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# 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 hydroxyethylated 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,[1] 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,[2] 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 fixating 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.

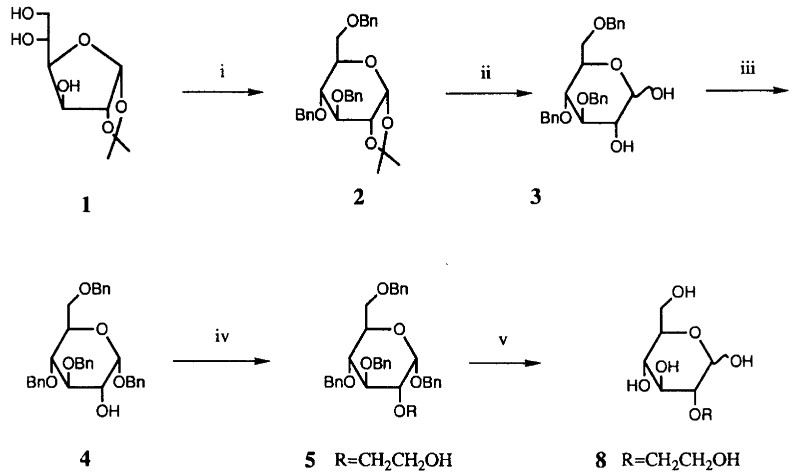
One way of obtaining hyaluronate gels is to react it with a bifunctional epoxy compound, such as 1,2:3,4-diepoxybutane,[3] 1,4-butanediol diglycidyl ether,[4] or epichlorohydrin, to give ether linkages that are reasonably stable under physiological conditions. Relatively stable crosslinks can also be introduced using divinyl sulfone.[4,5]

To gain knowledge of the substitution pattern of alkylated hyaluronate[6] 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-*O*-(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.[7,8] The GLC–MS analysis of alkylated hyaluronate will be reported separately.[6]

## Results and discussion

The 2-*O*-(2-hydroxyalkyl) derivatives of D-glucose were made from 1,2-*O*-isopropylidene-α-D-glucofuranose in five steps (Scheme 1). Compound **2** was hydrolysed with sulfuric acid in dioxane–water and crude **3**, with an $R_f$ value well in accordance with that reported earlier,[9] was used in the next step after separation of the starting ma-



1 2 3

4

5 R=$CH_2CH_2OH$
6 R=$(CH_2CH_2O)_2H$
7 R=$CH_2CH(OH)CH_3$

8 R=$CH_2CH_2OH$
9 R=$CH_2CH(OH)CH_3$

*Scheme 1.* i, BnBr–DMF–NaH; ii, dioxane–$H_2SO_4$(aq); iii, BnOH–BzCl; iv, ethylene or propylene oxide–DMF–$H_2O$–KOH; v, Pd–C–$H_2$(g).

terial. 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.*[10] 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 trace amounts of the 2-*O*-[2-(2-hydroxypropoxy)propyl] derivative were obtained.

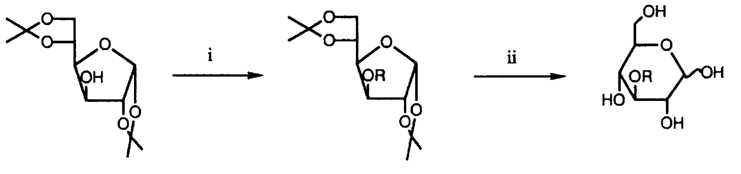
Compounds **5** and **7** were treated with Pd–$H_2$ to give **8** and **9** which were identified by NMR spectroscopy using data of Reuben[7] and Lee *et al.*,[8] respectively. It was observed that for dilute solutions it was possible to retain the glycosidic benzyl group. Compound **6** was not processed 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.[11] 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.[8]

The 3-*O*-(2-hydroxyalkyl) derivatives of D-glucose were prepared by alkylation of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**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)

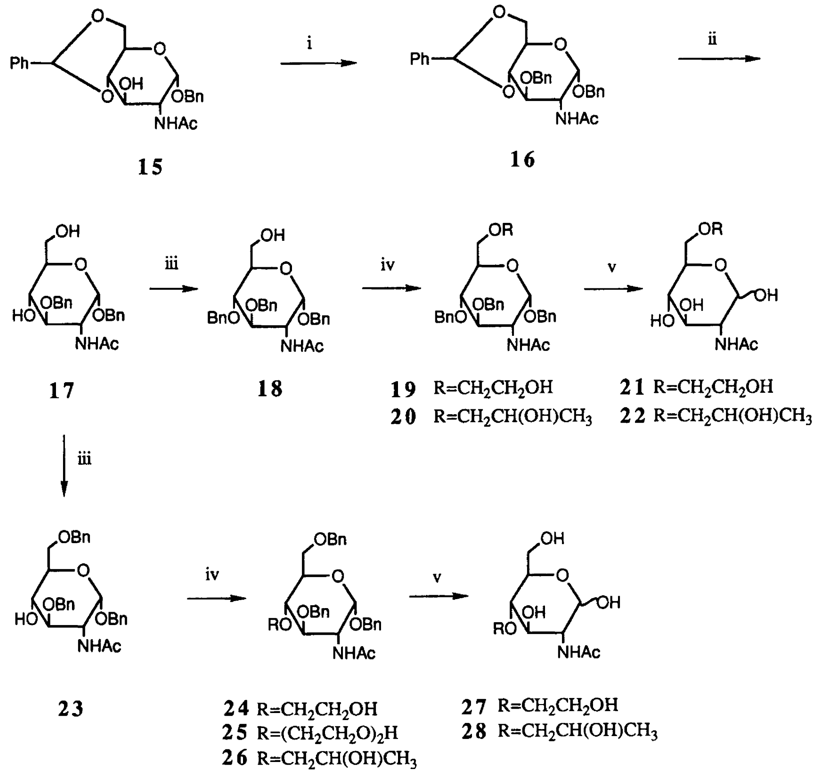


10

11 R=$CH_2CH_2OH$
12 R=$CH_2CH(OH)CH_3$

13 R=$CH_2CH_2OH$
14 R=$CH_2CH(OH)CH_3$

*Scheme 2.* i, Ethylene or propylene oxide–DMF–$H_2O$–KOH; ii, $H_2SO_4$(aq.).




*Scheme 3.* i, BnBr–DMF–KOH; ii, HOAc (conc.)–$H_2O$; iii, BnBr–DMF–KOH; iv, ethylene or propylene oxide–DMF–$H_2O$–KOH; v, Pd–C–$H_2$(g).

was performed essentially as described[13] 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.[14] Compounds **18** and **23** were separated by silica gel flash chromatography.

The product mixtures from hydroxyalkylation of **18** and **23** were analysed by GLC–MS as their alditol acetates. Using the same alkylation 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 hydroxyalkylations of sodium hyaluronate.[6] This may be due to differences in acidity between HO-4 and HO-6. Hydroxyethylation of **18** gave **19** and a small amount of the hydroxyethoxyethyl derivative. Hydroxypropylation of **18** gave **20** with both the *R* and the *S* isomers of the substituent shown by the $^{13}C$ NMR spectrum. The NMR spectrum indicated a 2-hydroxypropyl 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.[15] The hydroxyethylation of **23** yielded several compounds, mainly **24** and some **25**. After hydroxypropylation 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-hydroxypropyl 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 $^{13}C$ NMR data for 2-acetamido-2-deoxy-D-glucose.[15] Compound **25** was not further processed.

## Experimental

NMR spectra were recorded using a Jeol FX 200 instrument and a Varian Unity 500 instrument with $Me_4Si$ ($\delta = 0.00$) as an internal reference. For $D_2O$ solutions sodium 3-trimethylsilyltetradeuteriopropionate, 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° $min^{-1}$). 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-glucofuranose (**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, $R_f$ 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 partitioned between toluene and water. 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, $R_f$ 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%). $^1$H NMR ($CDCl_3$): δ 1.30 and 1.47 (2 s, 6 H, $CH_3$), 3.64–4.85 (m, 12 H, H2–H6 and $CH_2$Ph), 5.90 (d, 1 H, H1), 7.18–7.36 (m, 15 H, Ph). $^{13}$C NMR ($CDCl_3$): δ 26.3 and 26.7 ($CH_3$), 71.3 (C6), 71.9 (C5), 72.6, 73.3 and 75.5 ($CH_2$Ph), 79.0 (C4), 81.8 (C2) and (C3), 105.1 (C1), 111.7 [$O_2C(CH_3)_2$], 127.3–128.3 (aromatic CH), 137.5, 138.5 and 138.7 (aromatic C). Found: C 73.3; H 7.2. Calc. for $C_{30}H_{34}O_6$: C 73.4; H 7.0.

*3,4,6-Tri-O-benzyl-D-glucose* (**3**) *and benzyl 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 $R_f$ value of 0.25 in toluene–acetone 4:1 and 0.14 in ether–petroleum ether 2:1.[9] Benzoyl 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%), $R_f$ (toluene–ethyl acetate 9:1) 0.32. **3**: $^1$H NMR ($CDCl_3$); δ 3.58–4.84 (m, 12 H, H2-H6, $CH_2$Ph), 5.10 (d, 0.5 H, H1β), 5.44 (s, 0.5 H, H1α), 7.24–7.33 (m, 15 H, Ph). $^{13}$C NMR ($CDCl_3$): δ 70.6 (C2α), 71.0 (C2β), 72.0 (C6α), 72.3 (C6β), 72.5 (C5α), 72.8 (C5β), 73.4, 73.5, 74.3, 76.1, 76.8 and 77.1 ($CH_2$Ph), 77.9 (C4α), 80.2 (C4β), 82.3 (C3β), 83.6 (C3α), 96.8 (C1α), 103.5 (C1β), 127.4–128.7 (aromatic CH), 136.9–138.7 (6 aromatic C). Found: C 70.8; H 7.0. Calc. for $C_{27}H_{30}O_6$: C 72.0; H 6.7. **4**: $^1$H NMR ($CDCl_3$): δ 3.64–4.84 (m, 15 H,H2–H6 and $CH_2$Ph), 5.22 (d, 1 H, H1), 7.20–7.42 (m, 20 H, Ph). $^{13}$C NMR ($CDCl_3$)[10]: δ 70.0 (C6), 71.2 (C5), 71.6 (C2), 72.6, 73.4, 76.1 and 76.4 ($CH_2$Ph), 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 $C_{34}H_{36}O_6$: 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-(2-hydroxyethoxy)ethyl]-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, $R_f$ (toluene–ethyl acetate 3:2) 0.39 and 0.31, respectively. **5**: $^1$H NMR ($CDCl_3$): δ 3.40–4.82 (m, 18 H, H2–H6, $CH_2$Ph, H1′ and H2′), 5.13 (d, 1 H, H1), 7.15–7.32 (m, 20 H, Ph). $^{13}$C NMR ($CDCl_3$): δ 61.7 (C2′), 69.5 (C6), 71.1 (C5), 72.3 (C1′), 72.5, 73.4 and 76.6 ($CH_2$Ph), 77.0 (C4) and ($CH_2$Ph), 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 $C_{36}H_{40}O_7$: C 73.9; H 6.9. **6**: $^1$H NMR ($CDCl_3$): δ 3.54–4.81 (m, 22 H, H2–H6, H1′–H4′ and $CH_2$Ph), 5.12 (d, 1 H, H1), 7.23–7.48 (m, 20 H, Ph). $^{13}$C NMR ($CDCl_3$): δ 61.7 (C4′), 69.3 (C6), 70.2 (C3′), 70.4 (C2′), 71.3 (C5), 72.2 ($CH_2$Ph), 72.5 (C1′), 73.4 ($CH_2$Ph), 76.7 ad 77.0 (C4 and 2 $CH_2$Ph), 82.0 (C3), 85.2 (C2), 99.1 (C1), 127.3–128.3 (aromatic CH), 137.6–138.9 (4 aromatic C). Found: C 67.2; H 6.7. Calc. for $C_{38}H_{44}O_8$; C 72.6; H 7.0.

*Benzyl 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 followed by silica gel chromatography, to yield a total of 190 mg (43%), $R_f$ 0.43 (toluene–ethyl acetate 7:3). $^1$H NMR ($CDCl_3$): δ 1.07 (d, 3 H, H3′), 2.83–4.82 (m, 17 H, H2–H6, $CH_2$Ph), H1′ and H2′), 5.10–5.14 (2 d, 1 H, H1), 7.26–7.31 (m, 20 H, Ph). $^{13}$C NMR ($CDCl_3$): 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 ($CH_2$Ph), 76.5, 76.6, 76.8, 76.9 and 77.1 (C4) and ($CH_2$Ph), 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 $C_{37}H_{42}O_7$: C 74.2; H 7.1.

*2-O-(2-Hydroxyethyl)-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. $^1$H NMR ($D_2O$): δ 3.08–4.03 (m, 10 H, H2-H6, H1′ and H2′), 4.71 (d, 0.5 H, H1β), 5.44 (d, 0.5 H, H1α). $^{13}$C NMR ($D_2O$):[7] δ 63.4 and 63.6 (C2′), 63.8 and 63.9 (C6), 72.4 (C4β), 72.5 (C4α), 74.1 (C1′α), 74.2 (C5α), 74.9 (C1′β), 76.3 (C3α), 78.2 (C3β), 78.7 (C5β), 82.6 (C2α), 85.6 (C2β), 92.7 (C1α), 98.7 (C1β). No satisfactory elemental analysis could be obtained.

*2-O-(2-Hydroxypropyl)-D-glucose* (**9**). Compound **7** (150 mg, 0.25 mmol) was hydrogenolysed as described for **5** to yield **9** (50 mg, 84%). $^1$H NMR ($D_2O$): 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, H1β), 5.37 (d, 0.5 H, H1α). $^{13}$C NMR ($D_2O$)[8]: 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 (C5α), 72.2 and 74.1 (C3α), 77.4, 77.5, 77.6 and 77.9 (C1′β), (C3β) and (C5β), 79.4 and 79.8 (C1′α), 81.8 and 82.1 (C2α), 84.8 and 85.2 (C2β), 91.9 and 92.0 (C1α), 97.9 and 98.0 (C1β). Found: C 45.4; H 7.7. Calc. for $C_9H_{18}O_7$: C 45.4; H 7.6.

*3-O-(2-Hydroxyethyl)-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose*(**11**). 1,2:5,6-Di-*O*-isopropylidene-α-D-glucofuranose (**10**) (500 mg, 1.92 mmol) was hydroxylated essentially as described for **4** using 30 equivalents of ethylene oxide. The reaction mixture consisted of, according to GLC analysis of the corresponding alditol acetates,[16] the starting material (5%), compound **11** (66%), 3-*O*-[2-(2-hydroxyethoxy)ethyl]-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (25%) and 3-*O*-{2-[2-(2-hydroxyethoxy)-ethoxy]ethyl}-1,2:5,6-isopropylidene-α-D-glucofuranose (4%). Compound **11** (230 mg) was isolated by silica gel chromatography in ethyl acetate, $R_f$ 0.44. $^1$H NMR: ($CD_3OD$): δ 1.40, 1.42, 1.49 and 1.54 (4 s, 12 H, $CH_3$), 3.67–4.74 (m, 10 H, H2–H6, H1′ and H2′), 5.95 (d, 1 H, H1). $^{13}$C NMR ($CD_3OD$): δ 25.8, 26.7, 27.3 and 27.4 ($CH_3$), 62.5 (C2′), 68.2 (C6), 73.3 (C5), 74.4 (C1′), 82.6 (C4), 84.1 (C2) and (C3), 107.0 (C1), 110.3 and 113.2 [$O_2C(CH_3)_2$]. Found: C 54.8; H 7.9. Calc. for $C_{14}H_{24}O_7$: C 55.2; H 8.2].

*3-O-(2-Hydroxypropyl)-1,2:5,6-di-O-isopropylidene-α-D-glucofuranose* (**12**). Compound **12** was prepared from 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**10**) as described for **7**. The solvent was evaporated off to yield a syrup (550 mg). The reaction mixture contained, according to GLC analysis of the corresponding alditol acetates, starting material (19%), (**12**) (80%) and 3-*O*-[2-(2-hydroxypropoxy)propyl-1,2:5,6-di-*O*-isopropylidene-D-glucose (1%). Some **12** (220 mg) was isolated by silica gel chromatography in ethyl acetate, $R_f$ 0.52 (ethyl acetate). $^1$H NMR ($CDCl_3$): δ 1.12 (t, 3 H, H3′), 1.32, 1.37, 1.44 and 1.50 (4 s, 12 H, $CH_3$), 3.40–4.56 (m, 9 H, H2-H6, H1′ and H2′), 5.89–5.92 (2 d, 1 H, H1). $^{13}$C NMR ($CDCl_3$): δ 17.7 and 18.3 (C3′), 25.1, 26.2, 26.7 and 26.8 ($CH_3$), 64.36 and 67.2 (C2′), 67.8 and 67.9 (C6), 72.8 and 72.9 (C5), 74.8 and 77.1 (C1′), 81.2 and 81.3 (C4), 82.5 and 82.6 (C2), 84.5 (C3), 105.60 and 105.62 (C1), 109.4 and 111.9 [$O_2C(CH_3)_2$]. An extraneous signal at 81.4 ppm was also observed. Found: C 56.1; H 8.2. Calc. for $C_{15}H_{26}O_7$: C 56.6; H 8.2.

*3-O-(2-Hydroxyethyl)-D-glucose* (**13**). Compound **11** (138 mg, 0.45 mmol) was treated with 1% sulfuric acid (2.75 ml) at 100°C for 1 h. After removal of the acid as its barium salt the solution was evaporated to yield **13** (92 mg, 90%). $^1$H NMR ($D_2O$): δ 3.25–3.94 (m, 10 H, H2–H6, H′1 and H′2), 4.64 (d, 0.5 H, H1β), 5.20 (d, 0.5 H, H1α). $^{13}$C NMR ($D_2O$)[7]: δ 63.3 (C2′α), 63.5 (C2′β), 63.9 (C6), 72.0 (C4β), 72.1 (C4α), 74.0 (C2α), 74.2 (C5α), 76.4 (C1′α), 76.5 (C1′β), 76.6 (C2β), 78.6 (C5β), 84.8 (C3α), 87.5 (C3β), 94.9 (C1α) and 98.6 (C1β). Found: C 40.2; H 6.8. Calc. for $C_8H_{16}O_7$: C 42.8; H 7.2.

*3-O-(2-Hydroxypropyl)-D-glucose* (**14**). The hydrolysis of **12** (105 mg, 0.33 mmol) was performed as described for **11** to give **14** (59 mg, 75%). $^1$H NMR ($D_2O$): δ 1.14 (d, 3 H, H3′), 3.34–3.91 (m, 9 H, H2–H6, H1′ and H2′), 4.64 (d, 0.5 H, H1β), 5.21 (d, 0.5 H, H1α). $^{13}$C NMR ($D_2O$)[8]: δ 20.8 and 21.2 (C3′), 63.4 and 63.5 (C6), 69.6–69.7 (4 C, C2′), 72.0, 72.1 and 72.2 (C4), 74.0, 74.1, 74.2 and 74.3 (C2α) and (C5α), 76.6 and 76.7 (C2β), 78.5 and 78.6 (C5β), 80.4, 80.5, 80.6 and 80.7 (C1′), 85.0 and 85.1 (C3α), 87.7 and 87.8 (C3β), 94.9 and 95.0 (C1α), 98.6 and 98.7 (C1β). Found: C 43.3; H 6.9. Calc. for $C_9H_{18}O_7$: C 45.4; H 7.6.

*Benzyl 2-acetamido-3-O-benzyl-4,6-O-benzylidene-2-deoxy-α-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*-benzylidene-2-deoxy-α-D-glucopyranoside (**15**) (4.0 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** (3.78 g, 77%). $^1$H NMR (DMSO-$d_6$): δ 1.97 (s, 3 H, $CH_3$), 3.89–4.95 (m, 12 H, H2–H6, NH, $CH_2$Ph, $O_2CH$Ph), 5.82 (s, 1 H, H1), 7.38–7.54 (m, 15 H, Ph). $^{13}$C NMR (DMSO-$d_6$): δ 22.4 ($CH_3$), 52.7 (C2), 62.8 (C5), 67.9 and 68.8 (C6) and ($CH_2$Ph), 73.4 ($CH_2$Ph), 76.2 (C3), 81.5 (C4), 97.2 (C1), 100.2 ($O_2CH$Ph), 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 $C_{29}H_{31}NO_6$: C 71.1; H 6.4; N 2.9.

*Benzyl 2-acetamido-3-O-benzyl-2-deoxy-α-D-glucopyranoside* (**17**). To **16** (1.2 g, 2.45 mmol) in acetic acid (40 ml) at 100°C was added water (25 ml) in 5 ml portions.[17] After 30 min, the solution was cooled and evaporated. Treatment of the residue with sodium methoxide in methanol, ion exchange with Dowex 50W-X8($H^+$) and concentration of the solution yielded **17** (890 mg, 90%) as a syrup, $R_f$ 0.2 (chloroform–ethanol 95:5). $^1$H NMR ($CD_3OD$): δ 1.89 (s, 3 H, $CH_3$), 3.56–3.87 (m, 11 H, H2–H6, NH and $CH_2$Ph), 7.24–7.42 (m, 10 H, Ph), 8.06

and 8.11 (d, 1 H, H1). $^{13}$C NMR ($CD_3OD$): δ 22.7 ($CH_3$), 54.3 (C2), 62.7 (C6), 70.2 (C4), 72.1 (C5), 74.3 and 75.6 ($CH_2$Ph), 81.6 (C3), 97.8 (C1), 128.4–129.4 (aromatic CH), 138.9 and 140.4 (aromatic C),173.3 (CO). Found: C 65.0; H 6.8; N 3.5. Calc. for $C_{22}H_{27}NO_6$: C 65.8; H 6.8; N 3.5.

*Benzyl 2-acetamido-3,4-di-O-benzyl-2-deoxy-α-D-glucopyranoside* (**18**) *and benzyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-α-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-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranoside, $R_f$ 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**: $^1$H NMR ($CDCl_3$): δ 1.79 (s, 3 H, $CH_3$), 3.67–4.88 (13 H, H2–H6, NH and $CH_2$Ph), 5.34 (d, 1 H, H1), 7.24–7.38 (m, 15 H, Ph). $^{13}$C NMR ($CDCl_3$): δ 23.2 ($CH_3$), 52.6 (C2), 61.6 (C6), 69.6 (C5), 71.8, 74.7 and 75.1 ($CH_2$Ph), 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 $C_{29}H_{33}NO_6$: C 70.8; H 6.8; N 2.8. **23**: $^1$H NMR ($CDCl_3$): δ 1.83 (s, 3 H, $CH_3$), 2.93–4.89 (m, 13 H, H2–H6, NH and $CH_2$Ph), 5.45 (d, 1 H, H1), 7.24–7.38 (m, 15 H, Ph). $^{13}$C NMR ($CDCl_3$): δ 23.2 ($CH_3$), 51.8 (C2), 69.5 (C5), 70.1 and 70.5 (C4) and ($CH_2$Ph), 72.0 (C6), 73.6 and 73.7 ($CH_2$Ph), 79.9 (C3), 97.1 (C1), 127.6–128.4 (aromatic CH), 137.1–138.5 (aromatic C), 169.7 (CO). Found: C 70.8; H 6.7; N 2.6. Calc. for $C_{29}H_{33}NO_6$: C 70.8; H 6.8; N 2.8.

*Benzyl 2-acetamido-3,4-di-O-benzyl-2-deoxy-6-O-(2-hydroxyethyl)-α-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 as before to yield a syrup, 290 mg, shown on TLC (chloroform–2-propanol 9:1) to consist of one product in addition to **18**, $R_f$ 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. $^1$H NMR ($CDCl_3$): δ 1.80 (s, 3 H, $CH_3$), 3.55–4.88 (m, 17 H, H2–H6, H1′, H2, NH and $CH_2$Ph), 5.35 (d, 1 H, H1), 7.25–7.63 (m, 15 H, Ph). $^{13}$C NMR ($CDCl_3$): δ 23.3 ($CH_3$), 52.5 (C2), 61.8 (C2′), 69.8 (C5) and (C6), 71.2 ($CH_2$Ph), 72.8 (C1′), 74.8 and 75.1 ($CH_2$Ph), 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 $C_{31}H_{37}NO_7$: C 69.5; H 7.0; N 2.6.

*2-Acetamido-2-deoxy-6-O-(2-hydroxyethyl)-D-glucose* (**21**). Compound **19** (20 mg, 0.04 mmol) was hydrogenolysed as described for **5** to yield **21** (9 mg, 91%). $^1$H NMR ($D_2O$): δ 2.13 (s, 3 H, $CH_3$), 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). $^{13}$C NMR ($D_2O$): δ 24.7 ($CH_3$α), 25.0 ($CH_3$β), 56.9 (C2α), 59.5 (C2β), 63.2 (C2′), 72.3 (C6α), 72.5 (C6β), 72.8 (C4β), 73.0 (C4α), 73.2 (C3α), 73.5 (C5α), 75.0 (C1′α), 75.1 (C1′β), 76.7 (C3β), 77.6 (C5β), 93.7 (C1α), 97.8 (C1β). Found: C 43.5; H 6.9; N 4.6. Calc. for $C_{10}H_9NO_7$: C 45.3; H 7.2; N 5.3.

*Benzyl 2-acetamido-3,4-di-O-benzyl-2-deoxy-6-O-(2-hydroxypropyl)-α-D-glucopyranoside* (**20**). The preparation of **20** from **18** (145 mg, 0.30 mmol) 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 $R_f$ 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 **20** (17 g, 10%) was obtained. $^1$H NMR ($CDCl_3$): δ 1.14 (d, 3 H, H3′), 3.20–4.95 (m, 16 H, H2–H6, NH, H1′, H2′,$CH_2$Ph), 5.28–5.32 (m, 1 H, H1), 7.30–7.44 (m, 15 H, Ph). $^{13}$C NMR ($CDCl_3$): δ 18.6 (C3′), 23.4 ($CH_3$), 52.5 (C2), 66.4 and 66.5 (C2′), 69.7 and 70.0 (C5) and (C6), 71.2, 74.9 and 75.1 ($CH_2$Ph), 77.3 (C1′), 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 $C_{32}H_{39}NO_7$: C 69.9; H 7.2; N 2.6.

*2-Acetamido-2-deoxy-6-O-(2-hydroxypropyl)-D-glucose* (**22**). Compound **22** (5 mg, 66%) was obtained through hydrogenolysis of **20** (15 mg, 0.03 mmol) as described for **5**. $^1$H NMR ($D_2O$): δ 1.13 (d, 3 H, H3′), 2.03 (d,3 H, $CH_3$), 3.30–4.10 (m, 10 H, H2–H6, NH, H1′, H2′), 5.17 (d, 0.5 H, H1α). H1β under HDO peak. $^{13}$C NMR ($D_2O$): δ 20.9 (C3′), 24.7 ($CH_3$α), 25.0 ($CH_3$β), 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, C4β, C5α, C6α, C6β), 76.7 (C3β), 77.6 (C5β), 79.0 and 79.1 (C1′), 93.6 (C1α), 97.8 (C1β), 177.3 (CO α), 177.6 (CO β). A small extraneous signal at 72.8 ppm was also observed. Found: C 44.3; H 7.1; N 4.4. Calc. for $C_{11}H_{21}NO_7$: C 47.3; H 7.6; N 5.0.

*Benzyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-4-O-(2-hydroxyethyl)-α-D-glucopyranoside* (**24**) *and benzyl 2-acetamido-3,6-di-O-benzyl-2-deoxy-4-O-[2-(2-hydroxyethoxy)-ethyl]-α-D-glucopyranoside* (**25**). The hydroxyethylation 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-(2-hydroxyethoxy)ethoxy]ethyl derivative

(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 $R_f$ values of 0.33 and 0.20, respectively. **24**: $^1$H NMR ($CDCl_3$): δ 1.78 (s, 3 H, $CH_3$), 3.58–4.87 (m, 17 H, H2–H6, NH, H1′, H2′ and $CH_2$Ph), 5.45 (d, 1 H, H1), 7.24–7.39 (m, 15 H, Ph). $^{13}$C NMR ($CDCl_3$): δ 23.2 ($CH_3$), 52.4 (C2), 62.3 (C2′), 68.4 (C5), 69.5 ($CH_2$Ph), 71.2 (C6), 73.4 ($CH_2$Ph), 73.9 (C1′), 74.8 ($CH_2$Ph), 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 $C_{31}H_{37}NO_7$: C 69.5; H 7.0; N 2.6. **25**: $^1$H NMR ($CDCl_3$): δ 1.80 (s, 3 H, $CH_3$), 3.48–4.89 (m, 21 H, H2-H6, H1′–H4′, NH and $CH_2$Ph), 5.30 (d, 1 H, H1), 7.28–7.36 (m, 15 H, Ph). $^{13}$C NMR ($CDCl_3$): δ 23.3 ($CH_3$), 52.4 (C2), 61.7 (C4′), 68.3 (C5), 69.6 ($CH_2$Ph), 70.6 (C3′), 71.3 (C6), 72.0 (C2′), 72.2 (C1′), 73.4 and 74.7 ($CH_2$Ph), 78.8 (C3), 80.1 (C4), 97.1 (C1), 127.5–128.5 (aromatic CH), 137.1, 138.1 and 138.5 (aromatic C), 169.6 (CO). Found: C 68.4; H 7.4; N 2.1. Calc. for $C_{33}H_{41}NO_8$: C 68.4, H 7.1; N 2.4.

*Benzyl 2-acetamido-3,6-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**, $R_f$ 0.33, in addition to the starting material. Analysis of the mixture as their alditol 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%). $^1$H NMR ($CDCl_3$): δ 0.94 (d, 3 H, H3′), 1.78 (d, 3 H, $CH_3$), 3.26–4.87 (m, 16 H, H2–H6, NH, $CH_2$Ph), 5.34–5.38 (2 d, 1 H, H1), 7.31–7.34 (m, 15 H, Ph). $^{13}$C NMR ($CDCl_3$): δ 18.4 and 18.5 (C3′), 23.3 ($CH_3$), 52.4 (C2), 66.9 and 67.1 (C2′), 68.5 and 68.6 (C5), 69.6 ($CH_2$Ph), 71.2 and 71.3 (C6), 73.5, 74.7 and 74.8 ($CH_2$Ph), 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 $C_{32}H_{39}NO_7$: C 69.9; H 7.2; N 2.6.

*2-Acetamido-2-deoxy-4-O-(2-hydroxyethyl)-D-glucose (***27***)*. Compound **24** (80 mg, 0.15 mmol) was hydrogenolysed in the same way as **5**. After evaporation of the solvent and drying, **27** (33 mg, 83%) was obtained. $^1$H NMR ($D_2O$): δ 2.04 (s, 3 H, $CH_3$), 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, H1α). $^{13}$C NMR ($D_2O$): δ 24.7 ($CH_3$α), 25.0 ($CH_3$β), 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 $C_{10}H_{19}NO_7$: C 45.3; H 7.2; N 5.3.

*2-Acetamido-2-deoxy-4-O-(2-hydroxypropyl)-D-glucose (***28***)*. The hydrogenolysis of **26** (26 mg, 0.05 mmol) was performed as described for **5** to yield **28** (12 mg, 91%). $^1$H NMR ($D_2O$): δ 1.15 (d, 3 H, H3′), 2.05 (s, 3 H, $CH_3$), 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). $^{13}$C NMR ($D_2O$): δ 20.8 (C3′β), 20.9 (C3′α), 24.7 ($CH_3$α), 25.0 ($CH_3$β), 57.0 (C2α), 59.6 (C2β), 63.2 (C6α), 63.3 (C6β), 69.4 (C2′α), 69.7 (C2′β), 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, C1′), 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 $C_{11}H_{21}NO_7$; C 47.3; H 7.6; N 5.0.

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