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Synthesis, DNA-Binding Properties, and Antitumor Activity of Novel Distamycin Derivatives

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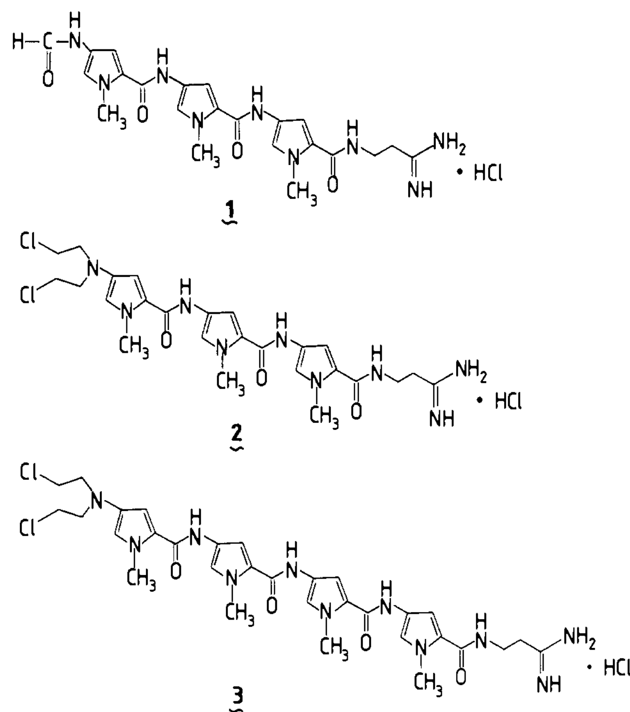
A group of potential alkylating agents have been synthesized that are structurally related to the oligopeptide antiviral antibiotic distamycin. All derivatives form complexes with native calf-thymus DNA but compounds **2**, **3**, and **6** give rise to covalent adducts. Cytostatic activity against both human and murine tumor cell lines in vitro is displayed by the new compounds. Compounds **3** and **4** are active on melphalan-resistant L1210 leukemia in mice.

Compounds endowed with the property of interacting directly with the nucleic acids are of great importance in cancer chemotherapy.[1] They may be divided into (a) compounds that are able to bind covalently to cell DNA (such as the alkylating agents), (b) agents that cause the breakage of the DNA molecule (such as the bleomycins and streptonigrin), and (c) drugs that bind reversibly to double-helical B DNA either by intercalation (such as the anthracyclines and the acridines) or by an external binding mode in the minor groove (such as the distamycins and netropsin).[2] Also in the case of reversible complexing agents, however, chemical reaction leading to irreversible damage of the DNA has been considered as the basis of the high antiproliferative effect of the same.[3] It appears therefore that the final effect exerted by drugs at the DNA level may be the result of different mechanisms related with the target recognition properties and the chemical reactivity of the different compounds. Distamycin (distamycin A, stallimycin, 1), an antiviral compound originally isolated from the cultures of *Streptomyces distallicus*,[4] is endowed with high affinity for AT-rich sequences of B DNA and the corresponding drug–DNA complex has been the object of extended studies.[5] We wish to report the synthesis and some properties of distamycin derivatives and analogues containing a chemically reactive appendage at the N-terminus. The compounds **2**–**6** represent a novel series of potential anticancer agents as it is shown by their ability to inhibit tumor cell proliferation in vitro and in vivo.



Compound **2** was obtained from *N*-deformyldistamycin (**7**), which was prepared from distamycin according to the already reported procedure.[6] Compound **7** was converted to **2** by reaction with ethylene oxide to give *N*,*N*-bis(2-hydroxyethyl) derivative **8**, which gave the corresponding *N*,*N*-bis(2-chloroethyl) derivative when treated with mesyl chloride in pyridine (Scheme I). Synthesis of **3** was similarly accomplished with **9a** as the starting material (ref 7). Compounds **4** and **5** were obtained from **7** with respectively **12** and **13a** as acylating agents. Analogue **6** was synthesized from **9b** (ref 7), which was reduced to the amine **10b** and converted to the *N*,*N*-bis(2-chloroethyl) derivative according to the procedure shown in Scheme

(1) Neidle, S., Waring, M. J., Eds. *Molecular Aspects of Anticancer Drug Action*; Macmillan: London, 1983.
(2) Waring, M. J. *Drugs Exp. Clin. Res.* **1986**, *12*, 441.
(3) Zwelling, L. A. *Cancer Metastasis Rev.* **1985**, *4*, 263.
(4) Arcamone, F.; Penco, S.; Orezzi, P. G.; Nicolella, V.; Pirelli, A. *Nature* **1964**, *203*, 1064.
(5) For a comprehensive review on the structure and physicochemical properties of the distamycin–DNA complex and the applications of the antibiotic in molecular biology and in genetic analysis, see: Zimmer, Ch.; Waehnert, V. *Prog. Biophys. Mol. Biol.* **1986**, *47*, 31.
(6) Arcamone, F.; Orezzi, P. G.; Barbieri, W.; Nicolella, V.; Penco, S. *Gazz. Chim. Ital.* **1967**, *97*, 1097.
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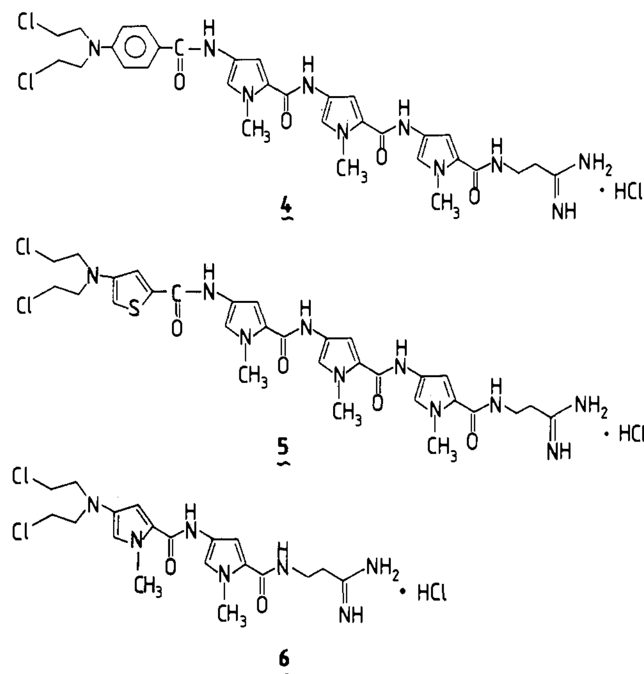
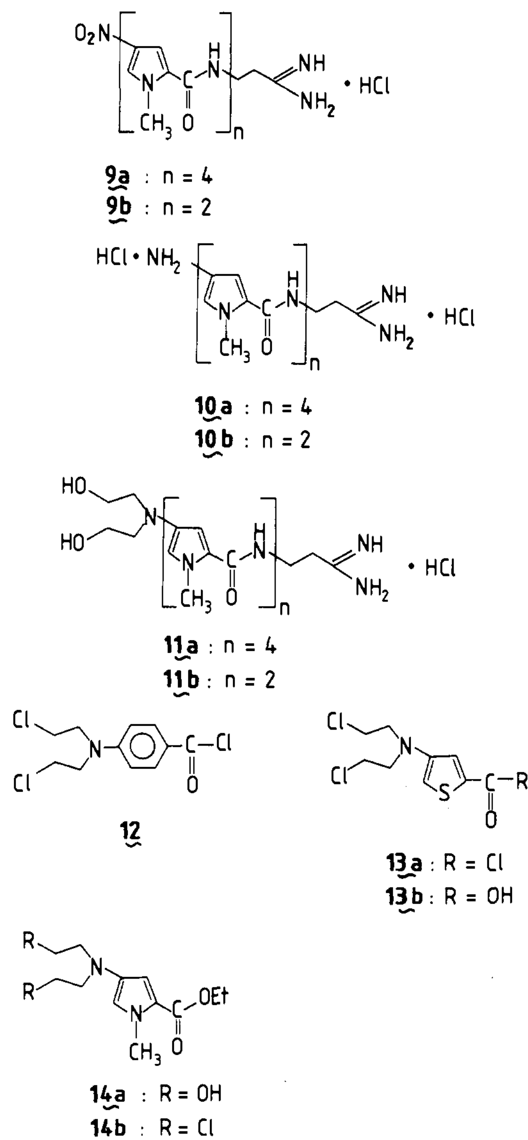


I. The monopyrrole analogue **14b** was also synthesized by using a similar reaction sequence starting from the corresponding amino ester.[8]



Scheme I

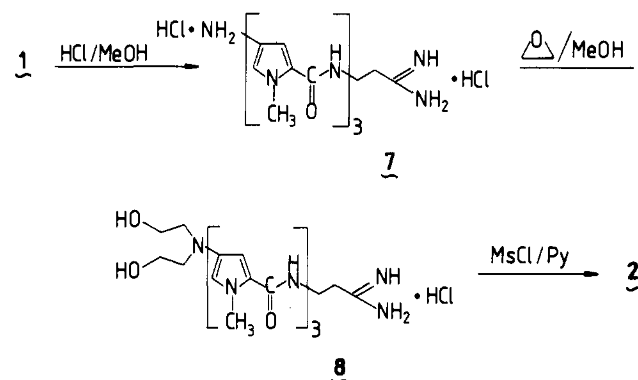


Table I. Circular Dichroism[a] Induced by Calf Thymus DNA[b] on Compounds 1–6

no.	ΔA before dilution	after DMF treatment[c] ΔA	after DMF treatment[c] undissociated fraction,[d] %	after SDS treatment[c] ΔA	after SDS treatment[c] undissociated fraction,[d] %
1	24.68	0	0	0.87	7
2	11.12	2.94	52.8	4.70	85
3	24.75	6.44	52.0	9.70	78
4	25.18	0.06	0.5	0	0
5	17.32	0.97	11.2	1.09	12.7
6	3.68	0.78	42.7	1.42	77

[a] $A \times 10^{-4}$ measured at 344–355 nm. [b] Concentration of DNA was 2.6×10^{-4} M (as DNA-P); total drug concentration was $(5.2 \pm 1) \times 10^{-5}$ M. [c] Drug and DNA concentrations were halved during these treatments. [d] Undissociated fraction was calculated by $(2\Delta A^* \times 100/\Delta A)$, where ΔA is the starting CD absorbance of the complexes and ΔA^* is a CD absorbance of the complexes after dilution with dimethylformamide (DMF) or sodium dodecyl sulfate (SDS).

On the basis of their structural relationship with 1, compounds 2–6 were all considered as potential DNA-binding agents. In order to evaluate their binding, experiments were performed at constant drug and DNA concentrations. The induced Cotton effect directly reflects bound drug molecules and is therefore suitable for quantitative determination of bound drug (Table I). Compounds 1 and 2, having the same chromophoric moiety, should have comparable chiroptical properties in DNA-bound form. The circular dichroism of 1 in the presence of DNA is twice that of 2, suggesting a comparatively higher DNA affinity. In order to ascertain whether binding of compounds bearing the *N,N*-bis(2-chloroethyl) group was reversible or not, solutions containing the drugs were exhaustively dialyzed against buffer. In these conditions the different behavior of 2 and 3 with respect to 1 (Figure 1) suggests that 2 and 3 bind to DNA in a different manner most probably with formation of an irreversible adduct. On the contrary, derivative 5 was, at least in part, in dialyzable form, and 4 appeared to be washed out from the dialysis bag as rapidly as the reversibly binding 1.

In addition, the dissociation of reversible drug–DNA complexes is obtained in the presence of dimethylformamide (DMF) or sodium dodecyl sulfate (SDS).[9] The solutions of the complexes of compounds 1–6 with calf thymus DNA were kept at room temperature for 24 h and then treated with an equal volume of DMF or SDS (2% in Tris buffer), and the residual dichroic band of the solution was measured. The amounts of undissociated complex (Table I) expressed as the percent of the total

(8) Grehn, L.; Ragnarsson, V. *J. Org. Chem.* 1981, *46*, 3492.
(9) Fox, K. R.; Brasset, C.; Waring, M. J. *Biochim. Biophys. Acta* 1985, *840*, 383.

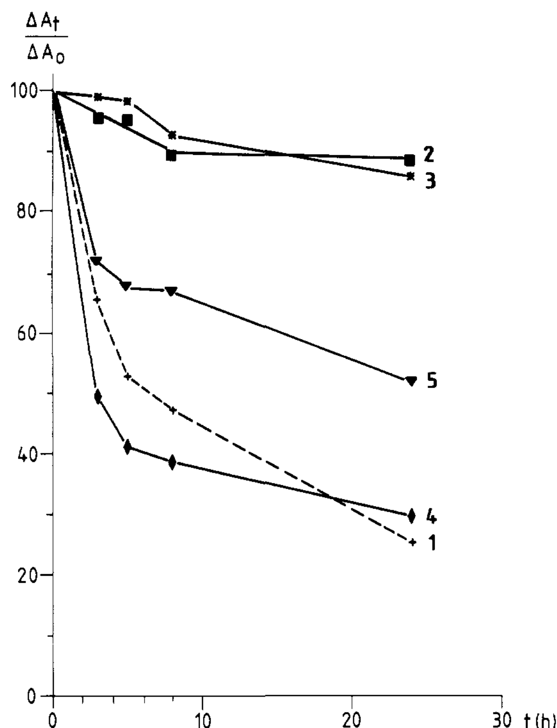


Figure 1. Dialysis of drug–DNA complexes. Solutions of the drug, 10^{-5} M, and calf thumus DNA, 10^{-4} M, in pH 7, 0.05 M Tris buffer + 0.15 M NaCl and 1 mM EDTA were dialyzed against buffer solution. The ratio of CD absorption at time *t* over that at time zero is plotted vs time.

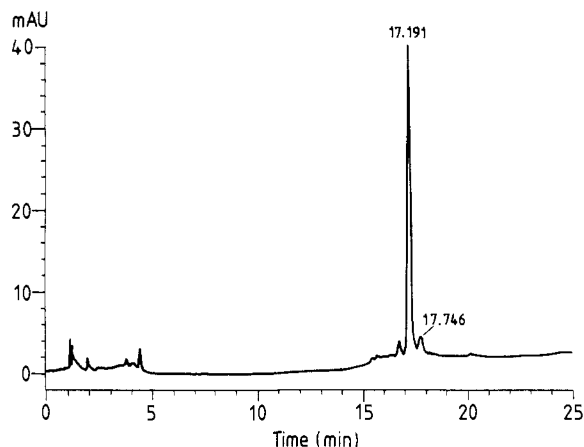


Figure 2. HPLC analysis of the butanol extract from 2–DNA (calf thymus) adduct. Retention time of 2 is 26.9 min. The peak at 17.746 corresponds to traces of compound 8.

complexed drug before treatment was high for compounds 2, 3, and 6, but low for 1, 4, and 5. The underestimation of the undissociated fraction resulting from the DMF treatment may be attributed to the denaturing effect of this reagent. With use of poly[d(A-T)] in place of calf thymus DNA and a prolonged reaction time (24 h), 2 and 3 showed a residual 65% of CD absorption after dilution with DMF, in contrast, for 2, with previous determinations performed after a short period of incubation.[10] Under the same conditions, 6 showed 32% of residual CD absorption, whereas the band of the complex formed by 4 and 5 completely disappeared when DMF was added to the solution.

The "irreversible" interaction of nitrogen mustards 2, 3, and 6 with DNA was further investigated. As was the case with CC-1065, an antibiotic known to bind covalently

(10) Arcamone, F.; Animati, F.; Menozzi, M.; Mongelli, N.; Penco, S.; Valentini, L. *Chim. Ind.* (*Milan*) **1986**, *68*, 63.

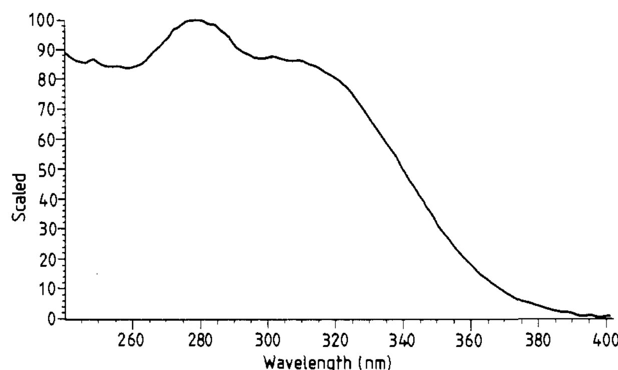


Figure 3. Ultraviolet spectrum of HPLC peak at 17.191 min from the butanol extract of 2–DNA (calf thymus) adduct.

Table II. In Vitro Activity of Distamycin Derivatives and Melphalan (L-PAM) on Different Cell Lines

	ID_{50},[a] M × 10^{-6}				
compd	HeLa[b]	Hep-2[c]	L1210[c]	L1210/LPAM[c]	RI[d]
distamycin	48	97	488	343	0.7
2	1.95	1.22	3.53	4.56	1.30
3	0.06	0.1	0.08	0.07	0.87
4	0.02	0.19	1.22	0.36	0.30
5	0.05	0.09	1.93	1.32	0.69
13b	1.6	1.25	10	21	2.1
14b	0.18	6.25	0.2	0.84	4.1
L-PAM	1.77	0.98	1.7	13.1	7.7

[a] ID_{50} (dose inhibiting cell proliferation by 50%). [b] Cytotoxicity evaluated as inhibition of colony growth after 24-h treatment. [c] Cytotoxicity evaluated as number of viable cells after 72-h treatment (Hep-2) or 4-H treatment and 48-h incubation in drug-free medium (L1210-L1210/L-PAM). [d] RI (resistance index ratio): ID_{50} (L1210/L-PAM)/(L1210). Culture conditions: all cell lines were maintained in vitro at 37 °C in a humidified atmosphere of 5% CO_2. Growth medium was MEM (HeLa, Hep-2) or RPMI 1640 (L1210, L1210/L-PAM). Medium was supplemented with 10% fetal calf serum, 1% glutamine solution (200 mM), and antibiotics (100 μg/mL^{-1} streptomycin and 100 units mL^{-1} penicillin).

in the minor groove of B-form DNA to AT-rich regions,[11] 1-butanol extraction of 2–calf thymus DNA adducts at 100 °C allowed the separation, among others, of a main fraction differing from 2 and 8 in the reverse-phase HPLC behavior (Figure 2) and showing the ultraviolet spectrum presented in Figure 3. The molecular weight of the compound at retention time 17.191 min was determined by LC/MS to be 658 mass units, which corresponds to structure **15**. The studies related to the separation and structure determination of the addition product will be reported elsewhere.[12] The same retention time and electronic spectrum were exhibited by the degradation product present in the butanolic extract of the 2–poly[d(A-T)] adduct. When the hot butanol extraction procedure was performed on the 3–DNA (calf thymus) adduct, a similar main peak with a retention time of 14.9 min was isolated (standard 3 was eluted at 26.7 min), but only the starting compound (retention time 26.0 min) was found in the butanol extract of the solution containing the 5–DNA (calf thymus) complex.

Compounds 2–6 as well as 1 and nitrogen mustards **13b** and **14b**, and clinically useful *p*-[*N*,*N*-bis(2-chloroethyl)-amino]phenylalanine (melphalan, L-PAM) were tested

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(12) Gioia, B.; Arlandini, E.; Borghi, D.; Configliacchi, E.; Marazzi, G.; Menozzi, M.; Mongelli, N.; Arcamone, F. M. 11th International Conference of Mass Spectrometry, Bordeaux, France, October 29–November 2, 1988.

Table III. In Vivo Activity of Distamycin Derivatives

	L1210		L1210/L-PAM	
compd	OD,[a] mg/kg	T/C,[b] %	OD, mg/kg	T/C, %
distamycin	200.00	113	200.00	106
2	1.56	138	1.56	122
3	0.39	188	0.39	167
4	3.12	175	3.12	144
5	1.56	132	1.56	111
L-PAM	10.00	192	10.00	104

[a] OD optimal nontoxic dose < LD_{10} (lethal dose 10%). [b] T/C % = median survival time of treated mice/median survival time of controls × 100. Evaluation of activity: compounds are considered active if T/C % values are ≥125. Experimental conditions: cells were inoculated intraperitoneally in CDF_1 mice (10^5 cells/mouse). Treatment was performed intraperitoneally after tumor transplant.

against different tumor cell cultures in vitro. All compounds were distinctly more cytotoxic than 1, with those containing the complete tris(1-methylpyrrolecarboxamide) structural system, that is, three pyrrole units and four carboxamido groups, being clearly the most potent (Table II). Interestingly, 3, 4, and 5 were also active against the melphalan-resistant line of L1210 murine leukemia. Although chemical reactivity with DNA in solution cannot be correlated with cytotoxicity, it appears clearly that the bioactivity of 3, 4, and 5 is due to both the alkylating group and to the DNA-recognizing oligo(*N*-methylpyrrolecarboxamide) moiety. In fact, the compounds inhibit cell viability much more effectively than 1 and their *N*-formyl congeners,[13] and are generally 1 order of magnitude more potent (and even more in the case of 3) when compared with the simple nitrogen mustards **14b** and 18 and with melphalan itself. The results of these studies would therefore suggest that specific association with AT-rich regions of the genome might be involved in the mechanism of action of these new agents whose antitumor activity is also demonstrable in vivo as is shown in Table III. In fact, compounds 2–5 were active against L1210 leukemia in the mouse, 3 and 4 being also effective against the melphalan-resistant disease.

Experimental Section

Synthesis. Melting points were taken with a Büchi apparatus and are uncorrected. Ultraviolet spectra were taken with a C.E. Spectracomp 601. Mass spectra were determined with a Varian-MAT 311A spectrometer, and unless specified otherwise, the field desorption technique was used. High-resolution mass spectra were determined on a DG7070 instrument with use of the positive ion FAB technique. ^1H NMR spectra were determined with a Bruker WP 80 or a Varian XL 200 instrument in DMSO-d_6 unless specified otherwise. Infrared spectra were recorded with a Perkin-Elmer 297 spectrometer.

Solvent evaporation was performed under reduced pressure. Kieselgel 60 (230–400 mesh, Merck) and neutral aluminum oxide (C. Erba) were used for flash chromatography. Thin-layer chromatography on silica gel 60 F254 and alumina F254 type E (Merck) was used for identification purposes and for homogeneity tests.

3-[1-Methyl-4-[1-methyl-4-[1-methyl-4-[*N*,*N*-bis(2-chloroethyl)amino]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine Hydrochloride (2). Cold ethylene oxide (20 mL) was added to a cooled (−10 °C) and stirred solution of 2 g (3.8 mmol) of deformyldistamycin dihydrochloride (7) (ref 6) in methanol (100 mL). The reaction flask was sealed and allowed to reach room temperature overnight. Methanol and excess ethylene oxide were removed by evaporation. The residue was chromatographed on acid-washed silica gel with chloroform/methanol (7:3 v/v) as the eluant to give 1.52 g (2.3 mmol, 60% yield) of 8: UV λ_{max} (95% EtOH) (ϵ) 244 (24140), 306 (27142) nm; MS, *m/z* 542 ($M^+ + 1$), 524 ($M^+ - NH_3$), 471 ($M^+ + 1 - CH_2CH_2C(NH)NH_2$); NMR δ 2.63 (2 H, t), 3.00–3.30 (4 H, m), 3.3–3.7 (6 H, m), 4.6 (2 H, t), 6.25–7.25 (6 H, m), 7.20 (1 H, t), 7.62 (2 H, s), 7.95 (2 H, s), 9.62 (1 H, s), 9.86 (1 H, s). Anal. ($C_{25}H_{26}ClN_9O_5$) C, H, N, Cl. This material (0.68 g, 1.11 mmol) was dissolved in pyridine (7 mL), cooled to 0–5 °C, and treated with 0.21 mL of methanesulfonyl chloride in 2 mL of pyridine. After 1 h methanol (7 mL) was added and the reaction mixture warmed to room temperature. Solvent was evaporated and the residue chromatographed on silica gel with chloroform/methanol (6:4 v/v) as the eluant. 2: yield 0.31 g (0.48 mmol) (43%); UV λ_{max} (95% EtOH) (ϵ) 244 (24568), 313 (28046) nm; MS; *m/z* 578 ($M^+ + 1$), 559 ($M^+ - NH_4^+$), 505 ($M^+ - CHCl$), 452 ($M^+ + 1 - 2CH_2CH_2Cl$); MS, *m/z* for $C_{25}H_{34}Cl_2N_9O_3$ 578.2151 ($M^+ + 1$); NMR 2.64 (2 H, t), 3.2–3.8 (10 H, m), 6.40–7.25 (6 H, m), 8.20 (1 H, t), 8.62 (2 H, s), 8.90 (2 H, s), 9.78 (2 H, s), 9.88 (1 H, s).

3-[1-Methyl-4-[1-methyl-4-[1-methyl-4-[1-methyl-4-[*N*,*N*-bis(2-chloroethyl)amino]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine Hydrochloride (3). Starting from 3 g (4.68 mmol) of **9a**, which was reduced to **10a** (ref 7) and treated with ethylene oxide as described above, 1.39 g (1.99 mmol) of **11a** was obtained (yield 42%): NMR δ 2.67 (2 H, t), 3.00–3.70 (10 H, m), 3.79 and 3.81 (3 H, s), 3.85 (6 H, s), 4.65 (2 H, dd), 6.30–7.25 (8 H, m), 8.26 (1 H, t), 8.92 and 9.18 (2 H, dd), 9.72 (1 H, s), 9.81 (2 H, s). Treatment of **11a** with methanesulfonyl chloride as described for 2 gave 0.33 g (0.45 mmol, 23%) of 3: UV λ_{max} (ϵ) 244 (29741) and 316 (39729) nm; NMR δ 2.63 (2 H, t), 3.20–3.90 (10 H, m), 3.80, 3.84, and 3.85 (3 H, s), 6.46 and 6.58 (1 H, d), 6.90–7.30 (6 H, m), 8.20 (1 H, t), 8.73 and 9.00 (2 H, dd), 9.70 (1 H, s), 9.90 (2 H, s); MS, *m/z* for $C_{31}H_{40}Cl_2N_{11}O_4$ 700.2623 ($M^+ + 1$).

3-[1-Methyl-4-[1-methyl-4-[1-methyl-4-[4-[*N*,*N*-bis(2-chloroethyl)amino]benzenecarboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]-propionamidine Hydrochloride (4). A solution of 0.195 g of sodium bicarbonate in 3 mL of water was added to a cooled solution of 0.4 g (0.76 mmol) of deformyldistamycin (7) in 21 mL of ethanol. To this mixture was added dropwise a solution of 0.32 g (1.14 mmol) of 4-[*N*,*N*-bis(2-chloroethyl)amino]benzoyl chloride[14] in 3 mL of benzene. The mixture was stirred for 3 h at 5 °C and then for 12 h at room temperature. Evaporation gave a solid from which after chromatography (silica gel, chloroform/methanol 9:1 and 7:3 v/v) 4 was obtained (0.22 g, 0.30 mmol, 39% yield): mp 295 °C dec (from iPrOH/Et_2O); MS, *m/z* 697 (MH^+), 679 ($M^+ - NH_3$); NMR (200 MHz) δ 2.64 (2 H, t), 3.51 (2 H, m), 3.78 (8 H, s), 3.81, 3.84, and 3.85 (9 H, s), 6.94, 7.05, 7.07, 7.15, 7.20, 7.26 (6 H, d), 6.82, 7.85 (4 H, 2 d), 8.11 (1 H, t), 8.61 and 8.94 (4 H, b), 9.79 and 9.82 (2 H, s), 9.91 (1 H, s). Anal. ($C_{32}H_{45}Cl_3N_{10}O_7$) C, H, N, Cl.

4-[*N*,*N*-Bis(2-chloroethyl)amino]thiophene-2-carboxylic Acid (13b). A solution of methyl 4-nitrothiophene-2-carboxylate (1.75 g, 9.36 mmol) in 18 mL of methanol was saturated at 0 °C with dry HCl, and with maintainance of the gas stream, 4.35 g of zinc in lumps was added in 30 min with external cooling. The mixture was stirred at room temperature until the zinc was consumed and evaporated. The residue was dissolved in 10% aqueous HCl, extracted with methylene chloride in order to recover unreacted starting material (0.45 g, 2.41 mmol), then cooled, basified with concentrated ammonium hydroxide, and extracted with toluene. Evaporation of the organic solvent gave 0.9 g (5.73 mmol, 83% yield) of methyl 4-aminothiophene-2-carboxylate: mp 81–82 °C after recrystallization from $(iPr)_2O$: NMR (200 MHz) δ 3.74 (3 H, s), 5.04 (2 H, b, NH_2), 6.35 (1 H, d), 7.20 (1 H, d); EI-MS, *m/z* 157 ($M^{\cdot+}$). This material (0.9 g, 5.73 mmol) in 12.5 mL of 40% aqueous AcOH was treated with cold ethylene oxide (4 g) at 5 °C with stirring. After one night at room temperature in a sealed flask, the mixture was concentrated to a small volume, diluted with water (25 mL), and extracted with ethyl acetate in the presence of sodium bicarbonate. Evaporation of the organic solvent gave 1.3 g (5.31 mmol, 93%) of methyl 4-[*N*,*N*-bis(2-

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(14) Elderfield, R. C.; Liao, T. K. *J. Org. Chem.* **1961**, *26*, 4996.

hydroxyethyl)amino]thiophene-2-carboxylate as a light brown oil, which solidified on standing: mp 68–69 °C (from EtAc/Hexane); EI-MS, m/z 245 (M^+), 214, 170. The solution of this material (1.3 g, 5.31 mmol) in 1.96 mL of phosphorus oxychloride was refluxed for 45 min. After evaporation the residue was treated with 7.75 mL of concentrated HCl at 100 °C for 3 h. After cooling, 21 mL of cold water was added and the resulting solution extracted with EtOAc. Evaporation of the extract and chromatography of the residue (silica gel, EtOAc/MeOH) afforded **13b** (0.49 g, 1.83 mmol, 34%): mp 135–137 °C (from benzene/hexane 6:4 v/v); EI-MS, m/z 267 (M^+), 218, 57; NMR ($CDCl_3$) δ 3.63 (8 H, s), 6.30 (1 H, d), 7.45 (1 H, d), 9.50 (1 H, b). Anal. ($C_9H_4Cl_2NO_2S$) C, H, N, Cl.

3-[1-Methyl-4-[1-methyl-4-[1-methyl-4-[4-[*N*,*N*-bis(2-chloroethyl)amino]thiophene-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine Hydrochloride (5). Triethylamine (0.1 mL) in DMF (0.5 mL) was added to a solution of 0.45 g (0.68 mmol) of **7** in DMF (4.5 mL) at room temperature and under N_2. The mixture was treated with 0.23 g (0.86 mmol) of **13b** and 0.77 g of dicyclohexylcarbodiimide in small portions and stirred overnight. Filtration and evaporation gave a residue that was chromatographed (silica gel, EtOAc/EtOH 9:1 and 8:2 v/v) to afford **5** (0.22 g, 0.30 mmol, 34% yield: mp 199–204 °C (from iPrOH); MS, m/z 703 (MH^+); NMR (200 MHz) δ 2.57 (2 H, m), 3.47 (2 H, m), 3.6–3.8 (8 H, m), 3.79, 3.83, and 3.85 (9 H, s), 6.92, 7.04, 7.07, 7.18, and 7.24 (6 H, d), 6.44 and 7.67 (2 H, 2 d), 8.24 (1 H, t), 8.9–9.3 (4 H, b), 9.94 and 9.98 (2 H, s), 10.28 (1 H, b). Anal. $C_{30}H_{36}Cl_2N_{10}O_4S \cdot HCl \cdot 1.5H_2O$) C, H, N, Cl.

3-[1-Methyl-4-[1-methyl-4-[*N*,*N*-bis(2-chloroethyl)-amino]pyrrole-2-carboxamido]pyrrole-2-carboxamido]-propionamidine Hydrochloride (6). Compound **9b** (900 mg, 2.26 mmol) was converted to **10b** (ref 7), which was treated with ethylene oxide as described above for the synthesis of **2** to give 800 mg (1.76 mmol, 78% yield) of **11b**: UV λ_{max} (95% EtOH) (ϵ) 245 (16 352), 292 (15 070) nm; MS, m/z 419 (M^+), 420 (MH^+); MS, m/z for $C_{19}H_{28}Cl_2N_7O_2$ 456.1680 ($M^+ + 1$); NMR δ 2.63 (2 H, t), 2.90–3.80 (10 H, m), 4.55 (2 H, b), 6.30 (1 H, d), 6.52 (1 H, d), 6.92 (1 H, d), 7.12 (1 H, d), 8.20 (1 H, t), 8.70 (2 H, bs), 9.01 (2 H, bs), 9.63 (1 H, s). This material (717 mg, 1.57 mmol) was treated with mesyl chloride to give 440 mg (0.89 mmol, 57% yield) of **6**: mp 140 °C dec; UV λ_{max} (95% EtOH) (ϵ) 245 (17 373), 293 (15 450); MS, m/z 456 (MH^+), 438 ($M^+ - NH_3$); NMR δ 2.63 (2 H, t), 3.30–3.80 (10 H, m), 3.78 (3 H, s), 3.81 (3 H, s), 6.42 (1 H, d), 6.55 (1 H, d), 6.92 (1 H, d), 7.17 (1 H, d), 8.20 (1 H, t), 8.70 (2 H, s), 9.02 (2 H, b), 9.68 (1 H, s).

Ethyl 1-Methyl-4-[*N*,*N*-bis(2-chloroethyl)amino]-pyrrole-2-carboxylate (14b). A solution of ethyl 1-methyl-4-aminopyrrole-2-carboxylate hydrochloride[15] (500 mg, 2.44 mmol) in MeOH (50 mL) was cooled to −20 °C and treated with ethylene oxide (12 mL). The reaction flask was sealed and the mixture allowed to stand at room temperature overnight. Evaporation gave a residue, which was taken up in EtOH containing 10% HCl. The solution was evaporated and the residue crystallized from EtAc to give 410 mg (1.40 mmol, 57% yield) of **14a**: mp 88–90 °C; NMR δ 1.29 (3 H, t), 3.52 (8 H, m), 3.88 (3 H, s), 4.22 (2 H, q), 7.03 (1 H, d), 7.34 (1 H, d). This material (713 mg, 2.44 mmol) in dry pyridine (7 mL) was cooled in an ice bath and treated, under N_2, with a 1.27 M solution of mesyl chloride in pyridine (9.6 mL). The mixture was allowed to warm to room temperature and, after 15 min, heated at 70 °C for 1 h. The resulting brown solution was poured in 150 mL of 2 N HCl and extracted with EtOAc. The organic layer was washed with aqueous $NaHCO_3$ followed by brine and then dried over sodium sulfate and evaporated. The residue was chromatographed (silica gel, benzene/hexane 6:4 v/v) to give **14b** as a wax (120 mg, 0.41 mmol, 17% yield): UV λ_{max} (95% EtOH) (ϵ) 250 (12 695), 329 (4662) nm; NMR δ 1.27 (3 H, t), 3.2–3.7 (8 H, m), 3.77 (3 H, s), 4.16 (2 H, q), 6.32 (1 H, d), 6.63 (1 H, d). Anal. ($C_{12}H_{18}O_2N_2Cl_2$) C, H.

DNA Complexes. Circular dichroism spectra typical of drug–DNA complexes were determined with a Jobin Yvon dichrograph. Optical density of all samples was maintained in the range 0.5–1.5 absorption units at the wavelength of maximal absorption. A values were measured at the wavelength corresponding to the CD maximum. Apparent values of ϵ were obtained as the ratio between the ΔA value and the total drug concentration. Calf thymus (Sigma) and poly[d(A-T)] (Boehringer Mannheim) were used. The buffer was pH 7, 0.05 M Tris + 0.15 M NaCl + 0.001 M EDTA. Dialysis experiments were performed with predialyzed DNA and dialysis tubing (Thomas), each tube containing 3 mL of sample solution. The magnetically stirred external compartment was represented by 3 L of buffer solution. All experiments were performed in duplicate.

Adduct Formation, Degradation, and Analysis. Samples (5 mL) of solutions containing the drug (10^{-4} M) and DNA (7.10^{-4} M) were left at room temperature for 24 h and then heated with an equal volume of 1-butanol at 100 °C in an oil bath under reflux for 30 min. The samples were centrifuged, and the organic layer was analyzed by high-performance liquid chromatography (HPLC). For HPLC analysis a Varian LC 5020 apparatus equipped with a Rheodyne Model 7120 injection valve, a spectrophotometric monitor HP 1040 M controlled by HP 9000 series 300, data treatment system HP 79994 A on a HP 310 (1MB) computer, Winchester disk HP 3153A, printer, and two-pen HP 7470A graphic plotter was used. The column was filled with Novapak-C18 (spherical, 4 μm; 3.9 mm × 15 cm). The flow rate was 1 mL/min at t = 37 °C. Gradient elution was performed, starting with eluant A (0.006 M tetrapropylammonium bromide in pH 4.5, 0.05 M KH_2PO_4) and adding eluant B (same as A but containing 40% MeOH by volume) at 10 min (50% of B) and at 12 min (100% of B). For the analysis of compounds **3** and **4**, tetraethylammonium bromide was used as the ion-exchange salt.

Biology. In Vitro Activity. The compounds were tested in vitro on different tumor cell lines (HeLa, human cervix hepithelioid carcinoma;[16] Hep-2, human larinx carcinoma;[17] L1210 murine leukemia and its melphalan-resistant sublime L1210/L-PAM[18]). Cytotoxic activity is reported in Table II: all distamycin-derived compounds demonstrate a remarkably increased potency when compared to distamycin. Moreover, when compared with melphalan, the same cytotoxic activity is observed both on L1210 and L1210/L-PAM.

In Vivo Activity. The antitumor activity of the compounds was tested in vivo on L1210 and L1210/L-PAM murine leukemias implanted intraperitoneally in CDF_1 mice. Results are reported in Table III. A good antileukemic effect is observed with compounds **3** and **4** on L1210 and, to a slightly lower degree, on L1210/L-PAM. Compounds **2** and **5** show a borderline activity on L1210 and are inactive on L1210/L-PAM.[19]

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Registry No. **2**·HCl, 118438-44-1; **2** (free base), 109858-32-4; **3**·HCl, 107580-27-8; **3** (free base), 107561-29-5; **4**·HCl, 118438-45-2; **4** (free base), 115308-98-0; **5**·HCl, 118438-46-3; **5** (free base), 115308-97-9; **6**·HCl, 107580-26-7; **6** (free base), 107561-77-3; **7**, 17165-10-5; **8**, 118438-47-4; **9a**, 23999-82-8; **9b**, 14555-80-7; **10a**, 118438-48-5; **10b**, 14555-90-9; **11a**, 118438-49-6; **11b**, 107580-25-6; **12**, 15944-88-4; **13b**, 115309-00-7; **14a**, 118438-52-1; **14b**, 118438-53-2; methyl 4-nitrothiophene-2-carboxylate, 24647-78-7; methyl 4-aminothiophene-2-carboxylate, 89499-43-4; methyl 4-[*N*,*N*-bis(2-hydroxyethyl)amino]thiophene-2-carboxylate, 118438-50-9; ethyl 1-methyl-4-aminopyrrole-2-carboxylate hydrochloride, 118438-51-0.

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