Articles

Antiproliferative Properties of Polyamine Analogues: A Structure–Activity Study

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A basis set of polyamine analogues was designed and synthesized. These compounds were used to initiate a systematic investigation of the role of chain length, terminal nitrogen alkyl group size, and symmetry of the methylene backbone in the antineoplastic properties of polyamine analogues. New synthetic methods predicated on our earlier polyamine fragment synthesis are described for accessing the tetraamines of interest. An unsymmetrically substituted diamine reagent, *N*-(*tert*-butoxycarbonyl)-*N*,*N*′-bis(mesitylenesulfonyl)-1,4-diaminobutane, was developed for entry into unsymmetrical tetraamines. All of the tetraamines synthesized were first evaluated in a murine leukemia L1210 cell IC_{50} assay at 48 and 96 h. In an attempt to correlate this behavior with some aspect of polyamine metabolism, each compound was tested for its ability to compete with spermidine for the polyamine uptake apparatus, its impact on the polyamine biosynthetic enzymes ornithine decarboxylase (ODC) and *S*-adenosylmethionine decarboxylase (AdoMetDC), and its effect on the polyamine-catabolizing enzyme spermidine/spermine N^1-acetyltransferase (SSAT) and on polyamine pools. While there was no obvious correlation between the 48 and 96 h IC_{50}'s and the impact of the analogues on polyamine metabolism, there were other structure–activity relationships. Correlations were observed to exist between chain length and IC_{50}'s and between terminal alkyl substituents and impact on K_i, ODC, and AdoMetDC. Also, preliminary studies suggest a relationship may exist between the 48 and 96 h IC_{50} activities and the analogue's chronic toxicity *in vivo*. Finally, when the overall length of the polyamine backbone was held constant, the symmetry of the methylene chains of the polyamine fragments was shown to be unimportant to the compound's activity.

Introduction

N-Alkylated polyamines, especially terminally dialkylated analogues and homologues of the naturally occurring tetraamine spermine, exhibit antineoplastic activity against a number of murine and human tumor lines both *in vitro* and *in vivo*.[1,2] Although these compounds have been clearly shown to utilize the polyamine transport apparatus for incorporation,[3,4] deplete polyamine pools,[5] drastically reduce the level of ornithine decarboxylase (ODC)[6,7] and *S*-adenosylmethionine decarboxylase (AdoMetDC) activities,[8] and in some cases upregulate spermidine/spermine N^1-acetyltransferase (SSAT),[9–12] the precise mechanism by which they induce death in tumor cells remains somewhat of a mystery.[13] Interestingly, very small structural alterations in these polyamine analogues can cause pronounced changes in their biological activity.[5] For example, while the tetraamines N^1,N^{12}-diethylspermine (DESPM), N^1,N^{11}-diethylnorspermine (DENSPM), and N^1,N^{14}-diethylhomospermine (DEHSPM) (Table 1) suppress ODC and AdoMetDC to about the same level at equimolar concentrations, the impact of both DESPM and DEHSPM on cell growth is much faster than that observed for DENSPM. Furthermore, while the K_i values of DESPM and DEHSPM for the polyamine transport system are within error of each other, the DENSPM value is over 10 times as great.[5] However, the most notable differences between the three analogues are related to their ability to stimulate SSAT.[10–12] The tetraamine DENSPM upregulates SSAT by 15 fold in L1210 cells, while DESPM and DEHSPM stimulate SSAT by 4.6- and 1.4-fold, respectively.[11] The SSAT stimulation is even more pronounced in other cell lines.[14,15] On the basis of these findings, we were compelled to carry out a more systematic investigation of the impact of chain length and terminal alkyl group alteration on the biological properties of the polyamine analogues.

In the initial studies, the terminal alkyl groups of the analogues, e.g., DENSPM, were held constant while the length of the polyamine backbone was altered in a symmetrical fashion.[5] In the current investigation, the analogues, all tetraamines, were altered in two different ways: either the polyamine backbone was held constant and the terminal alkyl groups were changed or the alkyl substituents were static while the methylene backbone was altered. When the methylene backbone was held constant, the terminal alkyl groups were increased in size (i.e., methyl, ethyl, propyl, isopropyl, and in one

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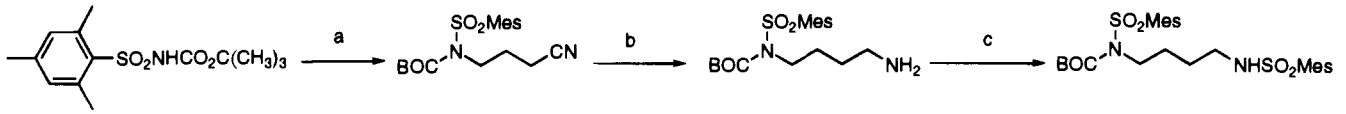
Scheme 1. Synthesis of Triprotected Putrescine Reagent **21**[a]



18 ($MesSO_2NHBOC$) **19** **20** **21**

[a] (a) 4-Chlorobutyronitrile/NaH/DMF; (b) H_2/Ra Ni/NH_3/CH_3OH; (c) mesitylenesulfonyl chloride/NaOH (aq)/CH_2Cl_2.

instance *tert*-butyl), a symmetrical modification, since both terminal alkyls were the same. In one example, an unsymmetrical dialkylation alteration was made on the terminal nitrogens: a *tert*-butyl group was fixed to one nitrogen and an ethyl group to the other. The other example of unsymmetrical substitution of the terminal nitrogens examined the monoethyl derivatives in which one terminal amine was left unmodified. The second alteration, holding the alkyl groups constant as ethyls, focused on DEHSPM and included either removing a single methylene from one of the terminal aminobutyl fragments or from the central aminobutyl insulator or adding a methylene to the central aminobutyl fragment. The principal directive of this work was to develop a "basis set" of polyamine analogues in which differences in IC_{50} properties could be ultimately correlated with some biochemical properties of the drugs.

While dimethyl- and diethylnorspermine and -spermine were prepared by the symmetrical terminal alkylation of the appropriate tetrasulfonamides, the corresponding symmetrical diisopropyl compound and all of the homospermine compounds were accessed by a fragment synthesis.[1] However, it was necessary to develop entirely new synthetic methodologies for the preparation of tetraamines with unsymmetrical backbones or unequal terminal alkyl groups. All of the compounds were screened for their 48 and 96 h IC_{50} values in L1210 cell culture assays. The same cell line was utilized to evaluate the tetraamine's competition with radiolabeled spermidine (SPD) for the polyamine uptake apparatus (K_i) and the effects of the analogues on ODC, AdoMetDC, SSAT, and polyamine pools.

Synthesis

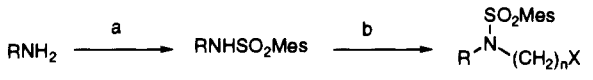
Three different families of tetraamines were synthesized (1) those with both symmetrical methylene backbones and the same alkyl group at each terminal nitrogen, referred to as the SS set, (2) those with symmetrical methylene backbones and different alkyl groups at the termini, or the SU set, and (3) those with unsymmetrical methylene backbones and symmetrical substitution at the terminal nitrogens, referred to as the US set. The symmetrical linear polyamines (SS) dimethyl- and diethylspermine and -norspermine, Table 1, were accessed by bis-alkylation of the corresponding tetrasulfonamide dianion. However, SS tetraamines with terminal secondary or tertiary alkyl substituents as well as the homospermine analogues were accessed via the "fragment synthesis" described in an earlier work.[1] In this case, the central diamine segment as its *N,N'*-bis-sulfonamide was alkylated at each end with the fragmenting reagent *N*-(ω-halomethylene)-*N*-alkanesulfonamide. This less direct method was employed for these compounds because alkylation of the tetrasulfonamide dianion with nonprimary halides, e.g., isopropyl bromide, proceeds poorly and homospermine is not commercially available. However, entirely new synthetic methods were required to access SU and US tetraamines.

The reagent designed for this study was an unsymmetrically protected diamine, **21**, Scheme 1. The protecting groups, a mesitylenesulfonyl at each end and a *tert*-butoxycarbonyl (BOC), although not completely orthogonal, can be selectively removed. This triprotected diamine can be deprotonated on the monosubstituted end and alkylated with fragmenting reagents. The BOC group can then be selectively removed with trifluoroacetic acid (TFA), resulting in only gaseous byproducts. The second labile hydrogen can be abstracted and the anion alkylated with any polyamine-fragmenting reagent that one chooses. All four of the mesitylenesulfonyl amine blocking groups could be cleanly removed under reductive conditions with 30% HBr in HOAc/PhOH to generate the polyamine analogue.[16,17] In previous work in the preparation of dialkylated polyamines,[1] the *p*-toluenesulfonyl protecting group was employed. Unmasking of the amines was accomplished by a dissolving metal reduction (Na/NH_3/THF); however, much insoluble byproduct was formed, and yields were variable. None of these problems occurred with the mesitylenesulfonyl protecting group.

Putrescine reagent **21** (Scheme 1) was prepared starting with *N*-(*tert*-butoxycarbonyl)-*N*-mesitylenesulfonamide (**18**), which was made by acylation of mesitylenesulfonamide[18] with oxalyl chloride, pyrolysis to the sulfonyl isocyanate,[19] and addition of *tert*-butyl alcohol. These conditions are more convenient than the literature synthesis of **18**,[20] which requires phosgene. Disubstituted ammonia derivative **18** was N-alkylated with 4-chlorobutyronitrile (NaH/DMF), generating nitrile **19.** The cyano group of **19** was hydrogenated with Raney nickel in methanolic ammonia, resulting in primary amine **20**. Both the *tert*-butoxycarbonyl and mesitylenesulfonyl amine protecting groups were stable to these alkaline hydrogenation conditions. Attachment of a second sulfonyl functionality to amine **20** under biphasic Schotten–Baumann conditions generated the versatile reagent *N*-(*tert*-butoxycarbonyl)-*N,N'*-bis(mesitylenesulfonyl)putrescine (**21**). It is noteworthy that this route is flexible, in that an ω-haloalkanenitrile of any length can be employed in the alkylation of reagent **18**; thus the length of the central chain of the polyamine target can be varied.

The synthesis of polyamine-segmenting reagents **26-31**, Scheme 2, began by reacting a primary amine, even if hindered, with mesitylenesulfonyl chloride. *N*-Alkylsulfonamides **22-25** were deprotonated (NaH/DMF) followed by alkylation with the appropriate dihalide in excess to generate synthons **26-31**. Thus *N*-(ω-halomethylene) *N*-alkylsulfonamides of any length can be prepared. These polyamine synthons eliminate limitations associated with the availability of the starting

Scheme 2. Synthesis of Polyamine Segmenting Reagents **26**–**31**[a]



22 R = Me
23 R = Et
24 R = *i*-Pr
25 R = *tert*-Bu

26 R = Me, n = 4, X = Br
27 R = Et, n = 3, X = Br
28 R = Et, n = 4, X = Br
29 R = *i*-Pr, n = 3, X = Cl
30 R = *i*-Pr, n = 4, X = Br
31 R = *tert*-Bu, n = 4, X = Br

[a] (a) Mesitylenesulfonyl chloride/NaOH (aq)/CH_2Cl_2; (b) NaH/DMF/terminal dihaloalkane.

tetraamine. In addition, while earlier methods were limited to accessing polyamine analogues with terminal primary alkyl groups, this approach allows for fixing primary, secondary, and tertiary alkyl groups to the terminal amines of polyamines. Thus triprotected diamine **21** can be elaborated in a stepwise manner by any pair of segmenting reagents to furnish a panel of polyamine analogues, including SS, SU, US, and completely unsymmetrical (UU) types.

Applications of the triprotected reagent and the terminal fragments to unsymmetrical polyamine preparation are shown in Schemes 3 and 4. The anion (NaH/DMF) of reagent **21** was alkylated with *N*-(4-bromobutyl)-*N*-ethylmesitylenesulfonamide (**28**), Scheme 3. The resulting *tert*-butoxy compound **32** was cleanly converted to *N*-alkyltrisulfonamide **33** under mild acidic conditions (TFA/CH_2Cl_2). The second nitrogen of reagent **21** was then elaborated by alkylation of the anion of **33** with *N*-(3-bromopropyl)-*N*-ethylmesitylenesulfonamide (**27**). The amino groups of **34** were efficiently unmasked using 30% HBr in acetic acid and phenol in CH_2Cl_2, generating 3,7,12,17-tetraazanonadecane, DE-(3,4,4) (**15**), a diethyl derivative of the natural product (aminopropyl)homospermidine[21] in which the outer chains are of unequal length (US set).

Although the same synthetic methodologies were employed to access N^1-(*tert*-butyl)-N^{14}-ethylhomospermine (ETBHSPM), **13**, an example of the SU set, Scheme 4, a rather unexpected elimination occurred during the acid-promoted deprotection step. *N*-Ethyltris(mesitylenesulfonamide) **33** was alkylated with *N*-(4-bromobutyl)-*N*-(*tert*-butyl)mesitylenesulfonamide (**31**). The resulting unsymmetrical derivative **35**, when exposed to 30% HBr in HOAc and PhOH in hopes of generating ETBHSPM (**13**), resulted in N^1-ethylhomospermine, MEHSPM (**10**). This turns out to be a useful method for the production of N^1-alkyl polyamines, which are proposed as metabolic products of the corresponding dialkyl polyamines in whole animals. Alternatively, the sulfonamides of **35** were cleaved using sodium and liquid ammonia in THF to provide the unsymmetrically dialkylated homospermine derivative **13** in moderate yield.

Finally, it is important to point out that with the segment approach, primary, secondary, or tertiary alkyl groups can be fixed to either terminal nitrogen of a polyamine. Also, the length of all three methylene spacers may be independently varied to provide convenient access to polyamines of the SS, SU, US, and UU sets. It is simply a matter of choosing the appropriate terminally mesitylenesulfonated central diamine, triprotected diamine reagent (Scheme 1), or alkylating fragment (Scheme 2).

Biological Evaluations

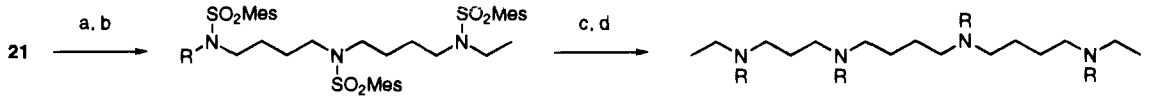
In summarizing the biological properties of the polyamine analogues, the results will be separated into three sets of measurements: the 48 and 96 h IC_{50} values against L1210 cells and the corresponding K_i values for the polyamine transport apparatus, Table 1; the effect on polyamine pools, Table 2; and the impact on ornithine decarboxylase, *S*-adenosylmethionine decarboxylase, and spermidine/spermine N^1-acetyltransferase activities, Table 3. These tables are arranged to describe each set of compounds: norspermines, spermines, homospermines, and homospermine homologues in turn. Finally, while we had reported the IC_{50} and K_i values, and the impact on polyamine pools, ODC, AdoMetDC, and SSAT of DENSPM, DESPM, and DEHSPM, the studies on these three compounds were repeated in order that the appropriate positive controls would be in place.

IC_{50} Studies. Whether or not both terminal nitrogens of norspermine were symmetrically substituted with methyl, ethyl, or isopropyl groups or had a single ethyl fixed to one of the terminal nitrogens was insignificant relative to the 48 and 96 h IC_{50}'s. All of the 48 h IC_{50} values were in excess of 100 μM, while the 96 h IC_{50} values were very similar, around 2 μM.

Unlike the norspermines, two of the spermine analogues, N^1,N^{12}-di-*n*-propylspermine (DPSPM) and DESPM, had a significant effect on cell growth at 48 h. The IC_{50} concentrations ranged from 3 to >100 μM with DPSPM < DESPM < N^1-ethylspermine (MESPM) < N^1,N^{12}-dimethylspermine (DMSPM). There was little difference in the 96 h IC_{50} values when the terminal nitrogens of spermine were symmetrically substituted with either a single methyl, ethyl, or propyl group or when a single ethyl was fixed to one of the terminal nitrogens.

There were considerable differences between the various alkylated homospermines at both 48 and 96 h. Unlike the norspermines, all of which had 48 h IC_{50}'s > 100 μM, the corresponding homospermine analogues,

Scheme 3. Synthesis of US Set Tetraamine **15**, DE(3,4,4)[a]

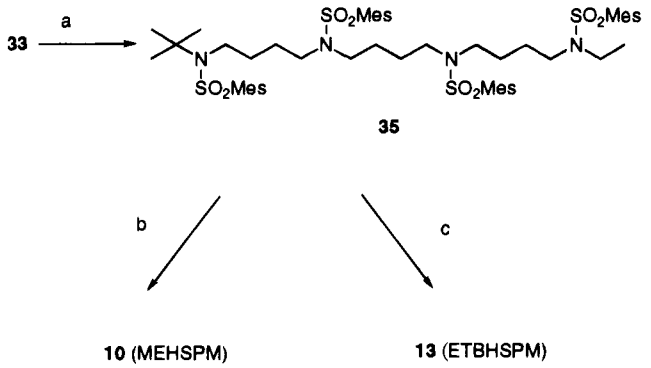


32 R = BOC
33 R = H

34 R = SO_2Mes
15 DE(3,4,4) R = H HCl

[a] (a) **28**/NaH/DMF; (b) TFA/CH_2Cl_2; (c) **27**/NaH/DMF; (d) 30% HBr in HOAc/PhOH/CH_2Cl_2, HCl.

Scheme 4. Synthesis of SU Set Tetraamines **10** (MEHSPM) and **13** (ETBHSPM)[a]



[a] (a) **31**/NaH/DMF; (b) 30% HBr in HOAc/PhOH/CH_2Cl_2, HCl; (c) Na/NH_3/THF, HCl.

except N^1,N^{14}-dimethylhomospermine (DMHSPM), had 48 h IC_{50}'s < 25 μM. The most notable differences in activity were between the diethyl and dimethyl compounds and between ETBHSPM and N^1,N^{14}-di-*tert*-butylhomospermine (DTBHSPM). At 48 h, DEHSPM, with a 0.2 μM IC_{50}, is the most active of all the homospermines, 70 times more effective than MEHSPM, 100 times more active than N^1,N^{14}-diisopropylhomospermine (DIPHSPM), and at least 500 times more active than DMHSPM. While replacement of a single ethyl group of DEHSPM with a *tert*-butyl group, ETBHSPM, resulted in a compound very similar in 48 h activity to DEHSPM, the DTBHSPM compound had an IC_{50} > 100 μM. It would seem that one sterically unhindered end of the homospermine analogues is required for significant 48 h activity. At 96 h, although there are differences in potency among the various homospermine analogues, they were not as impressive. The most notable difference, 100-fold, occurred between the *tert*-butyl-substituted homospermines ETBHSPM and DTBHSPM.

The outcome of holding the terminal alkyl groups and the overall length of the polyamine constant while changing the distance between the central and terminal nitrogens is interesting at an IC_{50} level. When comparing the homospermine homologues DE(3,4,4) and 3,8,-12,17-tetraazanonadecane, DE(4,3,4), the 48 and 96 h IC_{50}'s are the same within experimental error. The compounds' behavior is almost identical to that of DEHSPM. This was also true of 3,8,14,19-tetraazaheneicosane, DE(4,5,4), in which the length of DEHSPM is increased by one methylene. It is also notable that on going from the shortest diethylated tetraamine, DENSPM, to the longest, DE(4,5,4), the activity increases by a factor of at least 333 at 48 h and by 57 at 96 h. Thus an increase in the size of the methylene backbone proceeding from a (3,3,3), the number of methylenes between nitrogens for DENSPM, through (3,4,3), (4,3,4), (3,4,4), and (4,4,4) to a (4,5,4) array corresponds to a general decrease in the 48 and 96 h IC_{50} values.

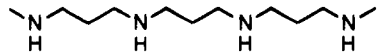
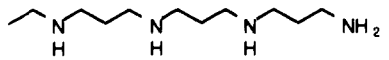
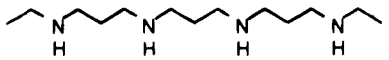
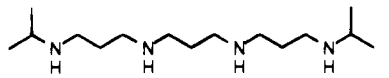
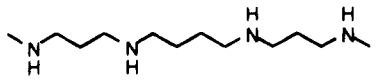
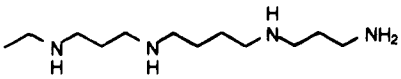
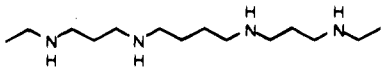
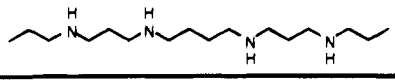
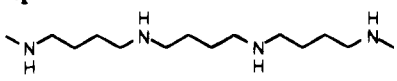
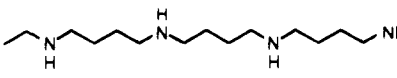
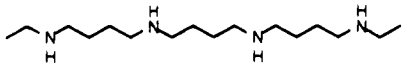
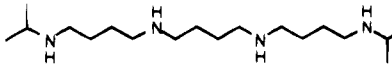
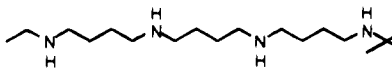
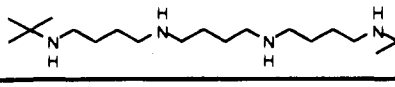
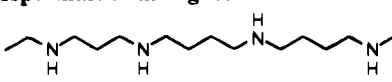
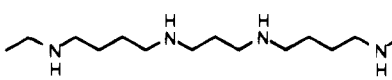
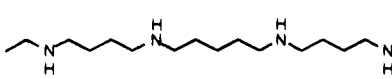
K_i Studies. The ability of the alkylated norspermines, spermines, homospermines, and homospermine homologues to compete with radiolabeled SPD for uptake was evaluated. The general trend within the norspermine and homospermine series was the larger the terminal alkyl group the larger the K_i value, Table 1. With the norspermines, the K_i went from 6 μM for DMNSPM to 40 μM for DIPNSPM. There was comparatively little variation of K_i in the spermine series. The lowest K_i, 1 μM for DMSPM, was about half the value seen for DPSPM. It is important to point out, however, that this is an *n*-propyl and not an isopropyl analogue. The *n*-propyl group is not as sterically bulky as the isopropyl alkyl. The most interesting series was the homospermines. As the terminal alkyl groups went from methyl to *tert*-butyl, a rather impressive change in K_i occurred.

The differences between the ETBHSPM and DTBHSPM K_i values are also notable. The ETBHSPM K_i is slightly higher than that of DEHSPM, while the K_i for DTBHSPM is 40 times higher than the DEHSPM value. This is in keeping with the idea that the polyamine transport apparatus requires only three cationic centers for polyamine recognition and transport.[4] Thus, one might expect ETBHSPM to bind a little more poorly than DEHSPM as DEHSPM has two ways it could present a nonsterically hindered tricationic array. In DEHSPM, an ethyl group, a relatively nonsterically hindered alkyl, is fixed to both ends of the tetraamine. ETBHSPM has an ethyl on one end and a sterically bulky *tert*-butyl group on the other. DTBHSPM has two *tert*-butyl groups, one on each terminus. If the tetracation, or fully protonated tetraamine, binds to the transport apparatus electrostatically, any *tert*-butyl group attached to a cationic center could compromise this interaction by increasing the distance between the charged sites.

Polyamine Pools. Because the IC_{50} curves for the polyamine analogues are sometimes flat near the 50% growth line, it can be difficult to determine the 48 h IC_{50} concentration with great accuracy. Thus 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 generally at least two different concentrations of analogue were evaluated. With analogues whose IC_{50} concentration exceeded 100 μM at 48 h, the determination was made at 100 and 500 μM except for DENSPM, which was run at 10 and 100 μM. For all other analogues, the effect on polyamine pools was evaluated at the 48 h IC_{50} concentration and at 5 times this number; ETBHSPM was also run at a third concentration, 25 times its 48 h IC_{50}. This was done in hopes of demonstrating that analogues which were not active at 48 h were not effective at depleting polyamine pools in this time frame. We wished to identify analogues which were not effective at suppressing polyamine pools at 48 h.

At 100 μM, the effect of DMNSPM, DENSPM, and MENSPM 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 36% with DMNSPM and to 11% with MENSPM. Increasing the concentration of the latter two drugs by a factor of 5 had little influence on the pools. DIPNSPM was not as effective as the other norspermine analogues at polyamine pool depletion. At 100 μM, putrescine was lowered to 10%, spermidine to 26%, and spermine to only 64%. At 500 μM DIPNSPM, spermidine was reduced to 12% of control, while putrescine essentially disappeared and spermine remained at 53%.

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

	Structure	Abbreviation	IC_{50} (μM) 48 h	IC_{50} (μM) 96 h	K_i (μM)
Norspermines					
1		DMNSPM	>100	2.5	5.6
2		MENSPM	>100	2.5	7.7
3		DENSPM	>100	2	17
4		DIPNSPM	>100	1.2	40
Spermines					
5		DMSPM	>100	0.75	1.1
6		MESPM	99	0.33	1.7
7		DESPM	30	0.18	1.6
8		DPSPM	3	0.2	2.3
Homospermines					
9		DMHSPM	>100	0.32	0.97
10		MEHSPM	14	0.17	1.1
11		DEHSPM	0.2	0.07	1.4
12		DIPHSPM	20	0.06	8.1
13		ETBHSPM	0.4	0.03	3
14		DTBHSPM	>100	3	56
Homospermine homologues					
15		DE(3,4,4)	0.3	0.08	8
16		DE(4,3,4)	1	0.18	4
17		DE(4,5,4)	0.3	0.035	6.0

[a] K_i values and IC_{50} concentrations at 48 and 96 h. K_i determinations were made by following analogue inhibition of spermidine transport.

The spermine analogues were slightly more effective than the norspermines. 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%.

At their 48 h IC_{50} concentrations, the homospermine analogues DMHSPM and MEHSPM were similar to the

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

	compd	conc (μM)	Put	SPD	SPM	analogue
		Norspermines				
1	DMNSPM	100	0	5	36	2.14
		500	0	3	27	1.84
2	MENSPM	100	0	5	11	1.69
		500	0	2	11	1.72
3	DENSPM	10	30	14	31	1.59
		100	0	6	30	2.44
4	DIPNSPM	100	10	26	64	
		500	0	12	53	
		Spermines				
5	DMSPM	100	0	0	21	1.26
		500	0	0	24	1.24
6	MESPM	100	0	1	21	1.24
		500	0	1	19	1.23
7	DESPM	30	0	0	12	0.40
		150	0	0	14	1.13
8	DPSPM	3	0	18	64	1.12
		15	0	9	43	1.09
		Homospermines				
9	DMHSPM	100	0	0	30	1.49
		500	0	0	27	1.03
10	MEHSPM	14	0	0	26	3.22
		70	0	0	26	2.99
11	DEHSPM	10	0	0	61	2.94
12	DIPHSPM	20	0	17	83	
		100	0	7	67	
13	ETBHSPM	0.4	133	96	75	
		2.0	78	68	63	
		10	0	10	77	
14	DTBHSPM	100	83	85	94	
		500	55	58	72	
		Homospermine Homologues				
15	DE(3,4,4)	0.3	40	47	65	0.27
		1.5	0	0	18	0.64
16	DE(4,3,4)	1	81	41	52	0.76
		5	0	0	27	1.50
17	DE(4,5,4)	0.3	44	61	70	0.26
		1.5	0	5	31	0.72

[a] Putrescine (Put), spermidine (SPD), and spermine (SPM) levels after 48 h of treatment are given as % polyamine found in untreated controls. Analogue amount is expressed as nmol/10^6 cells.

spermines in their ability to deplete the polyamines. DEHSPM and DIPHSPM were somewhat less effective at suppressing spermine pools. The homospermines with the largest terminal substituents, ETBHSPM and DTBHSPM, were the least effective at depletion of polyamine pools. At 10 μM, ETBHSPM reduced putrescine to below detectable limits, spermidine to 10% of control, and spermine to 80%. Even at 500 μM, DTBHSPM only reduced putrescine, spermidine, and spermine to 55%, 58%, and 72%, respectively. Finally, the homospermine homologues DE(3,4,4), DE(4,3,4), and DE(4,5,4), all of which demonstrated low 48 h IC_{50} values, did not show substantial depletion of polyamines except at the higher concentration. At this level, their behavior was similar to that of DEHSPM, although they were more effective at depleting spermine.

Although it was not possible to accurately measure the level of the isopropyl or *tert*-butyl analogues in the cells because of method limitations, the concentrations of all of the other analogues could be determined, Table 2. The data are not consistent with the idea that the most active compounds are accumulated to the greatest extent.

Impact of Analogues on ODC, AdoMetDC, and SSAT. As observed earlier and supported by these studies, there is little correlation between the ODC and AdoMetDC levels and the compound's 48 and 96 h IC_{50} values. Previous studies[11,13] suggested that the effect of the polyamine analogues on ODC and AdoMetDC is fairly rapid while analogue-induced upregulation of SSAT activity is somewhat slower. For example, DESPM reduction in ODC activity plateaued at 4 h and AdoMetDC at 12 h, while DENSPM induction of SSAT upregulation was maximized at 72 h of exposure to the analogues. On the basis of these studies, we elected to evaluate ODC and AdoMetDC at 4 and 6 h, respectively, and SSAT at 48 h, Table 3.

Table 3. Effect of Polyamine Homologues on Ornithine Decarboxylase (ODC), *S*-Adenosylmethionine Decarboxylase (AdoMetDC), and Spermidine/Spermine N^1-Acetyltransferase (SSAT) in L1210 Cells[a]

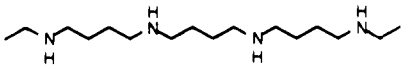
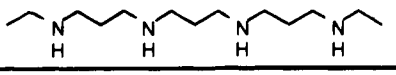
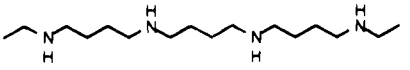
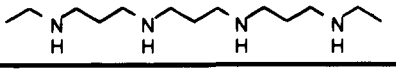
	compd	ODC	AdoMetDC	SSAT
		Norspermines		
1	DMNSPM	6	49	200
2	MENSPM	5	33	410
3	DENSPM	10	42	1500
4	DIPNSPM	100 (35)[b]	100	180
		Spermines		
5	DMSPM	3	40	300
6	MESPM	10	27	150
7	DESPM	3	28	460
8	DPSPM	52	72	500
		Homospermines		
9	DMHSPM	4	45	140
10	MEHSPM	3	41	110
11	DEHSPM	7 (1)	41	140
12	DIPHSPM	90 (10)	78	130
13	ETBHSPM	79 (9)	81	–
14	DTBHSPM	100 (60)[b]	100	–
		Homospermine Homologues		
15	DE(3,4,4)	5	37	370
16	DE(4,3,4)	5	28	120
17	DE(4,5,4)	20	39	120

[a] Enzyme activity is expressed as percent of untreated control for ODC (1 μM at 4 h (or 48 h)), AdoMetDC (1 μM at 6 h), and SSAT (2 μM at 48 h). [b] 100 μM at 48 h.

Reduction in ODC activity by all of the norspermines except DIPNSPM was rapid. In 4 h, 1 μM DMNSPM, MENSPM, or DENSPM reduced ODC activity to nearly the same extent, to approximately 7% of control, while DIPNSPM had no effect on this enzyme. The same phenomenon was observed for AdoMetDC. While 1 μM DMNSPM, MENSPM, or DENSPM all reduced the activity to approximately 42% at 6 h, DIPNSPM, again, had no effect on the enzyme. While DENSPM increased SSAT activity by 1500%, MENSPM increased SSAT by 410%, but DMNSPM and DIPNSPM only induced about a 200% upregulation. Except for acetylase activity, similar trends were observed for the spermine analogues.

At 1 μM DMSPM, MESPM, or DESPM, ODC activity was reduced to less than 10% of control, while ODC in DPSPM-treated cells was only lowered to 52% of controls. DMSPM, MESPM, or DESPM at 1 μM almost paralleled the ability of the corresponding norspermine analogues to suppress AdoMetDC. DPSPM at 1 μM reduced AdoMetDC activity to 72% of that seen in untreated cells. Although none of the spermine analogues was as effective as DENSPM at stimulating SSAT activity, nevertheless, there was a notable increase in activity by three of the four analogues tested. DESPM increased the acetylase activity by 460%, DPSPM by 500%, and DMSPM by 300%.

Table 4. Diethylnorspermine (DENSPM) and Diethylhomospermine (DEHSPM) Acute Single-Dose ip (od × 1 day) and Chronic Multiple-Dose ip (tid × 6 days) Toxicity Studies

Structure	Abbreviation	Lethal dose (mg/kg) LD_{10}	LD_{50}	LD_{90}
Acute				
	DEHSPM	250	325	450
	DENSPM	225	325	550
Chronic				
	DEHSPM	32.5	37.5	50
	DENSPM	180	196.5	225

Except for the impact on SSAT, the properties of the corresponding homospermine analogues were very similar to those of the spermines. DMHSPM, MEHSPM, or DEHSPM at 1 μM all reduced ODC to approximately 4% of control. All three of these analogues reduced AdoMetDC to 40%. Once again, DIPHSPM, the diisopropyl compound, had little impact on either ODC or AdoMetDC. ODC was reduced to 90% of control and AdoMetDC to 80%. While ETBHSPM diminished ODC and AdoMetDC to 80% of controls, DTBHSPM had no effect on either enzyme. None of the homospermine analogues, MEHSPM, DEHSPM, DMHSPM, or DIPHSPM, at 2 μM approached the effects of DENSPM on SSAT activity. MEHSPM had essentially no effect, while DEHSPM, DMHSPM, and DIPHSPM all increased SSAT to about 140%. Of the homospermine homologues, only DE(3,4,4) induced significant upregulation of SSAT activity, that is, 370%.

A comparison of the homospermine homologues DE-(3,4,4) and DE(4,3,4) with DEHSPM itself revealed that moving the three methylene bridges around had little effect on ODC. Interestingly, adding a methylene unit to DEHSPM to produce DE(4,5,4) resulted in a notable decrease in ODC-suppressing activity. ODC was only lowered to 20% of control, while other DE homologues reduced ODC to 5% of control. All three of the analogues were about as effective at reducing AdoMetDC, to about 40%.

The fact that DIPNSPM, DIPHSPM, and ETBHSPM had so little effect on ODC and AdoMetDC under the conditions of the experiment but yet depleted polyamines at 48 h compelled us to look at the effects of these analogues, at least on ODC, after extended exposure and/or at higher concentrations. It is important to recall that all previous experiments were carried out at 1 μM for a 4 h period. This is, of course, in many instances below the 48 h IC_{50} level and in some instances approaches the K_i for the analogue. At 1 μM and 48 h, DIPHSPM reduced ODC to 10% of control. At 1 μM for 48 h, ETBHSPM lowered ODC to 9% of control. At 100 μM DIPNSPM, ODC was lowered to 35% of control, while DTBHSPM had a minimal effect (60%) on ODC under the same conditions.

There was little correlation between the differences in 48 and 96 h IC_{50} concentrations among the analogues and the various biochemical parameters measured. However, a preliminary investigation suggested that the 48 vs 96 h IC_{50} values may be useful in selecting analogues for animals studies. When acute and chronic LD_{50} studies were carried out on DENSPM vs DEHSPM, some rather surprising results were encountered. The acute toxicity of DENSPM and DEHSPM were identical: both had an LD_{50} of 325 mg/kg at 4 h, Table 4. However, in chronic LD_{50} experiments in which animals were given the drug three times a day for 6 days, the DENSPM LD_{50} was more than 5 times that of DEHSPM. Further, both the acute and chronic LD_{10} and LD_{90} data reflected the LD_{50} numbers. The analogue with the lower 48 h IC_{50}, DEHSPM, had the greater chronic toxicity. Whether or not the 48 and 96 h IC_{50} values can indeed be utilized to predict chronic toxicity will clearly involve further investigation. Nevertheless, this initial toxicity study was further substantiated in later applications of DENSPM and DEHSPM in a MALME-3 animal model.[2]

Discussion

While all of the polyamine analogues did show some ability to suppress cell growth, at 48 h there were marked differences between the norspermine, spermine, homospermine, and homospermine homologues. At 48 h, the norspermines were the least active followed by the spermines. The homospermines and the homospermine homologues were the most effective; DTBHSPM and DMHSPM were exceptions. In the DE(3,4,4), -(4,3,4), and -(4,5,4) series, for which DEHSPM was regarded as the positive control, neither the molecules' symmetry nor the number of methylenes had a significant impact on their IC_{50} values, Table 1. At 96 h, all of the analogues, even DTBHSPM, had IC_{50}'s below 3

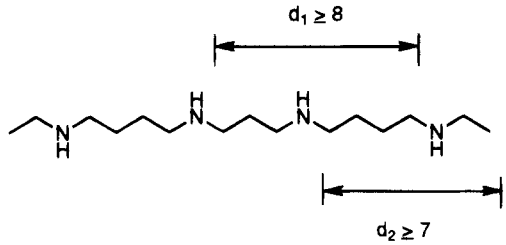


Figure 1. Structural parameters that define which analogues demonstrate 48 h IC_{50} activity < 25 μM.

μM. At 96 h, the differences in IC_{50} values between the polyamine analogues were compressed both within and between the respective families. Nevertheless, it is quite clear that some of the analogues are much faster acting than others. The tetraamines which very quickly shut down cell growth, 48 h IC_{50} < 25 μM, do have certain common structural features. These structural commonalities are related to both the distance between a terminal and internal nitrogen and the distance between the terminal carbon and the second closest nitrogen. This structure–activity relationship can be best understood from Figure 1, in which the terms d_1 and d_2 refer to the indicated distances along the backbone of the analogues. Thus d_1 is the number of chain atoms between a terminal nitrogen and the nitrogen second closest to it. Further, d_2 is the distance from a terminal carbon to the second nitrogen from that terminus; the terminal carbon is included in the count. If a molecule conforms to a $d_1 \geq 8$, $d_2 \geq 7$ structure, it is active at 48 h. This observation holds true except when the terminal alkyl groups become very bulky as *tert*-butyl, e.g., DTBHSPM. It is interesting to note that DE(3,4,4), in which only one side of the molecule conforms to $d_1 \geq 8$, $d_2 \geq 7$, is still active. Furthermore, although ETBHSPM contains a bulky terminus, both sides of the molecule conform to $d_1 \geq 8$, $d_2 \geq 7$, and therefore the analogue has rapid antiproliferative activity. There was also a notable trend regarding the size of the terminal alkyl group fixed to the norspermines and homospermines, Table 1. As the alkyl groups increased in size, the K_i value also increased. Although there were some differences among the spermine K_i's as the terminal alkyls were modified, the differences were not as pronounced.

Of the polyamines evaluated, DENSPM was distinct in its ability to increase SSAT activity, Table 3. While DESPM and DPSPM were the next most active compounds in SSAT upregulation, they were still far below DENSPM. The dimethyl, monoethyl, and diethyl tetraamines were all very effective at suppressing ODC and AdoMetDC. However, the analogues with larger terminal alkyl groups, DIPNSPM, DIPHSPM, ETBHSPM, and DTBHSPM, were less effective at ODC and AdoMetDC suppression. Once again, with DE(3,4,4) and DE(4,3,4), the symmetry of the molecule had little effect on how these compounds suppressed ODC or AdoMetDC. While it is tempting to simply ascribe these differences in activity to the fact that the larger molecules do not "fit" into a particular "receptor", there is an alternative explanation. It may be that while the analogues can all "fit" reasonably well into a receptor, for electrostatic reasons, they may not all bind as tightly.

On the basis of pK_a values measured for DENSPM and MINSQ calculations using published pK_a values for homospermine and spermine,[22] all of the tetraamines investigated are highly protonated at physiological pH. At pH 7.2, spermine and homospermine are 85% and 97% tetracation, respectively, while DENSPM is 74% in this form, the remainder being the trication. Their ability to interact with biological counterions, e.g., a group of anions fixed to a platform, as in nucleic acids and thus to be "read" by the cell as "normal" polyamines could well be related to these cationic properties. While the tetraamines with the largest alkyl groups, e.g., DTBHSPM, are still tetracations, the bulky substituents at the terminal nitrogens would compromise any electrostatic interaction between these compounds and a biological counteranion. In the simplest terms, the force vector between charged centers falls off as the inverse square of the distance between centers. A bulky substituent at a cationic center would force the cation away from the anion. This concept is now being further explored by maintaining alkyl substituents at the same size while changing the pK_a and thus altering the fraction of charged tetraamine.

The effectiveness with which the various polyamine analogues depleted polyamine pools, Table 2, did not parallel their differences in 48 and 96 h IC_{50} values. Furthermore, the level of accumulation of the analogues in the cells did not reflect the variation in the drug's activity. While the investigation has clearly defined a "test set" of polyamine analogues which will help to facilitate an understanding of the mechanism of action of polyamine analogues, additional work is clearly necessary.

Whether or not the analogue's impact on polyamine metabolism, e.g., ability to suppress polyamine pools, is solely responsible for IC_{50} properties is still not at all clear. In fact, the lack of correlation between analogue activity and parameters like polyamine depletion suggests that the drugs could owe their antineoplastic activity, at least in part, to something other than impacting on the polyamine metabolic network,[23] e.g., mitochondrial effects[1] and protein synthesis.[24] A further study of the effect of lower analogue concentration and shorter cell exposure times on polyamine metabolism is currently underway. This may filter out the differences in activity among the analogues. Finally, whether those compounds which demonstrate high 48 h IC_{50} values uniformly present with less chronic toxicity must also be explored. Clearly if this were the case, the 48 vs 96 h IC_{50} values would be of value in electing which polyamine analogues should be carried forward to animal studies.

Experimental Section

Polyamine analogues **5**, **7**, and **8** were previously prepared in these laboratories.[1] N^1-Acetylspermine trihydrochloride was obtained from Sigma Chemical Co., and other reagents were purchased from Aldrich Chemical Co. Reactions using hydride reagents were run in distilled DMF under a nitrogen 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 60 (70–230 mesh) obtained from EM Science (Darmstadt, Germany) or silica gel 32–63 (40 μm "flash") from Selecto, Inc. (Kennesaw, GA) was used for 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 given 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. Murine L1210 Leukemia cells were maintained in logarithmic growth as a suspension culture in RPMI-1640 medium containing 10% NuSerum (Collaborative Research, Bedford, MA), 2% HEPES–MOPS buffer, and 1 mM aminoguanidine (Sigma) at 37 °C in a water-jacketed 5% CO_2 incubator.

IC_{50} Determinations. Cells were grown in 25 cm^2 tissue culture flasks in a total volume of 10 mL. Cultures were treated while in logarithmic growth ($0.5-1.0 \times 10^5$ cells/mL) with the polyamine derivatives diluted in sterile water and filtered through a 0.2 μm filter. Following a 48 h period, cells were reseeded and incubated for an additional 48 h.

After the indicated time periods, cells were removed from flasks for counting. Cell number was determined by electronic particle counting (Model Z_F coulter counter, Coulter Electronics, Hialeah, FL) and confirmed periodically with hemocytometer measurements.

The percentage of control growth was determined as follows:

$$\% \text{ of control growth} = \left(\frac{\text{final treated cell no.} - \text{initial inoculum}}{\text{final untreated cell no.} - \text{initial inoculum}}\right) \times 100$$

The IC_{50} is defined as the concentration of compound necessary to reduce cell growth to 50% of control growth after defined intervals of exposure.

Polyamine Pool Analysis. While in logarithmic growth, cells were treated with the polyamine derivatives. At the end of the treatment period, cell suspensions were sampled, washed twice in cold medium RPMI-1640, and pelleted for extraction using 0.6 N perchloric acid.[5] Each supernatant was frozen at −20 °C until analysis by HPLC.

Uptake Determinations. The polyamine derivatives were studied for their ability to compete with [^{3}H]SPD or [^{14}C]SPD for uptake into L1210 leukemia cells *in vitro*.[5] Cell suspensions were incubated in 1 mL of RPMI-1640 containing 1, 2, 4, 6, 8, and 10 μM radiolabeled SPD alone or with the additional presence of 10, 25, and 50 μM polyamine analogue for 20 min at 37 °C. At the end of the incubation period, tubes were centrifuged at 900g for 5 min at 0–4 °C. The pellet was washed twice with 5 mL of cold RPMI-1640 containing 1 μM SPD, dissolved in 200 μL of 1 N NaOH at 60 °C for 1 h, and neutralized with 1 N HCl. The material was transferred to a vial for scintillation counting. Lineweaver–Burke plots indicated a simple competitive inhibition with respect to SPD.

Enzyme Assays. ODC and AdoMetDC activities were determined according to the procedures of Seely and Pegg[25] and Pegg and Pösö,[26] respectively, on the basis of quantitation of $^{14}CO_2$ released from [^{14}C]carboxyl-labeled L-ornithine or S-adenosyl-L-methionine.

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.[12]

Potentiometric Measurements. DENSPM (117.1 mg, 0.300 mmol) was dissolved in carbon dioxide-free 0.1 M KCl (20.0 mL). The amine tetrahydrochloride was then converted to the free base by addition of a slight excess of 1 N NaOH (1.25 mequiv). This solution was then *immediately* titrated with 1 N HCl using a Radiometer-Copenhagen DTS833 digital titration system. The pK_a's were estimated to be 10.83, 9.91, 8.51, and 7.68 from a computer fit of the DENSPM titration data using MINSQ v. 2.0 nonlinear parameter estimation software (Micromath Scientific Software, Salt Lake City, UT). The fraction of polyamine present in tetracationic, etc., forms was calculated by MINSQ in the simulation mode given the four pK_a values.

DEHSPM and DENSPM Toxicity Studies. Acute single-dose ip (od × 1 day) and chronic multiple-dose ip (tid × 6 days) toxicity studies were performed in CD-1 female mice (average weight, 35 g). The analogues tested were made up fresh daily in sterile water for injections and filtered through a 0.2 μm filter. The injection volume was equivalent to 1 mL/100 g of body weight. The acute toxicity doses ranged from 200 to 600 mg/kg in increments of 50 mg. In the case of the chronic toxicity investigation (three doses a day for 6 days), doses ranging from 5 to 20 mg/kg in increments of 5 mg were studied. Since no signs of toxicity in the case of DENSPM were observed at these doses, the drug was increased to a range of 40 to 90 mg/kg, again at increments of 5 mg. Severe weight loss (>20% of original weight) was observed on the last day of treatment in all animals at toxic doses, and at these toxic doses, animals either completely recovered or died within 1 week of the last injection.

***N*-(*tert*-Butoxycarbonyl)mesitylenesulfonamide (18).** Mesitylenesulfonamide[18] (5.0 g, 25 mmol) and oxalyl chloride (20 g, 0.16 mol) were stirred for 24 h at reflux to give a solid, which was heated at 170 °C for 1 h in *o*-dichlorobenzene (85 mL). After cooling to room temperature, *tert*-butyl alcohol (2.4 mL, 25 mmol) was added by syringe and stirring continued at room temperature for 8 h. The mixture was cooled to 0 °C, and 1 N NaOH (150 mL) was added. The aqueous phase was extracted with $CHCl_3$ (3 × 100 mL), cooled to 0 °C, and then acidified to pH = 2 with cold 1 N HCl (150 mL). Extraction with $CHCl_3$ (4 × 150 mL), removal of solvent, and purification by flash chromatography with 1:5 EtOAc/hexane gave 4.83 g (64%) of **18** as a white solid: mp 132–135 °C (lit.[20] mp 133–135 °C); NMR δ 1.26 (s, 9 H), 2.25 (s, 3 H), 2.60 (s, 6 H), 6.90 (s, 2 H), 7.55 (s, 1 H).

***N*-(*tert*-Butoxycarbonyl)-*N*-(3-cyanopropyl)mesitylenesulfonamide (19).** NaI (96 mg, 0.64 mmol) and NaH (80%, 0.52 g, 17.3 mmol) were added to **18** (4.14 g, 13.8 mmol) in DMF (85 mL), and the mixture was stirred for 25 min until hydrogen evolution ceased. 4-Chlorobutyronitrile (3.8 mL, 42 mmol) was added by syringe, and the contents of the flask were heated at 70 °C under nitrogen for 1 day. The reaction was quenched with water (10 mL) at 0 °C, and the solvents were removed under high vacuum. Distilled water (100 mL) was added to the residue, and product was extracted with $CHCl_3$ (3×). Organic extracts were washed with 100 mL portions of 1 N NaOH, 1% Na_2SO_3, and water, and solvent was removed by rotary evaporation. Silica gel column chromatography, eluting with 1% EtOH/$CHCl_3$, gave 3.79 g (75%) of **19** as a solid: NMR δ 1.23 (s, 9 H), 2.0–2.6 (m, 13 H), 3.91 (t, 2 H, J = 7), 6.91 (s, 2 H). Anal. ($C_{18}H_{26}N_2O_4S$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-*N*-(4-aminobutyl)mesitylenesulfonamide (20).** W-2 grade Raney nickel (5.90 g) and concentrated NH_4OH (5 mL) were successively added to a solution of **19** (3.74 g, 10.2 mmol) in CH_3OH (200 mL) in a 500 mL Parr bottle, and a slow stream of NH_3 was bubbled through the mixture for 30 min at 0 °C. After hydrogenation was carried out at 50–55 psi for 6 h, the suspension was filtered through Celite and the solvents were removed in vacuo. Silica gel column chromatography, eluting with 1% concentrated NH_4OH/CH_3OH, afforded 4.02 g (quantitative) of **20** as an oil: NMR δ 1.21 (s, 9 H), 1.4–2.1 (m, 6 H), 2.28 (s, 3 H), 2.51 (s, 6 H), 2.75 (t, 2 H, J = 7), 3.82 (t, 2 H, J = 7), 6.90 (s, 2 H). Anal. ($C_{18}H_{30}N_2O_4S$) C, H, N.

***N*-(*tert*-Butoxycarbonyl)-*N*,*N*'-bis(mesitylenesulfonyl)-1,4-diaminobutane (21).** A solution of mesitylenesulfonyl chloride (2.30 g, 10.5 mmol) in CH_2Cl_2 (100 mL) was added dropwise over 70 min to a rapidly stirred mixture of **20** (3.91 g, 10.6 mmol) in CH_2Cl_2 (50 mL) and 1 N NaOH (50 mL) at 0 °C. The mixture was stirred for 1 day (0 °C to room temperature). Water (50 mL) was added, the layers were separated, and the aqueous layer was extracted with $CHCl_3$ (2 × 50 mL). The combined organic extracts were washed with water (50 mL), and the solvent was removed. Silica gel column chromatography, eluting with 2% EtOH/$CHCl_3$, generated 5.06 g (87%) of **21** as a solid: mp 109–109.5 °C; NMR δ 1.18 (s, 9 H), 1.49–1.75 (m, 4 H), 2.26, 2.48, and 2.61 (3 s, 18 H), 2.78–3.08 (m, 2 H), 3.73 (t, 2 H, J = 7), 4.6 (m, 1 H), 6.89 (s, 4 H). Anal. ($C_{27}H_{40}N_2O_6S_2$) C, H, N.

***N*-Isopropylmesitylenesulfonamide (24).** A solution of mesitylenesulfonyl chloride (36.5 g, 0.167 mol) in CH_2Cl_2 (200 mL) was added dropwise to isopropylamine (9.87 g, 0.167 mol)

in 1 N NaOH (200 mL) at 0 °C, and the mixture was stirred at room temperature overnight. Product isolation by the procedure of **25** and recrystallization from aqueous EtOH gave 33.5 g (83%) of **24** as colorless prisms: mp 97–98 °C; NMR δ 1.00 (d, 6 H, J = 7), 2.20 (s, 3 H), 2.55 (s, 6 H), 3.16–3.55 (m, 1 H), 4.35 (d, 1 H, J = 7), 6.85 (s, 2 H). Anal. ($C_{12}H_{19}NO_2S$) C, H, N.

***N*-*tert*-Butylmesitylenesulfonamide (25).** Mesitylenesulfonyl chloride (10.0 g, 45.7 mmol) in CH_2Cl_2 (100 mL) was added dropwise with stirring to *tert*-butylamine (9.6 mL, 91 mmol) in 1 N NaOH (160 mL) at 0 °C. The ice bath was removed, and the mixture was stirred for 16 h at room temperature. The layers were separated, and the aqueous portion was further extracted with CH_2Cl_2 (3 × 80 mL). The organic extracts were washed with 1 N NaOH, 1 N HCl, and H_2O and evaporated *in vacuo*. Recrystallization with 15% aqueous EtOH produced 10.03 g (86%) of **25**: mp 141–143 °C; NMR δ 1.20 (s, 9 H), 2.27 (s, 3 H), 2.63 (s, 6 H), 4.53 (br s, 1 H), 6.90 (s, 2 H). Anal. ($C_{13}H_{21}NO_2S$) C, H, N.

***N*-(4-Bromobutyl)-*N*-methylmesitylenesulfonamide (26).** NaH (80%, 3.73 g, 0.124 mol) was added to a solution of **22**[18] (20.4 g, 95.7 mmol) in DMF (200 mL) at 0 °C. The mixture was stirred at room temperature for 30 min, and 1,4-dibromobutane (137 mL, 1.15 mol) was added at 0 °C. After stirring at 70 °C overnight, product was isolated following the procedure of **29**. Purification by column chromatography with 7:1 hexane/EtOAc produced 9.55 g (30%) of **26** as an oil: NMR δ 1.54–1.85 (m, 4 H), 2.25 (s, 3 H), 2.56 (s, 6 H), 2.68 (s, 3 H), 3.00–3.35 (m, 4 H), 6.88 (s, 2 H). Anal. ($C_{14}H_{22}BrNO_2S$) C, H, N.

***N*-(3-Bromopropyl)-*N*-ethylmesitylenesulfonamide (27).** NaH (80%, 0.58 g, 19 mmol) was added to **23**[18] (4 g, 17.6 mmol) in DMF (100 mL) at 0 °C. After the mixture was stirred at 0 °C for 30 min, 1,3-dibromopropane (21.4 mL, 211 mmol) was added. The mixture was stirred at 0 °C for 15 min and at 80 °C overnight. Following workup by the method of **29**, column chromatography with 8:1 hexane/EtOAc gave 3.26 g (53%) of **27** as an oil: NMR δ 1.10 (t, 3 H, J = 6), 2.05 (m, 2 H), 2.28 (s, 3 H), 2.60 (s, 6 H), 3.10–3.43 (m, 6 H), 6.92 (s, 2 H). Anal. ($C_{14}H_{22}BrNO_2S$) C, H, N.

***N*-(4-Bromobutyl)-*N*-ethylmesitylenesulfonamide (28).** Sodium hydride (80%, 3.71 g, 0.124 mol) was added to a solution of **23** (21.6 g, 95.0 mmol) in DMF (100 mL) at 0 °C. The mixture was stirred at 0 °C for 1 h, and 1,4-dibromobutane (140 mL, 1.17 mol) was added. The mixture was stirred at room temperature for 30 min and at 80 °C for 12 h. After workup by the procedure of **29**, purification by column chromatography with 6:1 hexane/EtOAc produced 33.2 g (96%) of **28** as an oil: NMR δ 1.04 (t, 3 H, J = 7), 1.4–1.8 (m, 4 H), 2.25 (s, 3 H), 2.55 (s, 6 H), 3.03–3.38 (m, 6 H), 6.87 (s, 2 H). Anal. ($C_{15}H_{24}BrNO_2S$) C, H, N.

***N*-(3-Chloropropyl)-*N*-isopropylmesitylenesulfonamide (29).** NaH (80%, 1.7 g, 57 mmol) was added to **24** (9.46 g, 32.9 mmol) in DMF (120 mL) at 0 °C. The mixture was stirred at room temperature for 30 min, and 1,3-dichloropropane (38 mL, 0.40 mol) was added at 0 °C. The mixture was stirred at 52 °C overnight and cooled and the reaction cautiously quenched with water (100 mL) followed by mixture extraction with Et_2O (3 × 100 mL). The combined organic fraction was washed with H_2O (4 × 100 mL) and brine (2 × 50 mL). The solvents were removed, and the crude oil was purified by column chromatography with 12:1 hexane/EtOAc to produce 7.48 g (60%) of **29** as an oil: NMR δ 1.10 (d, 6 H, J = 7), 1.72–2.02 (m, 2 H), 2.22 (s, 3 H), 2.52 (s, H), 3.80 (septet, 1 H, J = 7), 6.80 (s, 2 H). Anal. ($C_{15}H_{24}ClNO_2S$) C, H, N.

***N*-(4-Bromobutyl)-*N*-isopropylmesitylenesulfonamide (30).** NaH (80%, 1.54 g, 51.2 mmol) was added to **24** (9.5 g, 39 mmol) in DMF (120 mL) at 0 °C. The mixture was stirred at room temperature for 30 min, and 1,4-dibromobutane (56.5 mL, 0.473 mol) was added at 0 °C. The mixture was stirred at 60 °C overnight and worked up following the procedure of **29**. Purification by column chromatography with 10:1 hexane/EtOAc furnished 11.34 g (77%) of **30** as an oil: NMR δ 1.16 (d, 6 H, J = 7), 1.50–1.85 (m, 4 H), 2.27 (s, 3 H), 2.50 (s, 6 H), 3.00–3.40 (m, 4 H), 3.75–4.05 (septet, 1 H), 6.90 (s, 2 H). Anal. ($C_{16}H_{26}BrNO_2S$) C, H, N.

***N*-(*tert*-Butyl)-*N*-(4-bromobutyl)mesitylenesulfonamide (31).** NaH (80%, 0.43 g, 14 mmol) was added to a solution of **25** (2.98 g, 11.7 mmol) in DMF (90 mL). After the mixture was stirred for 17 min, gas evolution ceased and 1,4-dibromobutane (17.5 mL, 147 mmol) was added rapidly. After heating at 70 °C for 8 h, the reaction mixture was cooled to 0 °C and worked up by the method of **29**. Column chromatography on silica gel, eluting with 12.5% EtOAc/hexane, produced 1.87 g (41%) of **31**: NMR δ 1.33 (s, 9 H), 1.64–1.85 (m, 4 H), 2.25 (s, 3 H), 2.59 (s, 6 H), 3.17–3.40 (m, 4 H), 6.88 (s, 2 H). Anal. ($C_{17}H_{28}BrNO_2S$) C, H, N.

1-(*tert*-Butoxycarbonyl)-1,6,11-tris(mesitylenesulfonyl)-1,6,11-triazatridecane (32). NaH (80%, 0.143 g, 4.78 mmol) was added to a solution of **21** (2.40 g, 4.34 mmol) in DMF (40 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min, and a solution of **28** (1.73 g, 4.78 mmol) in DMF (10 mL) was slowly added. The solution was stirred at 0 °C for 15 min and at 70 °C overnight. Workup by the procedure of **29** and column chromatography with 4:1 hexane/EtOAc gave 3.41 g (94%) of **32** as an oil: NMR δ 1.06 (t, 3 H, J = 7), 1.20 (s, 9 H), 1.30–1.63 (m, 8 H), 2.30 (s, 9 H), 2.5 (s, 6 H), 2.57 (s, 12 H), 2.90–3.30 (m, 8 H), 3.67 (t, 2 H, J = 6), 6.92 (s, 6 H). Anal. ($C_{42}H_{63}N_3O_8S_3$) C, H, N.

1,6,11-Tris(mesitylenesulfonyl)-1,6,11-triazatridecane (33). TFA (15 mL) was slowly dripped into **32** (1.67 g, 2.00 mmol) in CH_2Cl_2 (60 mL) at 0 °C. Stirring was continued at 0 °C for an additional 20 min and at room temperature for 30 min. Solvents were removed by rotary evaporation, and the residue was cooled to 0 °C, basified to pH > 9 with saturated $NaHCO_3$, and extracted with CH_2Cl_2 (4 × 60 mL). Combined extracts were concentrated *in vacuo* to give 1.42 g (97%) of **33** as an oil: NMR δ 1.00 (t, 3 H, J = 6), 1.30–1.50 (m, 6 H), 1.60–1.73 (m, 2 H), 2.30 (s, 9 H), 2.57 (s, 18 H), 2.83 (t, 2 H, J = 6), 2.93–3.23 (m, 8 H), 4.53 (br s, 1 H), 6.90 (s, 6 H). Anal. ($C_{37}H_{55}N_3O_6S_3$) C, H, N.

3,7,12,17-Tetrakis(mesitylenesulfonyl)-3,7,12,17-tetraazanonadecane (34). NaH (80%, 65 mg, 2.15 mmol) was added to a solution of **33** (1.42 g, 1.95 mmol) in DMF (40 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min, and a solution of **27** (747 mg, 2.15 mmol) in DMF (25 mL) was added dropwise. The reaction mixture was then stirred at 0 °C for 15 min and at 70–80 °C overnight. Workup by the procedure of **29** and column chromatography with 2:1 hexane/EtOAc as eluant produced 0.97 g (50%) of **34** as an oil: NMR δ 0.97 (t, 3 H, J = 6), 1.00 (t, 3 H, J = 6), 1.23–1.50 (m, 8 H), 1.73 (m, 2 H), 2.30 (s, 12 H), 2.53 (s, 24 H), 2.90–3.27 (m, 16 H), 6.90 (s, 8 H). Anal. ($C_{51}H_{76}N_4O_8S_4$) C, H, N.

3,7,12,17-Tetraazanonadecane Tetrahydrochloride (15). Phenol (2.0 g, 21 mmol) and 30% HBr in HOAc (35 mL) were added successively to a solution of **34** (770 mg, 0.769 mmol) in CH_2Cl_2 (35 mL) at room temperature. The solution was stirred at room temperature for 48 h and worked up following the procedure of **1**. Recrystallization with 20% aqueous EtOH gave 244 mg (76%) of **15** as a crystalline solid: NMR (D_2O) δ 1.30 (t, 6 H, J = 6), 1.63–1.90 (m, 8 H), 1.93–2.30 (m, 2 H), 2.93–3.27 (m, 16 H). Anal. ($C_{15}H_{40}Cl_4N_4$) C, H, N.

***N*^{1},*N*^{5},*N*^{10},*N*^{14}-Tetrakis(mesitylenesulfonyl)-*N*^{1}-(*tert*-butyl)-*N*^{14}-ethylhomospermine (35).** NaH (80%, 51.2 mg, 1.71 mmol) was added to **33** (1.13 g, 1.55 mmol) in DMF (35 mL) at 0 °C, and the mixture was stirred at 0 °C for 30 min. A solution of **31** (668 mg, 1.71 mmol) in DMF (15 mL) was slowly added at 0 °C, and the reaction mixture was stirred at 70–80 °C for 8 h. Workup by the procedure of **29** and column chromatography with 2:1 hexane/EtOAc provided 1.20 g (74%) of **35** as an oil: NMR δ 1.00 (t, 3 H, J = 6), 1.27 (s, 9 H), 1.20–1.53 (m, 12 H), 2.30 (s, 12 H), 2.57 (s, 24 H), 2.87–3.30 (m, 14 H), 6.90 (s, 8 H). Anal. ($C_{54}H_{82}N_4O_8S_4$) C, H, N.

***N*^{1}-Ethylhomospermine Tetrahydrochloride (10).** Phenol (2.0 g, 21 mmol) and 30% HBr/HOAc (30 mL) were added to a solution of **35** (1.00 g, 0.958 mmol) in CH_2Cl_2 (25 mL). The solution was stirred at room temperature for 48 h and worked up following the procedure of **1**. Recrystallization from 20% aqueous EtOH gave 202 mg (50%) of **10** as a

crystalline solid: NMR (D_2O) δ 1.27 (t, 3 H, J = 6), 1.63–1.93 (m, 12 H), 2.90–3.23 (m, 14 H). Anal. ($C_{14}H_{38}Cl_4N_4$) C, H, N.

N^1-(*tert*-Butyl)-N^{14}-ethylhomospermine Tetrahydrochloride (13). Sodium (0.5 g, 21.7 mmol) was added to liquid NH_3 (150 mL) at −78 °C. After the solution was stirred at −78 °C for 30 min, a solution of **35** (860 mg, 0.824 mmol) in THF (150 mL) was slowly added dropwise. The reaction mixture was stirred at −78 °C for an additional 2 h, refluxed for 4 h, and stirred at room temperature overnight to drive off the ammonia. After the reaction was quenched with EtOH (50 mL), solvents were removed by rotary evaporation. The residue was dissolved in H_2O (15 mL) and extracted with $CHCl_3$ (5 × 40 mL). The solvent was removed, and the residue was dissolved in EtOH (60 mL) and treated with concentrated HCl (2 mL). The solvent was removed *in vacuo*, and the residue was recrystallized from 20% aqueous EtOH to produce 72 mg (20%) of **13** as a crystalline solid: NMR (D_2O) δ 1.30 (t, 3 H, J = 6), 1.37 (s, 9 H), 1.63–1.93 (m, 12 H), 2.90–3.23 (m, 14 H). Anal. ($C_{18}H_{46}Cl_4N_4$) C, H, N.

N^1,N^4,N^8,N^{11}-Tetrakis(mesitylenesulfonyl)norspermine (36). A solution of mesitylenesulfonyl chloride (24.2 g, 0.110 mol) in CH_2Cl_2 (50 mL) was added dropwise to a solution of *N*,*N*′-bis(3-aminopropyl)-1,3-propanediamine (5.2 g, 28 mmol) in 1 N NaOH (150 mL) at 0 °C. The mixture was stirred at room temperature overnight and treated by the procedure of **25**. Recrystallization from 50% EtOAc/hexane afforded 20.1 g (80%) of **36** as a crystalline solid: mp 135–136 °C; NMR δ 1.45–1.80 (m, 6 H), 2.25 (s, 12 H), 2.50 and 2.55 (2 s, 24 H), 2.63–3.27 (m, 12 H), 4.97 (t, 2 H, J = 7), 6.90 (s, 8 H). Anal. ($C_{45}H_{64}N_4O_8S_4$) C, H, N.

N^1,N^4,N^8,N^{11}-Tetrakis(mesitylenesulfonyl)-N^1,N^{11}-dimethylnorspermine (37). Sodium hydride (80%, 2.5 g, 0.083 mol) was added in portions to a solution of **36** (24.3 g, 0.026 mol) in DMF (200 mL) at 0 °C. The suspension was stirred for 1 h at room temperature until hydrogen evolution ceased and was cooled to 0 °C. Iodomethane (8.1 g, 57 mmol) was introduced, and the reaction mixture was heated at 60 °C for 20 h. After cooling, the reaction mixture was worked up by the method of **29**. Flash chromatography with 5:1 hexane/EtOAc as eluant produced 15.0 g (60%) of **37** as a white solid: NMR δ 1.55–1.81 (m, 6 H), 2.25 (s, 12 H), 2.50 and 2.56 (2 s, 30 H), 2.85–3.15 (m, 12 H), 6.87 (s, 8 H). Anal. ($C_{47}H_{68}N_4O_8S_4$) C, H, N.

N^1,N^{11}-Dimethylnorspermine Tetrahydrochloride (1). HBr (30%) in HOAc (300 mL) was added slowly to a solution of **37** (14.8 g, 15.7 mmol) and phenol (58.9 g, 0.626 mol) in CH_2Cl_2 (150 mL) at 0 °C. After the reaction mixture was stirred for 1 day at room temperature, H_2O (200 mL) was added followed by extraction with CH_2Cl_2 (3 × 200 mL). The aqueous portion was evaporated under high vacuum, and the residue was taken up in 1 N NaOH (10 mL) and 19 N NaOH (10 mL) followed by extraction with $CHCl_3$ (14 × 50 mL). After removal of $CHCl_3$, the residue was taken up in EtOH (100 mL) and acidified with concentrated HCl (10 mL). After the solvents were removed, the solid was recrystallized from aqueous EtOH to give 4.84 g (85%) of 1 as crystals: NMR (D_2O) δ 1.80–2.35 (m, 6 H), 2.70 (s, 6 H), 3.05–3.30 (m, 12 H). Anal. ($C_{11}H_{32}Cl_4N_4$) C, H, N.

N^1,N^4,N^8,N^{11}-Tetrakis(mesitylenesulfonyl)-N^1-ethylnorspermine (38). Sodium hydride (80%, 0.20 g, 6.5 mmol) was added in portions to a solution of **36** (6.0 g, 6.5 mmol) in DMF (50 mL) at 0 °C. The suspension was stirred for 8 h at room temperature and cooled to 0 °C. Iodoethane (0.52 mL, 6.5 mmol) was introduced slowly, and the reaction mixture was heated at 70 °C for 48 h. After workup by the method of **29**, purification by flash chromotograpy, using 5:3:2 hexane/CH_2Cl_2/EtOAc, produced 2.17 g (30%) of **38** as a white foam: NMR δ 0.85 (t, 3 H, J = 7), 1.50–1.70 (m, 6 H), 2.23 (s, 12 H), 2.47 and 2.50 (2 s, 24 H), 2.80–3.20 (m, 14 H), 4.80–5.00 (br s, 1 H), 6.85 (s, 8 H). Anal. ($C_{47}H_{68}N_4O_8S_4 \cdot H_2O$) C, H, N.

N^1-Ethylnorspermine Tetrahydrochloride (2). HBr (30%) in HOAc (50 mL) was added slowly to **38** (0.86 g, 0.91 mmol) and phenol (4.7 g, 50 mmol) at 0 °C. The reaction mixture was stirred overnight at 74 °C and worked up by the procedure of 1. Recrystallization from aqueous EtOH generated 60.5 mg of **2** (18%) as crystals: NMR (D_2O) δ 1.25 (t, 3 H, J = 7), 1.90–2.30 (m, 6 H), 2.95–3.30 (m, 14 H). Anal. ($C_{11}H_{32}Cl_4N_4$) C, H, N.

N^1,N^4,N^8,N^{11}-Tetrakis(mesitylenesulfonyl)-N^1,N^{11}-diethylnorspermine (39). Sodium hydride (80%, 1.91 g, 63.7 mmol) was added in two portions to a solution of **36** (26.6 g, 29.0 mmol) in DMF (200 mL) at 0 °C. The suspension was stirred for 1 h at room temperature and cooled to 0 °C. Iodoethane (20 g, 0.13 mol) was introduced, and the reaction mixture was heated at 80 °C for 12 h. After cooling, water (50 mL) was cautiously added, and solvents were removed under high vacuum. Water (200 mL) was combined with the residue followed by extraction with $CHCl_3$ (3 × 200 mL). The combined organic phase was washed with 1% $NaHSO_3$ (150 mL), H_2O (2 × 150 mL), and brine (150 mL) and evaporated *in vacuo*. Column chromatography on silica gel, eluting with 20:30:50 EtOAc/$CHCl_3$/hexane, produced 25.9 g (92%) of **39** as a colorless glass: NMR δ 0.95 (t, 6 H, J = 7), 1.39–1.87 (m, 6 H), 2.27 (s, 12 H), 2.52 (s, 24 H), 2.80–3.20 (m, 16 H), 6.90 (s, 8 H). Anal. ($C_{49}H_{72}N_4O_8S_4$) C, H, N.

N^1,N^{11}-Diethylnorspermine Tetrahydrochloride (3). A solution of **39** (5.00 g, 5.14 mmol) in CH_2Cl_2 (75 mL) was slowly added to phenol (19.3 g, 0.205 mol) and 30% HBr in acetic acid (100 mL) at 0 °C. After stirring for 1 day (0 °C to room temperature), the reaction mixture was worked up by the procedure of 1. Crude tetrahydrochloride salt was recrystallized from aqueous EtOH to furnish 1.67 g (84%) of **3** as white plates: NMR (D_2O) δ 1.28 (t, 6 H, J = 7), 1.91–2.35 (m, 6 H), 2.97–3.31 (m, 16 H); FABMS calcd for $C_{13}H_{32}N_4$ (free amine) 244.3, found 245.2 (M + 1, base peak). Anal. ($C_{13}H_{36}Cl_4N_4$) C, H, N.

***N*,*N*′-Bis(mesitylenesulfonyl)-1,3-propanediamine (40).** A solution of mesitylenesulfonyl chloride (28.4 g, 0.130 mol) in CH_2Cl_2 (150 mL) was added dropwise to a solution of 1,3-diaminopropane (4.84 g, 65 mmol) in 0.5 N NaOH (300 mL). The biphasic mixture was stirred at room temperature overnight, and the white solid was filtered and washed with 1 N HCl (3 × 25 mL) and H_2O (3 × 50 mL). Recrystallization from EtOAc furnished 26.1 g (91%) of **40** as crystals: mp 214–215 °C; NMR δ 2.20 (s, 6 H), 2.45 (s, 12 H), 2.60 (q, 4 H), 3.25 (s, 2 H), 6.93 (s, 4 H). Anal. ($C_{21}H_{30}N_2O_4S_2$) C, H, N.

N^1,N^4,N^8,N^{11}-Tetrakis(mesitylenesulfonyl)-N^1,N^{11}-diisopropylnorspermine (41). Sodium hydride (80%, 0.74 g, 23.6 mmol) was added in portions to a solution of **40** (3.11 g, 7.14 mmol) in DMF (40 mL) at 0 °C. The suspension was stirred for 1 h at room temperature and cooled to 0 °C. After addition of **29** (5 g, 15.7 mmol) in DMF (40 mL), the reaction mixture was heated at 60 °C for 20 h and worked up following the procedure of **29**. Column chromatography with 12:1 toluene/EtOAc as eluant produced 5.3 g (74%) of **41** as an oil: NMR δ 1.05 (d, 12 H, J = 7), 1.50–1.70 (m, 6 H), 2.25 (s, 12 H), 2.50 (s, 24 H), 2.80–3.05 (m, 12 H), 3.75 (septet, 2 H, J = 7), 6.90 (s, 8 H). Anal. ($C_{51}H_{76}N_4O_8S_4$) C, H, N.

N^1,N^{11}-Diisopropylnorspermine Tetrahydrochloride (4). A solution of **41** (5.2 g, 5.19 mmol) and phenol (18.4 g, 0.196 mol) in CH_2Cl_2 (80 mL) was cooled to 0 °C, and 30% HBr in HOAc (100 mL) was added slowly to the mixture. After the reaction mixture was stirred for 1 day at room temperature, workup was performed following the procedure of 1. Recrystallization from aqueous EtOH gave 1.5 g (69%) of **4** as crystals: NMR (D_2O) δ 1.30 (t, 12 H, J = 7), 1.90–2.30 (m, 6 H), 3.05–3.60 (m, 14 H). Anal. ($C_{15}H_{40}Cl_4N_4$) C, H, N.

N^1-Ethylspermine Tetrahydrochloride (6). Lithium aluminum hydride (1.2 g, 32 mmol) was added to N^1-acetylspermine trihydrochloride (0.500 g, 1.41 mmol) in THF (300 mL) at 0 °C, and the mixture was heated at reflux for 12 h. The reaction was quenched at 0 °C with H_2O (1.2 mL), 15% NaOH (1.2 mL), and H_2O (3.6 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 ($T \leq 180$ °C), and the distillate was dissolved in EtOH (40 mL) and treated with concentrated HCl (1 mL). Recrystallization of the concentrate from aqueous EtOH gave 0.34 g (65%) of **6** as crystals: NMR (D_2O) δ 1.30 (t, 3 H, J = 7), 1.77–1.84 (m, 4 H), 2.05–2.18 (m, 4 H), 3.08–3.22 (m, 14 H). Anal. ($C_{12}H_{34}Cl_4N_4$) C, H, N.

N,N'-Bis(mesitylenesulfonyl)-1,4-butanediamine (42). Mesitylenesulfonyl chloride (54.4 g, 0.249 mol) in CH_2Cl_2 (300 mL) was added to 1,4-diaminobutane (11.34 g, 0.129 mol) in 1 N NaOH (300 mL) at 0 °C, and the mixture was stirred for 1 day at room temperature. Organic solvent was evaporated, and 2.4 N HCl (250 mL) was added. Solid was filtered, washed with water (250 mL), and recrystallized from aqueous EtOH to give 50.46 g (90%) of **42** as needles: mp 156.5–157.5 °C; NMR δ 1.36–1.60 (m, 4 H), 2.27 (s, 6 H), 2.57 (s, 12 H), 2.69–2.96 (m, 4 H), 4.65 (t, 2 H, J = 6), 6.89 (s, 4 H). Anal. ($C_{22}H_{32}N_2O_4S_2$) C, H, N.

N^1,N^5,N^{10},N^{14}-Tetrakis(mesitylenesulfonyl)-N^1,N^{14}-dimethylhomospermine (43). Sodium hydride (80%, 1.19 g, 39.7 mmol) was added in portions to a solution of **42** (5.60 g, 12.4 mmol) in DMF (50 mL) at 0 °C. The suspension was stirred for 1 h at room temperature and cooled to 0 °C. A solution of **26** (9.55 g, 27.4 mmol) in DMF (50 mL) was introduced, and the reaction mixture was heated at 70 °C for 20 h and then worked up following the procedure of **29**. Purification by flash chromatography with 5:1 toluene/EtOAc as eluant gave 8.61 g (70%) of **43** as a white solid: NMR δ 1.25–1.50 (m, 12 H), 2.25 (s, 12 H), 2.53 and 2.59 (2 s, 30 H), 2.90–3.15 (m, 12 H), 6.90 (s, 8 H). Anal. ($C_{50}H_{74}N_4O_8S_4$) C, H, N.

N^1,N^{14}-Dimethylhomospermine Tetrahydrochloride (9). A solution of **43** (8.58 g, 8.61 mmol) and phenol (32.6 g, 0.347 mol) in CH_2Cl_2 (70 mL) was cooled to 0 °C. HBr (30%) in HOAc (170 mL) was added slowly to the mixture. After the reaction mixture was stirred for 1 day at room temperature and worked up following the procedure of 1, recrystallization from aqueous EtOH generated 2.54 g (72%) of **9** as white crystalline plates: NMR (D_2O) δ 1.62–1.90 (m, 12 H), 2.68 (s, 6 H), 2.95–3.20 (m, 12 H). Anal. ($C_{14}H_{38}Cl_4N_4$) C, H, N.

N^1,N^5,N^{10},N^{14}-Tetrakis(mesitylenesulfonyl)-N^1,N^{14}-diethylhomospermine (44). Sodium hydride (80%, 1.8 g, 60 mmol) was added in two portions to a solution of **42** (12.16 g, 26.9 mmol) in DMF (100 mL). The suspension was stirred for 1 h at room temperature, and **28** (20.45 g, 56.4 mmol) in DMF (50 mL) was added. The reaction mixture was stirred for 30 min and then heated at 72 °C for 16 h. After cooling, workup was carried out as for **39**. Column chromatography on silica gel, eluting with 1% $CH_3OH/CHCl_3$, produced 21.6 g (79%) of **44** as a glass: NMR δ 0.95 (t, 6 H, J = 7), 1.15–1.46 (m, 12 H), 2.23 (s, 12 H), 2.50 (s, 24 H), 2.85–3.25 (m, 16 H), 6.86 (s, 8 H). Anal. ($C_{52}H_{78}N_4O_8S_4$) C, H, N.

N^1,N^{14}-Diethylhomospermine Tetrahydrochloride (11). A solution of **44** (24.0 g, 23.65 mmol) in CH_2Cl_2 (250 mL) was slowly added to phenol (89.2 g, 0.946 mol) and 30% HBr/acetic acid (200 mL) at 0 °C. The reaction mixture was stirred for 1 day (0 °C to room temperature) and treated by the method of 1. Recrystallization from aqueous EtOH furnished 5.85 g (63%) of 11 as white plates: NMR (D_2O) δ 1.27 (t, 6 H, J = 7), 1.66–1.84 (m, 12 H), 3.01–3.15 (m, 16 H); FABMS calcd for $C_{16}H_{38}N_4$ (free amine) 286, found 287 (M + 1). Anal. ($C_{16}H_{42}Cl_4N_4$) C, H, N.

N^1,N^5,N^{10},N^{14}-Tetrakis(mesitylenesulfonyl)-N^1,N^{14}-diisopropylhomospermine (45). Sodium hydride (80%, 1.21 g, 40.3 mmol) was added in portions to a solution of **42** (5.70 g, 12.6 mmol) in DMF (50 mL) at 0 °C. The suspension was stirred for 1 h at room temperature and cooled to 0 °C. A solution of **30** (11.34 g, 30.1 mmol) in DMF (75 mL) was introduced, and the reaction mixture was heated at 60 °C for 20 h. Treatment by the method of **29** and flash column chromatography using 10:1 toluene/EtOAc gave 7.61 g (58%) of **45** as a white solid: NMR δ 1.10–1.45 (m, 24 H), 2.25 and 2.35 (2 s, 12 H), 2.55 (s, 24 H), 3.65–3.95 (m, 2 H), 6.85 (s, 8 H). Anal. ($C_{54}H_{82}N_4O_8S_4$) C, H, N.

N^1,N^{14}-Diisopropylhomospermine Tetrahydrochloride (12). A solution of **45** (7.57 g, 7.25 mmol) and phenol (27.2 g, 0.289 mol) in CH_2Cl_2 (100 mL) was cooled to 0 °C, and 30% HBr/HOAc (140 mL) was added slowly to the mixture. After the reaction mixture was stirred for 1 day at room temperature and worked up following the procedure of 1, recrystallization from aqueous EtOH gave 2.52 g (75%) of **12** as crystals: NMR (D_2O) δ 1.25 (d, 12 H, J = 7), 1.70–1.90 (m, 12 H), 2.95–3.15 (m, 12 H), 3.40 (septet, 2 H, J = 7). Anal. ($C_{18}H_{46}Cl_4N_4$) C, H, N.

N^1,N^5,N^{10},N^{14}-Tetrakis(mesitylenesulfonyl)-N^1,N^{14}-di-*tert*-butylhomospermine (46). NaH (80%, 138 mg, 4.60 mmol) was added to a solution of **42** (947 mg, 2.1 mmol) in DMF (20 mL) at 0 °C. After the mixture was stirred for 15 min, a solution of **31** (1.50 g, 4.2 mmol) in DMF (20 mL) was added dropwise at 0 °C. The mixture was stirred at 0 °C for 15 min and at 70 °C overnight. The reaction mixture was worked up by the method of **39**. Purification by silica gel column chromatography, using 4:1:2 hexane/EtOAc/$CHCl_3$, produced 1.40 g (66%) of **46** as an oil: NMR δ 1.27 (s, 18 H), 1.13–1.50 (m, 12 H), 2.27 (s, 12 H), 2.53 (s, 24 H), 2.90–3.30 (m, 12 H), 6.87 (s, 4 H), 6.90 (s, 4 H). Anal. ($C_{56}H_{68}N_4O_8S_4 \cdot H_2O$) C, H, N.

N^1,N^{14}-Di-*tert*-butylhomospermine Tetrahydrochloride (14). Sodium (0.60 g, 26.1 mmol) was added to liquid NH_3 (150 mL) at −78 °C. After the mixture was stirred for 30 min, a solution of **46** (1.34 g, 1.25 mmol) in THF (20 mL) was slowly added to the suspension at −78 °C. The mixture was stirred at −78 °C for 2 h, refluxed for 4 h, and stirred at room temperature overnight. The tetrahydrochloride salt was isolated by the method of **13** and recrystallized from aqueous EtOH to give 115 mg (19%) of **14** as a solid: NMR (D_2O) δ 1.36 (s, 18 H), 1.68–1.87 (m, 12 H), 3.04–3.16 (m, 12 H). Anal. ($C_{20}H_{50}Cl_4N_4 \cdot H_2O$) C, H, N.

3,8,12,17-Tetrakis(mesitylenesulfonyl)-3,8,12,17-tetraazanonadecane (47). NaH (80%, 0.205 g, 6.83 mmol) was added to **40** (1.25 g, 2.85 mmol) in DMF (25 mL) at 0 °C followed by the addition of **28** (2.46 g, 5.68 mmol) in DMF. The reaction mixture was stirred at 76 °C for 5 h and worked up by the procedure of **39**. Silica gel column chromatography with 8:1 and then 1:1 hexane/EtOAc gave 1.70 g (52%) of **47** as an oil: NMR δ 0.98 (t, 6 H, J = 7), 1.23–1.38 (m, 8 H), 1.58–1.81 (m, 2 H), 2.27 (s, 12 H), 2.52 and 2.54 (2 s, 24 H), 2.85–3.29 (m, 16 H), 6.90 (s, 8 H). Anal. ($C_{51}H_{76}N_4O_8S_4$) C, H, N.

3,8,12,17-Tetraazanonadecane Tetrahydrochloride (16). HBr (30%) in HOAc (30 mL) was added to **47** (1.7 g, 1.49 mmol) and phenol (2.42 g, 26 mmol) in CH_2Cl_2 (30 mL). The reaction mixture was stirred at room temperature for 1 day and worked up by the procedure of 1. Recrystallization from aqueous EtOH provided 0.38 g (61%) of **16** as plates: NMR (D_2O) δ 1.30 (t, 6 H, J = 7), 1.64–2.35 (m, 10 H), 2.98–3.33 (m, 16 H). Anal. ($C_{15}H_{40}Cl_4N_4$) C, H, N.

N,N'-Bis(mesitylenesulfonyl)-1,5-pentanediamine (48). A solution of mesitylenesulfonyl chloride (12.6 g, 57.6 mmol) in CH_2Cl_2 (150 mL) was added to 1,5-diaminopentane dihydrochloride (5.05 g, 28.8 mmol) in 1 N NaOH (150 mL) at 0 °C. After 1 day at room temperature, the reaction mixture was worked up by the method of **25**. Recrystallization from aqueous EtOH gave 12.21 g (91%) of **48** as crystals: mp 117–120 °C; NMR δ 1.18–1.49 (m, 6 H), 2.25 (s, 6 H), 2.58–2.97 (m + s, 16 H), 4.66 (t, 2 H, J = 6), 6.87 (s, 4 H). Anal. ($C_{23}H_{34}N_2O_4S_2$) C, H, N.

3,8,14,19-Tetrakis(mesitylenesulfonyl)-3,8,14,19-tetraazaheneicosane (49). NaH (80%, 0.35 g, 12 mmol) was cautiously added to a mixture of **48** (2.29 g, 4.90 mmol) and NaI (63 mg, 0.42 mmol) in DMF (90 mL). After hydrogen evolution ceased, **28** (5.90 g, 16.3 mmol) in DMF (10 mL) was added by syringe. After heating the reactants for 1 day at 53 °C, workup was performed by the method of **39**. Silica gel column chromatography, eluting with 1.5% $CH_3OH/CHCl_3$, gave 4.40 g (87%) of **49**: NMR δ 0.97 (t, 6 H, J = 7), 1.2–1.5 (m, 14 H), 2.25 (s, 12 H), 2.52 (s, 24 H), 2.8–3.2 (m, 16 H), 6.87 (s, 8 H). Anal. ($C_{53}H_{80}N_4O_8S_4$) C, H, N.

3,8,14,19-Tetraazaheneicosane Tetrahydrochloride (17). A solution of **49** (4.29 g, 4.17 mmol) and phenol (14.8 g, 0.157 mol) in CH_2Cl_2 (60 mL) was cooled to 0 °C, and 30% HBr/HOAc (80 mL) was added slowly to the mixture. The reaction mixture was stirred for 1 day at room temperature followed by workup by the method of 1. Recrystallization from aqueous EtOH gave 1.31 g (70%) of **17** as plates: NMR (D_2O) δ 1.25 (t, 6 H, J = 7), 1.55–1.85 (m, 14 H), 2.90–3.20 (m, 16 H). Anal. ($C_{17}H_{44}Cl_4N_4$) C, H, N.

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