Mannose-substituted PPEs detect lectins: A model for Ricin sensing†

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The interaction of a mannose-substituted poly(*para* phenyleneethynylene) (mPPE) with a lectin, Concanavalin A (ConA), is reported; the ConA causes fluorescence quenching of the mPPE with a K_{SV} of 5.6 × 10^5.

Sugar binding proteins, lectins, play a crucial role in cell-surface recognition, cell signalling and pathogen and toxin docking.[1–3] While lectins such as Concanavalin A (ConA) are harmless, Ricin, a toxic protein is perhaps the best known representative of its class, due to a bizarre assassination episode involving a toxin-spiked umbrella.[4] To detect pathogens[5] and toxins[2] on a broader base, it would be of interest to have simple fluorescence sensing methods for Ricin, *Botulinum* toxin, *E. coli* toxin(s), and other lectins of importance.[5] At the moment, lectin–sugar interactions are studied by agglutination of erythrocytes,[1] by surface plasmon resonance studies of carbohydrate-carrying polynorbornene derivatives,[6,7] or by colorimetric reaction with sugar-coated polydiacetylene vesicles.[8,9] We disclose herein the synthesis of the fluorescent mannose-substituted poly(*para* phenyleneethynylene) (mPPE) **5** and its interaction with Concanavalin A (ConA), the lectin of the jack bean. Detection of ConA by fluorescence quenching of the multivalent mannoside **5** is effective and sensitive.

Reaction of **1** with 8-chloro-3,6-dioxaoctanol in the presence of potassium carbonate in DMF furnished the diiodide **2** (see Scheme 1). Coupling of **2** to trimethylsilylacetylene in the presence of a Pd-catalyst gave rise to the formation of the monomer **3** after removal of the trimethylsilyl groups by tetrabutylammonium fluoride in THF. To attach the mannose substituents to the monomer core, **2** was treated with mannose pentaacetate and BF_3-etherate in dichloromethane analogous to a preparation described by van Doren for glycosylation of phenols.[10] The mannosylation of **2** is stereospecific under these conditions *and furnishes **4** as the double α-anomer*. In the last step **3** and **4** are coupled in a piperidine/THF mixture with copper iodide and $(Ph_3P)_2PdCl_2$ to form the PPE **5** in excellent yield and with a high degree of polymerization, according to gel permeation chromatography (yield 95%, P_n = 19, M_n = 22 × 10^3 by ^{1}H NMR; M_n = 62 × 10^3, M_w/M_n = 1.5 by gel permeation chromatography).[11–14] The nucleophilic solvent, piperidine, leads to the convenient *in situ* stripping of the acetate groups and the deacetylated polymer **5** is directly obtained. For a monomeric model, **4** was coupled under standard conditions to 4-methoxyphenyl acetylene; **6** formed in excellent yield after washing with an ethyl acetate–hexane mixture (see Scheme 2). As for **5**, the acetate groups are removed by piperidine in the course of the reaction.

† Electronic Supplementary Information (ESI) available: Synthesis of polymer **5** and model compound **6**, and details of the quenching experiments. See http://www.rsc.org/suppdata/cc/b4/b416587j/

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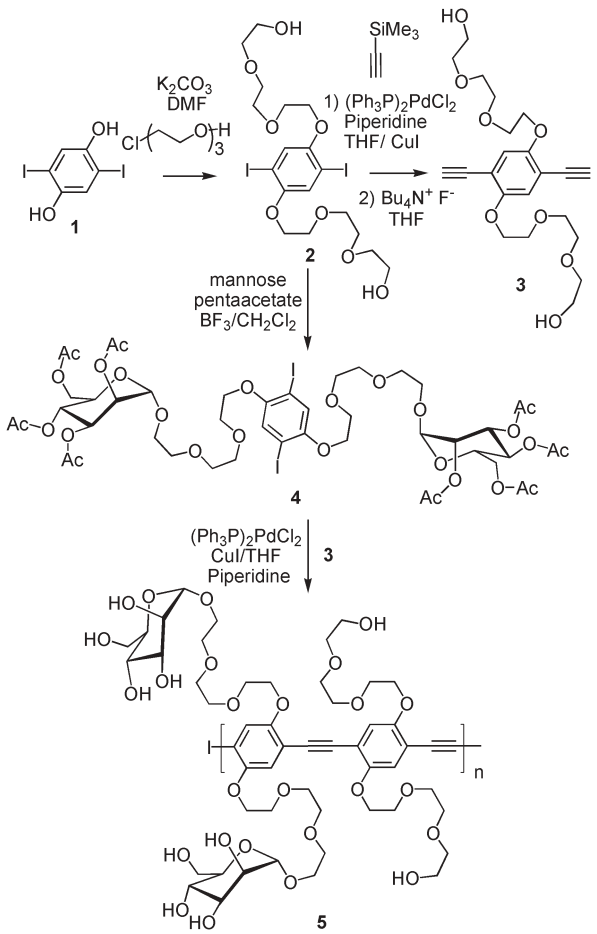
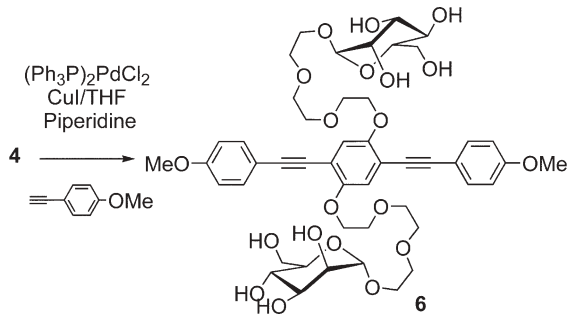
An aqueous solution of **6** was exposed to ConA but no distinct change in the fluorescent properties of **6** were observed, suggesting only weak binding of **6** to ConA. When an aqueous solution of polymer **5** in phosphate buffer was exposed to ConA, efficient fluorescence quenching occured at low concentrations of the lectin. The Stern–Volmer relationship[15]

$$(F_0/F_{[Q]}) = 1 + K_{SV}\,[Q] \text{ or } K_{SV} = \{(F_0/F_{[Q]}) - 1\}/[Q]$$

quantitatively correlates the loss of fluorescence ($F_0/F_{[Q]}$) with the concentration [Q] of added quencher. The slope of the graph of [Q] *vs.* ($F_0/F_{[Q]}$) is the Stern–Volmer constant. There are broadly two mechanisms for quenching of fluorophores: a static and a dynamic one. In dynamic (collisional) quenching, the excited state of the fluorophore forms a complex with the quencher, and the excited



Scheme 1 Synthesis of the mannose-substituted polymer **5** by Pd-catalyzed coupling of **3** to **4**.



Scheme 2 Synthesis of the mannose-substituted model compound **6** by Pd-catalyzed coupling of methoxyphenylacetylene to **4**.

state is quenched. In static quenching the ground state of the fluorophore forms a complex with the quencher, and K_{SV} represents the stability constant of the ground state complex. For PPEs as fluorophores static quenching is prevalent.[16–18] The short (0.3 ns) emissive lifetime of PPEs[12,16,17] makes dynamic quenching of PPEs difficult; K_{SV} here therefore equals the constant of complex formation between PPE **5** and the quencher, ConA.

Fig. 1 shows fluorescence spectra and Stern–Volmer plot of the exposure of **5** to ConA. The quenching of **5**'s fluorescence is linear at low ConA concentrations (Fig. 1 inset), but deviates from linearity at higher quencher concentrations. The K_{SV} based on the linear part of the curve is 5.6×10^6; **5** binds tightly to ConA. A control experiment with bovine serum albumin shows no quenching of the fluorescence and therefore no unspecific binding of **5** occurs; neither does the galactose-binding lectin jacalin (see ESI†) elicit a response, showing that **5** *is a specific sensor for mannose binding lectins*. The interaction of ConA with **5** leads finally to the precipitation of the complex. We examined the aggregates of ConA and **5** by transmission electron microscopy. Fig. 2 shows the spherical fluorescent aggregates that are approximately 300–500 nm in diameter. To make the assay more sensitive we induced aggregation of **5** with biotinylated ConA (Fig. 3) and find a similar fluorescence quenching as in Fig. 1. Upon addition of commercially available streptavidin-coated

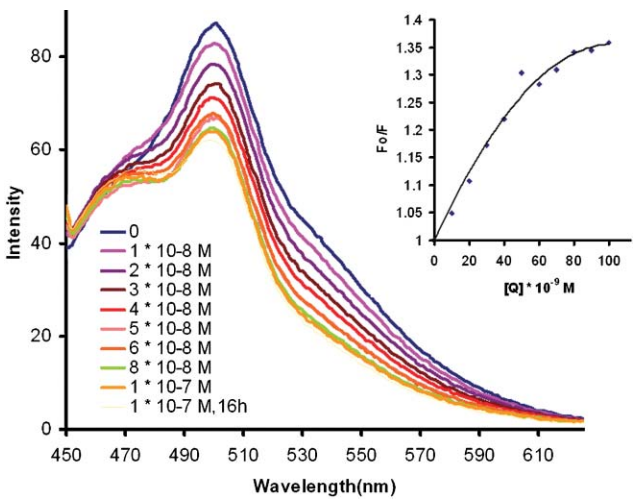


Fig. 1 Emission spectrum and Stern–Volmer plot (inset) of **5** in the presence of ConA.

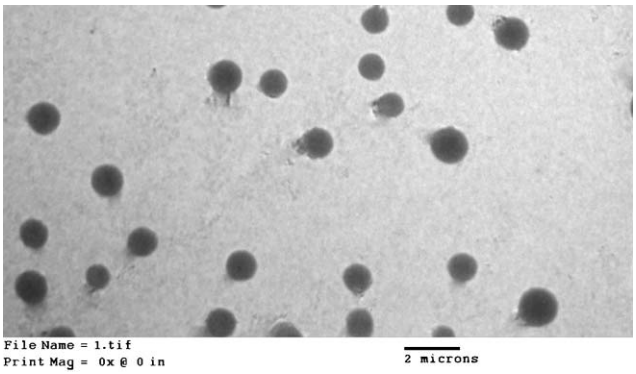


Fig. 2 Aggregates of **5** and ConA shown in the TEM.

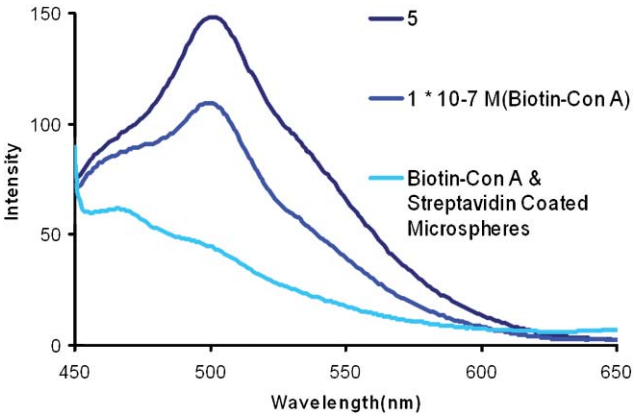


Fig. 3 Fluorescence of aggregates of **5** and biotinylated ConA before and after addition of streptavidin-coated microspheres.

polystyrene spheres, however, the fluorescence decreases significantly further, by precipitating the ConA·**5**-complex through the formation of a super-aggregate. The formation of the super-aggregate is important, because it significantly enhances the sensitivity of the assay. At the moment we are exposing solutions of **5** and its biotinylated congener to a mannose-binding strain of *E. coli* to examine the agglutination of bacteria by **5**.

In conclusion we have demonstrated that PPE **5** is an excellent fluorescent biosensor for lectins and multivalent interactions can be exploited in this scheme to obtain high sensitivities for lectin sensing by sugar-substituted PPEs, particularly when using the formation of a super-complex. Our results complement a recent study[19] that utilizes a postfunctionalization route to sugar-coated carboxy-substituted PPEs to sense ConA and *E. coli*. Our approach uses well-defined and anomerically pure building blocks and avoids problems such as partial functionalization and introduction of defects that are common in postfunctionalization schemes. The polymer **5** is well characterized by NMR and IR and is easily available on a 300-mg-scale.

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