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Developing Cellulosic Waste Products as Platform Chemicals: Protecting Group Chemistry of \( \alpha \)-Glucoisosaccharinic Acid.

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**Abstract**

Alpha and beta-glucoisosaccharinic acids (\((2S,4S)-2,4,5\)-trihydroxy-2-(hydroxymethyl)pentanoic acid) and \((2R,4S)-2,4,5\)-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 (\(\alpha\)-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 \(\alpha\)-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 \(\alpha\)-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 2S-epimer, alpha-glucoisosaccharinic acid (α-GISA (1); (2S,4S)-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 2S-epimer precipitates whilst the 2R-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. Acid catalysed lactonisation of α-GISA(1) to generate α-GISAL(2)
Despite the ease of preparation of $\alpha$-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 $\alpha$-GISA (1) into the synthesis of a range of anthracycline analogues. Monneret et al have incorporated $\alpha$-GISA (1) into the synthesis of nucleoside analogues with antiviral or antitumor activity[15]. Hanessian and Roy have utilised $\alpha$-GISA (1) in the synthesis of the antibiotic spectinomycin[16]. Thomassigny et al have incorporated $\alpha$-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 starting 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 $\alpha$-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-GISAL (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. 2,5,6-Tri-O-protected (3-5a) and 5,6-di-O-protected-α-GISAL (3b, 6b-10b).](image)

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 α-glucosacosaccharino-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. Giordan 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 TIPDSCI 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).

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 FMOCCI, 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 (7R:7S; 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. 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).

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-dibenzy1-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. Addition of orthogonal protecting groups to the primary versus tertiary alcohol groups.
Reaction of the 2,6-O-isopropylene-α-GISALs 11b with FMOCCI provided the opportunity to place orthogonal protecting groups onto the primary alcohols, 5-OH versus 6-OH, and gave the 2,6-O-isopropylene-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-benzylidene-5-O-benzoyl-α-GISALs 12c also in low yield (20%).

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

![Chemical structure](image)

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 TBSDMSCI 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. 2) 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

[Diagram]

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

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 °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$_2$SO$_4$/H$_2$O 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$_2$O, deuterated acetone or CDCl$_3$ and referenced to either internal tetramethylsilane ($\delta = 0$ ppm), internal CDCl$_3$ ($^1$H $\delta = 7.23$ ppm and $^{13}$C $\delta = 77.00$ ppm) or internal HOD ($^1$H $\delta = 4.65$ 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 $\alpha$-glucoisosaccharinic acid 1 and $\alpha$-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 (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 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) ν 2959 (C-H), 1781 & 1737 (C=O), 1437, 1370 (C-H), 1202, 1045 (C-O).

1H NMR (400 MHz, CDCl₃): 5.01-4.95 (m, 1H, H-4), 4.30 (s, 2H, H-6s), 4.27 (dd, 1H, J₅',₄ = 3.4 Hz, J₅,₅ = 12.3 Hz, H-5'), 4.13 (dd, 1H, J₅,₄ = 6.7 Hz, J₅,₅' = 12.3 Hz, H-5'), 2.50 (dd, 1H, J₃,₄ = 9.0 Hz, J₃,₅ = 14.7 Hz, H-3), 2.25 (dd, 1H, J₅',₄ = 6.3 Hz, J₅,₅' = 14.7 Hz, H3'), 2.11, 2.10, 2.08 (3s, 9H, 3 x CH₃CO);

13C NMR (100 MHz, CDCl₃): δ 172.0 (C1), 170.6, 170.0, 169.9 (3 x CH₃-CO), 77.9 (C2), 74.7 (C4), 65.3 (C6), 64.8 (C5), 32.1 (C3), 20.7, 20.6, 20.5 (3 x Me-CO).


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) ν 1771 & 1722 (C=O), 1451 (Ar C-C), 1262, 1233, 1092 & 1062 (C-O), 701 & 684 (Ar C-H). 1H NMR (400 MHz, CDCl₃): δ 8.08-7.48 (m, 15H, 3 x Ph), 5.40 (m, 1H, H-4), 4.93 (d, 1H, J₆,₆' = 11.2 Hz, H-6), 4.70 (d, 1H, J₆,₆' = 11.2 Hz, H-6'), 4.65 (dd, 1H, J₅,₄ = 3.4 Hz, J₅,₅' = 12.3 Hz, H-5),
4.53 (dd, 1H, J6,5 = 6.5 Hz, J5,5 = 12.3 Hz, H-5'), 2.82 (dd, 1H, J3,4 = 8.8 Hz, J3,3' = 15.2 Hz, H-3), 2.62 (dd, 1H, J3,4 = 8.8 Hz, J3,3' = 15.2 Hz, H-3'). 13C NMR (100 MHz, CDCl3): \[ \delta \]

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 C27H22O8 (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 °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 °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 x 25 mL). The organic extracts were combined, washed with 5 % sodium bicarbonate (2 x 25 mL) and saturated brine (2 x 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) v 773.6 (CO), 1347.5, 1172.0 (SO2). 1H NMR (400 MHz, d-DMSO): \[ \delta \] 5.02 (m, 1H, H-4), 4.52 (dd, 1H, J5,5' = 11.7 Hz, J5,4 = 2.6 Hz, H-5), 4.37 (dd, 1H, J5',4 = 6.3 Hz, J5,5' = 11.7 Hz, H-5'), 4.01 (2 x d, 2H, J8,8' = 7.0 Hz, H-6,6'), 3.24, 3.29, 3.39 (3s, 9H, 3 x Me-SO3), 2.89-2.47 (m, 2H, H-3,3'). 13C 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-SO₃). HRMS (m/z) Calcd for C₁₉H₁₆O₁₁S₃ (M+NH₄)⁺: 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) ν 3079 (O-H), 1781 & 1743 (C=O), 1482, 1373 (C-H), 1233, 1196 (C-O).¹H NMR (400 MHz, CDCl₃) δ 4.82-4.76 (m, 1H, H-4), 4.22 (dd, 1H, J₅,₄ = 2.88 Hz, J₅,₅ = 12.4 Hz, H-5), 4.20 (2d, 2H, J₆,₆ = 1.16 Hz, H-6 & 6'), 4.04 (dd, 1H, J₅,₄ = 6.28 Hz, H-5'), 2.23 (dd, 1H, J₃,₄ = 6.20 Hz, J₃,₃' = 13.54 Hz, H-3), 2.07 (dd, 1H, J₅,₄ = 9.32 Hz, J₃,₃' = 13.52 Hz, H-3') 1.94 & 1.89 (2s, 6H, 2 x CH₃CO); ¹³C NMR (100 MHz, CDCl₃): 175.4 (C1), 170.4 & 170.1 (2 x CH₃CO), 74.9 (C4), 74.0 (C2), 65.0 (C6), 64.6 (C5), 35.1 (C3), 20.6 & 20.5 (2 x CH₃CO). HRMS (m/z): Calcd for C₁₀H₁₄O₇ (M+NH₄)⁺: 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) δ 3460.1 (OH), 1782.1 (CO) 1597.1, 1354.3, 1171.3, 810.6. 1H NMR (400 MHz, CDCl3): 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, J6,6' = 10.6Hz, H-6), 4.07 (d, 1H, J6',6 = 10.6 Hz, H6'), 2.48-2.44 (m, 6H, CH3-Ph), 2.37 (m, 1H, H-3), 2.22 (m, 1H, H-3'). 13C NMR (100 MHz, CDCl3): 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), 21.72 (2 x CH3Ar); HRMS (m/z): Calcd for C20H22O5S2 (M+NH4)+: 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 °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 x 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) v 1779 (CO) 762.2, 745. 1H NMR (400 MHz, CDCl3) δ 7.48-7.27 (m, 30H, 6 x
PhH), 4.82 (m, 1H, H-4), 3.41 (d, 1H, J6,6 = 9.1 Hz, H-6), 3.30 (d, 1H, J6,6 = 9.1 Hz, H-6'),
3.36 (dd, 1H, J5,4 = 6.0 Hz, J5,5' = 10. 5 Hz, H-5), 3.28 (dd, 1H, J5,4 = 3.8 Hz, J5,5 = 10.5
Hz, H-5'), 2.20 (m, 2H, H-3); 13C NMR (100 MHz, CDCl3): 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 C44H38O5 (M+Na)+: 669.2611, Found:
669.2592.

7e IR (ATR) v 3353.1 (OH) 1774.0 (CO) 763.4, 745.8, 697.7. 1H NMR (400 MHz, CDCl3) δ
7.46-7.27 (m, 15H, 3 x PhH), 4.77 (m, 1H, H-4), 3.91 (dd, 1H, J5,4 = 2.8 Hz, J5,5' = 12.7 Hz,
H-5), 3.65 (dd, 1H, J5,4 = 5.1 Hz, J5,5 = 12.7 Hz, H-5'), 3.42 (d, 1H, J6,6' = 9.2 Hz, H-6),
3.32 (d, 1H, J6,6 = 9.2 Hz, H-6'), 2.33 (dd, 1H, J3,4 = 7.1 Hz, J3,3' = 13.8 Hz, H-3), 2.21 (dd,
1H, J3,4 = 8.5 Hz, J3,3' = 13.8 Hz, H-3'); 13C NMR (100 MHz, CDCl3): 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).

7f IR (ATR) v 3365.8 (OH) 1772.8. (CO) 763.6, 746.0, 697.2. 1H NMR (400 MHz, CDCl3):
δ 7.46-7.27 (m, 15H, 3 x PhH,), 4.89 (m, 1H, H-4), 3.84 (d, 1H, J6,6' = 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, $J_{3,3'} = 13.7$ Hz, H-3'), 13C 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-glucosioisosaccharino-1,4-lactone (8b)

The dibenzyl derivative 8b was synthesised using a method adapted from that described by Giordano and Iadonisi [29]. Dried α-glucosioisosaccharino-1,4-lactone 2 (1.0 g, 6.17 mmol) was dissolved in N,N-diisopropylethylamine (DIPEA) (2.3 mL, 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 °C. A second portion of BnBr and DIPEA (2 eqs each) were added and the reaction continued for further 2 h at 90 °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%. 1H 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, $J_{3,4} = 3.48$ 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'); 13C 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-butylidimethylsilyl 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% CuSO₄ 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) $\nu$ 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, CDCl₃) 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, $J_{5',4} = 7.40$ Hz, $J_{5',3} = 14.02$ Hz, H-3'), 0.86 (2s, 18H, 2 x TBDMS), 0.05 (m, 12H, 2 x TBDMS); $^{13}$C NMR (100 MHz, CDCl₃): 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 C₁₈H₃₈Si₂O₅ [M+Na]$^+$: 413.2150, Found: 413.2152.

4.3.6 (1',1',3',3'-Tetraisopropylidisiloxane-1,3-diyl)-5,6-α-D-glucoisosaccharino-1,4-lactone (14)
Dried α- D-glucosiosaccharino-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-Cl₂) (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 CuSO₄ 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) ν 2945, 2867, 1771 (C=O), 1464, 1387, 1084, 1042, 1012. ¹H NMR (400 MHz, CDCl₃) 4.70-4.62 (m, 1H, H-4), 4.62 (dd, 1H, J₆,₆' = 10.6 Hz, H-6) 3.94-3.85 (m, 2H, H-5, H-5'), 3.83 (d, 1H, J₆,₆' = 10.6 Hz, H-6'), 2.82 (dd, 1H, J₃,₃' = 13.9 Hz, J₃,₄ = 2.4 Hz, H-3), 2.30 (dd, 1H, J₃,₃' = 13.9 Hz, J₃,₄ = 2.4 Hz, H-3'), 1.1-0.9 (m, 28H, TIPDS).

¹³C (100 MHz, CDCl₃) 178.2 (C1), 1464, 1387, 1084, 1042, 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(CH₃)DS)

HRMS (m/z) calculated mass for C₁₈H₃₆O₆Si₂[M+NH₄]⁺ 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) ν 2944.6, 2867.5, 1779.5 & 1742.1 (C=O), 1463.9, 1369.8, 1084, 1252.1, 1215.1, 1082.5, 1043.2 (RsSi-O-SiR₃), 883.1. ¹H NMR (400 MHz, CDCl₃) 4.82-4.78 (m, 1H, H-4), 4.09 (dd, 1H, J₅,₅' = 12.02 Hz, J₅,₄ = 3.56 Hz, H-5), 4.05 (d, 1H, J₆,₆' = 11.6 Hz, H-6), 4.00 (d, 1H, J₆,₆' = 11.6 Hz, H-6'), 3.85 (dd, 1H, J₅,₅' = 12.0 Hz, J₅,₄ = 2.16 Hz, H-5'), 2.75
460 (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-
461 3'). 2.12 (s, 3H, OCH\(_3\)) 1.1-1.0 (m, 28H, TIPDS).

462 \(^{13}\)C (100 MHz, CDCl\(_3\)) 173.6 (C1), 170.4 (COCH\(_3\)) 81.7 (C2), 77.0(C4), 64.7 (C5), 64.5
463 (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
464 (TIPDS)
465 HRMS (m/z) calculated mass for C\(_{20}\)H\(_{38}\)O\(_7\)Si\(_2\) [M+NH\(_4\)]\(^+\) 464.2494 found 464.2503.

466 4.3.7. 5,6-Di-O-fluorenylmethoxycarbonyl-\(\alpha\)-D-glucoisosaccharino-1,4-lactone (10b)
467 \(\alpha\)-D-Glucoisosaccharino-1,4-lactone 2 (2.01 g, 12.4 mmol) and dimethylaminopyridine
468 (DMAP, 0.50 g) were dissolved in anhydrous pyridine (40 mL) and stirred under an
469 atmosphere of nitrogen for 20 min. The mixture was slowly added to a second reaction
470 vessel, cooled to 0\(^\circ\)C, containing fluorenylmethoxycarbonyl chloride (7.05 g, 273 mmol,
471 2.2 eq). After the addition was complete, the reaction was allowed to reach room
472 temperature and was stirred, under an atmosphere of nitrogen, for a further 3 h. During
473 this time a large quantity of colourless pyridinium hydrochloride precipitated from solution.
474 The reaction was quenched by adding ice-cold water (100 mL), followed by ice-cold diethyl
475 ether (100 mL). The organic layer was separated and the aqueous phase was extracted
476 with diethyl ether (3 x 100 mL). The combined organic fractions were washed with a large
477 quantity of brine (3 x 100 mL) to remove pyridine. The resulting solution was dried over
478 anhydrous sodium sulphate, before being concentrated under reduced pressure. The
479 crude product was a bright yellow crystalline syrup (3 g) The product was separated via
480 chromatography (eluting with a mobile phase compose of Hexane/EtOAc 1:1 v/v). The
481 target compound 10b (\(R_f = 0.47\) Hexane/EtOAc; 1:1v/v) was recovered as a pale yellow
482 solid (yield: 1.47 g, 2.45 mmol, 19.8 %). IR (ATR) \(\nu\) 2945, 2867, 1771 (C=O), 1464, 1387,
483 1084, 1042 (R\(_3\)Si-O-SiR\(_3\)), 1012.\(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.90-7.84 (m, 4H, ArH),
484 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-
4.03-4.52 (m, 10H, 2 x H-5s, 2 x H-6s, 4 x H-8s & 2 x H-9s), 2.44 (dd, 1H, J₃,₄ = 6.95 Hz, J₃,₃' = 14.2 Hz, H-3'), 2.24 (dd, 1H, J₃,₄ = 5.67 Hz, J₃,₃' = 14.2 Hz, H-3); ¹³C NMR (100 MHz, CDCl₃): 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 °C. HRMS (m/z): Calcd for C₃₆H₃₀O₉ [M+NH₄]⁺ 624.2228, Found: 624.2228.

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

4.4.1 5-Ο-Fluorenylmethoxycarbonyl-2,6-Ο-isopropylène-α-D-glucoisosaccharino-1,4-lactone (11c)

2,6-Ο-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°C, containing crystalline FMOCCI (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) ν: 2945, 2867, 1771 (C=O), 1464, 1387, 1084, 1042 (R₃Si-Ο-SiR₃), 1012.

¹H NMR (400 MHz, CDCl₃) 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, J₃,₃' = 14.38 Hz, J₃,₄ = 7.05 Hz, H-3); 2.20 (dd, 1H, J₃,₃' = 14.07 Hz, J₃,₄ = 7.47 Hz, H-3'); 1.49 (bs, 6H, 2 x CH₃). ¹³C NMR (100 MHz, CDCl₃): 174.8 (C1), 154.6 (FMOCCO): 142.8, 141.3, 127.9, 127.1, 125.0, 119.9 (ArC), 112.7
(C7), 80.8 (C2), 74.4 (C4), 72.0 (C6), 70.1 (C5), 67.7 (FMOCCH) 46.4 (FMOCCH$_2$) 36.5 (C3), 26.7 (C8), 25.3 (C9). HRMS (m/z) Calcd for C$_{24}$H$_{24}$O$_7$ [M+Na$^+$]: 447.1414, Found: 447.1415.

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

Synthesis of (7S)- and (7R)-2,6-O-benzylidene-α-D-glucoisosaccharino-1,4-lactone 12b -

Freshly distilled benzaldehyde (50 mL; 492 mmol) was added to a round bottomed flask (100 mL) containing α-glucoisosaccharino-1,4-lactone 2 (1.02 g; 6.27 mmol), p-TSA (20 mg) and ~ 30 4Å molecular sieves. The mixture was left to reflux under a slight vacuum for 4 h at 85 °C. After cooling to room temperature, the mixture was gravity filtered to remove the molecular sieves and excess benzaldehyde was removed by vacuum distillation to give the crude product as a semi-crystalline syrup. The crude mixture was purified by column chromatography (fractions were eluted with chloroform with increasing portions of methanol: 1-10%). The product eluted in two distinct bands which, after evaporating to dryness gave 0.90 g and 0.26 g of the required diastereoisomers with a combined yield of 78 %. Using NOESY NMR spectra, it was determined that the first fraction (Rf: 0.17, CHCl$_3$/MeOH 95:5 v/v) was the 7R-diastereomer of 12b whilst the second fraction (Rf: 0.26, CHCl$_3$/MeOH 95:5 v/v) contained the 7S-diastereomer of 12b.

$^1$H NMR 7S-diastereomer of 12b (400 MHz, d$_2$-DMSO): 7.35-7.55 (m, 5H, ArH), 5.98 (s, 1H, PhCH), 5.25 (s, 1H, OH), 4.71 (m, 1H, H-4), 4.33 (d, 1H, J$_{5,6}$ = 9.0 Hz, H-6), 4.16 (d, 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$_{5',6}$ = 3.2 Hz, J$_{5',5}$ = 12.2 Hz, H-5') 2.49 (m, 2H, H-3,3'). $^{13}$C NMR (100 MHz, d$_2$-DMSO): 176.4 (C1), 136.7, 127.4, 128.8, 130.2 (ArC), 104.9 (C7), 81.2 (C2), 78.7 (C4), 35.4 (C3), 62.5 (C5), 72.9 (C6).

$^1$H NMR 7R-diastereoisomer 12b (400 MHz, d$_2$-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$_{5,6}$ = 9.5 Hz, H-6), 4.04 (d,
1H, J\textsubscript{6',6} = 9.5 Hz, H-6'), 3.68 (m, 1H, H-5), 3.49 (dd, 1H, J\textsubscript{5',4} = 3.4 Hz, J\textsubscript{5',5} 12.3 Hz, H-5'), 2.60 (dd, 1H, J\textsubscript{3',4} = 7.7 Hz, J\textsubscript{3,3'} = 13.8 Hz, H-3'), 2.33 (dd, 1H, J\textsubscript{3',4} = 6.0 Hz, J\textsubscript{3',5'} 12.3 Hz, H-3'), 13C 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 °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) ν 1766.9 & 1727.2 (CO) 759.4, 708.6., 695.0. 1H 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\textsubscript{5,4} = 6.7 Hz, J\textsubscript{5,5'} = 12.4 Hz, H-5), 4.57 (dd, 1H, J\textsubscript{5',4} = 2.7 Hz, J\textsubscript{5',5'} = 12.4 Hz, H-5'), 4.33-4.31 (2 x d, 2H, J\textsubscript{6,6'} = 8.8 Hz, H-6, H-6'), 2.64 (m, 2H, H-3, H-3'). 13C NMR (100 MHz, d-DMSO): 175.2 (C1), 165.9 (PhCO), 136.4, 134.1,130.3, 129.8, 129.7, 129.3,
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) ν 2866, 1775 & 1740 (C=O), 1453, 1369, 1205 & 1096 (C–O), 736, 697. 1H NMR (400 MHz, CDCl3) 7.33–7.26 (m, 10H, ArH), 4.96–4.90 (m, 1H, H-4), 4.52-4.49 (2d, 4H, J7,7′ = 4.72 Hz, H-7s), 3.70 (m, 2H, H-6), 3.63 (dd, 1H, J5,4 = 3.96 Hz, J5,5′ = 10.7 Hz, H-5), 3.57 (dd, 1H, J5,4 = 5.04, J5,5′ = 10.7 Hz, H-5′), 2.60 (dd, 1H, J5,4 = 5.84 Hz, J3,3′ = 14.3 Hz, H-3), 2.42 (dd, 1H, J3,4 = 5.12 Hz, J3′,3 = 14.3 Hz, H-3′), 2.10 (s, 3H, CH3CO). 13C NMR (100 MHz, CDCl3): 173.66 (C1), 170.00 (CH3CO), 137.72 & 137.20 (PhCq), 128.48, 128.46, 127.89, & 127.78 (PhC), 79.44 (C2), 76.51 (C1), 73.86 & 73.46 (PhCH2), 71.59 (C6), 71.10 (C5), 31.96 (C3), 20.63 (CH3CO). HRMS (m/z) Calcd for C22H24O6 [M+NH4]+: 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) ν 2954, 2929, 2857, 1783 & 1747 (C=O), 1472, 1369, 1251, 1209 (C-O), 832, 776. 1H NMR (400 MHz, CDCl3)
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_{5,4} = 6.30 Hz, J_{5,3} = 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): 177.30 (C1), 169.74 (CH_3CO), 80.31 (C2), 77.57 (C4), 65.36 (C6), 64.54 (C5), 31.57 (C3), 20.43 (CH_3CO), -5.23, -5.55, -5.60 (TBDMS).

HRMS (m/z) Calcd for C_{45}H_{61}O_{10}Si_{2}O_{6} [M+Na]^+: 648.1995, found 648.1992.

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

5,6-di-O-FMOC-\alpha-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 °C and the reaction was allowed to proceed for 4 h at 100 °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) v 1784, 1745 & 1709 (C=O), 1253, 1206 (C=O), 784, 759, 739. \(^1H NMR (400 MHz, CDCl_3)\) 7.77-7.73 (m, 4H, ArH), 7.61-7.56 (m, 4H, ArH), 7.42-7.36 (m, 4H, ArH), 2.60 (dd, 1H, J = 9.38 Hz, J = 14.32 Hz, H-3), 2.43 (dd, 1H, J = 3.3', 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 (ArG), 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_{45}H_{61}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 TBDMSI (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% CuSO₄, 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) v 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. ¹H NMR (400 MHz, CDCl₃) 4.72–4.69 (m, 1H, H-4), 3.87 (dd, 1H, J₅,₄ = 3.20 Hz, J₅,₅ = 11.70 Hz, H-5) 3.78 (d, 1H, J₆,₆' = 11.80 Hz, H-6), 3.69 (d, 1H, J₆',₆ = 11.83 Hz, H-6'), 3.66 (dd, 1H, J₅',₄ = 3.76 Hz, J₅',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). ¹³C NMR (100 MHz, CDCl₃): 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 C₁₂H₂₄SiO₅ [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 °C crystalline
9-fluorenymethyloxycarbonyl 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). 1H NMR
(400 MHz, CDCl3, 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'). 13C (100 MHz, CDCl3) 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). 1H NMR
(400 MHz, CDCl3, 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'). 13C (100 MHz, CDCl3)
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
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 1 h, 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) ν 3420 (O-H), 2954, 2930, 2857, 1750 (C=O), 1463, 1377 (C-H), 1203, 1129 (C-O), 1044 (Si-OR), 1011, 833, 777.

1H NMR (400 MHz, CDCl3) 4.72-4.68 (m, 1H, H-4), 4.37 (d, 1H, J6,6′ = 11.56 Hz, H-6), 4.19 (d, 1H, J6′,6 = 11.56 Hz), 3.92 (dd, 1H, J5,5′ = 11.72, J5,4 = 3.12 Hz, H-5′), 3.66 (dd, 1H, J5′,5 = 11.72, J5′,4 = 3.36 Hz, H-5), 2.38 (dd, 1H, J3,3′ = 13.83, J3′,4 = 8.08 Hz, H-3), 2.23 (dd, 1H, J5,3 = 13.83, J5′,4 = 6.88 Hz, H-3′), 2.08 (CH3CO), 0.87 (s, 9H, TBDMS), 0.06 & 0.05 (2s, 6H TBDMS). 13C (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 C14H26O6Si [M+Na]+ 341.1391, Found: 341.1390.
References


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