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2,7-Bis(1*H*-pyrrol-2-yl)ethynyl-1,8-naphthyridine: An Ultrasensitive Fluorescent Probe for Glucopyranoside

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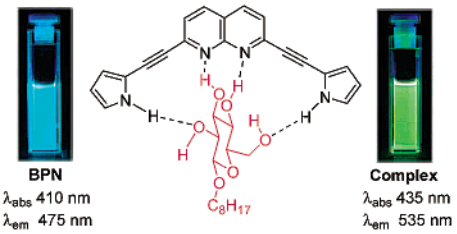
ABSTRACT



A push−pull conjugated molecule, 2,7-bis(1*H*-pyrrol-2-yl)ethynyl-1,8-naphthyridine (BPN), has been designed to bind selectively with octyl glucopyranoside (OGU). The BPN/OGU quadruple hydrogen-bonding complex adopts a rigid BPN conformation in which the *proton donor (d) and acceptor (a)* relays (*daad*) are resonantly conjugated through the ethynyl bridge, inducing π-electron delocalization, i.e., a charge transfer effect. The excellent photophysical properties make BPN a highly sensitive probe for monitoring glucopyranoside to a detection limit of ∼100 pM.

The hydrogen-bonding recognition for carbohydrates[1,2] affords an advantage of geometrical flexibility over that of the covalent type.[3] Multiple hydrogen bonding interactions provide a general method for the protein-carbohydrate recognition, in which most water molecules are often excluded from the cleft of the protein in order to avoid their possible hydrogen-bonding competition. Thus, many artificial receptors have been designed to mimic biotic carbohydrate recognition by using hydrogen bondings in aprotic solvents.[1b] Unfortunately, the detecting method generally relies on ^{1}H NMR chemical shift changes and hence is limited in its sensitivity. To circumvent this problem, fluorescence spectroscopic and circular dichroic methods have been utilized as the detection tools in several examples.[4] We report here

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a novel saccharide probe, 2,7-bis(l*H*-pyrrol-2-yl)ethynyl-l,8-naphthyridine (BPN, Figure 1).[5] BPN exhibits a unique

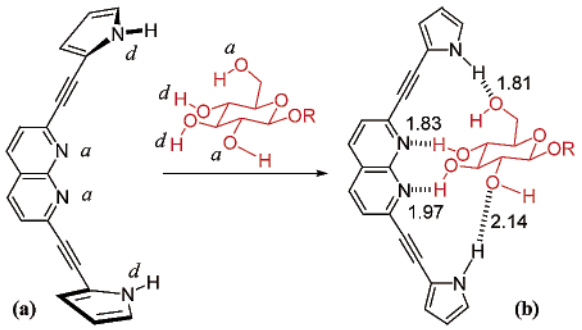
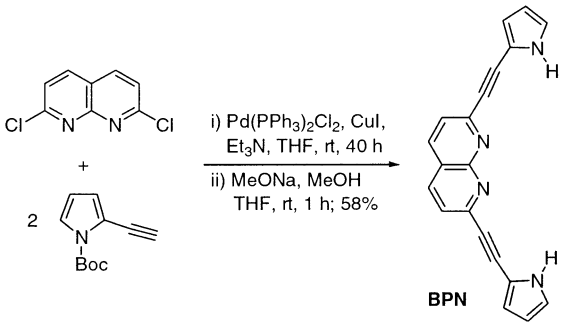


Figure 1. (a) Free BPN showing the conjugated *daad* array with two pyrrole rings slightly tilted toward opposite directions. (b) BPN and its associated BPN/β-D-glucoside hydrogen-bonding complex. The geometrically optimized complex (R = CH_3) derived by the B3LYP/6-31G** method shows the distances (Å) of the hydrogen bonds.

property among the hydrogen-bonding type of artificial receptors in which the conjugated *daad* array acts intrinsically as a saccharide receptor as well as a sensing chromophore. As an alternative to the extensively studied boronic acid receptors,[3] we brought noncovalent recognition and direct visual detection of carbohydrate into an integral system. The new concept evokes monosaccharide as an *adda* molecule complementary to the *daad* array of BPN to form a complex with quadruple hydrogen bondings.

Synthesis of BPN incorporated the coupling reaction of 2,7-dichloro-1,8-naphthyridine with 2 equiv of (1-*tert*-butoxycarbonyl-pyrrol-2-yl)acetylene, followed by removal of the Boc protective group (Scheme 1). Two pyrrole rings

Scheme 1. Synthetic of 2,7-Bis(1*H*-pyrrol-2-yl)ethynyl-1,8-naphthyridine (BPN)



in BPN are slightly tilted toward opposite directions (X-ray analysis, Figure 2), but reorganize to a rigid inward conformation upon binding with a chairlike pyranoside (Figure 1). The pyrrole and naphthyridine moieties function as the proton donor (*d*) and acceptor (*a*), respectively, and also develop an ideal V-shaped cleft to provide as many as

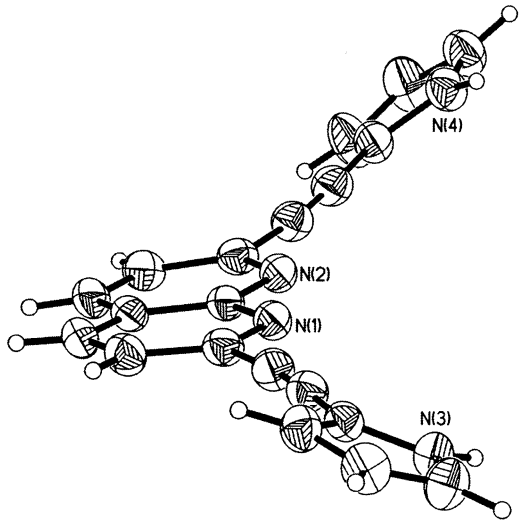


Figure 2. ORTEP drawing of BPN (side view).

four hydrogen-bonding sites. Through the multiple hydrogen bonds effect the unusual push−pull *daad* relay conjugated through the ethynyl bridge induces the π-electron delocalization.[7b] Furthermore, rigidification upon formation of the BPN/pyranoside complex might also contribute a role in enhanced fluorescence, as shown in previous studies on the bindings of ions[6] and carbohydrates.[7] In combination, ultrasensitive detection can thus be achieved via the remarkable change of the electronic spectroscopy.

Upon adding octyl β-D-glucopyranoside (OGU), the 410-nm free BPN band (log $\epsilon \approx 4.65$) in CH_2Cl_2 solution decreased significantly along with the growth of a new absorption band at ~435 nm (Figure 3). An isosbestic point at ~415 nm throughout the titration was attributed to the formation of BPN/OGU hydrogen-bonding complexes in equilibrium with free BPN. The measured absorbance [$A_0/(A - A_0)$] as a function of the inverse of OGU concentrations fit a linear relationship, indicating the 1:1 stoichiometry of the BPN/OGU complex.[8] On the basis of 1:1 stoichiometry of BPN/OGU, the relationship between the measured ab-

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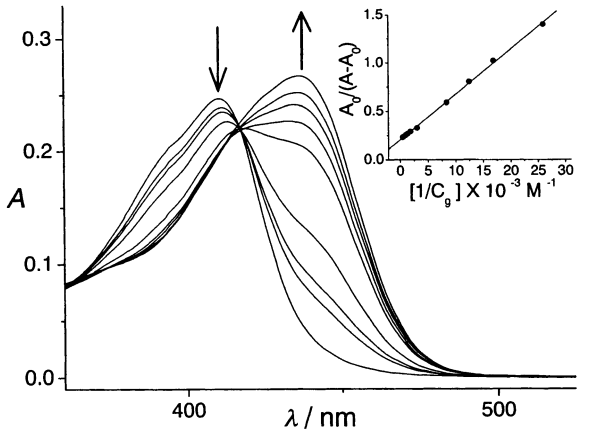

Figure 3. Absorption spectra of BPN (5.4×10^{-6} M) in CH_2Cl_2 by adding various concentrations (C_g) of octyl glucopyranoside. Insert: the plot at 435 nm shows a linear relationship of $[A_0/(A - A_0)]$ vs $1/C_g$, indicating the 1:1 stoichiometry of BPN/OGU.

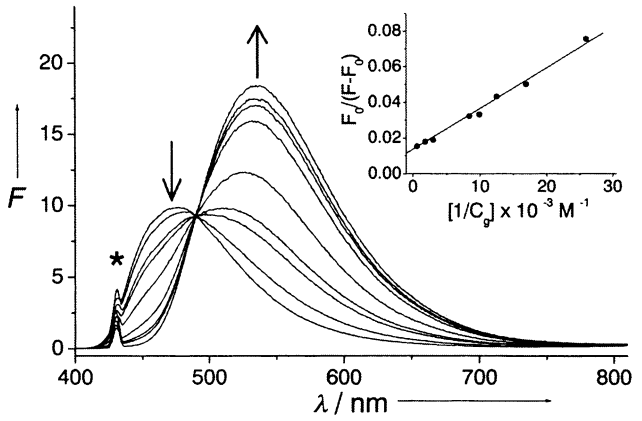

Figure 4. Fluorescence spectra of BPN (1.2×10^{-5} M) in CH_2Cl_2 by adding various concentrations (C_g) of octyl glucopyranoside. The fluorescence spectra ($\lambda_{ex} = 430$ nm) show the growth of a 535-nm emission band on complexation, accompanied by decrease of the 475-nm band for uncomplexed BPN. $*$ denotes Raleigh scattering. Insert: the plot at 535 nm shows a linear relationship of $[F_0/(F - F_0)]$ vs $1/C_g$, indicating the 1:1 stoichiometry of BPN/OGU.

sorbance A as a function of the added OGU concentration, C_g, can be expressed by $A_0/(A - A_0) = [\epsilon_M/(\epsilon_C - \epsilon_M)](K_a^{-1}C_g^{-1} + 1)$ where ϵ_M and ϵ_C are molar extinction coefficients of the BPN monomer and hydrogen-bonding complex, respectively, at a selected wavelength.[8] A_0 denotes the absorbance of the free BPN at that specific wavelength. The ratio for the intercept versus slope deduced the association constant K_a of $(4.8 \pm 0.8) \times 10^3$ M^{-1}.

Dual spectral features were observed in the fluorescence titration study. The characteristic uncomplexed BPN emission band at 475 nm ($\tau_f \approx 1.25$ ns) decreased, accompanied by the growth of a 535-nm ($\tau_f \approx 0.95$ ns) emission band. The results in combination with different excitation spectra between monitoring at, e.g., 450 and 550 nm led us to conclude that dual fluorescence originates from different ground-state precursors, namely, the uncomplexed BPN and BPN/OGU complex, respectively (Figure 4). The plot of $[F_0/(F - F_0)]$ at 535 nm versus the inverse of OGU concentrations reconfirmed a 1:1 BPN/OGU complex.[8] The relationship between the measured fluorescence intensity F and C_g in a selected wavelength can be expressed by $F_0/(F - F_0) = [\Phi_M\epsilon_M/(\Phi_M\epsilon_M - \Phi_C\epsilon_C)](K_a^{-1}C_g^{-1} + 1)$, where F_0 denotes the fluorescence intensity of free BPN. Φ_M and Φ_C are fluorescence quantum yields of the free BPN and complex, respectively, and are assumed to be constant throughout the titration.[8] The ratio for the intercept versus slope gave a K_a value of $(5.5 \pm 0.5) \times 10^3$ M^{-1}, which within experimental error is consistent with the value deduced from the absorption titration.

The fluorescence yields for BPN (i.e., the 475 nm band) and the BPN/OGU complex (i.e., the 535-nm band) were measured to be as high as 0.35 ± 0.01 and 0.23 ± 0.02 in CH_2Cl_2, respectively. By selecting the excitation wavelength at >460 nm where the absorbance was solely attributed to the BPN/OGU complex, a detection limit as low as ~100 pM OGU could be achieved on the basis of the nearly background (i.e., the uncomplexed BPN)-free 535-nm emission. The ratiometric fluorescence proved to be a reliable and ultrasensitive method for the real time detection of BPN/OGU complexation.

The 1:1 BPN/OGU complexation was further supported by the NMR study using a continuous variation method (Job plot),[9] in which the chemical shift changes of pyrrol-NH as a function of sugar concentrations were monitored. The temperature-dependent (285−315 K) ^{1}H NMR study indicated that the formation of the BPN/OGU complex was thermodynamically favored ($\Delta G_{300} = -5.79$ kcal/mol), in agreement with the proposed quadruple hydrogen bond formation. The association constants and thermodynamic parameters are collected in Table 1. A molecular modeling of BPN/methyl β-D-glucoside complex (Figure 1b) implied

Table 1. Association Constants and Thermodynamic Parameters for the Complexes of BPN with Octyl Glucoside (OGU), Octyl Galactoside (OGA), and Octyl Furanoside (OFU)

complex	K_a (M^{-1})	ΔH_{300} (kcal/mol)	ΔS_{300} (cal/mol)	ΔG_{300} (kcal/mol)
BPN/OGU	5000[a] (20000)[b]	−12.3[c]	−21.8[c]	−5.79[c]
BPN/OGA	1600[a] (6200)[b]			−5.21[d]
BPN/OFU	190[a] (490)[b]			−3.69[d]

[a] The association constant was derived from the absorption and fluorescence titrations in CH_2Cl_2 solution. Estimated errors are within ±20%. [b] The association constant was derived from the ^{1}N MR titrations in $CDCl_3$ solution. [c] The thermodynamic data were deduced from the temperature-dependent ^{1}H NMR studies. $\Delta G = \Delta H - T\Delta S$. [d] The value was derived from $\Delta G = -RT \ln K_a$ (in $CDCl_3$ solution).

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the importance of multiple hydrogen bond formation as well as the geometrical fitness to accommodate each hydrogen bond. All calculated OH···N and NH···O distances are significantly shorter than the sum of van der Waals radii of H and N (2.7 Å) and H and O (2.6 Å).

The intrinsic conjugated *daad* type chromophore[2d,j,k,10] in BPN is of key importance to account for the remarkable spectral differences. The acidity of pyrrole and basicity of naphthyridine could be enhanced at the excited state through the conjugated dual hydrogen-bonding effect.[8] The complexation with saccharide thus provides multiple hydrogen bond induced charge transfer to account for a drastic alternation on the fluorescent property. To support this viewpoint, a nonconjugated *daad* molecule of 2,7-bis(3-hydroxy-3-methylbutynyl)-1,8-naphthyridine (BHN) was prepared (Figure 5). Upon forming the BHN/OGU complex (K_a ≈ 1000 M^{-1} measured by ^1H NMR in $CDCl_3$) the fluorescence intensity (λ_{max} 387 nm) did increase, albeit no significant spectral shift was observed in either absorption or fluorescence spectra. The enhanced fluorescence could be rationalized by the conformational rigidification, whereas the lack of a continuous push−pull π-system prohibits the multiple hydrogen bond induced charge-transfer effect.

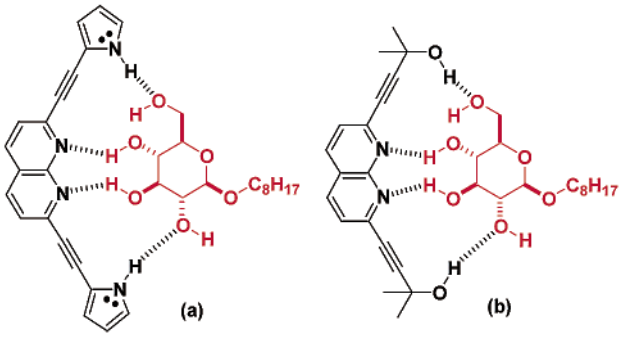

Figure 5. (a) A large Stoke shift (60 nm) in the fluorescence spectrum of BPN/OGU complex is attributed to the multiple hydrogen bond induced charge transfer. (b) Complexation of BHN with OGU does not show significant spectral shift because BHN lacks a continuous push−pull π-system to account for charge transfer.

The preferable 1:1 complexation of BPN with octyl β-D-galactopyranoside (OGA) or octyl β-L-fucopyranoside (OFU) was similarly determined by absorption, fluorescence, and ^1H NMR titrations (see Table 1 and Supporting Information). While the only structural difference between OGA and OGU is the spatial orientation of 4-OH, the association constant for OGA is smaller than that of OGU by ca. 3-fold. This binding selectivity of BPN/OGU over BPN/OGA is comparable or superior to those reported in the literature.[2,4e] For OFU in which one hydrogen bond is eliminated by modifying the C(6)CH_2OH to CH_3 group, K_a decreases by a factor of ~35 in comparison with that of the BPN/OGU complex.

In summary, our results demonstrate the selective affinity and ultrasensitivity for probing the BPN/OGU complex. Visual change of free BPN (cyanic color) in CH_2Cl_2 (1.2×10^{-5} M) to green color upon addition of octyl glucopyranoside (5.2×10^{-4} M) was obvious (see Abstract graphic). Since the synthesis of BPN was straightforward, chemical modification including the preparation of water-soluble derivatives would be feasible. We are currently engaged in the research of such aspects in order to develop practical carbohydrate sensors.

Acknowledgment. We thank the National Science Council for financial support.

Supporting Information Available: Detailed experimental procedures, absorption, fluorescence, and ^1H NMR spectra, molecular calculations, and X-ray diffraction data. This material is available free of charge via the Internet at http://pubs.acs.org.

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