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Synthesis of carbohydrate–amino acid conjugates related to the capsular antigen K54 from *Escherichia coli* O6:K54:H10 and artificial antigens therefrom*

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ABSTRACT

The disaccharides α-L-Rha*p*-(1→3)-β-D-Glc*p*A and β-D-Glc*p*A-(1→3)-α-L-Rha*p* bearing amide-linked L-serine or L-threonine, which represent the repeating unit(s) of the capsular polysaccharide from *E. coli* O6:K54:H10, have been synthesised. *O-tert*-Butyl-protected amino acid *tert*-butyl esters were condensed with the corresponding biouronic acid as the 2-acrylamidoethyl or 2-azidoethyl glycosides. The azido function was replaced by the acrylamido group by catalytic hydrogenation followed by *N*-acryloylation. The *tert*-butyl groups were removed by treatment with trifluoroacetic acid to give the target monomers which were copolymerised with acrylamide to give neoglycoconjugates that are potentially useful for immunochemical studies.

INTRODUCTION

One of the main features of microbial capsular antigens is the presence of negatively charged groups[1]. The acidic components of capsular antigens may be hexuronic acids, neuraminic acid, or amino acids. Amino acids amidically linked to the carboxyl group of uronic acid residues are constituents of several capsular antigens[2–8], often with non-stoichiometric substitution of uronic acid residues.

There have been few syntheses of uronic acids amidically substituted with amino acids, namely, the amides of D-galacturonic acid and methyl β-D-glucopyranosiduronic acid with glycine[9], L-alanine, L-serine, and L-threonine[2]. Synthetic fragments of capsular polysaccharides containing the aforementioned constituents would be of potential interest for immunochemical studies. Most promising, although more complicated, would be the synthesis of these fragments as glycosides with an aglycon suitable for further transformation into artificial antigens, using the traditional approach (conjugation to protein carrier) or *via* copolymerisation with acrylamide[10–18].

Jann *et al.*[5] showed that the K54 antigenic polysaccharide (K-54 antigen) from

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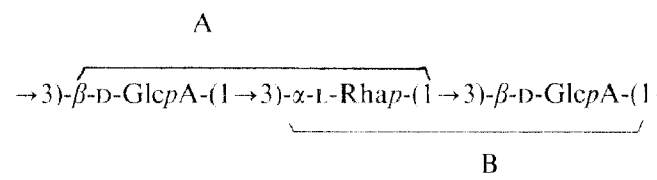
uropathogenic *E. coli* O6:K54:H10 consists of disaccharide repeating units of types A and B:



Of the repeating units, ~85% are substituted in the ratio 1:9 with L-serine and L-threonine amide-linked to the carboxyl group of the glucuronic acid residues.

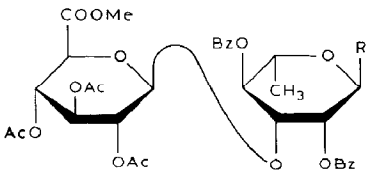
We now describe syntheses of all of the possible fragments of this capsular polysaccharide and their transformation into artificial antigens by copolymerisation with acrylamide.

RESULTS AND DISCUSSION

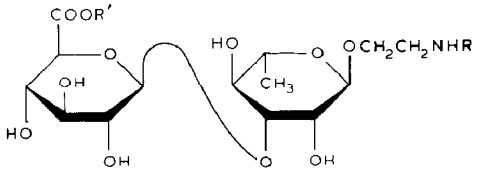
As an aglycon group that can be polymerised at a later stage, we chose the acrylamido function, which has been used in syntheses of artificial antigens[19-23]. The group was introduced by *N*-acryloylation of an aminoethyl aglycon at a late stage in the synthesis. The amino group in the aglycon was blocked initially as the *N*-benzyloxycarbonyl derivative or masked as the 2-azidoethyl group for fragments A and B, respectively. The free amino function could be generated under mild conditions of catalytic hydrogenation without degradation of the uronic acid residues or cleavage of the amide linkages.

2-(Benzyloxycarbonylamino)ethyl glycosides of neutral saccharides can be prepared in high yield[22] and this aglycon was used to prepare a glycoside of fragment A with an L-rhamnose residue as the reducing moiety. This synthesis, which was reported recently[23], involved coupling of methyl 2,4-di-*O*-benzoyl-α-L-rhamnopyranoside[24] with methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate (**7**) in acetonitrile, promoted by mercury(II) salts, to give the disaccharide derivative **1**. Acetolysis of **1** afforded the α-acetate **2**, which was transformed into the α-glycosyl bromide **3** and coupled with 2-(benzyloxycarbonylamino)ethanol in acetonitrile–dichloromethane in the presence of mercury(II) salts, to give the glycoside **4**. Deacylation of **4** followed by catalytic hydrogenation and *N*-acryloylation then gave the 2-acrylamidoethyl glycoside **5**, saponification of which with 0.2M sodium hydroxide in aqueous methanol afforded the disaccharide repeating unit A as the glycoside **6**.

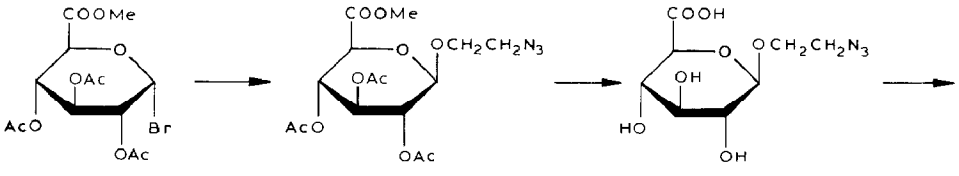
Since the glycosylation of 2-(benzyloxycarbonylamino)ethanol by the glycosyl bromide **7** proceeds in low yield[25], a new procedure was applied to introduce the aminoethyl aglycon by way of an azido precursor in the synthesis of the disaccharide fragment B. In the coupling reaction of **7** and 2-azidoethanol, the latter compound was also used as a solvent at elevated temperature as for the coupling reactions of **7** and benzyl[26] or allyl alcohol[27]. The reaction of **7** and 2-azidoethanol, promoted by mercury-



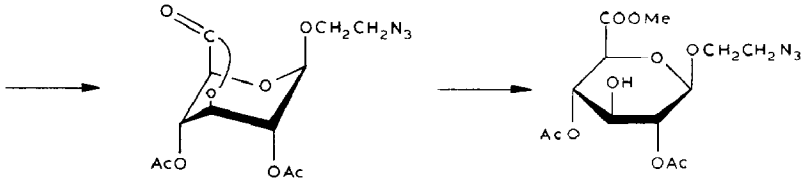
1 R = OMe
2 R = OAc
3 R = Br
4 R = $OCH_2CH_2NHCOOCH_2Ph$



5 R = $COCH{=}CH_2$, R′ = Me
6 R = $COCH{=}CH_2$, R′ = H



7 8 9



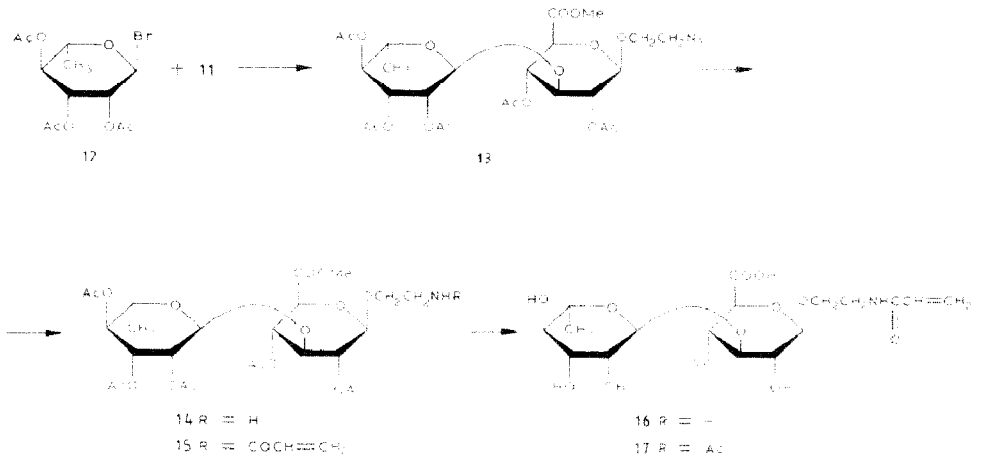
10 $^1C_4$ 11

(II) cyanide (10 min at 105°), gave the pure β-glycoside **8** (68%). The excess of 2-azidoethanol could be distilled from the reaction mixture *in vacuo* and reused.

For the selective liberation of HO-3 in **8**, an approach[28] based on the formation of the 6,3-lactone followed by selective alcoholysis was used. Thus, saponification of **8** with 0.17M sodium hydroxide in aqueous methanol at 4° afforded the glucuronoside **9**, the ^{13}C-n.m.r. spectrum of which (Table II) coincided essentially with those of allyl and methyl β-D-glycopyranosiduronic acids[27,29]. Lactonisation of **9** on heating with acetic anhydride[27,28], followed by reacetylation (acetic anhydride in pyridine), gave the 6,3-lactone **10** (66% from **8**). That the lactone ring in **10** stabilised the $^1C_4$ conformation was confirmed by ^{1}H-n.m.r. data (Table IV). The low ^3J values ($J_{1,2}$ 3.8, $J_{3,4}$ 4.6, $J_{4,5}$ 3.5 Hz) indicated all the substituents to be axial.

The lactone ring in **10** was opened selectively by methanolysis at 20° to give the monohydroxy derivative **11** (76%), the ^{1}H- (Table IV) and ^{13}C-n.m.r. spectra (see Experimental) of which were similar to those of the allyl glycoside analogue[27].

Coupling of **11** and acetobromorhamnose (**12**) in dichloromethane, in the presence of silver triflate at −40° → +20°, afforded the α-L-linked disaccharide derivative **13** (48%). Hydrogenation (Pd/C) of **13** in ethyl acetate in the presence of 1.1 equiv. of



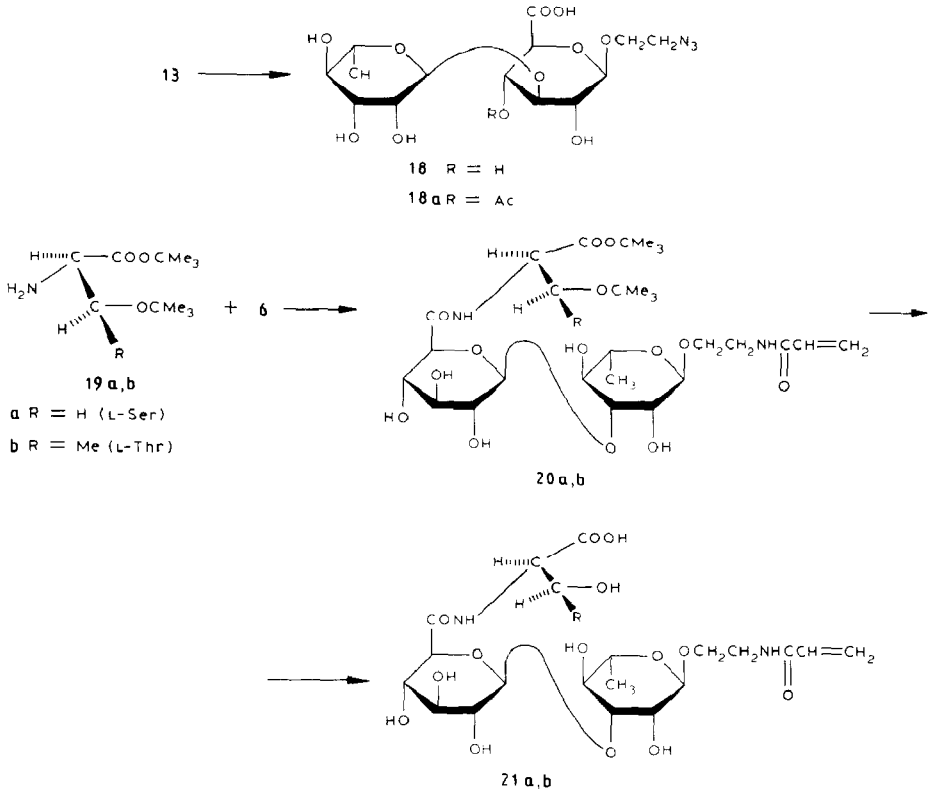
acetic acid gave the aminoethyl glycoside **14** (positive ninhydrin test on t.l.c.). Acylation of **14** with acryloyl chloride in the presence of poly(4-vinylpyridine) afforded the 2-acrylamidoethyl glycoside **15** (44% from **13**), which was identified from the ^{1}H- (Table IV) and ^{13}C-n.m.r. data (see Experimental).

Saponification of **15** with 0.14M sodium hydroxide in aqueous methanol (2 h at 2) gave a mixture of the disaccharide glycoside **16** (36%) and its 4-acetate **17** (52%), which were isolated by ion-exchange chromatography on DEAE-Spheron (AcO form). Treatment of **17** with 0.25M sodium hydroxide (22 h at) gave **16** (83%, total yield). The structure of the synthetic fragment B in the form of **16** was confirmed from ^{1}H- (Table IV) and ^{13}C-n.m.r. data (Table II). The resonances due to C-3′ (δ 71.7) and C-5′ (δ 70.3) were indicative of an α-L-rhamnosidic bond[30,31], and the $J_{1,2}$ value of 7.8 Hz indicated the D-glucuronoside moiety to be β. The structure of the 4-acetate **17** was indicated by the ^{1}H-n.m.r. data (Table IV); the signal of AcO-4 was recognised readily at high field (δ 2.1) and that of H-4 at low field (δ 4.39).

The disaccharide glycosides **6** and **16** are suitable for transformation into neoglycoconjugates *via* copolymerisation, and for the introduction of amino acid residues.

The possibility that self-polymerisation due to the presence of the acryloyl group in the aglycon[32] could cause difficulties at later stages in the synthesis prompted an examination (using fragment B) of the scope for introducing amino acid residues by way of the 2-azidoethyl glycoside **18** followed by transformation into the 2-acrylamidoethyl glycoside. Compound **18** was isolated (91%) after saponification of the disaccharide derivative **13** with 0.2M sodium hydroxide at 4 .

In order to convert the disaccharide fragments A and B into amides of hydroxy-amino acids, **6** and **18** were each coupled with *O*-(*tert*-butyl)-L-serine (**19a**) and *O*-(*tert*-butyl)-L-threonine *tert*-butyl esters (**19b**), using ethyl 2-ethoxy-1,2-dihydroquinoline-1-carboxylate (EEDQ), an effective and mild condensing reagent used in the synthesis of glycopeptides[33-37]. *tert*-Butyl ether and ester groups, which require mild acidic conditions for cleavage (trifluoroacetic acid at room temperature), were used to protect the



hydroxyl and carboxyl functions, respectively, in the amino acids. Glycosidic and amide bonds are stable under these mild acidic conditions[34,37], whereas, on treatment with a base, racemisation of hydroxyamino acids can occur easily[38].

The L-serine (**19a**) and L-threonine (**19b**) derivatives were each condensed (EEDQ) with **6** or **18**, to give the amides **20a** (73%) and **20b** (95%), and **22a** (85%) and **22b** (92%), respectively. The ^{13}C- and ^{1}H-n.m.r. spectra of these compounds indicated the structures assigned. In t.l.c., **20a** appeared to be homogeneous, but h.p.l.c. yielded two fractions (**20a-I** and **20a-II** in the ratio ~1:2) with $[\alpha]_D$ −30° and −50°, respectively (Table I). The spectral features of these fractions are discussed below.

Catalytic hydrogenation (Pd/C) of the 2-azidoethyl glycosides **20a** and **20b** afforded the corresponding 2-aminoethyl glycosides in yields of 95 and 78%, respectively, which were each treated with acryloyl chloride in aqueous methanol in the presence of Dowex 1-X8 (HCO_3^-) resin to give 2-acrylamidoethyl glycosides **23a** (90%) and **23b** (51%), respectively.

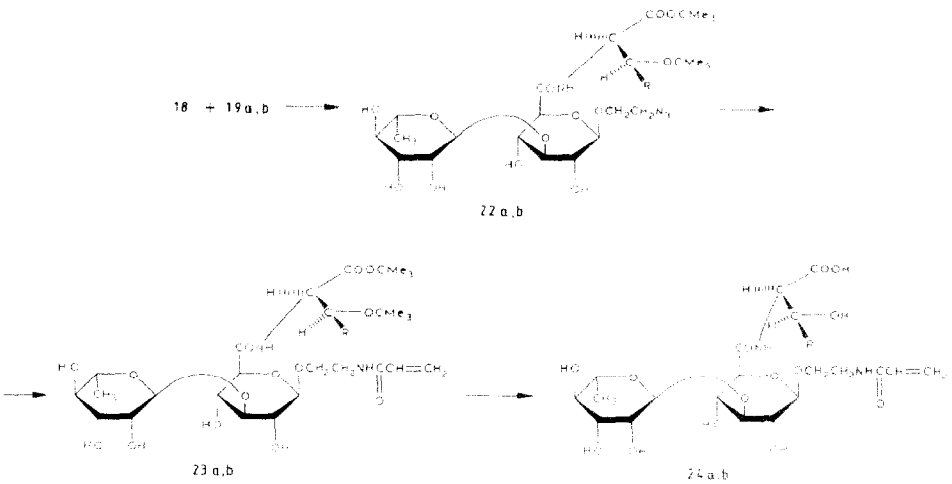
Deprotection of the amides **20a** (major component **20a-II**), **20b**, **23a**, and **23b** by brief treatment with trifluoroacetic acid at 20° afforded the target amino acid-containing disaccharide fragments A and B as the 2-acrylamidoethyl glycosides (**21a** and **21b**, and **24a** and **24b**, respectively). All of the expected signals were observed in the ^{13}C-n.m.r. spectra of these compounds. However, for **21b** and **24a**, line broadening and

TABLE I

Synthesis of **20**–**24**

Starting compound	*Procedure*	*Product*	*Yield (%)*	$[\alpha]_D$ *(degrees)*[a]
6 + **19a**	A	**20a-I**	73	−30 (chloroform)
		20a-II		−50 (chloroform)
6 + **19b**	A	**20b**	95	−67 (chloroform)
20a	C	**21a**	100	−54 (water)
20b	C	**21b**	100	−56 (water)[b]
18 + **19a**	A	**22a**	85	−50 (chloroform)
18 + **19b**	A	**22b**	92	−50 (chloroform)
22a	B	**23a**	90	−33 (chloroform)
22b	B	**23b**	51	−32 (chloroform)
23a	C	**24a**	96	−41 (water)
23b	C	**24b**	100	−40 (water)

[a] At 25–30° (*c* 0.6–1.0). [b] NH_4^+ salt.



diminished intensities of the signals at δ 61.3 (Thr α-C) and 56.6 (Ser α-C) were observed. This effect could reflect an equilibrium between conformers with interconversion at a rate comparable with the n.m.r. time-scale[39].

Each of the 2-acrylamidoethyl glycosides (**6**, **16**, **21a**, **21b**, **24a**, and **24b**) was copolymerised with acrylamide under standard conditions[10,11] (see Experimental) to give the neoglycoconjugates **26**–**31**. Copolymers isolated by gel filtration on Sephadex G-50 in yields of 80–90% consisted of unsubstituted acrylamide units and those *N*-substituted by a sugar moiety in the ratio 10–11:1 as deduced by integration of the appropriate ^{13}C signals or comparison of the $[\alpha]_D$ values for the copolymers and the corresponding carbohydrate monomers.

The Thr α-C signal of the copolymer **29** (prepared from **21b**) appeared as a

broader line with a diminished intensity. No Ser α-C signal was observed for the copolymer **30** (prepared from **24a**). These facts are also consistent with conformational interconversions, as noted above for **21b** and **24a**.

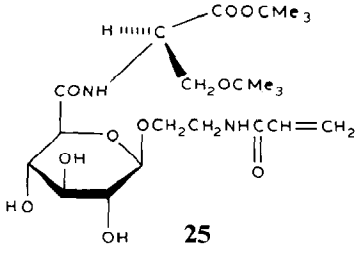
The immunochemical studies of neoglycoconjugates obtained will be reported elsewhere.

Spectral features of the fractions isolated from **20a**. — The serine amide derivative **20a** appeared to be a mixture of at least two components of similar structure but differing in the aglycon moieties. The ^{13}C-n.m.r. spectrum of **20a** contained all the expected signals but, in the double-bond region, there were two pairs of signals (δ 127.9 and 128.5, and δ 126.5 and 131.1) in the ratio ~1:2, which were distinct from two resonances (δ 128.7 and 131.1) for **6**. H.p.l.c. of **20a** gave the fractions **20a-I** and **20a-II** in the ratio ~1:2, the ^{13}C-n.m.r. spectra of which were similar except for the resonances of the aglycon (Table III). Thus, **20a-II** gave signals (δ 126.6 and 131.2) usually indicative[21,22] of the acrylamido group. The ^1H-n.m.r. spectra of **20a-I** and **20a-II** accord with the presence of a terminal double bond (AMX spin system), but the parameters were different (Table III). The spectrum of **20a-I**, recorded after keeping a sample at 4° for 3 months, showed some shifts of the resonances of the acrylamido group without any changes of their intensities and coupling constants (Table III).

From the results of n.O.e. experiments performed on the 2-acrylamidoethyl β-D-glucopyranosiduronamide of L-serine (**25**)* as a model compound, **20a-II** is considered to be the *Z* isomer, and **20a-I** the *E* isomer(s)†. This type of isomerism about the amide bond is known[41].

EXPERIMENTAL

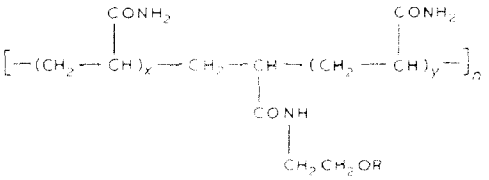
General methods. — T.l.c. was performed on Silica Gel $60F_{254}$ (Merck), using *A*, EtOAc–AcOH–HCOOH–water (18:4:1:3); *B*, EtOH–*n*-BuOH–pyridine–water–AcOH (100:10:10:10:3); *C*, $CHCl_3$–acetone (95:5); benzene–acetone (*D*, 8:2; and *E*, 6:4); hexane–EtOAc (*F*, 6:4; *G*, 1:1; and *H*, 4:6); *J*, $CHCl_3$–ether (7:3); *K*, $CHCl_3$–MeOH (85:15); and *L*, $CHCl_3$–EtOH (9:1); with detection by u.v. light, charring with sulfuric acid, 1%



25

* Synthesis of **25** and separation of isomers **25-I** and **25-II** have been described[40].

† Two sets of signals for H_A, H_M, and H_X in the spectrum of **20a-I** are due to isomerism about the uronamide bond.



26 R = α-L-Rhap-(1→3)-β-D-GlcpA-(1→

27 R = β-D-GlcpA-(1→3)-α-L-Rhap-(1→

28 R = β-D-GlcpA-[6(N)-L-Ser]-(1→3)-α-L-Rhap-(1→

29 R = β-D-GlcpA-[6(N)-L-Thr]-(1→3)-α-L-Rhap-(1→

30 R = α-L-Rhap-(1→3)-β-D-GlcpA-[6(N)-L-Ser]-(1→

31 R = α-L-Rhap-(1→3)-β-D-GlcpA-[6(N)-L-Thr]-(1→

potassium permanganate in aqueous sodium carbonate (for unsaturated compounds), Bromocresol Green (for uronic acids), 0.5M potassium iodide (after exposure to chlorine, for NH-containing compounds), or 0.3% ninhydrin in acetone (for amines). Column chromatography was performed on Silica Gel L (40–100 μm, 100–160 μm), Silpearl (25–40 μm, Czechoslovakia), and LiChroprep Si 60 (40–63 μm, Merck) with solvents that were distilled before use. H.p.l.c. was performed on columns (analytical, 6 × 150 mm; semi-preparative, 16 × 250 mm; preparative, 25 × 250 mm) of Silasorb 600 (5 μm, Czechoslovakia). Eluates were monitored with a differential refractometer (Knauer) or a u.v. detector ISCO, model UA-5 (254 nm) (U.S.A.). For g.l.c., a Hewlett–Packard 5890 instrument equipped with flame-ionisation detector and integrator HP 3393A was used. Separations were performed on a glass capillary column (0.2 mm × 25 m) coated with Ultra-1 (0.33-μm layer) at 200° with nitrogen as the carrier gas at 140 kPa. Elemental analyses were not obtained for syrupy or amorphous compounds, which were purified by column chromatography and characterised by n.m.r. spectroscopy.

^{1}H-N.m.r. (250 MHz) and ^{13}C-n.m.r. (75.43 MHz) spectra were recorded with Bruker WM-250 and AM-300 spectrometers, respectively. Chemical shifts (δ) are reported relative to that of Me_4Si with J values in Hz. The n.m.r. data are given in Tables II–IV. Optical rotations were determined with a DIP-360 (JASCO) polarimeter. Melting points, obtained on a Kofler apparatus, are uncorrected. Acetonitrile was boiled under reflux with $KMnO_4$ and $NaHCO_3$, then distilled over P_2O_5, and over CaH_2 prior to use. Benzene was distilled over CaH_2, toluene over $LiAlH_4$, and acetic anhydride over P_2O_5. Solvents were removed from organic extracts under vacuum with a rotary evaporator at <40° (bath) (or at <30° for unsaturated compounds). 2-Azidoethanol was synthesised according to the method described[42].

Methyl (2-azidoethyl 2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate (**8**). — Crystalline methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate[11] (7; 1.19 g, 3.0 mmol) was added in one portion to a solution of mercury(II) cyanide (780 mg, 3.1 mmol) in 2-azidoethanol[42] (3.27 mL, 43.2 mmol) at 105°. The mixture was stirred at 105–110° for 10 min, when t.l.c. (solvent *F*) revealed **8** (R_f 0.30) but no **7** (R_f 0.59).

Stirring was continued overnight at room temperature and the excess of 2-azidoethanol was then removed *in vacuo* (< 1 mm). The residue was partitioned between chloroform (50 mL) and water (50 mL), the aqueous layer was extracted with chloroform (3 × 50 mL), the combined organic phases (~200 mL) were washed with M NaI (4 × 200 mL), aqueous $NaHCO_3$ (200 mL), and water (200 mL), and dried (Na_2SO_4), and the solvent was evaporated. Column chromatography (light petroleum–EtOAc, 65:35) of the residue (1.25 g) gave crystalline **8** (840 mg, 69%) that was homogeneous in h.p.l.c. (hexane–EtOAc, 65:35), but g.l.c. revealed 3% of the α anomer. Recrystallisation from ether gave an analytical sample with m.p. 96–98°, $[\alpha]_D^{24}$ −58° (*c* 1, chloroform); ν_{max} 2120 cm^{-1} (N_3). ^{13}C-N.m.r. data ($CDCl_3$): δ 170.1, 169.4, 167.1 (C=O), 100.6 (C-1), 72.6, 72.0, 71.0, 69.4, 68.8 (C-2,3,4,5 and OCH_2), 52.9 ($COOCH_2$), 50.5 (CH_2N_3), 20.6 ($OCOCH_3$). The ^{1}H-n.m.r. data are given in Table IV.

Anal. Calc. for $C_{15}H_{21}N_3O_{10}$: C, 44.67; H, 5.25; N, 10.42. Found: C, 44.43; H, 5.32; N, 10.56.

*2-Azidoethyl 2,4-di-*O*-acetyl-β-*D*-glucopyranosidurono-6,3-lactone* (**10**). — Cold M NaOH (1.67 mL, 1.67 mmol) was added dropwise to a cooled solution of **8** (130 mg, 323 mmol) in methanol (10 mL). The mixture was kept at 4°, then neutralised with KU-2 (H^+) resin, and concentrated. Toluene was evaporated (4 times) from the residue which was then dried *in vacuo* over KOH. The resulting uronic acid **9** (R_F 0.63, solvent *A*) was heated with acetic anhydride (6 mL) at 70° for 1.5 h. The mixture was cooled, pyridine (6 mL) was added, and the mixture was kept at 20° for 12 h. T.l.c. (solvent *G*) then revealed no **8** (R_F 0.49), but a product with R_F 0.42. The mixture was concentrated, and toluene and heptane were evaporated from the residue, a solution of which in chloroform was washed through a column of SEP-PAK Si (Millipore) with ethyl acetate (20 mL). Concentration of the eluate gave a colorless syrup (110 mg) which was purified by chromatography (hexane–ethyl acetate, 8:2) on a column (10 × 250 mm) of LiChroprep Si 60, to give **10** (70.5 mg, 66.5%), $[\alpha]_D^{25}$ −154° (*c* 4, chloroform), R_F 0.57 (solvent *H*). The ^{1}H-n.m.r. data are given in Table IV.

*Methyl (2-azidoethyl 2,4-di-*O*-acetyl-β-*D*-glucopyranosid)uronate* (**11**). — A solution of **10** (50.7 mg, 154 μmol) in MeOH (2.5 mL) was kept at 22° for 92 h, then concentrated, and benzene was evaporated twice from the residue. Column chromatography (solvent *E*) gave **11** (42.4 mg, 76%), m.p. 105–106° (from ethanol), $[\alpha]_D^{25}$ −83° (*c* 1, chloroform), R_F 0.26 (solvent *H*). ^{13}C-N.m.r. data ($CDCl_3$): δ 170.5, 170.3, 167.7 (C=O), 100.6 (C-1, $J_{C\text{-}1,H\text{-}1}$ 161.1 Hz), 73.3, 72.7, 72.6, 71.8 (C-2,3,4,5), 68.5 (OCH_2), 52.8 ($COOCH_3$), 50.5 (CH_2N_3), 20.8, 20.6 ($OCOCH_3$). The ^{1}H-n.m.r. data are given in Table IV.

Anal. Calc. for $C_{13}H_{19}N_3O_9$: C, 43.22; H, 5.30; N, 11.63. Found: C, 43.10; H, 5.30; N, 11.68.

*Methyl [2-azidoethyl 2,4-di-*O*-acetyl-3-*O*-(2,3,4-tri-*O*-acetyl-α-*L*-rhamnopyranosyl)-β-*D*-glucopyranosid]uronate* (**13**). — A suspension of 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl bromide [**12**; prepared[43] from 1,2,3,4-tetra-*O*-acetyl-L-rhamnopyranose (157.5 mg, 474 μmol)] and **11** (108.6 mg, 300 μmol) in toluene (2 mL) was stirred with molecular sieves 4Å for 1 h under argon. To the stirred mixture at −40° was added

TABLE II

^{13}C-N.m.r. data[a] (δ in p.p.m., $J_{C-1,H-1}$ in Hz)

Compound	*Sugar residue*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*C=CH$_2$CH$_2$*	*C=CH$_2$CH$_2$*	*OCH$_2$CH$_2$N*	*OCH$_2$CH$_2$N*	*Amino acid residue*			*CH$_2$=CH*	*CH$_2$=CH*
												α-C	*β-C*	*γ-C*		
5[b]	β-D-GlcA	104.9	73.9	76.0	72.1	75.5			–	66.5	39.6	–			126.8	131.0
	α-L-Rha	100.3	70.4	82.9	71.7	68.6	17.8									
6	β-D-GlcA	105.0 (164)	74.1	76.3	72.1	75.8			–	67.0	40.2	–			128.7	131.0
	α-L-Rha	100.6 (171)	70.9	81.8	72.5	69.7	17.8									
9	β-D-GlcA	103.2 (165)	73.5	76.0	72.0	75.4	–	–		68.5	51.3	–	–			–
16	α-L-Rha	102.4	71.6	71.7	73.4	70.3	17.9		–	70.1	40.8				128.8	131.4
	β-D-GlcA	103.7	74.7	83.2	71.4	77.2	–									
18[b]	α-L-Rha	102.5 (171)	71.5	71.6	73.3	70.1	17.8		–	70.1	51.8			–		
	β-D-GlcA	103.5 (161)	74.6	82.9	71.1	76.5	–									
20a-11[c]	β-D-GlcA	103.9	73.3	76.7	71.5	75.8	–	73.3	27.4	66.8	39.5	53.2	62.0		126.6	131.2
	α-L-Rha	100.0	70.7	81.7	72.1	68.5	17.8	82.8	28.1							
21a	β-D-GlcA	105.3	74.3	76.5	72.1	76.1			–	67.2	40.4	55.8	62.5		128.9	131.3
	α-L-Rha	100.8	71.3	82.2	72.8	69.9	18.0									
21b	β-D-GlcA	105.3	74.4	76.6	72.2	76.2		–		67.3	40.5	61.2	69.4	20.7	128.9	131.4
	α-L-Rha	101.1	71.3	82.3	73.1	70.0	18.1		–							
22a[c]	β-D-GlcA	102.8	73.1	81.4	70.8	73.4	–	73.3	27.3	68.7	50.8	52.9	62.0			–
	α-L-Rha	101.1	70.9	71.4	72.7	68.8	17.5	82.1	28.1							

22b[b]	β-D-GlcA	102.8	73.3	81.4	70.9	73.5	–	74.0	28.2	68.7	50.8	56.2	67.2	21.0	–	–
	α-L-Rha	101.1	71.2	71.5	72.8	68.8	17.6	82.2	28.7							
23a[b]	β-D-GlcA	102.9	73.3	82.3	70.8	73.3	–	73.3	27.4	69.4	39.7	53.1	61.9	–	126.7	131.0
	α-L-Rha	101.3	70.8	71.3	72.7	68.9	17.6	82.3	28.1							
24a	β-D-GlcA	103.7	74.7	83.2	71.3	76.5	–	–	–	70.3	40.8	56.6	62.7	–	128.9	131.5
	α-L-Rha	102.5	71.4	71.8	73.5	70.4	18.0									
24b	β-D-GlcA	103.8	74.7	83.2	71.2	76.4	–	–	–	70.2	40.8	59.2	68.6	20.2	128.9	131.4
	α-L-Rha	102.5	71.6	71.8	73.4	70.3	17.9									
26	α-L-Rha	102.3	71.7	71.7	73.4	70.2	17.9	–	–	69.5	40.8	–	–	–	–	–
	β-D-GlcA	103.5	74.7	83.3	71.5	77.8	–									
27	β-D-GlcA	104.8	74.3	76.4	72.2	77.1	–	–	–	69.7	40.8	–	–	–	–	–
	α-L-Rha	102.3	70.7	81.6	72.8	69.7	18.0									
29	β-D-GlcA	105.3	74.3	76.6	72.3	76.2	–	–	–	67.1	40.4	60.1	69.0	20.5	–	–
	α-L-Rha	101.0	71.2	82.3	73.1	70.0	18.2									
30	α-L-Rha	102.5	71.6	71.8	73.5	70.3	18.0	–	–	70.3	40.8	–	63.0	–	–	–
	β-D-GlcA	103.9	74.7	83.2	71.3	76.5	–									

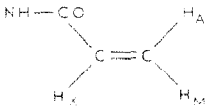
[a] For solutions in D_2O; other chemical shifts: δ 35.4–37.3 (CH_2, polyacrylamide), 42.8–43.6 (CH, polyacrylamide), 52.9 ($COOCH_3$), 166.6–174.4 (C=O), 180.9 (CONH, polyacrylamide). [b] For solutions in $CDCl_3$.

TABLE III

N.m.r. data for the acrylamido fragments[a] of the isomers **20a** and **25**

Compound	^1H (δ *in p.p.m.*)			*J* (*Hz*)			^{13}C (δ *in p.p.m.*)	
	H_A	H_X	H_M	J_{AM}	J_{AX}	J_{MX}	CH_2=CH	CH_2=CH
20-a-I	6.32dd	5.71dd	6.61dd	2.0	17.0	10.5	127.9	128.3
	6.35dd	5.71dd	6.71dd	2.0	17.0	10.5	127.9	128.4
20-a-I	6.17dd	5.60dd	6.50dd	2.0	17.0	10.5		
after 3 months	6.18dd	5.63dd	6.51dd	2.0	17.0	10.5		
20-a-II	6.31dd	5.61dd	6.21dd	3.0	17.5	9.0	126.6	131.2
25-I	6.27dd	5.69dd	6.55dd	2.0	17.0	10.5	127.5	129.5
25-II	6.22dd	5.56dd	6.12dd	3.0	17.0	8.8	126.6	131.0

[a]



, AMX spin system.

dropwise during 20 min a solution of silver trifluoromethanesulfonate (173.5 mg, 675 μmol) in toluene (2.1 mL). The mixture was stirred at −40° for 1 h under argon, then allowed to attain room temperature, and stirring was continued for 12 h. The mixture was filtered through Celite-545, the filter cake was washed with chloroform, and the combined filtrate and washings were washed successively with saturated aqueous $NaHCO_3$, aqueous $Na_2S_2O_3$, and water, dried (Na_2SO_4), and concentrated. Column chromatography (gradient of 0 → 15% of acetone in toluene) of the residue followed by h.p.l.c. (hexane–ethyl acetate, 63:37) gave **13** (92 mg, 48%), $[\alpha]_D^{20}$ −46° (*c* 1, chloroform), R_f 0.28 (solvent *G*). ^{13}C-N.m.r. data ($CDCl_3$): δ 170.1, 170.0, 169.4, 167.4 (C=O), 100.6 (C-1, $J_{C-1,H-1}$ 158.7 Hz), 99.2 (C-1', $J_{C-1',H-1'}$ 168.5 Hz), 80.1 (C-3), 72.9, 71.3, 70.8, 70.7, 70.0, 68.9, 67.5 (C-2',3',4',5', and C-2,4,5), 68.2 (OCH_2), 52.9 ($COOCH_3$), 50.7 (CH_2N_3), 20.8, 20.7 ($OCOCH_3$), 17.3 (C-6'). The ^{1}H-n.m.r. data are given in Table IV.

*Methyl [2-acrylamidoethyl 2,4-di-*O*-acetyl-3-*O*-(2,3,4-tri-*O*-acetyl-*α*-*L*-rhamnopyranosyl)-β-*D*-glucopyranosid]uronate* (**15**). A solution of **13** (88 mg, 139 μmol) in ethyl acetate (2 mL) and MeOH (2 mL) was hydrogenated at room temperature over 10% Pd/C. After 1 h, t.l.c. (solvent *D*) showed the complete conversion of **13** (R_f 0.43) into **14** (positive ninhydrin test) (R_f 0.54, solvent *B*). The mixture was filtered, then concentrated, and benzene was evaporated from the residue, which was dried *in vacuo*. A solution of the residue in ethyl acetate (2 mL) was cooled (ice–water) and stirred with poly(4-vinylpyridine) (Fluka). Acryloyl chloride (51 μL, 627 μmol) was added in three equal portions at intervals of 30 min. The mixture was then stirred at 20° for 12 h, when t.l.c. (solvent *E*) showed the conversion of **14** into a single product (R_f 0.40). The mixture

was filtered, the solids were washed with ethyl acetate, and the combined filtrate and washings were concentrated. Column chromatography (ethyl acetate) of the residue gave **15** (40.4 mg, 44%), $[\alpha]_D^{24}$ −24° (*c* 1, chloroform), R_F 0.31 (ethyl acetate). ^{13}C-N.m.r. data ($CDCl_3$): δ 169.8, 169.5 (C=O), 130.9 (*C*H=CH_2), 126.5 (CH=*C*H_2), 100.8 (C-1), 99.1 (C-1′), 79.7 (C-3), 72.5, 71.8, 70.6, 70.3, 69.9, 68.9, 67.6 (C-2′,3′,4′,5′ and C-2,4,5), 69.4 (OCH_2), 53.0 ($COOCH_3$), 39.4 (CH_2NH), 21.0, 20.8, 20.7 ($OCOCH_3$), 17.3 (C-6′). The ^{1}H-n.m.r. data are given in Table IV.

2-Acrylamidoethyl 3-O-(α-L-rhamnopyranosyl)-β-D-glucopyranosiduronic acid (**16**). — To a cooled (ice–water) solution of **14** (40 mg, 60.6 μmol) in MeOH (3.5 mL) was added M NaOH (608 μL, 608 μmol) dropwise during 10 min. The mixture was kept at 4° for 2 h, then diluted with equal volume of water, and eluted with water from a column (10 × 130 mm) of KU-2 (H^+) resin. The eluate was applied to a column (15 × 120 mm) of DEAE-Spheron (AcO^- form) and eluted with a linear gradient of aqueous acetic acid (0 → 20%; total volume, 200 mL) at 3 mL/min, to give **16** (9.6 mg, 36%) and the 4-acetate (**17**; 15.7 mg, 52%), R_F 0.34 (solvent *A*). To a cooled (ice–water) solution of **17** in water (2 mL) was added M NaOH (0.5 mL) dropwise during 10 min. The mixture was kept at 1° for 22 h and worked-up, as described above, to give **16** (12.2 mg; 82.5% total yield), $[\alpha]_D^{25}$ −43° (*c* 1, water). The ^{13}C-n.m.r. data for **16** are given in Table II, and the ^{1}H-n.m.r. data for **16** and **17** are given in Table IV.

2-Azidoethyl 3-O-(α-L-rhamnopyranosyl)-β-D-glucopyranosiduronic acid (**18**). — To a cooled (ice–water) solution of **13** (300 mg, 474 μmol) in MeOH (40 mL) was added M NaOH. The mixture was kept at 1° for 2 h, then neutralised with KU-2 (H^+) resin, and filtered, and the resin was washed with water. The combined filtrate and washings were applied to a column (1.5 × 12.5 cm) of DEAE-Spheron (AcO^- form). The column was irrigated with water and then eluted with a linear gradient of aqueous acetic acid (0 → 20%; total volume, 200 mL) at 3 mL/min, to give **18** (87.2 mg) and the 4-acetate (**18a**, 108.5 mg). To a cooled (ice–water) solution of **18a** in water (9 mL) was added M NaOH (2.25 mL). The mixture was kept at 8° for 24 h and worked-up, as described above, to give **18** (90.6 mg; total yield, 91%), $[\alpha]_D^{25}$ −69.5° (*c* 2, methanol). The ^{13}C-n.m.r. data for **18** are given in Table II.

Condensation of uronic acids with hydroxyamino acid derivatives (procedure A). — A mixture of uronic acid (0.01–0.6 mmol), *O-tert*-butyl amino acid *tert*-butyl ester (1.5 equiv.), and EEDQ (2 equiv.) in *N,N*-dimethylformamide (2 mL, freshly distilled *in vacuo* over ninhydrin) was kept at 20° for 24–72 h until disappearance of uronic acid was complete (t.l.c.; $CHCl_3$–MeOH–AcOH, 85:15:1). The mixture was then concentrated and toluene was evaporated from the residue, a solution of which in MeOH (3–5 mL) was treated with KU-2 (H^+) resin (20–30 mL) in order to remove quinoline and unreacted amino component. The mixture was filtered, the resin was washed with MeOH (50–100 mL), and the combined filtrate and washings were concentrated. The residue was purified by column chromatography (0 → 15% of methanol in chloroform). The yields and $[\alpha]_D$ values of the products (**20a,b** and **22a,b**) are given in Table I, and the ^{13}C-n.m.r. data are given in Table II. ^{1}H-N.m.r. data ($CDCl_3$, selected signals): **20b**, δ 6.30 (dd, 1 H, $J_{A,M}$ 3.0, $J_{A,X}$ 17.5 Hz, H_A), 6.24 (dd, 1 H, $J_{A,X}$ 17.5 Hz, H_X), 5.60 (dd, 1 H,

TABLE IV

^{1}H-N.m.r. data[a]

Compound	*Sugar residue*	*Chemical shifts (δ) (J in Hz)*										
		H-1 ($J_{1,2}$)	*H-2* ($J_{2,3}$)	*H-3* ($J_{3,4}$)	*H-4* ($J_{3,4}$)	*H-5* ($J_{4,5}$)	*H-6* ($J_{5,6}$)	CH_3CO	$COOCH_3$	OCH_AH_B-CH_CH_DN	OCH_AH_B-CH_CH_DN	$CH_2=CH$ $CH_2=CH$
8	β-D-GlcA	4.64d (7.8)	5.04m	5.25m (2 H)		4.05d (9.6)	–	2.03s 2.04s 2.07s	3.86s	3.69ddd (A) 4.09ddd (B) $J_{A,B}$ 15.2 $J_{A,C}$ 8.6 $J_{B,D}$ 4.9	3.29ddd (D) 3.51dddd (C) $J_{C,D}$ 13.6 $J_{B,C}$ 3.5 $J_{A,D}$ 3.5	–
10	β-D-GlcA	4.94bs (~ 1)	5.11m (3.8)	5.09m	4.86 dddd (4.6) $J_{3,4}$ 1 $J_{4,5}$ 1	4.23m (3.5) $J_{5,6}$ 1	–	2.10s 2.18s	–	3.58ddd (A) 3.94ddd (B) $J_{A,B}$ 10.3 $J_{A,C}$ 8.3 $J_{B,D}$ 4.8	3.28ddd (D) 3.46ddd (C) $J_{C,D}$ 12.9 $J_{B,C}$ 3.6 $J_{A,D}$ 3.6	
11	β-D-GlcA	4.56d (7.8)	4.92dd (9.5)	3.77bt	5.08dd (9.3)	3.96d (9.8)		2.08s 2.11s	3.73s	3.66ddd (A) 4.04ddd (B) $J_{A,B}$ 10.6 $J_{A,C}$ 8.5 $J_{B,D}$ 4.8	3.25ddd (D) 3.48ddd (C) $J_{C,D}$ 12.7 $J_{B,C}$ 3.3 $J_{A,D}$ 3.3	–
13	α-L-Rha	4.85d (1.6)	5.07dd (3.6)	4.95–5.11m (2 H)		3.88dq (9.0)	1.13d (6.0)	1.96s 2.03s 2.08s 2.13s 2.17s	3.75s	3.55ddd (A) 4.04ddd (B) $J_{A,B}$ 10.5 $J_{A,C}$ 8.0 $J_{B,D}$ 4.5	3.29ddd (D) 3.49ddd (C) $J_{C,D}$ 13.2 $J_{A,D}$ 3.2 $J_{B,C}$ 3.5	
	β-D-GlcA	4.54d (7.5)	5.09dd (9.0)	3.87t (9.0)	5.17t (9.5)	3.94d (9.7)						

15	α-L-Rha	4.87d (2.0)	5.02–5.14m (2 H)		5.07dd (9.5)	3.88dq (9.5)	1.15d (6.0)	1.98s 2.07s 2.11s 2.18s (6 H)	3.77s	3.70–3.85m (2 H)	3.45–3.65m (2 H)	5.65dd 6.31dd $J_{A,M}$ 2.0 $J_{A,X}$ 16.5 $J_{M,X}$ 9.5	6.16dd
	β-D-GlcA	4.49d (7.1)	5.10dd	3.88t (8.5)	5.02t (8.5)	3.97d (9.3)	–						
16[b]	α-L-Rha	5.06d (1.5)	3.99dd (3.4)	3.73dd	3.38dd (9.6)	3.95dq (9.6)	1.19d (6.1)	–	–	3.70–3.95m (2 H)	3.40–3.50m (2 H)	5.70dd, 6.13dd $J_{A,M}$ 2.1 $J_{A,X}$ 16.9 $J_{M,X}$ 9.2	6.23dd
	β-D-GlcA	4.47d (7.8)	3.39m	3.52–3.64m (2 H)		3.86d (9.5)	–						
17[b]	α-L-Rha	5.06d (1.8)	3.95dd (3.1)	3.65dd	3.36dd (9.5)	3.57dq (9.0)	1.21d (6.0)	2.10s	–	3.70–3.85m (1 H)	3.40–3.50m (2 H)	5.70dd. 6.13dd $J_{A,M}$ 2.4 $J_{A,X}$ 17.0 $J_{M,X}$ 9.3	6.13dd
	β-D-GlcA	4.51d (8.0)	3.49dd (9.2)	3.87dd	4.93dd (9.2)	4.07d (9.8)				3.85–4.00m (1 H)			

[a] For solutions in $CDCl_3$. [b] For solutions in D_2O.

$J_{A,M}$ 3.0, $J_{M,X}$ 9.3 Hz, H_M), 4.79 (d, 1 H, $J_{1',2'}$ 7.0 Hz, H-1'), 4.72 (bs, 1 H, $J_{1,2}$ ~1 Hz, H-1), 4.33 (dd, 1 H, $J_{\alpha,\beta}$ 2.2, $J_{\alpha,NH}$ 8.7 Hz, Thr α-H), 4.18 (dq, 1 H, $J_{\alpha,\beta}$ 2.2, $J_{\beta,\gamma}$ 6.2 Hz, Thr β-H), 1.44 (s, 9 H, ^{t}Bu), 1.28 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6,6,6), 1.17 (d, 3 H, 3 Thr γ-H), 1.13 (s, 9 H, ^{t}Bu); **22b**, δ 5.08 (bs, 1 H, $J_{1,2}$ ~1 Hz, H-1'), 4.32 (d, 1 H, $J_{1,2}$ 7.7 Hz, H-1), 4.23 (dd, 1 H, $J_{\alpha,\beta}$ 2.0, $J_{\alpha,NH}$ 5.6 Hz, Thr α-H), 4.14 (dq, 1 H, $J_{\beta,\gamma}$ 6.2 Hz, Thr β-H), 1.38 (s, 9 H, ^{t}Bu), 1.18 (d, 1 H, $J_{5,6}$ 6.2 Hz, H-6'), 1.09 (d, 3 H, 3 Thr γ-H), 1.08 (s, 9 H, ^{t}Bu).

Conversion of 2-azidoethyl glycosides into 2-acrylamidoethyl glycosides (procedure B). — A solution of 2-azidoethyl glycoside (0.1 mmol) in methanol (2 mL) was hydrogenated at atmospheric pressure over 10% Pd/C. After 1–2 h, t.l.c. (solvent *B*) showed the absence of the starting material and presence of a single product (positive ninhydrin test). The mixture was filtered, the solids were washed with methanol (100 mL), the combined filtrate and washings were concentrated, and the residue was dried *in vacuo* to give the 2-aminoethyl glycoside (70–100%). To a solution of the aminoethyl glycoside in methanol (4 mL) was added 2,6-di-*tert*-butyl-4-methylphenol (1–2 mg) as radical inhibitor, and then water (0.5 mL). The solution was stirred with Dowex 1-X8 (HCO_3^-) resin, acryloyl chloride (3 equiv.) was added, stirring was continued for 18 h, and more acryloyl chloride (3 equiv.) was added if necessary. After 2 h, the mixture was filtered, the solids were washed with methanol (100 mL), and the combined filtrate and washings were concentrated. Column chromatography (0 → 15% of methanol in chloroform) of the residue gave the protected amino acid–saccharide derivative. The yields and $[\alpha]_D$ values for the glycosides obtained (**23a,b**) are given in Table I, and the ^{13}C-n.m.r. data in Table II. ^{1}H-N.m.r. data ($CDCl_3$, only selected signals): **23b**, δ 6.27 (m, 2 H, H_A and H_X), 5.61 (dd, 1 H, $J_{A,M}$ 4.0, $J_{M,X}$ 7.3 Hz, H_M), 4.35 (d, 1 H, $J_{1,2}$ 7.0 Hz, H-1), 4.30 (dd, 1 H, $J_{\alpha,NH}$ 8.5 Hz, Thr α-H), 4.00 (dd, 1 H, $J_{\alpha,\beta}$ 2.1, $J_{\beta,\gamma}$ 6.2 Hz, Thr β-H), 1.48 (s, 9 H, ^{t}Bu), 1.29 (d, 3 H, $J_{5,6}$ 6.0 Hz, H-6',6',6'), 1.18 (d, 3 H, Thr 3 γ-H), 1.15 (s, 9 H, ^{t}Bu).

Removal of tert-*butyl ether and ester protecting groups (procedure C).* — A solution of *tert*-butyl-protected amino acid–saccharide derivative (0.05 mmol) in trifluoroacetic acid (1–2 mL; distilled over P_2O_5) was kept at 20° for 20–40 min, then concentrated. Tetrachloromethane and then methanol were evaporated from the residue, which was dried *in vacuo* over KOH. Water (2 mL) was added to the residue, and the suspension was filtered through a nylon filter (pore diameter, 0.45 μm; Nucleopore Corp.) and then concentrated to give the target monomer (**21a,b** and **24a,b**). The yields and $[\alpha]_D$ values are given in Table I, and the ^{13}C-n.m.r. data in Table II.

Copolymerisation of **16** *with acrylamide.* — A solution of **16** (21.8 mg, 50 μmol) and acrylamide (35.4 mg, 500 μmol) in distilled water (1 mL) was deaerated using a water pump. An aliquot (20 μL) of a solution of *N,N,N',N'*-tetramethylethylenediamine (10 μL) in water (90 μL) and ammonium persulfate (1 mg) were added, and the mixture was stirred at 20° under argon. After 10 min, more water (1 mL) was added to the viscous solution, and stirring was continued for 24 h. The mixture was then diluted with water (2 mL), applied to a column (2.6 × 40 cm) of Sephadex G-50, and eluted with 0.05/0.03M pyridine–acetate buffer (pH 5.5) at 1 mL/min. The higher-molecular-weight fraction (detected using a differential refractometer) was collected and lyophilised to give the copolymer **26** (46.8 mg, 82%), $[\alpha]_D^{28}$ −21° (*c* 1, water). The ^{13}C-n.m.r. data are given in Table II.

Copolymer **27**. — Copolymerisation of **6** (20 mg, 45.7 μmol) with acrylamide (32.5 mg, 457 μmol) gave **27** (44.9 mg, 85%), $[\alpha]_D^{28}$ −18° (*c* 0.5, water). The ^{13}C-n.m.r. data are given in Table II.

Copolymer **28**. — Copolymerisation of **21a** (12.8 mg, 24.7 μmol) with acrylamide (12.1 mg, 170.5 μmol) gave **28** (23 mg, 93%), $[\alpha]_D^{28}$ −17° (*c* 1, water).

Copolymer **29**. — Copolymerisation of **21b** (as the NH_4^+ salt, 32 mg, 57.5 μmol) and acrylamide (28.6 mg, 403 μmol) gave copolymer **29** (54 mg, 89%), $[\alpha]_D^{30}$ −23° (*c* 1, water). The ^{13}C-n.m.r. data are given in Table II.

Copolymer **30**. — Copolymerisation of **24a** (48.7 mg, 92.7 μmol) and acrylamide (46.1 mg, 648.8 μmol) gave **30** (77.4 mg, 82%), $[\alpha]_D^{29}$ −19° (*c* 1, water). The ^{13}C-n.m.r. data are given in Table II.

Copolymer **31**. — Copolymerisation of monomer **24b** (25.5 mg, 47.2 μmol) and acrylamide (23.5 mg, 331 μmol) gave copolymer **31** (40 mg, 82%), $[\alpha]_D^{25}$ −19° (*c* 1, water).

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