Water-soluble cyanine dyes for biological microchip technology

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Novel indodicarbocyanine dyes were obtained and their spectroscopic characteristics were determined. For equal concentrations of the dyes, the relative fluorescence efficiency was measured at the excitation wavelengths λ = 635 and 655 nm and the emission wavelengths λ = 670 and 690 nm, respectively.

Key words: hybridization analysis, cyanine dyes, oligonucleotides, fluorescence.

Hybridization analysis with fluorescence labeling is widely used in biological microchip technology. A variety of methodological techniques of analysis and recording of results necessitates a search for novel efficient fluorescent markers.

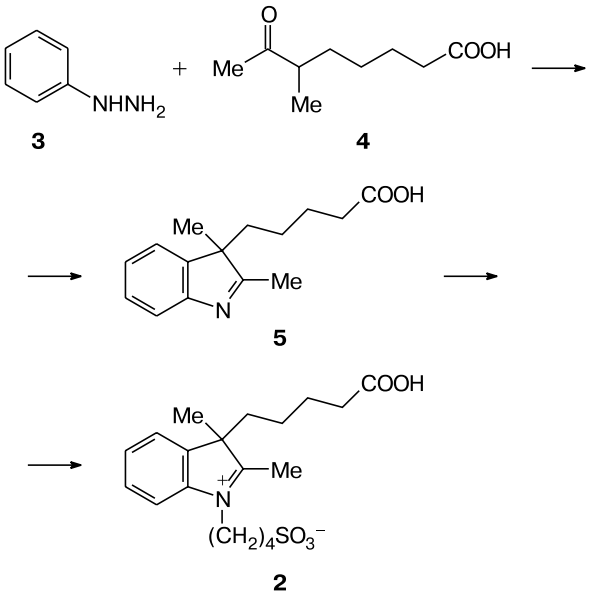
We proposed and conducted the synthesis of a novel series of water-soluble fluorescent dyes **1a**—**h** of the near-IR range for labeled primers technique using a polymerase chain reaction.[1] Dyes **1a**—**h** are characterized by the presence of a reactive carboxy group that is attached to position 3 of the indolenine fragment through the tetramethylene chain and is designed to label biomolecules. Because of such a structure, the fluorophore part of the dye is distant from a molecule to be labeled, which enhances its ability to form specific complexes.

Indoleninium salt **2** containing the carboxybutyl group was prepared from phenylhydrazine (**3**) and 6-methyl-7-oxooctanoic acid[2—6] (**4**) by the Fischer cyclization followed by quaternization of indolenine **5** with 4-hydroxybutane-1-sulfonic acid[7—9] (Scheme 1).

The synthesis of cyanine dyes **1a**—**h** involves sequential condensation reactions of appropriate indoleninium salts (**6a**—**h**) with malonaldehyde dianil (**7**) and of intermediate **8** with indoleninium salt **2** (Scheme 2).

Depending on indoleninium salt **6a**—**h** added in the first step, the synthesis of indodicarbocyanines was carried out either in acetic anhydride under heating or in acetic anhydride—acetic acid.[10—12] In the second step, ethyl(diisopropyl)amine or anhydrous potassium acetate was employed as a condensation agent.[12] The use of anhydrous sodium acetate sharply lowered the yield of the target product.

Scheme 1

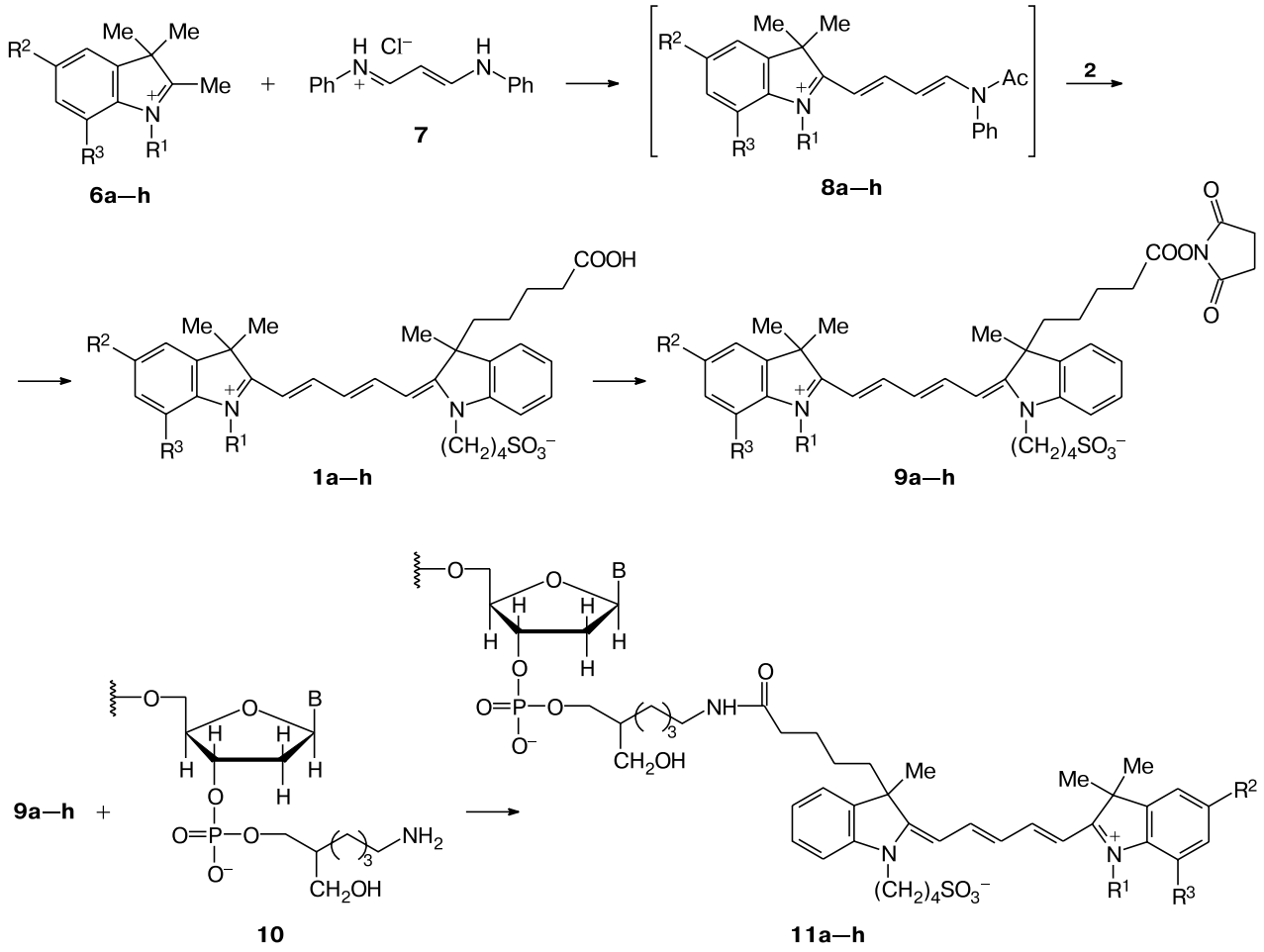


All the indodicarbocyanines **1a**—**h** obtained were isolated by reverse phase chromatography (RP-18 column, MeCN—0.05 *M* triethylammonium acetate buffer, gradient elution from 0 to 50% MeCN) and converted into sodium salts. The yields of the indodicarbocyanines varied from 5 to 48%. It should be noted that lithium salts of dyes **1a**—**h** are very unstable in storage. The structures of intermediates and target products were confirmed by elemental analysis, electronic absorption spectroscopy, MALDI-TOF mass spectrometry, and ^{1}H NMR spectroscopy.

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 12, pp. 2355—2359, December, 2007.

1066-5285/07/5612-2438

Scheme 2



1, 6, 8, 9, 11: R^1 = Et, R^2 = R^3 = H (**a**); R^1 = $(CH_2)_4SO_3^-$, R^2 = R^3 = H (**b**); R^1 = Et, R^2 = Me, R^3 = H (**c**); R^1 = $(CH_2)_4SO_3^-$, R^2 = Me, R^3 = H (**d**); R^1 = Et, R^2 = SO_3^-, R^3 = H (**e**); R^1 = $(CH_2)_4SO_3^-$, R^2 = SO_3^-, R^3 = H (**f**); R^1 = Et, R^2 = R^3 = Me (**g**); R^1 = $(CH_2)_4SO_3^-$, R^2 = R^3 = Me (**h**)

The indodicarbocyanines obtained have different absorption and fluorescence wavelengths, which was attained by introducing additional methyl substituents into indoleninium salts **6a**—**h**. The water solubility and total charge of the dye depends on the number of sulfo groups in its molecule.

For all indodicarbocyanine dyes **1a**—**h**, we determined molar absorption coefficients and fluorescence quantum yields in methanol and an aqueous phosphate salt buffer (PBS) (0.01 *M* K_3PO_4, 0.9% NaCl, pH 7.4) (Table 1).

The sensitivity of fluorescence detection is determined by the absolute sensitivity of a label defined as the product of the molar absorption coefficient and the fluorescence quantum yield. Thus, we determined the absolute detection sensitivity for indodicarbocyanines **1a**—**h** in terms of the corresponding absorption and fluorescence peaks.

In biological microchip technology, analysis is carried out on a fluorescence biochip analyzer fitted with commercial semiconducting laser light sources with wavelengths of 635 (20 mV) and 655 nm (40 mV) that provide fluorescence at λ = 670 and 690 nm, respectively. We measured the relative fluorescence efficiencies of dyes **1a**—**h** at the above wavelengths (see Table 1). The results obtained were normalized to the maximum value found for dye **1b** in methanol.

Succinimide esters **9a**—**h** were obtained in quantitative yields under the action of *N*-hydroxysuccinimide in the presence of dicyclohexylcarbodiimide in anhydrous DMF.[13] Synthetic oligonucleotides **10** containing an amino linker with a C_6 spacer were labeled with activated esters **9a**—**h** in aqueous solutions (see Scheme 2).

Fluorescent oligonucleotides proved to be completely suitable for labeled primers technique using a polymerase chain reaction followed by hybridization on oligonucleotide biochips. For aqueous solutions, the sensitivity of dye **1a** is best at λ = 655/690 nm and that of dye **1b** is best at λ = 635/670 nm.

Table 1. Fluorescence properties of the cyanine dyes*

Compound	Solvent	λ_{max}^{abs} / nm	λ_{max}^{em} / nm	$\varepsilon \cdot 10^{-5}$ /L mol^{-1} cm^{-1}	Φ	Relative fluorescence efficiency**		
						$\lambda_{max}^{abs}/\lambda_{max}^{em}$	655/690	635/670
1a	PBS	643	657	2.06±0.02	0.13	0.444	0.183	0.147
	MeOH	644	662	2.47±0.02	0.25	0.881	0.030	0.714
1b	PBS	647	661	2.43±0.02	0.17	0.677	0.102	0.427
	MeOH	648	664	2.40±0.02	0.27	1	0.222	0.681
1c	PBS	647	662	1.99±0.03	0.09	0.312	0.081	0.220
	MeOH	649	667	2.01±0.01	0.17	0.528	0.178	0.382
1d	PBS	649	665	2.16±0.02	0.11	0.428	0.121	0.283
	MeOH	651	669	2.29±0.01	0.19	0.667	0.260	0.471
1e	PBS	645	660	2.17±0.02	0.14	0.491	0.087	0.325
	MeOH	646	664	2.29±0.03	0.27	0.830	0.225	0.673
1f	PBS	646	660	2.20±0.02	0.14	0.522	0.096	0.325
	MeOH	648	665	2.43±0.02	0.25	0.958	0.283	0.706
1g	PBS	650	665	1.84±0.01	0.08	0.238	0.082	0.158
	MeOH	652	670	1.93±0.01	0.12	0.375	0.165	0.251
1h	PBS	650	668	1.46±0.02	0.05	0.082	0.039	0.057
	MeOH	651	669	1.54±0.02	0.08	0.167	0.092	0.109

* ε is the molar absorption coefficient; Φ is the fluorescence quantum yield; PBS is a 0.01 *M* potassium phosphate buffer, 0.9% NaCl, pH 7.4.

** The relative fluorescence efficiencies are given at different excitation/emission wavelengths (λ/nm).

Experimental

^{1}H NMR spectra were recorded on a Bruker AMX-400 pulse Fourier radiospectrometer (Germany) (400 MHz) in $CDCl_3$, D_2O, and DMSO-d_6 with Me_4Si as the external standard. Mass spectra were recorded on a Compact MALDI 4 MALDI-TOF 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 Kofler 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 a column 10×200 mm.

Indolenine bases **6a**—**h**,[7,10,12,14], malonaldehyde dianil,[15] and 6-methyl-7-oxooctanoic acid (**4**)[2—6] were prepared as described earlier.

3-(4-Carboxybutyl)-2,3-dimethylindolenine (5). A mixture of 6-methyl-7-oxooctanoic acid (**4**) (720 mg, 4.2 mmol), phenylhydrazine (**3**) (420 μL, 4.2 mmol), and glacial AcOH (4.2 mL) was heated in an inert atmosphere at 118 °C for 3.5 h. The solvent was removed and the oily residue was dissolved in chloroform (10 mL). The resulting solution was washed with water and brine and dried with $MgSO_4$. The solvent was removed and the residue was recrystallized from EtOAc—hexane (0.6 : 2). The yield of indolenine **5** was 0.8 g (78%), a yellow orange powder, m.p. 112—113 °C. Found (%): C, 73.49; H, 7.76; N, 5.72. $C_{15}H_{19}NO_2$. Calculated (%): C, 73.44; H, 7.81; N, 5.71. UV (MeOH), λ_{max}/nm: 256. MS, *m/z* 246.3 $[M]^+$. $C_{15}H_{19}NO_2$. Calculated: M = 245.32.

^{1}H NMR ($CDCl_3$), δ: 0.56—0.81 (m, 2 H, $CH_2CH_2C\underline{H}_2CH_2COOH$); 1.28 (s, 3 H, $H_3C(3)$); 1.43—1.51 (m, 2 H, $C\underline{H}_2CH_2CH_2CH_2COOH$); 1.76—1.92 (m, 2 H, $CH_2C\underline{H}_2CH_2CH_2COOH$); 2.13—2.21 (m, 2 H, $CH_2CH_2CH_2C\underline{H}_2COOH$); 2.24 (s, 3 H, $H_3C(2)$); 7.18—7.54 (m, 4 H, Ar).

3-(4-Carboxybutyl)-2,3-dimethyl-1-(4-sulfonatobutyl)indoleninium (2). A mixture of indolenine **5** (640 mg, 2.6 mmol), 4-hydroxybutane-1-sulfonic acid (530 μL, 5.2 mmol), and 1,2-dichlorobenzene (4.2 mL) was heated at 118 °C for 25 h. The solvent was decanted and the oily residue was triturated with diethyl ether. The precipitate that formed was filtered off, washed with diethyl ether, and dried in a vacuum desiccator over P_2O_5. The yield of indoleninium salt **2** was 0.98 g (99%), a dark cherry powder, m.p. 48—50 °C. Found (%): C, 59.78; H, 7.16; N, 3.69. $C_{19}H_{27}NO_5S$. Calculated (%): C, 59.82; H, 7.13; N, 3.67. UV (MeOH), λ_{max}/nm: 280. MS, *m/z* 382.0 $[M]^+$. $C_{19}H_{27}NO_5S$. Calculated: M = 381.49.

^{1}H NMR (D_2O), δ: 0.38—0.68 (m, 2 H, $CH_2CH_2C\underline{H}_2CH_2COOH$); 1.23—1.35 (m, 2 H, $C\underline{H}_2CH_2CH_2CH_2COOH$); 1.43 (s, 3 H, $H_3C(3)$); 1.71—1.81 (m, 2 H, $CH_2CH_2C\underline{H}_2CH_2SO_3$); 1.92—2.20 (m, 6 H, $CH_2C\underline{H}_2CH_2C\underline{H}_2COOH$, $CH_2C\underline{H}_2CH_2CH_2SO_3$); 2.84 (t, 2 H, $CH_2CH_2CH_2C\underline{H}_2SO_3$, *J* = 7.5 Hz); 4.41 (t, 2 H, $C\underline{H}_2CH_2CH_2CH_2SO_3$, *J* = 7.5 Hz); 7.18—7.70 (m, 4 H, Ar).

3-(4-Carboxybutyl)-1′-ethyl-3,3′,3′-trimethyl-1-(4-sulfonatobutyl)indodicarbocyanine (1a). A mixture of 1-ethyl-2,3,3-trimethylindoleninium iodide (**6a**) (63 mg, 0.2 mmol), malonaldehyde dianil hydrochloride (**7**) (52 mg, 0.2 mmol), acetic anhydride (700 μL), and acetic acid (300 μL) was heated at 118 °C for 2 h. Then the solvent was removed *in vacuo*. The residue (**8**) was dissolved in acetic anhydride (1.7 mL). A solution of

3-(4-carboxybutyl)-2,3-dimethyl-1-(4-sulfonatobutyl)indoleninium (**2**) (80 mg, 0.21 mmol) in a mixture of ethyl(diisopropyl)amine (200 μL, 2.1 mmol) and acetic anhydride (800 μL, 7.2 mmol) was added. The reaction mixture was kept at ~20 °C for a day. The reaction products were isolated by reverse phase chromatography on an RP-18 column in MeCN—0.05 *M* triethylammonium acetate buffer with gradient elution from 0 to 50% MeCN. The solvents were removed and the residue was diluted with water, put again on the top of the RP-18 column, washed with 0.1 *M* NaCl and water, and 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 **1a** was 46 mg (37.5%), m.p. 172—173 °C. Found (%): C, 69.55; H, 7.28; N, 4.64. $C_{35}H_{44}N_2O_5S$. Calculated (%): C, 69.51; H, 7.33; N, 4.63. The fluorescence characteristics of the dye are given in Table 1. MS, *m/z* 606.6 $[M]^+$. $C_{35}H_{44}N_2O_5S$. Calculated: M = 604.80.

^{1}H NMR (DMSO-d_6), δ: 0.43, 0.78 (both m, 1 H each, $CH_2CH_2C\underline{H}_2CH_2COOH$); 1.24—1.31 (m, 5 H, $CH_2C\underline{H}_3$, $CH_2C\underline{H}_2CH_2CH_2COOH$); 1.64 (s, 9 H, $H_3C(3)$, $H_3C(3')$); 1.73 (m, 4 H, $CH_2C\underline{H}_2C\underline{H}_2CH_2SO_3$); 1.89 (m, 2 H, $C\underline{H}_2CH_2CH_2CH_2COOH$); 2.18 (m, 1 H, $CH_2CH_2CH_2C\underline{H}_2COOH$); 2.41 (m, 3 H, $CH_2CH_2CH_2C\underline{H}_2COOH$, $CH_2CH_2CH_2C\underline{H}_2SO_3$); 4.12 (m, 4 H, $C\underline{H}_2CH_3$, $C\underline{H}_2CH_2CH_2CH_2SO_3$); 6.26 (d, 1 H, α′-CH, *J* = 13.5 Hz); 6.41 (d, 1 H, α-CH, *J* = 13.5 Hz); 6.57 (t, 1 H, γ-CH, *J* = 12.0 Hz); 7.21—7.61 (m, 8 H, Ar); 8.32 (m, 2 H, β-CH, β′-CH).

3-(4-Carboxybutyl)-3,3′,3′-trimethyl-1,1′-bis(4-sulfonatobutyl)indodicarbocyanine, sodium salt (1b). A mixture of 2,3,3-trimethyl-1-(4-sulfonatobutyl)indoleninium (**6b**) (59 mg, 0.2 mmol), malonaldehyde dianil hydrochloride (**7**) (52 mg, 0.2 mmol), acetic anhydride (700 μL), and acetic acid (160 μL) was heated at 118 °C for 2 h. Then 3-(4-carboxybutyl)-2,3-dimethyl-1-(4-sulfonatobutyl)indoleninium (**2**) (80 mg, 0.21 mmol), anhydrous potassium acetate (117 mg, 1.2 mmol), acetic anhydride (700 μL), and acetic acid (350 μL) were added and the reaction mixture was heated at 118 °C for 2 h. Compound **1b** was isolated as described for compound **1a**. The yield of dye **1b** was 71 mg (48%), m.p. 184—186 °C. Found (%): C, 60.41; H, 6.42; N, 3.84. $C_{37}H_{47}N_2NaO_8S_2$. Calculated (%): C, 60.47; H, 6.45; N, 3.81. For the fluorescence characteristics, see Table 1. MS, *m/z* 712.1 $[M]^+$. $C_{37}H_{47}N_2O_8S_2^-$. Calculated: M = 711.91.

^{1}H NMR (DMSO-d_6), δ: 0.45, 0.77 (both m, 1 H each, $CH_2CH_2C\underline{H}_2CH_2COOH$); 1.32 (m, 2 H, $CH_2C\underline{H}_2CH_2CH_2COOH$); 1.65—1.80 (s, 17 H, $H_3C(3)$, $H_3C(3')$, $CH_2C\underline{H}_2C\underline{H}_2CH_2SO_3$); 1.91 (m, 2 H, $C\underline{H}_2CH_2CH_2CH_2COOH$); 2.17 (m, 1 H, $CH_2CH_2CH_2C\underline{H}_2COOH$); 2.42 (m, 5 H, $CH_2CH_2CH_2C\underline{H}_2COOH$, $CH_2CH_2CH_2C\underline{H}_2SO_3$); 4.09 (m, 4 H, $C\underline{H}_2CH_2CH_2CH_2SO_3$); 6.37 (m, 2 H, α-CH, α′-CH); 6.57 (t, 1 H, γ-CH, *J* = 12.0 Hz); 7.21—7.6 (m, 8 H, Ar); 8.30 (m, 2 H, β-CH, β′-CH).

3-(4-Carboxybutyl)-1′-ethyl-3,3′,3′,5′-tetramethyl-1-(4-sulfonatobutyl)indodicarbocyanine (1c) was obtained as described for compound **1a**. The yield was 40 mg (32%), m.p. 151—153 °C. Found (%): C, 69.91; H, 7.47; N, 4.55. $C_{36}H_{46}N_2O_5S$. Calculated (%): C, 69.87; H, 7.49; N, 4.53. For the fluorescence characteristics, see Table 1. MS, *m/z* 619.3 $[M]^+$. $C_{36}H_{46}N_2O_5S$. Calculated: M = 618.83.

^{1}H NMR (DMSO-d_6), δ: 0.48, 0.78 (both m, 1 H each, $CH_2CH_2C\underline{H}_2CH_2COOH$); 1.22—1.35 (m, 5 H, $CH_2C\underline{H}_3$, $CH_2C\underline{H}_2CH_2CH_2COOH$); 1.64 (s, 9 H, $H_3C(3)$, $H_3C(3')$); 1.73 (m, 4 H, $CH_2C\underline{H}_2C\underline{H}_2CH_2SO_3$); 2.01 (m, 2 H, $C\underline{H}_2CH_2CH_2CH_2COOH$); 2.18 (m, 1 H, $CH_2CH_2CH_2C\underline{H}_2COOH$); 2.37 (s, 3 H, $H_3C(5')$); 2.45 (m, 3 H, $CH_2CH_2CH_2C\underline{H}_2COOH$, $CH_2CH_2CH_2C\underline{H}_2SO_3$); 4.12 (m, 4 H, $C\underline{H}_2CH_3$, $C\underline{H}_2CH_2CH_2CH_2SO_3$); 6.32 (m, 1 H, α-CH, α′-CH); 6.55 (t, 1 H, γ-CH, *J* = 12.0 Hz); 7.21—7.55 (m, 7 H, Ar); 8.27 (m, 2 H, β-CH, β′-CH).

3-(4-Carboxybutyl)-3,3′,3′,5′-tetramethyl-1,1′-bis(4-sulfonatobutyl)indodicarbocyanine, sodium salt (1d) was obtained as described for compound **1a**. The yield was 54 mg (36%), m.p. 120—122 °C. Found (%): C, 60.98; H, 6.55; N, 3.77. $C_{38}H_{49}N_2NaO_8S_2$. Calculated (%): C, 60.94; H, 6.59; N, 3.74. For the fluorescence characteristics, see Table 1. MS, *m/z* 726.9 $[M]^+$. $C_{38}H_{49}N_2O_8S_2^-$. Calculated: M = 725.94.

^{1}H NMR (DMSO-d_6), δ: 0.47, 0.79 (both m, 1 H each, $CH_2CH_2C\underline{H}_2CH_2COOH$); 1.32 (m, 2 H, $CH_2C\underline{H}_2CH_2CH_2COOH$); 1.65—1.81 (s, 17 H, $H_3C(3)$, $H_3C(3')$, $CH_2C\underline{H}_2C\underline{H}_2CH_2SO_3$); 2.03 (m, 2 H, $C\underline{H}_2CH_2CH_2CH_2COOH$); 2.14 (m, 1 H, $CH_2CH_2CH_2C\underline{H}_2COOH$); 2.36 (s, 4 H, $H_3C(5')$, $CH_2CH_2CH_2C\underline{H}_2COOH$); 2.48 (m, 4 H, $CH_2CH_2CH_2C\underline{H}_2SO_3$); 4.08 (m, 4 H, $C\underline{H}_2CH_2CH_2CH_2SO_3$); 6.35 (m, 2 H, α-CH, α′-CH); 6.57 (t, 1 H, γ-CH, *J* = 12.5 Hz); 7.19—7.54 (m, 7 H, Ar); 8.30 (m, 2 H, β-CH, β′-CH).

3-(4-Carboxybutyl)-1′-ethyl-3,3′,3′-trimethyl-5′-sulfo-1-(4-sulfonatobutyl)indodicarbocyanine, sodium salt (1e) was obtained as described for compound **1b**. The yield was 32 mg (22%), m.p. 139—141 °C. Found (%): C, 59.51; H, 6.11; N, 3.98. $C_{35}H_{43}N_2NaO_8S_2$. Calculated (%): C, 59.47; H, 6.13; N, 3.96. For the fluorescence characteristics, see Table 1. MS, *m/z* 684.3 $[M]^+$. $C_{35}H_{43}N_2O_8S_2^-$. Calculated: M = 683.86.

^{1}H NMR (DMSO-d_6), δ: 0.47, 0.77 (both m, 1 H each, $CH_2CH_2C\underline{H}_2CH_2COOH$); 1.24—1.38 (m, 5 H, $CH_2C\underline{H}_3$, $CH_2C\underline{H}_2CH_2CH_2COOH$); 1.68 (m, 13 H, $H_3C(3)$, $H_3C(3')$, $CH_2C\underline{H}_2C\underline{H}_2CH_2SO_3$); 2.00 (m, 2 H, $C\underline{H}_2CH_2CH_2CH_2COOH$); 2.19 (m, 1 H, $CH_2CH_2CH_2C\underline{H}_2COOH$); 2.40 (m, 3 H, $CH_2CH_2CH_2C\underline{H}_2COOH$, $CH_2CH_2CH_2C\underline{H}_2SO_3$); 4.13 (m, 4 H, $C\underline{H}_2CH_3$, $C\underline{H}_2CH_2CH_2CH_2SO_3$); 6.26 (d, 1 H, α′-CH, *J* = 13.5 Hz); 6.43 (d, 1 H, α-CH, *J* = 13.5 Hz); 6.57 (t, 1 H, γ-CH, *J* = 12.0 Hz); 7.23—7.79 (m, 7 H, Ar); 8.33 (m, 2 H, β-CH, β′-CH).

3-(4-Carboxybutyl)-3,3′,3′-trimethyl-5′-sulfo-1,1′-bis(4-sulfonatobutyl)indodicarbocyanine, disodium salt (1f) was obtained as described for compound **1b**. The yield was 54 mg (32%), m.p. >250 °C. Found (%): C, 53.15; H, 5.51; N, 3.37. $C_{37}H_{46}N_2Na_2O_{11}S_3$. Calculated (%): C, 53.10; H, 5.54; N, 3.35. For the fluorescence characteristics, see Table 1. MS, *m/z* 789.6 $[M]^+$. $C_{37}H_{46}N_2O_{11}S_3^{2-}$. Calculated: M = 790.97.

^{1}H NMR (DMSO-d_6), δ: 0.46, 0.78 (both m, 1 H each, $CH_2CH_2C\underline{H}_2CH_2COOH$); 1.33 (m, 2 H, $CH_2C\underline{H}_2CH_2CH_2COOH$); 1.65—1.75 (s, 17 H, $H_3C(3)$, $H_3C(3')$, $CH_2C\underline{H}_2C\underline{H}_2CH_2SO_3$); 2.02 (m, 2 H, $C\underline{H}_2CH_2CH_2CH_2COOH$); 2.16 (m, 1 H, $CH_2CH_2CH_2C\underline{H}_2COOH$); 2.39 (m, 5 H, $CH_2CH_2CH_2C\underline{H}_2COOH$, $CH_2CH_2CH_2C\underline{H}_2SO_3$); 4.09 (m, 4 H, $C\underline{H}_2CH_2CH_2CH_2SO_3$); 6.38 (m, 2 H, α-CH, α′-CH);

6.59 (t, 1 H, γ-CH, J = 12.0 Hz); 7.22—7.79 (m, 7 H, Ar); 8.32 (m, 2 H, β-CH, β′-CH).

3-(4-Carboxybutyl)-1′-ethyl-3,3′,3′,5′,7′-pentamethyl-1-(4-sulfonatobutyl)indodicarbocyanine (1g) was obtained as described for compound **1a**. The yield was 19 mg (20%), m.p. 110—112 °C. For the fluorescence characteristics, see Table 1. MS, m/z 633.6 $[M]^+$. $C_{37}H_{48}N_2O_5S$. Calculated: M = 632.85.

^{1}H NMR (DMSO-d_6), δ: 0.49, 0.78 (both m, 1 H each, $CH_2CH_2C\underline{H}_2CH_2COOH$); 1.33 (m, 5 H, $CH_2C\underline{H}_3$, $CH_2C\underline{H}_2CH_2CH_2COOH$); 1.64 (s, 9 H, $H_3C(3)$, $H_3C(3')$); 1.72 (m, 4 H, $CH_2C\underline{H}_2C\underline{H}_2CH_2SO_3$); 2.01 (m, 2 H, $C\underline{H}_2CH_2CH_2CH_2COOH$); 2.14 (m, 1 H, $CH_2CH_2CH_2C\underline{H}_2COOH$); 2.31 (s, 3 H, $H_3C(5')$); 2.4 (m, 3 H, $CH_2CH_2CH_2C\underline{H}_2COOH$, $CH_2CH_2CH_2C\underline{H}_2SO_3$); 2.59 (s, 3 H, $H_3C(7')$); 4.06 (m, 2 H, $C\underline{H}_2CH_3$); 4.27 (m, 2 H, $C\underline{H}_2CH_2CH_2CH_2SO_3$); 6.35 (m, 1 H, α-CH, α′-CH); 6.56 (d, 1 H, α-CH, J = 12.0 Hz); 6.99—7.54 (m, 6 H, Ar); 8.29 (m, 2 H, β-CH, β′-CH).

3-(4-Carboxybutyl)-3,3′,3′,5′,7′-pentamethyl-1,1′-bis(4-sulfonatobutyl)indodicarbocyanine, sodium salt (1h) was obtained as described for compound **1b**. The yield was 8 mg (5%), m.p. 108—110 °C. For the fluorescence characteristics, see Table 1. MS, m/z 741.2 $[M]^+$. $C_{39}H_{51}N_2O_8S_2^-$. Calculated: M = 739.96.

^{1}H NMR (DMSO-d_6), δ: 0.48, 0.79 (both m, 1 H each, $CH_2CH_2C\underline{H}_2CH_2COOH$); 1.32 (m, 2 H, $CH_2C\underline{H}_2CH_2CH_2COOH$); 1.67—1.81 (s, 17 H, $H_3C(3)$, $H_3C(3')$, $CH_2C\underline{H}_2C\underline{H}_2CH_2SO_3$); 2.05 (m, 2 H, $C\underline{H}_2CH_2CH_2CH_2COOH$); 2.18 (m, 1 H, $CH_2CH_2CH_2C\underline{H}_2COOH$); 2.36 (s, 4 H, $H_3C(5')$, $CH_2CH_2CH_2C\underline{H}_2COOH$); 2.47 (m, 4 H, $CH_2CH_2CH_2C\underline{H}_2SO_3$); 2.61 (s, 3 H, $H_3C(7')$); 4.12 (m, 4 H, $C\underline{H}_2CH_2CH_2CH_2SO_3$); 6.37 (m, 2 H, α-CH, α′-CH); 6.59 (t, 1 H, γ-CH, J = 12.5 Hz); 7.21—7.55 (m, 6 H, Ar); 8.33 (m, 2 H, β-CH, β′-CH).

Succinimide esters of indodicarbocyanine dyes 9a—h. *N*-Hydroxysuccinimide (69 mg, 0.6 mmol) and a solution of dicyclohexylcarbodiimide (62 mg, 0.3 mmol) in anhydrous DMF (500 μL) were added to a solution of a sodium salt of indodicarbocyanine **1a—h** (0.15 mmol) in anhydrous DMF (11 mL) cooled to −18 °C. The mixture was stirred at ~20 °C for 5 days, diluted with water, and filtered. The filtrate was put on the top of an RP-18 column, washed with water (100 mL), and eluted with MeCN—water (1 : 1). The solvent was removed and the residue was dried in a vacuum desiccator over P_2O_5. The yields of succinimide esters **9a—h** were 85—90%.

Oligonucleotide labeling. A 0.1 *M* carbonate-bicarbonate buffer (15 μL) and acetonitrile (15 μL) were added to the oligonucleotide 5′-CTCAGTTT-NH_2-3′ (**10**) (200 nmol). The mixture was thoroughly stirred and cooled to −18 °C. The succinimide ester of dye **9a—h** (~0.6 mg) was added and the mixture was stirred and kept at ~20 °C for 24 h. Then a 0.1 *M* triethylammonium acetate buffer (0.5 mL) was added and the product was extracted with butan-1-ol until the upper layer decolorized completely. Colored oligonucleotide **11a—h** was isolated by HPLC.

Monitoring was carried out at λ = 254 nm, flow rate 1 mL min^{-1}, gradient elution from *A—B* (20%) to *B* (100%) over 30 min (*A* is a 0.1 *M* triethylammonium acetate buffer and *B* is a 50% solution of acetonitrile in *A*). The yield of labeled oligonucleotide was 75—80%. The structures of the conjugates obtained were confirmed by MALDI-TOF mass spectrometry.

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

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