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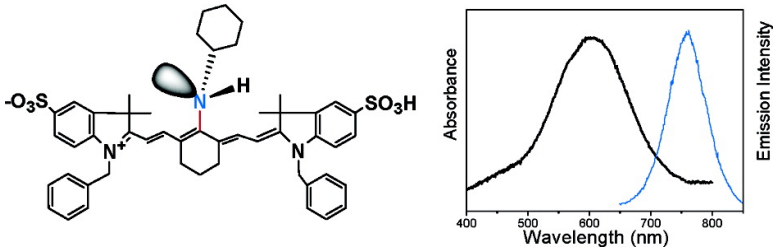

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Heptamethine Cyanine Dyes with a Large Stokes Shift and Strong Fluorescence: A Paradigm for Excited-State Intramolecular Charge Transfer

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Heptamethine cyanine dyes employed as fluorescence labels and sensors of biomolecules in vivo[1] have attracted immense interest because their spectra reach the near-infrared (NIR) region, where a biological matrix exhibits the least absorption and autofluorescence background. However, most polymethine cyanine dyes have the fatal disadvantage that their Stokes shifts are less than 25 nm. A small Stokes shift can cause self-quenching and measurement error by excitation light and scattered light.[2,3] Both of these can decrease the detection sensitivity to a great extent. Therefore, NIR dyes with a larger Stokes shift are very promising for NIR fluorescence bioassays.

Gabor Patonay et al. developed heptamethine cyanine dyes with a rigid chlorocyclohexenyl ring in the methine chain[4] which can increase the photostability and enhance the fluorescence quantum yield.[3] This structure also provides the dye with a reactive site for chemical substitution at the central ring. Recently, many heptamethine cyanine dyes used as biosensors and fluorescent probes have been obtained by nucleophilic substitution reactions at the central position. Phenol[1] and thiophenol[5] are often employed to replace the chlorine atom in these dyes, but the resulting enol or thioenol ether bond in these molecules is chemically unstable, which will result in the failure to detect fluorescence and will be responsible for the appearance of smearing in the sequence ladder when the dyes are used in DNA sequencing.[6]

In this paper, new heptamethine cyanine dyes (**1a**,**b**) containing robust C−N bonds were synthesized from dye **2** by an apparent $S_{NR}1$ reaction (Figure 1). Surprisingly, compared with common cyanine dyes, such as **2**, the new dyes have some important features, such as a large Stokes shift (>140 nm) and considerably stronger fluorescence. This might be attributed to an excited-state intramolecular charge transfer (ICT) between the donor and acceptor in the dyes.

Better photostability has been found previously in their parent dye **2** when linear alkyl groups were displaced with benzyl groups on the nitrogen atoms of 3H-indo rings.[7] Dyes (**1a**,**b**) contain two sulfonate groups that can provide a sphere of solvation in aqueous solvents. This brings better water solubility, which can prevent dyes from aggregating. Better water solubility is very useful when they are applied to biological analysis as probes in an aqueous environment.

The new dyes (**1a**,**b**) are completely different from dye **2** in their spectral properties (Figure 2 and Table 1). They have broader spectra and much stronger fluorescence than dye **2**. The maximum absorption wavelengths of the dyes (**1a**,**b**) exhibit a large blue shift (from 783 to ~600 nm in water), and a large Stokes shift (>140 nm) occurs. These phenomena were not found in other heptamethine dyes.

There are three features in the spectra of the new dyes: a large Stokes shift, broad and fairly structureless fluorescence spectra, and no mirror image relationship between the absorption and fluorescence spectra. These features may result from an intramolecular charge transfer (ICT)[10] or excited-state proton transfer.[11] However, further investigation proved that an excited-state ICT should take place in this case.[12]

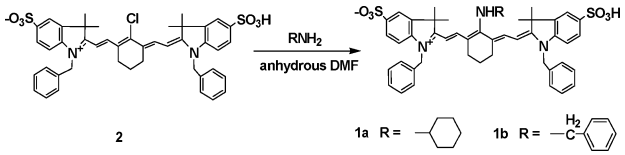

Figure 1. Synthetic scheme for the heptamethine cyanine dyes.

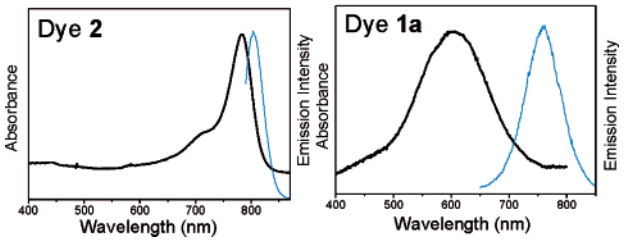

Figure 2. Absorption (black) and emission (blue) spectra of dye **2** (left) and **1a** (right) in water.

Table 1. Photophysical Characteristics of Dyes in Water at 1 × 10^{-6} M

dye	absorption λ_{ab} (nm)	emission λ_{em} (nm)	Stokes shift (nm)	ϵ ($\times 10^5$)[a]	ϕ_f[b]
1a	602	757	155	0.5	0.47
1b	617	757	140	0.7	0.38
2	783	803	20	2.0	0.17

[a] Molar extinction coefficients are in cm^{-1} M and in the maximum of the highest peak. [b] The fluorescence quantum yields of **1a**,**b** were determined in methanol in reference to rhodamine B ($\phi_f = 0.69$ in methanol),[8] while dye **2** in methanol was in reference to IR-125 ($\phi_f = 0.13$ in DMSO).[9]

A widely used criterion to identify a charge-transfer state is whether dyes have a strong solvatochromism.[13] In contrast with dye **2**, a marked negative solvatochromism (58 nm shift from water to acetone) in the absorption spectrum of dye **1a** was observed with increasing polarity of the solvents, but no apparent solvatochromism in the emission spectra (see Table 2). These properties might be due to hydrogen-bonding interaction between the solvents and the dye molecule. Similar results were also found in styryl pyridinium dyes, in which the ICT interaction was reported to exist between the dimethylamino group and the pyridinium moiety.[14]

One important structural change accompanying ICT[15] is that the pyramidal arrangement of the bridgehead amine in the ground state (Figure 3) is considerably flattened in the ICT state. In detail, a locally excited (LE) state (pyramidal geometry) is formed after excitation (Figure S4 in the Supporting Information), and then it is transformed into an ICT state (planar configuration).[16]

It is suggested that the rate of the transfer from LE to ICT is lowered in viscous polar solvents, such as glycerol, and LE emission is dominant.[17] Here, the transfer was observed in the changes of the fluorescence spectrum versus glycerol percent in ethanol when dye **1a** was excited at 490 nm (Figure 4). When "coagulated" in glycerol, the amine configuration still remains pyramidal in the

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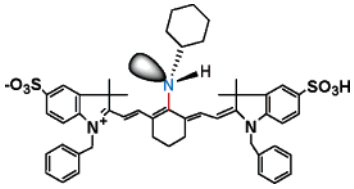

Figure 3. Pyramidal geometry of the central nitrogen atom when dye **1a** is in the ground state and LE state.

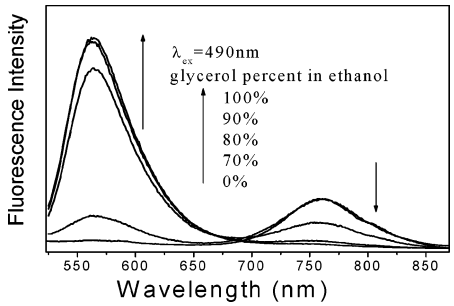

Figure 4. Fluorescence emission spectra of dye **1a** excited at 490 nm in glycerol/ethanol.

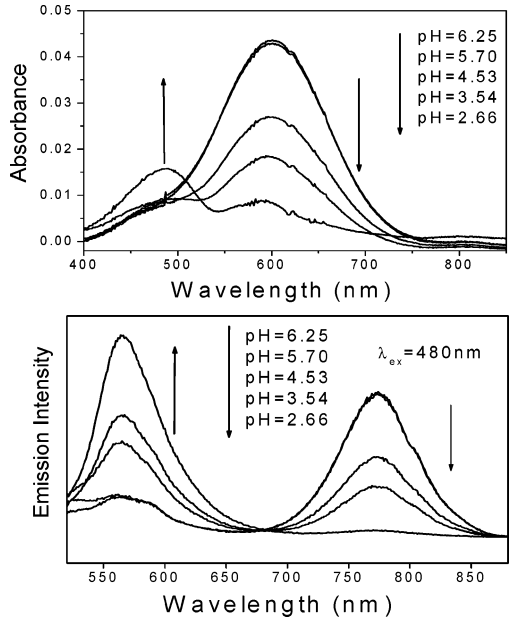

Figure 5. Absorption (above) and emission (below, $\lambda_{ex} = 480$ nm) spectra of dye **1a** in different pH buffer water solution. The pK_a is 4.49 (Figure S8 in the Supporting Information).

excited state, and emission is only derived from the LE state. However, no apparent change was observed in the absorption spectra.

To further investigate the configuration change at the bridgehead amine, another protonation experiment was carried out. It was found that in the absorption spectra, the longer wavelength band (ICT transition) near 600 nm became weak and a shoulder peak at 490 nm appeared when pH was decreased. At the same time, the transfer from ICT emission to LE emission occurred again (Figure 5). It can be easily understood that the amine is quaternarized in strong acid conditions, and the pyramidal arrangement does not change at the whole process. So, ICT emission disappears.

Though the ICT emission becomes weak in the acid and viscous media, such high quantum yields of ICT emission in the polar solvent and the neutral buffer solution are exceptional. Moreover,

Table 2. Solvatochromism Data of Dyes **1a** and **2** (in parentheses) at 1×10^{-6} M

solvents	$E_T(30)^a$	absorption λ_{ab} (nm)	emission λ_{em} (nm)	Stokes shift	$\phi_f{}^b$
water	63.1	602 (783)	757 (803)	155	0.07
methanol	55.4	623 (791)	743 (810)	120	0.47
ethanol	51.9	631 (796)	749 (813)	118	0.44
2-propanol	48.4	640 (800)	752 (815)	112	0.43
acetonitrile	45.6	640 (793)	753 (813)	113	0.31
acetone	42.2	660 (803)	761 (819)	101	0.25

[a] $E_T(30)$ is the polarity parameter of the solvent. [b] The fluorescence quantum yields were determined in methanol in reference to rhodamine B ($\phi_f = 0.69$ in methanol).

for negative solvatochromic dyes, strong fluorescence like this case (Table 2) is rarely found, too.[18]

In conclusion, we have synthesized two heptamethine cyanine dyes by introducing alkylamino groups at the central position. The new dyes are suggested to be a new paradigm for excited-state ICT. Significantly, they have a large Stokes shift (>140 nm) and strong fluorescence which make them more suitable for use as fluorescence probes than do common cyanine dyes.

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Supporting Information Available: Synthetic details and other spectroscopy data. This material is available free of charge via the Internet at http://pubs.acs.org.

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