $J_{3',4}$ = 6.72 Hz, $J_{3',3}$ = 13.73 Hz, H-3'), 0.87 (s, 9H, TBDMS), 0.07 & 0.05 (2s, 6H, TBDMS).

^{13}C **NMR** (100 MHz, $CDCl_3$ **15**):175.54 (C1), 166.34 (PhCO), 133.42 (ArCδ), 129.83 (ArCβ), 129.33 (ArCα), 128.49 (ArCγ), 77.94 (C4), 75.12 (C2), 66.32 (C6), 63.63 (C5), 34.12 (C3), 25.83 & 18.40 (TBDMS), -5.37 & -5.47 (TBDMS).

HRMS (m/z) Calculated mass for $C_{19}H_{28}O_6Si$ $[M+NH_4]^+$ 398.1993, found 398.2000.

FT-IR (cm^{-1}): 3434.8 (O-H), 2953.0, 2929.0 & 2856.8 (C-H), 1777.4 & 1723.6 (C=O), 1451.7 (Ar C-C), 1268.6 & 1107.9 (C-O), 833.3 & 708.0 (Ar C-H).

^{1}H **NMR** (400 MHz, $CDCl_3$ **16,** β): 8.01 (d, 2H, *J* = 7.08 Hz, ArHβ), 7.57 (t, 1H, *J* = 3.72 Hz, ArHδ), 7.44 (t, 2H, *J* = 3.87 Hz, ArHγ), 4.68-4.65 (m, 1H, H-4), 4.59 (d, 1H, $J_{6,6'}$ =

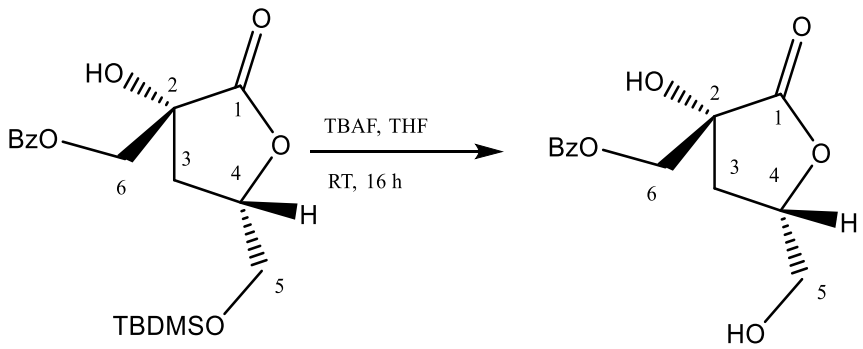
11.24 Hz, H-6), 4.48 (d, 1H, $J_{6',6}$ = 11.20 Hz, H-6'), 4.02 (dd, 1H, $J_{5,4}$ = 2.64 Hz, $J_{5,5'}$ = 11.60 Hz, H-5), 3.73 (dd, 1H, $J_{5',4}$ = 2.56 Hz, $J_{5',5}$ = 11.60 Hz, H-5'), 2.67 (dd, 1H, $J_{3,4}$ = 8.32 Hz, $J_{3,3'}$ = 14.13 Hz, H-3), 2.32 (dd, 1H, $J_{3',4}$ = 4.64 Hz, $J_{3',3}$ = 14.13 Hz, H-3'), 0.92 (s, 9H, TBDMS), 0.14 & 0.13 (2s, 6H, TBDMS).

^{13}C **NMR** (100 MHz, $CDCl_3$):175.69 (C1), 165.94 (PhCO), 133.47 (ArCδ), 129.79 (ArCβ), 129.26 (ArCα), 128.52 (ArCγ), 76.80 (C4), 73.70 (C2), 68.86 (C6), 64.46 (C5), 34.79 (C3), 25.83 & 18.60 (TBDMS), -5.49 & -5.55 (TBDMS).

HRMS (m/z) Calculated mass for $C_{19}H_{28}O_6Si$ $[M+H]^+$ 381.1728, found 381.1726.

FT-IR (cm^{-1}): 3435.5 (O-H), 2954.1, 2929.7 & 2857.4 (C-H), 1777.8 & 1723.9 (C=O), 1451.7 (Ar C-C), 1267.2 & 1095.0 (C-O), 833.3 & 708.5 (Ar C-H).

4.4.2.7 Preparation of 6-*O*-benzoyl-β-D-glucoisosaccharino-1,4-lactone (**17**) *via* deprotection of 5-*O*-tert-butyldimethylsilyl-6-*O*-benzoyl-β-D-glucoisosaaharino-1,4-lactone (**16**)



16 **17**

5-TBDMS-6-benzoyl-β-GISAL **16** (240 mg, 0.63 mmol) was dissolved in THF (2 mL) then tetrabutylammonium fluoride (180 mg, 0.069 mmol, 1.1 eq) was added cautiously. The reaction was stirred for 16 h at room temperature, after which time, DCM (30 mL) followed by water (30 mL) were added. The aqueous layer was separated and further extracted with DCM (2 x 30 mL) and the combined organic layer was dried over anhydrous sodium sulphate and concentrated to give a yellow crude product (110 mg)

which was purified via column chromatography to give a pale brown crystalline syrup (60 mg, 0.23 mmol, 37% yield) (Rf = 0.40, Hexane/EtOAc 1/2).

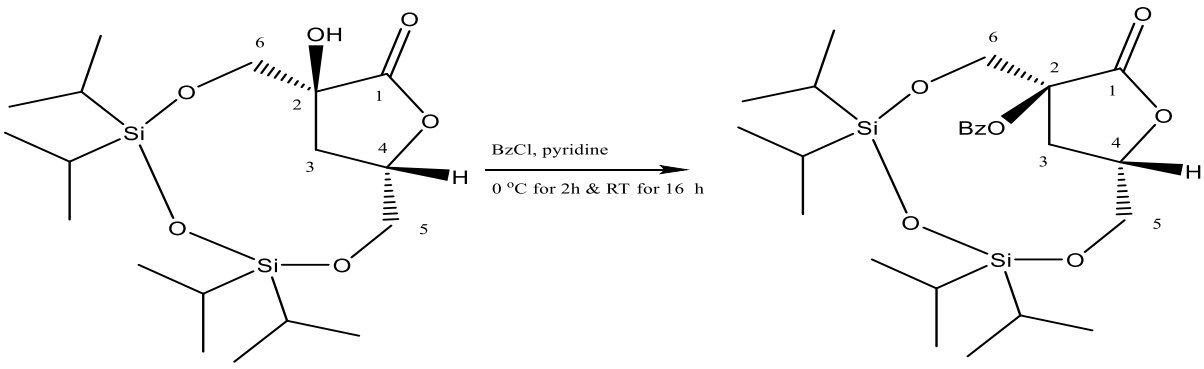
^{1}H **NMR** (400 MHz, $CDCl_3$): 8.00 (d, 2H, *J* = 7.12 Hz, ArHβ), 7.57-7.52 (m, 1H, ArHδ), 7.44-7.39 (m, 2H, ArHγ), 4.71-4.67 (m, 1H, H-4), 4.56 (d, 1H, $J_{6,6'}$ = 11.28 Hz, H-6) 4.48 (d, 1H, $J_{6',6}$ = 11.28 Hz, H-6'), 4.04 (dd, 1H, $J_{5,4}$ = 2.28 Hz, $J_{5,5'}$ = 12.56 Hz, H-5), 3.70 (dd, 1H, $J_{5',4}$ = 3.36 Hz, $J_{5',5}$ = 12.59 Hz, H-5'), 2.65 (dd, 1H, $J_{3,4}$ = 7.92 Hz, $J_{3,3'}$ = 14.05 Hz, H-3), 2.36 (dd, 1H, $J_{3',4}$ = 5.80 Hz, $J_{3',3}$ = 14.08 Hz, H-3').

^{13}C **NMR** (100 MHz, $CDCl_3$):176.5 (C1), 166.0 (PhCO), 133.6 (ArCδ), 129.8 (ArCβ), 129.1 (ArCα), 128.6 (ArCγ), 77.4 (C4), 74.3 (C2), 66.2 (C6), 63.3 (C5), 34.5 (C3).

HRMS (m/z) Calculated mass for $C_{13}H_{14}O_6$ $[M+Na]^+$ 289.0683, found 289.0688.

FT-IR (cm^{-1}): 3353.4 (O-H), 1773.7 & 1720.6 (C=O), 1451.1 (Ar C-C), 1266 & 1201.4 (C-O), 726.4 & 707.6 (Ar C-H).

4.4.2.8 Preparation of 2-*O*-benzoyl-(1',1',3',3'-tetraisopropyldislixone-1,3-diyl)-5,6-α-glucoisosaccharino-1,4-lactone (**18**) [134]



31 **18**

TIPDS-α-GISAL **31** (1.53 g, 3.79 mmol) (see 4.4.5.1) was dissolved in pyridine (3 mL) then cooled to 0 °C before benzoyl chloride (500 µL) was added slowly while stirring at 0 °C. The reaction was allowed to proceed at 0 °C for 2 h and then for 16 h at room

temperature. After 16 h, the work-up procedure for the isolation of the product **18** as a crude syrup (3.13 g) was carried out as described in 2.4.2.3 above to give, after chromatography, the product as a white crystalline syrup (850 mg; 1.67 mmol, 44% yield) (Rf: 0.61, Hexane/EtOAc 4/1).

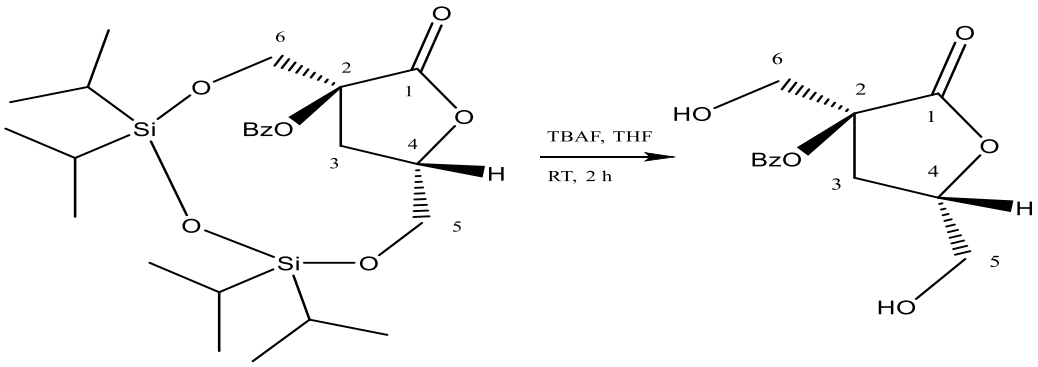
^{1}H **NMR** (400 MHz, $CDCl_3$): 8.07 (d, 2H, *J* = 7.16 Hz, ArHβ), 7.59-7.54 (m, 1H, ArHδ), 7.45-7.40 (m, 2H, ArHγ), 4.96-4.91 (m, 1H, H-4), 4.21 (s, 2H, H-6 & 6'), 4.16 (dd, 1H, $J_{5,4}$ = 3.32 Hz, $J_{5,5'}$ = 11.74 Hz, H-5), 3.92 (dd, 1H, $J_{5',4}$ = 2.52 Hz, $J_{5',5}$ = 11.76 Hz, H-5'), 2.88 (dd, 1H, $J_{3,4}$ = 3.52 Hz, $J_{3,3'}$ = 14.01 Hz, H-3), 2.58 (dd, 1H, $J_{3',4}$ = 10.04 Hz, $J_{3',3}$ = 14.01 Hz, H-3'), 1.10-1.03 (m, 28H, TIPDS)

^{13}C **NMR** (100 MHz, $CDCl_3$):173.6 (C1), 165.8 (PhCO), 133.7 (ArCδ), 130.0 (ArCβ), 129.0 (ArCα), 128.5 (ArCγ), 81.9 (C2), 77.4 (C4), 64.9 (C6), 64.5 (C5), 29.2 (C3), 17.4, 17.3, 17.2, & 17.1 (CH-TIPDS), 13.7, 13.6, 12.8 & 12.3 (CH_3-TIPDS).

HRMS (m/z) Calculated mass for $C_{25}H_{40}O_7Si$ $[M+H]^+$ 509.2385, found 509. 2389

FTIR (cm^{-1}): 2946.2, 2867.8 (C-H), 1774.8, 1717.2 (C=O), 1464.0 (C-H), 1452.5 (Ar C-C), 1281.0, 1220.8 (C-O), 1082.9, 1045.1 (R_3Si-O-SiR_3), 906.0 884.2 (Si-CH), 728.3, 710.5 (Ar C-H).

4.4.2.9 Preparation of 2-O-benzoyl-α-D-glucoisosaccharino-1,4-lactone (**19**) *via* deprotection of 2-O-benzoyl-(1',1',3',3'-tetraisopropyldislixone-1,3-diyl)-5,6-α-glucoisosaccharino-1,4-lactone (**18**).



18 **19**

Benzoyl-TIPDS-α-GISAL **18** (800 mg, 1.58 mmol) was dissolved in tetrahydrofuran THF (5 mL) whilst stirring for 5 min and then tetrabutylammonium fluoride TBAF (900 mg) was added gently while continuing to stir at room temperature. The reaction was allowed to proceed for 2 h at room temperature after which time it was quenched by the addition of EtOAc (30 mL) followed by water (30 mL). The organic layer was separated and the aqueous layer was further extracted with EtOAc (2 x 30 mL). The combined organic layer was dried over anhydrous sodium sulphate and concentrated to give a yellow crude syrup (500 mg). The desired product was purified using column chromatography to a give the desired product a white solid **19** (100 mg, 0.38 mmol, 24% yield) which eluted in the methanol wash.

^{1}H **NMR** (400 MHz, $CDCl_3$): 8.01 (d, 2H, J = 7.78 Hz, ArHβ), 7.57-7.52 (m, 1H, ArHδ), 7.43-7.38 (m, 2H, ArHγ), 4.85-4.79 (m, 1H, H-4), 4.67 (d, 1H, $J_{6,6'}$ = 11.64 Hz, H-6), 4.48 (d, 1H, $J_{6',6}$ = 11.60 Hz, H-6'), 4.00 (dd, 1H, $J_{5,4}$ = 2.56 Hz, $J_{5,5'}$ = 12.74 Hz, H-5), 3.65

(dd, 1H, $J_{5',4}$ = 4.12 Hz, $J_{5',5}$ = 12.80 Hz, H-5'), 2.47 (dd, 1H, $J_{3,4}$ = 8.28 Hz, $J_{3,3'}$ = 13.93 Hz, H-3), 2.36 (dd, 1H, $J_{3',4}$ = 6.92 Hz, $J_{3',3}$ = 13.95 Hz, H-3').

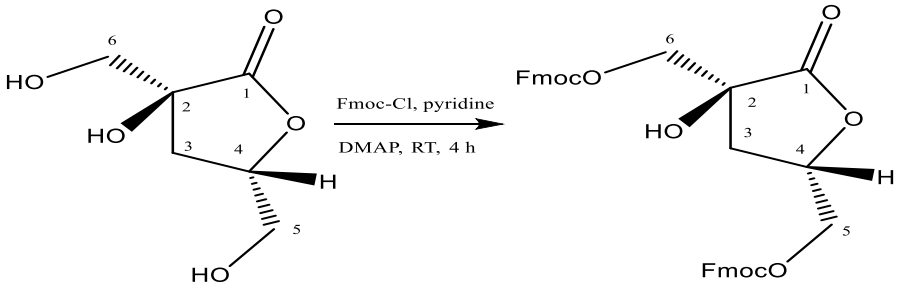
^{13}C **NMR** (100 MHz, $CDCl_3$):175.61 (C1), 166.39 (PhCO), 133.56 (ArCδ), 129.82 (ArCβ), 129.15 (ArCα), 128.55 (ArCγ), 78.37 (C4), 75.32 (C2), 66.15 (C6), 63.04 (C5), 33.68 (C3).

HRMS (m/z) Calculated mass for $C_{13}H_{14}O_6$ $[M+H]^+$ 267.0863, found 267.0866.

FT-IR (cm^{-1}): 3515 (O-H), 2951 & 2857 (C-H), 1784 & 1715 (C=O), 1449 (Ar C-C), 1268 & 1108 (C-O), 705 & 685 (Ar C-H).

4.4.3 Synthesis of Fmoc derivatives of α-glucoisosaccharino-1,4-lactone.

4.4.3.1 Preparation of 5,6-di-*O*-Fmoc-α-D-glucoisosaccharino-1,4-lactone (5,6-di-*O*-Fmoc-α-GISA$_L$). (20)[113]



2 **20**

Dried α-glucoisosaccharino-1,4-lactone **2** (2.01 g, 12.4 mmol) and dimethylaminopyridine DMAP (0.50 g) were dissolved in anhydrous pyridine (40 mL) and stirred under nitrogen for 20 mins. The mixture was added gently to a second reaction vessel, cooled to 0°C, containing fluorenylmethoxycarbonyl chloride (Fmoc-Cl) (7.05 g, 273 mmol, 2.2 eq). After the addition was complete, the reaction was allowed to reach room temperature and was stirred, under an atmosphere of nitrogen, for a further 3 h. The reaction was halted by adding ice-cold water (100 mL), followed by

diethyl ether (100 mL). The organic layer was separated, and the aqueous phase was extracted with diethyl ether (3 x 100 mL). The combined organic layer was washed with brine (3 x 100 mL) to remove pyridine, dried over anhydrous sodium sulphate and filtered before being concentrated under reduced pressure. The crude product was a bright yellow crystalline syrup (3 g). The crude material was separated via chromatography, and the desired product was recovered as a pale yellow solid (1.47 g, 2.45 mmol, 20 % yield), (Rf = 0.47 Hexane/EtOAc; 6:1-1:1 v/v)

^{1}H **NMR** (400 MHz, DMSO): δ 7.90-7.84 (m, 4H, ArHα), 7.65-7.59 (m, 4H, ArHδ), 7.43-7.37 (m, 4H, ArHβ), 7.34-7.28 (m, 4H, ArHγ), 4.85-4.77 (m, 1H, H-4), 4.56-4.50 (m, 3H, Fmoc C$\underline{H}_2$ & C$\underline{H}$), 4.37-4.26 (m, 4H, H6, H6' & Fmoc C$\underline{H}_2$'), 4.22-4.13 (m, 3H, Fmoc C$\underline{H}$', H5 & H5'), 2.20 (dd, 1H, $J_{3,4}$= 6.71 Hz, $J_{3,3'}$=14.08 Hz, H-3), 2.05 (dd, 1H, $J_{3',4}$= 9.39 Hz, $J_{3',3}$=14.08 Hz, H-3')

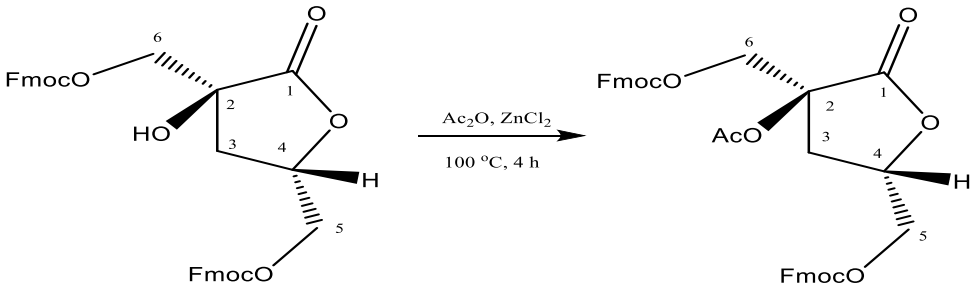
^{13}C **NMR** (100 MHz, DMSO): δ 174.5 (C1), 154.9 & 154.8 (Fmoc $\underline{CO}$), 143.2 & 141.3 (Fmoc $\underline{Cq}$'s), 128.0 (4 x ArCα), 127.2 (4 x ArCδ), 125.2 (4 x ArCβ), 120.1 (4 x ArCγ), 75.6 (C2), 75.0 (C4), 70.5 & 70.4 (Fmoc $\underline{C}H_2$'s), 68.9 (C6), 67.8 (C5), 46.6 (Fmoc $\underline{C}$H's), 34.6 (C3)

HRMS (m/z) Calculated mass for $C_{36}H_{30}O_9$ $[M+NH_4]^+$ 624.2228 found 624.2228.

FT-IR (cm^{-1}): 3399 (O-H), 2956 & 2925 (C-H), 1780 & 1746 (C=O), 1449 (Ar C-C), 1251 & 1194 (C-O), 736 (Ar C-H).

MP: 76-77^{o} C.

4.4.3.2 Preparation of 2-*O*-acetyl-5,6-di-*O*-Fmoc-α-D-glucoisosaccharino-1, 4-lactone (2-*O*-acetyl-5,6-di-*O*-Fmoc-α-GISAL). (21)[147]



20 **21**

5,6-di-*O*-Fmoc-α-GISAL **20** (2.34 g, 3.86 mmol) was dissolved in acetic anhydride (12.5 mL, 0.13 mmol) and then anhydrous $ZnCl_2$ (0.5 g) was added as catalyst. The reaction was allowed to proceed for 4 h at 100 °C. After 4 h, the contents of the flask were poured into ice cool water (100 mL) and the mixture was stirred at 0 °C for 30 min; the oil solidified while stirring. The resulting solid was filtered and the residue dried in a dessicator at room temperature overnight to give **21** (1.5 g, 2.14 mmol, 55 % yield) as a fine pale brown powder.

^{1}H **NMR** (400 MHz, $CDCl_3$): δ 7.77-7.73 (m, 4H, ArHα), 7.61-7.56 (m, 4H, ArHδ), 7.42-7.36 (m, 4H, ArHβ), 7.34-7.27 ppm (m, 4H, ArHγ), 5.12-5.04 (m, 1H, H-4), 4.53-4.40 (m, 7H, Fmoc (CH_2)$_2$, H-6, H-6' & H-5), 4.32-4.22 (m, 3H, H-5' & Fmoc CHs), 2.60 (dd, 1H, $J_{3,4}$= 9.38 Hz, $J_{3,3'}$=14.32 Hz, H-3), 2.42 (dd, 1H, $J_{3'4}$= 5.93 Hz, $J_{3'3}$=14.32 Hz, H-3'), 2.17 (s, 3H, CH_3CO).

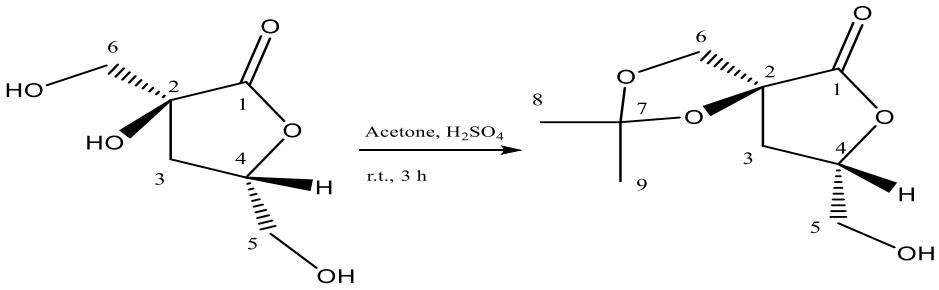
^{13}C **NMR** (100 MHz, $CDCl_3$): δ 177.3 (C1), 170.1 (CH_3CO), 154.8 & 154.4 (Fmoc CO), 143.2 & 141.3 (4 x Fmoc Cqs), 128.0 (4 x ArCα), 127.3 (4 x ArCδ), 125.1 (4 x ArCβ), 120.1 (4 x ArCγ), 77.9 (C2), 74.6 (C4), 70.6 & 70.4 (Fmoc CH_2), 68.5 (C6), 67.9(5), 46.63 & 46.60 (Fmoc CH) 31.8 (C3), 20.8 (CH_3CO).

HRMS (m/z): Calculated mass for $C_{38}H_{32}O_{10}$ $[M+Na]^+$ 648.1995 found 648.1992.

FT-IR: 1783.9, 1745.1 & 1708.5 (C=O), 1253.1, 1206.3 (C-O stretching), 784.0, 758.8, 738.5 (C-H).

MP: 90-91 ^oC.

4.4.3.3 Preparation of 2,6-*O*-isopropylidene-α-D-glucoisosaccharino-1,4-lactone (2,6-isopropylidene-α-GISAL) (22) [152, 153]



2 **22**

Lactone α-GISAL (8.09 g, 0.05 mol) was dissolved in anhydrous acetone (400 mL) and conc. H_2SO_4 (40 mL) was added. The reaction was kept stirring at room temperature for 3 h and dried amberlite IR-45 (OH^-) anion exchange resin (230 mL) was added. After filtration, the filtrate was concentrated under reduced pressure to give a partially crystalline yellow syrup (5.48 g).

NMR analysis of the dried syrup showed a large proportion of unreacted starting material. The syrup (5.48 g) was re-dissolved in freshly distilled anhydrous acetone (180 mL), and *p*-toulenesulfonic acid (PTSA) (60 mg) was added along with dried molecular sieves (60 g). The mixture was refluxed for 12 h. It was filtered, and the filtrate was reduced to dryness using a rotary evaporator, resulting in a pale yellow crystalline syrup. (5.22 g). The desired product was separated *via* column chromatography to give **22** (1.83 g, 9.06 mmol, 27 % yield) (Rf: 0.43; Pet. Ether/EtOAc 3/1, v/v)

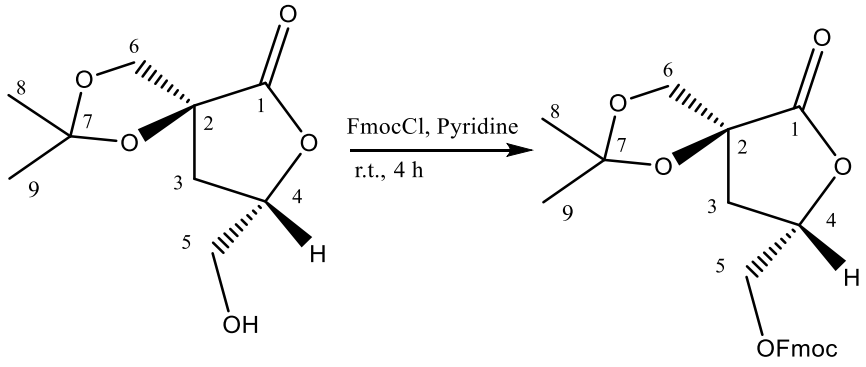
^{1}H **NMR** (400 MHz, $CDCl_3$): δ 4.68-4.64 (m, 1H, H-4), 4.27 (d, 1H, $J_{6,6'}$= 9.24 Hz, H-6), 4.08 (d, 1H, $J_{6',6}$= 9.24 Hz, H-6'), 3.91 (dd, 1H, $J_{5,4}$= 2.32 Hz, $J_{5,5'}$= 12.60 Hz, H-5), 3.55 (dd, 1H, $J_{5',4}$= 3.32 Hz, $J_{5',5}$= 12.64 Hz, H-5'), 2.41 (dd, 1H, $J_{3,4}$= 7.44 Hz, $J_{3',3}$= 13.83 Hz, H-3), 2.30 (dd, 1H, $J_{3',4}$= 6.48 Hz, $J_{3',3}$= 13.83 Hz, H-3'), 1.41 (2s, 6H, 2 x $C\underline{H}_3$).

^{13}C **NMR** (100 MHz, CDCl3): δ 176.3 (C1), 112.2 (C7), 80.9 (C2), 78.1 (C4), 71.7 (C6), 62.9 (C5), 35. 7 (C3), 26.3 & 25.7 (2 x CH_3).

HRMS (m/z) Calculated mass for $C_9H_{10}O_5$ [M+H$^+$] 203.0914 found 203.0916.

FT-IR (cm^{-1}): 3462.0 (O-H); 2989.2, 2937.4 (C-H); 1770.0 (C=O); 1372.9 (C-H).

4.4.3.3 Preparation of 5-*O*-Fmoc-2, 6-*O*-isopropylidene-α-D-glucoisosaccharino-1,4-lactone (5-*O*-Fmoc-2,6-isopropylidene-α-GISAL) (23) [113]



22 **23**

2,6-Isopropylidene-α-GISAL **22** (1.38 g, 6.83 mmol) was dissolved in anhydrous pyridine (20 ml). The solution was cautiously added to a flask, maintained at 0 ºC, containing crystalline Fmoc-Cl (2.66 g, 0.01 mmol) under an atmosphere of nitrogen while stirring. The reaction was stirred and allowed to proceed for 4 h at room temperature after which time it was carefully added to a beaker containing ice cold water (60 ml) and diethyl ether (60 ml). The organic layer was separated and the aqueous phase was extracted with diethyl ether (3 x 60 mL). The combined organic extracts were

washed with a saturated solution of brine (50 mL), water (50 mL) and then dried over anhydrous sodium sulphate before removing the solvent at reduced pressure to give a crude mixture (3.82 g) which after column chromatography, gave the desired product **23** as a yellow solid (570 mg, 1.34 mmol; Yield: 20 %) (Rf: 0.52 Pet. ether/EtOAc 3:1 v/v).

^{1}H **NMR** (400 MHz, $CDCl_3$): δ 7.78-7.68 (m, 2H, ArH), 7.59-7.50 (m, 2H, ArH), 7.45-7.40 (m, 2H, ArH), 7.36-7.31 (m, 2H, ArH), 4.88-4.82 (m, 1H, H-4), 4.50-4.37 (m, 4H, 2 x H-5 & 2 x H-6), 4.28-4.08 (m, 3H, 2 x H-11 & H-12), 2.20 (dd, 1H, $J_{3,4}$ = 7.05 Hz, $J_{3,3'}$ = 14.38 Hz, H-3), 2.55 (dd, 1H, $J_{3',4}$ = 7.47 Hz, $J_{3,3'}$ = 14.33 Hz, H-3'), 1.49 (2s, 6H, 2 x CH_3).

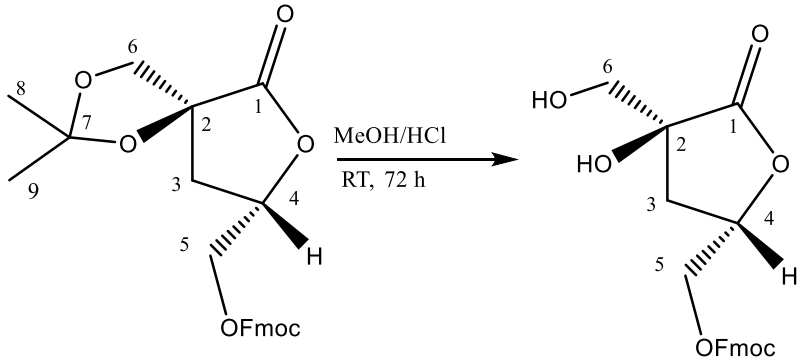
^{13}C **NMR** (100 MHz, $CDCl_3$): δ 174.8 (C1), 154.6 (FmocCO): 142.8, 141.3, 127.9, 127.1, 125.0, 119.9 (2 x ArC), 112.7 (C7), 80.8 (C2), 74.4 (C4), 72.0 (C6), 70.1 (C5), 67.7 (C11) 46.4 (C12) 36.5 (C3), 26.7 (C8), 25.3 (C9).

HRMS (m/z) Calculated mass for $C_{24}H_{24}O_7$ $[M+Na]^+$ 447.1414, found: 447.1415.

FT-IR (cm^{-1}): 1782 & 1746 (C=O), 1450 (Ar C-C), 1374 (C-H) 1246 & 1192 (C-O), 758 & 737. (Ar C-H).

MP: 75-77 ^{o}C

4.4.3.4 Preparation of 5-*O*-Fmoc-α-D-glucoisosaccharino-1, 4-lactone (24) via deprotection of 5-*O*-Fmoc-2,6-isopropylidene-α-D-glucoisosaccharino-1,4-lactone (5-*O*-Fmoc-α-GISAL). (23) [154]



23 **24**

5-*O*-Fmoc-2,6-isopropylidene-α-GISAL **23** (150 mg 0.35 mmol) was dissolved in an acidified methanol solution (40 mL; 1 M HCl in MeOH) and the solution was stirred at room temperature for 72 h after which time EtOAc (40 mL) was added and the two layers were separated. The aqueous layer was further extracted with EtOAc (3 x 40 ml) then the combined organic layer was dried overnight over anhydrous sodium sulphate. It was then filtered and the solvent were removed to give a crystalline white syrup **24** (100 mg, 0.26 mmol, 74% yield).

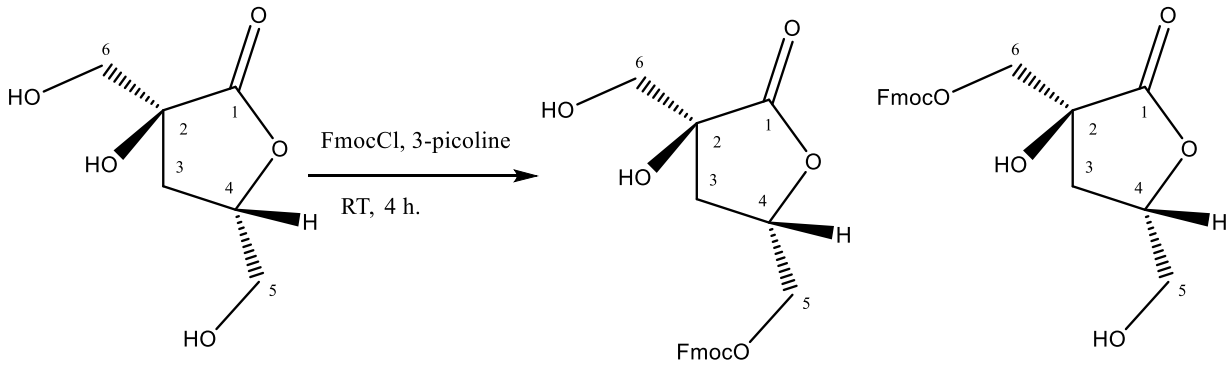
^{1}H **NMR** (400 MHz, $CDCl_3$): δ 7.78 (d, 2H, *J*= 8.0 Hz, ArHα), 7.60 (d, 2H, *J*= 8.0 Hz, ArHδ), 7.42 (t, 2H, *J*=4.0 Hz, ArHβ), 7.33 (t, 2H, *J*= 8.0 Hz, ArHγ), 5.00-4.93 (m, 1H, H-4), 4.47-4.42 (m, 3H, H-8, H-8' & H-9), 4.29-4.24 (m, 2H, H-5 & H-5'), 3.86 (d, 1H, $J_{6',6}$= 11.89 Hz, H-6'), 3.73 (d, 1H, $J_{6,6'}$= 11.89 Hz, H-6), 2.35 (dd, 1H, $J_{3',4}$= 7.0 Hz, $J_{3',3}$=13.17 Hz, H-3'), 2.07 (dd, 1H, $J_{3,4}$= 8.56 Hz, $J_{3,3'}$= 13.17 Hz, H-3).

^{13}C **NMR** (100 MHz, $CDCl_3$): δ 177.4 (C1), 155.1 (C7), 143.3 (ArCq), 141.7 (ArCq), 128.3 (ArCα), 127.2 (ArCδ), 125.6 (ArCβ), 120.5 (ArCγ), 76.0 (C2), 75.2 (C4), 70.9 (C8), 67.6 (C5), 65.2 (C6), 46.7 (C9), 33.6 (C3).

HRMS (m/z) Calculated mass for $C_{21}H_{20}O_7$ $[M+Na]^+$ 407.1101, found 407.1100.

FT-IR (cm^{-1}): 3460 (O-H), 1746.5 (C=O), 1450 (Ar C-C), 1193 & 1256 (C-O), 737.5 (Ar C-H).

4.4.3.5 Preparation of 5-*O*-Fmoc-α-D-glucoisosaccharino-1,4-lactone (5-*O*-Fmoc-α-GISAL) (24) and 6-O-Fmoc-α-D-glucoisosaccharino-1,4-lactone (6-*O*-Fmoc-α-GISAL). (25) [113]



2 **24** **25**

Freeze dried α-GISAL **2** (1 g, 6.17 mmol) was dissolved in 3-picoline (20 mL) and was added cautiously whilst stirring to cooled (0 ^oC) crystalline 9-flourenylmethyoxycarbonyl chloride (Fmoc Cl) (3.35 g, 13 mmol) under an atmosphere of nitrogen. The reaction was allowed to proceed for 3 h at room temperature. After which time, ice-cold water (60 mL) followed by diethyl ether (60 mL) were added. The organic layer was separated, and the aqueous layer was extracted with diethyl ether (2 x 60 mL) then, the combined extract was washed with 2 M HCl (2 x 100 mL), brine (2 x 100 mL) and dried over anhydrous sodium sulphate before being concentrated to dryness to give the crude product as a pale yellow crystalline crude syrup (3.62 g). The desired products were obtained from the crude using column chromatography (Silica 100 g; 60 × 3 cm) to give **24** (0.56 g, 1.46 mmol, 24% yield, R_f= 0.12) and **25** (1.32 g, 3.44 mmol, 56% yield, R_f= 0.17).

^{1}H **NMR** (400 MHz, $CDCl_3$, **25**): δ 7.74 (d, 2H, *J*= 8.0 Hz, ArHα), 7.58 (d, 2H, *J*=7.0 Hz, ArHδ), 7.39 (t, 2H, *J*= 4.0 Hz, ArHβ), 7.30 (d, 2H, *J*= 8.0 Hz, 7.0 Hz, ArHγ), 4.83-4.75 (m, 1H, H-4), 4.49 (d, 1H, $J_{6,6'}$= 11.45 Hz, H-6), 4.41 (dd, 2H, $J_{8,9}$= 3.41Hz, $J_{8,8}$= 7.31 Hz, H-8 & H-8'), 4.33 (d, 1H, $J_{6',6}$= 11.45 Hz, H-6'), 4.23 (m, 1H, H-9), 3.92 (dd, 1H, $J_{5,4}$= 2.50 Hz, $J_{5,5'}$= 12.98, H-5), 3.62 (dd, 1H, $J_{5',4}$= 4.12 Hz, $J_{5',5}$= 12.98 Hz, H-5'), 2.31 (2d, 2H, $J_{3,3}$= 7.31 Hz, H-3 & H-3').

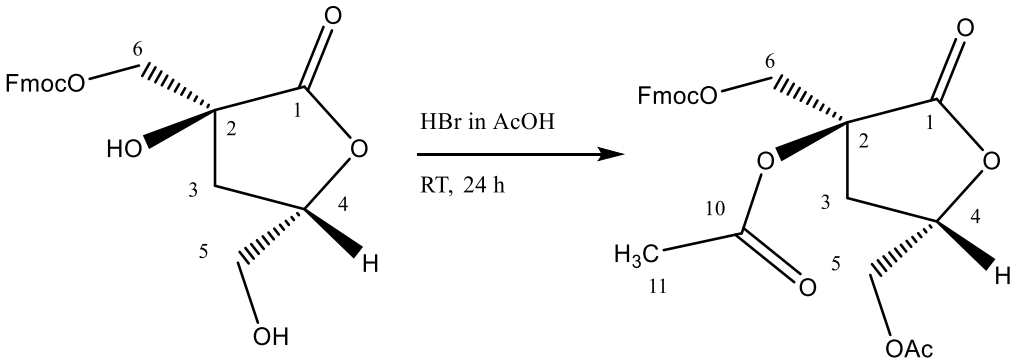
^{13}C **NMR** (100 MHz, $CDCl_3$): δ 175.8 (C1) 154.9 (Fmoc CO) 143.1 (ArCq), 141.7 (ArCq), 128.6 (ArCα), 127.2 (ArCδ), 125.4 (ArCβ), 120.3 (ArCγ), 79.2 (C2), 74.9 (C4), 70.6 (Fmoc CH_2's), 69.0 (C6), 63.6 (C5), 46.4 (Fmoc CH's), 33.8 (C3).

HRMS (m/z) Calculated mass for $C_{21}H_{20}O_7$ $[M+K]^+$ 423.0841, found 423.0854.

FT-IR (cm^{-1}): 3442 (O-H), 1748 (C=O), 1450 (Ar C-C), 1256 & 1195 (C-O), 727 (Ar C-H).

The characterisation data for **24** were the same as those reported on the previous page

4.4.3.6 Preparation of 2,5-di-O-acetyl-6-Fmoc-α-D-glucoisosaccharino-1,4-lactone (GISAL(6-Fmoc)OAc_2) (26)



25 **26**

6-*O*-Fmoc-α-GISAL **25** (1.0 g, 2.60 mmol) was added slowly to hydrogen bromide in acetic acid (33% v/v, 5 mL) at 0 °C. The mixture was allowed to reach room temperature,

during which time a deep yellow colour developed, and then the reaction was allowed to proceed for 24 h. After 24 h, the reaction was halted with $CHCl_3$ (30 mL) followed by addition of water (30 mL) and the two layers were separated. The aqueous phase was further extracted with $CHCl_3$ (2 x 50 mL) and the combined organic layer was dried over sodium sulphate and concentrated to give the pure product as a viscous, pale brown syrup (980 mg; 2.09 mmol; 80 %)

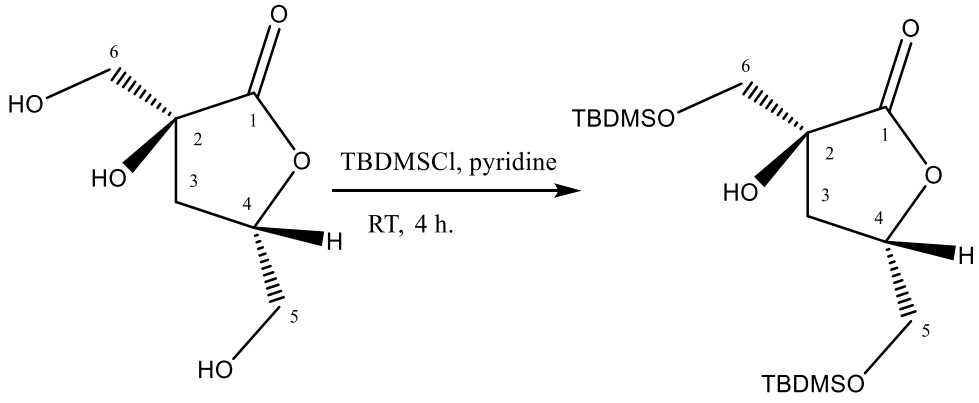
^1H **NMR** (400 MHz, $CDCl_3$, **25**): δ 7.78 (d, 2H, *J*= 7.55 Hz, ArHα), 7.60 (d, 2H, *J*=7.59 Hz, ArHδ), 7.41 (t, 2H, *J*= 3.58 Hz, ArHβ), 7.32 (d, 2H, *J*= 7.64 Hz, 1.07 Hz, ArHγ), 4.97-4.90 (m, 1H, H-4), 4.78 (d, 1H, $J_{6,6'}$= 11.98 Hz, H-6), 4.12-4.50 (m, 6H = H5, H5', H6', H8, H8' & H9), 2.87 (dd, 1H, $J_{3,4}$ = 7.03 Hz, $J_{3,3'}$ = 14.75 Hz, H-3), 2.34 (dd, 1H, $J_{3',4}$ = 7.40 Hz, $J_{3',3}$ = 14.75 Hz, H-3'), 2.15 (s, 3H, $COC\underline{H}_3$), 2.10 (s, 3H, $COC\underline{H}_3$).

^{13}C **NMR** (100 MHz, $CDCl_3$): δ 171.2 (C1), 170.9 & 170.0 (2 x $\underline{CO}CH_3$) 153.0 (Fmoc $\underline{CO}$) 144.1 (ArCq), 141.0 (ArCq), 128.0 (ArCα), 127.0 (ArCδ), 125.0 (ArCβ), 120.0 (ArCγ), 80.2 (C2), 75.1 (C4), 70.3 (Fmoc $\underline{C}H_2$'s), 66.0 (C6), 64.0 (C5), 46.7 (Fmoc $\underline{C}$H's), 35.1 (C3). 21.1 & 20.6 ($CO\underline{C}H_3$)

HRMS (m/z) Calculated mass for $C_{21}H_{20}O_7$ $[M+NH_4]^+$ 468.1420, found 468.1415.

4.4.4 Synthesis of tert-butyldimethylsilyl derivatives of α- and β-glucoisosaccharino-1,4-lactone.

3.4.4.1 Preparation of 5,6-di-*O*-tert-butyldimethylsilyl-α-D-glucoisosaccharino-1,4-lactone (27) [151]



2 **27**

Dried α-GISAL **2** (1.0 g, 6.17 mmol) was dissolved in anhydrous pyridine (5 mL) whilst stirring for 20 min at room temperature. It was then added cautiously to TBDMSCl (2.1 g, 13.93 mmol, 2.2 eq) while stirring at room temperature. The reaction was allowed to proceed for 4 h after which time DCM (50 mL) and water (50 mL) were added. The organic layer was separated and the aqueous layer was further extracted with DCM (2 x 50 mL). The combined organic layer was washed with a 1% $CuSO_4$ solution (2 x 50 mL), dried over anhydrous sodium sulphate and concentrated to give a crude sample of **27** as a semi-crystalline syrup. The product was purified by chromatography to give a semi-crystalline syrup (1.66 g; 4.26 mmol; 69 %) (Rf = 0.72; Hexane/EtOAc 3:1 v/v).

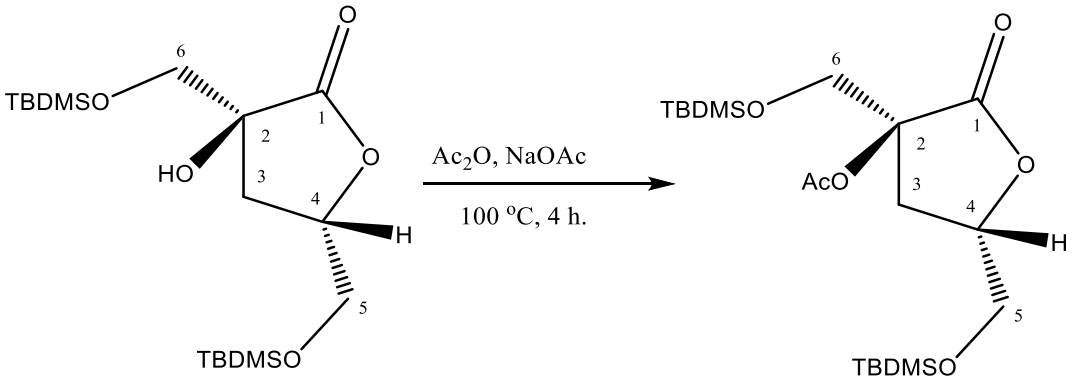
^{1}H **NMR** (400 MHz, $CDCl_3$): δ 4.68-4.60 (m, 1H, H-4), 3.78 (dd, 1H, $J_{5,4}$ = 3.79 Hz, $J_{5,5'}$ = 11.55 Hz, H-5), 3.76 (d, 1H, $J_{6,6'}$ = 9.85 Hz, H-6), 3.69 (dd, 1H, $J_{5',4}$ = 4.74 Hz, $J_{5',5}$ = 11.55 Hz, H-5'), 3.65 (d, 1H, $J_{6',6}$ = 9.85 Hz, H-6'), 2.32 (dd, 1H, $J_{3,4}$ = 8.30 Hz, $J_{3,3'}$ = 14.02 Hz, H-3), 2.17 (dd, 1H, $J_{3',4}$ = 7.40 Hz, $J_{3',3}$ = 14.02 Hz, H-3'), 0.88 & 0.86 (2s, 18H , 2 x TBDMS), 0.06 & 0.05 (2s, 12H, 2 x TBDMS).

^{13}C **NMR** (100 MHz, $CDCl_3$): δ 176.92 (C1), 77.77 (C4), 76.35 (C2), 65.42 (C6), 64.30 (C5), 33.72 (C3), 25.82, 25.78, 18.31& 18.24 (TBDMS), -3.67, -5.44, -5.55 & 5.61 (TBDMS).

HRMS (m/z): Calculated mass for $C_{18}H_{38}Si_2O_5$ $[M+Na]^+$ 413.2150, found 413.2152.

FT-IR (cm^{-1}): 3258.7 (O-H), 2951.9, 2928.0, 2885.7, 2856.8 (C-H), 1770.1 (C=O), 1471.1, 1461.5, 1359.5 (C-H), 1254.6, 832.8, 814.2, 774.6 ($SiMe_2$), 1200.1, 1167.7 (C-O), 1097.3, 1044.0 (Si-OR).

4.4.4.2 Preparation of 2-*O*-acetyl-5,6-di-*O*-tert-butyldimethylsilyl-α-D-glucoisosaccharino-1,4-lactone (28)



27 **28**

The 5,6-di-tert-butyldimethylsilyl lactone **27** (1.0 g, 2.56 mmol) was dissolved in acetic anhydride (10 mL) and a catalytic amount of sodium acetate (0.5 g) was added while stirring at room temperature. The reaction was allowed to continue uninterrupted for 4 h at 100 °C. After 4 h, the contents of the reaction vessel were transferred onto ice cold water (100 ml) and stirred for 1 h to convert excess acetic anhydride to acetic acid. The organic product was extracted with DCM (2 x 100 mL), and the organic layer was dried over anhydrous sodium sulphate and concentrated to give a brown crude semi-crystalline syrup (5.65 g). After chromatography, the product **28** was recovered as a

white crystalline semi-solid (900 mg, 2.08 mmol; 81%); (Rf: 0.82, Hexane/EtOAc 3:1, v/v).

^{1}H **NMR** (400 MHz, $CDCl_3$): δ 4.77-4.70 (m, 1H, H-4), 3.79 (d, 1H, $J_{6,6'}$ = 9.80 Hz, H-6), 3.72 (d, 1H, $J_{6',6}$ = 9.80 Hz, H-6'), 3.70-3.64 (m, 2H, H-5), 2.48 (dd, 1H, $J_{3,4'}$ = 6.30 Hz, $J_{3,3'}$ = 14.50 Hz, H-3), 2.24 (dd, 1H, $J_{3',4}$ = 5.65 Hz, $J_{3',3}$ = 14.48 Hz, H-3'), 2.01 (s, 3H, CH_3CO), 0.82 (2s, 18H, 2 x TBDMS), 0.00 (4s, 12H, 2 x TBDMS).

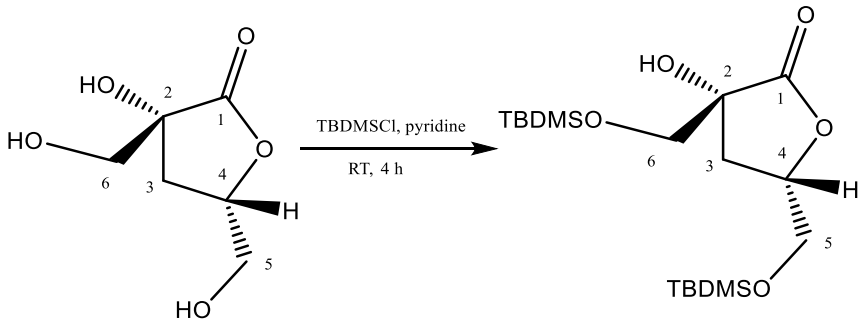
^{13}C **NMR** (100 MHz, $CDCl_3$): δ 173.70 (C1), 169.74 (CH_3CO), 80.31 (C2), 77.57 (C4), 65.36 (C6), 64.54 (C5), 31.57 (C3), 25.70, 25.63, 25.57, 18.18 & 18.16 (TBDMS), 20.43 (CH_3CO), -5.23, -5.55, -5.60 (TBDMS).

HRMS (m/z) Calculated for $C_{20}H_{40}Si_2O_6$ $[M+Na]^+$ 455.2256, found 455.2257.

FT-IR (cm^{-1}): 2954.0, 2929.3, 2857.2 (C-H), 1782.5, 1747.4 (C=O), 1471.7, 1369.1 (C-H), 1251.2, 832.4, 775.8 ($SiMe_2$), 1208.9 (C-O).

MP: 36 °C.

4.4.4.3 Preparation of 5,6-di-*O*-tert-butyldimethylsilyl-β-D-glucoisosaccharino-1,4-lactone (29) [151]



3 **29**

A mixture of dried α & β-GISAL (1:3 ratio; 1.63 g, 10.06 mmol) was dissolved in anhydrous pyridine (10 mL) and DMAP (0.5 g) was added whilst stirring for 20 min at

room temperature. Then, TBDMSCl (3.0 g, 0.02 mol, 2.2 eq) was added cautiously while stirring at room temperature. The reaction was allowed to proceed for 4 h after which time DCM (50 mL) and water (50 mL) were added. The organic layer was separated and the aqueous layer was further extracted with DCM (2 x 50 mL). The combined organic layer was washed with a 1% $CuSO_4$ solution (2 x 50 mL), dried over anhydrous sodium sulphate and concentrated to give the product mixture (2.20 g) as a semi-crystalline syrup. The target product **29** was isolated using column chromatography (180 mg; 0.46 mmol; 5 %) (Rf = 0.722; Hexane/EtOAc 4/1 v/v).

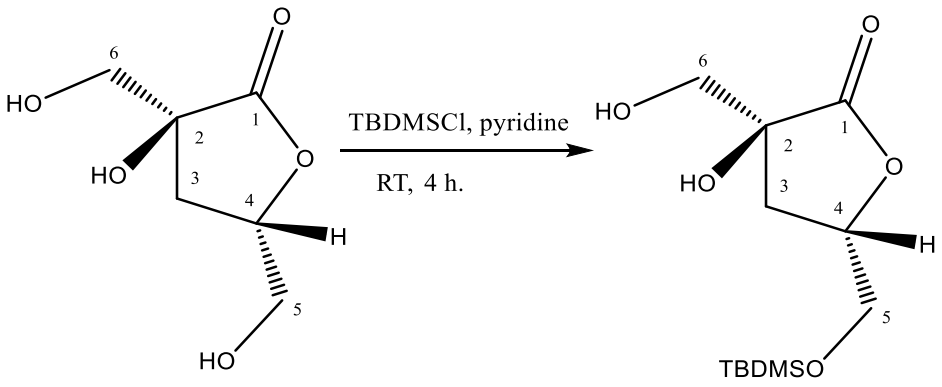
^{1}H **NMR** (400 MHz, $CDCl_3$ **29**): δ 4.56-4.54 (m, 1H, H-4), 3.94 (dd, 1H, $J_{5,4}$ = 2.84 Hz, $J_{5,5'}$ = 12.00 Hz, H-5), 3.85 (d, 1H, $J_{6,6'}$ = 9.48 Hz, H-6), 3.69 (dd, 1H, $J_{5',4}$ = 2.96 Hz, $J_{5',5}$ = 12.00 Hz, H-5'), 3.65 (d, 1H, $J_{6',6}$ = 9.48 Hz, H-6'), 2.68 (dd, 1H, $J_{3,4}$ = 8.12 Hz, $J_{3,3'}$ = 13.63 Hz, H-3), 2.14 (dd, 1H, $J_{3',4}$ = 5.28 Hz, $J_{3',3}$ = 13.63 Hz, H-3'), 0.89 & 0.86 (2s, 18H , 2 x TBDMS), 0.10, 0.09(7), 0.06 & 0.05 (4s, 12H, 2 x TBDMS).

^{13}C **NMR** (100 MHz, $CDCl_3$): δ 177.35 (C1), 76.89 (C4), 75.52 (C2), 65.78 (C6), 64.63 (C5), 34.24 (C3), 25.82, 25.76, 18.43 & 18.19 (TBDMS), -5.49, -5.53, -5.56 & -5. 63 (TBDMS).

HRMS (m/z): Calculated mass for $C_{18}H_{38}Si_2O_5$ $[M+Na]^+$ 413.2150, found 413.2154.

FT-IR (cm^{-1}): 3432.7 (O-H), 2953.8, 2929.0, & 2857.2 (C-H), 1778.3 (C=O), 1471.5, 1361.2 (C-H), 1253.6, 832.6, 775.7, 732.6 ($SiMe_2$), 1206.2, 1167.7 (C-O), 1104.2, 1005.4 (Si-OR).

4.4.4.4 Preparation of 5-*O*-tert-butyldimethylsilyl-α-D-glucoisosaccharino-1,4-lactone (30). [151]



2 **30**

Dried α-GlSAL (1.0 g 6.17 mmol) was dissolved in pyridine (5 mL) and the resulting solution was cautiously added dropwise to TBDMSCl (1.02 g, 6.79 mmol, 1.1 eq) while stirring. The reaction was allowed to proceed for 4 h at room temperature. After 4 h the contents of the flask were added to DCM (50 mL) and water (50 mL) and the two layers were separated. The aqueous layer was further extracted with DCM (2 x 50 mL) and the combined organic layer was washed with 1% $CuSO_4$, dried over anhydrous sodium sulphate and concentrated to give the desired product **30** as a crystalline syrup (780 mg, 46%; Rf: 0.35, Hexane/EtOAc 3:1 v/v).

^{1}H **NMR** (400 MHz, $CDCl_3$): δ 4.72-4.69 (m, 1H, H-4), 3.87 (dd, 1H, $J_{5,4}$ = 3.20 Hz, $J_{5,5'}$ = 11.70 Hz, H-5) 3.78 (d, 1H,$J_{6,6'}$ = 11.80 Hz, H-6), 3.69 (d, 1H, $J_{6',6}$ = 11.83 Hz, H-6'), 3.66 (dd, 1H, $J_{5',4}$ = 3.76 Hz, $J_{5',5}$ = 11.74 Hz, H-5'), 2.25-2.17 (m, 2H, H-3 & H3'), 0.85 (s, 9H, TBDMS), 0.04 & 0.03 (2s, 6H, TBDMS).

^{13}C **NMR** (100 MHz, $CDCl_3$): δ 177.76 (C1), 78.57 (C4), 75.61(C2), 65.36 (C6), 63.56 (C5), 33.31 (C3), 25.79 & 18.29 (TBDMS), -5.42 & -5.49 (TBDMS).

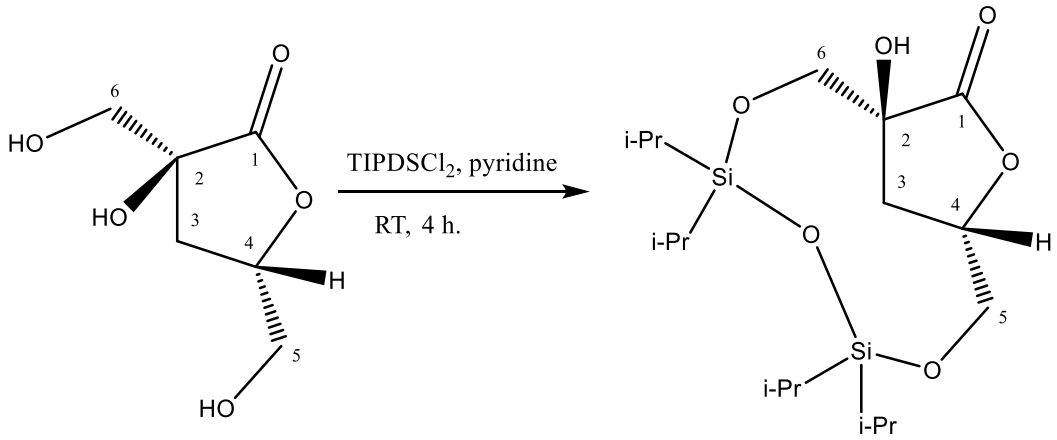
HRMS (m/z) Calculated mass for $C_{12}H_{24}SiO_5$ $[M+Na]^+$ 299.1285, found 299.1284.

FT-IR (cm^{-1}): 3407.1 (O-H), 2952.4, 2929.2, 2856.7 (C-H), 1760.7 (C=O), 1462.7, 1361.2 (C-H), 1253.7, 833.0, 775.9 ($SiMe_2$), 1201.4, 1121.5 (C-O), 1034.3 (Si-OR)

MP: 42 ºC.

4.4.5 Synthesis of triisopropylsilyl and tetraisopropyldisilyl derivatives of glucoisosaccharino-1,4-lactone.

4.4.5.1 Preparation of (1',1',3',3'-tetraisopropyl-1.3-diyl)-5,6-α-D-glucoisosaccharino-1,4-lactone (31).



2 **31**

Dried α-GISAL **2** (1.0 g 6.17 mmol) was dissolved in pyridine (6 mL) while stirring for 10 min at room temperature and then was added cautiously to 1,3-dichloro-1,1,3,3-tetraisopropyl-1,3-disiloxane (TIPDS-Cl_2; 2.17 mL; 6.78 mmol; 1.1 eq) whilst continuing to stir at room temperature. The reaction was allowed to proceed for 4 h at room temperature and was subsequently halted by the addition of DCM (60 mL) followed by water (60 mL). The organic layer was separated, and the aqueous layer was further extracted with DCM (2 x 50 mL). The combined organic layer was washed with 1% $CuSO_4$ (2 x 50 mL) and then dried over anhydrous Na_2SO_4 and concentrated to give crude **31** (4.14 g) as a pale brown crystalline syrup. The crude was purified using column chromatography (silica) to give the pure product as a white viscous liquid (2.05 g; 5.07 mmol; 82% yield; Rf: 0.68, hexane/EtOAc 4/1 v/v).

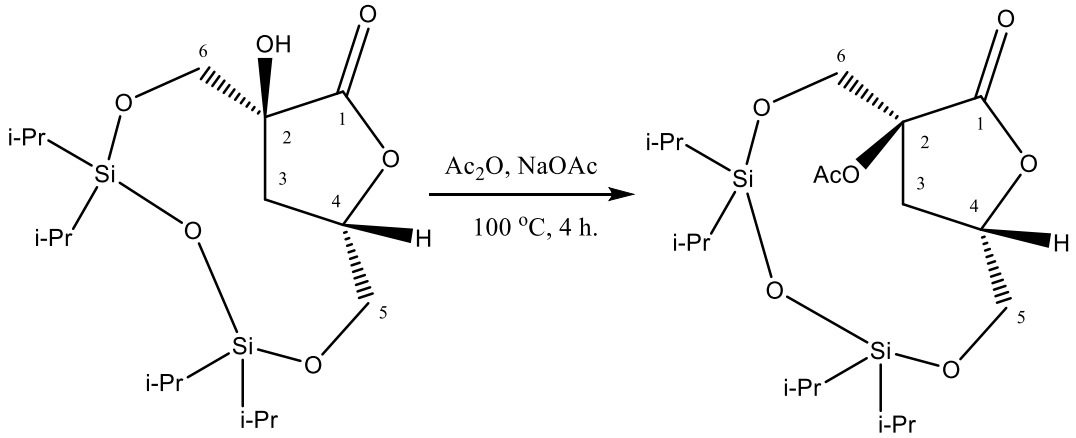
^{1}H **NMR** (400 MHz, $CDCl_3$): δ 4.87-4.66 (m, 1H, H-4), 4.08 (d, 1H, $J_{6,6'}$ = 10.72, H-6), 3.91 (d, 2H, *J*= 10.72 Hz, H-5 & 5'), 3.85 (d, 1H, $J_{6',6}$ = 10.80 Hz, H-6'), 2.84 (dd, 1H, $J_{3,4}$ = 1.88 Hz, $J_{3,3'}$ = 14.05, H-3), 2.32 (dd, 1H, $J_{3',4}$= 10.00 Hz, $J_{3'-3}$ = 14.05, H-3'), 1.05, 1.04, 1.02 & 1.00 (4s, 28H, TIPDS).

^{13}C **NMR** (100 MHz, $CDCl_3$): δ 178.2 (C1), 76.7 (C2), 76.3 (C4), 66.9 (C6), 63.6 (C5), 31.8 (C3), 17.19, 17.11, 17.09 & 17.07 (CH-TIPDS), 13.5, 13.1, 12.6 & 12.4 (CH_3-TIPDS)

HRMS (m/z): calculated mass for $C_{18}H_{36}O_6Si_2$ $[M+NH_4]^+$ 422.2389 found 422.2407

FT-IR (cm^{-1}): 2944.8, 2867.1 (C-H), 1771.1 (C=O), 1463.6, 1386.7 (C-H), 1084.4, 1041.9 (R_3Si-O-SiR_3), 1011.7 (Si-CH)

4.4.5.2 Preparation of 2-*O*-acetyl-(1',1',3',3'-tetraisopropyl-1.3-diyl)-5,6-α-D-glucoisosaccharino-1,4-lactone (32).



31 **32**

(TIPDS-1,3-diyl)-5,6-GISAL **31** (1.5 g, 3.71 mmol) was dissolved in acetic anhydride (10 mL) and a catalytic amount of sodium acetate (0.5 g) was added while stirring at room temperature. The reaction was allowed to continue uninterrupted for 4 h at 100 °C after which time the whole content of the reaction vessel was transferred into ice cold

water (100 ml) and stirred for 1 h to convert excess acetic anhydride to acetic acid. The organic product was extracted with DCM (2 x 100 mL), and the organic layer was dried over anhydrous sodium sulphate and concentrated to give the crude product as a pale brown crude semi-crystalline syrup (2.11 g). After chromatography, the product **32** was recovered as a white semi-crystalline syrup (680 mg, 1.53 mmol; 41% yield); (Rf: 0.721, Hexane/EtOAc 3:1, v/v).

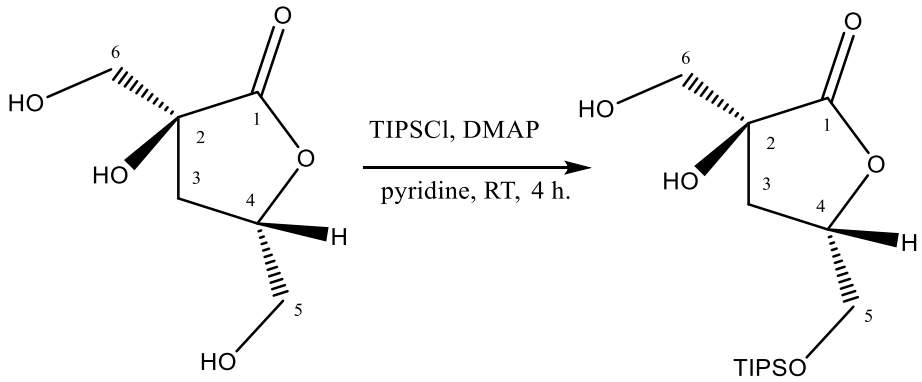
^{1}H **NMR** (400 MHz, $CDCl_3$): δ 4.82-4.78 (m, 1H, H-4), 4.09 (dd, 1H, $J_{5,4}$= 3.56 Hz, $J_{5,5'}$ = 11.78 Hz, H-5), 4.04 (d, 1H, $J_{6,6'}$ = 11.30 Hz, H-6), 4.00 (d, 1H, $J_{6,6}$ = 11.30 Hz, H-6'), 3.85 (dd, 1H, $J_{5',5}$ = 11.78, $J_{5',4}$ =2.61 Hz, H-5'), 2.75 (dd, 1H, $J_{3,4}$ =3.52 Hz, $J_{3,3'}$ = 13.91 Hz, H-3), 2.41 (dd, 1H, $J_{3',4}$= 9.56 Hz, $J_{3',3}$ = 13.91 Hz, H-3') 2.12 (s, 3H, COC**H**$_3$) 1.05, 1.02, 1.01(5) & 1.00 (4s, 28H, TIPDS).

^{13}C **NMR** (100 MHz, $CDCl_3$): δ 173.6 (C1), 170.4 (COCH_3), 81.7 (C2), 77.0 (C4), 64.7 (C5), 64.5 (C6), 30.0 (C3), 20.8 (COCH_3), 17.19, 17.15, 17.12 & 17.08 (TIPDS), 13.6, 13.5, 12.6 & 12.3 (TIPDS)

HRMS (m/z): calculated mass for $C_{20}H_{38}O_7Si_2$ $[M+NH_4]^+$ 464.2494 found 464.2503.

FT-IR (cm^{-1}): 2944.6, 2867.5 (C-H), 1779.5, 1742.1 (C=O), 1463.9, 1369.8 (C-H), 1252.1, 1215.1 (C-O), 1082.5, 1043.2 (R_3Si-O-SiR_3), 883.1 (Si-CH).

4.4.5.3 Preparation of 5-*O*-triisopropylsilyl-α-D-glucoisosaccharino-1,4-lactone (33)



2 **33**

Dried α-glucoisosaccharino-1,4-lactone **2** (500 mg, 3.09 mmol) was dissolved in pyridine (6 mL) whilst stirring for 10 min at room temperature, then DMAP (0.5 g) was added. The homogenous solution was then added cautiously to triisopropylsilyl chloride (TIPSCl; 3.0 ml; 14.02 mmol; 5.0 eq) while stirring at room temperature and the reaction was allowed to proceed for 4 h at room temperature. It was then halted with the addition of DCM (60 ml) followed by water (60 mL), the organic layer was separated and the aqueous layer was further extracted with DCM (2 x 50 mL). The combined organic layer was washed with 1% $CuSO_4$ (2 x 50 mL) and then dried over anhydrous sodium sulphate and concentrated to give crude **33** (3.56 g) as a semi-crystalline syrup which was purified using column chromatography to give a pure product as a colorless syrup (580 mg; 1.82 mmol; 59% yield) (Rf: 0.32, Hexane/EtOAc 5:1 v/v).

^{1}H **NMR** (400 MHz, $CDCl_3$): δ 4.79-4.76 (m, 1H, H-4), 4.02 (dd, 1H, $J_{5,5'}$ = 11.44, $J_{5,4}$ = 3.20 Hz, H-5), 3.85 (d, 1H, $J_{6,6'}$ = 11.84 Hz, H-6), 3.78 (dd, 1H, $J_{5',5}$ = 11.42, $J_{5',4}$ = 3.24 Hz, H-5'), 3.70 (d, 1H, $J_{6',6}$ = 11.88 Hz, H-6'), 2.24,2.25 (2 × d, 2H, J= 15.81 Hz, H-3), 1.06 & 1.04 (2s, 21H, TIPS).

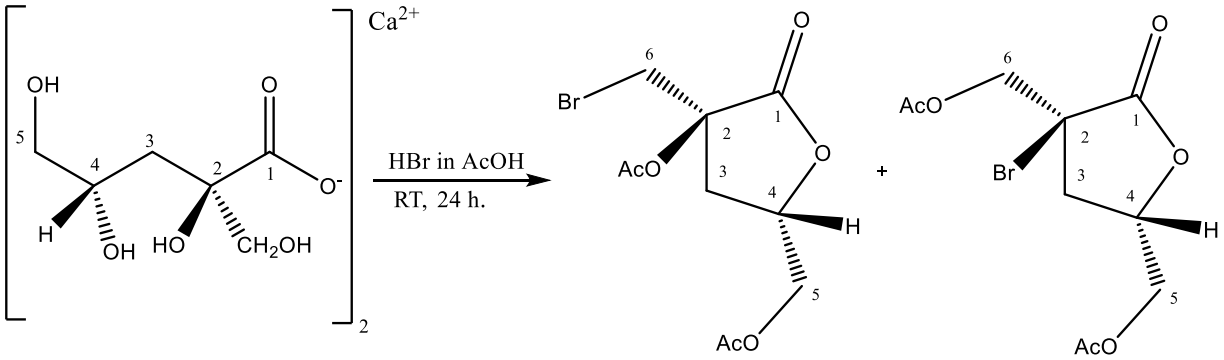
^{13}C **NMR** (100 MHz, $CDCl_3$): δ 177.9 (C1), 78.8 (C4), 65.7 (C6), 63.5 (C5), 32.9 (C3), 17.88 & 17.86 (TIPS)

HRMS (m/z) calculated mass for $C_{15}H_{30}O_5Si$ $[M+Na]^+$ 341.1755 found 341.1753.

FT-IR (**c**m^{-1}): 3374.8 (O-H), 2942.1, 2865.5 (C-H), 1763.5 (C=O), 1462.2, 1383.3, 1366.3 (C-H), 1202.3, 1128.2 (C-O), 1058.8 (Si-OR), 1012.1, 918.0 (Si-CH).

4.5 Synthesis of β-GISAL derivative from α-GISAL derivative(s) as starting material via a dihydroxylation reaction

4.5.1 Preparation of a mixture of 2, 5-di-*O*-acetyl-6-bromo-α-D-glucoisosaccharino-1, 4-lactone (33) and 5,6-di-*O*-acetyl-2-bromo-α-D-glucoisosaccharino-1,4-lactone (34) diastereoisomers (GISAL(OAc)$_2$Br) [139]



1 **34** **35**

Hydrogen bromide in acetic acid (33 % v/v, 40 mL) was added to Ca(GISA)$_2$ (5.0 g, 12.55 mmol) and the mixture was stirred at room temperature for 24 h. The reaction mixture was then diluted with $CHCl_3$ (100 mL) followed by water (100 mL). The aqueous phase was washed with $CHCl_3$ (2 x 100 mL) and then the combined organic layer was dried over sodium sulphate and concentrated to give a brown syrup of **34** and **35** in a 9:2 ratio (2.69 g; 8.71 mmol; 69 %).

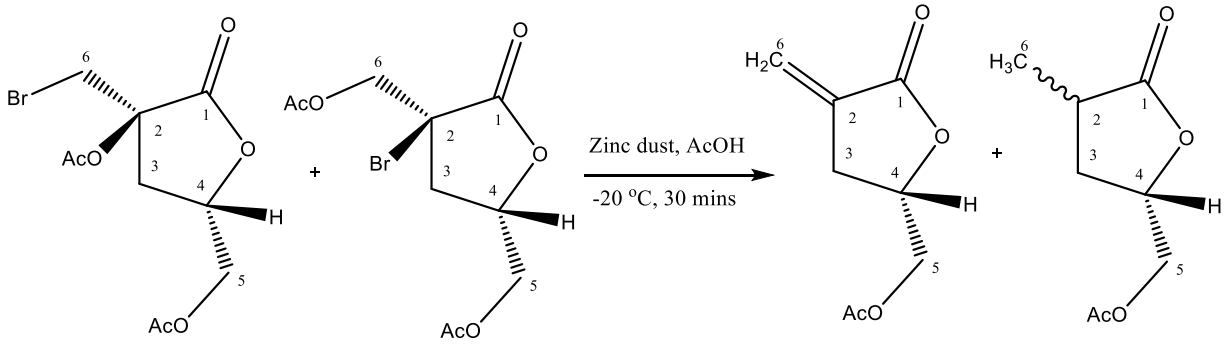
^{1}H **NMR** (400 MHz, $CDCl_3$, **34**): δ 5.02-4.96 (m, 1H, H-4), 4.31 (dd, 1H, $J_{5,4}$= 5.14 Hz, $J_{5,5'}$= 12.45 Hz, H-5), 4.18 (dd, 1H, $J_{5',4}$= 5.23 Hz, $J_{5',5}$= 12.45 Hz, H-5), 3.68 (d, 1H, $J_{6,6'}$=10.91 Hz, H-6), 3.57 (d, 1H, $J_{6',6}$= 10.91 Hz, H-6'), 2.48-2.53 (m, 2H, H-3 & H-3'), 2.11 (s, 3H, $\underline{C}H_3CO$), 2.08 (s, 3H, $\underline{C}H_3CO$),

^{13}C **NMR** (100 MHz, $CDCl_3$): δ 171.5 (C1), 170.5 ($CH_3\underline{C}O$), 170 ($CH_3\underline{C}O$), 79.2 (C2), 74.8 (C4), 64.5 (C5), 35.0 (C6), 33.4 (C3), 20.5 ($\underline{C}H_3CO$).

FT-IR (cm^{-1}): 2969.4 (C-H), 1713.5 and 1759.2 (C=O), 1370.4 (C-H), 1193 and 1045.4 (C-O), 605.2 cm^{-1} (C-Br).

^{1}H & ^{13}C NMR signal **35** were too small. The mixtures were in a 9:2 ratio.

4.5.2 Preparation of 5-*O*-acetyl-2-methylene-α-D-glucoisosaccharino-1, 4-lactone (methylene-GISAL(OAc)) (**36**) and 2(*R* & *S*)-methyl-5-*O*-acetyl-α-D-glucoisosaccharino-1,4-lactone (methyl-GISAL(OAc)) (**37**) [155, 156]



34 **35** **36** **37**

The mixture of the diastereomers **34** and **35** (2.60 g; 8.41 mmol) was dissolved in ethyl acetate (10 mL) and cooled to -20 °C, zinc dust (3 g) was suspended in 50 % aqueous acetic acid (50 mL) and the mixture was cooled to -20 °C, the reaction vessel was then covered in foil and kept stirring for 5 mins. The solution of the diastereomers was added slowly and drop-wise, to the suspended zinc solution. The reaction was allowed to proceed for 30 min at -20 °C. The suspended solid was removed by filtration under vacuum and was then washed with DCM (3 x 50 mL) and water (50 mL). The organic layer was washed with cold water (100 mL) and then with saturated aqueous sodium hydrogen carbonate (2 x 50 mL). The organic layer was then dried over anhydrous sodium sulphate, concentrated to dryness on a rotary evaporator to give the crude product as a pale brown syrup (0.96 g) as a mixture of **36** (0.58 g; 3.39 mmol; 40 %

yield; Purity: 60%) and **37**, all attempts to separate **36** & **37** failed. The yield was calculated using an estimated purity derived from inspection of the ^{1}H NMR spectrum.

^{1}H **NMR** (400 MHz, $CDCl_3$, **36**): δ 6.14 (t, 1H, $J_{6'6}$= 5.83 Hz, H-6'), 5.60 (t, 1H, $J_{6,6'}$= 5.23 Hz, H-6), 4.71-4.65 (m, 1H, H-4), 4.19 (dd, 1H, $J_{5,4}$= 5.40 Hz, $J_{5,5'}$= 12.0, H-5), 4.09 (dd, 1H, $J_{5'4}$= 3.36 Hz, $J_{5',5}$=12.0 Hz, H-5'), 2.96-3.05 (m, 1H, H-3'), 2.63-2.71 (m, 1H, H-3), 1.97 (s, 3H, $C\underline{H}_3CO$).

^{13}C **NMR** (100 MHz, $CDCl_3$, **36**): δ 170.7 ($CH_3\underline{C}O$), 169.8 (C1), 133.6 (C2), 122.9 (C6), 74.2 (C4), 65.2 (C5), 29.5 (C3), 20.7 ($\underline{C}H_3CO$).

HRMS (m/z) Calculated mass for $C_8H_{10}O_4$ $[M+H]^+$ 171.0652 found 171.0651.

FT-IR (cm^{-1}): 2970.7 (C-H), 1736.6 (C=O), 1665.3 (C=C), 1370.3 (C-H), 1224.3 and 1042.3 (C-O).

^{1}H **NMR** (400 MHz, $CDCl_3$, **37α**): δ 4.54-4.47 (m, 1H, H-4), 4.02 (dd, 1H, $J_{5,4}$= 3.28 Hz, $J_{5,5'}$= 12.21 Hz, H-5), 3.90 (dd, 1H, $J_{5,4}$= 5.32 Hz, $J_{5,5'}$=12.21 Hz, H-5'), 2.60-2.47 (m, 1H, H-2), 2.13-2.05 (m, 1H, H-3'), 1.88-1.79 (m, 1H, H-3), 1.04 (s, 3H, $C\underline{H}_3$).

^{13}C **NMR** (100 MHz, $CDCl_3$, **37α**): δ 179.5 (C1), 170.3 ($CH_3\underline{C}O$), 74.9 (C4), 65.3 (C5), 33.6 (C2), 31.7 (C3), 20.6 ($\underline{C}H_3CO$), 15.8 (C6).

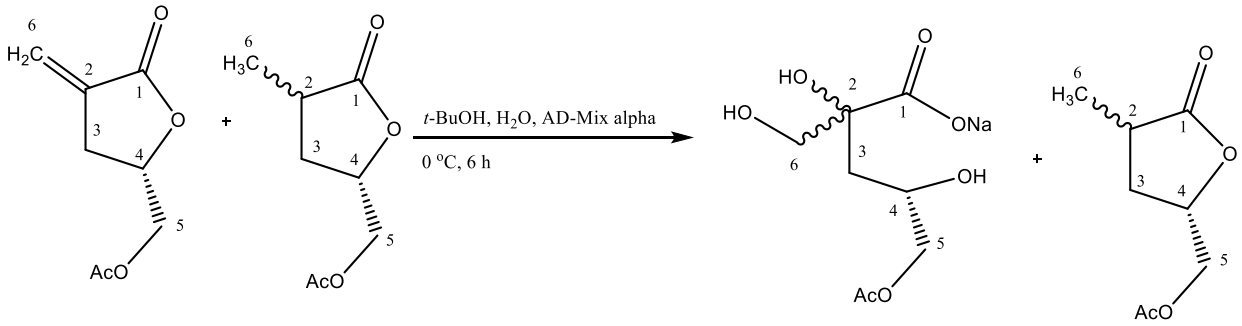
^{1}H **NMR** (400 MHz, $CDCl_3$, **37β**): δ 4.41-4.34 (m, 1H, H-4), 4.08 (dd, 1H, $J_{5,4}$= 2.89 Hz, $J_{5,5'}$=6.84 Hz, H-5), 3.85 (dd, 1H, $J_{5',4}$ = 2.28 Hz, $J_{5',5}$= 6.84 Hz, H-5'), 2.60-2.47 (m, 1H, H-2), 2.33-2.25 (m, 2H, H3 & H3'), 1.02 (s, 3H, $C\underline{H}_3$).

^{13}C (100 MHz, $CDCl_3$, **37**): δ 178.8 (C1), 170.3 ($CH_3\underline{C}O$), 75.3 (C4), 64.8 (C5), 34.9 (C2), 32.3 (C3), 20.40 ($\underline{C}H_3CO$), 14.9 (C6).

HMRS (m/z): Calculated mass for $C_8H_{12}O_4$ $[M+H]^+$ 173.0808 found 173.0809.

FT-IR (cm^{-1}): 2975.6 (C-H); 1768.5 & 1736.6 (C=O); 1455.4 & 1370.3 (C-H), 1031.3, 1167.8 & 1229.2 (C-O).

4.5.3 Preparation of 5-*O*-acetyl-β-D-glucoisosaccharino-1,4-lactone (β-GISA$_L$(OAc)) (**38**) [157]



36 **37** **38** **37α & 37β**

AD-mix-α (40 g) was dissolved in *tert*-butyl alcohol (150 mL) and water (150 mL). The mixture was stirred at room temperature until two bright yellow phases were observed. The mixture was then cooled to 0 °C, followed by the addition of the mixture of methylene-GISAL(OAc) **36** (5.0 g, 29.4 mmol) and **37** slowly and drop-wise. The progress of the reaction was monitored using GC for 6 h at 0 °C and the reaction was allowed to proceed for 17 h at room temperature while stirring vigorously until all the alkene had reacted. Sodium sulphite (45 g) was added, and the mixture was stirred at room temperature for 1 h. After 1 h, ethyl acetate (60 mL) was added to the mixture and the two layers were separated. The aqueous layer was further extracted with ethyl acetate (3 x 100 mL).

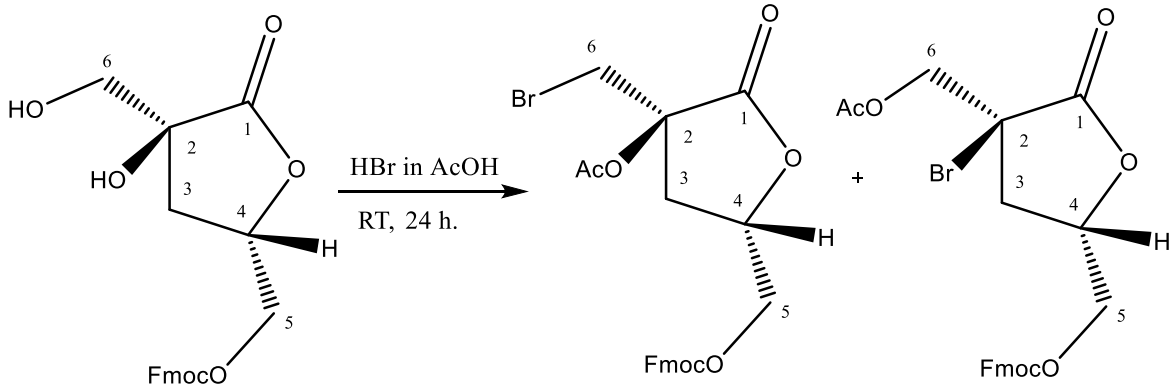
The unreacted, further-reduced product **37** was recovered from the combined organic layer which was concentrated to total dryness using a rotary evaporator to give crude a pale yellow semi-crystalline syrup (3.76 g) which was purified by column

chromatography to give a mixture of (780 mg; **37α (**62%) & **37β** (38%) pure) their purity was estimated using NMR and GC analyses.

The desired dihydroxylated product was recovered from the aqueous phase which was passed through a column (5 cm x 60 cm) filled 15% of its capacity with silica gel 63-200 µm as the stationary phase, and water (800 mL) was passed through the column. The eluents were separated into 3 fractions based on their appearance and analyses using GCMS, the fraction (3) that contained the desired product was concentrated to dryness to give a pale yellow solid (62.90 g) which included the desired products, ligands and sodium sulphite. It was then re-dissolved in water (200 mL) and extracted using ethanol (200 mL) and, under the conditions employed, the two layers which formed were separated. The ethanol layer was dried to give a crude solid **38** and salts (9.92 g). The presence of the desired product was confirmed using HPAEC-PAD to be Na(β-GISA) (20%) and Na(α-GISA).(80%).

In an attempt to optimise the enantiomeric excess of the β-GISA derivative, this reaction was repeated several times with different species of methylene lactone (**36**, **41** & **47**) (See Table 4 and discussion 2.3).

4.5.4 Preparation of a mixture of 2-O-acetyl-5-O-Fmoc-6-bromo-α-D-glucoisossacharino-1,4-lactone (**39**) and 6-O-acetyl-5-O-Fmoc-2-bromo-α-D-glucoisossacharino-1,4-lactone (GISAL (5-Fmoc) (OAc)Br) (**40**). [139]



24 **39** **40**

GISAL (5-Fmoc) **24** (1 g, 2.98 mmol) was added slowly to hydrogen bromide in acetic acid (33% v/v, 10 mL). The solution was stirred and the reaction was allowed to proceed for 24 h at room temperature. The work-up procedure was similar to that of 3.5.1 with the solvents quantities scaled appropriately. The diastereomers **39** and **40** were obtained in a 9:2 ratio as a dark reddish-brown syrup (1.30 g, 2.66 mmol, 89%).

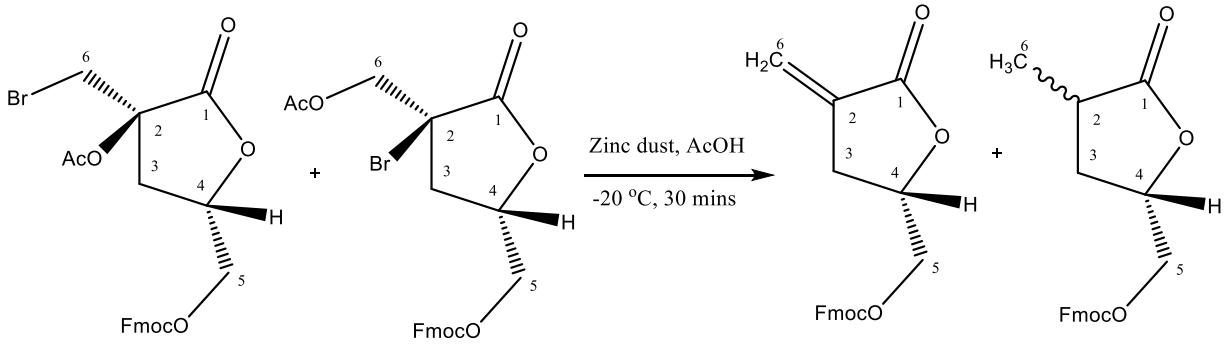
^{1}H **NMR** (400 MHz, $CDCl_3$, **39**): 7.77 (d, 2H, *J*= 7.45 Hz, ArHα), 7.68-7.57 (m, 2H, ArHδ), 7.41 (t, 2H, *J*= 3.67 Hz, ArHβ), 7.33 (t, 2H, *J*= 3.60 Hz, ArHγ), 5.02-4.97 (m, 1H, H-4), 4.52-4.19 (m, 5H, H-5, H-5', H-8, H-8' & H-9), 3.68 (d, 2H, *J*= 5.36 Hz, H-6 & H-6'), 2.57 (d, 2H, $J_{3,3}$= 6.81 Hz, H-3 & H-3'), 2.14 (s, 3H, CH_3CO).

^{13}C **NMR** (100 MHz, $CDCl_3$): 171.0 (C1), 169.9 ($CH_3\underline{C}O$), 154.9 (Fmoc $\underline{C}O$), 144.1 & 141.3 (ArCq), 128.0 (ArCα), 127.3 (ArCδ) 125.2 (ArCβ), 120.3 (ArCγ), 79.2 (C2), 74.4 (C4), 70.3 (Fmoc $\underline{C}H_2$'s), 67.6 (C5), 45.6 (Fmoc $\underline{C}H$'s), 35.2 (C6), 33.4 (C3), 20.6 ($\underline{C}H_3CO$).

HRMS (m/z) Calculated mass for $C_{23}H_{21}BrO_7$ $[M+NH_4]^+$ 506.0809 found 506.0824.

FT-IR (cm^{-1}): 3020 (C-H), 1785, 1744 (C=O), 1259, 1215 (C-O), 660.9 (C-Br)

4.5.5 Preparation of 5-*O*-Fmoc-2-methylene-α-D-glucoisossacharino-1, 4-lactone (methylene-GISA$_L$(5-Fmoc)) (**41**) and 5-*O*-Fmoc-2-methyl-α-D-glucoisossacharino-1,4-lactone. (methyl-GISAL(5-Fmoc)) (**42**) [155, 156]



39 **40** **41** **42**

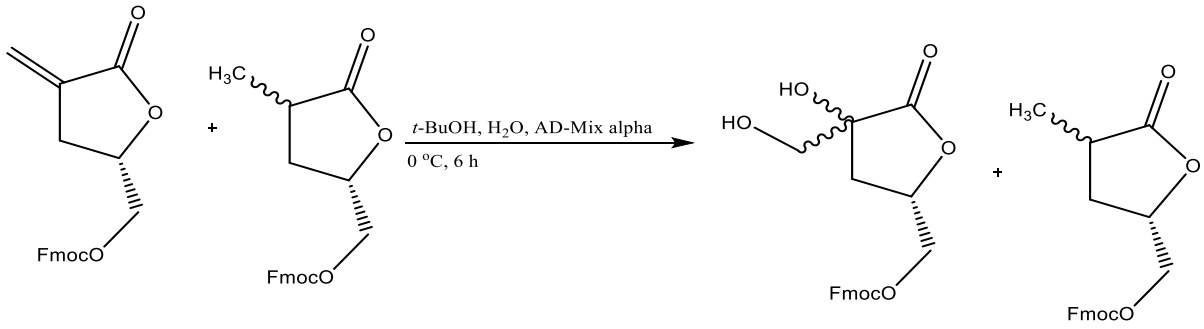
The mixture of GISAL(5-Fmoc)OAcBr diastereoisomers **39** and **40** (1.10 g, 2.99 mmol) were dissolved in a minimum amount of ethyl acetate and zinc dust (1.5 g) was suspended in 50% aqueous acetic acid (30 mL) in a separate flask. The two solutions were cooled to -20 ° C and the stereoisomers (**39** & **40**) was added to the suspended zinc solution cautiously and drop-wise. The work-up procedure was similar to that of 3.5.2, with solvent quantities scaled appropriately. The organic extract was dried over sodium sulphate and concentrated under reduced pressure, to give a crude syrup mixture of **41** and **42** (190 mg; **41** (55%), **42** (31%)).

^{1}H **NMR** (400 MHz, $CDCl_3$, **41**): 7.77 (d, 2H, *J*= 7.52 Hz, ArHα), 7.63-7.58 (m, 2H, ArHδ), 7.42 (t, 2H, *J*= 7.44 Hz, ArHβ), 7.33 (t, 2H, *J*= 7.44 Hz, ArHγ), 4.82-4.76 (m, 1H, H-4), 4.45-4.38 (m, 5H, H-5, H-5', H-8, H-8' & H-9), 3.11-3.04 (m, 1H, H-3), 2.83-2.80 (m, 1H, H-3')

^{13}C **NMR** (100 MHz, $CDCl_3$): 169.6 (C1), 154.9 (Fmoc CO), 143.1 & 141.3 (ArCq), 133.2 (C2), 128.0 (ArCα), 127.3 (ArCδ) 125.2 (ArCβ), 123.2 (C6), 120.5 (ArCγ), 73.8 (C4), 70.3 (Fmoc CH_2's), 68.4 (C5), 47.0 (Fmoc CH's), 29.8 (C3).

HRMS (m/z) Calculated mass for $C_{21}H_{18}O_5$ $[M+NH_4]^+$ 368.1492 found 368.1497.

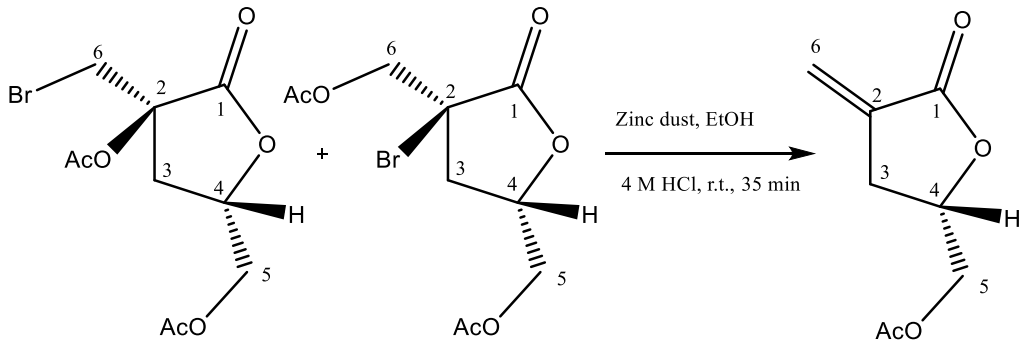
4.5.6 Preparation of 5-*O*-Fmoc-β-D-glucoisosaccharino-1,4-lactone (**38**) [157]



41 **42** **43** **44**

AD-mix-α (1.4 g) was dissolved in *tert*-butyl alcohol (5 mL) and water (5 mL). The mixture was stirred at room temperature until two bright yellow phases were observed. The mixture was then cooled to 0 ºC, followed by the addition of the mixture **41** & **42** (190 mg) slowly and drop-wise. The reaction was allowed to proceed for 6 h at 0º C, then, continued for 17 h at room temperature while stirring vigorously. Sodium sulphite (1.5 g) was added, and the mixture stirred at room temperature for an hour. Ethyl acetate (25 mL) was added to the mixture and the two layers separated. The aqueous layer was further extracted with ethyl acetate (2 x 25 mL). The combined organic layer was dried over sodium sulphate and concentrated to dryness to give a complex mixture of products (80 mg). Attempts to separate the mixture using column chromatography after a lactonisation step (See 4.3.2) were not successful.

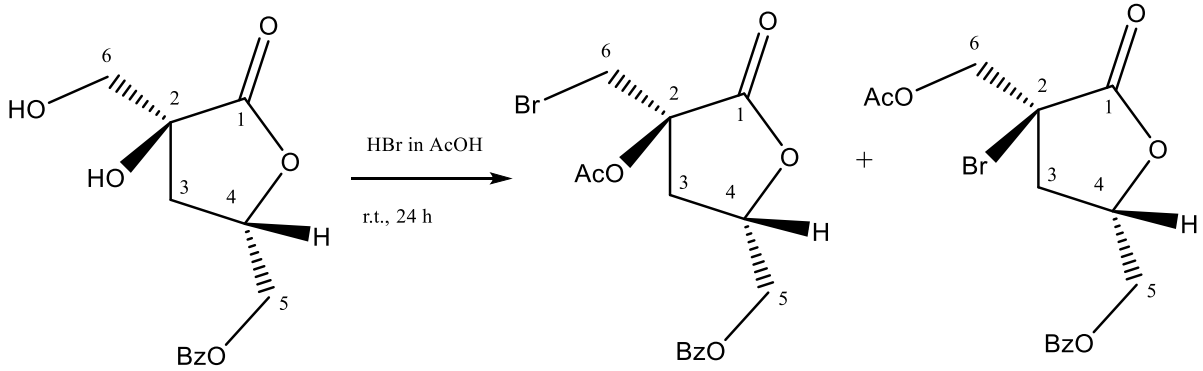
4.5.7 Preparation of 2-methylene-α-D-glucoisosaccharino-1, 4-lactone. (**36**) [139]



33 **34** **36**

The mixture of **33** & **34** (4 g, 13.44 mmol) was dissolved in absolute ethanol (50 mL) and zinc dust (16 g) was added gently while stirring at room temperature. The mixture was allowed to stir for 30 mins before the zinc was filtered under gravity, then 4 M HCl (5 mL) was added to the filtrate drop-wise and the mixture was further stirred for 5 min at room temperature before the filtrate was concentrated to total dryness to give the crude syrup (8.63 g) which contains **36** (6.82 g, 40.11 mmol, 34% yield) of the desired product with no further reduced product.

4.5.8 Preparation of 2-*O*-acetyl-5-*O*-benzoyl-6-bromo-α-D-glucoisosaccharino-1,4-lactone (**43**) and 6-*O*-acetyl-5-*O*-benzoyl-2-bromo-α-D-glucoisosaccharino-1,4-lactone. (**44**) [139]



13 **45** **46**

Hydrogen bromide in acetic acid (33 % v/v, 40 mL) was added cautiously to a 60/40 mixture of 5 & 6-benzoyl-α-GISAL (3.0 g; 1.8 g, 6.77 mmol) and the mixture was stirred

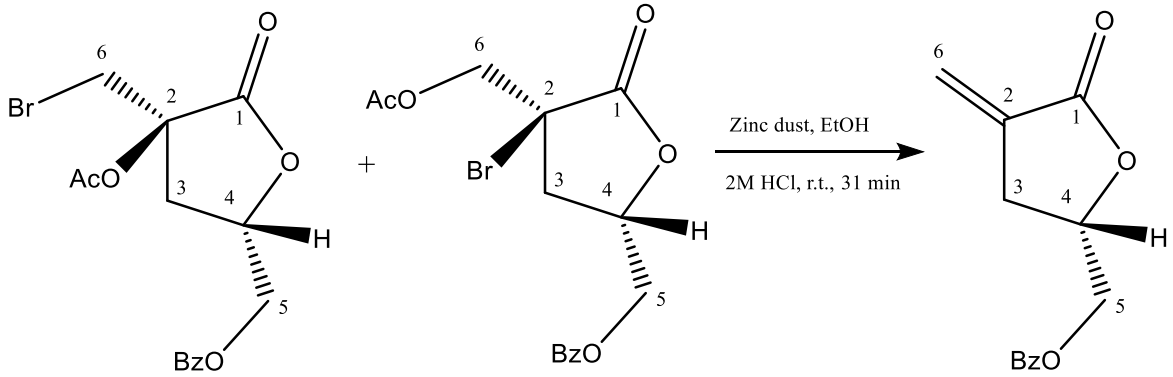
at room temperature for 24 h. The reaction mixture was then diluted with $CHCl_3$ (50 mL) followed by water (50 mL). The aqueous phase was washed with $CHCl_3$ (2 x 100 mL) and then, the combined organic layer was dried over sodium sulphate and concentrated to give a brown syrup of **45** and **46** (5.93 g; 20.38 mmol; 33 % yield)

^{1}H **NMR** (400 MHz, $CDCl_3$, **45**): 8.02 (d, 2H, *J* = 7.12 Hz, ArHβ), 7.57-7.53 (m, 1H, ArHδ), 7.44-7.41 (m, 2H, ArHγ), 5.16-5.11 (m, 1H, H-4), 4.62 (dd, 1H, $J_{5,4}$ = 3.16 Hz, $J_{5,5'}$ = 9.88 Hz, H-5), 4.46 (dd, 1H, $J_{5',4}$ = 5.84 Hz, $J_{5',5}$ = 9.88 Hz, H-5'), 3.69 (d, 1H, $J_{6,6'}$ = 10.76 Hz, H-6), 3.55 (d, 1H, $J_{6',6}$ = 10.76 Hz, H-6'), 2.59 (d, 2H, *J*= 7.84 Hz, H-3 & 3'), 2.07 (s, 3H, C$\underline{H}_3$CO).

^{13}C **NMR** (100 MHz, $CDCl_3$):171.5 (C1), 170.1 ($CH_3\underline{CO}$), 166.39 (Ph$\underline{CO}$), 133.5 (ArCδ), 129.7 (ArCβ), 129.3 (ArCq), 128.6 (ArCγ), 79.2 (C2), 75.2 (C4), 64.8 (C5), 35.1 (C6), 33.7 (C3), 20.8 ($\underline{C}H_3CO$).

HRMS (m/z) Calculated mass for $C_{15}H_{15}BrO_6$ $[M+NH_4]^+$ 388.0390, found 388.0398.

4.5.9 Preparation of 5-*O*-benzoyl-2-methylene-α-D-glucoisosaccharino-1, 4-lactone. (**47**) [139]



45 **46** **47**

The mixture of **45** & **46** (2.93 g, 10.07 mmol) was dissolved in absolute ethanol (30 mL) and zinc dust (20 g) was added gently while stirring at room temperature. The mixture was allowed to stir for 30 min before the zinc was filtered under gravity, then 2 M HCl (3

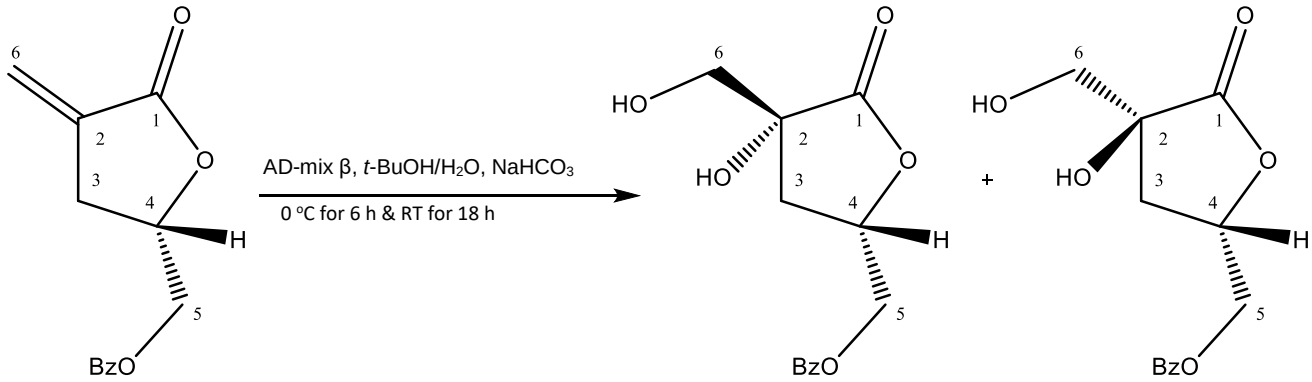
mL) was added to the filtrate drop-wise and the reaction mixture was stirred for an additional 1 min at room temperature before the filtrate was concentrated to total dryness to give (3.64 g) of a crude syrup (**47**; 2.08 g, 8.97 mmol, 89% yield).

^{1}H **NMR** (400 MHz, $CDCl_3$): δ 7.97 (d, 2H, *J* = 7.24 Hz, ArHβ), 7.59-7.54 (m, 1H, ArHδ), 7.47-7.40 (m, 2H, ArHγ), 6.29 (d, 1H, $J_{6',6}$= 5.76 Hz, H-6), 5.70 (d, 1H, $J_{6,6'}$= 5.76 Hz, H-6'), 4.94-4.92 (m, 1H, H-4), 4.53 (dd, 1H, $J_{5,4}$= 3.12 Hz, $J_{5,5'}$= 12.22, H-5), 4.45 (dd, 1H, $J_{5'4}$= 4.80 Hz, $J_{5',5}$=12.22 Hz, H-5'), 3.18-3.11 (m, 1H, H-3), 2.90-2.83 (m, 1H, H-3').

^{13}C **NMR** (100 MHz, $CDCl_3$): δ 170.3 (C1), 166.3 (PhCO), 133.5 (ArCδ) 133.4(7) (C2), 129.8 (ArCβ), 129.1 (ArCq), 128.5 (ArCγ), 123.0 (C6), 74.4 (C4), 65.8 (C5), 29.7 (C3).

HRMS (m/z) Calculated mass for $C_{13}H_{12}O_4$ $[M+NH_4]^+$ 250.1074, found 250.1080.

4.5.10 Preparation of 5-*O*-benzoyl-β-D-glucoisosaccharino-1, 4-lactone. (**48**) [142]



47 **48** **13**

AD-mix-α (12.6 g) was dissolved in *tert*-butyl alcohol (50 mL) and water (50 mL). The mixture was stirred at room temperature until two bright yellow phases were observed. After the addition of $NaHCO_3$ (3.69) the mixture was cooled to 0° C and **47** (2.08 g, 8.97 mmol) was added slowly and drop-wise. The reaction was allowed to proceed for 6 h at 0° C, then, continued for 17 h at room temperature while stirring vigorously. Sodium sulphite (5.3 g) was added, and the mixture was stirred at room temperature for 1 h.

Ethyl acetate (100 mL) was added to the mixture and the two layers were separated. The aqueous layer was further extracted with ethyl acetate (2 x 100 mL). The combined organic layer was dried over sodium sulphate and concentrated to dryness to give a mixture of four products (780 mg), which after chromatography gave a mixture of products (170 mg; 5-benzoyl-α-GISAL (19%) **13**, 5-benzoyl-β-GISAL (44%) **48** and (37%) of opened ring compounds **48** & **13**. The yield was calculated using a measure of purity derived from inspection of the ^{1}H NMR spectrum.

Publication

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Appendix

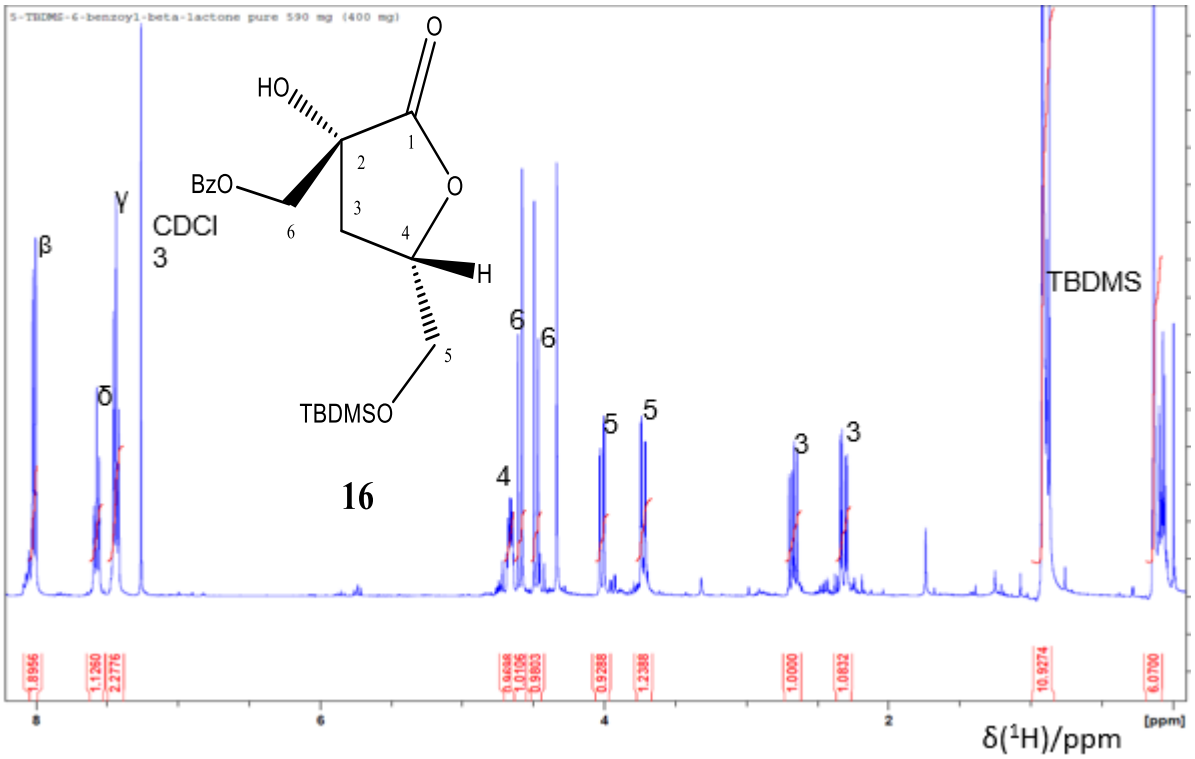

Figure A: ^{1}H NMR spectrum 6-*O*-benzoyl-5-*O*-TBDMS-β-GISAL **16**.

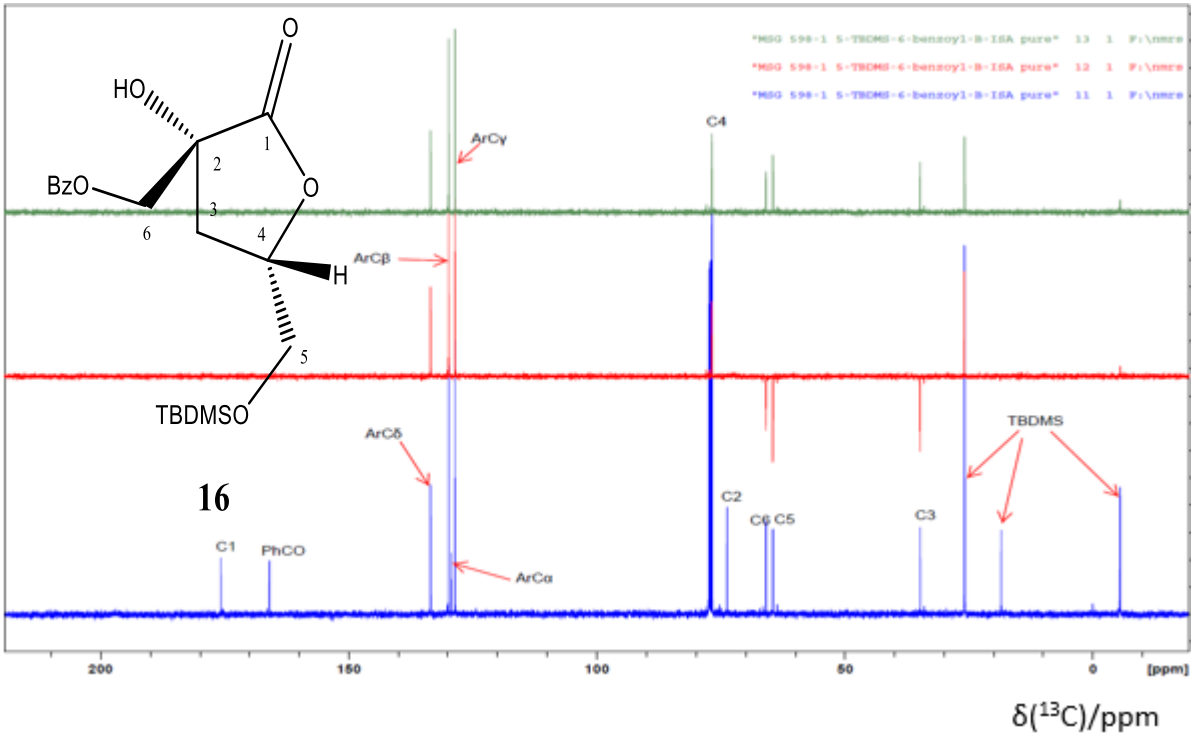

Figure B: ^{13}C NMR spectra of 6-*O*-benzoyl-5-*O*-TBDMS-β-GISAL **16**

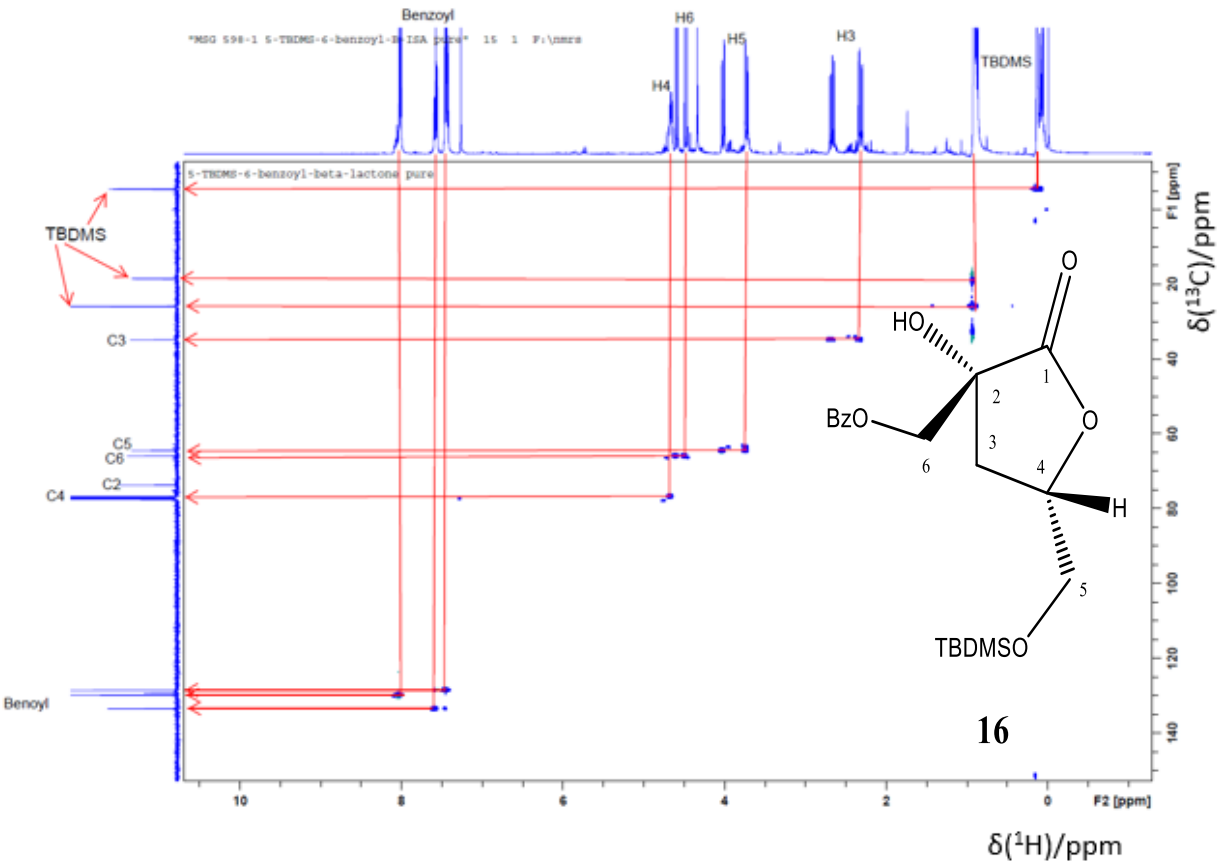

Figure C: HSQC spectrum of 6-*O*-benzoyl-5-*O*-TBDMS-β-GISAL **16**

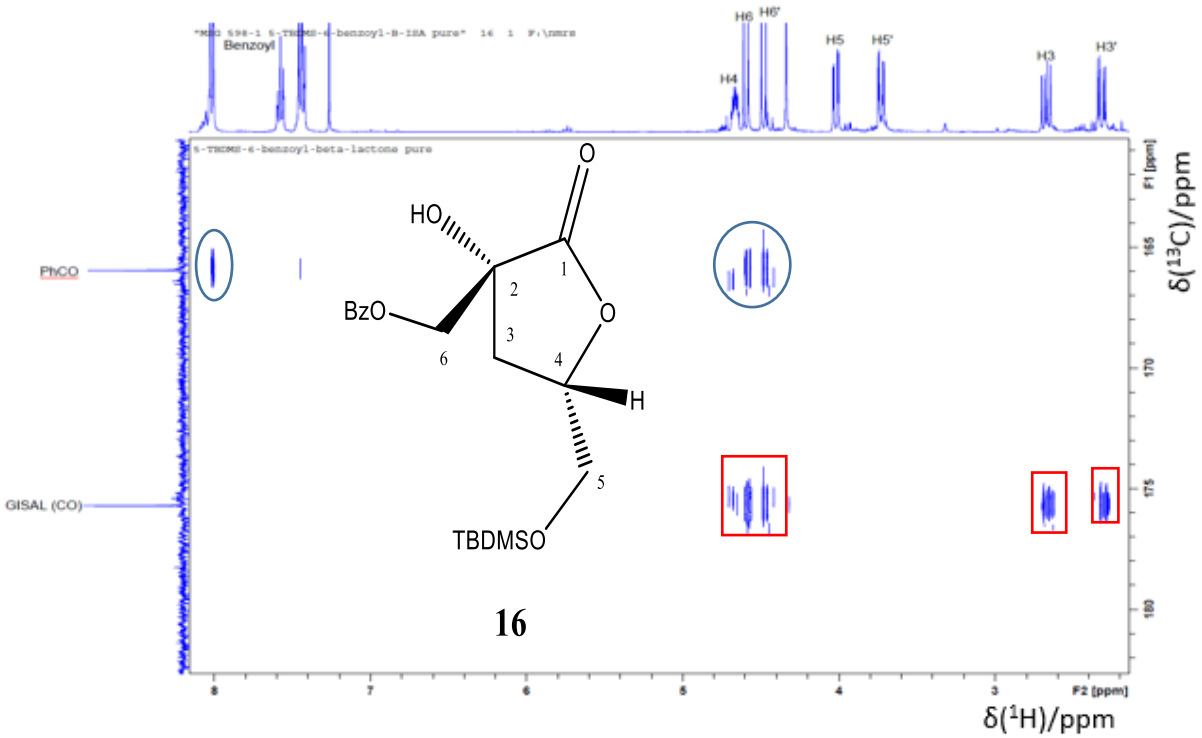

Figure D: HMBC spectrum of 6-*O*-benzoyl-5-*O*-TBDMS-β-GISAL **16**

Expansion 360 – 465 m/z:

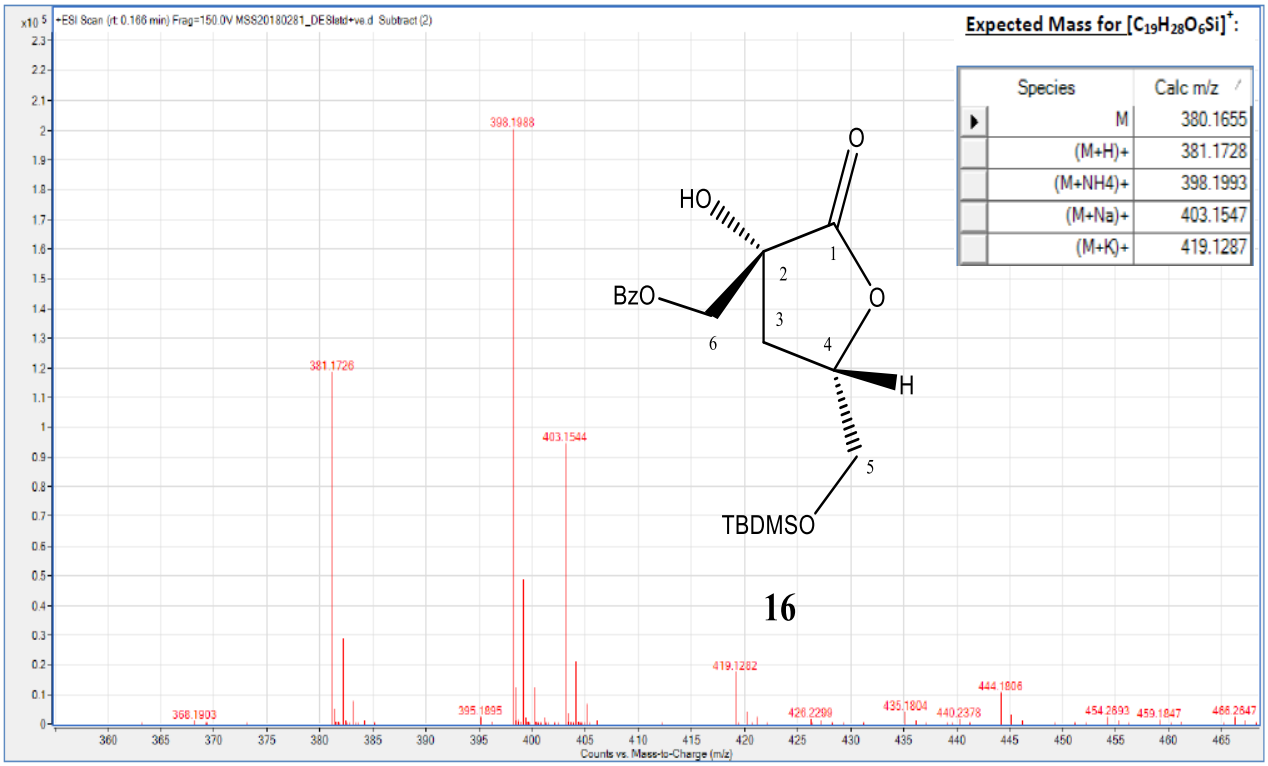

Figure E: HRMS spectrum of 6-*O*-benzoyl-5-*O*-TBDMS-β-GISAL **16**

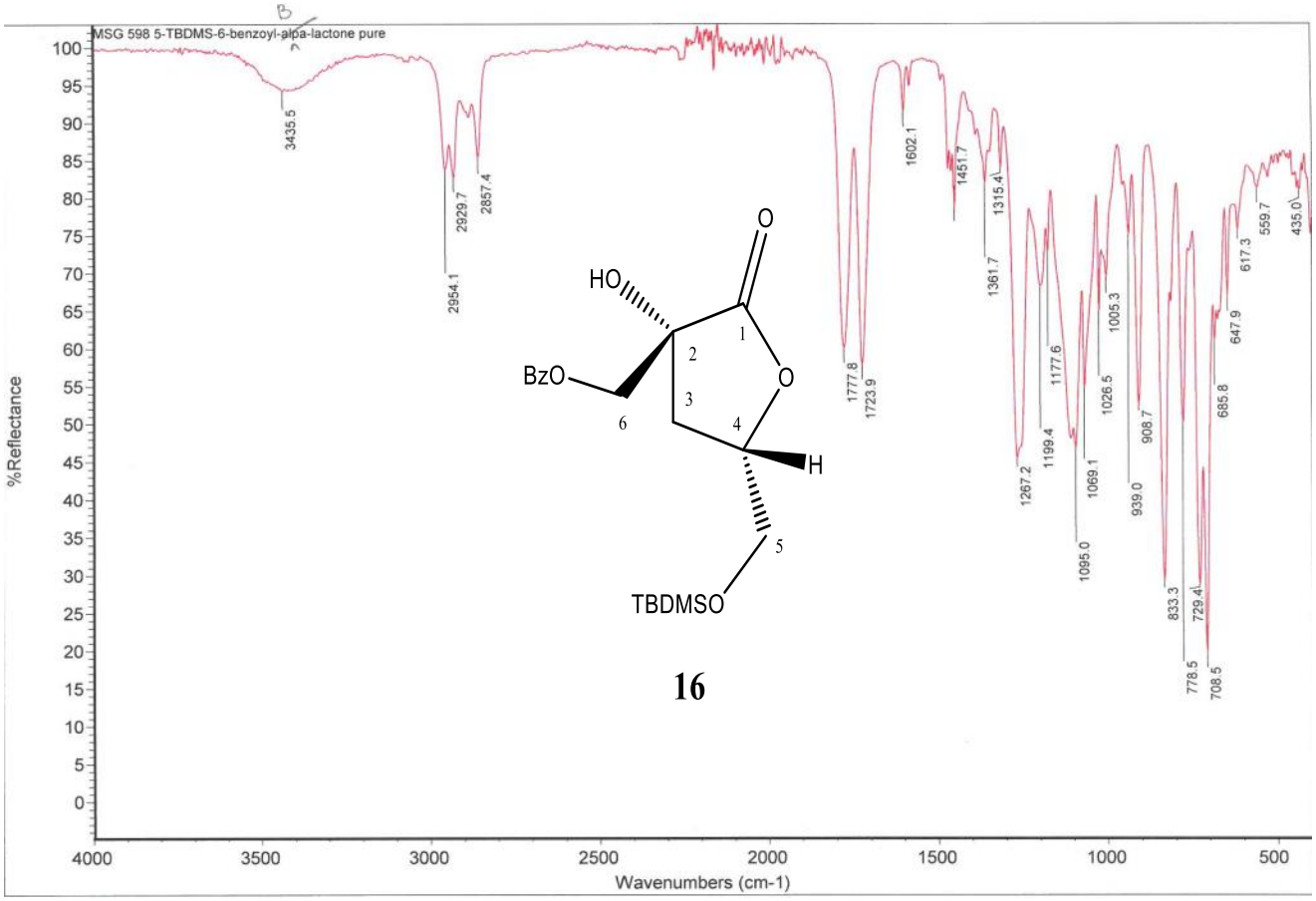

Figure F: IR spectrum of 6-*O*-benzoyl-5-*O*-TBDMS-β-GISAL 16