SYNTHESIS OF BISNETROPSIN-LINKED HYDROXAMIC ACIDS AND THEIR DNA CLEAVAGE STUDY IN THE PRESENCE OF TRANSITION OR LANTHANIDE METAL IONS

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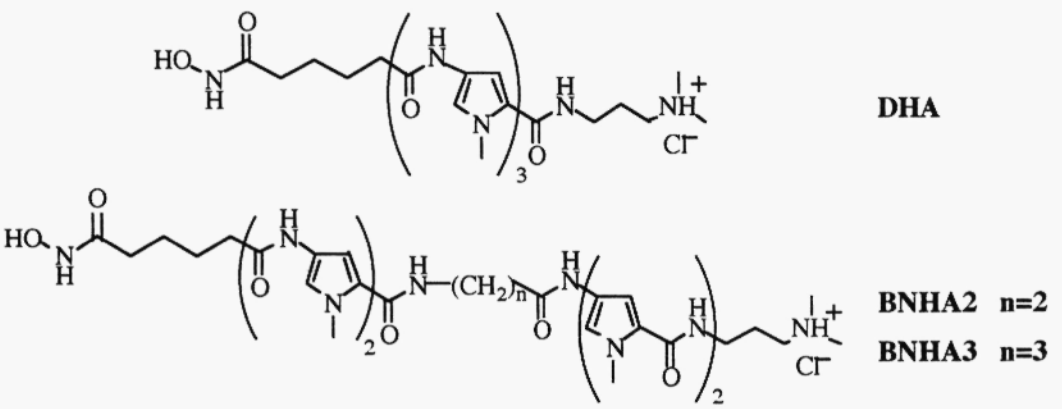
Abstracts

A novel class of hydroxamic acids (**BNHA2** and **BNHA3**) with two netropsin units connected by a polymethylene tether have been synthesized. Plasmid DNA cleavage assay have indicated that **BNHA2** induced effective DNA cleavage in the presence of Fe(II) and Ce(III) ions, but **BNHA3** exhibited significant cleavage only in the former case.

Introduction

The development of small synthetic molecules which promote hydrolytic cleavage of DNA is recently an area of intensive investigation.[1] In an effort to construct an efficient hydrolytic system, the lanthanide cation and its complexes have been increasingly employed because of their high catalytic activity.[2]

Hydroxamic acid is a bidentate oxygen ligand which strongly chelates with lanthanide cations and may be easily tethered to the known DNA binding molecules. Our previous studies have indicated that the intercalator-linked hydroxamic acid-lanthanide system can effectively cleave the double-stranded DNA.[3] Generally, DNA-cleaving metal complexes with an intercalation moiety induce non-sequence specific DNA cleavage. Conjugation of the hydroxamic acid-lanthanide system with a sequence specific DNA binding agent might lead to an artificial restriction enzyme model.



Distamycin and netropsin are naturally occurring agents that have been shown to bind in the minor groove of AT rich sequences. We have recently prepared distamycin linked-hydroxamic acid (**DHA**), and its Ce(III) complex was shown to induce effective plasmid DNA cleavage.[4, 5] Although this complex was expected to cleave nucleotide sequences adjacent to the five base pair AT sites, it exhibited a very low sequence-specific DNA cleavage pattern.[6] DNA binding analysis also revealed that it has dramatically reduced AT preference compared to natural distamycin.

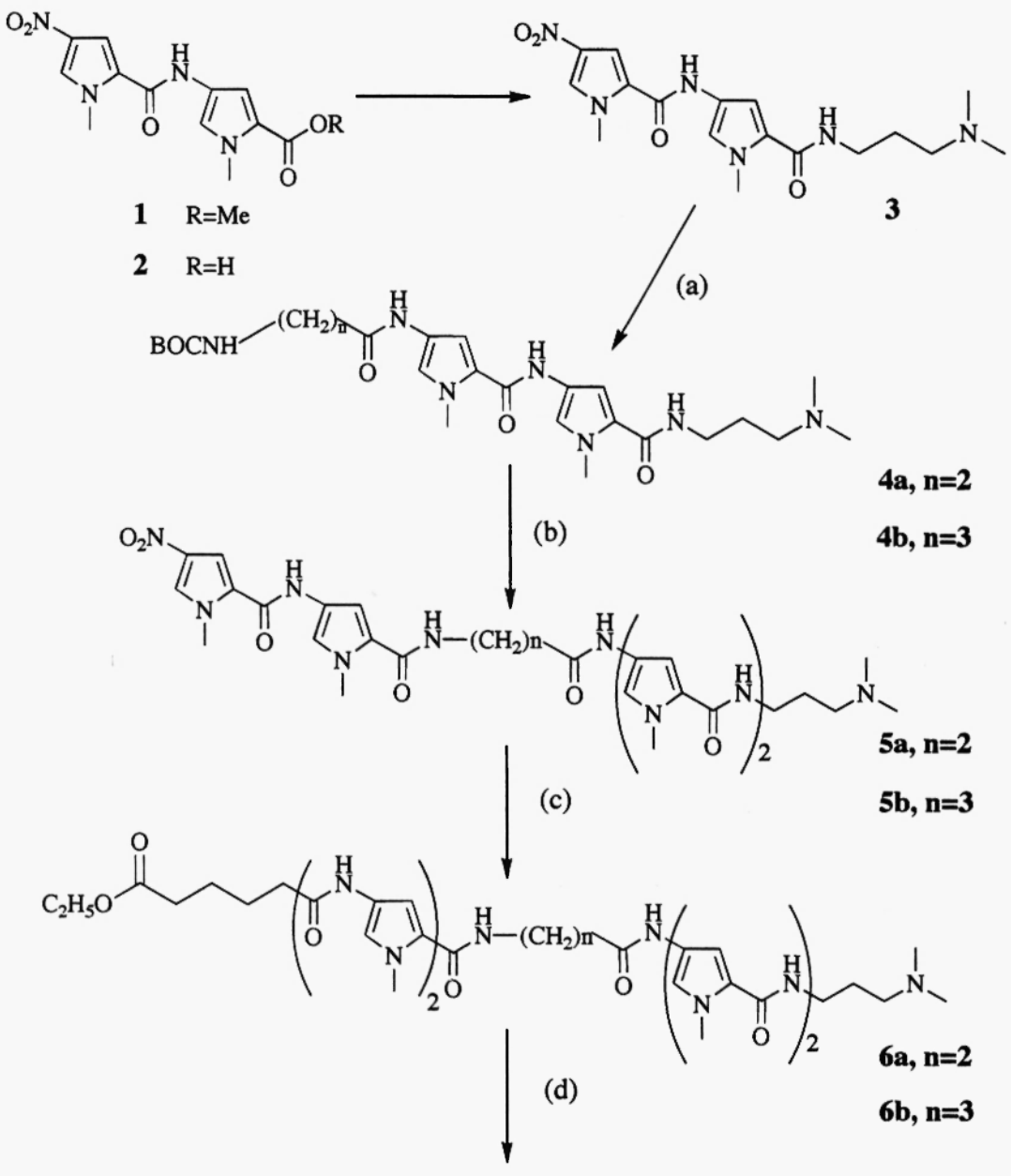
As one approach for the improvement of the DNA cleavage specificity of the *N*-methylpyrroleoligopeptide-type hydroxamic acid–lanthanide complex, bisnetropsin-linked hydroxamic acids (**BNHA**), which possess a greater number of hydrogen bonding sites than **DHA**, were newly designed. Two netropsin units were connected by the β-alanine or γ-aminobutyrate group to explore simultaneous binding of the units to contiguous AT regions in the minor groove of DNA.[7] The binding domain and hydroxamic acid was connected by a tetrakismethylene tether, because this linkage offered maximum DNA cleavage activity by the **DHA**–Ce(III) system.[5] In this paper, we present the synthesis of bisnetropsin-linked hydroxamic acids and their preliminary DNA cleavage studies in the presence of Fe(II) and Ce(III) ions.

Results and Discussion

Syntheses of BNHA. The syntheses of **BNHA2** and **BNHA3** are outlined in Scheme 1. Compound **1** was previously synthesized by Bialer and co-workers.[8] We employed their route for the preparation of this compound, but used a different precursor, 1-methyl-4-nitro-2-trichloroacetylpyrrole, which was prepared from *N*-methylpyrrole according to the method of Shibuya.[9] The overall yield from the *N*-methylpyrrole to compound **1** was 24% in 4 steps. The dipyrrole carboxylic acid **2** (obtained in 83% yield by saponification of **1**) was coupled with 3-dimethylaminopropylamine using the DCC–HOBt method to give the known compound **3** in 64% yield. Catalytic reduction of the nitro group of **3** gave the corresponding amine, which was immediately reacted with the activated Boc-protected alanine or aminobutyric acid to give Boc-protected netropsin **4a** (35% yield) and **4b** (20% yield), respectively. The Boc protective group of **4a** and **4b** was removed by the treatment with trifluoroacetic acid (TFA) and coupling of the amine components with the acid moiety **2** was facilitated by the DCC–HOBt method to give the bisnetropsin **5a** (40% yield) and **5b** (50% yield), respectively. The nitro group of **5a** and **5b** was reduced by catalytic hydrogenation and the resulting amine was immediately coupled with the activated tetrakismethylene tether to produce the bisnetropsin ester **6a** (34% yield) and **6b** (40% yield), respectively. The ester groups of **6a** and **6b** were transformed into the hydroxamic acids according to a published procedure.[3] The final purification was achieved by chromatography on HP 20. Elution with 70% MeOH–H_2O followed by evaporation of the appropriate fractions gave the hydroxamic acid **BNHA2** (59% yield) and **BNHA3** (33% yield). Both **BNHA2** and **BNHA3** showed a positive Fe(III) test which is characteristic of the hydroxamic acid group.

DNA Cleavage Studies. The ability of *N*-methylpyrrole oligopeptide-linked hydroxamic acids to induce DNA cleavage in the presence of metal ions was examined by monitoring the conversion of Col El supercoiled DNA (form I) to open circular DNA (form II). Previous experiments have indicated that **DHA** induced effective plasmid DNA cleavage in the presence of Fe(II) and Ce(III) ions,[5] and we compared the DNA cleavage activity of the **BNHA** complex with that of the **DHA** complex. As shown in Figure 1, both **BNHA2** and **BNHA3** were effective for DNA cleavage in the presence of Fe(II) ion and their activities were almost comparable to the activity

Scheme 1



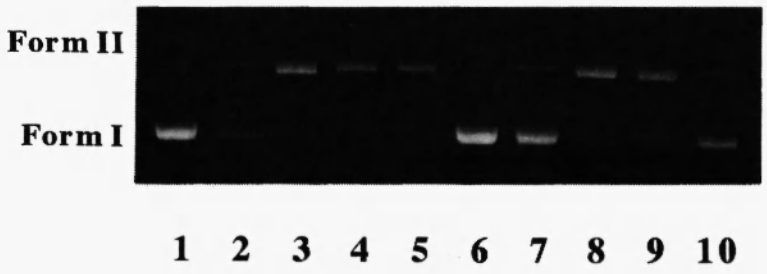
(a) 1)Pt, H_2; 2) *p*-NO_2-$C_6H_4OCO(CH_2)_nNHBoc$, DMF

(b) 1) 35% TFA-CH_2Cl_2; 2) Diaion HP-20; 3) compound **2**, DCC, DMF

(c) 1) Pt, H_2; 2) *p*-NO_2-$C_6H_4OCO(CH_2)_4CO_2Et$, DMF

(d) 1)$HONH_2HCl$, KOH, MeOH; 2) Diaion HP-20

(a)



(b)

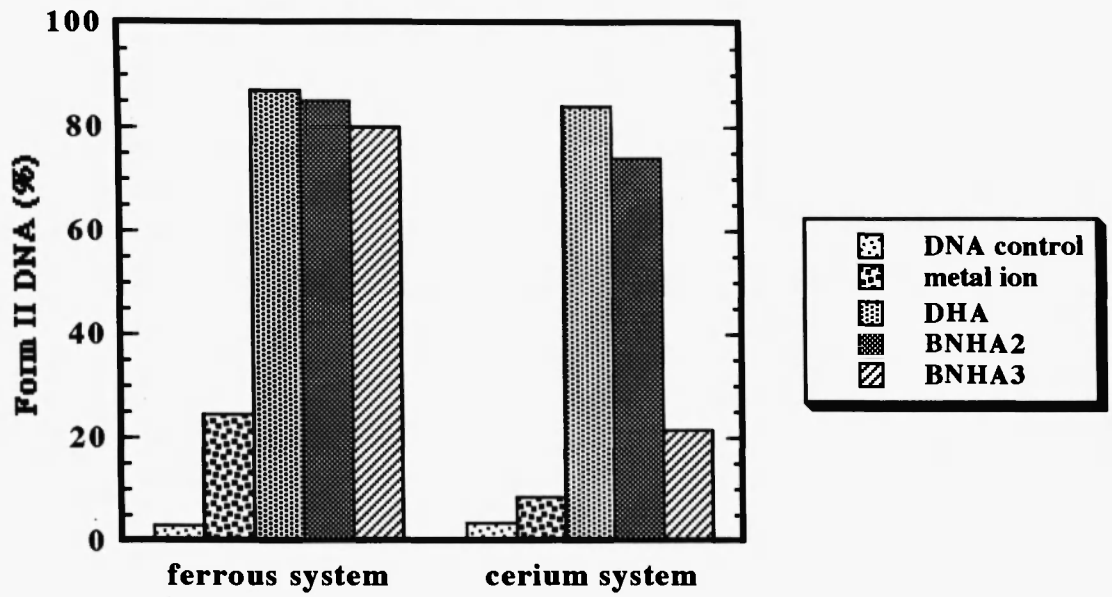


Figure 1. (a) Agarose gel electrophoretic patterns of Col E1 DNA cleavage by *N*-methylpyrrole oligopeptide-linked hydroxamic acids in the presence of Fe(II) or Ce(III) ions. Col E1 DNA (0.6 μg) was incubated with hydroxamic acid derivatives in the presence of metal ions in 40 mM Tris–HCl buffer (pH 8.0, total volume 30 μl) at 37 °C for 1.0 h (ferrous-mediated cleavage) or 3.0 h (cerium-mediated cleavage). After the reaction, the DNA samples were extracted with a mixture of phenol–chloroform to remove the DNA-bound hydroxamic acid–metal complexes and recovered by ethanol precipitation. Lane assignment: lane 1, DNA control; lane 2, 10 μM $FeSO_4$; lane 3, 10 μM $FeSO_4$+10 μM **DHA**; lane 4, 10 μM $FeSO_4$+10 μM **BNHA2**; lane 5, 10 μM $FeSO_4$+10 μM **BNHA3**; lane 6, DNA control; lane 7, 30 μM $CeCl_3$; lane 8, 30 μM $CeCl_3$+30 μM **DHA**; lane 9, 30 μM $CeCl_3$+30 μM **BNHA2**; lane 10, 30 μM $CeCl_3$+30 μM **BNHA3**. (b) Densitometric result of the gel electrophoretic patterns.

of the **DHA**–Fe(II) complex. The **BNHA2**–Ce(III) complex induced a slightly reduced DNA cleavage, while the **BNHA3** complex did not bring about significant cleavage as compared to the **DHA** complex. Only the Ce(III) ion was effective among the lanthanide cations tested, consistent with the cleavage by the **DHA**–Ce(III) system.[5] Control experiments verified that hydroxamic acid derivatives alone showed no DNA cleavage (data not shown). It has been shown that the hydroxamic acid–lanthanide system involves the metal-ligated hydroxide as the active species in the hydrolytic chemistry, whereas the transition system would require reactive oxygen species such as a hydroxyl radical. Hence, the **BNHA3**–Ce(III) complex is suggested not to correctly bring the hydroxide close to the phosphorus atom of DNA and the mode of interaction with DNA might be different from the corresponding **BNHA2** complex. A possible explanation for the reduced cleavage activity of the **BNHA2**–Ce(III) complex as compared with the **DHA** one may be fewer available cleavage sites by recognizing the longer AT rich sequences of DNA.[10] We are currently undertaking an end-labeled fragment cleavage study of **BNHA**–metal complexes in order to gain information about the sequence specificity of the DNA cleavage.

Conclusions

Novel hydroxamic acids containing two *N*-methylpyrrole dipeptide units coupled by a β-alanine (**BNHA2**) or γ-aminobutyrate (**BNHA3**) group were successfully synthesized. Both **BNHA2** and **BNHA3** were as effective as **DHA** in the ferrous-mediated DNA cleavage. In contrast, the cleavage activity increased in the order **DHA** > **BNHA2** > **BNHA3** in the cerium-mediated cleavage.

Experimental

General. 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 on 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-resolution FAB mass spectra were determined on a JEOL JMS-SX102A. TLC was performed on precoated aluminum sheets of silica gel $60F_{254}$ (Merck, No. 5554). TLC systems were as follows: system A, 0.49% concentrated aqueous ammonia in 33% MeOH–$CHCl_3$; system B, 0.49% concentrated aqueous ammonia in 33% MeOH–CH_2Cl_2; system C, 1-BuOH/AcOH/H_2O with 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. The Col E1 plasmid DNA was ethanol-precipitated to remove EDTA contained in the solution prior to the reaction.

4-[[[4-[[4-[*N*-(*tert*-butoxycarbonyl)amino]propionyl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-*N*-[3-(*N*, *N*-dimethylamino)propyl]-2-pyrrolecarboxamide 4a. A solution of compound **3** (1.0 g, 2.6 mmol) in MeOH (12 ml) was hydrogenated over PtO_2 (20 mg) 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 to completely remove the MeOH. After the residue was cooled to 0 °C, *p*-nitrophenyl 3-[*N*-(*tert*-

butoxycarbonyl)amino]propionate (0.65 g, 2.1 mmol) in dry DMF (5.0 ml) was added. The reaction mixture was stirred at 0 °C for 1 hr and at room temperature overnight. DMF was evaporated in vacuo, and the resulting residue was dissolved in AcOEt. The organic phase was washed with 5% aqueous K_2CO_3 and dried over anhydrous Na_2SO_4. After removal of the solvent, chromatography of the residue was done on a silica gel column (0.33% concentrated aqueous ammonia in 50% MeOH–$CHCl_3$) to provide pure **4a** (0.48 g, 35%); R_F=0.29 (system A); δ (DMSO-d_6) 1.38 (9H, s), 1.61 (2H, m), 2.14 (6H, s), 2.25 (2H, t, J=6.8), 2.40 (2H, t, J=7.2), 3.18–3.20 (4H, m), 3.80, 3.82 (3H×2, s), 6.81 (2H, d, J=1.2), 6.86 (1H, d, J=1.2), 7.16 (1H, s), 7.17 (1H, d, J=1.6), 8.06 (1H, t, J=6.6), 9.85 (1H, s), 9.86 (1H, s); m/z (FAB) 518 (MH^+, 100%), 418 (MH^++1–$CO_2C(CH_3)_3$, 17), 58 ($(CH_2N(CH_3)_2)^+$, 66).

4-[[[4-[[4-[*N*-(*tert*-butoxycarbonyl)amino]butyryl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-*N*-[3-(*N*, *N*-dimethylamino)propyl]-2-pyrrolecarboxamide 4b. This compound was prepared using a similar procedure to that described for compound **4a** in 20% yield; R_F=0.28 (system A); δ (DMSO-d_6) 1.38 (9H, s), 1.61 (2H, m), 1.67 (2H, m), 2.15 (6H, s), 2.20–2.27 (4H, m), 2.94 (2H, t, J=6.4), 3.18 (2H, t, J=5.6), 3.81, 3.82 (3H×2, s), 6.81 (2H, s), 6.85 (1H, s), 7.14 (1H, s), 7.17 (1H, s), 8.06 (1H, t, J=5.6), 9.78 (1H, s), 9.84 (1H, s); m/z (FAB) 532 (MH^+, 100%), 432 (MH^++1–$CO_2C(CH_3)_3$, 15), 245 ($(pyrroleCONH)_2^+$, 18), 129 ($(CONH(CH_2)_3N(CH_3)_2)^+$, 32), 58 ($(CH_2N(CH_3)_2)^+$, 67).

4-[[[4-[[4-[[[4-[[[4-nitro-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]propionyl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-*N*-[3-(*N*, *N*-dimethylamino)propyl]-2-pyrrolecarboxamide 5a. To a solution of compound **4a** (0.70 g, 1.4 mmol) in CH_2Cl_2 (6.0 ml) was added TFA (3.0 ml). The resulting solution was stirred for 30 min at room temperature and evaporated in vacuo. The residue dissolved in H_2O was adsorbed using a HP 20 column, washed with 5% aqueous K_2CO_3, deionized H_2O and eluted with 100% MeOH to give the corresponding amine. To the residue, compound **2** (0.47 g, 1.5 mmol) and HOBt (0.31 g, 2.0 mmol) were successively added in dry DMF (5.0 ml). The mixture was cooled to 0 °C and DCC (0.30 g, 1.5 mmol) was added dropwise to the solution. The reaction mixture was stirred at room temperature 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 then washed with 5% aqueous K_2CO_3 and dried over anhydrous Na_2SO_4. After removal of the solvent, chromatography of the residue was done on a silica gel column (0.38% concentrated aqueous ammonia in 20% MeOH–$CHCl_3$) to provide pure **5a** (0.38 g, 40%); R_F=0.24 (system A); δ (DMSO-d_6) 1.66 (2H, m), 2.25 (6H, s), 2.38 (2H, t, J=6.8), 3.20 (2H, t, J=6.4), 3.46 (2H, t, J=6.0), 3.81, 3.82, 3.84 (3H×3, s), 3.96 (3H, s), 6.84 (2H, d, J=2.0), 6.87 (1H, s), 7.18 (2H, d, J=1.2), 7.23 (1H, d, J=1.6), 7.58 (1H, d, J=2.0), 8.16 (3H, m), 9.86 (1H, s), 9.92 (1H, s), 10.25 (1H, S); m/z (FAB) 692 (MH^+, 25%), 290 ($(O_2N(pyrroleCONH)_2)^+$, 30), 58 ($(CH_2N(CH_3)_2)^+$, 100).

4-[[[4-[[4-[[[4-[[[4-nitro-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]butyryl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-*N*-[3-(*N*, *N*-dimethylamino)propyl]-2-pyrrolecarboxamide 5b. This compound was prepared using a similar procedure to that described for compound **5a** in 50% yield; R_F=0.23 (system A); δ (DMSO-d_6) 1.69 (2H, m), 1.79 (2H, m), 2.27–2.31 (4H, m), 2.37 (6H, s), 3.20–3.25 (4H, m), 3.80, 3.82 (9H, s), 3.96 (3H, s), 6.85 (1H, d, J=1.6), 6.86 (1H, d, J=2.0), 6.89 (1H, d, J=2.0), 7.17 (2H, s), 7.21 (1H, d, J=1.2), 7.58 (1H, d, J=1.6), 8.10 (2H, q, J=5.6), 8.17 (1H, s), 9.84 (1H, s), 9.85 (1H, s), 10.25 (1H, S); m/z (FAB) 706 (MH^+, 5.9%), 58 ($(CH_2N(CH_3)_2)^+$, 77).

4-[[[4-[[4-[[[4-[[[4-[[5-(ethoxycarbonyl)valeryl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]propionyl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-*N*-[3-(*N*, *N*-dimethylamino)propyl]-2-pyrrolecarboxamide 6a. A suspension of compound **5a** (0.20 g, 0.29 mmol) in MeOH (8.0 ml) was hydrogenated over PtO_2 (50 mg) 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 to completely remove the MeOH. After the residue was cooled to 0 °C, ethyl 5-[(*p*-nitrophenoxy)carbonyl]valerate (0.084 g, 0.28 mmol) in dry DMF (3.0 ml) was added. The reaction mixture was stirred at 0 °C for 1 hr and at room temperature overnight. DMF was evaporated in vacuo, and the resulting residue was dissolved in AcOEt. The organic phase was washed with 5% aqueous K_2CO_3 and dried over anhydrous Na_2SO_4. After removal of the solvent, chromatography of the residue was done on a silica gel column (0.38% concentrated aqueous ammonia in 20% MeOH–CH_2Cl_2) to provide pure **6a** (0.082 g, 34%); light-yellow crystalline solid; mp 108–110.5 °C; R_F=0.22 (system B); ν_{max}(KBr)/cm^{-1} 3300, 2950, 1715, 1650, 1635; δ (DMSO-d_6) 1.17 (3H, t, J=6.8), 1.59–1.64 (6H, m), 2.15–2.18 (8H, m), 2.28–2.33 (6H, m), 3.17–3.21 (4H, m), 3.80, 3.82, 3.83, 3.84 (3H×4, s), 4.04 (2H, q, J=6.8), 6.82 (1H, d, J=1.6), 6.83 (2H, s), 6.85 (1H, d, J=2.4), 7.14 (1H, d, J=1.2), 7.17 (3H, s), 8.07 (2H, br s), 9.78 (1H, s), 9.85 (3H, s); m/z (FAB) 818 (MH^+, 14%), 58 ($(CH_2N(CH_3)_2)^+$, 100).

4-[[[4-[[4-[[[4-[[[4-[[5-(ethoxycarbonyl)valeryl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]butyryl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-*N*-[3-(*N*, *N*-dimethylamino)propyl]-2-pyrrolecarboxamide 6b. This compound was prepared using a similar procedure to that described for compound **6a** in 40% yield; yellow microcrystalline; mp 123–126 °C; R_F=0.23 (system B); ν_{max}(KBr)/cm^{-1} 3300, 2950, 1715, 1650, 1635; δ (DMSO-d_6) 1.18 (3H, t, J=6.8), 1.57–1.64 (6H, m), 1.78–1.81 (2H, m), 2.18 (6H, s), 2.22–2.32 (8H, m), 3.17–3.27 (4H, m), 3.80, 3.81, 3.82 (12H, s), 4.05 (2H, q, J=7.2), 6.82 (1H, d, J=1.6), 6.86 (2H, s), 6.89 (1H, d, J=1.6), 7.15 (1H, d, J=1.2), 7.17 (3H, s), 8.06 (2H, q, J=6.1), 9.79 (1H, s), 9.84 (1H, s), 9.85 (2H, br s); m/z (FAB) 832 (MH^+, 46%), 503 (MH^++1–(pyrroleCONH)$_2$$(CH_2)_3N(CH_3)_2$, 6.1), 123 ((CONHpyrrole+H)$^+$, 64), 58 ($(CH_2N(CH_3)_2)^+$, 100).

4-[[[4-[[4-[[[4-[[[4-[[5-(hydroxyaminocarbonyl)valeryl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]propionyl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-*N*-[3-(*N*, *N*-dimethylamino)propyl]-2-pyrrolecarboxamide BNHA2. MeOH as the solvent was degassed with argon gas to remove atmospheric oxygen prior to the reaction. Separate solutions of hydroxylamine hydrochloride (0.18 g, 2.6 mmol) in MeOH (5.0 ml), KOH (0.32 g, 5.7 mmol) in MeOH (6.0 ml) and compound **6a** (0.090 g, 0.11 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 **6a** 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 (1.6 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 adsorbed using a HP 20 column, washed with deionized H_2O, 20% MeOH–H_2O and eluted with 70% MeOH–H_2O. The appropriate fractions were combined and evaporated in vacuo to give pure **BNHA2** (0.052g, 59%); light-yellow microcrystalline; mp 160–163 °C; R_F=0.37 (system C); ν_{max}(KBr)/cm^{-1}

3250, 2950, 1650, 1640; $\lambda_{max}(H_2O)$/nm 296 (log ε=4.53); δ(DMSO-d_6) 1.53 (4H, m), 1.75 (2H, m), 1.95 (2H, t, J=6.8), 2.21 (2H, t, J=6.8), 2.70 (2H, t, J=7.2), 3.21–3.24 (4H, m), 3.80, 3.81, 3.83 (12H, s), 6.84 (1H, s), 6.85 (1H, s), 6.87 (2H, s), 7.15 (1H, s), 7.18 (3H, br s), 8.07 (1H, t, J=6.9), 8.12 (1H, t, J=7.7), 9.79 (1H, s), 9.86 (1H, s), 9.88 (1H, s), 9.92 (1H, s), 10.38 (1H, s); δ(CD_3OD) 1.67 (4H, m), 1.92 (2H, m), 2.13 (2H, t, J=6.8), 2.33 (2H, t, J=8.0), 2.61 (2H, t, J=6.8), 2.71 (6H, s), 2.94 (2H, t, J=6.8), 3.35–3.39 (4H, m), 3.86, 3.87, 3.88, 3.89 (3H× 4, s), 6.76 (1H, s), 6.80 (1H, s), 6.84 (2H, s), 7.12 (1H, s), 7.14 (1H, s), 7.16 (2H, s); m/z (FAB) 805 (MH^+–Cl, 11%), 281 ($(HONHCO(CH_2)_4CONHpyrroleCONH)^+$, 6.8), 58 ($(CH_2N(CH_3)_2)^+$, 54) (Found: MH^+–Cl, 805.4116. $C_{38}H_{53}N_{12}O_8$ requires *MH^+*–Cl, 805.4109).

4-[[[4-[[4-[[[4-[[[4-[[5-(hydroxyaminocarbonyl)valeryl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]butyryl]amino]-1-methyl-2-pyrrolyl]carbonyl]amino]-1-methyl-*N*-[3-(*N*, *N*-dimethylamino)propyl]-2-pyrrolecarboxamide BNHA3. This compound was prepared using a similar procedure to that described for **BNHA2** in 33% yield; yellow microcrystalline; mp 149–152 °C; R_F=0.35 (system C); ν_{max}(KBr)/cm^{-1} 3300, 2950, 1650, 1635; $\lambda_{max}(H_2O)$/nm 299 (log ε=4.51); δ (DMSO-d_6) 1.53 (4H, m), 1.68 (2H, m), 1.79 (2H, m), 1.96 (2H, t, J=6.6), 2.21–2.29 (6H, m), 2.34 (6H, s), 3.20–3.22 (4H, m), 3.78, 3.80, 3.81, 3.82 (3H×4, s), 6.85 (1H, s), 6.86 (2H, s), 6.89 (1H, s), 7.15 (1H, s), 7.17 (3H, s), 8.05 (1H, br t), 8.10 (1H, br t), 9.78 (1H, s), 9.84 (1H, s), 9.86 (2H, s), 10.36 (1H, s); m/z (FAB) 819 (MH^+–Cl, 17%), 489 (MH^+–Cl–$(CONHpyrrole)_2(CH_2)_3N(CH_3)_2$, 5.4), 73 ($(((CH_2)_2N(CH_3)_2+H)^+$, 76) (Found: MH^+–Cl, 819.4263. $C_{39}H_{55}N_{12}O_8$. requires *MH^+*–Cl, 819.4265).

Plasmid DNA Cleavage. Each reaction mixture (30 μl total volume) contained 0.6 μg of Col El DNA, 40 mM Tris–HCl (pH 8.0), hydroxamic acid (0.3% maximum final DMF concentration) and metal ion. After incubation at 37 °C, the reaction was terminated by adding 60 μl of TE (10 mM Tris–HCl/1 mM EDTA, pH 8.0), 10 μl of 3N sodium acetate (pH 5.2) and 100 μl of phenol/chloroform (1:1, v/v). The mixture was shaken by vortex and centrifuged. To the aqueous phase, 250 μl of cold ethanol was added and precipitated. The recovered DNA pellet was washed with 600 μl of 70% ethanol, dried in vacuo, and redissloved in 6 μl of TE and 6 μl of loading solution (50% glycerol/25 mM EDTA/0.025% bromophenol blue). The resulting solution 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 form I and form II of the DNA were determined with a Shimadzu dual-wavelength flying spot scanner CS-9000. The area under form I DNA was multiplied by a factor of 1.07 to correct for reduced binding of the ethidium bromide by the form I DNA.

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4. Spectroscopic data of **DHA**: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 (light-brown glassy solid); TLC (silica gel, 1-BuOH/AcOH/H_2O=4:1:2) R_F=0.44; mp 144–147.5 °C; IR (KBr disc) 3250, 1650 and 1635 cm^{-1}; UV (H_2O) 240 nm (log ε=4.39); ^{1}H-NMR (400 MHz, DMSO-d_6) δ 1.53 (4H, m), 1.84 (2H, m), 1.98 (2H, t, J=6.6), 2.22 (2H, t, J=6.4), 2.69 (6H, s), 2.98 (2H, t, J=7.6), 3.24 (2H, t, J=5.6), 3.82, 3.84, 3.85 (3H×3, s), 6.89 (1H, d, J=2.0), 6.93 (1H, d, J=1.6), 7.06 (1H, d, J=1.6), 7.16 (1H, d, J=1.6), 7.19 (1H, s), 7.24 (1H, s), 8.18 (1H, t, J=5.3), 8.70 (1H, bs), 9.84 (1H, s), 9.92 (2H, s) and 10.41 (1H, s); MS (FAB) m/z 612 (M^+–Cl, 68%); HRMS (FAB) m/z 612.3244 ($C_{29}H_{42}N_9O_6$ requires 612.3258).
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