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ORIGINAL PAPER

Study on Synthesis and Spectrum of Novel Styryl Cyanine Dyes with a Carbazole Bridged Chain

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Abstract Based on the frequently-used cyanine dye probe thiazole orange (TO), a novel kind of cyanine dye was designed and synthesized. Carbazole was inserted into the methylidyne structure of TO as a bridge to afford a kind of novel styryl cyanine dye with carbazole bridged chain. The dyes were characterized by HNMR and MS. The spectra of the novel dyes were also studied and the results showed that the fluorescent wavelength of novel carbzole dye shifted red for 100 nm, stock shift increased by 70 nm and the fluorescent intensity enhanced by 70 times compared to that of TO. When the novel dye was labeled by BSA, its fluorescent wavelength changed little and the intensity enhanced. It is indicated that it can be used as an excellent fluorescent probe in biological labeling.

Keywords Carbazole · Bridge chain · Novel styryl cyanine dye · Fluorescence · Probe

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Introduction

With the properties of electron rich system, carbazole and its derivatives are widely used in the fields of dyes, medicine, biology, photoelectricity and so on owing to their advantages of large rigidity plan conjugated system, aromatic structures with strong fluorescence and easily being modified by the introduction of different kinds of functions.

The indolocarbazole alkaloids are a kind of natural products, a structurally rare, but biological activity class. The indolocarbazole alkaloids are extremely interesting owing to the wide range of biological activities including antimicrobial, hypotensive, antifungal, and inhibition of platelet aggregation. For example, Ellipticine is usually used to treat thyroid carcinoma, breast cancer and renal carcinoma.

NB-560 can inhibit the production of cancer cells. When it was conjugated with DNA, indolocarbazole was embedded into the two base pair of the double DNA and the residues located in the spiral groove [1].

Zeng et al. designed and synthesized 5-[2-(8-Hydroxy-quinolin-2-yl)-vinyl]-2-methyl-quinolin-8-ol [2] .The stem cell activity and antioxidation activity of the compound were investigated. The results showed that the title compound had a strong antioxidation activity and could induce the proliferation of mesenchymal stem cell under a low concentration condition. (E)-9-p-Tolyl-3-[2-(8-hydroxy-qui-nol-2-yl)vinyl]-carbazole and (E)-9-(p-Anisyl)-3-[2-(8-hydroxy-qui-nol-2-yl)vinyl]-carbazole [3] were also synthesized and the antioxidation activity and stem cell

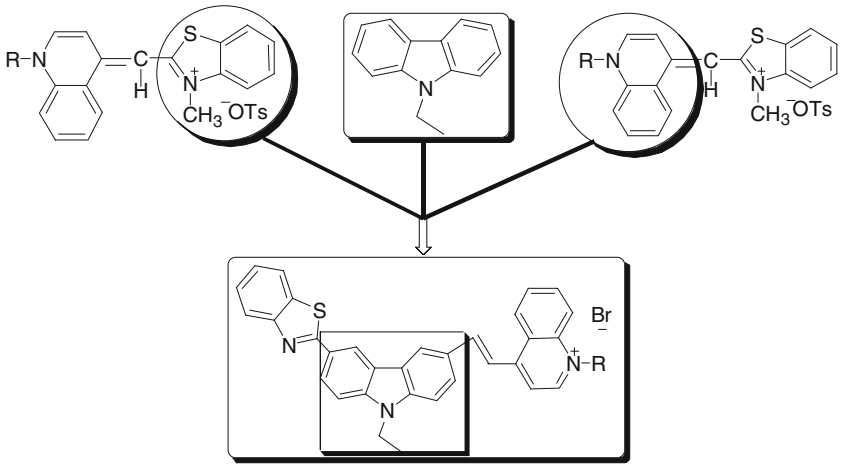


Fig. 1 Design of styryl cyanine dye with carbazole on the bridge chain

activity of the compounds were investigated too. Results showed that one compound could induce effectively the proliferation of mesenchymal stem cells under a low concentration condition.

Thiazole Orange (TO), an embedded cyanine dye, is comprised of a benzothiazole ring covalently linked to a quinoline via a monomethine bridge. It has been widely used as an embedded cyanine dye for labeling nucleic acids, which allows the detection of DNA and RNA in gels by flow cytometry or microscopy [4]. Although the fluorescence of free TO is extremely low in aqueous solution, when bound to nucleic acids, the viscosity of the dye's local environment is markedly increased, resulting in a drastic increase in fluorescence [5–8]. The large difference in fluorescence between free dye and nucleic acid-bound dye suggests an excellent way to image, label and detect cancer cells [9–17].

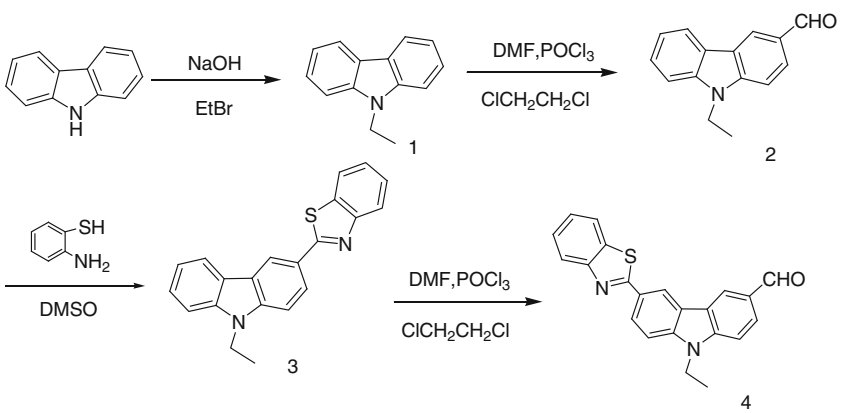
The fluorescent properties of dyes mainly depend on their chemical structures, such as conjugate system, coplanarity and riqidity. The longer the conjugate system is, the stronger the fluorescent intensity and the fluorescent emission wavelength would shift red, the background disturbing of fluorescent probe would reduce and the fluorescent lifetime would prolong. If the conjugated system was lengthened, the stability of probe would decrease.

Recently, we studied on the synthesis and properties of cyanine dye TO and its derivatives [18–21]. In order to make the conjugated system lengthen without the stability decrease, carbazole was inserted into the methylidyne structure of TO as a bridge to give a kind of novel carbazole styryl cyanine dye with carbazole bridged chain. With the introduction of carbazole, the stability of the novel probes increased, fluorescent wavelength shifted red, stock shift increased and the fluorescent intensity was enhanced. The design of novel probe was shown in Fig. 1.

Materials and Methods

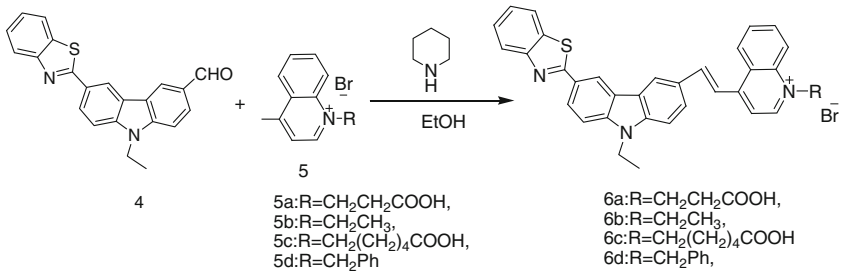
Materials and Instruments

Fluorescence spectra were scanned on a Cary Eclipse fluorescence spectrophotometer (Varian,American). The UV/Vis spectra were recorded on a Shimazu 2550



Scheme 1 Synthesis of carbazole bridge

Scheme 2 Synthesis of styryl cyanine dye with carbazole on the bridge chain



spectrophotometer (Jap.). American Mass spectral analyses were obtained using an electrospray ionization (ESI) mass spectrometer. ^{1}HNMR spectra were recorded on a Bruker (300 MHz or 400 MHz) spectrometer. Chemical shifts were reported in parts per million (ppm) downfield from TMS (tetramethylsilane), using D_2O or DMSO-d_6 as a solvent.

Synthesis

Synthesis of 3-Benzothiazole-6-Formyl Carbazole(4)

3-benzothiazole-6-formyl Carbazole was synthesized by alkylation, formylation, ring closing reaction and formylation from carbazole (Scheme 1).

Synthesis of N-ethyl carbazole (compound 1) A mixture of 25 mL DMSO, NaOH (1.30 g, 33 mol) and carbazole (5 g, 30 mol) in a 100 mL flask was stirred at room temperature for 1 h. Bromoethane (3.60 g, 33 mol) was added dropwise over 45 min into the mixture. After that, the mixture was stirred for another 6 h. The mixture was poured into cool water to precipitate the white crude product. The N-ethyl carbazole compound 1 was then filtered, washed and dried under vacuum. Yield: 85%, m.p. 68–70°C.

Synthesis of 3-formyl-N-ethyl carbazole (compound 2) To a stirred solution of DMF (15 mL) in a 100 mL flask under ice-cold condition, $POCl_3$ (7.70 g, 50 mmol) was added dropwise. When the addition was over, the reaction was stirred for another 30 min and brought to room temperature to react for 1 h. Compound 1(5.90 g, 30 mmol) of 1,2-dichloroethane (15 mL) was added dropwise and then stirred for 1 h, refluxed for 8 h and then cooled. When the former work was up, the mixture was poured into cool water and extracted with CH_2Cl_2 (3×10 mL). The organic phase was washed with water, dried with anhydrous $MgSO_4$. The solvent was distilled off and the residue was chromatographed over silica gel to give compound 2. Yield: 40%. m.p. 84–86°C.

Synthesis of 3-benzothiazole-N-ethyl carbazole (compound 3) A mixture of compound 2 (1.20 g, 5.40 mmol) , amino-thiopheno (0.70 g, 5.60 mmol) and DMSO (10 mL) was kept at 195°C for 3 h under an atmosphere of nitrogen and then cooled, poured into cool water and filtered. The filtrate was washed with water and acetone to give compound 3. Yield: 78%, m.p. 142–145°C. ^{1}HNMR (400 MHz,$CDCl_3$)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), 8.08(d, J=8.00 Hz, 1H), 8.21(d, J=7.60 Hz, 2H), 8.86(s, 1H). ESI-MS (m/z): 329.9 [M^++1].

3-benzothiazole-6-formyl-N-ethyl carbazole (compound 4) The synthetic method was similar to that of compound 2. Yield: 35%, m.p. 171–173°C. ^{1}HNMR(400 MHz,$CDCl_3$) δ: 1.43–1.46: (t, J=7.20z, 3H), 4.35–4.40(m, 2H), 7.30–7.34 (t, J=7.20 Hz, 1H), 7.42–7.48(m, 3H), 7.86(d, J=8.00 Hz, 1H), 7.99–8.04(t, J=9.20 Hz, 2H), 8.22(d, J=8.40 Hz, 1H),

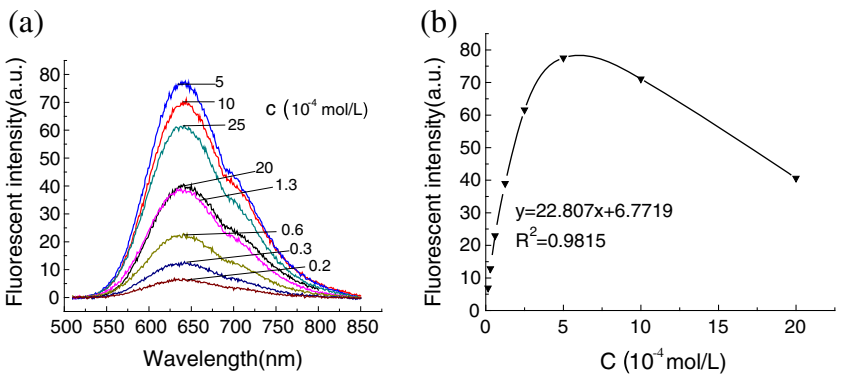


Fig. 2 (**a**) Fluorescent spectra of 6a with different concentrations, (**b**) Fluorescent intensities of 6a with different concentrations

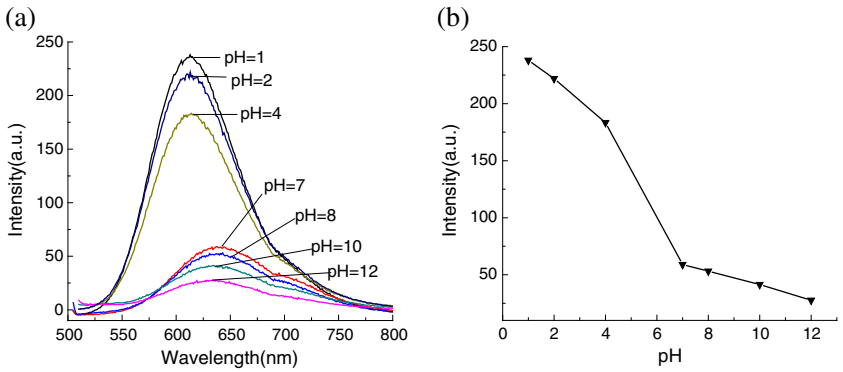


Fig. 3 (**a**) Fluorescent spectra of 6a at different pH, (**b**) Fluorescent intensities of 6a with the change of pH

8.64(s, 1H), 8.82(s, 1H), 10.06(s, 1H). ESI-MS (m/z): 357.8[M^++1].

Synthesis of Compounds 5a-5d

The compounds 5a-5d were synthesized according to literature methods [18].

5a (400 MHz, $CDCl_3$) δ: 1.82(t, J=7.20 Hz, 2H), 3.04 (s, 3H), 5.45–5.50(m, 2H), 7.98–8.05(m, 2H), 8.21–8.25(t, J=7.80 Hz, 1H), 8.39(d, J=8.40 Hz, 1H), 8.45 (d, J=9.20 Hz, 1H), 10.52(d, J=6.00 Hz, 1H). ESI-MS (m/z): 216.1[M^+], 217.1 [M^++1].

5b (400 MHz, $CDCl_3$) δ: 1.23(t, J=5.25 Hz, 3H), 3.04 (s, 3H), 5.46–5.51(m, 2H), 7.99–8.07(m, 2H), 8.21–8.34(t, J=7.60 Hz, 1H), 8.38–8.423(t, J=9.80 Hz, 2H), 10.58(d, J=5.60Hz, 1H). ESI-MS (m/z): 172.0[M^+], 173.1 [M^++1].

5c (400 MHz, CD_3OD) δ: 1.39–1.44(m, 2H), 1.52–1.57(m, 2H), 1.92–1.97(m, 2H), 2.19–2.23(t, J=9.60 Hz, 2H), 3.01(s, 3H), 5.00–5.05(m, 2H), 8.05–8.09(t, J=8.00 Hz, 2H), 8.40(d, J=11.60 Hz, 1H), 8.59(d, J=11.2 Hz, 1H), 8.60(d, J=11.6 Hz, 1H), 9.57(d, J=8.00 Hz, 1H). ESI-MS(m/z): 258.1[M^+], 259.1 [M^++1].

5d (400HMz,$CDCl_3$) δ: 3.03(s, 3H), 6.68(s, 2H), 7.28 (d, J=7.60 Hz, 3H), 7.41(d, J=9.60 Hz, 2H), 7.89–7.93(t,J=8.20 Hz, 1H), 8.04–8.10(m ,2H), 8.32(d, J=9.60 Hz, 1H), 8.52(d, J=9.60 Hz, 1H), 10.56(d, J=6.80 Hz,1H). ESI-MS (m/z): 234.0[M^+], 235.1 [M^++1].

Syntehsis of the Title Compounds 6a-6d

The compound 6a-6d was obtained by Knoecenaget condensation (Scheme 2).

The typical procedure for 6a-6d was: aldehyde 4 (0.10 g, 0.28 mmol) in 30 mL CH_2CH_3OH and salt 5 (0.12 g, 0.42 mmol) in 20 mL CH_2CH_3OH were added to a 100 mL flask, followed by catalytic piperdine (1–3 drop). The resulting mixture was allowed to be refluxed and stirred for 12 h. When it was cooled to room temperature, CH_3COOH was added and stirred for 2 h. Aether was added to give a red solid. The crude product was filtered, washed and the residue was chromatographed over silica gel to give compound 6.

6a (400 MHz, DMSO-d_6) δ: 1.35(m, 3H), 2.68–2.73 (m,2H), 4.44–4.45(m, 2H), 5.05–5.06(m, 2H), 7.40–7.43(m, 3H), 7.51–7.58(m, 6H), 7.73–7.76(m, 2H), 7.96(d, J=7.60 Hz, 2H), 8.11(d, J=7.20 Hz, 2H), 8.28(s, 1H), 8.48(s, 1H), 8.92(d, H=9.60 Hz,1H), 10.17(s, COOH). ESI-MS (m/z): 554.3[M^+], 555.4 [M^++1].

6b (300 MHz, CD_3OD) δ1.38–1.43(t, J=7.05 Hz, 3H), 1.58–1.63(t, J=7.20 Hz, 3H), 4.21–4.28(m, 2H), 4.65–4.72(m, 2H), 7.17–7.27(m, 2H), 7.31–7.42(m, 3H), 7.66(d, J=9.90 Hz,4H), 7.79–7.94(m, 4H), 8.04 (d, J=8.70 Hz, 1H), 8.28(s, 1H), 8.45(s, 1H), :8.63–8.71(m, 2H). ESI-MS (m/z): 510.3[M^+], 511.4 [M^++1].

6c (300 MHz, DMSO-d_6) δ: 1.17–1.20(m, 5H), 1.52–1.56(m, 2H), 1.94–1.96(m, 2H), 2.07–2.13(m,

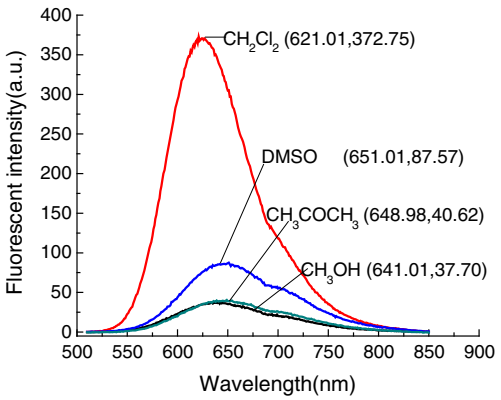


Fig. 4 Fluorescent spectra of 6a in different solvents

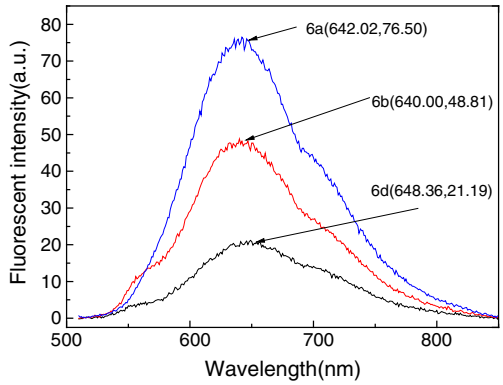


Fig. 5 Fluorescent spectra of styryl cyanine dye with carbazole bridge chain

2H), 4.52–4.54(m, 2H), 4.91–4.93(m, 2H), 7.39–7.44 (t, J=7.35 Hz, 1H), 7.50–7.55(t, J=7.50 Hz, 1H), 7.75–7.83(m, 2H), 8.00–8.01(m, 3H), 8.11(d, 8.10 Hz,1H), 8.17–8.23(m, 2H), 8.37(s, 2H), 8.48(d, J=8.70 Hz, 2H), 8.99(s, 1H), 9.11(d, J=10.5 Hz, 2H), 9.35(s, 1H). ESI-MS (m/z):596.4[M^+], 597.5[M^++1]. 6d (400 MHz, DMSO-d_6) δ: 1.12–1.08(t, J=7.20 Hz,3H), 3.84–3.90(m, 2H), 5.54(s, 2H), 6.76–6.83(m, 3H), 6.87–6.91(m, 4H), 6.94(d, J=8.40 Hz, 1H), 7.03(d, J=8.80 Hz, 1H), 7.23–7.36 (m, 4H), 7.42–7.45(t, J=7.80 Hz, 1H), 7.50–7.56(m, 3H), 7.66(d, J=8.80 Hz, 1H), 7.73(d, J=6.40 Hz, 1H), 8.25(s, 1H), 8.10(s, 1H), 8.32(d, J=8.80 Hz, 1H), 8.54 (d, J=6.80 Hz, 1H). ESI-MS (m/z): 572.2[M^+], 573.2 [M^++1].

Results and Discussion

Synthesis

In order to enhance the fluorescent intensity, increase the Stock shift and increase the stability of TO, carbazole was inserted into the methylidyne structure of TO as a bridge to give a kind of novel carbazole styryl cyanine dye with carbazole bridged chain. First, 3-substituted of carbazole was formylated, reacted with aminothiopheno to afford 3-benzothiazoleN-ethyl carbazole, which was formylated to give 3-benzothiazole-6-formyl-N-ethyl carbazole. The formyl reacted with active methylene compounds by Knoevenaget condensation to afford C = C and the title styryl cyanine dye with carbazole bridged chain obtained.

Spectral Properties of Cyanine Dye Probe to with Carbazole Bridged Chain

Effect of Concentration on the Fluorescence

Concentration was a factor exerting effect on the fluorescence. In this paper, the fluorescent spectra of a series 6a samples in CH_3OH were scanned at 500 nm. The concentrations of 6a were geminated from 0.2×10^{-4} mol/L to 20×10^{-4} mol/L.

The fluorescent spectra were shown in Fig. 2. Peak characteristics of fluorescence and the maximum emission wavelength of samples with different concentrations were of little difference. The florescence intensity increased with the increasing of concentration between 0.2×10^{-4} mol/L–2.5×10^{-4} mol/L, but decreased when the concentration exceeded 5×10^{-4} mol/L. With the increasing of the concentration, the absorbent excited quantum number increased and the emissive quantum number increased too, leading to the enhancement of the fluorescent intensity of the fluorescent compound. When the fluorescent intensity enhanced to the maximum, it decreased, which may result from fluorescence self-quenching.

ph Effect on the Fluorescence of 6a

Although the fluorescent properties of compounds depend on the chemical molecular structure, environmental factors where the molecules were in can also affect the fluorescence.

Five 6a solutions of 1×10^{-4} mol/L) in CH_3OH were prepared, and then the pH were adjusted from 1 to 12 respectively by addition of dilute HCl or NaOH. Because of the addition quantity were small, the changes of total volume could be ignored. The fluorescent spectra of five 6a solutions excited at 500 nm were shown in Fig. 3.

In the spectra of the five solutions, the fluorescent intensities of samples in acid medium were stronger than the others. The fluorescent intensities decreased with the pH added and decreased obviously in the basic medium with a red shift of the fluorescent wavelength. As an organic weak acid, its fluorescent intensity was affected by

Table 1 Degradation curve of cyanine 6a and 6b

Time/h		0	2	4	6	8	10	12	Rate of decline
Fluorescent intensity	6a	34.50	33.05	32.80	32.01	31.73	31.36	31.01	10.38%
	6b	34.60	31.20	30.01	29.36	29.17	29.03	28.20	18.50%

Table 2 Physical constants of solvents and the max absorption wavelengths of 6a

Sovlent	Dielectric Constant	refractive index (n)	f(n)*	λ_{max}(nm)	R*10^{-3}(cm^{-1})
CH_3OH	32.6	1.329	0.1690	479.00	2.0876
CH_3COCH_3	20.7	1.358	0.1801	480.00	2.0833
CH_3CH_2OH	24.3	1.361	0.1812	485.00	2.0619
CH_2Cl_2	9.1	1.424	0.2033	493.00	2.0284
$CHCl_3$	5.5	1.443	0.2092	493.50	2.0263
CH_2ClCH_2Cl	10.45	1.445	0.2102	495.00	2.0202

pH obviously, that's because they were different in electronic configuration under different pH values.

Solvent Effect on the Fluorescent Property of 6a

Solution obviously affects on the fluorescent properties. The fluorescent spectra of 6a in different solutions were shown in Fig. 4.

The maximum fluorescent emission wavelength shifted red with the solvent polarity added. The maximum emission wavelength in DMSO was at 651 nm, which shifted 13 nm, 7 nm and 12 nm compared to those in CH_3OH, CH_3COCH_3 and CH_2Cl_2 respectively. It may because that when the dye molecule is excited, the electronic excited state has bigger polarity than the ground state. The increased polarity makes the electronic excited state much more stable than the ground state, resulting in the red shift of wavelength.

Substitutional Groups on Quinoline Side Chain Effect on the Fluorescent Properties of Carbazole Styryl Dyes

Fluorescent properties of styryl dyes with carbazole bridged chain could also be affected by substitutional groups on quinoline side chain. In this paper, the fluorescent spectra of 6a, 6b, 6d with the same concentration in CH_3OH were obtained and shown in Fig. 5

The maximum emission wavelengths of the dyes were at about 640 nm. The maximum emission wavelengths of 6d with benzyl group on on quinoline side chain shifted 8 nm compared to that of 6b. The fluorescent intensity of 6a was the strongest while that of 6d was the weakest.

Photoreduction Stability

6a and 6b with the same concentration of 2.5×10^{-5} mol /L were prepared and irradiated by 250 w high-pressure mercury lamp at a distance of 10 cm. The fluorescence intensities were measured per 2 h and the data were shown in Table 1.

The fluorescent intensities of 6a and 6b were declined by only 10.38% and 18.50% respectively after irradiated for 12 h. The fluorescent intensities of 6a and 6b weakened only a little in the process, which indicated that the photoreduction stabilities of 6a and 6b were good.

Solvent Effect on the UV–vis Spectra of 6a

The UV–vis spectra of 6a in different solvent with same concentration were scanned and the results were shown in Table 2.

Solvent affected the UV–vis spectra of 6a. The absorption wavelength was longer with the refractive index increased. In CH_3OH, the absorption wavelength was 479 nm and 495 nm in CH_2ClCH_2Cl, which shifted red of 16 nm.

In order to investigate if the physical constants of solution affected the maximum absorption wavelength, the $r=1/\lambda_{max}$ was calculated by $f(n) = (n^2-1)/(2n^2+1)$ from the measured λ_{max}. The line of Bayliss f(n) was calculated by equation $f(n) = (n^2-1)/(2n^2+1)$. The relationship between r and Bayliss function f(n) was shown in Fig. 6.

There was some relativity between r and Bayliss function f (n). The relativity was satisfying from the data $R^2=0.9518$. It indicated that shift of absorption peak depend on the refractive index of the solvent used. The maximum absorption wavelength became longer with the increase of n value.

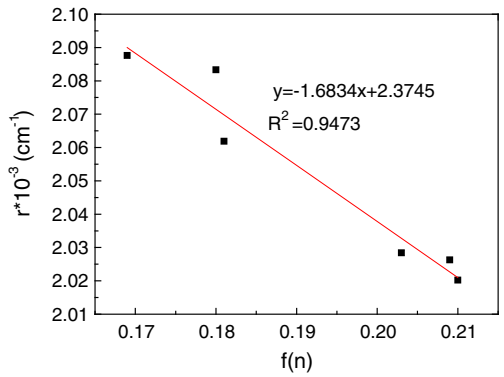


Fig. 6 Curve of f (n) with absorption wave number of 6a in different solvents

Table 3 Data of carzabole styryl cyanine dye and TO

Dye	Excition wavelength (nm)	Emission wavelength (nm)	Stocks shift(nm)	Intensity (a.u.)
TO	480	547	67	0.77
6a	500	638	138	44.94

Properties Compared with TO

In order to compare the fluorescent properties of dye 6a and TO, the solutions of them with the same concentration were prepared and scanned at 480 nm. The data results were shown in Table 3. The fluorescent wavelength of 6a shifted red of 91 nm compared to that of TO and Stocks shift increased 71 nm. When 6a was used in biological chemical, overlap between the excitation and emission wavelength could be reduced and the background interference could be decreased, leading to the resolution improvement.

In comparison with the structure of TO, the novel carbazole styryl dyes has extended conjugated system. The π electrons are excited easier, resulting that the fluorescent intensity enhances and the wavelength shifts red. The novel carbazole styryl dyes also has large rigidity plan, which make the reciprocity and conjugation of π electrons increase. Consequently, the wavelength of the novel carbazole styryl dyes shifted red to near the near-infrared region, the fluorescent intensity enhanced and the Stocks shift increased. The novel dyes have the better performance than TO, which can be used as an excellent cyanine dye probe in biological labeling.

Fluorescent Properties of Carbazole Styryl Dyes Labeled by BSA

25 mg BSA was dissolved in 0.05 mol/L Tris–HCl buffer (*pH*=7.7) as a stock solution. TO and carbazole styryl dyes solutions in CH_3OH with the same concentration of 1×10^{-4} mol/L were prepared as standard solutions respectively. 5 mL of standard solution was diluted to 15 mL by BSA stock solution to afford TO-BSA and carbazole styryl dye-BSA respectively and the fluorescent spectra were shown in Fig. 7.

The fluorescent intensity of TO enhanced and the wavelength shifted blue after labeled by BSA compared to that of TO. Under the same concentration, the fluorescent intensity of carbazole styryl dyes enhanced and the wavelength shifted little, exhibiting preferable fluorescent stability.

Conclusion

Based on the structure of the excellent cyanine dye TO, carbazole was inserted into the methylidyne structure of TO as a bridge to afford a series novel carbazole styryl cyanine dyes. The compounds were characterized by HNMR and MS.

Fluorescent results showed that the fluorescent wavelength of novel carbzole styryl cyanine dye shifted red of 100 nm compared to that of TO, stock shift increased 80 nm ,the fluorescent intensity enhanced by 70 times and the photoreduction stability was excellent. The novel carbazole styryl cyanine dye kept the same conjugated plane structure of its precursors TO and the embedded fluorescent properties. When the novel dye was labeled by BSA, its fluorescent wavelength changed little and the intensity enhanced.

The novel carbazole styryl cyanine dye has the excellent fluorescent properties, for the fluorescent wavelength shifted red, intensity enhanced and Stock shift increased. The fluorescent owned better performance than TO, therefore, it can be used as an excellent cyanine dye probe in biological labeling.

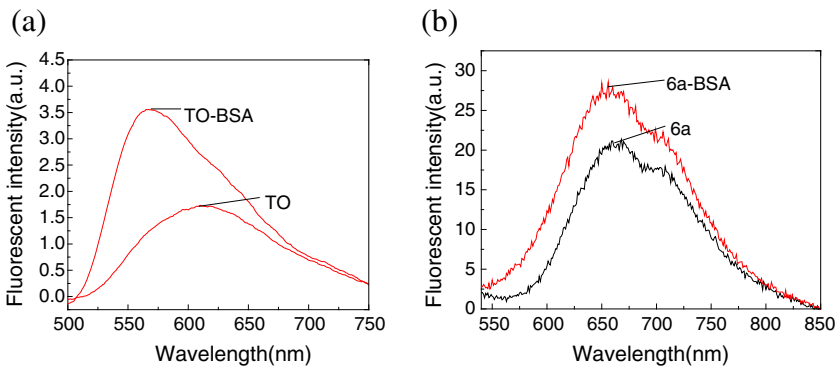


Fig. 7 Fluorescent spectra of carbazole styryl cyanine dye labeled by BSA and TO labeled by BSA

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