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Note

Synthesis and characterization of the hexenuronic acid model methyl 4-deoxy-β-L-*threo*-hex-4-enopyranosiduronic acid

Immanuel Adorjan,* Anna-Stiina Jääskeläinen and Tapani Vuorinen

Helsinki University of Technology, Department of Forest Products Technology, PO Box 6300, FIN-02015 HUT, Finland

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Abstract—A facile synthetic scheme for the preparation of methyl 4-deoxy-β-L-*threo*-hex-4-enopyranosiduronic acid utilizing the commercially available methyl α-D-galactopyranoside as starting material has been developed. The synthesis sequence comprises six high yielding reaction steps: TEMPO oxidation, acetylation, methanolysis of the lactone, acetylation, β-elimination, and final removal of the protecting groups. Only one column chromatographic purification is needed throughout the whole sequence. The overall yield is 60%. The final product has been characterized by NMR, Raman, UVRR, FTIR, and HRMS.

Keywords: TEMPO oxidation; β-Elimination; Characterization by FTIR, Raman, UVRR, NMR

Hexenuronic acid (HexA) is widely distributed among natural polysaccharides such as heparin, chondroitin, and lepidimoide.[1–3] In lepidimoide, for example, the HexA moiety is important for its growth promoting activity.[2,3] During alkaline pulping 4-*O*-methylglucuronic acid units, attached to xylan, are converted to HexA by β-elimination.[4,5] HexA was suspected, and has recently been confirmed, to play an important role in the brightness reversion of pulp[6] as well as to increase the consumption of bleaching chemicals[7,8] and contribute considerably to the kappa number of oxygen-delignified kraft pulp.[9] Despite the importance of HexA, no fast and easy chemical synthesis using a commercially available starting material has been published so far. Many HexA preparations use 4-*O*-methylglucuronoxylan as starting material.[10,11] Hot alkali treatment yields hexenuronoxylan, which is subjected to enzymatic hydrolysis to afford HexA oligosaccharides after tedious purification procedures. These procedures yield only small amounts of the final oligomeric product. Thus the aim of this work was to establish a fast, simple, and high yield synthetic pathway involving as few laborious purification steps as possible. The HexA model described here was designed to be utilized to investigate in detail all reactions in connection with bleaching and brightness revision of pulp. Further on the model should be applied as a calibration standard for analytical applications. An important key feature of the HexA model is the α-methoxy substituent at C-1. First of all the reducing end is protected and thus purely the reactions associated with the double bond in conjugation to the carboxyl group can be investigated. Second the α-substituent simulates the α-bonding in xylan, where the HexA is attached in pulp.

The commercially available methyl α-D-galactopyranoside was chosen as starting material for the synthesis of methyl 4-deoxy-β-L-*threo*-hex-4-enopyranosiduronic acid (**4**). In principle the cheaper methyl α-D-glucopyranoside could have been used as starting material as well, but the β-elimination step will be more efficient when the acidic proton and the leaving group are antiperiplanar (axial–axial), as in the galacto derivative, than when they are syn-periplanar (axial–equatorial) as in the gluco derivative. The synthetic scheme (Scheme 1) comprises six high yielding reaction steps: TEMPO oxidation, acetylation, lactone opening to yield a methyl ester, second acetylation, β-elimination and finally removal of all protecting groups. At first a carboxylic

* Corresponding author. Tel.: +358 9 451 4250; fax: +358 9 451 4259; e-mail: iadorjan@cc.hut.fi

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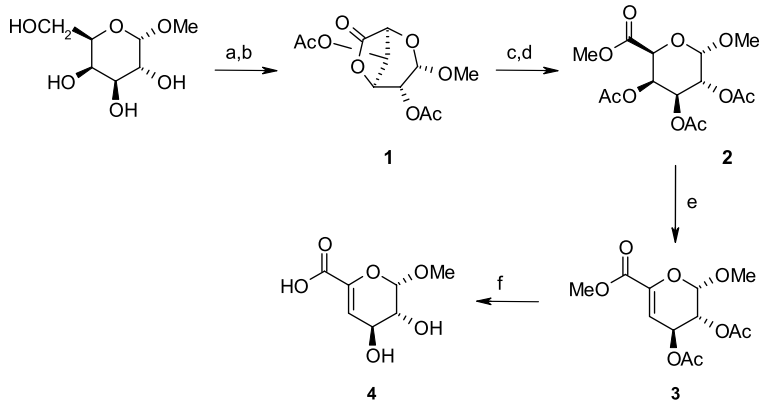

Scheme 1. Synthesis of **4**. Reagents and conditions: (a) TEMPO, NaBr, NaOCl, 0 °C; (b) Ac_2O, NaOAc, reflux; (c) MeOH, DBU; (d) Ac_2O, Py, DBU; (e) CH_2Cl_2, DBU; (f) LiOH (MeOH–H_2O–THF 5:4:1), Dowex 50 (H^+).

group was generated at C-6 by TEMPO oxidation. In this reaction the stable nitroxyl radical TEMPO (2,2,6,6-tetramethylpiperidine 1-oxyl) is used as catalyst to selectively oxidize the primary hydroxyl group at C-6 via the aldehyde to the carboxylic group.[12–14] A sodium hypochlorite/sodium bromide mixture is acting as regenerating oxidant in aqueous solution. After the complete conversion of methyl α-D-galactopyranoside to methyl α-D-galactopyranosiduronic acid and removal of the solvent, no attempt was made to separate the highly hydrophilic methyl α-D-galactopyranosiduronic acid from the salts stemming from the TEMPO oxidation as these do not hinder the following acetylation and are easily removed after acetylation by extraction. The acetylation of the crude methyl α-D-galactopyranosiduronic acid was carried out with acetic anhydride/sodium acetate under reflux for 10 min[15] and afforded methyl 2,4-di-*O*-acetyl-α-D-galactopyranosiduronic acid, γ-lactone (**1**)[16] in high yield. The lactone **1** was converted to methyl-(methyl 2,4-di-*O*-acetyl-α-D-galactopyranosid)-uronate by stirring with methanol and catalytic amount of DBU. Cleavage of acetyl groups and acetyl group migration occur as side reactions. As these side products will yield methyl-(methyl 2,3,4-tri-*O*-acetyl-α-D-galactopyranosid)uronate (**2**) as well upon acetylation, the crude product obtained after removal of the solvent was used without further purification. For the second acetylation reaction, crude methyl-(methyl 2,4-di-*O*-acetyl-α-D-galactopyranosid)uronate was dissolved in a mixture of acetic anhydride–pyridine = 1:1 at room temperature and catalytic amounts of DBU were added to accelerate the reaction. These smoother reaction conditions were chosen as they afforded **2** in very high yield. The two last reaction steps are a β-elimination[17,18] and the removal of the protecting groups. Both reactions require alkaline conditions so in theory they could be combined in one step. In this reaction scheme it was decided to separate these two reactions in order to have a better control over the β-elimination, which is in concurrence to the deacetylation. Additionally the fully protected β-elimination product methyl-(methyl 2,3-di-*O*-acetyl-4-deoxy-β-L-*threo*-hex-4-enopyranosid)uronate (**3**) is much easier to purify than the final product **4**. Thus reaction conditions, which facilitate the β-elimination, but at the same time do not lead to deacetylation had to be chosen. Unfortunately the method using pyridine as solvent and DBU as base[15] led to instant deacetylation upon DBU addition and no β-elimination product was obtained. Changing the solvent to dry dichloromethane[19,20] or toluene afforded **3** in high yield. It has to be noted that reaction control by TLC using anisaldehyde–sulfuric acid as dyeing reagent is complicated as the color intensity of **3** is considerably lower than that of **2**, but **3** exhibits strong UV activity due to the conjugated double bond. As there was no difference in yield between dichloromethane and toluene, dichloromethane was finally chosen as solvent. The final step in this synthetic scheme is the cleavage of the acetyl protecting groups and the methyl ester to yield methyl 4-deoxy-β-L-*threo*-hex-4-enopyranosiduronic acid **4**. This is accomplished by dissolving **3** in a 0.3 M lithium hydroxide solution (5:4:1 MeOH–water–THF) at 0 °C.[21] After complete conversion, Dowex 50 (H^+) is added to remove lithium hydroxide and to convert **4** into the acidic form. The solvent is removed by lyophilization to afford the final product **4** in quantitative yield. It has to be noted that elemental analysis was performed from the stable lithium salt. The overall yield of this synthetic scheme is 60%. One target of this synthetic scheme was to minimize laborious purifications steps. During scale up it was discovered that all but the column chromatographic purification of **3** can be omitted. It is essential to obtain **3** in high purity as after the deprotection no facile purification is available. The presented reaction sequence was successfully scaled up from 1 g to 5 g starting material without significant loss in overall yield.

1. Experimental

1.1. General methods

Optical rotations were measured with a Perkin–Elmer 341 polarimeter. NMR spectra were recorded with a Bruker Avance™ DPX400 instrument operating at 400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C using $CDCl_3$ or D_2O as the solvents. Tetramethylsilane was used as internal standard for ^{1}H spectra in $CDCl_3$, ^{13}C spectra in $CDCl_3$ were calibrated to the solvent peak (77.00 ppm), 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (DSS) was used as internal standard for ^{1}H spectra in D_2O, ^{13}C spectra in D_2O were externally calibrated to 1,4-dioxane (67.40 ppm). ^{1}H and ^{13}C NMR spectra were measured at 298.2 K. Homo- and heteronuclear 2D NMR spectroscopy was performed with Bruker standard software. Chemical shifts are given in ppm, coupling constants in hertz. FTIR spectra were acquired on a Bio-Rad 6000 spectrometer equipped with a MTEC 300 photoacoustic detector. UVRR spectra were collected with a Renishaw 1000 UV Raman spectrometer (Renishaw, Gloucestershire, UK) equipped with a Leica DMLM microscope (Leica Microsystems, Wetzlar, Germany). The light source was a frequency-doubled Ar^+ ion laser (Coherent Inc., CA, USA) tuned to an excitation wavelength of 244 nm. The laser beam was focused on the sample with a 15× objective lens. During measurements, the sample was spun and the measurements were repeated to obtain average spectra. Raman spectra were recorded with a dispersive Kaiser Optical Systems HoloLab Raman spectrometer equipped with a 785 nm GaAlAs diode laser and an Olympus BX 60 microscope. TLC was performed on Merck precoated plates (5 × 10 cm, layer thickness 0.25 mm, silica gel 60 F_{254}); detection was affected by dipping into anisaldehyde–H_2SO_4 followed by charring. For column chromatography, silica gel VWR Si60 (230–400 mesh) was used. Concentration of solutions was performed at reduced pressure and 40 °C. Elemental analyses were provided by Professor Ari Koskinen, Laboratory of Organic Chemistry, Helsinki University of Technology, Espoo. HRMS measurements were performed with a Waters LCT Premier by Dr. Jari Koivisto, Laboratory of Organic Chemistry, Helsinki University of Technology, Espoo.

1.2. Methyl 2,4-di-*O*-acetyl-α-D-galactopyranosiduronic acid, γ-lactone (1)

Methyl α-D-galactopyranoside (1.00 g, 5.15 mmol), NaBr (265 mg), and TEMPO (8 mg) were dissolved in distilled water (50 mL) and cooled to 0 °C. NaOCl solution (30 mL, 2.5%) was added dropwise while keeping the pH within 10–11 with sodium hydroxide solution (0.5 M). After complete conversion excess of NaOCl was deactivated by addition of MeOH (10 mL) and the solvent was removed under diminished pressure. Traces of water were removed by coevaporation with toluene. NaOAc (500 mg) and Ac_2O (16 mL) were added to crude methyl α-D-galactopyranosiduronic acid and heated for 10 min under reflux. After cooling to room temperature, MeOH (20 mL) was added under ice/water cooling to deactivate excess of Ac_2O. The reaction mixture was neutralized by saturated $NaHCO_3$ solution and extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4, filtrated, and the solvent was removed under reduced pressure. The crude product was purified by column chromatography (2:1 toluene–ethyl acetate) to yield **1** (1.14 g, 4.16 mmol, 81%) as a colorless syrup. $[\alpha]_D^{21}$ +30 (*c* 0.4, $CHCl_3$), lit.:[16] $[\alpha]_D^{20}$ +28 (*c* 1.2, $CHCl_3$); R_f 0.53 (1:1 toluene–ethyl acetate); ^{1}H NMR ($CDCl_3$): δ 5.47 (d, 1H, $J_{4,5} = 1.2$ Hz, H-5), 5,42 (dd, 1H, $J_{1,2} = 2.9$ Hz, $J_{2,3} = 4.8$ Hz, H-2), 4.88 (dd, 1H, $J_{3,4} = 1.7$ Hz, H-3), 4.7 (d, 1H, H-1), 4.30–4.29 (m, 1H, H-4), 3.54 (s, 3H, 1-OMe), 2.19 (s, 3H, –OAc), 2.13 (s, 3H, –OAc). ^{13}C NMR ($CDCl_3$): δ 170.25 (C-6), 169.40 (–OAc), 169.29 (–OAc), 97.43 (C-1), 78.73 (C-3), 71.13, 71.11 (C-4, C-5), 67.91 (C-2), 58.06 (1-OMe), 20.61 (–OAc), 20.56 (–OAc); ESI-MS Anal. Calcd for $C_{11}H_{15}O_8$ $[M+H]^+$: 275.0767. Found: 275.0780.

1.3. Methyl-(methyl 2,3,4-tri-*O*-acetyl-α-D-galacto-pyranosid)uronate (2)

1.3.1. Methyl-(methyl 2,4-di-*O*-acetyl-α-D-galacto-pyranosid)uronate. Methyl 2,4-di-*O*-acetyl-α-D-galactopyranosiduronic acid, γ-lactone **1** (1.14 g, 4.16 mmol) was dissolved in MeOH (50 mL), DBU (50 μL) was added and the reaction mixture was stirred for 17 h at room temperature. After complete conversion the solvent was removed under reduced pressure and the crude product (1.23 g, 4.02 mmol, 97%) was used without further purification for the next step. R_f 0.14 (1:1 toluene–ethyl acetate); ^{1}H NMR ($CDCl_3$): δ 5.65 (dd, 1H, $J_{3,4} = 3.6$ Hz, $J_{4,5} = 1.5$ Hz, H-4), 5.10–5.04 (m, 2H, H-1, H-2), 4.54 (d, 1H, H-5), 4.27–4.24 (m, 1H, H-3), 3.76 (s, 3H, –COOMe), 3.44 (s, 3H, –OMe), 2.69 (br s, 1H, –OH), 2.15 (s, 3H, –OAc), 2.13 (s, 3H, –OAc). ^{13}C NMR ($CDCl_3$): δ 171.04 (–OAc), 170.56 (–OAc), 167.79 (C-6), 97.67 (C-1), 71.46 (C-4), 70.62 (C-2), 68.45 (C-5), 66.42 (C-3), 56.11 (1-OMe), 52.57 (–COO*Me*), 20.83 (–OAc), 20.58 (–OAc).

1.3.2. Methyl-(methyl 2,3,4-tri-*O*-acetyl-α-D-galacto-pyranosid)uronate (2). Crude methyl-(methyl 2,4-di-*O*-acetyl-α-D-galactopyranosid)uronate (1.23 g, 4.02 mmol) was dissolved in a pyridine/Ac_2O mixture (1:1, 10 mL), DBU (50 μL) was added and the reaction mixture was stirred for 5 h at room temperature. After complete conversion, Ac_2O was deacticvated by adding ice to the reac-

tion mixture and stirring for 30 min. The reaction mixture was neutralized with saturated $NaHCO_3$ solution and extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4, filtered, and the solvent was removed under diminished pressure. The crude product was purified by column chromatography (4:1 toluene–ethyl acetate) to yield **2** (1.29 g, 3.70 mmol, 92%) as colorless foam. $[\alpha]_D^{21}$ 155 (*c* 1.2, $CHCl_3$), lit.:[15] $[\alpha]_D^{22}$ +160 ($CHCl_3$); R_f 0.36 (1:1 toluene–ethyl acetate); ^1H NMR ($CDCl_3$): δ 5.77 (dd, 1H, $J_{3,4} = 3.4$ Hz, $J_{4,5} = 1.6$ Hz, H-4), 5.41 (dd, 1H, $J_{2,3} = 10.9$ Hz, H-3), 5.21 (dd, 1H, $J_{1,2} = 3.6$ Hz, H-2), 5.13 (d, 1H, H-1), 4.61 (d, 1H, H-5), 3.76 (s, 3H, COO*Me*), 3.45 (s, 3H, 1-OMe), 2.11 (s, 3H, –OAc), 2.09 (s, 3H, –OAc), 2.00 (s, 3H, –OAc); ^{13}C NMR ($CDCl_3$): δ 170.16 (–OAc), 169.81 (–OAc), 169.73 (–OAc), 167.34 (C-6), 97.55 (C-1), 69.13 (C-4), 68.32 (C-5), 67.55 (C-2), 67.04 (C-3), 56.17 (1-OMe), 52.63 (–COO*Me*), 20.72 (–OAc), 20.57 (–OAc), 20.50 (–OAc); ESI-MS Anal. Calcd for $C_{14}H_{20}O_{10}Na$ $[M+Na]^+$: 371.0954. Found: 371.0953. Anal. Calcd for $C_{14}H_{20}O_{10}$: C, 48.28; H, 5.79. Found: C, 48.62; H, 5.54.

1.4. Methyl-(methyl 2,3-di-*O*-acetyl-4-deoxy-β-L-*threo*-hex-4-enopyranosid)uronate (3)

Methyl-(methyl 2,3,4-tri-*O*-acetyl-α-D-galactopyranosid)-uronate **2** (1.29 g, 3.70 mmol) was dissolved in dry CH_2Cl_2 (5 mL), DBU (600 μL) was diluted in dry CH_2Cl_2 (5 mL) and added to the reaction mixture at room temperature. The reaction mixture was stirred for 20 h and subjected directly to column chromatography (50:1 CH_2Cl_2–MeOH) to afford **3** (897 mg, 3.11 mmol, 84%) as colorless syrup. $[\alpha]_D^{21}$ +255 (*c* 1, $CHCl_3$), lit.:[22] $[\alpha]_D^{29}$ +259.9 (*c* 1.124, MeOH); R_f 0.49 (1:1 toluene–ethyl acetate); ^1H NMR ($CDCl_3$): δ 6.07 (d, 1H, $J_{3,4} = 3.0$ Hz, H-4), 5.62–5.58 (m, 1H, H-3), 5.31–5.15 (m, 2H, H-1, H-2), 3.83 (s, 3H, –COOMe), 3.54 (s, 3H, 1-OMe), 2.12 (s, 3H, –OAc), 2.09 (s, 3H, –OAc). ^{13}C NMR ($CDCl_3$): δ 170.04 (–OAc), 170.00 (–OAc), 161.90 (C-6), 141.64 (C-5), 108.49 (C-4), 98.33 (C-1), 68.73 (C-2), 66.38 (C-3), 56.84 (–OMe), 52.52 (–COO*Me*), 20.84 (–OAc), 20.74 (–OAc); ESI-MS Anal. Calcd for $C_{12}H_{16}O_8Na$ $[M+Na]^+$: 311.0743. Found: 311.0753.

1.5. Methyl 4-deoxy-β-L-*threo*-hex-4-enopyranosiduronic acid (4)

Methyl-(methyl 2,3-di-*O*-acetyl-4-deoxy-β-L-*threo*-hex-4-enopyranosid)uronate **3** (897 mg, 3.11 mmol) was dissolved in LiOH solution (50 mL, 0.3 M, MeOH/water/THF = 5:4:1) at 0 °C and stirred for 2 h. After complete conversion the reaction mixture was diluted with distilled water (100 mL) and Dowex 50 (H^+) was added to adjust the pH to 2.7. The ion exchanger was removed by filtration and the filtrate was lyophilized to afford **4** (586 mg, 3.08 mmol, 99%) as colorless amorphous solid. $[\alpha]_D^{21}$ +218 (*c* 0.2, H_2O); R_f 0.13 (5:10:1 MeOH–CH_2Cl_2–water); UV: λ_{max} 234; UVRR: *v* 1653; IR (KBr): *v* 1720, 1655; Raman (cm^{-1}): 860, 828; ^1H NMR (D_2O): δ 6.13 (d, 1H, $J_{3,4} = 3.1$ Hz, H-4), 5.08 (d, 1H, $J_{1,2} = 2.6$ Hz, H-1), 4.34 (dd, 1H, $J_{2,3} = 7.6$ Hz, H-3), 3.85 (dd, 1H, H-2), 3.55 (s, 3H, 1-OMe); ^{13}C NMR (D_2O): δ 166.19 (C-6), 141.19 (C-5), 113.35 (C-4), 101.45 (C-1), 70.40 (C-2), 66.42 (C-3), 57.71 (1-OMe); ESI-MS Anal. Calcd for $C_7H_9O_6$ $[M-H]^-$: 189.0399. Found: 189.0398. Anal. Calcd for $C_7H_9LiO_6$: C, 42.88; H, 4.63. Found: C, 43.21; H, 4.91.

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Supplementary data

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