$C_{24}H_{27}N_2O_5Cl$, found): 459.1687 (M + H monoisotopic), 459.1725. Elemental Anal. (C, H, N).

Biology. Uteri were removed from Sprague-Dawley rats (Charles River Laboratories, Inc., Wilmington, DE) weighing 200–250 g; the rate were anesthetized with 80 mg/kg i.p. sodium pentobarbital. The estrous cycles of rats were not controlled. All procedures were at 4 °C. Uteri were scraped free of mucosa and endometrium and homogenized in 20 volumes of ice-cold phosphate-buffered saline containing 5 mM EDTA. The homogenate was centrifuged at 1500*g* for 20 min. and the supernatant was recentrifuged at 100000*g* for 60 min. The pellet was resuspended in buffer consisting of 2 mM EGTA and 50 mM Tris-HCl, pH 7.5, to a final protein concentration of 4 mg/mL. Protein concentration was measured by using the Bio-Rad protein assay kit (Cambridge, MA). The assay for the receptor binding consisted of 0.25 mL of a solution containing 5 mM $MgCl_2$, 2 mM EGTA, 0.5% bovine serum albumin, 50 mM Tris-HCl, pH 7.5, and [^{125}I]Ang II (approximately 105 cpm) in the presence or absence of unlabeled peptide. The reaction was initiated by the addition of 50 μg of membrane protein, and the mixture was incubated at 25 °C for 30 min. The incubation was terminated with ice-cold 50 mM Tris-HCl, pH 7.5, and the mixture was filtered to separate membrane-bound labeled peptide from free ligand. The incubation tubes and filters were washed with ice-cold buffer. Filters were assayed for radioactivity in a Micromedic gamma counter (Micromedic Systems, Horsham, PA). Nonspecific binding was defined as binding in the presence of 10 μM unlabeled Agn II and was 15% or less of total binding. Specific binding was calculated as total binding minus nonspecific binding. Binding data were analyzed by a nonlinear least-squares curve-fitting program.

The compounds were tested for agonist and antagonist activity in rabbit aortic rings. Male New Zealand white rabbits (2–2.5 kg) were sacrificed using an overdose of pentobarbital and exsanguinated via the carotid arteries. The thoracic aorta was removed, cleaned of adherent fat and connective tissue, and then cut into 3-mm ring segments. The endothelium was removed from the rings by gently sliding a rolled-up piece of filter paper into the vessel lumen. The rings were then mounted in a water-jacketed tissue bath, maintained at 37 °C, between a moveable and a fixed stainless steel wire, with the moveable end attached to an FT03 Grass transducer coupled to a Model 7D Grass polygraph for recording isometric force responses. The bath was filled with 20 mL of oxygenated (95% oxygen/5% carbon dioxide) Krebs solution of the following composition (nM): 130 NaCl, 15 $NaHCO_3$, 15 KCl, 1.2 NaH_2PO_4, 1.2 $MgSO_4$, 2.5 $CaCl_2$, and 11.4 glucose. The preparations were equilibrated for 1 h before approximately 1 g of passive tension was placed on the rings. For the agonist assay, the rings were exposed to increasing concentrations of the test compound, at 30-min intervals, during which time the tissue was washed three times with 20 mL of fresh Krebs solution. For the measurement of antagonistic activity, paired rings from the same rabbits were used: one was exposed to increasing concentrations of AII, (at 30-min intervals), and a second ring was exposed to increasing concentrations of AII in the presence of the test compound, which was added 5 min prior to the addition of AII. The concentration–response curves for AII in the presence of the antagonist were evaluated in terms of the percent of the maximal contraction of the control ring exposed only to AII. pD_2 values for AII were calculated from the AII concentration response curves while pA_2s were determined according to Schild.[18]

Acknowledgment. We acknowledge Drs. E. Kolodziej and P. Toren for mass spectroscopy and wish to thank Prof. P. Beake for his suggestions and Drs. R. E. Manning and E. H. Blaine for their support.

Registry No. 1, 19815-03-3; 2, 40683-94-1; 4, 134360-53-5; 5, 134360-54-6; 6, 134360-55-7; 7, 134360-56-8; 8, 134388-43-5; 9, 134360-57-9; 2-butyl-4-chloro-5-(hydroxymethylene)imidazole, 79047-41-9; 2-butyl-5-chloro-4-(hydroxymethylene)imidazole, 79047-41-9; angiotension II, 11128-99-7.

Synthesis and DNA-Binding Properties of Polyamine Analogues

Michael L. Edwards,* Ronald D. Snyder, and David M. Stemerick

Marion Merrell Dow Research Institute, 2110 E. Galbraith Road, Cincinnati, Ohio 45215. *Received November 29, 1990*

The synthesis of a series of novel polyamine analogues is reported. The DNA binding of these compounds and a variety of other polyamines were compared with their IC_{50} values against HeLa cell. There seemed to be no apparent correlation between the DNA binding and toxicity against HeLa cells.

Introduction

Studies of the interactions between polyamines and nucleic acids in vitro have been reported,[1] but the biological relevance to in vivo situations is unclear. A growing amount of evidence suggests that polyamines do play a role in both modulation of cellular response to DNA reactive agents[2,3] and in maintenance of chromatin structure.[4] Recently, we reported the synthesis of polyamine analogues possessing antitumor[5,6] or antiprotozoal[7a] activity. The mechanism of action of these compounds is unknown; however, they may act through the displacement of the naturally occurring polyamines from DNA binding sites.

In this study the structure–activity relationships of a series of polyamine analogues toward binding to DNA is examined. If such an interaction is relevant to the antitumor activity of these compounds, one would expect some correlation between DNA-binding ability and growth inhibition, information useful in the design of more effective antitumor agents.

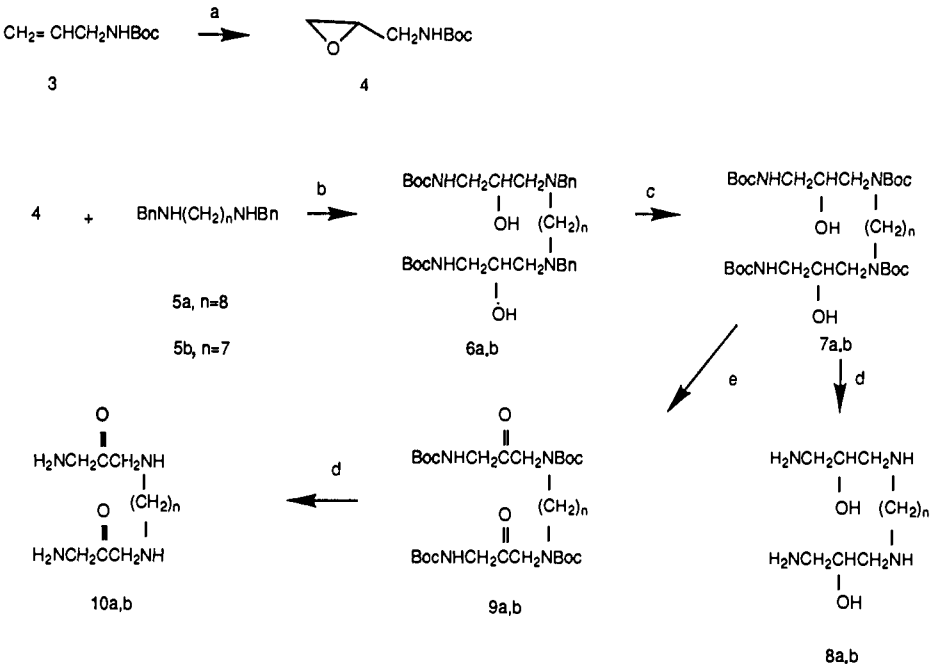
Chemistry

The synthesis of polyamine analogues 8 and 10 with hydroxyl or carbonyl substitution in the aminopropyl moiety followed the route outlined in Scheme I. Heating a mixture of the epoxide 4 and a *N,N'*-dibenzyldiamino-

(1) Marton, L. J.; Feuerstein, B. G. *Pharm. Res.* **1986**, *3*, 311.
(2) Porter, C. W.; Jänne, J. *Inhibition of Polyamine Metabolism. Biological Significance and Basis for New Therapies.* McCann, P. P., Pegg, A. E., Sjoerdsma, A. Eds. Academic Press: Orlando, 1987; pp 203–248.
(3) Snyder, R. D. *Int. J. Radiation Biol.* **1989**, *55*, 773.
(4) Snyder, R. D. *Biochem. J.* **1989**, *260*, 697.
(5) Prakash, N. J.; Bowlin, T. L.; Davis, G.; Sunkara, P. S.; Sjoerdsma, A. American Association for Cancer Research, May, 1987, Abstract 1260.
(6) Edwards, M. L.; Prakash, N. J.; Stemerick, D. M.; Sunkara, S. P.; Bitonti, A. J.; Davis, G. F.; Dumont, J. A.; Bey, P. *J. Med. Chem.* **1990**, *33*, 1369.
(7) (a) Edwards, M. L.; Stemerick, D. M.; Bitonti, A. J.; Dumont, J. A.; McCann, P. P.; Bey, P.; Sjoerdsma, A. *J. Med. Chem.* **1991**, *34*, 569. (b) Israel, M.; Zoll, E. C.; Muhammad, N.; Modest, E. J. *J. Med. Chem.* **1973**, *16*, 1. (c) Brown, D.; Woodcock, D. *Pestic. Sci.* **1973**, *4*, 485.

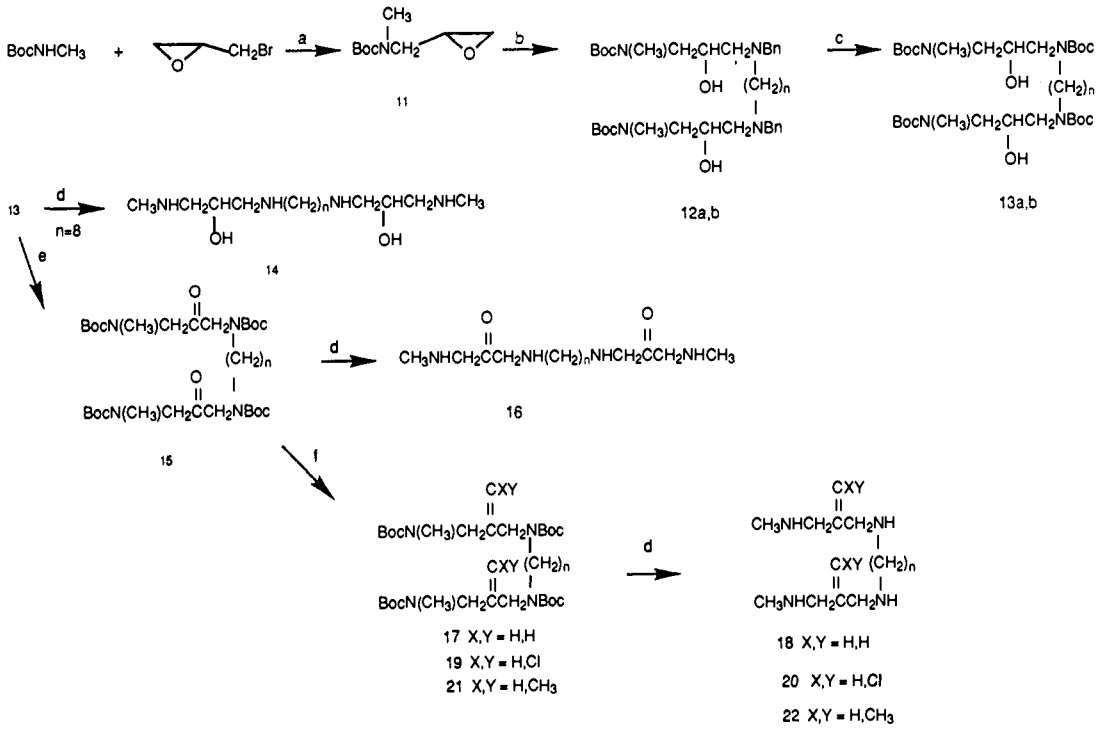
0022-2623/91/1834-2414$02.50/0

Scheme I[a]



[a] Reagents: (a) *m*-chloroperbenzoic acid; (b) neat, 50 °C; (c) Pd, H_2; $(CH_3)_3(COCO)_2O$; (d) HCl, methanol; (e) CrO_3, pyridine.

Scheme II[a]

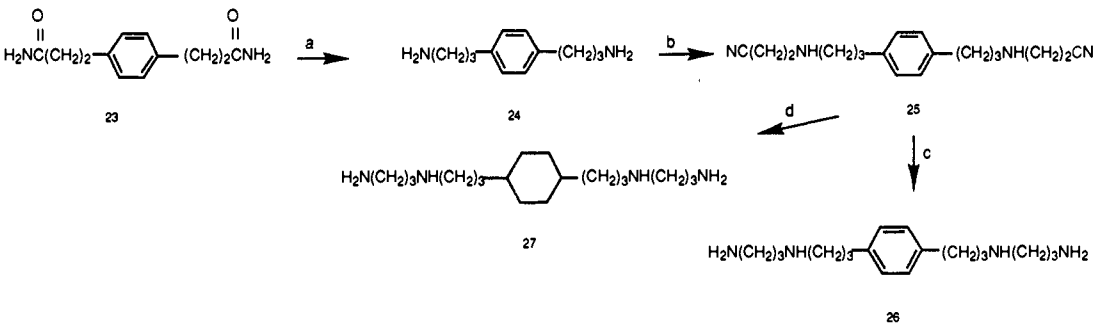


[a] Reagents: (a) NaH, DMF; (b) $BnNH(CH_2)_nNHBn$, 50 °C; (c) Pd/H_2; $(CH_3)_3(COCO)_2O$; (d) HCl, methanol; (e) CrO_3, pyridine; (f) Ph_3PCHXY, BuLi, THF.

alkane (**5a,b**) gave the bis-substituted tetraamines **6a,b** which were debenzylated by hydrogenolysis. The hydrogenolysis product was converted to the tetrakis-Boc derivative **7** which on oxidation (CrO_3) gave the diketone **9a,b**. Treatment of the tetrakis-Boc derivatives **7a,b** or **9a,b** with methanolic HCl removed the Boc-protecting groups providing the tetraamines **8** and **10** as tetrahydrochloride salts. Reaction of the tetrakis-Boc protected diketone **9** with Wittig reagents was unsuccessful, perhaps due to the presence of the ionizable amide NH. Therefore, a route to the analogous *N*-methyl compounds (**15**) was devised (Scheme II). In this case, the diketone **15** reacted with Wittig reagents to give, after removal of Boc-protecting groups with methanolic HCl, the desired tetraamine tetrahydrochloride salts **18**, **20**, and **22**. Compounds **20** and **22** were mixtures of isomers about the C–C double bond.

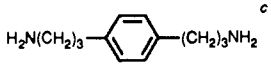
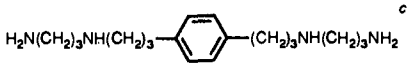
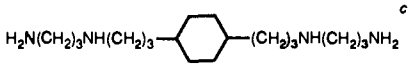
Those compounds (**23–29**, Scheme III) which incorporated a phenyl or cyclohexyl group as part of the central carbon chain were synthesized from the commercially available 1,4-benzenedipropionic acid which was converted to the dicarboxamide **23**, reduced (LAH) to the diamine **24**, and reacted with acrylonitrile, and the dinitrile product

Scheme III[a]



[a] Reagents: (a) $LiAlH_4$, THF; (b) acrylonitrile; (c) PtO_2, H_2, 4 h; (d) PtO_2, H_2, 24 h.

Table I. Ethidium Bromide Displacement, HeLa DNA Aggregation, and HeLa Growth Inhibition for Selected Derivatives[a]

compd no.	structure	ethidium bromide displacement: IC_{50}, μM CT[b]DNA	AT/TA	GC/CG	HeLa DNA aggreation: IC_{50}, μM	He La: IC_{50}, μM
	putrescine	4500	5100	5200	12000	8000
	norspermidine	220	140	370	3000	100
	spermidine	130	110	300	1500	100
	spermine	4.3	2.8	6.0	70	50
	6,6-difluorospermidine	875	1000	900	7000	
	7,7-difluorospermidine	275			3100	150
	$H_2N(CH_2)_3NHCH_2CF_2CH_2NH_2$	>10000				
	6,6-difluorospermine	140			2500	
24		400			3000	400
26		5			250	160
27		7.5				90
33	$H_2N(CH_2)_3S(CH_2)_4NH_2$[d]	1450			>15000	350
34	$H_2N(CH_2)_3NH(CH_2)_7NH(CH_2)_3NH_2$[e]	3.8	2.6	5.4	37	200
35	$H_2N(CH_2)_3NH(CH_2)_8NH(CH_2)_3NH_2$[f]	4.9	3.8	7.8	35	50
36	$H_2N(CH_2)_3NH(CH_2)_9NH(CH_2)_3NH_2$[e]	12	4.5	16		30
37	$H_2N(CH_2)_3NH(CH_2)_{10}NH(CH_2)_3NH_2$[e]	8	4.2	11.0	170	10
38	$H_2N(CH_2)_3NH(CH_2)_3O(CH_2)_3NH(CH_2)_3NH_2$[d]	6.0	4.6	6.8	60	

[a] See Materials and Methods for experimental procedures. [b] Calf thymus. [c] Preparation in Experimental Section. [d] Preparation in ref 6. [e] Preparation in ref 7a. [f] Preparation in ref 7c.

25 was reduced (PtO_2, H_2) (4 h) to give the tetraamine **26** (Scheme III). When reduction of the dinitrile **25** was allowed to proceed for 24 h, the benzene ring was reduced concomitantly, and the cyclohexane derivative **27** was obtained.

Synthesis of the other polyamine analogues in this study has been described[6,7a] with the exception of compounds **29** and **32**. Reaction of 1,7-diaminoheptane with methacrylonitrile gave the dinitrile **28** which was catalytically reduced to the tetraamine **29**. Conversion of **29** to the tetrakis-Boc derivative **30**, alkylation (NaH, CH_3I) and cleavage of the Boc groups (methanolic HCl) gave the *N*-methyl derivative **32** (Scheme IV).

Results and Discussion

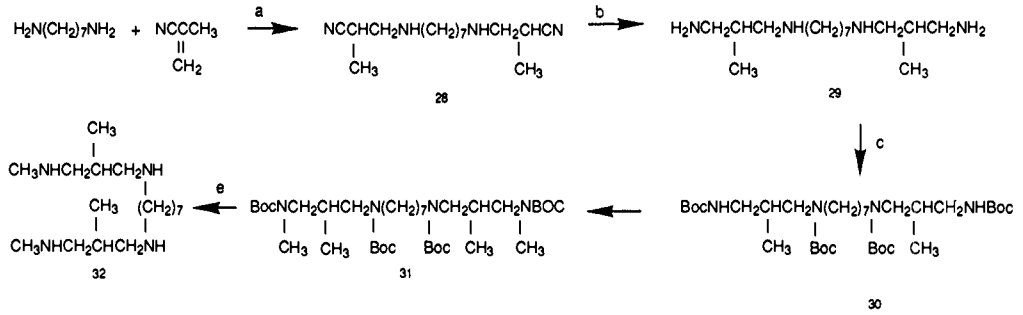
Two tests were used as a measure of DNA binding. The first measured the ability of drug to displace the intercalating dye ethidium bromide from calf thymus DNA or double stranded synthetic poly(dG-dC) and poly(dA-dT). Measurement of the ability of a drug to displace ethidium from DNA has been shown to be a valid measurement of DNA binding ability of both intercalative and nonintercalative (e.g., polyamines) drugs.[8-10]

In the second assay, the ability of polyamine analogues to cause aggregation of purified HeLa cell DNA was monitored. The collapse of DNA into compact structural forms due to charge neutralization is thought to be required for many cellular DNA metabolic processes including DNA packaging and various enzymatic reactions.[11] Inhibition of HeLa cell growth is also reported as an indicator of antiproliferative activity.

For discussion of the data, the compounds in this study are separated into five groups (Tables I–V). These data divide the discussion of SAR relationships of the polyamine analogues into analysis of the effect of central chain variation (Table I), α,ω-*N*-alkyl substitution (Table II), branching along the alkyl chain (Table III), C-3 modified derivatives (Table IV), and the distance between α,ω-nitrogen atoms (Table V). In Table I, data are presented for a group of compounds in which the terminal amino groups are primary. DNA binding was found to increase with chain length within the series putrescine to spermine. Within this group of compounds it was also found that replacement of a methylene group with a difluoro-

(8) Cain, B. F.; Baguley, B. C.; Denny, W. A. *J. Med. Chem.* **1978**, *21*, 658.
(9) Braithwaite, A. W.; Baguley, B. C. *Biochemistry* **1980**, *19*, 1101.
(10) Stewart, K. D. *Biochem. Biophys. Res. Commun.* **1988**, *152*, 1441.
(11) Feuerstein, B. G.; Basu, H. S.; Pellarin, M.; Marton, L. J. *Proc. Am. Assoc. Cancer Res.* **1989**, *30*, 585.

Scheme IV[a]



[a] Reagents: (a) EtOH, 50 °C; (b) PtO_2, H_2; (c) $(CH_3)_3(CO)_2O$; (d) CH_3I, NaH, DMF; (e) HCl, MeOH.

Table II. Ethidium Bromide Displacement, HeLa DNA Aggregation, and HeLa Growth Inhibition for *N*-Alkyl Tetraamines[a]

	$RNH(CH_2)_3NH$— C—$NH(CH_2)_3NHR$		ethidium bromide displacement: IC_{50}, μM			HeLa DNA aggregation:	HeLa:
compd no.	C	R	CT[b] DNA	AT/TA	GC/CG	IC_{50}, μM	IC_{50}, μM
34	$(CH_2)_7$	H[c]	3.8	2.6	5.4	37	200
40		CH_3[e]	6	3.5	11.0	150	500
41		C_2H_5[e]	11.0	4.0	20.0[f]	200	100
42		C_3H_7[e]	10.0	3.8	20.5[f]	250	300
43		C_6H_5[c]	80				
44		$CH_2C_6H_5$[c]	6.0	4.6	31[f]	150	5
45		$(CH_2)_2C_6H_5$[c]	8.0	2.8	10.7	85	20
46		$(CH_2)_3C_6H_5$[c]	7.5	4.7	12.0	100	20
35	$(CH_2)_8$	H[d]	4.9	3.8	7.8	35	50
48		CH_3[c]					
49		C_2H_5[c]	15.0	3.9	19.5[f]	150	200
50		C_3H_7[c]	17.0	3.8	25.0[f]	220	>500
51		C_4H_9[c]	20.0	4.2	40.0[f]	340	>500
52		$CH_2C_6H_5$[c]	13	4.2	32[f]	300	15

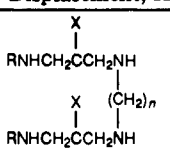
[a] See Material and Methods for experimental procedures. [b] Calf thymus. [c] Described in ref 7a. [d] Described in ref 7c. [e] Described in supplementary material. [f] Poor GC binding relative to AT binding as compared to spermine.

Table III. Ethidium Bromide Displacement, HeLa DNA Aggregation, and HeLa IC_{50} for Branched Chain Derivatives[a]

	$RNHCHCHCHNH(CH_2)_nNHCHCHCHNHR$ $R_1\ R_2\ R_3$ $R_3\ R_2\ R_1$		ethidium bromide displacement: IC_{50}, μM			HeLa DNA aggregation:	HeLa:
compd no.	CH_3	*n*	CT[b] DNA	AT/TA	GC/CG	IC_{50}, μM	IC_{50}, μM
29[c]	R_2	7	8	3.0	10.5[e]		>500
32[c]	R,R_2	7	12.8	4.5	20[e]		>500
53[d]	R,R_1	7	6.5	5.0	15[e]	100	
54[d]	R_1	8	7.5	4.2	12.5		
55[d]	R_3	8	7.0	3.6	12.6[e]	180	100

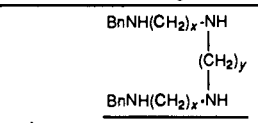
[a] See Materials and Methods for experimental procedures. [b] Calf thymus. [c] Described in supplementary material. [d] Described in ref 6. [e] Poor GC binding relative to AT binding as compared to spermine.

Table IV. Ethidium Bromide Displacement, HeLa DNA Aggregation, and HeLa IC_{50} for C-3 Modified Derivatives[a]

				ethidium bromide displacement: IC_{50}, μM			HeLa DNA aggregation:	HeLa:
compd no.[b]	R	X	*n*	CT[c] DNA	AT/TA	GC/CG	IC_{50}, μM	IC_{50}, μM
8a	H	H,OH	7	4.9	4.5	23	110	
8b[d]	H	H,OH	8	5.2	4.3	27.0[e]	200	
10a	H	O	7					
10b	H	O	8	8.2	10.5	18.0	400	
14a	CH_3	H,OH	7					
16a	CH_3	O	7					
16b	CH_3	O	8					
18a	CH_3	CH_2	7					
18b	CH_3	CH_2	8	19.1	4.5	30.0[e]		125
20a	CH_3	CHCl	7	12.0	5.0	34.5[e]		125
20b	CH_3	CHCl	8					
22a	CH_3	$CHCH_3$	7	12	5.0	34.5[e]		>500

[a] See Materials and Methods for experimental procedures. [b] All compounds described in Experimental Section. [c] Calf thymus. [d] DNA binding for this compound are given in ref 15. [e] Poor GC binding relative to AT binding compared with spermine.

Table V. Ethidium Bromide Displacement, HeLa DNA Aggregation, and HeLa IC_{50} for *N*-Benzyl Derivatives[a]



compd no.[b]	x	y	ethidium bromide displacement: IC_{50}, μM CT[c] DNA	AT/TA	GC/CG	HeLa DNA aggregation: IC_{50}, μM	HeLa: IC_{50}, μM
56	2	6	800			2400	75
57	2	7	600	170	316	600	60
58	2	8	130	40	100	750	20
59	2	10	600	700	1600	>8000	2
60	3	4	10	4.9	17	40	10
61	3	5	12	4.5	22.0[d]		80
62	3	6	5.5	10	25	85	60
44	3	7	6.0	4.6	31.0[d]	150	5
52	3	8	13.0	4.2	32.0[d]	300	15
63	3	9	31.0	2.9	24.5[d]	200	10
64	3	10	33.0	3.1	26.0[d]	400	1
65	4	7	10.0	3.2	20.0[d]	95	>500
66	4	8	12.0	3.4	20.0[d]	105	>500

[a] See Materials and Methods for experimental procedures. [b] All compounds described in ref 7a. [c] Calf thymus. [d] Poor GC binding relative to AT binding as compared to spermine.

methylene gave analogues with greatly diminished DNA-binding ability. Similar results have been reported by Feuerstein et al.[11] The two diamines **23** and **24** were relatively poor DNA-binding agents, similar to putrescine, while the tetraamines **26**, **27**, and **34–38** exhibited DNA-binding values similar to or superior to those of spermine.

Since the ability of ethidium to bind AT-rich DNA is likely to be different from its ability to bind GC-rich DNA, it cannot be concluded that polyamines, such as spermine, display a 2+-fold preference for AT over GC. What is evident upon examination of all analogues in the five tables is that none appears to bind GC DNA significantly better than spermine. That is, the ratio of GC/AT dye displacement is equal to or less than spermine. It is shown that certain analogues, notably those containing terminal benzyl groups or longer chain aliphatic groups, show a markedly lowered affinity for GC relative to AT DNA (marked with asterisks on tables). It is interesting to note that several series of nonintercalating minor groove binding agents have been previously shown to prefer AT binding[12] and this preference may, in part, be dependent on the width of the minor groove in the drug–DNA complex. Thus, the longer atomic distances in GC–drug complexes would tend to weaken the interaction energy and thus destabilize the complex. In addition, constraints placed upon the maximal permissible minor groove width for complex formation might limit GC binding under conditions in which too much helical distortion occurs during the hydrogen-bonding process, resulting in a still larger minor groove. No correlation of binding data with HeLa IC_{50} was noted.

Table II presents the data for *N*-alkyl tetraamines. The effects of N-alkylation on ethidium displacement were minimal, with an increase in the size of the alkyl group resulting in lowered DNA binding of calf thymus and GC DNA. The HeLa DNA aggregation data also suggest less tolerance for *N*-alkyl substitution, with IC_{50} values increasing with increasing size of the alkyl group. Methyl branching in the aminopropyl moiety also had a minimal effect on displacement of ethidium bromide (Table III).

There is tolerance for a wide variety of other functional groups (alcohol, ketone, alkene) within the aminopropyl chain (Table IV). The data in Table V indicate that replacement of the aminopropyl chain with aminoethyl diminishes the DNA binding, but the compounds retain growth inhibitory activity (**58** and **59**). Binding of polyamines to DNA appears to be a function more of total charge than charge distribution. The order of binding is diamines < triamines < tetraamines. In addition, it has been reported that pentaamines bind DNA much better than tetraamines[13] as measured both by DNA aggregation and ability to convert right handed (B) DNA into the left hand (Z) configuration. We have also tested certain of these pentaamines with the ethidium displacement and aggregation assays (data not shown) and have confirmed a nearly 10-fold increased binding by these compounds.

A nearly exact correspondence exists between the results of the ethidium displacement and DNA aggregation assays with respect to the dose of polyamine required to affect these parameters (aggregation assays employed 10-fold higher DNA concentration than displacement assays). A careful analysis indicates that ethidium displacement begins to occur at polyamine concentrations which do not cause measurable aggregation (not shown). However, Hard et al.[14] have demonstrated that the binding constant of ethidium bromide for DNA is dependent on the molecular flexibility of DNA in linker regions of chromatin and that this flexibility is altered through cationic compaction. Thus, DNA condensation, which precedes aggregation, might be expected to lower the affinity of ethidium and decrease fluorescence. Therefore, it is not clear that the ethidium displacement assay measures drug binding in the same direct manner as it does with intercalating drugs. However, the assay is still a useful indicator of the relative ability of drugs to interact with DNA, changing, in some manner, ethidium interactions with that DNA.

In conclusion, no correlation appeared evident between DNA binding and antiproliferative activity within the compounds in this study. Recently, a lack of relationship between DNA binding and polyamine cytotoxicity has been suggested by the preliminary studies of Feuerstein et al.[11] and Basu et al.[15]

Experimental Section

Melting points were determined in a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analyses were performed by the Merrell Dow Research Institute Analytical

(12) Gago, F.; Reynolds, C. A.; Richards, W. G. *Mole. Pharmacol.* 1989, *35*, 232.
(13) Basu, H. S.; Marton, L. J. *Biochem. J.* 1987, *244*, 243.
(14) Härd, T.; Nielsen, P. E.; Norden, B. *Eur. J. Biophys.* 1988, *16*, 231.
(15) Basu, H. S.; Feuerstein, B. G.; Deen, D. F.; Lubich, W. P.; Bergeron R. J.; Samejina, K., Marton, L. J. *Proc. Am. Assoc. Cancer Res.* 1989, *49*, 5591.

Department and, unless otherwise indicated, agree with theoretical values within 0.4%. NMR spectra were obtained on a Varian VXR-300 or a Varian EM-360L spectrometer. Chemical shifts are reported downfield from TMS in spectra obtained in $CDCl_3$. IR spectra were obtained on a Finnigan MAT 4600 spectrophotometer. All spectra were consistent with structure. Thin-layer chromatography (TLC) was done on Merck silica gel 60 F254 analytical plates, visualized with I_2 and/or UV. The preparation on compounds **3**, **4**, **12a,b**, **13a,b**, **14a**, and **23–40** is included in the supplementary material.

5,14-Dibenzyl-1,18-bis(*tert*-butoxycarbonyl)-3,16-dihydroxy-1,5,14-18-tetraazaoctadecane (6a). A mixture of *N,N'*-dibenzyl-1,8-diaminooctane[16] (3.2 g, 0.01 m) and the epoxide **4** (4 g, 0.023 m) was heated at 50 °C under a N_2 atmosphere for 18 h. The residue was chromatographed (EtOAc) to give the product (4.4 g, 66%) as a thick oil: IR (film) 3420, 3350, 2920, 1720, 1510, 1370, 1250, and 1170 cm^{-1}; NMR (Me_2SO-d_6) 7.34–7.2 (m, 10 H), 6.59 (t, J = 6.4 Hz, 2 H), 4.55 (s, 2H), 3.7–2.2 (m, 22 H), 1.37 (s, 22 H), and 1.2–1.1 (m, 4 H); MS (CI/CH_4) 671 (M + H). Anal. ($C_{36}H_{62}N_4O_6$) C, H, N.

Similarly prepared from *N,N'*-dibenzyl-1,7-diaminoheptane[7a] and **4** was **6b** (oil): IR (film) 3428, 3356, 3028, 3004, 2976, 2932, 2858, 2812, 1716, 1510, 1496, 1454, 1392, 1366, 1270, 1250, 1172, 1116, 1076, 1028, 978, 872, 736, and 700 cm^{-1}; NMR ($CDCl_3$) 7.35–7.2 (m, 10 H), 4.95 (bs, 2 H), 3.8–3.65 (m, 6 H), 3.47–3.25 (m, 4 H), 3.05–2.92 (m, 2H), 2.6–2.48 (m, 2 H), 2.45–2.25 (m, 6 H), 1.42 (s, 22 H), and 1.2 (s, 6H); MS (CI/CH_4) 657 (M + H). Anal. ($C_{32}H_{60}N_4O_6$) H, N; C: calcd, 67.65; found, 68.50.

1,5,14,18-Tetraaza-1,5,14,18-tetrakis(*tert*-butoxycarbonyl)-3,16-dihydroxyoctadecane (7a). A solution of **6** (24 g, 36 mmol) in ethanol (200 mL) was hydrogenated for 20 h on a Parr hydrogenation apparatus in the presence of Pearlman's catalyst[17] (0.6 g). The mixture was filtered and evaporated. The residue was dissolved in dichloromethane (700 mL) and di-*tert*-butyl dicarbonate (23 g, 100 mmol) was added in portions. The mixture was stirred for 24 h and extracted with aqueous HCl. The organic layer was dried and evaporated, and the residue was chromatographed ($PhCH_3$/EtOAc 1/1) to give the product (19.3 g, 78%) as an oil: IR (film) 3370, 2960, 2930, 2350, 1670, 1510, 1470, 1420, 1370, 1250, and 1160 cm^{-1}; NMR ($CDCl_3$) 5.15–5.05 (bs, 2 H), 4.25–3.7 (m, 6 H), 3.6–3.0 (m, 10 H), 1.8 (s, 4 H), 1.45 (s, 36 H), and 1.25 (s, 8 H); MS (CI/CH_4) 691 (M + H). Anal. ($C_{34}H_{66}N_4O_{10}$) H, N; C: calcd, 59.11; found, 60.73.

Similarly prepared was **7b** (oil): IR (film) 3372, 2978, 2932, 1690, 1512, 1480, 1456, 1418, 1392, 1368, 1276, 1250, 1170, and 756 cm^{-1}; NMR ($CDCl_3$) 5.3 (bs, 2 H), 4.21 (bs, 2 H), 3.8 (bs, 2 H), 3.4–3.0 (m, 12 H), 1.55 (s, 4 H), 1.48 (d, J = 7 Hz, 36 H), and 1.28 (bs, 6 H); MS (CI/CH_4) 677 (M + H). Anal. ($C_{33}H_{64}N_4O_{10}\cdot{}^2/_3PhCH_3$) C, H, N.

1,5,14,18-Tetraaza-3,16-dihydroxyoctadecane Tetrahydrochloride (8a). A solution of **7** (2.5 g, 3.6 mmol) in ethanol (50 mL) was treated with a solution of anhydrous HCl in ethanol (50 mL, 2 N). After 18 h, the mixture was concentrated to 25 mL, chilled, and filtered to give a white solid (1.3 g, 82%): mp 288–90 °C; IR (KBr) 3350, 2920, 1580, 1520, and 1460 cm^{-1}; NMR (Me_2SO-d_6) 8.4–8.25 (bs, exchange D_2O, 10 H), 6.1 (bs, exchange D_2O, 2H), 4.1 (bs, 2 H), 2.6–2.4 (m, 12 H), 1.7 (bs, 4 H), and 1.3 (s, 8 H); MS (CI/CH_4) 291 (M + H). Anal. ($C_{14}H_{34}N_4O_2\cdot4HCl$) C, H, N.

Similarly prepared was **8b**: mp 287–89 °C; IR (KBr) 3400, 2940, 1590, 1520, 1510, and 1460 cm^{-1}; NMR (D_2O) 4.3–4.2 (m, 2 H), 3.3–3.0 (m, 12 H), 1.72 (bs, 4 H), and 1.4 (s, 6 H); MS (CI/CH_4) 277 (M + H). Anal. ($C_{13}H_{32}N_4O_2\cdot4HCl$) C, H, N, Cl.

1,5,14,18-Tetraaza-1,5,14,18-tetrakis(*tert*-butoxycarbonyl)-3,16-diketooctadecane (9a). A solution of **7** (28.6 g, 41 mmol) in pyridine (350 mL) was added to a chilled solution of CrO_3 (44 g, 390 mmol) in pyridine (1.2 L), and the mixture was stirred 72 h at ambient temperature. The mixture was poured into water (3 L), and the aqueous mixture was extracted with ether. The combined extracts were washed with water and brine, dried, and evaporated. The residue was chromatographed ($PhCH_3$/EtOAc 2.5/1) to give the product (12 g, 42%) as a thick oil which solidified on standing: mp 65–69 °C; IR (film) 2980, 2920, 1700, 1370, 1250, and 1170 cm^{-1}; NMR ($CDCl_3$) 5.15 (bs, 2 H), 4.03–3.87 (m, 8 H), 3.25–3.15 (m, 4 H), 1.5–1.3 (m, 40 H), and 1.22 (s, 8 H); MS (CI/CH_4) 687 (M + H). Anal. ($C_{34}H_{62}N_4O_{10}$) C, H, N.

Similarly prepared was **9b** (oil): IR (film) 2978, 2932, 1696, 1506, 1480, 1458, 1424, 1394, 1368, 1282, 1259, 1168, and 758 cm^{-1}; NMR ($CDCl_3$) 5.2 (s, 2 H), 4.1–3.9 (m, 8 H), 3.3–3.17 (bs, 4 H), 1.5–1.4 (m, 40 H), and 1.3–1.2 (m, 6 H); MS (CI/CH_4) 673 (M + H). Anal. ($C_{33}H_{60}N_4O_{10}\cdot{}^1/_3PhCH_3$) C, H, N.

1,5,14,18-Tetraaza-3,16-diketooctadecane Tetrahydrochloride (10a). A solution of **8a** (0.9 g, 1.3 mmol) in methanolic HCl (150 mL, 1 N) was stirred for 1 h, chilled, and filtered to give a white solid (250 mg, 41%): mp >330 °C; IR (KBr) 2960, 1745, 1470, and 1430 cm^{-1}; NMR (D_2O) 4.3 (s, 4 H), 4.2 (s, 4 H), 3.07 (t, J = 9 Hz, 4 H), 1.7 (bs, 4 H), and 1.36 (s, 8 H); MS (FA/Xe) 287 (M + H). Anal. ($C_{14}H_{30}N_4O_2\cdot4HCl$) C, H, N.

Similarly prepared was **10b**: mp 280 °C; IR (KBr) 3458, 2933, 2796, 2760, 2732, 2630, 2590, 2460, 1744, 1528, 1428, 1410, and 1396 cm^{-1}; NMR (D_2O) 4.32 (s, 4 H), 4.22 (s, 4 H), 3.1 (t, J = 9 Hz, 4 H), 1.72 (bs, 4 H), and 1.4 (s, 6 H); MS (FAB/Xe–glycerol) 273 (M + H). Anal. ($C_{13}H_{25}N_4O_2\cdot4HCl$) C, H, N; Cl: calcd, 33.91; found, 32.88.

***N*-(*tert*-Butoxycarbonyl)-*N*-methyl-2,3-epoxypropylamine (11).** A solution of *N*-(*tert*-butoxycarbonyl)methylamine (20 g, 0.15 mol) in DMF (500 mL) was chilled in an ice bath and NaH (6 g, 60% mineral oil dispersion, 0.16 mol) was added in portions over 30 min. The mixture was stirred an additional hour and a solution of epibromohydrin (20.6 g, 0.15 mol) in DMF (50 mL) was added dropwise. The mixture was stirred 20 h and evaporated, and the residue was dissolved in EtOAc. The solution was washed with water and brine, the organic layer was dried and evaporated, and the residue was chromatographed ($PhCH_3$/EtOAc 6/1) to give the product (13.8 g, 49%) as an oil: IR (film) 2980, 1480, 1460, 1400, 1370, 1250, 1170, and 870 cm^{-1}; NMR ($CDCl_3$) 3.12–3.05 (m, 2 H), 2.93 (s, 3 H), 2.8–2.7 (m, 2 H), 2.55–2.5 (m, 1 H), and 1.46 (s, 9 H); MS (CI/CH_4) 188 (M + H). Anal. ($C_9H_{17}NO_3$) C, H, N.

2,6,15,19-Tetraaza-2,6,15,19-tetrakis(*tert*-butoxycarbonyl)-4,17-diketoeicosane (15a) was prepared by CrO_3 oxidation of **13a** by a procedure previously described: IR (film) 2970, 2930, 1750, 1700, 1480, 1460, 1390, 1370, 1250, and 1160 cm^{-1}; NMR ($CDCl_3$) 4.1–3.9 (m, 8 H), 3.3–3.15 (m, 4 H), 2.92 (s, 6 H), 1.53–1.4 (m, 40 H), and 1.3–1.23 (s, 8 H); MS (CI/CH_4) 715 (M + H). Anal. ($C_{36}H_{66}N_4O_{10}$) C, H, N.

Similarly prepared was **16b**: mp 235–37 °C; IR (KBr) 3462, 2948, 2928, 2860, 2768, 2738, 2702, 2456, 2364, 1738, 1478, 1426, 1410, and 1382 cm^{-1}; NMR (D_2O) 4.3 (s, 8 H), 3.15–3.05 (m, 4 H), 2.78 (s, 6 H), 1.7 (bs, 4 H), and 1.38 (s, 6 H); MS (CI/CH_4) 301 (M + H). Anal. ($C_{15}H_{32}N_4O_2\cdot4HCl$) C, H, N, Cl.

2,6,15,19-Tetraaza-2,6,15,19-tetrakis(*tert*-butoxycarbonyl)-4,17-dimethyleneeicosane (17a). Potassium *tert*-butoxide (1.3 g, 12 mmol) was added to a suspension of methyltriphenylphosphonium bromide (4.3 g, 12 mmol) in toluene (100 mL) and the mixture was heated at 50 °C for 2 h. The mixture was cooled to ambient temperature, a solution of compound **15a** (1.1 g, 1.5 mmol) in toluene (10 mL) was added, and the mixture was stirred at ambient temperature for 18 h. The reaction mixture was extracted with water, and the organic layer was dried and evaporated. The residue was chromatographed ($PhCH_3$/EtOAc, 4/1) to give the product (1 g, 90%) as a thick oil: IR (film) 2980, 2930, 1700, 1480, 1460, 1390, 1370, 1250, 1180, and 1150 cm^{-1}; NMR ($CDCl_3$) 4.9 (s, 4 H), 3.95–3.75 (m, 8 H), 3.12–3.08 (bs, 4 H), 2.8 (s, 6 H), 1.47 (s, 40 H), and 1.25 (s, 8 H); MS (CI/CH_4) 711 (M + H). Anal. ($C_{36}H_7ON_4O_6$) C, H, N.

Treatment of **17a** with methanolic HCl gave **18a**: mp 216–218 °C; IR (KBr) 2940, 2880, 2500, 2400, 1465, 1455, and 950 cm^{-1}. NMR (D_2O) 5.65 (d, J = 7.5 Hz, 4 H), 3.75 (s, 8 H), 3.12–3.05 (m, 4 H), 2.75 (s, 6 H), 1.7 (bs, 4 H), and 1.35 (s, 8 H); MS (CI/CH_4) 311 (M + H). Anal. ($C_{18}H_{38}N_4\cdot4HCl$) C, H, N, Cl.

2,6,15,19-Tetraaza-2,6,15,19-tetrakis(*tert*-butoxycarbonyl)-4,17-bis(chloromethylene)eicosane (19a). To a chilled (−30 °C) solution of LDA (6 mmol) in THF (50 mL) was added (chloromethyl)triphenylphosphonium chloride (2 g, 6 mmol), and the mixture was stirred at −20 °C for 1 h. A solution

(16) Wittbecker, E.; Houtz, R. C.; Watkins, W. W. *J. Am. Chem. Soc.* **1947**, *69*, 579.
(17) Pearlman, W. M. *Tetrahedron Lett.* **1967**, 1663.

of **15a** (0.7 g, 1 mmol) in THF (10 mL) was added, and the mixture was stirred for an additional 18 h. The mixture was poured into water, and the aqueous mixture was extracted with CH_2Cl_2. The organic extracts were dried and evaporated and the residue chromatographed (hexane/EtOAc 3/1) to give the product (0.3 g, 37%) as an oil: IR (film) 2980, 2940, 1700, 1415, 1390, 1370, 1250, and 1155 cm^{-1}; NMR ($CDCl_3$) 5.95 (bs, 2 H), 4.1 (s, 4 H), 3.8 (s, 4 H), 3.1 (bs, 4 H), 2.8 and 2.87 (s, 6 H total), 1.4 (s, 40 H), and 1.35 (s, 6 H); MS (CI/CH_4) 779 (M + H). Anal. ($C_{36}H_{65}Cl_2N_4O_8$) C, H, N.

Similarly prepared was **19b** (oil): IR (film) 2976, 2932, 1698, 1479, 1457, 1414, 1392, 1367, 1250, and 1152 cm^{-1}; NMR ($CDCl_3$) 5.95 (s, 2 H), 4.12 (s, 4 H), 3.82 (s, 4 H), 3.1 (bs, 4 H), 2.8 and 2.85 (s, 6 H), 1.48 (s, 40 H), and 1.35 (s, 6 H); MS (CI/CH_4) 765 (M + H). Anal. ($C_{37}H_{66}Cl_2N_4O_5$) C, H, N.

Deprotection of **19a** and **19b** (methanolic HCl) as previously described gave compounds **20a** and **20b**, respectively. Compound **20a**: mp 210-212 °C; IR (KBr) 2920, 2780, 2680, 2430, and 1460 cm^{-1}; NMR (D_2O) 7.05 (s, 2 H), 4.0 (s, 4 H), 3.9 (s, 4 H), 3.2-3.05 (m, 4 H), 2.8 and 2.73 (s, 6 H), 1.7 (bs, 4 H), and 1.4 (s, 8 H); MS (CI/CH_4) 378 (M + H). Anal. ($C_{18}H_{36}Cl_2N_4 \cdot 4HCl \cdot H_2O$) C, H, N; Cl: calcd, 39.16; found, 38.53. Compound **20b**: mp 228-230 °C; IR (KBr) 2940, 2854, 2754, 2690, 2510, 2456, 2412, 1608, 1468, and 824 cm^{-1}; NMR (D_2O) 7.05 (s, 2 H), 4.0 (s, 4 H), 3.9 (s, 4 H), 3.15-3.1 9 (m, 4 H), 2.8 and 2.75 (s, 6 H), 1.7 (bs, 4 H), and 1.4 (s, 6 H): MS (CI/CH_4) 365 (M + H). Anal. ($C_{17}H_{34}Cl_2N_4 \cdot 4HCl$) C, H, Cl, N.

By utilizing conditions similar to those used in the synthesis of **19**, compound **15b** was reacted with ethyltriphenylphosphonium bromide to give compound **21b** as an oil: IR (film) 1696, 1456, 1416, 1392, 1366, 1250, 1172, 1148, 936, 880, and 758 cm^{-1}; NMR ($CDCl_3$) 5.4 (bs, 2 H), 3.92 (s, 4 H), 3.7 (s, 4 H), 3.08 (s, 4 H), 2.82 and 2.72 (s, 6 H), 1.7 (d, J = 7.5 Hz, 6 H), 1.45 (s, 40 H), and 1.25 (s, 6 H); MS (CI/CH_4) 725 (M + H). Anal. ($C_{39}H_{72}N_4O_6 \cdot {}^1/_2PhCH_3$) C, H, N.

Deprotection (methanolic HCl) gave **22b**: mp 224-226 °C; IR (KBr) 2982, 2940, 2852, 2786, 2688, 2582, 2434, 1598, 1468, and 1456 cm^{-1}; NMR (D_2O) 6.4-6.3 (m, 2 H), 3.85 (s, 4 H), 3.75 (s, 4 H), 3.1-3.0 (m, 4 H), 2.75 and 2.73 (s, 6 H), 1.85 (d, J = 7.5 Hz, 6 H), 1.7 (s, 4 H), and 1.4 (s, 6 H); MS (CI/CH_4) 325 (M + H). Anal. ($C_{15}H_{40}N_4 \cdot 4HCl$) C, H, N, Cl.

Materials and Methods. HeLa Growth Inhibition Studies. HeLa cells were seeded in 60-mm tissue culture dishes at an initial density of 1×10^5 cells/dish. After 2 days growth, the average number of cells per dish was determined and this number was used as the baseline from which growth was calculated. Polyamine analogues were added to cells in complete medium and incubated for 2 days. At the end of this time, cells were counted, and the increase in cell number in drug-treated cultures was compared to that in untreated control cultures. The IC_{50} was that drug concentration that resulted in a 50% decrease in cell growth relative to controls.

Ethidium Bromide Displacement Assays. Measurement of polyamine analogue binding to calf thymus (CT) DNA by displacement of bound ethidium bromide was conducted as previously described.[7] Briefly, ethidium bromide (1.6 μM final concentration) (Sigma Chemical Co., St. Louis, MO) was added to 3 mL of buffer (2 mM HEPES, 10 μM EDTA, 9.4 mM NaCl, pH 7.0), and the fluorescence was recorded on an SLM-Aminco SPF-500C spectrofluorometer. Emission and excitation wavelengths were 598 and 546 nm, respectively. Upon the addition of calf thymus DNA, poly(dG-dC)·poly(dG-dC), or poly(dA-dT)·poly(dA-dT) (final concentration 2 μM, DNA phosphate), fluorescence increased on average 8-fold. Polyamine analogue was added in 10-μL aliquots and the decrease in fluorescence recorded. The IC_{50} value was defined as the concentration of polyamine required to decrease the fluorescence of the ethidium-DNA complex to 50%. None of the compounds tested absorbed or fluoresced at the critical wavelengths. Variability of multiple trials was generally less than 5%.

Aggregation of HeLa DNA. HeLa cell DNA was radioactively labeled by treatment of cultures with 4 μCi [^{3}H]thymidine (ICN, 60 Ci/mmol) for 3 days. DNA was then isolated by phenol/chloroform extraction and washed and the labeling quantitated. To 100 μL of assay buffer (25 mM Tris, 4.5 mM KCl, 3 mM $MgCl_2$, pH 7.6) were added 1.5 μg (10 μL) of HeLa DNA (approximately 20 000 cpm) and 10 μL of polyamine analogue at various concentrations. The mixture was incubated at 37 °C for 10 min, spun in an Eppendorf centrifuge for 2.5 min, and 50 μL of the resulting supernatant was counted for radioactivity. The IC_{50} value was defined as that concentration of polyamine analogue required to cause 50% of the radioactivity to aggregate and thus sediment out of the mixture. Due to cooperative binding of polyamines, aggregation profiles were very steep, the concentrations showing no aggregation and complete aggregation usually spanning only about 100 μM. For this reason, the error associated with IC_{50} determinations is estimated to be ±25%.

Supplementary Material Available: Preparation and analytical data for compounds **3**, **4**, **12a,b**, **13a,b**, **14a**, and **23-40** (6 pages). Ordering information is given on any current masthead page.

Synthesis of Structural Analogues of Lyngbyatoxin A and Their Evaluation as Activators of Protein Kinase C

Alan P. Kozikowski,*,† Patrick W. Shum,† Alakananda Basu,‡ and John S. Lazo‡

Neurochemistry Research, Mayo Clinic Jacksonville, 4500 San Pablo Road, Jacksonville, Florida 32224, and Department of Pharmacology, School of Medicine, Scaife Hall, University of Pittsburgh, Pittsburgh, Pennsylvania 15261.
Received March 14, 1991

Syntheses of several new analogues of lyngbyatoxin A from a single common intermediate are described. These compounds bear a carbon chain at the 7-position of the indolactam V (ILV) nucleus which contains either a hydrophilic or a lipophilic group. The effect of these minor structural alterations on the ability of the ILV analogues to activate the enzyme protein kinase C (PKC) was determined by measuring the extent of phosphorylation of calf thymus histone (III-S). Introduction of a hydroxyl group on the C-7 appendage was found to dramatically decrease compound **3**'s ability to activate PKC. This result is interpreted in terms of the decreased ability of **3** to associate with the membrane bilayer.

The enzyme protein kinase C (PKC) was discovered in 1977.[1] Over the intervening years, PKC was found to be a Ca^{2+}-activated, phospholipid-dependent enzyme[2] and was shown to play an important role in signal transduction. When a ligand binds to certain receptors on the cell surface thereby stimulating the cell, inositol phospholipids are

† Mayo Clinic Jacksonville.
‡ University of Pittsburgh.

(1) Inoue, M.; Kishimoto, A.; Takai, Y.; Nishizuka, Y. Studies on a Cyclic Nucleotide-independent Protein Kinase and Its Proenzyme in Mammalian Tissues. *J. Biol. Chem.* **1977**, *252*, 7610-7616.

0022-2623/91/1834-2420$02.50/0