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Chemoenzymatic Oligosaccharide Synthesis on a Soluble Polymeric Carrier

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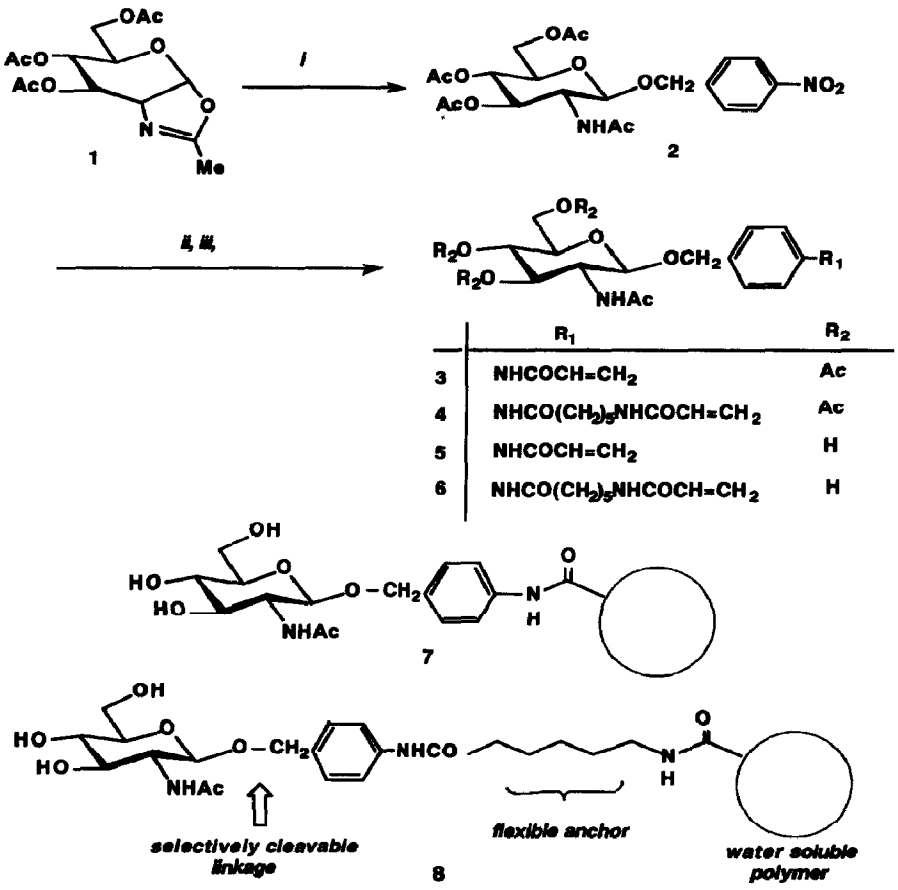
Abstract: A facile and efficient method for the chemical and enzymatic syntheses of oligosaccharides using a new type of soluble polymeric sugar acceptor substrate is described. The water soluble glycopolymers having N-acetyl-D-glucosamine (GlcNAc) branches derived from a p-substituted benzyl glycosides were galactosylated with bovine milk galactosyl transferase. The flexible GlcNAc branches of the polymer chains allow quantitative galactosylation and subsequent hydrogenolysis proceeded smoothly to release the desired N-acetyl-lactosamine from the polymer support in high yield.

Glycoconjugates as subjects of basic research and as potential sources for therapeutic ligands are receiving ever increasing attention recently, because of their important roles in biological functions. Efficient assembly of carbohydrates of intricate structures is of central importance in biological sciences and in their application in medicine. Chemical synthesis of oligosaccharides has made dramatic advances during the past few years. Chemical syntheses on polymer-support both in solid-phase synthesis[1] and in solution[2] have developed as efficient methods for preparation of oligosaccharides. Enzyme-assisted technique is an excellent alternative methodology in oligosaccharide synthesis because of its high stereo- and regioselectivity.[3] A recent study on enzymatic syntheses of glycopeptides utilized derivatized silica support particles.[4]

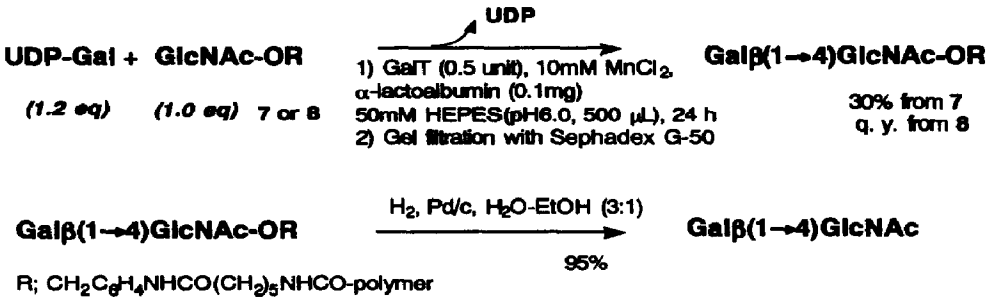
Enzymatic extension of the carbohydrate chains on water soluble polymer with flexible anchors has dual advantages of facilitating efficient separation of the product from the reactants and permiting high reactivity expected of simple soluble molecules. In the course of our synthetic studies on glycoconjugate polymers (neoglycopolymers),[5] we demonstrated that GlcNAc residues attached to a water soluble acrylamide polymer backbone by a 3-carbon spacer-arm allowed quantitative galactosylation with bovine galactosyl transferase, and partial sialylation of the galactosyl residue by *Trypanosoma cruzi trans*-sialidase.[6] The success of the enzymatic assembly of oligosaccharide on soluble polymer supports is critically dependent on the flexible spacer-arm, suitably distanced from the backbone, rendering the acceptor sugar available to the enzymes. We now describe a facile and efficient enzymatic preparation of *N*-acetyl-lactosamine on a specially designed polymeric carrier, which allows release of the product by catalytic hydrogenolysis.



Scheme 1 i) $HOCH_2C_6H_4NO_2$ (5 eq), 10-Camphorsulfonic acid, $(ClCH_2)_2$, 80°C, 2 h, 89%, ii) $HCOONH_4$ (20 eq), Pd/c, MeOH, 20 min, then, a; CH_2=CHCOCl (1.1 eq), Et_3N (pH 9), MeOH, 54% from **2**, b; CH_2=CHCONH$(CH_2)_5$COOH (1.0 eq), Et_3N (1.0 eq), 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1.0 eq), $(ClCH_2)_2$-DMF, 51% from **2**, iii) NaOMe (cat.), MeOH, q. y.

Scheme 1 describes the synthetic route for the new polymers having multivalent GlcNAc side chains. We selected a *p*-nitrobenzyl glycoside (**2**) which can be easily prepared by glycosidation of *p*-nitrobenzyl alcohol with the oxazoline derivative **1**. Selective hydrogen transfer reaction and subsequent condensation with acryloyl chloride or 6-acrylamidocaproic acid[7] afforded derivatives **3** and **4** in moderate yields. Finally, de-*O*-acetylation under the Zemplen condition gave polymerizable glycosides **5** and **6**. Radical copolymerization of these monomers with acrylamide proceeded smoothly under the usual condition[8] and yielded water soluble GlcNAc acceptor polymers **7** (61%) and **8** (83%).[9]

Enzymatic galactosylation of these GlcNAc acceptor polymers was carried out with bovine milkgalactosyl transferase as described previously.[6] As anticipated, the effect of the length of the spacer-arm on the degree of the glycosylation was evident, and almost quantitative galactosylation of the polymer



Scheme 2 Galactosylation and release of N-acetyl-lactosamine from polymer support

substrate **8** was achieved using only a small excess of UDP-Gal (1.2 molar equivalent to the acceptor GlcNAc residues). On the other hand, only 30% galactosylation was observed in the case of the short-armed polymeric glycosyl acceptor **7**. ^{1}H-NMR spectrum of the product clearly showed that a complete substitution of the GlcNAc residues with galactose residues on the polymeric acceptor **8** occurred.[10] Two anomeric protons at 4.45 and 4.50 ppm were observed concomitant with disappearance of a signal at 3.42 ppm attributable to the H-4 of an unsubstituted GlcNAc residue. Zehavi et al.[11] had previously showed only 27% of galactose-transfer reaction using a polyvinyl alcohol derivative having 2-nitrobenzyl glycoside of glucose as an acceptor substrate. These results indicate that longer and more flexible spacer-arms may be the key to the successful glycosylation with enzymes. Hydrogenolysis in the presence of palladium on carbon generated the desired product, *N*-acetyl-lactosamine, which was purified on Sephadex LH-20 with 95:5 (v/v) ethanol-water as eluant with a high recovery.[12] The ^{1}H- and ^{13}C-NMR spectrum of this material closely agree with those of published values.[13,14]

In summary, a water soluble polymer having pendant GlcNAc residues through a selectively cleavable *p*-substituted benzyl group has been shown to be a suitable glycosyl acceptor for chemoenzymic oligosaccharide synthesis. Our results clearly suggest that a flexible spacer-arm of suitable length is necessary to render the acceptor sugar available to the galactosyl transferase.

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7. 6-Acrylamidocaproic acid was prepared by the reaction of 6-aminocaproic acid with acryloyl chloride in water (pH 9). m. p. 65-66°C
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9. Compound **7**: δH (D_2O) 7.37 (br d, 4 H, aromatic), 4.70 (br d, 2 H, OCH_2Ph), 4.48 (d, 1 H, *J* 8 Hz, H-1) 3.89 (d, 1 H, *J* 12 Hz, H-6b), 3.70 (br d, 1 H, *J* 14 Hz, H-6a), 3.64 (t, 1 H, *J* 10 Hz, H-3),

3.53 (m, 1 H, H-2), 3.43 (br d, 2 H, H-4 and H-5), 2.3-2.1 (m, 10.7 H, -CH-), 1.90 (s, 3 H, $COCH_3$), and 1.7-1.4 (m, 26.9 H, $-CH_2-$). Compound **8**: δH (D_2O) 7.36 (br d, 4 H, aromatic), 4.60 (m, 2 H, OCH_2Ph), 4.49 (br d, 1 H, H-1), 3.93 (br d, 1 H, *J* 12 Hz, H-6b), 3.74 (br d, 1 H, *J* 12 Hz, H-6a), 3.68 (t, 1 H, *J* 10 Hz, H-3), 3.48 (m, 1 H, H-2), 3.42 (m, 2 H, H-4 and H-5), 3.17 (br s, 2 H, NCH_2), 2.4-2.1 (br m, 10.9 H, $COCH_2$ and -CH-), 1.92 (s, 3 H, $COCH_3$), and 1.8-1.4 (br m, 20.5 H, $-CH_2-$).

10. A typical procedure: Acceptor glycopolymer **8**, (30 mg, 27 μmol GlcNAc), UDP-Gal (18.0 mg, 32.4 μmol), α-lactalbumin (200 μg), and GalT (0.5 unit) were incubated in 50 mM HEPES (500 μL, pH 6.0) for 24 h at 37°C. The mixture was directly purified by chromatography on a Sephadex G-50 column (2.5 x 100 cm) eluted with 50 mM CH_3COONH_4. The polymer fractions were collected and lyophilized to give a glycopolymer having *N*-acetyllactosamine (32 mg). δH (D_2O) 7.38 (br d, 4 H, aromatic), 4.60 (m, 2 H, OCH_2Ph), 4.50 (br d, 1 H, H-1), 4.47 (d, 1 H, *J* 8 Hz, H-1'), 4.0 (br d, 1 H, H-6b), 3.91 (d, 1 H, J<3 Hz, H-4'), 3.75-3.65 (m, H-3 and H-4), 3.55-3.50 (m, 2 H, H-2 and H-3'), 3.42 (br s, 1 H, H-5), 3.17 (br s, 2 H, NCH_2), 2.4-2.1 (br m, 12.8 H, $COCH_2$ and -CH-), 1.91 (s, 3 H, $COCH_3$), and 1.8-1.35 (br m, 18.0 H, $-CH_2-$).
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12. Alternatively, oxidative cleavage with 2,3-dichloro-5,6-dicyanobenzoquinone can be used for the effective release of the disaccharide from the polymer supports. For DDQ oxidation, see: Oikawa, Y.; Yoshioka, T.; Yonemitsu, O. *Tetrahedron Lett.* **1982**, *23*, 885, and Fukase, K.; Tanaka, H.; Torii, S.; Kusumoto, S. *Tetrahedron Lett.* **1990**, *31*, 389.
13. δH (D_2O) 5.20 (d, *J* 2.3 Hz, H-1α), 4.46 (d, *J* 7.7 Hz, H-1β and H-1'), and 2.04 (s, 3 H, $COCH_3$). δC (D_2O) 177.1 (C=O), 105.6 (C-1), 97.5 (C-1β), 93.2 (C-1α), 81.4 (C-4α), 81.0 (C-4β), 78.0 (C-5'), 77.5 (C-5β), 75.1 (C-3β), 73.6 (C-2'), 72.9 (C-5α), 71.9 (C-3α), 71.2 (C-4'), 63.6 (C-6'), 62.6 (C-6), 58.8 (C-2β), 56.3 (C-2α), and 24.8 (CH_3).
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