Synthesis and Evaluation of Unsymmetrically Substituted Polyamine Analogues as Modulators of Human Spermidine/Spermine-N1-Acetyltransferase (SSAT) and as Potential Antitumor Agents[1]

Nada H. Saab,[†] Edward E. West,[†] Nella C. Bieszk,[†] Charles V. Preuss,[†] Amy R. Mank,[‡] Robert A. Casero, Jr.,[‡] and Patrick M. Woster*,[†]

Department of Pharmaceutical Sciences, College of Pharmacy and Allied Health Professions, Wayne State University, Detroit, Michigan 48202, and The Johns Hopkins Oncology Center, Johns Hopkins University, Baltimore, Maryland 21231

Received May 14, 1993[*]

Spermidine/spermine-N1-acetyltransferase (SSAT), the rate-limiting step in polyamine catabolism, is critical for the interconversion and modulation of cellular polyamines. Inhibitor-initiated induction of this enzyme also appears to correlate with the sensitivity of tumor cells to a class of novel polyamine analogues, the bis(ethyl)polyamines. Thus, terminally alkylated polyamines which modulate the cellular level of SSAT could be of great value for understanding the role of this enzyme both in analogue-mediated cytotoxicity and in overall cellular polyamine metabolism. Such analogues could also become important therapeutic agents by disrupting cellular polyamine metabolism. The structure–activity relationships defining the interaction of polyamine analogues with SSAT have not been fully elucidated, and, in particular, unsymmetrically alkylated polyamines have not been synthesized and evaluated as modulators of SSAT. To this end, we now report the synthesis and preliminary biological evaluation of N1-ethyl-N11-propargyl-4,8-diazaundecane and N1-ethyl-N11-((cyclopropyl)methyl)-4,8-diazaundecane *via* a synthetic pathway which represents an efficient route to a variety of unsymmetrically substituted polyamine analogues. The title compounds act as effective inhibitors of isolated human SSAT and produce a differential superinduction of SSAT *in situ* which appears to be associated with a cell specific cytotoxic response in two human lung cancer cell lines. In so doing, these analogues exhibit promising antitumor activity against cultured human lung cancer cells.

Introduction

The enzymes involved in the polyamine metabolic pathway have been the subject of intensive study, and a number of specific inhibitors for these enzymes have been designed as potential antitumor or antiparasitic agents.[2] Despite this effort, only one of these inhibitors, α-difluoromethylornithine, has become a clinically useful agent.[3] To date, most of the studies involving inhibitors of polyamine metabolism have focused on the enzymes involved in the biosynthetic pathway. Recently, however, there has been considerable interest generated in the enzyme spermidine/spermine-N1-acetyltransferase (SSAT),[4] the rate-limiting step in the back conversion of polyamines. SSAT, in conjunction with polyamine oxidase (PAO), allows for reversal of the biosynthetic pathway and attenuation of the levels of individual polyamines.

The induction of SSAT in two human lung cancer cell lines which respond differently to treatment with inhibitors of polyamine biosynthesis has recently been studied.[5] In these cell lines, the level of induction of SSAT appears to correlate inversely with the degree of resistance to cytotoxicity following treatment with the polyamine analogue N1,N12-bis(ethyl)spermine (BESpm, Figure 1).[5] Rate of growth and cellular polyamine content in the human small cell lung carcinoma (SCLC) line NCI H82 are minimally affected by BESpm, which appears to down regulate polyamine biosynthesis by the same mechanism as the natural polyamines.[6] By contrast, BESpm was found to be markedly cytotoxic at a concentration of 10 μM in the large cell lung carcinoma (LCLC) line NCI H157, accom-
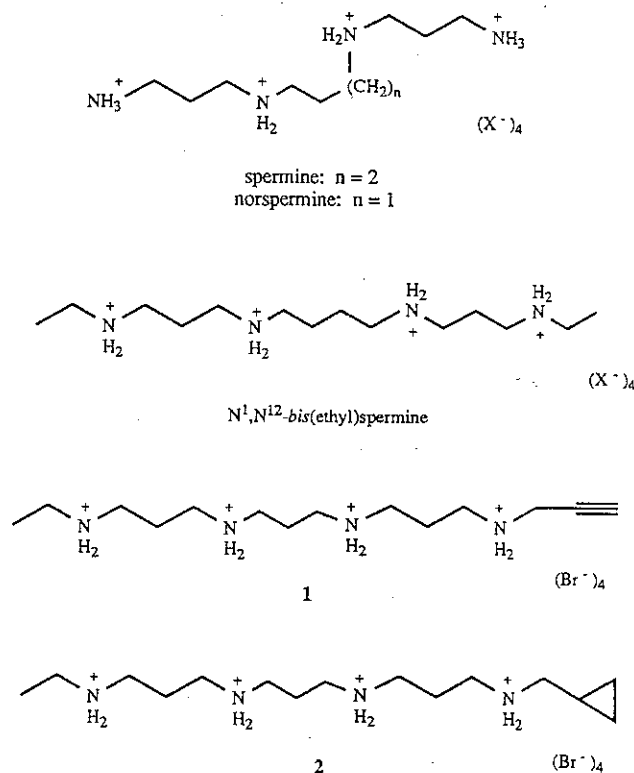

Figure 1. Structures of spermine, norspermine, N1,N12-bis(ethyl)spermine, and analogues **1** and **2**.
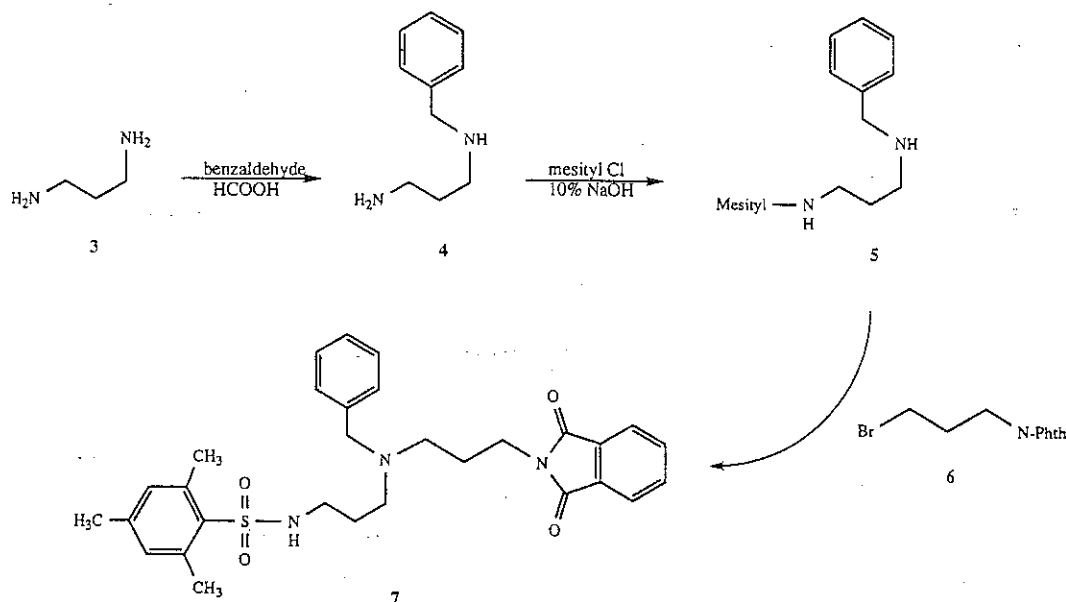
panied by nearly complete depletion of all intracellular polyamines and a decrease in ornithine decarboxylase (ODC) activity to undetectable levels. In the responsive LCLC line, BESpm was found to induce SSAT in a time- and dose-dependent manner to maximum levels greater

Scheme I

than 1000-fold above baseline. Conversely, in the unresponsive SCLC cell line, minimal (less than 7-fold) induction of SSAT was observed, regardless of the time period or the concentration of BESpm employed. These results suggest that the differential induction of SSAT may play a role in determining cell specific sensitivity to polyamine antimetabolites. Additional inhibitors of SSAT would be of great value in the study of this phenomenon.

The structural requirements for interaction with the SSAT active site have not been fully investigated, but some structure/affinity data are available.[7] The natural substrates for the enzyme, spermidine, and spermine, have K_m values of 130 and 34 μM, while *sym*-norspermidine and *sym*-norspermine exhibit K_m values of 9 and 10 μM, respectively. N^1-Acetylspermine is also a substrate (K_m = 51 μM), while N^1-acetylspermidine is not, since it does not possess the requisite free aminopropyl moiety. It has been suggested that bis-monoalkylation of both terminal nitrogens of spermidine or spermine is optimal for antiproliferative activity and that alkylation at an internal nitrogen reduces *in vitro* activity.[8] Elongation of the central alkyl chain of spermine or bis-alkylated spermine to C-8 produces analogues which have dramatic effects on *in vitro*[9] and *in vivo*[10] tumor cell growth, levels of intracellular polyamines,[9,11] and DNA conformation and aggregation.[9] The terminal bis(benzyl)-substituted C-8 spermine homologues have been shown to down regulate ornithine decarboxylase and S-adenosylmethionine decarboxylase in rat hepatoma cells, resulting in depletion of cellular polyamines and complete abolition of cell division.[11] However, this effect appears to be related to metabolism of the bis(benzyl) derivative to the corresponding bis-primary amine. The bis(ethyl)polyamines, and in particular bis(ethyl)norspermine, have excellent affinity for SSAT and serve as competitive inhibitors but can dramatically induce the enzyme to levels that are as much as 2000% above baseline.[5] This effect, combined with the down regulation of polyamine biosynthesis by bis(ethyl)polyamine analogues,[6,12] leads to a depletion of cellular polyamines and cytotoxicity in some cell lines. In recent studies, the bis(ethyl)polyamines have shown great promise *in vitro* and *in vivo* as antitumor agents.[13–16] To date, polyamine analogues with unsymmetrical bis(alkyl)

substitutions have not been synthesized and evaluated as inhibitors or inducers of SSAT or as antitumor agents. Such analogues, if active, would be of great value in terms of elucidating additional structure–activity relationships for optimal interaction with SSAT and could also prove to be effective antitumor agents.

In light of the potential value of terminally alkylated polyamines as therapeutic agents, the unsymmetrically substituted polyamine analogues 1 and 2 (Figure 1) have been proposed. The syntheses described for the symmetrically bis-alkylated polyamine derivatives such as BESpm, which involve as little as three steps,[8] are not adaptable for the production of unsymmetrically substituted polyamine analogues, since these routes depend on simultaneous alkylation of both terminal nitrogens of a protected tri- or tetraamine intermediate. Therefore, the synthetic route to the unsymmetrically alkylated polyamines 1 and 2 (shown in Schemes I and II) has been proposed. This route is intended to be of sufficient efficiency and versatility to allow for the eventual production of a variety of analogues in suitable quantities for biological evaluation. We now report the synthesis of the title compounds 1 and 2 *via* this synthetic route and describe the results of their preliminary biological evaluation.

Chemistry

The synthetic route leading to compounds 1 and 2 is depicted in Schemes I and II. 1,3-Diaminopropane (3) (Scheme I) was treated with benzaldehyde in formic acid using the method of Bergeron[17] to produce the monobenzylated diamine 4, which was then selectively protected at the remaining primary nitrogen using 2-mesitylenesulfonyl chloride[18] to afford the corresponding mesityl analogue 5. Addition of a phthalimidopropyl group was then accomplished using 3-bromopropylphthalimide (6) and lithium iodide in *n*-butyl alcohol, yielding the triprotected analogue 7. Compound 7 represents a versatile synthetic intermediate, since each of the three protecting groups can be removed individually, allowing for the possibility of selective functionalization at any of the three nitrogen atoms.

Scheme II
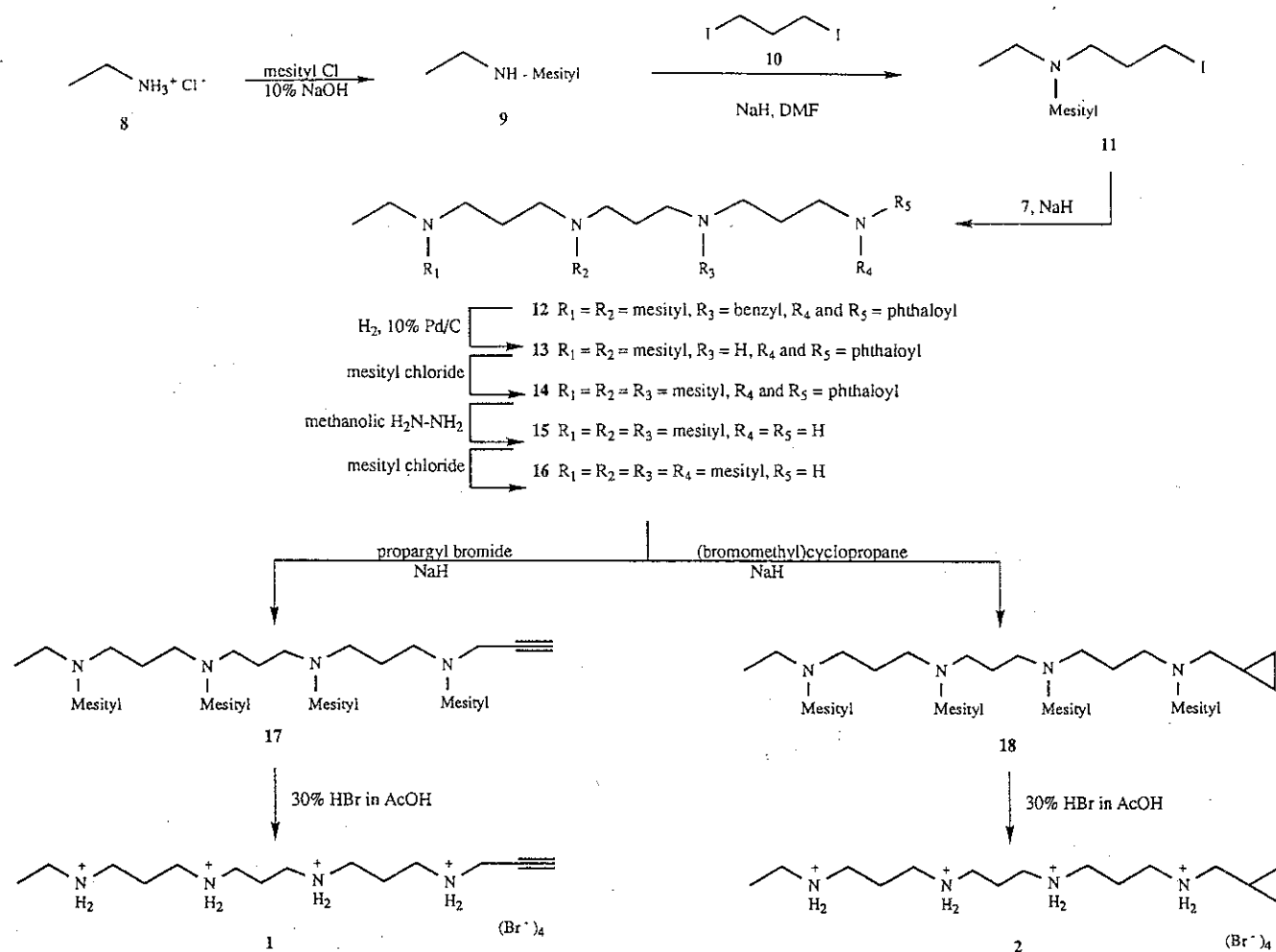
The elaboration of synthon **7** to the desired target molecules **1** and **2** is shown in Scheme II. Ethylamine hydrochloride (**8**) was treated with 2-mesitylenesulfonyl chloride[18] to afford the corresponding mesityl-protected analogue **9**. Treatment of **9** with sodium hydride in dimethylformamide, followed by dropwise addition of 1,3-diiodopropane **10**, then produced the monoalkylated iodide **11**. Analogue **11** was then added to a mixture of synthon **7** and sodium hydride to provide the fully protected tetramine **12**.

In order to reduce the number of deprotection steps necessary to reach the final compound, it became necessary to convert the *N*-benzyl and *N*-phthaloyl protected nitrogens to their corresponding *N*-mesityl moieties. Our attempts to sequentially remove the benzyl and phthalimide protecting groups, followed by dimesitylation in a single step, were unsuccessful, since the yield for the dimesitylation was low, and because attempts to remove the phthalimide in the presence of the debenzylated secondary amine resulted in the formation of undesired side products. Therefore, these transformations were accomplished in a stepwise fashion beginning with intermediate **12**. The benzyl protecting group was removed by hydrogenolysis[19] (H₂, 10% Pd/C) to yield the free secondary amine analogue **13**, which was reprotected by treatment with 2-mesitylenesulfonyl chloride[18] to afford intermediate **14**. Hydrazinolysis of the phthalimide[20] (methanolic NH₂NH₂) then produced primary amine **15**, which was reprotected as described above to yield the tetramesitylated compound **16**. The presence of an acidic

hydrogen at this terminal mesitylated nitrogen provides a means for facile selective functionalization at this position. Thus, deprotonation of **16** in DMF in the presence of sodium hydride, followed by the addition of either propargyl bromide or (bromomethyl)cyclopropane, resulted in the formation of the fully protected, unsymmetrically alkylated intermediates **17** or **18**, which were deprotected in 30% HBr in acetic acid[21] to afford the respective target molecules **1** and **2** as their tetrabromide salts.

Results and Discussion

Compounds **1** and **2** were evaluated as inhibitors of human SSAT using a crude lysate from H157 cells as previously described.[5] Both compounds **1** and **2** were found to be effective inhibitors in this assay system, exhibiting similar potency to the known inhibitor BESpm, as shown in Figure 2. To determine whether analogues **1** and **2** exhibit cytotoxic specificity similar to that previously observed for BESpm, 96-h dose-response experiments were performed on both the H157 LCLC and H82 SCLC cell lines. Preliminary results indicate that compounds **1** and **2** are also selectively cytotoxic. Treatment of H157 cells with either **1** or **2** at levels above 5 μM resulted in cytotoxicity, as shown in Figure 3, with IC$_{50}$ values of 0.79 and 0.57 μM, respectively. Both compounds are more potent against LCLC than the known inhibitor BESpm.[22] By contrast, compounds **1** and **2** are minimally cytostatic in the H82 SCLC cell line, exhibiting IC$_{50}$ values of 4.74 and 0.84 μM, respectively, as shown in Figure 4.
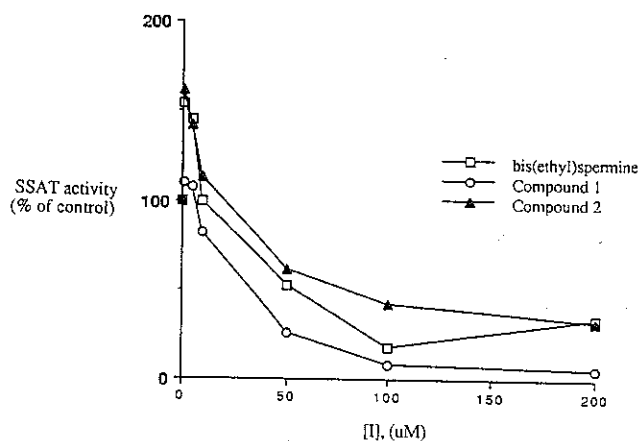
Figure 2. Inhibition of SSAT by BESpm and compounds 1 and 2. Each data point shown represents the average of two determinations.
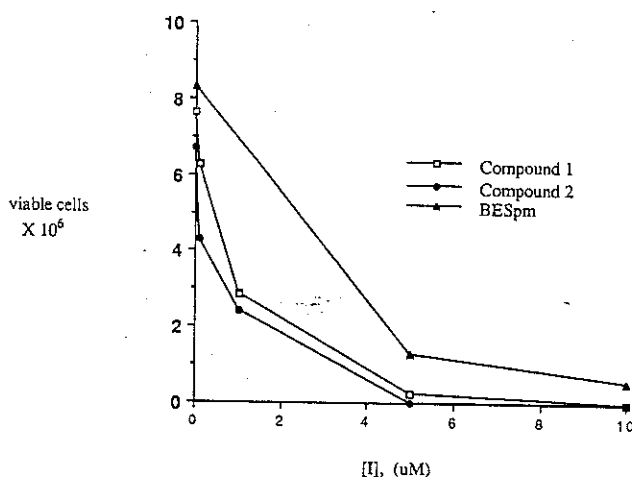

Figure 4. The cytostatic effect of compounds 1 and 2 on H82 small cell lung carcinoma (SCLC) following 96-h treatment.

Figure 3. The cytotoxic effect of BESpm and compounds 1 and 2 on H157 large cell lung carcinoma (LCLC) following 96-h treatment.

Table I. Induction of SSAT Activity in H157 LCLC or H82 SCLC Cells Following Treatment with BESpm, 1 or 2

treatment	cell line	SSAT activity (pmol/min/mg of protein)
none	H157	32.34
10 μM BESpm	H157	38330.08
10 μM 1	H157	19098.68
10 μM 2	H157	42780.40
none	H82	28.08
10 μM BESpm	H82	22.73
10 μM 1	H82	19.81
10 μM 2	H82	47.09

This observation is similar to the results of studies involving BESpm, in which no significant cytotoxicity was observed at concentrations of up to 100 μM in the H82 cell line.[22]

Compounds 1 and 2 were also evaluated *in vitro* for their ability to induce SSAT in both H157 LCLC cells and H82 SCLC cells. These data are outlined in Table I. Cells were exposed to a 10 μM concentration of either BESpm, 1 or 2 for 24 h, after which the cells were lysed and assayed for SSAT activity. Analogues 1 and 2 were found to be highly effective inducers of the human enzyme in the responsive H157 LCLC cell line, producing SSAT levels comparable to or greater than those observed following treatment with BESpm. It is equally important to determine whether compounds 1 and 2 induce SSAT in the unresponsive SCLC cell line, since the specificity of
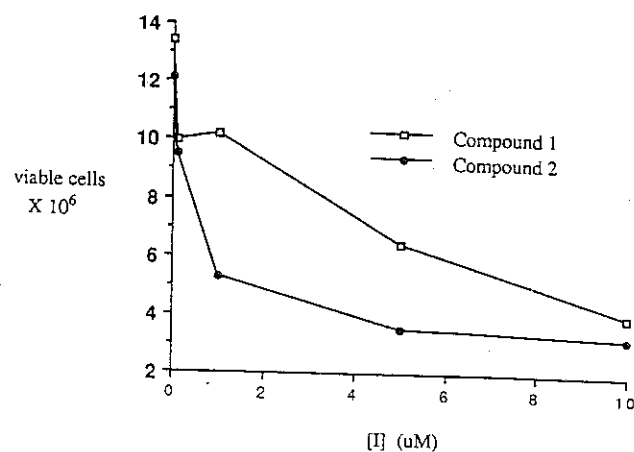
cytotoxicity observed with polyamine analogues such as BESpm has been postulated to be associated with their ability to superinduce SSAT.[23] Neither compound 1 or 2 caused significant induction of SSAT in the treated H82 SCLC cell line, an observation which is consistent with previous studies involving BESpm.[5,22,23] These findings, therefore, support the assertion that there is a positive correlation between analogue-initiated superinduction of SSAT and the development of cytotoxicity. Investigations are currently underway to determine the molecular mechanism responsible for the induction of SSAT by 1 and 2 in the H157 cell line and for the lack of induction in the unresponsive H82 cell line. Other polyamine analogues have demonstrated the ability to increase the steady-state levels of mRNA specific for SSAT, resulting in an increase in SSAT protein.[4,23,24] However, the precise mechanism underlying the induction of the SSAT protein has not been elucidated. Thus, compounds 1 and 2 should provide additional tools to facilitate the understanding of the regulation of SSAT gene expression.

Finally, the synthetic route leading to compounds 1 and 2 is sufficiently versatile and efficient to allow for the synthesis of a wide variety of unsymmetrically substituted polyamine analogues. In our laboratories, the synthesis of additional analogues for use in a structure–activity study is underway, as are additional biological experiments involving 1 and 2. The results of these studies will be reported in a future communication.

Experimental Section

Compound 4, 1-(benzylamino)-3-aminopropane, was synthesized according to the procedure of Bergeron.[17] All other reagents were purchased from Aldrich Chemical Co. (Milwaukee, WI) or Sigma Chemical Co. (St. Louis, MO) and were used without further purification except as noted below. Pyridine was dried by passing it through an aluminum oxide column and then stored over KOH. Triethylamine was distilled from potassium hydroxide and stored in a nitrogen atmosphere. Methanol was distilled from magnesium and iodine under a nitrogen atmosphere and stored over molecular sieves. Methylene chloride was distilled from phosphorus pentoxide, and chloroform was distilled from calcium sulfate. Tetrahydrofuran was purified by distillation from sodium and benzophenone. Dimethylformamide was dried by distillation from anhydrous calcium sulfate and was stored under nitrogen. Preparative scale chromatographic procedures were carried out using E. Merck silica gel 60, 230–440 mesh. Thin-layer chromatography was conducted on Merck precoated silica gel 60 F-254.

All ¹H and ¹³C NMR spectra were recorded on a General Electric QE-300 spectrometer, and all chemical shifts are reported

as δ values referenced to tetramethylsilane (TMS) or sodium 3-(trimethylsilyl)-1-propanesulfonate (DSS). Infrared spectra were recorded on a Nicolet 5DXB FT-IR spectrophotometer and are referenced to polystyrene. In all cases, ^{1}H NMR, ^{13}C NMR, and IR spectra were consistent with assigned structures. Microanalyses were performed by Galbraith Laboratories, Knoxville, TN, and are within 0.4% of calculated values.

1-[[(2-Mesitylene)sulfonyl]amino]-3-(benzylamino)propane (5). A 0.92-g portion of 2-mesitylenesulfonyl chloride (0.0042 mol) in dry dichloromethane (15 mL) was added dropwise with rapid stirring to a solution of 4 (0.70 g, 0.0042 mol) in 60 mL of 10% NaOH at 0 °C, and the mixture was allowed to stir at 0 °C for 90 min. The organic layer was separated, the aqueous layer was further extracted with 15 mL of chloroform, and the combined organic layers were dried over MgSO$_4$, filtered, and concentrated *in vacuo* to afford 1.69 g of a yellow oil. This crude product was purified on silica gel (chloroform/methanol/NH$_4$-OH 900:50:3) to give 1.35 g (92% yield) of the pure product as a yellow oil: ^{1}H NMR (CDCl$_3$) δ 7.20 (m, 5H, benzyl aromatic), 6.94 (s, 2H, mesityl aromatic H-3 and H-5), 3.74 (s, 2H, benzylic CH$_2$), 3.00 (t, 2H, H-1), 2.71 (t, 2H, H-3), 2.64 (s, 6H, mesityl 2-CH$_3$ and 6-CH$_3$), 2.3 (s, 3H, mesityl 4-CH$_3$), 1.66 (q, 2H, H-2); ^{13}C NMR (CDCl$_3$) δ 142.4 (mesityl aromatic C-2 and C-6), 139.3 (mesityl aromatic C-3 and C-5), 139.3 (benzyl aromatic C-1), 131.8 (mesityl aromatic C-4), 128.5 (benzyl aromatic C-2 and C-6), 128.2 (benzyl aromatic C-3 and C-5), 127.2 (benzyl aromatic C-4), 53.9 (benzylic CH$_2$), 48.6 (C-3), 42.9 (C-1), 27.8 (C-2), 22.9 (mesityl 2-CH$_3$ and 6-CH$_3$), 20.9 (mesityl 4-CH$_3$); IR cm^{-1} (KBr) 3296 (sulfonamide NH), 1602 (aromatic), 1461, 1314, 1152 (SO$_2$). Anal. (C$_{19}$H$_{26}$N$_2$O$_2$S), C, H, N.

1-Phthalimido-4-(N-benzyl)-7-[[(2-mesitylene)sulfonyl]-amino]-4-azaheptane (7). A solution of N-(3-bromopropyl)-phthalimide (6) (3.82 g, 0.014 mol) in 7 mL of hot n-butyl alcohol was slowly added to a hot (80 °C) suspension of 5 (2.00 g, 0.0057 mol), Na$_2$CO$_3$ (0.64 g, 0.006 mol), and LiI (0.18 g, 0.0014 mol) in 16 mL of n-butyl alcohol. The reaction mixture was allowed to stir at 80 °C for 24 h, and was then cooled and filtered. The filtrate was evaporated (35 °C at 0.02 mmHg) to give the crude product as yellow oil, which was chromatographed on silica gel (hexane/ethyl acetate, 4:6) to give 2.30 g (75%) of pure 7 as white crystals: ^{1}H NMR (CDCl$_3$) δ 7.82 (m, 2H, phthalimido H-3 and H-6), 7.73 (m, 2H, phthalimido H-4 and H-5), 7.20 (m, 5H, benzyl aromatic), 6.90 (s, 2H, mesityl aromatic H-3 and H-5), 5.82 (s, 1H, sulfonamide NH), 3.63 (t, 2H, H-1), 3.49 (s, 2H, benzylic CH$_2$), 3.00 (t, 2H, H-7), 2.60 (s, 6H, mesityl 2-CH$_3$ and 6-CH$_3$), 2.44 (m, 4H, H-3 and H-5), 2.23 (s, 3H, mesityl 4-CH$_3$), 1.8 (q, 2H, H-2), 1.65 (q, 2H, H-6); ^{13}C NMR (CDCl$_3$) δ 168.3 (phthalimido C=O), 141.8 (mesityl aromatic C-2 and C-6), 139.0 (mesityl aromatic C-3 and C-5), 139.3 (benzyl aromatic C-1), 133.9 (phthalimido C-1 and C-2), 132.1 (phthalimido C-3 and C-6), 131.8 (mesityl aromatic C-4), 128.5 (benzyl aromatic C-2 and C-6), 128.2 (benzyl aromatic C-3 and C-5), 127.2 (benzyl aromatic C-4), 123.2 (phthalimido C-4 and C-5), 58.4 (benzylic CH$_2$), 51.9 (C-3), 51.0 (C-5), 41.6 (C-7), 36.1 (C-1), 26.3 (C-2), 25.6 (C-6), 22.9 (mesityl 2-CH$_3$ and 6-CH$_3$), 20.9 (mesityl 4-CH$_3$); IR cm^{-1} (KBr) 3296 (sulfonamide NH), 1771, 1700 (phthalimido C=O), 1602 (aromatic), 1461, 1314, 1152 (SO$_2$). Anal. (C$_{30}$H$_{35}$N$_3$O$_4$S), C, H, N.

N-Ethyl-(2-mesitylene)sulfonamide (9). A 1.34-g portion of 2-mesitylenesulfonyl chloride (0.00613 mol) in 6 mL of dry dichloromethane was added dropwise with rapid stirring to ethylamine hydrochloride (8) (0.50 g, 0.00613 mol) in 6.1 mL of 10% NaOH (0.0153 mol) at 0 °C. After 1 h the mixture was allowed to warm to room temperature and stirred for an additional 2 h. The layers were separated, the aqueous phase was further extracted with two 10-mL portions of chloroform, and the combined organic extracts were dried over MgSO$_4$. Filtration and removal of the solvent *in vacuo* then afforded 1.454 g of a white amorphous solid, which was purified on silica gel (hexane/ethyl acetate, 6:2) to give 1.25 g (90% yield) of the product as a white solid: ^{1}H NMR (CDCl$_3$) δ 6.96 (s, 2H, mesityl aromatic H-3 and H-5), 4.39 (br s, 1H, NHSO$_2$), 2.96 (q, 2H, ethyl CH$_2$), 2.64 (s, 6H, mesityl 2-CH$_3$ and 6-CH$_3$), 2.3 (s, 3H, mesityl 4-CH$_3$), 1.09 (t, 3H, ethyl CH$_3$); ^{13}C NMR (CDCl$_3$) δ 142.4 ((mesityl aromatic C-2 and C-6), 139.3 (mesityl aromatic C-3 and C-5), 139.3 (benzyl aromatic C-1), 131.8 (mesityl aromatic C-4), 37.6

(ethyl CH$_2$), 22.9 (mesityl 2-CH$_3$ and 6-CH$_3$), 20.9 (mesityl 4-CH$_3$), 15.1 (ethyl CH$_3$); IR cm^{-1} (KBr) 3296 (sulfonamide NH), 1602 (aromatic), 1461, 1314, 1152 (SO$_2$). Anal. (C$_{11}$H$_{17}$NO$_2$S), C, H, N.

N-Ethyl-N-(3-iodopropyl)-(2-mesitylene)sulfonamide (11). A 0.116-g portion of sodium hydride (60% mineral oil dispersion, 0.0029 mol) was added to a solution of 9 (0.50 g, 0.0022 mol) in 8 mL of dry DMF in a nitrogen atmosphere at 0 °C. After the evolution of the gas subsided, the resulting mixture was added slowly *via* a transfer needle to another flask containing 1,3-diiodopropane 10 (3.91 g, 0.0132 mol) in 2 mL of dry THF. The reaction was then allowed to stir at room temperature for 4 h. Removal of the solvent by rotary evaporation (35 °C at 0.2 mm Hg) gave the crude product, which was purified on silica gel (hexane/ethyl acetate, 9:1) to give 0.317 g (36%) of pure 11 as a yellow oil: ^{1}H NMR (CDCl$_3$) δ 6.94 (s, 2H, mesityl aromatic H-3 and H-5), 3.22–3.27 (m, 4H, ethyl CH$_2$ and propyl H-1), 3.06 (t, 2H, H-3), 2.64 (s, 6H, mesityl 2-CH$_3$ and 6-CH$_3$), 2.3 (s, 3H, mesityl 4-CH$_3$), 2.29 (q, 2H, H-2), 1.09 (t, ethyl CH$_3$); ^{13}C NMR (CDCl$_3$) δ 142.4 (mesityl aromatic C-2 and C-6), 139.3 (mesityl aromatic C-3 and C-5), 139.3 (benzyl aromatic C-1), 131.8 (mesityl aromatic C-4), 45.8 (C-1), 40.7 (ethyl CH$_2$), 31.4 (C-2), 22.8 (mesityl 2-CH$_3$ and 6-CH$_3$), 20.9 (mesityl 4-CH$_3$), 12.9 (ethyl CH$_3$), 2.2 (C-3); IR cm^{-1} (neat) 3296 (sulfonamide), 1602 (aromatic), 1461, 1314, 1152 (SO$_2$). Anal. (C$_{14}$H$_{22}$NO$_2$SI), C, H, N.

1-Phthalimido-4-(N-benzyl)-8-[N-(2-mesitylene)sulfonyl]-11-[N-ethyl-N-[(2-mesitylene)sulfonyl]amino]-4,8-diazaundecane (12). A 0.047-g portion of sodium hydride (60% mineral oil dispersion, 0.00119 mol) in 2 mL of dry DMF was added slowly *via* syringe to a cooled (0 °C) solution of compound 7 (0.408 g, 0.000 76 mol) and compound 11 (0.604 g, 0.001 53 mol) in 3 mL of dry DMF under a nitrogen atmosphere. The reaction was allowed to stir at 0 °C for 30 min, after which cold water (1 mL) and chloroform (2 mL) were added with stirring. Removal of the solvents *in vacuo* followed by chromatography on silica gel (hexane/ethyl acetate, 6:4) then afforded pure 12 (0.584 g, 96%) as a yellow oil: ^{1}H NMR (CDCl$_3$) δ 7.82 (m, 2H, phthalimido H-3 and H-6), 7.73 (m, 2H, phthalimido H-4 and H-5), 7.20 (m, 5H, benzyl aromatic), 6.90 (s, 4H, mesityl aromatic H-3 and H-5), 3.63 (t, 2H, H-1), 3.39 (s, 2H, benzyl CH$_2$), 3.13 (q, 2H, ethyl CH$_2$), 3.13 (m, 6H, H-7, H-9 and H-11), 2.53 (s, 12H, mesityl 2-CH$_3$ and 6-CH$_3$), 2.35 (t, 2H, H-3), 2.27–2.24 (s, 6H, mesityl 4-CH$_3$), 1.72 (m, 4H, H-2 and H-6), 1.72 (q, 2H, H-10), 1.0 (t, 3H, ethyl CH$_3$); ^{13}C NMR (CDCl$_3$) δ 168.3 phthalimido C=O), 141.8 (mesityl aromatic C-2 and C-6), 139.0 (mesityl aromatic C-3 and C-5), 139.3 (benzyl aromatic C-1), 133.9 (phthalimido aromatic C-1 and C-2), 132.1 (phthalimido aromatic C-3 and C-6), 131.8 (mesityl aromatic C-4), 128.5 (benzyl aromatic C-2 and C-6), 128.2 (benzyl aromatic C-3 and C-5), 127.2 (benzyl aromatic C-4), 123.2 (phthalimido aromatic C-4 and C-5), 58.4 (benzylic CH$_2$), 51.0 (C-3), 51.9 (C-5), 44.2 (C-7), 43.5 (C-9), 42.7 (C-11), 40.1 (ethyl CH$_2$), 36.2 (C-1), 25.9, 25.6, 25.0 (C-2, C-6, C-10), 22.8 (mesityl 2-CH$_3$ and 6-CH$_3$), 20.9 (mesityl 4-CH$_3$), 12.7 (ethyl CH$_3$); IR cm^{-1} (neat) 1771, 1714 (phthalimido C=O), 1602 (aromatic), 1461, 1314, 1152 (SO$_2$). Anal. (C$_{44}$H$_{56}$N$_4$O$_6$S$_2$), C, H, N.

1-Phthalimido-8-[N-(2-mesitylene)sulfonyl]-11-[N-ethyl-N-[(2-mesitylene)sulfonyl]amino-4,8-diazaundecane (13). A 0.40 g portion of 12 (0.000 49 mol) was dissolved in 1 mL of chloroform and added to a suspension of 10% Pd/C which had been previously wetted with 1 mL of dry ethanol. An 8-mL portion of methanol was added, and the resulting suspension was hydrogenated at 50 psi in a Parr apparatus at room temperature for 2 days. The catalyst was filtered off (0.45 μ Zetapore filter) and washed with methanol, and the combined filtrate was concentrated *in vacuo* to give 0.263 g (75.5%) of 13 as a white solid. This product was used immediately and without further purification in the subsequent step: ^{1}H NMR (CDCl$_3$) δ 7.82 (m, 2H, phthalimido aromatic H-3 and H-6), 7.73 (m, 2H, phthalimido aromatic H-4 and H-5), 6.94 (s, 2H, mesityl aromatic H-3 and H-5), 6.90 (s, 2H, mesityl H-3 and H-5), 3.85 (t, 2H, H-1), 3.13 (m, 8H, H-7, H-9 and H-11 and ethyl CH$_2$), 2.54–2.49 (d, 12H, mesityl 2-CH$_3$ and 6-CH$_3$), 2.29 (s, 6H, mesityl 4-CH$_3$), 2.17 (m, 4H, H-2 and H-6), 1.65 (q, 2H, H-10), 0.86 (t, 3H, ethyl CH$_3$); IR cm^{-1} (neat) 3310 (NH), 1700 (phthalimido C=O), 1461, 1314, 1152 (SO$_2$).

1-Phthalimido-4,8-bis[*N*-(2-mesitylene)sulfonyl]-11-[*N*-ethyl-*N*-[(2-mesitylene)sulfonyl]amino]-4,8-diazaundecane (14). A 0.08-g portion of 2-mesitylenesulfonyl chloride (0.000 369 mol) in 3 mL of dry dichloromethane was added dropwise with rapid stirring to a solution of 13 (0.263 g, 0.003 69 mol) and 10% NaOH (0.5 mL, 0.001 25 mol) at 0 °C, and the mixture was allowed to stir for 1 h at 0 °C and then 2 h at room temperature. The layers were separated, the aqueous phase was further extracted with two 10-mL portions of chloroform, and the combined organic extracts were dried over MgSO$_4$. Filtration, removal of the solvent *in vacuo*, and chromatography of the residue on silica gel (hexane/ethyl acetate, 6:4) then afforded pure 14 (0.200 g, 61%) as a yellow oil: ^{1}H NMR (CDCl$_3$) δ 7.82 (m, 2H, phthalimido aromatic H-3 and H-6), 7.73 (m, 2H, phthalimido aromatic H-4 and H-5), 6.92 (s, 4H, mesityl aromatic H-3 and H-5), 6.78 (s, 2H, mesityl aromatic H-3 and H-5), 3.43 (t, 2H, H-1), 3.03–3.12 (m, 12H, H-3, H-5, H-7, H-9, H-11 and ethyl CH$_2$), 2.55–2.47 (d, 12H, mesityl 2-CH$_3$ and 6-CH$_3$), 2.29–2.24 (d, 6H, mesityl 4-CH$_3$), 1.67 (m, 6H, H-2, H-6 and H-10), 0.99 (t, 3H, ethyl CH$_3$); ^{13}C NMR (CDCl$_3$) δ 168.3 (phthalimido C=O), 141.8 (d, mesityl aromatic C-2 and C-6), 140.1 and 139.0 (mesityl aromatic C-3 and C-5), 133.9 (phthalimido aromatic C-1 and C-2), 132.1 (phthalimido aromatic C-3 and C-6), 131.8 (mesityl aromatic C-4), 123.2 (phthalimido aromatic C-4 and C-5), 43.4, 43.3, 43.2, 42.6 (C-3, C-5, C-7, C-9 and C-11), 40.2 (ethyl CH$_2$), 26.5 (C-2), 25.5 and 25.4 (C-6 and C-10), 22.8 (mesityl 2-CH$_3$ and 6-CH$_3$), 20.9 (mesityl 2-CH$_3$ and 6-CH$_3$), 12.7 (ethyl CH$_3$); IR cm^{-1} (neat) 1700 (phthalimido C=O), 1602 (aromatic), 1461, 1314, 1152 (SO$_2$). Anal. (C$_{46}$H$_{60}$N$_4$O$_8$S$_3$), C, H, N.

1-Amino-4,8-bis[*N*-(2-mesitylene)sulfonyl]-11-[*N*-ethyl-*N*-[(2-mesitylene)sulfonyl]amino]-4,8-diazaundecane (15). A 0.013-g portion of dry hydrazine (0.000 405 mol) was added to a solution of 14 (0.173 g, 0.000 193 mol) in 1.5 mL of dry methanol under a nitrogen atmosphere. The solution was heated at 50 °C for 18 h, cooled, and concentrated *in vacuo*. The residue was dissolved in 4 mL of dichloromethane, the solution was filtered, and the solvent was removed *in vacuo* to afford 0.146 g (100%) of the crude amine 15. This product was used immediately and without further purification in the subsequent step: ^{1}H NMR (CDCl$_3$) δ 6.92 (s, 6H, mesityl aromatic H-3 and H-5), 3.15–2.98 (m, 12H, H-3, H-5, H-7, H-9, H-11 and ethyl CH$_2$), 2.54 (s, 18H, mesityl 2-CH$_3$ and 6-CH$_3$), 2.28 (s, 9H, mesityl 4-CH$_3$), 1.66 (m, 4H, H-6 and H-10), 1.52 (m, 2H, H-2), 0.99 (t, 3H, ethyl CH$_3$); ^{13}C NMR (CDCl$_3$) δ 142.4 (mesityl aromatic C-2 and C-6), 140.1 (mesityl aromatic C-3 and C-5), 131.8 (mesityl aromatic C-4), 43.0, 42.6, 42.1 (C-7, C-9 and C-11), 40.1 (ethyl CH$_2$), 39.1 (C-3), 30.9 (C-2), 25.4 and 25.2 (C-6 and C-10), 22.7 (mesityl 2-CH$_3$ and 6-CH$_3$), 20.9 (mesityl 2-CH$_3$ and 6-CH$_3$), 12.6 (ethyl CH$_3$); IR cm^{-1} (neat) 3300 (NH$_2$), 1602 (aromatic), 1461, 1314, 1152 (SO$_2$).

1-[[*N*-(2-mesitylene)sulfonyl]amino]-4,8-bis[*N*-(2-mesitylene)sulfonyl]-11-[*N*-ethyl-*N*-[(2-mesitylene)sulfonyl]amino]-4,8-diazaundecane (16). A 0.163-g portion of 15 (0.000 21 mol) was mesitylated exactly as described for the synthesis of 14 to afford crude 16, which was purified on silica gel (hexane/ethyl acetate, 5:5) to give 0.100 g (50% yield) of the product 16 as a white foam: ^{1}H NMR (CDCl$_3$) δ 6.92 (s, 8H, mesityl aromatic H-3 and H-5), 3.18 (t, 2H, H-3), 2.97–3.10 (m, 10H, H-5, H-7, H-9, H-11 and ethyl CH$_2$), 2.58 (s, 6H, mesityl 2-CH$_3$ and 6-CH$_3$), 2.51 (s, 18H, mesityl 2-CH$_3$ and 6-CH$_3$), 2.28 (s, 12H, mesityl 4-CH$_3$), 1.61 (m, 6H, H-2, H-6 and H-10), 0.9 (t, 3H, ethyl CH$_3$); ^{13}C NMR (CDCl$_3$) δ 1424 (mesityl aromatic C-2 and C-6), 142.7 and 141.9 (mesityl aromatic C-2 and C-6), 140.1 and 138.9 (mesityl aromatic C-3 and C-5), 132.9 (mesityl aromatic C-4), 131.9 (mesityl aromatic C-4), 43.4, 43.3, 42.9, 42.7 (C-5, C-7, C-9 and C-11), 40.1 (ethyl CH$_2$), 39.3 (C-3), 27.58 (C-1), 25.53 and 25.24 (C-6 and C-10), 22.7 (mesityl 2-CH$_3$ and 6-CH$_3$), 20.9 (mesityl 2-CH$_3$ and 6-CH$_3$), 14.2 (C-2), 12.6 (ethyl CH$_3$); IR cm^{-1} (KBr) 3296 (sulfonamide NH), 1602 (aromatic), 1461, 1314, 1152 (SO$_2$). Anal. (C$_{47}$H$_{68}$N$_4$O$_8$S$_4$), C, H, N.

1-[*N*-Propargyl-*N*-[(2-mesitylene)sulfonyl]amino]-4,8-bis[*N*-(2-mesitylene)sulfonyl]-11-[*N*-ethyl-*N*-[(2-mesitylene)sulfonyl]amino]-4,8-diazaundecane (17). A 0.028-g portion of sodium hydride (60% mineral oil dispersion, 0.000 42 mol) in 4 mL of dry DMF was added slowly *via* a dry syringe to a solution of 16 (0.2 g, 0.000 21 mol) and propargyl bromide (0.074 g, 0.000 63 mol) in 4 mL of dry DMF at 0 °C under a nitrogen

atmosphere. The reaction was allowed to stir at 0 °C for 30 min, and then cold water (1 mL) and chloroform (3 mL) were added with stirring. Removal of the solvent *in vacuo* (35 °C at 0.2 mmHg) and chromatography of the residue on silica gel (hexane/ethyl acetate, 5:5) then afforded pure 17 (0.152 g, 73%) as a yellow oil: ^{1}H NMR (CDCl$_3$) δ 6.85 (s, 8H, mesityl aromatic H-3 and H-5), 3.78 (s, 2H, propargyl CH$_2$), 2.91–2.98 (m, 12H, H-3, H-5, H-7, H-9, H-11 and ethyl CH$_2$), 2.45–2.46 (m, 25H, mesityl 2-CH$_3$, mesityl 6-CH$_3$ and propargyl CH), 2.28 (s, 12H, mesityl 4-CH$_3$), 1.61 (m, 6H, H-2, H-6 and H-10), 0.9 (t, 3H, ethyl CH$_3$); ^{13}C NMR (CDCl$_3$) δ 142.5 (mesityl aromatic C-2 and C-6), 140.1 (mesityl aromatic C-3 and C-5), 132.9 (mesityl aromatic C-4), 131.9 (mesityl aromatic C-4), 73.5 (propargyl quaternary C), 60.4 (propargyl CH), 43.4, 43.3, 42.6 (C-1, C-3, C-5, C-7, C-9 and C-11), 40.1 (ethyl CH$_2$), 34.8 (propargyl CH$_2$), 25.4 and 24.9 (C-6 and C-10), 22.7 (mesityl 2-CH$_3$ and 6-CH$_3$), 20.9 (mesityl 2-CH$_3$ and 6-CH$_3$), 14.2 (C-2), 12.6 (ethyl CH$_3$); IR cm^{-1} (neat) 3282, 2253 (alkyne CH), 1602 (aromatic), 1461, 1314, 1152 (SO$_2$). Anal. (C$_{50}$H$_{70}$N$_4$O$_8$S$_4$), C, H, N.

1-(Propargylamino)-11-(ethylamino)-4,8-diazaundecane Tetrahydrobromide (1). A 0.72-g portion of phenol (0.0076 mol) of phenol was dissolved in 7.2 mL of 30% HBr/HOAc in a stoppered flask, and to this mixture a solution of 17 (0.06 g, 0.000 06 mol) in 2.4 mL of ethyl acetate was added in three portions over a period of 3 h. After the addition was complete, the reaction mixture was stirred for an additional 15 h at room temperature, then cooled to 0 °C, and diluted with 10 mL of water. The aqueous phase was washed with two 6-mL portions of ethyl acetate before being lyophylized to give the crude product as dark yellow solid. This crude product was washed with methanol and filtered to yield the tetrahydrobromide salt of 1 (0.03 g, 85%) as a white solid. An analytical sample of 1 was prepared by recrystallization from aqueous ethanol: ^{1}H NMR (D$_2$O) δ 3.96 (s, 2H, propargyl CH$_2$), 3.2 (m, 14H, H-1, H-3, H-5, H-7, H-9, H-11 and ethyl CH$_2$), 2.13 (q, 6H, H-2, H-6 and H-10), 1.23 (t, 2H, ethyl CH$_3$).

1-[*N*-[(Cyclopropyl)methyl]-*N*-[(2-mesitylene)sulfonyl]amino]-4,8-[*N*-(2-mesitylene)sulfonyl]-11-[N-ethyl-*N*-[(2-mesitylene)sulfonyl]amino]-4,8-diazaundecane (18). A 0.10-g portion of 16 (0.0001 mol) was reacted with (bromomethyl)cyclopropane (0.04 g, 0.0003 mol) exactly as described for the synthesis of 17 to afford crude 18, which was chromatographed on silica gel (hexane/ethyl acetate 6:4) to yield pure 18 (0.05 g, 56%) as a yellow oil: ^{1}H NMR (CDCl$_3$) δ 6.85 (s, 8H, mesityl aromatic H-3 and H-5), 3.11–3.21 (m, 12H, H-3, H-5, H-7, H-9, H-11 and ethyl CH$_2$), 2.86 (d, 2H, C$_3$H$_5$-CH$_2$), 2.55–2.56 (m, 24H, mesityl 2-CH$_3$ and 6-CH$_3$), 2.31 (s, 12H, mesityl 4-CH$_3$), 1.66 (m, 6H, H-2, H-6 and H-10), 0.96 (t, 3H, ethyl CH$_3$), 0.71 (m, 1H, cyclopropyl CH), 0.42 (m, 2H, cyclopropyl *exo*-CH$_2$), -0.02 (m, 2H, cyclopropyl *endo*-CH$_2$); ^{13}C NMR (CDCl$_3$) δ 142.5 (mesityl aromatic C-2 and C-6), 140.1 (mesityl aromatic C-3 and C-5), 132.9 (mesityl aromatic C-4), 131.9 (mesityl aromatic C-4), 50.0 (mesityl 4-CH$_3$), 43.3 and 42.6 (C-1, C-3, C-5, C-7, C-9 and C-11), 40.1 (ethyl CH$_2$), 25.4, 25.3, 25.2 (C-2, C-6 and C-10), 22.7 (mesityl 2-CH$_3$ and 6-CH$_3$), 20.9 (mesityl 2-CH$_3$ and 6-CH$_3$), 12.7 (ethyl CH$_3$), 9.0 (cyclopropyl CH), 3.81 (cyclopropyl CH$_2$); IR cm^{-1} (neat) 1602 (aromatic), 1461, 1314, 1152 (SO$_2$). Anal. (C$_{51}$H$_{74}$N$_4$O$_8$S$_4$), C, H, N.

1-[[(Cyclopropyl)methyl]amino]-11-(ethylamino)-4,8-diazaundecane Tetrahydrobromide (2). A 0.05-g portion of 18 (0.000 05 mol) was deprotected exactly as described for the synthesis of 1 to yield the tetrahydrobromide salt of 2 (0.021 g, 71%) as a white solid. An analytical sample of 2 was prepared by recrystallization from aqueous ethanol: ^{1}H NMR (D$_2$O) δ 3.11 (m, 14H, H-1, H-3, H-5, H-7, H-9, H-11 and ethyl CH$_2$), 2.08 (q, 6H, H-2, H-6 and H-10), 1.25 (t, 3H, ethyl CH$_3$).

Enzyme Assay Procedure and SSAT Induction. Direct measurement of the effect of compounds 1 and 2 on SSAT activity was accomplished using a modification of an existing assay which utilizes a cell free lysate as the source of enzyme.[5] The cells were incubated with 10 μM bis(ethyl)spermine for 24 h to induce SSAT, then harvested, washed, and lysed by brief sonication. The lysate was centrifuged (12 000 X g, 30 min), and the supernatant was used as a source of SSAT. Aliquots of the cytosol were incubated in 100 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (pH 7.8), along with 3.0 mmol spermidine and 0.5 nmol 1-[^{14}C]-

acetyl CoA in a final volume of 50 mL for 5 min at 37 °C. The reaction was stopped by the addition of 20 mL of 0.5 M hydroxylamine hydrochloride, followed by heating in boiling water for 3 min. The resulting samples were centrifuged and aliquots were spotted onto P-81 phosphocellulose discs and scintillation counted. Enzyme activity is expressed as pmol of [^{14}C]acetylspermidine formed/min/mg of protein, and protein was determined by the method of Bradford.[25]

Cell Culture. The NCI H157 (LCLC) and NCI H82 (SCLC) cell lines were maintained in culture as previously described.[26] Each cell line was refed with fresh medium (RPMI 1640 with 9% fetal calf serum, 100 units/mL of penicillin, 100 units/mL of streptomycin) every 3 days to maintain log phase growth. In experiments where the natural polyamines were added to cultures, aminoguanidine was included in the medium at a concentration of 1 mM to inhibit production of the toxic byproducts of the amine oxidases present in the fetal calf serum. For treatment, both cell types were manipulated as indicated in the Discussion section. After treatment, cells were harvested and assayed for cell growth and SSAT activity as described. Cell viability was measured by standard trypan blue exclusion.

References

(1) Presented in part at the 205th National Meeting of the American Chemical Society, March 28–April 2, 1993, in Denver, CO (Abstract MEDI 30).

(2) Sunkara, P. S.; Baylin, S. B.; Luk, G. D. Inhibitors of Polyamine Biosynthesis: Cellular and *In Vivo* Effects on Tumor Proliferation. In *Inhibition of Polyamine Metabolism: Biological Significance and Basis for New Therapies*; (McCann, P. P., Pegg, A. E., Sjoerdsma, A., Eds.; Academic Press: New York, 1987; pp 121–140.

(3) Metcalf, B. W.; Bey, P.; Danzin, C.; Jung, M. J.; Casara, P.; Vevert, J. P. Catalytic, Irreversible Inhibition of Mammalian Ornithine Decarboxylase (E.C. 4.1.1.17) by Substrate and Product Analogues. *J. Am. Chem. Soc.*, 1978, *100*, 2551–2553.

(4) Casero, R. A., Jr.; Pegg, A. E. Spermidine/Spermine-N^1-Acetyltransferase-The Turning Point in Polyamine Metabolism. *FASEB J.*, in press.

(5) Casero, R. A., Jr.; Celano, P.; Ervin, S. J.; Porter, C. W.; Bergeron, R. J.; Libby, P. R. Differential Induction of Spermidine/Spermine-N^1-Acetyltransferase in Human Lung Cancer Cells by the Bis(ethyl)polyamine Analogues. *Cancer Res.* 1989, *49*, 3829–3833.

(6) Porter, C. W.; McManis, J.; Casero, R. A., Jr.; Bergeron, R. J. The Relative Abilities of the Bis(ethyl) Derivatives of Putrescine, Spermidine and Spermine to Regulate Polyamine Biosynthesis and Inhibit Cell Growth. *Cancer Res.* 1987, *47*, 2821–2825.

(7) Della Ragione, F.; Pegg, A. E. Studies of the Specificity and Kinetics of Rat Liver Spermidine/Spermine-N^1-Acetyltransferase. *Biochem. J.* 1983, *213*, 701–706.

(8) Bergeron, R. J.; Neims, A. H.; McManis, J. S.; Hawthorne, T. R.; Vinson, J. R. T.; Bortell, R.; Ingeno, M. J. Synthetic Polyamine Analogues as Antineoplastics. *J. Med. Chem.* 1988, *31*, 1183–1190.

(9) Basu, H. S.; Feuerstein, B. G.; Deen, D. F.; Lubich, W. P.; Bergeron, R. J.; Samejima, K.; Marton, L. J. Correlation Between the Effects of Polyamine Analogues on DNA Conformation and Cell Growth. *Cancer Research* 1989, *49*, 5591–5597.

(10) Edwards, M. L.; Prakash, N. J.; Stemerick, D. M.; Sunkara, S. P.; Bitonti, A. J.; Davis, G. F.; Dumont, J. A.; Bey, P. Polyamine Analogues With Antitumor Activity. *J. Med. Chem.* 1990, *33*, 1369–1375.

(11) Bitonti, A. J.; Bush, T. J.; McCann, P. P. Regulation of Polyamine Biosynthesis in Rat Hepatoma (HTC) Cells by a Bisbenzyl Polyamine Analogue. *Biochem. J.* 1989, *257*, 769–774.

(12) Porter, C. W.; Pegg, A. E.; Ganis, B.; Madhabala, R.; Bergeron, R. J. Combined Regulation of Ornithine and S-Adenosylmethionine Decarboxylases by the Spermine Analogue N^1,N^{12}-bis(ethyl)-spermine. *Biochem. J.* 1990, *268*, 207–212.

(13) Chang, B. K.; Bergeron, R. J.; Porter, C. W.; Liang, Y. Antitumor Effects of N-Alkylated Polyamine Analogues in Human Pancreatic Adenocarcinoma Models. *Cancer Chemother. Pharmacol.* 1992, *30*, 179–182.

(14) Chang, B. K.; Bergeron, R. J.; Porter, C. W.; Vinson, J. R. V.; Liang, Y.; Libby, P. R. Regulatory and Antiproliferative Effects of N-Alkylated Polyamine Analogues in Human and Hamster Pancreatic Adenocarcinoma Cell Lines. *Cancer Chemother. Pharmacol.* 1992, *30*, 183–188.

(15) Porter, C. W.; Bernacki, R. J.; Miller, J.; Bergeron, R. J. Antitumor Activity of N^1,N^{11}-Bis(ethyl)norspermine Against Human Melanoma Xenografts and Possible Biochemical Correlates of Drug Action. *Cancer Res.* 1993, *53*, 581–586.

(16) Bernacki, R. J.; Bergeron, R. J.; Porter, C. R. Antitumor Activity of *N,N′*-Bis(ethyl)spermine Homologues Against Human MALME-3 Melanoma Xenografts. *Cancer Res.* 1992, *52*, 2424–2430.

(17) Bergeron, R. J.; Garlich, J. R.; Stolowich, N. J. Reagents for the Stepwise Functionalization of Spermidine, Homospermidine and Bis(3-aminopropyl)amine. *J. Org. Chem.* 1984, *49*, 2997–3001.

(18) Yajima, H.; Takeyama, M.; Kanaki, J.; Nishimura, O.; Fujino, M.: Studies on Peptides. LXXX. N^G-Mesitylene-2-sulfonylarginine. *Chem. Pharm. Bull.* 1978, *26*, 3752–3757.

(19) Rylander, P. N. In *Hydrogenation Methods*; Rylander, P.N., Ed.; Academic Press: New York, 1985; pp 163–165.

(20) Jasys, V. J.; Kelbaugh, P. R.; Nason, D. M.; Phillips, D.; Rosnack, K.J.; Forman, J. T.; Saccomano, N.A.; Stroh, J. G.; Volkmann, R. A. Novel Quaternary Ammonium Salt-Containing Polyamines from the *Agelenopsis aperta* Funnel-Web Spider. *J. Org. Chem.* 1992, *57*, 1814–1820.

(21) Roemmele, R.C.; Rapoport, H. Removal of N-Arylsulfonyl Groups from Hydroxy α-Amino Acids. *J. Org. Chem.* 1988, *53*, 2367–2371.

(22) Casero, R. A., Jr.; Ervin, S. J.; Celano, P.; Baylin, S. B.; Bergeron, R.J.: Differential Response to Treatment with Bis(ethyl)polyamine Analogues Between Human Small Cell Lung Carcinoma and Undifferentiated Large Cell Lung Carcinoma In Culture. *Cancer Res.* 1989, *49*, 639–643.

(23) Casero, R. A., Jr.; Mank, A. R.; Xiao, L.; Smith, J.; Bergeron, R. J.; Celano, P. Steady State Messenger RNA and Activity Correlates with Sensitivity to N^1,N^{12}-Bis(ethyl)spermine in Human Cell Lines Representing the Major Forms of Lung Cancer. *Cancer Res.* 1992, *52*, 5359–5363.

(24) Casero, R. A., Jr.; Celano, P.; Ervin, S. J.; Applegren, N. B.; Weist, L.; Pegg, A. E. Isolation and Characterization of a cDNA Clone that Codes for Human Spermidine/Spermine-N^1-Acetyltransferase. *J. Biol. Chem.* 1991, *266*, 810–814.

(25) Bradford, C.A. A Rapid and Sensitive Method for the Quantitation of Protein Using the Principle of Protein Dye Binding. *Anal. Biochem.* 1976, *72*, 248–254.

(26) Casero, R. A., Jr.; Go, B.; Theiss, H. W.; Smith, J.; Baylin, S. B.; Luk, G.D. Cytotoxic Response of the Relatively Difluoromethylornithine-Resistant Human Lung Tumor Cell Line NCI H157 to the Polyamine Analogue N^1,N^8-Bis(ethyl)spermidine. *Cancer Res.* 1987, *47*, 3964–3967.