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### Communication: A Facile Synthesis of a Glycoconjugate Cationic Polymer Carrying the 3,6-Branched $\alpha$ -D-Mannosyl Trisaccharide Cluster

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COMMUNICATION

A FACILE SYNTHESIS OF A GLYCOCONJUGATE  
CATIONIC POLYMER CARRYING  
THE 3,6-BRANCHED  $\alpha$ -D-MANNOSYL  
TRISACCHARIDE CLUSTER

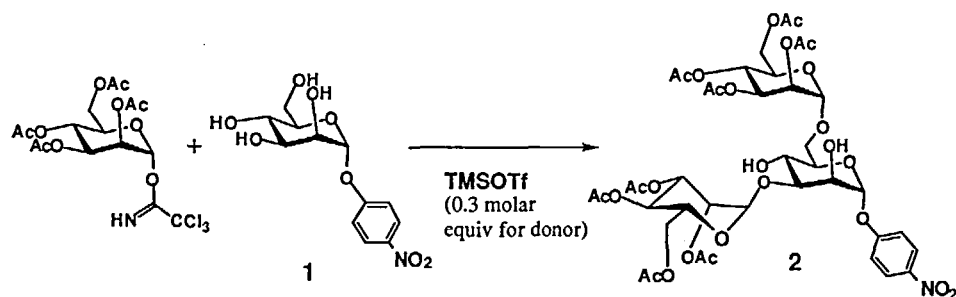
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Receptor-mediated gene transfection<sup>1</sup> has gained central interests since Wu and Wu<sup>2</sup> demonstrated successful gene transfection into hepatocytes by using an asialoglycoprotein-poly(L-lysine) complex as a nonviral gene vector. Besides the hepatoma cells possessing galactose-specific receptor proteins, macrophages bearing mannose-specific receptors can also become a target of the gene transfection.<sup>3-5</sup> Though partially mannosylated poly(L-lysine)<sup>3,4</sup> and mannosylated avidin<sup>5</sup> have been tested for this purpose, development of more effective vectors with higher cytoselectivity is required for gene therapies. As a part of our research efforts to develop and apply artificial glycoconjugate polymers carrying biologically active oligosaccharides,<sup>6-8</sup> our interest has been directed to the potential utility of 3,6-branched  $\alpha$ -D-mannoside for the receptor mediated gene transfer targeting macrophages. The 3,6-branched mannosyl trisaccharide is reported to be the much better ligand of mannose-specific binding proteins than mono- and linear oligomannosides.<sup>9</sup> In this communication, we report a facile synthesis of a glycoconjugate cationic polymer carrying the 3,6-branched  $\alpha$ -D-mannoside cluster<sup>10,11</sup> and evaluate its binding affinity to a mannose-specific lectin, concanavalin A.

Several synthetic approaches have already been reported for the 3,6-branched mannosides,<sup>12-15</sup> though they have not yet been extended to the synthesis of glycoconjugate polymers. Kaur and Hindsgaul<sup>14</sup> and Peretz and Bencomo<sup>15</sup> reported a common way based on random glycosylation for octyl- and 5-azido-3-oxapentyl- $\alpha$ -D-mannopyranosides, respectively. They used per-*O*-acetyl- $\alpha$ -D-mannopyranosyl bromide as the glycosyl donor and mercury (II) cyanide and mercury (II) bromide as the catalysts. We examined a similar but more convenient glycosylation of commercially available *p*-nitrophenyl (*p*NP)  $\alpha$ -D-mannopyranoside using per-*O*-acetyl- $\alpha$ -D-mannopyranosyl imidate<sup>16,17</sup> as the donor (Scheme).



Scheme

Table 1. Products of Random Mannosylation on *p*NP  $\alpha$ -D-Mannopyranoside.

Products <sup>a</sup>	Yields (%) <sup>b</sup>		
	Donor 2 mol equiv.	Donor 3 mol equiv.	
Disaccharides	$\alpha$ 1,3+ $\alpha$ 1,6	17	4
	$\alpha$ 1,3 (6-OAc) <sup>c</sup>	11	8
Trisaccharides	$\alpha$ 1,3 $\alpha$ 1,6	42	30
	$\alpha$ 1,4 $\alpha$ 1,6	11	1
Tetrasaccharides	$\alpha$ 1,3 $\alpha$ 1,4 $\alpha$ 1,6	6	39
Unidentified products		13	18

a. Determined by <sup>1</sup>H NMR spectroscopy.

b. Determined from the HPLC peak area (UV detection at 260 nm, ODS column, elution with CH<sub>3</sub>CN:H<sub>2</sub>O=6:4)

c. Determined by <sup>1</sup>H NMR and FAB-MS spectroscopy

This combination allows us to circumvent the use of the mercury salts as well as to prepare the desired glycoconjugate polymer in a facile manner, since the *p*NP can be converted into a polymerizable group as described below. The *p*NP group also functions

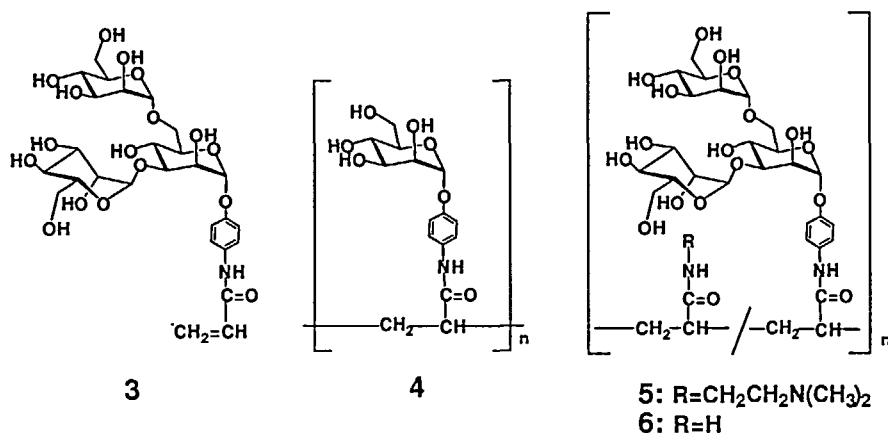
as a UV chromophore useful for the HPLC analysis to quantify the glycosylation products (Table 1).

After the investigation of several reaction conditions that involved changing solvents, Lewis acids, and temperatures, we set the reaction conditions using the imidate (2 equiv), the *p*NP mannoside **1** (1 equiv) and TMSOTf (0.6 equiv)<sup>18</sup> in CH<sub>3</sub>CN at -40 °C to 20 °C. The reaction gave a desired 3,6-branched trisaccharide **2** (42 %) as the major product together with other di-, tri- and tetrasaccharide products. The use of the Lewis acid in less than 0.3 equiv amounts resulted in the increase of orthoester formation, while the amounts more than 1 mol equiv caused a partial cleavage of the *p*NP mannoside linkage. The use of 3 mol equiv of the imidate donor afforded the 3,4,6-tetraoside (39 %) as the major product in addition to the desired **2** (30 %). These results enabled us to determine that the reactivity of OH-groups of **1** was in the order 6-OH,3-OH>4-OH>>2-OH under the present conditions. The poor reactivity at the position 2-OH may arise from the high electronegativity of the adjacent *p*NP group as well as from steric hindrance due to the axial orientation of the 2-OH bond. The 3,6-branched sugar **2** could be purified easily by silica gel column chromatography (chloroform/*tert*-BuOH) and identified from its <sup>1</sup>H NMR spectrum when compared with those of authentic  $\alpha$  and  $\beta$ (1-6)-mannobiosides.<sup>18,19</sup> The FAB-MS spectrum of **2** was consistent with the reported structure.

Catalytic hydrogenation of the *p*NP group [Pd(OH)<sub>2</sub> at room temperature] followed by the *N*-acryloylation in Et<sub>3</sub>N/CH<sub>2</sub>Cl<sub>2</sub> and Zemplen's de-*O*-acetylation gave a polymerizable *N*-acrylamido monomer **3**. Radical copolymerization of **3** with 2-acrylamido-*N,N*-dimethylethylamine (4 mol equiv) as a cationic source was carried out in the presence of *N,N,N',N'*-tetramethylethylenediamine (TMEDA, 10 mol%) and ammonium peroxodisulfate ((NH<sub>4</sub>)<sub>2</sub>S<sub>2</sub>O<sub>8</sub>, 5 mol%)<sup>20,21</sup> in degassed water. The desired polycationic glycoconjugate polymer **5** was obtained in 60 % yield after being reprecipitated from methanol and dialyzed in water. Molar % of the sugar component was estimated to be ca. 20 % by <sup>1</sup>H NMR analysis, and the number average molecular weight (*M*<sub>n</sub>) to be ca. 1.5 × 10<sup>5</sup> by size exclusion column chromatography in water. As a reference polymer to investigate the effect of the cationic species on lectin binding, a similar redox radical copolymerization was carried out with acrylamide to afford a non-cationic glycoconjugate copolymer **6** (molar % of the sugar = 20 %, *M*<sub>n</sub> = ca. 1.8 × 10<sup>5</sup>).

Monomeric and polymeric 3,6-branched  $\alpha$ -D-mannosides, thus derived, were subjected to a lectin binding assay using FITC-labeled concanavalin A (a glucose /mannose binding lectin).<sup>22</sup> The fluorescence assay<sup>23</sup> revealed that the lectin binding is increased in the order *p*NP mannoside < 3,6-branched mannoside monomer < 3,6-branched mannose polymers. The polycationic species in the polymer **5** did not impair

the binding affinity significantly. Thus, this binding assay shows that the polymer **5** is a good candidate as an effective gene vector possessing a macrophage targeting ability.



**Table 2.** Association Constants ( $K_a$ ) of Glycoconjugate Polymers Carrying 3,6-Branched  $\alpha$ -D-Mannoside to FITC-ConA.

Carbohydrates		$K_a \times 10^5$ [ $M^{-1}$ ] <sup>a</sup>
Monomeric	D-Man $\alpha$ -pNP	2
	Man $\alpha$ 1-6Man $\alpha$ -pNP	62
	Man $\alpha$ 1-3	
Polymeric	<b>4</b>	36
	<b>5</b> (polycationic)	340 (2400) <sup>b</sup>
	<b>6</b>	570 (4200) <sup>b</sup>

a. Determined from Stern-Volmer plots for the fluorescence intensity (Ex. 490 nm, Em. 518 nm) against five diluted solutions of each carbohydrate ligand.

b. Determined for another lot of FITC-ConA.

A preliminary test using human cells with mannose receptors has shown that the polycationic polymer can assist the gene internalization quite effectively. Details of that study will be reported in due course elsewhere.

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23. The fluorescence assay showed considerable deviation in the binding constants ( $K_a$ ) values depending on the product number of the commercially available lectin. This may arise from the partial denaturation during the storage or the FITC-labeling. The  $K_a$  values in the parentheses gave the value for more freshly prepared lectin.