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Synthesis of glycuronamides of amino acids, constituents of microbial polysaccharides and their conversion into neoglycoconjugates of copolymer type

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Glycopyranosiduronic acids, amidically linked to amino acids (alanine, serine, threonine, and lysine) were prepared. *O-tert*-Butyl and *Nε-tert*-butyloxycarbonyl protected amino acid *tert*-butyl esters were used in ethyl 2-ethoxy-1,2-dihydroquinoline-1-carboxylate promoted condensation with 2-azidoethyl glycosides of glucuronic and galacturonic acid. Reduction of the azido-function followed by *N*-acryloylation and removal of blocking groups with trifluoroacetic acid gave the target monomers. These were converted into neoglycoconjugates of copolymer type, potentially useful for immunochemical studies.

Keywords: amino acid-glycuronamides, synthesis, neoglycoconjugates

Amino acids were reported [1–12] to be non-carbohydrate constituents of microbial polysaccharides and are involved in ester linkages in teichoic acids [1]. Amide linkages involving the carboxyl group of amino acids are encountered with aminocarbohydrate components in the cell wall polysaccharide of *Staphylococcus aureus* [2] and *Escherichia coli* O114 [3]. Amino acids amidically linked to carboxyl groups of carbohydrates were found to be constituents of capsular polysaccharides from *Haemophilus influenza* type d [4, 5], *E. coli* O6:K54:H10 [6] and O8:K40:H9 [7] and lipopolysaccharides from *Rhodopseudomonas sphaeroides* ATCC 17023 [8], *Proteus mirabilis* S1959 [9, 10], *P. mirabilis* O27 [11], and *Serratia marcescens* O23 [12]. The frequent occurrence of amino acid residues (mainly lysine and alanine) was reported (cf. [9] and references therein) for O-antigens in most of *P. mirabilis* O-serogroups.

In the synthesis of the amide-linked amino acid–uronic acid derivatives described, uronic acids were used as free monosaccharides [13] or methyl glycosides [4]. For immunochemical applications derivatives would be more promising in the form of glycosides with a chemically reactive aglycone, which can easily be transformed into neoglycoconjugates.

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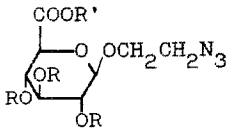
We describe herein D-gluco- and D-galactopyranosiduronamides of amino acids (L-alanine, L-serine, L-threonine, and L-lysine) which are components (or anomers) of capsular polysaccharides from *E. coli* [6, 7] and O-specific chains of *P. mirabilis* [9–11], and their transformation into neoglycoconjugates via copolymerization.

Results and discussion

As an aglycone we used the 2-azidoethyl group [14] containing a masked amino function. The latter is suitable for coupling to carrier proteins to prepare neoglycoproteins [15] or after *N*-acryloylation for transformation into neoglycoconjugates via copolymerization with acrylamide [16–20].

Synthetic sequence

2-Azidoethyl glycoside **2** was obtained starting from glucuronic acid. Condensation of methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate with 2-azidoethanol promoted by mercury(II) cyanide afforded tri-*O*-acetate **1**. Saponification of **1** with cold aqueous sodium hydroxide in methanol gave azidoethyl glycoside **2** isolated by ion-exchange chromatography on a Dowex 1-X8 column (AcO^--form).



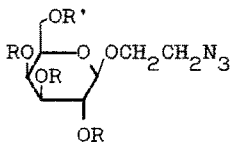
1 R = Ac, R' = Me

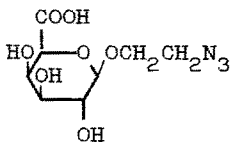
2 R = R' = H

Oxidation of an appropriate galactose derivative was used to prepare the 2-azidoethyl glycoside of galacturonic acid. Reaction between 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide and 2-azidoethanol in acetonitrile in the presence of mercury(II) salts resulted in stereoselective glycosylation, thereby affording acetylated galactoside **3** (90%). Treatment of **3** with sodium methoxide in methanol followed by conventional tritylation gave the 6-*O*-trityl ether **4**, which was then treated with acetic anhydride in pyridine to give the acetylated 6-*O*-trityl-galactoside **5**. Galactoside **5** was converted to 2-azidoethyl β-D-galactopyranosiduronic acid **6** by oxidation with Jones reagent [21] (directly, without a detritylation step for generation of a primary alcoholic function [22]) followed by *O*-deacetylation with aqueous sodium hydroxide in methanol at low temperature. Ion-exchange chromatography was used for purification of uronic acid glycoside **6**, the ^{13}C-NMR spectrum of which indicated the structure assigned.

Glycuronosides **2** and **6** were then coupled to amino acid *tert*-butyl esters (L-alanine, L-threonine, L-serine, and L-lysine). The *tert*-butyl ether was chosen for hydroxyl protection in serine and threonine, and the *tert*-butyloxycarbonyl (BOC) group was used as the ε-amino-blocking group in lysine. These groups in conjunction with the *tert*-butyl ester can be smoothly removed under mild conditions (trifluoroacetic acid at room temperature) which do not split glycosidic linkages or lead to racemization of the amino acid moiety. Ethyl 2-ethoxy-1,2-dihydroquinoline-1-carboxylate (EEDQ) was used as an effective and mild condensing reagent [23].

Coupling of glycuronosides **2** and **6** with protected amino acid derivatives in *N*,*N*-dimethylformamide in the presence of EEDQ afforded glycuronamides **7**–**10** and **23** (in 80–100% yields) which were identified from ^{13}C-NMR data. Catalytic hydrogenation of amino acid derivatives **7**–**10** and **23** followed by treatment with acryloyl chloride in aqueous



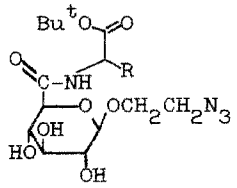


6

3 R = R' = Ac

4 R = H, R' = Tr

5 R = Ac, R' = Tr

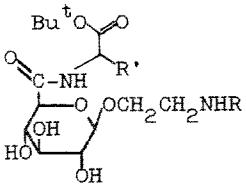


7 R = Me (L-Ala)

8 R = CH(OBu^t)Me (L-Thr)

9 R = CH_2OBu^t (L-Ser)

10 R = $(CH_2)_4NHBoc$ (L-Lys)



11 R = H, R' = Me (L-Ala)

12 R = H, R' = CH(OBu^t)Me (L-Thr)

13 R = H, R' = CH_2OBu^t (L-Ser)

14 R = H, R' = $(CH_2)_4NHBoc$ (L-Lys)

15 R = $COCH{=}CH_2$, R' = Me (L-Ala)

16 R = $COCH{=}CH_2$, R' = CH(OBu^t)Me (L-Thr)

17 R = $COCH{=}CH_2$, R' = CH_2OBu^t (L-Ser)

18 R = $COCH{=}CH_2$, R' = $(CH_2)_4NHBoc$ (L-Lys)

methanol in the presence of anion-exchange resin (HCO_3^--form) gave 2-acrylamidoethyl glycosides **15**–**18** and **25**.

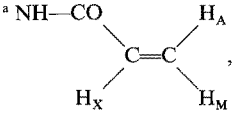
It should be noted that glycuronamide **17** was obtained as a mixture of two compounds and resolved by column chromatography to give two components, **17-I** and **17-II** in a ratio of ≈6:1. The ^{13}C-NMR spectrum of the major component **17-I** matched that of the minor one, distinctions were only found for resonances in the aglycon moiety (Table 1). The double bond C-signals at 126.6 and 131.0 ppm (major component) indicative for acrylamido group (cf. [14, 18]) were shifted in the spectrum of the minor component **17-II** (Table 1).

The ^{1}H-NMR spectral characteristics of both components **17-I** and **17-II** are in agreement with a presence of a terminal double bond (AMX-spin system) but some changes in the chemical shifts of the H_A-, H_M-, and H_X-signals were observed (Table 1). It seems that compound **17** was a mixture of two isomers, which could be *Z*/*E*-isomers about the amide bond and/or s-*cis*/s-*trans*-isomers of the α,β-unsaturated carbonyl group.

In an nOe (nuclear Overhauser effect) experiment on the major isomer **17-I**, irradiation of any protons of the double bond did not cause enhancement of the signals of the CH_2N-group protons. From assessment of geometrical relationships on molecular models (Fig. 1) it is evident that

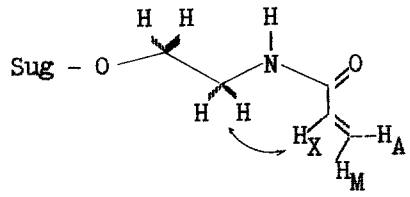
Table 1. ^{13}C-NMR δ, and ^{1}H-NMR δ and J values for the acrylamido fragment[a] in isomers **17-I** and **17-II**.

Compound	CH_2=CH	CH_2=CH	H_A	H_M	H_X	$J_{A,M}$	$J_{A,X}$	$J_{M,X}$
17-I	126.6	131.0	6.22 (dd)	5.56 (dd)	6.12 (dd)	3.0	17.0	8.8
17-II	127.5	129.5	6.27 (dd)	5.69 (dd)	6.55 (dd)	2.0	17.0	10.5

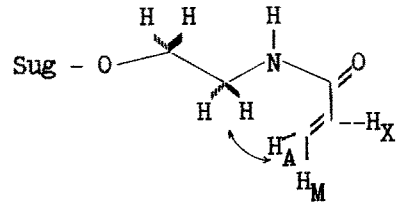
[a]  , AMX spin system.

this ^{1}H-NMR spectral feature is only consistent with the *Z*-isomer of glycoside **17**, having double bond protons (H_A, H_M, and H_X) remote from that of the CH_2N-group. However, it is impossible to discriminate between s-*cis* and s-*trans*-isomers from the nOe data. Irradiation of H_X (but not H_A or H_M) in the minor isomer **17-II** resulted in enhancements of proton signals belonging to the CH_2N-group. From this nOe data it is evident that the aforementioned protons are located in close proximity, which is consistent only with the (E)-s-*cis* isomer (Fig. 1).

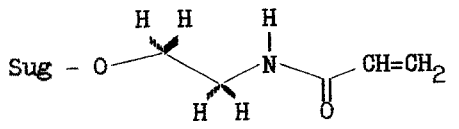
Z-Stereochemistry of the major isomer **17-I** as well as of 2-acrylamidoethyl glycosides **15**, **16**, **18**, and **25** described herein is in agreement with the known greater stability of *Z*-amides [24]. Only *Z*-isomers (with "normal" spectral characteristics) were found earlier in the syntheses of 2-acrylamidoethyl glycosides [14, 18].



(a) (E)-s-*cis*

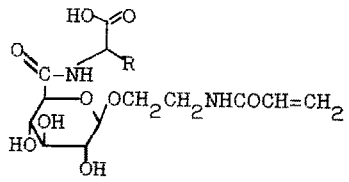


(b) (E)-s-*trans*

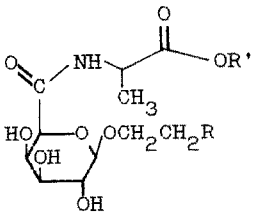


(c) (Z)-s-*cis*/(Z)-s-*trans*

Figure 1. Geometrical relationships for isomers of 2-acrylamidoethyl glycosides: (**a**) selective irradiation of H_X caused enhancement of the CH_2N-protons; (**b**) selective irradiation of H_A should cause enhancement of the CH_2N-protons; (**c**) no nOe enhancement for the CH_2N-protons on irradiation of the CH=CH_2-protons.



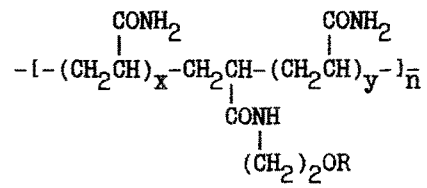
19 R = Me (L-Ala)
20 R = CH(OH)Me (L-Thr)
21 R = CH_2OH (L-Ser)
22 R = $(CH_2)_4NH_2$ (L-Lys)



23 R = N_3, R' = Bu^t
24 R = NH_2, R' = Bu^t
25 R = $NHCOCH=CH_2$, R' = Bu^t
26 R = $NHCOCH=CH_2$, R' = H

Glycosides **15**, **16**, **17-I**, **18**, and **25** were separately subjected to brief treatment with trifluoroacetic acid, to give deprotected amino acid-glycuronamides **19**–**22** and **26** in the form of glycosides suitable for copolymerization. The ^{13}C-NMR spectra of these compounds indicated the structures assigned. However, for threonine and serine derivatives **20** and **21** the line broadening and diminished intensities of signals at 60.5 ppm (Thr α-CH) and 56.0 ppm (Ser α-CH) were observed. This could be explained by the existence of conformers in equilibrium, undergoing interconversion at a rate comparable with the NMR time.

2-Acrylamidoethyl glycosides were copolymerized with acrylamide under conditions used earlier [14, 16–20] to give copolymers **27**–**31**. Copolymeric neoglycoconjugates were



27 R = [6(N)-L-Ala]GlcA(β1-
28 R = [6(N)-L-Thr]GlcA(β1-
29 R = [6(N)-L-Ser]GlcA(β1-
30 R = [6(Nα)-L-Lys]GlcA(β1-
31 R = [6(N)-L-Ala]GalA(β1-

isolated by gel filtration on Bio-Gel P-4 or Sephadex G-50. The yield of copolymers varied from 78 to 95%. The ratio of glycuronosidamide/$CH_2CHCONH_2$ in the polymers coincided well with that in the pre-polymerization mixture (≈1:7). This could be followed from integration of appropriate signals in the ^{13}C-NMR spectra.

It should be noted that the Thr α-CH signal of copolymer **28** (prepared from monomer **20**) also appeared as a broader line at 60.1 ppm with diminished intensity. No lines corresponding to Ala α-CH and Ser α-CH-signals were observed in spectra of copolymers **27** and **29** prepared from monomers **19** and **21** respectively. This fact could also be explained by considering the existence of conformational interconversions of polymers comparable in duration with the NMR-time. Immunochemical studies of neoglycoconjugates obtained will be published elsewhere.

Materials and methods

Concentrations were performed at <40°C (bath). Optical rotations were recorded at 24–32°C using a DIP-360 spectropolarimeter (Jasco, Japan), and i.r. spectra were recorded with a Perkin-Elmer 577 instrument. NMR spectra were recorded at 25°C with Bruker WM-250 (^{1}H-NMR spectra) and Bruker AM-300 (^{13}C-NMR spectra) spectrometers. TLC was performed on aluminium sheets, Silica Gel 60 F_{254} (Merck, Darmstadt, Germany) using appropriate eluent systems, detection by u.v. and/or by charring with 10% aqueous sulfuric acid. The spots were also visualized by spraying with a 1% solution of potassium permanganate in aqueous sodium carbonate (for double bond containing compounds), or with 0.3% ninhydrin in ethanol (for amines), followed by heating. Column chromatography was performed on Silica Gel L 40/100 μm, L 100/160 μm, and Silpearl (20–40 μm) (Czechoslovakia), or LiChroprep Si 60 (40–63 μm, Merck, Germany). HPLC was done with Silasorb 600 (5 μm) analytical (6 mm × 150 mm) and Silasorb 600 (10 μm) semi-preparative (16 mm × 250 mm) columns, using differential refractometer (Knauer, Germany) or u.v. detector ISCO, model UA-5 (254 nm) (USA). For GLC, a Hewlett-Packard 5890 instrument equipped with flame-ionization detector and integrator HP 3393A was used. Separations were performed on a glass capillary column (0.2 mm × 25 m) coated with Ultra-1 (layer thickness 0.33 μm) at 200°C and gas-carrier pressure 140 kPa. Elemental analyses were not obtained for syrupy or amorphous compounds. These were purified by column chromatography and characterized by NMR spectroscopy. Organic solvents were of *pro analysi* quality and distilled over appropriate drying agents. L-Alanine *tert*-butyl ester hydrochloride was purchased from Fluka, Buchs, Switzerland. *O-tert*-Butyl-L-threonine *tert*-butyl ester was from Serva, Heidelberg, Germany, *O-tert*-butyl-L-serine *tert*-butyl ester hydrochloride was from Reanal, Hungary, and *Nε*-BOC-lysine *tert*-butyl ester hydrochloride was from Bachem, Heidelberg, Germany.

2-Azidoethanol

2-Chloroethanol (25.2 ml, 375 mmol) was added to a solution of sodium azide (30 g, 461 mmol) and sodium hydroxide (1.5 g, 37.5 mmol) in water (112 ml). The mixture was stirred at 20°C for two days. Then sodium sulphate (35 g) was added, after 10 min the reaction mixture was extracted with dichloromethane (3 × 70 ml). The organic layer dried over anhydrous sodium sulphate was concentrated. Distillation of the residue (≈30 ml) *in vacuo* gave pure 2-azidoethanol (28.7 g, 88%), b.p. 76–78°C/25, n_D^{21} 1.4615; ν_{max} 2120 cm^{-1} (N_3). Lit. [25, 26]: b.p. 73°C/20, n_D^{25} 1.4578.

Methyl(2-azidoethyl 2,3,4-tri-O-acetyl-β-D-glucopyranosid)uronate (**1**)

Crystalline methyl(2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate [27] (1.19 g, 3 mmol) was added in one portion to a hot (105°C) solution of mercury(II) cyanide (780 mg, 3.1 mmol) in 2-azidoethanol (3.27 ml, 43.2 mmol). The mixture was stirred at 105–110°C for 10 min, plus overnight at room temperature. TLC (hexane:ethyl acetate, 6:4 by vol, 10 min) indicated that no starting material (R_F 0.59) remained and a product (R_F 0.30) appeared. An excess of 2-azidoethanol was evaporated *in vacuo* (<1 mm). A solution of the residue in chloroform (50 ml) was washed with water (50 ml). The aqueous layer was washed with chloroform (3 × 50 ml). The combined organic phases (≈200 ml) were then washed with 1 M sodium iodide (4 × 20 ml), and water (200 ml), dried over sodium sulfate and concentrated. The residue (1.25 g) was purified by chromatography on a Silpearl column (25 × 250 mm) in petroleum ether:ethyl acetate, 65:35 by vol, to give crystalline **1** (840 mg, 69%), homogeneous according to HPLC (hexane:ethyl acetate, 65:35 by vol). GLC analysis of the crude product showed the presence of the α-anomer, 3%. Recrystallization from ether gave material with m.p. 96–98°C, $[\alpha]_D$ −58° (*c* = 1.0, chloroform); ν_{max} 2120 cm^{-1} (N_3).

NMR data: ^{13}C, δ 20.6 ($COCH_3$), 50.45 (CH_2N_3), 52.9 ($COOCH_3$), 68.8, 69.35, 71.0, 72.0, 72.55 (C-2,3,4,5, OCH_2), 100.6 (C-1), 167.1, 169.4, 170.1 (CH_3CO, $COOCH_3$); ^{1}H, δ 2.03, 2.04, 2.07 (3s, CH_3CO), 3.29 (ddd, $J_{C,D}$ 13.6, $J_{A,D}$

3.5 Hz, H-D, $CH_CH_DN_3$), 3.51 (ddd, $J_{B,C}$ 3.5 Hz, H-C), 3.69 (ddd, $J_{A,B}$ 15.2, $J_{A,C}$ 8.6 Hz, H-A, OCH_AH_B), 3.86 (s, $COOCH_3$), 4.05 (d, $J_{4,5}$ 9.6 Hz, H-5), 4.09 (ddd, $J_{B,D}$ 4.9 Hz, H-B), 4.64 (d, $J_{1,2}$ 7.8 Hz, H-1), 5.04 (m, H-2), 5.25 (m, H-3,4).

Analytical data. Calculated for $C_{15}H_{21}N_3O_{10}$: C, 44.7; H, 5.2; N, 10.4. Found: C, 44.4; H, 5.3; N, 10.6.

2-Azidoethyl β-D-glucopyranosiduronic acid (**2**)

Cold 1 M sodium hydroxide (28 ml, 28 mmol) was added to a solution of methyl uronate **1** (1 g, 2.48 mmol) in methanol (160 ml) at 0°C. The reaction mixture was left at 4°C for 12 h, then decationized with KU-2 (H^+) ion-exchange resin and concentrated. The residue was applied to a Dowex 1-X8 (AcO^-) column (2.5 × 11 cm). Elution with water (150 ml) and then with 20% acetic acid (350 ml) gave **2** (595 mg, 91%). The compound, isolated by lyophilization, had $[\alpha]_D$ −37° (c = 3.0, methanol).

NMR data (^2H_2O): ^{13}C, δ 51.3 (CH_2N_3), 68.5 (OCH_2), 72.0 (C-4), 73.5 (C-2), 75.4 (C-5), 76.0 (C-3), 103.2 (C-1, $J_{C,H}$ 165 Hz), 173.1 (COOH).

Analytical data. Calculated for $C_8H_{13}N_3O_7$: C, 36.5; H, 5.0; N, 16.0. Found: C, 36.2; H, 5.2; N, 16.2.

2-Azidoethyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (**3**)

A solution of mercury(II) cyanide (252 mg, 1 mmol), mercury(II) bromide (360 mg, 1 mmol) and 2-azidoethanol (0.15 ml, 2 mmol) in freshly distilled (over calcium hydride) acetonitrile (5 ml) was stirred with 4 Å molecular sieves for 15 min under argon. A solution of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (822 mg, 2 mmol) in acetonitrile (5 ml) was added and the mixture was then stirred at 20°C for 12 h in the dark. TLC (ethyl acetate:heptane, 3:2 by vol, R_F 0.5) showed complete reaction. The mixture was diluted with chloroform (50 ml), and the solids (mercury salts) were filtered off. The filtrate was washed, successively, with cold water, sodium iodide, sodium hydrogen carbonate, and water, dried over magnesium sulfate, and concentrated. Column chromatography (heptane:ethyl acetate, 1:1 by vol) of the residue gave **3** as a syrup (750 mg, 90%), $[\alpha]_D$ −11.9° (c = 1.75, chloroform).

NMR data (C^2HCl_3): ^{13}C, δ 20.4, 20.5, 20.6 (CH_3COO), 50.4 (CH_2N_3), 61.2 (C-6), 68.3 (OCH_2), 66.9, 68.4, 70.7, 70.8 (C-2,3,4,5), 101.0 (C-1), 169.4, 170.1, 170.2 (CH_3COO).

2-Azidoethyl 6-O-trityl-β-D-galactopyranoside (**4**)

Sodium methoxide (1 M, 1.5 ml) was added dropwise to a solution of **3** (3.75 g, 8.99 mmol) in methanol (40 ml). The mixture was heated at 50°C for 45 min and then neutralized with a solution of acetic acid in methanol (1 M, 1.5 ml), and concentrated. The residue was dissolved in pyridine (30 ml). Trityl chloride (3.69 g, 13.2 mmol) was added and the mixture was then heated at 50°C. After 7 h TLC (benzene: ethyl acetate:methanol, 25:25:5 by vol, R_F 0.45) showed complete conversion. Ethanol (10 ml) was added, the mixture was concentrated and co-evaporated with a mixture of toluene, heptane and ethanol. Column chromatography (heptane:ethyl acetate, 5:1 by vol, then ethyl acetate) of the residue gave **4** (2.25 g, 52%) as a semi-solid material with $[\alpha]_D$ −13.6° (c = 1.4, chloroform).

NMR data (C^2HCl_3): ^{13}C, δ 50.7 (CH_2N_3), 62.7 (C-6), 68.1 (OCH_2), 69.2, 71.3, 73.5, 73.9 (C-2,3,4,5), 86.8 (CPh_3), 103.2 (C-1), 127.1, 127.9, 128.7 (Aromatic CH), 143.7 (Aromatic C).

2-Azidoethyl 2,3,4-tri-O-acetyl-6-O-trityl-β-D-galactopyranoside (**5**)

Acetic anhydride (4 ml) was added to a solution of **4** (2 g, 4.07 mmol) in pyridine (8 ml). After 12 h the reaction mixture was concentrated to give **5** (2.38 g, 95%) as a syrup, $[\alpha]_D$ −40.15° (c = 1.27, chloroform), R_F 0.66 (ethyl acetate: heptane, 3:2 by vol).

NMR data (C^2HCl_3): ^{13}C, δ 20.2–20.7 (CH_3COO), 50.5 (CH_2N_3), 68.1 (OCH_2), 67.2, 68.8, 71.1, 72.2 (C-2,3,4,5), 86.9 (CPh_3), 101.05 (C-1), 127.1–128.9 (Aromatic CH), 143.3 (Aromatic C), 169.4, 169.8, 170.05 (CH_3COO).

2-Azidoethyl β-D-galactopyranosiduronic acid (**6**)

Jones reagent [21] (6.96 ml); prepared from chromic anhydride (2.5 g), conc. sulfuric acid (2 ml), and water (7.5 ml) was added, while cooling to 0°C and stirring, to a solution of **5** (2.15 g, 3.48 mmol) in a mixture of dichloromethane (7 ml) and acetone (10.5 ml). The mixture was stirred at 20°C for 1.5 h, then poured into ice-water and extracted with chloroform (75 ml). The organic layer was washed with water (3 × 30 ml) and concentrated. The residue (1.35 g) was dissolved in methanol (210 ml) and after cooling to 0°C mixed with 1 M sodium hydroxide (34.7 ml). The mixture was kept at 0°C for 24 h and then decationized with KU-2 (H^+) ion exchange resin. Filtration and concentration gave crude **6**, which was purified by chromatography on a Dowex 1-X8 (AcO^-) column (elution with 20% acetic acid). After lyophilization pure **6** was obtained as a white powder (523 mg, 57%), $[\alpha]_D$ −31° (c = 1.33, water).

NMR data (^2H_2O): ^{13}C, δ 51.85 (CH_2N_3), 69.85 (OCH_2), 70.9 (C-4), 71.4 (C-2), 73.5 (C-5), 75.3 (C-3), 103.8 (C-1), 173.15 (COOH).

Analytical data. Calculated for $C_8H_{13}N_3O_7$: C, 36.5; H, 5.0; N, 16.0. Found: C, 36.3; H, 5.1; N, 16.3.

N-(2-Azidoethyl β-D-glucopyranosiduronoyl)-L-alanine tert-butyl ester (**7**)

A solution of L-alanine *tert*-butyl ester (43 mg, 0.3 mmol) (prepared from the corresponding hydrochloride by treatment with an equimolar quantity of triethylamine in ethyl acetate) in anhydrous *N,N*-dimethylformamide (2.7 ml, freshly distilled over ninhydrin *in vacuo*) was added, while stirring, to a solution of **2** (52 mg, 0.2 mmol) and EEDQ

(98 mg, 0.4 mmol) in *N,N*-dimethylformamide (3 ml). The mixture was stirred at 20°C for 12 h. More EEDQ (98 mg, 0.4 mmol) was added and stirring was continued for a further 12 h. TLC (chloroform:methanol, 4:1 by vol, R_F 0.69) showed completion of reaction. The solvent was evaporated *in vacuo* and a solution of the residue in methanol was treated with KU-2 (H^+) ion exchange resin to remove basic components. Filtration and concentration gave crude **7**, which was purified by column chromatography (gradient elution with methanol, 0–5%, in chloroform). Pure, syrupy **7** was obtained (75 mg, 97%), $[\alpha]_D$ −38.3° (*c* = 1.65, chloroform).

NMR data (C^2HCl_3): ^{13}C, δ 18.3 (β-CH_3), 27.9 [C(**C**H_3)$_3$], 48.3 (α-CH), 50.8 (CH_2N_3), 68.85 (OCH_2), 72.1 (C-4), 72.7 (C-2), 72.8 (C-5), 75.65 (C-3), 82.3 (CMe_3), 102.8 (C-1), 169.9, 171.5 (COOBu^t, CONH).

N-(2-Azidoethyl β-D-glucopyranosiduronyl)-O-(tert-butyl)-L-threonine tert-butyl ester (**8**)

Reaction of **2** (52 mg, 0.2 mmol) with *O-tert*-butyl-L-threonine *tert*-butyl ester (69 mg, 0.3 mmol) performed as described above gave **8** (94 mg, 99%) as an amorphous solid, $[\alpha]_D$ −29.2° (*c* = 1.47, chloroform), R_F 0.69 (chloroform:methanol, 4:1 by vol).

NMR data (C^2HCl_3): ^{13}C, δ 21.2 (γ-CH_3), 28.1, 28.6 [C(**C**H_3)$_3$], 50.7 (CH_2N_3), 58.1 (α-CH), 67.0 (β-CH), 68.6 (OCH_2), 73.6 (CHO**C**Me_3), 72.3 (C-4), 72.8 (2C) (C-2,5), 75.7 (C-3), 82.3 (COO**C**Me_3), 102.7 (C-1), 169.3, 170.9 (COOBu^t, CONH).

N-(2-Azidoethyl β-D-glucopyranosiduronoyl)-O-(tert-butyl)-L-serine tert-butyl ester (**9**)

Reaction of **2** (174 mg, 0.66 mmol) with *O*-(*tert*-butyl)-L-serine *tert*-butyl ester (215 mg, 0.99 mmol) performed as described above afforded **9** (280 mg, 93%) as a syrup, $[\alpha]_D$ −21.6° (*c* = 2, chloroform), R_F 0.52 (chloroform:methanol:acetic acid, 90:10:1 by vol).

NMR data (C^2HCl_3): ^{13}C, δ 27.3, 28.0 [C(**C**H_3)$_3$], 50.7 (CH_2N_3), 52.8 (α-CH), 61.8 (β-CH), 68.8 (OCH_2), 72.2 (C-4), 72.5 (C-2), 72.8 (C-5), 73.3 (CHO**C**Me_3), 75.6 (C-3), 82.2 (COO**C**Me_3), 102.7 (C-1), 168.8, 170.3 (COOBu^t, CONH).

Nε-(tert-Butyloxycarbonyl)-Nα-(2-azidoethyl β-D-glucopyranosiduronoyl)-L-lysine tert-butyl ester (**10**)

Reaction of **2** (190 mg, 0.72 mmol) with *N*ε-BOC-L-lysine *tert*-butyl ester (262 mg, 0.87 mmol) in the presence of EEDQ (267 mg, 1.08 mmol) performed as described above after 48 h gave **10** (324 mg, 82%) as a syrup, $[\alpha]_D$ −17.7 (*c* = 2, chloroform).

NMR data (C^2HCl_3): ^{13}C, δ 22.4 (γ-CH_2), 27.9, 28.4 [C(**C**H_3)$_3$], 29.5 (β-CH_2), 32.2 (δ-CH_2), 40.3 (ε-CH_2), 50.7 (CH_2N_3), 52.1 (α-CH), 68.9 (OCH_2), 72.3 (C-4), 72.8 (2C) (C-2,5), 75.7 (C-3), 82.5 (COO**C**Me_3), 102.8 (C-1), 170.0, 170.8 (COOBu^t, CONH).

N-(2-Acrylamidoethyl β-D-glucopyranosiduronoyl)-L-alanine tert-butyl ester (**15**)

A solution of **7** (75 mg, 0.192 mmol) in methanol (6 ml) was hydrogenated over 10% Pd/C. After 2 h TLC (chloroform:methanol, 4:1 by vol) showed complete conversion. The catalyst was filtered off, and the filtrate was concentrated to give **11** (70 mg, 99%). Acryloyl chloride (23 μl, 0.288 mmol) was added, while stirring with Dowex 1-X8 (HCO_3^-) ion exchange resin, to a solution of **11** (70 mg, 0.192 mmol) in a mixture of methanol and water, 8:1 by vol. After 2 h TLC (chloroform:methanol, 4:1 by vol, R_F 0.55) showed complete reaction. Filtration and concentration gave crude **15**, which was purified by column chromatography (gradient elution with methanol, 0–3%, in chloroform) to give pure, syrupy **15** (61 mg, 76%), $[\alpha]_D$ −21.65° (*c* = 1.33, chloroform).

NMR data (C^2HCl_3): ^{13}C, δ 18.2 (β-CH_2), 28.0 [C(**C**H_3)$_3$], 39.5 (OCH_2CH_2NH), 48.4 (α-CH), 68.9 (OCH_2), 72.0 (C-4), 72.6 (C-2), 72.8 (C-5), 75.7 (C-3), 82.2 (COO**C**Me_3), 102.6 (C-1), 126.65 (CH=**C**H_2), 131.1 (**C**H=CH_2), 166.4, 169.95, 171.6 (COOBu^t, CONH).

N-(2-Acrylamidoethyl β-D-glycopyranosiduronoyl)-O-(tert-butyl)-L-threonine tert-butyl ester (**16**)

Catalytic hydrogenation of **8** (95 mg, 0.2 mmol) followed by *N*-acryloylation as described above gave **16** (91 mg, 92%) as a syrup, $[\alpha]_D$ −20.75° (*c* = 1.33, chloroform), R_F 0.64 (chloroform:methanol, 8:1 by vol).

NMR data (C^2HCl_3): ^{13}C, δ 21.4 (γ-CH_3), 28.2, 28.7 [C(**C**H_3)$_3$], 39.6 (OCH_2CH_2NH), 58.3 (α-CH), 66.9 (β-CH), 69.5 (OCH_2CH_2NH), 72.2 (C-4), 73.0 (2C) (C-2,5), 74.2 (CHO**C**Me_3), 76.0 (C-3), 82.7 (COO**C**Me_3), 103.0 (C-1), 126.8 (CH=**C**H_2), 131.0 (**C**H=CH_2).

N-(2-Acrylamidoethyl β-D-glucopyranosiduronoyl)-O-(tert-butyl)-L-serine tert-butyl ester (**17**)

Hydrogenation of **9** (110 mg, 0.24 mmol) followed by *N*-acryloylation as described above afforded **17** as a mixture of two isomers. Chromatography on a LiChroprep Si 60 (40–63 μm) column (10 × 240 mm) (gradient elution with methanol, 1–5%, in chloroform) with u.v. detection (254 nm) gave first the isomer **17-II** (17 mg, 14.6%), $[\alpha]_D$ −16.6° (*c* = 1, chloroform), R_F (chloroform:methanol:acetic acid, 90:10:1 by vol).

NMR data (C^2HCl_3): ^{13}C, δ 27.4, 28.0 [C(**C**H_3)$_3$], 47.8 (OCH_2CH_2NH), 53.0 (α-CH), 61.8 (β-CH_2), 69.0 (OCH_2CH_2NH), 72.2 (C-4), 72.7 (C-2), 72.9 (C-5), 73.4 (CHO**C**Me_3), 75.9 (C-3), 82.4 (COO**C**Me_3), 103.4 (C-1), 127.5 (CH=**C**H_2), 129.5 (**C**H=CH_2).

The next fraction was **17-I** (98 mg, 84.5%), $[\alpha]_D$ −11.2° (*c* = 1, chloroform), R_F 0.16 (chloroform/methanol/acetic acid, 90/10/1 by vol).

NMR data (C^2HCl_3): ^{13}C, δ 27.3, 28.0 [C(**C**H_3)$_3$], 39.5 (OCH_2CH_2NH), 53.0 (α-CH), 61.7 (α-CH_2), 69.4 (OCH_2CH_2NH), 72.1 (C-4), 72.8 (C-2), 73.0 (C-5), 73.3

($CHOCMe_3$), 75.8 (C-3), 82.4 ($COOCMe_3$), 102.9 (C-1), 126.6 ($CH{=}CH_2$), 131.0 ($CH{=}CH_2$), 166.4, 168.9, 170.2 (COOBu^t, CONH).

Nε-(tert-Butyloxycarbonyl)-Nα-(2-acrylamidoethyl β-D-glucopyranosiduronoyl)-L-lysine tert-butyl ester (**18**)

Hydrogenation of **10** (174 mg, 0.317 mmol) followed by *N*-acryloylation (in the presence of 2,6-di-*tert*-butyl-4-methylphenol as a radical inhibitor) afforded crude **18** (187 mg). Part of the material obtained (87 mg) was purified by HPLC on a Silasorb 600 (10 μm) column (1.6 × 25 cm) using gradient elution with methanol (0–11%) in chloroform to give pure, syrupy **18** (50 mg), $[\alpha]_D$ −11.0° (c = 1, chloroform), R_F 0.5 (chloroform:methanol:acetic acid, 90:10:1 by vol).

NMR data (C^2HCl_3): ^{13}C, δ 22.6 (γ-CH_2), 28.1, 28.55 [$C(CH_3)_3$], 29.6 (α-CH_2), 32.1 (δ-CH_2), 39.65 (CH_2NHCO), 40.4 (ε-CH_2), 52.4 (α-CH), 69.7 (OCH_2), 72.3 (C-4), 73.1 (2C) (C-2,5), 76.1 (C-3), 82.7 (CMe_3), 103.2 (C-1), 126.7 ($CH{=}CH_2$), 131.0 ($CH{=}CH_2$), 166.5, 170.1, 171.1 (COOBu^t, CONH).

N-(2-Acrylamidoethyl β-D-glucopyranosiduronoyl)-L-alanine (**19**)

Compound **15** (60 mg, 0.166 mmol) was dissolved in trifluoroacetic acid (3 ml). The mixture was kept at 20°C for 20 min. TLC (chloroform:methanol, 4:1 by vol, R_F 0.15) showed complete conversion. The reaction mixture was concentrated, co-evaporated with methanol and tetrachloromethane. Water (2 ml) was added to the residue and the suspension obtained was filtered through nylon filter (pore diameter 0.45 μm, Nucleopore Corp., USA) followed by concentration to give pure, syrupy **19** (51 mg, 99%), $[\alpha]_D$ −44.5° (c = 1.33, water).

NMR data (C^2HCl_3): ^{13}C, δ 17.6 (β-CH_3), 40.8 (CH_2NH), 49.5 (α-CH), 70.3 (OCH_2), 72.7 (C-4), 74.1 (C-2), 76.4 (C-5), 76.8 (C-3), 104.1 (C-1), 128.9 ($CH{=}CH_2$), 131.4 ($CH{=}CH_2$), 170.2, 171.5, 177.4 (COOH, CONH).

N-(2-Acrylamidoethyl β-D-glucopyranosiduronoyl)-L-threonine (**20**)

Deprotection of **16** (91 mg, 0.23 mmol) as described above gave **20** (70 mg, 99%), as a syrup, $[\alpha]_D$ −22.85° (c = 1.33, water).

NMR data (^2H_2O): ^{13}C, δ 20.2 (γ-CH_3), 40.8 (CH_2NH), 59.1 (α-CH), 68.6 (β-CH), 70.2 (OCH_2), 72.8 (C-4), 74.1 (C-2), 76.2 (C-5), 76.7 (C-3), 104.0 (C-1), 128.9 ($CH{=}CH_2$), 131.4 ($CH{=}CH_2$), 170.15, 172.2, 174.8 (COOH, CONH).

N-(2-Acrylamidoethyl β-D-glucopyranosiduronoyl)-L-serine (**21**)

Deprotection of **17-I** (98 mg, 0.26 mmol) as described above afforded **21** (69 mg, 91%) as a syrup, $[\alpha]_D$ −19° (c = 1, water).

NMR data (^2H_2O): ^{13}C, δ 40.7 (CH_2NH), 56.0 (α-CH), 62.3 (β-CH_2), 70.1 (OCH_2), 72.6 (C-4), 73.9 (C-2), 76.1 (C-5), 76.5 (C-3), 103.9 (C-1), 128.8 ($CH{=}CH_2$), 131.2 ($CH{=}CH_2$), 170.1, 171.8 (COOH, CONH).

Nα-(2-Acrylamidoethyl β-D-glucopyranosiduronoyl)-L-lysine (**22**)

Deprotection of **18** (51 mg, 0.09 mmol) as described above after 1.5 h gave syrupy **22** (37 mg, 99%), $[\alpha]_D$ 34.6° (c = 1, water), R_F 0.35 (ethanol:*n*-butanol:pyridine:acetic acid: water, 100:10:10:3:10 by vol).

NMR data (^2H_2O): ^{13}C, δ 23.1 (γ-CH_2), 27.3 (β-CH_2), 31.2 (δ-CH_2), 40.5 (2C) (ε-CH_2, CH_2NH), 53.5 (α-CH), 69.9 (OCH_2), 72.6 (C-4), 73.9 (C-2), 78.1 (C-5), 78.55 (C-3), 103.9 (C-1), 128.4 ($CH{=}CH_2$), 131.4 ($CH{=}CH_2$), 171.47, 171.54, 176.0 (COOH, CONH).

N-(2-Azidoethyl β-D-galactopyranosiduronoyl)-L-alanine tert-butyl ester (**23**)

Reaction of **6** (52 mg, 0.2 mmol) with L-alanine *tert*-butyl ester (43 mg, 0.3 mmol) as described for the preparation of **7** afforded **23** (75 mg, 99%), R_F 0.33 (chloroform:methanol, 9:1 by vol), $[\alpha]_D$ −40.1° (c = 1.33, chloroform).

NMR data (C^2HCl_3): ^{13}C, δ 18.4 (β-CH_3), 28.0 [$C(CH_3)_3$], 48.6 (α-CH), 50.9 (CH_2N_3), 68.6 (OCH_2), 69.1, 70.8, 73.1, 75.2 (C-2,3,4,5), 82.0 (CMe_3), 103.35 (C-1), 168.1, 171.7 (COOBu^t, CONH).

N-(2-Acrylamidoethyl β-D-galactopyranosiduronoyl)-L-alanine tert-butyl ester (**25**)

Compound **23** (75 mg, 0.192 mmol) was subjected to hydrogenation followed by *N*-acryloylation as described for preparation of **15** to give **25** (41 mg, 51%) as a syrup, $[\alpha]_D$ −23° (c = 1.33, chloroform), R_F 0.55 (chloroform:methanol, 4:1 by vol).

NMR data (C^2HCl_3): ^{13}C, δ 18.3 (β-CH_3), 28.0 [$C(CH_3)_3$], 39.5 (CH_2NH), 48.7 (α-CH), 69.5 (OCH_2), 69.1, 70.9, 73.1, 75.1 (C-2,3,4,5), 82.2 ($COOCMe_3$), 103.6 (C-1), 126.5 ($CH{=}CH_2$), 131.2 ($CH{=}CH_2$), 166.3, 168.25, 171.8 (COOBu^t, CONH).

N-(2-Acrylamidoethyl β-D-galactopyranosiduronoyl)-L-alanine (**26**)

Deprotection of **25** (41 mg, 0.11 mmol) as described above gave **26** (35 mg, 98%) as a syrup, $[\alpha]_D$ −47.8 (c = 1.33, water), R_F 0.18 (chloroform:methanol, 4:1 by vol).

NMR data (^2H_2O): ^{13}C, δ 17.8 (β-CH_3), 40.9 (OCH_2CH_2NH), 51.4 (α-CH), 70.3 (OCH_2), 70.3, 71.6, 73.7, 75.9 (C-2,3,4,5), 103.9 (C-1), 128.9 ($CH{=}CH_2$), 131.4 ($CH{=}CH_2$), 168.5, 171.9, 177.2 (COOH, CONH).

Copolymerization of monomer **19** *with acrylamide*

A solution of **19** (50 mg, 0.138 mmol) and acrylamide (67 mg, 0.94 mmol) in distilled water (1 ml) was deaerated using a water pump. *N,N,N′,N′*-Tetramethylenediamine

(TEMED) (2 µl) and ammonium persulfate (1 mg) were added and the mixture was stirred at 20°C for 2 h under argon. The reaction mixture was diluted with 0.05/0.03 pyridine-acetate buffer pH 5.5 (1 ml) and applied to a Bio-Gel P-4 column (2.5 × 70 cm). The same buffer was used as eluent. The higher molecular weight fraction detected using a differential refractometer was collected and lyophilized to yield copolymer **27** (111 mg, 94.5%), $[\alpha]_D$ −12.3° (c = 1.33, water).

NMR data (^2H_2O): ^{13}C, δ 18.7 (β-CH_3), 34.0–38.0 (CH_2, polyacrylamide), 40.7 (OCH_2CH_2NH), 42.0–45.0 (CH, polyacrylamide), 69.8 (OCH_2CH_2NH), 72.8 (C-4), 74.1 (C-2), 76.5 (2C) (C-3,5), 104.0 (C-1), 171.0, 178.0 (COOH, CONH), 180.9 (CONH, polyacrylamide).

Copolymer **28**

Copolymerization of **20** (55.5 mg, 0.14 mmol) and acrylamide (70.5 mg, 0.92 mmol) as described above afforded copolymer **28** (97 mg, 78%), $[\alpha]_D$ −5.6° (c = 1, water).

NMR data (^2H_2O): ^{13}C, δ 20.5 (γ-CH_3), 35.0–38.0 (CH_2, polyacrylamide), 40.7 (OCH_2CH_2NH), 42.0–44.0 (CH, polyacrylamide), 60.1 (α-CH), 68.8 (β-CH), 69.8 (OCH_2CH_2NH), 72.8 (C-4), 74.1 (C-2), 76.3 (C-5), 76.7 (C-3), 103.9 (C-1), 172.0, 178.2 (COOH, CONH), 180.9 (CONH, polyacrylamide).

Copolymer **29**

Copolymerization of **21** (34 mg, 0.09 mmol) and acrylamide (45 mg, 0.63 mmol) as described above gave copolymer **29** (74 mg, 94%), $[\alpha]_D$ −3.6° (c = 0.5, water).

NMR data (^2H_2O): ^{13}C, δ 34.0–38.0 (CH_2, polyacrylamide), 40.7 (OCH_2CH_2NH), 42.0–45.0 (CH, polyacrylamide), 62.8 (β-CH_2), 69.9 (OCH_2CH_2NH), 72.8 (C-4), 73.1 (C-2), 76.3 (C-5), 76.6 (C-3), 103.9 (C-1), 171.7, 178.2 (COOH, CONH), 180.9 (CONH, polyacrylamide).

Copolymer **30**

Monomer **22** (28 mg, 0.066 mmol) was copolymerized with acrylamide (33 mg, 0.46 mmol) in 0.2 M acetate buffer (pH 5.8) as described above to yield copolymer **30** (51 mg, 84%), $[\alpha]_D$ −7° (c = 1, water).

NMR data (^2H_2O): ^{13}C, δ 22.7 (γ-CH_2), 30.0 (β-CH_2), 31.5 (δ-CH_2), 35.5–36.5 (CH_2, polyacrylamide), 39.9 (2C) (ε-CH_2, OCH_2CH_2NH), 42.5–42.8 (CH, polyacrylamide), 55.4 (α-CH), 69.0 (OCH_2CH_2NH), 72.1 (C-4), 73.4 (C-2), 75.85 (2C) (C-3,5), 103.2 (C-1), 170.5 (COOH), 180.2 (CONH, polyacrylamide).

Copolymer **31**

Copolymerization of monomer **26** (34 mg, 0.094 mmol) and acrylamide (47 mg, 0.66 mmol) as described above afforded copolymer **31** (66 mg, 81.5%), $[\alpha]_D$ −9.5° (c = 0.4, water).

NMR data (^2H_2O): ^{13}C, δ 19.1 (β-CH_3), 35.0–37.1 (CH_2, polyacrylamide), 40.6 (OCH_2CH_2NH), 42.8–43.8 (CH, polyacrylamide), 40.6 (OCH_2CH_2NH), 42.8–43.8 (CH, polyacrylamide), 51.75 (α-CH), 70.3 (OCH_2CH_2NH), 70.3, 71.6, 73.7, 75.9 (C-2,3,4,5), 103.9 (C-1), 170.7 (COOH), 180.6 (CONH, polyacrylamide).

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