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Note

Syntheses of the 4-nitrophenyl glycosides of hyalobiuronic acid and chondrosine

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Abstract

4-Nitrophenyl [sodium β-D-glucopyranosyluronate]-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (**1**) and 4-nitrophenyl [sodium β-D-glucopyranosyluronate]-(1→3)-2-acetamido-2-deoxy-β-D-galactopyranoside (**2**) were prepared from the zwitterions hyalobiuronic acid [β-D-glucopyranuronic acid-(1→3)-2-amino-2-deoxy-D-glucopyranose] and chondrosine [β-D-glucopyranuronic acid-(1→3)-2-amino-2-deoxy-D-galactopyranose], respectively. Compounds **1** and **2** were not hydrolysed by bovine testicular hyaluronidase. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: 4-Nitrophenyl glycosides; Zwitterions; Bovine testicular hyaluronidase; Hyalobiuronic acid; Hyaluronic acid; Chondrosine

1. Introduction

Hyalobiuronic acid [β-D-glucopyranuronic acid-(1→3)-2-amino-2-deoxy-D-glucopyranose, zwitterion, **3**] and chondrosine [β-D-glucopyranuronic acid-(1→3)-2-amino-2-deoxy-D-galactopyranose, zwitterion] are derived from the repeating units of the glycosaminoglycans (GAGs) hyaluronic acid and chondroitin sulfate, respectively. Hyaluronic acid is widely distributed among the organs and tissues in the body where it exerts profound physiological effects.[1] Chondroitin sulfate is of particular importance in the connective tissue where it contributes to the organisation and resilience of the extracellular matrix.[2] Hyalobiuronic acid and chondrosine can be readily obtained from the parent polysaccharides by acid catalysed hydrolysis,[3–5] a process that also results in desulfation and *N*-deacetylation of the disaccharides. The disaccharide products are obtained after neutralisation as zwitterionic salts with low cold-water solubility.

Despite considerable interest in chondroitin sulfate and hyaluronic acid,[6–10] the use of chondrosine and hyalobiuronic acid as starting materials for glycoside synthesis has not been reported in the literature. Chondrosine has been derivatised as the methyl ester and the methyl ester peracetate.[4,11] In addition hyalobiuronic acid and chondrosine have been *N*-acetylated and the products characterised.[12]

The *N*-acetylated 4-nitrophenyl glycosides of hyalobiuronic acid and chondrosine were prepared as potential chromogenic substrates for hyaluronidase, a matrix degrading enzyme involved in morphogenesis, angiogenesis and metastasis.[13]

2. Results and discussion

Methyl esterification of hyalobiuronic acid (**3**) was carried out with cold methanolic HCl[11] to give the new compound **4** as a deliquescent solid (Scheme 1). Peracetylation of **4** was carried out under standard conditions to give **5** in 86% yield. For the formation of the 4-nitrophenyl β-D-glycoside, the corresponding α-D-bromide of **5** was generated but not isolated. As was found for the preparation of 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl bromide[14] concentration of the bromide of **5** to dryness resulted in excessive degradation and very low yields of the desired glycoside. A two-phase system similar to that developed for the preparation of 4-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside was used for the preparation of **6** from **5**.[15] Deesterification and deacetylation pro-

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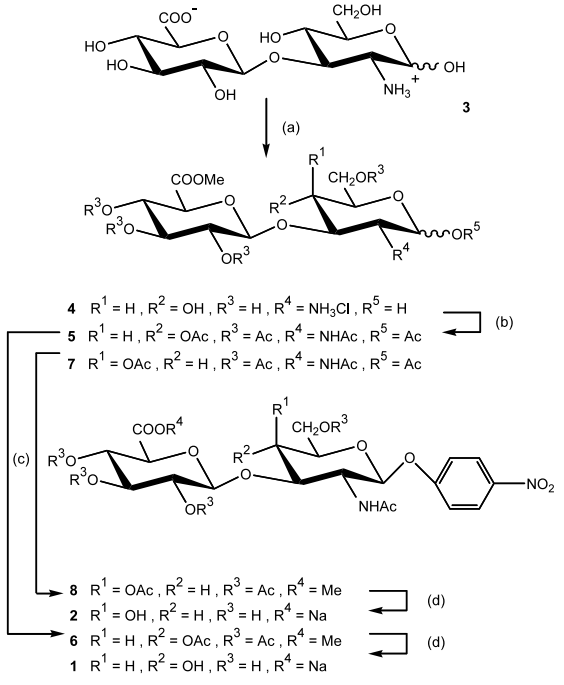
ceeded successfully using lithium hydroxide–hydrogen peroxide followed by sodium hydroxide–methanol.[16]

The preparation of the 4-nitrophenyl glycoside of chondrosine (**2**) was carried out in similar fashion to that of hyalobiuronic acid from the known compound **7**.[4] For convenience **7** was prepared from chondrosine by the method reported here for the conversion of hyalobiuronic acid to **4** rather than by the literature procedure.

The derivatives were tested as possible chromogenic substrates for hyaluronidase. 4-Nitrophenol is a relatively good leaving group and both **1** and **2** slowly hydrolyse in water at ambient temperature. No increase in the rate of their hydrolyses was detected on addition of hyaluronidase to the incubation mixture under conditions which resulted in the complete degradation of hyaluronic acid, as shown by the turbimetric assay of Tolksdorf.[17] The smallest hyaluronic acid oligosaccharide that has been shown to be a substrate for hyaluronidase is a hexasaccharide. Hydrolyses of the hexasaccharide by hyaluronidase liberates *N*-acetylated hyalobiuronic acid from the non-reducing end.[18] The failure of hyaluronidase to accelerate the hydrolyses of **1** and **2** confirms the inactivity of the enzyme towards smaller substrates.



Scheme 1. (a) MeOH–HCl, 5 °C, 64 h; (b) 1:1 pyridine–Ac_2O, 0 °C, 1 h, then rt, 16 h; (c) HBr–AcOH–CH_2Cl_2, rt, 2 h, then 4-nitrophenol–K_2CO_3 (1 M)–tetrabutylammonium hydrogensulfate–CH_2Cl_2 (two-phase), rt, 2 h; (d) THF–water–LiOH–H_2O_2, 0 °C, 2 h, then rt, 16 h, then THF–water–LiOH–H_2O_2–MeOH–NaOH, rt, 8 h.

3. Experimental

General methods.—Melting points were measured on a Reichert hot stage microscope and are uncorrected. Optical rotations were determined with a Perkin–Elmer 214 polarimeter and are in units of 10^{-1} deg cm^2 g^{-1} (conventionally °). TLC was performed on aluminium backed silica gel 60 F254 (E. Merck) with detection by UV absorption and/or by heating after dipping in $(NH_4)_6Mo_7O_{24}{\cdot}6H_2O$ (5 g) and $Ce(SO_4)_2$ (100 mg) in 5% aqueous H_2SO_4 (100 mL) solution. Flash column chromatography was performed on Scharlau silica gel (40–60 μm). Chromatography solvents were distilled prior to use. All solvents were evaporated at reduced pressure. Anhydrous solvents were those commercially available. NMR spectra were recorded on a Varian Unity 500 NMR spectrometer (operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C NMR) using a 5 mm inverse multinuclear probe. Solutions in D_2O were referenced to internal $(CH_3)_3COH$ (^{1}H δ 1.245, ^{13}C δ 30.715). Solutions in $CDCl_3$ were referenced to internal Me_4Si (^{1}H δ 0) and the centre line of the solvent (^{13}C δ 77.40). Assignments of ^{1}H and ^{13}C resonances were unambiguous and based on ^{1}H–^{1}H DQF–COSY, HMQC inverse ^{13}C–^{1}H HSQC, HMQC–TOCSY (mixing time 40 ms) and DEPT experiments. High-resolution electrospray mass spectra were obtained on a Biosystems Mariner System 5158 spectrometer.

Assessment of hyaluronidase action on the 4-nitrophenyl glycosides.—Solutions containing substrates (**1** or **2**, 2 mM), NaCl (0.2 M), NaOAc buffer (pH 5.0, 50 mM), Gelatine (0.03%, Type B from bovine skin) and hyaluronidase (5 mg/mL, Sigma Chemical Co., Type 1-S from Bovine Testes, 330 units/mg) were incubated for 1 h at 37 °C. An equal volume of glycine–NaOH buffer (pH 10.5, 0.3 M) was added to stop the reaction and the absorbance at 405 nm was measured. The activity of the hyaluronidase under the same conditions was checked using the turbidimetric assay of Tolksdorf[17] with rooster–comb hyaluronic acid (Sigma Chemical Company).

(Methyl β-D-glucopyranosyluronate)-(1→3)-2-amino-2-deoxy-D-glucopyranose hydrochloride salt (**4**).—Acetyl chloride (0.75 mL, 10.5 mmol) and hyalobiuronic acid **3** (3.00 g, 8.44 mmol) were added to stirred anhyd MeOH (90 mL) at 0 °C then left for 64 h at 5 °C. *tert*-BuOH (20 mL) was added and the solution evaporated. The *tert*-BuOH addition and evaporation step was repeated and the product was dissolved in water (150 mL) and decolourised with charcoal (3 g). The charcoal was removed by filtration through Celite and the solution was freeze-dried. The dried product was dissolved in MeOH (20 mL) and 1:1 MeOH–2-propanol (40 mL) was added followed by the addition of more 2-propanol (120 mL). The solids were removed by filtration and discarded and the filtrate was evapo-

rated. The resulting solid was dissolved in MeOH and **4** crystallised on the addition of Et_2O. The crystalline solid was filtered off and dried in vacuo. In order to eliminate trapped solvents, the crystals were dissolved in water and the resulting solution freeze-dried to give **4** as a deliquescent amorphous solid which analysed as the hemihydrate (1.76 g, 50%); $[\alpha]_D$ +21.7° (*c* 1, H_2O, 24 h); ^1H NMR (D_2O): δ 5.47 (d, 0.7 H, $J_{1\alpha,2\alpha}$ 3.4 Hz, H-1α), 4.97 (d, 0.3 H, $J_{1\beta,2\beta}$ 8.6 Hz, H-1β), 4.77 (d, 0.3 H, $J_{1'\beta,2'\beta}$ 8.0 Hz, H-1′β), 4.74 (d, 0.7 H, $J_{1'\alpha,2'\alpha}$ 8.0 Hz, H-1′α), 4.10–4.18 (m. 1.7 H), 3.95–4.12 (m, 0.6 H), 3.88–3.95 (m, 1 H), 3.73–3.88 (m, 1.4 H), 3.84 (s, 3 H, CH_3O), 3.42–3.70 (m, 5 H), 3.52 (dd, 0.7 H, $J_{2\alpha,3\alpha}$ 10.5 Hz, H-2α), 3.23 (dd, 0.3 H, $J_{2\beta,3\beta}$ 10.5 Hz, H-2β); ^{13}C NMR ($CDCl_3$): δ 171.7 (C-6′), 103.5 (C-1′β), 103.1 (C-1′α), 93.5 (C-1β), 90.2 (C-1α), 82.1 (C-3β), 81.1 (C-3α), 77.0 (C-5β), 76.1 (C-3′α), 76.0 (C-3′β), 75.6 (C-5′β), 75.6 (C-5′α), 73.9 (C-2α), 73.7 (C-2β), 72.5 (C-5α), 72.0 (H-4′), 69.2 (C-4β), 69.0 (C-4α), 61.5 (C-6β), 61.3 (C-6α), 57.0 (C-2β), 54.7 (C-2α), 54.3 (OCH_3); MS (ES): *m*/*z* $(M-Cl+H)^+$ 370.134 (100%, found) 370.134 (calcd). Anal. Calcd for $C_{13}H_{24}ClNO_{11}\cdot 0.5H_2O$: C, 37.64; H, 6.07; N, 3.38. Found: C, 37.74; H, 6.12; N, 3.30.

(Methyl 2,3,4-tri-O-*acetyl-β*-D-*glucopyranosyluronate)-(1→3)-2-acetamido-1,4,6-tri*-O-*acetyl-2-deoxy-α*-D-*glucopyranose* (**5a**).—The ester hydrochloride **4** (4.93 g, 11.9 mmol) was added to a 1:1 mixture of cold Ac_2O and pyridine (60 mL) and stirred for 1 h at 4 °C then overnight at ambient temperature. Toluene (150 mL) was added and the mixture was evaporated. The toluene addition and evaporation was repeated three times to give a syrup. Chromatography (9:1 EtOAc–hexanes) followed by crystallisation from CH_2Cl_2–Et_2O gave impure (methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-(1→3)-2-acetamido-1,4,6-tri-*O*-acetyl-2-deoxy-D-glucopyranose (**5**) as a mixture of anomers (6.65 g). TLC (9:1 EtOAc–hexanes): R_f 0.30 (β anomer), 0.24 (α anomer). Some of the product (0.94 g) was crystallised from MeOH by cooling on a dry-ice–acetone bath to give **5a** as a crystalline solid (0.54 g, 48% from **4**); mp 118–120 °C; $[\alpha]_D$ +27.6° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 6.06 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 5.60 (d, 1 H, $J_{NH,2}$ 9.5 Hz, NH), 5.25 (dd, 1 H, $J_{2',3'}$ 9.3 Hz, $J_{3',4'}$ 9.5 Hz, H-3′), 5.13 (dd, 1 H, $J_{4',5'}$ 10 Hz, H-4′), 5.07 (dd, 1 H, $J_{3,4}$ 9.3 Hz, $J_{4,5}$ 10.3 Hz, H-4), 4.82 (dd, 1 H $J_{1',2'}$ 7.8 Hz, H-2′), 4.75 (d, 1 H, H-1′), 4.53 (ddd, 1 H, $J_{2,3}$ 10.5 Hz, H-2), 4.19 (dd, 1 H, $J_{5,6a}$ 4.4 Hz, $J_{6a,6b}$ 12.5 Hz, H-6a), 4.07 (dd, 1 H, $J_{5,6b}$ 2.4 Hz, H-6b), 4.06 (d, 1 H, H-5′), 3.98 (ddd, 1 H, H-5), 3.95 (dd, 1 H, H-3), 3.75 (s 3 H, OCH_3), 2.19, 2.12, 2.08, 2.06, 2.03, 2.01, 2.00 (all s, each 3 H, 7 × Ac); ^{13}C NMR ($CDCl_3$): δ 171.2, 170.5, 170.0, 170.0, 169.8, 169.7, 169.0, 167.4, 100.8 (C-1′), 91.7 (C-1), 76.8 (C-3), 72.9 (C-5′), 72.3 (C-3′), 71.9 (C-2′), 70.3 (C-5′), 70.0 (C-4′), 68.2 (C-4), 62.2 (C-6), 53.2 (OCH_3), 51.6 (C-2), 23.7, 21.3, 21.1, 20.94, 20.90 (2 C), 20.8 (7 × Ac); MS (ES): *m*/*z* $(M+H_2O)^+$ 681.235 (61%, found) 681.211 (calcd), $(M+H)^+$ 664.216 (5%, found) 664.208 (calcd). Anal. Calcd for $C_{27}H_{37}NO_{18}$: C, 48.87; H, 5.62, N, 2.11. Found: C, 48.50; H, 5.60, N, 2.02.

4-Nitrophenyl (methyl 2,3,4-tri-O-*acetyl-β*-D-*glucopyranosyluronate)-(1→3)-2-acetamido-4,6-di*-O-*acetyl-2-deoxy-β*-D-*glucopyranoside* (**6**).—A solution of the impure **5** (1.00 g, 1.5 mmol) in 5 mL CH_2Cl_2 was treated with 1.85 mL of 30% HBr in AcOH at ambient temperature for 2 h. The mixture was diluted with CH_2Cl_2 (100 mL, pre-cooled to 0 °C) and washed vigorously and quickly with ice-cold water (100 mL), ice-cold $NaHCO_3$ (aq satd, 3 × 100 mL), dried and evaporated to ~5 mL. The resulting solution was added to a vigorously stirred aqueous solution of K_2CO_3 (1 M, 5 mL, 5 mmol) to which had just been added 4-nitrophenol (1.07 g, 7.7 mmol) and tetrabutylammonium hydrogensulfate (0.92 g, 2.7 mmol). The two-phase mixture was stirred vigorously at ambient temperature for 2 h, diluted with CH_2Cl_2 (100 mL), washed with water (3 × 100 mL) and concentrated. The residue was co-evaporated with 3:2 toluene–acetone (10 mL), then chromatographed to give impure **6** (3:2 toluene–acetone). Further chromatography (7:3 toluene–acetone) produced **6** which was crystallised from acetone–ether–hexanes (0.600 g, 0.81 mmol, 54%); mp 195 °C; $[\alpha]_D$ −31.7° (*c* 1, $CHCl_3$); TLC (3:2 toluene–acetone): R_f 0.15; ^1H NMR ($CDCl_3$): δ 8.18 (d, 2 H, *J* 9.3 Hz, ArH), 7.06 (d, 2 H, *J* 9.3 Hz, ArH), 6.19 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1), 5.74 (d, 1 H, $J_{NH,2}$ 7.6 Hz, NH), 5.26 (t, 1 H, $J_{3',4'}$ 9.3 Hz, $J_{2',3'}$ 9.0 Hz, H-3′), 5.18 (t, 1 H, $J_{4',5'}$ 9.8 Hz, H-4′), 5.06 (t, 1 H, $J_{3,4}$ 9.2 Hz, $J_{4,5}$ 9.2 Hz, H-4), 4.89 (dd, 1 H, $J_{1',2'}$ 7.6 Hz, $J_{2',3'}$ 9.0 Hz, H-2′), 4.79 (d, 1 H, H-1′), 4.61 (t, 1 H, $J_{2,3}$ 9.2 Hz, H-3), 4.23 (dd, 1 H, $J_{6a,6b}$ 12.2 Hz, $J_{5,6a}$ 5.9 Hz, H-6a), 4.14 (dd, 1 H, $J_{5,6b}$ 2.7 Hz, H-6b), 4.05 (d, 1 H, H-5′), 3.91 (ddd, 1 H, H-5), 3.75 (s, 3 H, OCH_3), 3.61 (ddd, 1 H, H-2), 2.11, 2.08, 2.04, 2.03 (4 s, 12 H, 4 Ac), 2.02 (s, 6 H, 2 Ac); ^{13}C NMR ($CDCl_3$): δ 171.5, 170.8, 170.4, 169.9, 169.7, 169.4, 167.5, 161.9, 143.4, 126.1, 117.0, 99.9 (C-1′), 96.9 (C-1), 76.9 (C-3), 72.8 (C-5), 72.6 (C-5′), 72.5 (C-3′), 72.3 (C-2′), 69.7 (C-4′), 68.4 (C-4), 62.7 (C-6), 57.3 (C-2), 53.2 (OCH_3), 23.9, 21.1, 21.0, 21.0, 20.9, 20.8 (6 Ac). Anal. Calcd for $C_{31}H_{38}N_2O_{19}$: C, 50.14; H, 5.16; N, 3.77. Found: C, 50.11; H, 5.07; N, 3.74; MS (ES): *m*/*z* $(M+H_2O)^+$ 760.236 (100%, found) 760.217 (calcd), $(M+H)^+$ 743.214 (13%, found) 743.214 (calcd), 604.187 (80%, M − O-Ph-NO_2, found) 604.187 (calcd).

4-Nitrophenyl (sodium β-D-*glucopyranosyluronate)-(1→3)-2-acetamido-2-deoxy-β*-D-*glucopyranoside* (**1**).—A 7:3 THF–water solution (20 mL) of the acetate **6** (540 mg, 0.73 mmol) was stirred at 0 °C with 30% H_2O_2 (2.2 mL) and LiOH (1 M, 4.4 mL) for 2 h, then at ambient temperature for 16 h. MeOH (80 mL) was added and the resulting solution was cooled to 0 °C.

NaOH (4 M, 4 mL) was added and the solution was stirred at ambient temperature for 8 h. Water (160 mL) was added and the solution eluted through a column of Amberjet 1200 (H^+) (150 mL bed volume). The column was then eluted with water (750 mL). The eluants from the column were combined and concentrated to ~50 mL and the pH adjusted to 6.5 with NaOH (0.05 M). The resulting solution was freeze-dried. Two recrystallisations from water–THF–CH_3CN–Et_2O gave **1** as the crystalline monohydrate (326 mg, 80%); mp 201–203 °C; $[\alpha]_D$ −54.4° (*c* 1, H_2O); ^1H NMR (D_2O): δ 8.22 (d, 2 H, *J* 9.3 Hz, ArH), 7.19 (d, 2 H, *J* 9.3 Hz, ArH), 5.35 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1), 4.54 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1′), 4.19 (dd, 1 H, $J_{2,3}$ 10.3 Hz, H-2), 3.97 (dd, 1 H, $J_{6a,6b}$ 12.5 Hz, $J_{5,6a}$ 2.2 Hz, H-6a), 3.92 (dd, 1 H, $J_{3,4}$ 8.5 Hz, H-3), 3.83 (dd, 1 H, $J_{5,6b}$ 5.1 Hz, H-6b), 3.76 (d, 1 H, $J_{4',5'}$ 9.5 Hz, H-5′), 3.71–3.40 (m, 1 H, H-5), 3.67 (dd, 1 H, $J_{4,5}$ 9.8 Hz, H-4), 3.50–3.56 (m, 2 H, H-3′ and H-4′), 3.38 (br dd, 1 H, $J_{2',3'}$ 8.8 Hz, H-2′), 2.02 (s, 3 H, Ac); ^{13}C NMR (D_2O): δ 176.6, 176.0, 162.8, 143.8, 127.2, 117.7, 104.0 (C-1′), 99.4 (C-1), 83.2 (C-3), 77.0 (C-5), 76.9 (C-5′), 76.4 (C-3′), 73.8 (C-2′), 72.8 (C-4′), 69.5 (C-4), 61.6 (C-6), 55.4 (C-2), 23.3 (Ac); MS (ES): *m*/*z* 517.132 $(M - Na)^-$ (100%, found) 517.131 (calcd). Anal. Calcd for $C_{20}H_{25}N_2NaO_{14}\cdot H_2O$: C, 43.02; H, 4.87; N, 5.02. Found: C, 43.25; H, 4.80; N, 4.85.

4-Nitrophenyl (methyl 2,3,4-tri-O-*acetyl-β*-D-*glucopyranosyluronate)-(1→3)-2-acetamido-4,6-di*-O-*acetyl-2-deoxy-β*-D-*galactopyranoside* (**8**).—Starting from **7** (1.75 g, 2.63 mmol) the procedure for the preparation of **6** was followed. Two crystallisations from acetone–ether–hexanes gave **8** as a crystalline solid (0.845 g, 43%); mp 208–209 °C; $[\alpha]_D$ −13.8° (*c* 1, $CHCl_3$); TLC (7:3 toluene–acetone): R_f 0.14; ^1H NMR ($CDCl_3$): δ 8.16 (d, 2 H, *J* 9.3 Hz, ArH), 7.06 (d, 2 H, *J* 9.3 Hz, ArH), 6.23 (d, 1 H, $J_{1,2}$ 7.0 Hz, H-1), 5.65 (d, 1 H, $J_{NH,2}$ 8.2 Hz, NH), 5.46 (d, 1 H, $J_{3,4}$ 3.4 Hz, H-4), 5.24 (t, 1 H, $J_{2',3'}$ 9.2 Hz, $J_{3',4'}$ 9.2 Hz, H-3′), 5.18 (t, 1 H, $J_{4',5'}$ 9.5 Hz, H-4′), 5.01 (dd, 1 H, $J_{1',2'}$ 7.8 Hz, H-2′), 4.81 (d, 1 H, H-1′), 4.71 (dd, 1 H, $J_{2,3}$ 10.7 Hz, H-3), 4.18 (m, 1 H, H-6a), 4.04–4.11 (m, 3 H, H-6b, H-5, H-5′), 3.86 (ddd, 1 H, H-2, 3.76 (s, 3 H, OCH_3), 2.12, 2.08, 2.07, 2.03, 2.02, 1.97 (all s, each 3 H, 6 Ac); ^{13}C NMR ($CDCl_3$): δ 171.6, 170.7, 170.3, 170.3, 169.8, 169.7, 167.4, 162.0, 143.3, 126.0, 116.9, 99.5 (C-1′), 97.6 (C-1), 74.2 (C-3), 72.7 (C-5′), 72.5 (C-3′), 72.2 (C-5), 69.3 (C-4′), 68.0 (C-4), 62.5 (C-6), 54.1 (C-2), 53.2 (OCH_3), 23.8, 21.1, 21.0, 21.0, 20.9, 20.8 (6 Ac); MS (ES): *m*/*z* $(M + H)^+$ 743.215 (38%, found) 743.214 (calcd), 604.185 (100%, M − O-Ph-NO_2, found) 604.187 (calcd). Anal. Calcd for $C_{31}H_{38}N_2O_{19}$: C, 50.14; H, 5.16; N, 3.77. Found: C, 50.06; H, 5.20; N, 3.71.

4-Nitrophenyl (sodium β-D-*glucopyranosyluronate)-(1→3)-2-acetamido-2-deoxy-β*-D-*galactopyranoside* (**2**).—Starting from **8** (500 mg, 0.67 mmol) the same procedure as for the deprotection of **6** was followed. The product was crystallised from water–MeOH–2-propanol–acetone then recrystallised from water–MeOH–acetone–Et_2O, dissolved in water and lyophilised. Further crystallisation from a small volume of MeOH gave **2** as the hemihydrate as a crystalline solid (203 mg, 55%); mp 229–230 °C; $[\alpha]_D$ −28.2° (*c* 1, H_2O); ^1H NMR (D_2O): δ 8.25 (d, 2 H, *J* 9.3 Hz, ArH), 7.21 (d, 2 H, *J* 9.3 Hz, ArH), 5.30 (d, 1 H, $J_{1,2}$ 8.5 Hz, H-1), 4.56 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1′), 4.36 (dd, 1 H, $J_{2,3}$ 10.8 Hz, H-2), 4.29 (d, 1 H, $J_{3,4}$ 3.1 Hz, H-4), 4.01 (dd, 1 H, H-3), 3.94 (br t, 1 H, $J_{5,6}$ 5.9 Hz, H-5), 3.82 (d, 2 H, H-6), 3.71 (br d, 1 H, $J_{4',5'}$ 9.3, H-5′), 3.47–3.54 (m, 2 H, H-3′ and H-4′), 3.38 (m, 1 H, H-2′), 2.00 (s, 3 H, Ac); ^{13}C NMR (D_2O): δ 177.0, 176.2, 162.9, 143.7, 127.2, 117.7, 105.2 (C-1′), 100.0 (C-1), 80.6 (C-3), 77.2 (C-5′), 76.6 (C-3′), 76.4 (C-5), 73.8 (C-2′), 72.9 (C-4′), 68.6 (C-4), 61.9 (C-6), 52.0 (C-2), 23.3 (Ac); MS (ES): *m*/*z* 517.131 $(M - Na)^-$ (100%, found) 517.131 (calcd). Anal. Calcd for $C_{20}H_{25}N_2NaO_{14}\cdot 0.5H_2O$: C, 43.72; H, 4.77; N, 5.10. Found: C, 43.80; H, 4.73, N, 5.04.

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