DNA Oligomers Having a Diazapyrenium Dication (DAP^{2+}); Synthesis and DNA Cleavage Activities

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Aiming at the creation of functionalized antisense DNA oligomers possessing site-selective DNA cleaving activity, viologen and a related compound, diazapyrenium dication (DAP^{2+}), were selected and introduced into oligodeoxyribonucleotides as a functionalized molecule. The conjugation of these functionalized molecules with DNA proceeded smoothly by using standard H-phosphonate chemistry. A part of the DAP^{2+}-tethered DNA oligomers was synthesized by a combination of solid support method and liquid phase technique. Viologen-tethered DNA oligomers showed no significant activity toward DNA cleavage in spite of their characteristic ESR spectra. On the other hand, it was observed that the DAP^{2+}-tethered DNA oligomers formed more stable duplexes with their complementary strands than the corresponding wild type, and these molecules effectively cleaved the complementary strands at the specific site of 2—3 bases away from the modified phosphoramidate linkage. The effect of position and length of the linker arm on the selectivity in the cleavage reaction was also investigated, and it was found that introduction at the 3′- or 5′-end phosphate site is more favorable, probably due to duplex stabilization.

Key words antisense DNA oligomer; H-phosphonate chemistry; DNA cleavage; 4,4′-dipyridinium dication; 2,7-diazapyrenium dication

Antisense DNA strategy[1] has received much attention as one of the most efficient methods for gene therapies based on the specific control of gene expression. Recently, functionalized antisense DNA oligomers, hybridized with some kind of functionalized molecules, have been developed for this purpose.[2] These DNA oligomers are believed to act more effectively than molecules for simply masking antisense strategy. For example, antisense DNA oligomers linked to phenanthridine,[3] acridine[4] and ellipticine[5] were prepared for the stabilization and the specific interaction with duplex DNA, and the oligomers linked to EDTA–Fe(II),[6] Porphyrin–Fe(III)[7] and phenanthroline–Cu(II)[8] were also prepared for cleaving DNA. Especially interesting is an oligomer linked to endonuclease[9] having a specific DNA cleaving ability.

For the design of effective functionalized antisense DNA oligomers, we considered the molecule which satisfied the following four factors: Firstly, the molecule has a catalytic ability for DNA cleavage. Secondly, the molecule acts without any metals which cleave DNA directly. Thirdly, the functionalized molecule, which is introduced into DNA oligomer, is a simple skeleton, and is also prepared easily and economical. Finally, the molecule has a cationic feature preferring the interaction with native anionic DNA. It is well documented that 4,4′-dipyridyl (commonly referred to as viologen[10]) and related compounds have redox potential and activate molecular oxygen in the absence of any metallic ion. Viologen has been used as an herbicide.[11] In connection with its mode of action, the role of viologen as an electron-mediator[12] has received considerable attention as well as the reactivity of superoxide toward biological substrates. One-electron reduction of the viologen dication gives a cation radical which transfers one electron to molecular oxygen to yield superoxide radical concomitant with regeneration of the dication. Degradation of nucleic acids by active oxygen species is a current topic which is intimately related to mutagenesis[13] and the function of DNA targeted anti-tumor drugs.[14] Viologen molecule is believed to have a nearly orthogonal π–π system, whereas DAP^{2+} has a planar π-framework which contains the viologen moiety as a built-in unit. The DAP^{2+} dication might be anticipated to contribute favorably to binding to DNA in terms of π–π stacking and electrostatic interaction. DAP^{2+} is also known to cleave DNA effectively under irradiation of light.[15] Consequently, in this study both the bis-quaternary salt of 4,4′-dipyridyl and its analog, 2,7-diazapyrenium dication[16] (DAP^{2+}), were selected as small functionalized compounds, which were introduced into DNA molecules through covalent bonding.

We have previously reported viologen- or DAP^{2+}-tethered DNA oligomers, into which the viologen or DAP^{2+} molecule was introduced through a phosphoramidate linkage, which were prepared for investigating the specific cleavage of DNA.[17] In this paper, we describe the synthesis and characterization of viologen- or DAP^{2+}-tethered DNA oligomers and the effect of the length and position of the linker arm which contributed to both the thermal stability of the duplexes and DNA cleavage in detail. We also discuss the stability of the DAP^{2+}-tethered heterooligomers.

Alkylamino linkers **1**, **2** and **3** were firstly prepared and connected with 4,4′-dipyridyl to give viologen derivatives **6**, **7** and **10** (Chart 1). Thus, 6-Amino-1-hexanol was treated successively with ethyl trifluoroacetate and *p*-toluenesulfonyl chloride (*p*-TsCl) to give 1-(*p*-toluenesulfonyloxy)-6-(trifluoroacetylamino)hexane **1**.[18] 3-Amino-1-propanol was transformed to 1-(*p*-toluenesulfonyloxy)-3-(trifluoroacetylamino) propane **2** in a similar manner and then to 1-iodo-3-(trifluoroacetylamino)propane **3**[19] with sodium iodide. 4,4′-Dipyridyl was treated with **1** or **3** to give viologen derivative **4** or **5**, respectively. Viologen derivatives **4** and **5** are soluble in protic polar solvents, but insoluble in CCl_4, which is an essential solvent and an oxidant for the H-phosphonate protocol.[20] Consequently, the preparation of more lipophilic viologen derivatives having improved solubility toward CCl_4 was necessary. 4,4′-Dipyridyl was successively transformed to 1-*n*-octyl-4,4′-dipyridinium bromide[21] using 1-*n*-octyl

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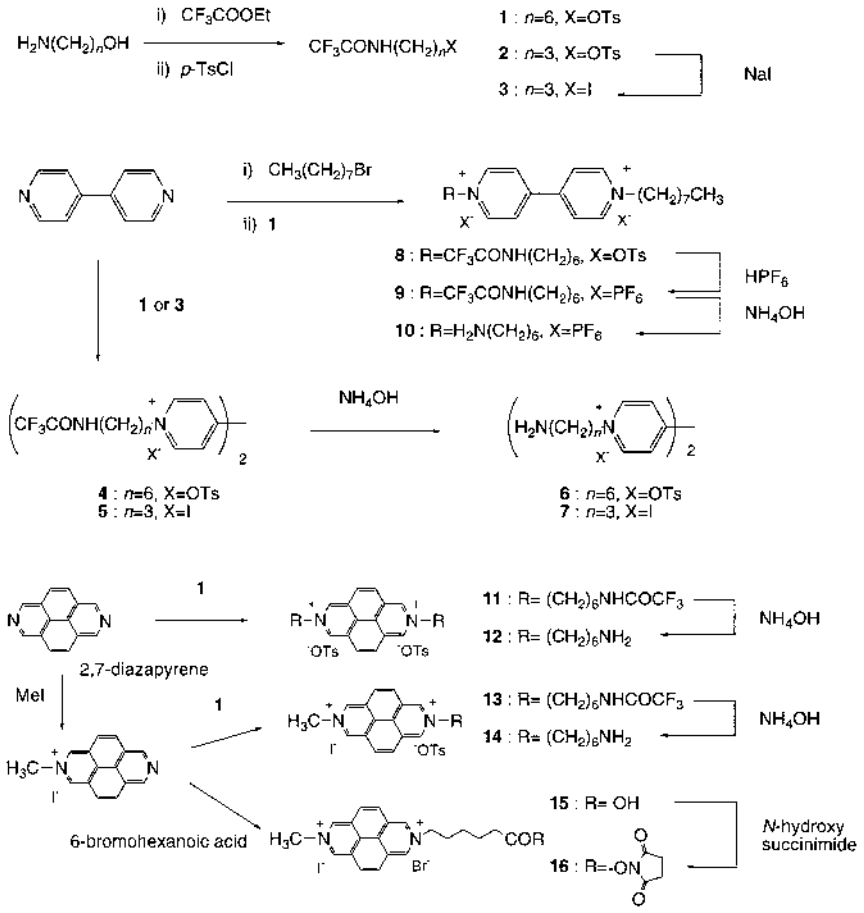


Chart 1. Synthesis of Viologen and DAP^{2+} Derivatives

bromide and to 1-*n*-octyl-1′-[6-(trifluoroacetylamino)hexyl]-4,4′-dipyridinium bis[*p*-toluenesulfonate] **8** using **1**. The counter anion of **8** was exchanged to PF^{6-} from OTs^{-} using hexafluorophosphoric acid[22] to give 1-*n*-octyl-1′-[6-(trifluoroacetylamino)hexyl]-4,4′-dipyridinium bis[hexafluorophosphonate] **9**. Deprotection of **4**, **5** and **9** with NH_4OH gave the corresponding viologen derivatives **6**, **7** and **10**, respectively.

The preparation of DAP^{2+} derivatives **12**, **14** and **16** is also shown in Chart 1. 2,7-Diazapyrene was prepared from 1,4,5,8-naphthalenetetracarboxylic dianhydride according to the reported procedure,[23] and then treated with tosylate **1** to give **12** after deprotection. Additionally, 2,7-diazapyrene was mono-methylated with MeI,[24] and then converted to **14** in the same way. Furthermore, the mono-methylated compound was alkylated with 6-bromohexanoic acid and then converted to an active ester, *N*-hydroxy succinimidate **16**.

Viologen derivatives **4**, **5** and **9** exhibited similar UV spectra to that of methylviologen.[22] Viologen dication has an absorption at 260 nm while viologen cation radical, generated by one-electron reduction with $Na_2S_2O_4$, has absorption at around 400 and 600 nm. The ESR spectrum of the cation radical of **6** showed characteristic splitting with $g=2.0012$ (Fig. 1). These results suggested that ESR spectra are indicative of the introduction of a viologen moiety into a nucleic acid, even when the UV spectra of the viologen derivatives overlapped with those of the nucleic acid at around 260 nm. On the other hand, DAP^{2+} derivatives **11**, **13** and **15** exhibited a characteristic color absorbing at 340 and 420 nm and their

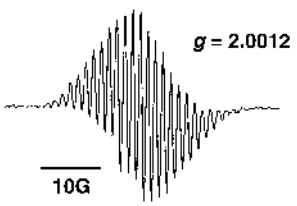


Fig. 1. ESR Spectrum of Cation Radical of **6**

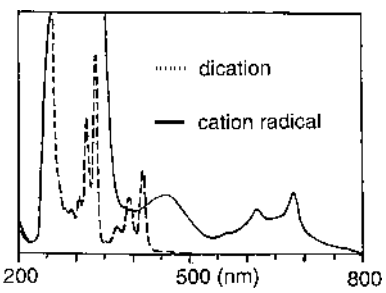


Fig. 2. UV Spectra of **13**

cation radicals at 450 and 650 nm (Fig. 2). These results imply the easy confirmation of the introduction of the DAP^{2+} molecule into DNA by taking the UV spectra of the DAP^{2+}-tethered DNA oligomer. The cation radicals, showing the same color as above, were also generated under irradiation of light in the presence of EDTA. From these experiments, it

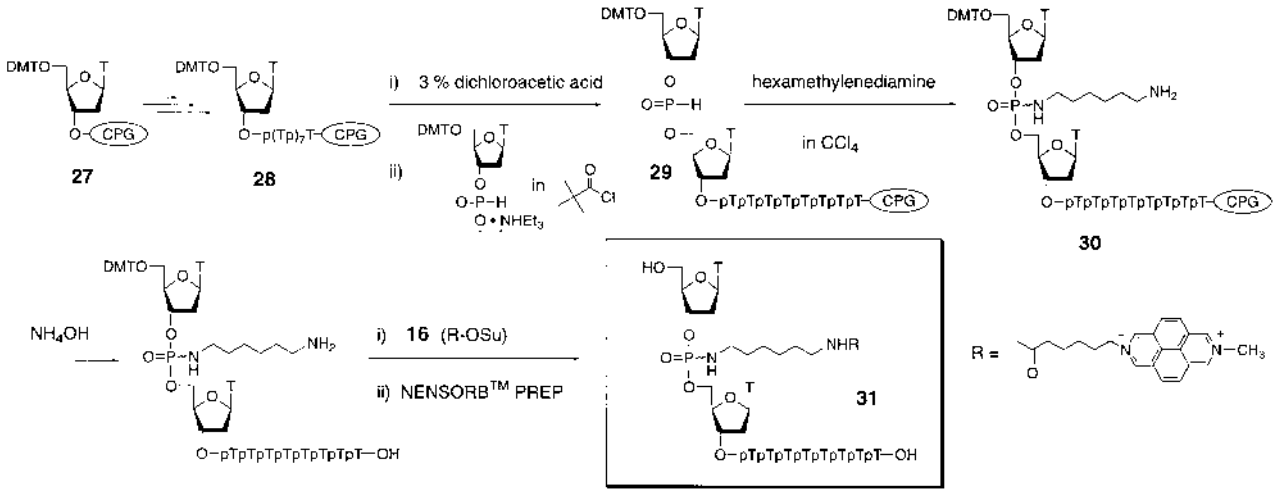


Chart 2. Conjugation of DAP^{2+} Molecules with DNA Oligomers

might be expected that DNA oligomers incorporating with **11**, **13** and **15**, are effectively converted into cation radicals, which cleave DNA under irradiation conditions in the presence of electron donor.[17)]

Several DNA oligomers **19**—**32**, which were conjugated with viologen or the DAP^{2+} molecule (Table 1), were prepared on solid supports using syringe technique[25)] by a standard protocol for H-phosphonate chemistry.[26)] A part of the DAP^{2+}-tethered DNA oligomers was synthesized by a combination of solid support method and liquid phase technique. The preparation of the DAP^{2+}-tethered DNA oligomer as shown in Chart 2 is representative of the protocol for introducing viologen or the DAP^{2+} molecule into the DNA oligomer. Briefly, thymidine–long chain alkylamino (LCAA)–controled pore glass (CPG) support **27** was firstly detritylated and coupled with a thymidine H-phosphonate unit. This process was repeated eight times, and then the H-phosphonates on support were oxidized with I_2–H_2O to give nonamer **28** possessing native internucleotide linkages. After additional detritylation of **28** and coupling with a thymidine H-phosphonate unit, **29** was oxidized with CCl_4 in the presence of excess hexamethylenediamine[27)] to give phosphoramidate **30**.[20)] The phosphoramidate **30** was detached from the CPG support by NH_4OH treatment and condensed with **16**. The final treatment with NENSORB™ PREP[28)] which can undergo simultaneous detritylation and purification was carried out to give the modified decamer **31**. In this way, modified DNA oligomers **19**—**22**, **24**—**32** were prepared and isolated as a diastereomeric mixture by reverse-phase HPLC. HPLC analyses of these compounds exhibited two peaks due to the diastereomeric phosphoramidate links and the tendency of slightly slower mobility compared to native T_{10} **17** owing to its lipophilic property. When viologen-tethered oligomers **19**—**22**, **24** were reduced with alkaline $Na_2S_2O_4$, cation radicals exhibited characteristic UV and ESR spectra similar to viologen derivatives **4**, **5** and **6** (Fig. 3).

The presence of the phosphoramidate linkage was also confirmed by ^{31}P-NMR spectroscopy.[29)] For example, 32 showed two signals of equal height at *ca*. 11 ppm and a broad signal at -0.58 ppm in a 2 : 5 ratio in connection to a diastereomeric phosphoramidate and native phosphodiesters, respectively. HPLC analyses of the products in enzymatic digestion[30)] of the modified oligomers with a combination of snake venom phosphodiesterase and alkaline phosphatase showed the formation of the monomeric nucleoside and the corresponding viologen- or the DAP^{2+}-tethered dimers (Fig. 4),[31)] indicating the resistance of the phosphoramidate bond toward nucleases.[32)] The polyacrylamide gel electrophoresis (PAGE, 20% denaturing gel) analysis showed slower mobility for the modified single strands which is attributable to a neutral phosphoramidate linkage and the cationic nature of the viologen or DAP^{2+} derivatives.

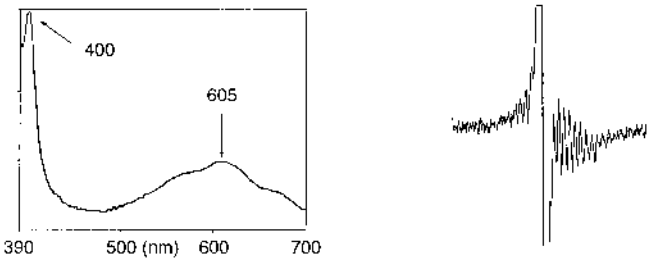
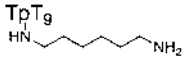
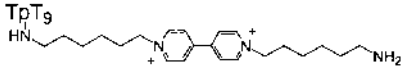
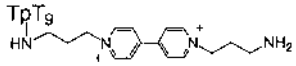
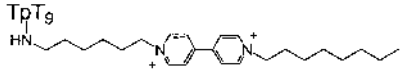
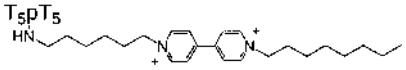
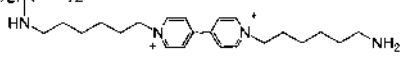
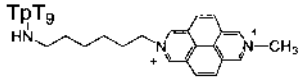
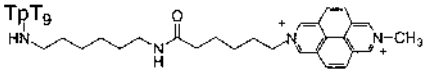
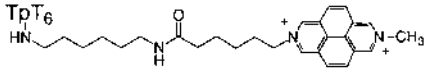
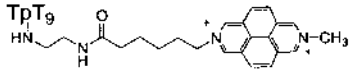
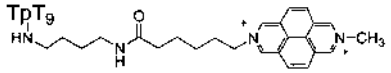
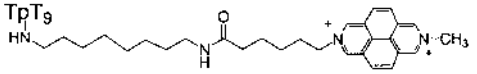
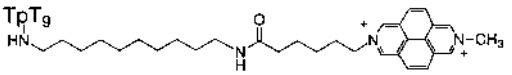
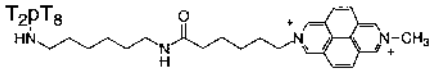
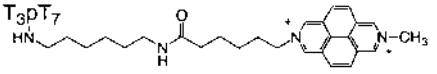
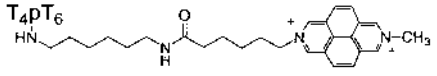
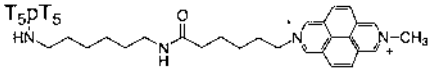
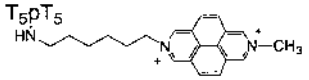


Fig. 3. UV Spectrum of Cation Radical Derived from **19** (A) and ESR Spectrum of Duplex of Its Cation Radical with Poly dA (B)

As mentioned above, viologen or the DAP^{2+} molecule could be introduced into a DNA oligomer. The thermal stabilities of the modified oligomers with complementary strands were next examined by taking their CD spectra and melting temperatures (Tm, Table 1). CD spectra of **25**, **26** and **32** are shown in Fig. 5. There is observed no significant difference between duplex of native T_{10} **17** and those of DAP^{2+}-tethered oligomers **25**, **26** or **32**, suggesting little influence of the introduction of viologen derivatives into DNA oligomers on duplex formation.

Tm values are also indicative for duplex stabilization. As can be seen from Table 1, viologen-tethered decamers have similar Tm values to that of native T_{10} **17**. Tm values of DAP^{2+}-tethered DNA oligomers are higher than those of **17** or viologen-tethered DNA oligomers, suggesting that the planar structural feature of DAP^{2+} molecules is favorable for stable duplex formation by π–π stacking. These experiments suggested that the viologen- or DAP^{2+}-tethered DNA oligomers might serve as functionalized antisense DNA oligomers.

Table 1. The Melting Temperature for Duplexes **17**—**41** with Their Complementary Strands

Strand		NaCl (M)	Tm (°C)
T_{10} (native)	**17**	0	5
		0.1	24
		1.0	41
TpT_9 	**18**	0	8
		0.1	22
		1.0	38
TpT_9 	**19**	0	5
		0.1	22
		1.0	38
TpT_9 	**20**	0	9
		0.1	25
		1.0	40
TpT_9 	**21**	0	5
		0.1	23
		1.0	39
T_5pT_5 	**22**	0.1	23
		1.0	27
$d(CG)_4$ (native)	**23**	0	35
		0.1	63
		1.0	63
$d(CG)_2p(CG)_2$ 	**24**	0	37
		0.1	53
		1.0	53
TpT_9 	**25**	0	11
		0.1	28
		1.0	43
TpT_9 	**26**	0	9
		0.1	29
		1.0	46
TpT_9 	**31**	0	11
		0.1	31
		1.0	45
TpT_6 	**32**	0.1	8
		1.0	24
TpT_9 	**33**	0	9
		0.1	25
		1.0	38
TpT_9 	**34**	0	10
		0.1	25
		1.0	40
TpT_9 	**35**	0	6
		0.1	22
		1.0	38
TpT_9 	**36**	0	6
		0.1	22
		1.0	37
T_2pT_8 	**37**	0	9
		0.1	24
		1.0	38
T_3pT_7 	**38**	0	9
		0.1	24
		1.0	36
T_4pT_6 	**39**	0	11
		0.1	23
		1.0	38
T_5pT_5 	**40**	0	9
		0.1	22
		1.0	38
T_5pT_5 	**41**	0	9
		0.1	19
		1.0	33

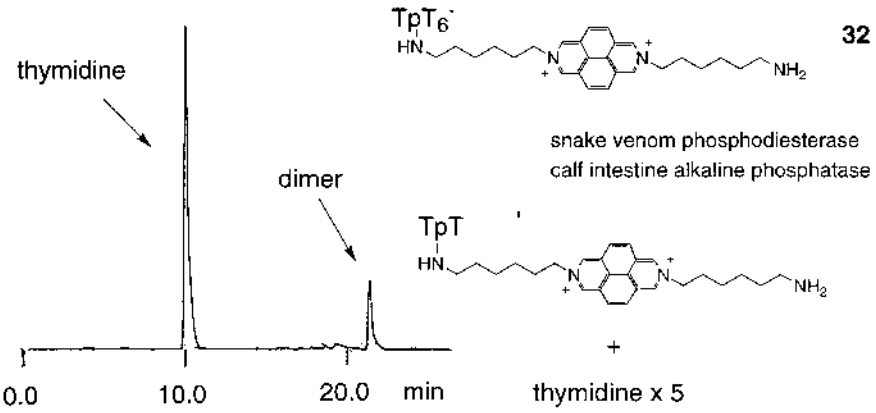


Fig. 4. HPLC Profile for Enzymatically Digested **32**

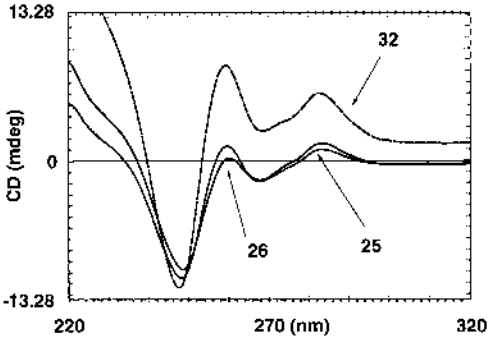


Fig. 5. Circular Dichroism Spectra of **25**, **26** and **32**

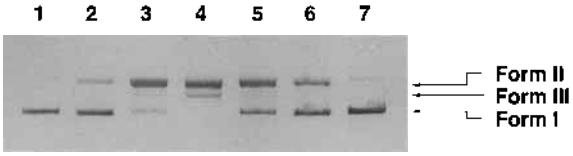


Fig. 6. Photochemical Cleavage of pBR322 Plasmid DNA by **13**

A solution of supercoiled (form I) pBR322 plasmid DNA (0.4 μg) and **13** in 10 mM Tris–HCl (pH 8.0) including 1 mM EDTA was photoirradiated at 254 nm at 4 °C for 20 min: (Lane 1) control DNA; (lane 2) irradiated DNA without **13**; (lane 3) **13** (100 μM); (lane 4) **13** (10 μM); (lane 5) **13** (1 μM); (lane 6) **13** (100 nM); (lane 7) **13** (100 μM), no irradiation.

Prior to the investigation using viologen- or DAP^{2+}-tethered DNA oligomers, we examined the DNA cleavage activities of the parent viologen derivative **5** and DAP^{2+} derivative 6 toward pBR322 plasmid DNA by monitoring the scission states of pBR322 between supercoiled (form I), nicked circular (form II) and linear (form III). Viologen derivative **5** did not work as a DNA cleavage agent even at high concentration (1 mM; data not shown). Takagi and coworkers have reported the viologen derivative conjugated with acridine,[33] which strongly binds to DNA molecules, cleaves DNA molecules effectively. These experiments suggest that an affinity to DNA is required for DNA cleavage. On the other hand, DAP^{2+} derivative **13** showed that form I disappeared concomitant with the appearance of form II with a trace of form III at 10—100 μM of DAP^{2+} solution (Fig. 6). Plasmid DNA pBR322 was also transformed into forms II and III with DAP^{2+} under photoirradiation conditions in the presence of EDTA. This kind of photocleavage ability of DNA with DAP^{2+} derivatives has been reported previously.[24] The DAP^{2+} derivative proved to be superior to viologen as a functionalized molecule which can effectively interact with DNA.

The DNA photocleavage activity of DAP^{2+}-tethered DNA oligomer **32**[34] was examined by using a 5′-^{32}P labeled 24-mer, 5′-d(TGAGTGAGTAAAAAAATGAGTGAC)-3′ (**42**),[35] which contains the complementary region (Fig. 7A). 15% PAGE analysis showed a single band at slower mobility than the 24-mer itself (lane 3). This indicated that **32** bound covalently to **42** although the bonding pattern was obscured. Piperidine treatment[36] of this sample (lane 3) suggested that **32** interacted with the duplex region in a site-selective manner (Fig. 7B). Therefore, DAP^{2+}-tethered DNA oligomer forms a stable duplex and interacted site-specifically with its complementary strand resulting in local DNA scission. From these experiments, the DAP^{2+}-tethered DNA oligomer can be regarded as a potentially promising candidate as a functionalized DNA oligomer possessing selective DNA photocleavage. Contrary to this, DNA cleavage experiments with viologen-tethered oligomers gave no satisfactory results, even though the viologen molecule generates active oxygen species by a redox cycle (data not shown).[37] This can be explained by the poor affinity to complementary strand probably due to the sterically and electronically unfavorable orthogonal conformational feature of the two pyridinium rings of the viologen molecule.

Since DAP^{2+}-tethered DNA oligomers, such as **31**, proved to be a candidate as a functionalized antisense DNA oligomer having specific photocleaving ability, several other types of DAP^{2+}-tethered oligomers, **33**—**41**, (Table 1) which have both different lengths of linkers and different positions of phosphorous linkages were prepared in order to obtain more refined molecules as chemical nucleases. **33**—**41** were prepared according to the previous method (Chart 2) and the introduction of DAP^{2+} molecules into DNA was confirmed by the characteristic UV absorption of the DAP^{2+} moiety.

The effect of length and position of linker was investigated by measuring and comparing Tm values of the corresponding strand (Table 1). The duplex stability did not depend on the length of linker. Thus, no remarkable difference was observed in Tm values of **31**, **33**—**36**. In a similar way, Tm values of oligomers **31**, **37**—**40** bearing the same linker arm at any phosphate linkage were compared. The Tm value of **31** bearing a linker arm at the end of the phosphate linkage was

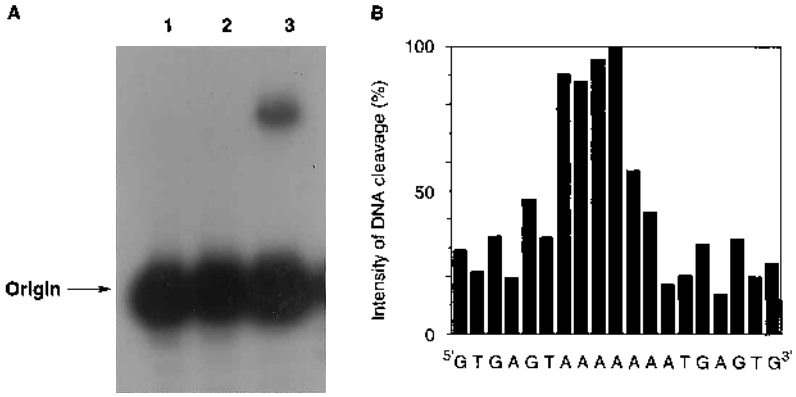


Fig. 7. Analysis of DNA Photocleavage by **32**

A: Sample solution, which contained ^{32}P 5′-end-labeled 24 mer **42** (20000 cpm), non-labeled **42** and **32** (each 0.1 OD_{260}/ml) in 20 μl of 10 mM Tris–HCl (pH 8.0) including 1 mM EDTA and 1 N NaCl, was photoirradiated at 254 nm at 4 °C for 20 min. After treatment under the same conditions as described in Fig. 6, the crude reaction mixture was dried, subjected to electrophoresis on a 7 M urea-containing 15% polyacrylamide gel (2500 V, 2.5 h), and visualized by autoradiography: (Lane 1) irradiated **42** with **13** (100 μM); (lane 2) irradiated **42** without **32**; (lane 3) irradiated **42** with **32**. B: Piperidine treatment (1 M aqueous piperidine, 90 °C, 30 min) of the crude photooxidation mixture of **42** with **32** derived from lane 3. Relative DNA cleavage intensity (%) of each base is shown.

slightly higher than others. In the case of **26** and **41** bearing another linker arm, the Tm value of **26** was significantly higher than that of **41**. These results indicate that the introduction of a linker arm at the middle position of phosphate linkages tends to perturb the stability of duplex. Therefore, it can be concluded that the introduction of the linker arm at the end of phosphonate backbone in a DNA oligomer is more favorable than at any inside position of phosphorous backbone. The effect of phosphorous chirality on the duplex stability was next examined. Generally, modified oligomer having a phosphoramidate bond are prepared usually as a mixture of two diastereomers due to *R*p and *S*p chirality at the phosphorous atom. Consequently, each diastereomer forms a duplex with the complementary strand in a different way. For example, the linker of one isomer places the arm inside of the duplex, while that of the other isomer outward. This suggests that the Tm value of each diastereoisomer with the complementary strand should be different. Each diastereoisomer of **26** was separated by the usual HPLC protocol, and the isolated isomers were subjected to duplex formation. The thermal stability of each duplex showed a remarkable difference (Fig. 8). This result indicates the importance of chirality control at the phosphorous atom.

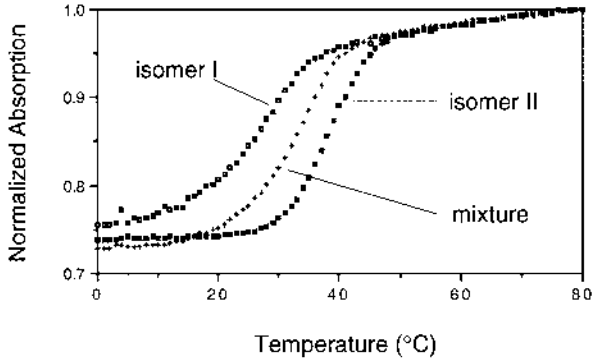


Fig. 8. The Melting Curves of the Complexes of Each Isomer I, II and Diastereomixture of **33** with Poly dA

Fig. 9. Base Selectivity Observed in DNA Photocleavage by **13**

Relative DNA photocleavage frequencies were obtained from densitometric scans of the autoradiogram of ^{32}P 5′-end-labeled *Eco*RI-*Bam*HI DNA fragment with **13** (100 nM). The height of each bar indicates the relative DNA cleavage intensity at the indicated base.

Base selectivity for DNA photocleavage with DAP^{2+} was also investigated. If the DAP^{2+} molecule has a function of base-specific DNA cleavage, the precise molecular design of the DAP^{2+} incorporated into functionalized antisense DNA oligomers might lead to a molecular weapon which can cleave the target DNA at a predictable site. According to this idea, a DNA cleavage experiment using isotope *Eco*RI-*Bam*HI DNA fragment was carried out with DAP^{2+} molecule **13** (Fig. 9). From this experiment, it turned out that the DAP^{2+} molecule selectively cleaves the DNA strand at G bases, especially at a GG site.

Based on the results of base-selectivity with the DAP^{2+} molecule observed above, the photochemical cleavage of *Eco*RI-*Eco*RV DNA fragment, which includes GG bases, was investigated. For this study, four DAP^{2+}-tethered oligomers **43**—**46** having complementary regions were prepared according to the same method which was previously described (Chart 2). Using 5′-^{32}P labeled *Eco*RI-*Eco*RV fragment derived from pBR322 plasmid DNA, the DNA photocleavage experiment was carried out and these results are shown in Fig. 10. Among the DNA photocleavage activities of **43**—**46**, the cleavage intensity caused by **44** (lane 3) is largest at the GG site. It also showed that the DAP^{2+}-tethered oligomers exhibited more efficient and selective cleaving activity than the parent DAP^{2+} molecule itself (lane 1). Thus, DAP^{2+}-tethered oligomers photochemically and site-selectively cleaved DNA at the GG site 2—3 bases away from the corresponding phosphoramidate site.

In conclusion, the present study shows that the DAP^{2+} molecule acts as a one electron oxidant under photoirradiation conditions and selectively cleaves DNA at G sites without any help from metals. Furthermore, the conjugation of the DAP^{2+} molecule with DNA through covalent bonding causes a thermodynamically stable duplex formation, and therefore, this approach may open a new way to a possible

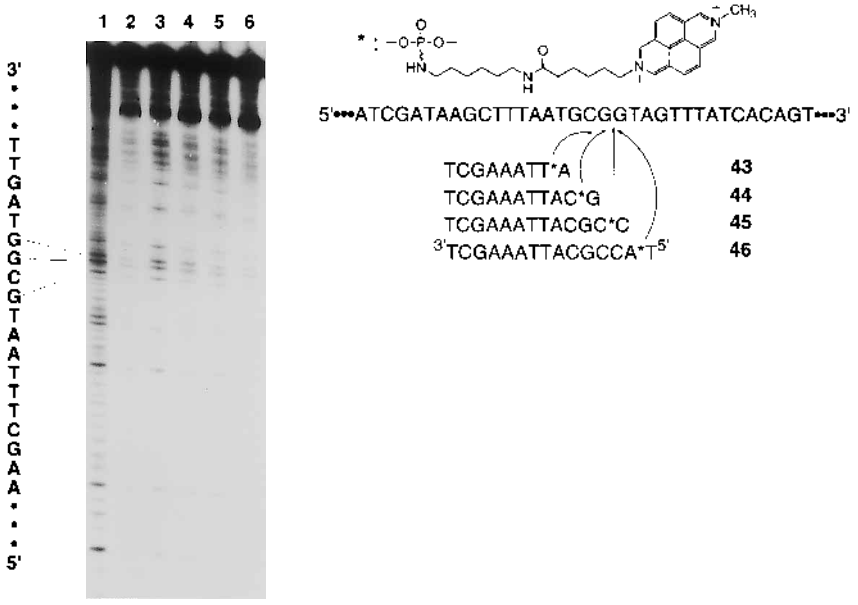


Fig. 10. Autoradiogram of ^{32}P 5′-End-Labeled *Eco*RI-*Eco*RV Fragment with DAP^{2+}-Tethered Heterooligomers **43**—**46**

Each sample solution, which contained ^{32}P 5′-end-labeled *Eco*RI-*Eco*RV fragment (20000 cpm), calf thymus (5 mM/base), and drug in 20 μl of 10 mM Tris–HCl (pH 7.6), was photoirradiated using UV/VIS lamp (300 W) at 4 °C for 1 h. After piperidine treatment (1 M aqueous piperidine, 90 °C, 30 min), the sample was dried, subjected to electrophoresis on a 7 M urea-containing 15% polyacrylamide gel (2000 V, 2 h), and visualized by autoradiography: (Lane 1) **13** (100 μM); (lane 2) **43** (2 OD_{260}/ml); (lane 3) **44** (2 OD_{260}/ml); (lane 4) **45** (2 OD_{260}/ml); (lane 5) **46** (2 OD_{260}/ml); (lane 6) control, no irradiation.

antisense strategy. DAP^{2+}-tethered DNA oligomer, such as **44**, can be regarded as a promising candidate for a functionalized antisense DNA molecule aimed at suppression of harmful gene expression such as viral disease. More precise molecular design as well as elucidation of mechanism for DNA cleavage are in progress.

Experimental

^{1}H- and ^{13}C-NMR spectra were recorded on a Varian Gemini-200 spectrometer (200 and 80 MHz, respectively) using tetramethylsilane as an internal standard. ^{31}P-NMR spectra were recorded on a Varian VXR-4000 spectrometer (80 MHz) or a Brucker AC-300 spectrometer (120 MHz) using H_2O as solvent and 80% aqueous H_3PO_4 as the external standard. Chemical shifts are recorded in δ values and coupling constants are in Hz. UV spectra were recorded on a Shimadzu UV-2200 spectrophotometer and CD spectra were measured with a JASCO J-720 spectrophotometer. IR spectra were recorded on a JASCO IR-810 spectrometer in Nujol or KBr disks. ESR spectra were measured by JEOL FE2XG. TLC was performed on Silica gel $60F_{254}$ (Merck). Flash chromatography was conducted on Silica Gel 60 (spherical, 150—325 mesh). All HPLC separations were carried out on a Shimadzu LC-6AD using either Chemcosorb 5-ODS-H (4.6×150 mm) or Nucleosil (6.0×150 mm) and eluting products were detected by UV at 260 nm. 0.1 M Triethylammonium acetate (TEAA, pH 7.0) and acetonitrile were used for the solvent system for HPLC. In all cases, linear gradient programs were employed.

1-Iodo-3-(trifluoroacetylamino)propane (3) *p*-TsCl (6.5 g, 25.2 mmol) was added portionwise to 3-(trifluoroacetylamino)-1-propanol[38] (3.32 g, 19.4 mmol) in pyridine (2.5 ml) and CH_3CN (20 ml) and the mixture was stirred for 3 h at 0 °C and concentrated under reduced pressure. The residue was partitioned between 5% aqueous HCl and Et_2O. The organic layer was dried with Na_2SO_4 and evaporated *in vacuo*. The residue was purified by flash chromatography with CH_2Cl_2 to give 1-(*p*-toluenesulfonyloxy)-3-(trifluoroacetylamino)propane (**2**) (3.9 g, 63%) as an oil. ^{1}H-NMR ($CDCl_3$): δ 7.79, 7.38 (each 2H, d, J=8, PhH), 6.93 (1H, br s, NH), 4.11 (2H, t, J=6, OCH_2), 3.34 (2H, q, J=6, NCH_2), 2.46 (3H, s, $PhCH_3$), 1.97 (2H, m, CH_2). ^{13}C-NMR ($CDCl_3$): δ 145.99, 132.74, 130.59, 130.59, 128.30, 128.30, 8.07, 36.60, 28.10, 21.79. UV λ_{max} nm: 273, 262, 233. MS *m/z*: 325 (M^+). HR-MS *m/z*: Calcd for $C_{12}H_{14}F_3NO_4S$ 325.309. Found 325.058. *Anal*. Calcd for $C_{12}H_{14}F_3NO_4S$: C, 44.31; H, 4.34; N, 4.31. Found: C, 43.75; H, 4.25; N, 4.36.

2 (1.3 g, 4 mmol) and sodium iodide (3 g, 20 mmol) were dissolved in 5 ml of acetone and the solution was stirred for 6 h at room temperature. The reaction mixture was evaporated and the residue partitioned between 5% aqueous HCl and Et_2O. The organic layer was dried with Na_2SO_4 and evaporated *in vacuo* to give **3**[19] (0.80 g) in 71% yield.

1,1′-Bis[6-(trifluoroacetylamino)hexyl]-4,4′-dipyridinium Bis(*p*-toluene-sulfonate) (4) 4,4′-Dipyridyl (410 mg, 2 mmol) and 1-(*p*-toluenesulfonyloxy)-6-(trifluoroacetyl-amino)hexane[18] (**1**) (2 g, 10 eq) were dissolved in 10 ml of CH_3CN and the mixture was refluxed for 5 d. The resulting mixture was filtered and the precipitates were treated with MeOH to give **4** (550 mg, 96%) as an amorphous powder. IR (KBr) cm^{-1}: 3450, 3290, 3120, 3050, 2945, 2865, 1720, 1690, 1640, 1560, 1450, 1380, 1200—1155, 1030, 1010, 850, 820, 680, 560. ^{1}H-NMR (DMSO-d_6): δ 9.38, 8.78 (each 4H, d, J=6.5, PhH), 7.48, 7.11 (each 4H, d, J=7.8, PhH), 4.68 (4H, t, J=6.9, NCH_2), 3.17 (4H, dd, J=12, 6, $NHCH_2$), 2.28 (6H, s, CH_3), 1.96, 1.48 (each 4H, m, CH_2), 1.30 (8H, m, CH_2). ^{13}C-NMR (DMSO-d_6): δ 156.20, 148.88, 146.14, 145.83, 138.13, 128.43, 126.88, 125.77, 107.36, 60.88, 40.77, 30.68, 27.99, 25.58, 25.00, 20.73; UV λ_{max} nm: 262 (cation radical 731, 675, 605, 398, 388, 370). *Anal*. Calcd for $C_{40}H_{48}F_6N_4O_8S_2$: C, 53.92; H, 5.43; F, 12.79; N, 6.29. Found: C, 52.66; H, 5.50; F, 12.54; N, 6.23.

1,1′-Bis[3-(trifluoroacetylamino)propyl]-4,4′-dipyridinium Bis(*p*-toluene-sulfonate) (5) 4,4′-Dipyridyl (32 mg, 0.2 mmol) and **3** (400 mg, 7 eq) were dissolved in 5 ml of CH_3CN and the mixture was refluxed for 1 d. The reaction mixture was filtered and the precipitates were treated with MeOH to give **5** as an amorphous powder in 96% yield. IR (KBr) cm^{-1}: 3235, 1700, 1640, 1550, 1200, 1155, 845, 810. ^{1}H-NMR (D_2O): δ 9.17, 8.59 (each 4H, d, J=6.5, PhH), 4.79 (4H, m, NCH_2), 3.50 (4H, t, J=5, $NHCH_2$), 2.43 (4H, dt, J=10, 5, CH_2). *Anal*. Calcd for $C_{20}H_{22}F_6I_2N_4O_2$: C, 33.45; H, 3.09; F, 15.87; I, 35.34; N, 7.80. Found: C, 32.84; H, 2.96; F, 15.43; I, 35.52; N, 7.62.

1-*n*-Octyl-1′-[6-(trifluoroacetylamino)hexyl]-4,4′-dipyridinium Bis(*p*-toluene-sulfonate) (8) A mixture of 1-*n*-octyl-4,4′-dipyridinium bromide[21] (800 mg, 2.3 mmol), **1** (2.5 mg, 3 eq) and 10 ml of CH_3CN was refluxed for 3 d with stirring. The reaction mixture was filtered and the precipitates were treated with MeOH to give **8** (1.45 g, 88%) as an amorphous powder. IR (KBr) cm^{-1}: 2925, 2860, 1640, 1600, 1550, 1525, 1480, 1410, 1220, 1180, 870, 820, 720. ^{1}H-NMR (DMSO-d_6): δ 9.38, 8.78 (each 4H, d, J=6.7, PhH), 7.47, 7.10 (each 4H, d, J=8.2, PhH), 4.68 (4H, t, J=7.3, NCH_2), 3.17 (2H, q, J=6.3, $NHCH_2$), 2.28 (6H, s, $PhCH_3$), 1.96 (4H, br t, CH_2), 1.48 (2H, br t, CH_2), 1.29—1.25 (14H, m, CH_2), 0.86 (3H, t, J=6.6, CH_3). ^{13}C-NMR (DMSO-d_6): δ 152.45, 151.30, 145.64, 141.13, 125.63, 122.18, 60.32, 31.12, 30.75, 28.44, 28.36, 25.38, 22.00, 13.87. UV λ_{max} nm: 261 (cation radical 730, 670, 602, 555, 398, 387). *Anal*. Calcd for

$C_{40}H_{52}F_3N_3O_7S_2$: C, 59.46; H, 6.49; N, 5.20. Found.: C, 58.85; H, 6.56; N, 5.12.

1-*n*-Octyl-1′-[6-(trifluoroacetylamino)hexyl]-4,4′-dipyridinium Bis (hexa-fluorophosphonate) (9) The compound was prepared according to the procedure previously reported.[22] **8** was dissolved in MeOH (*ca*. 1 ml) and the mixture was cooled with ice bath. Hexafluorophosphoric acid was added to the solution at 0 °C and the precipitates were filtered and dried to give **9** quantitatively as waxy solids. ^{1}H-NMR (DMSO-d_6): δ 9.37, 8.77 (each 4H, d, J=5.5, PhH), 4.67 (4H, br t, NCH_2), 3.17 (2H, dd, J=13, 7.5, $NHCH_2$), 1.97 (4H, m, CH_2), 1.48 (2H, m, CH_2), 1.30 (14H, m, CH_2), 0.85 (3H, m, CH_3). UV λ_{max} nm: 263 (cation radical 731, 670, 604, 555, 398, 388, 370).

***N*,*N*′-Bis[6-(trifluoroacetylamino)hexyl]-2,7-diazapyrenium Bis[*p*-toluene-sulfonate] (11)** A mixture of 2,7-diazapyrene[23] (410 mg, 2 mmol), **1** (2 g, 10 eq) and 10 ml of CH_3CN was refluxed for 5 d. The hot reaction mixture was filtered and the precipitates were treated with MeOH to give **11** (550 mg, 30%) as an yellow amorphous powder. ^{1}H-NMR (DMSO-d_6): δ 10.41, 8.91 (each 4H, s, PhH), 7.46, 7.09 (each 4H, d, J=8, PhH), 5.15 (4H, br t, NCH_2), 3.17 (4H, m, NCH_2), 2.28 (6H, s, Ar CH_3), 2.4—2.0 (4H, m, CH_2), 1.6—1.3 (12H, m, CH_2). UV λ_{max} nm: 420, 395, 370, 335, 320, 305, 255 (cation radical 683, 618, 569, 459). *Anal*. Calcd for $C_{44}H_{48}F_6N_4O_8S_2$: C, 56.28; H, 5.15; N, 5.97. Found: C, 55.88; H, 5.26; N, 5.88.

***N*-Methyl-*N*′-[6-(trifluoroacetylamino)hexyl]-2,7-diazapyrenium Iodide *p*-Toluenesulfonate (13)** A solution of *N*-methyl-2,7-diazapyrenium iodide[24] (700 mg, 2 mmol) and **1** (1 g, 5 eq) in 10 ml of CH_3CN was refluxed for 5 d. The hot reaction mixture was filtered and the precipitates were treated with MeOH to give **13** (712 mg, 49%) as an yellow amorphous powder. ^{1}H-NMR (DMSO-d_6): δ 10.15, 9.94 (each 2H, s, PhH), 8.80, 8.62 (each 2H, d, J=9, PhH), 5.06 (2H, t, J=7, NCH_2), 4.51 (3H, br s, NCH_3), 3.18 (2H, q, J=6, NCH_2), 2.19 (2H, m, CH_2), 1.6—1.3 (6H, m, CH_2). UV λ_{max} nm: 418, 393, 372, 333, 320, 306 (cation radical 683, 617, 563, 409). *Anal*. Calcd for $C_{30}H_{31}IF_3N_3O_4S$: C, 58.64; H, 5.05; N, 5.55. Found: C, 56.63; H, 4.88; N, 5.38.

***N*-Methyl-*N*′-(5-carboxypentyl)-2,7-diazapyrenium Bromide Iodide (15)** A solution of *N*-methyl-2,7-diazapyrenium iodide[24] (153 mg, 0.44 mmol) and 6-bromohexanoic acid (4 g, 40 mmol) in 10 ml of CH_3CN was refluxed for 10 d. The reaction mixture was purified by Sephadex LH-20 column chromatography (20×350 mm) using MeOH as an eluent to give **15** (216 mg, 91%) as a yellow amorphous powder. ^{1}H-NMR (CD_3OD): δ 10.40, 10.39 (each 2H, s, PhH), 9.01 (4H, s, PhH), 5.29 (2H, d, J=6.8, NCH_2), 5.07 (3H, s, NCH_3), 2.40 (4H, m,CH_2), 1.75—1.5 (4H, m, CH_2). UV λ_{max} nm: 419, 395, 372, 336, 320, 306, 252 (cation radical 624, 459, 317).

***N*-Methyl-*N*′-[5-(succinimidooxycarbonyl)pentyl]-2,7-diazapyrenium Bromide Iodide (16)** *N*-Hydroxysuccinimide (0.6 g, 12 eq) and dicyclohexylcarbodiimide (1.1 g, 12 eq) were added to a solution of **15** (216 mg, 0.4 mmol) in 10 ml of CH_3CN at 0 °C and the mixture was stirred for 1 h at the same temperature and then at room temperature for 10 h. The mixture was filtered and the precipitates were treated with H_2O to give **16** (196 mg, 77%) as a yellow amorphous powder. ^{1}H-NMR (DMSO-d_6): δ 10.08, 9.93 (each 2H, s, PhH), 8.79, 8.63 (each 2H, d, J=9, PhH), 5.35 (2H, t, J=6, NCH_2), 4.83 (3H, s, NCH_3), 2.49 (4H, d, J=6, $(CH_2)_2$), 2.25 (4H, m, CH_2), 1.61—1.45 (4H, m, CH_2).

General Procedure for the Preparation of Viologen or Diazapyrene-Tethered Oligodeoxyribonucleotides 17—46 Preparation of the oligomers was based on H-phosphonate chemistry for solid support DNA synthesis[26] using syringe work.[25] For example, the synthesis of **31** is illustrative and shown in Chart 2. Thymidine–(LCAA)–CPG support (1 μmol scale) was detritylated by 3% dichloroacetic acid in CH_2Cl_2 and coupled with a dC H-phosphonate unit (10 mg) in the presence of pivaloyl chloride (10 μl) in a solvent system of pyridine/CH_3CN (1 : 1) 1 ml for 2 min. This process was repeated eight times and then the nonamer on the support was oxidized with a 0.15 M solution of I_2 in 1 ml of pyridine/H_2O (98 : 2) for 10 min. After successive detritylation and coupling with a Thymidine H-phosphonate unit, the H-phosphonate link was oxidized to the phosphoramidate with 1.5 ml of carbon tetrachloride[26] in the presence of hexamethylenediamine (50 mg) for 10 min. Further couplings with Thymidine H-phosphonate units, hydrolytic treatment with 1.0 ml of NH_4OH for 1 h at room temperature and lyophilization gave the residue. The residue and **16** (0.5 mg) were dissolved in 2 ml of water and the mixture was stirred for 1 h at room temperature and then lyophilized to give the residue. The residue in 4 ml of 0.1 M TEAA buffer was applied to a NENSORB^{TM} PREP cartridge and the cartridge was washed successively with CH_3CN/0.1 M TEAA buffer (1 : 9, 10 ml), 0.5% aqueous trifluoroacetic acid (25 ml) and 0.1 M TEAA buffer (10 ml). Elution with 35% aqueous MeOH gave crude **31**, which was purified by reverse-phase HPLC to give pure **31** [conditions : 0.5 ml/min; CH_3CN/0.1 M TEAA buffer (pH 7.0), 5/95→100/0, 95 min].

Experiments for the Confirmation of Incorporation of Viologen or DAP^{2+} into DNA Oligomer The absorption of viologen- or DAP^{2+}-tethered DNA oligomers (*ca*. 1 OD_{260}) in water was measured by a UV spectrophotometer (800—200 nm) at room temperature. The UV-visible spectra of the cation radicals generated by the addition of aqueous $NaHCO_3$ and $Na_2S_2O_4$ (100 and 50 mM, respectively) were also taken. The ^{31}P-NMR spectra of the synthesized oligomers were recorded in D_2O using 85% H_3PO_4 as the external standard. The enzymatic digestion was carried out as follows: the sample (each 1 OD_{260}) was treated with snake venom phosphodiesterase, calf intestine phosphodiesterase and calf intestine alkaline phosphatase (each 10 unit) in Tris buffer (pH 7.6), incubation was continued for 1 h at 37 °C and the mixture was analyzed by HPLC.

Measurement of Tm and CD Spectra of the Duplexes of the Modified Oligomers with their Complementary Strands The thermal stabilities of the modified oligomers were measured with a UV spectrophotometer. Thus, the modified oligomers and poly dA (0.40, 0.42 OD_{260}, respectively) were mixed well and adjusted to a solution of 1.0 ml of 0.01 M Tris buffer (pH 7.0) with three different salt concentrations (0, 0.1, 1.0 M NaCl). The absorption of the sample at the different salt concentrations (0, 0.1, 1.0 M NaCl) was continuously measured as the cell temperature was increased from 0 to 80 °C at the rate of 1 °C per 1 min. CD spectra were taken using **25**, **26**, and **32** (each 0.40 OD_{260}) with poly dA (0.42 OD_{260}) in 1 ml of 0.01 M Tris buffer (pH 7.0) containing 0.1 m NaCl: **25**.

Preparation of 5′ End-Labeled *Eco*RI-*Bam*HI Fragment Derived from pBR322 Plasmid DNA Linear DNA formed by *Eco*RI digestion of pBR322 plasmid DNA was labeled using [γ-^{32}P]ATP and T4 polynuclease kinase, digested by *Bam*HI and purified using 15% polyacrylamide gel to give the desired fragment.

Assay of DNA Cleavage Activity of Viologen or Diazapyrene-Tethered Oligomers The assay mixture in a microtube (1.5 ml) consisted of 10000 cpm labeled *Eco*RI-*Bam*HI fragment, 0.5 OD_{260} of viologen derivative, 10 mM Tris–HCl (pH 8.0), 1 mM EDTA (pH 8.0), and 1 μg Calf Thymus DNA. After the addition of 10 mM aqueous $Na_2S_2O_4$ the mixture was incubated for 1 h at 37 °C, and then heated for 30 min at 90 °C in 1 M piperidine. Electrophoresis of the mixture was carried out for 2.5 h at room temperature at 2500 V and the result was elucidated by comparison of their radioactivities as detected on X-ray film.

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