Facile fabrication of a red AIE luminogen with simple structure and bright emissions for cell imaging

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Abstract. Fluorescence imaging in red region is of great interest in both practical application and fundamental research. However, the complicated synthetic approaches and low emission efficiency of the fluorescence materials are still implement their biological applications. Herein, a facile synthesis method for a red luminogen with aggregation-induced emission (AIE) is reported. The AIE probe possesses simple structure and high red brightness, favoring its applications toward in vitro and in vivo imaging. This study offers an ideal architecture for the construction of red AIEgens with a simple structure.

1. Introduction

Fluorescence imaging, which relies on emission color and quantum efficiency from fluorophores, presents a new method for real-time monitoring of specific biological processes and complicated molecular events. \cite{1-3} Efficient red fluorescent probes with maximum emission above 620 nm are promising candidates for fluorescence imaging, owing to their merits of overcoming the interferential drawbacks of biological absorption, light scattering, autofluorescence and so on. \cite{4-6} However, conventional fluorescence bioprobes usually exhibit low emission efficiency resulting from the aggregation-caused quenching (ACQ) effect in the aggregated state, which severely limited their practical applications. \cite{7} In contrast to ACQ effect, aggregation-induced emission (AIE) phenomenon reported by Tang group, which is non-emissive or weakly emissive in solutions but are induced to florescent intensely in aggregates, can promote the fluorescence quantum efficiency in solid state. \cite{8} The compounds with AIE-active nature exhibit an intrinsic capability to be used perfectly at high concentrations or in aggregation state with bright fluorescence and a high photobleaching threshold, which is favourable for wide-ranging imaging application. \cite{9} However, the complicated synthetic approaches for red AIEgens still remain a challenging task to implement these applications. Therefore, constructing efficient AIEgens with simple structures and low-energy emissions is attractive and useful.\cite{10}

Herein, fluorescence luminogen with excellent AIE nature and high emission brightness in the solid state is prepared by facile synthesis routes. The designed AIEgen, namely DBP are successfully utilized as a bioprobes in cell imaging with high photostability and brightness.
2. Experimental section

Scheme. 1. Synthesis route of the DBP. (I) Pd(OAc)₂, t-Bu₃P, NaOBu-t and PhMe. (II) Pd(dppf)Cl₂, KOAc and 1,4-dioxane. (III) Pd(PPh₃)₂, K₂CO₃ and THF/H₂O.

2.1 General Information

A 10 mM PBS buffer solution with pH of 7.4 was prepared with double distilled (deionized) water. The photophysical characteristics including photoluminescence and absorption spectra of DBP in dimethyl sulfoxide (DMSO) were performed on the F-4600 fluorescent spectrophotometer and Cary 50 UV-Vis-NIR spectrophotometer, respectively. The NMR characteristics of intermediates and final product were carried out on the Varian NMR spectrometer.

2.2 Synthesis

2.2.1 Synthesis of 7-bromo-N,N-diphenylbenzo[c][1,2,5]thiadiazol-4-amine (Br-NSPh)

A mixture including diphenylamine (1.80 g, 10.68 mmol), 4,7-dibromobenzo[c][1,2,5]-thiadiazole (3.14 g, 10.68 mmol), Pd(OAc)₂ (0.06 g, 0.26 mmol), t-Bu₃P (1.1 g, 5.34 mmol) and NaOBu-t (1.30 g, 17.36 mmol) in 20 mL toluene (PhMe) was heated to reflux under nitrogen atmosphere for 12 hours. Then the mixture was extracted with dichloromethane (DCM) and organic phase was dried over anhydrous Na₂SO₄. The solvent was concentrated under reduced pressure. The concentrate was purified by silica gel column chromatography with DCM and petroleum ether (PE) (1:5, v/v) to obtain the orange powder with a yield of 60%. ¹H NMR (600 MHz, DMSO-d₆) δ 7.92 (d, J = 7.8 Hz, 1H), 7.28 (t, J = 7.2 Hz, 2H), 7.10-7.05 (m, 3H), 7.02-6.98 (m, 4H).

2.2.2 Synthesis of DBB

The solution composing Pd(dppf)Cl₂ (0.15 g, 0.2 mmol), Br-NSPh (0.76 g, 2.0 mmol), bis(pinacolato)diboron (0.75 g, 3.0 mmol) and potassium acetate (0.59 g, 5.0 mmol) in 50 mL 1,4-dioxane was heated to 115°C under nitrogen atmosphere for 12 hours. After that, the mixture was extracted with DCM for three times and dried with anhydrous Na₂SO₄. The filtrate was removed to leave a white powdered form containing DBB, a portion of which was used in the next reaction without purification.

2.2.3 Synthesis of DBP

Under nitrogen atmosphere, the mixture of DDB (0.86 g, 2.0 mmol), Pd(PPh₃)₃ (0.29 g, 0.3 mmol), 2-bromopyridine (0.63 g, 4.0 mmol) and K₂CO₃ (3.45 g, 25.0 mmol) in 40 mL THF/water (3:1, v/v) was reacted at 65°C for 12 hours. After that, the mixture was extracted with DCM for three times, and the organic solution was dried over anhydrous Na₂SO₄ as well as concentrated under reduced pressure. The concentrate was purified by silica gel column chromatography with DCM and PE (1:1, v/v) to obtain the red solid with a yield of 45%. ¹H NMR (600 MHz, DMSO-d₆) δ 8.74-8.71 (m, 2H), 8.47 (d, J = 8.8Hz, 1H), 7.98 (t, J = 7.8 Hz, 1H), 7.42-7.41 (m, 1H), 7.35-7.27 (m, 5H), 7.10 (t, J = 7.2 Hz, 2H), 7.07-7.01 (m, 4H). ¹³C NMR (125 MHz, DMSO-d₆) δ 154.34, 153.63, 151.41, 150.10, 147.64, 140.45, 137.27, 130.68, 129.89, 126.54, 124.51, 124.43, 124.07, 123.54, 123.23.

2.2.4 Cell culture and fluorescence imaging

A549 cells were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) and 10% fetal bovine serum (FBS) with 5% CO₂ at 37°C. For imaging studies, HeLa cells (1 × 10⁴ cells/well) incubated on culture dishes for 24 hours. The DBP (5 μM) was added and was incubated with A549 cells for 30 min.
Then the culture dishes were washed with PBS for three times, and imaging experiments were performed on a EVOS M5000 fluorescence microscope (Thermo Fisher).

Fig. 1 $^1$H NMR spectrum of DBP in DMSO (top) and $^{13}$C NMR spectrum of DBP in DMSO (bottom).

2.3 Characterization and photophysical properties of DBP

DBP was simply obtained with three simple couplings reaction and characterized by NMR in details. The diphenylamine segment of DBP is employed as an electron donor (D), while pyridine-based benzothiadiazole is acted as electron acceptors (A), as shown in Fig. 2A. Meanwhile, the rotation of diphenylamine can endow the molecule with AIE properties. The PL spectrum of DBP in solid state exhibit red emission with peak at 626 nm (Fig. 2B). In addition, the intense absorption at 479 nm with a molar extinction coefficient of $0.89 \times 10^4$ M$^{-1}$ cm$^{-1}$ can be attributed to charge transfer state.(Fig. 2C).

Fig. 2. (A) Molecular structure of DBP. (B) The PL spectrum of DBP powder. (C) The absorption curve of DBP in DMSO.
The AIE properties of DBP were examined in DMSO/water solution system. The PL spectra of DBP molecule in different water fractions ($f_w$) were shown in Fig. 3. In DMSO solution, the PL spectra of DBP exhibited weak emission with the maximum peak at 623 nm. With the increasing water fractions up to 50%, the emission of DBP was reduced a little along with the red-shifted peaks, which probably due to the TICT property.[11, 12] While the water fraction exceeds 70%, the emitting intensities of DBP were obviously increased, and maximum peaks displayed blue-shifted, suggesting that DBP molecule is AIE-active. The significant increase of emissive intensity is caused by the formation of aggregates, which limits the rotation of the phenyl rings and then activates the radiative decay channels.

Fig. 3. (A) PL spectra of DBP (10 mM) in DMSO/water system with different water fractions ($f_w$). (B) Plot of the relative PL intensity ($\alpha_{AIE}$) at 623 nm and the peak wavelength versus $f_w$, where $\alpha_{AIE}$ is equal to $I/I_0$, $I$ represents PL intensity in the DMSO/water mixture and $I_0$ represents the PL intensity in the DMSO solution.

2.4 Fluorescence imaging of DBP

The fluorescence imaging of DBP is first determined by widely used A549 cells and captured with a fluorescence microscope (Fig. 4). After incubating A549 cells with DBP solution for 30 min, an intense emissions localizing in spherical organelles among the cytoplasm were obtained under the excitation of 488 nm. In consideration of both hydrophobicity property of the DBP molecule and the spherical shape of illuminated organelles, we speculate that the DBP molecules probably existed in lipid droplets.

Fig. 4. Fluorescence imaging ($\lambda_{ex} = 488$ nm) of A549 cells co-stained with DMSO (A, B), and 5 $\mu$M DBP (C, D).
3. Conclusions

In summary, a fluorescence AIEgen DBP have been feasibly developed by a simple synthetic approach with emission wavelengths falling in red region. The AIE-active molecule exhibits several impressive advantages such as simple structure, good solubility, excellent bright emission and high processability. Inspired by this, the DBP molecule is successfully applied in fluorescence imaging of cancer cells. These results indicated that it is a simple and feasible design strategy for construction and functionalization of bioprobes with AIE feature and long emission wavelengths, which shed light on the development of AIE study in the areas of fluorescent imaging applications.

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