Cyclopropane-Containing Polyamine Analogues Are Efficient Growth Inhibitors of a Human Prostate Tumor Xenograft in Nude Mice

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Polyamine analogues **7**, **10**, **18**, **27**, and **32** containing cyclopropane rings were obtained by chemical synthesis. Their antineoplastic activities were assessed against the cultured human prostate tumor cell lines DU-145, DuPro, and PC-3. Decamines **32** and **27** exhibited variable levels of cytotoxicity against all three cell lines, while **7**, **10**, and **18** were efficacious against DU-145 and DuPro. Maximum tolerated doses (MTD) for all five compounds in a NCr-nu mouse model were determined at dosing schedules of q1d × 5 (ip) in two cycles with a break of 10 days between cycles. Their antitumor efficacies were then tested against DU-145 tumor xenografts in mice treated with all five agents at their respective MTDs. In addition, the efficacies of **7** and **10** against the same tumor xenograft were assessed at doses below their respective MTDs. In all experiments, administration began two weeks after tumor implantation. All compounds efficiently inhibited tumor growth for up to 50 days postimplantation, with negligible animal body weight loss. Tetramine **10** and hexamine **18** were the most efficient among the five analogues in arresting tumor growth. Tetramine **10** containing two cyclopropane rings had the lowest systemic toxicity as reflected in animal body weight loss. It was further assessed at a weekly administration regimen of (q1w × 4) in two cycles with a four-week break between the cycles. At this dosing schedule, **10** again efficiently arrested tumor growth with negligible effect on animal body weight. Tetramine **10** also arrested the growth of large tumors (ca. 2000 mm^3) treated 66 days postimplantation. Studies on the metabolism of **10** showed that it accumulates in tumor within 6 h after the end of administration and reached a maximum level 72 h after cessation of dosing. Intracellular concentrations of **10** in liver and kidney were much smaller when compared to those in the tumor when measured 72 h after cessation of dosing. In liver and kidney, the deethyl metabolites of **10** accumulated over a 96 h period after cessation of dosing.

Introduction

Cancer cells accumulate the polyamines spermidine and spermine and their diamine precursor putrescine. Cancer-bearing animals have elevated levels of polyamines in their extracellular fluids.[1] The role of polyamines in cell division and the potential usefulness of polyamine analogues as antiproliferative agents against many tumor cell lines have been extensively discussed.[2,3] Prostate cancer shares with other cancers the above-mentioned intracellular accumulation of polyamines, but is different in the sense that the prostate is by itself a uniquely rich factory of polyamine production. The semen of healthy men contains large amounts of spermine (ca. 3 mM) that originates mainly from prostatic secretion.[4] No other human organ has such high polyamine concentrations.[5] Therefore, targeting prostatic polyamines has been a tempting approach for the therapy of prostatic carcinoma.[6]

Prostate cancer is the single most common cancer in American males.[7] During the year 2003, it is estimated that ca. 220,900 new cases of prostate cancer will be detected in the USA and about 28 800 men will die of this disease.[7] Human prostate cancer cell lines were found to be very sensitive to in vitro treatment with conformationally restricted $^{\alpha}N,^{\omega}N$-bisethylspermine analogues, as well as with their higher tetramine and pentamine homologues.[8−10] As is the case with most anticancer drugs, the aforementioned analogues are not devoid of systemic toxicity, and their cytotoxicities must be balanced against their toxic side effects.[11,12] A series of maximum tolerated dose (MTD) studies in animals helped to identify one of the least toxic among them; namely, SL-11093 (3,8,13,18-tetraaza-10,11-[(*E*)-1,2-cyclopropyl]eicosane tetrahydrochloride): a $^{\alpha}N,^{\omega}N$-bisethyl polyamine analogue that strongly inhibits the growth of cultured human prostate tumor cell lines.[9] When assayed in nude mice xenografted with the human prostate cancer cell line DU-145, SL-11093 showed a strong inhibitory effect on tumor growth at doses that had minimal systemic toxicity in the animals.[13]

The synthesis of a cyclopropane-substituted polyamine such as SL-11093, showing good in vivo antineoplastic effects and minimal systemic toxicity, opened a new vista for further synthesis of polyamine analogues. The cyclopropane ring, due to its unusual bonding and

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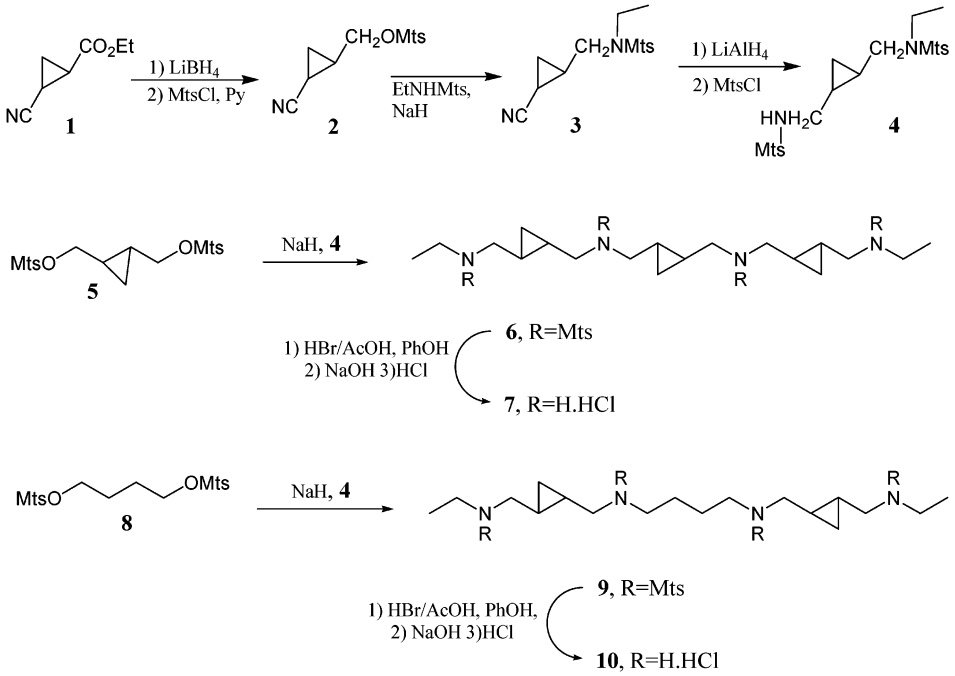
Scheme 1



inherent ring strain (275 kcal/mol), is unique among carbocycles in both its properties and reactions.[14] Substituted cyclopropanes are endowed with a large spectrum of biological properties, ranging from enzyme inhibitions, to antibiotic, antiviral, antitumor, and neurological properties.[15] They, therefore, seem quite suited for the design of novel polyamine analogues. The design of efficacious polyamine analogues poses challenges while offering tempting advantages. The main challenge is that the precise mechanisms by which polyamine analogues kill tumor cells and cause systemic toxicity are still not entirely clear.[16] It has been established that polyamines and their analogues bind to nucleic acids and alter their conformations.[17] It was also shown that in mammalian tissues, polyamine homeostasis involves a complex of several sensitive feedback systems regulating the synthesis, degradation, and transport of polyamines.[18] Disruption of this homeostasis by polyamine analogues leads to polyamine deprivation and can result in cell death. Cell death can also result from all these effects acting in tandem with the condensation of chromatin resulting from polyamine analogues binding to the phosphodiester bonds of DNA.[19] Polyamine analogues have recently been shown to induce the small regulatory protein antizyme, and this antizyme-inducing potential may maximize polyamine deprivation and growth inhibition.[20] There is also the interesting observation that high levels of intracellular polyamines promote hyperacetylation of histones,[21] and highly acetylated histones are known to signal the start of cell regulatory events.[22] On the other hand, the main advantage in designing new polyamine analogues is that relatively small structural changes in the aliphatic skeleton of the polyamines can cause pronounced differences in their pharmacological behavior and toxic side effects, as well as in their antineoplastic activities both at the cellular level and in animal models.[23]

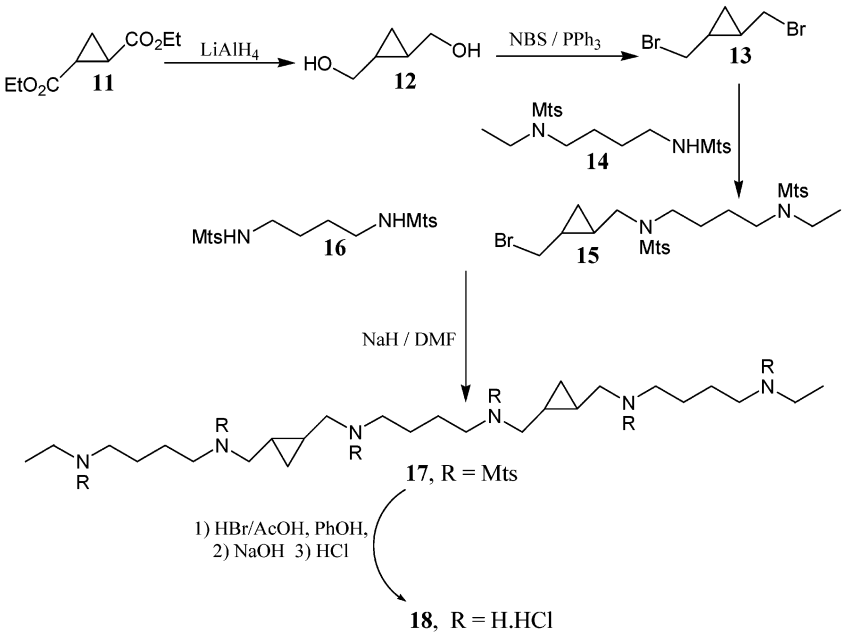
Structural variations of SL-11093[9] led to the synthesis of new $^{\alpha}N,^{\omega}N$-bis(ethyl)tetramines substituted with three cyclopropane rings (**7**, Scheme 1) as well as with two cyclopropane rings (**10**, Scheme 1). A $^{\alpha}N,^{\omega}N$-bis(ethyl)hexamine substituted with two cyclopropane rings (**18**, Scheme 2) was also prepared, and two $^{\alpha}N,^{\omega}N$-bis(ethyl)decamines substituted with one cyclopropane ring (**27**, Scheme 3) and with three cyclopropane rings (**32**, Scheme 4) were obtained by total syntheses. The cyclopropane-containing polyamine analogues are mixtures of stereoisomers. Systemic toxicities and cytotoxic effects of the aforementioned analogues were assessed in vivo in nude mice grafted with the human prostate cancer DU-145 cell line. Treatment with any of these analogues resulted in marked antiproliferative effects.

Chemistry

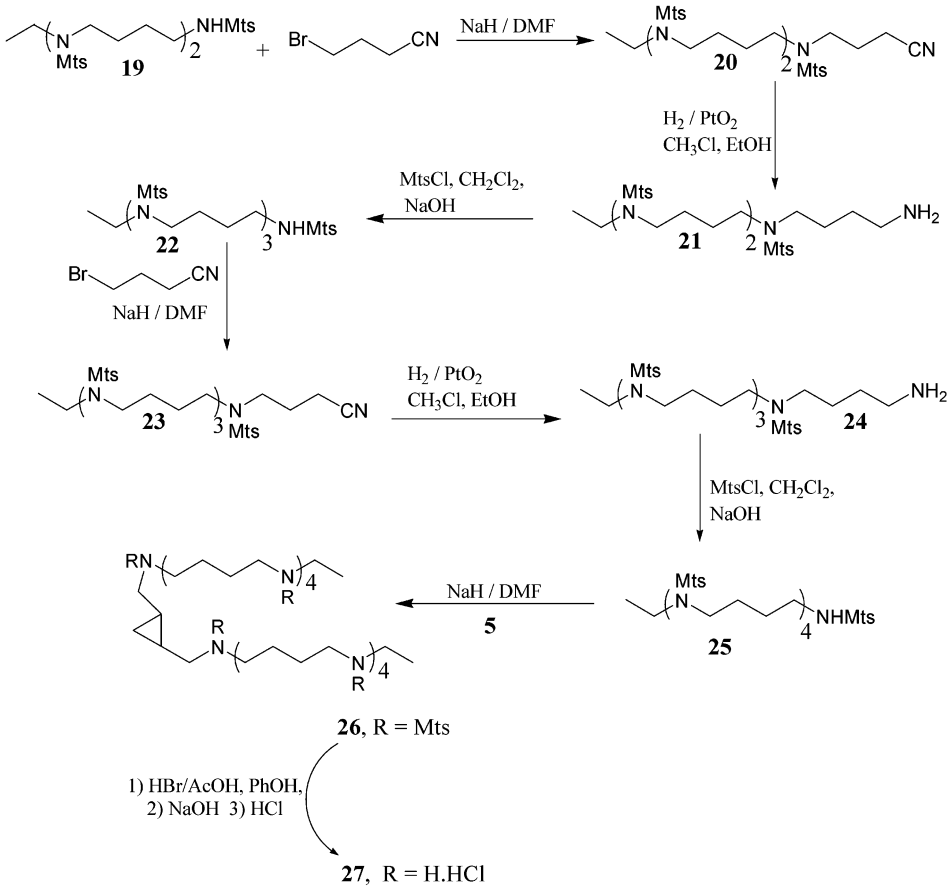
The synthesis of **7** made use of the known ethyl (*E*)-2-cyanocyclopropanecarboxylate **1** as a first intermediate (Scheme 1). It was reduced with lithium borohydride to the hydroxymethyl derivative and the latter esterified with mesitylenesulfonyl chloride to give **2**. This ester was used to alkylate mesitylenesulfonyl ethylamide, and **3** was thus prepared. Reduction of the nitrile residue followed by a reaction with mesitylenesulfonyl chloride gave **4**. It was brought into reaction with the mesitylenesulfonic diester of *trans*-1,2-bis(hydroxymethyl)-cyclopropane **5** to give the tetramide **6**. Cleavage of the protecting residues allowed the synthesis of the tetrahydrochloride **7**. Condensation of **4** with the bis-(mesitylenesulfonyl) ester of 1,4-butanediol **8** gave the tetramide **9**. Deprotection of the amine groups gave tetrahydrochloride **10**.

The synthesis of the hexamine **18** started with the known *trans*-diester **11** (Scheme 2). It was reduced to the dialcohol **12**, and the latter treated with *N*-bromosuccinimide and triphenylphosphine to convert it into the dibromomethyl derivative **13**. The reaction of **13** with the known diamide **14** allowed the synthesis of the bromomethyl derivative **15**. The latter was used to alkylate the diamide **16**, a reaction that allowed the synthesis of **17**. Cleavage of the mesitylenesulfonyl residues gave hexahydrochloride **18**.

Scheme 2



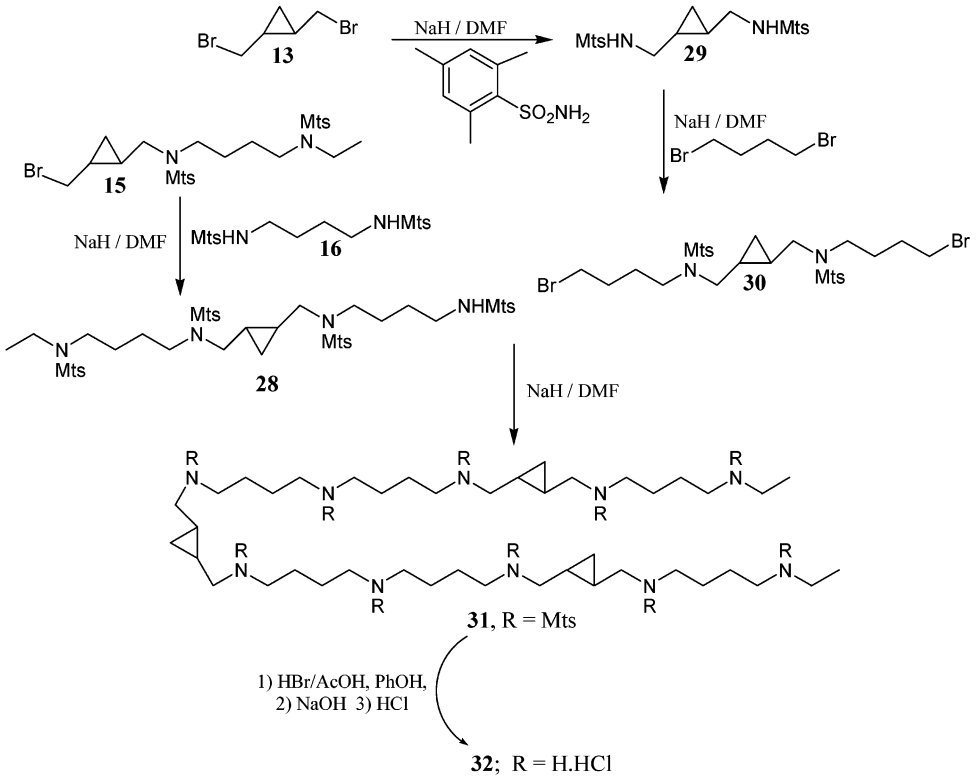
Scheme 3



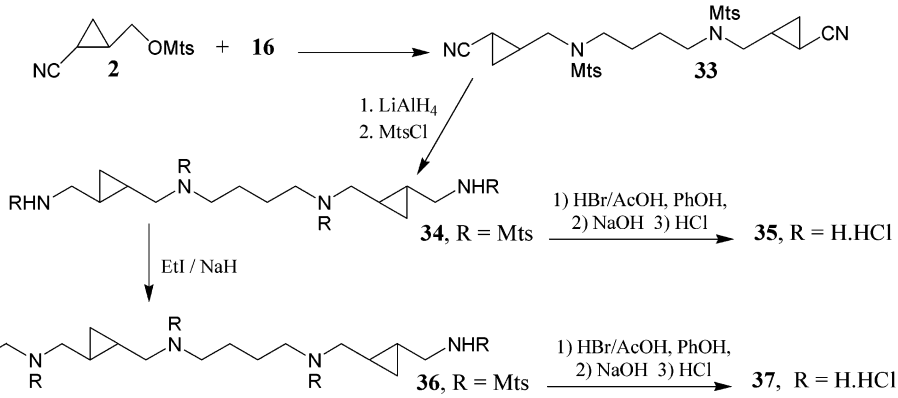
For the synthesis of decamine **27**, the pentamide intermediate **25** was constructed starting with the known triamide **19** (Scheme 3). The latter was alkylated with 4-bromobutyronitrile to give nitrile **20**, the nitrile was reduced to the amine **21** and the latter acylated with mesitylenesulfonyl chloride to give **22**. By repeating the aforementioned sequence on **22**, the nitrile **23** was obtained. It was then reduced to **24**, and the latter acylated to give **25**. This amide was alkylated with the bis-mesitylenesulfonyloxy derivative **5** to give the decamide **26**. Cleavage of the protecting groups in **26** gave the decahydrochloride **27**.

The synthesis of **32** started with the condensation of the bromomethyl intermediate **15** with 1,4-bis (mesitylenesulfonyl)putrescine **16** to give **28** (Scheme 4). In tandem, the dibromomethyl intermediate **13** was used to alkylate mesitylenesulfonylamide to give **29**. Alkylation of **29** with 1,4-dibromobutane gave **30**; condensa-

Scheme 4



Scheme 5



tion of **30** with **28** gave decamide **31**. Cleavage of the protecting residues of **31** gave decahydrochloride **32**.

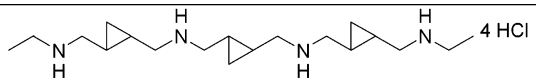
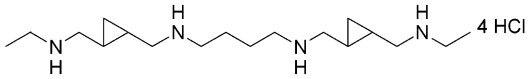
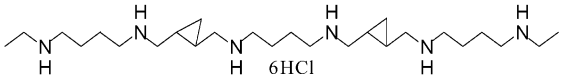
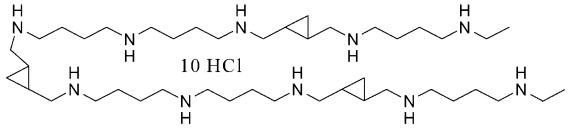
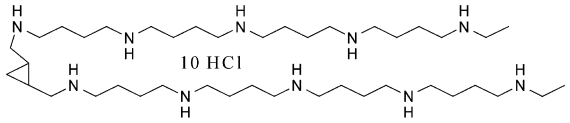
The synthesis of possible metabolites of **10** (see below), namely, the monoethyl derivative **37** and the dideethyl derivative **35**, were obtained as outlined in Scheme 5. Alkylation of 1,4-bis(mesitylenesulfonyl)putrescine **16** with **2** gave the dicyanocyclopropyl derivative **33**. Reduction of the nitrile residues with $LiAlH_4$ followed by sulfonylation of the free amine residues with mesitylenesulfonyl chloride gave **34**. Cleavage of the sulfonyl protecting groups gave **35** tetrahydrochloride. Alkylation of **34** with 1 equiv of ethyl iodide gave **36**; the deprotection of the amino residues on **36** gave tetrahydrochloride **37**.

Biological Results and Discussion

The growth inhibitory effects of the five cyclopropane-containing polyamine analogues were assessed against the human prostate tumor cell lines PC-3, DuPro, and DU-145 using a MTT assay. The ID_{50} value was defined as the concentration of analogue required to inhibit cell growth by 50%. The structures of the analogues and their ID_{50} values are listed in Table 1. All compounds efficiently inhibited the growth of DuPro and DU-145 cell lines at nanomolar concentrations. While **32** and **27** were effective against all three cell lines, **7**, **10**, and **18** have no appreciable effect on PC-3 cell growth within the concentration range assayed. The DU-145 cell line was the most sensitive of the cell lines assayed. Antitumor efficacies of the five synthetic polyamine analogues were assessed in vivo in athymic mice grafted with the DU-145 cell line.

Toxicity studies were carried out in male athymic NCr-nu mice housed in microisolator cages (see Experimental Section). The compounds were dissolved in water and administered ip daily for 5 consecutive days (q1d × 5) in a first cycle. The animals were then rested for 10 days, and a second cycle of treatment also followed the q1d × 5 schedule. Body weights were measured twice a week. Tetramine **7** at 75 mg/kg was

Table 1

Polyamines	Structure of Polyamines	$ID_{50}(\mu M)$ values		
		PC-3	DuPro	DU145
(**7**)		>31.25	0.63	0.08
(**10**)		>31.25	0.26	0.06
(**18**)		>31.25	0.38	0.12
(**32**)		0.30	0.20	0.22
(**27**)		0.40	0.34	0.20

lethal, resulting in the death of all treated mice. Lower dosages of 50, 25, and 12.5 mg/kg were well tolerated without animal deaths. Administration of 50 mg/kg was accompanied by maximum average body weight losses of 8% (2 g) and 17% (4 g) after the first and second rounds of treatment, respectively. Administration of two cycles of 25 mg/kg produced only 1 g (4%) body weight loss only after the second cycle of treatment, while administration of cycles of 12.5 mg/kg did not cause any body weight loss. The maximum tolerated dosage (MTD) for **7** was, therefore, 50 mg/kg for this treatment protocol.

Tetramine **10** at 75 mg/kg was also lethal, resulting in the death of all three mice. Deaths occurred during (on day 10) and after the end (day 15) of the second cycle of treatment. Dosages of 50, 25, and 12.5 mg/kg were well tolerated without deaths. The 50 mg/kg dose was associated with maximum average body weight losses of 8% and 17% as a result of the first and second cycles of treatment, respectively. The lower dosages of 25 and 12.5 mg/kg caused average body weight losses of 13% and 4%, respectively, only after the second cycle of treatment. The 50 mg/kg dose was the MTD for this treatment protocol.

Hexamine **18** was toxic at 25 mg/kg, resulting in two treatment-related deaths out of three animals. The deaths occurred after the end of the first treatment cycle. Dosages of 15 mg and 10 mg/kg were well tolerated without deaths or body weight loss during the first treatment cycle. The second treatment cycle, at 15 mg/kg resulted in an average maximum body weight loss of 8% (2 g). Thus, 15 mg/kg was the MTD for this treatment protocol.

Decamine **32** was lethal at a dosage of 25 mg/kg. Dosages of 12.5 mg and 6.25 mg/kg were well tolerated without deaths or body weight loss during the first treatment cycle. Administration of the second treatment cycle at 12.5 mg/kg was associated with an average 13% body weight loss (3 g). The second treatment cycle at 6.25 mg/kg did not produce any body weight loss. A 12.5 mg/kg dose was the MTD for this protocol.

Decamine **27** was toxic during the first treatment cycle at 25 mg/kg causing 100% lethality. At 12.5 mg/kg, the first treatment cycle was well tolerated. The second cycle, however, was lethal for two out of three animals. Dosages of 6.25 mg and 3.13 mg/kg were well tolerated, without deaths or body weight loss. 6.25 mg/kg was the MTD for this treatment protocol.

The aforementioned data showed that the systemic toxicities of the cyclopropane-containing polyamine analogues increased with the chain length. The shorter chain tetramines (**7** and **10**) were the least toxic, and their toxicity was comparable to that of the tetramine SL-11093.[13] The longer the chain, as in hexamine **18**, the more the systemic toxicity; the MTD values dropped from 50 mg/kg to 15 mg/kg when the drug was administered 5 days in a row (total 250 mg/kg/week for the tetramines versus 75 mg/kg/week for the hexamine). When the decamines were assessed, their systemic toxicity was found to be even higher. For a decamine with three cyclopropane rings as in **32**, the MTD values dropped to 62.5 mg/kg/week. If only one cyclopropane was present in the decamine, as in **27**, toxicity was even higher, and the MTD was only 13.2 mg/kg/week. It was therefore crucial to compare toxicities vs efficacies in this series of analogues to identify the most effective therapeutic analogue.

Efficacy studies were carried out using analogue doses near the MTD values against established DU-145 tumors (ca. 100−250 mm^3) initiating treatment about two weeks postimplantation. In addition, **7** and **10** were also studied at doses lower than their MTDs (33.5 mg/kg,

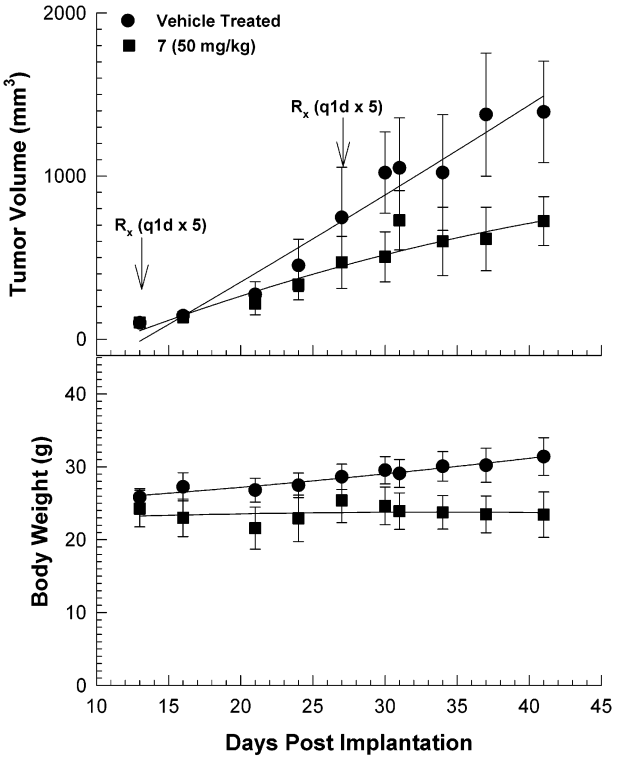


Figure 1. Effect of **7**, 50 mg/kg, q1d × 5 in two cycles on the growth of a DU-145 human prostate tumor xenograft and body weight in nude mice.

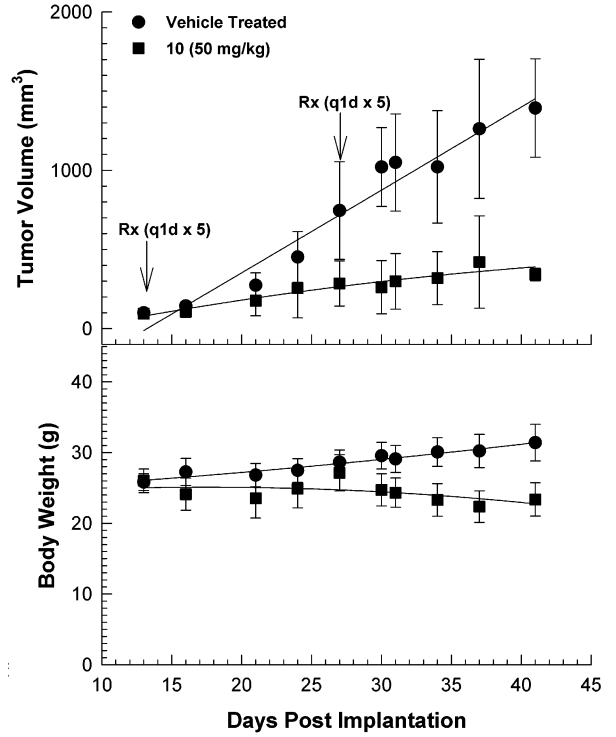


Figure 2. Effect of **10**, 50 mg/kg, q1d × 5 in two cycles on the growth of a DU-145 human prostate tumor xenograft and body weight in nude mice.

q1d × 5, see Figures 1 and 2, Supporting Information). The plot of tumor volumes vs time for all five polyamine analogues are shown in Figures 1−5. With the exception of **7** at 33.5 mg/kg (Figure 1, Supporting Information), all compounds showed marked inhibition of tumor growth within 10 days after the end of the first cycle

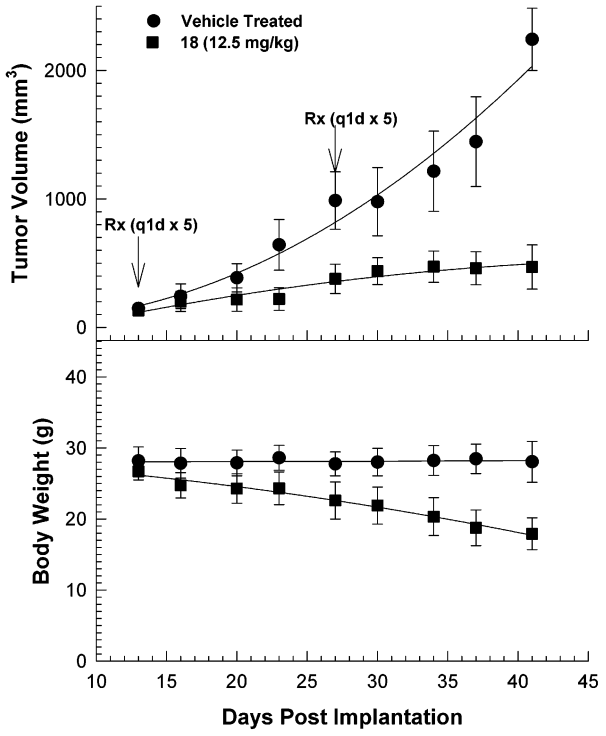


Figure 3. Effect of **18**, 12.5 mg/kg q1d × 5 in two cycles on the growth of a DU-145 human prostate tumor xenograft and body weight in nude mice.

and growth remained arrested for at least 10 days after the end of the second cycle. All experiments utilized weight mice in each group, including controls. No xenografted mice were lost when treated with **7**, **10**, and **18**. In the **32** treated group, one mouse died between days 37 and 41 postimplantation. In the **27** treated group, one mouse died at the end of first cycle of treatment and 50% of the mice (4 out of 8) died at the end of the second treatment cycle. The extent of tumor growth arrest and body weight loss 10 days after the end of the second treatment cycle for all the polyamine analogues are summarized in Figure 6. The mean tumor volumes in the treated mice as a percent of the mean tumor volume of controls (vehicle treated mice) at day 41 post-implantation are shown in the upper panel, and the corresponding mean body weight losses, as compared to the control mice are shown in the lower panel. While all the analogues are capable of arresting tumor growth, **10** at the daily 50 mg/kg dose and **18** at the daily 12.5 mg/kg dose were the two most efficacious drugs (tumor reductions of ca. 80% were achieved after two cycles of treatment). Of these two drugs, **10** was less toxic as judged from the smaller loss of body weight of animals treated with **10**, as compared to that of mice treated with **18** (Figure 6). Tetramine **10** was, therefore, chosen for a more detailed assessment of its antitumor efficacy in vivo.

Efficacy studies were carried out using a once-a-week administration protocol during a four weeks cycle (q1w × 4). For these studies, 12−14 mice were used to obtain statistically significant results. The data for dosages of 50 mg/kg/week and 75 mg/kg/week, along with their *p* values determined by using multivariant analysis,[24] are shown in Figure 7. Tetramine **10** was administered once a week in four- week cycles, inter-

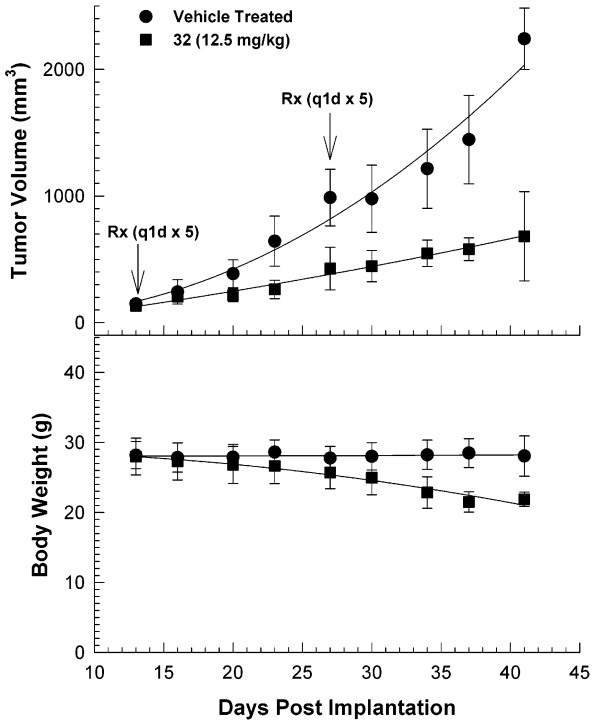


Figure 4. Effect of **32**, 12.5 mg/kg q1d × 5 in two cycles on the growth of a DU-145 human prostate tumor xenograft and body weight in nude mice.

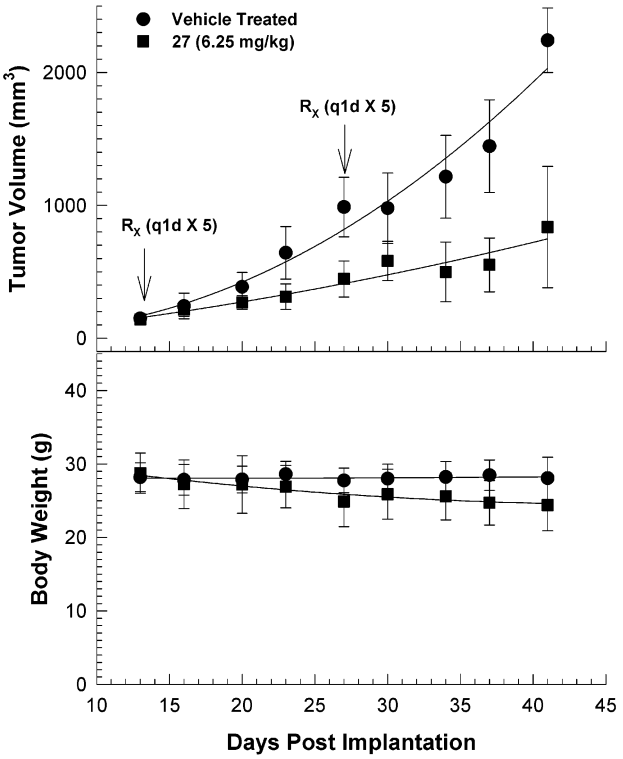


Figure 5. Effect of **27**, 6.25 mg/kg q1d × 5 in two cycles on the growth of a DU-145 human prostate tumor xenograft and body weight in nude mice.

rupted by four-week rest periods. After three cycles of treatment (a total of 120 days after implantation of the tumors), the weekly dosages of 50 mg/kg and 75 mg/kg exhibited statistically significant tumor growth inhibition (p values are between 0.06 and 0.00005). The tumor volumes in mice treated with 50 mg/kg/week were 70% of those in the control mice with negligible effect on body

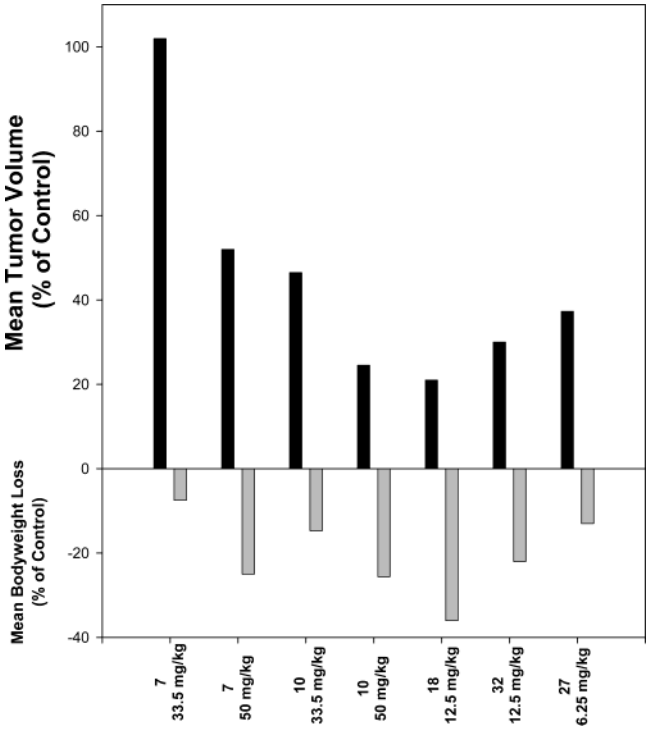


Figure 6. Summary of effects of polyamine analogues given q1d × 5 in two cycles on mean tumor volumes and body weights of nude mice xenografted with DU-145 human prostate tumor cells.

weight. During the same time period, tumor volumes of mice treated with 75 mg/kg/week were less than 25% of those in controls. Although an average 20% of body weight loss was observed at the end of the second cycle of treatment with this dosage, most of it was regained within 10 days after the end of the treatment. Thus, in terms of efficacy, the protocol of prolonged treatment was as satisfactory as the one involving a more intense and short-term treatment. Despite the different time frames, comparable results were obtained with both regimes of administration; i.e., 75−80% reduction of tumor volumes with a ca. 20% of body weight loss. In the case of the short-term administration protocol (Figure 2), 250 mg/kg of **10** was injected per week, while in the case of the prolonged treatment (Figure 7) only 75 mg/kg/week was administered.

To assess the efficacy of **10** against large tumors, a group of seven animals were treated with 75 mg/kg/week (q1w × 4) 66 days after tumor implantation. During this period, tumors had grown large, ca. 2000 mm^3, as compared to tumor volumes of ca. 200 mm^3 that were the starting points for the data presented above. Tetramine **10** entirely arrested the growth of the large tumors (Figure 8). Some body weight loss was observed for these animals, but the loss was difficult to quantitate due to the lack of appropriate controls. Mice had to be sacrificed because of tumor size. Nonetheless, such marked arrest of the growth of large tumors was remarkable. After a month of rest and in the absence of further treatment there was tumor regrowth (Figure 8). The regrowth could be arrested again by a new four-week cycle of treatment.

Metabolism of Tetramine 10. Tumors harvested at the time intervals shown in Figure 9 after the administration of **10** already showed significant uptake of the

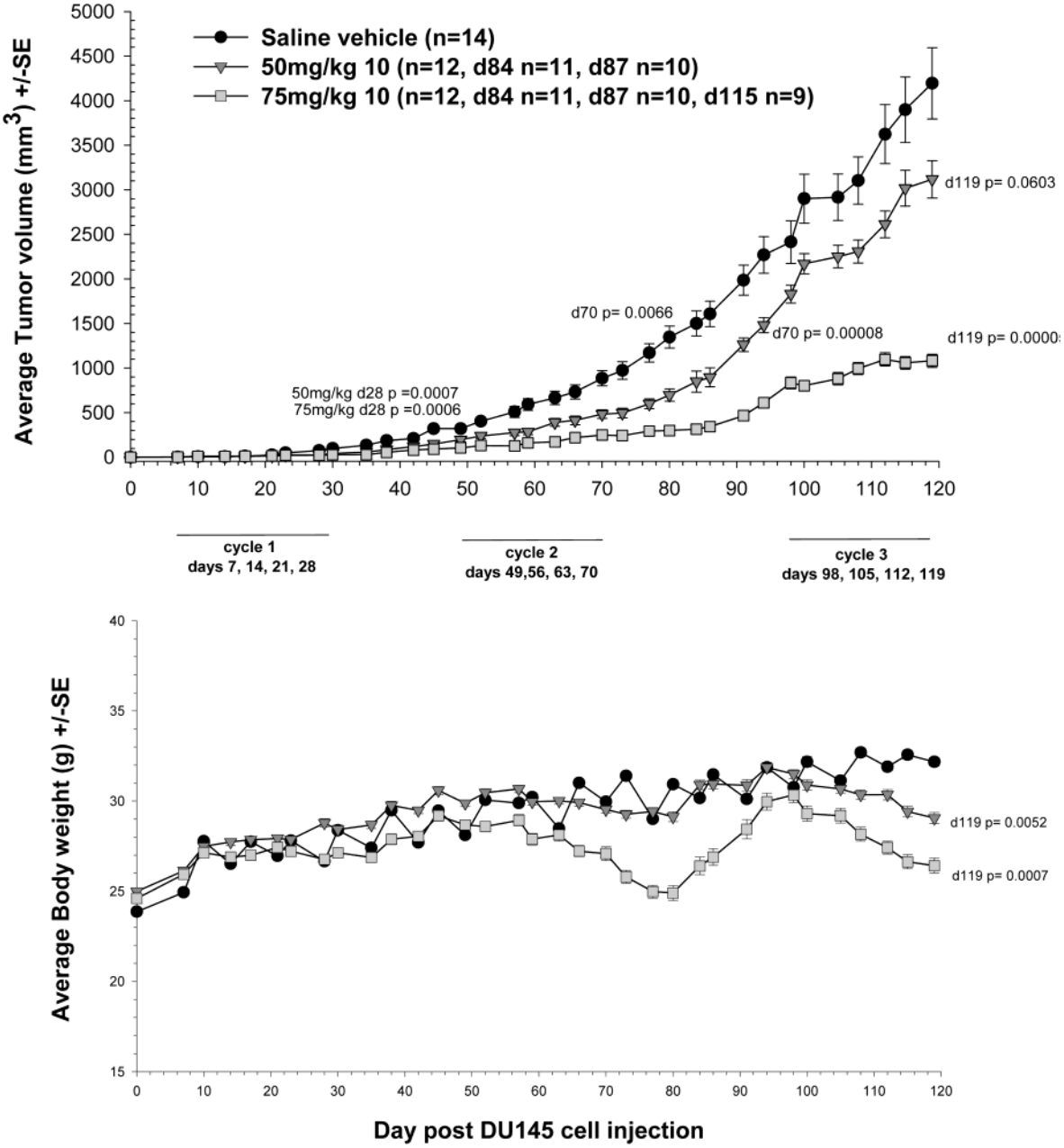


Figure 7. Growth of DU-145 human prostate tumor xenografts in nude mice and body weights of control untreated mice, mice treated in three cycles with **10** at a dose of 50 mg/kg q1w × 4, and 75 mg/kg q1w × 4.

compound 6 h after the last ip injection. Maximum drug uptake occurred 72 h after the last dose and decreased at 96 h. During the same time frame, two main metabolites were detected, the monodeethyl derivative **37** and the dideethyl derivative **35** (see Scheme 5 for structures). Deethylation of **10** is an oxidative dealkylation reaction analogous to that observed for SL-11093.[13] The monoethyl derivative **37** was the main metabolite. In the tumor, **37** peaked 48 h after the last dose, its levels decreased later, very likely due to excretion from the tumor. Tetramine **37** was also detected at high levels in the liver, where rather small amounts of the parent compound **10** were found 48 h after the last dosing (Figure 9). In kidney, the main metabolite was the dideethyl derivative **35** (Figure 9). Both **37** and **35** are much weaker inhibitors of DU-145 cell growth in culture (their IC_{50} is ca. 0.60 μM, a 10-fold decrease of activity as compared to **10**).

The effect of **10** on the intracellular pool of the natural polyamines (putrescine, spermidine, and spermine) in the tumor suggests it has a long lasting effect on tumor growth. Six hours after the last ip injection, **10** already caused a decrease in the polyamine pool present in the tumor (Figure 10). A burst in the intracellular concentrations of the polyamines (particularly spermine) followed, that peaked at 24 h after cessation of treatment. This increase originated very likely from an increased polyamine uptake from the media. Finally, 96 h after cessation of treatment, polyamine concentration in the tumor decreased and stayed low. This delayed strong inhibitory effect of **10** on polyamine concentration suggests that the compound has a lasting inhibitory effect on tumor growth. The intracellular polyamine pools in the livers of the treated mice were strongly inhibited by **10**. As could be expected from normal resting cells, the polyamine levels stayed very low after

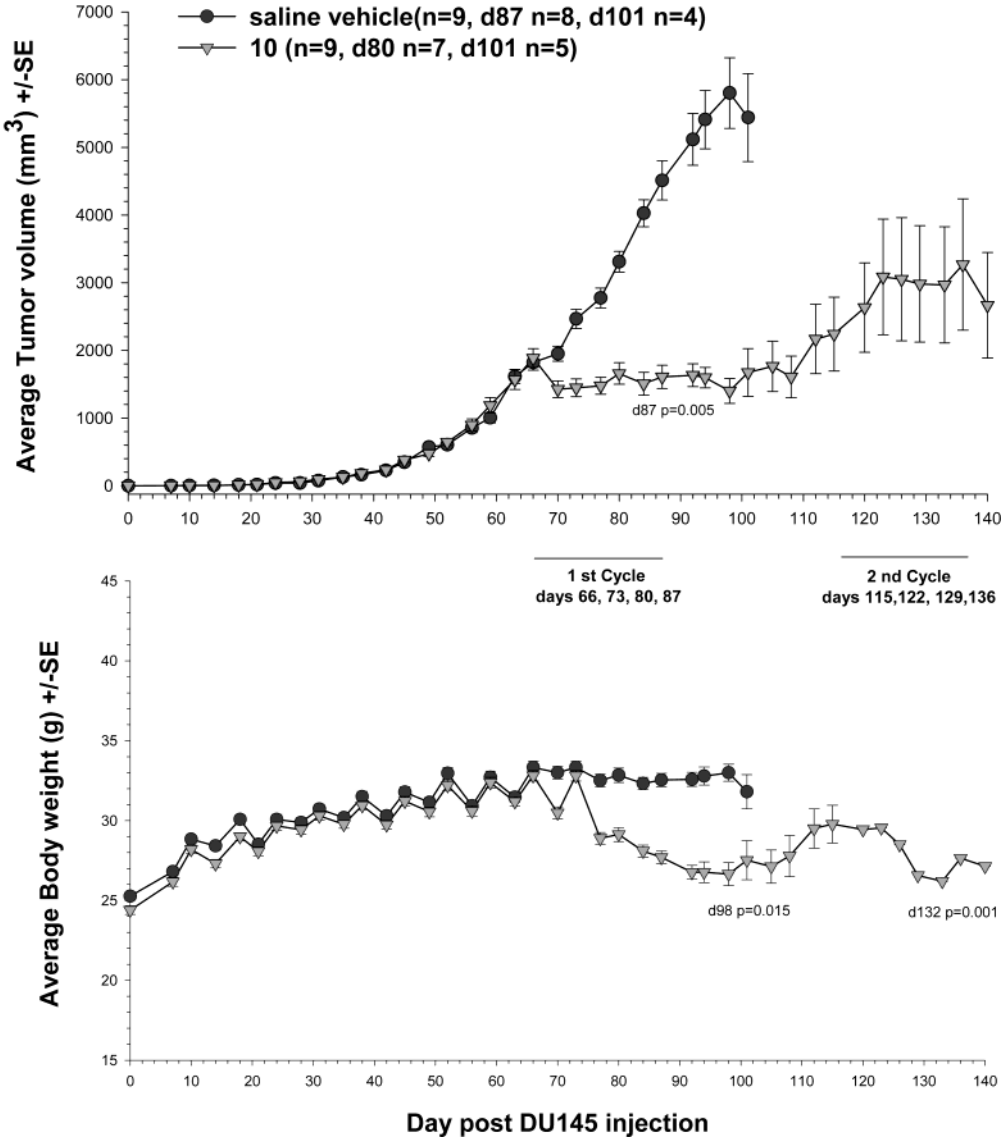


Figure 8. Growth of DU-145 human prostate tumor xenografts in nude mice and body weights of control untreated mice and mice treated in two cycles with **10** at a dose of 75 mg/kg q1w × 4; treatment started at day 66 after tumor implantation (n = number of mice).

treatment with **10** (Figure 10). Intracellular polyamine pools in the kidneys of the treated mice also remained lower than in control animals after treatment (Figure 10).

Conclusions

Five new cyclopropane-containing polyamine analogues, **7**, **10**, **18**, **27**, and **32**, have been assessed for their activity against DU-145 human prostate cancer tumors implanted in nude mice. Their structures were crafted to explore the therapeutic possibilities of polyamine analogues built around one or more cyclopropane rings. The model compound was considered to be SL-11093, an $^{\alpha}N,^{\omega}N$-bis(ethyl)homospermine analogue where the central butane segment had been replaced by a *trans*-1,2-dimethyl cyclopropyl ring.[13] SL-11093 was found to be an efficacious chemotherapeutic agent against human prostate cancer grown in nude mice. The new analogues were also, in general, effective in inhibiting human prostate cancer tumors grafted in nude mice. Tetramine **7**, a polyamine with a highly restricted rotation (three *trans*-cyclopropyl residues) was not as efficacious in inhibiting tumor growth as tetramine **10**, where the central butane residue confers considerable mobility to the structure. Hexamine **18**, although very efficacious in reducing tumor growth, was too toxic, as measured by the weight loss of the grafted mice. Decamines **32** and **27** had unfavorable ratios of efficacy vs toxicity in the grafted mice and were therefore not compounds of choice (Figure 6). Of the new analogues, the $^{\alpha}N,^{\omega}N$-bis(ethyl)tetramine tetrahydrochloride **10**, had a promising therapeutic index, comparable to that of SL-11093. When given ip (dissolved in saline) to grafted mice that carried established human prostate cancer tumors at 250 mg/kg/week every other week for two weeks, the inhibition of tumor growth (as compared to untreated mice) and mice weight loss was comparable to that of SL-11093 (Figure 6 and ref 13). There were some differences among both analogues; SL-11093 intratumoral levels[13] were higher than those of **10** (Figure 9). SL-11093 was also less N-deethylated to its metabolites in the tumor than was

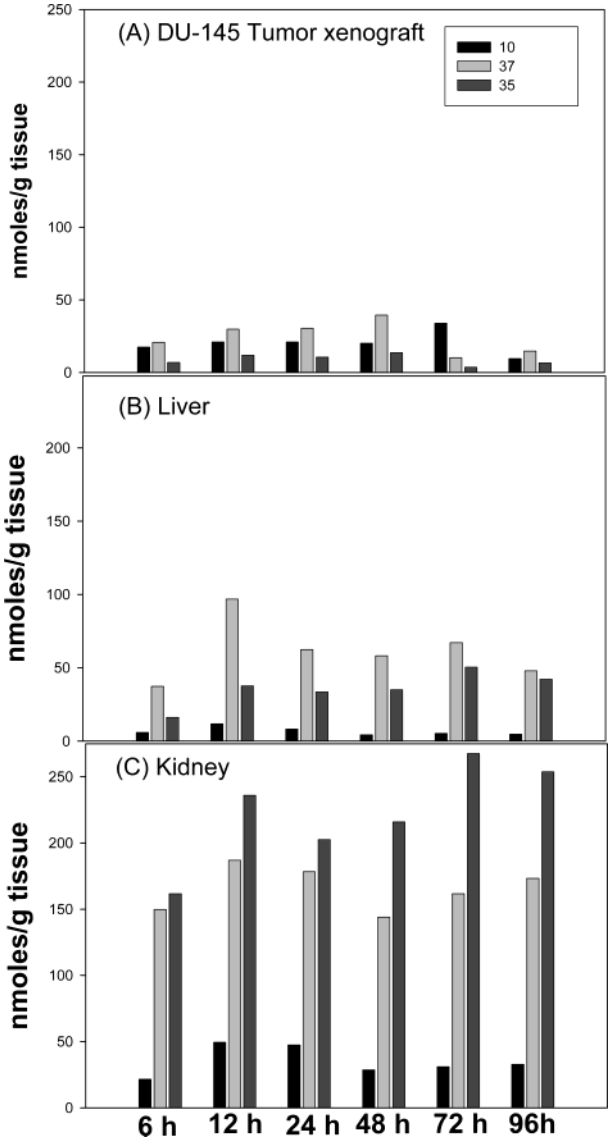


Figure 9. Levels of **10** and its metabolites **37** and **35** in the tumor xenografts, livers, and kidneys of nude mice 96 h after treatment with a single 50 mg/kg ip dose of **10**.

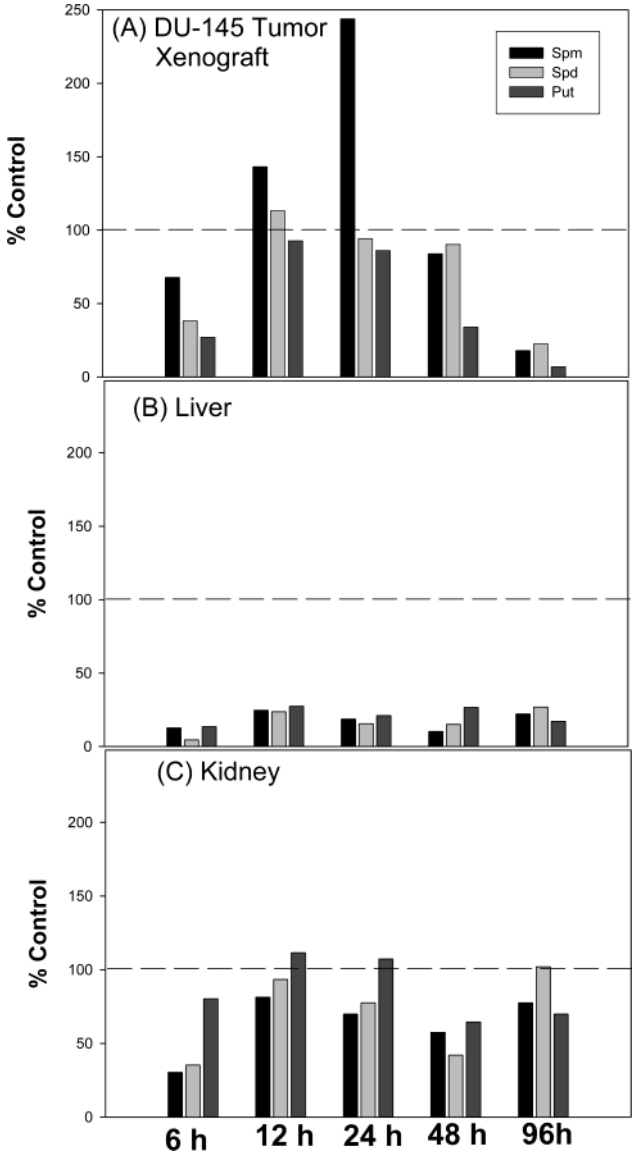


Figure 10. Levels of cellular polyamine putrescine, spermidine, and spermine in the tumor xenografts, livers and kidneys of nude mice 96 h after treatment with a single 50 mg/kg ip dose of **10**.

10. In general, however, both **10** and SL-11093 could be considered as having similar chemotherapeutic efficacies.

Tetramine **10** was also given to grafted mice at a regime of 75 mg/kg/week during monthly cycles, every other month for six months. This prolonged treatment also achieved inhibition of tumor growth of 80% as compared to untreated mice, and no animal was lost during or after the treatments. It showed that **10** can arrest tumor growth (even of large tumors) for long periods, when administered with the correct protocol. There was some weight loss (15%) in the treated mice during this regime, but the mice recovered most of the lost weight during the monthly resting periods, suggesting the absence of irreversible damage due to treatment.

The inhibition of tumor growth by **10** can be explained in part by its accumulation in tumor; as is the case with SL-11093.[13] In liver and kidney, concentrations of **10** remained relatively low, a fact that may account for its relatively low systemic toxicity. It was deethylated to its mono- and dideethyl- derivatives **37** and **35**, respectively. These metabolites (but not **10**) were present in high concentrations in liver and kidney, suggesting a catabolic pathway for **10** that leads to its excretion by dealkylation to more polar metabolites. In conclusion, tetramine **10** is an alternative choice to SL-11093 in the selection of potentially useful anticancer polyamine analogues of reduced toxicity and good efficacy.

Experimental Section

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; matrix-assisted laser desorption ionization (MALDI) mass spectra were obtained using a Bruker Biflex III spectrometer operating in the time-of-flight mode; mass spectra (FAB) were obtained on an Autospec (VG) spectrometer. HPLC analyses of the dansyl derivatives of the tetramines were routinely performed to check the purity of

the samples. A Vydac C-18 (300 μm pore) column was used for separations, and a fluorescence spectrometer (340-nm excitation, 515-nm emission) was used for detection.

***trans*-1-Cyano-2-(mesitylenesulfonyloxymethyl)cyclopropane (2).** A solution of ethyl (*E*)-2-cyanocyclopropanecarboxylate **1**[25] (8 g, 57.5 mmol) in isopropyl alcohol (8 mL) was added over 5 min into an ice-cold, N_2 swept solution of $LiBH_4$ (1.254 g, 57.5 mmol) in isopropyl alcohol (40 mL). The reaction mixture was stirred for 0.5 h in an ice bath. The bath was then removed, and the reaction was continued at 22 °C for another 8 h. The reaction mixture was concentrated in vacuo and evaporated three times from toluene. Et_2O (130 mL) and H_2O (2.5 mL) were added, and the reaction mixture was stirred for 10 h at 22 °C. It was dried (Na_2SO_4), filtered, and concentrated on a rotavapor. The residue of crude 2-cyano-1-hydroxymethylcyclopropane was dissolved in pyridine (50 mL) and cooled to 0 °C, and mesitylene sulfonyl chloride (18.9 g, 86.3 mmol) in pyridine (60 mL) was slowly added into the reaction mixture. Following 3 h of stirring at 0 °C, the reaction mixture was poured on ice (500 g) and acidified with 10% HCl, and the product was extracted with ethyl acetate. The organic extracts were washed with 3% HCl, a saturated $NaHCO_3$ solution, and brine. Column chromatography (silica gel, hexane:ethyl acetate, 4:1) of the residue left after evaporation of the organic extracts gave 5.25 g (33%) of **2**; ^{1}H NMR ($CDCl_3$) δ 0.8−1.1 (m, 1H), 1.25−1.40 (m, 2H), 1.70−1.90 (m, 1H), 2.33 (s, 3H), 2.63 (s, 6H), 3.80 (dd, $J^1 = 11.2$ Hz, $J^2 = 7.2$ Hz, 1H), 4.03 (dd, $J^1 = 11.2$ Hz, $J^2 = 5.9$ Hz, 1H, 1H), 7.00 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 2.28, 11.75, 19.35, 21.01, 22.53, 69.26, 119.90, 126.35, 131.88, 139.82, 143.75; MS-ESI (*m*/*z*) 280.2 ($M^+ + 1$).

***N*-(1-Cyanocyclopropylmethyl)-*N*-ethylmesitylenesulfonamide (3).** A suspension of NaH (60% in mineral oil, 508 mg, 12.7 mmol) in DMF (15 mL) was added to a stirred solution of **2** (2.3 g, 8.3 mmol) and *N*-ethylsulfonamide (2.1 g, 9.3 mmol) at 5 °C and the mixture stirred for 1 h. The cooling bath was removed, and the reaction mixture was stirred for additional 10 h. After the mixture was cooled in an ice bath, it was quenched with H_2O, neutralized to pH 7 with 2% HCl, concentrated in vacuo, suspended in $CHCl_3$, and washed twice with H_2O and brine. Column purification (silica gel, hexane: ethyl acetate, 4:1) of the residue left on evaporation of the $CHCl_3$ gave 2.42 g (95%) of the product **3**; mp 149−150 °C (ethyl acetate/hexane); ^{1}H NMR ($CDCl_3$) δ 0.85−1.00 (m, 1H), 1.1 (t, 3H), 1.20−1.30 (m, 1H), 1.60−1.75 (m, 1H), 2.31 (s, 3H), 2.59 (s, 6H), 3.06−3.26 (m, 2H), 3.25−3.34 (m, 2H), 7.00 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 2.75, 12.76, 12.98, 19.66, 20.88, 22.62, 41.06, 47.43, 120.62, 132.03, 132.63, 140.21, 142.76; MS-ESI (*m*/*z*) 307.4 ($M^+ + 1$).

***N*-Ethyl *N*,*N*′-Bis(mesitylenesulfonyl)-*trans*-1,2-bis(aminomethyl)cyclopropane (4).** A solution of nitrile **3** (2.42 g, 7.9 mmol) in Et_2O (15 mL) was slowly added into a stirred suspension of $LiAlH_4$ (368 mg, 9.7 mmol) in Et_2O (10 mL) at 0 °C. The mixture was stirred for 2.5 h, the cooling bath was removed, and the reaction mixture was left stirring for 20 h at 22 °C. The reaction mixture was quenched with 2 N NaOH at 0 °C, and the inorganic precipitate was filtered and washed thoroughly with Et_2O. The Et_2O solution was dried (Na_2SO_4) and concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (30 mL), mesitylenesulfonyl chloride (1.68 g, 7.7 mmol) was added, and the reaction mixture was cooled on an ice-bath and treated with 2 N NaOH (16 mL). After 4 h the reaction mixture was quenched with H_2O (60 mL), acidified to pH 1, the product was extracted with $CHCl_3$, washed with brine, dried (Na_2SO_4), and purified on a silica gel column (hexane:ethyl acetate, 4:1); 1.69 g (43%) of **4** were obtained; ^{1}H NMR ($CDCl_3$) δ 0.40−0.52 (m, 2H), 0.94 (t, 3H), 0.95−1.15 (m, 2H), 2.29 (s, 3H), 2.30 (s, 3H), 2.40−2.52 (m, 1H), 2.57 (s, 6H) 2.59 (s, 6H), 2.90−3.45 (m, 5H), 5.58 (t, 1H), 6.94 (4H); ^{13}C NMR ($CDCl_3$) δ 10.10, 12.33, 16.45, 17.51, 20.87, 20.93, 22.55, 22.93, 40.01, 46.61, 47.71, 131.81, 131.88, 132.49, 133.96, 139.08, 140.39, 141.75, 142.52. MS-ESI (*m*/*z*) 493.4 ($M^+ + 1$).

3,8,13,18-Tetrakis(mesitylenesulfonyl)-ter[(*E*)-5,6,(*E*)-10,11,(*E*)-15,16-cyclopropyl]-3,8,13,18-tetraazaeicosane (6). Sodium hydride (60% suspension in oil, 161 mg, 4.0 mmol) was added to a stirred mixture of diester **5**[9] (676 mg, 1.45 mmol) and diamide **4** (1.46 g, 2.95 mmol) in dry DMF (12 mL) at 0 °C. The cooling bath was removed, and stirring was continued for 10 h. The reaction mixture was quenched with water (1 mL), acidified to pH 6 with aq HCl (3%), and concentrated to dryness in vacuo at 40 °C. The residue was dissolved in ethyl acetate, washed three times with water and then with brine, and dried (Na_2SO_4). Column chromatography (silica gel, hexane:ethyl acetate, 3:1) of the residue left after evaporation of the organic solution gave tetrasulfonamide **6**; 1.26 g (83%). ^{1}H NMR ($CDCl_3$): 0.25−0.50 (m, 6H), 0.70−0.95 (m, 6H), 0.95−1.10 (m, 6H), 2.29 (s, 12H), 2.55 (s, 24H), 2.85−3.10 (m, 6H), 3.10−3.40 (m, 10H), 6.92 (s, 8H). ^{13}C NMR ($CDCl_3$): 10.92, 11.15, 12.64, 16.00, 16.16, 16.32, 20.92, 22.67, 40.26, 48.41, 49.04, 49.22, 131.88, 133.23, 133.36, 140.16, 142.21, 142.32. MS-MALDI (*m*/*z*) 1073.6 ($M^+ + Na$).

Ter[(*E*)-5,6,(*E*)-10,11,(*E*)-15,16-cyclopropyl]-3,8,13,18-tetraazaeicosane Tetrahydrochloride (7). Tetrasulfonamide **6** (1.26 g, 1.2 mmol) was stirred for 10 h in a mixture of phenol (4.5 g, 48 mmol), HBr (33% in AcOH, 26 mL), and CH_2Cl_2 (13 mL). The mixture was cooled in an ice bath, quenched with water (5.5 mL), washed two times with CH_2Cl_2, and concentrated in vacuo. The residue was neutralized with 2 N NaOH (1 mL) at 0 °C, made alkaline (pH 12) with 50% KOH (1 mL), extracted with $CHCl_3$ (five times), dried (Na_2SO_4), and concentrated in vacuo. The residue was dissolved in Et_2O and the hydrochloride precipitated with HCl gas. The precipitate was filtered, washed with Et_2O, and dried in vacuo; 374 mg (66%) of **7** were obtained; mp: 200 °C (dec). ^{1}H NMR (D_2O): δ 0.80−0.92 (m, 6H), 1.15−1.85 (m, 6H), 1.29 (t, $J = 7.3$, 6H), 6.28−3.00 (m, 6H), 3.12 (q, $J = 7.3$, 4H), 3.10−3.35 (m, 6H). ^{13}C NMR (D_2O): δ10.03, 10.14, 10.60, 13.78, 42.69, 50.13, 50.45. MS-ESI (*m*/*z*) 323.2 ($M^+ + 1$).

Bis[(*E*)-5,6-(*E*)-15,16-cyclopropyl]-3,8,13,18-tetraazaeicosane tetrahydrochloride (10) was prepared following the procedures described above for the synthesis of **7**. Tetrasulfonamide **9** was prepared in 93% yield by condensation of diester **8**[9] and amide **4**. ^{1}H NMR ($CDCl_3$): δ 0.25−0.45 (m, 4H), 0.65−0.85 (m, 4H), 0.99 (t, $J = 7.1$, 6H), 1.30−1.42 (m, 4H), 2.29 (s, 12 H), 2.56 (s, 24 H), 2.55−2.95 (m, 4H), 3.05−3.35 (m, 12H), 6.92 (s, 8H). ^{13}C NMR ($CDCl_3$): δ 10.88, 12.65, 15.99, 16.22, 20.91, 22.67, 22.71, 24.42, 40.30, 45.24, 48.41, 48.78, 131.90, 133.33, 140.10, 142.23, 142.30. MS-MALDI 1061.5 ($M^+ + Na$). Treatment of **9** with HBr/AcOH in the presence of phenol as described for **6** gave **10** (71%); mp: 200 °C (dec); ^{1}H NMR (D_2O) δ 0.85 (t, $J = 7.1$, 4H), 1.15−1.30 (m, 4H), 1.26 (t, $J = 7.4$, 6H), 1.75−1.90 (m, 4H), 2.82−3.00 (m, 4H), 3.05−3.30 (m, 12H). ^{13}C NMR (D_2O) δ: 10.00, 10.60, 13.75, 22.89, 42.68, 46.65, 50.12, 50.74. MS-ESI (*m*/*z*) 311.4 ($M^+ + 1$).

***trans*-1,2-Bis(bromomethyl)cyclopropane (13).** *trans*-1,2- Bis(hydroxymethyl)cyclopropane **12**[8] (2.6 g, 25 mmol) was dissolved in 60 mL of dry CH_2Cl_2 under argon, $P(Ph)_3$ (13 g, 50 mmol) was added, and the solution was cooled to 5 °C. *N*-Bromosuccinimide (9 g, 50 mmol) was added slowly with stirring. The mixture was kept at 22 °C for 18 h, the solution was evaporated to dryness, the residue was extracted with petroleum ether (bp 35−60 °C) (4 × 25 mL), the pooled extracts were cooled to 5°C, and the precipitated triphenylphosphine oxide was filtered off. The solution was evaporated to dryness, and the residual **13** (4.7 g) was directly used in the next step without further purification; ^{1}H NMR ($CDCl_3$) δ: 0.85 (t, $J =$ 6.76 Hz, 2H), 1.33 (t, $J = 6.30$ Hz, 2H), 3.35 (m, 4H); ^{13}C NMR ($CDCl_3$) δ: 17.27, 24.41, 36.75.

Bis(mesitylenesulfonyl)-12-bromo-10,11-[(*E*)-1,2-cyclopropyl]-3,8-diazadodecane (15). Amide **14**[9] (8.5 g, 18 mmol) was dissolved in dry DMF (150 mL), and NaH (60% in oil, 0.85 g) was added. The mixture was stirred for 30 min at 22 °C, when **13** (4.0 g, 18 mmol) dissolved in 40 mL of dry DMF was added and the mixture was further stirred during 18 h. Water (10 mL) was added, the solution was evaporated to dryness in vacuo, the residue partitioned between $CHCl_3$ and a saturated NH_4Cl solution, the organic layer separated, evaporated to dryness, and the residual oil was purifed by

column chromatography on silica gel using hexane/ethyl acetate, 1:1 as eluant; 2.7 g (26% yield) of **15** were recovered; ^{1}H NMR ($CDCl_3$) δ: 0.55 (m, 2H), 0.92(m,2H), 1.00 (t, $J = 7.05$ Hz, 3H), 1.40 (m, 4H), 2.30 (s, 6H), 2.58 (s, 12H), 2.90−3.20 (m, 10H), 6.94 (s, 4H); ^{13}C NMR ($CDCl_3$) δ: 12.75, 13.74, 19.77, 20.92, 21.07, 22.75, 24.74, 24.80, 37.59, 40.09, 44.62, 45.27, 48.61, 131.91, 140.13, 142.21, 142.43.

3,8,13,18,23,28-Hexakis(mesitylenesulfonyl)-10,11-[(*E*)-1,2-cyclopropyl]-20,21-[(*E*)-1,2-cyclopropyl]-3,8,13,18,23,-28-hexaazatriacontane (17). Amide **16**[26] (0.85 g, 1.8 mmol) was dissolved in 50 mL of dry DMF, NaH (60% in oil, 0.15 g) was added, the mixture was stirred for 30 min at 22 °C, and the bromomethyl derivative **15** (2.3 g, 3.7 mmol) dissolved in 25 mL of dry DMF was added. The mixture was stirred for 18 h at 22 °C, and the workup described for **15** was then followed. Column chromatography on silica gel using chloroform:ethyl acetate, 9:1 as eluant gave 2.0 g (34% yield) of **17**; ^{1}H NMR ($CDCl_3$) δ: 0.31 (m, 4H), 0.72 (m, 4H), 0.97 (t, $J =$ 7.12 Hz, 6H), 1.26 (m, 12H), 2.29 (s, 18H), 2.54−2.55 (s, 36H), 2.80 (m, 4H), 3.15 (m, 20H), 6.92 (s, 12H); MS-MALDI (*m*/*z*); 1567.39 ($M^+ + Na$).

Bis[(10,11-(*E*)-20,21-(*E*)-1,2-cyclopropyl)]-3,8,13,18,23,-28-hexaazatriacontane Hexahydrochloride (18). Hexamide **17** (2.0 g) was deprotected with hydrogen bromide in glacial acetic acid following the procedure described for **6**. SL-11231 hexachloride **18** was crystallized from aqueous ethanol; 0.5 g (60% yield) was obtained; ^{1}H NMR (D_2O) δ: 0.86 (t, $J =$ 6.8 Hz, 4H), 1.23 (m, 4H), 1.29 (t, $J = 7.3$ Hz, 6H), 1.78 (m, 12H), 2.89 (m, 4H), 3.15 (m, 20H); ^{13}C NMR (D_2O) δ: 12.48, 12.95, 16.14, 25.28, 45.36, 48.71, 49.05, 49.35, 53.12; MS-ESI (*m*/*z*): 453.6 ($M^+ + 1$).

3,8,13-Tris(mesitylenesulfonyl)-3,8,13-triazahexadecylcyanide (20). Triamide **19**[10] (44.1 g, 60 mmol) was dissolved in 700 mL of dry DMF, the solution cooled to 5 °C, and NaH (85% in oil, 3.25 g, 120 mmol) added portionwise with constant stirring. After 30 min, 4-bromobutyronitrile (13.34 g, 90.1 mmol) dissolved in 100 mL of dry DMF was added, and the stirring was kept for 18 h at 22 °C. The reaction was quenched with 10 mL of H_2O, and the mixture was evaporated to dryness in vacuo, leaving behind an oil that was purified by chromatography on silica gel using hexane/ethyl acetate, 4:1 as eluent; 47 g (97% yield) of **20** was thus obtained; ^{1}H NMR ($CDCl_3$) δ: 0.94 (t, 3H), 1.40 (m, 8H), 1.89 (m, 2H), 2.25 (t, 2H), 2.31 (s, 9H), 2.55 (s, 18H), 3.10 (m, 10H), 3.25 (t, 2H), 6.92 (s, 6H); ^{13}C NMR ($CDCl_3$) δ: 12.62, 14.57, 20.89, 22.70, 22.78, 23.68, 24.47, 24.72, 39.95, 44.49, 44.91, 119.00, 131.96, 132.12, 139.97, 140.00, 142.24, 142.34, 142.78.

5,10,15-Tris(mesitylenesulfonyl)-5,10,15-triaza-1-heptadecylamine (21). Nitrile **20** (47 g) was dissolved in 100 mL of $CHCl_3$ and 100 mL of ethanol and reduced with hydrogen over 4.5 g of PtO_2 during 5 h at 45 psi. The catalyst was then filtered over a Celite pad and the solvent evaporated to dryness. The residue was purified by chromatography on silica gel using hexane/ethyl acetate, 7:3 as first eluant, followed by methanol; 46.2 g (94% yield) of **21** were obtained; ^{1}H NMR ($CDCl_3$) δ: 0.98 (t, 3H), 1.35 (m, 12H), 2.28 (s, 9H), 2.55 (s, 20H), 3.08 (m, 12H), 6.90 (s, 6H); ^{13}C NMR ($CDCl_3$) δ: 12.67, 14.53, 20.86, 22.66, 22.73, 24.50, 24.57, 24.62, 24.71, 30.37, 40.12, 41.41, 44.54, 45.05, 45.32, 131.85, 133.30, 133.34, 133.43, 139.95, 139.99, 142.18, 142.25.

1,6,11,16- Tetrakis(mesitylenesulfonyl)-6,11,16-tetraaza-1-heptadecylamine (22). Heptadecylamine **21** (46.2 g, 57.4 mmol) was dissolved in a mixture of 270 mL of $CHCl_3$ and 137 mL of 2 N NaOH, the mixture was cooled to 5 °C, and a solution of mesitylenesulfonyl chloride (13.2 g, 60 mmol) in 80 mL of $CHCl_3$ was slowly added with stirring. The mixture was kept for further 18 h at 22 °C, the organic layer was then separated, the aqueous solution was further extracted with $CHCl_3$ (2 × 150 mL), and the organic solutions were combined, washed with H_2O (2 × 350 mL) and then with brine (200 mL), and evaporated to dryness. The residue was purified by column chromatography on silica gel, using hexane/ethyl acetate, 7:3, as a first eluant followed by ethyl acetate; 43.0 g (79% yield) of **22** was obtained; ^{1}H NMR (CDCl3) δ: 0.94 (t, 3H), 1.35 (m, 12H), 2.30 (s, 12H), 2.50 (s, 18H), 2.55 (s, 6H), 2.75 (m, 2H), 3.08 (m, 12H), 4.60 (br, 1H), 6.90 (s, 8H).

3,8,13,18-Tetrakis(mesitylenesulfonyl)-3,8,13,18-tetraazaheneicosanecyanide (23). It was obtained in 98% yield from **22** following the procedure described for **20**. ^{1}H NMR ($CDCl_3$) δ: 0.95 (t, 3H), 1.39 (m, 12H), 1.83 (m, 2H), 2.20 (t, 2H), 2.30 (s, 12H), 2.59 (s, 24H), 3.10 (m, 16H), 6.98 (s, 8H); ^{13}C NMR ($CDCl_3$) δ: 12.58, 14.47, 20.82, 22.62, 22.69, 23.60, 24.34, 24.42, 24.66, 39.92, 44.46, 44.84, 45.55, 118.64, 131.81, 131.89, 132.04, 133.23, 139.85, 139.90, 142.16, 142.26, 142.69.

5,10,15,20-Tetrakis(mesitylenesulfonyl)-5,10,15,20-tetraaza-1-docosamine (24). It was obtained in 98% yield from **23** following the procedure described for **21**. ^{1}H NMR ($CDCl_3$) δ: 0.98 (t, 3H), 1.32 (m, 14H), 1.40 (m,2H), 2.30 (s,12H), 2.55 (s, 24H), 2.60 (m, 2h), 3.05 (m, 16H), 6.95 (s,8H); ^{13}C NMR ($CDCl_3$) δ: 12.66, 20.87, 22.66, 22.73, 24.49, 24.58, 24.72, 29.58, 40.00, 41.11, 44.52, 44.95, 45.26, 131.92, 133.29, 139.92, 139.97, 142.20, 142.29.

1,5,10,15,20-Pentakis(mesitylenesulfonyl)-1,5,10,15,20-pentaazatricosane (25). It was obtained from **24** in 71% yield following the procedure described for **22**. ^{1}H NMR ($CDCl_3$) δ: 0.95 (t, 3H), 1.32 (m, 12H), 2.29 (s, 12H), 2.54 (s, 18H), 2.59 (s, 6H), 2.75 (m, 2H), 3.08 (m, 12H), 4.55 (br, 1H), 6.92 (s, 8H); ^{13}C NMR ($CDCl_3$) δ: 12.61, 20.82, 22.63, 22.71, 22.78, 24.35, 24.45, 24.68, 26.57, 39.96, 41.83, 44.47, 45.08, 131.88, 133.19, 133.28, 133.39, 138.86, 139.91, 141.97, 142.18, 142.25, 142.32; MS (*m*/*z*): 1279.0 ($M^+ + K$).

3,8,13,18,23,28,33,38,43,48-Decakis(mesitylenesulfonyl)-25,26-[(*E*)-1,2-cyclopropyl]-3,8,13,23,28,33,38,43,48-decaazapentacontane (26). Pentamide **25** (6.5 g, 5.25 mmol) was dissolved in 75 mL of dry DMF, and NaH (60%, 0.32 g) was added in several portions with constant stirring at 22 °C. After 10 min, diester **5**[8] (1.2 g, 2.6 mmol) dissolved in 45 mL of dry DMF was slowly added to the stirred solution and the latter kept for 18 h at 22 °C. Water (5 mL) was added, the solution adjusted to pH 7 with dil HCl, evaporated to dryness, the residue partitioned between $CHCl_3$ (200 mL) and water (100 mL), and the organic layer separated, washed with water (2 × 100 mL) and evaporated to dryness. The decamide **26** was purified by column chromatography on silica gel using hexane:ethyl acetate, 7:3, as eluant; 3.0 g (23%) of **26** was obtained; ^{1}H NMR ($CDCl_3$) δ: 0.30 (t, 2H), 0.70 (t, 2H), 0.98 (t, $J = 7.0$ Hz, 6H), 1.27 (m, 32H), 2.30 (s, 30H), 2.55 (s, 60H), 3.0 (m, 40H), 6.93 (s, 20H); ^{13}C NMR ($CDCl_3$) δ: 11.04, 12.25, 15.94, 20.93, 22.73, 24.39, 24.78, 40.08, 44.63, 45.19, 48.74, 131.94, 133.26, 140.07, 142.32; MS-MALDI (*m*/*z*): 2567.0 ($M^+ + Na$).

3,8,13,23,28,33,38,48-Decaaza-[(25,26(*E*)-1,2-cyclopropyl]-pentacontane Decahydrochloride (27). Decamide **26** (1 g) was deprotected using hydrogen bromide (33%) in glacial acetic acid in the presence of phenol as described for **6**, and decahydrochloride **27** was obtained in 85% yield. ^{1}H NMR (D_2O) δ: 0.88 (t, $J = 7.10$ Hz 2H), 1.20 (t, $J = 7.10$ Hz, 2H), 1.28 (t, $J = 7.13$ Hz, 6H), 1.78 (m, 32H), 3.10 (m, 40H); MS (*m*/*z*): 725.8 ($M^+ + 1$)

3,8,13,18-Tetrakis(mesitylenesulfonyl)-10,11-[(*E*)-cyclopropyl]-3,8,13,18-tetraazaoctadecane (28). 1,4-Bis-(mesitylenesulfonyl)putrescine **16** (5.6 g, 12.4 mmol) and the bromomethyl derivative **15** (3.9 g, 6.2 mmol) were dissolved in 150 mL of dry DMF, and NaH (60%, 0.92 g) was added in one portion. The mixture was stirred for 18 h at 22 °C, it was then quenched with 10 mL of 10% HCl, the solution evaporated to dryness, the residue dissolved in $CHCl_3$ (100 mL), the latter washed first with water (2 × 50 mL) and then with brine (50 mL), the organic solvent was evaporated to dryness, and the residue purified by column chromatography on silica gel using $CHCl_3$: ethyl acetate, 9:1, as eluant; 2.3 g (37% yield) of **28** were obtained; mp 63 °C; ^{1}H NMR ($CDCl_3$) δ: 0.35 (m, 2H), 0.80 (m, 2H), 0.95 (t, $J = 5.8$ Hz, 3H), 1.30−1.49 (m, 8H), 2.30 (s, 12H), 2.56 (s, 18H), 2.59 (s,6H), 2.70−3.30 (m, 14H), 4.66 (t, $J = 6.4$ Hz, 1H), 6.93 (s, 8H); MS-MALDI (*m*/*z*); 1021.0 ($M^+ + Na$).

Bis(mesitylenesulfonyl)-1,4-*trans*-1,2-diaminomethylcyclopropane (29). Bis(bromomethyl)cyclopropane **13** (1.14 g, 5 mmol) and mesitylenesulfonamide (2.19 g, 11 mmol) dissolved in 25 mL of anhydrous DMF were cooled to 5 °C, and NaH (60% in oil, 0.48 g) was added in several portions under an argon atmosphere. The mixture was stirred at 22°C during 18 h, after which it was evaporated to dryness in vacuo, and the residue was partitioned between ethyl acetate (50 mL) and water (50 mL). The organic layer was separated and evaporated to dryness, and the residue was purified by column chomathography on silica gel using ethyl acetate/hexane, 3:7, as eluant; 1.37 g (47%) of **29** were obtained; mp 152−153,°C (ethyl acetate/hexane); ^{1}H NMR ($CDCl_3$) δ: 0.06−0.10 (m, 1H), 0.62−0.75 (m, 1H), 1.00−1.16 (m, 2H), 2.29 (s, 6H), 2.62 (s, 12H), 2.69 (dd, J = 5.0, 8.6 Hz, 2H), 3.04 (dd, J = 5.0, 13.2 Hz, 2H), 3.70 (br, 2H), 6.04 (s, 4H).

5,10-Bis(mesitylenesulfonyl)-1,14-dibromo-7,8-[(*E*)-1,2-cyclopropyl]-5,10- diazatetradecane (30). Bis(mesitylenesulfonyl)-*trans*-1,2-diaminomethylcyclopropane **29** (1.4 g, 5.2 mmol) was dissolved in dry DMF (50 mL) under nitrogen, and NaH (60%, 0.4 g) was added to the solution. After the mixture was stirred for10 min at 22 °C, 1,4-dibromobutane (6.7 g, 31 mmol) dissolved in 25 mL of dry DMF was added in one portion. After the solution was stirred for further 18h, water was added (5 mL) and the reaction mixture was evaporated to dryness. The residue was purified by column chromatography on silica gel using hexane:ethyl acetate, 4:1, as eluant; 1.5 g (68% yield) of **30** were obtained; ^{1}H NMR ($CDCl_3$) δ: 0.41 (t, J = 6.9 Hz, 2H), 0.82 (t, J = 6.0 Hz, 2H), 1.66 (m, 8H), 2.29 (s, 6H), 2.58 (s, 12H), 2.94 (m, 2H), 3.21 (m, 10H), 6.94 (s, 4H); ^{13}C NMR ($CDCl_3$) δ: 11.04, 16.14, 20.93, 22,74, 25.70, 29.65, 32.88, 44.74, 48.88, 131.97, 160.12, 142.48; MS-MALDI (*m*/*z*): 757.09 (M^+ + Na).

3,8,13,18,23,28,33,38,43,48-Decakis(mesitylenesulfonyl)-10,11-25,26-40,41-ter[(*E*)-1,2-cyclopropyl]-3,8,13,23,28,33,-38,43,48-decaazapentacontane (31). Tetramide **28** (1.4 g, 1.4 mmol) was dissolved in 50 mL of dry DMF, and while kept under argon, NaH (60%, 75 mg) was added. The mixture was stirred for 10 min at 22 °C, a solution of the dibromo derivative **30** (0.5 g, 0.7 mmol) in 25 mL of dry DMF was then added, and the mixture was stirred for 18 h at 22 °C. Water (5 mL) was added, the solution adjusted to pH 7 with dilute HCl, evaporated to dryness in vacuo, and the residue purified by column chromatography on silica gel using chloroform:ethyl acetate, 9:1 as eluant; 1.0 g (28%) of **31** was obtained; ^{1}H NMR ($CDCl_3$) δ: 0.29 (m, 6H), 0.70 (m, 6H), 0.96 (t, J = 6.98 Hz, 6H), 1.26 (m, 24H), 2.28 (s, 30H), 2.53, 2.54, 2.55 (s,s,s, 60H), 2.72 (m, 6H), 3.09 (m, 34H), 6.91(s, 20H); ^{13}C NMR($CDCl_3$) δ:11.03,12.73, 15.88, 20.93, 22.72, 24.39, 40.07, 44.61, 44.89, 48.89, 48.72, 131.95, 133.27, 140.04, 142.34; MS-MALDI (*m*/*z*) 2594.1 (M^+ + Na).

Ter-[(10,11(*E*),25−26(*E*),40,41(*E*)-(1,2-cyclopropyl)]-3,8,-23,28,33,38,43,48-decaazapentacontane Decahydrochloride (32). Decamide **31**(1.0 g) was deprotected with hydrogen bromide (33%) in glacial acetic acid in the presence of phenol as described for **6**. Decahydrochloride **32** was obtained in 80% yield (0.3 g); ^{1}H NMR (D_2O) δ: 0.85 (t, 6.9 Hz, 6H), 1.23 (t, J = 6.9 Hz, 6H), 1.28 (t, J = 7.22 Hz, 6H), 1.78 (m, 24H), 2.89 (m, 6H), 3.10 (m, 34H); ^{13}C NMR (D_2O) δ: 12.65, 13.10, 16.28, 25.43, 45.51, 48.86, 49.21, 49.53, 53.28; MS-ESI (*m*/*z*) 750 (M^+ + 1).

***N*,*N*′-Bis(2-Cyanocyclopropylmethyl)-*N*,*N*′-bis(mesitylenesulfonyl)-1,4-butanediamine (33).** NaH (60% suspension in mineral oil, 640 mg,16 mmol) was added into a solution of **16** (3 g, 6.63 mmol) in DMF (200 mL) at 0 °C and stirred for 10 min. Ester **16** (3.9 g, 13.92 mmol) was introduced and stirred for 2 h, the cooling bath was removed, and the reaction was left overnight at 22 °C. The reaction was cooled on the ice bath, quenched with 1 mL of H_2O, concentrated to dryness in vacuo, and dissolved in EtOAc. The organic solution was washed with H_2O, 3% HCl, saturated $NaHCO_3$, and brine, dried (Na_2SO_4), and purified by column chromatography (silica gel, EtOAc:$CHCl_3$,1:9); 2.9 g (72%) of **33** was obtained. ^{1}H NMR ($CDCl_3$) δ 0.75−0.9 (m, 2H), 0.95−1.10 (m, 2H), 1.15−1.30 (m, 2H), 1.40−1.65 (m, 6H), 2.32 (s, 6H), 2.58 (s, 12H), 2.96 (dd, J^1 = 15.2 Hz, J^2 = 7.4 Hz, 2H), 3.15 (dd, J^1 = 15.4 Hz, J^2 = 6.2 Hz, 2H,), 3.18−3.38 (m, 4H), 6.98 (s, 4H); ^{13}C NMR ($CDCl_3$) δ 2.71, 12.77, 19.35, 20.95, 22.72, 24.41, 45.89, 47.90, 120.54, 132.17, 132.64, 140.16, 142.99; MS-ESI (*m*/*z*) 628.8 (MNH_4^+), 611.6 (MH^+).

***N*,*N*′-Bis[2-(Mesitylenesulfonylaminomethyl)cyclopropylmethyl]-*N*,*N*′-bis(mesitylenesulfonyl)-1,4-butanediamine (34).** A suspension of $LiAlH_4$ (365 mg, 9.6 mmol) in dry THF (10 mL) was added to an ice cold solution of dinitrile **33** (2.77 g, 4.54 mmol) in dry THF (20 mL) and the mixture stirred for 1 h at 0 °C followed by further stirring for 48 h at 22 °C. The reaction mixture was cooled in an ice-bath, quenched with 2 N NaOH until a white precipitate formed, and the mixture was diluted with 3 volumes of CH_2Cl_2. The suspension was dried (Na_2SO_4) and filtered through a Celite cake. The filtrate was concentrated in vacuo and the residue dissolved in light petroleum ether and evaporated to dryness. This operation was repeated three times; *N*,*N*′-bis[(aminomethyl)cyclopropylmethyl]-*N*,*N*′-bis(mesitylenesulfonyl)-1,4-butanediamine thus obtained was further utilized without additional purification. NaOH (2 N) (10 mL) was added at 5 °C to a mixture of the bisamine and mesitylsulfonyl chloride (2.1 g, 9.5 mmol) in CH_2Cl_2 (40 mL). The ice bath was removed, and the reaction was stirred overnight at 22 °C. It was quenched with aq NH_4Cl and diluted three times with $CHCl_3$, and the product was washed with H_2O, $NaHCO_3$, and brine. Column purification (silica gel,$CHCl_3$:EtOAc, 9:1) gave **34**, 1.86 g (42%), mp 90 °C (ethyl acetate/hexane). ^{1}H NMR ($CDCl_3$) δ 0.30−0.50 (m, 4H), 0.85−1.10 (m, 4H), 1.10−1.22 (m, 4H), 2.23 (s, 6H), 2.31 (s, 6H), 2.35−2.51 (m, 2H), 2.53 (s, 12H), 2.62 (s, 12H), 2.80−3.30 (m, 10H), 5.52 (t, J = 5.27 Hz, 2H,), 6.93 (s, 8H); ^{13}C NMR ($CDCl_3$) δ 10.06, 16.07, 17.62, 20.85, 20.94, 22.63, 22.89, 23.96, 44.88, 46.48, 48.30, 131.82, 131.94, 132.40, 133.95, 139.00, 140.25, 141.79, 142.65; MS-ESI (*m*/*z*) 1021.6 (MK^+), 1005.6 (MNa^+), 1001.0 (MNH_4^+), 983.6 (MH^+).

***N*″-Ethyl-*N*,*N*′-Bis[2-(Mesitylsulfonylaminomethyl)cyclopropylmethyl]-*N*,*N*′-bis(mesitylenesulfonyl)-1,4-butanediamine (36).** Sodium hydride (60% suspension in mineral oil, 38.4 mg, 0.96 mmol), was added into an ice-cold stirred mixture of **34** (900 mg, 0.915 mmol) in DMF (18 mL) and stirred for 10 min. Iodoethane (171.2 mg, 1.1 mmol) was added into the reaction mixture, the cooling bath was removed, and the reaction was stirred for 10 h at 22 °C. The reaction mixture was quenched with 0.5 mL of H_2O, concentrated in vacuo, dissolved in $CHCl_3$, washed with brine, and dried (Na_2SO_4). Column chromatography purification (silica gel, $CHCl_3$:EtOAc, 23:2) gave **36**, 413 mg (45%). ^{1}H NMR ($CDCl_3$) δ 0.2−0.4 (m, 1H), 0.38 (t, J = 6.9 Hz, 3H), 0.6−0.8 (m, 2H), 0.8−0.95 (m, 2H), 0.98 (t, J = 7.1 Hz, 3H), 1.15−1.40 (m, 4H), 2.29 (s, 12H), 2.30−2.50 (m, 2H), 2.54 (s, 6H), 2.54 (s, 6H), 2.56 (s, 6H), 2.62 (s, 6H), 2.70−3.35 (m, 12H), 5.52 (t, J = 5.4 Hz, 1H), 6.93 (s, 8H); ^{13}C NMR ($CDCl_3$) δ 10.01, 10.80, 12.58, 15.84, 16.17, 16.23, 17.60, 20.91, 22.65, 22.91, 24.06, 24.25, 40.23, 44.96, 45.08, 46.54, 48.31, 48.65, 131.89, 132.43, 133.25, 139.04, 140.03, 140.09, 140.32, 141.75, 142.27, 142.34, 142.60; MS-ESI (*m*/*z*)1033.6 (MNa^+), 1011.6 (MH^+).

Bis-[(*E*)-5,6,(*E*)-15,16-(1,2-cyclopropyl)]-3,8,13-heptadecamine Tetrahydrochloride (37). HBr in acetic acid (30%, 9 mL) was added to an ice-cold solution of **36** (410 mg, 0.41 mmol) and phenol (1.5 g, 16.2 mmol) in CH_2Cl_2 (4.5 mL), stirred for 15 h at 22 °C, and then cooled to 0 °C and quenched with 10 mL of H_2O. The aqueous phase was washed twice with CH_2Cl_2, concentrated in vacuo, cooled on the ice bath, and made basic with 2 N NaOH (5 mL). The product was extracted with $CHCl_3$ (six times), dried (Na_2SO_4), concentrated, dissolved in EtOH (2 mL), and cooled on an ice bath. HCl (37%, 2 mL) was added, and the precipitate was collected, washed with EtOH, and dried in vacuo; 142 mg (82%) of **37** were collected. ^{1}H NMR (D_2O) δ 0.84 (m, 4H), 1.12−1.25 (m, 4H), 1.29 (t, J = 7.3 Hz, 3H), 1.70−1.90 (m, 4H), 2.75−2.95 (m, 4H), 3.05−3.30 (m, 10H); ^{13}C NMR (D_2O) δ 9.83, 10.00, 10.62, 13.76, 15.02, 22.93, 42.71, 42.87, 46.67, 49.00, 50.15, 50.78; MS-ESI (*m*/*z*) 283.4 (MH^+).

1,14-Diamine-bis-[(*E*)-2,3,(*E*)-12,13(1,2-cyclopropyl)]5,-10-diazatetradecane tetrahydrochloride (35) was prepared by deprotection of **34** (400 mg, 0.407 mmol) in CH_2Cl_2 (4.5 mL) with phenol (1.523 g, 16.2 mmol) and HBr (9 mL, 30% in acetic acid) as described for **37**; 147 mg (90%) of **35** were obtained. ^{1}H NMR (D_2O) δ 0.83 (m, 4H), 1.15−1.30 (m, 4H), 1.75−1.90 (m, 4H), 2.75−2.95 (m, 4H), 3.05−3.30 (m, 8H); ^{13}C NMR (D_2O) δ 9.84, 13.75, 15.02, 22.94, 42.88, 46.67, 49.01, 50.85; MS-ESI (*m*/*z*) 255.6 (MH^+).

Cell Culture. DuPro cells were obtained from Dr. M. Eileen Dolan, University of Chicago, Department of Medicine. 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.

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 polyamine analogues. 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 and 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. 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 centrifuging 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 published elsewhere.[27] 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.[27] Peaks were detected and quantitated using a Shimadzu HPLC fluorescence monitor coupled to a Spectra-Physics peak integrator. Because polyamine levels vary with environmental conditions, control cultures were sampled for each experiment.

MTT Assay. 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. Twenty microliters of appropriately diluted stock solutions of each drug was added to the middle eight 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. Twenty five microliters of a 5 mg/mL solution of 3-(4,5-dimethythiazol-2yl)-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 contains: 100 g lauryl sulfate (SDS), 250 mL of *N*,*N*-dimethylformamide in 2 mL of glacial acetic acid, pH 4.8). The color that developed was read at room temperature at 570 nm in a E-max Precision Microplate Reader (Molecular Devices Corporation, Sunnyvale, CA), and data were analyzed using manufacturer supplied cell survival software.

Nude Mouse Xenografts. DU-145 human prostate tumors were maintained either in culture or by continuous in vivo passage in male athymic NCr-nu mice. Mice were housed in microisolator cages (five per cage) in a 12-h light/dark cycle in a barrier facility. Either tumor cell suspension (1×10^6) or tumor fragments (ca. 2×3 mm^3) were implanted subcutaneously with a 25-gauge regular needle. Tumor size was measured twice per week in two perpendicular dimensions with a vernier caliper and converted to tumor volume using the formula: $(l \times w^2)/2$, where l and w refer to the longer and shorter dimensions, respectively. Animal body weights were measured twice per week at the same time intervals as the tumor dimension measurements, and mortality was monitored daily. Effects of treatment on tumor growth, expressed as % growth delay, were calculated following the relation:

$$\% \text{ growth delay} = [(T - C)/C] \times 100\%$$

where T and C represented the median times post-staging for treated (T) and control (C) tumors to attain a prescribed size depending on the model. Analogues were administered intraperitoneally (ip) in water using either a chronic treatment schedule—administration for several cycles of once a day for five consecutive days (q1d × 5) with a rest period of ca. 10 days between each cycle, or a once a week administration schedule during monthly cycles with a month rest period in between. Treatments were usually initiated ca. 15 days after tumor implantation when the mice had established tumors ranging in size from 100 to 245 mm^3.

Metabolic Studies. Nude mice implanted with human prostate Du-145 cancer cell line were kept under the conditions described in the previous section. When tumor volumes reached sizes of ca. 200 mm^3, they were divided into two groups of three animals each; group 1 was given ip water for injection and group 2 was treated ip once a day for 5 days with 50 mg/kg/day of **10**. The animals were bled by retro-orbital puncture under CO_2/O_2 anesthesia 6 h after the last injection, and tumor, liver, and kidneys were harvested at 6, 24, 48, and 96 h after the last dose and snap frozen in liquid nitrogen. The tissues were homogenized in 1 mL of PBS using a Power Gen-700 homogenizer and centrifuged at 100 000 rpm for 10 min at 4 °C. After centrifugation, the pellet was resuspended in 0.5 mL of 2% perchloric acid and stored overnight at −20 °C. The pellet was sonicated with three 30 s pulses and then centrifuged at 10 000 rpm for 10 min at 4 °C. The supernatant was collected, and its polyamine composition was analyzed by derivatization with dansyl chloride followed by HPLC separation described above. Each dansyl polyamine derivative was collected and its molecular weight determined by mass spectrometry (MS-ESI).

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Supporting Information Available: Figures showing effect of **7** and **10** on growth of DU-145 human prostate tumor xenograft. This material is available free of charge via the Internet at http://pubs.acs.org.

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