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Substitution nitrogen for chlorine of heptamethine cyanines for large Stokes shift fluorescent probes

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ABSTRACT

On the basis of syntheses of three heptamethine indocyanines with methyl, ethyl, or *p*-carboxybenzyl groups on N atoms in the indole rings, nine novel aminoderivatives were designed and synthesized by butylamine, taurine, or benzylamine reacting with the indocyanines for substitution nitrogen for chlorine. The obtained products were purified by SiO_2 column chromatography, and confirmed by ESI-MS and ^1H NMR. Compared to the parents, the aminoderivatives showed blue shifts, larger Stokes shifts, and stronger fluorescence intensity, which were mainly related to the electron-donating ability of the amino substituents in the aminoderivatives. The stronger electron-donating ability of the amino substituents made the maximum absorption wavelengths show greater blue shifts.

Introduction

Cyanine dye with large molar extinction coefficients and broad wavelength tenability has been widely used as fluorescent probe in fluorescent labeling,[1–4] ion monitor[5–8] and medical imaging[9–12] because of its high sensitivity and unique selectivity. But the main challenges cyanine dyes facing have been further improvement of the detection sensitivity. If the maximum absorption and emission wavelengths are short, especially at the ultraviolet region, the detection sensitivity for biomass could be reduced due to the interface of biological autofluorescence. This will be effectively avoided by using near-infrared cyanines as the probes. The main method for making the wavelengths of cyanines in near-infrared region is to enlarge the conjugated polymethine chain.[13] Many near-infrared cyanines have been developed, such as indocyanine green (ICG, λ_{ab} = 800 nm), which is the classical heptamethine probe and applied in bioanalysis.

But with the increasing of the polymethine chain and the maximum absorption wavelength falling into near infrared region, the photostability of the cyanines decreases.[14] Patonay and coworkers,[15] and Chen et al.[16] improved the photostability of heptamethine cyanine dyes greatly through introducing a rigid ring into the polymethine chain and benzyl substitute on the nitrogen atom in the indole ring. On the basis, we introduced the *p*-carboxybenzyl group on the nitrogen atom in the indole ring to improve the photostability further,[17] and a novel heptamethine cyanine with the *p*-carboxybenzyl group (Scheme 1) was developed.

However, heptamethine cyanine had a small Stokes shift (<30 nm) and this might result in fluorescence quenching and decreased fluorescence detective sensitivity. In order to avoid the problems, new heptamethine cyanine fluorescent probes with large Stokes shifts should be developed. By substituting the chlorine atom at the cyclohexenyl bridge in the heptamethine chain with the amino reagents *p*-anisidine, aniline, cyclohexylamine, benzylamine, and γ-aminobutyric acid, Peng et al.[18] obtained five aminoderivatives with larger Stokes shifts based on the parent dye, *N*-benzyl heptamethine cyanine. In order to develop novel aminoderivatives with large Stokes shifts and investigate the corresponding action mechanism further, we substituted the chlorine atom by three amine reagents (butylamine, taurine, or benzylamine) to derive nine aminoderivatives (Scheme 2) based on three parent dyes (Scheme 1), which contained *N*-methyl, *N*-ethyl, or *p*-carboxybenzyl on the N-atoms in the indole rings.

The results showed that the aminoderivatives had the maximum absorption and emission wavelengths in 550–750 nm. Compared to their parents, the aminoderivatives had larger Stokes shifts, and this was influenced by the electron-donating ability of the amino substituents.

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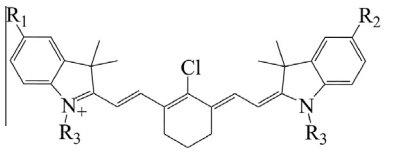


1: $R_1 = SO_3^-$, $R_2 = SO_3H$, $R_3 = H_2C$-C$_6$H$_4$-COOH
2: $R_1 = SO_3^-$, $R_2 = SO_3H$, $R_3 = CH_2CH_3$
3: $R_1 = R_2 = H$, $R_3 = CH_3$

Scheme 1. Structures of the parent dyes.

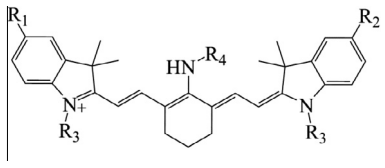
Results and discussion

Syntheses of the aminoderivatives

The synthetic route of the aminoderivatives and their dyes was shown in Scheme 3. Intermediates **4**–**6** and bisaldehyde compound **7** condensed to form parent dyes **1**–**3**,[17,19,20] and the dye**s** reacted with benzylamine, butylamine, or taurine, respectively, for substitution of nitrogen for chlorine to get nine novel aminoderivatives. The products were purified by SiO_2 column chromatography, and characterized by ^{1}H NMR and ESI-MS.

Synthetic conditions for the novel aminoderivatives were adopted according to the Ref. 21–23. But in the references, DMF was chosen as the solvent, so the post-treatment was complex due to the high boiling point. In this Letter, we tried to use other solvents for our syntheses, and found that ethanol could serve as a good solvent for the aminoderivatives (**1**–**2**)**a**–**c**, and DCM for **3a**, **3b**, and **3c**, so they made the post-treatment simple and cost less due to their lower boiling point and cheaper price. The synthetic route of **1a**, **1b**, and **1c** derived from dye **1** is shown in Scheme 4.

Liquid benzylamine and butylamine are bases and good amino reagents. In the synthetic process, they could act not only as strong nucleophilic reagents to react with the dyes for the substitution of the chlorine atoms, but also as deacid reagent to take away the HCl formed in the reactions, and this made the syntheses of aminoderivatives **1a**, **1c**, **2a**, **2c**, **3a**, and **3c** easy. While the amino group of taurine was in the form of an inner salt (ammonium salt) with the sulfonic acid group, and this decreased its nucleophilicity, we applied anhydrous sodium carbonate to accelerate the synthetic reaction by taking up the HCl formed and neutralize the sulfonic group to make the amino group free and the nucleophilicity increase. Meanwhile, sodium carbonate also could work as a deacid reagent to take up the HCl formed in the synthetic process of aminoderivatives **1b**, **2b**, and **3b**. So, aminoderivatives (**1**–**3**)**a**,**c** could be readily prepared at room temperature, aminoderivatives (**1**–**3**)**b** needed to be synthesized under heating. Furthermore, the nucleophilic activity of benzylamine and butylamine was higher than that of taurine. The optimal temperature for the syntheses of aminoderivatives (**1**–**2**)**b** was 50 °C, and that for **3b** was about 45 °C.



(1-3)a,b,c

(1-3)a : $R_4 = CH_2CH_2CH_2CH_3$,
(1-3)b : $R_4 = CH_2CH_2SO_3H$,
(1-3)c : $R_4 =$ H_2C-C$_6$H$_5$

Scheme 2. Structures of the aminoderivatives.

Spectral properties of the aminoderivatives and their parents

The data of maximum UV–Vis absorption and emission wavelengths of the aminoderivatives and their parents in different solvents are listed in Table 1. The maximum UV–Vis absorption and emission wavelengths of the aminoderivatives in 586–760 nm showed blue shifts compared to those of their parents in 770–820 nm, but large Stokes shifts were exhibited. For instance, the UV–Vis absorption and emission spectra of aminoderivative **1c** and its parent dye **1**, could be visually seen from Figure 1. The maximum absorption wavelengths of aminoderivative **1c** and its parent dye **1** were 614 nm and 792 nm in methanol, and their emission wavelengths were 733 nm and 818 nm in methanol, respectively. The Stokes shift of aminoderivative **1c** was 119 nm, much larger than that of its parent dye **1** (26 nm).

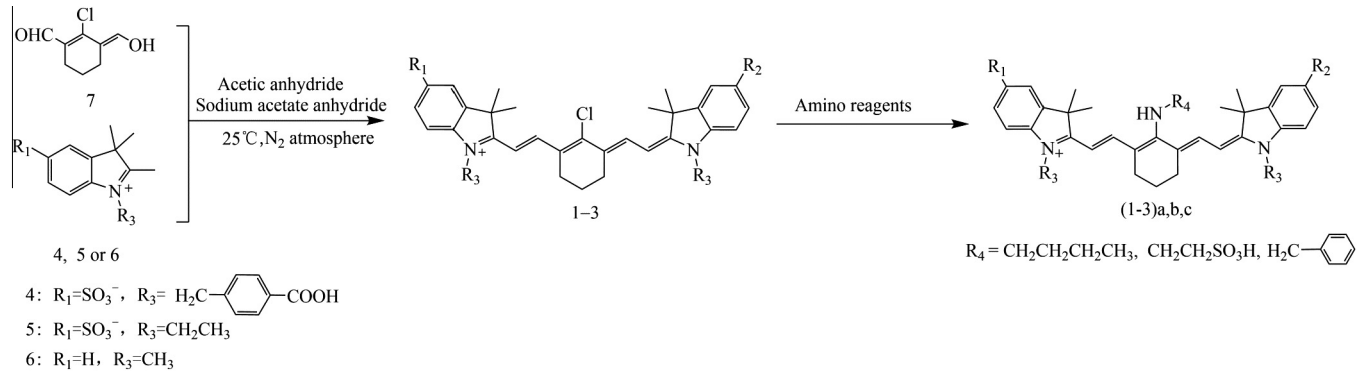
Compared to those of the parents, the shape and intensity of absorption and emission spectra of the aminoderivatives changed a lot. The absorption peaks of the aminoderivatives became broader and the absorption intensity at the corresponding maximum wavelengths turned weaker than those of their parents in methanol, such as **2a**, **2b**, **2c**, and their parent dye **2** in Figure 2; while the emission intensity at the corresponding maximum wavelengths of aminoderivatives were stronger than those of their parents, such as **3a**, **3b**, **3c**, and their parent dye **3** in Figure 3.

In different solvents, the aminoderivatives and their parents exhibited different absorption and emission wavelengths (Table 1). The wavelengths showed blue shifts with the increasing of polarity of the protic solvents and exhibited negative solvatochromism, the reason was that polarity of ground state molecule of the aminoderivatives and their parents was greater than that of the excited state, the ground state molecule was better stabilized by solvation than the molecule in the excited state, and made the energy band between the ground state and excited state enlarge with the increasing of the polarity of the solvents.[24,25] By the way, the *meso*-nitrogen-atom in the molecules of the aminoderivatives with a pair of lone electrons might form hydrogen-bonding with H-atom in protic solvents,[24–26] and the hydrogen-bonding lowered the energy level of the ground state, it could make the energy band enlarge and the wavelength shift enhance.

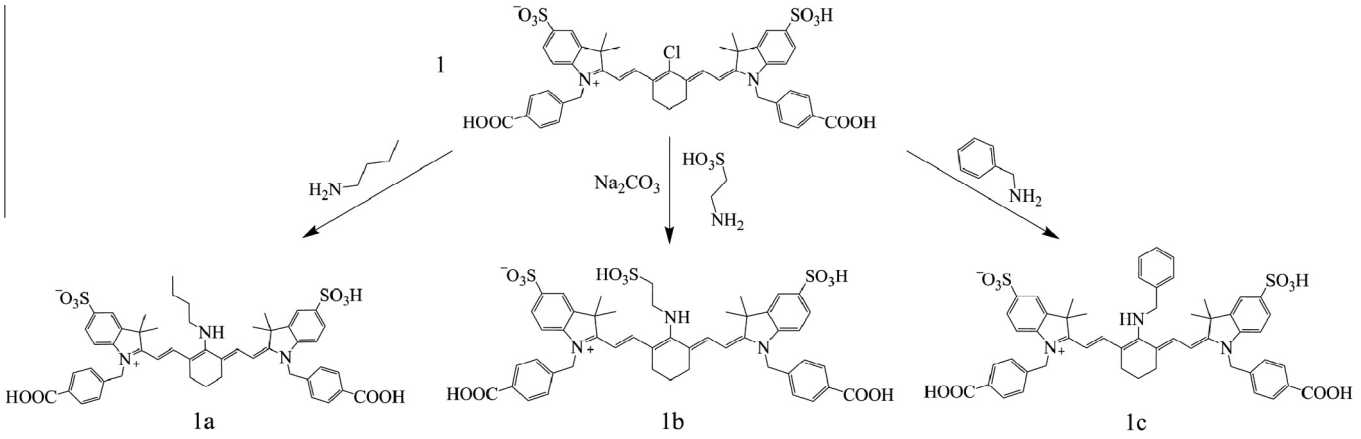
Influence of different N-substituents on spectral properties of the aminoderivatives

Compared to —Cl, the N atom in —NHR_4 had much more electron density, so substitution of —NHR_4 group for —Cl group at the polymethine chain of the cyanines remarkably changed the maximum UV–Vis absorption and emission wavelengths a lot. Because the substitution of —NHR_4 group for —Cl altered the energy of ground and excited states of the molecule, and made the aminoderivatives possess larger energy bands and greater blue shifts than those of their parents.[25] While an excited-state intramolecular charge transfer took place between the donor and acceptor in the aminoderivatives, the Stokes shift and fluorescence intensity would increase further.[18]

R_4 in —NHR_4 group of the aminoderivatives also affected the maximum absorption wavelengths due to their different electron-donating ability and space structures. Generally, stronger electron-donating ability of the —R_4 group made greater blue shifts of the maximum absorption wavelengths. Among them, the electron-donating ability of —$CH_2C_6H_5$ group was least, so the corresponding aminoderivatives (**1a**, **2a**, and **3a**) possessed the least blue shifts.



Scheme 3. Synthetic route of aminoderivatives and their parents.



Scheme 4. Syntheses of the aminoderivatives from dye **1**.

Table 1
Spectral data of the aminoderivatives and their parents in different solvents

	DCM	DMF	Ethanol	Water	Methanol		
	(λ_{ab}:λ_{em}) (nm)	(λ_{ab}:λ_{em}) (nm)	(λ_{ab}:λ_{em}) (nm)	(λ_{ab}:λ_{em}) (nm)	(λ_{ab}:λ_{em}) (nm)	Stokes shift (nm)	ε ($\times 10^5$) (L mol^{-1} cm^{-1})
1	—	808:820	804:815	784:805	792:818	26	1.77
2	—	800:819	789:815	778:809	784:813	29	2.4
3	785:817	784:814	777:808	—	774:801	27	2.96
1a	—	600:745	592:729	586:752	589:728	139	0.84
1b	—	620:749	615:735	607:750	609:731	122	1.15
1c	—	620:746	619:746	612:748	614:733	119	1.27
2a	—	657:754	627:742	605:755	618:737	119	0.8
2b	—	631:746	630:741	626:760	627:740	113	1.24
2c	—	651:752	640:737	625:755	633:735	102	1.56
3a	657:749	645:747	628:736	—	621:733	112	0.8
3b	648:744	644:744	636:735	—	635:735	100	0.94
3c	676:747	647:747	640:737	—	637:737	100	1.2

'—': insoluble.

Influence of substituents on photostability of the aminoderivatives and their parents

It had been proved that the photofading of heptamethine cyanines was associated with the attacking of singlet oxygen ($^1O_2$) and superoxide (O_2^-).[16] Substituents in the cyanines could affect the degree of the oxygen attacking due to different steric hindrance and electron effect on the N atoms in the substituents. Larger steric hindrance and lower electron density of N atoms in the substituents in the cyanines might decrease the attacking chance of singlet oxygen ($^1O_2$) and superoxide (O_2^-) to the cyanines and make the cyanines stable. We tested the photostability of the aminoderivatives and their parents in a closed cabinet with an UV lamp (Power = 100 W) 25 cm away from the testing solutions. The degradation constants were obtained by the equation below,[16] and exhibited in Figure 4.

$$\ln A_0/A_t = k \times t$$

The degradation constants of the parent dyes were 1.25×10^{-3} mol min^{-1} (**1**) < 4.38×10^{-3} mol min^{-1} (**2**) < 2.14×10^{-2} mol min^{-1} (**3**), so the order of photostability of them was **1** > **2** > **3**. It illustrated that the presence of the sulfonic group on the aromatic rings was of benefit to stabilize the parent dyes **1** and **2**, and the large group *p*-carboxybenzyl on nitrogen of the indole ring prevented dye **1**

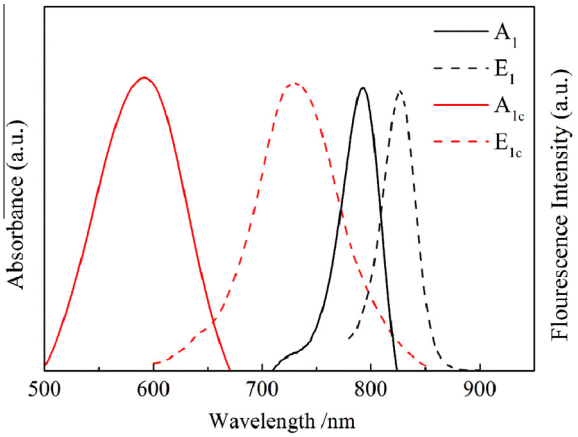


Figure 1. UV–Vis absorption (solid lines) and emission spectra (dash lines) of aminoderivative **1c** and its parent dye **1** in methanol.

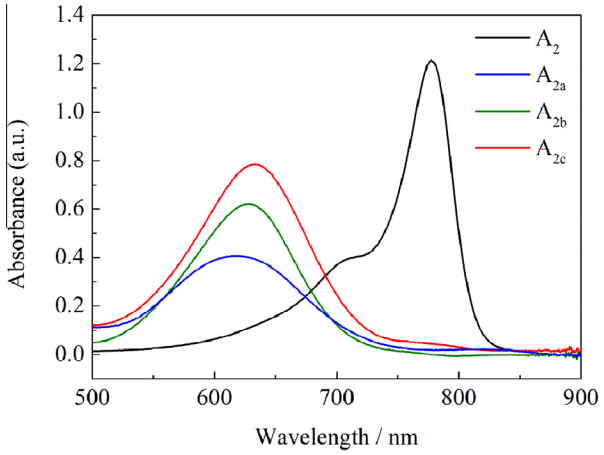


Figure 2. UV–Vis absorption spectra of aminoderivatives **2**(**a**–**c**) and dye **2** in methanol ($5 * 10^{-6}$ mol L^{-1}).

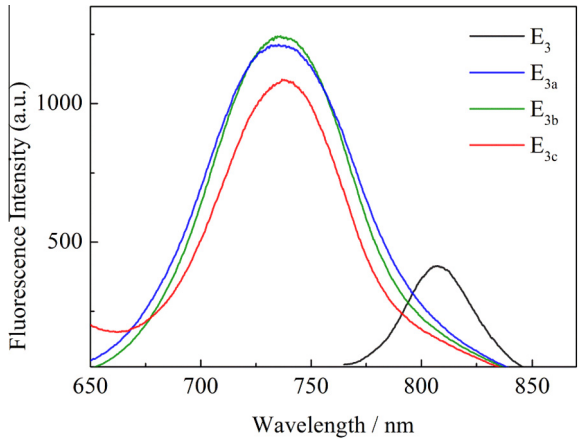


Figure 3. Emission spectra of aminoderivatives **3**(**a**–**c**) and their parent dye **3** in methanol ($5 * 10^{-6}$ mol L^{-1}, excitation wavelength for aminoderivatives was 630 nm, and that for dye **3** was 760 nm).

from being attacked by active oxidative species and improved its photostability further due to the larger steric hindrance. Compared to their parents, the aminoderivatives had bigger degradation constants, they were 8.68×10^{-3} mol min^{-1} (**1a**), 6.5×10^{-3} mol min^{-1} (**1b**), 1.5×10^{-3} mol min^{-1} (**1c**), 1.06×10^{-2} mol min^{-1} (**2a**), 1.04×10^{-2} mol min^{-1} (**2b**), 8.93×10^{-3} mol min^{-1} (**2c**), 6.19×10^{-2} mol min^{-1} (**3a**), 4.34×10^{-2} mol min^{-1} (**3b**) and 2.41×10^{-2} (**3c**) mol min^{-1}, respectively. So their photostability was weaker, and the order was about (**1**–**3**)**c** > (**1**–**3**)**b** > (**1**–**3**)**a**.

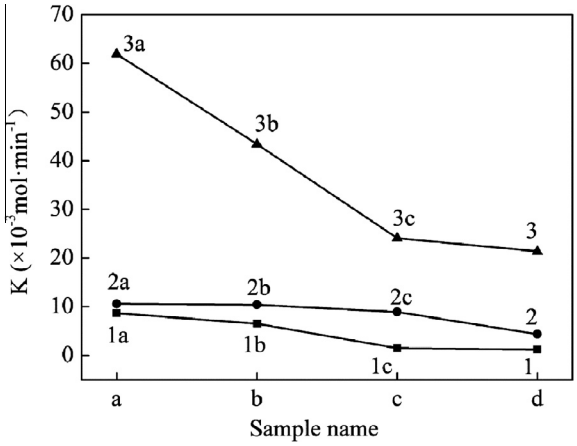


Figure 4. Degradation constants of the aminoderivatives and their parents.

This mainly attributed to the different electron-donating ability of —NHR_4 group in the aminoderivatives. With increasing of the electron-donating ability, the charge density of the *meso*-nitrogen increased, so the possibility of the lone pairs on the *meso*-nitrogen being attacked by the active oxidative species increased, and this made the photostability of the aminoderivatives turn weaker. The —$NHCH_2C_6H_5$ group possessed a weaker electron-donating ability, so its corresponding aminoderivatives had better photostability.

Conclusions

Nine novel aminoderivatives synthesized in ethanol or DCM through the substitution of nitrogen for the *meso*-chlorine of the parent cyanine dyes, had maximum absorption and emission wavelengths at around 620 nm and 750 nm in methanol. Compared to their parents, the aminoderivatives showed blue shifts in wavelengths, larger Stokes shifts (>100 nm), and stronger fluorescence intensity. Generally, the stronger the electron-donating ability and steric effect of —R_4, the greater the blue shifts of the wavelengths. The presence of the sulfonic and/or *p*-carboxybenzyl groups in the molecules benefited the photostability of the aminoderivatives and their parent dyes. These near infrared aminoderivatives with large Stokes shifts and fluorescence intensity were expected to be applied as fluorescent probes for bioanalysis.

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Supplementary data

Supplementary data (detailed experimental procedures and characterization data of compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2016.01.057.

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