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Solid state structure of sodium β-1-thiophenyl glucuronate identifies 5-coordinate sodium with three independent glucoronates.

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ABSTRACT: Glucuronic acid is a key component of the glycosaminoglycans (GAGs) Chrondroitin Sulfate (CS), Heparin/Heparan sulfate (HS) and Hyaluronic Acid (HA), as well an important metabolite derivative. In biological systems the carboxylate of uronic acids in GAGs is involved in important H-binding interactions, and the role of metal coordination, such as sodiated systems, has indications associated with a number of biological effects, and physiological GAG-related processes. In synthetic approaches to GAG fragments, thioglycoside intermediates, or derivatives from these, are commonly employed. Of the reported examples of sodium coordination in carbohydrates, 6-coordinate systems are usually observed often with water ligands involved, Herein we report an unexpected 5-coordinate sodiated GlcA crystal structure of the parent GlcA, but as a thioglucoside derivative, whose crystal coordination differs from previous examples, with no involvement of water as a ligand and containing a distorted trigonal bypramidal sodium with each GlcA having five of 6 oxygens sodium-coordinated.

1. Introduction

Sodium coordination plays roles in many areas of biological structure and function. The roles of sodium are manifold in nucleic acid structures[1] and their ligand interactions[2], and in biochemical roles of phosphate-containing biomolecules such as nucleotide phosphates and cyclic mononucleotides, and also in monophophate transport.[3

Glycosaminoglycans (GAGs) are complex carbohydrates constituted of repeating, typically sulfated, negatively charged, polysaccharide units. GAGs bind to a wide range of proteins, mediating diverse biological signalling and recognition and as such are implicated in numerous biological processes and diseases including cancer and pathogen infections, as well as relevance to tissue engineering applications.[4]

A number of literature examples have reported binding between glycosaminoglycans and sodium ions, evidencing different coordination patterns, typically hexavalent and often involving sulfates in sulfated GAGs.[5] The role of sodiated GAGs in biological systems, although often overshadowed by interests in sequences, conformations and sulfation-related interactions and selectivity, is of biological significance more widely too. Sodium salts of GAGs such as chondroitin and hyaluronic acid are relevant to the biomedical applications (as their sodium salts, eg clinical sodium hyaluronate) as well as evidence that GAG levels and functional are often related to physiological sodium levels,[6] all indicate that sodium-uronic acid interactions have biological relevance and functional significance in some cases. Sodium-GAG relationships have also been reported to be involved in heart failure mechanism.[7] This widely known association between sodium and GAGs in biology and physiology, is not matched by the scope of structural studies regarding sodium-uronic acid interactions.

Though sodium can display coordination numbers of 3, 4 and 5, the common coordination number of sodium is 6. With this coordination, sodium complexes display trigonal prism and octahedral geometries.[8] Complexes of Na with a coordination number of five are relatively rare and rarely exist in square pyramidal and trigonal bipyramidal geometries.[9]

There are a number of studies of X-ray structures of the sodium salts of various carbohydrates, of general structural context. Beijran (an anionic polysaccharide produced by microbes) forms an extended 2-fold helix with helices forming vertically extended thick sheets, 10.4 Å apart, with inter-sheet sodium and water ions. Two sodium atoms are located near carboxylate groups, one being pentavalent with four carbohydrate oxygen atoms and one water molecule as ligands, and the other tetravalently coordinated via three carbohydrate oxygen atoms and one water molecule.[10]

The crystal structure of the sodium salt of digeneaside (sodium 2-*O*-α-D-mannopyranosyl-D-glycerate monohydrate; isolated from red algae) shows three sodium ions bound to each digeneaside anion, with each sodium atom bound to six carbohydrate oxygen atoms. The average bond length between Na-O is found to be 2.5 Å, and Na ion chains with a Na-Na length averaging 3.98 Å.[11]

Several co-crystals of sodium halogen salts with carbohydrates have been reported, with most displaying hexavalent sodium with octahedral geometry.[12] Co-crystals of NaCl with D-(−)-ribose, D-(+)-glucose and

D-(+)-sucrose showed glucose formed monohydrated hexavalent sodiated co-crystals ((glucose)$_2$·NaCl·H_2O), whilst sucrose formed equimolar co-crystals (sucrose·NaCl·H_2O) with Na coordinated in a distorted octahedral geometry, coordinating to two oxygen atoms of water, one chloride and three sucrose hydroxyl oxygen atoms.[13] Ribose formed anhydrous co-crystals (ribose.NaCl) with heptavalent distorted square antiprismatic sodium ions and additional association with Cl as bridging ligands.

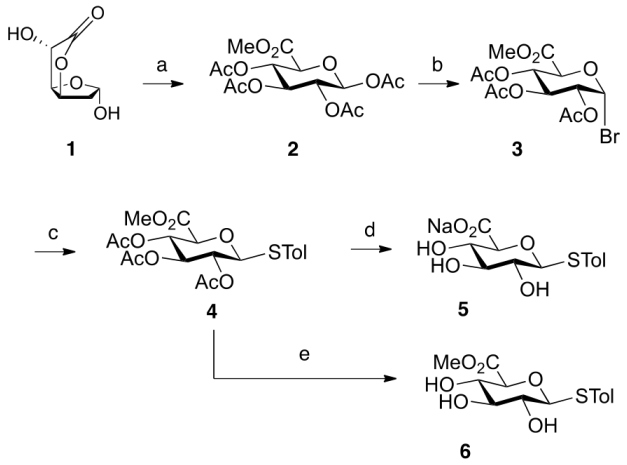
Gilli *et al* also reported structures of sucrose-NaCl.2H_2O and sucrose-NaBr.2H_2O. The NaBr complex showed pentavalent sodium with the 6-OH and 4-OH of different pyranoside residues, two water molecules and one bromide. Other similar examples have been reported with multiple halide salts viz Cl and Br. [14]

The crystal structure of sodium (1*S*)-D-lyxit-1-ylsulfonate ($NaC_5H_{11}O_8S$), the bisulfite adduct of D-lyxose, displayed sodium hexa-coordination with three oxygen atoms shared by the single D-lyxose sulfonate unit and the other three coordinating oxygens from two sulfonates and one hydroxyl group.[15]

2. Results and discussion

D-Glucuronic acid is a component of several glycosaminoglycans (GAGs), occurring in chondroitin sulfates (CS), hyaluronic acid (HA) and heparin/heparin sulfates (H/HS). In the latter, sequences are more diverse, with GlcA showing higher occurrence in HS with NA (*N*-acetylated) domains consisting largely of GlcNAcα(1→4)GlcAβ(1→4) disaccharide units (with little sulfation present).[16] Solid state examples of GlcA-sodium interactions thus offer potential value for those investigating such interactions in biology.

DeLucas and co-workers reported an X-ray structure of the parent glucuronic acid free sugar showing a hexavalent coordination of sodium cations to three symmetrical D-glucuronate anions and one water molecule.[17] Here, we report a solid-state sodium salt structure of a GlcA thioglycoside derivative (the β-1-thiotolyl glycoside). This displays a previously unseen example of ironic acid-5-coordinate Na interaction, unusually also in that all five ligating atoms are sugar oxygens.



Reagents and conditions: (a) (i) NaOH, MeOH, 0 ºC; (ii) Ac_2O, NaOAc, 90 ºC, 46%; (b) HBr, CH_2Cl_2, 96%; (c) TBAHS, MePhSH, EtOAc-aq.$NaHCO_3$, 85%; (d) MeOH, $NaOCH_3$, 67%; (e) MeOH, $NaOCH_3$, 70%.

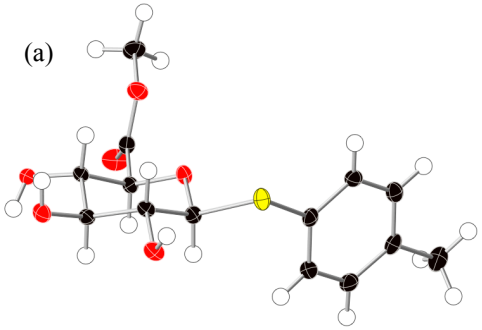
Scheme 1. Synthesis route for the Preparation of the Sodium Salt of the 1-thiotolyl D-glucuronate **5**

Synthesis of GlcA-containing GAG fragments and also of GlcA-conjugates, typically requires anomerically-reactive derivatives, and amongst these, thioglycosides are a valuable reagent type. The sodium thiotolyl GlcA in this report was obtained in four steps from D-glucuro-γ-lactone (**1**). Although the synthesis of the SPh analogue has been reported,[18] neither the STol parent, or crystal structures of either thioglycoside have been reported. The peracylated **2** was obtained as we have previously described [19] and then converted into the glycosyl bromide **3** with HBr in DCM, as we found that the conversion of this under phase-transfer catalysed conditions to the STol thioglycoside **4** was a more reliable route to the STol glucuronate **4**,[20] than the classical direct BF_3.OEt_2-mediated thioglycosylation of the 1-OAc substrate (**2**) (Scheme 1), which for example has been used to prepare the SEt analogue of **2**.[21]

The synthesis of the previously unknown sodium salt, **5**, from **4** was effected in good yield using NaOMe in methanol. We observed that the outcome was affected by the methoxide source, and that fresh newly opened bottles of commercial NaOMe in methanol in fact allowed alternatively for selective de-O-acetylation to yield the precursor glucuronate ester triol **6** in good yield, rather than acid **5**. In both cases, reaction times are substantively shorter, and at lower NaOMe concentration, than those reported to lead to epimerizaton to the L-Ido configuration.[18] Surprisingly, only the SEt analogue of the 2,3,4-hydroxy GlcA methyl ester analogue of **6** has been reported,[21] and the STol derivative is unknown. There are no prior crystal structures of such thioglycosides. We were able to determine the crystal structure of this STol glucuronate ester **6** and of the parent carboxylate **5**, both of which are novel.

Ester **6** crystallises in the chiral space group $P2_12_12_1$, with a single molecule of **6** in the asymmetric unit. There are no solvents of crystallisation present in the structure.

The structure shows that **6** packs in layers, with a head – head/tail - tail interactions between neighbouring layers, with a clear separation of the extensive hydrogen bonding of the hydroxy groups of the polar GlcA "head" group and the Van der Waal's non-polar interactions of the tolyl groups between the layers.



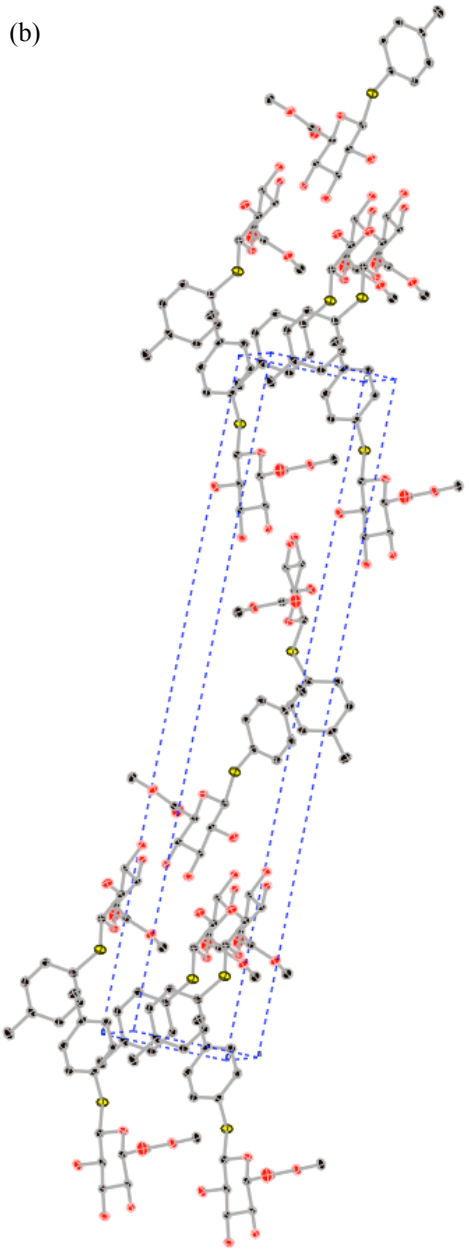


Figure 1: (a) ORTEP glucuronate **6** asymmetric unit. (b) the molecular cell with close neighbours.

Table 1

Crystal data and structure refinement data for **6**

Empirical formula	$C_{13}H_{15}NaO_6S$
Formula weight	322.30
Temperature/K	150.00(10)
Crystal system	orthorhombic
Space group	$P2_12_12_1$

a/Å	6.0756(5)
b/Å	7.9838(5)
c/Å	29.091(2)
α/°	90
β/°	90
γ/°	90
Volume/Å^3	1411.10(18)
Z	4
ρ_{calc}g/cm^3	1.517
μ/mm^1	0.284
F(000)	672.0
Crystal size/mm^3	0.35 × 0.3 × 0.09
Radiation	MoKα (λ = 0.71073)
2Θ range for data collection/°	5.292 to 50.684
Index ranges	-7 ≤ h ≤ 7, -9 ≤ k ≤ 9, -35 ≤ l ≤ 34
Reflections collected	14711
Independent reflections	2586 [R_{int}=0.0711, R_{sigma} = 0.0498]
Data/restraints/parameters	2586/3/194
Goodness-of-fit on F^2	1.104
Final R indexes [I>=2σ (I)]	R_1=0.0481, wR_2= 0.0926
Final R indexes [all data]	R_1=0.0544, wR_2= 0.0945
Largest diff. peak/hole / e Å^{-3}	0.26/-0.33
Flack parameter	0.03(7)

Each hydroxy group forms a hydrogen bond to an independent neighbour, while the carbonyl of the ester receives a hydrogen bond from a fourth independent neighbour. Such is the dominance of the hydrogen bonding that there is no obvious strong interactions between the neighbouring non-polar tolyl groups and the methyl of the ester; there is no evidence of any π-π, CH-π interactions within the layer.

The crystals of the sodium salt **5** were grown by slow evaporation of deuterated chloroform at room temperature, and thus, in contrast to most prior uronic acid crystal structures, the anhydrous crystallization conditions led to no water molecules in the complex.[13]

The sodium in this crystal is 5-coordinate, binding to three neighbouring GlcA neighbours, through different oxygens, involving the carboxylate and O4 of one GlcA unit (6-membered chelate), the carboxylate and the ring oxygen (O1) of another GlcA (5-membered chelate) and then to a single O-3 of the third GlcA. Each sodium is identical, and the coordination is of a distorted trigonal bipyramid with near perfect planarity of the three equatorial oxygens, but O-Na-O angles of 97.7º, 120.22º and 144.07º, and a O-Na-O angle for the two apical oxygens distorted to 158.96º.(Figure 2)

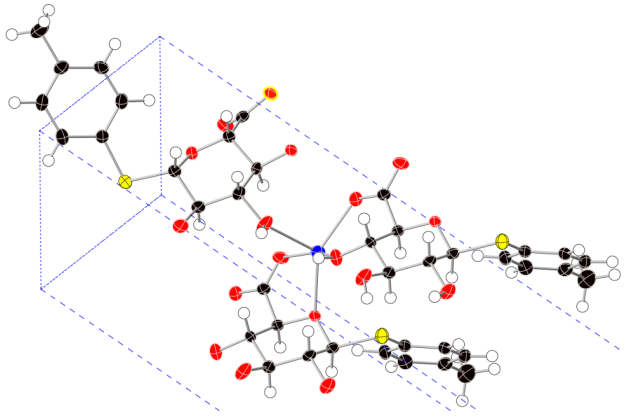


Figure 2: ORTEP of **5** showing single sodium associated with three different GlcA binding modes.

Table 2

Crystal data and structure refinement data for **5**

Empirical formula	$C_{14}H_{18}O_6S$
Formula weight	314.34
Temperature/K	100.00(10)
Crystal system	orthorhombic
Space group	$P2_12_12_1$
a/Å	5.82210(7)
b/Å	8.10357(10)
c/Å	31.2537(4)
α/°	90
β/°	90

γ/°	90
Volume/Å^3	1474.54(3)
Z	4
ρ_{calc}g/cm^3	1.416
μ/mm^1	2.188
F(000)	664.0
Crystal size/mm^3	0.153 × 0.129 × 0.119
Radiation	Cu Kα (λ = 1.54184)
2Θ range for data collection/°	5.656 to 152.278
Index ranges	-7 ≤ h ≤ 7, -10 ≤ k ≤ 8, -32 ≤ l ≤ 39
Reflections collected	14332
Independent reflections	3034 [R_{int}=0.0353, R_{sigma} = 0.0243]
Data/restraints/parameters	3034/0/193
Goodness-of-fit on F^2	1.031
Final R indexes [I>=2σ (I)]	R_1=0.0241, wR_2= 0.0623
Final R indexes [all data]	R_1=0.0246, wR_2= 0.0626
Largest diff. peak/hole / e Å^{-3}	0.20/-0.20
Flack parameter	-0.007(7)

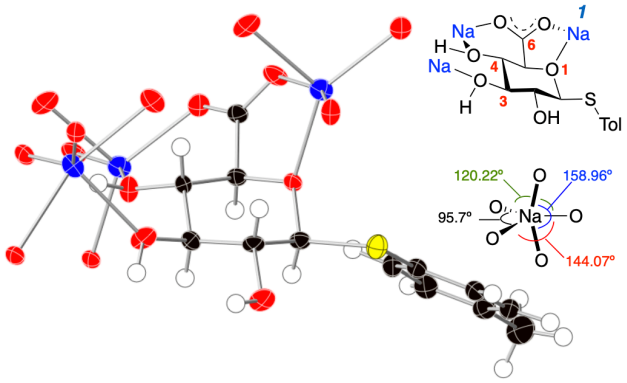


Figure 3: ORTEP showing single GlcA unit associated with three different sodiums.

Table 3

Selected distances/Å and angles/° **5**

Na(1)-O(6)	2.287(4)
Na(1)-O(1)	2.345(4)
Na(2)-O(6)	2.251(4)
Na(2)-O(4)	2.330(4)
Na(3)-O(3)	2.339(3)
Na(2)-Na(3)	5.742(3)
O(1)-Na(1)-O(6)	67.43(12)
O(6)-Na(2)-O(4)	81.37(13)

Although each sodium has three different binding modes to carbohydrate units, there is a single identical carbohydrate unit in the asymmetric unit. The C2-C2-Si-ArC torsion angle is 160.46º whilst the O1-C1-C6-carboxylate O angles are 165.54º and -15.37º. This GlcA unit is bound to three sodiums, through both carboxylate oxygens, O1, O3 and O4. Only O2 is thus not bound to a sodium.(Figure 2)

3. Conclusions

In conclusion, the majority of previously reported sodiated carbohydrate complexes exist in the solid state as 6-coordinate Na^+ with octahedral geometries, and sodium 5-coordinate structures are very rare. In particular, examples of other 5-coordinate Na^+-carbohydrate structures typically include at last one non-sugar ligand (typically water or a halide). Here, we report the X-ray structure of a 5-coordinate sodium structure with a new glucuronic acid thioglycoside ligand. This shows only pentavalent coordination of sodium, rather than hexavalent, and three different binding modes to the three associated GlcA molecules, and thus all ligating atoms from carbohydrate oxygens. This specific thioglycoside is chemically novel but also there are no examples

of crystal structures of GlcA thioglycosides previously reported. This is of interest as a new example of a sodium glucoronate binding arrangement, in the context of carbohydrate sodium salt solid state structure in general and in the context of Na^+-associated GAGs. Whilst the structure and functional roles of specific Na^+-GAG coordination is not yet clear, the relationship between Na^+ and GAG function in biological signalling and also physiological outcomes is well known. Knowledge regarding binding arrangements that may be accessible to GlcA may thus be of future interest in structure and functional studies of sodium and GlcA-containing biomolecules.

4. Experimental

4.1. General experimental

All reagents were either prepared or bought from external sources, and dried over 3 or 4 Å molecular sieves. All reactions were run under N2 atmosphere. Reactions were monitored using thin layer chromatography utilizing Merck silica gel plates 60 F254. NMR experiments were run using a 400 MHz Bruker NMR in $CDCl_3$, DMSO, or MeOD. All chemical shifts are reported in ppm values. Mass spectra (ES MS) were run on Acquity UPLC, and high-resolution mass spectra (HRMS) were obtained using Shimadzu Biotech Axima Confidence.

4.2 Methyl 1-Bromo-2,3,4-tri-*O*-acetyl-α-D-glucopyranuronate **3**

To methyl 1,2,3,4-tetra-O-acetyl-β-D-glucopyranuronate (**2**, 15.1 g, 40.1 mmol, 1 eq) in dry DCM (200 mL) was added hydrobromic acid (33% w/w in acetic acid, 80.0 mL, 1383.5 mmol, 34.5 eq). The reaction mixture was then stirred at room temperature for 19 h after which the reaction mixture was transferred to a separatory funnel, ice-cold water (700 mL) was added, the organic layer was separated, and the aqueous layer then washed with DCM, and organics combined. The crude product solution was neutralized with saturated aqueous sodium bicarbonate (650 mL), the organics separated and dried ($MgSO_4$), solvents removed *in vacuo* and the product dried under high vacuum to yield **3** (96%, 15.3 g).

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.66 (d, J = 4.0 Hz, 1H, H-1), 5.63 (t, J = 9.8 Hz, 1H, H-4), 5.26 (t, J = 9.8 Hz, 1H, H-3), 4.87 (dd, J = 4.0 Hz, 1H, H-2), 4.59 (d, J = 10.3 Hz, 1H, H-5), 3.78 (s, 3H, CO_2CH_3), 2.11 (s, 3H, OAc), 2.07 (d, J = 2.4 Hz, 6H, OAc); ^{13}C NMR (400 MHz, $CDCl_3$): δ 169.4 (C=O), 169.4 (C=O), 166.4 (C=O), 165.8 (C=O), 85.1 (C-1), 71.8 (C-5), 70.0 (C-2), 69.0 (C-4), 68.2 (C-3), 52.9 (CO_2CH_3), 20.3 (OAc), 20.2 (OAc). ^{13}C NMR (400 MHz, $CDCl_3$) δ 169.4 (C=O), 169.4 (C=O), 165.8 (C=O), 85.1 (C-1), 71.8 (C-5), 70.0 (C-2), 69.0 (C-4), 68.2 (C-3), 52.9 (CO_2CH_3), 20.3 (OAc), 20.2 (OAc). ES MS m/z 419 (MNa^+, 100%), HRMS calculated for $C_{13}H_{17}O_9BrNa^+$: 418.9953, found 418.9939. Melting point 85-87 °C, $[\alpha]_D$ = +181.5 (CH_2Cl_2) NMR and Mass spectrometry data in agreement with those reported in the literature. [22].

4.3. Methyl 1-Thiotolyl-2,3,4-tri-O-acetyl-β-D-glucopyranuronate **4**

To glycosyl bromide **3** (15.3 g, 38.72 mmol), dissolved in dry EtOAc (350 mL) was added tetrabutylammonium hydrogen sulfate (15.3 g, 45.07 mmol). Sat. aq. Na_2CO_3 (1 M, 400 mL) was then added and the biphasic mixture was then stirred vigorously on ice-salt bath. When the mixture had cooled to 2 °C, *p*-thiocresol (1.094 g, 64.75 mmol) was added portion-wise. The solution was allowed to continue stirring on the ice-salt bath for 1 h after which it was stirred at room temperature for 15 h, with TLC (3:2 Hex:EtOAc) confirming disappearance of starting material. Excess thiocresol was removed upon the treatment with molecular I_2 (7.9954 g, 31.52 mmol), and the excess I_2 was destroyed by addition of Na_2SO_4 (20.100 g, 141.52 mmol). The complete removal of I_2 was judged by the disappearance of the dark colour in the solution. The organic layer was then separated and the aqueous layer was washed with EtOAc, the organics combined, and washed with H_2O (400 mL), and brine (400 mL). The organics were dried ($MgSO_4$), filtered, concentrated, and purified by column chromatography (0-100 % EtOAc in pet ether) to give **4** as an off-white solid (14.4 g, 85 %, 32.69 mmol).

^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.40 (d, J = 8.0 Hz, 2 H, Ar-H), 7.14 (d, J = 8.0 Hz, 2 H, Ar-H), 5.32-5.25 (m, 1 H, H3), 5.15 (t, J = 9.6 Hz, 1 H, H4), 4.95 (t, J = 9.5 Hz, 1 H, H2), 4.67 (d, J = 10.0 Hz, 1 H, H1), 4.01 (d, J = 9.9 Hz, 1 H, H5), 3.76 (s, 3 H, OCH_3), 2.35 (s, 3 H, Ar-CH_3), 2.09 (s, 3 H, OAc), 1.99 (s, 3 H, OAc), 1.98 (s, 3 H, OAc). ^{13}C NMR ($CDCl_3$, 400 MHz) δ 170.1, 169.4, 169.2 [3xC=O], 166.9 (C6), 139.1 (Ar-H), 134.0 (2 x Ar-H), 132.3 (Ar), 129.9 (2 x Ar-H), 86.2 (C1), 76.1 (C5), 73.2 (C3), 69.6 (C2), 69.2 (C4), 53.0 (OCH_3), 21.2 (3 x Ar-CH_3), 20.8 (OAc), 20.6 (OAc), 20.5 (OAc). FTIR ν_{max}/cm^{-1} 1739 (C=O stretch), 1375 (acyl C-O stretch), 1210 (acyl C-O stretch), 1034 (alkoxy C-O stretch) cm^{-1}; MS m/z, HRMS calculated for $C_{20}H_{24}O_9KS$: 479.0778, found 479.0773. Rf = 0.86 in Pet Ether/Ethyl Acetate 1:1. Melting Point = 121 – 123 °C. $[\alpha]_D$ = –12.4 (MeOH).

4.4 Sodium 1-Thiotolyl D-glucuronate **5**

To **4** (0.5621 g, 1.27 mmol) under N_2 atmosphere dissolved in anhydrous MeOH (12 mL), sodium methoxide (25-30% w/w in MeOH, 0.23 mL) was added dropwise. The solution was then stirred at room temperature for 2 h whereupon TLC (product Rf = 0.26, 100% EtOAc) indicated completion. Amberlite IR-86 H-form ion exchange resin was then added to the reaction flask to neutralize the solution. The solution was

then filtered and concentrated *in vacuo* to yield crude **5** as a brown solid, which was then washed with MeOH. This is yielded product **5** as off-white solid (0.2644 g, 0.86 mmol, 67%).

^{1}H NMR (MeOD, 400 MHz) δ 7.53 (d, *J* = 8.1 Hz, 2 H, Ar-H), 7.13 (d, *J* = 8.2 Hz, 2 H, Ar-H), 4.89 (s, br, 3 H, -OH), 4.51 (d, *J* = 9.7 Hz, 1 H, H1), 3.59 (d, *J* = 9.4 Hz, 1 H, H5), 3.47-3.39 (m, 2 H, H3, H4), 3.23 (t, *J* = 9.5 Hz, 1 H, H2), 2.32 (s, 3 H, Ar-CH_3). ^{13}C-NMR (MeOD, 100 MHz) δ 175.2 (C6), 137.4 (Ar), 132.5 (2 x Ar-H), 129.7 (2 x Ar-H), 129.4 (Ar-H), 88.8 (C1), 79.4 (C5), 78.1 (C3), 72.2 (C2), 72.0 (C4), 19.8 ($ArCH_3$); Product Rf = 0.26, 100% EtOAc. Melting Point = 182 – 185 °C. $[\alpha]_D$ = –36.1 (H_2O).

4.5 Methyl 1-thiotolyl-β-D-glucopyranuronate **6**

To thioglycoside **6** (6.400 g, 20.29 mmol) under N_2 atmosphere dissolved in anhydrous MeOH (280 mL), was added sodium methoxide (25-30% w/w in MeOH, 2.5 mL, 43.73 mmol) dropwise. The solution was then stirred at room temperature for 90 min., whereupon TLC indicated completion of consumption of starting material. The reaction was neutralized with Amberlite IR-120 H-form ion exchange, filtered and concentrated to yield crude **6** as a brown solid which was purified via silica gel chromatography (100 % EtOAc) to furnish product 6 as an off-white solid (3.1877 g, 10.20 mmol, 70 %). Rf = 0.46 (100% EtOAc).

^{1}H NMR (MeOD, 400 MHz) δ 7.43 (d, *J* = 8.1 Hz, 2 H, Ar-H), 7.13 (d, *J* = 7.9 Hz, 2H, Ar-H), 4.56 (t, *J* = 9.6 Hz, 1 H, H1), 3.85 (d, *J* = 9.5 Hz, 1 H, H5), 3.79 (s, 3 H, -OCH_3), 3.50 (t, *J* = 9.2 Hz, 1 H, H4), 3.38 (d, *J* = 8.8 Hz, 1 H, H3), 3.19 (app.t, *J* = 9.7 Hz, 1 H, H2), 2.3 (s, 1 H, Ar-CH_3). ^{13}C NMR (MeOD, 100 MHz) δ 169.5 (C6), 137.9 (Ar), 132.7 (2 x Ar-H), 129.2 (2 x Ar-H), 128.9 (Ar-H), 88.7 (C1), 78.9 (C5), 77.5 (C3), 71.9 (C2), 71.3 (C4), 51.4 (OCH_3), 19.7 ($ArCH_3$); ES MS m/z 337 (MNa^+, 100%); HRMS Calculated for $C_{14}H_{18}O_6SNa^+$: 337.0721, Found: 337.0711; FTIR ν_{max}/cm^{-1} 3235 (br, O-H stretch), 1726 (C=O stretch) $cm^{-1;}$ Melting Point = 139 – 142 °C. $[\alpha]_D$ = –91.7 (MeOH).

4.6 Single Crystal Diffraction

Data for **5** were collected a Rigaku Fr-X DW diffractometer using CuKα at a temperature of 100K, cooled using an Oxford Cryostream 700. Data for **6** were collected a Rigaku Fr-X DW diffractometer using MoKα at a temperature of 150K, cooled using an Oxford Cryostream 700. The data were collected and reduced using Rigaku CrysAlisPro [23] and the structures solved and refined using the Shelx suite of programs (ShelXT and ShelXL, respectively)[24] implemented through Olex2[25].

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found at XXXX.

X-ray structural data have been deposited at the Cambridge Crystallographic Database.

Compound **5** CCDC: 2047322

Compound **6** CCDC: 2047323

References

1. Auffinger P.; D'Ascenzo L.; Ennifar E. (**2016**) Sodium and Potassium Interactions with Nucleic Acids. In: Sigel A.; Sigel H.; Sigel R. (eds) *The Alkali Metal Ions: Their Role for Life. Metal Ions in Life Sciences*, vol 16. Springer, Cham.
2. Zhang, X. X.; Brantley, S .L.; Corcelli, S.A.; Tokmakoff, A. *Commun. Biol.* **2020**, *3*, 525.
3. Tsai, J.-Y.; Chu, C.-H.;. Lin, M.-G; Chou, Y.-H.; Hong, R.-Y.; Yen, C.-Y.; Hsiao, C.-D.; Sun, Y.-J. *Science Advances* **2020**, *6*, eabb4024.
4. (a) Lindahl, U.; Kjell, L. *J. Intern. Med.* **2013**, *273*, 555–571; (b) Pomin, W. H.; Mulloy, B. *Pharmaceuticals* **2018**, *11*, 27. (c) Seeberger, P. H.; Werz, D. B. *Nature*. **2006**, *446*, 1046 – 1051. (d) Lima, M.; Rudd, T.; Yates, E. *Molecules* **2017**, *22*, 749; (d) DeAngelis, P.L.; Liu,J.; Linhardt, R. J. *Glycobiology* **2013**, *23*, 764−777. (e) Köwitsch, A.; Zhou, G.; Groth, T. *J. Tissue Eng. Regen. Med*. **2018**, *12*, e23– e41. (f) Jayson, G. C.; Miller, G. J.; Hansen, S. U.; Barath, M.; Gardiner, J. M.; Avizienyte, E. *Biochem. Soc. Trans.* **2014**, *42*, 1596-1600. (g) Zulueta, M. M. L.; Lin, S.-Y.; Hu, Y.-P.; Hung, S.-C. *Curr. Opin. Chem. Biol.* **2013**, *17*, 1023−1029. (h) Casu, B.; Naggi, A.; Torri, G. *Matrix Biol.* **2010**, *29*, 442−452. (i) Hallak, L. K.; Spillmann, D.; Collins, P. L.; Peeples, M. E. *J. Virol.* **2000**, *74*, 10508.
5. (a) Hricovíni, M. *J. Phys. Chem. B* **2011**, *115*, 1503–1511. (b) Hricovíni, M; Hricovíni, M. *Molecules* **2018**, *23*, 3042.
6. (a) SUGár, D.; Agócs, R.; Tatár, E.; Tóth, G.; Horváth, P.; Sulyok, E.; Szabó, A. J. *Physiol. Res.* **2018**, 67, 777-785. (b) Pan, W.; Roccabianca, S.; Basson, M. D. and Bush , T. R. *Royal Soc. Open Sci*. **2019**, *6*, 182076. http://doi.org/10.1098/rsos.182076.
7. Nijst, P.; Verbrugge, F. H.; Grieten, L.; Dupont, M.; Steels, P.; Tang, W. H. W.; Mullens, W. *J. Am. Coll. Cardiol.* **2015**, *65*, 378-388, https://doi.org/10.1016/j.jacc.2014.11.025]

8. (a) Kunert, M.; Wiegeleben, P.; Görls, H.; Dinjus, E. *Inorg. Chem. Commun.* **1998**, *1*, 131; (b) Belveren, S.; Poyraz, S.; Ülger, M.; Pask, C. M.; Döndaş, H. A.; Sansano, J. M. *Inorganica Chimica Acta* **2020**, *504*, 119456; (c) Zhang, Q.; Zhang, W.; Wang, S.; Solan, G. A.; Liang, T.; Rajendran, N. M.; Sun, W.-H. *Inorg Chem Front.* **2016**, *3*, 1178-1189. **YES**
9. (a) Bhowmik, P.; Chatterjee, S.; Chattopadhyay, S. *Polyhedron* **2013**, *63*, 214–221; (b) Shah, S. R.; Shah, Z.; Khan, A.; Ahmed, A.; Sohani; Hussain, J.; Csuk, R.; Anwar, M. U.; Al-Harrasi, A. *ACS Omega*, **2019**, *4*, 21559–21566.
10. Bian, W.; Chandrasekaran, R.; Ogawa, K. *Carbohydr. Res.*, **2002**, *337*, 305–314.
11. Claude, A.; Bondu, S.; Michaud, F.; Bourgougnon N.; Deslandes E. *Carbohydr. Res.* **2009**, *344*, 707–710.
12. Oertling, H.; Besnard, C.; Alzieu, T.; Wissenmeyer, M.; Vinay, C.; Mahieux , J.; Fumeaux, R. *Cryst. Growth & Des*. **2016**, *17*, 262–270.
13. Oertling, H. *CrystEngComm*. **2016**, *18*, 1676-1692.
14. Accorsi, C. A.; Bellucci, F.; Bertolasi, V.; Ferretti, V.; Gilli, G. *Carbohydr. Res*. **1989**, *191*, 105–116.
15. Haines, A. H.; Hughes, D. L. *Acta Cryst. E.* **2016**, *72*, 628–631.
16. (a) Velleman, S. G.; Liu, C. In *Chemistry and Biology of Heparin and Heparan Sulfate*; Garg, H. G.; Linhardt, R. J.; Hales, C. A. Eds.; Elsevier: Amsterdam, The Netherlands, **2005**; pp 29–54. (b) Stringer S. E.; Gallagher, J. T. *Int. J. Biochem. Cell Biol.* **1997**, *29*, 709-714. (c) Casu, B.; Lindahl, U. *Adv. Carbohydr. Chem. Biochem.* **2001**, 159. (d) Gandhi, N. S.; Mancera, R. L. *Glycobiology* **2009**, *19*, 1103–1115.
17. Delucas, J.; Gartland, G. L.; Bugg, C. E. *Carbohydr. Res.* **1978**, *62*, 213–221.
18. Cao, X.; Lv, Q.; Li, D.; Ye, H.; Yan, X.; Yang, X.; Gan, H.; Zhao, W.; Jin, L.; Wang, P.; Shen, J. *Asian J. Org. Chem.* **2015**, *4*, 899-902.
19. (a) Potter, G. T. PhD Thesis, University of Manchester, **2015**, p75; (b) Ní Cheallaigh, A.; Potter, G. T.; Gardiner, J. M.; Miller, G. J. *Org. Synth.* **2016**, *93*, 200-209.
20. Huang, L.; Huang, X. *Chem. – Eur. J.* **2007**, *13*, 529-540.
21. Lahmann, M.; Bergström, M. A.; Turek, D.; Oscarson, S. *J. Carbohydr. Chem.* **2004**, *23*, 123–132.
22. Jongkees, S. A. K.; Withers, S. G. *J. Am. Chem. Soc.* **2011**, *133*, 19334-19337.
23. Rigaku Oxford Diffraction, CrysAlisPro Software system, Rigaku Corporation, Oxford, UK **2020**
24. Sheldrick. G. M**.,** *Acta Crystallogr.* **2015**, *C71*, 3
25. O. V. Dolomanov, L. J. Bourhis, R. J. Gildea, J. A. K. Howard, H. Puschmann*, J. Appl. Cryst.* **2009** *42*, 339