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SCHIFF BASE FORMATION BETWEEN 5-FORMYL-2'-DEOXY-URIDINE AND LYSINE ε–AMINO GROUP AT MONOMER AND OLIGOMER LEVELS

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Abstract – Schiff base formation between the 5-formyl group of 5-formyl-2'-deoxyuridine, which is an oxidative thymidine lesion, and the ε-amino group of lysine at both monomer and oligomer levels is reported.

INTRODUCTION

In general, the complexes formed between nonspecific DNA binding proteins and DNA are intrinsically heterogenetic, and hence structural characterization of such type of complexes by X-Ray crystallography and NMR spectroscopy is not straightforward. In these circumstances, chemical cross-linking is a powerful tool for probing the contact points between proteins and DNA and shall afford important information on the structure of macromolecular complexes.

5-Formyl-2'-deoxyuridine (fdU, **1**), one of the oxidative thymidine lesions in DNA formed by ionizing radiation,[1-3] and photochemical sensitization,[4,5] has been known to be mutagenic and citotoxic. Its mutagenic effect is largely ascribed to mispairing during DNA replication[6-8,12] and the citotoxicity is likely to result from inhibition of thymidilate synthetase and thymidine kinase.[9-12] However, the 5-formyl group of **1** in DNA would be possible to form DNA-protein cross-links through Schiff bases

This paper is dedicated to Dr. Pierre Potier on the occasion of his 70th birthday.

with certain amino groups in the protein such as the ε-amino group of lysine.[2,6] Isolation of the adduct with the lysine side chain, however, has not been reported. First, we confirmed reactivity of the 5-formyl group with the lysine ε-amino group by a reductive amination reaction.[13] Next, we envisaged that the fdU-containing oligonucleotides can be a good cross-linking reagent for a system where cross-links can be stabilized by secondary interactions in the protein-DNA complex. To assess the cross-linking capability of fdU, we chose a RecA derived peptide as its target. *Escherichia coli* RecA protein promotes the strand exchange between two homologous DNA molecules.[14] RecA first polymerizes onto the ssDNA, producing a nucleoprotein filament. This complex then captures dsDNA to search the homologous region to the resident ssDNA. A twenty amino acid residue peptide spanning the RecA loop L2 region (FECO peptide in Figure 2**A**) has been shown not only bind to both ss- and dsDNA's but also catalyze the pairing reaction.[15] Determination of the structure of FECO-DNA complex should provide useful information on the mechanism of strand exchange reaction catalyzed by RecA protein. However, FECO-DNA complex formed by polymerization of FECO onto DNA is a heterogeneitic aggregate.[16] Owing to the heterogeneity, structural characterization of the complex has been a challenge to X-Ray crystallography and NMR spectroscopy. This peptide has one unique lysine, and hence seems to be a suitable target to assess the cross-linking activity of fdU in the oligonucleotide. Moreover, this chemical cross-linking experiment might reveal the accurate contact points between ssDNA and the peptide. Here we report the results of cross-linking experiments through Schiff base formation between fdU and the lysine ε-amino group at monomer and oligomer levels.

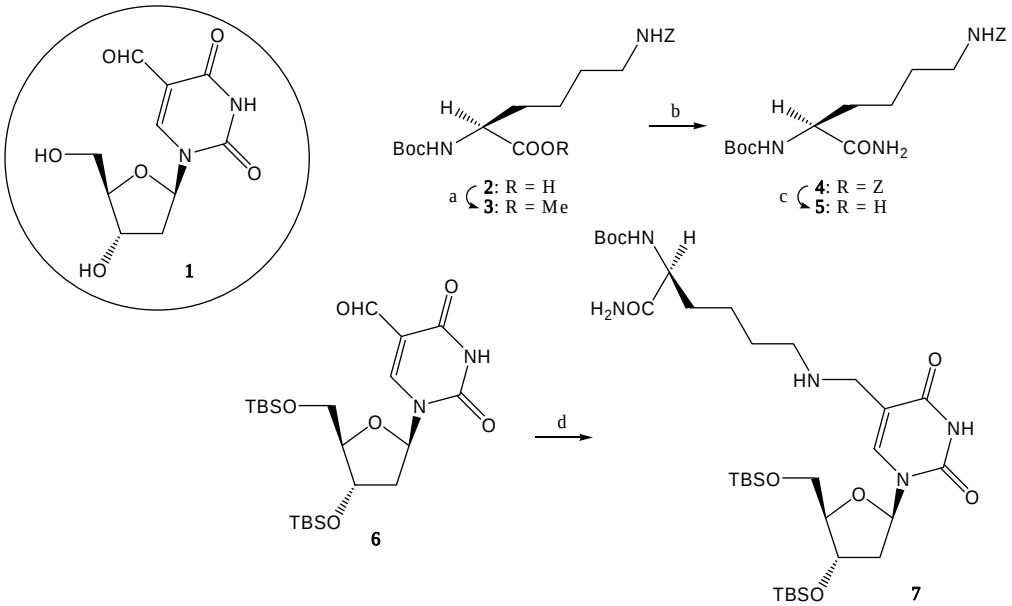
RESULTS AND DISCUSSION

N^{α}-Boc-N^{ε}-Z-L-lysine (**2**) was converted to its methyl ester (**3**) under Mitsunobu conditions in quantitative yield, and subsequent ammonolysis gave amide (**4**) in 53% yield. Deprotection of the N^{ε}-Z group by hydrogenolysis afforded **5**, which was used without complete purification for the next reductive amination reaction. Aldehyde (**6**) was obtained from the known compound (**10**) by periodate cleavage in 91% yield,[13,17] and coupled with the lysine derivative (**5**) in MeCN-DMF (6:1) at room temperature for 1 h, in the presence of MS 3Å. $NaBH_4$ in MeOH was added to the reaction mixture and the adduct (**7**) was isolated in 73% yield (Schemes 1 and 2).

Stable base conformation of 5-modified pyrimidine nucleosides is believed to be *anti*, and X-Ray crystallographic analysis of **6** showed that 5-formyluracil base adopts the standard *anti* conformation (Figure 1).

As shown above and from a previous report,[18] in organic solvents, fdU and its sugar-protected derivatives can form a Schiff base with some amines. However, until today direct observation of cross-linking via Schiff base formation between oligonucleotides containing fdU and lysine residue in proteins has not yet

been reported. Although Schiff bases of fdU can be observed spectroscopically in an aqueous solution, they are too unstable to be detected by HPLC.[6,19] In contrast, cysteine reacts with fdU to afford stable thiazolidine even in the aqueous solution.[19] In addition, phenylhydrazine also forms phenylhydrazone derivative with the oligonucleotides containing fdU in the aqueous solution.[6]



Scheme 1. *Reagents and conditions*: (a) DEAD (1.2 eq.), Ph_3P (1.2 eq.), MeOH (5 eq.), THF, 0 °C→rt. (b) NH_3/MeOH, rt, in a sealed tube. (c) H_2, 10% Pd/C, MeOH. (d) **5** (*ca.* 1 eq.), MeCN-DMF (6:1), MS 3Å, rt, 1 h; then $NaBH_4$ (3 eq), MeOH.

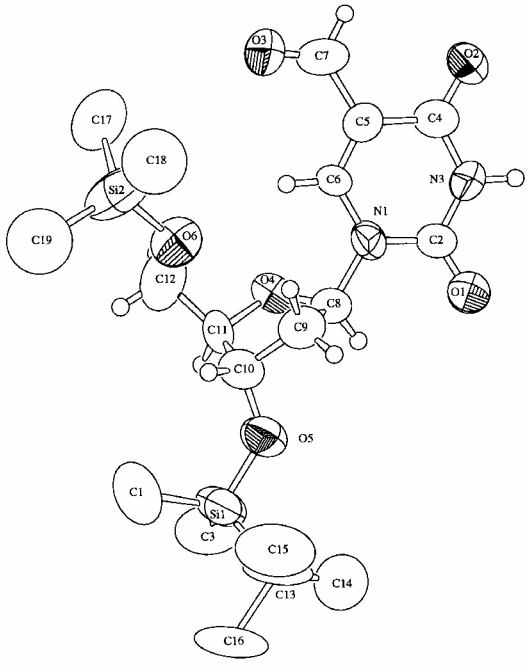
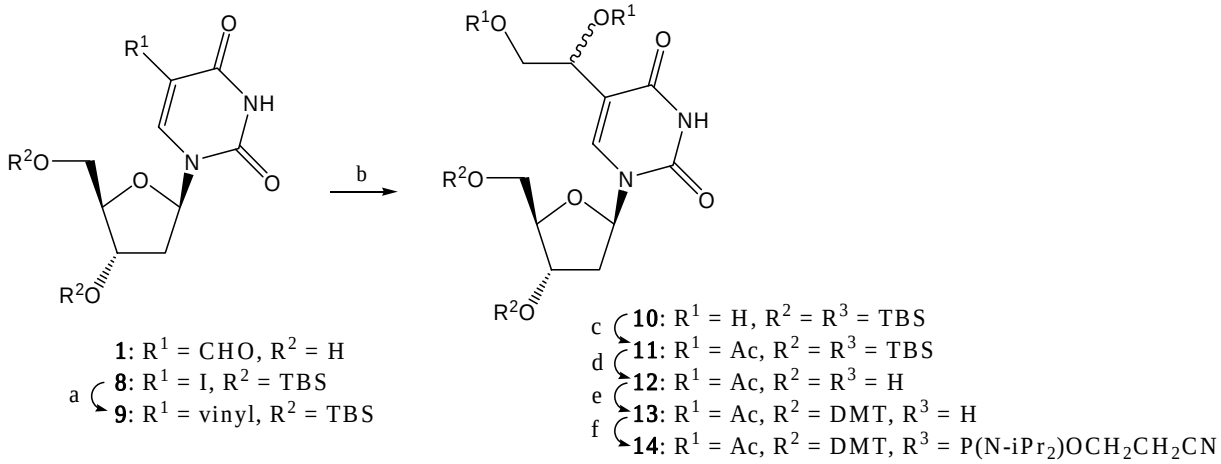


Figure 1. ORTEP drawing of the crystal structure on aldehyde (**6**). Crystal data of **6** are as follows: space group $P2_1$ (#4), Z = 4, a = 14.7872(6), b = 12.032(1), c = 16.5277(7) Å, V = 2910.9(3) $Å^3$, Dc = 1.106 g/cm^3. The *tert*-butyl group in 5'-*O*-TBDMS seems to be rotated and has not been determined owing to thermal vibrations.

Based on these facts, we envisaged that the fdU-containing oligonucleotides can be a good cross-linking reagent for a system where cross-links can be stabilized by secondary interactions in the protein-DNA complex. Oligonucleotides containing fdU should form Schiff base efficiently if the formyl group of fdU and the ε-amino group of lysine are placed in a stereochemically and electrically favourable manner. We incorporated fdU into the oligonucleotide sequences in Figure 2B through the following process. Stille coupling of protected 5-iodo-2'-deoxyuridine (**8**)[20] with tributyl(vinyl)tin[21] using $Pd(MeCN)_2Cl_2$ as a catalyst followed by dihydroxylation of the resulting vinyl group with *cat.* OsO_4/NMO gave **10** in 80% yield in two steps, along with a 17% recovery of **8**.[22] Acetylation of the vicinal diol and deprotection of 3',5'-bis-*O*-TBDMS groups afforded **12**, and subsequent dimethoxytritylation of the 5'-hydroxyl group lead to compound (**13**) (50% from **10**). 3'-*O*-Phosphitylation[23] afforded the known nucleoside phosphoramidite unit (**14**)[17] in 92% yield (Scheme 2). Oligonucleotides in Figure 2B were prepared with an automated DNA synthesizer.



Scheme 2. *Reagents and conditions*: (a) 5 mol% $Pd(MeCN)_2Cl_2$, $Bu_3SnCH{=}CH_2$ (1.5 eq.), MeCN, 80 °C. (b) *cat.* OsO_4, NMO (2.5 eq.), acetone-H_2O-*t*-BuOH (4:1:1). (c) Ac_2O (4 eq.), py. (d) TBAF (3 eq.), AcOH (2 eq.), THF. (e) DMTCl (1.5 eq.), py. (f) $[(i\text{-}Pr)_2N]_2POCH_2CH_2CN$ (1.8 eq.), DCI (0.7 eq.), MeCN-CH_2Cl_2 (1:10).

After ammonolysis, the oligonucleotides were purified by reverse phase HPLC. Oxidation with 100 eq. of $NaIO_4$ in H_2O for 5 min at 0 °C converted the 5-(1,2-dihydroxy)ethyl group to the 5-formyl at the X position in the sequence of Figure 2B.[13,17] Enzymatic hydrolysis and HPLC analysis proved the existence of **1** in the strands. As in the 5-formyl-2'-*O*-methyluridine case,[24] only aldehyde (**1**)[25] shows UV absorption over 325 nm in a neutral medium, and the detection of the minor component (**1**) was effective using both wavelengths at 260 nm (like normal nucleosides) and 304 nm (specific to **1**).[13]

Design and Synthesis of a Subset of RecA-derived Peptides: Published results have established that a RecA-derived peptide extending from residues 193 to 212, FECO, was able to not only bind to DNA but also catalyze the pairing reaction.[15] FECO binds to DNA in a non-sequence-specific manner. FECO

has one unique lysine residue which might react with aldehydes to form Schiff base. We synthesized a series of peptides (Figure 2A) whose amino acid residues spanning the center of the loop L2 of RecA were sequentially replaced with lysine to find out the appropriate position for Schiff base formation. Based on the results of mutation study of whole RecA,[26] the positions to be replaced with lysine were selected so that DNA binding activity of mutants would be retained. Further, peptides were designed to have a single lysine by replacing the congenital lysine with arginine to avoid the complexity arising from multiple reaction points. A mutant, K6R, in which single lysine of FECO was replaced with arginine, was synthesized as a reference to evaluate the effect of the substitution.

A peptides

FECO NQIRM**K**IGVMFGNPETTTGG
K6R NQIRM**R**IGVMFGNPETTTGG
G8K NQIRM**R**I**K**VMFGNPETTTGG
G8^{d}K NQIRM**R**I^{d}**K**VMFGNPETTTGG
V9K NQIRM**R**IG**K**MFGNPETTTGG
M10K NQIRM**R**IGV**K**FGNPETTTGG
F11K NQIRM**R**IGVM**K**GNPETTTGG
G12K NQIRM**R**IGVMF**K**NPETTTGG

B oligonucletotides

I* :5'-TT**X**TT**X**TT**X**TT**X**TT**X**TT**X**TT**X**TTU^{DIG}-3'
II* :5'-^{DIG}CATTATACTTA**X**TTTTATGTTCT-3'
III*:5'-^{DIG}CATTATACTTATTTTTATGTTCT-3'

X :5-formyl-2'-deoxyuridine unit

Figure 2. Sequences of the peptides and oligonucleotides used in this study.

Binding of mutant RecA peptides to ssDNA: First, we tested the binding of RecA peptides to ssDNA. As the binding of the peptides to natural DNA was too weak to cause gel shifts, it was assessed by CD spectroscopy. On binding to ssDNA, RecA peptides are known to change the conformation from a random coil to a β-structure, which can be easily monitored by CD in the far-UV region where the spectroscopic signals primarily arise from the peptide bonds.[15,16] The β-structure is characterized by a maximum at ~190 nm and a minimum at ~220 nm. The conformation of K6R changed from a random coil to a β-structure by adding ssDNA $(dT)_{21}$ (Figure 3A), showing that the replacement of lysine with arginine does not impair the ssDNA binding activity of the RecA peptide. Thus, K6R is a suitable reference. Effects of ssDNA on the CD spectra of six mutant peptides studied here are compared in Figure 3B. The order of the propensity to form the β-structure was found to be FECO > G8K > > M10K, G12K, and for V9K and F11K no β-structure was observed. This must reflect their affinity towards ssDNA. CD of DNA was negligible in the spectral region.

Interestingly, Gly8 is totally conserved among 64 eubacterial RecAs.[14,27] Since Gly has no side chain, the effect of amino acid stereochemistry at the 8th position on ssDNA-binding is of interest. We synthesized the peptide G8^{d}K in which L-Lys of G8K was replaced with D-Lys. Figure 3C shows that

the addition of ssDNA did not induce any conformational change in G8^{d}K, indicating the complete loss of DNA binding ability. The stereochemistry of Lys is strictly limited to L-configuration at the 8th position.

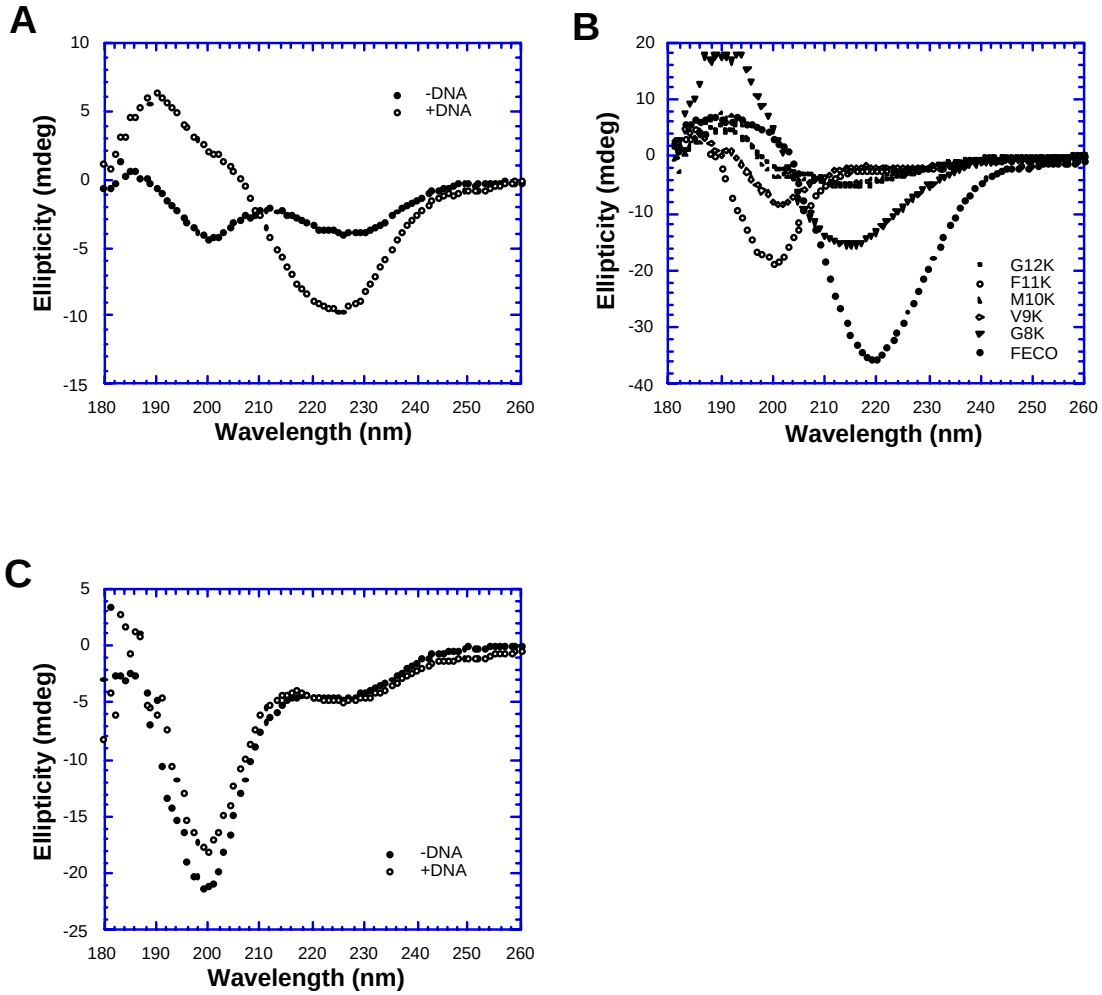


Figure 3. CD spectra of RecA-derived peptides (300 μM) in the presence and absence of $(dT)_{21}$ (15 μM). (**A**) CD of K6R in the presence (open circle) and absence (filled circle) of ssDNA. (**B**) CD of FECO and its mutant peptides in the presence of ssDNA. (**C**) CD of G8^{d}K in the presence (open circle) and absence (filled circle) of ssDNA.

Electrophoretic mobility shift assays

Cross-linking experiment (1): Figure 4A shows the results of cross-linking experiment for FECO and its lysine-scanned mutants. DIG-labeled DNA **I*** (Figure 2B), which has seven units of fdU, was incubated with 160 μM of peptide in PBS buffer containing 1 mM $MgCl_2$. The resultant Schiff bases were reduced by $NaBH_3CN$ to fix the cross-link and analyzed on 10% polyacrylamide gel electrophoresis. DNA was electroblotted from the gel to the nylon membrane. DIG-labeled DNA on the membrane was visualized by chemical luminescence. The reaction with FECO resulted in complete disappearance of **I*** on the gel (the reason for the absence of a band will be discussed in the next paragraph), showing that the cross-linking reaction proceeded efficiently. G8K also cross-linked with **I***, affording the high

molecular weight molecules. The other four peptides did not give any detectable cross-linked product. These results are consistent with those of CD, indicating that cross-linking through Schiff base formation proceeded efficiently only when the peptides bound to ssDNA. No gel shift was observed when natural ssDNA was employed (data not shown) and this supports the Schiff base formation through fdU.

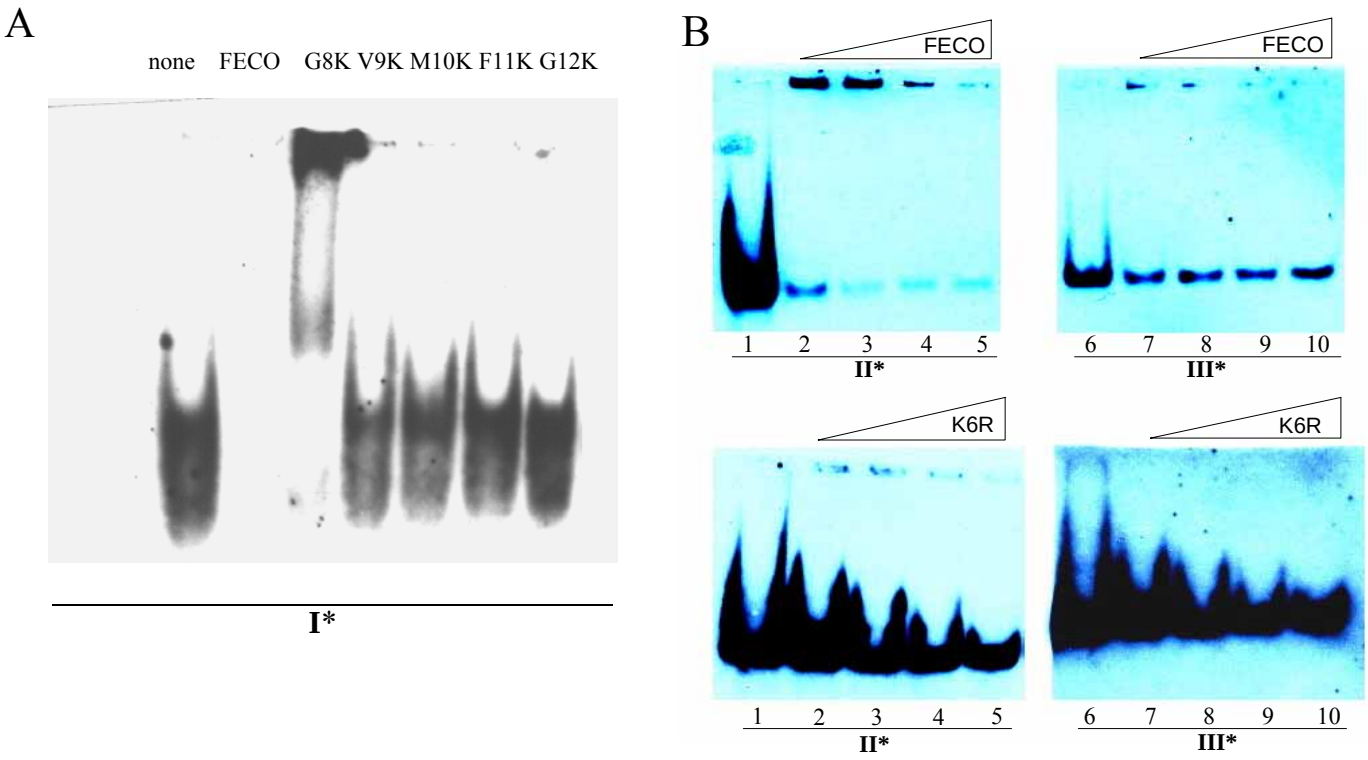


Figure 4. Electrophoretic mobility shift assays of cross-linking reactions. (**A**) DIG-labeled ssDNA **I*** was incubated with 160 μM of each peptide for 30 min at room temperature and reduced with $NaBH_3CN$ for 30 min. Each sample was resolved on a 10 % polyacrylamide gel and visualized by chemical luminescence. (**B**) DIG-labeled ssDNAs (**II*** and **III***) were reacted with serially diluted FECO or K6R. Lanes 1-5, **II***; lanes 6-10, **III***; Lanes 1 and 6, free probe; lanes 2-5 and lanes 7-10 contain 10, 20, 40, 80 μM of peptides, respectively.

Cross-linking experiment (2): Although FECO reacted with **I***, the reaction product could not be visualized in gel electrophoresis. We speculated that the cross-linking reaction between FECO and **I*** may have caused formation of insoluble very high molecular weight aggregates in the reaction mixture, and to prove this we conducted the second cross-linking experiments. In order to reduce the extent of aggregation, we synthesized 23 mer oligonucleotide **II** which has only one unit of fdU. The oligonucleotide **III** is identical to **II** except it contains natural T in the place of fdU. They were labeled with DIG at the 5'-end (Figure 2**B**). Figure 4B shows the results of the cross-linking experiment with **II*** and **III***. We examined four peptide-DNA combinations: FECO-**II***, FECO-**III***, K6R-**II***, and K6R-**III***. The DIG-labeled DNA was incubated with serially diluted peptides (10-80 μM) in PBS buffer containing 10 mM $MgCl_2$ and reduced by $NaBH_3CN$. The reaction mixture was separated on a 12% polyacrylamide gel and analyzed as described in *cross-linking (1)*. Although CD experiments have

demonstrated that both FECO and K6R can bind to ssDNA, only the combination of FECO-**II***, in which lysine and fdU coexisted, gave a new clear band. Since the new band was detected in the position of wells and there were no bands between free DNA and the well, the band must represent the product in an aggregate form. Interestingly, the reaction product exhibited a more intense band at the lower peptide concentrations compared with the higher ones. This is probably due to the fact that higher molecular weight aggregates which cannot get into the gel were formed at higher peptide concentrations, just like the case of FECO-**I*** (Figure 2A). The reaction did not proceed by binding of DNA to peptides alone, and coexistence of lysine and fdU was required. This strongly suggests that Schiff base was formed in the reaction.

Aggregation of ssDNA by RecA peptides: To find out whether the peptide-DNA complex detected on the PAGE is truly an aggregate, we assayed for aggregation[28] under the same condition of *cross-linking experiment (2)* using the same oligonucleotides (**II*** and **III***, Figure 2**B**) and 10 μM of peptides (FECO, K6R, G8K). When FECO or G8K was mixed with ssDNA **II***, most of the DNA was found in aggregates that sedimented to the bottom of a tube after centrifugation (Figure 5). Neither FECO nor G8K caused aggregation on ssDNA **III*** which lacks fdU. K6R did not form aggregates with **II*** nor **III***. Thus, the aggregation requires both lysine and fdU, and the fact also supports the formation of Schiff base. From these results as well as those of PAGE, we can conclude that the aggregation in FECO-**II*** and G8K-**II*** complexes is triggered by Schiff base formation between the lysine residue of the peptide and fdU of ssDNA.

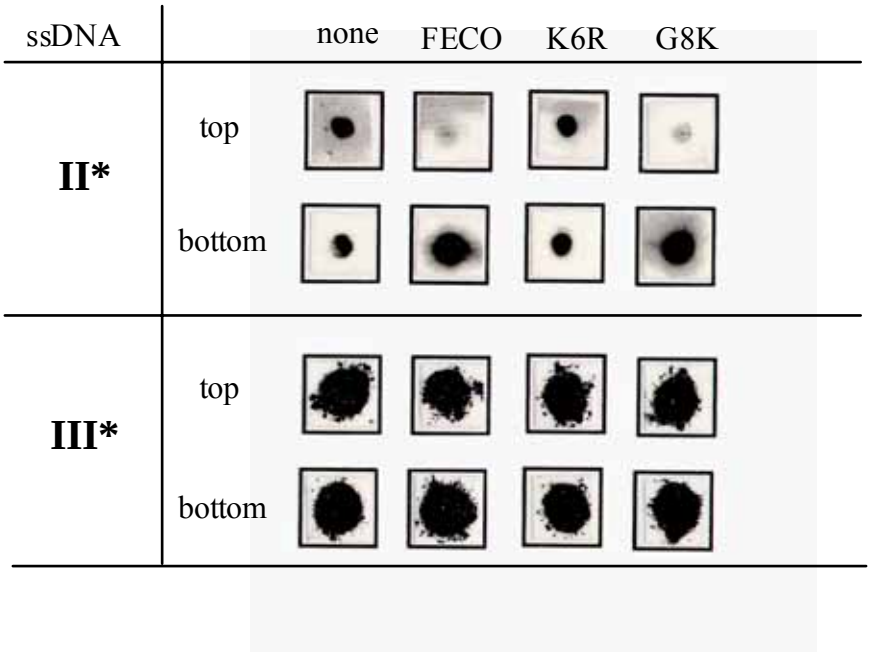


Figure 5. Assays for aggregation of ssDNA. DIG-labeled ssDNAs (**II*** and **III***) were reacted with 10 μM of FECO or K6R for 30 min at room temperature and reduced with $NaBH_3CN$ for 30 min. After centrifugation, aliquots of 2 μL were taken from the top of the supernatant and from the bottom of the tube and were spotted onto a positively charged nylon membrane to detect DIG-labeled ssDNA by chemical luminescence.

It has been believed that the reactivity of the formyl group of fdU was too low to cross-link the amino groups of proteins in an aqueous solution. Indeed, we have not recognized any evidence for cross-linking between dsDNA containing fdU and transcription factor NFκB so far.[13] However, this may not solely caused by low reactivity of fdU in aqueous solution. The binding of NFκB to decameric κB site in dsDNA is sequence specific and tight,[29] and this might impose some geometric restriction on the Schiff base formation between fdU and lysine of NFκB. In the present study, we showed that ssDNA containing fdU is capable of cross-linking RecA derived peptides. In contrast to NFκB, RecA-derived peptides bind to both ss- and dsDNA's without sequence specificity and polymerize onto the DNA. The loose recognition property of RecA-derived peptides seems to have allowed the ε-amino group of lysine to approach the formyl group of fdU in the correct direction to form Schiff base in the FECO-ssDNA and G8K-ssDNA complexes. RecA peptides bind to dsDNA as well as ssDNA, however, attempts to obtain a cross-link between dsDNA containing fdU and RecA peptides were unsuccessful (data not shown). Interactions of dsDNA with RecA peptide seem to be different from those of ssDNA, imposing unfavorable orientation or location of lysine in the complex. Thus, the cross-linking appears to depend on the DNA binding characteristics of proteins and peptides.

Formation of Schiff base reduces the dissociation rate of a bound RecA peptide from DNA. This facilitates the polymerization of the peptides on the DNA by promoting interpeptide interactions, which would result in aggregation (Figure 6).

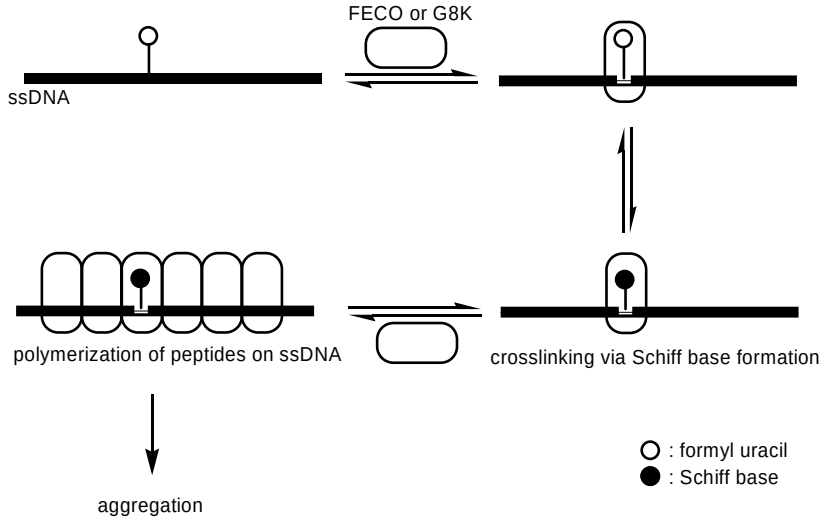


Figure 6. A plausible mechanism for Schiff base triggered aggregation of RecA-derived peptides onto fdU-containing ssDNA.

Since the Schiff base is likely to be stabilized in the aggregates, the ordinarily unfavorable equilibrium for the Schiff base formation in aqueous solution may be shifted towards the product side. In the cross-linking reaction using fdU, the background arising from nonspecific reaction would be very low by virtue of the low reactivity of formyl group of fdU in aqueous solution. Thus, oligonucleotides

containing fdU will be a novel probe which can specifically detect the lysines in the close proximity of fdU. Furhter, the cross-linking entraps kinetically unstable complexes which cannot survive during gel electrophoresis. Recently, another chemical cross-linking method in which trisubstituted pyrophosphate was used as a lysine trap has been reported.[30] These two methods appear to be complementary, that is, one targets nucleobase and another targets phosphates.

Based on the results of saturation mutagenesis of loop L2 region of whole RecA protein, we chose the lysine substitution positions so as not to impair the DNA binding ability (except for Phe11 which has been known to be indispensable for the function of FECO). However, among the 20 amino acid residue peptides used in this study, only G8K substitution was tolerated for DNA binding. This contrasts the fact that all the same substitutions on the whole RecA including Phe11 were known to cause no severe defects of their homologous recombination activity *in vivo*.[26] The difference is probably due to the lack of conformational constraints of the peptide. In the whole RecA protein the loop L2 region may be forced to adopt favorable conformation for DNA binding by the constraints at the both ends of the loop. Photo-cross-linking studies have mapped DNA-binding sites of RecA protein to loop L1 and L2.[31,32] RecA is thought to have two kinds of DNA binding sites, site I and site II,[33-35] but their locations in terms of amino acid sequences are not yet fully understood. Since only L2 not L1 has a lysine in the loop, direct application of this technique to whole RecA protein may be fruitful in elucidating the actual binding sites.

In summary, we demonstrated the usefulness of fdU (**1**) as a cross-linking reagent using RecA peptide based on high reactivity between the formyl group of fdU and the ε-amino group of Lys. PAGE as well as sedimentation experiments supported the hypothesis that the aggregation is triggered by Schiff base formation between lysine and fdU which are placed in close proximity. In fact, the aggregation is an important factor as it shifts the equilibrium towards the Schiff base formation and makes the Shiff base formation observable by PAGE. RecA-derived peptides are unique DNA binding peptides that form a β-structure and filament, resembling that of prions closely.[36] This type of cross-linking work may give an insight into the nature of DNA-protein and protein–protein interactions.[37,38]

EXPERIMENTAL

General. Melting points were determined with a Yanagimoto micro melting point apparatus and uncorrected. ^{1}H NMR spectra were measured on a JEOL JNM-GX 400 or a JEOL ramda 500 spectrometer, and chemical shifts were reported in ppm on the δ scale relative to the internal standard (Me_4Si). MS spectra were measured on a JEOL JMS D-300 or a JEOL SX-102A spectrometer in FAB mode (*m*-nitrobenzyl alcohol as a matrix). UV spectra were recorded on a JASCO Ubest-55 or a Shimadzu UV-1600 spectrophotometer. Elemental analyses were carried out in the Microanalytical Laboratory, School of Pharmaceutical Sciences, Showa University. A commercially available hexane solution of BuLi was titrated before use with diphenyl acetic acid in THF. THF was distilled from

benzophenone ketyl. Column chromatography was carried out on silica gel (silica gel 60, Merck). TLC was performed on silica gel (precoated silica gel plate F_{254}, Merck). Preparative HPLC was carried out on a Shimadzu LC-6AD with a Shim-pack Prep-Sil(H)·KIT column (2 X 25 cm). Oligonucleotides were synthesized in the 30 nmol scale with a Beckman Oligo 1000M DNA Synthesizer based on the Beckman standard protocol.

N^{ε}-Benzyloxycarbonyl-N^{α}-(*tert*-butoxycarbonyl)-L-lysine methyl ester (3). N^{ε}-Benzyloxycarbonyl-N^{α}-(*tert*-butoxycarbonyl)lysine (**2**, 540 mg, 1.42 mmol) was dissolved in THF (14.2 mL), and MeOH (0.29 mL, 7.10 mmol) and Ph_3P (0.45 mg, 1.70 mmol) were added. To the solution, DEAD (0.74 mL, 1.70 mmol) was added dropwise at 0 ºC. The mixture was stirred at rt for 2 h, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (10% → 33% EtOAc in hexane) to yield 600 mg of **3** as a colorless oil (quant.). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37-7.29 (5H, m), 5.09 (3H, br s), 4.83 (1H, s), 4.30-4.26 (1H, m), 3.73 (3H, s), 3.21-3.17 (2H, m), 1.80-1.78 (1H, m), 1.67-1.60 (2H, m), 1.55-1.48 (2H, m), 1.43 (9H, s), 1.39-1.30 (1H, m). FAB MS *m/z* 395 $(M + H)^+$. Anal. Calcd for $C_{20}H_{30}N_2O_6$: C, 60.90; H, 7.67; N, 7.10. Found: C, 60.95; H, 7.99; N, 7.10.

N^{ε}-Benzyloxycarbonyl-N^{α}-(*tert*-butoxycarbonyl)-L-lysinamide (4). Methyl ester (**3**) **(**530 mg, 1.34 mmol**)** was dissoleved in MeOH (10 mL) in a sealed tube, and liquid NH_3 (2 mL) was introduced at −78 ºC. After sealing, the mixture was stirred at rt for 3 days, and then cooled again to −78 ºC for opening the sealed tube. After concentration, the residue was purified by silica gel column chromatography (10% → 33% EtOAc in hexane) to yield 270 mg of **4** as a colorless needle (53%). mp 143.5-144.0 ºC (EtOAc-hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35-7.28 (5H, m), 6.21, 6.57, and 5.23 (3H, each br s), 5.09 (2H, s), 4.93 (1H, br s), 4.14-4.09 (1H, m), 3.20-3.17 (2H, m), 1.86-1.80 (1H, m), 1.69-1.46 (4H, m), 1.43 (9H, s), 1.41-1.37 (1H, m). FAB MS *m/z* 380 $(M + H)^+$. Anal. Calcd for $C_{19}H_{29}N_3O_5$: C, 60.14; H, 7.70; N, 11.07. Found: C, 60.40; H, 7.83; N, 11.23.

3',5'-Bis-(*O*-*tert*-butyldimethylsilyl)-5-iodo-2'-deoxyuridine (8). The mixture of 5-iodo-2'-deoxyuridine[20] (5.99 g, 16.9 mmol) and imidazole (5.76 g, 84.6 mmol) was dissolved in DMF (33.8 mL), and TBS-Cl (7.65 g, 50.8 mmol) was added. The solution was stirred at rt overnight. The mixture was partitioned between EtOAc (500 mL) and H_2O (100 mL), the organic layer was washed with H_2O and brine (100 mL each), and dried over Na_2SO_4. Silica gel column chromatography (10% → 25% EtOAc in hexane) gave 9.30 g of **8** as a white form (94%). UV (MeOH) λ_{max} 284 nm (ε 8000), λ_{min} 245 nm (ε 1700). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.56 (1H, br s), 8.09 (1H, s), 6.27 (1H, dd, *J* = 5.8, 7.9 Hz), 4.39 (1H, dt, *J* = 5.8, 2.3 Hz), 3.98 (1H, q, *J* = 2.3 Hz), 3.89 and 3.76 (2H, dd, *J* = 2.3, 11.6 Hz), 2.30 (1H, ddd, *J* = 2.3, 5.8, 13.1 Hz), 1.99 (1H, ddd, *J* = 5.8, 7.9, 13.1 Hz), 0.94 and 0.89 (18H, each s), 0.16, 0.15, 0.08, and 0.07 (12H, each s). FAB MS *m/z* 583 $(M + H)^+$, 525 $(M - Bu\text{-}t)^+$. Anal. Calcd for $C_{21}H_{39}N_2O_5ISi_2$: C, 43.29; H, 6.75; N, 4.81. Found: C, 43.44; H, 6.35; N, 4.70.

3',5'-Bis-(*O*-*tert*-butyldimethylsilyl)-5-vinyl-2'-deoxyuridine (9). To the MeCN (25 mL) solution of **8** (2.88 g, 4.94 mmol), $PdCl_2(MeCN)_2$ (71.1 mg, 0.27 mmol) and $Bu_3SnCH{=}CH_2$ (2.61 mL, 7.39 mmol) were added, and the mixture was refluxed for 12 h. After cooling to rt, the mixture was filtered through a filter paper, and the filtrate was concentrated *in vacuo*. Silica gel column chromatography (10% → 25% EtOAc in hexane) gave 2.28 g of crude **9**, which contained small amount of the starting material of **8**. This was used without further purification for the next step, while a portion of crude **9** was purified by HPLC (16% EtOAc in hexane) for physical data. Pure **9**: UV (MeOH) λ_{max} 279 nm (ε 6000), λ_{min} 256 nm (ε 4100). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.30 (1H, br s), 7.67 (1H, s), 6.35 (1H, dd, *J* = 11.3, 17.4 Hz),

6.30 (1H, dd, *J* = 5.8, 7.9 Hz), 6.01 (1H, dd, *J* = 1.5, 17.4 Hz), 5.24 (1H, dd, *J* = 1.5, 11.3 Hz), 4.40 (1H, ddd, *J* = 2.4, 2.8, 6.0 Hz), 3.97 (1H, q, *J* = 2.8 Hz), 3.87 and 3.77 (2H, dd, *J* = 2.8, 11.3 Hz), 2.31 (1H, ddd, *J* = 2.3, 5.8, 13.1 Hz), 2.02 (1H, ddd, *J* = 6.0, 7.9, 13.1 Hz), 0.91 and 0.90 (18H, each s), 0.10 and 0.08 (12H, each s). FAB MS (+KI) *m/z* 521 $(M + K)^+$. Anal. Calcd for $C_{23}H_{42}N_2O_5Si_2 \cdot H_2O$: C, 55.16; H, 8.86; N, 5.59. Found: C, 55.09; H, 8.48; N, 5.48.

3',5'-Bis-(*O*-*tert*-butyldimethylsilyl)-5-(1,2-dihydroxyethyl)-2'-deoxyuridine (10). To the acetone (94.5 mL) solution of crude **9** (2.28 g, ca. 4.72 mmol), H_2O, *tert*-butanol (23.6 mL each) and *N*-methylmorpholine *N*-oxide (1.38 g, 11.8 mmol) were added. To the resulting solution, 2%OsO_4 in H_2O (1.46 mL) was added with stirring, and the mixture was stirred at rt overnight. The mixture was partitioned between EtOAc (500 mL) and 0.1 M $Na_2S_2O_3$ (70 mL), and the organic layer was washed with H_2O and brine (70 mL each), and dried over Na_2SO_4. Silica gel column chromatography (10% → 25% EtOAc in hexane to remove **8**, and then 5%MeOH in $CHCl_3$ to obtain **10**) gave 1.94 g of **10** (a mixture of diastereomers of alcohol) as white foam (80% from **8**). UV (MeOH) λ_{max} 265 nm (ε 9700), λ_{min} 233 nm (ε 1900). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.34 (1H, br s), 7.44 and 7.42 (1H, each s), 6.16-6.12 (1H, m), 5.09-5.06 (1H, m), 4.63-4.57 (1H, m), 4.43-4.40 (1H, m), 4.37-4.35 (1H, m), 3.81-3.78 (1H, m), 3.71-3.64 (2H, m), 3.54-3.49 (1H, m), 3.28-3.23 (1H, m), 2.19-2.06 (2H, m), 0.88 (18H, s), 0.08 and 0.07 (12H, each s). FAB MS *m/z* 539 $(M + Na)^+$, 517 $(M + H)^+$, 459 $(M - Bu\text{-}t)^+$. Anal. Calcd for $C_{23}H_{44}N_2O_7Si_2$: C, 53.46; H, 8.58; N, 5.42. Found: C, 53.48; H, 8.70; N, 5.35.

3',5'-Bis-(*O*-*tert*-butyldimethylsilyl)-5-formyl-2'-deoxyuridine (6). To the MeOH-THF (2:3, 15.0 mL) solution of **10** (310 mg, 0.59 mmol), $NaIO_4$ (130 mg, 0.61 mmol) in H_2O (1 mL) was added dropwise with stirring, and the mixture was stirred at rt for 30 min. After quenching the reaction with ethylene glycol (33.1 μL), the mixture was concentrated *in vacuo*. The residue was partitioned between EtOAc (100 mL) and H_2O (30 mL), and the organic layer was washed with brine (30 mL), and dried over Na_2SO_4. Silica gel column chromatography (10% → 50% EtOAc in hexane) gave 260 mg of **6** as a white powder (91%). This was recrystallized from hexane-EtOAc. mp 139.1-140.1 ºC. UV (MeOH) λ_{max} 291 nm (ε 9800), λ_{min} 249 nm (ε 2000). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.00 (1H, s), 8.73 (1H, br s), 8.54 (1H, s), 6.30 (1H, dd, *J* = 5.7, 7.7 Hz), 4.41 (1H, dt, *J* = 5.7, 2.4 Hz), 4.07 (1H, q, *J* = 2.4 Hz), 3.88 and 3.78 (2H, dd, *J* = 2.4, 11.4 Hz), 2.44 (1H, ddd, *J* = 2.4, 5.7, 13.3 Hz), 2.04 (1H, ddd, *J* = 5.7, 7.7, 13.3 Hz), 0.90 and 0.89 (18H, each s), 0.11, 0.09, and 0.08 (12H, each s). FAB MS *m/z* 507 $(M + Na)^+$, 485 $(M + H)^+$, 427 $(M - Bu\text{-}t)^+$. Anal. Calcd for $C_{22}H_{40}N_2O_6Si_2$: C, 54.51; H, 8.32; N, 5.78. Found: C, 54.60; H, 8.48; N, 5.76.

5-[[[(S)-{5-(*tert*-Butoxycarbonyl)amino-5-carbamoyl}pentyl]amino]methyl]-3',5'-bis-*O*-(*tert*-butyldimethylsilyl)-2'-deoxyuridine (7). To the MeOH (4.8 mL) solution of **4** (190 mg, 0.48 mmol), 10% Pd/C (19.2 mg) was added, and the mixture was vigorously stirred at rt under H_2 atmosphere for 30 min. The mixture was passed through a paper filter, and the filtrate and washings were combined and concentrated in vacuo. The residual oil was used without further purification for the next reaction. The resulting amine was dissolved in DMF (2.3 mL), and MeCN (5.3 mL) and MS 3Å (250 mg) were added. To the mixture, the MeCN (4.8 mL) solution of aldehyde (**6**) (230 mg, 0.48 mmol) was added dropwise at rt with stirring. After 2 h, $NaBH_4$ (55 mg, 1.45 mmol) in MeOH (4.8 mL) was added, and the mixture was stirred for 30 min. The mixture was filtered through a celite pad, and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography (1% → 7% MeOH in CH_2Cl_2) to yield 250 mg of **7** as a pale yellow foam (73%). UV (MeOH) λ_{max} 264 nm (ε 9100), λ_{min} 233 nm (ε

2300). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.75 (1H, s), 6.73 (1H, br s), 6.27 (1H, dd, *J* = 5.8, 7.6 Hz), 5.86 (1H, br s), 5.37 (1H, br), 4.87 (2H, br s), 4.40 (1H, dt, *J* = 6.1, 2.8 Hz), 4.14 (1H, br s), 3.94 (1H, dt, 2.8, 3.7 Hz), 3.82 and 3.76 (2H, dd, *J* = 3.7, 11.3 Hz), 3.60 (2H, br s), 2.75-2.73 (2H, m), 2.26 (1H, ddd, *J* = 2.8, 5.8, 13.4 Hz), 2.09 (1H, ddd, *J* = 6.1, 7.6, 13.4 Hz), 1.84-1.78 (1H, m), 1.67-1.61 (3H, m), 1.47-1.41 (4H, m), 1.43 (9H, s), 0.91 and 0.89 (18H, each s), 0.10 and 0.08 (12H, each s). FAB MS *m/z* 714 $(M + H)^+$. Anal. Calcd for $C_{33}H_{63}N_5O_8Si_2 \cdot 3/2H_2O$: C, 53.48; H, 8.98; N, 9.45. Found: C, 53.78; H, 8.95; N, 9.06.

3',5'-Bis-(*O*-*tert*-butyldimethylsilyl)-5-(1,2-diacetoxyethyl)-2'-deoxyuridine (11). To the pyridine (38.5 mL) solution of **10** (1.99 g, 3.85 mmol), acetic anhydride (1.45 g, 15.4 mmol) was added dropwise, and the mixture was stirred at rt overnight. MeOH (10 mL) was added, and the mixture was concentrated *in vacuo*. The residue was partitioned between EtOAc (450 mL) and H_2O (150 mL), and the organic layer was washed with 0.5 M HCl, saturated $NaHCO_3$, H_2O and brine (120 mL each), successively, and dried over Na_2SO_4. Silica gel column chromatography (10% → 50% EtOAc in hexane) gave 2.30 g of **11** (a mixture of diastereomers) as colorless oil (99%). UV (MeOH) λ_{max} 265 nm (ε 10000), λ_{min} 231 nm (ε 1900). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.01 and 8.90 (1H, br s), 7.63 and 7.60 (1H, each s), 6.25-6.19 (1H, m), 5.87-5.79 (1H, m), 4.50-4.37 (3H, m), 4.97-4.94 (1H, m), 3.79-3.71 (2H, m), 2.34-2.27 (1H, m), 2.08, 2.07, 2.04, and 2.03 (6H, each s), 2.01-1.94 (1H, m), 0.91 and 0.89 (18H, each s), 0.10, 0.08, and 0.07 (12H, each s). FAB MS (+KI) *m/z* 639 $(M + K)^+$. Anal. Calcd for $C_{27}H_{48}N_2O_9Si_2$: C, 53.97; H, 8.05; N, 4.66. Found: C, 54.26; H, 8.17; N, 4.69.

5'-*O*-(4,4'-Dimethoxytrityl)-5-(1,2-diacetoxyethyl)-2'-deoxyuridine (13). To the THF (35 mL) solution of **11** (2.10 g, 3.49 mmol), acetic acid (0.40 mL, 6.98 mmol) and 1 M solution of Bu_4NF in THF were added, and the mixture was stirred at rt overnight. The mixture was concentrated *in vacuo*, and the residue was purified by silica gel column chromatography (1% → 4% MeOH in CH_2Cl_2) gave 1.30 g of **12** (a mixture of diastereomers) as colorless oil (quant.). This was used without further purification for the next step. Diol (**12**) (307.6 mg, 0.828 mmol) was dissolved in pyridine (8.3 mL), and DMTCl (420.8 mg, 1.24 mmol) was added. The mixture was stirred at rt for 3 h, and then EtOH (1 mL) was added, and concentrated *in vacuo*. The residue was partitioned between EtOAc (70 mL) and saturated $NaHCO_3$ (25 mL), and the organic layer was washed with brine, and dried over Na_2SO_4. Silica gel column chromatography (30% → 75% EtOAc in hexane) gave 279.2 mg of **13** (a mixture of diastereomers) as a white form (50%). The two diastereomers of **13** were separated by HPLC on a Shim-Pack Prep-SIL (H) column (2 X 25 cm, eluted with 20% hexane in EtOAc at a flow rate of 10 mL/min). Selected data of **13**[17] (retention time 15.1 min): UV (MeOH) λ_{max} 234 nm (ε 22500) and 267 nm (ε 11800), λ_{min} 227 nm (ε 21200) and 255 nm (ε 10100). Anal. Calcd for $C_{36}H_{38}N_2O_{11} \bullet H_2O$: C, 62.42; H, 5.82; N, 4.04. Found: C, 62.47; H, 5.85; N, 3.81. FAB MS *m/z* 675 $(M + H)^+$. For the other isomer (**13**) (retention time 16.8 min): UV (MeOH) λ_{max} 267 nm (ε 12800), λ_{min} 255 nm (ε 11000), $\lambda_{shoulder}$ 232 nm (ε 25300). Anal. Calcd for $C_{36}H_{38}N_2O_{11} \bullet 3/4H_2O$: C, 62.83; H, 5.79; N, 4.07. Found: C, 62.73; H, 5.86; N, 3.79. FAB MS *m/z* 675 $(M + H)^+$.

5'-*O*-(4,4'-Dimethoxytrityl)-5-(1,2-diacetoxyethyl)-2'-deoxyuridine-3'-*O*-(2-cyanoethyl-*N*,*N*,-diisopropyl)phosphoramidite (14). To the mixture of **13** and (523.2 mg, 0.775 mmol) and 2-cyanoethyl tetraisopropylphosphrodiamidite (0.443 mL, 1.40 mmol) in CH_2Cl_2 (12 mL), the solution of 4,5-dicyanoimidazole (64.1 mg, 0.543 mmol) in MeCN (1.2 mL) was added. The mixture was stirred at rt for 4 h, and then diluted with CH_2Cl_2 (80 mL). This was washed with saturated $NaHCO_3$ (25 mL),

and the organic layer was dried over Na_2SO_4. Silica gel column chromatography (30% → 150% EtOAc in hexane with 1% Et_3N) gave **14** as a colorless oil, which was dissolved in toluene (5 mL), and hexane (25 mL) was added with gentle stirring. After decantation and pump-up, 625.7 mg of the known amidite (**14**) was obtained as a white form (92%).[17]

Oligonucleotides. Nucleotide sequences of the oligonucleotides used in the present study are **I**: 5'-TT**X**TT**X**TT**X**TT**X**TT**X**TT**X**TT**X**TT-3', **II**: 5'-CATTATACTTA**X**TTTTATGTTCT-3', and **III***: 5'-^{DIG}CATTATACTTATTTTTATGTTCT-3', where X stands for fdU as the base moiety (Figure 2B). **I** and **II** were prepared at the 30 nmol scale on a Beckman Oligo 1000 DNA Synthesizer as described previously.[13,17] Oligonucleotides were non-radioactively labeled with DIG (Digoxigenin) according to the manufacture's manual (Roche Diagnostics). **I** was labeled at the 3'-end and **II** was labeled at the 5'-end, respectively. Labed **I** and **II** were termed **I*** and **II***. The position of DIG, whether 3' or 5'-end, did not affect the results in this study. Concentration of each oligonucleotide was estimated from UV-absorption at 260 nm. Oligomer $(dT)_{21}$ and 5'-DIG labeled oligonucleotide **III*** were purchased from Nihon Gene Research Lab's, Inc.

Peptides. All peptides used in this study (Figure 2A) were chemically synthesized by Fmoc (9-fluorenylmethyloxycarbonyl)-solid phase peptide synthesis on a PAL-PEG-PS resin (0.35-0.45 mmol/g, Applied Biosystems). The amino termini were acetylated with acetic anhydride and pyridine in DMF. The resin (20 mg) were treated with a cleavage mixture (1 mL), prepared from phenol (40 μL), thioanisole (40 μL), 1,2-ethanedithiol (40 μL), and water (40 μL) in trifluoroacetic acid (840 μL), for 6 h. Peptides were purified by reversed-phase HPLC using a Mightysil RP-18 GP 5 mm column (250 X 20 mm; Kanto Chemical co., inc.) and a linear gradient of MeCH-H_2O containing 0.1% trifluoroacetic acid at a flow rate of 7 mL/min. The fraction collected was lyophilized and dissolved in water as the stock solution and then stored at 4 °C. Concentration of each stock solution was estimated using the UV absorption at 205 nm (concentration (mg/mL) = 31 A units).[16] The identities of all peptides were confirmed by amino acid analysis and MALD-TOF (matrix-assisted laser desorption ionization time of flight) MS spectrometry. FECO calculated for $[MH^+]$ 2194.5, found 2194.2; K6R calculated for $[MH^+]$ 2222.5, found 2222.3; G8K calculated for $[MH^+]$ 2293.7, found 2293.6; G8^{d}K calculated for $[MH^+]$ 2293.7, found 2294.5; V9K calculated for $[MH^+]$ 2251.6, found 2251.7; M10K calculated for $[MH^+]$ 2219.5, found 2220.7; F11K calculated for $[MH^+]$ 2203.5, found 2203.4; G12K calculated for $[MH^+]$ 2293.7, found 2294.0. The stored peptides were heated at 65 °C for 20 min in the appropriate buffer and then were allowed to cool to room temperature for 30 min before use.

Circular dichroism spectroscopy. Circular dichroism (CD) measurements were performed in 10 mM sodium phosphate buffer, pH 7.4. All CD measurements were conducted at 25 °C on a J-720 spectropolarimeter using a 0.2 mm path length cell. Peptides (300 mM) with or without 15.2 mM of oligonucleotide $(dT)_{21}$ (304 mM per phosphate) were used. The peptide-DNA mixture was incubated at room temperature for 30 min before measurement.

Electrophoretic mobility shift assays. The ssDNA bindings to peptides were also studied by use of an electrophoretic mobility shift assays. Peptides were diluted from stock solutions into PBS binding buffer (1.47 mM KH_2PO_4, 8.10 mM Na_2HPO_4, 2.68 mM KCl, 137 mM NaCl, 1 or 10 mM $MgCl_2$, 0.1% IGEPAL CA-630 and 10% glycerol, pH 7.4) and each solution was heated at 65 °C for 20 min. After cooling to rt in 30 min, to the peptide solutions was added DIG-labeled ssDNA to a final concentration of <0.1 μM for ssDNA. Binding reactions were incubated for 30 min at rt and then reduced by $NaBH_3CN$

(0.7 mg/mL) for 30 min. Samples were then applied to a nondenaturing 10 or 12 % polyacrylamide gel (6.8 X 8.3 cm) and subjected to electrophoresis at 100 V for 1.5 h at 4 °C (22.25 mM Tris, 22.25 mM boric acid and 0.5 mM EDTA). DNA was electroblotted from the gel onto a nylon membrane (Millipore, ImmobilonTM-S) for 30 min at 20 mA. The membrane was baked for 20 min at 120 °C to fix the DNA and then treated with alkaline phosphatase conjugated anti-DIG antibodies (Roche Diagnostics) to visualize DNA bands on X-Ray film (Fuji RX-U). We used two different concentrations of $MgCl_2$ (1 or 10 mM) in the binding reaction and found that both gave the same results.

Assays for aggregation of DNA. Binding reactions were also assayed for aggregation. Peptides were diluted from the stock solutions into PBS with $MgCl_2$ (1.47 mM KH_2PO_4, 8.10 mM Na_2HPO_4, 2.68 mM KCl, 137 mM NaCl, 10 mM $MgCl_2$, pH 7.4) and each solution was heated at 65 °C for 20 min. After cooling to rt in 30 min, to the peptide solutions was added DIG-labeled ssDNA to a final concentration of 34 nM for ssDNA and 10 μM for peptides. Binding reactions (10 μL) were incubated for 30 min at rt and then reduced by adding 1 μL of $NaBH_3CN$ solution (1.5 mg in 200 μL PBS) for 30 min. The reaction mixture was centrifuged at the maximum speed in a TOMY Centrifuge MR-150 for 2 min. Aliquots of 2 μL were taken from the top of the supernatant and from the bottom of the tube and were spotted onto a positively charged nylon membrane (Millipore, ImmobilonTM-Ny+). The membrane was baked for 15 min at 120 °C and then treated with alkaline phosphatase conjugated anti-DIG antibodies (Roche Diagnostics) to visualize DNA spots on X-Ray film (Fuji RX-U).

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REFERENCES AND NOTES

1. J. Cadet and R. Teoule, *Bull. Soc. Chim. Fr.*, 1975, **3-4**, 891.
2. H. Kasai, A. Iida, Z. Yamaizumi, S. Nishimura, and H. Tonooka, *Mutat. Res.*,1990, **243**, 249.
3. T. Douki, T. Delatour, and J. Cadet, *Chem. Res. Toxicol.*, 1996, **9**, 1145.
4. C. Decarroz, J. R. Wagner, J. E. Van Lier, C. Murali Krishna, P. Riesz, and J. Cadet, *Int. J. Radiat. Biol.*, 1986, **50**, 491.
5. I. Saito, M. Takayama, and S. Kawanishi, *J. Am. Chem. Soc.*, 1995, **117**, 5590.
6. A. Ono, T. Okamoto, M. Inada, H. Nara, and A. Matsuda, *Chem. Pharm. Bull.*, 1994, **42**, 2231.
7. M. Yoshida, K. Makino, H. Morita, H. Terato, Y. Ohyama, and H. Ide, *Nucleic Acids Res.*, 1997, **25**, 1570.
8. H. Anensen, F. Provan, A. T. Lian, S.-H. S. Reinertsen, Y. Ueno, A. Matsuda, E. Seeberg, and S. Bjelland, *Mutat. Res.*, 2001, **476**, 99.
9. E. De Clercq, J. Barzarini, P. F. Torrence, M. P. Mertes, C. L. Schmidt, D. Shugar, P. J. Barr, A. S. Jones, G. Verhelst, and R. T. Walker, *Mol. Pharmacol.*, 1981, **19**, 321.
10. J. Balzarini, E. De Clercq, P. F. Torrence, M. P. Mertes, J. S. Park, C. L. Schmidt, D. Shugar, P. J. Barr, A. S. Jones, G. Verhelst, and R. T. Walker, *Biochem. Pharmacol.*, 1982, **31**, 1089.
11. J. Balzarini, E. De Clercq, M. P. Mertes, D. Shugar, and P. F. Torrence, *Biochem. Pharmacol.*, 1982, **31**, 3673.
12. A. Klungland, R. Paulsen, V. Rolseth, Y. Yamada, Y. Ueno, P. Wiik, A. Matsuda, E. Seeberg, and S.

Bjelland, *Toxicol. Lett.*, 2001, **119**, 71.
13. A. Kittaka, C. Horii, T. Kuze, T. Asakura, K. Ito, K. T. Nakamura, T. Miyasaka, and J. Inoue, *Synlett*, 1999, 869.
14. A. I. Roca and M. M. Cox, *Prog. Nucl. Acid Res. Mol. Biol.*, 1997, **56**, 129.
15. O. N. Voloshin, L. Wang, and R. D. Camerini-Otero, *Science*, 1996, **272**, 868.
16. L. Wang, O. N. Voloshin, A. Stasiak, and R. D. Camerini-Otero, *J. Mol. Biol.*, 1998, **277**, 1.
17. H. Sugiyama, S. Matsuda, K. Kino, Q. M. Zhang, S. Yonei, and I. Saito, *Tetrahedron Lett.*, 1996, **37**, 9067.
18. T. Takeda, K. Ikeda, Y. Mizuno, and T. Ueda, *Chem. Pharm. Bull.*, 1987, **35**, 3558.
19. H. Terato, H. Morita, Y. Ohyama, and H. Ide, *Nucleosides & Nucleotides*, 1998, **17**, 131.
20. J.-I. Asakura and M. J. Robins, *J. Org. Chem.*, 1990, **55**, 4928.
21. G. T. Crisp, *Synth. Commun.*, 1989, **19**, 2117.
22. Compounds (**8**) and (**9**) showed almost the same Rf value on a silica gel TLC plate (Rf 0.25 with 20% EtOAc in hexane); therefore, it was difficult to separate compounds (**8**) and (**9**) after the coupling reaction in a gram scale synthesis.
23. C. Vargeese, J. Carter, J. Yegge, S. Krivjansky, A. Settle, E. Kropp, K. Peterson, and W. Pieken, *Nucleic Acids Res.*, 1998, **26**, 1046.
24. A. Kittaka, T. Kuze, T. Asakura, K. Ito, T. Miyasaka, and J. Inoue, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 3207.
25. Authentic sample of aldehyde (**1**) was synthesized from **10** with TBAF followed by $NaIO_4$ oxidation in H_2O in 39% yield. The ^1H NMR spectral data were identical with those reported in ref. 6. UV spectral data of **1** in MeOH are as follows: λ_{max} 286 nm (ε 9900), λ_{min} 248 nm (ε 2400). Anal. Calcd for $C_{10}H_{12}N_2O_6$: C, 46.88; H, 4.72; N, 10.93. Found: C, 46.76; H, 4.58; N, 10.70.
26. K. Hortnagel, O. N. Voloshin, H. H. Kinal, N. Ma, C. Schaffer-Judge, and R. D. Camerini-Otero, *J. Mol. Biol.*, 1999, **286**, 1097.
27. S. Karlin and L. Brocchieri, *J. Bacteriol.*, 1996, **178**, 1881.
28. S. S. Tsang, S. A. Chow, and C. M. Radding, *Biochemistry*, 1994, **24**, 3226.
29. P. A. Baeuerle and T. Henkel, *Annu. Rev. Immunol.*, 1994, **12**, 141.
30. N. A. Naryshkin, M. A. Farrow, M. G. Ivanovskaya, T. S. Oretskaya, Z. A. Shabarova, and M. J. Gait, *Biochemistry*, 1997, **36**, 3496.
31. V. A. Malkov and R. D. Camerini-Otero, *J. Biol. Chem.*, 1995, **270**, 30230.
32. Y. Wang and K. Adzuma, *Biochemistry*, 1996, **35**, 3563.
33. M. Takahashi, M. Kubista, and B. Norden, *J. Mol. Biol.*, 1989, **205**, 137.
34. B. Müller, T. Koller, and A. Stasiak, *J. Mol. Biol.*, 1990, **212**, 97.
35. A. Zlotnick, R. S. Mitchell, and S. L. Brenner, *J. Biol. Chem.*, 1990, **265**, 17050.
36. H. Zhang, K. Kaneko, J. T. Nguyen, T. L. Livshits, M. A. Baldwin, F. E. Chen, T. L. James, and S. B. Prusiner, *J. Mol. Biol.*, 1995, **250**, 514.
37. T. Sugiyama, A. Kittaka, H. Takayama, M. Tomioka, Y. Ida, and R. Kuroda, *Bioorg. Med. Chem. Lett.*, 2003, **13**, 2847.
38. X-Ray crystallographic analysis of a fdU containing DNA duplex, see: M. Tsunoda, J. Kondo, N. Karino, Y. Ueno, A. Matsuda, A. Takenaka, *Biophys. Chem.*, 2002, **95**, 227.