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Design and Synthesis of Efficient Fluorescent Dyes for Incorporation into DNA Backbone and Biomolecule Detection

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Abstract

We report here the design and synthesis of a series of π -conjugated fluorescent dyes with D-A-D (D: donor; A: Acceptor), D- π -D, A- π -A, and D- π -A for applications as the signaling motif in biological-synthetic hybrid foldamers for DNA detection. Horner-Wadsworth-Emmons (HWE) reaction and Knoevenagel condensation were demonstrated as the optimum ways for construction of long π -conjugated systems. Such rod-like chromophores have distinct advantages, as their fluorescence properties are not quenched by the presence of DNA. To be incorporated into the backbone of DNA, the chromophores need to be reasonably soluble in organic solvent for solid-phase synthesis, and therefore a strategy of using flexible tetra(ethylene glycol) (TEG) linkers at either end of these rod-like dyes were developed. The presence of TEG facilitates the protection of the chain-growing hydroxyl group with DMTrCl (dimethoxy trityl chloride) as well as the activation of the coupling step with phosphoramidite chemistry on an automated DNA synthesizer. To form fluorescence resonance energy transfer (FRET) pairs, six synthetic chromophores with blue to red fluorescence have been developed and those with orthogonal fluorescent emission were chosen for incorporation into DNA-chromophore hybrid foldamers.

Introduction

Syntheses of deoxyribonucleic acid (DNA) and dye conjugates (1–3) have enabled many optical probes; these conjugate probes provide a means to study biomolecules within living cells in details via fluorescence imaging (4,5) and single molecules fluorescence spectroscopy (6–8). However, introducing multiple fluorescent dyes ($N > 2$) into the backbone of a single DNA chain remains largely an unsolved challenge for organic chemistry. To study complex biochemical assemblies and reactions, it is necessary in some systems to have multiple fluorescence probes on a single DNA chain. One such interesting architectural motif in supramolecular chemistry is to use the self-assembling amphiphilic properties of the dyes to induce folded nanostructures with alternating single strand DNA looping around the folded cores (9). Using this principle, hybrid biological and synthetic foldamers have been readily developed for DNA detection (10). The strategy is that organic fluorescence chromophores function as an optical reporter while the biological domain, in this case, a specific DNA sequence, functions as a high fidelity molecular recognition element. The rationale here is that when the built-in DNA sequences in the hybrid foldamer complement with the target DNA in completely or partially matched situation, the folded nanostructure of the foldamer will be converted to an extended one, a process in which fluorescence color of the foldamer will change dramatically because of FRET between two adjacent chromophores (11). Different mismatched DNA sequences lead to various degrees of emission color changes due to different

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levels of FRET between the organic chromophores at a given time. From the structural skeleton point of view, molecular beacons usually have organic dyes at both ends of a single DNA sequence and therefore their termini are dead end for incorporation into long DNA sequences (12–16), the hybrid foldamers have the organic dyes inserted between multiple DNA sequences, which allow further biological manipulation such that the DNA ends can be cut by endonucleases and incorporated into genes with a DNA polymerase or ligase. These special structural features make the hybrid foldamers unique and suitable candidates for studying biological mechanisms.

Since the foldamer probe with alternating hydrophobic and hydrophilic structures are constructed on solid-phase synthesis using an automated DNA synthesizer (Applied Biosystem 8909) (9), it is imperative to develop fluorescent building blocks with an activated linker which can be attached to the growing end of the DNA chain and a temporary blocking group to prevent non-desired coupling reactions and permit further chain extension after deprotection. The resulting hybrid foldamers have multiple fluorescent chromophores and its emission color is controlled by the distance between the chromophores separated by the DNA sequences (Figure 1). For completely mismatched sequences, no hybridization occurs between the foldamer and the mismatched target; therefore the flexible single stranded DNA chains allow individual chromophores to come to close proximity due to the hydrophobic and π -stacking effect and the net result is that fluorescence emission is in the long wavelength region because of the high degree of FRET between individual organic dyes. For perfect matched sequences, however, hybridization turns flexible single stranded DNA into rigid double helices, which in turn separates chromophores, and reduces the level of FRET between the organic dyes. In this case, the emission color from the foldamer will depend on the short wavelength chromophore. Therefore, we have designed and synthesized a series of fluorescent chromophores with emission wavelength ranging from blue to red; all fluorescent compounds bear tetraethylene glycol side chains with $-OH$ termini, which are amenable to DMTr protection and phosphoramidite chemistry.

As a class of bis(styryl)benzene derivatives, the donor- π -donor (D- π -D), acceptor- π -acceptor (A- π -A), donor-acceptor-donor (D-A-D), and acceptor-donor-acceptor (A-D-A) structural motifs have received a great deal of attention because they exhibit exceptional large two-photon absorption cross sections, up to ~ 400 times that of trans-stilbene (17). Compared to our prior perylene tetracarboxylic diimide unit, these new chromophores have desired features such as higher fluorescence quantum yield when incorporated into DNA, and adjustable excitation and emission wavelength via structural variations on similar molecular architectures. Meanwhile, a red fluorescence dye based on the symmetric structure of (2,6-dimethyl-4*H*-pyran-4-ylidene) malononitrile (DCM laser dye) has been developed according to a recent report (18), the large dipole moment and high decomposition temperature of this Y-shaped structure make it an attractive candidate for red fluorescence signaling reporter. Consequently, we have developed a series of organic chromophores with different fluorescent colors as molecular building blocks (Figure 2), which are suitable for construction of new hybrid foldamers as molecular probes (10).

Experimental Section

General methods: Solvents and reagents were purified where necessary using literature methods (31). In particular, *N,N*-dimethylformamide (DMF) was distilled from 4Å MS (Molecular Sieve) under reduced pressure and stored under argon. Acetonitrile (MeCN) was heated under reflux over calcium hydride and distilled under argon. MALDI Mass Spectra was obtained with an ABVS-2025 spectrometer. 1H NMR spectra were recorded with a Mercury 300 (300 MHz) spectrometer for solutions in $CDCl_3$ (CD_3OD) at ambient temperature. ^{13}C NMR spectra were recorded at 75.48 MHz with a Mercury 300 spectrometer for solutions in

CDCl_3 (CD_3OD) adopting 77.23 ppm for the central line of CDCl_3 . ^{31}P -NMR chemical shifts were reported in ppm using 85% H_3PO_4 as an external reference. Reactions were monitored by thin-layer chromatography (TLC) on a pre-coated plate of silica gel 60 F_{254} (EM Science). Column chromatography was performed on silica gel 60 (230–400 mesh, EM Science).

Compounds in tetraethylene chain optimization and modification: 4-(2-(2-(2-Hydroxyethoxy)-ethoxy)-ethoxy)-benzaldehyde (7)

A mixture of 2-(2-(2-Chloroethoxy)ethoxy)ethanol (commercial) (1.0 g, 5.9 mmol), 4-hydroxybenzaldehyde (0.8 g, 6.5 mmol, 1.1 equiv), and K_2CO_3 (1.8 g, 2.2 equiv) in dry N,N -dimethylformamide (DMF) (10 mL) was heated to 120 °C (oil bath 110 °C) under argon. After the stirring was continued at this temperature for 16 h the reaction mixture was cooled to room temperature. The solvent (DMF) was evaporated in vacuum, and the residue was diluted with CHCl_3 . The obtained suspension was suction-filtered and the filtrate was collected. After dried over Na_2SO_4 , the organic phase was concentrated, and the residue was purified by chromatography on a silica gel column eluted with EtOAc/MeOH (20:1) to give the title product (950 mg, yield, 63%) as a yellow oil: R_f 0.33 (EtOAc/MeOH 20/1). ^1H NMR (CDCl_3) δ (ppm): 9.89 (s, 1H, -CHO), 7.82 (dt, 2H, $J_1 = 9.0\text{Hz}$, $J_2 = 2.7\text{Hz}$, benzaldehyde), 7.02 (dt, 2H, $J_1 = 9.0\text{Hz}$, $J_2 = 2.7\text{Hz}$, benzaldehyde), 4.21 (t, 2H, $J = 4.8\text{Hz}$, triethylene glycol chain), 3.89 (t, 2H, $J = 4.8\text{Hz}$, triethylene glycol chain), 3.75–3.67 (m, 6H, triethylene glycol chain), 3.61 (bt, 2H, $J = 4.8\text{Hz}$, triethylene glycol chain), 2.40 (bt, 1H, $J = 6.0\text{Hz}$, -OH).

4-(2-(2-(2-Triethoxyethoxy)-ethoxy)-ethoxy)-benzaldehyde (8)

A mixture of **7** (0.5 g, 2.0 mmol), Trityl chloride (1.2 g, 4.3 mmol, 2.2 equiv), and a catalytic amount of 4-(Dimethylamino)pyridine (DMAP) in dry pyridine (5 mL) was heated to 40–50 °C under argon. After the stirring was continued at this temperature for 24 h the reaction mixture was cooled to room temperature. The solvent was removed in vacuum, and the residue was diluted with EtOAc. The obtained suspension was suction-filtered and the filtrate was collected. After dried over Na_2SO_4 , the organic phase was concentrated, and the residue was purified by chromatography on a silica gel column eluted with Cyclohexane/EtOAc/Pyridine (300/100/4, v/v) to give the title product (770 mg, yield 99%): R_f 0.31 (300/100/4, v/v). ^1H NMR (CDCl_3) δ (ppm): 9.87 (s, 1H, -CHO), 7.80 (dt, 2H, $J_1 = 8.7\text{Hz}$, $J_2 = 2.1\text{Hz}$, benzaldehyde), 7.48–7.43 (m, 6H, benzene ring of trityl group), 7.46–7.18 (m, 9H, benzene ring of trityl group), 6.98 (dt, 2H, $J_1 = 8.7\text{Hz}$, $J_2 = 2.1\text{Hz}$, benzaldehyde), 4.20 (bt, 2H, $J = 4.8\text{Hz}$, triethylene glycol chain), 3.92 (bt, 2H, $J = 4.8\text{Hz}$, triethylene glycol chain), 3.78–3.67 (m, 6H, triethylene glycol chain), 3.24 (t, 2H, $J = 4.8\text{Hz}$, triethylene glycol chain).

4-[2-[2-[2-(2-hydroxyethoxy)ethoxy]ethoxy]ethoxy]-benzaldehyde (10)

A mixture of 2-(2-(2-(2-Chloroethoxy)ethoxy)ethoxy)ethanol (**9**) (19) (2.0 g, 9.4 mmol), 4-Hydroxybenzaldehyde (1.4 g, 1.2 equal), and K_2CO_3 (2.5 g, 2 equal) in dry N,N -dimethylformamide (DMF) (10 mL) was heated to 100 °C (oil bath 110 °C) under argon. The stirring was continued at this temperature for 72 h. After the reaction mixture was cooled to room temperature, the solvent (DMF) was evaporated in vacuum and the residue was diluted with CHCl_3 . The obtained suspension was suction-filtered and the filtrate was collected. After dried over Na_2SO_4 , the organic phase was concentrated and the residue was purified by chromatography on a silica gel column eluted with EtOAc/MeOH (20:1) to give the title product (2.25 g, yield 80%) as a yellow oil: R_f 0.3 (EtOAc/MeOH 20/1). ^1H NMR (CDCl_3) δ (ppm): 9.87 (s, 1H, -CHO), 7.82 (dt, 2H, $J_1 = 9.0\text{Hz}$, $J_2 = 2.7\text{Hz}$, benzaldehyde), 7.01 (dt, 2H, $J_1 = 9.0\text{Hz}$, $J_2 = 2.7\text{Hz}$, benzaldehyde), 4.22 (bt, 2H, $J = 4.8\text{Hz}$, tetraethylene glycol chain), 3.89 (bt, 2H, $J = 4.8\text{Hz}$, tetraethylene glycol chain), 3.77–3.59 (m, 12H, tetraethylene glycol chain), 2.53 (t, 1H, $J = 6.0\text{Hz}$, -OH); ^{13}C NMR (CDCl_3) δ (ppm): 190.9, 163.9, 132.1, 130.1,

115.0, 72.65, 71.07, 70.86, 70.78, 70.53, 69.68, 67.92, 61.95. MS (MALDI-TOF): 299.01 [M+H]⁺, 320.99 [M+Na]⁺.

4-(2-(2-(2-(2-(Bis-(4-methoxy-phenyl)-phenyl-methoxy)-ethoxy)-ethoxy)-ethoxy)-ethoxy)-benzaldehyde (11)

A mixture of **10** (0.5 g, 1.7 mmol), DMTrCl (1.2 g, 3.5 mmol, 2 equiv), and a catalytic amount of 4-(Dimethylamino)pyridine (DMAP) in dry Pyridine (10 mL) was stirred at room temperature under argon. After 6 h, the reaction solvent was removed in vacuum and the residue was directly subject to a silica gel column eluted with Cyclohexane/EtOAc (gradient elution with 1% Pyridine: 500/150 → 300/150 → 150/150, v/v) to give the title product (1.056 g, yield 96%); *R_f* 0.42 (Cyclohexane/EtOAc 1/1, v/v). ¹H NMR (CDCl₃) δ (ppm): 9.87 (s, 1H, -CHO), 7.79 (dt, 2H, *J*₁ = 8.7 Hz, *J*₂ = 2.1 Hz, benzaldehyde), 7.45 (m, 2H, benzene ring of DMTr group), 7.36-7.15 (m, 3H, benzene ring of DMTr group), 7.34 (dt, 4H, *J*₁ = 9.0 Hz, *J*₂ = 2.7 Hz, methoxybenzene ring of DMTr group), 6.97 (dt, 2H, *J*₁ = 8.7 Hz, *J*₂ = 2.1 Hz, benzaldehyde), 6.81 (dt, 4H, *J*₁ = 9.0 Hz, *J*₂ = 2.7 Hz, methoxybenzene ring of DMTr group) 4.15 (bt, 2H, *J* = 4.8 Hz, tetraethylene glycol chain), 3.87 (bt, 2H, *J* = 4.8 Hz, tetraethylene glycol chain), 3.77 (s, 6H, -OCH₃), 3.77-3.64 (m, 10H, tetraethylene glycol chain), 3.22 (t, 2H, *J* = 4.8 Hz, tetraethylene glycol chain); ¹³C NMR (CDCl₃) δ (ppm): 191.0, 164.0, 158.5, 145.3, 136.5, 132.1, 130.3, 130.2, 128.4, 127.9, 126.8, 123.9, 115.1, 113.2, 86.2, 71.2, 71.1, 71.0, 69.7, 68.0, 63.4, 55.5, 27.3.

2-(2-(2-(2-N-n-Butylaminoethoxy)ethoxy)ethoxy)ethanol (12)

To a solution of 2-(2-(2-(2-Chloroethoxy)ethoxy)ethoxy)ethanol (2 g, 9.4 mmol) in n-Butylamine 10 mL at room temperature was added K₂CO₃ (1 g, 0.77 equal). The reaction mixture was heated to reflux for 24 h. Then another 10 mL of n-Butylamine was added into the mixture and the reaction was continued to reflux another 12 h. The reaction mixture was cooled to room temperature and the n-butylamine was removed in vacuum. The residue was diluted with CHCl₃ and the suspension was suction-filtered. The filtrate was collected and concentrated to give the title product as yellow syrup in quantitative yield, which could be used directly to the next step without further purification. ¹H NMR (CDCl₃) δ (ppm): 3.55-3.51 (m, 14H, tetraethylene glycol chain), 2.66 (bt, 2H, *J* = 5.1 Hz, tetraethylene glycol chain), 2.47 (t, 2H, *J* = 7.2 Hz, n-butyl chain), 1.43-1.31 (m, 2H, n-butyl chain), 1.30-1.16 (m, 2H, n-butyl chain), 0.80 (t, 3H, *J* = 7.2 Hz, n-butyl chain); ¹³C NMR (CDCl₃) δ (ppm): 72.9, 70.6, 70.4, 70.3, 70.2, 61.3, 49.5, 49.2, 32.1, 20.6, 14.1. MS (MALDI-TOF): 249.92 [M+H]⁺, 271.92 [M+Na]⁺, 287.86 [M+K]⁺.

4-N-n-Butyl-N-[2-[2-[2-(2-hydroxyethoxy)ethoxy]ethoxy]ethyl]amino benzaldehyde (13)

A mixture of 2-[2-[2-(2-N-n-Butylaminoethoxy)ethoxy]ethoxy]ethanol (**12**) (2.3 g, 9.4 mmol), 4-Fluorobenzaldehyde (1.0 mL, 9.4 mmol), and K₂CO₃ (1.5 g, 1.1 equal) in dry N,N-dimethylformamide (DMF) (10 mL) was heated with stirring to 120 °C (oil bath 128 °C) under argon. The stirring was continued at this temperature for 72 h, and the reaction mixture was cooled to room temperature. After the solvent (DMF) was evaporated in vacuum, the residue was diluted with CHCl₃. The obtained suspension was washed with brine (2 × 200 mL) and dried over Na₂SO₄. The organic layer was concentrated and the residue was purified by chromatography on a silica gel column eluted with EtOAc/MeOH (20:1) to give the title product (1.2 g, 36% in two steps based on **9**) as a yellow oil: *R_f* 0.4 (EtOAc/MeOH 20/1). ¹H NMR (CDCl₃) δ (ppm): 9.56 (s, 1H, -CHO), 7.57 (bd, 2H, *J* = 9.3 Hz, aromatic ring), 6.58 (bd, 2H, *J* = 8.7 Hz, aromatic ring), 3.65-3.40 (m, 16H, tetraethylene glycol chain), 3.29 (t, 2H, *J* = 7.8 Hz, n-butyl chain), 1.59-1.40 (m, 2H, n-butyl chain), 1.30-1.18 (m, 2H, n-butyl chain), 0.85 (t, 3H, *J* = 7.5 Hz, n-butyl chain); ¹³C NMR (CDCl₃) δ (ppm): 189.7, 152.4, 131.9, 124.5,

110.6, 72.4, 70.5, 70.4, 70.3, 70.1, 68.1, 61.4, 51.1, 50.3, 28.9, 20.0, 13.9. MS (MALDI-TOF): 353.23 [M]⁺.

4-((2-(2-(2-(2-(Bis-(4-methoxy-phenyl)-phenyl-methoxy)-ethoxy)-ethoxy)-ethoxy)-ethyl)-butyl-amino)-benzaldehyde (14)

A mixture of **13** (475 mg, 1.35 mmol), DMTrCl (912 mg, 2.7 mmol, 2 equiv), and a catalytic amount of 4-(Dimethylamino)pyridine (DMAP) in dry Pyridine (10 mL) was stirred at room temperature under argon. After the stirring was continued overnight, the reaction solvent was removed in vacuum and the residue was directly subject to a silica gel column eluted with Cyclohexane/EtOAc (gradient elution with 1% Pyridine: 500/150 → 300/150 → 150/150, v/v) to give the title product (800 mg, yield 90%): *R_f* 0.19 (Cyclohexane/EtOAc 1/1, v/v). ¹H NMR (CDCl₃) δ (ppm): 9.69 (s, 1H, -CHO), 7.68 (bd, 2H, J = 9.0Hz, benzaldehyde), 7.46 (m, 2H, benzene ring of DMTr group), 7.32-7.15 (m, 3H, benzene ring of DMTr group), 7.34 (dt, 4H, J₁ = 9.0Hz, J₂ = 3.0Hz, methoxybenzene ring of DMTr group), 6.81 (dt, 4H, J₁ = 9.0Hz, J₂ = 3.0Hz, methoxybenzene ring of DMTr group), 6.66 (bd, 2H, J = 9.0Hz, benzaldehyde), 3.77 (s, 6H, -OCH₃), 3.68-3.53 (m, 14H, tetraethylene glycol chain), 3.37 (bt, 2H, J = 7.9Hz, -NCH₂CH₂CH₂CH₃), 3.22 (t, 2H, J = 5.2Hz, tetraethylene glycol chain), 1.62-1.52 (m, 2H, -NCH₂CH₂CH₂CH₃), 1.40-1.28 (m, 2H, -NCH₂CH₂CH₂CH₃), 0.95 (t, 3H, J = 7.2Hz, -NCH₂CH₂CH₂CH₃).

Compounds in preparation of asymmetric pyridinium bistyrylbenzene: 4-(2-(2-(2-(2-(tert-Butyl-dimethyl-silyloxy)-ethoxy)-ethoxy)-ethoxy)-ethoxy)-benzaldehyde (20)

A mixture of **10** (1.4 g, 4.7 mmol), *tert*-Butyldimethylsilyl chloride (TBDMSCl) (0.85 g, 5.6 mmol, 1.2 equiv), and N,N-Diisopropylethylamine (DIPEA) (1.2 mL, 1.5 equiv) in dry CH₂Cl₂ (15 mL) was stirred at room temperature under argon. After 5 h, half of the starting material had gone. Another batch of DIPEA and TBDMSCl as well as a catalytic amount of DMAP was added. The reaction mixture was stirred overnight. The solvent (CH₂Cl₂) was evaporated in vacuum, and the residue was purified by chromatography on a silica gel column eluted with Cyclohexane/EtOAc (1/1) to give the title product (1.5 g, yield 77%): *R_f* 0.44 (Cyclohexane/EtOAc, 1/1). ¹H NMR (CDCl₃) δ (ppm): 9.88 (s, 1H, -CHO), 7.83 (dt, 2H, J₁ = 9.0Hz, J₂ = 2.1Hz, benzaldehyde), 7.02 (dt, 2H, J₁ = 9.0Hz, J₂ = 2.1Hz, benzaldehyde), 4.22 (t, 2H, J = 5.1Hz, tetraethylene glycol chain), 3.89 (t, 2H, J = 5.1Hz, tetraethylene glycol chain), 3.75 (bt, 2H, J = 5.1Hz, tetraethylene glycol chain), 3.71-3.59 (m, 8H, tetraethylene glycol chain), 3.49 (bt, 2H, J = 5.1Hz, tetraethylene glycol chain), 0.83 (s, 9H, -Si(CH₃)₂C(CH₃)₃), 0.06 (s, 6H, -Si(CH₃)₂C(CH₃)₃); ¹³C NMR (CDCl₃) δ (ppm): 190.9, 164.0, 132.1, 130.2, 115.1, 72.9, 71.2, 71.0, 70.99, 70.94, 69.7, 68.0, 63.0, 26.3, 18.7, -4.9.

4-(2-(4-(2-(2-(2-(2-(tert-Butyl-dimethyl-silyloxy)-ethoxy)-ethoxy)-ethoxy)-ethoxy)-phenyl)-vinyl)-benzonitrile (22)

A solution of Diisopropylamine (3.2 mL, 22.8 mmol, 4.3 equiv) in THF (40 mL) at room temperature under argon was cooled to -78 °C, and n-BuLi (12.5 mL, 1.6 M in Hexanes, 3.8 equiv) was added via syringe. The mixture was warmed to 0 °C for 30 min, and re-cooled to -78 °C. A solution of 4-Cyanobenzylphosphonic acid diethyl ester **21** (2.3 g, 9 mmol) in THF (15 mL) was pre-cooled, and transferred via cannula into the LDA solution at -78 °C, then the reaction mixture was warmed up to 0 °C. Another solution of **20** (2.2 g, 5.3 mmol) in THF (15 mL) was added into the reaction via cannula at 0 °C, and the reaction was warmed up to room temperature. After 24 h of stirring at room temperature, the reaction mixture was diluted with CH₂Cl₂ and the organic phase was washed with aqueous NaHCO₃ twice, then brine. The solvent (CH₂Cl₂) was evaporated in vacuum, and the residue was purified by chromatography on a silica gel column eluted with CH₂Cl₂/EtOAc (5/1, v/v) to give the title product (2.1 g, yield 84%): *R_f* 0.66 (DCM/EtOAc, 5/1). ¹H NMR (CDCl₃) δ (ppm): 7.61 (dt, 2H, J₁ = 8.7Hz,

$J_2 = 1.8\text{Hz}$, styryl benzene), 7.55 (dt, 2H, $J_1 = 8.7\text{Hz}$, $J_2 = 1.8\text{Hz}$, styryl benzene), 7.45 (dt, 2H, $J_1 = 8.7\text{Hz}$, $J_2 = 2.1\text{Hz}$, ethoxybenzene), 7.14 (d, 2H, $J = 16.2\text{Hz}$, double bond trans linkage), 6.94 (d, 2H, $J = 16.2\text{Hz}$, double bond trans linkage), 6.93 (dt, 2H, $J_1 = 8.7\text{Hz}$, $J_2 = 2.1\text{Hz}$, ethoxybenzene), 4.16 (t, 2H, $J = 4.8\text{Hz}$, tetraethylene glycol chain), 3.88 (t, 2H, $J = 4.8\text{Hz}$, tetraethylene glycol chain), 3.76 (bt, 2H, $J = 4.8\text{Hz}$, tetraethylene glycol chain), 3.76-3.64 (m, 8H, tetraethylene glycol chain), 3.56 (bt, 2H, $J = 4.8\text{Hz}$, tetraethylene glycol chain), 0.89 (s, 9H, $-\text{Si}(\text{CH}_3)_2\text{C}(\text{CH}_3)_3$), 0.06 (s, 6H, $-\text{Si}(\text{CH}_3)_2\text{C}(\text{CH}_3)_3$); ^{13}C NMR (CDCl_3) δ (ppm): 159.5, 142.4, 132.7, 132.1, 129.4, 128.4, 126.8, 124.8, 119.4, 115.2, 110.3, 72.9, 71.2, 71.0, 70.99, 70.95, 70.0, 67.8, 63.0, 26.3, 18.7, -4.7.

4-(2-(4-(2-(2-(2-(2-(tert-Butyl-dimethyl-silanyloxy)-ethoxy)-ethoxy)-ethoxy)-ethoxy)-phenyl)-vinyl)-benzaldehyde (23)

A solution of **22** (710 mg, 1.47 mmol) in Et_2O (40 mL) was cooled to -78°C . DIBALH (2.9 mL, in Hexane 1 M, 2 equiv) was added to the reaction solution dropwise via syringe in two equal portions. The reaction was stirred for 7 h at -78°C , then for 30 min at 0°C . The reaction mixture was transferred via cannula into 10% AcOH aqueous solution (150 mL) with stirring. The obtained solution was stirred vigorously till organic layer was separated from the aqueous layer. The organic layer was washed twice with aqueous NaHCO_3 (sat). Then the solvents were evaporated in vacuum, and the residue was purified by chromatography on a silica gel column eluted with $\text{CH}_2\text{Cl}_2/\text{EtOAc}$ (5/1, v/v) to give the title product (428 mg, yield 57%): R_f 0.50 ($\text{CH}_2\text{Cl}_2/\text{EtOAc}$, 5/1). ^1H NMR (CDCl_3) δ (ppm): 9.98 (s, 1H, $-\text{CHO}$), 7.85 (bd, 2H, $J = 8.4\text{Hz}$, benzaldehyde), 7.62 (bd, 2H, $J = 8.4\text{Hz}$, benzaldehyde), 7.47 (dt, 2H, $J_1 = 8.7\text{Hz}$, $J_2 = 1.8\text{Hz}$, ethoxybenzene), 7.19 (d, 2H, $J = 16.2\text{Hz}$, double bond trans linkage), 7.02 (d, 2H, $J = 16.2\text{Hz}$, double bond trans linkage), 6.93 (dt, 2H, $J_1 = 8.7\text{Hz}$, $J_2 = 1.8\text{Hz}$, ethoxybenzene), 4.16 (t, 2H, $J = 4.8\text{Hz}$, tetraethylene glycol chain), 3.88 (t, 2H, $J = 4.8\text{Hz}$, tetraethylene glycol chain), 3.76 (bt, 2H, $J = 5.1\text{Hz}$, tetraethylene glycol chain), 3.76-3.66 (m, 8H, tetraethylene glycol chain), 3.56 (bt, 2H, $J = 5.1\text{Hz}$, tetraethylene glycol chain), 0.89 (s, 9H, $-\text{Si}(\text{CH}_3)_2\text{C}(\text{CH}_3)_3$), 0.06 (s, 6H, $-\text{Si}(\text{CH}_3)_2\text{C}(\text{CH}_3)_3$); ^{13}C NMR (CDCl_3) δ (ppm): 191.7, 159.3, 144.0, 135.1, 131.9, 130.4, 129.6, 128.3, 126.7, 125.3, 115.1, 72.9, 71.1, 71.0, 70.93, 70.88, 69.9, 67.7, 62.9, 26.2, 18.7, -4.9.

4-(2-(4-(2-(2-(2-(2-Hydroxy-ethoxy)-ethoxy)-ethoxy)-ethoxy)-phenyl)-vinyl)-benzaldehyde (24)

To a solution of **23** (100 mg, 0.19 mmol) in THF (10 mL) was added Tetrabutylammonium fluoride (TBAF) (0.2 mL, 1.1 equiv, 1.0 M in THF) slowly at room temperature. After 5 min, another 0.1 mL TBAF was added. Then several drops of 10% AcOH/THF was added to quench the reaction. The solvent was evaporated in vacuum, and the crude product was purified by flash chromatography on a silica gel column eluted with $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (20/1) to give the title product (78 mg, yield 100%). From ^1H NMR the isomers (trans/cis double bond linkages) were observed. The isomerization was conducted: the isomers were dissolved in DMF (12 mL), and a catalytic amount of I_2 was added. The system was heated to 140°C for 36 h to give the trans product (ee $\geq 95\%$). R_f 0.26 ($\text{CH}_2\text{Cl}_2/\text{MeOH}$, 20/1). ^1H NMR (CDCl_3) δ (ppm): 9.93 (s, 1H, $-\text{CHO}$), 7.80 (bd, 2H, $J = 8.4\text{Hz}$, benzaldehyde), 7.57 (bd, 2H, $J = 8.4\text{Hz}$, benzaldehyde), 7.43 (bd, 2H, $J = 9.0\text{Hz}$, ethoxybenzene), 7.15 (d, 2H, $J = 16.5\text{Hz}$, double bond trans linkage), 6.96 (d, 2H, $J = 16.5\text{Hz}$, double bond trans linkage), 6.90 (bd, 2H, $J = 9.0\text{Hz}$, ethoxybenzene), 4.12 (t, 2H, $J = 4.8\text{Hz}$, tetraethylene glycol chain), 3.84 (t, 2H, $J = 4.8\text{Hz}$, tetraethylene glycol chain), 3.74-3.62 (m, 10H, tetraethylene glycol chain), 3.60-3.55 (m, 2H, tetraethylene glycol chain).

4-(2-(4-(2-(2-(2-(2-(Bis-(4-methoxy-phenyl)-phenyl-methoxy)-ethoxy)-ethoxy)-ethoxy)-ethoxy)-phenyl)-vinyl)-benzaldehyde (25)

A mixture of **24** (180 mg, 0.45 mmol), DMTrCl (0.5 g, 1.5 mmol, 3 equiv), and a catalytic amount of 4-(Dimethylamino)pyridine (DMAP) in dry pyridine (5 mL) was stirred at room temperature under argon. After the stirring was continued overnight, the reaction solvent was removed in vacuum and the residue was directly subject to a silica gel column eluted with CH₂Cl₂/EtOAc/pyridine (100/10/1, v/v) to give the title product (210 mg, yield 66%): *R_f* 0.36 (CH₂Cl₂/EtOAc 10/1, v/v). ¹H NMR (CDCl₃) δ (ppm): 9.98 (s, 1H, -CHO), 7.85 (bd, 2H, J = 8.4Hz, benzaldehyde), 7.62 (bd, 2H, J = 8.4Hz, benzaldehyde), 7.46 (m, 2H, benzene ring of DMTr group), 7.45 (bd, 2H, J = 8.7Hz, ethoxybenzene), 7.34 (dt, 4H, J₁ = 9.0Hz, J₂ = 2.4Hz, methoxybenzene ring of DMTr group), 7.32-7.15 (m, 4H, benzene ring of DMTr group and double bond trans linkage), 7.00 (d, 1H, J = 16.2Hz, double bond trans linkage), 6.91 (dt, 2H, J₁ = 8.7Hz, J₂ = 2.1Hz, ethoxybenzene), 6.81 (dt, 4H, J₁ = 9.0Hz, J₂ = 2.4Hz, methoxybenzene ring of DMTr group), 4.12 (bt, 2H, J = 4.8Hz, tetraethylene glycol chain), 3.86 (bt, 2H, J = 4.8Hz, tetraethylene glycol chain), 3.77 (s, 6H, -OCH₃), 3.74-3.65 (m, 10H, tetraethylene glycol chain), 3.22 (t, 2H, J = 5.1Hz, tetraethylene glycol chain).

4-(2-(4-(2-(2-(2-(2-(Bis-(4-methoxy-phenyl)-phenyl-methoxy)-ethoxy)-ethoxy)-ethoxy)-ethoxy)-phenyl)-vinyl)-phenyl)-vinyl)-1-(2-(2-(2-(2-hydroxy-ethoxy)-ethoxy)-ethoxy)-ethyl)-pyridinium chloride (26)

A mixture of **25** (200 mg, 0.28 mmol), 1-(2-(2-(2-(2-Hydroxy-ethoxy)-ethoxy)-ethoxy)-ethyl)-4-methyl-pyridinium chloride (90 mg, 0.29 mmol, 1 equiv), and a catalytic amount of Piperidine in absolute EtOH (30 mL) was heated to 80 °C for 12 h. After the system was cooled to room temperature, the reaction solvent was removed in vacuum and the residue was directly subject to a silica gel column eluted with CH₂Cl₂/MeOH/Pyridine/H₂O (400/60/5/5, v/v) to give the title product (85 mg, yield 30%): *R_f* 0.32 (CH₂Cl₂/MeOH/Pyridine/H₂O, 400/60/5/5, v/v). ¹H NMR (CDCl₃) δ (ppm): 9.27 (d, 2H, J = 6.6Hz, pyridinium), 7.91 (d, 2H, J = 6.6Hz, pyridinium), 7.64 (d, 1H, J = 16.8Hz, double bond trans linkage), 7.60 (d, 2H, J = 8.4Hz, styryl benzene), 7.53 (d, 2H, J = 8.4Hz, styryl benzene), 7.47-7.42 (m, 2H, benzene ring of DMTr group), 7.45 (bd, 2H, J = 8.7Hz, ethoxybenzene), 7.34 (dt, 4H, J₁ = 9.0Hz, J₂ = 2.1Hz, methoxybenzene ring of DMTr group), 7.28-7.15 (m, 3H, benzene ring of DMTr group), 7.16 (d, 1H, J = 16.5Hz, double bond trans linkage), 7.12 (d, 1H, J = 16.8Hz, double bond trans linkage), 6.97 (d, 1H, J = 16.5Hz, double bond trans linkage), 6.89 (bd, 2H, J = 8.7Hz, ethoxybenzene), 6.81 (dt, 4H, J₁ = 9.0Hz, J₂ = 2.1Hz, methoxybenzene ring of DMTr group), 5.07 (bt, 2H, tetraethylene glycol chain), 4.12 (bt, 4H, J = 4.8Hz, tetraethylene glycol chain), 3.85 (bt, 2H, J = 4.8Hz, tetraethylene glycol chain), 3.77 (s, 6H, -OCH₃), 3.74-3.55 (m, 22H, tetraethylene glycol chain), 3.22 (t, 2H, J = 5.1Hz, tetraethylene glycol chain).

Detritylation of 26 (34)

To a solution of **26** (20 mg, 0.02 mmol) in CHCl₃ (5 mL) was added Cl₂HCOOH (1 drops with a 9"-Pasteur pipet). After the completion of the reaction, the solvent was removed in vacuum and the residue was directly subject to a silica gel column eluted with CH₂Cl₂/MeOH/Pyridine/H₂O (400/60/5/5, v/v) to give the detritylated product **34** in quantitative yield: *R_f* 0.23 (CH₂Cl₂/MeOH/Pyridine/H₂O, 400/60/5/5, v/v). ¹H NMR (CDCl₃) δ (ppm): 9.11 (bs, 2H, pyridinium), 7.92 (bs, 2H, pyridinium), 7.60 (d, 1H, J = 16.5Hz, double bond trans linkage), 7.55 (d, 2H, J = 8.4Hz, styryl benzene), 7.47 (d, 2H, J = 8.4Hz, styryl benzene), 7.42 (d, 2H, J = 8.7Hz, ethoxybenzene), 7.10 (d, 1H, J = 16.2Hz, double bond trans linkage), 7.06 (d, 1H, J = 16.5Hz, double bond trans linkage), 6.91 (d, 1H, J = 16.2Hz, double bond trans linkage), 6.89 (d, 2H, J = 8.7Hz, ethoxybenzene), 4.91 (bs, 2H, tetraethylene glycol chain), 4.15 (bt, 2H, J = 4.8Hz, tetraethylene glycol chain), 4.02 (bs, 2H, tetraethylene glycol chain), 3.86 (bt, 2H, J = 4.8Hz, tetraethylene glycol chain), 3.78-3.55 (m, 24H, tetraethylene glycol chain).

1-(2-(2-(2-(2-Hydroxy-ethoxy)-ethoxy)-ethoxy)-ethyl)-4-methyl-pyridinium tetraphenylborate (27)

A mixture of Picoline (1 g, 11 mmol) and 2-(2-(2-(2-Chloro-ethoxy)-ethoxy)-ethoxy)-ethanol (1.5 g, 7.1 mmol) was heated to 140 °C for 4–6 h under Argon. After the system was cooled to room temperature, the excess picoline was removed in vacuum. The obtained brown oil was dissolved in Acetonitrile and Tetraphenylboron sodium (2.5 g, 7.3 mmol) was added into the solution. The mixture was refluxed for 20 min and concentrated to a residue which was directly subject to a silica gel column eluted with CH₂Cl₂/MeOH (10/1, v/v) to give the title product (4.2 g) in a quantitative yield. *R_f* 0.42 (CH₂Cl₂/MeOH, 10/1, v/v). ¹H NMR (CDCl₃) δ (ppm): 7.52-7.44 (m, 8H, benzene ring of BPh₄), 6.96 (t, 8H, J = 7.2Hz, benzene ring of BPh₄), 6.80 (bt, 4H, J = 7.2Hz, benzene ring of BPh₄), 6.63 (d, 2H, J = 7.5Hz, pyridinium), 6.60 (d, 2H, J = 7.5Hz, pyridinium), 3.67-3.34 (m, 12H, tetraethylene glycol chain), 3.22-3.16 (m, 4H, tetraethylene glycol chain), 2.49 (t, 1H, J = 6.0Hz, -OH), 2.21 (s, 3H, -CH₃); ¹³C NMR (CDCl₃) δ (ppm): 165.2, 164.5, 163.9, 163.2, 158.2, 143.2, 136.2, 128.0, 126.2, 126.1, 122.3, 72.6, 70.7, 70.5, 70.3, 70.27, 68.8, 61.8, 59.9, 22.2.

1-(2-(2-(2-(2-(Bis-(4-methoxy-phenyl)-phenyl-methoxy)-ethoxy)-ethoxy)-ethyl)-4-methyl-pyridinium tetraphenylborate (28)

A mixture of **27** (510 mg, 0.87 mmol), DMTrCl (600 mg, 1.77 mmol, 2 equiv), and a catalytic amount of 4-(Dimethylamino)pyridine (DMAP) in dry Pyridine (10 mL) was stirred at room temperature under argon. After 12 h, the reaction solvent was removed in vacuum and the residue was directly subject to a silica gel column eluted with CH₂Cl₂/MeOH/Pyridine (750/25/7, v/v) to give the title product (450 mg, yield 58%): *R_f* 0.33 (CH₂Cl₂/MeOH/Pyridine 750/25/7, v/v). ¹H NMR (CDCl₃) δ (ppm): 7.52-7.44 (m, 10H, benzene ring of BPh₄ and DMTr groups), 7.32 (bd, 4H, J = 9Hz, methoxybenzene ring), 7.34-7.15 (m, 3H, benzene ring of DMTr group) 6.96 (t, 8H, J = 7.2Hz, benzene ring of BPh₄), 6.80 (bt, 4H, J = 7.2Hz, benzene ring of BPh₄), 6.79 (bd, 4H, J = 9Hz, methoxybenzene ring), 6.67 (bd, 2H, J = 6.9Hz, pyridinium), 6.63 (bd, 2H, J = 6.9Hz, pyridinium), 3.75 (s, 3H, -OCH₃), 3.67-3.60 (m, 4H, tetraethylene glycol chain), 3.58-3.52 (m, 2H, tetraethylene glycol chain), 3.52-3.44 (m, 2H, tetraethylene glycol chain), 3.44-3.36 (m, 2H, tetraethylene glycol chain), 3.24-3.14 (m, 6H, tetraethylene glycol chain), 2.17 (s, 3H, -CH₃); ¹³C NMR (CDCl₃) δ (ppm): 165.2, 164.6, 163.9, 163.3, 158.6, 158.1, 145.2, 143.2, 136.3, 136.2, 130.3, 128.4, 128.00, 127.97, 127.3, 127.0, 126.2, 126.1, 122.3, 113.3, 86.2, 71.0, 70.9, 70.88, 70.7, 70.5, 68.8, 63.4, 59.9, 55.6, 22.1.

1-(2-(2-(2-(2-(Bis-(4-methoxy-phenyl)-phenyl-methoxy)-ethoxy)-ethoxy)-ethyl)-4-(2-(4-(2-4-(2-(2-(2-hydroxy-ethoxy)-ethoxy)-ethoxy)-ethoxy)-phenyl)-vinyl)-phenyl)-vinyl)-pyridinium tetraphenylborate (29)

A mixture of **28** (230 mg, 0.26 mmol), **24** (105 mg, 0.26 mmol, 1 equiv), and a catalytic amount (6 drops, 9''-Pasteur pipet) of Piperidine in absolute EtOH (30 mL) was heated to 100 °C for 12 h. After the reaction was cooled to room temperature, the reaction solvent was removed in vacuum and the residue was directly subject to a silica gel column eluted with CH₂Cl₂/MeOH/Pyridine/H₂O (800/60/4/3, v/v) to give the title product (73 mg, yield 22%): *R_f* 0.37 (CH₂Cl₂/MeOH/Pyridine/H₂O, 400/60/5/5, v/v). ¹H NMR (CDCl₃) δ (ppm): 7.56-7.43 (m, 16H, benzene ring of BPh₄ (8H), benzene ring of DMTr group (2H), styryl benzene (6H)), 7.36-7.18 (m, 4H, benzene ring of DMTr group (3H) and double bond trans linkage (1H)), 7.32 (bd, 4H, J = 9Hz, methoxybenzene ring), 7.17 (d, 1H, J = 15.9Hz, double bond trans linkage), 7.06 (d, 2H, J = 8.7Hz, pyridinium), 7.02 (t, 8H, J = 7.5Hz, benzene ring of BPh₄), 6.98 (d, 1H, J = 15.9Hz, double bond trans linkage), 6.94 (d, 2H, J = 8.7Hz, pyridinium), 6.85 (bt, 4H, J = 7.5Hz, benzene ring of BPh₄), 6.78 (bd, 4H, J = 9Hz, methoxybenzene ring), 6.74 (d, 1H, J = 16.2Hz, double bond trans linkage), 4.18 (bt, 2H, J = 4.8Hz, tetraethylene glycol chain),

3.88 (t, 2H, J = 4.8Hz, tetraethylene glycol chain), 3.73 (s, 6H, -OCH₃), 3.78-3.58 (m, 18H, tetraethylene glycol chain), 3.58-3.52 (m, 2H, tetraethylene glycol chain), 3.52-3.47 (m, 2H, tetraethylene glycol chain), 3.46-3.36 (m, 4H, tetraethylene glycol chain), 3.23 (bt, 2H, J = 4.8Hz, tetraethylene glycol chain); ¹³C NMR (CDCl₃) δ (ppm): 158.2, 152.5, 149.5, 144.8, 143.7, 141.1, 135.9, 134.5, 132.8, 130.3, 129.9, 129.5, 128.6, 128.0, 127.9, 127.6, 126.9, 126.7, 125.8, 125.7, 125.2, 123.6, 122.8, 121.9, 114.8, 112.9, 85.8, 72.4, 70.7, 70.6, 70.5, 70.3, 70.2, 69.6, 68.8, 67.4, 63.0, 61.7, 55.1.

2-(2-(2-(2-(4-(E-2-(4-(E-2-Pyridin-4-yl-vinyl)-phenyl)vinyl)-phenoxy)-ethoxy)-ethoxy)-ethoxy)-ethanol (30)

A mixture of **24** (180 mg, 0.45 mmol), 4-Picoline (1 mL, 10.3 mmol, 23 equiv), Acetic anhydride (1.3 mL), and Acetic acid (0.6 mL) was heated to 100 °C for 12 h. A complex was then obtained after chromatography. The improved procedure is as follows: a mixture of **24** (60 mg, 0.15 mmol), 4-Picoline (1.8 mL, 18 mmol), and 37% HCl (0.72 mL, 8.8 mmol) gave a yellow solution. The reaction mixture was heated to 100 °C for 48 h and cooled to room temperature. The reaction was diluted with CHCl₃, and washed with aqueous NaHCO₃, extracted with CHCl₃. The organic layer was collected, dried over Na₂SO₄, concentrated, and the residue was subject to a silica gel column eluted with CH₂Cl₂/MeOH (20/1, v/v) to give the title product (18 mg, yield 25%): *R*_f 0.28 (CH₂Cl₂/MeOH, 20/1, v/v). ¹H NMR (CDCl₃) δ (ppm): 8.57 (d, 2H, J = 6.0Hz, pyridinium), 7.51 (bs, 4H, styryl benzene), 7.44 (dt, 2H, J₁ = 8.7Hz, J₂ = 1.8Hz, ethoxybenzene), 7.37 (bd, 2H, J = 6.0Hz, pyridinium), 7.28 (d, 1H, J = 16.2Hz, double bond trans linkage), 7.10 (d, 1H, J = 16.5Hz, double bond trans linkage), 7.02 (d, 1H, J = 16.5Hz, double bond trans linkage), 6.98 (d, 1H, J = 16.2Hz, double bond trans linkage), 6.92 (dt, 2H, J₁ = 8.7Hz, J₂ = 1.8Hz, ethoxybenzene), 4.16 (bt, 2H, J = 4.8Hz, tetraethylene glycol chain), 3.87 (t, 2H, J = 4.8Hz, tetraethylene glycol chain), 3.77-3.64 (m, 10H, tetraethylene glycol chain), 3.61 (bt, 2H, J = 5.1Hz, tetraethylene glycol chain); ¹³C NMR (CDCl₃) δ (ppm): 158.8, 150.2, 145.0, 138.4, 135.1, 133.1, 130.3, 129.1, 128.0, 127.6, 126.9, 126.1, 125.6, 121.0, 115.1, 72.8, 71.1, 71.0, 70.9, 70.6, 70.0, 67.7, 62.1.

2-(2-(2-(2-(Bis-(4-methoxy-phenyl)-phenyl-methoxy)-ethoxy)-ethoxy)-ethoxy)-ethylene chloride (32)

A mixture of 2-(2-(2-(2-Chloro-ethoxy)-ethoxy)-ethoxy)-ethanol (1.5 g, 4.7mmol), DMTrCl (3.18 g, 9.4 mmol, 2 equiv), and a catalytic amount of 4-(Dimethylamino)pyridine (DMAP) in dry Pyridine (40 mL) was stirred at room temperature under argon. After 12 h, the reaction solvent was removed in vacuum. The reaction mixture was diluted with CH₂Cl₂ and washed with aqueous NaHCO₃ and brine. The organic layer was collected, dried over Na₂SO₄, concentrated, and the residue was subject to a silica gel column eluted with Cyclohexane/EtOAc (3/1 w/1% Pyridine, v/v) to give the title product in a quantitative yield. *R*_f 0.35 (Cyclohexane/EtOAc 3/1, v/v). ¹H NMR (CDCl₃) δ (ppm): 7.44 (bd, 2H, J = 7.2Hz, benzene ring of DMTr group), 7.36 (dt, 4H, J₁ = 9.0Hz, J₂ = 2.1Hz, methoxybenzene ring of DMTr group), 7.28 (bt, 2H, J = 7.2Hz, benzene ring of DMTr group), 7.20 (tt, 1H, J₁ = 7.2Hz, J₂ = 2.1Hz, benzene ring of DMTr group), 6.83 (dt, 4H, J₁ = 9.0Hz, J₂ = 2.4Hz, methoxybenzene ring of DMTr group), 3.78 (s, 6H, -OCH₃), 3.74 (bt, 2H, J = 5.1Hz, tetraethylene glycol chain), 3.71-3.66 (m, 10H, tetraethylene glycol chain), 3.60 (bt, 2H, J = 5.7Hz, tetraethylene glycol chain), 3.24 (t, 2H, J = 5.1Hz, tetraethylene glycol chain); ¹³C NMR (CDCl₃) δ (ppm): 158.5, 145.3, 136.5, 130.3, 128.4, 128.0, 126.9, 113.3, 86.2, 71.6, 71.1, 71.0, 70.99, 63.4, 55.5, 43.1, 27.3.

2-(2-(2-(2-(Bis-(4-methoxy-phenyl)-phenyl-methoxy)-ethoxy)-ethoxy)-ethoxy)-ethylene iodide (33)

A mixture of **32** (506 mg, 0.98 mmol), KI (1.4 g, 8.4 mmol, 8.6 equiv), and NaI (1.2 g, 8 mmol, 8 equiv) in dry Acetone (25 mL) was stirred at room temperature. During the reaction, another two batches of NaI (3 g) were added. After 72 h, the reaction solvent was removed in vacuum. The reaction mixture was diluted with CH₂Cl₂ and washed with aqueous NaHCO₃ and brine. The organic layer was collected, dried over Na₂SO₄, concentrated, and the residue was subject to a silica gel column eluted with Cyclohexane/EtOAc (3/1 w/1% Pyridine, v/v) to give the title product in a 90% yield. *R_f* 0.38 (Cyclohexane/EtOAc, 3/1, v/v). ¹H NMR (CDCl₃) δ (ppm): 7.46 (bd, 2H, J = 7.2Hz, benzene ring of DMTr group), 7.35 (dt, 4H, J₁ = 9.0Hz, J₂ = 2.4Hz, methoxybenzene ring of DMTr group), 7.28 (bt, 2H, J = 7.2Hz, benzene ring of DMTr group), 7.20 (tt, 1H, J₁ = 7.2Hz, J₂ = 2.4Hz, benzene ring of DMTr group), 6.82 (dt, 4H, J₁ = 9.0Hz, J₂ = 2.4Hz, methoxybenzene ring of DMTr group), 3.78 (s, 6H, -OCH₃), 3.74 (bt, 2H, J = 6.9Hz, tetraethylene glycol chain), 3.71-3.66 (m, 10H, tetraethylene glycol chain), 3.23 (bt, 2H, J = 6.9Hz, tetraethylene glycol chain); ¹³C NMR (CDCl₃) δ (ppm): 158.5, 145.3, 136.5, 130.3, 128.4, 128.0, 126.9, 113.3, 86.2, 71.6, 71.1, 71.0, 70.99, 63.4, 55.5, 43.1, 27.3.

1-(2-(2-(2-(2-(Bis-(4-methoxy-phenyl)-phenyl-methoxy)-ethoxy)-ethoxy)-ethoxy)-ethyl)-4-(2-(4-(2-(4-(2-(2-(2-hydroxy-ethoxy)-ethoxy)-ethoxy)-ethoxy)-phenyl)-vinyl)-phenyl)-vinyl)-pyridinium iodide (31)

A mixture of **30** (18 mg, 0.038 mmol) and **33** (60 mg, 0.1 mmol, 2.6 equiv) in anhydrous CH₂Cl₂ (10 mL) was heated to 60 °C for 24 h. After the system was cooled to room temperature, the reaction solvent was removed in vacuum and the residue was directly subject to a silica gel column eluted with CH₂Cl₂/MeOH/Pyridine/H₂O (400/60/5/5, v/v) to give the title product (20 mg, yield 49%): *R_f* 0.5 (CH₂Cl₂/MeOH/Pyridine/H₂O, 400/60/5/5, v/v). ¹H NMR (CDCl₃) δ (ppm): 9.07 (bs, 2H, pyridinium), 7.89 (bs, 2H, pyridinium), 7.52 (s, 4H, styryl benzene), 7.51 (d, 1H, J = 16.2Hz, double bond trans linkage), 7.46 (d, 2H, J = 9.0Hz, ethoxybenzene), 7.48-7.42 (m, 2H, benzene ring of DMTr group), 7.33 (dt, 4H, J₁ = 9.0Hz, J₂ = 2.1Hz, methoxybenzene ring of DMTr group), 7.28-7.15 (m, 3H, benzene ring of DMTr group), 7.16 (d, 1H, J = 15.3Hz, double bond trans linkage), 6.98 (d, 1H, J = 16.2Hz, double bond trans linkage), 6.97 (d, 1H, J = 15.3Hz, double bond trans linkage), 6.93 (bd, 2H, J = 9.0Hz, ethoxybenzene), 6.78 (dt, 4H, J₁ = 9.0Hz, J₂ = 2.1Hz, methoxybenzene ring of DMTr group), 4.17 (bt, 2H, J = 4.8Hz, tetraethylene glycol chain), 4.05 (bs, 2H, tetraethylene glycol chain), 3.88 (bt, 4H, J = 4.8Hz, tetraethylene glycol chain), 3.75 (s, 6H, -OCH₃), 3.76-3.55 (m, 22H, tetraethylene glycol chain), 3.24 (bt, 2H, J = 4.8Hz, tetraethylene glycol chain).

Compounds in preparation of *E,E*-1,4-Bis-4,4'-(2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethoxy)styryl benzene Derivatives: *E,E*-1,4-Bis-4,4'-(2-(2-(2-(2-tritylethoxy)ethoxy)ethoxy)ethoxy)styryl benzene (15 trans)

To a solution of 1,4-Phenylenebis(methylene)bis(chlorotriphenyl) phosphorane (500 mg, 0.715 mmol) in THF (5 mL) was added *n*-BuLi (0.89 mL, 1.4 mmol, 1.6 M in Hexanes) at 0 °C under argon. The mixture was stirred at room temperature for 0.5 h and cooled to -78 °C. A solution of **8** (770 mg, 1.55 mmol) in THF (4 mL) was pro-cooled and added dropwise to the cooled mixture via syringe. After 0.5 h the reaction mixture was warmed up to room temperature and stirred for another 1 h. The reaction solvents were removed in vacuum and the reaction mixture was diluted with CHCl₃. The organic phase was washed with brine, dried over Na₂SO₄, concentrated. Then the title product was precipitated with ethanol or methanol as a yellow solid. Isomerization: the solid was dissolved in DMF with a catalytic amount of I₂ and the mixture was refluxed overnight. After evaporation of the solvent, the residue was purified by chromatography on a silica gel column eluted with Cyclohexane/EtOAc (2/1, v/v) to give the double bond trans linked product in a yield of 17%. *R_f* 0.38 (Cyclohexane/EtOAc,

2/1). ¹H NMR (CDCl₃) δ (ppm): 7.51-7.46 (m, 16H, benzene ring of styryl benzene and trityl group), 7.44 (d, 4H, J = 8.7Hz, ethoxybenzene), 7.30 (bt, 12H, J = 6.9Hz, benzene ring of trityl group), 7.23 (bt, 6H, J = 7.2Hz, benzene ring of trityl group), 7.07 (d, 2H, J = 16.2Hz, double bond trans linkage), 6.97 (d, 2H, J = 16.2Hz, double bond trans linkage), 6.91 (d, 4H, J = 8.7Hz, ethoxybenzene), 4.16 (bt, 4H, J = 4.8Hz, tetraethylene glycol chain), 3.91 (t, 4H, J = 4.8Hz, tetraethylene glycol chain), 3.80-3.68 (m, 20H, tetraethylene glycol chain), 3.26 (bt, 2H, J = 5.1Hz, tetraethylene glycol chain). For the double bond cis linked product: **Z, Z-1,4-Bis-4,4'-(2-(2-(2-tritylethoxy)ethoxy)ethoxy)styryl benzene (15 cis)** ¹H NMR (CDCl₃) δ (ppm): 7.48-7.44 (m, 12H, benzene ring of trityl group), 7.26 (bt, 12H, J = 6.9Hz, benzene ring of trityl group), 7.19 (bt, 3H, J = 6.9Hz, benzene ring of trityl group), 7.15 (bd, 4H, J = 9.0Hz, ethoxybenzene), 7.12 (bs, 4H, styryl benzene), 6.74 (bd, 4H, J = 9.0Hz, ethoxybenzene), 6.48 (d, 2H, J = 12.3Hz, double bond cis linkage), 6.42 (d, 2H, J = 12.3Hz, double bond cis linkage), 4.07 (bt, 4H, J = 4.8Hz, tetraethylene glycol chain), 3.85 (bt, 4H, J = 4.8Hz, tetraethylene glycol chain), 3.76-3.64 (m, 20H, tetraethylene glycol chain), 3.23 (bt, 2H, J = 5.1Hz, tetraethylene glycol chain).

***E,E*-1,4-Bis-4,4'-(2-(2-(2-(2-(Bis-(4-methoxy-phenyl)-phenyl-ethoxy)ethoxy)ethoxy)ethoxy)ethoxy)styryl benzene (17)**

To a solution of bis-ylide precursor, 1,4-Phenylenebis(methylene)bis(chlorotriphenyl)phosphorane (550 mg, 0.79 mmol), in THF (5 mL) was added n-BuLi (1 mL, 1.6 mmol, 1.6 M in Hexanes) dropwise at 0 °C under argon. The mixture was stirred at room temperature for 0.5 h and cooled to -78 °C. A solution of **11** (1 g, 1.6 mmol) in THF (5 mL) was pre-cooled and added dropwise to the cooled mixture via syringe. After 0.5 h, the reaction mixture was warmed up to room temperature and stirred for another 4 h. The reaction solvents were removed in vacuum and the reaction mixture was diluted with CHCl₃. The organic phase was washed with brine, dried over Na₂SO₄, concentrated. Then the residue was dissolved with CHCl₃ and precipitated with ethanol. A yellow solid was collected as the title product with pure double bond trans linkages (150 mg, yield 15%). *R*_f 0.5 (Cyclohexane/EtOAc 1/2). Isomerization: the impure part (trans-cis double bond linkages) was deprotected (DMTr-group off) via Cl₂HCOOH in CH₂Cl₂. The obtained solid was dissolved in DMF with a catalytic amount of I₂ and the mixture was refluxed for 4 days. After evaporation of the solvent, the residue was purified by chromatography on a silica gel column eluted with CH₂Cl₂/MeOH (400/30, v/v) to give the double bond trans linked product (190 mg) in a yield of 35%. *R*_f 0.31 (CH₂Cl₂/MeOH, 20/1.5). For **17**: ¹H NMR (CDCl₃) δ (ppm): 7.49-7.43 (m, 8H, benzene ring of DMTr group and styryl benzene), 7.42 (d, 4H, J = 8.7Hz, ethoxybenzene), 7.34 (bd, 8H, J = 9.0Hz, methoxybenzene ring of DMTr group), 7.31-7.23 (m, 4H, benzene ring of DMTr group), 7.19 (bt, 2H, J = 7.2Hz, benzene ring of DMTr group), 7.05 (d, 2H, J = 16.2Hz, double bond trans linkage), 6.96 (d, 2H, J = 16.2Hz, double bond trans linkage), 6.89 (d, 4H, J = 8.7Hz, ethoxybenzene), 6.81 (bd, 4H, J = 9.0Hz, methoxybenzene ring of DMTr group), 4.12 (bt, 4H, J = 4.8Hz, tetraethylene glycol chain), 3.85 (bt, 4H, J = 4.8Hz, tetraethylene glycol chain), 3.77 (s, 12H, -OCH₃), 3.75-3.64 (m, 20H, tetraethylene glycol chain), 3.22 (t, 4H, J = 5.1Hz, tetraethylene glycol chain); ¹³C NMR (CDCl₃) δ (ppm): 158.7, 158.6, 145.3, 136.8, 136.5, 130.5, 130.3, 128.4, 128.1, 128.0, 127.9, 126.9, 126.8, 126.5, 115.1, 113.3, 86.2, 71.2, 71.1, 71.06, 70.0, 67.7, 63.5, 55.5.

***E,E*-1,4-Bis-4,4'-(2-(2-(2-(2-(Bis-(4-methoxy-phenyl)-phenyl-methoxy)-ethoxy)-ethoxy)-ethoxy)-ethyl)-butyl-amino)-styrylbenzene (18)**

To a solution of bis-ylide precursor, 1,4-Phenylenebis(methylene)bis(chlorotriphenyl)phosphorane (430 mg, 0.62 mmol), in THF (5 mL) was added n-BuLi (0.76 mL, 1.2 mmol, 1.6 M in Hexanes) dropwise at 0 °C under argon. The mixture was stirred at room temperature for 0.5 h and cooled to -78 °C. A solution of **14** (800 mg, 1.2 mmol) in THF (7 mL) was pre-cooled and added dropwise to the cooled mixture via syringe. After 0.5 h, the reaction mixture

was warmed up to room temperature and stirred for another 12 h. The reaction solvents were removed in vacuum and the reaction mixture was diluted with CHCl_3 . The organic phase was washed with brine, dried over Na_2SO_4 , concentrated, and the residue was purified by chromatography on a silica gel column eluted with Cyclohexane/EtOAc/Pyridine (300/200/5, v/v) to give the double bond trans linked product (220 mg, yield 26%). R_f 0.32 (Cyclohexane/EtOAc, 3/2). Isomerization: the impure part (*trans-cis* double bond linkages) was detritylated (DMTr-group off) via Cl_2HCOOH in CH_2Cl_2 within 3 h. The de-protection reaction then was diluted with CHCl_3 , washed with NaHCO_3 aqueous solution/brine, and concentrated. The obtained solid was dissolved in DMF with a catalytic amount of I_2 and the mixture was refluxed for 12 h. After evaporation of the solvent, the residue was purified by chromatography on a silica gel column eluted with $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (400/20, v/v) to give the double bond trans linked product **2** (27 mg) in a yield of 5.6%. R_f 0.31 ($\text{CH}_2\text{Cl}_2/\text{MeOH}$, 20/1). For **18**: ^1H NMR (CDCl_3) δ (ppm): 7.56-7.50 (m, 4H, benzene ring of DMTr group), 7.48-7.38 (m, 8H, styryl benzene and ethoxybenzene, 7.42 (bd, 8H, $J = 9.0\text{Hz}$, methoxybenzene ring of DMTr group), 7.36-7.20 (m, 6H, benzene ring of DMTr group), 7.06 (d, 2H, $J = 16.2\text{Hz}$, double bond trans linkage), 6.92 (d, 2H, $J = 16.2\text{Hz}$, double bond trans linkage), 6.86 (bd, 4H, $J = 9.0\text{Hz}$, methoxybenzene ring of DMTr group), 6.70 (d, 4H, $J = 8.7\text{Hz}$, methoxybenzene), 3.78 (s, 12H, $-\text{OCH}_3$), 3.76-3.64 (m, 24H, tetraethylene glycol chain), 3.61-3.54 (bt, 4H, $J = 5.4\text{Hz}$, tetraethylene glycol chain), 3.40-3.31 (m, 4H, $-\text{NCH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 3.29 (bt, 4H, $J = 5.1\text{Hz}$, tetraethylene glycol chain), 1.68-1.56 (m, 4H, $-\text{NCH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 1.46-1.31 (m, 4H, $-\text{NCH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 0.997 (t, 6H, $J = 7.2\text{Hz}$, $-\text{NCH}_2\text{CH}_2\text{CH}_2\text{CH}_3$); ^{13}C NMR (CDCl_3) δ (ppm): 158.6, 147.7, 145.4, 136.9, 136.6, 130.3, 128.5, 128.4, 128.0, 126.9, 126.5, 125.3, 124.0, 113.3, 112.0, 86.2, 71.1, 71.09, 68.9, 63.5, 55.5, 51.6, 50.9, 29.7, 21.0, 14.5.

HWE reagent tetraethyl 1,4-xylylenediphosphonate was obtained according to literatures (32,33).

***E,E*-1,4-Bis-4,4'-(2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethoxy)ethoxy)styryl benzene (5)**

A mixture of tetraethyl 1,4-xylylenediphosphonate (1.05 g, 2.78 mmol) and 3 equivalent of 4-[2-[2-[2-(2-hydroxyethoxy)ethoxy]ethoxy]ethoxy]-benzaldehyde (**10**) (2.45g, 8.22 mmol) was dried in vacuum overnight and dissolved in anhydrous DMF (25 mL) under argon. To the stirred solution of bisphosphonate and aldehyde at 0°C was added dropwise a solution of potassium tert-butoxide (KOtBu) (922 mg, 3 equal) in anhydrous DMF (15 mL) over 15 min by syringe. After stirring for 0.5 h at 0°C , the reaction mixture was slowly warmed up to room temperature and stirred for another 12 h, the reaction was quenched with water. The crude product was collected by suction filtration and the filtrate was evaporated to dryness. The residue was resolved with CHCl_3 and washed with water (2×100 mL). The organic layer was dried over Na_2SO_4 and concentrated to dryness. The crude product was then purified by being washed with tetrahydrofuran (THF) twice to give 1.69 g (91%) of the title compound (**5**). ^1H NMR (CDCl_3) δ (ppm): 7.47 (s, 4H, aromatic ring), 7.45 (d, 4H, $J = 8.7\text{Hz}$, aromatic ring), 7.06 (d, 2H, $J = 16.2\text{Hz}$, double bond trans-linkage), 6.97 (d, 2H, $J = 16.2\text{Hz}$, double bond trans-linkage), 6.92 (d, 4H, $J = 8.7\text{Hz}$, aromatic ring), 4.16 (bt, 4H, $J = 4.8\text{Hz}$, tetraethylene glycol chain), 3.87 (bt, 4H, $J = 4.8\text{Hz}$, tetraethylene glycol chain), 3.78-3.58 (m, 24H, tetraethylene glycol chain); ^{13}C NMR ($\text{CDCl}_3/\text{CD}_3\text{OD}$, 0.3mL/0.2mL) δ 15(ppm): 8.2, 136.5, 130.3, 127.6, 127.5, 126.4, 126.2, 114.6, 72.5, 70.55, 70.50, 70.38, 70.02, 69.63, 67.3, 61.2. MS (MALDI-TOF): m/z 666.8 $[\text{M}]^+$, 667.8 $[\text{M}+1]^+$, 689.7 $[\text{M}+\text{Na}]^+$.

Monotritylation of *E,E*-1,4-Bis-4,4'-(2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethoxy)ethoxy)styryl benzene (40)

E,E-1,4-Bis-4,4'-(2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethoxy)ethoxy)styryl benzene (**5**) (375 mg, 0.56 mmol) was dissolved in dry Pyridine (50 mL), followed by addition of DMTrCl (415 mg, 1.23 mmol) and DMAP (~10 mg). The mixture was stirred at room temperature under

argon overnight, and TLC monitoring (10/0.75, CH₂Cl₂/MeOH) showed the formation of monosubstituted (Rf 0.32) and disubstituted (Rf 0.74) products as well as the starting material (Rf 0.14). The reaction mixture was concentrated in vacuum and the residue was subject to a silica gel column with CH₂Cl₂/MeOH/Pyridine (100/5/0.5) as eluents affording the title product (160 mg, yield 30%) as a yellow powder. Meanwhile, disubstituted fraction was also collected and further monodetritylation was carried out as the following: A detritylation solution was made by mixing ZnCl₂ (1.5 g) into 110 mL of CH₂Cl₂/MeOH (10/1, v/v). Then the disubstituted compound (150 mg, 0.12 mmol) was dissolved by 50 mL of the detritylation solution and the reaction mixture was monitored by TLC (20/1, CH₂Cl₂/MeOH). Once the appearance of the spot corresponding to starting material (*E,E*-1,4-Bis-4,4'-(2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethoxy)styryl benzene) on the TLC plate, the reaction was quenched by pouring into a saturated aq. NaHCO₃ solution. The mixture was extracted by CHCl₃, and the organic layer was washed with brine, collected, dried over Na₂SO₄, concentrated, and subject to a silica gel column (100/5/0.5, CH₂Cl₂/MeOH/Pyridine) to give the title product **40** (48 mg, yield 42 %). Based on the starting material, the total yield of the monosubstituted product is 38 %. The product is stored with a stabilizer (Diisopropylethylamine) under argon at -80 °C. ¹H NMR (CDCl₃) δ (ppm): 7.46 (s, 4H, aromatic ring of styryl benzene), 7.49-7.38 (m, 6H, aromatic rings of styryl benzene and DMTr), 7.34 (dt, 4H, J₁ = 8.7Hz, J₂ = 2.3Hz, methoxybenzene ring), 7.31-7.15 (m, 3H, benzene ring of DMTr), 7.06 (d, 1H, J = 16.2Hz, double bond trans-linkage), 7.05 (d, 1H, J = 16.2Hz, double bond trans-linkage), 6.96 (d, 1H, J = 16.2Hz, double bond trans-linkage), 6.95 (d, 1H, J = 16.2Hz, double bond trans-linkage), 6.94-6.84 (m, 4 H, aromatic ring of styryl benzene), 6.81 (dt, 4H, J₁ = 8.7Hz, J₂ = 2.3Hz, methoxybenzene ring), 4.19-4.08 (m, 4H, tetraethylene glycol chain), 3.89-3.82 (m, 4H, tetraethylene glycol chain), 3.80-3.58 (m, 28H, CH₃O and tetraethylene glycol chain), 3.22 (t, 2H, J = 5.1Hz, DMTrOCH₂); ¹³C NMR (CDCl₃) δ (ppm): 158.57, 158.55, 158.43, 145.2, 136.74, 136.70, 136.4, 130.47, 130.40, 130.2, 128.3, 128.00, 127.96, 127.87, 127.79, 126.77, 126.67, 126.44, 126.38, 115.0, 113.2, 86.1, 72.7, 71.09, 71.04, 70.98, 70.95, 70.88, 70.81, 70.56, 69.95, 67.64, 63.4, 62.0, 55.4.

The Phosphoramidite of Monotritylated *E,E*-1,4-Bis-4,4'-(2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethoxy)styryl benzene (**43**)

To a solution of monotritylated *E,E*-1,4-Bis-4,4'-(2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethoxy)styryl benzene (**40**) (86 mg, 0.089 mmol) in 15 mL CH₂Cl₂ (dry) was added 5 equivalent of diisopropylethylamine (0.08 mL). Then Chloro-N,N-diisopropylaminocyclophosphane (0.04 mL, 0.18 mmol, ~2 equal) was added dropwise at room temperature under argon. After 20 min of stirring under argon at room temperature, the reaction mixture was diluted with 100 mL of CH₂Cl₂/Et₃N (300/15, v/v), and the organic phase was washed with a saturated aq. NaHCO₃ solution and brine. The organic layer was dried over Na₂SO₄, filtered, and evaporated to dryness. The residue was subject to a silica gel column (CH₂Cl₂/EtOAc/Et₃N, 3/6/1) to give the title product (88 mg, yield 85%) as a yellow powder, which should be used freshly for the next phosphotriester step in order to achieve a higher coupling yield.

E,E-1,4-Bis-4,4'-(2-(2-(2-(2-hydroxyethoxy)-ethoxy)-ethoxy)-ethyl)-butyl-amino-styrylbenzene (**2**)

To a solution of tetraethyl 1,4-xylylenediphosphonate (1.5 g, 3.9 mmol) and 4-N-n-Butyl-N-[2-[2-[2-(2-hydroxyethoxy)ethoxy]ethoxy]ethyl]amino benzaldehyde (**13**) (4 g, 11 mmol, 3 equiv) in anhydrous DMF (20 mL) was added KOBu (1.2 g, 11 mmol, 3 equiv) pre-dissolved in DMF (10 mL) dropwise via syringe at 0 °C under argon. After stirring for 0.5 h at 0 °C, the reaction mixture was slowly warmed up to room temperature and stirred for another 12 h, then the reaction was quenched with water. The crude product was collected by suction filtration and the filtrate was evaporated to dryness. The residue was re-dissolved with CHCl₃ and

washed with brine (2×100 mL). The organic layer was dried over Na_2SO_4 and concentrated to dryness. The crude product was then purified by chromatography on a silica gel column eluted with EtOAc/MeOH (20/1, v/v) to give the double bond trans linked product (1.54 g, yield 51%). R_f 0.33 (EtOAc/MeOH, 20/1). ^1H NMR (CDCl_3) δ (ppm): 7.42 (s, 4H, styryl benzene), 7.36 (bd, 4H, $J = 8.7\text{Hz}$, ethoxybenzene), 7.02 (d, 2H, $J = 16.2\text{Hz}$, double bond trans linkage), 6.88 (d, 2H, $J = 16.2\text{Hz}$, double bond trans linkage), 6.66 (d, 4H, $J = 8.7\text{Hz}$, ethoxybenzene), 3.76-3.51 (m, 28H, tetraethylene glycol chain), 3.34 (bt, 4H, $J = 7.5\text{Hz}$, $-\text{NCH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 1.65-1.52 (m, 4H, $-\text{NCH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 1.44-1.28 (m, 4H, $-\text{NCH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 0.96 (t, 6H, $J = 7.5\text{Hz}$, $-\text{NCH}_2\text{CH}_2\text{CH}_2\text{CH}_3$); ^{13}C NMR (CDCl_3) δ (ppm): 147.7, 136.8, 128.3, 127.9, 126.4, 125.3, 124.0, 111.9, 72.8, 71.0, 70.9, 70.88, 70.6, 68.8, 62.1, 51.5, 50.7, 29.6, 20.6, 14.4. MS (MALDI-TOF): 776.6 $[\text{M}]^+$.

***E*, 1-(4-((2-(2-(2-(2-(Bis-(4-methoxy-phenyl)-phenyl-methoxy)-ethoxy)-ethoxy)-ethoxy)-ethyl)-butyl-amino)-styryl)-*E*, 4-(4-((2-(2-(2-(2-(Bis-(4-methoxy-phenyl)-phenyl-methoxy)-ethoxy)-ethoxy)-ethoxy)-ethyl)-butyl-amino)-styryl) benzene (39)**

E,E-1,4-Bis-4,4'-((2-(2-(2-(2-hydroxyethoxy)-ethoxy)-ethoxy)-ethyl)-butyl-amino)-styrylbenzene (**2**) (1.116 g, 1.44 mmol) was dissolved in dry Pyridine (50 mL), followed by addition of DMTrCl (1.55 g, 4.3 mmol, 3 equiv) and DMAP (~10 mg). The mixture was stirred at room temperature under argon overnight, and TLC monitoring ($\text{CH}_2\text{Cl}_2/\text{MeOH}$, 400/15, v/v) showed the formation of monosubstituted (R_f 0.64) and disubstituted (R_f 0.88) products as well as the starting material (R_f 0.31). The reaction mixture was concentrated in vacuum and the residue was subject to a silica gel column with $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{pyridine}$ (400/15/2) as eluents to give the title product (390 mg, yield 25%) as a yellow powder. Meanwhile, disubstituted fraction was also collected and further monodetritylation was carried out as the following: a detritylation solution was made by mixing ZnCl_2 (1.5 g) with 110 mL of $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (10/1, v/v). Then the disubstituted compound (1150 mg, 0.83 mmol) was dissolved by 50 mL of the detritylation solution and the reaction mixture was monitored by TLC ($\text{CH}_2\text{Cl}_2/\text{MeOH}$, 400/15). Once the appearance of the spot corresponding to starting material **2** on the TLC plate, the reaction was quenched by pouring into a saturated aq. NaHCO_3 solution. The mixture was extracted by chloroform, and the organic layer was washed with brine, collected, dried over Na_2SO_4 , concentrated, and subject to a silica gel column ($\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{Pyridine}$, 400/15/2) to give the title product (470 mg, yield 53%). Based on the starting material, the total yield of the monosubstituted product is 55%. The product is stored with a stabilizer (Diisopropylethylamine) under argon at -80°C . ^1H NMR (CDCl_3) δ (ppm): 7.48-7.44 (m, 2H, benzene ring of DMTr group), 7.42 (bs, 4H, styryl benzene), 7.40-7.32 (m, 4H, butylaminobenzene), 7.34 (bd, 4H, $J = 9.0\text{Hz}$, methoxybenzene ring of DMTr group), 7.31-7.24 (m, 2H, benzene ring of DMTr group), 7.19 (bt, 1H, $J = 7.2\text{Hz}$, benzene ring of DMTr group), 7.02 (d, 1H, $J = 16.2\text{Hz}$, double bond trans linkage), 7.01 (d, 1H, $J = 15.9\text{Hz}$, double bond trans linkage), 6.88 (d, 2H, $J = 16.2\text{Hz}$, double bond trans linkage), 6.87 (d, 1H, $J = 15.9\text{Hz}$, double bond trans linkage), 6.81 (bd, 4H, $J = 9.0\text{Hz}$, methoxybenzene ring of DMTr group), 6.67 (d, 2H, $J = 8.7\text{Hz}$, butylaminobenzene), 6.64 (d, 2H, $J = 8.7\text{Hz}$, butylaminobenzene), 3.77 (s, 6H, $-\text{OCH}_3$), 3.76-3.50 (m, 30H, tetraethylene glycol chain), 3.38-3.26 (m, 4H, $-\text{NCH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 3.22 (bt, 2H, $J = 5.1\text{Hz}$, tetraethylene glycol chain), 1.64-1.52 (m, 4H, $-\text{NCH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 1.42-1.28 (m, 4H, $-\text{NCH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 0.96 (t, 3H, $J = 7.2\text{Hz}$, $-\text{NCH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 0.94 (t, 3H, $J = 7.2\text{Hz}$, $-\text{NCH}_2\text{CH}_2\text{CH}_2\text{CH}_3$); ^{13}C NMR (CDCl_3) δ (ppm): 158.5, 147.7, 145.3, 136.9, 136.9, 136.5, 130.3, 128.4, 128.2, 127.94, 127.91, 126.9, 126.4, 125.3, 125.3, 123.9, 123.9, 113.2, 111.92, 111.89, 86.2, 72.8, 71.1, 71.0, 70.98, 70.95, 70.88, 70.6, 68.8, 63.4, 62.1, 55.5, 51.5, 50.8, 29.6, 20.6, 14.4.

The Phosphoramidite of *E*, 1-(4-((2-(2-(2-(2-(Bis-(4-methoxy-phenyl)-phenyl-methoxy)-ethoxy)-ethoxy)-ethoxy)-ethyl)-butyl-amino)-styryl)-*E*, 4-(4-((2-(2-(2-(2-(Bis-(4-methoxy-phenyl)-phenyl-methoxy)-ethoxy)-ethoxy)-ethoxy)-ethyl)-butyl-amino)-styryl) benzene (41)

To a solution of *E*, 1-(4-((2-(2-(2-(2-(Bis-(4-methoxy-phenyl)-phenyl-methoxy)-ethoxy)-ethoxy)-ethoxy)-ethyl)-butyl-amino)-styryl)-*E*, 4-(4-((2-(2-(2-(2-(Bis-(4-methoxy-phenyl)-phenyl-methoxy)-ethoxy)-ethoxy)-ethoxy)-ethyl)-butyl-amino)-styryl) benzene (**39**) (770 mg, 0.71 mmol) in 80 mL CH₂Cl₂ (dry) was added 7 equivalent of diisopropylethylamine (0.8 mL). Then Chloro-N,N-diisopropylaminocycanoethoxyphosphane (0.47 mL, 2.1 mmol, ~3 equal) was added dropwise at room temperature under argon. After 20 min of stirring under argon at room temperature, the reaction mixture was diluted with 100 mL of CH₂Cl₂/Et₃N (300/15, v/v), and the organic phase was washed with a saturated aq. NaHCO₃ solution and brine. The organic layer was dried over Na₂SO₄, filtered, and evaporated to dryness. The residue was subject to a silica gel column (CH₂Cl₂/EtOAc/Et₃N, 3/6/1) to give the title product (870 mg, yield 96%) as a yellow powder, which should be used freshly for the next phosphotriester step in order to achieve a higher coupling yield. ¹H NMR (CDCl₃) δ (ppm): 7.48-7.44 (m, 2H, benzene ring of DMTr group), 7.42 (bs, 4H, styryl benzene), 7.40-7.32 (m, 4H, butylaminobenzene), 7.34 (bd, 4H, J = 9.0Hz, methoxybenzene ring of DMTr group), 7.31-7.15 (m, 3H, benzene ring of DMTr group), 7.01 (d, 1H, J = 16.2Hz, double bond trans linkage), 7.00 (d, 1H, J = 16.2Hz, double bond trans linkage), 6.88 (d, 2H, J = 16.2Hz, double bond trans linkage), 6.87 (d, 1H, J = 16.2Hz, double bond trans linkage), 6.81 (bd, 4H, J = 9.0Hz, methoxybenzene ring of DMTr group), 6.67 (d, 2H, J = 8.7Hz, butylaminobenzene), 6.64 (d, 2H, J = 8.7Hz, butylaminobenzene), 3.77 (s, 6H, -OCH₃), 3.90-3.75 (m, 2H, -OCH₂CH₂CN), 3.70-3.48 (m, 32H, -N(CH(CH₃)₂)₂ and tetraethylene glycol chain), 3.38-3.26 (m, 4H, -NCH₂CH₂CH₂CH₃), 3.22 (bt, 2H, J = 5.1Hz, tetraethylene glycol chain), 2.63 (bt, 2H, J = 6.5Hz, -OCH₂CH₂CN), 1.64-1.52 (m, 4H, -NCH₂CH₂CH₂CH₃), 1.42-1.28 (m, 4H, -NCH₂CH₂CH₂CH₃), 1.19 (d, 6H, J = 6.8Hz, -CH(CH₃)₂), 1.17 (d, 2H, J = 6.8Hz, -CH(CH₃)₂), 0.96 (t, 3H, J = 7.2Hz, -NCH₂CH₂CH₂CH₃), 0.94 (t, 3H, J = 7.2Hz, -NCH₂CH₂CH₂CH₃); ¹³C NMR (CDCl₃) δ (ppm): 158.6, 147.7, 145.3, 136.9, 136.6, 130.3, 128.4, 128.3, 128.3, 127.98, 127.95, 126.9, 126.4, 125.37, 125.32, 124.0, 123.95, 113.3, 111.9, 86.1, 71.5, 71.4, 71.0, 70.98, 70.94, 70.90, 70.8, 68.8, 63.4, 62.9, 62.7, 58.9, 58.6, 55.4, 51.4, 50.7, 43.4, 43.2, 29.6, 24.93, 24.86, 24.83, 24.77, 20.6, 20.5, 14.4, 14.3; ³¹P NMR (CDCl₃) δ (ppm): 149.6.

(4-(2-(4-(Butyl-(2-(2-(2-(2-hydroxy-ethoxy)-ethoxy)-ethoxy)-ethyl)-amino)-phenyl)-vinyl)-benzyl)-phosphonic acid diethyl ester (19)

To a solution of Tetraethyl 1,4-xylylenediphosphonate (466 mg, 1.2 mmol, 1.2 equiv) and 4-N-n-Butyl-N-[2-[2-[2-(2-hydroxyethoxy)ethoxy]ethoxy]ethyl]amino benzaldehyde (**13**) (360 mg, 1.1 mmol) in anhydrous DMF (15 mL) was added KOBu (123 mg, 1.1 mmol, 1 equiv) pre-dissolved in DMF (4 mL) dropwise via syringe at 0 °C under argon. After stirring for 0.5 h at 0 °C, the reaction mixture was slowly warmed up to room temperature and stirred for another 12 h. The crude product was then purified by chromatography on a silica gel column eluted with EtOAc/MeOH (20/1, v/v) to give the double bond trans linked product **2** (130 mg, yield 31%) and the title product (*trans/cis* 3/1) in a yield of 45% (281 mg). *R_f* 0.25 (EtOAc/MeOH, 20/1). ¹H NMR (CDCl₃) δ (ppm): double bond trans/cis linkage (3/1): 7.40 (d, 2H (3/4), J = 8.1Hz, aromatic ring), 7.35 (d, 2H (3/4), J = 8.7Hz, aromatic ring), 7.28 (d, 2H (1/4), J = 8.4Hz, aromatic ring), 7.24 (dd, 2H (3/4), J₁ = 8.1Hz, J₂ = 2.4Hz, aromatic ring), 7.16 (dd, 2H (1/4), J = 8.4Hz, aromatic ring), 7.10 (d, 2H (1/4), J = 8.7Hz, aromatic ring), 7.00 (d, 1H (3/4), J = 16.5Hz, double bond trans linkage), 6.84 (d, 1H (3/4), J = 16.5Hz, double bond trans linkage), 6.65 (d, 2H (3/4), J = 8.7Hz, aromatic ring), 6.48 (d, 2H (1/4), J = 8.7Hz, aromatic ring), 6.42 (d, 1H (1/4), J = 12.3Hz, double bond cis linkage), 6.33 (bd, 1H (1/4), J = 12.3Hz, double bond cis linkage), 4.06-3.95 (m, 4H, -OCH₂CH₃), 3.74-3.46 (m, 16H, tetraethylene glycol chain), 3.35 (bt, 2H (3/4), J = 7.5Hz, -NCH₂CH₂CH₂CH₃), 3.27 (bt, 2H (1/4), J = 7.5Hz,

-NCH₂CH₂CH₂CH₃), 3.17 (bs, 1H (3/4), benzyl), 3.16 (bs, 1H (1/4), benzyl), 3.10 (bs, 1H (3/4), benzyl), 3.08 (bs, 1H (1/4), benzyl), 1.63-1.50 (m, 2H, -NCH₂CH₂CH₂CH₃), 1.40-1.28 (m, 2H, -NCH₂CH₂CH₂CH₃), 1.24 (t, 6H, J = 7.1 Hz, -OCH₂CH₃), 0.95 (t, 3H (3/4), J = 7.2 Hz, -NCH₂CH₂CH₂CH₃), 0.92 (t, 3H (1/4), J = 7.2 Hz, -NCH₂CH₂CH₂CH₃). MS (MALDI-TOF): 576.6 [M]⁺.

***E*, 1-(4-((2-(2-(2-(2-hydroxyethoxy)-ethoxy)-ethoxy)-ethyl)-butyl-amino)-styryl)-*E*, 4-(4-(2-(2-(2-hydroxyethoxy)-ethoxy)-ethoxy)-ethoxy)styryl) benzene (3)**

To a solution of **19** (87 mg, 0.15 mmol) and **10** (90 mg, 0.3 mmol, 2 equiv) in anhydrous DMF (8 mL) was added KOBut (35 mg, 0.31 mmol, 2 equiv) pre-dissolved in DMF (4 mL) dropwise via syringe at 0 °C under argon. After stirring for 0.5 h at 0 °C, the reaction mixture was slowly warmed up to room temperature and stirred for another 12 h. Water was added to quench the reaction. After evaporation of the solvent, the crude product was purified by chromatography on a silica gel column eluted with EtOAc/MeOH (10/1, v/v) to give the title product (60 mg) in a yield of 55%. *R_f* 0.39 (EtOAc/MeOH, 10/1). ¹H NMR (CDCl₃) δ (ppm): 7.45 (bs, 4H, styryl benzene), 7.44 (bd, 2H, J = 9.0 Hz, ethoxybenzene), 7.38 (d, 2H, J = 8.7 Hz, butylaminobenzene), 7.04 (d, 1H, J = 16.2 Hz, double bond trans linkage), 7.03 (d, 1H, J = 16.2 Hz, double bond trans linkage), 6.97 (d, 1H, J = 16.2 Hz, double bond trans linkage), 6.92 (bd, 2H, J = 9.0 Hz, ethoxybenzene), 6.88 (d, 1H, J = 16.2 Hz, double bond trans linkage), 6.66 (d, 2H, J = 8.7 Hz, butylaminobenzene), 4.16 (bt, 2H, J = 4.8 Hz, tetraethylene glycol chain), 3.87 (bt, 2H, J = 4.8 Hz, tetraethylene glycol chain), 3.76-3.51 (m, 28H, tetraethylene glycol chain), 3.34 (bt, 2H, J = 7.5 Hz, -NCH₂CH₂CH₂CH₃), 2.53 (bt, 1H, J = 6.9 Hz, -OH), 2.47 (bt, 1H, J = 6.9 Hz, -OH), 1.65-1.52 (m, 2H, -NCH₂CH₂CH₂CH₃), 1.44-1.28 (m, 2H, -NCH₂CH₂CH₂CH₃), 0.96 (t, 3H, J = 7.5 Hz, -NCH₂CH₂CH₂CH₃); ¹³C NMR (CDCl₃) δ (ppm): 158.5, 147.7, 137.5, 136.1, 130.7, 128.7, 127.97, 127.8, 127.6, 126.7, 126.4, 125.2, 123.8, 115.0, 111.9, 72.4, 72.4, 70.7, 70.6, 70.56, 70.50, 70.2, 69.6, 68.4, 67.3, 61.7, 51.1, 50.3, 29.2, 20.2, 14.0. MS (MALDI-TOF): 721.3 [M]⁺. HWE reagent Tetraethyl-2,5-dicyano-1,4-xylylenediphosphonate was obtained according to literatures (27,28).

2,5-Dicyano-*E*,*E*-1,4-Bis-4,4'-((2-(2-(2-(2-hydroxy-ethoxy)-ethoxy)-ethoxy)-ethyl)-butyl-amino)-styrylbenzene (4)

To a solution of Tetraethyl-2,5-dicyano-1,4-xylylenediphosphonate (186 mg, 0.43 mmol) and 4-N-n-Butyl-N-[2-[2-[2-(2-hydroxyethoxy)ethoxy]ethoxy]ethyl]amino benzaldehyde (**13**) (460 mg, 1.3 mmol, 3 equiv) in anhydrous DMF (10 mL) was added KOBut (155 mg, 1.3 mmol, 3 equiv) pre-dissolved in DMF (10 mL) dropwise via syringe at 0 °C under argon. After stirring for 0.5 h at 0 °C, the reaction mixture was slowly warmed up to room temperature and stirred for another 12 h, then the reaction was quenched with water. The crude product was collected by suction filtration and the filtrate was evaporated to dryness. The residue was re-dissolved with CHCl₃ and washed with brine (2 × 100 mL). The organic layer was dried over Na₂SO₄ and concentrated to dryness. The crude product was then purified by chromatography on a silica gel column eluted with EtOAc/MeOH (20/1, v/v) to give the double bond trans linked product (233 mg, yield 65%). *R_f* 0.23 (EtOAc/MeOH, 20/1). ¹H NMR (CDCl₃) δ (ppm): 7.94 (bs, 2H, styryl benzene), 7.44 (d, 4H, J = 8.7 Hz, butylaminobenzene), 7.19 (d, 2H, J = 16.2 Hz, double bond trans linkage), 7.10 (d, 2H, J = 16.2 Hz, double bond trans linkage), 6.68 (d, 4H, J = 8.7 Hz, butylaminobenzene), 3.74 (bt, 4H, J = 4.5 Hz, tetraethylene glycol chain), 3.71-3.55 (m, 28H, tetraethylene glycol chain), 3.37 (bt, 2H, J = 7.5 Hz, -NCH₂CH₂CH₂CH₃), 2.62 (bs, 2H, -OH), 1.65-1.52 (m, 2H, -NCH₂CH₂CH₂CH₃), 1.37 (sext, 2H, J = 7.5 Hz, -NCH₂CH₂CH₂CH₃), 0.97 (t, 3H, J = 7.5 Hz, -NCH₂CH₂CH₂CH₃); ¹³C NMR (CDCl₃) δ (ppm): 149.1, 138.8, 134.6, 129.2, 129.1, 123.4, 117.4, 116.9, 114.2, 111.9, 72.7, 71.0, 70.89, 70.81, 70.6, 68.7, 62.0, 51.4, 50.6, 29.4, 20.5, 14.2. MS (MALDI-TOF): 827.0 [M]⁺.

2,5-Dicyano-*E,E*-1,4-bis-4,4'-(2-(2-(2-(2-hydroxy-ethoxy)ethoxy)ethoxy)ethoxy)ethyl)styryl benzene (1)

To a solution of Tetraethyl-2,5-dicyano-1,4-xylylenediphosphonate (200 mg, 0.47 mmol) and **10** (418 mg, 1.4 mmol, 3 equiv) in anhydrous DMF (15 mL) was added KOBut (160 mg, 1.4 mmol, 3 equiv) pre-dissolved in DMF (10 mL) dropwise via syringe at 0 °C under argon. After stirring for 0.5 h at 0 °C, the reaction mixture was slowly warmed up to room temperature and stirred for another 12 h, then the reaction was quenched with water. The crude product was collected by suction filtration and the filtrate was evaporated to dryness. The residue was re-dissolved with CHCl₃ and washed with brine (2 × 100 mL). The organic layer was dried over Na₂SO₄ and concentrated to dryness. The crude product was then purified by chromatography on a silica gel column eluted with EtOAc/MeOH (10/1, v/v) to give the double bond trans linked product (201 mg, yield 60%). *R_f* 0.20 (EtOAc/MeOH, 20/1). ¹H NMR (CDCl₃) δ (ppm): 7.97 (s, 2H, styryl benzene), 7.50 (bd, 4H, J = 8.7Hz, ethoxybenzene), 7.23 (d, 2H, J = 16.2Hz, double bond trans linkage), 7.18 (d, 2H, J = 16.2Hz, double bond trans linkage), 6.95 (d, 4H, J = 8.7Hz, ethoxybenzene), 4.16 (bt, 4H, J = 4.8Hz, tetraethylene glycol chain), 3.87 (bt, 4H, J = 4.8Hz, tetraethylene glycol chain), 3.78-3.64 (m, 20H, tetraethylene glycol chain), 3.60 (bt, 4H, J = 4.8Hz, tetraethylene glycol chain), 2.95 (bs, 2H, -OH); ¹³C NMR (CDCl₃) δ (ppm): 160.1, 138.9, 134.6, 129.6, 129.0, 128.7, 119.7, 117.0, 115.3, 114.8, 72.8, 71.0, 70.85, 70.77, 70.5, 69.8, 67.7, 61.9. MS (MALDI-TOF): 739.9 [M+Na]⁺.

Compounds in preparation of *E,E*-4-Dicyanomethylene-2,6-bis-*p*-N,N'-*n*-butyl-N,N'-(2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl)-aminostyryl pyran Derivatives: *E,E*-4-Dicyanomethylene-2,6-bis-*p*-N,N'-*n*-butyl-N,N'-(2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl)-aminostyryl pyran (6)

A mixture of 2,6-Dimethyl-4-(dicyanomethylene) pyran (220 mg, 1.28 mmol), 4-*N*-*n*-Butyl-N-[2-[2-[2-(2-hydroxyethoxy)ethoxy]ethoxy]ethyl]aminobenzaldehyde (1.0 g, 2.8 mmol), and Piperidine (1 mL, 10 mmol) in toluene (25 mL) was heated to reflux with a Dean-Stark trap under argon overnight. Then the reaction was cooled and the solvent was evaporated to dryness in vacuum. CHCl₃ was added to dilute the residue, and the organic layer was washed with water (100 mL) and brine (100 mL). After dried over Na₂SO₄, the organic phase was concentrated and the residue was purified by chromatography on a silica gel column eluted with EtOAc/MeOH (20/1) to give the title product (538 mg, yield 50%) as a red powder: *R_f* 0.14 (EtOAc/MeOH 20/1). ¹H NMR (CDCl₃) δ (ppm): 7.42 (d, 4H, J = 9.0Hz, aromatic ring), 7.41 (d, 2H, J = 16.2Hz, double bond trans-linkage), 6.69 (d, 4H, J = 9.0Hz, aromatic ring), 6.51 (s, 2H, pyran ring), 6.47 (d, 2H, J = 16.2Hz, double bond trans-linkage), 3.55-3.51 (m, 32H, tetraethylene glycol chain), 3.39 (t, 4H, J = 7.5Hz, *n*-butyl chain), 1.70-1.52 (m, 4H, *n*-butyl chain), 1.46-1.30 (m, 4H, *n*-butyl chain), 0.98 (t, 6H, J = 7.5Hz, *n*-butyl chain); ¹³C NMR (CDCl₃) δ (ppm): 159.4, 156.2, 149.8, 138.2, 129.9, 122.1, 116.5, 112.8, 111.7, 105.3, 72.6, 70.9, 70.8, 70.7, 70.4, 68.5, 61.8, 51.4, 50.5, 29.4, 20.4, 14.2. MS (MALDI-TOF): *m/z* 844.2 [M+1]⁺.

E,E-4-Dicyanomethylene-2,6-bis-4,4'-(2-(2-(2-(2-hydroxy-ethoxy)ethoxy)ethoxy)ethyl)styryl pyran (37)

A mixture of 2,6-Dimethyl-4-(dicyanomethylene) pyran (206 mg, 1.2 mmol), **10** (800 mg, 2.68 mmol), Acetic acid (0.5 mL, 8.5 mmol), and Piperidine (1 mL, 10 mmol) in Toluene (25 mL) was heated to reflux with a Dean-Stark trap under argon for 36 h. Then the reaction was cooled and the solvent was evaporated to dryness in vacuum. CHCl₃ was added to dilute the residue, and the organic layer was washed with brine (2×100 mL). After dried over Na₂SO₄, the organic phase was concentrated and the residue was purified by chromatography on a silica gel column eluted with EtOAc/MeOH (10:1) to give the title product (130 mg, yield 15%): *R_f* 0.23 (EtOAc/MeOH 20/1). ¹H NMR (CDCl₃) δ (ppm): 7.52 (bd, 4H, J = 9.0Hz, ethoxybenzene), 7.46 (d,

2H, $J = 16.2$ Hz, double bond trans linkage), 6.98 (d, 4H, $J = 9.0$ Hz, ethoxybenzene), 6.64 (s, 2H, pyran), 6.63 (d, 2H, $J = 16.2$ Hz, double bond trans linkage), 4.20 (bt, 4H, $J = 4.8$ Hz, tetraethylene glycol chain), 3.89 (bt, 4H, $J = 4.8$ Hz, tetraethylene glycol chain), 3.78-3.64 (m, 20H, tetraethylene glycol chain), 3.62 (bt, 4H, $J = 4.8$ Hz, tetraethylene glycol chain); ^{13}C NMR (CDCl_3) δ (ppm): 161.0, 158.8, 137.8, 129.7, 127.7, 116.5, 115.7, 115.5, 106.7, 72.7, 71.1, 70.88, 70.81, 70.5, 69.8, 67.8, 62.0. MS (MALDI-TOF): 731.7 $[\text{M}]^+$, 787.8 $[\text{M}+\text{K}+\text{H}_2\text{O}]^+$.

Monotrylation of *E,E*-4-Dicyanomethylene-2,6-bis-*p*-N,N'-*n*-butyl-N,N'-(2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl)-aminostyryl pyran (38)

E,E-4-Dicyanomethylene-2,6-bis-*p*-N,N'-*n*-butyl-N,N'-(2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl)-aminostyryl pyran (**6**) (538 mg, 0.64 mmol) was dissolved in dry Pyridine (50 mL), followed by addition of 3 equivalence of DMTrCl (684 mg) and DMAP (~10 mg). The mixture was stirred at room temperature under argon for 5 days, and TLC monitoring (20/1, $\text{CH}_2\text{Cl}_2/\text{MeOH}$) showed the formation of monosubstituted (Rf 0.25) and disubstituted (Rf 0.98) products as well as the starting material (Rf 0.09). The reaction mixture was concentrated in vacuum and the residue was directly subject to a silica gel column using the gradient elution technique with $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{Pyridine}$ (500/10/3 to 500/20/3) as eluents affording the monosubstituted product (250 mg, yield 35%); disubstituted product, and recovered starting material (123 mg). Meanwhile, disubstituted fraction was collected and further monodetrylation was carried out according to the following procedure: A detrylation solution was made by mixing ZnCl_2 (1.5 g) with 110 mL of $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (10/1, v/v). Then the crude disubstituted compound (~900 mg) was dissolved by 50 mL of the detrylation solution and the reaction mixture was monitored by TLC (20/1, $\text{CH}_2\text{Cl}_2/\text{MeOH}$). Once the appearance of the spot corresponding to the starting material (*E,E*-4-Dicyanomethylene-2,6-bis-*p*-N,N'-*n*-butyl-N,N'-(2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl)-aminostyryl pyran) on the TLC plate, the reaction was quenched by addition of a saturated aq. NaHCO_3 solution (100 mL). The mixture was extracted by chloroform, and the organic layer was washed with brine, collected, dried over Na_2SO_4 , concentrated, and subject to a silica gel column (500/10/3, $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{Pyridine}$) to give the title product **38** (128 mg, yield 17.5 %). If the recovered starting material was counted, the total yield of the monotrylation would be 67% based on the consumed starting material. ^1H NMR (CDCl_3) δ (ppm): 7.49-7.38 (m, 8H, aromatic rings and double bond trans-linkage), 7.34 (dt, 4H, $J_1 = 2.7$ Hz, $J_2 = 9$ Hz, methoxybenzene ring), 7.31-7.15 (m, 3H, benzene ring), 6.81 (dt, 4H, $J_1 = 2.7$ Hz, $J_2 = 9$ Hz, methoxybenzene ring), 6.69 (d, 2H, $J = 8.7$ Hz, benzene ring), 6.66 (d, 2H, $J = 9.0$ Hz, benzene ring), 6.54 (d, 1H, $J = 2.1$ Hz, pyran ring), 6.52 (d, 1H, $J = 2.1$ Hz, pyran ring), 6.48 (d, 1H, $J = 15.9$ Hz, double bond trans-linkage), 6.46 (d, 1H, $J = 15.9$ Hz, double bond trans-linkage), 3.77 (s, 6H, CH_3O), 3.56-3.50 (m, 30H, tetraethylene glycol chain), 3.44-3.31 (m, 4H, *n*-butyl chain), 3.22 (t, 2H, $J = 5.1$ Hz, tetraethylene glycol chain), 1.69-1.49 (m, 4H, *n*-butyl chain), 1.48-1.42 (m, 4H, *n*-butyl chain), 0.97 (t, 3H, $J = 7.5$ Hz, *n*-butyl chain), 0.96 (t, 3H, $J = 7.5$ Hz, *n*-butyl chain). ^{13}C NMR (CDCl_3) δ (ppm): 159.5, 158.4, 156.2, 149.8, 145.1, 138.2, 136.4, 130.2, 129.9, 128.3, 127.8, 126.7, 122.2, 122.1, 116.5, 113.1, 112.94, 112.88, 111.8, 111.7, 105.4, 86.1, 72.7, 71.0, 70.92, 70.84, 70.76, 70.5, 68.6, 63.3, 61.9, 56.1, 55.4, 51.4, 50.6, 29.4, 20.5, 14.3. MS (ESI): m/z 1145.6 $[\text{M}]^+$.

The Phosphoramidite of Monotrylated *E,E*-4-Dicyanomethylene-2,6-bis-*p*-N,N'-*n*-butyl-N,N'-(2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl)-aminostyryl pyran (42)

To a solution of monotrylated *E,E*-4-Dicyanomethylene-2,6-bis-*p*-N,N'-*n*-butyl-N,N'-(2-(2-(2-(2-hydroxyethoxy)ethoxy)ethoxy)ethyl)-aminostyryl pyran (**38**) (200 mg, 0.17 mmol) in dry CH_2Cl_2 (20 mL) was added 2.7 equivalent of diisopropylethylamine (0.08 mL). Then chloro-*N,N*-diisopropylaminocyanophosphane (0.05 mL, ~1.2 equal) was added dropwise at room temperature under argon. After 20 min of stirring under argon at room temperature, the reaction mixture was diluted with $\text{CH}_2\text{Cl}_2/\text{Et}_3\text{N}$ (300/15, v/v) 100 mL and the

organic phase was washed with a saturated aq. NaHCO₃ solution and brine. The organic layer was dried over Na₂SO₄, filtered, and evaporated to dryness. The residue was immediately subject to a silica gel column (CH₂Cl₂/EtOAc/Et₃N, 3/6/1) to give the title product (200 mg, yield 88%) as a red powder, which should be used freshly for the next phosphotriester step in order to achieve a higher coupling yield. ¹H NMR (CDCl₃) δ (ppm): 7.48-7.38 (m, 8H, aromatic rings and double bond trans-linkage), 7.34 (dt, 4H, J₁ = 2.4Hz, J₂ = 9Hz, methoxybenzene ring), 7.31-7.15 (m, 3H, benzene ring of DMTr group), 6.81 (dt, 4H, J₁ = 2.4Hz, J₂ = 9Hz, methoxybenzene ring), 6.69 (d, 2H, J = 8.7Hz, benzene ring), 6.67 (d, 2H, J = 9.0Hz, benzene ring), 6.56 (d, 1H, J = 2.1Hz, pyran ring), 6.54 (d, 1H, J = 2.1Hz, pyran ring), 6.50 (d, 1H, J = 15.9Hz, double bond trans-linkage), 6.48 (d, 1H, J = 15.6Hz, double bond trans-linkage), 3.91-3.70 (m, 4H, -CH₂CH₂CN, -CH(CH₃)₂), 3.77 (s, 6H, CH₃O), 3.70-3.50 (m, 30H, tetraethylene glycol chain), 3.44-3.31 (m, 4H, -NCH₂CH₂CH₂CH₃), 3.22 (t, 2H, J = 5.4Hz, tetraethylene glycol chain), 2.64 (t, 2H, J = 6.5Hz, -CH₂CH₂CN), 1.70-1.52 (m, 4H, -NCH₂CH₂CH₂CH₃), 1.48-1.30 (m, 4H, -NCH₂CH₂CH₂CH₃), 1.18 (d, 6H, J = 6.9Hz, -CH(CH₃)₂), 1.17 (d, 6H, J = 6.6Hz, -CH(CH₃)₂), 0.97 (t, 3H, J = 7.5Hz, -NCH₂CH₂CH₂CH₃) 0.96 (t, 3H, J = 7.5Hz, -NCH₂CH₂CH₂CH₃). ¹³C NMR, (CDCl₃) δ (ppm): 159.2, 158.1, 155.9, 149.5, 144.9, 137.9, 136.1, 129.9, 129.6, 128.0, 127.5, 126.5, 121.9, 121.8, 117.6, 116.2, 112.8, 112.7, 111.5, 105.1, 85.8, 71.2, 71.1, 70.7, 70.6, 70.53, 70.49, 68.3, 63.0, 62.6, 62.4, 58.5, 58.3, 55.9, 55.1, 51.1, 50.3, 46.1, 43.1, 42.9, 29.1, 24.65, 24.58, 24.55, 24.49, 20.3, 20.24, 20.18, 14.0.; ³¹P NMR (CDCl₃) δ (ppm): 149.5.

Solid phase synthesis of DNA-chromophore conjugates

The solid phase syntheses were carried out on an Expedite Nucleic Acid Syntheses System. The DNA bases were synthesized on either glass beads or polystyrene spheres using software protocol supplied by PerSeptive Biosystems (Framingham, MA) and the phosphoramidite reagents of DNA bases were obtained from Applied Biosystems. The phosphoramidite reagents of organic chromophores were synthesized according to procedures described in this report and their solid phase syntheses were carried out using custom protocol in order to improve the reaction yields. Typically, the phosphoramidite concentrations of the organic dyes used for the solid phase syntheses were greater than 40 mg/ml. Standard DNA base coupling delivers the phosphoramidite reagent with activator to the solid phase column immediately and allows for 47 sec for coupling reaction. This reaction time is not adequate for coupling chromophoric phosphoramidite to DNA bases and hence chromophoric coupling protocol was modified by delivering the phosphoramidite reagents to the column over 4 sec period (instead of immediately) and the reaction time was extended to 140 sec (instead of 47 sec). For high yield of coupling, phosphoramidite reagents must be prepared and purified with silica gel columns immediately before carrying out the solid phase syntheses. Using the modified protocol described here, the yield of the coupling reaction is typically greater than 80% as indicated by the trityl data.

Results and Discussion

The strategy for incorporating rigid dyes, which typically have poor solubility, into biomolecules is to functionalize them with very soluble ethylene glycol chains. In Scheme 1, all precursor compounds with flexible ethylene glycol chains were synthesized (19,20) as the appropriate benzaldehyde **7** – **14**, which can be further condensed with ylids to make large π -conjugated systems. The experimental procedures for the synthesis are described in the experimental section.

Our initial attempt was to use commercially available monochlorotriethylene glycol as the precursor for introducing soluble groups. Accordingly, in Scheme 2, compound **15** was synthesized using conventional Wittig reaction with poor yield and its detritylated derivative

16 is insoluble in organic solvents. The poor solubility of the final product **16** hinders further chemical manipulation and promotes us to make longer tetraethylene glycol chain for better soluble amphiphilic chromophores. Again, Wittig reaction was used in the initial coupling reaction between the desired aldehyde **11** and the bis-ylid precursor. The reaction yielded only 15% of all-*trans* **17**, mainly due to the formation of *cis-trans* isomers of **17** at low temperature ($-78\text{ }^{\circ}\text{C}$). Detritylation of **17** yielded the final product **5**, which could be dissolved in chloroform or dichloromethane. Following the above procedure, soluble compound **2** was also synthesized. Comparing the solubility difference between **16** and **2** or **5**, we have concluded that tetraethylene glycol was an excellent flexible spacer for enhancing solubility of rigid chromophores. Furthermore, tetraethylene glycol proved to be excellent hinges when inserted into the DNA sequence and the influence from the spacers to the folding or unfolding behaviors of resulting hybrid foldamers was minimum.

However, the above method for synthesizing all *trans* double bond π -conjugates still needs improvement. Thus, Horner-Wadsworth-Emmons (HWE) reaction was used instead of conventional Wittig reaction and all three bis(styryl)benzene derivatives were synthesized again by condensing phosphonate ylid with corresponding benzaldehyde derivatives as shown in Scheme 3. The HWE reagent (phosphonate ylid) was readily prepared from α,α' -dibromo-*p*-xylene according to the reported procedure (21). In dried DMF, 3 equiv of desired aldehyde **10** or **13** was reacted with phosphonate ylid using KOBu^t as the promoter at room temperature to give the final products **2** or **5** in a high yield. Even with the bulky tetraethylene glycol chain, the HWE condition still favored the formation of all *trans* double bond configuration. To prepare polar chromophore **3**, intermediate **19** was prepared following the same procedure for **2** and **5** except that 1 equiv of benzaldehyde **10** was used. Then final step-wise coupling reaction of **19** and **13** afforded the asymmetric π -conjugate **3**.

It was reported (22) that different donor or acceptor substituents on the ends of bis(styryl)benzene π -conjugate could significantly change their emission colors because of the electron donating or withdrawing effects. The oxygen substitute on both ends of the π -conjugate in **5** can be regarded as weak electron donors, involving in the symmetric electron transfer from both ends to the middle of the conjugated system; compound **5** has bright blue fluorescence. In **2**, *n*-butyl amino groups were used to end the π -conjugate instead of an oxygen atom as the electron donor to make a stronger donor- π -donor system, which gives green fluorescence. The trend appears that stronger electron donating groups will red-shift fluorescence emission. Following this trend, the featured asymmetric compound **3** has a fluorescence emission wavelength between **2** and **5**. The fact that asymmetric compound **3** does not have a fluorescence emission longer than the symmetric compound **2** may suggest that an acceptor is required in the asymmetric compound in order to have yellow to red fluorescence. With efficient blue fluorophores in hand, our efforts have shifted to prepare similar bis(styryl)benzene structures with red fluorescent emission; the strategy is to vary the structure of middle π -conjugated bridge and tune the electronic properties of the end substituents. In Scheme 4, three asymmetric bis(styryl)benzene structures were synthesized with the pyridinium ion as an extremely strong electron acceptor in the π -conjugated structure (**20–33**); the resulting D- π -A structures have yellow-orange emissions (23–26). However, the phosphoramidite products of all three precursors, **26**, **29**, **31**, were not isolated because of low mobility on silica column despite the fact that three different counter ions with various polarity and DMTr protection were attempted (experimental section).

Scheme 5 shows the syntheses of the chromophore blocks with red-orange fluorescence based on a donor-acceptor-donor (D-A-D) structure with electron rich-poor-rich distribution. Phosphonate ylid **36** was synthesized as reported previously (27,28). The same HWE procedures as described for preparation of **2** and **5** were carried out except that tetraethyl 1,4-xylylenediphosphonate was replaced by **36**, consequently **1** and **4** were synthesized in good

yields. Because of the electron withdrawing properties of the di-cyano groups, the hydrogen atoms on the methyl group of (2,6-dimethyl-4*H*-pyran-4-ylidene)malononitrile are acidic and in the presence of base the methyl groups can directly react with desired aldehyde in a Knoevenagel condensation. The simplicity of Knoevenagel condition leads to compounds **6** and **37** in one step (18). While compound **37** has low optical density and weak fluorescence properties, compound **6** emits impressive red fluorescence with peak maximum at 612 nm in organic solvents.

One important requirement for these fluorescent chromophores is to have asymmetric substitutions at either end of the rod-like or Y-shaped structures. This way, they can be protected with DMTr group on one end and activated with phosphoramidite group on the other. Such DMTr protected and phosphoramidite activated chromophores are suitable for incorporation into automated DNA synthesis.

Therefore one of the key steps is the phosphoramidite activation and its efficiency will depend on the yield and purity of its precursor—monotritylated derivative of the chromophoric building blocks. To improve the yield of monotritylated product, we have developed an efficient route as shown in Scheme 6. In a typical monotritylation process, two products were formed: monotritylated product and bistritylated product. The monotritylated product was purified as the desired product while the bistritylated product can be further converted to the monotritylated product using a weak Lewis acid zinc chloride as the detritylation reagent (29).

A typical detritylation solution consists of ZnCl₂ (1.5 g) with 110 mL of CH₂Cl₂/MeOH (10/1, v/v). The detritylation procedure involves treating the bistritylated product in the detritylation solution. The reaction can be monitored with TLC and typically detritylation finishes within an hour. Once the spot corresponding to the original, non-substituted chromophore appeared on the TLC plate, the reaction was quenched by pouring its content into an aqueous solution saturated with NaHCO₃. After chromatography, the yield of the monotritylated product exceeded 40% based on the bistritylated compound. While combining with the direct monotritylation product, the total yield of the mono-tritylated product had been significantly improved (from ~30% to 56–77%). This method can be applied to large-scale preparation of monotritylated derivatives of chromophoric building blocks and the resulting mono-substituted products can be kept in –80 °C freezer under basic atmosphere for several months without noticeable decomposition.

Finally, a few fluorescent chromophores, which have orthogonal fluorescence emission, were chosen for phosphoramidite activation. They are compound **38**, the precursor **6** of which emits red fluorescence with an intensity maximum at 612 nm, compound **40**, the precursor **5** of which emits blue fluorescence with an emission maximum at 430 nm, and compound **39**, the precursor **2** of which emits (at 473 nm) in blue-green region (Table 1). The fluorescence spectra of the red-blue or red-blue/green FRET pairs have little overlap, hence orthogonal in fluorescent emissions. For efficient FRET pairs, the absorption maximum of the red-fluorescence chromophore (488 nm) should overlap perfectly with the fluorescent emission of the blue-fluorescence donor.

The phosphoramidite derivatives were made according to our prior report (30). It is worth mentioning that methylene chloride or methylene chloride/acetonitrile (1/1) was employed as reaction solvents to ensure the maximum solubility of the chromophore precursor, as is the key point to reach the highest yield of the phosphoramidite derivatives. Also 5–10% triethylamine in the system was necessary to stabilize the phosphoramidite compounds. The procedure describing the synthesis of phosphoramidites is presented in the experimental section. The yields of crude phosphoramidites from the monotritylated derivatives usually exceeded 90%,

suitable for solid-state synthesis of phosphoramidite chemistry. The stability of these phosphoramidite chromophores was not optimum in solutions such that the phosphoramidite reagents must be prepared immediately before solid-phase synthesis. Because of the presence of tetra ethylene glycol units, the phosphoramidite reagents usually cannot be purified with crystallization, but flash chromatography was effectively for their final purification before usage. Additionally, the dumb-bell shaped spots on TLC plates, which correspond to the two steric isomers due to the chiral phosphorus atom in the phosphoramidite of normal DNA bases, were hardly observed in these phosphoramidite chromophores.

Solid phase synthesis was employed to incorporate the final chromophoric candidates into single strand DNA backbones as shown in Scheme 7. Our ability to synthesize asymmetrically substituted chromophoric building blocks has established a firm foundation for preparation of foldamers with a controlled molecular orientation, well-defined biological and synthetic sequences. All fluorescent chromophores have been functionalized with hydroxyl groups at their termini. Furthermore, one hydroxyl group will be protected with DMTr and the other will be activated with the β -cyanoethyl phosphoramidite group.

Foldamer synthesis employs the same strategy of DNA synthesis, i.e., one end of the chromophore is activated with phosphoramidite group for attacking the hydroxyl group at the growing chain terminal and the other end can be regenerated for chain growth by removing the DMTr protected hydroxyl group. The essential steps are coupling between a chain terminal hydroxyl group and a phosphoramidite group followed by detritylation to regenerate the chain terminal hydroxyl group for chain growth.

Unlike DNA synthesis that uses phosphoramidite on the *secondary* hydroxyl group of deoxyribose, the chromophoric blocks use phosphoramidite groups on *primary* hydroxyl groups, which have *completely different* inherent activities. Therefore, their stability and activity towards hydroxyl group at chain termini are critical to the success of solid phase synthesis. Nonetheless, we have achieved coupling yields as high as 95% by increasing the phosphoramidite concentration, reaction time, and by repeating the coupling step. The *solid-phase synthesis* of foldamer is compatible with DNA synthesis; fluorescent chromophores can be incorporated *anywhere* in the sequence, for example a juxtaposition of blue and red chromophores (**41-42-41**) embedded in the DNA main chains as shown in Figure 3 (51-mer). Using the trityl monitor reading of the five bases before the three chromophore sequence and five bases after the chromophore sequence, we obtained a *total yield* for coupling three chromophores consecutively is 92%, which corresponds to 97% yield per chromophore coupling. When the chromophore is coupled to a DNA base, followed by another DNA base (57-mer), the yield is also very high. For example, at the 48th position, the chromophore **42** is introduced and the yield of 95% is obtained using the three bases immediately before and after this position. Although trityl absorption does not provide extremely accurate yields for coupling reactions, but it should be a good indicator to gauge these high yield coupling reactions.

51-mer: 5'-GGC CAA CTC CAT CAC TAG GGG TTC CT-**41-42-41**-GGA GGG GTG GAG TCG TGA CGT G-3'

57-mer: 5'-TTT GAT GCT-**42**-AGT CCA TTT GTC AGT GCT-**41**-ACC AGA GAT GAG TTG ATG-**42**-TAC TGC CGA-3'

39-mer: 5'-AA-**42**-AA-**42**-AA-**42**-AA-**42**-AA-**42**-AA-**42**-AA-**42**-AA-**42**-AA-**42**-AA-**42**-AA-**42**-AA-**42**-AAG-3'

Another example of our state-of-the-art coupling syntheses is shown in Figure 3 with alternating sequences of **42** and double A bases (39-mer); again the yield of each individual

steps are high. For example, using the ratio of the two bases before and after position 13 (**42**), we obtained a yield of 92%. Because of such high coupling efficiency, the overall yield is at 40% at 39mer. In the finished sequence, all together 12 fluorescent dyes are introduced into the DNA backbone; this demonstrates the power of synthetic organic chemistry in biological applications.

As long as the dye number density in the DNA sequence is not too high, the resulting hybrid sequence behaves frequently like oligo-DNA. For example, the dye-DNA conjugates can be cleaved from the solid support using concentrated ammonium hydroxide and the crude product can be further purified with HPLC as shown in Figure 4. Monitoring at the absorption (λ^{\max} ~485 nm) of the red-fluorescence dye (**42**), the major isolated product is, typically, the desired full-length dye-DNA conjugates. The HPLC-purified dye-DNA conjugates have good purity and are suitable for most biological assays.

Conclusion

In conclusion, we have developed a new method for converting normal organic fluorescent dyes into asymmetrically functionalized phosphoramidite reagents, which has enabled bioconjugation of many fluorescent chromophores at multiple desired positions of DNA backbones. Using Horner-Wadsworth-Emmons (HWE) reaction and Knoevenagel condensation, a series of highly fluorescent amphiphilic compounds was constructed; these compounds are suitable for labeling DNA since they can be conveniently protected with DMTr and activated with phosphoramidite and their fluorescence is not quenched by the presence of DNA bases.

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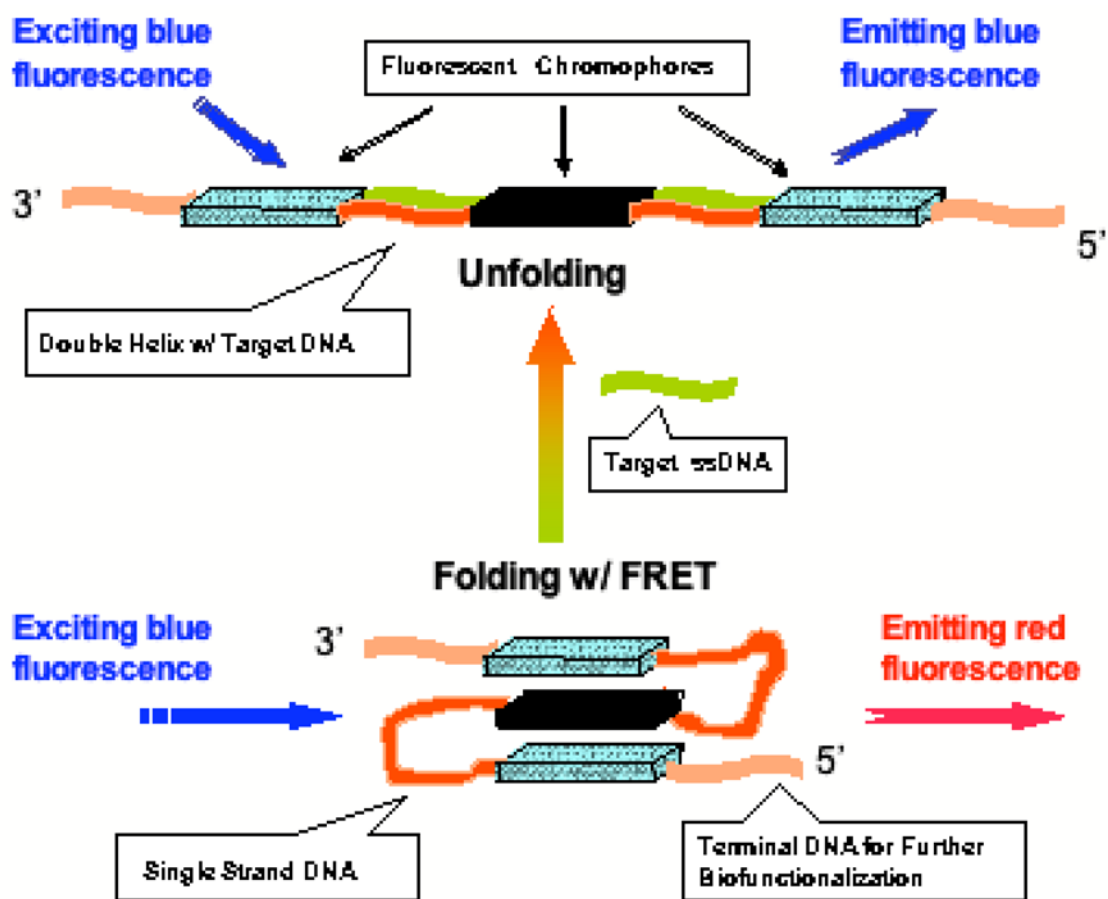


Figure 1. Incorporation of highly fluorescent chromophores into DNA main chains enables many biotechnologies, including DNA monitoring and detection.

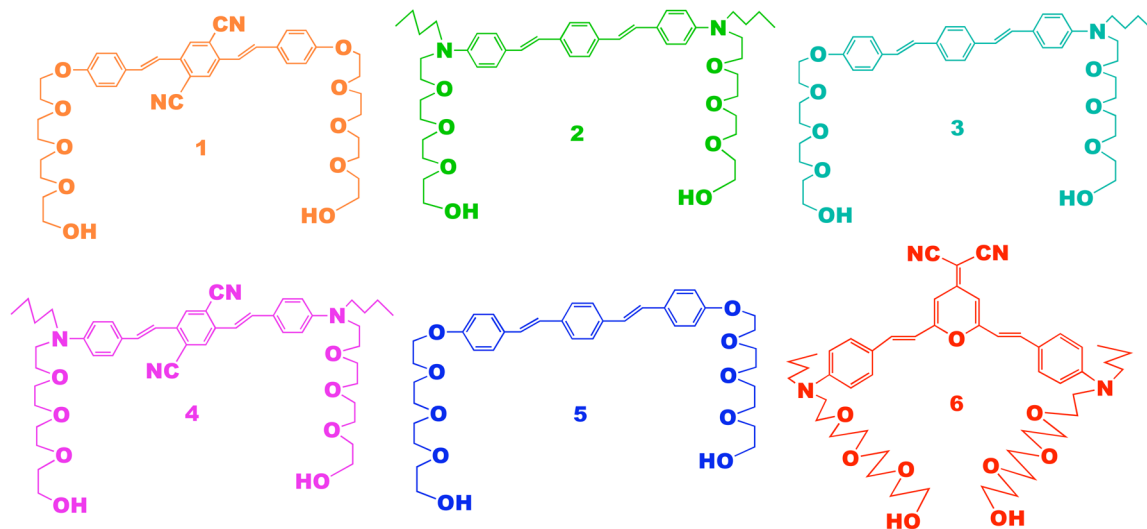


Figure 2. Chemical structures of highly fluorescent dye molecules that are amenable to the general strategy developed here: one hydroxyl group is protected with a dimethoxy trityl (DMTr) group and the other hydroxy group is activated with phosphoramidite.

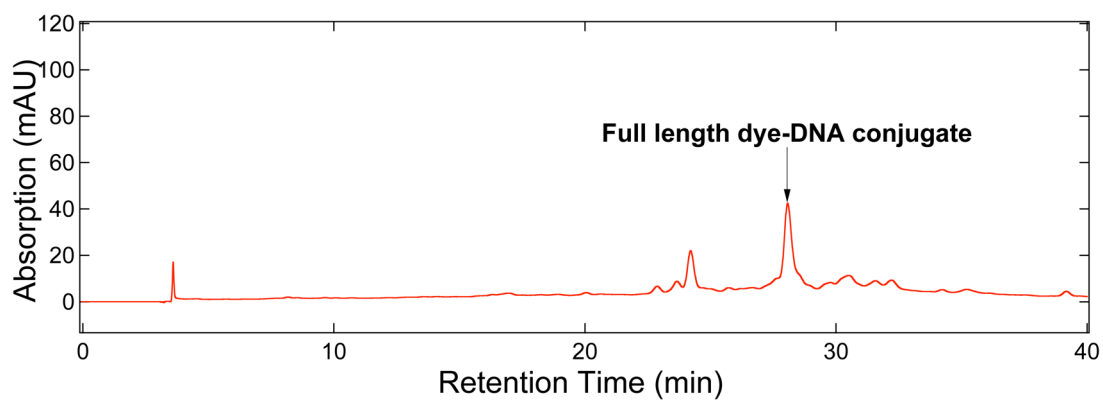
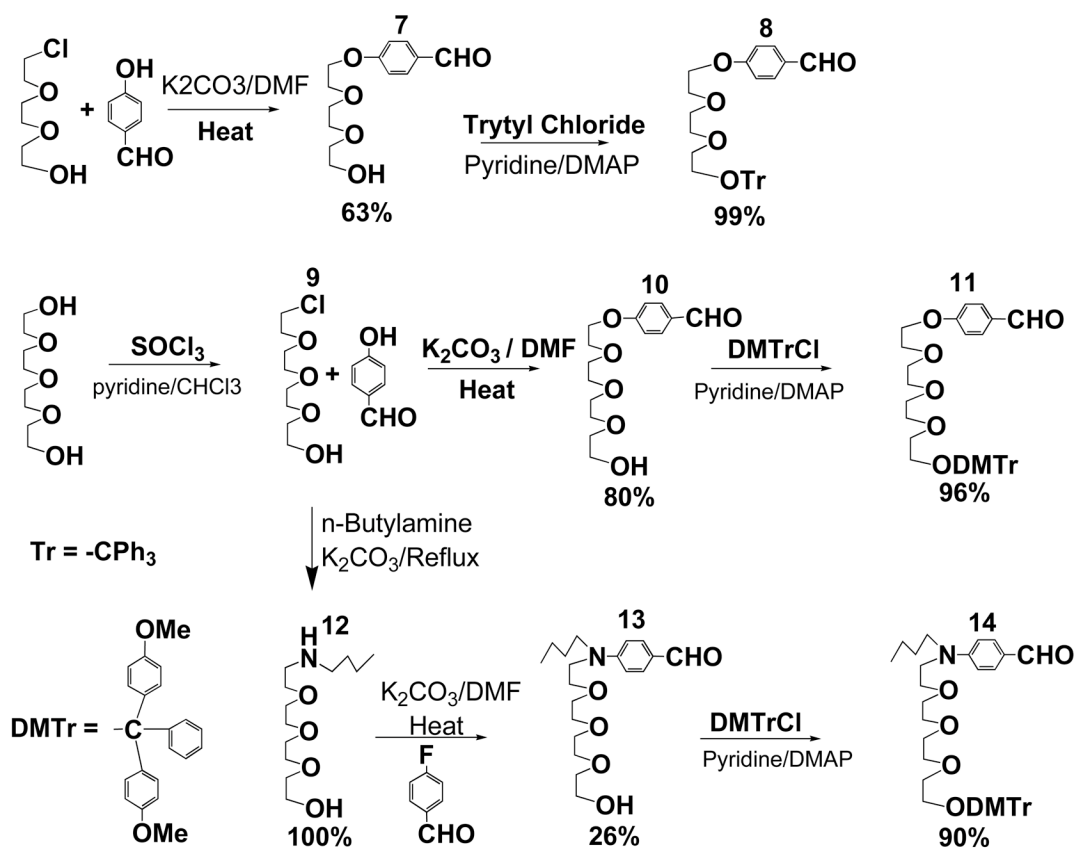
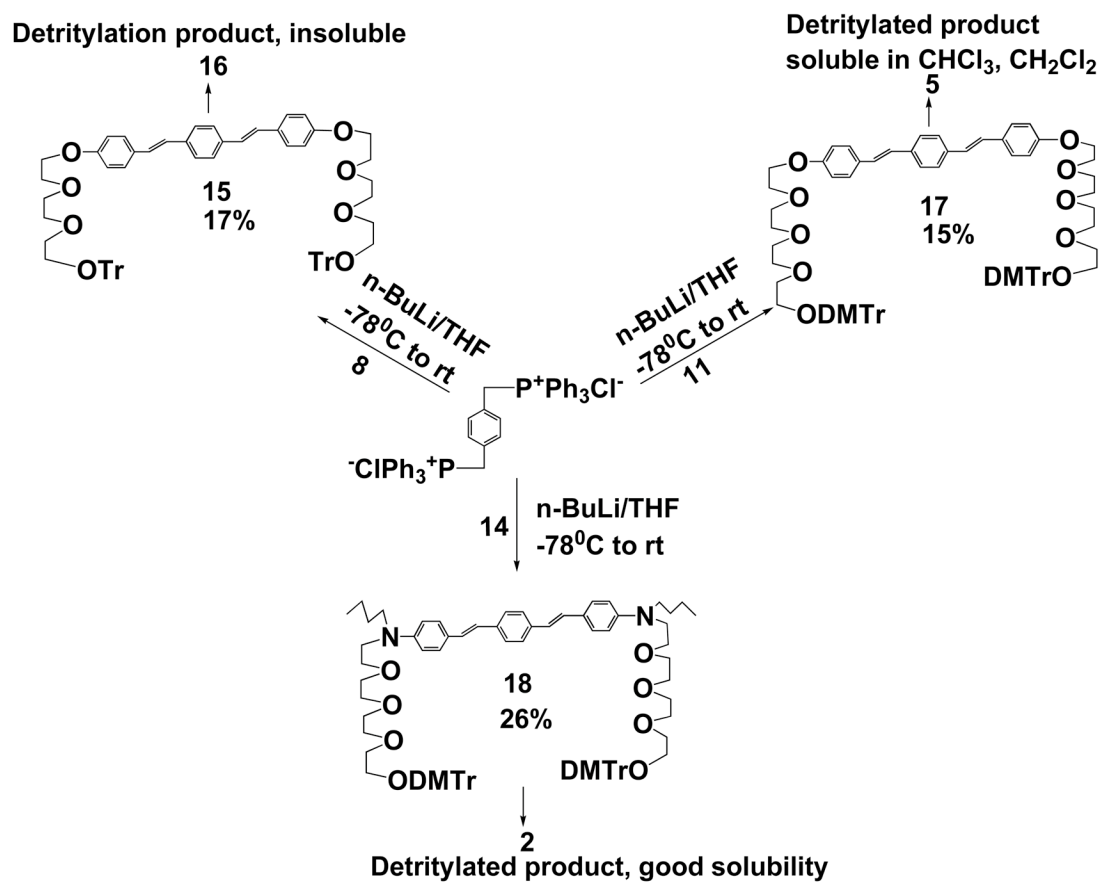


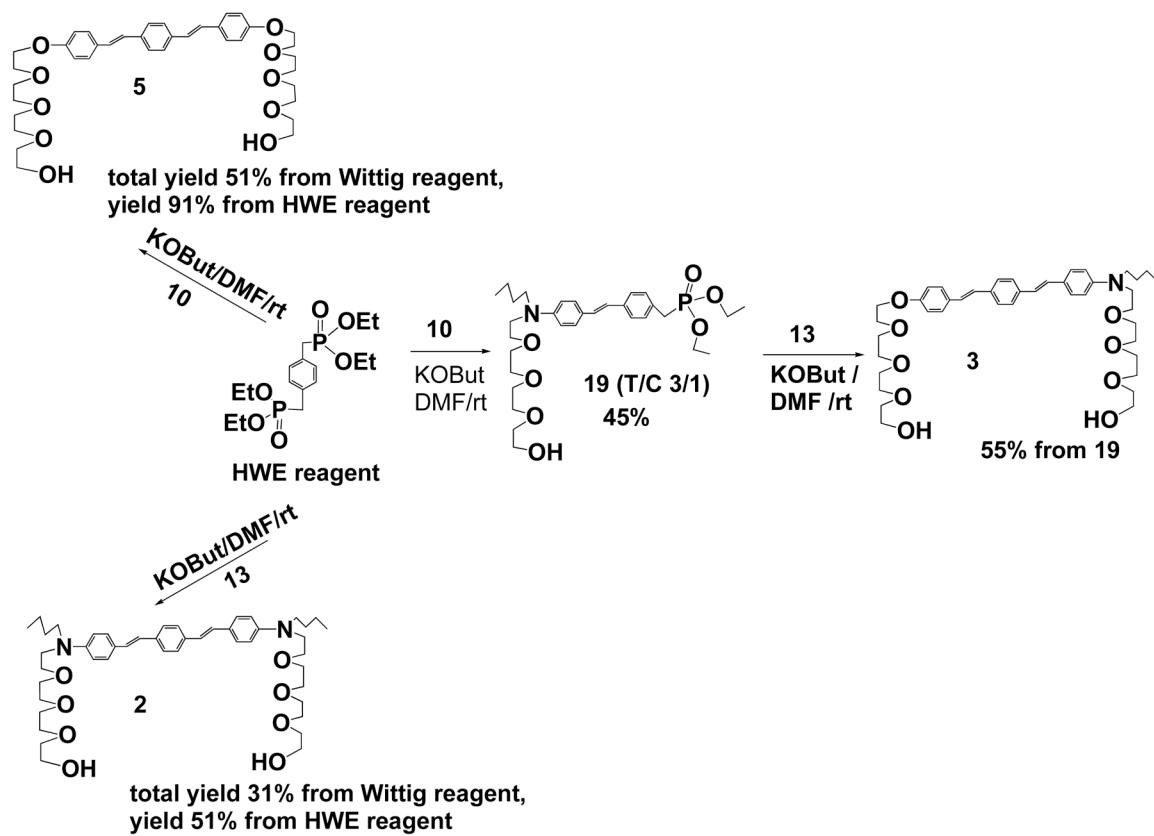
Figure 4. HPLC purification of a DNA 40 mer containing one orange-fluorescence dye (compound **42**) and two blue-fluorescence dyes (compound **43**) in the sequence. Full length DNA sequence containing three organic dyes has an elution time of approximate 28 min while using a linear gradient of H₂O and acetonitrile containing 0.1 M TEAA (triethylamine acetate).



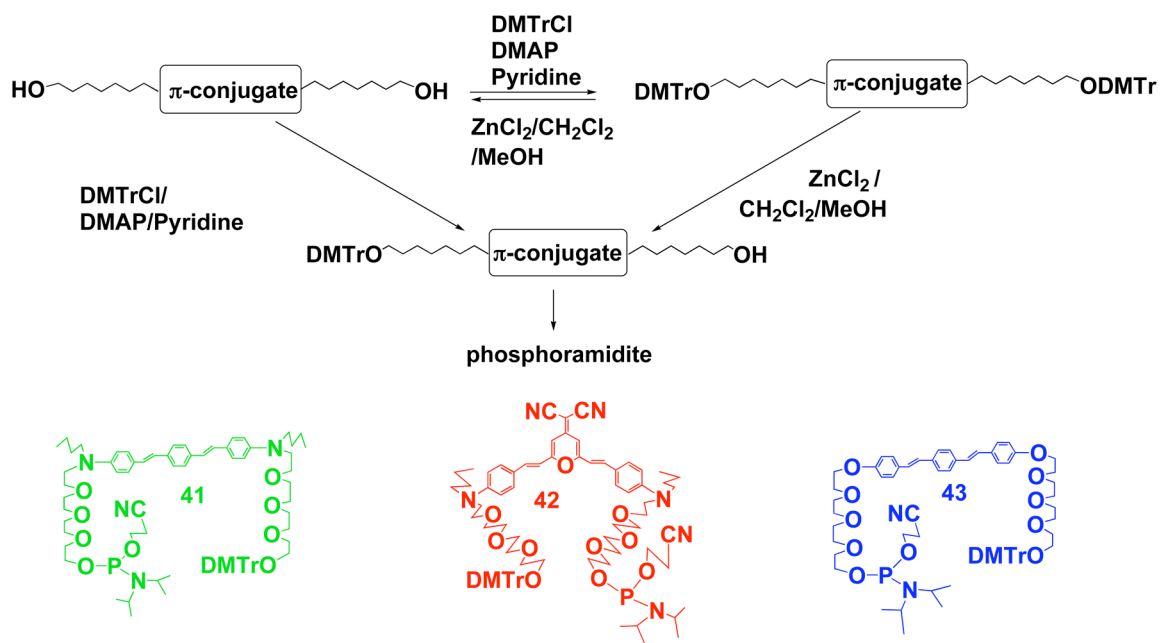
Scheme 1.



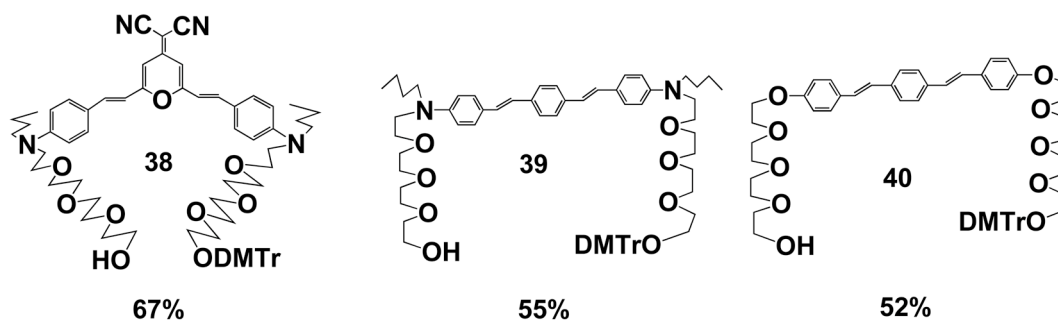
Scheme 2.



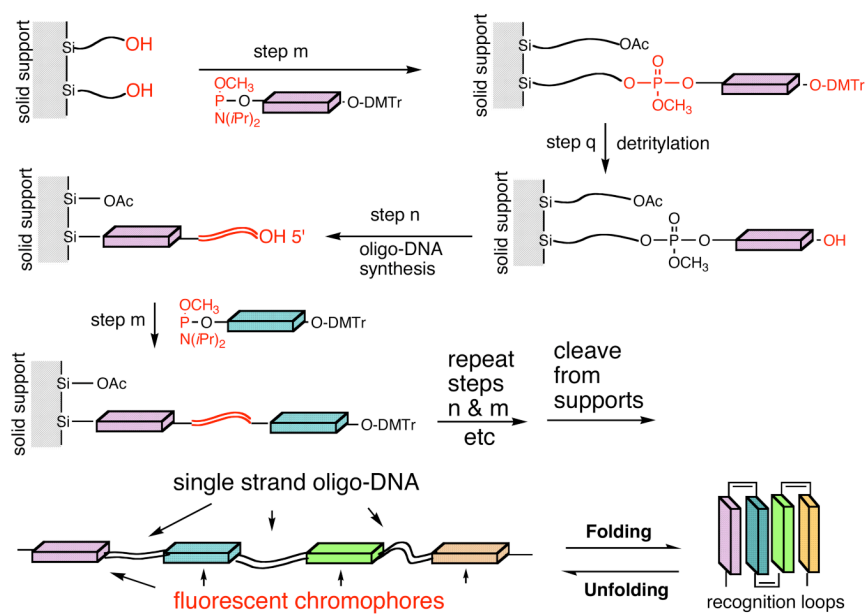
Scheme 3.



DMTr mono-substituted chromophores:



Scheme 6.



Scheme 7.

Table 1UV and fluorescence data. The solvent used is chloroform; the concentrations of various chromophores are ~10 μ M

Chromophores	Absorbance (λ_{\max}) Wavelength (nm)	Excitation Wavelength (nm)	Emission ($\lambda_{\max}^{\text{EM}}$) Wavelength (nm)
1	379	379	469
2	398	399	473
3	369	384	454
4	477	477	559
5	359	360	409, 430
6	488	490	612
29	433	418	581