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# Synthesis of Artifical Glycoconjugate Polymers Carrying Biologically Active Trisaccharides with α-D-Galactopyranosyl (1→3) and (1→4)-Linkage

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**Abstract**

Starting from *p*-nitrophenyl-β-lactoside, two types of neoglycoconjugate carrying either a trisaccharide segment of globotriaosyl ceramide (Gb3) or its regioisomeric Galα1,3Galβ1,4Glc were synthesized via a common intermediate. The former polymer carrying the Gb3 trisaccharide showed strong neutralization activity against Shiga-like toxin-I in the assay using human ACHN cells. 

*Keywords*: polymers; biologically active compounds; glycosidation; regiospecificity

In our continuous efforts to synthesize artificial glycoconjugate polymers, we have reported convenient synthetic ways to incorporate naturally or enzymatically available oligosaccharides into polystyrenes and polyacrylamides[1]. Owing to the multivalent binding or cluster effects[2], these glycoconjugate polymers show higher binding affinity with certain cells or lectin-like proteins than the corresponding monomeric sugars, and they have shown wide applicability in cell biology[3]. In the present study, our interest was focused on the syntheses of glycoconjugate polymers carrying α-D-galactopyranosyl-(1-4)-β-D-galactopyranosyl-(1-4)-D-glucopyranose and its regioisomeric triose, α-D-galactopyranosyl-

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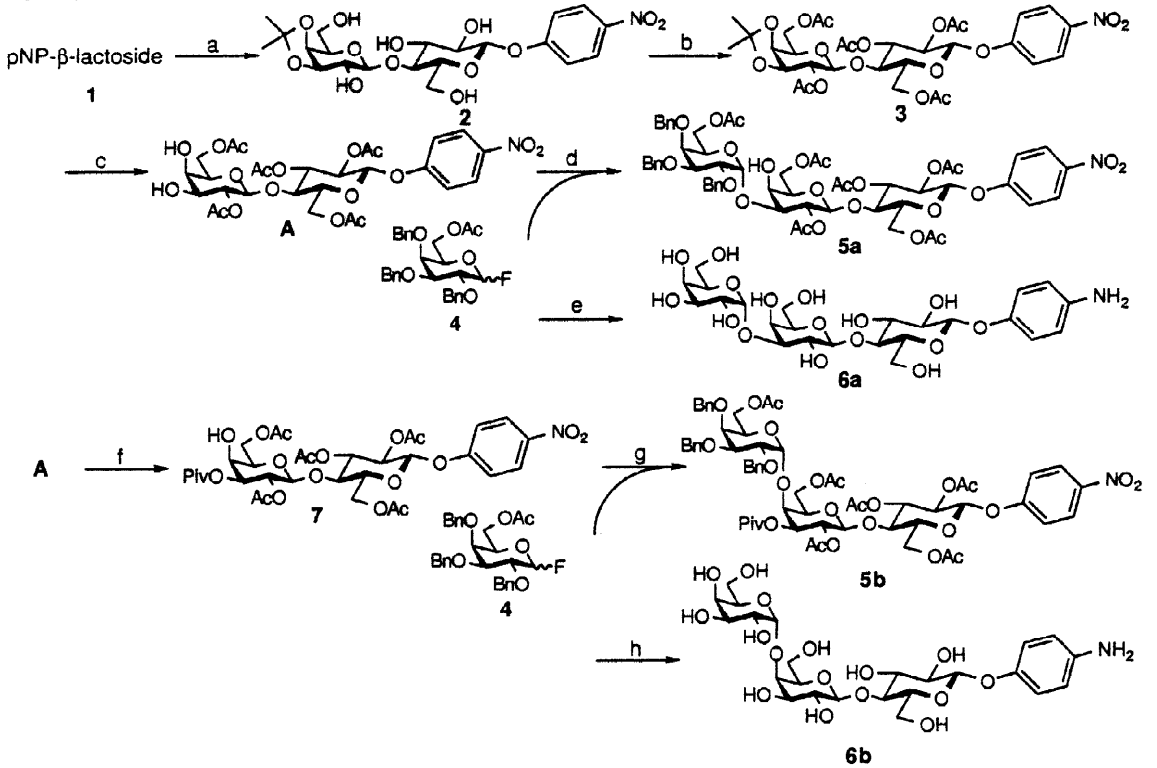
(1-3)-β-D-galactopyranosyl-(1-4)-D-glucopyranose. The former trisaccharide constitutes a globo-series glycosphingolipid, Gb3 ceramide, which is reported to be the ligand of Shiga-like toxins (Verotoxins) of *Escherichia coli* O-157[4]. The latter is an analog of Galα1,3Galβ1,4GlcNAc known as the antigenic triose involved in the pig to human hyperacute xenograft rejections[5] as well as the ligand of pathogenic *Clostridium difficile* toxin (toxin A)[6].

**Scheme 1**

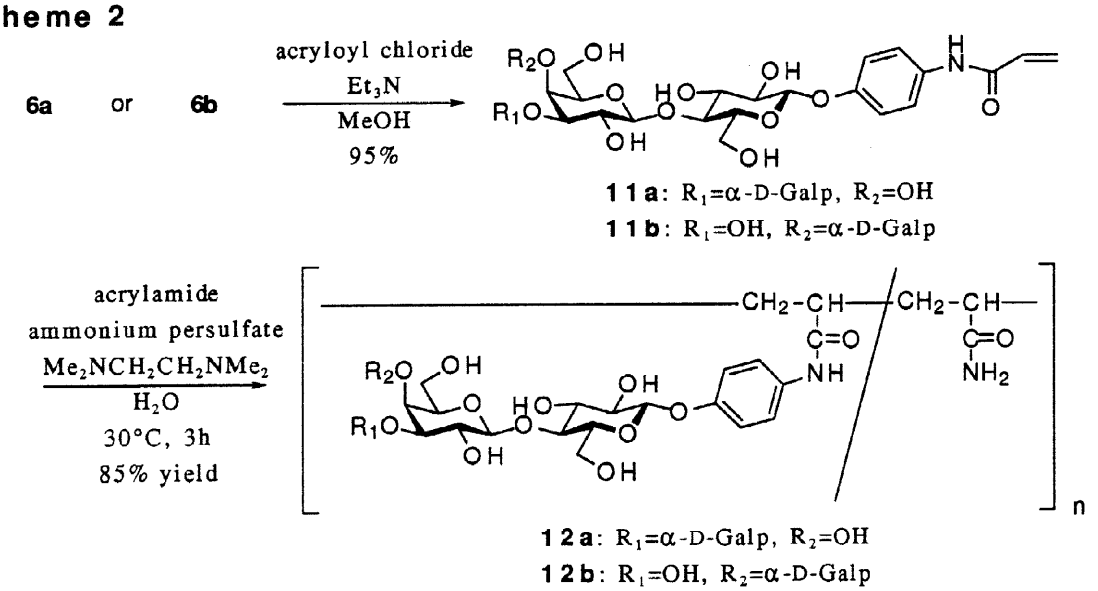



a) TMSCl/ acertone b) $Ac_2O$, pyridine, $Et_3N$, DMAP c) TMSCl, ethylene glycol/ $CH_2Cl_2$, MeOH d) $AgClO_4$, $SnCl_2$, MS4A/ $CH_2Cl_2$ e) 1) NaOMe/ MeOH 2) $Pd(OH)_2/C$, $H_2$, HCl/ MeOH f) PivCl, $Et_3N$, DMAP/ $CH_2Cl_2$ g) $AgClO_4$, $SnCl_2$, MS4A/ $CH_2Cl_2$ h) 1) NaOMe/ MeOH 2) $Pd(OH)_2/C$, $H_2$, HCl/ MeOH

Syntheses of Gb3 ceramide and its analogues have already been accomplished by several groups[7-8]. The reported synthetic methods, designed for the syntheses of glycosyl lipids, could not be applied conveniently for the synthesis of the titled glycoconjugate polymer. In the present study, we adopted a synthetic strategy starting from *p*-nitrophenyl-β-lactoside **1** (pNP-β-lactoside, Scheme 1) since the nitro group is accessible to glycoconjugate polymers after being reduced into an amino group[9]. Chemical manipulations of pNP-glycoside, however, were found to have to be carefully undertaken since the pNP ether group, particularly at the anomeric position, was labile under both basic and acidic conditions employed for the usual benzylation (benzyl bromide, NaH/DMF) and isopropylidenation

(refluxing in dimethoxy propane-acetone, *p*-toluenesulfonic acid) or deisopropylidenation (heating in 70% acetic acid) processes. The restricted reaction conditions led us to utilize the reactions and protecting groups as summarized in Scheme 1. Regioselective 3',4'-*O*-isopropylidenation of **1** was performed using trimethylsilyl chloride (TMSCl) and acetone at room temperature[10] to afford **2** in nearly quantitative yield. After per-*O*-acetylation, de-*O*-isopropylidenation was performed using TMSCl and ethylene glycol[11] in MeOH (room temperature) to afford a key compound **A** as a crystalline solid (74% yield mp= 105°C). Mono-galactosylation on **A** using $SnCl_2$ and $AgClO_4$ as activators and 6-*O*-acetylated glycosyl donor **4**[12] afforded 3'-*O*-α-D-galactosyl lactose **5a** (38% isolated yield, α/β=>99:1, $^1$H-NMR analysis) as the main trisaccharide product, which was also converted into a glycoconjugate polymer (Scheme 2). In order to obtain the desired 4'-*O*-α-D-galactosyl lactose, the OH-3' group of **A** was protected by a pivaloyl group and galactosylated in the same manner as that for **5a**. After purification on silica gel column chromatography, **5b** was obtained in 62% yield (α/β=>99:1, $^1$H-NMR analysis). The de-*O*-acylation of **5a** and **5b** using sodium methoxide in methanol (room temperature, 2 h) followed by hydrogenolysis with palladium hydroxide in methanol gave trisaccharides **6a** and **6b**, respectively, with a terminal amino group as the HCl salt.

**Scheme 2**




The amino group of each trisaccharide was converted into the N-acryloyl amide group (acryloyl chloride, $Et_3N$, MeOH, 0°C→r.t. for 2 h, Scheme 2). The derived acryloyl derivatives **11a** and **11b** were subjected to co-polymerization with acrylamide (feed molar ratio=1:4) in a redox radical manner using ammonium peroxodisulfate and *N, N, N', N'*-tetramethylethylenediamine in water[13]. After being precipitated twice in methanol, dialyzed in water (Mw. 3500 cut-off) and freeze-dried, neoglycoconjugate co-polymers

bearing the Gb3 trisaccharide segment **12b** and its regioisomer **12a** were derived (carbohydrate/acrylamide molar ratio = *ca.* 1 : 5, $^1$H-NMR analysis) as a water-soluble colorless powder.

The neutralization activities of the derived polymers against Shiga-like toxins (I and II) were preliminarily tested using human ACHN cells. The assay showed that the polymer **12b** has strong neutralization activity against Shiga-like toxin-I ($CD_{50}$ = 0.001~0.005 μg/ml) but no activity against Shiga-like toxin-II. The polymer **12a** has no apparent activity for either toxin in the present assay system. The results will be reported in more detail elsewhere.

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