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A robust method for the synthesis and isolation of β-gluco-isosaccharinic acid ((2*R*,4*S*)-2,4,5-trihydroxy-2-(hydroxymethyl)pentanoic acid) from cellulose and measurement of its aqueous pK_a

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ABSTRACT

In alkaline pulping wood pulp is reacted with concentrated aqueous alkali at elevated temperatures. In addition to producing cellulose for the manufacture of paper, alkaline pulping also generates large amounts of isosaccharinic acids as waste products. Isosaccharinic acids are potentially useful raw materials: they are good metal chelating agents and, in their enantiomerically pure form, they are valuable carbon skeletons with predefined stereochemistry that can be easily functionalised for use in synthesis. Despite this, there is no simple procedure for isolating pure beta-(gluco)isosaccharinic acid and very limited work has been undertaken to determine the chemical and physical properties of this compound. We report here a very simple but effective method for the synthesis of a mixture containing equal portions of the two isosaccharinic acids ((2*S*,4*S*)-2,4,5-trihydroxy-2-(hydroxymethyl)pentanoic acid and (2*R*,4*S*)-2,4,5-trihydroxy-2-(hydroxymethyl)pentanoic acid) and the separation of the two as their tribenzoate esters. We also report for the first time the aqueous pK_a of beta-(gluco)isosaccharinic acid (3.61).

1. Introduction

It has been known since the 1930s that treatment of cellulose with alkaline solution results in a change in morphology of cellulose and that prolonged exposure at elevated temperatures leads to a reduction in the degree of polymerisation of the cellulose chains.[1,2] Machell and Richards[3] proposed a mechanism for the degradation pathway applying the 'peeling' reaction, originally proposed by Isbell[4] to account for the alkaline degradation of substituted monosaccharides, to cellulose. In the peeling reaction hexose units, present at available reducing chain ends, are transformed initially into 4-deoxy-3-oxo-D-*glycero*-2-hexulose (**1**)[5,6] which subsequently undergoes a benzylic acid type rearrangement to generate (gluco)isosaccharinic acids: (2*R*,4*S*)-2,4,5-trihydroxy-2-(hydroxymethyl)pentanoic acid (**2**, β-GISA) and (2*S*,4*S*)-2,4,5-trihydroxy-2-(hydroxymethyl)pentanoic acid (**3**, α-GISA) and a new reducing chain end. The peeling reaction continues at the new chain end, slowly reducing the degree of polymerisation of the cellulosic substrate. The alkaline degradation of cellulosic materials generates a large number of hydroxyaliphatic acids with isosaccharinic acids being the most abundant.[7] Unfortunately 'peeling' is an unwanted side reaction in alkaline pulping[8–10] where it generates large amounts of by-products and this waste currently has little economic value. There is considerable commercial interest in developing new uses for isosaccharinic acids. Isosaccharinic acids are potentially useful raw materials: they are capable of acting as metal chelating agents[11–16] and, in their enantiomerically pure form, they are valuable carbon skeletons with predefined stereochemistry that can be easily functionalised for use in synthesis.[17–21] Their metal chelating properties are also of interest to organisations involved in the storage of cellulosic waste including municipal waste facilities[11] and the repositories currently being considered for the storage of nuclear waste;[22–27] it has been shown that isosaccharinic acids generated in cellulosic wastes can promote leaching of metals into water courses. Isosaccharinates are particularly effective at chelating radioactive actinides and increase their aqueous solubility, potentially threatening the integrity of nuclear waste repositories by providing a mechanism for the leaching of radioisotopes into the biosphere.[12–15,28,29]

It is now more than 50 years since the first methods were introduced for the direct synthesis of saccharinic acids,[30] Whistler and BeMiller demonstrated that a mixture of α-GISA & β-GISA could be prepared by reacting 1,4-linked oligosaccharides and polysaccharides with lime water.[30–32] Over the preceding decades these methods have been widely employed to prepare α-GISA for use in studies of its complexation of actinides.[12–15,28,29,33] In contrast, very little work has been carried out with β-GISA. The reason for this is that it is very difficult to isolate β-GISA free from α-GISA. A number of methods for the preparation of the calcium salt of β-GISA have been reported and these include the treatment of either lactose[34–36] cellulose[36,37] or guaran[32] with calcium

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hydroxide for extended periods and the separation of small quantities of β-GISA from α-GISA using either normal phase or anion exchange chromatography. Both Whistler and BeMiller[34] and Feast et al.[35] have reported the production of α-GISA & β-GISA from lactose, the conversion of the acids to their corresponding lactones and finally, the derivatisation of an analytical sample of the lactones as their tribenzoate esters (**4** and **5**). Feast et al.[35] also reported that they were able to isolate an analytical sample of the β-GISA tribenzoate ester using preparative thin layer chromatography.

We were therefore interested to see if we could optimise this sequence of steps with a view to providing a robust method for isolating gram quantities of β-GISA which could be used for determining its chemical and physical properties (Scheme 1).

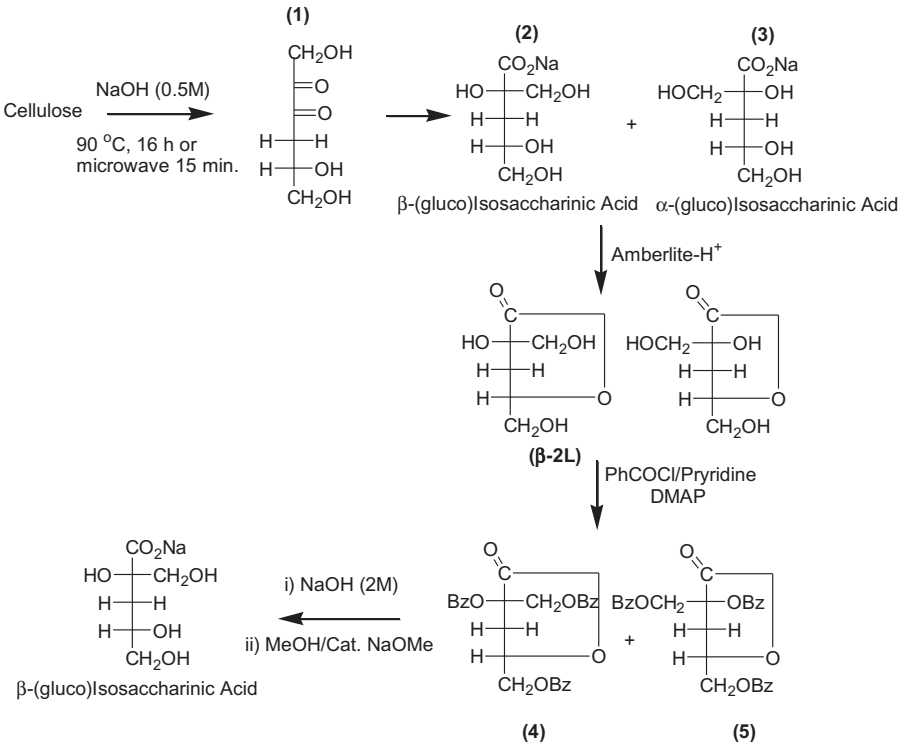
2. Results and discussion

In our initial experiments, attempts were made to optimise the yield of α- and β-GISA available from the treatment of micro-crystalline cellulose with a solution of sodium hydroxide. During these optimisation reactions, a range of reaction conditions were varied including: the reaction temperature (30, 50 and 90 °C), the length of the reaction (1–52 days) and a limited number of provisional experiments were undertaken using saturated solutions of calcium hydroxide. The progress of reactions was monitored using high performance anion exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD) and the maximum solution concentration of α- and β-GISA acids was obtained when a suspension of cellulose was treated with a solution of sodium hydroxide (0.5 M) at 90 °C for 16 h under an atmosphere of nitrogen (Fig. 1).

Historically, calcium hydroxide has been used as a catalyst for the synthesis of α-GISA. It has been reported that calcium promotes the benzylic acid rearrangement[3] of the 4-deoxy-3-oxo-D-*glycero*-2-hexulose intermediate (**1**) at the same time it has also been reported that the use of sodium hydroxide at elevated temperatures (>90 °C) results in fragmentation of the dicarbonyl-compound via retro-aldol type reactions[38] to give a range of C1–C4 hydroxyaliphatic acids in preference to GISAs. In the present studies, when a saturated solution of calcium hydroxide was used as a base, the concentration of the GISA acids reached a maximum and then fell; this was more noticeable when reactions were performed under aerobic conditions (results not shown). Similar findings have been reported by Glaus and Van Loon[39] who observed that when calcium hydroxide is used as a base GISAs are lost from the system by mechanisms involving absorption onto solid calcium hydroxide and via oxidation. Another reason for using sodium instead of calcium is that when calcium hydroxide is reacted with cellulose approximately equal amounts of α-GISA and β-GISA are produced, however, when sodium hydroxide was used as the base three times more of the desired β-GISA is present compared to that of the α-GISA.

Using the optimal reaction conditions (NaOH 0.5 M, 90 °C) the reaction was complete after 16 h and heating for extended periods of time did not increase the yield of GISAs. Interestingly, when a similar reaction was performed but using microwave heating, it was found that the reaction was complete after just 15 min. It is known that the peeling reaction takes place at accessible reducing chain ends and that these are normally within the amorphous regions of cellulosic substrates. The rate of the peeling reaction is reduced when the availability of chains end falls as amorphous cellulose reacts leaving crystalline cellulose. Moharram and Mahmooud[40] have reported that microwave heating reduces the time taken for the mercerization of cellulose; microwave heating is suspected to promote the transformation of cellulose (I) to cellulose (II) and the swelling of cellulose fibres. In the present system, the swelling of the cellulose fibres would be expected to increase the availability of the reducing ends and increase the rate of production of the isosaccharinic acids.

Unfortunately, the use of microwave heating does not increase the yield; using conventional and microwave heating the final concentrations of the two acids were very similar with the β-GISA being present in larger amount (8.1 gL^{-1} (**2**) & 2.7 gL^{-1} (**3**)). The



Scheme 1. Synthesis and isolation of the sodium salt of β-GISA (**2**).

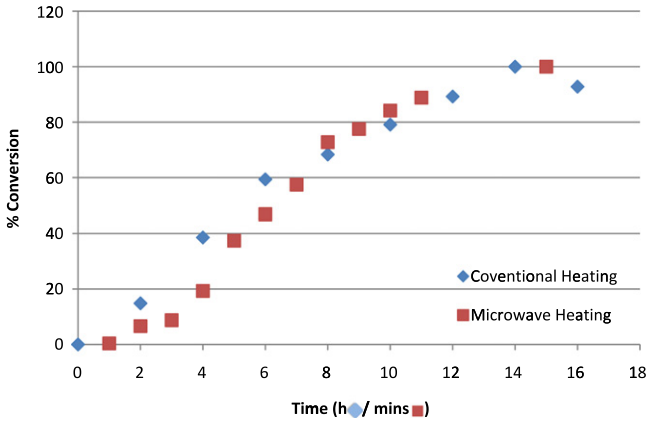


Figure 1. Time course for the production of β-GISA (**2**) expressed as a percentage of the final yield, for the sodium hydroxide catalysed decomposition of cellulose using conventional heating at 90 °C (diamonds) and microwave heating at reflux (squares).

yield of the isosaccharinic acid reaches a maximum after approximately a quarter of the cellulosic material has reacted. Under the conditions of the experiments being reported here, the reaction slows and stops. It is well known that cellulose that has been treated with alkaline solution becomes increasingly resistant to further degradation and this is explained by the so called stopping reactions: the number of available reducing chain ends falls as chains enter inaccessible crystalline regions or as chains are terminated by a stopping reaction in which *meta*-saccharinic is generated at the chain end.

After 16 h, the crude reaction mixture was hot filtered to remove unreacted cellulose and insoluble salts. The filtrate was passed through a cation exchange resin, in its H+ form, in order to convert the sodium salts of α-GISA and β-GISA into their free acids which, under the prevailing conditions (pH <2) undergo a rapid cyclization to form their corresponding isosacharino-1, 4-lactones.[41]

The isosaccharino-1,4-lactones were converted into their tribenzoate esters using the procedures described by Whistler and BeMiller.[30] The key step to isolating pure β-GISA was the separation of the two tribenzoate esters using normal phase chromatography. The crude mixture of triesters was applied to a large silica column and the products eluted using a linear gradient starting from 95% hexane and 5% ether and slowly rising to 30% ether (8 L) collecting 400 fractions (20 mL). NMR analysis identified that

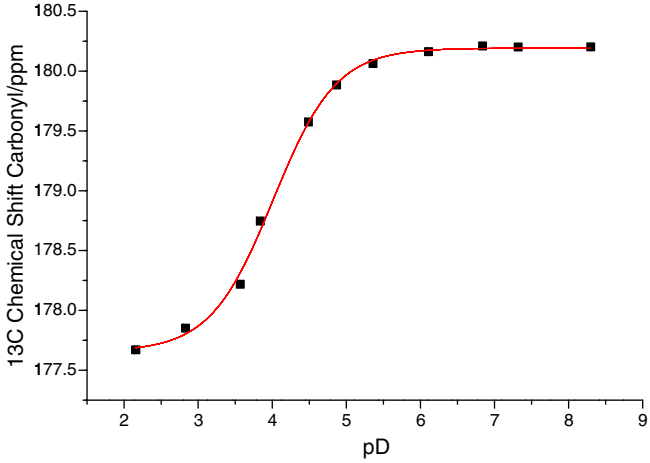


Figure 2. Variation of the ^{13}C chemical shifts of β-GISA's carbonyl carbon as a function of pD. Solid line represents the fit to the equation: $\delta(pD) = \delta_{acid} + \delta_{base}[10^{-pD}/(K_a + 10^{-pD})]$ giving a pK_a in D_2O of 4.01 ± 0.03.

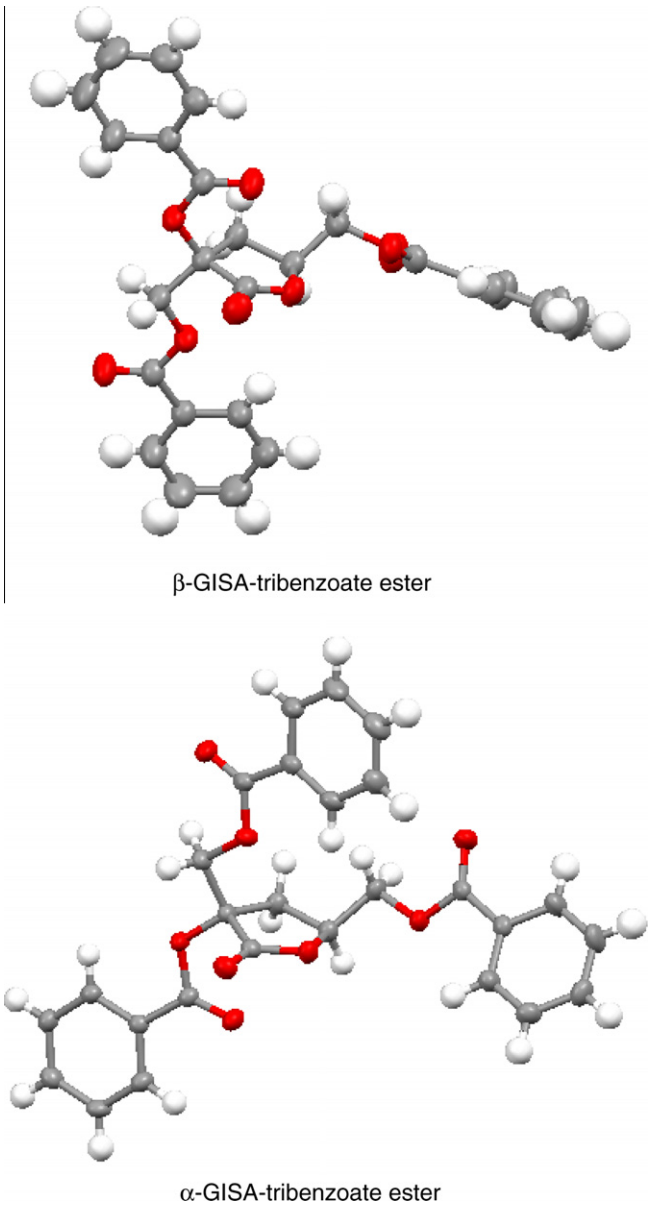


Figure 3. X-ray captions. Top: Solid-state structure of β-GISA-triester (thermal ellipsoids are shown at 50% probability level). Bottom: Solid-state structure of α-GISA-triester (thermal ellipsoids are shown at 50% probability level).

the early fractions contained pure α-tribenzoate **5** and the last fractions contained pure β-tribenzoate **4**; a number of the intermediate fractions contained mixtures of both α- and β-tribenzoates. Fractions containing single isomers were pooled and the solvent volume reduced to approximately one third to give crystals that were suitable for X-ray analysis. The crystal structures confirmed the *cis* and *trans* arrangement of the 2,5-hydroxymethyl groups in the α and β-GISA tribenzoate esters, respectively (Fig. 3).

Hydroxide catalyzed ring opening of the lactone ring followed by debenzoylation using Zemplén[42] methods afforded the sodium salt of the GISAs in greater than 95% purity.

The ability of β-GISA to complex metals will be influenced to a large extent by the basicity of the carboxylate group. Determination of the solution state properties of isosaccharinic acids is complicated by the fact that in acidic solution (pH <4) they undergo acid catalysed ring closure to form cyclic lactones and this prevents the direct measurement of the pK_a by acid–base titration.[43] In order to determine the pK_a of β-GISA we have measured the variation in the chemical shift of the carbons in β-GISA

as a function of pH using ^{13}C NMR spectroscopy following the procedures described by Cho et al.[43] Between pD 2 and pD 8 the resonance position of the carbonyl carbon follows a sigmoidal dependence on the hydrogen ion concentration (Fig. 2) with an apparent pK_a in D_2O of 4.01. The measured pK_a (H_2O) of 3.61 ± 0.03 (pK_a D_2O−0.4) is higher than the value reported in the literature for the α-GISA (3.27–3.36)[43] measured using the same technique and this is consistent with the elution of the β-GISA after the α-GISA in the HPAEC. These results suggest that β-GISA anion is more basic than the α-GISA anion. It should be noted that higher values for the pK_a for α-GISA have been reported using potentiometric methods.[41]

In summary, we have presented an optimised method for the synthesis and isolation of pure β-GISA and reported the pK_a of the acid group. It is hoped that these procedures will make β-GISA readily accessible and lead to studies of its complexation reactions with metals and with tetravalent actinides.

3. Experimental

3.1. Materials and chemicals

All organic solvents were dried before use, employing standard methods. The solvents used for column chromatography were GPR grade. Microcrystalline cellulose-Avicel PH-101 (Lot BCBB5909) was purchased from Fluka Analytical, all other reagents were purchased from Aldrich and were used without further purification.

3.2. General methods

Analytical TLC was performed on Silica Gel 60-F254 (Merck) and detection with either charring (lactones) following immersion in 5% H_2SO_4/H_2O and/or fluorescence. NMR spectra were recorded on either a Bruker Avance 400 MHz or 500 MHz spectrometer using Bruker pulse sequences, samples were dissolved in either D_2O or $CDCl_3$ and referenced to acetone (^1H) or internal $CDCl_3$- (capillary inset in D_2O samples). Chemical shifts are given in parts per million. High resolution mass spectra (HRMS) were recorded on a Bruker micrOTOF-Q with an ESI interface and operating in positive ion mode.

3.3. Monitoring production of GISA

The production of the α- and β-GISA in the alkali (sodium or calcium hydroxide) catalysed decomposition of cellulose was monitored using high performance anion exchange chromatography using the procedures described by Glaus el al.[36] At appropriate intervals (each hour for reactions performed using conventional heating and every minute for the reaction performed with microwave heating), small aliquots (5 mL) were removed from the reaction mixture. These samples were then centrifuged (25,000 g, 30 min) and a small portion of the supernatant liquid was diluted (×40) with an aqueous solution containing D-ribonic acid as an internal standard (5 mg L^{-1}). The resulting solutions were then analysed by high performance anion exchange chromatography using pulsed amperometric detection on a Dionex 3000 Ion chromatography system (Dionex, Camberly, UK) employing a Dionex Carbopac PA20 column (3 × 150 mm, 6.5 μm particle size) and eluting with aqueous sodium hydroxide (50 mM) operating at a flow rate of 0.5 mL min^{-1}. The column temperature was maintained at 25 °C. The isosacharinic acids were detected using a pulsed amperometric detector using a gold working electrode and a Ag/AgCl reference electrode. The retention times of two isosaccharinic acids were determined by comparison with analytical standards; see below for the preparation of standards.

3.4. Determination of the aqueous pK_a of β-GISA

The calcium salt of β-GISA (30 mg, 7.5 mmol) was dissolved in D_2O (1 mL) and the pH of the solution was varied between pD 1 and 8 using either DNO_3 or NaOD and reporting pD as the measured pH +0.4. Samples were quickly transferred to NMR tubes and spectra were recorded on a Bruker Avance 500 MHz (125 MHz ^{13}C) spectrometer using quantitative carbon pulse programmes. Each spectrum was recorded using 4000 scans employing a wait time of 12 s between scans. A capillary insert, containing $CDCl_3$, was used as an internal reference. The solution pH was measured in the NMR tube using a Beckman ø40 pH Meter (High Wycombe, UK) in combination with a Hanna instruments glass long reach NMR pH electrode (Mannheim, Germany). The pK_a of the acid was determined using the Henderson–Hasselbach equation: $pK_a = pD + \log\{[AD]/[A-]\}$

3.5. X-ray structure determination

Single crystal X-ray diffraction data were collected on a Bruker Apex Duo diffractometer equipped with a graphite monochromated Mo(Kα) radiation source. Preliminary scans were employed to assess crystal quality, lattice symmetry, ideal exposure time etc. prior to collecting a full sphere of diffraction intensity data using SMART operating software (*SMART Diffractometer Control Software*, Bruker Analytical X-ray Instruments Inc., Madison, WI, 1998). Intensities were then integrated from several series of exposures (each exposure covering 0.3° in ω), merged and corrected for Lorentz and polarisation effects using SAINT software (*SAINT Integration Software*, Siemens Analytical X-ray Instruments Inc., Madison, WI, 1994). Solutions were generated by direct methods and refined by full-matrix non-linear least squares on all F^2 data, using SHELXS-97 and SHELXL software, respectively (as implemented in the SHELXTL suite of programmes (*SHELXTL Program System*, *Vers. 5.1*, Bruker Analytical X-ray Instruments Inc., Madison, WI, 1998)). Empirical absorption corrections were applied based on multiple and symmetry-equivalent measurements using SADABS (Sheldrick, G. M. *SADABS*: *A Program for Absorption Correction with the Siemens SMART System*, University of Göttingen, Germany, 1996). All structures were refined until convergence (max shift/esd <0.01) and in each case, the final Fourier difference map showed no chemically sensible features.

3.6. Preparation of 2,5,6-tri-*O*-benzoyl-2-hydroxymethyl-2*R*,4*S*,5-trihydroxypentanoic acid-1,4-lactone (4) & 2,5,6-tri-*O*-benzoyl-2-hydroxymethyl-2*S*,4*S*,5-trihydroxypentanoic acid-1,4-lactone (5)

Avicel (200 g dry weight) was suspended in an aqueous solution of sodium hydroxide (2 L, 0.5 M) in a large round bottom flask (3 L) and an atmosphere of nitrogen was maintained above the reaction solution; the reaction mixture was stirred whilst heating at 90 °C for 16 h during which time the appearance of the suspension turned dark brown. After 16 h the reaction mixture was filtered hot to remove residual insoluble cellulose (142 g dry weight—29% conversion to soluble products) and the supernatant liquid, containing the crude mixture of the sodium salts of α- and β-GISA and impurities, was passed in several portions through a cation exchange column (Amberlite, 6 × 40 cm) which had previously been conditioned with dilute HCl (1 M, 6 bed volumes). NMR analysis of the material eluting from the amberlite column indicated that in addition to the exchange of Na+ for H+ the free acids had also been converted to their corresponding lactones (data not shown). After removal of water under vacuum at 40 °C, a crude mixture of products (57 g-estimated to contain approximately 18 g of GISAs based on analysis of the crude product by HPAEC 0.13 mol, 10.4% yield)

was obtained as a dark brown solid which was used directly in the next synthetic step.

To convert the lactones into their corresponding tribenzoate esters, the crude mixture was dissolved in pyridine (300 mL) a catalytic amount of 4-*N*,*N*-(dimethylamino)pyridine (1 g, 8.2 mmol) was added and the solution was cooled to 0 °C before slowly adding a large excess of benzoyl chloride (150 g, 1.2 mol). The mixture was stirred for 2 h at 0 °C and then at room temperature for a further 16 h. After 16 h the reaction mixture was poured onto an equal volume of water and the mixture stirred at room temperature for a further 1 h during which time the excess benzoyl chloride hydrolysed. The tribenzoate esters were extracted into chloroform (3 × 50 mL) and excess benzoic acid was removed from the chloroform layer by repeated extraction with a saturated solution of sodium hydrogen carbonate (3 × 50 mL). The chloroform layer was dried over anhydrous sodium sulfate and a large number of the highly coloured polar impurities were removed from the esters by passing the chloroform extract through a thin-bed of silica (6 cm deep × 20 cm od) and washing the bed with chloroform (100 mL). No attempt was made to recover the coloured impurities. Subsequent evaporation of the combined chloroform layers gave a pale-yellow syrup (27 g) which was shown by ^{1}H NMR to be rich in the tribenzoate esters.

The key step to isolating pure β-gluco-isosaccharinic acid was the separation of the two tribenzoate esters using normal phase chromatography. Part of the crude mixture (16 g) was applied to a large silica column (50 × 7 cm) and the products were eluted using a linear gradient starting with 95% hexane and 5% ether slowly rising to 30% ether (8 L) collecting 400 fractions each of approximately 20 mL. NMR analysis identified that a number of the early fractions contained pure α-tribenzoate **5** (<1 g, tubes 225–260), these were closely followed by mixed fractions containing increasing amounts of the β-tribenzoate **3** (<2 g, tubes 261–295), the third set of fractions contained only the β-tribenzoate **4** (<7 g, tubes −296–320, 14.7, 24%) a final fraction contained β-tribenzoate **4** contaminated with increasing amounts of the benzoate ester of 3,4-dihydroxybutanoic acid-1,4-lactone (<5 g).

3.6.1. 2,5,6-Tri-*O*-benzoyl-2-hydroxymethyl-2*R*,4*S*,5-trihydroxypentanoic acid-1,4-lactone (4)

^{1}H NMR (400 MHz, $CDCl_3$) 8.17–8.04 ppm (m, 6H) 7.72–7.60 ppm (m, 3H,) 7.52–7.44 ppm (m, 6H) 5.00–4.94 ppm (1H, m) 4.92 ppm (1H, d, J = 11.1 Hz) 4.73 ppm (1H, d J = 11.1 Hz) 4.74–4.65 (m 2H) 2.56 ppm (d, J = 8.1 Hz). ^{13}C NMR (100 MHz $CDCl_3$) 171.7, 166.3, 165.7, 164.8, 134.1, 133.9, 133.4, 130.1, 130.0, 129.8, 129.3, 128.8, 128.7, 128.5, 128.3, 78.7, 74.4, 65.6, 65.4, 32.2.

High Resolution Mass Spectrometry (HRMS) (ESI) Exact mass calculated for $C_{27}H_{22}O_8$ [MNa^+] 497.1213 found 497.1222.

3.6.2. 2,5,6-Tri-*O*-benzoyl-2-hydroxymethyl-2*S*,4*S*,5-trihydroxypentanoic acid-1,4-lactone (5)

^{1}H NMR (400 MHz, $CDCl_3$) 8.11–8.03 ppm (m, 6H) 7.67–7.56 ppm (m, 3H,) 7.52–7.42 ppm (m, 6H) 5.42–5.34 ppm (1H, m) 4.91 ppm (1H, d, J = 11.8 Hz) 4.68 ppm (1H, d J = 11.8 Hz) 4.64 (dd 1H J = 12.3 Hz, 3.4 Hz) 4.51 (dd 1H J = 12.3 Hz, 6.4 Hz)2.82 ppm (dd 1H J = 15.0 Hz, 8.6 Hz) 2.61 ppm (dd 1H J = 15.0 Hz, 7.0 Hz) ^{13}C NMR (100 MHz $CDCl_3$) 172.1, 166.3, 165.9, 165.6, 134.4, 134.0, 133.7, 130.3, 130.0, 129.9, 128.9 128.7, 78.6, 75.6, 66.2, 65.5, 32.9.

HRMS (ESI) Exact mass calculated for $C_{27}H_{22}O_8$ [MNa^+] 497.1213 found 497.1170.

3.7. X-Ray data for β-GISA-triester and α-GISA-triester

Crystal data for **β-GISA-triester** (1st structure) $C_{27}H_{22}O_8$: M = 474.45, monoclinic *Cc*, a = 13.581(5), b = 19.487(7), c = 10.066(7) Å, β = 118.715(6)°, V = 2336.(2), Z = 4, ρ_{calc} = 1.349 Mg m^{-3}, $F(000)$ = 992; crystal dimensions 0.60, 0.20, 0.10 mm; $\mu(MoK_\alpha)$ = 0.71073 mm^{-1}, T = 296.(2) K. A total of 10405 reflections measure in the range $2 \leq \theta \leq 27.87$ (*hkl* range indexes: $-16 \leq h \leq 17$, $-25 \leq k \leq 25$ $-13 \leq l \leq 11$), 4784 unique reflections (R_{int} = 0.0347). The structure was refined to F^2 to R_w = 0.0864, R = 0.0381 (3619 reflections with $I > 2\sigma(I)$) and GOF = 1.014 on F^2 for 316 refined parameters, 2 restraints. Largest peak and hole 0.155 and −0.131 eA^{-3}. The X-ray coordinates have been lodged with the Cambridge Crystallographic Data Centre (CCDC ID: 822401).

Crystal data for **α-GISA-triester** (2nd structure) $C_{27}H_{22}O_8$: M = 474.45, triclinic *P-1*, a = 9.7994(9), b = 10.4549 (10), c = 11.7106 (11) Å, α = 74.014 (2), β = 81.033 (2), γ = 86.059 (2)°, V = 1138.86 (19), Z = 2, ρ_{calc} = 1.384 Mg m^{-3}, $F(000)$ = 496; crystal dimensions 0.08, 0.06, 0.05 mm; $\mu(MoK_\alpha)$ = 0.71073 mm^{-1}, T = 162.(2) K. A total of 16452 reflections measure in the range $1.83 \leqslant \theta \leqslant 25.68$ (*hkl* range indexes: $-11 \leqslant h \leqslant 11$, $-12 \leqslant k \leqslant 12$ $-14 \leqslant l \leqslant 14$), 4310 unique reflections (R_{int} = 0.0347). The structure was refined to F^2 to R_w = 0.0849, R = 0.0378 (4310 reflections with $I > 2\sigma(I)$) and GOF = 1.010 on F^2 for 316 refined parameters, 0 restraints. Largest peak and hole 0.195 and −0.204 eA^{-3}. The X-ray coordinates have been lodged with the Cambridge Crystallographic Data Centre (CCDC ID: 822402).

3.8. Preparation of sodium (2*R*,4*S*)-2,4,5-trihydroxy-2-(hydroxymethyl)pentanoate (β-gluco-isosaccharinic acid β-GISA, 2)

Deprotection of the esters was accomplished in two stages: ring opening of lactone was achieved by dissolving the tribenzoylated lactone (**4**, 12 g) in an acetonitrile water mix (100:30 v/v) to which was added a solution of sodium hydroxide (2 M) maintaining the pH between 12 and 13. After the consumption of one equivalent of base the pH of the solution was brought back to pH 7 and the solvent removed under vacuum to give a solid product, this was directly dissolved in methanol (500 mL) and a catalytic amount of sodium methoxide (1 g) was added to remove the benzoate groups. After stirring for 2 h, the methanol was evaporated and the solid product was dissolved in water and extracted with ether to remove methyl benzoate. Removal of water under vacuum generated the sodium salt of β-GISA in greater than 95% purity (4.79 g, 94%).

^{1}H NMR (400 MHz, D_2O) 3.69 ppm (m 1H) 3.56 ppm (d, 1H, J = 11.5) 3.43 ppm (d 1H J = 11.5) 3.37 ppm (dd 1H, J = 11.7 Hz, 3.8 Hz) 3.26 ppm (dd 1H, J = 11.7 Hz, 6.8 Hz) 1.68 ppm (dd 1H, J = 14.7 Hz, 3.5 Hz) 1.68 ppm (dd 1H, J = 14.7 Hz, 8.5 Hz). ^{13}C NMR (100 MHz D_2O) 180.2, 80.1, 69.5, 67.8, 65.9, 37.5.

HRMS (ESI) Exact mass calculated for $C_6H_{12}O_6[M-H]^-$ 179.0561 found 179.05.

3.9. Preparation of 2-hydroxymethyl-2*R*,4*S*,5-trihydroxypentanoic acid-1,4-lactone (β-2L)

A pure sample of the β-lactone was prepared by passing the sodium salt of (2*R*,4*S*)-2,4,5-trihydroxy-2-(hydroxymethyl)pentanoate (1 g, 5 mmol) through a cation exchange column (Amberlite, 6 × 40 cm) which had previously been conditioned with dilute HCl (1 M, 6 bed volumes).The crude product was extracted into ether, dried with anhydrous sodium sulfate and the solvent removed under vacuum to give the lactone as a sticky clear liquid (0.8 g).

^{1}H NMR (400 MHz, $CDCl_3$) 4.52 ppm (m 1H) 3.72 ppm (dd, 1H, J = 13.0 Hz, 2.6 Hz) 3.61 ppm (d, 1H J = 11.7 Hz) 3.49 ppm (dd, 1H J = 13.0 Hz, 5.6 Hz) 2.43 ppm (dd 1H, J = 13.5 Hz, 6.7 Hz) 1.98 ppm (dd 1H, J = 13.5 Hz, 9.2 Hz). ^{13}C NMR (125 MHz D_2O) 179.8, 79.0, 77.0, 64.9, 64.9, 62.7, 34.0.

HRMS (ESI) Exact mass calculated for $C_6H_{10}O_5$ [MNa^+] 185.0426 found 185.0474.

3.10. Preparation of a crude mixture of (gluco)isosaccharinic acids using microwave heating

In a further attempt to optimise production of the cellulose decomposition products, a small scale reaction, 20 g of Avicel and 200 mL of 0.5 M NaOH, was heated using microwave heating in an adapted domestic microwave fitted with a condenser and an insulated magnetic stirrer bar (750 W). The production of α- and β-GISA was monitored using HPAEC-PAD and the reaction was complete after 15 min.

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