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Novel Heptamethine 3H-Indocyanines and their Spectral Properties

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Abstract: Novel heptamethine 3H-indocyanine dyes are synthesized and embedded into a matrix of silica gel derived from tetraethoxysilicane. The photophysical properties of these near infrared dyes in various solvents and in SiO_2 sol gel were investigated. The results show that the dyes containing cyclohexenylene bridge and N-(*p*-carboxy)benzyl groups have better photostability and longer absorption wavelength than those containing linear heptamethine bridge and/or N-(5-carboxy)pentanyl groups. The absorption maxima of these dyes are in reverse proportion to the polarity of the solvents. The microenvironment of the dyes in SiO_2 sol-gel characters medium polarity (between methanol and DMF) according to the absorption maxima.

Keywords: Near-infrared fluorescent cyanine, *p*-carboxybenzyl, spectral properties, bioanalysis.

Near-infrared dyes are becoming more and more important in modern technologies. In most of the applications, the dyes are used in solid-state matrix. To investigate some properties of the dyes, many dyes were embedded into silica matrix *via* the sol-gel[1,2]. Levy *et al*. had incorporated near-infrared fluorescent cyanine dye HITC into sol-gel glasses[3]. But stability of the dye was a problem. Patonay[4] has improved the stability with chlorocyclohexenylene, instead of linear "tricarbo-" bridge between two 3H-indo-moieties in dye molecules. In our recent research[5], we have discovered that the photo-stability have been further improved by introducing N-(*p*-carboxy)benzyl group (**Scheme 1**) in heptamethine indocyanines. In this paper, we report the synthesis of some novel heptamethine 3H-indocyanine dyes with this kind of N-(*p*-carboxy)benzyl group **1** and **2a** and their spectral properties in solutions and in SiO_2 sol-gel material.

Mass spectral determinations were made on HP1100 API-ES mass spectrometry. NMR spectra were recorded in D_2O on a VARIAN 400 MHz NMR spectrometer. Fluorescence measurements were performed using a PTI-C-700 Felix and Time-Master system. The purification of the dyes was performed by conventional column chromatography on C18-RP absorbent. Water-methanol mixtures were used for elution. Visible spectra were measured on a HP-8453 spectrophotometer. 6-Bromohexanoic acid and N-(5-anilino-2,4-pentadienylidene) aniline hydrochloride **a** were purchased from Acros Chemical Co..

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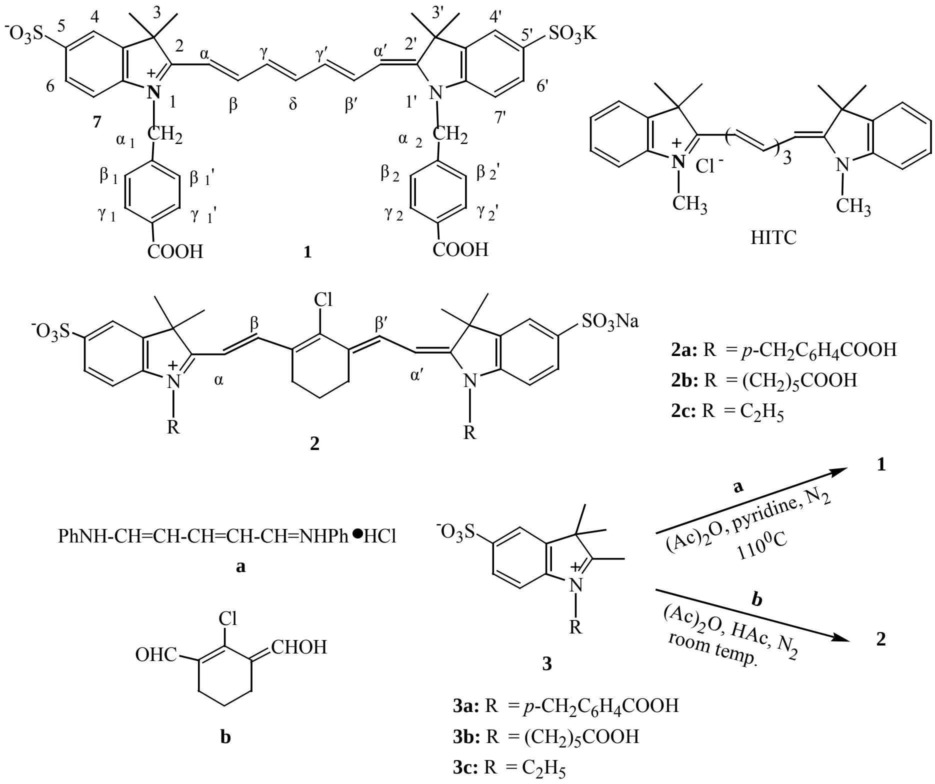
Scheme 1 Synthesis of the near infrared heptamethine indocyanines



As shown in **Scheme 1**, dyes **1** and **2** were synthesized by condensation of intermediates **3** with condensating agents **a** or **b**, respectively. **3a~3c** and condensating agents **b** were prepared according to the procedure described by reference 5 and 6. Compound **1**: ^{1}H NMR (D_2O, δ ppm) 7.91-7.83 (m, 6H, 4,4′, Ar-H in N-*p*-carboxybenzyl group), 7.72-7.67 (m, 4H, H- *β*, *β′* and H- 6,6′), 7.37-7.30 (m, 1H, H- *δ*), 7.22-7.20 (d, 4H, Ar-H in N-*p*-carboxybenzyl group), 7.10-7.08 (d, 2H, J = 8.0 Hz H- 7,7′), 6.17-6.10 (t, 2H, H- *γ*, *γ′*), 6.07-6.04 (d, 2H, H- *α*, *α′*), 5.24 (s, 4H, CH_2 in N-*p*-carboxybenzyl group), 1.56 (s, 12H, $C(CH_3)_2$). ESI-MS: $[M-K]^-$ (*m/z* 806.5), $[M-K-H]^{2-}$ (*m/z* 402.9), $[M-K-2H+Na]^{2-}$ (*m/z* 413.9), $[M-K-2H]^{3-}$ (*m/z* 268.3), $[M-K-2H+2Na]^-$ (*m/z* 850.9). Compound **2a**: ^{1}HNMR (D_2O, δ ppm) 8.01-7.97 (d, 2H, J = 13.6 Hz, H- *β*, *β′*), 7.85 (m, 6H, H- 4,4′, Ar-H in N-*p*-carboxybenzyl group), 7.75-7.73 (d, 2H, H- 6,6′), 7.26-7.23 (m, 6H, H- 7,7′, Ar-H in N-*p*-carboxybenzyl group), 6.03-5.99 (d, 2H, J = 14.0 Hz, H- *α,α′*), 5.31 (s, 4H, CH_2 in N-*p*-carboxybenzyl group), 2.09 (m, 4H, CH_2 in the cyclohexene), 1.51 (s, 12H, $C(CH_3)_2$), 1.38 (m, 2H, CH_2 in the cyclohexene). ESI-MS: $[M-Na]^-$ (*m/z* 881.8), $[M-Na-H]^{2-}$ (*m/z* 440.3), $[M-Na-2H]^{3-}$ (*m/z* 293.1).

Tetraethyl silicate, ethanol, distilled water (2: 4: 1 mol) and hydrogen chloride were mixed at room temperature, hydrolyzing for 1 h, then the aqueous solution of dye was added. The pH value of the solution was adjusted to 8 with diluted $NH_3 \cdot H_2O$, several weeks later, the homogeneous transparent dyes doped SiO_2 gel slices were prepared[4,5].

Table 1 Spectral data of the heptamethine indocyanines in solvents

Dye	Solvent	λ_{max}^{ab} (nm)	λ_{max}^{em} (nm)	Stock's shift $\Delta\lambda$(nm)	ε ($L{\cdot}mol^{-1}{\cdot}cm^{-1}$)
1b	water	749	787	38	170020
	methanol	755	790	35	
	DMF	767	803	36	
2a	water	784	805	19	104070
	methanol	795	818	23	
	DMF	809	820	11	
2b	water	783	812	29	170810
	methanol	791	825	34	
	DMF	804	821	17	
2c	water	778	809	31	220020
	methanol	785	815	30	
	DMF	800	819	19	

Table 2 Spectral data of the indocyanines in SiO_2 sol-gel

Dye	**1b**	**2a**	**2b**	**2c**
$\lambda^{ab}{}_{max}$ (nm)	760	794	793	789
$\lambda^{em}{}_{max}$ (nm)	788	821	811	808

The dyes showed absorption (λ_{max}^{ab}) and emission maxima (λ_{max}^{em}) in 745-830 nm (**Table 1**). The dyes **2a**, **2b** and **2c** with cyclohexenylene group in water have a 30-35 nm red-shift compared with the linear tricarbocyanine **1** (749 nm in water). Stockes shift is in the range 11-38 nm. The λ_{max} changed with the solvent polarity: more polar the solvent, shorter λ_{max} is. For example, λ_{max}^{ab} of **2a** in water appeared at 784 nm, 795 in methanol and 809 nm in DMF. The increase in λ_{max}^{ab} of the dyes with a decrease in solvent polarity might be due to the different solvation of the ground and excited states of these dyes. This suggests that the molecules have highly dipolar ground states and relatively low polar excited states.

Table 2 shows the λ_{max}^{ab} and λ_{max}^{em} of dye/SiO_2 sol-gel. There are red-shifts in λ_{max}^{ab} compared with those in water, but blue-shifts compared with in DMF. More intensive emissions were observed than in solvents. It can be attributed to the quantum size confinement effect of nanometer microcapsule in SiO_2 gel networks. It seems that the polarity of the microenvironment of the dyes in SiO_2 gel material is between methanol and DMF, as the main factor for the shift of λ_{max}^{ab} is the polarity of environment.

The photostabilities of **1**, **2a**, **2b** in water were tested under the irradiation of a 40 W iodine-tungsten lamp. The sample was placed 125 cm away in a glass holder, maintaining temperature at 25°C with circulating water. After irradiation for 75 h, the absorption intensity of **1** decreased 12 %, **2a** 4 % and **2b** 7 %, respectively. The dye **2a** was the most stable. Similar photo-stability order was observed in dye-doped in SiO_2 gel slices. The degradation is from the photo-oxidation of the dyes by singlet oxygen, superoxides, peroxides or other active oxidative species. With the change of N-(5-carboxy)pentanyl group on the nitrogen atom in 3H-indo-ring in **2b** into N-(*p*-carboxy)benzyl in **2a**, the sterichindrance in molecule must increase. This might help **2a** to prevent the attack of the active oxidative species. The rigid cyclohexenylene in the molecule of **2a** and **2b** made the dyes more photostable than linear polymethine in **1b**. Generally, as the quantum size confinement effect, dyes doped in SiO_2 gel material have better photostability than dissolved in solvents.

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