Polyamine Analogue Antidiarrheals: A Structure−Activity Study

Raymond J. Bergeron,* Jan Wiegand, James S. McManis, William R. Weimar, Richard E. Smith, Samuel E. Algee, Tammy L. Fannin, Michael A. Slusher, and Patti S. Snyder†

Department of Medicinal Chemistry, University of Florida, J. Hillis Miller Health Science Center, Gainesville, Florida 32610

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The syntheses of a group of spermine polyamine analogues and their evaluation as antidiarrheals are described. Each compound was assessed in a rodent castor oil-induced diarrhea model for its ability to reduce stool output and weight loss in a dose-dependent manner. The spermine pharmacophore is shown to be an excellent platform from which to construct antidiarrheals. The activity of the compounds is very dependent on both the nature of the terminal alkyl groups and the geometry of the methylene spacers separating the nitrogens. The toxicity profile is also quite dependent on these same structural features. On the basis of subcutaneous dose−response data and toxicity profiles, two compounds, N^1,N^{12}-diisopropylspermine and N^1,N^{12}-diethylspermine, were taken forward into more complete evaluation. These measurements included formal acute and chronic toxicity trials, drug and metabolic tissue distribution studies, and assessment of the impact of these analogues on tissue polyamine pools. Finally, the remarkable activity of *N*,*N*′-bis[3-(ethylamino)propyl]-*trans*-1,4-cyclohexanediamine underscores the need to further explore this framework as a pharmacophore for the construction of other antidiarrheal agents.

Introduction

In a series of elegant studies, Tansy was able to demonstrate that polyamines have a profound impact on the motility of the gastrointestinal (GI) tract.[1] The original work focused on poly(ethylenimine) and gastric emptying in rodents[2] and dogs.[3] Branched-chain poly(ethylenimine)s effected significant inhibition of gastric emptying in rodents;[2] however, they caused a severe retch response in dogs.[3] Because of the structural relationship between the poly(ethylenimine)s and natural polyamines, Tansy elected to evaluate the effect of spermidine, spermine, and a group of polyamine analogues on the gastric emptying of rodents.[4] It soon became clear that polyamines had a substantial influence on gastric emptying and that "endogenous spermine and spermidine may have some unrecognized GI secretomotor activity".[4] From a structure−activity perspective, it also became obvious that minor changes in the polyamine's structure could completely eradicate the molecule's ability to inhibit gastric emptying. These studies strongly suggested that the polyamine pharmacophore was an excellent candidate for the construction of antitransit, antidiarrheal drugs.

One polyamine analogue designed and synthesized in these laboratories, N^1,N^{14}-diethylhomospermine (DEHSPM), is a very potent antidiarrheal.[5] This has been demonstrated in a number of animal models and in the clinic against AIDS-related diarrhea.[6] However, DEHSPM is *N*-deethylated to homospermine (HSPM), which has a very protracted half-life: 2−3 weeks in mice and even longer in the dog.[7] Each succeeding dose of DEHSPM results in a further accumulation of HSPM until toxic levels of the latter tetraamine are reached, a troublesome metabolic property in animals.

In subsequent experiments, we attempted to circumvent the accumulation of HSPM by assembling a more metabolically labile DEHSPM analogue, (3*R*,12*R*)-N^1,N^{14}-diethyl-3,12-dihydroxyhomospermine [(*R*,*R*)-$(HO)_2$DEHSPM]. The design of this analogue was predicated on the idea that the hydroxyl groups would allow for further metabolic processing (e.g., oxidation or glucuronidation) and clearance. Indeed, this was what was initially observed − $(HO)_2$DEHSPM was as effective an antidiarrheal as DEHSPM.[8] Although the acute toxicity of the hydroxylated derivative was similar to that of DEHSPM, $(HO)_2$DEHSPM was less toxic to mice upon short-term chronic (5-day) exposure than was DEHSPM.[8] Its residence time in most mouse tissues was shorter than that of DEHSPM;[8] in addition, the induction of cardiac bigeminies observed in $(HO)_2$DEHSPM-treated dogs was minimal (R. J. Bergeron, J. Wiegand, W. R. Weimar, R. Müller, J. S. McManis, G. W. Yao, G. Huang, P. S. Snyder, C. Porter, R. Braylan, unpublished results). However, when dogs were exposed to either (*R*,*R*)- or (*S*,*S*)-$(HO)_2$DEHSPM at a dose of 4.3 mg/kg/day × 14 days or to (*R*,*R*)-$(HO)_2$DEHSPM at a dose of 2.15 mg/kg/day × 28 days, pancreatic insufficiency developed approximately 40 days post-final dose and became severe within an additional 14 days (R. J. Bergeron, J. Wiegand, W. R. Weimar, R. Müller, J. S. McManis, G. W. Yao, G. Huang, P. S. Snyder, C. Porter, R. Braylan, unpublished results). When the tissues from these animals were submitted for histopathology at the time of sacrifice, it was observed that the acinar-derived exocrine pancreas was essentially gone, yet the endocrine pancreas was intact (R. J. Bergeron, J. Wiegand, W. R. Weimar, R. Müller, J. S. McManis, G. W. Yao, G. Huang, P. S. Snyder, C. Porter, R. Braylan, unpublished results). Therefore, although the hydroxylated DEH-

* To whom correspondence should be addressed. Tel: (352) 846-1956. Fax: (352) 392-8406. E-mail: bergeron@mc.cop.ufl.edu.
† Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida.

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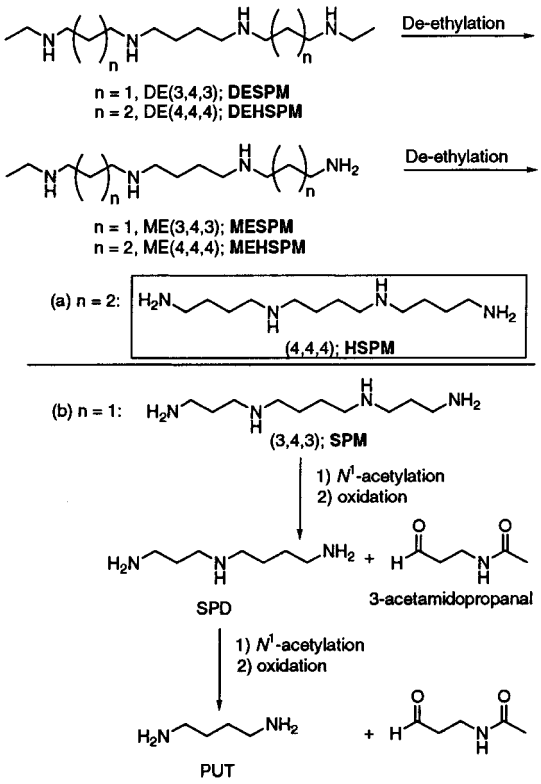


Figure 1. Metabolic breakdown of DEHSPM and DESPM. Although the *N*-deethylation pathway is operative for both compounds, the terminal 4-aminobutyl segments of HSPM are not recognized by the retrograde pathway, and further catabolism does not occur (a).[7] In contrast to MEHSPM, *N*-deethylation of MESPM yields SPM, which contains aminopropyl moieties and is further broken down via the retrograde pathway (b). SPD, spermidine; PUT, putrescine.

SPM analogue considerably decreased stool output with little buildup of either drug or metabolite in most tissues (with the exception of the pancreas), its long-term toxicity profile is unacceptable. Thus, the problem of designing a metabolically labile polyamine analogue still remains. The current work describes the design, synthesis, and testing of derivatives of N^1,N^{12}-diethylspermine (DESPM) in a castor oil-induced diarrhea model.[8]

Results

Design Concept. An initial comparative study among several polyamine analogues, including DEHSPM and N^1,N^{11}-diethylnorspermine (DENSPM), demonstrated that the longer tetraamine of these two, DEHSPM, was by far the most active of the compounds tested.[8] However, the practical utility of this polyamine was compromised by toxicity concerns, particularly cardiac adverse effects. This toxicity was compounded by, and likely related to, the analogue's metabolic profile.[7] In both mice and dogs, DEHSPM, a tetraamine with a (4,4,4) backbone (Figure 1), was shown to be first converted to N^1-monoethylhomospermine (MEHSPM) and then to HSPM which had a very protracted residence time in the tissues. The situation was quite the opposite with DENSPM, a compound with a (3,3,3) structure.[9] This tetraamine was first metabolized to monoethylnorspermine (MENSPM), which was taken further to either N^1-ethylnorspermidine (MENSPD) or to norspermine (NSPM). NSPM was subsequently deaminopropylated to norspermidine (NSPD); NSPD also derived from deethylation of MENSPD. In addition, MENSPD was deaminopropylated to N^1-ethyl-1,3-diaminopropane (MEDAP). Both MEDAP and NSPD were converted to 1,3-diaminopropane (DAP) via deethylation and deaminopropylation, respectively. Thus, the key in the processing of DENSPM was the removal of aminopropyl fragments.[9]

In the retrograde processing of both spermidine (SPD) and spermine (SPM) [(3,4,3); Figure 1], the aminopropyl ends of these systems are first acetylated by spermidine/spermine N^1-acetyltransferase (SSAT); the nearest internal carbon−nitrogen bond is oxidized to an imine; and the imine is hydrolyzed to 3-acetamidopropanal and the corresponding amine. Thus, SPM is converted to SPD and 1 mol of 3-acetamidopropanal, and SPD yields putrescine (PUT) and 3-acetamidopropanal (Figure 1).

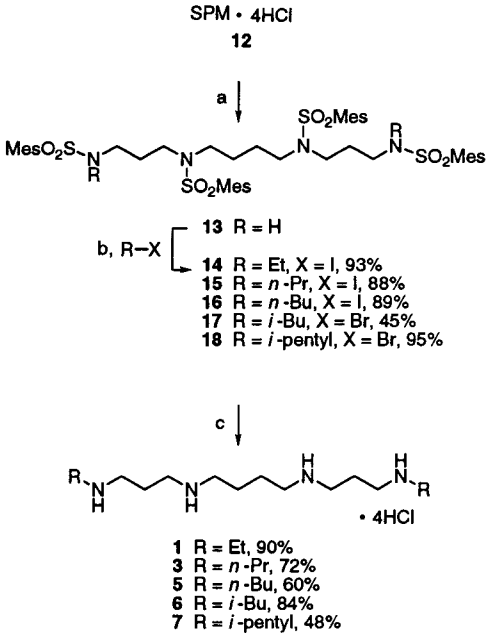
Thus, DEHSPM, the longer of the tetraamines, was the more active antidiarrheal but metabolically inert after deethylation to HSPM; however, DENSPM, although ineffective in reducing stooling, is less toxic and more metabolically labile by virtue of its aminopropyl components. On the basis of these observations, we were compelled to investigate a series of SPM analogues. If dialkylated SPMs ameliorated diarrhea and were subsequently dealkylated [e.g., N^1,N^{12}-diethylspermine (DESPM) to SPM, Figure 1], the issue of metabolite (i.e., SPM) buildup would not be problematic as with HSPM. The catabolic processing should be similar to what was observed with DENSPM. However, in this instance, three natural products − SPM, SPD, and PUT − are generated.

Once we established that DESPM was effective at reducing stool output in a castor oil-induced model of diarrhea, we undertook a systematic evaluation of how altering the terminal alkyl groups on SPM would impact the antidiarrheal properties of the SPM platform (Table 1). The parameters examined included charge, lipophilicity, and geometry on the activity of the SPM analogues in this model.

Synthesis. Terminally bis-alkylated SPMs, e.g., DESPM (**1**), were previously prepared in our laboratories by alkylation of tetratosylated SPM with primary halides.[10] An improved route (Scheme 1), suggested by our experience with alkylation of mesitylenesulfonyl-protected amines[11] and facilitated by the commercial availability of SPM tetrahydrochloride, was utilized to synthesize DESPM, N^1,N^{12}-dipropylspermine (DPSPM), N^1,N^{12}-dibutylspermine (DBSPM), N^1,N^{12}-diisobutylspermine (DIBSPM), and N^1,N^{12}-diisopentylspermine (DIPESPM) (**1**, **3**, and **5**−**7**, respectively).

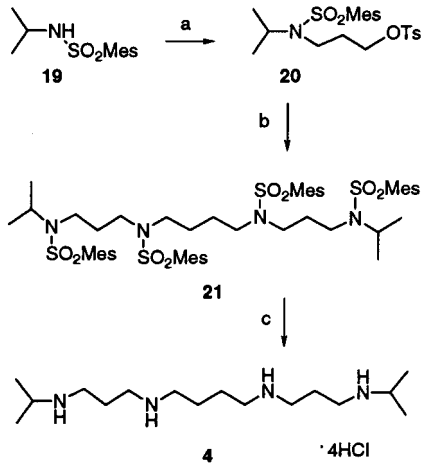
Each synthesis began with the reaction of SPM tetrahydrochloride (**12**) with 4 equiv of mesitylenesulfonyl chloride under biphasic conditions (CH_2Cl_2 and aqueous NaOH) to give the fully protected SPM **13** in 90% yield. Addition of 1-iodopropane (3 equiv) to a solution of the dianion of **13** (NaH) in DMF and heating at 45−50 °C resulted in dipropyl derivative **15** in 88% yield. Using inverse addition, a solution of deprotonated **13** was introduced to an excess of iodoethane, 1-iodobutane, 1-bromo-2-methylpropane, or 1-bromo-3-methylbutane in DMF at room temperature giving fully protected disubstituted SPM **14** (93%), **16** (89%), **17** (45%), or **18** (95%), respectively. Deprotection of **14**−**18** was accomplished with 30% HBr in acetic acid and

Scheme 1. Synthesis of Spermines That Are Terminally Disubstituted with Primary Alkyl Groups[a]



[a] Reagents: (a) mesitylenesulfonyl chloride, NaOH (aq), CH_2Cl_2, 90%; (b) NaH, RX, DMF; (c) 30% HBr in HOAc, PhOH, CH_2Cl_2; NaOH; HCl.

Scheme 2. Synthesis of DIPSPM[a]

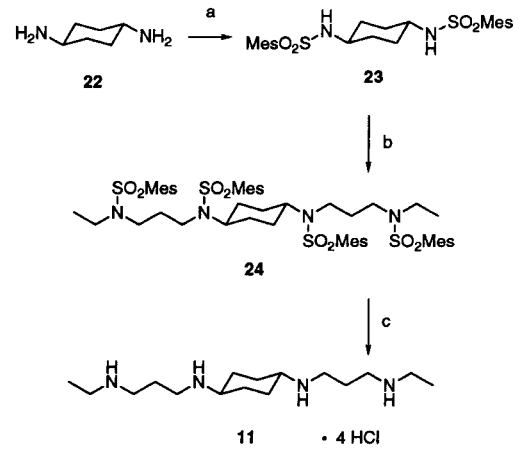


[a] Reagents: (a) NaH, 1,3-propanediol di-*p*-tosylate, DMF, 52%; (b) NaH, *N*,*N*′-bis(mesitylenesulfonyl)-1,4-butanediamine, DMF, 74%; (c) 30% HBr in HOAc, PhOH, CH_2Cl_2; NaOH; HCl, 80%.

phenol in CH_2Cl_2 at room temperature. Addition of water and concentration of the aqueous layer in vacuo afforded the crude tetrahydrobromide salts. Basification with aqueous NaOH, extraction with $CHCl_3$, concentration, and addition of HCl in ethanol, followed by recrystallization, gave the SPM analogues **1**, **3**, and **5**−**7**, respectively, as their tetrahydrochloride salts.

The synthesis of N^1,N^{12}-diisopropylspermine (DIPSPM, **4**) was accomplished by using segments (Scheme 2), as an attempt to dialkylate **13** by isopropyl halide would result in elimination. Treatment of *N*-isopropylmesitylenesulfonamide (**19**)[11] with a 10-fold excess of 1,3-propanediol di-*p*-tosylate gave the 1:1 adduct **20** in 52% yield. Addition of the dianion of *N*,*N*′-bis(mesitylenesulfonyl)-1,4-butanediamine in DMF[11] (NaH) to tosylate **20** in DMF generated fully protected SPM derivative **21** in 74% yield. Amino groups were unmasked with

Scheme 3. Synthesis of **11**[a]



[a] Reagents: (a) mesitylenesulfonyl chloride, NaOH (aq), CH_2Cl_2, 82%; (b) NaH, *N*-(3-bromopropyl)-*N*-ethylmesitylenesulfonamide, DMF, 73%; (c) 30% HBr in HOAc, PhOH, CH_2Cl_2; NaOH; HCl, 64%.

30% HBr in HOAc and phenol to provide DIPSPM (**4**) in 80% yield.

N,*N*′-Bis[3-(ethylamino)propyl]-*trans*-1,4-cyclohexanediamine tetrahydrochloride [CHX(3,4,3)-*trans*, **11**] was also assembled via a fragment synthesis (Scheme 3). Disulfonamide **23**, available in 90% yield from reaction of *trans*-1,4-diaminocyclohexane (**22**) with mesitylenesulfonyl chloride (2 equiv) under biphasic conditions, was alkylated with *N*-(3-bromopropyl)-*N*-ethylmesitylenesulfonamide[11] (2 equiv, NaH, DMF) to provide protected polyamine **24** in 73% yield. The amino groups of **24** were unmasked with 30% HBr in acetic acid and phenol, furnishing final product **11** in 64% yield.

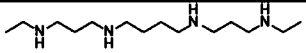
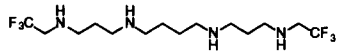
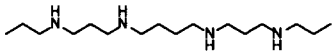
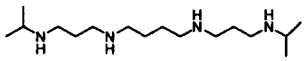
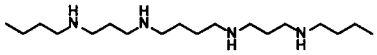
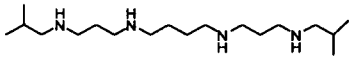
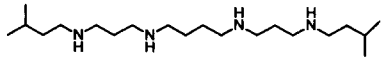
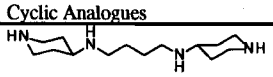
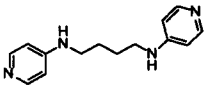
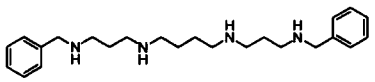
N^1,N^{12}-Dibenzylspermine (DBZSPM, **10**)[12] was synthesized by a reductive alkylation. SPM was treated with benzaldehyde (2.5 equiv) and $MgSO_4$ in CH_2Cl_2. The adduct was reduced with $NaBH_4$ in ethanol[13] to give DBZSPM as its tetrahydrochloride salt (**10**).

Biological Evaluations

Efficacy of SPM Derivatives Administered sc in a Castor Oil-Induced Diarrhea Model. The compounds were generally administered at doses equivalent (on a molar basis) to 1, 5, 10, or 25 mg/kg DEHSPM. Giving DESPM (**1**) to rodents at a dose of 4.7 mg/kg sc resulted in a significant decrease in stool output (60%) and reduction in weight loss (37%) ($P < 0.02$ and $P < 0.03$, respectively; Table 1). Doubling the dose reduced stool output by 92% and ameliorated the associated weight loss by 55% ($P < 0.004$ and $P < 0.008$, respectively), as did tripling the dose, which resulted in a 92% reduction in stool output and a 66% reduction in weight loss ($P < 0.004$ and $P < 0.003$, respectively), suggesting that we had reached the maximum effective dose at 9.4 mg/kg.

At physiological pH, DESPM is a tetracation; the corresponding N^1,N^{12}-bis(2,2,2-trifluoroethyl)spermine (FDESPM, **2**) is a dication.[14] At a dose of 5.9 mg/kg, administration of FDESPM did not result in any decrease in stool output relative to controls (Table 1). When the alkyl chains were lengthened to *n*-propyl groups, the resulting analogue, DPSPM (**3**), was about as active as the parent DESPM at reducing stool output and weight loss when the compounds were administered

Table 1. Antidiarrheal Activity of Polyamine Analogues Predicated on a (3,4,3) Backbone[a]

compd. no.	structure/abbreviation	dose per kg mg	dose per kg µmol	*n*	weight loss[b]	*P*-value[c]	% reduction[d]	stool output[e]	*P*-value[c]	% reduction[d]
	Linear Analogues									
1		0	0	5	11.9 ± 3.5	–	–	7.5 ± 3.2	–	–
	DESPM	4.7	11.6	10	7.5 ± 4.0	< 0.03	37	3.0 ± 3.0	< 0.02	60
		9.4	23.3	5	5.3 ± 3.3	< 0.008	55	0.6 ± 0.6	< 0.004	92
		14.3	35.4	5	4.4 ± 1.6	< 0.003	66	0.6 ± 0.9	< 0.004	92
2		0	0	4	18.2 ± 1.0	–	–	7.2 ± 1.9	–	–
	FDESPM	5.9	11.6	5	17.2 ± 2.5	> 0.05	NS	7.5 ± 0.7	> 0.05	NS
3		0	0	5	13.1 ± 1.4	–	–	6.0 ± 2.0	–	–
	DPSPM	1	2.3	5	9.1 ± 3.2	< 0.03	31	3.8 ± 2.4	> 0.05	37
		5	11.6	5	5.7 ± 2.1	< 0.001	56	2.7 ± 2.5	< 0.03	55
		25	57.8	5	4.0 ± 1.4	< 0.001	69	0 ± 0	< 0.002	100
4		0	0	15	11.8 ± 4.4	–	–	6.8 ± 2.7	–	–
	DIPSPM	1	2.3	10	6.9 ± 3.2	< 0.002	42	2.9 ± 3.3	< 0.003	57
		2.5	5.8	10	4.2 ± 1.2	< 0.001	64	0 ± 0	< 0.001	100
		5	11.6	10	4.4 ± 3.8	< 0.001	63	0.9 ± 2.5	< 0.001	87
5		0	0	5	13.6 ± 5.3	–	–	4.6 ± 2.8	–	–
	DBSPM	1.1	2.3	5	10.2 ± 2.3	> 0.05	NS	3.5 ± 1.1	> 0.05	NS
		2.7	5.8	5	11.2 ± 3.0	> 0.05	NS	4.2 ± 1.6	> 0.05	NS
		5.3	11.6	5	12.0 ± 3.8	> 0.05	NS	5.5 ± 2.9	> 0.05	NS
6		0	0	4	18.2 ± 1.0	–	–	7.2 ± 1.9	–	–
	DIBSPM	5.3	11.6	5	11.1 ± 3.6	< 0.005	39	5.1 ± 1.9	> 0.05	NS
		10.7	23.2	5	5.0 ± 3.8	< 0.001	78	1.6 ± 3.5	< 0.01	78
7		0	0	5	14.6 ± 5.0	–	–	4.6 ± 2.6	–	–
	DIPESPM	1.1	2.3	5	14.2 ± 4.9	> 0.05	NS	4.9 ± 3.1	> 0.05	NS
		5.7	11.6	5	18.3 ± 2.6	> 0.05	NS	7.8 ± 1.7	> 0.05	NS
		28.2	57.8	5	1.6 ± 1.1	< 0.002	89	0 ± 0	< 0.009	100
	Cyclic Analogues									
8		0	0	5	10.0 ± 5.4	–	–	4.2 ± 3.2	–	–
	PIP(3,4,3)	4.6	11.6	5	9.5 ± 4.3	> 0.05	NS	4.7 ± 2.3	> 0.05	NS
9		0	0	5	13.3 ± 3.4	–	–	5.6 ± 3.2	–	–
	PYR(3,4,3)	15	47.6	5	14.4 ± 3.4	> 0.05	NS	6.9 ± 3.9	> 0.05	NS
10		0	0	5	13.3 ± 2.2	–	–	6.2 ± 0.5	–	–
	DBZSPM	1.22	2.3	5	13.5 ± 3.2	> 0.05	NS	6.4 ± 2.4	> 0.05	NS
		6.11	11.6	5	13.1 ± 4.4	> 0.05	NS	6.3 ± 3.3	> 0.05	NS
		30.54	57.8	5	10.4 ± 5.8	> 0.05	NS	5.0 ± 3.3	> 0.05	NS
11		0	0	20	11.6 ± 3.7	–	–	4.9 ± 2.0	–	–
	CHX(3,4,3)-*trans*	0.0078	0.0181	5	8.8 ± 1.2	< 0.005	24	3.6 ± 1.3	> 0.05	NS
		0.0156	0.036	5	3.1 ± 1.2	< 0.001	73	0.4 ± 0.9	< 0.001	92
		0.03125	0.0726	5	4.2 ± 1.7	< 0.001	64	0 ± 0	< 0.001	100
		0.0625	0.145	5	3.7 ± 0.8	< 0.001	68	0 ± 0	< 0.001	100
		0.125	0.29	5	4.1 ± 0.7	< 0.001	65	0 ± 0	< 0.001	100
		0.25	0.58	4	2.7 ± 0.7	< 0.001	77	0 ± 0	< 0.001	100
		0.5	1.16	5	2.9 ± 0.1	< 0.001	75	0 ± 0	< 0.001	100
		0.99	2.3	9	2.7 ± 1.3	< 0.001	77	0 ± 0	< 0.001	100
		4.97	11.6	5	1.6 ± 1.1	< 0.001	86	0 ± 0	< 0.001	100
		24.87	57.8	5	0.8 ± 0.7	< 0.001	93	0 ± 0	< 0.001	100

[a] Polyamine analogues were administered sc to rats at the doses shown. 30 min later, the rats were given castor oil, 5 mL/kg, by gavage. Stool output was monitored for 6 h after castor oil administration. [b] Weight loss is expressed as g of weight lost/350 g of rat weight over the 6-h experimental period. [c] A one-tailed *t*-test assuming unequal variance was performed on the data of the treated vs control (0 mg/kg) animals for each compound. A value of $P < 0.05$ was considered significant. [d] Percent reduction was calculated by dividing the mean value from the treated animals (T) by the mean value from the control animals (C), subtracting the resulting quotient from 1.0, and multiplying by 100 [i.e., $(1.0 - T/C) \times 100$]. NS, not significant. [e] Stool output is expressed as the g of stool excreted/350 g of rat weight over the 6-h collection period. Data are not corrected for slight evaporative losses that occur during the assay. Such corrections do not significantly affect the results.

Table 2. Antidiarrheal Activity, when Given Orally, of Polyamine Analogues Predicated on a (3,4,3) Backbone[a]

		dose								
compd	abbreviation	mg/kg	μmol/kg	*n*	weight loss[b]	*P*-value[c]	% reduction[d]	stool output[e]	*P*-value[c]	% reduction[d]
2	DESPM	0	0	5	15.1 ± 4.7			7.2 ± 3.0		
		23.5	58.13	5	6.2 ± 2.8	<0.005	59	1.9 ± 2.2	<0.007	74
		47	116.25	5	3.8 ± 1.8	<0.002	75	0.6 ± 1.4	<0.003	92
		94	232.5	5	2.8 ± 0.5	<0.003	81	0 ± 0	<0.003	100
4	DIPSPM	0	0	5	17.2 ± 1.0			7.2 ± 1.5		
		12.5	28.9	5	8.7 ± 3.1	<0.001	49	3.6 ± 2.5	<0.02	50
		25	57.8	5	3.6 ± 0.8	<0.001	79	0 ± 0	<0.001	100
		50	115.6	5	3.2 ± 1.3	<0.001	81	0 ± 0	<0.001	100

[a] Polyamine analogues were administered to rats po by gavage at the doses shown in the table. 30 min later, the rats were given castor oil, 5 mL/kg, by gavage. Stool output was monitored for 6 h after castor oil administration. [b] Weight loss is expressed as g of weight lost/350 g of rat weight over the 6-h experimental period. [c] A one-tailed *t*-test assuming unequal variance was performed on the data of the treated vs control (0 mg/kg) animals for each compound. A value of $P < 0.05$ was considered significant. [d] Percent reduction was calculated by dividing the mean value from the treated animals (T) by the mean value from the control animals (C), subtracting the resulting quotient from 1.0, and multiplying by 100 [i.e., $(1.0 - T/C) \times 100$]. [e] Stool output is expressed as the g of stool excreted/350 g of rat weight over the 6-h collection period. Data are not corrected for slight evaporative losses that occur during the assay. Such corrections do not significantly affect the results.

at equivalent dosages and demonstrated an excellent dose response (Table 1). Interestingly, the corresponding branched-chain derivative, DIPSPM (**4**), was more efficacious at controlling diarrhea than either DPSPM at an equimolar dose or at one-fifth the effective dose of DESPM. At a dose of 1 mg/kg there was a 57% reduction in stool output relative to the controls ($P < 0.003$) and a 42% decrease in weight loss ($P < 0.002$). At a dose of 2.5 mg/kg sc, there was a 100% reduction in stool output ($P < 0.001$; Table 1) and a 64% decline in weight loss ($P < 0.001$). Extension of the propyl groups to *n*-butyl groups to generate DBSPM (**5**) resulted in a compound that did not diminish either diarrhea or weight loss (Table 1). However, the corresponding isobutyl compound, DIBSPM (**6**), although not as active as DESPM, exhibited an acceptable dose response (Table 1). At a dose of 5.3 mg/kg there was a significant amelioration of weight loss, 39% ($P < 0.005$), but the difference in stool output between controls and animals treated at this dose was not significant (7.2 ± 1.9 vs 5.1 ± 1.9 g/350 g rat weight, $P > 0.05$). A dose of 10.7 mg/kg elicited a significant antidiarrheal response as measured by both stool output and weight loss, a 78% reduction for each parameter ($P < 0.01$ and $P < 0.001$, respectively). The longer branched-chain polyamine, DIPESPM (**7**), completely eliminated stool output and its associated weight loss, 89%, only when administered at the highest dose tested, 28.2 mg/kg ($P < 0.009$ and $P < 0.002$, respectively); lower doses did not elicit any significant effect (Table 1).

When the ethyl groups of DESPM were folded back onto the aminopropyl fragment of the methylene backbone to produce *N*,*N*′-bis(4-piperidinyl)-1,4-diaminobutane [PIP(3,4,3), **8**], the compound was essentially inactive (Table 1). When the piperidine rings were converted to the corresponding aromatics to yield *N*,*N*′-bis(4-pyridyl)-1,4-diaminobutane [PYR(3,4,3), **9**], the molecule was still ineffective, even at a dose of 15 mg/kg sc. DBZSPM (**10**) was completely ineffective at preventing castor oil-associated weight loss and diarrhea, even when administered at a dose as high as 30.5 mg/kg ($P > 0.05$ for all doses).

However, CHX(3,4,3)-*trans* (**11**) was an effective antidiarrheal, the most potent one that has ever been tested in these laboratories (Table 1). When administered sc at a dose range of 0.0078−24.9 mg/kg, there was a significant reduction in weight loss at all dose levels (from $P < 0.005$ to $P < 0.001$, Table 1). In addition, the compound also significantly decreased stool output relative to controls at sc doses of 0.0156−24.9 mg/kg ($P < 0.001$ for all doses, Table 1).

Oral Activity of Selected Analogues. Since two of the SPM derivatives, DESPM and DIPSPM, showed remarkable activity without any apparent toxicity when administered sc, we evaluated the potency of these analogues when administered po (Table 2). Both DIPSPM and DESPM were effective antidiarrheals when given orally to fasted rats. DIPSPM was found to lessen diarrheal stooling at all three doses tested. At a dose of 12.5 mg/kg, DIPSPM reduced the castor oil-induced stool output by 50% ($P < 0.02$ vs controls) and diminished weight loss by 49% ($P < 0.001$). Increasing the dose to 25 or 50 mg/kg resulted in a 100% reduction in stool output relative to controls ($P < 0.001$ for both) and decreased associated weight loss by approximately 80% ($P < 0.001$).

DESPM was also effective when administered orally to fasted rats. At a dose of 23.5 mg/kg, stooling was reduced by approximately 74% relative to castor oil treated controls ($P < 0.007$), and weight loss was lessened by 59% ($P < 0.005$). Increasing the dose to 47 or 94 mg/kg resulted in a greater than 90% reduction in stooling compared to the controls ($P < 0.003$) and a significant decline in weight loss as well, 75% for the 47 mg/kg dose level and 81% for the 94 mg/kg group ($P < 0.002$ and $P < 0.003$, respectively). We have not yet explored the oral activity of the CHX(3,4,3)-*trans* analogue.

Acute Toxicity of DESPM and DIPSPM in Rodents. The acute toxicity of DESPM ip in female CD-1 mice is very similar to that observed for DEHSPM (Figure 2). In fact, the acute toxicities, as measured by LD_{50} values, of several *diethyl* tetraamines are very similar, between 325 and 375 mg/kg.[7−9,11] When allometrically scaled from mice to rats, this is approximately 20 times the maximum therapeutic dose of 9.4 mg/kg in rats. However, use of larger *N*-alkyl substituents, such as isopropyl in DIPSPM, results in enhanced acute toxicity. The LD_{50} of DIPSPM is about 125 mg/kg in mice, and there is a strong neurological component to these toxic effects not apparent in DESPM- or DEHSPM-treated animals, which usually display a generalized depression with respiratory failure. At toxic doses (≥100 mg/kg, single dose), the DIPSPM-treated

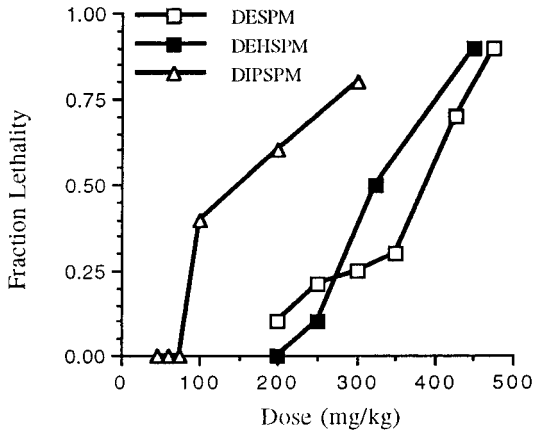


Figure 2. Acute toxicity of DIPSPM and DESPM vs that of DEHSPM. Groups of female CD-1 mice were administered DESPM (n = 10), DIPSPM (n = 5), or DEHSPM (n = 10) ip, and the mortality (shown as a fraction on the y-axis) was monitored over a 2-h period. The LD_{50} values for DESPM, DIPSPM, and DEHSPM[11] were 400, 150, and 300 mg/kg, respectively. The acute toxic effects of DEHSPM and DESPM presented as a generalized depression with respiratory failure, while toxic doses of DIPSPM resulted in CNS effects, including convulsions.

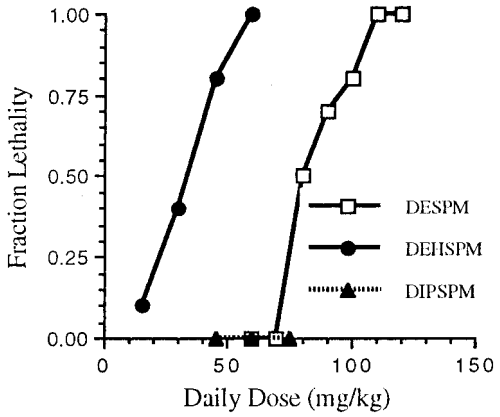


Figure 3. Chronic toxicity of DIPSPM (n = 5/dose) and DESPM (n = 10/dose) vs that of DEHSPM (n = 10/dose). Groups of female CD-1 mice were administered the compounds ip at the doses shown once daily for 5 days. The mortality (shown as a fraction on the y-axis) was monitored during dosing and for 10 days afterward. The LD_{50} values for DESPM and DEHSPM were 80 and 40 mg/kg, respectively; no lethalities were observed for DIPSPM at doses up to 75 mg/kg/day. Signs of toxicity included poor coat health, lassitude, and severe weight loss of 25−30% prior to death. When given at doses between 30 and 60 mg/kg/day for 5 days, those DEHSPM-treated animals that died did so between 4 and 10 days after the final dose (6.5 ± 2.2 days, n = 23/40). In animals treated with DESPM at doses between 80 and 110 mg/kg, deaths occurred from just prior to the final dose (day "0") to 5 days after the final dose (1.2 ± 1.1 days, n = 30/40).

mice displayed an ataxic gait, intention tremors, and severe motor dysfunction, especially of the hind limbs. However, it is critical to point out that this dose, allometrically scaled, is at least 20 times higher than a therapeutic dose of 2.5 mg/kg in the rat. At all the doses examined in the rat antidiarrheal studies (up to 15 mg/kg) and in other rodent models at doses up to 25 mg/kg, no signs of neurotoxicity were observed for DIPSPM. Thus, there is a large therapeutic window between a clinically effective dose of 1−2.5 mg/kg DIPSPM in rats and a neurotoxic dose. However, neurological signs were observed in the castor oil-treated rats that were treated with the longer chain analogues such as DIPESPM even at lower, therapeutic doses (28 mg/kg), suggesting that the longer, more lipophilic *N*-alkyl substituents result in increased neurotoxicity upon administration to animals. For example, signs of neurotoxicity similar to those described above in mice were observed in rats treated with DBSPM, DIBSPM, and DIPESPM at subtherapeutic doses equivalent to a 5 mg/kg dose of DEHSPM. Moderate to severe seizures were observed with DIPESPM at a dose of 28 mg/kg and with DBSPM at a dose of 26 mg/kg in another rodent model.

Although formal toxicity trials with the most active antidiarrheal, CHX(3,4,3)-*trans*, have not yet been initiated in mice, signs of motor dysfunction, including seizures, were observed in the castor oil-treated rats at doses ≥1 mg/kg. The 1 mg/kg dose is, however, greater than 60 times the lowest dose (0.0156 mg/kg) that significantly reduced both stool output and weight loss.

Chronic Toxicity of DEHSPM, DESPM, and DIPSPM in Mice. The marked difference in the chronic toxicities between DESPM and DEHSPM is indicated in Figure 3. Signs of chronic toxicity presented as poor coat health, lassitude, and severe weight loss of 25−30% prior to death. When given at doses between 30 and 60 mg/kg/day for 5 days, those DEHSPM-treated animals that died did so between 4 and 10 days after the final dose (6.5 ± 2.2 days, n = 23/40). In contrast, in animals treated with DESPM at doses between 80 and 110 mg/kg, deaths were observed in a range from just prior to the final dose (day "0") to 5 days after the final dose (1.2 ± 1.1 days, n = 30/40). This difference likely reflects the accumulation of the toxic metabolite HSPM during the course of DEHSPM treatment.[7] Surprisingly, no signs of toxicity, including loss of weight, were seen in the three groups of mice treated with 45, 60, or 75 mg/kg/day DIPSPM over a 5-day period, suggesting that this analogue was even less toxic than DESPM in a *chronic* regimen.

Chronic Toxicity of DESPM in Dogs. Ten young adult (1-year-old) male beagles were utilized. After a 2-week acclimation, the animals were weighed prior to starting drug administration and were weighed weekly during the drug-dosing period. A CBC and extensive chemistry panel were performed once a week during the drug-dosing period and during the recovery phase and/or prior to sacrifice. The dogs were given the drug sc twice daily at a daily dose of 1.87 (n = 3), 4 (n = 2), 6 (n = 2), or 8 (n = 2) mg/kg. The final dog served as a vehicle control.

The dogs given DESPM at a daily dose of 1.87 mg/kg/day × 28 days tolerated the drug well and had normal urine and feces production. No frank or occult blood was noted in the stool at any time during the study. Blood chemistries performed during the dosing period were unremarkable. One dog was sacrificed 10 days post-last dose (PLD). The remaining animals were not sacrificed until 75 days PLD; extensive tissues were taken for histology and for the determination of polyamine levels.

When the dose of the drug was increased to 4 mg/kg/day, both of the animals exhibited signs of toxicity that included vomiting, weight loss, and diarrhea that was positive for occult blood after 13 days of dosing. The drug

Table 3. Effects of DESPM Dosing and Time of Sacrifice on Liver Polyamine Pools and Metabolite Accumulation Compared with DEHSPM[a]

compd	dose (mg/kg/day)	no. days dosed	cum. dose (mg/kg)	sacrifice (no. days PLD)	DESPM/ DEHSPM[b]	MESPM/ MEHSPM[b]	HSPM[b]	native polyamines[b] PUT[c]	SPD[c]	SPM[c]
none[d]	0							51 ± 25	175 ± 9	901 ± 127
DESPM	1.87	28	52.4	10	18	7		86	179	850
	1.87	28	52.4	75	0	0		28	198	649
	4.0	13	52	10	36	34		82	176	462
	4.0	13	52	75	0	0		62	264	886
	6.0	4.5	27	10	30	23		87	181	848
	6.0	4.5	27	75	0	1		101	244	992
	8.0	4.5	36	10	29	21		109	187	937
	8.0	4.5	36	75	0	0		56	221	1058
DEHSPM	2.0	29	58	10	80	72	751	19	50	108
	2.0	11	22	75	0	13	668	34	68	386

[a] The compounds were given sc as shown; sacrifice followed at various times post-last dose (PLD) as set forth above. [b] Expressed as nmol/g wet weight of tissue. [c] PUT, putrescine; SPD, spermidine; SPM, spermine. [d] One naïve dog was sacrificed; another animal was given sterile saline vehicle sc for 13 days and was euthanized for tissues 13 days PLD. The native polyamine data are shown as mean ± range and are within the limits of individual animal variability.

administration was stopped at this point, and the dogs recovered quickly without any intervention. Blood chemistries collected during and after the drug dosing were unremarkable. Ten days PLD, one of the dogs was sacrificed; tissues were collected for polyamine analysis and histopathology. The second animal was not sacrificed until 75 days PLD.

When the dose of the drug was further increased to 6 or 8 mg/kg/day, dosing was stopped after 4.5 days due to weight loss, vomiting, and diarrhea with liquid, dark, tarry stool. The stool samples from each dog tested positive for occult blood. It was determined at this time that a toxic endpoint of DESPM had been reached at these dosage levels. Blood chemistries collected during and after the drug dosing were unremarkable. The dogs recovered without further intervention. One of the dogs from each of the groups was sacrificed 10 days PLD; the remaining animals were not sacrificed until 75 days PLD. Extensive tissue samples were taken for histopathology and polyamine levels.

At necropsy all tissues, including kidney, liver, lung, spleen, pancreas, and the others listed in the Experimental Section, appeared grossly normal. When these tissues were examined by an outside pathologist, the histopathologic changes in the tissues were similar to those of the untreated control dog; that is, there was no histological evidence of drug-induced toxicity in the tissues.

Chronic Toxicity of DIPSPM in Dogs. In a manner similar to the DESPM study, dogs received DIPSPM at a sc dose of 2.15 mg/kg/day (1.08 mg/kg twice daily) for 21 days. This dose was well-tolerated, and normal urine and feces production was observed. No frank or occult blood was noted in the stool at any time, and blood chemistries performed during the drug administration period were unremarkable. Because of the activity of this analogue, expanded pharmacokinetic, metabolism, and tissue distribution studies in dogs are ongoing; these will be the subject of a separate paper.

Ventricular Bigeminy Induced by DESPM and DIPSPM in Dogs. Ventricular bigeminy was found in all three of the dogs treated with DEHSPM at a dose of 2 mg/kg/day for 11−17 days (R. J. Bergeron, J. Wiegand, W. R. Weimar, R. Müller, J. S. McManis, G. W. Yao, G. Huang, P. S. Snyder, C. Porter, R. Braylan, unpublished results). The arrhythmia was apparent in a 6-lead EKG as early as 3 days into the dosing period. When continuous ambulatory electrocardiograms were obtained in the treated dogs for 5−6 h after 11−12 days of drug administration, one dog had the arrhythmia 99% of the time; another had the arrhythmia between 60% and 70% of the time. When the third dog was examined by continuous ambulatory electrocardiography 30 days post-drug, the arrhythmia was present between 30% and 40% of the time. Two of the three animals were allowed to recover to determine whether or when the arrhythmia would revert. The arrhythmia was present as detected by continuous ambulatory electrocardiography until approximately 60 days post-drug.

The results obtained after administration of DESPM are in sharp contrast to what was observed with the DEHSPM-treated dogs. Eight of the nine dogs had normal baseline continuous ambulatory electrocardiograms, but the final dog (EFV-7, 6 mg/kg/day) was found to have periods of sinus arrest followed by ventricular escape complexes. During the drug administration, all of the dogs retained their normal cardiac rhythms except for EFV-7, which continued to have sinus arrest and ventricular escape complexes as well as two short (<1 min) runs of ventricular bigeminy on day 4 of treatment. When a continuous ambulatory electrocardiogram was obtained 1 week PLD, three ventricular premature complexes were noted, but no evidence of bigeminy was apparent. Likewise, continuous ambulatory electrocardiograms from DIPSPM-treated dogs revealed no ventricular bigeminy during the drug-dosing period.

Metabolism and Tissue Distribution of DESPM in Dogs. The tissue distribution of DESPM and its monodeethylated metabolite, MESPM, are compared to that of DEHSPM and its metabolites, MEHSPM and HSPM, in dog liver, kidney, spleen, and lung (Tables 3 and 4, respectively, represent the liver and kidney data). The dogs that were treated with DESPM at doses of 1.87−8 mg/kg/day (total cumulative doses ranging from 27−52 mg/kg) and sacrificed at 10 and 75 days PLD were studied. For comparison, the DEHSPM tissue distribution data are taken from one dog treated at a dose of 2 mg/kg/day for 29 days (total cumulative dose, 58 mg/kg) and sacrificed 10 days PLD[7] and another dog treated at a dose of 2 mg/kg/day for 11 days (total cumulative dose, 22 mg/kg) and sacrificed 75 days PLD

Table 4. Effects of DESPM Dosing and Time of Sacrifice on Kidney Polyamine Pools and Metabolite Accumulation Compared with DEHSPM[a]

compd	dose (mg/kg/day)	no. days dosed	cum. dose (mg/kg)	sacrifice (no. days PLD)	DESPM/ DEHSPM[b]	MESPM/ MEHSPM[b]	HSPM[b]	native polyamines[b] PUT[c]	SPD[c]	SPM[c]
none[d]	0							0	71 ± 12	586 ± 39
DESPM	1.87	28	52.4	10	50	12		0	69	998
	1.87	28	52.4	75	0	0		16	87	540
	4.0	13	52	10	46	7		12	82	277
	4.0	13	52	75	0	0		7	117	534
	6.0	4.5	27	10	44	10		7	60	654
	6.0	4.5	27	75	0	0		8	90	584
	8.0	4.5	36	10	48	12		20	66	784
	8.0	4.5	36	75	0	0		6	80	624
DEHSPM	2.0	29	58	10	98	78	269	5	41	275
	2.0	11	22	75	0	3	43	0	68	611

[a] The compounds were given sc as shown; sacrifice followed at various times post-last dose (PLD) as set forth above. [b] Expressed as nmol/g wet weight of tissue. [c] PUT, putrescine; SPD, spermidine; SPM, spermine. [d] One naïve dog was sacrificed; another animal was given sterile saline vehicle sc for 13 days and was euthanized for tissues 13 days PLD. The native polyamine data are shown as mean ± range and are within the limits of individual animal variability.

(R. J. Bergeron, J. Wiegand, W. R. Weimar, R. Müller, J. S. McManis, G. W. Yao, G. Huang, P. S. Snyder, C. Porter, R. Braylan, unpublished results). Any monoethylated, deaminopropylated metabolite, i.e., N^1-ethylspermidine, was below detectable limits by 10 days PLD. The most remarkable finding was that at 10 days PLD low levels of DESPM and MESPM were observed in the liver and kidney (Tables 3 and 4) as well as in the spleen and lung (data not shown) relative to DEHSPM-treated dogs. In contrast, the summary concentration of DEHSPM, MEHSPM, and HSPM (Σ[DEHSPM + MEHSPM + HSPM]) exceeded the total native polyamine (SPM, SPD, and PUT) content (Σ[SPM + SPD + PUT]) at 10 days PLD. The percentages of total tissue polyamine N^+ equivalents derived from DEHSPM and its metabolites were 85%, 59%, 46%, and 71% in liver, kidney, spleen, and lung, respectively. Note that DEHSPM and its metabolites appear to displace the native polyamines in all of these tissues but especially in the kidney and liver.

At 75 days PLD, DESPM, and MESPM were not detected in most tissues (e.g., liver and kidney, Tables 3 and 4). SPM levels in liver and kidney had returned to control values 75 days post-treatment (Tables 3 and 4). These findings are in marked contrast to those for tissues from a DEHSPM-treated dog, which still contain a considerable amount of drug in the form of the metabolically inert metabolite HSPM. In fact, HSPM is the predominant polyamine in the liver even 75 days PLD. These observations validate our design concept: that is, the accumulation of metabolite, which is so significant with DEHSPM, is not an issue with DESPM.

Metabolism and Tissue Distribution of DIPSPM in Mice. The distribution of DIPSPM and its monoisopropyl metabolite MIPSPM was determined in the tissues of two groups of mice that had received chronic dosage regimens. The first group had received 60 mg/kg/day for 5 days and was sacrificed 1 day PLD. The second group received 75 mg/kg/day for 5 days and was sacrificed 14 days PLD. The contents of DIPSPM, MIPSPM, and native polyamines in kidney, liver, spleen, and lung are shown in Figure 4. These studies revealed several notable findings. First, high levels of DIPSPM were achieved in the kidney, and to a lesser extent the lung, at 1 day PLD. These high levels present at 1 day PLD were greatly diminished in all four tissues at 14 days PLD, even though some drug and metabolite were still present at this time. Second, whereas the metabolite MIPSPM was present in all tissues, there was no evidence of its accumulation. The ratio of metabolite to parent drug was similar for each tissue at both 1 and 14 days PLD: kidney, 0.10; liver, 0.71; spleen, 0.08; and lung, 0.09. Finally, the most interesting and unusual finding was that although substantial levels of DIPSPM and MIPSPM were present in these tissues, there was a very modest, if any, effect on the native polyamine pools. The effects of DIPSPM, DENSPM, and DEHSPM on polyamine pools in mouse kidney are compared in Figure 5. Although the tissue content of parent drug is much higher in the kidney of DIPSPM-treated animals, the native polyamine pools are not significantly different from control and differ significantly from native polyamine pools in DENSPM- and, especially, DEHSPM-treated animals. The native polyamine contents, expressed in terms of total equivalents of N^+, were: control, 4156 ± 287 equiv; DIPSPM, 3754 ± 403 equiv (not significant); DENSPM, 2975 ± 166 equiv ($P < 0.005$); and DEHSPM, 1560 ± 446 equiv ($P < 0.0005$). Considering that, normally as the concentration of analogue increases, native polyamines such as SPD and SPM are correspondingly reduced,[11,14] the behavior of DIPSPM is indeed atypical.

Discussion

Although DEHSPM proved to be a very potent antidiarrheal in animal models[8] and in the clinic,[15] its metabolic and toxicity profiles had several shortcomings. The final metabolite, HSPM, cannot be processed further by polyamine catabolic enzymes[7] and thus accumulates in tissues. The retrograde processing of polyamines (e.g., SPD and SPM) requires acetylation of the aminopropyl fragments of these molecules; however, there are only aminobutyl fragments in HSPM. The principal toxic manifestation of DEHSPM is cardiac in nature; animals develop ventricular bigeminies. On the basis of our previous studies with the hydroxylated analogue of DEHSPM, (*R*,*R*)-$(HO)_2$DEHSPM, the protracted tissue residence time plays a significant role in the toxicity of DEHSPM. Unfortunately, whereas the hydroxylated analogue did not appreciably affect cardiac function, it did compromise the exocrine pancreas (R. J. Bergeron, J. Wiegand, W. R. Weimar, R. Müller, J. S. McManis, G. W. Yao, G. Huang, P. S. Snyder, C. Porter, R. Braylan, unpublished results). Nevertheless,

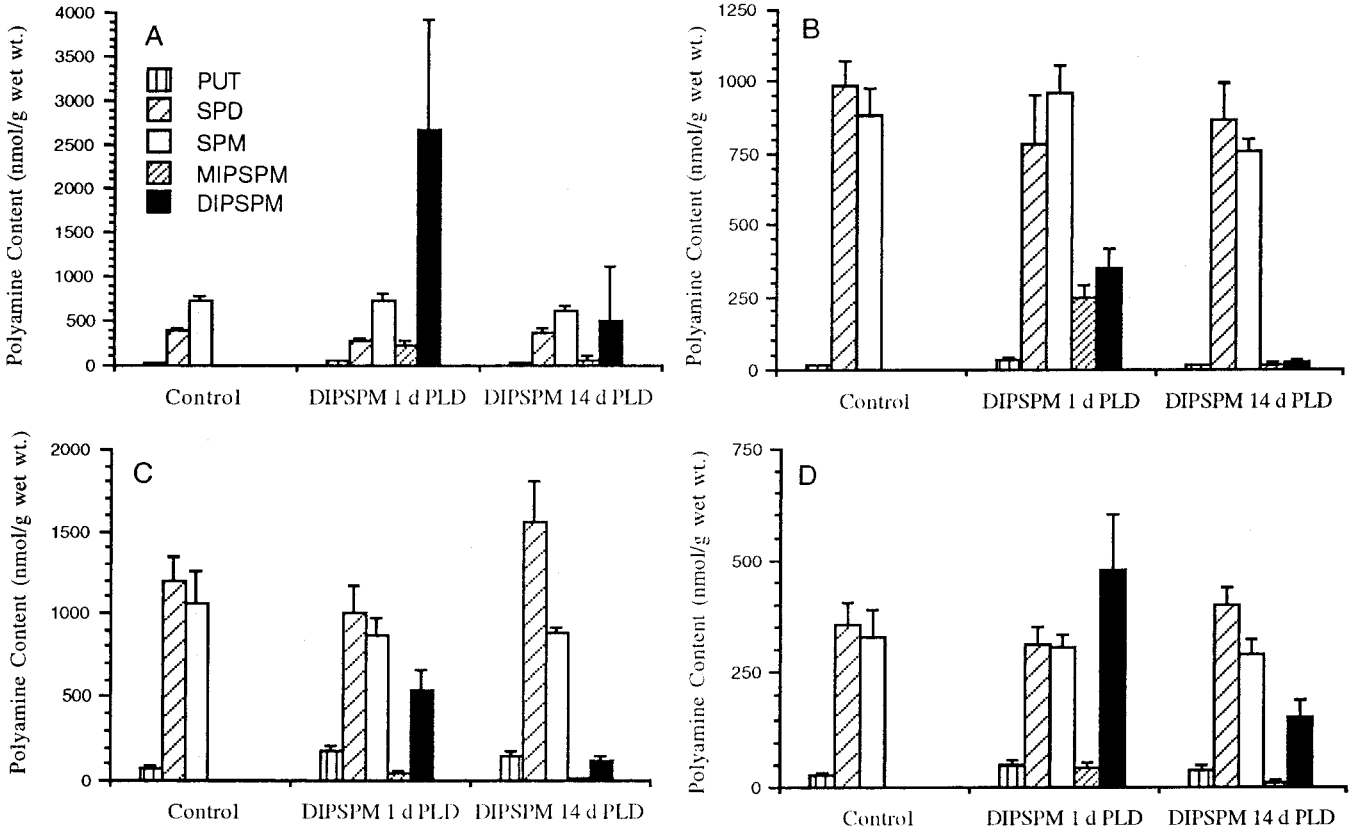


Figure 4. Concentrations of drug, metabolite, and native polyamines in kidney (A), liver (B), spleen (C), and lung (D) in DIPSPM-treated mice. The doses used were 60 mg/kg/day ip for 5 days followed by sacrifice 1 day PLD ("DIPSPM 1 d PLD") and 75 mg/kg/day ip for 5 days followed by sacrifice 14 days PLD ("DIPSPM 14 d PLD").

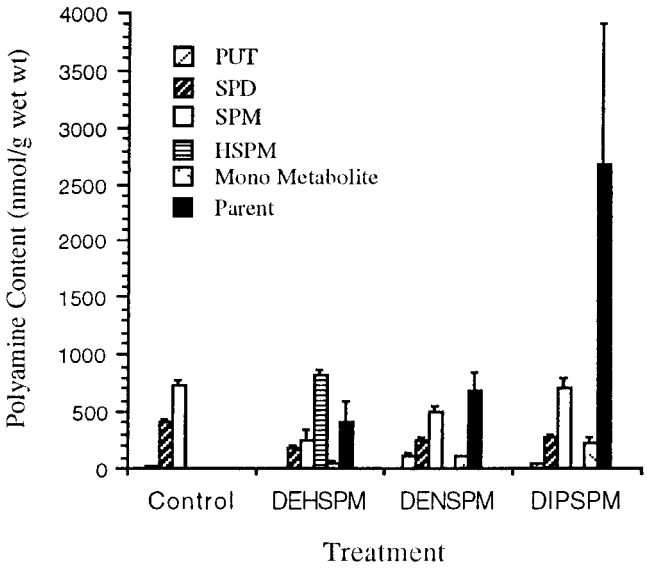


Figure 5. Polyamine contents of mouse kidney comparing treatments with DIPSPM·4HCl (60 mg/kg/day ip × 6 days; 1 day PLD), DENSPM·4HCl (60 mg/kg/day ip × 6 days; 1 day PLD), and DEHSPM·4HCl (15 mg/kg/day ip × 6 days; 1 day PLD). The native polyamine contents, expressed in terms of total equivalents of $N^+ \pm$ SD, were: control, 4156 ± 287 equiv ($n = 5$); DIPSPM, 3754 ± 403 equiv (not significant, $n = 5$); DENSPM, 2975 ± 166 equiv ($P < 0.005$, $n = 5$); and DEHSPM, 1560 ± 446 equiv ($P < 0.0005$, $n = 3$).

the importance of the metabolic issue in the design of therapeutics was clear.

Although HSPM, the major metabolite of DEHSPM, cannot be catabolized further in the polyamine biosynthetic network, dialkylspermines do not lead to this problem once they are dealkylated. Initial experiments with DESPM clearly demonstrated that it had excellent antidiarrheal activity. In an attempt to optimize this activity, we explored two structural alterations: modification of the terminal alkyl groups and a simple example of changing the geometry of the insulator between the two central nitrogens.

The synthetic approaches involved either alkylation of tetramesitylenesulfonated SPM with alkyl halides, followed by HBr/phenol-promoted protecting group removal, or a segment synthesis. The latter approach was employed in the assembly of both DIPSPM (**4**) and CHX-(3,4,3)-*trans* (**11**). The key fragments included 3-(*N*-isopropyl-*N*-mesitylenesulfonyl)aminopropyl tosylate and *N*-(3-bromopropyl)-*N*-ethylmesitylenesulfonamide. The tosylate was used to alkylate the dianion of $N^1,N^{4'}$-bis-(mesitylenesulfonyl)-1,4-butanediamine, and the bromide was used to alkylate *N*,*N*′-bis(mesitylenesulfonyl)-*trans*-1,4-cyclohexanediamine. All of these procedures lend themselves nicely to scaleup.

Six compounds were considered to show significant antidiarrheal activity when administered sc: CHX-(3,4,3)-*trans* (**11**) ≫ DIPSPM (**4**) > DESPM (**1**) ∼ DPSPM (**3**) > DIBSPM (**6**) ∼ DIPESPM (**7**). However, because of the neurological effects displayed by DPSPM, DIBSPM, and DIPESPM, their therapeutic window was too narrow for further consideration; these compounds were not taken into subsequent evaluations. When present, the neurological side effects in the analogue-treated animals included intention tremors, ataxia, motor dysfunction in the hind limbs, and seizures.

Although CHX(3,4,3)-*trans* (**11**) also elicited neurotoxicity, its activity was significant at a low enough dose to provide an acceptable therapeutic window: the neurological side effects described above disappeared at doses <1 mg/kg, yet a 92% reduction in stooling was observed at 0.0156 mg/kg, 1/64 of the toxic dose. However, the central *trans*-cyclohexanediamine fragment represents a metabolic unknown which requires further exploration (e.g., synthesis of suspected metabolites).

Two compounds, DESPM and DIPSPM, were moved into acute and chronic toxicity trials in mice. Whereas DIPSPM was acutely more toxic (LD_{50}, 150 mg/kg) than either DESPM (LD_{50}, 400 mg/kg) or DEHSPM (LD_{50}, 300 mg/kg[11]), in a chronic regimen, DIPSPM was less toxic than either DEHSPM (LD_{50}, 38 mg/kg[7]) or DESPM (LD_{50}, 80 mg/kg). At a dose of 75 mg/kg/day for 5 days, no deaths were observed. Most importantly, dogs treated either with DESPM at a dose of 1.87 mg/kg/day for 28 days or with DIPSPM at a dose of 2.15 mg/kg/day for 21 days did not present with any significant cardiac abnormalities when given continuous ambulatory electrocardiograms weekly during treatment. These doses were selected to be equimolar to the 2 mg/kg/day dose of DEHSPM at which dogs developed ventricular bigeminies as early as 3 days into treatment and still demonstrated the abnormality 30−40% of the time at day 30 PLD (R. J. Bergeron, J. Wiegand, W. R. Weimar, R. Müller, J. S. McManis, G. W. Yao, G. Huang, P. S. Snyder, C. Porter, R. Braylan, unpublished results).

A particularly noteworthy observation regarding DESPM and DIPSPM is related to their metabolism and impact on tissue polyamine pools relative to those of DEHSPM. Although the comparison was made in a number of tissues, for historical purposes we will focus on kidney, liver, spleen, and lung. In comparing DEHSPM with DESPM, two sets of data are available in dogs, 10 and 75 days PLD. The most outstanding feature in this comparison is the protracted residence time of HSPM. In DESPM-treated animals 10 days PLD, there was a measurable increase in both PUT and SPM, which was probably derived from the metabolism of the drug. In fact, at 75 days PLD, the PUT:SPD:SPM ratio is essentially the same as in control animals. In DEHSPM-treated animals at 10 days PLD, there was a significant suppression of PUT, SPD, and SPM. At 75 days PLD, native polyamines in kidney, spleen, and lung are returning to normal, yet liver polyamines remain diminished (R. J. Bergeron, J. Wiegand, W. R. Weimar, R. Müller, J. S. McManis, G. W. Yao, G. Huang, P. S. Snyder, C. Porter, R. Braylan, unpublished results).

Two tissue time points, 1 and 14 days PLD, were evaluated in rodents treated chronically with DIPSPM. These tissue samples were taken from animals in the chronic toxicity trial in which mice were given DIPSPM for 5 days at a dose of either 60 mg/kg/day and sacrificed 1 day PLD or 75 mg/kg/day and sacrificed 14 days PLD. The lack of impact of the drug on native polyamine pools is striking, particularly in the face of enormous tissue drug concentrations − 2700, 350, 600, and 500 nmol/g wet weight for kidney, liver, spleen, and lung, respectively − from mice given 60 mg/kg/day and sacrificed 1 day PLD. Typically, the cell or tissue tends to maintain a balance of charge contributions from polyamines, so that as the concentration of analogue increases, native polyamines such as SPD and SPM are correspondingly reduced.[11,14] This atypical action of DIPSPM may be related to some findings we previously reported of differences between two other diisopropyl analogues, N^1,N^{11}-diisopropylnorspermine (DIPNSPM) and N^1,N^{14}-diisopropylhomospermine (DIPHSPM), and their corresponding diethyl analogues, DENSPM and DEHSPM.[11] The diethyl analogues deplete polyamine pools and drastically reduce the levels of the key polyamine biosynthetic enzymes, ornithine decarboxylase and *S*-adenosylmethionine decarboxylase, in murine L1210 leukemia cells in vitro. In contrast, DIPNSPM and DIPHSPM had more modest effects on polyamine pools and almost no effect on ornithine decarboxylase and *S*-adenosylmethionine decarboxylase. We are currently exploring the nature of these distinctive properties that the N-terminal isopropyl group seems to confer on these polyamine analogues.

When evaluated orally in the rodent model, both DESPM and DIPSPM were active antidiarrheals; DIPSPM was significantly more so with a 100% reduction in fecal output at a dose of 25 mg/kg compared to a dose of 94 mg/kg DESPM to achieve the same effect. With allometric scaling, a 60-kg human would require a dose of 120 or 480 mg, respectively, for these compounds to ameliorate diarrhea. We are currently evaluating the pharmacokinetics and bioavailability of DESPM and DIPSPM as well as further assessing CHX(3,4,3)-*trans*, the internal cyclohexyl analogue.

Experimental Section

General. Our laboratory had previously synthesized **2**,[14] **8**,[16] and **9**.[16] Reagents were purchased from the Aldrich Chemical Co. and were used without further purification. Reactions using hydride reagents were run in distilled DMF under a nitrogen atmosphere. Fisher Optima-grade solvents were routinely used, and organic extracts were dried with sodium sulfate. Silica gel 32-63 from Selecto Scientific, Inc. (Suwanee, GA) was used for flash column chromatography. Melting points are uncorrected. Proton NMR spectra were obtained at 300 MHz and carbon spectra at 75 MHz in $CDCl_3$ (unless otherwise indicated) with chemical shifts given in parts per million downfield from tetramethylsilane (organic solvents) or 3-(trimethylsilyl)propionic-*2,2,3,3-d*$_4$ acid, sodium salt (D_2O, proton); dioxane at 67.19 ppm was the internal reference (D_2O, carbon). Coupling constants (*J*) are in Hz. FAB mass spectra were run in a glycerol/trifluoroacetic acid matrix. Elemental analyses were performed by Atlantic Microlabs, Norcross, GA.

***N*^{1},*N*^{12}-Dibenzylspermine Tetrahydrochloride (10).**[12] SPM was reductively alkylated with benzaldehyde (2.5 equiv) using $NaBH_4$ by a known method,[13] giving **10**: ^{1}H NMR (D_2O) δ 1.72−1.82 (m, 4 H), 2.06−2.19 (m, 4 H), 3.06−3.23 (m, 12 H), 4.28 (s, 4 H), 7.47−7.55 (m, 10 H). Anal. ($C_{24}H_{42}Cl_4N_4$) C, H, N.

***N*^{1},*N*^{4},*N*^{9},*N*^{12}-Tetrakis(mesitylenesulfonyl)spermine (13).** A solution of mesitylenesulfonyl chloride (42.8 g, 172 mmol) in CH_2Cl_2 (200 mL) was added dropwise to **12** (15.0 g, 43.1 mmol) in 2 N NaOH (200 mL) and CH_2Cl_2 (200 mL) with ice-bath cooling. The mixture was stirred overnight at room temperature, the layers were separated, and the aqueous portion was extracted with CH_2Cl_2 (2 × 100 mL). The combined organic layer was washed with H_2O (2 × 100 mL) and concentrated in vacuo. Recrystallization from 50% acetone in CH_2Cl_2 gave 36.09 g of **13** (90%) as a white solid: mp 160−161 °C; ^{1}H NMR δ 1.25−1.35 (m, 4 H), 1.57−1.70 (m, 4 H), 2.29 (s, 12 H), 2.52 (s, 12 H), 2.58 (s, 12 H), 2.73−2.83 (m, 4 H), 2.99−3.08 (m, 4 H), 3.12−3.23 (t, 4 H, J = 6.5), 4.88−4.94 (t, 2 H, J = 6.5), 6.92 (s, 4 H), 6.93 (s, 4 H); ^{13}C NMR δ 20.9,

22.2, 22.8, 24.3, 27.5, 39.3, 42.8, 45.1, 131.9, 132.0, 133.0, 133.7, 138.8, 139.9, 142.0, 142.6. Anal. ($C_{46}H_{66}N_4O_8S_4$) C, H, N.

***N*[1],*N*[12]-Dialkylation of 13. Bis-alkylation method 1:** Sodium hydride (60%, 2.5−3.0 equiv) was added to a stirred ice-bath-cooled solution of **13** (0.2 M in DMF). The mixture was stirred for 30 min (maintaining cooling) and for 30 min at room temperature, then added dropwise to a stirred solution of the indicated alkyl halide (3 equiv, 1 M in DMF) at room temperature. After stirring overnight, the mixture was cooled in an ice bath, quenched by dropwise addition of H_2O (volume about one-half that of the reaction mixture), and extracted with $CHCl_3$ (3−5 equal volumes). For alkyl iodide reactions the combined extracts were washed with an equal volume of 1% $NaHSO_3$. In all cases extracts were washed with H_2O and brine, and were concentrated in vacuo. The residue was purified by flash chromatography with the indicated solvent system to give **14** and **16**−**18**.

***N*[1],*N*[12]-Diethyl-*N*[1],*N*[4],*N*[9],*N*[12]-tetrakis(mesitylenesulfonyl)spermine (14).** Alkylation of **13** with iodoethane and chromatography (4% acetone in CH_2Cl_2) gave **14** in 93% yield as a white foam: mp 143−145 °C; ^{1}H NMR δ 0.95 (t, 6 H, J = 7.2), 1.28−1.36 (m, 4 H), 1.58−1.70 (m, 4 H), 2.29 (s, 12 H), 2.55 (s, 24 H), 2.94−3.12 (m, 16 H), 6.93 (s, 8 H); ^{13}C NMR δ 12.58, 20.88, 22.66, 22.73, 22.74, 24.32, 25.25, 40.00, 42.51, 43.01, 44.97, 131.86, 131.92, 133.05, 133.08, 139.94, 139.97, 142.31, 142.39; HRMS *m*/*z* calcd for $C_{50}H_{75}N_4O_8S_4$ 987.4467 (M + H), found 987.4461. Anal. ($C_{50}H_{74}N_4O_8S_4$) C, H, N.

***N*[1],*N*[12]-Dibutyl-*N*[1],*N*[4],*N*[9],*N*[12]-tetrakis(mesitylenesulfonyl)spermine (16).** Alkylation of **13** with 1-iodobutane and chromatography (25% EtOAc in hexane) provided **16** in 89% yield as a viscous oil: ^{1}H NMR δ 0.77 (t, 6 H, J = 7.4), 1.10 (sextet, 4 H, J = 7.4), 1.26−1.37 (m, 8 H), 1.57−1.69 (m, 4 H), 2.30 (s, 12 H), 2.54 (s, 12 H), 2.55 (s, 12 H), 2.91−3.11 (m, 16 H), 6.92 (s, 4 H), 6.93 (s, 4 H); ^{13}C NMR δ 13.49, 19.75, 20.88, 22.70, 22.76, 24.28, 25.05, 29.11, 42.89, 42.97, 44.90, 45.04, 131.85, 131.94, 133.07, 133.09, 139.96, 139.98, 142.30, 142.39; HRMS *m*/*z* calcd for $C_{54}H_{83}N_4O_8S_4$ 1043.5093 (M + H), found 1043.5089.

***N*[1],*N*[12]-Diisobutyl-*N*[1],*N*[4],*N*[9],*N*[12]-tetrakis(mesitylenesulfonyl)spermine (17).** Alkylation of **13** with 1-bromo-2-methylpropane and chromatography (25% EtOAc in hexane) generated **17** in 45% yield as a white glass: mp 57−59 °C; ^{1}H NMR δ 0.69 (d, 12 H, J = 6.6), 1.26−1.38 (m, 4 H), 1.52−1.70 (m, 6 H), 2.29 (s, 12 H), 2.54 (s, 12 H), 2.55 (s, 12 H), 2.90 (d, 4 H, J = 7.2), 2.92−3.02 (m, 8 H), 3.02−3.11 (m, 4 H), 6.92 (s, 8 H); ^{13}C NMR δ 19.9, 20.9, 22.8, 24.2, 24.7, 26.0, 43.0, 43.3, 44.8, 53.2, 131.9, 132.0, 133.1, 133.2, 139.9, 140.0, 142.3, 142.4; HRMS *m*/*z* calcd for $C_{54}H_{83}N_4O_8S_4$ 1043.5093 (M + H), found 1043.5080.

***N*[1],*N*[12]-Diisopentyl-*N*[1],*N*[4],*N*[9],*N*[12]-tetrakis(mesitylenesulfonyl)spermine (18).** Alkylation of **13** with 1-bromo-3-methylbutane and chromatography (6% acetone in toluene) furnished **18** in 95% yield as a viscous oil: ^{1}H NMR δ 0.73 (d, 12 H, J = 6.6), 1.16−1.25 (m, 4 H), 1.28−1.41 (m, 6 H), 1.57−1.70 (m, 4 H), 2.29 (s, 12 H), 2.54 (s, 12 H), 2.55 (s, 12 H), 2.90−3.15 (m, 16 H), 6.92 (s, 8 H); ^{13}C NMR δ 20.93, 22.20, 22.73, 22.74, 22.81, 24.30, 25.10, 25.65, 35.76, 42.84, 42.98, 43.58, 44.90, 131.85, 131.96, 133.05, 133.10, 139.96, 140.03, 142.34, 142.39; HRMS *m*/*z* calcd for $C_{56}H_{87}N_4O_8S_4$ 1071.5407 (M + H), found 1071.5335.

Bis-alkylation method 2: *N*[1],*N*[12]-Dipropyl-*N*[1],*N*[4],*N*[9],*N*[12]-tetrakis(mesitylenesulfonyl)spermine (15). Sodium hydride (60%, 1.12 g, 27.9 mmol) was added in portions to an ice-bath-cooled solution of **13** (10.0 g, 10.7 mmol) in DMF (150 mL), then the mixture was stirred at room temperature for 30 min. 1-Iodopropane (5.58 g, 32.8 mmol) was added in one portion; the mixture was heated to 45−50 °C for 18 h. The reaction was quenched with EtOH (10 mL) and H_2O (20 mL) and was concentrated in vacuo. Water (100 mL) was added, and the mixture was extracted with $CHCl_3$ (2 × 100 mL). The organic portions were combined and washed with 1% $NaHSO_3$ (150 mL) and brine (100 mL); the solvent was removed in vacuo. Flash chromatography of the residue with 8% acetone in toluene gave 9.6 g of **15** (88%) as a white solid: mp 83−85 °C; ^{1}H NMR δ 0.70 (t, 6 H, J = 7.5), 1.24−1.42 (m, 8 H), 1.58−1.70 (m, 4 H), 2.29 (s, 12 H), 2.540 (s, 12 H), 2.544 (s, 12 H), 2.90−3.03 (m, 12 H), 3.03−3.10 (m, 4 H), 6.92 (s, 4 H), 6.93 (s, 4 H); ^{13}C NMR δ 11.12, 20.44, 20.94, 22.75, 22.77, 22.79, 22.81, 24.32, 25.11, 43.04, 44.94, 47.19, 131.89, 131.97, 133.10, 133.11, 139.98, 140.00, 142.31, 142.43; HRMS *m*/*z* calcd for $C_{52}H_{79}N_4O_8S_4$ 1015.4780 (M + H), found 1015.4771.

3-[*N*-(Mesitylenesulfonyl)isopropylamino]-*O*-*p*-tosyl-1-propanol (20). Sodium hydride (60%, 3.00 g, 75.0 mmol) was added to a solution of **19**[11] (12.98 g, 53.8 mmol) in DMF (150 mL) at 0 °C; the mixture was stirred for 15 min. Stirring was continued for 30 min at room temperature, and the solution was added dropwise to 1,3-propanediol di-*p*-tosylate (62.29 g, 162 mmol) in DMF (100 mL). The mixture was stirred overnight at room temperature and then quenched by dropwise addition of saturated $NaHCO_3$ (100 mL) with ice-bath cooling. The resulting suspension was filtered; solids were washed with 3:1 hexane:EtOAc (3 × 200 mL). The filtrate and washings were combined, and solvents were removed in vacuo. The residue was dissolved in $CHCl_3$ (300 mL) and washed with H_2O (100 mL); $CHCl_3$ was removed in vacuo. Flash chromatography of the residue with 2:1 hexane:EtOAc removed the remaining 1,3-propanediol di-*p*-tosylate; flash chromatography of the resulting material with 3:1 cyclohexane:EtOAc gave 12.8 g of **20** (52%) as a white foam: mp 84−85 °C; ^{1}H NMR δ 1.10 (d, 6 H, J = 6.8), 1.81−1.93 (m, 2 H), 2.29 (s, 3 H), 2.46 (s, 3 H), 2.56 (s, 6 H), 3.20 (m, 2 H), 3.82 (septet, 1 H, J = 6.8), 3.96 (t, 2 H, J = 5.8), 6.92 (s, 2 H), 7.35 (d, 2 H, J = 8.4), 7.77 (d, 2 H, J = 8.4); ^{13}C NMR δ 20.8, 20.9, 21.6, 22.7, 30.3, 38.6, 48.5, 68.4, 127.8, 129.9, 132.0, 132.7, 133.4, 140.0, 142.4, 144.9; HRMS *m*/*z* calcd for $C_{22}H_{32}NO_5S_2$ 454.1722 (M + H), found 454.1723.

***N*[1],*N*[12]-Diisopropyl-*N*[1],*N*[4],*N*[9],*N*[12]-tetrakis(mesitylenesulfonyl)spermine (21).** Sodium hydride (60%, 0.524 g, 13.1 mmol) was added to a solution of *N*,*N*′-bis(mesitylenesulfonyl)-1,4-butanediamine[11] (1.98 g, 4.4 mmol) in DMF (65 mL) at 0 °C, and the mixture was stirred for 20 min. Stirring was continued for 30 min at room temperature, and the solution was added dropwise to **20** (4.05 g, 8.9 mmol) in DMF (75 mL). The mixture was stirred overnight at room temperature, cooled to 0 °C, quenched by dropwise addition of H_2O (25 mL), and extracted with $CHCl_3$ (3 × 150 mL), which was removed in vacuo. Flash chromatography of the residue with 3:1 cyclohexane:EtOAc gave 3.29 g of **21** (74%) as a white foam: mp 56−58 °C; ^{1}H NMR δ 1.02 (d, 12 H, J = 6.7) 1.28−1.36 (m, 4 H), 1.58−1.69 (m, 4 H), 2.29 (s, 12 H), 2.54 (s, 12 H), 2.56 (s, 12 H), 2.83−2.92 (m, 4 H), 2.96−3.10 (m, 8 H), 3.74 (septet, 2 H, J = 6.7), 6.92 (s, 4 H), 6.94 (s, 4 H); ^{13}C NMR δ 20.8, 20.9, 22.7, 22.8, 24.2, 28.4, 39.5, 43.0, 44.7, 48.3, 131.9, 132.0, 133.2, 133.5, 139.9, 140.0, 142.3, 142.4; HRMS *m*/*z* calcd for $C_{52}H_{79}N_4O_8S_4$ 1015.4780 (M + H), found 1015.4749.

***N*,*N*′-Bis(mesitylenesulfonyl)-*trans*-1,4-cyclohexanediamine (23).** Mesitylenesulfonyl chloride (12.2 g, 55.6 mmol) in CH_2Cl_2 (100 mL) was added to **22** (2.92 g, 25.6 mmol) in 1 N NaOH (100 mL) at 0 °C. The mixture was stirred at 0 °C for 30 min and at room temperature overnight. The solid was filtered and washed with H_2O and EtOH to give 10 g (82%) of **23** as a solid: mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ 0.99−1.23 (m, 4 H), 1.40−1.52 (m, 4 H), 2.24 (s, 6 H), 2.51 (s, 12 H), 2.66−2.79 (m, 2 H), 7.00 (s, 4 H), 7.4 (br). Anal. ($C_{24}H_{34}N_2O_4S_2$) C, H, N.

Bis[3-(ethylamino)propyl]-*N*,*N*′,*N*″,*N*‴-tetrakis(mesitylenesulfonyl)-*trans*-1,4-cyclohexanediamine (24). Sodium hydride (80%, 0.157 g, 5.23 mmol) was added to **23** (1.14 g, 2.38 mmol) in DMF (40 mL) at 0 °C. The mixture was stirred at 0 °C for 20 min, and a solution of *N*-(3-bromopropyl)-*N*-ethylmesitylenesulfonamide[11] (1.74 g, 5.0 mmol) in DMF (20 mL) was added dropwise. The solution was stirred at 0 °C for 30 min and at 70 °C for 12 h. The solvent was removed in vacuo, H_2O (30 mL) was added, and the mixture was extracted with $CHCl_3$ (4 × 40 mL), which was removed in vacuo. Flash chromatography of the residue with 3:3:1 hexane:$CHCl_3$:EtOAc gave 1.75 g (73%) of **24** as a solid: mp 209−212 °C; ^{1}H NMR δ 0.96 (t, 6 H, J = 7), 1.31−1.41 (m, 4 H), 1.47−1.59 (m, 4 H),

1.66−1.78 (m, 4 H), 2.282 and 2.294 (2s, 12 H), 2.557 (s, 24 H), 2.89 (t, 4 H, J = 8), 2.98−3.13 (m, 8 H), 3.32−3.43 (m, 2 H), 6.915 and 6.944 (2s, 8 H). Anal. ($C_{52}H_{76}N_4O_8S_4$) C, H, N.

Deprotection: Hydrogen bromide in HOAc (30%, 8−10 mL/mmol substrate) was added dropwise to a solution of **14**−**18**, **21**, and **24** (0.07−0.10 M in CH_2Cl_2) and phenol (40 equiv) with ice-bath cooling. The reaction mixture was stirred overnight at room temperature and was cooled in an ice bath; an equal volume of H_2O was added dropwise. The layers were separated, and the aqueous portion was extracted with CH_2Cl_2 (3 equal volumes). The aqueous portion was concentrated in vacuo, 1 N NaOH was added to the resulting residue, and the mixture was basified to pH > 13 by addition of 19 N NaOH with ice-bath cooling. The aqueous phase was extracted several times with $CHCl_3$, which was removed in vacuo. The resulting oil was dissolved in EtOH and acidified to pH < 1 with 1 N HCl. Solvent removal in vacuo followed by recrystallization from aqueous EtOH gave **1**, **3**−**7**, and **11**.

***N*^{1},*N*^{12}-Diethylspermine Tetrahydrochloride (1).** The yield was 90% from **14** as white plates: mp > 290 °C; ^{1}H NMR (D_2O) δ 1.30 (t, 6 H, J = 7.5), 1.76−1.83 (m, 4 H), 2.06−2.18 (m, 4 H), 3.09−3.20 (m, 16 H); ^{13}C NMR (D_2O) δ 11.15, 23.33, 23.35, 43.71, 44.50, 45.12, 47.62; HRMS *m*/*z* calcd for $C_{14}H_{35}N_4$ 259.2862 (M + H), found 259.2872. Anal. ($C_{14}H_{38}Cl_4N_4$) C, H, N.

***N*^{1},*N*^{12}-Dipropylspermine Tetrahydrochloride (3).** The yield was 72% from **15** as white plates: mp > 290 °C; ^{1}H NMR (D_2O) δ 0.98 (t, 6 H, J = 7.5), 1.64−1.76 (m, 4 H), 1.76−1.86 (m, 4 H), 2.06−2.19 (m, 4 H), 3.01−3.21 (m, 16 H); ^{13}C NMR (D_2O) δ 10.8, 19.8, 23.3, 23.4, 44.9, 45.1, 47.6, 50.0; HRMS *m*/*z* calcd for $C_{16}H_{39}N_4$ 287.3175 (M + H), found 287.3175. Anal. ($C_{16}H_{42}Cl_4N_4$) C, H, N.

***N*^{1},*N*^{12}-Diisopropylspermine Tetrahydrochloride (4).**[17] The yield was 80% from **21** as white needles: mp > 290 °C; ^{1}H NMR (D_2O) δ 1.33 (d, 12 H, J = 6.6) 1.74−1.84 (m, 4 H), 2.02−2.18 (m, 4 H), 3.07−3.22 (m, 12 H), 3.44 (septet, 2 H, J = 6.6); ^{13}C NMR (D_2O) δ 18.8, 23.4, 23.5, 42.2, 45.2, 47.6, 51.7; HRMS *m*/*z* calcd for $C_{16}H_{39}N_4$ 287.3175 (M + H), found 287.3193. Anal. ($C_{16}H_{42}Cl_4N_4$) C, H, Cl, N.

***N*^{1},*N*^{12}-Dibutylspermine Tetrahydrochloride (5).** The yield was 60% from **16** as white plates: mp > 290 °C; ^{1}H NMR (D_2O) δ 0.93 (t, 6 H, J = 7.4), 1.39 (sextet, 4 H, J = 7.4), 1.67 (quintet, 4 H, J = 7.6), 1.74−1.86 (m, 4 H), 2.05−2.19 (m, 4 H), 3.02−3.22 (m, 16 H); ^{13}C NMR (D_2O) δ 13.4, 19.7, 23.3, 23.4, 28.1, 44.9, 45.1, 47.6, 48.3; HRMS *m*/*z* calcd for $C_{18}H_{43}N_4$ 315.3488 (M + H), found 315.3452. Anal. ($C_{18}H_{46}Cl_4N_4$) C, H, Cl, N.

***N*^{1},*N*^{12}-Diisobutylspermine Tetrahydrochloride (6).** The yield was 84% from **17** as white plates: mp > 290 °C; ^{1}H NMR (D_2O) δ 1.00 (d, 12 H, J = 6.6), 1.75−1.85 (m, 4 H), 1.95−2.08 (m, 2 H), 2.08−2.20 (m, 4 H), 2.91−2.96 (d, 4 H, J = 6.6), 3.10−3.21 (m, 12 H); ^{13}C NMR (D_2O) δ 19.7, 23.2, 23.4, 26.2, 45.1, 45.5, 47.6, 55.5; HRMS *m*/*z* calcd for $C_{18}H_{43}N_4$ 315.3488 (M + H), found 315.3471. Anal. ($C_{18}H_{46}Cl_4N_4$) C, H, N.

***N*^{1},*N*^{12}-Diisopentylspermine Tetrahydrochloride (7).** The yield was 48% from **18** as white plates: mp > 290 °C; ^{1}H NMR (D_2O) δ 0.93 (d, 12 H, J = 6.6), 1.53−1.65 (m, 4 H), 1.68 (septet, 2 H, J = 6.6), 1.80 (quintet, 4 H, J = 3.6), 2.06−2.18 (m, 4 H), 3.06−3.21 (m, 16 H); ^{13}C NMR (D_2O) δ 21.98, 23.32, 23.40, 25.83, 34.80, 44.93, 45.13, 47.02, 47.63; HRMS *m*/*z* calcd for $C_{20}H_{47}N_4$ 343.3800 (M + H), found 343.3809. Anal. ($C_{20}H_{50}Cl_4N_4$) C, H, N.

***N*,*N*′-Bis[3-(ethylamino)propyl]-*trans*-1,4-cyclohexanediamine Tetrahydrochloride (11).** The yield was 64% from **24** as a crystalline solid: ^{1}H NMR (D_2O) δ 1.30 (t, 6 H, J = 7.4), 1.48−1.59 (m, 4 H), 2.03−2.13 (m, 4 H), 2.22−2.33 (m, 4 H), 3.08−3.27 (m, 14 H). Anal. ($C_{16}H_{40}Cl_4N_4$) C, H, N.

Castor Oil-Induced Diarrhea. Male Sprague−Dawley rats (350−400 g; Harlan Sprague−Dawley, Indianapolis, IN) were fasted overnight in hanging wire cages and allowed free access to water. A typical experiment involved 20 rats: 5 untreated controls and 5 pretreated with polyamine analogues at each of three doses (typically equivalent on a molar basis to 1, 5, 10, or 25 mg/kg of DEHSPM) as either a sc injection or a po gavage 30 min prior to castor oil. All animals were then challenged with castor oil (purchased from a local drugstore) as a gastric gavage of 5 mL/kg of body weight at t = 0 and monitored for the onset and duration of diarrhea at 30-min intervals for a 6-h period during which they received no food or water.[18] Onset of diarrhea for the control rats was between 30 and 90 min and lasted for at least 6 h. The animal weight and stool weight were recorded at 2, 4, and 6 h.

Acute and Chronic Toxicity of Polyamine Analogues in Mice. For acute toxicity assessments, the polyamine analogues were administered to female CD-1 mice (Charles River, Wilmington, MA) as a single ip injection. The animals were scored 2 h after administration of drug. All survivors were observed for a period of 10 days after the treatment. Based on the results from the acute toxicity trials, the chronic toxicity dosing regimen was set up so that at least one test group should have a high fraction of lethalities. In the chronic toxicity regimen, mice were administered the polyamine analogue as 1 ip dose/day for 5 days. Body weight and observations of coat health and overall physical appearance were recorded at the time of each injection. After the treatment regimen had been completed, animals were monitored daily for an additional 10 days.

Chronic Toxicity and Tissue Distribution of DESPM in Dogs. Ten young adult (1-year-old) male beagles were obtained from Harlan Sprague−Dawley. They were provided with food and water ad libitum and were allowed to acclimate to their new surroundings for 2 weeks. The animals were weighed prior to starting drug administration and were weighed once a week during the drug-dosing period. Continuous ambulatory electrocardiograms were performed once a week during the drug dosing. A CBC and extensive chemistry panel were performed once weekly during the drug-dosing period and during the recovery phase and/or prior to sacrifice. Unless otherwise indicated, the DESPM was dissolved in saline to a concentration of 3.74, 8, 12, and 16 mg/mL (for the 1.87, 4, 6, and 8 mg/kg/day regimens, respectively). After sterilization by filtration through a 0.2-μm syringe filter, the dogs were given DESPM sc at a daily dose of 1.87 (n = 3), 4 (n = 2), 6 (n = 2), or 8 (n =2) mg/kg/day (0.97, 2, 3, and 4 mg/kg twice daily). The final dog served as a vehicle control.

The animals were bled before dosing began, during the drug administration period, and during the recovery phase and/or prior to sacrifice. The blood samples were analyzed by an outside clinical laboratory (Antech Diagnostics, Gainesville, FL). The assays performed included complete blood cell count (CBC: white blood cells, differential, red blood cells, hemoglobin, hematocrit, RBC indices, platelet count), and chemistry profile [glucose, sodium, potassium, chloride, carbon dioxide, blood urea nitrogen (BUN), creatinine, calcium, phosphorus, total protein, albumin, alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol, total bilirubin, globulin, γ-glutamyl transpeptidase], magnesium, amylase, lipase, and trypsin-like immunoreactivity. The evaluation of these parameters allowed us to assess the health of the animals going into the experiment and to measure changes that the test drug may induce in an animal.

The tissues taken for histological evaluation by an outside pathologist (Florida Veterinary Pathology, Bushnell, FL) included: kidney, liver, lung, heart, spleen, pancreas, adrenal gland, testicle, bone marrow, jugular vein, aorta, parotid and mandibular salivary glands, lymph node, skin, pericardium, prostate, diaphragm, bladder, stomach, small intestine, large intestine, thyroid, thymus, muscle, fat, esophagus, tongue, and gall bladder. Polyamine level determination was performed on these tissues after preparation by the methods described below.

Chronic Toxicity of DIPSPM in Dogs. Two young adult (1-year-old) male beagles were obtained from Harlan Sprague−Dawley. They were provided with food and water ad libitum and were allowed to acclimate to their new surroundings for two weeks. The animals were weighed prior to starting drug administration and were weighed once a week during the drug-

dosing period. Continuous ambulatory electrocardiograms were performed once a week during the drug dosing. A CBC and extensive chemistry panel were performed once weekly during the drug-dosing period and during the recovery phase and/or prior to sacrifice. The DIPSPM was dissolved in saline to a concentration of 4.3 mg/mL. After sterilization by filtration through a 0.2-μm syringe filter, the dogs were given DIPSPM sc at a daily dose of 2.15 mg/kg/day (1.075 mg/kg twice daily) for 21 days.

HPLC Analysis of Polyamines in Tissues. Various tissues, including the liver, spleen, lung, and kidney, were prepared for HPLC analysis of polyamine content. To facilitate their handling, the organs were frozen in liquid nitrogen, weighed, and homogenized (Tissuemizer, Tekmar, Cincinnati, OH) in 1.2 N perchloric acid (containing 1,7-diaminoheptane internal standard) in a 1:20 (w/v) ratio.[7] The tissue homogenates were then freeze−thawed three times and stored in a −70 °C freezer until HPLC analysis was performed.

Each chromatographic assay included calibration standards, which were treated in the same manner as the samples. The calibration standards (typical retention times in min are indicated) were prepared by adding known amounts of PUT (9.6), 1,6-diaminohexane (13.0), 1,7-diaminoheptane (15.4), SPD (20.9), SPM (26.8), HSPM (27.6), MESPM (28.8), MIPSPM (29.2), MEHSPM (29.8), DESPM (30.8), DIPSPM (31.6), and DEHSPM (31.9) to a matrix that resembled the sample matrix. The concentration of each polyamine was calculated from the peak area by calibration curves obtained by nonweighted least-squares linear regression. Peak area and linear regression calculations were performed on a Macintosh Centris 650 or G3 with Rainin Dynamax HPLC Method Manager software (Rainin Instrument Co., Ridgefield, NJ). The method had a detection limit of ≤0.1 nmol/mL and was reproducible and linear over the range 0.5−100 nmol/mL.

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Supporting Information Available: Tables summarizing characteristic data and elemental analytical results. This material is available free of charge via the Internet at http://pubs.acs.org.

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