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Developing cellulosic waste products as platform chemicals: protecting group chemistry of  $\alpha$ -glucoisosaccharinic acid

### Original Citation

Almond, Michael, Suleiman, Mustapha G., Hawkins, Matthew, Winder, Daniel, Robshaw, Thomas, Waddoups, Megan, Humphreys, Paul and Laws, Andrew P. (2018) Developing cellulosic waste products as platform chemicals: protecting group chemistry of  $\alpha$ -glucoisosaccharinic acid. *Carbohydrate Research*, 455. pp. 97-105. ISSN 0008-6215

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2 **Developing Cellulosic Waste Products as Platform Chemicals:**  
3 **Protecting Group Chemistry of  $\alpha$ -Glucoisosaccharinic Acid.**  
4

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7

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28

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 ( $\alpha$ -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  $\alpha$ -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  $\alpha$ -GISAL derivatives  
41 where a number of the products contain orthogonal protecting groups.

42

43 **Key words:**

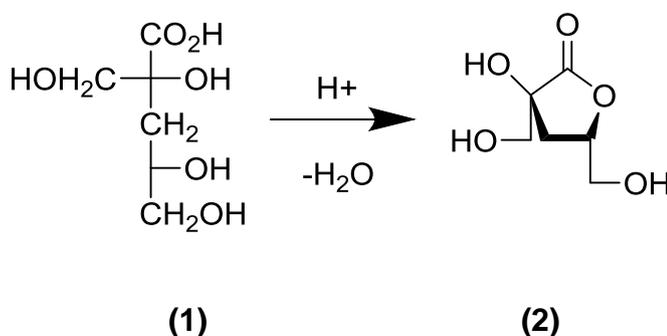
44 Saccharinic acids; Isosaccharinic acid; Glucoisosaccharinic acid; protecting groups.

45

46

## 47 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 2S-epimer, alpha-  
57 glucoisosaccharinic acid ( $\alpha$ -GISA **(1)**; (2S,4S)-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 2S-epimer precipitates whilst the 2R-epimer  
60 and other impurities remain in solution. The salts of  $\alpha$ -GISA are highly polar and have  
61 limited solubility in most organic solvents. However, in the presence of mild acids  $\alpha$ -GISA  
62 **(1)** undergoes an internal esterification reaction to give the less polar  $\alpha$ -  
63 glucoisosaccharino-1,4-lactone ( $\alpha$ -GISAL **(2)**):



66 **Scheme 1.** Acid catalysed lactonisation of  $\alpha$ -GISA(**1**) to generate  $\alpha$ -GISAL(**2**)

67 Despite the ease of preparation of  $\alpha$ -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 al*  
69 *al*[13] and Monneret *et al* [14] have incorporated  $\alpha$ -GISA (**1**) into the synthesis of a range  
70 of anthracycline analogues. Monneret *et al* have incorporated  $\alpha$ -GISA (**1**) into the  
71 synthesis of nucleoside analogues with antiviral or antitumor activity[15]. Hanessian and  
72 Roy have utilised  $\alpha$ -GISA (**1**) in the synthesis of the antibiotic spectinomycin[16].  
73 Thomassigny *et al* have incorporated  $\alpha$ -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 starting 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  $\alpha$ -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).

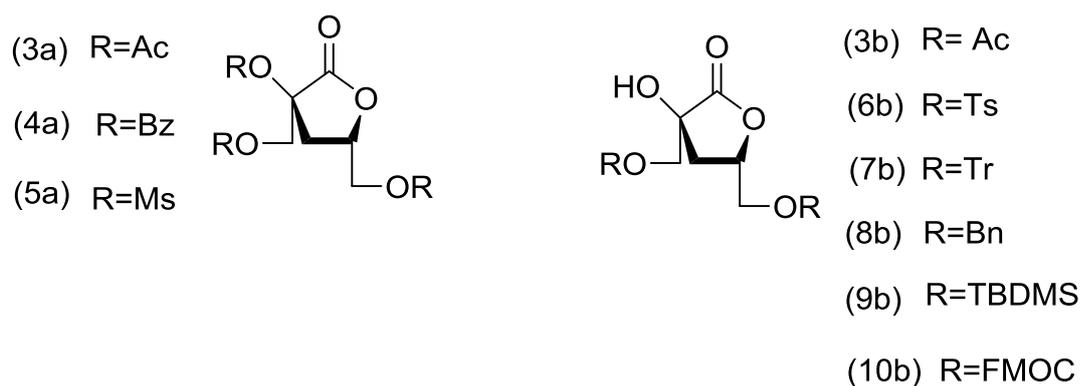
90

91 **2. Results and Discussion**

92 **2.1 Preparation of 2,5,6-tri-O-protected- $\alpha$ -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  $\alpha$ -GISAL (**2**) which was achieved by reaction of  $\alpha$ -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- $\alpha$ -GISAL (**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



105

106 **Figure 1.** 2,5,6-Tri-O-protected (**3-5a**) and 5,6-di-O-protected- $\alpha$ -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

110 large excess of *p*-toluenesulfonyl chloride a crude product was isolated which, after  
111 column chromatography, gave the 5,6-di-*O*-tosylated derivative **6b** (55%) and only a small  
112 amount (<10%) of the desired 2,5,6-trisubstituted  $\alpha$ -GISAL was produced. Further  
113 attempts to form triprotected derivatives of **2**, as either benzyl, trityl or silyl ethers, all led to  
114 the isolation of 5,6-di-*O*-protected derivatives (see section 2.2).

115 It is clear that derivatisation of all three hydroxyl groups in a single step procedure was  
116 only possible when using either forcing conditions (large excess of reagent), or when small  
117 sterically undemanding protecting groups (acetyl or mesyl) were employed. It is of note  
118 that Kumar and Alen have reported the synthesis of mixtures of mono and di-esters in the  
119 of  $\alpha$ -glucoisosaccharino-1,4-lactone with tall oil fatty acids[28].

## 120 **2.2. Preparation of 5,6-di-*O*-protected- $\alpha$ -GISALs in single step procedures.**

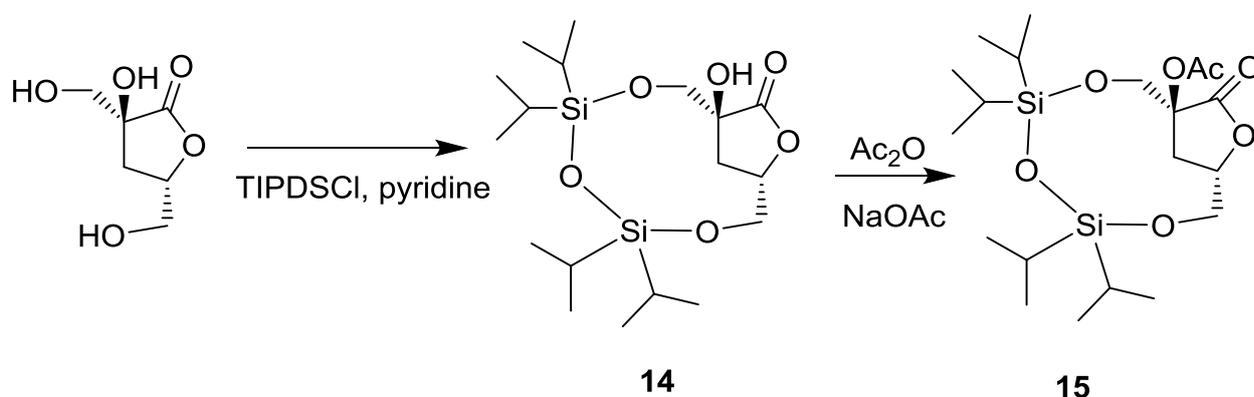
121 It was expected that the greater reactivity of the hydroxymethylene groups compared with  
122 that of the tertiary alcohol in **2** would allow direct access to the 5,6-di-*O*-protected- $\alpha$ -GISAL  
123 derivatives. Reaction of the lactone with two equivalents of acetyl chloride in pyridine and  
124 also the reaction of the lactone with two equivalents of *p*-toluenesulphonyl chloride in  
125 pyridine produced the desired 5,6-di-*O*-protected lactones **3b** (63%) & **6b** (55%) in  
126 reasonable yields. Reaction of the lactone with the larger trityl chloride generated a  
127 mixture of di-*O*-protected and mono-*O*-protected products which were easy to separate by  
128 column chromatography to give a very low yield of the desired 5,6-di-*O*-trityl- $\alpha$ -GISAL **7b**  
129 (13%), a similar amount of the 5-mono-*O*-trityl- $\alpha$ -GISAL **7e** (12%) and a very small  
130 amount of the 6-mono-*O*-trityl- $\alpha$ -GISAL **7f** (<2%).

131 Attempts to prepare the 5,6-di-*O*-benzylated derivative **8b** using sodium hydride as a base  
132 in DMF failed and only ring opened lactone products were obtained. Giordano and  
133 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- $\alpha$ -GISAL **9b** as the major product (69%). In a similar reaction, treatment  
140 of the lactone with TIPDSCI in pyridine afforded a high yield (82%) of the 5,6-TIPDS- $\alpha$ -  
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



146

147 **Scheme 2.** Synthesis of 5,6-cyclic-*O*-TIPDS- $\alpha$ -GISAL (**14**) and its conversion to 2-*O*-  
148 acetyl-5,6-TIPDS- $\alpha$ -GISAL(**15**).

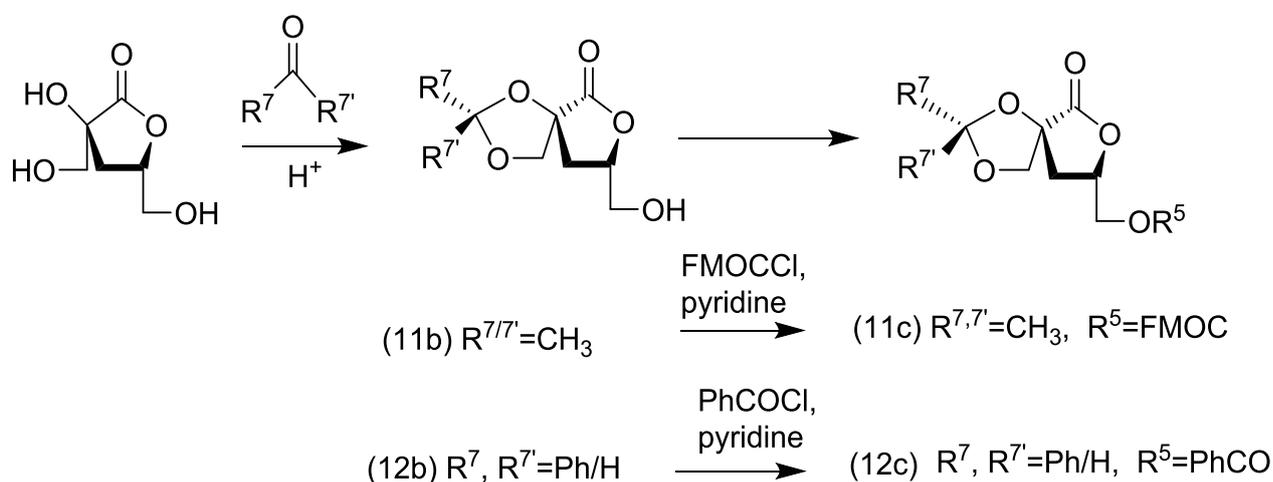
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150 In order to expand the range of protecting groups, an attempt was made to introduce acid  
151 stable carbonates at the 5 and 6-positions. Gioeli and Chattopadhyaya[30] have reported  
152 the use of the Fmoc-carbonate group to protect the hydroxyl groups of ribose, however,  
153 when the lactone **2** was reacted with a large excess of FMOCCI, 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 FMOCCI were  
 159 all present. As was the case with trityl-*O*-protection, pure samples of the desired 5,6-di-*O*-  
 160 Fmoc- $\alpha$ -GISAL**10b**, the 5-mono-*O*-protected **10e** and small amounts of the 6-mono-*O*-  
 161 protected- $\alpha$ -GISAL **10f** were isolated by column chromatography.

### 162 2.3. Preparation of 2,6-di-*O*-protected- $\alpha$ -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 FMOCCI 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

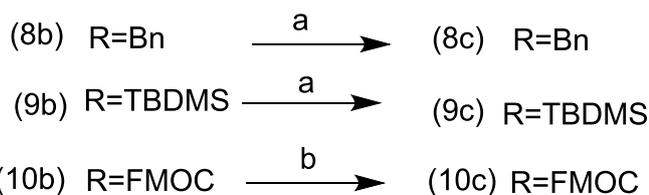
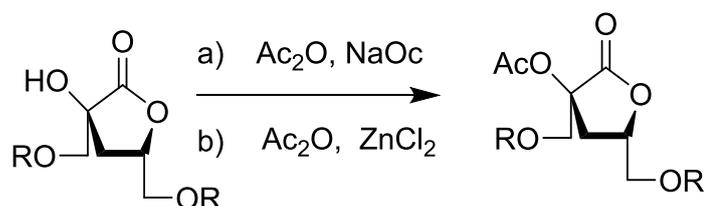
174 **Scheme 3.** Synthesis of 2,6-cyclic-O-acetals(**11b** & **12b**) and their further elaboration  
175 through addition of orthogonal protecting groups at the 5-OH: synthesis of 5,6-orthogonally  
176 protected  $\alpha$ -GISAL derivatives (**11c** and **12c**).

177

#### 178 **2.4 Preparation of 2,5,6-tri-O-protected- $\alpha$ -GISALs in two step procedures.**

179 The ease of formation of the 5,6-di-O-protected- $\alpha$ -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- $\alpha$ -GISAL  
182 **8b** and the 5,6-O-diTBDMS- $\alpha$ -GISALs **9b** were converted in variable but not optimised  
183 yields to their 2-O-acetyl-5,6-di-O-protected- $\alpha$ -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- $\alpha$ -GISAL **10b** with acetic anhydride in the  
186 presence of zinc dichloride afforded the 2-O-acetyl-5,6-di-O-protected- $\alpha$ -GISAL **10c** (Fig.  
187 2; reagents b, 55%).

188

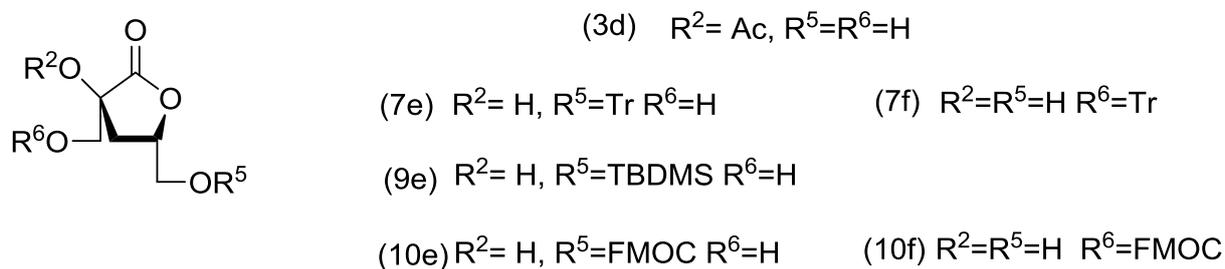


189

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

192 Reaction of the 2,6-O-isopropylidene- $\alpha$ -GISALs **11b** with FMOCCI 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-isopropylidene-5-O-FMOC- $\alpha$ -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-benzylidene-5-  
 196 O-benzoyl- $\alpha$ -GISALs **12c** also in low yield (20%).

197 **2.5 Preparation of the mono-O-protected  $\alpha$ -GISAL derivatives.**

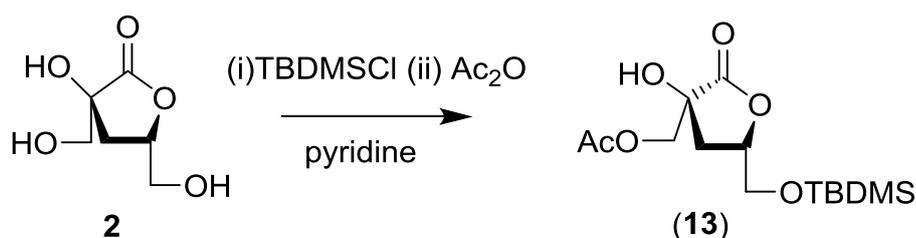


198  
 199 **Figure 2.** Mono-O-protected  $\alpha$ -GISAL derivatives (**3d**, **7e**, **7f**, **9e**, **10e** and **10f**).

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- $\alpha$ -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 TBSDMSCI 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- $\alpha$ -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- $\alpha$ -GISAL with triethylamine generated the 2-O-acetyl- $\alpha$ -  
 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- $\alpha$ -GISALs **10e** in  
 218 quantitative yield.

## 219 2.6 Preparation of a 5,6-di-O-protected- $\alpha$ -GISALs in a two-step one pot procedure



221 **Scheme 5.** Synthesis of a 5,6-orthogonally protected  $\alpha$ -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  $\alpha$ -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- $\alpha$ -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  $\alpha$ -glucoisosaccharinio-1,4-lactone derivatives which we hope will be  
242 employed in the synthesis of value added products.

243

244

## 245 **4. Experimental**

### 246 **4.1 General Methods**

247 All reagents were purchased from commercial sources unless otherwise stated and were  
248 used without further purification. Anhydrous solvents were dried over molecular sieves  
249 (activated under vacuum at 200 °C) and stored under an inert atmosphere before use. The  
250 solvents used for column chromatography were GPR grade. Analytical TLC was  
251 performed on Silica Gel 60-F254 (Merck) and detection was either by charring following  
252 immersion in 5% H<sub>2</sub>SO<sub>4</sub>/H<sub>2</sub>O and/or fluorescence. 1D <sup>1</sup>H and <sup>13</sup>C-NMR spectra were  
253 recorded on a Bruker Avance 400 MHz spectrometer operating at ambient temperature.  
254 2D-NMR (COSY, HSQC, HMBC or NOESY spectra) were recorded at 500 MHz using  
255 Bruker pulse sequences. NMR samples were dissolved in either D<sub>2</sub>O, deuterated acetone  
256 or CDCl<sub>3</sub> and referenced to either internal tetramethylsilane ( $\delta = 0$  ppm), internal CDCl<sub>3</sub> (<sup>1</sup>H  
257  $\delta = 7.23$  ppm and <sup>13</sup>C  $\delta = 77.00$  ppm) or internal HOD (<sup>1</sup>H  $\delta = 4.65$  ppm, 303K). Chemical  
258 shifts are given in parts per million.

259 High resolution mass spectra (HRMS) were recorded either by direct injection on an  
260 Agilent 6210 ToF spectrometer or by HPLC-MS (Agilent 1200 series HPLC coupled to an  
261 Agilent 6210 ToF Spectrometer). The HPLC employed a Phenomenex Luna 5 $\mu$  C18 2.4 x  
262 250 mm column and samples were eluted using an acetonitrile and water mobile phase  
263 operating with gradient elution: starting at 30% acetonitrile climbing to 95% acetonitrile  
264 over 15 mins. The mobile phase flow rate was 0.2 ml.min<sup>-1</sup>.

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

### 267 **4.2 Synthesis of tri-O-protected lactone derivatives: 3a, 4a and 5a.**

268 **4.2.1 2,5,6-Tri-O-acetyl- $\alpha$ -D-glucoisosaccharino-1,4-lactone (3a).**

269  $\alpha$ -D-Glucoisosaccharino-1,4-lactone (1.0 g; 6.17 mmol) was added whilst stirring to an ice  
270 cooled solution of acetic anhydride (10 mL), once the lactone had dissolved sodium acetate  
271 (0.5 g) was added and the reaction was heated to 100 °C for 4 h. The reaction was halted  
272 by addition of the contents of the round bottom flask to ice cold water (100 mL) and the  
273 solution was stirred at room temperature for a further 1 h. The organic products were then  
274 extracted into chloroform (3 x 60 mL) and the combined organic extracts were dried over  
275 anhydrous magnesium sulphate and concentrated at reduced pressure to give a golden  
276 crystalline syrup (1.77 g; 6.14 mmol; Yield: 99%). IR (ATR)  $\nu$  2959 (C-H), 1781 & 1737  
277 (C=O), 1437, 1370 (C-H), 1202, 1045 (C-O).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ): 5.01-4.95 (m, 1H,  
278 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,  $J_{5,4}$   
279 = 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,  
280  $J_{3',4} = 6.3$  Hz,  $J_{3',5} = 14.7$  Hz, H-3'), 2.11, 2.10, 2.08 (3s, 9H, 3 x  $\text{CH}_3\text{CO}$ );  $^{13}\text{C}$  NMR (100 MHz,  
281  $\text{CDCl}_3$ ):  $\delta$  172.0 (C1), 170.6, 170.0, 169.9 (3 x  $\text{CH}_3\text{-CO}$ ), 77.9 (C2), 74.7 (C4), 65.3 (C6),  
282 64.8 (C5), 32.1 (C3), 20.7, 20.6, 20.5 (3 x  $\text{Me-CO}$ ). HRMS (m/z) Calcd for  $\text{C}_{12}\text{H}_{16}\text{O}_8$   
283  $[\text{M}+\text{NH}_4]^+$ : 306.1183, Found:306.1187.

284 **4.2.2 2,5,6-Tri-O-benzoyl- $\alpha$ -D-glucoisosaccharino-1,4-lactone (4a).**

285 The procedure used to prepare **4a** was identical to that used to prepare 2,5,6-tri-O-  
286 benzoyl- $\beta$ -D-glucoisosaccharino-1,4-lactone reported by Shaw *et al*[27] and the product  
287 was recovered from a crude mixture by column chromatography (pale yellow syrup, 4.93g  
288 starting from 20g of GISAL(2)) (TLC Hex/EtOAc 1:1; RF 0.34). IR (ATR)  $\nu$  1771 & 1722  
289 (C=O), 1451 (Ar C-C), 1262, 1233, 1092 & 1062 (C-O), 701 & 684 (Ar C-H).  $^1\text{H}$  NMR (400  
290 MHz,  $\text{CDCl}_3$ ):  $\delta$  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,  
291 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),

292 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,  $J_{3,3'} = 15.2$   
293 Hz, H-3), 2.62 (dd, 1H,  $J_{3',4} = 7.2$  Hz,  $J_{3,3'} = 15.2$  Hz, H-3').  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ):  $\delta$   
294 171.96 (C1), 166.20, 165.74, 165.47 (3 x PhCO) 130.00, 129.96, 128.90, 134.24, 134.07,  
295 130.12, 129.96, 129.20, 128.90, 128.71 (ArC), 78.46 (C2), 75.45 (C4), 66.09 (C6), 65.39  
296 (C5), 32.65 (C3). HRMS (m/z) Calcd for  $\text{C}_{27}\text{H}_{22}\text{O}_8$  (M+Na) $^+$ : 497.1207, Found: 497.1236.

### 297 **4.2.3 2,5,6-Tri-O-methylsulphonyl- $\alpha$ -D-glucoisosaccharino-1,4-lactone (5a).**

298 The method used to prepare **5a** was adapted from that reported by Kabalka *et al*[31]. A  
299 solution of  $\alpha$ -D-glucoisosaccharino-1,4-lactone (1.0 g; 6.17 mmol) in anhydrous pyridine  
300 (10 mL) was added to a round bottomed flask and cooled to 0 °C whilst stirring.  
301 Methanesulphonyl chloride (3 mL; 38.8 mmol) was added cautiously over a period of 10  
302 min. The reaction mixture was kept at 0 °C for a further 5 min before continuing to stir at  
303 room temperature for 16 h. The reaction was halted by addition of ice cold water (25 mL)  
304 and dichloromethane (50 mL). The organic and aqueous layers were separated and any  
305 remaining organic product in the aqueous layer was extracted with dichloromethane (2 x  
306 25 mL). The organic extracts were combined, washed with 5 % sodium bicarbonate (2 x  
307 25 mL) and saturated brine (2 x 25 mL) before being dried over anhydrous magnesium  
308 sulphate. The solvent was removed at room temperature on a rotary evaporator to give a  
309 cream-orange coloured solid as the crude product (2.44 g). The product was purified by  
310 column chromatography (100% EtOAc). Fractions containing the desired product **5a** were  
311 combined and reduced by rotary evaporation to give **5a** as a white solid (1.50 g; yield: 61  
312 %) (Rf: 0.48, EtOAc). IR (ATR)  $\nu$  773.6 (CO), 1347.5, 1172.0 ( $\text{SO}_2$ ).  $^1\text{H}$  NMR (400 MHz,  
313  $d$ -DMSO):  $\delta$  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,  
314 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,  
315 3.39 (3s, 9H, 3 x Me-SO<sub>3</sub>), 2.89-2.47 (m, 2H, H-3,3').  $^{13}\text{C}$  NMR (100 MHz,  $d$ -DMSO):

316  $\delta$  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<sub>3</sub>). HRMS (m/z) Calcd for C<sub>9</sub>H<sub>16</sub>O<sub>11</sub>S<sub>3</sub> (M+NH<sub>4</sub>)<sup>+</sup>: 414.0193, Found: 414.0188.

318

### 319 **4.3 Synthesis of 5,6-di-O-protected lactone derivatives (3b, 6b-10b)**

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

321  $\alpha$ -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  $\mu$ L; 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)  $\nu$  3079 (O-H), 1781 & 1743 (C=O), 1482, 1373 (C-H), 1233,  
330 1196 (C-O). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  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<sub>3</sub>CO); <sup>13</sup>C NMR (100  
334 MHz, CDCl<sub>3</sub>): 175.4 (C1), 170.4 & 170.1 (2 x CH<sub>3</sub>CO), 74.9 (C4), 74.0 (C2), 65.0 (C6),  
335 64.6 (C5), 35.1 (C3), 20.6 & 20.5 (2 x CH<sub>3</sub>CO). HRMS (m/z): Calcd for C<sub>10</sub>H<sub>14</sub>O<sub>7</sub> (M+NH<sub>4</sub>)<sup>+</sup>:  
336 269.0748, Found: 269.0740.

337

338 **4.3.2. 5,6-Di-O-*p*-toluenesulphonyl- $\alpha$ -D-glucoisosaccharino-1,4-lactone (6b)**

339 *p*-Toluenesulphonyl chloride (2.58 g; 13.6 mmol; 2.1 eq.) was reacted with  $\alpha$ -D-  
340 glucoisosaccharino-1,4-lactone (1.06 g; 6.51 mmol) in anhydrous pyridine (5 mL) using the  
341 same procedure described in section 4.3.1 except that after the addition was complete, the  
342 solution was stirred at room temperature for a further 60 h. The crude product 5,6-di-O-  
343 tosyl- $\alpha$ -glucoisosaccharino-1,4-lactone was purified by column chromatography eluting  
344 with a solvent system with a starting composition of hexane and EtOAc (3:1) rising to 100  
345 % EtOAc. The purified compound **6b** (RF= 0.35; hexane/ether, 1:1) was isolated as a pale  
346 yellow syrup (1.69 g; yield: 55 %). IR (ATR)  $\nu$  3460.1 (OH), 1782.1 (CO) 1597.1, 1354.3,  
347 1171.3, 810.6.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.80-7.78 (m, 4H, 2 x Ar-H), 7.39-7.36 (m,  
348 4H, 2 x Ar-H), 4.83 (m, 1H, H-4), 4.24-4.11 (m, 2H, H-5s), 4.16 (d, 1H,  $J_{6,6'} = 10.6\text{Hz}$ , H-  
349 6), 4.07 (d, 1H,  $J_{6,6'} = 10.6\text{ Hz}$ , H6'), 2.48-2.44 (m, 6H,  $\text{CH}_3\text{-Ph}$ ), 2.37 (m, 1H, H-3), 2.22  
350 (m, 1H, H-3').  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ): 173.3 (C1), 145.6, 145.7, 130.2, 130.2, 131.8,  
351 132.0, 128.1, 128.1, (8 x ArC), 74.7 (C4), 74.3 (C2), 70.2 (C6), 68.7 (C5), 34.2 (C3), 21.7  
352 (2 x  $\text{CH}_3\text{Ar}$ ); HRMS (m/z): Calcd for  $\text{C}_{20}\text{H}_{22}\text{O}_9\text{S}_2$  ( $\text{M}+\text{NH}_4$ ) $^+$ : 488.1043, Found: 488.1049.

353 **4.3.3. 5,6-Di-O-triphenylmethyl- $\alpha$ -D-glucoisosaccharino-1,4-lactone (7b) , 6-O-**  
354 **triphenylmethyl- $\alpha$ -D-glucoisosaccharino-1,4-lactone (7e) and 5-O-triphenylmethyl- $\alpha$ -**  
355 **D-glucoisosaccharino-1,4-lactone (7f)**

356 The following synthetic procedure was adapted from the work by Choudhary and  
357 Hernandez[32]. Triphenylmethyl chloride (25.07 g; 89.9 mmol) and  $\alpha$ -D-  
358 glucoisosaccharino-1,4-lactone **2** (6.82 g; 41.9 mmol) were dissolved in pyridine (300 mL)  
359 and a catalytic amount of DMAP (1 g; 8.19 mmol) was added. The resulting solution was  
360 stirred at 25 °C for 12 h under an atmosphere of nitrogen. After the reaction was complete,  
361 the solution was added to an equal volume of water and then extracted into chloroform (2  
362 x 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- $\alpha$ -GISAL **7b** (Rf 0.79; Hex/EtOAc (2:1));  
368 3.34 g; yield: 12 %, followed by the 6-mono-*O*-trityl- $\alpha$ -GISAL **7e** (Rf 0.29;  
369 Hex/EtOAc, 1:2 v/v)); 0.20 g; yield: <2 % and 5-mono-*O*-trityl- $\alpha$ -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)  $\nu$  1779 (CO) 762.2, 745.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.48-7.27 (m, 30H, 6 x  
372 PhH), 4.82 (m, 1H, H-4), 3.41 (d, 1H,  $J_{6,6'} = 9.1\text{ Hz}$ , H-6), 3.30 (d, 1H,  $J_{6',6} = 9.1\text{ Hz}$ , H-6'),  
373 3.36 (dd, 1H,  $J_{5,4} = 6.0\text{ Hz}$ ,  $J_{5,5'} = 10.5\text{ Hz}$ , H-5), 3.28 (dd, 1H,  $J_{5',4} = 3.8\text{ Hz}$ ,  $J_{5',5} = 10.5$   
374 Hz, H-5'), 2.20 (m, 2H, H-3);  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ): 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  $\text{C}_{44}\text{H}_{38}\text{O}_5$  (M+Na) $^+$ : 669.2611, Found:  
377 669.2592.

378 **7e** IR (ATR)  $\nu$  3353.1 (OH) 1774.0 (CO) 763.4, 745.8, 697.7.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$   
379 7.46-7.27 (m, 15H, 3 x PhH), 4.77 (m, 1H, H-4), 3.91 (dd, 1H,  $J_{5,4} = 2.8\text{ Hz}$ ,  $J_{5,5'} = 12.7\text{ Hz}$ ,  
380 H-5), 3.65 (dd, 1H,  $J_{5',4} = 5.1\text{ Hz}$ ,  $J_{5',5} = 12.7\text{ Hz}$ , H-5'), 3.42 (d, 1H,  $J_{6,6'} = 9.2\text{ Hz}$ , H-6),  
381 3.32 (d, 1H,  $J_{6',6} = 9.2\text{ Hz}$ , H-6'), 2.33 (dd, 1H,  $J_{3,4} = 7.1\text{ Hz}$ ,  $J_{3,3'} = 13.8\text{ Hz}$ , H-3), 2.21 (dd,  
382 1H,  $J_{3',4} = 8.5\text{ Hz}$ ,  $J_{3',3} = 13.8\text{ Hz}$ , H-3');  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ): 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  $\text{C}_{25}\text{H}_{24}\text{O}_5$  [M+Na] $^+$ : 427.1516, Found: 427.1513.

385 **7f** IR (ATR)  $\nu$  3365.8 (OH) 1772.8. (CO) 763.6, 746.0, 697.2.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ):  
386  $\delta$  7.46-7.27 (m, 15H, 3 x PhH,), 4.89 (m, 1H, H-4), 3.84 (d, 1H,  $J_{6,6'} = 11.7\text{ Hz}$ , 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');  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ): 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  $\text{C}_{25}\text{H}_{24}\text{O}_5$  (M+Na) $^+$ : 427.1516, Found: 427.1506.

#### 392 4.3.4. 5,6-Di-O-dibenzyl- $\alpha$ -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  $\alpha$ -glucoisosaccharino-1,4-lactone **2** (1.0 g, 6.17 mmol)  
395 was dissolved in *N,N*-diisopropylethylamine (DIPEA) (2.3 mL, 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 were 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%.  $^1\text{H}$  NMR  
405 (400 MHz,  $\text{CDCl}_3$ ) 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');  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ ) 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  $\text{C}_{20}\text{H}_{22}\text{O}_5$  [M+Na] $^+$ : 365.1359, Found:  
411 365.1358.

412 **4.3.5. 5,6-Di-O-tert-butyldimethylsilyl- $\alpha$ -D-glucoisosaccharino-1,4-lactone (5a)**

413 The di-tert-butyldisilyl derivative **9b** was synthesised using a method adapted from that  
414 described by Iadonisi et al[33] employing only a minimal amount of solvent. Dried  $\alpha$ -  
415 glucoisosaccharino-1,4-lactone **2** (1.0 g, 6.17 mmol) was suspended in anhydrous pyridine  
416 (5 mL) whilst stirring for 20 min at room temperature. It was then added cautiously to a  
417 mixture of tert-butyldimethylsilyl chloride (TBDMSCl) (2.1 g, 13.93 mmol, 2.2 eq) while  
418 stirring at room temperature. The reaction was allowed to proceed for 4 h after which time  
419 DCM (50 mL) and water (50 mL) were added. The organic layer was separated and  
420 aqueous layer was further extracted with DCM (2 x 50 mL). The combined organic layer  
421 was washed with a 1% CuSO<sub>4</sub> solution (2 x 50 mL), dried over anhydrous sodium sulphate  
422 and concentrated to give a crude sample of **9b** as a white solid. The product was purified  
423 by chromatography (elution with EtOAc/Hexane; 3:1 v/v) and the early fractions contained  
424 pure **9b** (1.66 g; 4.26 mmol; 69 %) (R<sub>f</sub> = 0.722; Hexane/EtOAc 3:1 v/v) were combined  
425 and the solvent evaporated. IR (ATR)  $\nu$  3259 (O-H), 2952, 2928, 2886, 2857 (C-H), 1770  
426 (C=O), 1471, 1462, 1360 (C-H), 1255, 1200, 1168 (C-O), 1097, 1044 (Si-OR) 833, 814,  
427 775. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) 4.68-4.60 (m, 1H, H-4), 3.78 (dd, 1H, J<sub>5,4</sub> = 3.79 Hz, J<sub>5,5'</sub> =  
428 11.55 Hz, H-5), 3.76 (d, 1H, J<sub>6,6'</sub> = 9.85 Hz, H-6), 3.69 (dd, 1H, J<sub>5',4</sub> = 4.74 Hz, J<sub>5',5</sub> = 11.55  
429 Hz, H-5'), 3.65 (d, 1H, J<sub>6',6</sub> = 9.85 Hz, H-6'), 2.32 (dd, 1H, J<sub>3,4</sub> = 8.30 Hz, J<sub>3,3'</sub> = 14.02 Hz,  
430 H-3), 2.17 (dd, 1H, J<sub>3',4</sub> = 7.40 Hz, J<sub>3',3</sub> = 14.02 Hz, H-3'), 0.86 (2s, 18H, 2 x TBDMS), 0.05  
431 (m, 12H, 2 x TBDMS); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): 176.92 (C1), 77.77 (C4), 76.35(C2),  
432 65.42 (C6), 64.30 (C5), 33.72 (C3), 25.82 & 25.78 (TBDMS), 18.31 & 18.24 (TBDMS).  
433 HRMS (m/z) Calcd for C<sub>18</sub>H<sub>38</sub>Si<sub>2</sub>O<sub>5</sub> [M+Na]<sup>+</sup> : 413.2150, Found: 413.2152.

434 **4.3.6 (1',1',3',3'-Tetraisopropylidisiloxane-1,3-diyl)-5,6- $\alpha$ -D-glucoisosaccharino-1,4-**  
435 **lactone (14)**

436 Dried  $\alpha$ -D-glucosiosaccharino-1,4-lactone **2** (1.0 g, 6.17 mmol) was dissolved in pyridine (6  
437 mL) at room temperature and the solution was added cautiously to 1,3-dichloro-1,1,3,3-  
438 tetraisopropyl-1,3-disiloxane (TIPDS-Cl<sub>2</sub>) (2.17 mL; 6.78 mmol; 1.1 eq) whilst stirring at room  
439 temperature. The reaction was allowed to proceed for 4 h. After 4 h it was halted with the  
440 addition of DCM (60 mL) and water (60 mL). The organic layer was separated and the  
441 aqueous layer was further extracted with DCM (2 x 50 mL). The combined organic layer was  
442 washed with an aqueous CuSO<sub>4</sub> solution (1%, 2 x 50 mL) dried over anhydrous sodium  
443 sulphate and concentrated to give crude **14** (4.14 g) as a brown crystalline syrup which was  
444 purified using column chromatography to give the desired product as a pale yellow syrup  
445 (2.05 g; 5.07 mmol; 82% yield) (RF: 0.68, Hexane/EtOAc 4/1 v/v). IR (ATR)  $\nu$  2945, 2867,  
446 1771 (C=O), 1464, 1387, 1084, 1042 (R<sub>3</sub>Si-O-SiR<sub>3</sub>), 1012. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) 4.70-  
447 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,  
448 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,  $J_{3,3}$   
449 = 13.9 Hz,  $J_{3',4} = 10.1$  Hz, H-3'), 1.1-0.9 (m, 28H, TIPDS).

450 <sup>13</sup>C (100 MHz, CDCl<sub>3</sub>) 178.2 (C1), 76.7(C2), 76.3 (C4), 66.9 (C6), 63.6 (C5), 31.8 (C3),  
451 17.19, 17.11, 17.09 & 17.07 (TIP(CH)DS), 13.5, 13.1, 12.6 & 12.4 (TIP(CH<sub>3</sub>)DS)

452 HRMS (m/z) calculated mass for C<sub>18</sub>H<sub>36</sub>O<sub>6</sub>Si<sub>2</sub> [M+NH<sub>4</sub>]<sup>+</sup> 422.2389 found 422.2407

453 To confirm the location of the protecting group, **14** (1.5g, 3.71mmol) was acetylated using  
454 the procedure described in section 4.5.1 to give, after chromatography, the product **15** as a  
455 white semi-crystalline syrup (680 mg, 1.53 mmol; 41% yield); (Rf: 0.721, Hexane/EtOAc 3:1,  
456 v/v). IR (ATR)  $\nu$  2944.6, 2867.5, 1779.5 & 1742.1 (C=O), 1463.9, 1369.8, 1084, 1252.1,  
457 1215.1, 1082.5, 1043.2 (R<sub>3</sub>Si-O-SiR<sub>3</sub>), 883.1. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) 4.82-4.78 (m, 1H,  
458 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),  
459 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

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<sub>3</sub>) 1.1-1.0 (m, 28H, TIPDS).

462 <sup>13</sup>C (100 MHz, CDCl<sub>3</sub>) 173.6 (C1), 170.4 (COCH<sub>3</sub>), 81.7 (C2), 77.0(C4), 64.7 (C5), 64.5  
463 (C6), 30.0 (C3), 20.8 (COCH<sub>3</sub>), 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<sub>20</sub>H<sub>38</sub>O<sub>7</sub>Si<sub>2</sub> [M+NH<sub>4</sub>]<sup>+</sup> 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°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<sub>3</sub>Si-O-SiR<sub>3</sub>), 1012.<sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>),  $\delta$  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-

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);  $^{13}\text{C}$  NMR (100  
487 MHz,  $\text{CDCl}_3$ ): 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 °C. HRMS  
489 (m/z): Calcd for  $\text{C}_{36}\text{H}_{30}\text{O}_9$   $[\text{M}+\text{NH}_4]^+$  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-isopropylidene- $\alpha$ -D-glucoisosaccharino-** 493 **1,4-lactone (11c)**

494 2,6-O-Isopropylidene- $\alpha$ -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 FMOCCI (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)  $\nu$  2945, 2867, 1771 (C=O), 1464, 1387, 1084, 1042 ( $\text{R}_3\text{Si-O-SiR}_3$ ), 1012.  
505  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$ : 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  $\text{CH}_3$ ).  $^{13}\text{C}$  NMR (100 MHz,  
509  $\text{CDCl}_3$ ): 174.8 (C1), 154.6 (FMOCCO): 142.8, 141.3, 127.9, 127.1, 125.0, 119.9 (ArC), 112.7

510 (C7), 80.8 (C2), 74.4 (C4), 72.0 (C6), 70.1 (C5), 67.7 ( FMOCCH) 46.4 (FMOCCH<sub>2</sub>) 36.5  
511 (C3), 26.7 (C8), 25.3 (C9). HRMS (m/z) Calcd for C<sub>24</sub>H<sub>24</sub>O<sub>7</sub> [M+Na<sup>+</sup>]: 447.1414, Found:  
512 447.1415.

#### 513 **4.4.2 5-O-Benzoyl-2,6-O-benzylidene- $\alpha$ -D-glucoisosaccharino-1,4-lactone (12c)**

514 *Synthesis of (7S)- and (7R)-2,6-O-benzylidene- $\alpha$ -D-glucoisosaccharino-1,4-lactone 12b -*

515 Freshly distilled benzaldehyde (50 mL; 492 mmol) was added to a round bottomed flask  
516 (100 mL) containing  $\alpha$ -glucoisosaccharino-1,4-lactone **2** (1.02 g; 6.27 mmol), *p*-TSA (20  
517 mg) and ~ 30 4Å molecular sieves. The mixture was left to reflux under a slight vacuum for  
518 4 h at 85 °C. After cooling to room temperature, the mixture was gravity filtered to remove  
519 the molecular sieves and excess benzaldehyde was removed by vacuum distillation to  
520 give the crude product as a semi-crystalline syrup. The crude mixture was purified by  
521 column chromatography (fractions were eluted with chloroform with increasing portions of  
522 methanol: 1-10%). The product eluted in two distinct bands which, after evaporating to  
523 dryness gave 0.90 g and 0.26 g of the required diastereoisomers with a combined yield of  
524 78 %. Using NOESY NMR spectra, it was determined that the first fraction (Rf: 0.17,  
525 CHCl<sub>3</sub>/MeOH 95:5 v/v) was the 7*R*- diastereomer of **12b** whilst the second fraction (Rf:  
526 0.26, CHCl<sub>3</sub>/MeOH 95:5 v/v) contained the 7*S*-diastereomer of **12b**.

527 <sup>1</sup>H NMR 7*S*-diastereomer of **12b** (400 MHz, *d*-DMSO): 7.35-7.55 (m, 5H, ArH), 5.98 (s,  
528 1H, PhCH), 5.25 (s, 1H, OH), 4.71 (m, 1H, H-4), 4.33 (d, 1H, *J*<sub>6,6'</sub> = 9.0 Hz, H-6), 4.16 (d,  
529 1H, *J*<sub>6',6</sub> = 9.0 Hz, H-6'), 3.67 (dd, 1H, *J*<sub>5,4</sub> = 2.0 Hz, *J*<sub>5,5'</sub> = 12.1 Hz, H-5), 3.49 (dd, 1H, *J*<sub>5',4</sub>  
530 = 3.2 Hz, *J*<sub>5',5</sub> = 12.2 Hz, H-5') 2.49 (m, 2H, H-3,3') . <sup>13</sup>C NMR (100 MHz, *d*-DMSO): 176.4  
531 (C1), 136.7, 127.4, 128.8, 130.2 (ArC), 104.9 (C7), 81.2 (C2), 78.7 (C4), 35.4 (C3), 62.5  
532 (C5), 72.9 (C6).

533 <sup>1</sup>H NMR 7*R*-diastereoisomer **12b** (400 MHz, *d*-DMSO): 7.38-7.60 (m, 5H, ArH), 5.91 (s,  
534 1H, PhCH), 5.21 (s, 1H, OH), 4.68 (m, 1H, H-4), 4.44 (d, 1H, *J*<sub>6,6'</sub> = 9.5 Hz, H-6), 4.04 (d,

535 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'),  
536 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,  
537 H-3').  $^{13}\text{C}$  NMR (100 MHz, *d*-DMSO): 175.8 (C1), 136.9, 130.2, 127.9, 128.7 (ArC), 105.0  
538 (C7), 81.0 (C2), 78.5 (C4), 73.3 (C6), 62.5 (C5), 34.5 (C3).

539 *Synthesis of 5-O-benzoyl-(7R)-2,6-O-benzylidene- $\alpha$ -D-glucoisosaccharino-1,4-lactone*  
540 **12c**. Compound **12b** (0.90 g; 3.60 mmol) was dissolved in pyridine (50 mL) and benzoyl  
541 chloride (1.5 g; 1.3 mL; 10.7 mmol) and a catalytic quantity of DMAP (20 mg) were added.  
542 The reaction was stirred at room temperature for 2 h. The pyridine was removed by rotary  
543 evaporation and the resulting brown residue was dissolved in diethyl ether (50 mL) and  
544 washed with a saturated sodium hydrogen carbonate solution (2 x 20 mL) and then with  
545 saturated sodium chloride (20 mL). The organic layer was reduced to dryness, the crude  
546 product was dissolved in sodium dried ether (20 mL) and this was once again dried on the  
547 rotary evaporator. This process was repeated with sodium dried ether until the odour of  
548 pyridine had disappeared to give a mixture of the desired product and pyridinium  
549 hydrochloride as a semi-solid syrup. Finally, a small amount of the desired product was  
550 obtained by recrystallization from petroleum ether, the residue was dissolved in petroleum  
551 ether (bpt 40-60 °C, 10 mL) and the volume of the solvent was reduced slowly until a white  
552 cloudy solution was first observed. After cooling to room temperature, the mixture was  
553 chilled at 5 °C for 3 h until white crystals were visible which were filtered under gravity and  
554 dried at room temperature in a desiccator to isolate the crystalline product **12c** as white  
555 needles (0.26 g; yield: 20 %). IR (ATR)  $\nu$  1766.9 & 1727.2 (CO) 759.4, 708.6., 695.0.  $^1\text{H}$   
556 NMR (400 MHz, *d*-DMSO): 8.05-7.35 (m, 10H, ArH), 5.98 (s, 1H, PhCH), 5.06 (m, 1H, H-  
557 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,  $J_{5',5} = 12.4$   
558 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').  $^{13}\text{C}$  NMR  
559 (100 MHz, *d*-DMSO): 175.2 (C1), 165.9 (PhCO), 136.4, 134.1, 130.3, 129.8, 129.7, 129.3,

560 128.8 & 127.5 (ArC), 104.9 (C7), 80.9 (C2), 76.1 (C4), 71.4 (C6), 65.7 (C5), 34.9 (C3).

561 HRMS (m/z) Calcd for C<sub>20</sub>H<sub>18</sub>O<sub>6</sub> [M+K]<sup>+</sup>: 393.0735, Found: 393.0735.

## 562 **4.5 Preparation of orthogonally protected trisubstituted 2- $\alpha$ -D-glucoisosaccharino-** 563 **1,4-lactone**

### 564 **4.5.1 2-O-Acetyl-5,6-di-O-benzyl- $\alpha$ -D-glucoisosaccharino-1,4-lactone (8c)**

565 5,6-di-O-Dibenzyl-D-glucoisosaccharino-1,4-lactone **8b** (1.0 g, 2.92 mmol) was reacted with  
566 acetic anhydride (10 m) and sodium acetate (0.5 g) employing the procedure described in  
567 section 4.2.1 to give a brown crystalline syrup which was purified by column  
568 chromatography ( EtOAc/hexane 5/1-1:1 v/v ) providing **8c** as a colourless oil (330 mg; 0.86  
569 mmol; 29.4%); (Rf: 0.211; EtOAc/hexane 1:1 v/v). IR (ATR)  $\nu$  2866, 1775 & 1740 (C=O),  
570 1453, 1369, 1205 & 1096 (C-O), 736, 697. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) 7.33-7.26 (m, 10H,  
571 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),  
572 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-  
573 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,  $J_{3',3} = 14.3$   
574 Hz, H-3'), 2.10 (s, 3H, CH<sub>3</sub>CO). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): 173.66 (C1), 170.00 (CH<sub>3</sub>CO),  
575 137.72 & 137.20 (PhCq), 128.48, 128.46, 127.89, & 127.78 (PhC), 79.44 (C2), 76.51 (C1),  
576 73.86 & 73.46 (PhCH<sub>2</sub>), 71.59 (C6), 71.10 (C5), 31.96 (C3), 20.63 (CH<sub>3</sub>CO). HRMS (m/z)  
577 Calcd for C<sub>22</sub>H<sub>24</sub>O<sub>6</sub> [M+NH<sub>4</sub>]<sup>+</sup>: 402.1911, Found: 402.1910.

### 578 **4.5.2. 2-O-Acetyl-5,6-di-O-tert-butyltrimethylsilyl- $\alpha$ -D-glucoisosaccharino-1,4-** 579 **lactone (9c)**

580 The same procedure as described above for the synthesis of **8c** was used to prepare **9c**.  
581 After chromatography, the product **9c** was recovered as a white crystalline semi-solid (900  
582 mg, 2.08 mmol; 81%; Rf: 0.821, Hexane/EtOAc 3:1, v/v). IR (ATR)  $\nu$  2954, 2929, 2857,  
583 1783 & 1747 (C=O), 1472, 1369, 1251, 1209 (C-O), 832, 776. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)

584 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'),  
585 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,  $J_{3',4}$   
586 = 5.65 Hz,  $J_{3',3} = 14.48$  Hz, H-3'), 2.01 (s, 3H, CH<sub>3</sub>CO), 0.82 (2s, 18H, 2 x TBDMS), 0.00  
587 (4s, 12H, 2 x TBDMS). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): 173.70 (C1), 169.74 (CH<sub>3</sub>CO), 80.31  
588 (C2), 77.57 (C4), 65.36 (C6), 64.54 (C5), 31.57 (C3), 25.70, 25.63, 25.57 (TBDMS), 20.43  
589 (CH<sub>3</sub>CO), -5.23, -5.55, -5.60 (TBDMS). HRMS (m/z) Calcd for C<sub>20</sub>H<sub>40</sub>Si<sub>2</sub>O<sub>6</sub> [M+Na]<sup>+</sup>:  
590 455.2256, Found: 455.2257.

591  
592 **4.5.3 2-O-Acetyl-5,6-di-O-fluorenylmethoxycarbonyl- $\alpha$ -D-glucoisosaccharino-1,4-**  
593 **lactone (10c).**

594  
595 5,6-di-O-FMOC- $\alpha$ -GISA<sub>L</sub> (**10b**, 2.34 g, 3.86 mmol) was added to a round bottom flask  
596 containing acetic anhydride (12.5 ml, 0.13 mol) and ZnCl<sub>2</sub> (0.5 g). The solution was heated  
597 to 100 °C and the reaction was allowed to proceed for 4 h at 100 °C. After 4h the sample  
598 was cooled to room temperature and the contents of the flask were poured cautiously onto  
599 ice cool water (100mL) to give the product as a semisolid. The suspension was stirred for  
600 30 min over which time the product solidified. The solid was filtered and the residue dried  
601 at room temperature overnight to give **10c** as a white powder (1.5 g; 2.14 mmol, 55%). IR  
602 (ATR)  $\nu$  1784, 1745 & 1709 (C=O), 1253, 1206 (C-O), 784, 759, 739. <sup>1</sup>H NMR (400 MHz,  
603 CDCl<sub>3</sub>) 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  
604 (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,  
605 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,  $J_{3',4} =$   
606 5.93,  $J_{3',3} = 14.32$  Hz, H-3'), 2.17 (s, 3H, CH<sub>3</sub>CO). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): 177.6,  
607 177.1 (FMOCO), 171.7 (C1), 170.1 (CH<sub>3</sub>CO), 143.2 & 141.1 (ArCq), 128.5, 127.2, 125.1 &  
608 120.5 (ArC), 77.6 (C2), 74.7 (C4), 70.7 (C8), 68.8 (C6), 67.7 (C5), 31.7 (C3), 21.1  
609 (COCH<sub>3</sub>). HRMS (m/z) Calcd for C<sub>38</sub>H<sub>32</sub>O<sub>10</sub> [M+ Na]<sup>+</sup> 648.1995, found 648.1992.

610 **4.6 Preparation of mono-protected lactone derivatives (7e-f, 9e and 10e-10f)**

611  
612 **4.6.1** The single step preparation of the mono-protected lactones **7e** and **7f** was described  
613 in section 4.3.3

614 **4.6.2 5-O-tert-Butyldimethylsilyl- $\alpha$ -D-glucoisosaccharino-1,4-lactone (9e).**

615  $\alpha$ -D-Glucoisosaccharino-1,4-lactone **2** (1.0 g 6.17 mmol) was dissolved in pyridine (5 mL)  
616 and the resulting solution was cautiously added dropwise to TBDMSCl (1.02 g, 6.79 mmol,  
617 1.1 eq) while stirring. The reaction was allowed to proceed for 4 h at room temperature. After  
618 4h the contents of the flask were added to DCM (50 mL) and water (50 mL) and the two  
619 layers were separated. The aqueous layer was further extracted with DCM (2 x 50 mL) and  
620 the combined organic layer was washed with 1% CuSO<sub>4</sub>, dried over anhydrous sodium  
621 sulphate and concentrated to give a white crystalline syrup **9e** (780 mg; 2.83 mmol; Yield:  
622 46%); ( RF: 0.35, Hexane/EtOAc 3:1 v/v). IR (ATR)  $\nu$  3407 (O-H), 2952, 2929, 2856 (C-H),  
623 1761 (C=O), 1463, 1361 (C-H), 1254, 1201, 1122 (C-O), 1034 (Si-OR) 833, 776. <sup>1</sup>H NMR  
624 (400 MHz, CDCl<sub>3</sub>) 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-  
625 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,  $J_{5',4} =$   
626 3.76 Hz,  $J_{5',5} = 11.74$  Hz, H-5'), 2.21 (m, 2H, H-3, H3'), 0.85 (s, 9H, TBDMS), 0.04 & 0.03  
627 (2s, 6H, TBDMS). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): 177.76 (C1), 78.57 (C4), 75.61(C2), 65.36  
628 (C6), 63.56 (C5), 33.31 (C3), 25.70 (TBDMS), -5.42, -5.49 (TBDMS). HRMS (m/z)  
629 Calculated mass for C<sub>12</sub>H<sub>24</sub>SiO<sub>5</sub> [M+Na]<sup>+</sup> 299.1285, found 299.1284.

630 .

631 **4.6.3 5-O-Flourenylmethoxycarbonyl- $\alpha$ -D-glucoisosaccharino-1,4-lactone (10e)**  
632 **and 6-O-flourenylmethoxycarbonyl- $\alpha$ -D-glucoisosaccharino-1,4-lactone (10f)**  
633

634 Dry  $\alpha$ -D-Glucoisosaccharino-1,4-lactone (1.0 g, 6.17 mmol) was dissolved in 3-picoline (20  
635 mL) and the resulting solution was added cautiously, whilst stirring, to cooled 0 °C crystalline

636 9-flourenylmethyloxycarbonyl chloride (FMOCCI) (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)  $\nu$

644 (**10 e**) IR (ATR)  $\nu$  3460 (O-H), 1747 (C=O), 1450, 1193 & 1256 (C-O), 738 (Ar C-H).  $^1\text{H}$  NMR  
645 (400 MHz,  $\text{CDCl}_3$ , **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').  $^{13}\text{C}$  (100 MHz,  $\text{CDCl}_3$ ) 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  $\text{C}_{21}\text{H}_{20}\text{O}_7$   $[\text{M}+\text{Na}]^+$ : 407.1101, Found: 407.1101.

651 (**10 f**) IR (ATR)  $\nu$  3442 (O-H), 1747.5 (C=O), 1450, 1195 & 1256, (C-O), 727 (Ar C-H).  $^1\text{H}$   
652 NMR (400 MHz,  $\text{CDCl}_3$ , **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').  $^{13}\text{C}$  (100 MHz,  $\text{CDCl}_3$ )  
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  $\text{C}_{21}\text{H}_{20}\text{O}_7$   
658  $[\text{M}+\text{K}]^+$ : 423.0841, Found: 423.0854.

659 **4.7 Preparation of 5,6-diprotected lactone derivative (13) in a one pot sequential**  
660 **reactions**

661 **4.7.1 5-O-tert-Butyldimethylsilyl-6-O-acetyl- $\alpha$ -D-glucoisosacharino-1,4-lactone (13)**

662 Dried  $\alpha$ -D-glucoisosaccharino-1,4-lactone **2** (500 mg, 3.09 mmol) was dissolved in pyridine  
663 (6 mL) whilst stirring for 10 min at room temperature. It was then added cautiously to tert-  
664 butyldimethylsilyl chloride (TBDMSCl) (520 mg; 3.45 mmol; 1.1 eq) while stirring at room  
665 temperature. The reaction was allowed to proceed for 1h, then acetyl chloride (250  $\mu$ L;  
666 3.40 mmol; 1.1 eq) was added cautiously. The reaction was allowed to continue for a  
667 further 2 h at room temperature. After 2 h, the reaction was halted with DCM (50 mL),  
668 followed by water (50 mL). The aqueous layer was further extracted with DCM (2 x 30 mL)  
669 and the combined organic layer was dried over anhydrous sodium sulphate and  
670 concentrated to give a crude **13** (3.30 g) as a brown syrup which was purified using  
671 column chromatography to give the desired product as a white solid (300 mg; 0.754 mmol;  
672 Yield: 24 %); (RF: 0.42; Hexane/EtOAc 5:1 v/v). IR (ATR)  $\nu$  3420 (O-H), 2954, 2930, 2857,  
673 1750 (C=O), 1463, 1377 (C-H), 1203, 1129 (C-O), 1044 (Si-OR), 1011, 833, 777.  
674  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ) 4.72-4.68 (m, 1H, H-4), 4.37 (d, 1H,  $J_{6,6'} = 11.56\text{Hz}$ , H-6), 4.19  
675 (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,  $J_{5',5}$   
676 = 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,  
677  $J_{3',3} = 13.83$ ,  $J_{3',4} = 6.88$  Hz, H-3'), 2.08 ( $\text{CH}_3\text{CO}$ ), 0.87 (s, 9H, TBDMS), 0.06 & 0.05 (2s, 6H  
678 TBDMS).  $^{13}\text{C}$  (100 MHz,  $\text{CDCl}_3$ ) 175.5 (C1), 170.8 (C7), 77.97(C4), 74.9 (C2), 65.6 (C6),  
679 63.3 (C5), 33.7 (C3), 25.8 (TBDMS), 20.7 (C8), -5.4, -5.5 (TBDMS). HRMS (m/z): Calculated  
680 mass for  $\text{C}_{14}\text{H}_{26}\text{O}_6\text{Si}$   $[\text{M}+\text{Na}]^+$  341.1391, Found: 341.1390.

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- 684 1. Gakhokidze RA: **Saccharinic Acids**. *Russian Chemical Reviews* 1980, **49**(3):222-236.  
685 2. Sowden JC: **The Saccharinic Acids**. In: *Advances in Carbohydrate Chemistry*. vol. 12;  
686 1957: 35-79.  
687 3. Knill CJ, Kennedy JF: **Degradation of cellulose under alkaline conditions**. *Carbohydrate*  
688 *Polymers* 2002, **51**(3):281-300.  
689 4. Isbell HS: **Interpretation of some reactions in the carbohydrate field in terms of**  
690 **consecutive electron displacement**. *Journal of Research National Bureau of Standards*  
691 1944, **32**:45-59.  
692 5. Nef JU: **Dissociationsvorgänge in der Zuckergruppe. (Erste Abhandlung.): Ueber das**  
693 **Verhalten der Zuckerarten gegen die Fehling'sche Lösung sowie gegen andere**  
694 **Oxydationsmittel**. *Justus Liebigs Annalen der Chemie* 1907, **357**(2-3):214-312.  
695 6. Machell G, Richards GN: **384. Mechanism of saccharinic acid formation. Part I.**  
696 **Competing reactions in the alkaline degradation of 4-O-methyl-D-glucose, maltose,**  
697 **amylose, and cellulose**. *Journal of the Chemical Society (Resumed)* 1960:1924-1931.  
698 7. Machell G, Richards GN: **386. Mechanism of saccharinic acid formation. Part III. The**  
699 **[small alpha]-keto-aldehyde intermediate in formation of D-glucometasaccharinic**  
700 **acid**. *Journal of the Chemical Society (Resumed)* 1960:1938-1944.  
701 8. Machell G, Richards GN: **385. Mechanism of saccharinic acid formation. Part II. The**  
702 **[small alpha][small beta]-dicarbonyl intermediate in formation of D-**  
703 **glucoisosaccharinic acid**. *Journal of the Chemical Society (Resumed)* 1960:1932-1938.  
704 9. Feast AAJ, Lindberg B, Theander O: **Studies on the D-glucosaccharinic acids**. *Acta Chem*  
705 *Scand* 1965, **19**:1127-1134.  
706 10. Pavasars I: **Characterisation of organic substances in waste materials under alkaline**  
707 **conditions**. Sweden: Linköping University; 1999.  
708 11. Pavasars I, Hagberg J, Boren H, Allard B: **Alkaline degradation of cellulose: Mechanisms**  
709 **and kinetics**. *Journal of Polymers and the Environment* 2003, **11**(2):39-47.  
710 12. Whistler RL, BeMiller JN:  **$\alpha$ -D-Isosaccharino-1,4-lactone**, vol. II. New York: Academic;  
711 1963.  
712 13. Florent JC, Ughetto-Monfrin J, Monneret C: **Anthracyclinones. 2. Isosaccharinic acid as**  
713 **chiral template for the synthesis of (+)-4-demethoxy-9-deacetyl-9-**  
714 **hydroxymethyl-daunomycinone and (-)-4-deoxy- $\gamma$ -rhodomycinone**. *J Org Chem* 1987,  
715 **52**(Copyright (C) 2014 American Chemical Society (ACS). All Rights Reserved.):1051-1056.  
716 14. Bertounesque E, Millal F, Meresse P, Monneret C: **Synthesis of a highly functionalized  $\gamma$ -**  
717 **lactone as a precursor of 9- pentyl anthracyclines**. *Tetrahedron Asymmetry* 1998,  
718 **9**(17):2999-3009.  
719 15. Wolf J, Jarrige JM, Florent JC, Grierson DS, Monneret C: **New 2'-branched-chain sugar**  
720 **nucleoside analogs with potential antiviral or antitumor activity**. *Synthesis* 1992(8):773-  
721 778.  
722 16. Hanessian S, Roy R: **Chemistry of spectinomycin: Its total synthesis, stereocontrolled**  
723 **rearrangement, and analogs**. *Canadian Journal of Chemistry* 1985, **63**(1):163-172.  
724 17. Bennis K, Gelas J, Thomassigny C: **Synthesis from lactose of new enantiomerically pure**  
725 **polyhydroxylated pyrrolidines with branched structures**. *Carbohydr Res* 1995,  
726 **279**(C):307-314.  
727 18. Thomassigny C, Bennis K, Gelas J: **Synthesis of enantiomerically pure heterocycles:**  
728 **Access to hydroxylated piperidines from a sugar lactone**. *Synthesis* 1997(2):191-194.  
729 19. Niemela K, Alén R: **Characterization of pulping liquors**. In: *Analytical methods in wood*  
730 *chemistry, pulping, and papermaking* Edited by Sjöström E, Alén R. Berlin: Springer; 1999:  
731 193-231.  
732 20. Niemela K, Alén R, Sjöström E: **The formation of carboxylic acids during kraft and kraft-**  
733 **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  $\alpha$ -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  $\beta$ -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  $\alpha$ -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.

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