Synthesis and Evaluation of Hydroxylated Polyamine Analogues as Antiproliferatives

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A new means of accessing N^1-cyclopropylmethyl-N^{11}-ethylnorspermine (CPMENSPM) and the first synthesis of (2*R*,10*S*)-N^1-cyclopropylmethyl-2,10-dihydroxy-N^{11}-ethylnorspermine [(2*R*,10*S*)-$(HO)_2$CPMENSPM] are described. Both of these polyamine analogues are shown to be more active against L1210 murine leukemia cell growth than either N^1,N^{11}-diethylnorspermine (DENSPM) or (2*R*,10*R*)-N^1,N^{11}-diethyl-2,10-dihydroxynorspermine [(2*R*,10*R*)-$(HO)_2$DENSPM] after 96 h of treatment; the activity was comparable to that of (2*S*,10*S*)-N^1,N^{11}-diethyl-2,10-dihydroxynorspermine [(2*S*,10*S*)-$(HO)_2$DENSPM] at 96 h. Both cyclopropyl compounds reduced putrescine and spermidine pools, but less effectively than did DENSPM and its derivatives. Only CPMENSPM, and not (2*R*,10*S*)-$(HO)_2$CPMENSPM, lowered spermine pools. As with DENSPM and (2*R*,10*R*)-$(HO)_2$DENSPM, both cyclopropyl analogues diminished ornithine decarboxylase and *S*-adenosylmethionine decarboxylase activity. Unlike the hydroxylated DENSPM compounds, both cyclopropyl norspermines substantially upregulated spermidine/spermine N^1-acetyltransferase. The most interesting effect of hydroxylating CPMENSPM is the profound reduction in toxicity compared with that of the parent drug. The same phenomenon had been observed for the DENSPM/(2*R*,10*R*)-$(HO)_2$DENSPM pair. Thus, hydroxylation of norspermine analogues appears to be a way to maintain the compounds' antiproliferative activity while reducing their toxicity.

Introduction

The observed increases in polyamine biosynthesis in neoplastic tissues have made the polyamine biosynthetic network an attractive target in cancer chemotherapeutic strategies.[1−3] There is abundant evidence that *N*-alkylated polyamines exhibit activity against a number of murine and human tumor lines both in vitro and in vivo.[4−8] The phase II clinical trials with N^1,N^{11}-diethylnorspermine (DENSPM) have further highlighted interest in these molecules. It is certainly well established that these polyamine analogues utilize the polyamine transport apparatus for incorporation into cells,[9−11] reduce polyamine pools,[12] diminish the level of ornithine decarboxylase (ODC)[13,14] and *S*-adenosylmethionine decarboxylase (AdoMetDC) activities,[14,15] and in some cases upregulate spermidine/spermine N^1-acetyltransferase (SSAT).[16−19] However, the precise mechanism by which the molecules induce death in tumor cells has not been completely defined.[20] Systematic structure−activity studies have shown that very small structural alterations in these compounds can cause pronounced differences in their biological properties at both cellular and whole animal levels.[12,21,22] On a cellular level, for example, whereas the tetraamines DENSPM, N^1,N^{12}-diethylspermine (DESPM), and N^1,N^{14}-diethylhomospermine (DEHSPM) 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. The most profound difference among the three analogues is related to their ability to stimulate SSAT.[17−19] The tetraamine DENSPM upregulates SSAT by 15-fold in L1210 murine leukemia cells, yet DESPM and DEHSPM stimulate SSAT by 4.6- and 1.4-fold, respectively.[17] The SSAT stimulation is even more striking in other cell lines.[23] Furthermore, although the K_i values of DESPM and DEHSPM for the polyamine transport system are within error of each other, the DENSPM value is >10 times as great.[21]

Minor changes in an analogue's structure also cause a significant difference in pharmacological behavior in whole animals. DENSPM has little gastrointestinal antitransit activity; DEHSPM is a potent antidiarrheal.[24] DEHSPM also decreases blood pressure in rats, whereas neither DENSPM[25] nor DESPM possesses this property. All three analogues are first metabolized in vivo by *N*-deethylation to the corresponding tetraamine.[26−29] Spermine and norspermine are then processed by deaminopropylation[26,28,29] through the polyamine metabolic network. However, because of the presence of the aminobutyl fragments in homospermine,[27] it is not terminally *N*-acylated and cannot be further degraded. Thus, it remains unmetabolized for a protracted period of time in animals. These differences in susceptibility to catabolism correlate with the toxicity profiles: DENSPM is the least toxic,[26] and DEHSPM is the most.[27]

Although DENSPM's chronic dose-limiting toxicity is gastrointestinal, its subacute toxicity is neurological. Considering the enormous difference between the dose given to patients and the dose at which mice exhibited neurological side effects, including pronounced ataxia,

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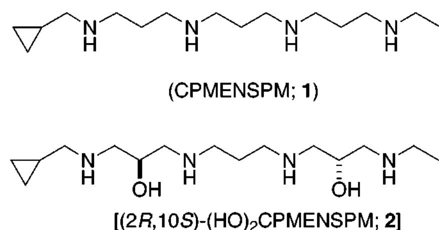


Figure 1. Structures of N^1-cyclopropylmethyl-N^{11}-ethylnorspermine (CPMENSPM, **1**) and its hydroxylated derivative, (2*R*,10*S*)-N^1-cyclopropylmethyl-2,10-dihydroxy-N^{11}-ethylnorspermine [(2*R*,10*S*)-$(HO)_2$CPMENSPM, **2**].

intention tremors, and motor dysfunction,[30] it seemed that neurotoxicity would be an unlikely problem in the clinic. Nevertheless, this represented an easily observable means to help evaluate how structural alterations in the polyamine backbone affect toxicity. In a recent study,[30] we examined the effect of hydroxylation of DENSPM on both the activity of the compound against L1210 cells in culture and adverse effects in vivo. In this paper, we have carried out these observations further to include an unsymmetrically substituted analogue, N^1-cyclopropylmethyl-N^{11}-ethylnorspermine [CPMENSPM (CPENSpm in the nomenclature of Woster and co-workers),[31] **1**; Figure 1]. This tetraamine is cytotoxic to several tumor cell lines in vitro, increasing SSAT activity[31−33] and initiating apoptosis.[31,32,34−36] However, its activity and toxicity in vivo have not been reported. We describe an improved method of generating CPMENSPM and the synthesis of a hydroxylated derivative, (2*R*,10*S*)-N^1-cyclopropylmethyl-2,10-dihydroxy-N^{11}-ethylnorspermine [(2*R*,10*S*)-$(HO)_2$CPMENSPM, **2**; Figure 1]. Initial evaluations of these analogues in L1210 cells are performed; the toxicity of these compounds in mice is also examined. Once again, hydroxylation is shown to substantially reduce toxicity.

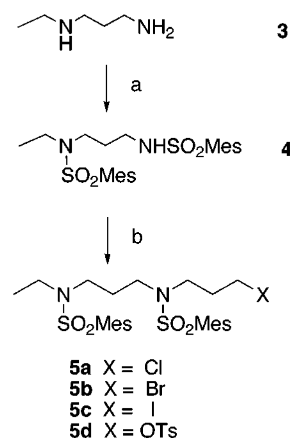
Results

Synthesis. The first synthesis of the polyamine analogue CPMENSPM (**1**) was carried out in 2% overall yield.[31] Specifically, *N*-benzyl-1,3-diaminopropane[37] was converted into 21 mg of **1** as its tetrahydrobromide salt in 10 steps.[31] To generate sufficient quantities of CPMENSPM for biological testing in our laboratories, dialkylated polyamine **1** as its tetrahydrochloride salt was accessed by a segmented route (Schemes 1 and 2). *N*-Ethyl-1,3-diaminopropane (**3**) was treated with mesitylenesulfonyl chloride (2 equiv) under biphasic conditions (CH_2Cl_2/dilute NaOH), giving **4** in 88% recrystallized yield (Scheme 1). Dropwise addition of the anion of **4** (NaH/DMF) to 1,3-dichloro-, 1,3-dibromo-, or 1,3-diiodopropane in 12-fold excess in DMF gave each electrophilic reagent (**5a**, **5b**, and **5c**) in 66, 71, and 40% yield, respectively. However, addition of deprotonated **4** to excess 1,3-propanediol-di-*p*-tosylate led to a mixture from which tosylate **5d** could not be easily purified.

Synthesis of the segment that contains the small ring began with modification of (aminomethyl)cyclopropane hydrochloride (**6**) using mesitylenesulfonyl chloride (CH_2Cl_2/1 N NaOH) in 97% yield (Scheme 2). Proton removal from the resulting **7** with NaH in DMF and alkylation with *N*-(3-bromopropyl)phthalimide in DMF provided intermediate **8** in 84% yield. Unmasking the primary amino functionality of **8** with hydrazine in refluxing ethanol generated primary amine **9** in 87% yield. Treatment of **9** with mesitylenesulfonyl chloride as before produced *N*,*N*-bis(mesitylenesulfonyl)-*N*-cyclopropylmethyl-1,3-diaminopropane (**10**) in 84% yield.

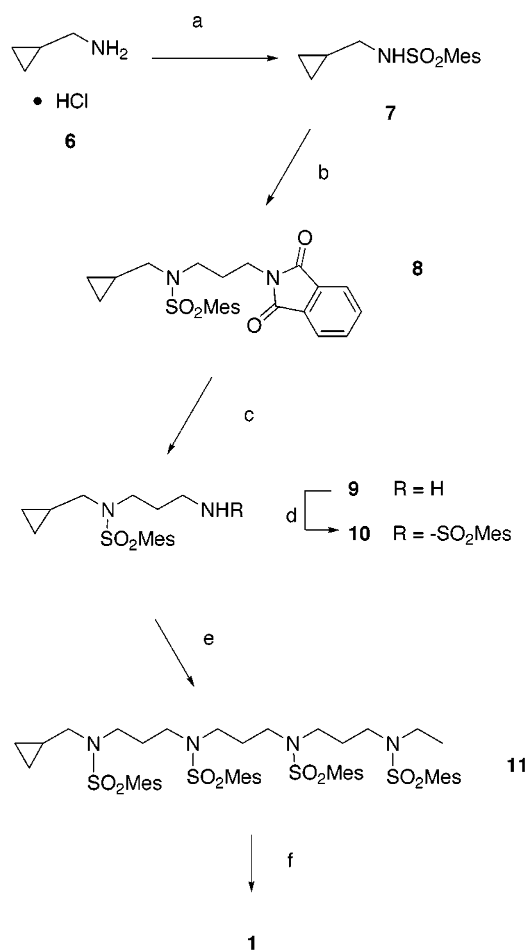
Connecting disulfonamide **10**, which contains the cyclopropylmethylamino unit, with halides **5a**−**c**, which

Scheme 1. Synthesis of Diamine Reagents (**5a**−**c**)[a]



[a] Reagents: (a) mesitylenesulfonyl chloride, NaOH (aq), CH_2Cl_2, 88%; (b) NaH, DMF, $X(CH_2)_3X$, X = Cl (66%), X = Br (71%), X = I (40%), X = OTs (0%).

Scheme 2. Synthesis of CPMENSPM (**1**)[a]



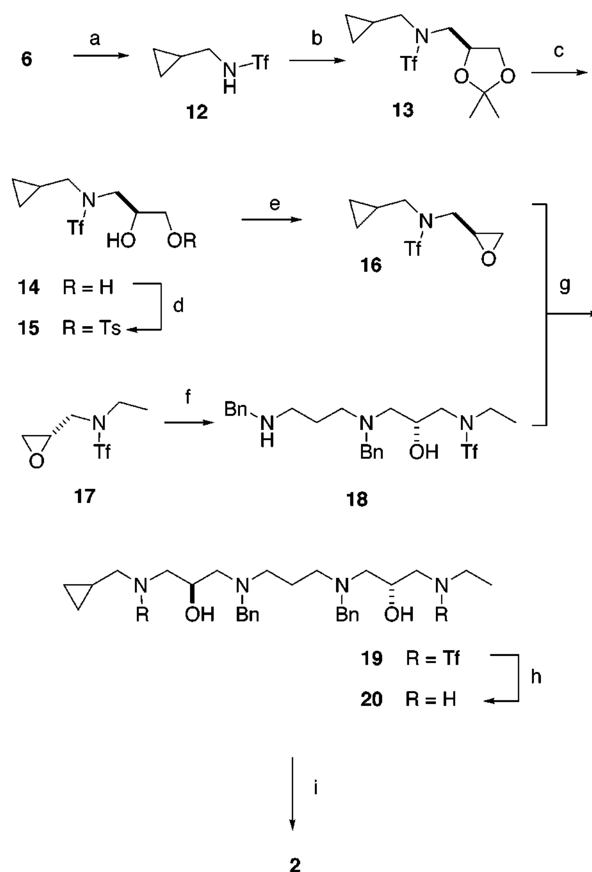
[a] Reagents: (a) mesitylenesulfonyl chloride, NaOH (aq), CH_2Cl_2, 97%; (b) *N*-(3-bromopropyl)phthalimide, NaH, DMF, 84%; (c) hydrazine hydrate, EtOH, reflux, 87%; (d) a, 84%; (e) **5b**, NaH, DMF, 73%; (f) 30% HBr in HOAc, PhOH, CH_2Cl_2; NaOH; HCl, 36%.

possess the ethylamino functionality, would complete the framework of unsymmetrically dialkylated norspermine **1**. A solution of the anion of **10** (NaH/DMF) was added to a solution of each alkylating agent—**5a**, **5b**, or **5c**—in DMF to optimize production of masked tetraamine **11**. With alkyl chloride **5a**, difficulties in purification prevented isolation of **11**. Utilizing iodo compound **5c** resulted in **11** in only 6% yield. Treatment of **10** with primary bromide **5b** led efficiently to tetramesitylenesulfonamide **11** (73% yield). Removal of the protective groups from **11** using 30% HBr in acetic acid and phenol in CH_2Cl_2 and conversion to the tetrahydrochloride salt furnished CPMENSPM (**1**) in 36% recrystallized yield.

We report the first synthesis of (2*R*,10*S*)-$(HO)_2$-CPMENSPM (**2**) in which both outer trimethylene chains of CPMENSPM (**1**) are hydroxylated. Two pairs of enantiomeric dihydroxylated, terminally diethylated tetraamines have been synthesized by way of fragments in this laboratory: (2*R*,10*R*)- and (2*S*,10*S*)-N^1,N^{11}-diethyl-2,10-dihydroxynorspermine [$(HO)_2$DENSPMs] and (3*R*,12*R*)- and (3*S*,12*S*)-N^1,N^{14}-diethyl-3,12-dihydroxyhomospermine.[30] The final steps in the assembly of (2*S*,10*S*)-$(HO)_2$DENSPM involved alkylation of *N*,*N'*-dibenzyl-1,3-propanediamine with *N*-[(2*S*)-2,3-epoxypropyl]-*N*-ethyltrifluoromethanesulfonamide (2 equiv) and removal of the trifluoromethanesulfonyl (Tf)[38] and benzyl (Bn) protecting groups with $LiAlH_4$ (THF, reflux, 19 h) and H_2 (1 atm, 10% Pd−C, 1 N HCl, EtOH), respectively.[30] However, because a cyclopropyl group may undergo ring opening under reductive conditions,[39−41] it was not initially clear that we could use this methodology to make (2*R*,10*S*)-$(HO)_2$CPMENSPM (**2**). The stability of the cyclopropane ring to these deprotection conditions was verified in two model systems before the synthesis of hydroxylated tetraamine **2** was undertaken. First, *N*-benzyl-*N*-cyclopropylmethyl-*N*-propylamine was prepared by successive alkylation of primary amine **6** with benzaldehyde ($NaBH_4$/EtOH)[42] and propyl iodide (K_2CO_3/1-butanol). Mild hydrogenolysis of this tertiary amine (1 atm, 10% Pd−C, 1 N HCl, EtOH) gave *N*-cyclopropylmethyl-*N*-propylamine in 91% yield. Second, heating the triflamide of *N*-cyclopropylmethyl-*N*-propylamine with $LiAlH_4$ (THF, reflux, 3 days) or sodium bis(2-methoxyethoxy)aluminum hydride (Red-Al) in boiling toluene for 12 h[43] removed the sulfonyl group to give back the secondary amine in 60 or 75% yield, respectively. These model reactions demonstrated that the three-membered ring would be stable to the reductive amine deprotection steps during the synthesis of **2**.

The key reaction in the preparation of (2*R*,10*S*)-$(HO)_2$CPMENSPM (**2**) was alkylation of norspermidine derivative **18** with *N*-cyclopropylmethyl-*N*-[(2*S*)-2,3-epoxypropyl]trifluoromethanesulfonamide (**16**), which was prepared according to the method of *N*-ethylamine-derived epoxide **17**.[30] The cyclopropyl terminus of analogue **2** originated from conversion of cyclopropylmethylamine hydrochloride (**6**) to *N*-cyclopropylmethyltriflamide (**12**) in 50% distilled yield using trifluoromethanesulfonic anhydride (NEt_3/CH_2Cl_2) (Scheme 3). Sulfonamide **12** was alkylated with (*S*)-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol under Mitsunobu conditions (diisopropyl azodicarboxylate, triphenylphosphine, THF) to generate adduct **13** in 96% yield. Cleavage of the ketal

Scheme 3. Synthesis of (2*R*,10*S*)-$(HO)_2$CPMENSPM (**2**)[a]



[a] Reagents: (a) Tf_2O, Et_3N, CH_2Cl_2, 50%; (b) (*S*)-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol, PPh_3, diisopropyl azodicarboxylate, THF, 96%; (c) 1 N HCl, acetone, reflux, 98%; (d) TsCl, CH_2Cl_2, pyridine, 88%; (e) K_2CO_3, CH_3OH, 74%; (f) *N*,*N'*-dibenzyl-1,3-diaminopropane, EtOH, reflux, 64%; (g) EtOH, reflux, 94%; (h) Red-Al, toluene, reflux, 60%; (h) H_2, 10% Pd-C, 1 N HCl (8 equiv), EtOH, 70%.

of **13** in refluxing 1 N HCl (acetone) gave diol **14** in 98% yield; activation of the primary hydroxyl group of **14** with tosyl chloride in pyridine led to monotosylate **15** in 88% yield. Intramolecular displacement of the tosylate by the secondary alcohol of **15** under basic conditions (K_2CO_3/CH_3OH) at room temperature furnished epoxide **16** in 74% yield.

Alkylation of *N*,*N'*-dibenzyl-1,3-diaminopropane (DBnDAP) with chiral epoxide **17** (2.0 equiv) previously resulted in fully *N*-protected (2*S*,10*S*)-$(HO)_2$DENSPM in 80% yield.[30] Heating only 0.6 equiv of electrophile **17** with DBnDAP in EtOH led to 1:1 adduct **18** as the major product (64%). The secondary amino function of **18** added efficiently to epoxide **16** at its less substituted carbon, affording masked tetraamine **19** (94%). Deprotection of **19** to analogue **2** was achieved in two stages. Removal of the trifluoromethanesulfonyls from **19** was accomplished with Red-Al in toluene at reflux,[38] furnishing (2*S*,10*S*)-N^1-cyclopropylmethyl-N^4,N^8-dibenzyl-2,10-dihydroxy-N^{11}-ethylnorspermine (**20**) in 60% yield. Hydrogenation (1 atm) in 1 M HCl (8 equiv) and EtOH over 10% Pd−C removed the *N*-benzyl moieties of **20**, giving (2*R*,10*S*)-$(HO)_2$CPMENSPM (**2**) as its tetrahydrochloride salt in 70% recrystallized yield. The overall yield was 8% for unsymmetrically dialkylated, outer-chain-dihydroxylated norspermine analogue **2**.

Table 1. L1210 Cell Growth Inhibition and Transport for DENSPM, Dihydroxylated DENSPMs, CPMENSPM, and (2*R*,10*S*)-(HO)$_2$CPMENSPM

compound abbreviation	IC$_{50}$[a] (μM)		K_i[b] (μM)
	48 h	96 h	
DENSPM[c]	20	2.0	17
(2*R*,10*R*)-(HO)$_2$DENSPM[c]	3.0	2.0	68
(2*S*,10*S*)-(HO)$_2$DENSPM[c]	1.0	0.6	43
CPMENSPM	16	0.55	13
(2*R*,10*S*)-(HO)$_2$CPMENSPM	4.5	0.97	40

[a] The IC$_{50}$ was estimated from growth curves for L1210 cells grown in the presence of nine different concentrations of drug spanning four logarithmic units: 0, 0.03, 0.1, 0.3, 1.0, 3, 10, 30, and 100 μM. IC$_{50}$ data are presented as the mean of at least two experiments with variation from the mean typically 10−25% for the 96-h IC$_{50}$ values. [b] K_i determinations were made by following analogue inhibition of spermidine transport. All polyamine analogues exhibited simple substrate-competitive inhibition of [^{3}H]SPD transport by L1210 cells. Values reported in the table represent the mean of at least two or three experiments with a variation typically <10%. [c] These results are from ref 30.

Table 2. Effect of DENSPM, Dihydroxylated DENSPMs, CPMENSPM, and (2*R*,10*S*)-(HO)$_2$CPMENSPM on Polyamine Pools in L1210 Cells

compound	concn (μM)	PUT[a]	SPD[a]	SPM[a]	analogue[b]
DENSPM[c]	10	30	14	31	1.59
	100	0	6	30	2.44
(2*R*,10*R*)-(HO)$_2$DENSPM[c]	3	9	14	63	1.88
	15	5	9	54	1.99
(2*S*,10*S*)-(HO)$_2$DENSPM[c]	1	66	46	94	1.18
	5	5	7	56	2.02
CPMENSPM	20	22	25	66	2.17 ± 0.11
	100	13	16	56	2.59 ± 0.19
(2*R*,10*S*)-(HO)$_2$CPMENSPM	4	43	48	119	1.94 ± 0.03
	20	20	32	123	3.05 ± 0.37

[a] Putrescine (PUT), spermidine (SPD), and spermine (SPM) levels after 48 h of treatment are given as percent of the polyamine found in untreated controls. Typical control values in pmol/10^6 L1210 cells are PUT = 260 ± 59, SPD = 3354 ± 361, and SPM = 658 ± 119. [b] Analogue amount is expressed as nmol/10^6 cells. Untreated L1210 cells (10^6) correspond to ~1 μL volume; therefore, the concentration can be estimated as mM. [c] These results are from ref 30.

Biological Evaluations

The biological studies encompass both in vitro and in vivo assessments of the polyamine analogues. In each instance, the findings are compared to historical data for DENSPM and its hydroxylated counterparts, (2*R*,10*R*)-(HO)$_2$DENSPM and (2*S*,10*S*)-(HO)$_2$DENSPM.[30] The in vitro measurements, conducted in L1210 cells, include IC$_{50}$ and K_i determinations (Table 1), as well as evaluations of how the analogues accumulate intracellularly (Table 2), affect the native polyamine pools (Table 2), and impact the polyamine enzymes ODC, AdoMetDC, and SSAT (Table 3). The in vivo assessment involves a comparison of the acute and chronic toxicities (Tables 4 and 5, respectively) of the parent and hydroxylated compounds in mice.

IC$_{50}$ Measurements. As with DENSPM (20 μM) and its hydroxylated analogues [(2*R*,10*R*)-(HO)$_2$DENSPM, 3.0 μM, and (2*S*,10*S*)-(HO)$_2$DENSPM, 1.0 μM] at 48 h, the hydroxylated analogue of CPMENSPM, (2*R*,10*S*)-(HO)$_2$CPMENSPM (4.5 μM), was more active than its parent (16 μM) (Table 1). At 96 h, again in a manner similar to that of the DENSPMs, the large differences in IC$_{50}$ values between hydroxylated and parent drug were absent.

Table 3. Impact of DENSPM, Dihydroxylated DENSPMs, CPMENSPM, and (2*R*,10*S*)-(HO)$_2$CPMENSPM on Ornithine Decarboxylase (ODC), *S*-Adenosylmethionine Decarboxylase (AdoMetDC), and Spermidine/Spermine-*N*^{1}-acetyltransferase (SSAT) in L1210 Cells[a]

compound	ODC	AdoMetDC	SSAT
DENSPM	7[b]	42[b]	3877
(2*R*,10*R*)-(HO)$_2$DENSPM[b]	35	59	177
(2*S*,10*S*)-(HO)$_2$DENSPM[b]	61	51	816
CPMENSPM	26	48	3258
(2*R*,10*S*)-(HO)$_2$CPMENSPM	37	59	2497

[a] Enzyme activity is expressed as percent of untreated control for ODC (1 μM at 4 h), AdoMetDC (1 μM at 6 h), and SSAT (10 μM at 48 h for all of the above analogues except DENSPM, which is 2 μM). Each experiment included a positive control, which had a known, reproducible impact on enzyme activities (mean ± SD): 1 μM DEHSPM lowered ODC to 6.7 ± 2.6% of untreated control; 1 μM DEHSPM decreased AdoMetDC to 40.7 ± 6.2% of untreated control; and 2 μM DENSPM increased SSAT to 3877 ± 76% of untreated control. Data shown in the table represent the mean of at least three experiments and have variances consistent with those suggested by the positive control data presented above. [b] These results are from ref 30.

Table 4. Survival of CD-1 Mice Treated with DENSPM, (*R*,*R*)-(HO)$_2$DENSPM, CPMENSPM, or (2*R*,10*S*)-(HO)$_2$CPMENSPM under an Acute Toxicity Regimen[a]

compound	dose (mg/kg)	no. of deaths/no. of mice injected
DENSPM[b]	325	5/10
(2*R*,10*R*)-(HO)$_2$DENSPM	325	0/6
CPMENSPM	100	1/5[c]
CPMENSPM	200	5/5[c]
(2*R*,10*S*)-(HO)$_2$CPMENSPM	100	0/5
(2*R*,10*S*)-(HO)$_2$CPMENSPM	200	0/5
(2*R*,10*S*)-(HO)$_2$CPMENSPM	400	0/5[d]

[a] The polyamine analogues were administered to female CD-1 mice at the doses indicated as a single ip injection. The mice were monitored for 2 h after administration of drug, after which scoring took place. Their overall health was then checked daily during a 10-day postdosing observational period. [b] These results are from ref 21. [c] Those animals that died did so in a matter of minutes of convulsions and asphyxiation; all mice exhibited pronounced signs of CNS toxicity, including splayed hind limbs, intention tremors, and ataxia. The survivors appeared to be healthy throughout the 10-day postdosing observational period. [d] These animals exhibited significant CNS signs during the first several hours after injection but had recovered completely by the next morning.

***K*$_i$ Studies.** The ability of CPMENSPM and (2*R*,10*S*)-(HO)$_2$CPMENSPM to compete with radiolabeled spermidine for the polyamine transport apparatus (K_i) was determined. In accord with the results for (2*R*,10*R*)-(HO)$_2$DENSPM (68 μM) and (2*S*,10*S*)-(HO)$_2$DENSPM (43 μM) versus that for DENSPM (17 μM), the K_i value for (2*R*,10*S*)-(HO)$_2$CPMENSPM (40 μM) was also higher than that of its parent drug, CPMENSPM (13 μM). The most likely explanation is that hydroxylated norspermines simply do not bind as well to the polyamine transport apparatus as do the nonhydroxylated compounds.

Impact of Analogues on Polyamine Pools. The intracellular accumulations of CPMENSPM and (2*R*,10*S*)-(HO)$_2$CPMENSPM as well as the impact of these compounds on polyamine pools in L1210 cells were measured after 48 h of incubation. Two different concentrations were used, the approximate 48-h IC$_{50}$ concentration and 5 times this value (Table 2). In the case of DENSPM and its hydroxylated analogues, at either concentration assessed, similar intracellular accumulations of the compound generated comparable

Table 5. Survival of CD-1 Mice Treated with a 5-Day Course of Hydroxylated versus Nonhydroxylated Norspermine Analogues[a]

compound	dose (mg/kg/day)	no. of days	no. of deaths/no. of mice injected
DENSPM[b]	200	6	10/12
(2*R*,10*R*)-$(HO)_2$DENSPM[c]	217	6	0/15
CPMENSPM	10	5	0/7
	25	5	0/7
	50	5	0/7
	75	5	0/5
	100	5	0/5[d]
(2*R*,10*S*)-$(HO)_2$CPMENSPM	100	5	0/6
	200	5	0/6
	300	5	0/5[e]

[a] Female CD-1 mice were administered the polyamine analogues as 1 ip dose/day for the number of days indicated. Animals were monitored daily for an additional 10 days. [b] These results are from ref 21. During the course of treatment, all of the animals displayed severe neurological signs, which included severe ataxia, intention tremors, and motor dysfunction. By day 3, two of the animals had died of seizures; by the end of the dosing period, the number of animals indicated had died from neurological sequelae. [c] The data are from ref 30. Neither weight loss nor any other adverse effects were observed. [d] Animals in the 100 mg/kg group exhibited pronounced signs of CNS toxicity, such as splayed hind limbs, tremors, and ataxia, immediately after the dose, but the mice recovered after several hours. No signs of chronic toxicity, such as weight loss or changes in coat appearance, were observed during the 5-day treatment period; all mice appeared to be healthy throughout the 10-day postdosing observational period. [e] Animals in the 300 mg/kg group exhibited mild signs of CNS toxicity, such as ataxia, immediately after the dose, but the mice completely recovered after several hours. No signs of chronic toxicity, such as weight loss or changes in coat appearance, were observed during the 5-day treatment period; all mice appeared to be healthy throughout the 10-day postdosing observational period.

impacts on polyamine pools. This was not quite the case with CPMENSPM and its hydroxylated counterpart. At its 48-h IC_{50} concentration, CPMENSPM diminished the putrescine (PUT), spermidine (SPD), and spermine (SPM) levels to 22, 25, and 66%, respectively, of control values; CPMENSPM reached an intracellular concentration of 2.17 ± 0.11 mM. At its approximate 48-h IC_{50} concentration (4 μM), (2*R*,10*S*)-$(HO)_2$CPMENSPM attained a concentration of 1.94 ± 0.03 mM and concomitantly lowered PUT and SPD to 43 and 48%, respectively, of controls, but SPM was slightly elevated (119% of control value). The reduction of polyamine pools was not as significant with (2*R*,10*S*)-$(HO)_2$CPMENSPM, particularly considering that SPM was unaffected or slightly enhanced at both drug concentrations tested.

Effect of Compounds on ODC, AdoMetDC, and SSAT. In each case, ODC and AdoMetDC measurements were made in L1210 cells after exposure to 1 μM polyamine analogue. Unlike DENSPM (7% of control value) and its hydroxylated analogues [(2*R*,10*R*)-, 35% of control value; (2*S*,10*S*)-, 61%, Table 3], there was not a substantial difference in ODC activity after treatment with (2*R*,10*S*)-$(HO)_2$CPMENSPM (37%) or CPMENSPM (26%). However, the moderate impact of both CPMENSPM (48% of control value) and (2*R*,10*S*)-$(HO)_2$-CPMENSPM (59%) on AdoMetDC resembled that of DENSPM (42%) and its hydroxylated derivatives [(2*R*,10*R*)-, 59% of control value; (2*S*,10*S*)-, 51%].

The most notable difference between the DENSPMs and the CPMENSPMs is related to their stimulation of SSAT after a 48-h incubation with 10 μM polyamine analogue. Whereas DENSPM dramatically upregulated SSAT (3877% of control value) at 2 μM, neither (2*R*,10*R*)-$(HO)_2$DENSPM (177%) nor (2*S*,10*S*)-$(HO)_2$DENSPM (816%) achieved this level of stimulation, even at 5 times this concentration. In contrast, the increase in SSAT activity elicited by CPMENSPM (3258% of control value) is analogous to that observed with DENSPM; however, unlike either of the hydroxylated DENSPMs, (2*R*,10*S*)-$(HO)_2$CPMENSPM increased SSAT activity by 2497% of control value, a level comparable to that of its parent drug.

Toxicity of CPMENSPM and (2*R*,10*S*)-$(HO)_2$-CPMENSPM in Mice. The toxicity of CPMENSPM is acute in nature, including a strong neurological component. All mice treated with CPMENSPM at an ip dose of $\geq$100 mg/kg exhibited pronounced signs of central nervous system (CNS) toxicity. These included an ataxic gait, intention tremors, and severe motor dysfunction, especially of the hindlimbs (Table 4). Those animals that died did so in a matter of minutes of convulsions and asphyxiation. The survivors appeared to be healthy throughout the 10-day postdosing observational period. When animals were treated in a chronic toxicity protocol (daily ip injections for 5 days), the only signs of toxicity were observed immediately after injection. Again, these consisted of acute CNS signs, which were moderate at 75 mg/kg and severe at 100 mg/kg. No signs of chronic toxic effects, such as weight loss or poor coat health, were observed during the treatment period or during a 10-day post-treatment followup (Table 5). In marked contrast, only minimal toxic effects (ataxia of 15−20-min duration) were observed in the five mice treated with (2*R*,10*S*)-$(HO)_2$CPMENSPM at 200 mg/kg, a dose 93% on a molar basis of that which was lethal to five of five mice treated with CPMENSPM (Table 4). Moreover, even at a dose of 400 mg/kg of (2*R*,10*S*)-$(HO)_2$CPMENSPM, there were no deaths among the five mice injected, although there were significant neurological signs at this dose. Even when the mice received (2*R*,10*S*)-$(HO)_2$CPMENSPM at doses of 100, 200, or 300 mg/kg/day under a 5-day chronic dosing regimen, there was no apparent toxicity beyond what was found during the acute study. In fact, the neurological signs that were observed in the mice treated with the hydroxylated analogue at 300 mg/kg/day were much milder than those in the animals given the nonhydroxylated compound at approximately one-third the dose (Table 5). Thus, the introduction of hydroxyls reduced the toxicity at least severalfold.

Discussion

The current synthetic route to CPMENSPM (**1**) offers a number of advantages over the previously described approach.[31] The scheme is shorter (8 versus 10 steps) and generates a higher yield (10 versus 2%), offering facile access to crystalline product. The key step involved alkylation of the anion of *N*,*N'*-bis(mesitylenesulfonyl)-*N*-cyclopropylmethyl-1,3-diaminopropane with *N*,*N'*-bis(mesitylenesulfonyl)-*N*-(3-bromopropyl)-*N'*-ethyl-1,3-diaminopropane, followed by removal of the mesitylenesulfonamide protecting groups.

The seminal step in the assembly of the hydroxylated analogue (**2**) required two different alkylating agents, *N*-[(2*S*)-2,3-epoxypropyl]-*N*-ethyltrifluoromethanesulfonamide and *N*-cyclopropylmethyl-*N*-[(2*S*)-2,3-epoxypropyl]trifluoromethanesulfonamide. The reagents were

coupled in a stepwise fashion to *N,N'*-dibenzyl-1,3-diaminopropane, and the triflyl and benzyl protecting groups were removed. Once again, the chemistry is relatively simple (nine steps) and effective, accomplished in nearly the same overall yield (8%) as that of (2*S*,10*S*)-(HO)$_2$DENSPM (10%),[30] despite potential stability problems with the cyclopropyl ring[39,40] in **2**.

In a comparison of how hydroxylation of DENSPM and CPMENSPM influences their respective biological properties, the similarities are far more common than the differences. In both instances, hydroxylation has only a minor effect on the 96-h IC_{50} values, and the K_i values are increased. Regarding the impact of the analogues on polyamine pools, when similar intracellular levels of drug are considered, hydroxylation exerts little effect on DENSPM's reduction of polyamine pools. Although hydroxylation of CPMENSPM moderately reduces the molecule's ability to diminish PUT and SPD, this modification significantly alters CPMENSPM's depletion of SPM. Hydroxylation of DENSPM also attenuates its ability to decrease ODC and AdoMetDC as well as its capability to stimulate SSAT. This was not the case with CPMENSPM hydroxylation. There was little change in ODC and AdoMetDC and only a very modest effect on SSAT stimulation.

With both DENSPM and CPMENSPM, the most interesting observation was that hydroxylation profoundly reduced the compounds' neurologic toxicity. Thus, hydroxylation of norspermine analogues may be a means for increasing their therapeutic indices. However, the question regarding the generality of this approach remains to be answered. In particular, as the lipophilicity of the molecule increases (i.e., by adding larger terminal alkyl groups), will it be necessary to likewise increase the number of appended hydroxyls to reduce toxicity? Experiments designed to define the possible relationships among hydrophobicity, the ability to penetrate the blood−brain barrier, and CNS toxicity of norspermine analogues are ongoing.

Experimental Section

General. Compound **3** was obtained from Karl Industries, Aurora, OH. Other reagents were purchased from the Aldrich Chemical Co., Milwaukee, WI. Fisher Optima-grade solvents were routinely used, and organic extracts were dried with sodium sulfate. DMF and THF were distilled, the latter from sodium and benzophenone. Reactions in nonaqueous solvents were run under a nitrogen atmosphere. Silica gel 32−63 from Selecto Scientific, Inc. (Suwanee, GA) was used for flash column chromatography. Melting points are uncorrected. NMR spectra were recorded at 300 MHz (^{1}H) or at 75 MHz (^{13}C) on a Varian Unity 300. Compounds **1** and **2** were measured in D_2O with sodium *2,2,3,3-d$_4$*-3-(trimethylsilyl)propionate (TSP, δ 0.0) for ^{1}H or dioxane (δ 67.19) for ^{13}C as references. Other spectra were run in $CDCl_3$ with tetramethylsilane (δ 0.0) for ^{1}H or the solvent (δ 77.0) for ^{13}C as standards. Coupling constants (*J*) are in hertz. Optical rotations were measured in $CHCl_3$ (unless specified) at 589 nm (sodium D line) with a Perkin-Elmer 341 polarimeter; *c* equals grams of compound per 100 mL of solution. FAB mass spectra were run in a 3-nitrobenzyl alcohol or glycerol (**1** and **2**) matrix. Elemental analyses were performed by Atlantic Microlabs, Norcross, GA.

***N,N'*-Bis(mesitylenesulfonyl)-*N*-ethyl-1,3-diaminopropane (4).** A solution of mesitylenesulfonyl chloride (85.43 g, 0.391 mol) in CH_2Cl_2 (260 mL) was added to **3** (20.07 g, 0.196 mol) in 2 N NaOH (210 mL) with ice-bath cooling. The mixture was stirred for 18 h at room temperature, the layers were separated, and the aqueous portion was extracted with $CHCl_3$ (2 × 200 mL). The combined organic layers were washed with 0.5 N HCl (200 mL) and 1:1 H_2O/brine (200 mL) and were concentrated in vacuo. Recrystallization from aqueous EtOH gave 80.57 g of **4** (88%) as a solid: mp 103.5−104 °C; ^{1}H NMR δ 0.95 (t, 3 H, *J* = 7.2), 1.68 (quintet, 2 H, *J* = 6.6), 2.30 (s, 6 H), 2.56 (s, 6 H), 2.62 (s, 6 H), 2.92 (quartet, 2 H, *J* = 6.6), 3.12 (quartet, 2 H, *J* = 7.2), 3.31 (t, 2 H, *J* = 6.6), 4.92 (t, 1 H, *J* = 6.6), 6.94 (s, 2 H), 6.95 (s, 2 H); ^{13}C NMR δ 12.51, 20.84, 22.74, 22.82, 27.73, 39.23, 39.94, 42.23, 131.87, 131.97, 133.20, 133.94, 138.82, 139.91, 141.95, 142.50; HRMS, *m/z* calcd for $C_{23}H_{35}N_2O_4S_2$ 467.2038 (M + H), found 467.2057. Anal. Calcd ($C_{23}H_{34}N_2O_4S_2$) C, H, N.

***N,N'*-Bis(mesitylenesulfonyl)-*N*-(3-bromopropyl)-*N'*-ethyl-1,3-diaminopropane (5b).** Sodium hydride (60%, 2.23 g, 55.7 mmol) was added to **4** (20.0 g, 42.9 mmol) in DMF (300 mL) and stirred for 30 min in an ice bath and for 30 min at room temperature. The solution was added slowly to 1,3-dibromopropane (104 g, 0.514 mol) in DMF (200 mL). The mixture was stirred for 18 h at room temperature. The reaction was quenched with EtOH (10 mL) and H_2O (20 mL) after 40 h, and solvents were removed in vacuo. The residue was dissolved in $CHCl_3$ (200 mL), washed with brine (200 mL), and concentrated in vacuo. Flash chromatography (15−33% EtOAc/hexane) afforded 17.8 g of **5b** (71%) as a white glassy solid: ^{1}H NMR δ 1.00 (t, 3 H, *J* = 7.2), 1.71 (quintet, 2 H, *J* = 7.2), 1.96 (quintet, 2 H, *J* = 7.2), 2.30 (s, 6 H), 2.56 (s, 6 H), 2.57 (s, 6 H), 3.01−3.09 (m, 4 H), 3.14 (quartet, 2 H, *J* = 7.2), 3.21−3.28 (m, 4 H), 6.93 (s, 2 H), 6.94 (s, 2 H); ^{13}C NMR δ 12.67, 20.91, 22.72, 22.77, 25.43, 30.18, 30.38, 40.12, 42.54, 43.81, 44.35, 131.90, 132.01, 132.71, 133.09, 140.04, 140.09, 142.37, 142.68; HRMS, *m/z* calcd for $C_{26}H_{40}BrN_2O_4S_2$ 587.1613 (M + H), found 587.1603.

***N*-Cyclopropylmethylmesitylenesulfonamide (7).** Mesitylenesulfonyl chloride (22.4 g, 0.102 mol), **6** (10.0 g, 0.093 mol), and 1 N NaOH (250 mL) were reacted according to the method of **4**. Recrystallization of the crude product from EtOH gave 23.0 g of **7** (97%) as a solid: mp 69−70 °C; ^{1}H NMR δ 0.04−0.11 (m, 2 H), 0.42−0.50 (m, 2 H), 0.79−0.94 (m, 1 H), 2.30 (s, 3 H), 2.65 (s, 6 H), 2.76 (dd, 2 H, *J* = 6.9, 6.0), 4.57 (br s, 1 H), 6.96 (s, 2 H); ^{13}C NMR δ 3.45, 10.59, 20.89, 22.89, 47.76, 131.87, 133.69, 139.00, 142.05; HRMS, *m/z* calcd for $C_{13}H_{20}NO_2S$ 254.1215 (M + H), found 254.1198. Anal. Calcd ($C_{13}H_{19}NO_2S$) C, H, N.

***N*-Cyclopropylmethyl-*N*-(3-phthalimidopropyl)mesitylenesulfonamide (8).** Sodium hydride (60%, 4.74 g, 0.118 mol) was added to **7** (20.0 g, 78.9 mmol) in DMF (300 mL) under ice-bath conditions. The mixture was stirred for 30 min and then at room temperature for an additional 30 min. *N*-(3-Bromopropyl)phthalimide (31.7 g, 0.118 mol) was added followed by heating at 40 °C for 4 h. The reaction was quenched with EtOH (10 mL) and H_2O (20 mL), and solvents were removed in vacuo. The residue was dissolved in $CHCl_3$ (1 L) and washed with H_2O (500 mL). Flash chromatography (4% acetone/toluene) gave 29.2 g of **8** (84%) as a white solid: mp 93−96 °C; ^{1}H NMR δ 0.08−0.16 (m, 2 H), 0.45−0.53 (m, 2 H), 0.85−1.00 (m, 1 H), 1.84 (quintet, 2 H, *J* = 7.5), 2.25 (s, 3 H), 2.55 (s, 6 H), 3.12 (d, 2 H, *J* = 7.2), 3.32 (t, 2 H, *J* = 7.5), 3.54 (t, 2 H, *J* = 7.5), 6.83 (s, 2 H), 7.70−7.78 (m, 2 H), 7.80−7.88 (m, 2 H); ^{13}C NMR δ 3.93, 9.31, 20.92, 22.68, 26.51, 35.33, 43.32, 50.13, 123.17, 131.80, 131.92, 132.79, 133.96, 140.08, 142.31, 168.06; HRMS, *m/z* calcd for $C_{24}H_{29}N_2O_4S$ 441.1848 (M + H), found 441.1816.

***N*-(3-Aminopropyl)-*N*-cyclopropylmethylmesitylenesulfonamide (9).** Hydrazine hydrate (55%, 3.46 g, 59.4 mmol) and **8** (11.9 g, 27.0 mmol) were heated at reflux in EtOH (270 mL) for 12 h, the mixture was filtered through Celite, and solvents were removed in vacuo. Flash chromatography (2% concentrated NH_4OH/CH_3OH) afforded 7.3 g of **9** (87%) as a thick oil: ^{1}H NMR δ 0.03−0.10 (m, 2 H), 0.43−0.51 (m, 2 H), 0.79−0.93 (m, 1 H), 1.70 (quintet, 2 H, *J* = 7), 1.86 (br s, 2 H), 2.29 (s, 3 H), 2.60 (s, 6 H), 2.65 (t, 2 H, *J* = 7), 3.03 (d, 2 H, *J* = 7), 3.40 (t, 2 H, *J* = 7), 6.93 (s, 2 H); ^{13}C NMR δ 3.83, 8.99, 20.90, 22.75, 30.57, 39.08, 43.10, 49.84, 131.84, 133.24, 140.11,

142.25; HRMS, *m/z* calcd for $C_{16}H_{27}N_2O_2S$ 311.1793 (M + H), found 311.1776.

***N,N*-Bis(mesitylenesulfonyl)-*N*-cyclopropylmethyl-1,3-diaminopropane (10).** Mesitylenesulfonyl chloride (7.75 g, 35.4 mmol), **9** (10.0 g, 32.2 mmol), and 1 N NaOH (50 mL) were reacted according to the method of **4**. Flash chromatography (8% acetone/toluene) generated 13.4 g of **10** (84%) as a white solid: mp 92−94 °C; ^{1}H NMR δ −0.06 to +0.05 (m, 2 H), 0.39−0.48 (m, 2 H), 0.67−0.80 (m, 1 H), 1.72 (quintet, 2 H, *J* = 6.3), 2.30 (s, 6 H), 2.57 (s, 6 H), 2.62 (s, 6 H), 2.87−2.96 (m, 4 H), 3.45 (t, 2 H, *J* = 6.3), 4.95 (t, 1 H, *J* = 6.3), 6.93 (s, 2 H), 6.95 (s, 2 H); ^{13}C NMR δ 3.85, 8.89, 20.94, 22.79, 22.87, 27.71, 39.24, 42.93, 50.01, 131.91, 132.00, 133.15, 134.02, 138.87, 140.05, 141.97, 142.56; HRMS, *m/z* calcd for $C_{25}H_{37}N_2O_4S_2$ 493.2195 (M + H), found 493.2132.

***N*¹-Cyclopropylmethyl-*N*¹¹-ethyl-*N*¹,*N*⁴,*N*⁸,*N*¹¹-tetrakis-(mesitylenesulfonyl)norspermine (11).**[31] Sodium hydride (60%, 0.39 g, 9.8 mmol) was added to **10** (3.7 g, 7.5 mmol) in DMF (50 mL) under ice-bath conditions. The mixture was stirred for an additional 30 min each at 0 °C and at room temperature and was added dropwise to **5b** (5.3 g, 9.0 mmol) in DMF (75 mL). The mixture was stirred overnight at room temperature and was quenched under ice-bath conditions with EtOH (10 mL) and H_2O (10 mL). The solvents were removed in vacuo, and the concentrate was taken up in $CHCl_3$ (50 mL) and H_2O (50 mL). The chloroform layer was washed with brine (50 mL); the solution was concentrated in vacuo. Flash chromatography (3:1 hexane/EtOAc) gave 5.47 g of **10** (73%) as a glassy white solid: ^{1}H NMR δ −0.08 to −0.02 (m, 2 H), 0.38−0.45 (m, 2 H), 0.64−0.78 (m, 1 H), 0.95 (t, 3 H, *J* = 7.2), 1.56−1.73 (m, 6 H), 2.29 (s, 12 H), 2.53 (s, 12 H), 2.54 (s, 12 H), 2.86 (d, 2 H, *J* = 6.6), 2.91−3.17 (m, 14 H), 6.93 (s, 8 H); ^{13}C NMR δ 3.77, 8.90, 12.61, 20.90, 22.68, 22.69, 22.74, 22.76, 25.08, 25.18, 25.28, 40.04, 42.49, 43.20, 49.92, 131.88, 131.96, 132.91, 132.96, 133.02, 133.10, 140.00, 140.02, 140.03, 142.32, 142.49, 142.52; HRMS, *m/z* calcd for $C_{51}H_{75}N_4O_8S_4$ 999.4468 (M + H), found 999.4382.

***N*¹-Cyclopropylmethyl-*N*¹¹-ethylnorspermine Tetrahydrochloride (1).**[31] Hydrogen bromide in HOAc (30%, 50 mL) was added to **11** (5.3 g, 5.3 mmol) and phenol (19.9 g, 0.212 mol) in CH_2Cl_2 (50 mL) at 0 °C. The reaction mixture was stirred overnight at room temperature and was quenched with H_2O (100 mL) under ice-bath conditions. The aqueous layer was washed with CH_2Cl_2 (4 × 100 mL), and the former was concentrated in vacuo. The residue was treated with 1 N NaOH (20 mL) and 19 N NaOH (10 mL) under ice-bath cooling and was extracted with $CHCl_3$ (4 × 50 mL). After concentration of the $CHCl_3$ extracts, the oil was dissolved in EtOH (50 mL) and acidified with concentrated HCl (10 mL). Solvent removal in vacuo followed by recrystallization from aqueous EtOH gave 0.800 g of **1** (36%) as white flakes: mp >290 °C; ^{1}H NMR δ 0.34−0.41 (m, 2 H), 0.66−0.74 (m, 2 H), 1.02−1.17 (m, 1 H), 1.30 (t, 3 H, *J* = 7.5), 2.07−2.21 (m, 6 H), 2.98 (d, 2 H, *J* = 7.5), 3.08−3.26 (m, 14 H); ^{13}C NMR δ 4.04, 7.22, 11.14, 23.34, 43.71, 44.47, 45.22, 45.31, 53.29; HRMS, *m/z* calcd for $C_{15}H_{35}N_4$ 271.2862 (M + H), found 271.2861. Anal. Calcd ($C_{15}H_{38}Cl_4N_4$) C, H, N.

***N*-Cyclopropylmethyltrifluoromethanesulfonamide (12).** Trifluoromethanesulfonic anhydride (27.00 g, 95.7 mmol) in CH_2Cl_2 (50 mL) was added dropwise to **6** (10.30 g, 95.7 mmol) and Et_3N (40 mL, 0.29 mol) in CH_2Cl_2 (100 mL) at −78 °C, and the reaction mixture was stirred overnight at room temperature. The reaction solution was washed with 1 N HCl (3 × 100 mL) and brine and was concentrated under reduced pressure. Vacuum distillation afforded 9.74 g of **12** (50%): bp 81−84 °C at 2.6 mmHg; ^{1}H NMR δ 0.27 (m, 2 H), 0.63 (m, 2 H), 1.08 (m, 1 H), 3.17 (t, 2 H, *J* = 6.3), 4.91 (br, 1 H).

***N*-Cyclopropylmethyl-*N*-[(4*S*)-2,2-dimethyl-1,3-dioxolan-4-ylmethyl]trifluoromethanesulfonamide (13).** (*S*)-(+)-2,2-Dimethyl-1,3-dioxolane-4-methanol (2.64 g, 20.0 mmol) and triphenylphosphine (6.29 g, 24.0 mmol) were added to **12** (4.06 g, 20.0 mmol) in THF (100 mL). Diisopropyl azodicarboxylate (4.85 g, 24.0 mmol) in THF (40 mL) was added dropwise at room temperature, and the reaction mixture was stirred overnight. The solvent was removed in vacuo, and the crude product was purified by flash chromatography ($CHCl_3$) to give 6.10 g of **13** (96%) as a colorless oil: ^{1}H NMR δ 0.27 (m, 1 H), 0.35 (m, 1 H), 0.60 (m, 2 H), 1.04 (m, 1 H), 1.32 (s, 3 H), 1.40 (s, 3 H), 3.43 (m, 1 H), 3.51 (dd, 2 H, *J* = 15.0, 7.2), 3.65 (dd, 1 H, *J* = 8.7, 6.3), 3.69 (m, 1 H), 4.08 (dd, 1 H, *J* = 8.7, 6.3), 4.31 (m, 1 H); ^{13}C NMR δ 3.39, 4.58, 9.21, 25.25, 26.67, 54.22, 67.16, 75.26, 77.20, 110.06, 120.01 (q, *J* = 328); HRMS, *m/z* calcd for $C_{11}H_{19}F_3NO_4S$ 318.0987 (M + H), found 318.0985; $[\alpha]^{25}_D$ −20.1 (*c* 2.33).

***N*-Cyclopropylmethyl-*N*-[(2*S*)-2,3-dihydroxypropyl]trifluoromethanesulfonamide (14).** A solution of **13** (5.91 g, 18.6 mmol) in acetone (40 mL) and 1 N HCl (80 mL) was heated at reflux for 2 h. The solvent was removed in vacuo, and the residue was purified by flash chromatography (15:1 $CHCl_3/CH_3OH$) to give 5.07 g of **14** (98%) as an oil: ^{1}H NMR δ 0.24 (m, 2 H), 0.57 (m, 2 H), 0.97 (m, 1 H), 2.60 (s, 2 H), 3.30 (s, 2 H), 3.52 (m, 3 H), 3.67 (dd, 1 H, *J* = 11.7, 3.6), 3.93 (m, 1 H); ^{13}C NMR δ 4.11, 9.42, 50.00, 54.69, 63.54, 70.03, 120.05 (q, *J* = 323); HRMS, *m/z* calcd for $C_8H_{15}F_3NO_4S$ 278.0674 (M + H), found 278.0673; $[\alpha]^{24}_D$ −4.94 (*c* 1.74).

***N*-Cyclopropylmethyl-*N*-[(2*S*)-2-hydroxy-3-(*p*-toluenesulfonato)propyl]trifluoromethanesulfonamide (15).** *p*-Toluenesulfonyl chloride (3.70 g, 19.4 mmol) in anhydrous CH_2Cl_2 (20 mL) was added dropwise to **14** (4.89 g, 17.7 mmol) in anhydrous pyridine (25 mL) at 0 °C. After the reaction mixture had been stirred at room temperature overnight, it was poured into a slurry of 1 N HCl (300 mL) and ice and then extracted with $CHCl_3$ (300 mL). The organic layer was washed with H_2O (300 mL) and brine (300 mL), concentrated under reduced pressure, and purified by flash chromatography (40:1 $CHCl_3/CH_3OH$) to give 6.70 g of **15** (88%) as an oil: ^{1}H NMR δ 0.28 (m, 2 H), 0.61 (m, 2 H), 0.98 (m, 1 H), 2.44 (s, 3 H), 2.63 (br, 1 H), 3.33 (m, 2 H), 3.49 (dd, 1 H, *J* = 15.3, 7.8), 3.58 (dd, 1 H, *J* = 15.3, 4.2), 4.02 (m, 2 H), 4.14 (m, 1 H), 7.35 (d, 2 H, *J* = 8.1), 7.78 (d, 2 H, *J* = 8.1); ^{13}C NMR δ 3.96, 4.27, 9.32, 21.65, 49.94, 54.90, 68.48, 71.03, 119.97 (q, *J* = 323), 127.96, 130.06, 132.09, 145.47; HRMS, *m/z* calcd for $C_{15}H_{21}F_3NO_6S_2$ 432.0762 (M + H), found 432.0765; $[\alpha]^{22}_D$ −3.83 (*c* 1.07).

***N*-Cyclopropylmethyl-*N*-[(2*S*)-2,3-epoxypropyl]trifluoromethanesulfonamide (16).** A suspension of **15** (6.59 g, 15.3 mmol) and K_2CO_3 (2.24 g, 16.2 mmol) in CH_3OH (100 mL) was stirred at room temperature for 4 h. Solids were filtered, and brine (100 mL), water (150 mL), and CH_2Cl_2 (150 mL) were added to the filtrate. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 100 mL). The organic phase was evaporated under reduced pressure and purified by flash chromatography (4:1 cyclohexane/EtOAc) to give 2.93 g of **16** (74%) as an oil: ^{1}H NMR δ 0.27 (m, 1 H), 0.35 (m, 1 H), 0.60 (m, 2 H), 1.07 (m, 1 H), 2.56 (dd, 1 H, *J* = 4.5, 2.4), 2.82 (t, 1 H, *J* = 4.5), 3.16 (m, 1 H), 3.31 (m, 2 H), 3.43 (m, 1 H), 3.95 (dd, 1 H, *J* = 15.0, 3.0); ^{13}C NMR δ 3.49, 4.52, 9.45, 44.67, 50.54, 54.22, 119.99 (q, *J* = 323); HRMS, *m/z* calcd for $C_8H_{13}F_3NO_3S$ (M + H), 260.0568, found 260.0560; $[\alpha]^{22}_D$ −32.1 (*c* 1.00).

(2*R*)-*N*¹,*N*⁷-Dibenzyl-*N*¹-ethyl-2-hydroxy-*N*¹-trifluoromethanesulfonylnorspermidine (18). A solution of **17** (11.36 g, 50.89 mmol)[30] and *N,N*-dibenzyl-1,3-diaminopropane (21.80 g, 85.70 mmol)[42] in EtOH (200 mL) was heated at reflux overnight, and the solvent was removed under reduced pressure. The residue was purified using flash chromatography (60:39.5:0.5 toluene/acetone/concentrated NH_4OH) to afford 15.80 g of **18** (64%) as an oil: ^{1}H NMR δ 1.13 (t, 3 H, *J* = 7.2), 1.64 (m, 2 H), 2.37 (m, 2 H), 2.52 (m, 1 H), 2.65 (m, 3 H), 3.17 (m, 2 H), 3.40 (d, 1 H, *J* = 13.5), 3.46 (m, 2 H), 3.64 (d, 1 H, *J* = 13.5), 3.68 (s, 2 H), 3.73 (m, 1 H), 5.31 (br s, 1 H), 7.20 (m, 10 H); ^{13}C NMR δ 13.49, 26.86, 44.80, 47.06, 51.19, 52.24, 53.97, 57.63, 59.46, 68.21, 120.00 (q, *J* = 324), 127.03, 127.21, 128.28, 128.37, 128.41, 129.00, 138.64, 139.73; HRMS, *m/z* calcd for $C_{23}H_{33}F_3N_3O_3S$ 488.2195 (M + H), found 488.2206; $[\alpha]^{24}_D$ +12.6 (*c* 1.00).

(2*R*,10*R*)-*N*[1],*N*[11]-Bis(trifluoromethanesulfonyl)-*N*[1]-cyclopropylmethyl-*N*[4],*N*[8]-dibenzyl-2,10-dihydroxy-*N*[11]-ethylnorspermine (19). A solution of **16** (2.82 g, 10.9 mmol) and **18** (5.31 g, 10.9 mmol) in EtOH was heated at reflux for 3 days. The reaction mixture was concentrated under reduced pressure, and the residue was purified by flash chromatography (3:1 then 1:1 cyclohexane/EtOAc) providing 7.67 g of **19** (94%) as a viscous oil: ^{1}H NMR δ 0.27 (m, 2 H), 0.54 (m, 2 H), 1.00 (m, 1 H), 1.20 (t, 3 H, $J = 7.2$), 1.67 (m, 2 H), 2.39 (m, 6 H), 2.57 (m, 2 H), 3.14 (m, 1 H), 3.38 (m, 7 H), 3.52 (m, 4 H), 3.75 (m, 4 H), 7.17 (m, 4 H), 7.27 (m, 6 H); ^{13}C NMR δ 3.46, 4.37, 9.17, 13.52, 24.27, 45.14, 51.28, 51.82, 54.31, 57.14, 57.30, 58.84, 67.24, 120.02 (q, $J = 320$), 127.45, 128.50, 129.09, 137.81; HRMS, *m/z* calcd for $C_{31}H_{45}F_6N_4O_6S_2$ 747.2685 (M + H), found 747.2687; $[\alpha]^{22}_D$ +30.5 (*c* 1.30).

(2*S*,10*S*)-*N*[1]-Cyclopropylmethyl-*N*[4],*N*[8]-dibenzyl-2,10-dihydroxy-*N*[11]-ethylnorspermine (20). Red-Al (65% in toluene, 19.3 mL, 64.2 mmol) was added slowly to a solution of **19** (6.00 g, 8.04 mmol) in toluene (300 mL), and the reaction mixture was heated at reflux overnight. The reaction mixture was cautiously quenched with H_2O (100 mL) and 10% NaOH (100 mL) at room temperature. The layers were separated, and the aqueous portion was extracted with CH_2Cl_2 (3 × 200 mL). The combined organic layers were washed with brine (500 mL) and concentrated under reduced pressure. Purification using flash chromatography (20:1 and then 10:1 CH_3CN/concentrated NH_4OH) furnished 2.32 g of **20** (60%) as an oil: ^{1}H NMR δ 0.08 (m, 2 H), 0.44 (m, 2 H), 0.91 (m, 1 H), 1.07 (t, 3 H, $J = 7.2$), 1.64 (quintet, 2 H, $J = 6.9$), 2.43 (m, 12 H), 2.60 (m, 4 H), 3.00 (br s, 4 H), 3.45 (d, 2 H, $J = 13.5$), 3.64 (d, 2 H, $J = 13.5$), 3.79 (m, 2 H), 7.23 (m, 10 H); ^{13}C NMR δ 2.85, 2.89, 10.64, 14.67, 23.91, 43.59, 51.53, 53.16, 54.47, 57.78, 58.46, 66.30, 66.39, 126.51, 127.74, 128.47, 135.25; HRMS, *m/z* calcd for $C_{29}H_{47}N_4O_2$ 483.3699 (M + H), found 483.3702; $[\alpha]^{24}_D$ +57.6 (*c* 1.18).

(2*R*,10*S*)-*N*[1]-Cyclopropylmethyl-*N*[11]-ethyl-2,10-dihydroxynorspermine Tetrahydrochloride (2). A solution of **20** (2.32 g, 4.81 mmol) in EtOH (200 mL) and 1 N HCl (38 mL) was degassed with N_2, and 10% Pd−C (0.66 g) was introduced. After the flask had been purged with N_2 (three times), the reaction mixture was stirred overnight under an H_2 atmosphere (1 atm). Solids were filtered through Celite, and the filtrate was concentrated under reduced pressure. The residue was recrystallized from aqueous EtOH to give 1.50 g of **2** (70%) as white crystals: ^{1}H NMR δ 0.39 (m, 2 H), 0.71 (m, 2 H), 1.11 (m, 1 H), 1.32 (t, 3 H, $J = 7.2$), 2.20 (m, 2 H), 3.19 (m, 16 H), 4.33 (m, 2 H); ^{13}C NMR δ 4.11, 7.05, 10.98, 23.01, 43.93, 45.38, 49.91, 49.99, 50.73, 50.79, 53.40, 63.52; HRMS, *m/z* calcd for $C_{15}H_{35}N_4O_2$ 303.2760 (M + H), found 303.2762; $[\alpha]^{24}_D$ −1.6 (*c* 1.00, 1 N HCl). Anal. Calcd ($C_{15}H_{38}Cl_4N_4O_2$) C, H, N.

Cell Culture. Murine L1210 leukemia cells were maintained in logarithmic growth as a suspension culture in RPMI-1640 medium (Gibco) containing 10% fetal bovine serum (Sigma), 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 during logarithmic growth (0.5−1.0 × 10^5 cells/mL) with the polyamine derivatives, reseeded, and incubated as described previously.[30] Cell counting and calculation of percent of control growth were also carried out as given in an earlier publication.[30] 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. During logarithmic growth, cells were treated with the polyamine derivatives. At the end of the treatment period, cell suspensions were sampled, washed twice in ice-cold, incomplete medium, pelleted for extraction using 0.6 N perchloric acid,[21] and then freeze-fractured in liquid nitrogen/hot water three times. Each supernatant was frozen at −20 °C until analysis of polyamine content by HPLC.[26]

Uptake Determinations. The polyamine derivatives were studied for their ability to compete with [^{3}H]SPD for uptake into L1210 leukemia cell suspensions in vitro as given in detail in previous publications.[12,21,30] Briefly, cell suspensions were incubated in 1 mL of culture medium containing radiolabeled SPD alone or radiolabeled SPD in the presence of graduated concentrations of polyamine analogue. At the end of the incubation period, the tubes were centrifuged; the pellet was washed, digested, and neutralized prior to scintillation counting. Lineweaver−Burk 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[44] and Pegg and Pösö,[45] respectively, on the basis of quantitation of $^{14}CO_2$ released from [^{14}C]carboxyl-labeled L-ornithine or *S*-adenosyl-L-methionine. Included in each assay were untreated L1210 cells as negative controls as well as cells treated with DEHSPM, a drug having a known reproducible effect on each enzyme, as positive controls.

Spermidine/spermine *N*[1]-acetyltransferase activity was based on quantitation of [^{14}C]-*N*[1]-acetylspermidine formed by acetylation of SPD with [^{14}C]-acetyl coenzyme A according to the method of Libby et al.[18] Cells treated with DENSPM were positive controls.

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.[27] The animals were scored 2 h after administration of drug; survivors were observed for a period of 10 days after the treatment. On the basis of 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.

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