Conformationally Restricted Analogues of $^{1}N,^{12}N$-Bisethylspermine: Synthesis and Growth Inhibitory Effects on Human Tumor Cell Lines

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Eight analogues of $^{1}N,^{12}N$-bisethylspermine (BES) with restricted conformations were synthesized in the search for new spermine mimetics with cytotoxic activities. By replacing the central butane segment of BES with a 1,2-disubstituted cyclopropane ring, a pair of cis/trans-isomers was obtained that introduced a spatial constraint in the otherwise freely mobile butane chain. An analogous pair of isomers was obtained when the butane segment was replaced with a 1,2-disubstituted cyclobutane ring or with a 2-butene residue. The six new BES analogues thus obtained (three pairs of cis/trans-isomers) were growth inhibitory at low-micromolar concentrations against four human tumor cell lines (A549, HT-29, U251MG, and DU145) but were less growth inhibitory against two other human tumor cell lines (PC-3 and MCF7). $^{1}N,^{12}N$-Bisethylspermyne, where the central butane segment of BES was replaced by the rigid 2-butyne segment, was devoid of growth inhibitory activity against five of the six human cell lines studied (DU145 being the only exception), a clear indication of the importance of conformational mobility at the $^{4}N,^{9}N$-butane segment of BES for its biological activity. When the butane segment was replaced by a benzene-1,2-dimethyl residue, the resulting BES analogue was devoid of growth inhibitory activity despite its cisoid conformation. The cytotoxicity of the analogues does not seem to be directly related to their uptake by the cells or to their effects on cellular polyamine levels. BES analogues with restricted conformations but which contained the equivalent of a two-carbon unit, rather than the natural four-carbon unit, at the central segment, such as 1,2-diaminocyclopropyl or 1,2-diaminocyclobutyl derivatives, were devoid of growth inhibitory effects at the concentrations studied. The development of conformationally restricted polyamine analogues appears to show promise in the further quest for polyamine-related therapeutic agents with specificity of action.

Introduction

The polyamines (putrescine, spermidine, and spermine) increase in proliferating tissues and are essential for cellular growth and division.[1,2] A number of polyamine analogues show promise as anticancer agents. They are able to kill cells and inhibit cell growth both in vitro and in vivo.[1] Most successful among these analogues have been the $^{\alpha}N,^{\omega}N$-alkyl derivatives of spermine's higher and lower homologues, although several alkylated diamines also show promise as inhibitors of tumor cell proliferation.[3] Of the many hypotheses advanced to explain the biological effects of the polyamines,[1,2] the ones that explore their binding to nucleic acids and to receptor targets are the most compelling. Since spermidine and spermine are strong bases, they are protonated at physiological pH[1,2] and can therefore bind to the negatively charged nucleic acids either by electrostatic interactions or by hydrogen bonding. Polyamines are known to interact with and induce structural changes in DNA in cell-free systems.[4] Spermidine and spermine can cause DNA to condense and aggregate and can induce both B-to-Z and B-to-A transitions in certain DNA sequences.[5] Molecular mechanics studies of spermine−DNA interactions have shown that, in a minimum energy conformation, spermine is bound in a cisoidal conformation that wraps around the major groove of the double helix.[6] Spermine and its $^{\alpha}$N,$^{\omega}$N-bisethylated higher and lower homologues, as well as spermidine, have been shown to bind to t-RNA.[7] Using ^{1}H NMR analysis spermine and $^{1}N,^{12}N$-bisethylspermine (BES) were found to bind at the TψC loop of t-RNA^{Phe}. The binding is not of an electrostatic nature, but rather a consequence of the different hydrogen-bonding modes that can be established between both types of molecules. Finally, polyamines have recently been shown to bind to receptors such as the *N*-methyl-D-glutamate (NMDA) receptor and the glutamate receptor (Glu-R) and to block and modulate a number of ion channels:[8] results that open new vistas in the pharmacology of the polyamines.

DNA-interacting drugs have long been of interest as anticancer agents. Mechanical models of the DNA double helix have created an image of a rigid structure; however, experimental evidence suggests that DNA has considerable flexibility.[9] When designing polyamine analogues that can bind to DNA, considered structural

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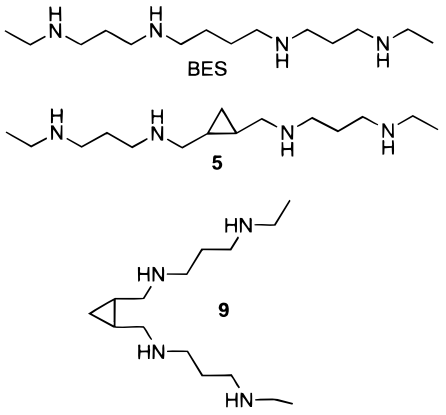


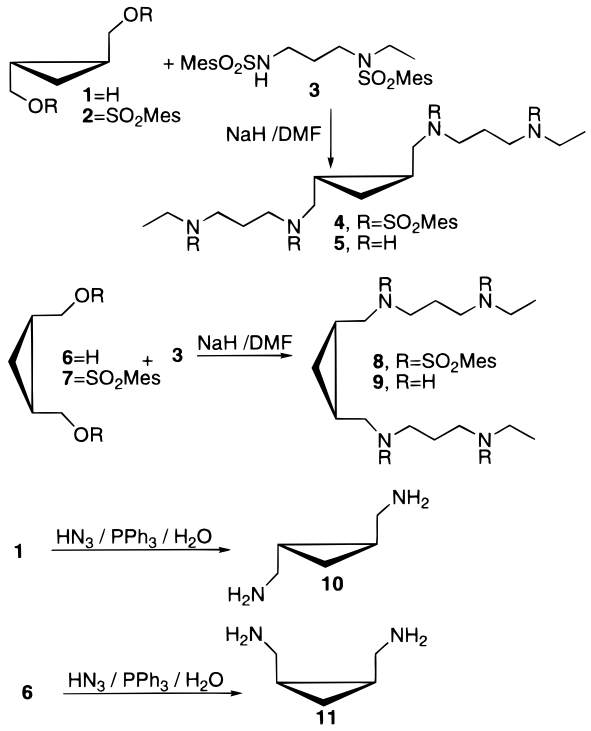
Figure 1. Conformations of $^{1}N,^{12}N$-bisethylspermine (BES) and the trans/cis-isomer pair **5** and **9** (see text) resulting from replacing the central butane segment in BES with *trans*- and *cis*-1,2-dimethylcyclopropyl residues.

modifications include variations in the number and distance of carbons between nitrogens and/or terminal N-substitutions of different types.[3] We report below the design and synthesis of chiral analogues of spermine that might bind DNA, t-RNA, or other polyamine binding sites and due to their increased conformational rigidity might do so in a modified and selective fashion. The introduction of restriction in the free rotation about the single bonds in a flexible molecule such as spermine (which has myriad potential conformations) can conceivably result in spatial rigidity that could introduce bends, kinks, or loops at their binding domains. The introduction of conformational restriction has been very fruitful in the design of peptidomimetics,[10] and a recent report described the synthesis of chiral pyrrolidyl polyamines.[11] We followed the principle that in order to be able to bind to biologically relevant sites, it would be best to construct rigid analogues of spermine in which the added atoms or bonds would affect the size and molecular weight of the parent compound as little as possible. The simple addition of a cyclopropyl ring to the butane segment of spermine introduces chirality and conformational restriction in an otherwise flexible molecule (Figure 1). The same is true for the introduction of a cyclobutyl ring and of a double bond. The synthesis of the $^{\alpha}N,^{\omega}N$-bisethyl derivatives of the aforementioned partially rigid spermine analogues is described below.

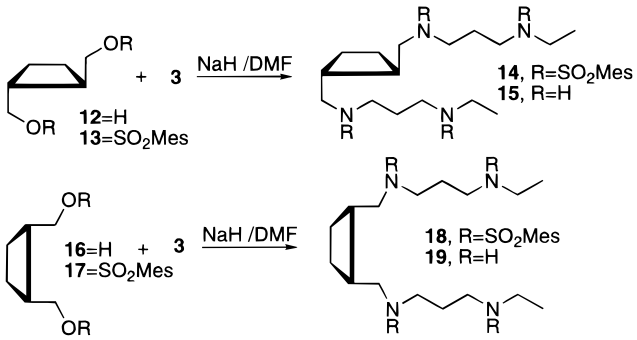
Chemistry

The synthesis of the cyclopropyl analogues of spermine was achieved using the *trans*- and *cis*-1,2-bis-(dihydroxymethyl)cyclopropanes as starting intermediates. Thus, the trans-isomer **1** was acylated with mesitylenesulfonyl chloride to give **2** (Scheme 1). The ester was then reacted with the sodium salt of N^1-ethylpropane-1,3-diamine dimesitylate (**3**). Displacement of the mesitylenesulfonate residues on **2** gave the tetramide **4**. Deprotection of the amine groups using established procedures[3b,12] resulted in a 75% yield of the tetrahydrochloride of **5**. This approach was also found to be useful in obtaining the cis-isomer **9**. Starting with the *cis*-bis(dihydroxymethyl) **6**, esterification with mesitylenesulfonyl chloride gave **7**, reaction with the sodium salt of **3** gave **8,** and deprotection in acid medium gave the tetrahydrochloride of **9**. This sequence of reactions

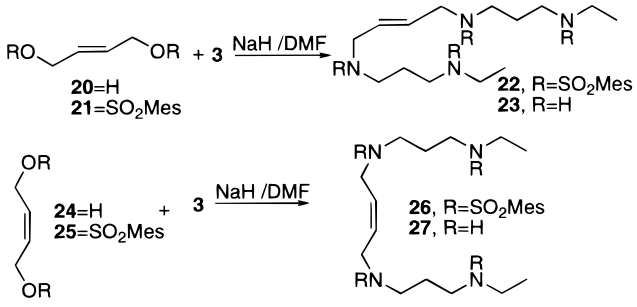
Scheme 1



Scheme 2



Scheme 3

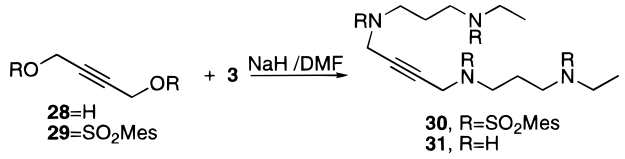


also lent themselves to upscaling when larger amounts of polyamines were needed.

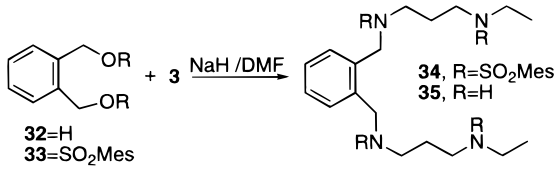
The synthetic outline also allowed for the synthesis of the cyclobutyl analogues (Scheme 2). The *trans*- and *cis*-diols **12** and **16** were esterified to **13** and **17** and the ester residues displaced with the sodium salt of **3** to give **14** and **18**. Deprotection of the amino groups gave the tetrahydrochlorides of **15** and **19**.

The same outline led to the synthesis of the geometrical isomers **23** and **27** (Scheme 3). Starting with the *trans*- and *cis*-2-butenediols **20** and **24**, by converting them to the esters **21** and **25**, and by reacting the latter

Scheme 4



Scheme 5



with **3** as described above, it was possible to obtain **22** and **26**. Deprotection of the amine groups yielded the tetrahydrochlorides of **23** and **27** in good overall yields (56% from the diol to the tetramine).

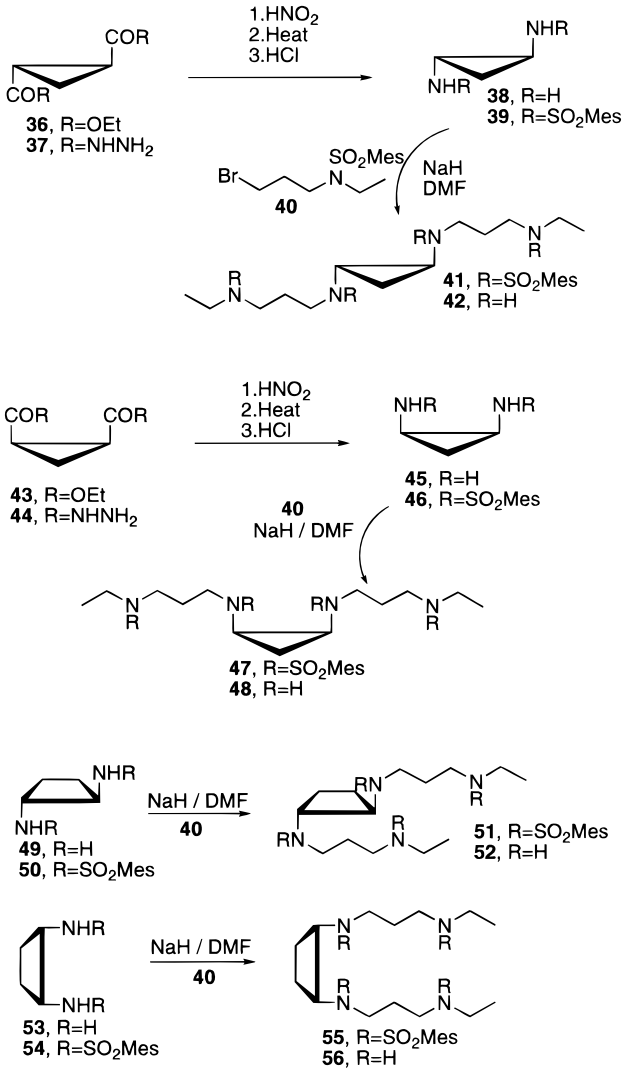
Of the synthetic approaches to the conformationally restricted spermine analogues that were tried and discarded, the use of the Mitsonobu reaction[13] is worth mentioning. The latter was used in an attempt to convert the bis(hydroxymethyl) derivatives (e.g., **1**, **6**, **12**, **16**, **20**, **24**) into the corresponding bis(aminomethyl) derivatives. As exemplified for **1** and **6** (Scheme 1), the diols were brought into reaction with hydrazoic acid in the presence of diisopropyl azodicarboxylate, followed by triphenylphosphine. Finally the phosphoranes were hydrolyzed with water, and the bases **10** and **11** were isolated as their hydrochlorides. Yields never exceeded 20−35% with any of the diols. Moreover, in the case of the diols **12** and **16,** the reaction also led to the formation of mixed 1-hydroxymethyl-2-aminomethyl derivatives that further decreased the overall yield of the reaction; hence, this approach was not pursued further.

To further examine the influence of conformation on the central butane segment, the synthesis of the bisethyl derivative of spermyne (the triple-bond analogue of bisethylspermine) **31** was carried out (Scheme 4). In **31** the four-carbon backbone is entirely rigid due to the sp bond at the central carbons and allows no conformational isomerism. It was prepared by starting with the commercially available butyne-1,4-diol (**28**) which was mesitylated to **29**, the latter brought into reaction with **3** to give the tetramide **30**. Deprotection to **31** followed the procedure mentioned above.

To further examine the influence of a bulky substituent on the butane segment of the analogues, the bisethyl derivative **35** was prepared (Scheme 5). In **35** the central four carbons are part of a 1,2-benzyldiamine structure and are therefore restricted by the aromatic sp^2 bonds to a cisoid conformation analogous to that present in **27** (Scheme 3). This analogue was constructed starting with commercially available benzene-1,2-dimethanol (**32**) which was mesitylated to **33**, the latter brought into reaction with **3** to give **34**. Deprotection to **35** followed the usual procedure.

When the isomeric cyclopropyl and cyclobutyl *cis*- and *trans*-1,2-diamines were used as starting materials, they were found to be easily alkylated to give the tetramines (Scheme 6). By using the Curtius rearrangement on the hydrazides of the *trans*- and *cis*-1,2-bis(ethoxycarbonyl)-

Scheme 6



cyclopropanes **36** and **43**, it was possible to convert them into the 1,2-diaminocyclopropanes **38** and **45**. The syntheses of the latter by other procedures have been reported,[14−16] but we found it more convenient to use the above-mentioned method. The trans- and cis-1,2-cyclobutane-diamines **49** and **53** are known.[17] The diamines were converted into the corresponding sulfonylmesitylene derivatives **39**, **46**, **50**, and **54**, and the latter were alkylated with *N*-ethyl-*N*-(3-bromopropyl)-mesitylenesulfonamide (**40**) in the presence of sodium hydride. The tetramides **41**, **47**, **51**, and **55** thus obtained were cleaved with 33% HBr in glacial acetic acid in the presence of phenol, and the corresponding tetramines **42**, **48**, **52**, and **56** were isolated as their tetrahydrochlorides.

Biological Results and Discussion

Six human tumor cell lines were used to assay for the growth inhibitory activities of the new polyamine analogues (Table 1). As a reference, the growth inhibitory activity of N^1,N^{12}-bisethylspermine (BES) (the flexible chain polyamine analogue) was assayed on lung (A549), colon (HT-29), prostate (PC-3 and DU145), breast (MCF7), and brain (U251MG NCI) human tumor cells. BES was effective against all cell lines (Table 1). The

Table 1. Inhibition of Cell Growth by Polyamine Analogues in Human Tumor Cell Lines[a]

	ID_{50} (μM)[b]					
drug	A549	HT-29	PC-3	MCF7	U251MG NCI	DU145
BES	0.20	0.43	0.24	0.55	0.20	0.04
9	0.12	1.60	7.40	>31.25	0.10	0.02
5	0.25	1.40	12.40	>31.25	0.10	0.06
19	0.10	1.50	>31.25	25.50	0.10	0.05
15	0.30	1.60	>31.25	>31.25	0.12	0.01
27	0.25	1.60	3.60	9.50	0.55	0.05
23	0.26	1.40	1.40	>31.25	2.00	0.14
31	>31.25	>31.25	>31.25	>31.25	>31.25	1.33
35	>31.25	>31.25	>31.25	>31.25	>31.25	12.60

[a] Exponentially growing cells were exposed to graded concentrations of polyamine analogues for 6 days. The MTT assay was performed to determine cell survival. ID_{50} values were determined by extrapolation from a plot of relative changes in absorbance at 570 nm. Each value is an average of experiments performed in quadruplicate. [b] ID_{50} represents the drug concentration that killed 50% of the cells.

conformationally restricted analogues **9**, **5**, **19**, **15**, **27**, and **23** were found to have good growth inhibitory effects against A549, HT-29, U251MG NCI, and DU145 cells. The ID_{50} values were of the same order of magnitude as those found for BES (in the range from 0.01 to 2.00 μM). No significant differences in cytotoxicity were observed between the trans- and cis-isomeric pairs (i.e., **9** and **5**, **19** and **15**, **27** and **23**).

To check whether the cytotoxicities of the analogues are due to their cellular uptake and/or effects on cellular polyamine levels, we measured the cellular polyamine and analogue concentrations in DU145 prostate tumor cells treated with some of the polyamine analogues used in our studies. The DU145 cells were chosen because this cell line has shown good sensitivity to most polyamine analogues used in our studies (Table 1). The cellular polyamines and analogue concentrations are listed in Table 2. With the exception of the *trans*-cyclopropyl analogue **5**, all other analogues are readily taken up by the cells. At concentrations between 2 and 5 μM, the intracellular concentrations of analogues (except **5**) are comparable with the intracellular spermine level in control untreated cells. While compounds **5, 9**, **15**, and **27** are highly cytotoxic to DU145 cells ($ID_{50} \leq 0.06$ μM), compounds **31** and **35** have very little cytotoxic effect ($ID_{50} > 1$ μM). Thus, cytotoxicities of the analogues do not seem to be directly related to intracellular analogue concentrations. While compounds **9**, **15**, and **27** at concentrations between 2 and 5 μM deplete cellular putrescine, spermidine, and spermine levels within 5 days of treatment, compounds **5**, **31**, and **35** have no appreciable effect on cellular polyamine levels.

With PC-3 and MCF7 cell lines the restricted analogues did not exhibit strong growth inhibition (with the exception of **23** in PC-3), at variance with the results for BES. In PC-3 cells slightly different ID_{50} values were observed for the *trans*-cyclopropyl analogue **5** and for the cis-isomer **9**. Both cyclobutyl isomers **15** and **19** were minimally growth inhibitory. The double bond isomers **23** and **27** were active with slightly different ID_{50} values. In MCF7 cells most of the isomers were inactive; however, the cis-double-bond isomer **27** was moderately active, while the trans-isomer **23** was inactive. It is conceivable that the difference in growth inhibition between the isomeric pairs may increase when the analogues are assayed in vivo and additional pharmacokinetic factors come into play.

Table 2. Polyamine and Analogue Levels in DU145 Cells Treated with Polyamine Analogues[a]

			concentration (nmol/10^6 cells)			
drug	concn (μM)	day of treatment	PU	SD	SP	analogue
control	0	1	0.19	2.44	3.18	
		2	0.19	2.40	3.04	
		3	0.11	0.15	2.63	
		5	0.04	1.61	1.52	
9	0.02	1	0.18	1.64	2.16	0.90
		3	0.05	1.01	1.97	0.15
	2.00	1	NQ	0.10	0.40	4.08
		3	NQ	NQ	0.06	3.97
5	0.02	1	0.20	1.92	2.48	0.14
		3	0.08	1.41	2.73	0.06
	2.00	1	0.55	2.63	2.64	0.33
		3	0.05	1.38	2.28	0.18
15	0.02	3	0.162	2.13	3.54	0.07
		5	0.081	1.6	2.10	0.05
	5.00	3	0.036	0.15	0.49	1.75
		5	0.031	0.06	0.20	1.84
27	0.02	3	0.11	1.79	2.99	0.04
		5	0.04	1.73	1.71	0.03
	5.00	3	0.06	0.02	0.09	2.76
		5	0.03	0.02	0.07	2.54
31	0.	3	0.11	1.73	3.13	0.06
		5	0.03	1.55	1.55	0.03
	5.00	3	0.04	0.90	2.56	2.16
		5	0.06	0.59	1.33	1.71
35	10.00	3	0.12	1.50	2.70	0.82
		5	0.06	1.84	2.13	1.25

[a] Each number is an average of two separate experiments. NQ, not quantifiable; PU, putrescine; SD, spermidine; SP, spermine.

The total lack of activity of bisethylspermyne (**31**) in five cell lines (A549, HT-29, PC-3, MCF7, and U251MG) is noteworthy. This is the rigid analogue of BES at its central four-carbon segment, and its inability to adopt any spatial compromise related to binding might relate to its lack of growth inhibitory effects. Although **31** showed some growth inhibition in the DU145 prostate cell line, its ID_{50} value was almost 100-fold higher than that of several of the other analogues. These results lend support to previous reports that suggested that the hydrogens carried by the central nitrogen atoms may be a major binding force in spermine−nucleic acid interactions.[6,7] The lack of growth inhibition found for **35** is also revealing. Although in **35** the *N*-ethylamino-^{1}*N*-propyl side chains are bound to the central four-carbon chain by sp^2 bonds, as is the case with **27** and **23,** thus conferring to **35** a cisoid conformation, the analogue carrying the aromatic ring is biologically inactive. This again stresses the need not to depart too greatly from the structures of the natural polyamines when designing conformationally restricted analogues.

It is evident from the data shown in Table 2 that the least active polyamines, i.e., **31** and **35**, were taken up by cells as efficiently as the most active ones. Thus, cellular uptake does not seem to be a factor that influences the cell growth inhibition found with the new polyamine analogues. There appears to be a differential effect in cell uptake related to cis/trans-isomerism: the cis-isomer **9** entered the cells at much higher concentra-

tions than its trans-isomer **5**. This uptake effect clearly influenced the intracellular pools of the natural polyamines, which were more depleted in the presence of **9** than in the presence of **5**. Cellular polyamine pools were not depleted by the uptake of the weak cell growth inhibitory **31** and **35**. Thus, no clear-cut relationship between the depletion of intracellular polyamine pools and inhibition of cell growth can be drawn from the data shown in Table 2, further evidence of the complexity of the biological effects of polyamines (see Introduction).

The tetramines **42**, **48**, **52**, and **56** did not exhibit growth inhibitory effects on the aforementioned cell lines, as might be expected from polyamines having a 1,2-diaminoethane backbone.[18]

Conclusions

We report on the synthesis of conformationally restricted analogues of ^{1}N,^{12}N-bisethylspermine (BES). Restriction was achieved by introducing a cyclopropyl, a cyclobutyl, or a double bond in the central butane segment of BES. The cis/trans-isomeric pairs thus obtained showed growth inhibition in several human tumor cell lines. The difference between these isomer pairs was not experimentally significant. This could be due to an insufficient degree of restriction imposed on the isomer pairs. Introduction of further restrictions at other sites of the spermine chain might lead to more pronounced differential effects among the isomers. Nevertheless, the significant growth inhibition achieved with several cell lines with these new synthetic polyamines opens a new vista in the development of polyamine analogues with antiproliferative effects.

Experimental Section

NMR spectra were obtained using a Bruker MSL 300 spectrometer. Reactions were monitored using TLC on silica gel plates (0.25 mm thick). Flash chromatography was performed on columns packed with EM Science silica gel, 230−400 mesh ASTM. Melting points were determined on an Electrothermal IA 9100 digital melting point apparatus. Mass spectra (EI) were obtained on a Kratos MS-80RFA; mass spectra (FAB) were obtained on a Autospec (VG) spectrometer that was also used to secure ESI (electrospray) spectra. Matrix assisted laser desorption ionization (MALDI) mass spectra were obtained using a Bruker Reflex II spectrometer equipped with a 337-nm laser and delayed extraction. MALDI and ESI spectra of polyamines were secured by injecting their hydrochlorides.

***N*-Ethyl-*N*-(3-(mesitylenesulfonylamino)propyl)mesitylenesulfonamide (3).** ^{1}N-Ethylpropane-1,3-diamine[19] (5.8 g, 56.8 mmol) was dissolved in a mixture of 50 mL of 4 N NaOH and 25 mL at dioxane, and a solution of mesitylenesulfonyl chloride (27.22 g, 124.9 mmol) in 100 mL of dioxane was added dropwise to the stirred solution kept at 5 °C. Once the addition was completed the solution was kept at 20 °C during a further 18 h. It was then poured over an excess of ice water; the precipitate was filtered, dried, and crystallized from ethanol−water: 19.8 g (73%) of **3** obtained as a white solid; mp 101−102 °C; ^{1}H NMR ($CDCl_3$) δ 0.95 (t, J = 6.99 Hz, 3H, CH_3), 1.68 (pent, J = 6.25 Hz, 2H, $NCCH_2$), 2.29 (s, 6H, $2CH_3$), 2.56 (s, 6H, $2CH_3$), 2.62 (s, 6H, $2CH_3$), 2.91 (m, 2H, $NHCH_2$), 3.12 (q, J = 7.0 Hz, 2H, NCH_2), 3.31 (t, J = 6.44 Hz, 2H, NCH_2), 4.93 (br, NH), 6.93 (s, 2H, aromatic), 6.95 (s, 2H, aromatic); ^{13}C NMR ($CDCl_3$) δ 12.57, 20.93, 22.81, 22.89, 27.84, 39.26, 39.99, 42.29, 131.95, 132.05, 133.32, 138.90 140.00, 142.02, 142.57. Anal. Calcd for $C_{23}H_{34}N_2O_4S_2$: C, 59.23; H, 7.30; N, 6.01. Found: C, 59.15; H, 7.26; N, 6.59.

***trans*-1,2-Bis((mesitylenesulfonyloxy)methyl)cyclopropane (2).** Diol **1**[20] (1.65 g, 16.18 mmol) was dissolved in 10 mL of pyridine, the solution cooled to 0 °C, and mesitylenesulfonyl chloride (9.13 g, 41.75 mmol) in 23 mL of pyridine added slowly over a span of 15 min. The mixture was stirred at 25 °C for 3 h and then poured onto ice (75 g), and the white solid precipitate was filtered and recrystallized from ethanol: 2.8 g (37%); mp 81−82 °C; ^{1}H NMR ($CDCl_3$) δ 0.50−0.65 (m, 2H, $cyPrH_2$), 1.10 (m, 2H, cyPrH), 2.31 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 2.60 (s, 6H, $2CH_3$), 2.61 (s, 6H, $2CH_3$), 3.80 (m, 4H, $2CH_2$), 6.97 (s, 2H, Ph), 6.99 (s, 2H, Ph); ^{13}C NMR ($CDCl_3$) δ 9.47, 16.36, 21.04, 22.57, 72.13, 130.62, 131.76, 139.79, 143.37; MS-EI m/z 466 (M^+), 452, 386, 118 (100%). Anal. Calcd for $C_{23}H_{30}O_3S_2$: C, 66.03; H, 7.18. Found: C, 66.05; H, 7.15.

3,7,13,17-Tetrakis(mesitylenesulfonyl)-9,10-[(*E*)-1,2-cyclopropyl]-3,7,13,17-tetraazaoctadecane (4). Amide **3** (2.5 g, 5.37 mmol) was dissolved in 40 mL of anhydrous DMF, NaH (95%, 300 mg) was slowly added under argon and the mixture was stirred at 25 °C for 0.5 h, after which a solution of **2** (1.16 g, 2.49 mmol) in 35 mL of anhydrous DMF was added over a period of 10 min. The stirred reaction mixture was heated to 70 °C for 4 h, then cooled to 0 °C, and quenched by the addition of 10 mL of water. The mixture was extracted with ether (3 × 30 mL); the combined organic layers were washed with water (4 × 30 mL) and then brine (2 × 25 mL), dried (Na_2SO_4), and evaporated to dryness. The oily residue was purified by flash chromatography on silica gel using hexanes−ethyl acetate (8:2) as eluant to give **4** as a low-melting white semisolid (1.0 g, 40% yield): ^{1}H NMR ($CDCl_3$) δ 0.34 (m, 2H, $cyPrH_2$), 0.75 (m, 2H, cyPrH), 0.95 (t, J = 7.1 Hz, 6H, $2CH_3$), 1.70 (m, 4H, $2CH_2$), 2.30 (s, 12H, $4CH_3$), 2.54 (s, 24H, $8CH_3$), 2.75 (m, 2H, NCH), 3.10 (m, 14H, NCH_2), 6.90 (s, 4H, Ph), 6.93 (s, 4H, Ph); ^{13}C NMR ($CDCl_3$) δ 12.69, 15.69, 20.95, 22.75, 25.29, 40.17, 42.64, 43.40, 48.90, 131.92, 131.98, 132.99, 133.26, 140.08, 140.12, 142.34, 142.49; MS-FAB m/z 999.4 ($M + 1$)$^+$, 815.3, 633.3, 533.2.

3,7,13,17-Tetraaza-9,10-[(*E*)-1,2-cyclopropyl]octadecane (5) Tetrahydrochloride. Phenol (1.68 g, 17.8 mmol) and hydrogen bromide (33%) in glacial acetic acid (8.9 mL) were added in tandem to a solution of **4** (447 mg, 0.45 mmol) in methylene chloride (5 mL) at 25 °C. The solution was stirred for 48 h, after which water (6 mL) was added and the aqueous layer was separated and extracted with methylene chloride (3 × 8 mL). The aqueous layer was evaporated under reduced pressure, the residue was taken up in 10 N sodium hydroxide (3 mL), and the solution was extracted with chloroform (12 × 6 mL). The pooled extracts were evaporated to dryness, leaving behind a thick gum that was taken up in dry ethyl ether. Hydrogen chloride was bubbled through the solution to precipitate the tetrahydrochloride of **5** as a white solid; 140 mg (75% yield). The product was recrystallized from ethanol/ether: mp 270 °C dec; ^{1}H NMR (D_2O) δ 0.88 (dd, J = 6.8, 7.0 Hz, 2H, $cyPrH_2$), 1.25 (t, J = 6.1 Hz, 2H, cyPrH), 1.30 (t, J = 7.3 Hz, 6H, $2CH_3$), 2.12 (pent, J = 7.9 Hz, 4H, $NCCH_2$), 2.89 (dd, J = 8.5, 12.1 Hz, 2H, NCH_2), 3.08−3.30 (m, 14H, NCH_2); ^{13}C NMR (D_2O) δ 13.62, 14.10, 17.25, 26.35, 46.70, 47.49, 47.81, 54.41; MS-ESI m/z 271.3 ($M + 1$)$^+$, 190.1, 145.0 (100%). Anal. Calcd for $C_{15}H_{40}Cl_4N_4$: C, 43.06; H, 9.57; N, 13.40. Found: C, 42.99; H, 10.00; N, 13.35.

***cis*-1,2-Bis((mesitylenesulfonyloxy)methyl)cyclopropane (7)** was obtained from **6**[20] as described above for **2**: mp 90−91 °C (ethanol−water); ^{1}H NMR ($CDCl_3$) δ 0.35 (m, 1H, $cyPrH_2$), 0.93 (m, 1H, $cyPrH_2$), 1.32 (m, 2H, cyPrH), 2.32 (s, 6H, $2CH_3$), 2.60 (s, 12H, $4CH_3$), 3.01 (m, 4H, $2CH_2$), 6.99 (s, 4H, Ph); ^{13}C NMR ($CDCl_3$) δ 9.71, 15.25, 21.08, 22.61, 69.61, 130.63, 131.79, 139.82, 143.39; MS-EI m/z 466 (M^+), 452, 266, 200, 185, 171, 119 (100%). Anal. Calcd for $C_{23}H_{30}O_3S_2$: C, 66.03; H, 7.18. Found: C, 66.01; H, 7.20.

3,7,13,17-Tetrakis(mesitylenesulfonyl)-9,10-[(*Z*)-1,2-cyclopropyl]-3,7,13,17-tetraazaoctadecane (8) was obtained by the reaction of **7** with **3** as described for **4**: mp 56−58 °C (ethyl acetate−hexane) (64%); ^{1}H NMR ($CDCl_3$) δ 0.07 (m, 1H, $cyPrH_2$), 0.76 (m, 1H, $cyPrH_2$), 0.95 (t, J = 7.1 Hz, 8H, $2CH_3$ and cyPrH), 1.71 (m, 4H), 2.28 (s, 6H), 2.29 (s, 6H), 2.54 (s,

12H), 2.55 (s, 12H), 2.69−2.90 (m, 2H), 3.00−3.20 (m, 12H), 3.29−3.40 (m, 2H), 6.92 (s, 4H), 6.93 (s, 4H); ^{13}C NMR ($CDCl_3$) δ 12.63, 13.92, 20.96, 22.73, 22.77, 25.50, 40.07, 42.65, 43.49, 45.17, 131.92, 133.00, 146.06, 142.35; MS-FAB, *m/z* 999.40 (M^+), 815.30, 533.20, 167.10, 119.00 (100%). Anal. Calcd for $C_{51}H_{76}N_4O_8S_4$: C, 61.20; N, 7.60; N, 5.60. Found: C, 61.18; H, 7.62; N, 5.56.

3,7,13,17-Tetraaza-9,10-[(*Z*)-1,2-cyclopropyl]octadecane (9) tetrahydrochloride was prepared following the procedure described for **5**: mp 240 °C dec (75%); ^{1}H NMR (D_2O) δ 0.43 (m, 1H), 1.0 (m, 1H), 1.29 (t, J = 6.3 Hz, 8H), 2.04 (m, 4H), 2.97 (m, 8H), 3.11 (m, 8H); ^{13}C NMR (D_2O) δ 12.63, 13.53, 15.74, 26.89, 45.82, 47.30, 47.39, 50.58; MS-ESI *m/z* 271.3 ($M + 1$)$^+$. Anal. Calcd for $C_{15}H_{80}Cl_4N_4$: C, 43.06; H, 9.57; N, 13.40. Found: C, 43.08; H, 10.01; N, 13.45.

***trans*-1,2-Bis((mesitylenesulfonyloxy)methyl)cyclobutane (13)** was obtained (83%) from the diol **12**[21] following the procedure described for **2**: mp 77−78 °C; ^{1}H NMR ($CDCl_3$) δ 1.65 (m, 2H, cyBuH$_2$), 1.95 (m, 2H, cyBuH$_2$), 2.30 (s, 6H, 2CH$_3$), 2.40 (m, 2H, 2cyBuH), 2.60 (s, 12H, 4CH$_3$), 3.88 (d, 4H, CH$_2$O), 6.96 (s, 4H, Ph); ^{13}C NMR ($CDCl_3$) δ 20.67, 20.86, 22.39, 36.37, 71.28, 130.35, 131.59, 131.62, 139.60, 143.17; MS-EI *m/z* 480 (M^+), 281, 183, 119 (100%). Anal. Calcd for $C_{24}H_{32}O_3S_2$: C, 66.67; H, 7.41. Found: C, 66.63; H, 7.38.

3,7,13,17-Tetrakis(mesitylenesulfonamido)-9,10-[(*E*)-1,2-cyclobutyl]-3,7,13,17-tetraazaoctadecane (14). Amide **3** (4.6 g, 10 mmol) was dissolved in 25 mL of DMF, and 300 mg (10 mmol) of an 85% dispersion of sodium hydride in oil was added while the solution was kept under N_2 at 5 °C with constant stirring. After 1 h a solution of 2.2 g (4.5 mmol) of **13** in 25 mL of DMF was slowly added, and the mixture was kept at 75−80 °C for 4 h. The solution was then evaporated to dryness in vacuo, the solid partitioned between chloroform and water, and the organic layer separated, washed with water, dried (Na_2SO_4), and evaporated to dryness. The residue was finally purified by chromatography through a silica gel column using hexane−ethyl acetate (8:2) as the eluant. Tetramide **14** was recovered as a glassy oil: 3.0 g (66%); ^{1}H NMR ($CDCl_3$) δ 0.93 (t, 6H, 2CH$_3$), 1.30 (m, 2H, cyBuH$_2$), 1.70 (m, 6H, cyBuH$_2$, CH$_2$), 2.10 (br, 2H, 2cyBuH), 2.28 (s, 12H, CH$_3$), 2.52 (s, 24H, CH$_3$), 2.85−3.20 (m, 14H, CH$_2$), 3.30 (m, 2H, −CHN-), 6.90, 6.92 (s, s, 8H, Ph); ^{13}C NMR ($CDCl_3$) δ 12.63, 20.93, 22.72, 22.81, 23.28, 24.97, 37.45, 39.95, 42.29, 43.68, 50.01, 131.91, 133.70, 139.97, 140.03, 142.31; MS-FAB *m/z* 1012.4546.

3,7,13,17-Tetraaza-9,10-[(*E*)-1,2-cyclobutyl]octadecane (15) Tetrahydrochloride. This was obtained following the procedure described for **5**. Tetramide **14** (1.0 g, 0.98 mmol) was treated with phenol (2.6 g, 27.6 mmol) and 45 mL of 33% hydrogen bromide. After the usual workup the tetrahydrochloride **15** (355 mg, 87%) was crystallized from aqueous ethanol: mp 288−289 °C dec; ^{1}H NMR (D_2O) δ 1.30 (t, 6H, CH$_3$), 1.80 (m, 2H), 2.02−2.28 (m, 6H), 2.50 (br, 2H), 3.02−3.30 (m, 16H); ^{13}C NMR (D_2O) δ 14.22, 26.43, 38.77, 46.82, 47.60, 48.40, 55.03; MS-ESI *m/z* 286.31 (M^+). Anal. Calcd for $C_{16}H_{42}Cl_4N_4$: C, 44.44; H, 9.72; N, 12.96. Found: C, 44.48; H, 9.69; N, 12.93.

***cis*-1,2-Bis((mesitylenesulfonyloxy)methyl)cyclobutane (17)** was obtained (80%) from the diol **16**[21] following the procedure described for **2**: mp 92−93 °C; ^{1}H NMR ($CDCl_3$) δ 1.72 (m, 2H), 2.05 (br, 2H), 2.31 (s, 6H), 2.57, 2.59 (s, 12H), 2.78 (br, 2H), 3.85−4.10 (m, 4H), 6.96 (s, 4H); ^{13}C NMR ($CDCl_3$) δ 20.69, 20.89, 21.49, 22.41, 34.94, 69.14, 130.41, 131.66, 139.62, 139.65, 143.20, 143.25; MS-EI *m/z* 480 (M^+), 281, 199, 183. Anal. Calcd for $C_{24}H_{32}O_3S_2$: C, 66.67; H, 7.41. Found: C, 66.70; H, 7.49.

3,7,13,17-Tetrakis(mesitylenesulfonamido)-9,10-[(*Z*)-1,2-cyclobutyl]-3,7,13,17-tetraazaoctadecane (18) was obtained (70%) as an oil from the condensation of **17** with **3** following the procedure described for **14**: ^{1}H NMR ($CDCl_3$) δ 0.95 (t, 6H), 1.30 (m, 4H), 1.70 (m, 6H), 2.30 (s, 12H), 2.58 (s, 24H), 3.10 (m, 16H), 6.95 (s, 8H); ^{13}C NMR ($CDCl_3$) δ 12.53, 20.86, 22.65, 22.72, 22.89, 25.37, 33.95, 39.98, 42.60, 43.31, 45.46, 131.92, 133.17, 139.94, 142.25, 142.45; MS-FAB *m/z* 1012.4546.

3,7,13,17-Tetraaza-9,10-[(*Z*)-1,2-cyclobutyl]octadecane (19) tetrahydrochloride was obtained (82%) from **18** following the procedure described for the synthesis of **15**: mp 271−272 °C dec; ^{1}H NMR (D_2O) δ 1.30 (t, 6H), 1.85 (m, 2H), 2.08−2.25 (m, 6H), 2.50 (br, 2H), 3.10−3.30 (m, 16H); ^{13}C NMR (D_2O) δ 14.24, 26.44, 38.77, 46.80, 47.60, 48.41, 55.05; MS-ESI *m/z* 286.31 (M^+). Anal. Calcd for $C_{16}H_{42}Cl_4N_4$: C, 44.44; H, 9.72; N, 12.96. Found: C, 44.39; H, 9.69; N, 12.90.

(*E*)-2-Butene-1,4-diyl Bis[mesitylenesulfonate] (21). Diol **20**[22] (1.76 g, 20 mmol) and benzyltriethylammonium bromide (270 mg, 1 mmol) were dissolved in 30 mL of a 50% potassium hydroxide solution and 30 mL of dioxane. The mixture was stirred at 5 °C, and mesitylenesulfonyl chloride (8.72 g, 40 mmol) dissolved in 30 mL of dioxane was added dropwise. When the addition was over, stirring was continued for 2 h, water was then added, and the white precipitate was filtered and crystallized from chloroform−hexane: 7.0 g (77%); mp 119−120 °C; ^{1}H NMR ($CDCl_3$) δ 2.35 (s, 6H), 2.60 (s, 12H), 4.45 (d, 4H), 5.75 (br, 2H), 6.95 (s, 4H); ^{13}C NMR ($CDCl_3$) δ 20.96, 22.52, 67.96, 127.67, 131.69, 131.74, 139.79, 143.45; MS-EI *m/z* 452 (M^+), 253, 200, 183. Anal. Calcd for $C_{22}H_{28}O_6S_2$: C, 58.40; H, 6.19. Found: C, 58.35; H, 6.22.

(*E*)-3,7,12,16-Tetrakis(mesitylenesulfonyl)-3,7,12,16-tetraazaoctadec-9-ene (22). This was obtained following the procedure described for **14**. Amide **3** (4.15 g, 9.2 mmol) was transformed into its sodium salt by reaction with 276 mg (9.2 mmol) of NaH (85% dispersion in oil) and the latter brought into reaction with 1.94 g (4.2 mmol) of diester **21**. The residue was obtained after evaporation of the chloroform solution and crystallized from ethyl acetate−hexane: 3.9 g (92%); mp 145−146 °C; ^{1}H NMR ($CDCl_3$) δ 0.95 (t, J = 7.1 Hz, 6H, 2CH$_3$), 1.65 (m, 4H, NCCH$_2$), 2.29 (2, 12H, 4CH$_3$), 2.53 (s, 12H, 4CH$_3$), 2.55 (s, 12H, 4CH$_3$), 2.99 (t, J = 7.3 Hz, 8H, NCH$_2$), 3.08 (q, J = 7.1 Hz, 4H, NCH$_2$), 3.65 (d, J = 5 Hz, 4H, C=C−CH$_2$), 5.45 (m, 2H, CH=CH), 6.92 (s, 4H, aromatic), 6.93 (s, 4H, aromatic); ^{13}C NMR ($CDCl_3$) δ 12.62, 20.90, 22.68, 22.77, 25.56, 40.02, 42.53, 43.79, 43.83, 128.51, 131.88, 132.00, 132.71, 133.00, 140.02, 140.17, 142.30, 142.66; MS-FAB *m/z* 985.4 ($M + 1$)$^+$.

(*E*)-3,7,12,16-Tetraazaoctadec-9-ene (23) tetrahydrochloride was obtained (86%) from **22** following the procedure used for **15**: mp 303−304 °C dec; ^{1}H NMR (D_2O) δ 1.30 (t, J = 7.4 Hz, 6H, 2CH$_3$), 2.10 (m, 4H, NCCH$_2$), 3.13 (m, 12H, NCH$_2$), 3.77 (m, 4H, NCH$_2$), 6.07 (m, 2H, CH=CH); ^{13}C NMR (D_2O) δ 13.34, 25.57, 45.90, 46.71, 46.80, 51.04, 131.25; MS-MALDI *m/z* 257.2 ($M + 1$)$^+$. Anal. Calcd for $C_{15}H_{40}Cl_4N_4$: C, 43.06; H, 9.57; N, 13.40. Found C, 43.09; H, 9.60; N, 13.48.

(*Z*)-2-Butene-1,4-diyl bis[mesitylenesulfonate] (25) was obtained (75%) from **24** (Aldrich) by following the procedure described for **21**: mp 71−72 °C; ^{1}H NMR ($CDCl_3$) δ 2.25 (s, 6H), 2.50 (s, 19H), 4.40 (d, 4H), 5.62 (br, 2H), 6.90 (s, 4H); ^{13}C NMR ($CDCl_3$) δ 20.90, 22.40, 63.66, 127.54, 131.71, 139.69, 143.46; MS-EI *m/z* 452 (M^+), 253, 199, 183. Anal. Calcd for $C_{22}H_{28}O_6N_2$: C, 58.40; H, 6.19. Found: C, 58.45; H, 6.25.

(*Z*)-3,7,12,16-Tetrakis(mesitylenesulfonyl)-3,7,12,16-tetraazaoctodec-9-ene (26) was prepared (88%) by condensation of **25** with **3** following the procedure described for **22**: mp 143−144 °C; ^{1}H NMR ($CDCl_3$) δ 0.93 (t, J = 7.1 Hz, 6H), 1.60−1.70 (m, 4H), 2.29 (s, 6H), 2.30 (s, 6H), 2.52 (s, 12H), 2.55 (s, 12H), 2.99 (m, 8H), 3.07 (q, J = 7.1 Hz, 4H), 3.74 (d, J = 4.6 Hz), 6.90 (s, 4H, aromatic), 6.93 (s, 4H, aromatic); ^{13}C NMR ($CDCl_3$) δ 12.69, 20.93, 22.71, 22.80, 25.20, 40.12, 42.56, 43.25, 47.06, 129.50, 131.93, 132.03, 132.86, 133.25, 140.07, 140.12, 142.36, 142.62; MS-FAB *m/z* 985.4 ($M + 1$)$^+$.

(*Z*)-3,7,12,16-Tetraazaoctadec-9-ene (27) tetrahydrochloride was obtained (87%) from **26** following the procedure used for **5**: mp above 300 °C dec; ^{1}H NMR (D_2O) δ 1.30 (t, J = 7.3 Hz, 6H), 2.10−2.59 (m, 4H), 3.05−3.25 (m, 12H), 3.87 (d, J = 4.8 Hz, 4H), 5.98 (t, J = 4.8 Hz, 2H); ^{13}C NMR (D_2O) δ 13.35, 25.69, 45.93, 46.70, 46.96, 47.02, 129.31; MS-MALDI *m/z* 257.1 ($M + 1$)$^+$. Anal. Calcd for $C_{15}H_{40}Cl_4N_4$: C, 43.06; H, 9.57; N, 13.40. Found: C, 43.02; H, 9.51; N, 13.45.

2-Butyne-1,4-diyl bis[mesitylenesulfonate] (29) was obtained (80%) from **28** (Aldrich) by following the procedure described for **21**: mp 105−106 °C (ethyl acetate−hexane); ^{1}H NMR ($CDCl_3$) δ 2.31 (s, 6H), 2.60 (s, 12H), 4.52 (s, 4H), 6.98 (s, 4H); ^{13}C NMR $CDCl_3$ δ 20.93, 22.48, 56.13, 80.41, 130.65, 131.67, 139.98, 143.67; MS-EI *m/z* 450 (M^+). Anal. Calcd for $C_{22}H_{26}O_6S_2$: C, 58.67; H, 5.78. Found: C, 58.64; H, 5.75.

3,7,12,16-Tetrakis(mesitylenesulfonyl)-3,7,12,16-tetraazaoctadec-9-yne (30) was prepared (61%) by condensation of **29** with **3** following the procedure described for **22**: mp 165−166 °C (ethyl acetate−hexane); ^{1}H NMR ($CDCl_3$) δ 1.08 (t, 6H), 1.75 (m, 4H), 2.28 (s, 12H), 2.55 (brs, 24H), 3.10 (m, 12H), 3.98 (s, 4H), 6.95 (m, 8H); ^{13}C NMR ($CDCl_3$) δ 12.70, 20.86, 22.64, 25.14, 34.85, 40.22, 42.62, 43.37, 78.80, 131.99, 132.26, 133.21, 140.26, 142.28, 142.71; MS-FAB *m/z* 982 (M^+). Anal. Calcd for $C_{50}H_{70}N_4O_8S_4$: C, 61.10; H, 7.13; N, 9.33. Found: C, 61.08; H, 7.15; N, 9.38.

3,7,12,16-Tetraazaoctadec-9-yne (31) tetrahydrochloride was obtained (86%) from **30** following the procedure described for **5**:[23] mp dec above 250 °C; ^{1}H NMR (D_2O) δ 1.29 (t, 6H), 2.13 (m, 4H), 3.14 (m, 12H), 4.06 (s, 4H); ^{13}C NMR (D_2O) δ 13.34, 25.52, 39.45, 45.90, 45.64, 46.71, 81.32; MS-MALDI *m/z* 255 $(M + 1)^+$. Anal. Calcd for $C_{14}H_{34}N_4Cl_4$: C, 42.00; H, 8.50; N, 14.00. Found: C, 42.15; H, 8.68; N, 14.25.

1,2-Dimethylbenzene 1,2-bis(mesitylenesulfonate) (33) was obtained (66%) from **32** (Aldrich) by following the procedure described for **21**: mp 128−129 °C (ethyl acetate−hexane); ^{1}H NMR ($CDCl_3$) δ 2.31 (s, 6H), 2.57 (s, 12H), 5.00 (s, 4H), 6.95 (s, 4H), 7.26 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 20.97, 22.49, 67.78, 129.49, 130.19, 131.74, 132.39, 138.82, 143.43; MS-FAB *m/z* 502 (M^+). Anal. Calcd for $C_{26}H_{30}O_6S_2$: C, 62.15; H, 5.98. Found: C, 62.11; H, 5.94.

9,10-Benzo-3,7,12,16-tetrakis(mesitylenesulfonyl)-3,7,12,16-tetraazaoctodecane (34) was prepared (72%) by condensation of **33** with **3** following the procedure described for **22**: mp 120−121 °C (ethyl acetate−hexane); ^{1}H NMR ($CDCl_3$) δ 0.92 (t, 6H), 1.45 (m, 4H), 2.32, 2.38 (s, 12H), 2.48 (s, 12H), 2.65 (s, 12H), 2.75 (m, 4H), 2.85 (t, 4H), 2.95 (q, 4H), 4.30 (s, 4H), 6.88 (s, 4H), 7.02 (s, 4H), 7.28 (s, 4H); MS-FAB *m/z* 1034 (M^+), 851. Anal. Calcd for $C_{54}H_{74}N_4O_8S_4$: C, 62.67; H, 7.16; N, 5.42. Found: C, 62.63; H, 7.20; N, 5.40.

9,10-Benzo-3,7,12,16-tetraazaoctadecane (35) tetrahydrochloride was obtained (72%) from **35** following the procedure described for **5**: mp dec above 260 °C; ^{1}H NMR (D_2O) δ 1.31 (t, 6H), 2.18 (m, 4H), 3.18 (m, 8H), 3.27 (m, 4H), 4.46 (s, 4H), 7.62 (s, 4H); ^{13}C NMR (D_2O) δ 13.31, 13.40, 25.71, 45.90, 46.75, 47.39, 50.91, 133.16, 133.38, 134.36; MS-MALDI *m/z* 307 $(M + 1)^+$. Anal. Calcd for $C_{18}H_{38}N_4Cl_4$: C, 47.79; H, 8.41; N, 12.39. Found: C, 47.73; H, 8.38; N, 12.19.

***trans*-1,2-Bis(aminomethyl)cyclopropane (10) Dihydrochloride.** A 0.4 M solution of hydrazoic acid in toluene (102 mL) was added to a solution of diol **1** (1.7 g, 17.01 mmol) in anhydrous THF (10 mL). This was followed by the addition of a solution of diisopropyl azidodicarboxylate (7.48 g, 36.99 mmol) in THF (15 mL) and then by a solution of triphenylphosphine (21.6 g, 92.35 mmol) in THF (30 mL). The temperature of the mixture was kept at 25 °C by regulating the rate of addition of the latter solution. Stirring was maintained for 1 h at 25 °C and then for 18 h at 50 °C. Water (3.5 mL) was then added, the mixture was stirred at 50 °C for a further 6 h, the solvent was then removed under vacuum, and the residue was partitioned between methylene chloride (100 mL) and 1 N HCl (100 mL). The aqueous phase was separated and repeatedly washed with methylene chloride (4 × 100 mL). Evaporation of the aqueous solution under reduced pressure (40 °C) left behind **10** as a white solid that was recrystallized from methanol/ether (0.650 g, 25%): mp 230 °C dec; ^{1}H NMR (D_2O) δ 0.40−0.53 (m, 1H, cyPrCH), 1.00−1.15 (m, 1H, cyPrCHH), 1.30−1.48 (m, 2H, cyPrCH), 2.75−2.95 (m, 2H, NCH_2), 3.20−3.35 (m, 2H, NCH_2). Anal. Calcd for $C_5H_{10}Cl_2N_2$: C, 35.50; H, 5.92; N, 16.57. Found: C, 35.56; H, 6.01; N, 16.60.

***cis*-1,2-Bis(aminomethyl)cyclopropane (11) dihydrochloride** was obtained (35%) from **6** following the procedure described for the synthesis of **10**: mp 230 °C dec; ^{1}H NMR (D_2O) δ 0.71−0.87 (m, 2H), 1.10−1.29 (m, 2H), 2.85 (m, 2H), 3.09 (m, 2H); ^{13}C NMR (D_2O) δ 13.19, 18.55, 46.53. Anal. Calcd for $C_5H_{10}Cl_2N_2$: C, 35.50; H, 5.92; N, 16.57. Found: C, 35.47; H, 5.89; N, 16.54.

***trans*-1,2-Cyclopropanedicarboxydihydrazide (37).** Hydrazine monohydrate (3.38 mL, 80 mmol) was added to a solution of diethyl ester **36**[20] (3.5 mL, 20 mmol) in ethanol (10 mL), and the mixture was heated to reflux overnight. The reaction was cooled and diluted with chloroform (20 mL); the precipitate was filtered, dried, and recrystallized from ethanol: 2.53 g (80%); mp 227 °C (lit.[23] mp 228 °C); ^{1}H NMR (D_2O) δ 1.35 (dd, J = 7.8 Hz, 2H, cyPrCH), 2.04 (dd, J = 7.8 Hz, 1H, cyPrCH); ^{13}C NMR (D_2O) δ 15.5, 24.0, 176.

***trans*-1,2-Cyclopropanediamine (38) Dihydrochloride.** Hydrazide **37** (1.58 g, 10 mmol) dissolved in a mixture of 4.5 mL of concentrated HCl and 9 g of ice was topped with 10 mL of ethyl ether, and a solution of sodium nitrite (1.73 g, 25 mmol) in 4 mL of water was slowly added at 10 °C. The organic layer was then separated, the aqueous layer was extracted with ether (3 × 20 mL), and the ether extracts were pooled, dried ($CaCl_2$), and diluted with anhydrous toluene (30 mL). The ethyl ether was distilled off using a fractionation column condenser, and the remaining toluene solution was heated to 85 °C until the evolution of nitrogen ceased and then for an additional 10 min. While still hot, the solution was poured into preheated (60 °C) concentrated HCl (8 mL). Toluene was distilled off under vacuum, and anhydrous ethanol (15 mL) was added to the flask and then distilled off. This process was repeated twice to obtain a cream-colored solid that was digested with cold ethanol and suction-filtered to afford 0.645 g (45%) of **38**: mp 220 °C dec (from ethanol) (lit.[14] mp 210 °C); ^{1}H NMR (D_2O) δ 1.48 (dd, 2H), 3.13 (dd, 2H). Anal. Calcd for $C_3H_{10}Cl_2N_2$: C, 24.82; H, 6.89; N, 19.31. Found: C, 24.79; H, 6.85; N, 19.28.

***trans*-1,2-Cyclopropanediylbis(mesitylenesulfonamide) (39).** Dihydrochloride **38** (145 mg, 1 mmol) was dissolved in 4 mL of dioxane/water (1:1), while maintaining a pH of ca. 11 by the addition of 5% potassium hydroxide. A solution of mesitylenesulfonyl chloride (875 mg, 4 mmol) in 5 mL of dioxane was then slowly added, the upper layer of the mixture was decanted, and the gummy residue was triturated with hexane to afford 330 mg (76%) of **39** as a white solid that was recrystallized from chloroform/hexane: mp 189−191 °C; ^{1}H NMR ($CDCl_3$) δ 0.9 (t, J = 8.0 Hz, 2H, cyPrCH_2), 2.24 (t, J = 8 Hz, 2H, cyPrCH), 2.30 (s, 6H, 2CH_3), 2.55 (s, 12H, 4CH_3), 5.00 (s, 2H, NH), 6.75 (s, 4H, aromatic); ^{13}C NMR ($CDCl_3$) δ 14.16, 20.98, 22.92, 30.92, 132.11, 132.89, 139.20, 142.79; MS-EI *m/z* 408 (M^+). Anal. Calcd for $C_{21}H_{28}N_2S_2O_4$: C, 61.76; H, 6.86; N, 6.86. Found: C, 61.70; H, 6.91; N, 6.90.

3,7,10,14-Tetrakis(mesitylenesulfonyl)-8,9-[(*E*)-1,2-cyclopropyl]-3,7,10,14-tetraazahexadecane (41). Sodium hydride (0.111 g, 4.4 mmol) was added to a solution of **39** (0.872 g, 2 mmol) in anhydrous DMF (40 mL) at 0 °C, the mixture was stirred for 30 min, a solution of **40**[3b] (1.531 g, 4.4 mmol) in anhydrous DMF (50 mL) was then slowly added, and the mixture was further stirred at 25 °C for 18 h. The reaction was quenched with water (8 mL) and extracted with ether (3 × 25 mL); the combined organic layers were washed with water (4 × 30 mL) and then with brine (2 × 25 mL), dried (Na_2SO_4), and evaporated to dryness. The residue was filtered through a silica gel column using 8:1 hexane−ethyl acetate as the eluant: 1.7 g (77%) of **41** recovered from the eluates; mp 60−62 °C; ^{1}H NMR ($CDCl_3$) δ 0.57 (dd, J = 6 and 8 Hz, 2H, cyPrCH_2), 0.99 (t, J = 8 Hz, 6H, 2CH_3), 1.7−1.9 (m, 4H, NCH_2CH_2), 2.25 (s, 6H, 2CH_3), 2.29 (s, 6H, 2CH_3), 2.50 (s, 12H, 4CH_3), 2.55 (s, 12H, 4CH_3), 2.56−2.67 (m, 2H, cyPrCH), 2.87−3.19 (m, 12H, 6NCH_2), 6.86 (s, 4H, aromatic), 6.90 (s, 4H, aromatic); MS-FAB *m/z* 971.4 $(M + 1)^+$, 787, 605, 295, 119 (100%). Anal. Calcd for $C_{49}H_{70}N_4S_4O_8$: C, 60.61; H, 7.22; N, 5.77. Found: C, 60.59; H, 7.18; N, 5.70.

3,7,10,14-Tetraaza-8,9-[(*E*)-1,2-cyclopropyl]hexadecane (42) tetrahydrochloride was obtained (77%) from **41** following the procedure described for **5**: mp 240 °C dec (from

ethanol); ^{1}H NMR (D_2O−acetone-d_6) δ 1.23 (t, J = 6.8, 2H, cyPrCH_2), 1.31 (t, J = 7.3 Hz, 6H, 3CH_3), 2.00−2.10 (m, 4H, NCCH_2), 2.81 (t, J = 6.6 Hz, 2H cyPrC**H**), 3.00−3.20 (m, 12H); ^{13}C NMR (D_2O−acetone-d_6) δ 13.33, 13.73, 26.64, 37.63, 45.76, 47.27, 47.83; MS-ESI m/z 243.4 (M + 1)$^+$, 163.3, 141.3. Anal. Calcd for $C_{13}H_{34}Cl_4N_4$: C, 40.21; H, 8.76; N, 14.43. Found: C, 40.27; H, 8.80; N, 14.49.

***cis*-1,2-Cyclopropanedicarboxydihydrazide (44)** was obtained (87%) from **43**[20] following the procedure described for **37**: mp 196−198 °C (lit.[24] mp 196 °C); ^{1}H NMR (D_2O) δ 1.25 (m, 1H, cyPrCH_2), 1.45 (ddd, J = 5.0, 6.0, 5.5 Hz, 1H, cyPrCH_2), 2.05 (dd, J = 6.0, 9.0 Hz, 2H, cyPrCH); ^{13}C NMR (D_2O) δ 12.97, 23.88, 175.0.

***cis*-1,2-Cyclopropanediamine (45) dihydrochloride** was obtained (58%) from **44** following the procedure described for **38**: mp 220 °C (lit.[15] mp 220 °C); ^{1}H NMR (D_2O) δ 1.25 (m, 2H, cyPrCH_2), 2.9 (dd, 2H, J = 6.0, 8.0 Hz, cyPrCH). Anal. Calcd for $C_3H_{10}Cl_2N_2$: C, 24.82; H, 6.89; N, 19.31. Found: C, 24.89; H, 7.02; N, 19.50.

***cis*-1,2-Cyclopropanediylbis(mesitylenesulfonamide) (46)** was obtained (95%) from **45** following the procedure described for **39**: mp 175−177 °C; ^{1}H NMR ($CDCl_3$) δ 0.80 (m, 1H), 0.95 (m, 1H), 2.25 (m, 2H), 2.34 (s, 6H), 2.65 (s, 12H), 5.25 (br, 2H), 7.00 (s, 4H, aromatic); ^{13}C NMR ($CDCl_3$) δ 13.5, 20.96, 22.96, 28.00, 132.08, 133.00, 139.45, 142.09. Anal. Calcd for $C_{21}H_{28}N_2O_4S_2$: C, 61.76; N, 6.86; N, 6.86. Found: C, 61.80; H, 6.83; N, 6.92.

3,7,10,14-Tetrakis(mesitylenesulfonyl)-8,9-[(*Z*)-1,2-cyclopropyl]-3,7,10,14-tetraazahexadecane (47) was obtained (30%) from **46** following the procedure described for **41**: mp 74−75 °C; ^{1}H NMR ($CDCl_3$) δ 0.31 (m, 1H), 0.99 (t, J = 8 Hz, 6H), 1.06 (m, 1H), 1.60 (m, 4H), 2.30 (s, 12H), 2.55 (s, 12H), 2.58 (s, 12H), 2.63 (m, 2H), 3.10 (m, 12H, 6NCH_2), 6.90 (s, 4H, aromatic), 6.93 (s, 4H, aromatic); MS-FAB m/z 971.4 (M + 1)$^+$, 787, 605, 154 (100%), 199. Anal. Calcd for $C_{49}H_{70}N_4S_4O_8$: C, 60.61; H, 7.22; N, 5.77. Found: C, 60.68; H, 7.28; N, 6.00.

3,7,10,14-Tetraza-8,9-[(*Z*)-1,2-cyclopropyl]hexadecane (48) tetrahydrochloride was obtained (48%) from **47** following the procedure described for **42**: mp 240 °C dec; ^{1}H NMR (D_2O−acetone-d_6) δ 0.75 (m, 1H), 1.16 (ddd, J = 7.2, 8.1, 7.2 Hz, 1H), 1.29 (t, J = 7.4 Hz, 6H), 2.04 (pent, J = 7.5 Hz, 4H), 2.69 (dd, J = 6.8, 7.4 Hz, 2H), 2.98−3.24 (m, 12H); ^{13}C NMR (D_2O−acetone-d_6) δ 12.44, 13.43, 27.12, 35.86, 45.89, 47.48, 48.06; MS-ESI m/z 243.3 (M + 1)$^+$ (100%), 163, 142. Anal. Calcd for $C_{13}H_{34}Cl_4N_4$: C, 40.21; H, 8.76; N, 14.43. Found: C, 40.01; H, 8.69; N, 13.39.

***trans*-1,2-Cyclobutanediylbis(mesitylenesulfonamide) (50)** was obtained (65% yield) from **49**[17] following the procedure described for **39**: mp 201−202 °C (methanol−water); ^{1}H NMR ($CDCl_3$) δ 1.35 (m, 2H), 1.95 (m, 2H), 2.30 (s, 6H), 2.60 (s, 6H), 2.60 (s, 12H), 3.32 (m, 2H), 5.18 (brd, 2H), 6.95 (s, 4H); ^{13}C NMR ($CDCl_3$) δ 20.96, 22.88, 23.80, 54.68, 132.09, 134.10, 139.16, 142.80; MS-EI m/z 451 (M + 1)$^+$, 267, 183. Anal. Calcd for $C_{22}H_{30}N_2O_4S_2$: C, 58.67; H, 6.67; N, 6.22. Found: C, 58.61; H, 6.71; N, 6.27.

***cis*-1,2-Cyclobutanediylbis(mesitylenesulfonamide) (54)** was obtained (70%) from **53**[17] following the procedure described for **50**: mp 192−193 °C (methanol−water); ^{1}H NMR ($CDCl_3$) δ 1.90−2.18 (m, 4H), 2.35 (s, 6H), 2.60 (s, 12H), 3.63 (br, 2H), 5.41 (br, 2H), 6.96 (s, 4H); ^{13}C NMR ($CDCl_3$) δ 20.95, 22.89, 25.32, 51.20, 132.04, 134.08, 139.30, 142.51; MS-EI m/z 451 (M + 1)$^+$, 267, 183. Anal. Calcd for $C_{22}H_{30}N_2O_4S_2$: C, 58.67; H, 6.67; N, 6.22. Found: C, 58.72; H, 6.70; N, 6.00.

3,7,10,14-Tetrakis(mesitylenesulfonyl)-8,9-[(*E*)-1,2-cyclobutyl]-3,7,10,14-tetraazahexadecane (51) was prepared (60%) from **50** following the procedure described for **41**: colorless, glassy oil; ^{1}H NMR ($CDCl_3$) δ 0.98 (t, 6H), 1.55 (br, 6H), 1.82 (br, 2H), 2.28, 2.30 (s, 12H), 2.56−2.58 (s, 24H), 3.00 (m, 12H), 4.20 (br, 2H), 6.93−6.96 (s, 8H); ^{13}C NMR ($CDCl_3$) δ 12.70, 20.94, 21.70, 22.76, 22.90, 28.20, 40.20, 41.54, 43.16, 55.48, 131.91, 132.10, 133.02, 133.18, 140.23, 142.80, 143.17; MS-FAB m/z 985.4 (M + 1)$^+$, 801.4, 619.3.

3,7,10,14-Tetraaza-8,9-[(*E*)-1,2-cyclobutyl]hexadecane (52) tetrahydrochloride was obtained (40%) from **51** following the procedure described for **5**: mp 275 °C dec; ^{1}H NMR (D_2O) δ 1.20 (t, 6H), 1.80−2.21 (m, 6H), 2.35 (m, 2H), 3.08 (m, 12H), 4.00 (m, 2H); ^{13}C NMR (D_2O) δ 14.20, 22.57, 26.34, 46.26, 46.76, 47.44, 57.57; MS-ESI m/z 257.2 (M + 1)$^+$, 172.2. Anal. Calcd for $C_{14}H_{36}Cl_4N_4$: C, 65.62; H, 8.95; N, 13.93. Found: C, 65.68; H, 9.00; N, 13.90.

3,7,10,14-Tetrakis(mesitylenesulfonyl)-8,9-[(*Z*)-1,2-cyclobutyl]-3,7,10,14-tetraazahexadecane (55) was prepared in 50% yield from **54** following the procedure described for **41**: glassy oil; ^{1}H NMR ($CDCl_3$) δ 0.98 (t, 6H), 1.50 (m, 4H), 1.80 (br, 2H), 2.12 (br, 2H), 2.30 (s, 6H), 2.52, 2.54 (s, 12H), 2.82 (m, 4H), 2.93−3.23 (m, 8H), 4.20 (br, 2H), 6.93 (s, 8H); ^{13}C NMR ($CDCl_3$) δ 12.69, 20.94, 22.75, 22.82, 24.63, 25.94, 40.14, 42.16, 43.25, 55.43, 131.89, 132.23, 133.51, 135.08, 139.52, 140.16, 142.22, 142.88; MS-FAB m/z 985.4 (M + 1)$^+$, 801.3, 619.3.

3,7,10,14-Tetraaza-8,9-[(*Z*)-1,2-cyclobutyl]hexadecane (56) tetrahydrochloride was obtained from **55** in 37% yield following the procedure described for **5**: mp dec above 270 °C; ^{1}H NMR (D_2O) δ 2.10−2.25 (m, 4H), 2.35−2.55 (m, 4H), 3.08−3.30 (m, 12H), 4.15 (m, 2H); ^{13}C NMR (D_2O) δ 14.12, 25.04, 26.28, 46.72, 47.31, 47.46, 57.98; MS-ESI m/z 257.3 (M + 1)$^+$, 172.3. Anal. Calcd for $C_{14}H_{36}Cl_4N_4$: C, 65.62; H, 8.95; N, 13.93. Found: C, 65.70; H, 8.99; N, 13.97.

Cell Culture. The human lung adenocarcinoma cell line, A549, was a gift from Dr. M. Eileen Dolan, University of Chicago, Department of Medicine, and was grown in Ham's F-12K medium (Fisher Scientific, Itasca, IL) supplemented with 10% fetal bovine serum and 2 mM l-glutamine. The human colon carcinoma cell line, HT-29, and the human breast carcinoma cell line, MCF7, were obtained from the American Type Culture Collection, Rockville, MD. HT-29 cells were grown in McCoy's 5A medium (Gibco, BRL, Gaithersburg, MD) supplemented with 10% fetal bovine serum. MCF7 cells were grown in Richter's improved modified Eagle's medium supplemented with 10% fetal bovine serum and 2.2 g/L sodium bicarbonate. The human prostate adenocarcinoma cell lines, PC-3 and DU145, were gifts from Dr. George Wilding, University of Wisconsin Comprehensive Cancer Center and the Department of Medicine, and were grown in Dulbecco's modified Eagle's medium supplemented with 5% fetal bovine serum. The malignant glioma cell line, U251MG NCI, was obtained from the brain tumor tissue bank at the University of California, San Francisco Department of Neurosurgery, and was grown in Dulbecco's modified Eagle's medium supplemented wth 10% fetal bovine serum. Both A549 and MCF7 cells were grown in 100 units/mL penicillin and 100 μg/mL streptomycin. HT-29 and U251MG NCI cells were grown in 50 μg/mL gentamycin. PC-3 and DU145 cells were maintained in 1% antibiotic antimycotic solution (Sigma, St. Louis, MO). All cell cultures were maintained at 37 °C in 5% CO_2/95% humidified air.

MTT Assay.[25] Exponentially growing monolayer cells were plated in 96-well plates at a density of 500 cells/well and allowed to grow for 24 h. Serially diluted drug solutions were added such that the final drug concentrations in the treatment media were between 0 and 35 μM. Six days after drug treatment, 25 μL of a Dulbecco's phosphate-buffered saline solution containing 5 mg/mL MTT (thiazolyl blue) (Sigma) was added to each well and incubated for 4 h at 37 °C. Then 100 μL of lysis buffer (20% sodium dodecyl sulfate, 50% *N*,*N*-dimethylformamide, and 0.8% acetic acid, pH 4.7) was added to each well and incubated for an additional 22 h. A microplate reader (E max, Molecular Devices, Sunnyvale, CA) set at 570 nm was used to determine the optical density. Results were plotted as a ratio of the optical density in drug-treated wells to the optical density in wells treated with vehicle alone. The ID_{50} values were determined by fitting the plot of optical density vs concentration of analogues to a sigmoidal function using Softmax Pro 2.0 4-parameter curve fitting software that was developed based on Marquadt−Levenberg algorithm (Molecular Devices, Sunnyvale, CA).

Polyamine Analysis. Cells were incubated with the polyamine analogues for the appropriate number of days (see Biological Results and Discussion). Approximately $0.5-1.0 \times 10^6$ cells were harvested by trypsinization and washed twice with cold (4 °C) isotonic phosphate buffer (pH 7.4). Cells were sonicated in 100−200 μL of 0.6 N perchloric acid and centrifuged at 8000*g* for 5 min, and the supernatant was carefully separated for analysis. Between 50 and 100 μL of the supernatant was fluorescence-labeled by derivatizing with dansyl chloride. Labeled polyamines were loaded onto a C-18 high-performance liquid chromatography column and separated by gradient elution with acetonitrile/water at 50 °C following a published procedure.[26] Peaks were detected and quantitated using a Perkin-Elmer HPLC fluorescence monitor coupled with a Spectra Physics peak integrator. Because polyamine levels vary with environmental conditions, control cultures were sampled for each experiment.

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