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# Synthesis, characterization and detection of Concanavalin A based on a mannose-substituted conjugated polymer through aggregation-enhanced FRET

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ABSTRACT

A conjugated polymer (**P1**) with pendant mannose linkages has been designed and synthesized, which is a highly efficient ratiometric probe for Concanavalin A (Con A) detection. **P1** is doped with a low-bandgap fluorophore, porphyrin, which in conjunction with favorable spectral overlap between the poly(fluorene–phenylene) and porphyrin facilitates efficient FRET process in aqueous solutions in presence of Con A. UV/vis spectroscopy and dynamic light scattering studies indicate that **P1** and Con A form into nanoparticles by carbohydrate–lectin interactions. Addition of Con A results in the quenching of the poly(fluorene–phenylene) emission at 456 nm with simultaneous enhancement of porphyrin emission at 625 nm, exhibiting visual emission color change from blue to purple. The intensity ratio of $I_{625}/I_{456}$ is enhanced 9.3-fold when the concentration of Con A reaches 10 μmol/L. Nonspecific proteins including bovine serum albumin (BSA), pepsin (Pep), lysozyme (Lys) show poor fluorescence response, thus highlighting the selectivity and sensitivity of the FRET-based sensing of Con A by **P1**. Moreover, **P1** shows low cytotoxicity by CCK-8 assay, which makes its potential application as fluorescent agent for cellular imaging.



## 1. Introduction

Identification and quantification of specific proteins is an important issue in medical and clinical research as many diseases have a specific change in protein expression. Lectins are sugar-recognition proteins that widely distribute in natural plants and mammalian cells and tissues [1]. Specific sugar–lectin interactions modulate a number of physiological and pathological events. For example, the hemagglutinin and neuraminidase expressed on the influenza viral particle surface can recognize and interact with sialic acid-terminated trisaccharide motifs on the membrane of blood cells and epithelial cells of the upper respiratory trait, leading to virus invasion and spread. As a consequence, the sensitive and selective probing of sugar–lectin interactions could offer substantial insights into the advancement of disease theranostics. In this regard, conjugated polymers (CPs) comprising π-conjugated fluorescent backbones well meet these criteria, providing a unique optical platform for protein sensing [2]. In particular, their large absorption extinction coefficients and rapid intramolecular/intermolecular exciton migration make CPs light-harvesting antennae in optical transduction, ultimately imparting amplified signals and improved sensitivity relative to traditional small fluorophores [3].

To date, most CPs used for protein biosensors generally include two categories of detection mechanisms, namely, fluorescence intensity (one-channel signal) [4–8] and fluorescence ratiometry (generally based on Förster fluorescence resonance energy transfer (FRET), dual-channel signal) [9–11]. Compared with fluorescence intensity, which is significantly influenced by variable factors, such as photobleaching, probe concentration, intracellular microenvironment or light source stability, ratiometry is highly preferred because of its built-in correction property, which could efficiently reduce the influence of the external environment and possibility of false-positive signals. For example, Liu's group reported label-free protein detection by anionic polyfluorene derivatives containing long-wavelength emissive 2,1,3-benzothiadiazole (BT) units by FRET [12–14]. However, the electrostatic interaction lacks specificity, where ionic CPs usually combine nontarget molecules with opposite charges. Moreover, the intrinsic hydrophobic backbones are still prone to aggregation and interaction with hydrophobic substrates, thereby resulting in decreased quantum yield and unexpected nonspecific binding. To overcome these drawbacks, mannose, a protein-recognition sugar [15], was introduced into

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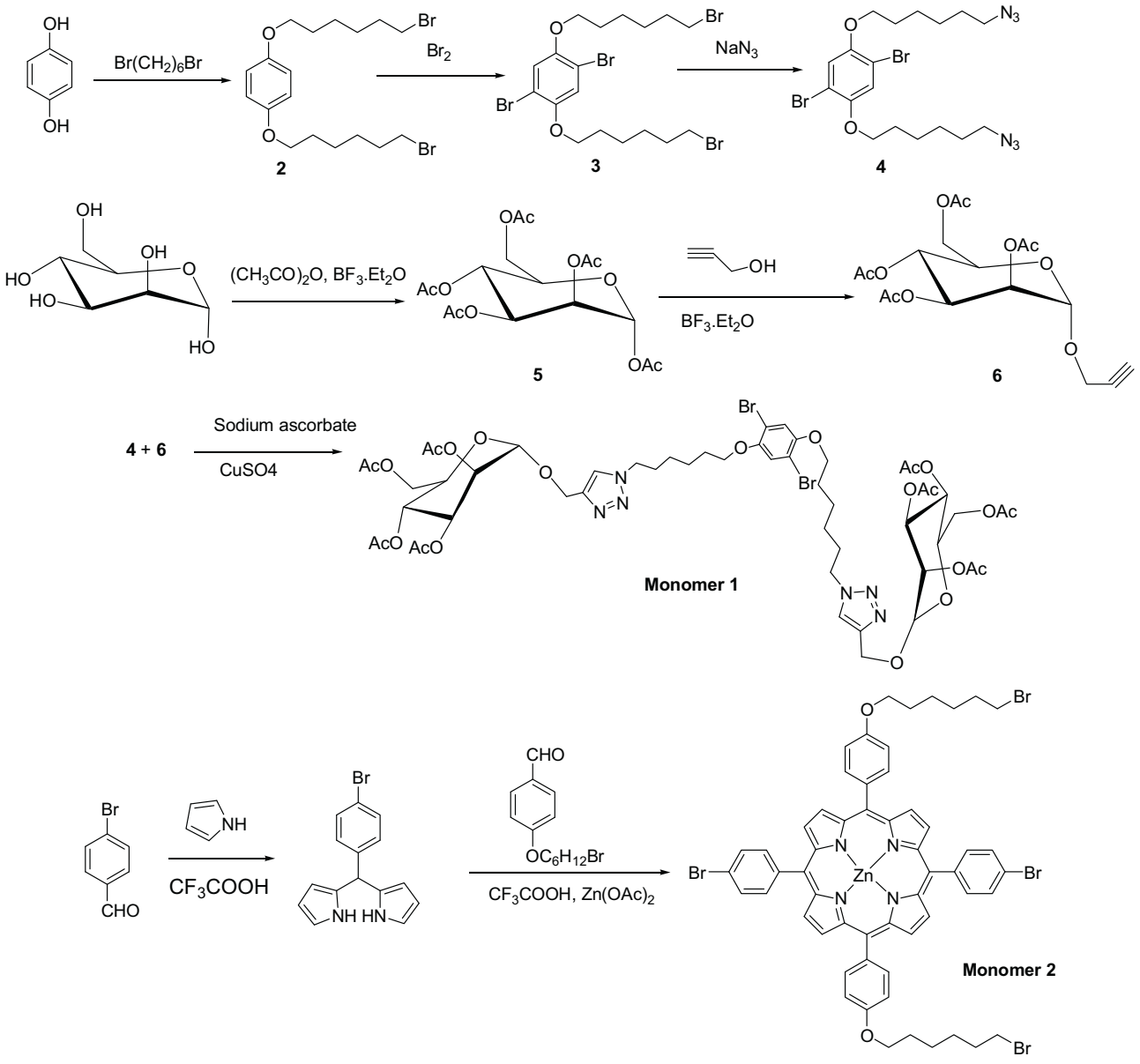


**Scheme 1.** Synthetic routes of **monomers 1** and **2**.

CPs would be desirable. Thus, developing neutral CPs by sugar-recognition with ratiometric signals would achieve superiority in the detection of lectins.

We are particularly interested in developing CPs with energy donor-acceptor backbones for chemical and biological detection [16–18]. In this paper, we report a new design of conjugated polymer (**P1**) containing porphyrin, fluorene and phenylene moieties in the main chain, constructing a FRET system. Low-bandgap fluorophore, porphyrin was introduced into polyfluorene at 5 mol% to generate a pair of ratiometric signals. Considering the high affinity between mannose and Con A, mannose side chains as the recognition groups were used to increase the water solubility, reduce undesirable aggregation, and eliminate nonspecific interactions with biomacromolecules or cells. By virtue of its hydrophobic aromatic backbone and nonionic nature, **P1** should have weak or no interactions with nonspecific proteins. When interaction between mannose and Con A occurred, we anticipated aggregation of the conjugated backbone. So, shortening of intramolecular and intermolecular distances enhanced the energy transfer efficiency between conjugated backbone and low-bandgap groups and resulted in a significant ratiometric change of fluorescence intensity as well as an intuitionistic conversion in the color of fluorescence. Thus, the detection of Con A can be achieved by using **P1**. To study the effect of porphyrin units in CPs, the neutral mannose-substituted conjugated polymer **P0** was also synthesized for comparison (Scheme 1).

## 2. Experimental

### 2.1. Chemicals and instruments

Nuclear magnetic resonance spectra were recorded on Bruker Avance III 400 MHz and chemical shifts are expressed in ppm using TMS as an internal standard. Fourier transform infrared (FTIR) spectra were obtained on an RFX-65A (Analect Co.) spectrometer. The molecular weights of the polymers were determined by gel permeation chromatography (GPC) relative to polystyrene standards, using THF at room temperature at a flow rate of 1.0 mL/min. The