Effect of Polymethylene and Phenylene Linking Groups on the DNA Cleavage Specificity of Distamycin-Linked Hydroxamic Acid–Vanadyl Complexes

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Two types of distamycin-linked hydroxamic acids (DHA), which contain various lengths of polymethylene chains (PM-DHA) and relatively rigid phenylene ones (Ph-DHA), have been synthesized for the first time. Their DNA cleavage specificities were investigated by an end-labeled fragment cleavage experiment in the presence of vanadyl ion and hydrogen peroxide. The DNA cleavage by the PM-DHA·VO(II) complexes was shown to be very dependent on the length of the chain and the AT sequences. The tetramethylene DHA (1b) complex exhibited highly specific cleavage patterns flanking the 8 and 10 AT sites. Interestingly, the Ph-DHA complexes selectively cleaved the 5′ end-labeled strand at the AT sites, but did not cleave the 3′ end-labeled strand. The vanadyl complexing moieties and the local sequence conformation of the AT tract are suggested to contribute significantly to the DNA recognition of the PM-DHA·VO(II) complexes.

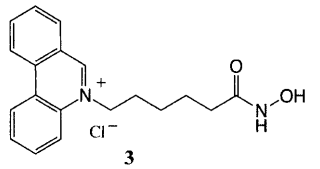
Key words DNA cleavage; sequence-specific cleavage; distamycin; hydroxamic acid; vanadyl ion; pyrrolepeptide

The antitumor antibiotic, bleomycin, efficiently degrades DNA strands in 5′-GC-3′ and 5′-GT-3′ sequences.[1] For the specific cleavage to occur, the metal binding domain of the antibiotic should be positioned near the C4′-H of the pyrimidine residue, which leads to the C4′-H abstraction responsible for DNA cleavage.[2] On the other hand, most of the conventional artificial DNA-cleaving metal complexes fail to successfully direct the complexing moiety to the reaction site of DNA. These complexes usually afford multiple cleavage patterns flanking the DNA recognition site.[3,4] One strategy for controlling the DNA cleavage is to choose the proper linkage group.[5]

Hydroxamic acids have been used in calorimetric analyses of vanadium[6] and the complex formed has been characterized.[7] In the course of our continuing research into the DNA cleavage properties of the hydroxamic acid–metal system, we have recently found that hydroxamic acid conjugated to the tripyrrolepeptide, distamycin, induced highly specific DNA cleavage in the presence of vanadyl ion.[8] This highly specific DNA cleavage was not observed for other metal complexes of the ligand. To explore the optimum distance and relative orientation between the distamycin and hydroxamic acid complexing moieties, we have evaluated the effect of the group linking them on the DNA cleavage specificity. We have recently designed two types of distamycin-linked hydroxamic acid (DHA) which contain various lengths of polymethylene chains (PM-DHA) (**1a**—**d**) and relatively rigid phenylene ones (Ph-DHA) (**2a**—**c**) (Fig. 1).[9] The distances between the carbonyl carbons of the chain of these hydroxamic acids increase in the order **2a**<**1a**<**2b**<**1b**<**2c**<**1c**<**1d**. In the present study, the effect of the linker length of the PM-DHA·VO(II) complexes on DNA cleavage specificity was investigated using two restriction DNA fragments. The DNA cleavage specificities of comformationally restricted Ph-DHA complexes were also compared with that of the **1b** complex.

Results

Synthesis of Hydroxamic Acids The syntheses of PM-DHA (**1a**—**d**) and Ph-DHA (**2a**—**c**) are outlined in Chart 1. The tripyrrole compound **4** was synthesized from *N*-methylpyrrole following a previously described procedure.[8a,10] The nitro compound **4** was catalytically hydrogenated to afford the unstable aromatic amines, which immediately reacted with the *p*-nitrophenol-activated alkyl esters to give the ester compounds **5a**—**d** in 31—37% yield. Similarly, the ester compounds **6a**—**c** were prepared in 40—53% yield from compound **4** by condensation with the phenylene monoesters *via* dicyclohexylcarbodiimide (DCC). The transformation of the ester groups of compounds **5a**—**d** and **6a**—**c** into the corresponding hydroxamic acids afforded **1a**—**d**



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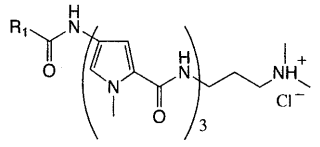
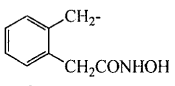


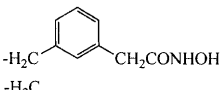
1a R_1= -$(CH_2)_3$CONHOH

1b R_1= -$(CH_2)_4$CONHOH

1c R_1= -$(CH_2)_5$CONHOH

1d R_1= -$(CH_2)_6$CONHOH

2a R_1= 

2b R_1= 

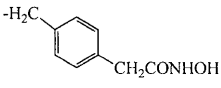
2c R_1= 

Fig. 1. DNA Cleaving Hydroxamic Acids

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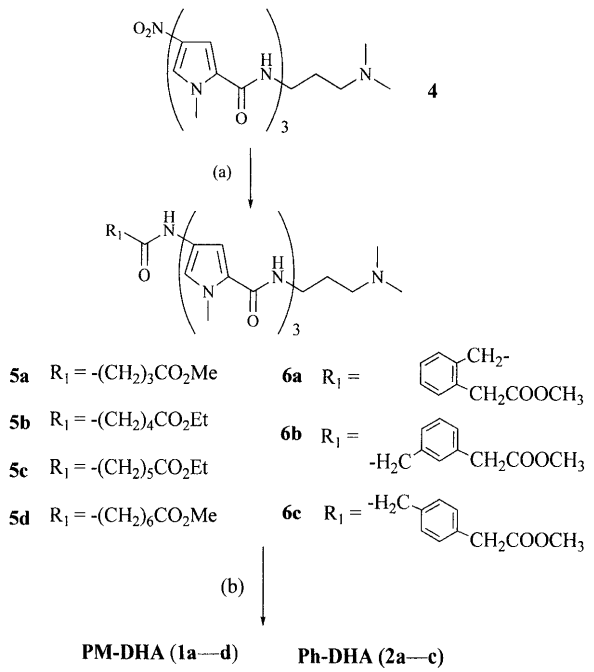

(a) i) H_2, PtO_2, MeOH; ii) For compound **5** series, *p*-$O_2NC_6H_4OCO(CH_2)_nCO_2R$(R=Me or Et), DMF, For compound **6** series, $MeO_2CCH_2C_6H_4CH_2CO_2H$ (*o*, *m*, or *p*), DCC, HOBt, DMF
(b) $HONH_2HCl$, KOH, MeOH

Chart 1

(54—70% yield) and **2a—c** (66—75% yield), respectively. All the hydroxamic acids were characterized by spectroscopic examination and gave a positive Fe(III) test which is characteristic of the hydroxamic acid group.

DNA Cleavage Patterns Produced by PM-DHA·VO(II) Complexes The DNA cleaving ability of PM-DHA·VO(II) complexes in the presence of hydrogen peroxide was initially examined using supercoiled Col E1 plasmid DNA. At a 10 μM concentration of the complexes, maintained at 37 °C for 30 min, all PM-DHA·VO(II) complexes showed almost the same DNA cleavage activity (data not shown). A comparative study of the DNA cleavage patterns produced by the PM-DHA·VO(II) complexes was carried out on the end-labeled 167 and 517 bp fragments of pBR 322 plasmid DNA. The 5′ end-labeled DNA fragments were prepared by digesting pBR 322 DNA with *Eco*RI and successive treatment with calf intestine alkaline phosphatase, (γ-^{32}P)ATP and T4 polynucelotide kinase.[11] A second enzymatic digestion with *Rsa* I yielded the 167 and 517 bp fragments labeled at the 5′ end. The 3′ end-labeled DNA fragments were prepared by digesting pBR 322 DNA with *Eco*RI and labeled with (α-^{32}P)dATP using the Klenow fragment of DNA polymerase I.[11] A second enzymatic digestion with *Rsa*I yielded two 3′ end-labeled fragments, 167 and 517 bp. Figure 2a shows the 517 bp fragment cleavage patterns produced by the PM-DHA·VO(II) complexes. The sequenced portion of this DNA fragment contains 8 or 10 base pair AT contiguous sites (sites 1 and 2, respectively).[12] The DNA cleavage specificity of the PM-DHA complexes was compared with that of the phenanthridine-linked hydroxamic acid (**3**) complex. This hydroxamic acid ligand affords a sequence-neutral cleavage pattern in the presence of metal ions due to the intercalative binding of the phenanthridine ring.[12] Distinct cleavage patterns were observed for cleavage by the **1b** and **1d** complexes. Noticeably, the **1b** complex produced doublet bands both at the labeled and non-labeled sides of site 2. In contrast to the DNA cleavage produced by this complex, no DNA cleavage patterns were observed for cleavage by the **1a** and **1c** complexes. These results indicate that the linker length is an important factor which strictly controls the DNA cleavage by the vanadyl complexes of PM-DHA at the 8 and 10 AT contiguous sites. We then examined the 167 bp fragment cleavage patterns produced by the PM-DHA·VO(II) complexes (Fig. 2b). The sequenced portion of this DNA fragment contains 7, 5, and 6 base pair AT contiguous sites (sites 3, 4, and 5, respectively). As can be seen from Fig. 2b, the complexes of **1b—d** with chains longer than **1a** produced almost similar cleavage patterns on both the 5′ and 3′ end-labeled strands. Doublet and triplet bands produced by these complexes were observed only for the 5′ end-labeled strand at sites 3 and 4. No complexes produced a DNA cleavage at site 5. These results indicate that the DNA cleavage by the vanadyl complexes of PM-DHA at 5 and 7 AT contiguous sites is not significantly influenced by the linker length. Figure 3 shows a histogram of the 517 bp fragment cleavage pattern produced by the **1b**·VO(II) complex. Although the cleavage patterns at sites 1 and 2 are asymmetric and shifted to the 3′ side of each strand, cleavage occurs almost exclusively at two nucleotide residues on both strands of site 2. This highly specific cleavage contrasts with the behavior of the affinity cleaving agent, distamycin-EDTA·Fe(II), which produces asymmetric multiple cleavage patterns.[3] We chose to use **1b** for comparison with the phenylene hydroxamic acids in the following experiments because of its high activity and specificity.

DNA Cleavage Patterns Produced by Ph-DHA·VO(II) Complexes We investigated the effect of the rigid linking group of DHA on the DNA cleavage specificity.[13] It has been proposed that the diffusible hydroxyl radical is involved in the DNA cleavage by vanadyl and hydrogen peroxide *via* a Fenton-like mechanism.[14] If the phenylene linkage can restrict the position and orientation of the vanadyl complexing moieties directing them toward the affected nucleotide and increase the local concentration of the cleaving species, single-site DNA cleavage might be induced by the phenylene complexes. Figure 4 shows the 517 bp fragment cleavage patterns produced by Ph-DHA·VO(II) complexes. As shown in Fig. 4a, the **2a** complex produced the same doublet band as that seen for cleavage by the **1b** complex at the 3′ side of site 2. The **2b** and **2c** complexes, which have a linker length close to the optimum **1b** complex, produced the expected singlet band at site 1. This single-site cleavage may be the effect of the restrained orientation of the vanadyl complexing moiety of these complexes. No single-site cleavage, however, was observed in the opposite strand analysis (Fig. 4b). All the phenylene hydroxamic acid complexes produced no significant DNA cleavage on the 3′ end-labeled 517 bp fragment. The DNA cleavage specificities of Ph-DHA·VO(II) complexes were also examined using the 167 bp fragment (Fig. 5). Although the **2a** complex produced no cleavage at sites 3, 4 and 5, much less specific cleavage patterns were observed for cleavage by the **2b** and **2c** complexes at these sites (Fig. 5a). To our surprise, the 3′ end-labeled fragment was not cleaved by all the phenylene hydroxamic acid complexes (Fig. 5b). This result is consistent with the cleavage of the 3′

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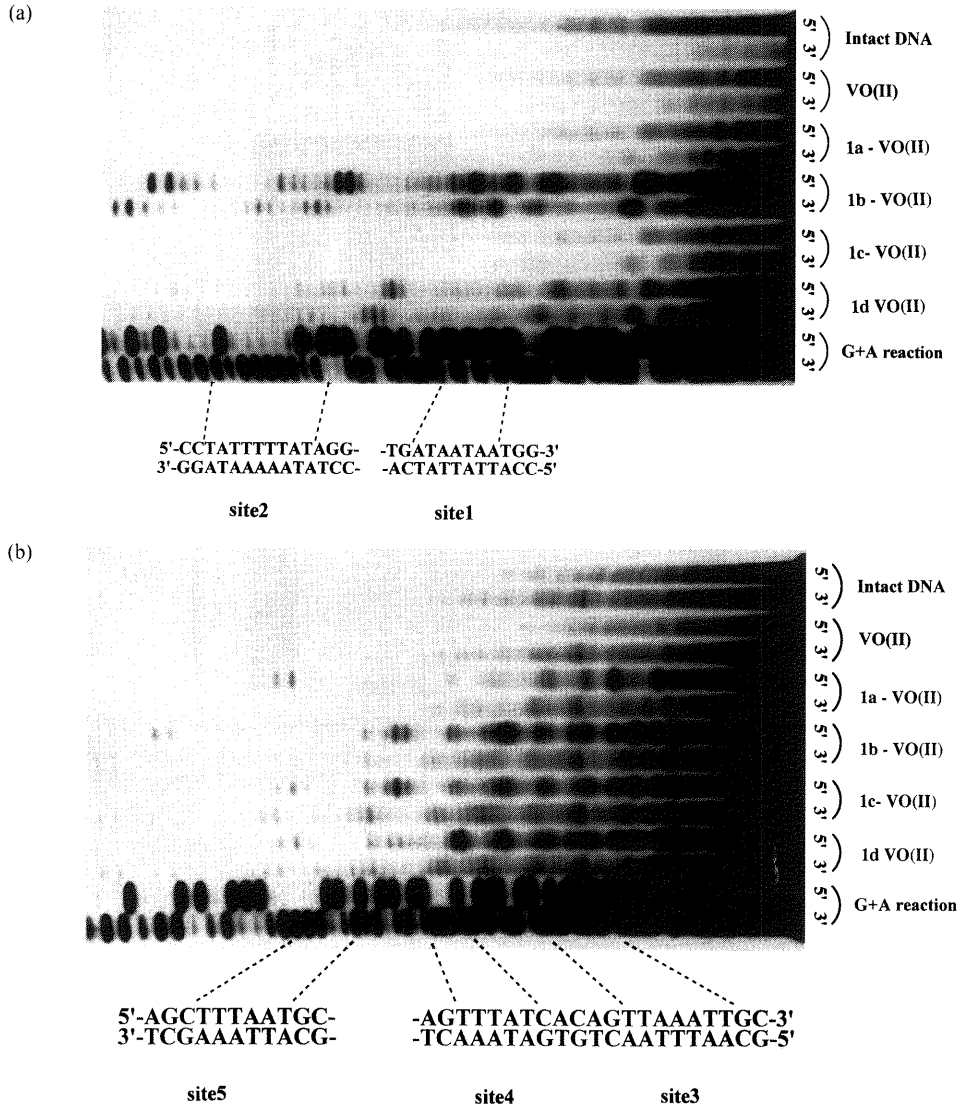

Fig. 2. Autoradiograms of a 10% Polyacrylamide Gel Showing Cleavage of End-Labeled 517 and 167 bp DNA Fragments by PM-DHA·VO(II) Complexes

In autoradiogram (a), the cleavage of the 5′ end- and 3′ end-labeled 517 bp fragments is alternatively sequenced. In autoradiogram (b), the cleavage of the 5′ end- and 3′ end-labeled 167 bp fragments is alternatively sequenced. End-labeled fragments were incubated with each hydroxamic acid (10 μM) in the presence of vanadyl ion (10 μM) and hydrogen peroxide (500 μM) in 40 mM Tris–HCl (pH 8.0) at 37 °C for 3.0 h. In each reaction, sonicated calf thymus DNA was added to give a final concentration of 10 μM (nucleotide concentration).

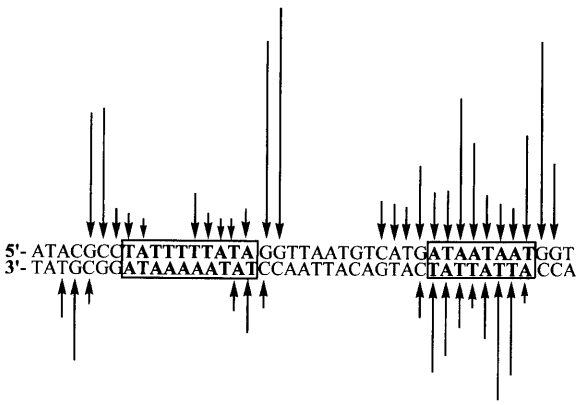

Fig. 3. Histograms of the Vanadyl Complex of the **1b** Cleavage Pattern Flanking Both Sites 1 (Right) and 2 (Left)

Bars indicate sites and extent of DNA cleavage.

end-labeled 517 bp fragment in Fig. 4b. It seems that the phenylene hydroxamic acid complexes bind tightly to one of the strands of pBR 322 DNA and not to the opposite strand.

Discussion

It was found that DNA cleavage by the vanadyl complexes of PM-DHA at the 8 and 10 AT sites was significantly influenced by the length of the polymethylene chain (Fig. 2a). At these AT contiguous sites, the vanadyl complex of tetramethylene compound **1b** afforded asymmetric and highly specific DNA cleavage patterns (Fig. 3). Asymmetric multiple cleavage patterns produced by the affinity cleaving agent show no association of the negatively charged Fe(II)·EDTA moiety with DNA and the generation of freely diffusible hydroxyl radical.[3] To attain highly specific DNA cleavage, the monohydroxamic acid vanadyl complex should associate with the DNA nucleotide. Vanadyl ion is known to strongly coordinate to the triphosphate group of ATP at neutral pH.[15] So, it is feasible to envisage the formation of a transient vanadyl

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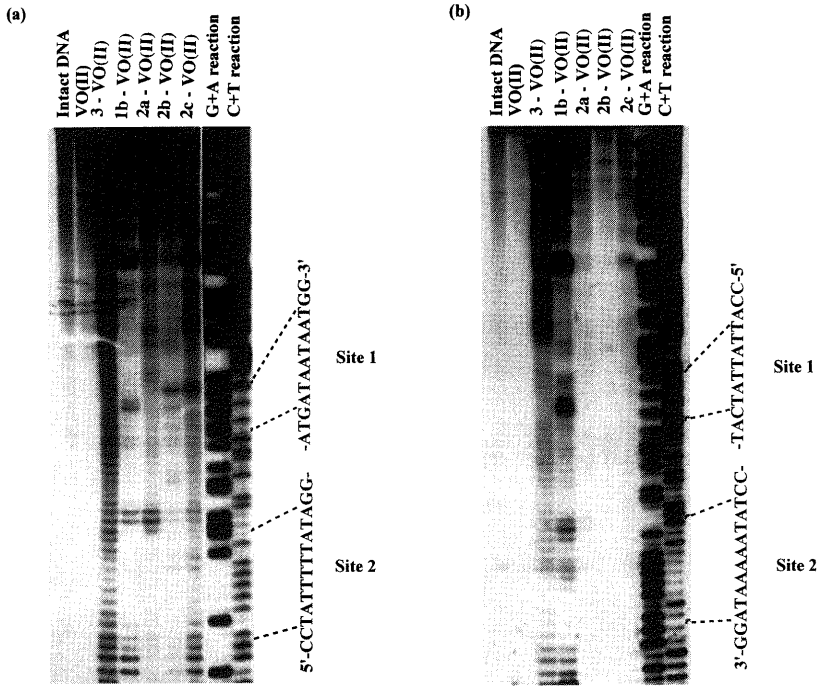

Fig. 4. Autoradiograms of a 10% Polyacrylamide Gel Showing Cleavage of the End-Labeled 517 bp DNA Fragment by Ph-DHA · VO(II) Complexes

Autoradiograms (a) and (b) show DNA fragments labeled at the (a) 5′ end or (b) 3′ end, respectively. End-labeled fragments were incubated with each hydroxamic acid (20 μM) in the presence of vanadyl ion (20 μM) and hydrogen peroxide (500 μM) in 40 mM Tris–HCl (pH 8.0) at 37 °C for 5.0 h. In each reaction, sonicated calf thymus DNA was added to give a final concentration of 10 μM (nucleotide concentration).

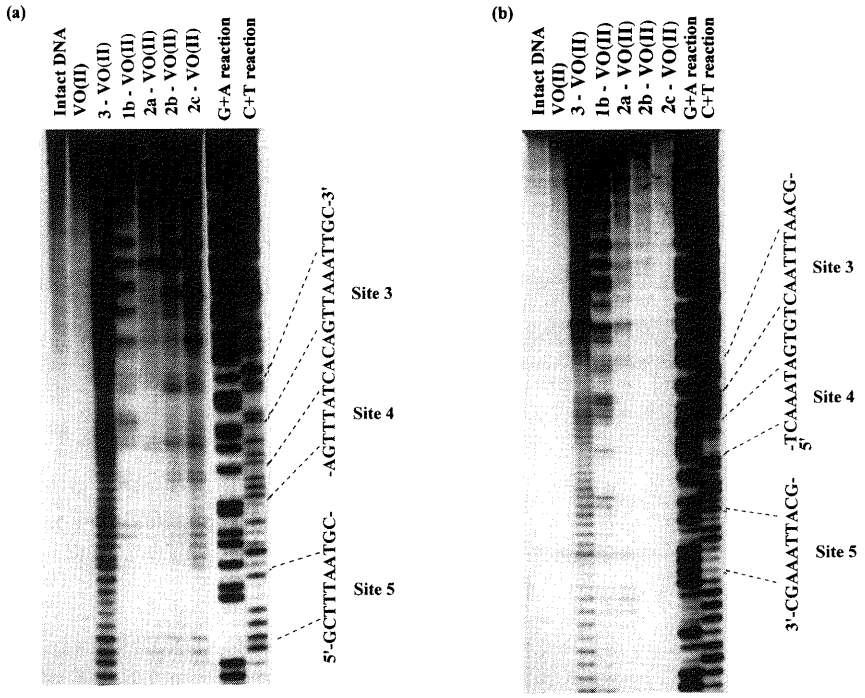

Fig. 5. Autoradiograms of a 10% Polyacrylamide Gel Showing Cleavage of the End-Labeled 167 bp DNA Fragment by Ph-DHA · VO(II) Complexes

Autoradiograms (a) and (b) show DNA fragments labeled at the (a) 5′ end or (b) 3′ end, respectively. The reaction conditions were the same as in Fig. 4.

complex with monohydroxamic acid and the phosphodiester of DNA.[16] The tetramethylene chain of **1b** may allow the transient complex to maintain a suitable position for the abstraction of the deoxyribose proton of DNA nucleotide. DNA cleavage by the vanadyl complex of **1b**—**d** at the 5 and 7 AT sites was almost independent of the length of the polymethylene chain (Fig. 2b). The vanadyl complex of **1b**, which showed highly specific DNA cleavage at the 8 and 10 AT sites, afforded moderately specific cleavage patterns at these sites. The narrowness of the minor groove in B-DNA is known to depend on the local DNA sequence.[3b,d,17] This variation in local DNA structure will affect the groove binding mode of the distamycin moiety of the vanadyl complex of **1b**. We conclude that the DNA cleavage specificity of the vanadyl complex of distamycin-linked hydroxamic acid is a function of the binding affinity, which is based on a combina-

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tion of tripyrrolepeptides and the monohydroxamic acid complex, and a sequence-dependent DNA structure.

It was expected that the vanadyl complexes of conformationally rigid Ph-DHA would exhibit more specific cleavage than the flexible PM-DHA complexes. Although the **2b** and **2c** complexes, which have a linker length close to the optimum **1b** complex, produced a single-site DNA cleavage at the 8 AT site (Fig. 4a), these complexes produced much less specific cleavage patterns at other AT sites (Fig. 5a). In addition, these complexes failed to cleave the 3′ end-labeled sequences of AT-rich DNA (Fig. 4b, 5b). These complexes seem to selectively recognize one of the strands of AT-rich DNA and we believe that the van der Waals interaction between the benzene rings of phenylene hydroxamic acids and the Waals of the minor groove allows the monohydroxamic acid complex to direct itself away from the opposite strand of DNA. This unfavorable interaction may prevent the distamycin and vanadyl complexing moieties from synergistically binding to the AT sequence, which would lead to a reduction in the binding selectivity of the vanadyl complex of phenylene hydroxamic acid for the AT sequence.

In conclusion, we have synthesized DHAs and evaluated the effect of the length and rigidity of their chains on the DNA cleavage specificity in the presence of vanadyl ion. The tetramethylene chain was found to be the optimal linking group for connecting the distamycin and hydroxamic acid moieties. A suitably designed linking moiety would allow the creation of a more potent and sequence-specific DHA system.

Experimental

General Methods The evaporation of solvents was carried out in a rotary evaporator under reduced pressure. Dimethylformamide (DMF) was dried over anhydrous magnesium sulfate overnight and distilled under reduced pressure. Melting points were determined using a Yanagimoto micromelting point apparatus and are uncorrected. The IR spectra were obtained using KBr discs on a Shimadzu IR-470 and only the principal peaks are reported. The UV spectra were recorded on a Shimadzu UV-2100 instrument. The ^{1}H-NMR spectra were recorded on a JEOL JNM-EX 400 (400 MHz) spectrometer using tetramethylsilane (TMS) as the internal reference. The low-resolution (LR) and high-resolution (HR) FAB-MS spectra were recorded on a JEOL JMS-SX102A instrument. TLC was performed on precoated aluminum sheets of Silica gel 60F_{254} (Merck, No. 5554). The TLC systems were as follows: system A, 0.49% concentrated aqueous ammonia in 15% MeOH–$CHCl_3$; system B, 0.49% concentrated aqueous ammonia in 20% MeOH–$CHCl_3$; system C, *n*-BuOH–AcOH–H_2O in a 4 : 1 : 2 ratio. Silica gel column chromatography was carried out using a Fuji Silysia Chemical BW-127ZH. Polystyrene resin DIAION HP 20 was purchased from the Mitsubishi Chemical Co. Yields refer to chromatographically and spectroscopically (^{1}H-NMR) homogeneous materials.

Plasmid pBR 322 DNA was isolated from *Escherichia coli* strain JM109 by alkali lysis and was purified by precipitation with polyethylene glycol.[18] [γ-^{32}P]ATP (3000 Ci/mmol) and [α-^{32}P]dATP (3000 Ci/mmol) were obtained from NEN-Dupont. The microfine glass beads and chaotropic salt buffer were from Bio 101. Chemical DNA sequencing was carried out according to the method of Maxam and Gilbert.[19] All enzymes used were from commercial sources. Vanadyl sulfate trihydrate was purchased from Aldrich Chem. Co. and used without further purification.

4-[[[4-[[[4-[[4-(Methoxycarbonyl)butyryl]amino]-1-methyl-2-pyrrolyl]-carbonyl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-*N*-[3-(*N*,*N*-dimethylamino)propyl]-2-pyrrolecarboxamide (5a) A solution of compound **4** (1.0 g, 2.0 mmol) in MeOH (5.0 ml) was hydrogenated over PtO_2 (0.015 g) at room temperature and atmospheric pressure. After the calculated amount of hydrogen was taken up, the mixture was filtered through Celite. The filtrate was concentrated *in vacuo*, and the evaporation was repeated with some DMF. After the residue was cooled to 0 °C, methyl 5-[(*p*-nitrophenoxy)carbonyl]butyrate (0.53 g, 2.0 mmol) in dry DMF (3.0 ml) was added. The reaction mixture was stirred at 0 °C for 1 h and then at room temperature overnight. The DMF was evaporated *in vacuo*, and the resulting residue was dissolved in $CHCl_3$. The organic phase was washed with two portions of 5% aqueous K_2CO_3 and dried over anhydrous Na_2SO_4. After removal of the solvent, the residue was purified by silica gel chromatography. Elution with a stepwise gradient of 5% and 10% MeOH in 0.49% concentrated aqueous ammonia–$CHCl_3$ provided pure **5a** as a yellow crystalline solid (0.44 g, 37%), Rf=0.34 (system A), mp 115—118 °C. IR (KBr) cm^{-1}: 3290, 2950, 1730 (C=O ester), 1640, 1580, 1530. ^{1}H-NMR (DMSO-d_6) δ: 1.62 (2H, t, J=7.2 Hz), 1.83 (2H, t, J=7.2 Hz), 2.14 (6H, s), 2.23—2.30 (4H, m), 2.35 (2H, t, J=7.6 Hz), 3.20 (2H, t, J=6.4 Hz), 3.60 (3H, s), 3.80, 3.84, 3.85 (3H×3, s), 6.83 (1H, s), 6.88 (1H, s), 7.04 (1H, s), 7.16 (1H, s), 7.19 (1H, s), 7.24 (1H, s), 8.07 (1H, s), 9.82 (1H, s), 9.89 (1H, s), 9.90 (1H, s). FAB-LR-MS *m/z*: 597 (MH^+, 84%), 251 ((CONHpyrroleCONH$(CH_2)_3$-$N(CH_3)_2)^+$, 36), 73 ($(H_3COCOCH_2)^+$, 99).

4-[[[4-[[[4-[[5-(Ethoxycarbonyl)valeryl]amino]-1-methyl-2-pyrrolyl]-carbonyl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-*N*-[3-(*N*,*N*-dimethylamino)propyl]-2-pyrrolecarboxamide (5b) A synthetic procedure similar to that for compound **5a** was followed for the preparation of **5b**: yellow crystalline solid (yield: 35%), Rf=0.37 (system A), mp 100—105 °C. IR (KBr) cm^{-1}: 3290, 2940, 1730 (C=O ester), 1640, 1580, 1530. ^{1}H-NMR (DMSO-d_6) δ: 1.18 (3H, t, J=7.2 Hz), 1.54—1.64 (6H, m), 2.15 (6H, s), 2.24—2.27 (4H, m), 2.31 (2H, t, J=6.8 Hz), 3.20 (2H, t, J=6.8 Hz), 3.81, 3.84, 3.86 (3H(3, s), 4.05 (2H, q, J=7.2 Hz), 6.83 (1H, d, J=1.6 Hz), 6.89 (1H, d, J=1.6 Hz), 7.04 (1H, d, J=2.0 Hz), 7.16 (1H, d, J=1.2 Hz), 7.19 (1H, d, J=1.2 Hz), 7.24 (1H, d, J=1.2 Hz), 8.07 (1H, s), 9.78 (1H, s), 9.88 (1H, s), 9.90 (1H, s). FAB-LR-MS *m/z*: 625 (MH^+, 26%), 279 ($(H_5C_2OCO(CH_2)_4CONHpyrroleCO)^+$, 60).

4-[[[4-[[[4-[[6-(Ethoxycarbonyl)hexanoyl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-*N*-[3-(*N*,*N*-dimethylamino)propyl]-2-pyrrolecarboxamide (5c) A synthetic procedure similar to that for compound **5a** was followed for the preparation of **5c**: yellow crystalline solid (yield: 32%), Rf=0.40 (system A), mp 110.5—112.5 °C. IR (KBr) cm^{-1}: 3290, 2930, 1730 (C=O ester), 1650, 1580, 1530. ^{1}H-NMR (DMSO-d_6) δ: 1.17 (3H, t, J=7.2 Hz), 1.23—1.32 (2H, m), 1.51—1.63 (6H, m), 2.14 (3H, s), 2.21—2.30 (6H, m), 3.20 (2H, t, J=6.6 Hz), 3.80, 3.81, 3.83 (3H×3, s), 4.04 (2H, q, J=7.2 Hz), 6.84 (1H, s), 6.88 (1H, s), 7.04 (1H, s), 7.16 (1H, s), 7.19 (1H, s), 7.23 (1H, s), 8.08 (1H, s), 9.79 (1H, s), 9.89 (1H, s), 9.90 (1H, s). FAB-LR-MS *m/z*: 639 (MH^+, 100%), 293 ($(H_5C_2OCO(CH_2)_5CONHpyrroleCO)^+$, 45).

4-[[[4-[[[4-[[7-(Methoxycarbonyl)heptanoyl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-*N*-[3-(*N*,*N*-dimethylamino)propyl]-2-pyrrolecarboxamide (5d) A synthetic procedure similar to that for compound **5a** was followed for the preparation of **5d**: yellow solid (yield: 31%), Rf=0.41 (system A), mp 91—93.5 °C. IR (KBr) cm^{-1}: 3290, 2950, 1730 (C=O ester), 1640, 1575, 1525. ^{1}H-NMR (DMSO-d_6) δ: 1.28 (4H, m), 1.51—1.63 (6H, m), 2.10, 2.14 (3H×2, s), 2.21—2.31 (6H, m), 3.19 (2H, t, J=6.4 Hz), 3.58 (3H, s), 3.82, 3.83, 3.85 (3H×3, s), 6.83 (1H, d, J=1.6 Hz), 6.88 (1H, d, J=1.2 Hz), 7.03 (1H, d, J=2.0 Hz), 7.16 (1H, d, J=1.2 Hz), 7.19 (1H, s), 7.24 (1H, s), 8.07 (1H, s), 9.77 (1H, s), 9.88 (1H, s), 9.90 (1H, s). FAB-LR-MS *m/z*: 639 (MH^+, 92%), 293 ($(H_3COCO(CH_2)_6CONHpyrroleCO)^+$, 23).

4-[[[4-[[[4-[[*o*-(Methoxycarbonyl)phenylenecarbonyl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-2-pyrrolyl]carbonyl]-amino]-1-methyl-*N*-[3-(*N*,*N*-dimethylamino)propyl]-2-pyrrolecarboxamide (6a) To a solution of [*o*-(methoxycarbonylmethyl)phenyl]acetic acid (0.083 g, 0.40 mmol) in dry DMF (3 ml) was added 1-hydroxybenzotriazole (HOBt) (0.070 g, 0.52 mmol). The mixture was cooled to 0 °C, and DCC (0.090 g, 0.44 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 4 h. Separately, a solution of compound **4** (0.20 g, 0.40 mmol) in MeOH (5 ml) was hydrogenated over PtO_2 (0.010 g) at room temperature and atmospheric pressure. After the calculated amount of hydrogen was taken up, the mixture was filtered through Celite. The filtrate was concentrated *in vacuo*, and the concentration was repeated with some DMF. Immediately, the solution of DCC-activated phenylene monoester was cooled to 0 °C and the aromatic amine was added dropwise. The reaction mixture was stirred at 0 °C for 30 min and then at room temperature overnight. The DMF was evaporated *in vacuo*, and the resulting residue was dissolved in AcOEt. The organic phase was washed with two portions of 5% aqueous K_2CO_3 and dried over anhydrous Na_2SO_4. After removal of the solvent, the residue was purified by flash chromatography. Elution with 0.49% concentrated aqueous ammonia in 5% MeOH–$CHCl_3$ provided pure **6a** as pale-yellow microcrystals (0.14 g, 53%), Rf=0.55 (system B), mp 122—127 °C. IR (KBr) cm^{-1}: 3290, 2940, 1730 (C=O ester), 1650, 1580, 1530. ^{1}H-NMR (DMSO-d_6) δ: 1.64 (2H, m), 2.21 (6H, s), 2.33 (2H, t, J=6.8 Hz),

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3.19 (2H, t, J=6.8 Hz), 3.61 (5H, s), 3.81 (2H, s), 3.83, 3.84, 3.85 (3H×3, s), 6.84 (1H, d, J=2.0 Hz), 6.90 (1H, d, J=1.6 Hz), 7.03 (1H, d, J=2.0 Hz), 7.14 (1H, d, J=2.0 Hz), 7.18 (1H, d, J=1.6 Hz), 7.21—7.30 (5H, m), 8.07 (1H, s), 9.87 (1H, s), 9.89 (1H, s), 10.04 (1H, s).

4-[[[4-[[[4-[[*m*-(Methoxycarbonyl)phenylenecarbonyl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-2-pyrrolyl]carbonyl]-amino]-1-methyl-*N*-[3-(*N*,*N*-dimethylamino)propyl]-2-pyrrolecarboxamide (6b) A synthetic procedure similar to that for compound **6a** was followed for the preparation of **6b**: Pale-yellow microcrystals (yield: 40%), Rf=0.55 (system B), mp 115—119 °C. IR (KBr) cm^{-1}: 3290, 2920, 2850, 1730 (C=O ester), 1640, 1580, 1530. ^{1}H-NMR (DMSO-d_6) δ: 1.61 (2H, m), 2.14 (6H, s), 2.25 (2H, t, J=7.2 Hz), 3.18 (2H, t, J=6.4 Hz), 3.55 (2H, s), 3.61 (3H, s), 3.66 (2H, s), 3.80, 3.83, 3.84 (3H×3, s), 6.82 (1H, d, J=1.6 Hz), 6.90 (1H, d, J=1.6 Hz), 7.02 (1H, d, J=1.6 Hz), 7.13—7.27 (7H, m), 8.07 (1H, s), 9.89 (1H, s), 9.90 (1H, s), 10.08 (1H, s).

4-[[[4-[[[4-[[*p*-(Methoxycarbonyl)phenylenecarbonyl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-2-pyrrolyl]carbonyl]-amino]-1-methyl-*N*-[3-(*N*,*N*-dimethylamino)propyl]-2-pyrrolecarboxamide (6c) A synthetic procedure similar to that for compound **6a** was followed for the preparation of **6c**: Pale-yellow microcrystals (yield: 41%), Rf=0.55 (system B), mp 117—121 °C. IR (KBr) cm^{-1}: 3290, 2950, 1730 (C=O ester), 1640, 1580, 1530. ^{1}H-NMR (DMSO-d_6) δ: 1.62 (2H, m), 2.17 (6H, s), 2.29 (2H, t, J=6.8 Hz), 3.18 (2H, t, J=6.8 Hz), 3.54 (2H, s), 3.60 (3H, s), 3.65 (2H, s), 3.80, 3.82, 3.84 (3H×3, s), 6.83 (1H, d, J=1.6 Hz), 6.89 (1H, d, J=2.0 Hz), 7.02 (1H, d, J=1.6 Hz), 7.15—7.28 (7H, m), 8.08 (1H, s), 9.89 (1H, s), 9.90 (1H, s), 10.06 (1H, s).

4-[[[4-[[[4-[[4-(Hydroxyaminocarbonyl)butyryl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-*N*-[3-(*N*,*N*-dimethylamino)propyl]-2-pyrrolecarboxamide (1a) Separate solutions of hydroxylamine hydrochloride (0.20 g, 2.9 mmol) in MeOH (2.5 ml), KOH (0.35 g, 6.2 mmol) in MeOH (3.0 ml) and compound **5a** (0.30 g, 0.48 mmol) in MeOH (3.0 ml) were prepared. The solution containing alkali was added *via* a syringe to the stirred hydroxylamine solution, and the mixture was allowed to stand in ice-water for 3 min under an argon atmosphere. To the alkaline mixture was added the solution of compound **5a** *via* a syringe, and the reaction mixture was then stirred overnight at room temperature under an argon atmosphere. The reaction was terminated by the addition of 2.0 M aqueous HCl (1.7 ml, 3.4 mmol) to the mixture which was then filtered to remove the salt. The filtrate was evaporated and the residue was taken up in 50% MeOH–EtOH and filtered. This procedure was repeated once more and then the filtrate was evaporated. The residue dissolved in H_2O was applied to an HP 20 column, which was successively washed with deionized H_2O. Elution with 50% MeOH–H_2O followed by evaporation of the appropriate fractions provided pure **1a** as a light-brown glassy solid (0.22 g, 70%), Rf=0.47 (system C), mp 151.5—154 °C. IR (KBr) cm^{-1}: 3250, 1650, 1635. λ_{max}(H_2O)/nm 300 (log ε=4.32). ^{1}H-NMR (DMSO-d_6) δ: 1.80 (4H, m), 1.99 (2H, t, J=7.2 Hz), 2.24 (2H, t, J=6.8 Hz), 2.70 (3H, s), 2.72 (3H, s), 3.01 (2H, t, J=6.0 Hz), 3.24 (2H, m), 3.80, 3.83, 3.84 (3H×3, s), 6.89 (1H, s), 6.92 (1H, d, J=2.0 Hz), 7.05 (1H, s), 7.16 (1H, d, J=1.2 Hz), 7.19 (1H, s), 7.23 (1H, s), 8.18 (1H, s), 8.70 (1H, br s), 9.87 (1H, s), 9.92 (2H, s), 10.41 (1H, s). FAB-LR-MS m/z: 598 (M^+−Cl, 82%). FAB-HR-MS m/z: 598.3111 (Calcd for $C_{28}H_{40}N_9O_6$ M^+−Cl: 598.3101).

4-[[[4-[[[4-[[5-(Hydroxyaminocarbonyl)valeryl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-*N*-[3-(*N*,*N*-dimethylamino)propyl]-2-pyrrolecarboxamide (1b) A synthetic procedure similar to that for **1a** was followed for the preparation of **1b**: Light-brown glassy solid (yield: 53%), Rf=0.44 (system C), mp 144—147.5 °C. IR (KBr) cm^{-1}: 3250, 1650, 1635. λ_{max}(H_2O)/nm 300 (log ε=4.27). ^{1}H-NMR (DMSO-d_6) δ: 1.53 (4H, m), 1.84 (2H, m), 1.98 (2H, t, J=6.6 Hz), 2.22 (2H, t, J=6.4 Hz), 2.69 (6H, s), 2.98 (2H, t, J=7.6 Hz), 3.24 (2H, t, J=5.6 Hz), 3.82, 3.84, 3.85 (3H×3, s), 6.89 (1H, d, J=2.0 Hz), 6.93 (1H, d, J=1.6 Hz), 7.06 (1H, d, J=1.6 Hz), 7.16 (1H, d, J=1.6 Hz), 7.19 (1H, s), 7.24 (1H, s), 8.18 (1H, t, J=5.3 Hz), 8.70 (1H, br s), 9.84 (1H, s), 9.92 (2H, s), 10.41 (1H, s). FAB-LR-MS m/z: 612 (M^+−Cl, 68%). FAB-HR-MS m/z: 612.3258 (Calcd for $C_{29}H_{42}N_9O_6$ M^+−Cl: 612.3244).

4-[[[4-[[[4-[[6-(Hydroxyaminocarbonyl)hexanoyl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-*N*-[3-(*N*,*N*-dimethylamino)propyl]-2-pyrrolecarboxamide (1c) A synthetic procedure similar to that for **1a** was followed for the preparation of **1c**: Yellow crystalline solid (yield: 57%), Rf=0.45 (system C), mp 151—154 °C. IR (KBr) cm^{-1}: 3250, 1640. λ_{max}(H_2O)/nm 299 (log ε=4.34). ^{1}H-NMR (DMSO-d_6) δ: 1.27 (2H, m), 1.50—1.58 (4H, m), 1.88 (2H, m), 1.95 (2H, t, J=7.2 Hz), 2.23 (2H, t, J=7.0 Hz), 2.74, 2.77 (3H×2, s), 3.04 (2H, t, J=7.8 Hz), 3.27 (2H, m), 3.82, 3.84, 3.85 (3H×3, s), 6.90 (1H, d, J=1.2 Hz), 6.94 (1H, d, J=1.6 Hz), 7.06 (1H, d, J=1.6 Hz), 7.16 (1H, s), 7.19 (1H, s), 7.24 (1H, s), 8.18 (1H, br s), 8.68 (1H, br s), 9.82 (1H, s), 9.92 (2H, s), 10.38 (1H, s). FAB-LR-MS m/z: 626 (M^+−Cl, 73%). FAB-HR-MS m/z: 626.3411 (Calcd for $C_{30}H_{44}N_9O_6$ M^+−Cl: 626.3414).

4-[[[4-[[[4-[[7-(Hydroxyaminocarbonyl)heptanoyl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-*N*-[3-(*N*,*N*-dimethylamino)propyl]-2-pyrrolecarboxamide (1d) A synthetic procedure similar to that for **1a** was followed for the preparation of **1d**: Light-yellow crystalline solid (yield: 63%), Rf=0.46 (system C), mp 149.5—152.5 °C. IR (KBr) cm^{-1}: 3250, 1645, 1635. λ_{max}(H_2O)/nm 300 (log ε=4.32). ^{1}H-NMR (DMSO-d_6) δ: 1.26 (4H, m), 1.48 (2H, m), 1.55 (2H, m), 1.86 (2H, t, J=7.6 Hz), 1.94 (2H, t, J=7.2 Hz), 2.22 (2H, t, J=7.2 Hz), 2.72 (6H, s), 3.02 (2H, t, J=7.6 Hz), 3.24 (2H, m), 3.81, 3.83, 3.84 (3H×3, s), 6.89 (1H, d, J=2.0 Hz), 6.92 (1H, d, J=1.6 Hz), 7.05 (1H, d, J=2.0 Hz), 7.16 (1H, s), 7.19 (1H, s), 7.24 (1H, s), 8.18 (1H, s), 8.68 (1H, br s), 9.83 (1H, s), 9.93 (2H, s), 10.37 (1H, s). FAB-LR-MS m/z: 640 (M^+−Cl, 27%). FAB-HR-MS m/z: 640.3582 (Calcd for $C_{31}H_{46}N_9O_6$ M^+−Cl: 640.3571).

4-[[[4-[[[4-[[*o*-(Hydroxyaminocarbonyl)phenylenecarbonyl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-2-pyrrolyl]carbonyl]-amino]-1-methyl-*N*-[3-(*N*,*N*-dimethylamino)propyl]-2-pyrrolecarboxamide (2a) Separate solutions of hydroxylamine hydrochloride (0.062 g, 0.89 mmol) in MeOH (2.0 ml), NaOH (0.071 g, 1.8 mmol) in MeOH (2.0 ml) and compound **6a** (0.10 g, 0.15 mmol) in MeOH (2.0 ml) were prepared and reacted as described in the procedure for **1a**. Purification using a Serva XAD-II polystyrene resin columun and elution with 50% MeOH–H_2O provided pure **2a** as light-brown microcrystals (0.074 g, 75%), Rf=0.50 (system C), mp 138—144 °C. λ_{max}(H_2O)/nm 303 (log ε=4.51). ^{1}H-NMR (DMSO-d_6) δ: 1.87 (2H, m), 2.75 (6H, s), 3.05 (2H, t, J=6.4 Hz), 3.25 (2H, t, J=5.6 Hz), 3.27 (2H, s), 3.74 (2H, s), 3.81, 3.82, 3.84 (3H×3, s), 6.91 (1H, s), 6.93 (1H, s), 7.04 (1H, s), 7.16 (1H, s), 7.17 (1H. s), 7.18 (1H, s), 7.23—7.29 (4H, m), 8.17 (1H, t, J=5.6 Hz), 9.91 (2H, s), 10.05 (1H, s), 10.80 (1H, s). FAB-LR-MS m/z: 660 (M^+−Cl, 33%), 245 ((pyrroleCONH)$_2$+H)$^+$, 12). FAB-HR-MS m/z: 660.3262 (Calcd for $C_{33}H_{42}N_9O_6$ M^+−Cl: 660.3258).

4-[[[4-[[[4-[[*m*-(Hydroxyaminocarbonyl)phenylenecarbonyl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-2-pyrrolyl]carbonyl]-amino]-1-methyl-*N*-[3-(*N*,*N*-dimethylamino)propyl]-2-pyrrolecarboxamide (2b) A synthetic procedure similar to that for **2a** was followed for the preparation of **2b**: yellow microcrystals (yield: 66%), Rf=0.50 (system C), mp 135—143 °C. λ_{max}(H_2O)/nm 302 (log ε=4.43). ^{1}H-NMR (DMSO-d_6) δ: 1.86 (2H, m), 2.74 (6H, s), 3.03 (2H, t, J=7.6 Hz), 3.24 (2H, t, J=5.6 Hz), 3.27 (2H, s), 3.55 (2H, s), 3.82, 3.83, 3.84 (3H×3, s), 6.92 (1H, d, J=2.0 Hz), 6.93 (1H, d, J=1.6 Hz), 7.04 (1H, d, J=2.0 Hz), 7.12—7.26 (7H, m), 8.17 (1H, s), 8.83 (1H, s), 9.92 (2H, s), 10.13 (1H, s), 10.69 (1H, s). FAB-LR-MS m/z: 660 (M^+−Cl, 31%). FAB-HR-MS m/z: 660.3260 (Calcd for $C_{33}H_{42}N_9O_6$ M^+−Cl: 660.3258).

4-[[[4-[[[4-[[*p*-(Hydroxyaminocarbonyl)phenylenecarbonyl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-2-pyrrolyl]carbonyl]-amino]-1-methyl-*N*-[3-(*N*,*N*-dimethylamino)propyl]-2-pyrrolecarboxamide (2c) A synthetic procedure similar to that for **2a** was followed for the preparation of **2c**: yellow microcrystals (yield: 69%), Rf=0.50 (system C), mp 139—146 °C. λ_{max}(H_2O)/nm 301 (log ε=4.28). ^{1}H-NMR (DMSO-d_6) δ: 1.84 (2H, m), 2.73 (6H, s), 3.02 (2H, t, J=7.2 Hz), 3.25 (4H, s), 3.53 (2H, s), 3.81, 3.82, 3.84 (3H×3, s), 6.90 (1H, d, J=1.6 Hz), 6.93 (1H, d, J=2.0 Hz), 7.04 (1H, d, J=1.6 Hz), 7.14 (1H, d, J=1.6 Hz), 7.18—7.25 (6H, m), 8.17 (1H, s), 9.90 (1H, s), 9.91 (1H, s), 10.06 (1H, s), 10.65 (1H, s). FAB-LR-MS m/z: 660 (M^+−Cl, 10%). FAB-HR-MS m/z: 660.3267 (Calcd for $C_{33}H_{42}N_9O_6$ M^+−Cl: 660.3258).

Preparation of 5′-^{32}P End-Labeled Restriction Fragments Plasmid pBR 322 DNA was linearized with restriction endonuclease *Eco*RI, dephosphorylated with calf intestine alkaline phosphatase, then 5′-^{32}P end-labeled with T4 polynuleotide kinase and [γ-^{32}P]ATP.[11] Digestion with *Rsa*I afforded the 167 and 517 bp fragments, which were purified on 4% sieve agarose gel. The gel was visualized by autoradiography, the bands of interest were excised from the gel, and the DNAs were isolated by the glass matrix method.[20]

Preparation of 3′-^{32}P End Labeled Restriction Fragments Plasmid pBR 322 was digested with *Eco*RI and the linearized DNA was recovered by ethanol precipitation. The DNA was 3′-^{32}P end-labeled with the Klenow fragment of DNA polymerase I and [α-^{32}P]dATP.[11] Digestion with the second enzyme *Rsa* I afforded the singly end-labeled 167 and 517 bp fragments, which were isolated as described in the preparation of the 5′-^{32}P labeled

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fragments.

Cleavage Reactions of Labeled DNA Fragments by the Vanadyl Complexes of Hydroxamic Acids The reaction mixtures contained 10 μM (nucleotide concentration) sonicated calf thymus DNA and 10000—50000 cpm of the 5′- or 3′-^{32}P labeled DNA fragment in 40 mM Tris–Cl (pH 8.0). The reactions were initiated by the addition of the appropriate concentration of hydroxamic acid, vanadyl sulfate and hydrogen peroxide. The reaction mixtures were maintained at 37 °C for 3 or 5 h and stopped by the addition of stopping buffer (0.3 M AcONa, pH 7.0, 0.1 mM EDTA, 25 μg/ml tRNA) followed by ethanol precipitation. The precipitated DNA was washed with 70% cold ethanol and dried *in vacuo*. The recovered DNA was dissolved in 5 μl of loading buffer (80% v/v formamide, 10 mM NaOH, 1 mM EDTA, 0.1% bromophenol blue, 0.1% xylene cyanol). All DNA samples were heated at 90 °C for 3 min and loaded on a 10% polyacrylamide, 8 M urea sequencing gel. Electrophoresis was performed at 1500 V for approximately 2.5 h. Autoradiography of the gel was carried out at −80 °C with an intensifying screen on Fuji medical X-ray film. Densitometry of the gel was performed with a computer program, NIH image.

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