Carbohydrate Research, 103 (1982) 176–180
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

Note

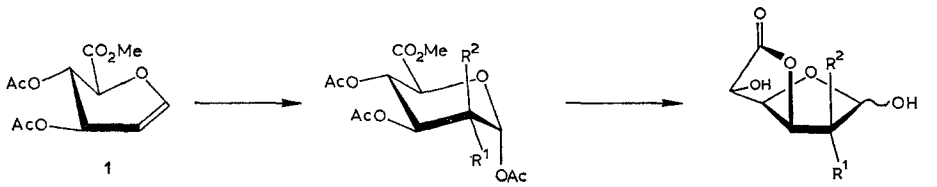
Synthesis of 2-acetamido-2-deoxyhexuronic acids with the D-*gluco*, D-*manno*, and D-*galacto* configurations

EFTHYMIOS DARAKAS, HANS HULTBERG, KARIN LEONTEIN, AND JÖRGEN LÖNNGREN
Department of Organic Chemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm (Sweden)

(Received August 25th, 1981; accepted for publication, October 19th, 1981)

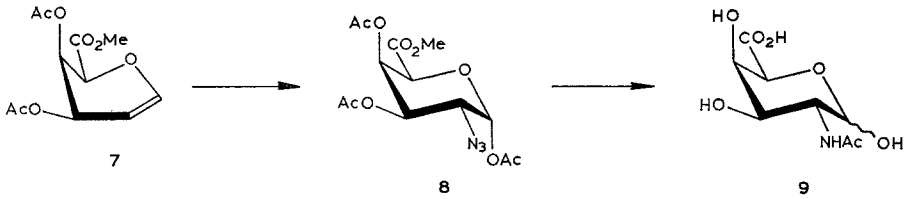
2-Acetamido-2-deoxyhexuronic acids have been found in Nature as constituents of bacterial polysaccharides, and several syntheses have been reported[1–7]. The principal step in these syntheses was catalytic oxidation of an amino sugar derivative to its uronic acid. However, in our hands, these reactions gave unsatisfactory yields. We now report on alternative syntheses of the title compounds, using azidonitration[8] of the corresponding glycal methyl esters as the key step. The D-*gluco* and D-*manno* analogues were isolated as their 6,3-lactones.

Methyl 3,4-di-*O*-acetyl-2,6-anhydro-5-deoxy-D-*lyxo*-hex-5-enonate[9] (**1**) was treated with ceric ammonium nitrate and sodium azide in acetonitrile[8], to give a crude mixture of azidonitrates in 90% yield. The product was not fractionated, but was treated with sodium acetate in glacial acetic acid. The ^{13}C-n.m.r. spectrum of the resulting, crude product indicated the presence of methyl 1,3,4-tri-*O*-acetyl-2-azido-2-deoxy-α-D-glucuronate (**2**), methyl 1,3,4-tri-*O*-acetyl-2-azido-2-deoxy-β-D-glucuronate, and methyl 1,3,4-tri-*O*-acetyl-2-azido-2-deoxy-α-D-mannuronate (**3**) in the proportions ~5:2:1. After chromatography on silica gel, **2** and **3** were obtained in yields of 42 and 6%, respectively. The structures of **2** and **3** were determined from their ^{1}H- and ^{13}C-n.m.r. spectra (see Experimental). Attempted saponification of **2** caused excessive degradation. However, treatment of **2** with aqueous hydrogen chloride gave crystalline 2-azido-2-deoxy-D-glucofuranurono-6,3-lactone (**4**). For



preparative purposes, however, it was more convenient to hydrogenate the crude substance, in methanol–acetic anhydride, using palladium-on-charcoal as the catalyst. In this reaction, the amino group is formed and simultaneously acetylated, and 2-acetamido-2-deoxy-D-glucofuranurono-6,3-lactone (**5**) was obtained in 72% yield from **2**. When **3** was subjected to the de-esterification–reduction–*N*-acetylation sequence, 80% of crystalline 2-acetamido-2-deoxy-D-mannofuranurono-6,3-lactone (**6**) was obtained

Methyl 3,4-di-*O*-acetyl-2,6-anhydro-5-deoxy-D-*arabino*-hex-5-enonate[10] (**7**) was subjected to azidonitration and the resulting syrup was treated with sodium acetate in acetic acid. After chromatography on silica gel, methyl 1,3,4-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galacturonate (**8**) was obtained in 44% yield. As described above, **8** was transformed into 2-acetamido-2-deoxy-D-galacturonic acid (**9**), which was obtained in 27% yield after chromatography. As expected, this acid does not form a lactone; in order to prevent decomposition, **9** was stored as its sodium salt.



EXPERIMENTAL

General methods. — Melting points are corrected. Concentrations were performed at bath temperatures below 40°. Optical rotations were measured with a Perkin–Elmer 241 polarimeter. N.m.r. spectra were recorded in the pulse-F.t.-mode with a JEOL FX-100 instrument at 25°. Chemical shifts are given in p.p.m. relative to internal Me_4Si (^1H and ^{13}C, chloroform and acetone solutions), external Me_4Si (^{13}C, aqueous solutions), or internal sodium 1,1,2,2,3,3-hexadeuterio-4,4-dimethyl-4-silapentane-1-sulphonate (^1H, aqueous solutions). All ^1H-n.m.r. spectra were interpreted on a first-order basis. For t.l.c., Merck plates (silica gel 60 F_{254}) were used. Compounds were located by quenching of u.v. fluorescence, or by charring with sulphuric acid. For column chromatography, Merck silica gel 60 (0.040–0.063 mm) was used. The loadings on columns were usually 1:50–100 and separations were run as flash-chromatography[11].

*Methyl 1,3,4-tri-*O*-acetyl-2-azido-2-deoxy-α-D-glucuronate* (**2**) *and methyl 1,3,4-tri-*O*-acetyl-2-azido-2-deoxy-α-D-mannuronate* (**3**). — A solution of **1** (6.0 g) in acetonitrile (126 mL) was added to a mixture of sodium azide (2.25 g) and ceric ammonium nitrate (38.0 g). The mixture was stirred for 16 h at −15° under nitrogen. Cold ether (150 mL) and ice-cold water (150 mL) were added and the mixture was shaken. The organic phase was washed with water (3 × 150 mL) and concentrated to dryness, yielding a syrup (7.6 g, 90%). A solution of this product (7.0 g) in glacial

acetic acid (44 mL) containing anhydrous sodium acetate (3.0 g) was heated at 100° for 1.5 h and then cooled, and chloroform (200 mL) was added. The mixture was successively washed with water, aqueous sodium hydrogencarbonate, and water. Concentration gave a syrup (6.0 g, 86%) which was purified on silica gel with light petroleum–ethyl acetate (2:1). Crystallisation from acetone yielded **2** (2.9 g, 42%), m.p. 107–108°, $[\alpha]_{578}^{21}$ +113° (*c* 1.7, chloroform); ^{1}H-n.m.r. data (acetone-d_6): δ 2.04, 2.12, 2.26 (3 s, 3 × 3 H, 3 OAc), 3.70 (s, 3 H, OMe), 4.05 (dd, 1 H, $J_{1,2}$ 4.0, $J_{2,3}$ 11.0 Hz, H-2), 4.48 (d, 1 H, $J_{4,5}$ 10.0 Hz, H-5), 5.13 (dd, 1 H, $J_{3,4}$ 10.0 Hz, H-4), 5.48 (dd, 1 H, H-3), and 6.35 (d, 1 H, H-1); ^{13}C-n.m.r. ($CDCl_3$): δ 20.5, 20.7, 20.9 (3 C, 3 OAc), 52.9 (OMe), 60.2 (C-2), 69.3, 70.0, 70.5 (3 C, C-3,4,5), 89.8 (C-1), and 167.0, 167.9, 169.1, 169.5 (C=O).

Anal. Calc. for $C_{13}H_{17}N_3O_9$: C, 43.46; H, 4.77; N, 11.70. Found: C, 43.61; H, 4.76; N, 11.54.

Fractions containing **3** were concentrated and the residue was crystallised from acetone, to give **3** (0.4 g, 6%), m.p. 144–145°, $[\alpha]_{578}^{21}$ +58° (*c* 2, chloroform); ^{1}H-n.m.r. data (acetone-d_6): δ 2.02, 2.06, 2.16 (3 s, 3 × 3 H, 3 OAc), 3.68 (s, 3 H, OMe), 4.28 (dd, 1 H, $J_{1,2} \simeq J_{2,3}$ 3 Hz, H-2), 4.44 (d, 1 H, $J_{4,5}$ 8.0 Hz, H-5), 5.36 (m, 2 H, H-3,4), and 6.14 (d, 1 H, H-1); ^{13}C-n.m.r. data ($CDCl_3$): δ 20.4, 20.6, 20.8 (3 C, 3 OAc), 52.8 (OMe), 59.2 (C-2), 66.7, 69.3, 72.0 (3 C, C-3,4,5), 90.3 (C-1, $J_{C\text{-}1,H\text{-}1}$ 177.0 Hz), and 166.9, 167.7, 168.9, 169.1 (C=O).

Anal. Calc. for $C_{13}H_{17}N_3O_9$: C, 43.46; H, 4.77; N, 11.70. Found: C, 43.36; H, 4.76; N, 11.69.

*2-Azido-2-deoxy-*D*-glucofuranurono-6,3-lactone* (**4**) *and 2-acetamido-2-deoxy-*D*-glucofuranurono-6,3-lactone* (**5**). — Compound **2** (360 mg) was dissolved with stirring in 4M hydrochloric acid (20 mL) at 40° and the solution was kept at room temperature for 3 h, diluted and then freeze-dried. In order to ensure complete removal of acid, the residue was dissolved in water and the solution freeze-dried. An aliquot of the crude product was crystallised from methanol, to yield **4**, m.p. 135–136, $[\alpha]_D^{21}$ +39° (*c* 0.3, water; no mutarotation); ^{1}H-n.m.r. data (D_2O): δ 4.57 (s, 0.7 H, H-1β) and 5.73 (d, 0.3 H, $J_{1,2}$ 4.0 Hz, H-1α); ^{13}C-n.m.r. data (D_2O): δ 66.1 (C-2α), 68.2 (C-2β), 69.5, 76.4, 83.3 (C-3α,4α,5α), 69.3, 77.9, 82.6 (C-3β,4β,5β), 98.7 (C-1α), 101.5 (C-1β), 176.9 (C=O α), and 177.1 (C=O β); ν_{max}^{KBr} 2110 cm^{-1} (azide) and 1785 cm^{-1} (γ-lactone).

Crude, de-esterified material (200 mg) was dissolved in methanol (8 mL) containing acetic anhydride (0.3 mL) and hydrogenated at room temperature and atmospheric pressure over 10% palladium-on-charcoal (20 mg). After work-up, the product was crystallised from water, to yield **5** (100 mg, 55%). Purification of the mother liquor on a column (2.5 × 80 cm) of Sephadex G-15 irrigated with water gave more **5** (32 mg, 17%). Compound **5** had m.p. 175–178°, $[\alpha]_D^{21}$ +44° (*c* 0.6, water; no mutarotation observed); lit.[7] m.p. 177–178°, $[\alpha]_D^{20}$ +43.6°; ^{1}H-n.m.r. data (D_2O): δ 2.02 (NHAc β), 2.08 (NHAc α), 5.50 (s, 0.7 H, H-1β), and 5.70 (d, 0.3 H, $J_{1,2}$ 5.0 Hz, H-1α); ^{13}C-n.m.r. data (D_2O): δ 22.1 (NHAc), 58.7 (C-2α), 60.4 (C-2β), 69.4, 76.0, 84.9 (C-3α,4α,5α), 69.4, 77.7, 83.2 (C-3β,4β,5β), 96.8 (C-1α), 101.9

(C-1β), 173.7 (CONH β), 173.8 (CONH α), 176.9 (C=O α), and 177.0 (C=O β); ν_{max}^{KBr} 1785 (γ-lactone), 1650 and 1545 cm^{-1} (sec. amide).

Anal. Calc. for $C_8H_{11}NO_6$: C, 44.24; H, 5.11; N, 6.45. Found: C, 44.01; H, 5.08; N, 6.21.

*2-Acetamido-2-deoxy-*D*-mannofuranurono-6,3-lactone* (**6**). — Compound **3** (135 mg) was de-esterified and transformed into the *N*-acetyl derivative **6** as described for **5**. Crystallisation from water gave **6** (65 mg, 80%), m.p. 198–201°, $[\alpha]_D^{21}$ +121° (*c* 0.4, water; no mutarotation observed); ^{1}H-n.m.r. data (D_2O): δ 2.06 (NHAc β), 2.08 (NHAc α), 5.34 (d, 0.2 H, $J_{1,2}$ 6.0 Hz, H-1β), 5.53 (d, 0.8 H, $J_{1,2}$ 5.0 Hz, H-1α); ^{13}C-n.m.r. data (H_2O): δ 22.0 (NHAc), 54.8 (C-2α), 58.9 (C-2β), 69.8, 76.3, 79.3 (C-3α,4α,5α), 70.2, 77.4, 79.8 (C-3β,4β,5β), 95.7 (C-1α), 100.8 (C-1β), 174.4 (CONH), and 177.5 (C=O); ν_{max}^{KBr} 1790 (γ-lactone), 1620 and 1540 cm^{-1} (sec. amide).

Anal. Calc. for $C_8H_{11}NO_6$: C, 44.24; H, 5.11; N, 6.45. Found: C, 42.65; H, 5.08; N, 6.08.

*Methyl 1,3,4-tri-*O*-acetyl-2-azido-2-deoxy-*α*-*D*-galacturonate* (**8**). — Compound **7** (4.7 g) was subjected to azidonitration and acetolysis as described for **1**. The product was purified on silica gel with toluene–ethyl acetate (2:1) and re-crystallised from ethyl acetate–ether, to give **8** (2.89 g, 44%), m.p. 116–117°, $[\alpha]_{578}^{21}$ +120° (*c* 1, chloroform); ^{1}H-n.m.r. data (acetone-d_6): δ 2.02–2.18 (9 H, 3 OAc), 3.68 (s, 3 H, OMe), 4.16 (dd, 1 H, $J_{1,2}$ 4.0, $J_{2,3}$ 11.0 Hz, H-2), 5.00 (d, 1 H, $J_{4,5}$ 1.0 Hz, H-5), 5.34 (dd, 1 H, $J_{3,4}$ 4.0 Hz, H-3), 5.72 (dd, 1 H, H-4), and 6.38 (d, 1 H, H-1); ^{13}C-n.m.r. data ($CDCl_3$): δ 20.4, 20.6, 20.8 (3 C, 3 OAc), 52.8 (OMe), 56.6 (C-2), 68.1, 68.4, 70.6 (C-3,4,5), 90.1 (C-1), and 166.4, 168.1, 169.4, 169.9 (C=O).

Anal. Calc. for $C_{13}H_{17}N_3O_9$: C, 43.46; H, 4.77; N, 11.70. Found: C, 43.42; H, 4.77; N, 11.71.

*2-Acetamido-2-deoxy-*D*-galacturonic acid* (**9**). — Compound **8** (200 mg) was de-esterified and transformed into the *N*-acetyl derivative **9** as described for **5**. After work-up, the product was purified on a column (2.5 × 80 cm) of Biogel P 2 irrigated with water. After freeze-drying of appropriate fractions, **9** (36 mg, 27%) was obtained as an amorphous powder, $[\alpha]_D^{22}$ +29° (*c* 1.4, water). Since it was impracticable to store **9** in the acid form, it was converted into its sodium salt, $[\alpha]_D^{22}$ +23° (*c* 1.5, water, pH $\simeq$ 7); ^{1}H-n.m.r. data (D_2O, pD $\simeq$ 7): δ 2.04 (s, 3 H, NHAc) and 5.27 (d, 0.7 H, $J_{1,2}$ 2.5 Hz, H-1α); ^{13}C-n.m.r. data (D_2O, pD $\simeq$ 7): δ 23.2 (NHAc α), 23.4 (NHAc β), 51.2 (C-2α), 54.5 (C-2β), 68.7 (C-3α), 70.6 (C-4β), 71.2 (C-4α), 72.4, 72.6 (C-3β,5α), 76.7 (C-5β), 92.1 (C-1α), 96.2 (C-1β), 175.6, 175.9 (CONH), and 176.2, 177.1 (C=O).

ACKNOWLEDGMENTS

We thank Professor B. Lindberg for his interest, and the Swedish Natural Science Research Council for financial support.

REFERENCES

1 K. HEYNS AND W. KOCH, *Chem. Ber.*, 86 (1953) 110–114.
2 K. HEYNS AND H. PAULSEN, *Chem. Ber.*, 88 (1955) 188–195.
3 K. HEYNS AND M. BECK, *Chem. Ber.*, 90 (1957) 2443–2447.
4 K. HEYNS AND P. KÖLL, *Methods Carbohydr. Chem.*, 6 (1972) 342–347.
5 H. WEIDMAN AND H. K. ZIMMERMAN, *Justus Liebigs Ann. Chem.*, 684 (1965) 226–231.
6 N. G. KUNDU, J. F. CRAWFORD, B. PRAJSUAR, E. J. REED, AND S. ROSENTHAL, *Carbohydr. Res.*, 12 (1970) 225–232.
7 H. WEIDMAN, E. FAULAND, R. HELBIG, AND H. K. ZIMMERMAN, *Justus Liebigs Ann. Chem.*, 694 (1966) 183–189.
8 R. U. LEMIEUX AND R. M. RATCLIFFE, *Can. J. Chem.*, 57 (1979) 1244–1251.
9 F. B. ANDERSON AND D. H. LEABACK, *Chem. Ind. (London)*, (1960) 967–968.
10 T. KONDO AND T. GOTO, *Agric. Biol. Chem.*, 35 (1971) 912–917.
11 W. C. STILL, M. KAHN, AND A. MITRA, *J. Org. Chem.*, 43 (1978) 2923–2925.