Novel asymmetric indodicarbocyanine dyes

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Novel indodicarbocyanine dyes functionalized at position 5 of the indolenine system were obtained and characterized by spectroscopic methods.

Key words: indodicarbocyanine dyes, indolenines, 4-(2-carboxyethyl)phenylhydrazine, fluorescence.

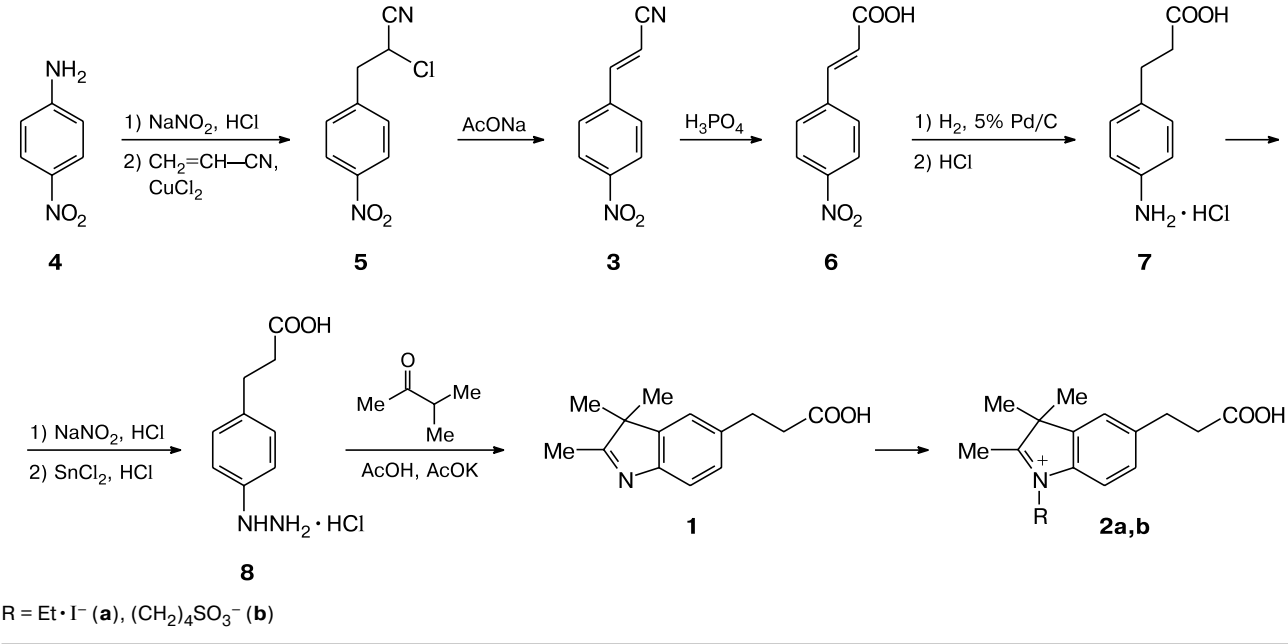
High-intensity fluorescence of indodicarbocyanine dyes in the near-IR range (600—800 nm) makes them most suitable markers for analysis of oligonucleotides and proteins in biological microchip technology.[1]

Indocyanine dyes with the carboxy group attached to the indolenine fragment in position 1 (through a polymethylene chain[2—4]) and position 5 (directly and through a methylene unit[5—8]) have been documented. However, these dyes have some drawbacks. A probe attached to the carboxy and carboxymethyl groups in position 5 of the indolenine ring is in close vicinity of the bulky fluorophore, which prevents complete formation of specific complexes between a compound to be analyzed and the fluorescent probe. The nature of the substituents and the locations of their binding to the fluorophore also have a strong effect on the properties of dyes and their complexes with biomolecules and, consequently, on the whole bioanalysis.[1,6]

The goal of the present work was to obtain a novel series of indodicarbocyanine dyes containing a 5-carboxyethylindolenine fragment. The synthesis of 5-(2-carboxyethyl)-2,3,3-trimethylindolenine (**1**) and its derivatives **2a,b** is shown in Scheme 1.

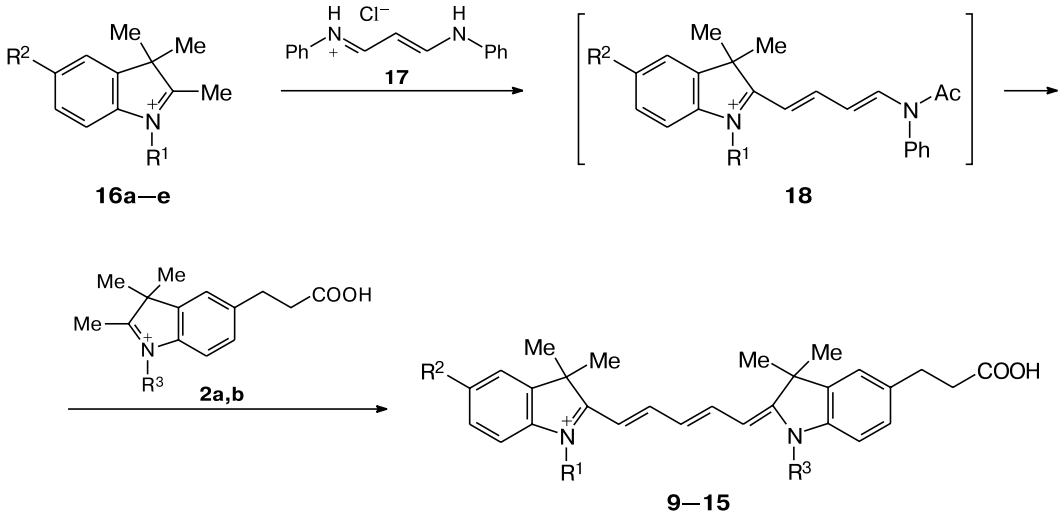
4-Nitrocinnamonitrile (**3**) was synthesized[9] from 4-nitroaniline (**4**) through 2-chloro-3-(4-nitrophenyl)propionitrile (**5**). Hydrolysis of compound **3** in conc. H_3PO_4 gave 4-nitrocinnamic acid (**6**). Catalytic hydrogenation of acid **6** on 5% Pd/C in AcOH followed

Scheme 1



Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 11, pp. 2186—2190, November, 2007.
1066-5285/07/5611-2263 © 2007 Springer Science+Business Media, Inc.

Scheme 2



16: R^1 = Et, R^2 = H (**a**); R^1 = Et, R^2 = SO_3^- (**b**); R^1 = $(CH_2)_4SO_3^-$, R^2 = H (**c**); R^1 = $(CH_2)_4SO_3^-$, R^2 = Me (**d**); R^1 = $(CH_2)_4SO_3^-$, R^2 = SO_3^- (**e**)

by hydrochlorination afforded 3-(4-aminophenyl)propionic acid hydrochloride (**7**) in 96% yield. Diazotization of compound **7** and reduction[10] of the resulting diazonium salt with $SnCl_2 \cdot 2H_2O$ in HCl gave 4-(2-carboxyethyl)phenylhydrazine hydrochloride (**8**).

Indolenine **1** was obtained by the Fischer cyclization[11] of compound **8** with isopropyl methyl ketone in AcOH in the presence of AcOK without isolating intermediate hydrazone. Alkylation of indolenine **1** with ethyl iodide or 4-hydroxybutane-1-sulfonic acid yielded the corresponding derivatives **2a,b**.

Dyes **9**—**15** were obtained by two-step condensation of appropriate indoleninium salts **16a**—**e** and **2a,b** with malonaldehyde dianil hydrochloride (**17**) in Ac_2O (Scheme 2, Table 1).

The first step involved condensation of indolenines **16a**—**e** (containing no carboxy group) with dianil hydrochloride **17**. Addition of acetyl chloride to the reaction mixture largely lowered the fraction of the symmetric dye. In the second step, indolenine **2a** or **2b** was added and condensation was carried out in the presence of ethyl(diisopropyl)amine or potassium acetate as con-

Table 1. Spectroscopic characteristics of indodicarbocyanine dyes **9**—**15**

Compound	R^1	R^2	R^3	Solvent	λ_{max}^{abs} / nm	λ_{max}^{em} / nm	$\varepsilon \cdot 10^{-5}$ /L mol^{-1} cm^{-1}	Φ
9	Et	H	$(CH_2)_4SO_3^-$	PBS	646	658	1.46±0.02	0.11
				MeOH	648	666	1.92±0.02	0.14
10	Et	SO_3^-	Et	PBS	648	662	1.54±0.02	0.09
				MeOH	650	670	1.43±0.03	0.11
11	$(CH_2)_4SO_3^-$	H	Et	PBS	646	660	1.52±0.03	0.11
				MeOH	648	665	1.56±0.02	0.16
12	$(CH_2)_4SO_3^-$	Me	$(CH_2)_4SO_3^-$	PBS	652	662	1.34±0.03	0.11
				MeOH	654	673	1.89±0.05	0.19
13	$(CH_2)_4SO_3^-$	H	$(CH_2)_4SO_3^-$	PBS	647	662	1.32±0.01	0.11
				MeOH	649	669	1.97±0.03	0.17
14	Et	SO_3^-	$(CH_2)_4SO_3^-$	PBS	648	656	1.72±0.01	0.08
				MeOH	650	668	1.62±0.02	0.14
15	$(CH_2)_4SO_3^-$	SO_3^-	$(CH_2)_4SO_3^-$	PBS	648	664	1.37±0.03	0.10
				MeOH	650	670	1.69±0.04	0.14

Note. λ_{max}^{abs} and λ_{max}^{em} are the absorption and fluorescence peak wavelengths, respectively; ε is the molar absorption coefficient; Φ is the fluorescence quantum yield; PBS is a 0.01 *M* solution of a potassium phosphate buffer, 0.9% NaCl, pH 7.4.

densation agents. All the dyes obtained were isolated by reverse phase chromatography (RP-18 column, MeCN—0.05 *M* triethylammonium acetate buffer, gradient elution from 0 to 50% MeCN) and converted into sodium salts. The structures of all the compounds were confirmed by elemental analysis data, MALDI-TOF mass spectra, and ^{1}H NMR spectra.

For dyes **9**—**15**, we determined absorption and fluorescence peaks, molar absorption coefficients, and relative quantum yields of fluorescence in MeOH and a phosphate-salt buffer (see Table 1). Dyes **9**—**15** have good fluorescent characteristics, are thermally and chemically stable, and are resistant to light under bioanalysis conditions. Because of this, they can be successfully used as fluorescent markers in hybridization analysis. At present, dyes **9**—**15** are being additionally tested through biological microchip technology.

Experimental

^{1}H NMR spectra were recorded on a pulse Bruker AMX-400 Fourier spectrophotometer (Germany) (400 MHz) in $CDCl_3$ (with Me_4Si as the external standard) and DMSO-d_6.

MALDI-TOF mass spectra were recorded on a Compact MALDI 4 instrument (Kratos Analytical, USA).

UV spectra were recorded on a Jasco V-550 spectrophotometer (Japan); fluorescence spectra were recorded on a Shimadzu RF 5000 spectrofluorimeter (Japan). Melting points were determined on a Koffler Boetius hot stage (Germany).

All solvents were purified to meet specific requirements.

Column chromatography was carried out on Lichroprep RP-18 (particle size 0.040—0.063 nm; Merck, Germany) in two columns 16×400 mm and 12×100 mm.

Indolenine bases **16a**—**e**,[2,4,8] malonaldehyde dianil,[12] 2-chloro-3-(4-nitrophenyl)propionitrile (**5**),[8] and 4-nitrocinnamonitrile (**3**)[8] were prepared as described earlier.

4-Nitrocinnamic acid (6). A mixture of 4-nitrocinnamonitrile (**3**) (17.4 g, 0.1 mol) and conc. H_3PO_4 (350 mL) was heated at 150 °C for 2 h. On cooling to ~20 °C, the mixture was poured into ice water (4 L). The precipitate of crude product **6** (18.3 g) was filtered off, dissolved in 0.12 *M* NaOH (1 L), and treated with activated charcoal (3.5 g). The resulting solution was filtered and the filtrate was acidified to pH ~2 by adding dropwise conc. HCl. The precipitate that formed was filtered off, washed with water, and dried at 90 °C. The yield of 4-nitrocinnamic acid (**6**) was 17.7 g (92%), yellow needles, m.p. >250 °C (*cf.* Ref. 9: m.p. 283—284 °C). Found (%): C, 55.92; H, 3.71; N, 7.28. $C_9H_7NO_4$. Calculated (%): C, 55.96; H, 3.65; N, 7.25. ^{1}H NMR (DMSO-d_6), δ: 6.74 (d, 1 H, CHC<u>H</u>COOH, J = 16.5 Hz); 7.69 (d, 1 H, C<u>H</u>CHCOOH, J = 16.5 Hz); 7.96—8.25 (d, 4 H, Ar, J = 9 Hz).

3-(4-Aminophenyl)propionic acid hydrochloride (7). A vigorously stirred mixture of the catalyst 5% Pd/C (400 mg), acetic acid (80 mL), and 4-nitrocinnamic acid (**6**) (3.9 g, 20 mmol) was hydrogenated in a hydrogen flow at ~1 atm and ~20 °C for 2.5 h. The reaction mixture was filtered and the filtrate was concentrated. The oily residue was dissolved in a mixture of 5 *M* HCl (7 mL) and water (30 mL) and treated with activated carbon under heating. The yield of 3-(4-aminophenyl)propionic acid hydrochloride (**7**) was 3.9 g (96%), m.p. 186—187 °C. Found (%): C, 53.68; H, 5.95; N, 7.02. $C_9H_{12}ClNO_2$. Calculated (%): C, 53.61; H, 6.00; N, 6.95. ^{1}H NMR (DMSO-d_6), δ: 2.53 (t, 2 H, $CH_2C\underline{H}_2COOH$, J = 7.5 Hz); 2.83 (t, 2 H, $C\underline{H}_2CH_2COOH$, J = 7.5 Hz); 7.28—7.35 (d, 4 H, Ar, J = 8.5 Hz); 10.31 (s, 2 H, NH_2).

4-(2-Carboxyethyl)phenylhydrazine hydrochloride (8). A three-neck flask fitted with a mechanical stirrer and a thermometer was charged with 3-(4-aminophenyl)propionic acid hydrochloride (**7**) (2.0 g, 10 mmol) and conc. HCl (35 mL), and the mixture was cooled. Then a solution of $NaNO_2$ (0.7 g, 10 mmol) in water (0.9 mL) was added dropwise under the layer of the liquid. The addition rate was such as to prevent a rise of the reaction temperature above −5 °C. The mixture was warmed to 0 °C for 30 min and then recooled to −5 °C. A solution of $SnCl_2 \cdot 2H_2O$ (10 g, 44 mmol) in conc. HCl (15 mL) was added precooled to −18 °C. The reaction temperature was maintained at no higher than −5 °C by regulating the addition rate. The mixture was additionally stirred at −5 °C for 30 min and kept at 5 °C for ~24 h. The crystals that formed were filtered off and washed with conc. HCl (6 mL) cooled to −18 °C. The yield of compound **8** was 1.62 g (75%), m.p. 191—192 °C. Found (%): C, 49.95; H, 6.09; N, 12.87. $C_9H_{13}ClN_2O_2$. Calculated (%): C, 49.89; H, 6.05; N, 12.93. ^{1}H NMR (DMSO-d_6), δ: 2.47 (t, 2 H, $CH_2C\underline{H}_2COOH$, J = 7.5 Hz); 2.73 (t, 2 H, $C\underline{H}_2CH_2COOH$, J = 7.5 Hz); 6.91—7.13 (d, 4 H, Ar, J = 8.5 Hz); 10.27 (s, 3 H, $N\underline{H}N\underline{H}_2$).

5-(2-Carboxyethyl)-2,3,3-trimethylindolenine (1). A mixture of compound **8** (216 mg, 1 mmol), anhydrous AcOK (196 mg, 2.0 mmol), and 3-methylbutan-2-one (150 μL, 1.37 mmol) was heated in acetic acid (1 mL) at 118 °C for 1 h. Then the solvent was removed and the residue was dissolved in $CHCl_3$, washed with water and brine, and dried with Na_2SO_4. The solvent was removed and the oily residue was recrystallized from ethyl acetate—hexane (1 : 10). The yield of 5-(2-carboxyethyl)-2,3,3-trimethylindolenine (**1**) was 118 mg (55%), m.p. 175—176 °C. Found (%): C, 72.64; H, 7.39; N, 6.01. $C_{14}H_{17}NO_2$. Calculated (%): C, 72.70; H, 7.41; N, 6.06. UV (MeOH), λ_{max}/nm: 262. MS, *m/z*: 232.3 $[M]^+$. $C_{14}H_{17}NO_2$. Calculated: M = 231.29. ^{1}H NMR ($CDCl_3$), δ: 1.27 (s, 6 H, 2 C(3)H_3); 2.27 (s, 3 H, C(2)H_3); 2.68 (t, 2 H, $CH_2C\underline{H}_2COOH$, J = 8 Hz); 3.00 (t, 2 H, $C\underline{H}_2CH_2COOH$, J = 8 Hz); 7.12—7.46 (d, 3 H, Ar, J = 8.5 Hz).

5-(2-Carboxyethyl)-1-ethyl-2,3,3-trimethylindoleninium iodide (2a). A mixture of compound **1** (230 mg, 1 mmol) and ethyl iodide (500 μL, 5.5 mmol) was heated in acetonitrile (2 mL) at 80 °C for 15 h. On cooling to ~20 °C, anhydrous diethyl ether (5 mL) was added and the mixture was kept at −18 °C for 24 h. The crystals that formed were filtered off and dried in a vacuum desiccator over P_2O_5 and KOH. The yield of compound **2a** was 377 mg (97%), m.p. 194—195 °C. Found (%): C, 49.68; H, 5.71; N, 3.58. $C_{16}H_{22}INO_2$. Calculated (%): C, 49.62; H, 5.73; N, 3.62. UV (MeOH), λ_{max}/nm: 284. MS, *m/z*: 259.8 $[M]^+$. $C_{16}H_{21}NO_2^+$. Calculated: M = 259.34. ^{1}H NMR (DMSO-d_6), δ: 1.44 (t, 3 H, $CH_2C\underline{H}_3$, J = 7.0 Hz); 1.52 (s, 6 H, 2 C(3)H_3); 2.62 (t, 2 H, $CH_2C\underline{H}_2COOH$, J = 7.5 Hz); 2.80 (s, 3 H, C(2)H_3); 2.96 (t, 2 H, $C\underline{H}_2CH_2COOH$, J = 7.5 Hz); 4.47 (q, 2 H, $C\underline{H}_2CH_3$, J = 7.0 Hz); 7.47—7.86 (m, 3 H, Ar).

5-(2-Carboxyethyl)-2,3,3-trimethyl-1-(4-sulfonatobutyl)indoleninium (2b). A mixture of compound **1** (230 mg, 1 mmol) and 4-hydroxybutane-1-sulfonic acid (160 μL, 1.6 mmol) was

heated in butyronitrile (2.2 mL) at 118 °C for 22 h. The reaction mixture was cooled to ~20 °C and triturated with anhydrous diethyl ether. The precipitate that formed was filtered off and dried in a vacuum desiccator over P_2O_5 and KOH. The yield of compound **2b** was 300 mg (86%), m.p. >250 °C. Found (%): C, 58.75; H, 6.92; N, 3.82. $C_{18}H_{25}NO_5S$. Calculated (%): C, 58.83; H, 6.86; N, 3.81. UV (MeOH), λ_{max}/nm: 284. MS, *m/z*: 368.2 $[M]^+$. $C_{18}H_{25}NO_5S$. Calculated: M = 367.46. ^{1}H NMR (DMSO-d_6), δ: 1.51 (s, 6 H, 2 C(3)H_3); 1.73 (m, 2 H, $CH_2CH_2C\underline{H}_2CH_2SO_3$); 1.96 (m, 2 H, $CH_2C\underline{H}_2CH_2CH_2SO_3$); 2.61 (t, 2 H, $CH_2C\underline{H}_2COOH$, *J* = 7.5 Hz); 2.81 (s, 3 H, C(2)H_3); 2.95 (t, 2 H, $C\underline{H}_2CH_2COOH$, *J* = 7.5 Hz); 4.46 (m, 2 H, $C\underline{H}_2CH_2CH_2CH_2SO_3$); 7.47—7.93 (m, 3 H, Ar).

5-(2-Carboxyethyl)-1′-ethyl-3,3,3′,3′-tetramethyl-1-(4-sulfonatobutyl)indodicarbocyanine (9). A mixture of compound **16a** (54 mg, 0.2 mmol) and malonaldehyde dianil hydrochloride (**17**) (59 mg, 0.23 mmol) was heated in acetic anhydride (1 mL) containing acetyl chloride (64 μL) at 118 °C for 2 h. Then the solvent was removed *in vacuo* and the residue was dissolved in acetic anhydride (2 mL). Compound **2b** (81 mg, 0.22 mmol) and a mixture (550 μL) of ethyl(diisopropyl)amine (200 μL, 2.1 mmol) and acetic anhydride (800 μL, 7.2 mmol) were added. The reaction mixture was kept at ~20 °C for 24 h. Reaction products were prepurified by flash chromatography on an RP-18 column. Then the target compound was isolated by reverse phase chromatography on an RP-18 column in MeCN—0.05 *M* $Et_3NH^+AcO^-$ buffer by gradient elution from 0 to 50% MeCN. The solvents were removed and the residue was diluted with water, placed again in the RP-18 column, and washed with 0.1 *M* NaCl and water. The product was isolated by reverse phase chromatography in MeCN—H_2O. The solvent was removed *in vacuo* and the residue was dried in a vacuum desiccator over P_2O_5. The yield of dye **9** was 57 mg (48%), m.p. >250 °C. Found (%): C, 69.18; H, 7.12; N, 4.78. $C_{34}H_{42}N_2O_5S$. Calculated (%): C, 69.12; H, 7.17; N, 4.74. The spectroscopic characteristics of the dye are given in Table 1. MS, *m/z*: 591.0 $[M]^+$. $C_{34}H_{42}N_2O_5S$. Calculated: M = 590.77. ^{1}H NMR (DMSO-d_6), δ: 1.26 (t, 3 H, $CH_2C\underline{H}_3$, *J* = 7.0 Hz); 1.63 (s, 12 H, 2 C(3)H_3, 2 C(3′)H_3); 1.73 (m, 4 H, $CH_2C\underline{H}_2C\underline{H}_2CH_2SO_3$); 2.48 (m, 2 H, $CH_2CH_2CH_2C\underline{H}_2SO_3$); 2.58 (t, 2 H, $CH_2C\underline{H}_2COOH$, *J* = 7.5 Hz); 2.88 (t, 2 H, $C\underline{H}_2CH_2COOH$, *J* = 7.5 Hz); 4.10 (m, 4 H, $C\underline{H}_2CH_2CH_2CH_2SO_3$, $C\underline{H}_2CH_3$); 6.37 (d, 2 H, α-CH, α′-CH, *J* = 13.5 Hz); 6.56 (t, 1 H, γ-CH, *J* = 12.0 Hz); 7.19—7.63 (m, 7 H, Ar); 8.31 (m, 2 H, β-CH, β′-CH).

5-(2-Carboxyethyl)-1,1′-diethyl-3,3,3′,3′-tetramethyl-5′-sulfoindodicarbocyanine (10). A mixture of compound **16b** (53 mg, 0.2 mmol) and malonaldehyde dianil hydrochloride (**17**) (59 mg, 0.23 mmol) was heated in acetic anhydride (1.5 mL) and acetic acid (0.5 mL) at 118 °C for 2 h. Then the solvents were removed *in vacuo*. The residue was heated with compound **2a** (85 mg, 0.22 mmol) and anhydrous AcOK (120 mg, 1.2 mmol) in acetic anhydride (1.5 mL) and acetic acid (0.5 mL) at 118 °C for 2 h. Compound **10** was isolated as described for compound **9**. The yield of dye **10** was 60 mg (53%), m.p. 245—247 °C. Found (%): C, 68.35; H, 6.78; N, 4.95. $C_{32}H_{38}N_2O_5S$. Calculated (%): C, 68.30; H, 6.81; N, 4.98. The spectroscopic characteristics of the dye are given in Table 1. MS, *m/z*: 563.2 $[M]^+$. $C_{32}H_{38}N_2O_5S$. Calculated: M = 562.72. ^{1}H NMR (DMSO-d_6), δ: 1.25 (t, 6 H, $CH_2C\underline{H}_3$, *J* = 7.0 Hz); 1.69 (s, 12 H, 2 C(3)H_3, 2 C(3′)H_3); 2.32 (t, 2 H, $CH_2C\underline{H}_2COOH$, *J* = 7.5 Hz); 2.86 (t, 2 H, $C\underline{H}_2CH_2COOH$, *J* = 7.5 Hz); 4.95 (m, 4 H, $C\underline{H}_2CH_3$); 6.30 (d, 2 H, α-CH, α′-CH, *J* = 13.5 Hz); 6.58 (t, 1 H, γ-CH, *J* = 12.0 Hz); 7.21—7.63 (m, 6 H, Ar); 8.31 (m, 2 H, β-CH, β′-CH).

5-(2-Carboxyethyl)-1-ethyl-3,3,3′,3′-tetramethyl-1′-(4-sulfonatobutyl)indodicarbocyanine (11). Compound **11** was obtained as described for compound **10**. The yield was 59 mg (50%), m.p. 185—187 °C. Found (%): C, 69.15; H, 7.21; N, 4.71. $C_{34}H_{42}N_2O_5S$. Calculated (%): C, 69.12; H, 7.17; N, 4.74. The spectroscopic characteristics of the dye are given in Table 1. MS, *m/z*: 592.8 $[M]^+$. $C_{34}H_{42}N_2O_5S$. Calculated: M = 590.77. ^{1}H NMR (DMSO-d_6), δ: 1.28 (t, 3 H, $CH_2C\underline{H}_3$, *J* = 7.0 Hz); 1.67 (s, 12 H, 2 C(3)H_3, 2 C(3′)H_3); 1.74 (m, 4 H, $CH_2C\underline{H}_2C\underline{H}_2CH_2SO_3$); 2.27 (t, 2 H, $CH_2C\underline{H}_2COOH$, *J* = 7.5 Hz); 2.48 (m, 2 H, $CH_2CH_2CH_2C\underline{H}_2SO_3$); 2.82 (t, 2 H, $C\underline{H}_2CH_2COOH$, *J* = 7.5 Hz); 4.13 (m, 4 H, $C\underline{H}_2CH_2CH_2CH_2SO_3$, $C\underline{H}_2CH_3$); 6.30 (d, 2 H, α-CH, α′-CH, *J* = 13.5 Hz); 6.56 (t, 1 H, γ-CH, *J* = 12.0 Hz); 7.24—7.58 (m, 7 H, Ar); 8.28 (m, 2 H, β-CH, β′-CH).

5-(2-Carboxyethyl)-3,3,3′,3′,5′-pentamethyl-1,1′-bis(4-sulfonatobutyl)indodicarbocyanine, sodium salt (12). Compound **12** was obtained as described for compound **9**. The yield was 81 mg (55%), m.p. 150—152 °C. Found (%): C, 60.51; H, 6.49; N, 4.85. $C_{37}H_{47}N_2NaO_8S_2$. Calculated (%): C, 60.47; H, 6.45; N, 3.81. The spectroscopic characteristics of the dye are given in Table 1. MS, *m/z*: 713.0 $[M]^+$. $C_{37}H_{47}N_2O_8S_2^-$. Calculated: M = 711.91. ^{1}H NMR (DMSO-d_6), δ: 1.66 (s, 12 H, 2 C(3)H_3, 2 C(3′)H_3); 1.74 (m, 8 H, $CH_2C\underline{H}_2C\underline{H}_2CH_2SO_3$); 2.36 (s, 3 H, C(5)$H_3$); 2.42 (m, 4 H, $CH_2CH_2CH_2C\underline{H}_2SO_3$); 2.86 (t, 2 H, $CH_2C\underline{H}_2COOH$, *J* = 7.5 Hz); 3.44 (t, 2 H, $C\underline{H}_2CH_2COOH$, *J* = 7.5 Hz); 4.06 (m, 4 H, $C\underline{H}_2CH_2CH_2CH_2SO_3$); 6.31 (d, 2 H, α-CH, α′-CH, *J* = 13.5 Hz); 6.56 (t, 1 H, γ-CH, *J* = 12.0 Hz); 7.17—7.47 (m, 6 H, Ar); 8.26 (m, 2 H, β-CH, β′-CH).

5-(2-Carboxyethyl)-3,3,3′,3′-tetramethyl-1,1′-bis(4-sulfonatobutyl)indodicarbocyanine, sodium salt (13). Compound **13** was obtained analogously. The yield was 104 mg (72%), m.p. 218—219 °C. Found (%): C, 60.04; H, 6.25; N, 3.92. $C_{36}H_{45}N_2NaO_8S_2$. Calculated (%): C, 59.98; H, 6.29; N, 3.89. The spectroscopic characteristics of the dye are given in Table 1. MS, *m/z*: 699.0 $[M]^+$. $C_{36}H_{45}N_2O_8S_2^-$. Calculated: M = 697.88. ^{1}H NMR (DMSO-d_6), δ: 1.67 (s, 12 H, 2 C(3)H_3, 2 C(3′)H_3); 1.75 (m, 8 H, $CH_2C\underline{H}_2C\underline{H}_2CH_2SO_3$); 2.36 (t, 2 H, $CH_2C\underline{H}_2COOH$, *J* = 7.5 Hz); 2.43 (m, 4 H, $CH_2CH_2CH_2C\underline{H}_2SO_3$); 2.85 (t, 2 H, $C\underline{H}_2CH_2COOH$, *J* = 7.5 Hz); 4.06 (m, 4 H, $C\underline{H}_2CH_2CH_2CH_2SO_3$); 6.33 (d, 2 H, α-CH, α′-CH, *J* = 13.5 Hz); 6.58 (t, 1 H, γ-CH, *J* = 12.0 Hz); 7.19—7.57 (m, 7 H, Ar); 8.28 (m, 2 H, β-CH, β′-CH).

5-(2-Carboxyethyl)-1′-ethyl-3,3,3′,3′-tetramethyl-5′-sulfo-1-(4-sulfonatobutyl)indodicarbocyanine, sodium salt (14). Compound **14** was obtained analogously. The yield was 39 mg (28%), m.p. 230—232 °C. Found (%): C, 58.98; H, 5.92; N, 4.06. $C_{34}H_{41}N_2NaO_8S_2$. Calculated (%): C, 58.94; H, 5.96; N, 4.04. The spectroscopic characteristics of the dye are given in Table 1. MS, *m/z*: 670.8 $[M]^+$. $C_{34}H_{41}N_2O_8S_2^-$. Calculated: M = 669.83. ^{1}H NMR (DMSO-d_6), δ: 1.23 (t, 3 H, $CH_2C\underline{H}_3$, *J* = 7.0 Hz); 1.67 (s, 12 H, 2 $H_3C(3)$, 2 C(3′)H_3); 1.76 (m, 4 H, $CH_2C\underline{H}_2C\underline{H}_2CH_2SO_3$); 2.25 (t, 2 H, $CH_2C\underline{H}_2COOH$, *J* = 7.5 Hz); 2.47 (m, 2 H, $CH_2CH_2CH_2C\underline{H}_2SO_3$); 2.83 (t, 2 H, $C\underline{H}_2CH_2COOH$, *J* = 7.5 Hz); 4.90 (m, 4 H, $C\underline{H}_2CH_2CH_2CH_2SO_3$, $C\underline{H}_2CH_3$); 6.32 (d, 2 H, α-CH, α′-CH, *J* = 13.5 Hz); 6.56 (t, 1 H, γ-CH, *J* = 12.0 Hz); 7.23—7.62 (m, 6 H, Ar); 8.28 (m, 2 H, β-CH, β′-CH).

5-(2-Carboxyethyl)-3,3,3′,3′-tetramethyl-5′-sulfo-1,1′-bis(4-sulfonatobutyl)indodicarbocyanine, disodium salt (15). Compound **15** was obtained as described for compound **10**. The yield was 55 mg (33%), m.p. >250 °C. Found (%): C, 52.59; H, 5.33; N, 3.42. $C_{36}H_{44}N_2Na_2O_{11}S_3$. Calculated (%): C, 52.54; H, 5.39; N, 3.40. The spectroscopic characteristics of the dye are given in Table 1. MS, *m/z*: 777.6 $[M]^+$. $C_{36}H_{44}N_2O_{11}S_3^{2-}$. Calculated: M = 776.94. ^{1}H NMR (DMSO-d_6), δ: 1.67 (s, 12 H, 2 C(3)H_3, 2 C(3′)H_3); 1.75 (m, 8 H, $CH_2C\underline{H}_2C\underline{H}_2CH_2SO_3$); 2.28 (t, 2 H, $CH_2C\underline{H}_2COOH$, J = 7.5 Hz); 2.44 (m, 4 H, $CH_2CH_2CH_2C\underline{H}_2SO_3$); 2.84 (t, 2 H, $C\underline{H}_2CH_2COOH$, J = 7.5 Hz); 4.09 (m, 4 H, $C\underline{H}_2CH_2CH_2CH_2SO_3$); 6.28 (d, 2 H, α-CH, α′-CH, J = 13.5 Hz); 6.58 (t, 1 H, γ-CH, J = 12.0 Hz); 7.26—7.75 (m, 6 H, Ar); 8.25 (m, 2 H, β-CH, β′-CH).

This work was financially supported by the International Scientific and Technical Center (Grant 2906).

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Received May 25, 2007;
in revised form August 2, 2007