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Preparation and characterisation of solid state forms of paracetamol-*O*-glucuronide

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ABSTRACT

The synthesis and crystallisation of the pharmaceutically important metabolite, paracetamol-*O*-glucuronide, is described. Hydrated and anhydrous forms of the target molecule have been characterised by PXRD, DSC and TGA. In addition, a methanol solvate has been analysed, including single crystal analysis, which represents the first structure solution for this system.

Keywords: Paracetamol-*O*-glucuronide, Solvates, Hydrates, X-ray diffraction.

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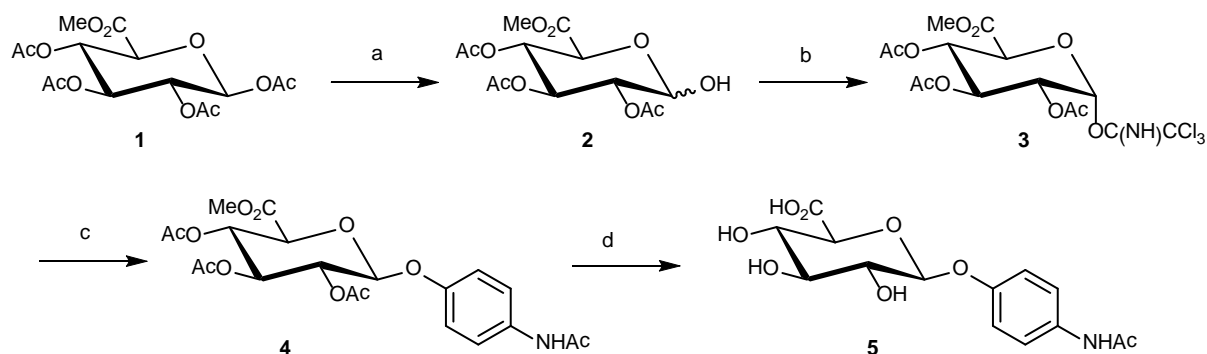
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Glucuronides are a group of biologically active molecules formed *in vivo* as a result of phase II metabolism.¹ This metabolic pathway is primarily concerned with the bioconjugation of non-polar xenobiotics to glucuronic acid resulting in more water soluble forms of the parent compound which then can be readily excreted. For example, paracetamol, one of the world's most widely used analgesic and antipyretics, is metabolized extensively in the body, mainly in the liver. The main metabolites from this pathway are paracetamol-*O*-sulfate, *N*-acetyl-*p*-benzoquinone imine and paracetamol-*O*-glucuronide **5**. Formation of paracetamol-*O*-glucuronide accounts for up to two thirds of this metabolic pathway.²

Glucuronides are highly polar molecules which have traditionally been synthesized on milligram scales for the purposes of pharmacological testing, testing for drug substances of abuse and investigation of possible metabolites of new APIs during clinical trials.³ Many synthetic methods exist for the synthesis of *O*-linked glucuronides and their various derivatives. Acyl protected intermediates such as methyl tetra-*O*-acetyl- β -D-glucopyranuronate **1** seem to be the most prevalent intermediates for the synthesis of the glucuronide series, although higher priority alkyl and aryl protecting groups such as the isobutyryl,⁴ pivaloyl⁵ and benzyl⁶ groups have also been employed.

Based on Etter's rules,⁷ the ratio of hydrogen bond donors : acceptors for a typical glucuronide suggest that it is possible to incorporate one or more hydrate or solvate molecule within the hydrogen bonding framework in the solid state.^{8,9} This guideline for the formation of hydrates and solvates is in agreement with Etter's rules.⁹ Hydrate forms of both the parent acid and sodium salt of paracetamol-*O*-glucuronide **5** have been reported^{10,11} as well as an anhydrous form.¹² To date no solid state forms of this biologically important molecule have been fully elucidated. Herein we report the preparation, crystallisation and crystal forms of 4-acetamidophenyl- β -D-glucopyranosiduronic acid **5** (Scheme 1) and for the first time the

crystal structure of a methanol solvate of the target compound. The only reported crystal structures of a glucuronide is that of estrone glucuronide.¹³ (CSD¹⁴ ref. code RESKAB)



Scheme 1. (a) Bu_3SnOMe , CH_2Cl_2 , 87%; (b) CCl_3CN , DBU, CH_2Cl_2 , 65%; (c) paracetamol, $\text{BF}_3\cdot\text{Et}_2\text{O}$, CH_2Cl_2 , 76%; (d) 2 eq. K_2CO_3 in 5:5:1 MeOH:THF:H₂O, followed by Amberlyst[®] 15 H⁺ ion exchange resin, 47% (for sodium salt, Amberlyst[®] 15 H⁺ resin, then aq. NaHCO_3 soln, MeOH, 85%).

A number of syntheses of compound **5** have been reported.^{10, 12, 15} Brown et al¹⁵ used a tri-*O*-isobutyryl protected trichloroacetimidate as glucuronidating agent. However, we found that use of the tri-*O*-acetyltrichloroacetimidate **3**¹⁶ provided the most practical route to useful quantities of compound **5** (Scheme 1). Treatment of methyl tetra-*O*-acetyl- β -D-glucopyranuronate **1** with tributyltin methoxide in dichloromethane¹⁷ yielded the hemiacetal **2** as a mixture of diastereoisomers (approximately 75:25 α : β) in 87% yield. Coupling **2** to trichloroacetimidate with DBU resulted in the exclusive formation of the α -imidate **3**. Methyl (4-acetamidophenyl-2,3,4-tri-*O*-acetyl- β -D-glucopyranosid)uronate **4** was obtained using the glucuronide donor **3**, paracetamol and $\text{BF}_3\cdot\text{OEt}_2$ in dichloromethane. Base hydrolysis of **4** was achieved using 2 equivalents of K_2CO_3 in 5:5:1 MeOH:THF:H₂O.¹⁸

Following neutralisation with a strongly acidic ion-exchange resin and column chromatography, 4-acetamidophenyl- β -D-glucopyranosiduronic acid **5** was isolated in 47% yield. A coupling constant of 7.5Hz for the ^1H doublet was assigned to the hydrogen on C-1 of the glucopyranose, indicative of β stereochemistry, which was also confirmed by single crystal analysis (see below).

In our hands, complete evaporation to dryness of solutions of **5** gave amorphous material, displaying typical amorphous halos in their powder X-ray diffraction (PXRD) patterns. Attempts to crystallize these batches from solvents also gave amorphous material. Crystalline material could be obtained by clarifying the product solution with charcoal, reducing to approximately half volume and cooling to between 0-5 °C. This resulted in the formation of two distinct crystalline forms of target compound **5**, an anhydrous form and a hydrated form. The anhydrous form of **5** was characterised by PXRD (Supplementary Information) and shows no significant weight loss in the corresponding TGA traces (Supplementary Information). The other crystalline form of **5** was isolated as a monohydrate with a needle-like habit (Supplementary Information). The TGA traces (Figure 1) revealed two events, the first in the region of 20-60 °C which is due to residual solvent tightly held on the crystal surfaces, the second in the region of 100-120 °C, being due to the hydrate. On no occasion was both of these forms observed together. Concomitant polymorphism, in which two or more crystal forms are observed to be present simultaneously, is well reported¹⁹. Formation of either one particular form or another, but not both simultaneously, as observed in this case, is more unusual but still would not be a particularly surprising crystallisation phenomenon. It is likely that sub-critical nuclei of both forms are present in solution but that once one form nucleates, growth of that form is exclusive at least on the occasions these

crystallisation have carried out by us. There was no obvious difference in how these crystallisations were carried which would lend a bias toward one form or the other.

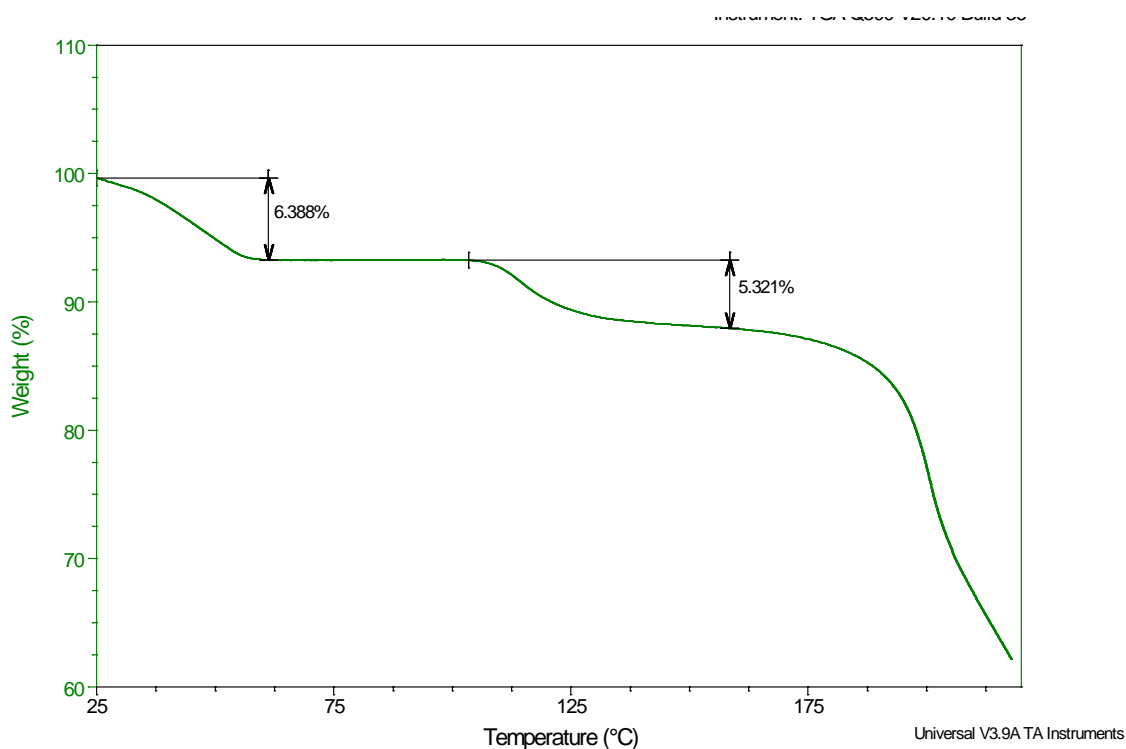


Figure 1: TGA Trace **5.H₂O**.

The crystalline sodium salt of **5** was achieved by reaction of the free acid with aqueous sodium bicarbonate in ethanol. However, recrystallisation of the sodium salt from aqueous ethanol yields microcrystalline **5** sodium salt as an agglomerated solid which was not suitable for structural analysis.

The only form observed in our studies suitable for single crystal analysis was a methanol solvate of **5** which was crystallized from methanol-dichloromethane. This form was found to be orthorhombic, crystallizing in the $P2_12_12_1$ space group (See Table 1). Comparison of the

PXRD pattern of the bulk material with the theoretically generated pattern based on the crystal structure we have obtained shows that the structure is representative of the bulk material (Supplementary Information).

Table 1: Crystal data for 5.MeOH.

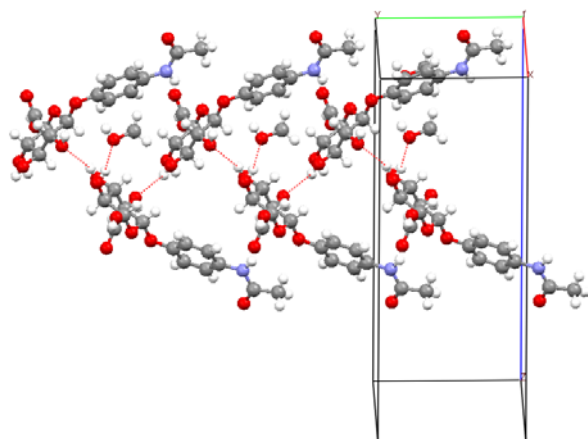
| | |
|---|---|
| Chemical formula | C ₁₅ H ₂₁ NO ₉ |
| Formula Mass | 359.33 |
| Crystal system | Orthorhombic |
| <i>a</i> /Å | 8.3590(4) |
| <i>b</i> /Å | 8.4792(4) |
| <i>c</i> /Å | 22.8663(10) |
| Unit cell volume/Å ³ | 1620.71(13) |
| Temperature/K | 296(2) |
| Space group | <i>P</i> 2 ₁ 2 ₁ 2 ₁ |
| No. of formula units per unit cell, <i>Z</i> | 4 |
| Radiation type | CuK _α (1.5418 Å) |
| Absorption coefficient, μ/mm ⁻¹ | 1.054 |
| No. of reflections measured | 12824 |
| No. of independent reflections | 2699 |
| <i>R</i> _{int} | 0.032 |
| Final <i>R</i> _{<i>I</i>} values (<i>I</i> > 2σ(<i>I</i>)) | 0.033 |
| Final <i>wR</i> (<i>F</i> ²) values (<i>I</i> > 2σ(<i>I</i>)) | 0.085 |
| Final <i>R</i> _{<i>I</i>} values (all data) | 0.038 |
| Final <i>wR</i> (<i>F</i> ²) values (all data) | 0.088 |
| Goodness of fit on <i>F</i> ² | 1.03 |
| Flack parameter | 0.3(2) |

Face indexing experiments of the needle-like crystals observed for the methanol solvate indicate that the preferred growth direction is along the *b* axis of the unit cell (Figure 2).

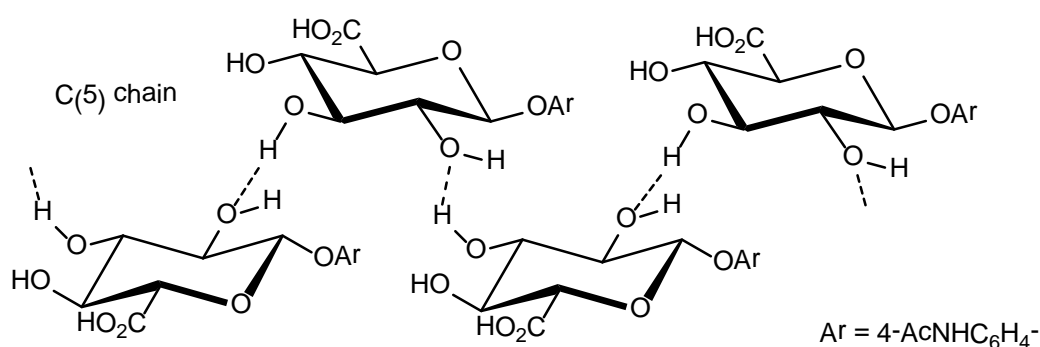


Figure 2: Lattice orientation of **5.MeOH** showing preferential growth along the crystallographic *b* axis.

This can be rationalised by inspection of the single crystal data which shows C(5) chains⁷ along the crystallographic *b* axis (Figure 3). These C(5) chains are made by the diol functionality on C-2 and C-3 of the glucopyranose ring, and are related by a 2_1 screw axis as represented in Figure 3. Unitary graph set analysis⁷ along this crystallographic axis shows a discrete (capping) H-----O-Me hydrogen bond between the OH group on C-4 of the glucopyranose ring and the interstitial MeOH molecule is also observed (Figure 3).



(a)



(b)

Figure 3: (a) Unit cell of **5.MeOH** along the crystallographic *b* axis, showing C(5) chains and interstitial MeOH molecules, along the preferential growth axis (some molecules have been removed for clarity). (b) Graphical representation of the C(5) chains along the *b* axis of unit cell.

1. Experimental

1.1. General

All commercial reagents were purchased from Sigma-Aldrich and were used without further purification. All solvents were either of a HPLC grade or distilled prior to use. Methyl tetra-*O*-acetyl- β -D-glucopyranuronate **1** was prepared as described by Bollenback et al.²⁰ Thin

layer chromatography (TLC) was conducted on coated silica plates (Merck silica gel 60, F24). Column chromatography was conducted using Merck silica gel 60, typically with a 30:1 ratio of silica to sample. TLC plates were visualized either under ultraviolet (UV) light or an anisaldehyde stain. Infrared spectra were recorded on a Perkin-Elmer Paragon 1000 FT-IR spectrometer. The ^1H NMR spectra were recorded on a Bruker AVANCE 300 MHz or 400 MHz spectrometers. Spectra were recorded using either deuterated chloroform (CDCl_3), deuterated dimethyl sulfoxide (d_6 -DMSO) or deuterated water (D_2O) using tetramethylsilane as the internal standard. Chemical shift values (δ_{H} and δ_{C}) are expressed as parts per million (ppm). Elemental analyses were performed by the Microanalysis Laboratory, University College Cork, using a Perkin-Elmer 240 and an Exeter Analytical CE440 elemental analyzer.

1.2. Synthesis

1.2.1. Methyl 2,3,4-triacetyl- α,β -glucopyranuronate (2)

Methyl tetra-*O*-acetyl- β -D-glucopyranuronate (1) (15.0 g, 39 mmol) was dissolved in dry CH_2Cl_2 (120 mL) and tributyltin methoxide (12.6 mL, 43.8 mmol) was added. The solution was refluxed for 4 h until TLC indicated consumption of the starting material. The solution was cooled to room temperature and then washed with 10% aq HCl (2 x 20 mL), water (20 mL), dried and concentrated *in vacuo*. The resulting syrup was triturated with hexane (3 x 40 mL) to yield a solid which was recrystallised from EtOAc:hexane to give a white crystalline solid (11.6 g, 87%) ratio of α to β anomers (by ^1H NMR) = 75:25 (α : β) mp 89-90 °C, lit.²¹ 91-92 °C; IR (KBr) ν 3469 (O-H) 2958 (C-H) 1752 (C=O) cm^{-1} ; m/z (ESI): 357 ($\text{M}^+ + \text{Na}$, 15%). ^1H NMR (CDCl_3) (α anomer) δ 5.57 (t, 1H, J 9.5 Hz, H-3), 5.54 (d, 1H, d, J 3.6 Hz, H-1), 5.17 (t, 1H, J 9.5 Hz, H-4), 4.90 (dd, 1H, J 3.6, 9.5 Hz, H-2), 4.59 (d, 1H, J 9.5 Hz, H-

5), 4.24 (br d, 1H, J 3.6 Hz, **OH**) 3.75 (s, 1H, **CO₂Me**), 2.09 (s, 3H, **OAc**), 2.04 (s, 3H, **OAc**), 2.03 (s, 3H, **OAc**). ¹³C NMR (CDCl₃) (α anomer) δ 170.2 (C=O), 170.1 (C=O), 168.5 (C=O), 166.7 (C=O), 90.2 (C-1), 70.78 (C-H), 69.6 (C-H), 69.2 (C-H), 67.99 (C-H), 52.90 (CO₂CH₃), 20.6 (3 x OAc). ¹H NMR (CDCl₃) (β anomer) δ 5.29 (t, 1H, J 9.5 Hz), 5.21 (t, 1H, J 9.5 Hz), 4.95 (d, 1H, J 7.8 Hz, H-1), 4.82 (t, 1H, J 9.3 Hz), 4.36 (br d, 1H, J 7.8 Hz, **OH**), 4.12 (d, 1H, J 9.6 Hz, H-5), 3.76 (s, 3H, **CO₂Me**), 2.09 (s, 3H, **OAc**), 2.04 (s, 3H, **OAc**), 2.03 (s, 3H, **OAc**). ¹³C NMR (β anomer) δ 170.52 (C=O), 170.10 (C=O), 169.59 (C=O), 167.61 (C=O), 95.45 (C-1), 72.85 (C-H), 72.53 (C-H), 71.6 (C-H), 69.43 (C-H), 53.10 (CO₂CH₃), 20.51 (3 x OAc). Anal. Calcd for C₁₃H₁₈O₁₀: C, 46.71; H, 5.43. Found: C, 46.36; H, 5.70.

1.2.2. Methyl 2,3,4-triacetyl-1-*O*-(trichloroacetimidoyl)- α -D-glucopyranuronate (**3**)

Methyl 2,3,4 triacetyl- α,β -glucopyranuronate (**2**) (12.0 g, 36 mmol) and trichloroacetonitrile (18 mL, 180 mmol) was stirred in dry CH₂Cl₂ at 0 °C for 30 min. DBU (1.5 mL, 10 mmol) was added dropwise and the solution was allowed warm to room temperature and stirred overnight. The solvent was removed *in vacuo* and the residue was subjected to flash chromatography (40:59:1 EtOAc: hexane: Et₃N). Appropriate fractions were pooled and the solvent removed under reduced pressure to yield a syrup which was triturated with diethyl ether. Following recrystallisation with EtOAc:hexane (50:50) to yield 8.5g (65%) of an off white solid. mp 108-109 °C, lit.²¹ 109-110 °C; IR (KBr) ν 3320 (N-H), 2958 (C-H), 1755 (C=O) cm⁻¹. ¹H NMR (CDCl₃) δ 8.73 (s, 1H, **NH**), 6.64 (d, 1H, J 3.6 Hz, H-1), 5.63 (t, 1H, J 10 Hz, H-3), 5.27 (t, 1H, J 10 Hz, H-4), 5.16 (dd, 1H, J 3.6 Hz, 10 Hz, H-2), 4.49 (d, 1H, J 10 Hz, H-5), 3.75 (s, 3H, **CO₂Me**), 2.05 (s, 3H, **OAc**), 2.04 (s, 3H, **OAc**), 2.02 (s, 3H, **OAc**). ¹³C NMR (CDCl₃) δ 169.78 (C=O), 169.72 (C=O), 169.47 (C=O), 167.14 (C=O), 160.58

(C=N), 92.64 (C-H), 70.49 (C-H), 69.47 (C-H), 69.10 (C-H), 68.96 (C-H), 53.04 (CO₂Me), 21.04, 20.66, 20.48, 20.39 (3 x OAc and 1 x CCl₃).

1.2.3. Methyl (4-acetamidophenyl-2,3,4-tri-*O*-acetyl- β -D-glucopyranosid)uronate (4)

Methyl 2,3,4-triacetyl-1-*O*-(trichloroacetimidoyl)- α -D-glucopyranouronate (3) (10.0 g, 21.0 mmol), paracetamol (3.5 g, 23 mmol) and 4Å molecular sieves were stirred in dry CH₂Cl₂ for 30min. BF₃.Et₂O (4 mL, 23 mmol) was added dropwise at 0 °C and the solution was allowed to stir overnight. The solvent volume was reduced, washed with sat. aq Na₂CO₃ (2 x 30mls), water (2 x 30ml) dried with MgSO₄ and concentrated in *vacuo* to yield a pale white solid. Column chromatography (40:1 CHCl₃:MeOH) followed by recrystallisation from IPA gave a white crystalline solid (7.5g, 76%). mp 214-216 °C, lit.²³ 213.5-214.5 °C; IR (KBr) ν 3300 (N-H), 3146-2969 (C-H), 1758 (C=O), 1509 (Ar-H) cm⁻¹. m/z (ESI): 468 (M⁺, 100%)
¹H NMR (CDCl₃) δ 7.42 (d, 2H, *J* 9 Hz, Ar-H), 7.2 (br s, 1H, NH), 6.96 (d, 2H, *J* 9 Hz, Ar-H), 5.35-5.23 (m, 3H, H-2, 3, 4), 5.07 (d, 1H, *J* 7.2 Hz, H-1), 4.14 (d, 1H, *J* 9.6 Hz, H-5), 3.74 (s, 3H, CO₂Me), 2.16 (s, 3H, NAc), 2.06 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.04 (s, 3H, OAc). ¹³C NMR (CDCl₃) δ 170.08 (C=O), 169.35 (C=O), 169.25 (C=O), 168.29 (C=O), 166.91 (C=O), 153.28 (C_q, Ar) 133.28 (C_q, Ar), 121.66 (C-H, Ar), 117.82 (C-H, Ar), 99.64 (C¹-H), 72.62 (C-H), 71.91 (C-H), 71.1 (C-H), 69.16 (C-H), 52.97 (CO₂Me), 24.37 (NHAc), 20.75, 20.6, 20.49 (3 x OAc).

1.2.4. Acetamidophenyl- β -D-glucopyranosiduronic Acid (5)

Methyl (4-acetamidophenyl-2,3,4-tri-*O*-acetyl- β -D-glucopyranosid)uronate (4) (1.00 g, 2.15 mmol) was dissolved in a solution of MeOH: THF: H₂O (5:5:1, 40-50 mL) and allowed to stir under an nitrogen atmosphere at 0 °C. K₂CO₃ (0.6g, 4.3mmol) was added and the reaction mixture was heated to 40 °C for 5h. The reaction mixture was cooled to room

temperature and neutralized with strongly acidic ion exchange resin (Amberlyst[®] 15 H⁺ Form). The exchange resin was removed and the solution clarified with charcoal. The solvent volume was removed *in vacuo* and the residue subjected to column chromatography using EtOAc:MeOH:H₂O (60:30:10). Appropriate fractions were pooled and the solvent removed *in vacuo*. Recrystallisation of the residue yielded 0.34g (47%) of crystalline material. mp 135 °C (MeOH:CH₂Cl₂), lit.¹⁵ 134-137 °C; IR (KBr) ν 3347 (O-H), 2925 (C-H), 1728 (C=O), 1663 (C=C), 1510 (C=C) cm⁻¹. m/z (ESI): 328 (M⁺, 10%), 326 (M⁻, 20%). ¹H NMR (d₆-DMSO) δ 9.89 (s, 1H, NH), 7.52 (d, 2H, *J* 8.8 Hz, Ar-H), 7.02 (d, 2H, *J* 8.8 Hz, Ar-H), 5.41 (br s, 1H, OH) 5.22 (br s, 1H, OH), 4.92 (d, 1H, *J* 7.5 Hz, H-1), 4.42 (br s, 1H, OH), 4.17 (br s, 1H, OH), 2.06 (s, 3H, NAc). δ _H (D₂O shake) 7.25 (d, 2H, *J* 9Hz, Ar-H), 7.03 (d, 2H, *J* 9Hz, Ar-H), 5.06 (d, 1H, *J* 7.5Hz, H-1), 4.06 (d, 1H, *J* 9Hz, H-5), 3.64-3.53 (m, 3H, H-2, 3, 4), 2.04 (s, 3H, NAc). ¹³C NMR (D₂O) δ 172.7 (C=O), 171.9 (C=O), 153.8 (C_q), 132.18 (C_q), 123.7, 117.2, 100.4 (C-H), 75.0 (C-H), 74.5 (C-H), 72.5 (C-H), 71.1 (C-H), 23.7 (NHCOCH₃).

1.2.5. 4-Acetamidophenyl- β -D-glucopyranosiduronic acid (5) sodium salt.

Methyl (4-acetamidophenyl 2,3,4-tri-*O*-acetyl- β -D-glucopyranosid)uronate (4) (1.00 g, 2.15 mmol) was dissolved in a solution of MeOH: THF: H₂O (5:5:1, 40-50 mL) and the solution stirred under an N₂ atmosphere at 0 °C. K₂CO₃ (0.6g, 4.3mmol) was added and the reaction mixture was heated to 40 °C for 5h. The reaction mixture was cooled to room temperature and neutralized with strongly acidic ion exchange resin (Amberlyst[®] 15 H⁺ Form). The exchange resin was removed and the solution was clarified with charcoal. After removal of the charcoal on a bed of Celite[®] the solvent was removed and the residue was dissolved in

MeOH (50 mL). Aq. NaHCO₃ solution (20 ml) was added and the solution heated to boiling. After heating for 10 min. the solution was allowed to cool to room temperature and left to stir for 1 h. The resulting solid was isolated and recrystallised from aq. EtOH to give the product as a white crystalline solid 0.63 g (85% yield). mp 220-230 °C, (decomp.). IR (KBr) ν 3298 (O-H), 2935 (C-H), 1578 (C=O), 1510 (C=C), 1414 (C=C), 1043(C-O) cm⁻¹; ¹H NMR (D₂O) δ 7.36 (d, 2H, *J* 9.2Hz, Ar-**H**), 7.14 (d, 2H, *J* 9.2Hz, Ar-**H**), 5.10 (d, 1H, *J* 6.8Hz, H-1), 3.89 (d, 1H, *J* 9.6Hz, H-5), 3.64-3.55 (m, 3H, H-2, 3, 4), 2.16 (s, 3H, **NAc**).

1.3. Crystal Growth

1.3.1. Hydrated and anhydrous forms. Crystalline **5** was obtained as follows. Following neutralization of the reaction mixture with acidic ion exchange resin, removal of the resin and clarification of the solution with charcoal, the filtered solution was reduced to approximately half volume and cooled to between 0-5 °C. This procedure provided either the hydrate or the anhydrous form of compound **5** with no obvious bias towards either. Mixtures of the two forms were not observed on any occasion.

1.3.2. Amorphous Form. Amorphous **5** was obtained by allowing a saturated solution of paracetamol-*O*-glucuronide, from a variety of solvents, including MeOH, EtOH and H₂O, to slowly evaporate at room temperature.

1.3.3. MeOH Solvate. Compound **5** (100 mg) was suspended in a minimum of dichloromethane (approx 10 mL), a minimum of hot methanol (approx 5-6 mL) was added to affect dissolution, and following cooling and slow evaporation of the solvent crystalline **5** was isolated with a needle-like crystal habit.

1.3.4. Sodium Salt. Compound **5** sodium salt (200 mg) was completely dissolved in a minimum of aqueous ethanol (approx 15 mL) and following cooling and slow evaporation of the solvent microcrystalline **5** sodium salt was isolated as an aggregated solid.

1.4. Solid state characterisation

DSC was performed using a TA Q1000 DSC with RSC 40 cooling system. The sample was placed into an aluminum DSC pan, and the weight accurately recorded. The pan was covered with a lid and then crimped. The sample cell was heated under a nitrogen purge at a rate of 5 °C min⁻¹, from 25 °C up to a final temperature of 160 °C. TGA analysis was performed using a TA Instruments Q500 thermogravimetric analyzer. The sample was placed in an aluminum sample pan and inserted into the TG furnace. The furnace was heated under nitrogen at a rate of 5 °C min⁻¹ from 25 °C up to a final temperature of 160 °C. Single-crystal X-ray diffraction measurements were made on a Bruker APEX II DUO diffractometer using graphite monochromatized CuK_α (1.5418 Å). All calculations were made using the APEX2 v2009.3-0 software^{24,25} and the diagrams prepared using Mercury.²⁶

1.5 Supplementary data

Complete crystallographic data for the structural analysis have been deposited with the Cambridge Crystallographic Data Centre, CCDC number 841974. Copies of this information may be obtained free of charge from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK. (fax: +44-1223-336033, e-mail: deposit@ccdc.cam.ac.uk or via: www.ccdc.cam.ac.uk). PXRD data on the crystalline forms and TGA data on the anhydrous forms are also provided.

Acknowledgments

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