CH_2CH), 2.8-3.0 (m, 8, $CH_2C_6H_5$, CH_2CO, CH_2NH_2), 3.2-4.6 (m, 24, CONHCHCO, $NHCH_2CO$, CH_2N, COCHN, CH_2NH), 7.2 (m, 10, aryl), 7.7-8.5 (m, 9, NH_2, =NH).

FMOC-δ-aminovaleric Acid. A mixture of 1.56 g of δ-aminovaleric acid, 3.2 g of $NaHCO_3$, 25 mL of H_2O, 5 g of FMOC-OSu, and 30 mL of dioxane was treated with Na_2CO_3 to bring the pH to 7.8 and stirred overnight. Addition of 80 mL of distilled water effected complete solution. Three washes with 50-mL portions of ether removed excess acylating agent, and the remaining aqueous solution was acidified with concentrated HCl to pH 8. The precipitate was washed three times with 30-mL portions of 1 N HCl and three 30-mL portions of water. The residue was dissolved in acetone; the solution was dried (Na_2SO_4), filtered, evaporated, and dried in a vacuum dessicator. The filtrate was acidified to pH 3, and the new precipitate was treated similarly. Both fractions were dissolved in 125 mL of acetone; the solution was dried (Na_2SO_4) and evaporated to give 3.5 g (84.5%) of the acid as a white solid, mp 136-7 °C. The analytical sample was recrystallized from EtOAc/hexane to give white crystals: mp 135-6 °C; IR (KBr) 3349 (NH), 1693 (COOH, OCONH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1-1.9 (m, 4, CH_2CH_2), 2.2 (t, 2, CH_2CO), 2.9 (m, 2, CH_2NH), 4.1-4.4 (m, 3, $CHCH_2O$), 5.6 (d, 1, NH), 7.3-8.0 (m, 8, aryl). Anal. Calcd for $C_{20}H_{21}NO_4$: C, 70.77; H, 6.23; N, 4.12. Found: C, 70.55; H, 6.11; N, 3.96.

FMOC-δ-aminovaleric Acid Pentafluorophenyl Ester. To a solution of 824 mg of DCC and 736 mg of pentafluorophenol in 25 mL of CH_2Cl_2 was added after 5 min 1.24 g of FMOC-δ-Ava-OH. After adding 25 mL of CH_2Cl_2, stirring was continued for 10 h, and the precipitated urea was filtered and washed with another 25 mL of CH_2Cl_2. The filtrate gave after recrystallization from CH_2Cl_2-hexane 1.69 g (87%) of the ester: mp 118-9 °C; IR (KBr) 3387 (NH), 1790, 1699 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.5-1.9 (m, 4, CH_2CH_2), 2.7 (t, 2, CH_2CO), 3.3 (q, 2, CH_2NH), 4.25 (t, 1, $CHCH_2O$), 4.45 (d, 2, CH_2O), 4.9 (m, 1, NH), 7.3-7.9 (m, 8, aryl). Anal. Calcd for $C_{26}H_{20}F_5NO_4$: C, 61.78; H, 3.96; N, 2.77. Found: C, 61.63; H, 3.96; N, 2.77.

Acknowledgment. We are indebted to the National Institutes of Health (GM-09706), the National Science Foundation (NSF CHE-8609176), the U.S. National Academy of Sciences, and the Akademie der Wissenschaften der DDR for support of this work. The National Science Foundation is thanked for grants used to purchase the high-field NMR spectrometers used in this research. We thank U. Kertscher and H. Apelt for HPLC investigations, Dr. S. Katzwinkel, Ch. Salewski, and Rick Neves for amino acid analyses, and H. Hans and M. Dreissigaker for skillful technical assistance. We are indebted to Messrs. Kenton E. Stephens, Jr., and Denton Langridge for initial experiments on the direct addition of 4-AMP to the coupled reaction mixture to effect scavenging of excess acid chloride as well as deblocking of the FMOC function. We thank Dr. Robert Cotter of the Middle Atlantic Mass Spectrometry Laboratory of Johns Hopkins University for MS/FAB data.

Registry No. 3·HCl, 68759-90-0; 4·2HCl, 123622-30-0; 5, 97559-40-5; 6, 123622-31-1; 7, 123622-32-2; 8, 111422-33-4; 9, 42001-58-1; 10, 123622-33-3; 10·3 TFA, 123622-35-5; 11, 78326-84-8; 12, 123622-34-4; 13, 61243-38-7; 4-AMP, 7144-05-0; Fmoc-PNA, 123622-29-7; $4\text{-}O_2NC_6H_4NH_2$, 100-01-6; H-Nle-OBn·TsOH, 63219-55-6; H-Met-OBn·TsOH, 68739-90-2; Fmoc-Gly-Cl, 103321-49-9; Fmoc-Leu-Cl, 103321-59-1; Fmoc-Phe-Cl, 103321-57-9; Fmoc-Glu(OBn)-Cl, 12362-36-6; BOC-Gln-OPfp, 50903-58-7; Fmoc-Lys(Z)-Cl, 103321-56-8; Fmoc-δ-Ava-Cl, 123622-37-7; Fmoc-Pro-Cl, 103321-52-4; Z-Arg(NO_2)-OPfp, 17543-52-1; H-Pro-OBu-*t*, 2812-46-6; H-Pro-$NHC_{12}H_{25}$·HCl, 123622-38-8; H-Leu-Nle-OBn, 123622-39-9; Fmoc-Phe-Phe-Gly-Leu-Nle-OBn, 123622-40-2; H-Lys(Z)-Phe-Phe-Gly-Leu-Met-OBn, 123622-41-3; H-Phyr-Phe-Phe-Gly-Leu-Nle-OBn, 123622-42-4; H-Lys(Z)-Pro-OBn, 68280-75-1; H-Leu-Nle-OBn·HCl, 123622-43-5; H-Leu-Met-OBn·HCl, 123622-44-6; H-Lys(Z)-Pro-Lys(Z)-Pro-$NHC_{12}H_{25}$·HCl, 123622-45-7; H-Pro-Lys(Z)-Pro-OBu-*t*, 123622-46-8; Z-Arg(NO_2)-Pro-Lys(Z)-Pro-OBu^t, 123639-60-1; Z-Arg(NO_2)-Pro-Lys(Z)-Pro-δ-Ava-Phe-Phe-Gly-Leu-Nle-NH_2, 123622-47-9; H-δ-Ava-OH, 660-88-8; Fmoc-δ-Ava-OH, 123622-48-0; Fmoc-δ-Ava-OPfp, 123622-49-1; Fmoc-Gly-OH, 29022-11-5; Fmoc-Leu-OH, 35661-60-0; Fmoc-Phe-OH, 35661-40-6; Fmoc-Glu(OBn)-OH, 123639-61-2; Fmoc-Lys(Z)-OH, 86060-82-4; Fmoc-Pro-OH, 71989-31-6; H-δ-Ava-Phe-Phe-Gly-Leu-Nle-OBn, 123622-50-4; H-Pro-OBn·HCl, 16652-71-4.

Synthesis of Novel Thiazole-Containing DNA Minor Groove Binding Oligopeptides Related to the Antibiotic Distamycin

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The synthesis of novel thiazole-bearing oligopeptides related to the antibiotic distamycin is described. These lexitropsins, or information reading agents, are designed to test the principle of DNA base site exclusion or avoidance as well as base pair acceptance in molecular recognition of oligopeptides for DNA. The first group of compounds, therefore, has the sulfur atom aligned inward to the DNA minor groove, whereas the second group of agents has the sulfur atom directed away from the DNA minor groove. All six compounds synthesized bind to double helical DNA, and their binding constants to calf thymus DNA and relative to distamycin and netropsin are reported.

Introduction

Because of current interest in the control of gene expression,[1] synthetic chemists have been attracted to the problem of developing DNA sequence-specific agents. Conceptually there are a number of approaches to this problem, e.g., using β-oligonucleotides[2-4] or their backbone-modified counterparts[5-8] which take advantage of inherent Watson-Crick base pairing to target single-strand sequences or with hybrid probes incorporating an intercalator.[9-11] Another approach is to take advantage of the ability of certain oligonucleotide sequences to form triplex

* Author to whom correspondence should be addressed.

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0022-3263/90/1955-0728$02.50/0

structures and thereby target double-strand nucleic acid sequences.[12] These and other related approaches are being actively pursued by many groups in efforts that are international in scope.

An alternative, and in some respects, complementary approach is to develop sequence-specific probes based on natural DNA groove binding agents. Groove binding agents have several advantages over intercalators for this purpose in that, unlike the latter, groove binders in general cause minimal structural distortion of the DNA and correspondingly less disturbance of the information inherent in the DNA sequence.[13] We have reported an approach based on the naturally occurring oligopeptide antibiotics netropsin and distamycin.[14,15] Rational structural modification led to the development of lexitropsins, or information-reading oligopeptides, some of which are capable of recognizing unique sequences and exhibit no memory for the home site of the parent antibiotic.[16-21]

Encouraged by these results, we have begun to explore systematically the several structural factors in the ligands that might be expected to contribute to the processes of molecular recognition. One legitimate aspect of molecular design in the context of lexitropsins is the introduction of heterocyclic moieties capable of specific DNA recognition by hydrogen bond acceptance, as exemplified by imidazole[16,17] or oxazole[18] moieties in the prototype lexitropsins. Other important components of molecular recognition include steric hindrance,[21] electrostatic interactions,[14,15,22-24] and ligand chirality.[18,20]

The structural consideration we examine in the present paper is the complementary one of site avoidance, i.e., whether a given heterocyclic moiety can actually exclude binding at a particular base site. The corollary question then is whether this property can be incorporated into the design of agents capable of recognizing and binding to a unique sequence. A necessary prerequisite is the synthesis of the appropriate minor groove binding agents. We report the synthesis of novel thiazole-containing oligopeptides including those designed so that the sulfur atom is aligned toward the DNA minor groove and those with the sulfur oriented away from the groove. The comparison of the sequence-reading properties of such lexitropsins with CH, N, or S at the key position 3 of the heterocyclic moiety then becomes possible. Additional interest attaches to the DNA base recognizing properties of the thiazole group owing to the presence of the bithiazole moiety in the side chain of the glycopeptide antitumor antibiotic bleomycin[25] and which is thought to determine the sequence-recognizing properties of the latter.[26]

Synthetic Strategy

The prototype lexitropsins were based on the doubly cationic netropsin **1** (Scheme I), the structure of which was established in 1963 by Julia and Preau-Joseph.[27] Following the recognition of the importance of electrostatic effects in the molecular recognition of ligands by DNA,[14,15,22-24] later generations of lexitropsins have been based instead on the monocationic natural antibiotic distamycin **2**, the structure of which was established by Arcamone et al. in 1967.[28] Since that time the syntheses of certain analogues have been reported. These included those bearing additional pyrrole units or in which the pyrrole rings were replaced by benzene, pyridine, thiophene,[29] or trimethylpyrrole or by changing the mode of pyrrole ring substitution from 2,4 to 2,5.[30] The syntheses of these analogues were based largely on the original method of Julia and Preau-Joseph. We recently reported new, more efficient, total syntheses of netropsin and distamycin in which the synthetic strategy involved significant changes in the methods and order of introduction of the terminal amidinium, guanidinium, and *N*-formyl groups.[15,31] These changes in procedure resulted in improved yields and purity of the final products and were therefore adopted, where appropriate, in the present study.

Synthesis of Thiazole Lexitropsins with Sulfur Directed Inward to the DNA Minor Groove

The required key starting material ethyl 2-amino-4-methylthiazole-5-carboxylate (**9a**) was prepared following the reported procedure[32] by condensing thiourea and ethyl acetoacetate in the presence of iodine.

Acylation of the aminothiazole **9a** with the acid chloride of 1-methyl-4-nitropyrrolecarboxylic acid[31] in dichloromethane in the presence of triethylamine afforded **10** in 79% yield. The preparation of the pyrrolecarboxylic acid chloride in THF with thionyl chloride is very sensitive to the length of the reaction time.[15,31] This acid chloride was

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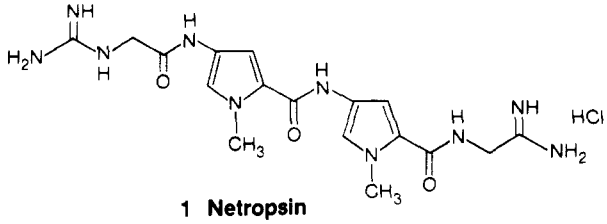
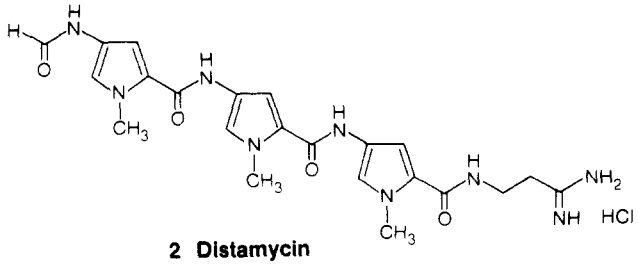
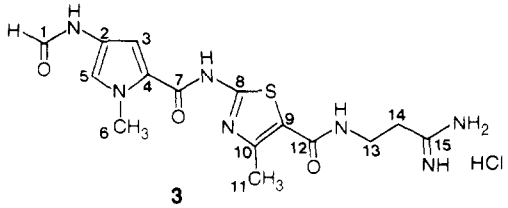
Scheme I. Structures of Natural Antibiotics Netropsin and Distamycin and the Novel Synthetic Thiazole–Containing Oligopeptide DNA Minor Groove Binding Agents.



1 Netropsin



2 Distamycin



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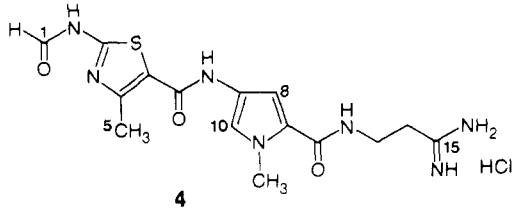

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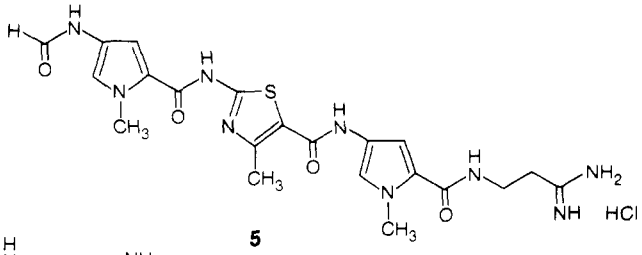

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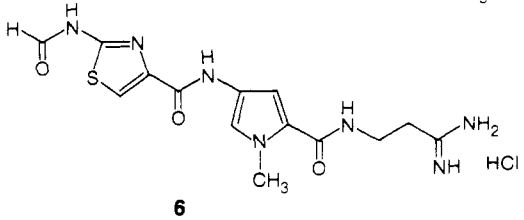
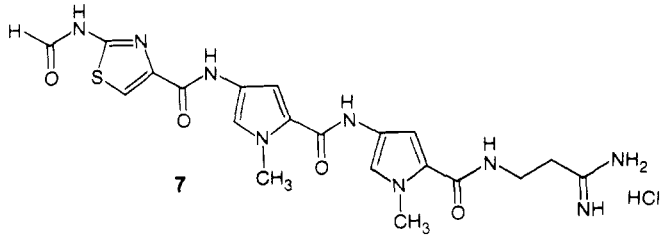
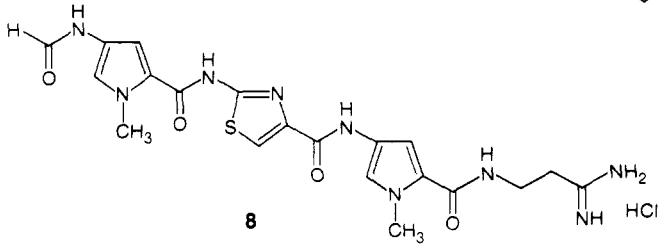


6



7



8

obtained in quantitative yield when the pyrrole acid was refluxed in thionyl chloride for 1.0–1.5 h. Alkaline hydrolysis of ester **10** gave the acid **11**. Condensation of β-aminopropionitrile with acid **11** was attempted using dicyclohexylcarbodiimide (DCC), as well as carbonyldiimidazole (CDI) in dimethylformamide employing different temperatures (−10 to 60 °C) and different reaction times (2–48 h). However, the yield was consistently low (<10%) by this method. Therefore, treatment of acid **11** with thionyl chloride in benzene afforded the acid chloride **12**, which was condensed with β-aminopropionitrile in the presence of triethylamine to give **13** in 83% yield. Pinner reaction[33] of **13** with hydrogen chloride gas in absolute ethanol followed by treatment of the intermediate imidate ester with ammonia gave the amidinium hydrochloride **14** in quantitative yield. Catalytic reduction of the nitro group of **14** gave **15**, which followed by immediate N-formylation with formic acetic anhydride gave the desired thiazole-lexitropsin **3** in 72% yield (Scheme II).

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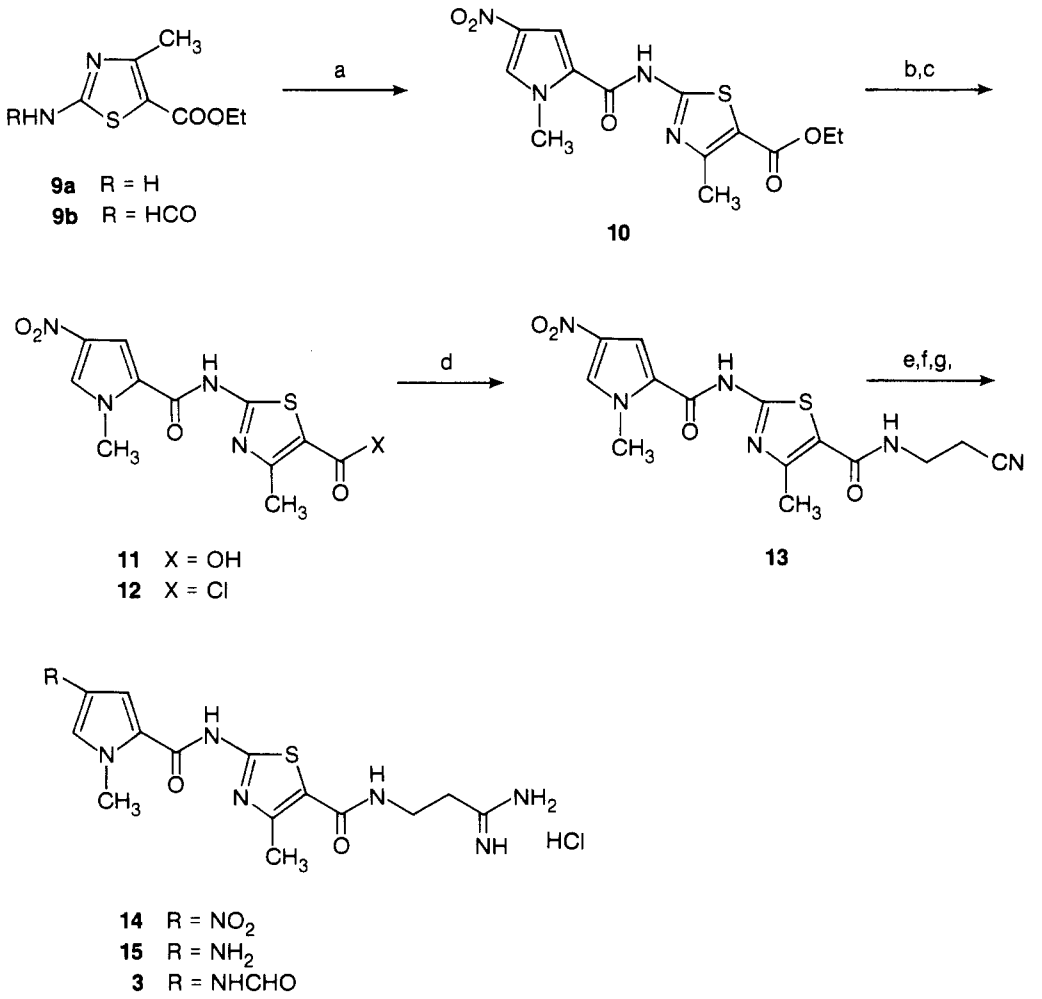
Scheme II[a]



[a] Reaction conditions: (a) acid chloride of 1-methyl-4-nitropyrrole-2-carboxylic acid, Et_3N, CH_2Cl_2; (b) ethanolic NaOH, heat; (c) $SOCl_2$, C_6H_6, heat; (d) 3-aminopropionitrile, Et_3N, CH_2Cl_2; (e) HCl in dry EtOH, then dry NH_3, EtOH; (f) H_2, Pd/C, MeOH; (g) HCO_2H, Ac_2O.

The synthesis of the lexitropsin **4** bearing an N-terminal thiazole residue was carried out by condensation of the known pyrrole–amine derivative **18**[15,31] with 2-(formylamino)-4-methylthiazole-5-carboxylic acid (**17**). The required acid **17** could, in principle, be obtained from **9a** either by N-formylation followed by alkaline hydrolysis of the ester function or by the reverse order of steps. Formylation of **9a** with a mixture of formic acid and acetic anhydride gave **9b** in 48%. Attempted hydrolysis of **9b** to the acid **17** with 0.25 M sodium hydroxide and 0.5 M lithium hydroxide resulted in competing hydrolysis of the N-formyl group. Alternatively treatment of ester **9a** with 0.5 M ethanolic sodium hydroxide solution at room temperature followed by neutralization with acetic acid afforded acid **16** in quantitative yield. N-Formylation of **16** with a mixture of formic acid and acetic anhydride gave the required *N*-formylcarboxylic acid **17** in 47% yield. Pinner reaction[33] on **19** gave the corresponding nitroamidine hydrochloride,[34] and catalytic hydrogenation of the latter at atmospheric pressure gave the pyrrole amidinium amine **18**.[34] Finally condensation of the acid **17** and the amine **18** with dicyclohexylcarbodiimide gave the desired thiazole lexitropsin **4** in 55% yield (Scheme IIIA).

The last agent required in this series, **5**, which contains a thiazole moiety centrally placed, was synthesized as outlined in Scheme IIIB). Catalytic hydrogenation of the nitropyrrole **19** gave the amine **20**,[15,31] which was acylated with the acid chloride **12** to give **21** in 62% yield. The nitrile group in **21** was converted to the amidinium hydrochloride **22** under Pinner reaction conditions.[33] Catalytic hydrogenation of **22** to give the amine **23**, followed by N-formylation of **23** with formic–acetic mixed anhydride gave the desired tripeptide thiazole lexitropsin **5** in 61% yield.

Synthesis of Thiazole Lexitropsins with Sulfur Directed Outward away from the DNA Minor Groove

The requirements in this series of compounds were to synthesize both dipeptidic and tripeptidic lexitropsins bearing a thiazole moiety with an orientation opposite to that in the first group of agents and with the thiazole placed either terminally or centrally in the oligopeptide. The convergent synthesis of the dipeptide **6** is outlined in Scheme IV. Pyruvic acid was brominated and condensed with thiourea to obtain the hydrobromide salt of 2-aminothiazole-4-carboxylic acid, which was then esterified and neutralized to provide compound **24**.[35] The *N*-formyl derivative **25** was obtained by treating **24** with the formic acid–carbonyldiimidazole reagent at room temperature. Compound **25** was hydrolyzed with 2 M NaOH in 1:1

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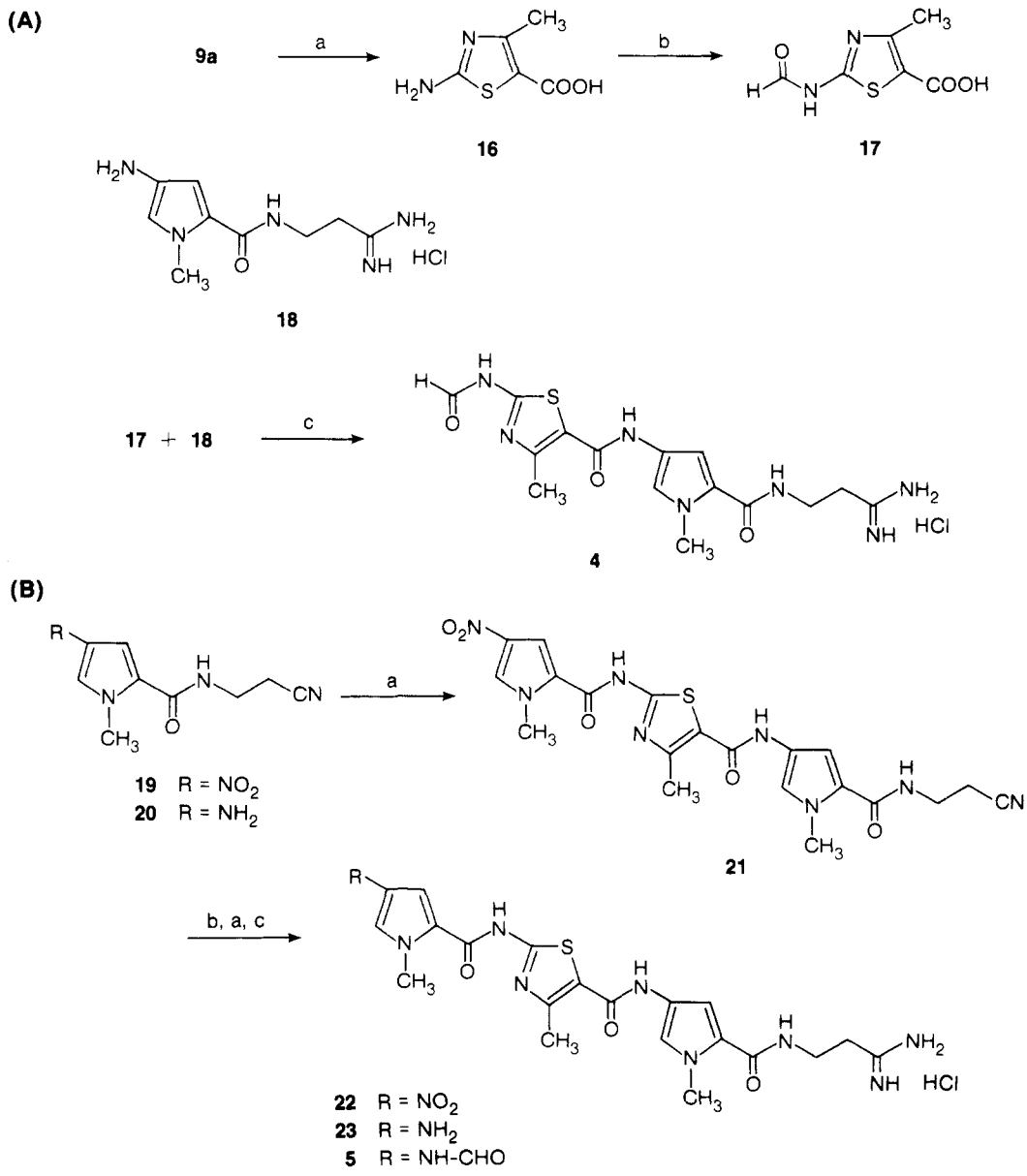
Scheme III[a]



[a] Reaction conditions: A (a) NaOH, EtOH; (b) HCO_2H, Ac_2O; (c) DCC, DMF. B (a) **19** to **20**, H_2, Pd/C, MeOH; then **12**, Et_3N, THF; (b) HCl, dry EtOH; (a) H_2, Pd/C, MeOH; (c) HCO_2H, Ac_2O.

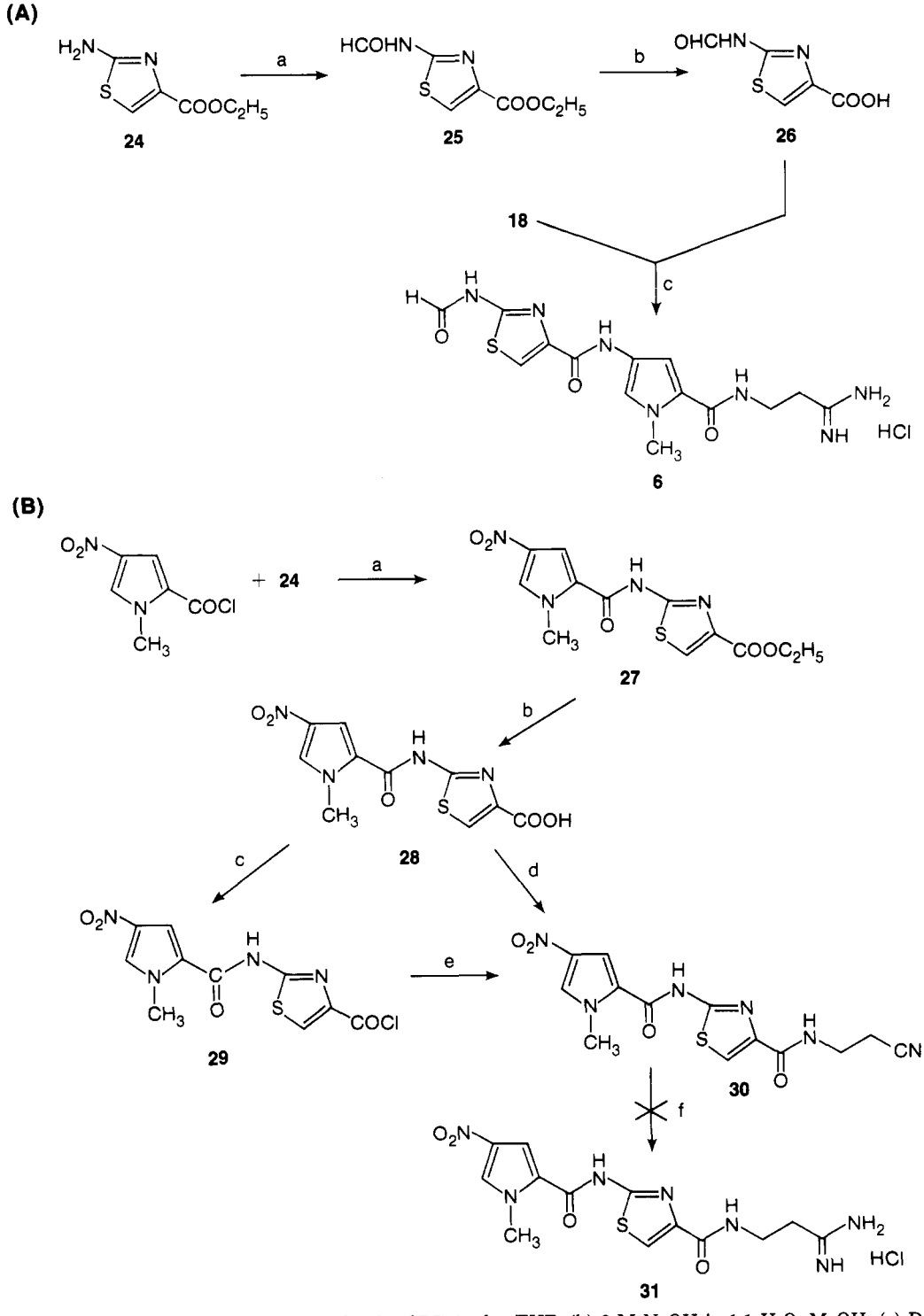
methanol–water mixture at room temperature to give **26**. Treatment of the freshly prepared amine **18** with acid **26** in the presence of dicyclohexylcarbodiimide and 1-hydroxybenzotriazole hydrate (HOBT) in DMSO–DMF afforded compound **6** in 67% yield.

Synthesis of **6** initiated attempts to synthesize the isomeric structure bearing a carboxyl terminus thiazole group (Scheme IVB). Condensation of the acid chloride of 1-methyl-4-nitropyrrole-2-carboxylic acid with **24** proceeded normally to give **27**, as did hydrolysis to the corresponding acid **28** and formation of acid chloride **29**. Conversion of either **28** or **29** to the propionitrile derivative **30** proceeded satisfactorily, employing reaction conditions similar to those described in connection with the syntheses of **13** and **21**. However **30** proved to be so insoluble that neither the Pinner reaction[33] (to give **31**) nor catalytic reduction of the nitro group could be effected. This problem of extreme insolubility of certain intermediates encountered in the synthesis of lexitropsins represents a severe limitation especially for the projected design and synthesis of lexitropsins capable of recognizing longer gene sequences. However recent encouraging results from the introduction of appropriate 1-pyrrole substituents in longer lexitropsins suggest that problem is not insurmountable (data not shown).

The tripeptide **7** was synthesized according to Scheme VA. Reaction of **20** with 1-methyl-4-–nitropyrrol-2-carboxylic acid chloride in the presence of Hunig's base as reported earlier[31] gave bispyrrole propionitrile **32**. Subsequent Pinner reaction[33] on **32** to convert to nitrile to amidinium hydrochloride **33**, catalytic reduction of the nitro group in **33** to give the aminopyrrole **34**, and condensation of **34** with **26** in the presence of DCC and HOBT gave the desired tripeptide **7** in 49% yield.

The third structure required was the tripeptide **8** in which the thiazole moiety is centrally located in the molecule. The compound was synthesized as shown in Scheme VB. The nitropyrrole thiazole acid **28** was condensed with **18** in the presence of DCC and HOBT to afford **35**. Subsequent catalytic reduction of the nitro group in **35** to give **36** and immediate N-formylation on **36** with formic acid–carbonyldiimidazole reagent afforded

Scheme IV[a]



[a] Reaction conditions: A (a) HCO_2H, carbonyldiimidazole (CDI) in dry THF; (b) 2 M NaOH in 1:1 H_2O, MeOH; (c) DCC, 1-hydroxybenzotriazole hydrate (HOBT) in dry DMSO–DMF. B (a) Pyridine in dry THF, (b) 2 M NaOH in 1:1 H_2O, MeOH; (c) oxalyl chloride in dry THF; (d) β-aminopropionitrile, DCC, HOBT in dry DMSO; (e) β-aminopropionitrile, Hunig's base in dry THF; (f) dry HCl in dry EtOH, or dry MeOH then dry NH_3 in EtOH.

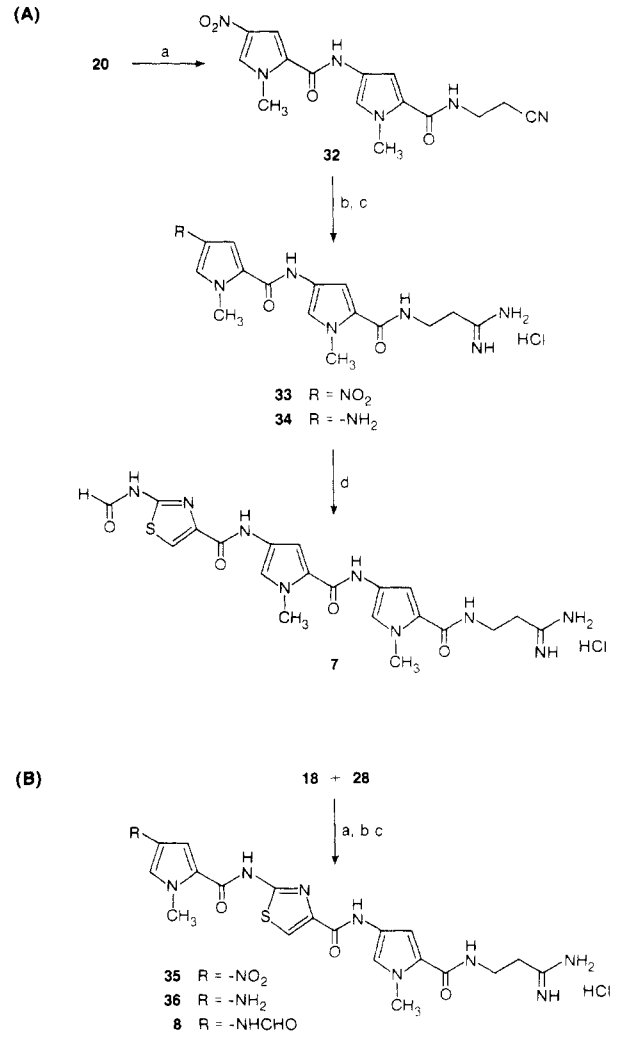
the desired tripeptide **8** in 40% yield.

DNA Binding Characteristics of Novel Thiazole Containing Oligopeptides Compared with Netropsin and Distamycin

The relative binding constants of the novel oligopeptide structures **3** to **8** to calf thymus DNA were determined and compared with those of distamycin and netropsin by the ethidium displacement method. The results are summarized in Table I. The new compounds **3–8** should strictly be compared with the monocationic distamycin **2** rather than the biscationic netropsin. It may be seen that the binding of **3–8** is generally comparable with, and in the cases of **5–7** greater than, that of distamycin for calf thymus DNA. Other things being equal, the binding of the dipeptidic examples **3**, **4**, and **6** would be expected to be lower than that of the tripeptidic distamycin, but, in fact, both **5** and **6** bind somewhat more strongly than **2**.

A more detailed analysis of the base and sequence preferential binding of these novel thiazole-containing

Scheme V[a]



[a] Reaction conditions: A (a) 1-methyl-4-nitropyrrole-2-carboxyl chloride, Hunig's base in dry THF; (b) dry HCl in dry EtOH, then dry NH_3 in EtOH; (c) H_2, Pd/C, MeOH; (d) **26**, HOBT, DCC in DMSO–DMF. B (a) HOBT, DCC in dry DMSO–DMF; (b) H_2, Pd/C; (c) HCOOH, CDI, cold conditions.

Table I. Relative Binding Constants of Thiazole-Containing Oligopeptides to DNA Compared with Distamycin and Netropsin[a]

ligand	$10^{-6}K_{app}$ to calf thymus DNA, M^{-1}	ligand	$10^{-6}K_{app}$ to calf thymus DNA, M^{-1}
netropsin (**1**)	8.0	**5**	7.1
distamycin (**2**)	3.3	**6**	7.8
3	3.0	**7**	6.7
4	1.3	**8**	4.1

[a] Ethidium bromide to DNA base pair ratio was 1.26. Experiments performed in 20 mM NaCl, pH 7.1.

oligopeptides by MPE footprinting on restriction fragments of plasmid DNAs and by high-field NMR analysis of their complexes with double helical oligonucleotides will be reported in due course.

Experimental Section

Melting points were determined on an Electrothermal melting point apparatus and are uncorrected. The IR spectra were recorded on a Nicolet 7199 FT spectrophotometer, and only the principal bands are reported. The ^1H NMR spectra were recorded on Bruker WH-200 and WH-400 spectrometers. FAB (fast atom bombardment) mass spectra with glycerol as the matrix were determined on an Associate Electrical Ind. (AEI) MS-9 and MS-50 focusing high-resolution mass spectrometer. Kieselgel 60 (230–400 mesh) of E. Merck and florisil (60–100 mesh) was used for chromatography, and precoated silica gel 60F-254 sheets of E. Merck were used for TLC, with the solvent system indicated in the procedure. TLC plates were visualized by using UV light or 2.5% phosphomolybdic acid in methanol with heating.

All compounds obtained commercially were used without further purification unless otherwise stated. Ethanol and methanol were freshly distilled from magnesium turnings; tetrahydrofuran was distilled from sodium/benzophenone under an atmosphere of dry argon; ether was dried over sodium; methylene chloride was distilled from phosphorous pentoxide and stored over 3A molecular sieves; triethylamine was treated with potassium hydroxide then distilled from barium oxide and stored over 3A molecular sieves; dimethylformamide was distilled from barium oxide and stored over 3A molecular sieves.

Ethyl 4-Methyl-2-(1-methyl-4-nitropyrrole-2-carboxamido)thiazole-5-carboxylate (10). 1-Methyl-4-nitropyrrole-2-carboxylic acid (850 mg, 5 mmol) was refluxed in 12 mL of thionyl chloride for 1.5 h. The reaction mixture was concentrated under reduced pressure, and the resulting residue was coevaporated with hexane (3 × 25 mL) to remove the last traces of thionyl chloride. The resulting acid chloride was used without further characterization.

To a cooled solution of thiazole amine **9a** (930 mg, 5 mmol) and triethylamine (2 mL) in dichloromethane (40 mL), the above acid chloride in methylene chloride (10 mL) was added dropwise. After 1 h at 0 °C, the reaction mixture was allowed to rise to ambient temperature and stirred for 12 h. The reaction mixture was diluted with 30 mL of methylene chloride and washed successively with dilute hydrochloric acid and brine. Solvent was evaporated, and the resulting solid was purified on a column of fluorisil (EtOAC:hexane) to give **10**, 1.340 g (79% yield); mp 235–6 °C; IR (KBr) ν_{max} 3420, 3120, 1700, 1680, 1540, 1520 cm^{-1}; ^1H NMR ($CDCl_3$ + TFA) δ 1.34 (t, 3 H, OCH_2CH_3), 2.7 (s, 3 H, $ArCH_3$), 4.0 (s, 3 H, NCH_3), 4.36 (q, 2 H, OCH_2CH_3), 7.7 (d, 1 H, Ar-H), 7.92 (d, 1 H, Ar-H); MS, m/z (rel intensity) $C_{13}H_{14}N_4O_5S$ found 338.0683 (23) (calcd 338.0685), 310 (3), 153 (100), 107 (28).

4-Methyl-2-(1-methyl-4-nitropyrrole-2-carboxamido)thiazole-5-carboxylic Acid (11). A solution of ester **10** (676 mg, 2 mmol) in 0.5 M ethanolic sodium hydroxide (30 mL) was heated under reflux for 2 h, at which time TLC analysis indicated complete hydrolysis of the ester. The reaction mixture was cooled in an ice bath and acidified with concentrated HCl. The precipitated solid was filtered and washed with water and ether to give acid **11**, 540 mg (87% yield); mp 262–4 °C; IR (KBr) ν_{max} 3420, 3130, 2920, 1680, 1520 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.56 (s, 3 H, $ArCH_3$), 4.0 (s, 3 H, NCH_3), 7.96 (d, 1 H, Ar-H), 8.30 (d, 1 H, Ar-H); MS, m/z (rel intensity) $C_{11}H_{10}N_4O_5S$ found 310.0372 (3), calcd 310.0372, 266 (M − 44, 11), 153 (100).

4-Methyl-2-(1-methyl-4-nitropyrrole-2-carboxamido)thiazole-5-carboxamidopropionitrile (13). To a suspension of acid **11** (310 mg, 1 mmol) in benzene (30 mL) was added 2 mL of thionyl chloride. The reaction mixture was heated under reflux for 4 h. Solvent was removed under reduced pressure, and the resulting solid was coevaporated with hexane (3 × 20 mL) to give the acid chloride **12**. This was used for a further step without characterization.

To a cooled solution of 3-aminopropionitrile (140 mg, 2 mmol) and triethylamine (0.5 mL) in methylene chloride (10 mL) was added dropwise the above acid chloride in tetrahydrofuran (15 mL). After the reaction mixture was stirred at room temperature for 12 h, the solvent was removed under reduced pressure, and the resulting residue was dissolved in a 1:1 mixture of tetrahydrofuran:ethyl acetate. The solution was washed with brine, dried, and evaporated, and the resulting solid was purified on a column of silica gel (hexane:ethyl acetate 1:1, flash chromatography) to give **13**, 300 mg (83% yield); mp 228–9 °C; IR (KBr) ν_{max} 3250, 3120, 2240, 1710, 1680, 1630, 1560, 1530, 1500, 1420 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.54 (s, 3 H, $ArCH_3$), 2.74 (t, 2 H, CH_2), 3.42 (q, 2 H, CH_2), 3.98 (s, 3 H, NCH_3), 7.94 (d, 1 H, Ar-H), 8.3 (d, 1 H, Ar-H), 8.34 (t, 1 H, N*H*), 12.80 (br s, 1 H, N*H*); D_2O exchange resulted in the disappearance of peaks at δ 8.34 and 12.80, and quartet at 3.42 became a triplet; MS, m/z (rel intensity)

$C_{14}H_{14}N_6O_4S$ found 362.0802 (16) (calcd 362.0797), 293 (3), 153 (100), 107 (32).

3-[4-Methyl-2-(1-methyl-4-nitropyrrole-2-carboxamido)-thiazole-5-carboxamido]propionamidine Hydrochloride (14). A solution of **13** (253 mg, 0.7 mmol) in anhydrous ethanol (25 mL) was cooled to −15 °C and saturated with dry HCl gas. The resulting solution was stirred at 0 °C for 1 h and at room temperature another hour. Solvent was evaporated under reduced pressure, and the resulting residue was washed with dry ether (2 × 15 mL). The solid was suspended in anhydrous ethanol (20 mL), and then 15 mL of dry ammonia was condensed. After stirring the reaction mixture for 4 h at room temperature, solvent was removed under reduced pressure, and the resulting solid was recrystallized from methanol–ethyl acetate to give amidine hydrochloride **14**, 250 mg (86% yield); mp 243–5 °C; IR (Nujol) ν_{max} 3130, 3040, 1700, 1680, 1625, 1540, 1520, 1450 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.5 (s, 3 H, $ArCH_3$), 2.64 (t, 2 H, CH_2), 3.54 (t, 2 H, CH_2), 4.0 (s, 3 H, NCH_3), 7.7 (d, 1 H, Ar-H), 8.2 (d, 1 H, Ar-H); FAB-MS, m/z (rel intensity) 380 ($(M - Cl)^+$) anal. calcd for $C_{14}H_{18}ClN_7O_4S$: C, 40.43; H, 4.33; N, 23.58. Found: C, 40.1; H, 4.46; N, 23.7.

3-[4-Methyl-2-(1-methyl-4-(formylamino)pyrrole-2-carboxamido)thiazole-5-carboxamido]propionamidine Hydrochloride (3). A solution of **14** (208 mg, 0.5 mmol) in methanol (20 mL) was hydrogenated over 10% palladium on charcoal (42 mg) at room temperature and atmospheric pressure. The catalyst was removed by filtration, and the filtrate was concentrated to give amine **15**. This amine developed a dark color on exposure to atmosphere. It was used immediately for next step.

To the amine **15** in 98% formic acid (6 mL), acetic anhydride (2 mL) was added, and the reaction mixture was stirred at room temperature for 12 h. Solvent was evaporated to dryness in vacuo, and the resulting residue was crystallized in methanol and ethyl acetate to give **3**, 150 mg (72% yield); mp 202–4 °C; IR (KBr) ν_{max} 3390, 3240, 3120, 3040, 1660, 1520, 1400 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.5 (s, 3 H, $ArCH_3$), 2.6 (t, 2 H, CH_2), 3.52 (q, 2 H, CH_2), 3.88 (s, 3 H, NCH_3), 7.26 (d, 1 H, Ar-H), 7.44 (d, 1 H, Ar-H), 8.14 (s, 1 H, CHO), 8.2 (t, 1 H, NH), 8.62 (s, 2 H, amidine), 9.0 (s, 2 H, amidine), 10.2 (s, 1 H, NH), 12.3 (br s, 1 H, NH); D_2O exchange resulted in the disappearance of peaks at δ 8.2, 8.62, 9.0, 10.2, and 12.3, and the quartet at δ 3.52 became a triplet; FAB-MS, m/z (rel intensity) 378 (M − Cl, 66). Anal. Calcd for $C_{15}H_{20}ClN_7O_3S$: C, 43.53; H, 4.83; N, 23.7. Found: C, 43.7; H, 4.68; N, 23.45.

Ethyl 2-(Formylamino)-4-methylthiazole-5-carboxylate (9b). To a solution of **9a** (930 mg, 5 mmol) in 98% formic acid (12 mL), acetic anhydride (4 mL) was added dropwise at room temperature. After the reaction mixture was stirred for 12 h, the solvent was removed under reduced pressure, and water (25 mL) was added to the resulting residue. This material was extracted with ethyl acetate (3 × 30 mL), and the combined organic extract was washed successively with 2% hydrochloric acid and brine and evaporated to give **9b**, 510 mg, 48%); mp 203–4 °C; IR (KBr) ν_{max} 3400, 3160, 2880, 1720, 1700, 1580, 1540 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.3 (t, 3 H, CH_2CH_3), 2.54 (s, 3 H, $ArCH_3$), 4.24 (q, 2 H, OCH_2CH_3), 8.54 (s, 1 H, CHO), 10.6 (br s, 1 H, exchanged with D_2O); MS, m/z (rel intensity) $C_8H_{10}N_2O_3S$ found 214.0412 (39) (calcd 214.0412), 186 (100), 158 (21), 141 (37), 112 (39).

2-Amino-4-methylthiazole-5-carboxylic Acid (16). The thiazole ester **9a** (930 mg, 5 mmol) was treated with 0.5 M ethanolic sodium hydroxide solution (60 mL) at room temperature for 24 h, at which time TLC analysis indicated complete hydrolysis of the ester. The reaction mixture was cooled in an ice bath and neutralized very cautiously with acetic acid (neutralization with HCl gave a very poor yield). Precipitated acid **16** was collected, 670 mg (85% yield), mp 172–3 °C.

2-(Formylamino)-4-methylthiazole-5-carboxylic Acid (17). To a solution of **9b** (632 mg, 4 mmol) in 98% formic acid (8 mL) was added dropwise acetic anhydride (2.7 mL), and the reaction mixture was stirred for 18 h at room temperature. Solvent was evaporated under reduced pressure, and water (30 mL) was added to the resulting residue. The resulting mixture was extracted with 1:1 THF:ethyl acetate (3 × 30 mL). The combined organic extract was washed successively with 2% HCl (2 × 20 mL) and brine (2 × 20 mL) and then dried (Na_2SO_4). Evaporation of the solvent gave **17**, 350 mg (47% yield); mp 220 °C; IR (KBr) ν_{max} 3400, 3200, 2920, 1700, 1540 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.5 (s, 3 H, $ArCH_3$), 8.52 (s, 1 H, CHO), 12.53 (br s, 1 H, exchanged with D_2O); MS, m/z (rel intensity) $C_6H_6N_2O_3S$ found 186.0096 (40) (calcd 186.0099), 186 (41), 158 (100), 141 (6), 130 (3), 112 (40).

3-[1-Methyl-4-(2-(formylamino)-4-methylthiazole-5-carboxamido)pyrrole-2-carboxamido]propionamidine Hydrochloride (4). A solution of 3-(1-methyl-4-nitropyrrole-2-carboxamido)propionamidine hydrochloride (138 mg, 0.5 mmol) in methanol (15 mL) was hydrogenated over 10% Pd on charcoal (28 mg) at room temperature and atmospheric pressure until TLC analysis indicated completion of the reduction. The catalyst was removed by filtration. The filtrate was concentrated, and the residue was coevaporated with dry CH_2Cl_2 (2 × 20 mL) to give amine **18**. Due to instability of the amine **18**, it was used immediately in the following step.

Acid **17** (93 mg, 0.5 mmol) was added to the above amine. The mixture was dissolved in dry DMF (8 mL) and cooled to −10 °C, and then a solution of dicyclohexylcarbodiimide (155 mg, 0.75 mmol) in 4 mL of dry DMF was added dropwise during 30 min. The reaction mixture was allowed to reach room temperature and stirred overnight. Solvent was removed under reduced pressure, and the resulting residue was triturated with ether and then dissolved in water (10 mL). This aqueous solution was washed with ethyl acetate (3 × 30 mL) and evaporated in vacuo, and the resulting solid was crystallized from methanol:ethyl acetate to give **4**, 115 mg (56% yield), no distinct melting point (decomposes at 250–5 °C); IR (KBr) ν_{max} 3320, 2920, 1680, 1630, 1520, 1435 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.5 (s, 3 H, $ArCH_3$), 2.6 (t, 2 H, CH_2), 3.5 (q, 2 H CH_2), 3.82 (s, 3 H, NCH_3), 6.93 (d, 1 H, ArH), 7.2 (d, 1 H, ArH), 8.26 (t, 1 H, NH), 8.54 (s, 1 H, CHO), 8.7 (br s, 2 H, amidine), 9.0 (br s, 2 H, amidine), 10.0 (s, 1 H, NH), 12.4 (br s, 1 H, NH); D_2O exchange resulted in the disappearance of peaks at δ 8.26, 8.7, 9.0, 10.0, and 12.4, and the quartet at δ 3.5 became a triplet; FAB-MS, m/z (rel intensity) 378 (M − Cl, 19). Anal. Calcd for $C_{15}H_{20}ClN_7O_3S$: C, 43.53; H, 4.83; N, 23.7. Found: C, 43.85; H, 4.55; N, 23.85.

3-[1-Methyl-4-[4-methyl-2-(1-methyl-4-nitropyrrole-2-carboxamido)thiazole-5-carboxamido]pyrrole-2-carboxamido]propionitrile (21). A solution of **19** (222 mg, 1 mmol) in methanol (25 mL) was hydrogenated over 10% Pd on charcoal (25 mg) at room temperature and atmospheric pressure. The catalyst was removed by filtration, and the filtrate was concentrated to give an oily residue, which was coevaporated with CH_2Cl_2 to give amine **20**. This amine was used immediately in the following step.

To the solution of above amine **20** and triethylamine (0.5 mL) in CH_2Cl_2 (20 mL) was added acid chloride **12** (obtained from 310 mg of acid **11**) in tetrahydrofuran (20 mL) dropwise at 0 °C. The reaction mixture was allowed to attain room temperature and stirred for 12 h. The solvent was evaporated to dryness under reduced pressure, and water (30 mL) was added to the resulting residue. This was extracted with a 1:1 mixture of ethyl acetate and THF (3 × 30 mL), and the combined organic extract was dried and evaporated. The resulting solid was purified on a column of silica gel (flash chromatography, EtOAc:hexane, 2:1) to give **21**, 300 mg (62% yield); mp 205–6 °C; IR (KBr) ν_{max} 3260, 3120, 2930, 2240, 1640, 1510, 1440, 1420, 1400 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.58 (s, 3 H, $ArCH_3$), 2.74 (t, 2 H, CH_2), 3.40 (q, 2 H, CH_2), 4.0 (s, 3 H, NCH_3), 6.94 (d, 1 H, Ar-H), 7.24 (d, 1 H, Ar-H), 7.96 (d, 1 H, Ar-H), 8.32 (d, 1 H, Ar-H), 8.36 (t, 1 H, NH), 10.2 (s, 1 H, NH), 12.8 (br s, 1 H, NH); D_2O exchange resulted in the disappearance of peaks at δ 8.36, 10.2, and 12.8, and the quartet at δ 3.40 became a triplet; MS, m/z (rel intensity) $C_{20}H_{20}N_8O_5S$ found 484.1270 (7) (calcd. 484.1277), 332 (5), 213 (15), 192 (74), 153 (100), 107 (37).

3-[1-Methyl-4-[4-methyl-2-(1-methyl-4-nitropyrrole-2-carboxamido)thiazole-5-carboxamido]pyrrole-2-carboxamido]propionamidine Hydrochloride (22). A solution of **21** (242 mg, 0.5 mmol) in anhydrous ethanol (30 mL) was cooled to −15 °C and saturated with dry HCl gas. The reaction mixture was stirred at 0 °C for 1 h and at room temperature another hour. Solvent was removed in vacuo and the resulting residue was washed with dry ether (2 × 15 mL) to give imidate ester as a colorless solid. This solid was suspended in anhydrous ethanol (20 mL), and then 15 mL of ammonia was condensed. After the reaction mixture was stirred for 6–8 h at room temperature, solvent

was removed, and the resulting residue was recrystallized from methanol–ethyl acetate to give **22** as light yellow crystalline solid, 230 mg (86% yield); mp 228–30 °C; IR (Nujol) ν_{max} 3130, 3045, 1680, 1645, 1540, 1520, 1460 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.54 (s, 3 H, $ArCH_3$), 2.64 (t, 2 H, CH_2), 3.52 (q, 2 H, CH_2), 3.8 (s, 3 H, NCH_3), 4.0 (s, 3 H, NCH_3), 7.0 (d, 1 H, Ar-H), 7.24 (d, 1 H, Ar-H), 7.86 (d, 1 H, Ar-H), 8.2 to 8.3 (d merged with t, 2 H, Ar-H, NH), 8.4 (br s, 4 H, amidine), 10.0 (s, 1 H, NH), 11.45 (br s, 1 H, NH); FAB-MS, m/z (rel intensity) 502 (M – Cl, 32). Anal. Calcd for $C_{20}H_{24}ClN_9O_5S$: C, 44.65; H, 4.46; N, 23.44. Found: C, 44.80; H, 4.25; N, 23.70.

3-[1-Methyl-4-[4-methyl-2-(1-methyl-4-(formylamino)-pyrrole-2-carboxamido)thiazole-5-carboxamido]pyrrole-2-carboxamido]propionamidine Hydrochloride (5). A suspension of **22** (161 mg, 0.3 mmol) in methanol (25 mL) was hydrogenated over 10% Pd on charcoal (32 mg) at room temperature and atmospheric pressure. The catalyst was removed by filtration, and the filtrate was concentrated to give amine **23**. Owing to the instability of the amine, it was used immediately in the following reaction.

To the amine **23** in 98% formic acid (5 mL), acetic anhydride (1.5 mL) was added, and the reaction mixture was stirred at room temperature for 12 h. Solvent was evaporated to dryness in vacuo, and the resulting residue was crystallized from methanol and ethyl acetate to give **5**, 98 mg (61% yield); mp 230 °C (dec); IR (KBr) ν_{max} 3130, 3040, 1690, 1660, 1520, 1400 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.54 (s, 3 H, $ArCH_3$), 2.6 (t, 2 H, CH_2), 3.5 (t, 2 H, CH_2), 3.8 (s, 3 H, NCH_3), 3.9 (s, 3 H, NCH_3), 6.94 (d, 1 H, Ar-H), 7.2 (d, 1 H, Ar-H), 7.28 (d, 1 H, Ar-H), 7.46 (d, 1 H, Ar-H), 8.12 (s, 1 H, CHO), 8.24 (br s, 1 H, NH), 8.5 (br s, 2 H, amidine), 9.0 (br s, 2 H, amidine), 9.1 (s, 1 H, NH), 9.94 (s, 1 H, NH), 10.2 (s, 1 H, NH); FAB-MS, m/z (rel intensity) 500 (M–Cl, 10). Anal. Calcd for $C_{21}H_{26}ClN_9O_4S$: C, 47.05; H, 4.85; N, 23.52. Found: C, 47.36; H, 4.63; N, 23.20.

Ethyl 2-Aminothiazole-4-carboxylate (24). Synthesis of this compound was essentially based on the reported method:[35] mp 171–4 °C (lit.[35] mp 174.5–5.5); IR (Nujol) ν_{max} 3450, 1690, 1620, 1540, 1460 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.1 (t, 3 H, CH_3), 4.1 (q, 2 H, CH_2), 7.13 (br s, 2 H, NH_2), 7.35 (s, 1 H, Ar-H); MS, m/z (rel intensity) calcd for $C_6H_8N_2O_2S$ 172.0306, found 172.0306 (M^+, 96), 126 (M – C_2H_5O, 74), 100 (M^+ – $C_3H_4O_2$, 100).

Ethyl 2-(Formylamino)thiazole-4-carboxylate (25). To a solution of 3.89 g (24 mmol) of carbonyldiimidazole in 6 mL of dry THF was added 1 mL of 98% formic acid in 1 mL of dry THF, and this was stirred at room temperature for 15 min. **24** (1.032 g, 6 mmol) was dissolved in 6 mL of dry THF with little warming, and HCOOH-CDI reagent was added slowly and stirred at room temperature for 12 h. The THF was evaporated under reduced pressure, and the solid was washed with cold 1 N HCl and water and dried in vacuo, 1.145 g (95% yield); mp 215–18 °C; IR (Nujol) ν_{max} 3400, 1725, 1685, 1560, 1500, 1460 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.34 (t, 3 H, CH_3), 4.3 (q, 2 H, CH_2), 8.12 (s, 1 H, Ar-H), 8.58 (s, 1 H, CHO), 10.48 (br s, 1 h, NH); MS, m/z (rel intensity) calcd for $C_7H_8N_2O_3S$ 200.0256, found 200.0254 (M^+, 43), 172 (M – CO, 100), 128 (M – $C_3H_4O_2$, 6), 100 (M – $C_4H_4O_3$, 15), 76 (CH_4N_2S, 31).

2-(Formylamino)thiazole-4-carboxylic Acid (26). A suspension of 1.0 g (5 mmol) of **25** in 5 mL of 2 M NaOH in 1:1 methanol–water was stirred at room temperature for 4 h. The methanol was evaporated and the remaining water solution was cooled to <5 °C and neutralized with cold 1 N HCl until the pH was 2.5. A white jelly like precipitate was collected by centrifugation and the solid was washed with water and dried in vacuo, 800 mg (93% yield); mp 287–9 °C; IR (Nujol) ν_{max} 3300, 1720, 1625, 1540, 1505, 1460 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.38 (br s, 1 H, OH), 8.05 (s, 1 H, Ar-H), 8.6 (s, 1 H, CHO), 12.3 (br s, 1 H, NH); MS, m/z (rel intensity) calcd for $C_5H_4N_2O_3S$ 171.9943, found 171.9943 (M^+, 60), 125 (M – CH_2O_2, 100).

3-[1-Methyl-4-(2-(formylamino)thiazole-4-carboxamido)-pyrrole-2-carboxamido]propionamidine Hydrochloride (6). To a solution of 172 mg (1 mmol) of **26** in 8 mL of dry DMSO, 189 mg (1.4 mmol) and HOBT and 245.5 mg (1 mmol) of **18** were added. To this mixture 288.4 (1.4 mmol) of DCC in 2 mL of dry DMF was added in portions over 2 h. Stirring continued at room temperature for 12 h under argon atmosphere. The solvents were evaporated in vacuo to a small volume, and water was added to precipitate dicyclohexylurea (DCU). The DCU was removed by filtration, and the filtrate was evaporated to dryness under reduced pressure. The residue was dissolved in a small volume of dry methanol, precipitated with ethyl acetate, and filtered. The solid was collected, dissolved in a small volume of dry methanol, and precipitated with dry ether. The solid was collected and dried in vacuo, 270 mg (67% yield); mp 176–8 °C; IR (Nujol) ν_{max} 3240, 1640, 1540, 1460, 1375 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.62 and 3.5 (2 t, 4H, CH_2), 3.8 (s, 3 H, CH_3), 7.05 and 7.26 (2 d, 2 H, Ar-H), 7.9 (s, 1 H, Ar-H), 8.28 (br s, 1 H, NH), 8.58 (s, 1 H, CHO), 8.8 (br s, 2 H, NH_2), 9.0 (br s, 3 H, NH_2, NH), 9.88 (s, 1 H, NH); FAB-MS, m/z (rel intensity) 399.5, 364 (M^+ – Cl, 13). Anal. Calcd for $C_{14}H_{18}ClN_7O_3S$: C, 42.05; H, 4.51; N, 24.53. Found: C, 41.94; H, 4.46; N, 24.78.

Ethyl 2-(1-Methyl-4-nitropyrrole-2-carboxamido)thiazole-4-carboxylate (27). The acid chloride was made from 425 mg (2.5 mmol) of 4-nitro-1-methylpyrrole-2-carboxylic acid as described above. This was dissolved in 5 mL of dry THF and added slowly to a mixture of 430 mg (2.5 mmol) of **24** and 0.21 mL of dry pyridine in 10 mL of dry THF at –5 °C. The reaation mixture was allowed to come to room temperature and left stirring for 10 h. The THF was evaporated in vacuo, and water was added to precipitate a solid. This solid was collected by filtration, washed with acid, alkali, and water, and dried in vacuo, 730 mg (90% yield); mp 215–7 °C; IR (Nujol) ν_{max} 3250, 1720, 1660, 1540, 1510, 1460 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.26 (t, 3 H, CH_3), 3.96 (s, 3 H, CH_3), 4.28 (q, 2 H, CH_2), 8.1 (s, 1 H, Ar-H), 8.02 and 8.3 (2 d, 2 H, Ar-H), 10.58 (s, 1 H, NH); MS, m/z (rel intensity) calcd for $C_{12}H_{12}N_4O_5S$ 324.0528, found 324.0528 (M^+, 24), 153.0303 (100).

2-(1-Methyl-4-nitropyrrole-2-carboxamido)thiazole-4-carboxylic Acid (28). A suspension of 685 mg (2.1 mmol) of **27** in 25 mL of 2 M NaOH in 1:1 water–methanol was stirred for 90 min or until the solid dissolved. Insoluble solids, if any, were removed by filtration, and the methanol was evaporated in vacuo. The remaining water solution was cooled to <5 °C and acidified with cold 6 N HCl (until the pH was 2.5). The solid was collected by centrifugation, washed with water, and dried in vacuo, 594 mg (95% yield); mp 313–5 °C; IR (Nujol) ν_{max} 3600, 1850, 1680, 1570, 1540, 1500, 1460 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 3.99 (s, 3 H, CH_3), 8.03 (s, 1 H, Ar-H), 8.01 and 8.31 (2 d, 2 H, Ar-H), 10.52 (br s, 1 H, NH); MS, m/z (rel intensity) calcd for $C_{10}H_8N_4O_5S$ 296.0215, found 296.0219 (M^+, 13), 153 (100), 107 (33), 79 (5).

3-[2-(1-Methyl-4-nitropyrrole-2-carboxamido)thiazole-4-carboxamido]propionitrile (30). (a) A solution of 89 mg (0.3 mmol) of **28** in 2 mL of dry THF and 2 mL of oxalyl chloride was refluxed for 2 h. Excess oxalyl chloride and THF were removed under reduced pressure. The resultant acid chloride (**29**) was dissolved in 1 mL of dry THF and added slowly to a mixture of 21 mg (0.3 mmol) of β-aminopropionitrile and 40 mg of Hunig's base in 3 mL of dry THF at –10 °C. The reaction was allowed to come to room temperature and stirred for 3 h. The THF was removed in vacuo, water added, and the solid collected and dried, 77 mg (75% yield).

(b) A solution of 444 mg (1.5 mmol) of **28**, 119 mg (1.7 mmol) of β-aminopropionitrile, and 205 mg (1.5 mmol) of HOBT in 5 mL of dry DMSO was treated with 412 mg (2 mmol) of DCC in 1 mL of dry DMF in portions over a 1 h. The reaction was left at room temperature under argon atmosphere with stirring for 10 h. The solvents were evaporated to a small volume under reduced pressure and water was added. The solid was collected and washed with a 9:1 ratio of methanol–acetone, 345 mg (66% yield); mp 248–50 °C; IR (Nujol) ν_{max} 3300, 2320, 1650, 1540, 1490, 1460, 1420 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.8 (t, 2 H, CH_2), 3.55 (q, 2 H, CH_2), 4.01 (s, 3 H, CH_3), 7.9 (s, 1 H, Ar-H), 7.99 and 8.33 (2 d, 2 H, Ar-H), 10.32 (s, 1 H, NH); MS, m/z (rel intensity) calcd for $C_{13}H_{12}N_6O_4S$ 348.064, found 348.0643 (M^+, 16), 153 ($C_6H_5N_2O_3$, 100), 107 (C_6H_5NO, 25).

3-[1-Methyl-4-[1-methyl-4-(2-(formylamino)thiazole-4-carboxamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine Hydrochloride (7). A solution of 139 mg (0.35 mmol) of **33** in 10 mL of a 1:1 mixture of dry DMF and dry methanol at <5 °C was treated with 40 mg of 10% Pd/C and hydrogenated at room temperature and atmospheric pressure until the calculated amount of hydrogen was taken up. The catalyst was filtered off under argon atmosphere and the solvent was evaporated in vacuo to obtain **34**.

To a solution of 61 mg (0.35 mmol) of **26** and 68 mg (0.5 mmol) of HOBT in 3 mL of dry DMSO was added **34** in 1 mL of dry DMF. A solution of 103 mg (0.5 mmol) of DCC in 2 mL of dry DMF was then added in portions over 90 min and left at room temperature with stirring for 12 h under argon. The solvents were evaporated to a small volume under reduced pressure, and water was added to precipitate DCU. The solid was filtered off, and the filtrate was evaporated to dryness in vacuo. The residue was dissolved in methanol and precipitated with ethyl acetate and the solid was collected, 89.5 mg (49% yield), no distinct melting point, decomposed about 207 °C; IR (Nujol) ν_{max} 3255, 1680, 1533, 1464, 1377 cm^{-1}; ^1H NMR (CD_3OD) δ 2.64 and 3.58 (2 t, 4 H, CH_2), 3.82 and 3.88 (2 s, 6 H, CH_3), 6.84, 6.96, 7.14 and 7.26, (4 d, 4 H, Ar-H), 7.82 (s, 1 H, Ar-H), 8.52 (s, 1 H, CHO); FAB-MS, m/z (rel intensity) 521.5, 486 (M − Cl, 16). Anal. Calcd for $C_{20}H_{24}ClN_9O_4S$: C, 46.02; H, 4.60; N, 24.16. Found: C, 46.11; H, 4.69; N, 24.02.

3-[1-Methyl-4-[2-(1-methyl-4-nitropyrrole-2-carboxamido)thiazole-4-carboxamido]pyrrole-2-carboxamido]propionamidine Hydrochloride (35). To a solution of 110 mg (0.37 mmol) of **28** and 54 mg (0.4 mmol) of HOBT in 3 mL of dry DMSO was added 91 mg (0.37 mmol) of **18** in 1 mL of dry DMF. A solution of 103 mg (0.5 mmol) of DCC in 1 mL of dry DMF was then added over a period of 90 min in portions and left at room temperature with stirring for 10 h. The solvents were removed in vacuo, and the residue was dissolved in dry DMF, cooled to −20 °C, and filtered free of DCU. The filtrate was evaporated to dryness in vacuo, and the solid was dissolved in a small volume of dry methanol (~2 mL) and precipitated with ethyl acetate. This process of dissolving in methanol and precipitation with ethyl acetate was done five times. Finally the solid was washed with dry ether and hexane and dried in vacuo, 131 mg (68% yield); mp 236 °C mp (dec); IR (Nujol) ν_{max} 3250, 1690, 1660, 1540, 1500, 1460 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.68 (t, 2 H, CH_2), 3.54 (q, 2 H, CH_2), 3.84 and 4.02 (2 s, 6 H, CH_3), 7.97 (s, 1 H, Ar-H), 7.01, 7.3, 8.02 and 8.34 (4 d, 4 H, Ar-H), 8.44 (t, 1 H, NH), 8.72 and 9.08 (2 br s, 4 H, NH_2), 9.78 (s, 1 H, NH), 12.88 (br s, 1 H, NH); FAB-MS, m/z (rel intensity) 523.5, 487 (M^+ − Cl, 9). Anal. Calcd for $C_{19}H_{22}ClN_9O_5S$: C, 43.55; H, 4.20; N, 24.07. Found: C, 43.63; H, 4.44; N, 24.17.

3-[1-Methyl-4-[2-[1-methyl-4-(formylamino)pyrrole-2-carboxamido]thiazole-4-carboxamido]pyrrole-2-carboxamido]propionamidine Hydrochloride (8). A cold solution of 105 mg (0.2 mmol) of **35** in 5 mL of dry methanol was treated with 32 mg of 10% Pd/C and hydrogenated at room temperature and atmospheric pressure until the calculated amount of hydrogen was taken up. The catalyst was removed by filtration and the filtrate was evaporated to dryness. The solid was washed with dry ether and hexane and dried in vacuo to obtain **36**.

A solution of 38 μL (1 mmol) of 98% formic acid in 12 mL of dry THF was added to 163 mg (1 mmol) of carbonyldiimidazole in 1 mL of dry THF and left at room temperature for 15 min. The amine **36** was dissolved in 4 mL of dry methanol and cooled to −40 °C. To this solution was added formic acid–carbonyldiimidazole reaction mixture, and this was stirred at −40 °C for 30 min. The solvent was evaporated to a small volume and the compound was precipitated with ethyl acetate. The crude compound was collected and purified by preparative TLC using methanol with a few drops of acetic acid as solvent system, 42 mg (40% yield); no distinct melting point, decomposed at 219 °C; IR (Nujol) ν_{max} 3260, 1700, 1660, 1540, 1460 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.52 and 3.62 (2 t, 4 H, CH_2), 3.98 and 4.09 (2 s, 6 H, CH_3), 7.32 (s, 1 H, Ar-H), 6.66, 7.07, 7.14 and 7.4 (4 d, 4 H, Ar-H), 8.12 (s, 1 H, CHO), 8.38 (br t, 3 H, NH, NH_2), 10.0 (s, 1 H, NH), 11.6 (br s, 1 H, NH); FAB-Ms, m/z (rel intensity) 486 (M − Cl, 7). Anal. Calcd for $C_{20}H_{24}ClN_9O_4S$: C, 46.11; H, 4.69; N, 24.12. Found: C, 45.98; H, 4.73; N, 24.28.

Estimation of Drug–DNA Binding Constants. The binding constants were measured by the ability of the new compounds to compete with the binding of ethidium bromide on ct DNA.[36-38] The fluorescence measurements were done on a Turner 430 spectrofluorometer. All the experiments were done in 20 mM NaCl buffer, pH 7.1, and at a room temperature of 21 ± 1 °C. The EtBr; DNA ratio was 1.26. The excitation and emission wavelengths were 525 and 600 nm, respectively. Controls were performed to show that the new compounds themselves did not interfere with the fluorescence measurements at the levels employed. IC_{50} (concentration of the compound required to reduce fluorescence of DNA bound EtBr to 50%) values were determined from the best computed fit of the competitive sigmoid curve. K_{app} values were calculated by using IC_{50} values assuming K_{app} of EtBr as 1×10^7 M^{-1} for ct DNA.

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Registry No. 1, 1438-30-8; 2, 39389-47-4; 3, 123724-77-6; 3 (free base), 123724-99-2; 4, 123724-78-7; 4 (free base), 123725-00-8; 5, 123724-79-8; 5 (free base), 123725-01-9; 6, 123724-80-1; 6 (free base), 123725-02-0; 7, 123724-81-2; 7 (free base), 123725-03-1; 8, 123724-82-3; 8 (free base), 123725-04-2; 9a, 7210-76-6; 9b, 51755-34-1; 10, 123724-83-4; 11, 123751-02-0; 12, 123724-84-5; 13, 123724-85-6; 14, 123724-86-7; 15, 123724-87-8; 16, 67899-00-7; 17, 123724-88-9; 18, 78395-16-1; 19, 3185-95-3; 20, 97950-77-1; 21, 123724-89-0; 22, 123751-03-1; 23, 123724-90-3; 24, 5398-36-7; 25, 123724-91-4; 26, 123724-92-5; 27, 123724-93-6; 28, 123724-94-7; 29, 123724-95-8; 30, 123724-96-9; 32, 3185-94-2; 33, 14555-80-7; 34, 97950-75-9; 35, 123724-97-0; 36, 123724-98-1; $H_2NCH_2CH_2CN$, 151-18-8; 1-methyl-4-nitropyrrole-2-carboxylic acid, 13138-78-8; 1-methyl-4-nitropyrrole-2-carboxylic chloride, 28494-51-1; 3-(1-methyl-4-nitropyrrole-2-carboxamido)propionamidine hydrochloride, 24064-13-9.

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