Transfer of Ganglioside GM3 Oligosaccharide from a Water Soluble Polymer to Ceramide by Ceramide Glycanase. A Novel Approach for the Chemical-Enzymatic Synthesis of Glycosphingolipids

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It has become a popular topic in recent years that cell surface carbohydrates play central roles in many biological recognition processes.[1] Of particular interest to glycobiology and medicinal chemistry is easy, versatile, and practical methodology for the construction of glycoconjugates of higher structural complexity and in a combinatorial fashion. Enzyme-assisted strategy for the synthesis of oligosaccharides is recognized as one of the promising practical alternatives to chemical synthesis because of highly stereo- and regioselective reactions with no tedious protection/deprotection steps.[2] As part of an ongoing project on the feasible and efficient methods for enzymatic syntheses of carbohydrates using water-soluble glycopolymers as *primers*,[3] our attention was then focused on new synthetic technology for the construction of sphingoglycolipids on a water-soluble polymer support having a specific linker that can be recognized by ceramide glycanase.[4]

Ganglioside GM3 (**1**)[5] was selected as a target for evaluation of the present synthetic methodology. Although this trisaccharide sequence (αNeuAc2→3βGal1→4βGlc) was synthesized previously from methyl lactoside and CMP-NeuAc[6] by use of rat liver βGal1→3/4GlcNAc α-2,3-sialyltransferase[7] in modest yield (39%), the advent of new technologies that amplifies efficacy of enzymatic strategies is still very much in demand. This communication describes a facile and efficient enzymatic synthesis of GM3 using water-soluble polymer support. The described strategy combines (a) application of water-soluble and amphiphilic glycopolymer in enzymatic glycolipid synthesis, (b) synthesis and utilization of a new lactosyl ceramide-mimetic monomer in polymer-supported glycoconjugate synthesis, (c) high efficiency in glycosylation reactions by glycosyltransferases based on "polymeric glycoside-cluster effect"[8] and easy purification of the polymer bearing oligosaccharides by simple gel filtration, and (d) versatile and practical procedure for the transfer of oligosaccharide products from polymer-support to ceramide by unique transglycosylation activity of ceramide glycanase.[4b]

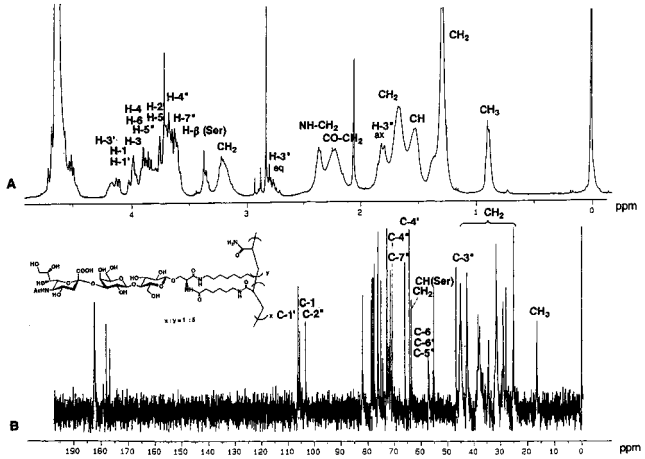

Figure 1. (A) ^{1}H- and (B) ^{13}C-NMR spectra of compound **8**.

For the purpose of the present technology, polymerizable lactose derivative **6**[9] was designed to allow simple preparation of water-soluble polyacrylamide having lactose residues through a ceramide mimetic linker derived from a readily available L-serine derivative **3** (Scheme 1). The lactosyl ceramide (LacCer) mimetic glycopolymer **7** was obtained in 92% yield by radical copolymerization of monomeric precursor **6** with acrylamide in deoxygenated water according to the procedure previously described.[10] This primer support was employed for sialylation reaction using rat liver βGal1→3/4GlcNAc α-2,3-sialyltransferase in the presence of CMP-NeuAc according to the previous report.[7] The product **8** (*quantitative yield*) was conveniently isolated by a simple column of Sephadex G-25 gel, which can be monitored by NMR measurements (Figure 1). Finally, treatment of **8** with leech ceramide glycanase in the presence of excess of ceramide as an acceptor and subsequent chromatographic purifications gave ganglioside GM3 (**1**) in 61% yield.

The polymer-assisted enzymatic process described above afforded GM3 *in three steps with 56% overall yield from a readily available precursor* ***6***, a remarkable improvement in both ease of synthesis and overall yield compared to those of chemical synthesis.[5] It should also be noted that each step for enzymatic sugar-elongations of water-soluble polymer primers can be clearly characterized by NMR spectroscopy. Since large-scale preparation of both recombinant glycosyltransferases[11] and sugar nucleotides[12] are now possible, the present methodology

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(12) Some recombinant glycosyl transferases and sugar nucleotides are now obtainable from Calbiochem.

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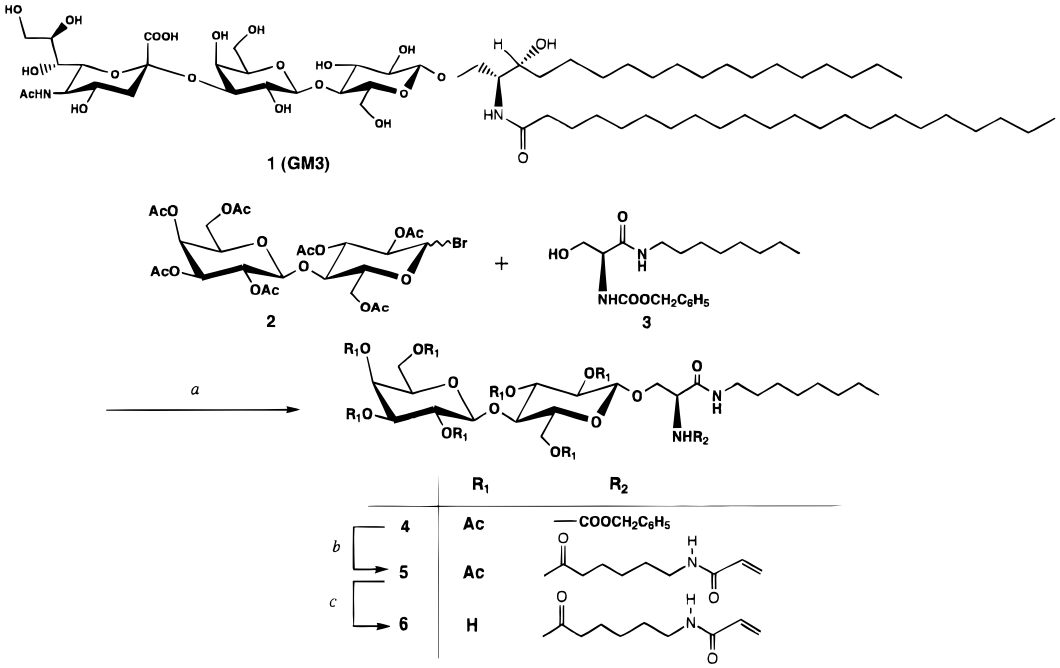
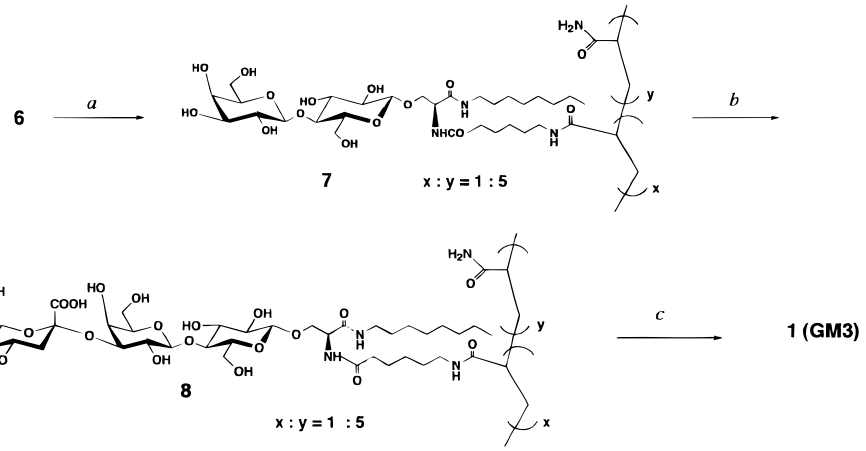
Scheme 1.[a] Synthesis of LacCer Mimetic Monomer **6**



[a] Reagents and conditions: (a) AgOTf (1.5 equiv), CH_2Cl_2, −20 °C, 24 h, 48%; (b) i. H_2, Pd-c, MeOH, room temperature, 24 h; ii. $HOOC(CH_2)_5NHCOCH{=}CH_2$ (0.91 equiv), EEDQ (1.0 equiv), EtOH-C_6H_6, 50 °C, 24 h, 78% from **4**; (c) MeONa (0.4 equiv), 25 °C, 2 h, >99%. EEDQ = *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline.

Scheme 2.[a] Preparation of Primer and Enzymatic Synthesis of GM3



[a] Reagents and conditions: (a) acrylamide (4.0 equiv), TEMED (0.4 equiv), APS (0.16 equiv), DMSO−H_2O, 50 °C, 48 h, 92%; (b) CMP-NeuAc (1.2 equiv), α-2,3-sialyltransferase (0.3 unit), BSA, $MnCl_2$, CIAP (20 unit), 50 mM sodium cacodylate buffer (pH 7.49), 37 °C, 3 days, >99%; (c) ceramide (4.85 equiv), ceramide glycanase (0.01 unit), Triton CF-54 (1 drop), 50 mM sodium citrate buffer (pH 6.0), 37 °C, 17 h, 61%. TEMED = *N*,*N*,*N*′,*N*′-tetramethylethylene-diamine; APS = ammonium peroxodisulfate; BSA = bovine serum albumin; CIAP = carf intestinal alkaline phosphate.

using LacCer mimetic polymer should be widely applicable both for the synthesis of various naturally occurring sphingoglycolipids and combinatorial synthesis of libraries of glycolipids varying in the lipid portion.

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Supporting Information Available: Experimental details and spectral and analytical data for all new compounds (16 pages). See any masthead page for ordering and Internet access instructions.

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