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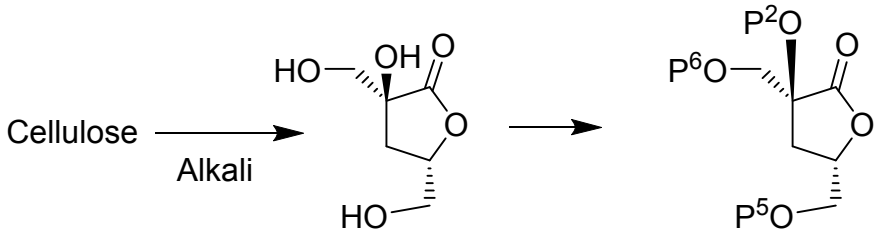
Graphical Abstract



Saccharinic acid

P2/P5/P6= H or Protecting Group

Developing Cellulosic Waste Products as Platform Chemicals: Protecting Group Chemistry of α-Glucoisosaccharinic Acid.

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Abstract

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) which are produced when cellulosic materials are treated with aqueous alkali are potentially valuable platform chemicals. Their highly functionalised carbon skeleton, with fixed chirality at C-2 and C-4, makes them ideal starting materials for use in synthesis. In order to assess the potential of these saccharinic acids as platform chemicals we have explored the protecting group chemistry of the lactone form of alpha-glucoisosaccharinic acid (α-GISAL). We report here the use of single and multiple step reaction pathways leading to the regioselective protection of the three different hydroxyl groups of α-GISAL. We report strategies for protecting the three different hydroxyl groups individually or in pairs. We also report the synthesis of a range of tri-O-protected α-GISAL derivatives where a number of the products contain orthogonal protecting groups.

Key words:

Saccharinic acids; Isosaccharinic acid; Glucoisosaccharinic acid; protecting groups.

1. Introduction

Saccharinic acids[1, 2] are a group of branched-chain polyhydroxyl acids which are generated in large quantities when cellulosic materials are treated with aqueous alkali[3]. The mechanism for saccharinic acid production has been studied in detail and the base catalysed depolymerisation of cellulose is known to proceed via a 'peeling' reaction[4, 5] [6-8]. Depending on the reaction conditions (type of alkali, length of reaction and temperature) a large number of different hydroxy acids can be formed but the main saccharinic acids formed from cellulose, accounting for up to 80% of the total organic matter, are a pair of C-2 epimeric six carbon glucoisosaccharinic acids (GISA) [9-11]. Whistler and Bemiller have reported that the calcium salt of the 2*S*-epimer, alpha-glucoisosaccharinic acid (α-GISA **(1)**; (2*S*,4*S*)-2,4,5-trihydroxy-2-(hydroxymethyl)pentanoic acid) can be economically manufactured by heating lactose with a saturated aqueous calcium hydroxide solution[12]; on cooling, the 2*S*-epimer precipitates whilst the 2*R*-epimer and other impurities remain in solution. The salts of α-GISA are highly polar and have limited solubility in most organic solvents. However, in the presence of mild acids α-GISA **(1)** undergoes an internal esterification reaction to give the less polar α-glucoisosaccharino-1,4-lactone (α-GISAL **(2)**):

Scheme 1.

Despite the ease of preparation of α-GISA **(1)** and its ready conversion to its less polar lactone **(2)** the two have rarely been exploited as starting materials in synthesis. Florent *et al[13]* and Monneret et al [14] have incorporated α-GISA **(1)** into the synthesis of a range of anthracycline analogues. Monneret *et al* have incorporated α-GISA **(1)** into the synthesis of nucleoside analogues with antiviral or antitumor activity[15]. Hanessian and

Roy have utilised α-GISA **(1)** in the synthesis of the antibiotic spectinomycin[16]. Thomassigny *et al* have incorporated α-GISA **(1)** into the synthesis of a small number of heterocycles including variously protected pyrrolidines[17] and piperidines[18].

It has been estimated that many millions of metric tons of saccharinic acids are produced each year as by products in the alkaline pulping of wood[19-22]. Currently, this large reservoir of potentially valuable organic molecules is combusted within pulping mills to recover their calorific value. Ideally, wood pulping companies would like to be able to extract extra value from these saccharinic acids and one way this could be achieved is by employing them as staring materials in synthetic chemistry. For this ambition to be realised and to determine the true synthetic utility of GISAs it will be necessary to develop strategies for the regioselective protection of the different hydroxyl groups, either individually or in groups. In this paper we report our studies of the protecting group chemistry of α-GISAL (**2**)**,** including the regioselective protection of different combinations of the three hydroxyl groups.

It should be noted that whilst the gluco-prefix identifies GISAs as being derived from a 1,4-glucan such as cellulose , in the early scientific literature and also in current literature describing environmental aspects of GISA's properties[23-26] these molecules are frequently referred to as isosaccharinic acids (ISA).

2. Results and Discussion

2.1 Preparation of 2,5,6-tri-*O*-protected-α-GISALs in a single step procedure.

In the first set of experiments, attempts were made to protect all three hydroxyls of GISA as ester derivatives (Fig. 1, 3a-5a). We have previously reported the synthesis of the tribenzoyl-ester of α-GISAL **(2)** which was achieved by reaction of α-GISAL **(2)** with a large excess of benzoyl chloride with pyridine as solvent and employing dimethylaminopyridine as an acyl-transfer catalyst[27]. When an acetylation reaction was performed with an excess of acetic anhydride with sodium acetate as a base a near quantitative yield of the 2,5,6-tri-*O*-acetyl-α-$GISA_L$ (**3a,** 99%) was recovered. However, when an attempt was made to reduce the quantity of the bulkier acylating reagents to nearer stoichiometric amounts (3.3 equivalents) a mixture of di and triacylated products was obtained. The trisubstituted derivative **4a** could only be produced as a single compound when a large excess of benzoyl chloride was used (10 equivalents).

Figure 1.

A similar picture emerged with the attempted synthesis of sulfonate esters. Reaction of **2** with six equivalents of methanesulfonyl chloride in the presence of pyridine gave the trimesylated product **5a** in reasonable yield (61%). In contrast, when **2** was reacted with a large excess of *p*-toluenesulfonyl chloride a crude product was isolated which, after column chromatography, gave the 5,6-di-*O*-tosylated derivative **6b** (55%) and only a small amount (<10%) of the desired 2,5,6-trisubstituted α-GISAL was produced. Further attempts to form triprotected derivatives of **2,** as either benzyl, trityl or silyl ethers, all led to the isolation of 5,6-di-*O*-protected derivatives (see section 2.2).

It is clear that derivatisation of all three hydroxyl groups in a single step procedure was only possible when using either forcing conditions (large excess of reagent), or when small

sterically undemanding protecting groups (acetyl or mesyl) were employed. It is of note that Kumar and Alen have reported the synthesis of mixtures of mono and di-esters in the of a-glucoisosaccharino-1,4-lactone with tall oil fatty acids[28].

2.2. Preparation of 5,6-di-*O*-protected-α-GISALs in single step procedures.

It was expected that the greater reactivity of the hydroxymethylene groups compared with that of the tertiary alcohol in **2** would allow direct access to the 5,6-di-*O*-protected-α-GISAL derivatives. Reaction of the lactone with two equivalents of acetyl chloride in pyridine and also the reaction of the lactone with two equivalents of *p*-toluenesulphonyl chloride in pyridine produced the desired 5,6-di-*O*-protected lactones **3b** (63%) **& 6b** (55%) in reasonable yields. Reaction of the lactone with the larger trityl chloride generated a mixture of di-*O*-protected and mono-O-protected products which were easy to separate by column chromatography to give a very low yield of the desired 5,6-di-*O*-trityl-α-GISAL **7b** (13%), a similar amount of the 5-mono-*O*-trityl-α-GISAL **7e** (12%) and a very small amount of the 6-mono-*O*-trityl-α-GISAL **7f** (<2%).

Attempts to prepare the 5,6-di-*O*-benzylated derivative **8b** using sodium hydride as a base in DMF failed and only ring opened lactone products were obtained. Giordano and Iadonisi[29] have recently reported the regioselective benzylation of primary alcohols in carbohydrate based polyols using a combination of benzyl bromide and the base diisopropylethylamine in the presence of a di-*tert*-butyltin oxide catalyst. When the reaction was applied to the lactone **2** a reasonable yield of the desired 5,6-di-*O*-benzylated product **8b** (59%) was recovered.

Reaction of **2** with an excess of TBDMSCl in pyridine gave, after column chromatography, 5,6-di-*O*-TBDMS-α-GISAL **9b** as the major product (69%). In a similar reaction, treatment of the lactone with TIPDSCl in pyridine afforded a high yield (82%) of the 5,6-TIPDS-α-GISAL (**14**) in which the protecting group bridges between the 5 and 6-positions. The 5,6-

arrangement of the protecting group was confirmed by acetylating the remaining hydroxyl group and identifying strong NOE contacts between the protons of the isopropyl groups and the methylene protons at 5 and 6 in the acetylated product (**15**).

Scheme 2.

In order to expand the range of protecting groups, an attempt was made to introduce acid stable carbonates at the 5 and 6-positions. Gioeli and Chattopadhyaya[30] have reported the use of the FMOC-carbonate group to protect the hydroxyl groups of ribose, however, when the lactone **2** was reacted with a large excess of FMOCCl, either in the presence or absence of an acyl transfer catalyst, a mixture of di-protected and mono-protected products were obtained. Despite using longer reaction times and up to ten equivalents of the 9-fluorenylmethoxycarbonyl chloride, the maximum yield of the desired di-protected product **10b** never exceeded 27%. From these studies, it was clear that the reaction had reached equilibrium in which the diprotected, monoprotected and unreacted FMOCCl were all present. As was the case with trityl-*O*-protection, pure samples of the desired 5,6-di-*O*-FMOC-α-GISAL**10b**, the 5-mono-*O*-protected **10e** and small amounts of the 6-mono-O-protected-α-GISAL **10f** were isolated by column chromatography.

2.3. Preparation of 2,6-di-*O*-protected-α-GISALs in single step procedures.

The combined protection of the primary alcohol at the 6-position and the tertiary alcohol at the 2-position using an isopropylidene group has previously been reported by Florent et al[13]. In a similar reaction, the lactone **2** was condensed with freshly distilled benzaldehyde in the presence of an acid catalyst to give the 2,6-*O*-benzylidene protected lactone **12b** (78%) as a pair of diastereoisomers in a 1:3.5 ratio (7*R*:7*S*; scheme 2). Reaction of the 2,6-acetal protected substrates with either FMOCCl or benzoyl chloride in pyridine provided mixtures of starting materials and products, with only moderate yields of the desired products being obtained after column chromatography (**11c** 14% and **12c**

20%). The low yields are consistent with steric crowding reducing access to tri-O-protected products, especially when bulky protecting groups are employed.

Scheme 3,

2.4 Preparation of 2,5,6-tri-*O*-protected-α-GISALs in two step procedures.

The ease of formation of the 5,6-di-*O*-protected-α-GISALs (**6b-10b**) provided an opportunity to introduce orthogonal protection at the tertiary hydroxyl groups albeit with the requirement for the use of a small protecting group. Both the 5,6-di-*O*-dibenzyl-α-GISAL **8b** and the 5,6-*O*-diTBDMS-α-GISALs **9b** were converted in variable but not optimised yields to their 2-*O*-acetyl-5,6-di-*O*-protected-α-GISALs (**8c** 30%**, 9c** 80%) on reaction with acetic anhydride using sodium acetate as a base catalyst (Fig 2; reagents a). In a similar manner, treatment of the 5,6-*O*-diFMOC-α-GISAL **10b** with acetic anhydride in the presence of zinc dichloride afforded the 2-*O*-acetyl-5,6-di-*O*-protected-α-GISAL **10c** (Fig. 2; reagents b, 55%).

Scheme 4.

Reaction of the 2,6-*O*-isopropyliene-α-GISALs **11b** with FMOCCl provided the opportunity to place orthogonal protecting groups onto the primary alcohols, 5-OH versus 6-OH, and gave the 2,6-*O*-isopropyliene-5-*O*-FMOC-α-GISAL **11c** but in low yield (14%). In a similar reaction, treatment of **12b** with benzoyl chloride in pyridine gave the 2,6-*O*-benzilydene-5-*O*-benzoyl-α-GISALs **12c** also in low yield (20%).

2.5 Preparation of the mono-*O*-protected α-GISAL derivatives.

Figure 2.

In most cases, attempts to directly add a single protecting group to the lactone **2** did not give single products: the similar reactivity of the two primary hydroxyls meant that in the

majority of cases mixtures of the 5,6-di-*O*-protected, 5-mono-*O*-protected and small amounts of the 6-mono-*O*-protected-α-GISALs were recovered. However, in the majority of the reactions, more of the 5-mono-*O*-protected product was obtained and when using the relatively bulky TBSDMSCl as reagent the reaction took place exclusively at the 5-position. As the starting lactone was easy to prepare and because it proved to be relatively straight forward to separate the different mono-O-protected lactones, this route provided an opportunity to prepare a range of mono-O-protected-α-GISAls (Fig. 3) including the mono-substituted trityl-ethers (**7f,** 13% & **7e**, 2%) the silyl ether (**9e,** 46%) and the carbonates (**10e,** 24% and **10f,** 56%).

A number of additional mono-protected products were synthesised by three step procedures in which the required regioselective protection was achieved by first generating a di-O-protected product, followed by the addition of a small orthogonal protecting group at the remaining free-hydroxyl and then removal of the original protecting group. Treatment of the 5,6-di-*O*-FMOC-2-*O*-acetyl-α-GISAL with triethylamine generated the 2-O-acetyl-α-GISAL **3d** in near quantitative yield. Likewise, treatment of the 5,6-*O*-isopropylidene-2-O-FMOC lactone **12c** with aqueous acid generated the 5-FMOC-α-GISALs **10e** in quantitative yield.

2.6 Preparation of a 5,6-di-*O*-protected-α-GISALs in a two-step one pot procedure

Scheme 5.

The greater reactivity of 5-OH towards the silylating agent TBDMSCl meant that it is was possible to add orthogonal protecting groups onto the primary alcohols in a sequential reaction series in a one pot reaction (Scheme 3). Reaction of α-GISAL **2** with one equivalent of TBDMSCl in pyridine followed by the addition of 1.1 equivalent of acetic

anhydride led to the isolation, after column chromatography, of the 6-*O*-acetyl-5-*O*-TBDMS-α-GISAL (**13**).

3. Conclusion:

Many of the reactions used in this study to generate protected glucoisosaccharinic acids derivatives are the same as those that are applied to protect hydroxyls in monosaccharides. The main difference in their outcome is related to the steric demands of trying to put bulky protecting groups on a tertiary alcohol which is alpha to a carbonyl carbon. In order to get reaction at the tertiary alcohol either forcing conditions or the use of small sterically undemanding protecting groups was required. Unsurprisingly, the attempted synthesis of mono-protected glucoisosaccharinic acids led to the isolation of mixtures of products. However, the higher reactivity of the C-5 primary hydroxyl group makes this the preferred initial point of reaction and this was particularly true when reaction was with a bulky-silylating agent. Despite these difficulties, the use of multiple steps and the employment of orthogonally protected hydroxyls have provided access to a wide range of novel α-glucoisosaccharinio-1,4-lactone derivatives which we hope will be employed in the synthesis of value added products.

4. Experimental

4.1 General Methods

All reagents were purchased from commercial sources unless otherwise stated and were used without further purification. Anhydrous solvents were dried over molecular sieves (activated under vacuum at 200 ^{o}C) and stored under an inert atmosphere before use. The solvents used for column chromatography were GPR grade. Analytical TLC was performed on Silica Gel 60-F254 (Merck) and detection was either by charring following immersion in 5% H_2SO_4/H_2O and/or fluorescence. 1D ^{1}H and ^{13}C-NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer operating at ambient temperature. 2D-NMR (COSY, HSQC, HMBC or NOESY spectra) were recorded at 500 MHz using Bruker pulse sequences. NMR samples were dissolved in either D_2O, deuterated acetone or $CDCl_3$ and referenced to either internal tetramethylsilane (δ = 0 ppm), internal $CDCl_3$ (^{1}H δ = 7.23 ppm and ^{13}C δ = 77.00 ppm) or internal HOD (^{1}H δ = 4.65 ppm, 303K). Chemical shifts are given in parts per million.

High resolution mass spectra (HRMS) were recorded either by direct injection on an Agilent 6210 ToF spectrometer or by HPLC-MS (Agilent 1200 series HPLC coupled to an Agilent 6210 ToF Spectrometer). The HPLC employed a Phenomenex Luna 5µ C18 2.4 x 250 mm column and samples were eluted using an acetonitrile and water mobile phase operating with gradient elution: starting at 30% acetonitrile climbing to 95% acetonitrile over 15 mins. The mobile phase flow rate was 0.2 ml.min^{-1}.

Stocks of the calcium salt of α-glucoisosaccharinic acid **1** and α-glucoisosaccharino-1,4-lactone **2** were prepared using the procedures described by Whistler and Bemiller[12].

4.2 Synthesis of tri-*O*-protected lactone derivatives: 3a, 4a and 5a.

4.2.1 2,5,6-Tri-*O*-acetyl-α-D-glucoisosaccharino-1,4-lactone (3a).

α-D-Glucoisosaccharino-1,4-lactone (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 sodium acetate (0.5 g) was added and the reaction was heated to 100 ^{o}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 x 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%).IR (ATR) υ 2959 (C-H), 1781 & 1737 (C=O), 1437, 1370 (C-H), 1202, 1045 (C-O). ^{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, $J_{3,4}$ = 9. 0 Hz, $J_{3,5}$ = 14.7 Hz, H-3), 2.25 (dd, 1H, $J_{3',4}$ = 6.3 Hz, $J_{3',5}$ = 14.7 Hz, H3'), 2.11, 2.10, 2.08 (3s, 9H, 3 x C$\underline{H_3}$CO); ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.0 (C1), 170.6, 170.0, 169.9 (3 x CH_3-$\underline{CO}$), 77.9 (C2), 74.7 (C4), 65.3 (C6), 64.8 (C5), 32.1 (C3), 20.7, 20.6, 20.5 (3 x $\underline{Me}$-CO). HRMS (m/z) Calcd for $C_{12}H_{16}O_8$ $[M+NH_4]^+$: 306.1183, Found:306.1187.

4.2.2 2,5,6-Tri-*O*-benzoyl-α-D-glucoisosaccharino-1,4-lactone (4a).

The procedure used to prepare **4a** was identical to that used to prepare 2,5,6-tri-*O*-benzoyl-β-D-glucoisosacharino-1,4-lactone reported by Shaw *et al[27]* and the product was recovered from a crude mixture by column chromatography (pale yellow syrup, 4.93g starting from 20g of GISAL(**2**)) (TLC Hex/EtOAc 1:1; RF 0.34). IR (ATR) υ 1771 & 1722 (C=O), 1451 (Ar C-C), 1262, 1233, 1092 & 1062 (C-O), 701 & 684 (Ar C-H). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08-7.48 (m, 15H, 3 x Ph), 5.40 (m, 1H, H-4), 4.93 (d, 1H, $J_{6,6'}$ = 11.2 Hz, H-6), 4.70 (d, 1H, $J_{6,6'}$ = 11.2 Hz, H-6'), 4.65 (dd, 1H, $J_{5,4}$ = 3.4 Hz, $J_{5,5'}$ = 12.3 Hz, H-5),

4.53 (dd, 1H, $J_{5',4}$ = 6.5 Hz, $J_{5',5}$ = 12.3 Hz, H-5'), 2.82 (dd, 1H, $J_{3,4}$ = 8.8 Hz, $J_{3,3'}$ = 15.2 Hz, H-3), 2.62 (dd, 1H, $J_{3',4}$ = 7.2 Hz, $J_{3,3'}$ = 15.2 Hz, H-3'). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.96 (C1), 166.20, 165.74, 165.47 (3 x PhCO) 130.00, 129.96, 128.90, 134.24, 134.07, 130.12, 129.96, 129.20, 128.90, 128.71 (ArC), 78.46 (C2), 75.45 (C4), 66.09 (C6), 65.39 (C5), 32.65 (C3). HRMS (m/z) Calcd for $C_{27}H_{22}O_8$ $(M+Na)^+$: 497.1207, Found: 497.1236.

4.2.3 2,5,6-Tri-*O*-methylsulphonyl-α-D-glucoisosaccharino-1,4-lactone (5a).

The method used to prepare **5a** was adapted from that reported by Kabalka *et al[31]*. A solution of α-D-glucoisosaccharino-1,4-lactone (1.0 g; 6.17 mmol) in anhydrous pyridine (10 mL) was added to a round bottomed flask and cooled to 0 ^{o}C whilst stirring. Methanesulphonyl chloride (3 mL; 38.8 mmol) was added cautiously over a period of 10 min. The reaction mixture was kept at 0 ^{o}C for a further 5 min before continuing to stir at room temperature for 16 h. The reaction was halted by addition of ice cold water (25 mL) and dichloromethane (50 mL). The organic and aqueous layers were separated and any remaining organic product in the aqueous layer was extracted with dichloromethane (2 × 25 mL). The organic extracts were combined, washed with 5 % sodium bicarbonate (2 × 25 mL) and saturated brine (2 × 25 mL) before being dried over anhydrous magnesium sulphate. The solvent was removed at room temperature on a rotary evaporator to give a cream-orange coloured solid as the crude product (2.44 g). The product was purified by column chromatography (100% EtOAc). Fractions containing the desired product **5a** were combined and reduced by rotary evaporation to give **5a** as a white solid (1.50 g; yield: 61 %) (Rf: 0.48, EtOAc). IR (ATR) υ 773.6 (CO), 1347.5, 1172.0 (SO_2). ^{1}H NMR (400 MHz, *d*-DMSO): δ 5.02 (m, 1H, H-4), 4.52 (dd, 1H, $J_{5,5'}$ = 11.7 Hz, $J_{5,4}$ = 2.6 Hz, H-5), 4.37 (dd, 1H, $J_{5',4}$ = 6.3 Hz, $J_{5',5}$ = 11.7 Hz, H-5'), 4.01 (2 x d, 2H, $J_{6,6'}$ = 7.0 Hz, H-6,6'), 3.24, 3.29, 3.39 (3s, 9H, 3 x Me-SO_3), 2.89-2.47 (m, 2H, H-3,3'). ^{13}C NMR (100 MHz, *d*-DMSO):

δ 169.9 (C1), 83.7 (C2), 32.1 (C3), 76.1 (C4), 69.8 (C5), 70.0 (C6), 41.0, 37.4, 37.3 (3 x Me-SO_3). HRMS (m/z) Calcd for $C_9H_{16}O_{11}S_3$ $(M+NH_4)^+$: 414.0193, Found: 414.0188.

4.3 Synthesis of 5,6-di-O-protected lactone derivatives (3b, 6b-10b)

4.3.1. 5,6-Di-*O*-acetyl-α-D-glucoisosaccharino-1,4-lactone (3b)

α-D-Glucoisosaccharino-1,4-lactone (**2**, 500 mg; 3.09 mmol) was dissolved in pyridine (5 mL) while stirring at room temperature for 10 min. 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 x 30 mL). The combined organic layer was washed with 1% copper sulphate solution (2 x 50 mL) and dried over anhydrous magnesium sulphate, then concentrated to give 3b (1.20 g; 5.61 mmol; Yield: 55%) IR (ATR) υ 3079 (O-H), 1781 &1743 (C=O), 1482, 1373 (C-H), 1233, 1196 (C-O).^{1}H NMR (400 MHz, $CDCl_3$) δ 4.82-4.76 (m, 1H, H-4), 4.22 (dd, 1H, $J_{5,4}$ = 2.88 Hz, $J_{5',5}$ = 12.4 Hz, H-5), 4.20 (2d, 2H, $J_{6,6'}$ = 1.16 Hz, H-6 & 6'), 4.04 (dd, 1H, $J_{5',4}$ = 6.28 Hz, $J_{5',5}$ = 12.4 Hz, H-5'), 2.23 (dd, 1H, $J_{3,4}$ = 6.20 Hz, $J_{3,3'}$ = 13.54 Hz, H-3), 2.07 (dd, 1H, $J_{3',4}$ = 9.32 Hz, $J_{3',3}$ = 13.52 Hz, H-3') 1.94 & 1.89 (2s, 6H, 2 x CH_3CO); ^{13}C NMR (100 MHz, $CDCl_3$): 175.4 (C1), 170.4 & 170.1 (2 x CH_3CO), 74.9 (C4), 74.0 (C2), 65.0 (C6), 64.6 (C5), 35.1 (C3), 20.6 & 20.5 (2 x $\underline{C}H_3CO$). HRMS (m/z): Calcd for $C_{10}H_{14}O_7$ $(M+NH_4)^+$: 269.0748, Found: 269.0740.

4.3.2. 5,6-Di-*O*-*p*-toluenesulphonyl-α-D-glucoisosaccharino-1,4-lactone (6b)

p-Toluenesulphonyl chloride (2.58 g; 13.6 mmol; 2.1 eq.) was reacted with α-D-glucoisosaccharino-1,4-lactone (1.06 g; 6.51 mmol) in anhydrous pyridine (5 mL) using the same procedure described in section 4.3.1 except that after the addition was complete, the solution was stirred at room temperature for a further 60 h. The crude product 5,6-di-*O*-tosyl-α-glucoisosaccharino-1,4-lactone was purified by column chromatography eluting with a solvent system with a starting composition of hexane and EtOAc (3:1) rising to 100 % EtOAc. The purified compound **6b (**RF= 0.35; hexane/ether, 1:1) was isolated as a pale yellow syrup (1.69 g; yield: 55 %). IR (ATR) υ 3460.1 (OH), 1782.1 (CO) 1597.1, 1354.3, 1171.3, 810.6. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.80-7.78 (m, 4H, 2 x Ar-H), 7.39-7.36 (m, 4H, 2 x Ar-H), 4.83 (m, 1H, H-4), 4.24-4.11 (m, 2H, H-5s), 4.16 (d, 1H, $J_{6,6'}$ = 10.6Hz, H-6), 4.07 (d, 1H, $J_{6',6}$ = 10.6 Hz, H6'), 2.48-2.44 (m, 6H, CH_3-Ph), 2.37 (m, 1H, H-3), 2.22 (m, 1H, H-3'). ^{13}C NMR (100 MHz, $CDCl_3$): 173.3 (C1), 145.6, 145.7, 130.2, 130.2, 131.8, 132.0, 128.1, 128.1, (8 x ArC), 74.7 (C4), 74.3 (C2), 70.2 (C6), 68.7 (C5), 34.2 (C3), 21.7 (2 x $\underline{C}H_3$Ar); HRMS (m/z): Calcd for $C_{20}H_{22}O_9S_2$ $(M+NH_4)^+$: 488.1043, Found: 488.1049.

4.3.3. 5,6-Di-*O*-triphenylmethyl-α-D-glucoisosaccharino-1,4-lactone (7b) , 6-*O*-triphenylmethyl-α-D-glucoisosaccharino-1,4-lactone (7e) and 5-*O*-triphenylmethyl-α-D-glucoisosaccharino-1,4-lactone (7f)

The following synthetic procedure was adapted from the work by Choudhary and Hernandez[32]. Triphenylmethyl chloride (25.07 g; 89.9 mmol) and α- D-glucoisosaccharino-1,4-lactone **2** (6.82 g; 41.9 mmol) were dissolved in pyridine (300 mL) and a catalytic amount of DMAP (1 g; 8.19 mmol) was added. The resulting solution was stirred at 25 ^{o}C for 12 h under an atmosphere of nitrogen. After the reaction was complete, the solution was added to an equal volume of water and then extracted into chloroform (2 × 200 mL). The two layers were separated and the organic layers were washed with

saturated brine (100 ml) a saturated solution of sodium bicarbonate (100 ml) and dried over anhydrous sodium sulphate. Evaporation of solvent produced a beige coloured solid (13.9 g). Subsequent TLC analysis showed the presence of three compounds of interest. Following separation by column chromatography eluting with Hex/EtOAc (2:1), the desired compounds were identified as 2,5-di-*O*-trityl-α-GISAL **7b** (Rf 0.79; Hex/EtOAc ((2:1)); 3.34 g; yield: 12 %, followed by the 6-mono-*O*-trityl-α-GISAL **7e** (Rf 0.29; Hex/EtOAc,1:2 v/v)); 0.20 g; yield: <2 % and 5-mono-*O*-trityl-α-GISAL **7f** was recovered from a chloroform wash (Rf 0.16; Hex/EtOAc, (1:2)); 2.25 g; yield: 13 %.

7b IR (ATR) υ 1779 (CO) 762.2, 745. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48-7.27 (m, 30H, 6 x PhH), 4.82 (m, 1H, H-4), 3.41 (d, 1H, $J_{6,6'}$ = 9.1Hz, H-6), 3.30 (d, 1H, $J_{6',6}$ = 9.1 Hz, H-6'), 3.36 (dd, 1H, $J_{5,4}$ = 6.0 Hz, $J_{5,5'}$ = 10. 5 Hz, H-5), 3.28 (dd, 1H, $J_{5',4}$ = 3.8 Hz, $J_{5',5}$ = 10.5 Hz, H-5'), 2.20 (m, 2H, H-3); ^{13}C NMR (100 MHz, $CDCl_3$): 176.4 (C1), 143.3, 143.6, 128.7, 128.7, 128.0, 128.0, 127.3, 127.2, 86.9 & 87.2 (TrC*), 77.4 (C4), 75.5 (C2), 65.4 (C5), 65.3 (C6), 35.0 (C3).HRMS (m/z) Calcd for $C_{44}H_{38}O_5$ $(M+Na)^+$: 669.2611, Found: 669.2592.

7e IR (ATR) υ 3353.1 (OH) 1774.0 (CO) 763.4, 745.8, 697.7. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46-7.27 (m, 15H, 3 x PhH), 4.77 (m, 1H, H-4), 3.91 (dd, 1H, $J_{5,4}$ = 2.8 Hz, $J_{5,5'}$ = 12.7 Hz, H-5), 3.65 (dd, 1H, $J_{5',4}$ = 5.1 Hz, $J_{5',5}$ = 12.7 Hz, H-5'), 3.42 (d, 1H, $J_{6,6'}$ = 9.2 Hz, H-6), 3.32 (d, 1H, $J_{6',6}$ = 9.2 Hz, H-6'), 2.33 (dd, 1H, $J_{3,4}$ = 7.1 Hz, $J_{3,3'}$ = 13.8 Hz, H-3), 2.21 (dd, 1H, $J_{3',4}$ = 8.5 Hz, $J_{3',3}$ = 13.8 Hz, H-3'); ^{13}C NMR (100 MHz, $CDCl_3$): 176.2 (C1), 143.2 , 128.7, 128.0, 127.3, 87.3 (TrC), 75.9 (C2), 78.2 (C4), 65.3 (C6), 63.6 (C5), 33.6 (C3). HRMS (m/z) Calcd for $C_{25}H_{24}O_5$ $[M+Na]^+$: 427.1516, Found: 427.1513.

7f IR (ATR) υ 3365.8 (OH) 1772.8. (CO) 763.6, 746.0, 697.2. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.46-7.27 (m, 15H, 3 x PhH,), 4.89 (m, 1H, H-4), 3.84 (d, 1H, $J_{6,6'}$ = 11.7 Hz, H-6), 3.71

(d, 1H, $J_{6',6}$= 11.7 Hz, H-6'), 3.43 (dd, 1H, $J_{5,4}$ = 3.3 Hz, $J_{5,5'}$ = 10.5 Hz, H-5), 3.22 (dd, 1H, $J_{5',4}$ = 5.0 Hz, $J_{5',5}$ = 10.5 Hz, H-5'), 2.22 (dd, 1H, $J_{3,4}$ = 6.7 Hz, $J_{3,3'}$ = 13.7 Hz, H-3), 2.12 (dd, 1H, $J_{3',4}$ = 8.6 Hz, $J_{3,3'}$ = 13.7Hz, H-3'); ^{13}C NMR (100 MHz, $CDCl_3$): 177.7 (C1) 143.4, 128.6, 128.0, 127.3, 86.9 (TrC), 77.7 (C4), 73.6 (C2), 65.5 (C6), 64.2 (C5), 34.0(C3). HRMS (m/z) Calcd for $C_{25}H_{24}O_5$ $(M+Na)^+$: 427.1516, Found: 427.1506.

4.3.4. 5,6-Di-*O*-dibenzyl-α-D-glucoisosaccharino-1,4-lactone (8b)

The dibenzyl derivative **8b** was synthesised using a method adapted from that described by Giordano and Iadonisi [29]. Dried α-glucoisosaccharino-1,4-lactone **2** (1.0 g, 6.17 mmol) was dissolved in *N,N*-diisopropylethylamine (DIPEA) (2.3mL, 4 eq), and a catalytic amount 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, 8 eq), was added slowly and the reaction was allowed to proceed for 4 h at 90 ^{o}C. A second portion of BnBr and DIPEA (2 eqs each) were added and the reaction continued for further 2 h at 90 ^{o}C. 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 x 50 mL). The combined organic extracts was dried over anhydrous sodium sulphate and concentrated to dryness to give crude **8b** as a golden syrup which was purified by column chromatography (EtOAc:Hexane 1/1 v/v); to give the product as a transparent oil 1.24 g; yield: 59% . ^{1}H NMR (400 MHz, $CDCl_3$) 7.34-7.29 (m, 10H, ArH), 4.83-4.77 (m, 1H, H-4), 4.54 (AB, 4H, $J_{7,7'}$ = 6.08 Hz, H-7, 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 (2 x 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) Calcd for $C_{20}H_{22}O_5$ $[M+Na^+]$: 365.1359, Found: 365.1358.

4.3.5. 5,6-Di-*O*-tert-butyldimethylsilyl-α-D-glucoisosaccharino-1,4-lactone (5a)

The di-tert-butyldisilyl derivative **9b** was synthesised using a method adapted from that described by Iadonisi et al[33] employing only a minimal amount of solvent. Dried α-glucoisosaccharino-1,4-lactone **2** (1.0 g, 6.17 mmol) was suspended in anhydrous pyridine (5 mL) whilst stirring for 20 min at room temperature. It was then added cautiously to a mixture of tert-butyldimethylsilyl chloride (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 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 **9b** as a white solid. The product was purified by chromatography (elution with EtOAc/Hexane; 3:1 v/v) and the early fractions contained pure **9b** (1.66 g; 4.26 mmol; 69 %) (Rf = 0.722; Hexane/EtOAc 3:1 v/v) were combined and the solvent evaporated. IR (ATR) υ 3259 (O-H), 2952, 2928, 2886, 2857 (C-H), 1770 (C=O), 1471, 1462, 1360 (C-H), 1255, 1200, 1168 (C-O), 1097, 1044 (Si-OR) 833, 814, 775. ^{1}H NMR (400 MHz, $CDCl_3$) 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'), 0.86 (2s, 18H , 2 x TBDMS), 0.05 (m, 12H, 2 x TBDMS); ^{13}C NMR (100 MHz, $CDCl_3$): 176.92 (C1), 77.77 (C4), 76.35(C2), 65.42 (C6), 64.30 (C5), 33.72 (C3), 25.82 & 25.78 (TBDMS), 18.31& 18.24 (TBDMS). HRMS (m/z) Calcd for $C_{18}H_{38}Si_2O_5$ $[M+Na]^+$: 413.2150, Found: 413.2152.

4.3.6 (1',1',3',3'-Tetraisopropyldisiloxane-1,3-diyl)-5,6-α-D-glucoisosaccharino-1,4-lactone (14)

Dried α- D-glucoisosaccharino-1,4-lactone **2** (1.0 g, 6.17 mmol) was dissolved in pyridine (6 mL) at room temperature and the solution 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 stirring at room temperature. The reaction was allowed to proceed for 4 h. After 4 h it was halted with the addition of DCM (60 mL) and 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 an aqueous $CuSO_4$ solution (1%, 2 x 50 mL) dried over anhydrous sodium sulphate and concentrated to give crude **14** (4.14 g) as a brown crystalline syrup which was purified using column chromatography to give the desired product as a pale yellow syrup (2.05 g; 5.07 mmol; 82% yield) (RF: 0.68, Hexane/EtOAc 4/1 v/v). IR (ATR) υ 2945, 2867, 1771 (C=O), 1464, 1387, 1084, 1042 (R_3Si-O-SiR_3), 1012. ^{1}H NMR (400 MHz, $CDCl_3$) 4.70-4.62 (m, 1H, H-4), 4.07 (d, 1H, $J_{6,6'}$ = 10.6 Hz, H-6) 3.94-3.85 (m, 2H, H-5, H-5'), 3.83 (d, 1H, $J_{6',6}$ = 10.6 Hz, H-6'), 2.82 (dd, 1H, $J_{3,3'}$ = 13.9 Hz, $J_{3,4}$ = 2.4 Hz, H-3), 2.30 (dd, 1H, $J_{3',3}$ = 13.9 Hz, $J_{3',4}$ =10.1 Hz, H-3'), 1.1-0.9 (m, 28H, TIPDS).

^{13}C (100 MHz, $CDCl_3$) 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 (TIP(CH)DS), 13.5, 13.1, 12.6 & 12.4 (TIP(CH_3)DS)

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

To confirm the location of the protecting group, **14** (1.5g, 3.71mmol) was acetylated using the procedure described in section 4.5.1 to give, after chromatography, the product **15** as a white semi-crystalline syrup (680 mg, 1.53 mmol; 41% yield); (Rf: 0.721, Hexane/EtOAc 3:1, v/v). IR (ATR) υ 2944.6, 2867.5, 1779.5 & 1742.1 (C=O), 1463.9, 1369.8, 1084, 1252.1, 1215.1, 1082.5, 1043.2 (R_3Si-O-SiR_3), 883.1. ^{1}H NMR (400 MHz, $CDCl_3$) 4.82-4.78 (m, 1H, H-4), 4.09 (dd, 1H, $J_{5,5'}$ = 12.02 Hz, $J_{5,4}$ = 3.56 Hz, H-5), 4.05 (d, 1H, $J_{6,6'}$ = 11.6 Hz, H-6), 4.00 (d, 1H, $J_{6,6'}$ = 11.6 Hz, H-6'), 3.85 (dd, 1H, $J_{5',5}$ = 12.0 Hz, $J_{5',4}$ = 2,16

Hz, H-5'), 2.75 (dd, 1H, $J_{3,3'}$ = 13.61 Hz, $J_{3,4}$ = 3.52 Hz, H-3), 2.41 (dd, 1H, $J_{3',3}$ = 13.61 Hz, $J_{3',4}$ = 9.9 Hz, H-3') 2.12 (s, 3H, OC**H**$_3$) 1.1-1.0 (m, 28H, TIPDS).

^{13}C (100 MHz, CDCl3) 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.

4.3.7. 5,6-Di-*O*-fluorenylmethoxycarbonyl-α-D-glucoisosaccharino-1,4-lactone (10b)

α-D-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 an atmosphere of nitrogen for 20 min. The mixture was slowly added to a second reaction vessel, cooled to 0°C, containing fluorenylmethoxycarbonyl chloride (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. During this time a large quantity of colourless pyridinium hydrochloride precipitated from solution. The reaction was quenched by adding ice-cold water (100 mL), followed by ice-cold 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 fractions were washed with a large quantity of brine (3 x 100 mL) to remove pyridine. The resulting solution was dried over anhydrous sodium sulphate, before being concentrated under reduced pressure. The crude product was a bright yellow crystalline syrup (3 g) The product was separated via chromatography (eluting with a mobile phase compose of Hexane/EtOAc 1:1 v/v). The target compound **10b** (R_F = 0.47 Hexane/EtOAc; 1:1v/v) was recovered as a pale yellow solid (yield: 1.47 g, 2.45 mmol, 19.8 %). IR (ATR) υ 2945, 2867, 1771 (C=O), 1464, 1387, 1084, 1042 (R_3Si-O-SiR_3), 1012.^{1}H NMR (400 MHz, $CDCl_3$), δ 7.90-7.84 (m, 4H, ArH),

7.65-7.59 (m, 4H, ArH), 7.43-7.37 (m, 4H, ArH), 7.34-7.28 (m, 4H, ArH), 4.92 (m, 1H, H-4), 4.03-4.52 (m, 10H, 2 x H-5s, 2 x H-6s, 4 x H-8s & 2 x H-9s), 2.44 (dd, 1H, $J_{3',4}$ = 6.95 Hz, $J_{3,3'}$ = 14.2 Hz, H-3'), 2.24 (dd, 1H, $J_{3,4}$ = 5.67 Hz, $J_{3,3'}$ = 14.2 Hz, H-3); ^{3}C NMR (100 MHz,$CDCl_3$): 175 (C1), 155 (C7), 143,141,128,127,125,120 (ArC), 75.0 (C2), 74.5 (C4), 70.4 (C8), 68.9 (C6), 67.8 (C5), 46.8 (C9), 34.7 (C3). Melting point: 76-77 ^{O}C. HRMS (m/z): Calcd for $C_{36}H_{30}O_9$ $[M+NH_4]^+$ 624.2228 ,Found: 624.2228.

4.4 Synthesis of 2,6-di-*O*-protected lactone derivatives (11b and 12b) and their conversion to 2,5,6-tri-*O*-protected lactone derivatives (11c and 12c).

4.4.1 5-*O*-Fluorenylmethoxycarbonyl-2,6-*O*-isopropyliene-α-D-glucoisosaccharino-1,4-lactone (11c)

2,6-*O*-Isopropylidene-α-D-glucoisosaccharino-1,4-lactone **11b,** prepared using the procedures described by Florent *et al[13]* (1.38 g, 6.83 mmol), was dissolved in anhydrous pyridine (20 ml). The solution was cautiously added to a flask, maintained at 0^{o} C, containing crystalline FMOCCl (2.66 g, 0.01 mmol). The reaction was 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 the desired product **11c** as a yellow solid (570 mg, 1.34 mmol; Yield: 19.68%); (Pet. ether/EtOAc 3:1 v/v). IR (ATR) υ 2945, 2867, 1771 (C=O), 1464, 1387, 1084, 1042 (R_3Si-O-SiR_3), 1012. ^{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, H-8 & H-9), 2.20 (dd, 1H, $J_{3,3'}$ = 14.38 Hz, $J_{3,4}$ = 7.05 Hz, H-3), 2.55 (dd, 1H, $J_{3,3'}$ = 14.07 Hz, $J_{3',4}$ = 7.47 Hz, H-3'); 1.49 (bs, 6H,

493 2 x CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): 174.8 (C1), 154.6 (FMOC<u>C</u>O): 142.8, 141.3, 127.9,
494 127.1, 125.0, 119.9 (ArC), 112.7 (C7), 80.8 (C2), 74.4 (C4), 72.0 (C6), 70.1 (C5), 67.7 (
495 FMOC<u>C</u>H) 46.4 (FMOC<u>C</u>H_2) 36.5 (C3), 26.7 (C8), 25.3 (C9). HRMS (m/z) Calcd for
496 $C_{24}H_{24}O_7$ [M+Na^+]: 447.1414, Found: 447.1415.

497 **4.4.2 5-*O*-Benzoyl-2,6-*O*-benzylidene-α-D-glucoisosaccharino-1,4-lactone (12c)**

498 *Synthesis of (7S)- and (7R)-2,6-O-benzylidene-α-D-glucoisosaccharino-1,4-lactone* **12b** -
499 Freshly distilled benzaldehyde (50 mL; 492 mmol) was added to a round bottomed flask
500 (100 mL) containing α-glucoisosaccharino-1,4-lactone **2** (1.02 g; 6.27 mmol), *p*-TSA (20
501 mg) and ~ 30 4Å molecular sieves. The mixture was left to reflux under a slight vacuum for
502 4 h at 85 °C. After cooling to room temperature, the mixture was gravity filtered to remove
503 the molecular sieves and excess benzaldehyde was removed by vacuum distillation to
504 give the crude product as a semi-crystalline syrup. The crude mixture was purified by
505 column chromatography (fractions were eluted with chloroform with increasing portions of
506 methanol: 1-10%). The product eluted in two distinct bands which, after evaporating to
507 dryness gave 0.90 g and 0.26 g of the required diastereoisomers with a combined yield of
508 78 %. Using NOESY NMR spectra, it was determined that the first fraction (Rf: 0.17,
509 $CHCl_3$/MeOH 95:5 v/v) was the 7*R*- diastereomer of **12b** whilst the second fraction (Rf:
510 0.26, $CHCl_3$/MeOH 95:5 v/v) contained the 7*S*-diastereomer of **12b**.

511 ^{1}H NMR 7*S*-diastereomer of **12b** (400 MHz, *d*-DMSO): 7.35-7.55 (m, 5H, ArH), 5.98 (s,
512 1H, PhC<u>H</u>), 5.25 (s, 1H, OH), 4.71 (m, 1H, H-4), 4.33 (d, 1H, $J_{6,6'}$ = 9.0 Hz, H-6), 4.16 (d,
513 1H, $J_{6',6}$ = 9.0 Hz, H-6'), 3.67 (dd, 1H, $J_{5,4}$ = 2.0 Hz, $J_{5,5'}$= 12.1 Hz, H-5), 3.49 (dd, 1H, $J_{5',4}$
514 = 3.2 Hz, $J_{5',5}$ = 12.2 Hz, H-5') 2.49 (m, 2H, H-3,3') . ^{13}C NMR (100 MHz, *d*-DMSO): 176.4
515 (C1), 136.7, 127.4, 128.8, 130.2 (ArC), 104.9 (C7), 81.2 (C2), 78.7 (C4), 35.4 (C3), 62.5
516 (C5), 72.9 (C6).

^{1}H NMR 7*R*-diastereoisomer **12b** (400 MHz, *d*-DMSO): 7.38-7.60 (m, 5H, ArH), 5.91 (s, 1H, PhCH), 5.21 (s, 1H, OH), 4.68 (m, 1H, H-4), 4.44 (d, 1H, $J_{6,6'}$ = 9.5 Hz, H-6), 4.04 (d, 1H, $J_{6',6}$ = 9.5 Hz, H-6'), 3.68 (m, 1H, H-5), 3.49 (dd, 1H, $J_{5',4}$ = 3.4 Hz, $J_{5',5}$ 12.3 Hz, H-5'), 2.60 (dd, 1H, $J_{3,4}$ = 7.7 Hz, $J_{3,3'}$ = 13.8 Hz, H-3), 2.33 (dd, 1H, $J_{3',4}$ = 6.0 Hz, $J_{3',3}$ = 14.0 Hz, H-3'). ^{13}C NMR (100 MHz, *d*-DMSO): 175.8 (C1), 136.9, 130.2,127. 9, 128.7 (ArC), 105.0 (C7), 81.0 (C2), 78.5 (C4), 73.3 (C6), 62.5 (C5), 34.5 (C3).

Synthesis of 5-O-benzoyl-(7R)-2,6-O-benzylidene-α-D-glucoisosaccharino-1,4-lactone **12c.** Compound **12b** (0.90 g; 3.60 mmol) was dissolved in pyridine (50 mL) and benzoyl chloride (1.5 g; 1.3 mL; 10.7 mmol) and a catalytic quantity of DMAP (20 mg) were added. The reaction was stirred at room temperature for 2 h. The pyridine was removed by rotary evaporation and the resulting brown residue was dissolved in diethyl ether (50 mL) and washed with a saturated sodium hydrogen carbonate solution (2 x 20 mL) and then with saturated sodium chloride (20 mL). The organic layer was reduced to dryness, the crude product was dissolved in sodium dried ether (20 mL) and this was once again dried on the rotary evaporator. This process was repeated with sodium dried ether until the odour of pyridine had disappeared to give a mixture of the desired product and pyridinium hydrochloride as a semi-solid syrup. Finally, a small amount of the desired product was obtained by recrystallization from petroleum ether, the residue was dissolved in petroleum ether (bpt 40-60 ^{o}C,10 mL) and the volume of the solvent was reduced slowly until a white cloudy solution was first observed. After cooling to room temperature, the mixture was chilled at 5 °C for 3 h until white crystals were visible which were filtered under gravity and dried at room temperature in a desiccator to isolate the crystalline product **12c** as white needles (0.26 g; yield: 20 %). IR (ATR) υ 1766.9 & 1727.2 (CO) 759.4, 708.6., 695.0. ^{1}H NMR (400 MHz, *d*-DMSO): 8.05-7.35 (m, 10H, ArH), 5.98 (s, 1H, PhCH), 5.06 (m,1H, H-4), 4.46 (dd, 1H, $J_{5,4}$ = 6.7 Hz, $J_{5,5'}$ = 12.4 Hz, H-5), 4.57 (dd, 1H, $J_{5',4}$ = 2.7 Hz, $J_{5',5}$ = 12.4

Hz, H-5'), 4.33-4.31 (2 x d, 2H, $J_{6,6'}$ = 8.8 Hz, H-6, H-6'), 2.64 (m, 2H, H-3, H-3'). ^{13}C NMR (100 MHz, *d*-DMSO): 175.2 (C1), 165.9 (PhCO), 136.4, 134.1,130.3, 129.8, 129.7, 129.3, 128.8 & 127.5 (ArC), 104.9 (C7), 80.9 (C2), 76.1 (C4), 71.4 (C6), 65.7 (C5), 34.9 (C3). HRMS (m/z) Calcd for $C_{20}H_{18}O_6$ $[M+K]^+$: 393.0735, Found: 393.0735.

4.5 Preparation of orthogonally protected trisubstituted 2-α-D-glucoisosaccharino-1,4-lactone

4.5.1 2-*O*-Acetyl-5,6-di-*O*-benzyl-α-D-glucoisosaccharino-1,4-lactone (8c)

5,6-di-*O*-Dibenzyl-D-glucoisosaccharino-1,4-lactone **8b** (1.0 g, 2.92 mmol) was reacted with acetic anhydride (10 m) and sodium acetate (0.5 g) employing the procedure described in section 4.2.1 to give a brown crystalline syrup which was purified by column chromatography (EtOAc/hexane 5/1-1:1 v/v) providing **8c** as a colourless oil (330 mg; 0.86 mmol; 29.4%); (Rf: 0.211; EtOAc/hexane 1:1 v/v). IR (ATR) υ 2866, 1775 & 1740 (C=O), 1453, 1369, 1205 & 1096 (C-O), 736, 697. ^{1}H NMR (400 MHz, $CDCl_3$) 7.33-7.26 (m, 10H, ArH), 4.96-4.90 (m, 1H, H-4), 4.52-4.49 (2d, 4H, $J_{7,7'}$ = 4.72 Hz, H-7s), 3.70 (m, 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, CH_3CO). ^{13}C NMR (100 MHz, $CDCl_3$): 173.66 (C1), 170.00 (CH_3CO), 137.72 & 137.20 (PhCq), 128.48, 128.46, 127.89, & 127.78 (PhC), 79.44 (C2), 76.51 (C1), 73.86 & 73.46 ($PhCH_2$), 71.59 (C6), 71.10 (C5), 31.96 (C3), 20.63 (CH_3CO). HRMS (m/z) Calcd for $C_{22}H_{24}O_6$ $[M+NH_4]^+$: 402.1911, Found: 402.1910.

4.5.2. 2-*O*-Acetyl-5,6-di-*O*-tert-butyldimethylsilyl-α-D-glucoisosaccharino-1,4-lactone (9c)

The same procedure as described above for the synthesis of **8c** was used to prepare **9c**. After chromatography, the product **9c** was recovered as a white crystalline semi-solid (900 mg, 2.08 mmol; 81%; Rf: 0.821, Hexane/EtOAc 3:1, v/v). IR (ATR) υ 2954, 2929,

2857, 1783 &1747 (C=O), 1472, 1369, 1251, 1209 (C-O), 832, 776. ^{1}H NMR (400 MHz, $CDCl_3$) 4.37-4.70 (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, $CDCl_3$): 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 (TBDMS), 20.43 (CH_3CO), -5.23, -5.55, -5.60 (TBDMS). HRMS (m/z) Calcd for $C_{20}H_{40}Si_2O_6$ $[M+Na]^+$: 455.2256, Found: 455.2257.

4.5.3 2-*O*-Acetyl-5,6-di-*O*-fluorenylmethoxycarbonyl-α-D-glucoisosaccharino-1,4-lactone (10c).

5,6-di-*O*-FMOC-α-GISA$_L$ (**10b,** 2.34 g, 3.86 mmol) was added to a round bottom flask containing acetic anhydride (12.5 ml, 0.13 mol) and $ZnCl_2$ (0.5 g). The solution was heated to 100 ^{o}C and the reaction was allowed to proceed for 4 h at 100 ^{o}C. After 4h the sample was cooled to room temperature and the contents of the flask were poured cautiously onto ice cool water (100mL) to give the product as a semisolid. The suspension was stirred for 30 min over which time the product solidified. The solid was filtered and the residue dried at room temperature overnight to give **10c** as a white powder (1.5 g; 2.14 mmol, 55%). IR (ATR) υ 1784, 1745 & 1709 (C=O), 1253, 1206 (C-O), 784, 759, 739. ^{1}H NMR (400 MHz, $CDCl_3$) 7.77-7.73 (m, 4H, ArH), 7.61-7.56 (m, 4H, ArH), 7.42- 7.36 (m, 4H, ArH), 7.34-7.27 (m, 4H, ArH), 5.13-5.05 (m, 1H, H-4), 4.53-4.40 (m, 6H, 4 x H-8 & 2 x H-5), 4.32-4.22 (m, 3H, 2 x H-6 & H-9), 2.60 (dd, 1H, $J_{3,4}$ = 9.38 Hz, $J_{3,3'}$ = 14.32 Hz, H-3), 2.43 (dd, 1H, $J_{3',4}$ = 5.93, $J_{3',3}$ =14.32 Hz, H-3'), 2.17 (s, 3H, CH_3CO). ^{13}C NMR (100 MHz, $CDCl_3$): 177.6, 177.1 (FMOCO), 171.7 (C1), 170.1 (CH_3CO), 143.2 & 141.1 (ArCq), 128.5, 127.2, 125.1 & 120.5 (ArC), 77.6 (C2), 74.7 (C4), 70.7 (C8), 68.8 (C6), 67.7 (C5), 31.7 (C3), 21.1 ($COCH_3$). HRMS (m/z) Calcd for $C_{38}H_{32}O_{10}$ $[M+ Na]^+$ 648.1995, found 648.1992.

4.6 Preparation of mono-protected lactone derivatives (7e-f, 9e and 10e-10f)

4.6.1 The single step preparation of the mono-protected lactones **7e** and **7f** was described in section 4.3.3

4.6.2 5-*O*-*tert*-Butyldimethylsilyl-α-D-glucoisosaccharino-1,4-lactone (9e).

α-D-Glucoisosaccharino-1,4-lactone **2** (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 4h 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 a white crystalline syrup **9e** (780 mg; 2.83 mmol; Yield: 46%); (RF: 0.35, Hexane/EtOAc 3:1 v/v). IR (ATR) υ 3407 (O-H), 2952, 2929, 2856 (C-H), 1761 (C=O), 1463, 1361 (C-H), 1254, 1201, 1122 (C-O), 1034 (Si-OR) 833, 776. ^{1}H NMR (400 MHz, $CDCl_3$) 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'), 2.21 (m, 2H, H-3, H3'), 0.85 (s, 9H, TBDMS), 0.04 & 0.03 (2s, 6H, TBDMS). ^{13}C NMR (100 MHz, $CDCl_3$): 177.76 (C1), 78.57 (C4), 75.61(C2), 65.36 (C6), 63.56 (C5), 33.31 (C3), 25.70 (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.

.

4.6.3 5-*O*-Flourenylmethoxycarbonyl-α-D-glucoisosaccharino-1,4-lactone (10e) and 6-*O*-flourenylmethoxycarbonyl-α-D-glucoisosaccharino-1,4-lactone (10f)

Dry α-D-Glucoisosaccharino-1,4-lactone (1.0 g, 6.17 mmol) was dissolved in 3-picoline (20 mL) and the resulting solution was added cautiously, whilst stirring, to cooled 0 ^{o}C crystalline 9-flourenylmethyloxycarbonyl chloride (FMOCCl) (3.35 g, 13 mmol). The reaction was allowed to proceed for 3 h at room temperature. 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). The combined extracts was washed with 2M HCl (2 x 100 mL), brine (2 x 100 mL) and dried over sodium sulphate, concentrated to dryness to give a pale yellow crystalline crude syrup (3.62 g). The crude was separated using column chromatography to give **10e** (0.56 g, 1.46 mmol, 24% yield, R_F = 0.120) and **10f** (1.32 g, 3.44 mmol, 56% yield, R_F= 0.170). IR (ATR) υ

(**10 e**) IR (ATR) υ 3460 (O-H), 1747 (C=O), 1450, 1193 & 1256 (C-O), 738 (Ar C-H). ^{1}H NMR (400 MHz, $CDCl_3$, **10e**) 7.78-7.33 (m, 8H, ArH), 5.0-4.93 (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, $J_{6,6'}$= 11.9 Hz, H-6), 3.73 (d, 1H $J_{6',6}$ = 11.9 Hz, H-6), 2.35 (dd, 1H, $J_{3,3'}$ =13.17 Hz, $J_{3,4}$ = 7.0 Hz, H-3), 2.07 (dd, 1H, $J_{3',3}$ = 13.17 Hz, $J_{3',4}$ =8.56 Hz, H-3').^{13}C (100 MHz, $CDCl_3$) 177.4 (C1), 155.1 (C7), 143.3 , 141.7 , 128.3 , 127.2 , 125.6, 120.5 (ArC), 76.0 (C2), 75.2 (C4), 70.9 (C8), 67.6 (C5), 65.2 (C6), 46.7 (C9), 33.6 (C3). HRMS (m/z) Calcd for $C_{21}H_{20}O_7$ $[M+Na]^+$: 407.1101, Found: 407.1101.

(**10 f**) IR (ATR) υ 3442 (O-H), 1747.5 (C=O), 1450, 1195 & 1256, (C-O), 727 (Ar C-H).^{1}H NMR (400 MHz, $CDCl_3$, **10f**) 7.74-7.30 (m, 8H, ArH), 4.83-4.75 (m, 1H, H-4) 4.49 (d, 1H, $J_{6,6'}$ = 12.0 Hz, H-6), 4.41 (m, 2H, H-8, H-8'), 4.33 (d, 1H, $J_{6',6}$ = 12.0 Hz, H-6), 4.23 (t, 1H, $J_{9,8}$= 8.37 Hz H-9), 3.92 (dd, 1H, $J_{5,5'}$ = 12.98, $J_{5,4}$ =2.50 Hz, H-5) 3.62 (dd, 1H, $J_{5',5}$ = 12.98 Hz, $J_{5',4}$ = 4.12 Hz, H-5'), 2.31 (2 x d, 2H, $J_{3,3'}$ = 7.31 Hz, H3, H3'). ^{13}C (100 MHz, $CDCl_3$) 175.8 (C1), 154.9 (C7), 143.1, 141.7, 128.6, 127.2, 125.4, 120.3 (ArC), 79.2 (C2),

74.9 (C4), 70.6 (C8), 69.0 (C6), 63.6 (C5), 46.4 (C9), 33.8 (C3). HRMS (m/z) Calcd for $C_{21}H_{20}O_7$ $[M+K]^+$: 423.0841, Found: 423.0854.

4.7 Preparation of 5,6-diprotected lactone derivative (13) in a one pot sequential reactions

4.7.1 5-*O*-*tert*-Butyldimethylsilyl-6-*O*-acetyl-α-D-glucoisosacharino-1,4-lactone (13)

Dried α-D-glucoisosaccharino-1,4-lactone **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 1h, then acetyl chloride (250 µL; 3.40 mmol; 1.1 eq) was added cautiously. The reaction was allowed to continue for a further 2 h at room temperature. After 2 h, the reaction was halted with DCM (50 mL), followed by water (50 mL). The aqueous layer was further extracted with DCM (2 x 30 mL) and the combined organic layer was dried over anhydrous sodium sulphate and concentrated to give a crude **13** (3.30 g) as a brown syrup which was purified using column chromatography to give the desired product as a white solid (300 mg; 0.754 mmol; Yield: 24 %); (RF: 0.42; Hexane/EtOAc 5:1 v/v). IR (ATR) υ 3420 (O-H), 2954, 2930, 2857, 1750 (C=O), 1463, 1377 (C-H), 1203, 1129 (C-O), 1044 (Si-OR), 1011, 833, 777. ^{1}H NMR (400 MHz, $CDCl_3$) 4.72-4.68 (m, 1H, H-4), 4.37 (d, 1H, $J_{6,6'}$ = 11.56Hz, H-6), 4.19 (d, 1H, $J_{6',6}$ = 11.56 H-6'), 3.92 (dd, 1H, $J_{5,5'}$ = 11.72, $J_{5,4}$ = 3.12 Hz, H-5), 3.66 (dd, 1H, $J_{5',5}$ = 11.72, $J_{5',4}$ = 3.36 Hz, H-5'), 2.38 (dd, 1H, $J_{3,3'}$ = 13.83, $J_{3',4}$ = 8.08 Hz, H-3), 2.23 (dd, 1H, $J_{3',3}$ = 13.83, $J_{3',4}$ = 6.88 Hz, H-3'), 2.08 ($\underline{CH_3}CO$), 0.87 (s, 9H, TBDMS), 0.06 & 0.05 (2s, 6H TBDMS). ^{13}C (100 MHz, CDCl3) 175.5 (C1), 170.8 (C7), 77.97(C4), 74.9 (C2), 65.6 (C6), 63.3 (C5), 33.7 (C3), 25.8 (TBDMS), 20.7 (C8), -5.4, -5.5 (TBDMS). HRMS (m/z): Calculated mass for $C_{14}H_{26}O_6Si$ $[M+Na]^+$ 341.1391, Found: 341.1390.

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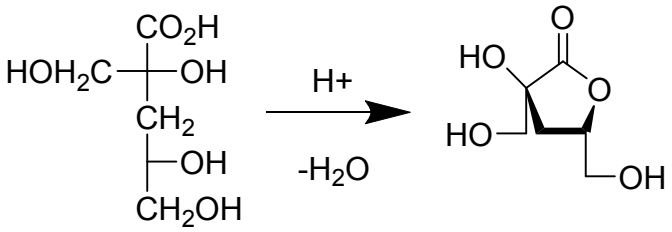
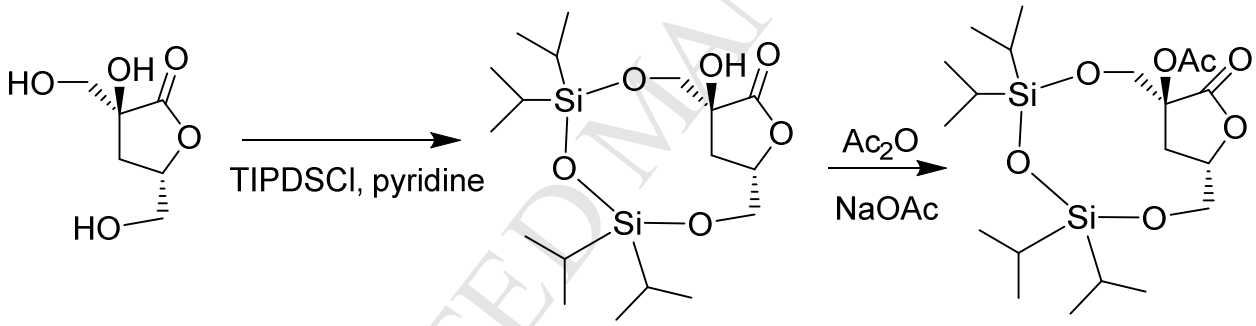
Scheme 1.



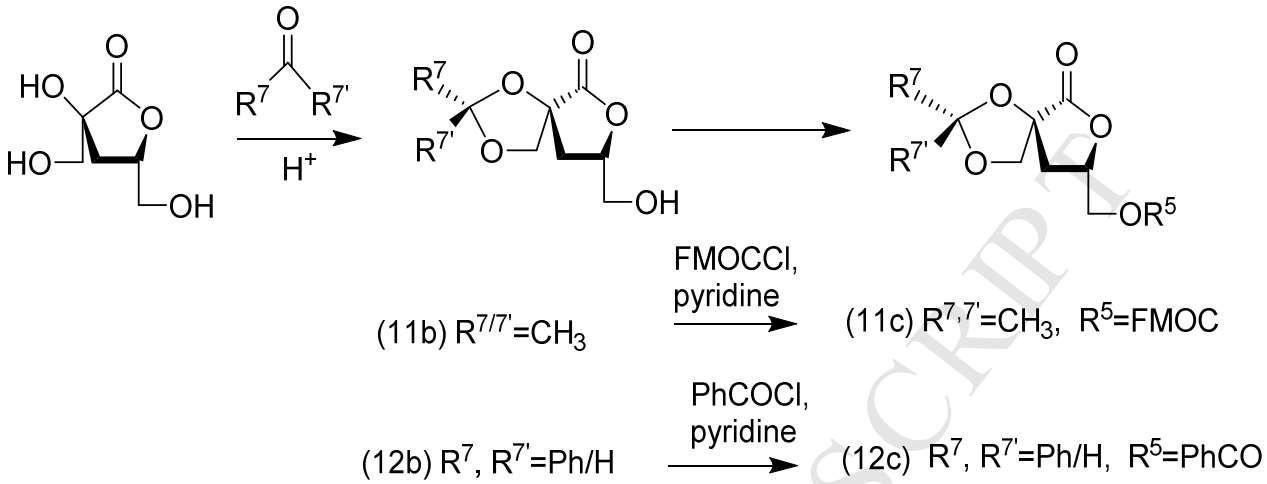
(1) **(2)**

Scheme 2.

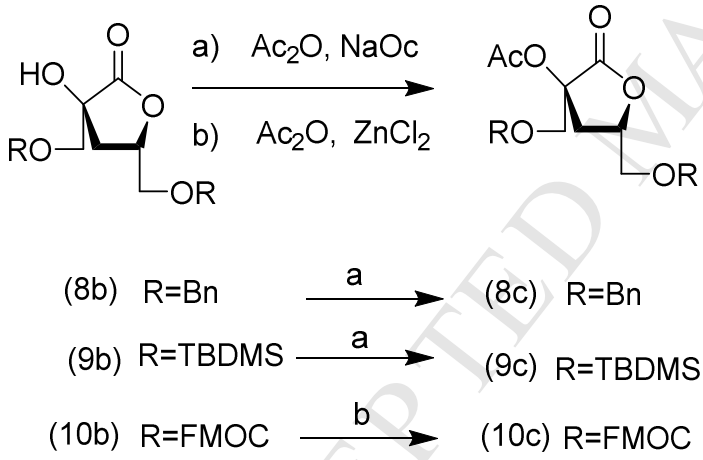


14 **15**

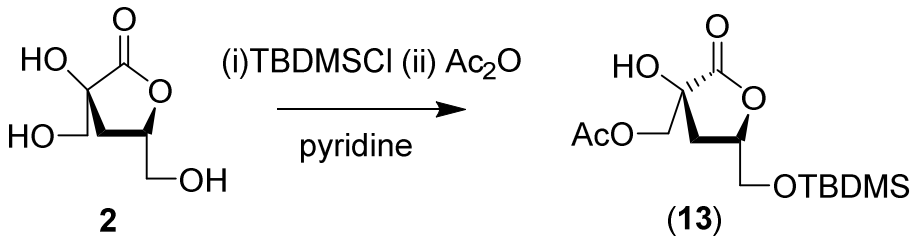
Scheme 3.



Scheme 4.



Scheme 5.



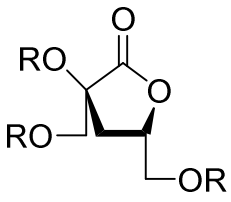
2 (**13**)

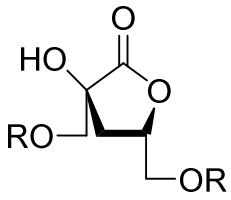
Figure 1.

(3a) R=Ac

(4a) R=Bz

(5a) R=Ms





(3b) R= Ac

(6b) R=Ts

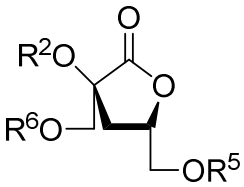
(7b) R=Tr

(8b) R=Bn

(9b) R=TBDMS

(10b) R=FMOC

Figure 2.



(3d) R^2= Ac, R^5=R^6=H

(7e) R^2= H, R^5=Tr R^6=H

(7f) R^2=R^5=H R^6=Tr

(9e) R^2= H, R^5=TBDMS R^6=H

(10e) R^2= H, R^5=FMOC R^6=H

(10f) R^2=R^5=H R^6=FMOC

Legends for Figures and Schemes:

Scheme 1. Acid catalysed lactonisation of α-GISA(**1**) to generate α-GISAL(**2**)

Scheme 2. Synthesis of 5,6-cyclic-*O*-TIPDS-α-GISAL (**14**) and its conversion to 2-*O*-acetyl-5,6-TIPDS-α-GISAL(**15**).

Scheme 3. Synthesis of 2,6-cyclic-O-acetals(**11b** & **12b**) and their further elaboration through addition of orthogonal protecting groups at the 5-OH: synthesis of 5,6-orthogonally protected α-GISAL derivatives (**11c** and **12c**).

Scheme 4. Addition of orthogonal protecting groups to the primary versus tertiary alcohol groups.

Scheme 5. Synthesis of a 5,6-orthogonaslly protected α-GISAL derivative (**13**) in a one pot sequential reaction sequence.

Figure 1. 2,5,6-Tri-*O*-protected (**3-5a**) and 5,6-di-O-protected-α-GISAL (**3b, 6b-9b**).

Figure 2. Mono-*O*-protected α-GISAL derivatives (**3d, 7e, 7f, 9e, 10e** and **10f**).

Highlights.

Synthesis of novel bis-5,6-di-*O*-protected-α-glucoisosaccharinic acid derivatives;

Synthesis of novel tris-2,5,6-tri-O-protected-α-glucoisosaccharinic acid derivatives;

Synthesis of 5,6-di-*O*-protected α-glucoisosaccharinic acids with orthogonal protection;

Synthesis of novel cyclic-2,6-di-O-protected α-glucoisosaccharinic acid derivatives;

Synthesis of novel cyclic-5,6-di-O-protected α-glucoisosaccharinic acid derivatives;