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Probing sugar–lectin recognitions in the near-infrared region using glyco-diketopyrrolopyrrole with aggregation-induced-emission

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ABSTRACT

This study describes the construction of aggregation-induced-emission (AIE)-based glycosyl probes for the sensitive and selective detection of sugar–lectin interactions in the near-infrared (NIR) region. Mannosyl and galactosyl diketopyrrolopyrrole (DPP) derivatives were effectively synthesized by the Cu (I)-catalyzed azide-alkyne 1.3-dipolar cycloaddition reaction. We observed that these glycodyes had typical AIE behaviors in a semi-aqueous solution with a strong fluorescence (FL) emission in the NIR region. In a buffer solution, the glycosyl DPPs at the quenching state showed sharply increased FL upon addition of a selective lectin that recognizes the glycosyl moiety of the compounds with nanomolar limits of detection. In contrast, addition of unselective lectins, proteins and ions did not fluctuate the FL. Scanning electron microscopy analyses suggested that the FL generation could probably be a result of AIE of the glyco-DPPs upon complexation with lectins. These glyco-DPPs, to the best of our knowledge, represent the first fluorogenic AIE-based probes that can sense lectins in the *NIR region*, providing insights for the further extension towards low-background *in vivo* targeted imaging of tissues that express a lectin.

1. Introduction

Lectins are sugar-recognition proteins that widely distribute in natural plants and mammalian cells and tissues (Lis and Sharon, 1998). Specific sugar–lectin interactions modulate a number of physiological and pathological events (Bertozzi and Kiessling, 2001; Hart and Copeland, 2010; Zhang et al., 2013). 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. On the basis of the perturbation of these lectin-sugar interactions, ingenious virus probes (Marin et al., 2013; Lee et al., 2013; Wei et al., 2014) as well as drugs that combat influenza (such as tamiflu) (Magano, 2009) have been developed. Galectins and the asialoglycoprotein receptors (ASGPr) are galactoside-recognition lectins encoded by the human genome. While these lectins are important regulators for maintaining normal cellular functions, recent investigations suggest that they also play key roles in human fatal diseases such as inflammation, cardiopathy, and cancer (Jain et al., 2012; Liu and Rabinovich, 2005). As a consequence, the sensitive and selective probing of sugar–lectin interactions could offer substantial insights into the advancement of basic science as well as disease theranostics.

However, conventional approaches for probing sugar–lectin interactions rely on the use of the immunofluorescence technique, which requires heavy workload for sensor fabrication (surface derivatization and functionalization), long detection time (repetitive blocking and rinsing procedures) and high detection cost (use of expensive antibodies). Although many convenient label-free (no need to label the lectins) solid-phase methods for lectin detection have been developed (Chen et al., 2013; He et al., 2011; Li et al., 2013), they possess some potential problems involving the difficulty in sensor standardization and in laboratorial popularization because of the requirement for heavy detection facilities and the inability for imaging a target *in vivo*.

The aggregation-induced-emission (AIE) is a unique photophysical phenomenon observed by Tang and colleagues, where non-planar fluorogens fluoresce through aggregation, probably due to the restriction of intramolecular rotations (Luo et al., 2001). This mechanism, being completely opposite to the conventional aggregation-caused-quenching, has been actively exploited in the construction of fluorogenic biological probes (Hong et al., 2011). The merit of using these probes lies in the following factors: (1) these molecules are structurally unified, facilitating sensor standardization, (2) they provide a “turn-on” optical signal only

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upon formation of AIE species with the biological target, and (3) they can be used for *in vivo* tissue imaging (Wang et al., 2014; Shi et al., 2013).

1,4-Diketo-3,6-diphenylpyrrolo[3,4-c]pyrrole (DPP) and derivatives have been extensively applied in the construction of fluorescence (Ftouni et al., 2013), two-photon absorption sensors (Guo et al., 2009), polymer solar cells (PSCs) (Kirkus et al., 2013a), OLEDs and field effect transistors (FET) (Kirkus et al., 2013b) because of their brilliant red-fluorescence emission and exceptional light, weather, and heat stability. DPP derivatives functionalized with electron-donor groups can exhibit red to near-infrared (NIR) emission and large Stokes shift that are advantageous in bioapplications.

Considering the polymeric feature of lectins that renders the formation of sugar–lectin complexes with high avidities (Lis and Sharon, 1998; Kiessling and Grim, 2013), multivalent glycosyl AIE probes for lectin detection have been prepared (Shiraishi et al., 2010; Takanobu et al., 2010; Sanji et al., 2009; Hu et al., 2011; Wang et al., 2011). The rationale on which to construct these probes depends on the aggregation of the glyco-AIE dyes with the lectins, producing a strong emissive signal. Nevertheless, the emissions of these probes are mainly in the UV–vis or visible-light region, which largely hampers their extension towards *in vivo* imaging. We report here what is to our knowledge the first glyco-AIE probe that can sensitively and selectively detect lectins in the *near infrared region*. This study might provide insights into the development of chemical tools useful for the target-specific imaging of cells and tissues expressing lectins (Wang et al., 2014; Shi et al., 2013; Ma et al., 2014).

2. Experimental section

2.1. General

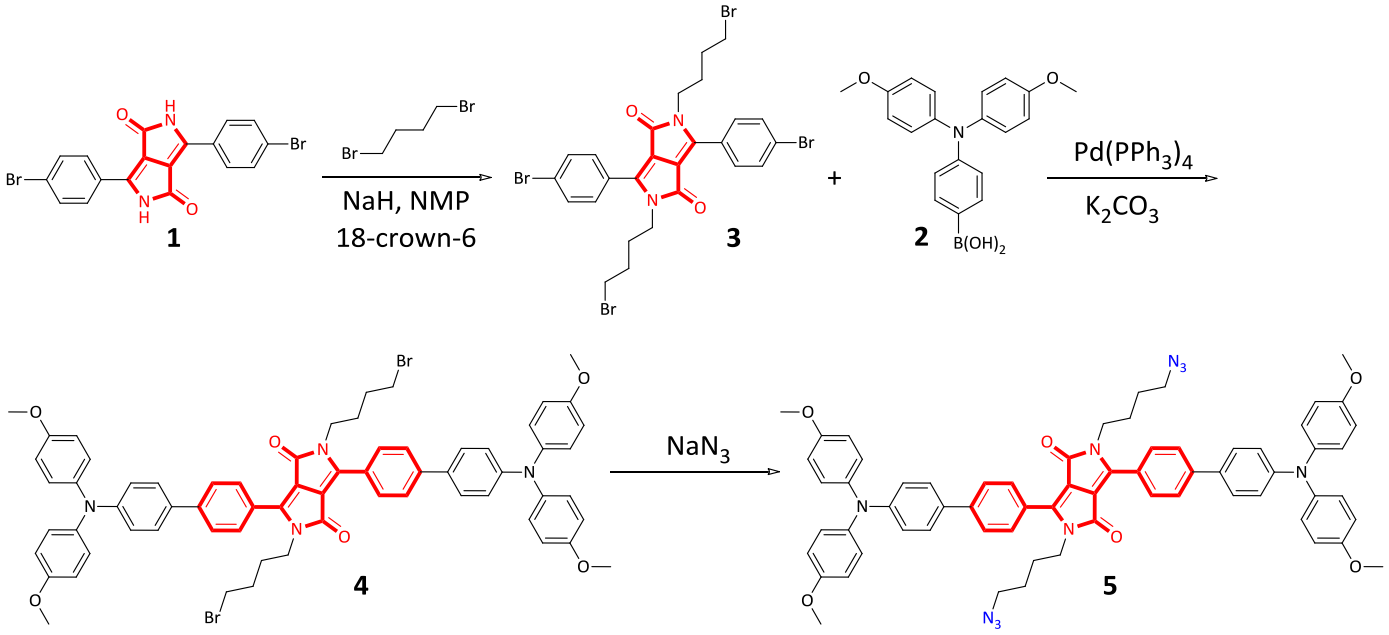
N, *N*-dimethylformamide (DMF) was refluxed with calcium hydride and distilled before use. Tetrahydrofuran (THF) was predried over 4 A° molecular sieves and distilled under an argon atmosphere from sodium benzophenone ketyl immediately before use. 3,6-bis(4-bromophenyl)pyrrolo[3,4-c]pyrrole-1,4(2H,5H)-dione (compound **1**), 4-(bis(4-methox-yphenyl)-amino)phenyl boronic acid (compound **2**) (Wang et al., 2012) and sugar alkynes (compounds **6** and **8**) (Hasegawa et al., 2007) were prepared according to previous literature protocols. All other reagents, proteins and fetal bovine serum were purchased from Sigma-Aldrich and used as received. The solutions for analytical studies were prepared with deionized water treated with a Milli-Q System (Billerica, MA, USA).

2.2. UV–vis and fluorescence (FL) spectra

Stock solutions of **DPPM** and **DPPG** were prepared in dimethyl sulfoxide (DMSO) (1 mM). Aliquots of the stock solution were transferred to 10 mL volumetric flasks. After addition of appropriate amounts of DMSO, water was added dropwise under vigorous stirring to furnish 1.0×10^{-5} M solutions with different water fractions (0–90 vol%). The UV and FL measurements of the resulting solutions were then performed immediately. *For sensitivity measurements,* Stock solutions of **DPPM** and **DPPG** were prepared in DMSO (1 mM), which were diluted to 0.1 mM with DMSO before use. Lectins (concanavalin A (Con A) and lentil agglutinin (LcA) are selective for **DPPM** and peanut agglatinin (PNA) is selective for **DPPG**) were dissolved in a 0.5 mM, pH 7.2 Tris–HCl buffer (containing 50 mM Tris, 0.1 mM Ca^{2+} and 0.1 mM Mn^{2+}).

Fluorescence titrations were carried out by sequentially adding 0, 2.0, 4.0, 6.0, 10.0, 14.0 20.0 and 30.0 μL aliquots of Con A, 0, 2.0, 4.0, 6.0, 10.0, 14.0, 20.0 30.0 and 60.0 μL aliquots of LcA solution or 0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0 and 16.0 μL aliquots of PNA solution to a 10 μL diluted solution of **DPPM** or **DPPG**, followed by addition of an aqueous Tris–HCl buffer to obtain a solution of 1.0 mL. The resulting concentrations of Con A were 0, 1.0, 2.0, 3.0, 5.0, 7.0, 10.0, and 15.0 μM. The resulting concentrations of LcA were 0, 1.0, 2.0, 3.0, 5.0, 7.0, 10.0, 15.0 and 30.0 μM. The resulting concentrations of PNA were 0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 8.0 μM. The mixtures were stirred for half an hour prior to FL measurement.

For calculation of limit of detection (*LOD*), 0.6, 1.2, 1.8, 2.4, 3.0, 3.6 and 4.2 μL aliquots of Con A or LcA solutions were added sequentially to 10 μL diluted solution of **DPPM** followed by addition of an aqueous Tris–HCl buffer to obtain a solution of 1.0 mL. The resulting concentrations of Con A or LcA were 0.3, 0.6, 0.9, 1.2, 1.5, 1.8 and 2.1 μM. The mixtures were stirred for half an hour prior to



Scheme 1. Synthesis of the azido DPP-based intermediate.