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Spectral study, stability and protein labeling of two Carbazole–Benzothiazole derivatives

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ABSTRACT

3-benzothiazole-9-ethyl-carbazole (BEC) and 3,6-di-benzothiazole-9-ethyl-carbazole (DBEC) were synthesized by using carbazole as base and then characterized by ^{1}H NMR. The optical properties of them were investigated and compared with carbazole and 9-ethyl-carbazole. The photostability and thermostability of the compounds were studied and the results indicated they are both very stable. Effects of solvent properties including polarity, viscosity and refractive index on the spectra properties of the compounds were investigated. Quantum yield of the compounds in organic solvent was also determined and it is found the most complex compound has the highest quantum yield up to 0.68. Finally the synthesized compounds were used to label bovine serum albumin (BSA) protein and simultaneously stability constant (K_s) between them is calculated and compared with other similar dyes. The results showed that the fluorescent intensity of the compound is increased significantly after labeling with BSA protein.

1. Introduction

Fluorescent labeling has become one of the most used technologies for application in bio-analytical detection and medical diagnoses etc. [1–3]. The materials composing the fluorescent labeling can be different ranging from organic molecules [4,5] to semiconductor nanocrystals [6] to meet the demand of broad emission wavelength range. After they interact with biomolecules, like peptides, proteins and nucleic acids through covalent or noncovalent interaction, the fluorescent intensity of these molecules normally will increase significantly [4,7,8].

Carbazole derivative is a kind of promising compound and has been applied for fluorescent labeling due to its outstanding properties, such as biological activity as well as chemical and light stability [9–12]. The biological activity of the small molecules used as fluorescent labeling is important because in the case they can act as both fluorescent probe and functional compound [13–15]. But relatively low quantum yield limits its application. For designing fluorescent molecules with high quantum yield, the molecules should possess both electron-donating moiety and electron-accepting moiety for charge carrier injection [16–19]. As we know carbazole has large conjugated system and high electron cloud density, so it can be an excellent electron-donating moiety. Simultaneously benzothiazole derivatives are good electron-acceptor moieties due to its heterocyclic system [20,21]. For this reason researchers have combined carbazole with benzothiazole and prepared many carbazole–benzothiazole derivatives with different fluorescent properties [22–24]. Wan et al. [24] synthesized a bipolar molecule with carbazole as electron-donor and benzothiazole as electron-accepter. The bipolar molecule has high fluorescence quantum yield and has potential application as luminescence material in organic light emitting diode.

Here in this study we designed and synthesized two novel carbazole–benzothiazole derivatives via a series processes. The synthesized compounds possessed higher quantum yields and we estimated their fluorescent properties in different solvent and pH value. Furthermore the interactions between these compounds with bovine serum albumin protein were studied and the stability constants between them were determined and the results were compared with each other.

2. Experimental

2.1. Chemicals and instruments

All the reagents and solvents were commercial and used without further purification. Melting points were measured by using a melting point apparatus made by Beijing Tech. Instrument Co. UV–vis absorption spectra were recorded on a Perkin Elmer LAMBDA 35 spectrometer. Fluorescent spectra were recorded on a Hitachi F-7000 spectrometer. The detailed syntheses of compound 2–6 were described below and an illustration was shown in Scheme 1.

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2.2. Synthesis of 9-ethyl-carbazole (2)

10 g (60 mmol) carbazole and 2.6 g (6 5 mmol) sodium hydroxide were dissolved in 100 ml dimethylsulfoxide (DMSO) and stirred for 30 min. Then 7.1 g (6.5 mmo1) bromoethane was added dropwise into the mixture and reacted for another 8 h at room temperature. The solution was poured into ice-water and stirred for 1 h. White crude product was obtained by filtration and washed with water. The crude product was further recrystallized from ethanol to get purified white crystals. Melting point: 68–70 °C, yield: 85%.

2.3. Synthesis of 3-benzothiazole -9-ethyl-carbazole (BEC) (4)

The synthesis procedure of compound 4 was as follows based on the Ref. [25] with a minor revision. 15 ml of anhydrous DMF was added slowly to 15.2 g of $POCl_3$ (0.1 mol) under stirring in an ice bath at room temperature until the solution became red. Then 3.155 g of 9-ethylcarbazole (16 mmol) solved in 25 ml 1,2-dichloro-ethane were added. The mixture was heated to 353 K and kept reacting at this temperature for 8 h and then cooled to room temperature. The resultant was poured into a mixture of ice-water and followed by extraction with dichloromethane (3×100 ml). The resultant dichloromethane solution was washed three times with 100 ml of water and dried over anhydrous magnesium sulfate. Afterwards the solvent was evaporated under reduced pressure and the residue was dissolved in a minimum amount of dichloromethane and then purified by silica-gel column chromatography to get compound 3. Melting point: 84–86 °C, yield: 50%.

0.25 g (2.5 mmol) of compound 3 was dissolved in 25 ml DMSO and then 0.27 ml (2.5 mmol) of 2-Aminobenzenethiol was added. The mixture was slowly heated to 333 K and reacted at this temperature for 4 h and then cooled to room temperature. The resultant was poured into a mixture of ice-water and faint yellow precipitate was obtained. After filtration, the crude product was purified by silica-gel column chromatography to get compound 4. Melting point: 142–145 °C, yield: 80%. ^{1}H NMR δ:1.45–1.49 (t, J=7.20 Hz, 3H), 4.37–4.43 (m, 2H), 7.28–7.38 (m, 2H), 7.43–7.54 (m, 4H), 7.91 (d, J=8.00 Hz, 1H), 8.21 (d, J=7.60 Hz, 2H), 8.86 (s, 1H).

2.4. Synthesis of 3.6-di-2-benzothiazole-9-ethyl-carbazole (6)

The synthesis procedure of compound 6 was as follows based on the Ref. [26,27] with a minor revision. 22 ml (0.3 mol) of anhydrous DMF was added slowly to 28 ml of $POCl_3$ (0.3 mol) under stirring in an ice bath. After 30 min a white precipitate was obtained and a solution of 3.155 g 9-ethylcarbazole (16 mmol) in 20 ml of DMF was added. The mixture was slowly heated to 373 K and reacted at this temperature for 30 h and then cooled to room temperature. The brown viscous oily resultant was poured into a mixture of ice-water and followed by extraction with dichloromethane (3×100 ml). The resultant dichloromethane solution was washed three times with 100 ml of water and dried over anhydrous magnesium sulfate. Afterwards the solvent was evaporated under reduced pressure and the residue was dissolved in a minimum amount of dichloromethane and then purified by silica-gel column chromatography using dichloromethane as eluent to get compound 5. Yield: 50%.

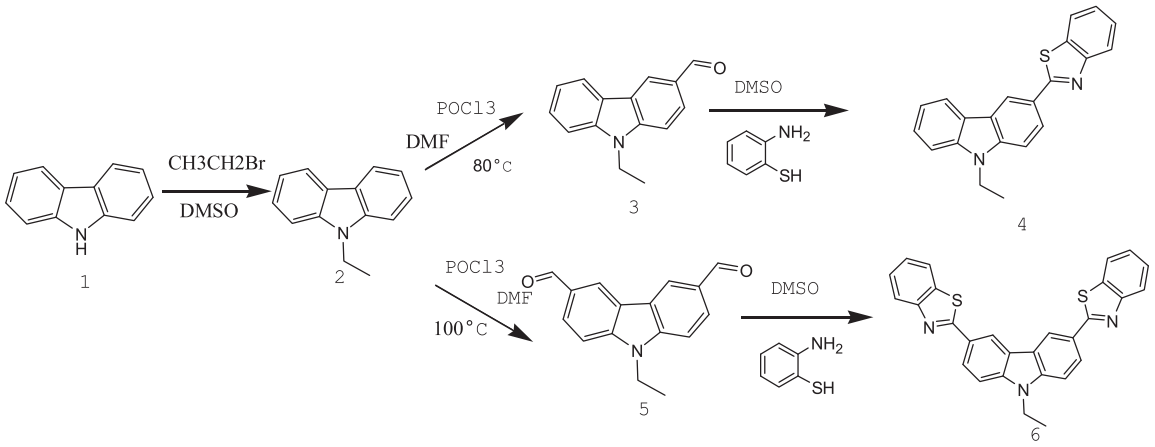
0.25 g (2.5 mmol) of compound 5 was dissolved in 25 ml DMSO and then 0.27 ml (2.5 mmol) of 2-Aminobenzenethiol was added. The reaction mixture was slowly heated to 333 K and reacted at this temperature for 4 h and then cooled to room temperature. The resultant was poured into a mixture of ice-water and faint yellow precipitate was obtained. After filtration, the crude product was purified by silica-gel column chromatography to get compound 6. Melting point: >250 °C. yield: 80%. ^{1}H NMR δ: 1.51–1.59 (t, 3H), 4.43–4.52 (m, 2H), 7.41–7.45 (t, 2H), 7.53–7.58 (m, 4H), 7.96–7.98 (d, 2H), 8.16–8.18 (d, 2H), 8.36–8.38 (d, 2H), 8.99 (s, 2H).

3. Results and discussion

3.1. Spectral properties

The absorption and fluorescence spectra of carbazole (1), 9-ethyl-carbazole (2), 3-benzothiazole -9-ethyl-carbazole (4) and 3.6-di-2-benzothiazole -9-ethyl-carbazole (6) were measured in methanol solution respectively and the results are shown in Fig. 1 and Table 1. Compounds 1 and 2 have only one absorption peak at ca. 290 nm, while compounds 4 and 6 both have two absorption peaks. The absorption peaks between 292 and 318 nm for all the 4 compounds are originated from the π–π^* transition of the conjugated system in carbazole moiety. It is interesting to find that there is absorption peak in 345 nm and 358 nm for compound 4 and 6, respectively, which can be attributed to the π donator–acceptor structure formed by the introduction of benzothiadiazole moiety. The possible mechanism is that a π–π^* charge transition is occurred from HOMO to LUMO [27,28], which is located at electron-donating carbazole moiety and electron-accepting benzothiazole moiety, respectively.

With the enlargement of the molecule from 1 to 4, the maximum absorption wavelength that comes from the π–π^* transition inside the carbazole moiety is red-shifted from 292 to 318 nm. From compound 1 to 2 the shift is because the ethyl group



Scheme 1. General procedure for the synthesis of compounds 2–6.