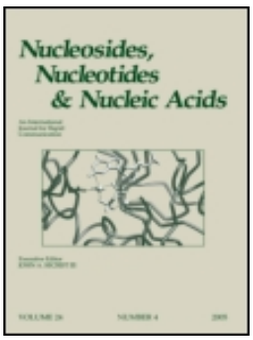

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EFFECT OF ACRIDINE WITH VARIOUS LINKER ARMS ATTACHED TO C5 POSITION OF 2'-DEOXYURIDINE ON THE STABILITY OF DNA/DNA AND DNA/RNA DUPLEXES

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ABSTRACT: Acridine-modified oligodeoxyribonucleotides (ODNs) at the C5-position of a 2'-deoxyuridine via different lengths of linker arms were synthesized. Reaction of 5-(N-aminoalkyl)carbamoylmethyl-2'-deoxyuridines with 9-phenoxyacridine gave the acridine-modified 2'-deoxyuridines which were incorporated into ODNs. The duplexes containing the acridine-modified strands and their complementary DNA or RNA were thermally more stable than that containing the unmodified strand. Thermal stability of the duplexes of the modified ODNs varied depending on the length of the linker arms.

INTRODUCTION

Various kinds of functional groups have been incorporated into oligodeoxyribonucleotides (ODNs) for antisense strategies and for DNA probes. Functions required for the antisense oligonucleotides to regulate a gene expression efficiently are the stability of a duplex with a target strand, nuclease resistance, and cell permeability. For the efficient hybridization to the target strand, many functional groups such as acridine[1], psoralen[2], polyamine[3], Hoechst 33258[4], and anthraquinone[5] were incorporated into ODN by chemical modification. The stability of the duplex depends on not only the functional group but also the length of the linker arm and the modified position of the ODN. The ODNs modified at various positions have been synthesized by several synthetic techniques of ODN.[6] Recently, Asseline *et al.* reported the modification of ODN via a linker to various positions, 3'-position, 5'-position, internucleotide phosphate, or C5-position of a 2'-deoxyuridine, with 2-methoxy-6-

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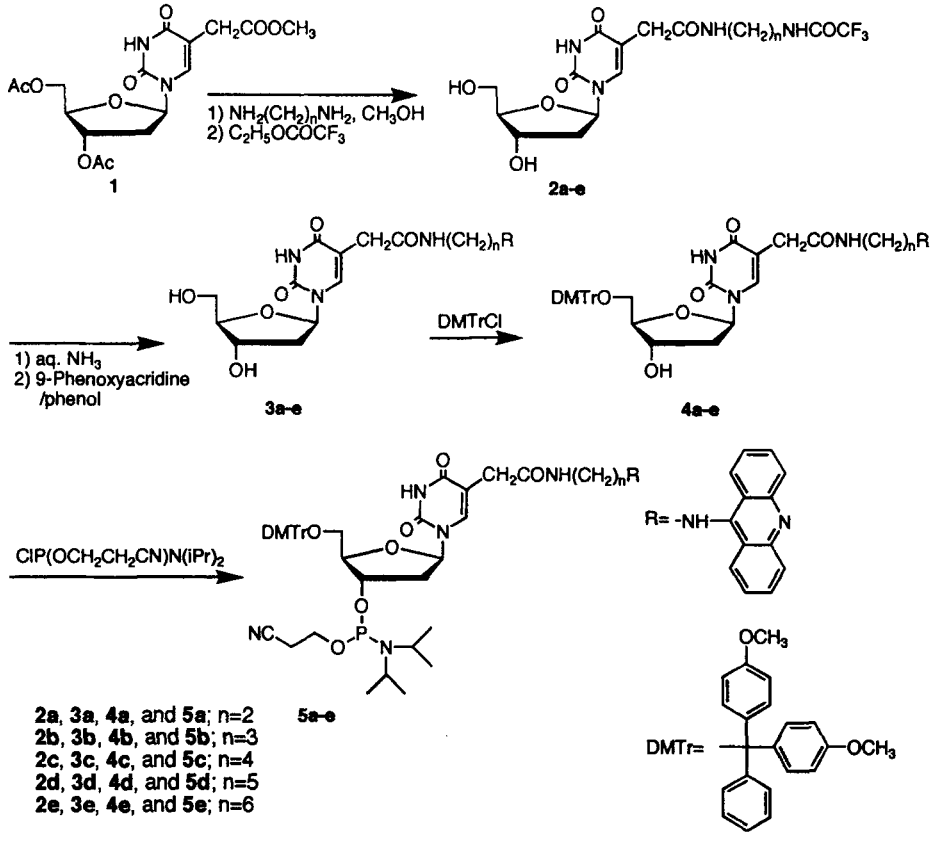
This paper is dedicated to the late Professor Tsujiaki Hata.

chloro-9-aminoacridine.[7] They have shown that the acridine modification of ODN at the 5'-position or 3'-position of ODN leads to stabilization of the duplex. Also, the attachment of acridine at an internucleotide phosphate group increases the stability of the duplex. On the other hand, when the acridine is introduced in the C5-position of 2'-deoxyuridine in the middle of the ODN, the melting temperature of the duplex decreases. They suggested that the modification at the C5-position changed the hydration spin inside the groove and intercalation of acridine from the major groove side of base-pair had an undesirable effect on stabilization of the duplex. However, the effect of the linker between the acridine and the C5-position of 2'-deoxyuridine is still unclear. We have developed a synthetic procedure of C5-substituted 2'-deoxyuridine and reported the hybridization property of the amino-linker arms in the DNA duplex.[8] Here, we report synthesis of acridine-modified ODNs at the C5-position of a pyrimidine nucleoside with different linker arms, and the effect of the modification and the length of the linker arm on the thermal stability of the duplexes.

RESULTS AND DISCUSSION

Syntheses of C5 substituted pyrimidine nucleosides.

C5-Substituted pyrimidine nucleosides were synthesized from 5-methoxycarbonylmethyl-2'-deoxyuridine (**1**) which was prepared from arabinoaminooxazoline and dimethyl α-bromomethylfumarate. Each diaminoalkane from ethylenediamine to 1,6-hexanediamine was easily reacted with a methoxycarboxymethyl group of the nucleoside. The terminal amino group of the C5 substituted nucleosides was protected with trifluoroacetyl group without purification. After protection of the terminal amino group, the 2'-deoxyuridine derivatives (**2a-e**) obtained were purified by conventional silica-gel column chromatography. C5-Substituted 2'-deoxyuridine derivatives bearing several amino-linker arms were isolated in 60-93 % yield. An acridine residue was further incorporated into the nucleoside derivatives in high yields, after deprotection of the amino group, by the reaction of the terminal amino group of the 2'-deoxyuridine derivatives with 9-phenoxyacridine.[9] These modified nucleosides (**3a-e**) were used for the synthesis of the modified oligodeoxyribonucleotides after 5'-protection with a dimethoxytrityl group (**4a-e**) and 3'-phosphitylation (**5a-e**). The efficiencies of both reactions were modest, 39-45% for the 5'-protection and 17-52% for the 3'-phosphitylation. This low reactivity may be due to steric hindrance of the C5-substituent group. The retention times of the acridine-modified nucleosides on a reversed-phase HPLC column were longer than that of thymidine and also increased with increasing the number of methylenes in the linker arm.



SCHEME 1

Oligodeoxyribonucleotide synthesis.

The modified ODNs (5'd(CATAGGAGAXGCCTA)3', X=**3a-e**) were synthesized on a DNA synthesizer using the modified nucleoside phosphoramidites. The abbreviations for the modified ODNs are shown in Table 1 with coupling yields and their retention times of reversed-phase HPLC. Normal deoxynucleoside phosphoramidites bearing easily removable protecting groups (base protecting group; phenoxyacetyl for dA, acetyl for dC, isopropylphenoxyacetyl for dG) were used for the syntheses of acridine-modified ODNs because N^9-alkylated-9-aminoacridine was unstable under the normal deprotection conditions of conc. aq. ammonia at 55 ℃ for 12h. The use of deoxynucleosides bearing easily removable protecting groups made the deprotection complete under extremely mild conditions (50 mM K_2CO_3 at r.t. for 2h)

TABLE 1. Abbreviations, coupling yields and retention times of modified ODNs with retention times of the uridine derivatives

Abbr.	X	Coupling yield(%)[a]	T_R/min[b]	
			nucleosides	ODN
N-ODN	T	99	11.1	16.5
ODN-2A	3a	87	22.3	19.0
ODN-3A	3b	24	23.2	18.8
ODN-4A	3c	75	24.6	19.4
ODN-5A	3d	77	27.1	19.4
ODN-6A	3e	97	30.0	19.8

ODN sequence; 5'd(CATAGGAGAXGCCTA)3'
a) Coupling yields are for incorporation of the modified nucleosides.
b)HPLC condition is given in experimental section.

without the removal of the acridine moiety. The chromatographic behavior of the ODNs reflects not only the hydrophobicity of the linker arm but also the structural change since the retention times of the ODNs did not correlate to the length of the linker arm (see Table 1). The characterization of the modified ODNs was carried out by a nuclease digestion. The ratio of the nucleosides obtained (dC:dG:T:dA:X = 3:4:2:5:1) was consistent with the calculated ratio (dC:dG:T:dA:X = 3:4:2:5:1) of the composition of ODNs.

The effect of C5-modification on the structure and stability of the duplexes.

FIG. 1 shows the typical CD spectra for unmodified (**N-ODN**) and acridine-modified ODN (**ODN-5A**) duplexes formed with DNA and RNA. The CD profiles of the duplexes formed with other acridine-modified ODN are almost the same as those of duplexes formed with **ODN-5A**. Similar CD spectra between **N-ODN**/DNA and **ODN-5A**/DNA indicate that the conformations of both duplexes are B-form and the modification of ODN with acridine had slight effect on global conformation. Also, the CD spectra of **N-ODN**/RNA and **ODN-5A**/RNA suggest an A-form-like conformation and little effect of the acridine modification on the duplex conformation.

The melting temperatures (Tm's) of each duplex of the modified ODN with its complementary DNA or RNA were measured by the temperature-dependent UV absorption change. The Tm values are summarized in TABLE 2. Incorporation of acridine led to the stabilization of the DNA duplex with any linker arm. **ODN-2A** has six atoms between the C5-position of uracil and the C9-position of acridine and **ODN-6A** has ten atoms between these positions. This result shows that the acridine ring tethered with ODN can interact with the duplex DNA even for the **ODN-2A** which has the shortest linker arm in this study. The length of the linker will affect the local hydrophobicity around the linker arm and freedom of the acridine moiety. However,

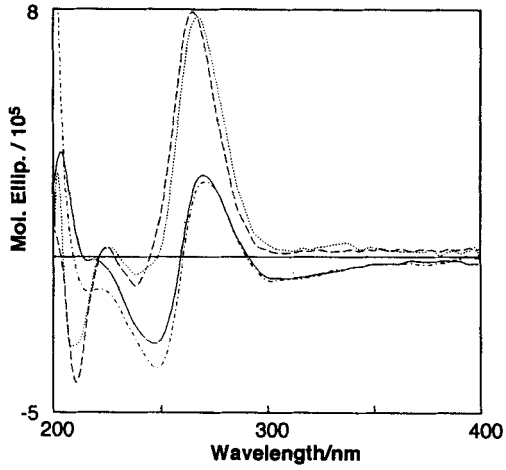


FIG. 1. CD spectra of unmodified and modified ODN duplexes. Solid line, **N-ODN**/DNA; dot-dash line, **ODN-5A**/DNA; broken line, **N-ODN**/RNA; dotted line, **ODN-5A**/RNA. Measurement condition is in experimental section.

TABLE 2. Melting temperatures of the duplex of modified ODNs with unmodified complementary ODN(DNA or RNA)

	ODN/DNA		ODN/RNA	
ODN	Tm/°C[a)]	ΔTm/°C[b)]	Tm/°C[a)]	ΔTm/°C[b)]
N-ODN	49.6	---	51.3	---
ODN-2A	59.3	9.7	52.2	0.9
ODN-3A	59.5	9.9	54.7	3.4
ODN-4A	60.7	11.1	56.4	5.1
ODN-5A	56.4	6.8	57.8	6.5
ODN-6A	61.1	11.5	57.0	5.7

a) Measurement condition is given in experimental section.

b) ΔTm is obtained by subtracting the Tm's of the modified ODN from those of the normal ODN with a complementary strand.

Tm's of these duplexes were affected little by the length of the linker arm except in the case of **ODN-5A**. It is unclear why the duplex containing **ODN-5A** and the complementary DNA is unstable compared with those containing other acridine-tethered ODNs.

For the ODN/RNA heteroduplex, which has different conformation from that of the DNA duplex (see Figure 1) and is important for the translation-arrest effects on the mRNA target, the modification with acridine also resulted in the stabilization of the ODN/RNA heteroduplex. But the stabilization effect of the heteroduplexes was smaller

than that for the DNA duplexes. This is consistent with a recent report by Haginoya *et al.*, which showed the smaller effect of a intercalating group at C5 position of pyrimidine nucleoside on the stabilization of the DNA/RNA heteroduplex compared with that of the DNA/DNA duplexes.[10] In our study, the ODNs bearing an acridine moiety through a short linker (**ODN-2A** and **ODN-3A**) showed small increment of Tm values in the ODN/RNA heteroduplexes. This result suggests that the acridine can not well interact with the duplex due to the narrow major groove in the DNA/RNA heteroduplex when the linker arm between the ODN and the acridine moiety is short. However, when the acridine was tethered to the ODN via a relatively long linker arm, the duplex was stabilized more effectively than that via a short linker arm. This suggests that a degree of freedom of the acridine moiety is important for the interaction of acridine with the DNA/RNA heteroduplex. It is noteworthy that the Tm value of the **ODN-5A**/DNA duplex is lower than those of any other modified ODN/DNA duplexes, while that of the **ODN-5A**/RNA heteroduplex is higher than any other. The mode of the interaction of the acridine moiety tethered at C5 of pyrimidine to DNA is not characterized in this study. We reported previously that the alkyl linker at the C5 position of the pyrimidine results in some disruption of the duplex form.[8] Hence, it is suggested that the stabilization of the duplexes by the acridine in this study overcomes the destabilization effect of the alkyl linker arm.

In conclusion, the modification of ODN at the C5-position of pyrimidine with acridine in our study stabilized both the ODN/DNA and ODN/RNA duplexes. Additionally, it was found that a relatively long linker arm is preferred for the ODN/RNA duplex. This kind of modification of ODN at the C5 position of a pyrimidine nucleoside will be useful for the design of antisense molecules or nucleic acid probes.

EXPERIMENTAL

General. Thin-layer chromatography (TLC) was performed on Kieselgel $60F_{254}$ (Art. 5554, E. Merck). Silica-gel column chromatography was performed on Wako gel C-200 (Wako Pure Chemical Industries Ltd.) and Silica gel 60 (63-200 μm or 40-63 μm, Merck). High-performance liquid chromatography (HPLC) was carried out on a Wakosil 5C18 column (4 mmϕ x 250 mm, Wako) by use of a system consisting of JASCO 880-PU pump, 875-UV UV/VIS detector, 801-SC system controller, and Shimadzu C-R5A chromatopac. The eluent was a linear gradient from 2.1 % to 37.1 % acetonitrile in 50 mM triethylammonium acetate (TEAA, pH 7.0) within 35 min for the unprotected ODNs. ^{1}H NMR spectra and ^{31}P NMR spectra were obtained with a Varian Gemini 200 or a JEOL α-500 spectrometer. ^{1}H NMR spectra were recorded relative to internal tetramethylsilane and ^{31}P NMR spectra were recorded relative to external 85 %

H_3PO_4. Mass spectra were measured by a Perkin Elmer Sciex API-100 instrument in ESI mode. Oligodeoxyribonucleotides were synthesized by phosphoramidite chemistry on an Applied Biosystems 381A DNA synthesizer. An oligoribonucleotide was purchased from Genset Corp. and purified by a Mono Q HR5/5 anion-exchange column. Normal nucleoside phosphoramidites were purchased from Wako or Glen Research. 9-Phenoxyacridine was synthesized by the described method[11]. Dichloro(2-cyanoethoxy)phosphine were prepared by the described method[12]. 3',5'-Di-*O*-acetyl-5-(methoxycarbonylmethyl)-2'-deoxyuridine (**1**) was prepared by the method described previously.[13] Snake venom phosphodiesterase (SVPD) was purchased from Worthinton. Nuclease P1 was from Yamasa Co.. Alkaline phosphatase (AP) was purchased from Boehringer Mannheim BmbH. All other reagents were purchased from Wako or Kanto Chemical Co.. All organic solvents for reactions were dried and distilled in the usual manner.

Synthesis of 5-(*N*-substituted carbamoylmethyl)-2'-deoxyuridine (2a-e). 5-[*N*-(2-Trifluoroacetylaminoethyl)]carbamoylmethyl-2'-deoxyuridine (2a). A mixture containing **1** (0.568 g, 1.48 mmol), ethylenediamine (1.19 mL, 17.8 mmol), and 4-dimethylaminopyridine (0.012 g, 0.098 mmol) in methanol (2 mL) was stirred at 50 °C for 23 h. After the reaction solution was evaporated and coevaporated with methanol, the residue was dissolved in a small amount of methanol and added dropwise to benzene to precipitate 5-[*N*-(2-aminoethyl)]carbamoylmethyl-2'-deoxyuridine (**2a'**) as an oily residue. Then the terminal amino group of **2a'** was protected by a trifluoroacetyl group without further purification. Ethyl trifluoroacetate (0.5 mL, 4.18 mmol) was added dropwise to a solution of the crude product (**2a'**) in methanol (2 mL) containing 4-dimethylaminopyridine (0.012 g, 0.098 mmol) and the reaction mixture was stirred overnight at room temperature. After the reaction solution was evaporated, the residue was dissolved in a small amount of methanol and added dropwise to dichloromethane to precipitate 5-[*N*-(2-trifluoroacetylamidoethyl)]-carbamoylmethyl-2'-deoxyuridine (**2a**) as a white precipitate. Yield of **2a** was 0.581 g (92.5%) from **1**. High-purity samples for the analyses were obtained from recrystallization in ethanol. mp >220 °C (decomp.). ^{1}H NMR (D_2O) δ 7.82 (s, 1H, H6), 6.31 (t, 1H, J = 6.6 Hz, H1'), 4.48 (m, 1H, H3'), 4.06 (m, 1H, H4'), 3.81 (m, 2H, H5'), 3.44 (m, 4H, -CH_2CH_2-), 3.30 (s, 2H, C5-CH_2-), 2.42 (m. 2H, H2'); UV (H_2O, pH 7.0) λmax 266.5 nm (ε 8900 $M^{-1}cm^{-1}$); Mass *m/z* 425.1 (MH^+, 425.1 calcd for $C_{15}H_{20}F_3N_4O_7$). Anal. Calcd for $C_{15}H_{19}F_3N_4O_7$ 1/2H_2O: C, 41.58; H, 4.65; N, 12.93. Found: C, 41.33; H, 4.47; N, 12.54.

5-[*N*-(3-Trifluoroacetylaminopropyl)]carbamoylmethyl-2'-deoxyuridine (2b). Compound **2b** was similarly prepared from **1** (0.607 g, 1.58

mmol) and 1,3-propanediamine (1.58 mL, 19.0 mmol). Yield 0.577 g (83.4% as a white precipitate from dichloromethane-methanol); mp 163-166 ℃; ^{1}H NMR (CD_3OD) δ 7.93 (S, 1H, H6), 6.28 (t, 1H, J = 6.7 Hz, H1'), 4.40 (dd, 1H, H3'), 3.92 (dd, 1H, H4'), 3.77 (m, 2H, H5'), 3.22 (m, 6H, C5-CH_2- and -NH$\underline{CH_2}$- x 2), 2.27 (m, 2H, H2'), 1.94-1.71 (m, 2H, -CH_2-); Mass m/z 439.3 (MH^+, 439.1 calcd for $C_{16}H_{22}F_3N_4O_7$).

5-[*N*-(4-Trifluoroacetylaminobutyl)]carbamoylmethyl-2'-deoxyuridine (2c). Compound **2c** was similarly prepared from **1** (0.600 g, 1.56 mmol) and 1,4-butanediamine (1.23 g, 18.7 mmol). Yield 0.566 g (80.3% as a white precipitate from dichloromethane-methanol); mp 155 ℃; ^{1}H NMR (CD_3OD) δ 7.91 (s, 1H, H6), 6.28 (t, 1H, J = 6.7 Hz, H1'), 4.40 (dd, 1H, H3'), 3.92 (dd, 1H, H4'), 3.78 (m, 2H, H5'), 3.21 (m, 6H, C5-CH_2- and -NH$\underline{CH_2}$- x 2), 2.26 (m, 2H, H2'), 1.61-1.50 (m, 4H, -$(CH_2)_2$-); Mass m/z 453.3 (MH^+, 453.2 calcd for $C_{17}H_{24}F_3N_4O_7$).

5-[*N*-(5-Trifluoroacetylaminopentyl)]carbamoylmethyl-2'-deoxyuridine (2d). Compound **2d** was similarly prepared from **1** (1.01 g, 2.63 mmol) and 1,5-pentanediamine (3.08 g, 26.3 mmol). Yield 0.740 g (60.2% as a white precipitate from dichloromethane-methanol); mp 105-107 ℃; ^{1}H NMR (CD_3OD) δ 7.92 (s, 1H, H6), 6.29 (t, 1H, J = 6.7 Hz, H1'), 4.41 (dd, 1H, H3'), 3.93 (dd, 1H, H4'), 3.79 (m, 2H, H5'), 3.21 (m, 6H, C5-CH_2- and -NH$\underline{CH_2}$- x 2), 2.28 (m, 2H, H2'), 1.63-1.36 (m, 6H, -$(CH_2)_3$); Mass m/z 467.2 (MH^+, 467.2 calcd for $C_{18}H_{26}F_3N_4O_7$).

5-[*N*-(6-Trifluoroacetylaminohexyl)]carbamoylmethyl-2'-deoxyuridine (2e). Compound **2e** was similarly prepared from **1** (0.600 g, 1.56 mmol) and 1,6-hexanediamine (2.17 g, 18.7 mmol). Yield 0.642 g (85.7% as a white precipitate from dichloromethane-methanol); mp 159-163 ℃; ^{1}H NMR (CD_3OD) δ 7.91 (s, 1H, H), 6.29 (t, 1H, J = 6.7 Hz, H1'), 4.40 (dd, 1H, H3'), 3.92 (dd, 1H, H4'), 3.77 (m, 2H, H5'), 3.20 (m, 6H, C5-CH_2- and -NH$\underline{CH_2}$- x 2), 2.24 (m, 2H, H2'), 1.65-1.36 (m, 8H, -$(CH_2)_4$-); Mass m/z 481.3 (MH^+, 481.2 calcd for $C_{19}H_{28}F_3N_4O_7$).

Synthesis of 5-[(*N*-acridinylaminoalkyl)carbamoylmethyl]-2'-deoxyuridine (3a-e). 5-[*N*-(2-(9-Acridinyl) aminoethyl)]carbamoylmethyl-2'-deoxyuridine (3a). The trifluoroacetyl group was removed by treatment of **2a**(0.130 g, 0.396 mmol) with conc. aq. ammonia solution (2 mL) at r.t. for 1.5 h. The reaction mixture was evaporated to dryness. A mixture of 9-phenoxyacridine (0.269 g, 0.992 mmol) and phenol (3.70 g) was added to the residue and stirred at 80℃ for 3.5h. The reaction mixture was added dropwise to diethylether and the precipitate was collected by filtration. The precipitate was dissolved in water and washed with ethyl acetate. The aqueous layer was evaporated to dryness. Yield of **3a** was 0.160 g (79.6%). ^{1}H NMR (D_2O) δ 8.07-7.44 (m, 9H, Ar and H6), 6.30 (t, 1H, H1'), 4.46 (m, 1H, H3'), 4.22 (m, 1H, H4'), 4.03 (m, 2H, H5'), 3.90-3.71 (m, 6H, C5-CH_2- and -NH$\underline{CH_2}$- x 2), 2.41 (t, 2H, H2'); Mass m/z 506.3 (MH^+, 506.2 calcd for $C_{26}H_{28}N_5O_6$).

5-[*N*-(3-(9-Acridinyl)aminopropyl)]carbamoylmethyl-2'-deoxyuridine (3b). Compound **3b** was similarly prepared from **2b** (0.400 g, 0.912 mmol) and 9-phenoxyacridine (0.619 g, 2.28 mmol). Yield 86.1% (0.474 g as a yellow solid from water); mp 136-137 °C; ^{1}H NMR (D_2O) δ 8.01-7.35 (m, 9H, Ar and H6), 6.14 (t, 1H, H1'), 4.41 (m, 1H, H3'), 3.98-3.63 (m, 3H, H4' and H5'), 3.38-3.10 (m, 6H, C5-CH_2- and -NH$\underline{CH_2}$- x 2), 2.42-1.94 (m, 4H, H2' and -CH_2-); Mass *m/z* 520.3 (MH^+, 520.2 calcd for $C_{27}H_{30}N_5O_6$).

5-[*N*-(3-(9-Acridinyl)aminobutyl)]carbamoylmethyl-2'-deoxyuridine (3c). Compound **3c** was similarly prepared from **2c** (0.500 g, 1.11 mmol) and 9-phenoxyacridine (0.754 g, 2.78 mmol). Yield 92.9% (0.550 g as a yellow solid from water); mp 123-125 °C; ^{1}H NMR (D_2O) δ 7.99-7.34 (m, 9H, Ar and H6), 5.98 (t, 1H, H1'), 4.36 (m, 1H, H3'), 3.87-3.67 (m, 3H, H4' and H5'), 3.38-3.27 (m, 6H, C5-CH_2- and -NH$\underline{CH_2}$- x 2), 2.27-1.55 (m, 6H, H2' and -$(CH_2)_2$-); Mass *m/z* 534.3 (MH^+, 534.2 calcd for $C_{28}H_{32}N_5O_6$).

5-[*N*-(3-(9-Acridinyl)aminopentyl)]carbamoylmethyl-2'-deoxyuridine (3d). Compound **3d** was similarly prepared from **2d** (0.500 g, 1.11 mmol) and 9-phenoxyacridine (0.894 g, 3.30 mmol). Yield 92.2% (0.540 g as a yellow solid from water); mp 123-126 °C; ^{1}H NMR (D_2O) δ 7.96-7.32 (m, 9H, Ar and H6), 6.10 (t, 1H, H1'), 4.42 (m, 1H, H3'), 3.95-3.72 (m, 3H, H4' and H5'), 3.40-3.25 (m, 6H, C5-CH_2- and -NH$\underline{CH_2}$- x 2), 2.34-1.40 (m, 8H, H2' and -$(CH_2)_3$-); Mass *m/z* 548.3 (MH^+,548.2 calcd for $C_{29}H_{34}N_5O_6$).

5-[*N*-(3-(9-Acridinyl)aminohexyl)]carbamoylmethyl-2'-deoxyuridine (3e). Compound **3e** was similarly prepared from **2e** (0.600 g, 1.25 mmol) and 9-phenoxyacridine (0.894 g, 3.30 mmol). Yield 97.0% (0.681 g as a yellow solid from water); mp 104-106 °C; ^{1}H NMR (D_2O) δ 7.87-7.03 (m, 9H, Ar and H6), 5.91 (t, 1H, H1'), 4.33 (m, 1H, H3'), 3.85-3.63 (m, 3H, H4' and H5'), 3.34-3.10 (m, 6H, C5-CH_2- and -NH$\underline{CH_2}$- x 2), 2.22-1.27 (m, 10H, H2' and -$(CH_2)_4$-); Mass *m/z* 562.4 (MH^+, 562.3 calcd for $C_{30}H_{36}N_5O_6$).

Synthesis of 5'-DMTr-nucleoside derivatives (4a-e). 5'-*O*-(4,4'-Dimethoxytrityl)-5-[*N*-(2-(9-acridinyl)aminoethyl)]carbamoylmethyl-2'-deoxyuridine (4a). Compound **3a** (0.090 g, 0.178 mmol) was reacted with 4,4'-dimethoxytrityl chloride (DMTr-Cl, 0.090 g, 0.267 mmol) in pyridine (3.5 mL) containing 4-dimethylaminopyridine (3 mg, 0.024 mmol) overnight at room temperature. The reaction mixture was poured into cold water and extracted 5 times with dichloromethane (5 x 50 mL). After the organic layer was dried with anhydrous sodium sulfate, the solvent was removed by evaporation and coevaporation with toluene. The product was purified by silica-gel column chromatography using 10% methanol in

dichloromethane containing 0.2% triethylamine as an eluent. The appropriate fractions were collected, evaporated, and precipitated with a small amount of dichloromethane into hexane to give yellow precipitates, **4a**. Yield of **4a** was 0.061 g (42.4%). ^{1}H NMR ($CDCl_3$) δ 8.42 (s, 1H, imido-NH), 8.16-6.89 (m, 23H, H6 and Ar), 6.06 (t, 1H, H1'), 4.68 (m, 1H, H3'), 4.29 (m, 1H, H4'), 4.20 (m, 2H, H5'), 3.81 (s, 6H, CH_3O-), 3.62-3.44 (m, 6H, -NHC$\underline{H_2}$- x 2, and C5-CH_2-), 2.48-2.35 (m, 2H, H2') ; Mass *m/z* 808.4 (MH^+, 808.3 calcd for $C_{47}H_{46}N_5O_8$).

5'-*O*-(4,4'-Dimethoxytrityl)-5-[*N*-(3-(9-acridinyl)aminopropyl)]-carbamoylmethyl-2'-deoxyuridine (4b). Protection of the 5'-OH of **3b** (0.628 g, 1.21 mmol) with the 4,4'-dimethoxytrityl group was carried out in a similar manner. Yield 0.409 g (41.1%); ^{1}H NMR ($CDCl_3$) δ 8.20-6.82 (m, 23H, H6 and Ar), 6.24 (m, 1H, H1'), 4.56 (m, 1H, H3'), 4.03 (m, 1H, H4'), 3.75 (s, 6H, CH_3O- x 2), 3.42 (m, 6H, -NHC$\underline{H_2}$- x 2, and C5-CH_2-), 2.70 (m, 2H, H5'), 2.48-2.21 (m, 2H, H2'), 1.86 (m, 2H, -CH_2-); Mass *m/z* 822.5 (MH^+, 822.3 calcd for $C_{48}H_{48}N_5O_8$).

5'-*O*-(4,4'-Dimethoxytrityl)-5-[*N*-(4-(9-acridinyl)aminobutyl)]-carbamoylmethyl-2'-deoxyuridine (4c). Protection of the 5'-OH of **3c** (0.400 g, 0.750 mmol) with the 4,4'-dimethoxytrityl group was carried out in a similar manner. Yield 0.248 g (39.4%); ^{1}H NMR ($CDCl_3$) δ 8.10-6.80 (m, 23H, H6 and Ar), 6.25 (m, 1H, H1'), 4.50 (m, 1H, H3'), 4.00 (m, 1H, H4'), 3.77 (s, 6H, CH_3O- x 2), 3.40-3.17 (m, 6H, -NHC$\underline{H_2}$- x 2, and C5-CH_2-), 2.67 (m, 2H, H5'), 2.32-2.18 (m, 2H, H2'), 1.78-1.53 (m, 4H, -$(CH_2)_2$-); Mass *m/z* 836.5 (MH^+, 836.4 calcd for $C_{49}H_{50}N_5O_8$).

5'-*O*-(4,4'-Dimethoxytrityl)-5-[*N*-(5-(9-acridinyl)aminopentyl)]-carbamoylmethyl-2'-deoxyuridine (4d). Protection of the 5'-OH of **3d** (0.400 g, 0.730 mmol) with the 4,4'-dimethoxytrityl group was carried out in a similar manner. Yield 0.268 g (43.2%); ^{1}H NMR ($CDCl_3$) δ 8.12-6.80 (m, 23H, H6 and Ar), 6.22 (m, 1H, H1'), 4.52 (m, 1H, H3'), 3.98 (m, 1H, H4'), 3.77 (s, 6H, CH_3O- x 2), 3.38-3.10 (m, 6H, -NHC$\underline{H_2}$- x 2, and C5-CH_2-), 2.69 (m, 2H, H5'), 2.38-2.13 (m, 2H, H2'), 1.83-1.27 (m, 6H, -$(CH_2)_3$-); Mass *m/z* 850.5 (MH^+, 850.4 calcd for $C_{50}H_{52}N_5O_8$).

5'-*O*-(4,4'-Dimethoxytrityl)-5-[*N*-(6-(9-acridinyl)aminohexyl)]-carbamoylmethyl-2'-deoxyuridine (4e). Protection of the 5'-OH of **3e** (0.400 g, 0.712 mmol) with the 4,4'-dimethoxytrityl group was carried out in a similar manner. Yield 0.278 g (45.2%); ^{1}H NMR ($CDCl_3$) δ 8.13-6.75 (m, 23H, H6 and Ar), 6.2 7(m, 1H, H1'), 4.50 (m, 1H, H3'), 3.99 (m, 1H, H4'), 3.78 (s, 6H, CH_3O- x 2), 3.42-3.10 (m, 6H, -NHC$\underline{H_2}$- x 2, and C5-CH_2-), 2.72 (m, 2H, H5'), 2.41-2.18 (m, 2H, H2'), 1.85-1.27 (m, 6H, -$(CH_2)_4$-); Mass *m/z* 864.6 (MH^+, 864.4 calcd for $C_{51}H_{54}N_5O_8$).

Phosphitylation of 5'-DMTr-nucleoside derivatives (5a-f). 3'-*O*-[(2-Cyanoethyl)(diisopropylamino)]phosphino-5'-*O*-(4,4'-dimethoxy-

trityl)-5-[*N*-(2-(9-acridinyl)aminoethyl)]carbamoylmethyl-2'-deoxyuridine (5a). Phosphitylating reagent, chloro-(2-cyanoethoxy)-diisopropylaminophosphine, was prepared *in situ* immediately before use from dichloro-(2-cyanoethoxy)phosphine and diisopropylamine. Diisopropylamine (244 μL, 1.74 mmol) was added dropwise into a solution of dichloro-(2-cyanoethoxy)phosphine (110 μL, 0.870 mmol) in dry dichloromethane (3 mL) under N_2 atmosphere at 0 ℃ and the solution was stirred at room temperature for 30 min. This reaction solution was added dropwise to a solution of **4a** (70 mg, 0.087 mmol) in dry dichloromethane (5 mL) containing *N*-ethyl-*N*,*N*-diisopropylamine (303 μL, 1.74 mmol) under N_2 atmosphere at room temperature. After this mixture was stirred for 1 h, 63 μL of dry methanol was added to the reaction mixture and the reaction mixture was poured into cold ethyl acetate (pre-washed with 5% aq. sodium hydrogencarbonate solution). The solution was washed with 5% aq. sodium hydrogencarbonate solution and water, dried with anhydrous sodium sulfate, and evaporated to dryness. The crude product was purified by silica-gel column chromatography using ethyl acetate/methanol/triethylamine (88/2/10, v/v/v) as an eluent. The appropriate fractions were collected, evaporated, and precipitated with a small amount of dichloromethane into hexane to give precipitates. Yield of **5a** was 0.015 g (17.0%). ^{31}P NMR ($CDCl_3$) δ 149.41 and 149.57 ppm.

3'-*O*-[(2-Cyanoethyl)(diisopropylamino)]phosphino-5'-*O*-(4,4'-dimethoxytrityl)-5-[*N*-(3-(9-acridinyl)aminopropyl)]carbamoylmethyl-2'-deoxyuridine (5b). The phosphitylation of **4b** (0.142 g, 0.173 mmol) was carried out in the same manner as described above. Yield 0.088 g (49.7%); ^{31}P NMR ($CDCl_3$) δ 144.49 and 149.60 ppm.

3'-*O*-[(2-Cyanoethyl)(diisopropylamino)]phosphino-5'-*O*-(4,4'-dimethoxytrityl)-5-[*N*-(4-(9-acridinyl)aminobutyl)]carbamoylmethyl-2'-deoxyuridine (5c). The phosphitylation of **4c** (0.253 g, 0.303 mmol) was carried out in the same manner as described above. Yield 0.163 g (51.9%); ^{31}P NMR ($CDCl_3$) δ 144.39 and 149.61 ppm.

3'-*O*-[(2-Cyanoethyl)(diisopropylamino)]phosphino-5'-*O*-(4,4'-dimethoxytrityl)-5-[*N*-(5-(9-acridinyl)aminopentyl)]carbamoylmethyl-2'-deoxyuridine (5d). The phosphitylation of **4d** (0.269 g, 0.316 mmol) was carried out in the same manner as described above. Yield 0.168 g (50.8%); ^{31}P NMR ($CDCl_3$) δ 144.36 and 149.60 ppm.

3'-*O*-[(2-Cyanoethyl)(diisopropylamino)]phosphino-5'-*O*-(4,4'-dimethoxytrityl)-5-[*N*-(6-(9-acridinyl)aminohexyl)]carbamoylmethyl-2'-deoxyuridine (5e). The phosphitylation of **4e** (0.325 g, 0.376 mmol) was carried out in the same manner as described above. Yield 0.140 g (35.0%); ^{31}P NMR ($CDCl_3$) δ 144.38 and 149.63 ppm.

Oligodeoxyribonucleotide synthesis. Oligodeoxyribonucleotides and their analogs were prepared using normal phosphoramidite coupling procedure on a DNA synthesizer. The oligodeoxyribonucleotides bearing the C5-substituted 2'-deoxyuridine in place of thymidine were synthesized along with the normal and complementary oligodeoxyribonucleotides. The modified nucleoside phosphoramidite was incorporated into the oligodeoxyribonucleotides at the appropriate position by using the normal synthetic cycle except that the reaction time for the coupling step was 6 min. For the synthesis of an acridine-modified oligodeoxyribonucleotide, normal nucleoside phosphoramidites bearing easy-removal protecting groups (Pac-dA-CE phosphoamidite, Ac-dC-CE phosphoamidite, and iPr-Pac-dG-CE phosphoamidite; Glen Research Corp.), 5'-protected thymidine phosphoramidite, the modified nucleoside phosphoramidite(**5a**, **5b**, **5c**, **5d**, or **5e**), and Pac-dA-CPG (Glen Research Corp.) were used. The deprotection and cleavage from CPG support was carried out by the treatment with 50 mM potassium carbonate at r.t. for 2h. All modified oligodeoxyribonucleotides with 5'-(4,4'-dimethoxytrityl) group were isolated by reversed-phase HPLC on a Wakosil 5C18 column (4 mmϕ x 250 mm length) using 50 mM TEAA (pH 7.0) with a gradient of 15.0-40.0% acetonitrile in 25 min. The isolated compound was treated with 10% acetic acid by the usual procedure to remove a 4,4'-dimethoxytrityl group followed by desalting on a Sephadex G-25 column. The modified oligodeoxyribonucleotides were further purified by reversed-phase HPLC after deprotection of the 5'-(4,4'-dimethoxytrityl) group. Isolated yields: **ODN-2A**, 13%; **ODN-3A**, 5.5%; **ODN-4A**, 10%; **ODN-5A**, 4.5%; **ODN-6A**, 4.2%; **N-ODN**, 29%; **C-ODN**, 57%. The abbreviations are shown in Table 1 and **C-ODN** is a complementary strand (DNA) to **N-ODN**.

Nuclease digestion of oligodeoxyribonucleotides containing the modified base. The modified oligomers (ca. 0.5 $OD_{260\,nm}$) were treated overnight with snake venom phosphodiesterase (0.5 units) and alkaline phosphatase (1 units) in 20 mM Tris-HCl (pH 8.0) containing 10 mM $MgCl_2$ at 37℃. To the reaction mixtures were added nuclease P1 (ca. 4 units) and 0.1 M sodium acetate (pH 4.75), then the reaction mixtures were incubated at 37℃. The reaction mixtures were analyzed by reversed-phase HPLC. The nucleoside composition ratio was calculated from areas of the peaks in the HPLC chart.

Tm measurements. UV absorbance was measured with a Hitachi UV-3000 spectrophotometer equipped with a Hitachi Temperature Controller SPR-10. The solution temperature in a cuvette was measured directly with a temperature data collector AM-7002 (Anritsu Meter Co., Ltd.). Absorbance and temperature data were recorded on NEC personal computer PC-9821. The rate of heating or cooling was 0.5 ℃/min. Tm values were obtained in 10 mM sodium phosphate buffer (pH 7.0) containing 150 mM

sodium chloride for the duplex with DNA or in 10 mM sodium phosphate buffer (pH 7.0) containing 150 mM sodium chloride and 0.01 mM EDTA for the duplex with RNA at a duplex concentration of 2 x 10^{-6} M. Tm values were determined by the described method[14] from the absorbance *vs.* temperature plot using an Igor graphing and data analysis program (WaveMatrics, Inc.) and a PowerMacintosh 8500 computer (Apple computer, Inc.).

CD spectra. CD spectra were recorded on a JASCO J-720 spectropolarimeter equipped with a PTC-343 temperature controller, using the same solution for the Tm measurements at 20 ℃.

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