Macrocyclic Polyamines Deplete Cellular ATP Levels and Inhibit Cell Growth in Human Prostate Cancer Cells

Benjamin Frydman,*,† Subhra Bhattacharya,† Aparajita Sarkar,† Konstantin Drandarov,‡ Sergiy Chesnov,‡ Armin Guggisberg,‡ Kasim Popaj,‡ Sergey Sergeyev,‡ Aysil Yurdakul,‡ Manfred Hesse,‡ Hirak S. Basu,† and Laurence J. Marton†

SLIL Biomedical Corp., 535 Science Dr., Suite C, Madison, Wisconsin 53711-1066, and Organisch-chemisches Institut der Universität Zürich, Winterthurerstrasse 190, 8057 Zürich, Switzerland

Received September 4, 2003

In solid tumors, when O_2 partial pressure drops below 10 mmHg, ATP levels rapidly decrease due to the Warburg effect. It is known that certain macrocyclic polyamines catalyze the chemical hydrolysis of ATP with release of inorganic phosphate. Since tumor cells have diminished ATP levels as compared to normal cells, we attempted to deplete cellular ATP with macrocyclic polyamines in an effort to inhibit tumor cell proliferation. Five macrocyclic polyamines, related to the budmunchamine family of alkaloids, were prepared by total synthesis. They were the [17]-N_4 macrocycle **1**, the [16]-N_4 macrocycle **20**, the [18]-N_4 macrocycle **13**, the [20]-N_5 macrocycle **8**, and the [13]-N_3 macrocycle **17**. Each one of them hydrolyzed ATP in vitro with release of P_i; the largest ring macrocycle **8** was the most efficient catalyst, while the smallest ring macrocycle **17** was the least efficient (P_i released in these runs was on the order of 40−100 μM). The linear polyamine spermine had no hydrolytic effect on ATP. The macrocycles were found to be cytotoxic when assessed by means of a MTT assay against two human prostate cell lines, DuPro and PC-3, with resultant ID_{50} values ranging between 0.5 and 1.8 μM. Colony forming efficiency (CFE) assays performed on DuPro cells, where the macrocycles were used in a concentration range of 1−8 μM, confirmed the cytotoxic effect of each macrocycle. Each killed 3−4 log of DuPro cells. The smallest ring **17** was the least cytotoxic after 24 h of incubation, although after 144 h of incubation it showed significant cytotoxicity at 8 μM. The macrocycles were equally efficient in depleting the intracellular ATP pools; after a 24 h incubation with each macrocycle other than **17** at 1−8 μM concentrations, cellular ATP concentrations were decreased by 3 orders of magnitude. The decrease in ATP levels was more pronounced after a 72 h incubation, when even **17** reduced ATP by 2 orders of magnitude. A linear pentamine of established cytotoxicity was without effect on the ATP pools. The macrocycles depleted almost entirely the intracellular pools of polyamines and were efficiently taken up by cells. A rough correlation could be established between the cytotoxic effect of the macrocyclic polyamines and their ATP-ase like activity in the DuPro cell line. As ATP is a scarce metabolite in cancer cells, where it can only be replenished through the very ATP-inefficient glycolytic pathway; macrocyclic polyamines appear to be promising new anticancer agents.

Almost 80 years ago, Otto Warburg showed that cancer cells are characterized by a marked increase in glycolytic metabolism, even when cultured in the presence of high O_2 concentrations.[1,2] This phenomenon of aerobic glycolysis in tumor cells (the Warburg effect) is one of the fundamental problems of tumor biochemistry; the switch from oxidative metabolism in normal cells to glycolytic metabolism in cancer cells appears to be hard-wired into the program of cellular proliferation. Virtually all solid tumors manifest the Warburg effect, regardless of their genomic makeup.[3] Extended investigations in a variety of cancer cells confirmed the high rate of lactate production from glucose, despite the presence of O_2 and mitochondria.[4] Increases in glycolytic enzyme capacities correlate well with carcinogenesis and with cell proliferation.[4c]

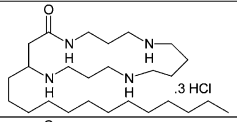
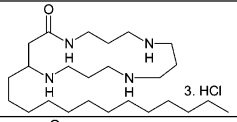
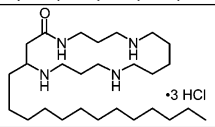
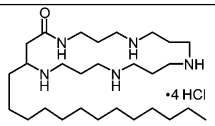
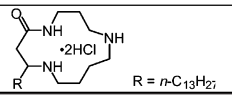
Therefore, tumor cells meet their energy requirements mainly from glucose oxidation by the anaerobic glycolytic pathway, even though this pathway is far less effective in ATP production than is aerobic glucose oxidation (2 mol ATP/mol of glucose metabolized glycolytically, and 38 mol ATP/mol of glucose metabolized aerobically). Indeed, it has been shown that quiescent thymocytes cultured in the absence of mitogens derive ca. 80% of their ATP from oxidative phosphorylation, while mitogen-stimulated thymocytes derive ca. 80% of their ATP from glycolysis.[5] Thus, the relationship between proliferation and glycolysis is seen even in normal cells.

In solid tumors, ATP synthesis is strongly dependent on O_2 partial pressure. It is well-known that O_2 delivery to neoplastic cells is reduced or abolished by a deteriorating diffusion geometry, by structural abnormalities of tumor microvessels, and by disturbed microcirculation.[6] It has been shown, in grafts of murine fibrosarcoma in mice,[7] that as long as O_2 partial pressure in

* Corresponding author. Phone: (608) 231-3875 x 12. Fax: (608) 231-3892. E-mail: bjfrydman@ slil.com.
† SLIL Biomedical Corp.
‡ Universität Zürich.

10.1021/jm030437s CCC: $27.50
Published on Web 01/14/2004

Table 1. Structures and Inhibitory Effects of Macrocyclic Polyamines on the Growth of Human Prostate Cancer Cell Lines Measured by a MTT Assay

Macrocyclic polyamine	Structure	ID_{50} (μM)	
		DuPro	PC-3
1		0.83	0.6
20		0.58	0.5
13		1.2	1.14
8		1.4	1.3
17		1.8	1.7

the tumor stays above 10 mmHg, ATP concentrations remain constant, but ATP concentrations rapidly drop below this median O_2 partial pressure, and total inorganic phosphate concentrations increase proportionally. ATP concentrations are therefore very much at a premium in tumor cells, and it is tempting to try to deplete cellular ATP in an effort to inhibit tumor cell proliferation. There are reports that depletion of cellular ATP can be related to growth inhibition of tumor cell lines and to drug resistance in MDR cells.[8]

The affinity of polyamine macrocycles for ATP was first observed by Dietrich et al. in 1981.[9] Different polyamine macrocycles have varying degrees of affinity for ATP, and several of them were found to be very efficient in catalyzing the hydrolysis of ATP.[10] This surprising ATP-ase mimicry shown by several polyamine macrocycles is entirely dependent on the ring size of the macrocycle.[11]

When searching for macrocyclic polyamines that could deplete ATP levels in tumor cells, we were aware of a report that the macrocyclic polyamine alkaloids from *Albizia amara*, the so-called budmunchiamines, behaved as cytotoxic agents when assayed against a battery of cultured tumor cells.[12] The budmunchiamines are formally derived from the [17]-membered polyamine macrocycle **1** (Table 1). We therefore prepared by synthesis several analogous macrocycles of different ring sizes and assessed their efficacy against cultures of two human prostate cancer cell lines. They were found to be very efficacious both as cytotoxic agents and in depleting the endogenous ATP levels of the tumor cells. Both effects appear to correlate, and cytotoxicity is dependent on the efficacy of the macrocycles in catalyzing ATP hydrolysis in vivo.

Chemistry

Syntheses of the Macrocyclic Polyamines. The five macrocycles shown in Table 1 were prepared by synthesis. The basic structure of the budmunchamines is the [17]-N_4 tetraaza macrocycle **1**; its synthesis has been reported elsewhere.[13] Macrocycle **20** and macrocycle **13** are the [16]-N_4 and the [18]-N_4 analogues of **1**. Macrocycle **8** is the [20]-N_5 pentaaza analogue, and macrocycle **17** is the smaller [13]-N_3 triaza analogue of **1**. The synthesis of the four new macrocycles is described below.

Preparation of the pentamine **5** (caldopentamine, 4,8,-12-triaza-1,15-diaminopentadecane; Scheme 1) has already been reported by Niitsu and Samejima.[14] They prepared the compound in six steps in an overall yield of approximately 32%.

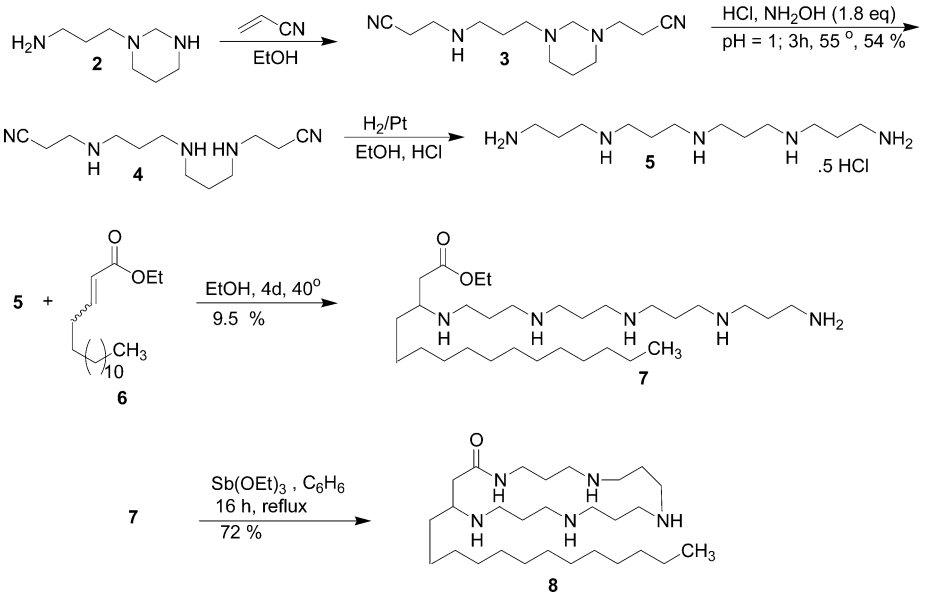
Another procedure reported by Osvath et al.[15] gave caldopentamine **5** in 39% overall yield. Both syntheses are too complex and laborious for the obtention of larger amounts of **5**. A new approach was therefore developed. Starting with 1-(3-aminopropyl)hexahydropyrimidine **2**, a partly protected bis(3-aminopropyl)amine,[16] and following alkylation with acrylonitrile, the dialkylated hexahydropyrimidine derivative **3** was obtained in almost quantitative yield. Its deprotection in the presence of hydrochloric acid and hydroxylamine led (54%) to the open chain dinitrile **4**; after catalytic hydrogenation of **4** in EtOH/HCl, the desired pentamine **5** was obtained (Scheme 1). The preparation of ethyl 2-hexadecenoate **6** was achieved by a Wittig reaction between (ethoxycarbonylmethyl)triphenylphosphonium bromide and myristinaldehyde.[13] The reaction product was a (*E*/*Z*) mixture in a ratio of 2:1. Ester **6** and free base **5** were mixed in an 1:1 molar ratio and kept in an ethanol solution for 4 days at 40 °C. The resulting aminoethyl ester **7**, obtained in low yield, was purified by chromatography. Cyclization was achieved using triethoxyantimony ($Sb(OEt)_3$), a very effective macrocyclization reagent for α,ω-tetraamino esters developed by Yamamoto and co-workers.[17] Lactam **8** was thus obtained in 72% yield after a prolonged reflux in dry benzene.

N,*N*′-Bis(3-aminopropyl)-1,5-diaminopentane **11** was prepared from 1,5-diaminopentane **9** by treatment with acrylonitrile. The resulting dinitrile **10** (Scheme 2), was reduced with hydrogen over Raney Ni to give the expected tetramine **11** as a colorless oil in 56% yield. In the following reactions it was not necessary to protect the polyamine **11**, because of the symmetrical nature of the expected **12**. Tetramine **11** was condensed with **6** to give **12,** and the latter cyclized to **13** as described above.

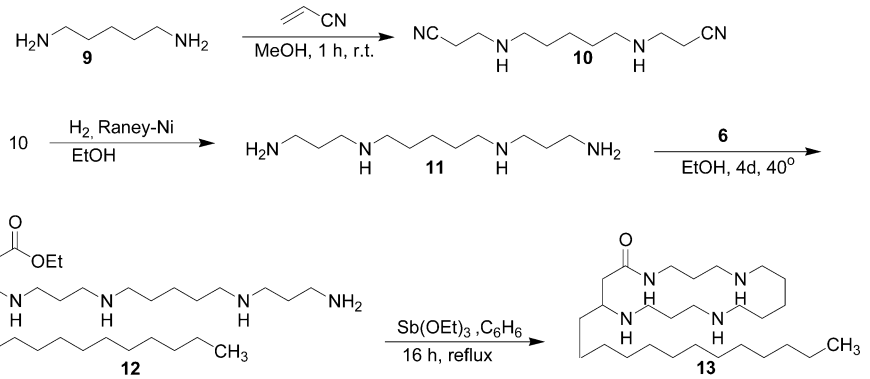
The reaction of **6** with the partly protected spermidine derivative **14**[16] lead to the amino ethyl ester **15**, which by deprotection and transesterification gave the amino methyl ester **16** (Scheme 3)**.** The cyclization was performed in boiling xylene and in the presence of the boronamide $B(NMe_2)_3$. The yield of the cyclization product **17** was only 50%.

The lower homologue of **1**, with one methylene group less in the central part of the ring, was prepared starting with the commercially available *N*,*N*′-bis(3-aminopropyl)-1,3-diaminopropane **18**, which was condensed with **6** under the usual conditions to give **19** in 26% yield (Scheme 4). The cyclization reaction in the presence of the antimony catalyst gave the macrocycle **20** in 54% yield.

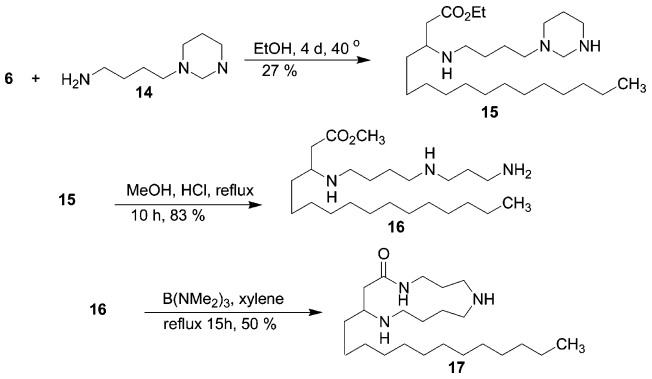
Scheme 1



Scheme 2



Scheme 3

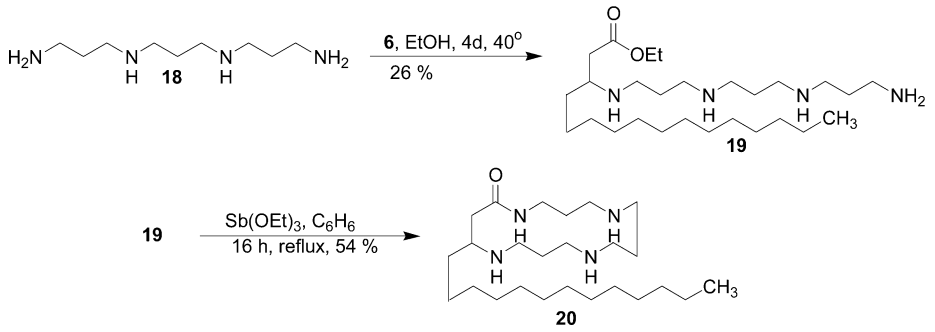


Results and Discussion

Chemical ATP Hydrolysis Catalyzed by the Macrocyclic Polyamines. Spermine, a linear polyamine, failed to catalyze any ATP hydrolysis under our experimental conditions. The cyclic analogues, on the contrary, hydrolyzed ATP with release of inorganic phosphate (Figure 1). Reactions were run at low pH (pH ca. 3) to ensure that the amino groups were protonated, since the ATP-ase effect was previously achieved with polyammonium macrocycles.[9−11] The amounts of inorganic phosphate released were determined from a standard curve generated with each set of experiments. To account for any spontaneous ATP hydrolysis, inorganic phosphate data were normalized to phosphate released in the absence of the cyclic polyamines. The macrocycle with the smallest ring, the [13]-member **17**, released the smallest amount of inorganic phosphate from ATP. The larger [20]-member macrocycle **8**, released the highest amount of inorganic phosphate from ATP. The [18]-, [17]-, and [16]-member macrocycles **13**, **1**, and **20** were also very efficient in releasing inorganic phosphate from ATP; their efficiency was halfway between those of **8** and **17**. Inorganic phosphate released was in the range of 40−100 μM. No hydrolysis of ATP catalyzed by the above-mentioned macrocyclic polyamines was observed at higher pH.

Inhibitory Effects of Macrocyclic Polyamines on Growth of Human Prostate Cancer Cells. The growth inhibitory activities of the five macrocycles **1**, **20**, **13**, **8**, and **17** were assessed against two androgen-independent human prostate cancer cell lines, DuPro and PC-3. Growth inhibition was determined with a MTT assay after incubating cells with the various macrocycles for 144 h incubations. The ID_{50} values determined for the macrocycles, along with their structures, are shown in Table 1. ID_{50} values are defined as the macrocycle concentration required to inhibit cell growth by 50%. Both cell lines are sensitive to the macrocyclic polyamines, with ID_{50} values that range

Scheme 4



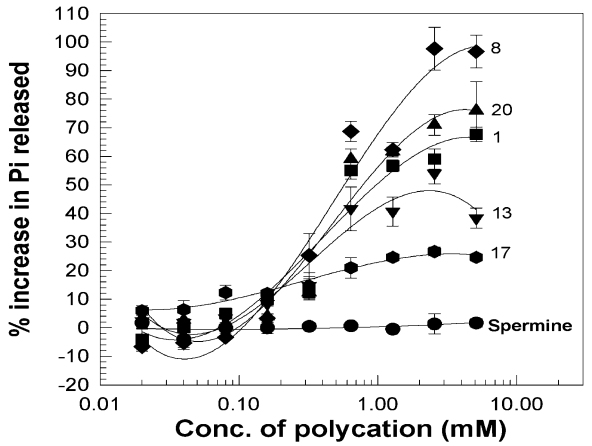


Figure 1. Chemical hydrolysis of ATP with release of inorganic phosphate catalyzed by macrocyclic polyamines. Symbols for each macrocycle are shown in the figure. Each data point is an average of at least four separate experiments. Controls were run containing macrocycle or spermine without ATP for each experiment. Control readings were subtracted from experimental runs, and all data were normalized for ATP hydrolysis at zero concentration of macrocycles or spermine. Error bars that were smaller than symbol sizes and are not shown.

between 500 nM and 1.8 μM. The DuPro cell line was selected for further studies, since it was known to be a more sensitive cell line than PC-3 to treatment with polyamines.[18]

Inhibition of growth of DuPro cells was assessed after treatment with the macrocycles for 3, 4, and 5 days (Figure 2). All five macrocyclic polyamines showed significant growth inhibitory effects within the concentration range of 5−10 μM. Near normal cellular proliferation was observed after 5 days of treatment with macrocyclic polyamines at 0.5 and 1 μM. At 5 μM, both **20** and **13** showed significant growth inhibitory effects after 3 days of treatment, while **1** and **8** showed similar growth inhibitory effects at 10 μM (Figure 2A). The growth inhibitory effect of **17**, the smallest ring, was weaker than those of the other macrocycles and required 10 μM for a 4 day treatment to reach results comparable to those of the other four macrocycles (Figure 2B).

The expected cytotoxic effects of the macrocycles were further investigated using the colony forming efficiency (CFE) assay. The CFE data after incubating the macrocycles with DuPro cells for 72 and 144 h are shown, respectively, in parts A and B of Figure 3. It is evident that the macrocycles are cytotoxic; the smallest macrocycle **17** is the least cytotoxic when assessed 72 h after treatment (Figure 3A); however, after 144 h even it

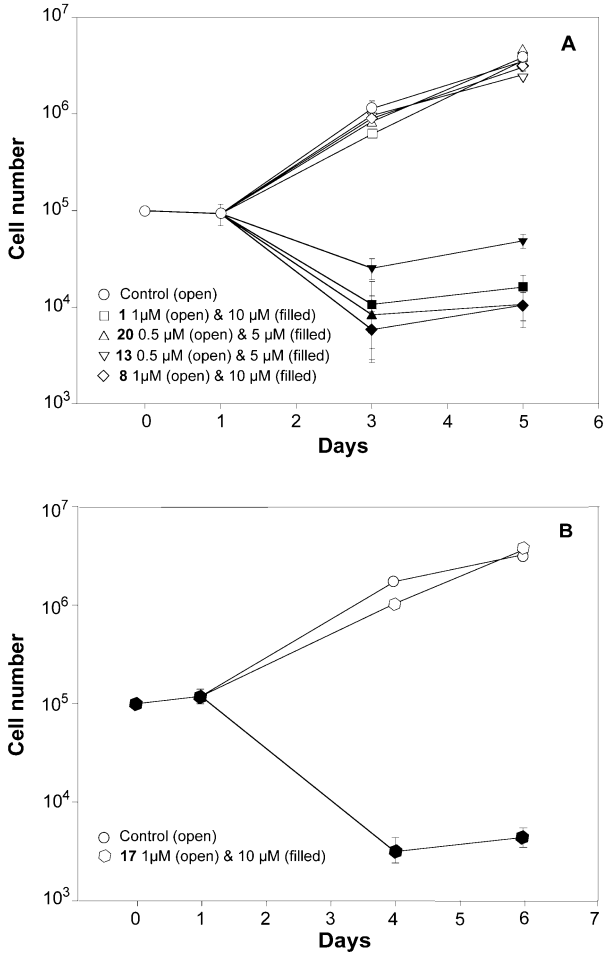


Figure 2. Effects of macrocyclic polyamines on DuPro cell growth. (A) Effect of macrocycles **1**, **20**, **13**, and **8**; symbols for each macrocycle and concentrations are shown in the inset. Each data point is an average of at least three separate experiments. Each experiment was run in duplicate. Error bars, where not shown, are smaller than the symbol bars. (B) Effect of macrocycle **17**; all the rest as in part A.

showed significant cytotoxicity at 8 μM (Figure 3B). The larger ring macrocycle **8** is only moderately cytotoxic at the shorter incubation time (Figure 3A), but becomes strongly cytotoxic at longer incubation periods (Figure 3B). The other macrocycles showed significant cytotoxicities after 72 h that increased at the longer incubation time (Figure 3B).

Macrocyclic Polyamines Deplete Cellular ATP Pools in DuPro Cells. Cellular ATP levels in DuPro cells were measured 24 and 72 h after incubation with the macrocyclic polyamines (Figure 4A,B). Cellular ATP

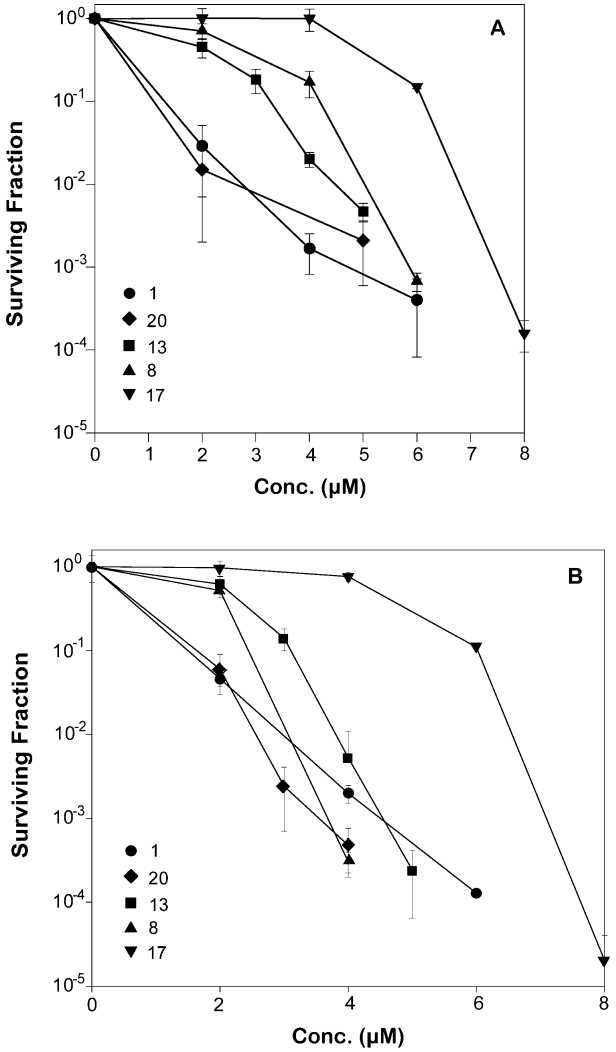


Figure 3. Effect of macrocyclic polyamines on the survival of DuPro cells: (A) after 72 h treatment and (B) after 144 h treatment. Each data point and corresponding error bars are, respectively, an average and the standard deviation of six independent observations.

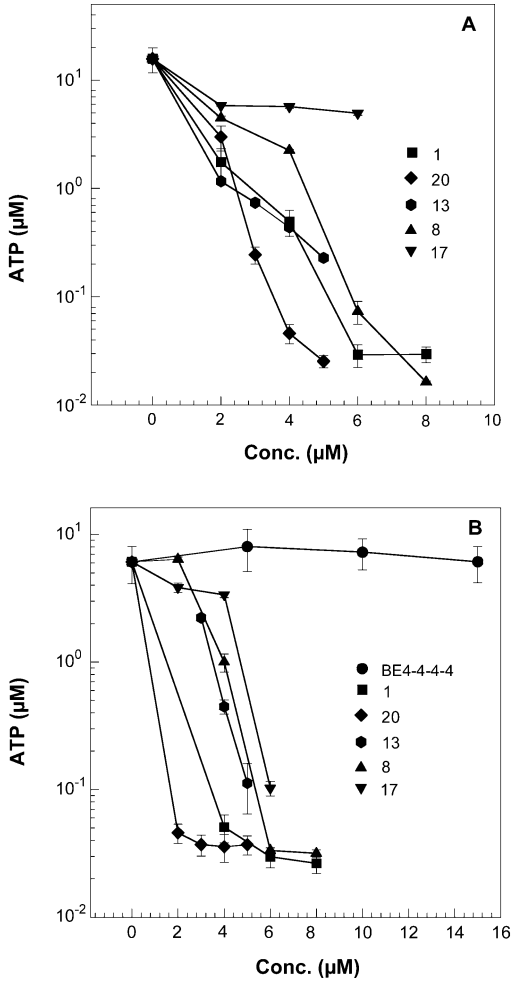


Figure 4. Effect of macrocyclic polyamines on the intracellular ATP levels of DuPro cells after incubation for (A) 24 h and (B) 72 h. Treated cells for each concentration of macrocycle or pentaamine BE-4-4-4-4 were run in quadruplicate. ATP concentrations were determined using Enlitten reagent (Promega), and were read in a luminometer after addition of the luciferase−luciferin reagent. Luminescence was read after a 5 s mixing time. Relative light units were converted to ATP concentrations using a reference plot.

concentrations were measured using the luciferase−luciferin system. ATP levels remained unchanged after a 4 h treatment with the polycations (data not shown). However, after a 24 h treatment, all the macrocycles, with the exception of the smallest ring **17**, efficiently depleted cellular ATP pools. After a 72 h incubation, all the macrocyclic polyamines were found to deplete intracellular ATP pools, while an open chain polyamine analogue known for its cytotoxic effect (BE4-4-4-4[19]) and used as reference did not produce any reduction in cellular ATP levels when assayed under similar incubation conditions. The [17]- and [20]-member macrocycles **1** and **20** required 2−4 μM concentrations and 72 h incubations to deplete ATP intracellular levels to the lowest values obtained, while the [13]-member macrocycle **17** required a 6 μM concentration to achieve comparable inhibitions (Figure 4B).

Macrocyclic Polyamines Uptake and Depletion of Polyamine Intracellular Pools in DuPro Cells. Because of the poor cell yields resulting from incubating cells with 5 μM of the macrocycles, smaller concentrations (0.5−4 μM) were used to collect cells for cellular polyamine determinations. Incubations were run for 3 and 5 days. Cellular polyamine levels were determined by a standard HPLC method[18] using a modified dansylation procedure to achieve full dansylation of the ring NH residues (see Experimental Section). On treatment with the macrocycles, DuPro cells showed significant depletions of their cellular polyamine levels, as well as a efficient uptake of the macrocycles (Table 2). The largest uptake was that of the [16]-member macrocycle **20**; its intracellular levels were already high after a 3 day incubation. The [20]-member macrocycle **8**, while having a slower initial uptake, reached high intracellular levels after 5 days of incubation. Similar results were obtained for the smallest [13]-member macrocycle **17**. The [17]-member macrocycle **1** reached high intracellular concentrations only if incubated with 2 μM. The uptake of the [18]-member macrocycle **13** was favored when using the lower concentration (0.5 μM) rather than the higher one (2 μM) (Table 2). The mechanism of transport of the macrocyclic polyamines into the DuPro cells was not explored further.

Table 2. Polyamine Levels in DuPro Cells Treated with Macrocyclic Polyamines

macrocyclic polyamines used	concn (μM)	treatment time (days)	polyamines (nmol/10^6 cells)			
			Put	Spd	Sm	macrocycle
control		3	0.832	1.579	2.484	
		5	0.314	0.385	1.067	
1	1	3	ND	ND	ND	2.79
		5	ND	ND	ND	17.20
	2	3	ND	ND	ND	25.07
		5	ND	ND	ND	21.47
20	0.5	3	ND	ND	0.003	47.57
		5	ND	ND	ND	35.91
	2	3	ND	ND	ND	49.07
		5	ND	ND	ND	42.34
13	0.5	3	ND	ND	ND	26.96
		5	ND	0.005	ND	26.84
	2	3	ND	ND	ND	17.20
		5	ND	ND	ND	10.24
8	1	3	0.004	0.029	0.088	10.09
		5	ND	0.014	0.043	46.77
	2	3	0.020	0.027	0.094	5.99
		5	0.011	0.018	0.053	49.31
17	1	3	ND	ND	ND	17.33
		5	ND	ND	ND	24.75
	4	3	ND	ND	ND	22.10
		5	ND	ND	ND	32.00

Conclusions

Five macrocyclic polyamines structurally related to the budmunchiamine alkaloids were prepared by total synthesis. All of them had pronounced inhibitory effects (ID_{50} = 0.5−1.8 μM) on the growth of two human prostate cancer cell lines, DuPro and PC-3 (Table 1 and Figure 2). They were found to hydrolyze ATP in a chemical reaction, with release of inorganic phosphate. The most efficient ATP-ase mimic was the [20]-N_5 macrocycle **8** and the least efficient was the [13]-N_3 macrocycle **17**, while macrocycles [16]-N_4 **20** and [17]-N_4 **1** were efficient catalysts of ATP hydrolysis and macrocycle [18]-N_4 **13** was less so. A linear polyamine, such as spermine, did not catalyze ATP hydrolysis (Figure 1). More interesting, from the biological standpoint, the macrocyclic polyamines also depleted the intracellular pools of ATP in DuPro cells. At a shorter incubation time (24 h), the [13]-N_3 macrocycle **17** was not very efficient in depleting ATP intracellular levels (Figure 4A), as could be expected from the poor ATP-ase activity it exhibited in the ATP chemical hydrolysis experiment. The other macrocycles exhibited good ATP-ase activities in vivo after a 24 h incubation time. The effect was more pronounced after a 72 h incubation (Figure 4B), when even **17** reduced ATP levels by 2 orders of magnitude. The most efficient macrocycles in depleting cellular ATP were **20**, **1**, and **8**; they were also efficiently taken up by the cells (Table 2). The macrocycles were cytotoxic to DuPro cells. After incubating DuPro cells for 24 h, both **20** and **1**, at 2 μM, inhibited colony formation by 2 log, while **17**, **8**, and **13** had smaller effects (Figure 3A). These results could be roughly correlated with the ATP-ase activity of these macrocycles in the cells; at 2−4 μM and after 72 h incubation, both **20** and **1** had depleted ATP by more than 2 orders of magnitude, while **17**, **8**, and **13** had not yet depleted ATP levels to that point. At longer incubation times (144 h), the cytotoxic effect of the polyamine macrocycles increased; however, differences remained among them that could be attributed to their efficacy in catalyzing ATP hydrolysis. The [13]-N_3 cycle **17** had the lowest cytotoxicity, requiring higher concentrations (8 μM) to achieve a 5 log inhibition of cell growth (Figure 3B). The efficent ATP-ase mimics **20** and **1** killed the cells at 4 and 6 μM; while **8**, which was less efficient in reducing ATP levels, killed the cells at 4 μM and after 144 h. (Figure 4B). In the latter case, the significant uptake of **8** by the cells undoubtedly favored its cytotoxic effect (Table 2). All five of the polyamine macrocycles strongly depleted intracellular polyamine pools in DuPro cells (Table 2), hence, this mechanism cannot be the rational behind the differential inhibitions found for the macrocycles. On the other hand, the experimental data suggests that the depletion of the cellular ATP levels produced by the macrocycles could be correlated with their cytotoxic properties. The macrocyclic polyamines therefore represent a new and promising class of cytotoxic agents; by disposing of cellular ATP, they destroy a metabolite that is a scarce commodity in cancer cells.

Experimental Part

General. Unless otherwise stated, all organic solvents were distilled prior to use. Ethanol, benzene, acetonitrile, methylene chloride, and ethyl ether were dried over molecular sieves. All reactions were carried out under an Ar atmosphere. Solutions of salts and acids for workup procedures were prepared in deionized water. Extracts were dried (Na_2SO_4) and evaporated in vacuo. Column chromatography was performed on Merck silica gel 60 (40−63 μm), TLC was carried out using precoated silica plates (Merck 60 F_{254}), spot detection was carried out using UV at 254 nm, Fluram (Fluka AG, Buchs, Switzerland), or Schlittler reagent.[20] IR spectra ($CHCl_3$) were carried out on a Perkin-Elmer 781 or Perkin-Elmer 297; data are in cm^{-1}. ^1H NMR spectra were obtained at 300 MHz in $CDCl_3$ (Bruker AC-300 or Bruker ARX-300); δ are in ppm relative to $CHCl_3$ (=7.26 ppm), and J are in hertz. ^{13}C NMR spectra were obtained at 75.6 MHz in $CDCl_3$; Bruker ARX-300; δ are in ppm relative to $CDCl_3$ (=77.0 ppm); multiplicities are from DEPT experiments. CI-MS spectra were collected with ammonia as the reactant gas; EI-MS spectra were obtained with a Finnigan MAT 90 or Finnigan SSQ 700 at 70 eV; ESI-MS spectra were obtained on a Finnigan TSQ 700; data are in m/z (rel %, where appropriate).

1-(4-Azaheptanenitrilo)-3-propionitrilohexahydropyrimidine (3). To a solution of 1-(3-aminopropyl)hexahydropyrimidine (**2**, 30 g, 0.21 mol) in 185 mL of ethanol was added acrylonitrile (27.9 mL, 0.42 mol) under Ar at 0 °C. The mixture was stirred for 24 h at room temperature. After evaporation, **3** (54.73 g, 0.22 mol) was isolated in almost quantitative yield. ^1H NMR ($CDCl_3$): 3.22 (s, br, 2 H, NCH_2N), 2.92−2.87 (m, 2 H), 2.78−2.70 (m, 2 H), 2.69−2.62 (m, 4 H), 2.60−2.42 (m, 6 H), 2.41−2.38 (m, 2 H), 1.74−1.60 (m, 6 H). ^{13}C NMR ($CDCl_3$): 118.8, 118.7 (2s, 2 CN), 75.1 (t, NCH_2N), 52.8, 52.2, 51.7, 49.8, 47.5, 44.9, 27.0, 22.5, 18.4, 16.5 (10 t, 10 CH_2). ESI-MS: 249 ([M + H]$^+$).

***N*(1),*N*(9)-Bis(4-azahexanenitrilo)amine (4).** A solution of **3** (54.73 g, 0.22 mol) in 50 mL of water was treated with concentrated hydrochloric acid to pH = 1. The mixture was stirred in the presence of hydroxylamine hydrochloride (18.3 g, 0.26 mol) for 3 h at 55 °C. A second portion of 9 g of hydroxylamine hydrochloride was added and the mixture stirred until completion of the reaction. After cooling to room temperature, **4** crystallized as its trihydrochloride (41 g, 0.17 mol, 54%). ^1H NMR ($CDCl_3$): 2.91 (t, J = 6.6, 4 H), 2.71 (t, J = 6.8, 4 H), 2.69 (t, J = 6.8, 4 H), 2.52 (t, J = 6.6, 4 H), 2.01 (s, br, 3 NH), 1.73−1.64 (m, 4 H). ^{13}C NMR ($CDCl_3$): 118.7 (s, 2 CN), 48.2, 47.6, 44.9, 29.7, 18.5 (5t, 10 CH_2). ESI-MS: 238 ([M + H]$^+$).

4,8,12-Triaza-1,15-diaminopentadecane (5, Caldopentamine). Concentrated aqueous hydrochloric acid (13 mL) and 400 mg of PtO_2 were added to **4** (15 g, 0.043 mol) dissolved in

a mixture of 120 mL of water and 75 mL of ethanol. The mixture was hydrogenated in a Parr apparatus at 55 psi in the presence of H_2 for 24 h at room temperature. After neutralization with a solution of potassium hydroxide and evaporation, the residue was dissolved in a minimum amount of water, saturated with Na_2CO_3, and extracted with chloroform/ethanol. After evaporation, the oily **7** (9.85 g, 0.04 mol, 93%) was obtained. ^{1}H NMR ($CDCl_3$): 2.85−2.78 (m, 4 H, CH_2NH$_2$), 2.76−2.64 (m, 12 H, CH_2NH), 1.71−1.58 (m, 8 H, CH$_2$CH_2-CH$_2$), 1.43 (s, br, 7 H, NH). ^{13}C NMR ($CDCl_3$): 48.6 (2t, NCH$_2$), 48.0 (t, NCH$_2$), 40.6 (t, 2 NCH$_2$), 33 .9, 30.4 (2t, 4 CH$_2$CH$_2$-CH$_2$). ESI-MS: 246 ([M + H]$^+$). A sample of the free base **5** was evaporated with hydrochloric acid to give the pentahydrochloride after crystallization from ethanol/water. ^{1}H NMR (D_2O): 3.15−2.95 (m, 16 H), 2.15−1.85 (m, 8 H). ^{13}C NMR (D_2O): 44.7, 44.6, 44.4, 36.6, 23.7, 22.6 (6t, 6 CH$_2$).

Ethyl 19-Amino-3-tridecyl-4,8,12,16-tetraazanonadecanoate (7). A mixture of **5** (9.85 g, 0.04 mol) and ester **6** (11.34 g, 0.04 mol) in ethanol (1500 mL) was stirred for 4 days at 40 °C. After evaporation, the residue was purified by column chromatography (chloroform/methanol/25% ammonium hydroxide, 7:3:1) to give 2 g of **7** (3.80 mmol, 9.5%). ^{1}H NMR ($CDCl_3$): 4.18−4.15 (q, 2 H, OCH_2CH$_3$), 3.19−3.08 (m, 1 H, C*H*NH), 3.05−2.67 (m, 12 H, CH_2NH), 2.52−2.42 (m, 2 H, CH$_2$-COO), 2.10−1.84 (m, 6 H, CH$_2$CH_2CH$_2$), 1.65−1.10 (m, 27 H), 0.95−0.85 (m, 3 H, CH$_2$CH_3). ^{13}C NMR ($CDCl_3$): 172.5 (s, CO), 60.3 (t, OCH$_2$CH$_3$), 54.9 (d, *C*HNH), 47.7, 47.0, 44.8, 39.2, 38.6, 38.5, 34.0, 31.8, 29.6, 29.5, 29.2, 27.6, 27.2, 26.0, 25.8, 22.5 (16t, 22 CH$_2$), 14.1, 14.0 (2d, 2 CH$_3$). ESI-MS: 528 ([M + H]$^+$).

4-Tridecyl-1,5,9,13,17-pentaazacycloeicosan-2-one (8). A dry 250 mL round-bottom flask fitted with a stirbar and a 50 mL pressure-equalized dropping funnel (containing a glass cotton plug and ca. 10 g of 4 Å molecular sieves and functioning as a extractor) surmounted by a reflux condenser was loaded with **7** (2.9 g, 5.50 mmol) and dry benzene (327 mL). An Ar atmosphere was secured and the solution brought to reflux. After 1 h, it was cooled to 22 °C and antimony(III) ethoxide (1.5 mL, 8.25 mmol, in 12 mL benzene) was added. The reaction mixture was stirred for 16 h under reflux. After cooling to 5 °C, it was quenched with methanol and evaporated to dryness. Purification by column chromatography (chloroform/methanol/25% ammonium hydroxide, 7:3:1) gave **8** (1.9 g, 3.96 mmol, 72%). ^{1}H NMR ($CDCl_3$): 8.40 (s br, CONH), 3.45−3.20 (m, 2 H, CONHCH_2), 2.88−2.65 (m, 12 H, CH_2NH), 2.40−2.30 (m, 1 H, C*H*NH), 2.28−2.10 (m, 4 H, CH_2CO), 1.80−1.65 (m, 6 H, CH$_2$CH_2CH$_2$), 1.55−1.15 (m, 24 H, CH$_2$), 0.95−0.82 (m, 3 H, CH$_3$). ^{13}C NMR: 172.0 (s, CO), 55.0 (d, *C*HNH), 49.4, 49.1, 48.9, 48.6, 48.0, 44.4, 39.5, 37.3, 33.8, 31.8, 30.5, 29.6, 29.5, 29.2, 28.9, 28.7, 25.6, 22.6 (18t, 25 CH$_2$), 14.0 (d, CH$_3$). ESI-MS: 482 ([M + H]$^+$).

***N,N′*-Bis(3-propionitrilo)-1,5-diaminopentane (10).** A solution of acrylonitrile (5.45 g, 102.7 mmol) in 50 mL of methanol was added dropwise during 1 h to a solution of 1,5-diaminopentane (5 g, 48.9 mmol) in 50 mL of methanol. The mixture was stirred at 22 °C for 2 h and evaporated. The residue was purified by column chromatography on silica gel (chloroform/methanol/25% ammonium hydroxide, 85:14:1) to give **10** (8.34 g, 40.1 mmol, 82%) as a colorless oil. IR (film): 3310br, 2920s, 2850s, 2250s, 1650w, 1470m, 1420m, 1360m, 1130s, 740m. ^{1}H NMR ($CDCl_3$): 2.95−2.90 (m, 4H, CH$_2$N), 2.66−2.61 (m, 4 H, CH$_2$N), 2.54−2.50 (m, 4 H, CH$_2$CH_2CH$_2$), 1.43−1.38 (m, 6 H, CH$_2$CH_2CH$_2$), 1.23 (s, br, 2 H, NH). ^{13}C NMR ($CDCl_3$): 118.7 (s, CN), 49.05, 49.11, 29.9, 26.9, 24.8 (5t, 10 CH$_2$), 18.8 (1t, 2 CH$_2$). CI-MS: 209 ([M + H]$^+$).

***N,N′*-Bis(3-aminopropyl)-1,5-diaminopentane (11).** A solution containing **10** (7 g, 33.7 mmol) and freshly prepared Raney Ni (approximately 5 g) in 100 mL of absolute ethanol that was previously saturated with ammonia was shaken under hydrogen under a pressure of 3.87 kg/cm^3 (55 psi) on a Parr hydrogenator. After the pressure remained constant during 0.5 h, the suspension was filtered through Celite, the residue was washed with ethanol, and the pooled filtrates were evaporated under vacuum. Pure **11** (4.95 g, 22.9 mmol, 68%) was obtained, and it solidified to a white solid on cooling. IR ($CHCl_3$): 2930s, 2500w, 1580w, 1450m, 1370w, 1260w, 1115m, 1050w. ^{1}H NMR ($CDCl_3$): 2.79−2.74 (m, 4 H, CH$_2$NH), 2.69−2.64 (m, 4 H, CH$_2$NH), 2.63−2.58 (m, 4 H, CH$_2$NH), 1.66−1.61 (m, 4 H, CH$_2$CH_2CH$_2$), 1.58−1.53 (m, 4 H, CH$_2$CH_2CH$_2$), 1.27 (s, br, 6 H, NH). ^{13}C NMR ($CDCl_3$): 50.1, 48.0, 40.7 (3t, 6 CH$_2$N), 34.0, 30.1, 25.2 (3t, 5 CH$_2$CH_2CH$_2$). CI-MS: 217 ([M + H]$^+$).

4-Tridecyl-1,4,8,14-tetraazacyclooctadecan-2-one (13) via Ethyl 17-Amino-3-tridecyl-4,8,14-triazaheptadecanoate (12). The synthesis was carried out by condensation of polyamine **11** and ethyl 2-hexadecenoate **6** to give **12**, followed by cyclization of **12** to **13** trihydrochloride as described above for the preparation of compounds **7** and **8** . Trihydrochloride **13** is a colorless solid. ^{1}H NMR (D_2O): 4.78 (s, br, CHN), 3.55−3.10 (m, 12 H, CH$_2$N), 2.97, 2.87 (AB of ABX, J_{AB} = 16, J_{AX} = 1.0, J_{BX} = 7.0, CH$_2$CON), 2.46−2.23 (m, CH), 2.14−1.61 (m, 1 OH), 1.59−1.25 (m, 24 H), 0.95 (t-like m, CH$_3$). ^{13}C NMR (D_2O): 172.8 (s, CON), 55.3 (d, CHN), 45.9, 45.2, 44.0, 43.0, 41.7, 35.7, 33.4, 31.8, 30.9,29.7, 2963, 29.57, 29.3, 29.1, 25.7, 25.2, 23.3, 22.8, 22.5, 21.9, 21.8 (21 t, partially overlapping signals), 13.9 (q, CH$_3$). ESI-MS (acetonitrile): 453 ([M+H]$^+$), 475 ([M + Na]$^+$).

***N*(4)-[(2-Ethoxycarbonyl-1-tridecyl)ethyl]aminobutyl)-hexahydropyrimidine (15).** Ester **6** (2.82 g, 10 mmol) and 1-(4-aminobutyl)hexahydropyrimidine (**14**, 1.57 g, 10 mmol[14]) were dissolved in 400 mL of absolute EtOH and the solution stirred for 4 days at 40 °C. After evaporation, the residue was purified by column chromatography on silica gel (methylene chloride/methanol/25% ammonium hydroxide, 100:10:1) to give **15** (1.21 g (27%)), as a colorless oil. R_f = 0.44 (methylene chloride/methanol/25% ammonium hydroxide, 40:6:1). ^{1}H NMR ($CDCl_3$): 4.13 (q, J = 7.0, OCH$_2$), 3.48 (s, N−CH$_2$−N), 2.92 (m, CH), 2.80 (t, J = 5.6, CH$_2$), 2.59 (m, 2 CH$_2$), 2.41 (d, J = 10.0, CH$_2$CO), 2.25 (m, CH$_2$), 1.70−1.45 (m, 10 H, 4 CH$_2$ + 2 NH), 1.35−1.15 (m, 11 CH$_2$), 0.98 (m, 2 CH$_3$). CI-MS: 440 ([M + H]$^+$).

Methyl 12-Amino-3-tridecyl-4,9-diazadodecanoate (16). A solution of **15** (663 mg, 1.51 mmol) in 50 mL of methanol saturated with dry hydrogen chloride was heated under reflux for 10 h. After evaporation, the residue was dried in a vacuum and converted to the free base by column chromatography on silica gel (methylene chloride/methanol/25% ammonium hydroxide, 70:30:5) to give 517 mg (83%) of **16** as a colorless oil. R_f = 0.60 (chloroform/methanol/25% ammonium hydroxide, 7:3:1). ^{1}H NMR ($CDCl_3$): 3.64 (s, OCH$_3$), 3.38 (s, br, NH), 2.91 (m, CH), 2.74 (m, CH$_2$), 2.70−2.50 (m, 4 CH$_2$), 2.47 (d, J = 6.2, CH$_2$CO), 1.90−1.75 (m, 2 CH$_2$ + NH$_2$), 1.63 (m, CH$_2$), 1.55−1.45 (m, 2 CH$_2$), 1.35−1.15 (m, 9 CH$_2$), 0.88 (t, J = 6.6, CH$_3$). CI-MS: 414 ([M + H]$^+$).

4-Tridecyl-1,5,10-triazacyclotridecan-2-one (17). To a solution of **16** (190 mg, 0.46 mmol) in anhydrous xylene were added B(NMe$_2$)$_3$ (0.09 mL, 75 mg, 0.5 mmol) and NH_4Cl (5 mg). The mixture was heated under reflux in an Ar atmosphere for 15 h; after cooling to room temperature, 5 mL of ethanol was added. After evaporation, the residue was purified by column chromatography on silica gel (methylene chloride/methanol/25% ammonium hydroxide, 70:30:3) to give 88 mg (50%) of **17**as a white solid. R_f = 0.28 (chloroform/methanol/25% ammonium hydroxide, 70:30:5). ^{1}H NMR ($CDCl_3$): 8.56 (br s, CONH), 3.60−3.43 (m, 1 H), 3.30−3.10 (m, 1 H), 2.90−2.42 (m, 3 CH$_2$), 2.41 (dd, J = 15.1, 2.9, 1 H, COCH$_2$), 2.14 (dd, J = 15.1, 9.2, 1 H, COCH$_2$), 1.80−1.10 (m, 15 CH$_2$), 0.87 (t, J = 6.5, CH$_3$). ^{13}C NMR ($CDCl_3$): 172.1 (s, CO), 55.7 (d, CH), 49.4, 48.7, 45.1, 41.0, 39.4, 33.8, 31.9 (7t, 7 CH$_2$), 29.66−29.76 (several t, 7 CH$_2$), 29.6, 29.4, 28.1, 27.7, 26.9, 25.7 (6t, 6 CH$_2$), 14.1 (q, CH$_3$). ESI-MS: 382 ([M + H]$^+$).

Ethyl 15-Amino-3-tridecyl-4,8,12-triazapentadecanoate (19). A solution of commercial (Fluka AG, Buchs, Switzerland) *N,N′*-bis(3-aminopropyl)-1,3-diaminopropane **18** (500 mg, 2.66 mmol) and ethyl 2-hexadecenoate **6** (751 mg, 2.66 mmol) in ethanol (105 mL) was stirred for 4 days at 40 °C. After evaporation, the residue was purified by column chromatography on silica gel (chloroform/methanol/25% ammonium hydroxide, 7:3:1) to give **19** (328 mg, 0.70 mmol, 26%).

IR: 3270br, 1730s, 1600m, 1470m, 1370m, 1260s, 1100m, 1020m, 860m. ^{1}H NMR: 4.18−4.15 (q, 2 H, OCH_2CH$_3$), 3.19−3.08 (m, 1 H, C*H*NH), 3.05−2.67 (m, 12 H, CH_2N), 2.52−2.42 (m, CH$_2$CO), 2.10−1.84 (m, 6 H, CH$_2$CH_2CH$_2$), 1.65−1.10 (m, 32 H), 0.95−0.85 (m, 3 H, CH$_2$CH_3). ^{13}C NMR: 172.5 (s, CO), 60.3 (t, O*C*H$_2$CH$_3$), 54.9 (d, *C*HNH), 47.7, 47.0, 44.8, 39.2, 38.6, 38.5, 34.0, 31.8, 29.6, 29.5, 29.2, 27.6, 27.2, 26.0, 25.8, 22.5 (16t, 22 CH$_2$), 14.1, 14.0 (2q, 2 CH$_3$). ESI-MS: 471 ([M + H]$^+$).

4-Tridecyl-1,5,9,13-tetraazacyclohexadecan-2-one (20). A dry 250 mL round-bottom flask fitted with a stirbar and a 50 mL pressure-equalized dropping funnel (containing a glass cotton plug and ca. 10 g of 4 Å molecular sieves and functioning as a extractor) surmounted by a reflux condenser was loaded with **19** (198 mg, 0.42 mmol) and dry benzene (22 mL). An Ar atmosphere was secured, and the solution was brought to reflux. After 1 h, the solution was cooled to room temperature and antimony(III) ethoxide (0.1 mL, 0.55 mmol, in 1 mL benzene) was added; the mixture was stirred under reflux for a further 16 h. After cooling to 5 °C, it was quenched with methanol and evaporated to dryness. Purification by column chromatography on silica gel (chloroform/methanol/25% ammonium hydroxide, 15:4:1) gave **20** (94 mg, 0.22 mmol, 54% yield) as a colorless oil. IR: 3659br, 1648s, 1550m, 1470m, 1130m. ^{1}H NMR: 8.40 (s br, CONH), 3.75−3.62 (q, 2 H, OCH_2-CH$_3$), 3.41−3.38 (m, 2 H, CONHCH_2), 2.88−2.70 (m, 10 H, CH_2NH), 2.70−2.60 (m, 1 H, C*H*NH), 2.48−2.25 (m, 2 H, CH_2-CO), 1.82−1.60 (m, 6 H, CH$_2$CH_2CH$_2$), 1.57−1.15 (m, 24 H, CH$_2$), 0.93−0.82 (m, 3 H, CH$_3$). ^{13}C NMR: 172.3 (s, CO), 55.7 (d, *C*HNH), 48.3, 47.9, 47.4, 46.2, 44.9, 39.1, 36.1, 33.9, 31.8, 29.5, 29.5, 29.4, 29.2, 28.9, 28.4, 28.2, 25.8, 22.5 (18t, 22 CH$_2$), 14.0 (d, CH$_3$). ESI-MS: 425 (60, [M + H]$^+$), 213 (100, [M + 2H]$^{2+}$).

Materials. DuPro cells were obtained from Prof. M. Eileen Dolan (University of Chicago, Department of Medicine). All other cell lines used in this study were obtained from American Type Cell Culture Collection (Rockville, MD). Tissue culture medium RPMI 1640, penicillin, and streptomycin were obtained from Fisher Scientific (Itasca, IL). Fetal calf serum was obtained from Gemini Bioproducts, Inc (Calabasas, CA). Adenosine 5′-triphosphate (ATP) was obtained from Pharmacia Biotech (Piscataway, NJ). Enlitten total ATP rapid biocontamination kit was obtained from Promega, Madison, WI. The other special chemicals were obtained from Sigma Chemical Co. (St. Louis, MO). All other reagents were of analytical grade. Deionized double distilled water was used in all studies.

ATP Hydrolysis Assay. A Corning 96-well microtiter plate was used; the first two columns were routinely used for generating a standard curve. For the standard curve, 40 μL of 0−70 μM phosphate buffer was used by serially diluting a 1 mM NaH_2PO_4 solution in 1 N HCl. For assessment of ATP hydrolysis, the rest of the microtiter plate was equally divided into two sections to run two macrocycles simultaneously. Each section was divided into an appropriate number of columns to serially dilute each macrocycle from 0 to 5 mM in 20 μL of HCl for a final concentration of 2 N HCl. Each macrocycle concentration was run in quadruplicate. Equal volumes (20 μL) of a 200 μM ATP solution (pH 7.5) were added to each well and the plates were incubated at 37 °C for the specific times. After incubation, 160 μL of the coloring reagent (0.045% Malachite Green in water and 4.2% ammonium molybdate in 4 N HCl (3:1 v/v)) was added, and the plates were incubated at 37 °C for an additional 30 min. The plates were read at 595 nm using an E-max precision microplate reader (Molecular Device, San Jose, CA). A control plate containing macrocycle solutions without ATP was created for each experiment. The control plate readings were subtracted, and all data were normalized for ATP hydrolysis at zero concentration of the macrocyclic polyamines. The average and standard deviations for quadruplicate runs were plotted.

Tissue Culture. The cells (3×10^5) were seeded into 75 cm^2 culture flasks in 15 mL RPMI 1640 with L-glutamine medium supplemented with 10% fetal calf serum and penicillin−streptomycin (100 units/mg). 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. The flasks were incubated in a humidified 95%/5% CO_2 atmosphere. They were then treated with the macrocyclic polyamines. Cells were harvested by treatment for 5 min with STV (saline A, 0.05% trypsine, 0.02% EDTA) at 37 °C. The flasks were rapped on a laboratory bench and pipetted several times, and aliquots of cell suspension were withdrawn and counted using a Coulter particle counter that has been standardized for each cell line using a hematocytometer.

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 two times by resuspending in chilled Dulbecco's isotonic phosphate buffer (pH 7.4) and centrifuged at 1000 rpm at 4 °C. The supernatant was decanted and 250 μL of 2% perchloric acid (v/v) was added to the cell pellet. The cells were then sonicated, and the lysates were kept at 4 °C for at least 1 h. The lysates were centrifuged at 8000*g* for 5 min. The supernatant was removed for analysis and an appropriate volume of the supernatant (50−100 μL) was fluorescence-labeled by derivatizing with dansyl chloride. In an Eppendorf tube, 100 μL of cell lysate was incubated with 100 μL of 50 mM aqueous lithium carbonate at 37 °C. The mixture was further incubated with 100 μL of 10 mg/mL dansyl chloride in acetonitrile at 70 °C for 10 min. The reaction mixture was purified through a Bond elute C-18 octadecyl precolumn (Varian) and eluted with 1 mL of HPLC grade acetonitrile. Labeled polycations were loaded onto a C-18 high-performance liquid chromatography column and separated using a previously published procedure.[21] 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 in 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 atm. From the diluted stock solutions of each macrocycle, 20 μL was added to the middle eight columns of 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 drug for 6 days. Twenty-five microliters of a 5 mg/mL solution of 3-(4,5-dimethylthiazolyl-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to each well. The plates were incubated for 4 h. Cells were then lysed by incubating overnight with 100 μL of lysis buffer [containing 20% (w/v) lauryl sulfate (SDS), 50% (v/v) *N*,*N*-dimethylformamide, and 0.4% (v/v) glacial acetic acid, pH 4.8]. The color developed was read at room temperature at 570 nm in an E-max Precision Microplate Reader (Molecular Devices Corp., Sunnyvale, CA), and data were analyzed using manufacturer-supplied cell survival software.

Cellular ATP Measurement. Approximately 3×10^5 cells were taken from harvested samples and centrifuged at 1500 rpm for 15 min at 4 °C. Cells were washed twice with chilled Dulbecco's isotonic phosphate buffer (pH 7.4, Ca^{2+} and Mg^{2+} free) and were collected by centrifugation at 1500 rpm for 15 min at 4 °C. After the final centrifugation, the supernatant was decanted and the cell pellets were resuspended in 1.5 mL of Enlitten treatment reagent. The first two columns of a 96-well microtiter plate were used for positive and negative control supplied by the manufacturer. The rest of the plate was divided into four equal parts. Each division was used for four different concentrations of a macrocyclic polyamine. An aliquot of 140 μL of cell suspension was dispensed in each column of the plate, so that the cell suspension with increasing concentration of macrocycles progressed from the left to the right of the plate. Treated cells for each compound concentration were run in quadruplicate. Forty microliters of the Extractant solution containing the cell lysis buffer was added and thoroughly mixed. The plate was placed in a luminometer (EG &G Berthold microplate luminometer LB96V, Germany)

that automatically added 40 μL of rL/L (luciferase−luciferin) reagent and read the luminescence after a 5 s mixing dead time.

Colony Forming Efficiency Assay. The cell line used in this assay was previously optimized with respect to the number of feeder cells and lengths of incubation times necessary for observable colony formation. Both floating and attached cells were harvested and centrifuged at 1000 rpm for 10 min at 4 °C. The cell pellets were resuspended and replated in quadruplicate at appropriate dilutions into 60 mm plastic Petri dishes. The Petri dishes were prepared not more than 24 h in advance with 4 mL of supplemented RPMI 1640 with L-glutamine containing 10% fetal bovine serum (standardized for each cell line used). Cells were incubated for the previously standarized number of days in a 95% air/5% CO_2 atmosphere. The plates were stained with 0.125% crystal violet in ethanol and counted. Results were expressed as a surviving fraction of an appropriate control.

Acknowledgment. The investigators at the University of Zuerich are grateful for the support of the University, the Swiss National Science Foundation, the Dr. Helmut Legerlotz-Stiftung, and to Prof. St. Bienz for help. We are also grateful to Ms. Amy C. Harms and Mr. James F. Brown (Madison) and Dr. L. Bigler and N. Bild (Zuerich) for securing the mass spectra and to Mr. Herbert Grimek for performing the HPLC analyses. These assays were performed at the Mass Spectrometry−Bioanalytical Facility of the Biotechnology Center of the University of Wisconsin−Madison.

References

(1) Warburg, O.; Posener, K.; Negelein, E. Über den Stoffwechsel der Carzinomzelle. *Biochem. Z.* **1924**, *152*, 309−354.
(2) Warburg, O. Ist die aerobe Glykolyse spezifisch für die Tumoren? *Biochem. Z.* **1929**, *204*, 482−483.
(3) (a) Eigenbrodt, E.; Glossmann, H. Glycolysis−One of the keys to cancer? *Trends Pharmacol. Sci.* **1980**, *1*, 240−245. (b) Argiles, J. M.; Lopez-Soriano, F. J. Why do cancer cells have such a high glycolytic rate? *Med. Hypotheses* **1990**, *32*, 151−155. (c) Baggetto, L. G. Deviant energetic metabolism of glycolytic cancer cells. *Biochimie* **1992**, *74*, 959−974.
(4) (a) Schwickert, G.; Walenta, S.; Sundfor, K.; Rofstad, E. K.; Mueller-Klieser, W. Correlation of high lactate levels in human cervical cancer with incidence of metastasis. *Cancer Res.* **1995**, *55*, 4757−4759. (b) Walenta, S.; Salameh, A.; Lyng, H.; Evensen, J. F.; Mitze, M.; Rofstad, E. K.; Mueller-Klieser, W. Correlation of high lactate levels in head and neck tumors with incidence of metastasis. *Am. J. Pathol.* **1997**, *150*, 409−415. (c) Semenza, G. L.; Hypoxia, clonal selection, and the role of HIF-1 in tumor progression. *Crit. Rev. Biochem. Mol. Biol.* **2000**, *35*, 71−103.
(5) Brand, K. A.; Hermfisse, U. Aerobic glycolysis by proliferating cells: A protective strategy against reactive oxygen species. *FASEB J.* **1997**, *11*, 388−395.
(6) (a) Vaupel, P.; Kallinowski, F.; Okunieff, P. Blood flow, oxygen and nutrient supply, and metabolic microenvironment of human tumors: A review. *Cancer Res.* **1989**, *49*, 6449−6465. (b) Kallinovski, F.; Schlenger, K. H.; Runkel, S.; Kloes, M.; Stohrer, M.; Okunieff, P.; Vaupel, P. Blood flow, metabolism, cellular microenvironment, and growth rate of human tumor xenografts. *Cancer Res.* **1989**, *49*, 3759−3764. (c) Vaupel, P.; Schaefer, C.; Okunieff, P. Intracellular acidosis in murine fibrosarcomas coincides with ATP depletion, hypoxia, and high levels of lactate and total P_i. *NMR Biomed.* **1994**, *7*, 128−136.
(7) Hoeckel, M.; Vaupel, P. Tumor hypoxia: Definitions and current clinical, biologic, and molecular aspects. Review. *J. Natl. Cancer Inst.* **2001**, *93*, 266−276.
(8) (a) Lu, X.; Errington, J.; Chen, V. J.; Curtin, N. J.; Boddy, A. V.; Newell, D. R. Cellular ATP depletion by LY 309887 as predictor of growth inhibition in human tumor cell lines. *Clin. Cancer Res.* **2000**, *5*, 271−277.(b) Batrakova, E. V.; Li, S.; Elmquist, W. F.; Miller, D. W.; Alakhov, V. Y.; Kabanov, A. V. Mechanism of sensitization of MDR cancer cells by pluronic block copolymers: Selective energy depletion. *Br. J. Cancer* **2001**, *85(12)*, 1987−1997.
(9) Dietrich, B.; Hosseini, M. W.; Lehn, J.-M.; Sessions, R. B. Anion receptor molecules. Synthesis and anion-binding properties of polyammonium macrocycles. *J. Am. Chem. Soc.* **1981**, *103*, 1282−1283.
(10) (a) Hosseini, M. W.; Lehn, J.-M.; Maggiora, L.; Mertes, K. B.; Mertes, M. P. Supramolecular catalysis in the hydrolysis of ATP facilitated by macrocyclic polyamines: Mechanistic studies. *J. Am. Chem. Soc.* **1987**, *109*, 537−544. (b) Mertes, M. P.; Mertes, K. B. Polyammonium macrocycles as catalysts for phosphoryl transfer: The evolution of an enzyme mimic. *Acc. Chem. Res.* **1990**, *23*, 413−418. (c) Hosseini, M. W.; Lehn, J.-M.; Mertes, M. P. Efficient molecular catalysis of ATP-hydrolysis by protonated macrocyclic polyamines. *Helv. Chim. Acta*, **1983**, *66*, 2454−2466.
(11) (a) Bencini, A.; Bianchi, A.; Garcia-Espana, E.; Scott, E. C.; Morales, L.; Wang, B.; Deffo, T.; Takusagawa, F.; Mertes, M. P.; Mertes, K. B.; Paoletti, P. Potential ATPase mimics by polyammonium macrocycles: Criteria for catalytic activity. *Bioorganic Chem.* **1992**, *20*, 8−29. (b) Prakash, T. P.; Pattuparanbil, R.; Ganesh, K. W. Macrocyclic polyamines [16]-N_3 and [21]-N_4: Synthesis and study of their ATP complexation by ^{31}P nuclear magnetic resonance spectroscopy. *J. Chem. Soc., Perkin Trans. 1*, **1991**, 1273−1278.
(12) Mar, W.; Tan, G. T.; Cordell, G. A.; Pezzuto, J. M.; Jurcic, K.; Offerman, F.; Redp, K.; Steinke, B.; Wagner, H. Biological activity of novel macrocyclic alkaloids (budmunchiamines) from *Albizia amara* detected on the basis of interaction with DNA. *J. Nat. Prod.* **1991**, *54*, 1531−1542.
(13) (a)Popaj, K.; Hesse, M. Synthesis of the macrocyclic spermine alkaloids budmunchiamines A−C. *Helv. Chim. Acta*, **2001**, *84*, 180−186. (b) Li, Y.; Hesse, M.; The synthesis of cyclic spermine alkaloids: Analogues of buchnerine and budmunchiamine C. *Helv. Chim. Acta* **2003**, *86*, 310−323.
(14) Niitsu, M.; Samejima, K. Syntheses of a series of linear pentaamines with three and four methylene chain intervals. *Chem. Pharm. Bull. Jpn.* **1986**, *34(3)* 1032−1038.
(15) Osvath, P.; Curtis, N. F.; Weatherburn, D. C. Copper(II) and Nickel(II) complexes of pentaaza macrocyclic ligands. *Aust. J. Chem.* **1987**, *40*, 347−360.
(16) McManis, J. S.; Ganem, B. Chemistry of naturally occurring polyamines. 1. Total synthesis of celacinnine, celabenzine, and maytenine. *J. Org. Chem.* **1980**, *45*, 2041−2042.
(17) (a) Ishihara, K.; Kuroki, Y.; Hanaki, N.; Ohara, S.; Yamamoto, H. Antimony-templated macrolactamization of tetraamino esters. Facile synthesis of macrocyclic spermine alkaloids, buchnerine, verbacine, verbaskine, and verbascenine. *J. Am. Chem. Soc.* **1996**, *118*, 1569−1570. (b) Kuroki, Y.; Ishihara, K.; Hanaki, N.; Ohara, S.; Yamamoto, H. Metal-templated macrolactamization of triamino and tetramino esters. Facile synthesis of macrocyclic spermidine and spermine alkaloids, (S)-(+)-dihydroperiphylline, (±)-buchnerine, (±)-verbacine, (±)-verbaskine, and (±)- verbascenine. *Bull. Chem. Soc. Jpn.* **1998**, *71*, 1221−1230.
(18) Frydman, B.; Blokhin, A. V.; Brummel, S.; Wilding, G.; Maxuitenko, Y.; Sarkar, A.; Bhattacharya, S.; Church, D.; Reddy, V. K.; Kink, J. A.; Marton, L. J.; Valasinas, A.; Basu, H. S. Cyclopropane-containing polyamine analogues are efficient growth inhibitors of a human prostate tumor xenograft in nude mice. *J. Med. Chem.* **2003**, *46*, 4586−4600; and references therein.
(19) Dolan, M. E.; Fleig, M. J.; Feuerstein, B. G.; Basu, H. S.; Luk, G. D.; Casero, R. A., Jr.; Marton, L. J. Effect of 1,19-bis-(ethylamino)-5-10-15-triazanonadecane on human tumor xenografts. *Cancer Res* **1994**, *54*, 4698−4702.
(20) Schlittler, E.; Hohl, J. Über die alkaloide aus Strychnos melinoniana Baillon. *Helv. Chim. Acta* **1952**, *35*, 29−42.
(21) Kabra, P. M.; Lee, H. K.; Lubich, W. P.; Marton, L. J. Solid-phase extraction and determination of dansyl derivatives of unconjugated and acetylated polyamines by reversed-phased liquid chromathography. *J. Chromatogr. Biomed. Appl.* **1986**, *380*, 19−32.

JM030437S