Carbohydrate Research 425 (2016) 35–39

Contents lists available at ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Methyl tetra-O-acetyl-α-D-glucopyranuronate: crystal structure and influence on the crystallisation of the β anomer

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ARTICLE INFO

Article history:
Received 29 October 2015
Received in revised form 14 January 2016
Accepted 21 January 2016
Available online 14 March 2016

Keywords:
Methyl tetra-O-acetyl-D-glucopyranuronate
Crystallisation of anomers

ABSTRACT

Methyl tetra-O-acetyl-β-D-glucopyranuronate (**1**) and methyl tetra-O-acetyl-α-D-glucopyranuronate (**3**) were isolated as crystalline solids and their crystal structures were obtained. That of the β anomer (**1**) was the same as that reported by Root et al., while anomer (**3**) was found to crystallise in the orthorhombic space group $P2_12_12_1$ with two independent molecules in the asymmetric unit. No other crystal forms were found for either compound upon recrystallisation from a range of solvents. The α anomer (**3**) was found to be an impurity in initially precipitated batches of β-anomer (**1**) in quantities <3%; however, it was possible to remove the α impurity either by recrystallisation or by efficient washing, i.e. the α anomer is not incorporated inside the β anomer crystals. The β anomer (**1**) was found to grow as prisms or needles elongated in the *a* crystallographic direction in the absence of the α impurity, while the presence of the α anomer (**3**) enhanced this elongation.

1. Introduction

Methyl tetra-O-acetyl-β-D-glucopyranuronate[1] (**1**) (Fig. 1) has been very widely used as a protected intermediate in the synthesis of glucuronides.[2–16] Glucuronides are of great importance as metabolites of active pharmaceuticals.[17–22] Compound (**1**) is prepared from D-glucurono-6,3-lactone (**2**) (Fig. 1) by reaction with methanol and sodium hydroxide followed by either acetic anhydride in pyridine or acetic anhydride with perchloric acid.[1] The product is isolated by cooling of the final reaction mixture from which the product crystallises. This is a convenient method of obtaining compound (**1**) as good quality crystalline solid on a preparative laboratory scale, i.e. in batches of up to 0.5 kg. Yields are typically around 40%. It would be expected that the preparation of methyl tetra-O-acetyl-β-D-glucopyranuronate (**1**) from glucuronolactone (**2**) would also yield the corresponding α-anomer (compound (**3**), Fig. 1) and that this compound would constitute a major part of the material making up the mass balance. Processing of the mother liquors from preparations of compound (**1**) has provided the α-anomer (**3**), i.e. both the α and β anomers are formed.[1,23]

The presence of mixtures of α and β anomers in solution[24] can affect the crystallisation of specific saccharides. This is well illustrated by the case of α-D-lactose monohydrate, which is obtained by crystallisation from cheese whey and followed by recrystallisation.[25] The crystallisation media always contains β-D-lactose in significant quantities that affect the nucleation and growth of the α-D-lactose monohydrate crystals.[26] For example, the characteristic morphology of the α-D-lactose monohydrate crystals has been shown to be a result of face-selective growth inhibition by β-lactose molecules.[27] Adjustment of the quantity of β-lactose present in solution results in a corresponding alteration in the morphology of the α-D-lactose monohydrate crystals.[28] Another example of the effect of an anomer on crystallisation of a sugar is provided by β-melibiose; the presence of trace quantities of the α anomer of this disaccharide has been proposed to be responsible for the complete conversion of crystalline β-melibiose to the α form on certain occasions.[29]

In the case of methyl tetra-O-acetyl-β-D-glucopyranuronate (**1**), one specific anomer is obtained as a crystalline solid; however, the α anomer is also formed and is present in the crystallisation medium. The α anomer may therefore be affecting aspects of crystal nucleation and growth such as the degree of supersaturation necessary for nucleation, nucleation induction time, the crystal form obtained, crystal habit and crystal size distribution. We have found that the crystallisation of methyl tetra-O-acetyl-β-D-glucopyranuronate (**1**) from the final reaction medium gives variable results in the hands of different researchers with variation in yield and in the time taken for crystallisation to occur. The α anomer may also be present in some quantity as an impurity in batches of

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http://dx.doi.org/10.1016/j.carres.2016.01.012
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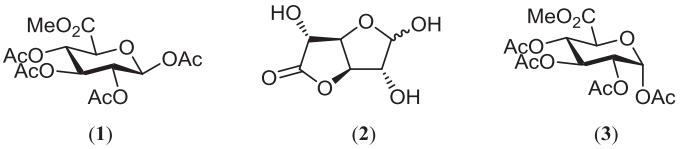

Fig. 1. Structures of methyl tetra-O-acetyl-β-D-glucopyranuronate (**1**), D-glucurono-6,3-lactone (**2**) and methyl tetra-O-acetyl-α-D-glucopyranuronate (**3**).

compound (**1**) that would affect further synthetic steps such as glucuronidation and deprotection of the final glucuronide product. Poor yield, presence of impurities and possible formation of traces of the incorrect glucuronide isomer are possible consequences. These are specific issues for compound (**1**) and glucuronide products synthesised from it, but these are also examples of general issues affecting synthetic routes to pharmaceutical compounds. Manufacturing scale pharmaceutical synthesis invariably gives rise to impurities arising from the process chemistry that impact on the solid product obtained in terms of purity, crystal form and morphology, and regulatory compliance.[30,31] In this paper, both anomers of methyl tetra-O-acetyl-D-glucopyranuronate are isolated and their crystal structures determined. The crystal structure of the β-anomer has previously been reported.[23] The occurrence of the α-anomer as an impurity in batches of the β-anomer is examined as well as the impact on growth morphology and phase.

2. Results and discussion

The α and β anomers of methyl tetra-O-acetyl-D-glucopyranuronate were synthesised as previously reported,[1,23] i.e. by opening of D-glucurono-6,3-lactone (**2**) with sodium hydroxide in methanol followed by acetylation using acetic anhydride in pyridine. The β-anomer (**1**) was obtained as a crystalline solid in 39% yield, while the mother liquor was subjected to silica gel chromatography to provide the α-anomer (**3**) as a crystalline solid in 37% yield. The $J_{H1\text{-}H2}$ for the β anomer was found to be 7.6 Hz and for the α anomer to be 3.6 Hz, consistent with the expected geometry of the H1-H2 torsional angle in both anomers. Single crystals suitable for crystal structure determination were grown from ethanol in the case of β-anomer (**1**) and from ethyl acetate/THF in the case of α-anomer (**3**). Both anomers crystallised in the orthorhombic space group $P2_12_12_1$ and their crystallographic data are presented in Table 1. ORTEP diagrams are shown in Figs. 2 and 3.

Table 1
Crystallographic data for compounds **1** and **3**

Compound reference	**1**	**3**
Chemical formula	$C_{15}H_{20}O_{11}$	$C_{15}H_{20}O_{11}$
Formula mass	376.31	376.31
Crystal system	Orthorhombic	Orthorhombic
a/Å	7.5193(3)	8.8074(3)
b/Å	13.8777(5)	13.7144(4)
c/Å	17.0439(6)	29.1375(9)
Unit cell volume/Å^3	1778.54(11)	3519.47(19)
Temperature/K	150(2)	150(2)
Space group	$P2_12_12_1$	$P2_12_12_1$
No. of formula units per unit cell, *Z*	4	8
Radiation type	Cu Kα	Cu Kα
Absorption coefficient, μ/mm^{-1}	1.058	1.070
No. of reflections measured	18573	58010
No. of independent reflections	3093	6292
R_{int}	0.0216	0.0233
Final R_1 values ($I > 2\sigma(I)$)	0.0242	0.0247
Final $wR(F^2)$ values (all data)	0.0637	0.0647
Goodness of fit on F^2	1.060	1.059
Flack parameter	0.04(12)	0.03(9)
CCDC number	1433823	1433822

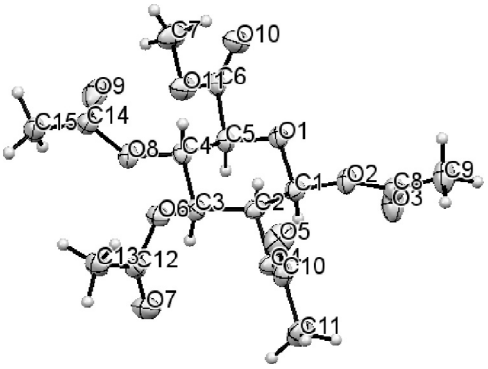

Fig. 2. ORTEP diagram of the methyl tetra-O-acetyl-β-D-glucopyranuronate (**1**) molecule in the asymmetric unit of the crystal structure.

The unit cell dimensions obtained for the β-anomer (**1**) are commensurate with those reported by Root et al.[23] The α-anomer (**3**) crystallised in orthorhombic $P2_12_12_1$ with two independent molecules in the asymmetric unit (Figs. 3 and 4). In both molecules, the 1-acetoxy group is in the α anomeric position, forming torsional angles with the axial hydrogen at ring position 2 of 165.05(13)° in the first molecule and 177.62(10)° in the second molecule. Acetal C—O bond lengths for the exocyclic acetoxy are 1.4219(16) Å for the first molecule and 1.4177(16) Å for the second molecule, while those for the endocyclic bonds are 1.4075(16) Å and 1.4086(16) Å

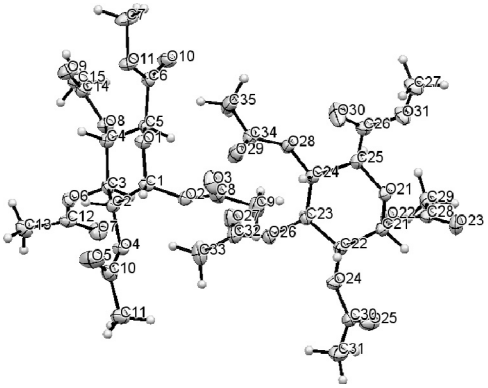

Fig. 3. ORTEP diagram of the two independent methyl tetra-O-acetyl-α-D-glucopyranuronate (**3**) molecules in the asymmetric unit of the crystal structure.

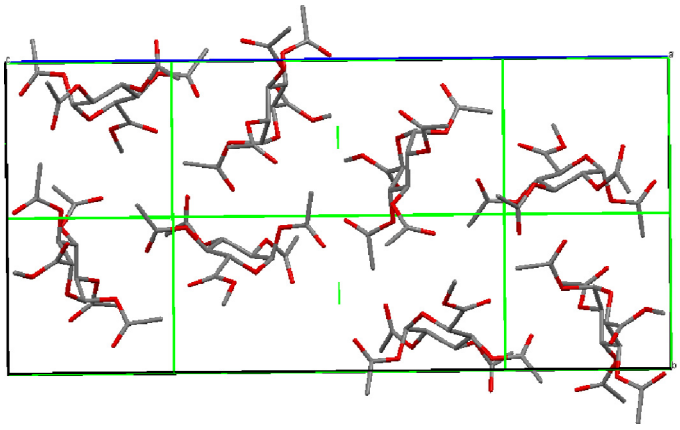

Fig. 4. View of the crystal structure of methyl tetra-O-acetyl-α-D-glucopyranuronate (**3**) along the *a* axis showing molecules related by a 2_1 screw axis.

Table 2
Cremer–Pople parameters for glucopyranose rings of compounds (**1**) and (**3**)

	Q	θ	ϕ
(**1**)	0.59 Å	6.4°	310.5°
(**3**) 1st molecule in asy. unit	0.60 Å	12.7°	292.1°
(**3**) 2nd molecule in asy. unit	0.54 Å	11.0°	41.4°

respectively. These compare with the C—O bond lengths in the β anomer of 1.414 Å and 1.416 Å reported for the exocyclic and endocyclic acetal[23] (1.4141(15) Å and 1.41189(16) Å respectively in our determination) indicating a lengthening of the exocyclic C—O in the α anomer. Relative to the β anomer, the methoxy and carbonyl group of the carbomethoxy group are orientated in the opposite direction, giving torsional angles between the ring oxygen and the methoxy oxygen of –32.16(14)° for the first molecule and 57.41(14)° for the second molecule, compared with ~180° for the β anomer.[23] The conformations of the acetoxy groups at ring positions 2, 3 and 4 are similar in both molecules and in the β anomer.

In both structures, the glucopyranose rings are in $^4C_1$ conformations. Calculated Cremer–Pople parameters[32] for our structure of compound (**1**) are given in Table 2. The puckering amplitude (Q) of 0.59 Å with distortion (θ) of 6.4° from a perfect chair is consistent with a degree of distortion, as was also noted by Root et al.[23] For example, the corresponding values for the glucopyranose ring of sucrose are 0.56 Å and 5.2° respectively.[32]

Samples of anomers (**1**) and (**3**) were recrystallised from methanol, ethanol, isopropanol, acetonitrile, diethyl ether, THF, acetone, dichloromethane, chloroform and ethyl acetate, and the recrystallised samples were analysed by DSC and PXRD to screen for multiple crystal forms. DSC analysis of all recrystallised samples showed a melting endotherm as the only thermal event, at 178 °C for β-anomer (**1**) and 107 °C for the α-anomer (**3**). (Examples of DSC traces for both anomers are given in the Supplementary Material.) PXRD patterns of all recrystallised samples of both anomers were also consistent and consistent with the theoretical patterns generated from the crystal structures. (The theoretical patterns and examples of experimental PXRD patterns for both anomers are given in the Supplementary Material.) Unground samples of β-anomer (**1**) recrystallised from ethyl acetate or from THF gave PXRD patterns from which the diffraction peaks at 8° and 20° 2θ were absent. These peaks were found to be present upon re-recording the patterns after grounding the samples, i.e. the absence of the peaks was due to a preferred orientation effect.[33]

HPLC analysis of batches of the β anomer (**1**) obtained directly from the reaction mixture (i.e. crystallised from the acetic anhydride/pyridine mixture following concentration and refrigeration) showed 2.5–3.0% of the material to be the α anomer (**3**). No α anomer was detected in any of the recrystallised batches of β anomer (**1**). Thorough washing of the directly obtained β anomer material with methanol was also found to completely remove the α anomer. While both α and β anomers are clearly present in solution, the β anomer crystallises from the reaction medium without inclusion of α anomer impurity into the β anomer crystal structure. The 2.5–3.0% of the α anomer found as an impurity in the precipitated material is likely to arise from evaporation of traces of reaction medium liquid adhered to the precipitated particles. Hence this impurity can be removed by efficient washing. In manufacturing scale process chemistry and crystallisations, compounds from the crystallisation mother liquor adhering on crystal surfaces is a major source of impurities,[34] of which the behaviour found in this case is another example. The initially precipitated β anomer material and the batches of recrystallised β anomer were all found to be of the same crystal structure described above, hence the presence or absence of the α anomer does not appear to have any impact on the crystal phase of the β material.

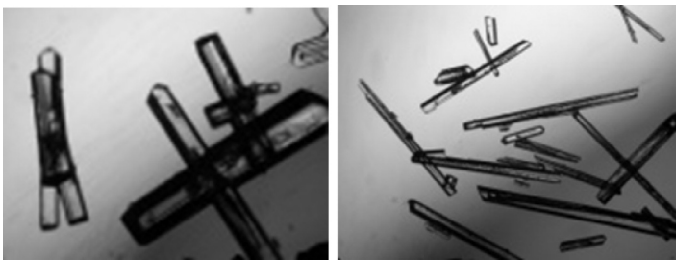

Fig. 5. Micrographs of crystals of methyl tetra-O-acetyl-β-D-glucopyranuronate (**1**) grown from ethanol (left) in the absence of methyl tetra-O-acetyl-α-D-glucopyranuronate (**3**) and (right) with 0.1:1 α:β ratio.

In addition, a series of 'spiking' experiments were carried out in which samples of the β anomer (**1**) were recrystallised from ethanol containing the α anomer (**3**) in α:β ratios of 0.01:1, 0.1:1 and 1:1. (A ratio of 1:1 is approaching the relative proportions of anomers obtained from the reaction medium.) At all three ratios, exclusively the β anomer (**1**) crystallised, in the crystal phase previously observed. The β anomer crystallises from ethanol as elongated prisms (Fig. 5). The β anomer crystals grown from ethanol spiked with the α anomer were found to have a more acicular habit (Fig. 5). Examination in an X-ray goniometer of crystals obtained from both the spiked and α free ethanol solutions showed that all the crystals were elongated in the *a* crystallographic direction (see Supplementary Material). This is consistent with the unit cell dimensions (Table 1) in which *a* is a short axis. Hence, presence of the α anomer does not fundamentally change the morphology of the β anomer crystals but enhances the tendency towards elongation and formation of needles, possibly by further disfavouring growth in the *b* and *c* directions. Examination of the crystal structure of the β-anomer shows that the anomeric acetoxy group is orientated in the *bc* plane, so it could be postulated that addition of α-anomer molecules at growing crystal faces could further decrease the relative growth rate in these directions.

The formation of mixtures of anomers (**1**) and (**3**) from the methanolysis and acetylation of (**2**) is an obvious disadvantage in terms of product yield, therefore we examined the possibility of interconverting the anomers to possibly provide a better yield of either anomer. We investigated conversion of the more readily accessible β anomer to give mixtures of α and β anomers. Samples of the β anomer (**1**) were subjected to a variety of conditions reported to interconvert anomeric 1-acetoxypyranoses in acetic anhydride solution,[35] as listed in Table 3. Catalytic quantities of iodine, 4-toluenesulfonic acid or $SnCl_4$ induced no epimerisation. Some degree of epimerisation was observed with $ZnCl_2$ with microwaves. Use of catalytic H_2SO_4, $FeCl_3$ or $ZnCl_2$ at elevated or reflux temperature gave significant interconversion, but also in each case some degree of degradation. Attempts to use any of these conditions

Table 3
Interconversion of α and β anomers of methyl tetra-O-acetyl-D-glucopyranuronate, compounds (**1**) and (**3**) respectively, in Ac_2O with 100 % β anomer (**1**) as starting material. The ratio of α to β anomer was determined by ^1H NMR

Catalyst	Conditions	Ratio α/β
I_2 (10%)	Δ, 12 h	0:1
H_2SO_4 (10%)	60 °C, 4 h	1.3:1
4-TSA (10%)	100 °C, 12 h	0:1
$FeCl_3$ (10%)	Δ, 12 h	1.2:1
$SnCl_4$ (10%)	300 W, 10 min	0:1
$ZnCl_2$ (10%)	Δ, 12 h	1.9:1
$ZnCl_2$ (10%)	250 W, 1 min	0.2:1
$ZnCl_2$ (10%)	300 W, 5 min	0.6:1
$ZnCl_2$ (20%)	300 W, 15 min	0.5:1

as a crystallisation medium for mixtures of α and β anomers (**1**) and (**3**) were unsuccessful.

Batches of up to 50 g of methyl tetra-O-acetyl-β-D-glucopyranuronate (**1**) could be recrystallised from ethanol or THF giving good yield (ca. 80%) for material with or without seeding. In particular, batches crystallised from THF under controlled conditions (65 g L^{-1} compound (**1**) in THF; agitation at 100 rpm; cooling at –0.5 °C min^{-1} from 38 to 18 °C) gave very evenly grown and uniform prisms of methyl tetra-O-acetyl-β-D-glucopyranuronate (**1**). Under these conditions, spiking of batches of β-anomer (**1**) with quantities of α-anomer (**3**) had no impact on the time of appearance of crystalline material during controlled linear cooling, i.e. gave no detectible variation in nucleation induction time under controlled conditions.

3. Conclusions

Both methyl tetra-O-acetyl-β-D-glucopyranuronate (**1**) and methyl tetra-O-acetyl-α-D-glucopyranuronate (**3**) were isolated as crystalline solids from the methanolysis and subsequent acetylation of D-glucurono-6,3-lactone (**2**). Crystal structures were obtained on crystals of both anomers. That of methyl tetra-O-acetyl-β-D-glucopyranuronate (**1**) was the same as that reported by Root et al.,[23] while methyl tetra-O-acetyl-α-D-glucopyranuronate (**3**) was found to be orthorhombic $P2_12_12_1$ with two independent molecules in the asymmetric unit. Both crystal structures were found to be representative of the bulk material. DSC analysis found, for both materials, a single melting endotherm as the only thermal event, and no other crystal forms were found for either compound upon recrystallisation from a range of solvents. The α-anomer (**3**) was found to be an impurity in initially precipitated batches of β-anomer (**1**) in quantities <3%, but could be removed by recrystallisation or efficient washing, suggesting the α-anomer was not significantly incorporated inside the β-anomer crystals. The morphology of the initially precipitated β-anomer crystals was that of elongated prisms. In recrystallisation experiments spiked with α-anomer impurity, the morphology of the β-anomer crystals became more elongated, approaching needles. All crystals for the β-anomer were found to be of the same crystal structure and to be elongated in the α crystallographic direction, which corresponds to the shortest unit cell axis. The presence of the α-anomer further enhances crystal growth in the *a* direction, most likely by further inhibiting addition of β-anomer molecules at faces orientated in the *b* and or *c* directions. Attempts to bias the crystallising solutions to produce predominately one anomer in solution by the use of Lewis acidic acetylation conditions to generate α/β mixtures from the β anomer did not result in interconversion of anomers free from significant hydrolytic degradation that had a negative impact on crystal yield and purity. Recrystallisation of the batches of the β-anomer (**1**) from ethanol or THF could be scaled up to ca. 50 g quantities. Seeding with samples of β-anomer (**1**) or spiking with quantities of α-anomer (**3**) had no significant impact on crystal habit, size distribution or nucleation induction time.

4. Experimental

All commercial reagents were purchased from Sigma-Aldrich and were used without further purification. All solvents were either of HPLC grade or were distilled prior to use. Infrared spectra were recorded on a PerkinElmer Paragon 1000 FT-IR spectrometer. Optical activities were measured on a PerkinElmer model 341 polarimeter. NMR spectra were recorded on a Bruker AVANCE 300 spectrometer at 300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR. High resolution mass spectra (HRMS) were recorded on a Waters LCT Premier LC-MS instrument in electrospray ionisation (ESI) positive mode using 50 % MeCN-H_2O containing 0.1 % HCO_2H as eluant; samples were made up in MeCN. Crystal habits were observed using a Nikon Polarizing Microscope Eclipse 50i POL and photomicrographs were taken on a Nikon Digital Sight DS-Fi1 digital camera. DSC was carried out on a TGA Q1000 Calorimeter with an RCS 40 cooling system at 2 °C/min.

PXRD was performed at ambient temperature using a Stoe Stadi MP PXRD operating in transmission mode with a linear PSD detector with an anode current of 40 mA, an accelerating voltage of 40 kV and Cu $K\alpha_1$ radiation (λ = 1.5406 Å) scanning in steps of 2° for 90 s per step. Samples were held between acetate foils.

Single crystal diffraction was conducted on a BRUKER APEX DUO using monochromated Cu Kα (λ = 1.54056 Å) as described previously.[36] Calculations and refinement were made using the APEX software,[37] containing the SHELX suite of programs,[38] and diagrams were prepared using Mercury version 3.3.[39]

HPLC was carried out on an Agilent Technologies 1260 Infinity chromatography system using a YMC-Pack ODS-A C-18 column (250 × 4.5 mm, 5 μm, 120 Å) with 80:20 water:acetonitrile mobile phase at a flow rate of 1.5 mL min^{-1} and UV detection at 210 nm. Methyl tetra-O-acetyl-β-D-glucopryranuronate (**1**) eluted at 37.6 min and methyl tetra-O-acetyl-α-D-glucopryranuronate (**3**) at 39.9 min.

4.1. *Methyl tetra-O-acetyl-β-D-glucopyranuronate (**1**) and methyl tetra-O-acetyl-α-D-glucopyranuronate (**3**)*

D-(+)-Glucurono-6,3-lactone (20.0 g, 113.55 mmol) was dissolved in methanol (150 mL) containing NaOH (0.03 g, 0.75 mmol) and the solution was stirred until the lactone had completely dissolved. The solvent was removed under vacuum. The resulting syrup was dissolved in acetic anhydride (65 mL, 688.90 mmol) and pyridine (10 mL) was added dropwise over 30 minutes. The solution was stirred for 1 hour and the solvent volume was reduced to 40 mL under reduced pressure. The solution was refrigerated for 12 hours after which crystalline material precipitated. This was isolated and recrystallised from ethanol to yield methyl tetra-O-acetyl-β-D-glucopyranuronate (**1**) (16.65 g, 39%) as a white crystalline solid. M.p. = 177–179 °C (ethanol) (Lit.[1] = 177–178 °C), $[\alpha]_D^{20}$ +9.0 (c 1.0 $CHCl_3$), ν_{max}/cm^{-1} (KBr disc) 2956 (C—H), 1749 (C═O), 1379 (CH), 1216 (O—C═O), 1019 (C—O—C). $\delta_H(CDCl_3)$ (β anomer). 2.04 (3H, s, OAc), 2.05 (6H, s, 2 × OAc), 2.12 (3H, s, OAc), 3.75 (3H, s, CO_2Me), 4.17 (1H, d, ^3J = 9.2 Hz, H-5), 5.15 (1H, dd, ^3J = 9.2 & 7.6 Hz, H-2), 5.24 (1H, t, ^3J = 9.2 Hz, H-4), 5.31 (1H, t, ^3J = 9.2 Hz, H-3), 5.76 (1H, d, ^3J = 7.6 Hz, H-1). δ_C ($CDCl_3$) (β anomer) 20.40 (CH_3CO), 20.47 (CH_3CO), 20.49 (CH_3CO), 20.70 (CH_3CO), 52.94 (CH_3O), 68.89 (*C*H), 70.14 (*C*H), 71.78 (*C*H), 72.94 (*C*H), 91.33 (*C*H), 166.78 (*C*═O), 168.76 (*C*═O), 169.11 (*C* = O), 169.35 (*C*═O), 169.83 (*C*═O). HRMS (ESI): calcd. for $C_{15}H_{24}NO_{11}$ $[M + NH_4]^+$ 394.1349; found 394.1337. The mother liquor was evaporated and the residue was subjected to flash chromatography (50:50 ethyl acetate: hexane) to yield an oil. Trituration with ethanol gave methyl tetra-O-acetyl-α-D-glucopryranuronate (**3**) (15.8 g, 37%) as a white crystalline solid. M.p. = 106–108 °C, $[\alpha]_D^{20}$ +61.0 (c 2.0 $CHCl_3$), δ_H ($CDCl_3$) (α anomer) 1.98 (3H, s, OAc), 2.05 (6H, s, 2 × OAc), 2.12 (3H, s, OAc), 3.76 (3H, s, CO_2Me), 4.34 (1H, d, ^3J = 10.0 Hz, H-5), 5.21 (1H, dd, ^3J = 8.0, 3.6 Hz H-2), 5.24 to 5. 33 (2H, m, H-3 & H-4), 6.34 (1H, d, ^3J = 3.6 Hz, H-1). δ_C ($CDCl_3$) (α anomer) 20.50 (CH_3CO), 20.64 (CH_3CO), 20.71 (CH_3CO), 20.79 (CH_3CO), 52.95 (CH_3O), 71.45 (*C*H), 72.70 (*C*H), 73.07 (*C*H), 73.15 (*C*H), 91.34 (*C*H), 167.17 (*C*═O), 169.02 (*C*═O), 169.57 (*C*═O), 170.24 (*C*═O), 170.37 (*C*═O). HRMS (ESI): calcd. for $C_{15}H_{24}NO_{11}$ $[M + NH_4]^+$ 394.1349; found 394.1328.

4.2. *Recrystallisation scale-up*

Batches of up to 50 g of methyl tetra-O-acetyl-β-D-glucopyranuronate (**1**) were recrystallised from ethanol or THF using a HEL Autolab 1L jacketed reactor vessel. A PTFE PT100 thermocouple gave *in situ* temperature measurements of the crystallisation medium. The

temperature of the jacket fluid (Huber DW-Therm thermal fluid, operating range –90 °C to 200 °C) was controlled by a Huber unistat 815 circulation thermostat. The system was entirely controlled from one PC using HEL WinISO software, allowing control of stirring rates and heating and cooling rates.

Acknowledgments

This publication has emanated from research conducted with the financial support of Science Foundation Ireland under Grant Numbers 07/SRC/B1158, 05/PICA/B802/EC07 and 12/RC/2275, IRCSET (PD/2012/2652) and the University College Cork 2013 Strategic Research Fund.

Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.carres.2016.01.012.

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