Microgonotropens and Their Interactions with DNA. 1.[1] Synthesis of the Tripyrrole Peptides Dien-Microgonotropen-a, -b, and -c and Characterization of Their Interactions with dsDNA

Gong-Xin He, Kenneth A. Browne, Jay C. Groppe, Andrei Blaskó, Houng-Yau Mei, and Thomas C. Bruice*

Contribution from the Department of Chemistry, University of California at Santa Barbara, Santa Barbara, California 93106

Received August 5, 1992

Abstract: Exploration of the novel idea to employ a pyrrole nitrogen of a tripyrrole peptide minor groove binding agent to carry catalytic entities to the phosphates and major groove of DNA has been initiated with the synthesis of dien-microgonotropen-a, -b, and -c (**5a**, **5b**, and **5c**). Replacing the carboxyl terminal amidine and amino terminal formyl functionalities of distamycin (Dm) by $CH_2N(CH_3)_2$ and acetyl substituents, respectively, provides **2**, which has greater stability in water than does Dm. The synthetic design allows the *N*-methyl substituent on the central pyrrole of **2** to be replaced by connectors terminating in a dien ligand [$-(CH_2)_3N\{(CH_2)_3N(CH_3)_2\}_2$ (**5a**), $-(CH_2)_4N\{(CH_2)_3N(CH_3)_2\}_2$ (**5b**), $-(CH_2)_5N\{(CH_2)_3N(CH_3)_2\}_2$ (**5c**)]. The binding of **2** is about 20-fold weaker than the binding of **5a**, **5b**, and **5c** to calf thymus DNA, poly(dA-dT), and poly(dI-dC) due to the contribution of the polyamine substituents of the latter. The specificity and affinity of binding of **5a**, **5b**, and **5c** to the 5′-[^{32}P] 167-bp *Eco*RI/*Rsa*I restriction fragment of pBR322 was determined by DNase I footprint analysis. Specific inhibition of cleavage was observed at each of the four potential A+T-rich binding sites after preincubation with **5a**, **5b**, and **5c** at concentrations as high as 50 μM. At 250 μM, binding at short heteropolymeric A+T secondary sites distal to the cluster of A+T-rich primary binding sites was observed. At such higher concentrations of **5a**, **5b**, and **5c**, increased rates of enzymatic cleavage at specific sequences were observed. DNase I footprinting analysis of the 3′-labeled fragment provided complementary results. Electrophoretic migration of *Hae*III restriction digest fragments of ϕX-174-RF DNA after preincubation with **5a**, **5b**, and **5c** was used to assess induction of gross conformational changes in DNA molecules. As the concentration of the agents increases, the effect of the agents in changing the conformation of larger DNA fragments decreases in the order **5c** > **5b** > **5a** ≫ Dm > Hoechst 33258. The electrophoretic mobilities of smaller DNA fragments are unaltered in the presence of the various agents. The dien-microgonotropens are much more effective in inducing changes than the sum of the Dm and bis[3-(dimethylamino)propyl]methylamine parts. This is due to the unique relationship between the minor groove binding portion of the dien-microgonotropens and the accompanying electrostatic complexing of the covalently attached dien moiety to the phosphodiester backbone of DNA.

Introduction

A subject which has gained considerably in general interest is the design of reagents capable of sequence-selective complexation to DNA.[4] A class of reagents whose specificity for binding to the minor groove of DNA is accommodated by the crescent shape created by peptide linkages between two or three 1-methyl-4-aminopyrrole-2-carboxylic acid residues is shown in Chart I. The most common of these reagents are distamycin (with antibiotic, antiviral, and oncolytic properties; from *Streptomyces distallicus*[5]) and netropsin (with a wide range of antimicrobial activity; from *Streptomyces netropsis*[6]).[7] Analogues (lexitropsins) which contain, in place of a pyrrole ring, imidazoles, furans, thiophenes, and thiazoles have been synthesized and studied in interactions with DNA.[8] Other crescent-shape DNA minor groove binders which have attracted particular attention are Hoechst 33258,[9] 4′,6′-diamidino-2-phenylindole (DAPI),[10] and (+)-CC-1065 (Chart I) as well as many related structures.[11]

(1) (a) Chemistry of Phosphodiesters, DNA and Models. 3. Part 1: Reference 2. Part 2: Reference 3. (b) Microgonotropens are defined as DNA minor groove binding agents with substituents which project outward from the minor groove and terminate with either interactions with the phosphodiester backbone or with interactions in the major groove. A prefix denotes the nature of the projecting substituent (in this study, dien-microgonotropens). Suffixes (–a, –b, etc.) are used to differentiate among related analogues (i.e., that vary by methylene linker chain length).

(2) Bruice, T. C.; Mei, H.-Y.; He, G.-X.; Lopez, V. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 1700.

(3) Browne, K. A.; Bruice, T. C. *J. Am. Chem. Soc.* **1992**, *114*, 4951.

(4) For reviews see: (a) Nielsen, P. E. *Bioconjugate Chem.* **1991**, *2*, 1. (b) Bailly, C.; Hénichart, J.-P. *Bioconjugate Chem.* **1991**, *2*, 379.

(5) *The Merck Index*, 11th ed.; Merck & Co., Inc.: Rahway, NJ, 1989; pp 1383.

(6) *The Merck Index*, 11th ed.; Merck & Co., Inc.: Rahway, NJ, 1989; pp 1024.

(7) Kopka, M. L.; Yoon, C.; Goodsell, D.; Pjura, P.; Dickerson, R. E. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 1376.

(8) (a) Lown, J. W.; Krowicki, K.; Bhat, U. G.; Skorobogaty, A.; Ward, B.; Dabrowski, J. C. *Biochemistry* **1986**, *25*, 7408. (b) Kissinger, K.; Krowicki, K.; Dabrowiak, J. C.; Lown, J. W. *Biochemistry* **1987**, *26*, 5590. (c) Lee, M.; Pon, R. T.; Krowicki, K.; Lown, J. W. *J. Biomol. Struct. Dyn.* **1988**, *5*, 939. (d) Zakrzewska, K.; Randrianarivelo, M.; Pullman, B. *J. Biomol. Struct. Dyn.* **1988**, *6*, 331. (e) Lee, M.; Krowicki, K.; Shea, R. G.; Lown, J. W. *J. Mol. Recognit.* **1989**, *2*, 84. (f) Rao, K. E.; Bathini, Y.; Lown, J. W. *J. Org. Chem.* **1990**, *55*, 728. (g) Lee, M.; Chang, D. K.; Hartley, J. A.; Pon, R. T.; Krowicki, K.; Lown, J. W. *Biochemistry* **1988**, *27*, 445. (h) Kumat, S.; Jaseja, M.; Zimmermann, J.; Yadagiri, B.; Pon, R. T.; Sapse, A.-M.; Lown, J. W. *J. Biomol. Struct. Dyn.* **1990**, *8*, 331. (i) Rao, K. E.; Shea, R. G.; Yadagiri, B.; Lown, J. W. *Anti-Cancer Drug Des.* **1990**, *5*, 3. (j) Bailly, C.; Helbecque, N.; Hénichart, J.-P.; Colson, P.; Houssier, C.; Rao, K. E.; Shea, R. G.; Lown, J. W. *J. Mol. Recognit.* **1990**, *3*, 26. (k) Montecucco, A.; Fontana, M.; Focher, F.; Lestingi, M.; Spadari, S.; Ciarrocchi, G. *Nucleic Acids Res.* **1991**, *19*, 1067. (l) Singh, M. P.; Kumar, S.; Joseph, T.; Pon, R. T.; Lown, J. W. *Biochemistry* **1992**, *31*, 6453. (m) Bailly, C.; Colson, P.; Houssier, C.; Houssin, R.; Mrani, D.; Gosselin, G.; Imbach, J.-L.; Waring, M. J.; Lown, J. W.; Hénichart, J.-P. *Biochemistry* **1992**, *31*, 8349. (n) Dwyer, T. J.; Geierstanger, B. H.; Bathini, Y.; Lown, J. W.; Wemmer, D. E. *J. Am. Chem. Soc.* **1992**, *114*, 5911. (o) Mrksich, M.; Wade, W. S.; Dwyer, T. J.; Geierstanger, B. H.; Wemmer, D. E.; Dervan, P. B. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 7586.

(9) (a) Harshman, K. D.; Dervan, P. B. *Nucleic Acids Res.* **1985**, *13*, 4825. (b) Pjura, P. E.; Grzeskoiak, K.; Dickerson, R. E. *J. Mol. Biol.* **1987**, *197*, 257.

0002-7863/93/1515-7061$04.00/0

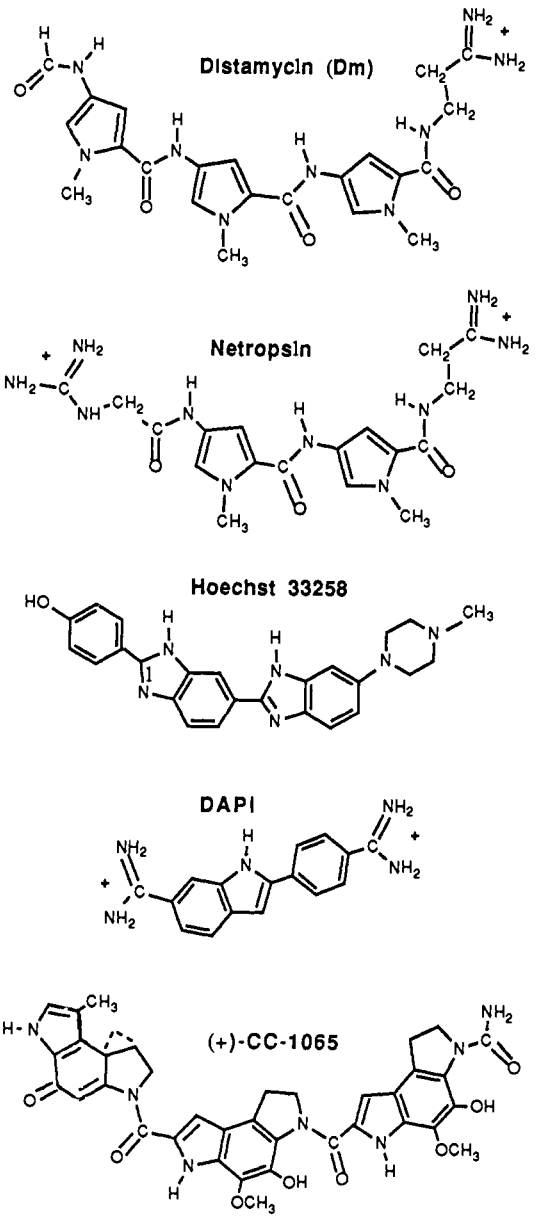
Use of DNA minor groove binding molecules as covalent carriers of chemical agents capable of modifying or cleaving DNA was initiated in the laboratories of Sigman[12] and Dervan.[13] An entire subfield now deals with the cleavage of DNA by sequence-selective degradation of deoxyribose or base entities using redox chemistry[12-15] and photochemistry.[15-17] In Dervan's early designs of rather site-specific agents for DNA oxidative cleavage via Fenton chemistry,[13] a linker to an EDTA functional group was attached at either the NH_2 or CO_2H terminus of a lexitropsin molecule. Once bound within the minor groove, the complexation of iron(III) by the EDTA substituent promoted regiospecific generation of HO·. In a somewhat similar manner, the Dervan group succeeded in sequence-specific alkylation of mainly one adenine in duplex DNA by attachment of $COCH_2Br$ in place of the EDTA substituent.[18]

Our goals are to design selective DNA minor groove binding molecules which (i) approach the phosphodiester linkages and the major groove and (ii) are selective catalysts of the hydrolysis of DNA. For this purpose, we introduce the tren- and dien-microgonotropens[1b] (**3**, **4**, and **5** in Chart II). Our approach is to replace the *N*-methyl substituent of a pyrrole moiety of a lexitropsin with linkers of varying chain length which terminate in a recognition site or warhead. This design has certain advantages including the pointing of the recognition or putative warhead substituents toward the phosphodiester groups and the major groove while leaving the terminal carboxyl and amino ends of the lexitropsin portion free for the purpose of building in greater specificity and/or stronger binding to DNA.[2]

Results and Discussion

Aqueous solutions of distamycin begin to turn yellow in a short time after preparation at room temperature. Exchange of the carboxyl terminal amidine and the amino terminal formyl substituents present in the structure of distamycin (Chart I) by $CH_2N(CH_3)_2$ and acetyl substituents, respectively (as in **2**[19] of Chart II), provides much greater stability of lexitropsin-type molecules. Target molecules (**3** and **4**) have the *N*-methyl group of the central pyrrole replaced by $(CH_2)_nNHCH_2CH_2N(CH_2CH_2NH_2)_2$. Additional target compounds **5a,b,c** have the *N*-methyl group of the central pyrrole of **2** replaced by the side chains $(CH_2)_nN\{(CH_2)_3NMe_2\}_2$. Tren $[NH_2CH_2CH_2N(CH_2CH_2NH_2)_2]$[20] and dien $[HN(CH_2CH_2CH_2NMe_2)_2]$[21] are ex-

Chart I



cellent ligating agents for a number of metal ions. The tren–Ni^{2+} binding constant is about 10^{15} M^{-1}, and the dien–Ni^{2+} binding constant is about 10^8 M^{-1}. Tren–Ni^{2+}, – Co^{3+}, etc. complexes are known to be catalysts for the hydrolysis of the phosphodiester bis(*p*-nitrophenyl) phosphate.[22,23] Also, the polyamine side chains of **3**, **4** and **5a,b,c** are expected to associate with the negative charge of several DNA phosphodiester linkages much as spermine does.[24] We consider here the synthesis and study of dien-microgonotropen-a, -b, and -c (**5a**, **5b**, and **5c**). Tren-microgonotropens (agents with tren substituent groups) will be described separately.

Synthesis. Our synthesis (Scheme I) begins with the preparation of the central pyrrole units (**6a,b,c**) which are attached to the desired linker arms. In the presence of KI the reaction of the 1-pyrrole potassium salt, prepared from ethyl 4-nitro-2-pyrrolecarboxylate and potassium metal in dry DMF, with the appropriate $Cl(CH_2)_mCH(OEt)_2$ gives the desired pyrrole units **6a,b,c**, which bear linker arms that terminate in a protected aldehyde

(10) (a) Wilson, W. D.; Tanious, F. A.; Barton, H. J.; Jones, R. L.; Fox, K.; Wydra, R. L.; Strekowski, L. *Biochemistry* **1990**, *29*, 8452. (b) Tanious, F. A.; Veal, J. M.; Buczak, H.; Ratmeyer, L. S.; Wilson, W. D. *Biochemistry* **1992**, *31*, 3103.

(11) (a) Hurley, L. H.; Needham-VanDevanter, D. R. *Acc. Chem. Res.* **1986**, *19*, 230. (b) Boger, D. L.; Coleman, R. S.; Invergo, B. J.; Sakya, S. M.; Ishizaki, T.; Munk, S. A.; Zarrinmayeh, H.; Kitos, P. A.; Thompson, S. C. *J. Am. Chem. Soc.* **1990**, *112*, 4623. (c) Boger, D. L.; Wysocki, R. J., Jr.; Ishizaki, T. *J. Am. Chem. Soc.* **1990**, *112*, 5230. (d) Boger, D. L.; Munk, S. A.; Zarrinmayeh, H. *J. Am. Chem. Soc.* **1991**, *113*, 3980. (e) Hurley, L. H.; Warpehoski, M. A.; Lee, C.-S.; McGovren, J. P.; Scahill, T. A.; Kelly, R. C.; Mitchell, M. A.; Wicnienski, N. A.; Gebhard, I.; Johnson, P. D.; Bradford, V. S. *J. Am. Chem. Soc.* **1990**, *112*, 4633.

(12) Sigman, D. S. *Acc. Chem. Res.* **1986**, *19*, 180.

(13) (a) Schultz, P. G.; Taylor, J. S.; Dervan, P. B. *J. Am. Chem. Soc.* **1982**, *104*, 6861. (b) Dervan, P. B. *Science* **1986**, *232*, 464.

(14) (a) Takenaka, S.; Ihara, T.; Takaki, M. *J. Mol. Recognit.* **1990**, *3*, 156. (b) Ding, L.; Etemad-Moghadam, G.; Meunier, B. *Biochemistry* **1990**, *29*, 7868. (c) Nagai, K.; Carter, B. J.; Xu, J.; Hecht, S. M. *J. Am. Chem. Soc.* **1991**, *113*, 5099. (d) Chatterjee, M.; Rokita, S. E. *J. Am. Chem. Soc.* **1991**, *113*, 5116. (e) Grover, N.; Thorp, H. H. *J. Am. Chem. Soc.* **1991**, *113*, 7030. (f) Chen, X.; Burrows, C. J.; Rokita, S. E. *J. Am. Chem. Soc.* **1992**, *114*, 322.

(15) Nielsen, P. E. *J. Mol. Recognit.* **1990**, *3*, 1.

(16) (a) Barton, J. K. *Science* **1986**, *233*, 727. (b) Sitlani, A.; Long, E. C.; Pyle, A. M.; Barton, J. K. *J. Am. Chem. Soc.* **1992**, *114*, 2303.

(17) (a) Woo, J.; Hopkins, P. B. *J. Am. Chem. Soc.* **1991**, *113*, 5457. (b) Nielsen, P. E.; Hiort, C.; Sonnichsen, S. H.; Buchardt, O.; Dahl, O.; Norden, B. *J. Am. Chem. Soc.* **1992**, *114*, 4967.

(18) Baker, B. F.; Dervan, P. B. *J. Am. Chem. Soc.* **1989**, *111*, 2700.

(19) Compound **2** has been reported by Wade and Dervan, but no synthetic details were given. Wade, S. W.; Dervan, P. B. *J. Am. Chem. Soc.* **1987**, *109*, 1574.

(20) Prue, J. E.; Schwarzenbach, G. *Helv. Chim. Acta* **1950**, *33*, 963.

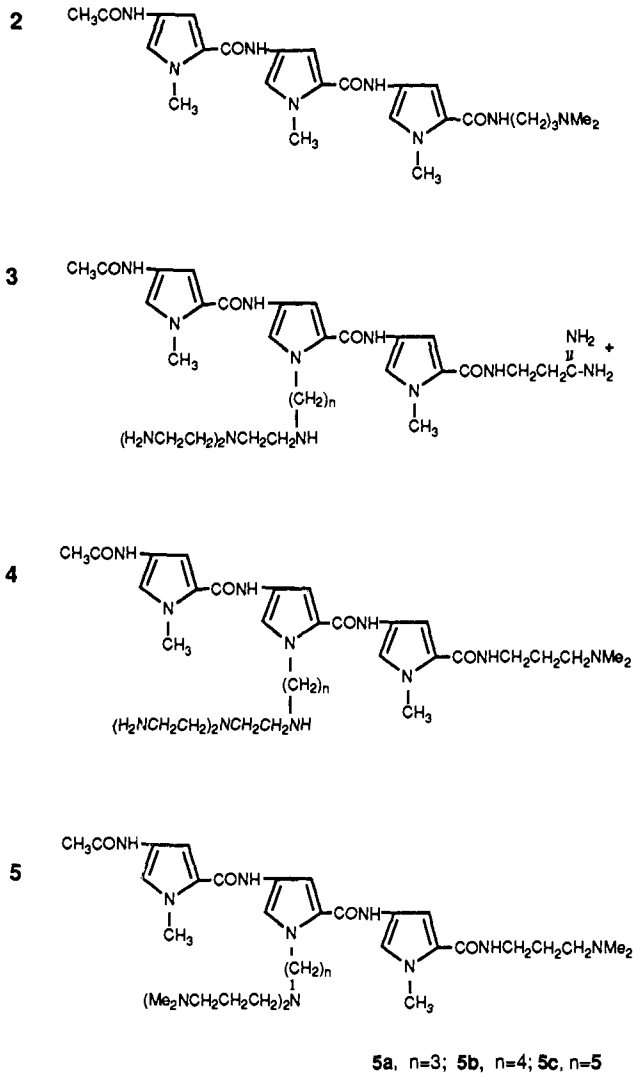
(21) Golgberg, D. E.; Fernelius, W. C. *J. Phys. Chem.* **1959**, *63*, 1328.

(22) De Rosch, M. A.; Trogler, W. C. *Inorg. Chem.* **1990**, *29*, 2409.

(23) Chin, J. *Acc. Chem. Res.* **1991**, *24*, 145.

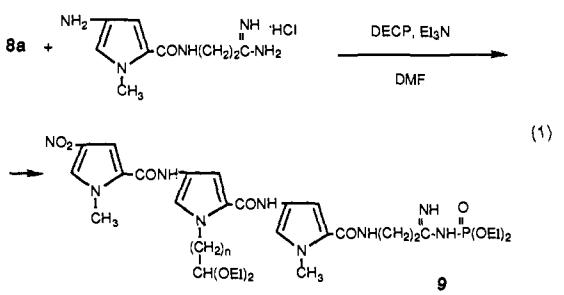
(24) Egli, M.; Williams, L. D.; Gao, Q.; Rich, A. *Biochemistry* **1991**, *30*, 11388.

Chart II



group on the 1-nitrogen. Desired products were not obtained in the absence of KI as catalyst.

We have employed diethyl cyanophosphonate (DECP)[25] as the coupling reagent for peptide synthesis. Our use of DECP is predicated on a simplification of older procedures to obviate the required preparation of acid chlorides from carboxylic acid intermediates. The use of DECP provides higher yields under mild conditions. Unfortunately, with the use of DECP in the coupling of triethylammonium 1-(3,3-diethoxypropyl)-4-(1-methyl-4-nitro-2-pyrrolecarboxamido)-2-pyrrolecarboxylate (**8a**) with 3-(1-methyl-4-nitropyrrole-2-carboxamido)propionamidine hydrochloride,[26] the major product was identified as compound **9** by ^{1}H NMR ($CDCl_3$) and MS (FAB). From eq 1 under the

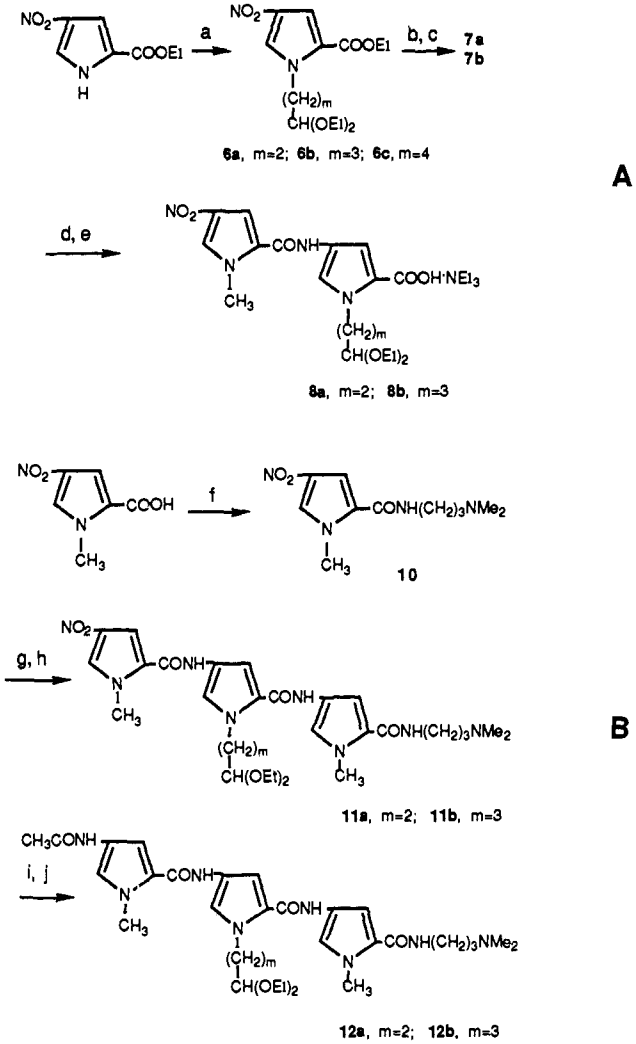


(1)

conditions used, the propionamidine hydrochloride group reacts

(25) Yamada, S.-I.; Kasai, Y.; Shioiri, T. *Tetrahedron Lett.* **1973**, 1595.
(26) Lee, M.; Lown, J. W. *J. Org. Chem.* **1987**, *52*, 5717.

Scheme I[a]

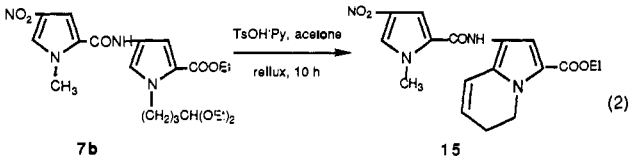


[a] Reagent and conditions: (a) K, KI, $Cl(CH_2)_mCH(OEt)_2$, m = 2, 3, 4, DMF, 80 °C, 5 h; (b) H_2, 10% Pd/C, MeOH, room temperature 1 atm; (c) *N*-methyl-4-nitro-2-pyrrolecarboxylic acid, DECP, Et_3N, THF, room temperature 10 h; (d) NaOH, 80% aqueous EtOH, reflux, 24 h; (e) Et_3N·HCl, 0 °C; (f) $H_2N(CH_2)_3NMe_2$, DECP, THF, room temperature 10 h; (g) H_2 10% Pd/C, MeOH, room temperature 1 atm; (h) **8a** or **8b**, DECP, Et_3N, DMF, room temperature 10 h; (i) H_2, 10% Pd/C, MeOH, room temperature 1 atm; (j) CH_3COCl, Et_3N, DMF, room temperature 10 h.

with DECP to form a phosphoramide. Although the possibility of acidic hydrolysis of the phosphoramide group of **9** to regenerate the required amidine was considered, we decided to change the terminal amidino group to a dimethylamino group[27] as in compound **4** (Chart II). This decision was based on the greater ease of purification with a neutral substituent and the greater solution stability of a dimethylamino as compared to an amidino group. The pK_a value for the terminal tertiary amino group is about 9.5, such that it should be protonated like the amidine moiety of Dm at neutral pH.

As shown in Scheme II, attempts at deprotecting the aldehyde function of **12a,b** failed to provide the aldehydes **13a,b**. With an excess of CF_3COOH in refluxing THF/H_2O (1:1), **12a,b** provide polymeric products. By the use of **12b** with 1 equiv of CF_3-COOH in refluxing THF/H2O (1:1), the major product is **14**. The model reaction of **7b** in refluxing acetone/H_2O (10:1) in the presence of pyridinium *p*-toluenesulfonate (TsOH·Py) gave compound **15** in a 66% yield (eq 2). The ability to form a six-membered ring makes the intramolecular condensation so facile that no detectable aldehyde product is formed. This finding is

(27) Taylor, J. S.; Schultz, P. G.; Dervan, P. B. *Tetrahedron* **1984**, *40*, 457.



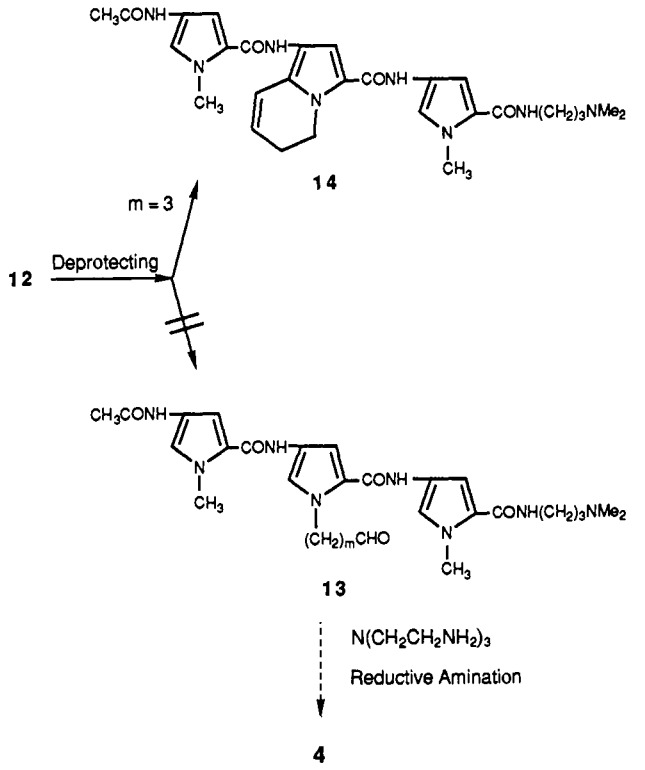
rather remarkable, since the two weakly electron withdrawing CO_2Et and NHC(O)Ar substituents should attenuate the enamine character of the pyrrole ring. Further studies show that this condensation reaction does not occur if the carbamide substituent is replaced by a nitro group. To obviate the condensation of free aldehyde group and pyrrole ring, we chose to condense the aldehyde and polyamino group by reductive amination before assembling the peptide backbone. (Synthetic procedures and spectral data for compounds **7–9**, **11**, **12**, **14**, and **15** are available as supplementary material.) The preparation of the target compounds **5a**,**b**,**c** (Chart II) was based on this concept, as shown in Scheme III. In order to evaluate the influence of the linkers and the bis[3-(dimethylamino)propyl]amino polyamine ligand[28,29] in **5a**,**b**,**c** upon reaction with DNA, a tripyrrole peptide is required wherein the polyamine side chain is replaced by a methyl group. Compound **2** (Chart II) was synthesized using synthetic procedures similar to those employed with **5a**,**b**,**c** (Scheme IV).[19]

The identifications of all final products (**5a**,**b**,**c** **12a**,**b**, and **2**) were carried out by ^{1}H NMR, MS, IR, and elemental analysis. ^{1}H NMR assignments for **5a** and **5b** were obtained by comparing the spectra of **5a** and **5b** with that of **5c**. For **5c** the assignment was based on 500-MHz 2D ^{13}C–^{1}H (HETCOR) and ^{1}H–^{1}H (DQF-COSY) experiments.[30]

Complexing of 2 and 5a,b,c by DNA. The binding affinities for **2**, **5a**, **5b**, and **5c** by sonicated calf thymus DNA (ctDNA) and synthetic polymers poly(dA-dT), poly(dG-dC), and poly(dI-dC) have been compared by using the ethidium bromide displacement method.[31] Binding indices of **5a**, **5b**, and **5c** are, in all cases, comparable.[2] The binding of **2** is about 20-fold weaker than the binding of **5a**, **5b**, and **5c** to ctDNA, poly(dA-dT), and poly(dI-dC) while the binding of poly(dG-dC) with **2**, **5a**, **5b**, and **5c** is comparable. [Poly(dI-dC) is the same as poly(dG-dC) except there is no amino group on position 2 of each guanine base protruding in the minor groove.] These results suggest that the polyamine substituents of **5a**, **5b**, and **5c** contribute to their preferential DNA binding at other than dG-dC sequences. That binding is in the minor groove is further supported by the finding that **5a** is strongly bound to T4 coliphage DNA.[2] The latter can only bind in its minor groove, since the major groove is glycosylated throughout.[32]

DNase I Footprint Analysis. Footprinting analyses, by Dervan and co-workers, of the 167-bp *Eco*RI/*Rsa*I restriction fragment of plasmid pBR322 with distamycin (Dm)[9a] and the analogue, (bromoacetyl)distamycin,[18] have identified four A+T-rich binding sites: 5'TTTAA3', 5'GTTA3', 5'AAATT3', and 5'GAAAT3' (Figure 1). Common to each of the five base pair sites is a *homopolymer* tract of three (A·T) or (T·A) base pairs. In the studies described here, the specificity and affinity of binding of **5a**, **5b**, and **5c** to the 167-bp *Eco*RI/*Rsa*I restriction fragment were determined by DNase I footprint analysis.[33] DNase I has the advantage over Tullius' HO•[34] and Dervan's MPE·Fe(II)[35] in that it cleaves

Scheme II

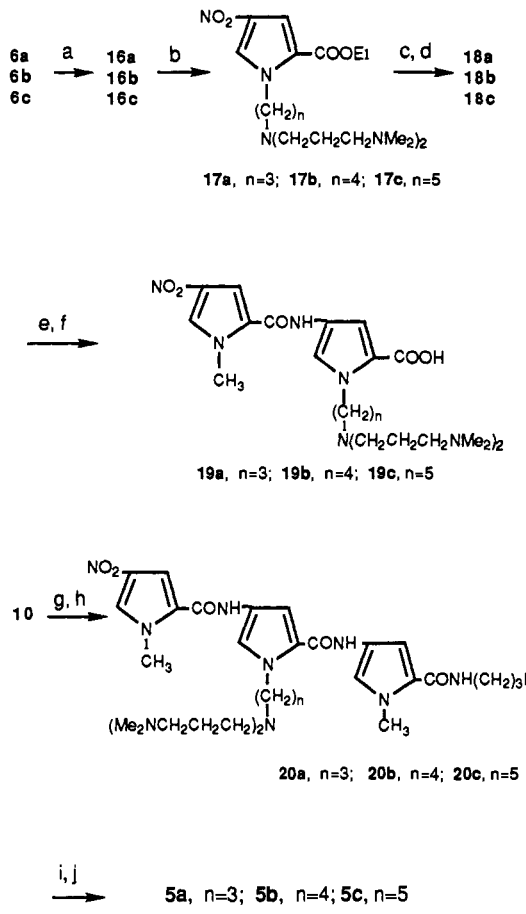


precisely at the 5′ edge of an agent's minor groove binding site, which produces a protected region with a sharp, well-defined 5′ border.[36] On the other hand, DNase I cleavage is not observed precisely at the 3′ edge of the lexitropsin-binding site, due to a small polypeptide loop of the enzyme which enters the minor groove and spans 3 or 4 base pairs of DNA. As a result, protection from DNase I cleavage is expected to extend 3–4 base pairs from the actual 3′ border,[36] yielding an inhibition pattern 8–9 nucleotides in length for a 5-bp binding site. The disadvantage produced by the polypeptide loop is removed if DNase I footprint analysis is extended to both 3′- and 5′-labeled strands. This has been done (*vida infra*).

Preincubation of the 167-bp 5′-[^{32}P]-labeled restriction fragment with 5 μM **5a**, **5b**, **5c**, or distamycin (0.05 ligand/bp DNA) did not produce detectable inhibition of DNase I cleavage at any of the four A+T-rich binding sites (Figure 2). In contrast, specific inhibition of cleavage was observed at each of the four sites after preincubation with 50 μM **5a**, **5b**, **5c**, or distamycin (0.5 ligand/bp DNA). The patterns of inhibition were similar for all three dien-microgonotropens and were essentially indistinguishable from the patterns produced by protection with distamycin. Preincubation of the restriction fragment with 250 μM ligand (2.5 ligand/bp DNA) resulted in additional protection from DNase I cleavage within the spacer regions which flank the A+T-rich binding sites. Dervan and co-workers have observed a similar binding isotherm for distamycin on the 516-bp *Rsa*I/*Eco*RI restriction fragment of pBR322. At higher concentrations of distamycin (3.1 ligand/bp DNA), spacer regions which flanked A+T-rich binding sites coalesced into a single, broad, protected zone.[35] At 250 μM, binding of **5a**, **5b**, **5c**, and distamycin at secondary sites distal to the cluster of A+T-rich primary binding sites also was observed. The secondary sites, proximal to the unlabeled end of the fragment (upper one-third of the gel), contain short, heteropolymeric A+T sequences only.

(28) Bis[3-(dimethylamino)propyl]amine is available from Aldrich as 3,3′-iminobis(*N*,*N*-dimethylpropylamine).
(29) Recently, the distamycin analogue with a tren group on the central pyrrole, compound **4** in Chart II, has been synthesized in this laboratory by use of a suitable protective group, and the detailed procedure will be published in following papers.
(30) 2D NMR spectra {^{13}C–^{1}H (HETCOR) and ^{1}H–^{1}H (DQF-COSY)} for compound **5c** in $CDCl_3$ are available as supplementary material.
(31) LePecq, J.-B.; Paoletti, C. *J. Mol. Biol.* **1967**, *27*, 87.
(32) Mokulśkii, M. A.; Kaiptarova, K. A.; Mokulśkaya, T. D. *Mol. Biol. (Moscow)* **1972**, *6*, 714.
(33) Galas, D. J.; Schmitz, A. *Nucleic Acids Res.* **1978**, *5*, 3157.
(34) Burkhoff, A. M.; Tullius, T. D. *Cell* **1987**, *48*, 935.
(35) Van Dyke, M. W.; Hertzberg, R. P.; Dervan, P. B. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 5470.
(36) (a) Dabrowiak, J. C.; Goodisman, J. In *Chemistry & Physics of DNA–Ligand Interactions*; Kallenbach, N. R., Ed.; Adenine Press: New York, 1989; pp 143–174. (b) Goodisman, J.; Dabrowiak, J. C. *Biochemistry* **1992**, *31*, 1058.

Scheme III[a]

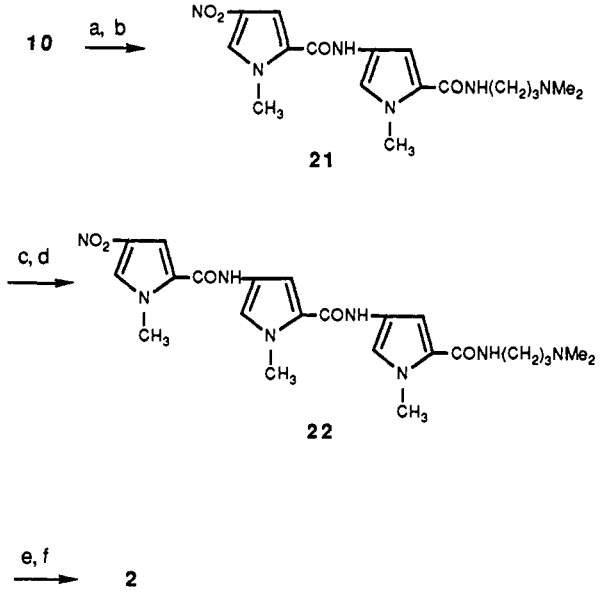


[a] Reagent and conditions: (a) 90% aqueous acetone, TsOH·Py, reflux, 10 h; (b) MeOH, $HN(CH_2CH_2CH_2NMe_2)_2$, AcOH, $NaBH_3CN$, room temperature, 72 h; (c) H_2, 10% Pd/C, MeOH, room temperature 1 atm; (d) *N*-methyl-4-nitro-2-pyrrolecarboxylic acid, DECP, Et_3N, DMF, room temperature 10 h; (e) NaOH, 80% aqueous EtOH, reflux, 24 h; (f) 10% HCl, 0 °C, to pH = 7; (g) H_2, 10% Pd/C, MeOH, room temperature 1 atm; (h) **19**, DECP, Et_3N, DMF, room temperature 10 h; (i) H_2, 10% Pd/C, MeOH, room temperature 1 atm; (j) CH_3COCl, Et_3N, DMF, room temperature 10 h.

DNase I footprinting analysis of the 3′-[^{32}P]-labeled 167-bp *Eco*RI/*Rsa*I restriction fragment with **5a**, **5b**, and **5c** (data not shown), when compared to results with the 5′-labeled material, defined binding sites similar to those for distamycin. The four A+T-rich binding sites (Figure 1) were protected specifically by dien-microgonotropen-a, -b, and -c and by distamycin at intermediate concentration (50 μM), while, at higher concentration (250 μM), sequences flanking the cluster of A+T-rich sites were protected, producing one long protected region. Secondary binding sites were detected distal to the clustered A+T-rich sites as binding approached saturation. Comparison of the DNase I footprinting analyses with **5a**, **5b**, **5c**, and distamycin demonstrates that replacement of the central pyrrole *N*-methyl group with the varying chain length triamine $(CH_2)_nN\{(CH_2)_3N(CH_3)_2\}_2$ linkers does not alter the specificity of binding to specific A+T-rich sites in naturally occurring duplex DNA.

With higher concentrations of each compound, enhancements in or increased rates of DNase I cleavage were observed at specific sequences for both the 5′- and the 3′-[^{32}P]-labeled restriction fragments. In a study of netropsin binding to DNA, Dabrowiak and Goodisman proposed that the enhancements in cleavage may be due to induced structural changes or simply from mass action effects.[36] As binding to the DNA approaches saturation, the ratio of unprotected cleavage sites to enzyme decreases, leading to an expected increased rate of cleavage at all available sites. The same cleavage sites that show an apparent hypersensitivity

Scheme IV[a]



[a] Reagent and conditions: (a) H_2, 10% Pd/C, MeOH, room temperature 1 atm; (b) *N*-methyl-4-nitro-2-pyrrolecarboxylic acid, DECP, Et_3N, DMF, room temperature 10 h; (c) H_2, 10% Pd/C, MeOH, room temperature 1 atm; (d) *N*-methyl-4-nitro-2-pyrrolecarboxylic acid, DECP, Et_3N, DMF, room temperature 10 h; (e) H_2, 10% Pd/C, MeOH, room temperature 1 atm; (f) CH_3COCl, Et_3N, DMF, room temperature 10 h.

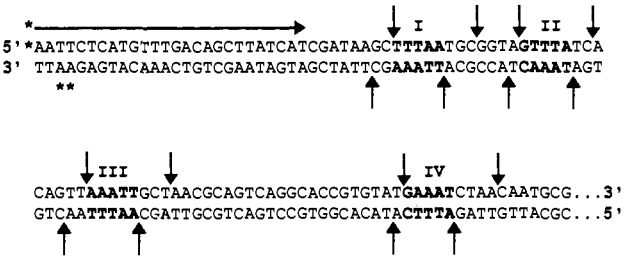


Figure 1. Nucleotide sequence of the 167-bp *Eco*RI/*Rsa*I restriction fragment from plasmid pBR322 containing four A + T-rich binding sites (bold type). The oligonucleotide primer for synthesis of dideoxynucleotide sequencing products is depicted by a horizontal arrow adjacent the annealing site. Positions of incorporated radiolabel in the oligonucleotide primer, 5′-labeled strand, and 3′-labeled strand are indicated with asterisks. Vertical arrows depict cleavages proximal to the protected sites. These were determined by the analysis of both 5′- (downward arrows) and 3′-(upward arrows) labeled restriction fragments.

in the control DNase I treatment of the 167-bp fragment (lane 2, Figure 2) show dramatically increased cleavage with increasing concentration of the dien-microgonotropens. The apparent hypersensitivity to DNase I cleavage is likely the result of not only mass action effects on the entire sequence but, more importantly, induced structural changes by the dien-microgonotropens' and distamycin's binding to sequences flanking the hypersensitive sites.

Electrophoretic Mobility Shift Assay. Dependence of DNA conformation on secondary structure and on alteration of DNA conformation by various binding agents has been studied by a variety of techniques which include electron microscopy,[37] hydroxyl radical cleavage of the DNA backbone,[34] computer modeling,[38] and electrophoretic behavior.[39] Various investigators have determined that DNA with repeating tracts of ten bases each having a run of adenosine residues leads to an anomalous

(37) Griffith, J.; Bleyman, M.; Rauch, C. A.; Kitchin, P. A.; Englund, P. T. *Cell* **1986**, *46*, 717.
(38) Levene, S. D.; Crothers, D. M. *J. Biomol. Struct. Dyn.* **1983**, *1*, 429.
(39) (a) Marini, J. C.; Levene, S. D.; Crothers, D. M.; Englund, P. T. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 7664. (b) Wu, H.-M.; Crothers, D. M. *Nature* **1984**, *308*, 509.

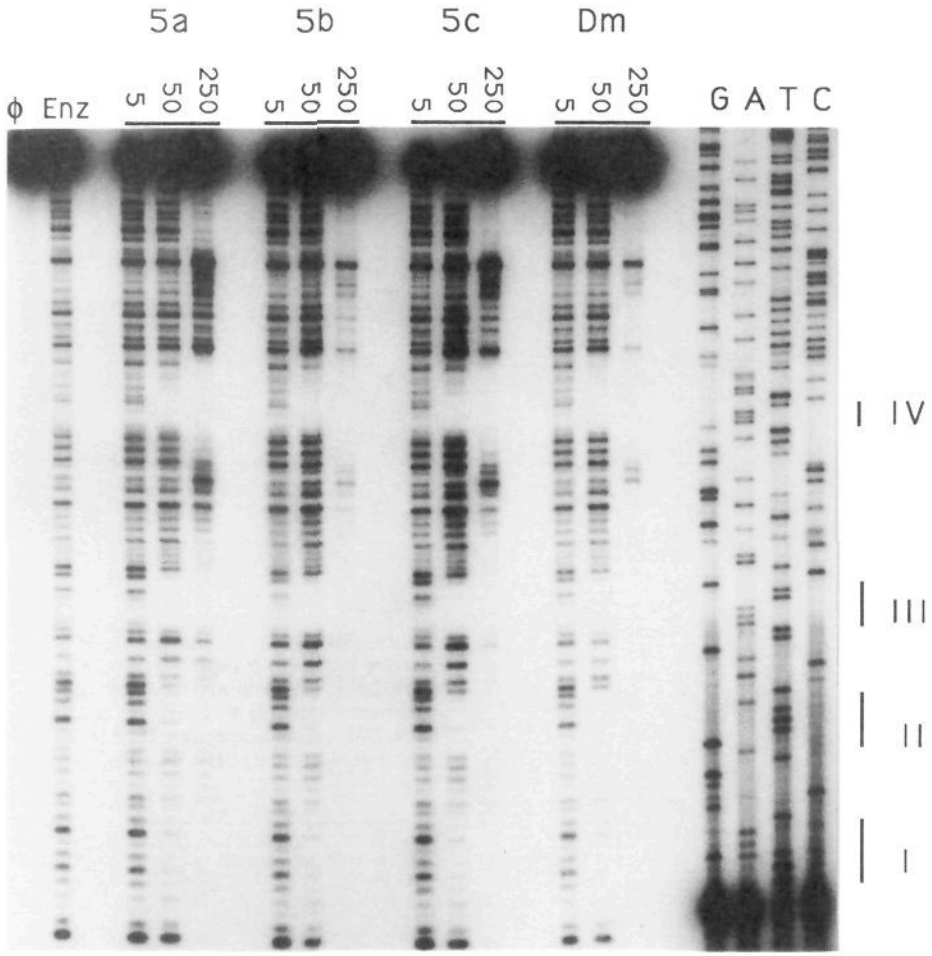


Figure 2. DNase I footprint analysis of the binding of the dien-microgonotropens and distamycin (**Dm**) to the 5′-labeled 167-bp *Eco*RI/*Rsa*I restriction fragment: lane 1, ϕ indicates intact DNA; lane 2, Enz is the abbreviation for DNase I cleavage of unprotected DNA; lanes 3–14, DNase I footprinting reactions containing **5a**, **5b**, **5c**, and **Dm**, respectively, are at the concentrations indicated in μM. Products of the four dideoxynucleotide sequencing reactions (GATC) were synthesized by extension of the 5′-labeled oligonucleotide primer depicted in Figure 1. The four A+T-rich binding sites, also depicted in Figure 1, are indicated adjacent to the DNA sequence ladder.

increase in apparent size of a given DNA fragment[39,40] and generally assume that the apparent increase is a function of the extent of DNA bending. Wu and Crothers observed that distamycin (Dm) normalizes the electrophoretic mobility of "slow" (i.e., bent) kinetoplast DNA fragments and decreases the electrophoretic mobility of some "normally" migrating (essentially linear) DNA.[39b] These studies form the basis for the ensuing discussion.

The effect of **5a**, **5b**, and **5c** binding to DNA on the electrophoretic migration of ϕX-174-RF DNA *Hae*III restriction digest fragments can be seen in Figure 3. Employment of ϕX-174-RF DNA is predicated on its use as a molecular weight size standard and its slight A+T-richness (55%).[41] We have calculated 246 A-tracts (AAAA, AAAT, or TAAA; independent or overlapping), which are the most preferred binding sites for distamycin analogues.[18] These sites are approximately evenly distributed, being separated on the average by $\sim 29 \pm 14$ bp. This frequency is common to fragments of all sizes. With increasing concentrations of **5b**, **5b**, and **5c**, the electrophoretic mobility of the DNA restriction fragments decreases. The decrease in mobility is most pronounced for the largest restriction fragments and not perceptible for the smallest. This suggests

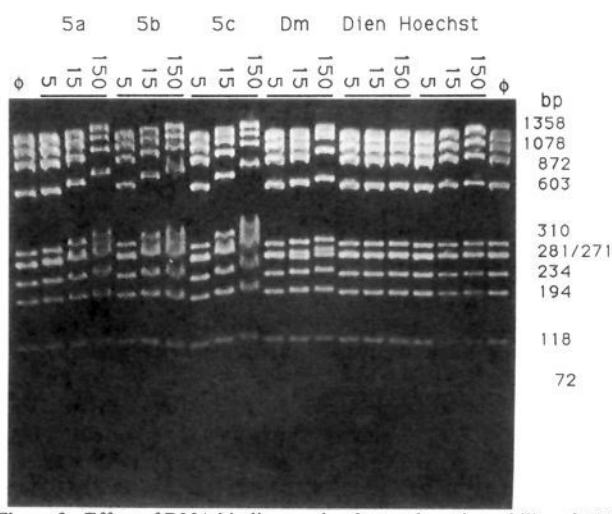


Figure 3. Effect of DNA binding on the electrophoretic mobility of ϕX-174-RF DNA *Hae*III restriction digest fragments (sizes indicated to the right side of the figure): lanes 1 and 20, ϕ indicates control DNA; lanes 2–19, the concentrations of **5a**, **5b**, **5c**, distamycin (**Dm**), bis[3-(dimethylamino)propyl]methylamine (dien), and Hoechst 33258 are in μM.

that the binding of the dien-microgonotropens to A+T-rich sites changes the DNA conformation at these sites and that a critical number of sites are required before conformational changes are great enough to influence electrophoretic mobility. Complexing

(40) (a) Koo, H.-S.; Wu, H.-M.; Crothers, D. M. *Nature* **1986**, *320*, 501. (b) Hagerman, P. J. *Nature* **1986**, *321*, 449. (c) Ulanovsky, L. E.; Bodner, M.; Trifonov, E. N.; Choder, M. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 862.

(41) (a) Sanger, F.; Air, G. M.; Barrell, B. G., Brown, N. L.; Coulson, A. R.; Fiddes, J. C.; Hutchison, C. A., III.; Slocombe, P. M.; Smith, M. *Nature* **1977**, *265*, 687. (b) Sanger, F.; Coulson, A. R.; Friedmann, T.; Air, G. M.; Barrell, B. G.; Brown, N. L.; Fiddes, J. C.; Hutchison, C. A., III; Slocombe, P. M.; Smith, M. *J. Mol. Biol.* **1978**, *125*, 225.

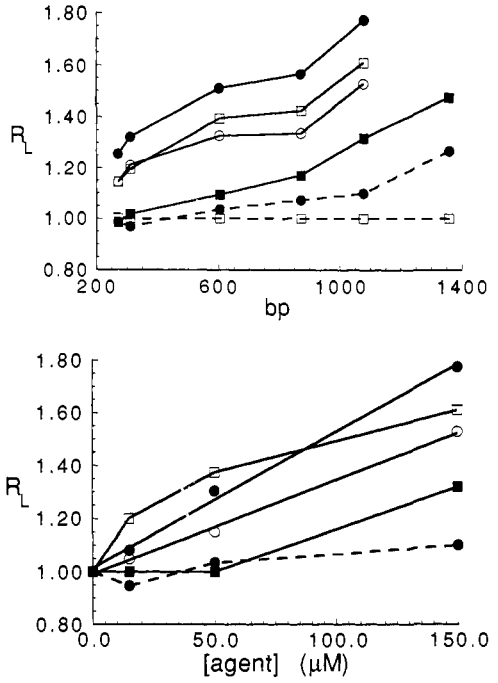


Figure 4. (A, top) Ratio of DNA apparent to real length (R_L) vs the number of base pairs (bp) in the longest and intermediate-sized DNA fragments in the presence of 150 μM **5a** (—○—), **5b**, (—□—), **5c** (—●—), distamycin (—■—), Hoechst 33258 (- ● - -), and no added agent (- - □- -). (B, bottom) R_L vs [agent] {**5a** (—○—), **5b** (—□—), **5c** (—●—), distamycin (—■—), and Hoechst 33258 (- -●- -)} when examining the 1078-bp DNA fragment. The curves are interpolations between the data points for **5b**, distamycin, and Hoechst 33258, while the straight lines for **5a** and **5c** are linear least-squares optimized to the data points. The data for Figures 4A and 4B were generated from Figure 3 as explained in the text.

of **5a**, **5b**, and **5c** to DNA results in the interaction of the positive charges of the protonated polyamine moieties of the dien-microgonotropens with the negatively charged phosphate backbone. The resultant decrease in the net negative charge does not provide an explanation for the decrease in electrophoretic mobility. This is so, since the shortest fragments would be expected to exhibit the greatest change in mobility (at a constant charge to mass ratio, mobility is a logarithmic function of mass). Instead it is the longest fragments (1358, 1078, and 872 bp) whose mobility shows the greatest change. Meanwhile, a "smearing" of the bands is evident in the intermediate-sized fragments (603, 310, 281/271, 234, and 194 bp), especially at 50 and 150 μM dien-microgonotropen. This indicates not only a conformational change but perhaps an aggregation of DNA:dien-microgonotropen complexes. The intercomplex interactions create a distribution of multiple sizes (larger effective molecular weights) for a given fragment and, hence, less sharply defined bands. Hoechst 33258 and distamycin bring about much smaller mobility changes. Bis-[3-(dimethylamino)propyl]methylamine, essentially the dien moiety of the dien-microgonotropens, produces little apparent change in electrophoretic behavior compared with the control lanes. Insight into the structural characteristics necessary for bending cannot be made from this qualitative overview alone. Therefore, we have carried out a more quantitative analysis of the migration data.

The decrease in electrophoretic mobility has been calculated for each compound as the R_L values [ratio of the apparent length to real length where apparent length is the length of uncomplexed double-stranded DNA (interpolated or slightly extrapolated from the control standard fragments) with the same mobility].[39b] The representative plot of R_L vs bp at 150 μM **5a**, **5b**, **5c**, Hoechst 33258, and distamycin (Figure 4A) shows that as the size of the fragment increases, the effect of these agents is to increase the apparent size of DNA fragments relative to the control (φX-174-RF DNA with no added agent). The order of decrease in apparent length is **5c** > **5b** > **5a** ≫ distamycin > Hoechst 33258 > dien. The R_L value does not vary as a simple function with increasing DNA fragment size. Instead, variation in migration patterns is probably contingent on the number of A+T-rich sequences in each fragment and their relative positions within the fragment.[42] In addition, the plot of R_L vs [agent] for the 1078-bp fragment in Figure 4B shows that R_L is very nearly linearly dependent on dien-microgonotropen-a and -c concentration. Dien-microgonotropen-b is different in that its influence on the DNA conformation increases very rapidly initially and then almost levels off. This may be due to the relative affinities of **5a**,**c** vs **5b** for the sequences found in this DNA.[3] Hoechst 33258 and distamycin do not demonstrate very marked changes even at the highest concentrations examined. As is evident from the above discussion, the dien-microgonotropens are much more effective in inducing structural changes than are the sum of their parts (Dm and bis-[3-(dimethylamino)propyl]methylamine).

Conclusions Concerning Interactions of DNA with 5a,b,c. The dien-microgonotropens **5a**,**b**,**c** bind in the minor groove of B-DNA. At low concentrations the primary binding is to homopolymer tracts of at least three (A·T) or (T·A) base pairs, and at higher concentration complexation occurs at secondary sites which are distal to the primary sites and contain short heteropolymeric A+T-rich sequences. The sequence specificity of minor groove binding is determined entirely by the lexitropsin nature of these substances, but the strength of binding is significantly enhanced by the interaction of the spacer $(CH_2)_n$ sequence and its terminal dien substituent $N\{(CH_2)_3N(CH_3)_2\}_2$. Distortion of the DNA conformation brought about by **5a**,**b**,**c** binding can be observed as sites hypersensitive to DNase I cleavage and by shifts in electrophoretic mobility which increases with increasing DNA fragment size. The distortion of DNA follows the order **5c** > **5b** > **5a** ≫ distamycin. We conclude that both minor groove binding and the accompanied polyamine complexing of the phosphate backbone are required to account for the structural alterations that appear as marked changes in relative electrophoretic migration.

In the following paper in this series, the determination of the association constants of the dien-microgonotropens with a single site of the hexadecamer d(GGCGCAAATTTGGCGG)/d(C-CGCCAAATTTGCGCC) is reported. In the third manuscript of this series, we examine the 2D ^{1}H NMR generated solution structure of a complex of the dodecamer $d(CGCAAATTTGCG)_2$ with dien-microgonotropen-c (**5c**) in a 1:1 ratio.

Experimental Section

Materials. (a) DNA Binding Studies. Enzymes were obtained commercially from the following sources: *Eco*RI and *Rsa*I restriction endonucleases, New England Biolabs; *E. coli* DNA polymerase I (Klenow fragment), Pharmacia; deoxyribonuclease I (bovine pancreas) and T4 polynucleotide kinase, Bethesda Research Laboratories; calf intestinal alkaline phosphatase (in ethanolamine), Boehringer Mannheim; modified T7 DNA polymerase (Sequenase), US Biochemical. Other reagents obtained commercially included the following: [α-^{32}P]dATP (800 Ci/mmol) and [γ-^{32}P]ATP (6000 Ci/mmol), Du Pont–New England Nuclear; φX-174-RF DNA *Hae*III restriction digest fragments, ultrapure 2′-deoxynucleoside triphosphates, and sonicated calf thymus DNA (phenol extracted), Pharmacia; tRNA (brewer's yeast), Boehringer Mannheim; 2′,3′-dideoxynucleoside triphosphates, US Biochemical; pBR322 plasmid DNA, Promega; distamycin and ethidium bromide, Sigma; Hoechst 33258, Alrich; Bio-Gel P-6 spin columns (Bio-Spin 6), BioRad; modified acrylamide monomer, AT Biochem; (hydroxyethyl)agarose (NuSieve 3:1), FMC; microfine glass beads and chaotropic salt buffer (Mermaid), Bio 101. Doubly distilled water was used for all biological reactions and dilutions.

(b) Organic Synthesis. Reagent-grade chemicals were used without purification unless otherwise stated. Methanol was refluxed and distilled

(42) Levene, S. D.; Wu, H.-W.; Crothers, D. M. *Biochemistry* **1986**, *25*, 3988.

from CaH_2. Dimethylformamide (DMF) was dried over CaH_2 overnight and distilled under reduced pressure. Triethylamine was dried over KOH and distilled. Methanol, DMF, and trimethylamine were stored over 4A molecular sieves. Tetrahydrofuran (THF) was refluxed with Na metal and distilled before use. Mucobromic acid, 1-methyl-2-pyrrolecarboxylic acid, 3-(dimethylamino)propylamine, 3,3'-iminobis(*N*,*N*-dimethylpropylamine), pyridinium *p*-toluenesulfonate (TsOH·Py), and diethyl cyanophosphonate (DECP) were purchased from Aldrich. Ethyl 4-nitro-2-pyrrolecarboxylate was synthesized by condensation of equimolar quantities of sodium nitromalonic aldehyde, prepared from mucobromic acid and $NaNO_2$, and glycine ethyl ester hydrochloride.[43] 1-Methyl-4-nitro-2-pyrrolecarboxylic acid was prepared by nitration of 1-methyl-2-pyrrolecarboxylic acid according to the published methods.[23,44]

Procedures (DNA Experiments). (a) Preparation of 5'- and 3'-[^{32}P]-Labeled Restriction Fragments. Aliquots of supercoiled pBR322 plasmid (5 pmol) were linearized by digestion with the restriction endonuclease *Eco*RI and radiolabeled by enzymatic incorporation of ^{32}P at either 5' or 3' termini. The 3' recessed ends were filled-in by primer extension with the Klenow fragment of *E. coli* DNA polymerase I in reactions containing dTTP and [α-^{32}P]dATP (800 Ci/mmol). The radioactive phosphoryl group from [γ-^{32}P]ATP was transferred to the 5' termini with T4 polynucleotide kinase after dephosphorylation of 5' ends with calf intestinal alkaline phosphatase (CIAP). Labeled DNA was isolated from the bulk of the unincorporated nucleoside triphosphates by spin column chromatography through Bio-Gel P6 in a clinical centrifuge. Digestion with a second restriction endonuclease, *Rsa*I, yielded two end-labeled fragments (516 bp and 167 bp in length), which were fractionated by electrophoresis through a 4% NuSieve 3:1 (hydroxyethylated) agarose gel in 50 mM Tris–acetate, pH 8.2. The radiolabeled 167-bp products (5'- or 3'-labeled) were detected by autoradiography, and the DNA-containing slices were excised from the gel. After dissolution of the agarose in a chaotropic salt buffer, the DNA fragments were bound to microfine glass beads, washed extensively, and eluted with water. The yields of end-labeled products (in cpm) were estimated by measurement of Cerenkov radiation, assuming a counting efficiency of 40%.

(b) Footprint Reactions. The final concentrations of all reactions (10 μL) were 100 μM (bp) sonicated calf thymus DNA in 10 mM Tris-HCl, pH 7.5, 50 mM KCl, 1 mM $CaCl_2$, and 10 mM $MgCl_2$. After preincubation with the appropriate DNA-binding ligand (**5a**, **5b**, **5c**, or Dm) and concentration (0, 5, 50, or 250 μM) for 15 min at 24 °C, DNase I digestions were carried out for 2 min at 24 °C. The reactions were stopped with the addition of 2 μL of a 0.25 M EDTA, pH 7.5, 0.12 M thiourea, and 600 μg/mL tRNA solution. Ammonium acetate and ethanol were used to precipitate the DNA fragments prior to drying and subsequent suspension in gel loading buffer. A negative control (no DNase I) was incubated for 30 min at 24 °C before being worked up exactly as above.

(c) Sequencing Primer. An oligonucleotide primer (26mer: 5'AATTCTCATGTTTGACAGCTTATCAT3') complementary to nucleotides 4361–24 of pBR322 was synthesized (1.0 μmol scale) from β-cyanoethyl phosphoramidates with an Applied Biosystems 380A automated oligonucleotide synthesizer, without hydrolysis of the final 5'-dimethoxytrityl group. Full-length oligonucleotide was purified free of failure sequences and desalted by reverse-phase chromatography (OPC cartridges, Applied Biosystems). The bound oligonucleotide was deprotected with 2% trifluoroacetic acid, eluted with 20% methanol (HPLC grade), and lyophilized during centrifugation in a Speed-Vac concentrator. An aliquot of the purified oligonucleotide was dissolved in water and the concentration estimated spectrophotometrically by absorbance at 260 nm (1 μg/mL equal to 0.03 A_{260} unit).

(d) Dideoxy DNA Sequencing. Approximately 15 pmol of oligonucleotide primer, 50 μCi of [γ-^{32}P]ATP (6000 Ci/mmol), and 5 units of T4 polynucleotide kinase were incubated in a total volume of 10 μL for 1 h at 37 °C. One microliter of the kinase reaction was mixed with 2 pmol of supercoiled pBR322 plasmid, denatured in 0.2 M NaOH, 0.2 mM EDTA (20 μL). After neutralization with 2 M ammonium acetate, pH 5.4 (2 μL), plasmid and oligonucleotide were precipitated with ice-cold ethanol (55 μL), collected by centrifugation, and resuspended in 10 mM Tris-HCl, pH 7.5. The 5'-labeled primer, annealed to pBR322, was extended with modified T7 DNA polymerase (Sequenase) in four separate reactions, each containing a 2',3'-dideoxynucleotide (following recommendations of enzyme supplier for chain termination).

(e) Sequencing Gel Electrophoresis. The DNase I footprinting and dideoxy sequencing products were coelectrophoresed at ca. 50 °C (75 W, constant power) through 8% (w/v) polyacrylamide gels (30 × 40 cm, 0.4 mm in width) which contained 50% (w/v) urea. After electrophoresis, gels were fixed in 10% methanol, 10% glacial acetic acid and dried at 80 °C under vacuum. Autoradiography was for 12–72 h without intensifing screens.

(f) Electrophoretic Mobility Shift Assay. The final concentrations (10 μL) of all reactions were 0.15 mg/mL ϕX-174-RF DNA *Hae*III digest in 10 mM Tris-HCl, pH 7.5, 50 mM KCl. Incubation of DNA with each of six compounds [**5a**, **5b**, **5c**, Dm, bis[3-(dimethylamino)-propyl]methylamine,[45] or Hoechst 33258] at three concentrations (15, 50, or 150 μM) was for 120 min at 24 °C (control reactions were exactly the same except that additional agents were not added). At this time, 1.1 μL of 10% (w/v) glycerol, 0.1% (w/v) sodium dodecyl sulfate, and 0.1% (w/v) bromophenol blue loading buffer[46] was added to each sample. Samples were electrophoresed through a 4% NuSieve 3:1 (hydroxyethylated) agarose gel in 89 mM Tris–borate, 1 mM EDTA, pH 8.0 (1 × TBE)[46] for 4.5 h at 3 V/cm. The gel was stained with a 0.5 μg/mL solution of ethidium bromide in deionized water for 30 min, destained for 15 min in deionized water, and photographed on a UV (302 nm) transilluminator with Polaroid type 667 film.

Procedures (Organic Synthesis). (a) General. Melting points were determined on a Bristoline hot-stage microscope and are uncorrected. Infrared (IR) spectra were obtained in KBr or neat on a Perkin-Elmer monochromator grating spectrometer (Model 1330) and in $CHCl_3$ on a Perkin-Elmer FT-IR spectrometer (Model 1600). Low-resolution mass spectra (LRMS) were recorded on a VG Analytical spectrometer (Model VGII-250) by electron impact (EI), chemical ionization (CI) with CH_4, or fast atom bombardment (FAB) using *m*-nitrobenzyl alcohol (NBA) or glycerol as a matrix. High-resolution mass spectrometry (HRMS) was performed at the University of California, Riverside (laser desorption). ^{1}H and ^{13}C NMR spectra were obtained in $CDCl_3$ or in DMSO-d_6 with Nicolet NT-300 and General Electric GN-500 spectrometers. Chemical shifts are reported in δ (ppm) relative to Me_4Si with s, d, t, q, and m signifying singlet, doublet, triplet, quartet, and multiplet; coupling constants J are reported in Hz. Chromatographic Silica Gel (Fisher-Chemical, 200–425 mesh) was used for flash chromatography and glass-backed plates of 0.25-mm SiO_2 60-F_{254} (Merck) were used for thin-layer chromatography (TLC). For reversed-phase TLC, Whatman plates $KC_{18}F$ (0.2 mm × 20 cm × 20 cm) and $PLKC_{18}F$ (1 mm × 20 cm × 20 cm) were used. Elemental analysis was carried out by Galbraith Laboratories, Inc. (Knoxville, TN). All nonaqueous reactions were run under argon with rigorous exclusion of water unless otherwise noted. 3-Chloropropionaldehyde diethyl acetal is commercially available (Aldrich). Synthetic procedures and spectral data for compounds **7**, **8**, **9**, **11**, **12**, **14**, and **15** are available as supplementary material.

4-Chlorobutyraldehyde diethyl acetal and 5-chlorovaleraldehyde diethyl acetal were synthesized from 4-chlorobutyryl chloride and 5-chlorovaleryl chloride, respectively, by hydrogenation at room temperature and 1 atm in the presence of 10% Pd/C and 2,6-lutidine in THF,[47] followed by the reaction with triethyl orthoformate catalyzed by Amberlyst 15 ion-exchange resin.[48]

(b) 4-Chlorobutyraldehyde: colorless oil, 43%; bp 50–53 °C/20 mmHg; IR (neat) $\nu_{C=O}$ = 1730 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.08 (m, C–CH_2–C, 2H), 2.65 (t, J = 6.5, CH_2–CO, 2H), 3.57 (t, J = 6, $ClCH_2$, 2H), 9.79 (s, HCO, 1H).

(c) 4-Chlorovaleraldehyde: colorless oil, 66%; bp 85–90 °C/20 mmHg; IR (neat) $\nu_{C=O}$ = 1730 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.78 (m, C–CH_2–CH_2–C, 4H), 2.47 (m, CH_2–CO, 2H), 3.53 (t, J = 6, $ClCH_2$, 2H), 9.76 (t, J = 1, HCO, 1H).

(d) 4-Chlorobutyraldehyde diethyl acetal: colorless oil, 72%; bp 88–92 °C/20 mmHg; IR (neat) ν_{C-O} = 1080 and 1140 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.18 (t, J = 7, C–CH_3, 6H), 1.70–1.90 (m, C–CH_2CH_2–C, 4H), 3.40–3.70 (m, C–OCH_2–C + C–CH_2Cl, 6H), 4.49 (t, J = 5, OCHO, 1H).

(e) 4-Chlorovaleraldehyde diethyl acetal: colorless oil, 87%; bp 110–112 °C/20 mmHg; IR (neat) ν_{C-O} = 1070 and 1130 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.18 (t, J = 7, C–CH_3, 6H), 1.46–1.52 (m, C–CH_2–C, 2H), 1.59–1.64 (m, C–CH_2–C, 2H), 1.75–1.81 (m, C–CH_2–C, 2H), 3.44–

(43) Hale, W. J.; Hoyt, W. V. *J. Am. Chem. Soc.* **1915**, *37*, 2538.

(44) Youngquist, R. S. Ph.D. Dissertation, California Institute of Technology, Pasadena, CA, 1988.

(45) Bis[3-(dimethylamino)propyl]methylamine was prepared by methylation of bis[3-(dimethylamino)propyl]amine. Borch, R. F.; Hassid, A. I. *J. Org. Chem.* **1972**, *37*, 1673.

(46) Sambrook, J.; Fritsch, E. F.; Maniatis, T. *Molecular Cloning, A Laboratory Manual*, 2nd ed.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, New York, 1989.

(47) Burgstahler, A. W.; Weigel, L. O.; Shaefer, C. G. *Synthesis* **1976**, 767.

(48) Patwardhan, S. A.; Dev, S. *Synthesis* **1974**, 348.

3.50 (m, C–OCH_2–C, 2H), 3.52 (t, J = 7, C–CH_2Cl, 2H), 3.59–3.65 (m, C–OCH_2–C, 2H), 4.46 (t, J = 6, OCHO, 1H).

(f) Ethyl 1-(3,3-Diethoxypropyl)-4-nitro-2-pyrrolecarboxylate (6a). To a solution of ethyl 4-nitro-2-pyrrolecarboxylate (1.84 g, 10 mmol) in 50 mL of DMF, K metal (0.63 g, 16 mmol) was added. After the reaction mixture was stirred at room temperature for 3 h, the metal was completely dissolved and the yellow solution turned orange. KI (1.7 g, 10 mmol) and 3-chloropropionaldehyde diethyl acetal were added at room temperature, and the solution was warmed to 100 °C and stirred for 5 h at 100 °C. The reaction was followed by TLC (SiO_2, hexane/EtOAc = 3:1). Usually the color of the solution turns back to yellow and some white precipitate forms during the reaction. After cooling, the reaction mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was dissolved in 200 mL of CH_2Cl_2, and the organic phase was washed with 100 mL of 5% aqueous Na_2CO_3 and dried over K_2CO_3. Removal of the solvent gave the crude product, which was purified by a flash column (SiO_2, hexane/EtOAc = 3:1). **6a**: 2.2 g, 70%; pale-yellow oil; TLC (SiO_2, hexane/EtOAc = 3:1) R_f = 0.63; IR (neat) $\nu_{C=O}$ = 1720 cm^{-1}, ν_{N-O} = 1510, 1320 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.18 (t, J = 7, C–O–C–CH_3, 6H), 1.35 (t, J = 7, COO–C–CH_3, 3H), 2.07–2.13 (m, C–CH_2–C, 2H), 3.39–3.50 (m, C–O–CH_2–C, 2H), 3.56–3.67 (m, C–O–CH_2–C, 2H), 4.29 (q, J = 7, $COOCH_2$–C, 2H), 4.42 (t, J = 7, pyrrole N–CH_2–C, 2H), 4.44 (t, J = 5.5, C–CH(O–C–C)$_2$, 1H), 7.41 (d, J = 2, pyrrole Ar-H, 1H), 7.62 (d, J = 2, pyrrole Ar-H, 1H); LRMS (EI) m/z (relative abundance) 314 (M^+, 12), 269 ($M^+ - OC_2H_5$, 17), 198 ($M^+ - CH_2CH(OC_2H_5)_2$, 60), 103 ($(C_2H_5O)_2CH^+$, 64).

(g) Ethyl 1-(4,4-Diethoxybutyl)-4-nitro-2-pyrrolecarboxylate (6b). The procedure used for the synthesis of **6b** was much the same as that employed for **6a**. **6b**: 2.9 g, 88%; pale-yellow oil; TLC (SiO_2, hexane/EtOAc = 3:1) R_f = 0.56; IR (neat) $\nu_{C=O}$ = 1720 cm^{-1}, ν_{N-O} = 1510, 1320 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.18 (t, J = 7, C–O–C–CH_3, 6H), 1.34 (t, J = 7, COO–C–CH_3, 3H), 1.58–1.62 (m, C–CH_2–C, 2H), 1.86–1.89 (m, C–CH_2–C, 2H), 3.44–3.47 (m, C–O–CH_2–C, 2H), 3.58–3.62 (m, C–O–CH_2–C, 2H), 4.28 (q, J = 7, $COOCH_2$–C, 2H), 4.37 (t, J = 7, pyrrole N–CH_2–C, 2H), 4.46 (t, J = 5.5, C–CH(O–C–C)$_2$, 1H), 7.41 (d, J = 2, pyrrole Ar-H, 1H), 7.63 (d, J = 2, pyrrole Ar-H, 1H); LRMS (EI) m/z (relative abundance) 328 (M^+, 13), 283 ($M^+ - OC_2H_5$, 16), 211 ($M^+ - CH_3CH(OC_2H_5)_2$, 21), 181 ($M^+ - CH_3CH(OC_2H_5)_2 - C_2H_6$, 49), 103 ($(C_2H_5O)_2CH^+$, 100).

(h) Ethyl 1-(5,5-Diethoxypentyl)-4-nitro-2-pyrrolecarboxylate (6c). The procedure used for the synthesis of **6c** was much the same as that employed for **6a**. **6c**: 2.4 g, 70%; pale-yellow oil; TLC (SiO_2, hexane/EtOAc = 3:1) R_f = 0.45; IR (neat) $\nu_{C=O}$ = 1720 cm^{-1}, ν_{N-O} = 1510, 1320 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.17 (t, J = 7, C–O–C–CH_3, 6H), 1.35 (t, J = 7, COO–C–CH_3, 3H), 1.35–1.43 (m, C–CH_2–C, 2H), 1.60–1.66 (m, C–CH_2–C, 2H), 1.76–1.86 (m, C–CH_2–C, 2H), 3.41–3.51 (m, C–O–CH_2–C, 2H), 3.56–3.66 (m, C–O–CH_2–C, 2H), 4.29 (q, J = 7, $COOCH_2$–C, 2H), 4.33 (t, J = 7, pyrrole N–CH_2–C, 2H), 4.46 (t, J = 5.5, C–CH(O–C–C)$_2$, 1H), 7.41 (d, J = 2, pyrrole Ar-H, 1H), 7.60 (d, J = 2, pyrrole Ar-H, 1H); LRMS (EI) m/z (relative abundance) 342 (M^+, 4), 296 ($M^+ - OC_2H_5$, 12), 103 ($(C_2H_5O)_2CH^+$, 100).

(i) *N,N*-Dimethyl-3-(1-methyl-4-nitro-2-pyrrolecarboxamido)propylamine (10). To a solution of 1-methyl-4-nitro-2-pyrrolecarboxylic acid (1.7 g, 10 mmol) in 50 mL of dry THF was added 3-(dimethylamino)-propylamine (1.02 g, 10 mmol). After the reaction mixture was cooled to 0 °C, DECP (2.3 g, 14 mmol) and Et_3N (2.02 g, 20 mmol) were added dropwise to the solution. The solution was stirred at 0 °C for 2 h and at room temperature for another 10 h. Solvent was evaporated to dryness in vacuo, and the resulting residue was dissolved in 200 mL of CH_2Cl_2. The organic phase was washed with 2% aqueous Na_2CO_3 and dried over K_2CO_3. Removal of the solvent gave the crude product, which was purified by recrystallization from EtOAc. **10**: 60%; pale-yellow plates; mp 125.5–127.5 °C; TLC (SiO_2/MeOH) R_f = 0.26; IR (KBr) ν_{N-H} = 3260 cm^{-1}, $\nu_{C=O}$ = 1660 cm^{-1}, ν_{N-O} = 1500, 1300 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.68–1.73 (m, C–CH_2–C, 2H), 2.30 (s, N–CH_3, 6H), 2.48 (t, J = 6, C–CH_2–N, 2H), 3.34–3.47 (m, CO–N–CH_2–C, 2H), 3.98 (s, pyrrole N–CH_3, 3H), 6.90, 7.50 (4d, J = 2, pyrrole Ar-H, 4H), 8.71 (bs, CONH, 1H); LRMS (EI) m/z (relative abundance) 254 (M^+, 1.2), 153 ($M^+ - NHC_3H_6NMe_2$, 4). Anal. Calcd for $C_{11}H_{18}N_4O_3$: C, 51.96; H, 7.13; N, 22.03. Found: C, 51.90; H, 7.27; N, 22.09.

(j) Ethyl 1-(3-Oxopropyl)-4-nitro-2-pyrrolecarboxylate (16a). A solution of **6a** (2.2 g, 7.0 mmol) and pyridinium *p*-toluenesulfonate (0.2 g, 0.8 mmol) in 100 mL of acetone and 10 mL of H_2O was refluxed for 10 h, and the reaction was followed by TLC (SiO_2, hexane/EtOAc = 3:1). After cooling, the solution was concentrated and the residue obtained was dissolved in 200 mL of CH_2Cl_2. The organic phase was washed with saturated aqueous NaCl and dried over $MgSO_4$. Removal of the solvent gave the product as a colorless oil which became solid on standing. **16a**: 1.4 g, 83%; white solid; mp 65–67 °C; TLC (SiO_2, hexane/EtOAc = 3:1) R_f = 0.20; IR (KBr) $\nu_{C=O}$ = 1710 cm^{-1}, ν_{N-O} = 1500, 1320 cm^{-1}, ν_{C-O} = 1100, 1080 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.37 (t, J = 7, COO–C–CH_3, 3H), 3.09 (t, J = 6.5, C–CH_2–CO, 2H), 4.31 (q, J = 7, $COOCH_2$–C, 2H), 4.64 (t, J = 6.5, pyrrole N–CH_2–C, 2H), 7.43, 7.77 (2d, J = 2, Ar-H, 2H), 9.78 (s, C–CHO, 1H); LRMS (EI) m/z (relative abundance) 240 (M^+, 22), 212 ($M^+ - CO$, 100), 198 ($M^+ - COCH_2$, 57). Anal. Calcd for $C_{10}H_{12}N_2O_5$: C, 50.00; H, 5.40; N, 11.66. Found: C, 50.02; H, 5.05; N, 11.46.

(k) Ethyl 1-(4-Oxobutyl)-4-nitro-2-pyrrolecarboxylate (16b). The procedure used for the synthesis of **16b** was much the same as that employed for **16a**. **16b**: 92%; white solid; mp 82–84 °C; TLC (SiO_2, hexane/EtOAc = 3:1) R_f = 0.21; IR (KBr) $\nu_{C=O}$ = 1720 cm^{-1}, ν_{N-O} = 1500, 1320 cm^{-1}, ν_{C-O} = 1120, 1090 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.35 (t, J = 7, COO–C–CH_3, 3H), 2.07–2.17 (m, C–CH_2–C, 2H), 2.51 (t, J = 7, C–CH_2–CO, 2H), 4.31 (q, J = 7, $COOCH_2$–C, 2H), 4.39 (t, J = 7, pyrrole N–CH_2–C, 2H), 7.42, 7.61 (2d, J = 2, Ar-H, 2H), 9.67 (s, C–CHO, 1H); LRMS (EI) m/z (relative abundance) 254 (M^+, 5), 226 ($M^+ - CO$, 16), 181 ($M^+ - COOC_2H_5$, 100). Anal. Calcd for $C_{11}H_{14}N_2O_5$: C, 51.95; H, 5.55; N, 11.02. Found: C, 51.93; H, 5.64; N, 10.82.

(l) Ethyl 1-(5-Oxopentyl)-4-nitro-2-pyrrolecarboxylate (16c). The procedure used for the synthesis of **16c** was much the same as that employed for **16a**. **16c**: 96%; white solid; mp 69–71 °C; TLC (SiO_2, hexane/EtOAc = 3:1) R_f = 0.21; IR (KBr) $\nu_{C=O}$ = 1720 cm^{-1}, ν_{N-O} = 1500, 1320 cm^{-1}, ν_{C-O} = 1120, 1090 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.35 (t, J = 7, COO–C–CH_3, 3H), 1.59–1.69 (m, C–CH_2–C, 2H), 1.78–1.88 (m, C–CH_2–C, 2H), 2.50 (t, J = 7, C–CH_2–CO, 2H), 4.29 (q, J = 7, $COOCH_2$–C, 2H), 4.35 (t, J = 7, pyrrole N–CH_2–C, 2H), 7.41, 7.60 (2d, J = 2, Ar-H, 2H), 9.75 (s, C–CHO, 1H); LRMS (EI) m/z (relative abundance) 268 (M^+, 10), 198 ($M^+ - COC_3H_6$, 23), 167 ($M^+ - COOC_2H_5 - C_2H_4$, 77). Anal. Calcd for $C_{12}H_{16}N_2O_5$: C, 53.71; H, 6.01; N, 10.45. Found: C, 54.01; H, 6.03; N, 10.47.

(m) Ethyl 1-{3-[*N,N*-Bis(3-(*N,N*-dimethylamino)propyl)amino]propyl}-4-nitro-2-pyrrolecarboxylate (17a). To a solution of 3,3′-iminobis(*N,N*-dimethylpropylamine) (2.2 g, 12 mmol) and acetic acid (0.36 g, 6 mmol) in 50 mL of methanol was added **16a** (1.2 g, 5 mmol), followed by sodium cyanoborohydride (0.4 g, 6 mmol). The solution was stirred at room temperature for 36 h, by which time TLC (SiO_2, MeOH/concentrated aqueous NH_3 = 95:5) showed complete reaction. Concentrated HCl was added to pH = 2, and the solution was basified to pH = 12 by adding solid NaOH after gas evolution stopped. Methanol was removed under reduced pressure, the residue was dissolved in 200 mL of CH_2Cl_2, and the organic phase was washed with 5% Na_2CO_3 and dried over K_2CO_3. After removal of the solvent, flash chromatography on silica gel (SiO_2, MeOH/concentrated aqueous NH_3 = 95:5) provided 1.1 g (54%) of **17a** as a pale-yellow oil. **17a**: TLC (SiO_2, MeOH/concentrated aqueous NH_3 = 95:5) R_f = 0.20; IR (KBr) $\nu_{C=O}$ = 1710 cm^{-1}, ν_{N-O} = 1500, 1320 cm^{-1}, ν_{C-O} = 1100, 1080 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.34 (t, J = 7, COO–C–CH_3, 3H), 1.55–1.67 (m, C–CH_2–C, 4H), 1.88–1.95 (m, C–CH_2–C, 2H), 2.27 (s, NCH_3, 12H), 2.30–2.45 (m, CH_2–N, 10H), 4.28 (q, J = 7, $COOCH_2$–C, 2H), 4.38 (t, J = 7, pyrrole N–CH_2–C, 2H), 7.40, 7.71 (2d, J = 2, Ar-H, 2H); LRMS (FAB) m/z 412 (calcd for $C_{20}H_{38}N_5O_4$ (M + H^+) 412).

(n) Ethyl 1-{4-[*N,N*-Bis(3-(*N,N*-dimethylamino)propyl)amino]butyl}-4-nitro-2-pyrrolecarboxylate (17b). The procedure used for the synthesis of **17b** was much the same as that employed for **17a**. **17b**: 89%; pale-yellow oil; TLC (SiO_2, MeOH/concentrated aqueous NH_3 = 95:5) R_f = 0.20; IR (KBr) $\nu_{C=O}$ = 1720 cm^{-1}, ν_{N-O} = 1510, 1320 cm^{-1}, ν_{C-O} = 1100, 1080 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.34 (t, J = 7, COO–C–CH_3, 3H), 1.40–1.44 (m, C–CH_2–C, 2H), 1.50–1.58 (m, C–CH_2–C, 4H), 1.77–1.80 (m, C–CH_2–C, 2H), 2.18 (s, NCH_3, 12H), 2.20–2.41 (m, CH_2–N, 10H), 4.28 (q, J = 7, $COOCH_2$–C, 2H), 4.34 (t, J = 7, pyrrole N–CH_2–C, 2H), 7.41, 7.61 (2d, J = 2, Ar-H, 2H); LRMS (FAB) m/z 426 (calcd for $C_{21}H_{40}N_5O_4$ (M + H^+) 426).

(o) Ethyl 1-{5-[*N,N*-Bis(3-(*N,N*-dimethylamino)propyl)amino]pentyl}-4-nitro-2-pyrrolecarboxylate (17c). The procedure used for the synthesis of **17c** was much the same as that employed for **17a**. **17c**: 95%; pale-yellow oil; TLC (SiO_2, MeOH/concentrated aqueous NH_3 = 95:5) R_f = 0.19; IR (KBr) $\nu_{C=O}$ = 1720 cm^{-1}, ν_{N-O} = 1510, 1320 cm^{-1}, ν_{C-O} = 1110, 1080 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.22–1.30 (m, C–CH_2–C, 2H), 1.34 (t, J = 7, COO–C–CH_3, 3H), 1.41–1.45 (m, C–CH_2–C, 2H), 1.53–1.59 (m, C–CH_2–C, 4H), 1.75–1.80 (m, C–CH_2–C, 2H), 2.19 (s, NCH_3, 12H), 2.20–2.40 (m, CH_2–N, 10H), 4.28 (q, J = 7, $COOCH_2$–C, 2H),

4.32 (t, J = 7, pyrrole N–CH_2–C, 2H), 7.41, 7.61 (2d, J = 2, Ar-H, 2H); LRMS (FAB) m/z 440 (calcd for $C_{22}H_{42}N_5O_4$ (M + H^+) 440).

(p) Ethyl 1-{3-[*N*,*N*-Bis(3-(*N*,*N*-dimethylamino)propyl)amino]propyl}-4-(1-methyl-4-nitro-2-pyrrolecarboxylamido)-2-pyrrolecarboxylate (18a). A solution of **17a** (1.0 g, 2.4 mmol) in 100 mL of MeOH was hydrogenated over 10% palladium on charcoal (500 mg) at room temperature and atmospheric pressure. The catalyst was removed by filtration, and the filtrate was concentrated. To the residue were added dry DMF (80 mL) and 1-methyl-4-nitro-2-pyrrolecarboxylic acid (0.53 g, 3 mmol). After the reaction mixture was cooled to 0 °C, DECP (0.65 g, 4 mmol) and Et_3N (2.0 g, 20 mmol) were added. The solution was stirred at 0 °C for 2 h and at room temperature overnight. Solvent was evaporated to dryness in vacuo, and the resulting residue was dissolved in 200 mL, CH_2Cl_2. The organic phase was washed with 5% aqueous Na_2CO_3 and dried over K_2CO_3. After removal of the solvent, flash chromatography on silica gel (SiO_2, MeOH/concentrated aqueous NH_3 = 90:10) provided 1.0 g (78%) of **18a** as a yellow oil. **18a**: TLC (SiO_2, MeOH/concentrated aqueous NH_3 = 90:10) R_f = 0.38; IR (neat) ν_{N-H} = 3380 cm^{-1}, $\nu_{C=O}$ = 1680, 1660 cm^{-1} ν_{N-O} = 1500, 1320 cm^{-1}, ν_{C-O} = 1100 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.31 (t, J = 7, COO–C–CH_3, 3H), 1.54–1.60 (m, C–CH_2–C, 4H), 1.86–1.89 (m, C–CH_2–C, 2H), 2.24 (s, NCH_3, 12H), 2.28–2.39 (m, C–CH_2–N, 10H), 4.00 (s, pyrrole N–CH_3, 3H), 4.23 (q, J = 7, $COOCH_2$–C, 2H), 4.30 (t, J = 6.5, pyrrole N–CH_2–C, 2H), 7.12, 7.17, 7.25, 7.55 (4d, J = 2, pyrrole Ar-H, 4H), 8.87 (bs, CONH, 1H); LRMS (FAB) m/z 534 (calcd for $C_{26}H_{44}N_7O_5$ (M + H^+) 534).

(q) Ethyl 1-{4-[*N*,*N*-Bis(3-(*N*,*N*-dimethylamino)propyl)amino]butyl}-4-(1-methyl-4-nitro-2-pyrrolecarboxamido)-2-pyrrolecarboxylate (18b). **18b** was prepared similarly to **18a**. **18b**: 69%; yellow oil; TLC (SiO_2, MeOH/concentrated aqueous NH_3 = 90:10) R_f = 0.37; IR (neat) ν_{N-H} = 3380 cm^{-1}, $\nu_{C=O}$ = 1680, 1660 cm^{-1}, ν_{N-O} = 1500, 1320 cm^{-1}, ν_{C-O} = 1100 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.32 (t, J = 7, COO–C–CH_3, 3H), 1.35–1.43 (m, C–CH_2–C, 2H), 1.50–1.60 (m, C–CH_2–C, 4H), 1.69–1.79 (m, C–CH_2–C, 2H), 2.19 (s, NCH_3, 12H), 2.22–2.39 (m, C–CH_2–N, 10H), 4.00 (s, pyrrole N–CH_3, 3H), 4.24 (q, J = 7, $COOCH_2$–C, 2H), 4.28 (t, J = 6.5, pyrrole N–CH_2–C, 2H), 6.90, 7.23, 7.32, 7.55 (4d, J = 2, pyrrole Ar-H, 4H), 8.20 (bs, CONH, 1H); LRMS (FAB) m/z 548 (calcd for $C_{27}H_{46}N_7O_5$ (M + H^+) 548).

(r) Ethyl 1-{5-[*N*,*N*-Bis(3-(*N*,*N*-dimethylamino)propyl)amino]pentyl}-4-(1-methyl-4-nitro-2-pyrrolecarboxamido)-2-pyrrolecarboxylate (18c). **18c** was prepared similarly to **18a**. **18c**: 44%; yellow oil; TLC (SiO_2, MeOH/concentrated aqueous NH_3 = 90:10) R_f = 0.35; IR (neat) ν_{N-H} = 3400 cm^{-1}, $\nu_{C=O}$ = 1680, 1660 cm^{-1}, ν_{N-O} = 1500, 1320 cm^{-1}, ν_{C-O} = 1100 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.20–1.29 (m, C–CH_2–C, 2H), 1.32 (t, J = 7, COO–C–CH_3, 3H), 1.32–1.41 (m, C–CH_2–C, 2H), 1.50–1.60 (m, C–CH_2–C, 4H), 1.72–1.80 (m, C–CH_2–C, 2H), 2.19 (s, NCH_3, 12H), 2.22–2.42 (m, C–CH_2–N, 10H), 4.00 (s, pyrrole N–CH_3, 3H), 4.24 (q, J = 7, $COOCH_2$–C, 2H), 4.26 (t, J = 6.5, pyrrole N–CH_2–C, 2H), 6.86, 7.19, 7.33, 7.57 (4d, J = 2, pyrrole Ar-H, 4H), 8.07 (bs, CONH, 1H); LRMS (FAB) m/z 562 (calcd for $C_{28}H_{48}N_7O_5$ (M + H^+) 562).

(s) 1-{3-[*N*,*N*-Bis(3-(*N*,*N*-dimethylamino)propyl)amino]propyl}-4-(1-methyl-4-nitro-2-pyrrolecarboxamido)-2-pyrrolecarboxylic acid (19a). To a solution of **18a** (0.8 g, 1.5 mmol) in 100 mL of EtOH was added NaOH (0.2 g, 5 mmol) in 30 mL of H_2O. The resulting solution was refluxed for 10 h, and the reaction was followed by TLC (SiO_2, MeOH/EtOAc/Et_3N = 3:3:1). After cooling, concentrated HCl (0.5 mL, 6 mmol) was added and the color of the solution turned from orange to pale yellow (pH = 7). The solution was concentrated to dryness under reduced pressure, and the residue was extracted by MeOH (3 × 50 mL). Removal of the solvent gave the product as a dark-yellow glassy solid (very hygroscopic). **19a**: 100%; TLC (SiO_2, MeOH/EtOAc/Et_3N = 3:3:1) R_f = 0.10; IR (neat) ν_{N-H} = 3380 cm^{-1}, ν_{N^+-H} = 2400–2800 cm^{-1}, $\nu_{C=O}$ = 1660 cm^{-1}, ν_{N-O} = 1530, 1320 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.70–1.75 (m, C–CH_2–C, 4H), 1.75–1.84 (m, C–CH_2–C, 2H), 2.55 (s, NCH_3, 12H), 2.41–2.87 (m, C–CH_2–N, 10H), 3.96 (s, pyrrole N–CH_3, 3H), 4.30 (t, J = 7, pyrrole N–CH_2–C, 2H), 6.85, 7.44, 7.70, 8.19 (4d, J = 2, pyrrole Ar-H, 4H), 10.45 (s, CONH, 1H).

(t) 1-{4-[*N*,*N*-Bis(3-(*N*,*N*-dimethylamino)propyl)amino]butyl}-4-(1-methyl-4-nitro-2-pyrrolecarboxamido)-2-pyrrolecarboxylic acid (19b). **19b** was prepared by a procedure similar to that used for **19a**. **19b**: 100%; yellow glassy solid; TLC (SiO_2, MeOH/EtOAc/Et_3N = 3:3:1) R_f = 0.10; IR (neat) ν_{N-H} = 3400 cm^{-1}, ν_{N^+-H} = 2400–2800 cm^{-1}, $\nu_{C=O}$ = 1650 cm^{-1}, ν_{N-O} = 1530, 1320 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.35–1.45 (m, C–CH_2–C, 2H), 1.60–1.70 (m, C–CH_2–C, 2H), 1.80–1.90 (m, C–CH_2–C, 4H), 2.70 (s, NCH_3, 12H), 2.50–3.04 (m, C–CH_2–N, 10H), 3.98 (s, pyrrole N–CH_3, 3H), 4.30 (t, J = 7, pyrrole N–CH_2–C, 2H), 6.96, 7.51, 7.80, 8.20 (4d, J = 2, pyrrole Ar-H, 4H), 10.61 (s, CONH, 1H).

(u) 1-{5-[*N*,*N*-Bis(3-(*N*,*N*-dimethylamino)propyl)amino]pentyl}-4-(1-methyl-4-nitro-2-pyrrolecarboxamido)-2-pyrrolecarboxylic acid (19c). **19c** was prepared by a procedure similar to that used for **19a**. **19c**: 100%; yellow glassy solid; TLC (SiO_2, MeOH/EtOAc/Et_3N = 3:3:1) R_f = 0.10; IR (neat) ν_{N-H} = 3400 cm^{-1}, ν_{N^+-H} = 2400–2800 cm^{-1}, $\nu_{C=O}$ = 1650 cm^{-1}, ν_{N-O} = 1530, 1320 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.16–1.22 (m, C–CH_2–C, 2H), 1.39–1.46 (m, C–CH_2–C, 2H), 1.61–1.70 (m, C–CH_2–C, 2H), 1.71–1.80 (m, C–CH_2–C, 4H), 2.60 (s, NCH_3, 12H), 2.40–2.92 (m, C–CH_2–N, 10H), 3.96 (s, pyrrole N–CH_3, 3H), 4.35 (t, J = 7, pyrrole N–CH_2–C, 2H), 6.80, 7.38, 7.69, 8.18 (4d, J = 2, pyrrole Ar-H, 4H), 10.42 (s, CONH, 1H).

(v) *N*,*N*-Dimethyl-3-{1-methyl-4-[1-(3-(*N*,*N*-bis(3-(*N*,*N*-dimethylamino)propyl)amino)propyl)-4-(1-methyl-4-nitro-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]-2-pyrrolecarboxamido}propylamine (20a). A solution of **10** (0.38 g, 1.5 mmol) in 100 mL of MeOH was hydrogenated over 10% palladium on charcoal (500 mg) at room temperature and atmospheric pressure. The catalyst was removed by filtration, and the filtrate was concentrated. To the residue was added **19a** (0.8 g, 1.4 mmol) in dry DMF (50 mL). After the reaction mixture was cooled to 0 °C, DECP (0.33 g, 2.0 mmol) and Et_3N (1.0 g, 10 mmol) were added dropwise to the solution. The solution was stirred at 0 °C for 2 h and at room temperature for another 10 h. Solvent was removed to dryness in vacuo, and the resulting residue was dissolved in 200 mL of CH_2Cl_2. The organic phase was washed with 2% aqueous Na_2CO_3 and dried over K_2CO_3. The crude product was purified with a flash column (SiO_2, MeOH/concentrated NH_3 aqueous = 90:10) to give **20a** as a yellow glassy solid (0.7 g, 70%). **20a**: TLC (SiO_2, MeOH/concentrated NH_3 aqueous = 90:10) R_f = 0.19; IR (neat) ν_{N-H} = 3280 cm^{-1}, $\nu_{C=O}$ = 1650 cm^{-1}, ν_{N-O} = 1520, 1310 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.55–1.60 (m, N–C–CH_2–C–N, 4H), 1.72–1.76 (m, CON–C–CH_2–C–N, 2H), 1.89–1.92 (m, C–CH_2–C, 2H), 2.27 (s, N–CH_3, 12H), 2.25 (s, N–CH_3, 6H), 2.33–2.39 (m, CH_2–N, 8H), 2.48 (t, J = 6, CH_2–N, 2H), 3.41–3.44 (m, CON–CH_2–C, 2H), 3.87 (s, pyrrole N–CH_3, 3H), 4.01 (s, pyrrole N–CH_3, 3H), 4.35 (t, J = 7, pyrrole N–CH_2–C, 2H), 6.45, 6.97, 7.08, 7.16, 7.46, 7.54 (6d, J = 2, pyrrole Ar–H, 6H), 7.71, 7.87, 9.83 (3bs, CONH, 3H); LRMS (FAB) m/z 712 (calcd for $C_{35}H_{58}N_{11}O_5$ (M + H^+) 712).

(w) *N*,*N*-Dimethyl-3-{1-methyl-4-[1-(4-(*N*,*N*-bis(3-(*N*,*N*-dimethylamino)propyl)amino)butyl)-4-(1-methyl-4-nitro-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]-2-pyrrolecarboxamido}propylamine (20b). **20b** was prepared by a method similar to that used for **20a**. **20b**: 40%; yellow glassy solid; TLC (SiO_2, MeOH/conentrated aqueous NH_3 = 90:10) R_f = 0.21; IR (KBr) ν_{N-H} = 3300 cm^{-1}, $\nu_{C=O}$ = 1650 cm^{-1}, ν_{N-O} = 1520, 1310 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.36–1.41 (m, C–CH_2–C, 2H), 1.48–1.58 (m, N–C–CH_2–C–N, 4H), 1.70–1.76 (m, CON–C–CH_2–C–N + C–CH_2–C, 4H), 2.17 (s, N–CH_3, 12H), 2.20–2.24 (m, CH_2–N, 4H), 2.25 (s, N–CH_3, 6H), 2.31–2.37 (m, CH_2–N, 4H), 2.42 (t, J = 6, CH_2–N, 2H), 3.44–3.51 (m, CON–CH_2–C, 2H), 3.81 (s, pyrrole N–CH_3, 3H), 4.01 (s, pyrrole N–CH_3, 3H), 4.29 (t, J = 7, pyrrole N–CH_2–C, 2H), 6.40, 6.46, 7.04, 7.31, 7.42, 7.55 (6s, pyrrole Ar–H, 6H), 7.66, 7.93, 9.40 (3bs, CONH, 3H); LRMS (FAB) m/z 726 (calcd for $C_{36}H_{60}N_{11}O_5$ (M + H^+) 726).

(x) *N*,*N*-Dimethyl-3-{1-methyl-4-[1-(5-(*N*,*N*-bis(3-(*N*,*N*-dimethylamino)propyl)amino)pentyl)-4-(1-methyl-4-nitro-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]-2-pyrrolecarboxamido}propylamine (20c). **20c** was prepared in a fashion similar to that used for **20a**. **20c**: 43%; yellow glassy solid; TLC (SiO_2, MeOH/concentrated aqueous NH_3 = 90:10) R_f = 0.22; IR (KBr) ν_{N-H} = 3300 cm^{-1}, $\nu_{C=O}$ = 1650 cm^{-1}, ν_{N-O} = 1520, 1310 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.20–1.26 (m, C–CH_2–C, 2H), 1.34–1.40 (m, C–CH_2–C, 2H), 1.50–1.56 (m, N–C–CH_2–C–N, 4H), 1.69–1.77 (m, CON–C–CH_2–C–N + C–CH_2–C, 4H), 2.17 (s, N–CH_3, 12H), 2.19–2.22 (m, CH_2–N, 4H), 2.25 (s, N–CH_3, 6H), 2.31–2.36 (m, CH_2N, 4H), 2.42 (t, J = 6, CH_2–N, 2H), 3.44–3.51 (m, CON–CH_2–C, 2H), 3.81 (s, pyrrole N–CH_3, 3H), 4.02 (s, pyrrole N–CH_3, 3H), 4.28 (t, J = 7, pyrrole N–CH_2–C, 2H), 6.44, 6.47, 7.05, 7.27, 7.46, 7.55 (6s, pyrrole Ar-H, 6H), 7.61, 7.86, 9.31 (3bs, CONH, 3H); LRMS (FAB) m/z 740 (calcd for $C_{37}H_{62}N_{11}O_5$ (M + H^+) 740).

(y) *N*,*N*-Dimethyl-3-{1-methyl-4-[1-(3-(*N*,*N*-bis(3-(*N*,*N*-dimethylamino)propyl)amino)propyl)-4-(1-methyl-4-acetylamido-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]-2-pyrrolecarboxamido}propylamine (5a). A solution of **20a** (0.5 g, 0.7 mmol) in 100 mL of MeOH was hydrogenated over 10% palladium on charcoal (500 mg) at room temperature and atmospheric pressure. The catalyst was removed by filtration, and the filtrate was concentrated and placed on a high-vacuum to remove MeOH.

The residue was dissolved in dry DMF (50 mL). After cooling to 0 °C, CH_3COCl (0.1 g, 1.1 mmol) and Et_3N (0.3 g, 3 mmol) were added dropwise to the solution. The solution was stirred at 0 °C for 2 h and at room temperature for another 10 h. Solvent was evaporated to dryness in vacuo, and the resulting residue was dissolved in 200 mL of CH_2Cl_2. The organic phase was washed with 2% aqueous Na_2CO_3 and dried over K_2CO_3. The crude product was purified with a flash column (SiO_2, MeOH:concentrated aqueous NH_3 = 90:10) to give **5a** as a pale-yellow glassy solid (0.2 g, 40%). **5a**: TLC (SiO_2, MeOH/concentrated NH_3 aqueous = 90:10) R_f = 0.26; FT-IR ($CHCl_3$) ν_{N-H} = 3304 cm^{-1}, $\nu_{C=O}$ = 1647 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.50–1.55 (m, N–C–CH_2–C–N, 4H), 1.68–1.71 (m, CON–C–CH_2–C–N, 2H), 1.84–1.90 (m, pyrrole N–C–CH_2–C–N, 2H), 2.07 (s, CH_3CO–N, 3H), 2.17 (s, N–C–C–C–N–CH_3, 12 H), 2.24 (s, CON–C–C–C–N–CH_3, 6H), 2.21–2.27 (m, pyrrole N–C–C–C–N–CH_2, 4H), 2.31–2.37 (m, pyrrole N–C–C–CH_2–N, 4H), 2.40 (t, J = 6.5, CON–C–C–CH_2–N, 2H), 3.39–3.42 (m, CON–CH_2–C, 2H), 3.85 (s, pyrrole N–CH_3, 3H), 3.86 (s, pyrrole N–CH_3, 3H), 4.28 (t, J = 6.5, pyrrole N–CH_2–C, 2H), 6.54, 6.63, 6.76, 7.06, 7.13, 7.18 (6s, pyrrole Ar–H, 6H), 7.73, 8.09, 8.23, 8.25 (4bs, CONH, 4H); HRMS (laser desorption) m/z 724.4892 (calcd for $C_{37}H_{62}N_{11}O_4$ (M + H^+) 724.4986). Anal. Calcd for $C_{38}H_{61}N_{11}O_4 + 1.5H_2O$: C, 59.18; H, 8.59; N, 20.52. Found: C, 58.94; H, 8.29; N, 20.16.

(z) *N,N*-Dimethyl-3-{1-methyl-4-[1-(4-(*N,N*-bis(3-(*N,N*-dimethylamino)propyl)amino)butyl)-4-(1-methyl-4-acetylamido-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]-2-pyrrolecarboxamido}propylamine (5b). **5b** was prepared by the general procedure used for **5a**. **5b**: 40%, pale-yellow glassy solid; TLC (SiO_2, MeOH/concentrated NH_3 aqueous = 85:15) R_f = 0.28; FT-IR ($CHCl_3$) ν_{N-H} = 3302 cm^{-1}, $\nu_{C=O}$ = 1650 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.33–1.41 (m, pyrrole N–C–C–CH_2–C, 2H), 1.49–1.55 (m, N–C–CH_2–C–N, 4H), 1.67–1.75 (m, CON–C–CH_2–C–N + pyrrole N–C–CH_2–C–C–N, 4H), 2.07 (s, CH_3CO–N, 3H), 2.16 (s, N–C–C–C–N–CH_3, 12H), 2.17–2.21 (m, pyrrole N–C–C–C–C–C–N–CH_2, 4H), 2.24 (s, CON–C–C–C–N–CH_3, 6H), 2.32–2.37 (m, pyrrole N–C–C–CH_2–N, 4H), 2.40 (t, J = 6.5, CON–C–C–CH_2–N, 2H), 3.39–3.42 (m, CON–CH_2–C, 2H), 3.85 (s, pyrrole N–CH_3, 3H), 3.86 (s, pyrrole N–CH_3, 3H), 4.27 (t, J = 6.5, pyrrole N–CH_2–C, 2H), 6.52, 6.61, 6.66, 7.02, 7.15, 7.17 (6s, pyrrole Ar–H, 6H), 7.74, 7.95, 8.08, 8.10 (4bs, CONH, 4H); HRMS (laser desorption) m/z 738.5086 (calcd for $C_{38}H_{64}N_{11}O_4$ (M + H^+) 738.5142). Anal. Calcd for $C_{38}H_{63}N_{11}O_4 + 4H_2O$: C, 56.34; H, 8.83; N, 19.02. Found: C, 56.54; H, 8.63; N, 18.72.

(aa) *N,N*-Dimethyl-3-{1-methyl-4-[1-(5-(*N,N*-bis(3-(*N,N*-dimethylamino)propyl)amino)pentyl)-4-(1-methyl-4-acetylamido-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]-2-pyrrolecarboxamido}propylamine (5c). **5c** was prepared by the general procedure used for **5a**. **5c**: 53%; pale-yellow glassy solid; TLC (SiO_2, MeOH/concentrated aqueous NH_3 = 90:10) R_f = 0.21; FT-IR ($CHCl_3$) ν_{N-H} = 3305 cm^{-1}, $\nu_{C=O}$ = 1649 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.15–1.22 (m, pyrrole N–C–C–CH_2–C–C–N, 2H), 1.30–1.36 (m, pyrrole N–C–C–C–CH_2–C–N, 2H), 1.48–1.55 (m, N–C–CH_2–C–N, 4H), 1.64–1.72 (m, CON–C–CH_2–C–N + pyrrole N–C–CH_2–C–C–C–N, 4H), 2.00 (s, CH_3CO–N, 3H), 2.14 (s, N–C–C–C–N–CH_3, 12H), 2.15–2.20 (m, pyrrole N–C–C–C–C–C–N–CH_2, 4H), 2.18 (s, CON–C–C–C–N–CH_3, 6H), 2.27–2.37 (m, pyrrole N–C–C–C–C–CH_2–N + CON–C–C–CH_2–N, 4H), 3.39–3.42 (m, CON–CH_2–C, 2H), 3.78 (s, pyrrole N–CH_3, 3H), 3.80 (s, pyrrole N–CH_3, 3H), 4.18 (t, J = 6.5, pyrrole N–CH_2–C, 2H), 6.57, 6.67, 6.68, 6.97, 7.15, 7.18 (6s, pyrrole Ar–H, 6H), 7.70, 8.24, 8.45, 8.70 (4bs, CONH, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 168.4 (CONH–Ar), 161.9 (Ar–CONH), 159.0 (2C in ArCONHAr), 123.3, 122.8, 122.3 ($3C_2$ of pyrrole), 121.6 (C_4 of pyrrole), 119.2, 118.6, 117.8 ($3C_5$ of pyrrole), 104.3, 103.8, 103.4 ($3C_3$ of pyrrole), 58.1 (CONH–C–C–*C*–N), 57.7 (N–*C*–C–C–NMe$_2$), 53.7 (pyrrole N–C–C–C–C–*C*–N), 51.8 (N–C–C–*C*–NMe$_2$), 48.5 (pyrrole N–*C*–C–C–C–C–N), 45.2 (6C of NCH_3), 38.6 (CO–NH–*C*), 36.3 (2C of pyrrole N–CH_3), 31.4 (pyrrole N–C–*C*–C–C–C–N), 26.3 (pyrrole N–C–C–C–C–C–N), 26.2 (CONH–C–*C*–C–N), 24.8 (2C of N–C–*C*–C–NMe$_2$), 24.4 (pyrrole N–C–C–*C*–C–C–N), 24.0 (*C*H_3CONH); LRMS (FAB) m/z 752 (calcd for $C_{39}H_{66}N_{11}O_4$ (M + H^+) 752). Anal. Calcd for $C_{39}H_{65}N_{11}O_4 + 2.5H_2O$: C, 58.77; H, 8.85; N, 19.33. Found: C, 58.79; H, 8.86; N, 18.76.

(bb) *N,N*-Dimethyl-3-[1-methyl-4-(1-methyl-4-nitro-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]propylamine (21). A solution of **10** (0.5 g, 2 mmol) in 100 mL of MeOH was hydrogenated over 10% palladium on charcoal (500 mg) at room and atmospheric pressure. The catalyst was removed by filtration, and the filtrate was concentrated. To the residue, 1-methyl-4-nitro-2-pyrrolecarboxylic acid (0.34 g, 2 mmol) was added in dry THF (50 mL). After the reaction mixture was cooled to 0 °C, DECP (0.41 g, 2.5 mmol) and Et_3N (1.0 g, 10 mmol) were added dropwise to the solution. The solution was stirred at 0 °C for 2 h and at room for another 10 h. Solvent was evaporated to dryness in vacuo, and the resulting residue was dissolved in 200 mL of CH_2Cl_2. The organic phase was washed with 2% aqueous Na_2CO_3 and dried over K_2CO_3. The crude product was purified with a flash column (SiO_2, MeOH/concentrated NH_3 aqueous = 99:1) to give **21** as a yellow solid (0.61 g, 80%). **21**: mp 181–183 °C; TLC (SiO_2, MeOH/concentrated NH_3 aqueous = 99:1) R_f = 0.20; IR (KBr) ν_{N-H} = 3280 cm^{-1}, $\nu_{C=O}$ = 1670, 1630 cm^{-1}, ν_{N-O} = 1500, 1310 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.68–1.72 (m, CON–C–CH_2–C–N, 2H), 2.24 (s, N–CH_3, 6H), 2.37 (t, J = 6, CH_2–N, 2H), 3.31–3.36 (m, CON–CH_2–C, 2H), 3.88, 4.03 (2s, pyrrole N–CH_3, 6H), 6.79, 7.19, 7.59, 8.06 (4s, pyrrole Ar–H, 4H), 7.86, 10.08 (2bs, CONH, 2H); LRMS (FAB) m/z 377 (calcd for $C_{17}H_{25}N_6O_4$ (M + H^+) 377).

(cc) *N,N*-Dimethyl-3-{1-methyl-4-[1-methyl-4-(1-methyl-4-nitro-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]-2-pyrrolecarboxamido}propylamine (22). **22** was prepared by a method similar to that used for **21**. **21** (0.6 g, 1.6 mmol) in 100 mL of MeOH was hydrogenated over 10% palladium on charcoal (500 mg) at room temperature and atmospheric pressure. The amine thus obtained reacted with 1-methyl-4-nitro-2-pyrrolecarboxylic acid (0.27 g, 2 mmol) in 50 mL of DMF in the presence of DECP (0.41 g, 2.5 mmol) and Et_3N (1.0 g, 10 mmol). Flash chromatography (SiO_2, MeOH/concentrated aqueous NH_3 = 98:2) gave **22** as a yellow glassy solid (0.5 g, 63%). **22**: TLC (SiO_2, MeOH/concentrated aqueous NH_3 = 98:2) R_f = 0.42; IR (KBr) ν_{N-H} = 3350 cm^{-1}, $\nu_{C=O}$ = 1650, 1630 cm^{-1}, ν_{N-O} = 1500, 1310 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.58–1.64 (m, CON–C–CH_2–C–N, 2H), 2.15 (s, N–CH_3, 6H), 2.26 (t, J = 6, CH_2–N, 2H), 3.18–3.24 (m, CON–CH_2–C, 2H), 3.80, 3.86, 3.97 (3s, pyrrole N–CH_3, 9H), 6.82, 7.03, 7.19, 7.27, 7.59, 8.19 (6d, J = 1.5, pyrrole Ar–H, 6H), 8.07 (t, J = 5, CONH–C, 1H), 9.93, 10.28 (2s, CONH–Ar, 2H); LRMS (FAB) m/z 499 (calcd for $C_{23}H_{31}N_8O_5$ (M + H^+) 499).

(dd) *N,N*-Dimethyl-3-{1-methyl-4-[1-methyl-4-(1-methyl-4-acetylamido-2-pyrrolecarboxamido)-2-pyrrolecarboxamido]-2-pyrrolecarboxamido}propylamine (2). **2** was prepared by a method similar to that used in the synthesis of **21**. **22** (0.3 g, 0.6 mmol) in 100 mL of MeOH was hydrogenated over 10% palladium on charcoal (500 mg) at room temperature and atmospheric pressure. The amine thus obtained reacted with 0.1 g (1.1 mmol) of CH_3COCl in 50 mL of DMF in the presence of Et_3N (1.0 g, 10 mmol) at 0 °C. Flash chromatography (SiO_2, MeOH/concentrated aqueous NH_3 = 98:2) gave **2** as a pale-yellow glassy solid (0.2 g, 65%). **2**: TLC (SiO_2, MeOH/concentrated aqueous NH_3 = 98:2) R_f = 0.27; IR (KBr) ν_{N-H} = 3300 cm^{-1}, $\nu_{C=O}$ = 1670, 1650 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.58–1.64 (m, CON–C–CH_2–C–N, 2H), 1.97 (s, CH_3CO–N, 3H), 2.15 (s, N–CH_3, 6H), 2.26 (t, J = 6, CH_2–N, 2H), 3.17–3.21 (m, CON–CH_2–C, 2H), 3.80, 3.83, 3.84 (3s, pyrrole *N*-CH_3, 9H), 6.82, 6.86, 7.03, 7.15, 7.18, 7.23 (6d, J = 1.5, pyrrole Ar–H, 6H), 8.06 (t, J = 5, CONH–C, 1H), 9.81, 9.88, 9.89 (3s, CONH–Ar, 3H); LRMS (FAB) m/z 511 (calcd for $C_{25}H_{35}N_8O_4$ (M + H^+) 511). Anal. Calcd for $C_{25}H_{34}N_8O_4 + 2H_2O$: C, 54.93; H, 7.01; N, 20.50. Found: C, 54.74; H, 6.86; N, 20.11.

Acknowledgment. We express appreciation to the Office of Naval Research for a grant (N000 14-90-J-4132) to support this work. In addition, we would like to thank Professor John Carbon for use of his laboratory facilities in conducting biochemical assays and for helpful comments.

Supplementary Material Available: Synthetic procedures and spectral data for compounds **7–9**, **11**, **12**, **14**, and **15** and 2D NMR spectra [^{13}C–^1H (HETCOR) and ^1H–^1H (DQF–COSY)] in $CDCl_3$ for compound **5c** (14 pages). Ordering information is given on any current masthead page.