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Synthesis of Glycopolymers Containing GM_3-Saccharide

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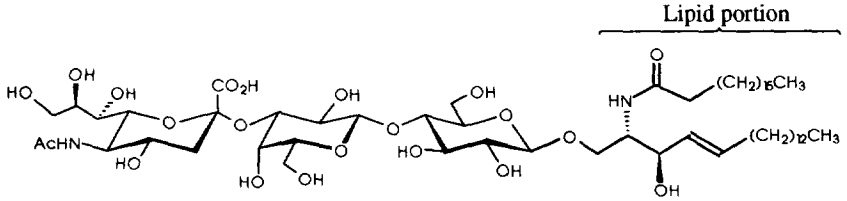
Abstract: GM_3-trisaccharide [Neu5Acα-(2→3)-Galβ-(1→4)-Glcβ-(1→OR)] was synthesized using our "active-latent" thioglycosylation strategy. The trisaccharide was prepared with a *p*-acrylamidophenyl aglycon for copolymerization with acrylamide. The resulting water-soluble sialyl α-(2→3)-lactoside copolyacrylamide with different carbohydrate incorporation was shown to possess antigenic properties. Copyright © 1996 Elsevier Science Ltd

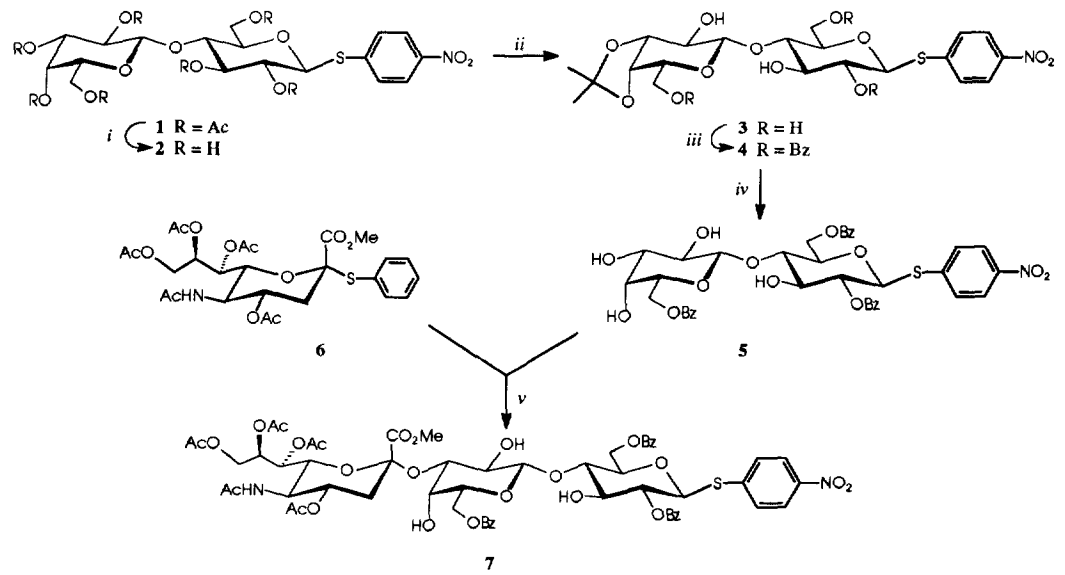
Sialic acid-containing glycoconjugates play critical roles in numerous biological phenomena such as cell-cell adhesion,[1] malignancy,[2] and cell growth regulation.[3] Of concern, and under active investigation, is ganglioside GM_3 which is over expressed on tumor cells.[4] GM_3 is also known to modulate epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) receptors.[5] It is therefore of prime interest to have access to glycoconjugates containing only the trisaccharide portion of the glycolipid in order to evaluate the intrinsic biological function played by the trisaccharide itself. Ideally, the glycoconjugates should be prepared in forms suitable for bio- and immuno-logical investigations. Carbohydrate-containing polymers ("glycopolymers"),[6] because of their high binding affinities to protein receptors,[7] constitute reliable tools for a wide variety of bioassays. Several successful chemo-enzymatic syntheses of ganglioside GM_3 and GM_3-trisaccharide derivatives have been reported.[8] However, there has been no report on the chemical synthesis of water-soluble glycopolymers containing GM_3 trisaccharide units. In addition, most of the reported syntheses of GM_3 trisaccharide lack the necessary anomeric functionality at the reducing glucosyl residue for direct modification into glycoconjugates. Hence, lengthy anomeric group activations-transformations were necessary for useful functionalization.



Structure of Ganglioside GM_3

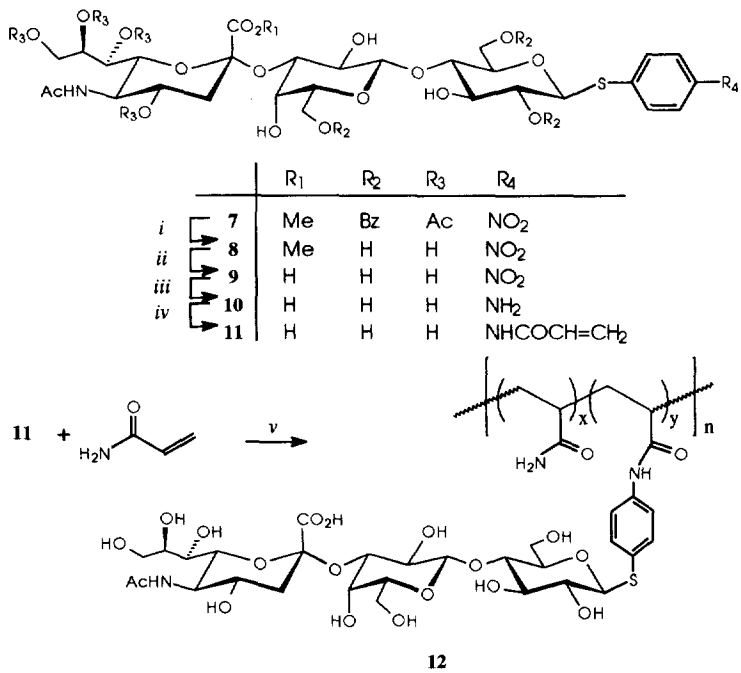
As part of ongoing projects on the syntheses of biologically active glycoconjugates,[6] we describe herein another useful application of our "active-latent" glycosylation strategy[9] for the synthesis of GM_3 trisaccharide containing a polymerizable aglycon. The above strategy makes use of aryl 1-thioglycosides that can act as glycosyl donors or acceptors depending on the electron density of the aryl substituents. For instance, "active" thiosialosyl donor **6** can be chemoselectively activated with suitable thiophilic promotors in the presence of *p*-nitrophenyl thiolactoside **5** acting as "latent" acceptor (Scheme 1). The resulting "latent" thiooligosaccharide (**7**) can be further transformed into an "active" form by a mild reduction-acetylation sequence. This new glycosylation strategy has been very useful in blockwise oligosaccharide synthesis.[10]

Suitably protected lactosyl acceptor **5** was synthesized from readily available *p*-nitrophenyl hepta-*O*-acetyl-1-thio-β-D-lactoside (**1**)[11] obtained under phase transfer catalysis (PTC), according to Scheme 1. Zemplén de-*O*-acetylation (NaOMe, MeOH) of **1** afforded **2** in 98% yield. Treatment of **2** with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid gave 3,4-O-isopropylidene derivative **3** in 80% yield after cleavage of mixed 6'-acetal also formed in the process. Initial attempts of regioselective benzoylation at 2-, 6-, 6'-OH of **3** using benzoyl chloride (3.6 eq.) in pyridine-dichloromethane (1:1, v/v) at -50°C gave a mixture of di-, tri- and tetra-*O*-benzoylated products. However, dibutyltin oxide-mediated regioselective benzoylation afforded the desired *p*-nitrophenyl 2,6,6'-tri-*O*-benzoylated-1-thio-β-D-lactoside derivative **4** in 74% yield. Hydrolysis of isopropylidene group in **4** afforded acceptor **5** quantitatively.



Scheme 1. i) NaOMe, MeOH, 25°C, 45 min, 98%; ii) a. DMP, cat. p-TsOH, 25°C, 25 min; b. MeOH-H_2O (10:1), reflux, 2 h, 80%; iii) a. $(Bu_3Sn)_2O$, $PhCH_3$, reflux, 24 h; b. BzCl, C_5H_5N, 45°C, 24 h, 74%; iv) MeOH, p-TsOH, reflux, quant.; v) NIS/TfOH, CH_3CH_2CN, -60°C, 47%.

Active thiosialyl donor **6** (0.189 mmol, 1.5 equiv.), also prepared under PTC conditions,[9b] was glycosidated with latent acceptor **5** (0.126 mmol) in the presence of *N*-iodosuccinimide (NIS, 0.252 mmol) and triflic acid (TfOH, 0.126 mmol) in propionitrile (5 mL, -60°C, 50 min) to provide partially protected GM_3 trisaccharide **7** in 47% yield. No cross-coupling, nor β-linked trisaccharide by-products were detected under these glycosylation conditions. The α-configuration at the newly introduced anomeric center (NeuAc residue) was confirmed by ^1H- and ^{13}C-NMR spectroscopy which showed H-3e at δ 2.46 ppm ($J_{3e,4}$ = 4.6, $J_{3e,3a}$ = 12.9 Hz) and C-1 at δ 168.1 ppm ($^3J_{C1,H3a}$ = 4.2 Hz).



Scheme 2. i) NaOMe, MeOH, 25°C, quant.; ii) 0.1M aq. NaOH, 25°C, quant.; iii) Zn, AcOH, MeOH, 25°C, 2 h, 90%; iv) CH_2=CHCOCl (1.2 eq), Et_3N, MeOH, 25°C, 30 min, 83%; v) $(NH_4)_2S_2O_8$, H_2O, 90°C, 30 min, 50-70%.

The ester groups of GM_3 trisaccharide **7** were hydrolyzed (NaOMe, MeOH, followed by 0.1 M NaOH) to provide **9** quantitatively. The *p*-nitrophenyl group was then reduced with zinc dust in a mixture of AcOH-MeOH (1:6, v/v) to give *p*-aminophenyl derivative **10** in 90% yield. *N*-acryloylation of **10** (CH_2=CHCOCl, Et_3N, MeOH) afforded **11** in 83% yield.[12] It is worth mentioning that if the reduction/*N*-acryloylation sequence was performed prior to the hydrolysis reactions, extensive conjugate addition (1:2) of methoxide anions was observed.

The monomeric GM_3 precursor **11** was copolymerized ($(NH_4)_2S_2O_8$, 90 °C, 30 min) with acrylamide in deoxygenated water using various molar ratios. GM_3 copolyacrylamides **12** with molar ratios ranging from 1:5, 1:10, 1:24, to 1:48 (^1H-NMR) were obtained in good yields (50-70%). The water-soluble GM_3

copolymers were purified by dialysis and freeze drying. They had molecular weights in the range of 100 kDa based on copolyacrylamide standards.[11] Preliminary binding studies with the plant lectin wheat germ agglutinin showed high binding affinities.

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11. Selected data for **11**: $[\alpha]_D$ -68.2° (c 0.8, D_2O); ^{1}H-NMR (500 MHz, D_2O) δ (ppm): 6.50 (dd, 1H, J_{cis} 10.1, J_{trans} 17.1 Hz, COCH=), 6.41 (dd, 1H, J_{gem} 1.3 Hz, C=CH_2, trans), 5.95 (dd, 1H, C=CH_2, cis), 4.86 (d, 1H, $J_{1,2}$ 9.8 Hz, H-1), 4.59 (d, 1H, $J_{1',2'}$ 7.9 Hz, H- 1'), 2.82 (dd, 1H, $J_{3e''',4'''}$ 4.6, $J_{3e''',3a'''}$ 12.5 Hz, H'''-3e), 2.10 (s, NHAc), 1.86 (t, $J_{3a''',4'''}$ = 11.7 Hz, H-3a'''); ^{13}C-NMR (125.8 MHz, D_2O) δ (ppm): 174.6 ($NCOCH_3$) 173.4 (C-1'''), 166.4 ($COCH=CH_2$), 128.2 (CH=), 127.5 (=CH_2), 102.1 (C-1'), 99.4 (C-2'''), 86.8 (C-1),
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