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Synthesis of lysine-containing fragments of the *Proteus mirabilis* O27 O-specific polysaccharide and neoglycoconjugates therefrom

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ABSTRACT

Amide-linked lysine mono- and di-uronic acid fragments of the O-specific polysaccharide from *P. mirabilis* O27 have been synthesised. N^{ε}-Boc-L-lysine *tert*-butyl ester was condensed with 2-azidoethyl glycosides of glucuronic acid and β-D-Glc*p*NAc-(1→3)-β-D-Glc*p*A. Transformation of the products into 2-acrylamidoethyl glycosides, followed by deprotection using trifluoroacetic acid, gave the target monomers that were converted into high-molecular-weight copolymer-type neoglycoconjugates.

INTRODUCTION

Amino acids, amide-linked to the carboxyl groups of uronic acid residues in the polysaccharide chain, are constituents of some capsular and O-specific polysaccharides[1]. The frequent occurrence of amino acid residues (mainly lysine and alanine) has been reported for O-antigens in most of the *P. mirabilis* O-serogroups which cause mainly infections of the urinary tract and wounds[2]. The immunodominant role of lysine residues in the serological properties was shown for the LPS of some *P. mirabilis* serotypes[2]. The repeating unit of *P. mirabilis* O27 O-antigen comprises alanine and lysine, linked through the α-amino group to the carboxyl group of D-galacturonic and D-glucuronic acid, respectively[3].

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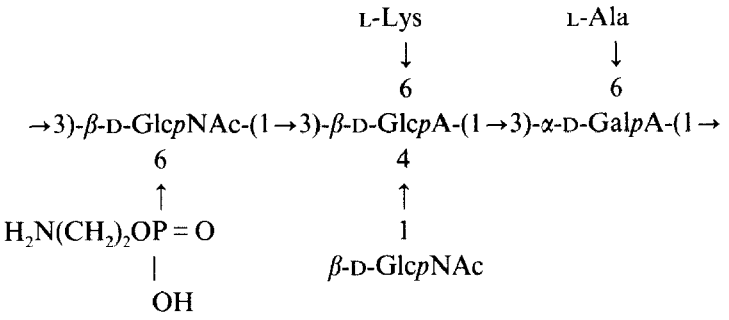
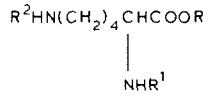
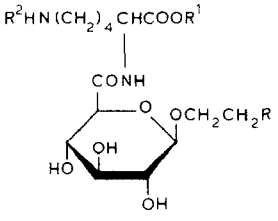


Synthesis of amino acid-containing fragments of the polysaccharide followed by transformation into neoglycoconjugates could be useful for defining the immunochemically important details of this O-antigen. A synthesis of the [6(*N*)-L-Ala]-Gal*p*A fragment (as the β-glycoside) of the *P. mirabilis* O27 O-antigen and its conversion into a copolymer-type neoglycoconjugate has been reported[1].

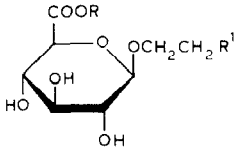
We now describe the synthesis of mono- and di-saccharide fragments of this O-antigen, which contain L-lysine.





1 R = H, R^1 = Z, R^2 = Boc
2 R = Me, R^1 = Z, R^2 = Boc
3 R = Me, R^1 = H, R^2 = Boc
4 R = Bu^t, R^1 = H, R^2 = Boc

9 $R = N_3$, R^1 = Me, R^2 = Boc
10 R = NHCOCH=CH_2, R^1 = Me, R^2 = Boc
11 R = NHCOCH=CH_2, R^1 = R^2 = H
12 $R = N_3$, R^1 = Bu^t, R^2 = Boc
13 R = NHCOCH=CH_2, R^1 = Bu^t, R^2 = Boc



5 R = H, R^1 = N_3
6 R = Me, R^1 = N_3
7 R = Me, R^1 = NHCOCH=CH_2
8 R = H, R^1 = NHCOCH=CH_2

RESULTS AND DISCUSSION

The fragments were synthesised as 2-azidoethyl glycosides which contain a masked terminal amino function[4,5] suitable for the preparation of neoglycoconjugates by covalent coupling to protein carriers[6] or via *N*-acryloylation followed by copolymerisation with acrylamide[7,8].

In the synthesis of the [6(N^{α})-L-Lys]-Glc*p*A fragment, the partially protected L-lysine derivatives **3** and **4** were used. Conversion[9] of the partially protected amino acid **1**[9] into the *tert*-butyl ester **4** gave a low yield, and a commercial preparation was used.

The methyl ester **3** was obtained by treatment of **1** with diazomethane followed by hydrogenolysis. Condensation of 2-azidoethyl β-D-glucopyranosiduronic acid[1,4,5] (**5**) with **3**, promoted[1,10] by ethyl 2-ethoxy-1,2-dihydroquinoline-1-carboxylate (EEDQ), afforded the amide **9** (66%), the structure of which was confirmed by the ^{13}C-n.m.r. data. However, hydrogenation of the azide group in **9** followed by *N*-acryloylation gave only a low yield of the target compound **10**.

In order to avoid *N*-acryloylation in the final stages of the synthesis, **5** was treated with diazomethane to give the methyl ester **6**, catalytic hydrogenation of which followed by *N*-acryloylation afforded the 2-acrylamidoethyl glycoside **7** (58% from **5**). Saponification of **7** gave the acid derivative **8** (89%), which was condensed with **3** to give the amide **10** in low yield (17%). The structure of **10** was confirmed from ^{13}C-n.m.r. data (see Experimental). Mild treatment (20°, 45 min) of **9** with trifluoroacetic acid followed by saponification afforded the target [6(N^{α})-L-Lys]-GlcA fragment as the 2-acrylamidoethyl glycoside **11** (55%).

An improved synthesis of **11** was achieved by using the *tert*-butyl ester **4**. The *tert*-butyl ester group requires mild acidic conditions for cleavage (similar to that for cleavage of the Boc group) and is more resistant to bases and amines than the methyl ester group[11] (see Experimental, *cf.* the conditions of *N*-acryloylation).

Coupling of the 2-azidoethyl glycoside **5** with **4** gave the amide **12** (82%). The azidoethyl group in **12** was transformed into the 2-acrylamidoethyl group to give the glycoside **13** (59%). Further deprotection of **13** under mild acidic conditions afforded the target monomer **11** (99%).

The synthesis of the lysine-containing disaccharide fragment **20** involved glycosylation of the partially protected glucuronic acid derivative **15**, prepared from **5** by the formation of the 6,3-lactone followed by selective alcoholysis[12]. Attempted glycosylation of **15** with 2-methyl-(1,2-dideoxy-α-D-glucopyrano)[2,1-*d*]-2-oxazoline (**14**) failed. No reaction occurred in the 1,2-dichloroethane–*p*-toluenesulfonic acid system at 70°[13] for 2 weeks, and **16** (35%) was obtained together with an unidentified unsaturated sugar (*cf.* ref. 13) when trimethylsilyl triflate was used as promoter[14].

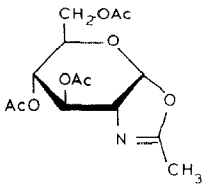
However, the glycosyl bromide **17**[15] reacted with **15** under conditions described[16] [mercury(II) cyanide, acetonitrile, 20°], to give the disaccharide derivative **18** (27%). When silver trifluoromethanesulfonate was used as the promoter in dichloromethane in the presence of *sym*-collidine and molecular sieves 3 Å at −30°→20°, 68% of **18** was obtained. The structure of **18** was confirmed from ^{1}H- and ^{13}C-n.m.r. data (see Experi-

mental); the $J_{1',2'}$ and $J_{C\text{-}1',H\text{-}1'}$ values of 8.4 and 166 Hz, respectively, indicated the 2-deoxy-2-phthalimido-D-glucopyranoside moiety to be β.

Saponification of **18** followed by treatment[17] with hydrazine hydrate removed the protecting groups. *N*-Acetylation (acetic anhydride–methanol) of the product gave the target azidoethyl glycoside **19**, the ^{1}H- and ^{13}C-n.m.r. spectra of which confirmed the structure assigned.

Coupling of the disaccharide glycoside **19** and the L-lysine *tert*-butyl ester **4**, under the conditions described above, afforded the amide **20** (62%), which was identified from the ^{13}C-n.m.r. data (see Experimental). Catalytic hydrogenation (Pd/C) of the azidoethyl group in **20** gave the aminoethyl derivative which, without purification, was treated with acryloyl chloride in aqueous methanol in the presence of Dowex 1-X8 (HCO_3^-) resin and 2,6-di-*tert*-butyl-4-methylphenol as a radical inhibitor (to avoid polymerisation[18]). H.p.l.c. then gave **21a** (60%), **21b** (16%), and **21c** (12%), which were isomeric at the 2-acrylamidoethyl group as concluded from the n.m.r. spectra. Similar isomers were isolated during the synthesis of the glucuronamide of L-serine[1].

In the ^{1}H- and ^{13}C-n.m.r. spectra of **21a**, the resonances of the aglycon moiety coincided with those observed for the *Z* isomers of 2-acrylamidoethyl glycosides (*cf.* ref. 1). The resonances of **21b** and **21c**, in the region for the signals associated with the



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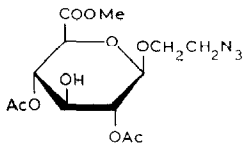

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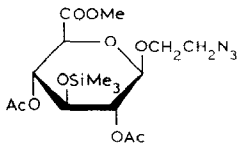

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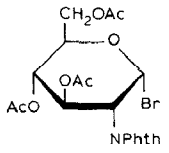

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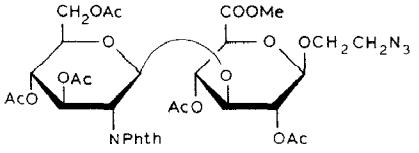

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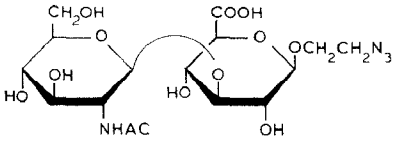

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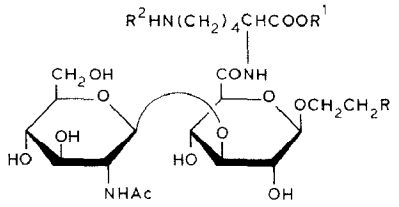
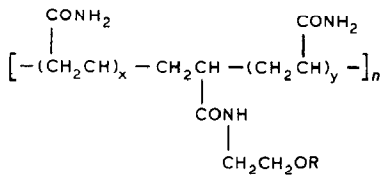


20 R = N_3, R^1 = Bu^t, R^2 = Boc

21 R = NHCOCH=CH_2, R^1 = Bu^t, R^2 = Boc

22 R = NHCOCH=CH_2, R^1 = R^2 = H



23 R = β-D-GlcpA-[6(N^{α})-L-Lys]-(1→

24 R = β-D-GlcpNAc-(1→3)-β-D-GlcpA-[6(N^{α})-L-Lys]-(1→

double bond, were similar to those of *E* isomers of 2-acrylamidoethyl glycosides described earlier (*cf.* refs. 1 and 10). Deprotection of **21a** by brief treatment with trifluoroacetic acid afforded the target lysine-containing fragment as the 2-acrylamidoethyl glycoside **22** (97%), the structure of which was indicated by the ^{13}C-n.m.r. data (see Experimental).

The lysine-containing mono- (**11**) and di-saccharide (**22**) fragments were transformed into high-molecular-weight neoglycoconjugates *via* copolymerisation with acrylamide promoted by ammonium persulfate and *N,N,N',N'*-tetramethylethylenediamine in 0.2M acetate buffer (pH 5.8) as described[1,10]. The copolymers **23** and **24**, isolated by gel-filtration on Sephadex G-50 in yields of 80–90%, contained both unsubstituted acrylamide units and those *N*-substituted by a sugar moiety, in the ratio 10–11:1 as indicated by integration of the appropriate ^{13}C signals or comparison of the $[\alpha]_D$ values for the copolymers and the corresponding sugar monomers.

The immunochemical studies of the neoglycoconjugates prepared will be reported elsewhere.

EXPERIMENTAL

General methods. — The methods and the instrumental and chromatographic procedures used have been described[1,5,10].

N$^{\alpha}$-*Benzyloxycarbonyl*-N$^{\varepsilon}$-*(*tert-*butyloxycarbonyl)*-L-*lysine methyl ester* (**2**). — To a solution of *N*$^{\alpha}$-benzyloxycarbonyl-*N*$^{\varepsilon}$-(*tert*-butyloxycarbonyl)-L-lysine[9] (**1**; 200 mg, 0.526 mmol) in ether (10 mL) was added cold ethereal diazomethane until a yellow colour persisted. The solvent was evaporated and h.p.l.c. (80:20 hexane–EtOAc) of the syrupy residue (219 mg) on a column (2.5 × 25 cm) of Silasorb 600 (10 μm) gave **2** (175 mg, 84%), $[\alpha]_D^{20}$ +3.8° (*c* 2, chloroform), R_F 0.53 (1:1 hexane–EtOAc). ^{13}C-N.m.r. data ($CDCl_3$): δ 172.9, 156.1 (C=O), 136.3, 128.5, 128.2 (Ph), 79.2 (O*C*Me$_3$), 67.0 (OCH_2), 53.8 (α-CH), 52.3 (COO*C*H$_3$), 40.1 (ε-CH_2), 32.2 (δ-CH_2), 29.6 (β-CH_2), 28.4 [OC(*C*H$_3$)$_3$], 22.4 (γ-CH_2).

N$^{\varepsilon}$-*(*tert-*Butyloxycarbonyl)*-L-*lysine methyl ester* (**3**). — A solution of **2** (98 mg, 0.248 mmol) in MeOH (20 mL) was stirred with 10% Pd/C under hydrogen at atmospheric pressure. After 1.5 h, t.l.c. (1:1 hexane–EtOAc) showed complete conversion of **2** into **3** (R_F 0). The mixture was filtered and concentrated to give **3** (64 mg, 99%), which was sufficiently pure for the next stage.

2-Acrylamidoethyl β-D-glucopyranosiduronic acid (**8**). — To a solution of **5**[1,4,5] (150 mg, 0.57 mmol) in MeOH (5 mL) was added cold ethereal diazomethane until a yellow colour persisted. The solvent was evaporated and the residue was hydrogenated over 10% Pd/C in MeOH (5 mL) containing acetic acid (36 μL, 0.062 mmol). After 2 h, t.l.c. (90:10:1 $CHCl_3$–MeOH–AcOH) showed complete conversion of the methyl ester **6** into the corresponding aminoethyl glycoside. The mixture was filtered and concentrated. To a solution of the residue (140 mg) in MeOH–water (8:1, 8 mL) were added Dowex 1-X8 (HCO_3^-) resin and acryloyl chloride (0.13 mL, 0.16 mmol), and the mixture was stirred at room temperature. After 18 h, t.l.c. (90:10:1 $CHCl_3$–MeOH–AcOH) showed complete conversion into the acrylamidoethyl glycoside **7**. The mixture was filtered, the solids were washed with MeOH (50 mL), and the combined filtrate and washings were concentrated. Column chromatography (95:5 $CHCl_3$–MeOH) of the residue gave syrupy **7** (100 mg, 57.5% from **5**), R_F 0.4 (90:10:1 $CHCl_3$–MeOH–AcOH), 0.33 (100:10:10:10:3 EtOH–1-BuOH–pyridine–water–AcOH).

Cold M NaOH (4.5 mL) was added to a solution of **7** (100 mg, 0.328 mmol) in MeOH (18 mL), and the mixture was kept at 0°. After 15 min, t.l.c. (80:20:1 $CHCl_3$–MeOH–AcOH) showed the absence of **7**. The mixture was neutralised with KU-2 (H^+) resin and concentrated to give syrupy **8** (85 mg, 89%), $[\alpha]_D^{22}$ −40° (*c* 1, MeOH). ^{13}C-N.m.r. data (D_2O): δ 173.1 (COOH), 131.3 (*C*H = CH_2), 128.8 (CH = *C*H_2), 104.0 (C-1), 76.7 (C-3), 76.3 (C-5), 74.0 (C-2), 72.7 (C-4), 70.2 (OCH_2), 40.6 (CH_2N).

N$^{\alpha}$-*(2-Azidoethyl β-D-glucopyranosiduronoyl)*-N$^{\varepsilon}$-*(*tert-*butyloxycarbonyl)*-L-*lysine methyl ester* (**9**). — To a solution of **3** (64 mg, 0.246 mmol) and 2-azidoethyl β-D-glucopyranosiduronic acid[1,4,5] (**5**; 40 mg, 0.152 mmol) in anhydrous *N,N*-dimethylformamide (3 mL, freshly distilled *in vacuo* over ninhydrin) was added EEDQ (60 mg, 0.242 mmol) in one portion. The mixture was stirred for 48 h at 20° until **5** disappeared (t.l.c.; 90:10:1 $CHCl_3$–MeOH–AcOH). The mixture was concentrated, and toluene was evaporated from the residue, a solution of which in MeOH (3 mL) was treated with KU-2 (H^+) resin to remove quinoline and unreacted amino acid. The mixture was filtered and concentrated, and column chromatography (95:5 $CHCl_3$–MeOH) of the residue gave syrupy **9** (51 mg, 66%), $[\alpha]_D^{28}$ −21.5° (*c* 2, $CHCl_3$), R_F 0.46. ^{13}C-N.m.r. data ($CDCl_3$): δ 172.3, 170.3 (C = O), 102.9 (C-1), 79.2 (O*C*Me_3), 75.9 (C-3), 72.9 (C-2,5), 72.3 (C-4), 69.2 (OCH_2), 52.7 (α-CH), 51.7 ($COOCH_3$), 50.8 (CH_2N_3), 40.3 (ε-CH_2), 32.1 (δ-CH_2), 29.6 (β-CH_2), 28.5 [C(*C*H_3)$_3$], 22.6 (γ-CH_2).

N$^{\alpha}$-*(2-Acrylamidoethyl β-D-glucopyranosiduronoyl)*-N$^{\varepsilon}$-*(*tert-*butyloxycarbonyl)*-L-*lysine methyl ester* (**10**). — To a solution of **3** (75 mg, 0.29 mmol) and **8** (85 mg, 0.29 mmol) in anhydrous *N,N*-dimethylformamide (3 mL) was added EEDQ (108 mg, 0.44 mmol) in one portion, and the mixture was kept at 20°. After 48 h, t.l.c. (80:20:1 $CHCl_3$–MeOH–AcOH) showed complete conversion of **8** into **10** (R_F 0.54). The mixture was concentrated, and toluene was evaporated from the residue, chromatography (96:4 $CHCl_3$–MeOH) of which on a column (10 × 250 mm) of LiChroprep Si 60 (40–63 μm, Merck) gave syrupy **10** (27 mg, 17%), $[\alpha]_D^{28}$ −20° (*c* 2, $CHCl_3$). ^{13}C-N.m.r. data ($CDCl_3$): δ 172.5, 170.3, 166.5, 156.3 (C = O), 131.0 (*C*H = CH_2), 126.7 (CH = *C*H_2), 103.2 (C-1), 79.2 (O*C*Me_3), 75.9 (C-3), 73.2 (C-5), 73.0 (C-2), 72.1 (C-4), 69.5 (OCH_2), 52.7 (α-CH),

52.0 ($COOCH_3$), 40.3 (ε-CH_2), 39.6 (OCH_2CH_2N), 31.6 (δ-CH_2), 29.6 (β-CH_2), 28.5 [$C(CH_3)_3$], 22.8 (γ-CH_2).

N^{α}-*(2-Acrylamidoethyl β-*D*-glucopyranosiduronoyl)*-L-*lysine* (**11**). — (*a*) A solution of **10** (30 mg, 0.056 mmol) in trifluoroacetic acid (2 mL) was kept for 45 min at 20°, then concentrated, and CCl_4 and then MeOH were evaporated from the residue (R_F 0.33, t.l.c.; 100:10:10:10:1 EtOH–1-BuOH–pyridine–water–AcOH). To a cooled (0°) solution of the residue in MeOH (6 mL) was added cold M NaOH (1.5 mL), and the mixture was stirred at 0°. After 45 min, t.l.c. (100:10:10:10:3 EtOH–1-BuOH–pyridine–water–AcOH) showed complete conversion into **11** (R_F 0.23). The mixture was neutralised with AcOH and concentrated. The residue was desalted by elution from a column (1.5 × 73 cm) of Bio Gel P-2 (50–100 mesh) with pyridine–acetate buffer (pH 6.5) and then purified on a column (2.5 × 35 cm) of Bio Gel P-2 (200–400 mesh) by elution with pyridine–acetate buffer (pH 3.5) to give **11** (13 mg, 55%), $[\alpha]_D^{28}$ − 34° (*c* 1, water), R_F 0.35 (100:10:10:10:3 EtOH–1-BuOH–pyridine–water–AcOH). ^{13}C-N.m.r. data (D_2O): δ 176.0, 171.5 (CONH and COOH), 131.4 (CH_2 = *C*H), 128.4 (*C*H_2 = CH), 103.9 (C-1), 76.6 (C-3), 76.1 (C-5), 73.9 (C-2), 72.6 (C-4), 69.9 (OCH_2), 53.5 (α-CH), 40.5 (ε-CH_2 and OCH_2CH_2NHCO), 31.2 (δ-CH_2), 27.3 (β-CH_2), 23.1 (γ-CH_2).

(*b*) A solution of **13** (51 mg, 0.09 mmol) in trifluoroacetic acid (3 mL) was kept at 20° for 1.5 h and then worked-up as in (*a*) to give **11** (37 mg, 99%).

N^{α}-*(2-Azidoethyl β-*D*-glucopyranosiduronoyl)*-N^{ε}-*(*tert*-butyloxycarbonyl)*-L-*lysine* tert-*butyl ester* (**12**). — Condensation of **5** (190 mg, 0.72 mmol) with N^{ε}-Boc-L-lysine *tert*-butyl ester (Bachem, **4**; 262 mg, 0.87 mmol) in the presence of EEDQ (267 mg, 1.08 mmol), as described above, for 48 h gave syrupy **12** (324 mg, 82%), $[\alpha]_D^{25}$ − 18° (*c* 2, $CHCl_3$). ^{13}C-N.m.r. data ($CDCl_3$): δ 170.8, 170.0 (*C*OO^{t}Bu and CONH), 102.8 (C-1), 82.5 ($OCMe_3$), 75.7 (C-3), 72.8 (C-2,5), 72.3 (C-4), 68.9 (OCH_2), 52.1 (α-CH), 50.7 (CH_2N_3), 40.3 (ε-CH_2), 32.2 (δ-CH_2), 29.5 (β-CH_2), 28.4 and 27.9 [$C(CH_3)_3$], 22.4 (γ-CH_2).

N^{α}-*(2-Acrylamidoethyl β-*D*-glucopyranosiduronoyl)*-N^{ε}-*(*tert*-butyloxycarbonyl)*-L-*lysine* tert-*butyl ester* (**13**). — Catalytic hydrogenation of **12** (174 mg, 0.317 mmol), as described above, followed by *N*-acryloylation in the presence of 2,6-di-(*tert*-butyl)-4-methylphenol (as a radical inhibitor), afforded crude **13** (187 mg). H.p.l.c. (gradient of 0→11% of MeOH in $CHCl_3$ of a portion (87 mg) on a column (1.6 × 25 cm) of Silasorb 600 (10 μm) gave syrupy **13** (50 mg), $[\alpha]_D^{25}$ − 11° (*c* 1, $CHCl_3$), R_F 0.5 (90:10:1 chloroform–methanol–AcOH). ^{13}C-N.m.r. data ($CDCl_3$): δ 171.1, 170.1, 166.5 (*C*OO^{t}Bu and *C*ONH), 131.0 (*C*H = CH_2), 126.7 (CH = *C*H_2), 103.2 (C-1), 82.7 ($OCMe_3$), 76.1 (C-3), 73.1 (C-2,5), 72.3 (C-4), 69.7 (OCH_2), 52.4 (α-CH), 40.4 (ε-CH_2), 39.7 (OCH_2CH_2N), 32.1 (δ-CH_2), 29.6 (β-CH_2), 28.6 and 28.1 [$C(CH_3)_3$], 22.6 (γ-CH_2).

Reaction of azidoethyl glycoside **15** *with the oxazoline* **14** *promoted by trimethylsilyl triflate.* — A solution of **15**[12] (91 mg, 0.126 mmol) and **14**[19] (63 mg, 0.190 mmol) in dry toluene (2 mL) was stirred with molecular sieves 3 Å for 2 h at 20° under argon. Trimethylsilyl trifluoromethanesulfonate (35 μL, 0.190 mmol) was injected with a syringe into the mixture, which was heated at 60° for 4 h, then filtered through Celite-545. The filtrate was washed successively with saturated aq. $NaHCO_3$ and water,

dried (Na_2SO_4), and concentrated. Column chromatography (85:15 hexane–EtOAc) of the syrupy residue (99 mg) gave methyl [2-azidoethyl 2,4-di-*O*-acetyl-3-*O*-trimethylsilyl-β-D-glucopyranosid]uronate (**16**; 19 mg, 35%), R_F 0.78 (95:5 ether–MeOH), 0.40 (6:4 hexane–EtOAc). ^{1}H-N.m.r. data ($CDCl_3$): δ 5.04 (dd, 1 H, $J_{3,4}$ 9.1, $J_{4,5}$ 9.6 Hz, H-4), 4.98 (dd, 1 H, $J_{1,2}$ 7.9, $J_{2,3}$ 9.1 Hz, H-2), 4.51 (d, 1 H, H-1), 4.05 (ddd, 1 H, OCH_A), 3.92 (d, 1 H, H-5), 3.85 (t, 1 H, H-3), 3.73 (s, 3 H, COOMe), 3.65 (ddd, 1 H, OCH_B), 3.49 (ddd, 1 H, CH_AN), 3.28 (ddd, 1 H, CH_BN), 2.12 (s, 3 H, Ac), 2.08 (s, 3 H, Ac), 0.10 (s, 9 H, $SiMe_3$).

Also isolated was an unidentified unsaturated sugar component (23 mg), R_F 0.40 (95:5 ether–MeOH).

*Methyl [2-azidoethyl 2,4-di-*O*-acetyl-3-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-β-D-glucopyranosid]uronate* (**18**). — (*a*) A solution of the glycosyl bromide **17** [prepared[15] from 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-D-glucopyranose (540 mg, 1.15 mmol)] in acetonitrile (4 mL) was added dropwise under argon during 1 h to a solution of **15**[12] (106 mg, 0.294 mmol) and mercury(II) cyanide (319 mg, 1.26 mmol) in freshly distilled acetonitrile (3 mL). The mixture was stirred for 18 h at 20° and then concentrated, the residue was extracted with $CHCl_3$, and the combined extracts (250 mL) were washed successively with M KBr (3 × 200 mL), saturated aq. $NaHCO_3$ (200 mL), and water (200 mL), dried (Na_2SO_4), and concentrated. The residue was subjected to column chromatography (3:7 hexane–EtOAc), and the fractions were monitored by h.p.l.c. (4:6 hexane–EtOAc) on a column (6 × 150 mm) of Silasorb 600 (5 μm). The appropriate fractions were combined and concentrated to give **18** (62 mg, 27%), $[\alpha]_D^{25}$ −101° (*c* 1, $CHCl_3$). N.m.r. data ($CDCl_3$): ^{1}H, δ 7.66–7.88 (m, 5 H, Ar), 5.78 (dd, 1 H, $J_{2',3'}$ 11.0, $J_{3',4'}$ 8.8 Hz, H-3'), 5.41 (d, 1 H, $J_{1',2'}$ 8.4 Hz, H-1'), 5.16 (dd, 1 H, $J_{4',5'}$ 10.2 Hz, H-4'), 5.02 (dd, 1 H, $J_{3,4}$ 8.8 Hz, H-4), 4.80 (dd, 1 H, $J_{2,3}$ 8.8 Hz, H-2), 4.48 (dd, 1 H, $J_{6'a,6'b}$ 12.5, $J_{5',6'b}$ 4.3 Hz, H-6'b), 4.42 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 4.21 (dd, 1 H, H-2'), 4.09 (dd, 1 H, $J_{5',6'a}$ 2.5 Hz, H-6'a), 4.07 (t, 1 H, H-3), 4.03 (d, 1 H, $J_{4,5}$ 10.0 Hz, H-5), 3.89 (m, 1 H, H-5'), 3.73 (s, 3 H, CO_2Me), 3.54 (m, 2 H, OCH_2), 3.30 (m, 2 H, CH_2N_3), 2.14, 2.11, 2.08, 2.02 (4 s, 15 H, 5 OAc), 1.84 (s, 3 H, NAc); ^{13}C, δ 170.3, 169.8, 169.2, 168.9, and 167.1 (C=O), 134.0, 131.2, and 123.3 (Ar), 100.5 (C-1, $J_{C-1,H-1}$ 161 Hz), 97.4 (C-1', $J_{C-1',H-1'}$ 166 Hz), 76.5 (C-3), 72.4 (C-5'), 72.0 (C-5), 71.5 (C-3'), 70.1 (C-4), 69.4 (C-2), 68.5 (C-4'), 67.6 ($OCH_2CH_2N_3$), 61.6 (C-6'), 54.3 (C-2'), 52.5 (CO_2CH_3), 50.3 ($OCH_2CH_2N_3$), 20.8, 20.5, 20.3, 20.2, and 20.1 ($COCH_3$).

(*b*) A solution of **15**[12] (116 mg, 0.32 mmol), silver trifluoromethanesulfonate (289 mg, 1.12 mmol), and 2,4,6-collidine (0.127 mL, 0.96 mmol) in freshly distilled CH_2Cl_2 (3 mL) was stirred with powdered molecular sieves 3 Å for 1 h under argon. To the stirred mixture at −30° was added dropwise a solution of **17**[15] [prepared from 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-phthalimido-D-glucopyranose (540 mg, 1.15 mmol)] in dichloromethane (2 mL) under argon during 1 h. Stirring at −30° was continued for 30 min, the mixture was allowed to attain room temperature, and stirring was continued for 18 h. The mixture was filtered through Celite-545, the filter cake was washed with $CHCl_3$, and the combined filtrate and washings were washed successively with saturated aq. $NaHCO_3$ (200 mL), M sodium thiosulfate (3 × 200 mL), water (200 mL), aq. 10% citric acid (200 mL), saturated aq. $NaHCO_3$ (200 mL), and water (200 mL), dried (Na_2SO_4), and concentrated. Column chromatography of the residue as in (*a*) gave **18** (170 mg, 68%).

2-Azidoethyl 3-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-glucopyranosiduronic acid (**19**). — Cold M NaOH (2 mL) was added to a cooled (ice–water) solution of **18** (51 mg, 0.066 mmol) in MeOH (8 mL). The mixture was kept at 0° for 2.5 h, then neutralised with AcOH (0.6 mL), and concentrated. Water was evaporated from the residue, to a solution of which in EtOH (6 mL) was added hydrazine hydrate (1 mL). The mixture was boiled under reflux for 7 h, then concentrated. 1-Butanol and then EtOH were evaporated from the residue, to a cooled (ice–water) solution of which in MeOH (2 mL) was added acetic anhydride (0.5 mL) dropwise. The mixture was kept for 1 h at 0°, then concentrated. The residue was desalted by elution from a column (1.5 × 70 cm) of Bio Gel P-2 (50–100 mesh) with pyridine–acetate buffer (pH 6.5), then purified by reverse-phase h.p.l.c. on a column (2.5 × 25 cm) of Silasorb C8 (10 μm) by elution with aq. 0.4% trifluoroacetic acid and then by ion-exchange chromatography by elution from a column (1.6 × 15 cm) of DEAE-Spheron (25–40 μm; AcO^--form) with aq. acetic acid (0→20%, 200 mL) to give **19** (16 mg, 52%), $[\alpha]_D^{26}$ −24° (*c* 1, water). N.m.r. data (D_2O), selected signals: ^1H, δ 4.78 (d, 1 H, $J_{1',2'}$ 8.25 Hz, H-1'), 4.50 (d, 1 H, $J_{1,2}$ 7.75 Hz, H-1), 2.01 (s, 3 H, $NHCOCH_3$); ^{13}C, δ 103.9 (C-1), 103.1 (C-1'), 84.8 (C-3), 77.3 (C-5'), 74.9 (C-3',5), 74.0 (C-2), 71-2 (C-4,4'), 70.2 ($OCH_2CH_2N_3$), 62.1 (C-6'), 57.1 (C-2'), 51.9 ($OCH_2CH_2N_3$), 23.5 ($NHCOCH_3$).

N^{α}-*[2-Azidoethyl 3-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-glucopyranosiduronoyl]*-N^{ε}-(tert-*butyloxycarbonyl*)-L-*lysine* tert-*butyl ester* (**20**). — To a solution of **19** (41 mg, 0.088 mmol) and N^{ε}-Boc-L-lysine *tert*-butyl ester [generated from the corresponding hydrochloride (40 mg, 0.106 mmol) by treatment with an equimolar amount of triethylamine in EtOAc] in anhydrous *N,N*-dimethylformamide (3 mL) was added EEDQ (33 mg, 0.132 mmol) in one portion. The mixture was kept for 48 h at 20° until **19** disappeared (t.l.c.; 80:20:1 $CHCl_3$–MeOH–acetic acid). The mixture was then concentrated and toluene was evaporated from the residue *in vacuo* at 30° (bath). Column chromatography (94:6 $CHCl_3$–MeOH) of the residue gave syrupy **20** (43 mg, 62%), $[\alpha]_D^{28}$ −27° (*c* 1, $CHCl_3$). ^{13}C-N.m.r. data ($CDCl_3$): δ 173.7, 173.6, 171.1, and 169.4 (C=O), 102.9 (C-1), 101.7 (C-1'), 82.4 (C-3), 77.7 (C-5'), 76.7 (C-5), 76.5 (C-3'), 73.8 (C-2), 70.9 and 70.8 (C-4,4'), 68.9 ($OCH_2CH_2N_3$), 61.8 (C-6'), 56.9 (C-2'), 52.5 (α-CH), 50.8 ($OCH_2CH_2N_3$), 40.4 (ε-CH_2), 32.1 (δ-CH_2), 29.6 (β-CH_2), 28.6 and 28.1 [$C(CH_3)_3$], 23.3 ($NHCOCH_3$), 22.5 (γ-CH_2).

N^{α}-*[2-Acrylamidoethyl 3-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-glucopyranosiduronoyl]*-N^{ε}-(tert-*butyloxycarbonyl*)-L-*lysine* tert-*butyl ester* (**21**). — A solution of **20** (49 mg, 0.062 mmol) in MeOH (10 mL) was hydrogenated over 10% Pd/C. After 3.5 h, t.l.c. (100:10:10:10:3 EtOH–1-butanol–pyridine–water–acetic acid) showed complete conversion into the aminoethyl glycoside (positive ninhydrin test), and the mixture was filtered and concentrated. To a solution of the residue (45 mg) in 8:1 MeOH–water (4.5 mL) containing 2,6-di-*tert*-butyl-4-methylphenol (3–5 mg, as an inhibitor of polymerisation) was added acryloyl chloride (30 μL, 0.37 mmol), and the mixture was stirred with Dowex 1-X8 (HCO_3^-) resin. After 18 h, t.l.c. (8:2 $CHCl_3$–MeOH) showed complete conversion into **21** (R_F 0.28), and the mixture was filtered and concentrated. H.p.l.c. (9:1 $CHCl_3$–MeOH) of the residue on a column (1.6 × 25 cm) of

Silasorb 600 (10 μm) gave **21a** (60%), $[\alpha]_D^{27}$ −9.7° (*c* 1, $CHCl_3$), **21b** (16%), and **21c** (12%). N.m.r. data ($CDCl_3$): ^{13}C, **21a** δ 173.6, 171.1, 169.8, and 166.5 (C=O), 131.2 ($CH_2=CH$), 126.5 ($CH_2=CH$), 103.0 (C-1), 101.9 (C-1′), 84.9 [$C(CH_3)_3$], 82.4 (C-3), 77.5, 76.4, 74.7, 74.0, 73.1, 70.7 (C-2,4,5,3′,4′,5′), 69.5 (OCH_2CH_2NH), 61.7 (C-6′), 56.8 (C-2′), 52.7 (α-CH), 40.5 (ε-CH_2), 39.7 (OCH_2CH_2NH), 32.0 (δ-CH_2), 29.6 (β-CH_2), 28.6 and 28.1 [$C(CH_3)_3$], 23.4 ($NHCOCH_3$), 22.6 (γ-CH_2); ^1H (selected signals), **21a** δ 1.42 and 1.48 (2 s, 18 H, 2 ^{t}Bu), 5.60 (m, 1 H, H_M), 6.20–6.30 (m, 2 H, H_X and H_A); **21b** δ 1.43 and 1.46 (2 s, 18 H, 2 ^{t}Bu), 5.75 (dd, 1 H, $J_{A,M}$ 2.0, $J_{M,X}$ 10.6 Hz, H_M), 6.33 (m, 1 H, H_A), 6.62 (dd, 1 H, $J_{A,X}$ 17.0 Hz, H_X); **21c** δ 1.44 and 1.48 (2 s, 18 H, 2 ^{t}Bu), 5.74 (dd, 1 H, $J_{A,M}$ 2.0, $J_{M,X}$ 10.5 Hz, H_M), 6.28 (dd, 1 H, $J_{A,X}$ 17.0 Hz, H_A), 6.62 (dd, 1 H, H_X).

N$^{\alpha}$-*[2-Acrylamidoethyl 3-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-glucopyranosiduronoyl]-L-lysine* (**22**). — A solution of **21a** (31 mg, 0.038 mmol) in trifluoroacetic acid (2 mL) was kept for 45 min at 20°, then concentrated. Tetrachloromethane and then MeOH were evaporated from the residue. Water (2 mL) was added to the residue, and the suspension was filtered through a Nylon 66 membrane filter (pore diameter, 0.45 μm; Nucleopore Corp.) and then concentrated to give **22** (30 mg, 97%) as the trifluoroacetate salt, $[\alpha]_D^{26}$ −18° (*c* 1, water). ^{13}C-N.m.r. data (D_2O): δ 175.8, 175.7, 170.8, and 169.5 (C=O), 130.7 ($CH_2=CH$), 128.2 ($CH_2=CH$), 103.5 (C-1), 102.7 (C-1′), 84.3 (C-3), 76.6 (C-5′), 75.5 (C-5), 74.3 (C-2), 73.4 (C-3′), 70.7 (C-4), 70.6 (C-4′), 69.8 (OCH_2CH_2NH), 61.4 (C-6′), 56.5 (C-2′), 53.0 (α-CH), 40.0 (ε-CH_2), 39.9 (OCH_2CH_2NH), 30.7 (δ-CH_2), 26.9 (β-CH_2), 22.9 ($NHCOCH_3$), 22.7 (γ-CH_2).

Copolymerisation of **11** *with acrylamide.* — A solution of **11** (28 mg, 0.066 mmol) and acrylamide (33 mg, 0.464 mmol) in 0.2M acetate buffer (pH 5.8, 2 mL) was deaerated, then *N,N,N′,N′*-tetramethylethylenediamine (5 μL) and ammonium persulfate (1 mg) were added. The mixture was stirred for 18 h at 20° under argon, then diluted with 0.05–0.03M pyridine–acetate buffer (pH 5.4, 3 mL), and eluted from a column (2.5 × 37 cm) of Sephadex G-50 with the same buffer. The high-molecular-weight fraction (detected using a differential refractometer) was collected and lyophilised to give the copolymer **23** (51 mg, 84%), $[\alpha]_D^{26}$ −7° (*c* 1, water). ^{13}C-N.m.r. data (D_2O): δ 180.2 (CONH, polyacrylamide), 170.5 (C=O), 103.2 (C-1), 75.8 (C-3,5), 73.4 (C-2), 72.1 (C-4), 69.0 (OCH_2CH_2NH), 55.4 (α-CH), 42.5–42.8 (CH, polyacrylamide), 39.9 (OCH_2CH_2NHCO and ε-CH_2), 35.5–36.5 (CH_2, polyacrylamide), 31.5 (δ-CH_2), 30.0 (β-CH_2), 22.7 (γ-CH_2).

Copolymer **24**. — Copolymerisation of **22** (28.25 mg, 45.4 μmol) with acrylamide (22.57 mg, 317 μmol), as described above, gave **24** (31.6 mg, 62%), $[\alpha]_D^{28}$ −8.4° (*c* 1, water). ^{13}C-N.m.r. data (D_2O): δ 180.8 (CONH, polyacrylamide), 104.2 (C-1), 103.1 (C-1′), 84.9 (C-3), 77.3 (C-5′), 76.2 (C-5), 75.0 (C-2), 74.0 (C-3′), 71.4 (C-4′), 71.2 (C-4), 62.0 (C-6′), 57.2 (C-2′), 56.0 (α-CH), 42.0–44.0 (CH, polyacrylamide), 40.6 (OCH_2CH_2NH and ε-CH_2), 34.0–38.0 (CH_2, polyacrylamide), 32.3 (δ-CH_2), 27.7 (β-CH_2), 23.7 ($NHCOCH_3$), 23.3 (γ-CH_2).

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