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Fluorescent Nano-Assembly of Organic Conjugate Molecules with Benzoxazole Moiety and Its Application in Sensor

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Fluorescence labeling or sensing is a very useful analytical technique for investigating the structure of living cells or for detecting ionic metals. Numerous materials including inorganic quantum dots and organic fluorescent dyes have been used and investigated for further development so far. However, they are inherited natural discrepancies of cyto-toxicity or easy degradation. Thus, developing highly emissive, biocompatible, and chemically readily modifiable luminescent materials is strongly desired. Here, we report the enhanced photoluminescence of an oxazole derivative for possible use in the field of fluorescent sensor.

Keywords: Fluorescence Sensor, Nano Aggregate, ESIPT, Benzoxazole, Zinc.

1. INTRODUCTION

Nitrogen-containing heterocycles have been known to haved b high electron mobility and ability to support a number of charge carriers.¹ Among those heterocyclic molecules, the compounds having the hydroxyphenyl-benzoxazole (HBO) unit have an interesting behavior of the excited state intramolecular proton transfer (ESIPT) upon electromagnetic wave excitation.²⁻⁴ ESIPT has received considerable attention due to the characteristic four-level photo-physical state transition between the two different tautomers. Based on this unique property, ESIPT molecules are gaining interest for potential application in organic light emitting diodes, chemosensor, photostabilizer, and etc.

Detection and quantification of transition metal, especially, Zn^{2+} is crucial to human health. Zinc is very important in biological processes. Failure to maintain zinc level causes in a number of severe neurological diseases such as Alzheimer's disease, hypoxia ischemia, and certain Parkinson's disease.^{5,6} Therefore, highly selective and sensitive detection of Zn^{2+} is of toxicological and environmental concern.

Here, we report a new fluorescent molecule and demonstrate fluorescence sensor application.

2. EXPERIMENTAL DETAILS

2.1. Materials and Characterization

All chemical materials were purchased from Sigma Aldrich and used without further purification.

1P: 94.158.22.47 On: **Tue** 25 Dec 2018 12:35:22 H nuclear magnetic resonance (NMR) and ¹³C NMR have been known to have y to support a number of terocyclic molecules, the nenyl-benzoxazole (HBO) of the excited state intra-T) upon electromagnetic ceived considerable attenlevel photo-physical state **25** Dec 2018 12:35:22 H nuclear magnetic resonance (NMR) and ¹³C NMR spectra were obtained in CDCl₃ at 400 MHz and 100 MHz on a Bruker Avance 500. High resolution mass spectra were obtained with HP5890 series II. Elemental analysis was carried with Thermo Electron Flash EA 1112. UV/Vis absorption spectra were recorded on a Varian Cary50 spectrometer. Luminescence spectra were collected on a Photon Technology International QM4 spectrometer with a xenon lamp as a light source.

2.2. Synthesis

2.2.1. 2-(benzoxazole-2-yl)-4-Bromophenol (1)

2-Aminophenol (23.0 mmol) and 5-bromosalicylic acid (23.0 mmol) were added in 30 ml of poly-phosphoric acid and the mixture was heated to 130 °C and stirred for 4 h. After cooling to room temperature, the reaction mixture was precipitated in ice-water, filtered and washed with DI water. The product was recrystallized from acetic acid and dried in vacuo. 400 MHz ¹H NMR (CDCl₃): δ 7.06 (*d*, 1H), 7.44 (*m*, 2H), 7.53 (*d*, 1H), 7.65 (*m*, 1H), 7.77 (*m*, 1H), 8.18 (*d*, 1H), 11.50 (*s*, 1H).

2.2.2. Tert-Butyl-2-(benzoxazole-2-yl)-4-Bromoph-Enyl Carbonate (2)

1 (2.76 mmol) is dissolved in dry, distilled tetrahydrofuran (THF) with di-tert-butyldicarbon-ate and (4-dimethylamino)pyridine (5 mol%). When the reaction appeared complete by TLC, the solution was concentrated

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Figure 1. Synthetic scheme of DBO.

and precipitated in DI water, washed with ethanol. The product was dried in vacuo. 400 MHz ¹H NMR (CDCl₃): δ 1.63 (*s*, 9H), 7.04 (*d*, 1H), 7.42 (*m*, 2H), 7.51 (*d*, 1H), 7.62 (*m*, 1H), 7.75 (*m*, 1H), 8.16 (*d*, 1H).

2.2.3. 4,4'-(9,9-dihexyl-9H-fluorene-2,7-diyl) Bis(2-(benzo[d]oxazol-2-yl)phenol) (DBO)

2 (0.8 mmol) and 9,9-dihexylfluorene-2,7-di-boronic acid (0.4 mmol), palladium catalyst (5 mol%) are placed in a two-necked round bottom flask charged with 7 ml of THF under argon. 1 M Na₂CO₃ solution 4 ml is added and stirred for 48 h at 80 °C. After cooling, the reaction mixture was poured into methanol. The precipitate is isolated by filtration and washed with DI water and methanol and dried under vacuo.

Above synthesized oligomer is dissolved in chloroform (3 ml) and trifluoroacetic acid (2 ml) was added. After stirring for 12 h, solvents was removed by evaporation and dried under vacuo. The compound, DBO, was purified by chromatography and recrystallization to yield yellow needle shape crystals (yield 81%). ¹H NMR(400 MHz, CDCl₃): δ 0.80 (*m*, 10H), 1.13 (*m*, 12H), 2.13 (*m*, 4H), 7.26 (*d*, 4H), 7.41–7.48 (*m*, 4H), 7.72–7.60 (*m*, 6H), 7.77–86 (*m*, 6H), 8.36 (*s*, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 14.1, 22.8, 24.5, 30, 31.8, 44, 53.7, 110.4, 123.8, 124.7, 127.3, 127.4, 127.9, 128.9, 129, 129.1, 130.6, 136.6, 139.5, 141.2, 141.8, 148.6, 150.5, 162.9; Anal. Calcd. for: C₅₁H₄₈N₂O₄: C, 81.35; H, 6.43; N, 3.72 Found C, 81.31; H, 6.41; N, 3.70. HRMS (FAB): calculated *m/z* 752.36; observed *m/z* 752.39.

3. RESULTS AND DISCUSSION

UV/Vis absorption spectrum of 10^{-5} M DBO in THF solution has its maximum absorption at 332 nm as shown

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Figure 2. UV/vis absorption (left) and fluorescence intensity (right) spectrum of DBO 10^{-5} M THF solution (black lines), DBO dispersion (blue broken lines), and its solid film (red dots).

in Figure 2, having slight hypsochomic shift from that of the monomer 1, whose absorption maxima is 340 nm attributed to the syn-enol structure.³ Halogen atom of 1 extends conjugation length of benzoxazole to yield a slight red-shift by about 8 nm.⁷ The photoluminescence spectrum of DBO solution has emission at the wavelength of 527 nm. This large Stokes' shift over 190 nm is caused by the ESIPT phenomena of the enol-keto stautomerization.^{2,3,8} The relative quantum efficiency (Φ) of DBO 10⁻⁵ M THF solution was 0.08.

It is worth to point out that the photo-luminescence of the fluorene is suppressed, whose usual photophysical emission at ~410 nm.¹¹ One possible explanation is the radiationless decay pathway increase due from the energy loss via vibrational or rotational motions of the molecules.^{2–4,10} The other culprit would be that the excited spin density is popular not on the center fluorene, but on the benzoxazole pendant groups. From the density functional theory calculation using B3LYP/6-311G+(d),¹¹ excited state dipoles have been moved toward the pendant benzoxazole groups to excite enol moieties rather than to excite the fluorene group.

The torsional angle of ca $\sim 39^{\circ}$ between the main fluorene and the benzoxozole play a key role to reduce $\pi - \pi$ interaction and thus, widens the radiative decay pathway. As a result, huge increase in the fluorescence is observed for the solid film and dispersion of DBO by more than 4 times from its solution (black solid lines) as shown in Figure 2 (red dots and blue dashes) with relative quantum efficiency of 0.42. Moreover, DBO solid film has another small Stokes' shift at ~ 430 nm, since the vibration restriction of the phenyl rings and the hydrocharbon side chains give birth to radiative decay of the center fluorene groups leading structural vibrations. This emission color change is further utilized to detect ions and further application onto biosensor has been performed.



Figure 3. UV/vis absorption (left) and PL (right) spectra of 10^{-5} M DBO THF solution (black solid lines) upon addition of 10 equiv. metal ions of Zn²⁺(red dots), Fe²⁺ (black broken lines), and Cu²⁺. (Blue dash-dot-dash).

Spectroscopic analysis was performed by adding 10 equivalent amounts of cations onto 10^{-5} M DBO THF solutions. Absorption changes after addition of metal cations onto DBO solution are not significant. Cu²⁺ or Fe²⁺ ions show just a small hump at 400 nm in Figure 3. The solution turned to pale-yellow due to a new absorption band at ~400 nm.¹² Meanwhile, their photo-luminescence intensity decreased, but no spectral change is observed. The lack of a spectral change indicates a fluorescence transition is from an identical excited state regardless of the presence of metal cations. Thus, the formation of new absorption species prohibits radiation decay pathway, such as intermolecular packing or π - π * quenching.

To the contrary, added Zn^{2+} ions move the absorption spectrum by 7 nm to longer wavelength, suggesting extended conjugation length. Fluorescence spectrum shows significant increase with a spectral blue-shift. The photoluminescence response of DBO is different from the usual ESIPT process and displays ratiometric responses.^{13, 14} The weak fluorescence of DBO in the absence of Zn^{2+} might be attributed to irradiative channels from the $n-\pi^*$ state.¹⁵ In the presence of Zn²⁺ that coordinates with the lone pair of the oxygen, the energy of the $n-\pi^*$ state would be raised so that the $\pi - \pi^*$ state becomes the lowest excited state, leading a substantial increase in the fluorescence intensity.^{16,17} In addition, Zn²⁺ chelating to DBO could not only induce a conformation restriction¹⁸ but also block photoinduced electron transfer quenching of the singlet excited state of benzoxazole moiety, thus, a big fluorescence enhancement could be observed.¹⁹

It is very interesting for solid DBO to have a bright fluorescence as the radiative pathway increase upon vibration restriction. As organic fluorescence nanoparticle possessing fine optical properties will be applied in the field of the immunofluorescence labeling. DBO/THF solution



Figure 4. (A) Illustration of selective fluorescence labeling with DBO nanoparticles. (B) Fluorescence microscopy image after selective binding of biotinylated DBO-PCDA nanoparticles on the patterned avidin surface (diameters are 500 μ m). (C) A fluorescence image of DBO THF solution, DBO-PCDA nanoparticle dispersion, and DBO solid film. (D) A SEM image of DBO-PCDA nano-assembly diameter of ~100 nm.

is injected to the pentacosadiynoic acid (PCDA) vesicles of diameter of ~100 nm with green emission as seen on Figure 4(C).³ As expected, synthesized DBO nano-assembly has the fascinating optical property. Generally, organic nanoparticles are poor fluorescent materials because of self-quenching between absorption and their emission. These highly emissive and highly photo-stable organic nanoparticles have the possible application in the immunofluorescence labeling as shown in Figure 4.

4. CONCLUSION

Highly emissive photo-stable organic materials have many applications in the bio-imaging. New benzoxazole molecule was synthesized and its photophysical behavior has been studied. DBO shows peculiar ESIPT mechanism and fluorescence enhancement upon aggregation, whose solid state quantum yield of 42%. Further fluorescent labeling and metal ion, especially Zn²⁺ cation sensing have been demonstrated with the nanoparticles assembled with functionalized diacetylene molecules for application in bio-imaging.

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