Cis-Unsaturated Analogues of 3,8,13,18,23-Pentaazapentacosane (BE-4-4-4-4): Synthesis and Growth Inhibitory Effects on Human Prostate Cancer Cell Lines

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From the results of our previous physicochemical studies of polyamine−nucleic acid interactions, we concluded that polyamine analogues in cisoidal conformation are capable of wrapping around the major groove of the double helix, of displacing natural polyamines from their nucleic acid binding sites, and of inhibiting cell division. On the basis of this hypothesis, nine unsaturated pentamines, formally derived from the cytotoxic pentamine 3,8,13,18,23-pentaazapentacosane (BE-4-4-4-4), were prepared in an attempt to increase antineoplastic activity. *Cis*-double bonds were introduced in all possible sites in the saturated pentaazapentacosane structure of BE-4-4-4-4 to yield two pentacosenes, four pentacosadienes, two pentacosatrienes, and one pentacosatetraene. *Cis*-double bonds should also provide good targets for mixed-function oxidases that might eliminate the accumulation of unsaturated pentamines in serum, thereby reducing systemic toxicity in animals. We determined the ability of these new pentamines to inhibit growth in four cultured human prostate cancer cell lines (LnCap, DU145, PC-3, and DuPro) using a MTT assay. LnCap and DU145 cells were very sensitive, PC-3 cells were relatively resistant, and DuPro cells were intermediate in sensitivity to most of these synthetic pentamines. In all cell lines, pentamines that had unsaturation(s) at the end of the chain showed the highest cell growth inhibitory effects. The cellular uptake, effects on cellular polyamine levels, and cytotoxicity of these pentamines on one representative prostate cancer cell line (DuPro) were further examined with a colony-forming efficiency (CFE) assay. The pentamines with unsaturation(s) at the end of the chain were once again the most cytotoxic among both the saturated (BE-4-4-4-4) and unsaturated analogues. Appreciable amounts of all pentamines entered DuPro cells and depleted cellular polyamine pools by day 6 of treatment. For most pentamines, however, cell growth inhibitory and cytotoxic effects could not be directly correlated either with their cellular uptake or with their ability to deplete cellular polyamine pools. The position of the double bonds in the aliphatic backbone seems to be the most important determinant of cytotoxicity. For some pentamines, however, depletion of cellular polyamines may add to their efficacy.

Introduction

The polyamines putrescine, spermidine, and spermine are organic cations found in all mammalian cells and are required for cell proliferation.[1] A number of polyamine analogues have shown promise as anticancer agents.[2,3] They inhibit cell growth and even kill cancer cells both in tissue culture as well as in experimental animal models. Most successful among these analogues have been the $^{\alpha}N,^{\omega}N$-bisethyl derivatives of spermine and its higher and lower homologues.[4,5] Of the many theories advanced to explain the biological effects of the polyamines, the hypothesis centering on their binding to nucleic acids is the most compelling.[1] Both spermine and spermidine are strong bases that are protonated at physiological pH.[2,3] These cationic polyamines can bind to the negatively charged nucleic acids either by electrostatic interactions or by hydrogen bonding. DNA-binding compounds have always been of interest as anticancer agents. Although the model of the DNA double helix has created an image of a rigid structure, experimental evidence suggests that DNA has considerable flexibility.[6] The structural changes that take place in DNA as well as in model oligo- and polynucleotides upon interaction with polyamines have been extensively studied by us and by others using both experimental methods as well as theoretical models.[7,8] It has been conclusively shown that polyamines, including the pentamine 3,8,13,18,23-pentaazapentacosane (BE-4-4-4-4), interact with anionic DNA, induce changes in DNA secondary structure, and thus alter the flexibility of the DNA chain.[7] It is also known that spermine and spermidine bind to t-RNA, are needed to stabilize the Γ-shaped structure of the t-RNAs, and are required for the fidelity of transcription.[1] $^{\alpha}N,^{\omega}N$-Bisethyl- and -bismethylspermine and its higher and lower homologues were found to bind regioslectively to t-RNA as a consequence of different hydrogen-bonding modes that are formed between both types of molecules.[9] In an attempt to apply these extensive studies on polyamine−nucleic acid interactions to drug discovery, pentamines were designed where changes were made in the lengths of the carbon chains separating the central NH_2^+ groups, in the distribution of the positive charges along

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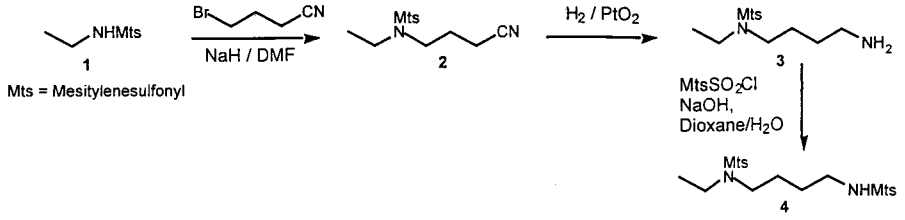
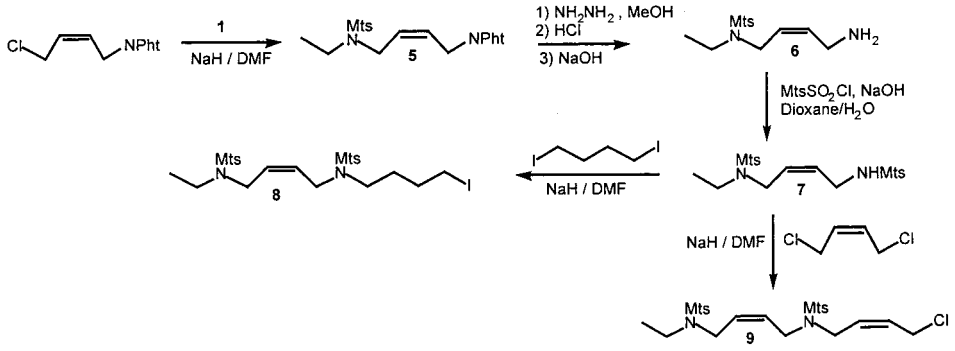
Scheme 1



Scheme 2



the molecule's backbone, and in the degree of rotational freedom of the polyamine backbone.[5,7−13] One of our most successful designs was BE-4-4-4-4, a higher homologue of bisethylspermine. BE-4-4-4-4 has a relatively higher affinity for DNA than has spermine, it is more efficient than is spermine in inducing structural changes in DNA, and unlike spermine, it inhibits nucleosome formation in cell free systems.[12] It also enters cells and produce alterations both in chromatin structure as well as in gene expression in cellular systems.[14,15] It is cytotoxic to many human tumor cell lines both in culture and in nude mouse xenografts.[12,13] It is presently under consideration for clinical trials as an antineoplastic agent.

In our search for polyamine analogues with enhanced cytotoxic effects and decreased systemic toxicity, we decided to refine the structure of BE-4-4-4-4 by introducing unsaturation(s) in the pentacosane backbone. Our previous molecular dynamic studies on spermine−DNA interactions had suggested that in a minimum energy conformation, spermine binds to DNA in a cisoidal conformation that wraps around the major groove of the double helix.[16] *Cis*-double bonds in the alkane chain should also provide good targets for mixed-function oxidases that could oxidize the unsaturation(s) to diols,[17] thus facilitating the elimination of the potentially toxic pentamines from animals. Here we report the synthesis of mono-, di-, tri-, and tetraunsaturated derivatives of BE-4-4-4-4 (see Table 1 for structures). The resulting nine pentamines were evaluated in tissue culture against human prostate cancer cells. As the prostate gland is one of the major sites of polyamine biosynthesis in mammals and since prostate cancers are generally sensitive to inhibitors of polyamine biosynthesis,[18] four human prostate cancer cell lines (LnCap, DU-145, DuPro, and PC-3) were selected for this study. We studied the effects of the nine pentamines on the growth of the four cell lines with a MTT assay. We selected DuPro as a representative cell line and further studied the effects of the pentamines on growth, intracellular polyamine levels, and cell survival, using a colony-forming efficiency (CFE) assay. Our results suggest that pentamines with *cis*-double bonds in the terminal segments of the pentacosane chain are not only more cytotoxic than are those pentamines with *cis*-double bonds in the central segments but are also more cytotoxic than is the saturated parent pentamine BE-4-4-4-4.

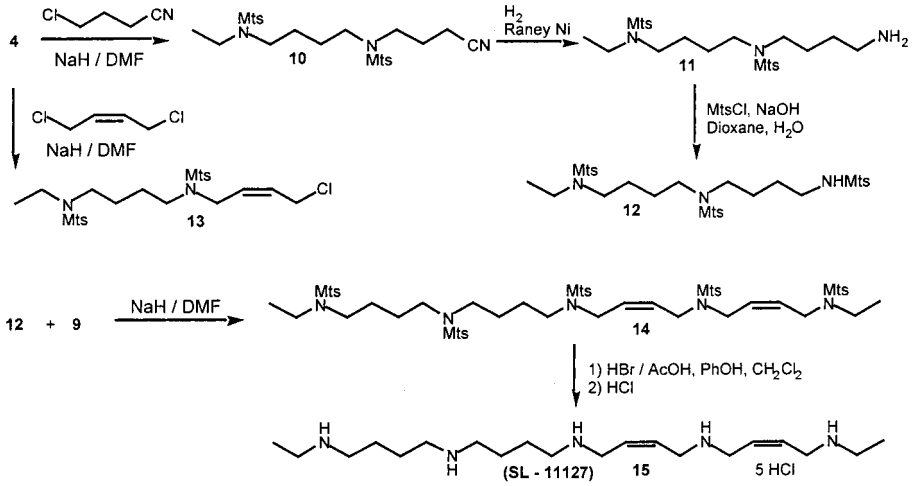
Chemistry

The synthesis of the *cis*-unsaturated pentamines required the prior availability of the diamide **4** (Scheme 1). Alkylation of *N*-ethylmesityleneamide (**1**) with 4-bromobutyronitrile gave the nitrile **2**. The latter was then reduced to the mesitylenediamine **3** and converted to the diamide **4**. Alkylation of **1** with *N*-pthaloyl-*cis*-4-chloro-2-butenylamine gave phthaloylamide **5**. The phthaloyl protecting group was cleaved with hydrazine, and the resulting amine was protected by acylation with mesitylenesulfonyl (Mts) chloride to give **7**. Monoalkylation of **7** with *cis*-1,4-dichloro-2-butene gave the expected diene **9** (Scheme 2). Alkylation of **7** with 1,4-diiodobutane gave the monounsaturated diamide **8**. Monoalkylation of **4** with 4-chlorobutylnitrile gave the diamide **10**, and on reduction of **10** over Raney Ni, amine **11** was obtained. Amidation of **11** gave triamide **12** (Scheme 3). Alkylation of **4** with *cis*-1,4-dichloro-2-butene gave diamide **13**.

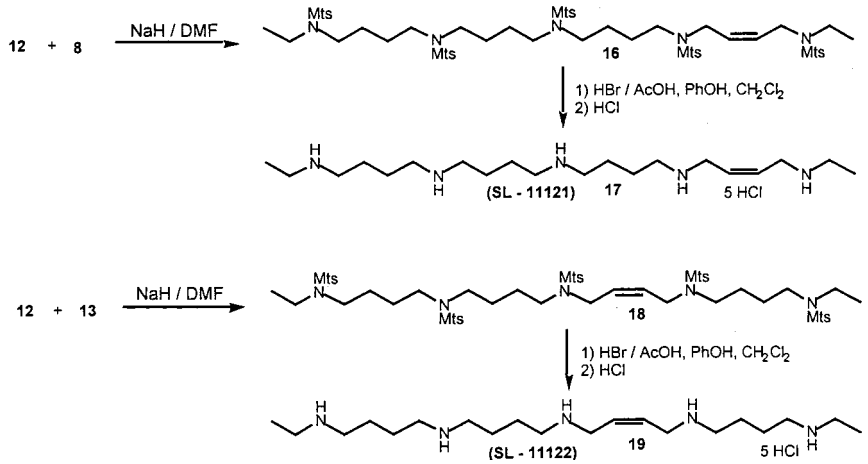
Using the abovementioned intermediates it was possible to build the backbones of several unsaturated pentamines. Thus, the condensation of **12** and **9** gave pentamide **14** that after deprotection from its mesitylenesulfonyl residues gave pentamine **15** that was isolated as its pentahydrochloride (Scheme 3). Condensation of **12** and **8** gave pentamide **16**; after cleavage of the amide residues, pentamine **17** was isolated as its pentahydrochloride. Condensation of **12** and **13** gave pentamide **18**; on deprotection pentamine **19** was obtained (Scheme 4). Good reaction yields were obtained for all the reaction sequences.

A second approach to the unsaturated pentamines was based on the dialkylation of mesitylenesulfonamide.

Scheme 3



Scheme 4



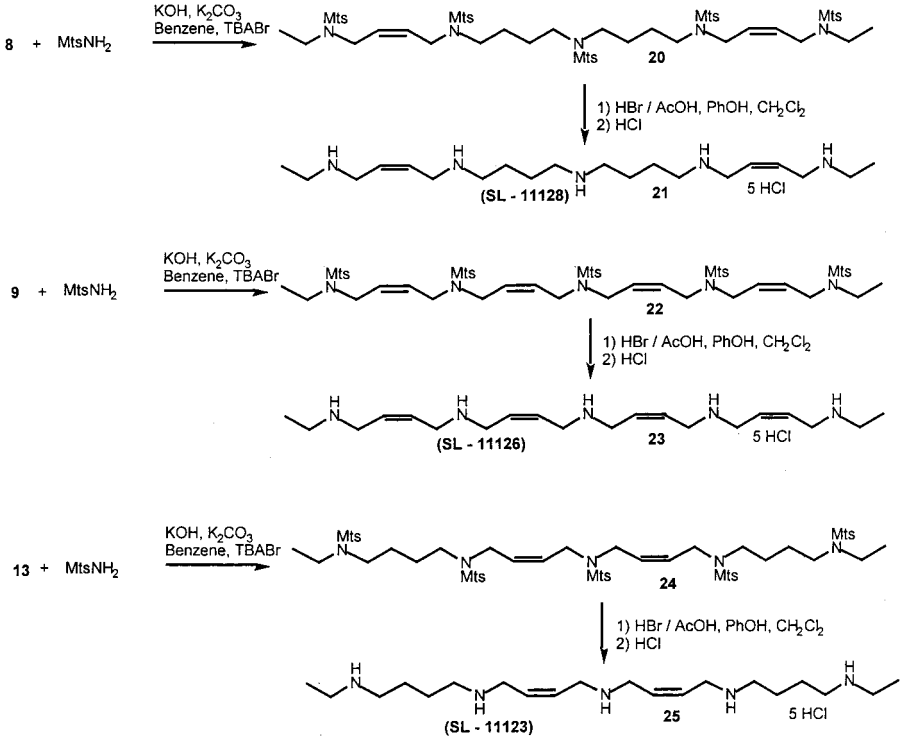
Thus, by alkylation of the amide with iodoalkyl diamide **8** in the presence of a phase-transfer reagent it was possible to obtain the pentamide **20** (Scheme 5). Deprotection gave the *cis*-diene **21**. Dialkylation of mesitylenesulfonamide with **9** gave pentamide **22**; on deprotection the completely unsaturated pentamine **23** was obtained. Dialkylation of mesitylenesulfonamide with **13** gave pentamide **24** that on deprotection gave pentamine **25**. Monoalkylation of mesitylenesulfonamide with chloroalkyl diamide **13** gave triamide **26**; by further alkylation of **26** with iodoalkyl diamide **8** it was possible to obtain pentamide **27**. Deprotection gave pentamine **28** (Scheme 6). When mesitylenesulfonamide was first alkylated with chloroalkyl diamide **9** to give **29**, and the latter then alkylated with **8**, it was possible to obtain pentamide **30**. Deprotection of **30** gave pentamine **31**. By condensation of triamide **29** and diamide **13**, it was possible to obtain pentamide **32**. Deprotection of **32** gave pentamine **33**. These synthetic procedures allowed the obtention of all the possible *cis*-unsaturated isomers of the pentamines in preparative amounts when needed.

Biological Results and Discussion

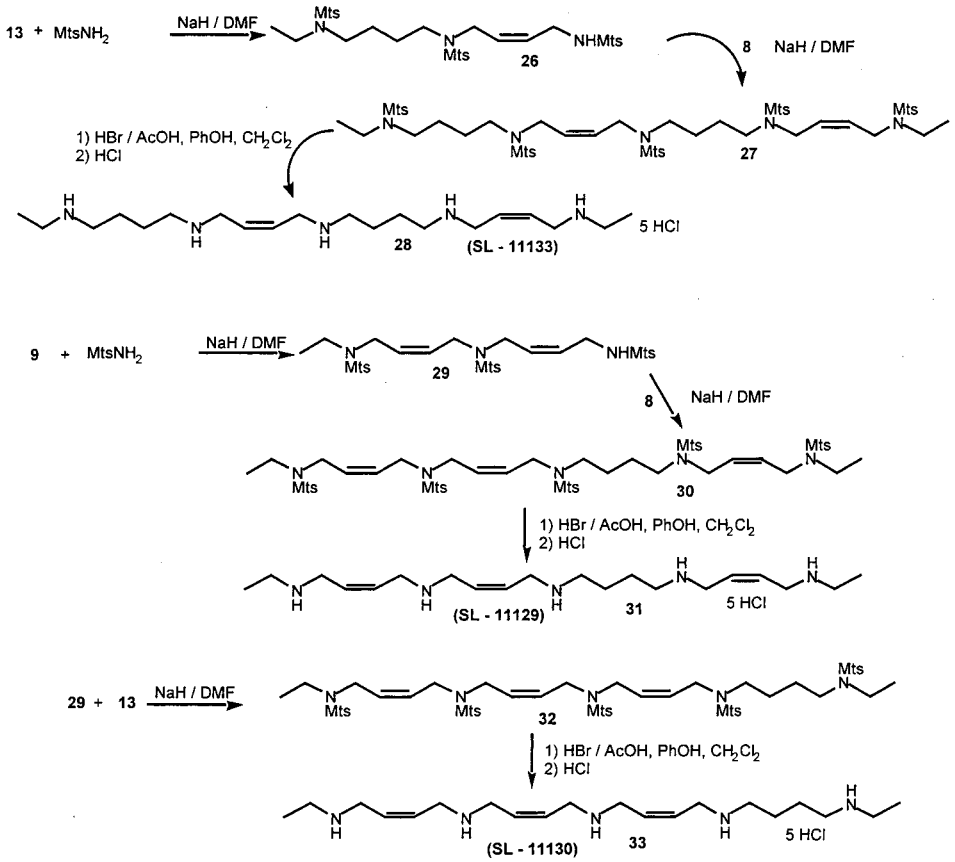
The concentration of a pentamine required to inhibit 50% cell growth (ID_{50}) after 6 days of treatment was determined by a MTT assay. The ID_{50} values for each pentamine in four human prostate cancer cell lines (LnCap, DU145, DuPro, and PC-3) together with their SLIL identification numbers and chemical structures arranged in increasing order of unsaturation are shown in Table 1. The cell lines displayed different sensitivities toward the pentamines. LnCaP and DU145 cells were the most sensitive with ID_{50} values for all pentamines of less than 1 μM. In contrast, DuPro and PC-3 cells were resistant to many of the pentamines with ID_{50} values for some of them above the highest drug concentration used (31.25 μM). Analysis of the data shown in Table 1 indicates that those pentamines that had ID_{50} values less than 1 μM in all four cell lines were either completely saturated (BE-4-4-4-4) or carried at least one unsaturated site in the terminal segment of the hydrocarbon chain (**17** and **21**). Pentamines with double bonds in the mid-section of the polyamine chain showed markedly less growth inhibitory activity, particularly in DuPro and PC-3 cell lines. PC-3 cells are generally more resistant to the pentamines than are many other human tumor cell lines, perhaps due to a diminished capacity of the polyamine transport system in PC-3 cells.[19]

We selected the DuPro cell line that exhibits an intermediate level of sensitivity to the pentamines (Table 1) as a representative cell line for detailed study. All nine pentamines plus BE-4-4-4-4 at 1 and 10 μM were assayed for their effects on changes in cell number and in cellular polyamine levels over time. The effects of the pentamines on DuPro cell growth along with the

Scheme 5

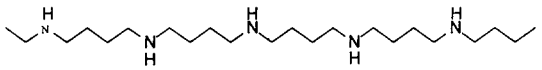
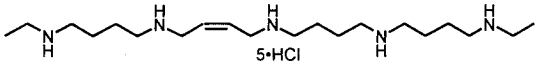
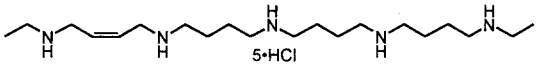
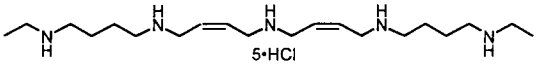
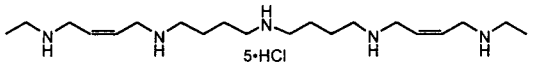
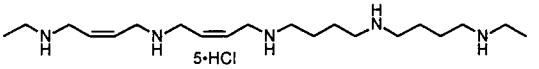
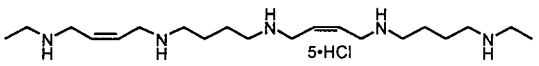
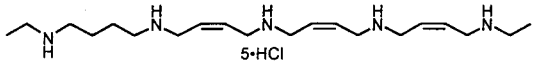
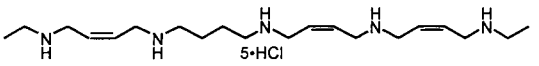
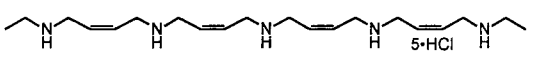


Scheme 6



cellular polyamine levels are shown in Figures 1−4. At 1 μM, BE-4-4-4-4 inhibited growth by 50% on day 6 of treatment (Figure 1A). No other pentamine showed any observable growth inhibitory effect at 1 μM (Figures 1A and 2A). While all pentamines were detectable in cell lysates, **31** and **33** were present at higher concentrations than were the other pentamines (Figures 1B,C and 2B,C). At 1 μM, no pentamine was able to completely deplete any of the natural polyamines. In most cases, cellular polyamine levels were within 50% of those in control cells and for **25**, **23**, **33**, and **28**, the polyamine levels were actually higher than those of the controls

Table 1. Chemical Structures and Effects of the Pentamines on Human Prostate Tumor Cell Growth as Determined by MTT Assay

Polyamine Analogs	Structures of Polyamine Analogs with Unsaturated Groups	ID_{50} (μM) values for Human Prostate Tumor Cell Lines			
		LNCAP	DU145	DuPro	PC-3
BE-4-4-4-4		0.14	0.07	0.2	0.6
19		0.09	0.08	0.56	>31.25
17		0.14	0.08	0.4	0.52
25		0.15	0.51	>31.25	>31.25
21		0.3	0.14	0.25	0.5
15		0.15	0.22	1.3	>31.25
28		0.54	0.34	>31.25	>31.25
33		0.24	0.43	>31.25	>31.25
31		0.32	0.32	>31.25	1.7
23		0.55	0.51	1.1	0.2

on day 6 of treatment (Figure 2C). At 10 μM, however, a marked decrease in cell growth was observed not only for BE-4-4-4-4 but also for **17** and **21**, particularly on day 6 of treatment (Figure 3). Interestingly, **19** and **15**, which showed good growth inhibition as assessed with the MTT assay (Table 1), showed little effect, even at 10 μM, on day 6 after treatment. Cell growth was near that of control after an initial delay. This underscores the necessity of confirming the MTT data with assays that follow a time course before drawing definitive conclusions about growth inhibitory activities. At 10 μM, appreciable amounts of all pentamines were detected in cell lysates (Figures 3B,C and 4B,C). While none of the pentamines was able to completely deplete the cellular polyamine pools, BE-4-4-4-4, **17**, **19**, **15**, and **21** were more efficient in decreasing cellular polyamines than were the other pentamines (compare Figure 3 with Figure 4). Similar to the treatment with 1 μM, treatment with 10 μM **25**, **23**, **31**, and **33** resulted in an increase in the cellular polyamine levels, particularly on day 6 of treatment (Figure 4C).

The abilities of various pentamines to deplete cellular polyamine pools correlate poorly with their growth inhibitory effects. For example, at 10 μM, **19** and **15** failed to arrest cell growth but depleted cellular polyamine levels to the same extent as did **17** and **21** (Figure 3), two very efficient growth inhibitors. On the other hand, 10 μM **33** inhibited cell growth to the same extent as did 10 μM BE-4-4-4-4 (compare Figure 3 with Figure 4), but **33** had little effect on putrescine and spermidine levels and actually increased the cellular spermine level by day 6 of the treatment, while BE-4-4-4-4 markedly decreased all natural polyamine pools. Although there are some variations in the intracellular pentamine levels, no correlation could be observed between intracellular pentamine levels and growth inhibitory activities. For example, on day 6 of treatment with 10 μM pentamine, the intracellular level of the weak growth inhibitor **28** was approximately 3−5-fold higher than were those of the strong growth inhibitors **17** and **21** (compare Figures 3 and 4). Therefore, we had to conclude that the growth inhibitory activities of the pentamines are in general related neither to their intracellular levels nor to their abilities to deplete cellular polyamine pools. As was the case with other strong DNA-binding polyamine analogues,[20] their cytotoxicity may relate to their DNA-binding abilities (Basu et al., unpublished results). If the intracellular pentamine concentration is sufficient to displace cellular polyamines from their DNA binding site(s), the pentamine can act as a growth inhibitor even in the presence of cellular polyamines. The depletion of cellular polyamines, however, may help enhance the growth inhibitory activities of a few of the pentamines.

The cytotoxic effects of pentamines that exhibit significant growth inhibition was further studied using a CFE assay. The CFE data for most of the active pentamines on day 6 of treatment are shown in Figure 5; **28** was used as a control to confirm that compounds that do not exhibit growth inhibition do not exhibit cytotoxicity. Only two pentamines, **17** and **21**, killed more cells than did BE-4-4-4-4. Both pentamines have

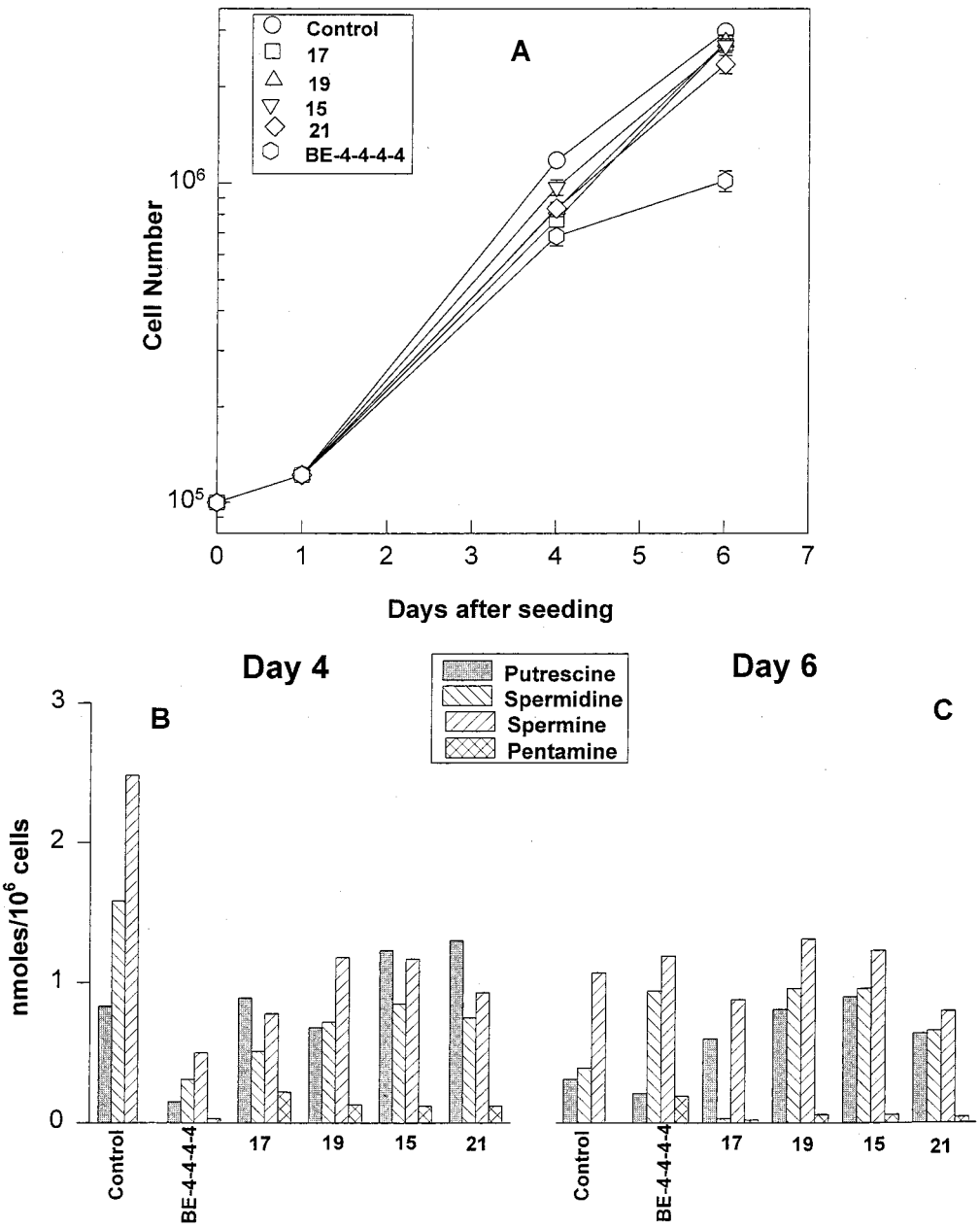


Figure 1. (A) Effects of 1 μM BE-4-4-4-4, **17**, **19**, **15**, and **21** on the growth of DuPro cells. Symbol for each pentamine is shown in the inset. Each data point is an average of at least three separate experiments run in duplicate. Error bars where not shown are smaller than the symbol size. (B) Effects of 1 μM BE-4-4-4-4, **17**, **19**, **15**, and **21** on the polyamine levels of DuPro cells on day 4 of treatment. Symbol for each polyamine is shown in the inset. Each data point is an average of two separate experiments. (C) Effects of 1 μM BE-4-4-4-4, **17**, **19**, **15**, and **21** on the polyamine levels of DuPro cells on day 6 of treatment. Symbol for each polyamine is shown in the inset. Each data point is an average of two separate experiments.

their unsaturated site(s) at the terminal segment of the hydrocarbon chain(s). Introduction of an unsaturated site in the middle of the chain markedly reduced the pentamine's cytotoxicity, as compared to that of BE-4-4-4-4, even though some of these pentamines may induce initial growth delay.

The rationale behind the strong cytotoxic activities of **21** and **17** is difficult to identify with precision, since the mechanism by which polyamines induce cell death is still controversial.[1] As mentioned before (see Introduction), we favor explanations that are based on the binding of the polyamine analogues to nucleic acids. It has been shown that DNA bending is necessary for nucleosome formation during cell division.[21] Therefore, interference with DNA bending should inhibit chromatin condensation and cell division. We suggested that a flexible polyamine backbone is necessary for efficient polyamine−DNA interactions.[7] The flexibility of the polyamine chain will facilitate its interaction with DNA and help in displacing the natural polyamines from their DNA binding site(s). Pentamines with unsaturated site(s) at the end segments of the carbon chain (as in **17** and **21**) could therefore be more efficient binders than pentamines with unsaturated sites at the center of the chain. Once bound, the partially restricted conformation of the active pentamines might affect DNA function more than their saturated counterpart by introducing unwanted steric effects in its spatial arrangement. We are, however, aware that subtler biochemical effects such as induction of enzymes of the cellular apoptotic

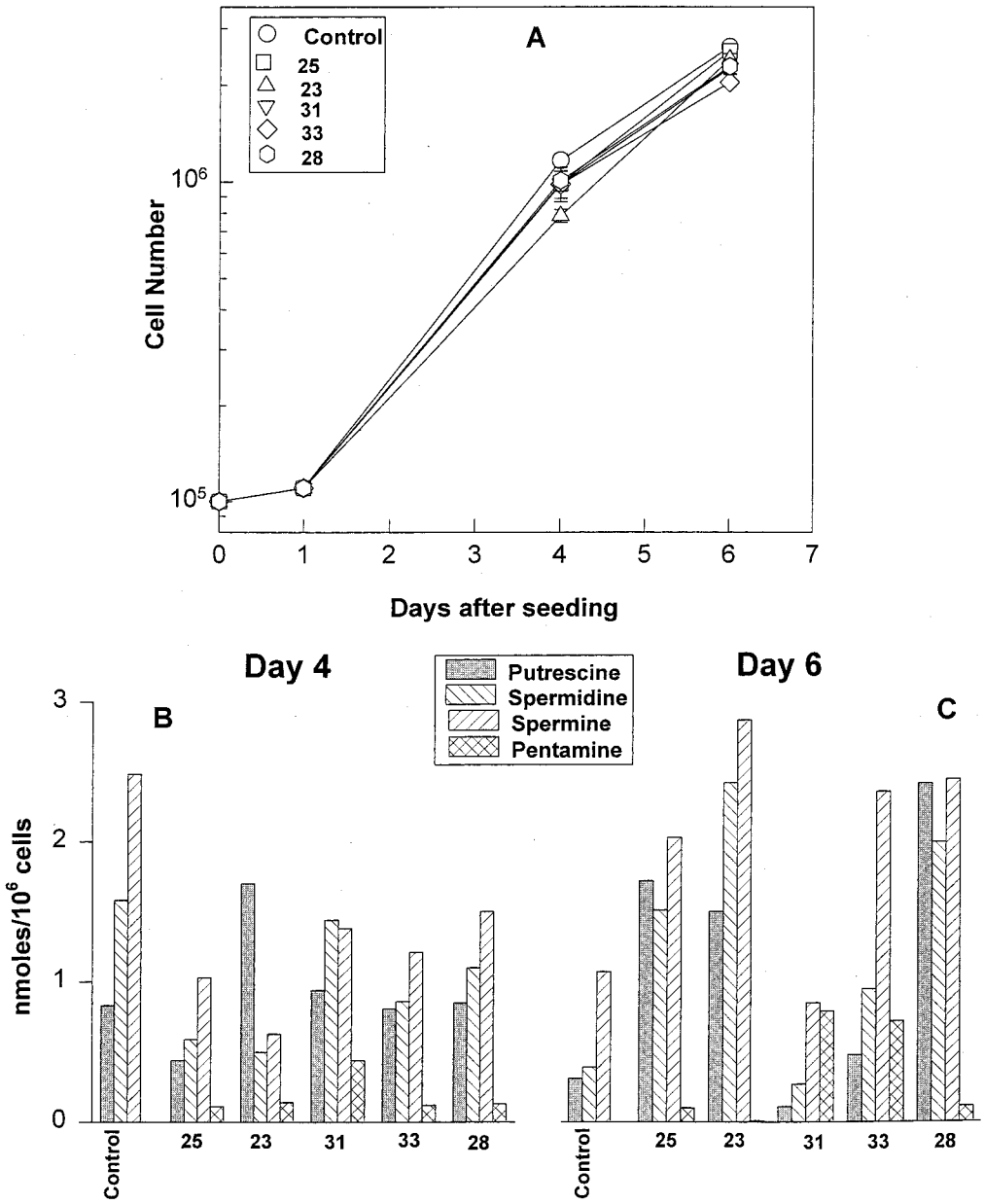


Figure 2. (A) Effects of 1 μM **25**, **23**, **31**, **33**, and **28** on the growth of DuPro cells. Symbol for each pentamine is shown in the inset. Each data point is an average of at least three separate experiments run in duplicate. Error bars where not shown are smaller than the symbol size. (B) Effects of 1 μM **25**, **23**, **31**, **33**, and **28** on the polyamine levels of DuPro cells on day 4 of treatment. Symbol for each polyamine is shown in the inset. Each data point is an average of two separate experiments. (C) Effects of 1 μM **25**, **23**, **31**, **33**, and **28** on the polyamine levels of DuPro cells on day 6 of treatment. Symbol for each polyamine is shown in the inset. Each data point is an average of two separate experiments.

pathways[22] or inhibition of the synthesis of cell cycle-related proteins[23] may also contribute to the cytotoxic effects of the unsaturated pentamines. Very recent evidence in yeast cells has shown that several of these subtle effects, such as the expression of certain cell cycle-related proteins, can be regulated by cellular polyamine-induced changes in chromatin structure.[24]

Conclusions

The synthesis of nine new unsaturated pentamines formally derived from the strong cytotoxic agent BE-4-4-4-4 was reported. The unsaturated pentamines carry *cis*-double bonds at all possible positions of the pentaazapentacosane chain and are the mono-, di-, tri-, and tetraunsaturated derivatives. When assayed against four human prostate cancer cell lines, two pentamines, one a monounsaturated and one a diunsaturated pentamine, where the double bonds are placed at the terminal butane segments of the pentaazapentacosane chain, were nearly as growth inhibitory as the fully saturated analogue (BE-4-4-4-4). Among all the pentamines, they were the most efficient inhibitors of cell growth at 10 μM when assayed by cell counting in the DuPro cell line and were about 1 order of magnitude more cytotoxic than was BE-4-4-4-4 as determined by the CFE assay. It can be expected that the introduction of *cis*-double bonds in the polyamine backbone would favor their in vivo catabolism by mixed-function oxidases and facilitate their elimination by conjugation with metabolic transporters. Only after completing the in vivo assays will it become clear if the new unsatur-

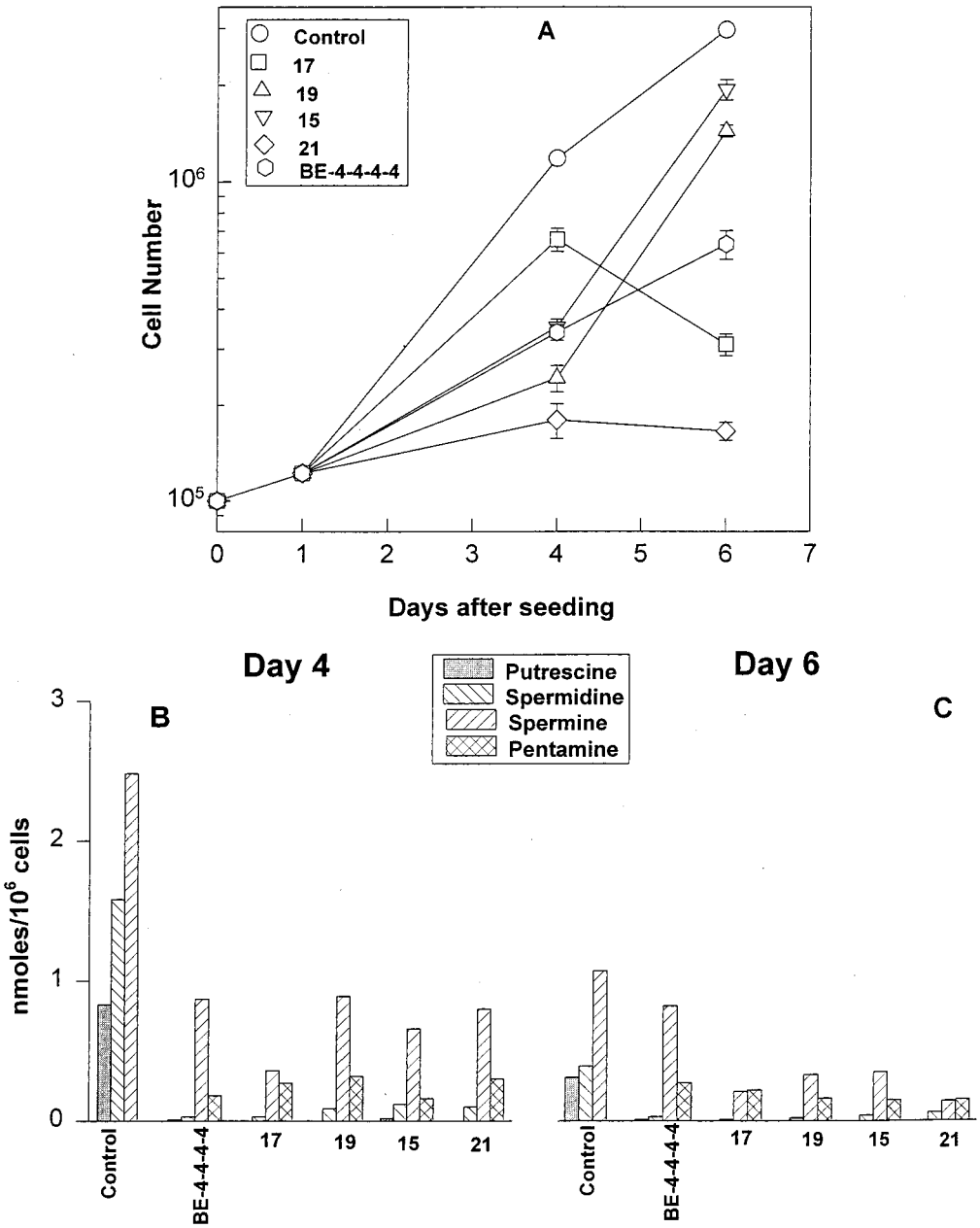


Figure 3. (A) Effects of 10 μM BE-4-4-4-4, **17**, **19**, **15**, and **21** on the growth of DuPro cells. Symbol for each pentamine is shown in the inset. Each data point is an average of at least three separate experiments run in duplicate. Error bars where not shown are smaller than the symbol size. (B) Effects of 10 μM BE-4-4-4-4, **17**, **19**, **15**, and **21** on the polyamine levels of DuPro cells on day 4 of treatment. Symbol for each polyamine is shown in the inset. Each data point is an average of two separate experiments. (C) Effects of 10 μM BE-4-4-4-4, **17**, **19**, **15**, and **21** on the polyamine levels of DuPro cells on day 6 of treatment. Symbol for each polyamine is shown in the inset. Each data point is an average of two separate experiments.

ated pentamines are indeed a major step toward the development of effective cancer chemotherapeutic agents.

Experimental Section

Chemistry. NMR spectra were obtained using a Bruker AM-250 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 (ESI) were obtained on a PE Sciex API 365 electrospray triple quadrupole spectrometer; mass spectra (FAB) were obtained on an Autospec (VG) spectrometer. HPLC analyses of the dansyl derivatives of the polyamine pentamines were routinely performed to check the purity of the samples. A Vydac C-18 (300-μm pore) column for separations and a fluorescence spectrometer (340-nm excitation, 515-nm emission) for detection were used.

***N*-Ethyl-*N*-(3-cyanobutyl)mesitylenesulfonamide (2).** NaH (80%, 1.08 g, 36 nmol) was added under nitrogen to a solution of amide **1**[11b] (6.8 g, 30 mmol) in DMF (50 mL). The mixture was stirred for 1 h and a solution of 4-bromobutyronitrile (4.88 g, 33 mmol) in DMF (10 mL) was slowly added. The mixture was stirred overnight at 75 °C, the solvent was distilled off, the residue taken up in chloroform, washed with a saturated solution of ammonium chloride, dried (Na_2SO_4), and evaporated. The residue was purified by flash chromatography on silica gel (hexane−ethyl acetate 3:1); 8.0 g (90%) of **2** was obtained as a colorless oil: ^{1}H NMR ($CHCl_3$) δ 1.05 (t, 3H), 1.90 (m, 2H), 2.30 (bm, 5H), 2.60 (s, 6H), 3.20 (q, 2H), 3.35 (t, 2H), 6.95 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 12.50, 20.61, 22.43, 23.60, 31.05, 36.12, 40.39, 43.78, 118.62, 131.79, 132.67, 139.71, 142.41; MS-ESI (*m*/*z*) calcd 294.41 (M^+), found 294.40.

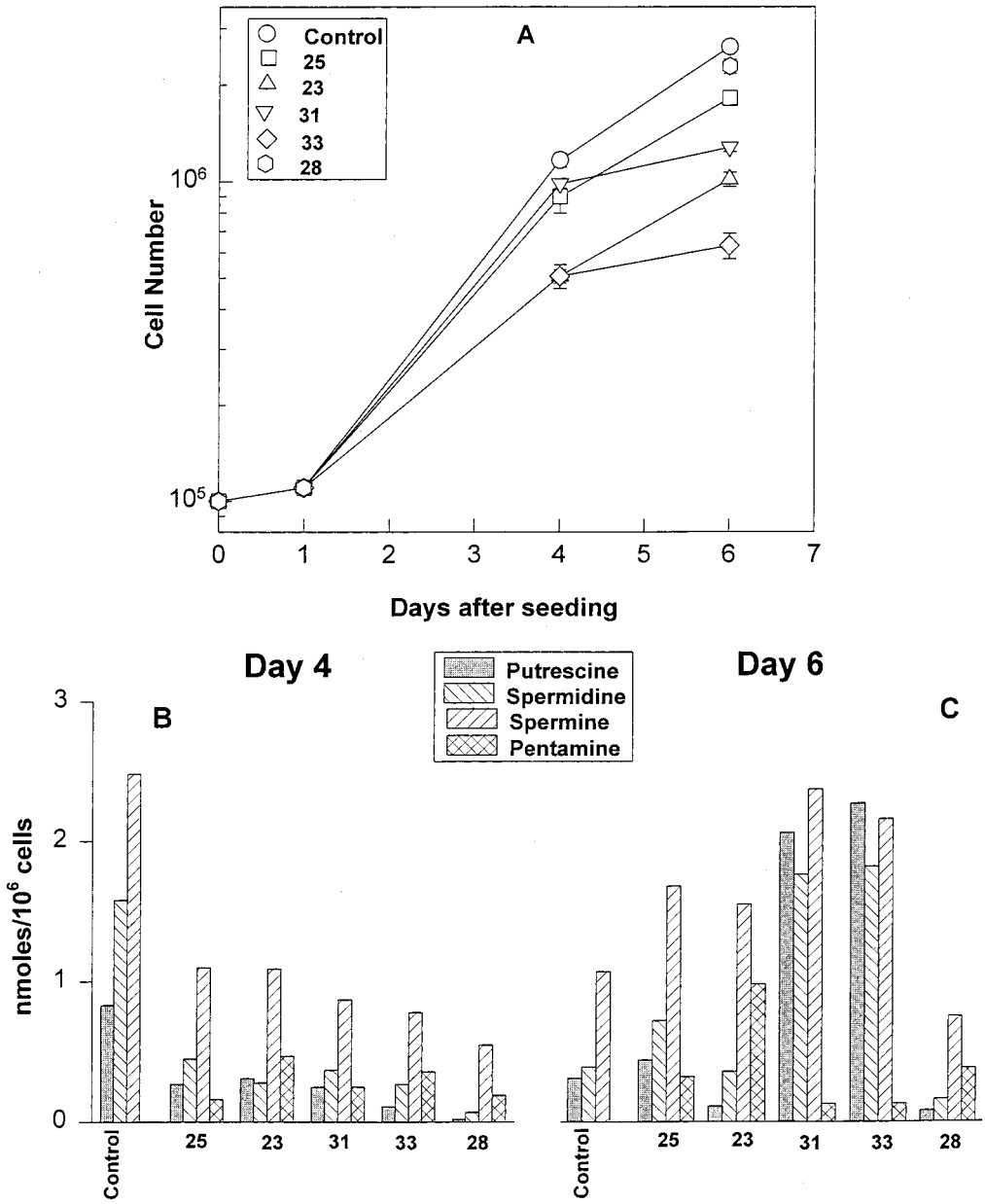


Figure 4. (A) Effects of 10 μM **25**, **23**, **31**, **33**, and **28** on the growth of DuPro cells. Symbol for each pentamine is shown in the inset. Each data point is an average of at least three separate experiments run in duplicate. Error bars where not shown are smaller than the symbol size. (B) Effects of 10 μM **25**, **23**, **31**, **33**, and **28** on the polyamine levels of DuPro cells on day 4 of treatment. Symbol for each polyamine is shown in the inset. Each data point is an average of two separate experiments. (C) Effects of 10 μM **25**, **23**, **31**, **33**, and **28** on the polyamine levels of DuPro cells on day 6 of treatment. Symbol for each polyamine is shown in the inset. Each data point is an average of two separate experiments.

^1N,^4N-Bis(mesitylenesulfonyl)-^4N-ethyl-1,4-diaminobutane (4). Nitrile **2** (7.8 g, 27 mmol) was dissolved in a mixture of ethanol (150 mL) and concentrated HCl (1.5 mL), PtO_2 was added (700 mg), and the mixture was hydrogenated at 50 psi overnight. The catalyst was filtered off, the solvent was evaporated and the amine **3** (7.8 g, 98%) was used in the next step without further purification: ^{1}H NMR ($CDCl_3$, free base) δ 1.00 (t, 3H), 1.55 (m, 4H), 2.25 (s, 3H), 2.80 (t, 2H), 3.20 (m, 4H), 6.95 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 12.54, 20.69, 22.53, 24.72, 27.65, 39.92, 40.29, 44.59, 131.71, 133.21, 139.82, 142.09; MS-FAB (*m*/*z*) calcd 299.94 ($M^+ + 1$), found 299.45.

Mesitylenesulfonyl chloride (8.8 g, 40.5 mmol) in dioxane (30 mL) was added dropwise to a stirred mixture of **3** (7.8 g, 27 mmol) dissolved in dioxane (60 mL) and 50% KOH (30 mL) at 5 °C. The reaction mixture was allowed to reach 20 °C and then kept overnight. An excess of water was added and the mixture was extracted with chloroform, dried (Na_2SO_4) and evaporated. The oily residue was crystallized from ethyl acetate−hexane; 10.9 g (82%) of **4** was obtained: mp 71.5−72 °C; ^{1}H NMR ($CDCl_3$) δ 1.00 (t, 3H), 1.10−1.50 (m, 4H), 2.30 (s, 6H), 2.55, 2.60 (s, 12H), 2.85 (q, 2H), 3.15 (m, 4H), 4.70 (t, 1H), 6.95, 7.00 (s, 4H); ^{13}C NMR ($CDCl_3$) δ 12.70, 20.92, 21.04, 22.73, 22.92, 24.58, 26.68, 40.04, 42.02, 44.42, 131.91, 133.31, 133.64, 138.99, 140.05, 142.15, 142.35; MS-FAB (*m*/*z*) calcd 480.68 (M^+), found 480.69. Anal. ($C_{24}H_{36}N_2O_4S_2$) C, H, N.

***N*-Ethyl-*N*-(4-phthalimido-2-butenyl)mesitylenesulfonamide (5).** Amide **1** (3.1 g, 13.65 mmol) was dissolved in anhydrous DMF (30 mL), NaH (85%, 0.42 g) was slowly added, and the mixture was stirred at room temperature for 1 h. *N*-(4-Chloro-2-butenyl)phthalimide[25] (3.06 g, 13 mmol) dissolved in 20 mL of DMF was added and the mixture stirred at 80 °C for 18 h. It was then cooled to room temperature, quenched with water (10 mL), and the solution was evaporated to dryness in vacuo. The aqueous layer was extracted with chloroform (3 × 25 mL), the organic layers were washed with brine (35 mL), dried ($MgSO_4$), and the solvent was evaporated leaving behind

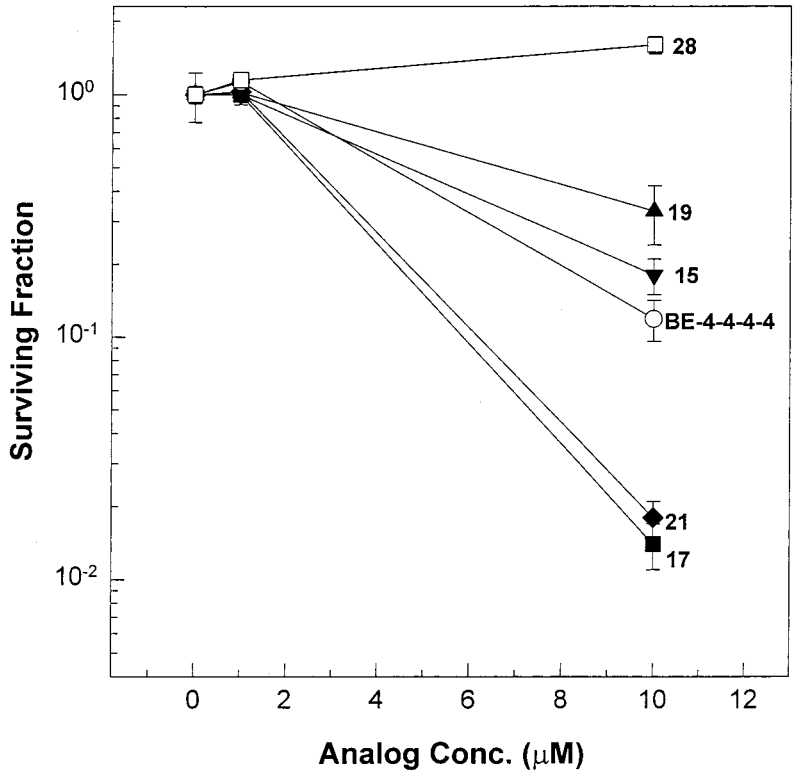


Figure 5. Effects of BE-4-4-4-4 (○), **17** (■), **19** (▲), **15** (▼), **21** (◆), and **28** (□) on the survival of DuPro cells on day 6 of treatment. Each data point and corresponding error bars are, respectively, an average and the standard deviation of six independent observations.

a viscous residue that solidified upon titration with hexane to give **5**. It was pure enough to be used in the next step without purification: 4.75 g (82%); ^{1}H NMR ($CDCl_3$) δ 1.16 (t, J = 7.11 Hz, 3H), 2.29 (s, 3H), 2.63 (s, 6H), 3.29 (q, J = 7.11 Hz, 2H), 4.06 (d, J = 5.24 Hz, 2H), 4.26 (d, J = 5.72 Hz, 2H), 5.59 (m, 2H), 6.95 (s, 2H), 7.71 (m, 2H), 7.83 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 13.06, 20.89, 22.72, 34.35, 40.68, 42.01, 123.27, 126.69, 129.47, 131.90, 134.00, 140.24; MS-FAB (*m*/*z*) calcd 426.53 (M^+), found 426.54.

***N*-Ethyl-*N*-(4-amino-2-butenyl)mesitylenesulfonamide (6).** Amide **5** (20 g, 47 mmol) was dissolved in methanol, hydrazine monohydrate (50 mL, 98.5 mmol) was added and the solution stirred at 55 °C for 24 h. Initially it was a homogeneous solution; after several hours a white solid precipitated. The mixture was cooled to 20 °C, 300 mL of concentrated HCl was slowly added (exothermic reaction), stirring was continued for additional 12 h, the solution was evaporated, and the resulting solid was dissolved in 200 mL of water and extracted with chloroform (3 × 150 mL). The combined organic layers were pooled, dried ($MgSO_4$),and evaporated to dryness. The residue was purified by flash chromatography using hexanes−ethyl acetate (7:3) as eluant; 9.0 g (65%) of **6** was obtained: ^{1}H NMR ($CDCl_3$) δ 1.0 (t, J = 7.1 Hz, 3H), 2.28 (s, 3H), 2.56 (s, 6H), 2.62 (br, 2H), 3.12 (q, J = 7.1 Hz, 3H), 3.73 (br, 2H), 3.94 (d, J = 6.0 Hz, 2H), 5.80 (m, 2H), 6.92 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 12.97, 20.93, 22.74, 36.43, 40.94, 42.08, 124.29, 131.89, 132.00, 132.62, 140.21, 142.67; MS-FAB (*m*/*z*) calcd 480.68 (M^+), found 296.44. It was used without further purification in the next step.

^{1}*N*,^{4}*N*-Bis(mesitylenesulfonyl)-^{4}*N*-ethyl-1,4-diamino-2-butene (7) was obtained from **6** as described for **4** in 96% yield. It was purified by flash chromatography using hexane−ethyl acetate (4:1.5) as eluant: mp 98−99 °C; ^{1}H NMR ($CDCl_3$) δ 0.93 (t, J = 5.85 Hz, 3H), 2.23 (s, 3H), 2.24 (s, 3H), 2.50 (s, 6H), 2.56 (s, 6H), 3.06 (q, J = 7.15 Hz, 2H), 3.48 (t, J = 5.99 Hz, 2H), 3.68 (d, J = 5.72 Hz, 2H), 4.58 (t, J = 6.24 Hz, 1H), 5.44 (m, 2H), 6.87 (s, 2H), 6.89 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 12.80, 20.89, 22.64, 22.89, 39.01, 40.59, 41.41, 128.14, 128.46, 131.91, 139.08, 140.19, 142.26, 142.54; MS-FAB (*m*/*z*) calcd 480.68 (M^+).

^{3}*N*,^{8}*N*-Bis(mesitylenesulfonyl)-12-chloro-3,8-diaza-(5*Z*,-10*Z*)-dodecadiene (9). Amide **7** (4.8 g, 10 mmol) was dissolved in anhydrous DMF (40 mL); NaH (0.37 g) was added in portions while the mixture was stirred at room temperature for 2 h. *cis*-1,4-Dichloro-2-butene (7.5 g, 60 mmol) in 10 mL DMF was then added, and stirring was continued at 50 °C for 18 h. The mixture was cooled to 20 °C, quenched with 10 mL water, the solvents evaporated, and the contents were partitioned between water (50 mL) and chloroform (50 mL). The aqueous layer was further extracted with chloroform (3 × 50 mL), the pooled organic layers dried ($MgSO_4$), evaporated to dryness, and **9** was purified by flash chromatography using hexane−ethyl acetate (8.5:1.5) as eluant: 5.5 g (97%); mp 106−108 °C; ^{1}H NMR ($CDCl_3$) δ 1.03 (t, 3H), 2.30 (s, 6H), 2.57 (s, 12H), 3.17 (q, 2H), 3.71 (m, 4H), 3.81 (d, 2H), 3.95 (d, 2H), 5.50 (m, 3H), 5.74 (m, 1H), 6.93 (s, 2H), 6.95 (s, 2H); ^{13}CNMR ($CDCl_3$) δ 12.91, 22.70, 22.74, 38.20, 40.45, 41.60, 42.11,42.33, 128.17, 128.95, 129.34, 129.40, 131.94, 132.08, 140.23, 140.34, 142.91. Anal. ($C_{28}H_{39}ClN_2O_4S_2$) C, H, N.

^{3}*N*,^{8}*N*-Bis(mesitylenesulfonyl)-12-iodo-3,8-diaza-(5*Z*)-dodecene (8) was prepared (70%) from **7** and 1,4-diiodobutane as described above for **9**. The product was purified by column chromatography using hexanes−ethyl acetate (4:1) as eluant: ^{1}H NMR($CDCl_3$) δ 1.04 (t, 3H), 1.63 (m, 4H), 2.30 (s, 6H), 2.58 (s, 12H), 3.04 (t, 2H), 3.16 (m, 4H), 3.78 (d, 4H), 5.55 (m, 2H), 6.94 (s, 2H), 6.95 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 5.69, 12.92, 20.95, 22.72, 22.78, 28.25, 30.36, 40.47, 41.59, 42.11, 44.71, 128.34, 129.00, 131.94, 132.06, 132.60, 132.89, 140.15, 140.21, 142.50, 142.71; MS-FAB (*m*/*z*) calcd 661.67 (M^+ +1), found 661.68.

^{3}*N*,^{8}*N*-Bis(mesitylenesulfonyl)-3,8-diazalauronitrile (10) was prepared (94%) from **4** and 4-bromobutyronitrile as described for **2**: ^{1}H NMR ($CDCl_3$) δ 0.97 (t, 3H), 1.40 (m, 4H), 1.85 (pent, 2H), 2.27 (t, 2H), 2.30 (s, 6H), 2.57 (s, 6H), 2.58 (s, 6H), 3.13 (m, 6H), 3.28 (t, 2H), 6.94 (s, 2H), 6.96 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 12.55, 14.54, 20.84, 22.64, 22.73, 23.65, 24.43, 24.57, 39.88,44.31, 44.54, 45.58, 118.69, 131.84, 132.05, 132.73, 133.36, 139.94, 142.20, 142.71; MS-FAB (*m*/*z*) calcd 548.77 (M^+ + 1), found 548.78.

^{3}*N*,^{8}*N*-Bis(mesitylenesulfonyl)-3,8-diazalaurylamine (11) was prepared (93%) from **10** as described above for **3**: ^{1}H NMR

($CDCl_3$) δ 1.00 (t, 3H), 1.40 (m, 10H), 2.29 (s, 6H), 2.57 (b, 14H), 3.13 (m, 8H), 6.93 (s, 4H); ^{13}C NMR ($CDCl_3$) δ 12.72, 20.90, 22.72, 22.78, 24.67, 24.80, 30.80, 40.02, 41.61, 44.56, 45.10, 45.38, 131.87, 140.04, 142.21, 142.28; MS-FAB (*m*/*z*) calcd 552.80 ($M^+ + 1$), found 552.81.

1,6,11-Tris(mesitylenesulfonyl)-1,6,11-triazatridecane (12) was prepared (97%) from **11** as described for **4**: ^{1}H NMR ($CDCl_3$) δ 0.96 (t, 3H), 1.38 (m, 8H), 2.29 (s, 9H), 2.55 (s, 6H), 2.56 (s, 6H), 2.59 (s, 6H), 2.80 (m, 2H), 3.10 (m, 8H), 4.67 (t, 1H), 6.93 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 12.56, 20.87, 22.70, 22.74, 22.84, 24.40, 26.45, 24.67, 26.62, 39.87, 41.88, 44.45, 45.02, 45.09, 131.86, 131.90, 133.12, 133.12, 133.32, 133.68, 138.91, 139.97, 142.02, 142.02, 142.21, 142.38; MS-FAB (*m*/*z*) calcd 734.04 (M^+), found 734.05.

^3N,^8N-Bis(mesitylenesulfonyl)-12-chloro-3,8-diaza-(10*Z*)-dodecene (13) was prepared (99%) from **4** and 1,4-dichloro-2-butene as described for **9**: ^{1}H NMR ($CDCl_3$) δ 1.01 (t, 3H), 1.38 (m, 4H), 2.29 (s, 3H), 2.30 (s, 3H), 2.57 (s, 6H), 2.61 (s, 6H), 3.11 (m, 4H), 3.16 (q, 2H), 3.81 (d, 2H), 3.98 (d, 2H), 5.51 (m, 1H), 5.77 (m, 1H), 6.93 (s, 2H), 6.95 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 12.76, 20.91, 22.71, 22.76, 24.74, 38.12, 40.08, 41.85, 44.59, 129.14, 129.25, 131.88, 132.02, 140.19, 142.21, 142.63; MS-FAB (*m*/*z*) calcd 569.22 (M^+), found 569.23.

^3N,^8N,^{13}N,^{18}N,^{23}N-Pentakis(mesitylenesulfonyl)-3,8,13,-18,23-pentaaza-(15*Z*,20*Z*)-pentacosadiene (14) was prepared (78%) from **12** and **9** following the procedure described above for **9**. It was purified by column chromatography using hexane−ethyl acetate (7:3) as eluant: ^{1}H NMR ($CDCl_3$) δ 0.97 (t, 3H), 0.99 (t, 3H), 1.29 (m, 8H), 2.29 (s, 15H), 2.54, 2.55, 2.59 (s, 3H), 3.06 (m, 12H), 3.65 (m, 8H), 5.48 (m, 4H), 6.92 (s, 10H); ^{13}C NMR ($CDCl_3$) δ 12.70, 12.83 20.88, 20.91, 22.65, 22.68, 22.72, 22.74, 24.48, 24.72, 40.04, 40.47, 41.53, 42.07, 42.22, 42. 34, 44.54, 44.96, 127.94, 128.27, 129.20, 131.92, 132.05, 139.96, 140.00, 140.12, 140.16, 140.27, 142.19, 142.25, 142.47, 142.58, 142.87; MS-FAB (*m*/*z*) calcd 1264.78 (M^+), found 1264.77.

3,8,13,18-Tetraaza-(15*Z*,20*Z*)-pentacosadiene Pentahydrochloride (15). Pentamide **14** (0.93 g, 0.735 mmol) was dissolved in 20 mL anhydrous methylene chloride; phenol (3.5 g,36.8 mmol) was added, followed by 30% HBr in glacial acetic acid (17.6 mL). The mixture was stirred overnight at 25 °C. Water (10 mL) was added, the aqueous layer was separated, the organic layer extracted with 5 mL of water, and the combined aqueous layers were washed with methylene chloride (6 × 15 mL). The aqueous layer was evaporated under vacuum to a solid residue that was dissolved in 1 mL 1 N sodium hydroxide followed by 1 mL 50% potassium hydroxide. The solution was extracted with chloroform (10 × 5 mL), the combined organic layers were dried ($MgSO_4$), evaporated, and the residue dissolved in dry ethyl ether. Dry hydrogen chloride was passed through the solution kept at 0 °C; pentahydrochloride **15** (0.33 g, 84%) precipitated, it was filtered and washed with ether: ^{1}H NMR (D_2O) δ 1.29 (t, 3H), 1.31 (t, 3H), 1.79 (m, 8H), 3.12 (m, 12H), 3.87 (m, 8H), 5.98 (m, 4H); ^{13}C NMR (D_2O) δ 13.36, 13.46, 25.66, 25.77, 45.44, 45.74, 46.24, 46.41, 46.84, 49.09, 49.41, 49.70, 129.02, 12916, 129.47, 129.66; MS-ESI (*m*/*z*) calcd 354.59 ($M^+ + 1$), found 354.60. Anal. ($C_{20}H_{48}Cl_5N_5$) C, H, N.

^3N,^8N,^{13}N,^{18}N,^{23}N-Pentakis(mesitylenesulfonyl)-3,8,13,-18,23-pentaaza-(20*Z*)-pentacosene (16) was prepared (51%) from **12** and **8** as described above for **14**: ^{1}H NMR ($CDCl_3$) δ 0.97 (t, 3H), 0.99 (t, 3H), 1.29 (m, 12H), 2.29 (s, 15H), 2.55 (s), 2.56 (s), 2.57 (s), 3.10 (m, 16H), 3.70 (m, 4H), 5.47 (m, 2H), 6.93 (s, 10H); ^{13}C NMR ($CDCl_3$) δ 12.69, 12.83, 20.91, 22.69, 22.71, 22.76, 24.43, 24.70, 40.48, 41.11, 41.48, 44.50, 44.91, 128.13, 128.90, 131.88, 131.94, 132.01, 133.29, 139.95, 140.00, 140.15, 142.22, 142.29, 142.60; MS-FAB (*m*/*z*) calcd 1266.80 (M^+), found 1266.79.

3,8,13,18,23-Pentaza-(20*Z*)-pentacosene pentahydrochloride (17) was prepared (79%) from **16** as described above for **15**: ^{1}H NMR (D_2O) δ 1.29 (t, 3H), 1.30 (t, 3H), 1.78 (m, 12H), 3.12 (m, 16H), 3.83 (m, 4H), 5.96 (m, 2H); ^{13}C NMR (D_2O) δ 13.31, 13.42, 25.62, 25.75, 45.38, 45.71, 46.18, 46.76, 49.07, 49.32, 49.69, 129.11, 129.39; MS-ESI (*m*/*z*) calcd 356.61 ($M^+ + 1$), found 356.60.

^3N,^8N,^{13}N,^{18}N,^{23}N-Pentakis(mesitylenesulfonyl)-3,8,13,-18,23-pentaza-(15*Z*)-pentacosene (18) was prepared (52%) from **12** and **13** as described for **14:** ^{1}H NMR ($CDCl_3$) δ 0.95 (m, 6H), 1.32 (m, 12H), 2.29 (s, 15H), 2.55 (s, 30H), 3.06 (m, 16H), 3.70 (m, 4H), 5.47 (m, 2H), 6.92 (s, 10H); ^{13}C NMR ($CDCl_3$) δ 12.67, 20.90, 22.71, 22.76, 24.43, 24.68, 39.97, 42.08, 44.48, 44.90, 45.61, 128.28, 128.45, 131.87, 131.93, 132.01, 139.96, 140.00, 140.12, 142.21, 142.28, 142.58; MS-FAB (*m*/*z*) calcd 1266.80 (M^+), found 1266.81.

3,8,13,18-Pentaaza-(15*Z*)-pentacosene pentahydrochloride (19) was prepared (96%) from **18** as described above for **15**: ^{1}H NMR (D_2O) δ 1.28 (t, 6H), 1.78 (m, 12H), 3.09 (m, 16H), 3.84 (m, 4H), 5.96 (m, 2H); ^{13}C NMR (D_2O) δ 13.31, 25.61, 25.73, 45.70, 46.79, 49.05, 49.36, 49.65, 129.19; MS-ESI (*m*/*z*) calcd 356.61 ($M^+ + 1$), found 356.60.

^3N,^8N,^{13}N,^{18}N,^{23}N-Pentakis(mesitylenesulfonyl)-3,8,13,-23-pentaza-(5*Z*,20*Z*)-pentacosadiene (20). A mixture of potassium hydroxide (0.25 g), potassium carbonate (0.25 g) and tetrabutylammonium bromide (0.05 g) was suspended in 15 mL benzene, mesitylenesulfonamide (0.2 g, 1 mmol) was added to the suspension and the mixture was heated at 50 °C. Iodide **14** (2.0 g, 3 mmol) in 10 mL benzene was added, the mixture heated under reflux for 18 h, then cooled to 20 °C, the inorganic solids filtered off, washed with benzene (2 × 20 mL), and the combined benzene layers washed several times with water until the washings were neutral. The benzene was dried ($MgSO_4$), evaporated to dryness and the residue purified by column chromatography using hexanes−ethyl acetate (7.5:2.5) as eluant; 0.95 g (25%) of **20** was obtained: ^{1}H NMR ($CDCl_3$) δ 1.00 (t, 6H), 1.28 (m, 8H), 2.29 (s, 15H), 2.53 (s), 2.55 (s), 2.57 (s), 3.03 (m, 8H), 3.12 (q, 4H), 3.70 (m, 8H), 5.47 (m, 4H), 6.93 (s, 10H); ^{13}C NMR ($CDCl_3$) δ 12.78, 20.85, 22.63, 22.69, 24.32, 24.58, 40.41, 41.43, 42.00, 44.76, 45.43, 128.08, 128.83, 131.88,131.95, 132.77, 132.85, 133.23, 139.90. 140.00, 140.08, 142.22, 142.43,142.53; MS-FAB (*m*/*z*) calcd 1264.78 (M^+), found 1264.79.

3,8,13,18,23-Tetraaza-(5*Z*,20*Z*)-pentacosadiene pentahydrochloride (21) was prepared (57%) from **20**: ^{1}H NMR (D_2O) δ 1.31 (t, 6H), 1.78 (m, 8.H), 3.15 (m, 12H), 3.83 (m, 8H), 5.96 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 13.43, 25.64, 25.76, 45.39, 46.19, 46.77, 49.35, 49.72, 129.11, 129.41; MS-ESI (*m*/*z*) calcd 354.59 ($M^+ + 1$), found 354.60. Anal. ($C_{20}H_{48}Cl_5N_5$) C, H, N.

^3N,^8N,^{13}N,^{18}N,^{23}N-Pentakis(mesitylenesulfonyl)-3,8,13,-18,23-pentaaza-(5*Z*,10*Z*,15*Z*,20*Z*)-pentacosatetraene (22) was prepared (24%) from **9** and mesitylenesulfonamide as described for **20**: mp 58 °C; ^{1}H NMR ($CDCl_3$) δ 0.99 (t, 6H), 2.29 (s, 15H), 2.53 (s), 2.55 (s), 3.12 (q, 4H), 3.63 (m, 16H), 5.49 (m, 8H), 6.93 (s, 10H); ^{13}C NMR ($CDCl_3$) δ 12.85, 20.89, 20.92, 22.66, 40.47, 41.53, 42.19, 128.00, 128.47, 128.58, 129.11, 131.92, 132.05, 140.17, 140.30, 142.46, 142.87; MS-FAB (*m*/*z*) calcd 1260.75 (M^+), found 1260.76.

3,8,13,18,23-Pentaza-(5*Z*,10*Z*,15*Z*,20*Z*)-pentacosatetraene pentahydrochloride (23) was prepared (81%) from **22** as described above for **15**: ^{1}H NMR (D_2O) δ 1.31 (t, 6H), 3.15 (q, 4H), 3.84 (m, 4H), 3.90 (m, 12H), 5.98 (m, 8H); ^{13}C NMR (D_2O) δ 13.42, 45.41, 46.22, 46.44, 129.07, 129.37, 129.42, 129.58; MS-MALDI (*m*/*z*) calcd 350.56 ($M^+ + 1$), found 350.55. Anal. ($C_{20}H_{44}Cl_5N_4$) C, H, N.

^3N,^8N,^{13}N,^{18}N,^{23}N-Pentakis(mesitylenesulfonyl)-3,8,13,-18,23-pentaaza-(10*Z*,15*Z*)-pentacosadiene (24) was prepared (25%) from **13** as described above for **20**: mp 62 °C; ^{1}H NMR ($CDCl_3$) δ 0.95 (quint, 6H), 1.33 (m,8H), 2.29 (s, 15H), 2.54 (s), 2.55 (s), 3.07 (m, 12H), 3.65 (m, 8H), 5.48 (m,4H), 6.92 (s, 10H); ^{13}C NMR ($CDCl_3$) δ 12.69, 20.90,22.69, 22.73, 24.70, 40.03, 42.13, 42.30, 44.53, 45.59, 128.11, 128.79, 131.87, 132.00, 140.02, 140.14, 140.28, 142.17, 142.58, 142.85; MS-FAB (*m*/*z*) calcd 1264.78 (M^+), found 1264.79.

3,8,13,18,23-Pentaaza-(10*Z*,15*Z*)-pentacosadiene pentahydrochloride (25) was prepared from **24** (87%) as described above for **15**: ^{1}H NMR (D_2O) δ 1.28 (t, 6H), 1.79 (m, 8H), 3.10 (m, 12H), 3.87 (m, 8H), 5.98 (m, 4H); ^{13}C NMR (D_2O)

δ 12.70, 25.00 25.13, 45.10, 45.81, 46.21, 48.44, 48.78, 128.44, 128.85; MS-ESI (*m*/*z*) calcd 354.59 ($M^+ + 1$), found 354.60. Anal. ($C_{20}H_{48}Cl_5N_5$) C, H, N.

1,6,11-Tris(mesitylenesulfonyl)-1,6,11-triaza-(3*Z*)-tridecene (26). Mesitylenesulfonamide (1.47 g, 7.38 mmol) was dissolved in 50 mL anhydrous DMF, and NaH (85%, 0.3 g) was added under nitrogen. The mixture was stirred at 20 °C, **13** (1.40 g, 2.46 mmol) dissolved in 25 mL dry DMF was added, and the solution was heated at 65 °C during 18 h. The mixture was then cooled, 10 mL water was added, the solvent evaporated to dryness, and the solid residue partitioned between 40 mL water and 40 mL chloroform. The aqueous layer was extracted with chloroform (2 × 30 mL), the combined organic layers were washed with water (3 × 50 mL), dried ($MgSO_4$), and evaporated. The residue was purified by column chromatography using hexanes−ethyl acetate (7.5:2:5); 1.7 g (97%) of **26** was obtained: mp 50 °C; ^{1}H NMR ($CDCl_3$) δ 0.94 (t, 3H), 1.30 (m, 4H), 2.29 (s), 2.30 (s), 2.55 (s, 12H), 2.65 (s, 6H), 3.11 (m, 6H), 3.52 (m, 1H), 3.65 (m, 2H), 3.71 (m, 1H), 4.82 (br, 1H), 5.47 (m, 2H), 6.93 (s, 4H), 6.96 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 12.50, 20.91, 22.71, 22.76, 22.83, 22.91, 24.66, 38.98, 39.85, 42.15, 42.26, 44.50, 128.06, 128.51, 131.86, 131.91, 138.18, 140.00, 140.14, 140.28, 142.17, 142.65. Anal. ($C_{37}H_{53}N_3O_6S_3$) C, H, N.

^{3}*N*,^{8}*N*,^{13}*N*,^{18}*N*,^{23}*N*-Pentakis(mesitylenesulfonyl)-3,8,13,-18,23-pentaaza-(10*Z*,20*Z*)-pentacosadiene (27) was prepared (50%) from **26** and **8** as described above for **16**: ^{1}H NMR ($CDCl_3$) δ 0.99 (quint, 6H), 1.33 (m, 8H), 2.29 (s, 15H), 2.55 (s), 2.57 (s), 3.10 (m, 12H), 3.70 (m, 4H), 3.77 (m, 4H), 5.42 (m, 4H), 6.93 (s, 10H); ^{13}C NMR ($CDCl_3$) δ 12.70, 12.71, 20.89, 22.66, 22.72, 22.78, 22.81, 24.60, 26.53, 40.39, 41.37,41.87, 42.20, 45.47, 128.26, 128.62, 131.78, 131.84, 131.86,131.92, 132.77, 138.92, 139.96, 140.09 140.17, 142.57, 142.63. Anal. ($C_{65}H_{92}N_5O_{10}S_5$) C, H, N.

3,8,13,18,23-Pentaaza-(10*Z*,20*Z*)-pentacosadiene pentahydrochloride (28) was prepared (40%) from **27** following the procedure described for **15**: ^{1}H NMR (D_2O) δ 1.28 (t, 3H), 1.30 (t, 3H), 1.70−1.89 (m, 8H), 3.00−3.20 (m, 12H), 3.78−3.80 (m, 12H), 3.78−3.90 (m, 8H), 5.90−6.10 (m, 4H); ^{13}C NMR (D_2O) δ 13.45, 25.71, 25.76, 26.77, 45.40, 46.20, 46.78, 49.10, 49.40, 129.10, 129.40. Anal. ($C_{20}H_{48}Cl_5N_5$) C, H, N.

1,6,11-Tris(mesitylenesulfonyl)-1,6,11-triaza-(3*Z*,8*Z*)-tridecatriene (29) was prepared (94%) from **9** following the procedure described for **16**: ^{1}H NMR ($CDCl_3$) δ 0.97 (t, 3H), 2.30 (s, 9H), 2.53 (s, 6H), 2.58 (s,6H), 2.64 (s, 6H), 3.10 (q, 2H), 3.49 (m, 2H), 3.59 (m, 2H), 3.67 (m, 4H), 5.46 (m, 4H), 6.93 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 12.70, 20.86, 20.89, 22.67, 22.81, 22.88, 39.01, 40.49, 41.51, 42.23, 42.52, 127.71, 127.97, 128.95, 129.18, 131.84, 131.93, 131.96, 131.99, 132.05, 139.08, 140.17, 140.21, 140.30. Anal. ($C_{37}H_{51}N_3O_6S_3$) C, H, N.

^{3}*N*,^{8}*N*,^{13}*N*,^{18}*N*,^{23}*N*-Pentakis(mesitylenesulfonyl)-3,8,13,-18,23-pentaaza-(5*Z*,10*Z*,20*Z*)-pentacosatriene (30) was prepared (82%) by the condensation of **29** and **8** as described for **16**: ^{1}H NMR ($CDCl_3$) δ 0.99 (t, 6H), 1.33 (4H), 2.29 (s, 15H), 2.55 (s), 2.57 (s), 3.07 (m, 8H), 3.70 (m, 12H), 5.46 (m, 6H), 6.92 (s, 10H); ^{13}C NMR ($CDCl_3$) δ 12.69, 12.80, 20.84, 22.62, 22.68, 22.73, 22.77, 24.58, 26.55, 40.44, 41.51, 41.86, 42.04, 42.24, 45.49, 128.10, 128.25, 128.52, 128.62, 128.82, 131.89, 131.95, 132.79, 138.89, 140.07, 140.14, 140.23, 141.94, 142.44, 142.53, 142.82; MS-FAB (*m*/*z*) calcd 1262.77 (M^+), found 1262.78.

3,8,13,18,23-Pentaaza-(5*Z*,10*Z*,20*Z*)-pentacosatriene pentahydrochloride (31) was obtained from **30** (65%) following the procedure described above for **15**: ^{1}H NMR (D_2O) δ 1.31 (t, 6H), 1.79 (m, 4H), 3.12 (m, 8H), 3.83 (m, 12H), 5.96 (m, 6H); ^{13}C NMR (D_2O) δ 13.48, 25.69, 26.76, 41.67, 45.44, 46.24, 46.45, 46.80, 49.41, 129.00, 129.12, 129.45, 129.71; MS-ESI (*m*/*z*) calcd 352.58 ($M^+ + 1$), found 352.59. Anal. ($C_{20}H_{46}Cl_5N_5$) C, H, N.

^{3}*N*,^{8}*N*,^{13}*N*,^{18}*N*,^{23}*N*-Pentakis(mesitylenesulfonyl)-3,8,13,-18,23pentaza-(5*Z*,10*Z*,15*Z*)-pentacosatriene (32) was obtained (89%) by condensation of **29** and **13** following the procedure described for **16**: ^{1}H NMR ($CDCl_3$) δ 0.95 (m, 6H), 1.33 (m, 4H), 2.29 (s, 15H), 2.54 (s), 2.55 (s), 2.57 (s), 3.09 (m, 8H), 3.66 (m, 12H), 5.48 (m, 6H), 6.93 (s, 10H); ^{13}C NMR ($CDCl_3$) δ 12.51, 12.63, 20.84, 20.86, 22.63, 22.65, 22.84, 24.61, 38.92, 40.40, 41.40, 42.11, 42.18, 44.44, 45.48, 127.95, 128.07, 128.49, 128.62, 128.80, 131.76, 131.83, 131.85, 131.88, 132.01, 138.05, 139.01, 140.07, 140.13, 140.24, 142.15, 142.21, 142.87; MS-FAB (*m*/*z*) calcd 1262.77 (M^+), found 1262.76. Anal. ($C_{65}H_{91}N_5O_{10}S_5$) C, H, N.

3,8,13,18,23-Pentaaza-(5*Z*,10*Z*,15*Z*)-pentacosatriene pentahydrochloride (33) was obtained (54%) from **32** following the procedure described for **15**: mp 270 °C dec; ^{1}H NMR (D_2O) δ 1.31 (m, 6H), 1.80 (m, 4H), 3.10 (m, 8H), 3.86 (m, 12H), 5.98 (m, 6H); ^{13}C NMR (D_2O) δ 13.30, 13.42, 25.58, 25.70, 45.69, 46.21, 46.43, 46.81, 49.02, 49.37, 129.00, 129.15, 129.37, 129.59; MS-ESI (*m*/*z*) calcd 352.58 ($M^+ + 1$), found 352.59. Anal. ($C_{20}H_{46}N_5Cl_5$) C, H, N.

Biology. Materials: DuPro cells were obtained from M. Eileen Dolan of the University of Chicago, Chicago, IL. All other cell lines used in this study were obtained from the American Type Culture Collection (Rockville, MD). Tissue culture medium was obtained from Fisher Scientific (Itasca, IL) and fetal bovine serum was obtained from Gemini Bioproducts, Inc. (Calabasas, CA). All other reagents were analytical grade. Deionized double-distilled water was used in all studies.

Tissue culture:[12] Cells were seeded into 75-cm^2 culture flasks with 15 mL of Eagle's minimal essential medium supplemented with 10% fetal calf serum and nonessential amino acids. The flasks were incubated in a humidified 95% air/5% CO_2 atmosphere. The cells were grown for at least 24 h to ensure that they were in the log phase of growth. They were then treated with the pentamines. Cells were harvested by treatment for 5 min with STV (saline A, 0.05% trypsin, 0.02% EDTA) at 37 °C. The flasks were rapped on a lab bench, pipetted several times and aliquots of the cell suspension were withdrawn and counted using a Coulter particle counter that was standardized for each cell line using a hemacytometer.

Polyamine analysis:[26] Approximately 1 × 10^6 cells were taken from harvested samples and centrifuged at 1000 rpm at 4 °C for 5 min. The cells were washed twice by resuspending them in chilled Dulbecco's isotonic phosphate buffer (pH 7.4) and centrifuged at 1000 rpm at 4 °C. The supernatant was decanted and 250 μL of 2% perchloric acid was added to the cell pellet. The cells were then sonicated and the lysates were kept at 4 °C for at least 1 h. After centrifugation at 8000*g* for 5 min, the supernatant was removed for analysis. An appropriate volume of the supernatant (50−100 μL) was fluorescence-labeled by derivatization with dansyl chloride following procedures described elsewhere.[26] Each sample was loaded onto a C-18 high-performance liquid chromatography column and separated at the analytical laboratory of the University of Wisconsin Comprehensive Cancer Center (UWCCC) using a previously published procedure.[26] Peaks were detected and quantitated using a Shimadzu HPLC fluorescence monitor that was coupled to a Spectra-Physics peak integrator. Because polyamine levels vary with environmental conditions, control cultures were sampled for each experiment.

MTT assay:[5] Trypsinized cell suspensions were diluted to seed a 80-μL suspension of 500 cells into each well of a 96-well Corning microtiter plate. The plates were incubated overnight at 37 °C in a humidified 95% air/5% CO_2 atmosphere. 20 μL of appropriately diluted stock solutions of each drug was added to the middle 8 columns of the microtiter plates. Each drug concentration was run in quadruplicate. The outer columns of the plates were used for buffer controls. Cells were incubated with the drug for 6 days. 25 μL of a 5 mg/mL solution of 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to each well and the plates were incubated at 37 °C for 4 h. Cells were then lysed by incubating at 37 °C overnight with 100 μL of lysis buffer (500 mL of the lysis buffer contained: 100 g lauryl sulfate (SDS), 250 mL of *N*,*N*-dimethylformamide in 2 mL of glacial acetic acid, pH 4.8). The color developed was read at room temperature at 570 nm in a E-max precision microplate reader (Molecular Devices

Corp., Sunnyvale, CA) and data were analyzed using manufacturer supplied cell survival software.

CFE assay:[12] All of the cell lines that were used in this assay have previously been optimized with respect to the necessary incubation times for observable colony formation. Both floating and attached cells were harvested and centrifuged at 1000 rpm for 10 min at 4 °C. The cell pellets were resuspended and replated in quadruplicate at appropriate dilution into 60-mm plastic Petri dishes. The Petri dishes were prepared not more than 24 h in advance with 4 mL of supplemented Eagle's minimum essential medium containing 5−10% fetal bovine serum (standardized for each cell line). Cells were incubated for the previously standardized number of days in a 95% air/5% CO_2 atmosphere. The plates were stained with 0.125% crystal violet in methanol and counted. Results are expressed as a surviving fraction of an appropriate control.

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