Design, Synthesis, DNA Binding, and Biological Activity of a Series of DNA Minor-Groove-Binding Intercalating Drugs

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Abstract □ A group of pseudopeptides, molecular combination of the natural antitumor agents distamycin or netropsin and the anilinoacridine chromophore (which is related to the synthetic antileukemic drug amsacrine) has been synthesized. Their DNA binding properties were determined and discussed in terms of their structural differences and in relation to their observed base-dependent binding. Binding data are consistent with a model in which the acridine nucleus occupies an intercalation site and the netropsin or distamycin residue resides in the DNA minor groove. Cytostatic and cytotoxic activities against a murine cell line are reported, as well as significant differences in the inhibition of DNA synthesis.

The majority of antitumor agents in current clinical use are thought to exert their cytotoxic action by interfering with DNA metabolism.[1] Most of them bind to DNA by a noncovalent binding process and act either by inhibition of nucleic acid synthesis or by initiation of DNA breakage. This group of compounds is subdivided into agents which bind by a classical intercalative process[2] and agents which form a noncovalent nonintercalative complex with DNA.[3]

Among intercalating compounds[4] used, acridine derivatives stand as the archetypes. Amsacrine[5] (*m*-Amsa), a synthetic antitumor drug used in the treatment of acute leukemia,[6] is the leader of this group and has attracted attention with the aim of developing analogues with an improved broad spectrum of activity. The second class is represented by netropsin (Nt) and distamycin (Dst-A), naturally occurring nonintercalative binding compounds forming highly ordered complexes with DNA.[7] Both drugs were shown to lie across the minor groove of DNA and recognize specific DNA nucleotides sequences.[7] These natural compounds constitute models for the design of sequence-reading oligopeptides.[8] This approach is attractive from a fundamental point of view and now permits the synthesis of peptides which bind to a predetermined sequence specifically.[9]

For that purpose, we decided to introduce intercalating moieties into the structure of groove-selective binding agents. Netropsin (Nt) and Dst-A demand binding sites consisting of $(AT)_4$ and $(AT)_5$, respectively,[10] while the anilinoacridine moiety intercalates between GC base pairs.[11] The linkage of peptides with strict sequence specificity to an intercalating ring with a different base specificity can lead to a new class of sequence-reading oligopeptides with enhanced antitumor activity.

In this study, we report the design, synthesis, characterization, DNA binding properties, and biological evaluation of a series of hybrid molecules in the structure of which a minor-groove-binding peptide (polyaminopyrrole carboxylic acid) is linked to a classical intercalating moiety, the anilinoacridine ring (see Figure 1). These studies have been carried out in order to develop more effective anticancer agents and contribute to the general preoccupation of pharmacologist and chemist to explore more systematically the structure–biological activity relationship of sequence-directed DNA effectors.

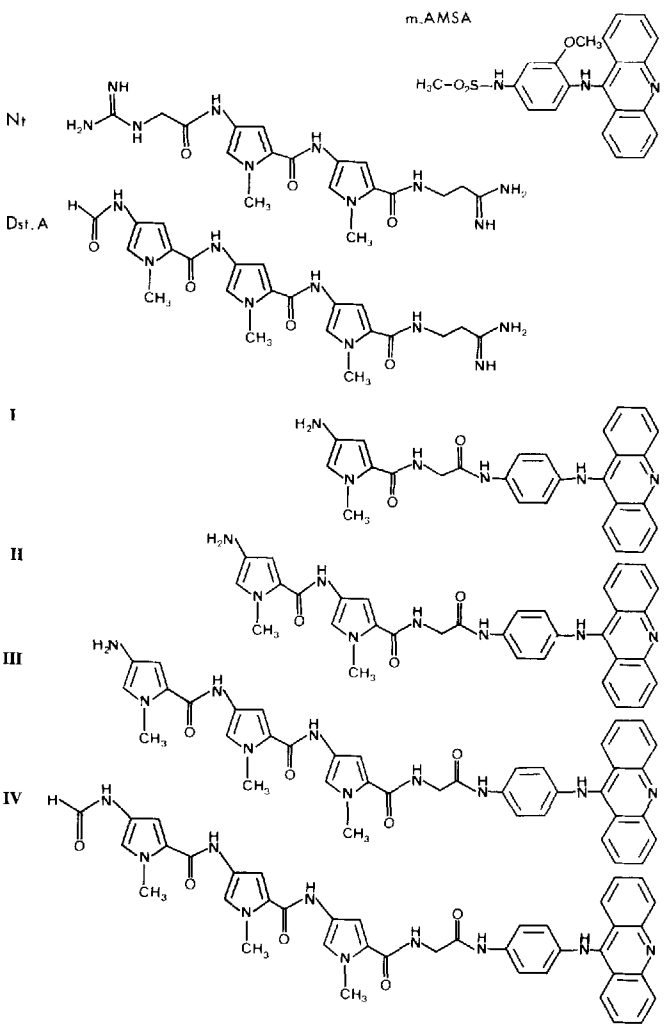

Figure 1—Structure of amsacrine (*m*-Amsa), netropsin (Nt), distamycin (Dst-A), and related synthetic hybrid compounds **I**, **II**, **III**, and **IV**.

Results and Discussion

Chemistry—N-Methyl-2-pyrrole carboxylic acid was esterified with methyl iodide, and then nitrated with nitric acid in acetic anhydride (see Scheme I). Separation by column chromatography gave the methyl *N*-methyl-4-nitro-2-pyrrole carboxylate (**2**), the 5-nitro isomer, and the dinitro derivative. The nitro ester **2** was reduced to the amine **3**, which was condensed with ditertbutyldicarbonate [$(BOC)_2O$] to give the BOC-protected ester **4**. However, it should be pointed out here that the unstable air-sensitive amino acid **3** could be easily

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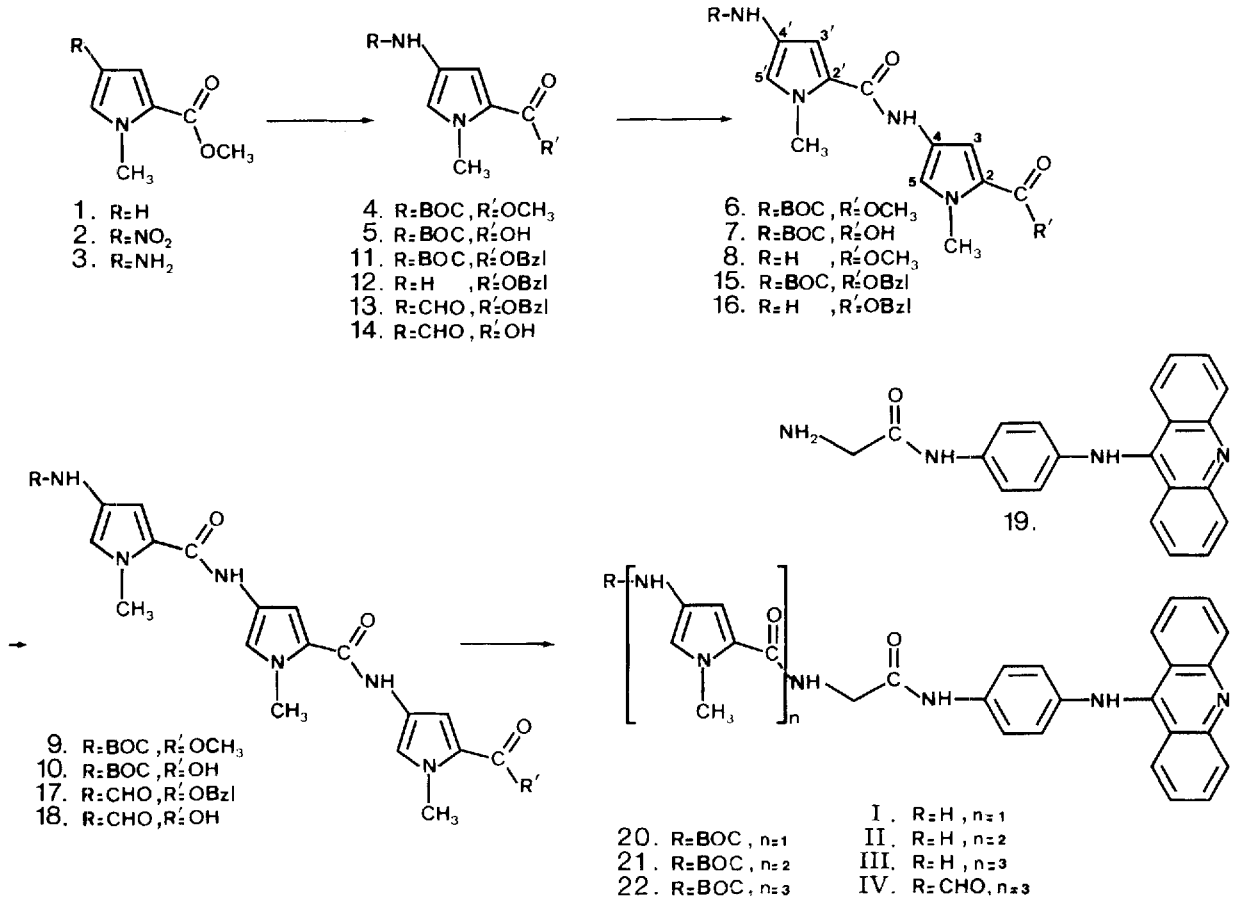

Scheme I

stored as the stable trifluoroacetate salt in a high yield, making it a convenient precursor for synthesis of different oligomers. Alkaline hydrolysis of 4 afforded the acid 5 after acidification. This acid was used for the synthesis of I (see below and see Figure 1). Coupling of 5 with the amine 3 was readily achieved with dicyclohexylcarbodiimide (DCC) in the presence of catalytic amounts of dimethylaminopyridine (DMAP). Alternatively, the amide linkage could be realized via an HOBt-activated ester, but the presence of unreacted products also needed a chromatographic purification.

The dimeric compound 6 was classically saponified with a hydromethanolic sodium hydroxide solution to give the corresponding acid 7. This acid was used in the final synthesis of II.

The protecting BOC group in 6 was classically cleaved off with trifluoroacetic acid (TFA). Due to the light decomposition observed during the extraction, the aqueous solution of the salt 8 was immediately lyophilized. The building of the trimeric block 9 was effected by coupling the acid 5 with the appropriate amine 8 using DCC:DMAP as condensing agent. In this case, the procedure with DMAP was found to be better than with HOBt (80% versus 45%). Compound 9 has also been synthesized starting from the acid 7 and the amine 3 in a lower yield. In this series, the sensitivity of the coupling reaction, as well as the saponification and the BOC cleavage procedures, are decreasing from the monomer to the trimer. Conventional conversion of the methyl ester 9 into its corresponding acid 10 preceeded the coupling with the glycylanilinoacridine moiety to give III.

The synthesis of the formylated trimeric compound IV, mimicking more precisely the distamycin antibiotic, presented difficulties and needed another strategy. The *N*-terminal group of 3 could be easily formylated in a satisfactory yield using formic acid, DCC, and HOBt, but the so-obtained *N*-formyl moiety was cleaved off during the saponification of the methyl ester. The degradation of the *N*-formyl derivative in alkaline saponification conditions led us to prepare the benzyl ester 11 (Cs salt of 5, then benzyl bromide in DMF, as described[11]). After deprotection with TFA, aqueous extraction gave essentially pure 12. The subsequent *N*-formylation (formic acid, DCC:HOBt) was proved to be easier than with formic anhydride.[12] The coupling of the acid 5 with the amine 12 (DCC:DMAP) yielded the protected dimer 15. After removal of the BOC group, the creation of the trimeric building block 17 was effected by condensing the amine 16 with the appropriate acid 14 using EDC as condensing agent.[12] The conversion of the benzyl ester into acids 13 and 17 was effected in a straightforward way by catalytic hydrogenation (Pd on C) in ethanol. The final incorporation of the anilinoacridine moiety into one, two, or three pyrrole residues was a crucial step. The DCC:HOBt coupling procedure was used and gave, after chromatography, the corresponding *N*-protected compounds 20, 21, 22, and IV. Final deprotection of 20, 21, and 22 by acid hydrolysis was accomplished with dry HCl in acetic acid medium. The four hybrid compounds, I, II, III, and IV were used as hydrochloride salts. The analytical data and NMR characteristics of all described compounds are reported in Tables I and II.

DNA Binding—Distamycin (Dst-A) and netropsin (Nt) bind specifically to the minor groove of DNA,[13] while amsacrine intercalates between adjacent base pairs.[14] The intercalative process for amsacrine concerns only the acridine

chromophore, whereas the anilino ring lodges in the minor groove with a nearly orthogonal orientation with respect to the mean plane of the acridine ring.[15] Thus, the linkage of a minor groove binding residue to the anilino ring via a short spacer should lead to compounds which bind strongly to DNA by two well-distinct processes (i.e., intercalation and minor groove binding).

As shown in Table III, there is apparently no significant difference in the ΔTm and Q values observed for the **I**, **II**, and **III**. All of the compounds produce positive ΔTm increase in nucleic acids melting temperatures. These compounds also stabilize GC-containing synthetic polynucleotide against thermal denaturation even at high temperatures (up to 100 °C). If the extent to which ligands stabilize the helix coil thermal transition of double-stranded DNA is representative of the strength and extent of binding, it does not presume of the mode of interaction. For example, polyamines, which bind to DNA by bridging the minor groove and by inter- or intramolecular cross-linking,[16] stabilize double-helical DNA against thermal melting by neutralization of the phosphate; such an interaction could not be taken into account in view of fluorescence experiments. However, in quenching assays employing an excess of poly[d(AT)·d(AT)], nonintercalative binding agents can be distinguished from intercalating agents.[17] Because of their different site size for DNA, intercalating agents have much smaller effects than nonintercalative binding agents (*m*-AMSA, Q = 25 μM; Dst-A, Q = 1.6 μM). The Q values observed for **I**–**IV** with poly[d(AT)·d(AT)] are in the range 3.3–6.7 μM, just greater than those observed for a minor groove binding. Moreover, the high ΔTm values are not in favor of a strict intercalative process. Thus, it seems obvious that both binding modes are involved. One can conclude the stronger binding of **IV** relative to its deformylated analogue **III**, considering the Q and ΔTm: the Q value with calf thymus DNA for **IV** is more than 25-fold lower than with **III**. With the synthetic poly[d(GC)·d(GC)], the difference is lower but significant.

Previous studies[18] have shown similar differences in the binding of Dst-A and its deformylated analogue. This result demonstrates the crucial role played by hydrogen acceptor (or donor) sites in the binding to DNA. This property was not observed for the potential hydrogen acceptor and donor carboxamide groups in pyrrole residues since no binding potency increase was detected when going from one (**I**) to three (**III**) methylpyrrole units. The Q values and the effects of the studied compounds on ΔTm of native or synthetic DNAs of different base composition give information on their base preferential binding. The corresponding results indicate that **I**, **III**, and **IV** do not exhibit any base selectivity. On the other hand, values observed with **II** could be ascribed to its enhanced affinity for poly(AT) sequence, as observed for Dst-A. These results could indicate the dominating role of the structure portion closely related to Nt, which exhibits an AT specificity,[10] versus the anilino-9-aminoacridine ring, which was described as having a GC specificity.[11] However, recent results[19] obtained by computation of the intercalative interaction energy of *m*-AMSA with B-DNA have revealed AT binding site preference as a possibility.

Biological Activity—The cytotoxicity and the growth inhibition and DNA synthesis inhibition abilities of the four hybrid molecules were determined on L1210 murine leukemia cells (Table IV). Increasing the length of the pseudopeptidic moiety with one, two, or three *N*-methylpyrrole residues does not induce large changes in their antitumor activities. Among the pseudopeptide derivatives tested for their inhibitory effects on the proliferation of murine L1210, after only 24 h of incubation, **III** proved to be the most active (ID_{50} = 0.5 μM or 0.39 μg/mL). Surprisingly, its formylated analogue **IV** was fourfold less active. On the other hand, their DNA synthesis inhibitory effects reflect the stronger binding of **IV** than **III** to DNA. This would suggest that DNA is not the only target of **III** and of the other drugs. The two other simpler molecules **I** and **II** are less active (ID_{50} = 3 and 3.8 μM or 1.6 and 2.5 μg/mL, respectively), but remain in the range of the clinically used drugs, except for the highly toxic antileukemic agent *m*-AMSA (ID_{50} = 0.05 μM or 0.02 μg/mL). Compound **II** seems to be interesting in another way. As shown in Figure 2, a 10 μM concentration completely inhibited the proliferation of the tumor cells, without killing >15% of them (LD_{50} = 31 μM). Its poor cytotoxic capacity, coupled with its relative cytostatic power, could make it a convenient compound for reiterative treatments.

Thus, the series of compounds shows interesting biological activities. However no correlation has emerged in attempts to relate the pharmacological activity of the compounds to their nucleic acid binding ability.

Experimental Section

Equipment—Melting points were determined in capillary tubes and are uncorrected. The IR spectra were obtained on a Perkin-Elmer 177 spectrophotometer, in KBr pellets. The ^{13}C and ^{1}H NMR spectra were recorded on a Brücker WP 80 SY or AM 400 WB spectrophotometer. The EI mass spectra were recorded on a Ribermag R10.10 (combined with Riber 400 data system) mass spectrophotometer at 70 eV by using direct insertion. The FAB mass spectra were determined on a Kratos MS-50 RF mass spectrometer arranged in an EBE geometry. The sample was bombarded using a beam of xenon with a kinetic energy of 7 keV. The mass spectrometer was operated at 8 kV accelerating voltage with mass resolution of 3000. Thin layer chromatography (TLC) was carried out using UV-sensitive plates precoated with silica gel 60F-254 Merck (0.25 mm thick). Spots were visualized by inspection under UV light at 254 nm and after exposure to vaporized I_2 and/or ninhydrin. Kieselgel 60 Merck (230–400 mesh) was used for flash chromatography according to the procedure of Still et al.[20]

Syntheses—*Methyl 1-methyl-pyrrole-2-carboxylate* (**1**)—1-Methyl-pyrrole-2-carboxylic acid (Aldrich, 5 g, 0.04 mol), sodium carbonate (21.2 g), and 99% methyl iodide (10 mL) were refluxed in dry acetone (400 mL) for 18 h. The insoluble material was discarded and the filtrate evaporated. The resulting mixture was taken in ether before being filtered to remove the remaining NaI. Removal of ether under reduced pressure afforded a yellow oil. Distillation gave pure **1**; bp: 62 °C/1 mm Hg.

Methyl 1-methyl-4-nitro-pyrrole-2-carboxylate (**2**)—The ester **1** (5 g, 36 mmol) was dissolved in acetic anhydride (30 mL). The solution was cooled to −20 °C and cold nitric acid (65%, 7 mL) was added very slowly to avoid rapid elevation of temperature. Strict temperature control was essential to obtain a pure product in an acceptable yield. Slightly elevated reaction temperature increased the formation of side products and the total yield fell significantly. The mixture was stirred at −20 °C for 6 h, and then at room temperature for 4 h. Removal of the solvent from the red mixture gave a solid. The nitro derivatives were separated by chromatography in the system solvent CH_2Cl_2:petroleum ether (7:3, v/v). The 5-nitro isomer eluted first to yield 1.4 g (21.1%); mp: 109 °C; *Rf* (CH_2Cl_2): 0.70, *Rf* (CH_2Cl_2:petroleum ether, 7:3, v/v): 0.58; ^{1}H NMR ($CDCl_3$): δ 3.89 (s, 3H, $COOCH_3$), 4.31 (s, 3H, NCH_3), 6.89 (d, 1H, CH, J = 4.5 Hz), and 7.13 ppm (d, 1H, CH, J = 4.5 Hz); ^{13}C NMR ($CDCl_3$): δ 34.96 (NCH_3), 51.96 (OCH_3), 111.80 (C_4), 115.47 (C_3), 126.78 (C_2), and 141.04 ppm (C_5), 160.39 (CO); MS:*m/e* (rel. intensity) 184 (100), 153 (52), 122 (67), 107 (47), 79 (84).

Further elution with the same system solvent gave 150 mg (1.8% yield) of the 4,5-dinitro derivative; *Rf* (CH_2Cl_2): 0.67, *Rf* (CH_2Cl_2:petroleum ether, 7:3, v/v): 0.49; MS:*m/e* (rel. intensity) 229 (3.3), 212 (1.0), 199 (1.0), 167 (31.6).

The final elution with CH_2Cl_2 gave 2.3 g of the 4-nitro isomer (**2**); *Rf* (CH_2Cl_2): 0.53; *Rf* (CH_2Cl_2:petroleum ether, 7:3 v/v): 0.36.

Methyl 4-amino-1-methyl-pyrrole-2-carboxylate (**3**)—The 4-nitro derivative **2** (2 g, 0.01 mol) in absolute ethanol (50 mL) was poured into a stainless steel bomb and allowed to be hydrogenated under 50 kg of pressure for 12 h at 50 °C over a Ni Raney catalyst. The catalyst was filtered off, and the colorless filtrate containing the very unstable amino ester **3** was evaporated under reduced pressure in the dark.

Table I—Analytical Data for Compounds 1–22 and I–IV

Compound	mp, °C	TLC *Rf*[a]	Yield, %	IR ν, cm^{-1}	MS M^+ (rel. int.)
1	oil	0.93 (A)	90	1710 ($COOCH_3$)	—[e]
2	112	0.97 (A) 0.53 (B)	35	3120 (CH), 1700 ($COOCH_3$), 1480 (NO_2)	184 (100)[f]
3[b]	185	0.72 (A)	92	2900–3100 (NH_3^+) 1730 ($COOCH_3$)	155 (100)[f]
4	109	0.95 (A) 0.56 (B)	84	3360 (NH), 1725 ($COOCH_3$), 1685 (OCO)	254 (58)[f]
5	146	0 (A) 0.71 (C)	93	3330 (NH), 3000 (CH), 1725 (COOH), 1620 (OCO)	240 (25)[f]
6	125	0.92 (A)	80	3340 (NH), 1770 ($COOCH_3$), 1715 (OCO), 1680 (CONH)	376 (7)[f]
7	100	0 (A) 0.84 (C)	86	1705 (COOH), 1690 (OCO), 1655 (CONH)	—[e]
8[b]	111	0.65 (A)	93	2900–3100 (NH_3^+), 1730 ($COOCH_3$), 1670 (CONH)	276 (35)[f]
9	—[d]	0.86 (A)	96	3340 (NH), 1720 ($COOCH_3$), 1695 (OCO), 1650 (CONH)	498 (66)[f]
10	170	0.41 (A) 0.82 (C)	72	3200 (NH), 1700 (OCO) 1650, 1665 (CONH)	483 (48)[g]
11	139	0.90 (A) 0.54 (B)	86	3380 (NH), 1720 (COOBzl), 1680 (OCO)	330 (12)[f]
12[b]	—[d]	0.78 (A) 0.29 (B)	87	3050–3150 (NH_3^+), 1720 (COOBzl)	230 (58)[f]
13	206	0.83 (A)	78	1715 (COOBzl), 1655 (CHO)	258 (100)[f]
14	186	0.1 (A) 0.72 (C)	95	3150 (NH), 1700 (COOH), 1660 (CHO)	168 (100)[f]
15	158	0.8 (A)	87	3340 (NH), 2980 (CH_3) 1700 (OCO), 1660 (CONH)	452 (4)[f]
16[b]	187	0.8 (A)	83	3200–3600 (NH), 1720 (COOBzl), 1670 (CONH)	353 (36)[h]
17	261	0.63 (A)	59	1720 (COOBzl), 1700 (CHO), 1640, 1650, 1670 (CONH)	503 (43)[h]
18	210- 212	0 (A) 0.82 (C)	77	1710–1700 (CHO, COOH) 1640–1660 (CONH)	413 (17)[h]
20	247	0.77 (A)	65	2920, 2850 (NH), 1700 (OCO), 1645, 1635 (CONH)	565 (17)[h]
21	195	0.61 (A)	52	3340 (NH), 2920 (CH), 1700 (OCO), 1630–1650 (CONH)	687 (45)[h]
22	260	0.75 (A)	72	3400–3000 (NH, CH) 1690 (OCO), 1650 (CONH)	809 (52)[h]
I[c]	220- 223	0 (A) 0.15 (C)	67	3600–3200 (NH_3^+, NH), 2900–3000 (CH)	466 (28)[h]
II[c]	242	0 (A) 0.22 (C)	43	3600–3200 (NH_3^+, NH), 1630, 1640 (CONH)	587 (63)[h]
III[c]	210- 213	0 (A) 0.18 (C)	29	3600–3200 (NH_3^+, NH), 1625–1650 (CONH)	709 (42)[h]
IV[c]	235- 239	0 (A) 0.18 (C)	43	3600–3200 (NH_3^+, NH), 1700 (CHO), 1650 (CONH)	737 (6)[h]

[a] Solvents: A: $CHCl_3$:MeOH (80:20, v/v) in a saturated NH_3 atmosphere; B: CH_2Cl_2; C: CH_2Cl_2:MeOH (20:80 v/v). [b] TFA salt. [c] Hydrochloride. [d] Decomposes upon heating. [e] No molecular ion peak in the 70-eV mass spectrum. [f] Determined by electronic impact (EI). [g] M^+-1. [h] M^++1, determined by fast atom bombardment (FAB).

The resulting residue was not isolated due to its air sensitivity (light exposure provide a rapid decomposition into a purple mixture) and was immediately used for the next step.

To prevent degradation, the amine was dissolved in CH_2Cl_2 (5 mL), and trifluoroacetic acid (1 mL) was added in a dropwise manner, with rapid stirring, for 15 min. After evaporation of the solution, the residue was washed with CH_2Cl_2 (5 × 30 mL), precipitated with ether:petroleum ether (1:4), and collected.

Methyl 4-[[(tert-butyloxy)carbonyl]amino]-1-methyl-pyrrole-2-carboxylate (**4**)—A solution of ditertbutyldicarbonate (3.27 g, 0.015 mmol) in CH_2Cl_2 (150 mL) was added to **3** (1.54 g, 0.01 mol) in CH_2Cl_2 (100 mL) for 12 h under reflux. The solution was then extracted with water (3 × 30 mL), dried over Na_2SO_4, evaporated, washed with cold petroleum ether, and finally afforded the desired compound **4**. Recrystallization from CH_2Cl_2:petroleum ether (1:25) gave a white microcrystalline product; *Rf* (CH_2Cl_2): 0.56.

4-[[(tert-Butyloxy)carbonyl]amino]-1-methyl-pyrrole-2-carboxylic acid (**5**)—A solution of **4** (2.54 g, 0.01 mol) in MeOH (50 mL) and 1.6 g (0.04 mol) of sodium hydroxide in water (5 mL) was boiled under reflux with stirring. The progress of the reaction was monitored by TLC and was thereby judged to be complete after 18 h. The resulting solution was cautiously acidified to pH 3.0 with a few drops of dilute HCl. Evaporation of the solvent, trituration in absolute ethanol, elimination of sodium chloride by filtration, and evaporation of ethanol yielded 2.23 g of a white solid. The product was isolated by precipitation with a mixture of ether and petroleum ether; *Rf* (MeOH:CH_2Cl_2, 80:20, v/v): 0.71.

Methyl 4-[[[4-[[(tert-butyloxy)carbonyl]amino]-1-methyl-pyrrol-2-yl]carbonyl]amino]-1-methyl-pyrrole-2-carboxylate (**6**)—*The DCC:DMAP Coupling Procedure*—The acid **5** (0.61 g, 2.54 mmol) in 20 mL of CH_2Cl_2 reacted with the amine **3** (0.4 g, 2.54 mmol). After evolution of the reaction (2 to 4 h), the solution was filtered off and polar compounds were successively removed by extraction with 1 M HCl, H_2O, and 1 M $NaHCO_3$ (30 mL). After drying and evaporation of the CH_2Cl_2, the crude residue was purified by chromatography with CH_2Cl_2:acetone (20:1, v/v) as eluent.

Table II—Nuclear Magnetic Resonance Chemical Shift Assignments

Compound	^{1}H NMR (δ), ppm/TMS	^{13}C NMR (δ), ppm/TMS
1	3.72 (s,3,OCH_3), 3.84 (s,3,NCH_3), 6.07, 6.82 and 7.0 (3m,3,3CH)[a]	37.78 (NCH_3), 51.25 (OCH_3), 110.83 (C_4), 116.71 (C_3), 124.0 (C_2), 125.52 (C_5), 160.86 (CO)[b]
2	3.78 (s,3.OCH_3), 3.92 (s,3,NCH_3), 7.27 and 8.17 (2d,2,2CH,J = 4.7Hz)[a]	37.73 (NCH_3), 51.63 (OCH_3), 112.42 (C_3), 122.71 (C_2), 127.51 (C_5), 135.01 (C_4), 160.35 (CO)[b]
3[e]	3.55 (s,3,OCH_3), 3.60 (s,3,NCH_3), 6.71 and 6.89 (2d,2,2CH)[c]	37.89 (NCH_3), 53.0 (OCH_3), 113.46 (C_3), 122.97 (C_5), 124.83 (C_4), 125.19 (C_2), 163.21 (CO), 113.05 (CF_3), 163.63 (COO^-)[c]
4	1.42 (s,9,$(CH_3)_3$), 3.68 (s,3,OCH_3), 3.76 (s,3,NCH_3), 6.64 and 7.0 (2d,2,2CH), 8.85 (s,1,NH)[a]	28.26 (CH_3,BOC), 36.54 (NCH_3), 50.93 (OCH_3), 80.08 (C,BOC), 108.0 (C_3),119.81 (C_5,C_4), 122.12 (C_2), 153.21 (CO,BOC), 161.45 (COO)[b]
5	1.41 (s,9,$(CH_3)_3$), 3.76 (s,3,NCH_3), 6.58 and 7.0 (2d,2,2CH), 8.87 (s,1,NH)[a]	28.22 (CH_3,BOC), 36.50 (NCH_3), 80.0 (C,BOC), 107.91 (C_3), 119.72 (C_5), 120.17 (C_4), 122.35 (C_2), 153.20 (CO,BOC), 164.81 (COOH)[b]
6	1.44 (s,9,$(CH_3)_3$), 3.67 (s,3,OCH_3), 3.74 and 3.79 (2s,6,2NCH_3), 6.80, 6.81, 6.88 and 7.4 (4d,4,4CH), 8.88 and 9.70 (2s,2,2NH)[a]	28.31 (CH_3,BOC), 36.51, 36.63 (2NCH_3), 51.02 (OCH_3), 79.56 (C,BOC), 103.63, 108.26 (C_3,C_3'), 118.43, 118.73 (C_5,C_5'), 120.88, 121.05 (C_4,C_4'), 121.74, 121.93 (C_2,C_2'), 153.51 (CO,BOC), 160.94 (CONH), 161.47 (COO)[b]
7	1.50 (s,9,$(CH_3)_3$), 3.83 (s,6,2NCH_3), 6.85, (m,3,3CH), 7.37 (d,1,CH), 8.89 and 9.68 (2s,2,2NH)[a]	28.32 (CH_3,BOC), 36.54 36.92 (2NCH_3), 80.92 (C,BOC), 103.94, 110.04 (C_3,C_3'), 118.19, 122.10 (C_5,C_5'), 119.26, 121.74 (C_4,C_4'), 121.96, 123.20 (C_2C_2'), 153.56 (CO,BOC), 158.90 (CONH), 164.73 (COOH)[b]
8[e]	3.72 (s,3,OCH_3), 3.79 and 3.85 (2s,6,2NCH_3), 6.88 (m,2,2CH), 7.05 and 7.40 (2d,2,2CH,J = 4.7Hz), 9.90 (s,1,NH)[a]	37.13, 37.25 (2NCH_3), 52.79 (OCH_3), 113.40, 113.57 (C_3,C_3'), 123.06, 125.92 (C_5,C_5'), 124.83 126.35 (C_4,C_4'), 125.11, 125.60 (C_2,C_2'), 161.13 (CONH), 163.30 (CO), 113.20 (CF_3), 163.50 (COO^-)[c]
9	1.48 (s,9,$(CH_3)_3$), 3.76 (s,3,OCH_3), 3.83 (s,9,3NCH_3), 4.96 (m,1,NH), 6.53, 6.64, 6.72, 6.79, 7.12, and 7.38 (6d,6,6CH), 7.79 and 8.17 (2s,2,2NH)[a]	28.34 (CH_3,BOC), 33.87 (NCH_3), 36.58 (2NCH_3), 50.71 (OCH_3), 80.34 (C,BOC), 103.73, 103.91, 108.39, (C_3,C_3',C_3''), 115.43, 116.32, 118.53, 119.33, 119.77, 119.97, 121.42, 121.78, 123.10 (C_2,C_2',C_2'', C_4,C_4',C_4'', C_5,C_5',C_5''), 153.64 (CO,BOC), 158.01 159.01 (2CONH), 161.53 (COO)[b]
10	1.50 (s,9,$(CH_3)_3$), 3.88, 3.89 and 3.92 (3s,9,3NCH_3), 6.85, 6.90, 7.01, 7.10 and 7.17 (5s,6,6CH), 7.62 (m,1,NH)[a]	26.50 (CH_3,BOC), 34.35, 34.39, 34.47 (3NCH_3), 76.54 (C,BOC), 102.18, 103.08, 106.63 ($C_3C_3'C_3''$), 115.19, 116.58, 118.11, 118.37, 120.60, 120.77, 120.91, 120.98, 121.15 (C_2,C_2',C_2'', C_4,C_4',C_5'', C_5,C_5',C_5''), 151.17 (CO,BOC), 156.85 (2CONH), 160.50 (COOH)[a]
11	1.42 (s,9,$(CH_3)_3$), 3.83 (s,3,NCH_3), 5.22 (s,2,CH_2), 6.83 and 7.11 (2s,2,2CH), 7.37 (s,5,5CH), 7.45 (m,1,NH)[a]	28.25 (CH_3,BOC), 36.58 (NCH_3), 65.37 (CH_2), 79.98 (C,BOC), 108.15 (C_3) 119.30 (C_4), 121.52 (C_5), 127.32 128.90 (CH,arom.), 136.26 (C_2), 153.11 (CO,BOC), 160.66 (CO,Bzl)[b]
12[e]	3.87 (s,3,NCH_3), 5.25 (s,2,CH_2), 6.83 and 7.06 (2d,2,2CH), 7.35 (s,5,5CH), 8.31 (m,3,NH_3^+)[a]	37.58 (NCH_3), 52.72 (CH_2), 108.56 (C_3), 120.62 (C_5), 122.0 (C_4), 123.38 (C_2), 126.0 (CH,arom), 163.35 (CO), 113.15 (CF_3), 163.50 (COO^-)[c]
13	3.88 (s,3,NCH_3), 5.24 (s,2,CH_2), 6.65 and 6.75 (2d,2,2CH), 7.32 (s,5,5CH), 7.57 (m,1,NH), 8.2 (d,1,CHO)[b]	36.62 (NCH_3), 65.46 (CH_2), 108.15 (C_3), 119.32 (C_4), 121.52 (C_5), 127.48, 129.05 (CH,arom.), 135.95 (C_2), 160.80 (CO,Bzl), 162.50 (CHO)[a]
14	3.78 (s,3,NCH_3), 5.52 (d,1,NH), 6.67 and 7.24 (2d,2,2CH), 8.1 (s,1,CHO), 9.87 (s,1,COOH)[a]	36.68 (NCH_3), 107.95 (C_3), 119.40 (C_4), 122.35 (C_5), 133.56 (C_2), 162.55 (CHO), 162.90 (COOH)[a]
15	1.50 (s,9,$(CH_3)_3$), 3.91 (s,6,2NCH_3), 5.25 (s,2,CH_2), 6.32 and 6.50 (2d,2,2CH), 6.82 (m,2,2CH), 7.30 (s,5,5CH), 7.55 and 7.64 (2s,2,2NH)[b]	28.23 (CH_3,BOC), 36.41, 36.56 (2NCH_3), 65.44 (CH_2), 80.15 (C,BOC), 103.86, 108.61 (C_3,C_3'), 118.60, 119.55 (C_5,C_5'), 121.07, 121.53 (C_4,C_4'), 121.81, 122.99 (C_2,C_2'), 127.81, 128.39 (CH,arom.), 136.27 (C,arom.), 153.51 (CO,BOC), 158.82 (CONH), 160.71 (COO)[b]
16[e]	3.90 (2s,6,2NCH_3), 5.28 (s,2,CH_2), 6.79, 6.82, 6.90 and 6.33 (4d,4,4CH), 7.35 (s,5,5CH), 7.40 (m,1,NH), 8.35 (m,3,NH_3^+)[c]	37.58 (2NCH_3), 52.75 (CH_2), 108.47, 110.83 (C_3,C_3'), 120.65 (C_5,C_5'), 122.17 (C_4,C_4'), 123.27 (C_2,C_2'), 125.98 (CH,arom.), 160.95 (CONH), 113.15 (CF_3), 163.95 (COO^-)[c]
17	3.89, 3.91 and 3.92 (3s,9,3NCH_3), 5.24 (s,2,CH_2), 6.92, 7.00, 7.08, 7.13, 7.20 and 7.33 (6s,6,6CH), 7.4 (m,2,2NH), 7.82 (s,5,5CH), 8.16 (s,1,CHO), 9.75 (m,1,NH)[a]	—[d]
18	3.89 (m,9,3NCH_3), 6.91, 6.93 and 6.97 (3d,3,3CH), 7.12 (m,2,2CH), 7.18 (d,1,CH), 7.55 and 7.6 (m,2,2NH), 8.09 (m,1,CHO)[a]	—[d]
20	1.45 (s,9,$(CH_3)_3$), 3.76 (s,3,NCH_3), 3.95 (d,2,CH_2, J = 7.6Hz), 6.86 (d,1,NH), 7.10 (d,1,CH), 6.60–8.20 (m,14,NH,13CH), 8.90 and 9.76 (2s,2,2NH)	—[d]
21	1.45 (s,9,$(CH_3)_3$), 3.81 and 3.82 (2s,6,2NCH_3), 3.96 (d,2,CH_2,J = 5.7Hz), 6.71–7.63 (m,16,16CH), 8.28 (m, 1, NH), 9.08 (m, 1, NH), 9.75, 9.86 and 9.95 (3 s, 3, 3NH)[a]	—[d]
22	1.42 (s,9,$(CH_3)_3$), 3.70 (d,2,CH_2), 3.79 (m,12,3NCH_3, OCH_3), 6.66–8.11 (m,23,18CH,5NH), 9.70 (m,1,NH)[a]	—[d]
I[f]	3.80 (s,3,NCH_3), 4.05 (d,2,CH_2), 6.94–8.33 (m,16,14CH,2NH), 8.56 (m,1,NH), 10.42 (s,3,NH_3^+)[a]	—[d]
II[f]	3.68 and 3.79 (2s,6,2NCH_3), 4.0 (d,2,CH_2,J = 7.7Hz), 6.23 (s,1,NH), 6.37–7.95 (m,18,16CH,2NH), 9.37 (s,1,NH), 9.79 (s,1,NH), 10.35 (s,3,NH_3^+)[a]	—[d]

(Continued)

Table II—Continued

Compound	^{1}H NMR (δ), ppm/TMS	^{13}C NMR (δ), ppm/TMS
III[f]	3.70, 3.75 and 3.77 (3s,9,3NCH$_3$), 4.10 (d,2,CH$_2$,J = 7.7Hz), 6.50 (m,1,NH), 6.32–8.05 (m,21,18CH,3NH), 9.45 (s,1,NH), 9.65 (s,1,NH), 10.42 (s,3,NH$_3^+$)[a]	—[d]
IV[f]	3.86, 3.87 and 3.88 (3s,9,3NCH$_3$), 4.10 (d,2,CH$_2$), 6.40–7.88 (m,CH,arom, CHO)[c]	—[d]

[a] Solvent: Me_2SO-d_3. [b] Solvent: $CDCl_3$. [c] Solvent: D_2O. [d] Not determined. [e] TFA salt. [f] Hydrochloride.

Table III—DNA Binding Parameters of the Hybrid Compounds and Parent Molecules

Compound	DNA								Duplex Native and Synthetic DNA					
	poly[d(AT)·d(AT)]				*Clostridium perfringens* (26.5% GC)				Calf Thymus (42% GC)				poly[d(GC)·d(GC)]	
	ΔTm[a] (D/P)[b]			Q[c]	ΔTm D/P			Q	ΔTm (D/P)			Q	ΔTm[d]	Q
	0.1	0.5	1		0.1	0.5	1		0.1	0.5	1			
I	4	14	17	5.3	3	6	9	4.7	7	13	18	5	—	9
II	5	18	19	5.5	2	4	6	6	3	7	8	7	—	12
III	1	6	11	6.7	1	4	7	10.8	1	5	8	71	—	12.5
IV	6	19	23	3.3	5	12	15	2.7	9	18	20	2.7	—	3.6
Dst-A	18	34	38	1.6	15	19	29	22.2	14	22	24	39.5	—	130
m-AMSA	4	10	14	25	2	5	10	2.8	1	5	8	3.8	—	4.5

[a] Elevation in thermal denaturation temperature (°C). [b] Drug to phosphate residue ratio. [c] Concentration (μM) of drug to give 50% of quenching of fluorescence of bound ethidium at an added D/P ratio of 0.1; see text. [d] Temperature elevation too high to be measured because of instrumental limitations.

Table IV—Biological Activity of Hybrid Compounds and Parent Molecules

Compound	Leukemia Murine L 1210 cells		
	ID_{50}[a]	LD_{50}[b]	I_{DNA50}[c]
I	3	13.5	13.5
II	8	31	12.5
III	0.5	9	26
IV	2	7	15
m-AMSA	0.05	0.5	2.7

[a] Concentration (μM) to inhibit cell growth by 50% following a 24-h exposure. [b] Concentration (μM) to kill 50% of cells following a 24-h exposure. [c] Concentration (μM) to inhibit DNA synthesis by 50%.

The DCC:HOBt coupling procedure—Compound **5** (0.76 g, 3.16 mmol) in cold dry CH_2Cl_2 (50 mL) was stirred with DCC (0.72 g, 3.5 mmol) and HOBt (0.54 g, 3.5 mmol) for 1 h. Then a cold solution of **3** (0.49 g, 3.16 mmol), plus TEA for the TFA salt, in CH_2Cl_2 (50 mL) was added. Stirring was continued for 2 h at 0 °C and for at least 20 h at 20 °C. The precipitated dicyclohexylurea (DCU) was filtered off and the CH_2Cl_2 solution was washed with 30 mL of 1 M HCl, H_2O, and 1 M $NaHCO_3$. After drying over Na_2SO_4, the solvent was removed under reduced pressure. The remaining DCU was discarded by precipitation with acetone. Trituration with ether:petroleum ether gave a crude product containing ~80% of the desired dimeric **6** and 20% of a side compound; Rf(A): 0.92; Rf(MeOH:CH_2Cl_2, 0.5:9.5, v/v): 0.74. The ^{1}H NMR and MS (337, M^+) analyses allowed identification of the compound as 1,2,3-benzotriazol-1-yl 4-[[(*tert*-butyloxy)-carbonyl]amino]-1-methyl-pyrrole-2-carboxylate.[12] This active ester of HOBt was obtained even with longer reaction times and had to be separated from **6** by flash chromatography with MeOH:CH_2Cl_2 (0.5:9.5 v/v) as eluent before the next step; Rf(MeOH:CH_2Cl_2, 0.5:9.5, v/v): 0.45.

4-[[[4-[[(tert-Butyloxy)carbonyl]amino]-1-methyl-1-pyrrol-2-yl] carbonyl] amino]-1-methyl-pyrrole-2-carboxylic acid (**7**)—The methyl ester **6** (1.2 g, 3.2 mmol) was converted to the corresponding acid **7** according to the method of preparation of **5**. The saponification was stopped after 72 h (longer reaction times did not afford higher yields). Evaporation of the solvent gave a white powder which was partitioned between H_2O and CH_2Cl_2 to remove the remaining ester **6**. Acidification of the aqueous layer to pH 3 with dilute HCl and extraction with ethyl acetate (3 × 25 mL) afforded 0.88 g of the chromatographically pure acid **7**; Rf (MeOH:$CHCl_3$, 8.0:2.0, v/v): 0.84.

Methyl 4-[[[4-[[[4-[[(tert-butyloxy)carbonyl]amino]-1-methyl-pyrrol-2-yl]carbonyl]amino]-1-methyl-pyrrol-2-yl]carbonyl] amino]-1-methyl-pyrrole-2-carboxylate (**9**)—*Amine Component* (**8**)—A 30-mL aliquot of CH_2Cl_2:TFA (2:1, v/v) was slowly added to **6** (0.83 g, 2.2 mmol), and the brownish solution was left at ambient temperature for 1 h and then evaporated. The resulting hygroscopic residue was washed several times with ether or CH_2Cl_2 (acetone or methanol were not used to avoid a rapid decomposition). The dry residue was then partitioned between H_2O (20 mL) and CH_2Cl_2 (20 mL). After centrifugation, the clear aqueous layer was collected and lyophilized. The amine **8** was obtained pure in a good yield. The trimeric compound **9** was synthesized using DCC:DMAP as coupling agents, as described for **6**. The crude product obtained after the washing steps was directly used for the next step without chromatography. However, an analytical sample could be obtained by dissolution of the crude material in a minimum volume of acetone, followed by addition of sufficient cold dry ether with rapid stirring.

4-[[[4-[[[4-[[(tert-Butyloxy)carbonyl]amino]-1-methyl-pyrrol-2-yl]-carbonyl]amino]-1-methyl-pyrrol-2-yl]carbonyl]amino]-1-methyl-pyrrole-2-carboxylic acid (**10**)—The methyl ester **9** was saponified to give the acid **10** as described for the preparation of **7**; Rf (MeOH:CH_2Cl_2, 80:20, v/v): 0.82.

Benzyl 4-[[(tert-butyloxy)carbonyl]amino]-1-methyl-pyrrole-2-carboxylate (**11**)—This compound was prepared from **5** by modification of a previous procedure:[12] 1 g of **5** (4.16 mmol) in EtOH:H_2O (3:1, v/v, 50 mL) was treated dropwise with a solution of Cs_2CO_3 (0.68 g, 2.08 mmol) in H_2O (10 mL). After complete addition, the solution was evaporated to dryness. The resulting semisolid residue was dissolved in absolute EtOH (3 × 40 mL) and evaporation of the solvent gave the cesium salt of **5**. Benzyl bromide (0.5 mL, 4.17 mmoL) was slowly added to the solution of the cesium salt in DMF (20 mL) and stirring was maintained overnight at 40 °C. The DMF was evaporated and the residue was dissolved with water (10 mL). After three extractions with CH_2Cl_2 (3 × 30 mL), the combined organic layers were concentrated and diluted 10-fold with cold petroleum ether. The precipitated material was filtered, rinsed with petroleum ether and dried; Rf (CH_2Cl_2): 0.54.

Benzyl 4-(formylamino)-1-methyl-pyrrole-2-carboxylate (**13**)—*Amine Component* (**12**)—To 0.75 g (2.27 mmol) of **11** in 50 mL of CH_2Cl_2, 10 mL of pure TFA were added. After 1 h of stirring, the solution was evaporated. Careful elimination of the TFA gave a crude

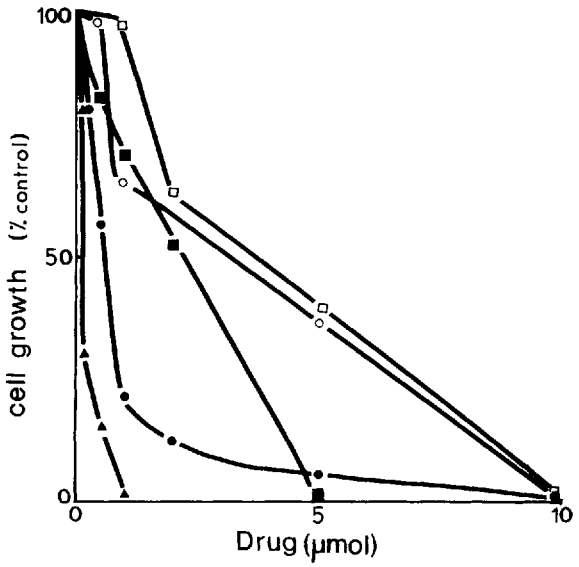

Figure 2—Effects of *m*-Amsa (▲) and of the synthetic hybrid compounds **I** (○), **II** (□), **III** (●), and **IV** (■) on the proliferation of murine leukemia (L1210) cells after a 24-h exposure.

residue which was dissolved in acetone (3 mL), ether (20 mL), and petroleum ether (100 mL), giving a white precipitate. This product was filtered, washed, and dried, and **12** was obtained without further purification. An analytically pure sample was obtained after two successive precipitations in CH_2Cl_2:petroleum ether (1:15) in the cold; *Rf* (CH_2Cl_2): 0.29.

Formylation—Formic acid (83 μL, 2.56 mmol) in cold CH_2Cl_2 was stirred with DCC (0.53 g, 2.56 mmol) and HOBt (0.4 g, 2.56 mmol) for 1 h. A cold solution of **12** (0.8 g, 2.32 mmol) and TEA (0.32 mL, 2.32 mmol) in CH_2Cl_2 was added, and stirring was maintained for 2 h at 0 °C and 15 h at 20 °C. The DCU was discarded by precipitation with acetone. The remaining powder was dissolved in ether (insoluble material discarded) and addition of petroleum ether led to precipitation. Pure **13** precipitated as a microcrystalline solid after 1 d in the cold.

4-(Formylamino)-1-methyl-pyrrole-2-carboxylic acid (**14**)—Compound **13** (0.4 g, 1.55 mmol) dissolved in absolute ethanol (40 mL) was reduced by hydrogen (atmospheric pressure) for 12 h in the presence of a Pd catalyst (5% on C). The mixture was filtered and the clear solution concentrated to <4 mL. Addition of cold ether with rapid stirring give the desired acid **14**; *Rf* (MeOH:CH_2Cl_2, 80:20, v/v): 0.72.

Benzyl 4-[[[4-[[(tert-butyloxy)carbonyl]amino]-1-methyl-pyrrol-2-yl]carbonyl]amino]-1-methyl-pyrrole-2-carboxylate (**15**)—The acid **5** was coupled with the amine **12** in the presence of DCC and DMAP according to a well-established procedure.[12] Compound **15** was obtained pure, as judged by TLC, after purification by chromatography with CH_2Cl_2:acetone (20:1, v/v) as eluent; *Rf* (solvent of chromatography): 0.33.

Benzyl 4-[[[4-[[[4-[[(formylamino)-1-methyl-pyrrol-2-yl]carbonyl]-amino-1-methyl-pyrrole-2-yl]carbonyl]amino-1-methyl-pyrrole-2-carboxylate (**17**)—*Amine* **16**—The BOC protected amine **15** (0.3 g, 0.66 mmol) was deprotected with TFA to give the corresponding free amine **16**. Purification was accomplished according to the method of preparation of **8**.

A solution of **14** (57 mg, 0.34 mmol), **16** (142 mg, 0.31 mmol), and TFA (43 μL, 0.31 mmol) in DMF (20 mL) was immediately treated with EDC (65 mg, 0.34 mmol). The resulting solution was stirred at ambient temperature overnight and then evaporated. The brownish residue was washed with 1 M $KHSO_4$, 1 M $NaHCO_3$, H_2O, and absolute ethanol. The remaining insoluble material was dissolved in CH_2Cl_2 (2 mL) and precipitated with ether and petroleum ether. The crude product was suitable for the preparation of **18**. An analytical specimen could be obtained as white crystals by recrystallization from CH_2Cl_2:ether:petroleum ether (1:10:100).

4-[[[4-[[[4-[[(Formylamino)-1-methyl-pyrrol-2-yl]carbonyl]amino]-1-methyl-pyrrol-2-yl]carbonyl]amino-1-methyl-pyrrole-2-carboxylic acid (**18**)—Crude **17** (140 mg, 0.28 mmol) in DMF (10 mL) was totally converted into the corresponding acid by catalytic hydrogenation (H_2, atmospheric pressure, Pd on C) at room temperature for 6 h. After filtration of the catalyst, the solution was evaporated, and the residue was dissolved in EtOH, clarified with decolorizing carbon and finally taken to dryness; *Rf* (MeOH:CH_2Cl_2, 80:20, v/v): 0.82.

4-(9-Acridinylamino)-N-[4-[[(tert-butyloxy)carbonyl]amino]-1-methyl-pyrrol-2-carbonyl]-glycylaniline (**20**)—*Amine* **19**—4-(9-Acridinylamino)-*N*-glycylaniline hydrochloride trifluoro acetate, synthesized as previously described,[21] was dissolved in water, dilute $NaHCO_3$ was added to pH 8–8.5, and the amine **19** was extracted with ethyl acetate. Drying over Na_2SO_4 and evaporation of the organic layer afforded **19**.

A solution of **5** (95 mg, 0.39 mmol), DCC (89 mg, 0.43 mmol), and HOBt (66 mg, 0.43 mmol) in 40 mL of CH_2Cl_2:DMF (1:1, v/v) was added to 0 °C to a solution of **19** (134 mg, 0.39 mmol) in 10 mL of DMF. After 18 h of stirring, the solution was evaporated, the DCU was discarded by precipitation with acetone, and the concentrated filtrate was precipitated with ether. Filtration gave **20** suitable for the next and last step. An analytical sample was obtained by chromatography with CH_2Cl_2:MeOH, (80:20, v/v) as solvent; *Rf* (CH_2Cl_2:MeOH, 80:20, v/v): 0.66.

4-(9-Acridinylamino)-N-[4-[[[4-[[(tert-butyloxy)carbonyl]amino]-1-methyl-pyrrol-2-yl]carbonyl]amino]-1-methyl-pyrrol-2-carbonyl-]glycylaniline (**21**)—Compound **7** (105 mg, 0.29 mmol) was coupled to **19** (100 mg, 0.29 mmol) by the DCC:HOBt procedure, as described for **20**. The reaction mixture was stirred at 0 °C for 4 h and at room temperature overnight. The solution was evaporated and washed with CH_2Cl_2 to eliminate the DMF. The crude red residue was triturated with CH_2Cl_2 (10 mL), filtered, and washed with CH_2Cl_2 (4 × 20 mL). Compound **21** was then purified by flash chromatography with CH_2Cl_2:MeOH (80:20, v/v) as eluent; *Rf* (CH_2Cl_2:MeOH, 80:20, v/v): 0.63.

4-(9-Acridinylamino)-N-[4-[[[4-[[[[(tert-butyloxy)carbonyl]amino]-1-methyl-pyrrol-2-yl]carbonyl]amino]-1-methyl-pyrrol-2-yl]carbonyl]amino]-1-methyl-pyrrol-2-carbonyl]-glycylaniline (**22**)—Compound **22** was obtained by coupling the acid **10** (0.4 g, 0.83 mmol) with the amine **19** (0.26 g, 0.76 mmol) using DCC:HOBt as described for **20**. The purification was accomplished by the same technique used for **21**.

4-(9-Acridinylamino)-N-[4-amino-1-methyl-pyrrol-2-carbonyl]-glycylaniline Hydrochloride (**I**)—The BOC-protected amine **20** (100mg, 0.17 mmol) in acetic acid (30 mL) was flushed with dry HCl for 10 min and stirring was maintained for 50 min. The acid solution was evaporated (below 45 °C) and washed with acetone and ether. The resulting residue was dissolved in water and extracted with CH_2Cl_2 (2 × 20 mL) and ethyl acetate (2 × 20 mL). Final chromatography in EtOH:$CHCl_3$ (80:20, v/v) gave **I**; *Rf* (solvent of chromatography): 0.15.

4-(9-Acridinylamino)-N-[4-[[[4-amino-1-methyl-pyrrol-2-yl]-carbonyl]amino]-1-methyl-pyrrol-2-carbonyl]-glycylaniline Hydrochloride (**II**)—The BOC-protecting group in **21** (160 mg, 0.24 mmol) was cleaved off with dry HCl in acetic acid medium for 1 h and then purified as described for **I**.

4-(9-Acridinylamino)-N-[4-[[[4-amino-1-methyl-pyrrol-2-yl]carbonyl]amino]-1-methyl-pyrrol-2-yl]carbonyl]amino]-1-methyl-pyrrol-2-carbonyl]glycylaniline Hydrochloride (**III**)—Cleavage of the BOC group and final purification were accomplished as described for **I**.

4-(9-Acridinylamino)-N-[4-[[[-4-[[[4-(formylamino)-1-methyl-pyrrol-2-yl]carbonyl]amino]-1-methyl-pyrrol-2-yl]carbonyl]amino]-1-methyl-pyrrol-2-carbonyl]-glycylaniline Hydrochloride (**IV**)—A 60-mg amount of the acid **18** (0.145 mmol) was coupled to 580 mg of the amine **19** (0.145 mmol) via a DCC:HOBt (0.16 mmol) procedure as adopted for **20**. The compound obtained after chromatography was acidified with diluted HCl. The acid aqueous layer was lyophilized, and the lyophilized material was dissolved in water (50 mL). This procedure was repeated five times to assure complete elimination of excess HCl.

Drug-DNA Binding—Poly[d(AT) · d(AT)], poly[d(GC) · d(GC)], calf thymus DNA, and *Clostridium perfringens* DNA were purchased from Sigma Chemical and used without purification. Concentrations of the DNAs were determined spectroscopically from their extinction coefficients (in M^{-1} cm^{-1}), ε_{260} = 6700, ε_{254} = 8400, ε_{260} = 6600, and ε_{260} = 6500, respectively.

The *Q* values for quenching were determined employing 20 μM DNA in 0.01 M ionic strength buffer (9.3 mM NaCl, 2 mM NaOAc buffer, pH 5, plus 0.1 mM EDTA) containing 2 μM ethidium in such a way that there was minimal ethidium displacement and maximum drug-induced quenching.[22] All measurements were made in 4-mL, 10-mm pathlength quartz cuvettes, at 20 °C on a Jobin-Yvon J-Y-3 spectrofluorometer equipped with an X-Y recorder (excitation at 546 nm and measurement at 595 nm). The *Q* value is defined as the drug

concentration which reduces the fluorescence of initially DNA-bound ethidium by 50%.

Melting temperature studies were made in 0.1 SSC buffer (0.15 NaCl, 0.015 M sodium citrate, pH 7.0) as previously described.[23]

Biological Testing—*Cell Culture*—Murine L1210 leukemia cells were maintained in logarithmic growth as suspension cultures in RPMI-1640 medium (GIBCO) containing 10% fetal calf serum. Cells were grown in 25-cm^2 tissue culture flasks (Corning) in a total volume of 10 mL in a water-saturated atmosphere containing 5% CO_2 at 37 °C.

Growth and Viability Assays (ID_{50} and LD_{50} Determinations—The cells were treated, while in logarithmic growth (10^6 cells/mL), with the hybrid derivatives diluted in sterile water and filtered through a 0.2-μm filter immediately prior to use. Following a 24-h incubation, cells samples were removed for counting. Cell growth and viability was estimated by counting the cells after dilution with trypan blue solution at 0 and 24 h. The cytotoxic effects on cellular growth were expressed as a function of drug concentration. For each compound, we determined the inhibitory dose (ID_{50}) reducing cell growth to 50% of control growth, and the lethal dose (LD_{50}) producing 50% of death cells in the culture.

DNA Synthesis Inhibition Assays—The L1210 cells in exponential growth were incubated for 1 h at 37 °C in growth medium containing various doses of the hybrid compounds. The cells were incubated for 15 h at 37 °C in growth medium containing 10 μCi/mL of [^{3}H]thymidine (43 Ci/mM, CEA). The radioactive medium was removed, and the cells were washed twice in saline buffer and allowed to swell for 10 min in ice in 1 mL of hypotonic buffer (TNE: 0.01 M Tris-HCl, pH 8.1; 0.05 M NaCl; 0.001 M EDTA). The cells were then disrupted by congelation-decongelation (three times) and digested by proteinase K (100 μg/mL, 4 h at 37 °C). The TCA that precipitated was collected on a filter and counted in a liquid scintillation counter. For each compound, we determined the in vitro inhibitory dose (I_{DNA50}) that reduced DNA synthesis by 50%.

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