

SYNTHESIS AND DUPLEX-FORMING PROPERTY OF OLIGONUCLEOTIDES BEARING A NOVEL POLYAMINE-MODIFIED INTERCALATOR AT THE TERMINAL OR THE INTERNAL POSITION

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□ *Novel oligonucleotides bearing a polyamine-intercalator conjugate modified at the terminal or the internal position were reported. These modified oligonucleotides showed duplex-stabilization effect, and the thermodynamic analysis and the salt concentration dependency of the duplex stability revealed that the polyamine moiety also acted as the duplex stabilizer by neutralization of the phosphate negative charge.*

Keywords Anthraquinone; Antisense DNA; Intercalator

INTRODUCTION

Oligonucleotides capable of forming a stable duplex with its complementary mRNA have been attracting great interest since they would prevent the expression of certain gene functions responsible for disease through the antisense-mechanism.^[1,2] Although the antisense effect would depend on various factors, such as the nuclease-resistant property and cell permeability of the antisense oligomer, the most basic feature to bring about the effect is, naturally, the stability of the complementary duplex formed between the antisense oligomer and the target mRNA.^[3,4] Thus, the duplex-stabilizing ability of the antisense oligomer toward its complement is the vital factor to achieve efficient gene-silencing in the antisense methodology.

One common method to increase the duplex-stabilizing ability of an antisense oligomer is to covalently introduce an intercalative moiety to the oligomer.^[5,6] For example, several modified oligonucleotides bearing an

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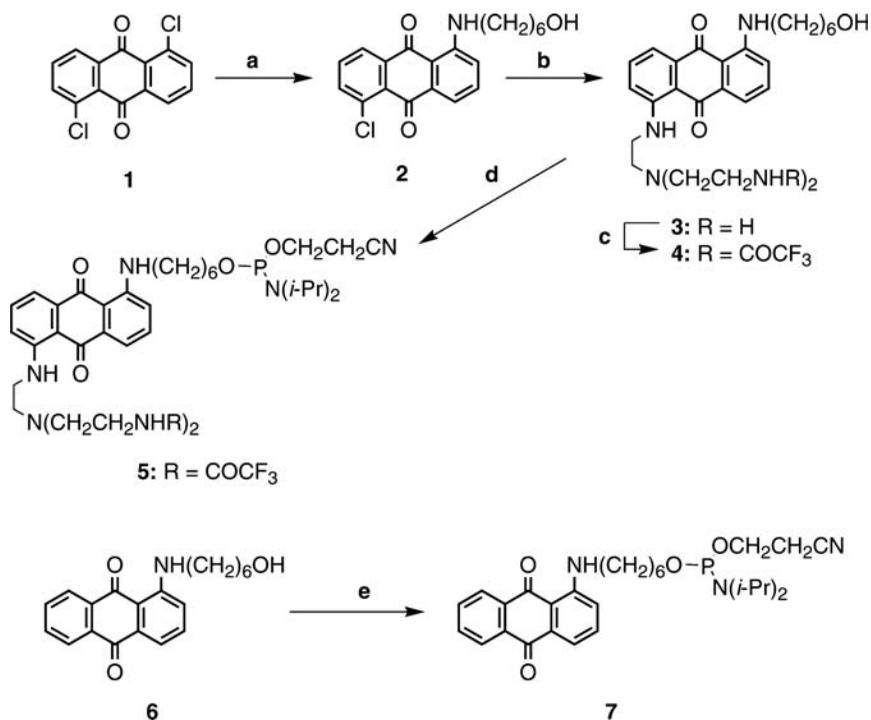
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This article is dedicated to Professor Eiko Ohtsuka on the occasion of her 70th birthday.

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anthraquinone moiety have been synthesized to show improved duplex-stabilizing ability (higher T_m values) compared to their unmodified counterparts.^[7] Meanwhile, it is also well known that the incorporation of some cationic moieties, such as polyamine moieties, to oligonucleotide brings about enforcement of the ability.^[8–14] These facts suggest that the simultaneous introduction of both an intercalative moiety and a polyamine moiety to oligonucleotide would bring about substantial enhancement of its duplex-stabilizing ability. However, reports dealing with the combination of both an intercalative moiety and a polyamine moiety on the duplex stability are quite rare. This prompted us to synthesize novel anthraquinone-polyamine conjugates. The conjugates were successfully incorporated into either the C-5 position of certain pyrimidine nucleoside locating at the middle portion of the sequence or the 5'-OH terminus of oligonucleotide. Here, we would like to report the synthesis of the modified oligodeoxynucleotides bearing the conjugates and their duplex-stabilizing ability including the results of thermodynamic analysis of the duplex.

The 5'-terminal modified oligonucleotide was prepared by the successive solid-phase DNA synthesis strategy, and we needed to prepare the phosphoramidite of the anthraquinone-polyamine derivatives (Scheme 1).



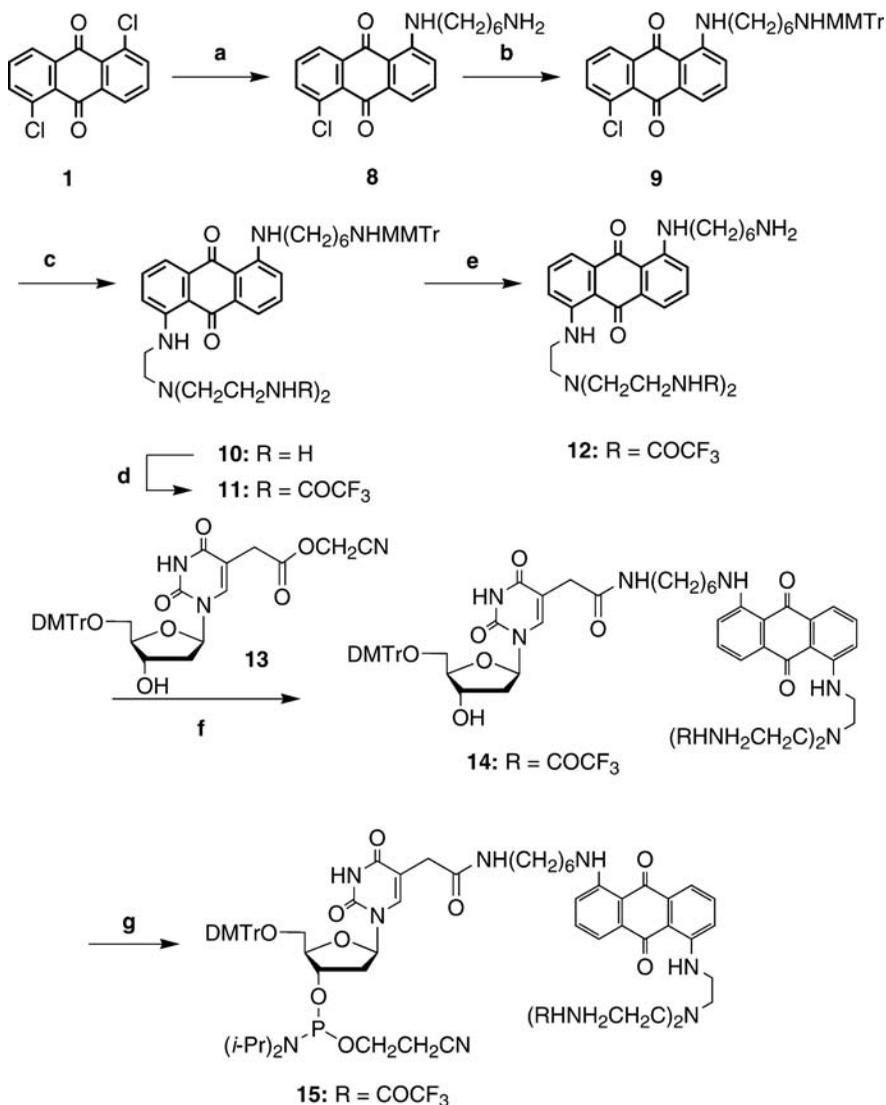
SCHEME 1 a. H₂N(CH₂)₆OH, xylene-pyridine (3:1, v/v), reflux, 11 h, 67%. b. (NH₂CH₂CH₂)₃, pyridine, 100°C, 3 h, 83%. c. CF₃C(O)OEt, Et₃N, MeOH, r.t., 5 h, 98%. d. NCCH₂CH₂OPCIN(*i*-Pr)₂, (*i*-Pr)₂NEt, CH₂Cl₂, r.t., 30 min, 35%. e. NCCH₂CH₂OPCIN(*i*-Pr)₂, (*i*-Pr)₂NEt, CH₂Cl₂, r.t., 30 min, 55%.

The anthraquinone-polyamine conjugate (**3**) was synthesized from 1,5-dichloroanthraquinone (**1**). In brief, 1,5-dichloroanthraquinone was reacted with 6-amino-1-hexanol in a mixture of pyridine/toluene to give the mono-reacted compound (**2**) as the main product. **2** was reacted with tris(2-aminoethyl)amine in pyridine to give the anthraquinone-polyamine conjugate (**3**). After protection of the primary amine moieties with trifluoroacetyl function by the reaction of trifluoroacetic acid ethyl ester, compound **3** was converted to the corresponding phosphoramidite derivative (**5**) in the usual manner. It should be noted that the phosphoramidite **5** is somewhat unstable under storage; therefore, it was used immediately after the purification with silica-gel column chromatography. For the synthesis of ODN-2, non-polyamine conjugated anthraquinone derivative^[15] (**6**) was also converted to the corresponding phosphoramidite derivative (**7**) in the usual manner. The incorporation of **5** and **7** into oligonucleotide were carried out the same as the incorporation of normal nucleoside phosphoramidites. However, extended coupling period (360 s) was needed to achieve the satisfactory coupling yield estimated by the usual trityl-color assay.

Another modified oligonucleotide, namely the anthraquinone-polyamine conjugate incorporated at the middle position of oligonucleotide, was prepared by the post-synthetic procedure according to our previous report (Scheme 2). The reaction of 1,5-dichloroanthraquinone with hexamethylenediamine in xylene gave 5-(6-aminohexylamino)-1-chloroanthraquinone (**8**) as the main product. After protection of the primary amine moiety with a monomethoxytrityl group (MMTr), **9** was reacted with tris(2-aminoethyl)amine in toluene to give the anthraquinone-polyamine conjugate (**10**). The primary amine moieties of **10** were protected with the trifluoroacetyl function as described above and the resultant **11** was treated with trichloroacetic acid to remove the MMTr group to afford compound **12**.

The incorporation of **11** into the C-5 position of the pyrimidine base was hardly achievable, and was carried out by treatment with a 0.15 M solution of compound **12** in dimethylacetamide after standard DNA synthesis. However, no satisfactory result was given in spite of the investigation of various reaction conditions. Therefore, the modified nucleoside, namely the conjugate introduced at the 5-position of the deoxyuridine derivative, was prepared in advance, and the phosphoramidite of this modified nucleoside derivative was applied to the DNA synthesizer.

The previously prepared compound **12** was allowed to react with the 5-cyanomethoxycarbonylmethyl-2'-deoxyuridine derivative (**13**) to give the successfully introduced product (**14**) in 72% yield. The phosphitylation of **14** by the standard method gave the phosphoramidite derivative **15**; however, purification of **15** by silica gel column chromatography was difficult due to the rapid decomposition in the purification step. Therefore, the given



SCHEME 2 a. H₂N(CH₂)₆NH₂, xylene, 110°C, 2 h, 63%. b. MMTTrCl, CH₂Cl₂, r.t., 1 h, 98%. c. (H₂NCH₂CH₂)₃N, toluene, reflux, 2 h, 77%. d. CF₃C(O)OEt, Et₃N, CH₂Cl₂, r.t., 5 h, 98%. e. 10% TCA/CH₂Cl₂, 1 h, 89%. f. **13**, triazole, DMA, 40°C, 24 h, 72%. g. NCCH₂CH₂OPCIN(*i*-Pr)₂, (*i*-Pr)₂NEt, CH₂Cl₂, r.t., 87%.

phosphoramidite derivative was applied to the DNA synthesizer after the simple purification, reprecipitation by hexane.

The incorporation into DNA was carried out following the modified procedure on DNA synthesizer, the extended coupling period (360 s) and the twice coupling reaction. The modified DNA was given after the standard deprotection procedure, and the structure of the synthesized modified DNA was confirmed by the ESI-mass,^[16] and the introduction of the

TABLE 1 Melting Temperature of the Modified DNA

	ODN-N	ODN-1	ODN-2	ODN-3	ODN-4
T_m ($^{\circ}\text{C}$)	63.3	68.4	68.3	67.7	68.5
ΔT_m ($^{\circ}\text{C}$)	—	+5.1	+5.0	+4.4	+5.2

anthraquinone moiety was also confirmed by the comparison of the ratio of the absorption maximum of the 260 nm and 554 nm, the absorption maximum of anthraquinone, with the calculated value. The structure and sequence of the synthesized oligonucleotides are shown in Figure 1. ODN-1 and -2 represent the oligonucleotides bearing non-trisamine conjugated anthraquinone derivatives. ODN-3 and 4 represent the oligonucleotides bearing the anthraquinone-trisamine conjugates. Moreover, ODN-1 and -3 are introduced the intercalative moiety at the 5'-terminus of oligonucleotides, whereas ODN-2 and -4 are introduced the intercalative moiety at the central position of the oligonucleotides.

Next, to evaluate the stabilizing effect of the anthraquinone-polyamine conjugates toward the duplex DNA, the thermal property and the thermodynamic property were discussed. At first, the thermal property was evaluated by the comparison of the melting temperature (T_m) of the duplex of the modified DNA and the corresponding complementary DNA. The thermal stability of the duplexes containing modified oligonucleotides was measured under the following physiological conditions; each oligonucleotide was dissolved in 10 mM phosphate buffer containing 100 mM NaCl, and the oligonucleotide concentration was 2.5 μM . Table 1 shows that all the modified ODN stabilized the duplex formed with the complementary DNA, and the magnitude of the stabilization effects was almost the same as that of the non-polyamine conjugates. Moreover, no difference in thermal stability of the

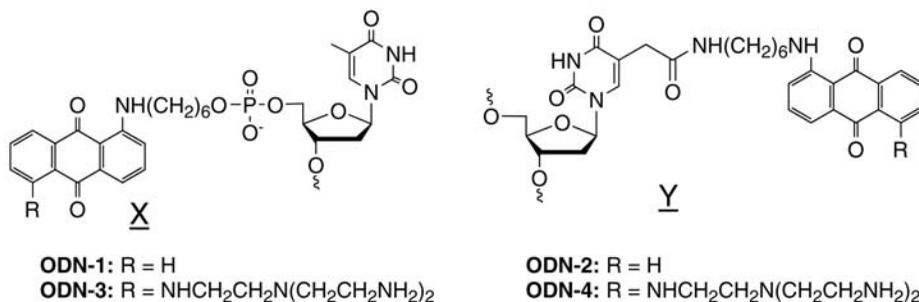
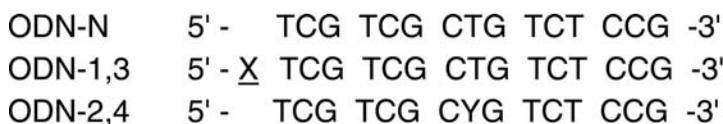
**FIGURE 1** Sequence and structure of the oligonucleotides in this work.

TABLE 2 Thermodynamic Parameter of the Modified DNA

	ODN-N	ODN-1	ODN-2	ODN-3	ODN-4
ΔH (kcal/mol)	-85.9	-101.4	-108.8	-133.6	-126.3
ΔS (cal/mol · K)	-227.1	-274.2	-290.0	-363.2	-341.6
ΔG (kcal/mol)	-18.7	-19.6	-22.3	-25.3	-24.4

duplexes between the positions of the incorporation of the anthraquinone moiety was observed. This enhanced stabilization was due to the introduction of the anthraquinone moiety into the oligonucleotide; however, in this thermal profile experiment, no effect of the polyamine moiety was observed.

Next, in order to discuss the effect of the polyamine moiety on the duplex stability, the thermodynamic analysis was explored (Table 2). The thermodynamic parameter was calculated from the slope of the $1/T_m$ vs. $\ln(C_i/4)$ after the T_m experiment at various oligonucleotide concentrations (0.63–10 μM). All data was approximated to the first functional lines by high correlation coefficient (Figure 2). The ΔH values were reduced about 30 kcal/mol with the introduction of the anthraquinone moiety, and this indicated the anthraquinone moiety stabilized the duplex DNA. Moreover, the additive increase of the ΔH values by the introduction of the polyamine moiety was also observed. However, the loss of the ΔS values was also observed. The reason for the ΔS loss seemed to be the enhancement of the structural rigidity by the introduction of the polyamine moiety.

Totally, the introduction of an anthraquinone moiety and a polyamine moiety seems to promote the stabilization of the duplex DNA thermodynamically.

In order to estimate the effect of the polyamine moiety, the salt concentration dependency against duplex stability was discussed. The relationship between $1/T_m$ and $\ln(\text{Na}^+)$ of all duplexes is shown in Figure 3. The thermal

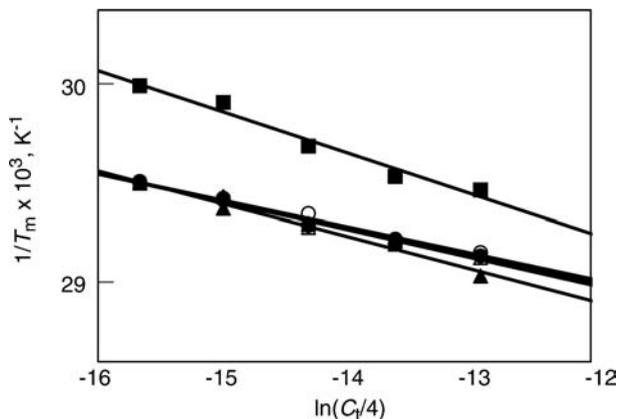


FIGURE 2 Plots of $1/T_m$ versus $\ln(C_i/4)$. Closed squares, closed circles, closed triangles, open circles, and open triangles represent ODN-N, -1, -2, -3, and -4, respectively.

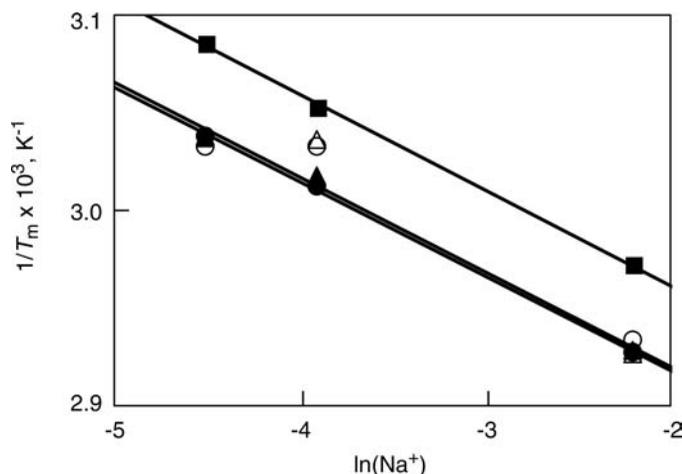


FIGURE 3 Plots of T_m^{-1} versus $\ln(\text{Na}^+)$. Closed squares, closed circles, closed triangles, open circles, and open triangles represent ODN-N, -1, -2, -3, and -4, respectively.

stability of no polyamine-tethering ODNs (ODN-N, -1, and -2) decreased according to the decrease of the NaCl concentration. However, the polyamine-tethering ODNs (ODN-3 and -4) showed the independency of the duplex stability against low salt concentration. Therefore, the polyamine moiety stabilizes the duplex DNA by the neutralization of the negative charge of the phosphate backbone.

In conclusion, we have synthesized a novel modified DNA bearing the anthraquinone-polyamine conjugate attached at the 5'-terminus or internal position of the DNA. The modified DNA stabilized the DNA duplex forming with the complementary DNA, and the thermodynamic analysis indicated that the intercalative moiety and the cationic moiety act as stabilizers for the duplex formation.

EXPERIMENTAL

General Procedures

CH_2Cl_2 , MeCN and triethylamine were freshly distilled from CaH_2 , after being refluxed several hours. Pyridine was distilled from CaH_2 , after being refluxed several hours, and stored over KOH pellet. ^1H NMR was obtained at 300 MHz with a JEOL-AL-300 spectrometer with tetramethylsilane (TMS) as an internal standard in CDCl_3 . ^{31}P NMR was obtained at 121 MHz with a JEOL-AL-300 spectrometer with 85% H_3PO_4 as an external standard in all solvents. UV spectra were recorded with a Shimadzu UV-2450 spectrophotometer. Reverse-phase HPLC was performed on a JASCO PU-2089 gradient pump system with a Wakosil 5C18(10 ml \times 250 mm). ESI-TOF mass spectrometry was obtained with a Perkin Elmer Sciex API-100.

1-Chloro-5-(6-hydroxyhexylamino)anthraquinone (2). 1,5-Dichloroanthraquinone (800 mg, 2.89 mmol) and 6-aminohexanol (778 mg, 6.63 mmol) were dissolved in xylene-pyridine (8 ml, 3:1 (v/v)), and the mixture was refluxed for 11 h. After cooling to room temperature, the reaction mixture was diluted with CH₂Cl₂ (50 ml), washed twice with 0.1 M KOH and once with brine, and the organic layer was dried over Na₂SO₄, filtered, and concentrated to dryness. The residue was purified by silica gel column chromatography (10% MeOH/CH₂Cl₂) to give the desired compound **2** (696 mg, 67%) as a dark red solid. ¹H NMR (CDCl₃): 8.10–8.18 (1H, d, ArH), 7.38–7.63 (4H, m, ArH), 6.85–6.92 (1H, m, ArH), 3.55–3.65 (2H, t, CH₂-OH), 3.15–3.25 (2H, q, CH₂-NH) 1.30–1.75 (8H, m, (CH₂)₄).

1-[2-[N,N-Bis-[(2-aminoethyl)amino]ethylamino]-5-(6-hydroxyhexylamino)-anthraquinone (3). Compound **2** (450 mg, 1.25 mmol) was dissolved in dry pyridine (15 ml), and to this solution was added *tris*(2-aminoethyl)amine (1.0 ml, 6.68 mmol), and stirred at 100°C for 3 h. After cooling to room temperature, the reaction mixture was diluted with CH₂CH₂ and washed twice with H₂O, and the organic layer was dried over Na₂SO₄, filtered, and concentrated to dryness. The residue was purified by silica gel column chromatography (CH₂Cl₂:MeOH:NH₃aq = 4:1:0.2 (v/v/v)) to give the desired compound **3** (483 mg, 83%) as a dark red solid. ¹H NMR (CDCl₃): 7.40–7.56 (4H, m, ArH), 6.84–6.91 (2H, m, ArH), 3.55–3.65 (2H, t, CH₂-OH), 3.30–3.36 (2H, q, TAEA), 3.20–3.24 (2H, q, CH₂-NH), 2.75–2.83 (6H, m, TAEA), 2.55–2.60 (4H, m, TAEA), 1.30–1.75 (8H, m, (CH₂)₄).

1-[2-[N,N-Bis-[(2-trifluoroacetamidoethyl)amino]ethylamino]-5-(6-hydroxyhexylamino)anthraquinone (4). Compound **3** (229 mg, 0.49 mmol) was dissolved in MeOH (7 ml), and to this solution were added ethyl trifluoroacetate (0.30 ml, 2.52 mmol) and triethylamine (0.35 ml, 2.52 mmol). After being stirred at room temperature for 5 h, the reaction mixture was concentrated to dryness. The residue was purified by silica gel column chromatography (10% MeOH/CH₂Cl₂) to give the desired compound **4** (315 mg, 98%) as a dark red solid. ¹H NMR (CDCl₃): 7.50–7.66 (4H, m, ArH), 6.98–7.05 (1H, m, ArH), 6.89–6.93 (1H, m, ArH), 3.64–3.71 (2H, t, CH₂-OH), 3.42–3.49 (4H, m, TAEA), 3.29–3.34 (2H, m, CH₂-NH,TAEA), 2.85–2.92 (2H, t, TAEA), 2.74–2.79 (4H, m, TAEA), 1.22–1.84 (8H, m, (CH₂)₄).

2-Cyanoethyl 6-[1-[2-[N,N-Bis-[(2-trifluoroacetamidoethyl)amino]ethylamino]anthraquinone-5-yl]amino]hexylN,N-Diisopropylphosphoramidite (5). Compound **4** (224 mg, 0.34 mmol) and *N,N*-diisopropylethylamine (0.20 ml, 1.18 mmol) were dissolved in dry CH₂Cl₂ (6 ml), and to this solution was added 2-cyanoethyl *N,N*-diisopropyl phosphorochloridite (0.20 ml, 0.90 mmol) at 0°C. After being stirred at room temperature for 30 min, the reaction mixture was diluted with CH₂Cl₂, washed twice with 5% NaHCO₃aq and once with brine, and the organic layer was dried over Na₂SO₄, filtered, and concentrated to dryness. The residue was purified by silica gel column chromatography (CH₂Cl₂:ethyl acetate: triethylamine = 45:45:10, (v/v/v))

to give the desired compound **5** (103 mg, 35%) as a dark red solid. ^{31}P NMR (CDCl_3):147.83.

2-Cyanoethyl 6-(Anthraquinone-1-yl)aminohexyl N,N-Diisopropylphosphoramidite (7). 1-(6-hydroxyhexylamino)anthraquinone^[15] (**6**) (120 mg, 0.37 mmol) and *N,N*-diisopropylethylamine (0.12 ml, 0.74 mmol) were dissolved in dry CH_2Cl_2 (3 ml), and to this solution was added 2-cyanoethyl *N,N*-diisopropyl phosphorochloridite (0.12 ml, 0.56 mmol) at 0°C . After being stirred at room temperature for 30 min, the reaction mixture was diluted with CH_2Cl_2 , washed twice with 5% NaHCO_3 aq and once with brine, and the organic layer was dried over Na_2SO_4 , filtered, and concentrated to dryness. The residue was purified by silica gel column chromatography (CH_2Cl_2 :ethyl acetate:triethylamine = 45:45:10, (v/v/v)) to give the desired compound **7** (107 mg, 55%) as a dark red solid. ^{31}P NMR (CDCl_3):148.01.

1-Chloro-5-(6-aminohexylamino)anthraquinone (8). 1,5-Dichloroanthraquinone (**1**) (1.00 g, 3.61 mmol) and 1,6-hexamethylenediamine (965 mg, 8.30 mmol) were dissolved in xylene (7 ml), and this solution was stirred at 110°C for 2 h. After cooling to room temperature, the reaction mixture was diluted with CH_2Cl_2 , washed twice with 0.1 M KOH and once with brine, and the organic layer was dried over Na_2SO_4 , filtered, and concentrated to dryness. The residue was purified by silica gel column chromatography (CH_2Cl_2 : MeOH: NH_3 aq = 4:1:0.2 (v/v/v)) to give the desired compound **6** (814 mg, 63%) as a dark red solid. ^1H NMR (CDCl_3): 7.50–7.60 (1H, m, ArH), 7.25–7.30 (4H, m, ArH), 6.94–6.98 (2H, dd, ArH), 3.30–3.30 (2H, q, CH_2 -NH), 2.65–2.75 (2H, m, CH_2 -NH), 1.30–1.75 (8H, m, $(\text{CH}_2)_4$).

1-Chloro-5-[6-[(monomethoxytrityl)amino]hexylamino]anthraquinone (9). Compound **8** (726 mg, 2.03 mmol) and monomethoxytrityl chloride (972 mg, 3.15 mmol) were dissolved in dry CH_2Cl_2 (10 ml), and this solution was stirred at room temperature for 1 h. The reaction mixture was diluted with CH_2Cl_2 , washed three times with H_2O , and the organic layer was dried over Na_2SO_4 , filtered, and concentrated to dryness. The residue was purified by silica gel column chromatography (CH_2Cl_2 : MeOH: hexane: triethylamine = 90:3:6:1 (v/v/v/v)) to give the desired compound **9** (1.25 g, 98%) as a dark red solid. ^1H NMR (CDCl_3): 8.24–8.27 (1H, dd, ArH), 7.16–7.70 (16H, m, ArH), 6.97–7.02 (1H, m, ArH), 6.77–6.82 (2H, d, ArH), 3.76 (3H, s, OCH_3), 3.25–3.32 (2H, q, CH_2 -NH), 2.12–2.17 (4H, m, CH_2 -NH), 1.43–1.73 (8H, m, $(\text{CH}_2)_4$).

1-[2-[N, N-Bis-(2-aminoethyl)amino]ethylamino]-5-[6-[(monomethoxytrityl)amino]hexylamino]anthraquinone (10). Compound **9** (456 mg, 0.73 mmol) and tris(2-aminoethyl)amine (1.0 ml, 6.7 mmol) were dissolved in toluene (12 ml), and this solution was refluxed for 2 h. After cooling to room temperature, the reaction mixture was diluted with CH_2Cl_2 , washed three times with H_2O , and the organic layer was dried over Na_2SO_4 , filtered, and concentrated to dryness. The residue was purified by silica gel column chromatography (CH_2Cl_2 : MeOH: NH_3 aq = 4:1:0.2 (v/v/v)) to give the desired

compound **10** (408 mg, 77%) as a dark red solid. $^1\text{H NMR}$ (CDCl_3) δ 7.16–7.54 (16H, m, ArH), 6.90–6.93 (2H, m, ArH), 6.78–6.81 (2H, m, ArH), 3.76 (3H, s, OCH_3), 3.34–3.40 (2H, q, TAEA), 3.23–3.29 (2H, q, $\text{CH}_2\text{-NH}$), 2.78–2.85 (6H, m, TAEA), 2.57–2.61 (4H, m, TAEA), 2.10–2.16 (2H, t, $\text{CH}_2\text{-NH}$), 1.40–1.72 (8H, m, $(\text{CH}_2)_4$).

1-[2-[N,N-Bis-(2-trifluoroacetamidoethyl)amino]ethylamino]-5-[6-(monomethoxytritylamino)hexylamino]anthraquinone (11). Compound **10** (408 mg, 0.55 mmol) was dissolved in dry CH_2Cl_2 (12 ml), and to this solution were added ethyl trifluoroacetate (0.34 ml, 2.8 mmol) and triethylamine (0.39 ml, 2.8 mmol). After being stirred at room temperature for 5 h, the reaction mixture was concentrated to dryness. The residue was purified by silica gel column chromatography (10% MeOH/ CH_2Cl_2) to give the desired compound **11** (501 mg, 98%) as a dark red solid. $^1\text{H NMR}$ (CDCl_3): 7.13–7.61 (16H, m, ArH), 6.98–7.01 (1H, m, ArH), 6.88–6.91 (1H, m, ArH), 3.80 (3H, s, (OCH_3)), 3.42–3.47 (4H, t, $\text{CH}_2\text{-NH}$), 3.27–3.36 (4H, m, $\text{CH}_2\text{-NH}$), 2.86–2.90 (2H, t, $\text{CH}_2\text{-NH}$), 2.74–2.77 (4H, t, $\text{CH}_2\text{-N}$), 2.10–2.16 (2H, t, $\text{CH}_2\text{-NH}$), 1.40–1.72 (8H, m, $(\text{CH}_2)_4$).

1-[2-[N,N-Bis-(2-trifluoroacetamidoethyl)amino]ethylamino]-5-(6-aminohexylamino)anthraquinone (12). Compound **11** was treated with 10% trichloroacetic acid in CH_2Cl_2 (10 ml) solution at room temperature for 1 h. The reaction mixture was diluted with CH_2Cl_2 , and washed twice with 5% NaHCO_3 and once with brine, and the organic layer was dried over Na_2SO_4 , filtered, and concentrated to dryness. The residue was purified by silica gel column chromatography (CH_2Cl_2 : MeOH: NH_3aq = 4:1:0.2 (v/v/v)) to give the desired compound **12** (314 mg, 89%) as a dark red solid. $^1\text{H NMR}$ (CDCl_3): 7.40–7.65 (4H, m, ArH) 6.88–6.91 (1H, m, ArH), 6.78–6.84 (1H, m, ArH), 3.44–3.45 (4H, m, TAEA), 3.26–3.33 (4H, m, $\text{CH}_2\text{-NH}$, TAEA), 2.85–2.88 (2H, t, TAEA), 2.73–2.76 (6H, m, TAEA, $\text{CH}_2\text{-NH}$), 1.41–1.74 (8H, m, $(\text{CH}_2)_4$).

1-[N-[6-[5-[2-[N,N-Bis-(2-trifluoroacetamidoethyl)amino]ethylamino]anthraquinone-5-yl]amino]hexyl]carbamoylmethyl]-2'-deoxyuridine (14). Compound **12** (674 mg, 1.02 mmol), 1*H*-1,2,4-triazole (64 mg, 0.93 mmol) and 5-cyanomethoxycarbonylmethyl-2'-deoxyuridine (**13**) (150 mg, 0.24 mmol) were dissolved in *N,N*-dimethylacetamide (10 ml), and this solution was stirred at 40°C for 24 h. The reaction mixture was diluted with ethyl acetate, and washed three times with H_2O , and the organic layer was dried over Na_2SO_4 , filtered, and concentrated to dryness. The residue was purified by silica gel column chromatography (10% 2-propanol in ethyl acetate containing 2% triethylamine) to give the desired compound **14** (210 mg, 72%) as a dark red solid. $^1\text{H NMR}$ (CDCl_3): 7.82 (1H, s, *H*6), 7.40–7.65 (4H, m, ArH), 7.18–6.92 (13H, m, DMTr), 6.88–6.91 (1H, m, ArH), 6.78–6.84 (1H, m, ArH), 6.45 (1H, t, *H*1'), 4.70–4.65 (1H, m, *H*3'), 4.15 (1H, m, *H*4'), 3.80 (6H, s, DMTr- OCH_3), 3.44–3.45 (4H, m, TAEA), 3.40 (2H, dd, *H*5'),

3.26–3.33 (4H, m, $\text{CH}_2\text{-NH}$, TAEA), 2.85–2.88 (2H, t, TAEA), 2.73–2.76 (4H, m, TAEA), 2.65–2.75 (2H, m, $\text{CH}_2\text{-NH}$), 2.58–2.40 (4H, m, $\text{H}2'$ and $\text{-CH}_2\text{-}$), 1.41–1.74 (8H, m, $(\text{CH}_2)_4$).

2-Cyanoethyl 1-[*N*-[6-[5-[2-[*N,N*-Bis-(2-trifluoroacetamidoethyl)amino]ethylamino]anthraquinone-5-yl]amino]hexyl]carbamoylethyl]-2'-deoxyuridine-3'-yl *N,N*-diisopropylphosphoramidite (**15**). Compound **14** (166 mg, 0.14 mmol) and *N,N*-diisopropylethylamine (69 μl , 0.41 mmol) were dissolved in dry CH_2Cl_2 (6 ml), and to this solution was added 2-cyanoethyl *N,N*-diisopropyl phosphorochloridite (75 μl , 0.34 mmol) at 0°C . After being stirred at room temperature for 30 min, the reaction mixture was diluted with CH_2Cl_2 , washed twice with 5% NaHCO_3 aq and once with brine, and the organic layer was dried over Na_2SO_4 , filtered, and concentrated to dryness. The residue was dissolved in small amounts of CH_2Cl_2 , reprecipitated into hexane, and the resultant precipitate was collected by filtration to give the desired compound **15** (167 mg, 87%) as a dark red solid. ^{31}P NMR (CDCl_3): 148.7, 149.0.

Oligonucleotides Synthesis

Oligonucleotides were synthesized on an Applied Biosystems 392A DNA/RNA synthesizer on a 1 μmol scale, using the canonical phosphoramidites from Glen Research. A 0.1-M solution of anthraquinone derivatives **5**, **7**, and **15** (for the synthesis of ODN-1, -3, and -4) was used, and the coupling time was set up to be 360 s. For the synthesis of ODN-2, 5-methoxycarbonylmethyl-2'-deoxyuridine was used at the modified nucleoside position, and after synthesis of oligonucleotide on the DNA synthesizer, CPG-bound oligonucleotide was treated with 0.15 M 1-(6-aminohexylamino)anthraquinone solution in *N,N*-dimethylacetamide for 24 h. The CPG was washed several times with CH_2Cl_2 , and was further treated with 25% NH_3 at 55°C for 24 h. All DMTr-on oligonucleotides were purified by the reversed-phase HPLC, and were detritylated by the treatment with 10% AcOH. All the purified oligonucleotides were given after EtOH precipitation and gel filtration.

Melting Temperature Analysis

A solution of the appropriate concentration of the oligonucleotides and the complementary DNA was prepared in 10 mM sodium phosphate buffer (pH 7.2) containing 100 mM NaCl. The T_m experiments were done on a Shimadzu UV-2450 spectrophotometer and TMSPC system. The solution was heated from 15 to 80°C , and the heating rate was $0.1^\circ\text{C}/\text{min}$. Each melting curve was calculated by use of Igor Pro. Software (WaveMetrics, Inc.). For the ion strength experiment, the concentration of each oligomer was performed on 2.5 μM under the same buffer conditions.

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