Synthesis of Novel Clustered Glycopolymers Containing Triantennary Glycosides of *N*-Acetyllactosamine[1]

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An efficient method for the first synthesis of novel glycopolymers containing triantennary glycosides of *N*-acetyllactosamine [Gal*p*β(1→4)Glc*p*NAc] is described. Radical copolymerization of the triantennary glycosides with acrylamide proceeded smoothly in an aqueous solution and gave water-soluble clustered glycopolymers.

A biological significance of oligosaccharides of asparagine-linked type glycoproteins existing on the cell surfaces has been rapidly investigated. Unique functions of multi-antennary sugar chains were referred to "cluster effect" by Lee et al.[2] In particular, this cluster effect, in the sugar moiety of the relatively non-reducing terminal, has been recently noted in biochemical phenomena represented by carbohydrate-receptor bindings in relation to cell-cell interactions.[3]

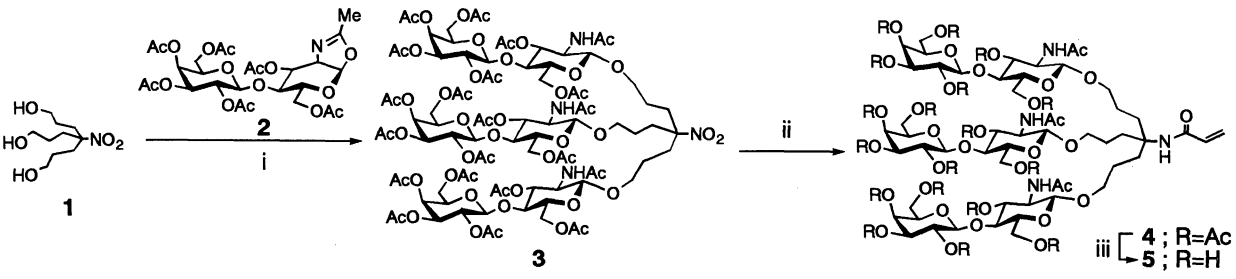
In the preceding paper, we have reported an efficient and facile method for the syntheses of biochemically useful glycoprotein models based on the radical copolymerization of a variety of *n*-pentenyl glycosides with acrylamide.[4] In addition, aglycones of ω-acryloylamino-type structure have been proved to permit homopolymerization of polymerizable sugar derivatives in order to produce highly clustered glycoprotein models.[5] Although useful methods for the synthesis of "triantennary glycosides" having galactose residues were reported using tris(hydroxymethyl)aminomethane by Lee et al.[6] and Polidori et al.,[7] there is no attempt to prepare polymers bearing clustered "triantennary glycoside" which is expecting to show specific affinity with liver lectins.

In the present communication, we report a simple and versatile synthesis of a new glycoprotein model having clustered triantennary *N*-acetyllactosamine (LacNAc) using 4-nitro- 4-[1-(3-hydroxypropyl)]- 1,7- heptandiol **1**[8] as a key starting material.

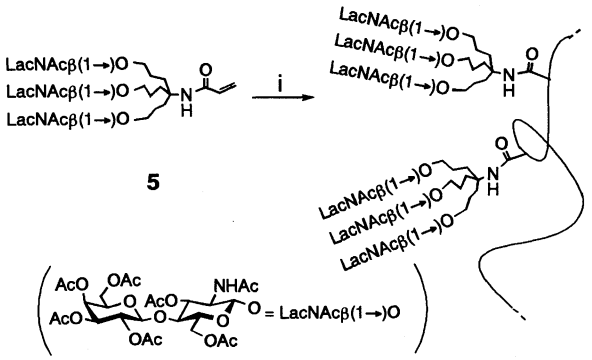
Firstly, oxazoline derivative **2**,[4] readily obtainable from *N*-acetyllactosamine octaacetate, was coupled with triol **1** in the presence of 10-camphorsulfonic acid (CSA) as the promoter in 1,2-dichloroethane to give nitromethane tris[propyl *O*-(2',3',4',6'- tetra- *O*-acetyl- β- D-galactopyranosyl)- (1→4)- 2-acetamido- 3,6- di-*O*- acetyl-2- deoxy-β- D-glucopyranoside] **3**[9] {mp 132 °C, $[\alpha]_D^{25}$ -19.8°(c 0.238, chloroform)} in 75% yield (Scheme 1). The tris-glycoside **3** was hydrogenated in the presence of Raney Ni (T-1)[10] as catalyst followed by *N*-acryloylation to afford *O*-protected tris-LacNAc monomer **4**[9] {$[\alpha]_D^{25}$ -21.2°(c 0.287, chloroform)} in 65% overall yield from **3**. After deacetylation by Zemplen method, a desired key glycomonomer **5**[9,11] {$[\alpha]_D^{25}$ -28.3°(c 0.231, water)} was obtained in 95% yield. Next, copolymerization of this triantennary glycomonomer **5** with acrylamide in deionized water was carried out at room temperature in the presence of *N,N,N'N'*-tetramethylethylenediamine (TEMED) and ammonium peroxodisulfate (APS) as initiators (Scheme 2). After 6 h, the viscous solution was diluted with 0.1 M pyridine-acetic acid buffer (pH 5.1), subjected to Sephadex G-50 column chromatography with 10 mM ammonium acetate solution as elutant, and the crude polymer fractions were dialyzed against deionized water. Finally, the solution was lyophilized to afford water-soluble copolymers in 52-91% yield. The sugar content of these copolymers was determined according to the previous papers.[4,5]

The results of copolymerization are summarized in Table 1 together with physical data. Fully assigned ^{13}C-NMR spectra of copolymer (the ratio of copolymerization ; monomer : acrylamide = 1 : 20)[12] in D_2O is shown in Figure 1 and notes. The spectrum shows a disappearance of signals due to C-C double bond of glycosides after polymerization and characteristic signals attributed to the ring carbons of sugar residues besides the carbons of polyacrylamide. The method described in this paper using Newkome's building block of cascade molecules[8] as a polymerizable aglycon seems to be a simpler and more convenient method in terms of the sugar content (41.2-68.2%) than those previously published.[4,5]

In conclusion, an efficient synthetic procedure of triantennary glycopolymers having pendant *N*-acetyllactosamine residues was established. Binding-interaction of these glycopolymers



Scheme 1. *Reagents and conditions*: i. CSA, $ClCH_2CH_2Cl$, 70 °C, 75%; ii. H_2, Raney Ni(T-1), EtOAc-EtOH, r.t., then CH_2=CHCOCl, Et_3N, THF, 0 °C→r.t., 65% from **3**, iii. NaOMe, MeOH, r.t., 95%.



Scheme 2. *Reagents and conditions*: i. CH_2=$CHCONH_2$, APS, TEMED, H_2O, r.t., 6 h.

Table 1. Polymerizations of triantennary glycomonomers with acrylamide

monomer ratio[a]	total yield (%)	polymer compos[a]	sugar (wt%)	$[\alpha]_D^{25}$ (deg)	Mw[b] (kDa)
1 : 0[c]	—	—	—	—	—
1 : 4	52.8	1 : 5	68.2	-18.1	>300
1 : 10	83.7	1 : 9	58.7	-15.8	>300
1 : 20	91.4	1 : 20	41.2	-11.3	>300

[a] Ratio of carbohydrate monomer to acrylamide was determined by ^{1}H-NMR spectrum. [b] Mw's were determined by the GPC method 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

with liver lectins that specifically recognize clustered *N*-acetyllactosamine residues is now under investigation and the results will be reported in the near future.

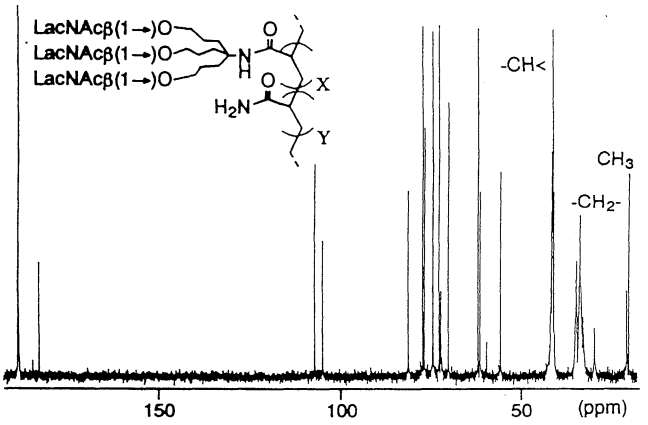


Figure 1. ^{13}C-NMR spectrum of copolymer (X : Y=1 : 20) measured in D_2O at 50 °C.

References and notes

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9 ^{1}H-NMR spectroscopic data : **3** ; ($CDCl_3$) δ 1.45(m, 6H, 3×$OCH_2CH_2CH_2$), 1.90(m, 6H, 3×OCH_2CH_2), 1.96-2.14(all s, 63H, 21×$OCOCH_3$), 3.42(m, 3H, 3×OCH_2), 3.80(m, 3H, 3×OCH_2), 4.49(d, 3H, $J_{1,2}$ 7.8 Hz, 3×H-1), 4.55(d, 3H, $J_{1',2'}$ 7.9 Hz, 3×H-1'), 5.35(d, 3H, 3×H-4'), 6.36(d, 3H, 3×NH). **4** ; ($CDCl_3$) δ 1.43(m, 6H, 3×$OCH_2CH_2CH_2$), 1.90(m, 6H, 3×OCH_2CH_2), 1.97-2.15(all s, 63H, 21×$OCOCH_3$), 3.57(m, 3H, 3×OCH_2), 3.80(m, 3H, 3×OCH_2), 4.41(d, 3H, $J_{1,2}$ 7.8 Hz, 3×H-1), 4.54(d, 3H, $J_{1',2'}$ 7.7 Hz, 3×H-1'), 5.36(d, 3H, 3×H-4'), 5.69[dd, 1H, -CH=CH_2(cis)], 5.86[s, 1H, NH (aglycon)], 6.22[dd, 1H, -CH=CH_2(trans)], 6.43(dd, 1H, -CH=CH_2), 6.56(d, 3H, 3×NH). **5** ; (D_2O) δ 1.49(m, 6H, 3×$OCH_2CH_2CH_2$), 1.74(m, 6H, 3×OCH_2CH_2), 2.13(s, 9H, 3×$OCOCH_3$), 4.48(d, 3H, $J_{1,2}$ 7.8 Hz, 3×H-1), 4.52(d, 3H, $J_{1',2'}$ 7.5 Hz, 3×H-1'), 5.71[d, 1H, -CH=CH_2(cis)], 6.15[d, 1H, -CH=CH_2(trans)], 6.29(dd, 1H, -CH=CH_2).

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11 ^{13}C-NMR spectroscopic data : **5** ; (D_2O) δ 25.2(CH_3), 25.7, 33.8(-$OCH_2CH_2CH_2$), 58.0, 61.7, 63.1, 63.9[C-2, $C(CH_2CH_2)_3$, C-6, 6'], 71.4, 73.5, 73.8, 75.3, 75.4, 77.6, 78.2, 81.7(C-4', -OCH_2, C-2', 3, 5', 3', 5, 4), 103.8(C-1), 105.8(C-1'), 129.5, 133.8(CH=CH_2), 170.4[C=O(aglycon)], 177.0 [C=O (GlcNAc)].

12 ^{13}C-NMR spectroscopic data : copolymer (the ratio of copolymerization ; monomer : acrylamide = 1 : 20) of **5** with acrylamide ; (D_2O) δ 25.1(CH_3), 25.7, 34.0(-$OCH_2CH_2CH_2$), 36.6-38.6(-CH_2-), 44.2-45.5(-CH<), 71.3, 73.2, 73.7, 75.2, 75.3, 77.5, 78.0, 81.7(C-4', -OCH_2, C-2', 3, 5', 3', 5, 4), 57.9, 61.4, 63.1, 63.6[C-2, -$C(CH_2CH_2)_3$, C-6, 6'], 103.6(C-1), 105.6(C-1'), 176.6[C=O(GlcNAc)], 178.3[C=O (aglycon)], 182.0[C=O(acrylamide)].