The incubation was continued for another 10 min. The enzyme reaction was terminated and the activity was measured in the same way as for the CKS-KLT assay. If no difference in enzyme activity was observed with or without an active compound, this indicates that the test compound inhibited the CMP-KDO synthetase.

Determination of Minimum Inhibitory Concentration (MIC). Twofold serial dilutions of the test compound were made in Mueller-Hinton broth (Oxoid) in the wells of a Microtitre plate, giving a final volume of 100 μL/well. Bacteria (10^3), from an overnight bacterial culture, were inoculated into each well, and the Microtitre plate was incubated at 37 °C overnight. The MIC was judged to be the lowest drug concentration at which no turbidity could then be detected.

Acknowledgment. We thank Susanna Wingborg for skillful technical assistance and Margareta Berg and Monica Thedvall for carrying out MIC determinations.

Registry No. 3, 106174-63-4; 4, 116337-35-0; 5, 116337-38-3; 5 (8-*o*-trityl derivative), 116337-36-1; 5 (8-*o*-tosyl derivative), 116337-43-0; 6, 116337-39-4; 6 (8-*o*-trityl derivative), 116337-37-2; 7, 116337-29-2; 8, 116337-30-5; 9, 116337-24-7; 10, 116337-31-6; 10 [8-(de-iodo) derivative], 116337-32-7; 11, 107584-46-3; 11 (methyl ester), 116337-33-8; 12, 116337-41-8; 13, 106174-48-5; 14, 106174-79-2; 15, 107584-44-1; 16, 116337-34-9; 17, 116363-83-9; 17 (methyl ester), 116337-47-4; EC 2.7.7.38, 37278-28-7.

Synthesis, DNA Binding, and Biological Evaluation of Synthetic Precursors and Novel Analogues of Netropsin[1]

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A series of oligopeptides have been synthesized that are structurally related to the natural agent netropsin. The binding constants to double-stranded polynucleotides as well as the cytostatic activity against both murine human tumor cell lines and the in vitro activity against a range of DNA and RNA viruses have been determined for these novel compounds and some of their synthetic precursors. 1-Methyl-5-nitropyrrole-2-carboxylic acid methyl ester (**4**), *N*-[[1-methyl-4-(1-methyl-4-nitropyrrole-2-carboxamido)pyrrol-2-yl]carbonyl]-L-alanine *tert*-butyl ester (**28**), and *N*-[[1-methyl-4-(1-methyl-4-nitropyrrole-2-carboxamido)pyrrol-2-yl]carbonyl]-L-alanyl-L-alanine *tert*-butyl ester (**29**) showed modest inhibitory effect on tumor cell proliferation (CD_{50} = 26–85 μg/mL). Of all the compounds that were evaluated, **28** proved the most potent antiviral agent. It was inhibitory to parainfluenza-3 virus and Coxsackie virus B4 in Vero cells at a concentration of 20 μg/mL.

Netropsin[2] (synonym congocidin) is a highly basic oligopeptide containing two 4-amino-1-methylpyrrole-2-carboxylate residues that belongs to a family of natural substances including distamycin,[3] anthelvencin,[4] kikumycin,[5] amidomycin,[6] and noformycin[7] (Figure 1).

To date, netropsin has attracted considerable attention because of its noteworthy biological activities and DNA binding capacity.[8] Thus netropsin appeared to be primarily active against Gram-positive and Gram-negative bacteria[9,10] and it was also endowed with antiparasitic properties.[11-13] As an antiviral drug, it was reported to inhibit the multiplication of DNA viruses such as vaccinia virus,[14] herpes simplex virus,[15] swine fever virus,[16] and several retroviruses such as Rous Sarcoma virus,[17] murine leukemogenic Rauscher virus,[18] and feline leukemia virus.[19] Netropsin is also of prime interest in molecular biology owing to its strong minor groove nonintercalative binding to double-stranded B-DNA and its $(A{\cdot}T)_4$ sequence selectivity.[20] Through it has not been conclusively proven, it is generally believed that its DNA binding property is responsible for its biological activity.

Netropsin was first isolated from the fermentation medium of various *Streptomyces* species.[11,12,21] Since the report of its definite structure,[22] several total syntheses of the parent compound[24,29] as well as of dimeric derivatives[30-32] and certain analogues[33-46] of netropsin have been reported. These analogues consisted of compounds in which (i) the number of pyrrole units varied from one to three and/or the aminopropionamidine side chain was replaced by various aminoalkylamidine side chain was replaced by various aminoalkylamidine moieties,[33-36] (ii) the mode of pyrrole ring substitution was changed from

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[§] Rega Institute for Medical Research.

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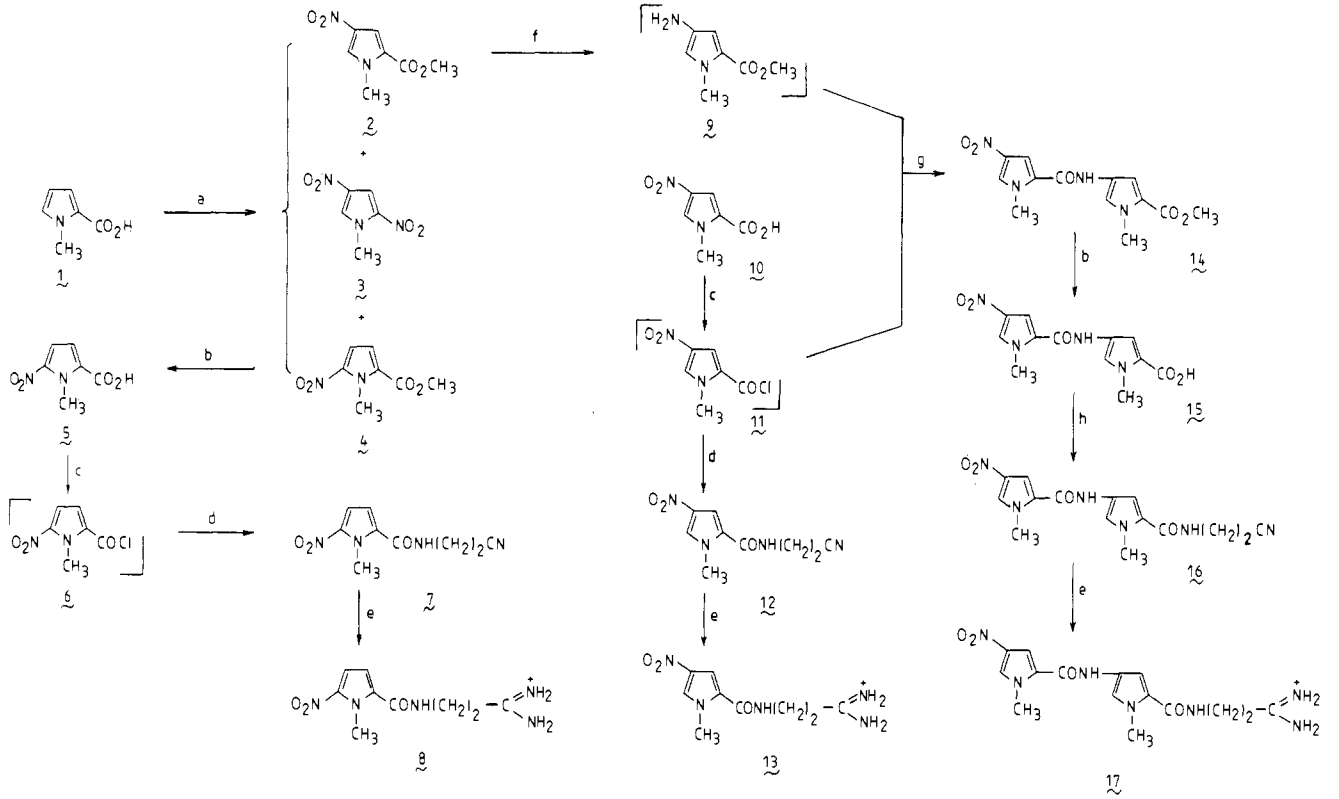
Scheme I[a]



[a] Reactions conditions: (a) HNO_3, Ac_2O, then CH_3OH, H_2SO_4; (b) aqueous NaOH in EtOH, then HCl; (c) $SOCl_2$, THF, Δ; (d) 3-aminopropionitrile, $(C_3H_7)_2N(C_2H_5)$, THF; (e) HCl, EtOH, then dry NH_3, EtOH; (f) H_2; Pd/C in MeOH; (g) $(C_3H_7)_2N(C_2H_5)$ in THF; (h) 3-aminopropionitrile in DMF, then HOBT and DCC or BOP and $N(C_2H_5)_3$.

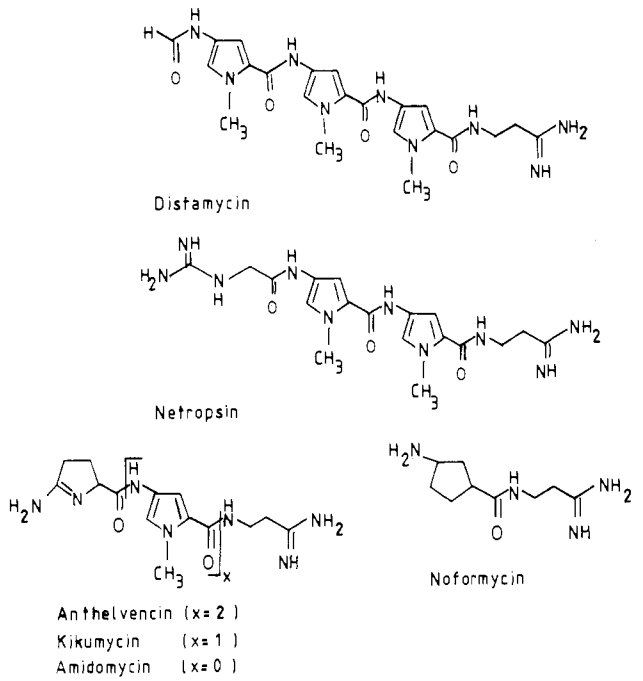


Figure 1.

2,4 to 2,5,[37] (iii) the pyrrole rings were replaced by benzene,[38,39] pyridine,[39] thiophene,[39] triazine arylamidine,[40] and imidazole,[41,42] (iv) the guanidinoacetamido side chain was substituted by an amine[29,43] or a quaternary ammonium

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end group,[44] a formamido,[45] acetamido,[46] benzamido,[46] and dansylglycylamido residues[46] or various alkylating functions.[47] Although these modifications have not been successful in significantly improving the biological activity of the parent compound, it is noteworthy that the replacement of one or more pyrrole rings by imidazole resulted in an alteration of DNA base recognition from A·T to G·C.[41,48-52]

In the present study we report the synthesis of novel analogues of netropsin showing substitution of the aminopropionamidine side chain either by spermidine or by mono-, di-, and tri-L-alanyl residues. The rationale for considering these structural changes results from an attempt to assess the role of the nature and size of the charged end groups on binding to DNA and on biological activity. Thus, basic spermidine is known to interact with phosphate groups of DNA and to bind in its minor groove, sharing some common characteristics with netropsin,[53] whereas alanine residues have been suggested to display preferential interaction with the G·C base pairs according to a proposed code that might control protein–nucleic acid recognition.[54] It was therefore expected that the preference of the parent compound for $(A\cdot T)_4$ base sequences should be altered for analogues bearing L-alanyl residues. We also report biological data on these altered side chain compounds and some of their synthetic precursors and attempts to relate their biological properties to their potential interaction with double-stranded polynucleotides.

Chemistry

The approach employed for the synthesis of the novel netropsin analogues required the preparation of 1-methyl-4-(1-methyl-4-nitropyrrole-2-carboxamido)-pyrrole-2-carboxylic acid (**15**). The preparation of this key intermediate was carried out from the commercial 1-methyl-2-pyrrolecarboxylic acid (**1**) essentially according to the strategy recently reported by Lown et al.,[29] and the main features are outlined in Scheme I. But the anticipated need for a number of derivatives of 1-methylpyrrole in order to evaluate their biological activity prompted us to investigate closely the first step of the synthesis. Thus esterification of the filtrate resulting of the direct precipitation of 1-methyl-4-nitropyrrole-2-carboxylic acid (**10**)[27,29,55-57] (32% yield) from the nitration reaction of **1**[29] allowed us to isolate 1-methyl-2,4-dinitropyrrole (**3**)[55,56] as side product and the two isomeric 4- and 5-nitro methyl ester derivatives **2** and **4**.[55,58] From the latter compounds the key intermediate **15**[29] as well as two monomeric compounds **8**[37] and **13**[59,60] bearing a propionamidine function were synthesized with the use of previously published methods.[29]

Concerning the introduction of the various side chains at carboxyl on **15**, two one-step active ester procedures implementing either 1-hydroxybenzotriazole (HOBT)/carbodiimide[61] or benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP)[62] reagents were applied interchangeably for amide bond formation. On the one hand, condensation of **15** with 3-aminopropionitrile by either procedure afforded **16**[24,29,63] in almost quantitative yield. Pinner reaction[64] of **16** with ethanol in the presence of hydrogen chloride and subsequent ammonolysis gave the already known amidine hydrochloride **17**[24,29,59] (Scheme I). On the other hand, condensation of **15** with N^4,N^8-bis(*tert*-butoxycarbonyl)spermidine[65] using BOP reagent or with L-alanine *tert*-butyl ester and its corresponding di- and tripeptides using the HOBT/[(dimethylamino)propyl]-N'-ethylcarbodiimide (EDC) procedure afforded the desired fully protected derivatives **24** and **28–30**, respectively, in lower but still satisfactory yields. Additionally we also prepared in another way the protected monopyrrole spermidine derivative **18** (Scheme II).

With the aim of introducing the guanidinoacetyl function, the nitro compounds **18**, **23**, and **28–30** were reduced catalytically to give the amines **20**, **25**, and **31–33**, respectively. Owing to their instability, these amines were not purified but allowed to react with guanidinoacetic acid

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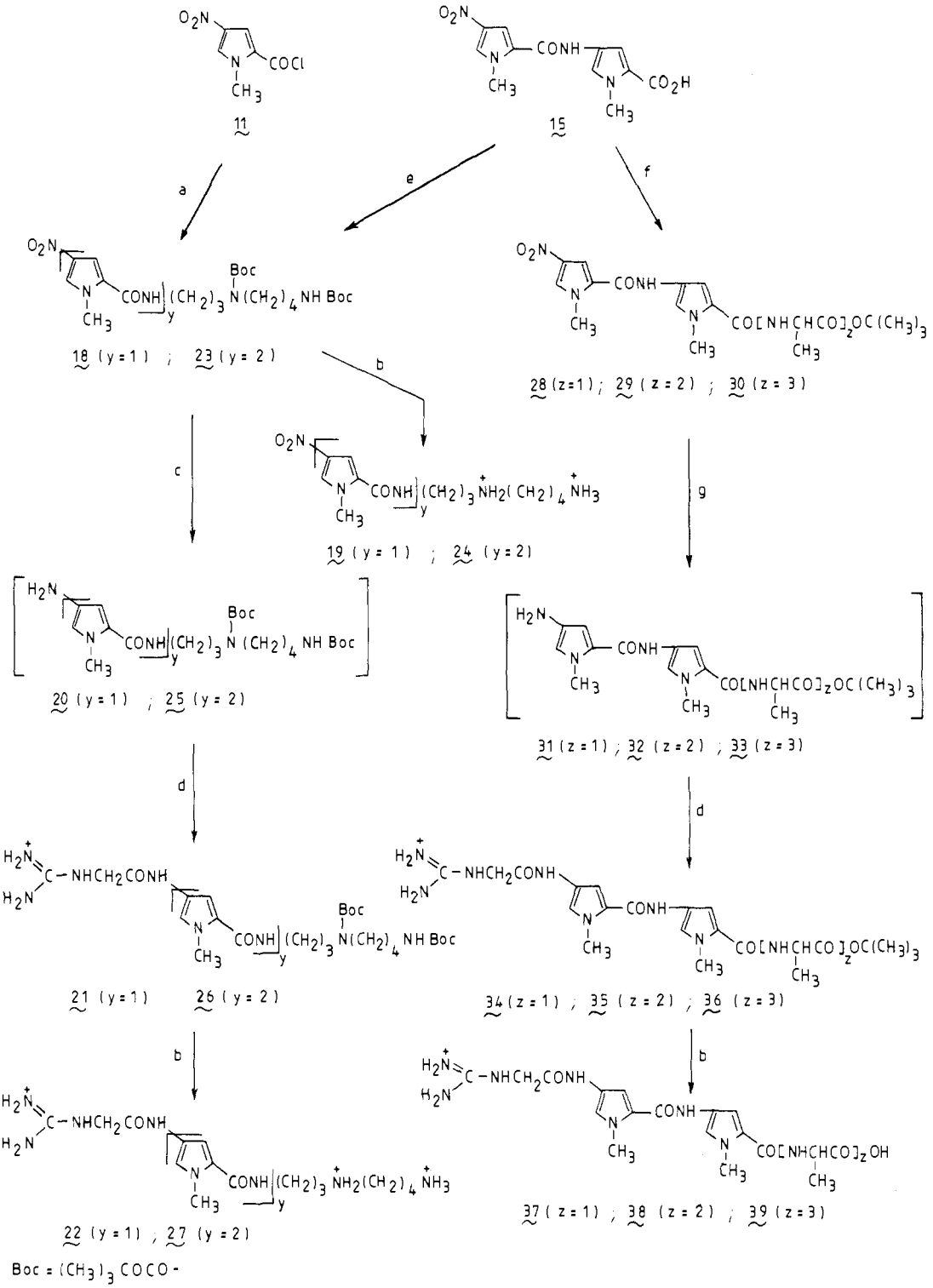
Scheme II[a]



[a] Reactions conditions: (a) $NH_2(CH_2)_3N(BOC)(CH_2)_4NH$ BOC, $(C_3H_7)_2N(C_2H_5)$, THF; (b) CF_3COOH; (c) H_2, Pd/C in MeOH; (d) guanidinoacetic acid hydrochloride, DCC or EDC in DMF; (e) $NH_2(CH_2)_3N(BOC)(CH_2)_4NHBOC$ in DMF, then BOP and $N(C_2H_5)_3$; (f) H-$[NHCH(CH_3)CO]_zOC(CH_3)_3$·HCl, 1,8-bis(dimethylamino)naphthalene, HOBT, EDC in DMF; (g) H_2, Pd/C in MeOH–DMF.

hydrochloride in the presence of a carbodiimide [EDC or dicyclohexylcarbodiimide (DCC)] to afford **21**, **26**, and **34–36**. The low yields observed in these condensations were caused by a known side reaction[66] between the carbodiimide and aminopyrrole moieties as exemplified by the isolation of N^1-[[1-methyl-4-(N',N'-dicyclohexylguanilyl)pyrrol-2-yl]carbonyl]-N^4,N^8-bis(*tert*-butoxycarbonyl)spermidine during the purification of the crude reaction mixture resulting from the coupling of the amine **20** with guanidinoacetic acid in the presence of DCC.

The final step of the synthesis, removal of the *tert*-butoxycarbonyl or *tert*-butyl groups, respectively, from **18**, **21**, **23**, **26**, or **34–36** proceeded satisfactorily on treatment of these compounds with trifluoroacetic acid to afford the target compounds **19**, **22**, **24**, **27**, and **37–39**, respectively.

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Table I. Relevant Data for the Novel Netropsin Analogues

compd	HPLC retention time, min	^{1}H NMR, δ	mass spectrum (FAB > 0)
19	10.0[a]	8.53 (t, 1 H, CONH; J = 5.8 Hz), 8.14 and 7.42 (2 d, 2 × 1 H, H-3 and H-5; J = 1.9 Hz), 8.4–7.5 (br s, 5 H, $^+NH_3$ and $^+NH_2$), 3.91 (s, 3 H, CH_3), 3.3, 2.9, and 2.8 [3 m, 2, 4, and 2 H, 4 N(CH_2)C], 1.8 and 1.6 [2 m, 2 and 4 H, 3 C(CH_2)C]	298 (M + H − 2 CF_3CO_2H)$^+$
22	10.6[a]	10.07 (s, 1 H, NH-Pyr), 8.5, 7.8, and 7.3 [3 br s, 2, 3, and 4 H, $^+NH_2$, $^+NH_3$, and C(NH_2)$^+NH_2$], 8.19 (t, 1 H, CONH, J = 5.5 Hz), 7.59 [t, 1 H, $^+NH_2$(NH_2)CN*H*, J = 5.7 Hz], 7.13 and 6.72 (2 d, 2 × 1 H, H-3 and H-5, J = 1.8 Hz), 3.97 (d, 2 H, NCH_2CO, J = 6.0 Hz), 3.80 (s, 3 H, CH_3), 3.3, 2.9 and 2.8 [3 m, 2, 4, and 2 H, 4 N(CH_2)C], 1.8 and 1.6 [2 m, 2 and 4 H, 3 C(CH_2)C]	367 (M + H − 3 CF_3CO_2H)$^+$
24	15.0[b]	10.23 (s, 1 H, NH-Pyr), 8.20 (t, 1 H, CON*H*CH_2), 8.17, 7.57, 7.20, and 6.91 (4 d, 4 × 1 H, 2 H-3 and 2 H-5, J = 1.6 Hz), 8.5 and 7.8 (2 br s, 2 and 3 H, $^+NH_2$ and $^+NH_3$), 3.95 and 3.83 (2 s, 2 × 3 H, 2 CH_3), 3.3, 2.9, and 2.8 [3 m, 2, 4, and 2 H, 4 N(CH_2)C], 1.8 and 1.6 [2 m, 2 and 4 H, 3 C(CH_2)C]	420 (M + H − 2 CF_3CO_2H)$^+$
27	10.2[b]	10.11 and 9.87 (2 s, 2 × 1 H, 2 NH-Pyr), 8.5, 7.8, and 7.3 [3 br s, 2, 3, and 4 H, $^+NH_2$, $^+NH_3$, and C(NH_2)$^+NH_2$], 8.17 (t, 1 H, CON*H*CH_2, J = 5.7 Hz), 7.61 [t, 1 H, $^+NH_2$(NH_2)CN*H*, J = 6.0 Hz], 7.17, 7.16, 6.93, and 6.90 (4 d, 4 × 1 H, 2 H-3 and 2 H-5, J = 1.7 Hz), 3.99 (d, 2 H, NCH_2CO, J = 6.0 Hz), 3.84 and 3.82 (2 s, 2 × 3 H, 3 CH_3), 3.3, 2.9, and 2.8 [3 m, 2, 4, and 2 H, 4 N(CH_2)C], 1.8 and 1.6 [2 m, 2 and 4 H, 3 C(CH_2)C]	489 (M + H − 3 CF_3CO_2H)$^+$
28	9.6[c]	10.20 (s, 1 H, NH-Pyr), 8.22 [d, 1 H, CON*H*CH(CH_3), J = 7.3 Hz], 8.15, 7.56, 7.21, and 6.97 (4 d, 4 × 1 H, 2 H-3 and 2 H-5 $J \simeq$ 1 Hz), 4.26 [m, 1 H, C*H*(CH_3)], 3.95 and 3.79 (2 s, 2 × 3 H, 2 NCH_3), 1.40 [s, 9 H, C(CH_3)$_3$], 1.33 [d, 3 H, CH(C*H*$_3$), J = 7.4 Hz]	420 (M + H)$^+$
29	8.5[c]	10.20 (s, 1 H, NH-Pyr), 8.15, 7.56, 7.20, and 6.96 (4 d, 4 × 1 H, 2 H-3 and 2 H-5; $J \simeq$ 1 Hz), 8.11 and 7.98 [2 d, 2 × 1 H, 2 CON*H*CH(CH_3), J = 7.0 and 7.7 Hz], 4.41 and 4.11 [2 m, 2 × 1 H, 2 C*H*(CH_3)], 3.95 and 3.79 (2 s, 2 × 3 H, 2 NCH_3), 1.38 [s, 9 H, C(CH_3)$_3$], 1.31 and 1.26 [2 d, 2 × 3 H, 2 CH(C*H*$_3$), J = 7.2 Hz]	491 (M + H)$^+$
30	7.8[c]	10.21 (s, 1 H, NH-Pyr), 8.15, 7.56, 7.20, and 6.97 (4 d, 4 × 1 H, 2 H-3 and 2 H-5; $J \simeq$ 1 Hz), 8.12, 8.05, and 7.84 [3 d, 3 × 1 H, 3 CON*H*CH(CH_3), J = 7.0, 7.5, and 7.7 Hz], 4.37, 4.31, and 4.09 [3 m, 3 × 1 H, 3 C*H*(CH_3)], 3.95 and 3.79 (2 s, 2 × 3 H, 2 NCH_3), 1.37 [s, 9 H, C(CH_3)$_3$], 1.29 and 1.23 [2 d, 3 and 6 H, 3 CH(CH_3), J = 7.2 Hz]	562 (M + H)$^+$
34	23.4[d]	10.3 and 9.9 (2 br s, 2 × 1 H, 2 NH-Pyr), 8.2 [br s, 1 H, CON*H*CH(CH_3)], 8.5–6.5 [br, 5 H, $^+NH_2$(NH_2)CNH], 7.19, 7.18, 6.98, and 6.91 (4 d, 4 × 1 H, 2 H-3 and 2 H-5, $J \simeq$ 1 Hz), 4.27 [m, 1 H, C*H*(CH_3)], 3.9 (br s, 2 H, NCH_2CO), 3.84 and 3.78 (2 s, 2 × 3 H, 2 NCH_3), 1.66 (s, 3 H, CH_3COO^-), 1.41 [s, 9 H, C(CH_3)], 1.33 [d, 3 H, CH(C*H*$_3$), J = 7.2 Hz]	489 (M + H − CH_3CO_2H)$^+$
35	22.6[d]	10.3 and 9.9 (2 br s, 2 × 1 H, 2 NH-Pyr), 8.8–7.2 [br s, 5 H, $^+NH_2$(NH_2)CNH], 8.12 and 7.92 [2 d, 1 H, 2 CON*H*CH(CH_3), J = 7.0 and 7.8 Hz], 7.20, 7.17, 6.96, and 6.91 (4 d, 4 × 1 H, 2 H-3 and 2 H-5; $J \simeq$ 1 Hz), 4.43 and 4.13 [2 m, 2 × 1 H, 2 C*H*(CH_3)], 3.9 (br s, 2 H, NCH_2CO), 3.84 and 3.79 (2 s, 2 × 3 H, 2 NCH_3), 1.71 (s, 3 H, CH_3COO^-), 1.39 [s, 9 H, (CH_3)$_3$], 1.31 and 1.27 [2 d, 2 × 3 H, 2 CH(C*H*$_3$), J = 7.2 and 7.4 Hz]	560 (M + H − CH_3CO_2H)$^+$
36	22.3[d]	10.3 and 9.9 (2 br s, 2 × 1 H, 2 NH-Pyr), 8.8–7.0 [br s, 5 H, NH_2(NH_2)CNH], 8.16, 8.01, and 7.88 [3 d, 3 × 1 H, 3 CON*H*CH(CH_3), J = 6.6, 7.4, and 7.5 Hz], 7.20, 7.18, 6.98, and 6.91 (4 d, 4 × 1 H, 2 H-3 and 2 H-5; $J \simeq$ 1 Hz), 4.3 and 4.10 [2 m, 2 and 1 H, 3 C*H*(CH_3)], 3.9 (br s, 2 H, NCH_2CO), 3.84 and 3.78 (2 s, 2 × 3 H, 2 NCH_3), 1.67 (s, 3 H, CH_3COO^-), 1.38 [s, 9 H, C(CH_3)$_3$], 1.29, 1.25, and 1.24 [3 d, 3 × 3 H, 3 CH(C*H*$_3$), J = 7.1, 7.5, and 7.0 Hz]	631 (M + H − CH_3CO_2H)$^+$
37	13.0[d]	12.4 (br s, 1 H, CO_2H), 10.1 and 9.8 (2 br s, 2 × 1 H, 2 NHPyr), 8.14 [d, 1 H, CON*H*CH(CH_3), J = 7.3 Hz], 7.48 [t, 1 H, $^+NH_2$(NH_2)CN*H*], 7.6–7.0 [br s, 4 H, C(NH_2)$^+NH_2$], 7.17, 7.16, 6.98, and 6.88 (4 d, 4 × 1 H, 2 H-3 and 2 H-5, $J \simeq$ 1 Hz], 4.34 [m, 1 H, C*H*(CH_3)], 3.97 (d, 2 H, NCH_2CO, J = 5.9 Hz), 3.83 and 3.98 (2 s, 2 × 3 H, 2 NCH_3), 1.34 [d, 3 H, CH(C*H*$_3$), J = 7.3 Hz]	433 (M + H − CF_3CO_2H)$^+$
38	13.3[d]	12.5 (br s, 1 H, CO_2H), 10.1 and 9.8 (2 br s, 2 × 1 H, 2 NH-Pyr), 8.06 and 7.91 [2 d, 2 × 1 H, 2 CON*H*CH(CH_3), J = 7.0 and 7.7 Hz], 7.5 [br s, 1 H, $^+NH_2$(NH_2)CN*H*], 7.7–6.9 [br s, 4 H, C(NH_2)$^+NH_2$], 7.19, 7.17, 6.94, and 6.88 (4 d, 4 × 1 H, 2 H-3 and 2 H-5, $J \simeq$ 1 Hz), 4.43 and 4.20 [2 m, 2 × 1 H, 2 C*H*(CH_3)], 3.98 (d, 2 H, NCH_2CO, J = 5.7 Hz), 3.84 and 3.78 (2 s, 2 × 3 H, 2 NCH_3), 1.30 and 1.28 [2 d, 2 × 3 H, 2 CH(C*H*$_3$), J = 6.3 Hz]	504 (M + H − CF_3CO_2H)$^+$
39	13.4[d]	12.5 (br s, 1 H, COOH), 10.1 and 9.9 (2 br s, 2 × 1 H, 2 NH-Pyr), 8.10, 8.00, and 7.87 [3 d, 3 × 1 H, 3 CON*H*CH(CH_3), J = 7.3, 7.4, and 7.6 Hz], 7.51 [t, 1 H, $^+NH_2$(NH_2)CN*H*, J = 5.9 Hz], 7.6–7.0 [br s, 4 H, C(NH_2)$^+NH_2$], 7.20, 7.18, 6.97, and 6.90 (4 d, 4 × 1 H, 2 H-3 and 2 H-5; $J \simeq$ 1 Hz), 4.39, 4.31, and 4.19 [3 m, 3 ×1 H, 3 C*H*(CH_3)], 3.98 (d, 2 H, NCH_2CO, J = 6.0 Hz), 3.85 and 3.79 (2 s, 2 × 3 H, 2 NCH_3), 1.30, 1.27 and 1.23 [3 d, 3 × 3 H, 3 CH(C*H*$_3$), J = 7.2, 7.4, and 7.0 Hz]	575 (M + H − CF_3CO_2H)$^+$

[a] Apparatus A; column μBondapak C_{18} Waters, 150 × 19 mm i.d., 10 μm particle size, protected by a precolumn C_{18} "Guard Pak"; solvent 50% of acetonitrile in 0.1 M ammonium acetate buffer, pH 5.7; isocratic conditions, flow rate 6.75 mL/min. [b] Apparatus B; column Ultrasphere XLODS cartridge Beckman, 70 × 4.6 mm i.d., 3 μm particle size, protected by prefilters and a XLODS precolumn (5 × 4.6 mm i.d., 3 μm); solvent A, 100% 0.1 M ammonium acetate buffer, pH 5.9; solvent B, 100% acetonitrile; linear gradient 0–50% B in 20 min, flow rate 1 mL/min. [c] Apparatus A; column C_{18} "Radial Pak" (100 × 8 mm i.d., 10 μm particle size) in a Waters Associates Radial compression module RCM 100, protected by a precolumn C_{18} "Guard Pak"; solvent A, 25% acetonitrile in 0.1 M ammonium acetate buffer, pH 5.9; solvent B, 100% acetonitrile; linear gradient 0–100% B in 15 min; flow rate 3.0 mL/min. [d] Same apparatus and column as in the above C; solvent A, 0.1 M ammonium acetate buffer, pH 5.9; solvent B, 25% acetonitrile in the same buffer; solvent C, 50% acetonitrile in the same buffer; linear gradient 0–100% B in 15 min, then 100% B for 1 min, then linear gradient 0–100% C in 5 min; flow rate 3.0 mL/min.

Structural assignment for the reported novel compounds are based on their physical properties (Table I). Unless otherwise noted, our data were in accord with literature values for previously described compounds.

Binding of Compounds to Polynucleotides

The binding constants of novel oligopeptides and of some of their synthetic precursors to poly(dA-dT)·poly(dA-dT), and poly(dG-dC)·poly(dG-dC) are presented in Table II. All the spermidine derivatives **19**, **22**, **24**, and **27** keep the ability of the parent compound, netropsin, to bind poly(dA-dT)·poly(dA-dT) although with a reduced affinity concerning the former compounds. The association constant values were relatively higher for **22** and **27**, which possess a cationic guanidinoacetamido function. In addition, these derivatives are also able to better bind poly(dG-dC)·poly(dG-dC) as compared to netropsin (increase of K_{app}: about 2 orders of magnitude). The loss of

Table II. Association Constants (K_{app}) of Compounds with Polynucleotides

compound	K_{app}, M^{-1} poly(dA-dT)	K_{app}, M^{-1} poly(dG-dC)	ratio K_{app}: poly(dA-dT)/poly(dG-dC)
7	$<10^2$	$<10^2$	
8	$<10^2$	$<10^2$	
12	$<10^2$	$<10^2$	
13	$<10^2$	$<10^2$	
16	$<10^2$	$<10^2$	
17	1.2×10^4	$<10^2$	>120
19	1.0×10^6	4.2×10^5	2.38
22	1.0×10^7	1.9×10^6	5.26
24	4.4×10^5	8.2×10^4	5.37
27	2.0×10^7	7.6×10^6	2.63
28	2.0×10^2	4.0×10^2	0.50
29	6.5×10^2	2.0×10^4	0.033
30	$<10^2$	7.5×10^2	<0.13
34	8.7×10^3	1.0×10^4	0.87
35	4.4×10^3	7.0×10^2	6.29
36	2.0×10^4	3.3×10^3	6.06
37	<100	<100	
38	1.2×10^3	1.2×10^3	1.00
39	$<10^2$	5.0×10^3	<0.02
netropsin	7.3×10^8	3.1×10^4	23548
distamycin	8.5×10^7	2.5×10^4	3400

sequence selectivity recognition for **27** and **22** and the disappearance of significant binding capacity for **34–39**, all these compounds differing from netropsin only by a change of the original propionamido right function, emphasize the importance of the integrity of netropsin structure for its A·T specificity of binding. It can be also noted that compound **17**, which only differs from netropsin by absence of the guanidinoacetamido function, retains preference for A·T sites although it displays a lessened polynucleotide binding capacity. The di- and trialanyl derivatives **29**, **30**, and **39** display a low affinity constant for double-stranded polynucleotides. However, it is interesting to note that, as expected from the rationale that directed the synthesis of these compounds (see introduction), their affinity for poly(dG-dC)·poly(dG-dC) is higher than that for poly(dA-dT)·poly(dA-dT) by at least 1 order of magnitude. These findings provide relevant information in the design of future sequence specific agents structurally related to netropsin and retaining some of its DNA-recognition characteristics.

Biological Evaluation

Cytostatic Activity. Among the compounds tested, **4**, **28**, and **29** showed inhibitory effects on the proliferation of murine (L1210 and FM3A) and human (Raji and Molt/4F) cells in culture at substantial lower concentrations than the parent compound netropsin (Table III). Their CD_{50} (50% inhibitory dose) values for the different tumor cell lines varied from 26 to 66 μg/mL. In contrast with netropsin, **28** and **29** consist of a *N*-[1-methyl-4-(1-methyl-4-nitropyrrole-2-carboxamido)pyrrole] moiety instead of a *N*-[1-methyl-4-(1-methyl-4-(guanidinoacetamido)pyrrole-2-carboxamido)pyrrole] moiety. However, replacement of the guanidinylacetamido part by a nitro function does not necessarily impart cytostatic activity, because compounds **15**, **19**, and **24** are devoid of any antiproliferative effect and compound **30**, which contains one additional L-alanyl moiety compared to **29**, is also without any cytostatic effect at 200 μg/mL. Compound **35** is only modestly inhibitory to human Raji cell proliferation (CD_{50} = 55 μg/mL). None of the other compounds showed any cytostatic effect at the highest concentration tested (100 or 200 μg/mL).

Antiviral Activity. The compounds listed in Table IV were evaluated for their inhibitory effects on the replication of a number of DNA viruses [herpes simplex virus type 1 (HSV-1), HSV-2, and vaccinia virus (VV)] and RNA viruses [vesicular stomatitis virus (VSV), Coxsackie virus B4, polio virus-1, parainfluenza-3 virus, reovirus-1, Sindbis virus, and Semliki forest virus] in three cell systems (primary rabbit kidney, Hela, and Vero B cells) [Table IV; for comparative purposes, the antiviral data of the well-known antivirals tubercidin, (*S*)-DHPA, ribavirin, and carbocyclic 3-deazaadenosine are included]. With a few exceptions, none of the synthesized compounds proved effective against the replication of any of the DNA and RNA viruses when evaluated at nontoxic concentrations. However, **5** was active at 70 μg/mL against Coxsackie virus B4 in Hela cells; **12** was effective at 150 μg/mL against Coxsackie virus B4 in vero cells; **17** was active at 300 μg/mL against Sindbis virus and Semliki forest virus in Vero cells, and **34** inhibited VV replication at 150 μg/mL. Furthermore compound **28** showed a minimum inhibitory concentration (MIC) of 20 μg/mL against parainfluenza-3 virus and Coxsackie virus B4 in Vero cells; this concentration was 5-fold below the toxicity threshold of the compound for Vero cells, and at least 20-fold lower than the MIC obtained for the parent compound (netropsin) against these viruses. In fact in our studies netropsin itself showed only marginal activity, mainly against VV (MIC = 70 μg/mL). Finally, in contrast to previously published results,[37] compound **8** showed no antiviral effect at the highest concentration tested.

Table III. Inhibitory Effects of Compounds on the Proliferation of Murine Leukemia (L1210), Murine Mammary Carcinoma (FM3A), Human B-Lymphoblast (Raji), and Human T-Lymphoblast (Molt/4F) Cells

compd	CD_{50},[a] (μg/mL) L1210	FM3A	Raji	Molt/4F
2	>100	>100	>100	>100
4	28.0 ± 3.1	84.7 ± 10.1	62.4 ± 34.9	66.0 ± 33.1
5	>50	ND[b]	>50	>50
7	>50	ND	>50	>50
8	>50	ND	>50	>50
10	>100	>100	>100	>100
12	>50	ND	>50	>50
13	>50	ND	>50	>50
14	>100	>100	>100	>100
15	>100	>100	>100	>100
16	>100	ND	>100	>100
17	>100	ND	>100	>100
19	>200	>200	>200	>200
22	>200	>200	>200	>200
24	>100	ND	>100	>100
27	>200	>200	>200	>200
28	37.8 ± 8.6	27.7 ± 4.6	33.8 ± 2.5	26.6 ± 7.0
29	28.8 ± 5.8	27.2 ± 6.5	33.0 ± 1.4	29.0 ± 8.3
30	>100	>100	>100	>100
34	>100	>100	>100	>100
35	>100	>100	54.7 ± 10.4	>100
36	>100	>100	>100	>100
37	>100	>100	>100	>100
38	>100	>100	>100	>100
39	>100	>100	>100	>100
netropsin[c]	295 ± 92	321 ± 18	139 ± 63	>100

[a] 50% cytotoxic dose or dose required to inhibit cell proliferation by 50%. [b] Not determined. [c] Data taken from ref 47.

Experimental Section

Chemical Synthesis. General Procedures. Evaporation of solvents was done with a rotary evaporator under reduced pressure. Melting points were determined on a Büchi 510 apparatus and are uncorrected. Proton nuclear magnetic resonances were determined in DMSO-d_6 at ambient temperature on a Bruker WM 360 WB spectrometer. Chemical shifts are expressed in parts per million in DMSO set at 2.49 ppm as reference. Fast-atom-bombardment mass spectra (FAB-MS) were recorded in the

Table IV. Antiviral Activity of Compounds against Different Viruses in Different Cell Systems

	minimum cytotoxic concn,[b] μg/mL			minimum inhibitory concentration,[a] μg/mL										
				primary rabbit kidney (PRK) cells			HeLa cells			African green monkey kidney (Vero B) cells				
compd	PRK cells	HeLa cells	Vero B cells	herpes simplex virus-1 (KOS)	herpes simplex virus-2 (G)	vaccinia virus	vesicular stomatitis virus	polio virus-1	Coxsackie virus B4	Sindbis virus	parainfluenza virus-3	reovirus-1	Semliki forest virus
2	≥400	>400	≥400	>400	>200	>200	>400	>400	>400	>400	200	>400	>200	>400	>400
4	≥400	400	400	>400	>400	>400	>400	>400	>400	>400	>200	>400	>100	>200	>400
5	>400	>400	≥400	>400	>400	>200	>400	>200	>400	70	>100	>100	>200	>100	>200
7	400	400	≥400	>200	>200	>200	300	>200	>200	>200	>200	>100	>100	>100	>100
8	≥400	≥400	≥400	>200	>100	>100	>400	>400	>400	>400	>100	>200	100	>100	>200
10	>400	>400	≥400	>400	>400	>400	>400	>400	>400	>400	>200	>400	>400	>400	>400
12	>400	≥400	>400	>400	>400	>200	>200	>400	>400	>400	150	>100	>100	>200	>100
13	≥400	≥400	≥400	>200	>200	>200	>400	>400	>400	>400	>200	>200	>200	>200	>200
14	≥40	>40	>40	>10	>10	>10	>40	>40	>40	>40	>10	>10	>10	>10	>10
15	>400	>400	≥400	>400	>400	>400	>400	>400	>400	>400	150	>400	>200	>400	>400
16	≥40	≥40	≥40	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10	>10
17	400	≥400	400	>200	>200	>100	>100	>400	>400	>400	>200	300	>200	>100	300
19	>400	>400	≥400	>200	>200	>200	>400	>400	>400	>400	>200	>400	>200	>100	>400
22	>400	≥400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>200	>200	>400
24	≥200	>400	≥400	>200	>200	>200	>100	>400	>400	>400	>200	>400	>200	>200	>400
27	≥400	>200	≥200	>200	>200	>200	>200	>200	>200	>200	>100	>200	>100	>100	>200
28	≥100	≥100	≥100	>40	>40	>40	>40	>100	>40	>100	20	>40	20	>40	>40
29	≥400	≥400	≥200	>100	>200	>100	>100	>200	>200	>200	>200	>100	>200	>200	>200
30	≥400	≥400	≥400	>100	>200	>100	>100	>200	>400	>200	>200	>200	>400	>200	>200
34	>400	>400	>400	>400	>400	150	>400	>400	>400	>400	>400	>400	>400	>400	>400
35	≥200	>400	>400	>200	>200	>200	>200	>400	>400	>400	>400	>400	>400	>200	>400
36	400	>400	>400	>100	>100	>100	>200	>400	>400	>400	>200	>400	>400	>200	>400
37	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200	>200
38	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>200	>400
39	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>400	>200	>400
netropsin	>400	>100	>400	>400	>400	70	>400	>100	>100	>100	>400	>400	>400	>400	>400
tubercidin	≥0.4	≥0.4	≥0.4	>0.1	>0.1	0.07	0.2	0.07	0.07	0.07	0.07	>0.1	0.07	0.07	>0.4
(*S*)-DHPA	>400	>400	>400	>400	>400	70	70	20	>400	>400	200	>400	7	20	>400
ribavirin	>400	≥200	≥400	>400	>400	2	>400	20	70	70	70	70	20	20	150
carbocyclic 3-deazaadenosine	≥400	>400	>400	>400	>400	2	2	2	>400	>400	70	>400	0.4	0.2	>400

[a] Required to reduce virus-induced cytopathogenicity by 50%. Viral cytopathogenicity was recorded as soon as it reached completion in the control virus-infected cell cultures. The multiplicity of infection (MOI) was invariably 100 × $CCID_{50}$, that is 100 times the virus dose needed to infect 50% of the cell. [b] Required to cause a microscopically detectable alteration of normal cell morphology, when incubated with cells for the same duration as required to measure antivirial activity.

positive-ion mode on a JEOL DX 300 mass spectrometer, with a JMA-DA 5000 mass data system; xenon was used for the atom gun at 3 KeV with a total discharge current of 20 μA and the matrix was glycerol. Elemental analyses were determined by the Service de Microanalyse du CNRS, Division de Vernaison. Thin-layer chromatography (TLC) was performed on precoated aluminum sheets of silica gel 60 F_{254} (Merck, No. 5554), visualization of products being accomplished by UV absorbance; amino acid containing and amine-containing compounds were detected by spraying with ethanolic ninhydrin solution,[67] aromatic primary amines were visualized with Bratton-Marshall reagent,[68] and guanidine-containing derivatives were detected with Sakaguchi reagent.[69] Short-column chromatography was performed with silica gel 60 H (Merck No. 7736) under weak nitrogen pressure (≃4 psi). High-pressure liquid chromatographic (HPLC) studies were carried out on two Waters Associate Units: one (apparatus A) equipped with two Model 510 EF solvent delivery systems, a Model 720 solvent programmer, a Model U6K sample injector, a Model 481 UV-absorbance detector operating at 254 nm, a Model R 401 differential refractometer, and a M-730 microprocessor-controlled data system; the other (apparatus B) equipped with two Model 6000 A solvent delivery systems, a Model 680 solvent programmer, a Model U6K sample injector and (i) a Model 440 UV-absorbance detector operating at 254 nm, a Model R401 differential refractometer, a two-way Omniscribe recorder; (ii) a Philips instrument composed of a PU 4021 multichannel detector, a PU 4850 videochromatography center, and a PU 4900/20 printer/plotter.

L-Alanine *tert*-butyl ester hydrochloride and its corresponding di- and tripeptides were purchased from Novabiochem (Switzerland).

1-Methyl-4-nitropyrrole-2-carboxylic Acid (10) and Its Methyl Ester (2), 1-Methyl-2,4-dinitropyrrole (3), and 1-Methyl-5-nitropyrrole-2-carboxylic Acid Methyl Ester (4). 1-Methyl-2-pyrrolecarboxylic acid (1) (20.0 g, 0.16 mol) was nitrated with nitric acid–acetic anhydride mixture as previously described by Lown.[29] 1-Methyl-4-nitropyrrole-2-carboxylic acid (10) precipitated from the reaction mixture at low temperature as a pure isomer in a yield of 32% (8.7 g). The filtrate was extracted with chloroform, dried over sodium sulfate, filtered, and evaporated to dryness. A cold solution of sulfuric acid (23 mL) in methanol (230 mL) was added and the mixture was boiled under reflux for 12 h. Water was added after cooling and the mixture was extracted with chloroform. The organic layer was dried over sodium sulfate, filtered, and evaporated to dryness. Chromatography of the residue on a silica gel column using as eluent a stepwise gradient of chloroform (0–15%) in toluene afforded successively pure 4 (2.95 g, 10%, crystallized from methanol–petroleum ether), 3 (0.27 g, 1%, crystallized from methylene chloride–cyclohexane) and 2 (4.13 g, 14%, crystallized from methanol). The physical data of 10,[27,29,55-57] 2,[55,58] 3,[55,56] and 4[55,58] were in accordance with those of previously described compounds and elemental analyses agreed with calculated values within ±0.4%. Alternatively 10 was obtained in 95% yield by treating 2 with an aqueous alkaline solution.

1-Methyl-5-nitropyrrole-2-carboxylic Acid (5), 3-(1-Methyl-5-nitropyrrole-2-carboxamido)propionitrile (7), 3-(1-Methyl-5-nitropyrrole-2-carboxamido)propionamidine Hydrochloride (8), the Corresponding 4-Nitro Isomers 12 and 13, and the 4-Nitro Dimeric Derivatives 14, 15, and 17. These compounds were synthesized in good yields following similar reaction conditions (Scheme I) as those used by Lown during his synthesis of netropsin.[29] Their analyses agreed with calculated values and their physical properties were in accord with literature data: 5,[37] 7,[37] 8,[37] 12,[24,29,63] 13,[2,60] 14,[29,58] 15,[29] and 17.[24,29,59]

3-[1-Methyl-4-(1-methyl-4-nitropyrrole-2-carboxamido)-pyrrole-2-carboxamido]propionitrile (16). Method A. To a solution of 1-methyl-4-(1-methyl-4-nitropyrrole-2-carboxamido)pyrrole-2-carboxylic acid (15) (50 mg, 0.17 mmol) in anhydrous dimethylformamide (DMF; 1.3 mL) were added consecutively 3-aminopropionitrile (0.025 mL, 0.34 mmol), 1-hydroxybenzotriazole (HOBT; 46.21 mg, 0.34 mmol), and dicyclohexylcarbodiimide (DCC; 35.28 mg, 0.17 mmol). The reaction mixture was stirred for 1 h at 0 °C. After an additional 12 h of stirring at room temperature, the formed precipitate of dicyclohexylurea was removed by filtration and water was slowly added. The resulting yellow precipitate was filtered off, washed with cold water, and dried to give pure 16 (55.4 mg, 94% yield).

Method B. To a solution of the acid 15 (550 mg, 1.88 mmol), 3-aminopropionitrile (0.138 mL, 1.88 mmol), and benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate[62] (BOP; 831 mg, 1.88 mmol) in anhydrous DMF (29 mL) was added triethylamine (0.52 mL, 3.76 mmol). The mixture was stirred at room temperature for 1.5 h and then saturated sodium chloride solution (100 mL) was added. Compound 16 was extracted with ethyl acetate (3 × 100 mL). The combined organic layers were washed successively with 2 N hydrochloric acid, water, 5% sodium hydrogen carbonate, and water and then dried over sodium sulfate and filtered. The filtrate was evaporated to dryness to afford pure crystalline 16 (629 mg, 97% yield), which was recrystallized from cold methanol: 249–250 °C (lit. mp 248 °C,[24] mp 254–255 °C,[63] mp 230–232 °C[29]). This compound was identical in all respects with that obtained by using method A, and its physical properties (^{1}H NMR, IR) were in close agreement with literature data.[29] Anal. ($C_{15}H_{16}N_6O_4$) C, H, N.

N^1-[(1-Methyl-4-nitropyrrol-2-yl)carbonyl]-N^4,N^8-bis-(*tert*-butoxycarbonyl)spermidine (18). Acyl chloride 11[29] (565.7 mg, 3.0 mmol) was dissolved in anhydrous tetrahydrofuran (THF; 1.7 mL) and cooled to −25 °C. A solution of N^4,N^8-bis-(*tert*-butoxycarbonyl)spermidine[65] (1.04 g, 3.0 mmol) and *N*-ethyldiisopropylamine (0.51 mL, 3.0 mmol) in THF (2.3 mL) was added, and the mixture was stirred at ambient temperature for 19 h. After dilution with methylene chloride, the mixture was poured into saturated sodium hydrogen carbonate. The organic phase was separated, twice washed with water, dried over sodium sulfate, and evaporated. Chromatography of the residue on a silica gel column using diethyl ether as eluent led, after evaporation of the appropriate fractions, to the isolation of 18 (612 mg, 41% yield). This compound was sufficiently pure (TLC and ^{1}H NMR analyses) to be used without further purification for the preparation of 19 and 21.

N^1-[(1-Methyl-4-nitropyrrol-2-yl)carbonyl]spermidine Bis(trifluoroacetate) (19). Compound 18 (100 mg, 0.20 mmol) was dissolved in trifluoroacetic acid (TFA; 1.5 mL). After 20 min, addition of diethyl ether (150 mL) led to the precipitation of crude compound 19. The solid was washed with ether (4 × 50 mL) and dried in vacuo to give pure 19 (95 mg, 90% yield). The properties of the hygroscopic trifluoroacetate salt 19 are presented in Table I.

N^1-[[1-Methyl-4-(guanidinoacetamido)pyrrol-2-yl]-carbonyl]-N^4,N^8-bis(*tert*-butoxycarbonyl)spermidine Hydrochloride (21). Methanol (8 mL) cooled to −20 °C was added to 10% palladium on charcoal (127 mg) and 18 (380 mg, 0.76 mmol) was added. The solution was hydrogenated at atmospheric pressure and room temperature. After the calculated amount of hydrogen was taken up (≃12 h), the catalyst was removed by filtration, the methanol was removed in vacuo, and the evaporation was repeated with some DMF. The presumed intermediate amine 20 was not further purified due to instability. DMF (13 mL) and guanidinoacetic acid hydrochloride[2] (116.7 mg, 0.76 mmol) were added. The mixture was cooled to 0 °C and treated with a solution of DCC (173.3 mg, 0.84 mmol) in DMF (1.3 mL) in portions during 2 h under argon. After the mixture was stirred for 24 h at room temperature, the formed precipitate of dicyclohexylurea was removed by filtration, and the filtrate was evaporated. Chromatography of the residue on a silica gel column using as eluent a stepwise gradient of methanol (0–10%) in methylene chloride led to the isolation of pure N^1-[[1-methyl-4-(N',N'-dicyclohexylguanilyl)pyrrol-2-yl]carbonyl]-N^4,N^8-bis(*tert*-butoxycarbonyl)spermidine (160 mg, 31% yield) and 21 (65 mg, 14% yield). Compound 21 was sufficiently pure (TLC, ^{1}H NMR, and HPLC analyses) to be used without further purification for the preparation of 22.

N^1-[[1-Methyl-4-(guanidinoacetamido)pyrrol-2-yl]-carbonyl]spermidine Tris(trifluoroacetate) (22). Compound 21 (60 mg, 0.1 mmol) was deprotected with TFA essentially as described for the preparation of 19 to afford pure 22 (63 mg, 89%

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yield). The properties of the hygroscopic tris(trifluoroacetate) salt **22** are presented in Table I.

N^1-[[1-Methyl-4-(1-methyl-4-nitropyrrole-2-carboxamido)pyrrol-2-yl]carbonyl]-N^4,N^8-bis(*tert*-butoxycarbonyl)spermidine (23). This compound was synthesized (following a similar procedure as described in method B for the preparation of **16**) from the acid **15**[29] (672 mg, 2.3 mmol) in DMF (35 mL) and N^4,N^8-bis(*tert*-butoxycarbonyl)spermidine (795 mg, 2.3 mmol), BOP (1.02 g, 2.3 mmol), and triethylamine (0.64 mL, 4.6 mmol). After a similar workup, the residue obtained by evaporation of the ethyl acetate layer was chromatographed on a silica gel column using as eluent a stepwise gradient of acetone (0–12%) in methylene chloride to afford **23** (741 mg, 52% yield). This compound was sufficiently pure (TLC, ^{1}H NMR, and mass spectral analyses) to be used without further purification for the preparation of **24** and **26**.

N^1-[[1-Methyl-4-(1-methyl-4-nitropyrrole-2-carboxamido)pyrrol-2-yl]carbonyl]spermidine Bis(trifluoroacetate) (24). Compound **23** (62 mg, 0.1 mmol) was deprotected with TFA essentially as described for the preparation of **19** to afford **24** (57 mg, 88% yield). Additional purification was obtained by dissolving the salt in a mixture of water and dioxane, followed by lyophilization. The properties of the bis(trifluoroacetate) salt **24** are presented in Table I.

N^1-[[1-Methyl-4-[1-methyl-4-(guanidinoacetamido)pyrrole-2-carboxamido]pyrrol-2-yl]carbonyl]-N^4,N^8-bis(*tert*-butoxycarbonyl)spermidine Acetate (26). This compound was prepared in a manner analogous to that for **21**. Thus, **23** (372 mg, 0.6 mmol) was hydrogenated over 10% palladium on charcoal (100 mg) in methanol (6 mL). Due to its instability, amine **25** was used directly in the following step. DMF (10 mL) and guanidinoacetic acid hydrochloride (92.2 mg, 0.6 mmol) were added, and the mixture was treated at 0 °C with a solution of DCC (136.2 mg, 0.66 mmol) in DMF (1 mL) in portions during 1.5 h under argon. After usual workup, purification of **26** was accomplished by silanized silica gel column chromatography [RP-2, Merck No. 7719, eluent: linear gradient of methanol (0–100%) in water]. Further purification of **26** was accomplished by HPLC (apparatus B; column μBondapak C_{18} Waters, 150 × 19 mm i.d., 10 μm particle size, protected by a precolumn C_{18} "Guard Pak"; solvent 50% of acetonitrile in 0.1 M ammonium acetate buffer, pH 5.9; isocratic conditions, flow rate 6.75 mL/min). After evaporation of the appropriate fractions, methylene chloride was added and ammonium acetate was removed by repetitive filtration on HV-4 Millipore filters. Evaporation of methylene chloride and lyophilization from dioxane gave the acetate salt **26** (50 mg, 11% yield). This compound was sufficiently pure (TLC, ^{1}H NMR, and mass spectra analyses) to be used without further purification for the preparation of **27**.

N^1-[[1-Methyl-4-[1-methyl-4-(guanidinoacetamido)pyrrole-2-carboxamido]pyrrol-2-yl]carbonyl]spermidine Tris(trifluoroacetate) (27). Compound **26** (37.4 mg, 0.05 mmol) was deprotected with TFA essentially as described for the preparation of **19** to afford pure **27** (36.1 mg, 87% yield). The properties of the tris(trifluoroacetate) salt **27** are presented in Table I.

General Method for the Synthesis of *N*-[[1-Methyl-4-(1-methyl-4-nitropyrrole-2-carboxamido)pyrrol-2-yl]carbonyl]-L-alanine *tert*-Butyl Ester (28), -L-alanyl-L-alanine *tert*-Butyl Ester (29), and -L-alanyl-L-alanyl-L-alanine *tert*-Butyl Ester (30). To a solution of the acid **15** (410 mg, 1.4 mmol) in DMF (4.2 mL) were added successively L-alanine *tert*-butyl ester hydrochloride (254.3 mg, 1.4 mmol) [or the corresponding dipeptide (353.6 mg, 1.4 mmol) and tripeptide (453.3 mg, 1.4 mmol)], HOBT (189.2 mg, 1.4 mmol), and 1,8-bis(dimethylamino)naphthalene (330 mg, 1.54 mmol). The mixtures were stirred at room temperature under argon while solid *N*-[(dimethylamino)propyl]-*N'*-ethylcarbodiimide hydrochloride (EDC) was added in small portions. The progress of the reactions was monitored by means of TLC and HPLC and was thereby judged to be complete after between 2 and 4 h, when the added quantity of EDC reached between 2 and 4 equiv. Water was added, and the precipitates formed were filtered off to give crude **28–30**. Chromatography on a silica gel column using as eluent a stepwise gradient of methanol (0–3%) in methylene chloride led to the isolation of pure **28** (440 mg, 75% yield), **29** (522 mg, 76% yield), and **30** (598 mg, 76% yield) as powders after evaporation of the appropriate fractions. The properties of **28–30** are presented in Table I.

General Method for the Synthesis of *N*-[[1-Methyl-4-[1-methyl-4-(guanidinoacetamido)pyrrole-2-carboxamido]-pyrrol-2-yl]carbonyl]-L-alanine *tert*-Butyl Ester Acetate (34), -L-alanyl-L-alanine *tert*-Butyl Ester Acetate (35), and -L-alanyl-L-alanyl-L-alanine *tert*-Butyl Ester Acetate (36). The above-described derivatives **28** (335 mg, 0.8 mmol), **29** (390 mg, 0.8 mmol), or **30** (450 mg, 0.8 mmol) were dissolved at room temperature in a mixture of methanol–DMF (4 mL, 3:1, v/v) and were hydrogenated at atmospheric pressure with 10% palladium on charcoal (56 mg). The progress of the reactions was monitored by means of HPLC and was thereby judged to be complete after between 20 and 22 h. The catalyst was removed by filtration, the solvent was evaporated under reduced pressure, and the evaporation was repeated with some DMF. The presumed amines **31–33** were not further purified due to instability. DMF (3.2 mL) and guanidinoacetic acid hydrochloride (123 mg, 0.8 mmol) were added. The mixtures were stirred at room temperature under argon while solid EDC was added in small portions. The progress of the reactions was monitored by means of HPLC, and disappearance of the amines **31–33** was judged to be complete after between 1 and 2 h, when the added quantity of EDC reached between 1.5 and 4 equiv. Water was added, the solutions were washed with ethyl acetate, and the aqueous phases were evaporated under reduced pressure. Purification of **34–36** was accomplished by HPLC (apparatus A; column μBondapak C_{18} Waters, 150 × 19 mm i.d., 10 μm particle size, protected by a precolumn C_{18} "Guard Pak"; solvent A, 25% acetonitrile in 0.1 M ammonium acetate buffer, pH 5.9; solvent B, 100% acetonitrile; linear gradient 0–30% B in 30 min, flow rate 6.75 mL/min). After evaporation of the appropriate fractions, ammonium acetate was removed by silanized silica gel column chromatography [RP-2, Merck No. 7719; eluent, linear gradient of methanol (0–100%) in water]. Additional purification by lyophilization from water–dioxane mixtures afforded the pure acetate salt **34** (154 mg, 35% yield), **35** (153 mg, 31% yield), and **36** (205 mg, 37% yield). The properties of **34–36** are presented in Table I.

***N*-[[1-Methyl-4-[1-methyl-4-(guanidinoacetamido)pyrrole-2-carboxamido]pyrrol-2-yl]carbonyl]-L-alanine Trifluoroacetate (37), -L-alanyl-L-alanine Trifluoroacetate (38), and -L-alanyl-L-alanyl-L-alanine Trifluoroacetate (39).** Compounds **34** (100 mg, 0.18 mmol), **35** (100 mg, 0.16 mmol), and **36** (140 mg, 0.20 mmol) were deprotected with TFA essentially as described for the preparation of **19** to afford **37** (86 mg, 89% yield), **38** (91 mg, 91% yield), and **39** (114 mg, 82% yield). Additional purification was obtained by dissolving the salts in a mixture of water and dioxane, followed by lyophilization. The properties of the trifluoroacetate salts **37–39** are presented in Table I.

Determination of Association Constant Values to Polynucleotides. The binding constants (K_{app}) of compounds to poly(dA-dT)·poly(dA-dT) and poly(dG-dC)·poly(dG-dC) were determined with use of their ability to compete with the binding of ethidium bromide according to Baguley et al.[70] Measurements were done at 25 °C in 0.01 M cacodylate buffer, pH 7.0, containing 1.26 μM ethidium bromide and 1 μM polynucleotide. Ethidium bound to polynucletotide was estimated from the fluorescence increase of the chromophore upon binding assuming that fluorescence quenching is negligible and using the following relation: $Db = \Delta IF/k(V - 1)$ where, ΔIF is the difference between the fluorescence of ethidium in the presence and in the absence of polynucleotide, V is the fluorescence increment resulting from the binding to DNA, and k is the factor that links the concentration of free compound to the fluorescence intensity of the solution. In our operating conditions, V values were 34 for poly(dA-dT) and 25 for poly(dG-dC). IC_{50} (the concentration of tested drug required to displace 50% ethidium bromide from its binding sites) was estimated from the best computed fit of the competition sigmoid curve. Association constants K_{app} were calculated from IC_{50} assuming (i) a binding size of four base pairs

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for two cycles containing compounds and two base pairs for one cycle containing compounds and (ii) K_{app} values of ethidium bromide of 9.5×10^6 M^{-1} for poly(dA-dT) and 9.9×10^6 for poly(dG-dC).

Biological Methods. Cytostatic Activity. The cytostatic assays were performed according to previously established procedures.[71,72]

Antiviral Activity. The antiviral assays were performed as reported previously.[73,74]

Acknowledgment. The investigations were supported by grants from the French "Centre National de la Recherche Scientifique" (ARI Chimie-Biologie and PNR-SIDA), the Belgian "Fonds voor Geneeskundig Wetenschappelijk Onderzoek" (Project No. 3.0010.83), and the Belgian "Geconcerteerde Onderzoeksacties" (Project No. 85/90–79). We thank Professor J. W. Lown (Edmonton, Canada) for his interest and helpful discussions and for kindly providing a sample of netropsin. We are indebted to Anita Van Lierde, Frieda De Meyer, and Lizette Van Berckelaer for excellent technical assistance with the biological assays. The assistance of C. Duguet in typing this manuscript is also greatly appreciated.

Registry No. 1, 6973-60-0; 2, 13138-76-6; 3, 2948-69-8; 4, 13138-75-5; 5, 1873-12-7; 7, 2877-46-5; 8, 2853-18-1; 10, 13138-78-8; 11, 28494-51-1; 12, 3185-95-3; 13, 24064-13-9; 14, 69910-20-9; 15, 97950-76-0; 16, 3185-94-2; 17, 14555-80-7; 18, 119436-86-1; 19, 119436-88-3; 19 (free base), 119436-87-2; 20, 119454-85-2; 21, 119436-89-4; 22, 119436-91-8; 22 (free base), 119436-90-7; 23, 119454-86-3; 24, 119436-93-0; 24 (free base), 119436-92-9; 25, 119436-94-1; 26, 119436-96-3; 27, 119436-98-5; 27 (free base), 119436-97-4; 28, 119436-99-6; 29, 119437-00-2; 30, 119437-01-3; 31, 119437-02-4; 32, 119454-87-4; 33, 119437-03-5; 34, 119437-05-7; 34 (free base), 119437-04-6; 35, 119437-07-9; 35 (free base), 119437-06-8; 36, 119437-09-1; 36 (free base), 119437-08-0; 37, 119437-11-5; 37 (free base), 119437-10-4; 38, 119437-13-7; 38 (free base), 119437-12-6; 39, 119437-15-9; 39 (free base), 119437-14-8; $H_2NCH_2CH_2CN$, 151-18-8; $(BOC)NH(CH_2)_4N(BOC)(CH_2)_3NH_2$, 68076-39-1; $H_2NC(NH)NHCH_2COOH$·HCl, 14901-20-3; H-Ala-OBu-*t*·HCl, 13404-22-3; H-Ala-Ala-OBu-*t*·HCl, 60417-38-1; H-Ala-Ala-Ala-OBu-*t*·HCl, 119437-17-1; poly(dA-dT), 26966-61-0; poly(dG-dC), 36786-90-0; N^1-[[1-methyl-4-(N',N'-dicyclohexylguanilyl)pyrrol-2-yl]carbonyl]-N^4,N^8-bis(*tert*-butoxycarbonyl)-spermidine, 119437-16-0.

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New Hydrogen-Bond Potentials for Use in Determining Energetically Favorable Binding Sites on Molecules of Known Structure

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Received May 31, 1988

An empirical energy function designed to calculate the interaction energy of a chemical probe group, such as a carbonyl oxygen or an amine nitrogen atom, with a target molecule has been developed. This function is used to determine the sites where ligands, such as drugs, may bind to a chosen target molecule which may be a protein, a nucleic acid, a polysaccharide, or a small organic molecule. The energy function is composed of a Lennard-Jones, an electrostatic and a hydrogen-bonding term. The latter is dependent on the length and orientation of the hydrogen bond and also on the chemical nature of the hydrogen-bonding atoms. These terms have been formulated by fitting to experimental observations of hydrogen bonds in crystal structures. In the calculations, thermal motion of the hydrogen-bonding hydrogen atoms and lone-pair electrons may be taken into account. For example, in a alcoholic hydroxyl group, the hydrogen may rotate around the C–O bond at the observed tetrahedral angle. In a histidine residue, a hydrogen atom may be bonded to either of the two imidazole nitrogens and movement of this hydrogen will cause a redistribution of charge which is dependent on the nature of the probe group and the surrounding environment. The shape of some of the energy functions is demonstrated on molecules of pharmacological interest.

Predictions of how ligands, such as drugs, might bind to biological molecules can be made by examining the energetic and steric properties of the system. In the method described here, the energies are calculated by a program called GRID,[1] which can be made available for use. They are displayed as energy contours around the chosen molecular target, using three-dimensional computer graphics with program FRODO[2] on an Evans and Sutherland PS300 display system. The energy is calculated between a probe group, which could be, for example, a water molecule or an amine nitrogen atom or an hydroxyl group, and a molecule of known structure, which is called the target molecule, at regular intervals throughout a target region. Large target molecules such as proteins or nucleic acids in an aqueous environment may be studied, and ligand molecules are treated by studying a number of probe groups individually. Other force fields have been developed in order to determine the interaction between whole molecules, but in order to calculate the interaction between a probe and a molecule, it was necessary to develop an independent force field.

Previous approaches to the calculation of binding energies have used either quantum mechanics[3–5] or molecular mechanics methods.[6–9] The latter approach is adopted

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