experiment. ED_{50} values were determined from individual plots.

Isolated Fat Cells. Epididymal fat tissue obtained from nonfasted male Sprague–Dawley rats weighing 200–250 g was used. Fat cells were isolated by the method of Rodbell[14] after digestion of adipose tissue with crude collagenase (Worthington) in a Krebs bicarbonate buffer containing 3% bovine serum albumin.

Incubation mixtures contained 0.2 ml of fat cell suspension, test drug, and Krebs bicarbonate–albumin solution in a total volume of 2.5 ml. Drugs were tested in the concentration range of 1×10^{-8}–3×10^{-4} M. Flasks were incubated in air at 37° for 1 h. All reactions were terminated by the addition of an equal volume of 10% TCA and the amount of glycerol released was measured by procedures described previously.[15,16]

In each experiment, a maximal release of glycerol was obtained in the presence of 10^{-6} M isoproterenol and this maximal figure was used to calculate the dose–response relationships obtained in this study.

Drugs. All drugs were prepared in normal saline containing 0.05% sodium metabisulfite.

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Synthesis and Some Properties and Antitumor Effects of the Actinomycin Lactam Analog, [Di(1'-L-α,β-diaminopropionic acid)]actinomycin D[1]

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A lactam analog of actinomycin D (AMD) has been synthesized as a potential antitumor chemotherapeutic agent. Both L-threonine residues were replaced by L-α,β-diaminopropionic acid. Starting with N^{α}-benzyloxycarbonyl-N^{β}-*tert*-butyloxycarbonyl-L-α,β-diaminopropionic acid methyl ester hydrochloride the linear intermediate N^{α}-benzyloxycarbonyl-N^{β}-(*tert*-butyloxycarbonylsarcosyl-L-*N*-methylvalyl)-L-α,β-diaminopropionyl-D-valyl-L-proline *p*-nitrophenyl ester was prepared by conventional methods of peptide synthesis in solution. Selective cleavage of the N^{β}-*tert*-butyloxycarbonyl group and lactam formation afforded the desired cyclic pentapeptide derivative. The chromophore precursor, N^{α}-(2-nitro-3-benzyloxy-4-methylbenzoyl) substituent, was introduced *via* its symmetric anhydride. Catalytic reduction followed by ferricyanide-mediated phenoxazinone formation provided the lactam analog, [di(1'-L-α,β-diaminopropionic acid)]actinomycin D ($[Dpr^1]_2$-AMD). Its binding to natural and synthetic DNA and that of an analogous L-*threo*-α,β-diaminobutyric acid containing lactam ($[Dbu^1]_2$-AMD) compared with the binding of AMD (in which the peptides are in lactone form) was studied by circular dichroic (CD) spectroscopy. The visible and uv CD spectra of free AMD differed from those of the free lactam analogs, indicating that the asymmetric environment of the pentapeptide rings in the region of the chromophore differs in free actinomycin lactone and lactams. In the presence of calf thymus DNA, PM2 DNA, and the synthetic d(A-T)-like copolymers containing 2,6-diaminopurine (DAP), poly[d(DAP-T)], and poly[d(DAP-A-T)], the rotational strengths of the optically active transitions in the visible region of the actinomycins increased, and the CD spectra in the presence of the various DNA duplexes were qualitatively similar. The CD spectra of bound actinomycin lactams resembled the spectrum of bound AMD. This suggests that the lactone and lactam actinomycins acquire a similar environment when bound to DNA. $[Dpr^1]_2$-AMD was less cytotoxic than AMD in antibacterial assays but exhibited somewhat higher toxicity in mice than AMD. At optimal dose levels the lactam analog had little or no antitumor activity in three murine tumor systems.

Actinomycin D (AMD), Figure 1 (a), is a clinically used chemotherapeutic agent capable of effecting cures in treatment of gestational choriocarcinoma[3] and Wilms' tumor.[4] Although being one of the few antitumor drugs that exhibit curative effects in two different tumors (of comparatively infrequent occurrence), the overall spectrum of actinomycin antitumor activity in man remains narrow.[5,6] At present, clinical use extends to only a few other cancers as trophoblastic tumors,[7] rhabdomyosarcoma,[8] and melanoma.[9] Moreover, its high host toxicity[10] and difficult administration have not encouraged wider application. Thus, the search for modified actinomycins of a broader range of activity and/or improved therapeutic index remains to be a worthwhile effort.[11]

The well-known importance of the two peptide lactone moieties in actinomycin molecules for the manifestation

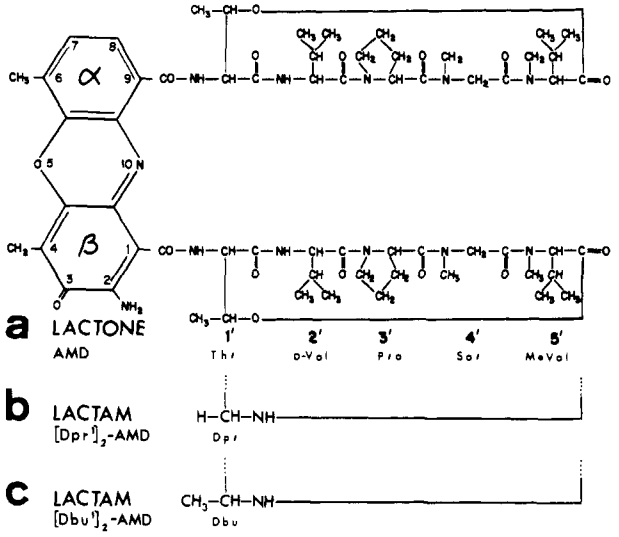


Figure 1. Structures of actinomycin and lactam analogs. (a) Actinomycin D (C_1) [H. Brockmann et al., *Angew. Chem.*, 68, 70 (1956)]; (b) [di(1'-L-α,β-diaminopropionic acid)]actinomycin D lactam analog, in which the threonine residues of both cyclic peptides have been replaced by Dpr; (c) [di(1'-L-*threo*-α,β-diaminobutyric acid)]-actinomycin D lactam analog (replacement of threonine residues by Dbu).

of high biological activity[12,13] (for a review, see ref 14) led us to investigate the effects of replacing the lactone functions by lactams on the therapeutic efficacy of AMD.[15] In this communication we report details of a synthesis and some biological and physicochemical properties of a lactam analog of AMD, [di(1'-L-α,β-diaminopropionic acid)]actinomycin D (**16**, $[Dpr^1]_2$-AMD). In this analog both L-threonine residues of AMD are substituted by L-α,β-diaminopropionic acid, Figure 1 (b).

The biological action of actinomycin D is derived from its ability to bind to DNA in a manner that selectively inhibits DNA-dependent RNA synthesis.[12] The interaction of $[Dpr^1]_2$-AMD (**16**) with natural and synthetic DNA duplexes was deconvoluted into five separate rate processes.[16] The rate constants of the three slower processes were lower than the corresponding rate constants for interaction of actinomycins D, X_2, and $X_{0\beta}$ with DNA, possibly reflecting conformational differences in the cyclic peptides of the actinomycin lactones and the $[Dpr^1]_2$-AMD lactam analog. To provide further characterization of the actinomycin–DNA complexes, in this communication we compare the circular dichroism (CD) spectra of the free and bound lactone (AMD) and lactam ($[Dpr^1]_2$-AMD) actinomycins. CD spectra of free and bound $[Dbu^1]_2$-AMD, a previously described synthetic lactam analog which contains L-*threo*-α,β-diaminobutyric acid in the 1' positions[17] [Figure 1 (c)], have been included for comparison.

The synthesis of the $[Dpr^1]_2$-AMD lactam analog **16** was conducted by conventional methods of peptide synthesis in solution. The required N^{α}-protected starting material, N^{α}-benzyloxycarbonyl-L-α,β-diaminopropionic acid methyl ester hydrochloride (**9**), was synthesized by two different approaches; see Scheme I. Via route A, the known L-α-benzyloxycarbonylamino-β-chloropropionic acid (**3**)[18] was obtained from L-serine methyl ester (**1**) by treatment with PCl_5–AcCl–HCl followed by acid saponification of **2**[19] and benzyloxycarbonylation. Treatment of **3** with concentrated ammonia resulted in conversion to N^{α}-benzyloxycarbonyl-L-α,β-diaminopropionic acid (**4**) which was esterified[20] to form **9**.

Scheme I. Preparation of N^{α}-Benzyloxycarbonyl-L-α,β-diaminopropionic Acid Methyl Ester Hydrochloride (**9**)

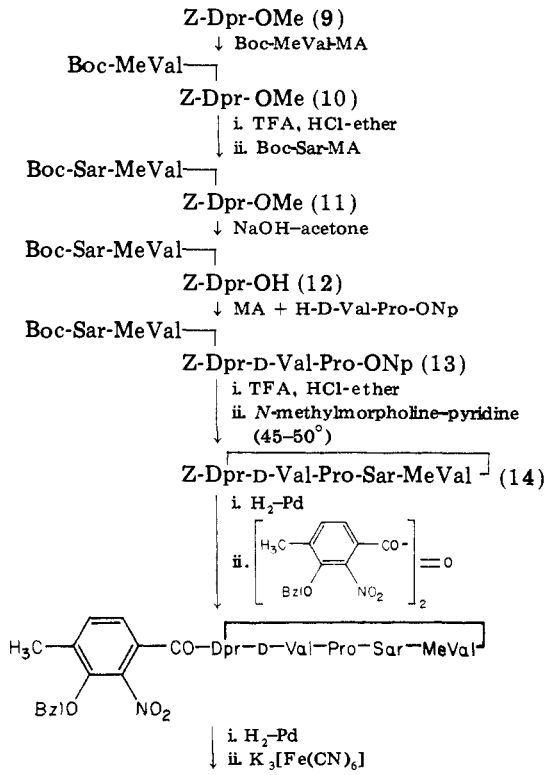
A	B
H-Ser-OMe·HCl	Tos-Asn-OH
↓	↓
H-Ala(Cl)-OMe·HCl (**1**)	Tos-Dpr-OH (**5**)
↓	↓
H-Ala(Cl)-OH·HCl (**2**)	Tos-Dpr(Boc)-OH (**6**)
↓	↓
Z-Ala(Cl)-OH (**3**)	Z-Dpr(Boc)-OH (**7**)
↓	↓
Z-Dpr-OH (**4**)	Z-Dpr(Boc)-OMe (**8**)
↘	↙
Z-Dpr-OMe·HCl (**9**)	

Low yields in several steps of route A led us to adopt route B (Scheme I), in which **9** was obtained through a series of four efficient processes starting from the known N^{α}-*p*-toluenesulfonyl-L-α,β-diaminopropionic acid[21] (**5**, derived from Tos-Asn-OH by Hofmann rearrangement). Compound **5** was converted to the N^{β}-*tert*-butyloxycarbonyl derivative **6** by the NaOH pH-stat method of Schnabel.[22] The N^{α}-*p*-toluenesulfonyl group was removed by sodium in liquid ammonia reduction[23] which was directly followed by benzyloxycarbonylation to afford N^{α}-benzyloxycarbonyl-N^{β}-*tert*-butyloxycarbonyl-L-α,β-diaminopropionic acid (**7**).[24] Attempted selective cleavage of the *tert*-butyloxycarbonyl group of **7** with trifluoroacetic acid (TFA) was accompanied by partial cleavage of the benzyloxycarbonyl moiety. Therefore, **7** was converted to N^{α}-benzyloxycarbonyl-N^{β}-*tert*-butyloxycarbonyl-L-α,β-diaminopropionic acid methyl ester (**8**) by reaction with diazomethane. Treatment of **8** with anhydrous TFA gave N^{α}-benzyloxycarbonyl-L-α,β-diaminopropionic acid methyl ester hydrochloride (**9**) without detectable cleavage of the benzyloxycarbonyl group.

[Di(1'-L-α,β-diaminopropionic acid)]actinomycin D (**16**) was synthesized according to Scheme II. *tert*-Butyloxycarbonyl-L-*N*-methylvaline was coupled to **9** by the mixed carbonic anhydride procedure[25] to yield N^{α}-benzyloxycarbonyl-N^{β}-(*tert*-butyloxycarbonyl-L-*N*-methylvalyl)-L-α,β-diaminopropionic acid methyl ester (**10**). Cleavage of the *tert*-butyloxycarbonyl group followed by mixed carbonic anhydride coupling yielded N^{α}-benzyloxycarbonyl-N^{β}-(*tert*-butyloxycarbonylsarcosyl-L-*N*-methylvalyl)-L-α,β-diaminopropionic acid methyl ester (**11**). Purification was achieved by Sephadex LH-20 chromatography in 95% ethanol. The methyl ester **11** was saponified with 1 M sodium hydroxide in acetone to yield the free acid **12**. *p*-Nitrophenyl-D-valyl-L-prolinate hydrobromide[1] was coupled to **12** to yield N^{α}-benzyloxycarbonyl-N^{β}-(*tert*-butyloxycarbonylsarcosyl-L-*N*-methylvalyl)-L-α,β-diaminopropionyl-D-valyl-L-proline *p*-nitrophenyl ester (**13**). After purification of **13** by Sephadex LH-20 chromatography in ethyl acetate the *tert*-butyloxycarbonyl group was removed by treatment with trifluoroacetic acid and the ensuing trifluoroacetate salt converted to the hydrochloride with HCl–ether.

For the cyclization of the open-chain pentapeptide the hydrochloride was dissolved in dimethylformamide–acetic acid (9:1) and the reaction carried out at high dilution.[26] After 6 h at 55°, two products were obtained, the desired compound **14** and a closely related side product. They were very difficult to separate, requiring twice repeated Sephadex LH-20 chromatography. At a reaction temperature of 60° this side product was the major product. Cyclizations performed at temperatures between 45 and 50° gave the desired product as the major product, i.e.,

Scheme II. Synthesis of [Di(1'-L-α,β-diaminopropionic acid)]actinomycin D Lactam Analog (16)



[di(1'-L-α,β-diaminopropionic acid)]actinomycin D (16)

N^{α}-benzyloxycarbonyl-L-α,β-diaminopropionyl-D-valyl-L-prolylsarcosyl-L-*N*-methylvaline (N^{β}-diaminopropionic acid) lactam (14). The benzyloxycarbonyl group of 14 was removed by catalytic hydrogenation and the 2-nitro-3-benzyloxy-4-methylbenzoyl group introduced via its symmetrical anhydride.[27] The ensuing N^{α}-(2-nitro-3-benzyloxy-4-methylbenzoyl)-L-α,β-diaminopropionyl-D-valyl-L-prolylsarcosyl-L-*N*-methylvalyl(N^{β}-diaminopropionic acid) lactam (15) was hydrogenated, followed by oxidative phenoxazinone formation using ferricyanide[28] to give [di(1'-L-α,β-diaminopropionic acid)]actinomycin D (16). After purification by Sephadex LH-20 chromatography a crystalline product was obtained.

Although the visible absorption spectrum of the $[Dpr^1]_2$-AMD lactam analog 16 was very similar to that of AMD (spectra not shown), their visible and uv CD spectra displayed marked differences. The visible and near-uv CD spectrum of uncomplexed AMD (Figure 2) is characterized by weak molecular ellipticity in the region of the long-wavelength absorption maximum (ca. 440 nm) and stronger optical activity at shorter wavelengths (ca. 380 nm). These bands are thought to arise from steric interactions between the two pentapeptide lactones.[29] In the presence of DNA, the ellipticities of these bands increase. Similar spectra have been reported in previous studies of the optical activity of the AMD–DNA complex.[30,31]

In contrast to AMD, uncomplexed $[Dpr^1]_2$-AMD lactam (16) displays bands of positive ellipticity at wavelengths removed from the principal absorption band (Figure 3). In the presence of DNA, there is an enhancement in the negative ellipticity of the long-wavelength CD band and a reversal in sign of the shorter wavelength bands, with the appearance of a strong negative CD band at about 380 nm. Figure 3 also shows the visible CD and near-uv spectrum of uncomplexed $[Dbu^1]_2$-AMD lactam [Figure 1 (c)] which differs from the spectra of AMD and

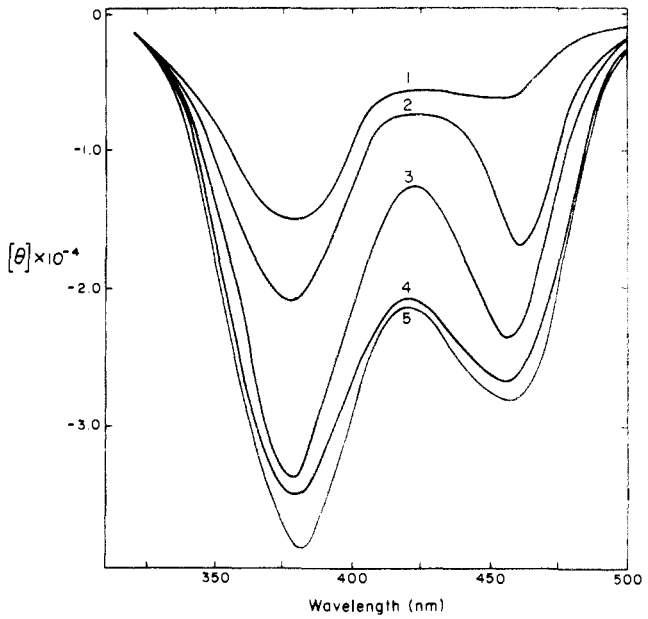


Figure 2. Visible and near-uv CD spectra of free actinomycin D (AMD), and of AMD in the presence of DNA duplexes, in 0.01 M Na phosphate buffer. The curves shown are 1, free AMD, 72.2 μM; 2, AMD, 14.3 μM, in the presence of poly[d(DAP-A-T)], 0.12 mM; 3, AMD, 14.3 μM, in the presence of poly[d(DAP-T)], 0.27 mM; 4, AMD, 34.5 μM, in the presence of calf thymus DNA, 0.50 mM; 5, AMD, 31.0 μM, in the presence of PM2 DNA, 0.54 mM.

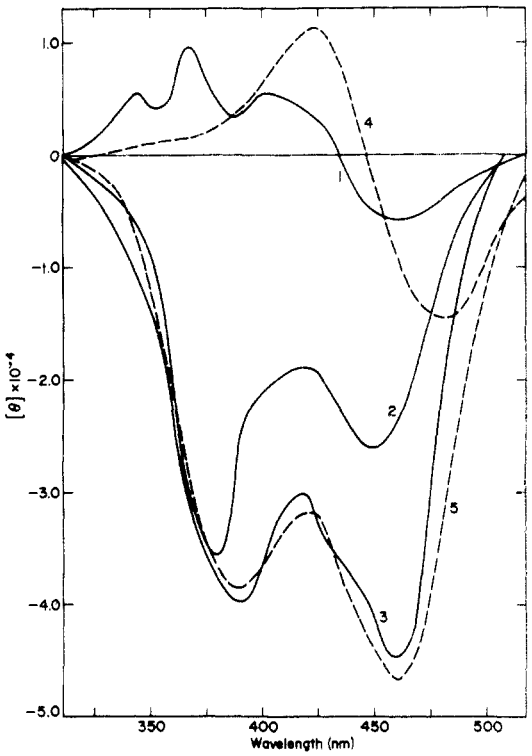


Figure 3. Visible and near-uv CD spectra of free actinomycin lactam analogs, i.e., $[Dpr^1]_2$-AMD and $[Dbu^1]_2$-AMD, and of the analogs in the presence of calf thymus DNA and PM2 DNA. The curves shown are 1, free $[Dpr^1]_2$-AMD lactam, 25.0 μM; 2, $[Dpr^1]_2$-AMD lactam, 25.0 μM, in the presence of calf thymus DNA, 0.52 mM; 3, $[Dpr^1]_2$-AMD lactam, 25.0 μM, in the presence of PM2 DNA, 0.52 mM. The broken lines shown are 4, free $[Dbu^1]_2$-AMD lactam, 37.1 μM; 5, $[Dbu^1]_2$-AMD lactam, 37.1 μM, in the presence of calf thymus DNA, 0.53 mM. Solvent, 0.01 M Na phosphate buffer.

Table I. In Vivo Antitumor Activity of the [Dpr^1]$_2$-AMD Lactam Analog of Actinomycin D against Leukemia L1210[a]

Drug	Dosage range, mg/kg/inj	Optimal dose, mg/kg/inj	MST (range), days	ILS, %
Untreated control			9.6 (8–15)	
	Daily Treatment, Days 1–9			
Actinomycin D	0.1–0.0125	0.05	13.6 (12–17)	42
[Dpr^1]$_2$-AMD lactam	0.2–0.00625	0.0125	11.2 (10–13)	17
	Intermittent Treatment, Days 1, 5, 9			
Actinomycin D	0.6–0.075	0.3	14.0 (12–17)	45
[Dpr^1]$_2$-AMD lactam	1.2–0.0375	0.15	11.0 (7–13)	14
	Single Dose, Day 1 only			
Actinomycin D	1.6–0.2	0.4	12.6 (9–18)	31
[Dpr^1]$_2$-AMD lactam	3.2–0.1	0.2	11.8 (6–14)	22

[a] 10^5 L1210 cells implanted ip on day 0 into groups of eight BDF_1 male mice. Drugs administered ip. MST (range), mean survival time in days (range of individual animal deaths). ILS, percent increase in life-span.

[Dpr^1]$_2$-AMD lactam (**16**). The similarity of the visible and near-uv CD spectra of the AMD–DNA and the two lactam analog–DNA complexes suggests that the lactone and lactam actinomycins acquire a similar environment when bound to DNA. Since optical activity is highly sensitive to the arrangement of atoms in a molecule, we conclude that the lactone and lactam analogs of actinomycin D are bound to DNA in a similar manner. Furthermore, analysis of binding isotherms indicates that their affinities for calf thymus DNA and the number of binding sites on DNA are not markedly different.[16]

Figure 4 shows the uv CD spectra of uncomplexed AMD and [Dpr^1]$_2$-AMD lactam. They differ in the magnitude of the ellipticities of the 260- and 230-nm bands; furthermore, the minimum of very strong molecular ellipticity observed at about 211 nm in the lactone was not found in [Dpr^1]$_2$-AMD lactam. The negative CD band of AMD at about 211 nm is believed to be associated with the pentapeptide rings.[29] The differences observed in the visible and uv CD spectra of the uncomplexed lactone and lactam analogs (Figures 2–4) suggest that their pentapeptide rings differ in conformation or in orientation with respect to the phenoxazinone group.

The biological activity of the [Dpr^1]$_2$-AMD lactam analog **16** was compared with that of actinomycin D by microbiological and in vivo antitumor assays. Microbiological assays were conducted according to literature.[32-34] Against *Bacillus subtilis* (Marburg) [Dpr^1]$_2$-AMD lactam (MIC, 0.9 μg/ml) was ten times less active than AMD (MIC, 0.09 μg/ml). Against *Staphylococcus aureus* (pen.-resistant) and *Sarcina subflava* the lactam (MIC, 0.35 and 0.05 μg/ml, respectively) had the same potency as AMD (MIC, 0.35 and 0.045 μg/ml). Inhibition of an exponentially growing culture of *B. subtilis* (Marburg) plotted against time[34] provided ID_{50} values of 0.05 μg/ml for AMD compared with 0.4 μg/ml for the lactam analog.

The in vivo antitumor activity of the lactam analog was compared with the activity of actinomycin D in three experimental murine tumor systems. The compounds were tested over four- to fivefold dosage ranges on three schedules of treatment employing standard screening methodology.[35] In leukemia L1210 (Table I) optimal doses of actinomycin D produced a moderate increase in life-span (31–45%) on the various treatment schedules. The lactam analog was inactive (ILS <25%) against L1210. Extensive increases in life-span (110–262%) and a number of long-term survivors were obtained on treatment of P388 leukemia with actinomycin D (Table II). Maximally tolerated doses of the lactam analog had only borderline activity (33–38% ILS) in this highly sensitive tumor system. The lactam analog had no antitumor activity (ILS <25%) in a solid tumor, B16 melanoma, which is sensitive to actinomycin D (Table III).

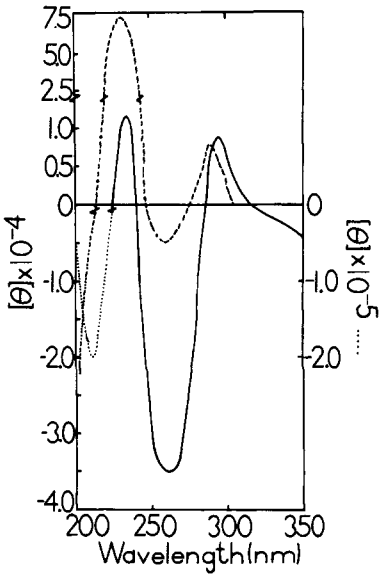


Figure 4. CD spectra of free actinomycin D (AMD) and free [Dpr^1]$_2$-AMD lactam: (——) AMD; (– – –) [Dpr^1]$_2$-AMD lactam; (· · ·) far-uv CD spectra of AMD and [Dpr^1]$_2$-AMD lactam (right-hand axis). Solvent, 0.01 M Na phosphate buffer.

These studies indicate that substitution of L-α,β-diaminopropionic acid for L-threonine in the 1' position of the cyclic pentapeptides of actinomycin, thus replacing the lactone by lactam rings and the Thr methyl groups by hydrogen, resulted in a decrease in antibacterial potency. The lactam analog was slightly more toxic to mice on a weight basis than the parent compound and at tolerated doses the lactam analog had little or no antitumor activity in a spectrum of tumors sensitive to actinomycin D.

Experimental Section

Materials and Methods. Actinomycin D was obtained from Merck Sharp & Dohme, Inc. Calf thymus DNA was purchased from Worthington Biochemical Corp. and dissolved, centrifuged, and dialyzed against buffer solution as described previously.[16] PM2 DNA was a gift from Dr. M. J. Waring and poly[d(DAP-A-T)] and poly[d(DAP-T)] were gifts from Drs. E. Reich and R. Klett. Concentrations of DNA solutions in terms of nucleotide phosphorus were determined as described previously.[16] Concentrations of actinomycin solutions were determined spectrophotometrically using extinction coefficients at 440 nm of 24450 M^{-1} cm^{-1} for AMD and [Dpr^1]$_2$-AMD and 23585 M^{-1} cm^{-1} for [Dbu^1]$_2$-AMD. Solutions were prepared in 0.01 M sodium phosphate buffer, pH 7.0. CD spectra were recorded at approximately 23° on a Cary 60 spectropolarimeter equipped with a 6001 CD attachment. Tandem cylindrical quartz cells of 1-cm

Table II. In Vivo Antitumor Activity of the [Dpr^1]$_2$-AMD Lactam Analog of Actinomycin D against P388 Leukemia[a]

Drug	Dosage range, mg/kg/inj	Optimal dose, mg/kg/inj	MST (range), days	ILS (surv), %
Untreated control			10.5 (9-19)	
	Daily Treatment, Days 1-9			
Actinomycin D	0.1-0.0125	0.05	28.5 (19-29)	171 (1/8)
[Dpr^1]$_2$-AMD lactam	0.1-0.0026	0.1	14.5 (10-28)	38
	Intermittent Treatment, Days 1, 5, 9			
Actinomycin D	0.6-0.075	0.3	38.0 (31-38)	262 (3/8)
[Dpr^1]$_2$-AMD lactam	0.6-0.0375	0.15	14.0 (9-28)	33
	Single Dose, Day 1 Only			
Actinomycin D	1.6-0.2	0.4	22.0 (8-31)	110 (1/8)
[Dpr^1]$_2$-AMD lactam	0.8-0.05	0.2	14.0 (12-19)	33

[a] 10^6 P388 cells implanted ip on day 0 into groups of eight BDF_1 male mice. Drugs administered ip. MST (range), median survival time in days (range of individual animal deaths). ILS (surv), percent increase in life-span (survivors/total at day 60).

Table III. In Vivo Antitumor Activity of the [Dpr^1]$_2$-AMD Lactam Actinomycin D against B16 Melanoma[a]

Drugs	Dosage range, mg/kg/inj	Optimal dose, mg/kg/inj	MST (range), days	ILS (surv), %
Untreated control			28.0 (21-53)	
	Daily Treatment, Days 1-9			
Actinomycin D	0.1-0.0125	0.025	45.0 (36-53)	61 (2/10)
[Dpr^1]$_2$-AMD lactam	0.2-0.0125	0.05	30.0 (8-24)	7
	Intermittent Treatment, Days 1, 5, 9			
Actinomycin D	0.6-0.075	0.3	41.5 (29-49)	48
[Dpr^1]$_2$-AMD lactam	0.6-0.0375	0.075	32.0 (24-43)	14
	Single Dose, Day 1 Only			
Actinomycin D	0.8-0.1	0.4	38.5 (33-43)	38 (1/10)
[Dpr^1]$_2$-AMD lactam	0.8-0.05	0.05	29.0 (25-34)	4

[a] 0.2 ml of a 1:5 (weight/volume) brei of B16 melanoma implanted ip on day 0 into groups of ten BDF_1 male mice. Drugs administered ip. MST (range), median survival time in days (range of individual animal deaths). ILS (surv), percent increase in life-span (survivors/total at day 60).

path length in each compartment were used. Molecular ellipticity, $[\theta]$, is reported in units of deg cm^2 dmol^{-1}.

Details on materials and methods used for synthesis have been described before.[36] Solvent systems for silica gel thin-layer chromatography were A, *sec*-BuOH–HCOOH–H_2O (75:13.5:11.5); B, *sec*-BuOH–10% NH_4OH (85:15); C, $CHCl_3$–MeOH (5:1); D, $CHCl_3$–MeOH–HOAc (8:1:1).

N^{α}-Benzyloxycarbonyl-L-α,β-diaminopropionic Acid (4). Benzyloxycarbonyl-L-β-chloroalanine (3, 7.71 g) was dissolved in concentrated ammonia. The solution was kept in a pressure bottle at 40° for 72 h. Evaporation and recrystallization from H_2O yielded colorless crystals: 2.1 g (29.5%); mp 243–245°; $[\alpha]^{20}D$ −14.2° (*c* 1, glacial HOAc). Anal. ($C_{11}H_{14}N_2O_4$) C, H, N.

N^{α}-*p*-Toluenesulfonyl-N^{β}-*tert*-butyloxycarbonyl-L-α,β-diaminopropionic Acid (6). To a solution of N^{α}-*p*-toluenesulfonyl-L-α,β-diaminopropionic acid (5, 8.3 g) in 1 M NaOH (40 ml) and dioxane (15 ml) was added *tert*-butyloxycarbonyl azide (23 ml) in dioxane (20 ml). The mixture was stirred at 25°. The pH was held constant, at 9.5, by pH-stat controlled addition of 4 M NaOH.[22] After 42 h, TLC (C) indicated complete acylation. Standard work-up and recrystallization of crude 6 from ether–hexane yielded colorless crystals: 10.7 g (93%); mp 127–128°; $[\alpha]^{25}D$ −72° (*c* 3.9, 1 M NaOH) [lit.[24] mp 125–129°; $[\alpha]^{22}D$ −71.1° (*c* 4, 1 M NaOH)]. Anal. ($C_{15}H_{22}N_2O_6S$) C, H, N.

N^{α}-Benzyloxycarbonyl-N^{β}-*tert*-butyloxycarbonyl-L-α,β-diaminopropionic Acid (7). To a well-agitated solution of 6 (35.8 g) in liquid NH_3 (1000 ml) at −50° were added small portions of Na until a blue color persisted for 10 min. Care was taken to avoid excessive frothing. Excess Na was neutralized by adding NH_4Cl. Evaporation of the ammonia left a residue which was dissolved in H_2O (500 ml). Filtration and evaporation left a residue which was redissolved in H_2O (1000 ml). TLC (A, B) showed a single ninhydrin-positive spot. The pH was adjusted to 7 with acetic acid and 25 g of $NaHCO_3$ and 100 ml of H_2O were added followed by dropwise addition over 45 min of benzyloxycarbonyl chloride (15.8 ml, 1.1 equiv) in ether (90 ml) with vigorous stirring. Another quantity of $NaHCO_3$ (18.8 g) was added and the mixture stirred vigorously for a further 2.5 h. The mixture was washed twice with ether (100 ml), cooled to 0°, acidified with solid citric acid, and then extracted with EtOAc (3 × 100 ml). The EtOAc solution was washed with 1 M citric acid, water and saline, dried, and evaporated to an oil, which crystallized from EtOAc–petroleum ether, as colorless crystals: 20 g (59%); mp 145–148°; $[\alpha]^{25}D$ −9.5° (*c* 1, MeOH); homogeneous on TLC (D) [lit.[24] mp 145–149°; $[\alpha]^{25}D$ −8.6° (*c* 0.99, MeOH)].

N^{α}-Benzyloxycarbonyl-N^{β}-*tert*-butyloxycarbonyl-L-α,β-diaminopropionic Acid Methyl Ester (8). To a solution of 7 (15 g) in EtOAc (50 ml) was added an etheral solution of diazomethane until a yellow color persisted. After 10 min excess diazomethane was discharged by adding a few drops of glacial HOAc. Repeated washing with 5% $NaHCO_3$, H_2O, and saline, drying ($MgSO_4$), and evaporation afforded an oil, which crystallized from EtOAc–hexane in colorless prisms: 15.0 g (96%); mp 74–77°; $[\alpha]^{25}D$ −6.5° (*c* 1.1, MeOH). Anal. ($C_{17}H_{24}N_2O_6$) C, H, N.

N^{α}-Benzyloxycarbonyl-L-α,β-diaminopropionic Acid Methyl Ester Hydrochloride (9). I. From Z-Dpr(Boc)-OMe (8). Compound 8 (3.0 g) was dissolved in redistilled anhydrous trifluoroacetic acid (TFA, 15 ml) at 0°. TLC (D) indicated complete cleavage of the Boc group after 45 min (single ninhydrin-positive spot). After evaporation of TFA the ensuing oil was dissolved in anhydrous MeOH (15 ml) and 2 M HCl in EtOAc (2 equiv) was added. Evaporation, precipitation, and recrystallization from MeOH–ether produced colorless crystals: 1.95 g (79.5%); mp 163–166°; $[\alpha]^{25}D$ −43.5° (*c* 1.1, MeOH). Anal. ($C_{12}H_{17}N_2O_4Cl$) C, H, N.

II. From Z-Dpr-OH (4). Compound 4 (4.15 g) was added to a precooled (−30°) mixture of anhydrous MeOH (60 ml) and $SOCl_2$ (1.4 ml, 1.1 equiv, freshly distilled from linseed oil). The mixture was stirred for 2 h at −30° and for 15 h at 25°. Evaporation and recrystallization from MeOH–ether yielded colorless crystals: 3.65 g (72.5%); mp 165–167°; $[\alpha]^{20}D$ −42.5° (*c* 1, MeOH).

Anal. C, H, N, Cl.

tert-**Butyloxycarbonyl-L-*N*-methylvaline** was prepared by pH-stat controlled reaction of L-*N*-methylvaline with *tert*-butyloxycarbonyl azide at pH 10.5 for 36 h[22] as described for **6** except that 4 M LiOH was used. The ensuing oil (56% yield) was converted (in ether) to the dicyclohexylammonium salt, which was recrystallized from hexane (81% yield): mp 107–110°; $[\alpha]^{20}D$ −54.1° (*c* 1, MeOH). Anal. ($C_{23}H_{44}N_2O_4$) C, H, N.

N^{α}-Benzyloxycarbonyl-N^{β}-(*tert*-butyloxycarbonyl-L-*N*-methylvalyl)-L-α,β-diaminopropionic Acid Methyl Ester (10). To a cooled (−15°) stirred solution of Boc-MeVal-OH (2.5 g) in freshly distilled THF (15 ml) was added *N*-methylmorpholine (1.2 ml) followed by isobutyl chloroformate (1.7 ml). After stirring for 2–3 min at −15°, a solution of **9** (3.1 g) in DMF (15 ml), cooled to −20° and mixed with NEt_3 (1.5 ml), was added to the mixed anhydride solution. After agitating for 2 h at −15° and for 15 h at 25°, the THF was evaporated and the residual DMF solution poured into H_2O (2000 ml) and repeatedly extracted with EtOAc. The organic phase was washed successively three times each with 1 N $NaHCO_3$, H_2O, 0.5 N H_2SO_4, H_2O, and saline, dried ($MgSO_4$), and concentrated to an oil which was purified by passage through a Sephadex LH-20 column (190 × 5 cm) in ethanol. Evaporation of the main peak fractions yielded a colorless oil: 4.05 g (84%); $[\alpha]^{25}D$ −70.8° (*c* 1, MeOH). Anal. ($C_{23}H_{35}N_3O_7$) C, H, N.

N^{α}-Benzyloxycarbonyl-N^{β}-(*tert*-butyloxycarbonyl-sarcosyl-L-*N*-methylvalyl)-L-α,β-diaminopropionic Acid Methyl Ester (11). Treatment of **10** (4.0 g) with anhydrous TFA (20 ml) for 1 h at 0°, evaporation, conversion to the hydrochloride by 2 N HCl in MeOH, and precipitation with ether provided a colorless powder, 2.5 g (73%), single ninhydrin-positive spot in TLC (A, B), which was thoroughly dried over KOH in vacuo, prior to dissolution in DMF (13 ml). After cooling to −20° and addition of NEt_3 (0.86 ml) the mixture was added to the mixed anhydride formed from Boc-Sar-OH (1.17 g) and isobutyl chloroformate (0.8 ml) at −15° in THF (13 ml) in the presence of *N*-methylmorpholine (0.69 ml) as described for **10**. After stirring for 2 h at −15° and 15 h at 25°, work-up and Sephadex LH-20 chromatography, as described for **10**, provided a colorless oil: 3 g (65%); $[\alpha]^{25}D$ −82.1° (*c* 1.12, MeOH). Anal. ($C_{26}H_{40}N_4O_8$) C, H, N.

N^{α}-Benzyloxycarbonyl-N^{β}-(*tert*-butyloxycarbonyl-sarcosyl-L-*N*-methylvalyl)-L-α,β-diaminopropionic Acid (12). To an agitated cooled (0°) solution of **11** (1.05 g) in acetone–water (3:2, 15 ml) was added 1 N NaOH (1.85 ml). The solution was stirred for 1 h at 0°, the acetone evaporated, and the aqueous phase washed twice with EtOAc and acidified with 1 M citric acid. Extraction with EtOAc, washing of the organic phase with water, drying ($MgSO_4$), and evaporation yielded a colorless foam: 0.9 g (88%); $[\alpha]^{25}D$ −78.5° (*c* 1, MeOH). Anal. ($C_{25}H_{38}N_4O_8$) C, H, N.

N^{α}-Benzyloxycarbonyl-N^{β}-(*tert*-butyloxycarbonyl-sarcosyl-L-*N*-methylvalyl)-L-α,β-diaminopropionyl-D-valyl-L-proline *p*-Nitrophenyl Ester (13). To an agitated THF solution (at −15°) of the mixed anhydride formed from **12** (0.9 g) with isobutyl chloroformate (0.227 ml) in THF (4 ml) in the presence of *N*-methylmorpholine (0.19 ml), as described for **10**, was added a DMF solution (4 ml) of H-D-Val-Pro-ONp·HBr (0.72 g) after cooling to −20° and addition of NEt_3 (0.24 ml). After stirring for 2 h at −15° and 2 h at 25°, work-up as described for **10**, Sephadex LH-20 column (87 × 5 cm) chromatography of the crude oil in EtOAc, and precipitation from ether–hexane yielded a cream-colored powder: 0.75 g (52%); mp 97–98°; $[\alpha]^{25}D$ −61.4° (*c* 1.1, MeOH); homogeneous on TLC (A, D). Anal. ($C_{41}H_{57}N_7O_{12}$) C, H, N.

N^{α}-Benzyloxycarbonyl-L-α,β-diaminopropionyl-D-valyl-L-prolylsarcosyl-L-*N*-methylvaline (N^{β}-Diaminopropionic acid) Lactam (14). Treatment of **13** (0.3 g) with anhydrous TFA (5 ml) for 1.5 h at 20°, evaporation, and conversion to the hydrochloride by ethereal 1 N HCl in benzene–THF (1:1) yielded an oil (0.28 g) which was homogeneous on TLC (A), R_f 0.3. It was dissolved in DMF (2 ml) containing HOAc (0.2 ml) and added dropwise over 2 h to stirred freshly distilled pyridine (700 ml) at 55° containing *N*-methylmorpholine (0.056 ml). Stirring was continued at 55° for an additional 3.5 h. The solvent was removed under reduced pressure. The residue was taken up in EtOAc and the solution washed, as described for **10**, dried ($MgSO_4$), and evaporated. The residue was dissolved in 95% ethanol and applied to a Sephadex LH-20 column (190 × 5.0 cm). Two slightly overlapping peaks were obtained. The desired product was contained in the fast-eluting peak (A) which was obtained as a colorless powder from ether–hexane: 57 mg (26.5%); $[\alpha]^{20}D$ −60° (*c* 1, MeOH); homogeneous on TLC (A), R_f 0.55. Anal. ($C_{30}H_{44}N_6O_7$) C, H, N. Amino acid analysis[37] (6 N HCl, 110°, 24 h) gave molar ratios of Dpr, 1.1; Sar, 1.1; MeVal, 1.0; Pro, 1.1; Val, 1.0.

Material from the second peak (B) [41 mg (19%); $[\alpha]^{25}D$ −30.4° (*c* 1, MeOH); TLC (A), R_f 0.65] showed no Dpr in amino acid analysis but some yet unknown basic component. When the cyclization was carried out at 45–50° formation of side product (peak B) was suppressed and 28% of desired product (peak A) was obtained.

N^{α}-(2-Nitro-3-benzyloxy-4-methylbenzoyl)-L-α,β-diaminopropionyl-D-valyl-L-prolylsarcosyl-L-*N*-methylvaline (N^{β}-Diaminopropionic acid) Lactam (15). The N^{α}-benzyloxycarbonyl group of **14** (0.13 g) was cleaved by Pd black catalyzed hydrogenolysis in methanol for 1 h (ref 36) in the presence of 1.2 equiv of HCl. Evaporation left the cyclic pentapeptide hydrochloride (**14a**) as an oil, homogeneous on TLC (A). The symmetric anhydride of 2-nitro-3-benzyloxy-4-methylbenzoic acid[28,38] was generated with exclusion of light by a 15-min treatment of a solution of the acid (0.253 g) in EtOAc (1.5 ml) with dicyclohexylcarbodiimide (88.7 mg in 4 ml of EtOAc) at 35°. Compound **14a** was dissolved in distilled DMF (3 ml), *N*-methylmorpholine (0.02 ml) added, and the solution added dropwise to the agitated symmetric anhydride solution at 25°. After 1 h an additional 0.01 ml of *N*-methylmorpholine was added and stirring continued for 15 h. After filtration the reaction mixture was poured into H_2O, which was twice extracted with EtOAc (50 ml, each). The organic phase was washed until neutral and dried ($MgSO_4$), and the solvent was evaporated. The residue was purified in the dark by Sephadex LH-20 chromatography (190 × 5 cm column) in ethanol and crystallized from benzene–hexane as cream-colored needles (light sensitive): 84 mg (53%); mp 155–156°; $[\alpha]^{25}D$ −11.2° (*c* 0.52, MeOH). Anal. ($C_{37}H_{49}N_7O_9$) C, H, N.

[Di(1'-L-α,β-diaminopropionic acid)]actinomycin D ([Dpr^1]$_2$-AMD, 16). A solution of **15** (82 mg) in MeOH (15 ml) was hydrogenated in the presence of Pd black for 1 h (ref 36) at 25° with exclusion of light. After filtration under N_2 an equal volume of 0.067 M phosphate buffer, pH 7.1, containing $K_3Fe(CN)_6$ (109 mg) was added. The pH was maintained at 7.1 by further addition of 0.067 M K_2HPO_4 and the mixture stirred at 25° for 2 h. It was then partitioned between EtOAc and H_2O (100 ml, each). On separation the aqueous phase was extracted with a further quantity of EtOAc (three 50-ml portions). The EtOAc solution was washed, as described for **10**, dried (Na_2SO_4), and evaporated. The residue was applied with exclusion of light to a Sephadex LH-20 column (190 × 5 cm) in 95% ethanol. The fractions containing [Dpr^1]$_2$-AMD (**16**) were combined and evaporated. The residue was recrystallized from EtOAc as red prisms: 41.7 mg (61%); mp 238–242°; $[\alpha]^{25}D$ −101.2° (*c* 0.08, MeOH); TLC R_f 0.27 (A); uv max (CH_3OH) 236–237 nm (ε 33450), 429 (19970), 448 (20830). Anal. ($C_{60}H_{84}N_{14}O_{14}$) C, H, N.

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Potential Antitumor Agents. 17. 9-Anilino-10-methylacridinium Salts

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9-Anilino-10-methylacridinium salts result from reaction of substituted anilines and 9-chloro-10-methylacridinium salts in turn prepared from the 10-methyl-9(10*H*)-acridones and $SOCl_2$ or $POCl_3$. Antileukemic (L1210) activities of the quaternary salts were uniformly depressed compared to their unquaternized counterparts. If drug–solvent partition properties (log *P*) of the cations were considered, log *P*–activity relationships were similar for both base and quaternary salt series. Substituent effects on antitumor activity were similar in both series.

Our entry into the series of 9-anilinoacridines[1,2] having broad-spectrum tumor activity[3] was via earlier described bisquaternary ammonium heterocycles[4] which had a considerably narrower action spectrum. Our interest in the effect of changing drug pharmacodynamic properties on the observable tumor spectrum of action prompted an examination of quaternary analogues of the 9-anilinoacridines. Additionally, the extensive ionization of quaternary salts at physiologic pH's serves to attenuate any p*K*-dependent phenomena induced by varying acridine ring substitution in the parent series. A study of the quaternary salts might therefore illuminate the role of changing p*K* in both observed structure–activity relationships (SAR) and drug pharmacodynamic properties. This communication details synthetic methods and L1210 screening results for a series of quaternary 9-anilinoacridines.

Chemistry. In our earlier experiences with N-quaternization of heterocycles[4,5] simple pyridines reacted readily, corresponding quinoline derivatives more slowly, and certain nitroquinolines with considerable difficulty. We have now concluded that the difficulties of obtaining complete reaction and the resulting problems of product purification make direct quaternization of the 9-anilinoacridines a synthetically unattractive reaction. Invariably a mixture of the quaternary salt and the salt of unreacted acridine resulted and these have marked tendency to cocrystallize and coprecipitate. Attempts to purify crude products from quaternization by direct crystallization and either selective precipitation or solvent partition at controlled pH's were of limited value. Products can be purified by tedious chromatography. Attention has already been drawn[6] to the difficulty of purifying simple acridinium quaternary salts such as 3,6-diamino-10-methyl-