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Synthesis and characterization of novel zwitterionic heptamethine indocyanine fluorophores

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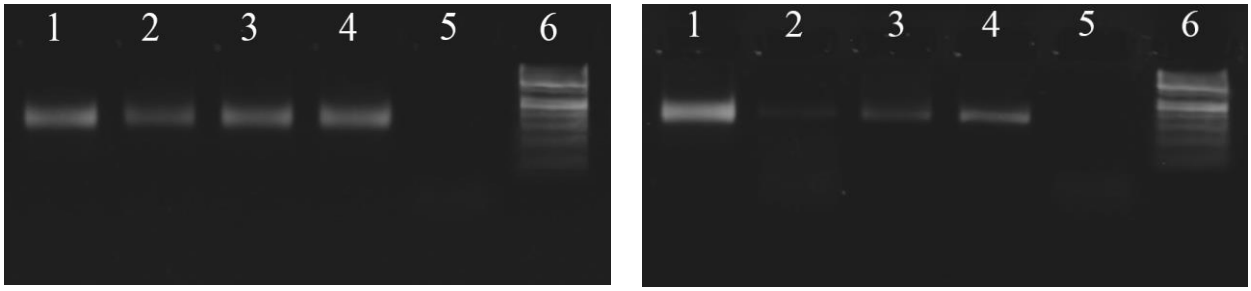

(A) (B)

Figure S1 Analysis of PCR products at different concentrations of fluorescently labeled nucleoside triphosphates in 5% (w/v) agarose gel with ethidium bromide staining: (A) 8 μM, (B) 80 μM. Lane 1: Cy3-labeled primer (without fluorescently labeled deoxynucleoside triphosphates); lane 2: nucleotide **10**; lane 3: nucleotide **9**; lane 4: nucleotide **11**; lane 5: negative control; lane 6: molecular ladder GeneRuler 50bp.

† The ^{1}H NMR spectra were recorded with a Bruker AMX-400 spectrometer (400 MHz) (Bruker, USA) in D_2O and DMSO-d_6 solutions. Chemical shifts (δ) are given in ppm. Coupling constants (J) are given in Hz. Multiplicities of the signals: s, singlet; d, doublet; t, triplet; m, multiplet. The mass spectroscopic analysis was performed with a 4800 Plus MALDI-TOF mass spectrometer (Applied Biosystems/MDS Sciex, Foster City, CA). Mass spectra were recorded in the linear mode for positive ions. Fluorescence spectra were recorded using a Cary Eclipse fluorescence spectrophotometer (Agilent Technologies, Santa Clara, CA). UV spectra were measured using a Jasco V-550 spectrophotometer (JASCO International Co., Tokyo, Japan). The pH values were determined using a Thermo Orion 330 pH-meter (Thermo Scientific, Waltham,

MA). «TB-Biochip» test system was purchased from the manufacturer (Biochip-EIMB, Moscow, Russia).

All reagents were obtained from Sigma-Aldrich or Fluka unless otherwise stated. 5-(3-Aminoallyl)-2′-deoxyuridine 5′-triphosphate lithium salt was purchased from Biosan (Russia). The NHS esters of Cy3 and Cy7 were purchased from GE Healthcare (Piscataway, NJ). Thin layer chromatography (TLC) was used as a quality control check of dyes and dye-labeled deoxyuridine triphosphates to ensure chemical purity >95%. TLC was performed using silica gel 60 RP-18 F254S plates from Merck (Darmstadt, Germany) with mobile phase: acetonitrile/0.1 M TEAHC 30/70 v/v. The GeneRuler 50bp molecular ladder was purchased from Thermo Fisher Scientific (Waltham, Massachusetts, USA).

The quantum yield of fluorophores was measured at 25°C using a reference compound with a known quantum yield.[18] Photo- and thermal stability were determined according to our previously published procedure.[13]

Sodium salt 5,5'-disulfo-1,1'-bis(5-carboxypentyl)-3,3,3',3'-tetramethylindotricarbocyanine **1** and sodium salt 5,5'-disulfo-1,1'-diethyl-3,3,3',3'-tetramethylindotricarbocyanine **4** were synthesized according to the literature.[13]

1'-[7-Aza-10-dimethylamino-6-oxodec-1-yl)-1-(5-carboxypentyl)-3,3,3′,3′-tetramethyl-5,5'-disulfoindotricarbocyanine sodium salt 2: A solution of dye **1** (31 mg, 41 μmol), *O*-(benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HBTU, 61 mg, 0.16 mmol) and *N*-hydroxysuccinimide (NHS, 29 mg, 0.24 mmol) in anhydrous DMF (0.6 ml) was stirred at 20ºC for 2 h. A solution of 3-dimethylamino-1-propylamine (14 mg, 82 μmol) in $NaHCO_3/Na_2CO_3$ buffer (0.5 ml, 0.1 M, pH 9.2) was added. The reaction mixture was stirred at 20°C for 24 h, diluted with 0.1 M triethylammonium acetate (TEAA, 5 ml), and purified in reverse-phase (C18) chromatography using a linear gradient from 0.1 M TEAA to 50% acetonitrile in 0.1 M TEAA. Triethylammonium salts of the dyes were dissolved in deionized water and desalted in the following manner: a solution of dye in Milli-Q water was loaded onto the C18-RP column, washed with 0.1 M NaCl, Milli-Q water, and then eluted with aqueous acetonitrile. The yield of dye **2** as dark-green powder was 16 mg (44%), mp 235°C. MS (MALDI-TOF) *m/z*: calcd. for $C_{44}H_{59}N_4O_9S_2^-$ 852.09; found 854.9 $[M]^+$. ^{1}H NMR (DMSO-d6) δ: 1.3, 1.58, 1.68, 1.78, 2.03 (5m, 18H, $CH_2CH_2CH_2CH_2CH_2COOH$, C(2)H_2, C(3)H_2, C(4)H_2, C(5)H_2, C(9)H_2), 1.63 [s, 12H, C(3,3)H_3, C(3',3')H_3], 2.24 (s, 6H, N(CH_3)$_2$), 2.41 (m, 2H, C(10)H_2), 3.01 (m, 2H, C(8)H_2), 4.08 (m, 4H, C(1)H_2, $CH_2CH_2CH_2CH_2CH_2COOH$), 6.34 (m, 2H, α,α'-C*H*), 6.52 (m, 2H, γ,γ'-C*H*), 7.29, 7.63, 7.74 (3m, 6H, Ar*H*), 7.87 (m, 3H, δ-C*H*, β,β'-C*H*). Anal. Calcd. for $C_{44}H_{59}N_4NaO_9S_2$ (%): C, 60.39; H, 6.80; N, 6.40. Found: C, 60.81; H,

6.96; N, 6.31.

1'-[7-Aza-10-trimethylammonio-6-oxodec-1-yl)-1-(5-carboxypentyl)-3,3,3′,3′-tetramethyl-5,5'-disulfoindotricarbocyanine 3: A solution of dye **2** (30 mg, 34 μmol) and methyl *p*-toluenesulfonate (19 mg, 0.1 mmol) in anhydrous DMF (1.4 ml) was stirred at 20°C for 3 h. The reaction mixture diluted with 0.1 M TEAA (5 ml), and purified in reverse-phase (C18) chromatography. The yield of dye **3** as dark-green powder was 22 mg (73%), mp 220°C. MS (MALDI-TOF) *m/z*: calcd. for $C_{45}H_{62}N_4O_9S_2$ 867.13; found 868.5 $[M]^+$. ^{1}H NMR (DMSO-d6) δ: 1.35, 1.53, 1.68, 1.75, 2.03 (5m, 16H, $CH_2CH_2CH_2CH_2CH_2COOH$, C(2)H_2, C(3)H_2, C(4)H_2, C(5)H_2), 1.63 [s, 12H, C(3,3)H_3, C(3',3')H_3], 3.02 [s, 11H, C(10)H_2, N(CH_3)$_3$], 3.25 (m, 2H, C(8)H_2, C(9)H_2), 4.07 (m, 4H, C(1)H_2, $CH_2CH_2CH_2CH_2CH_2COOH$), 6.38 (m, 2H, α,α'-C*H*), 6.51 (m, 2H, γ,γ'-C*H*), 7.27, 7.65, 7.78 (3m, 6H, Ar*H*), 7.85 (m, 3H, δ-C*H*, β,β'-C*H*). Anal. Calcd. for $C_{45}H_{62}N_4O_9S_2$ (%): C, 62.33; H, 7.21; N, 6.46. Found: C, 62.01; H, 7.52; N, 6.32.

1,1'-Diethyl-5-[*N*-(5-carboxypenthyl)]aminosulfonyl]-3,3,3',3'-tetramethyl-5′-sulfoindodicarbocyanine 6: A mixture containing sodium salt 1,1'-diethyl-3,3,3',3'-tetramethyl-5,5'-disulfoindodicarbocyanine **4** (20 mg, 32 μmol), phosphorus pentachloride (32 mg, 0.16 mmol) and trimethyl phosphate (20 ml) was stirred at 20°C for 5.5 h. The reaction mixture was cooled to -18°C, and solution of 6-aminocaproic acid (210 mg, 1.6 mmol) in 1 M $NaHCO_3$ (10 ml) was added dropwise. Stirring was continued at 0°C for 5 h and then at room temperature for 12 h. The reaction mixture was diluted with water (30 ml), neutralized with acetic acid, and the product was extracted with a toluene (100 ml). The combined extracts were washed with water (50 ml), brine (100 ml), and dried over $MgSO_4$. The solvent was evaporated, and the dye was purified by a procedure similar to that described above. The yield of dye **6** as dark-green powder was 7 mg (30%), mp 215°C (decomp.). MS (MALDI-TOF) *m/z*: calcd. for $C_{37}H_{47}N_3O_7S_2$ 709.92; found 711.2 $[M]^+$. ^{1}H NMR (DMSO-d_6) δ: 1.24 (m, 6H, CH_2CH_3), 1.10, 1.94, 2.85 (3m, 10H, $NHCH_2CH_2CH_2CH_2CH_2COOH$), 1.63 [s, 12H, C(3,3)$H_3$, C(3',3')$H_3$], 4.08 (m, 4H, CH_2CH_3), 6.41 (m, 2H, α,α'-C*H*), 6.53 (m, 2H, γ,γ'-C*H*), 7.32, 7.37, 7.78 (3m, 6H, Ar*H*), 7.82 (m, 3H, δ-C*H*, β,β'-C*H*). Anal. Calcd. for $C_{37}H_{47}N_3O_7S_2$ (%): C, 62.60; H, 6.67; N, 5.92. Found: C, 62.84; H, 6.36; N, 5.83.

5-[4-Aza-5-oxo-10-{1′-(7-aza-10-trimethylammonio-6-oxodec-1-yl)-3,3,3′,3′-tetramethyl-5,5′-disulfoindotricarbocyanin-1-yl}-dec-1-en-1-yl)]-2′-deoxyuridine-5′-triphosphate lithium salt 9 was synthesized according to our previously published procedure.[23a] The yield of modified nucleotide as dark-green powder was 0.22 μmol (22%). MS (MALDI-TOF) *m/z*: calcd. for $C_{57}H_{76}N_7O_{22}P_3S_2^{4-}$ 1368.30; found 1373.12 $[M]^+$. ^{1}H NMR (D_2O) δ: 1.25, 1.35, 1.54, 1.79 (4m, 12H, dye C(7)H_2, C(8)H_2, C(9)H_2, C(2')H_2, C(3')H_2, C(4')H_2], 1.65 [s, 12H,

C(3,3)H_3, C(3,3')H_3], 2.24 [m, 6H, C(2')H_2, dye C(6)H_2, C(5')H_2], 3.04 [s, 11H, dye C(10')H_2, N(CH_3)$_3$], 3.23 [m, 4H, dye C(8')H_2, C(9')H_2], 3.83 [m, 2H, dye C(3)H_2], 4.02 [br.m., 7H, C(3')*H*, C(5')H_2, dye C(10)H_2, C(1')H_2], 4.55 [m, 1H, C(4')*H*], 6.31 [m, 5H, C(1')*H*, dye α,α'-C*H*, C(1)*H*, C(2)*H*], 6.53 (m, 2H, dye γ,γ'-C*H*), 7.75, 7.31 (2m, 7H, C(6)*H*, dye Ar*H*), 7.85 (m, 3H, δ-C*H*, β,β'-C*H*). ^{31}P NMR (D_2O) δ: -21.60 (t, $^{\beta}$P), -10.76 (d, $^{\alpha}$P), -5.45 (d, $^{\gamma}$P).

5-[4-Aza-5-oxo-10-(1,1′-diethyl-3,3,3′,3′-tetramethyl-5′-sulfoindotricarbocyanin-5-sulfonamidyl)dec-1-en-1-yl]-2′-deoxyuridine-5′-triphosphate lithium salt 10 was synthesized according to our previously published procedure.[23a] The yield of modified nucleotide as dark-green powder was 0.28 μmol (28%). MS (MALDI-TOF) *m/z*: calcd. for $C_{49}H_{61}N_6O_{20}P_3S_2^{4-}$ 1211.09; found 1216.8 $[M]^+$. ^{1}H NMR (D_2O) δ: 1.21 (t, 6H, CH_2CH_3, J = 7.0 Hz), 1.29, 1.56, 2.2, 2.62 (4m, 12H, NHCH_2CH_2CH_2CH_2CH_2CO, C(2')H_2), 1.69 (s, 12H, C(3,3)H_3, C(3',3')H_3), 3.49 (s, 2H, CH=CH-CH_2NH), 3.74 (m, 2H, C(5')H_2), 3.88 (m, 1H, C(4')*H*), 4.12 (m, 4H, CH_2CH$_3$), 4.24 (m, 1H, C(3')*H*), 6.32 (m, 3H, dye α,α'-C*H*, C(1')*H*), 6.43 (m, 1H, CH=C*H*-CH$_2$NH), 6.52 (m, 2H, dye γ-C*H*, C*H*=CH-CH$_2$NH), 7.23, 7.35, 7.62 (3m, 6H, dye Ar*H*), 7.71 (m, 1H, δ-C*H*), 7.84 (m, 2H, β,β'-C*H*), 8.11 (s, 1H, C(6)*H*). ^{31}P NMR (D_2O) δ: -19.71 (t, $^{\beta}$P), -10.14 (d, $^{\alpha}$P), -4.35 (d, $^{\gamma}$P).

5-[4-Aza-5-oxo-10-(1′-ethyl-3,3,3′,3′-tetramethyl-5,5′-disulfoindotricarbocyanin-1-yl)dec-1-en-1-yl]-2′-deoxyuridine-5′-triphosphate lithium salt 11 was synthesized according to our previously published procedure.[23a] The yield of modified nucleotide as dark-green powder was 0.37 μmol (37%). MS (MALDI-TOF) *m/z*: calcd. for $C_{47}H_{55}N_5O_{21}P_3S_2^{5-}$ 1183.01; found 1189.72 $[M]^+$. ^{1}H NMR (D_2O) δ: 1.22, 1.36, 1.51, 1.74 (4m, 9H, dye C(7)H_2, C(8)H_2, C(9)H_2, CH_2CH_3], 1.62 [s, 12H, C(3,3)H_3, C(3,3')H_3], 2.27 [m, 4H, C(2')H_2, dye C(6)H_2], 3.81 [m, 2H, dye C(3)H_2], 4.07 [br.m., 7H, C(3')*H*, C(5')H_2, dye C(10)H_2, CH_2CH$_3$], 4.52 [m, 1H, C(4')*H*], 6.34 [m, 5H, C(1')*H*, dye α,α'-C*H*, C(1)*H*, C(2)*H*], 6.51 (m, 2H, dye γ,γ'-C*H*), 7.71, 7.53, 7.31 (2m, 7H, C(6)*H*, dye Ar*H*), 7.81 (m, 3H, δ-C*H*, β,β'-C*H*). ^{31}P NMR (D_2O) δ: -20.83 (t, $^{\beta}$P), -10.54 (d, $^{\alpha}$P), -5.11 (d, $^{\gamma}$P).

«TB-Biochip» analysis. DNA samples were analyzed according to the protocol provided by the manufacturer (Biochip-IMB, Moscow, Russia).[23b] DNA sample was amplified by a two-round PCR. The presence of PCR products was confirmed by agarose gel electrophoresis after each reaction. In the first round PCR 3 μl of genomic DNA was amplified in a total reaction volume of 30 μl. The first round PCR reaction mixture contained 3 μl dilution of Taq buffer; 2.5 U Taq polymerase; 0.2 μM each deoxynucleoside triphosphate (dNTP); primers; and a 3 μl of the DNA sample. PCR was performed according to the following protocol: 4 min at 95ºC, (36

cycles): 30 s at 95°C, 30 s at 67°C, and 30 s at 72°C, and then 5 min at 72°C. The volume of 1 µl after the first round of PCR was used for the second PCR round. The reaction mixture for the second round PCR contained a fluorescently labeled nucleotide (8 and 80 µM) and was carried out with an excess of forward Cy3-labeled primer to obtain single-stranded product for hybridization. Amplification was performed according to the following protocol: 5 min at 95°C, (37 cycles): 20 s at 95°C, 30 s at 65°C, and 30 s at 72°C, and then 5 min at 72°C. Single-stranded DNA was then hybridized with a microarray chip by dispensing 12 µl of the labeled PCR product in hybridization buffer into the hybridization chamber and incubating the chip for 18 h at 37°C. The chip was washed with deionized water and air dried. The hybridization results were analyzed using two portable chip analyzers. First is equipped with a green laser (532 nm, 50mW), and emission was detected at 607 ± 35 nm. Second analyzer contains near-IR laser (760 nm, 300mW), and detection was performed at 832 ± 37 nm. Both analyzers were equipped with a charge-coupled device camera (BIOCHIP-IMB, Russian Federation). Data processing and image analysis were performed using dedicated software (ImaGeWare version 3.50).