Synthesis and anti-influenza virus activity of novel glycopolymers having triantennary oligosaccharide branches[1]

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An efficient method for the synthesis of novel glycopolymers with triantennary sialooligosaccharides showing potent anti-influenza virus activity is described. Polymerisable glycoside of triantennary *N*-acetyllactosamine [β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranose, Galβ(1→4)GlcNAc] is synthesised from lactose and 4-(3-hydroxypropyl)-4-nitroheptane-1,7-diol as key starting materials, and converted into water-soluble glycopolymers by radical copolymerisation with acrylamide. Subsequent enzymic sialylation using cytidine 5′-monophospho-*N*-acetylneuraminic acid (CMP-Neu5Ac) with α-2,3-sialyltransferase from porcine liver or with α-2,6-sialyltransferase from rat liver gives novel glycoprotein mimics having potent inhibitory activity against influenza virus infection. It is demonstrated that the present triantennary glycoligands exhibit much higher biological activities than the effects by glycopolymers derived from the simple monovalent-type glycomonomers.

Introduction

Roles of carbohydrate moieties of glycoproteins in a variety of biological systems have been extensively investigated.[2] In particular, the significance of multivalency of oligosaccharides known as the 'glycoside-cluster effect' is one of the most specific mechanisms found in protein–carbohydrate interactions.[3,4] Recently, much attention has been paid toward this type of multiple binding of synthetic glycoligands with protein receptors in relation to cell–cell interactions.[5–7] On the other hand, it was reported that sialic acids existing on the non-reducing terminal moieties of many glycoconjugates have important functions in cellular recognition events such as cell adhesion and differentiation.[8,9] Therefore, it is of growing importance to construct sialic acid-containing oligosaccharides and glycoconjugates by means of efficient chemical and/or enzymic synthetic strategies.[10–14]

In preceding papers, we have reported some efficient and simple methods for the synthesis of convenient glycoprotein models based on common radical copolymerisation of simple ω-alkenyl glycosides with acrylamide.[15,16] In addition, glycosides with a terminal acrylamido group at the anomeric position have also been shown to permit the synthesis of high-density glycoligands, including their homopolymers.[17,18] It was demonstrated that these glycopolymers prepared from simple monovalent-type glycomonomers showed enhanced affinity with lectins[7] or glycosyltransferases[19] based on the macroscopic 'polymer-cluster effect.' Although some convenient multivalent galactosides showing specific affinity with lectins were synthesised from tris(hydroxymethyl)aminomethane as a key starting material by Lee *et al.*[20,21] and Polidori *et al.*,[22,23] there has been no attempt at the synthesis of glycopolymers bearing triantennary sugar branches. Thus, our attention was focused on the synthesis and biological functions of novel glycoprotein models having 'multivalency' in view both of macroscopic and microscopic properties. In this paper, we describe the synthesis and anti-influenza virus activity of novel sialoglycopolymers by means of a versatile trimeric *N*-acetyllactosamine (LacNAc) derivative as a key intermediate.

Results and discussion

Synthesis of triantennary *N*-acetyllactosamine derivatives

Scheme 1 indicates a synthetic route to a target glycomonomer having triantennary LacNAc residues. First, an oxazoline derivative, readily obtained from lactose,[15] was coupled with 4-(3-hydroxypropyl)-4-nitroheptane-1,7-diol, known as Newkome's building block,[24–26] in the presence of camphor-10-sulfonic acid (CSA) in 1,2-dichloroethane to give nitrotris{3-[*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyloxy]propyl}methane **1** in 75% yield. The tris-glycoside **4** was hydrogenated in the presence of Raney Ni (T-1)[27] as catalyst, followed by *N*-acryloylation to afford *O*-protected tris-LacNAc monomer **2** in 65% overall yield from **1**. A desired key glycomonomer **3** was obtained by de-*O*-acetylation under Zemplen conditions in 82% yield. All new compounds prepared here gave satisfactory analytical and spectroscopic data.

Radical copolymerisation of triantennary glycomonomer

As anticipated, homopolymer from the triantennary glycomonomer **3** could not be obtained under the common conditions for radical polymerisation due to steric hindrance by the bulky triantennary carbohydrates. Next, copolymerisation of this compound with acrylamide (AAm) in deionised water was

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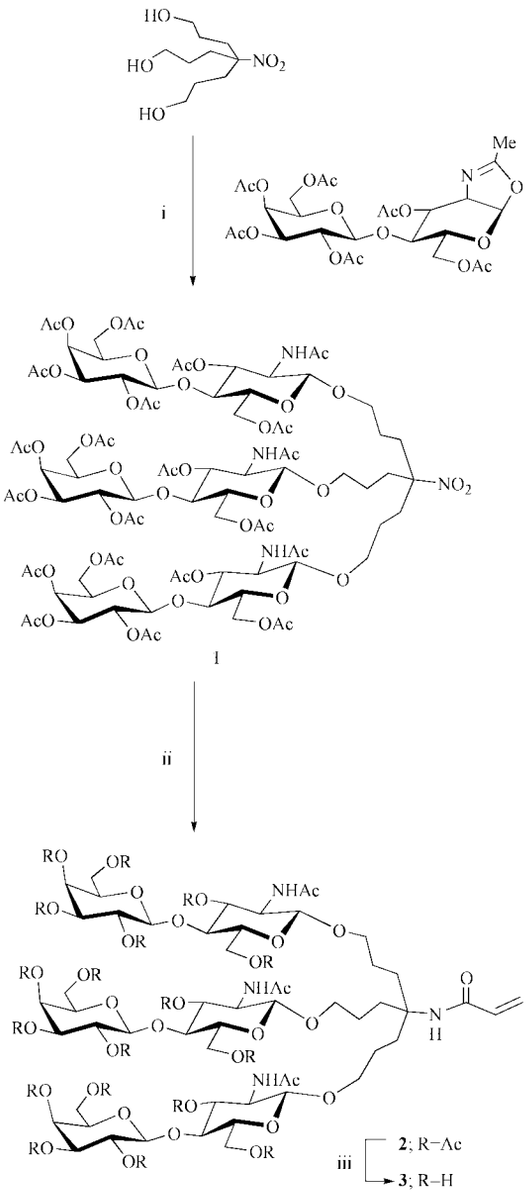


Scheme 1 *Reagents and conditions*: i, CSA, $ClCH_2CH_2Cl$, 70 °C; ii, H_2, Raney Ni (T-1), EtOAc–EtOH, rt; then $ClCOCH{=}CH_2$, Et_3N, THF, 0 °C ⟶ rt; iii, NaOMe, MeOH, rt.

carried out at room temperature in the presence of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) and ammonium peroxodisulfate (APS) as initiators (Scheme 2). Reactions proceeded smoothly and gave highly viscous solutions after several hours. The copolymers **4a**–**4c** were purified by gel filtration and dialysis, and each was isolated as a white power by lyophilisation (52–91%). The sugar content of these copolymers was determined by the integration ratio of the signals due to methyl protons (δ 1.9) of the *N*-acetyl groups and the methine protons (δ 2.0–2.4) of the main chain in the ^1H NMR spectra according to our previous papers.[15–18] The results of copolymerisation are summarised in Table 1 together with physical data. Fully assigned ^{13}C NMR spectra of trimeric glycoside **3** and copolymers (**4a**–**c**) in D_2O are shown in Fig. 1. These spectra clearly show the disappearance of signals due to the C=C double bond of glycosides after polymerisation. Although the bulky sugar-triantennas prevented this glycomonomer from undergoing homopolymerisation, all copolymers gave satisfactory relative molecular masses and sugar contents, suggesting that the synthetic strategy using Newkome's building block for cascade molecules was suitable for the preparation of multivalent glycocopolymers in the presence of AAm as appropriate

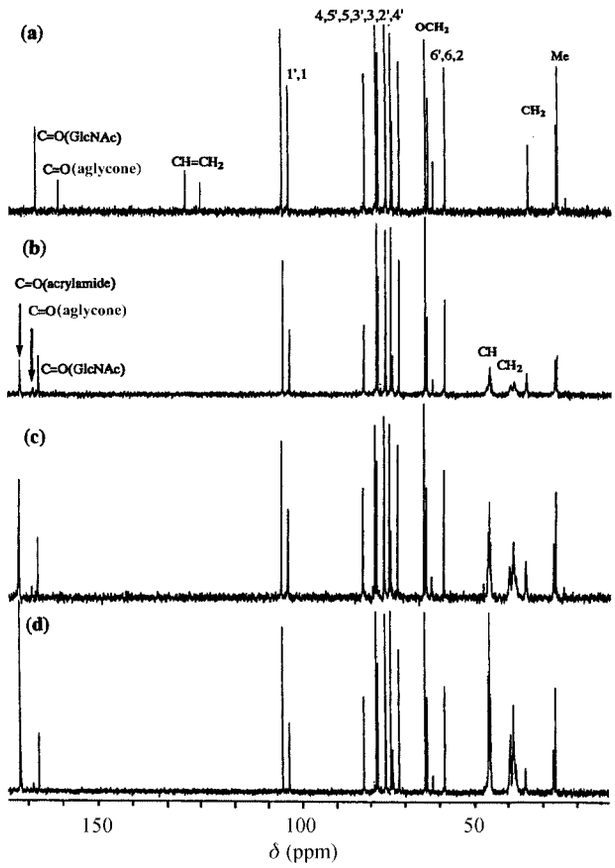
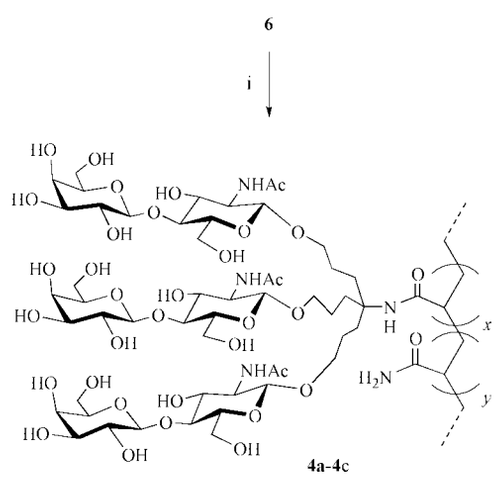


Fig. 1 ^{13}C NMR spectra of (a) triantennary glycoside derived from *N*-acetyllactosamine **3**, (b) the polymer **4a** (cluster unit–acryamide = 1 : 5), (c) the polymer **4b** (cluster unit–acrylamide = 1 : 9) and (d) the polymer **4c** (cluster unit–acrylamide = 1 : 20).



Scheme 2 *Reagents and conditions*: i, $CH_2{=}CHCONH_2$, APS, TMEDA, water, rt.

spacing molecule. All the chemical shifts of the glycomonomer **3** and the copolymer **4c** prepared here are summarised in Table 2.

Enzymic sialylation of triantennary *N*-acetyllactosamine copolymers

Polyacrylamide having triantennary *N*-acetyllactosamine **4c** (the ratio of LacNAc residue to AAm residue in the copolymer; LacNAc–AAm was 1 : 20) was employed for further enzymic modification study by α-2,3-sialyltransferase from porcine liver or α-2,6-sialyltransferase from rat liver. Reactions were performed in 50 mM sodium cacodylate buffer (pH 6.5) at 37 °C

Table 1 Polymerisation of triantennary glycoside **3** with acrylamide

Polymer number	Monomer ratio[a]	Total yield (%)	Polymer composition[a]	Sugar content (w%)	$[\alpha]_D^{25}$ (deg)	M[b] (kDa)
Homopolymer[c]	1:0					
4a	1:4	52.8	1:5	68.2	−18.1	>300
4b	1:10	83.7	1:9	58.7	−15.8	>300
4c	1:20	91.4	1:20	41.2	−11.3	>300

[a] Ratio of carbohydrate monomer to acrylamide. [b] M was determined by GPC with an Asahipack GS-510 column [pullulans (5.8, 12.2, 23.7, 48.0, 100, 186, and 380 kDa, Shodex Standard P-82) were used as standards]. [c] Homopolymer was not observed.

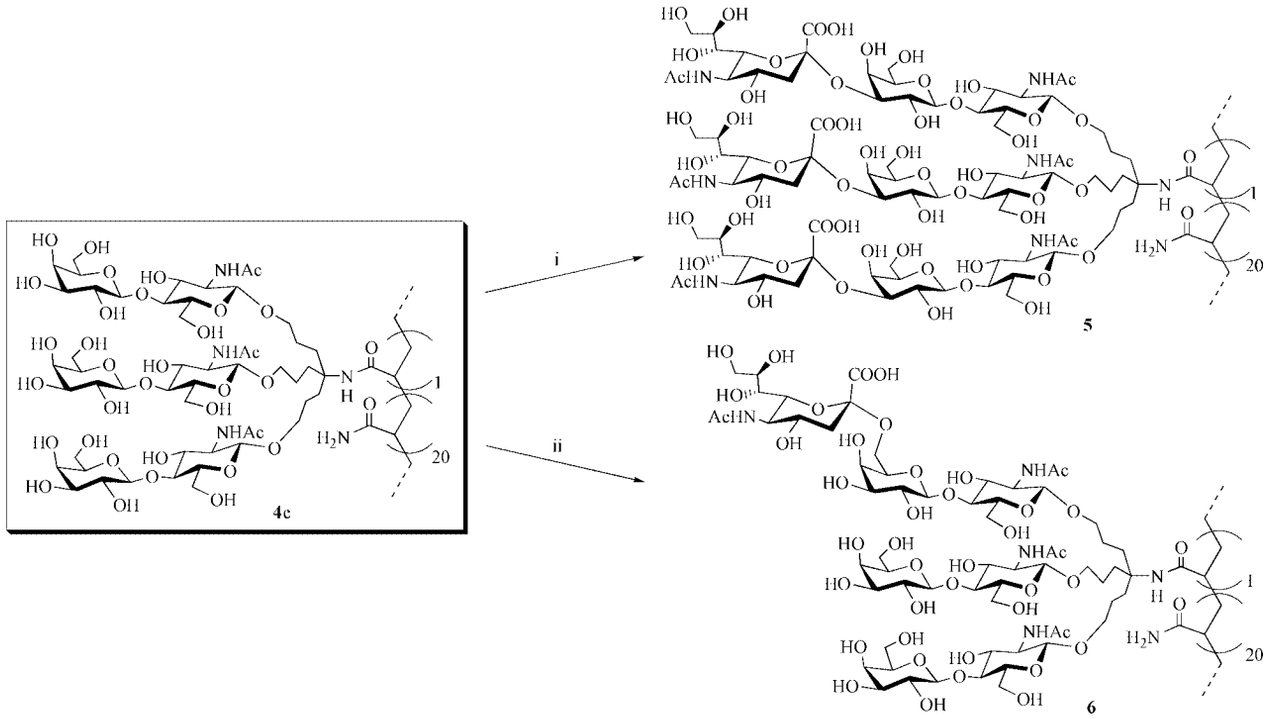
Table 2 ^{13}C NMR chemical shifts of triantennary glycoside and the polymers (in ppm from TSP[a])

				6	
Carbon	**3**	**4c**	**5**	Non-sialylated residue	Sialylated residue
C-1	102.1	102.0	101.9	101.9	102.0
C-2	56.3	56.2	55.9	56.0	55.7
C-3	73.6	73.5	73.3	73.4	72.5
C-4	79.9	80.0	79.2	79.4	81.7
C-5	75.9	75.8	75.6	76.2	75.3
C-6	61.3	61.4	61.8	61.0	61.8
C-1′	104.0	104.0	103.3	103.7	104.3
C-2′	71.7	71.5	70.2	71.5	71.8
C-3′	73.7	73.7	76.2	73.4	73.2
C-4′	69.7	69.6	69.1	69.4	69.3
C-5′	76.4	76.3	75.9	75.6	74.5
C-6′	62.1	62.0	61.8	61.8	64.0
C-1″			174.6		174.3
C-2″			100.6		101.0
C-3″			40.4		40.2
C-4″			68.9		69.0
C-5″			52.5		52.7
C-6″			73.6		73.4
C-7″			68.3		68.0
C-8″			72.5		72.5
C-9″			63.4		63.5
CH_2=CH	127.8				
CH_2=CH	132.1				
CH_2	24.8, 32.1	24.1, 32.4	23.8, 32.1	23.8, 32.0	
OCH_2	72.0	72.0	71.4	71.5	
tert-C	59.9	59.7	60.9	59.6	
CH[b]		43.0	42.6	42.6	
CH_2[b]		36.1	36.6	35.7	
C=O[c]	175.2	174.9	174.8	174.9	
C=O[d]	168.7	176.5	175.7	175.6	
C=O[e]			175.7	175.7	
$CONH_2$		180.3	180.2	180.3	
CH_3	23.4	23.5	23.2	23.3	
CH_3[f]			22.8	22.9	

[a] 3-(Trimethylsilyl)propanesulfonic acid sodium salt. [b] Methine and methylene carbons due to main chain were observed as multiplets. [c] Signals due to the carbonyl group of GlcNAc residue. [d] Signals due to the carbonyl groups of aglycones. [e] Signals due to carbonyl groups of Neu5Ac. [f] Signals due to methyl carbon of Neu5Ac residue.

for 48 h according to the conditions reported previously (Scheme 3).[28–34] The products **5** and **6** were isolated by the usual procedure reported previously[19] and the fully assigned ^{13}C NMR spectrum of the glycopolymer **5** measured in D_2O is shown in Fig. 2. All chemical shifts of the compounds **5** and **6** are summarised in Table 2 together with the data on glycomonomer **3**. As indicated in Fig. 2, the spectrum of compound **5** clearly shows perfect substitution with sialic acids at all C-3 positions of the galactose residues of the acceptor substrate **4c**. Typical chemical shifts of the signals of sialic acid introduced were observed in the ^{13}C NMR spectrum at δ_C 100.6 (C-2) and at δ_C 174.6 due to the carboxy group (C-1), in addition to the signal at δ 2.7 due to H-3^{eq} in the ^{1}H NMR spectrum. Furthermore, the integration data of H-3^{eq} of Neu5Ac in comparison with those of the anomeric protons (δ 4.3–4.5) of the galactose and *N*-acetyl-D-glucosamine residues in the ^{1}H NMR spectra also exhibited quantitative substitution of sialic acid residues by the α-2,3-sialyltransferase from porcine liver. On the other hand, α-2,6-sialyltransferase from rat liver transferred only one sialic acid residue to the trimeric *N*-acetyllactosamine unit even in the presence of a large excess of CMP-Neu5Ac or enzyme. These data clearly suggest that the introduction of the first α-2,6-sialyl linkage drastically inhibited further sialylation reaction to the glycopolymer **4c**. The formation of this α-2,6-sialyl linkage may cause a significant conformational change in the triantennary carbohydrate chains.

Interestingly, it has been well documented that there are few examples of naturally occurring glycoproteins carrying the trimeric α-2,6-sialyl-*N*-acetyllactosamine structure.[35,36]



Scheme 3 *Reagents and conditions*: i, CMP-Neu5Ac, α-2,3-sialyltransferase, calf intestine alkaline phosphatase, BSA, sodium azide, $MnCl_2 \cdot 4H_2O$, Triton CF-54, 50 mM sodium cacodylate buffer (pH 6.5), 37 °C; ii, CMP-Neu5Ac, α-2,6-sialyltransferase, calf intestine alkaline phosphatase, BSA, sodium azide, $MnCl_2 \cdot 4H_2O$, Triton CF-54, 50 mM sodium cacodylate buffer (pH 6.5), 37 °C.

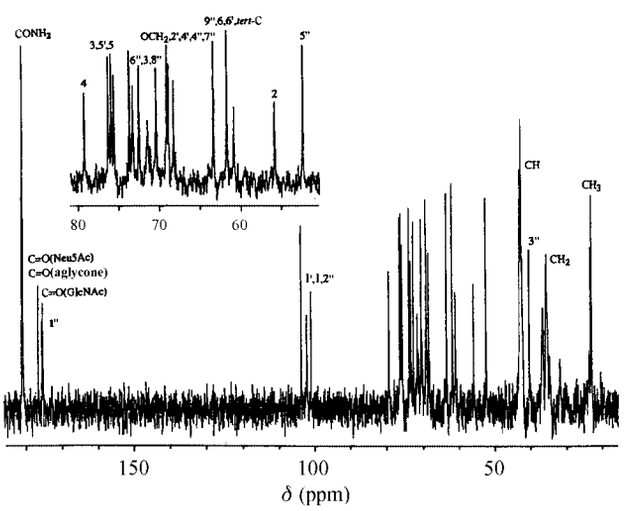


Fig. 2 ^{13}C NMR spectrum of trimeric sialyllactosamine polymer **5**.

Anti-influenza virus activity of glycopolymers having triantennary oligosaccharide branches

Next, our attention was focused on the biological activity of the novel glycopolymers synthesised herein. Thus, the inhibitory effect of these glycopolymers on haemagglutination by three types of influenza viruses was examined according to the method previously used.[37] The results are summarised in Table 3 in comparison with those by glycopolymers prepared from simple monovalent-type glycomonomers.[37,38] Compounds **5** and **6** showed much higher inhibitory effects on human influenza virus haemagglutination than did monovalent-type glycopolymers (Table 3). The triantennary α-2,3-sialic acids of compound **5** seem to be suitable for successful binding with carbohydrate-recognising domains of haemagglutinins of the viruses. Surprisingly, it was also suggested that compound **6**, having mono-2,6-sialylated trimeric *N*-acetyllactosamine branches, exhibited amplified anti-influenza virus activity. This polymer preferentially inhibited haemagglutination caused by A/Aichi/2/68 (H3N2) and A/Memphis/1/71 (H3N2) strains. This suggests the importance of topology of the sialo-oligosaccharide branches on the glycopolymers as well as of multivalency or the total numbers of sialic acid on the macromolecular scaffoldings. Conformational analysis of these trivalent-type glycoligands is under investigation and a more detailed discussion will be reported elsewhere.

In summary, a convenient glycomonomer bearing a triantennary *N*-acetyllactosamine residue was synthesised and converted into water-soluble glycopolymers. The polymer was employed for further sialylation reactions by 2,3- and 2,6-sialyltransferases to afford novel glycoprotein mimics showing potent anti-influenza virus activity.

Experimental

General procedures

Mps were determined with a Laboratory Devices melting point apparatus and are uncorrected. 4-(3-Hydroxypropyl)-4-nitroheptane-1,7-diol was purchased from Aldrich Co. Ltd. α-2,3-Sialyltransferase from porcine liver, α-2,6-sialyltransferase from rat liver, CMP-Neu5Ac and calf intestine alkaline phosphatases were from Sigma Co. Ltd. Optical rotations were determined with a Horiba SEPA-200 digital polarimeter at 25 °C. $[\alpha]_D$-Values are given in units of 10^{-1} deg cm^2 g^{-1}. ^1H and proton-decoupled ^{13}C NMR spectra were recorded at 400 and 100.4 MHz, respectively, on a JEOL α-400 spectrometer. *J*-Values are in Hz. Average relative molecular mass was estimated by gel-permeation chromatography (GPC) with an Asahipak GS-510 column, and pullulans (5.8, 12.2, 23.7, 48.0, 100, 186, and 380 kDa, Shodex Standard P-82) were used as standards. Reactions for the syntheses of glycomonomer **3** and the intermediates were monitored by thin-layer chromatography (TLC) on a precoated plate of silica gel $60F_{254}$ (layer thickness, 0.25 mm; E. Merck, Darmstadt). Column chromatography was performed on silica gel (Wakogel C-200; 100–200 mesh, Wako Pure Chemical Industries Co. Ltd.). Solvent extracts were dried with anhydrous magnesium sulfate and concentrated below 40 °C under reduced pressure.

Table 3 Inhibition of human influenza virus haemagglutination by glycoconjugate polymers[a]

	HAI titre, dilution (μg cm^{-3})		
	A/PR/8/34 (H1N1)	A/Aichi/2/68 (H3N2)	A/Memphis/1/71 (H3N2)
Neu5Ac α 2,3LacNAcPAAm	32 (31)	32 (31)	8 (125)
Neu5Ac α 2,6LacNAcPAAm	<4 (250)	128 (7.8)	32 (31)
(Neu5Ac α 2,3LacNAc)$_3$PAAm **5**	256 (3.9)	128 (7.8)	128 (7.8)
Neu5Ac α 2,6LacNAc(LacNAc)$_2$PAAm **6**	4 (250)	1024 (0.98)	256 (3.9)

[a] Conditions of the inhibitory assay are described in the text.

Nitrotris{3[*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyloxy]propyl}methane 1

A solution of the oxazoline derivative[16] shown in Scheme 1 (940 mg, 1.53 mmol) and 4-(3-hydroxypropyl)-4-nitroheptane-1,7-diol (120 mg, 0.510 mmol) in 1,2-dichloroethane (10 cm^3) was stirred at 70 °C in the presence of CSA (150 mg) under nitrogen. After 1 h, another solution of the oxazoline derivative (940 mg, 1.53 mmol) in 1,2-dichloroethane (10 cm^3) was added to the reaction mixture and the whole solution was stirred at 70 °C for 1 h. The solution was cooled to room temperature, diluted with chloroform, and poured into ice–water. The organic layer was washed successively with saturated aq. sodium hydrogen carbonate and brine, dried, filtered, and evaporated. The residue was purified by chromatography on silica gel with, at first, 50:1 (v/v) and then 20:1 (v/v) chloroform–methanol to afford compound **1** (800 mg, 75%); mp 132 °C (from EtOH) (Found: C, 50.02; H, 6.12; N, 2.94. $C_{88}H_{126}N_4O_{53}\cdot H_2O$ requires C, 50.19; H, 6.12; N, 2.66%); $[\alpha]_D^{25}$ −19.8 (*c* 0.238, chloroform); $\delta_H(CDCl_3)$ 1.45 (6 H, m, 3 × $OCH_2CH_2CH_2$), 1.90 (6 H, m, 3 × OCH_2CH_2), 1.96–2.14 (63 H, all s, 21 × $OCOCH_3$), 3.42 (3 H, m, 3 × OCHH), 3.62 (3 H, m, 3 × 5-H), 3.77 (3 H, t, $J_{3,4}$ 8.5, 3 × 4-H), 3.80 (3 H, m, 3 × OCHH), 3.88 (3 H, m, 3 × 5′-H), 3.92 (3 H, d, $J_{1,2}$ 7.6, 3 × 2-H), 4.05–4.30 (9 H, m, 3 × 6-H^a, 3 × 6′-H^a and 3 × 6′-H^b), 4.47 (3 H, m, 3 × 6-H^b), 4.49 (3 H, d, $J_{1,2}$ 7.8, 3 × 1–H), 4.55 (3 H, d, $J_{1',2'}$ 7.9, 3 × 1′-H), 4.99 (3 H, dd, $J_{3',4'}$ 3.4 and $J_{2',3'}$ 10.4, 3 × 3′-H), 5.09 (3 H, m, 3 × 2′-H), 5.35 (3 H, d, 3 × 4′-H) and 6.36 (3 H, d, $J_{2,NH}$ 9.3, 3 × NH).

***N*-(Tris{3-[*O*-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyloxy]propyl}methyl)acrylamide 2**

Compound **1** (470 mg, 0.225 mmol) was hydrogenated at room temperature in the presence of Raney Ni (T-1) (1.0 g) in ethanol (10 cm^3) and ethyl acetate (5 cm^3) for 48 h. The reaction was monitored by TLC in 5:4:1 (v/v/v) chloroform–ethyl acetate–methanol. The mixture was filtered through Celite and the solution was evaporated to give the crude trimeric glycosylamine.

To a solution of the crude trimeric glycosylamine in tetrahydrofuran (THF) (5.0 cm^3) were added triethylamine (47.1 mm^3) and acryloyl chloride (27.4 mm^3) at 0 °C and the solution was stirred for 3 h at room temperature. The mixture was evaporated *in vacuo*, and the residue obtained was poured into ice–water and extracted with chloroform. The organic layer was washed with brine, dried, filtered and concentrated. The residue was subjected to silica gel chromatography and eluted by, first, 50:1 and then 20:1 (v/v) chloroform–methanol as eluent. Crystallisation from chloroform–diethylether–*n*-hexane gave **2** (310 mg, 65%), mp 142–144 °C (from chloroform–ether–*n*-hexane) (Found: C, 51.65; H, 6.34; N, 2.72. $C_{91}H_{130}N_4O_{52}$ requires C, 51.75; H, 6.20; N, 2.65%); $[\alpha]_D^{25}$ −21.2 (*c* 0.287, chloroform); $\delta_H(CDCl_3)$ 1.43 (6 H, m, 3 × $OCH_2CH_2CH_2$), 1.90 (6 H, m, 3 × OCH_2CH_2), 1.97–2.15 (63 H, all s, 21 × $OCOCH_3$), 3.57 (3 H, m, 3 × OCHH), 3.69 (3 H, m, 3 × 5-H), 3.70 (3 H, m, 3 × OCHH), 3.78 (3 H, t, $J_{3,4}$ 8.6, 3 × 4-H), 3.89 (3 H, m, 3 × 5′-H), 3.96 (3 H, d, 3 × 2-H), 4.03 (3 H, d, $J_{6'a,6'b}$ 13.5, 3 × 6′-H^a), 4.09–4.17 (6 H, m, 3 × 6-H^a and 3 × 6′-H^b), 4.41 (3 H, d, $J_{1,2}$ 7.8, 3 × 1-H), 4.51 (3 H, m, 3 × 6-H^b), 4.54 (3 H, d, $J_{1',2'}$ 7.7, 3 × 1′-H), 4.99 (3 H, dd, $J_{3',4'}$ 3.3 and $J_{2',3'}$ 10.7, 3 × 3′-H), 5.05 (3 H, d, $J_{3,4}$ 8.7, 3 × 3-H), 5.11 (3 H, dd, $J_{1',2'}$ 7.7 and $J_{2',3'}$ 10.4, 3 × 2′-H), 5.13 (3 H, d, 3 × 3-H), 5.36 (3 H, d, 3 × 4′-H), 5.69 [1 H, dd, CH=CH_2(*Z*)], 5.86 [1 H, br s, NH(aglycone)], 6.22 [1 H, dd, CH=CH_2(*E*)], 6.43 (1 H, dd, C*H*=CH_2), 6.56 (3 H, d, 3 × NH).

***N*-(Tris{3-[*O*-(β-D-galactopyranosyl)-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyloxy]propyl}methyl)acrylamide 3**

To a solution of compound **2** (250 mg, 0.118 mmol) in dry methanol (10 cm^3) was added sodium methoxide (40 mg), and the mixture was stirred for 2 h at room temperature. The mixture was quenched by addition of water and the solution was neutralised with Dowex 50W-X8 (H^+) resin, filtered, and evaporated *in vacuo*. The residue was crystallised from 95% ethanol to give **3** (131 mg, 82%), mp 222 °C (decomp.) (Found: C, 48.05; H, 7.06; N, 4.01. $C_{55}H_{94}N_4O_{34}\cdot H_2O$ requires C, 48.10; H, 7.05; N, 4.08%); $[\alpha]_D^{25}$ −28.3 (*c* 0.231, water); $\delta_H(CDCl_3)$ 1.49 (6 H, m, 3 × $OCH_2CH_2CH_2$), 1.74 (6 H, m, 3 × OCH_2CH_2), 2.13 (9 H, s, 3 × $NHCOCH_3$), 4.48 (3 H, d, $J_{1,2}$ 7.8, 3 × 1-H), 4.52 (3 H, d, $J_{1',2'}$ 7.5, 3 × 1′-H), 5.71 [1 H, d, CH=CH_2(*Z*)], 6.15 [1 H, d, CH=CH_2(*E*)], 6.29 (1 H, dd, C*H*=CH_2).

Copolymerisation of 3 with acrylamide

A solution of trimeric glycomonomer **3** (100 mg, 0.0738 mmol) and 4, 10 or 20 mole equivalents of acrylamide in deionised water (1.0 cm^3) was deaerated using a water-pump for 20 min, and TMEDA (0.1 molar equivalent of the carbohydrate monomer) and APS (0.04 mole equivalent of the carbohydrate monomer) were then added. After being stirred for 4 h at room temperature, the reaction mixture was diluted with 0.1 M acetic acid–pyridine buffer (pH 5.1). The viscous solution was subjected to Sephadex G-50 column chromatography with 10 mM aq. ammonium acetate as eluent. The polymer fractions obtained were dialysed against deionised water and lyophilised to give water-soluble copolymers **4a**–**4c** as white powders.

The physical data and fully assigned ^{13}C NMR chemical shifts of all the polymers prepared above are summarised in Tables 1 and 2, respectively.

Enzymic sialylation of triantennary *N*-acetyllactosamine copolymers

(a) Reaction with α-2,3-sialyltransferase from porcine liver. To a solution of triantennary glycopolymer **4c** (13.6 mg; glycomonomer unit–acrylamide = 1:20), CMP-Neu5Ac (14.5 mg, 1.5 mole equivalent of a galactose residue), calf intestine alkaline phosphatase (10 unit), bovine serum albumin (BSA) (3.0 mg), sodium azide (1.0 mg), $MnCl_2\cdot 4H_2O$ (94.5 μg) and Triton CF-54 (5.0 mm^3) in sodium cacodylate buffer (50 mM, pH 6.5; 1.0 cm^3) was added α-2,3-sialyltransferase (0.3 unit), and the mixture was incubated at 37 °C for 48 h. The reaction

was terminated by boiling at 100 °C for 3 min, and the suspended solution was subjected to column chromatography on Sephadex G-25 with water as eluent. The polymer-containing fractions were collected and dialysed against deionised water and lyophilised to give white powdery product **5** (16.4 mg).

(b) Reaction with α-2,6-sialyltransferase from rat liver. Sialylation of triantennary glycopolymer **4c** (13.6 mg) using α-2,6-sialyltransferase (0.1 unit) was performed with CMP-Neu5Ac (14.5 mg), calf intestine alkaline phosphatase (10 unit), BSA (3.0 mg), sodium azide (1.0 mg), $MnCl_2 \cdot 4H_2O$ (94.5 μg) and Triton CF-54 (0.8 mm^3) in sodium cacodylate buffer (50 mM, pH 6.5; 1.0 cm^3). Isolation and purification of the product was carried out according to the procedure described above for the synthesis of compound **5**, and white powdery product **6** was obtained (15.4 mg).

All the ^{13}C NMR chemical shifts of sialylated materials and the results of the sialylation reaction are summarised in Tables 2 and 3, respectively.

Haemagglutination inhibition assay (HAI)

Glycopolymers (1 mg cm^{-3}) were successively diluted (2-fold) with 25 mm^3 of phosphate buffer saline (PBS) in microtitre wells (Immuno plate Nunc. Denmark). Influenza virus suspension [25 mm^3 of PBS containing 4 HAU (*h*aem*a*gglutination *u*nit)] was added to the wells. After incubation at 4 °C for 1 h, 50 mm^3 of 1% (v/v) guinea pig erythrocytes suspension was added to each well, mixed, and allowed to settle at 4 °C for 1 h. Haemagglutination inhibition titres are expressed as the maximum dilution required to give complete inhibition of haemagglutination.

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