Preparation of $O$-Hydroxyethyl and $O$-Hydroxypropyl Derivatives of $d$-Glucose and 2-Acetamido-2-deoxy-$d$-glucose for Studies of Modified Hyaluronic Acid

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Some hydroxyethyl and hydroxypropyl derivatives of $d$-glucose and of 2-acetamido-2-deoxy-$d$-glucose have been synthesized for use as reference substances for structural studies of hydroxymethylated and hydroxypropylated hyaluronic acid. Hydroxyethyl and hydroxypropyl substituents were introduced in the 2-O- or 3-O-position of $d$-glucose and in the 4-O- or 6-O-positions of 2-acetamido-2-deoxy-$d$-glucose by reaction of suitably protected sugars with either ethylene oxide or propylene oxide. For hydroxyethyl derivatives yields varied between 21 and 74%, and with a substantial portion of the doubly alkylated compounds. For hydroxypropyl derivatives yields varied between 15 and 80%. Only trace amounts of the doubly alkylated compounds were found. The proportions of the respective derivatives were estimated using GLC–MS. All products were characterized by $^1$H and $^{13}$C NMR spectroscopy.

Over the last decade sodium hyaluronate has found use in medical applications. Solutions of sodium hyaluronate are frequently used in eye surgery, both in cataract and in vitreous surgery. Hyaluronate solutions are also used in the knee joints of race horses, to replace synovial fluid and to relieve pain.

In various other types of surgery, hyaluronate solutions have been used in attempts to prevent postoperative adhesions. The results have not always been satisfactory, possibly because the hyaluronate did not have the ability to prevent contact between tissues long enough to inhibit the formation of adhesions. In some types of eye surgery, e.g., retinal detachment surgery, the duration of the hyaluronate solution and its tissue fixing action is sometimes too short.

In view of these limitations, there has been a growing interest in the modification of hyaluronic acid in order to influence the rheological properties to broaden its utility as a surgical tool and to create a more gel-like material.

One way of obtaining hyaluronate gels is to react it with a bifunctional epoxy compound, such as 1,2,3,4-diepoxobutane, 1,4-butanediol diglycidyl ether, or epichlorohydrin, to give ether linkages that are reasonably stable under physiological conditions. Relatively stable crosslinks can also be introduced using divinyl sulfone.

To gain knowledge of the substitution pattern of alkylated hyaluronate we chose the reaction of hyaluronate with ethylene oxide and propylene oxide as a model for the crosslinking reaction of hyaluronate with bifunctional epoxy compounds.

In this paper we report the synthesis of the monohydroxyethyl and monohydroxypropyl derivatives of $d$-glucose and 2-acetamido-2-deoxy-$d$-glucose that can arise from alkylation of hyaluronate. These compounds are 2-0(2-hydroxyethyl)-$d$-glucose, 3-O(2-hydroxyethyl)-$d$-glucose, 2-O(2-hydroxypropyl)-$d$-glucose, 3-O(2-hydroxypropyl)-$d$-glucose, 2-acetamido-2-deoxy-4-O(2-hydroxyethyl)-$d$-glucose, 2-acetamido-2-deoxy-6-O(2-hydroxyethyl)-$d$-glucose, 2-acetamido-2-deoxy-4-O(2-hydroxypropyl)-$d$-glucose, and 2-acetamido-2-deoxy-6-O(2-hydroxypropyl)-$d$-glucose. The glucose derivatives have been prepared previously using other methods.

The GLC–MS analysis of alkylated hyaluronate will be reported separately.

Results and discussion

The 2-O-(2-hydroxyalkyl) derivatives of $d$-glucose were made from 1,2-O-isopropylidene-$d$-glucofuranose in five steps (Scheme 1). Compound 2 was hydrolysed with sulfuric acid in dioxide–water and crude 3, with an $R_f$ value well in accordance with that reported earlier, was used in the next step after separation of the starting ma-
A small amount of pure 3 was analysed by NMR spectroscopy. Glycosidation was accomplished with 0.25 M hydrogen chloride in benzyl alcohol. The reaction was monitored by TLC, which indicated a 50% reaction. Treatment with molecular sieves to remove the water did not influence the equilibrium. The product, 4, was identified by NMR spectroscopy using the $^{13}$C data of Koto et al.[11] The alkylation reaction in step iv with ethylene oxide gave, besides 5, the 2-O-[2-(2-hydroxyethoxy)ethyl] derivative 6. In the reaction with propylene oxide, compound 7 and traces of the 2-O-[2-(2-hydroxypropoxy)propyl] derivative were obtained.

Compounds 5 and 7 were treated with Pd–H$_2$ to give 8 and 9 which were identified by NMR spectroscopy using data of Reuben[1] and Lee et al.[9] respectively. It was observed that for dilute solutions it was possible to retain the glycosidic benzyl group. Compound 6 was not processed further.

The reaction with propylene oxide results in both the R and S configurations of the substituent. This results in formation of two isomers, as observed by ‘twinning’ in the $^{13}$C NMR spectrum. It is expected that in basic solution attack occurs only at the primary carbon, it being the most accessible.[10] This is indicated by the close similarity of $^{13}$C NMR chemical shifts for the signals of hydroxypropyl carbons with data for 2-hydroxypropyl derivatives of D-glucose presented earlier.[9]

The 3-O-(2-hydroxyalkyl) derivatives of D-glucose were prepared by alkylation of 1,2:5,6-di-O-isopropylidene-α-D-glucopyranoside (10) and hydrolysis of the derivatives as outlined in Scheme 2. In the reaction mixture from the alkylation, different multiply substituted products were also obtained as demonstrated by GLC–MS. In the alkylation using propylene oxide the hydroxypropoxypropyl derivative was found in only small amounts in addition to the desired product, 12. Compound 12 was an RS-mixture as shown by the NMR spectrum in which there were ‘twinned’ signals. The propylene oxide thus reacted only on the primary carbon atom as discussed above. $^{13}$C NMR spectra of compounds 11 and 12 were assigned by comparison with $^{13}$C NMR data for 10.[12] The products were then hydrolysed using aqueous sulfuric acid to give 13 and 14, identified analogously to 8 and 9.

The 4-O-(2-hydroxyalkyl) and 6-O-(2-hydroxyalkyl) derivatives of 2-acetamido-2-deoxy-D-glucose were synthesized from benzyl 2-acetamido-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside in five steps as described in Scheme 3.

The benzylation of benzyl 2-acetamido-4,6-O-benzylidene-2-deoxy-α-D-glucopyranoside (Scheme 3, step i)
was performed essentially as described using benzyl bromide with potassium hydroxide in N,N-dimethylformamide which gave an easy work-up procedure. Benzylations using sodium hydride or silver oxide in N,N-dimethylformamide resulted in complex product mixtures, which were difficult to purify. The benzylidene group was removed in boiling aqueous acetic acid to give 17 which was partially benzylated using the method above with 1.3 equivalents of benzyl bromide. The reaction was monitored by TLC and interrupted before all 17 had reacted to allow for isolation of both 18 and 23. The preparation of these compounds has been reported. Compounds 18 and 23 were separated by silica gel flash chromatography.

The product mixtures from hydroxalkylation of 18 and 23 were analysed by GLC–MS as their alditol acetates. Using the same alklylation conditions, HO-4 in 23 was alkylated to a greater extent than HO-6 in 18. This was also observed for the 2-acetamido-2-deoxy-D-glucose moiety in hydroxalkylations of sodium hyduronate. This may be due to differences in acidity between HO-4 and HO-6. Hydroxymethylation of 18 gave 19 and a small amount of the hydroxymethoxymethyl derivative. Hydroxymethylation of 18 gave 20 with both the R and the S isomers of the substituted shown by the 13C NMR spectrum. The NMR spectrum indicated a 2-hydroxymethyl substituent as discussed earlier. Compounds 21 and 22 were obtained through hydrogenolysis of 19 and 20, respectively, and identified by comparison of the NMR spectra with that of 2-acetamido-2-deoxy-D-glucose.

The hydroxymethylation of 23 yielded several compounds, mainly 24 and some 25. After hydroxymethylation of 23, 26, with both the R and S forms of the substituent, were found and also in this case the propylene oxide had reacted giving the 2-hydroxymethyl derivative as shown by NMR spectroscopy and as discussed above. The benzyl protecting groups of 24 and 26 were removed by catalytic hydrogenolysis in acetic acid yielding 27 and 28. These compounds were identified by NMR spectroscopy using the 13C NMR data for 2-acetamido-2-deoxy-D-glucose. Compound 25 was not further processed.

**Experimental**

NMR spectra were recorded using a Jeol FX 200 instrument and a Varian Unity 500 instrument with Me$_4$Si ($\delta = 0.00$) as an internal reference. For D$_2$O solutions sodium 3-trimethylsilyltetradecyloproponate, TSP, ($\delta = 0.00$) was used as an internal reference. All spectra were obtained at ambient temperature. Assignments of NMR spectra were made by comparison with reference compounds. TLC was run on Merck precoated silica gel plates and developed by spraying with sulfuric acid (20% v/v) and heating. GLC and GLC–MS data were obtained with a DB 5 column (HP) using the temperature program 100°C (1.0 min)–220°C (12°C min$^{-1}$). Mass
spectra were recorded on a Finnigan MAT INCOS 50 Mass Spectrometer. Elemental analyses were performed by Mikro Kemi AB, Uppsala, Sweden.

3.4.6-Tri-O-benzyl-1,2-O-isopropylidene-α-D-glucose (2). A solution of 1,2-O-isopropylidene-α-D-glucopyranosuronic acid (1) (6 g, 27 mmol) in N,N-dimethylformamide (80 ml) and benzyl bromide (15 ml, 126 mmol) was added dropwise to sodium hydride (5 g) in a glass flask under nitrogen at 0°C. After 60 min, when all 1 had reacted according to TLC (ethyl acetate–ethanol 9:1, Rf 0.88 for 2), methanol (80 ml) was added to destroy the excess of benzyl bromide. Water (200 ml) was added and the product was benzylated. The organic phase was washed with water, dried with sodium sulfate, filtered and concentrated to yield a syrup (13.9 g), which gave one spot on TLC (toluene-ethyl acetate 3:1, Rf 0.65). Traces of benzyl alcohol were separated from the product on a silica gel column (toluene-ethyl acetate 3:1). Yield 10.9 g (82%). 1H NMR (CDCl₃): δ 3.10 and 1.47 (2 s, 6 H, CH₃). 3.64-4.85 (m, 12 H, H₂-H₆ and CH₃Ph), 5.90 (d, 1 H, H₁), 7.18-7.36 (m, 15 H, Ph). 13C NMR (CDCl₃): δ 76.3 and 26.7 (CH₃), 71.3 (C6), 71.9 (C5), 72.6, 73.3 and 75.9 (CH₃Ph), 79.0 (C4), 81.8 (C2) and (C3), 105.1 (C1), 111.7 [O=C(CH₃)₂], 127.3-128.3 (aromatic CH), 137.5, 138.5 and 138.7 (aromatic C). Found: C 73.3; H 7.2. Calc. for C₉₀H₁₃₀O₂: C 73.4; H 7.0.

3.4.6-Tri-O-benzyl-α-D-glucopyranoside (4). Sulfuric acid (0.5 M, 25 ml) was added to 2 (10 g, 20.4 mmol) in dioxane (50 ml) and the solution was refluxed at 100°C. After 4 h the reaction mixture was cooled and saturated sodium hydrogen carbonate was added to neutralize the acid. The product was extracted with dichloromethane. The organic phase was worked up as above and evaporated. Column chromatography (toluene-ethyl acetate 3:2) of the residue yielded crude 3 (5.9 g), the main spot having a TLC Rf value of 0.25 in toluene-acetone 4:1 and 0.14 in ether-petroleum ether 2:1. Benzyl chloride (900 µl, 7.8 mmol) was added to crude 3 (5.5 g) in benzyl alcohol (30 ml, 296 mmol). The mixture was stirred at 60°C for 20 h when 0.5 M sodium hydrogen carbonate was added and the reaction mixture partitioned between dichloromethane and water. The organic phase was washed with water and the solvents were evaporated off. The remaining benzyl alcohol was removed by column chromatography (toluene-ethyl acetate 4:1) to yield 4 (1.0 g, 15%). Rf (toluene-ethyl acetate 9:1) 0.32. 3H NMR (CDCl₃): δ 3.58-4.84 (m, 12 H, H₂-H₆ and CH₃Ph), 5.10 (d, 0.5 H, H1β), 5.44 (s, 0.5 H, H1α), 7.24-7.33 (m, 15 H, Ph). 13C NMR (CDCl₃): δ 70.6 (C2a), 71.0 (C2β), 72.0 (C6a), 72.3 (C6b), 72.5 (C5a), 72.8 (C5b), 73.4, 73.5, 74.3, 76.1, 76.8 and 77.1 (CH₃Ph), 77.9 (C4z), 80.2 (C4b), 82.3 (C3β), 83.6 (C3z), 96.8 (C1z). 1H NMR (CDCl₃): δ 3.64-4.84 (m, 15 H,H₂-H₆ and CH₃Ph), 5.22 (d, 1 H, H1), 7.20-7.42 (m, 20 H, Ph). 13C NMR (CDCl₃): δ 70.0 (C6), 71.2 (C5), 71.6 (C2), 72.6, 73.4, 76.1 and 76.4 (CH₃Ph), 77.8 (C4), 84.0 (C3), 100.2 (C1), 127.3-128.4 (aromatic CH), 137.1-138.9 (aromatic C). Found: C 73.1; H 6.6. Calc. for C₉₂H₁₅₀O₃: C 75.5; H 6.7.

Benzyl 3.4.6-tri-O-benzyl-2-O-2-hydroxyethyl-α-D-glucopyranoside (5) and benzyl 3.4.6-tri-O-benzyl-2-O-[2-hydroxyethoxyethyl]-α-D-glucopyranoside (6). Powered potassium hydroxide (1 g) and ethylene oxide (3.7 ml, 100 equiv.) were added to 4 (400 mg, 0.74 mmol) in N,N-dimethylformamide (23 ml) and water (800 µl). The reaction was stirred at 20°C for 16 h after which the reaction was stopped by addition of water and neutralization with aqueous acetic acid. The solution was extracted with dichloromethane and the organic phase was worked up as before to yield 0.5 g of products. Silica gel chromatography (toluene-ethyl acetate 3:2) gave 5 (188 mg, 43%) and 6 (89 mg, 19%) as syrups. Rf (toluene-ethyl acetate 3:2) 0.39 and 0.31, respectively. 1H NMR (CDCl₃): δ 3.40-4.82 (m, 18 H, H₂-H₆, CH₃Ph, H1 and H2', 5.13 (d, 1 H, H1), 7.15-7.32 (m, 20 H, Ph). 13C NMR (CDCl₃): δ 61.7 (C2'), 69.5 (C6), 71.1 (C5), 72.3 (C1'), 72.5, 73.4 and 76.6 (CH₃Ph), 77.0 (C4) and (CH₃Ph), 82.3 (C3), 85.1 (C2), 99.2 (C1), 127.3-128.3 (aromatic CH), 137.1-138.9 (4 aromatic C). Found: C 69.5; H 6.5. Calc. for C₉₀H₁₃₀O₂: C 73.9; H 6.9.

3.4.6-Tri-O-benzyl-2-O-(2-hydroxypropyl)-α-D-glucopyranoside (7). The preparation of 7 was carried out from 4 (400 mg, 0.74 mmol) essentially as described for 5 and 6 using propylene oxide. Silica gel chromatography yielded 7 and 4, the latter was reacted again following by silica gel chromatography, to yield a total of 190 mg (43%), Rf 0.43 (toluene-ethyl acetate 7:3). 1H NMR (CDCl₃): δ 1.07 (d, 3 H, H3'), 2.83-4.82 (m, 17 H, H₂-H₆, CH₂Ph), H1 and H2', 5.10-5.14 (2 d, 1 H, H1), 7.26-7.31 (m, 20 H, Ph). 13C NMR (CDCl₃): δ 18.1 and 18.2 (C3'), 65.8 and 66.5 (C2'), 69.4 and 69.5 (C6), 71.2 (C5), 72.2 and 72.3 (C1'), 72.5 and 73.4 (CH₃Ph), 76.5, 76.6, 76.8, 76.9 and 77.1 (C4) and (CH₃Ph), 82.1 and 82.2 (C3), 85.2 and 85.3 (C2), 98.9 and 99.3 (C1), 127.2-128.3 (aromatic CH), 137.2-138.9 (aromatic C). Found: C 71.4; H 7.1. Calc. for C₉₂H₁₃₀O₂: C 74.2; H 7.1.

2-O-[2-Hydroxyethyl]-α-D-glucose (8). Hydrogenolysis of 5 (104 mg, 0.18 mmol) in acetic acid (25 ml) with palladium-on-charcoal for 24 h at 2.7 atm gave 8 (17 mg, 43%) after work-up. 1H NMR (D₂O): δ 3.08-4.03 (m, 10 H,
H2-H6, H1' and H2'), 4.71 (d, 0.5 H, H1B), 5.44 (d, 0.5 H, H1x). 13C NMR (D2O): δ 63.4 and 63.6 (C2'), 63.8 and 63.9 (C6), 72.4 (C4B), 72.5 (C4x), 74.1 (C1'x), 74.2 (C5x), 74.9 (C1'B), 76.3 (C3x), 78.2 (C3B), 78.7 (C5B), 82.6 (C2x), 85.6 (C2B), 92.7 (C1z), 98.7 (C1'B). No satisfactory elemental analysis could be obtained.

2-O-(2-Hydroxypropyl)-p-glucose (9). Compound 7 (150 mg, 0.25 mmol) was hydrolysed as described for 5 to yield 9 (80 mg, 84%). 1H NMR (D2O): δ 1.18 (d, 3 H, H3'), 3.10~4.10 (m, 9 H, H2-H6, H1' and H2'). 4.59~4.66 (2 d, 0.5 H, H1B), 5.37 (d, 0.5 H, H1x). 13C NMR (D2O): δ 20.0 and 20.1 (C3'), 62.7 and 62.8 (C6), 68.5, 68.6 and 68.9 (C2'), 71.6~71.7 (3 C, C4), 73.3 and 73.4 (C5x), 72.2 and 74.1 (C3z), 77.4, 77.5, 77.6 and 77.9 (C1'B), 73.9 and 77.8 (C1'x), 81.8 and 82.1 (C2z), 84.8 and 85.2 (C2B), 91.9 and 92.0 (C1z), 97.9 and 98.0 (C1'B). Found: C 45.4; H 7.7. Calc. for C9H16O7: C 45.4; H 7.6.

3-O-(2-Hydroxyethyl)-2,5,6-di-O-isopropylidenex-x-d-glucofuranose (11): 1H NMR (D2O): δ 2.05 and 2.06 (2 d, 0.5 H, H1B), 5.39 (d, 0.5 H, H1x). 13C NMR (D2O): δ 20.8 and 21.2 (C3'), 62.8, 63.5 and 63.6 (C6), 69.6, 69.7 (4 C), 72.2, 72.1 and 72.2 (C4), 78.5, 74.1, 74.3 and 74.2 (C3z) and (C5z), 78.5 and 78.6 (C5B), 80.4, 80.5, 80.6 and 80.7 (C1), 85.0 and 85.1 (C3z), 87.7 and 87.8 (C3B), 94.9 and 95.0 (C1z), 98.6 and 98.7 (C1'B). Found: C 43.3; H 6.9. Calc. for C9H16O7: C 45.4; H 7.6.

Benzyl 2-acetamido-3-O-benzyl-4,6-O-benzylidenex-2-deoxy-x-d-glucopyranoside (16). Benzyl bromide (22.8 ml, 190 mmol) and ground potassium hydroxide (12 g, 0.2 mol) were added to a solution of benzyl 2-acetamido-4,6-O-benzylidenex-2-deoxy-x-d-glucopyranoside (15) (40 g, 10.0 mmol; Fluka) in N,N-dimethylformamide (85 ml) at 70°C. The temperature was slowly raised to 130°C and maintained for 2.5 h. The solution was cooled and poured into ice-water (550 ml). The precipitate formed was removed by filtration, washed with ice-cold water and ethanol to give 16 (3.7 g, 77%). 1H NMR (DMSO-d6): δ 1.97 (s, 3 H, CH3), 3.89~4.95 (m, 12 H, H2-H6, NH, CH2Ph, OCH2Ph), 5.82 (s, 1 H, H1), 7.38~7.54 (m, 15 H, Ph). 13C NMR (DMSO-d6): δ 22.4 (CH3), 52.7 (C2), 62.8 (C5), 67.9 and 68.6 (C6 and CH2Ph), 73.4 (CH2Ph), 76.2 (C3), 81.5 (C4), 97.2 (C1), 102.0 (O2CH2Ph), 125.9~128.7 (aromatic CH), 137.5~138.8 (aromatic C), 169.3 (CO). Found: C 70.5; H 6.3; N 2.7. Calc. for C26H31NO6: C 71.1; H 6.4; N 2.9.
and 8.11 (d, 1 H, H1). 13C NMR (CD3OD): 22.7 (CH3), 54.3 (C2), 62.7 (C6), 70.2 (C4), 72.1 (C5), 74.3 and 75.6 (CH2Ph), 81.6 (C3), 97.8 (C1), 128.4–129.4 (aromatic CH), 138.9 and 140.4 (aromatic C1), 173.3 (CO). Found: C 65.0; H 6.8; N 3.5. Calc. for C22H25NO6: C 65.8; H 6.8; N 3.5.

Benzyl 2-acetamido-3,4-di-O-benzyl-2-deoxy-β-D-glucopyranoside (18) and benzyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranoside (23). Benzyl bromide (310 µl, 2.62 mmol) and ground potassium hydroxide (8.65 g) were added to a solution of 17 (810 mg, 2 mmol) in N,N-dimethylformamide (25 ml). After 30 min at 70°C the temperature was slowly raised to 90°C. After cooling, ice-water was added to the reaction mixture and a product precipitated. The product was washed thoroughly with water and dried. The mixture was shown by TLC (chloroform–ethanol 95:5) to contain 18, 23 and benzyl 2-acetamido-3,6,4-tri-O-benzyl-2-deoxy-α-D-glucopyranoside, Rf 0.43, 0.52 and 0.66, respectively. Silica gel chromatography (chloroform–ethanol 95:5) gave 180 mg (18%), 210 mg (21%) and 292 mg (25%) respectively.

18: 1H NMR (CDCl3): 1.79 (s, 3 H, CH3), 3.67–4.88 (13 H, H2–H6, NH and CH2Ph), 5.34 (d, 1 H, H1), 7.24–7.38 (15, 15 H, Ph). 13C NMR (CDCl3): 23.2 (CH3), 52.6 (C2), 61.6 (C6), 69.6 (C5), 71.8, 74.7 and 75.1 (CH2Ph), 78.2 (C4), 80.0 (C3), 97.1 (C1), 127.7–128.5 (aromatic CH), 137.0–138.3 (aromatic C), 169.7 (CO). Found: C 70.7; H 6.9; N 2.8. Calc. for C23H26NO6: C 70.8; H 6.8; N 2.8.

Benzyl 2-acetamido-3,4-di-O-benzyl-2-deoxy-6-O-(2-hydroxyethyl)-β-D-glucopyranoside (19). Compound 19 was prepared from 18 (250 mg, 0.51 mmol) as described for 5 and 6. The organic phase was worked up before to yield a syrup, 290 mg, shown on TLC (chloroform–2-propanol 9:1) to consist of one product in addition to 18, Rf 0.50 (18) and 0.43 (19). The products, analysed as the corresponding alditol acetates by GLC–MS, was shown to be 18 (75%), 19 (21%) and the 2-(2-hydroxyethoxy)ethyl derivative (4%). The compounds were separated on a silica gel column using the solvents above and 19 (26 mg, 10%) was obtained. 1H NMR (CDCl3): 1.80 (s, 3 H, CH3), 3.55–4.88 (m, 17 H, H2–H6, H1', H2', NH and CH2Ph), 5.35 (d, 1 H, H1), 7.25–7.63 (15, 15 H, Ph). 13C NMR (CDCl3): 23.3 (CH3), 52.5 (C2), 61.8 (C2'), 69.8 (C5) and (C6), 71.2 (CH2Ph), 72.8 (C1'), 74.8 and 75.1 (CH2Ph), 78.4 (C4), 80.3 (C3), 97.2 (C1), 127.7–128.5 (aromatic CH), 137.1, 138.0 and 138.3 (aromatic C), 169.7 (CO). Found: C 69.0; H 6.9; N 2.5. Calc. for C23H26NO6: C 69.5; H 7.0; N 2.6.

2-Acetamido-2-deoxy-6-O-(2-hydroxyethyl)-β-D-glucopyranose (21). Compound 19 (20 mg, 0.04 mmol) was hydrolysed as described for 5 to yield 21 (9 mg, 91%). 1H NMR (D2O): δ 2.13 (s, 3 H, CH3), 3.58–3.98 (m, 11 H, H2–H6, H1', H2' and NH), 4.77 (d, 0.5 H, H1β), 5.27 (d, 0.5H, H1α). 13C NMR (D2O): δ 24.7 (CH2), 25.0 (CHβ), 56.9 (C2α), 59.5 (C2β), 63.2 (C2'), 72.3 (C6α), 72.5 (C6β), 72.8 (C4β), 73.0 (C4α), 73.2 (C3α), 75.0 (C5α), 75.1 (C1β), 76.7 (C3β), 77.6 (C5β), 93.7 (C1α), 97.8 (C1β). Found: C 43.5; H 6.9; N 4.6. Calc. for C9H14NO5: C 45.3; H 7.2; N 5.3.

Benzyl 2-acetamido-3,4-di-O-benzyl-2-deoxy-6-O-(2-hydroxypropyl)-β-D-glucopyranoside (20). The preparation of 20 was carried out mainly as described for 7 but the alkylation was repeated twice to increase the yield. The residue contained, according to TLC (dichloromethane–2-propanol 9:1), one product with an Rf value of 0.45 in addition to the starting material. The product mixture, analysed by GLC–MS as above, contained 18 (76%), and 20 (24%). The compounds were fractionated (dichloromethane–2-propanol 9:1 and 19:1) and obtained by H NMR (CDCl3): δ 1.14 (d, 3 H, H3'), 3.20–4.95 (m, 16 H, H2–H6, NH, H1', H2', CH2Ph), 5.28–5.32 (m, 1 H, H1), 7.30–7.44 (m, 15 H, Ph). 13C NMR (CDCl3): δ 18.6 (C3'), 23.4 (CH3), 52.5 (C2), 66.4 and 66.5 (C2'), 69.7 and 70.0 (C5) and (C6), 71.2, 74.9 and 75.1 (CH2Ph), 73.0 (C3'), 78.4 (C4), 80.4 (C3), 97.2 (C1), 127.8–128.5 (aromatic CH), 137.1–138.4 (aromatic C), 169.6 (CO). Found: C 69.8; H 6.6; N 2.6. Calc. for C23H30NO6: C 69.9; H 7.2; N 2.6.

2-Acetamido-2-deoxy-6-O-(2-hydroxypropyl)-β-D-glucopyranose (22). Compound 22 (5 mg, 66%) was obtained through hydrolysis of 20 (15 mg, 0.03 mmol) as described for 5. 1H NMR (D2O): δ 1.13 (d, 3 H, H3'), 2.03 (d, 3 H, CH3), 3.30–4.10 (m, 10 H, H2–H6, NH, H1', H2'), 5.17 (d, 0.5 H, H1α), H1β under HDO peak. 13C NMR (D2O): δ 20.9 (C3'), 24.7 (CH2), 25.0 (CHβ), 56.8 (C2α), 59.5 (C2β), 68.9 and 69.0 (C2'), 72.6, 72.7, 73.0, 73.1, 73.3 and 73.5 (C3α, C4α, C5α, C6α, C6β), 76.7 (C3β), 77.6 (C5β), 79.0 and 79.1 (C1α), 93.6 (C1α), 97.8 (C1β), 177.3 (CO α), 177.6 (CO β). A small extraneous signal at 28.2 ppm was also observed. Found: C 44.3; H 7.1; N 4.4. Calc. for C17H21NO5: C 47.3; H 7.6; N 5.0.

Benzyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-4-O-(2-hydroxyethyl)-β-D-glucopyranoside (24) and benzyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-4-O-[2-(2-hydroxyethoxymethyl)-β-D-glucopyranoside (25). The hydrolylation of 23 (280 mg, 0.57 mmol) was carried out as described for 4. After evaporation of the solvent the remaining syrup (360 mg) was shown to contain three products in addition to 23. The compounds, according to GLC–MS as above, were 23 (18%), 24 (74%), 25 (17%) and trace amounts of the 2-(2-hydroxyethoxymethyl) derivative.
PREPARATION OF GLC AND GCN DERIVATIVES

(1%). The products were separated by column chromatography (ethyl acetate–toluene 3:1) to yield 24 (100 mg, 33%) and 25 (30 mg, 9%) having TLC Rf values of 0.33 and 0.20, respectively. 24: 1H NMR (CDCl3): δ 1.78 (s, 3 H, CH3), 3.58–4.87 (m, 17 H, H2–H6, NH, H1′, H2′ and CH2Ph), 5.45 (d, 1 H, H1), 7.24–7.39 (m, 15 H, Ph). 13C NMR (CDCl3): δ 23.2 (CH2), 52.4 (C2), 62.3 (C2′), 68.4 (C5), 69.5 (CH2Ph), 71.2 (C6), 73.4 (CH3Ph), 73.9 (C1′), 74.8 (CH2Ph), 78.6 (C3), 80.2 (C4), 97.1 (C1), 127.6–128.4 (aromatic CH), 137.0–138.0 (aromatic C), 169.6 (CO). Found: C 69.4; H 7.0; N 2.8. Calc. for C31H36NO4: C 68.4; H 7.1; N 2.4.

Benzyl 2-acetamido-1,6-di-O-benzyl-2-deoxy-4-O-(2-hydroxypropyl)-α-D-glucopyranoside (26). The synthesis of 26 was performed from 23 (220 mg, 0.45 mmol) as described for 7. The syrup obtained (460 mg) contained, according to TLC (ethyl acetate–toluene 3:1), 26, Rf 0.33, in addition to the starting material. Analysis of the mixture as their aildot acetates showed 85% and 15% of 23 and 26, respectively. The products were separated by chromatography on silica gel using the solvent system above to give 26 (31 mg, 13%). 1H NMR (CDCl3): δ 0.94 (d, 3 H, H3′), 1.78 (s, 3 H, CH3), 3.26–4.87 (m, 16 H, H2–H6, NH, CH2Ph), 5.34–5.38 (2 d, 1 H, H1), 7.31–7.34 (m, 15 H, Ph). 13C NMR (CDCl3): δ 18.4 and 18.5 (C3′), 32.3 (CH3), 52.4 (C2), 66.9 and 67.1 (C2′), 68.5 and 68.6 (C5), 69.6 (CH2Ph), 71.2 and 71.3 (C6), 73.5, 74.7 and 74.8 (CH2Ph), 78.2 (C1′), 78.6 and 78.7 (C3), 80.3 and 80.4 (C4), 97.2 (C1′), 127.7–128.5 (aromatic CH), 137.2–138.0 (aromatic C), 169.6 (CO). Found: C 69.7; H 7.4; N 2.6. Calc. for C31H36NO4: C 68.4; H 7.1; N 2.4.

2-Acetamido-2-deoxy-4-O-(2-hydroxyethyl)-α-D-glucose (27). Compound 24 (80 mg, 0.15 mmol) was hydrolysed in the same way as 5. After evaporation of the solvent and drying, 27 (33 mg, 83%) was obtained. 1H NMR (D2O): δ 2.04 (s, 3 H, CH3), 3.38–3.96 (m, 11 H, H2–H6, NH, H1′ and H2′), 4.69 (d, 0.5 H, H1β), 5.18 (d, 0.5 H, H1β). 13C NMR (D2O): δ 24.7 (CH2α), 25.0 (CH2β), 57.0 (C2α), 59.6 (C2β), 63.2 (C6α), 63.3 (C6β), 63.8 (C2′), 73.4 (C3α), 73.5 (C5α), 76.5 (C1′), 76.6 (C3β), 77.9 (C5β), 81.3 (C4β), 81.6 (C4α), 93.6 (C1α), 97.7 (C1β), 177.3 (CO α), 177.6 (CO β). Found: C 45.1; H 7.4; N 5.1. Calc. for C19H19NO4: C 45.3; H 7.2; N 5.3.

2-Acetamido-2-deoxy-4-O-(2-hydroxypropyl)-α-D-glucose (28). The hydrogenolysis of 26 (26 mg, 0.05 mmol) was performed as described for 5 to yield 28 (12 mg, 91%). 1H NMR (D2O): δ 1.15 (d, 3 H, H3′), 2.05 (s, 3 H, CH3), 3.30–4.10 (m, 10 H, H2–H6, NH, H1′, H2′, 4.69 (d, 0.5 H, H1β), 5.19 (d, 0.5 H, H1α). 13C NMR (D2O): δ 20.8 (C3′), 20.9 (C3′), 24.7 (CH2α), 25.0 (CH2β), 57.0 (C2α), 59.6 (C2β), 63.2 (C6α), 63.3 (C6β), 69.4 (C2′), 69.7 (C2′), 73.3, 73.4, 73.5 and 73.6 (C3α) and (C5α), 76.5 and 76.6 (C3β), 77.9 and 78.0 (C5β), 80.3, 80.4–80.5 (3 C, C′), 81.3, 81.5, 81.6 and 81.9 (C4), 93.6 (C1α), 97.7 (C1β), 177.3 (CO α), 177.6 (CO β). Found: C 44.1; H 6.7; N 4.0. Calc. for C21H21NO4: C 47.3; H 7.6; N 5.0.

References


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