A Comparison of Structure−Activity Relationships between Spermidine and Spermine Analogue Antineoplastics

Raymond J. Bergeron,*,† Yang Feng,† William R. Weimar,† James S. McManis,† Hristina Dimova,† Carl Porter,‡ Brian Raisler,† and Otto Phanstiel§

Department of Medicinal Chemistry, University of Florida, Gainesville, Florida 32610, Grace Cancer Center, Roswell Park Memorial Institute, Buffalo, New York 14263, and Chemistry Department, University of Central Florida, Orlando, Florida 32816

Received December 16, 1996⊗

A systematic investigation of the impact of spermidine analogues both *in vitro* and *in vivo* is described. The study characterizes the effects of these analogues on L1210 cell growth, polyamine pools, ornithine decarboxylase, *S*-adenosyl-L-methionine decarboxylase, spermidine/spermine N^1-acetyltransferase, the maintenance of cellular charge, i.e., cationic equivalence associated with the polyamines and their analogues, and compares their ability to compete with spermidine for transport. The findings clearly demonstrate that the activity of the linear polyamine analogues is highly dependent on the length of the triamines and the size of the N^{α},N^{ω}-substituents. It appears that there is an optimum chain length for various activities and that the larger the N^{α},N^{ω}-alkyls, the less active the compound. Metabolic transformation including N-dealkylation of these compounds is also evaluated. While there is no monotonic relationship between chain length and the ability of the analogue to be metabolized, the dipropyl triamines are clearly more actively catabolized than the corresponding methyl and ethyl systems. A comparison of the triamines with the corresponding tetraamines is made throughout the text regarding both *in vitro* activity against L1210 cells and *in vivo* toxicity measurements, suggesting that several triamine analogues may offer therapeutic advantages over the corresponding tetraamines.

Introduction

Because of the sustained increases in polyamine biosynthesis in preneoplastic and neoplastic tissues, a great deal of attention has been given to the polyamine biosynthetic network as a target in antineoplastic strategies.[1,2] Initial work focused on the design and synthesis of compounds which would inhibit L-ornithine decarboxylase (ODC)[3] and *S*-adenosyl-L-methionine decarboxylase (AdoMetDC).[1,4] Some success was achieved through this approach in that difluoromethylornithine (DFMO), an ODC inhibitor, and methyl glyoxyl bis(guanylhydrazone) (MGBG), an AdoMetDC inhibitor, were effective against both *in vivo* and *in vitro* tumors.[5,6] However, clinical trials did not mirror the success realized in the model systems; the drug either was too toxic as with MGBG[7] or was unable to show significant impact on tumors in humans as with DFMO.[8] One of the problems with the target enzymes ODC and AdoMetDC is associated[9] with their very short half-lives, 20 min. Thus this can translate into a protracted exposure requirement for patients—a less than desirable situation. While there remains some clinical interest in strategies which target these enzymes, e.g., using DFMO and "chemopreventive" strategies for cancer prophylaxis[10] or as a chemotherapeutic agent in combination with radiation for certain head and neck tumors,[11] therapeutic approaches directly targeting ODC or AdoMetDC as a means of disrupting polyamine metabolism have been, for the most part, disappointing in the clinic. Nonetheless, both DFMO and MGBG served well as proof of principle that the polyamine biosynthetic network was an excellent mark in the design of anticancer drugs.

The idea was to devise polyamine analogues which would be incorporated via the polyamine transport apparatus, and once in the cell would find their way to the same subcellular distribution sites as the normal polyamines do but be unable to be further processed.[12,13] They would appear enough like the natural polyamines to shut down polyamine enzymes just as when the cells are exposed to exogenous spermine. Initially a small group of spermidine analogues and homologues were investigated but were abandoned for the more active tetraamines, spermine analogues.

Thus, a series of terminally N-alkylated tetraamines, which exhibit antineoplastic activity against a number of murine and human tumor lines both *in vitro* and *in vivo*, were assembled.[14−18] These analogues have been shown to utilize the polyamine transport apparatus for incorporation,[15,19] deplete polyamine pools,[20] drastically reduce the level of ODC and AdoMetDC activities,[21−23] and in some cases to upregulate spermidine/spermine N^1-acetyltransferase (SSAT).[24−30] Interestingly on incorporation of the tetraamine analogues the total picoequivalents of charge associated with the analogues, as well as the natural polyamines, is maintained for about 24 h. Thus as the cell is incorporating *n* picoequivalents of drug it is excreting *n* picoequivalents of natural polyamines.

Incremental structural alterations in these spermine analogues and homologues result in substantial differences in their biological activity.[20] For example while the tetraamines N^1,N^{12}-diethylspermine (DESPM), N^1,N^{11}-diethylnorspermine (DENSPM), and N^1,N^{14}-diethylhomospermine (DEHSPM) suppress ODC and AdoMetDC to about the same level at equimolar concentrations, the effect of both DESPM and DEHSPM

* To whom correspondence should be addressed.
† University of Florida.
‡ Roswell Park Memorial Institute.
§ University of Central Florida.
⊗ Abstract published in *Advance ACS Abstracts,* April 15, 1997.

S0022-2623(96)00849-7 CCC: $14.00

Table 1. Triamine Analogue Structures, Abbreviations, L1210 Growth Inhibition, and Transport[a]

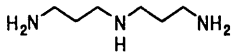
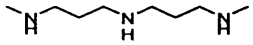
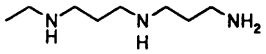
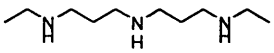
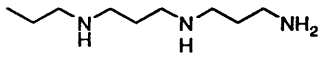
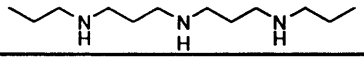
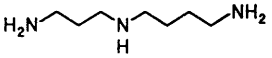
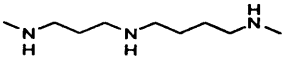
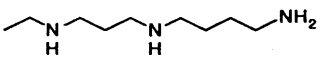
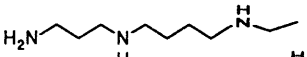
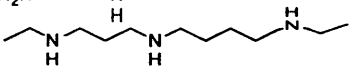
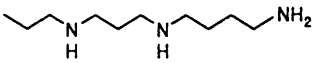
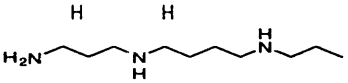
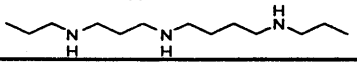
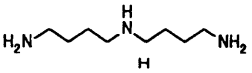
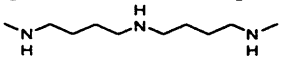
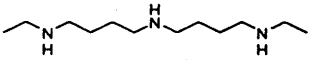
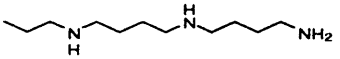
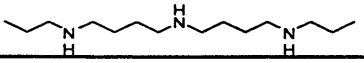
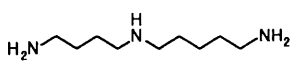
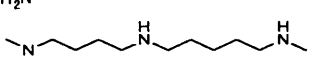
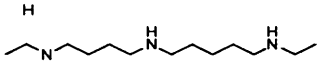
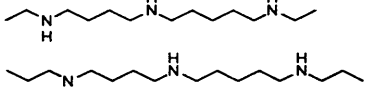
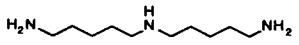
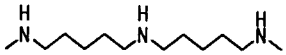
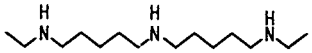
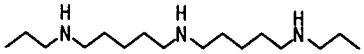
	Structure	Abbreviation	IC_{50} (μM) 48 h		96 h		K_i (μM)	
Norspermidines								
1		NSPD	0.9		0.5		7.2	
2		DMNSPD	>100	(>100)	4.8	(2.5)	60	(5.6)
3		MENSPD	>100	(>100)	>100	(2.5)	34	(7.7)
4		DENSPD	>100	(>100)	10	(2.0)	250	(17)
5		MPNSPD	>100		~100		33	
6		DPNSPD	>100	(>100)	60	(18)	125	(11)
Spermidine								
7		SPD	>100		>100		2.2	
8		DMSPD	>100	(>100)	1.7	(0.75)	5.1	(1.1)
9		MESPD(N^1)	>100	(99)	4.0	(0.33)	8.6	(1.7)
10		MESPD(N^8)	>100		40		7.0	
11		DESPD	~100	(30)	0.7	(0.18)	19	(1.6)
12		MPSPD(N^1)	>100		28		3.0	
13		MPSPD(N^8)	>100		55		8.5	
14		DPSPD	>100	(3)	33	(0.20)	26	(2.3)
Homospermidines								
15		HSPD	>100		3.0		3.4	
16		DMHSPD	>100	(>100)	0.9	(0.32)	5.5	(0.97)
17		DEHSPD	22	(0.20)	0.35	(0.07)	19	(1.4)
18		MPHSPD	100		0.60		5.0	
19		DPHSPD	>100		6.0		67	
4,5-triamines								
20		4,5-Triamine	>100		0.18		1.4	
21		DM(4,5)	2.0		0.12		21	
22		DE(4,5)	4.5	(0.30)	0.20	(0.04)	64	(6.0)
23		DP(4,5)	~100		1.2		75	

Table 1 (Continued)

5,5-triamines								
24		5,5-Triamine	~100		0.4		14	
25		DM(5,5)	15		0.4		130	
26		DE(5,5)	11	(0.4)	0.7	(0.03)	170	(16)
27		DP(5,5)	>100		6.0		87	

[a] IC_{50} was estimated from growth curves for L1210 cells grown in the presence of nine different concentrations of drug spanning 4 log units: 0, 0.03, 0.1, 0.3, 1.0, 3, 10, 30, and 100 μM. IC_{50} data are presented as the mean of at least two experiments with variation from the mean typically 10−25% for the 96 h IC_{50} values. All polyamine analogues exhibited simple substrate-competitive inhibition of [^{14}C]SPD transport by L1210 cells. Each experiment examined the rate of uptake of [^{14}C]SPD at six different SPD concentrations in the absence of inhibitor (analogue), and at three different inhibitor concentrations typically within the range $0.2K_i$ to $5K_i$. Values reported in the table represent the mean of at least two or three such experiments with a variation typically less than 10%. K_i determinations were made by following analogue inhibition of spermidine transport. The IC_{50} and K_i values of corresponding tetraamine analogues are shown in parentheses. The tetraamine corresponding to DE(4,5) (**22**) is DE(4,5,4), and to DE(5,5) (**26**), DE(5,4,5) (**29**).

on cell growth occurs earlier than that observed for DENSPM. The K_i value of DENSPM is about 10 times as great[20] as those of DESPM and DEHSPM for the polyamine transport system. However, the most notable difference between the three analogues is related to their ability to stimulate SSAT.[25−28] The tetraamine DENSPM upregulates SSAT by 1200-fold in MALME-3 cells, while DESPM and DEHSPM stimulate SSAT by 250- and 30-fold, respectively.[28] Thus the impact of the tetraamine compounds on cell growth was shown to be dependent on the distance between the nitrogens, the nature of the terminal alkyl substituents,[15] and most importantly the charge status of the molecules.[31]

What remains to be established is whether or not a similar structure−activity relationship exists for the triamines, analogues of spermidine. The importance of this issue is underscored by the tremendous difference in toxicity between the triamines and tetraamines in general. Triamines are much less toxic, thus making them of potentially useful therapeutic value.[32] Therefore this paper presents new evidence to suggest that the triamines may have been abandoned too quickly.

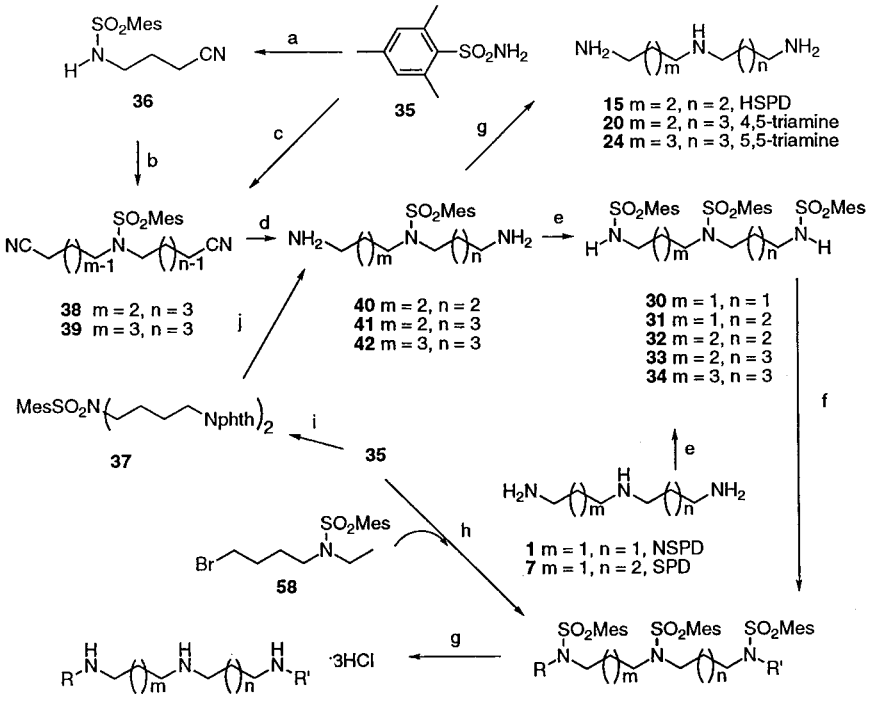
Synthesis of Triamines. Two families of triamines were synthesized: (1) those with symmetrical methylene backbones, i.e., derived from the parent polyamines norspermidine (3,3), homospermidine (4,4), or the longer triamine (5,5), with an alkyl group at one or both terminal nitrogens and (2) those with unsymmetrical methylene backbones, i.e., from the parent polyamines spermidine (3,4) or the (4,5) triamine, with an alkyl group at one or both terminal nitrogens (Table 1). The numbers in parentheses refer to the number of methylenes separating successive nitrogens. In the case of the N^{α},N^{ω}-disubstituted norspermidine ($m = 1$, $n = 1$) and spermidine ($m = 1$, $n = 2$) analogues, the commercially available triamines norspermidine (NSPD) (**1**) and spermidine (SPD) (**7**) were reacted with mesitylenesulfonyl chloride (3 equiv) under biphasic conditions (CH_2Cl_2/dilute NaOH) to give **30**[32] and **31**, respectively (step e) (Scheme 1). These trisulfonamides were deprotonated with NaH in DMF and treated with an excess of the appropriate primary alkyl iodide to make intermediates **43**, **44**, and **46**−**49** (step f). Finally the mesitylenesulfonyl blocking groups were cleanly removed under reductive conditions, utilizing 30% HBr in HOAc and phenol in CH_2Cl_2 (step g) to give terminal dimethyl (**2, 8**), diethyl (**4, 11**), and dipropyl (**6, 14**) NSPD and SPD, respectively, which were isolated as their recrystallized trihydrochloride salts.

The symmetrical triamines homospermidine (HSPD) (**15**) (4,4) and 1,7,13-triazatridecane (**24**) (5,5), which were not commercially available, and their terminally dialkylated derivatives were synthesized by a segmented synthesis (Scheme 1). Mesitylenesulfonamide (**35**)[33] was dialkylated with either *N*-(4-bromobutyl)-phthalimide to give **37** (step i) or with 5-chlorovaleronitrile to furnish **39** (step c). Hydrogenation of the cyano groups of **39** with Raney nickel in methanolic ammonia gave *N*,*N*-bis(5-aminopentyl)mesitylenesulfonamide (**42**) (step d), which provided (5,5) triamine **24** in good yield by treatment with 30% HBr in HOAc (step g). Use of the aromatic imide blocking group in **37** avoided the solubility problems during attempted hydrogenation (Raney nickel, methanolic NH_3) of *N*,*N*-bis(3-cyanopropyl)mesitylenesulfonamide. Hydrazinolysis of **37** in refluxing EtOH (step j) led to *N*,*N*-bis(4-aminobutyl)-mesitylenesulfonamide (**40**). HSPD (**15**) itself resulted from reductive deprotection of monosulfonamide **40** (step g). Terminal diamines **40** and **42** were converted to their mesitylenesulfonamides **32** and **34**, respectively (step e), and were alkylated with the appropriate primary halide (step f). Hydrogen bromide-promoted deprotection of masked analogues **50**, **52**, and **55**−**57** yielded DMHSPD (**16**), DPHSPD (**19**), DM(5,5) (**25**), DE-(5,5) (**26**), and DP(5,5) (**27**), respectively.

N^1,N^9-Diethylhomospermidine (DEHSPD) (**17**) was made by a convergent route (Scheme 1). Alkylation of sulfonamide **35** with *N*-(4-bromobutyl)-*N*-ethylmesitylenesulfonamide (**58**)[13] (2 equiv) led to triprotected analogue **51** (step h), which was unmasked with HBr/HOAc, giving DEHSPD (**17**) (step g).

3,8,14-Triazahexadecane [DE(4,5), **22**], the terminally diethylated analogue of the unsymmetrical (4,5) triamine, was assembled from *N*-(*tert*-butoxycarbonyl)-*N*-mesitylenesulfonamide (**59**), a diprotected ammonia synthon[15] (Scheme 2). Alkylation of reagent **59** with *N*-(4-bromobutyl)-*N*-ethylsulfonamide (**58**) (NaH/DMF) (step a) gave triprotected monoethylputrescine **62**. The BOC group of **62** was removed with trifluoroacetic acid (TFA) (step c). The resulting sulfonamide **63** was alkylated with *N*-(5-bromopentyl)-*N*-ethylmesitylenesulfonamide (**61**) (step a), which was made from ethylsulfonamide **60** and excess 1,5-dibromopentane (NaH/DMF) (step b), to generate fully protected triamine **64**.

Scheme 1. Synthesis of Analogues of NSPD, SPD, HSPD, and (4,5-) and (5,5-)Triamines[a]

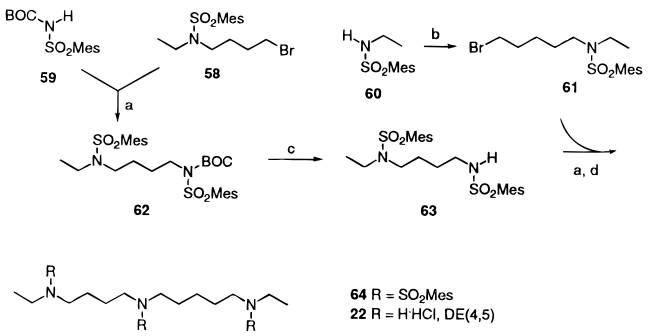


2 m = 1, n = 1, R = R' = Me, DMNSPD
4 m = 1, n = 1, R = R' = Et, DENSPD
5 m = 1, n = 1, R = n-Pr, R' = H, MPNSPD
6 m = 1, n = 1, R = R' = n-Pr, DPNSPD
8 m = 1, n = 2, R = R' = Me, DMSPD
11 m = 1, n = 2, R = R' = Et, DESPD
14 m = 1, n = 2, R = R' = n-Pr, DPSPD
16 m = 2, n = 2, R = R' = Me, DMHSPD
17 m = 2, n = 2, R = R' = Et, DEHSPD
19 m = 2, n = 2, R = R' = n-Pr, DPHSPD
21 m = 2, n = 3, R = R' = Me, DM(4,5)
23 m = 2, n = 3, R = R' = n-Pr, DP(4,5)
25 m = 3, n = 3, R = R' = Me, DM(5,5)
26 m = 3, n = 3, R = R' = Et, DE(5,5)
27 m = 3, n = 3, R = R' = n-Pr, DP(5,5)

43 m = 1, n = 1, R = R' = Me
44 m = 1, n = 1, R = R' = Et
45 m = 1, n = 1, R = n-Pr, R' = H
46 m = 1, n = 1, R = R' = n-Pr
47 m = 1, n = 2, R = R' = Me
48 m = 1, n = 2, R = R' = Et
49 m = 1, n = 2, R = R' = n-Pr
50 m = 2, n = 2, R = R' = Me
51 m = 2, n = 2, R = R' = Et
52 m = 2, n = 2, R = R' = n-Pr
53 m = 2, n = 3, R = R' = Me
54 m = 2, n = 3, R = R' = n-Pr
55 m = 3, n = 3, R = R' = Me
56 m = 3, n = 3, R = R' = Et
57 m = 3, n = 3, R = R' = n-Pr

[a] Reagents: (a) 4-bromobutyronitrile/NaH/DMF; (b) 5-bromovaleronitrile/NaH/DMF; (c) NaH/DMF/4-bromobutyronitrile or 5-chlorovaleronitrile; (d) H_2/Ra Ni/NH_3/CH_3OH; (e) mesitylenesulfonyl chloride/NaOH(aq)/CH_2Cl_2; (f) NaH/DMF/haloalkane; (g) 30% HBr in HOAc/PhOH/CH_2Cl_2, HCl; (h) NaH/DMF; (i) *N*-(4-bromobutyl)phthalimide/NaH/DMF; (j) $(H_2N)_2 \cdot H_2O$/EtOH.

Scheme 2. Synthesis of DE(4,5) (**22**)[a]



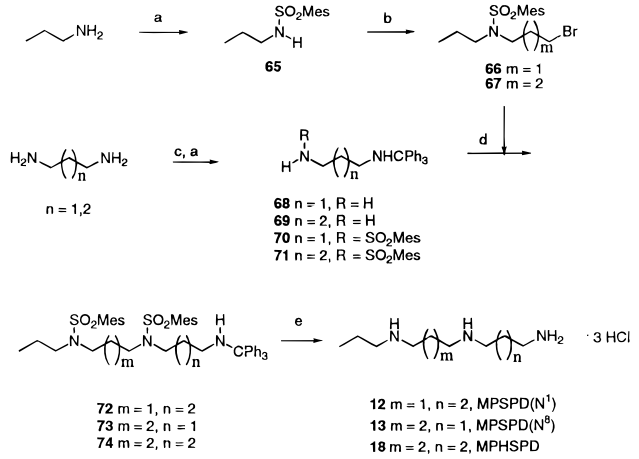
[a] Reagents: (a) NaH/DMF; (b) NaH/DMF/1,5-dibromopentane; (c) TFA/CH_2Cl_2; (d) 30% HBr/HOAc/PhOH, HCl.

Deprotection of the amino groups of **64** with HBr led to the diethylated analogue **22** (step d).

The (4,5) triamine 1,6,12-triazadodecane (**20**) and its dialkylated analogues 2,7,13-triazatetradecane [DM(4,5), **21**] and 4,9,15-triazaoctadecane [DP(4,5), **23**] were produced by a segmented synthesis (Scheme 1). Consecutive monoalkylation of sulfonamide **35** with 4-bromobutyronitrile (step a) and 5-bromovaleronitrile (step b) generated dinitrile **38**. The cyano groups of **38** were reduced in a Parr shaker with Raney nickel in methanolic ammonia (step d), resulting in primary amine **41**. Cleavage of the sulfonyl group of **41** with HBr (step g) produced the parent (4,5) triamine **20**. Treatment of **41** with mesitylenesulfonyl chloride (2 equiv) gave **33** (step e), which was terminally dialkylated with iodomethane to **53** or with 1-iodopropane to **54** (step f). Unmasking the amino groups led to dimethylated and dipropylated 4,5-analogues **21** and **23**, respectively (step g).

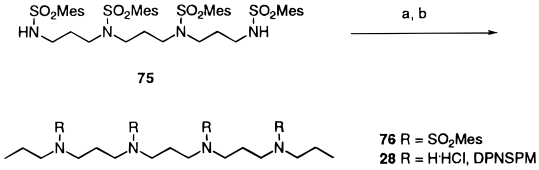
N^1-Propylnorspermidine (MPNSPD, **5**) was made by treating trimesitylenesulfonyl NSPD **30**[32] with 1-iodopropane (1 equiv/NaH/DMF), and isolating **45** from the statistical mixture of un- and dialkylated products by flash column chromatography (step f) (Scheme 1). Since SPD is unsymmetrical, reaction of its trisulfonamide **31** with a primary alkyl iodide (1 equiv) would lead to N^1- and N^8-monoalkylated products, which may be difficult to separate. Thus the synthesis of both SPD and the HSPD monopropyl analogues required a fragment synthesis (Scheme 3). *N*-Propylmesitylenesulfonamide (**65**) was converted to 3-bromopropyl **66** or 4-bromobutyl reagent **67**, with the required dibromoalkane in excess (NaH/DMF). Triphenylmethyl chloride was stirred at room temperature with either 1,3-diaminopropane or 1,4-diaminobutane (5 equiv) in CH_2Cl_2 (step c), resulting in N^1-tritylated trimethylenediamine **68** or putrescine **69**. Sulfonation of **68** and **69** occurred at the primary nitrogen rather than adjacent to the bulky triphenylmethyl group to give N,N′-disubstituted diamines **70**

Scheme 3. Synthesis of Monopropyl SPD and HSPD Analogues[a]



[a] Reagents: (a) mesitylenesulfonyl chloride/1 N NaOH(aq)/CH_2Cl_2; (b) NaH/DMF/1,3-dibromopropane (m = 1) or 1,4-dibromobutane (m = 2); (c) Ph_3CCl/CH_2Cl_2; (d) NaH/DMF/**66** or **67**; (e) 30% HBr in HOAc/PhOH/CH_2Cl_2; HCl.

Scheme 4. Synthesis of DPNSPM (**28**)[a]



[a] Reagents: (a) NaH/DMF/*n*-PrI; (b) 30% HBr in HOAc/PhOH/CH_2Cl_2 then HCl.

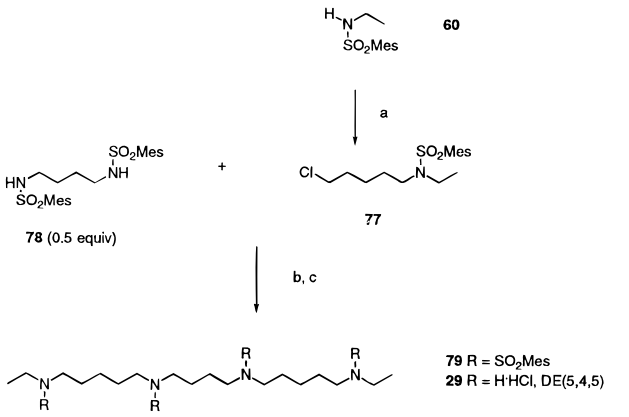
and **71**, respectively (step a). Reaction of the anions of **70** or **71** with the appropriate bromide **66** or **67** resulted in regiospecific N-alkylation at the sulfonamide terminus. Specifically, reaction of **70** with **67** gave **73**, and **71** plus **66** or **67** led to **72** or **74**, respectively. The protecting groups of **45** and **72**−**74** were removed simultaneously with HBr in HOAc/PhOH, resulting in MPNSPD (**5**), MPSPD(N^1) (**12**), MPSPD(N^8) (**13**), and MPHSPD (**18**), respectively.

Both N^1- (**9**) and N^8-ethylspermidine (**10**) were obtained from reduction of the requisite monoacetylspermidine with lithium aluminum hydride in hot THF, thus completing the synthesis of the triamine series.

Tetraamine analogue N^1,N^{11}-dipropylnorspermine (DPNSPM) (**28**) was accessed from commercially available norspermine (Scheme 4). Bisalkylation of the tetrasulfonamide dianion of **75**[15] with 1-iodopropane (step a) and facile removal of the mesitylenesulfonyl blocking groups of **76** with HBr (step b) generated DPNSPM (**28**).

The longer polyamine 3,9,14,20-tetraazadocosane [DE-(5,4,5), **29**], the terminally diethylated derivative of the unknown (5,4,5) tetraamine, was synthesized in three high-yield steps by the segmenting method (Scheme 5). *N*-Ethylmesitylenesulfonamide (**60**)[33] was deprotonated (NaH/DMF) and treated with 1,5-dichloropentane (10 equiv), resulting in alkyl chloride **77** (step a). N^1,N^4-Bis(mesitylenesulfonyl)putrescine (**78**)[15] was alkylated with synthon **77** to give masked tetraamine **79** (step b). The four blocking groups were removed with HBr (step c) to furnish DE(5,4,5) **29** as its crystalline tetrahydrochloride salt.

Scheme 5. Synthesis of DE(5,4,5) (**29**)[a]



[a] Reagents: (a) NaH/DMF/excess 1,5-dichloropropane; (b) NaH/DMF; (c) 30% HBr in HOAc/PhOH/CH_2Cl_2 then HCl.

Biological Evaluations

In summarizing the biological properties of the polyamine analogues, the results will be separated into three sets of measurements: the 48 and 96 h IC_{50} values against L1210 cells and the corresponding K_i values for the polyamine transport apparatus (Table 1); the effect on polyamine pools (Table 2); and the impact on ODC, AdoMetDC, and SSAT (Table 3). The compounds are arranged in sets by increasing length, e.g., norspermidine, spermidine, homospermidine, (4,5) and (5,5) triamines. Each set is ordered in terms of the size of the terminal alkyl groups. While we have previously reported the IC_{50} and K_i values of DESPD, and its impact on polyamine pools, ODC, AdoMetDC, and SSAT,[18] the measurements on this compound were repeated so that the appropriate positive control and not a historical control would be in place. In order to showcase the importance of the polyamine's overall chain length in structure−activity relationships, we will include a brief commentary of results of tetraamine analogues[15] where available. Thus numbers included in parentheses in the tables represent the values for the corresponding tetraamine analogues. A discussion will be presented on the metabolic profile of the triamines and on the cationic conservation of charge the cell maintains as defined by the polyamines. Finally a comparison of the acute and chronic *in vivo* toxicites of several key triamines and tetraamines is presented.

Antiproliferative Activity: IC_{50} of L1210 Cells. As shown in Table 1, all of the alkylated norspermidine analogues have IC_{50} values >100 μM at 48 h. At 96 h the IC_{50}s range from 5 to >100 μM with an order of DMNSPD < DENSPD < DPNSPD < MENSPD and MPNSPD (most to least active). Thus in this family terminal dialkylation with smaller groups increases the compound's activity, while triamines with a single alkyl group are less active than the corresponding compound with N^{α},N^{ω}-bisalkyl substitution. In contrast, analogues of the tetraamine norspermine, although also inactive at 48 h, were more active than the corresponding triamines at 96 h. Moreover, whether norspermine was symmetrically substituted with methyl or ethyl groups or had a single ethyl fixed to one of the terminal nitrogens was insignificant relative to the 96 h IC_{50} values, which were around 2 μM.

At 48 h, SPD and all of its analogues had an IC_{50} of at least 100 μM. SPD is the least active compound in its family with an IC_{50} value above 100 μM at 96 h as well. At 96 h, DMSPD and DESPD are substantially more active than DPSPD. When an ethyl group was removed from either end of DESPD, a monoalkylated analogue was produced with lower activity than DESPD. It is interesting that monoalkylation of SPD by ethyl

Table 2. Impact of Triamine Analogues on Polyamine Pools[a]

compd	concn (μM)	PUT	SPD	SPM	analogue[b]
		Norspermidines			
1, NSPD	0.9	38	44	113	1.09
	4.5	0	12	83	2.14
2, DMNSPD	100 (100)	0 (0)	9 (5)	58 (36)	5.00 (2.14)
	500 (500)	0 (0)	6 (3)	48 (27)	5.51 (1.84)
4, DENSPD	100 (10)	0 (30)	17 (14)	74 (31)	3.67 (1.59)
	500 (100)	0 (0)	7 (6)	47 (30)	3.77 (2.44)
5, MPNSPD	100	0	29	56	3.07
	500	0	15	49	4.78
6, DPNSPD	100 (100)	70 (61)	76 (35)	96 (77)	0.49 (0.89)
	500 (500)	68 (0)	71 (19)	102 (56)	1.24 (1.28)
		Spermidines			
7, SPD	100	0	117	118	
	500	0	145	108	
8, DMSPD	100 (100)	0 (0)	5 (0)	58 (21)	4.96 (1.26)
	500 (500)	0 (0)	0 (0)	54 (24)	4.89 (1.24)
9, MESPD(N^1)	100 (100)	0 (0)	25 (1)	80 (21)	4.20 (1.24)
	500 (500)	0 (0)	14 (1)	53 (19)	4.73 (1.23)
10, MESPD(N^8)	100	0	26	84	4.41
	500	0	15	61	4.96
11, DESPD	100 (30)	0 (0)	5 (0)	74 (12)	4.61 (0.40)
	500 (150)	0 (0)	0 (0)	55 (14)	4.20 (1.13)
12, MPSPD(N^1)	100	0	25	90	3.97
	500	0	15	72	4.95
13, MPSPD(N^8)	100	0	33	103	3.52
	500	0	16	95	5.16
14, DPSPD	100 (3)	6 (0)	35 (18)	135 (64)	3.26 (1.12)
	500 (15)	0 (0)	12 (9)	99 (43)	3.69 (1.09)
		Homospermidines			
15, HSPD	100	0	4	32	3.58
	500	0	2	21	4.44
16, DMHSPD	100 (100)	0 (0)	3 (0)	106 (30)	5.51 (1.49)
	500 (500)	0 (0)	0 (0)	106 (27)	5.85 (1.03)
17, DEHSPD	25 (10)	0 (0)	6 (0)	114 (61)	4.61 (2.94)
	125	0	3	97	4.69
18, MPHSPD	100	0	2	82	5.16
	500	0	0	66	5.86
19, DPHSPD	100	0	19	144	3.12
	500	0	7	111	3.68
		(4,5) Triamines			
20, 4,5	100	0	1	53	3.02
	500	0	0	35	3.20
21, DM(4,5)	2	0	33	112	2.79
	10	0	18	111	5.36
22, DE(4,5)	3 (0.3)	0 (44)	47 (61)	99 (70)	1.20 (0.26)
	15 (1.5)	0 (0)	18 (5)	98 (31)	3.40 (0.72)
23, DP(4,5)	100	0	40	119	1.40
	500	0	31	121	2.42
		(5,5) Triamines			
24, 5,5	100	0	0	33	2.58
	500	0	0	20	2.50
25, DM(5,5)	15	0	33	115	2.56
	75	0	20	101	3.61
26, DE(5,5)	15 (0.15)	0 (37)	55 (55)	97 (88)	1.09 (0.34)
	75 (0.75)	0 (0)	23 (10)	73 (58)	1.59 (1.48)
27, DP(5,5)	100	59	73	97	0.86
	500	51	69	103	1.33

[a] Putrescine (PUT), spermidine (SPD), and spermine (SPM) levels after 48 h of treatment are given as percent polyamine found in untreated controls. Typical control values in pmol/10^6 L1210 cells are PUT = 260 ± 59, SPD = 3354 ± 361, SPM = 658 ± 119. [b] Analogue amount is expressed as nmole/10^6 cells. Untreated L1210 cells (10^6) correspond to about 1 μL volume; therefore, concentration can be estimated as nmol/mM. Concentrations of the corresponding tetraamine analogues and the resulting percent polyamine and analogue are shown in parentheses. The effects of polyamine analogues on polyamine pools were examined after 48 h growth in the presence of two different concentrations of analogue. Each experiment included untreated controls and was done in triplicate, and thus represents nine flasks of cells. Native polyamine pools are presented as a percent of control. Control values for 63 flasks of cells representing 21 experiments were (pmol/10^6 cells; n = 63): PUT, 259 ± 64 (±25%); SPD, 3078 ± 275 (±9%); and SPM, 605 ± 86 (±14%). Of course the variation was somewhat lower for data within each individual experiment. The mean standard deviations for the 21 experiments were as follows: PUT, ±15.5%; SPD, ±6.5%; and SPM, ±7.9%. Evaluation of statistical significance between selected treatment groups was evaluated by ANOVA.

Table 3. Effect of Polyamine Homologues on Ornithine Decarboxylase (ODC), *S*-Adenosylmethionine Decarboxylase (AdoMetDC) and Spermidine/Spermine−Acetyltransferase (SSAT) in L1210 Cells[a]

compd	ODC	AdoMetDC	SSAT
	Norspermidines		
1, NSPD	11	62	150
2, DMNSPD	17 (6)	41 (49)	250 (200)
3, MENSPD	42 (5)	58 (33)	390 (410)
4, DENSPD	80 (10)	45 (42)	780 (1480)
5, MPNSPD	33	38	470
6, DPNSPD	100 (79)	99 (70)	220 (460)
	Spermidines		
7, SPD	16	43	160
8, DMSPD	22 (3)	68 (40)	270 (300)
9, MESPD(N^1)	10 (10)	58 (27)	430 (150)
10, MESPD(N^8)	17	54	400
11, DESPD	30 (3)	68 (28)	1380 (460)
12, MPSPD(N^1)	18	56	1200
13, MPSPD(N^8)	14	64	500
14, DPSPD	75 (52)	107 (72)	1030 (500)
	Homospermidines		
15, HSPD	11	54	430
16, DMHSPD	20 (4)	86 (45)	510 (140)
17, DEHSPD	47 (7)	90 (41)	640 (110)
18, MPHSPD	20	59	570
19, DPHSPD	86	123	420
	(4,5) Triamines		
20, 4,5	19	57	410
21, DM(4,5)	56	71	130
22, DE(4,5)	100 (20)	70 (39)	120 (120)
23, DP(4,5)	83	86	80
	(5,5) Triamines		
24, 5,5	16	88	90
25, DM(5,5)	105	97	90
26, DE(5,5)	100 (19)	109 (54)	90 (190)
27, DP(5,5)	73	123	90

[a] Enzyme activity is expressed as percent of untreated control for ODC (1 μM at 4 h, AdoMetDC (1 μM at 6 h), and SSAT (10 μM at 48 h for triamine analogues, and 2 μM at 48 h for tetraamine analogues). The ODC, AdoMet, and SSAT levels of corresponding tetraamine analogues were shown in parentheses. Each experiment included a positive "control" which had a known, reproducible impact on enzyme activities (mean ± sd): 1 μM DEHSPM reduced ODC to 6.7 ± 2.6% of untreated control (n = 29); 1 μM DEHSPM reduced AdoMetDC to 40.7 ± 6.2% of untreated control (n = 23); and 2 μM DENSPM induced SSAT levels to 1480 ± 120% (n = 8) of untreated control. Data presented in the table represent the mean of at least three experiments and have variances consistent with those suggested by the positive control data presented above.

or propyl at different ends result in very different activities. At 96 h, with an IC_{50} of 4 μM, MESPD(N^1) was about 10 times more active than MESPD(N^8). The same trend was found, although to a lesser degree, among the two monopropyl SPD analogues in that MPSPD(N^1) was about twice as active as MPSPD(N^8). Thus alkylation of SPD at the N^1 position results in a higher activity than alkylation at N^8 (Table 1). The spermine analogues had a significant effect on cell growth even at 48 h, and at 96 h the IC_{50} concentrations of tetraamines ranged from 0.2 to 0.8 μM, with DESPM < DPSPM < MESPM < DMSPM. Again in every instance the tetraamines were more active. It is interesting that 3.7% of intracellular N^1-MESPD and 6.1% of intracellular N^1-MPSPD are metabolically converted to the corresponding N^1-alkylated spermines (Table 4). Given the potent antiproliferative activity of the tetraamines in general, this may help explain the enhanced activity of the N^1-alkylspermidines in comparison to the N^8-alkylspermidines since the latter are not metabolically converted to tetraamines.

Table 4. Metabolic Transformation of Polyamine Analogues by L1210 Cells

Analogue#		N-Monodealkylation^				Deaminopropylation^				Elaboration^	
2- DM-[3,3] (100 μM)	5000(100%)	no N-demethylation									
MM-[3,3,3] (100 μM)	2633 (82.9%)	no N-demethylation				MM-[3,3]	523 (16.5%)	MM-[3]**	20 (0.6%)		
DE-[3,3,3] (500 μM)	2440 (95.3%)	ME-[3,3,3]	194 (4.7%)								
4- DE-[3,3] (500 μM)	3761 (90.7%)	ME-[3,3]	194 (4.7%)			ME-[3]	192 (4.6%)				
3- ME-[3,3] (100 μM)	3051 (78.2%)	[3,3]	20 (0.5%)			ME-[3]	831 (21.3%)			no elaboration of N-Monoalkyl [3,3]	
DP-[3,3,3] (100 μM)	893 (78.2%)	MP-[3,3,3]*	160 (14.0%)			MP-[3,3]	89 (7.8%)				
6- DP-[3,3] (100 μM)	404 (57.7%)	MP-[3,3]	214 (30.6%)	[3,3]***	64 (9.1%)	MP-[3]*	18 (2.6%)				
5- MP-[3,3] (100 μM)	3019 (94.3%)	[3,3]	144 (4.5%)			MP-[3]*	37 (1.2%)			no elaboration of N-Monoalkyl [3,3]	
8- DM-[3,4] (100 μM)	5000(100%)	no N-demethylation									
11- DE-[3,4] (100 μM)	4041 (96.3%)	N8-ME-[4,3]	32 (0.8%)	N1-ME-[3,4]	101 (2.4%)						
9- N1-ME-[3,4] (100 μM)	3946 (96.3%)	[3,4]	not determined							ME-[3,4,3]	150 (3.7%)
10- N8-ME-[4,3] (100 μM)	4825 (99.4%)	[3,4]	not determined			ME-[4]	29 (0.6%)			no elaboration of N8-Alkyl [4,3]	
DP-[3,4,3] (15 μM)	1568 (79.0%)	MP-[3,4,3]*	361 (18.2%)			N1-MP-[3,4]	57 (2.9%)				
14- DP-[3,4] (100 μM)	3260 (90.1%)	N8-MP-[4,3]	192 (5.3%)	N1-MP-[3,4]	166 (4.6%)						
12 -N1-MP-[3,4] (100 μM)	4238 (93.9%)	[3,4]	not determined							MP-[3,4,3]	275 (6.1%)
13- N8-MP-[4,3] (100 μM)	3549 (99.3%)	[3,4]	not determined			MP-[4]*	26 (0.7%)			no elaboration of N8-Alkyl [4,3]	
16- DM-[4,4] (100 μM)	5500 (100%)	no N-demethylation				no exposed primary aminopropyl terminal segment					
DE-[4,4,4] (100 μM)	4215 (100%)	no N-deethylation				"	"				
17- DE-[4,4] (100 μM)	4215 (100%)	no N-deethylation				"	"				
19- DP-[4,4] (500 μM)	3149 (87.7%)	MP-[4,4]	441 (12.3%)			"	"			no elaboration of N-Monoalkyl [4,4]	
21- DM-[4,5] (2 μM)	2790 (100%)	no N-demethylation				no exposed primary aminopropyl terminal segment					
22- DE-[4,5] (100 μM)	4215 (100%)	no N-deethylation				"	"				
23- DP-[4,5] (100 μM)	1325 (69.7%)	N10-MP-[5,4]*	576 (30.3%)			"	"			no elaboration of N-Monoalkyl [5,4]	
25- DM-[5,5] (15 μM)	2560 (100%)	no N-demethylation				no exposed primary aminopropyl terminal segment					
26- DE-[5,5] (75 μM)	1585 (100%)	no N-deethylation				"	"				
27- DP-[5,5] (500 μM)	1300 (100%)	no N-depropylation				"	"				

#L1210 cells were grown 48 h in medium containing polyamine analogue at the indicated concentration. Then the polyamine contents of the cells were analyzed by HPLC of the fluorescent DANSYL derivatives.

^Concentrations of parent drug and metabolites in L1210 cells are in pmoles/10^6 cells and as % of total drug in the cell.

*An authentic sample of these presumed metabolites was not available for analytical reference. All other metabolites were identified and quantitated by comparison to authenticated reference compounds.

**Formed by deaminopropylation of primary metabolite MM-[3,3].

***Formed by N-depropylation of MP-[3,3].

Among the HSPD analogues, DEHSPD was active at 48 h with an IC_{50} of ~22 μM. Other analogues' IC_{50}s are at least 100 μM at 48 h. At 96 h, all have IC_{50}s in the range 0.3−0.9 μM except for DPHSPD which has an IC_{50} of 6 μM. Compared to the norspermidine and spermidine analogues, the homospermidine analogues as a group are more active. Of the homospermines studied, diethyl was more active than dimethyl (Table 1).

The (4,5) series were the most effective triamines identified. Specifically DM(4,5) and DE(4,5) have IC_{50} values in the 2−5 μM range, while the DP(4,5) has an IC_{50} around 100 μM at 48 h. At 96 h the IC_{50} values substantially decrease; even DP(4,5) has an $IC_{50} < 2$ μM. As the triamine chain increases in length from (4,5) to (5,5), the activity decreases at both 48 and 96 h. The tetraamine analogues DE(4,5,4) and DE(5,4,5) are more active than triamines DE(4,5) and DE(5,5), respectively, at both time points.

Competitive Uptake Determinations in L1210 Cells. The ability of the norspermidines, spermidines, homospermidines, (4,5) and (5,5) triamines to compete with radiolabeled SPD for uptake was evaluated (Table 1). The general trend is that the terminally alkylated triamines have higher K_i values than the unalkylated triamines and are thus less easily taken up by the cell. In the dialkylated series of spermidines, homospermidines and (4,5) triamines, K_i values increase as the size of the terminal group increases. However, with the dialkylated norspermidines and (5,5) triamines, the ethyl analogues are taken up the least. Finally, the number of methylenes separating the amines plays a role in determining polyamine uptake properties. The effectiveness with which the diethylated analogues compete for uptake is spermidine ≈ homospermidine > (4,5) triamine > (5,5) triamine > norspermidine. Interestingly this same trend is observed with the diethylated tetraamines, spermine ≈ homospermine > DE(4,5,4) > DE(5,4,5) > norspermine.

Polyamine Pools. The following guidelines were adopted for studying the impact of the analogues on polyamine pools (Table 2). The measurements were made after a 48 h exposure to the analogue, and two different concentrations of analogue were evaluated in each case. For analogues whose IC_{50} concentration exceeded 100 μM at 48 h, the polyamine pools were determined at 100 and 500 μM. In general, the effect on polyamine pools was evaluated at the 48 h IC_{50} concentration and at 5 times this number for the other analogues.

At 500 μM, the effects of DMNSPD, DENSPD, and MPNSPD on polyamine pools were similar, i.e., PUT was depleted below detectable limits, and spermidine was reduced to 6−15% of controls, while spermine levels were diminished to below 50%. DPNSPD was not as effective as the other norspermidine analogues in depletion of polyamine pools, e.g., at 500 μM, PUT was only lowered to 68%, SPD to 71%, and there was no effect on SPM level. The corresponding norspermines were again more effective. At 100 μM, the effect of DMNSPM and DENSPM on polyamine pools was similar, i.e., putrescine was depleted to below detectable limits, and spermidine was reduced to around 5% of controls, while spermine levels were diminished to 30−36%.

DMSPD and DESPD at 100 μM depleted PUT to below detectable limits, SPD to 5%, SPM to 58% and 74% of control, respectively. The monoalkylated SPD analogues MESPD(N^1), MESPD(N^8), and MPSPD(N^1) gave a similar pattern of polyamine pool depletion. At 100 μM, putrescine was depleted to below detectable levels, spermidine to 25−26%, and spermine to 80%, 84%, and 90% of control value. At 100 μM, MPSPD-(N^1) was more effective than MPSPD(N^8) in reducing SPD ($p < 0.002$), although the suppression in SPM was not significant ($p < 0.07$). At 500 μM, DPSPD reduced PUT to below detectable levels and SPD to around 10% of control. Like DPNSPD, DPSPD showed little suppression of SPM levels and possibly even some upregulation at 100 μM. Interestingly, at the level of PUT and SPD suppression MPSPD (N^1) and MPSPD (N^8) behave very much like their MESPD counterparts. However the propyl analogues are slightly less effective at spermine suppression. The parent amine SPD suppresses PUT but not SPD or SPM. Again the corresponding spermines are more effective than the triamines. At 100 or 500 μM DMSPM or MESPM or at 30 and 150 μM DESPM, putrescine was reduced to below detectable limits, spermidine diminished to under 2% of control, and spermine to under 25%. At 3 μM, DPSPM reduced putrescine to below detection, and spermidine to 18%, while the spermine level remained at 64% of control. At 15 μM DPSPM, spermidine was further reduced to 9% and spermine to 43%.

Among the homospermidine analogues, the parent triamine, HSPD, was the most active at polyamine suppression. At 100 μM, PUT was depleted again to below detectable levels, SPD to 4%, and SPM to 32%. With all of the HSPD analogues, at 500 μM, the level of putrescine was diminished to below detectable limits and the SPD level below 10% of control. DMHSPD and DEHSPD had little impact on SPM level, while MPHSPD produced a mild decrease. In the case of cells grown in 100 μM and 500 μM DPHSPD, the level of SPM was increased compared to the controls. As is usual the homospermines were more effective than the corresponding triamine counterparts. At 100 μM the homospermine analogue DMHSPM was similar to the corresponding alkylspermine in its ability to deplete the polyamines. However, DEHSPM was somewhat less effective at suppressing spermine pools in comparison to DESPM.

Similar results were observed with homospermidine homologues, the (4,5) and (5,5) triamines. At 500 μM the (4,5) and (5,5) parent amines depleted both PUT and SPD below detectable level, and SPM to 35% and 20% of control, respectively. DM(4,5) at 10 μM and DE(4,5) at 15 μM reduce PUT below detectable limits and SPD to 18% of control. However, neither is effective at reducing SPM levels. DP(4,5) even at 500 μM while it depletes the cell of PUT only reduces SPD to 31% of control with possible stimulation of SPM. Finally DP-(5,5) is only marginally active, requiring a 500 μM concentration to even reduce PUT by 50% and SPD by 30% and with no impact on SPM. However the homospermine homologues DE(4,5,4) and DE(5,4,5) both of which demonstrated low 48 h IC_{50} values, 0.3 and 0.4 μM, respectively, were similar to the corresponding triamines at reducing polyamines.

Impact of Analogues on ODC and AdoMetDC Activities. A comparison of the effects of the triamine *vs* tetraamine polyamine analogues on ODC and AdoMetDC clearly demonstrates that in general the tetraamines are more effective at suppressing these enzymes than the corresponding triamines. Previous studies[34,35] suggested that the effect of the polyamine

analogues on ODC and AdoMetDC is fairly rapid. For example, DESPM induced reduction in ODC activity plateaued at 4 h and AdoMetDC at 6 h. On the basis of these studies, we elected to evaluate the impact of the triamines on ODC and AdoMetDC at 4 and 6 h, respectively.

The parent triamine norspermidine reduced ODC activity to 11% of control, the corresponding dimethyl, DMSPD, to 17%, the diethyl analogue, DENSPD, to 80%, and the dipropyl compound, DPNSPD, had no effect on this enzyme (Table 3). Monoethylnorspermidine, MENSPD, was more active than the corresponding dialkyl analogue, DENSPD, with reduction to 42 *vs* 80% of control, as was the monopropyl, MPNSPD, relative to its dipropyl counterpart DPNSPD, with reduction to 33 *vs* 100% of control. In 4 h, 1 μM DMNSPM, MENSPM, or DENSPM reduced ODC activity to nearly the same extent, to approximately 7% of control. The triamines are generally less effective than the corresponding tetraamines at suppressing AdoMetDC, although the differences are not as profound with the norspermidines *vs* the norspermines. The alkylated norspermidines reduce the AdoMetDC to 41−58% of control and the norspermines to 33−49% of control, except the dipropyls, which are at best marginally effective.

The spermidine analogues are less active than the corresponding spermines but are more effective overall at reducing ODC activity than the norspermidines. At 1 μM DMSPM, MESPM, or DESPM, ODC activity was reduced to 10% or less of control, while ODC in DPSPM treated cells was only lowered to 52% of controls. The parent triamine spermidine reduces ODC to 16% of control while the alkylated analogues except for DPSPD diminish ODC activity to between 10 and 30% of control. Again the monoalkyl analogues are more effective than the corresponding dialkyl compounds. MESPD(N^1) and MESPD(N^8) reduce ODC to 10 and 17% *vs* 30% for DESPD. This property of the monoalkylated analogue is even further accentuated with the propylated spermidines MPSPD(N^1) and MPSPD(N^8) *vs* DPSPD lowering ODC to 18 and 14% *vs* 75% of control. Again when comparing dialkylated compounds, the larger the alkyl substituent the less active the analogue.

The spermidine analogues except for DPSPD reduce AdoMetDC to under 70% of control activity. DPSPD has no impact on the enzyme. Again as with ODC, the monoalkylated spermidine compounds were more active than the dialkylated compounds. DMSPM, MESPM, or DESPM at 1 μM almost paralleled the ability of the corresponding norspermine analogues to suppress AdoMetDC with an average reduction to 32% of control, more active than the spermidines. DPSPM at 1 μM reduced AdoMetDC activity to 72% of that seen in untreated cells.

The homospermidines were less active than the corresponding homospermines at reducing ODC activity but similar in behavior to the spermidines. Also, consistent with the norspermidine and spermidine results, the dipropyltriamine, i.e., with the largest substituent, was least effective, and removal of one of the propyls markedly increased the activity. Finally, the homospermidine analogues, except for MPHSPD, were not effective at AdoMetDC inhibition and certainly less active than the corresponding tetraamines.

Interestingly, adding a methylene unit to DEHSPM to produce DE(4,5,4) resulted in a decrease in ODC-suppressing activity. ODC was lowered to only 7% of control with DEHSPM and to 20% of control with DE-(4,5,4). This methylene addition had little effect on reduction of AdoMetDC activity, to about 40% for both. The same phenomenon was observed on moving from DMHSPD and DEHSPD to the dialkyl (4,5) and dialkyl (5,5) compounds in that the ODC-suppressing capacity substantially decreased. It is noteworthy however that the parent (4,5) triamine demonstrates reasonably effective suppression of ODC and AdoMetDC. The parent (5,5) triamine is an effective and highly selective ODC antagonist, reducing ODC to 16% of control but with little effect on AdoMetDC. Other than this there is little effect on either ODC or AdoMetDC by (5,5) analogues. The tetraamine analogue DE(5,4,5) is far more active against both ODC and AdoMetDC than DE-(5,5).

Impact of Triamine Analogues on SSAT Activity. While the influence of chain length and terminal substituents are more monotonic regarding their effect on the analogues' suppression of ODC and AdoMetDC, there are nevertheless some notable structure−activity relationships for SSAT stimulation. The ability of triamine analogues to upregulate SSAT in L1210 cells is remarkably sensitive to small structural changes (Table 3; Figure 1a). For example, the diethyltriamines stimulate SSAT 780% for DENSPD to a peak of 1380% for DESPD, with a decrease to 640% for DE(4,4) and falling to essentially control values for DE(4,5), 120%, and DE(5,5), 90%. The DE triamine structure−activity curve appears to be shifted to the right from the corresponding DE tetraamine curve (Figure 1b). Thus the DE tetraamine curve is maximal at 1480% of control for DENSPM and falls to nearly control value for DEHSPM, DE(4,5,4), and DE(5,4,5).

Substituent changes on the triamines have a profound effect on SSAT stimulation only with the (3,3) and (3,4) compounds. The differences are more compressed for the (4,4) and (4,5) triamines and absent with (5,5) triamines. In the case of the tetraamines the (3,3,3) system is the only framework in which a marked effect in SSAT stimulation is observed with substituent changes. While there are some changes with the (3,4,3) backbone, these are again compressed.

With both the triamines and tetraamines, unlike with ODC and AdoMetDC, there is no relationship between substituent size and SSAT upregulation. However when clear differences exist between stimulatory abilities, i.e., (3,3), (3,4), (3,3,3), the ethyl group is clearly the superior.

Metabolism. In an experiment focused on the impact of DPNSPD (**DP-[3,3]** in Table 4) on polyamine pools, a substantial unexpected peak appeared in the chromatogram of treated cells. The suspicious peak was shown to correspond to MPNSPD (**MP-[3,3]** in Table 4) as confirmed by coelution with an authentic sample. Note that the polyamines in Table 4 are designated by their terminal substituents, e.g., MP = monopropyl, and by the number of methylenes between successive nitrogens; thus **[3,3]** = NSPD, **[3,4,3]** = SPM, **[4]** = putrescine, and so forth. The intracellular levels of MPNSPD after a 48 h exposure to DPNSPD was about 50% of intracellular level of the parent compound. Although N-dealkylation had been shown to be an important step in the metabolism of the alkylated tetraamines DENSPM[32] and DEHSPM[36] *in vivo* in rodents, dogs, and humans, previous *in vitro* studies with DEHSPM or DESPM in L1210 cells[15] revealed

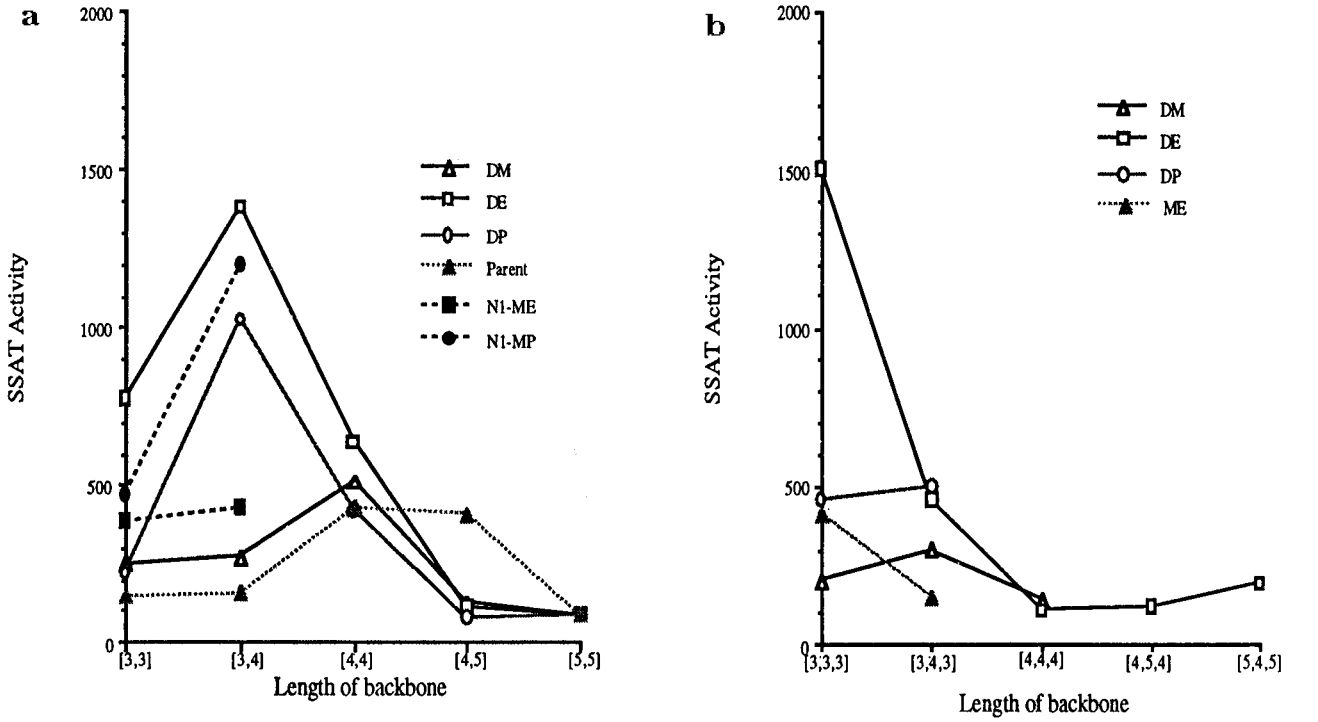


Figure 1. (a) The structure−activity relationship between the triamine analogues and SSAT upregulation. (b) The structure−activity relationship between the tetraamine analogues and SSAT upregulation.

Table 5. Metabolism of DPNSPD in Different Culture Systems[a]

assay no.	experiment treatments	metabolites (% of DPNSPD)
1	FBS[a] + L1210 (48 h)	MPNSPD (50)
2	Nuserum[b] + L1210 (48 h)	MPNSPD (50)
3	FBS (48 h)	MPNSPD (3)
4	albumin[c] + L1210 (4 h)	MPNSPD (50)
5	FBS + bathophenanthroline disulfonic acid (0.1 mM) (48 h)	MPNSPD (0)
6	FBS + L1210 + bathophenanthroline disulfonic acid (0.1 mM) (48 h)	MPNSPD (50)

[a] In all of the assays, RPMI-1640 was used as culture media. NuSerum IV is a semisynthetic FBS substitute, containing 25% of FBS. [b] At concentration of 10%. [c] At concentration of 1.5%.

either little or no N-dealkylation under the conditions of the experiments. The observation of N-depropylation of DPNSPD compelled us to look more closely at the metabolism of the polyamine analogues in L1210 cells. In particular we evaluated the significance of the nature of the *N*-alkyl groups on N-dealkylation in addition to the length and symmetry of the polyamine backbone.

In order to assure that the observation was not some artifact of the experimental conditions, we assessed whether or not components of the culture media itself were responsible for dealkylation (Table 5). Fetal bovine serum (FBS) for example, is well-known to contain amine oxidases.[37] Indeed, 1 mM aminoguanidine, an inhibitor[38] of bovine serum amine oxidase present in our standard L1210 cell culture media, did not totally eliminate such FBS-related amine oxidase activity. When the "complete" RPMI40 medium containing FBS and 1 mM aminoguanidine was incubated in the presence of 100 or 500 μM DPNSPD, a small amount (<3%) of the DPNSPD was metabolized to MPNSPD in the absence of L1210 cells. This corresponds to a comparatively low extracellular concentration of MPNSPD (~3 μM) and, given its relatively poor affinity (K_i = 33 μM) for the polyamine transport apparatus, argues against the extracellular medium as a major source of the high levels of MPNSPD (264 μM) seen intracellularly. This conclusion is further supported by experiments which partially or totally eliminate the source of extracellular metabolism. For example, when FBS was replaced with either NuSerum, a semisynthetic substitute, or purified bovine serum albumin, we still observed a high level of intracellular metabolite (50% of parent analogue, Table 5). The chelator bathophenanthroline disulfonic acid is a well-known inhibitor of the Cu-dependent amine oxidases present in plasma[39] and, given its comparatively high MW and anionic charge, does not cross the cell membrane.[40,41] As expected, bathophenanthroline disulfonic acid completely abolished the ability of RPMI40 + 10% FBS to convert DPNSPD to MPNSPD. However, when cells were grown in RPMI containing FBS and bathophenanthroline disulfonic acid, high intracellular concentrations of MPNSPD corresponding to ca. 50% of the intracellular DPNSPD content were observed. These results are in keeping with the idea that the dealkylation indeed takes place within L1210 cells.

Assured that we were indeed seeing the results of *intracellular* metabolic transformation of bisalkylated triamines, we undertook an examination of the influence of polyamine analogue structure on the metabolite pattern observed in L1210 cells. These results are detailed in Table 4 for the bisalkyltriamines and a number of their primary metabolites. Several representative tetraamine analogues are also included to demonstrate their similar metabolic fate to the corresponding triamines.

Three types of metabolic transformations explain the particular patterns observed (Figure 2). First bisalkyl-

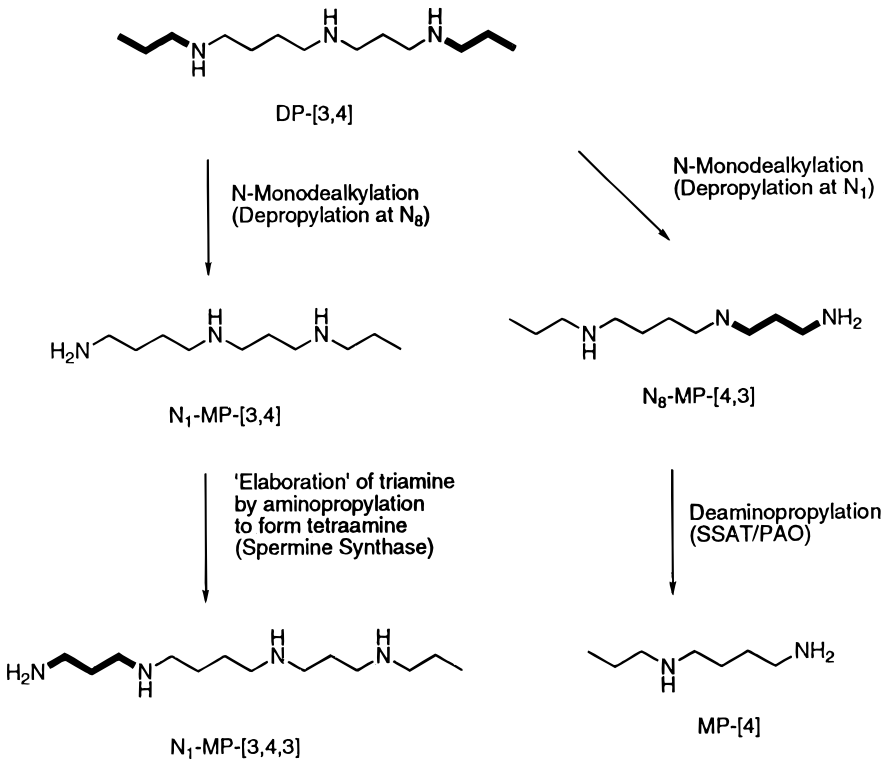


Figure 2. Three types of metabolic transformation of bisalkyltriamines in L1210 cells. (a) N-Dealkylation is a prerequisite for further metabolism. In L1210 cells: N-depropylation > N-deethylation. No N-demethylation was observed. (b) Deaminopropylation by SSAT/PAO can occur if N-dealkylation exposes a primary aminopropyl terminus. Deaminopropylation is most pronounced in analogues with a norspermine, [3,3,3], or norspermidine, [3,3], backbone. (c) Elaboration of a triamine to the corresponding tetraamine can occur when N-dealkylation exposes a primary aminobutyl terminus which can be aminopropylated by spermine synthase. Only analogues or metabolites with a spermidine, [3,4], backbone appear to serve as substrates (i.e. N^1-alkyl-[3,4]; not N^8-alkyl-[4,3], *N*-alkyl-[3,3], *N*-alkyl-[4,4], or N^{10}-alkyl-[5,4]).

polyamines must undergo *N-dealkylation* before any further metabolism can occur. If this N-dealkylation results in exposure of a primary aminopropyl segment, the primary metabolite(s) may undergo *deaminopropylation* by the SSAT/PAO polyamine degradation pathway. If this N-dealkylation results in exposure of a primary aminobutyl segment, then the triamine might undergo *elaboration* into a tetraamine by serving as a substrate for spermine synthase, which anneals an aminopropyl segment derived from *S*-adenosylmethionine (AdoMet) to the free aminobutyl end of the molecule. We will now detail the evidence as revealed in the metabolite patterns that support these three types of metabolic transformations and comment on the implications these data have with respect to the structural requirements of the corresponding enzyme systems *in vivo*.

A careful inspection of chromatograms from cells treated with **DM-[3,3]**, **DM-[3,4]**, **DM-[4,4]**, **DM-[4,5],** and **DM-[5,5]** revealed no N-demethylation (Table 4). The dimethyltetraamines **DM-[3,3,3]**, **DM-[3,4,3]**, and **DM-[4,4,4]** also showed no evidence of N-dealkylation (data not shown), and the unsymmetric tetraamine **MM-[3,3,3]** is only metabolized by deaminopropylation at the primary amine terminus end of the molecule.

Treatment of cells with **DE-[3,3]** or the corresponding tetraamine, **DE-[3,3,3]**, each resulted in the monodeethylated metabolite, **ME-[3,3]** or **ME-[3,3,3]**, respectively, in similar amounts: 4.7% on a mole percent basis of the total (parent drug + identified metabolites) in the cell. Interestingly, cells treated with the unsymmetric **DE-[3,4]** contain each of the two possible monodeethylated metabolites, **N1-ME-[3,4]** and **N8-ME-[4,3]**, with the total amount representing about 4% of the drug in the cell. Among the diethylated triamine analogues, only **DE-[3,3]** and **DE-[3,4]** showed N-deethylated metabolite(s); the analogues with longer backbones, i.e., **DE-[4,4]**, **DE-[4,5]**, and **DE-[5,5]**, do not show significant N-deethylation at all.

Of the five different dipropyltriamines which were evaluated, **DP-[3,3], DP-[3,4]**, **DP-[4,4]**, **DP-[4,5]**, and **DP-[5,5]**, all but **DP-[5,5]** showed significant N-depropylation. As suggested from the quantity of analogue present in cells as monodealkylated metabolite (Table 4), N-depropylation in general occurs to a greater extent than N-deethylation. For example, in L1210 cells treated with **DP-[3,3]**, 57.7% is present as the parent drug, **DP-[3,3]**, 30.6% as the mono-N-dealkylated metabolite, **MP-[3,3]**, 9.1% as the di-N-dealkylated metabolite, **[3,3]**, and 2.6% as the secondary metabolite, **MP-[3]**, formed by deaminopropylation of **MP-[3,3]**. The same general pattern holds for cells treated with the corresponding tetraamine, **DP-[3,3,3]**, where 14.0% of the total is present as **MP-[3,3,3]** and 7.8% as **MP-[3,3]** formed by secondary deaminopropylation of **MP-[3,3,3]**. Cells treated with the corresponding diethyl analogues contain substantially lower amounts of metabolites by comparison so that 90.7% of **DE-[3,3]** or 95.3% of **DE-[3,3,3]** is present as the unmetabolized parent compound.

The dipropyltriamine with the shortest backbone **DP-[3,3]** seemed most sensitive to metabolism with **MP-[3,3]** representing 31% of the total drug. With **DP-[3,4]** both possible monoalkylated products **N1-MP-[3,4]** and **N8-MP-[4,3]** were detected at levels corresponding to 4.6% and 5.3%, respectively, of the total drug in the cell. Cells exposed to **DP-[4,4]** contained the mono-N-dealkylated metabolite, **MP-[4,4]**, representing 12.3%

of the total drug in the cell. Interestingly, only one of the two possible N-dealkylated metabolites was apparent in cells treated with the unsymmetric triamine **DP-[4,5]**, and this **MP-[5,4]** metabolite represented 30.3% of the total drug in the cell. When **DP-[5,5]** was evaluated, no metabolic products were found, suggesting that the aminobutyl end of the **DP-[4,5]** system was selectively dealkylated to form the monodealkylated metabolite, **N10-MP-[5,4]**.

If mono-N-dealkylation exposes a primary aminopropyl terminus, this compound is subject to further metabolism by the SSAT/PAO system present in all cells. First SSAT acetylates the exposed primary amine end, then PAO oxidatively deaminates at the interior secondary amino nitrogen of the acetamidopropylamine segment to give 3-acetamidopropanal, i.e., net *deaminopropylation* of the substrate. PAO actively deaminopropylates N^1-acetylspermine and N^1-acetylspermidine, the native substrates, but does not recognize the acetamidobutyl segment of N^8-acetylspermidine or *N*-acetylputrescine as substrate. Table 4 demonstrates that in L1210 cells there is a strict adherence to this specificity for a primary aminopropyl segment for further metabolism of the monoalkylated triamines, i.e., only examples of deaminopropylation are observed. For example the tetraamine **MM-[3,3,3]** shows a substantial amount of the deaminopropylation metabolite, **MM-[3,3]** representing 16.5% of the total drug in the cell, and even some **MM-[3]** (0.6%), the product of deaminopropylation of **MM-[3,3]**. No examples of deaminobutylation are seen, e.g., **N1-alkyl-[3,4]**, **monoalkyl-[4,4]**, and **monoalkyl-[5,4]** do not give rise to such metabolites. In the case of cells treated with **ME-[3,3]** or **MP-[3,3]**, both N-dealkylation and deaminopropylation are available paths of primary metabolism. The deaminopropylation metabolite, **ME-[3]**, represented 21.3% of the total drug in the **ME-[3,3]**-treated cells compared to only 0.5% for the N-deethylation product, **[3,3]**. In contrast the N-depropylation product, **[3,3]** (4.5%), predominated compared to the deaminopropylation metabolite, **MP-[3]** (1.2%) in **MP-[3,3]**-treated cells.

In cells treated with the N^1-monoalkylated spermidines, **N1-ME-[3,4]** or **N1-MP-[3,4]**, peaks corresponding to the respective tetraamines **ME-[3,4,3]** (3.7% of total drug in cell) and **MP-[3,4,3]** (6.1% of total drug in cell) were observed in the HPLC chromatograms of the dansylated cell extract (Table 4). In the case of cells containing substantial amounts of triamine analogues with a free primary aminopropyl end (i.e. **ME-[3,3]**, **MP-[3,3]**, **N8-ME-[4,3]**, and **N8-MP-[4,3]**), no evidence of a tetraamine elaboration metabolite was observed. Only in those cases where a free aminobutyl end was available on a spermidine, **[3,4]**, backbone was a tetraamine metabolite produced. No such metabolite was produced from triamines with a free aminobutyl end on a longer backbone (i.e. **MP-[4,4]** or **N10-MP-[5,4]**).

Thus it is likely that at least two of the pathways responsible for metabolic transformation of these analogues involve enzymes of the polyamine metabolic cycle present in all cells. Spermine synthase is responsible for elaboration of an N^1-alkylspermidine to the corresponding N^1-alkylspermine by annealing an aminopropyl segment to the primary aminobutyl end of the triamine. The deaminopropylation observed in L1210 cells treated with triamine and tetraamine analogues is readily explained as a consequence of action by the SSAT/PAO polyamine degradative enzymes. The possibility that the N-dealkylation step required for further metabolic transformation of bisalkylpolyamines may also involve PAO is an interesting question raised by the metabolic patterns observed.

N-Dealkylation of analogues with a hydrophobic segment shorter than *N*-propyl appears to occur much less efficiently in the case of *N*-ethyl, or not at all in the case of *N*-methyl. Among the reported amine oxidases, polyamine oxidase (PAO) is the only one which usually attacks at a secondary amine center, three hydrophobic methylene carbons internal to the neutral N^1-acetamido nitrogen terminus of N^1-acetylspermidine, for example. The corresponding acetamidobutyl segment of N^8-acetylspermidine is not recognized and therefore not deaminobutylated. We are currently examining the possibility that purified polyamine oxidase may recognize such a similarity between its native substrate and *N*-propyl analogues and catalyze N-depropylation at a significant rate, similar to the rate of deaminopropylation of the native substrates, N^1-acetylspermine and N^1-acetylspermidine.

Conservation of Charge. In two earlier studies we noted that there was a conservation of charge with respect to the total tetraamine cationic picoequivalence in the cell.[20,36] For example if after 24 h of exposure to an alkylated polyamine each of the equivalent concentrations associated with charge on the amines of both the analogues and natural polyamine is added together, the numbers are fairly constant. For example, each picoequivalent of putrescine is associated with two picoequivalents of cationic charge, each picoequivalent of spermidine or its analogues with three, and each picoequivalent of spermine or its analogues with four. In order to maintain this balance of charge the cell processes the natural polyamines, e.g., exports them as it incorporates the analogues. The maintenance of total cellular charge holds for all of the triamines examined, except the (5,5) triamines (Table 6). The implication is that the cell will not incorporate analogue beyond a point where the charge balance is disrupted, at which time cell death may occur. We are currently exploring this issue further. In the case of the tetraamines the conservation of charge behavior seems to hold for 24 h but erodes after a period of time.[20] With the triamines the conservation of charge continues even at 48 h.

Acute and Chronic Toxicity of Triamines. In early studies of polyamine toxicity in laboratory animals, triamines were found to be less toxic than tetraamines. Spermidine was approximately one-twentieth as nephrotoxic as spermine, and putrescine was the least toxic.[42,43]

In the current study, the acute toxicity of six analogues and the chronic toxicity of two triamines were measured (Table 7). The value of all polyamine LD_{50}s are shown in both mg/kg and mmol/kg for comparison. For acute toxicities, the polyamine analogues were administered as a single ip injection to groups of five or six animals at each dose. The animals were scored 2 h after administration of drug. It is clear that the acute LD_{50}s for triamine analogues are approximately twice the acute LD_{50}s for the corresponding tetraamine analogues.

In the chronic toxicity regimen, mice were administered the polyamine analogue in three doses per day (tid) for 6 days for a total of 18 injections per animal

Table 6. Summation of Intracellular Levels of Analogues and Polyamines Analyzed for Amine Equivalence after Exposure to Polyamine Analogues[a]

polyamine analogues	picoequivalents of amine/10^6 cells ($\times 10^3$)	av ± SD
control cell	13.21	
2, DMNSPD	18.40	
4, DENSPD	13.70	
5, MPNSPD	17.71	
6, DPNSPD	15.01	16.21 ± 2.22
8, DMSPD	16.09	
9, MESPD(N^1)	16.99	
10, MESPD(N^8)	17.99	
11, DESPD	14.05	
12, MPSPD(N^1)	18.25	
13, MPSPD(N^8)	19.59	
14, DPSPD	15.33	16.90 ± 1.89
16, DMHSPD	20.34	
17, DEHSPD	16.92	
18, MPHSPD	20.55	
19, DPHSPD	15.99	18.45 ± 2.34
21, DM(4,5)	20.81	
22, DE(4,5)	14.59	
23, DP(4,5)	15.15	16.85 ± 3.44
25, DM(5,5)	15.5	
26, DE(5,5)	9.01	
27, DP(5,5)	13.91	12.81 ± 3.38
all analogues	mean	16.47 ± 2.08

[a] The L1210 cells were treated with polyamine analogues at 500 μM, except DEHSPD (125 μM), DM(4,5) (10 μM), DE(4,5) (15 μM), DM(5,5) (75 μM), and DE(5,5) (75 μM), for 48 h. Levels of amine equivalence for every analogue treated cells are averages from analysis of triplicate cell samples. Values are obtained by multiplying the number of moles of spermine by four, spermidine by three, putrescine by two, and analogue by three. The typical control values in nmol/million L1210 cells are PUT = 0.260 ± 0.059, SPD = 3.354 ± 0.361, SPM = 0.658 ± 0.119.

and observed for 10 days after the final dose for lethalities. DE(4,5) was the most active triamine against L1210 cells *in vitro*, and the spermidine analogue DE-(3,4) demonstrated much less toxicity in mice than the related tetraamines DE(4,5,4), DE(5,4,5), and DE(3,4,3). In our early study of tetraamines, a preliminary investigation suggested a direct ratio relationship between the IC_{50} and the chronic LD_{50} values.[15] However, in the triamine systems, the 96 h IC_{50} values of DE(4,5) suggested that this triamine should be 5−7 times less toxic than corresponding tetraamine analogue, DE-(4,5,4), or the longer DE(5,4,5) (Table 1), but in fact DE-(4,5) is almost *11-fold less toxic* than DE(4,5,4) and more than *14-fold less toxic* than DE(5,4,5) *in vivo* (Table 7). A similar difference is also observed in the chronic toxicity of DE(3,4). The ratio of the triamine to the tetraamine 96 h IC_{50}s suggests that DE(3,4) should be approximately 4 times less toxic than DE(3,4,3), but in fact DE(3,4) is greater than *6 times* less toxic than DE-(3,4,3) *in vivo*. These results suggest a potential widening of the therapeutic window, which renders the triamine analogues as promising antineoplastics of lower toxicity and encourages further pursuit of animal studies.

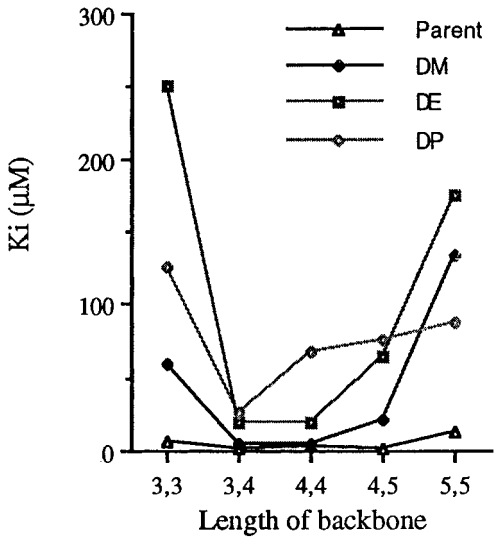


Figure 3. The structure−activity relationship between the triamine analogues and K_i values.

Discussion

The study serves to define the similarities and differences between triamine and tetraamine analogue antineoplastics. With both types of analogues, K_i values are sensitive to the size of the terminal substituents and the length of the backbone. This is illustrated for triamines in Figure 3. Generally the larger the terminal substituent, the more poorly the analogues are transported. In the triamine family spermidine analogues are the best transport competitors. Interestingly, the (3,3) and (5,5) triamine analogues are most sensitive to N-terminal substituent changes. Although a tetraamine competes better with SPD for uptake than the corresponding triamine, the triamines are more effectively accumulated in L1210 cells than the corresponding tetraamines. Once in the cell tetraamine analogues have a greater impact on lowering overall polyamine pools; however, the triamines are more selective at reducing spermidine. The total intracellular charge in picoequivalents associated with polyamines, both native and analogues, is maintained by cells exposed to both tetraamines and triamines. However, cells treated with triamines are able to maintain this charge balance for a more prolonged period of time. We were able to demonstrate that triamine analogue dealkylation was very specific for triamines with backbones of less than or equal to four methylenes and most effective for triamines and tetraamines with N^{α},N^{ω}-dipropyl substituents. We are currently trying to relate this finding to the effectiveness with which polyamine oxidase operates on the various analogues.

The tetraamine analogues are uniformly more active against L1210 cells than their triamine counterparts. With both the triamine and tetraamine analogues the

Table 7. Comparison of the Acute and Chronic Toxicity of Tetraamine and Triamine Analogues on Mice[a]

tetraamines			triamines		
compd	acute[b] LD_{50}, mg/kg (mmol/kg)	chronic[c] LD_{50} mg/kg/day (mmol/kg/day)	compd	acute LD_{50}, mg/kg (mmol/kg)	chronic LD_{50}, mg/kg/day (mmol/kg/day)
DE-[3,4,3]	340 (0.842)	87 (0.215)	DE-[3,4] (**11**)	>650[e] (>3.22)	426 (1.37)
DE-[4,5,4]	285 (0.638)	48 (0.104)	DE-[4,5] (**22**)	555 (1.64)	375 (1.11)
DE-[5,4,5] (**29**)	195 (0.424)	36 (0.078)	DE-[5,5] (**26**)	500 (1.42)	nd

[a] All of the polyamine analogues were administrated in the form of hydrochloride salts. [b] Single dose ip. [c] Multiple dose ip (tid × 6 days). [d] At a single dose of 250 mg/kg, no death within the initial 2 h, but all six animals were expired within seven days. [e] 15 mg/kg (tid × 3 day), 4/5 died on day 6 and 5/5 died on day 7. [f] At a single dose of 600 mg/kg, no death within the initial 2, but 5/5 died within seven days.

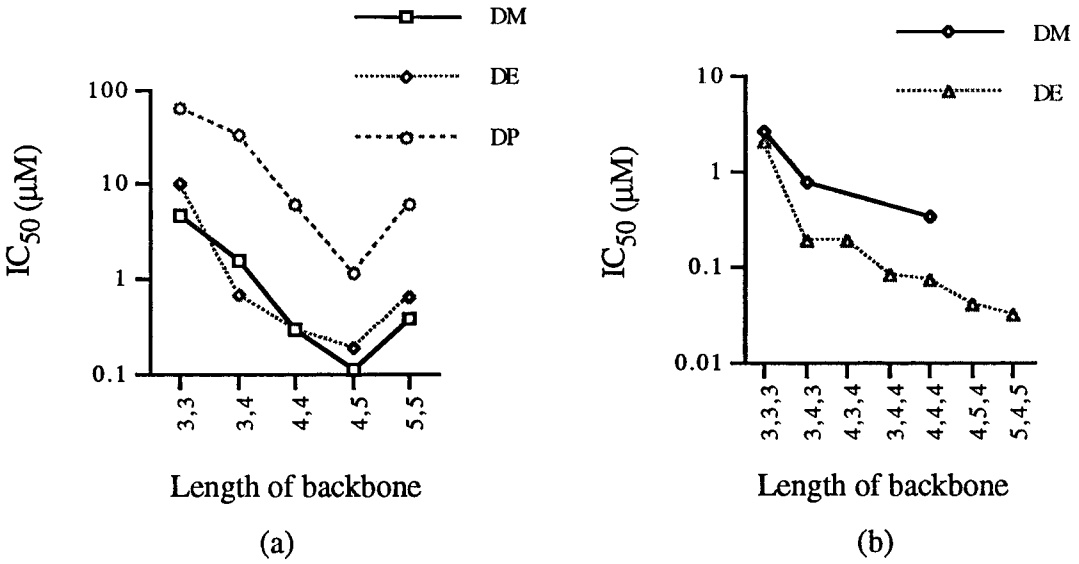


Figure 4. (a) The structure−activity relationship between the triamine analogues and 96 h IC_{50} values. (b) The structure−activity relationship between the tetraamine analogues and 96 h IC_{50} values.

compounds' IC_{50} values are also sensitive to the size of the terminal substituent and the length of the backbone. However, the overall length between the terminal nitrogens is the most critical issue in assessing this activity; Figure 4a illustrates the triamine case. When comparing N^1- with N^8-monoalkylspermidines, the N^1 compounds, both ethyl and propyl were more active than the N^8 compound. The fact that the N^1 compounds are elaborated by the cell to the corresponding and more active N^1-alkylspermines is in keeping with this observation. Recall that the N^8-alkylspermidines cannot be elaborated and thus are only degraded by the polyamine biosynthetic network. While we have not yet achieved the optimum length for the tetraamine activity (Figure 4b), evidence would suggest that we have identified the optimum length for the triamines as seen in the terminally dialkylated (4,5) methylene backbone series.

The triamine analogues are less toxic than the corresponding tetraamines. Furthermore, and most important when comparing the ratio of the 96 h IC_{50}/chronic LD_{50} values of the two triamines, DE(3,4) and DE(4,5), with the corresponding tetraamines, a kind of therapeutic window, the triamines appear more favorable. This is a critical issue in the choice of the best polyamine therapeutic. In fact this finding has compelled us to revisit the issue of triamine analogues in whole animal models.

Experimental Section

MENSPD (**3**)[32] and tetraamine analogues,[14,15] except for DPNSPM and DE(5,4,5),[29] were previously synthesized in this laboratory. N^1- and N^8-Acetylspermidine dihydrochlorides were obtained from Sigma Chemical Co. Chemical reagents were purchased from Aldrich Chemical Co.; *N*-(3-aminopropyl)-1,3-propanediamine (**1**) was converted to its trihydrochloride salt and recrystallized from aqueous ethanol. Sodium hydride reactions were run in distilled DMF under an inert atmosphere. THF was distilled from sodium and benzophenone. Fisher Optima grade solvents were routinely used, and organic extracts were dried with sodium sulfate. Silica gel 32−63 (40 μm "flash") from Selecto, Inc. (Kennesaw, GA) was used for flash column chromatography. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Proton NMR spectra were run at 90 or 300 MHz in $CDCl_3$ (not indicated) or D_2O with chemical shifts in parts per million downfield from tetramethylsilane or 3-(trimethylsilyl)propionic-*2,2,3,3-d*$_4$ acid, sodium salt, respectively. Coupling constants (*J*) are in hertz. FAB mass spectra were run in a glycerol/trifluoroacetic acid matrix. Elemental analyses were performed by Atlantic Microlabs, Norcross, GA.

Cell culture materials were obtained from Sigma Chemical Co. RPMI-1640 medium, fetal bovine serum, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), and 3-(*N*-morpholino)propanesulfonic acid (MOPS) were obtained from Gibco (Grand Island, NY). Cell numbers were determinated by electronic particle analysis (Coulter Counter, Model ZF, Coulter Electronics, Hialeah, FL). The solid phase extraction columns (SPE-3 mL-500 mg) were obtained from J. T. Baker Chemical Co. (Phillipsburg, NJ). Murine L1210 leukemia cells were obtained from the American Type Tissue Corp. (Bethesda, MD).

[^{3}H]Spermidine for uptake determinations and acetyl coenzyme A (acetyl-*1*-^{14}*C*) were purchased from New England Nuclear Res. (Boston, MA). L-[*Carboxyl*-^{14}C]ornithine and *S*-adenosyl-L-[*carboxyl*-^{14}C]methionine for enzyme assays were obtained from Amersham Corp. (Arlington Heights, IL).

Cell Culture and IC_{50} Determination. Murine L1210 leukemia cells (ATCC CCL 219) were maintained in logarithmic growth in RPMI-1640 medium containing 10% fetal calf serum or a semisynthetic equivalent, NuSerum (Collaborative Research, Bedford, MA), 2% HEPES-MOPS buffer, and 1 mM aminoguanidine. The IC_{50}s, the concentration of compound which reduces cell growth to 50% of untreated control cell growth, was determined after 48 and 96 h of exposure to polyamine analogue as detailed elsewhere.[15]

Polyamine Pool Analysis. L1210 cells in logarithmic growth were treated with polyamine analogue at the concentrations indicated in Table 2 for 48 h. The cells were washed twice with cold RPMI-1640, and the pellet was treated with 0.6 N $HClO_4$ (1 mL/10^7 cells). Polyamine contents of the perchloric acid extracts were quantitated by HPLC of the DANSYL derivatives.[15]

Uptake Determinations. The polyamine derivatives were studied for their ability to compete with [^{3}H]SPD for uptake into L1210 cells.[15] Lineweaver−Burk plots indicated a simple competitive inhibition with respect to SPD.

Enzyme Assays. ODC and AdoMetDC activities were determined as $^{14}CO_2$ released from [^{14}C]-carboxyl-labeled L-ornithine[44] or *S*-adenosyl-L-methionine,[45] respectively. Included in each assay were untreated L1210 cells as controls as well as cells treated with DEHSPM, a drug having a known reproducible effect on each enzyme, as positive controls.

Spermidine/spermine N^1-acetyltransferase activity was based on quantitation of [^{14}C]-N^1-acetylspermidine formed by acetylation of SPD with [^{14}C]acetyl coenzyme A according to the method of Libby et al.[27] Cells treated with DENSPM were positive controls.

Toxicity in Mice. Acute and chronic toxicities were

assessed in 10−12-week-old CD-1 female mice from Harlan Sprague−Dawley, Indianapolis, IN. For acute toxicities, the polyamine analogues were administered in a single ip injection to groups of five or six animals at each dose. The animals were scored 2 h after administration of the dose. All survivors were further observed for 10 days to assess late onset of toxicity from the single acute dose. In the chronic toxicity regimen, mice were administered polyamine analogue in three ip doses per day (tid) for 6 days, a total of 18 doses per animal. Appetite, weight, and overall appearance were monitored daily. Animals were observed for 10 days following the final dose, at which time the final score was registered. At least three test groups of five or six animals each, representing three different dose levels, were evaluated for each analogue tested. These dose levels were chosen so that at least two groups presented with lethalities, one with a high fraction of lethalities (>0.50, but <1.00).

***N*¹,*N*⁴,*N*⁷-Tris(mesitylenesulfonyl)-*N*¹,*N*⁷-dimethylnorspermidine (43).** NaH (60% in oil, 0.44 g, 11 mmol) was added to a solution of **30**[32] (3.39 g, 5 mmol) in DMF (70 mL) at 0 °C. After hydrogen evolution ceased (30 min), iodomethane (1.63 g, 11.5 mmol) was slowly added to the mixture. After being stirred for 12 h at room temperature, the reaction mixture was quenched with distilled water (10 mL). The solvents were removed under high vacuum, and the residue was combined with H_2O (30 mL) and extracted with $CHCl_3$ (4 × 40 mL). The organic portion was washed with brine (80 mL) and evaporated by rotary evaporation. Purification by column chromatography (8:1 toluene/EtOAc) gave 2.47 g (70%) of **43** as an oil: NMR δ 1.73 (quintet, 4 H), 2.29 (s, 6 H), 2.54−2.55 (2 s, 18 H), 2.60 (s, 6 H), 2.99−3.06 (2 t, 8 H, J = 7), 6.94 (s, 6 H). Anal. ($C_{35}H_{51}N_3O_6S_3$) C, H, N.

***N*¹,*N*⁷-Dimethylnorspermidine Trihydrochloride (2).** HBr (30% in HOAc, 30 mL) was added slowly to a mixture of **43** (2.13 g, 3.02 mmol) and phenol (12.27 g, 0.13 mol) in CH_2Cl_2 at 0 °C. After the reaction mixture was stirred for 1 day at room temperature, H_2O (20 mL) was added followed by extraction with CH_2Cl_2 (3 × 30 mL). The aqueous portion was concentrated under high vacuum, and the residue was basified to pH 14 with 1 N NaOH (4 mL) and 19 N NaOH (2 mL) and extracted with $CHCl_3$ (14 × 10 mL). The organic extracts were concentrated, and the residue was taken up in absolute EtOH (40 mL) and acidified with concentrated HCl (2 mL). After the solvents were removed, the solid was recrystallized from aqueous EtOH to generate 0.298 g (37%) of **2** as plates: NMR (D_2O) δ 2.08−2.19 (m, 4 H), 2.75 (s, 6 H), 3.13−3.22 (m, 8 H). Anal. ($C_8H_{24}Cl_3N_3$) C, H, N.

***N*¹,*N*⁴,*N*⁷-Tris(mesitylenesulfonyl)-*N*¹,*N*⁷-diethylnorspermidine (44).** NaH (60%, 9.0 g, 0.23 mol), **30**[32] (70.0 g, 0.103 mol), and iodoethane (19 mL, 0.24 mol) in DMF (400 mL) were reacted and worked up by the method of **43**. Column chromatography (1:4 EtOAc/petroleum ether) afforded 63.7 g (84%) of **44** as a viscous oil: NMR δ 0.97 (t, 6 H, J = 7), 1.60−1.72 (m, 4 H), 2.29 (s, 9 H), 2.54 and 2.55 (2 s, 18 H), 2.95−3.15 (m, 12 H), 6.92 (s, 6 H). Anal. ($C_{37}H_{55}N_3O_6S_3$) C, H, N. A sample was recrystallized from EtOAc/petroleum ether, mp 97 °C.

***N*¹,*N*⁷-Diethylnorspermidine Trihydrochloride (4).** HBr (30% in HOAc, 300 mL), **44** (23.0 g, 30.7 mmol), and phenol (116 g, 1.23 mol) in CH_2Cl_2 (300 mL) were reacted, and product was isolated using the procedure of **2** to give 6.45 g (71%) of **4** as colorless plates: NMR (D_2O) δ 1.30 (t, 6 H, J = 7), 2.05−2.18 (m, 4 H), 3.08−3.22 (m, 12 H). Anal. ($C_{10}H_{28}Cl_3N_3$) C, H, N.

***N*¹-Propyl-*N*¹,*N*⁴,*N*⁷-tris(mesitylenesulfonyl)norspermidine (45).** NaH (60%, 0.52 g, 13 mmol), **30**[32] (2.57 g, 3.8 mmol), and 1-iodopropane (0.46 mL, 4.7 mmol) in DMF were reacted and worked up by the method of **43**. Column chromatography (3:1 hexane/EtOAc) afforded 1.16 g (23%) of **45** as an oil: NMR δ 0.66 (t, 3 H, J = 7), 1.23−1.31 (m, 2 H), 1.58−1.62 (m, 4 H), 2.26−2.27 (2 s, 9 H), 2.51−2.52 (2 s, 12 H), 2.59 (s, 6 H), 2.82−2.99 (m, 8 H), 3.21 (t, 2 H, J = 7), 4.85 (br t, 1 H), 6.89−6.92 (m, 6 H). Anal. ($C_{36}H_{53}N_3O_6S_3$) C, H, N.

***N*¹-Propylnorspermidine Trihydrochloride (5).** HBr (30% in HOAc, 30 mL), **45** (1.14 g, 1.58 mmol), and phenol (6.4 g) in CH_2Cl_2 were reacted, and product was isolated using the procedure of **2** to give 87 mg (20%) of **5** as crystals: NMR (D_2O) δ 0.98 (t, 3 H, J = 7), 1.67−1.75 (m, 2 H), 2.07−2.16 (m, 4 H), 3.01−3.21 (m, 10 H). Anal. ($C_9H_{26}Cl_3N_3$) C, H, N.

***N*¹,*N*⁷-Dipropyl-*N*¹,*N*⁴,*N*⁷-tris(mesitylenesulfonyl)norspermidine (46).** NaH (60%, 0.44 g, 11 mmol), **30**[32] (3.39 g, 5 mmol), and 1-iodopropane (1.95 g, 11.5 mmol) in DMF (70 mL) were reacted and worked up by the method of **43**. Column chromatography (3:1 hexane/EtOAc) afforded 3.54 g (93%) of **46** as an oil: NMR δ 0.7 (s, 6 H), 1.20−1.65 (m, 8 H), 2.25 (s, 9 H), 2.50 (s, 18 H), 2.80−3.05 (m, 12 H), 6.87 (s, 6 H). Anal. ($C_{39}H_{59}N_3O_6S_3$) C, H, N.

***N*¹,*N*⁷-Dipropylnorspermidine Trihydrochloride (6).** HBr (30% in HOAc, 80 mL), **46** (3.475 g, 4.57 mmol), and phenol (15.8 g) in CH_2Cl_2 (30 mL) were reacted, and product was isolated by the procedure of **2** to provide 1.26 g (85%) of **6** as plates: NMR (D_2O) δ 0.87 (t, 6 H, J = 7), 1.60 (m, 4 H), 2.01 (m, 4 H), 2.93 (t, 4 H, J = 7), 3.06 (m, 8 H). Anal. ($C_{12}H_{32}Cl_3N_3$) C, H, N.

***N*¹,*N*⁴,*N*⁸-Tris(mesitylenesulfonyl)spermidine (31).** Mesitylenesulfonyl chloride (6.87 g, 31.4 mmol) in CH_2Cl_2 (30 mL) was added to spermidine trihydrochloride (2.5 g, 9.8 mmol) in 1 N NaOH (35 mL) at 0 °C, and the mixture was efficiently stirred at room temperature overnight. The layers were separated, and the aqueous phase was extracted with $CHCl_3$ (3 × 50 mL). The organic phase was the washed with brine (100 mL), evaporated, and purified by column chromatography (4:3 hexane/EtOAc) to give 3.73 g (55%) of **31** as a white foam: NMR δ 1.30 (m, 2 H), 1.44 (m, 2 H), 1.66 (m, 2 H), 2.30 (s, 9 H), 2.46 (s, 6 H), 2.60 (s, 12 H), 2.76 (q, 2 H), 2.84 (q, 2 H), 3.04 (t, 2 H, J = 7), 3.24 (t, 2 H, J = 7), 4.56 (br t, 1 H), 4.92 (br t, 1 H), 6.90 (s, 2 H), 6.95 (s, 4 H). Anal. ($C_{34}H_{49}N_3O_6S_3$) C, H, N.

***N*¹,*N*⁸-Dimethyl-*N*¹,*N*⁴,*N*⁸-tris(mesitylenesulfonyl)spermidine (47).** NaH (60%, 0.41 g, 10 mmol), **31** (2.15 g, 3.11 mmol), and iodomethane (0.62 mL, 10 mmol) in DMF (60 mL) were reacted and worked up as was **43**. Column chromatography (5:3 hexane/EtOAc) furnished 2.24 g (100%) of **47** as an oil: NMR δ 1.39−1.43 (m, 4 H), 1.69−1.78 (m, 2 H), 2.28 (s, 3 H), 2.30 (s, 6 H), 2.55 (s, 12 H), 2.57 (s, 6 H), 2.60 (s, 3 H), 2.62 (s, 3 H), 2.96−3.13 (m, 8 H).

***N*¹,*N*⁸-Dimethylspermidine Trihydrochloride (8).** HBr (30% in HOAc, 60 mL), **47** (2.24 g, 3.11 mmol), and phenol (12.3 g) in CH_2Cl_2 (30 mL) were reacted, and product was isolated by the procedure of **2** to give 0.658 g (75%) of **8** as crystals: NMR (D_2O) δ 1.77−1.82 (m, 4 H), 2.06−2.18 (m, 2 H), 2.73 (s, 3 H), 2.75 (s, 3 H), 3.06−3.19 (m, 8 H). Anal. ($C_9H_{26}Cl_3N_3$) C, H, N.

***N*¹,*N*⁸-Diethyl-*N*¹,*N*⁴,*N*⁸-tris(mesitylenesulfonyl)spermidine (48).** NaH (80%, 0.68 g, 23 mmol), **31** (7.06 g, 10.2 mmol), and iodoethane (2.5 mL, 31 mmol) in DMF (75 mL) were combined as in **43**. The mixture was heated at 65 °C for 12 h, cooled, and cautiously quenched with water (70 mL) and brine (100 mL), followed by extraction with EtOAc (5 × 100 mL). Combined organic extracts were washed with 100 mL of 1% Na_2SO_3, H_2O (2×), and brine. The solvents were removed, and the residue was purified by column chromatography (4.5% EtOAc/CH_2Cl_2) to produce 7.21 g (94%) of **48** as an oil: NMR δ 0.8−1.8 (m, 12 H), 2.28 (s, 9 H), 2.54 (s, 18 H), 2.8−3.3 (m, 12 H), 6.90 (s, 6 H). Anal. ($C_{38}H_{57}N_3O_6S_3$) C, H, N.

***N*¹,*N*⁸-Diethylspermidine Trihydrochloride (11).** HBr (30% in HOAc, 150 mL), **48** (7.16 g, 9.57 mmol), and phenol (28 g, 0.30 mol) in CH_2Cl_2 (125 mL) were reacted, and product was isolated utilizing the procedure of **2** to give 2.17 g (73%) of **11** as white plates: NMR (D_2O) δ 1.28 and 1.30 (2 t, 6 H, J = 7), 1.73−1.85 (m, 4 H), 2.06−2.18 (m, 2 H), 3.04−3.21 (m, 12 H). Anal. ($C_{11}H_{30}Cl_3N_3$) C, H, N.

***N*¹,*N*⁸-Dipropyl-*N*¹,*N*⁴,*N*⁸-tris(mesitylenesulfonyl)spermidine (49).** NaH (80%, 0.80 g, 27 mmol) was added to **31** (8.13 g, 11.7 mmol) in DMF (75 mL) at 0 °C. The mixture was stirred at room temperature for 1 h, and 1-iodopropane (3.5 mL, 36 mmol) was added by syringe. The mixture was stirred at 80 °C for 12 h and worked up as was **48**. Purification by column chromatography (3.5% EtOAc/CH_2Cl_2) resulted in 8.42 g (93%) of **49** as an oil: NMR δ 0.55−1.72 (m, 16 H), 2.25 (s, 9 H), 2.50 (s, 18 H), 2.7−3.3 (m, 12 H), 6.87 (s, 6 H). Anal. ($C_{40}H_{61}N_3O_6S_3$) C, H, N.

***N*¹,*N*⁸-Dipropylspermidine Trihydrochloride (14).** HBr (30% in HOAc, 150 mL), **49** (8.32 g, 10.7 mmol), and phenol (28 g, 0.29 mol) in CH_2Cl_2 (125 mL) were reacted, and product was isolated by the procedure of **2** to produce 2.58 g (71%) of **14** as white plates: NMR (D_2O) δ 0.97 and 0.98 (2 t, 6 H, J = 7), 1.63−1.83 (m, 8 H), 2.06−2.19 (m, 2 H), 2.97−3.21 (m, 12 H). Anal. ($C_{13}H_{34}Cl_3N_3$) C, H, N.

***N*,*N*′-Bis(4-phthalimidobutyl)mesitylenesulfonamide (37).** NaH (60%, 1.6 g, 40 mmol) was added to **35**[33] (2.72 g, 13.5 mmol) in DMF (60 mL) at 0 °C. After the mixture was stirred at 0 °C for 30 min, *N*-(4-bromobutyl)phthalimide (11.51 g, 40 mmol) in DMF (20 mL) was introduced. The mixture was stirred at room temperature for 1 h and at 60 °C overnight. Following the workup procedure of **43**, column chromatography (25:1 $CHCl_3$/acetone) gave 3.77 g (46%) of **37** as a white powder: NMR δ 1.51−1.54 (m, 8 H), 2.18 (s, 3 H), 2.57 (s, 6 H), 3.18−3.24 (m, 4 H), 3.55−3.60 (m, 4 H), 6.86 (s, 2 H), 7.69−7.25 (m, 4 H), 7.82−7.85 (m, 4 H); HRMS calcd for $C_{33}H_{36}N_3O_6S$ 602.2325 (M + H), found 602.2320 (M + H).

***N*,*N*′-Bis(4-aminobutyl)mesitylenesulfonamide (40).** Hydrazine monohydrate (0.82 g, 16 mmol) was added to a suspension of **37** (3.5 g, 5.8 mmol) in absolute EtOH (100 mL), and the mixture was stirred at 65 °C for 24 h. After cooling the solid was filtered and washed with EtOH (2 × 10 mL). The combined filtrate was concentrated and purified by column chromatography (6:1 MeOH/concentrated NH_4OH) to produce 1.50 g (76%) of **40** as a viscous oil: NMR δ 1.37 (quintet, 4 H), 1.52 (quintet, 4 H), 2.30 (s, 3 H), 2.54 (t, 4 H, J = 7), 2.58 (s, 6 H), 3.20 (t, 4 H, J = 7), 7.04 (s, 3 H).

Homospermidine Trihydrochloride (15). HBr (30% in HOAc, 30 mL), **40** (1.50 g, 4.39 mmol), and phenol (4.49 g, 48 mmol) in CH_2Cl_2 (20 mL) were reacted, and product was isolated by the procedure of **2** to afford 0.86 g (73%) of **15** as white crystals: NMR (D_2O) δ 1.73−1.80 (m, 8 H), 3.03−3.14 (m, 8 H). Anal. ($C_8H_{24}Cl_3N_3$) C, H, N.

***N*¹,*N*⁵,*N*⁹-Tris(mesitylenesulfonyl)homospermidine (32).** Mesitylenesulfonyl chloride (6.71 g, 30.7 mmol) and **40** (4.76 g, 14 mmol) in CH_2Cl_2 (30 mL) and 1 N NaOH (35 mL) were combined and worked up by the method of **31**. Column chromatography (4:1 toluene/EtOAc) produced 3.06 g (31%) of **32** as a white foam: NMR δ 1.32−1.38 (m, 4 H), 1.44−1.54 (m, 4 H), 2.28−2.29 (2 s, 9 H), 2.54 (s, 6 H), 2.60 (s, 12 H), 2.79 (quartet, 4 H), 3.09 (t, 4 H, J = 7), 4.70−4.80 (br s, 2 H), 6.90 (s, 2 H), 6.92 (s, 4 H). Anal. ($C_{35}H_{51}N_3O_6S_3$) C, H, N.

***N*¹,*N*⁹-Dimethyl-*N*¹,*N*⁵,*N*⁹-tris(mesitylenesulfonyl)homospermidine (50).** NaH (60%, 0.17 g, 4.2 mmol), **32** (1.28 g, 1.8 mmol), and iodomethane (0.25 mL, 4.0 mmol) in DMF (50 mL) were reacted and worked up as was **43**. Column chromatography (2:1 toluene/EtOAc) gave 1.14 g (86%) of **50** as an oil: NMR δ 1.38−1.44 (m, 8 H), 2.28 (s, 3 H), 2.30 (s, 6 H), 2.57 (s, 18 H), 2.62 (s, 6 H), 3.03−3.14 (m, 8 H), 6.93−6.94 (2 s, 6 H). Anal. ($C_{37}H_{55}N_3O_6S_3$) C, H, N.

***N*¹,*N*⁹-Dimethylhomospermidine Trihydrochloride (16).** HBr (30% in HOAc, 30 mL), **50** (1.12 g, 1.52 mmol), and phenol (5.4 g, 57 mmol) in CH_2Cl_2 (25 mL) were reacted, and product was isolated by the procedure of **2** to provide 354 mg (79%) of **16** as plates: NMR (D_2O) δ 1.78 (m, 8 H), 2.73 (s, 6 H), 3.08−3.12 (m, 8 H). Anal. ($C_{10}H_{28}Cl_3N_3$) C, H, N.

***N*¹,*N*⁹-Diethyl-*N*¹,*N*⁵,*N*⁹-tris(mesitylenesulfonyl)homospermidine (51).** NaH (80%, 0.264 g, 8.8 mmol) was added to **35**[33] (0.796 g, 4 mmol) in DMF (60 mL) at 0 °C. After the mixture was stirred at 0 °C for 30 min, **58**[15] (3.19 g, 8.8 mmol) in DMF (15 mL) was added. The mixture was heated at 75 °C overnight and worked up by the procedure of **43**. Column chromatography (3:1 hexane/EtOAc) gave 2.82 g (93%) of **51** as an oil: NMR δ 0.96 (t, 6 H), 1.20−1.40 (m, 8 H), 2.25 (s, 9 H), 2.55 (s, 18 H), 2.85−3.20 (m, 12 H), 6.90 (s, 6 H). Anal. ($C_{39}H_{59}N_3O_6S_3$) C, H, N.

***N*¹,*N*⁹-Diethylhomospermidine Trihydrochloride (17).** HBr (30% in HOAc, 20 mL), **51** (1.87 g, 2.45 mmol), and phenol (4.4 g, 49 mmol) in CH_2Cl_2 (20 mL) were reacted, and product was isolated by the procedure of **2** to give 493 mg (62%) of **17** as plates: NMR (D_2O) δ 1.30 (s, 6 H), 1.55−1.90 (m, 8 H), 2.95−3.20 (m, 12 H). Anal. ($C_{12}H_{32}Cl_3N_3$) C, H, N.

***N*¹,*N*⁹-Dipropyl-*N*¹,*N*⁵,*N*⁹-tris(mesitylenesulfonyl)homospermidine (52).** NaH (60%, 0.17 g, 4.2 mmol), **32** (1.28 g, 1.8 mmol), and 1-iodopropane (0.39 mL, 4.0 mmol) in DMF (50 mL) were reacted and worked up using the procedure of **43**. Column chromatography (4:1 hexane/EtOAc) gave 1.26 g (89%) of **52** as an oil: NMR δ 0.74 (t, 6 H, J = 7), 1.26−1.45 (m, 12 H), 2.29 (s, 9 H), 2.55 (s, 18 H), 2.98−3.13 (m, 12 H), 6.87 (s, 6 H). Anal. ($C_{41}H_{63}N_3O_6S_3$) C, H, N.

***N*¹,*N*⁹-Dipropylhomospermidine Trihydrochloride (19).** HBr (30% in HOAc, 30 mL), **52** (1.24 g, 1.56 mmol), and phenol (5.4 g, 57 mmol) in CH_2Cl_2 (25 mL) were reacted, and product was isolated by the procedure of **2** to give 430 mg (78%) of **19** as plates: NMR (D_2O) δ 0.98 (t, 6 H, J = 7), 1.70 (m, 4 H), 1.76−1.80 (m, 8H), 3.02 (t, 4 H, J = 7), 3.08−3.12 (m, 8 H). Anal. ($C_{14}H_{36}Cl_3N_3$) C, H, N.

***N*-(3-Cyanopropyl)mesitylenesulfonamide (36).** NaH (60%, 2.0 g, 50 mmol), **35**[33] (10.0 g, 50 mmol), and 4-bromobutyronitrile (4 mL, 40 mol) in DMF (100 mL) were combined. The mixture was heated at 80 °C overnight and worked up by the procedure of **43**. Column chromatography (4:3 hexane/EtOAc) gave 5.04 g (38%) of **36** as an oil: NMR δ 1.78 (s, 3 H), 2.25 (s, 3 H), 2.35 (t, 2 H, J = 7), 2.95 (q, 2 H), 5.05 (br t, 1 H), 6.90 (s, 2 H). Anal. ($C_{13}H_{18}N_2O_2S$) C, H, N.

***N*-(4-Cyanobutyl)-*N*-(3-cyanopropyl)mesitylenesulfonamide (38).** NaH (60%, 0.90 g, 23 mmol), **36** (5.02 g, 18.85 mmol), and 5-bromovaleronitrile (2.4 mL, 21 mmol) in DMF were combined and worked up by the method of **43**. Column chromatography (1:1 hexane/EtOAc) provided 5.20 g (79%) of **38** as an oil: NMR δ 1.49−1.66 (m, 4 H), 1.82 (m, 2 H), 2.22 (t, 2 H, J = 7), 2.25 (t, 2 H, J = 7), 2.29 (s, 3 H), 2.57 (s, 6 H), 3.19 (t, 2 H, J = 7), 3.29 (t, 2 H, J = 7), 6.95 (s, 2 H). Anal. ($C_{18}H_{25}N_3O_2S$) C, H, N.

6-(Mesitylenesulfonyl)-1,6,12-Triazadodecane (41). Raney nickel (W-2 grade, 7.60 g) and concentrated NH_4OH (10 mL) were successively added to **38** (5.06 g, 14.6 mmol) in CH_3OH (30 mL) and THF (30 mL) in a 200 mL Parr bottle, and a slow stream of NH_3 was bubbled through the mixture for 30 min at 0 °C. After hydrogenation in a Parr bottle was carried out at 50−55 psi for 8 h, the suspension was filtered through Celite, and the solvents were removed *in vacuo* to give 4.70 g (91%) of **41** as an oil: NMR δ 1.14−1.24 (m, 10 H), 2.25 (s, 3 H), 2.6 (s, 6 H), 3.05−3.25 (m, 8 H), 3.45 (s, 4 H), 6.9 (s, 2 H).

1,6,12-Triazadodecane Trihydrochloride (20). HBr (30% in HOAc, 33 mL), **41** (2.43 g, 6.83 mmol), and phenol (6 g, 60 mmol) in CH_2Cl_2 were reacted, and product was isolated by the procedure of **2** to give 0.97 g (50%) of **20** as a hygroscopic solid: NMR (D_2O) δ 1.47 (m, 2 H), 1.70−1.80 (m, 8 H), 3.00−3.10 (m, 8 H). Anal. ($C_9H_{26}Cl_3N_3$) C, H, N.

1,6,12-Tris(mesitylenesulfonyl)-1,6,12-Triazadodecane (33). Mesitylenesulfonyl chloride (4.29 g, 19.6 mmol) and **41** (3.17 g, 8.92 mmol) in CH_2Cl_2 (40 mL) and 1 N NaOH (20 mL) were combined and worked up by the method of **31**. Column chromatography (4:3 hexane/EtOAc) generated 5.66 g (88%) of **33** as an oil: NMR δ 1.12−1.17 (m, 2 H), 1.34−1.51 (m, 8 H), 2.29 (s, 3 H), 2.30 (s, 6 H), 2.55 (s, 6 H), 2.60−2.62 (2s, 12 H), 2.77−2.81 (m, 4 H), 3.06 (t, 2 H, J = 7), 3.11 (t, 2 H, J = 7), 4.50−4.60 (m, 2 H), 6.92 (s, 2 H), 6.95 (s, 2 H). Anal. ($C_{36}H_{53}N_3O_6S_3$) C, H, N.

2,7,13-Tris(mesitylenesulfonyl)-2,7,13-Triazatetradecane (53). NaH (60%, 0.28 g, 6.9 mmol), **33** (2.16 g, 3.0 mmol), and iodomethane (6.1 mL, 9.8 mmol) in DMF (30 mL) were combined and worked up by the method of **43**. Column chromatography (7:3 hexane/EtOAc) gave 1.90 g (85%) of **53** as an oil: NMR δ 1.08−1.16 (m, 2 H), 1.38−1.50 (m, 8 H), 2.28−2.29 (2s, 9 H), 2.57−2.58 (2 s, 18 H), 2.63 (s, 3 H), 2.65 (s, 3 H), 3.02−3.14 (m, 8 H), 6.95 (s, 6 H); HRMS calcd for $C_{38}H_{58}N_3O_6S_3$ 748.3487 (M + H), found 748.3483 (M + H).

2,7,13-Triazatetradecane Trihydrochloride (21). HBr (30% in HOAc, 45 mL), **53** (1.85 g, 2.47 mmol), and phenol (8.5 g) in CH_2Cl_2 (20 mL) were reacted, and product was isolated by the procedure of **2** to give 529 mg (69%) of **21** as crystals: NMR (D_2O) δ 1.42−1.52 (m, 2 H), 1.69−1.81 (m, 8 H), 2.73−2.74 (2 s, 6 H), 3.03−3.12 (m, 8 H). Anal. ($C_{11}H_{30}Cl_3N_3$) C, H, N.

4,9,15-Tris(mesitylenesulfonyl)-4,9,15-Triazaoctadecane (54). NaH (60%, 0.273 g, 6.84 mmol), **33** (2.24 g, 3.11 mmol), and 1-iodopropane (0.67 mL, 6.9 mmol) in DMF (30 mL) were combined and worked up by the method of **43**. Column chromatography (3:1 hexane/EtOAc) provided 2.01 g

(80%) of **54** as oil: NMR δ 0.71−0.78 (m, 6 H), 1.01−1.11 (m, 2 H), 1.34−1.48 (m, 12 H), 2.29 (s, 6 H), 2.57−2.58 (2 s, 18 H), 2.98−3.13 (m, 12 H), 6.92 (s, 6 H). Anal. ($C_{42}H_{65}N_3O_6S_3 \cdot H_2O$) C, H, N.

4,9,15-Triazaoctadecane Trihydrochloride (23). HBr (30% in HOAc, 45 mL), **54** (1.99 g, 2.47 mmol), and phenol (8.5 g) in CH_2Cl_2 (20 mL) were reacted, and product was isolated by the procedure of **2** to give 852 mg (83%) of **23** as plates: NMR (D_2O) δ 0.97 (s, 6 H), 1.40−1.51 (m, 2 H), 1.66−1.80 (m, 12 H), 2.98−3.15 (m, 12 H). Anal. ($C_{15}H_{38}Cl_3N_3$) C, H, N.

***N,N*-Bis(4-cyanobutyl)mesitylenesulfonamide (39).** NaH (80%, 1.22 g, 51 mmol), **35**[33] (5.0 g, 25 mmol), and 5-chlorovaleronitrile (6.5 g, 55 mmol) in DMF (50 mL) were combined. The mixture was heated at 60 °C overnight and worked up by the procedure of **43**. Column chromatography (7:3 hexane/EtOAc) yielded 6.31 g (70%) of **39** as an oil: NMR δ 1.57 (m, 4 H), 1.66 (m, 4 H), 2.26 (t, 4 H, J = 7), 2.60 (s, 6 H), 3.22 (t, 4 H, J = 7), 6.98 (s, 2 H). Anal. ($C_{19}H_{27}N_3O_2S$) C, H, N.

7-(Mesitylenesulfonyl)-1,7,13-triazatridecane (42). Raney nickel (W-2 grade, 2.9 g) and **39** (5.69 g, 15.8 mmol) in concentrated NH_4OH (10 mL) and CH_3OH (60 mL) were saturated with NH_3 as **41**. The mixture was shaken with hydrogen at 50−55 psi in a 200 mL Parr bottle for 42 h. The suspension was filtered through Celite, and the solvents were removed *in vacuo*. The residue was passed through a short silica gel column (EtOH then 5% concentrated NH_4OH/EtOH) to give 5.71 g (98%) of **42** as a light yellow oil: NMR δ 1.17 (m, 4 H), 1.47 (m, 8 H), 2.27 (s, 3 H), 2.57 (s, 6 H), 2.61 (m, 4 H), 3.13 (t, J = 7.5, 4 H), 6.91 (s, 2 H); HRMS calcd for $C_{19}H_{36}N_3O_2S$ 370.2528 (M + H), found 370.2530 (M + H).

1,7,13-Triazatridecane Trihydrochloride (24). HBr (30% in HOAc, 26 mL), **42** (2.0 g, 5.42 mmol), and phenol (4.8 g, 51 mmol) in $CHCl_3$ (40 mL) were reacted, and product was isolated by the method of **2** to give 0.97 g (61%) of **24** as white solid: NMR (D_2O) δ 1.45 (m, 4 H), 1.70 (m, 8 H), 3.01 (m, 8 H). Anal. ($C_{10}H_{28}Cl_3N_3$) C, H, N.

1,7,13-Tris(mesitylenesulfonyl)-1,7,13-triazatridecane (34). Mesitylenesulfonyl chloride (4.52 g, 20.7 mmol) and **42** (3.47 g, 9.4 mmol) in CH_2Cl_2 and 1 N NaOH (30 mL) were combined and worked up by the method of **31**. Column chromatography (3:2 hexane/EtOAc) gave 6.44 g (93%) of **34** as a white solid: NMR δ 1.16 (m, 4 H), 1.39 (m, 8 H), 2.30 (s, 3 H), 2.31 (s, 6 H), 2.57 (s, 6 H), 2.62 (s, 12 H), 2.81 (d of t, 4 H), 3.10 (t, 4 H, J = 7), 4.49 (br t, 2 H), 6.95 (s, 2 H), 6.97 (s, 4 H); HRMS calcd for $C_{37}H_{56}N_3O_6S_3$ 734.3331 (M + H), found 734.3351 (M + H).

2,8,14-Tris(mesitylenesulfonyl)-2,8,14-triazapentadecane (55). NaH (80%, 0.207 g, 6.9 mmol), **34** (1.58 g, 2.16 mmol), and iodomethane (0.30 mL, 4.8 mmol) in DMF (30 mL) were reacted and worked up as was **43**. Column chromatography (5:2 hexane/EtOAc) gave 1.51 g (92%) of **55** as an oil: NMR δ 1.06−1.18 (m, 4 H), 1.40−1.52 (m, 8 H), 2.29 (s, 9 H), 2.59 (s, 18 H), 2.66 (s, 6 H), 3.03−3.14 (m, 8 H), 6.95 (s, 6 H). Anal. ($C_{39}H_{59}N_3O_6S_3$) C, H, N.

2,8,14-Triazapentadecane Trihydrochloride (25). HBr (30% in HOAc, 30 mL), **55** (1.48 g, 1.94 mmol), and phenol (5.2 g, 55 mmol) in CH_2Cl_2 (30 mL) were reacted, and product was isolated by the method of **2** to produce 480 mg (76%) of **25** as needles: NMR (D_2O) δ 1.4−1.5 (quintet, 4 H), 1.7−1.8 (quintet, 8 H), 2.7 (s, 6 H), 3.05 (t, 8 H, J = 7). Anal. ($C_{12}H_{32}Cl_3N_3$) C, H, N.

3,9,15-Tris(mesitylenesulfonyl)-3,9,15-triazaheptadecane (56). NaH (80%, 0.52 g, 17 mmol), **34** (3.2 g, 4.36 mmol), and iodoethane (1.5 g, 9.6 mmol) in DMF (20 mL) were reacted and worked up by the method of **43**. Column chromatography (4:1 hexane/EtOAc) gave 2.91 g (85%) of **56** as a white solid: mp 60−62 °C; NMR δ 1.01 (t, 6 H, J = 7), 1.08 (m, 4 H), 1.42 (m, 8 H), 2.29 (s, 9 H), 2.57 (s, 6 H), 2.58 (s, 12 H), 3.07 (t, 4 H, J = 7), 3.11 (t, 4 H, J = 7), 3.17 (q, 4 H), 6.92 (s, 6 H). Anal. ($C_{41}H_{63}N_3O_6S_3$) C, H, N.

3,9,15-Triazaheptadecane Trihydrochloride (26). HBr (30% in HOAc, 20 mL), **56** (2.9 g, 3.67 mmol), and phenol (3.25 g, 34.5 mmol) in $CHCl_3$ (27 mL) were reacted, and product was isolated by the method of **2** to give 1.0 g (77%) of **26** as white crystals: NMR (D_2O) δ 1.28 (t, 6 H, J = 7), 1.45 (m, 4 H), 1.73 (m, 8 H), 3.08 (m, 12 H). Anal. ($C_{14}H_{36}Cl_3N_3$) C, H, N.

4,10,16-Tris(mesitylenesulfonyl)-4,10,16-triazanonadecane (57). NaH (80%, 198 mg, 6.6 mmol), **34** (1.51 g, 2.06 mmol), and 1-iodopropane (0.44 mL, 4.5 mmol) in DMF (40 mL) were reacted and worked up by the method of **43**. Column chromatography (7:2/hexane/EtOAc) provided 1.61 g (95%) of **57** as an oil: NMR δ 0.75 (t, 6 H, J = 7), 1.02−1.14 (m, 4 H), 1.36−1.52 (m, 12 H), 2.30 (s, 9 H), 2.60 (s, 18 H), 3.02−3.16 (m, 12 H), 6.95 (s, 6 H). Anal. ($C_{43}H_{67}N_3O_6S_3$) C, H, N.

4,10,16-Triazanonadecane Trihydrochloride (27). HBr (30% in HOAc, 30 mL), **57** (1.58 g, 1.93 mmol), and phenol (5.2 g, 55 mmol) were reacted, and product was isolated by the method of **2** to give 579 mg (79%) of **27** as plates: NMR (D_2O) δ 0.95 (t, 6 H, J = 7), 1.38−1.49 (m, 4 H), 1.62−1.77 (m, 12 H), 2.95−3.05 (m, 12 H). Anal. ($C_{16}H_{40}Cl_3N_3$) C, H, N.

***N*-(5-Bromopentyl)-*N*-ethylmesitylenesulfonamide (61).** NaH (80%, 1.26 g, 42 mmol), **60**[33] (6.82 g, 30.0 mmol), and 1,5-dibromopentane (49 mL, 0.36 mol) in DMF (100 mL) were combined. The mixture was heated at 74 °C overnight and worked up by the procedure of **43**. Column chromatography (7:1 hexane/EtOAc) produced 7.87 g (70%) of **61** as an oil: NMR δ 1.00 (t, 3 H, J = 7), 1.30−1.75 (m, 6 H), 2.20 (s, 3 H), 2.50 (s, 6 H), 3.02−3.30 (m, 6 H), 6.80 (s, 2 H); HRMS calcd for $C_{16}H_{27}BrNO_2S$ 376.0946 (M + H), found 376.0960 (M + H).

***N*^{1},*N*^{4}-Bis(mesitylenesulfonyl)-*N*^{1}-(*tert*-butoxycarbonyl)-*N*^{4}-ethyl-1,4-diaminobutane (62).** NaH (80%, 0.45 g, 23 mmol) was added to **59**[15] (3.45 g, 11.5 mmol) in DMF (100 mL) at 0 °C. After the mixture was stirred at 0 °C for 40 min, **58**[15] (5.00 g, 13.8 mmol) in DMF (10 mL) was added. The mixture was heated at 60 °C for 18 h and then worked up by the method of **43**. Column chromatography with (20:1 toluene/EtOAc) gave 6.44 g (96%) of **62** as an oil: NMR δ 1.12 (t, 3 H, J = 7), 1.20 (s, 9 H), 1.55−1.65 (m, 4 H), 2.29 (s, 2 H), 2.30 (s, 2 H), 2.52 (s, 4 H), 2.62 (s, 4 H), 3.16−3.24 (m, 2 H), 3.30 (q, 2 H), 3.70 (m, 2 H), 6.94 (s, 4 H).

***N*^{1},*N*^{4}-Bis(mesitylenesulfonyl)-*N*^{4}-ethyl-1,4-diaminobutane (63).** TFA (70 mL) was slowly dripped into a solution of **62** (6.20 g, 10.6 mmol) in CH_2Cl_2 (30 mL) at 0 °C. After the solution was stirred at 0 °C for 20 min and at room temperature for 30 min, solvents were removed by rotary evaporation. The residue was basified to pH > 8 with saturated $NaHCO_3$ and extracted with CH_2Cl_2 (4 × 100 mL). Removal of organic extracts led to 5.10 g (100%) of **63** as a foam: NMR δ 0.97 (t, 3 H, J = 7), 1.20−1.50 (m, 4 H), 2.25 (s, 6 H), 2.50−2.55 (2 s, 12 H), 2.95−3.25 (m, 6 H), 4.45 (t, 1 H), 6.85 (s, 4 H). Anal. ($C_{24}H_{36}N_2O_4S_2$) C, H, N.

3,8,14-Tris(mesitylenesulfonyl)-3,8,14-Triazahexadecane (64). NaH (80%, 0.41 g, 14 mmol) was added to **63** (5.10 g, 10.6 mmol) in DMF (50 mL) at 0 °C. After the mixture was stirred at 0 °C for 30 min, **61** (4.80 g, 12.7 mmol) in DMF (10 mL) was added. The mixture was heated at 89 °C overnight and then worked up by the method of **43**. Column chromatography (12:1 toluene/EtOAc) gave 5.43 g (66%) of **64** as an oil: NMR δ 0.9−1.1 (m, 6 H), 1.2−1.5 (m, 10 H), 2.25 (s, 6 H), 2.30 (s, 3 H), 2.55 (s, 18 H), 2.9−3.2 (m, 12 H), 6.85 (s, 6 H). Anal. ($C_{40}H_{61}N_3O_6S_3$) C, H, N.

3,8,14-Triazahexadecane Trihydrochloride (22). HBr (30% in HOAc, 100 mL), **64** (5.4 g, 7.0 mmol), and phenol (25 g, 0.28 mol) were reacted, and product was isolated by the method of **2** to give 1.63 g (69%) of **22** as plates: NMR (D_2O) δ 1.38 (t, 3 H, J = 7), 1.39 (t, 3 H, J = 7), 1.60−1.70 (m, 10 H), 3.02−3.15 (m, 12 H). Anal. ($C_{13}H_{34}Cl_3N_3$) C, H, N.

***N*^{1}-(Triphenylmethyl)-1,3-diaminopropane (68).** A solution of triphenylmethyl chloride (6.97 g, 25 mmol) in CH_2Cl_2 (100 mL) was added dropwise to a rapidly stirred solution of 1,3-diaminopropane (9.86 g, 133 mmol) in CH_2Cl_2 (100 mL). After the mixture was stirred at room temperature for 2 days, 1 N NaOH (50 mL) was added, and the mixture was extracted with $CHCl_3$ (3 × 50 mL). Organic extracts were washed with 100 mL of H_2O and brine. After solvent removal, column chromatography (3% concentrated NH_4OH/MeOH) gave 6.32 g (80%) of **68** a white solid: mp 59−61 °C (lit.[46] mp 59−61

°C); NMR δ 1.35−1.65 (m, 6 H), 2.18 (t, 2 H, J = 7), 2.77 (s, 1 H), 7.13−7.24 (m, 9 H), 7.44−7.46 (m, 6 H). Anal. ($C_{22}H_{24}N_2$) C, H, N.

***N*^{1}-(Mesitylenesulfonyl)-*N*^{3}-(triphenylmethyl)-1,3-diaminopropane (70).** Mesitylenesulfonyl chloride (5.25 g, 24 mmol) and **68** (6.30 g, 20 mmol) in CH_2Cl_2 (30 mL) and 1 N NaOH (27 mL) were combined and worked up by the method of **31**. Column chromatography (7:2 hexane/EtOAc) gave 8.37 g (84%) of **70** as a white solid: NMR δ 1.56−1.66 (m, 3 H), 2.17 (t, 2 H, J = 7), 2.29 (s, 3 H), 2.60 (s, 6 H), 3.08 (q, 2 H), 5.25 (br t, 1 H), 6.93 (s, 2 H), 7.15−7.28 (m, 9 H), 7.35−7.39 (m, 6 H). Anal. ($C_{31}H_{34}N_2O_2S$) C, H, N.

***N*^{1}-(Triphenylmethyl)-1,4-diaminobutane (69).** A solution of triphenylmethyl chloride (59.94 g, 0.215 mol) in CH_2Cl_2 (500 mL) was added dropwise to a rapidly stirred solution of 1,4-diaminobutane (96.47 g, 1.094 mol) in CH_2Cl_2 (1.1 L) over a period of 2 h. The reaction mixture was stirred at room temperature for 3 days and was worked up following the method of **68** to give a quantitative yield of **69** as an oil, which was used directly in the next step: NMR δ 1.39−1.54 (m, 7 H), 2.12 (t, 2 H, J = 7), 2.62 (t, 2 H, J = 7), 7.13−7.28 (m, 9 H), 7.41−7.47 (m, 6 H).

***N*^{1}-(Mesitylenesulfonyl)-*N*^{4}-(triphenylmethyl)-1,4-diaminobutane (71).** Mesitylenesulfonyl chloride (3.1 g, 14 mmol) and **69** (3.39 g, 10.3 mmol) in CH_2Cl_2 (20 mL) and 1 N NaOH (15 mL) were combined and worked up by the method of **31**. Column chromatography (1:3 hexane/EtOAc) furnished 3.79 g (72%) of **71** as a white solid: NMR δ 1.41−1.50 (m, 5 H), 2.03−2.08 (t, 2 H, J = 7), 2.27 (s, 3 H), 2.62 (s, 6 H), 2.87 (q, 2 H), 4.41 (br t, 1 H), 6.93 (s, 2 H), 7.15−7.28 (m, 9 H), 7.40−7.44 (m, 6 H). Anal. ($C_{32}H_{36}N_2O_2S$) C, H, N.

***N*-Propylmesitylenesulfonamide (65).** Mesitylenesulfonyl chloride (12.0 g, 55 mmol) and propylamine (2.96 g, 50 mmol) in CH_2Cl_2 (60 mL) and 1 N NaOH (60 mL) were combined and worked up by the method of **31**. Column chromatography (3:1 hexane/EtOAc) afforded 8.44 g (85%) of **65** as crystalline solid: mp 53−54 °C; NMR δ 0.86 (t, 3 H, J = 7), 1.44−1.51 (m, 2 H), 2.30 (s, 3 H), 2.64 (s, 6 H), 2.86 (q, 2 H), 4.40 (br t, 1 H), 6.96 (s, 2 H). Anal. ($C_{12}H_{19}NO_2S$) C, H, N.

***N*-(3-Bromopropyl)-*N*-propylmesitylenesulfonamide (66).** NaH (60%, 0.34 g, 8.4 mmol), **65** (1.7 g, 7.0 mmol), and 1,3-dibromopropane (17.0 g, 84 mmol) in DMF (30 mL) were combined and worked up by the method of **43**. Column chromatography (6:1 hexane/EtOAc) produced 1.82 g (80%) of **66** as an oil: NMR δ 0.79 (s, 3 H), 1.48−1.56 (m, 2 H), 2.04−2.10 (m, 2 H), 2.30 (s, 3 H), 2.60 (s, 6 H), 3.09−3.14 (m, 2 H), 3.29−3.36 (m, 4 H). Anal. ($C_{15}H_{24}BrNO_2S$) C, H, N.

***N*-(4-Bromobutyl)-*N*-propylmesitylenesulfonamide (67).** NaH (60%, 0.70 g, 17 mmol), **65** (3.5 g, 14.5 mmol), and 1,4-dibromobutane (37.6 g, 174 mmol) in DMF (40 mL) were combined and worked up by the method of **43**. Excess 1,4-dibromobutane was removed by a Kugelrohr apparatus under high vacuum. Column chromatography (7:1 hexane/EtOAc) produced 5.21 g (95%) of **67** as an oil: NMR δ 0.79 (t, 3 H, J = 7), 1.46−1.54 (m, 2 H), 1.64−1.78 (m, 4 H), 2.30 (s, 3 H), 2.60 (s, 6 H), 3.11 (t, 2 H, J = 7), 3.21 (t, 2 H, J = 7), 3.31 (t, 2 H, J = 7), 6.93 (s, 2 H). Anal. ($C_{16}H_{26}BrNO_2S$) C, H, N.

6,10-Bis(mesitylenesulfonyl)-1-(triphenylmethyl)-1,6,10-triazatridecane (72). NaH (60%, 0.24 g, 5.96 mmol) was added to **71** (2.55 g, 4.97 mmol) in DMF (40 mL) at 0 °C. After the mixture was stirred at 0 °C for 20 min, **66** (1.80 g, 4.97 mmol) in DMF (20 mL) was added. The mixture was stirred at room temperature for 1 day and then worked up following the method of **43**. Column chromatography (20:1 toluene/EtOAc) produced 3.72 g (94%) of **72** as an oil: NMR δ 0.73 (t, 3 H, J = 7), 1.26−1.70 (m, 8 H), 2.01 (t, 2 H, J = 7), 2.26 (s, 3 H), 2.27 (s, 3 H), 2.54 (s, 12 H), 2.96−3.04 (m, 8 H), 6.90 (s, 4 H), 7.18−7.45 (m, 15 H). Anal. ($C_{47}H_{59}N_3O_4S_2$) C, H, N.

5,10-Bis(mesitylenesulfonyl)-1-(triphenylmethyl)-1,5,-10-triazatridecane (73). NaH (60%, 0.22 g, 5.42 mmol), **70** (2.25 g, 4.52 mmol) in DMF (40 mL), and **67** (1.70 g, 4.52 mmol) in DMF (20 mL) were reacted and worked up following the method of **72**. Column chromatography (25:1 toluene/EtOAc) produced 2.87 g (80%) of **73** as an oil: NMR δ 0.75 (t, 3 H, J = 7), 1.41−1.46 (m, 9 H), 1.97 (t, 2 H, J = 7), 2.26 (s, 6 H), 2.53 (s, 6 H), 2.56 (s, 6 H), 3.04 (t, 2 H, J = 7), 3.10−3.15 (m, 6 H), 6.88 (s, 2 H), 6.90 (s, 2 H), 7.15−7.38 (m, 15 H). Anal. ($C_{47}H_{59}N_3O_4S_2$) C, H, N.

6,11-Bis(mesitylenesulfonyl)-1-(triphenylmethyl)-1,6,11-triazatetradecane (74). NaH (60%, 0.12 g, 2.90 mmol), **71** (1.24 g, 2.42 mmol) in DMF (30 mL), and **67** (0.91 g, 2.42 mmol) in DMF (10 mL) were reacted and worked up following the method of **72**. Column chromatography (25:1 toluene/EtOAc) gave 1.67 g (85%) of **74** as an oil: NMR δ 0.74 (t, 3 H, J = 7), 1.35−1.45 (m, 11 H), 2.00 (t, 2 H, J = 7), 2.25 (s, 3 H), 2.28 (s, 3 H), 2.55 (s, 6 H), 2.56 (s, 6 H), 2.98−3.08 (m, 8 H), 6.90 (s, 2 H), 6.91 (s, 2 H), 7.15−7.44 (m, 15 H). Anal. ($C_{48}H_{61}N_3O_4S_2$) C, H, N.

***N*^{1}-Propylspermidine Trihydrochloride (12).** HBr (30% in HOAc, 45 mL), **72** (3.70 g, 4.66 mmol), and phenol (8.4 g, 89 mmol) in CH_2Cl_2 (50 mL) were reacted, and product was isolated by the method of **2** to give 1.02 g (74%) of **12** as plates: NMR (D_2O) δ 0.98 (t, 3 H, J = 7), 1.66−1.79 (m, 6 H), 2.06−2.17 (m, 2 H), 3.01−3.19 (m, 10 H). Anal. ($C_{10}H_{28}Cl_3N_3$) C, H, N.

***N*^{8}-Propylspermidine Trihydrochloride (13).** HBr (30% in HOAc, 35 mL), **73** (2.85 g, 3.59 mmol), and phenol (6.5 g, 69 mmol) in CH_2Cl_2 (30 mL) were reacted, and product was isolated by the method of **2** to give 810 mg (76%) of **13** as plates: NMR (D_2O) δ 0.98 (t, 3 H, J = 7), 1.66−1.79 (m, 6 H), 2.01−2.12 (m, 2 H), 2.99−3.14 (m, 10 H). Anal. ($C_{10}H_{28}Cl_3N_3$) C, H, N.

***N*^{1}-Propylhomospermidine Trihydrochloride (18).** HBr (30% in HOAc, 20 mL), **74** (1.65 g, 2.0 mmol), and phenol (3.6 g, 38 mmol) in CH_2Cl_2 (20 mL) were reacted, and product was isolated by the method of **2** to give 268 mg (43%) of **18** as plates: NMR (D_2O) δ 0.98 (t, 3 H, J = 7), 1.66−1.80 (m, 10 H), 2.99−3.11 (m, 10 H). Anal. ($C_{11}H_{30}Cl_3N_3$) C, H, N.

***N*^{1}-Ethylspermidine Trihydrochloride (9).** Lithium aluminum hydride (1.6 g, 42 mmol) was added to *N*^{1}-acetylspermidine dihydrochloride (0.50 g, 1.9 mmol) in THF (300 mL) at 0 °C, and the mixture was heated at reflux for 17 h. The reaction was quenched at 0 °C with H_2O (1.6 mL), 15% NaOH (1.6 mL), and H_2O (4.8 mL). Salts were filtered and washed with THF, and solvent was removed by rotary evaporation. The residue was distilled in a Kugelrohr apparatus under high vacuum ($T \leq$ 60 °C), and the distillate was dissolved in EtOH (5 mL) and treated with concentrated HCl (0.5 mL). Recrystallization from aqueous EtOH gave 0.096 g (18%) of **9** as crystals: NMR (D_2O) δ 1.30 (t, 3 H, J = 7), 1.72−1.83 (m, 4 H), 2.05−2.16 (m, 2 H), 3.02−3.19 (m, 10 H). Anal. ($C_9H_{26}Cl_3N_3$) C, H, N.

***N*^{8}-Ethylspermidine Trihydrochloride (10).** Lithium aluminum hydride (1.73 g, 45.6 mmol) and *N*^{8}-acetylspermidine dihydrochloride (0.54 g, 2.1 mmol) in THF (300 mL) were reacted, and product was isolated by the method of **9** to furnish 0.164 g (28%) of **10** as crystals: NMR (D_2O) δ 1.29 (t, 3 H, J = 7), 1.73−1.83 (m, 4 H), 2.03−2.16 (m, 2 H), 3.05−3.20 (m, 10 H). Anal. ($C_9H_{26}Cl_3N_3$) C, H, N.

***N*^{1},*N*^{4},*N*^{8},*N*^{11}-Tetrakis(mesitylenesulfonyl)-*N*^{1},*N*^{11}-dipropylnorspermine (76).** NaH (60%, 3.60 g, 90.0 mmol), **75**[15] (27.5 g, 30.0 mmol), and 1-iodopropane (7.5 mL, 77 mmol) in DMF (200 mL) were combined, and the reaction was worked up by the method of **48**. Column chromatography (5:1 toluene/EtOAc) resulted in 27.79 g (92%) of **76** as a white foam: NMR δ 0.72 (t, 6 H, J = 7), 1.2−1.7 (m, 10 H), 2.30 (s, 12 H), 2.55 (s, 24 H), 2.92−3.03 (m, 16 H), 6.93 (s, 8 H). Anal. ($C_{51}H_{76}N_4O_8S_4$) C, H, N.

***N*^{1},*N*^{11}-Dipropylnorspermine Tetrahydrochloride (28).** HBr (30% in HOAc, 500 mL), **76** (27.54 g, 27.5 mmol), and phenol (105 g, 1.12 mol) in CH_2Cl_2 (250 mL) were reacted, and product was isolated by the method of **2** to give 7.82 g (68%) of **28** as white plates: NMR (D_2O) δ 0.98 (t, 6 H, J = 7), 1.64−1.78 (m, 4 H), 2.07−2.21 (m, 6 H), 3.00−3.25 (m, 16 H). Anal. ($C_{15}H_{40}Cl_4N_4$) C, H, N.

***N*-(5-Chloropentyl)-*N*-ethylmesitylenesulfonamide (77).** NaH (80%, 1.34 g, 44.7 mmol) was added to **60**[33] (7.68 g, 33.8 mmol) in DMF (130 mL) at 0 °C. The mixture was stirred at room temperature for 1 h and cooled to 0 °C. 1,5-Dichloropentane (45 mL, 0.35 mol) was added all at once. The reaction mixture was stirred at 55 °C for 12 h and was worked up by the method of **48**. Column chromatography (11.5% EtOAc/

hexane) gave 10.54 g (94%) of **77** as an oil: NMR δ 1.07 (t, 3 H, $J = 8$), 1.25−1.37 (m, 2 H), 1.47−1.71 (m, 4 H), 2.30 (s, 3 H), 2.60 (s, 6 H), 3.14−3.28 (m, 4 H), 3.44 (t, 2 H, $J = 7$), 6.94 (s, 2 H). Anal. ($C_{16}H_{26}ClNO_2S$) C, H, N.

3,9,14,20-Tetrakis(mesitylenesulfonyl)-3,9,14,20-tetraazadocosane (79). NaH (80%, 1.14 g, 38.0 mmol) was added to **78**[15] (5.77 g, 12.7 mmol) in DMF (75 mL) at 0 °C. The mixture was stirred at room temperature for 1 h, and **77** (10.51 g, 31.7 mmol) in DMF (55 mL) was added by cannula. The reaction mixture was stirred at 55 °C for 16 h and was worked up by the method of **48**. Column chromatography (30% EtOAc/hexane) afforded 12.67 g (96%) of **79** as an oil: NMR δ 0.97−1.12 (m, 10 H), 1.30−1.47 (m, 12 H), 2.29 (s, 12 H), 2.56 and 2.58 (2 s, 24 H), 2.97−3.22 (m, 16 H), 6.93 (s, 8 H). Anal. ($C_{54}H_{82}N_4O_8S_4$) C, H, N.

3,9,14,20-Tetraazadocosane Tetrahydrochloride (29). HBr (30% in HOAc, 195 mL), **79** (12.66 g, 12.1 mmol), and phenol (33.61 g, 0.357 mol) in CH_2Cl_2 (135 mL) were reacted, and product was isolated by the method of **2** to provide 4.50 g (81%) of **29** as white crystals: NMR (D_2O) δ 1.28 (t, 6 H, $J =$ 7), 1.40−1.52 (m, 4 H), 1.66−1.80 (m, 12 H), 3.00−3.14 (m, 16 H). Anal. ($C_{18}H_{46}Cl_4N_4$).

Acknowledgment. Support was provided by National Institutes of Health Grant No. R01 DK49108. We thank Mr. Richard E. Smith and Mr. Sam Algee for assistance in the preparation of compounds.

References

(1) Pegg, A. E. Polyamine Metabolism and Its Importance in Neoplastic Growth and as a Target for Chemotherapy. *Cancer Res.* **1988**, *48*, 759−774.
(2) Marton, L. J.; Pegg, A. E. Polyamines as Targets for Therapeutic Intervention. *Annu. Rev. Pharmacol. Toxicol.* **1995**, *35*, 55−91.
(3) Bey, P.; Danzin, C.; Jung, M. Inhibition of Basic Amino Acid Decarboxylases Involved in Polyamine Biosynthesis. In *Inhibition of Metabolism. Biological Significance and Basis for New Therapies*; McCann, P. P., Pegg, A. E., Sjoerdsma, A., Eds.; Academic Press: Orlando, 1987; pp 1−32.
(4) Williams-Ashman, H. G.; Schenone, A. Methyl Glyoxal Bis-(guanylhydrazone) as a Potent Inhibitor of Mammalian and Yeast *S*-Adenosylmethionine Decarboxylases. *Biochem. Biophys. Res. Commun.* **1972**, *46*, 288−295.
(5) 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: Orlando, 1987; pp 121−140.
(6) Pegg, A. E.; McCann P. P. *S*-Adenosylmethionine Decarboxylase as an Enzyme Target for Therapy. *Pharmacol. Ther.* **1992**, *56*, 359−377.
(7) Pegg, A. E.; McGill, S. M. *Biochem. Pharmacol.* **1978**, *27*, 1625−1629.
(8) Schecter, P. J.; Barlow, J. L. R.; Sjoerdsma, A. Clinical Aspects of Inhibition of Ornithine Decarboxylase with Emphasis on the Therapeutic Trials of Eflornithine (DFMO) in Cancer and Protozoan Diseases. In *Inhibition of Polyamine Metabolism. Biological Significance and Basis for New Therapies*; McCann, P. P., Pegg, A. E., Sjoerdsma, A., Eds.; Academic Press: Orlando, 1987; pp 345−364.
(9) Seiler, N.; Dezeure, F. Polyamine Transport in Mammalian Cells. *Int. J. Biochem.* **1990**, *22*, 211−218.
(10) Kelloff, G. J.; Crowell, J. A.; Boone, C. W.; Steele, V. E.; Lubet, R. A.; Greenwald, P.; Alberts, D. S.; Covey, J. M.; Doody, L. A.; Knapp, G. G.; Nayfield, S.; Parkinson, D. R.; Prasad, V. K.; Prorok, P. C.; Sausville, E. A.; Sigman, C. C. Strategy and Planning for Chemopreventive Drug Development: Clinical Development Plans. *J. Cell. Biochem.* **1994** (Suppl.), *20*, 55−62.
(11) Petereit, D. G.; Harari, P. M.; Contreras, L.; Pickart, M. A.; Verma, A. K.; Gerner, E. W.; Kinsella, T. J. Combining Polyamine Depletion with Radiation Therapy for Rapidly Dividing Head and Neck Tumors: Strategies for Improved Locoregional Control. *Int. J. Radiat. Oncol. Biol. Phys.* **1994**, *28* (4), 891−898.
(12) Jännë, J.; Pösö, H.; Raina, A. Polyamines in Rapid Growth and Cancer. *Biochim. Biophys. Acta* **1978**, *473*, 241.
(13) Porter, C.; Bergeron, R. J. Enzyme Regulation as an Approach to Interference with Polyamine Biosynthesis—an Alternative to Enzyme Inhibition. *Enzyme Regul.* **1988**, *27*, 57−79.
(14) 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.
(15) Bergeron, R. J.; McManis, J. S.; Liu, C. Z.; Feng, Y.; Weimar, W. R.; Luchetta, G. R.; Wu, Q.; Ortiz-Ocasio, J.; Vinson, J. R. T.; Kramer, D.; Porter, C. Antiproliferative Properties of Polyamine Analogues: a Structure-Activity Study. *J. Med. Chem.* **1994,** *37*, 3464−3476.
(16) Bernacki, R. J.; Bergeron, R. J.; Porter, C. W. Antitumor Activity of *N,N′*-Bis(ethyl)spermine Homologues Against Human MALME-3 Melanoma Xenografts. *Cancer Res.* **1992**, *52*, 2424−2430.
(17) Porter, C. W.; Bergeron, R. J.; Stolowich, N. J. Biological Properties of N^4-Spermidine Derivatives and Their Potential in Anticancer Chemotherapy. *Cancer Res.* **1982**, *42*, 4072−4078.
(18) Porter, C. W.; Cavanaugh, P. F., Jr.; Stolowich, N.; Ganis, B.; Kelly, E.; Bergeron, R. J. Biological Properties of N^4- and N^1,N^8-Spermidine Derivatives in Cultured L1210 Leukemia Cells. *Cancer Res.* **1985**, *45*, 2050−2057.
(19) Porter, C. W.; Miller, J.; Bergeron, R. J. Aliphatic Chain Length Specific of the Polyamine Transport System in Ascites L210 Leukemia Cells. *Cancer Res.* **1984**, *44*, 126−128.
(20) Bergeron, R. J.; Hawthorne, T. R.; Vinson, J. R. T.; Beck, D. E., Jr.; Ingeno, M. J. Role of the Methylene Backbone in the Antiproliferative Activity of Polyamine Analogues on L1210 Cells. *Cancer Res.* **1989**, *49*, 2959−2964.
(21) Porter, C. W.; Pegg, A. E.; Ganis, B.; Madhubala, R.; Bergeron, R. J. Combined Regulation of Ornithine and S-Adenosylmethionine Decarboxylase by Spermine and the Spermine Analogue N^1,N^{12}-Bis(ethyl)spermine. *Biochem. J.* **1990**, *268*, 207−212.
(22) Pegg, A. E.; Madhubala, R.; Kameji, T.; Bergeron, R. J. Control of Ornithine Decarboxylase Activity in α-Difluoromethylornithine-Resistant L1210 Cell by Polyamines and Synthetic Analogues. *J. Biol. Chem.* **1988**, *263*, 11008−11014.
(23) Porter, C. W.; McManis, J. S.; Casero, R. A.; Bergeron, R. J. Relative Abilities of Bis(ethyl) Derivatives of Putrescine, Spermidine, and Spermine to Regulate Polyamines Biosynthesis and Inhibit L1210 Leukemia Cell Growth. *Cancer Res.* **1987**, *47*, 2821−2825.
(24) Pegg, A. E.; Wechter, R.; Pakala, R.; Bergeron, R. J. Effect of N^1,N^{12}-Bis(ethyl)spermine and Related Compounds on Growth and Polyamine Acetylation, Content and Excretion in Human Colon Tumor Cell. *J. Biol. Chem.* **1989**, *264*, 11744−11749.
(25) 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.
(26) Libby, P. R.; Henderson, M.; Bergeron, R. J.; Porter, C. W. Major Increases in Spermidine/Spermine-N^1-Acetyltransferase by Spermine Analogues and Their Relationship to Polyamine Depletion and Growth Inhibition in L1210 Cells. *Cancer Res.* **1989**, *49*, 6226−6231.
(27) Libby, P. R.; Bergeron, R. J.; Porter, C. W. Structure-Function Correlations of Polyamine Analog-Induced Increases in Spermidine/Spermine Acetyltransferase Activity. *Biochem. Pharmacol.* **1989**, *38*, 1435−1442.
(28) Porter, C. W.; Ganis, B.; Libby, P. R.; Bergeron, R. J. Correlations Between Polyamine Analog-Induced Increases in Spermidine/Spermine N^1-Acetyltransferase Activity, Polyamine Pool Depletion, and Growth Inhibition in Human Melanoma Cell Lines. *Cancer Res.* **1991**, *51*, 3715−3720.
(29) Fogel-Petrovic, M.; Shappell, N. W.; Bergeron, R. J.; Porter, C. W. Polyamine and Polyamine Analog Regulation of Spermidine/Spermine N^1-Acetyltransferase in MALME-3M Human Melanoma Cells. *J. Biol. Chem.* **1993**, *268*, 19118−19125.
(30) Shappell, N. W.; Fogel-Petrovic, M. F.; Porter, C. W. Regulation of Spermidine/Spermine N^1-Acetyltransferase by Intracellular Polyamine Pools—Evidence for a Functional Role in Polyamine Homeostasis. *FEBS Lett.* **1993**, *321*, 179−183.
(31) Bergeron, R. J.; McManis, J. S.; Weimar, W. R.; Schreier, K. M.; Gao, F.; Wu, Q.; Ortiz-Ocasio, J.; Luchetta, G. R.; Porter, C.; Vinson, J. R. T. The Role of Charge in Polyamine Analogue Recognition. *J. Med. Chem.* **1995**, *38*, 2278−2285.
(32) Bergeron, R. J.; Weimar, W. R.; Luchetta, G.; Streiff, R. R.; Wiegand, J.; Perrin, J.; Schreier, K. M.; Porter, C.; Yao, G. W.; Dimova, H. Metabolism and Pharmacokinetics of N^1,N^{11}-Diethylnorspermine. *Drug Metab. Dispos.* **1995**, *23*, 1117−1125.
(33) Schreinemakers, F. A. H. Some Amides of Aromatic Sulfonic Acids. *Recl. Trav. Chim. Pays-Bas Belg.* **1897**, *16*, 411−424.
(34) Porter, C. W.; Berger, F. G.; Pegg, A. E.; Ganis, B.; Bergeron, R. J. Regulation of Ornithine Decarboxylase Activity by Spermidine and the Spermine Analogue N^1,N^8-Bis(ethyl)spermidine. *Biochem. J.* **1987**, *242*, 433−440.
(35) Porter, C. W.; Pegg, A. E.; Ganis, B.; Madhabala, R.; Bergeron, R. J. Combined Regulation of Ornithine and S-Adenosylmethionine Decarboxylase by Spermine and the Spermine Analogue N^1,N^2-Bis(ethyl)spermine. *Biochem. J.* **1990**, *268*, 207−212.
(36) Bergeron, R. J.; Weimar, W. R.; Luchetta, G.; Sninsky, C. A.; Streiff, R. R.; Wiegand, J. Metabolism and Pharmacokinetics of N^1,N^{14} -Diethylhomospermine. *Drug Metab. Dispos.* **1996**, *24*, 334−343.

(37) Morgan, D. M. Polyamine Oxidases and Oxidized Polyamines. In *The Physiology of Polyamines*; Bachrach, U., Heimer, Y. M., Eds.; CRC Press: Boca Raton, FL, 1989; Vol. I, Chapter 13, pp 203−229.
(38) Gahl, W. A.; Pitot, H. C. Reversal by Aminoguanidine of the Inhibition of Proliferation of Human Fibroblasts by Spermidine and Spermine. *Chem.-Biol. Interact.* **1978**, *22*, 91−98.
(39) Frieden, E. Complex Copper of Nature. In *Metamorphosis, A Problem in Developmental Biology*, 2nd ed.; Gibert, L. I., Frieden, E., Eds.; Plenum Press: New York, 1981; pp 478−483.
(40) Alcain, F. J.; Low, H.; Crane, F. L. Iron Reverses Impermeable Chelator Inhibition of DNA synthesis in CCl 39 cells. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 7903−6.
(41) Glahn, R. P.; Gangloff, M. B.; Van-Campen, D. R.; Miller, D. D.; Wien, E. M.; Norvell, W. A. Bathophenanthroline Disulfonic Acid and Sodium Dithionite Effectively Remove Surface-Bound Iron from Caco-2 Cell Monolayers. *J. Nutr.* **1995**, *125*, 1833−40.
(42) Tabor, C. W.; Rosenthal, S. M. Pharmacology of Spermine and Spermidine. Some Effects on Animals and Bacteria. *J. Pharmacol. Exp. Ther.* **1956**, *116*, 139−155.
(43) Shaw. S. S. Some Pharmacological Properties of the Polyamine Spermine and Spermidine—a Re-appraisal. *Arch. Int. Pharmacodyn. Ther.* **1972**, *198*, 36−48.
(44) Seely, J. E.; Pegg, A. E. Ornithine Decarboxylase (Mouse Kidney). *Methods Enzymol.* **1983**, *94*, 158−161.
(45) Pegg, A. E.; Pösö, H. S-Adenosylmethionine Decarboxylase (Rat Liver). *Methods Enzymol.* **1983**, *94*, 234−239.
(46) Parg, R. P.; Kilburn, J. D.; Petty, M. C.; Pearson, C.; Ryan T. G. A Semiconducting Langmuir-Blodgett Film of a Non-amphiphilic Bis-tetrathiafulvalene Derivative. *J. Mater. Chem.* **1995**, *5*, 1609−1615.

JM960849J