

## Novel Water Soluble Fluorescent Trimethine Indocyanines Containing N-*p*-Carboxybenzyl Group

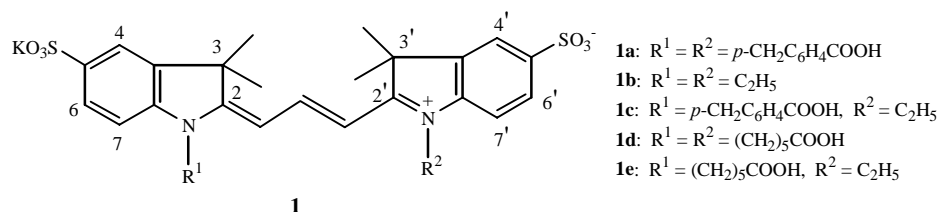
Li Qiu WANG, Xiao Jun PENG\*, Rong ZHANG, Guo Quan XU, Jing Nan CUI

State Key Laboratory of Fine Chemicals, Dalian University of Technology, Dalian 116012

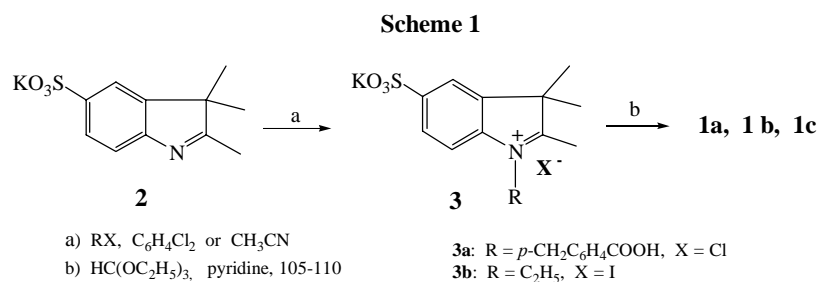
**Abstract:** Two fluorescent indocyanine dyes containing at least one *p*-carboxybenzyl group on the nitrogen atoms in the heterocyclic rings were designed and synthesized. Their absorption maxima were 549 nm and 551 nm in water respectively. They had good water solubility and photostability.

**Keywords:** Sulfoindocyanine, fluorescent dyes, biological label.

Indocyanine dyes as fluorescent labeling reagents have been used in bioanalysis<sup>1,2</sup>. Arylsulfonate indocyanine dyes **1d** and **1e** developed by Waggoner A. S. *et al.*<sup>3,4</sup> have excellent fluorescent properties combined with good aqueous solubility, are a new generation of fluorescent label compounds for proteins, oligonucleotides and other compounds containing hydroxyl, mercaptol or primary amine groups<sup>5</sup>. However, photostability of these dyes has been a problem. Z. G. YAO *et al.*<sup>6</sup> reported that polymethine indocyanine dyes containing N-benzyl substituents in the heterocyclic rings were more photostable than the indocyanine dyes containing N-*n*-alkyl. Combining the fluorescent probes **1d** and **1e** with Yao's research, we designed the substituent as *p*-carboxybenzyl (*p*-CH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>COOH) on the nitrogen atoms in the heterocyclic rings, and synthesized novel sulfoindocyanine dyes **1a** and **1c**. As a linker for combining with biological substrates in the next application reactions, the substituent of carboxyl group was introduced on the nitrogen atom. Spectral properties of these dyes were investigated.



\*E-mail: [visitor@dlut.edu.cn](mailto:visitor@dlut.edu.cn)



As shown in **Scheme 1**, compound **2** prepared by Fisher indole synthesis<sup>4</sup> reacted with ethyl iodide in acetonitrile or *p*-(chloromethyl)benzoic acid in 1,2-dichlorobenzene to form intermediate **3a** or **3b**. The symmetrical dyes **1a** and **1b** were obtained in 40-50% yield by selfcondensation of **3a** or **3b** with triethyl orthoformate in pyridine, respectively. Condensation of equimolar portions **3a** and **3b** with triethyl orthoformate afforded a mixture of three sulfoindocyanine dyes **1a**, **1b** and **1c**, their relative quantity was 1:2:2. The unsymmetrical dye **1c** was isolated in 35% yield by preparative HPLC on reverse-phase C18 using a water-methanol mixture as eluent.

<sup>1</sup>H NMR spectra were recorded in D<sub>2</sub>O on a Varian 400 MHz <sup>1</sup>H NMR spectrometer. Mass spectral determinations were made on HP1100API-ES mass spectrometry. **1a**: <sup>1</sup>H NMR (D<sub>2</sub>O ppm), δ 8.34 (m, 1H, βproton of the bridge), 7.93 (s, 2H, 4, 4'-H), 7.79-7.77 (d, 2H, *J* = 8.0 Hz, 6, 6'-H), 7.69-7.67 (d, 4H, *J* = 7.2 Hz, γ<sub>1</sub>, γ<sub>1</sub>'-H), 7.26-7.24 (d, 2H, *J* = 8.0 Hz, 7, 7'-H), 7.11-7.09 (d, 4H, *J* = 7.2 Hz, β<sub>1</sub>, β<sub>1</sub>'-H), 6.18-6.15 (d, 2H, *J* = 13.5 Hz, α, α' protons of the bridge), 5.30 (s, 4H, α<sub>1</sub>, α<sub>1</sub>'-H), 1.62 (s, 12H, C(CH<sub>3</sub>)<sub>2</sub>). ESI-MS: [M-K]<sup>-</sup> (*m/z* = 755.7), [M-K-H]<sup>2-</sup> (*m/z* = 377.0), [M-K-2H]<sup>3-</sup> (*m/z* = 251.4). **1c**: <sup>1</sup>H NMR (D<sub>2</sub>O ppm), δ 8.45 (m, 1H, β proton of the bridge), 7.93 (s, 2H, 4, 4'-H), 7.89-7.87 (d, 2H, *J* = 8.4 Hz, γ<sub>1</sub>-H), 7.80-7.78 (d, 2H, *J* = 8.4 Hz, 6, 6'-H), 7.37-7.35 (d, 2H, *J* = 8.4 Hz, β<sub>1</sub>-H), 7.30-7.28 (d, 2H, *J* = 8.4 Hz, 7, 7'-H), 6.34-6.26 (q, 2H, α, α' protons of the bridge), 5.43 (s, 2H, α<sub>1</sub>-H, N-CH<sub>2</sub>), 4.10 (q, 2H, α<sub>2</sub>-H, N-CH<sub>2</sub>), 1.91 (s, 12H, C(CH<sub>3</sub>)<sub>2</sub>), 1.31 (t, 3H, β<sub>2</sub>-H, -CH<sub>3</sub>). ESI-MS: [M-H]<sup>-</sup> (*m/z* = 687.0), [M-K]<sup>-</sup> (*m/z* = 649.0), [M-K-H]<sup>2-</sup> (*m/z* = 324.0).

**Table 1** Fluorescent spectral data of the indocyanine dyes in solvents

Dye No.	Solvent	λ <sup>ab</sup> max (nm)	λ <sup>em</sup> max (nm)	ε (L.mol <sup>-1</sup> .cm <sup>-1</sup> )
<b>1a</b>	water	551	565	112745
	methanol	561	576	
	ethanol	567	580	
<b>1b</b>	water	547	560	110076
	methanol	552	568	
	ethanol	557	570	
<b>1c</b>	water	549	561	125106
	methanol	554	570	
	ethanol	558	572	

Dyes **1a** and **1c** showed absorption and emission maxima in 540-590 nm (see **Table 1**). Generally, change of the substituent on the nitrogen atoms slightly changed the basic indocyanine absorption and fluorescence properties. Compared to the commercial dyes **1d** and **1e**<sup>4,5</sup>, the absorption and emission wavelengths of dyes **1a** and **1c** were relatively solvent sensitive, the bands of emission spectra exhibited a red shift of 16 nm for **1a** and 10 nm for **1c** from water to ethanol.

It was found that dyes **1a** and **1c** were more stable than **1d** by the qualitative test for their photostability. No noticeable change of absorption spectra of dye **1a** and **1c** was observed when they were stored in water solution saturated with N<sub>2</sub> under room-light irradiation in several weeks. However, the absorption intensity of **1d** decreased. Compared with **1d**, dyes **1a** and **1c** were with aromatic *p*-carboxybenzyl substituent on the nitrogen atoms in 3*H*-indole rings. The *p*-carboxybenzyl substituent may let the molecule more enduring or resistant to the attack by singlet oxygen than 5-carboxypentyl group in **1d**.

Dyes **1a** and **1c** dissolved easily in water, even at millimolar concentrations, with no evidence of new absorption bands that were indicative of dimers.

### Acknowledgments

The authors would like to thank the National Natural Science Foundation of China and The Ministry of Education of China for providing financial support for this project.

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Received 3 April, 2002