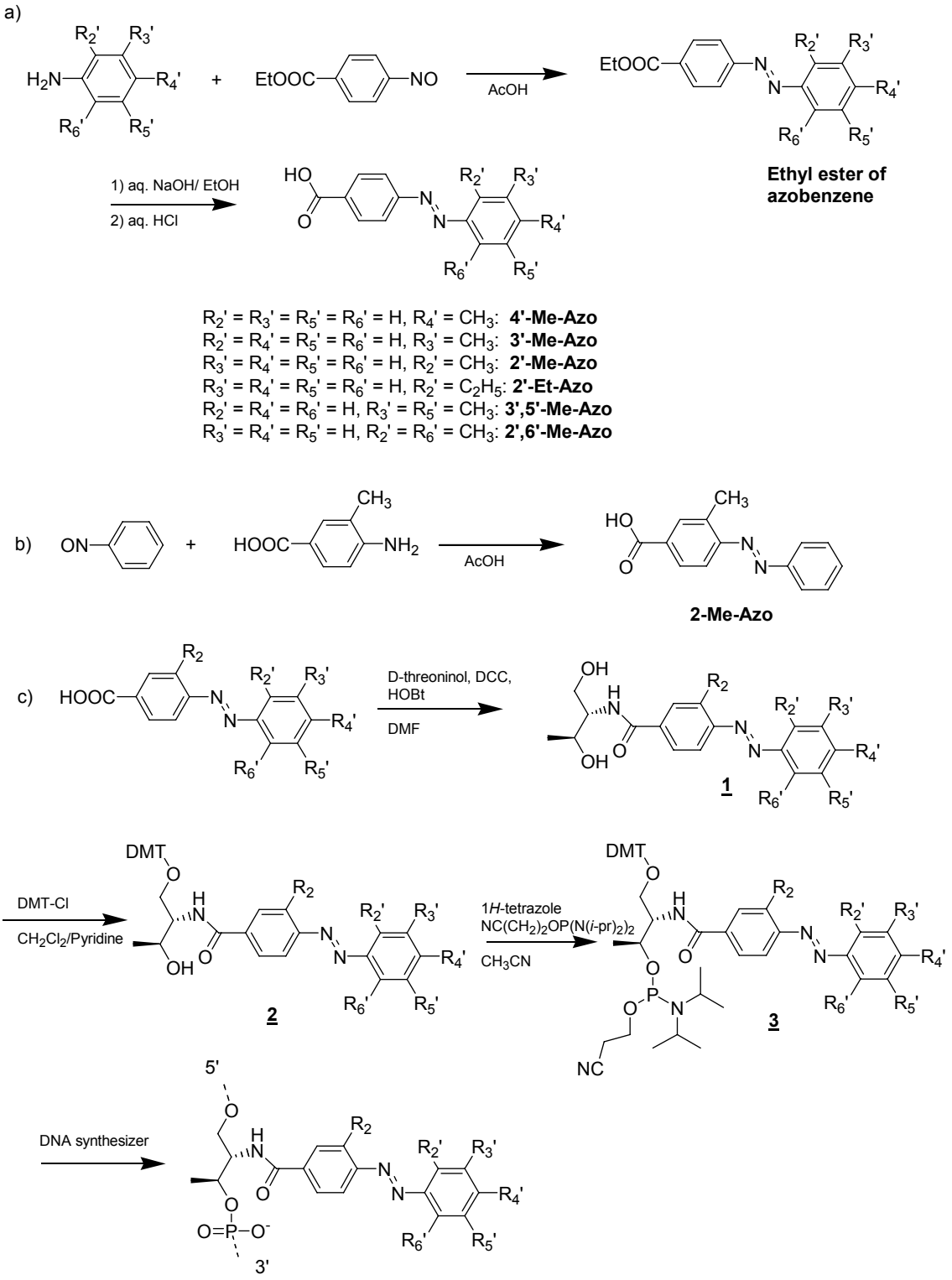


Supplemental Scheme 1. Synthetic procedures of the alkylated azobenzenes and their phosphoramidite monomers, and introduction to oligonucleotide.

Synthesis of the alkyl-substituted azobenzene (Scheme 1a)

All the alkyl-substituted azobenzenes except for **2-Me-Azo** were synthesized according to the Scheme 1a. Typical procedures are described by taking **3′-Me-Azo** as an example as follows: 0.33 g (3.08 mmol) of *m*-tolidine and 0.50g (2.79 mmol) of ethyl *p*-nitrosobenzoate were dissolved in 30 ml of acetic acid under nitrogen. The mixture was stirred at room temperature over night under dark. Then the solution was poured into water and extracted with ethyl acetate. The organic layer was washed with distilled water, saturated solutions of $NaHCO_3$ and NaCl, and dried over $MgSO_4$. After the removal the solvent, the crude mixture was subjected to silica gel column chromatography (hexane: AcOEt = 20 :1) to afford 0.38 g (1.42 mmol) of ethyl ester of 3′-Me-Azo (yield 50.7%).

0.38 g (1.42 mmol) of ethyl ester of **3′-Me-Azo** was dissolved in 15 ml of ethanol. Then 4 ml of aqueous NaOH solution (2 M) was added and the mixture was stirred overnight under dark. After the hydrolysis was completed, 10 ml of aqueous HCl solution (1M) was added to the mixture and extracted with ethyl acetate. The organic layer was washed with distilled water and saturated solution of NaCl and dried over $MgSO_4$. After removal the solvent, the crude product was used in the next step without further purification (0.33 g (1.37 mmol) yield 97.1%).

Synthesis of 2-Me-Azo (Scheme 1b)

2-methyl-substituted azobenzene was synthesized as follow: 0.50 g (3.31 mmol) of 4-amino-3-methylbenzoic acid and 0.30 g (2.80 mmol) of nitosobenzene were dissolved in 30 ml of acetic acid under nitrogen. The mixture was stirred at room temperature over night under dark, and was poured into water followed by the extraction with ethyl acetate. The organic layer was washed with distilled water (once), saturated solution of NaCl, and dried over $MgSO_4$. After the removal the solvent, the crude mixture was subjected to silica gel column chromatography (Hexane : AcOEt = 5 : 1) to afford 0.30 g (1.26 mmol) of **2-Me-Azo** (yield 44.8%).

Synthesis of the phosphoramidite monomer (Scheme 1c)

0.13 g (1.24 mmol) of D-threoninol was coupled with 0.33 g (1.37 mmol) of **3′-Me-Azo** in the presence of 0.28 g (1.36 mmol) of dicyclohexylcarbodiimide and 0.19 g (1.41 mmol) of 1-hydroxybenzotriazole in 40ml of DMF. After the reaction mixture was stirred at room temperature for 24 h under dark, the solvent was removed and the remained oil was subjected to silica gel column chromatography (chloroform : MeOH = 20 : 1) to afford 0.39 g (1.19 mmol) of **1** (yield 97.5%).

Dry pyridine solution (10 ml) containing 0.39g (1.19 mmol) of **1** (**3′-Me-Azo**) was cooled on ice under nitrogen, and 0.44 g (1.30 mmol) of 4,4-dimethoxytrityl chloride in 10 ml of dichloromethane was added to the above mixture. After 6 h of stirring, the solvent was removed, followed by silica gel column chromatography (Hexane : AcoEt : Et_3N = 50 : 50 : 3) to afford 0.34 g (0.54 mmol) of **2** (yield 45.3%).

In dry acetonitrile (4 ml) under nitrogen, 0.17 g (0.27 mmol) of **2** and 0.09 g (0.30 mmol) of 2-cyanoethyl *N,,N,,N′,N′* -tetraisopropylphosphordiamidite were reacted with 0.021 g (0.35 mmol) of 1*H*-tetrazole. Prior to the reaction, **2** and 1*H*-tetrazole were dried by coevaporation with dry acetonitrile

(twice). After 2 h, the solvent was removed by evaporation then the crude mixture was dissolved in ethyl acetate. The solvent was washed with distilled water and saturated solution of $NaHCO_3$ and NaCl and dried over $MgSO_4$. After removal the solvent, the oily product **3** was directly used for the DNA synthesis.

NMR and MS assignments

Ethyl ester of 2′-Me-Azo: Silica gel column chromatography (hexane: AcOEt = 20 :1) (yield 53.3%): ^{1}H NMR [DMSO, 500 MHz] δ = 8.17 – 6.94 (m, 8H, aromatic protons of azobenzene), 4.38 (q, ^3J(H,H) = 7.0, 2H, -O-CH_2-CH_3), 2.70 (s,3H, -NC_6H_4-CH_3), 1.36 (t.^3J(H,H) = 7.0 Hz, 3H, -O-CH_2-CH_3)

2′-Me-Azo (yield quant): ^{1}H NMR [DMSO, 500 MHz] δ = 8.16 – 6.93 (m, 8H, aromatic protons of azobenzene), 2.71 (s, 3H, -NC_6H_4-CH_3)

Compound 1 of 2′-Me-Azo: Silica gel column chromatography (chloroform : MeOH = 20 : 1) (yield quant): ^{1}H NMR [DMSO, 500 MHz] δ = 8.10- 6.92 (m, 9H, aromatic protons of azobenzene, -N*H*CO-), 4.70 (m, 2H, CH_3CH(O*H*)-, -(NH)CHCH_2(O*H*)), 3.98 (m, 2H, -CH_2(OH)C*H*(NH)CO-)-, C*H*(OH)CH_3), 3.66 and 3.59 (m, 2H, HOCH_2CH(NH)CO), 2.71 (s,3H, -NC_6H_4-CH_3), 1.10 (d, ^3J(H,H) = 6.0 Hz, CH(OH)CH_3)

Compound 2 of 2′-Me-Azo: Silica gel column chromatography (hexane : AcOEt : Et_3N = 50 : 50 : 3) (yield 36.5%): ^{1}H NMR [$CDCl_3$, 500 MHz] δ = 8.00 – 6.77 (m, 22H, aromatic protons of azobenzene, DMT, -N*H*CO-), 4.26 (m, 1H, -C*H*(OH)CH_3), 4.16 (m, 1H, -CH_2(ODMT)C*H*(NHCO-)-), 3.77 and 3.76 (s, 6H, -C_6H_4-OCH_3), 3.63 and 3.42 (dd, ^2J(H,H) = 9.5 Hz, ^3J(H,H) = 4.0 Hz, 2H, CH_2-ODMT), 2.76 (s, 3H, -NC_6H_4-CH_3), 1.24 (d, ^3J(H,H) = 6.5 Hz, 3H, -CH(OH)CH_3)

MS(FAB): m/z 630 $(MH)^+$ (calcd. 630)

Ethyl ester of 2′-Et-Azo: Silica gel column chromatography (hexane: AcOEt = 20 :1) (yield 64.6%): ^{1}H NMR [DMSO, 500 MHz] δ = 8.18 – 6.96 (m, 8H, aromatic protons of azobenzene), 4.38 (q, ^3J(H,H) = 7.00, 2H, -O-CH_2-CH_3), 3,17 (q, ^3J(H,H) = 7.5 Hz, 2H, -NC_6H_4-CH_2-CH_3) 1.37 (t, ^3J(H,H) = 7.00 Hz, 3H, -O-CH_2-CH_3), 1.26 (t, ^3J(H,H) = 7.5 Hz, 3H, -NC_6H_4-CH_2-CH_3)

2′-Et-Azo (yield quant): ^{1}H NMR [DMSO, 500 MHz] δ = 8.17 – 7.35 (m, 8H, aromatic protons of azobenzene), 3.17 (q, ^3J(H,H) = 7.5 Hz, 2H, -NC_6H_4-CH_2-CH_3), 1.27 (t, ^3J(H,H) = 7.5 Hz, 3H, -NC_6H_4-CH_2-CH_3)

Compound 1 of 2′-Et-Azo: Silica gel column chromatography (ethyl acetate only) (yield 91.9%): ^{1}H NMR [DMSO, 500 MHz] δ = 8.10- 7.35 (m, 9H, aromatic protons of azobenzene, -N*H*CO-), 4.67 (m, 2H, CH_3CH(O*H*)-, -(NH)CHCH_2(O*H*)), 3.96 (m, 2H, -CH_2(OH)C*H*(NH)CO-)-, C*H*(OH)CH_3), 3.64 and 3.54 (m, 2H, HOCH_2CH(NH)CO), 3.17 (q, ^3J(H,H) = 7.5 Hz, 2H, -NC_6N_4-CH_2-CH_3), 1.27 (t, ^3J(H,H) = 7.5 Hz, 3H, -NC_6H_4-CH_2-CH_3), 1.09 (d, ^3J(H,H) = 6.5 Hz, CH(OH)CH_3)

Compound 2 of 2′-Et-Azo: Silica gel column chromatography (hexane : AcOEt : Et_3N = 60 : 40 : 3) (yield 66.4%): ^{1}H NMR [$CDCl_3$, 500 MHz] δ = 8.00 – 6.78 (m, 22H, aromatic protons of azobenzene,

DMT, -N*H*CO-), 4.25 (m, 1H, -C*H*(OH)CH_3), 4.16 (m, 1H, -CH_2(ODMT)C*H*(NHCO-)-), 3.77 and 3.76 (s, 6H, -C_6H_4-OCH_3), 3.63 and 3.42 (dd, ^2J(H,H) = 10 Hz, ^3J(H,H) = 4.0 Hz, 2H, CH_2-ODMT), 3.22 (q, ^3J(H,H) = 7.5 Hz, 2H, -NC_6N_4-CH_2-CH_3), 1,34 (t, ^3J(H,H) = 7.5 Hz, 3H, -NC_6H_4-CH_2-CH_3) 1.24 (d, ^3J(H,H) = 6.5 Hz 3H, -CH(OH)CH_3)
MS(FAB): m/z 644 $(MH)^+$ (calcd. 644)

Ethyl ester of 3′-Me-Azo: Silica gel column chromatography (hexane: AcOEt = 20 :1) (yield 50.7%): ^{1}H NMR [DMSO, 500 MHz] δ = 8.18 – 6.95 (m, 8H, aromatic protons of azobenzene), 4.39 (q, ^3J(H,H) = 7.0, 2H, -O-CH_2-CH_3), 2.44 (s, 3H, -NC_6H_4-CH_3), 1.37 (t, ^3J(H,H) = 7.0 Hz, 3H, -O-CH_2-CH_3)
Compound 1 of 3′-Me-Azo: Silica gel column chromatography (chloroform : MeOH = 20 : 1) (yield 97.5%): ^{1}H NMR [DMSO, 500 MHz] δ = 8.10- 7.42 (m, 9H, aromatic protons of azobenzene, -N*H*CO-), 4.67 (m, 2H, CH_3CH(O*H*)-, -(NH)CHCH_2(O*H*)), 3.97 (m, 2H, -CH_2(OH)C*H*(NH)CO-)-, C*H*(OH)CH_3), 3.64 and 3.53 (m, 2H, HOCH_2CH(NH)CO), 2.44 (s,3H, -NC_6H_4-CH_3), 1.09 (d, ^3J(H,H) = 6.0 Hz, CH(OH)CH_3)
Compound 2 of 3′-Me-Azo: Silica gel column chromatography (hexane : AcOEt : Et_3N = 50 : 50 : 3) (yield 45.3%): ^{1}H NMR [$CDCl_3$, 500 MHz] δ = 8.00 – 6.78 (m, 22H, aromatic protons of azobenzene, DMT, -N*H*CO-), 4.26 (m, 1H, -C*H*(OH)CH_3), 4.16 (m, 1H, -CH_2(ODMT)C*H*(NHCO-)-), 3.77 and 3.76 (s, 6H, -C_6H_4-OCH_3), 3.62 and 3.42 (dd, ^2J(H,H) = 9.5 Hz, ^3J(H,H) = 4.0 Hz, 2H, CH_2-ODMT), 2.48 (s, 3H, -NC_6H_4-CH_3), 1.24 (d, ^3J(H,H) = 6.5 Hz 3H, -CH(OH)CH_3)
MS(FAB): m/z 630 $(MH)^+$ (calcd. 630)

Ethyl ester of 4′-Me-Azo: Silica gel column chromatography (hexane: AcOEt = 5 :1) (yield 41.5%): ^{1}H NMR [DMSO, 500 MHz] δ = 8.17 – 7.43 (m, 8H, aromatic protons of azobenzene), 4.39 (q, ^3J(H,H) = 7.0, 2H, -O-CH_2-CH_3), 2.43 (s, 3H, -NC_6H_4-CH_3), 1.37 (t,^3J(H,H) = 7.0 Hz, 3H, -O-CH_2-CH_3)
4'-Me-Azo (yield quant): ^{1}H NMR [DMSO, 500 MHz] δ = 8.15 – 7.43 (m, 8H, aromatic protons of azobenzene), 2.43 (s, 3H, -NC_6H_4-CH_3)
Compound 1 of 4′-Me-Azo: Silica gel column chromatography (chloroform : MeOH = 20 : 1) (yield 99.1%): ^{1}H NMR [DMSO, 500 MHz] δ = 8.08- 7.42 (m, 9H, aromatic protons of azobenzene, -N*H*CO-), 4.66 (m, 2H, CH_3CH(O*H*)-, -(NH)CHCH_2(O*H*)), 3.96 (m, 2H, -CH_2(OH)C*H*(NH)CO-)-, C*H*(OH)CH_3), 3.64 and 3.53 (m, 2H, HOCH_2CH(NH)CO), 2.42 (s,3H, -NC_6H_4-CH_3), 1.08 (d, ^3J(H,H) = 6.5 Hz, CH(OH)CH_3)
Compound 2 of 4′-Me-Azo: Silica gel column chromatography (hexane : AcOEt : Et_3N = 50 : 50 : 3) (yield 32.2%): ^{1}H NMR [$CDCl_3$, 500 MHz] δ = 7.99 – 6.79 (m, 22H, aromatic protons of azobenzene, DMT, -N*H*CO-), 4.25 (m, 1H, -C*H*(OH)CH_3), 4.14 (m, 1H, -CH_2(ODMT)C*H*(NHCO-)-), 3.77 and 3.76 (s, 6H, -C_6H_4-OCH_3), 3.62 and 3.42 (dd, ^2J(H,H) = 9.5 Hz, ^3J(H,H) = 4.0 Hz, 2H, CH_2-ODMT), 2.46 (s, 3H, -NC_6H_4-CH_3), 1.24 (d, ^3J(H,H) = 6.0 Hz 3H, -CH(OH)CH_3)

MS(FAB): m/z 630 $(MH)^+$ (calcd. 630)

2-Me-Azo: Silica gel column chromatography (Hexane : AcOEt = 5 : 1) (yield 44.8%) ^{1}H NMR [DMSO, 500 MHz] δ = 8.01 – 7.59 (m, 8H, aromatic protons of azobenzene), 2.72 (s, 3H, -NC_6H_3-C*H*$_3$)

Compound 1 of 2-Me-Azo: Silica gel column chromatography (ethyl acetate) (yield 88.9%):^{1}H NMR [DMSO, 500 MHz] δ = 7.96 - 7.60 (m, 9H, aromatic protons of azobenzene, -N*H*CO-), 4.66 (m, 2H, CH_3CH(O*H*)-, -(NH)CHCH_2(O*H*)), 3.96 (m, 2H, -CH_2(OH)C*H*(NH)CO-)-, C*H*(OH)CH_3), 3.64 and 3.57 (m, 2H, HOCH_2CH(NH)CO), 2.73 (s,3H, -NC_6H_3-C*H*$_3$), 1.08 (d, ^3J(H,H) = 6.5 Hz, CH(OH)C*H*$_3$)

Compound 2 of 2-Me-Azo: Silica gel column chromatography (hexane : AcOEt : Et_3N = 60 : 40 : 3) (yield 42.6%): ^{1}H NMR [$CDCl_3$, 500 MHz] δ = 7.97 – 6.78 (m, 22H, aromatic protons of azobenzene, DMT, -N*H*CO-), 4.26 (m, 1H, -C*H*(OH)CH_3), 4.16 (m, 1H, -CH_2(ODMT)C*H*(NHCO-)-), 3.77 and 3.76 (s, 6H, -C_6H_4-OC*H*$_3$), 3.62 and 3.40 (dd, ^2J(H,H) = 9.5 Hz, ^3J(H,H) = 4.0 Hz, 2H, CH_2-ODMT), 2.78 (s, 3H, -NC_6H_3-C*H*$_3$), 1.24 (d, ^3J(H,H) = 6.5 Hz 3H, -CH(OH)C*H*$_3$)

MS(FAB): m/z 630 $(MH)^+$ (calcd. 630)

Ethyl ester of 3′,5′-Me-Azo: Silica gel column chromatography (hexane: AcOEt = 20 :1) (yield 30.4%): ^{1}H NMR [$CDCL_3$, 500 MHz] δ = 8.20 – 7.14 (m, 7H, aromatic protons of azobenzene), 4.43 (q, ^3J(H,H) = 7.0, 2H, -O-CH_2-CH_3), 2.41 (s ,6H, -NC_6H_3(C*H*$_3$)$_2$), 1.43 (t.^3J(H,H) = 7.0 Hz, 3H, -O-CH_2-C*H*$_3$)

3′,5′-Me-Azo (yield 74.4%): ^{1}H NMR [$CDCl_3$, 500 MHz] δ = 8.28 – 7.17 (m, 7H, aromatic protons of azobenzene), 2.44 (s, 6H, -NC_6H_3(C*H*$_3$)$_2$)

Compound 1 of 3′,5′-Me-Azo: Silica gel column chromatography (chloroform : MeOH = 20 : 1) (yield quant): ^{1}H NMR [$CDCl_3$, 500 MHz] δ = 7.98 - 6.99 (m, 7H, aromatic protons of azobenzene), 4.34 (m, 1H, CH_3C*H*(OH)-), 4.11 (m, 1H, -CH_2(OH)C*H*(NH)CO-)-), 3.99 (m, 2H, HOCH_2CH(NH)CO-), 2.43 (s, 6H, -NC_6H_3(C*H*$_3$)$_2$), 1.31 (d, ^3J(H,H) = 6.5 Hz, CH(OH)C*H*$_3$)

Compound 2 of 3′,5′-Me-Azo: Silica gel column chromatography (hexane : AcOEt : Et_3N = 50 : 50 : 3) (yield 28.5%): ^{1}H NMR [$CDCl_3$, 500 MHz] δ = 7.99 – 6.78 (m, 21H, aromatic protons of azobenzene, DMT, -N*H*CO-), 4.26 (m, 1H, -C*H*(OH)CH_3), 4.16 (m, 1H, -CH_2(ODMT)C*H*(NHCO-)-), 3.77 and 3.76 (s, 6H, -C_6H_4-OC*H*$_3$), 3.62 and 3.42 (dd, ^2J(H,H) = 9.5 Hz, ^3J(H,H) = 4.0 Hz, 2H, CH_2-ODMT), 2.43 (s, 6H, -NC_6H_3(C*H*$_3$)$_2$), 1.24 (d, ^3J(H,H) = 6.5 Hz 3H, -CH(OH)C*H*$_3$)

Ethyl ester of 2′,6′-Me-Azo: Silica gel column chromatography (hexane: AcOEt = 20 :1) (yield 26.6%): ^{1}H NMR [DMSO, 500 MHz] δ = 8.22 – 7.24 (m, 7H, aromatic protons of azobenzene), 4.43 (q, ^3J(H,H) = 7.0, 2H, -O-CH_2-CH_3), 2.39 (s,6H, -NC_6H_3(C*H*$_3$)$_2$), 1.41 (t, ^3J(H,H) = 7.0 Hz, 3H, -O-CH_2-C*H*$_3$)

2′,6′-Me-Azo (yield 94.7%): ^{1}H NMR for **3** [DMSO, 500 MHz] δ = 8.20 – 7.00 (m, 7H, aromatic protons of azobenzene), 2.38 (s, 6H, -NC_6H_3(C*H*$_3$)$_2$)

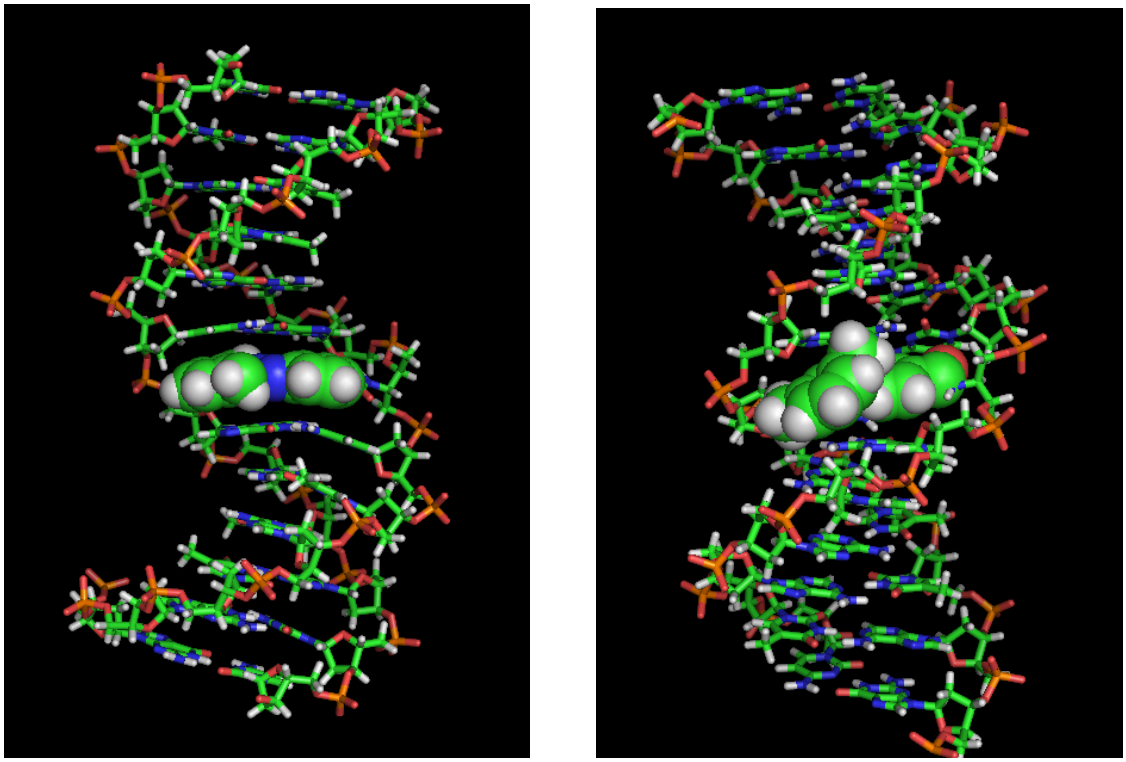
Compound 1 of 2′,6′-Me-Azo: Silica gel column chromatography (chloroform : MeOH = 10 : 1) (yield

95.5%): ^{1}H NMR [$CDCl_3$, 500 MHz] δ = 8.00 – 6.97 (m, 8H, aromatic protons of azobenzene, -N*H*CO-), 4.35 (m, 1H, CH_3C*H*(OH)-), 4.12 (m, 1H, -CH_2(OH)C*H*(NH)CO-)-), 4.00 (m, 2H, HOC*H_2*CH(NH)CO-), 2.39 (s, 6H, -NC_6H_3(C*H_3*)$_2$), 1.31 (d, ^{3}J(H,H) = 6.5 Hz, CH(OH)C*H_3*)

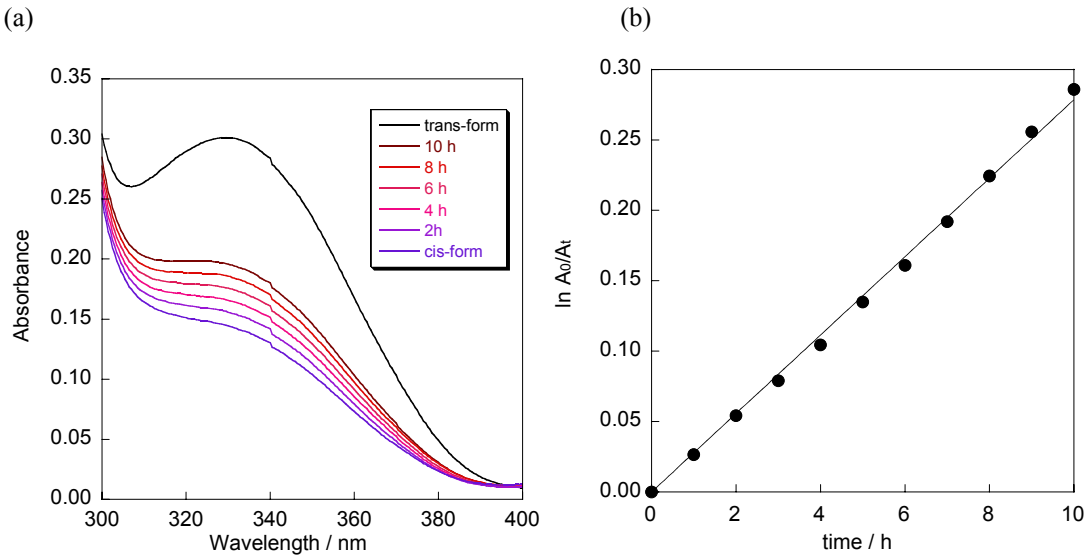
Compound 2 of 2′,6′-Me-Azo: Silica gel column chromatography (hexane : AcOEt : Et_3N = 50 : 50 : 3) (yield 53.2%): ^{1}H NMR [$CDCl_3$, 500 MHz] δ = 7.96 – 6.76 (m, 21H, aromatic protons of azobenzene, DMT, -N*H*CO-), 4.25 (m, 1H, -C*H*(OH)CH_3), 4.16 (m, 1H, -CH_2(ODMT)C*H*(NHCO-)-), 3.77 and 3.76 (s, 6H, -C_6H_4-OC*H_3*), 3.63 and 3.42 (dd, ^{2}J(H,H) = 9.5 Hz, ^{3}J(H,H) = 4.0 Hz, 2H, C*H_2*-ODMT), 2.41 (s, 6H, -NC_6H_3(C*H_3*)$_2$), 1.24 (d, ^{3}J(H,H) = 6.5 Hz 3H, -CH(OH)C*H_3*)

MS(FAB): m/z 644 $(MH)^+$ (calcd. 644)

(a) (b)



Supplemental Figure 1. Energy-minimized structures of **Da/Dc** duplex involving **2',6'-Me-Azo** (CPK part) either (a) in the *trans* or (b) *cis*-form calculated with InsightII/Discover.



Supplemental Figure 2. Change of UV-Vis spectra (a) by the thermal *cis*→*trans* isomerization of **2',6'-Me-Azo** tethered on **Da** ([**Da**] = 20 μM) at 60 °C in the presence of 0.1 M NaCl at pH 7.0 (10 mM phosphate buffer), and linear plots of *ln* A_0/A_t (b) as a function of time where A_0 represents initial absorbance at 334 nm and A_t represents the absorbance at a particular time.