# SYNTHESIS OF BIS(N-METHYLPYRROLE OLIGOPEPTIDE-LINKED HYDROXAMIC ACIDS) AND EFFECTIVE DNA CLEAVAGE BY THEIR VANADYL COMPLEXES

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Abstract - Dimeric N-methylpyrrole oligopeptide hydroxamic acids, which are connected at the carboxy termini *via* an alkylamino tether, have been newly synthesized. The plasmid DNA relaxation assay indicated that vanadyl-mediated DNA cleavage by these molecules is more effective than that by the corresponding monomeric ones. The cleavage activity of the dimeric complexes exhibited a maximum ratio for vanadyl / dimeric compounds of 2 whereas that of the monomeric ones exhibited a ratio of 1. Two vanadyl-hydroxamate complexing moieties are suggested to independently participate in the effective DNA cleavage by the dimeric complexes.

We have designed and synthesized DNA binding-hydroxamic acids which can induce effective DNA cleavage by forming metal complexes. [1] These molecules contain one hydroxamate group per molecule and induce only single-strand cleavage in the plasmid relaxation assay. For the creation of a practical and useful DNA-cleaving system, our objective was to design a more potent and sequence-specific metal-hydroxamic acid complex.
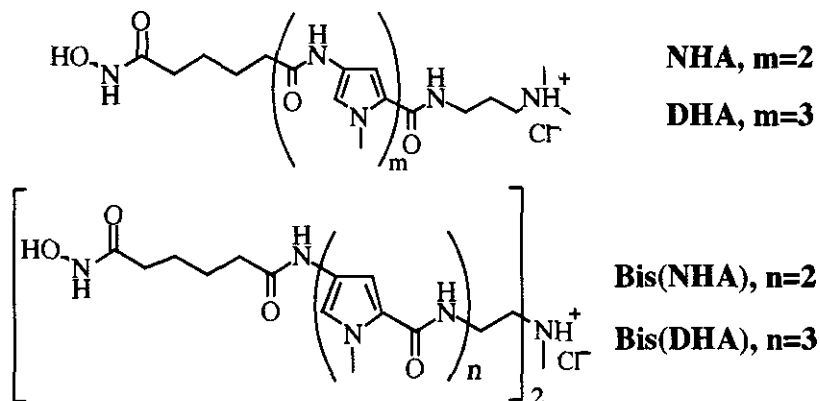
It has been reported that synthetic DNA-cleaving agents, which have two cleavage centers in the molecule, show more potent DNA cleavage than single-armed derivatives.[2] This strategy prompted us to design the dimeric hydroxamic acids, bis(netropsin-linked hydroxamic acid) **Bis(NHA)** and bis(distamycin-linked hydroxamic acid) **Bis(DHA)**. These molecules contain two N-methylpyrrole oligopeptide units coupled at the carboxy termini *via* an alkylamino tether with hydroxamic acid attached to both amino termini. If the two metal complexing moieties can each induce opposite strand cleavage of the duplex DNA before the oligopeptide moieties dissociate from the nicked AT contiguous site, a double strand cleavage of DNA would be also expected. We report the synthesis of **Bis(NHA)** and **Bis(DHA)**. In the presence of hydrogen peroxide, the VO^{II}—**Bis(NHA)** and **Bis(DHA)** complexes cleave more effectively the Col El plasmid DNA than the corresponding monomeric (**NHA**[3] and **DHA**, respectively) complexes at micromolar concentrations.

RESULTS AND DISCUSSION

Synthetic Chemistry. The synthesis of **Bis(NHA)** and **Bis(DHA)** are outlined in Scheme 1. Compound (**1a**) was synthesized from N-methylpyrrole following a previously described procedure.[1b] By repeating the reduction-condensation process, the tripyrrole compound (**1b**) was prepared from the dipyrrole compound (**1a**) (86% yield). The oligopyrrole carboxylic acids (**2a**) and (**2b**) (obtained in 83% and 90% yields, respectively) were connected together by N-methyl-2, 2´-diaminodiethylamine to produce the bis(netropsin) compound (**3a**) (78% yield) and bis(distamycin) compound (**3b**) (75% yield), respectively. Both **3a** and **3b** were purified by recrystallization from DMF—isopropanol. The nitro compounds (**3a**) and (**3b**) were catalytically hydrogenated to give the corresponding aromatic amines, which immediately reacted with the activated tetrakismethylene tether to give the bis(netropsin) ester (**4a**) (18% yield) and bis(distamycin) ester (**4b**) (77% yield), respectively. Finally, the ester compounds (**4a**) and (**4b**) were transformed into **Bis(NHA)** and **Bis(DHA)** in a way similar to that previously described for the transformation of the ester group to the hydroxamic acid (66% and 53% yield, respectively).[1b] Both **Bis(NHA)** and **Bis(DHA)** showed a positive Fe(III) test which is characteristic of the hydroxamic acid group.

DNA Cleavage Studies. We investigated the DNA cleavage by **Bis(NHA)** and **Bis(DHA)** in the presence of ferrous or vanadyl ion with Col El supercoiled DNA.[4] The DNA cleavage activity was estimated by monitoring the conversion of the covalently closed circular (form I) to the open circular (form II) and linear (form III) DNA. The cleavage activity of the **Bis(NHA)** and **Bis(DHA)** complexes was compared with those of the corresponding monomeric (**NHA** and **DHA**, respectively) complexes. The addition of hydrogen peroxide accelerated the cleavage efficiency of both systems.[5] In Fenton-type chemistry, hydrogen peroxide is postulated to be reduced to the hydroxyl radical by ferrous ion.[6] By the same chemistry, the hydroxyl radical is thought to be involved in the DNA cleavage by vanadyl and hydrogen peroxide.[7] Although the ferrous system is more efficient than the vanadyl one, superiority of the dimeric compounds over the monomeric ones was scarcely observed (data not shown). Figure 1 (a) shows the DNA cleavage pattern by the vanadyl system. Both the **Bis(NHA)** and **Bis(DHA)** complexes affected the DNA cleavage at micromolar concentrations. The order of the activity was found to be as follows:

Scheme 1
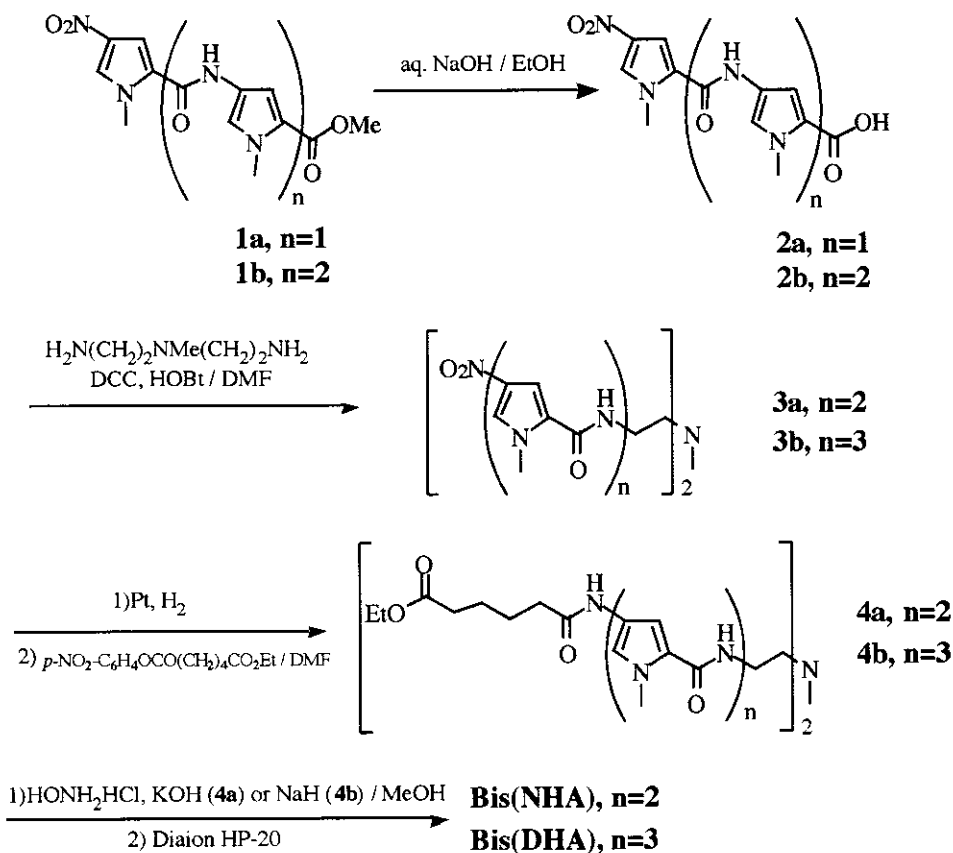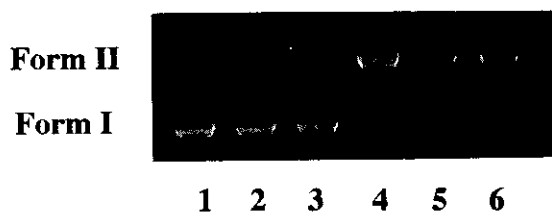
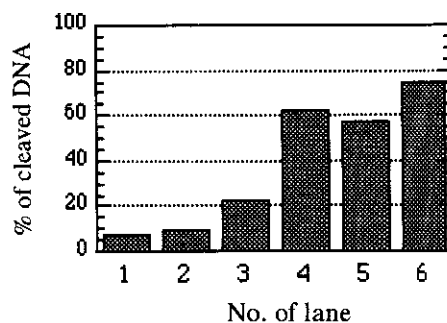Figure1. (a) Agarose gel electrophoretic patterns of vanadyl-mediated DNA cleavage by hydroxamic acid derivatives. Col E1 DNA (0.3 µg) was incubated with each hydroxamic acid (5 µM) in the presence of vanadyl ion (5 µM) and hydrogen peroxide (500 µM) in 40 mM Tris—HCl buffer (pH 8.0, total 15 µl) at 37 °C for 30 min. Lane assignment: lane 1, DNA control; lane 2, vanadyl control; lane 3, **NHA**; lane 4, **Bis(NHA)**; lane 5, **DHA**; lane 6, **Bis(DHA)**. Except for lane 1, all reactions contain vanadyl and hydrogen peroxide. (b) Densitometric results of the gel electrophoretic patterns.

Bis(DHA)>Bis(NHA)>DHA>NHA. The most effective DNA cleavage was brought about by Bis(DHA) and the lowest one by NHA. These observations are interpreted in terms of the number of recognition sites and cleaving centers.

In order to discover the stoichiometry of the hydroxamic acid-vanadyl interaction responsible for the DNA cleavage, the dependence of the cleavage activity on the increasing concentration of the hydroxamic acids was studied, while the vanadyl concentration remained constant; the results from this study are shown in Figure 2 (a). Although the activities of the monomeric complexes continued to increase up to a molar ratio of 1.0, those of the dimeric ones reached a maximum at 0.5. This result suggests that the 1:1 complex, in which one hydroxamic acid coordinates to vanadyl, is the active species. Similarly, the dependence on the increasing concentration of vanadyl was studied, while the hydroxamic acid concentrations remained constant. As can be seen from Figure 2 (b), the activities of the dimeric complexes almost leveled off at a molar ratio of 2.0, whereas those of the monomeric ones leveled off at 1.0. A control experiment verified that vanadyl itself showed no activity in the presence of hydrogen peroxide (at a concentration of 10 μM, form II DNA production was less than 5%). These results also suggest the formation of 1:1 vanadyl-hydroxamate complexes as the active species. Contrary to expectation, a small amount of double-strand cleavage was observed for the cleavage by the Bis(DHA) complex throughout the cleavage experiments (maximum form III DNA production was 7.8%). We speculate that the distamycin portions of this complex dissociate from the nicked-binding sites after nick formation due to the conformational change in the DNA minor groove. Alternatively, both distamycin portions may not simultaneously bind DNA in a bidentate fashion and thus monodentate binding may be taking place.[8] We are currently performing a DNA binding analysis of the dimeric complexes to elucidate the mode of interaction with DNA.

CONCLUSIONS
Novel DNA binding-hydroxamic acid derivatives (Bis(NHA) and Bis(DHA)) bearing two hydroxamate groups per molecule have been prepared and their DNA cleavage activities have been compared to monomeric compounds (NHA and DHA) in the presence of vanadyl and hydrogen peroxide. Among the derivatives, Bis(DHA) exhibited the most potent DNA cleavage activity. The most active vanadyl to dimeric compound molar ratio has been found to be 2 whereas that of the monomeric ones was found to be 1. Two hydroxamate complexing moieties of the dimeric compounds seem to be acting independently during DNA cleavage.

EXPERIMENTAL SECTION
Synthesis. The evaporation of solvents was done with 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 Yanaco micro melting-point apparatus and are uncorrected. The IR spectra were obtained as 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 taken on a JEOL JNM-EX 400 (400 MHz) spectrometer using TMS as the internal reference. Chemical shifts are expressed in ppm and J values were recorded in Hz. The low-resolution and high-
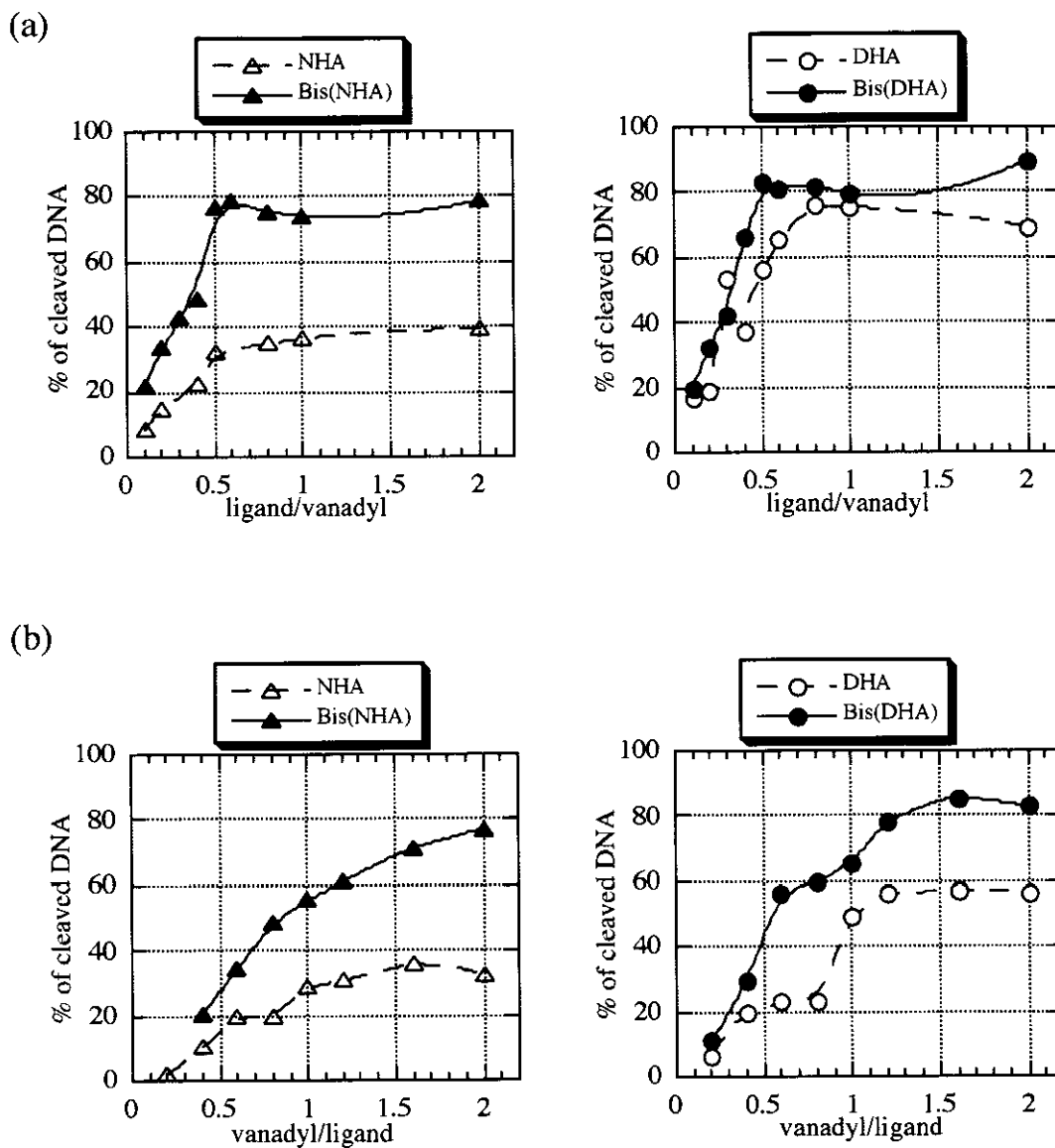
(a)

(b)

Figure 2. (a) The dependence of cleavage activities on the increasing concentration of hydroxamic acids to vanadyl ion. Col E1 DNA was incubated with increasing concentrations of the hydroxamic acids in the presence of a constant concentration (10 μM) of vanadyl ion and hydrogen peroxide (500 μM). (b) The dependence of cleavage activities on the increasing concentration of vanadyl ion to hydroxamic acids. Col E1 DNA was incubated at a constant concentration (5 μM) of hydroxamic acids in the presence of increasing concentrations of vanadyl ion and hydrogen peroxide (500 μM).

resolution FAB MS spectra were determined on a JEOL JMS-SX102A. Elemental analyses were carried out at Ibaraki Enviroment Research Center, Inc. using a Perkin-Elmer 2400 II 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 10% MeOH—CHCl$_3$; system B, 1-BuOH/AcOH/H$_2$O with 4:1:2 ratio. Silica gel column chromatography was carried out using a Fuji Silysia Chemical BW-127ZH. Fast-flow liquid chromatography was carried out on a Yamazen YFLC-540 system using a silica gel column. Polystyrene resin DIAION HP 20 was purchased from the Mitsubishi Chemical Co. The Col El plasmid DNA was ethanol-precipitated to remove EDTA contained in the solution prior to the reaction. Prior to the cleavage reaction, 30% (wt. %) hydrogen peroxide was diluted with deionized H$_2$O to 500 µM solution.

N-Methyl-2,2′-bis{[[[4-[[[4-nitro-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino] }diethylamine (3a). To an ice-cooled solution of compound (2a) (5.0 g, 17 mmol), N-methyl-2, 2′-diaminodiethylamine (0.95 g, 8.2 mmol) and HOBt (3.7 g, 24 mmol) in DMF (40 mL) DCC (3.7 g, 18 mmol) was added dropwise. The reaction mixture was allowed to warm to rt and stirred overnight. The formed precipitate of dicyclohexylurea was removed by filtration and the filtrate was evaporated *in vacuo*. The resulting residue was dissolved in AcOEt. The organic phase was washed with two portions of 5% aqueous K$_2$CO$_3$ and dried over anhydrous Na$_2$SO$_4$. After removal of the solvent, the residue was recrystallized from DMF—isopropanol to give pure **3a** (4.5 g, 78%); yellow microcrystals; mp 162—168 °C; R$_F$=0.33 (system A); ν$_{max}$(KBr)/cm^{-1} 3400, 3140, 2950, 2800, 1665, 1565, 1530; δ (DMSO-d_6) 2.29 (3H, s), 3.28—3.30 (4H, m), 3.80, 3.81 (6H, s), 3.95, 3.96 (6H, s), 6.83 (2H, s), 7.21 (2H, s), 7.57 (2H, s), 7.96 (2H, s), 8.16 (2H, s), 10.22 (2H, s); m/z (FAB) 666 (MH$^+$, 1.8%), 289 ((O$_2$N(pyrroleCONH)$_2$—H)$^+$, 4.4), 246 ((O$_2$NpyrroleCONHpyrrole—H)$^+$, 17), 154 ((O$_2$NpyrroleCO+H)$^+$, 79) (Found: C, 49.95; H, 5.94; N, 21.72. C$_{29}$H$_{35}$N$_{11}$O$_8$·2H$_2$O requires C, 49.64; H, 5.56; N, 21.97%).

N-Methyl-2,2′-bis{[[[4-[[[4-[[[4-nitro-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]}diethylamine (3b). By a similar procedure to that described for compound (3a), compound (3b) was prepared from tripyrrole-carboxylic acid (3.0 g, 8.0 mmol), N-methyl-2, 2-diaminodiethylamine (0.44 g, 3.8 mmol), HOBt (1.7 g, 11 mmol) in DMF (50 mL), and DCC (1.7 g, 8.3 mmol). Yield 2.4 g (75%); light-brown microcrystals; mp 194—198 °C; R$_F$=0.25 (system A); ν$_{max}$(KBr)/cm^{-1} 3350, 3120, 2950, 1635, 1575, 1530; δ (DMSO-d_6) 2.29 (3H, s), 3.28—3.30 (4H, m), 3.81, 3.86, 3.97 (6H×3, s), 6.85 (2H, s), 7.04 (2H, s), 7.20 (2H, s), 7.27 (2H, s), 7.60 (2H, s), 7.92 (2H, br s), 8.18 (2H, s), 9.94 (2H, s), 10.29 (2H, s); m/z (FAB) 910 (MH$^+$, 19%), 483 ((O$_2$N(pyrroleCONH)$_3$(CH$_2$)$_2$N(CH$_3$)CH$_2$)$^+$, 11), 440 ((O$_2$N(pyrroleCONH)$_3$(CH$_2$)$_2$)$^+$, 14), 275 ((O$_2$NpyrroleCONHpyrroleCO)$^+$, 23), 154 ((O$_2$NpyrroleCO+H)$^+$, 100) (Found: C, 51.52; H, 6.00; N, 21.50. C$_{41}$H$_{47}$N$_{15}$O$_{10}$·3H$_2$O requires C, 51.20; H, 5.52; N, 21.85%).

N-Methyl-2,2′-bis{[[[4-[[[4-[5-(ethoxycarbonyl)valeryl]amino]-1-methyl-2-pyrrolyl]-carbonyl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]}diethylamine (4a). A solution of compound (3a) (0.50 g, 0.63 mmol) in MeOH (8.0 mL) was hydrogenated over PtO$_2$ (60 mg) at rt 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, ethyl 5-[(*p*-nitrophenoxy)carbonyl]valerate (0.47 g, 1.6 mmol) in dry DMF (3.0 mL) was added. The reaction mixture was stirred at 0 °C for 1 h and at rt 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$_2$CO$_3$ and dried over anhydrous Na$_2$SO$_4$. After removal of the solvent, the residue was purified by silica gel fast flow liquid chromatography. Elution with 0.38% concentrated aqueous ammonia in 2% MeOH—CHCl$_3$ provided pure **4a** (0.12 g, 18%); light-brown microcrystals; mp 103—106 °C; R$_F$=0.35 (system A); ν$_{max}$(KBr)/cm^{-1} 3300, 2950, 1725, 1640, 1575, 1520; δ (CDCl$_3$) 1.24 (6H, t, J=7.0), 1.68 (8H, br s), 2.32—2.35 (12H, m), 2.60 (3H, br s), 3.41 (4H, br s), 3.80 (12H, s), 4.10 (4H, q, J=7.0), 6.50 (2H, s), 6.61 (2H, s), 6.75 (2H, br s), 7.01 (2H, s), 7.15 (2H, s), 7.27 (1H, s), 8.31 (1H, br s), 8.52 (2H, br s); δ (DMSO-d_6) 1.17 (6H, t, J=6.8), 1.55 (8H, br s), 2.23 (4H, t, J=6.8), 2.29—2.32 (7H, m), 3.79, 3.81 (12H, s), 4.04 (4H, q, J=6.8), 6.83 (2H, d, J=1.6), 6.85 (2H, d, J=1.6), 7.15 (2H, d, J=2.0), 7.18 (2H, d, J=1.2), 7.93 (2H, br s), 9.78 (2H, s), 9.85 (2H, s); m/z (FAB) 918 (MH$^+$, 41%), 640 (MH$^+$+1—EtO$_2$C(CH$_2$)$_4$CONHpyrroleCO, 3.1), 279 ((EtO$_2$C(CH$_2$)$_4$CONHpyrroleCO)$^+$, 65), 444 ((EtO$_2$C(CH$_2$)$_4$CONH(pyrroleCONH)$_2$(CH$_2$)$_2$)$^+$, 38).

***N*-Methyl-2,2′-bis{[[[4-[[[4-[[4-[[5-(ethoxycarbonyl)valeryl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-2-pyrrolyl]-carbonyl]amino]}diethylamine (4b).** A solution of compound (**3b**) (0.84 g, 1.0 mmol) in MeOH—dioxane (each 15 mL) was hydrogenated over PtO$_2$ (0.11 g) and the corresponding aromatic amine was reacted with ethyl 5-[(*p*-nitrophenoxy)carbonyl]valerate (0.63 g, 2.1 mmol) in dry DMF (5.0 mL) as described in the procedure for compound (**4a**). Purification by silica gel fast flow liquid chromatography with elution of 0.38% concentrated aqueous ammonia in 10% MeOH—CHCl$_3$ provided pure **4b** (0.84 g, 77%); yellow microcrystals; mp 186—193 °C; R$_F$=0.27 (system A); ν$_{max}$(KBr)/cm^{-1} 3280, 3120, 2930, 1725, 1650, 1580, 1520; δ (DMSO-d_6) 1.18 (6H, t, J=7.0), 1.57 (8H, br s), 2.23—2.26 (6H, m), 2.29—2.32 (6H, m), 3.81, 3.82, 3.83, 3.85 (18H, s), 4.05 (4H, q, J=7.2), 6.86 (2H, d, J=1.2), 6.87 (2H, d, J=1.6), 7.03 (2H, s), 7.16 (2H, d, J=1.2), 7.19 (2H, d, J=1.6), 7.23 (2H, d, J=1.6), 7.92 (2H, br s), 9.79 (2H, s), 9.89 (4H, s); δ (CD$_3$OD) 1.23 (6H, t, J=7.2), 1.67—1.68 (8H, br s), 2.31—2.37 (10H, m), 2.51 (2H, s), 2.78 (3H, br s), 3.47 (4H, t, J=6.0), 3.77 (6H, s), 3.85, 3.86, 3.88 (12H, s), 4.11 (4H, q, J=7.2), 6.74 (2H, d, J=2.0), 6.81 (2H, d, J=2.0), 6.87 (2H, d, J=2.0), 7.10 (4H, d, J=2.0), 7.12 (2H, d, J=1.6); m/z (FAB) 1162 (MH$^+$, 20%), 609 (M$^+$—EtO$_2$C(CH$_2$)$_4$CONH(pyrroleCONH)$_3$(CH$_2$)$_2$N(CH$_3$), 29), 279 ((EtO$_2$C(CH$_2$)$_4$CONHpyrroleCO)$^+$, 74).

***N*-Methyl-2,2′-bis{[[[4-[[[4-[[5-(hydroxyaminocarbonyl)valeryl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]}diethylamine Bis(NHA).** Separate solutions of hydroxylamine hydrochloride (0.18 g, 2.6 mmol) in MeOH (5.0 mL), KOH (0.32 g, 5.8 mmol) in MeOH (6.0 mL) and compound (**4a**) (0.12 g, 0.13 mmol) in MeOH (5.0 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 (**4a**) *via* a syringe, and the reaction mixture was then stirred overnight at rt under an argon atmosphere. The reaction was terminated by the addition of 2.0 M aqueous HCl (1.6 mL, 3.2 mmol) to the mixture which was 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

after which the filtrate was evaporated. The residue dissolved in H_2O was applied to a HP 20 column, which was successively washed with deionized H_2O and 20% MeOH–H_2O. Elution with 50% MeOH–H_2O followed by evaporation of the appropriate fractions provided pure **Bis(NHA)** (0.076 g, 66%); light-brown microcrystals; mp 159—164 °C; R_F=0.063 (system B); v_{max}(KBr)/cm^{-1} 3250, 2950, 1635, 1580, 1530; λ_{max}(H_2O)/nm 302 (log ε=4.62); δ (DMSO-d_6) 1.52 (8H, br s), 1.97 (4H, t, J=6.8), 2.22 (4H, t, J=6.6), 2.28 (3H, s), 3.25—3.29 (4H, m), 3.80, 3.82 (12H, s), 6.82 (2H, d, J=1.2), 6.85 (2H, d, J=2.0), 7.15 (2H, d, J=1.6), 7.18 (2H, d, J=1.6), 7.90 (2H, t, J=5.4), 8.68 (1H, br s), 9.77 (2H, s), 9.85 (2H, s), 10.36 (1H, br s); δ (CD$_3$OD) 1.67 (8H, br s), 2.13 (4H, t, J=7.5), 2.33 (4H, t, J=8.0), 3.12 (4H, s), 3.58 (3H, s), 3.66 (8H, s), 3.71—3.78 (2H, m), 3.86 (6H, s), 6.82 (2H, s), 6.89 (2H, s), 7.12 (4H, t, J=2.2); m/z (FAB) 892 (MH$^+$—Cl, 4.8%), 431 ((HONHCO(CH$_2$)$_4$CONH(pyrroleCONH)$_2$(CH$_2$)$_2$)$^+$, 2.0), (Found: MH$^+$—Cl, 892.4433. C$_{41}$H$_{58}$N$_{13}$O$_{10}$ requires *MH*$^+$—Cl, 892.4429).

N-Methyl-2,2′-bis{[[[4-[[4-[[[4-[[5-(hydroxyaminocarbonyl)valeryl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-2-pyrrolyl] carbonyl]amino]}diethylamine **Bis(DHA)**. Separate solutions of hydroxylamine hydrochloride (0.18 g, 2.6 mmol) in MeOH (2.0 mL), 66% (wt. %) NaH (0.21 g, 5.6 mmol) in MeOH (2.0 mL) and compound (**4b**) (0.19 g, 0.17 mmol) in MeOH (3.0 mL) were prepared and reacted as described in the procedure for **Bis(NHA)**. Purification by HP 20 polystyrene resin with elution of 70% MeOH—H_2O provided pure **Bis(DHA)** (0.10 g, 53%); pale-yellow microcrystals; mp 156—162 °C; R_F=0.047 (system B); v_{max}(KBr)/cm^{-1} 3250, 2930, 1640, 1575, 1520; λ_{max}(H_2O)/nm 310 (log ε=4.88); δ(DMSO-d_6) 1.53 (8H, br s), 1.97 (4H, t, J=6.8), 2.22 (4H, t, J=6.8), 3.80, 3.83, 3.85 (18H, s), 6.87 (4H, d, J=1.2), 7.04 (2H, d, J=1.6), 7.15 (2H, d, J=1.2), 7.19 (2H, d, J=1.6), 7.23 (2H, d, J=1.6), 7.95 (2H, br s), 8.68 (2H, s), 9.78 (2H, s), 9.90 (4H, s), 10.37 (2H, s); δ (pyridine-d_5) 1.87 (8H, s), 2.18 (3H, s), 2.35, 2.40 (4H×2, s), 2.57 (4H, t, J=6.0), 3.54, 3.55 (4H, s), 3.95 (18H, s); m/z (FAB) 1136 (MH$^+$—Cl, 13%), 596 (M$^+$—HONHCO(CH$_2$)$_4$CONH(pyrroleCONH)$_3$CH$_2$, 11),

553 ((HONHCO(CH$_2$)$_4$CONH(pyrroleCONH)$_3$(CH$_2$)$_2$)$^+$, 3.7); (Found: MH$^+$—Cl, 1136.5366. C$_{53}$H$_{70}$N$_{17}$O$_{12}$ requires *MH*$^+$—Cl, 1136.5389).

Plasmid DNA Cleavage. A standard reaction mixture (15 μL total volume) contained 0.3 μg of supercoiled Col E1 DNA, 40 mM Tris—HCl (pH 8.0), a varying concentration of hydroxamic acids (2.0% maximum final DMF concentration) and vanadyl ion. The reaction was started by the addition of hydrogen peroxide as the last component after which the mixture was kept at 37 °C for 30 min. The reaction was terminated by the addition of a solution (8 μL) of 50% glycerol/25 mM EDTA/0.025% bromophenol blue. The resulting sample was applied to 0.9% agarose gel containing ethidium bromide (0.5 μg/mL) and electrophoresed at 100 V for 1.2 h in TAE (40 mM Tris—HCl/5 mM sodium acetate/1 mM EDTA, pH 8.0). The gels were destained for 10 min in deionized water prior to being photographed with Polaroid film under UV light. The relative amounts of the form I and form II of DNA were determined using a Shimadzu dual-wavelength flying spot scanner CS-9000. The area under the form I DNA was multiplied by a factor of 1.07 to correct for the reduced binding of ethidium bromide by the form I DNA. Intact Col E1 plasmid DNA was on the average 95% form I and 5% form II DNA. The percent of cleaved DNA described in the present paper reflects the averages of the results of four (cleavage by monomeric hydroxamic acids) or six

(cleavage by dimeric hydroxamic acids) runs.

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3.   Spectroscopic data of **NHA**:4-[[[4-[[5-(hydroxyaminocarbonyl)valeryl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-*N*-[3-(*N*, *N*-dimethylamino)propyl]-2-pyrrolecarboxamide (brown solid); TLC (silica gel, 1-BuOH/AcOH/H$_2$O=4:1:2) R_F=0.42; mp 99—103 °C; IR (KBr disc) 3250, 2920, 1650, 1635, 1575 and 1535 cm^{-1}; UV (H$_2$O) 241 nm (log ε=4.31), 298 nm (log ε=4.34); ^{1}H-NMR (400 MHz, DMSO-*d$_6$*) δ 1.53 (4H, s), 1.62 (2H, m), 1.97 (2H, t, J=6.8), 2.16 (6H, s), 2.22 (2H, t, J=6.8), 2.29 (2H, t, J=7.2), 3.19 (2H, t, J=6.8), 3.80, 3.82 (3H×2, s), 6.81 (1H, d, J=1.6), 6.85 (1H, d, J=1.2), 7.15 (1H, d, J=1.6), 7.17 (1H, d, J=1.6), 8.07 (1H, d, J=5.6), 9.78 (1H, s), 9.84 (1H, s) and 10.37 (1H, br s); MS (FAB) m/z 490 (MH$^+$—Cl, 67%); HRMS (FAB) m/z 490.2762 (C$_{23}$H$_{36}$N$_7$O$_5$ requires 490.2775).

4.   No DNA cleavage was observed for the lanthanoide dimeric complexes, including the cerium (III) complex.

5.   The reaction was also accelerated by the addition of DTT, which was less effective than hydrogen peroxide.

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