Simple reagent for the synthesis of oligonucleotides labeled with 3,3,3′,3′-tetramethyl-2,2′-indodicarbocyanine

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Synthesis of a phosphoramidite reagent for the preparation of oligonucleotides labeled at the 5′-end with a fluorescent dye, 3,3,3′,3′-tetramethyl-2,2′-indodicarbocyanine, is described. The efficiency of this reagent is confirmed by the synthesis of several labeled oligonucleotides.

Key words: modified oligonucleotides, cyanine dyes, fluorescence.

Nucleic acids labeled with a fluorescent dye have found wide use in molecular biology as a convenient tool for specific detection of DNA sequences, in sequencing, and other applications.[1—3] Among cyanine dyes,[4] 3,3,3′,3′-tetramethyl-2,2′-indocyanine derivatives, whose methyl groups prevent dye intercalation or binding in the grooves of the DNA duplex, are used most often for covalent DNA labeling.[5] The fluorophore 3,3,3′,3′-tetramethyl-2,2′-indodicarbocyanine (Cy5) shows an absorption peak near 646 nm and can be excited using the 633 nm line of a helium-neon laser, the 647 nm line of a krypton laser, or affordable laser diodes. The fluorescence of indodicarbocyanine is observed in the red region where nonspecific fluorescence of biological specimens is negligibly low, which implies a good signal-to-noise ratio. The high sensitivity of fluorescence methods using cyanine dyes (in particular, Cy5) can be illustrated by monitoring the conformational changes in the DNA four-way junctions in a single molecule.[6] The fluorophore Cy5 proved to be perfect for primer labeling in automated DNA sequencers. To use an oligonucleotide as the primer, it is necessary to attach one Cy5 residue to its 5′-end; the ability of oligonucleotide to be involved in specific hybridization and elongation from the 3′-end is retained. Such conjugates can be synthesized using a non-nucleotide phosphoramidite reagent in a DNA synthesizer. Syntheses of a number of phosphoramidite reagents based on cyanine dyes have been described in patents,[7—11] and some of these reagents are commercially available, but very expensive.

In the present work, we describe the synthesis of simple and relatively inexpensive phosphoramidite reagent, which allows labeling of oligonucleotides with 3,3,3′,3′-tetramethyl-2,2′-indodicarbocyanine in an automated mode.

Results and Discussion

Alkyl substituents can easily be introduced into positions 1 and 1′ of the 3,3,3′,3′-tetramethyl-2,2′-indodicarbocyanine molecule. One alkyl group can contain a reactive phosphoramidite group suitable for condensation with the 5′-hydroxyl of the oligonucleotide immobilized on a polymeric support. It is reasonable to introduce a sulfonate into the second alkyl chain. This would result in overall neutrality of the molecule and additional weakening of the dye interaction with DNA due to the Coulomb repulsion between the sulfonate and the phosphates in the oligonucleotide chain.

2,3,3-Trimethylindolenine (**1**) served as the starting compound for the synthesis of the target dye (Scheme 1). It is readily alkylated with 1,4-butanesultone to give the inner salt **2**,[9] which is converted into hemicyanine **3** on treatment with malonaldehyde bis(phenylimine) in Ac_2O. Compound **3** is yellow-colored; however, the reaction yields also a minor amount of blue-colored symmetrical indodicarbocyanine, and the reaction mixture becomes green. The alkylation of indolenine **1** with 3-acetoxypropyl iodide results in product **4**,[7] which smoothly reacts with hemicyanine **3** to afford *N*,*N*′-disubstituted 3,3,3′,3′-tetramethyl-2,2′-indodicarbocyanine **5**. After its deacetylation by acid hydrolysis, the resulting alcohol **6** is phosphitylated with bis(diisopropylamino)-2-cyanoethoxyphosphine to yield phosphoramidite **7**.

The structures of compounds **2—7** were confirmed by ^{1}H NMR spectroscopy with the use of double resonance

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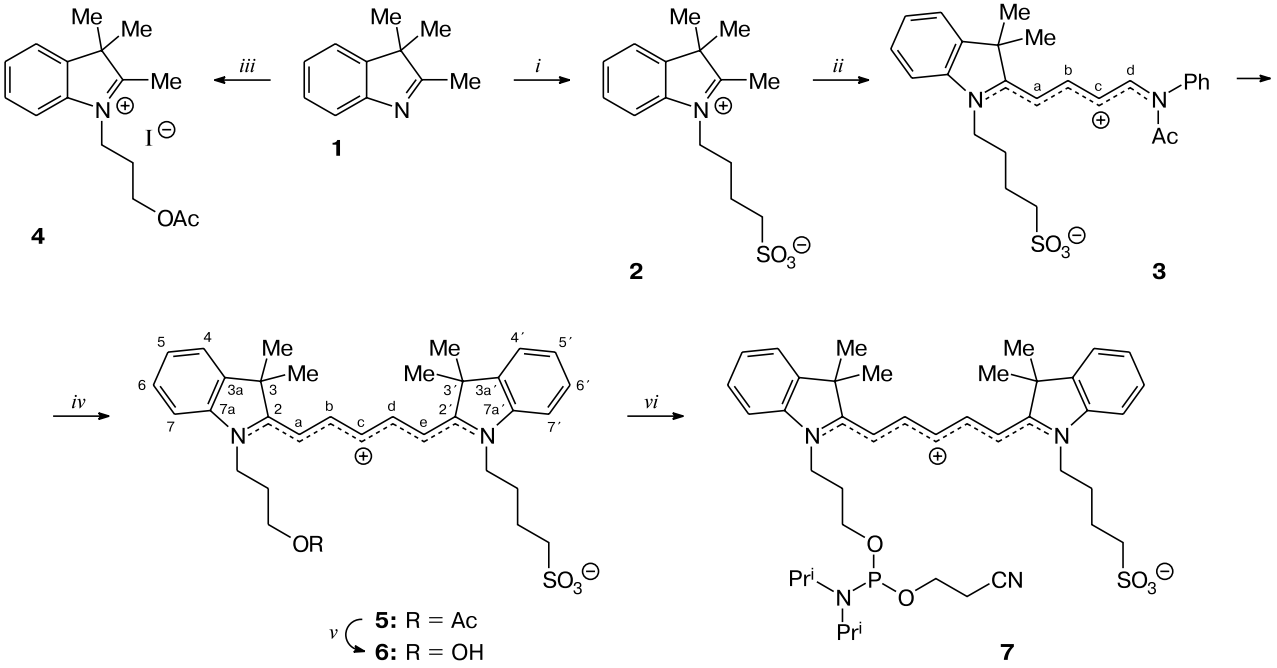
Scheme 1



Reagents and conditions: *i*. $\overline{O(CH_2)_4SO_2}$, 120 °C, 3 h; *ii*. $CH_2(CH{=}NPh)_2 \cdot HCl$, Ac_2O, 120 °C, 30 min; *iii*. $I(CH_2)_3OAc$, 110 °C, 4 h; *iv*. **4**, Py, ~20 °C, 3 h; *v*. HCl/MeOH, ~20 °C, 24 h; *vi*. $(Pr^i_2N)_2PO(CH_2)_2CN$, tetrazole, MeCN, 2 h.

and ^{13}C NMR spectroscopy. The 2D HMQC and HMBC heteronuclear ^1H-^{13}C correlation spectra were used to assign all of the ^{13}C NMR signals (except for the 3-Me and 3′-Me groups in compounds **5** and **6**). Phosphoramidite **7** gives only one signal in the ^{31}P NMR spectrum.

Cyanine-containing phosphoramidite **7** was used in an automated DNA synthesis for the preparation of 5′-labeled oligodeoxyribonucleotides. The coupling time with this reagent was increased from 20 s to 5 min. The resulting conjugates **8—13** were characterized by MALDI-TOF mass spectra and by UV and fluorescence spectra (Table 1, Fig. 1).

Labeled primers **8—13** were used in an automated DNA sequencer and showed a high efficiency of reading the nucleotide sequence (400—600 nucleotides on a gel).

Thus, we synthesized a simple phosphoramidite reagent for the preparation of oligonucleotide labeled at the 5′-end with the fluorescent dye 3,3,3′,3′-tetramethyl-2,2′-indodicarbocyanine.

Experimental

Commercial 2,3,3-trimethylindolenine, malonaldehyde bis(phenylimine) hydrochloride, and 1,4-butanesultone (Fluka) were used. Prior to use, pyridine was distilled from CaH_2 and acetic anhydride was distilled from P_4O_{10}. 3-Iodopropyl acetate[7] and bis(diisopropylamino)-2-cyanoethoxyphosphine[12] were synthesized by reported procedures. The reactions were monitored by TLC on Kieselgel 60 F_{254} plates (Merck); the spots were visualized in UV light at 256 nm. Column chromatography was performed using silica gel Kieselgel 60 (Merck)

Table 1. Properties of modified oligonucleotides synthesized using reagent **7** (**X** is the residue of **6**-*p*)

Compound	(5′→3′) sequence	MALDI-TOF mass spectrum, mass found/calculated	Peaks/nm	
			absorption	fluorescence
8	**X**-ATCACCACCAGCAGCAAAG	6376.2/6371.5	646	669
9	**X**-CGAATATTTTCACTCTGACTGGG	7652.8/7641.3	645	668
10	**X**-GGAAGCTGCATGATGAGACC	6811.9/6802.7	646	669
11	**X**-GAATTCTCGTCCCTTCATCTC	6904.1/6894.8	646	667
12	**X**-ATGGAGTTTCAGGACCACTT	6751.8/6743.7	647	670
13	**X**-ACAATTGAATGTCTGCACAGCCACTT	8538.2/8525.8	647	668

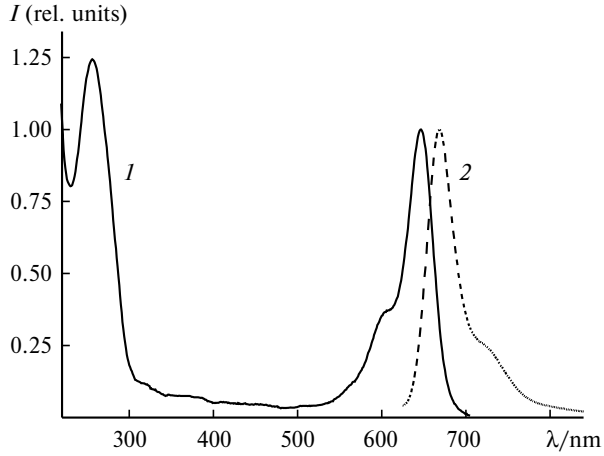


Fig. 1. Normalized absorption (*1*) and fluorescence (*2*) spectra of oligonucleotide **10**.

with a particle size of 40—63 μm. The solutions were concentrated on a rotary evaporator in the vacuum of a water-jet pump at 30—50 °C. The NMR spectra were recorded at 500 MHz (^{1}H), 125.7 MHz (^{13}C), and 202.4 MHz (^{31}P) on a Bruker AC-500 spectrometer in DMSO-d_6. The spectra were calibrated based on the residual proton signals of the solvent (δ_H 2.50, δ_C 39.60), the chemical shifts were referred to Me_4Si (^{1}H and ^{13}C) or 85% H_3PO_4 (^{31}P). Melting points were determined on a Boetius hot stage (not corrected). The oligonucleotide synthesis was carried out on a BiosSet ASM-700 instrument on a 200 nmol scale using standard manufacturer´s protocols. MALDI-TOF mass spectra were run on a Bruker Ultraflex spectrometer. The UV spectra of compound **6** and oligonucleotides were measured on a Varian Cary 300 spectrophotometer in doubly distilled water. The fluorescence spectra were recorded on a high-aperture optics[13] in doubly distilled water; the spectra were corrected for the spectral sensitivity of the instrument. For sequencing, an ALFexpress automated DNA sequencer was used (Amersham Pharmacia Biotech); the results were processed using the ALFwin Sequence Analyser 2.10 software.

2,3,3-Trimethyl-1-(4-sulfonatobutyl)-3*H*-indolium (2).[9] A mixture of 2,3,3-trimethylindolenine (**1**) (3.97 g, 25.0 mmol) and 1,4-butanesultone (3.47 g, 25.5 mmol) was heated for 4 h at 120 °C; during this period, the reaction mixture solidified. After cooling, the resulting solid was triturated with ether (30 mL), filtered off, and thoroughly washed with ether. The hygroscopic compound **2** thus obtained was dried *in vacuo*. Yield 6.20 g (83%), colorless crystals, R_f 0.11 (MeOH—CH_2Cl_2, 1 : 4), dec. >232 °C (CH_2Cl_2—ether). ^{1}H NMR (DMSO-d_6), δ: 1.53 (s, 6 H, 3-C$\underline{H}_3$); 1.75 (m, 2 H, $SCH_2C\underline{H}_2$); 1.98 (m, 2 H, $NCH_2C\underline{H}_2$); 2.51 (t, 2 H, SCH_2, J = 7.3 Hz); 2.85 (s, 3 H, 2-CH_3); 4.49 (t, 2 H, NCH_2, J = 7.6 Hz); 7.61 (m, 2 H, H(5), H(6)); 7.83 (m, 1 H, H(4)); 8.03 (m, 1 H, H(7)). ^{13}C NMR (DMSO-d_6), δ: 13.88 (2-CH_3); 22.07 (2 C, 3-CH_3); 22.20 ($NCH_2\underline{C}H_2$); 26.08 ($SCH_2\underline{C}H_2$); 47.37 (NCH_2); 50.20 (SCH_2); 54.17 (C(3)); 115.68 (C(7)); 123.46 (C(4)); 129.00 (C(6)); 129.41 (C(5)); 141.24 (C(7a)); 141.91 (C(3a)); 196.56 (C(2)).

2-[4-(*N*-Acetylanilino)buta-1,3-dienyl]-3,3-dimethyl-1-(4-sulfonatobutyl)-3*H*-indolium (3). A mixture of betaine **2** (1.588 g, 5.38 mmol) and malonaldehyde bis(phenylimine) hydrochloride (0.836 g, 6.46 mmol) in Ac_2O (20 mL) was heated for 30 min at 120 °C, cooled, and diluted with chloroform (20 mL), and the product was precipitated with cold ether (150 mL). The solid was filtered off, washed with ether, and reprecipitated with cold ether (150 mL) from a solution in CH_2Cl_2 (50 mL). The precipitate was re-dissolved in CH_2Cl_2 (50 mL), the solvent was evaporated, this procedure was repeated twice more, and the resulting foam was dried *in vacuo* to give 2.060 g (82%) of hemicyanine **3**, R_f 0.30 (EtOH—CH_2Cl_2, 1 : 4), which was used in the subsequent transformations without additional purification. ^{1}H NMR (DMSO-d_6), δ: 1.84—1.63 (m, 10 H, $CHC\underline{H}_3$, $NCH_2C\underline{H}_2C\underline{H}_2$); 2.03 (s, 3 H, $COCH_3$); 2.42 (t, 2 H, SCH_2, J = 7.3 Hz); 4.30 (t, 2 H, NCH_2, J = 7.4 Hz); 5.56 (dd, 1 H, H_c, $J_{b,c}$ = 11.4 Hz, $J_{c,d}$ = 13.0 Hz); 6.95 (d, 1 H, H_a, $J_{a,b}$ = 15.0 Hz); 7.83—7.36 (m, 8 H, H(4), H(6), H(7), Ph); 8.52 (dd, 1 H, H_b, $J_{a,b}$ = 15.0 Hz, $J_{b,c}$ = 11.4 Hz); 8.89 (d, 1 H, H_d, $J_{c,d}$ = 13.0 Hz).

1-(3-Acetoxypropyl)-2,3,3-trimethyl-3*H*-indolium iodide (4) (see Ref. 7). A mixture of 2,3,3-trimethylindolenine (**1**) (10.91 g, 68.6 mmol) and 3-iodopropyl acetate (15.65 g, 68.6 mmol) was heated for 4 h at 110 °C, cooled, dissolved in a mixture of MeOH (5.5 mL) and CH_2Cl_2 (50 mL), and precipitated with cold ether (200 mL). The precipitated oil crystallized over a period of several h at room temperature. The crystals were triturated with ether (100 mL), filtered off, washed with ether (100 mL), and dried *in vacuo*. Yield 13.02 g (49%), fine colorless crystals, R_f 0.55 (MeOH—CH_2Cl_2, 1 : 19), m.p. 153—154 °C ($CHCl_3$—ether). ^{1}H NMR (DMSO-d_6), δ: 1.55 (s, 6 H, 3-CH_3); 1.90 (s, 3 H, $COCH_3$); 2.22 (m, 2 H, $NCH_2C\underline{H}_2$); 2.86 (s, 3 H, 2-CH_3); 4.16 (t, 2 H, OCH_2, J = 6.1 Hz); 4.56 (t, 2 H, NCH_2, J = 7.0 Hz); 7.63 (m, 2 H, H(5), H(6)); 7.85 (m, 1 H, H(4)); 7.96 (m, 1 H, H(7)). ^{13}C NMR (DMSO-d_6), δ: 14.15 (2-CH_3); 20.56 ($CO\underline{C}H_3$); 22.02 (2 C, 3-CH_3); 26.39 ($NCH_2\underline{C}H_2$); 45.11 (NCH_2); 54.28 (C(3)); 61.21 (OCH_2); 115.32 (C(7)); 123.57 (C(4)); 128.99 (C(6)); 129.47 (C(5)); 141.28 (C(7a)); 141.82 (C(3a)); 170.21 (CO); 197.18 (C(2)).

2-{5-[1-(3-Hydroxypropyl)-3,3-dimethyl-2,3-dihydroindol-2-ylidene]-1,3-pentadienyl}-3,3-dimethyl-1-(4-sulfonatobutyl)-3*H*-indolium (6). ***A.*** A solution of compound **4** (1.258 g, 3.25 mmol) in pyridine (8 mL) was added to a stirred solution of hemicyanine **3** (1.317 g, 2.83 mmol) in Ac_2O (8 mL), and the mixture was stirred for 2 h (TLC monitoring, CH_2Cl_2—EtOH, 4 : 1, as the eluent). The reaction mixture was diluted with CH_2Cl_2 (50 mL), and the product was precipitated with cold petroleum ether (250 mL). The precipitate was filtered off, washed with petroleum ether, and dissolved in CH_2Cl_2 (2×50 mL). The solvent was evaporated. The resulting **2-{5-[1-(3-acetoxypropyl)-3,3-dimethyl-2,3-dihydroindol-2-ylidene]penta-1,3-dienyl}-3,3-dimethyl-1-(4-sulfonatobutyl)-3*H*-indolium (5)** was used in the next step without purification. An analytical sample was isolated by chromatography on silica gel (gradient elution with EtOH in CH_2Cl_2, 0→25%), R_f 0.43 (EtOH—CH_2Cl_2, 1 : 4). ^{1}H NMR (DMSO-d_6), δ: 1.68 (s, 12 H, CH_3); 1.71—1.88 (m, 6 H, $OCH_2C\underline{H}_2$, $SCH_2C\underline{H}_2C\underline{H}_2$); 2.50 (m, 2 H, SCH_2); 3.50 (m, 2 H, OCH_2); 4.12 (m, 4 H, NCH_2); 4.76 (t, 1 H, OH, J = 5.2 Hz); 6.31 (d, 1 H, H_e, $J_{d,e}$ = 13.0 Hz); 6.41 (d, 1 H, H_a, $J_{a,b}$ = 13.0 Hz); 6.58 (t, 1 H, H_c, $J_{b,c}$ = $J_{c,d}$ = 13.0 Hz); 7.24 (m, 2 H, H(5), H(5´)); 7.34—7.46 (m, 4 H, H(6), H(6´), H(7), H(7´)); 7.60 (m, 2 H, H(4), H(4´)); 8.32 (t, 2 H, H_b, H_d, $J_{a,b}$ = $J_{b,c}$ = $J_{c,d}$ = $J_{d,e}$ = 13.0 Hz). ^{13}C NMR (DMSO-d_6), δ: 22.51 ($SCH_2CH_2\underline{C}H_2$); 26.01 ($SCH_2\underline{C}H_2$); 27.21 (2 C), 27.23 (2 C) (3-CH_3, 3´-CH_3); 30.14 ($OCH_2\underline{C}H_2$); 40.74

($OCH_2CH_2\underline{C}H_2$); 43.50 ($SCH_2CH_2CH_2\underline{C}H_2$); 48.87 (C(3)); 48.96 (C(3′)); 50.69 (SCH_2); 57.72 (OCH_2); 102.98 (C_e); 103.61 (C_a); 110.93 (C(7)); 111.32 (C(7′)); 122.41 (2 C, C(4), C(4′)); 124.58 (C(5′)); 124.78 (C(5)); 125.72 (C_c); 128.43 (C(6)); 128.51 (C(6′)); 141.12 (C(3a′)); 141.21 (C(3a)); 142.04 (C(7a′)); 142.18 (C(7a)); 153.84 (C_d); 154.22 (C_b); 172.47 (C(2)); 172.85 (C(2′)).

Compound **5** was dissolved in MeOH (80 mL), conc. HCl (19 mL) was added, and the solution was kept for 24 h at room temperature. The solvent was evaporated, H_2O (100 mL) and $CHCl_3$ (100 mL) were added to the residue, the mixture was stirred, and the aqueous phase was extracted with $CHCl_3$ (4×50 mL). The combined organic phases were washed with a saturated solution of $NaHCO_3$ (2×100 mL), dried with anhydrous Na_2SO_4, and concentrated, and the residue was chromatoraphed on silica gel (elution with a gradient of EtOH in CH_2Cl_2, 0→25%). The product was dissolved in CH_2Cl_2, the solvent was evaporated (2×50 mL), and the residue was dried *in vacuo*. The yield of compound **6** was 0.375 g (38%, based on hemicyanine **3**), a gold-brown foam, R_f 0.18 (EtOH—CH_2Cl_2, 1 : 4), m.p. 192—194 °C (dec.) ($CHCl_3$—ether). UV (water), λ_{max}/nm, (log ε): 641 (5.26). Fluorescence (water), λ_{max}/nm: 663. ^{1}H NMR (DMSO-d_6), δ: 1.69 (s, 12 H, CCH_3); 1.70—1.84 (m, 4 H, $SCH_2C\underline{H}_2C\underline{H}_2$); 1.95 (s, 3 H, $COCH_3$); 2.04 (m, 2 H, $OCH_2C\underline{H}_2$); 2.50 (m, 2 H, SCH_2); 4.07 (m, 2 H, OCH_2); 4.16 (m, 4 H, NCH_2); 6.26 (d, 1 H, H_e, $J_{d,e}$ = 13.7 Hz); 6.45 (d, 1 H, H_a, $J_{a,b}$ = 14.0 Hz); 6.58 (t, 1 H, H_c, $J_{b,c} = J_{c,d}$ = 12.3 Hz); 7.22 (t, 1 H, H(5), J = 7.3 Hz); 7.27 (t, 1 H, H(5′), J = 7.3 Hz); 7.32—7.48 (m, 4 H, H(6), H(6′), H(7), H(7′)); 7.61 (m, 2 H, H(4), H(4′)); 8.33 (m, 2 H, H_b, H_d). ^{13}C NMR (DMSO-d_6), δ: 20.66 ($CO\underline{C}H_3$); 22.51 ($SCH_2CH_2\underline{C}H_2$); 26.01 (2 C, $OCH_2\underline{C}H_2$, $SCH_2\underline{C}H_2$); 27.13 (2 C), 27.29 (2 C) (3-CH_3, 3′-CH_3); 40.42 ($OCH_2CH_2\underline{C}H_2$); 43.61 ($SCH_2CH_2CH_2\underline{C}H_2$); 48.73 (C(3)); 49.12 (C(3′)); 50.62 (SCH_2); 61.33 (OCH_2); 102.64 (C_e); 104.07 (C_a); 110.68 (C(7)); 111.53 (C(7′)); 122.44 (2 C, C(4), C(4′)); 124.39 (C(5′)); 125.01 (C(5)); 125.72 (C_c); 128.39 (C(6)); 128.53 (C(6′)); 140.92 (C(3a′)); 141.34 (C(3a)); 141.95 (C(7a′)); 142.21 (C(7a)); 153.67 (C_d); 154.62 (C_b); 170.33 (CO); 171.95 (C(2)); 173.43 (C(2′)).

B. A mixture of betaine **2** (1.50 g, 5.08 mmol) and malonaldehyde bis(phenylimine) hydrochloride (0.790 g, 6.10 mmol) in Ac_2O (15 mL) was heated for 30 min on an oil bath at 120 °C and cooled with stirring. A solution of compound **4** (2.75 g, 7.11 mmol) in pyridine (15 mL) was added. The mixture was stirred at room temperature for 3 h and concentrated, the residue was dissolved in $CHCl_3$ (50 mL), and indodicarbocyanine **5** was precipitated by cold petroleum ether (200 mL). The precipitate was filtered off and dissolved in a minimum volume of MeOH, and a solution of HCl in MeOH (prepared by adding AcCl (15 mL) to MeOH (100 mL)) was added. The reaction mixture was stirred for ~16 h and carefully poured onto dry Na_2CO_3 (30 g), and the mixture was stirred for 10 min. The reaction mixture was filtered, the precipitate was washed with MeOH (50 mL), the filtrate was concentrated, the residue was suspended in CH_2Cl_2 (100 mL), the solution was filtered, and the residue was twice refluxed with CH_2Cl_2 (100 mL, 5 min) and filtered. The filtrates were combined, the solvent was removed, and the residue was dissolved in CH_2Cl_2 (5 mL) and chromatographed on silica gel as described in method *A*. The yield of compound **6** was 1.045 g (38%).

2-(5-{1-[3-(*N*,*N*-Diisopropylamino-2-cyanoethoxyphosphinyloxy)propyl]-3,3-dimethyl-2,3-dihydroindol-2-ylidene}penta-1,3-dienyl)-3,3-dimethyl-1-(4-sulfonatobutyl)-3*H*-indolium (7). Indocyanine **6** (0.208 g, 0.38 mmol) was dissolved in anhydrous MeCN, the solvent was evaporated (2×30 mL), the residue was dissolved in anhydrous MeCN (25 mL), and a 0.5 *M* solution of tetrazole in acetonitrile (76 μL, 0.38 mmol) and bis(diisopropylamino)-2-cyanoethoxyphosphine (178 μL, 0.56 mmol) were added. The mixture was concentrated twice at 35 °C and stirred at room temperature for 3 h, the solvent was evaporated, the residue was diluted with $CHCl_3$ (50 mL), the solution was washed with a mixture of brine and saturated $NaHCO_3$ (1 : 1, 30 mL), dried with Na_2SO_4 (1 h), and filtered, and the solvent was evaporated. The residue was dissolved in CH_2Cl_2 (5 mL) and precipitated with cold ether (50 mL). The resulting phosphoramidite was dissolved in dry CH_2Cl_2, the solvent was evaporated (2×50 mL), and the residue was dried *in vacuo*. Yield 0.213 g (75%), foam yield, R_f 0.62 (MeOH—CH_2Cl_2—Et_3N, 1 : 17 : 2). ^{1}H NMR (DMSO-d_6), δ: 1.14 (m, 12 H, $CHC\underline{H}_3$); 1.68 (s, 12 H, 3-CH_3, 3′-CH_3); 1.69—1.83 (m, 4 H, $SCH_2C\underline{H}_2C\underline{H}_2$); 2.00 (m, 2 H, $OCH_2C\underline{H}_2CH_2$); 2.50 (m, 2 H, SCH_2); 2.80 (t, 2 H, $NCCH_2$, J = 6.0 Hz); 3.54—3.80 (m, 6 H, $POCH_2$, PNCH); 4.11 (m, 4 H, NCH_2); 6.28 (m, 1 H, H_e); 6.37 (m, 1 H, H_a); 6.55 (m, 1 H, H_c); 7.24 (m, 2 H, H(5), H(5′)); 7.31—7.48 (m, 4 H, H(6), H(6′), H(7), H(7′)); 7.61 (m, 2 H, H(4), H(4′)); 8.32 (m, 2 H, H_b, H_d). ^{31}P NMR (DMSO-d_6), δ: 147.67.

Oligonucleotide synthesis was carried out in the automated mode using standard phosphoramidites dA^{Bz}, dC^{Bz}, and dG^{Ibu} according to instrument manufacturer′s protocols. The coupling of terminal phosphoramidite **7** was performed for 5 min (the capping and removal of the dimethoxytrityl group were not carried out). Oligonucleotide detachment from the substrate and deprotection were performed by treatment with concentrated (28%) ammonia (0.75 mL). After deprotection, the resulting solution was concentrated, the residue was dissolved in water (150 μL), and the oligonucleotide was precipitated with 0.5 *M* $LiClO_4$ in acetone (1.5 mL), centrifuged, washed with acetone (1 mL), and dissolved in deionised formamide (50 μL). The solution was purified by electrophoresis in 20% denaturing (7 *M* urea) polyacrylamide gel (550 V, 20 mA, monitoring using Bromophenol blue and Xylene cyanol). Oligonucleotides were visualized in the gel based on the dye absorption, eluted with 0.5 *M* $LiClO_4$ for 12 h, filtered to remove the gel, and desalted on NAP-10 columns (Amersham Biosciences). The oligonucleotide concentration was determined by spectrophotometry by measuring the absorption at 260 nm. The fluorescence spectra were recorded in water, with an excitation wavelength of 620 nm. When recording the mass spectra of oligonucleotides, a 1 : 1 (v/v) mixture of solutions of 2,6-dihydroxyacetophenone (40 mg in 1 mL of methanol) and diammonium citrate (80 mg in 1 mL of 50% aqueous acetonitrile) prepared directly prior to each measurement was used as the ionization matrix.

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