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Long-Chain Polyamines (Oligoamines) Exhibit Strong Cytotoxicities against Human Prostate Cancer Cells

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Abstract—$^{\alpha}N,^{\omega}N$-bis(ethyl) octamine SL-11160, decamine SL-11159, dodecamine SL-11226, and tetradecamine SL-11175 were chemically synthesized. We called this class of compounds 'oligoamines'. In these compounds, each $-NH_2^+$ residue is separated by four CH_2 residues. *trans*-Unsaturation was also introduced into the center of the oligoamine chain resulting in the *trans*-octamine SL-11158, *trans*-decamine SL-11144, *trans*-dodecamine SL-11172 and *trans*-tetradecamine SL-11227. *cis*-Unsaturation gave the *cis*-octamine SL-11157 and *cis*-decamine SL-11150. When assayed for their growth inhibitory effect against four human prostate cancer cell lines LnCap, DU-145, DuPro, and PC-3 by a MTT assay, the ID_{50} values were less than 1 μM in all four cell lines. On day 6 of treatment, 2 μM SL-11159, SL-11144 and SL-11175 killed over five logs of DuPro cells while SL-11172 killed over four logs as determined by a colony forming efficiency (CFE) assay. In addition, SL-11159, SL-11226 and SL-11227 killed four logs of PC-3 cells. PC-3 cells are generally resistant to shorter chain polyamine analogues. Such a level of cytotoxicity in any of the prostate tumor cell lines has not been observed for any other polyamine analogues tested thus far. The DU-145 cell line was too sensitive to oligoamines to perform a CFE analysis and the DuPro cell line was too sensitive to SL-11227 treatment to obtain reproducible CFE data. Interestingly, all 10 oligoamines were efficient DNA aggregators in a cell-free system and their cytotoxicities generally parallel their capacities to aggregate DNA.

Introduction

The polyamines putrescine, spermidine, and spermine are organic cations found in all mammalian cells and are required for cell proliferation.[1] Polyamine analogues inhibit cell growth and kill cancer cells both in tissue culture as well as in experimental animal models.[2,3] The $^{\alpha}N,^{\omega}N$-bisethyl derivatives of spermine and its higher and lower homologues are efficacious cytotoxic agents.[4-6] Of the many theories advanced to explain the biological effects of polyamine analogues,[1] the hypotheses centering on their binding to nucleic acids are the most compelling.[7,8] Polyamines are strong bases that are protonated at physiological pH, they bind to the negatively charged nucleic acids either by electrostatic interactions or by hydrogen bonding.[8] The polyamines induced structural changes in DNA or model oligo- and polynucleotides and their biological significance have been extensively studied using computer graphic modeling and physico-chemical, biochemical, cellular and molecular biological methods.[9,10] Relevant to polyamine action are the findings that a polyamine analogue (a pentamine) inhibited nucleosome formation in cell-free systems[11] and altered chromatin structure and gene expression in cellular systems.[12]

The cytotoxic polyamine analogues previously assayed as antiproliferative agents were either tetra- or pentamines.[4–6] It was, therefore, of interest to explore the antiproliferative activities of their higher homologues, such as octamines, decamines, dodecamines, and tetradecamines. We coined the name 'oligoamines' for these polycations since the name polyamine has been traditionally used for the shorter chain diamines, triamines and tetramines. We report here the synthesis and the strong antiproliferative effects of these novel oligoamines on human prostatic carcinoma cell lines. Prostate cancer is only second to skin tumors as the most common malignancy in American male population and is the second leading cause of cancer related deaths (after lung cancer) in US males. It is estimated that in 2002, ca. 189,000 men will be diagnosed with

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prostate cancer in the USA, and that ca. 30,000 of these men will die within a decade of diagnosis.[13] We have shown that conformationally restricted polyamine analogues are strong inhibitors of the proliferation of human prostatic carcinoma cell lines,[4–6] both in culture as well as in xenografts in nude mice.[14] The new oligoamines are efficacious in the 10–100 nM dose range. They are markedly more cytotoxic against human prostatic carcinoma cells in culture than any previously described polyamine analogues.

Chemistry

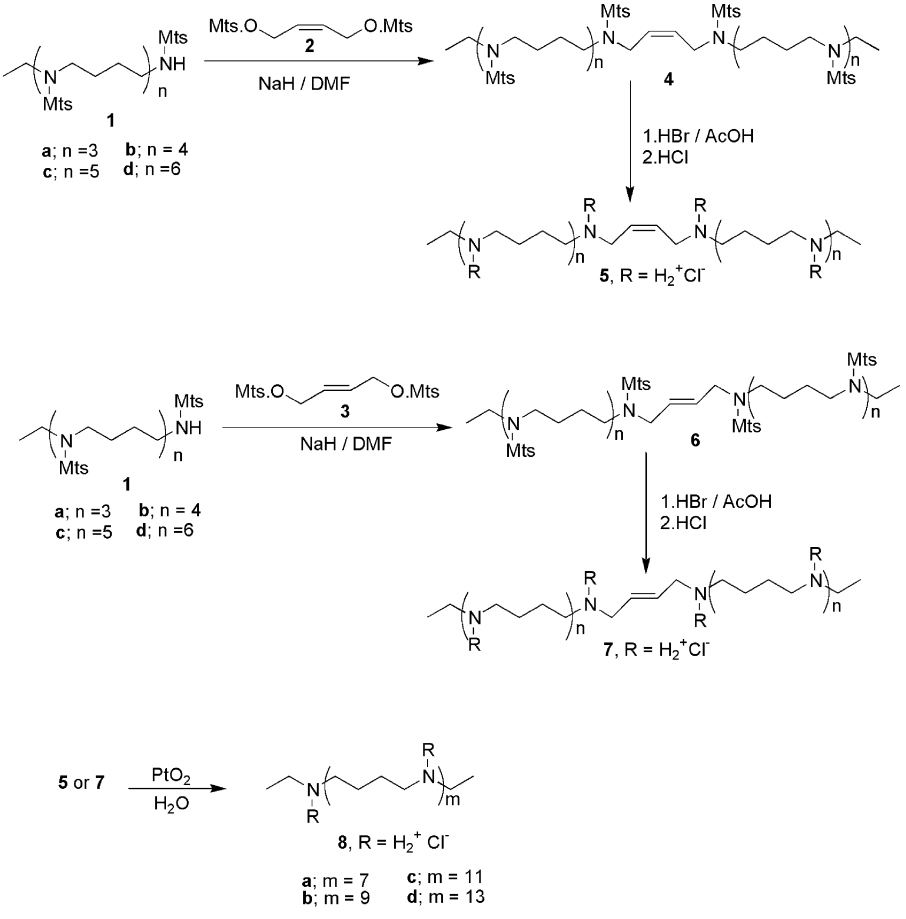
Oligoamine synthesis

Tetra-, penta-, hexa-, and heptapolyamides **1** (Scheme 1) were obtained following the procedures described elsewhere[5,6] with modifications. By condensation of **1** with the *cis*-diester **2** in the presence of sodium hydride,[4] it was possible to obtain the protected oligoamides **4**. Cleavage of the protecting mesitylenesulfonyl groups of **4** in acid media, allowed the synthesis of the *cis*-oligoamines **5**. An analogous series of reactions using the *trans*-ester **3** gave the oligoamides **6** in a first stage; they were then cleaved with acid to give the *trans*-oligoamines **7**. By hydrogenation of the double bonds in **5** or **7** it was possible to obtain the saturated oligoamines **8**. Analysis of oligoamines had to be performed using a LC/MS procedure (Fig. 1), since the classical dansylation method[15] used for polyamine analysis for some yet unknown reasons could not be applied to oligoamines.

Biology

The growth inhibitory activities of the 10 new oligoamines SL-11144, SL-11150, SL-11157, SL-11158, SL-11159, SL-11160, SL-11172, SL-11175, SL-11226 and SL-11227 against four human prostate cancer cell lines LnCap, DU-145, DuPro and PC-3 were determined after 96-h incubations using a MTT assay. The ID_{50} values of the oligoamines along with their structures are shown in Table 1. ID_{50} values are defined as the oligoamine concentration required for inhibiting cell growth by 50%. All oligoamines had ID_{50} values of less than 1 μM in all four cell lines. These are the lowest ID_{50} values for any polyamine analogues observed in these cell lines.[4–6] LnCap and DU-145 cell lines were slightly more sensitive to the growth inhibitory effects of oligoamines than were the other two cell lines. The differences in ID_{50} values were, however, small and no definitive conclusions about differences in growth inhibitory activities of the oligoamines based on the MTT assays could be drawn.

The androgen-independent prostate cancer cell lines DU-145, DuPro and PC-3 were selected for further



Scheme 1.

studies. Growth inhibitory activities of the oligoamines in these cell lines were determined by cell counting. Representative plots of the inhibitory effects of all 10 oligoamines on DuPro cell growth at 0.8 and 1.6 μM are shown in Figure 2. At both concentrations, oligoamines were growth inhibitory on day 4 of incubation and the effect became more pronounced on day 6 of incubation. The dodecamines and the tetradecamines SL-11172, SL-11175, SL-11226 and SL-11227 were strongly inhibitory at both concentrations. At 1.6 μM, these oligoamines reduced cell numbers by nearly 3 orders of magnitude as compared to cell numbers in untreated control. Such dramatic reduction in cell numbers at such low concentrations has not been previously observed with any other polyamine analogues. Representative plots of the inhibitory effects of the tetradecamine SL-11227 on the growth of DU-145, DuPro, and PC-3 cell lines are shown in Figure 3. SL-11227 was the most efficient growth inhibitor of all three cell lines among all 10 oligoamines.

The growth inhibitory effects of all oligoamines were determined from the cell numbers obtained by direct counting of trypsinized cell suspension and expressed as percent of control growth of DU-145 and PC-3 cells on day 4 and day 6 of treatment as summarized in Table 2. As observed in the MTT assay, the cell lines differed in their sensitivities to the growth inhibitory activity of oligoamines. DU-145 cells were the most sensitive and PC-3 cells were the least sensitive. In DU-145 cells, all oligoamines, with the exception of SL-11158, showed marked growth inhibition (cell number less than 10% of the control) on day 6 of incubation with 0.5 μM, while SL-11158 showed similar inhibition only at 1.0 μM. These data further corroborated the MTT assay data shown in Table 1, where all oligoamines showed ID_{50} values of less than 0.5 μM. Even though PC-3 cells were relatively resistant to inhibition by polyamine analogues,[5,6] decamines SL-11144, SL-11150, and SL-11159, dodecamines SL-11172 and SL-11226, and tetradecamines SL-11175 and SL-11227 at 5.0 μM reduced cell numbers to less than 1–2% of control by day 6 of incubation.

The cytotoxicities of the oligoamines were further tested by using a colony forming efficiency (CFE) assay. Two representative decamines SL-11144 and SL-11159 were initially chosen for their ability to kill DuPro cells at various treatment times. Cell survival on day 4 of treatment at increasing concentrations of the decamines is shown in Figure 4a, and cell survival on day 6 of treatment is shown in Figure 4b. Both decamines killed over three logs of cells at 1.5 μM on day 4 of treatment and ca. five logs on day 6 of treatment. Although growth inhibition (as determined by a MTT assay) was observed within 2 days of oligoamine treatment (data not shown), their cytotoxic effects were enhanced with longer incubation times.

The DuPro cell line that was sensitive and the PC-3 cell line that was resistant to treatment with polyamine

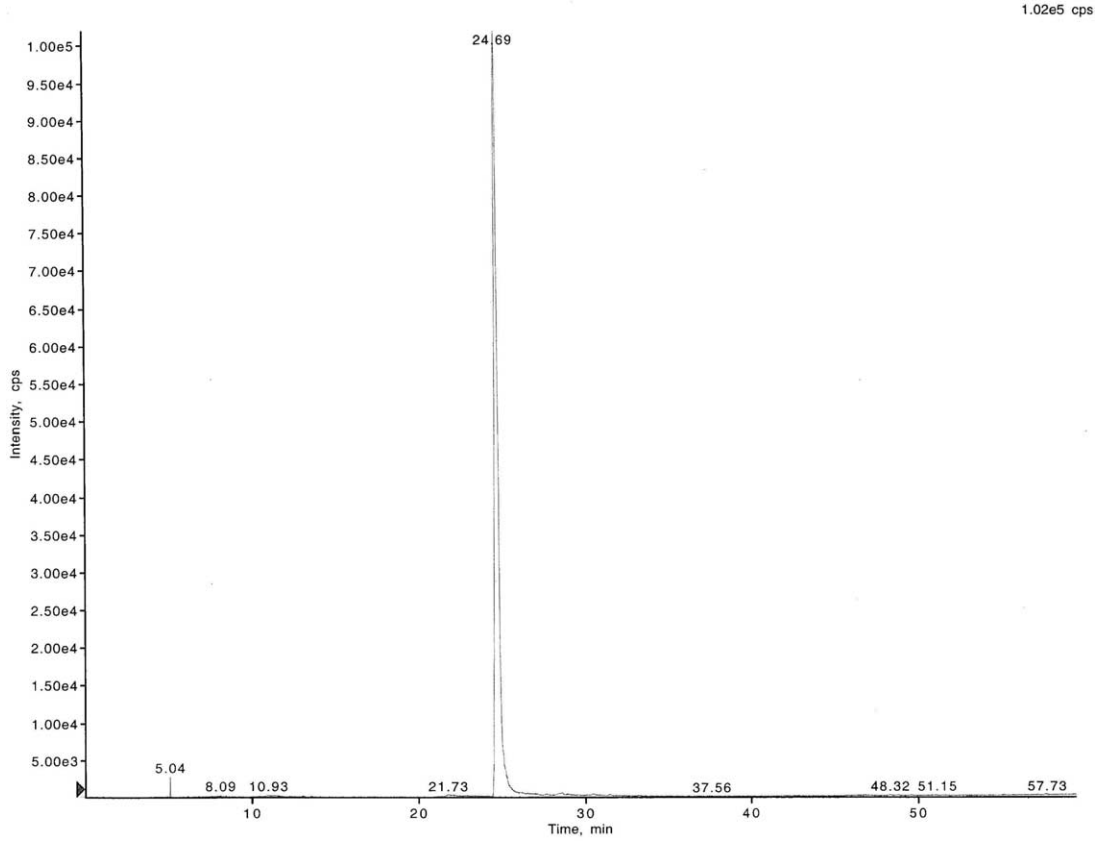
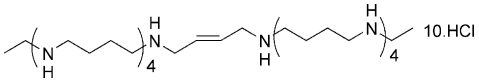
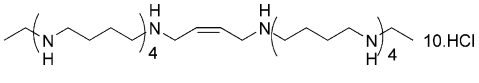
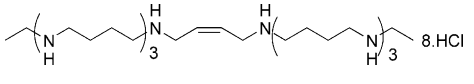
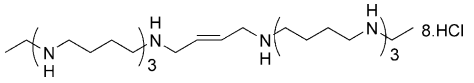
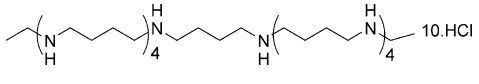
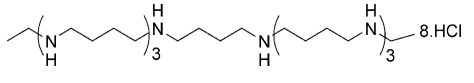
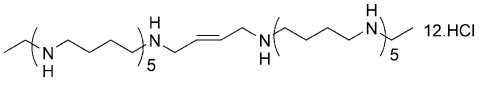
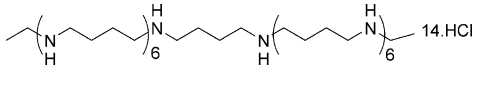
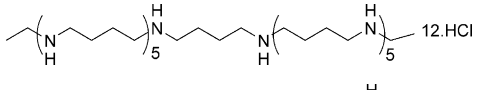
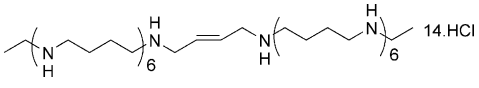


Figure 1. Oligoamine SL-11144 extracted from a cell pellet was isolated using the LC/MS procedure described in Methods and detected using the MRM (multiple reaction monitoring technique). A PE SCIEX API 365 LC/MS/MS system was used with an electrospray source. In this instrument SL-11144 has a $M_r = 711.8$ ($M^+ + 1$, free base). Scanning was set at 0.5 s. Retention time was 24.69 s. All other conditions as described in Methods.

Table 1. Effect of oligoamines on human prostate tumor cell growth by the MTT assay

Oligoamines	Structures of oligoamines	ID_{50} (μM) values for human tumor cell lines			
		LnCap	DU145	DuPro	PC-3
SL-11144		0.19	0.09	0.47	0.30
SL-11150		0.23	0.11	0.50	0.50
SL-11157		0.29	0.19	0.40	0.81
SL-11158		0.13	0.20	0.40	0.41
SL-11159		0.23	0.15	0.30	0.60
SL-11160		0.15	0.13	0.55	0.40
SL-11172		0.12	0.32	0.35	0.33
SL-11175		0.32	0.24	0.35	0.5
SL-11226		0.15	0.08	0.11	0.14
SL-11127		0.17	0.09	0.17	0.21

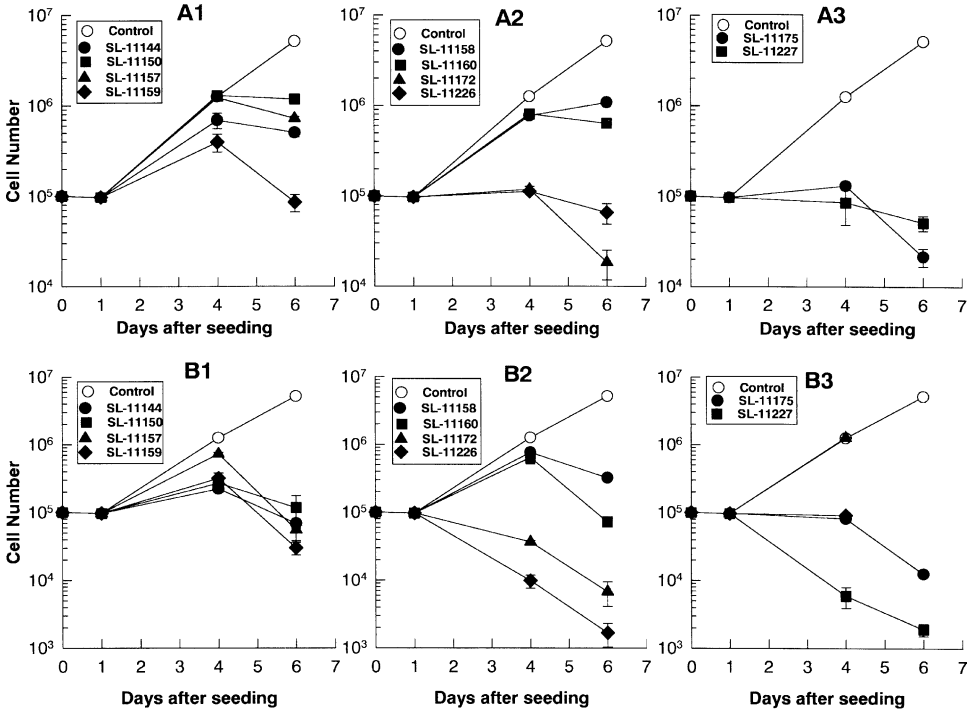


Figure 2. Effect of 12 oligoamines (symbol legends in the inset) at 0.8 μM (A1, A2, and A3) and 1.6 μM (B1, B2, and B3) on the growth of DuPro cells as determined by cell counting. Each data point is an average of at least three separate experiments. Error bars, where not shown, are smaller than the symbol size.

analogues[4–6] were chosen to study the cytotoxic effects of all 10 oligoamines. Because of the very strong growth inhibitory effects of most of the oligoamines against DU-145 cells (Table 2), resultant cell counts were too low to perform accurate CFE assays. Figure 5a shows the surviving fractions of DuPro cells, and Figure 5b shows the surviving fractions of PC-3 cells that remained attached to flasks on day 6 of treatment with varying concentrations of the ten oligoamines. The results were similar to those observed in the cell growth experiments (Fig. 2, and Tables 1 and 2). Decamines SL-11159 and SL-11144 at 2 μM, and tetradecamine SL-11175 at 1.5 μM killed over five logs of DuPro cells, while dodecamine SL-11172 at 1.5 μM killed more than four logs. At 2 μM SL-11227 treatment, DuPro cell yield was too low to obtain reproducible CFE data. Against the PC-3 cell line, SL-11227 was again the best oligoamine as it killed four logs of cells, while decamines SL-11159 and SL-11226 killed almost as efficiently. In both cell lines, cytotoxicities in general decreased in the order: tetradecamines = dodecamines > decamines > octamines.

Oligoamine uptake by the prostate cancer cell lines was measured by a LC/MS procedure (see Experimental) and their effect on the intracellular levels of natural polyamines (putrescine, spermidine, and spermine) were determined by a HPLC assay.[15] Representative data for DuPro and PC-3 cells on day 4 of treatment are shown in Table 3. The data show that there was considerable uptake of oligoamines by both cell lines. In most cases, however, only modest decreases in intracellular polyamine levels were observed in both cell lines even under conditions where the oligoamines exhibited considerable growth inhibition and cytotoxicity. In certain instances, however, there was a marked decrease in cellular polyamine level, particularly spermine level. In some cases spermine level went down below 50% of that of the control untreated cells.

The concentrations of oligoamines required for DNA aggregation and the concentration required to kill one log of DuPro cells were listed in Table 4. The effect of the oligoamines on DNA aggregation

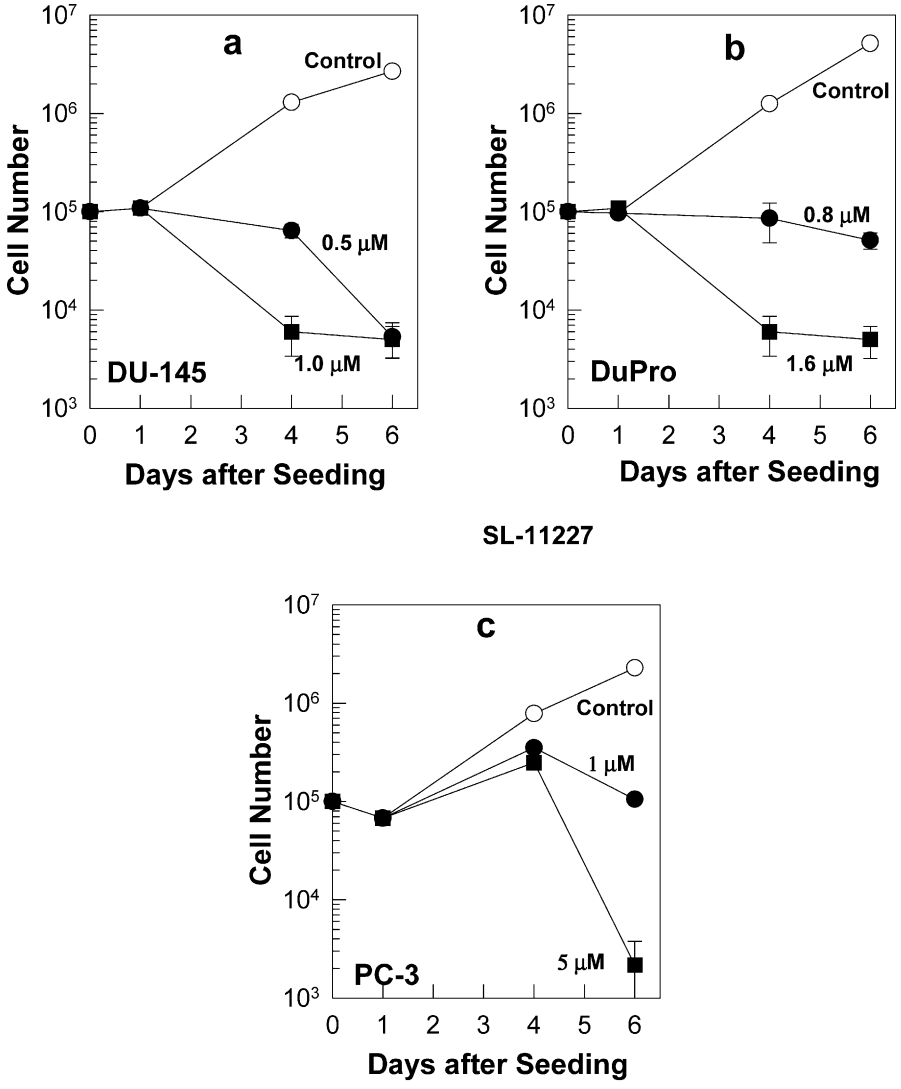


Figure 3. Effect of SL-11227 on the growth of DU-145 (a), DuPro (b), and PC-3 (c) cells. We used control (○), 0.5 μM (●) and 1.0 μM (■) in DU-145 cells, 0.8 μM (●) and 1.6 μM (■) in DuPro cells and 1.0 μM (●) and 5.0 μM (■) in PC-3 cells. Each data point is an average of at least three separate experiments. Error bars, where not shown, are smaller than the symbol size.

Table 2. Effects of oligoamines on the growth of DU-145 and PC-3 human prostate tumor cells

Oligoamines	Days after treatment	Cell number (% of control)			
		DU-145		PC-3	
		0.5 μM	1.0 μM	1.0 μM	5.0 μM
SL-11144	4	24.6±2.0	3.4±0.9	53.9±4.4	37.5±2.4
	6	6.9±0.8	2.5±0.4	27.9±2.6	1.4±0.5
SL-11150	4	31.2±1.6	2.7±0.6	58.7±2.4	42.6±4.0
	6	7.1±0.6	2.9±0.4	40.9±2.1	2.0±0.3
SL-11157	4	28.5±3.0	5.3±1.2	71.4±3.4	55.3±1.4
	6	6.7±0.5	5.9±0.3	64.3±3.5	37.5±7.1
SL-11158	4	28.4±1.8	9.8±1.1	86.6±7.3	61.5±5.5
	6	21.7±4.8	2.8±0.4	57.0±6.2	29.9±1.3
SL-11159	4	7.5±1.1	2.4±0.4	50.3±4.1	31.4±1.7
	6	1.5±0.2	1.4±0.4	16.3±0.8	0.6±0.3
SL-11160	4	13.4±0.8	3.1±0.4	61.5±6.5	33.9±1.8
	6	2.9±0.5	3.6±0.3	75.1±1.9	10.8±1.3
SL-11172	4	2.3±0.4	0.5±0.2	53.3±1.1	35.9±1.2
	6	0.9±0.3	0.2±0.1	9.8±0.5	0.2±0.06
SL-11175	4	8.1±1.6	0.7±0.3	55.6±3.2	24.3±3.4
	6	3.9±0.5	0.3±0.1	24.3±3.4	0.2±0.01
SL-11226	4	9.6±0.5	0.7±0.2	46.8±4.0	34.2±1.8
	6	0.7±0.2	0.2±0.06	5.2±0.4	0.2±0.09
SL-11227	4	6.5±0.9	0.6±0.2	44.9±4.0	31.6±3.3
	6	2.2±0.1	0.3±0.1	4.6±0.3	0.1±0.06

Table 3. Cellular polyamine and oligoamine levels of prostate tumor cells treated with oligoamines

Treatment	Analogue concn (μM)	Polyamine levels (nmol/10^6 cells) on day 4 of treatment in DuPro cells				Polyamine levels (nmol/10^6 cells) on day 4 of treatment in PC-3 cells			
		Put	Spd	Spm	Analogue	Put	Spd	Spm	Analogue
Control	—	0.966	3.870	1.006	—	0.567	2.790	0.660	—
SL-11144	0.8	0.399	0.457	1.006	0.275	0.467	0.730	ND	0.288
	1.6	0.352	0.385	0.171	0.131	0.589	ND	0.278	0.385
SL-11150	0.8	0.580	1.370	0.330	0.112	0.840	ND	0.280	0.155
	1.6	0.250	ND	0.144	0.221	*	*	*	*
SL-11157	0.8	0.430	2.320	0.450	0.563	0.737	0.830	0.520	0.650
	1.6	0.360	ND	0.170	0.810	0.670	0.750	0.430	0.900
SL-11158	0.8	0.725	1.821	0.450	0.812	ND	0.182	0.285	0.004
	1.6	0.695	ND	0.170	0.810	ND	0.122	0.192	0.008
SL-11159	0.8	0.560	ND	ND	0.525	0.525	0.531	0.300	0.513
	1.6	0.137	ND	ND	0.513	0.670	ND	0.240	0.388
SL-11160	0.8	0.430	0.897	0.222	0.575	ND	0.097	0.502	0.005
	1.6	0.412	ND	0.195	0.775	ND	0.077	0.525	0.011
SL-11172	0.8	1.701	ND	0.140	0.080	2.880	ND	ND	0.030
	1.6	4.241	ND	ND	0.890	1.230	ND	ND	0.026
SL-11175	0.8	0.157	ND	0.050	0.145	0.515	ND	ND	0.055
	1.6	0.410	ND	ND	0.150	0.519	ND	ND	0.128
SL-11226	0.8	0.593	0.017	0.067	0.032	0.573	ND	ND	0.021
	1.6	*	*	*	*	0.985	0.071	ND	0.026
SL-11227	0.8	0.366	0.022	0.090	0.019	1.121	ND	ND	0.011
	1.6	*	*	*	*	2.890	ND	ND	0.011

ND, not detected
* Cell yield is too low for accurate measurement.

was determined by the light scattering method. As expected, 20–40× less concentration of oligoamines than that of spermine were required to initiate DNA aggregation (Table 4). The efficiency of DNA aggregation was similar for the decamines SL-11144 and SL-11159, the dodecamines SL-11172 and SL-11226 and the tetradecamines SL-11175 and SL-11227, it was twice that of the octamines SL-11157, SL-11158 and SL-11160. An exception was the *cis*-decamine SL-11150 whose ability to aggregate DNA was similar to that of an octamine. Increasing the cationic charges above 10 did not result in an increase in DNA aggregating abilities.

Conclusions

The data reported here show that even though prostate cancer cell lines differ in their sensitivity to oligoamines, the oligoamines were at least one order of magnitude more cytotoxic against cultured human prostate tumor cells as compared to other polyamine analogues tested thus far.[4–6] There are differences among the oligoamines regarding their cytotoxicities. Tetradecamines and dodecamines were more cytotoxic than the less cationic octamines (SL-11157, SL-11158, and SL-11160) (Fig. 5). There is no apparent correlation between the cytotoxicity of oligoamines and their cellular uptake (Table 3).

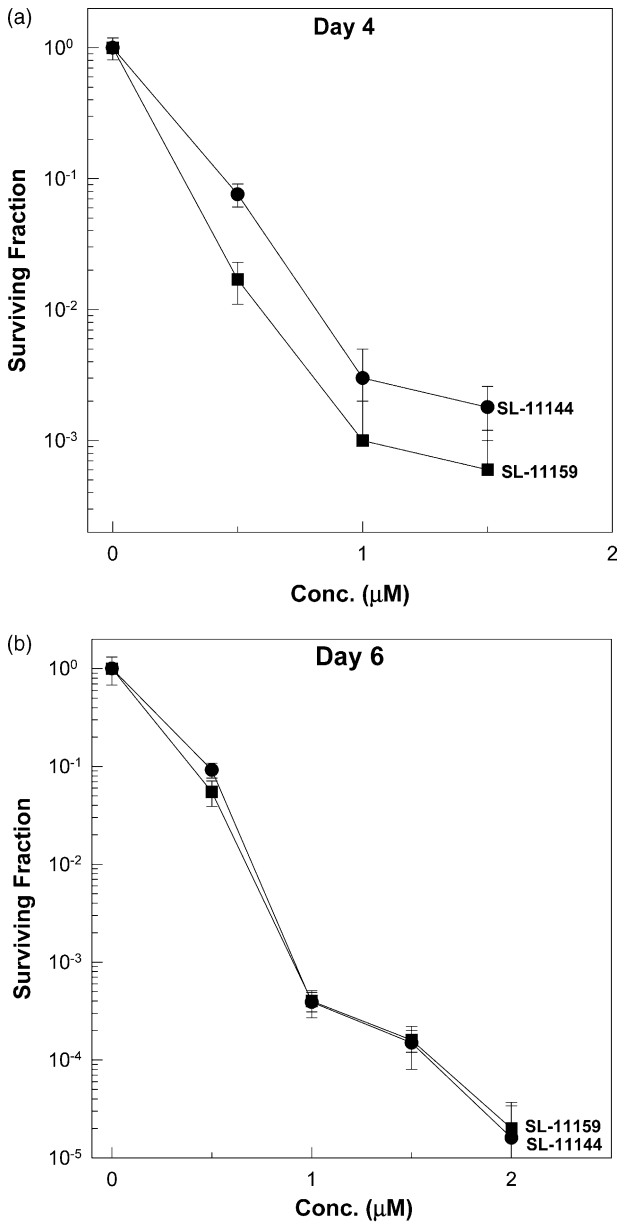


Figure 4. Effect of SL-11144 (●) and SL-11159 (■) on the survival of DuPro cells on day 4 (a) and day 6 (b) of treatment as determined by a colony forming efficiency (CFE) assay. Each data point is an average of six separate determinations.

Table 4. Correlation between DNA aggregating ability and cytotoxicity of the oligoamines

Oligoamine	Concn (μM) at the start of DNA aggregation	Concn (μM) to kill 1 log DuPro cells
Spermine	88.5	Non-cytotoxic
SL-11144	2.0	0.50
SL-11150	4.1	1.25
SL-11157	3.9	0.80
SL-11158	4.3	1.05
SL-11159	2.5	0.40
SL-11160	4.5	1.12
SL-11172	2.0	0.50
SL-11175	2.0	0.60
SL-11226	2.2	0.50
SL-11227	1.9	0.40

Although very few polyamine analogues or biosynthetic inhibitors can decrease cellular spermine level to below 50% of the control untreated cells, the lowering of the cellular polyamine levels does not correlate with the growth inhibitory effects of the oligoamines (compare data shown in Table 3 with that in Figs 2–4). In many cases, the cellular spermine levels increased on day 6 after an initial decrease on day 4, while the growth inhibitory and cytotoxic effects increased with an increase with the incubation time. Therefore, it is difficult to draw any conclusion about the growth inhibitory effects of oligoamines based on their effects on cellular polyamine levels.

There is, however, a general correlation between their effect on in vitro DNA aggregation and their cytotoxic effects. When the concentration of oligoamines required to initiate DNA aggregation was compared with the concentration necessary to kill one log DuPro cells (Table 4), it was evident that the oligoamines that are more efficacious in producing DNA aggregation are also the more cytotoxic. Thus, the octamines SL-11157, SL-11158 and SL-11160 that required relatively higher concentrations to initiate DNA aggregation than that required by other oligoamines had relatively lower cytotoxicities than the decamines (SL-11144 and SL-11159) or their higher homologues. The exception was the cis-decamine SL-11150 that was weaker in aggregating DNA and was also a weaker cytotoxic agent, when compared with the other decamines (Table 4). The departure from the stretched conformation in SL-11150 seems to be a factor in this anomalous behavior. These data corroborate our hypothesis that structures of polyamine analogues affect their DNA binding abilities and, in turn, their cytotoxicities by altering DNA/chromatin condensation during cell division.[7,9] Thus, oligoamines are more efficacious antiproliferative agents than any polyamine analogues described thus far and hold promise as novel chemotherapeutic drugs.

Experimental

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

All cell lines used in this study were obtained from the American Type Culture Collection (Rockville, MD, USA). Tissue culture medium was obtained from Fisher Scientific (Itasca, IL, USA) and fetal bovine serum was obtained from Gemini Bioproducts, Inc. (Calabasas, CA, USA).

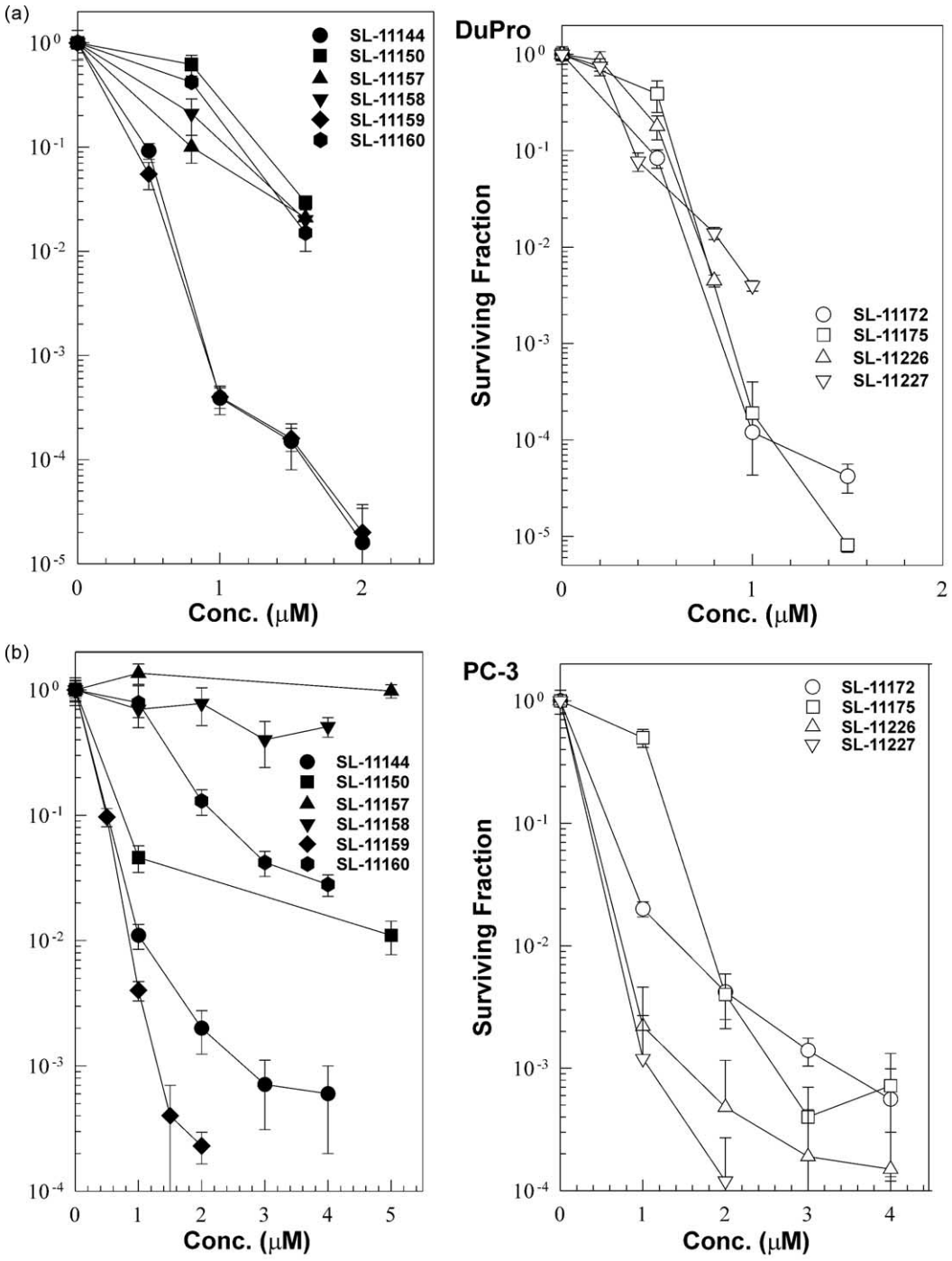


Figure 5. Effect of 12 oligoamines (symbol legends in the inset) on the survival of DuPro (a) and PC-3 (b) cells as determined by a colony forming efficiency (CFE) assay. Each data point is an average of six separate determination. Error bars, where not shown, are smaller than the symbol size.

All other reagents were analytical grade. Deionized double-distilled water was used in all studies.

Preparation of *cis*-oligoamides 4 and *trans*-oligoamides 6

General procedure. A solution of polyamide **1** (3 mmol) and of diesters **2** or **3** (1.5 mmol) in DMF (20 mL) was kept under a N_2 atmosphere at 5 °C while NaH (3.6 mmol, a 60% dispersion in oil was used) was slowly added. The stirred mixture was kept at 22 °C overnight. The solvent was then evaporated in vacuo, the residue partitioned between $CHCl_3$ and a concentrated solution of NH_4Cl, the organic layer was separated, washed thrice with concentrated NH_4Cl solution, then evaporated to dryness and the residue was purified by flash chromatography on a silica gel (EM Science, 230–400 mesh ASTM) column using hexane/ethyl acetate (6:4) as eluant. The following *cis*- and *trans*-oligoamides were thus prepared.

^{3}N,^{8}N,^{13}N,^{18}N,^{23}N,^{28}N,^{33}N,^{38}N-Octakis(mesitylenesulfonyl) - 3,8,13,18,23,28,33,38 - octaaza - (20*Z*) - tetracontene (4a). Condensation of tetramide **1a** with diester **2** gave **4a** in 83% yield; ^{1}H NMR ($CDCl_3$) δ 0.98 (t, 6H), 1.32

(br, 24H), 2.32 (s, 24H), 2.55 (s, 48H), 2.85–3.25 (br, 28H), 3.65 (d, 4H), 5.45 (t, 2H), 6.94 (s, 16H). MS-MALDI (*m*/*z*) calcd: 2026.87 (M^+), found: 2027.79 ($M^+ + H$).

^{3}N,^{8}N,^{13}N,^{18}N,^{23}N,^{28}N,^{33}N,^{38}N-Octakis(mesitylenesulfonyl)-3,8,13,18,23,33,38-octaaza-tetracontene (6a). Condensation of tetramide **1a** with diester **3** gave **6a** in 76% yield; ^{1}H NMR ($CDCl_3$) δ 0.98 (t, 6H), 1.30 (br, 24H), 2.25 (s, 24H), 2.55 (s, 48H), 2.90–3.20 (br, 48H), 3.65 (d, 4H), 5.40 (t, 2H), 6.95 (s, 16H). MS-MALDI (*m*/*z*) calcd: 2026.87 (M^+), found: 2027.80 ($M^+ + H$).

^{3}N,^{8}N,^{13}N,^{18}N,^{23}N,^{28}N,^{33}N,^{38}N,^{43}N,^{48}N-Decakis(mesitylenesulfonyl) - 3,8,13,18,23,28,33,38,43,48 - decaaza(25*Z*)-pentacontene (4b). Condensation of pentamide **1b** with diester **2** gave **4b** in 72% yield. ^{1}H NMR ($CDCl_3$) δ 0.95 (t, 3H), 1.10–1.40 (br, 32H), 2.30 (s, 30H), 2.40–2.60 (br, 60H), 3.65 (d, 4H), 5.40 (t, 2H), 6.95 (s, 20H). MS-MALDI (*m*/*z*): calcd: 2533.59 (M^+) found : 2566.80 ($M^+ + Na$).

^{3}N,^{8}N,^{13}N,^{18}N,^{23}N,^{28}N,^{33}N,^{38}N,^{43}N,^{48}N-Decakis (mesitylenesulfonyl) - 3,8,13,18,23,28,33,38,43,48 - decaaza (25*E*)-pentacontene (6b). Condensation of pentamide **1b** with diester **3** gave **6b** in 85% yield; mp 129.7 °C (ethyl acetate–hexane). ^{1}H NMR ($CDCl_3$) δ 0.95 (t, 6H), 1.15–1.40 (br, 32H), 2.30 (s, 30H), 2.40–2.55 (br s, 2H), 6.95 (s, 20H). MS-MALDI (*m*/*z*) calcd: 2533.59 (M^+), found: 2556.90 ($M^+ + Na$).

^{3}N,^{8}N,^{13}N,^{18}N,^{23}N,^{28}N,^{33}N,^{38}N,^{43}N,^{48}N,^{53}N,^{58}N - Dodecakis(mesitylenesulfonyl)-3,8,13,18,23,28,33,38,43,48,53,58-dodecaaza (30*E*)-hexacontene (6c). Condensation of hexamide **1c** with diester **3** gave **6c** in 83% yield; mp 128 °C. ^{1}H NMR ($CDCl_3$) δ (t, 6H), 1.10–1.40 (br, 40H), 2.25 (s, 36H), 2.50 (s, 72H), 2.85–3.20 (br, 44H), 3.60 (d, 4H), 5.40 (t, 2H), 6.95 (s, 24H). MS-MALDI (*m*/*z*) calcd: 3040.31 (M^+), found: 3063.66 ($M^+ + Na$).

^{3}N,^{8}N,^{13}N,^{18}N,^{23}N,^{28}N,^{33}N,^{38}N,^{43}N,^{48}N,^{53}N,^{58}N,^{63}N,^{68}N-Tetradecakis(mesitylenesulfonyl) - 3,8,13,18,23,28,33,38,43,48,58,63,68-tetradecaaza-(35*E*)-heptacontene (6d). Condensation of hexamide **1d** with diester **3** gave **6d** in 85% yield; mp 125 °C. ^{1}H NMR ($CDCl_3$) δ 1.95 (t, 6H), 1.0-1.40 (br, 48H), 2.30 (s,42H), 2.50 (s, 84H), 2.80–3.15 (br, 52H), 3.60 (d, 4H), 5.40 (t, 2H), 6.95 (s, 28H). MS-MALDI (*m*/*z*) calcd: 3547.03 (M^+), found: 3570.10 ($M^+ + Na$), 3586.20 ($M^+ + K$).

Preparation of *cis*- and *trans*-oligoamines from oligoamides 4 and 6

General procedure. Oligoamides **4** or **6** (0.5 mmol) were dissolved in CH_2Cl_2 (25 mL), phenol (35 mmol) was added, followed by a solution of 33% HBr in glacial acetic acid (25 mL), and the mixture was kept at 22 °C for 18 h. Water (25 mL) was then added, the aqueous layer was separated, washed with CH_2Cl_2 (3×10 mL), and evaporated to dryness. The residue was dissolved in 2 N NaOH (4 mL), a further 9 N KOH (4 mL) was added and the aqueous solution was extracted with $CHCl_3$ (4×10 mL). The pooled extracts were dried (Na_2SO_4), evaporated to dryness and the residue crystallized from ethanol to which a few drops of concd HCl were added. The following *cis*- and *trans*-oligoamines were thus prepared.

3,8,13,18,23,28,33,38-Octaaza-(20*Z*)-tetracontene octahydrochloride (5a). SL-11157 was obtained from **4a** in 89% yield; mp >250 °C (dec). ^{1}H NMR (D_2O) δ 1.28 (t, 6H), 1.58–1.90 (br, 24H), 2.90–3.25 (br, 28H), 3.85 (d, 4H), 5.95 (t,2H); ^{13}C NMR (D_2O) δ 13.29, 25.59, 25.75, 45.68, 49.03, 49.37, 49.68, 129.23. MS-ESI (*m*/*z*) calcd: 568.98 (M^+, free base), found: 569.88 ($M^+ + H$).

3,8,13,18,23,28,33,38-Octaaza-(20*E*)-tetracontene octahydrochloride (7a). SL-11158 was obtained from **6a** in 80% yield; mp >250 °C (dec). ^{1}H NMR (D_2O) δ 1.30 (t, 6H), 1.75 (br, 24H), 2.90–3.25 (br, 28H), 3.75 (d, 4H), 6.10 (t, 2H); ^{13}C NMR (D_2O) δ 13.29, 25.59, 45.68, 49.03, 49.17, 49.68, 50.88, 131.16; MS-ESI (*m*/*z*) calcd: 568.98 (M^+, free base), found: 569.81 ($M^+ + H$), 285.4 $(M/2 + H)^{++}$, 697.80 ($M^+ +$ HCl).

3,8,13,18,23,28,33,38,43,48-Decaaza-(25*Z*)-pentacontene decahydrochloride (5b). SL-11150 was obtained from **4b** in 89% yield; mp >250 °C (dec). ^{1}H NMR (D_2O) δ 1.30 (t, 6H), 1.60–1.90 (br, 32H), 2.90–3.25 (br, 36H), 3.85 (d, 4H), 5.95 (t, 2H); ^{13}C NMR (D_2O) δ 13.35, 25.12, 25.64, 25.77, 45.76, 46.87, 49.09, 49.43, 49.73, 129.24. MS-ESI (*m*/*z*) calcd: 711.22 (M^+, free base), found: 712.80 ($M^+ + H$), 894.10 ($M^+ +$ 5HCl), 356.40 $(M/2 + H)^{++}$.

3,8,13,18,23,28,33,38,43,48 - Decaaza(25*E*) - pentacontene decahydrochloride (7b). SL-11144 was obtained from **6b** in 90% yield. ^{1}H NMR (D_2O) δ 1.30 (t, 6H), 1.60-190 (br, 32H), 2.95–3.25 (br, 36H), 3.75 (d, 4H), 6.05 (t, 2H); ^{13}C NMR (D_2O) δ 13.28, 25.57, 45.66, 49.00, 4913, 49.64, 50.86, 131.15. MS-ESI (*m*/*z*) calcd: 711.22 (M^+, free base), found: 712.80 ($M^+ + H$), 747.80 ($M^+ +$ HCl), 356.40 $(M/2 + H)^{++}$.

3,8,13,18,23,28,33,38,43,48,53,58-Dodecaaza-(30*E*)-hexacontene dodecahydrochloride (7c). SL-11172 was obtained from **6c** in 89% yield; mp >250 °C (dec). ^{1}H NMR (D_2O) δ 1.30 (t, 6H), 1.60–1.90 (br, 40H), 2.90–3.30 (br, 44H), 3.80 (d, 4H), 6.05 (t, 2H); ^{13}C NMR (D_2O) δ 13.27, 25.58, 45.68, 49.02, 49.18, 49.68, 50.88, 131.15. MS-ESI (*m*/*z*) calcd: 853.47 (M^+, free base), found: 427.80 $(M/2 + H)^{++}$, 1035.80 ($M^+ + 5HCl$), 518.60 $(M/2 + 5HCl + H)^{++}$

3,8,13,18,23,28,33,38,43,48,58,63,68-Tetradecaaza-(35*E*)-heptacontene tetradecahydrochloride (7d). SL-11227 was obtained from **6d** in 88% yield; mp >250 °C (dec). ^{1}H NMR (D_2O) δ 1.30 (t, 6H), 1.60–1.95 (br, 48H), 2.85–3.25 (br, 52H), 3.75 (d, 4H), 6.05 (t, 2H); ^{13}C NMR (D_2O) δ 13.29, 19.62, 45.69, 49.04, 49.24, 49.71, 50.91, 131.18. MS-ESI (*m*/*z*) calcd: 995.71 (M^+, free base), found: 996.0 ($M^+ + H$), 1018.20 $(M + Na)^+$, 1032.00 ($M^+ +$ HCl).

Preparation of saturated oligoamines

General procedure. Hydrochlorides **5** or **7** (0.50 mmol) were dissolved in 20 mL of H_2O, PtO_2 (40 mg) was added, and the solution was reduced with hydrogen using Parr hydrogenator at 50 psi for 2 h. The catalyst was then filtered, the solution evaporated to dryness in vacuo and the residue crystallized from water/ethanol. The hydrochlorides of the saturated oligoamines were thus obtained.

3,8,13,18,23,28,33,38-Octaazatetracontane octahydrochloride (8a). SL-11160 was obtained from **5a** or **7a** in 85% yield; mp $>250\,^{\circ}C$ (dec). ^1H NMR (D_2O) δ 1.30 (t, 6H), 1.60–1.90 (br, 28H), 2.90, 3.25 (br, 32H); ^{13}C NMR (D_2O) δ 13.27, 25.60, 45.68, 49.03, 49.70. MS-ESI (*m*/*z*) calcd: 570.99 (M^+), found: 571.92 ($M^+ + H$), 286.46 $(M/2 + H)^{++}$

3,8,13,18,23,28,33,38,43,48-Decaazapentacontane decahydrochloride (8b). SL-11159 was obtained from either **5b** or **7b** in 94% yield; mp $>250\,^{\circ}C$. ^1H NMR (D_2O) δ 1.30 (t, 6H), 1.60–1.90 (br, 36H), 2.90–3.30 (br, 40H); ^{13}C NMR (D_2O) δ 13.28, 25.61, 45.67, 49.02, 49.69. MS-ESI (*m*/*z*) calcd: 713.24 (M^+, free base), found: 714.80 ($M^+ + H$), 859.80 ($M^+ +$ HCl), 357.60 $(M/2 + H)^{++}$

3,8,13,18,23,33,38,43,48,53,58 - Dodecaazahexacontane dodecahydrochloride (8c). SL-11226 was obtained from **7c** in 85% yield; mp $>250\,^{\circ}C$ (dec). ^1H NMR (D_2O) δ 1.30 (t, 6H), 1.55–2.05 (br, 44H), 2.90–3.30 (br, 48H); ^{13}C NMR (D_2O) δ 13.29, 25.62, 45.70, 49.04, 49.72. MS-ESI (*m*/*z*) calcd: 855.48 (M^+, free base), found: 856.00 ($M^+ + H$), 878.0 ($M^+ + Na$), 892.2 ($M^+ +$ HCl).

3,8,13,23,33,38,43,48,53,58,63,68 - Tetradecaazaheptacontane tetradecahydrochloride (8d). SL-11175 was obtained from **7d** in 80% yield; mp $>250\,^{\circ}C$ (dec). ^1H NMR (D_2O) δ 1.30 (t, 6H), 1.60–1.90 (br, 52H), 2.90–3.25 (br, 56H); ^{13}C NMR (D_2O) δ 13.28, 25.61, 45.69, 49.04, 49.71. MS-ESI (*m*/*z*) calcd: 997.73 (M^+, free base) found: 1180.20 ($M^+ +$ 5HCl), 1252.20 ($M^+ +$7HCl).

Biological assays

Tissue culture.[9] 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 non-essential 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, and were then treated with the oligoamines. The 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 the laboratory bench, pipetted several times, and aliquots of cell suspension were withdrawn and counted using a Coulter particle counter that has been standardized for counting each cell line using a hemacytometer.

Oligoamine analysis. Oligoamines cannot be analyzed by the dansylation method described above. Therefore, the following method for oligoamine analysis was developed. A pellet containing ca. 1×10^6 cells was suspended in 250 μL of 2% perchloric acid and the suspension was kept for 18 h at −20 °C. It was then sonicated twice with 30-s pulses, centrifuged at 10,000 rpm and the supernatant analyzed by LC/MS. An Applied Biosystems/MDS Sciex API 365 LC/MS/MS triple quadrupole with an electrospray source coupled to a Perkin–Elmer ABI 140D HPLC and a Perkin Elmer series 200 autosampler was used. A Hypersil BDS C-18 (3 μ, 120 Å) column was used (1×150 mm). Solvent A = water plus 0.05% TFA; solvent B = 90% acetonitrile, 10% water containing 0.044% TFA. The column had a flow rate of 25 μL/min; it was equilibrated during 20 min with solvent A, after which 10–20 μL of the solution containing ca. 50 pmol of oligoamine was injected using the autosampler. Solvent A ran for 5 min, changing to a gradient with up to 99% of solvent B over 35 min, then hold at solvent B for 5 min, after which it reverted to solvent A for 3 min. The entire flow was introduced into the mass spectrometer (no split) and submitted to electrospray. The triple quadrupole was operated in the SIM (select ion monitoring) mode while monitoring MH^+. The desolvation energy was set very high (OR = 200, RNG = 350) in order to remove acid adducts and to give the highest signal for the free base. The MS data were imported into 'mac quant' software, standard curves were constructed using dilutions series of the standards and the plots were used to calculate the concentration of oligoamines in cell extracts (see Fig. 1 for an elution profile of an oligoamine).

MTT assay.[4] A trypsin-treated cell suspension was diluted to seed 80 μL containing 500 cells in each well of a 96-well corning microtiter plate and incubated overnight at 37 °C in a humidified incubator under 5% CO_2. Twenty microliters of an appropriately diluted stock solution of each oligoamine was added to the middle eight columns of cell suspensions in the microtiter plates. Each drug concentration was run in quadruplicate. Outer columns of the plates were used for buffer controls. Cells were incubated with the oligoamine for 6 days at 37 °C in a 5% CO_2/H_2O atmosphere. Twenty-five microliters of 5 mg/mL solution of 3-(4,5-dimethythiazol-yl-2,5-diphenyl tetrazolium bromide (MTT)) were added to each well and incubated for 4 h at 37 °C in a 5% CO_2/H_2O incubator. Cells were lysed by incubating overnight with 100 μL lysis buffer [500 mL of the lysis buffer contained 100 g lauryl sulfate (SDS), and 250 mL of *N*,*N*-dimethylformamide in 2 mL of glacial acetic acid, pH 4.8]. Color was monitored at room temperature at 570 nm in an E-max Precision Microplate Reader (Molecular Devices Corporation, Sunnyvale, CA, USA) and the data were analyzed using cell survival software supplied by Molecular Devices Corporation.

Colony forming efficiency assay.[9] Cell lines used in this assay have already been optimized with respect to the number of feeder cells and length of incubation time for observable colony formation. Cells were washed, harvested, and replated in quadruplicate at appropriate dilutions 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) for all cell lines. Cells were incubated for 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 were expressed as a surviving fraction of an appropriate control.

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