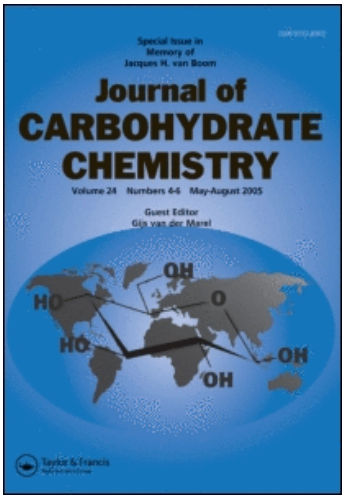

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Large-Scale Synthesis of a Lewis b Tetrasaccharide Derivative, its Acrylamide Copolymer, and Related DI- and Trisaccharides for Use in Adhesion Inhibition Studies with *Helicobacter Pylori*.

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LARGE-SCALE SYNTHESIS OF A LEWIS B TETRASACCHARIDE DERIVATIVE, ITS ACRYLAMIDE COPOLYMER, AND RELATED DI- AND TRISACCHARIDES FOR USE IN ADHESION INHIBITION STUDIES WITH *HELICOBACTER PYLORI.*

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ABSTRACT

The 2-aminoethyl glycoside of O-α-L-fucopyranosyl-(1→2)-O-β-D-galactopyranosyl-(1→3)-[O-α-L-fucopyranosyl-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranose (Lewis B tetrasaccharide) was synthesized on a large scale and acryloylated with acryloyl chloride. The obtained oligosaccharide 2-acrylamidoethyl glycoside was then copolymerized with acrylamide to form a water-soluble, high molecular weight polymer, suitable for use in adhesion inhibition studies with *Helicobacter pylori*. Also synthesized were the corresponding derivatives of O-α-L-fucopyranosyl-(1→2)-O-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranose and O-α-L-fucopyranosyl-(1→2)-β-D-galactopyranose.

INTRODUCTION

Carbohydrate determinants, present on mammalian cell walls in the form of glycolipids or glycoproteins, are important for bacterial adhesion to

host cells. This adhesion is often the first in a series of events that lead to bacterial infection. Bacterial adhesion has been shown in several instances[3] to involve highly specific interaction of the host cell surface carbohydrates with adhesion proteins on the bacteria. Recently, it was shown that *Helicobacter pylori* bacteria adhered in a specific manner to gastric epithelial cells, and the adhesion could be inhibited with glycoconjugates carrying H type 1 and Lewis B determinants.[4] The *in vitro* bacterial binding to oligosaccharide inhibitors was greatly enhanced, if they were presented in the form of multivalent neoglycoproteins. This finding suggested that inhibitors of adhesion could be used as therapeutic agents for preventing infections caused by *Helicobacter pylori*. However, although they are good inhibitors, neoglycoproteins are not well suited for use as therapeutics. Another type of multivalent glycoconjugates are the polyacrylamide copolymers.[5-7] It has been shown[8] that such copolymers bind to influenza virus in a specific and epitope-density related manner. These polymers should be attractive as drug candidates. We therefore now report synthesis of the polyacrylamide copolymers **8**, **17** and **22** (the latter containing the Lewis B determinant). The Lewis B oligosaccharide determinant in **22** has been synthesized before[9-14] in various forms, as have the determinants in **8** and **17** (for reference, see a comprehensive review[15] covering recently synthesized oligosaccharides).

The synthesized polyacrylamide copolymers were used, together with other similar structures derived from other sources, in a tissue slice[16,17] *Helicobacter pylori* adhesion inhibition assay. The most active inhibitor was a Lewis B hexasaccharide copolymer, followed by, in order of decreasing activity, the Lewis B tetrasaccharide copolymer **22**, the disaccharide copolymer **8**, and the trisaccharide copolymer **17**.

RESULTS AND DISCUSSION

Polyacrylamide copolymers of oligosaccharides have been prepared by copolymerizing *e.g.* allyl, *N*-acryloyl, or acrylamidoethyl glycosides with acrylamide. We have chosen the acrylamidoethyl glycosides for our syntheses as these are easily obtained, in two steps, from the corresponding aminoethyl glycosides. The starting point for our syntheses were the azidoethyl monosaccharide glycosides **4** and **9**. Glycoside **4** was prepared from the key intermediate **3**, which in turn was prepared from acetobromogalactose by a

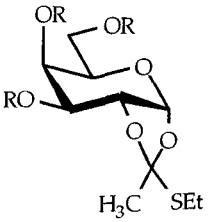
similar route as that reported for the analogous tri-*O*-*p*-chlorobenzyl derivative.[18] The following steps were carried out: acetobromogalactose was treated with ethanethiol, collidine, and tetraethylammonium bromide in nitromethane to give the acetylated thioorthoester **1**, which was treated, without isolation, with sodium methoxide in methanol followed by benzyl chloride and sodium hydroxide in *N,N*-dimethylformamide. The resulting benzylated thioorthoester **2** was directly rearranged by treatment with trimethylsilyl triflate in acetonitrile, to give **3** in crystalline form. Since **3** is a key intermediate, its preparation was scaled up to a 350 g batch size, the yield of **3** after some optimization work was 65 % (from acetobromogalactose, without chromatography).

Conversion (bromine treatment) of **3** to the corresponding bromide followed by silver triflate-mediated glycosylation with azidoethanol[19] gave, after treatment with sodium methoxide in methanol, compound **4** in 71 % yield. Halide-assisted glycosylation of **4** with 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide gave **5** in 78 % yield. Catalytic hydrogenation (Pd/C, ethanol-water) of **5** gave **6** in 44 % yield. The low yield was because hydrogenolysis of the benzyl groups was sluggish, a common phenomenon when amines are formed as initial hydrogenation products. In the later analogous hydrogenation of **18** to give **19**, hydrochloric acid was added to the reaction mixture to convert any amine formed to the hydrochloride form. This greatly increased the hydrogenolysis rate and yield. Another way to avoid hydrogenolysis problems caused by amines is to carry out hydrogenation in two steps, as in the conversion **13** - **15**, with intermediate trifluoroacetylation of the initially formed amine.

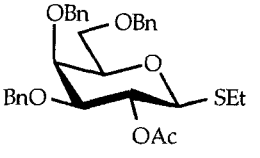
Crude compound **6** was acryloylated with acryloyl chloride to give **7** (57 %) which was then copolymerized with acrylamide to give the copolymer **8**.

For synthesis of the two other copolymers (**17** and **22**), the azidoethyl glycoside[20] **9** was the starting point. It was prepared by reacting 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-d]-2-oxazoline[21] with 2-azidoethanol and sulfuric acid in dichloromethane, giving **9** in 80 % crystalline yield. This is an improvement both in yield and purification method over the reported[20] procedure for **9**. Treatment of **9** with sodium methoxide in methanol, followed by α,α-dimethoxytoluene in *N,N*-dimethylformamide, gave **10** in 82 % crystalline yield. Methyl triflate promoted glycosylation of **10** with **3** gave a disaccharide product in high yield, which was not isolated.

Methanolic sodium methoxide was instead added directly to the glycosylation mixture, which caused deacetylation as well as efficient destruction of the excess methyl triflate (otherwise a serious health hazard at the multigram scale at which this synthesis was carried out). The yield of deacetylated disaccharide **11** after crystallization was 70 %.



1: R = Ac
2: R = Bn



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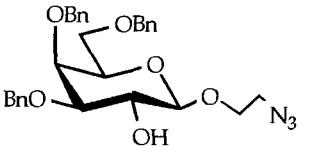

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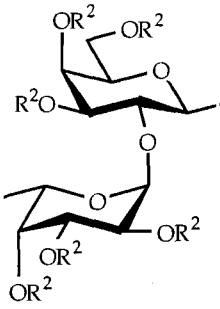
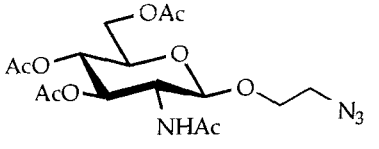


5; $R^1 = N_3$, R^2 = Bn
6; $R^1 = NH_2$, R^2 = H
7; R^1 = NHCOCH=CH_2, R^2 = H
8; R^1 = NH-polymer, R^2 = H



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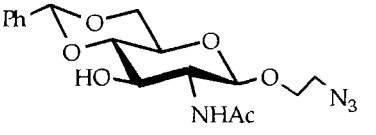

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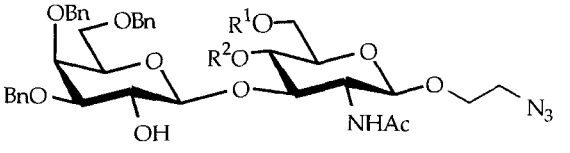
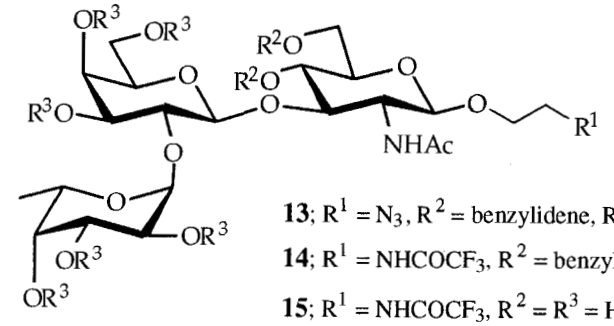


11; R^1, R^2 = benzylidene
12; R^1 = Bn, R^2 = H

Glycosylation of **11** with ethyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside,[22] using dimethyl(methylthio)sulfonium triflate[23] as promotor, gave trisaccharide **13** in 49 % yield. To avoid problems with sluggish hydrogenolysis of the benzyl groups, the azido group of **13** was selectively converted into an amino group by brief (Pd/C) hydrogenolysis, and the product was treated with trifluoroacetic anhydride to give the corresponding trifluoroacetamide derivative **14** in 52 % yield. Hydrogenolysis (Pd/C) of **14** gave **15**, which was directly treated with aqueous ammonia and then acryloyl chloride, to give **16** in 92 % yield (from **14**). Copolymerization of **16** with acrylamide then gave the copolymer **17**.

For synthesis of the Lewis B tetrasaccharide copolymer **22**, the benzylidene acetal in **11** was opened with sodium cyanoborohydride and tetrafluoroboric acid[24] to give diol **12** (90 %). The 4,2'-diol structure of **12** was confirmed by ^{1}H NMR spectroscopy of the di-*O*-acetylated derivative. Compound **12** was difucosylated by treatment with tetraethylammonium bromide and 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl bromide to give the syrupy tetrasaccharide derivative **18** (66 %). Hydrogenolysis of **18** with Pd/C in ethanol containing hydrogen chloride gave **19** as the only detectable (TLC, ^{1}H NMR) compound, acryloylation of which gave **21** (87 %). A small amount of **19** was *N*-acetylated with acetic anhydride and methanol (to give **20**) for NMR characterization. Copolymerization of **21** with acrylamide gave **22** (93.5 % of **21** was incorporated into the polymer, which showed, by NMR, an average incorporation of 1 oligosaccharide unit per 7.4 acrylamide units).

It should be noted that, in the syntheses of **8** and **17**, only small amounts of material was prepared within a tight time-frame, and the yields were not optimized. On the other hand, the synthesis of the Lewis B derivative **21** was carried out several times, finally on a relatively large scale, and some yield optimization work was performed. More than 50 g of this derivative was produced, which required more than 100 g of the intermediates **3** and **10**. The number of chromatographic purification steps (adsorption-desorption type) in the synthesis of **21** was minimized to three, all but one were on the tetrasaccharide stage. This work demonstrates again,[25] that chemical synthesis of oligosaccharide derivatives on a multigram scale is today a feasible task. Production of even larger quantities of complex oligosaccharides is possible. Especially advantageous here is the use of a combination of chemical and enzymatic methods, as previously demonstrated in the large-scale synthesis of a sialyl Lewis X derivative.[26]



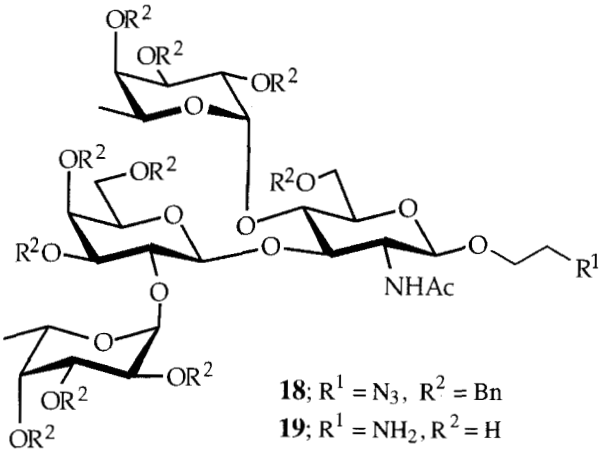
13; $R^1 = N_3$, R^2 = benzylidene, R^3 =Bn

14; $R^1 = NHCOCF_3$, R^2 = benzylidene, R^3 =Bn

15; $R^1 = NHCOCF_3$, $R^2 = R^3 = H$

16; $R^1 = NHCOCH{=}CH_2$, $R^2 = R^3 = H$

17; R^1 = NH-polymer, $R^2 = R^3 = H$



18; $R^1 = N_3$, R^2 = Bn

19; $R^1 = NH_2$, $R^2 = H$

20; $R^1 = NHCOCH_3$, $R^2 = H$

21; $R^1 = NHCOCH{=}CH_2$, $R^2 = H$

22; R^1 = NH-polymer, $R^2 = H$

EXPERIMENTAL

General Methods. Melting points are corrected. Concentrations were performed under reduced pressure at < 40 °C bath temperature. Optical rotations were measured at 23 °C (*c* = 0.5-1.0, chloroform) unless otherwise stated, using a Perkin-Elmer 241 polarimeter. NMR spectra were recorded at the specified temperature, using Varian Gemini 300 MHz or Varian Unity 400

MHz instruments, using, unless otherwise stated, $CDCl_3$ as solvent. The following reference signals were used: $CDCl_3$ δ 77.0 (^{13}C in $CDCl_3$); Me_4Si δ 0.00 (^1H in $CDCl_3$), TPS δ 0.00 (^1H in D_2O), acetone δ 31.0 (^{13}C in D_2O). HRMS spectra were recorded with a JEOL SX/102A mass spectrometer, using the FAB ion source and 3-nitrobenzyl alcohol as matrix. Column chromatography was performed in the "flash" mode on silica gel (0.035-0.070 mm, Matrex LC 60A, Grace, Helsingborg, Sweden). Reversed-phase C-18 silica ("Isolute") was from International Sorbent Technology Ltd (UK). High performance thin layer chromatography was performed on Merck HPTLC Fertigplatten (Kiselgel 60 F_{254}). After elution with appropriate eluants, spots were visualized by UV light and/or by dipping in 5 % sulfuric acid, followed by charring. Preparative TLC separations were performed on Chromatotron 7924T rotary TLC equipment, using 1-2 mm silica gel 60 PF_{254} with gypsum. All Biogel P2 columns were packed and eluted with 1% aqueous 1-butanol. Molecular sieves (4Å, powder, obtained from Fluka) were dried before use in an oven at 400 °C overnight. The prefix "dry" before a solvent implies the following: for dichloromethane; p.a. quality distilled over phosphorus pentoxide, for tetrahydrofuran; freshly opened bottle of "dry" quality (< 50 ppm water), for pyridine; p.a. quality distilled over CaH_2, for *N,N*-dimethylformamide; freshly opened bottle of p.a. quality (< 0.7 % water), for nitromethane; p.a. quality, distilled (100-102 °C fraction), for acetonitrile; freshly opened bottle of p.a. quality. Water-washed organic solutions were dried over magnesium sulfate. For ultrafiltration, an Ultra Pump II and a 10K Ultrasette radial filter (both from Pall-Filtron, Lund, Sweden) were used. Ethyl 2,3,4-tri-*O*-benzyl-1-thio-α-L-fucopyranoside[22] was purchased from Synthelec AB (Lund, Sweden) or from IRL (Lower Hutt, New Zealand).

Ethyl 2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-1-thio-β-D-galactopyranoside (**3**). A solution of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (389 g, 0.946 mol), ethanethiol (143 mL, 1.93 mol), tetraethylammonium bromide (19.9 g, 0.095 mol), and 2,6-dimethylpyridine (157 g, 1.29 mol) in dry nitromethane (620 mL) was stirred at rt under nitrogen in a sealed flask for 36 h, then the mixture was diluted with water (1.5 L) and ethyl acetate (1.5 L), the organic layer was washed with water and saturated aqueous sodium chloride solution. The resulting organic solution was concentrated using a rotavapor-pump system equipped with an aqueous potassium permanganate trap (for enclosement of the ethanethiol odor). The residue, containing crude thio-orthoester **1**, was

dissolved in methanol (500 mL), and sodium (3.38 g, 0.147 mmol) was added in small pieces. After 1 h, when TLC indicated no further change, the mixture was concentrated and the residue was co-concentrated with toluene (4 x 300 mL). The resulting oil was dissolved in dry *N,N*-dimethylformamide (4.2 L), and the solution was stirred and cooled in an ice-water bath (13-15 °C initial temperature) while freshly powdered sodium hydroxide (571 g, 14.2 mol) was added at such a rate, that the temperature was kept below 30 °C. Then benzyl chloride (490 mL, 4.25 mol) was added, during which the temperature was kept at 20 - 35 °C (in some runs, the reaction mixture initially had to be heated to 30 °C to start the reaction). When TLC indicated no further change (shortly after all benzyl chloride had been added) methanol (75 mL) was slowly added, and the mixture was stirred for 30 min, after which ice-cold water (5 L) was added while cooling, followed by toluene (4.5 L). After thorough stirring, the organic layer was separated and washed with, sucessively, ice-cold 1 *M* aqueous sulfuric acid, saturated aqueous sodium chloride, and saturated aqueous sodium bicarbonate. Drying, concentrating and vacuum suction (48 h, rt, 0.5 torr) left an oil consisting of crude benzylated thio-orthoester **2**. This material was dissolved in dry acetonitrile (650 mL), stirred and cooled to near 0 °C with an ice-bath while trimethylsilyl triflate (20 mL, 0.103 mol) was added dropwise. After 30 min, when TLC indicated no further change, saturated aqueous sodium bicarbonate solution (1.2 L) was added, and the mixture was then extracted with ethyl acetate (2.0 L). The organic layer was washed with saturated aqueous sodium chloride solution, dried, and concentrated. The residue was crystallized from ethanol (1 L) in the cold (-20 °C) to give, after filtration (at 4 °C) and washing with cold ethanol, **3** as a solid (354 g, 65 %, purity >95% by ^{1}H NMR). An analytical sample was recrystallized from ethanol and had mp 58-59 °C, $[\alpha]_D$ +1°. NMR data: ^{1}H (30 °C), δ 1.22 (t, CH_3CH_2S), 2.02 (s, CH_3CO), 2.68 (m, CH_2S), 3.54 (dd, H-3), 3.60 (m, H-6a,b), 3.99 (d, H-4), 4.33 (d, $J_{1,2}$ 9.9 Hz, H-1), 4.40 - 4.95 (6 d, CH_2Ph), 5.42 (t, H-2); ^{13}C (25 °C), δ 15.4 (CH_3CH_2S), 21.6 (CH_3CO), 24.1 (CH_3CH_2S), 69.2, 70.3, 72.6, 73.6, 74.1, 75.0, 78.1, 82.1, 84.2 (C-1-6, 3 x CH_2Ph), 138.4, 138.6, 139.2 (Ph), 170.2 (CO). HRMS data: Calc for $C_{31}H_{37}SO_6$: 537.2311. Found: 537.2305 $(M+H)^+$.

2-Azidoethyl 3,4,6-Tri-*O*-benzyl-β-D-galactopyranoside (4). To a solution of **3** (700 mg, 1.30 mmol) in dichloromethane (15 mL) was added bromine (208 mg, 1.30 mmol) at 0 °C. The solution was stirred at 0 °C for 30 min and the solvent was subsequently evaporated. After coevaporation with toluene, the

residue was dissolved in dichloromethane (5 mL) together with 2-azidoethanol[19] (227 mg, 2.60 mmol) and ground molecular sieves (100 mg, 4Å). The suspension was stirred for 15 min at room temperature and then cooled to -45 °C. To the cooled suspension was added a solution of silver trifluoromethanesulphonate (402 mg, 1.56 mmol) in toluene (1 mL). After one h, the reaction mixture was filtered through Celite, diluted with dichloromethane and washed twice with water. The organic layer was dried and concentrated. After dissolving the crude, oily residue in methanol (6 mL), sodium methoxide was added to pH 12. When TLC indicated no further reaction, the solution was neutralised with acetic acid. Concentration was followed by chromatography (toluene:ethyl acetate, 15:1) to give syrupy **4** (290 mg, 43%). NMR data (25 °C): ^{1}H, δ 4.29 ($J_{1,2}$ 7.7 Hz, H-1); ^{13}C, δ 50.7 (CH_2N_3), 68.4, 68.7 (C-6, CH_2O), 71.4, 73.0, 73.9, 81.7 (C-2,3,4,5), 72.6, 73.6, 74.5 (3 x $PhCH_2$), 103.4 (C-1), 125.3-129.0 (aromatic C-H), 137.8, 138.1, 138.4 (aromatic C-C). HRMS data: Calc for $C_{29}H_{34}N_3O_6$: 520.2448. Found: 520.2455 $(M+H)^+$.

2-Azidoethyl 3,4,6-Tri-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-β-D-galactopyranoside (**5**). To a solution of ethyl 2,3,4-tri-*O*-benzyl-1-thio-α-L-fucopyranoside[22] (400 mg, 0.836 mmol) in dichloromethane (10 mL), bromine (134 mg, 0.836 mmol) was added at 0 °C, the solution was allowed to attain room temperature and the solvent was evaporated. After coevaporation with toluene the residue was dissolved in dry dichloromethane (2 mL) and the solution added at room temperature to a mixture of tetraethylammonium bromide (176 mg, 0.836 mmol), compound **4** (290 mg, 0.558 mmol) and molecular sieves (3Å, 300 mg) in dichloromethane:*N*,*N*-dimethylformamide (4:1, 7 mL). TLC (toluene:ethyl acetate, 6:1) showed complete conversion after stirring for 20 h. The mixture was filtered, diluted with dichloromethane and washed with water. The organic phase was dried, filtered and concentrated. Preparative TLC yielded the title compound **5** as a viscous oil (407 mg, 78%). NMR data (25 °C): ^{1}H, δ 5.69 ($J_{1,2}$ 2.9 Hz, H-1'); ^{13}C, δ 16.5 (C-6'), 29.7, 50.8, 66.3, 66.9, 68.8, 71.4, 72.0, 72.8, 72.9, 73.5, 73.6, 74.4, 74.8, 75.6, 78.1, 79.6, 84.3 (C-2 - 6, C-2' - 5', CH_2N, CH_2O, 6 x CH_2Ph), 97.3, 102.0 (C-1', C-1), 126.4-129.0 (aromatic CH), 137.8, 137.9, 138.30, 138.31 138.9, 139.0 (aromatic C).

2-Acrylamidoethyl 2-*O*-α-L-Fucopyranosyl-β-D-galactopyranoside (**7**). The protected derivative **5** (50 mg, 85 μmol) was dissolved in ethanol (99%, 10 mL) and water (1 mL) and Pd/C (10%, 100 mg) was added. The mixture was hydrogenated at room temperature at 50 PSI. The reaction was not completed

within 60 h, however, the mixture was filtered and the product formed was isolated by column chromatography (ethyl acetate:methanol:acetic acid:water, 5:3:3:1). After concentration in vacuo, the residue was redissolved in a buffer of pyridine/acetic acid (2.5%/1%, aqueous, pH 5.4) and passed through a Bio-Gel P-2 column, eluted in the same buffer. Evaporation and lyophilization gave **6** (14 mg, 71%), NMR data (D_2O, 25 °C): ^{13}C, δ 16.8, 39.8, 61.1, 66.4, 67.1, 68.7, 69.6, 71.9, 73.0, 75.0, 78.4, 100.0, 101.7. The material (6.4 mg, 17 μmol) was dissolved in deaerated 0.5 *M* aqueous sodium borate buffer (0.3 mL, pH 8.5) and methanol (0.9 mL), the vessel was flushed with nitrogen and cooled to 0 °C. Acryloyl chloride (2.0 μL, 26 μmol) was added and stirring was continued for 10 min. The reaction mixture was then concentrated at room temperature to about a third of its original volume. Purification on a Bio-Gel P-2 column and lyophilization gave compound **7** (4 mg, 57% from **6**), NMR data: ^1H (D_2O, 25 °C): δ 1.16 (d, CH_3 fucose), 4.55 (d, H-1), 5.22 (d, H-1′), 5.80 (dd, CH=CH_2), 6.25 (m, CH=CH_2).

2-Acrylamidoethyl 2-*O*-α-L-Fucopyranosyl-β-D-galactopyranoside, copolymer with acrylamide (**8**). To a solution of **7** (4 mg, 9 μmol) and acrylamide (3.3 mg, 47 μmol) in deaerated water (0.75 mL) was added first *N,N,N′,N′* -tetramethylenediamine (2.0 μL) and then ammonium persulphate (1.5 mg). The mixture was stirred at room temperature in a sealed flask in the dark overnight. The material was purified on a Bio-Gel P2 column to give **8** (6.1 mg). ^1H NMR data (D_2O, 25 °C) showed an average incorporation of 1 oligosaccharide per 12.3 acrylamide units.

2-Azidoethyl 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (**9**). A solution of 2-azidoethanol[19] (65.4 g, 0.75 mol) and 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2,1-d]-2-oxazoline[21] (196 g, 0.60 mol) in dry dichloromethane (755 mL) containing molecular sieve (4 Å, 78 g) was stirred under nitrogen for 2 h, then sulfuric acid (concd, 7 mL) was added. After stirring for 48 h, TLC indicated no further reaction, and the mixture was diluted with dichloromethane, filtered through Celite, washed with aqueous sodium bicarbonate and water, dried, and concentrated. The residue was dissolved in boiling dichloromethane (200 mL), then diethyl ether (500 mL) was added. The precipitated material **9** (178.4 g, 80 %) had mp 153-155 °C, $[\alpha]_D$ -28° (lit.[20] -43.5). NMR data: ^1H (30 °C), δ 1.96, 2.030, 2.034, 2.09 (4 s, Acetyl CH_3), 3.28 (dq, CH_2N_3), 3.50 (dq, CH_2N_3), 3.7-3.8 (m, H-5, CH_2O), 3.83 (q, H-2), 4.04 (dq, CH_2O), 4.16 (dd, H-6a), 4.26 (dd, H-6b), 4.85 (d, $J_{1,2}$ 8.4 Hz, H-1), 5.07 (t, H-4), 5.37 (dd, H-3), 5.88 (d, NH); ^{13}C (25 °C), δ 20.60, 20.64, 20.71 (3 x $OCOCH_3$), 23.33 ($NHCOCH_3$), 50.62

(CH_2N_3), 54.91 (C-2), 62.02, 68.35 (C-6, CH_2O), 68.62, 71.99, 72.10 (C-3,4,5), 100.48 (C-1). An IR spectrum (KBr) of **9** showed a strong absorption band at 2110 cm^{-1}, assigned to the azido group.

2-Azidoethyl 2-Acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (10). Pieces of sodium metal (a total of 0.31 g, 0.013 mol) were added to a slurry of **9** (178.4 g, 0.429 mol) in methanol (700 mL). After 1 h, TLC indicated no further change, and the mixture was neutralized with Amberlite IR-120 (H^+) ion-exchange resin, filtered, and concentrated. The residue was dried in vacuum (0.1 torr) overnight. The resulting solid (123.7 g) was dissolved in a mixture of *N,N*-dimethylformamide (600 mL) and α,α-dimethoxytoluene (131 mL, 0.87 mol), to which was then added 4-toluenesulfonic acid monohydrate (18 g, 0.095 mol). The mixture was stirred at rt for 48 h, then poured into a rapidly stirred mixture of saturated aqueous sodium bicarbonate solution (1.8 L) and water (1.0 L). After continuous stirring for 5 h, the mixture was filtered, washed with water and petroleum ether, and dried (40 °C) to give crude, solid **10** (132.3 g, 82 %). An analytical sample was recrystallized from methanol, mp 231-32 °C (d.), $[\alpha]_D$ -88° (*c* 1.0, $CHCl_3$:CH_3OH 1:1). NMR data: (25 °C, $CDCl_3/CD_3OD$, ref. CD_3OD 3.30 ppm), δ 4.67 (d, $J_{1,2}$ 7.4 Hz, H-1), 5.61 (s, PhCH); ^{13}C (25 °C, $CDCl_3/CD_3OD$, ref. $CDCl_3$ 77.0 ppm), δ 22.07 (CH_3CO), 50.18, 55.56 (2 x CN), 65.93, 67.87, 68.09, 70.75, 81.13 (C-3,4,5,6,CH_2O), 100.97, 101.33 (C-1, PhCH), 125.80, 136.78 (aromatic C), 172.29 (CO). HRMS data: Calc for $C_{17}H_{23}N_4O_6$: 379.1618. Found: 379.1652 $(M+H)^+$.

2-Azidoethyl *O*-(3,4,6-Tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (11). A solution of **10** (69.7 g, 0.185 mol) and **3** (109 g, 0.203 mol) in dry dichloromethane (1.8 L) was stirred for 30 min under nitrogen at rt in the presence of molecular sieve (4 Å, 100 g). Then methyl triflate (50 g, 0.305 mol) was added, and stirring was continued at rt for 24 h, after which TLC indicated no further change. Then a solution of sodium methoxide in methanol (1.4 *M*, 400 mL) was added, and stirring was continued for 3 h, after which TLC indicated no further change. The mixture was neutralized with acetic acid (20 mL), and filtered through Celite. The solids were carefully washed with dichloromethane (600 mL) and the filtrate was further diluted with the same solvent (900 mL) and the filtrate was then washed with saturated aqueous sodium bicarbonate solution and water, dried, and concentrated. The residue was dissolved in boiling ethyl acetate (1 L), the solution was concentrated to 500 mL, and diethyl ether (1.5 L)

was added. After completion of the crystallization at 4 °C, the crude, solid **11** (106 g, 70 %, >90 % purity by ^{1}H NMR) was collected. An analytical sample was recrystallized from ethyl acetate-ether, mp 188-189 °C, $[\alpha]_D$ -35°. NMR data: ^{1}H (30 °C), δ 4.96 (d, $J_{1,2}$ 8.2 Hz, H-1), 4.36 (d, $J_{1',2'}$ 7.8 Hz, H-1'), 3.99 (dd, H-2'), 3.90 (d, H-4'), 3.34 (dd, H-3'); ^{13}C (25 °C), δ 23.59 (CH_3CO), 50.59 (CH_2N_3), 56.89 (C-2), 66.40, 70.55, 73.21, 73.53, 76.08, 79.75, 81.7 (C-3, 4, 5, C-2', 3', 4', 5'), 68.28, 68.32, 68.56, 72.44, 73.40, 74.60 (C-6, 6', CH_2O, 3 x CH_2Ph), 100.97, 101.25, 103.48 (C-1, 1', PhCH), 126.09-128.97 (aromatic CH), 136.92, 137.70, 138.37, 138.71 (aromatic C), 171.67 (CO). HRMS data: Calc for $C_{44}H_{51}N_4O_{11}$: 811.3554. Found: 811.3538 $(M+H)^+$.

2-Azidoethyl *O*-(3,4,6-Tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (12). A solution of crude **11** (111 g, 0.148 mol) and sodium cyanoborohydride (43.2 g, 0.687 mol) in dry tetrahydrofuran (795 mL) was stirred and cooled (3-4 °C, ice-bath) under nitrogen while tetrafluoroboric acid (54 % in diethyl ether, 93.5 mL, 0.685 mol) was added. The mixture was stirred under nitrogen while the temperature was allowed to gradually rise to room temperature. When TLC indicated no further formation of **12** (3-4 h), saturated aqueous sodium bicarbonate solution (800 mL), water (400 mL), and ethyl acetate (1 L) were added, and the mixture was shaken in a separatory funnel. The organic layer was washed with saturated aqueous sodium bicarbonate solution (12 L) and saturated aqueous sodium chloride solution (1 L), dried, and concentrated. The residue was dissolved in dichloromethane (600 mL), and mixed with triethylamine (60 mL) and silica gel (75 g). This slurry was applied to a silica gel column (315 g, packed in dichloromethane). Elution with, successively, dichloromethane (1.2 L), 2:1 dichloromethane-ethyl acetate (1.8 L), ethyl acetate (2.5 L), and methanol (1.5 L) gave fractions containing syrupy **12**, slightly contaminated with non-carbohydrate material (118 g, 85 % pure by NMR). NMR data: ^{1}H (30 °C), δ 4.23 (d, $J_{1,2}$ 7.8 Hz, H-1'), 4.94 ($J_{1,2}$ 8.4 Hz, H-1); ^{13}C (25 °C), δ 23.60 (CH_3CO), 50.60 (CH_2N_3), 56.74 (C-2), 68.37, 68.62, 69.69, 69.80, 70.74, 72.68, 72.94, 73.45, 73.56, 73.83, 74.57, 75.38, 81.70, 84.56 (C-3, 4, 5, 6, C-2', 3', 4', 5', 6', CH_2O, 3 x CH_2Ph), 99.64, 104.47 (C-1, 1'), 126.09-128.97 (aromatic CH), 137.52, 137.93, 138.14, 138.36 (aromatic C), 171.95 (CO).

For further proof of structure, an aliquot of **12** was acetylated with acetic anhydride/pyridine (1:2), concentrated, and purified by silica gel chromatography. In the product, the two downfield signals at 4.85 (dd, $J_{3,4}$ =$J_{4,5}$

9.3 Hz) and 5.16 (dd, $J_{1',2'}$ 7.7, $J_{2',3'}$ 10.2 Hz) were assigned to H-4 and H-2', respectively.

2-Azidoethyl *O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (13). Compound **11** (137 mg, 0.17 mmol) and ethyl 2,3,4-tri-*O*-benzyl-1-thio-α-L-fucopyranoside (162 mg, 0.34 mmol) were dissolved in dichloromethane (75 mL), molecular sieves were added, and the mixture was stirred for 20 min, then dimethyl(thiomethyl)sulfonium triflate (96 mg, 0.37 mmol) was added and stirring was continued for 1.5 h. Triethylamine (1.0 mL) was added and stirring was continued for another 20 min. Filtration through Celite, concentration and column chromatography (toluene: ethyl acetate 1:1) gave **13** (101 mg, 49%). NMR data (25 °C): ^{13}C , δ 16.83 (CH_3 fucose), 23.30 ($NHCOCH_3$), 50.66 (CH_2-N), 57.40 (C-2), 66.55, 67.17, 67.96, 68.60, 72.31, 72.91, 72.99, 73.94, 73.10, 73.51, 74.48, 74.65, 76.05, 76.29, 76.62, 77.60, 79.43, 79.53, 83.21, (C-3, 4, 5, 6, C-2', 3', 4', 5', 6', 2'', 3'' ,4'' ,5'' ; 6 x CH_2Ph), 97.67 (C-1''), 100.94, 101.07, 102.13 (C-1, C-1' , CHPh), 170.92 (C=O).

2-Trifluoracetamidoethyl *O*-(2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl)-(1→2)-*O*-(3,4,6-tri-*O*-benzyl-β-D-galactopyranosyl)-(1→3)-2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (14). Compound **13** (135 mg) was dissolved in ethanol (11 mL) and 10% Pd/C (140 mg) was added. The mixture was then stirred under an atmosphere of hydrogen for 15 min. Analysis by TLC (ethyl acetate:methanol:acetic acid:water 12:3:3:1) showed disappearance of starting material, and a new, ninhydrin positive product. The mixture was filtered through Celite, concentrated and dissolved in a mixture of dichloromethane (7 mL) and pyridine (3.5 mL), flushed with nitrogen and cooled to 0 °C. Trifluoroacetic anhydride (31 μL, 0.22 mmol) was added. After one hour, the mixture was concentrated and coevaporated with toluene (2 mL) twice. Column chromatography (toluene:ethyl acetate, 1:3) gave **14** (73 mg, 52%). NMR data (25 °C): ^{13}C, δ 17.06 (CH_3 fucose) 22.66 ($NHCOCH_3$), 39.59 (CH_2-N), 54.83 (C-2), 65.56, 66.77, 67.78, 68.44, 68.69, 72.80, 73.05, 73.24, 2x73.46, 74.46, 74.66, 76.38, 77.08, 77.80, 78.91, 79.96, 80.01, 82.07, (C-3, 4, 5, 6, C-2' ,3', 4', 5', 6', C-2'', 3'', 4'', 5''; 6xCH_2Ph) 98.61 (C-1''), 101.22, 101.99, 102.35 (C-1, C-1', CHPh), 171.64 ($NHCOCH_3$).

2-Acrylamidoethyl *O*-(α-L-Fucopyranosyl)-(1→2)-*O*-(β-D-galactopyranosyl)-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (16). Trisaccharide (**14**) (73 mg, 56.2 μmol) was dissolved in a mixture of absolute

ethanol (7 mL), water (0.25 mL) and acetic acid (2 mL). The solution was hydrogenolyzed over 10% Pd/C (152 mg) at room temperature/50 PSI for 1 h. When TLC (ethyl acetate:acetic acid:methanol:water 12:3:3:1; R_f = 0.14 for **15**) showed complete conversion, the reaction mixture was filtered through a layer of Celite and concentrated. The crude, solid **15** (46 mg) was used in the next reaction without further purification. NMR data (D_2O, 25 °C): ^{13}C, δ 16.59 (CH_3 fucose), 21.85 ($NHCOCH_3$), 39.44 (CH_2-N), 54.56 (C-2), 60.45-76.95 (C-3, 4, 5, 6, C-2′, 3′, 4′, 5′, 6′, C-2″, 3″, 4″, 5″) 99.29, 99.92, 101.28 (C-1, C-1′ , C-1″), 173.48 ($NHCOCH_3$).

The crude material (46 mg) was dissolved in aqueous ammonia (25%, 4 mL) at room temperature. The detrifluoroacetylation reaction was complete within 1 h (TLC ethyl acetate:acetic acid:methanol:water 5:3:3:1). Concentration and coconcentration with toluene was followed by purification on a Bond-Elut (SCX, H^+-form, 0.5 g cartridge) cation exchange resin. The sample was dissolved in water (3 mL) and the pH was adjusted to pH 6 with aqueous acetic acid. The sample was applied to the column, and then eluted with 2*M* ammonia in methanol:water, 1:1 (5 mL). The fractions containing free amine (ninhydrin positive on TLC) were pooled, concentrated and lyophilized to give crude amine (30 mg, 0.05 mmol). Deaerated 0.5 *M* aqueous sodium borate buffer (1 mL, pH 8.5) and deaerated methanol (3 mL) was added to the crude amine. The reaction mixture was flushed with nitrogen and cooled to 0 °C, acryloyl chloride (6.4 μL, 0.078 mmol) was added and stirring was continued for 10 min. The mixture was concentrated at room temperature to about a third of its original volume. Purification on a Bio-Gel P2 column and lyophilization gave **16** (30 mg, 92 % from **14**). NMR data: ^1H (D_2O, 25 °C): δ 4.30 (d, $J_{1,2}$ 8.4 Hz), 4.50 (d, $J_{1,2}$ 7.6 Hz), 5.02 (d, $J_{1,2}$ 4.1 Hz), 5.65 (dd, C*H*=CH_2), 6.10 (m, CH=C*H*$_2$); ^{13}C (D_2O, 25 °C), δ 14.99 (CH_3 fucose), 21.99 ($NHCOCH_3$), 39.10 (CH_2-N), 54.58 (C-2), 60.46, 60.90, 66.25, 67.75, 67.82, 68.48, 68.91, 69.21, 71.60, 73.26, 74.86, 75.23, 76.39, 76.99 (C-3,4,5,6; C-2′,3′,4′,5′,6′, C-2″,3″,4″,5″, CH_2O), 99.25, 99.93, 101.39 (C-1, C-1′, C-1″), 127.7, 129.65 (CH=CH_2).

2-Acrylamidoethyl *O*-(α-L-Fucopyranosyl)-(1→2)-*O*-(β-D-galactopyranosyl)-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside, copolymer with acrylamide (**17**). Compound **16** (18 mg, 29 μmol) was copolymerized with acrylamide (10 mg, 144 μmol) using *N,N,N′,N′*-tetramethylethylenediamine (6 μL) and ammonium persulphate (3.5 mg) in water (1 mL) essentially as described for compound **7**. The product was purified on a Bio-Gel P2 column.

Lyophilization of the polymeric fractions, eluted in the void volume, gave a first fraction (13.1 mg), where ^1H NMR (D_2O, 25 °C) showed an average incorporation of 1 oligosaccharide per 7.6 acrylamide units, and a second fraction (11.9 mg) containing 1 oligosaccharide per 10.3 acrylamide units.

2-Azidoethyl *O*-(2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl)-(1→2)-*O*-(3,4,6-tri-O-benzyl-β-D-galactopyranosyl)-(1→3)-[*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-(1→4)]-2-acetamido-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (18). A solution of **12** (90 %, 36.0 g, 40 mmol) in dry dichloromethane (600 mL) was added to a solution of ethyl 2,3,4-tri-*O*-benzyl-1-thio-α-L-fucopyranoside (66.4 g, 137 mmol) in dry *N,N*-dimethylformamide (100 mL) containing molecular sieve (4Å, 60 g). The mixture was stirred at rt for 15 min, it was then cooled in an ice bath while bromine (7.7 mL, 150 mmol) was added dropwise. After 10 min, cyclohexene (1.4 mL, 14 mmol) was added to destroy the excess bromine, followed by tetraethylammonium bromide (25 g, 119 mmol). Stirring was continued at rt under nitrogen for 24 h, after which TLC indicated no further change. Methanol (25 mL) was added, and the mixture was further stirred for 2 h, then filtered through Celite. The filtrate was diluted with dichloromethane, washed with saturated aqueous sodium bicarbonate solution, dried, and concentrated (1 torr final rotavapor pressure). The residue was dissolved in 2:1 toluene - ethyl acetate (200 mL) and applied to a silica gel column (14 x 18 cm). The column was eluted with the same solvent, followed by 1.5:1 toluene - ethyl acetate. Fractions containing pure **18** were pooled and concentrated to give a syrup (43.3 g, 66 %). NMR data (25 °C): ^{13}C, δ 16.30, 16.44 (2 x CH_3 fucose), 23.55 (CH_3CO), 50.60 (CH_2N_3), 58.05 (C-2), 66.68, 66.83, 67.58, 68.01, 68.18, 71.35, 71.81, 72.27, 72.73, 72.77, 72.86, 72.98, 72.981, 73.21, 73.50, 74.45, 74.78, 74.85, 75.29, 75.30, 75.44, 75.60, 75.70, 77.59, 78.21, 79.46, 80.26, 84.13 (C-3,4,5,6, C-2', 3', 4', 5', 6', 2 x C-2,3,4,5-fucose, 10 x CH_2Ph, CH_2O), 97.48, 97.64, 99.97, 101.9 (C-1, C-1', 2 x C-1-fucose), 125.26-128.99 (aromatic CH), 137.75-139.24 (10 x aromatic C), 170.49 (CO). HRMS data: Calc for $C_{98}H_{109}N_4O_{19}$: 1645.7686. Found: 1645.7817 $(M+H)^+$.

2-Acetamidoethyl *O*-(α-L-Fucopyranosyl)-(1→2)-*O*-(β-D-galactopyranosyl)-(1→3)-[*O*-(α-L-fucopyranosyl)-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranoside (20) and 2-Acrylamidoethyl *O*-(α-L-Fucopyranosyl)-(1→2)-*O*-(β-D-galactopyranosyl)-(1→3)-[*O*-(α-L-fucopyranosyl)-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranoside (21). A solution of **18** (38.3 g) in ethyl acetate (600 mL) was stirred at rt with activated carbon (20 g) for 3 h, then filtered and concentrated. The residue was dissolved in a mixture of ethyl acetate (70 mL), ethanol (95%,

200 mL) and hydrochloric acid (1*M*, 46 mL), to this solution was added a slurry of palladium on carbon (10%, 13 g), ethanol (95 %, 135 mL), and water (10 mL), then the mixture was hydrogenolyzed at room temperature and atmospheric pressure. After consumption of approximately 2/3 of the theoretical amount of hydrogen, the reaction became slow due to precipitated product. Therefore water (100 mL) was added, and hydrogenation was continued until the theoretical amount of hydrogen had been consumed. The mixture was then filtered with a fine glass filter, and the solids were washed thoroughly with water and ethanol. The filtrate was neutralized with aqueous sodium bicarbonate solution, concentrated to 150 mL, and lyophilized. The residue (28.8 g) contained **19** as the only detectable (TLC) sugar, contaminated with inorganic salts.

***N*-Acetylation**: an aliquot of crude **19** (0.5 g) was dissolved in acetic anhydride-methanol (1:10, 11 mL) and, after 16 h, the mixture was concentrated and the residue was purified by Bio-Gel P-2 gel filtration. Pooling and lyophilization of appropriate fractions gave **20** (0.4 g, 80 %) as a white powder. NMR data: ^{1}H (25 °C, D_2O), δ 1.25 (2 x CH_3-fucose), 1.99, 2.02 (2s, 2 x CH_3CO), 4.41 ($J_{1,2}$ 7.2 Hz, H-1 GlcNAc), 4.65 ($J_{1,2}$ 7.0 Hz, H-1 Gal), 5.01 ($J_{1,2}$ 5.0 Hz, H-1, 4-Fuc), 5.15 ($J_{1,2}$ 4.7 Hz, H-1, 2-Fuc); ^{13}C (25 °C, D_2O), δ 16.5, 16.24 (2 x CH_3-fucose), 22.76, 23.07 (2 x CH_3CON), 40.26, 56.49, 60.49, 62.45 , 67.11, 67.88, 68.72, 69.02, 69.15, 69.63, 70.00, 70.34, 72.84, 72.89, 73.01, 74.54, 75.52, 75.65, 76.28, 77.36 (C-2,3,4,5,6; C-2′,3′,4′,5′,6′, 2 x C-2,3,4,5-fucose, CH_2N, CH_2O), 98.71, 100.43, 101.48, 102.65 (4 x C-1), 174.49, 174.95 (2xCON).

HRMS data: Calc for $C_{30}H_{53}N_2O_{20}$: 761.3192. Found: 761.3129 $(M+H)^+$.

***N*-Acryloylation**: crude **19** (54 g, corresponding to approximately 34.3 g, 48 mmol, of pure material) and sodium bicarbonate (27 g) was dissolved in water (700 mL). When all had dissolved, methanol (350 mL) was added, and the mixture was cooled to 3-4 °C in an ice-bath and stirred while acryloyl chloride (15 mL, 185 mmol) was added during 1.5 h. After an additional 1 h, a solution of 2,6-di-*t*-butyl-4-methylphenol (5 mL, 1.0 % in methanol, a polymerization inhibitor) was added, and the mixture was concentrated to 300 mL and neutralized to pH 9 with concd hydrochloric acid. This solution was applied to a column of C-18 silica (800 g, packed in water), the column was then eluted with water, followed by 5 and 10 % methanol mixtures. This gave a pool of **21** that was passed through a column of Amberlite IR-910 (500 g, OH^- form) to remove remaining traces of acrylic acid. All fractions containing **21** were

pooled, pH was adjusted to 10 with hydrochloric acid, and the volume was reduced by lyophilization to 0.90 L. According to ^{1}H NMR quantification against added 1,2-*O*-isopropylidene-α-D-glucofuranose, the solution contained a total of 31.3 g of **21** (82%). This solution was used directly in the next step (polymerization). An aliquot was lyophilized to give a white powder for characterization, NMR data: ^{1}H (25 °C), δ 1.250, 1.255 (2 d, 2 x fucose CH_3), 2.00 (s, $NHCOCH_3$), 4.42, 4.64, 5.02, 5.15 (4 d, 4 x H-1), 5.78 (dd, CH=), 6.24 (m, CH_2=). HRMS data: Calc for $C_{31}H_{53}N_2O_{20}$: 773.3191. Found: 773.3279 $(M+H)^+$.

2-Acrylamidoethyl *O*-(α-L-Fucopyranosyl)-(1→2)-*O*-(β-D-galactopyranosyl)-(1→3)-[*O*-(α-L-fucopyranosyl)-(1→4)]-2-acetamido-2-deoxy-β-D-glucopyranoside, copolymer with acrylamide (22). A solution of **21** (57 g, 74.7 mmol) and acrylamide (32 g, 451 mmol) in water (1.6 L) was cooled in an ice-bath to 2-3 °C and deaerated by vacuum suction (aspirator) for 30 min. The solution was then blanketed with nitrogen, and *N,N,N',N'*-tetramethylethylene-diamine (15 mL) was added, followed by ammonium persulfate (7.5 g) in portions. A transient rise in temperature to 5-6 °C was observed, and the mixture soon became viscous. The cooling was discontinued, and stirring was continued for 16 h at rt, then the mixture was diluted with water and ultrafiltered (down to 1 L, refill x 5 with 2.5 L water). The retentate solution was lyophilized to give **22** (87.9 g). According to NMR quantification against added 1,2-*O*-isopropylidene-α-D-glucofuranose, the material contained 53.3 g of **21** (93.5 % yield), and showed an average incorporation of 1 oligosaccharide per 7.4 acrylamide units.

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REFERENCES AND NOTES

1. Present adress: Astra Production Chemicals, S-151 85 Södertälje, Sweden.

2. Present adress: Pharmacia Biotech AB, Björkgatan 30, S-751 82, Uppsala, Sweden.
3. S. J. Hultgren, S. Abraham, M. Caparon, P. Falk, J. W. Stgeme and S. Normark, *Cell*, **73**, 887 (1993).
4. T. Boren, P. Falk, K. Roth, G. Larson and S. Normark, *Science*, **262**, 1892 (1993).
5. V. Horejsi, P. Smolek and J. Kocourek, *Biochim. Biophys. Acta*, **538**, 293 (1978).
6. E. Kallin in *Methods in Enzymology*, Vol **242**; Y. C. Lee and R. T. Lee, Eds; Academic Press, San Diego 1994, p 221.
7. E. Kallin, H. Lönn, T. Norberg and M. Elofsson, *J. Carbohydr. Chem.*, **8**, 597 (1989).
8. W. J. Lees, A. Spaltenstein, J. E. Kingerywood and G. M. Whitesides, *J. Med. Chem.*, **37**, 3419 (1994).
9. R. U. Lemieux, *Chem. Soc. Rev.*, **7**, 423 (1978).
10. J. T. Randolph, K. F. McClure and S. J. Danishefsky, *J. Am. Chem. Soc.*, **117**, 5712 (1995).
11. U. Spohr and R. U. Lemieux, *Carbohydr. Res.*, **174** , 211 (1988).
12. S. Sato, Y. Ito and T. Ogawa, *Carbohydr. Res.*, **155** , C1 (1986).
13. N. Bovin and A. Khorlin, *Bioorg. Khim.*, **10** , 853 (1984).
14. S. S. Rana, J. J. Barlow and K. L. Matta, *Carbohydr. Res.*, **96**, 231 (1981).
15. F. Barresi and O. Hindsgaul, *J. Carbohydr. Chem.*, **14**, 1043 (1995), and references cited therein.
16. P. Falk, K. A. Roth, T. Boren, T. U. Westblom, J. I. Gordon, and S. Normark, *Proc. Natl. Acad. Sci. U.S.A.*, **90**, 2035 (1993).
17. U. Johansson, S. Lind, A.-K. Tidén, K. Eklind, R. Gustafsson, H. Lönn, and K. Stenberg, *Coll. of Abstracts, 8th European Carbohydrate Symposium*: Seville, Spain; 1995, and *J. Biol. Chem.*, submitted.
18. S. Nilsson, H. Lönn and T. Norberg, *Glycoconjugate J.*, **6**, 21 (1989).
19. A. Y. Chernyak, G. V. M. Sharma, L. O. Kononov, P. R. Krishna, A. B. Levinsky, N. K. Kochetkov and A. V. R. Rao, *Carbohydr.Res.*, **223**, 303 (1992).
20. A. Hasegawa, T. Terada, H. Ogawa and M. Kiso, *J. Carbohydr. Chem.*, **11**, 331 (1992).
21. R. U. Lemieux and H. Driguez, *J. Am. Chem. Soc.*, **97**, 4063 (1975).
22. H. Lönn, *Carbohydr. Res.*, **139**, 105 (1985).
23. P. Fügedi and P. J. Garegg, *Carbohydr. Res.*, **149**, C9 (1986).
24. J.-M. Mallet, G. Meyer, F. Yvelin, A. Jutand, C. Amatore and P. Sinaÿ, *Carbohydr. Res.*, **244**, 237 (1993).
25. P.-M. Åberg, L. Blomberg, H. Lönn and T. Norberg, *J. Carbohydr. Chem.*, **13**, 141 (1994).
26. R. Bayer, S. DeFrees, F. Gaeta, C. Jiang, T. Norberg, J. Ramphal, M. Ratcliffe and L. Walker, *Coll. of Abstracts, 2nd CHI Glycotechnology Conference:* La Jolla, CA; 1994.