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Efficient synthesis of non-natural ganglioside (pseudo-GM3) and fluorescent labelled lysoGM3 on the basis of polymer-assisted enzymatic strategy

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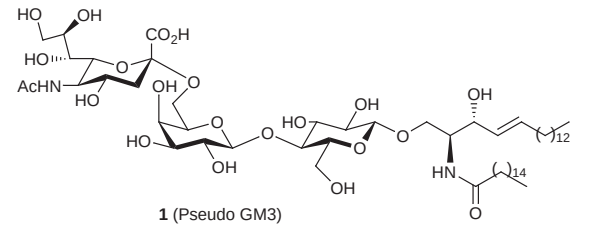
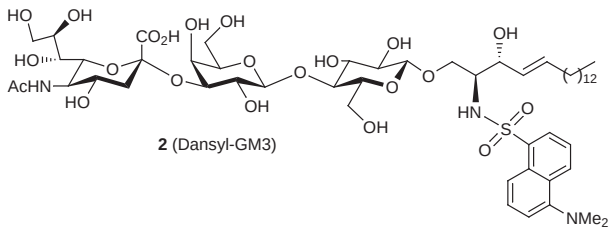
The versatility of polymer-assisted enzymatic synthesis of non-natural and biologically significant glycolipid derivatives was demonstrated by constructing pseudo-ganglioside GM3 1 having the trisaccharide sequence Neu5Acα-(2→6)Galβ(1→4)Glc and a fluorescent labelled lysoGM3 2.

Cell surface glycoconjugates such as glycoproteins and glycolipids are an important class of biomolecules providing a great deal of significant information on cell–cell interactions.[1] The advent of efficient and practical methods for glycoconjugate synthesis is urgently required. Enzymatic synthesis of carbohydrates with glycosyltransferases[2] and glycosidases[3] has been recognised as a promising practical alternative to chemical synthesis. Recently, we found an efficient synthetic strategy for glycoconjugates based on novel water-soluble polymer supports with specific linkers.[4–7] It was suggested that polymer **8** bearing ceramide-mimetic linkers might yield a practical procedure for the construction of natural sphingoglycolipid GM3.[6] In addition to the synthesis of naturally occurring gangliosides, our attention is now directed toward the applicability of this strategy to the synthesis of non-natural and biologically significant glycolipid derivatives. Here we report efficient syntheses of pseudo-ganglioside GM3 **1** having the



1 (Pseudo GM3)

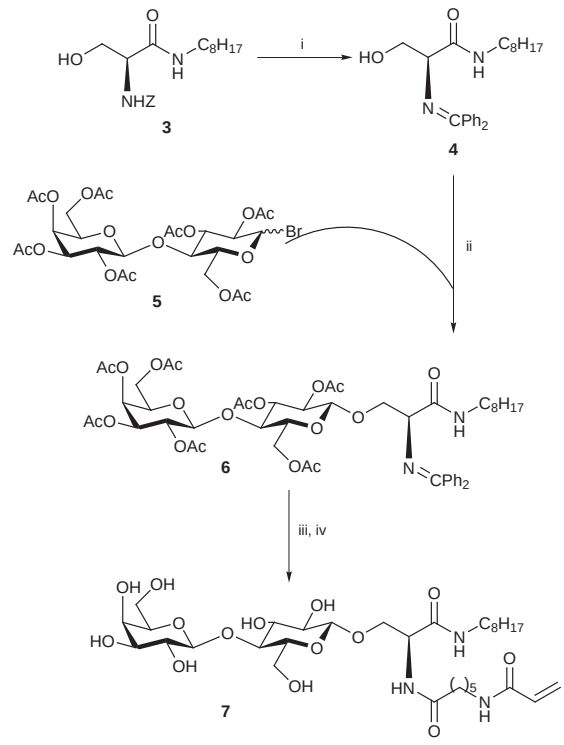


2 (Dansyl-GM3)

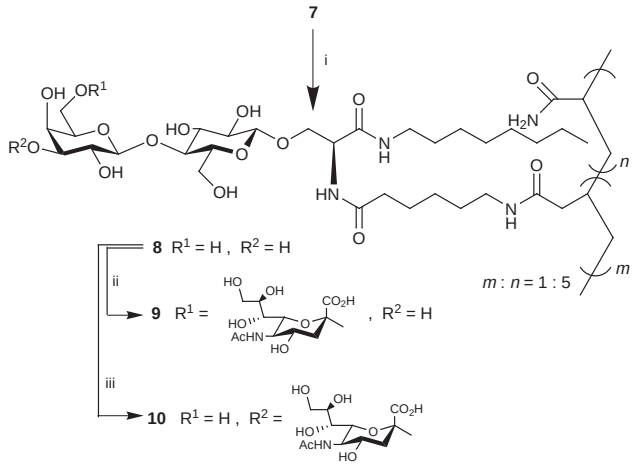
trisaccharide sequence Neu5Acα(2→6)Galβ(1→4)Glc and a fluorescent-labeled lysoGM3 **2** *via* enzymatic manipulations on the polymer support **8**.

Scheme 1 indicates an improved synthetic route to key glycomonomer **7**.[6] The nucleophilicity of the hydroxy group of glycosyl acceptor **4**,† *N′*-octyl-*N*²-diphenylmethylene-L-serinoamide, to the anomeric carbon of a glycosyl donor **5** was drastically enhanced by employing a Schiff base-type protective group[8] at the amino group, and the yield of the glycoside **6**†‡ was improved from 48 to 82%. Removal of the diphenylmethylene group by hydrogenation, subsequent coupling with 6-(*N*-acrylamido)hexanoic acid and *O*-deacetylation proceeded smoothly and afforded compound **7** in 78% yield from **6**. Water-soluble polymer **8** prepared from **7** by the usual radical copolymerisation with acrylamide was employed for further sugar elongation and subsequent transglycosylation reactions, as shown in Scheme 2.

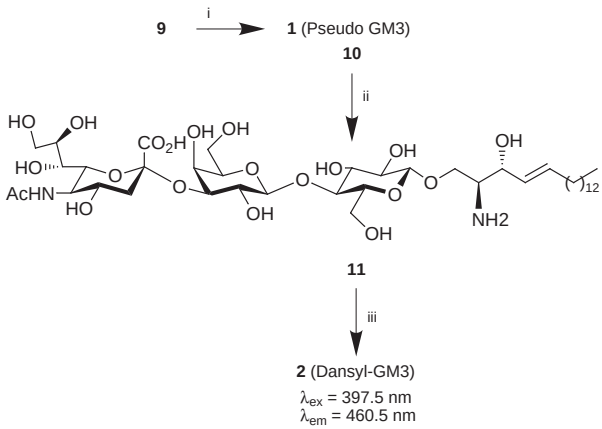
In the previous report,[6] it was demonstrated that rat recombinant α(2→3)-(N)-sialyltransferase (Calbiochem®), known as an enzyme catalysing sialylation reactions of glycoprotein biosynthesis in nature, successfully transferred sialic acid residues to the glycolipid-mimetic polymer **8**. In order to evaluate the versatility of this procedure in other glycosyltransferases, we decided to synthesise non-natural ganglioside isomer **1** (pseudo GM3) utilising rat liver α(2→6)-(N)-sialyltransferase (Boehringer Mannheim). As anticipated, this enzyme also recognised polymer **8** as a 'glycoprotein-mimetic polymer' rather than as 'glycolipid-type clusters'. Novel glycopolymer **9** carrying trisaccharide Neu5Acα-(2→6)Galβ(1→4)Glc was obtained in quantitative yield and this non-natural trisaccharide moiety was subsequently transferred from polymer **9** to C16 ceramide using ceramide glycanase (CGase, Boehringer Mannheim) to afford the target glycolipid **1**† in 60% yield (11 mg) from **9** (Scheme 3). This result shows clearly that CGase exhibits broad substrate



Scheme 1 *Reagents and conditions* : i, Pd-C, H_2 gas, MeOH, 25 °C, 6 h, then $Ph_2C{=}NH$ (1.0 equiv.), CSA (0.1 equiv.), CH_2Cl_2, 25 °C, 24 h, 82.3% from **3**; ii, lactosyl bromide **5** (1.2 equiv.), 4 Å molecular sieves, AgOTf (1.2 equiv.), CH_2Cl_2, 0 °C, 15 h, 82.0% from **4**; iii, Pd-C, H_2 gas, MeOH, 25 °C, 2 h, then 6-(*N*-acrylamido)hexanoic acid (1.1 equiv.), 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (1.1 equiv.), benzene, EtOH, 25 °C, 24 h, 78.0% ; iv, NaOMe (0.4 equiv.), THF, MeOH, 25 °C, 1 h, 100 % from **6**.



Scheme 2 *Reagents and conditions*: i, acrylamide monomer (5.0 equiv.), TMEDA (2.4 equiv.), ammonium persulfate (APS) (0.96 equiv.), DMSO, H_2O, 50 °C, 24 h, 92.0% from **7**; ii, CMP-Neu5Ac (1.2 equiv. for lactose), bovine serum albumin (BSA), calf intestinal alkaline phosphatase (CIAP) (20 units), 50 mM sodium cacodylate buffer (pH 7.40), $MnCl_2$, NaN_3, α-2,6 sialyltransferase (0.1 units, 2.7 munits per 1.0 μmol acceptor), 37 °C, 48 h, 100%; iii, CMP-Neu5Ac (1.2 equiv. for lactose), BSA, CIAP (20 units), 50 mM sodium cacodylate buffer (pH 7.40), $MnCl_2$, Triton CF-54, α-2,3 sialyltransferase (0.3 units, 8.0 munits per 1.0 μmol acceptor), 37 °C, 72 h, 100%.



Scheme 3 *Reagents and conditions* : i, C16 ceramide (5.0 equiv.), 50 mM sodium citrate buffer (pH 6.0), Triton CF-54, ceramide glycanase from leech (0.005 units), 37 °C, 17 h, 60% from **9**; ii, D-sphingosine (5.0 equiv.), 50 mM sodium citrate buffer (pH 6.0), Triton CF-54, ceramide glycanase from leech (0.005 units), 37 °C, 17 h; iii, dansyl chloride (5.0 equiv.), Et_3N (5.0 equiv.), $CHCl_3$, 25 °C, 2 h, 50% from **10**.

specificity of transglycosylation with regard to the carbohydrate structure of glycolipids.

Next, our interest focused on the substrate specificity of the transglycosylation reaction carried out by CGase against glycosyl acceptor substrates,[9] as it will significantly influence the versatility of the present technology in the combinatorial synthesis of 'libraries of glycolipids and their mimetics'. We selected D-sphingosine (*trans*-D-erythro-2-amino-4-octadecene-1,3-diol) as a potential candidate for the synthesis of functional sphingoglycolipid derivatives, since the amino group of D-sphingosine is convenient for further modification. We were pleased to find that CGase transferred the GM3 oligosaccharide [Neu5Acα(2→3)Galβ(1→4)Glc], to the primary hydroxy group of D-sphingosine from the polymer **10** and intermediate **11** was directly dansylated to afford a fluorescent-labelled lysoGM3 **2**† in 50% yield (12 mg) from compound **10**.

In conclusion, we have demonstrated the versatility of water-soluble polymer supports in the enzymatic synthesis of non-natural and biologically important glycolipid derivatives. It should be noted that this synthetic strategy should greatly accelerate efficient combinatorial synthesis of libraries of glycolipids varying both in the carbohydrate and in the lipid portions.

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Notes and references

† *Selected data* for **4**: δ_H(400 MHz, $CDCl_3$) 7.80–7.09 (m, 10 H, 2 × Ph), 6.92 (br s, 1 H, NH), 4.31–3.81 (m, 2 H, β-H), 4.01 (br s, 1 H, α-H), 1.32–1.24 (m, 12 H, 6 × CH_2) and 0.89 (t, 3 H, *J* 6.7, CH_3). For **6**: δ_H(400 MHz, $CDCl_3$) 7.60–7.03 (m, 10 H, 2 × Ph), 5.25 (d, 1 H, *J* 3.4, H-4′), 5.05 (dd, 1 H, *J* 8.4 and 9.0, H-3), 4.84 (dd, 1 H, *J* 3.5 and 9.2, H-2′), 4.81 (dd, 1 H, *J* 3.4 and 9.0, H-3′), 4.79 (dd, 1 H, *J* 7.6 and 9.0, H-2), 4.38 (d, 1 H, *J* 7.6, H-1), 4.32 (d, 1 H, *J* 7.8, H-1′), 4.29 (dd, 1 H, *J* 1.7 and 12.1, H-6a), 4.18 (br d, 1 H, *J* 6.5, α-H), 3.43 (dt, 1 H, *J* 1.7 and 5.2, H-5), 2.27 (s, 2 H, $NHCH_2$), 2.06–1.85 (each s, 21 H, OAc), 1.46–1.18 (m, 12 H, 6 × CH_2) and 0.79 (t, 3 H, *J* 6.7, CH_3). For **1**: δ_H(400 MHz, $CDCl_3$) 5.71 (dt, 1 H, *J* 6.7 and 14.7, Cer-5), 5.46 (dd, 1 H, *J* 7.6 and 14.7, Cer-4), 4.24 (d, 1 H, *J* 7.0, H-1′), 4.23 (d, 1 H, *J* 7.8, H-1), 2.78 (dd, 1 H, *J* 4.2 and 12.0, H-3″$_{eq}$), 2.01 (s, 3 H, NAc) and 0.81 (t, 6 H, *J* 6.7, 2 × CH_3). For **2**: δ_H(400 MHz, $CDCl_3$) 8.58–7.04 (m, 6 H, dansyl), 4.35 (d, 1 H, *J* 7.0, H-1′), 4.20 (d, 1 H, *J* 7.4, H-1), 2.73 (br d, 1 H, *J* 4.3, H-3″$_{eq}$), 1.90 (s, 3 H, NAc) and 0.88 (t, 3 H, CH_3); λ_{ex}/nm 397.5; λ_{em}/nm 460.5.

‡ In the previous report (ref. 6), the coupling reaction of **3** with **5** gave a glycoside intermediate in 48% yield.

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