1

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

Michael Almond[a], Mustapha G. Suleiman[a], Matthew Hawkins[a], Daniel Winder[a], Thomas Robshaw[a], Megan Waddoups[a], Paul N. Humphreys[b], Andrew P. Laws[a]*

[a]Department of Chemical Sciences, School of Applied Sciences, University of Huddersfield, Queensgate Huddersfield HD1 3DH, United Kingdom.

[b]Department of Biological Sciences, School of Applied Sciences, University of Huddersfield, Queensgate Huddersfield HD1 3DH, United Kingdom.

*Corresponding author. Tel.: +44(0) 1484 472668; fax: +44(0) 1484 472182
Email address: a.p.laws@hud.ac.uk

29   **Abstract**

30   Alpha and beta-glucoisosaccharinic acids ((2*S*,4*S*)-2,4,5-trihydroxy-2-

31   (hydroxymethyl)pentanoic acid and (2*R*,4*S*)-2,4,5-trihydroxy-2-(hydroxymethyl)pentanoic

32   acid) which are produced when cellulosic materials are treated with aqueous alkali are

33   potentially valuable platform chemicals. Their highly functionalised carbon skeleton, with

34   fixed chirality at C-2 and C-4, makes them ideal starting materials for use in synthesis. In

35   order to assess the potential of these saccharinic acids as platform chemicals we have

36   explored the protecting group chemistry of the lactone form of alpha-glucoisosaccharinic

37   acid (α-GISAL). We report here the use of single and multiple step reaction pathways

38   leading to the regioselective protection of the three different hydroxyl groups of α-GISAL.

39   We report strategies for protecting the three different hydroxyl groups individually or in

40   pairs. We also report the synthesis of a range of tri-O-protected α-GISAL derivatives

41   where a number of the products contain orthogonal protecting groups.

42

45

1. Introduction

48 Saccharinic acids[1, 2] are a group of branched-chain polyhydroxyl acids which are

49 generated in large quantities when cellulosic materials are treated with aqueous alkali[3].

50 The mechanism for saccharinic acid production has been studied in detail and the base

51 catalysed depolymerisation of cellulose is known to proceed via a 'peeling' reaction[4, 5]

52 [6-8]. Depending on the reaction conditions (type of alkali, length of reaction and

53 temperature) a large number of different hydroxy acids can be formed but the main

54 saccharinic acids formed from cellulose, accounting for up to 80% of the total organic

55 matter, are a pair of C-2 epimeric six carbon glucoisosaccharinic acids (GISA) [9-11].

56 Whistler and Bemiller have reported that the calcium salt of the 2*S*-epimer, alpha-

57 glucoisosaccharinic acid (α-GISA **(1)**; (2*S*,4*S*)-2,4,5-trihydroxy-2-(hydroxymethyl)pentanoic

58 acid) can be economically manufactured by heating lactose with a saturated aqueous

59 calcium hydroxide solution[12]; on cooling, the 2*S*-epimer precipitates whilst the 2*R*-epimer

60 and other impurities remain in solution. The salts of α-GISA are highly polar and have

61 limited solubility in most organic solvents. However, in the presence of mild acids α-GISA

62 **(1)** undergoes an internal esterification reaction to give the less polar α-

63 glucoisosaccharino-1,4-lactone (α-GISAL **(2)**):

64

65 **(1)** **(2)**
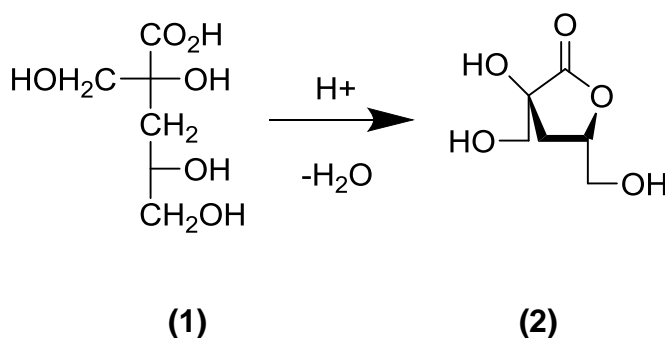
66 **Scheme 1.** Acid catalysed lactonisation of α-GISA(**1**) to generate α-GISAL(**2**)

67   Despite the ease of preparation of α-GISA **(1)** and its ready conversion to its less polar

68   lactone **(2)** the two have rarely been exploited as starting materials in synthesis. Florent *et*

69   *al[13]* and Monneret et al [14] have incorporated α-GISA **(1)** into the synthesis of a range

70   of anthracycline analogues. Monneret *et al* have incorporated α-GISA **(1)** into the

71   synthesis of nucleoside analogues with antiviral or antitumor activity[15]. Hanessian and

72   Roy have utilised α-GISA **(1)** in the synthesis of the antibiotic spectinomycin[16].

73   Thomassigny *et al* have incorporated α-GISA **(1)** into the synthesis of a small number of

74   heterocycles including variously protected pyrrolidines[17] and piperidines[18].

75   It has been estimated that many millions of metric tons of saccharinic acids are produced

76   each year as by products in the alkaline pulping of wood[19-22]. Currently, this large

77   reservoir of potentially valuable organic molecules is combusted within pulping mills to

78   recover their calorific value. Ideally, wood pulping companies would like to be able to

79   extract extra value from these saccharinic acids and one way this could be achieved is by

80   employing them as staring materials in synthetic chemistry. For this ambition to be realised

81   and to determine the true synthetic utility of GISAs it will be necessary to develop

82   strategies for the regioselective protection of the different hydroxyl groups, either

83   individually or in groups. In this paper we report our studies of the protecting group

84   chemistry of α-GISAL (**2**)**,** including the regioselective protection of different combinations

85   of the three hydroxyl groups.

86   It should be noted that whilst the gluco-prefix identifies GISAs as being derived from a 1,4-

87   glucan such as cellulose , in the early scientific literature and also in current literature

88   describing environmental aspects of GISA's properties[23-26] these molecules are

89   frequently referred to as isosaccharinic acids (ISA).

90

2. Results and Discussion

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

93   In the first set of experiments, attempts were made to protect all three hydroxyls of GISA

94   as ester derivatives (Fig. 1, 3a-5a). We have previously reported the synthesis of the

95   tribenzoyl-ester of α-GISAL **(2)** which was achieved by reaction of α-GISAL **(2)** with a

96   large excess of benzoyl chloride with pyridine as solvent and employing

97   dimethylaminopyridine as an acyl-transfer catalyst[27]. When an acetylation reaction was

98   performed with an excess of acetic anhydride with sodium acetate as a base a near

99   quantitative yield of the 2,5,6-tri-O-acetyl-α-GISA$_L$ (**3a,** 99%) was recovered. However,

100   when an attempt was made to reduce the quantity of the bulkier acylating reagents to

101   nearer stoichiometric amounts (3.3 equivalents) a mixture of di and triacylated products

102   was obtained. The trisubstituted derivative **4a** could only be produced as a single

103   compound when a large excess of benzoyl chloride was used (10 equivalents).

104
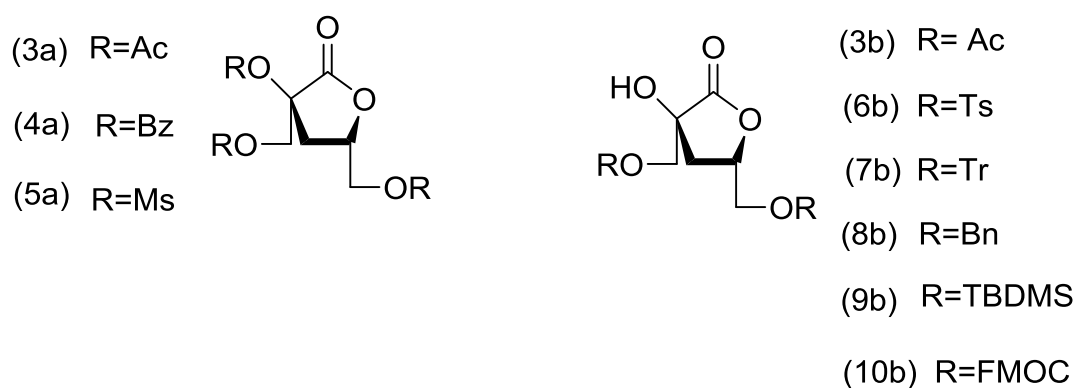
Figure 1. 2,5,6-Tri-*O*-protected (**3-5a**) and 5,6-di-O-protected-α-GISAL (**3b, 6b-10b**).

107   A similar picture emerged with the attempted synthesis of sulfonate esters. Reaction of **2**

108   with six equivalents of methanesulfonyl chloride in the presence of pyridine gave the

109   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

134 carbohydrate based polyols using a combination of benzyl bromide and the base

135 diisopropylethylamine in the presence of a di-*tert*-butyltin oxide catalyst. When the reaction

136 was applied to the lactone **2** a reasonable yield of the desired 5,6-di-*O*-benzylated product

137 **8b** (59%) was recovered.

138 Reaction of **2** with an excess of TBDMSCl in pyridine gave, after column chromatography,

139 5,6-di-*O*-TBDMS-α-GISAL **9b** as the major product (69%). In a similar reaction, treatment

140 of the lactone with TIPDSCl in pyridine afforded a high yield (82%) of the 5,6-TIPDS-α-

141 GISAL (**14**) in which the protecting group bridges between the 5 and 6-positions. The 5,6-

142 arrangement of the protecting group was confirmed by acetylating the remaining hydroxyl

143 group and identifying strong NOE contacts between the protons of the isopropyl groups

144 and the methylene protons at 5 and 6 in the acetylated product (**15**).

145

14　　　　　**15**
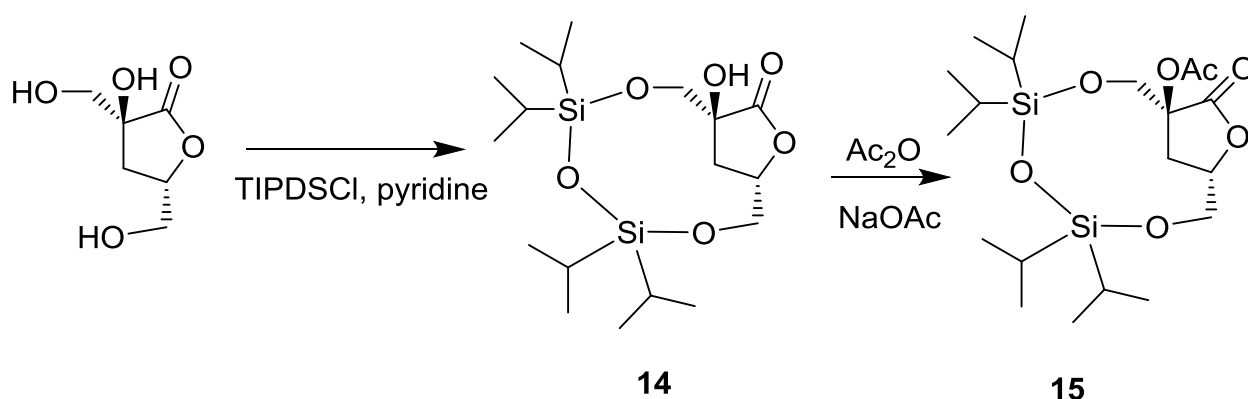
146

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

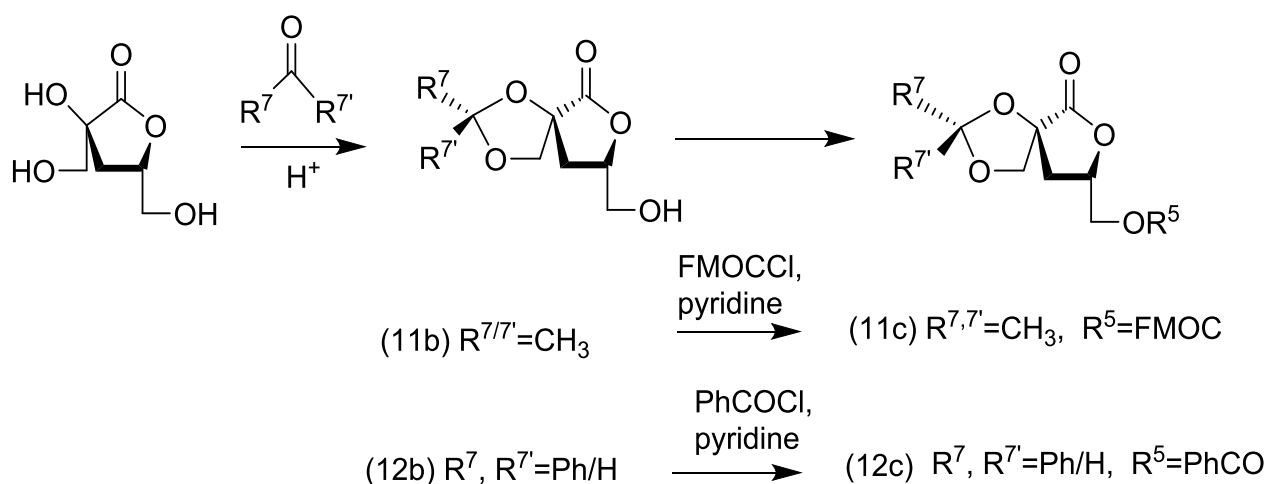148 acetyl-5,6-TIPDS-α-GISAL(**15**).

149

150 In order to expand the range of protecting groups, an attempt was made to introduce acid

151 stable carbonates at the 5 and 6-positions. Gioeli and Chattopadhyaya[30] have reported

152 the use of the FMOC-carbonate group to protect the hydroxyl groups of ribose, however,

153 when the lactone **2** was reacted with a large excess of FMOCCl, either in the presence or

154 absence of an acyl transfer catalyst, a mixture of di-protected and mono-protected

155 products were obtained. Despite using longer reaction times and up to ten equivalents of

156 the 9-fluorenylmethoxycarbonyl chloride, the maximum yield of the desired di-protected

157 product **10b** never exceeded 27%. From these studies, it was clear that the reaction had

158 reached equilibrium in which the diprotected, monoprotected and unreacted FMOCCl were

159 all present. As was the case with trityl-*O*-protection, pure samples of the desired 5,6-di-*O*-

160 FMOC-α-GISAL**10b**, the 5-mono-*O*-protected **10e** and small amounts of the 6-mono-O-

161 protected-α-GISAL **10f** were isolated by column chromatography.

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

163 The combined protection of the primary alcohol at the 6-position and the tertiary alcohol at

164 the 2-position using an isopropylidene group has previously been reported by Florent et

165 al[13]. In a similar reaction, the lactone **2** was condensed with freshly distilled

166 benzaldehyde in the presence of an acid catalyst to give the 2,6-*O*-benzylidene protected

167 lactone **12b** (78%) as a pair of diastereoisomers in a 1:3.5 ratio (7*R*:7*S*; scheme 2).

168 Reaction of the 2,6-acetal protected substrates with either FMOCCl or benzoyl chloride in

169 pyridine provided mixtures of starting materials and products, with only moderate yields of

170 the desired products being obtained after column chromatography (**11c** 14% and **12c**

171 20%). The low yields are consistent with steric crowding reducing access to tri-O-protected

172 products, especially when bulky protecting groups are employed.

173

(11b) $R^{7/7'} = CH_3$ FMOCCl, pyridine →   (11c) $R^{7,7'} = CH_3$, $R^5 = FMOC$

(12b) R^7, $R^{7'} = Ph/H$ PhCOCl, pyridine →   (12c) R^7, $R^{7'} = Ph/H$, $R^5 = PhCO$
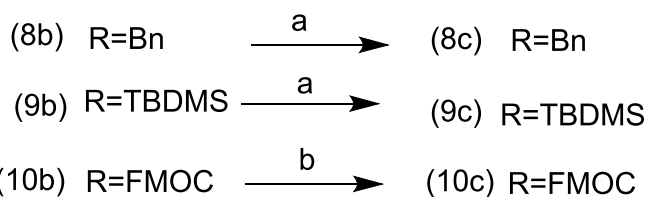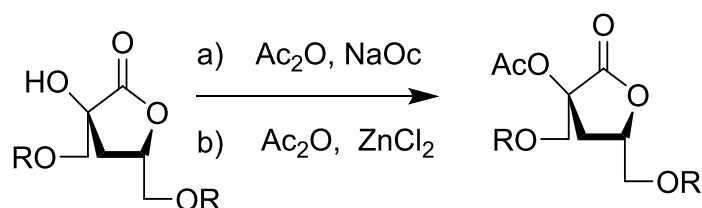
174   **Scheme 3.** Synthesis of 2,6-cyclic-O-acetals(**11b** & **12b**) and their further elaboration

175   through addition of orthogonal protecting groups at the 5-OH: synthesis of 5,6-orthogonally

176   protected α-GISAL derivatives (**11c** and **12c**).

177

178   **2.4 Preparation of 2,5,6-tri-*O*-protected-α-GISALs in two step procedures.**

179   The ease of formation of the 5,6-di-*O*-protected-α-GISALs (**6b-10b**) provided an

180   opportunity to introduce orthogonal protection at the tertiary hydroxyl groups albeit with the

181   requirement for the use of a small protecting group. Both the 5,6-di-*O*-dibenzyl-α-GISAL

182   **8b** and the 5,6-*O*-diTBDMS-α-GISALs **9b** were converted in variable but not optimised

183   yields to their 2-*O*-acetyl-5,6-di-*O*-protected-α-GISALs (**8c** 30%**, 9c** 80%) on reaction with

184   acetic anhydride using sodium acetate as a base catalyst (Fig 2; reagents a). In a similar

185   manner, treatment of the 5,6-*O*-diFMOC-α-GISAL **10b** with acetic anhydride in the

186   presence of zinc dichloride afforded the 2-*O*-acetyl-5,6-di-*O*-protected-α-GISAL **10c** (Fig.

187   2; reagents b, 55%).

188

(8b) R=Bn $\xrightarrow{a}$ (8c) R=Bn

(9b) R=TBDMS $\xrightarrow{a}$ (9c) R=TBDMS

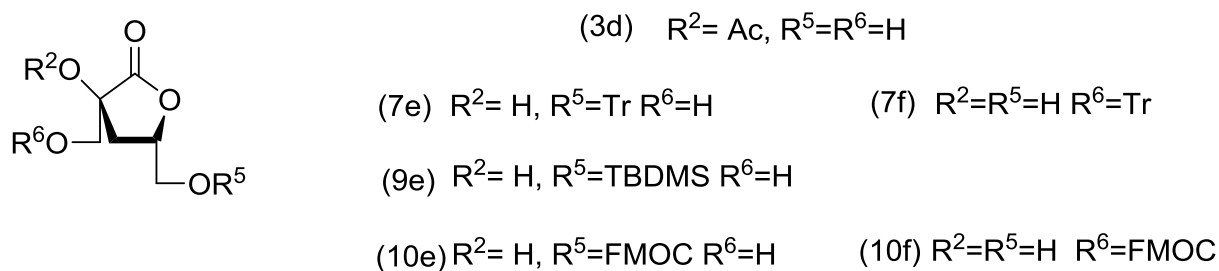(10b) R=FMOC $\xrightarrow{b}$ (10c) R=FMOC

189

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

191   groups.

192 Reaction of the 2,6-O-isopropyliene-α-GISALs **11b** with FMOCCl provided the opportunity

193 to place orthogonal protecting groups onto the primary alcohols, 5-OH versus 6-OH, and

194 gave the 2,6-O-isopropyliene-5-O-FMOC-α-GISAL **11c** but in low yield (14%). In a similar

195 reaction, treatment of **12b** with benzoyl chloride in pyridine gave the 2,6-O-benzilydene-5-

196 O-benzoyl-α-GISALs **12c** also in low yield (20%).

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

(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

198

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

200 In most cases, attempts to directly add a single protecting group to the lactone **2** did not

201 give single products: the similar reactivity of the two primary hydroxyls meant that in the

202 majority of cases mixtures of the 5,6-di-O-protected, 5-mono-O-protected and small

203 amounts of the 6-mono-O-protected-α-GISALs were recovered. However, in the majority

204 of the reactions, more of the 5-mono-O-protected product was obtained and when using

205 the relatively bulky TBSDMSCl as reagent the reaction took place exclusively at the 5-

206 position. As the starting lactone was easy to prepare and because it proved to be relatively

207 straight forward to separate the different mono-O-protected lactones, this route provided

208 an opportunity to prepare a range of mono-O-protected-α-GISAls (Fig. 2) including the

209 mono-substituted trityl-ethers (**7f,** 13% & **7e**, 2%) the silyl ether (**9e,** 46%) and the

210 carbonates (**10e,** 24% and **10f,** 56%).

211   A number of additional mono-protected products were synthesised by three step

212   procedures in which the required regioselective protection was achieved by first generating

213   a di-O-protected product, followed by the addition of a small orthogonal protecting group at

214   the remaining free-hydroxyl and then removal of the original protecting group. Treatment of

215   the 5,6-di-*O*-FMOC-2-*O*-acetyl-α-GISAL with triethylamine generated the 2-O-acetyl-α-

216   GISAL **3d** in near quantitative yield. Likewise, treatment of the 5,6-*O*-isopropylidene-2-O-

217   FMOC lactone **12c** with aqueous acid generated the 5-FMOC-α-GISALs **10e** in

218   quantitative yield.

219   **2.6 Preparation of a 5,6-di-*O*-protected-α-GISALs in a two-step one pot procedure**

220
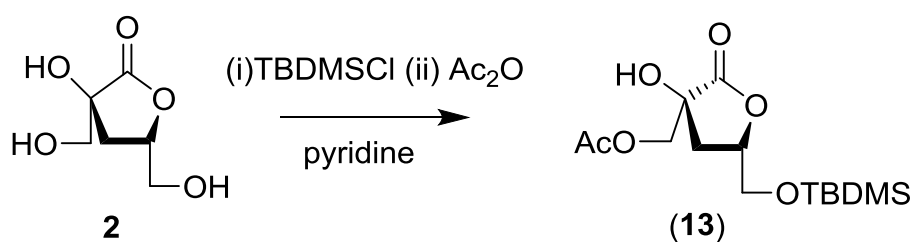
221   **Scheme 5.** Synthesis of a 5,6-orthogonally protected α-GISAL derivative (**13**) in a one pot

222   sequential reaction sequence.

223   The greater reactivity of 5-OH towards the silylating agent TBDMSCl meant that it is was

224   possible to add orthogonal protecting groups onto the primary alcohols in a sequential

225   reaction series in a one pot reaction (Scheme 3). Reaction of α-GISAL **2** with one

226   equivalent of TBDMSCl in pyridine followed by the addition of 1.1 equivalent of acetic

227   anhydride led to the isolation, after column chromatography, of the 6-*O*-acetyl-5-*O*-

228   TBDMS-α-GISAL (**13**).

229   **3. Conclusion:**

230   Many of the reactions used in this study to generate protected glucoisosaccharinic acids

231   derivatives are the same as those that are applied to protect hydroxyls in

232   monosaccharides. The main difference in their outcome is related to the steric demands of

233   trying to put bulky protecting groups on a tertiary alcohol which is alpha to a carbonyl

234   carbon. In order to get reaction at the tertiary alcohol either forcing conditions or the use of

235   small sterically undemanding protecting groups was required. Unsurprisingly, the

236   attempted synthesis of mono-protected glucoisosaccharinic acids led to the isolation of

237   mixtures of products. However, the higher reactivity of the C-5 primary hydroxyl group

238   makes this the preferred initial point of reaction and this was particularly true when

239   reaction was with a bulky-silylating agent. Despite these difficulties, the use of multiple

240   steps and the employment of orthogonally protected hydroxyls have provided access to a

241   wide range of novel α-glucoisosaccharinio-1,4-lactone derivatives which we hope will be

242   employed in the synthesis of value added products.

243

244

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_2SO_4/H_2O and/or fluorescence. 1D ^1H 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 $CDCl_3$ (^1H δ = 7.23 ppm and ^{13}C δ = 77.00 ppm) or internal HOD (^1H 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 °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<u>H$_3$</u>CO); ^{13}C NMR (100 MHz, CDCl$_3$): δ 172.0 (C1), 170.6, 170.0, 169.9 (3 x CH$_3$-<u>CO</u>), 77.9 (C2), 74.7 (C4), 65.3 (C6), 64.8 (C5), 32.1 (C3), 20.7, 20.6, 20.5 (3 x <u>Me</u>-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 Ph<u>C</u>O) 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 °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 ×

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):

316 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

317 Me-SO$_3$). HRMS (m/z) Calcd for $C_9H_{16}O_{11}S_3$ (M+NH$_4$)$^+$: 414.0193, Found: 414.0188.

318

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

320 **4.3.1.   5,6-Di-*O*-acetyl-α-D-glucoisosaccharino-1,4-lactone (3b)**

321 α-D-Glucoisosaccharino-1,4-lactone (**2**, 500 mg; 3.09 mmol) was dissolved in pyridine (5

322 mL) while stirring at room temperature for 10 min. Acetyl chloride (470 µL; 6.48 mmol, 2.1

323 eq) was added cautiously at room temperature. The reaction was allowed to proceed

324 uninterrupted for 3 h at room temperature. The reaction was halted by adding

325 dichloromethane (30 mL) followed by ultra-pure water (30 mL), the organic layer was

326 separated and the aqueous layer was further extracted with dichloromethane (2 x 30 mL).

327 The combined organic layer was washed with 1% copper sulphate solution (2 x 50 mL)

328 and dried over anhydrous magnesium sulphate, then concentrated to give 3b (1.20 g; 5.61

329 mmol; Yield: 55%) IR (ATR) υ 3079 (O-H), 1781 &1743 (C=O), 1482, 1373 (C-H), 1233,

330 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

331 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

332 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,

333 $J_{3',4}$ = 9.32 Hz, $J_{3',3}$ = 13.52 Hz, H-3') 1.94 & 1.89 (2s, 6H, 2 x CH$_3$CO); ^{13}C NMR (100

334 MHz, CDCl$_3$): 175.4 (C1), 170.4 & 170.1 (2 x CH$_3$CO), 74.9 (C4), 74.0 (C2), 65.0 (C6),

335 64.6 (C5), 35.1 (C3), 20.6 & 20.5 (2 x <u>C</u>H$_3$CO). HRMS (m/z): Calcd for $C_{10}H_{14}O_7$ (M+NH$_4$)$^+$:

336 269.0748, Found: 269.0740.

337

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$_9$S$_2$ (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 °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

363 saturated brine (100 ml) a saturated solution of sodium bicarbonate (100 ml) and dried

364 over anhydrous sodium sulphate. Evaporation of solvent produced a beige coloured solid

365 (13.9 g). Subsequent TLC analysis showed the presence of three compounds of interest.

366 Following separation by column chromatography eluting with Hex/EtOAc (2:1), the

367 desired compounds were identified as 2,5-di-*O*-trityl-α-GISAL **7b** (Rf 0.79; Hex/EtOAc (

368 (2:1)); 3.34 g; yield: 12 %, followed by the 6-mono-*O*-trityl-α-GISAL **7e** (Rf 0.29;

369 Hex/EtOAc,1:2 v/v)); 0.20 g; yield: <2 % and 5-mono-*O*-trityl-α-GISAL **7f** was recovered

370 from a chloroform wash (Rf 0.16; Hex/EtOAc, (1:2)); 2.25 g; yield: 13 %.

371 **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

372 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'),

373 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

374 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,

375 128.7, 128.0, 128.0, 127.3, 127.2, 86.9 & 87.2 (TrC*), 77.4 (C4), 75.5 (C2), 65.4 (C5),

376 65.3 (C6), 35.0 (C3).HRMS (m/z) Calcd for C$_{44}$H$_{38}$O$_5$ (M+Na)$^+$: 669.2611, Found:

377 669.2592.

378 **7e** IR (ATR) υ 3353.1 (OH) 1774.0 (CO) 763.4, 745.8, 697.7. ^{1}H NMR (400 MHz, CDCl$_3$) δ

379 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,

380 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),

381 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,

382 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 ,

383 128.7, 128.0, 127.3, 87.3 (TrC), 75.9 (C2), 78.2 (C4), 65.3 (C6), 63.6 (C5), 33.6 (C3).

384 HRMS (m/z) Calcd for C$_{25}$H$_{24}$O$_5$ [M+Na]$^+$: 427.1516, Found: 427.1513.

385 **7f** IR (ATR) υ 3365.8 (OH) 1772.8. (CO) 763.6, 746.0, 697.2. ^{1}H NMR (400 MHz, CDCl$_3$):

386 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

387 (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,

388 $J_{5',4}$ = 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

389 (dd, 1H, $J_{3',4}$ = 8.6 Hz, $J_{3,3'}$ = 13.7 Hz, H-3'); ^{13}C NMR (100 MHz, CDCl$_3$): 177.7 (C1) 143.4,

390 128.6, 128.0, 127.3, 86.9 (TrC), 77.7 (C4), 73.6 (C2), 65.5 (C6), 64.2 (C5), 34.0(C3).

391 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)

393 The dibenzyl derivative **8b** was synthesised using a method adapted from that described by

394 Giordano and Iadonisi [29]. Dried α-glucoisosaccharino-1,4-lactone **2** (1.0 g, 6.17 mmol)

395 was dissolved in *N,N*-diisopropylethylamine (DIPEA) (2.3mL, 4 eq), and a catalytic amount

396 of dibutyltin oxide (154 mg, 0.1 eq) and tetrabutylammonium bromide (597 mg, 0.3 eq) were

397 added while stirring. Benzyl bromide (BnBr) (6 mL, 8 eq), was added slowly and the reaction

398 was allowed to proceed for 4 h at 90 °C. A second portion of BnBr and DIPEA (2 eqs each)

399 were added and the reaction continued for further 2 h at 90 °C. The reaction was halted by

400 pouring the reaction solution into a mixture of DCM (50 mL) and water (50 mL). The organic

401 layer was separated, and the aqueous phase was extracted with DCM (2 x 50 mL). The

402 combined organic extracts was dried over anhydrous sodium sulphate and concentrated to

403 dryness to give crude **8b** as a golden syrup which was purified by column chromatography

404 (EtOAc:Hexane 1/1 v/v); to give the product as a transparent oil 1.24 g; yield: 59% . ^{1}H NMR

405 (400 MHz, CDCl$_3$) 7.34-7.29 (m, 10H, ArH), 4.83-4.77 (m, 1H, H-4), 4.54 (AB, 4H, $J_{7,7'}$ =

406 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-

407 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, $J_{3,3'}$

408 = 7.50 Hz, H-3, H-3'); ^{13}C NMR (100 MHz, CDCl$_3$) 176.79 (C1), 137.59, 137.39 (ArCq),

409 128.50, 127.89, 127.84, 127.79 (ArC), 76.78 (C4), 75.34(C2), 73.73, 73.56 (C7), 72.05 (C6),

410 70.88 (C5), 34.61 (C3). HRMS (m/z) Calcd for C$_{20}$H$_{22}$O$_5$ [M+Na$^+$]: 365.1359, Found:

411 365.1358.

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 <u>TB</u>DMS), 0.05
(m, 12H, 2 x TB<u>DMS</u>); ^{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 (<u>TB</u>DMS), 18.31& 18.24 (TB<u>DMS</u>).
HRMS (m/z) Calcd for C$_{18}$H$_{38}$Si$_2$O$_5$ [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$_3$Si-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, <u>TIPDS</u>).

^{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 (<u>TIP(CH)DS</u>), 13.5, 13.1, 12.6 & 12.4 (TIP(CH$_3$)<u>DS</u>)

HRMS (m/z) calculated mass for C$_{18}$H$_{36}$O$_6$Si$_2$ [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$_3$Si-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, <u>TIPDS</u>).

^{13}C (100 MHz, CDCl3) 173.6 (C1), 170.4 (<u>C</u>OCH$_3$), 81.7 (C2), 77.0(C4), 64.7 (C5), 64.5 (C6), 30.0 (C3), 20.8 (CO<u>C</u>H$_3$), 17.19, 17.15, 17.12 & 17.08 (<u>TIPDS</u>), 13.6, 13.5, 12.6 & 12.3 (TIP<u>DS</u>)

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$_3$Si-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-

485   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

486   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

487   MHz,CDCl$_3$): 175 (C1), 155 (C7), 143,141,128,127,125,120 (ArC), 75.0 (C2), 74.5 (C4),

488   70.4 (C8), 68.9 (C6), 67.8 (C5), 46.8 (C9), 34.7 (C3). Melting point: 76-77 ^{O}C. HRMS

489   (m/z): Calcd for $C_{36}H_{30}O_9$ [M+NH$_4$]$^+$ 624.2228 ,Found: 624.2228.

490   **4.4   Synthesis of 2,6-di-*O*-protected lactone derivatives (11b and 12b) and their**

491   **conversion to 2,5,6-tri-*O*-protected lactone derivatives (11c and 12c).**

492   **4.4.1 5-*O*-Fluorenylmethoxycarbonyl-2,6-*O*-isopropyliene-α-D-glucoisosaccharino-**

493   **1,4-lactone (11c)**

494   2,6-*O*-Isopropylidene-α-D-glucoisosaccharino-1,4-lactone **11b,** prepared using the

495   procedures described by Florent *et al[13]* (1.38 g, 6.83 mmol), was dissolved in anhydrous

496   pyridine (20 ml). The solution was cautiously added to a flask, maintained at 0° C, containing

497   crystalline FMOCCl (2.66 g, 0.01 mmol). The reaction was allowed to proceed for 4 h at

498   room temperature after which time it was carefully added to a beaker containing ice cold

499   water (60 ml) and diethyl ether (60 ml). The organic layer was separated and the aqueous

500   phase was extracted with diethyl ether (3 x 60 ml). The combined organic extracts were

501   washed with a saturated solution of brine (50 mL), water (50 mL) and then dried over

502   anhydrous sodium sulphate before removing the solvent at reduced pressure to give the

503   desired product **11c** as a yellow solid (570 mg, 1.34 mmol; Yield: 19.68%); (Pet. ether/EtOAc

504   3:1 v/v). IR (ATR) υ 2945, 2867, 1771 (C=O), 1464, 1387, 1084, 1042 (R$_3$Si-O-SiR$_3$), 1012.

505   ^{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,

506   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

507   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

508   (dd, 1H, $J_{3,3'}$ = 14.07 Hz, $J_{3',4}$ = 7.47 Hz, H-3'); 1.49 (bs, 6H, 2 x CH$_3$). ^{13}C NMR (100 MHz,

509   CDCl$_3$): 174.8 (C1), 154.6 (FMOC$\underline{C}$O): 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 (FMOC$\underline{C}$H) 46.4 (FMOC$\underline{C}$H$_2$) 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 7*R*- diastereomer of **12b** whilst the second fraction (Rf: 0.26, CHCl$_3$/MeOH 95:5 v/v) contained the 7*S*-diastereomer of **12b**.

^{1}H NMR 7*S*-diastereomer of **12b** (400 MHz, *d*-DMSO): 7.35-7.55 (m, 5H, ArH), 5.98 (s, 1H, PhC$\underline{H}$), 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, 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, $J_{5',4}$ = 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 (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 7*R*-diastereoisomer **12b** (400 MHz, *d*-DMSO): 7.38-7.60 (m, 5H, ArH), 5.91 (s, 1H, PhC$\underline{H}$), 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,

24

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, PhC<u>H</u>), 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 (Ph<u>C</u>O), 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$_3$CO). ^{13}C NMR (100 MHz, CDCl$_3$): 173.66 (C1), 170.00 (CH$_3$CO), 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$_3$CO). 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$_3$CO), 0.82 (2s, 18H, 2 x T$\underline{B}$DMS), 0.00

(4s, 12H, 2 x TB$\underline{DMS}$). ^{13}C NMR (100 MHz, CDCl$_3$): 173.70 (C1), 169.74 (CH$_3\underline{C}$O), 80.31

(C2), 77.57 (C4), 65.36 (C6), 64.54 (C5), 31.57 (C3), 25.70, 25.63, 25.57 ($\underline{TB}$DMS), 20.43

($\underline{CH_3}$CO), -5.23, -5.55, -5.60 (TB$\underline{DMS}$). HRMS (m/z) Calcd for C$_{20}$H$_{40}$Si$_2$O$_6$ [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 °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) ʋ 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$_3$CO). ^{13}C NMR (100 MHz, CDCl$_3$): 177.6,

177.1 (FMO$\underline{C}$O), 171.7 (C1), 170.1 (CH$_3\underline{C}$O), 143.2 & 141.1 (Ar$\underline{C}$q), 128.5, 127.2, 125.1 &

120.5 (Ar$\underline{C}$), 77.6 (C2), 74.7 (C4), 70.7 (C8), 68.8 (C6), 67.7 (C5), 31.7 (C3), 21.1

(CO$\underline{C}$H$_3$). 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, <u>TB</u>DMS), 0.04 & 0.03 (2s, 6H, TB<u>DMS</u>). ^{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 (<u>TB</u>DMS), -5.42, -5.49 (TB<u>DMS</u>). 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 °C crystalline

636   9-flourenylmethyloxycarbonyl chloride (FMOCCl) (3.35 g, 13 mmol). The reaction was

637   allowed to proceed for 3 h at room temperature. Cold water (60 mL) followed by diethyl ether

638   (60 mL) were added. The organic layer was separated and the aqueous layer was extracted

639   with diethyl ether (2 x 60 mL). The combined extracts was washed with 2M HCl (2 x 100

640   mL), brine (2 x 100 mL) and dried over sodium sulphate, concentrated to dryness to give a

641   pale yellow crystalline crude syrup (3.62 g). The crude was separated using column

642   chromatography to give **10e** (0.56 g, 1.46 mmol, 24% yield, R_F = 0.120) and **10f** (1.32 g,

643   3.44 mmol, 56% yield, R_F= 0.170). IR (ATR) υ

644   (**10 e**) IR (ATR) υ 3460 (O-H), 1747 (C=O), 1450, 1193 & 1256 (C-O), 738 (Ar C-H). ^{1}H NMR

645   (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-

646   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

647     $J_{6',6}$ = 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, $J_{3',3}$ =

648   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

649   , 128.3 , 127.2 , 125.6, 120.5 (ArC), 76.0 (C2), 75.2 (C4), 70.9 (C8), 67.6 (C5), 65.2 (C6),

650   46.7 (C9), 33.6 (C3). HRMS (m/z) Calcd for C$_{21}$H$_{20}$O$_7$ [M+Na]$^+$: 407.1101, Found: 407.1101.

651   (**10 f**) IR (ATR) υ 3442 (O-H), 1747.5 (C=O), 1450, 1195 & 1256, (C-O), 727 (Ar C-H).^{1}H

652   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,

653     $J_{6,6'}$ = 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,

654     $J_{9,8}$= 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

655   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$_3$)

656   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),

657   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$_7$

658   [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 (<u>CH$_3$</u>CO), 0.87 (s, 9H, <u>TBDMS</u>), 0.06 & 0.05 (2s, 6H TB<u>DMS</u>). ^{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 (<u>TBDMS</u>), 20.7 (C8), -5.4, -5.5 (TB<u>DMS</u>). HRMS (m/z): Calculated mass for C$_{14}$H$_{26}$O$_6$Si [M+Na]$^+$ 341.1391, Found: 341.1390.

References

1. Gakhokidze RA: **Saccharinic Acids**. *Russian Chemical Reviews* 1980, **49**(3):222-236.
2. Sowden JC: **The Saccharinic Acids**. In: *Advances in Carbohydrate Chemistry.* vol. 12; 1957: 35-79.
3. Knill CJ, Kennedy JF: **Degradation of cellulose under alkaline conditions**. *Carbohydrate Polymers* 2002, **51**(3):281-300.
4. Isbell HS: **Interpretation of some reactions in the carbohydrate field in terms of consecutive electron displacement.** *Journal of Research National Bureau of Standards* 1944, **32**:45-59.
5. Nef JU: **Dissociationsvorgänge in der Zuckergruppe. (Erste Abhandlung.): Ueber das Verhalten der Zuckerarten gegen die Fehling'sche Lösung sowie gegen andere Oxydationsmittel**. *Justus Liebigs Annalen der Chemie* 1907, **357**(2-3):214-312.
6. Machell G, Richards GN: **384. Mechanism of saccharinic acid formation. Part I. Competing reactions in the alkaline degradation of 4-O-methyl-D-glucose, maltose, amylose, and cellulose**. *Journal of the Chemical Society (Resumed)* 1960:1924-1931.
7. Machell G, Richards GN: **386. Mechanism of saccharinic acid formation. Part III. The [small alpha]-keto-aldehyde intermediate in formation of D-glucometasaccharinic acid**. *Journal of the Chemical Society (Resumed)* 1960:1938-1944.
8. Machell G, Richards GN: **385. Mechanism of saccharinic acid formation. Part II. The [small alpha][small beta]-dicarbonyl intermediate in formation of D-glucoisosaccharinic acid**. *Journal of the Chemical Society (Resumed)* 1960:1932-1938.
9. Feast AAJ, Lindberg B, Theander O: **Studies on the D-glucosaccharinic acids**. *Acta Chem Scand* 1965, **19**:1127-1134.
10. Pavasars I: **Characterisation of organic substances in waste materials under alkaline conditions.** Sweden: Linköping University; 1999.
11. Pavasars I, Hagberg J, Boren H, Allard B: **Alkaline degradation of cellulose: Mechanisms and kinetics**. *Journal of Polymers and the Environment* 2003, **11**(2):39-47.
12. Whistler RL, BeMiller JN: **a-D-Isosaccharino-1,4-lactone**, vol. II. New York: Academic; 1963.
13. Florent JC, Ughetto-Monfrin J, Monneret C: **Anthracyclinones. 2. Isosaccharinic acid as chiral template for the synthesis of (+)-4-demethoxy-9-deacetyl-9-hydroxymethyldaunomycinone and (-)-4-deoxy-γ-rhodomycinone**. *J Org Chem* 1987, **52**(Copyright (C) 2014 American Chemical Society (ACS). All Rights Reserved.):1051-1056.
14. Bertounesque E, Millal F, Meresse P, Monneret C: **Synthesis of a highly functionalized γ-lactone as a precursor of 9- pentyl anthracyclines**. *Tetrahedron Asymmetry* 1998, **9**(17):2999-3009.
15. Wolf J, Jarrige JM, Florent JC, Grierson DS, Monneret C: **New 2'-branched-chain sugar nucleoside analogs with potential antiviral or antitumor activity**. *Synthesis* 1992(8):773-778.
16. Hanessian S, Roy R: **Chemistry of spectinomycin: Its total synthesis, stereocontrolled rearrangement, and analogs**. *Canadian Journal of Chemistry* 1985, **63**(1):163-172.
17. Bennis K, Gelas J, Thomassigny C: **Synthesis from lactose of new enantiomerically pure polyhydroxylated pyrrolidines with branched structures**. *Carbohydr Res* 1995, **279**(C):307-314.
18. Thomassigny C, Bennis K, Gelas J: **Synthesis of enantiomerically pure heterocycles: Access to hydroxylated piperidines from a sugar lactone**. *Synthesis* 1997(2):191-194.
19. Niemela K, Alén R: **Characterization of pulping liquors**. In: *Analytical methods in wood chemistry, pulping, and papermaking* Edited by Sjöström E, Alén R. Berlin: Springer; 1999: 193-231.
20. Niemela K, Alén R, Sjöström E: **The formation of carboxylic acids during kraft amd kraft-anthraquinone pulping of birch wood**. *Holzforschung* 1985, **39**:167

735   21.   Nieminen K, Testova L, Paananen M, Sixta H: **Novel insight in carbohydrate degradation**
736   **during alkaline treatment**. *Holzforschung* 2015, **69**(6):667-675.
737   22.   Strand SE, Dykes J, Chiang V: **Aerobic microbial degradation of glucoisosaccharinic**
738   **acid**. *Applied and Environmental Microbiology* 1984, **47**(2):268-271.
739   23.   Kobayashi T, Teshima T, Sasaki T, Kitamura A: **Thermodynamic model for Zr solubility**
740   **in the presence of gluconic acid and isosaccharinic acid**. *Journal of Nuclear Science*
741   *and Technology* 2017, **54**(2):233-241.
742   24.   Kuippers G, Bassil NM, Boothman C, Bryan N, Lloyd JR: **Microbial degradation of**
743   **isosaccharinic acid under conditions representative for the far field of radioactive**
744   **waste disposal facilities**. *Mineralogical Magazine* 2015, **79**(6):1443-1454.
745   25.   Kyeremeh IA, Charles CJ, Rout SP, Laws AP, Humphreys PN: **Microbial community**
746   **evolution is significantly impacted by the use of calcium isosaccharinic acid as an**
747   **analogue for the products of alkaline cellulose degradation**. *PLoS ONE* 2016, **11**(11).
748   26.   Rai D, Kitamura A: **Evaluation of equilibrium constants for deprotonation and**
749   **lactonisation of α-D-isosaccharinic acid**. *Journal of Nuclear Science and Technology*
750   2016, **53**(4):459-467.
751   27.   Shaw PB, Robinson GF, Rice CR, Humphreys PN, Laws AP: **A robust method for the**
752   **synthesis and isolation of β-gluco- isosaccharinic acid ((2R,4S)-2,4,5-trihydroxy-2-**
753   **(hydroxymethyl)pentanoic acid) from cellulose and measurement of its aqueous pK a**.
754   *Carbohydr Res* 2012, **349**:6-11.
755   28.   Kumar H, Alén R: **Microwave-assisted catalytic esterification of α-glucoisosaccharino-**
756   **1,4-lactone with tall oil fatty acids**. *Sustainable Chemical Processes* 2016, **4**(1):4.
757   29.   Giordano M, Iadonisi A: **Tin-mediated regioselective benzylation and allylation of**
758   **polyols: Applicability of a catalytic approach under solvent-free conditions**. *Journal of*
759   *Organic Chemistry* 2014, **79**(1):213-222.
760   30.   Gioeli C, Chattopadhyaya JB: **The flouren-9-ylmethoxycarbonyl group for the protection**
761   **of hydroxy-groups; its application in the synthesis of an octathymidylic acid fragment**.
762   *Journal of the Chemical Society - Series Chemical Communications* 1982(12):672-674.
763   31.   Kabalka GW, Varma M, Varma RS, Srivastava PC, Knapp FF: **The tosylation of alcohols**.
764   *The Journal of Organic Chemistry* 1986, **51**(12):2386-2388.
765   32.   Chaudhary SK, Hernandez O: **A simplified procedure for the preparation of**
766   **triphenylmethylethers**. *Tetrahedron Letters* 1979, **20**(2):95-98.
767   33.   Traboni S, Bedini E, Iadonisi A: **Orthogonal protection of saccharide polyols through**
768   **solvent-free one-pot sequences based on regioselective silylations**. *Beilstein Journal of*
769   *Organic Chemistry* 2016, **12**:2748-2756.
770

771