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An Efficient Synthesis of Sialoglycoconjugates on a Peptidase-Sensitive Polymer Support[1]

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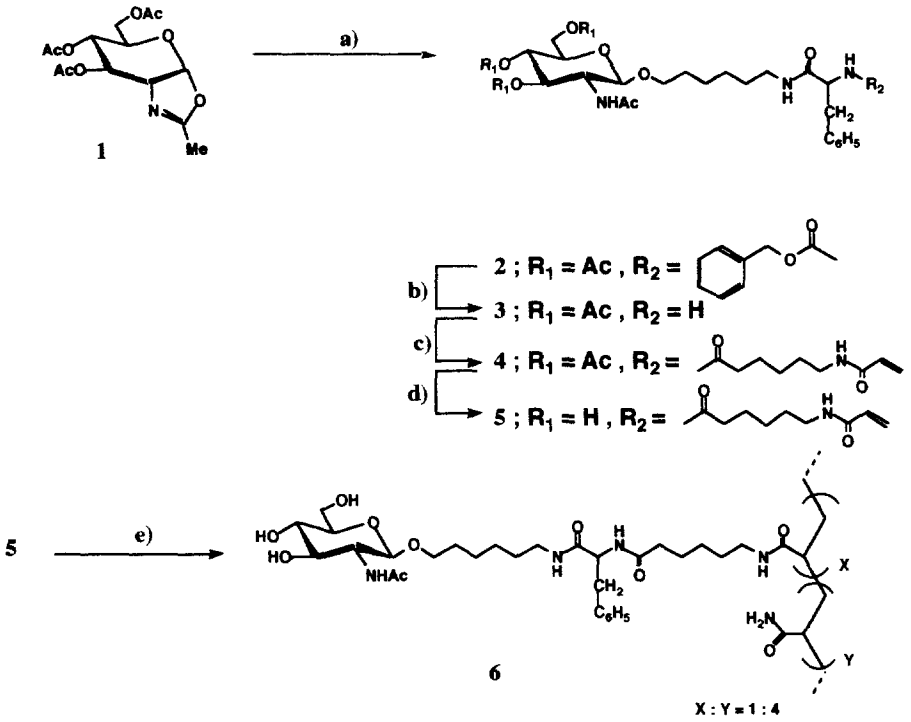
Abstract: A novel method for the enzymatic synthesis of oligosaccharide derivatives on a α-chymotrypsin-sensitive polymer support is described. The primer polymer having N-acetyl-D-glucosamine (GlcNAc) residue through a phenylalanine-containing spacer moiety was successfully elongated with galactosyl and sialyltransferases to give a glycopolymer bearing sialyl α(2→6) N-acetyllactosamine branches in high yield. Subsequent hydrolysis with α-chymotrypsin proceeded smoothly and afforded a versatile sialotrisaccharide derivative having a terminal amino group which can be used for creating neoglycoconjugates.

Enzymatic oligosaccharide synthesis based on glycosyltransferases[2] is a useful and powerful alternative to chemical synthesis since glycosyltransferases are highly stereo- and regioselective with regard to the glycoside bond formations, and no tedious protection/deprotection steps are required. On the other hand, water-soluble glycopolymers as high-performance primers for the enzymatic synthesis of glycoconjugates have been also receiving ever increasing attention recently, because of their high efficiency in glycosylation reactions attributed to "polymeric sugar-cluster effects"[3,4] and simple procedures for the purification of products.[5-8]

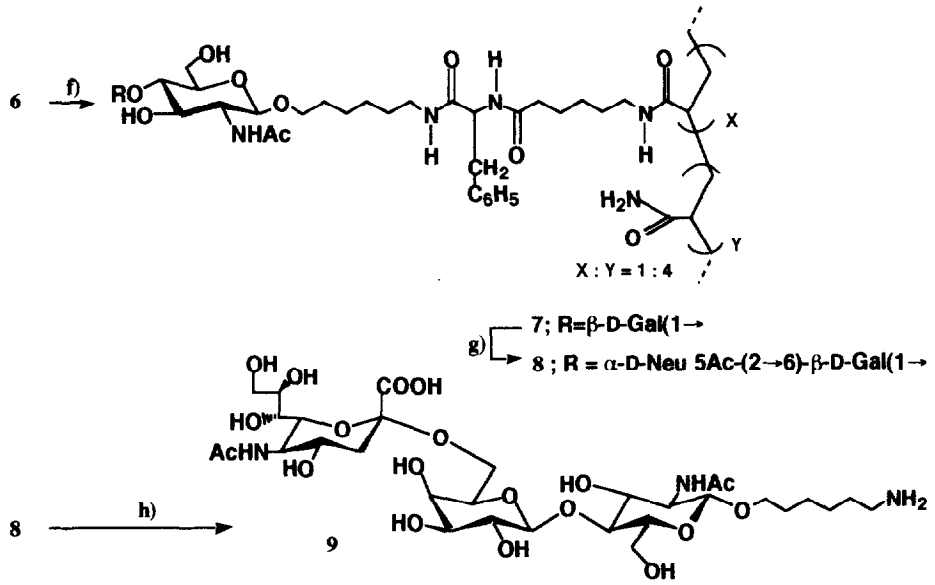
In our preceding paper,[5] we demonstrated using hydrogen-sensitive glycopolymers having flexible GlcNAc branches whereby the polymeric primers allow quantitative transfer of D-galactose from UDP-galactose (UDP-Gal) to the GlcNAc residues with bovine milk galactosyl transferase; subsequent hydrogenolysis with palladium on charcoal proceeded smoothly to release the targeted *N*-acetyllactosamine from the polymer support in high yield. The success of this methodology is critically dependent on the "polymeric sugar-cluster effects" which promote the successful binding of the sugar acceptor with the enzymes.[5] It was also suggested that the advantages of the enzymatic assembly of oligosaccharides on the water-soluble polymer supports seemed to be significantly affected by the flexible anchor moiety, rendering the primer sugar accessible to the enzymes and reagents. In an extension of this strategy, we report herein the synthesis and feasibility of a novel type of primer support bearing α-chymotrypsin-sensitive structure in the spacer-arm moieties and present a facile preparation of an available 6-aminohexyl glycoside of sialooligosaccharide in exemplification.



Scheme 1 a) Z-Phe-NH-$(CH_2)_6$-OH (2.0 eq), 10-camphorsulfonic acid, $(CHCl_2)_2$, 70 °C, 30 min, 36%, b) H_2, Pd/c, MeOH, 50 °C, q. y., c) CH_2=CHCONH$(CH_2)_5$COOH (1.1 eq), EtOH-C_6H_6, 77%, d) NaOMe (cat), MeOH-THF, q. y., e) i; CH_2=$CHCONH_2$ (4.0 eq), TEMED (0.4 eq), APS (0.16 eq), DMSO-H_2O, 50 °C,, ii; gel filtration on Sephadex G-25 with 0.05 M CH_3COONH_4 aq., 93%.

Scheme 1 indicates the synthetic route of the new primer GlcNAc-polymer having a peptidase-sensitive spacer moiety. Firstly, oxazoline derivative **1** was coupled with a readily preparable 6-(*N*-benzyloxycarbonyl L-phenylalanyl)-amino-1-hexanol[9] according to the method reported previously.[10] Next, *N*-deprotection of the phenylalanine segment and subsequent condensation with 6-acrylamidocaproic acid[5] afforded a polymerizable GlcNAc derivative **3** in moderate yield. Finally, usual de-*O*-acetylation and copolymerization of the sugar monomer **5**[11] proceeded smoothly in the presence of ammonium persulfate (APS) and *N,N,N',N'*-tetramethyl-ethylenediamine (TEMED) as promotors and gave the *primer polymer* **6** in high yield.[12]

Galactosylation and subsequent sialylation with the corresponding glycosyl transferases were carried out according to the conditions shown in Scheme 2. As expected, high efficiency in the sugar-transfer reactions was achieved using only a small excess of UDP-Gal or CMP-*N*-acetylneuramic acid (CMP-Neu5Ac), producing the polymer **8** bearing sialyl α(2→6)-LacNAc branches in excellent yield.[13] A useful sialyl α(2→6)-LacNAc derivative **9** having a terminal amino group at the reducing end was successfully released from the



Scheme 2 f) i; GalT (1.0 unit), UDP-Gal (1.3 eq), α-lactalbumin, HEPES buffer (pH 6.0), 37 °C, 24 h, ii; gel filtration on Sephadex G-25 with 0.05 M CH_3COONH_4 aq., g) i; α(2→6)-SialylT (0.1 unit), CMP-Neu5Ac (1.5 eq), phosphatase alkaline (20 unit), bovine serum albumin (2 mg), NaN_3 (14.38 μmole), $MnCl_2$ (1.58 μmole), sodium cacodylate buffer (pH 7.4), 37 °C, 48 h, ii; gel filtration on Sephadex G-25 with 0.05 M CH_3COONH_4 aq., h) i; α-chymotrypsin (1.0 mg), Tris-Hcl buffer (pH 7.8), 40 °C, 24 h, ii; gel filtration on Sephadex G-15 with 0.05 M CH_3COONH_4 aq., 72% from **6**.

polymer chains by treating with α-chymotrypsin at 40 °C for 24 h in 72% overall yield from the primer polymer **6**.[14] As anticipated from the ^{1}H-NMR spectra of compounds **7** and **8**, minor products could not be isolated by this purification procedure. Conjugation of the reactive sialooligosaccharide **9** with bovine serum albumin was successfully performed to give a novel type of neoglycoconjugates bearing sialooligosaccharide. This neoglycoprotrein at the concentration of 150 μg/mL showed inhibitory activity against hemagglutination induced by wheat germ agglutinin.

In conclusion, it was clearly demonstrated that a water-soluble primer having pendant GlcNAc residues through a selectively cleavable phenylalanyl moiety is a practicably available glycosyl acceptor for the enzyme-assisted synthetic strategy of this novel type of neoglycoconjugates.

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References and Notes

1. A preliminary account of this work was partly presented at 13th International Symposium on Glycoconjugates, Seattle, August 20-26, 1995.

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9. This compound was prepared by condensation of Z-Phe with 6-amino-1-hexanol in the presence of EEDQ as a promotor; 85%, m. p. 109-110 °C. δH ($CDCl_3$) 7.29 (m, 10 H, aromatic), 5.72 (br s, 1 H, NH), 5.44 (br s, 1 H, NH), 5.09 (s, 2 H, $OCH_2C_6H_5$), 4.34 (q, 1 H, CHα), 3.62 (t, 2 H, *J* 6.6 Hz, CH_2OH), 3.08 (m, 4 H, CH_2N, $CHCH_2C_6H_5$), and 1.40 (m, 8 H, 4 x CH_2).
10. Nishimura, S. -I.; Matsuoka, K.; Furuike, T. *Methods Enzymol.* **1994**, *242*, 235-246.
11. Selected spectral data for compound **5**: δH (DMSO-d_6) 7.95 (m, 2 H, $NHCOCH=CH_2$, NH_{Phe}), 7.85 (s, 1 H, *J* 8.4 Hz, NHAc), 7.18 (m, 5 H, C_6H_5), 6.18 (q, 1 H, $COCH=CH_2$), 6.03 (d, 1 H, *J* 16.8 Hz, $COCH=CH_{2\ trans}$), 5.52 (d, 1 H, *J* 9.6 Hz, $COCH=CH_{2\ cis}$), 4.87 (d, 2 H, OCH_2), 4.46 (m, 2 H, H-4 and $H\alpha_{Phe}$), 4.23 (d, *J* 7.5 Hz, H-1), 3.67 (s, 1 H, H-3), 3.40 (m, 2 H, H-2 and H-5), 2.96-2.71 (m, 8 H, $H\text{-}6_{a,b}$, 2 x CH_2, and $CH_2C_6H_5$), 2.00 (s, 2 H, $COCH_2$), 1.80 (s, 3 H, $NHCOCH_3$), and 1.34-1.10 (m, 16 H, 8 x CH_2).
12. Compound **6**: Mw=380000 (GPC), δH (D_2O) 7.29 (m, 5 H, aromatic), 4.50 (br t, 2 H, H-1 and $H\alpha_{Phe}$), 3.89 (m, 1 H, H-4), 3.71 (m, 1 H, H-2), 3.44 (br d, 3 H, H-3 and OCH_2), 3.12-2.86 (m, 4 H, CH_2N, $CHCH_2C_6H_5$), 2.20 (br s, 5 H, CH), and 1.80-1.15 (m, 26 H, 13 x CH_2).
13. Enzymatic elongations of sugar residues and subsequent purifications by simple gel filtration were carried out according to the procedures reported in the reference 5. Efficiency of the enzymatic glycosylations was determined by the integration data of the ring protons of each sugar residue. Although broadening of the signals was observed owing to the high molecular weights, the degree of sugar transferring reactions was preliminarily estimated to be more than 90% in both cases.
14. A typical procedure: Glycopolymer product (33 mg, 24.0 μmole of sialyl-LacNAc), α-chymotrypsin (1 mg, 100 units) were incubated in 0.08 M Tris-HCl buffer (1 mL, pH 7.8) for 24 h at 40 °C. The mixture was directly purified by chromatography on a Sephadex G-25 column (1.5 x 40 cm) eluted with 0.05 M CH_3COONH_4 solution. The sugar-containing fractions were collected and lyophilized to give pure compound **9** (15 mg, 72% from **6**). Compound **9**: δH (D_2O) 4.56 (d, 1 H, *J* 7.3 Hz, H-1), 4.45 (d, 1 H, *J* 8.0 Hz, H-1'), 4.00 (br d, 3 H, H-6b, H-4, and H-6'b), 3.97 (m, 1 H, H-4'), 3.92 (br d, 1 H, *J* 3.81 (br d, 1 H, H-5"), 3.72 (dd, 1 H, *J* 10 Hz, H-2), 3.67-3.62 (m, 3 H, H-3, H-3', and H-4"), 3.59-3.52 (m, 5 H, H-5, H-2', H-3', H-6'a, and H-7"), 3.00 (t, 2 H, *J* 7.5 Hz, NCH_2), 2.67 (dd, 1 H, *J* 4.7 and 12.5 Hz, H-3"eq), 2.06-2.03 (each s, 2 H, 2 x $COCH_3$), 1.74 (d, 1 H, *J* 12.4 Hz, H-3"ax), and 1.68-1.37 (m, 8 H, 4 x CH_2). δC (D_2O) 177.8, 177.3, and 176.4 (C=O), 106.4 (C-1'), 103.8 (C-1), 103.0 (C-2"), 83.7 (C-4), 77.4 (C-5), 76.6 (C-5'), 75.4 (C-6"), 75.3 (C-3'), 74.6 (C-3), 73.7 (C-8"), 73.2 (C-2'), 71.3 (C-4 and C-4"), 71.1(C-7"), 66.3 (C-6'), 65.5 (C-9"), 63.3 (C-6), 57.8 (C-2), 54.7 (C-5"), 43.0 (OCH_2), 42.3 (C-3"), 31.2, 29.5, 28.1, and 27.5 (CH_2), 25.2 and 24.9 (CH_3).

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