

Synthesis and Spectral Properties of Novel Water-soluble Near-infrared Fluorescent Indocyanines

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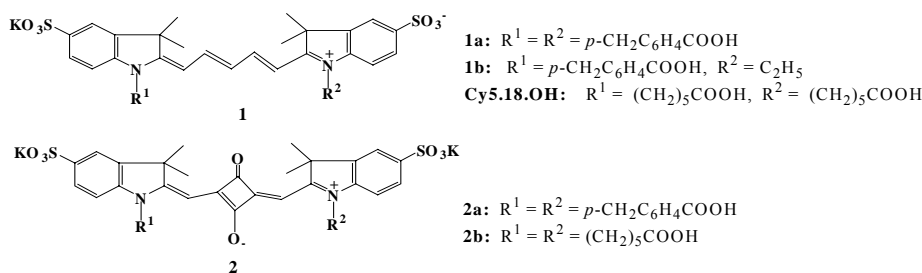
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Abstract: Two fluorescent pentamethine and a squarilium indocyanines containing at least one *p*-carboxybenzyl group on N atoms in the heterocyclic rings were synthesized. They had good water solubility and photostability. Their maximum absorption and maximum emission were 600-700 nm in water. When it was anchored onto nanostructured TiO₂ electrode, compared with in water, the squaraine showed double absorption peaks (one blue shifted and another red shifted) and absorption intensity of the red shift peak increased with the increase of the time of irradiation. The intensity of the blue one decreased simultaneously. We proposed that the presence of two electronic charge forms of squaraine anchored on the TiO₂ film might be the reason.

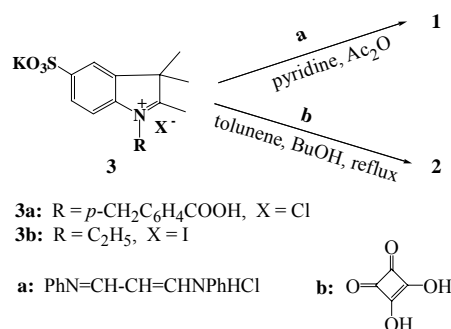
Keywords: Indocyanine, *p*-carboxybenzyl, near-infrared, fluorescence, bioanalysis, solar cell.

On the basis of the trimethine indocyanines synthesized before¹, we synthesized pentamethine indocyanines **1a** and **1b** containing at least one *p*-carboxybenzyl group on the nitrogen atoms in the heterocyclic rings, their absorption and emission maxima were in near-infrared range. For improving the photostability of these dyes further, we introduced rigid ring into the methine chain, and synthesized another squaraine **2a**. To introduce the *p*-carboxybenzyl group to N atom of the dye for that such group can link covalently with biological substrates, which contain NH₂ groups, and also for making the dye easierly anchor on the nanostructured TiO₂ electrodes in solar cells. Spectral properties of the dyes in solvents and dye-sensitized the TiO₂ film were investigated.



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Scheme 1



As shown in **Scheme 1**, dyes **1** and **2** were obtained by condensation of intermediates **3** with **a** or **b**, respectively. Intermediate **3** was prepared according to reference 1. The NHS-carboxyl squaraine was prepared from the esterification of N-hydroxysuccinimide with squaraine **2a**, and the formation of the succinimidyl active ester was confirmed by its reaction with taurine in bicarbonate buffer² (pH 9.5).

Dye-coating of TiO₂ film was carried out by soaking the film in the dye solution for 2-3 h, washed the film with water three times, then kept it dry³.

Tetraethyl silicate, ethanol, distilled water (2: 4: 1 mol) and hydrogen chloride were mixed at room temperature, hydrolyzing for 1h, then the aqueous solution of dye was added. The pH value of the solution was adjusted to 8 with diluted NH₃·H₂O, several weeks later, homogeneous transparent dyes doped SiO₂ gel slices were prepared^{4,5}.

¹H NMR spectra were recorded in D₂O on a VARIAN 400 MHz spectrometer. Mass spectral determinations were made on HP1100API-ES mass spectrometry. Fluorescence measurements were performed using a PTI-C-700 Felix (U.S.A.). Purification of the dyes was performed on a C18-RP powder (40-75 μm) column. Water-methanol mixtures (0-4:1) were used as elution in all experiments. UV-Vis spectra were measured on a HP-8453 spectrophotometer. Absorbances of dye adsorbed on TiO₂ films were recorded with a bare TiO₂ film as a reference. Squaric acid and 6-bromohexanoic acid were purchased from ACROS Chemical Co.(U.S.A.). The nanostructured TiO₂ film electrode was a gift from Professor Licheng Sun (Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm, Sweden). The thickness of the transparent TiO₂ film electrode, recorded by Dektak3 Surface Profile Measuring System, was about 7.6 μm.

1a: ¹H NMR (D₂O, δ ppm) 8.05-7.99 (t, 2H, *J* = 25.6 Hz, β,β' proton of the bridge), 7.90 (s, 2H, H-4,4'), 7.83-7.81 (d, 4H, *J* = 8.0 Hz, H-γ₁,γ₁'), 7.78-7.76 (d, 2H, *J* = 8.0 Hz, H-6,6'), 7.29-7.27 (d, 4H, *J* = 8.0 Hz, H-β₁,β₁'), 7.26-7.27 (d, 2H, *J* = 8.0 Hz, H-7,7'), 6.35 (t, 1H, γ proton of the bridge), 6.23-6.20 (d, 2H, *J* = 14.0 Hz, α,α' proton of the bridge), 5.34 (s, 4H, H-α₁,α₁'), 1.72 (s, 12H, C(CH₃)₂). ESI-MS: [M -2H+K]⁻ (*m/z* = 819.5), [M-H]⁻ (*m/z* = 781.7), [M-K-H]²⁻ (*m/z* = 390.7), [M-K-2H]³⁻ (*m/z* = 260.1). **1b:** ¹H NMR (D₂O, δ ppm) 8.08-8.03 (m, 2H, β, β' proton of the bridge), 7.92 (s, 2H, H-4,4'), 7.90-7.88 (d, 1H, *J* = 8.0 Hz, H-6), 7.85-7.83 (d, 2H, *J* = 8.0 Hz, H-γ₁,γ₁'), 7.76-7.74 (d,

1H, $J = 8.0$ Hz, H-6'), 7.42-7.40 (d, 1H, $J = 8.0$ Hz, H-7), 7.35-7.33 (d, 2H, $J = 8.0$ Hz, H- β_1, β_1'), 7.23-7.21 (d, 1H, $J = 8.0$ Hz, H-7'), 6.53-6.47 (t, 1H, γ proton of the bridge), 6.41-6.38 (d, 1H, $J = 12.0$ Hz, α proton of the bridge), 6.20-6.16 (d, 1H, $J = 16.0$ Hz, α' proton of the bridge), 5.34 (s, 2H, H- α_1), 4.12-4.05 (m, 2H, H- α_2), 1.76, 1.69 (s, 12H, C(CH₃)₂), 1.36-1.33 (m, 3H, H- β_2). ESI-MS: [M+K]⁺ ($m/z = 675.3$), [M-K-H]²⁺ ($m/z = 337.1$). **2a**: ¹H NMR (D₂O, δ ppm) 7.88 (s, 2H, H-4,4'), 7.84-7.82 (d, 4H, $J = 8.0$ Hz, H- γ_1, γ_2), 7.76-7.74 (d, 2H, $J = 8.0$ Hz, H-6,6'), 7.33-7.31 (d, 4H, $J = 8.0$ Hz, H- β_1, β_2), 7.25-7.23 (d, 2H, $J = 8.0$ Hz, H-7,7'), 5.87 (s, 2H, α, α' protons of the bridge), 5.39 (s, 4H, H- α_1, α_2), 1.69 (s, 12H, C(CH₃)₂). ESI-MS: [M+Na-H]⁺ ($m/z = 922.5$), [M-K]⁻ ($m/z = 861.0$), [M-2K]²⁻ ($m/z = 411.3$), [M-2K+Na]⁻ ($m/z = 846.2$), [M-2K-H]³⁻ ($m/z = 273.9$), [M-2K-2H]⁴⁻ ($m/z = 205.1$).

Table 1 Spectral data of the indocyanine dyes in solvents

Dye No.	Solvent	$\lambda^{ab}max$ (nm)	$\lambda^{em}max$ (nm)	ϵ (L.mol ⁻¹ .cm ⁻¹)
1a	water	649	669	180000
	methanol	655	680	
	DMF	666	691	
1b	water	645	664	170000
	methanol	648	671	
	DMF	658	680	
2a	water	631	641	280000
	methanol	639	650	
	DMF	656	675	

Figure 1 Absorption spectrum of **2a** in water

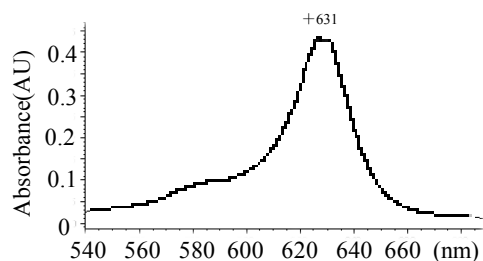
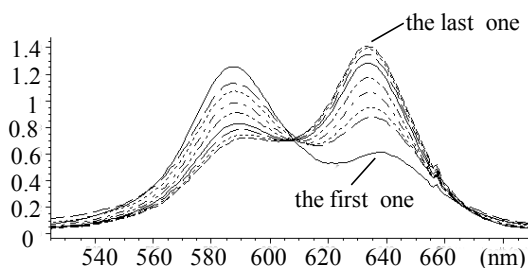


Figure 2 Absorption spectra of the TiO₂ film coated with **2a** for different times of irradiation.



Dyes showed absorption and emission maxima in 600-700 nm (**Table 1**). Compared with those of the trimethine dyes synthesized before, the absorption and emission maxima of **1a**, **1b** and **2a** were about 100 nm red shifts.

Absorption spectra of the nanostructured TiO₂ film coated with squaraine **2a** were found. Two very broad bands covering from 540 to 660 nm were observed, as shown in **Figure 2**. Compared with that in water (631 nm in **Figure 1**), one band blue shifted (587 nm), another red shifted (638 nm). The absorption intensity of the blue shift is stronger than that of the red shift at the beginning of the testing, then with the increase of the irradiation time the absorption intensity of the red shift peak increased, but the intensity of the blue shifted band decreased at the same quantity. The absorption spectrum of squaraine **2a** doped in SiO₂ gel only had a red shift.

The absorption spectra of **1a**, **1b** and **2b** adsorbed on TiO₂ film or doped in SiO₂ gel only exhibited a red shift compared with those in water.

These indicated that the double absorption peaks of **2a** adsorbed on TiO₂ film were produced by the result of the interactions among the nanostructured TiO₂ film, *p*-carboxybenzyl group and the squaric ring.

The formation of the squaraine **2a** NHS and its combination with taurine indicated that the dye could couple covalently to biomass containing free NH₂ group, and can be expected to use in bioanalysis as fluorescent labeling reagent.

The photostability of **1a**, **1b**, **2a** and **2b** was tested under irradiation (40 W light) about 125 cm away, being sealed in test tubes in air. After 75 h, the absorption intensity of **1a** decreased 8 %, **1b** 9 %, **2a** 5 % and **2b** 11 %, respectively. The dye **1a**, **1b** and **2a** become more stable when substituents on the nitrogen atoms in heterocyclic rings were *p*-carboxybenzyl and squaric ring was introduced in the methine chain, due to the steric hindrances of **1a**, **1b** and **2a** were increased to prevent the attack of singlet oxygen.

Acknowledgments

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