New Indocyanine Derivatives for the Synthesis of Fluorescently Labeled Oligonucleotides

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Abstract: New sulfonated Cy3 and Cy5 phosphoramidites and solid supports were synthesized. These building blocks were used in the synthesis of terminally or internally labeled oligodeoxynucleotides. Mild deprotection schemes were applied to the sulfocyanine-labeled oligonucleotides.

Keywords: Oligonucleotides, fluorescent label, phosphoramidites, controlled pore glass, indocyanines, deprotection.

INTRODUCTION

Many applications in biology, such as oligonucleotide microarray analyses, detection of PCR products and structural studies, require fluorescently labeled oligonucleotides [1-3]. In recent years, researchers have taken advantage of the excellent fluorescent properties of carbocyanines to label biological molecules [4-5]. Cyanine dyes have many desirable properties, including safe handling, absorbtion at longer wavelengths, high extinction coefficients, relatively high quantum efficiency, small molecular size, ease of chemical manipulation, and reasonable stability to reagents, pH and temperature. Because of a low background fluorescence of biological materials and a high absorbtion of cyanine dyes in the long-wave range of the spectrum, cyanine dyes provide excellent signal-to-noise ratios. The versatility of the functional groups that can be incorporated into cyanine dyes allows control over the solubility of the dye and labeled product, and helps reduce non-specific binding of the labeled materials to irrelevant components in an assay mixture [6-7].

At present, the labeling of oligonucleotides with cyanine dyes is performed either post-synthetically by aminoalkyl modification using activated derivatives of dyes [8] or using phosphoramidites during an automated oligonucleotide synthesis [9]. Currently available phosphoramidites of cyanine dyes allow the incorporation of a fluorescent label into the oligonucleotides at the 5′ end, or anywhere in the sequence except for the 3′-terminus. Some of these carbocyanine and dicarbocyanine amidites have a MMTr protected hydroxypropyl chain attached directly to indolenine and lack any sulfonate groups, which might increase their solubility in water and reduce their self-association [10]. Other products include di- and tricarbocyanine phosphoramidites, which are 5′-end terminators with a sulfobutyl substituent at the tertiary nitrogen [11].

RESULTS AND DISCUSSION

To extend the range of useful cyanine dye derivatives, make 3′-labeling possible, implement convenient DMT chemistry, and provide better solubility in aqueous media, we report here the synthesis of new sulfonated Cy3 (SCy3) and Cy5 (SCy5) phosphoramidites (**5a, 5b**) and solid supports (**6a, 6b**). These derivatives were synthesized by the coupling of activated dye acids **2a** and **2b** with the functional aminolink **3**, and subsequent conversion to phosphoramidites or alternative binding to a solid support. Linker **3** was derived from 1,2,6-hexanetriol using a transient 1,2-isopropylidene protecting group, followed by the conversion of the primary hydroxyl group to an amine through an azide, Scheme (**1**). The protected aminodiol **3** was synthesized with an overall yield of 27%. Asymmetric indocyanine dye acids **1a,b** were prepared following published procedures with modifications [12-14]. Sulfonated indocyanines **1a** and **1b** have absorbence (emission) maxima at 550 (565) and 643 (657) nm, respectively. Before reacting with aminolink **3**, free dye acids were converted to activated N-hydroxysuccinimide esters **2a** and **2b,** Fig. (**1**). Coupling of these activated dyes with the protected aminodiol gave amide dye conjugates **4a** and **4b**.

The separate synthesis of a functional linker, such as **3**, and carboxyalkyl substituted fluorescent synthons seems to have advantages over the functionalization of dyes. First, activated carboxyalkyl indocyanines are commonly used reagents for labeling biomolecules, including amino-modified oligonucleotides, nucleoside triphosphates, and proteins. Coupling of the same universal linker with different dyes would then provide a wide range of building blocks for the preparation of phosphoramidites or derivatized controlled pore glass (CPG) supports. Finally, a DMTr protected linker adds solubility to conjugates of sulfonated indocyanines in aprotic organic solvents, such as acetonitrile and dichloromethane. Indeed, conjugates **4a** and **4b** were found to have excellent solubility in dichloromethane. At the same time, it was possible to precipitate these compounds with ethyl acetate or diethyl ether.

The reaction of conjugates **4a** and **4b** with 2-cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropyl-phosphoramidite in the presence of pyridinium tetrazolide provided phosphoramidites **5a** and **5b**. We used 0.1 M phosphoramidite solutions in MeCN-DCM (1:1) for the preparation of oligonucleotides I–III, Table (**1**). The incorporation of SCy3 and SCy5 residues during

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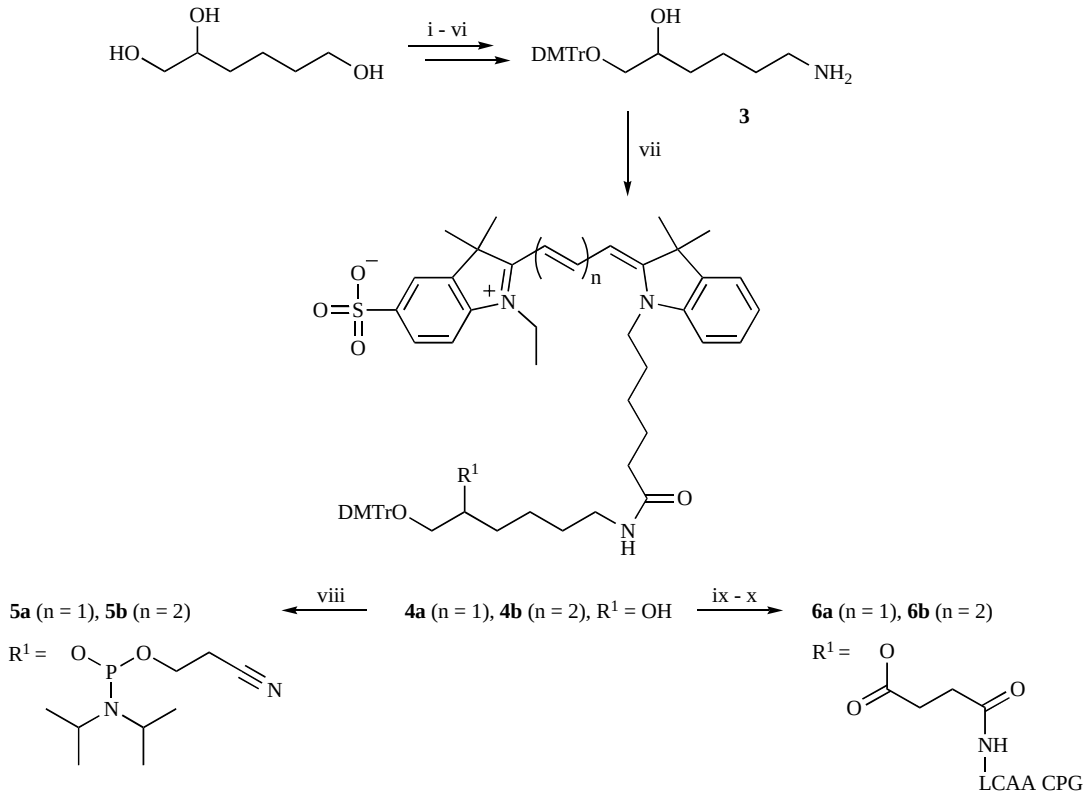

Scheme 1. Synthesis of sulfonated Cy3 and Cy5 phosphoramidites and solid supports. Reagents and conditions: (i) acetone, TsOH in $CHCl_3$, reflux with azeotropic removal of H_2O; (ii) MsCl in Py, 25ºC, 12h; (iii) LiN_3 in DMF, 12h, 70ºC; (iv) 80% AcOH, 15min, 60ºC; (v) DMTrCl in Py, 25ºC, 12h; (vi) Ph_3P in Py; (vii) (**2a,b**) in DMF, 25ºC, 2h; (viii) 2-Cyanoethyl-*N*,*N*,*N′*,*N′*-tetraisopropyl-phosphoramidite, tetrazole, Py in MeCN, 25ºC, 30min; (ix) succinic anhydride in Py, 25ºC, 4h; (x) long chain alkylamine controlled pore glass (LCAA CPG), diisopropyl-carbodiimide, Et_3N in DCM, 35ºC, 4h.

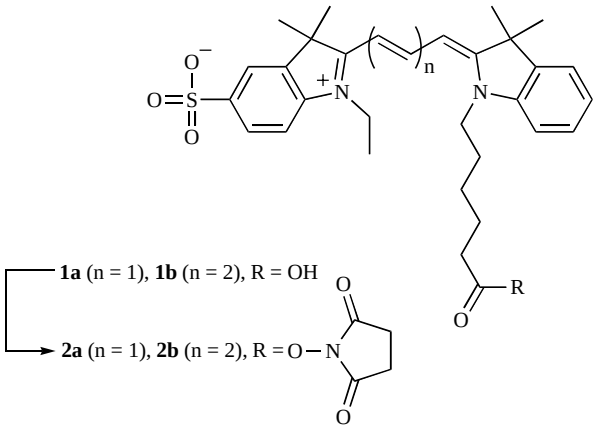

Fig. (1). Indocyanine dyes and their *N*-hydroxysuccinimide esters.

automated oligonucleotide synthesis was performed with a modification of the standard protocol: the coupling time was 3 min and 0.02 M solution of I_2 was used at the oxidation step. The efficiency of coupling for **5a** and **5b** was found to be at least 95%, as estimated by the DMTr cation absorbance at 495 nm.

The attachment of conjugates **4a** and **4b** to a 500Å long-chain alkylamine controlled pore glass *via* a succinate linker produced derivatized cyanine solid supports with loadings of 34 and 40 μmol/g for SCy3 and SCy5 respectively. These CPG supports were used to synthesize 3′-labeled oligonucleotides IV-VI, Table (**1**).

The deprotection conditions were found to differ considerably for SCy3- and SCy5-labeled oligonucleotides. The treatment of oligonucleotides with concentrated aqueous ammonia at room temperature for 4–48 hours was found to be non-destructive for the SCy3 unit. This deprotection procedure was successively used with both standard phosphoramidites (dA^{bz}, dG^{ibu}, dC^{bz}) and amidites with labile protecting groups (dA^{PAC}, $dG^{iPr\text{-}PAC}$, dC^{Ac}). By contrast, the SCy5 label underwent noticeable degradation in aqueous ammonia at room temperature. A solution of ammonia in anhydrous methanol (2 M) induced rapid destruction of the sulfonated indodicarbocyanine dye. We believe that the increased sensitivity of the SCy5 label to nucleophilic reagents originates from the aromatic sulfonate group substitution. The only deprotection scheme that did not induce the decomposition of the SCy5 label was treatment with 0.05 M K_2CO_3 in anhydrous methanol for 4–48 hours at room temperature. Shorter dye exposure to alkaline reagents is preferable and requires the use of mild deprotection phosphoramidites during oligonucleotide synthesis.

The incorporation of the SCy3 and SCy5 residues into oligonucleotides was confirmed by MALDI mass-spectrometry, Table (**1**).

We synthesized labeled oligonucleotides in both DMTr-ON and DMTr-OFF modes. The presence of the DMTr protecting group was useful for purification of the 3′-end or internally labeled oligonucleotides on reverse phase. Although the difference in hydrophobic properties for the 5’-DMTr-

Table 1. Sequences, MALDI-TOF Data and Spectroscopic Properties of Sulfocyanine Labeled Oligonucleotides

No.	Oligonucleotide*	MALDI-TOF MS calcd/found	$\lambda_{abs}/\lambda_{em}$, nm**
I	5'-SCy3-GCG-AGC-ATA-ATG-CCT-GCG-TCA-TCC-G-C7NH_2	8582/8587.8	552/566
II	5'-SCy3-CGG-ATG-ACG-CAG-GCA-TTA-TGC-TCG-C	8412/8419.9	551/566
III	5'-DMTr-CTG-ACT-CCA-SCy5-G-C7NH_2	4255/4256.5	645/662
IV	5'-ATG-ACG-CAG-GCA-TTA-SCy3	5330/5328.0	551/566
V	5'-ATA-ACC-G-SCy5	2843/2845.8	644/662
VI	5'-CTG-ACT-CCA-G-SCy5	3742/3742.1	645/661

* C7NH_2 – C7 aminolink; ** Measured in 0.1M NaCl, 0.05M sodium phosphate (pH 7.0).

capped and the completely deprotected labeled oligonucleotides may be insufficient for cartridge purification, the standard gradient in reverse-phase HPLC allowed clean separation of the DMTr-ON oligonucleotide. Due to the chiral nature of aminodiol **3,** we often observed two peaks in the separation profile.

In conclusion, we have developed new sulfonated indocyanine derivatives for the synthesis of fluorescently labeled oligonucleotides. Derivatization with dye solid supports allows the incorporation of sulfocyanines at the 3′-end while cyanine phosphoramidites can be introduced at the 5′-end or inside the oligonucleotide sequence. Depending on the nature of the dye, either aqueous ammonia or K_2CO_3 in anhydrous methanol should be used for the deprotection of labeled oligonucleotides at room temperature.

EXPERIMENTAL SECTION

General

TLC was performed using Merck $60F_{254}$ plates with the eluents $CHCl_3$/MeOH 9 : 1 (A); $CHCl_3$/MeOH/Hexane 9 : 1 : 10 (B); $CHCl_3$/MeOH 8 : 2 (C). Compounds were visualized by UV light (254 nm) or by spraying with 5% sulphuric acid in ethanol and heating. Reverse-phase TLC was performed using Merck RP-18 F_{254} plates in MeCN/H_2O 1 : 1 (D). Column chromatography was performed using Merck silica gel grade 60, 70-230 mesh. NMR spectra were recorded using a Bruker AMX-400 spectrometer at 300 K. Chemical shifts (δ) are given in ppm and referenced to the residual solvent peaks (^1H and ^{13}C) or relative to external standard (H_3PO_4, capil.) (^{31}P). Coupling constants (*J*) are given in Hz. MALDI mass spectra were acquired using a Bruker Reflex IV mass spectrometer with hydroxypicolinic acid or 2-amino-5-nitropyridine as a matrix. IUPAC names of new compounds were obtained using the ACD/ILab Web service at http://www.acdlabs.com/ilab. UV absorbane spectra were recorded using a Shimadzu UV-160A spectrophotometer. Fluorescence emission spectra were recorded using a Shimadzu RF-5301PC spectrofluorophotometer.

Synthesis of Compound 3

4-(2,2-Dimethyl-1,3-dioxolan-4-yl)butan-1-ol. A mixture of 1,2,6-hexanetriol (11.4 g, 85 mmol), chloroform (200 ml), acetone (30 ml) and *p*-toluenesulfonic acid (0.1 g) was refluxed with azeotropic removal of water until complete consumption of triol. Reaction progress was monitored by TLC (A). Cooled solution was diluted with chloroform (200 ml), washed with saturated aqueous sodium bicarbonate (200 ml) and with water (200 ml). After drying over sodium sulfate, the solution was concentrated under reduced pressure. The residue was distilled *in vacuo*, yielding 11.5 g (77.7%) of protected diol, b.p. 122–124 °C (15 mm Hg), R_f (A) = 0.64. ^1H NMR ($CDCl_3$, 400 MHz) δ 3.97–4.10 (2H, m, $\underline{CH_2}$-CH), 3.60 (2H, t, *J* 6.52, $\underline{CH_2}$-OH), 3.47 (1H, t, *J* 7.16, CH), 1.40–1.68 (6H, m, $(CH_2)_3$), 1.31 and 1.37 (6H, s, 2×CH_3). MS (EI): m/z = 159 $(M - CH_3)^+$, calcd. for $C_9H_{18}O_3$ 174.23.

4-(2,2-Dimethyl-1,3-dioxolan-4-yl)butyl methanesulfonate. Methane sulfonyl chloride (2.2 ml, 28 mmol) was added at 0 °C to a solution of 1,2-protected triol (4.75 g, 27 mmol) in pyridine (20 ml). The mixture was stirred for 16 h at room temperature then concentrated under reduced pressure. The residue was dissolved in chloroform (200 ml). Solution was washed with saturated aqueous sodium bicarbonate (200 ml) and with water (200 ml). Solvent was removed *in vacuo* to provide 4.49 g (65.3%) of yellow gum. R_f (B) 0.80. ^1H NMR ($CDCl_3$, 400 MHz) δ4.21 (2H, t, *J* 6.52, Ms-O$\underline{CH_2}$), 3.97–4.11 (2H, m, CH-CH_2), 3.49 (1H, t, *J* 7.16, CH), 2.99 (3H, s, SO_3-CH_3), 1.45–1.85 (6H, m, $(CH_2)_3$), 1.32 and 1.38 (6H, s, 2×CH_3). MS (EI): m/z = 237 $(M - CH_3)^+$, calcd. for $C_{10}H_{20}O_5S$ 252.33.

4-(2,2-Dimethyl-1,3-dioxolan-4-yl)butyl azide. Lithium azide (4 g, 82 mmol) was added to a solution of 4-(2,2-dimethyl-1,3-dioxolan-4-yl)butyl methanesulfonate (4.1 g, 16 mmol) in DMF (50 ml). The mixture was heated at 70 °C for 12 h. Cooled reaction mixture was poured into water (100 ml) and the product was extracted with ethyl ether (2 x 30 ml). Ether solution was dried over sodium sulfate and the solvent was removed *in vacuo*, yielding 2.95 g (91.2%) of azide. R_f (B) 0.85. ^1H NMR ($CDCl_3$, 400 MHz) δ3.98-4.10 (2H, m, CH-$\underline{CH_2}$), 3.49 (1H, t, *J* 6.84, CH), 3.26 (2H, t, *J* 6.56, CH_2N_3), 1.44–1.72 (6H, m, $(CH_2)_3$), 1.33 and 1.38 (6H, s, 2×CH_3). MS (EI): m/z = 184 $(M - CH_3)^+$, calcd. for $C_9H_{17}N_3O_2$ 199.25.

6-Azidohexane-1,2-diol. 4-(2,2-Dimethyl-1,3-dioxolan-4-yl)butyl azide (2.4 g, 7 mmol) was treated with 80% acetic acid (50 ml) at 60 °C for 15 min. The solution was concentrated under reduced pressure and residue was coevaporated with water (3 x 30 ml). Crude deprotected azide was purified by column chromatography. Yield 1.86 g (97%), R_f (A) 0.50. ^1H NMR (DMSO-d_6, 400 MHz) δ3.13–3.45 (5H, m, $\underline{CH,}$

$\underline{CH_2}OH$, $\underline{CH_2}N_3$), 1.14–1.65 (6H, m, $(CH_2)_3$). MS (EI): m/z = 159 M^+, calcd. for $C_6H_{13}N_3O_2$ 159.18.

6-Azido-1-[bis(4-methoxyphenyl)(phenyl)methoxy]hexane-2-ol. 6-Azidohexane-1,2-diol (2.1 g, 13.2 mmol) was treated with DMTrCl (4.7 g, 13.8 mmol) in pyridine (50 ml) at room temperature for 12 h. The mixture was then concentrated under reduced pressure and dissolved in ethyl acetate (100 ml). The solution was washed with saturated aqueous sodium bicarbonate (100 ml), dried over Na_2SO_4 and concentrated *in vacuo*. The product was purified by column chromatography on silica gel. Yield 5.23 g (86%). R_f = 0.85 (B). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 6.78–8.72 (13H, m, DMTr), 3.72 (6H, s, OCH_3), 2.74–3.64 (5H, m, $\underline{CH}$, $\underline{CH_2}$ODMTr, $\underline{CH_2}N_3$), 1.14–1.64 (6H, m, $(CH_2)_3$). MS (MALDI): m/z = 1038.7 $(M - N_2 + 2\times DMTr)^+$, 737.5 $(M - N_2 + DMTr)^+$; MS (EI): m/z = 461 $(M)^+$, 303 $(DMTr)^+$, calcd. for $C_{27}H_{31}N_3O_4$ 461.55.

6-Amino-1-[bis(4-methoxyphenyl)(phenyl)methoxy]hexane-2-ol (**3**). The solution of azide (5.1 g, 11.1 mmol) from previous step in pyridine (100 ml) was treated with PPh_3 (3.06 g, 12.2 mmol) for 12 h at room temperature. The reaction mixture was treated with concentrated aqueous NH_3 (20 ml) and left for a further 12 h. Then, it was concentrated under reduced pressure. The residue was dissolved in methanol (30 ml), washed with hexane (150 ml) and diluted with ethyl acetate (200 ml). The resulting solution was dried over Na_2SO_4 and concentrated *in vacuo*. The product was purified by column chromatography on silica gel. Yield 3.29 g (68.5%). R_f = 0.05 (A). ^{1}H NMR (DMSO-d_6, 400 MHz) δ6.87–7.42 (13H, m, DMTr), 3.73 (6H, s, OCH_3), 3.53–3.64 (1H, m, CH), 2.75–2.99 (2H, m, $\underline{CH_2}$ODMTr), 2.55 (2H, t, *J* 6.52 $\underline{CH_2}NH_2$), 1.15–1.55 (6H, m, $(CH_2)_3$). ^{13}C NMR (DMSO-d_6, 100MHz) δ 157.9, 145.1, 136.0, 135.9, 129.6, 127.7, 127.6, 126.4, 113.0, 85.0, 69.0, 67.6, 55.0, 40.7, 33.6, 31.5, 22.2. MS (EI): m/z = 435 $(M)^+$, 303 $(DMTr)^+$, calcd. for $C_{27}H_{33}NO_4$ 435.55. Anal. Calcd. for $C_{27}H_{33}NO_4$: C, 74.45; H, 7.64; N, 3.22. Found: C, 74.33; H, 7.71; N, 3.15.

Synthesis of Conjugates 4a and 4b

2-((1E,3Z)-3-{1-[6-({6-[Bis(4-methoxyphenyl)(phenyl)methoxy]-5-hydroxyhexyl}amino)-6-oxohexyl]-3,3-dimethyl-1,3-dihydro-2H-indol-2-ylidene}prop-1-en-1-yl)-1-ethyl-3,3-dimethyl-3H-indolium-5-sulfonate (**4a**). Dicyclohexylcarbodiimide (50 mg, 0.24 mmol) was added to a solution of carboxycyanine **1a** (100 mg, 0.18 mmol) and *N*-hydroxysuccinimide (30 mg, 0.27 mmol) in anhydrous DMF (2 ml). Additional portions of dicyclohexylcarbodiimide (20 mg, 0.097 mmol) were added every 12 h until complete conversion of **1a** to **2a**. Reaction progress was monitored by reverse-phase TLC (D). Then mixture was filtered and a solution of **3** (150 mg, 0.345 mmol) and *i*Pr_2EtN (50 µl, 0.287 mmol) in DMF (300 µl) was added. After 20 min stirring the reaction mixture was diluted with ethyl acetate (30 ml). Precipitate of dye conjugate **4a** was separated by centrifugation and dried. Yield 130 mg (73.9%). R_f = 0.45 (C). ^{1}H NMR (DMSO-d_6, 400 MHz) δ8.35 (1H, t, *J* 13.5, β-CH), 6.86–7.95 (20H, m, DMTr and dye aromatics), 6.51 (2H, d, *J* 13.5, α,α′-CH), 4.00–4.20 (6H, m., $CH_3\underline{CH_2}N^+$, $CH_2\underline{CH_2}N^+$, $\underline{CH_2}$NHCO), 3.73 (6H, s, OCH_3), 3.50–3.51 (1H, m, $\underline{CH}$OH), 2.70–3.05 (2H, m, $\underline{CH_2}$ODMTr), 2.04 (2H, t, *J* 7.16, $\underline{CH_2}$CONH), 1.69 and 1.70 (12H, s, 4×CH_3), 1.15–1.61 (15H, m, $(\underline{CH_2})_3CH_2$NH, $(\underline{CH_2})_3CH_2$CONH, $\underline{CH_3}CH_2$). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 173.9, 173.6, 171.5, 157.9, 149.9, 145.9, 145.1, 141.8, 141.3, 140.6, 140.0, 135.9, 135.8, 129.6, 128.6, 127.7, 127.6, 126.4, 126.2, 125.2, 122.4, 119.9, 113.0, 111.5, 110.3, 102.7, 102.4, 84.9, 68.9, 67.6, 54.9, 48.9, 48.8, 47.4, 45.6, 43.7, 35.1, 33.5, 33.3, 29.2, 27.4, 27.3, 26.7, 25.7, 25.3, 25.2, 24.8, 24.4, 22.4, 12.2. MS (MALDI): m/z = 968.4 (M^+), calcd. for $C_{58}H_{69}N_3O_8S$ 968.24. Anal. Calcd. for $C_{58}H_{69}N_3O_8S$: C, 71.95; H, 7.18; N, 4.34. Found: C, 71.89; H, 7.19; N, 4.25.

2-((1E,3E,5Z)-5-{1-[6-({6-[Bis(4-methoxyphenyl)(phenyl)methoxy]-5-hydroxyhexyl}amino)-6-oxohexyl]-3,3-dimethyl-1,3-dihydro-2H-indol-2-ylidene}penta-1,3-dien-1-yl)-1-ethyl-3,3-dimethyl-3H-indolium-5-sulfonate (**4b**) was synthesized from **1b** (100 mg, 0.173 mmol) as described for compound **4a**. Precipitation of dye conjugate **4b** was performed with a mixture of ethyl acetate and ether (1:1). Yield 130 mg (76.3%). R_f = 0.75 (C). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.34 (2H, t, J 13.4, β,β′-CH), 6.86–7.95 (20H, m, DMTr and dye aromatics), 6.54–6.61 (1H, m, γ-CH), 6.25–6.31 (2H, m, α,α′-CH), 4.00–4.20 (6H, m., $CH_3\underline{CH_2}N^+$, $CH_2\underline{CH_2}N^+$, $\underline{CH_2}$NHCO), 3.72 (6H, s, OCH_3), 3.50–3.51 (1H, m, $\underline{CH}$OH), 2.90–3.00 (2H, m, $\underline{CH_2}$ODMTr), 2.03 (2H, t, J 7.16, $\underline{CH_2}$CONH), 1.68 and 1.69 (12H, s, 4×CH_3), 1.15–1.57 (15H, m, $(\underline{CH_2})_3CH_2$NH, $(\underline{CH_2})_3CH_2$CONH, $\underline{CH_3}CH_2$). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 170.2, 170.1, 162.2, 157.9, 154.2, 149.5, 145.4, 141.9, 141.4, 141.1, 140.4, 136.0 135.9, 135.8, 129.6, 128.3, 126.4, 126.0, 124.6, 123.8, 122.3, 119.9, 113.0, 111.0, 109.7, 103.2, 102.7, 85.0, 68.9, 67.6, 59.6, 54.9, 48.8, 48.7, 43.3, 35.7, 35.0, 33.5, 33.2, 29.2, 27.0, 26.9, 26.6, 25.7, 25.3, 25.1, 24.8, 23.8, 22.3, 20.6, 14.0, 11.9. MS (MALDI): m/z = 994.3 (M^+), calcd. for $C_{60}H_{71}N_3O_8S$ 994.29. Anal. Calcd. for $C_{60}H_{71}N_3O_8S$: C, 72.48; H, 7.20; N, 4.23. Found: C, 72.38; H, 7.25; N, 4.19.

Synthesis of Phosphoramidites 5a and 5b

2-((1E,3Z)-3-{1-[12-{[Bis(4-methoxyphenyl)(phenyl)methoxy]methyl}-17-cyano-14-(diisopropylamino)-6-oxo-13,15-dioxa-7-aza-14-phosphaheptadec-1-yl]-3,3-dimethyl-1,3-dihydro-2H-indol-2-ylidene}prop-1-en-1-yl)-1-ethyl-3,3-dimethyl-3H-indolium-5-sulfonate (**5a**). Compound **4a** (60 mg, 62 µmol) was dissolved in anhydrous MeCN (300 µl). Tetrazole (22 mg, 310 µmol), dry pyridine, (30 µl), and molecular sieves (4 Å; approximately 5% by volume) were added. After 30 min, 2-cyanoethyl N,N,N´,N´-tetraisopropylphosphoramidite (92 µl, 310 µmol) was added to the mixture under intensive stirring. The reaction was completed in 15 min as evidenced by TLC (C). The reaction was quenched with cold saturated $NaHCO_3$ (30 ml), and the mixture was extracted with DCM (30 ml). The organic layer was dried over Na_2SO_4 , and the solution was concentrated in vacuo. The residue was diluted with ethyl aceteate (30 ml). Precipitate of **5a** was separated by centrifugation and dried. Yield 60 mg (86%).R_f = 0.5 (C). ^{1}H NMR (DMSO-d_6, 400 MHz), selected signals δ 8.36 (1H, t, J 13.4, β-CH), 6.85–7.82 (20H, m, DMTr and dye aromatics), 6.52 (2H, d, J 13.4, α,α′-CH), 4.10–4.20 (4H, m., $CH_3\underline{CH_2}N^+$, $CH_2\underline{CH_2}N^+$), 3.73 (6H, s, OCH_3), 2.04 (2H, m, $\underline{CH_2}$CONH), 1.69 and 1.70 (12H, s, 4×CH_3), 0.96-1.62 (15H, m, $(\underline{CH_2})_3CH_2$NH,

$(CH_2)_3CH_2CONH$, CH_3CH_2). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 173.9, 173.7, 171.5, 158.0, 149.9, 146.0, 144.9, 141.8, 141.2, 140.6, 140.1, 135.7, 135.6, 129.6, 128.6, 127.7, 126.5, 126.2, 125.2, 122.4, 119.9, 113.0, 111.5, 110.3, 102.7, 102.4, 85.2, 65.6, 58.0, 57.8, 55.0, 54.8, 48.8, 45.7, 43.7, 42.5, 42.4, 35.1, 33.0, 32.7, 29.1, 27.4, 27.3, 26.7, 25.7, 25.5, 24.9, 24.3, 24.3, 24.2, 24.1, 22.6, 21.9, 21.7, 19.7, 19.7, 19.0, 12.1. ^{31}P NMR (DMSO-d_6, 162 MHz) δ 150.2. MS (MALDI): m/z = 1168.4 (M^+), calcd. for $C_{67}H_{86}N_5O_9PS$ 1168.47. Anal. Calcd. for $C_{67}H_{86}N_5O_9PS$: C, 68.87; H, 7.42; N, 5.99. Found: C, 68.81; H, 7.53; N, 5.92.

2-((1E,3E,5Z)-5-{1-[12-{[Bis(4-methoxyphenyl)(phenyl) methoxy]methyl}-17-cyano-14-(diisopropylamino)-6-oxo-13, 15-dioxa-7-aza-14-phosphaheptadec-1-yl]-3,3-dimethyl-1,3-dihydro-2H-indol-2-ylidene}penta-1,3-dien-1-yl)-1-ethyl-3,3-dimethyl-3H-indolium-5-sulfonate (**5b**) was synthesized from **4b** (68 mg, 68 μmol) as described for compound **5a**. Yield 65 mg (80%).R_f = 0.6 (C). ^1H NMR (DMSO-d_6, 400 MHz) selected signals δ 8.34 (2H, t, J 13.4, β,β′-CH), 6.85–7.82 (20H, m, DMTr and dye aromatics), 6.54–6.61 (1H, m, γ-CH), 6.25–6.31 (2H, m, α,α′-CH), 4.00–4.20 (4H, m., $CH_3CH_2N^+$, $CH_2CH_2N^+$), 3.71 and 3.72 (6H, s, OCH_3), 2.01 (2H, m, CH_2CONH), 1.69 (12H, s, 4×CH_3), 0.96–1.62 (27H, m, $(CH_2)_3CH_2NH$, $(CH_2)_3CH_2CONH$, CH_3CH_2, $CH(CH_3)_2$). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 172.8, 172.3, 171.5, 158.0, 154.2, 154.0, 145.3, 144.9, 141.9, 141.4, 141.1, 140.5, 135.7, 129.6, 128.3, 127.7, 126.5, 126.0, 125.6, 124.7, 122.4, 119.9, 113.1, 111.1, 109.7, 103.3, 102.8, 85.2, 59.7, 58.0, 57.8, 55.0, 48.9, 48.8, 46.1, 45.7, 43.3, 42.5, 42.4, 35.1, 27.0, 27.0, 26.6, 25.7, 24.8, 24.4, 24.3, 24.2, 18.9, 12.0, 10.1. ^{31}P NMR (DMSO-d_6, 162 MHz) δ 150.2. MS (MALDI): m/z = 1195.1, calcd. for $C_{69}H_{88}N_5O_9PS$ 1194.50. Anal. Calcd. for $C_{69}H_{88}N_5O_9PS$: C, 69.38; H, 7.43; N, 5.86. Found: C, 69.35; H, 7.54; N, 5.78.

Synthesis of Solid Supports 6a,b

2-((1E,3Z)-3-{1-[6-({6-[Bis(4-methoxyphenyl)(phenyl) methoxy]-5-[(3-carboxypropanoyl)oxy]hexyl}amino)-6-oxo-hexyl]-3,3-dimethyl-1,3-dihydro-2H-indol-2-ylidene}prop-1-en-1-yl)-1-ethyl-3,3-dimethyl-3H-indolium-5-sulfonate. Succinic anhydride (20 mg, 200 μmol) and N,N-dimethylaminopyridine (25 mg, 200 μmol) were added to a solution of conjugate **4a** (40 mg, 41 μmol) in dry pyridine (1 ml). The reaction was complete in 12 h. The mixture was concentrated in vacuo, diluted with DCM (30 ml) and washed with brine. The organic layer was dried over Na_2SO_4 and concentrated. The residue was diluted with ethyl acetate (30 ml). Precipitate of succinate was separated by centrifugation and dried. Yield 40 mg (90%). R_f = 0.35 (C). ^1H NMR (DMSO-d_6, 400 MHz) δ 8.36 (1H, t, J 13.5, β-CH), 6.87–7.81 (20H, m, DMTr and dye aromatics), 6.51 (2H, d, J 13.5, α,α′-CH), 4.00–4.20 (6H, m., $CH_3CH_2N^+$, $CH_2CH_2N^+$, CH_2NHCO), 3.73 (6H, s, OCH_3), 2.90–3.04 (2H, m, CH_2ODMTr), 2.54–2.57 (4H, m, $COCH_2CH_2CO$), 2.04 (2H, t, J 7.2, CH_2CONH), 1.69 and 1.70 (12H, s, 4×CH_3), 1.15–1.59 (12H, m, $(CH_2)_3CH_2NH$, $(CH_2)_3CH_2CONH$), 0.98 (3H, t, J 6.8, CH_3CH_2). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 173.9, 173.6, 173.4, 171.9, 158.0, 149.9, 145.9, 144.7, 141.8, 141.3, 140.6, 140.1, 135.5, 129.5, 128.6, 127.8, 127.5, 126.6, 126.2, 125.2, 122.4, 119.9, 113.1, 111.5, 110.3, 102.7, 102.4, 85.1, 72.3, 64.2, 63.0, 57.7, 55.0, 48.9, 45.6, 43.7, 35.1, 30.0, 29.2, 28.7, 27.4, 27.3, 26.7, 25.7, 24.9, 21.9, 12.1, 11.2. MS (MALDI): m/z = 1068.3 (M^+), calcd. for $C_{62}H_{73}N_3O_{11}S$ 1068.32.

SCy3 derivatized controlled pore glass (**6a**). LCAA CPG (100 mg, 500Å, 120–200 mesh), pyridine (20 μl) and triethylamine(10 μl) were added to a solution of SCy3-succinate (30 mg, 28 μmol) in DCM (1 ml). The suspension was concentrated under reduced pressure. Diisopropyl carbodiimide (50 μl) was added to the reaction mixture. The suspension of CPG was agitated for 1 h at 37 ºC, then filtered, treated with acetic anhydride in pyridine (1:2 mixture, 5 ml), washed with DCM (30 ml) and ethyl ether (30 ml) and dried. Loading 34 μmol/g ($DMTr^+$ at 495 nm).

2-((1E,3E,5Z)-5-{1-[6-({6-[Bis(4-methoxyphenyl)(phenyl) methoxy]-5-[(3-carboxypropanoyl)oxy]hexyl}amino)-6-oxo-hexyl]-3,3-dimethyl-1,3-dihydro-2H-indol-2-ylidene}penta-1,3-dien-1-yl)-1-ethyl-3,3-dimethyl-3H-indolium-5-sulfonate was synthesized from **4b** (40 mg, 40 μmol) as described for SCy3-succinate. Yield 37 mg (84%).R_f = 0.45 (C). ^1H NMR (DMSO-d_6, 400 MHz) δ 8.31–8.38 (2H, m, β,β′-CH), 6.86–7.81 (20H, m, DMTr and dye aromatics), 6.54–6.61 (1H, m, γ-CH), 6.28 (2H, t, J 13.6, α,α′-CH), 4.00–4.20 (6H, m., $CH_3CH_2N^+$, $CH_2CH_2N^+$, CH_2NHCO), 3.72 (6H, s, OCH_3), 3.15–3.31 (1H, m, $CHOCO$), 3.00–3.05 (2H, m, CH_2ODMTr), 2.54–2.57 (4H, m, $COCH_2CH_2CO$), 2.02 (2H, t, J 7.2, CH_2CONH), 1.68 and 1.69 (12H, s, 4×CH_3), 1.15–1.61 (15H, m, $(CH_2)_3CH_2NH$, $(CH_2)_3CH_2CONH$) 0.99 (3H, t, J 7.2, CH_3CH_2). ^{13}C NMR (DMSO-d_6, 100 MHz) δ 173.3, 172.8, 172.3, 171.7, 171.6, 158.0, 145.3, 144.7, 141.9, 141.4, 141.1, 140.5, 135.5, 131.7, 131.5, 129.5, 128.6, 127.8, 127.5, 126.6, 126.0, 125.6, 124.7, 122.4, 119.9, 113.1, 111.1, 109.7, 103.3, 102.8, 85.1, 72.4, 67.4, 64.2, 57.7, 55.0, 48.9, 48.8, 43.3, 35.0, 33.2, 30.0, 29.7, 29.0, 28.9, 28.8, 28.3, 27.0, 27.0, 26.6, 25.7, 24.8, 23.2, 22.5, 21.0, 14.0, 12.0. MS (MALDI): m/z = 1094.3, calcd. for $C_{64}H_{75}N_3O_{11}S$ 1094.36.

SCy5 derivatized controlled pore glass (**6b**) was prepared as described for solid support **6a**. Loading 40 μmol/g ($DMTr^+$ at 495 nm).

Oligonucleotide Synthesis

Oligodeoxynucleotides were synthesized using an ASM-102U DNA synthesizer (Biosset Ltd., Russia) or an ABI 3400 (Applied Biosystems, Forster City, CA, USA) using phosphoramidite chemistry at the 0.2-μmol scale. Phosphoramidites with standard protecting groups (dA^{bz}, dG^{ibu}, dC^{bz} and T) or fast deprotection phosphoramidites (dA^{PAC}, dC^{Ac}, and $dG^{iPr\text{-}PAC}$) from Glen Research (Sterling, VA, USA) were used.

Fluorescently labeled (SCy3) oligodeoxynucleotides were deprotected in concentrated aqueous NH_3 for 4-48 h at 25 ºC. For deprotection of SCy5-labeled oligonucleotides, treatment with 50 mM K_2CO_3 in MeOH for 4–48 h at 25 ºC was used.

Purification of oligodeoxynucleotides performed using a Hypersil ODS column (5 μm; 4.6× 250 mm), 0.05 M TEAA (pH 7.0) and a linear gradient of MeCN (10–50%, 30 min for DMTr-protected oligodeoxynucleotides and 0–25%, 30 min

for fully deblocked oligodeoxynucleotides). The flow rate was 1 ml/min. Removal of the 5'-O-DMTr group was achieved by treatment with 2% aqueous trifluoroacetic acid for 1 min, followed by Et_3N neutralization and precipitation with 2% $LiClO_4$ in acetone.

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