# Helical Stereoregular Artificial Glycoconjugate Polymers Based on Poly(phenylacetylene) Backbone: Synthesis and Molecular Recognition with Lectin

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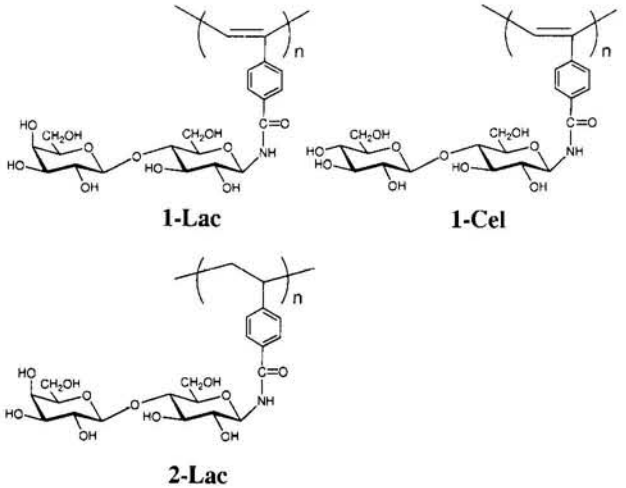
Stereoregular poly(phenylacetylene)s bearing pendant lactose and cellobiose moiety were synthesized by polymerization of $\beta$-*N*-glycosylated 4-ethynylbenzamides with $[Rh(nbd)Cl]_2$ (nbd=norbornadiene) as initiator. The glycoconjugate polymer possessed alternating double bond backbone with *cis-transoidal* regioregularity and helical conformation. Inhibition assay of hemagglutination revealed that galactose-specific lectin efficiently bound to the stereoregular helical glycopolymer bearing lactose, but the binding was not so strong as that of the corresponding flexible glycoconjugate polystyrene.

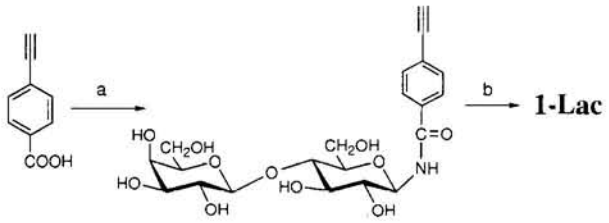
Recently, artificial glycoconjugate polymers, substituted with pendant oligosaccharide moieties as polyvalent recognition signals, have attracted much attention.[1] We also have developed artificial glycoconjugate polystyrenes,[2a,2b] polyacrylamides,[2c] polysaccharides[2d] and polypeptides.[2e] These polymers have been reported to bind strongly to carbohydrate-recognition proteins, toxins, viruses, and cells and utilized as cell-specific culture substrates, artificial antigens, and targeted drug delivery systems.[1,2] Multivalent or clustered saccharide ligands along the polymer backbones are involved in these specific recognition processes and these polymers can be regarded as a model of glycoprotein and glycolipid on membranes. On the other hand, optically active poly(phenylacetylene)s have recently aroused wide interests from synthetic, structural, and functional viewpoints.[3] In this paper, we report synthesis and molecular recognition of a new type artificial glycoconjugate polymer which possesses regioregular helical conformation based on poly(phenylacetylene) backbone. Chart 1 shows the structural unit of poly(phenylacetylene) bearing lactose (**1-Lac**) and



**1-Lac** **1-Cel**

**2-Lac**

**Chart 1.** Chemical structures of glycoconjugate polymers.



**3-Lac**

**Scheme 1.** Reagents and conditions: (a) (i) $SOCl_2$, cat. hydroquinone, 40 °C, 10 h. (ii) lactosylamine, $Na_2CO_3$, THF / methanol / water = 1 / 6 / 1 (v/v/v), rt, 20 h. (b) $[Rh(nbd)Cl]_2$, $Et_3N$, DMSO, 30 °C, 1 h.

cellobiose (**1-Cel**). Interaction of these helical glycopolymers with lectin is compared with the corresponding polystyrene derivative **2-Lac**[2b] which has atactic flexible polymer chain.

Scheme 1 shows the synthesis of the poly(phenylacetylene) bearing *N*-glycosylated disaccharide as the side chain. 4-Ethynylbenzoic acid was prepared according to the literature's procedure[3e] using Heck reaction, and converted to the acid chloride with thionyl chloride. Then the acid chloride was allowed to react with $\beta$-glycosylamines which were prepared by treating lactose and cellobiose with ammonium hydrogen carbonate.[2b,4] $\beta$-*N*-Glycosylated 4-ethynylbenzamide **3** (**Lac** and **Cel**) were obtained in 40~50% yield from 4-ethynylbenzoic acid. The monomers **3** (**Lac** and **Cel**) were polymerized with catalytic amount of $[Rh(nbd)Cl]_2$ in distilled DMSO at room temperature. Reprecipitation and dialysis of the reaction mixture afforded **1-Lac** and **1-Cel** as yellow solid in 60~70% yield. The polymers **1** were soluble in water, DMSO, and DMF. The molecular weight of **1-Lac** was estimated to be $M_n = 5.6 \times 10^5$ ($M_w / M_n = 1.7$) by size exclusion chromatography (SEC) using pullulan as standards and water as eluent. Other transition metal catalyst, $WCl_6$, could polymerize neither nonprotected monomer **3-Lac** in DMSO nor its heptaacetylated monomer in benzene. Copolymerization between **3-Lac** and 2-propyne-1-ol with $[Rh(nbd)Cl]_2$ afforded copolymer **4** with various lactose content (50, 15, and 8 mol%) which was determined by galactoseoxidase-peroxidase assay.[5]

The $^1H$ NMR spectrum (500 MHz) of **1** in DMSO-$d_6$ showed broad singlet peak at 5.7 ppm which was assigned to *cis-transoidal* main chain alkene proton. The *cis* content was estimated to be >95% by the integral curve. Specific optical rotation $[\alpha]_D^{25}$ of polymer **1-Lac** was -56.8° (c = 0.1 g/dL in DMSO), whereas that of monomer **3-Lac** was +31.0° (c = 0.1 g/dL in DMSO). Figure 1 shows the induced CD spectrum of **1** with negative Cotton effect at 350 nm, whereas lactose-carrying polystyrene **2-Lac** gave no induced CD in the wavelength range

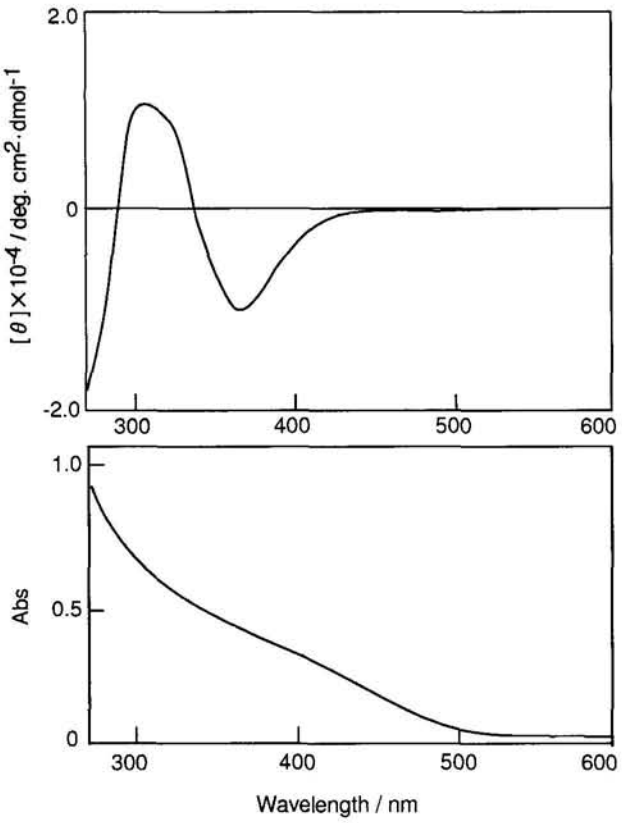



**Figure 1.** CD and UV-Vis spectra of homopolymer **1-Lac** in DMSO at 25°C.

**Table 1.** Inhibition of lectin[a] -induced hemagglutination by glycopolymers and sugars

| Inhibitor | | $IC_{min}$ / M[b] |
|---|---|---|
| Glycopolymer | **1-Lac** | $1 \times 10^{-4}$ |
| | **1-Cel** | N.I.[c] |
| | **2-Lac** | $9 \times 10^{-6}$ |
| Copolymer **4** | Lac 50% | $1 \times 10^{-4}$ |
| | Lac 15% | $8 \times 10^{-5}$ |
| | Lac 8% | $6 \times 10^{-5}$ |
| **3**-Lac | | $3 \times 10^{-3}$ |
| Lactose | | $4 \times 10^{-3}$ |
| Cellobiose | | N.I.[c] |

[a] $RCA_{120}$ (*Ricinus communis* ) lectin was used. [Lectin] = 4 × [Minimum concentration required for hemagglutination] = 7.4 μg/ml.

[b] Minimum inhibition concentration represented by the molarity (mol $L^{-1}$) of oligosaccharide unit.

[c] Not inhibited by 10 mg/ml.

above 200 nm. It is suggested that the *cis-transoidal* glycosylated poly(phenylacetylene)s **1** take stereoregular helical conformation, although the helix sence was not determined from the Cotton effect data of poly(phenylacetylene) derivatives.[3]

Table 1 compares the inhibitory effect of glycopolymer **1-Lac** on hemagglutination of human A-type blood cells by β-galactose specific lectin $RCA_{120}$. The agglutination was inhibited by **1-Lac** but not by **1-Cel**. The minimum inhibition concentration $IC_{min} = 1.3 \times 10^{-4}$ M of **1-Lac** was lower than those of lactose and the acetylene monomer **3-Lac**. The multivalent β-galactose residues of **1-Lac** bound more strongly to the lectin than their monovalent counterparts. However, the affinity of **1-Lac** to the lectin was lower than that of **2-Lac**. The helical cis-transoidal poly(phenylacetylene) backbone was more rigid than the atactic polystyrene backbone. Hence, the multivalent β-galactose ligands of **1-Lac** were too much closely packed to interact with the bindng sites of the lectin. On the other hand, the copolymers **4** with 2-propyne-1-ol showed the higher affinity to the lectin than the homopolymer **1-Lac**. The β-galactose ligands of **4** became more accessible to the lectin, since the ligands were more spaced and the cis-transoidal regularity as well as the rigidity of the polymer backbone were reduced.

In conclusion, we have developed a simple synthetic method of the first stereoregular helical glycopolymers based on poly(phenylacetylene) backbone. Lactose-carrying polymer **1-Lac** was recongnized by $RCA_{120}$ lectin more strongly than the corresponding monomeric **3-Lac**, but less strongly than the corresponding flexible polystyrene **2-Lac**. The increase of recognition due to the multivalent effect was partly compensated by the rigid helical cis-transoidal backbone structure.

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