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# A Convenient Synthesis of Cyanine Dyes: Reagents for the Labeling of Biomolecules

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Four tetramethylindo(di)carbocyanine-derived carboxylic acids have been prepared using a modified one-pot procedure for the dye assembly. The acids were converted into oxysuccinimide esters and phosphoramidite reagents. The efficiency of the reagents in the labeling of oligonucleotides and proteins has been demonstrated.


## Introduction

Cyanine dyes derived from 3,3-dimethylindole are poor DNA intercalators or groove binders owing to the steric bulk introduced by the geminal methyl groups[1] and therefore are very useful for the covalent labeling of nucleic acids. Tetramethylindo(di)-carbocyanines (Cy3, Cy3.5, Cy5, and Cy5.5) exhibit bright fluorescence in the visible region ($\lambda_{max}$ values are around 570, 615, 670, and 710 nm, respectively), and their spectroscopic and photophysical properties do not change significantly after covalent attachment to DNA. The application of cyanines as donors/acceptors in fluorescence resonance energy transfer (FRET) based methods is especially popular. The dyes are suitable for the imaging of single molecules in living cells[2] and are widely used as covalent labels for nucleic acids in sequencing,[3] array technologies,[4] PCR-based assays using energy transfer,[5] fluorescence in situ hybridization (FISH),[6] and the structural studies of nucleic acids,[7] including multiple FRET techniques.[8]

A convenient method for the synthesis of labeled DNA fragments is the use of cyanine dye-based phosphoramidites in an automated DNA synthesizer. Although several cyanine phosphoramidites have been described in the literature,[9,10] commercially available Cy™ phosphoramidites are rather expensive. With one exception,[9p] all the reported cyanine phosphoramidites are derived from primary alcohols. Recently, the lower stability of primary alkyl phosphoramidites in solution was confirmed by $^{31}P$ NMR spectroscopy, and *trans*-4-aminocyclohexanol was suggested as a precursor of more stable phosphoramidites.[11] Very recently, we reported the synthesis and successful use of *trans*-4-aminocyclohexanol-based fluorescein phosphoramidites.[12]

Cyanine dyes are useful reagents for the labeling of proteins and peptides.[13] These reagents, especially differential gel electrophoresis (DIGE) activated esters for proteomics,[14] are extremely expensive.

Our aim was to develop a procedure for the large-scale preparation of inexpensive reagents based on conventional tetramethylindo(di)carbocyanines that are suitable for the labeling of oligonucleotides and proteins.

## Results and Discussion

Symmetrical tetramethylindo(di)carbocyanines containing different alkyl groups at N and N′ were assembled from corresponding indolium salts using a stepwise process developed by Waggoner and co-workers.[15] Our modification[10] allowed us to perform the reaction as a convenient one-pot procedure.

The starting indolium salts were prepared by the alkylation of indoles **1a**,**b** with alkyl halides. Alkylation with methyl or butyl iodide gave salts **2a**,**b** at room temperature, whereas carboxypentyl derivatives **3a**,**b** were obtained after heating indoles **1** and 6-bromohexanoic acid in nitromethane. To synthesize cyanine acids **4**, the salts **3a**,**b** were heated first with diphenylformamidine or malondialdehyde bis(phenylimine) monohydrochloride in acetic anhydride to form hemicyanines. The latter were treated with the corresponding salts **2a**,**b** in pyridine at ambient temperature. The resulting mixtures were separated by column chromatography to afford derivatives **4a**–**d** in reasonably good yields (49–67% for the two-step procedure). The acids **4a**–**d**

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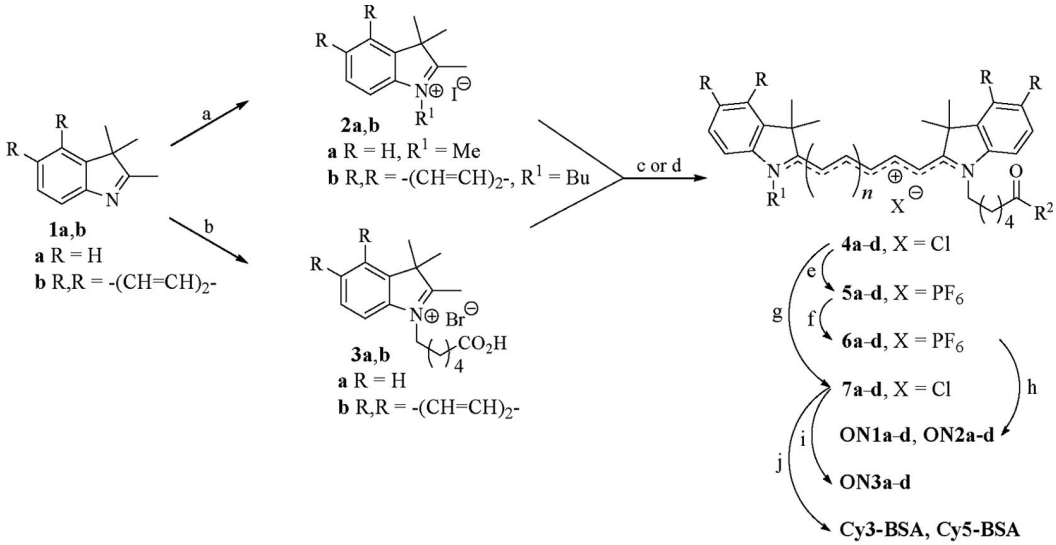
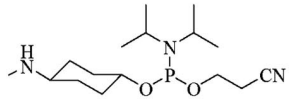
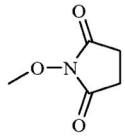
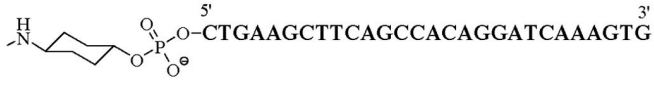
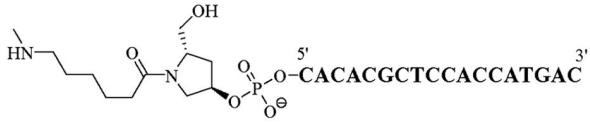



| # | *n* | R,R | $R^1$ | $R^2$ |
|---|---|---|---|---|
| **4a** | 0 | H,H | Me | OH |
| **4b** | 0 | -(CH=CH)$_2$- | Bu | |
| **4c** | 1 | H,H | Me | |
| **4d** | 1 | -(CH=CH)$_2$- | Bu | |
| **5a** | 0 | H,H | Me |  |
| **5b** | 0 | -(CH=CH)$_2$- | Bu | |
| **5c** | 1 | H,H | Me | |
| **5d** | 1 | -(CH=CH)$_2$- | Bu | |
| **6a** | 0 | H,H | Me |  |
| **6b** | 0 | -(CH=CH)$_2$- | Bu | |
| **6c** | 1 | H,H | Me | |
| **6d** | 1 | -(CH=CH)$_2$- | Bu | |
| **7a** | 0 | H,H | Me |  |
| **7b** | 0 | -(CH=CH)$_2$- | Bu | |
| **7c** | 1 | H,H | Me | |
| **7d** | 1 | -(CH=CH)$_2$- | Bu | |
| **ON1a** | 0 | H,H | Me |  |
| **ON1b** | 0 | -(CH=CH)$_2$- | Bu | |
| **ON1c** | 1 | H,H | Me | |
| **ON1d** | 1 | -(CH=CH)$_2$- | Bu | |
| **ON2a** | 0 | H,H | Me |  |
| **ON2b** | 0 | -(CH=CH)$_2$- | Bu | |
| **ON2c** | 1 | H,H | Me | |
| **ON2d** | 1 | -(CH=CH)$_2$- | Bu | |
| **ON3a** | 0 | H,H | Me |  |
| **ON3b** | 0 | -(CH=CH)$_2$- | Bu | |
| **ON3c** | 1 | H,H | Me | |
| **ON3d** | 1 | -(CH=CH)$_2$- | Bu | |
| **Cy3-BSA** | 0 | H,H | Me |  |
| **Cy5-BSA** | 1 | H,H | Me | (*m* is the dye/protein ratio) |

Scheme 1. Reaction conditions and yields: (a) RI, $MeNO_2$, room temp., 12 h, 96% (**2a**), 90% (**2b**); (b) $Br(CH_2)_5CO_2H$, $MeNO_2$, 80 °C, 7 h, 92% (**3a**), 89% (**3b**); (c) PhNHCH=NPh, $Ac_2O$, 120 °C, 30 min, then **2a** or **2b**, Py, room temp., 12 h, 60% (**4a**), 49% (**4b**); (d) PhN=CHCH$_2$CH=NPh·HCl, $Ac_2O$, 120 °C, 30 min, then **2a** or **2b**, Py, room temp., 12 h, 67% (**4c**), 51% (**4d**); (e) PyBOP, DIEA, DMF, room temp., 5 min, then *trans*-4-aminocyclohexanol, 4 h, 77% (**5a**), 80% (**5b**), 76% (**5c**), 66% (**5d**); (f) $(i\text{PrN})_2PO(CH_2)_2CN$, diisopropylammonium tetrazolide, DCM, room temp., 3 h, 93% (**6a**), 96% (**6b**), 93% (**6c**), 95% (**6d**); (g) DSC, DIEA, DCM, room temp., 2 h, 96% (**7a**), 98% (**7b**), 96% (**7c**), 97% (**7d**); (h) coupling in oligonucleotide synthesizer, then deprotection and purification; (i) aminoalkyl-modified oligonucleotide, DMF, aq. buffer, pH 8.5, 0 °C, 12 h; (j) bovine serum albumin, aq. buffer, pH 8.5, 0 °C, 12 h.

were used for the *N*-acylation of *trans*-4-aminocyclohexanol using (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) as a coupling reagent to afford the amides **5a**–**d** in good yields. As a result of this step, the cyanines acquire a lipophilic hexafluorophosphate counterion. The phosphitylation of the secondary alcohols **5a**–**d** with bis(diisopropylamino)-2-cyanoethoxyphosphane in the presence of diisopropylammonium tetrazolide gave the desired phosphoramidites **6a**–**d** as hexafluorophosphate salts ($^{31}$P NMR showed a 1:1 phosphoramidite/hexafluorophosphate ratio). The acids **4a**–**d** were also converted into the oxysuccinimide activated esters **7a**–**d** (Scheme 1). The structures of the compounds were confirmed by $^{1}$H, $^{13}$C, and $^{31}$P NMR spectra, and by high-resolution mass spectra (HRMS). For the cyanine derivatives **4**–**7** the $^{1}$H and $^{13}$C signals in the NMR spectra were assigned using 2D $^{1}$H–$^{13}$C HMQC and HMBC techniques.

Phosphoramidites **6a**–**d** were tested in automated oligonucleotide synthesis. They are readily soluble in acetonitrile and their 0.1 M solutions were stored at ambient temperature and used during the course of a week to synthesize 5′-labeled oligonucleotides. Over the testing period, no significant decrease in the coupling yields was observed (estimated by comparison of PAGE view and the HPLC profiles of the crude conjugates). The main factor effecting a decrease in the yields of the desired conjugates is, however, the chemical lability of cyanine dyes under conditions of iodine oxidation (one step in the oligonucleotide synthesis cycle) and ammonolysis (final oligonucleotide deprotection step).[16] In our experiments we used standard iodine oxidation after modified phosphoramidite coupling and ammonolysis for 72 h at 4 °C as the final deprotection method. Cyanine-labeled oligonucleotides were isolated/purified by PAGE or HPLC.[17] The yield of purified 5′-labeled conjugates from a 200-nmol-scale column was usually 15–20 nmol (7.5–10%), somewhat decreasing from Cy3 to Cy5.5. The **ON1a**–**d** and **ON2a**–**d** conjugates (Scheme 1) are representative examples of the approach. Cyanine-modified oligonucleotides were characterized by MALDI-TOF MS.[17]

The presence of cyanine dyes in the conjugates was confirmed by their UV/Vis spectra which contain distinctive long-wavelength bands of cyanine absorbance (550, 602, 647, and 688 nm, respectively, for conjugates **ON1a**–**d** in low-salt aqueous solutions) together with an oligonucleotide band at around 260 nm (Figure 1). The absorbance maxima and their intensities are close to the reported values.[16] The conjugates also showed standard cyanine fluorescence with emission maxima at 568, 617, 673, and 711 nm, respectively (Figure 2).

Despite the relatively low overall yield of fluorescent oligonucleotides prepared using the modifying phosphoramidites **6a**–**d**, the approach is effective in the case of the extensive production of conjugates on a small scale. There are minor labor inputs in comparison with the synthesis of nonmodified oligonucleotides: the dissolution of an additional phosphoramidite and a more prolonged cold ammonolysis step.

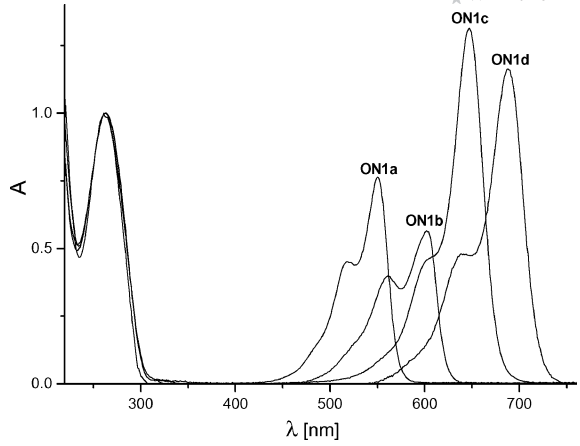



Figure 1. UV/Vis spectra of purified **ON1a**–**d** conjugates in water at pH 7.5.

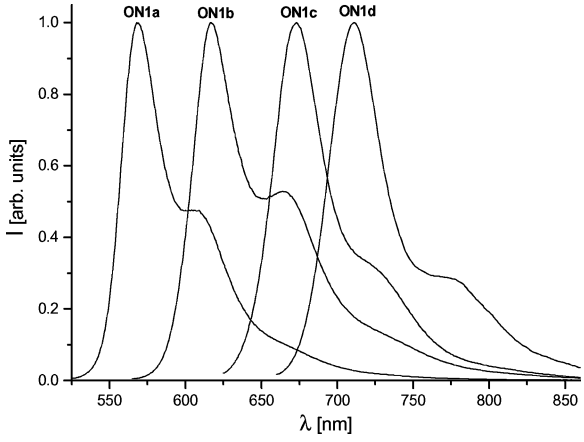



Figure 2. Normalized emission spectra of purified **ON1a**–**d** conjugates in water at pH 7.5. Excitation wavelengths: 520 (**ON1a**), 560 (**ON1b**), 620 (**ON1c**), and 650 nm (**ON1d**).

We assumed that to scale up the synthesis of cyanine-modified oligonucleotides, a postlabeling procedure could be the method of choice. This procedure includes the synthesis of amine-containing oligonucleotides followed by their reaction with the activated esters of cyanine dyes. To test the approach, a crude amino-modified oligomer[18] was acylated with esters **7a**–**d** to give the conjugates **ON3a**–**d** (Scheme 1). We found that after similar PAGE or HPLC purification the procedure usually gave 20–30 nmol isolated yield of the desired conjugate (10–15% total yield). Although the postlabeling procedure gave similar yields, the consumption of the cyanine reagent was about 10 times less than was the case with the amidite labeling procedure (ca. 0.5 mg of activated ester vs. 5–6 mg of amidite per reaction). Therefore the postlabeling method can be easily scaled up to several μmol of the starting amino-modified oligonucleotide without significant increase in the oligonucleotide cost.

Moreover, activated esters **7** can be used for the acylation of proteins. This has been demonstrated for bovine serum albumin (BSA) labeling with **7a** and **7c** (Scheme 1). The reaction was performed upon cooling and at pH 8.5, and the

labeled protein, **Cy3-BSA** or **Cy5-BSA**, was isolated using size-exclusion chromatography (see the Exptl. Sect.). BSA is a protein of around 66000 Da with $\varepsilon_{280}$ = 44000 L mol$^{-1}$ cm$^{-1}$; its labeling with cyanine dye Cy5 has already been studied.[19] The UV/Vis spectra of Cy3- and Cy5-labeled BSA show characteristic protein and dye absorbance bands (Figures 3 and 4).

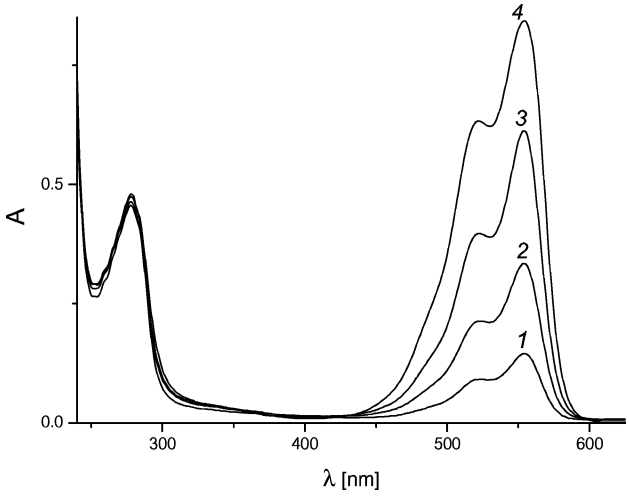



Figure 3. UV/Vis spectra of isolated **Cy3-BSA** [BSA(Cy3)$_m$ conjugates] in water. **7a**/BSA in ratios of 0.20, 0.50, 1.00, and 2.00 were used in the labeling reaction and gave conjugates with $m$ = 0.12 (*1*), 0.24 (*2*), 0.43 (*3*), and 0.59 (*4*), respectively.

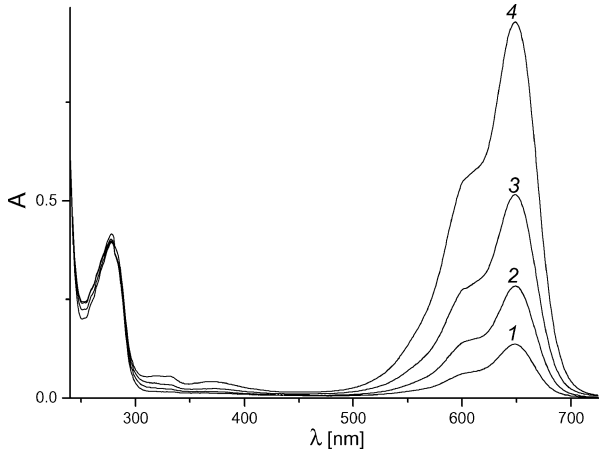



Figure 4. UV/Vis spectra of isolated **Cy5-BSA** [BSA(Cy5)$_m$ conjugates] in water. **7c**/BSA in ratios of 0.20, 0.50, 1.00, and 2.00 were used in the labeling reaction and gave conjugates with $m$ = 0.06 (*1*), 0.13 (*2*), 0.23 (*3*), and 0.42 (*4*), respectively.

We assumed $\varepsilon_{280,Cy3}$ = 5000, $\varepsilon_{554,Cy3}$ = 136000, $\varepsilon_{280,Cy5}$ = 6000, and $\varepsilon_{650,Cy5}$ = 250000 L mol$^{-1}$ cm$^{-1}$, and used these values to calculate approximate dye/protein ratios in prepared samples of **Cy3-BSA** and **Cy5-BSA**. The degree of dye attachment decreased with an increase in the starting **7**/BSA ratio and was lower for **7c** than for **7a** (Figures 3 and 4). By using **7a**(**7c**)/BSA = 2 we prepared labeled proteins BSA(Cy3)$_{0.59}$ and BSA(Cy5)$_{0.42}$, respectively. The results are superior to those described for Cy5 BSA labeling at pH 9.3 and room temperature,[19] probably due to the better stability of the oxysuccinimide ester under our conditions (pH 8.5, 0 °C).

## Conclusions

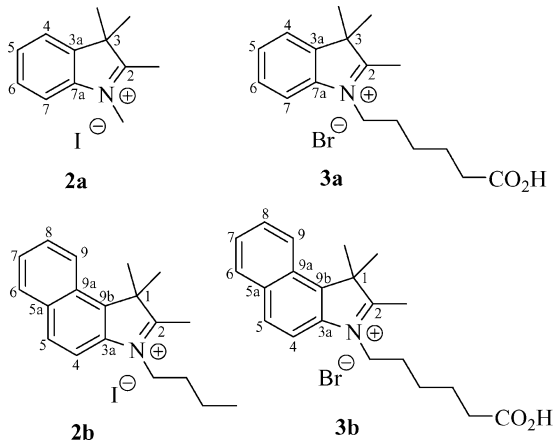
We report the convenient and scaleable one-pot synthesis of four cyanine acids (Cy3, Cy3.5, Cy5, and Cy5.5), compounds suitable for the preparation of both phosphoramidites or oxysuccinimide esters. The *trans*-4-aminocyclohexanol-based phosphoramidites **6a**–**d** can be easily synthesized on mmol scale to give a further 170–200 of 5′-labeled oligonucleotides per mmol of phosphoramidite. Alternatively, oxysuccinimide esters **7a**–**d** were prepared and used for the labeling of amino-modified oligonucleotides and bovine serum albumin.

## Experimental Section

**General Methods:** Reagents obtained from commercial suppliers were used without further purification; 1,1,2-trimethyl-1*H*-benzo[*e*]indole and 2,3,3-trimethyl-3*H*-indole were from Acros; *trans*-4-aminocyclohexanol hydrochloride, iodobutane, DSC, and BSA were from Sigma–Aldrich; 6-bromohexanoic acid, *N*,*N*′-diphenylformamidine, and malondialdehyde bis(phenylimine) monohydrochloride were from Alfa Aesar; DIEA was from Fluka and PyBOP was from NovaBiochem; bis(*N*,*N*-diisopropylamino)-2-cyanoethoxyphosphane,[20] and diisopropylammonium tetrazolide[21] were prepared as described previously. Solvents were from Chimmed (Russia), mainly HPLC grade, and were used without further purification unless otherwise noted. Dichloromethane (DCM) was always used freshly distilled from $CaH_2$. DMF was freshly distilled under reduced pressure. $^1$H (500 MHz), $^{13}$C (125.7 MHz), and $^{31}$P NMR (202.4 MHz) spectra were recorded with a Bruker DRX-500 spectrometer at 303 K and referenced to [D$_6$]DMSO ($\delta$ = 2.50 ppm for $^1$H and 39.70 ppm for $^{13}$C) and 85% aq. $H_3PO_4$, ($\delta$ = 0.00 ppm for $^{31}$P). $^1$H–$^{13}$C gradient-selected HMQC and HMBC spectra were obtained by using 2048 ($t_2$) × 256 ($t_1$) complex point data sets, zero filled to 2048 ($F_2$) × 1024 ($F_1$) points. The spectral widths were 13 and 200 ppm for $^1$H and $^{13}$C dimensions, respectively. HMBC spectra were measured with a 50 ms delay for the evolution of long-range couplings. $^1$H NMR coupling constants are reported in Hz and refer to apparent multiplicities. High-resolution mass spectra were recorded in positive ion mode using an IonSpec FT ICR (MALDI) or PE SCIEX QSTAR pulsar mass spectrometer (ESI). UV/Vis absorption spectra were recorded using a Varian Cary-300 spectrophotometer. Fluorescence studies were performed using a large-aperture apparatus, as described earlier.[22] Melting points were determined using a Boetius heating table and are uncorrected. Analytical thin-layer chromatography was performed on Kieselgel 60 $F_{254}$ precoated aluminium plates (Merck). Silica gel column chromatography was performed on Merck Kieselgel 60 0.040–0.063 mm. Oligonucleotide synthesis was carried out with a BiosSet ASM-800 DNA/RNA Synthesizer on a 200-nmol scale using standard manufacturer's protocols. The coupling step time for the terminal modifying phosphoramidites was extended to 7.5 min (the capping and removal of the dimethoxytrityl group were omitted). The oligonucleotides were isolated using 20% denaturing (7 M urea) PAGE in Tris-borate buffer, pH 8.3, and desalted by gel filtration through a Sephadex G-25 column eluting with saltless buffer.

**General Procedure for the Preparation of Indolium Salts 2 and 3:** An alkyl halide (0.10 mol) [or in the case of the synthesis of salts **3**, 6-bromohexanoic acid (0.10 mol)] was added to a solution of the corresponding indole **1a** or **1b** (0.10 mol) in $MeNO_2$ (60 mL) and the reaction mixture was magnetically stirred for 12 h at ambient temperature (for the synthesis of **2a** or **2b**. ***Caution:*** In case of an initial exothermic reaction the flask must be cooled by using a cold water bath) or for 6 h with heating (for the synthesis of **3a**,**b**; oil bath, 80 °C). The cooled reaction mixture was triturated with diethyl ether (400 mL) and the precipitate was filtered off, washed with diethyl ether (3 × 100 mL), and dried in vacuo. This procedure gave the following compounds.




**2a** **3a** **2b** **3b**

**1,2,3,3-Tetramethyl-3*H*-indolium Iodide (2a):**[23] Compound **2a** was prepared from 2,3,3-trimethyl-3*H*-indole (**1a**) and methyl iodide. Yield 96%, off-white crystals, m.p. >245 °C (dec.). $^1$H NMR ([D$_6$]-DMSO): $\delta$ = 7.91 (m, 1 H, ArH), 7.83 (m, 1 H, ArH), 7.62 (m, 2 H, ArH), 3.98 (s, 3 H), 2.77 (s, 3 H), 1.53 (s, 6 H, $CH_3$) ppm. $^{13}$C NMR ([D$_6$]DMSO): $\delta$ = 196.04, 142.13, 141.64, 129.34, 128.84, 123.31, 115.15, 53.95, 34.73 ($NCH_3$), 27.74 (2 C), 14.16 ppm.

**3-Butyl-1,1,2-trimethyl-1*H*-benzo[*e*]indolium Iodide (2b):**[24] Compound **2b** was prepared from 1,1,2-trimethyl-1*H*-benzo[*e*]indole (**1b**) and iodobutane. Yield 35.5 g (90%), pale-purple crystals, m.p. 165–168 °C (ref.[24] m.p. 114–115 °C). $^1$H NMR ([D$_6$]DMSO): $\delta$ = 8.37 (d, $J$ = 8.2 Hz, 1 H, 6-H or 9-H), 8.29 (d, $J$ = 8.8 Hz, 1 H, 4-H or 5-H), 8.22 (d, $J$ = 8.2 Hz, 1 H, 6-H or 9-H), 8.16 (d, $J$ = 8.8 Hz, 1 H, 4-H or 5-H), 7.79 (m, 1 H, 7-H or 8-H), 7.73 (m, 1 H, 7-H or 8-H), 4.59 (t, $J$ = 7.6 Hz, 2 H, $NCH_2$), 2.96 (s, 3 H, 2-$CH_3$), 1.88 (m, 2 H, $NCH_2CH_2$), 1.77 (s, 6 H, 1-$CH_3$), 1.47 (m, 2 H, $CH_2CH_3$), 0.96 (t, $J$ = 7.5 Hz, 3 H, $CH_2CH_3$) ppm. $^{13}$C NMR ([D$_6$]DMSO): $\delta$ = 196.31 (C-2), 138.52, 137.03, 133.08, 130.72, 129.75, 128.45, 127.31, 127.26, 123.46, 113.38, 55.53 (C-1), 47.77 ($NCH_2$), 29.53 ($NCH_2CH_2$), 21.68 (2 C, 1-$CH_3$), 19.36 ($CH_2CH_3$), 13.94 ($CH_2CH_3$), 13.65 (2-$CH_3$) ppm. HRMS (MALDI+): calcd. for $C_{19}H_{24}N^+$ [M – I]$^+$ 266.1903; found 266.1889.

**1-(5-Carboxypentyl)-2,3,3-trimethyl-3*H*-indolium Bromide (3a):**[9o] Compound **3a** was prepared from 2,3,3-trimethyl-3*H*-indole (**1a**) and 6-bromohexanoic acid. Yield 32.6 g (92%), pale-pink crystals, m.p. 127–129 °C. $^1$H NMR ([D$_6$]DMSO): $\delta$ = 11.98 (br. s, 1 H, $CO_2H$), 7.99 (m, 1 H, 7-H), 7.85 (m, 1 H, 4-H), 7.62 (m, 2 H, 5-H, 6-H), 4.46 (t, $J$ = 7.6 Hz, 2 H, $NCH_2$), 2.86 (s, 3 H, 2-$CH_3$), 2.23 (t, $J$ = 7.2 Hz, 2 H, $COCH_2$), 1.85 (m, 2 H, $NCH_2CH_2$), 1.56 (m, 2 H, $COCH_2CH_2$), 1.54 (s, 6 H, 3-$CH_3$), 1.43 (m, 2 H, $NCH_2CH_2CH_2$) ppm. $^{13}$C NMR ([D$_6$]DMSO): $\delta$ = 196.60 (C-2), 174.36 (CO), 141.95 (C-3a), 141.12 (C-7a), 129.47 (C-5), 129.02 (C-6), 123.59 (C-4), 115.58 (C-7), 54.24 (C-3), 47.53 ($NCH_2$), 33.44 ($COCH_2$), 27.02 ($NCH_2CH_2$), 25.49 ($NCH_2CH_2CH_2$), 24.09 ($COCH_2CH_2$), 22.09 (2 C, 3-$CH_3$), 14.12 (2-$CH_3$) ppm. HRMS (MALDI+): calcd. for $C_{17}H_{24}NO_2^+$ [M – Br]$^+$ 274.1802; found 274.1799.

**3-(5-Carboxypentyl)-1,1,2-trimethyl-1*H*-benzo[*e*]indolium Bromide (3b):**[25] Compound **3b** was prepared from 1,1,2-trimethyl-1*H*-benzo[*e*]indole (**1b**) and 6-bromohexanoic acid. Yield 36.1 g, (89%), pale-pink crystals, m.p. 211–213 °C. $^1$H NMR ([D$_6$]DMSO): $\delta$ = 8.37 (d, $J$ = 8.1 Hz, 1 H, 6-H or 9-H), 8.29 (d, $J$ = 8.8 Hz, 1 H, 4-H or 5-H), 8.22 (d, $J$ = 8.1 Hz, 1 H, 6-H or 9-H), 8.16 (d, $J$ = 8.8 Hz, 1 H, 4-H or 5-H), 7.79 (m, 1 H, 7-H or 8-H), 7.72 (m, 1 H, 7-H or 8-H), 4.60 (t, $J$ = 7.6 Hz, 2 H, $NCH_2$), 2.97 (s, 3 H, 2-$CH_3$), 2.23 (t, $J$ = 7.2 Hz, 2 H, $COCH_2$), 1.91 (m, 2 H, $NCH_2CH_2$), 1.77 (s, 6 H, 1-$CH_3$), 1.58 (m, 2 H, $COCH_2CH_2$), 1.47 (m, 2 H, $NCH_2CH_2CH_2$) ppm. $^{13}$C NMR ([D$_6$]DMSO): $\delta$ = 196.44 (C-2), 174.33 (CO), 138.55, 137.02, 133.09, 130.73, 129.77, 128.45, 127.30, 127.28, 123.47, 113.42, 55.55 (C-1), 47.77 ($NCH_2$), 33.43 ($COCH_2$), 27.21 ($NCH_2CH_2$), 25.45 ($NCH_2CH_2CH_2$), 24.11 ($COCH_2CH_2$), 21.67 (2 C, 1-$CH_3$), 13.96 (2-$CH_3$) ppm. HRMS (MALDI+): calcd. for $C_{21}H_{26}NO_2^+$ [M – Br]$^+$ 324.1958; found 324.1951.

**General Procedure for the Preparation of Cyanine Acids 4a–d:** A solution of indolium salt **3a** or **3b** (50.0 mmol) and *N*,*N*′-diphenylformamidine or malondialdehyde bis(phenylimine) monohydrochloride (60.0 mmol) in acetic anhydride (150 mL) was heated in an oil bath at 120 °C for 30 min. The reaction mixture was cooled to room temperature and a solution of the indolium salt **2a** or **2b** (70.0 mmol), respectively, in dry pyridine (150 mL) was added. The mixture was stirred at ambient temperature for 12 h, concentrated, and the residue was dissolved in chloroform (100 mL) and precipitated with hexane (1 L). The supernatant was decanted and the residual oil was dissolved in chloroform (500 mL) and successively washed with water (3 × 500 mL) and 0.1 M hydrochloric acid (500 mL). The organic layer was dried with $Na_2SO_4$, evaporated, and the residue was purified by chromatography on silica gel using ethanol in chloroform as eluent (gradient of 0→10%).

**2-[3-(1,3,3-Trimethyl-2,3-dihydro-1*H*-indol-2-ylidene)-1-propenyl]-3,3-dimethyl-1-(5-carboxypentyl)-3*H*-indolium Chloride (4a):** Compound **4a** was prepared from **3a**, *N*,*N*′-diphenylformamidine, and **2a**. Yield 14.8 g (60%), dark-purple foam. TLC: $R_f$ = 0.24 [10% MeOH in $CHCl_3$ (v/v)]. $^1$H NMR ([D$_6$]DMSO): $\delta$ = 12.05 (br. s, 1 H, $CO_2H$), 8.35 (t, $J_{a,b}$ = $J_{b,c}$ = 13.4 Hz, 1 H, b-H), 7.64 (m, 2 H, 4-H, 4′-H), 7.45 (m, 4 H, 6-H, 7-H, 6′-H, 7′-H), 7.30 (m, 2 H, 5-H, 5′-H), 6.53 (d, $J_{a,b}$ = 13.4 Hz, 1 H, a-H), 6.51 (d, $J_{b,c}$ = 13.4 Hz, 1 H, c-H), 4.12 (t, $J$ = 7.5 Hz, 2 H, $NCH_2$), 3.66 (s, 3 H, $NCH_3$), 2.22 (t, $J$ = 7.2 Hz, 2 H, $COCH_2$), 1.75 (m, 2 H, $NCH_2CH_2$), 1.69 (s, 12 H, 3-$CH_3$, 3′-$CH_3$), 1.57 (m, 2 H, $COCH_2CH_2$), 1.43 (m, 2 H, $NCH_2CH_2CH_2$) ppm. $^{13}$C NMR ([D$_6$]DMSO): $\delta$ = 174.54, 174.38 (C-2, C-2′), 173.71 (CO), 149.76 (C-b), 142.70 (C-7a′), 141.96 (C-7a), 140.64 (2 C, C-3a, C-3a′), 128.72, 128.64 (C-6, C-6′), 125.31, 125.18 (C-5, C-5′), 122.58, 122.47 (C-4, C-4′), 111.59, 111.54 (C-7, C-7′), 103.06, 102.42 (C-a, C-c), 48.92 (2 C, C-3, C-3′), 43.75 ($NCH_2$), 33.55 ($COCH_2$), 31.54 ($NCH_3$), 27.54 (2 C), 27.33 (2 C) (3-$CH_3$, 3′-$CH_3$), 26.80 ($NCH_2CH_2$), 25.75 ($NCH_2CH_2CH_2$), 24.30 ($COCH_2CH_2$) ppm. HRMS (MALDI+): calcd. for $C_{30}H_{37}N_2O_2^+$ [M – Cl]$^+$ 457.2850; found 457.2866.

**2-[3-(3-Butyl-1,1-dimethyl-1,2-dihydro-3*H*-benzo[*e*]indol-2-ylidene)-1-propenyl]-1,1-dimethyl-3-(5-carboxypentyl)-1*H*-benzo[*e*]indolium Chloride (4b):** Compound **4b** was prepared from **3b**, *N*,*N*′-diphenylformamidine, and **2b**. Yield 15.6 g (49%), dark-violet foam. TLC: $R_f$ = 0.20 [10% MeOH in $CHCl_3$ (v/v)]. $^1$H NMR ([D$_6$]DMSO): $\delta$ = 12.04 (br. s, 1 H, $CO_2H$), 8.59 (t, $J_{a,b}$ = $J_{b,c}$ = 13.5 Hz, 1 H, b-H), 8.29 (d, $J$ = 8.3 Hz, 2 H, 9-H, 9′-H), 8.11 (d, $J$ = 8.7 Hz, 2 H,

5-H, 5′-H), 8.08 (d, $J$ = 8.3 Hz, 2 H, 6-H, 6′-H), 7.79 (m, 2 H, 4-H, 4′-H), 7.68 (m, 2 H, 8-H, 8′-H), 7.54 (m, 2 H, 7-H, 7′-H), 6.66 (d, $J_{a,b} = J_{b,c}$ = 13.5 Hz, 2 H, a-H, c-H), 4.29 (m, 4 H, $NCH_2$), 2.24 (t, $J$ = 7.2 Hz, 2 H, $COCH_2$), 2.01 (s, 12 H, 1-$CH_3$, 1′-$CH_3$), 1.81 (m, 4 H, $NCH_2CH_2$), 1.60 (m, 2 H, $COCH_2CH_2$), 1.50 (m, 4 H, $NCH_2CH_2CH_2$, $CH_2CH_3$), 0.96 (t, $J$ = 7.3 Hz, 3 H, $CH_2CH_3$) ppm. $^{13}$C NMR ([D$_6$]DMSO): $\delta$ = 175.17, 175.13 (C-2, C-2′), 174.35 (CO), 148.56 (C-b), 139.62 (2 C, C-3a, C-3a′), 133.21, 133.17 (C-9b, C-9b′), 131.54 (2 C, C-5a, C-5a′), 130.51 (2 C, C-5, C-5′), 129.98 (2 C, C-6, C-6′), 127.93 (2 C, C-8, C-8′), 127.46 (2 C, C-9a, C-9a′), 125.09 (2 C, C-7, C-7′), 122.22 (2 C, C-9, C-9′), 111.82 (2 C, C-4, C-4′), 102.19, 102.16 (C-a, C-c), 50.63 (2 C, C-1, C-1′), 43.94 (2 C, $NCH_2$), 33.56 ($COCH_2$), 29.59 ($CH_2CH_2CH_3$), 28.38 ($COCH_2CH_2CH_2CH_2$), 27.18 (2 C), 27.17 (2 C) (1-$CH_3$, 1′-$CH_3$), 25.71 ($COCH_2CH_2CH_2$), 24.34 ($COCH_2CH_2$), 19.59 ($CH_2CH_3$), 13.90 ($CH_2CH_3$) ppm. HRMS (MALDI+): calcd. for $C_{41}H_{47}N_2O_2^+$ [M – Cl]$^+$ 599.3632; found 599.3635.

**2-[5-(1,3,3-Trimethyl-2,3-dihydro-1*H*-indol-2-ylidene)-1,3-pentadienyl]-3,3-dimethyl-1-(5-carboxypentyl)-3*H*-indolium Chloride (4c):** Compound **4c** was prepared from **3a**, malondialdehyde bis(phenylimine) monohydrochloride, and **2a**. Yield 17.4 g (67%), dark-blue foam. TLC: $R_f$ = 0.26 [10% MeOH in $CHCl_3$ (v/v)]. $^1$H NMR ([D$_6$]DMSO): $\delta$ = 12.07 (br. s, 1 H, $CO_2H$), 8.32 (t, $J_{a,b} = J_{b,c} = J_{c,d} = J_{d,e}$ = 13.0 Hz, 2 H, b-H, d-H), 7.60 (m, 2 H, 4-H, 4′-H), 7.39 (m, 4 H, 6-H, 7-H, 6′-H, 7′-H), 7.25 (m, 2 H, 5-H, 5′-H), 6.58 (t, $J_{b,c} = J_{c,d}$ = 13.0 Hz, 1 H, c-H), 6.31 (d, $J_{a,b}$ = 13.0 Hz, 1 H, a-H), 6.26 (d, $J_{d,e}$ = 13.0 Hz, 1 H, e-H), 4.09 (m, 2 H, $NCH_2$), 3.60 (s, 3 H, $NCH_3$), 2.20 (t, $J$ = 7.2 Hz, 2 H, $COCH_2$), 1.70 (m, 2 H, $NCH_2CH_2$), 1.68 (s, 12 H, 3-$CH_3$, 3′-$CH_3$), 1.55 (m, 2 H, $COCH_2CH_2$), 1.39 (m, 2 H, $NCH_2CH_2CH_2$) ppm. $^{13}$C NMR ([D$_6$]DMSO): $\delta$ = 174.30 (CO), 173.31 (C-2′), 172.56 (C-2), 154.03 (2 C, C-b, C-d), 142.80 (C-7a′), 142.06 (C-7a), 141.13 (C-3a), 141.06 (C-3a′), 128.45 ppm. 128.38 (C-6, C-6′), 125.41 (C-c), 124.77, 124.65 (C-5, C-5′), 122.46, 122.34 (C-4, C-4′), 111.08 (2 C, C-7, C-7′), 103.35 (C-e), 103.09 (C-a), 48.91 (2 C, C-3, C-3′), 43.28 ($NCH_2$), 33.52 ($COCH_2$), 31.18 ($NCH_3$), 27.22 (2 C), 27.05 (2 C) (3-$CH_3$, 3′-$CH_3$), 26.71 ($NCH_2CH_2$), 25.68 ($NCH_2CH_2CH_2$), 24.22 ($COCH_2CH_2$) ppm. HRMS (MALDI+): calcd. for $C_{32}H_{39}N_2O_2^+$ [M – Cl]$^+$ 483.3006; found 483.3018.

**2-[5-(3-Butyl-1,1-dimethyl-1,2-dihydro-3*H*-benzo[*e*]indol-2-ylidene)-1,3-pentadienyl]-1,1-dimethyl-3-(5-carboxypentyl)-1*H*-benzo[*e*]indolium Chloride (4d):** Compound **4d** was prepared from **3b**, malondialdehyde bis(phenylimine) monohydrochloride, and **2b**. Yield 16.7 g (51%), dark-green foam. TLC: $R_f$ = 0.25 [10% EtOH in DCM (v/v)]. $^1$H NMR ([D$_6$]DMSO): $\delta$ = 12.17 (br. s, 1 H, $CO_2H$), 8.46 (t, $J_{b,c} = J_{c,d}$ = 13.4 Hz, 2 H, b-H, d-H), 8.26 (d, $J$ = 8.2 Hz, 2 H, 9-H, 9′-H), 8.07 (m, 4 H, 5-H, 5′-H, 6-H, 6′-H), 7.74 (m, 2 H, 4-H, 4′-H), 7.68 (m, 2 H, 8-H, 8′-H), 7.51 (m, 2 H, 7-H, 7′-H), 6.66 (t, $J_{b,c} = J_{c,d}$ = 13.0 Hz, 1 H, c-H), 6.37 (d, $J_{a,b} = J_{d,e}$ = 13.4 Hz, 2 H, a-H, e-H), 4.24 (m, 4 H, $NCH_2$), 2.21 (m, 2 H, $COCH_2$), 1.97 (s, 12 H, 1-$CH_3$, 1′-$CH_3$), 1.74 (m, 4 H, $NCH_2CH_2$), 1.57 (m, 2 H, $COCH_2CH_2$), 1.44 (m, 4 H, $NCH_2CH_2CH_2$, $CH_2CH_3$), 0.95 (t, $J$ = 7.3 Hz, 3 H, $CH_2CH_3$) ppm. $^{13}$C NMR ([D$_6$]DMSO): $\delta$ = 174.31, 173.70, 171.31 (CO, C-2, C-2′), 152.87 (2 C, C-b, C-d), 139.76 (2 C, C-3a, C-3a′), 133.20 (2 C, C-9b, C-9b′), 131.33 (2 C, C-5a, C-5a′), 130.34 (2 C, C-5, C-5′), 129.95 (2 C, C-6, C-6′), 127.77 (2 C, C-8, C-8′), 127.64 (2 C, C-9a, C-9a′), 126.16 (C-c), 124.82 (2 C, C-7, C-7′), 122.09 (2 C, C-9, C-9′), 111.65 (2 C, C-4, C-4′), 103.01 (2 C, C-a, C-e), 50.75 (2 C, C-1, C-1′), 43.44 (2 C, $NCH_2$), 33.54 ($COCH_2$), 29.43 ($CH_2CH_2CH_3$), 27.01 ($COCH_2CH_2CH_2CH_2$), 26.86 (4 C, 1-$CH_3$, 1′-$CH_3$), 25.68 ($COCH_2CH_2CH_2$), 24.27 ($COCH_2CH_2$), 19.51 ($CH_2CH_3$), 13.82 ($CH_2CH_3$) ppm. HRMS (MALDI+): calcd. for $C_{43}H_{49}N_2O_2^+$ [M – Cl]$^+$ 625.3789; found 625.3812.

**General Procedure for the Preparation of Cyanine Amido Alcohols 5a–d:** The corresponding cyanine acid **4a**–**d** (15.0 mmol) and DIEA (4.96 mL, 30.0 mmol) were dissolved in DMF (60 mL). PyBOP (7.81 g, 15.0 mmol) was added in one portion and the reaction mixture was stirred for 5 min at ambient temperature. A solution of *trans*-4-aminocyclohexanol hydrochloride (2.28 g, 15.0 mmol) in DMF (30 mL) was added; the mixture was stirred for 4 h and then diluted with chloroform (500 mL), washed with water (2 × 200 mL) and brine (200 mL), dried with $Na_2SO_4$, and the solvents evaporated. The residue was purified by chromatography on silica gel using ethanol in chloroform as eluent (gradient of 0→5%) to give the desired amide.

**2-[3-(1,3,3-Trimethyl-2,3-dihydroindol-2-ylidene)-1-propenyl]-3,3-dimethyl-1-[5-(*trans*-4-hydroxycyclohexylaminocarbonyl)pentyl]-3*H*-indolium Hexafluorophosphate (5a):** Compound **5a** was prepared from **4a**. Yield 8.10 g (77%), dark-purple foam. TLC: $R_f$ = 0.42 [15% MeOH in $CHCl_3$ (v/v)]. $^1$H NMR ([D$_6$]DMSO): $\delta$ = 8.35 (t, $J_{a,b} = J_{b,c}$ = 13.4 Hz, 1 H, b-H), 7.63 (m, 2 H, 4-H, 4′-H), 7.57 (d, $J$ = 8.0 Hz, 1 H, NH), 7.45 (m, 4 H, 6-H, 7-H, 6′-H, 7′-H), 7.30 (m, 2 H, 5-H, 5′-H), 6.47 (d, $J_{a,b} = J_{b,c}$ = 13.4 Hz, 2 H, a-H, c-H), 4.49 (d, $J$ = 4.3 Hz, 1 H, OH), 4.10 (t, $J$ = 7.5 Hz, 2 H, $NCH_2$), 3.66 (s, 3 H, $NCH_3$), 3.40 (m, 1 H, 4′′-H), ca. 3.30 (m, 1 H, 1′′-H), 2.03 (t, $J$ = 7.0 Hz, 2 H, $COCH_2$), 1.80–1.60 (m, 18 H, 3-$CH_3$, 3′-$CH_3$, 2”$_{eq}$-H, 3′′$_{eq}$-H, 5′′$_{eq}$-H, 6′′$_{eq}$-H, $NCH_2CH_2$), 1.54 (m, 2 H, $COCH_2CH_2$), 1.37 (m, 2 H, $NCH_2CH_2CH_2$), 1.10 (m, 4 H, 2′′$_{ax}$-H, 3′′$_{ax}$-H, 5′′$_{ax}$-H, 6′′$_{ax}$-H) ppm. $^{13}$C NMR ([D$_6$]DMSO): $\delta$ = 174.54, 173.76 (C-2, C-2′), 171.07 (CO), 149.75 (C-b), 142.71 (C-7a′), 141.99 (C-7a), 140.64 (2 C, C-3a, C-3a′), 128.70, 128.66 (C-6, C-6′), 125.33, 125.20 (C-5, C-5′), 122.55, 122.47 (C-4, C-4′), 111.58 (2 C, C-7, C-7′), 103.00, 102.45 (C-a, C-c), 68.22 (C-1′′), 48.93 (2 C, C-3, C-3′), 47.04 (C-4′′), 43.78 ($NCH_2$), 35.21 ($COCH_2$), 34.04 (2 C, C-2′′, C-6′′), 31.43 ($NCH_3$), 30.40 (2 C) (C-3′′, C-5′′), 27.52 (2 C), 27.33 (2 C) (3-$CH_3$, 3′-$CH_3$), 26.84 ($NCH_2CH_2$), 25.74 ($NCH_2CH_2CH_2$), 25.00 ($COCH_2CH_2$) ppm. $^{31}$P NMR ([D$_6$]DMSO): $\delta$ = –144.18 (sept, $^1J_{P,F}$ = 711 Hz, $PF_6^-$) ppm. HRMS (MALDI+): calcd. for $C_{36}H_{48}N_3O_2^+$ [M – $PF_6$]$^+$ 554.3741; found 554.3760.

**2-[3-(3-Butyl-1,1-dimethyl-1,2-dihydrobenzo[*e*]indol-2-ylidene)-1-propenyl]-1,1-dimethyl-3-[5-(*trans*-4-hydroxycyclohexylaminocarbonyl)-pentyl]-1*H*-benzo[*e*]indolium Hexafluorophosphate (5b):** Compound **5b** was prepared from **4b**. Yield 10.16 g (80%), dark-violet foam. TLC: $R_f$ = 0.40 [15% MeOH in $CHCl_3$ (v/v)]. $^1$H NMR ([D$_6$]DMSO): $\delta$ = 8.59 (t, $J_{a,b} = J_{b,c}$ = 13.4 Hz, 1 H, b-H), 8.30 (m, 2 H, 9-H, 9′-H), 8.11 (m, 4 H, 5-H, 5′-H, 6-H, 6′-H), 7.79 (m, 2 H, 4-H, 4′-H), 7.69 (m, 2 H, 8-H, 8′-H), 7.55 (m, 3 H, 7-H, 7′-H, NH), 6.58 (m, 2 H, a-H, c-H), 4.44 (d, $J$ = 4.6 Hz, 1 H, OH), 4.27 (m, 4 H, $NCH_2$), 3.39 (m, 1 H, 4′′-H), 3.24 (m, 1 H, 1′′-H), 2.02 (m, 14 H, $COCH_2$, 1-$CH_3$, 1′-$CH_3$), 1.80 (m, 4 H, $NCH_2CH_2$), 1.71–1.37 (m, 10 H, $COCH_2CH_2CH_2$, 2′′$_{eq}$-H, 3′′$_{eq}$-H, 5′′$_{eq}$-H, 6′′$_{eq}$-H, $CH_2CH_3$), 1.05 (m, 4 H, 2′′$_{ax}$-H, 3′′$_{ax}$-H, 5′′$_{ax}$-H, 6′′$_{ax}$-H), 0.97 (t, $J$ = 7.3 Hz, 3 H, $CH_2CH_3$) ppm. $^{13}$C NMR ([D$_6$]DMSO): $\delta$ = 175.18 (2 C, C-2, C-2′), 171.03 (CO), 148.54 (C-b), 139.68, 139.64 (C-3a, C-3a′), 133.22, 133.15 (C-9b, C-9b′), 131.57 (2 C, C-5a, C-5a′), 130.55, 130.51 (C-5, C-5′), 129.99 (2 C, C-6, C-6′), 127.96 (2 C, C-8, C-8′), 127.48 (2 C, C-9a, C-9a′), 125.14 (2 C, C-7, C-7′), 122.25 (2 C, C-9, C-9′), 111.84 (2 C, C-4, C-4′), 102.11 (2 C, C-a, C-c), 68.21 (C-1′′), 50.68 (2 C, C-1, C-1′), 47.02 (C-4′′), 43.92 (2 C, $NCH_2$), 35.20 ($COCH_2$), 34.00 (2 C, C-2′′, C-6′′), 30.38 (2 C, C-3′′, C-5′′), 29.59 ($CH_2CH_2CH_3$), 27.19 (2 C), 27.13 (2 C) (1-$CH_3$, 1′-$CH_3$), 25.67 ($COCH_2CH_2CH_2$), 25.02 ($COCH_2CH_2$), 19.63

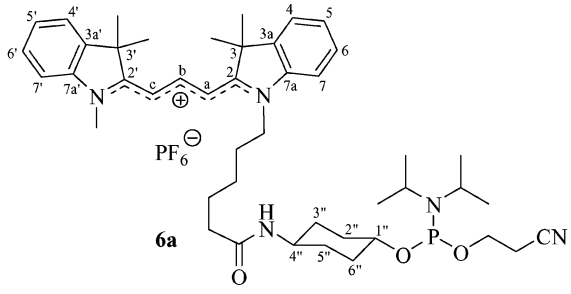
  

($CH_2CH_3$), 13.91 ($CH_2CH_3$) ppm. $^{31}$P NMR ([D$_6$]DMSO): $\delta$ = –144.19 (sept, $^1J_{P,F}$ = 710 Hz, $PF_6^-$) ppm. HRMS (MALDI+): calcd. for $C_{47}H_{58}N_3O_2^+$ [M – $PF_6$]$^+$ 696.4524; found 696.4509.

**2-[5-(1,3,3-Trimethyl-2,3-dihydroindol-2-ylidene)-1,3-pentadienyl]-3,3-dimethyl-1-[5-(*trans*-4-hydroxycyclohexylaminocarbonyl)pentyl]-3*H*-indolium Hexafluorophosphate (5c):** Compound **5c** was prepared from **4c**. Yield 8.25 g (76%), dark-blue foam. TLC: $R_f$ = 0.44 [15% MeOH in $CHCl_3$ (v/v)]. $^1$H NMR ([D$_6$]DMSO): $\delta$ = 8.32 (t, $J_{a,b} = J_{b,c} = J_{c,d} = J_{d,e}$ = 13.0 Hz, 2 H, b-H, d-H), 7.61 (m, 2 H, 4-H, 4′-H), 7.55 (d, $J$ = 8.0 Hz, 1 H, NH), 7.38 (m, 4 H, 6-H, 7-H, 6′-H, 7′-H), 7.25 (m, 2 H, 5-H, 5′-H), 6.56 (t, $J_{b,c} = J_{c,d}$ = 13.0 Hz, 1 H, c-H), 6.30 (d, $J_{d,e}$ = 13.0 Hz, 1 H, e-H), 6.24 (d, $J_{a,b}$ = 13.0 Hz, 1 H, a-H), 4.09 (m, 2 H, $NCH_2$), 3.60 (s, 3 H, $NCH_3$), 3.42 (m, 1 H, 4′′-H), ca. 3.30 (m, 1 H, 1′′-H), 2.01 (t, $J$ = 7.0 Hz, 2 H, $COCH_2$), 1.80–1.60 (m, 18 H, 2′′$_{eq}$-H, 3′′$_{eq}$-H, 5′′$_{eq}$-H, 6′′$_{eq}$-H, $NCH_2CH_2$, 3-$CH_3$, 3′-$CH_3$), 1.52 (m, 2 H, $COCH_2CH_2$), 1.31 (m, 2 H, $NCH_2CH_2CH_2$), 1.10 (m, 4 H, 2′′$_{ax}$-H, 3′′$_{ax}$-H, 5′′$_{ax}$-H, 6′′$_{ax}$-H) ppm. $^{13}$C NMR ([D$_6$]DMSO): $\delta$ = 173.27, 172.67 (C-2, C-2′), 170.98 (CO), 154.11, 153.98 (C-b, C-d), 142.82 (C-7a′), 142.06 (C-7a), 141.13 (C-3a), 141.05 (C-3a′), 128.43, 128.39 (C-6, C-6′), 125.40 (C-c), 124.75, 124.68 (C-5, C-5′), 122.44, 122.33 (C-4, C-4′), 111.14, 111.05 (C-7, C-7′), 103.23 (C-e), 103.17 (C-a), 68.21 (C-1′′), 48.92, 48.90 (C-3, C-3′), 47.01 (C-4′′), 43.35 ($NCH_2$), 35.14 ($COCH_2$), 34.04 (2 C, C-2′′, C-6′′), 31.10 ($NCH_3$), 30.40 (2 C, C-3′′, C-5′′), 27.20 (2 C), 27.06 (2 C) (3-$CH_3$, 3′-$CH_3$), 26.81 ($NCH_2CH_2$), 25.64 ($NCH_2CH_2CH_2$), 24.95 ($COCH_2CH_2$) ppm. $^{31}$P NMR ([D$_6$]DMSO): $\delta$ = –144.18 (sept, $^1J_{P,F}$ = 710 Hz, $PF_6^-$) ppm. HRMS (MALDI+): calcd. for $C_{38}H_{50}N_3O_2^+$ [M – $PF_6$]$^+$ 580.3898; found 580.3890.

**2-[5-(3-Butyl-1,1-dimethyl-1,2-dihydrobenzo[*e*]indol-2-ylidene)-1,3-pentadienyl]-1,1-dimethyl-3-[5-(*trans*-4-hydroxycyclohexylaminocarbonyl)pentyl]-1*H*-benzo[*e*]indolium Hexafluorophosphate (5d):** Compound **5d** was prepared from **4d**. Yield 8.66 g (66%), dark-green foam. TCL $R_f$ = 0.52 [10% EtOH in $CH_2Cl_2$ (v/v)]. $^1$H NMR ([D$_6$]-DMSO): $\delta$ = 8.46 (t, $J_{b,c} = J_{c,d}$ = 13.1 Hz, 2 H, b-H, d-H), 8.30 (d, $J_{8,9} = J_{8',9'}$ = 8.6 Hz, 2 H, 9-H, 9′-H), 8.08 (m, 4 H, 5-H, 5′-H, 6-H, 6′-H), 7.73 (m, 2 H, 4-H, 4′-H), 7.68 (m, 2 H, 8-H, 8′-H), 7.52 (m, 3 H, 7-H, 7′-H, NH), 6.63 (t, $J_{b,c} = J_{c,d}$ = 13.1 Hz, 1 H, c-H), 6.36 (m, 2 H, a-H, e-H), 4.43 (br. s, 1 H, OH), 4.23 (m, 4 H, $NCH_2$), 3.35 (m, 1 H, 4′′-H), 3.25 (m, 1 H, 1′′-H), 2.01 (t, $J$ = 7.0 Hz, 2 H, $COCH_2$), 1.97 (m, 12 H, 1-$CH_3$, 1′-$CH_3$), 1.74 (m, 4 H, $NCH_2CH_2$), 1.69 (m, 2 H), 1.60 (m, 2 H, 2′′$_{eq}$-H, 3′′$_{eq}$-H, 5′′$_{eq}$-H, 6′′$_{eq}$-H), 1.54 (m, 2 H), 1.43 (m, 2 H), 1.35 (m, 2 H, $COCH_2CH_2CH_2$, $CH_2CH_3$), 1.07 (m, 4 H, 2′′$_{ax}$-H, 3′′$_{ax}$-H, 5′′$_{ax}$-H, 6′′$_{ax}$-H), 0.96 (t, $J$ = 7.3 Hz, 3 H, $CH_2CH_3$) ppm. $^{13}$C NMR ([D$_6$]DMSO): $\delta$ = 173.80, 173.69 (C-2, C-2′), 170.97 (CO), 152.85 (2 C, C-b, C-d), 139.79 (2 C, C-3a, C-3a′), 133.20 (2 C, C-9b, C-9b′), 131.34 (2 C, C-5a, C-5a′), 130.37 (2 C, C-5, C-5′), 129.97 (2 C, C-6, C-6′), 127.80 (2 C, C-8, C-8′), 127.65 (2 C, C-9a, C-9a′), 125.83 (C-c), 124.84 (2 C, C-7, C-7′), 122.19 (2 C, C-9, C-9′), 111.71, 111.67 (C-4, C-4′), 103.11, 102.95 (C-a, C-e), 68.20 (C-1′′), 50.77 (2 C, C-1, C-1′), 47.01 (C-4′′), 43.53, 43.44 ($NCH_2$), 35.17 ($COCH_2$), 34.02 (2 C, C-2′′, C-6′′), 30.40 (2 C, C-3′′, C-5′′), 29.45 ($CH_2CH_2CH_3$), 27.12 ($COCH_2CH_2CH_2CH_2$), 26.89 (2 C), 26.84 (2 C) (1-$CH_3$, 1′-$CH_3$), 25.63 ($COCH_2CH_2CH_2$), 24.99 ($COCH_2CH_2$), 19.54 ($CH_2CH_3$), 13.85 ($CH_2CH_3$) ppm. $^{31}$P NMR ([D$_6$]DMSO): $\delta$ = –144.17 (sept, $^1J_{P,F}$ = 710 Hz, $PF_6^-$) ppm. HRMS (MALDI+): calcd. for $C_{49}H_{60}N_3O_2^+$ [M – $PF_6$]$^+$ 722.4680; found 722.4683.

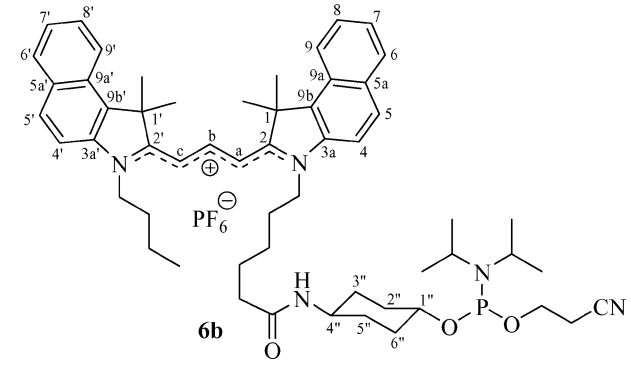
**General Procedure for the Preparation of Phosphoramidites 6a–d:** The corresponding alcohol **5a**–**d** (10.0 mmol) was solved in dry DCM and the solution was evaporated (2 × 150 mL); thereafter the residue was dissolved again in dry DCM (150 mL). Diisopropylammonium tetrazolide (1.70 g, 10.0 mmol) and bis(diisopropylamino)-2-cyanoethoxyphosphane (3.84 mL, 12.0 mmol) were added to the solution that was stirred under argon for 3 h. After conversion of the starting compound was complete [monitoring by TLC, $CHCl_3$/acetone (1:1) + 1% $Et_3N$ (v/v/v)], the mixture was diluted with $CHCl_3$ (150 mL) and washed with 5% $NaHCO_3$ (2 × 100 mL) and 20% NaCl (100 mL). The dried organic layer ($Na_2SO_4$) was evaporated to dryness. The residue was dissolved in dry DCM (30 mL) and precipitated in cold (–10 °C) diethyl ether (1 L). The solid was collected and dried in vacuo to afford compounds **6a**–**d** as amorphous solids.




**2-[3-(1,3,3-Trimethyl-2,3-dihydro-1*H*-indol-2-ylidene)-1-propenyl]-1-{5-[*trans*-4-(diisopropylamino-2-cyanoethoxyphosphanyloxy)-cyclohexylaminocarbonyl]pentyl}-3,3-dimethyl-3*H*-indolium Hexafluorophosphate (6a):** Compound **6a** was prepared from **5a**. Yield 8.38 g (93%), dark-purple foam. TLC $R_f$ = 0.28 [20% acetone in $CH_2Cl_2$ + 1% $Et_3N$ (v/v/v)]. $^1$H NMR ([D$_6$]DMSO): $\delta$ = 8.35 (t, $J_{a,b} = J_{b,c}$ = 13.4 Hz, 1 H, b-H), 7.62 (m, 2 H, 4-H, 4′-H), 7.59 (d, $J$ = 7.6 Hz, 1 H, NH), 7.45 (m, 4 H, 6-H, 7-H, 6′-H, 7′-H), 7.30 (m, 2 H, 5-H, 5′-H), 6.48 (d, $J_{a,b} = J_{b,c}$ = 13.4 Hz, 2 H, a-H, c-H), 4.10 (t, $J$ = 7.3 Hz, 2 H, $NCH_2$), 3.72–3.60 (m, 6 H, $NCH_3$, 1′′-H, $POCH_2$), 3.58–3.43 (m, 3 H, 4′′-H, $Me_2CHN$), 2.73 (t, $J$ = 5.8 Hz, 2 H, $CH_2CN$), 2.04 (t, $J$ = 7.0 Hz, 2 H, $COCH_2$), 1.93–1.68 (m, 18 H, 2′′$_{eq}$-H, 3′′$_{eq}$-H, 5′′$_{eq}$-H, 6′′$_{eq}$-H, $NCH_2CH_2$, 3-$CH_3$, 3′-$CH_3$), 1.56 (m, 2 H, $COCH_2CH_2$), 1.36 (m, 4 H, 2′′$_{ax}$-H, 3′′$_{ax}$-H, 5′′$_{ax}$-H, 6′′$_{ax}$-H), 1.12 (m, 14 H, $NCH_2CH_2CH_2$, $CHCH_3$) ppm. $^{13}$C NMR ([D$_6$]DMSO): $\delta$ = 174.51, 173.78 (C-2, C-2′), 171.10 (CO), 149.74 (C-b), 142.68 (C-7a′), 141.96 (C-7a), 140.63, 140.60 (C-3a, C-3a′), 128.67, 128.63 (C-6, C-6′), 125.30, 125.16 (C-5, C-5′), 122.51, 122.42 (C-4, C-4′), 119.05 (CN), 111.54 (2 C, C-7, C-7′), 102.96, 102.44 (C-a, C-c), 71.69 (d, $^2J_{P,C}$ = 18.4 Hz, C-1′′), 57.92 (d, $^2J_{P,C}$ = 18.4 Hz, $POCH_2$), 48.91 (2 C, C-3, C-3′), 46.45 (C-4′′), 43.77 ($NCH_2$), 42.51 (d, $^2J_{P,C}$ = 12.6 Hz, PNCH), 35.17 ($COCH_2$), 32.46 (m), 32.37 (m, C-2′′, C-6′′), 31.40 ($NCH_3$), 29.91, 29.87 (C-3′′, C-5′′), 27.49 (2 C), 27.32 (2 C) (3-$CH_3$, 3′-$CH_3$), 26.80 ($NCH_2CH_2$), 25.71 ($NCH_2CH_2CH_2$), 24.93 ($COCH_2CH_2$), 24.40 (d, $^3J_{P,C}$ = 6.9 Hz, 2 C), 24.28 (d, $^3J_{P,C}$ = 7.4 Hz, 2 C, $CHCH_3$), 19.87 (d, $^3J_{P,C}$ = 7.4 Hz, $CH_2CN$) ppm. $^{31}$P NMR ([D$_6$]DMSO): $\delta$ = 144.88 (s, 1 P, phosphoramidite), –144.18 (sept, $^1J_{P,F}$ = 711 Hz, 1 P, $PF_6^-$) ppm. HRMS (ESI+): calcd. for $C_{45}H_{65}N_5O_3P^+$ [M – $PF_6$]$^+$ 754.4820; found 754.4840.

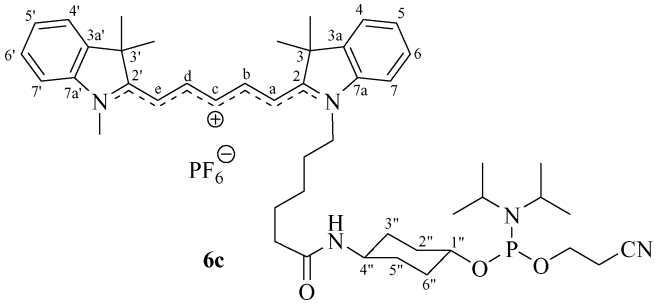
**2-[3-(3-Butyl-1,1-dimethyl-1,2-dihydro-3*H*-benzo[*e*]indol-2-ylidene)-1-propenyl]-1,1-dimethyl-3-{5-[*trans*-4-(diisopropylamino-2-cyanoethoxyphosphanyloxy)cyclohexylaminocarbonyl]pentyl}-1*H*-benzo[*e*]indolium Hexafluorophosphate (6b):** Compound **6b** was prepared from **5b**. Yield 9.98 g (96%), dark-violet foam. TLC: $R_f$ = 0.26 [20% acetone in $CH_2Cl_2$ + 1% $Et_3N$ (v/v/v)]. $^1$H NMR ([D$_6$]-DMSO): $\delta$ = 8.59 (t, $J_{a,b} = J_{b,c}$ = 13.6 Hz, 1 H, b-H), 8.30 (m, 2 H, 9-H, 9′-H), 8.11 (m, 4 H, 5-H, 5′-H, 6-H, 6′-H), 7.79 (m, 2 H, 4-H, 4′-H), 7.69 (m, 2 H, 8-H, 8′-H), 7.55 (m, 3 H, 7-H, 7′-H, NH), 6.58 (m, 2 H, a-H, c-H), 4.27 (m, 4 H, $NCH_2$), 3.70–3.41 (m,



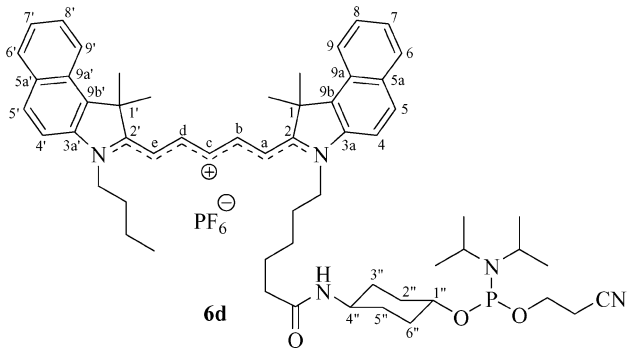
**6b**

6 H, 1′′-H, 4′′-H, $POCH_2$, $Me_2CHN$), 2.72 (t, $J$ = 5.8 Hz, 2 H, $CH_2CN$), 2.02 (m, 14 H, $COCH_2$, 1-$CH_3$, 1′-$CH_3$), 1.80 (m, 4 H), 1.74 (m, 2 H), 1.60 (m, 4 H), 1.48 (m, 2 H), 1.40 (m, 2 H), 1.25 (m, 2 H, 2′′$_{eq}$-H, 3′′$_{eq}$-H, 5′′$_{eq}$-H, 6′′$_{eq}$-H, 2′′$_{ax}$-H, 3′′$_{ax}$-H, 5′′$_{ax}$-H, 6′′$_{ax}$-H, $COCH_2CH_2CH_2CH_2$, $CH_2CH_2CH_3$), 1.08 (m, 14 H, $NCH_2CH_2CH_2$, $CHCH_3$), 0.98 (t, $J$ = 7.3 Hz, 3 H, $CH_2CH_3$) ppm. $^{13}$C NMR ([D$_6$]DMSO): $\delta$ = 175.24, 175.14 (C-2, C-2′), 171.06 (CO), 148.54 (C-b), 139.66, 139.61 (C-3a, C-3a′), 133.19, 133.16 (C-9b, C-9b′), 131.56 (2 C, C-5a, C-5a′), 130.53, 130.48 (C-5, C-5′), 129.95 (2 C, C-6, C-6′), 127.91 (2 C, C-8, C-8′), 127.47, 127.44 (C-9a, C-9a′), 125.10 (2 C, C-7, C-7′), 122.20 (2 C, C-9, C-9′), 119.02 (CN), 111.84, 111.78 (C-4, C-4′), 102.13, 102.06 (C-a, C-c), 71.64 (d, $^2J_{P,C}$ = 18.4 Hz, C-1′′), 57.89 (d, $^2J_{P,C}$ = 17.8 Hz, $POCH_2$), 50.65 (2 C, C-1, C-1′), 46.41 (C-4′′), 43.91 (2 C, $NCH_2$), 42.48 (d, $^2J_{P,C}$ = 12.6 Hz, PNCH), 35.15 ($COCH_2$), 32.33 (m, 2 C, C-2′′, C-6′′), 29.87 (2 C, C-3′′, C-5′′), 29.57 ($CH_2CH_2CH_3$), 27.18 (2 C), 27.12 (2 C) (1-$CH_3$, 1′-$CH_3$), 25.60 ($COCH_2CH_2CH_2$), 24.93 ($COCH_2CH_2$), 24.36 ($COCH_2CH_2$), 24.40 (d, $^3J_{P,C}$ = 6.9 Hz, 2 C), 24.23 (d, $^3J_{P,C}$ = 6.9 Hz, 2 C, $CHCH_3$), 19.86 (d, $^3J_{P,C}$ = 6.9 Hz, $CH_2CN$), 19.60 ($CH_2CH_3$), 13.87 ($CH_2CH_3$) ppm. $^{31}$P NMR ([D$_6$]-DMSO): $\delta$ = 144.90 (s, 1 P, phosphoramidite), –144.17 (sept, $^1J_{P,F}$ = 711 Hz, 1 P, $PF_6^-$) ppm. HRMS (ESI+): calcd. for $C_{56}H_{75}N_5O_3P^+$ [M – $PF_6$]$^+$ 896.5602; found 896.5612.



**6c**

**2-[5-(1,3,3-Trimethyl-2,3-dihydro-1*H*-indol-2-ylidene)-1,3-pentadienyl]-3,3-dimethyl-1-{5-[*trans*-4-(diisopropylamino-2-cyanoethoxyphosphanyloxy)cyclohexylaminocarbonyl]pentyl}-3*H*-indolium Hexafluorophosphate (6c):** Compound **6c** was prepared from **5c**. Yield 8.58 g (93%), dark-blue foam. TLC: $R_f$ = 0.30 [20% acetone in $CH_2Cl_2$ + 1% $Et_3N$ (v/v/v)]. $^1$H NMR ([D$_6$]DMSO): $\delta$ = 8.32 (t, $J_{a,b}$ = $J_{b,c}$ = $J_{c,d}$ = $J_{d,e}$ = 13.0 Hz, 2 H, b-H, d-H), 7.60 (m, 2 H, 4-H, 4′-H), 7.57 (d, $J$ = 7.6 Hz, 1 H, NH), 7.39 (m, 4 H, 6-H, 7-H, 6′-H, 7′-H), 7.24 (m, 2 H, 5-H, 5′-H), 6.56 (t, $J_{b,c}$ = $J_{c,d}$ = 13.0 Hz, 1 H, c-H), 6.31 (d, $J_{d,e}$ = 13.0 Hz, 1 H, e-H), 6.23 (d, $J_{a,b}$ = 13.0 Hz, 1 H, a-H), 4.09 (m, 2 H, $NCH_2$), 3.72–3.62 (m, 3 H, 1′′-H, $POCH_2$), 3.61 (s, 3 H, $NCH_3$), 3.58–3.43 (m, 3 H, 4′′-H, $Me_2CHN$), 2.73 (t, $J$ = 5.8 Hz, 2 H, $CH_2CN$), 2.02 (t, $J$ = 7.0 Hz, 2 H, $COCH_2$), 1.93–1.64 (m, 18 H, 2′′$_{eq}$-H, 3′′$_{eq}$-H, 5′′$_{eq}$-H, 6′′$_{eq}$-H, $NCH_2CH_2$, 3-$CH_3$, 3′-$CH_3$), 1.52 (m, 2 H, $COCH_2CH_2$), 1.32 (m, 4 H, 2′′$_{ax}$-H, 3′′$_{ax}$-H, 5′′$_{ax}$-H, 6′′$_{ax}$-H), 1.10 (m, 14 H, $NCH_2CH_2CH_2$, $CHCH_3$) ppm. $^{13}$C NMR ([D$_6$]DMSO): $\delta$ = 173.24, 172.69 (C-2, C-2′), 171.04 (CO), 154.11, 153.99 (C-b, C-d), 142.82 (C-7a′), 142.07 (C-7a), 141.14 (C-3a), 141.03 (C-3a′), 128.43, 128.39 (C-6, C-6′), 125.42 (C-c), 124.75, 124.67 (C-5, C-5′), 122.43, 122.31 (C-4, C-4′), 119.05 (CN), 111.16, 111.04 (C-7, C-7′), 103.24 (2 C, C-a, C-e), 71.73 (d, $^2J_{P,C}$ = 17.6 Hz, C-1′′), 57.93 (d, $^2J_{P,C}$ = 17.6 Hz, $POCH_2$), 48.92, 48.88 (C-3, C-3′), 46.44 (C-4′′), 43.34 ($NCH_2$), 42.50 (d, $^2J_{P,C}$ = 12.6 Hz, PNCH), 35.14 ($COCH_2$), 32.42 (m), 32.34 (m) (C-2′′, C-6′′), 31.10 ($NCH_3$), 29.95, 29.91 (C-3′′, C-5′′), 27.20 (2 C), 27.07 (2 C) (3-$CH_3$, 3′-$CH_3$), 26.79 ($NCH_2CH_2$), 25.66 ($NCH_2CH_2CH_2$), 24.88 ($COCH_2CH_2$), 24.40 (d, $^3J_{P,C}$ = 7.4 Hz, 2 C), 24.28 (d, $^3J_{P,C}$ = 6.9 Hz, 2 C, $CHCH_3$), 19.87 (d, $^3J_{P,C}$ = 7.5 Hz, $CH_2CN$) ppm. $^{31}$P NMR ([D$_6$]DMSO): $\delta$ = 144.90 (s, 1 P, phosphoramidite), –144.18 (sept, $^1J_{P,F}$ = 710 Hz, 1 P, $PF_6^-$) ppm. HRMS (ESI+): calcd. for $C_{47}H_{67}N_5O_3P^+$ [M – $PF_6$]$^+$ 780.4976; found 780.4991.



**6d**

**2-[5-(3-Butyl-1,1-dimethyl-1,2-dihydrobenzo[*e*]indol-2-ylidene)-1,3-pentadienyl]-1,1-dimethyl-3-{5-[*trans*-4-(diisopropylamino-2-cyanoethoxyphosphanyloxy)cyclohexylaminocarbonyl]pentyl}-1*H*-benzo-[*e*]indolium Hexafluorophosphate (6d):** Compound **6d** was prepared from **5d**. Yield 10.21 g (95%), dark-green foam. TLC: $R_f$ = 0.50 [20% acetone in $CH_2Cl_2$ + 1% $Et_3N$ (v/v/v)]. $^1$H NMR ([D$_6$]-DMSO): $\delta$ = 8.45 (t, $J_{b,c}$ = $J_{c,d}$ = 13.0 Hz, 2 H, b-H, d-H), 8.25 (d, $J_{8,9}$ = $J_{8',9'}$ = 8.6 Hz, 2 H, 9-H, 9′-H), 8.07 (m, 4 H, 5-H, 5′-H, 6-H, 6′-H), 7.73 (m, 2 H, 4-H, 4′-H), 7.68 (m, 2 H, 8-H, 8′-H), 7.53 (m, 3 H, 7-H, 7′-H, NH), 6.66 (t, $J_{b,c}$ = $J_{c,d}$ = 13.0 Hz, 1 H, c-H), 6.36 (m, 2 H, a-H, e-H), 4.23 (m, 4 H, $NCH_2$), 3.70–3.40 (m, 6 H, 1′′-H, 4′′-H, $POCH_2$, $Me_2CHN$), 2.72 (t, $J$ = 6.0 Hz, 2 H, $CH_2CN$), 2.02 (t, $J$ = 7.0 Hz, 2 H, $COCH_2$), 1.97 (m, 12 H, 1-$CH_3$, 1′-$CH_3$), 1.81 (m, 2 H), 1.74 (m, 4 H), 1.64 (m, 2 H) ($NCH_2CH_2$, 2′′$_{eq}$-H, 3′′$_{eq}$-H, 5′′$_{eq}$-H, 6′′$_{eq}$-H), 1.54 (m, 2 H), 1.44 (m, 4 H), 1.35 (m, 2 H) ($COCH_2CH_2$, $CH_2CH_3$, 2′′$_{ax}$-H, 3′′$_{ax}$-H, 5′′$_{ax}$-H, 6′′$_{ax}$-H), 1.09 (m, 14 H, $NCH_2CH_2CH_2$, $CHCH_3$), 0.96 (t, $J$ = 7.3 Hz, 3 H, $CH_2CH_3$) ppm. $^{13}$C NMR ([D$_6$]DMSO): $\delta$ = 173.64 (2 C, C-2, C-2′), 170.99 (CO), 152.82 (2 C, C-b, C-d), 139.77 (2 C, C-3a, C-3a′), 133.19 (2 C, C-9b, C-9b′), 131.34 (2 C, C-5a, C-5a′), 130.31 (2 C, C-5, C-5′), 129.96 (2 C, C-6, C-6′), 127.75 (2 C, C-8, C-8′), 127.64 (2 C, C-9a, C-9a′), 125.84 (C-c), 124.82 (2 C, C-7, C-7′), 122.15 (2 C, C-9, C-9′), 119.02 (CN), 111.62 (2 C, C-4, C-4′), 102.91 (2 C, C-a, C-e), 71.72 (d, $^2J_{P,C}$ = 18.6 Hz, C-1′′), 57.90 (d, $^2J_{P,C}$ = 19.2 Hz, $POCH_2$), 50.74 (2 C, C-1, C-1′), 46.40 (C-4′′), 43.50, 43.41 ($NCH_2$), 42.49 (d, $^2J_{P,C}$ = 13.0 Hz, PNCH), 35.13 ($COCH_2$), 32.47, 32.37 (C-2′′, C-6′′), 29.92 (2 C, C-3′′, C-5′′), 29.43 ($CH_2CH_2CH_3$), 27.07 ($COCH_2CH_2CH_2CH_2$), 26.87 (2 C), 26.83 (2 C) (1-$CH_3$, 1′-$CH_3$), 25.61 ($COCH_2CH_2CH_2$), 24.98 ($COCH_2CH_2$), 24.37 (d, $^3J_{P,C}$ = 6.8 Hz, 2 C), 24.26 (d, $^3J_{P,C}$ = 6.2 Hz, 2 C, $CHCH_3$), 19.86 (d, $^3J_{P,C}$ = 6.2 Hz, $CH_2CN$), 19.51

($CH_2CH_3$), 13.81 ($CH_2CH_3$) ppm. $^{31}P$ NMR ([D$_6$]DMSO): $\delta$ = 144.89 (s, 1 P, phosphoramidite), –144.18 (sept, $^1J_{P,F}$ = 710 Hz, 1 P, $PF_6^-$) ppm. HRMS (ESI+): calcd. for $C_{58}H_{77}N_5O_3P^+$ [M – $PF_6$]$^+$ 922.5759; found 922.5790.

**General Procedure for the Preparation of Cyanine Oxysuccinimide Esters 7a–d:** The corresponding cyanine acid **4a**–**d** (10.0 mmol) and DIEA (3.50 mL, 20 mmol) were dissolved in dry DCM (100 mL). DSC (2.82 g, 11.0 mmol) was added in one portion and the mixture was stirred for 2 h at room temp., then diluted with DCM (100 mL), and washed successively with water (400 mL), 1 M HCl (400 mL), and brine (400 mL). The solution was dried with $Na_2SO_4$, evaporated, and the residue was triturated in diethyl ether to give pure esters **7a**–**d** as amorphous solids.

**2-[3-(1,3,3-Trimethyl-2,3-dihydroindol-2-ylidene)-1-propenyl]-3,3-dimethyl-1-[5-(succinimidooxycarbonyl)pentyl]-3*H*-indolium Chloride (7a):** Compound **7a** was prepared from **4a**. Yield 5.64 g (96%), dark-purple foam. TLC: $R_f$ = 0.61 [20% MeOH in $CHCl_3$ (v/v)]. $^1H$ NMR ([D$_6$]DMSO): $\delta$ = 8.35 (t, $J_{a,b}$ = $J_{b,c}$ = 13.4 Hz, 1 H, b-H), 7.64 (m, 2 H, 4-H, 4′-H), 7.45 (m, 4 H, 6-H, 7-H, 6′-H, 7′-H), 7.30 (m, 2 H, 5-H, 5′-H), 6.52 (d, $J_{a,b}$ = 13.4 Hz, 1 H, a-H), 6.50 (d, $J_{b,c}$ = 13.4 Hz, 1 H, c-H), 4.12 (t, $J$ = 7.3 Hz, 2 H, $NCH_2$), 3.66 (s, 3 H, $NCH_3$), 2.81 (s, 4 H, $COCH_2CH_2CO$), 2.70 (t, $J$ = 7.3 Hz, 2 H, $COCH_2$), 1.82–1.67 (m, 4 H, $NCH_2CH_2CH_2CH_2$), 1.70 (s, 12 H, 3-$CH_3$, 3′-$CH_3$), 1.57 (m, 2 H, $NCH_2CH_2CH_2$) ppm. $^{13}C$ NMR ([D$_6$]DMSO): $\delta$ = 174.56 (2 C, C-2, C-2′), 173.73 (OCO), 170.29 (2 C, NCO), 149.78 (C-b), 142.70 (C-7a′), 141.93 (C-7a), 140.64 (2 C, C-3a, C-3a′), 128.71, 128.64 (C-6, C-6′), 125.32, 125.17 (C-5, C-5′), 122.57, 122.47 (C-4, C-4′), 111.59, 111.51 (C-7, C-7′), 103.05, 102.42 (C-a, C-c), 48.93 (2 C, C-3, C-3′), 43.71 ($NCH_2$), 31.53 ($NCH_3$), 30.04 ($COCH_2$), 27.53 (2 C), 27.33 (2 C) (3-$CH_3$, 3′-$CH_3$), 26.54 ($NCH_2CH_2$), 25.51 (2 C, $COCH_2CH_2CO$), 25.49 ($NCH_2CH_2CH_2$), 24.04 ($COCH_2CH_2$) ppm. HRMS (MALDI+): calcd. for $C_{34}H_{40}N_3O_4^+$ [M – Cl]$^+$ 554.3013; found 554.3027.

**2-[3-(3-Butyl-1,1-dimethyl-1,2-dihydrobenzo[*e*]indol-2-ylidene)-1-propenyl]-1,1-dimethyl-3-[5-(succinimidooxycarbonyl)pentyl]-1*H*-benzo[*e*]indolium Chloride (7b):** Compound **7b** was prepared from **4b**. Yield 7.14 g (98%), dark-violet foam. TLC: $R_f$ = 0.74 [20% MeOH in $CHCl_3$ (v/v)]. $^1H$ NMR ([D$_6$]DMSO): $\delta$ = 8.60 (t, $J_{a,b}$ = $J_{b,c}$ = 13.4 Hz, 1 H, b-H), 8.30 (m, 2 H, 9-H, 9′-H), 8.10 (m, 4 H, 5-H, 5′-H, 6-H, 6′-H), 7.80 (m, 2 H, 4-H, 4′-H), 7.69 (m, 2 H, 8-H, 8′-H), 7.54 (m, 3 H, 7-H, 7′-H, NH), 6.61 (m, 2 H, a-H, c-H), 4.44 (d, $J$ = 4.6 Hz, 1 H, OH), 4.29 (m, 4 H, $NCH_2$), 2.79 (s, 4 H, $COCH_2CH_2CO$), 2.71 (t, $J$ = 7.2 Hz, 2 H, $COCH_2$), 2.02 (s, 12 H, 1-$CH_3$, 1′-$CH_3$), 1.88 (m, 4 H, $NCH_2CH_2$), 1.67 (m, 2 H, $COCH_2CH_2$), 1.48 (m, 4 H, $NCH_2CH_2CH_2$, $CH_2CH_3$), 0.97 (t, $J$ = 7.3 Hz, 3 H, $CH_2CH_3$) ppm. $^{13}C$ NMR ([D$_6$]DMSO): $\delta$ = 175.20 (2 C, C-2, C-2′), 172.76 (OCO), 170.23 (2 C, NCO), 148.59 (C-b), 139.62 (2 C, C-3a, C-3a′), 133.21 (2 C, C-9b, C-9b′), 131.55 (2 C, C-5a, C-5a′), 130.51 (2 C, C-5, C-5′), 129.98 (2 C, C-6, C-6′), 127.93 (2 C, C-8, C-8′), 127.45 (2 C, C-9a, C-9a′), 125.10 (2 C, C-7, C-7′), 122.22 (2 C, C-9, C-9′), 111.82 (2 C, C-4, C-4′), 102.12 (2 C, C-a, C-c), 50.66 (2 C, C-1, C-1′), 43.92 (2 C, $NCH_2$), 30.06 ($COCH_2$), 29.57 ($CH_2CH_2CH_3$), 28.42 ($COCH_2CH_2CH_2CH_2$), 27.17 (4 C, 1-$CH_3$, 1′-$CH_3$), 25.48 (3 C, $COCH_2CH_2CH_2$,$COCH_2CH_2CO$), 24.08 ($COCH_2CH_2$), 19.60 ($CH_2CH_3$), 13.88 ($CH_2CH_3$) ppm. HRMS (MALDI+): calcd. for $C_{45}H_{50}N_3O_4^+$ [M – Cl]$^+$ 696.3796; found 696.3781.

**2-[5-(1,3,3-Trimethyl-2,3-dihydro-1*H*-indol-2-ylidene)-1,3-pentadienyl]-3,3-dimethyl-1-[5-(succinimidooxycarbonyl)pentyl]-3*H*-indolium Chloride (7c):** Compound **7c** was prepared from **4c**. Yield 5.93 g (96%), dark-blue foam. TLC: $R_f$ = 0.39 [20% MeOH in $CHCl_3$ (v/v)]. $^1H$ NMR ([D$_6$]DMSO): $\delta$ = 8.33 (t, $J_{a,b}$ = $J_{b,c}$ = $J_{c,d}$ = $J_{d,e}$ = 13.0 Hz, 2 H, b-H, d-H), 7.61 (m, 2 H, 4-H, 4′-H), 7.40 (m, 4 H, 6-H, 7-H, 6′-H, 7′-H), 7.24 (m, 2 H, 5-H, 5′-H), 6.57 (t, $J_{b,c}$ = $J_{c,d}$ = 13.0 Hz, 1 H, c-H), 6.30 (d, $J_{a,b}$ = 13.0 Hz, 1 H, a-H), 6.26 (d, $J_{d,e}$ = 13.0 Hz, 1 H, e-H), 4.09 (m, 2 H, $NCH_2$), 3.61 (s, 3 H, $NCH_3$), 2.82 (s, 4 H, $COCH_2CH_2CO$), 2.68 (t, $J$ = 7.2 Hz, 2 H, $COCH_2$), 1.78–1.69 (m, 4 H, $NCH_2CH_2CH_2CH_2$), 1.68 (s, 12 H, 3-$CH_3$, 3′-$CH_3$), 1.49 [m, 2 H, ($NCH_2CH_2CH_2$)] ppm. $^{13}C$ NMR ([D$_6$]DMSO): $\delta$ = 173.34 (C-2′), 172.75 (OCO), 172.53 (C-2), 170.25 (2 C, NCO), 154.03 (2 C, C-b, C-d), 142.80 (C-7a′), 142.04 (C-7a), 141.14 (C-3a), 141.07 (C-3a′), 128.44, 128.39 (C-6, C-6′), 125.45 (C-c), 124.78, 124.64 (C-5, C-5′), 122.45, 122.34 (C-4, C-4′), 111.09, 111.04 (C-7, C-7′), 103.38 (C-e), 103.05 (C-a), 48.91 (2 C, C-3, C-3′), 43.24 ($NCH_2$), 31.17 ($NCH_3$), 30.05 ($COCH_2$), 27.22 (2 C), 27.04 (2 C) (3-$CH_3$, 3′-$CH_3$), 26.43 ($NCH_2CH_2$), 25.50 (3 C, $COCH_2CH_2CH_2CO$, $NCH_2CH_2CH_2$), 23.96 ($COCH_2CH_2$) ppm. HRMS (MALDI+): calcd. for $C_{36}H_{42}N_3O_4^+$ [M – Cl]$^+$ 580.3170; found 580.3193.

**2-[5-(3-Butyl-1,1-dimethyl-1,2-dihydrobenzo[*e*]indol-2-ylidene)-1,3-pentadienyl]-1,1-dimethyl-3-[5-(succinimidooxycarbonyl)pentyl]-1*H*-benzo[*e*]indolium Chloride (7d):** Compound **7d** was prepared from **4d**. Yield 7.36 g (97%), dark-green foam. TLC: $R_f$ = 0.75 [20% EtOH in DCM (v/v)]. $^1H$ NMR ([D$_6$]DMSO): $\delta$ = 8.46 (t, $J_{b,c}$ = $J_{c,d}$ = 13.0 Hz, 2 H, b-H, d-H), 8.26 (d, $J$ = 8.2 Hz, 2 H, 9-H, 9′-H), 8.07 (m, 4 H, 5-H, 5′-H, 6-H, 6′-H), 7.74 (m, 2 H, 4-H, 4′-H), 7.68 (m, 2 H, 8-H, 8′-H), 7.51 (m, 2 H, 7-H, 7′-H), 6.66 (t, $J_{b,c}$ = $J_{c,d}$ = 13.0 Hz, 1 H, c-H), 6.37 (d, $J_{a,b}$ = $J_{d,e}$ = 13.0 Hz, 2 H, a-H, e-H), 4.24 (m, 4 H, $NCH_2$), 2.80 (s, 4 H, $COCH_2CH_2CO$), 2.20 (m, 2 H, $OCOCH_2$), 1.97 (s, 12 H, 1-$CH_3$, 1′-$CH_3$), 1.74 (m, 4 H, $NCH_2CH_2$), 1.57 (m, 2 H, $OCOCH_2CH_2$), 1.45 (m, 4 H, $NCH_2CH_2CH_2$), 0.95 (t, $J$ = 7.3 Hz, 3 H, $CH_2CH_3$) ppm. $^{13}C$ NMR ([D$_6$]DMSO): $\delta$ = 174.31, 173.70 (C-2, C-2′), 168.94 (OCO), 170.30 (2 C, NCO), 152.87 (2 C, C-b, C-d), 139.76 (2 C, C-3a, C-3a′), 133.20 (2 C, C-9b, C-9b′), 131.33 (2 C, C-5a, C-5a′), 130.34 (2 C, C-5, C-5′), 129.95 (2 C, C-6, C-6′), 127.77 (2 C, C-8, C-8′), 127.64 (2 C, C-9a, C-9a′), 126.16 (C-c), 124.82 (2 C, C-7, C-7′), 122.09 (2 C, C-9, C-9′), 111.65 (2 C, C-4, C-4′), 103.01 (2 C, C-a, C-e), 50.75 (2 C, C-1, C-1′), 43.44 (2 C, $NCH_2$), 33.54 ($COCH_2$), 29.43 ($CH_2CH_2CH_3$), 27.01 ($COCH_2CH_2CH_2CH_2$), 26.86 (4 C, 1-$CH_3$, 1′-$CH_3$), 25.68 ($COCH_2CH_2CH_2$), 24.27 ($COCH_2CH_2$), 22.47 (2 C, $NCOCH_2$), 19.51 ($CH_2CH_3$), 13.82 ($CH_2CH_3$) ppm. HRMS (MALDI+): calcd. for $C_{47}H_{52}N_3O_4^+$ [M – Cl]$^+$ 722.3952; found 722.3955.

**Synthesis of Cyanine-Labeled Oligodeoxynucleotides Using Modifying Phosphoramidite Reagents:** Modified phosphoramidites **6a**–**d** (0.1 M solutions in MeCN) were used in the last step of solid-phase oligodeoxyribonucleotide synthesis with their coupling time increased to 7.5 min. After assembly, the CPG-bound oligonucleotides were treated with conc. ammonium hydroxide at 4 °C for 72 h, evaporated, and precipitated from 1 M $LiClO_4$ (100 μL) by dilution with acetone (1.5 mL). The oligonucleotides were isolated by reversed-phase HPLC (C18 column) or by electrophoresis in 20% denaturing (7 M urea) polyacrylamide gel in Tris-borate buffer (pH 8.3), and desalted using a NAP-10 column and a standard purification procedure.

**Synthesis of Cyanine-Labeled Oligodeoxynucleotides Using Postsynthetic Modification with Cyanine Activated Esters:** Crude deprotected 5′-aminoalkyl oligonucleotides (from 200-nmol-scale synthesis) were evaporated and dissolved in 0.1 M sodium hydrogen carbonate buffer (pH 8.5, 420 μL). Activated esters **7a**–**d** (25 mM solutions in DMF, 80 μL, 2 μmol) were added, the mixtures were vortexed and kept at 0 °C for 12 h, and the solvents evaporated. Oligonucleotide conjugates were isolated by precipitation from 1 M $LiClO_4$ followed by PAGE and desalination as described above.

**Procedure for Protein Labeling with Cy3 and Cy5:** Activated esters **7a**,**c** (20 μL of 1.50, 3.75, 7.50, or 15.0 mM solutions in DMF) were added (Cy activated ester/BSA ratio 0.2, 0.5, 1.0, and 2.0, respectively) to a 0.15 mM solution of BSA (1.0 mL, 150 nmol) in 0.2 M sodium hydrogen carbonate buffer (pH 8.5). The mixtures were vortexed and kept at 0 °C for 12 h. Labeled protein was eluted as a colored band in the void volume upon filtration through a Sephadex G-25 column.[17]

**Supporting Information** (see also the footnote on the first page of this article): Examples of HPLC profiles of crude conjugates **ON1b** and **ON1c**, isolation of conjugates **ON3a**–**d** using 20% PAGE (gel scan), MALDI-TOF MS spectroscopic data for oligonucleotide conjugates, isolation of cyanine–BSA conjugates **Cy3-BSA** and **Cy5-BSA** using size-exclusion chromatography (color photos).

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