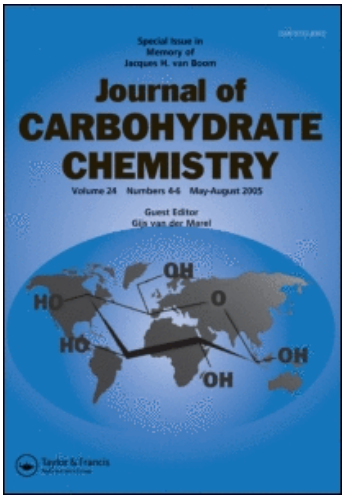

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GLYCOPOLYMERS FROM SYNTHETIC FRAGMENTS (AMIDES OF α-D-GALACTURONIC ACID WITH AMINO ACIDS) OF *PROTEUS* O-ANTIGENS

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ABSTRACT

Galacturonamides of amino acids (alanine, lysine, serine, and threonine), constituents of *Proteus* O-specific polysaccharides, have been synthesised. *O-tert*-Butyl and N^{ε}-*tert*-butyloxycarbonyl protected amino acid *tert*-butyl esters were condensed with the 2-azidoethyl α-glycoside of D-galacturonic acid, prepared by Fischer glycosidation. Reduction of the azido group followed by *N*-acryloylation and deprotection gave the target monomers. By copolymerisation with acryl-amide, these were converted into glycopolymers potentially useful for defining epitopes in *Proteus* O-antigens.

INTRODUCTION

Several *Proteus* O-antigens have been shown to comprise L-lysine[1-3,5] and L-alanine[3,4] amidically linked to the carboxyl group of β-D-glucuronic[3] or α-D-galacturonic acid.[1-5] Recently, two other amino acids, L-serine and L-threonine,

found earlier as constituents of capsular polysaccharides from *Escherichia coli*[6,7] and *Haemophilus influenzae* type d,[8] were identified as *N*-(α-D-galacturonoyl) derivatives in the O-antigens of *Proteus mirabilis* O28[5] and *P. mirabilis* 3/6.[9] Synthetic amino acid-uronic acid conjugates in the form of glycopolymers could be useful for defining epitopes of *Proteus* O-antigens. Syntheses of the [6(*N*)-R]-β-D-Glc*p*A amides (R = L-Ala, L-Lys, L-Ser, and L-Thr) as well as [6(*N*)-L-Ala]-β-D-Gal*p*A and derived glycopolymers have been reported.[10]

We now describe the synthesis of [6(*N*)-R]-α-D-Gal*p*A amides (where R is L- and D-alanine, L- and D-lysine, L-serine, and L-threonine) found in several *Proteus* O-specific polysaccharides. The reasons for synthesizing analogues with D-amino acids were:

1) To find distinctive properties of diastereomers derived from L- and D-amino acids. Using these findings, to make a conclusion, whether epimerisation of amino acid residue (so called "racemisation") takes place in the course of condensation and/or subsequent synthetic steps.

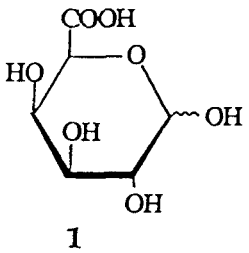
2) To have glycopolymers with enantiomeric amino acids residues in order to compare their immunochemical properties.

RESULTS AND DISCUSSION

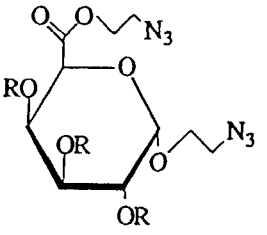
The amino acid-galacturonic acid conjugates were synthesized as 2-azidoethyl glycosides with a masked terminal amino function.[11] The latter is suitable for preparation of neoglycoproteins by coupling to protein carriers.[12] For preparation of glycopolymers, *N*-acryloylation of the amino group in the aglycon followed by copolymerisation with acrylamide was used.[13,14] Due to the presence of the second amino group (in the amino acid moiety), application of an alternative approach to oligosaccharide-polyacrylamide polymers by nucleophilic displacement in poly(4-nitrophenylacrylate)[15] is hindered.

Recently, syntheses of 2-azidoethyl glycosides by several methods have been described.[11] For the preparation of the α-anomer of 2-azidoethyl D-galactopyranosiduronic acid 8, Fischer glycosidation seemed to be the simplest way. Attempted glycosidation of D-galacturonic acid 1 with 2-azidoethanol[11,16] under the condition described in reference 17 [in DMSO in the presence of trifluoromethanesulfonic (triflic) acid] failed. 2-Azidoethyl glycosides could not be found in the mixture even after 3 days of reaction at 85 °C. When triflic acid

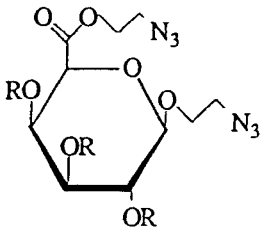
promoted glycosidation of **1** was performed in 2-azidoethanol as a solvent, a mixture of furanosides and pyranosides was obtained. A reaction in the presence of 20 mol % of triflic acid after 52 h at 85 °C afforded 5% of the α-pyranoside **2**. Also isolated were the β-pyranoside **4** (4%) and a mixture (~1:2) of the α,β-furanosides **6** (36%), the latter could not be separated by column chromatography on silica gel. With an increased concentration of triflic acid a ratio of **2** to **6** improved. Glycosidation of **1** using 34 mol % of the promoter at 70-80 °C (3 days) gave **2** in 18% yield.



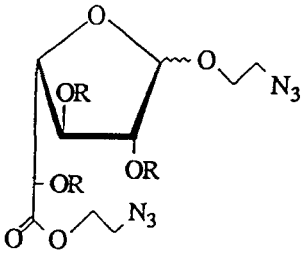
1



2 R = H
3 R = Ac



4 R = H
5 R = Ac

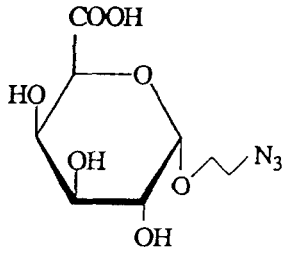


6 R = H
7 R = Ac

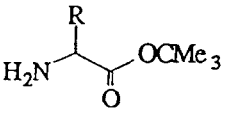
Initially, assignment of isomeric glycosides (**2**, **4**, and **6**) was made from ^{1}H NMR data for the acetylated derivatives **3**, **5**, and **7**. The spectra of **3** and **7** matched the proposed structures, whereas ^3J values ($J_{2,3}$ 2.0, $J_{3,4}$ 7.0, $J_{4,5}$ 7.0 Hz) in the spectrum of **5** were not typical for the β-D-galactopyranose ($^4C_1$). CI-MS (with CH_4 as a gas-reagent) of **5** showed a cluster molecular ion $[M+C_2H_5]^+$ of *m/z* 487, which is consistent with the mass of the tri-*O*-acetyl derivative of **4**. In NOE experiments selective irradiation of the OCH_2-protons in the aglycon of the α-pyranoside **3** caused enhancement of the H-5 and H-1 signals, whereas in the case of isomeric **5** enhancement of only the H-1 signal (indicative of β–configuration) was observed. However, the ^{1}H NMR characteristics of the

glycoside **4** itself are consistent with the β-D-galactopyranose in the $^4C_1$ conformation, which is disturbed in the case of **5** due to acetylation.

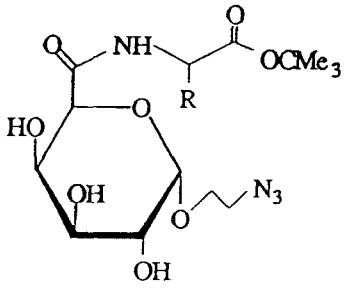
Saponification of **2** with 0.2M sodium hydroxide afforded the free acid **8** (70%) further used for coupling with amino acids. Condensation of **8** with *tert*-butyl esters of O- and N^{ε}-protected amino acids **9-14**, promoted by ethyl 2-ethoxy-1,2-dihydroquinoline-1-carboxylate (EEDQ) as described earlier,[10,18] gave the protected amides **15-20** respectively. The structures of **15-20** were confirmed by the ^{13}C NMR data (see Experimental). The spectra of the diastereomers **15, 16** (or **17, 18**) containing L- and D-alanine (L- and D-lysine) residues were practically indistinguishable. However, in each pair the diastereomers have close but clearly distinct mobilities in TLC (see Experimental). Therefore, TLC analysis was used to ascertain that no "racemisation" took place in the course of condensation and/or subsequent synthetic steps.



8



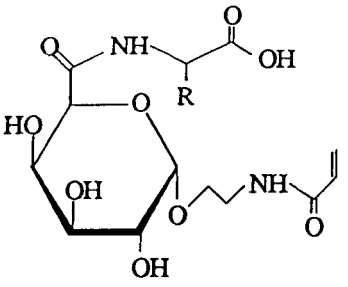
9-14



15-20

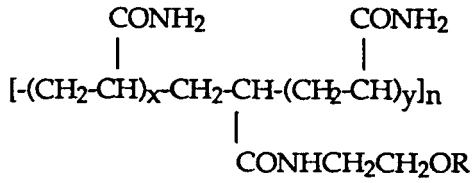
9, 15	R = Me	(L-Ala)
10, 16	R = Me	(D-Ala)
11, 17	R = $(CH_2)_4NHCOOMe_3$	(L-Lys)
12, 18	R = $(CH_2)_4NHCOOMe_3$	(D-Lys)
13, 19	R = CH_2OCMe_3	(L-Ser)
14, 20	R = $CH(Me)OCMe_3$	(L-Thr)

Catalytic hydrogenation of the azidoethyl group in **15-20** followed by *N*-acryloylation [acryloyl chloride in the presence of Dowex 1x8 (HCO_3^-) resin] afforded the corresponding protected 2-acrylamidoethyl glycosides. These were separately subjected to brief treatment with trifluoroacetic acid (45 min at 20 °C) to give the target monomers **21-26**. The ^{13}C NMR spectra of the monomers **21-26** confirmed the structures assigned.



21 R = Me (L-Ala)
22 R = Me (D-Ala)
23 R = $(CH_2)_4NH_2$ (L-Lys)
24 R = $(CH_2)_4NH_2$ (D-Lys)
25 R = CH_2OH (L-Ser)
26 R = CH(Me)OH (L-Thr)

It should be noted that in case of D-alanine and D-lysine, derivatives obtained by *N*-acryloylation were resolved using column chromatography to give two fractions. The major fraction, being most probably the *Z* isomer about the amide bond in the aglycon (*cf.* ref 10) was deprotected with trifluoroacetic acid to give the target monomer (**22** or **24**) with "normal" position of the double bond C signals at 128.7 (CH_2=CH) and 131.1 p.p.m. (CH_2=CH) (*cf.* ref. 10,19). Deprotection of the minor fraction (isolated in a yield of 20 and 23% for D-alanine and D-lysine, respectively), being a mixture of *E* isomers, resulted in a mixture of compounds with shifted C signals of the double bond (CH_2=CH, 129.1; CH_2=CH, 130.2 p.p.m.)



27 R = [6(*N*)-L-Ala]-D-Gal*p*A-(α1→
28 R = [6(*N*)-D-Ala]-D-Gal*p*A-(α1→
29 R = [6(N^{α})-L-Lys]-D-Gal*p*A-(α1→
30 R = [6(N^{α})-D-Lys]-D-Gal*p*A-(α1→
31 R = [6(*N*)-L-Ser]-D-Gal*p*A-(α1→
32 R = [6(*N*)-L-Thr]-D-Gal*p*A-(α1→

The amino acid containing galacturonamides **21-26** were transformed into high-molecular-weight glycopolymers *via* radical copolymerisation with acrylamide promoted by ammonium persulfate and *N,N,N′,N′*-tetramethylethylenediamine (TMEDA) in 0.2M acetate buffer (pH 5.8) as described earlier.[18] The copolymers **27-32** were isolated by gel-filtration on Sephadex G-50 in yields of 80-90%. The presence of both unsubstituted acrylamide residues and those *N*-

substituted by an amino acid-galacturonamide moiety in the ratio of 10-11:1 was deduced by integration of the appropriate ^{13}C signals.

The use of the glycopolymers obtained for defining epitopes in *Proteus* O-antigens will be reported elsewhere.

EXPERIMENTAL

General Methods. The methods and the instrumental and chromatographic procedures used in the present study have been described.[10,11] Elemental analyses were not obtained for syrupy or amorphous compounds, which were purified by column chromatography and characterised by NMR spectroscopy. Assignment of signals in ^1H NMR spectra were based on H_i-{H_j} homonuclear resonance technique. D-Galacturonic acid was from BDH Chemicals Ltd., Poole, England. L-Alanine *tert*-butyl ester hydrochloride was purchased from Fluka, Buchs, Switzerland. *O-tert*-Butyl-L-serine and *O-tert*-butyl-L-threonine *tert*-butyl esters (special order) were from Serva, Heidelberg, Germany. N^{ε}-BOC-L- and -D-lysine *tert*-butyl ester hydrochlorides, and D-alanine *tert*-butyl ester hydrochloride were from Bachem, Heidelberg, Germany. EEDQ was purchased from Merck, Darmstadt, Germany. Acrylamide (ultragrade) was from LKB, Sweden. MilliQ water was used throughout the preparation of 2-acrylamidoethyl glycosides.

2-Azidoethyl (2-Azidoethyl α- and β-D-galactopyranosid)uronates (2 and 4) and 2-Azidoethyl (2-Azidoethyl D-galactofuranosid)uronate (6). To a cold (ice-water) solution of D-galacturonic acid monohydrate (**1**, 2.12 g, 10 mmol) in 2-azidoethanol (5 mL) was dropwise added triflic acid (0.3 mL, 3.4 mmol) while stirring and cooling. The mixture was heated at 70-80 °C (bath) for 3 days and cooled. Then triethylamine (0.48 mL, 3.5 mmol) was added, and excess of 2-azidoethanol was distilled off *in vacuo* (< 1 mm).[20] The dark residue was dissolved in ethyl acetate and filtered through a silica gel column (ethyl acetate, then ethyl acetate/methanol, 85/15 by vol.). The eluate was concentrated and then purified by HPLC (Silasorb 600, 10 μm, 2.5 x 25 cm; ethyl acetate) to give pure **2** (602 mg, 18%), $[\alpha]^{23}_D$ +62° (*c* 1, $CHCl_3$), R_F 0.31 (ethyl acetate/methanol, 9/1); R_F 0.41, $R_{\alpha\text{-D-GalOMe}}$ 3.4 (chloroform/methanol, 85/15). NMR data ($CDCl_3$ - CD_3OD): ^1H, δ 3.30-3.38 (m, 2H, 2 x $CH_AH_BN_3$), 3.41-3.56 (m, 3H, $CO_2CH_AH_BCH_2N_3$, 2 x $CH_AH_BN_3$), 3.58-3.64 (m, 1H, $OCH_AH_BCH_2N_3$), 3.75 (dd, 1H, $J_{2,3}$ 10.0 Hz, H-2), 3.77-3.86 (m, 1H, $OCH_AH_BCH_2N_3$), 3.80 (dd, 1H, $J_{3,4}$

3.0 Hz, H-3), 3.96-4.10 (m, 1H, $CO_2CH_AH_BCH_2N_3$), 4.22 (dd, 1H, H-4), 4.50 (d, 1H, $J_{4,5}$ 1.5 Hz, H-5), 4.92 (d, 1H, $J_{1,2}$ 3.0 Hz, H-1); ^{13}C, δ 49.4 ($OCH_2CH_2N_3$), 50.5 ($CO_2CH_2CH_2N_3$), 63.9 ($OCH_2CH_2N_3$), 67.3 ($CO_2CH_2CH_2N_3$), 67.7 (C-2), 69.2 (C-3), 70.0 (C-4), 70.5 (C-5), 99.1 ($^1J_{C\text{-}1,H\text{-}1}$171 Hz, C-1), 168.9 (C=O).

By further elution were also isolated the β-pyranoside **4**, $[\alpha]^{25}D$ +33.2° (*c* 1, $CHCl_3$), R_F 0.45 (ethyl acetate/methanol, 9/1) and the furanoside **6**, R_F 0.69 (ethyl acetate/methanol, 9/1), the latter being a mixture of anomers (α/β ≅ 1/2, ^1H NMR data).

NMR data for **4** ($CDCl_3$ - CD_3OD): ^1H, δ 3.41-3.51 (m, 2H, $OCH_2CH_2N_3$), 3.52-3.60 (m, 2H, $CO_2CH_2CH_2N_3$), 3.65 (dd, 1H, $J_{2,3}$ 10.0 Hz, H-2), 3.71 (dd, 1H, H-3), 3.72-3.80 (m, 1H, $OCH_AH_BCH_2N_3$), 4.01-4.10 (m, 1H, $OCH_AH_BCH_2N_3$), 4.26-4.40 (m, 2H, $CO_2CH_2CH_2N_3$), 4.27 (dd, 1H, $J_{3,4}$ 3.0 Hz, H-4), 4.32 (d, 1H, $J_{4,5}$ 1.6 Hz, H-5), 4.39 (d, 1H, $J_{1,2}$ 7.1 Hz, H-1); ^{13}C, δ 48.2 ($OCH_2CH_2N_3$), 50.5 ($CO_2CH_2CH_2N_3$), 63.7 ($OCH_2CH_2N_3$), 68.1 ($CO_2CH_2CH_2N_3$), 69.4 (C-4), 70.2 (C-2), 72.5 (C-3), 73.8 (C-5), 102.9 (C-1), 168.0 (C=O).

NMR data for **6** ($CDCl_3$ - CD_3OD), selected signals: ^1H, δ 4.84 (d, 1H, $J_{1,2}$ 4.5 Hz, H-1, β-anomer), 4.97 (s, 1H, H-1, α-anomer); ^{13}C (there are two series of signals belonging to α- and β-anomer), **α-6**, δ 49.6 ($OCH_2CH_2N_3$), 51.0 ($CO_2CH_2CH_2N_3$), 64.0 ($OCH_2CH_2N_3$), 67.4 ($CO_2CH_2CH_2N_3$), 69.9 (C-5), 73.9 (C-3), 77.4 (C-2), 82.5 (C-4), 101.1 (C-1), 172.0 (C=O); **β-6**, δ 49.6 ($OCH_2CH_2N_3$), 50.6 ($CO_2CH_2CH_2N_3$), 64.4 ($OCH_2CH_2N_3$), 66.2 ($CO_2CH_2CH_2N_3$), 69.9 (C-5), 77.4 (C-3), 80.0 (C-2), 86.1 (C-4), 108.4 (C-1), 171.8 (C=O).

2-Azidoethyl (2-Azidoethyl 2,3,4-tri-*O*-acetyl-α- and β-D-galactopyranosid)-uronates (3 and 5) and 2-Azidoethyl (2-Azidoethyl 2,3,5-tri-*O*-acetyl-D-galacto-furanosid)uronate (7). Conventional acetylation (acetic anhydride, pyridine) of **2**, **4**, and **6** gave the corresponding acetates **3**, **5**, and **7**.

3, NMR data ($CDCl_3$): ^1H, δ 2.01, 2.10, and 2.12 (3s, 9H, OAc), 3.35-3.52 (m, 2H, $OCH_2CH_2N_3$), 3.44-3.58 (m, 2H, $CO_2CH_2CH_2N_3$), 3.63-3.72 (m, 1H, $OCH_AH_BCH_2N_3$), 3.78-3.96 (m, 1H, $OCH_AH_BCH_2N_3$), 4.20-4.40 (m, 2H, $CO_2CH_2CH_2N_3$), 4.71 (d, 1H, $J_{4,5}$ 1.8 Hz, H-5), 5.20 (dd, 1H, H-2), 5.31 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 5.43 (dd, 1H, $J_{2,3}$ 11.5 Hz, H-3), 5.81 (dd, 1H, $J_{3,4}$ 3.5 Hz, H-4).

5, NMR data ($CDCl_3$): ^1H, δ 2.12, 2.18, and 2.20 (3s, 9H, OAc), 3.37-3.55 (m, 4H, $OCH_2CH_2N_3$, $CO_2CH_2CH_2N_3$), 3.65-3.83 (m, 3H, $CO_2CH_2CH_2N_3$, $OCH_AH_BCH_2N_3$, $OCH_AH_BCH_2N_3$), 3.91-4.00 (m, 1H, $OCH_AH_BCH_2N_3$), 4.77 (dd, 1H, $J_{2,3}$ 2.0 Hz, $J_{3,4}$ 7.0 Hz, H-3), 4.83 (d, 1H, $J_{1,2}$ 7.5 Hz, H-1), 5.31 (t, 1H, H-4), 5.32 (dd, 1H, H-2), 5.56 (d, 1H, $J_{4,5}$ 7.0 Hz, H-5).

The MS-CI (CH_4) spectrum of **5** showed an $[M+C_2H_5]^+$ ion of *m/z* 487 and a series of derived ions of *m/z* 372 ($[M+H-HOCH_2CH_2N_3]^+$), 344 ([M+H-

$HOCH_2CH_2N_3$ -N_2]$^+$), 302 ([M+H-$HOCH_2CH_2N_3$ -N_2 -CH_2=C=O]$^+$), and 242 ([M+H -$HOCH_2CH_2N_3$ -N_2 -CH_2=C=O -CH_3COOH]$^+$).

7, NMR data ($CDCl_3$): ^1H (the spectrum contains series of both α- and β-anomers), α-7, δ 2.10, 2.13, and 2.25 (3s, 9H, OAc), 3.25-3.37 (m, 1H, $OCH_2CH_AH_BN_3$), 3.45-3.58 (m, 4H, $OCH_AH_BCH_2N_3$, $CO_2CH_2CH_2N_3$, $OCH_2CH_AH_BN3$), 3.93-4.01 (m, 1H, $OCH_AH_BCH_2N_3$), 4.29-4.43 (m, 2H, $CO_2CH_2CH_2N_3$), 4.42 (dd, 1H, H-2), 5.08 (dd, 1H, H-4), 5.24 (d, 1H, $J_{4,5}$ 4.5 Hz, H-5), 5.34 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 5.57 (dd, 1H, $J_{2,3}$ 6.5 Hz, $J_{3,4}$ 8.0 Hz, H-3); β-7, δ, 2.11, 2.12, and 2.23 (3s, 9H, OAc), 3.35-3.44 (m, $OCH_2CH_AH_BN_3$), 3.48-3.56 (m, 3H, $CO_2CH_2CH_2N_3$, $OCH_2CH_AH_BN_3$), 3.61-3.70 (m, 1H, $OCH_AH_BCH_2N_3$), 3.83-3.92 (m, 1H, $OCH_AH_BCH_2N_3$), 4.26-4.45 (m, 2H, $CO_2CH_2CH_2N_3$), 4.61 (dd, 1H, $J_{3,4}$ 5.4 Hz, H-3), 5.05 (dd, 1H, H-4), 5.13 (s, 1H, H-1), 5.14 (d, 1H, $J_{4,5}$ 1.8 Hz, H-5), 5.47 (d, 1H, $J_{2,3}$ 3.0 Hz, H-2).

2-Azidoethyl α-D-Galactopyranosiduronic acid (8). To a cooled (ice-water) solution of **2** (240 mg, 0.72 mmol) in methanol (8 mL) was added sodium hydroxide (2 mL, 1 M). After 30 min at 4 °C the mixture was treated with KU-2 (H^+) resin and filtered, the resin was washed with water. The combined filtrate and washings were applied to a column (1.5 x 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 **8** (133 mg, 70%), $[\alpha]^{27}_D$ +96° (*c* 2, H_2O). NMR data (D_2O): ^{13}C, δ 51.6 ($OCH_2CH_2N_3$), 66.4 ($OCH_2CH_2N_3$), 66.8 (C-2), 70.0 (C-3), 71.2 (C-4), 71.7 (C-5), 99.8 ($^1J_{C\text{-}1,H\text{-}1}$ 171 Hz, C-1), 173.7 (COOH).

***N*-(2-Azidoethyl α-D-galactopyranosiduronoyl)-L-alanine *tert*-Butyl ester (15).** To a solution of **8** (28 mg, 0.11 mmol) and **9** [generated from the corresponding hydrochloride (30 mg, 0.16 mmol) by treatment with an equivalent amount of triethylamine in ethyl acetate] in anhydrous *N,N*-dimethylformamide (3 mL, freshly distilled *in vacuo* over ninhydrin) was added EEDQ (54 mg, 0.22 mmol) in one portion. The mixture was kept at 20 °C and after 24 h another portion of EEDQ (54 mg, 0.22 mmol) was added. After 48 h TLC (chloroform/methanol/acetic acid, 85/15/1) showed complete conversion of **8** into the L-alanine-galacturonic acid derivative **15**, R_F 0.42. The mixture was concentrated, and toluene then water and finally toluene were evaporated from the residue, which was then purified by column chromatography (hexane followed by chloroform/methanol, 98/2) to give pure **15** (30 mg, 71%), $[\alpha]^{25}_D$ +33.6° (*c* 1, $CHCl_3$/MeOH, 5/1), R_F 0.44, $R_{\alpha\text{-D-GalOMe}}$ 3.7 (chloroform/methanol, 85/15). NMR data ($CDCl_3/CD_3OD$, 5/1): ^{13}C, δ 17.6 (β-CH_3), 27.6 [$OC(CH_3)_3$], 48.4 (α-

CH), 50.4 ($OCH_2CH_2N_3$), 67.3 ($OCH_2CH_2N_3$), 68.2 (C-2), 69.4 (C-3), 69.5 (C-4), 71.5 (C-5), 82.1 ($OCMe_3$), 99.3 (C-1). 168.7, 171.7 (C=O).

***N*-(2-Azidoethyl α-D-galactopyranosiduronoyl)-D-alanine *tert*-Butyl ester (16).** Condensation of **8** (44 mg, 0.17 mmol) with **10** [generated from the corresponding hydrochloride (75 mg, 0.41 mmol)] as described above gave **16** (65 mg, 100%), $[\alpha]^{23}_D$ +40.6° (*c* 1, $CHCl_3$), R_F 0.47, $R_{\alpha\text{-D-GalOMe}}$ 3.9 (chloroform/methanol, 85/15). NMR data ($CDCl_3$): ^{13}C, δ 17.9 (β-CH_3), 28.0 [$OC(CH_3)_3$], 48.8 (α-CH), 50.7 ($OCH_2CH_2N_3$), 67.6 ($OCH_2CH_2N_3$), 68.8 (C-2), 69.9 (C-3), 70.5 (C-4), 72.2 (C-5), 82.6 ($OCMe_3$), 99.5 (C-1), 168.9, 172.4 (C=O).

***N*$^{\alpha}$-(2-Azidoethyl α-D-galactopyranosiduronoyl)-*N*$^{\varepsilon}$-(*tert*-butyloxycarbonyl)-L-lysine *tert*-Butyl ester (17).** Condensation of **8** (102 mg, 0.39 mmol) with **11** [generated from the corresponding hydrochloride (285 mg, 0.76 mmol)] was performed as described above to give **17** (205 mg, 92%), $[\alpha]^{23}_D$ +31° (c 1, $CHCl_3$), R_F 0.53, $R_{\alpha\text{-D-GalOMe}}$ 4.4 (chloroform/methanol, 85/15). NMR data ($CDCl_3$): ^{13}C, δ 22.2 (γ-CH_2), 28.0 and 28.5 [$OC(CH_3)_3$], 29.1 (β-CH_2), 32.4 (δ-CH_2), 40.3 (ε-CH_2), 50.7 ($OCH_2CH_2N_3$), 52.0 (α-CH), 67.6 ($OCH_2CH_2N_3$), 68.8 (C-2), 69.5 (C-3), 70.2 (C-4), 71.9 (C-5), 82.2 ($OCMe_3$), 99.3 (C-1), 168.5, 171.3 (C=O).

***N*$^{\alpha}$-(2-Azidoethyl α-D-galactopyranosiduronoyl)-*N*$^{\varepsilon}$-(*tert*-butyloxycarbonyl)-D-lysine *tert*-Butyl ester (18).** Condensation of **8** (35 mg, 0.13 mmol) with **12** [generated from the corresponding hydrochloride (68 mg, 0.2 mmol)] was performed as described above to give **18** (66 mg, 85%), $[\alpha]^{25}_D$ +23.6° (*c* 1, $CHCl_3$), R_F 0.49, $R_{\alpha\text{-D-GalOMe}}$ 4.1 (chloroform/methanol, 85/15). NMR data ($CDCl_3$): ^{13}C, δ 22.6 (γ-CH_2), 28.0 and 28.4 [2 x $OC(CH_3)_3$], 29.6 (β-CH_2), 31.5 (δ-CH_2), 40.3 (ε-CH_2), 50.6 ($OCH_2CH_2N_3$), 52.8 (α-CH), 67.7 ($OCH_2CH_2N_3$), 68.9 (C-2), 69.8 (C-3), 70.1 (C-4), 72.1 (C-5), 82.8 ($OCMe_3$), 99.4 (C-1), 169.1, 171.6 (C=O).

***N*-(2-Azidoethyl α-D-galactopyranosiduronoyl)-*O*-(*tert*-butyl)-L-serine *tert*-Butyl ester (19).** Condensation of **8** (35 mg, 0.13 mmol) with **13** (44 mg, 0.2 mmol) was performed as described above to give **19** (62 mg, 100%), $[\alpha]^{23}_D$ +47° (*c* 1, $CHCl_3$), R_F 0.51, $R_{\alpha\text{-D-GalOMe}}$ 4.3 (chloroform/methanol, 85/15). NMR data ($CDCl_3$): ^{13}C, δ 27.4 and 28.1 [2 x $OC(CH_3)_3$], 50.6 ($OCH_2CH_2N_3$), 53.1 (α-CH), 62.3 (β-CH_2), 67.6 ($OCH_2CH_2N_3$), 68.7 (C-2), 69.5 (C-3), 70.1 (C-4), 71.8 (C-5), 73.4 (CH_2OCMe_3), 82.0 ($COOCMe_3$), 99.3 (C-1), 168.7, 169.3 (C=O).

***N*-(2-Azidoethyl α-D-galactopyranosiduronoyl)-*O*-(*tert*-butyl)-L-threonine *tert*-Butyl ester (20).** Condensation of **8** (35 mg, 0.13 mmol) with **14** (49 mg, 0.21 mmol) was performed as described above to give **20** (64 mg, 100%), $[\alpha]^{21}_D$

+41.8° (*c* 1, $CHCl_3$), R_F 0.50, $R_{\alpha\text{-D-GalOMe}}$ 4.2 (chloroform/methanol, 85/15). NMR data ($CDCl_3$): ^{13}C, δ 20.5 (γ-CH_3), 28.2 and 28.8 [2 x $OC(CH_3)_3$], 50.7 ($OCH_2CH_2N_3$), 58.1 (α-CH), 67.4 (β-CH), 67.6 ($OCH_2CH_2N_3$), 68.8 (C-2), 69.5 (C-3), 70.2 (C-4), 71.9 (C-5), 74.0 ($CHOCMe_3$), 82.0 ($COOCMe_3$), 99.4 (C-1), 169.1, 169.6 (C=O).

***N*-(2-Acrylamidoethyl α-D-galactopyranosiduronoyl)-L-alanine (21).** A solution of **15** (67 mg, 0.17 mmol) in methanol (6 mL) was hydrogenated over Pd/C (~50 mg, 10%). After 3 h, TLC (chloroform/methanol, 85/15) showed complete conversion into the aminoethyl glycoside (R_F 0, positive ninhydrin test). The mixture was filtered and concentrated. To a solution of the residue (55 mg, 88%) in methanol/MilliQ water, 8/1 (4.5 mL), containing 2,6-di-*tert*-butyl-4-methylphenol (3-5 mg, as an inhibitor of polymerisation) was added acryloyl chloride (42 μL, 0.52 mmol), and the mixture was stirred with Dowex 1x8 (HCO_3^-) resin. After 18 h, TLC (chloroform/methanol, 85/15) showed complete conversion into the acrylamidoethyl glycoside, R_F 0.40. The mixture was filtered and concentrated. Column chromatography (chloroform/methanol, 97/3) of the residue gave pure protected acrylamidoethyl glycoside (42 mg, 54% from **15**), which was then dissolved in trifluoroacetic acid (2 mL). After 45 min at 20 °C the mixture was concentrated, tetrachloromethane and then methanol were evaporated from the residue. MilliQ 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 **21** (26 mg, 42% from **15**), $[\alpha]^{24}D$ +47° (*c* 1, H_2O), R_F 0.44 (ethyl acetate/acetic acid/formic acid/water, 18/4/1/3 by vol.). NMR data (D_2O): ^{13}C, δ 17.6 (β-CH_3), 40.4 (OCH_2CH_2NH), 49.5 (α-CH), 68.3 (OCH_2CH_2NH), 69.1 (C-2), 70.4 (C-3), 71.0 (C-4), 72.2 (C-5), 99.8 (C-1), 128.8 (CH_2=CH), 131.1 (CH_2=CH), 170.0, 171.9, and 177.3 (C=O).

***N*-(2-Acrylamidoethyl α-D-galactopyranosiduronoyl)-D-alanine (22).** Transformation of **16** (70 mg, 0.18 mmol), as described above, gave the target monomer **22** (42 mg, 65%), $[\alpha]^{27}D$ +14.7° (*c* 2, H_2O), R_F 0.45 (ethyl acetate/acetic acid/formic acid/water, 18/4/1/3 by vol). NMR data (D_2O): ^{13}C, δ 17.4 (β-CH_3), 40.5 (OCH_2CH_2NH), 49.5 (α-CH), 68.2 (OCH_2CH_2NH), 69.0 (C-2), 70.4 (C-3), 70.9 (C-4), 72.2 (C-5), 99.8 (C-1), 128.7 (CH_2=CH), 131.0 (CH_2=CH), 169.9, 171.8, and 177.1 (C=O).

***N*$^{\alpha}$-(2-Acrylamidoethyl α-D-galactopyranosiduronoyl)-L-lysine (23).** Transformation of **17** (150 mg, 0.26 mmol), as described above, afforded the target monomer **23** (113 mg, 72%), as the trifluoroacetate salt, $[\alpha]^{26}D$ +35.8° (*c*

2.5, H_2O), R_F 0.18 (ethyl acetate/acetic acid/formic acid/water, 18/4/1/3 by vol.). NMR data (D_2O): ^{13}C, δ 23.1 (γ-CH_2), 27.3 (β-CH_2), 31.4 (δ-CH_2), 40.4 (ε-CH_2), 40.5 (OCH_2CH_2NH), 53.2 (α-CH), 68.4 (OCH_2CH_2NH), 69.1 (C-2), 70.4 (C-3), 70.9 (C-4), 72.3 (C-5), 99.8 (C-1), 128.7 (CH_2=CH), 131.1 (CH_2=CH), 169.9, 172.1, and 176.2 (C=O).

***N*$^{\alpha}$-(2-Acrylamidoethyl α-D-galactopyranosiduronoyl)-D-Lysine (24).** Hydrogenation of **18** (64 mg, 0.11 mmol) followed by *N*-acryloylation and deprotection, as described above, gave **24** (40 mg, 60%) as the trifluoroacetic salt, $[\alpha]^{25}_D$ +58.9° (*c* 2, H_2O), R_F 0.09 (ethyl acetate/acetic acid/formic acid/water, 18/4/1/3). NMR data (D_2O): ^{13}C, δ 23.3 (γ-CH_2), 27.5 (β-CH_2), 31.1 (δ-CH_2), 40.4 (2C, ε-CH_2, OCH_2CH_2NH), 53.5 (α-CH), 68.2 (OCH_2CH_2NH), 69.0 (C-2), 70.4 (C-3), 71.0 (C-4), 72.2 (C-5), 99.8 (C-1), 128.7 (CH_2=CH), 131.1 (CH_2=CH), 169.9, 172.1, and 176.3 (C=O).

***N*-(2-Acrylamidoethyl α-D-galactopyranosiduronoyl)-L-serine (25).** Reaction succession with **19** (68 mg, 0.15 mmol), as described above, afforded **25** (42 mg, 76%), $[\alpha]^{28}_D$ +62.2° (*c* 2.2, H_2O), R_F 0.29 (ethyl acetate/acetic acid/formic acid/water, 18/4/1/3). NMR data (D_2O): ^{13}C, δ 40.4 (OCH_2CH_2NH), 55.6 (α-CH), 62.4 (β-CH_2), 68.3 (OCH_2CH_2NH), 69.0 (C-2), 70.3 (C-3), 70.9 (C-4), 72.3 (C-5), 99.8 (C-1), 128.7 (CH_2=CH), 131.1 (CH_2=CH), 172.1, 174.2, and 178.2 (C=O).

***N*-(2-Acrylamidoethyl α-D-galactopyranosiduronoyl)-L-threonine (26).** Transformation of **20** (69 mg, 0.14 mmol), as described above, gave the target monomer **26** (45 mg, 79%), $[\alpha]^{28}_D$ +63.8° (*c* 2.4, H_2O), R_F 0.38 (ethyl acetate/acetic acid/formic acid/water, 18/4/1/3). NMR data (D_2O): ^{13}C, δ 20.0 (γ-CH_3), 40.3 (OCH_2CH_2NH), 58.6 (α-CH), 68.4 (OCH_2CH_2NH), 68.6 (β-CH), 69.0 (C-2), 70.4 (C-3), 70.8 (C-4), 72.5 (C-5), 99.8 (C-1), 128.9 (CH_2=CH), 131.0 (CH_2=CH), 169.8, 172.3, and 174.4 (C=O).

Copolymerisation of 21 with acrylamide. A solution of **21** (23 mg, 0.063 mmol) and acrylamide (31 mg, 0.436 mmol) in acetate buffer (2 mL, 0.2M, pH 5.8) was deaerated using a water pump. Then TMEDA (5 μL) and ammonium persulfate (1 mg) were added, and the mixture was stirred for 18 h at 20 °C under argon. The mixture was diluted with pyridine-acetate buffer (3 mL, 0.05M, pH 5.4) and eluted from a column (2.5 x 37 cm) of Sephadex G-50 with the same buffer. The high-molecular-weight fraction (detected using a differential refractometer) was pooled and lyophilised to give the glycopolymer **27** (48 mg, 89%), $[\alpha]^{23}_D$ +18° (*c* 1, H_2O). NMR data (D_2O): ^{13}C, δ 17.6 (β-CH_3), 35.2-36.6 (CH_2, polyacrylamide), 39.9 (OCH_2CH_2NH), 42.5-43.2 (CH, polyacrylamide), 49.6 (α-CH), 67.6 (OCH_2CH_2NH), 68.8 (C-2), 70.1 (C-3), 70.6 (C-4), 71.9 (C-5), 99.5 (C-1), 171.1, 177.5 (C=O), 180.2 ($CONH_2$, polyacrylamide).

Glycopolymer 28. Copolymerisation of **22** (42 mg, 0.116 mmol) with acrylamide (58 mg, 0.815 mmol), as described above, gave **28** (83 mg, 83%), $[\alpha]^{23}D$ +6° (*c* 1, H_2O). NMR data (D_2O): ^{13}C, δ 18.9 (β-CH_3), 35.2-36.7 (CH_2, polyacrylamide), 39.9 (OCH_2CH_2NH), 42.5-43.3 (CH, polyacrylamide), 51.5 (α-CH), 67.6 (OCH_2CH_2NH), 68.8 (C-2), 70.2 (C-3), 70.7 (C-4), 72.0 (C-5), 99.6 (C-1), 170.4, 177.5 (C=O), 180.2 ($CONH_2$, polyacrylamide).

Glycopolymer 29. Copolymerisation of **23** (77 mg, 0.126 mmol) and acrylamide (63 mg, 0.886 mmol) afforded **29** (103 mg, 74%), $[\alpha]^{23}D$ +15° (*c* 1, H_2O). NMR data (D_2O): ^{13}C, δ 22.6 (γ-CH_2), 27.1 (β-CH_2), 32.2 (δ-CH_2), 35.2-36.6 (CH_2, polyacrylamide), 39.8 (ε-CH_2), 40.3 (OCH_2CH_2NH), 42.5-43.3 (CH, polyacrylamide), 55.3 (α-CH), 67.5 (OCH_2CH_2NH), 68.8 (C-2), 70.2 (C-3), 70.6 (C-4), 72.0 (C-5), 99.5 (C-1), 170.6, 177.5, and 178.7 (C=O), 180.2 ($CONH_2$, polyacrylamide).

Glycopolymer 30. Copolymerisation of the monomer **24** (38 mg, 0.062 mmol) with acrylamide (31 mg, 0.436 mmol) gave **30** (51 mg, 75%). Solutions of the glycopolymer **30** were too viscous for optical rotation measurement and recording spectra.

Glycopolymer 31. The monomer **25** (42 mg, 0.111 mmol) was copolymerised with acrylamide (55 mg, 0.774 mmol), as described above, to yield the glycopolymer **31** (76 mg, 78%), $[\alpha]^{23}D$ +23° (*c* 1, H_2O). NMR data (D_2O): ^{13}C, δ 35.8-36.6 (CH_2, polyacrylamide), 39.9 (OCH_2CH_2NH), 42.5-43.3 (CH_2, polyacrylamide), 57.5 (α-CH), 63.3 (β-CH_2), 67.5 (OCH_2CH_2NH), 68.8 (C-2), 70.2 (C-3), 70.6 (C-4), 72.0 (C-5), 99.5 (C-1), 171.0 and 177.5 (C=O), 180.2 ($CONH_2$, polyacrylamide).

Glycopolymer 32. Copolymerisation of **26** (45 mg, 0.114 mmol) and acrylamide (57 mg, 0.802 mmol) according to the general protocol afforded the glycopolymer **32** (93 mg, 92%), $[\alpha]^{23}D$ +24° (*c* 1, H_2O). NMR data (D_2O): ^{13}C, δ 20.3 (γ-CH_3), 34.0-38.5 (CH_2, polyacrylamide), 40.3 (OCH_2CH_2NH), 43.0-44.2 (CH, polyacrylamide), 59.0 (α-CH), 67.9 (OCH_2CH_2NH), 68.7 (β-CH), 69.2 (C-2), 70.5 (C-3), 70.9 (C-4), 72.5 (C-5), 99.8 (C-1), 180.7 ($CONH_2$, polyacrylamide).

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20. Recovered 2-azidoethanol can be reused.