

the water in the perfusing sample. A Lorentzian-shaped water signal with half-height width of ca. 20 Hz was generally obtained. Proton decoupling was applied only during the acquisition period in order to suppress sample heating as well as possible differential nuclear Overhauser effects. Prior to Fourier transformation, the averaged FID (2000 transients were averaged) signal was multiplied by an exponential function so as to result in an additional 15-Hz line broadening in the frequency domain spectrum and then twice zero filled (to 16K data points). Spectra were obtained at ambient probe temperature (ca. $23 \pm 1^\circ\text{C}$).

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Structure-Activity Relationship of Novel Oligopeptide Antiviral and Antitumor Agents Related to Netropsin and Distamycin

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A group of oligopeptides have been synthesized that are structurally related to the natural antiviral antitumor agents netropsin and distamycin. Cytostatic activity against both human and murine tumor cell lines as well as their in vitro activity against a range of viruses is reported. The biological activity of these agents is discussed both in terms of their structural differences and, in particular, in relation to their observed base- and sequence-dependent minor-groove binding to duplex oligonucleotides.

The current dearth of clinically effective antiviral agents demands the exploration of new structural types of potential agents of this class. While the more familiar antiviral agents belong to the nucleoside class,¹ there exists a family of naturally occurring oligopeptides² including netropsin (1),³ distamycin (2),^{2,4} anthelvencin,⁵ amidinomycin,⁶ kikumycin B,⁷ and norformycin,⁸ some examples of which display antiviral and anticancer activities. The most active of these, netropsin and distamycin, are too toxic for clinical use,^{1,2} and yet relatively few reports exist of the examination of variants of the basic pyrrolo oligopeptide structure.⁹⁻¹⁷ Netropsin and distamycin bind within the minor groove of DNA where they demand binding sites consisting of four and five A-T base pairs, respectively.¹⁸ This firm and site-specific binding, which seems to underlie the biological activity,^{1,2} is the net result of specific hydrogen-bonding, electrostatic attraction, and van der Waals interactions.¹⁹ Analysis of the structural and spatial requirements for this specific binding led to the prediction that replacement of one or more pyrrole moieties by imidazole should alter the strict A-T base preference, leading to G-C preferences.^{20,21} A recent study requiring the synthesis of several novel oligopeptides showed that this prediction is, in fact, borne out.²⁰

In the present study we report the synthesis of structurally modified oligopeptides including those bearing quaternary ammonium end groups in place of the guanidine moiety in netropsin. These latter compounds, together with the free amino derivative, permit an assessment of the role of the nature and size of the charged end group on binding to DNA and in biological activity. We report biological data on these altered-base preferential minor-groove binding and other structurally modified oligopeptides and attempts to relate their biological properties to their specific interactions with DNA.

Synthesis

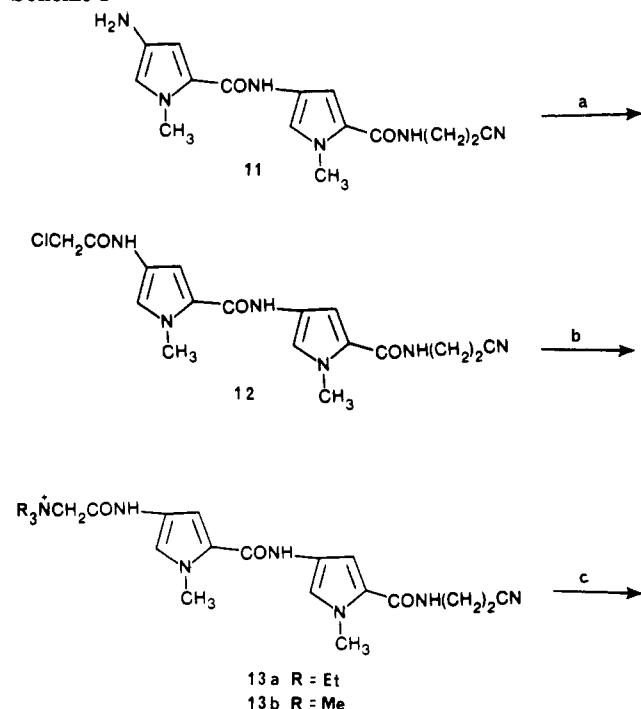
The structure of the antibiotic netropsin (1) was established in 1963 by Julia and Préau-Joseph.³ Since that time,

syntheses of both the parent antibiotic and distamycin²²⁻²⁴ as well as certain homologues and analogues^{9,10,13-17} have

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Scheme I^a

^a Reaction conditions: (a) ClCH_2COCl , $i\text{-Pr}_2\text{EtN}$, CH_3CN ; (b) Me_3N or Et_3N , EtOH , Δ ; (c) HCl in EtOH , then dry NH_3 , EtOH .

been reported. The syntheses of those latter compounds were based largely on the original method of Julia and Préau-Joseph.²² The anticipated need to examine a number of new structures in order to establish the molecular recognition characteristics for DNA binding and, possibly, for biological activity required development of an efficient and flexible general synthesis. Therefore, we recently reported new and efficient total syntheses of netropsin and distamycin that are sufficiently adaptable for the present requirements.²⁵

The new syntheses have the following advantages: (i) isolation of the starting 1-methyl-4-nitropyrrole-2-carboxylic acid in an isomerically pure state by recrystallization thus avoiding column chromatography; (ii) high-yield peptide linkage effected by coupling of amines with acyl chlorides in the presence of Hunig's base; (iii) more convenient and efficient procedure for attachment of side chains involving a change of sequence of their introduction with the Pinner reaction in the last step; (iv) tripeptide formation effected by coupling of a dipeptide acid with an amine bearing the amidopropionitrile moiety; (v) new, more efficient N-formylation using *N*-formyl-imidazole in methanolic solution.²⁵ For example, this permitted the synthesis of netropsin lacking the guanidinoacetyl group, 3, as well as deformyl-distamycin (4).²⁵ The strategy adopted involves significant changes in the methods and order of introduction of the end groups from

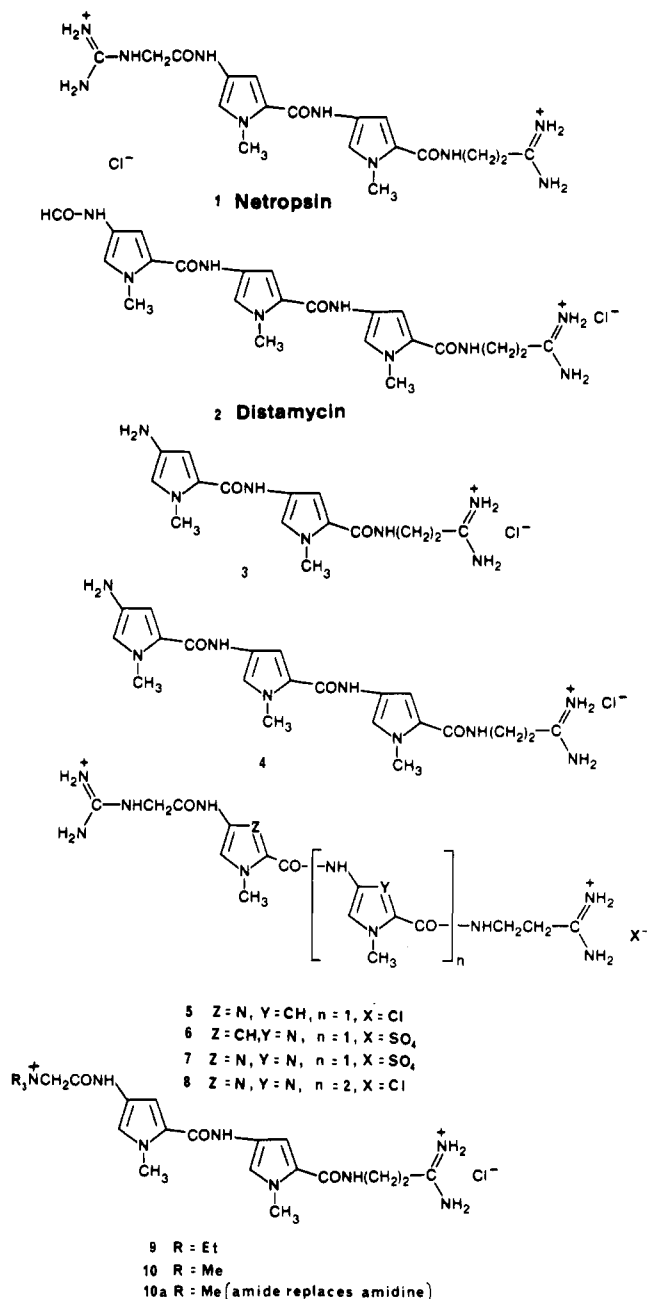


Figure 1. Structures of oligopeptide antiviral antibiotics netropsin, distamycin, and related synthetic agents.

those reported hitherto.^{4,22-24,26} This resulted in good yields of the final products. An additional important advantage was the avoidance of chromatography that had been used hitherto. That technique is unsuitable for such polar compounds as these oligopeptide antiviral agents, because of contamination of the final products with inorganic salts eluted from the absorbents.

The synthesis of mixed imidazole-pyrrole and pyrrole-imidazole dipeptides 5 and 6 as well as their di- and tri-imidazole counterparts 7 and 8 was adapted from the preceding method using the appropriate heterocyclic precursors.²⁰ Compounds 9 and 10 bearing alternative end groups to the guanidinoacetyl moiety present in netropsin were prepared as outlined on Scheme I. The amino compound 11 was allowed to react with chloroacetyl chloride in acetonitrile in the presence of Hunig's base. The resulting chloroacetyl derivative 12 was then treated with triethylamine or trimethylamine for several hours in

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Table I. Inhibitory Effects of the Netropsin- and Distamycin-Related Oligopeptides on the Proliferation of Murine Leukemia (L1210), Murine Mammary Carcinoma (FM3A), and Human Lymphoblast (Raji and Namalva) Cells

compd	ID ₅₀ ^a $\mu\text{g/mL}$			
	L1210	FM3A	Raji	Namalva
1 (netropsin)	245 $\pm$ 92	321 $\pm$ 18	139 $\pm$ 63	103 $\pm$ 46
2 (distamycin)	27 $\pm$ 4.7	31 $\pm$ 2.4	24 $\pm$ 3.7	22 $\pm$ 3.5
3	276 $\pm$ 38	327 $\pm$ 9.0	43 $\pm$ 10	36 $\pm$ 1.5
4	174 $\pm$ 22	454 $\pm$ 133	32 $\pm$ 6.1	29 $\pm$ 3.6
5	>100	>100	>100	>100
6	>100	>100	>100	>100
7	238 $\pm$ 77	>500	316 $\pm$ 14	261 $\pm$ 19
8	42 $\pm$ 0.5	46 $\pm$ 2.0	52 $\pm$ 2.0	100
9	222 $\pm$ 18	334 $\pm$ 56	252 $\pm$ 26	180 $\pm$ 32
10	543 $\pm$ 51	>1000	504 $\pm$ 61	340 $\pm$ 43
10a	>500	>500	>500	225 $\pm$ 11

^a 50% inhibitory dose $\pm$ standard deviation.

refluxing ethanol to give the quaternary salts **13a** and **13b**, respectively. Treatment of the latter under the modified Pinner reaction conditions gave **9** and **10**, respectively. The additional compound **10a** in which the amidine group is replaced by an amide moiety was also synthesized.

Results

Physical Properties of the Novel Oligopeptides.

The oligopeptides **1**, **2**, and **5–8** bind to duplex DNA but not to single-stranded DNA.²⁰ Significant binding is observed with T₄ DNA for compounds **5–8** in accord with minor-groove binding of the new agents, as is found for the parent antibiotics netropsin (**1**) and distamycin (**2**).²⁷ The exclusively pyrrole-containing oligopeptides **3**, **4**, **9**, and **10** are expected to show the strict A-T base preferential binding of the parent antibiotics **1** and **2**. In contrast, the imidazole-bearing counterparts **5–8** display a progressively increasing preference for binding to both native DNAs and synthetic repeating oligodeoxyribonucleotides with higher (G + C) content as each of the pyrrole units is successively replaced by imidazole.²⁰ The strongest trend toward lessened preference for A-T sites and a corresponding G-C preference is observed for the triimidazole **8**.²⁰ Since the imidazole-containing oligopeptides bind in the minor groove, then the acceptance of G-C pairs implicates hydrogen bonding to the 2-NH₂ group of guanine.²⁰

Cytostatic Activity. Among the oligopeptide derivatives tested for their inhibitory effects on the proliferation of murine L1210 and FM3A and human Raji and Namalva cells, distamycin (**2**) proved to be the most active (Table I). Its ID₅₀ values for the different tumor cell lines ranged between 22 and 31 $\mu\text{g/mL}$. Compound **4** (deformyl-distamycin) was equally inhibitory to the two human tumor cell lines, but 5–15-fold less active against the murine tumor cell lines. This proved also to be the case for compound **3**, which may be considered as an analogue of netropsin lacking the guanidinoacetyl group. Netropsin itself was slightly more active against the human cell lines (ID₅₀ = 103–139 $\mu\text{g/mL}$) than against the murine cell lines (ID₅₀ = 245–321 $\mu\text{g/mL}$). These data indicate that a terminal unsubstituted amino group may impart a greater selectivity of the oligopeptides toward human tumor cells. The imidazole-pyrrole, pyrrole-imidazole, and imidazole-

Table II. Antiviral Activity of the Netropsin- and Distamycin-Related Oligopeptides in Primary Rabbit Kidney Cell Cultures

compd	min inhib concn, ^b $\mu\text{g/mL}$				
	min, cytotoxic concn, ^a $\mu\text{g/mL}$	herpes simplex virus type 1 (strain KOS)	herpes simplex virus type 2, strain G	vaccinia virus	vesicular stomatitis virus
1 (netropsin)	40	≥ 40	≥ 40	2	≥ 40
2 (distamycin)	200	70	70	0.2	≥ 200
3	400	150	150	20	≥ 400
4	100	≥ 100	≥ 100	7	≥ 100
5	>400	>400	>400	40	>400
6	200	≥ 200	≥ 200	40	≥ 200
7	>400	>400	>400	300	>400
8	100	≥ 100	≥ 100	≥ 100	≥ 100
9	>400	300	300	300	>400
10	≥ 400	>200	>200	20	>400
10a	≥ 400	>400	>400	100	>400
tubercidin	0.4	≥ 0.4	≥ 0.4	0.07	0.07
(S)-DHPA	>400	>400	>400	70	20
ribavirin	>400	>400	>400	30	200
C-c ³ Ado	>400	200	≥ 400	0.3	0.3

^a Required to cause a microscopically detectable alteration of normal cell morphology. ^b Required to reduce virus-induced cytopathogenicity by 50%.

ole-imidazole dipeptides **5–7** were virtually inactive (ID₅₀ = $\gg 200$ $\mu\text{g/mL}$), whereas the triimidazole derivatives **8** showed a moderate inhibitory effect on tumor cell proliferation (ID₅₀ = 42–100 $\mu\text{g/mL}$). The (triethylamino)- and (trimethylamino)-acetyl derivatives of netropsin (**9**, **10a**, the latter bearing an amide group instead of the usual amidine) showed equal or lesser cytostatic activity than netropsin itself (**11**) whereas **10** had virtually no cytostatic activity.

Antiviral Activity. Several oligopeptides were specifically active against vaccinia virus; i.e., they inhibited the cytopathic effect of vaccinia virus at a concentration that was significantly lower than their minimum cytotoxic concentration. Thus, in order of decreasing antivaccinia potency, **2** > **1** > **4** > **3** = **10** > **5** = **6** > **10a** > **8** > **7** = **9**. None of the oligopeptide compounds exhibited a significant activity against herpes simplex virus type 1 or type 2, and they were completely devoid of activity against vesicular stomatitis virus (Table II). Various other RNA viruses, i.e. polio type 1, coxsackie type B4, reotype 1, parainfluenza type 3, measles, Sindbis, and Semliki forest, were also examined for their sensitivity to the oligopeptides (in either HeLa or Vero cell cultures), and again, no activity was noted against these RNA viruses with any of the compounds at subtoxic concentrations (data not shown).

The reference compounds included in the antiviral tests were tubercidin, (S)-DHPA ((S)-9-(2,3-dihydroxypropyl)adenine), ribavirin, and C-c³Ado (carbocyclic 3-deazaadenosine).^{28,29} The oligopeptides differed from these reference compounds, in that their antiviral activity was restricted to vaccinia virus and that they were quite specific in their antivaccinia activity. Tubercidin was active against vaccinia virus at a minimum antiviral concentration that was only slightly lower than its cytotoxic concentration. C-c³Ado inhibited vaccinia and vesicular stomatitis virus to the same extent, and (S)-DHPA, ribavirin, as well as C-c³Ado proved inhibitory to a broad

(27) Since T₄ DNA has glycosylation of the 5-(hydroxymethyl)cytidine residues that occlude the major groove the observed binding is consistent with attachment in the minor groove as has been established for netropsin:^{18,19} Erikson, R. L.; Szybalski, W. *Virology* 1964, 22, 111.

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spectrum of viruses, including several RNA viruses, i.e. reo, parainfluenza, and measles (data not shown).

In terms of the relationship between structure and antiviral activity, it appears that (i) pyrrole dipeptides (i.e., 1) are more active than mixed pyrrole-imidazole dipeptides (i.e., 5 and 6), which are, in turn, more active than the imidazole di- or tripeptides 7 and 8; (ii) pyrrole tripeptides are generally more active than pyrrole dipeptides (i.e., compare 3 with 4 but excepting 1 and 2); (iii) the formyl group may play an important part in the activity of the new oligopeptides, since, for example, deformylated distamycin (4) is considerably less active than distamycin. (iv) Smaller charged end groups replacing the guanidinium group confer greater activity against vaccinia virus.

Discussion

The results indicate some of the structural parameters that contribute to the cytostatic and antiviral activity of the novel oligopeptides. Comparison of the cytostatic activities of 2 with 4 and of 1 with 3 shows that at least one end group may be removed and that activity is retained. Indeed, the free amino group in both 3 and 4 may confer greater selectivity against human tumor cell lines compared with murine cells. In contrast, altering the semantophore units, or primary-sequence reading sites, which are primarily the heterocyclic residues,¹⁹ markedly decreases cytostatic activity in the cases of 5-7.

These compounds all bind to the minor groove of duplex DNA²⁰ like the parent compounds netropsin and distamycin¹⁸ and the binding constants to calf thymus DNA are as follows: netropsin, 1.87; 5, 1.87; 6, 1.98; 7, 1.76; 8, $1.06 \times 10^8 \text{ M}^{-1}$. Thus, the overall physical characteristics of target-site binding are comparable for this series of compounds. The imidazole-containing compounds 5-8, however, show a progressive trend toward G-C preferential binding.²⁰ Since their transport properties are likely to be similar to those of the natural products, then it appears that the strict sequence preference of netropsin and distamycin for AT-rich sites is an important determinant of their cytostatic and antiviral activities. The loss of cytostatic activity resulting from the increased G-C preference of 5-7 is offset somewhat by the introduction of the third heterocyclic moiety, a trend that is also reflected in the antiviral activity results.

Replacement of the guanidinium group by a bulky triethylammonium group in 9 or the smaller trimethylammonium in 10 reduces cytostatic activity perhaps due in part to the decreased overall binding to duplex DNA. However, replacement of the triethylammonium group in 9 by the smaller end group in 10 markedly increased activity against vaccinia virus (Table II). In contrast removal of the charged amidine in 10a, another important semantophoric group that recognizes an A-T site,¹⁹ markedly decreases the cytostatic activity.

The above conclusions on the effects of systematic structural changes on activity are borne out in the antiviral screens. Again, the dominant effect is seen in altering the base sequence specificity toward G-C preference, which reduces antiviral activity. However, this trend is offset somewhat in the case of 7 and 8 due to the increased length of the oligopeptide, an effect that is, as we have seen, also observed in the cytostatic activity data.

In conclusion the strict requirements of this class of novel oligopeptides for tight binding to the minor groove of duplex DNA and their predictable base preferences in the processes of molecular recognition and binding provide a framework for the interpretation of their structure-biological activity relations. The properties of additional classes of oligopeptide agents designed to further delineate

their molecular recognition requirement will be reported in due course.

Experimental Section

Melting points were determined on a Fisher-Johns apparatus and are uncorrected. The IR spectra were recorded on Nicolet 7199 FT spectrophotometer, and only the principal peaks are reported. The ¹H NMR spectra were recorded on Bruker WH-200 and WH-400 spectrometers. FAB (fast atom bombardment) mass spectra were determined on an Associated Electrical Industries (AEI) MS-9 and MS-50 focusing high-resolution mass spectrometers. Kieselgel 60 (230-400 mesh) of E. Merck was used for flash chromatography, and precoated sheets Silica gel 60F₂₅₄ of E. Merck were used for TLC. TLC systems: (i) covalent peptidic compounds, chloroform-methanol (9:1); (ii) ionic compounds with one ionic pair, methanol with some AcOH; (iii), ionic compounds with two ionic pairs, methanol with some formic acid.

3-[1-Methyl-4-[1-methyl-4-(chloroacetamido)pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionitrile (12). A solution of 3-[1-methyl-4-(1-methyl-4-aminopyrrole-2-carboxamido)pyrrole-2-carboxamido]propionitrile (7; 315 mg, 1 mmol) and ethyldiisopropylamine (191 μL , 1.1 mmol) in anhydrous acetonitrile (5 mL) was cooled to -15 °C, and a solution of chloroacetyl chloride (88 μL , 1.1 mmol) in 1 mL of anhydrous THF was added. The temperature was allowed to reach 20 °C, and the solvent was removed in vacuo. After the addition of water the resulting solid was collected and recrystallized from CH₃CN to give 300 mg (77% yield) of 12: mp 233-234 °C; ¹H NMR (DMF-*d*₇) δ 2.86 (t, 2 H), 3.93 and 3.96 (2 s, 6 H), 4.26 (s, 2 H), 7.07 (d, 2 H), 7.30 and 7.35 (2 d, 2 H), 8.46 (t, 1 H), 10.03 (s, 1 H), 10.42 (s, 1 H), the region 3.5-3.9 overlapped by H₂O peak; IR (Nujol) 1377, 1464, 1523, 1560, 1575, 1587, 1634, 2250, 3120, 3242, 3330 cm^{-1} ; MS (*m/z*, relative intensity) 392.1186 (11.54) M⁺ for ³⁷Cl, 390.1206 (33.5) M⁺ for ³⁵Cl.

3-[1-Methyl-4-[1-methyl-4-[(triethylammonio)acetamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionitrile Chloride (13a). A solution of compound 12 (195 mg, 0.5 mmol) in 10 mL of dry ethanol with an excess of triethylamine (1 mL) was heated under reflux. The progress of the reaction was monitored by means of TLC (SiO₂, MeOH) and was thereby judged to be complete after 6 h. The solution was evaporated to dryness in vacuo, and the residual solid was extracted with chloroform (3 $\times$ 25 mL). The combined CHCl₃ extracts were concentrated, and the addition of hexane precipitated 196 mg (80% yield) of 13a: mp 165 °C dec; ¹H NMR (CDCl₃) δ 1.28 (t, 9 H), 2.76 (t, 2 H), 3.43 (q, 6 H), 3.90 and 3.92 (2 s, 6 H), 4.33 (s, 2 H), 7.18 (d, 1 H), 7.24 (d, 1 H), 7.37 (d, 1 H), 7.46 (d, 1 H), 7.91 (t, 1 H), 9.49 (s, 1 H), 11.0 (s, 1 H); IR (Nujol) 1378, 1408, 1463, 1530, 1580, 1650, 1678, 2242, 3260 cm^{-1} ; MS FAB (*m/z*) 947 (2 M - Cl)⁺, 911 (2 M - Cl - HCl)⁺, 456 (M - Cl)⁺. Anal. Calcd for C₂₃H₃₄ClN₇O₃: C, 56.1; H, 7.0; Cl, 7.2; N, 19.9. Found: C, 55.7; H, 7.2; Cl, 7.0; N, 19.5.

3-[1-Methyl-4-[1-methyl-4-[(trimethylammonio)acetamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionitrile Chloride (13b). A solution of the chloroacetyl derivative 12 (420 mg, 1.07 mmol) and 2 mL of trimethylamine in 98% EtOH (40 mL) was heated for 1.5 h at 90 °C in a sealed tube. The reaction was shown to be complete by TLC analysis on silica gel developed with MeOH/AcOH. The mixture was evaporated to dryness and the residue taken up in CHCl₃ with a few drops of MeOH and hexane added to initial turbidity. The solution was decolorized with charcoal and the process repeated. The pure product was obtained by recrystallization from a small volume of MeOH: 213 mg (77% yield); mp 239-241 °C dec; ¹H NMR (me₂SO-*d*₆) δ 2.74 (t, 2 H), 3.28 (s, 9 H), 3.40 (q, 2 H), 3.81 and 3.86 (2 s, 6 H), 4.37 (s, 2 H), 6.93 and 6.97 (2 d, 2 H), 7.22 and 7.25 (2 d, 2 H), 8.37 (t, 1 H), 10.00 (s, 1 H), 11.01 (s, 1 H); IR (Nujol) ν_{max} 1220, 1252, 1376, 1403, 1437, 1464, 1503, 1557, 1564, 1654, 1680, 2240, 3190, 3270, 3350 cm^{-1} ; MS FAB (*m/z*) 414 (M - Cl)⁺.

3-[1-Methyl-4-[1-methyl-4-[(triethylammonio)acetamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamide Hydrochloride (9). A solution of 13a (173 mg, 0.35 mmol) in 10 mL of absolute ethanol was treated with dry hydrogen chloride with cooling. After 2 h the solvent was removed in vacuo and the residue dissolved in 10 mL of absolute ethanol and treated with an excess of dry ammonia. After

2 h at room temperature the solvent was removed in vacuo and the residue dissolved in 5 mL of isopropyl alcohol; then, the product was precipitated with ether. The solid was collected, washed with ether, and dried at 100 °C in vacuo to afford **9**: 103 mg (59% yield); mp 180 °C dec; ¹H NMR (Me₂SO-*d*₆) δ 1.32 (t, 9 H), 2.67 (t, 2 H), 3.54 (m, 8 H), 3.83 and 3.88 (2 s, 6 H), 4.32 (s, 2 H), 6.96 (d, 1 H), 7.01 (d, 1 H), 7.21 (d, 1 H), 7.30 (d, 1 H), 8.28 (t, 1 H), 8.80 and 9.10 (2 br s, 4 H), 10.03 (s, 1 H), 11.47 (s, 1 H); IR (Nujol) 1376, 1404, 1462, 1531, 1581, 1646, 1684, 3250 cm⁻¹; MS FAB (*m/z*) 981 (2 M - HCl - Cl)⁺, 473 (M - HCl - Cl)⁺.

3-[1-Methyl-4-[1-methyl-4-[(trimethylammonio)acet-amido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]-propionamide Chloride Hydrochloride (10). A solution of **13b** (347 mg, 0.7 mmol) in 5 mL of absolute ethanol was treated with dry hydrogen chloride with cooling. After 2 h the solvent was removed in vacuo, 5 mL of absolute ethanol was added, and dry NH₃ gas was passed into the solution. The solid dissolved during 2 h at room temperature and then the solution was evaporated to dryness and extracted with hot isopropyl alcohol (100 mL). The extract was concentrated to ca. 10 mL, acetone added, and the resulting precipitate collected, washed with acetone, and dried in vacuo to give **10**: 300 mg (85% yield), as an amorphous hygroscopic solid; no definite mp; ¹H NMR (Me₂SO-*d*₆) δ 2.67 (t, 2 H), 3.31 (s, 9 H), 3.52 (q, 2 H), 3.82 and 3.87 (2 s, 6 H), 4.44 (s, 2 H), 6.97 (d, 1 H), 7.02 (d, 1 H), 7.24 (d, 1 H), 7.29 (d, 1 H), 8.31 (t, 1 H), 8.82 (br s, 2 H), 9.72 (br s, 2 H), 10.06 (s, 1 H), 11.23 (s, 1 H); IR (Nujol) ν_{max} 1260, 1377, 1405, 1453, 1531, 1582, 1643, 1685, 3247 cm⁻¹; MS FAB (*m/z*) 430 (M - HCl - Cl)⁺.

Sulfate. The sulfate corresponding to **10** was prepared in order to obtain an analytically pure sample by precipitation from a methanolic solution of **10** by means of a large excess of tetraethylammonium sulfate: mp 295 °C; IR (Nujol) ν_{max} 1255, 1377, 1405, 1462, 1525, 1560, 1580, 1640, 1670, 1678, 3280 cm⁻¹; MS FAB (*m/z*) 431 (M - HSO₄)⁺, 529, MH⁺. Anal. Calcd for C₂₀H₃₂N₈O₇S (528.59): C, 45.4; H, 6.1; N, 21.1; S, 6.1. Found: C, 45.0; H, 6.0; N, 20.7; S, 5.8.

3-[1-Methyl-4-[1-methyl-4-[(trimethylammonio)acet-amido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]-propionamide Chloride (10a). A solution of **13b** (200 mg, 0.45 mmol) in 5 mL of absolute ethanol was saturated with dry HCl gas with cooling. After 2 h at room temperature the solvent was removed in vacuo, 5 mL of absolute ethanol was added, and dry NH₃ was condensed into the reaction vessel. After 10 min the solution was evaporated to dryness, some water added, and the

solution evaporated again. The residual solid was extracted with hot isopropyl alcohol, and then the extracts were concentrated to a small volume and acetone was added. The resulting precipitate was collected, washed with acetone, and quickly dried at 100 °C to give **10a**: 125 mg (60% yield); mp 177–179 °C; ¹H NMR (Me₂SO-*d*₆) δ 2.33 (t, 2 H), 3.30 (s, 9 H), 3.38 (q, 2 H), 3.81 and 3.86 (2 s, 6 H), 4.37 (s, 2 H), 6.84 (br s, 2 H), 6.98 (d, 1 H), 7.26 (d, 1 H), 7.38 (d, 1 H), 7.38 (br s, 1 H), 8.00 (t, 1 H), 9.97 (s, 1 H), 10.98 (s, 1 H); IR (Nujol) ν_{max} 1230, 1255, 1376, 1410, 1465, 1508, 1558, 1572, 1648, 1670, 3200, 3250, 3350, cm⁻¹; MS FAB (*m/z*) 432 (M - Cl)⁺.

Antitumor assays were performed according to previously established procedures.^{30,31}

Antiviral assays were performed as reported previously.^{28,32} The origin and preparation of the virus stocks have been documented in ref 28 and 32.

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