Synthesis and Evaluation of Hydroxylated Polyamine Analogues as Antiproliferatives

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The synthesis of four hydroxylated polyamine analogues, (2*R*,10*R*)-N^1,N^{11}-diethyl-2,10-dihydroxynorspermine, (2*S*,10*S*)-N^1,N^{11}-diethyl-2,10-dihydroxynorspermine, (3*S*,12*S*)-N^1,N^{14}-diethyl-3,12-dihydroxyhomospermine, and (3*R*,12*R*)-N^1,N^{14}-diethyl-3,12-dihydroxyhomospermine, is described along with their impact on the growth and polyamine metabolism of L1210 murine leukemia cells. Four different synthetic approaches are set forth, two each for the hydroxylated norspermines and for the hydroxylated homospermines. The key step in the assembly of the norspermines was the coupling of either *N*-[(2*R*)-2,3-epoxypropyl]-*N*-ethyl *p*-toluenesulfonamide or *N*-[(2*S*)-2,3-epoxypropyl]-*N*-ethyl trifluoromethanesulfonamide to *N*,*N*′-dibenzyl-1,3-diaminopropane. The key step with homospermines employed alkylation of putrescine with (3*S*)-*N*-(benzyloxycarbonyl)-*N*-ethyl-3,4-epoxybutylamine or of *N*,*N*′-bis(mesitylenesulfonyl)-1,4-butanediamine with (2*R*)-2-benzyloxy-4-[*N*-(mesitylenesulfonyl)ethylamino]-*O*-tosyl-1-butanol. All of the hydroxylated analogues were active against L1210 cells with 96-h IC_{50} values of ≤2 μM, and they also effectively reduced putrescine and spermidine, although the effect on spermine pools ranged from moderate to insignificant. Interestingly, the impact of the hydroxylated analogues on ornithine decarboxylase (ODC) was significantly less than that of unhydroxylated parent drug (e.g., N^1,N^{11}-diethylnorspermine [DENSPM]) at 1 μM; however, *S*-adenosylmethionine decarboxylase (AdoMetDC) depletion was nearly identical to what was observed in cells treated with parent drug. The most notable difference between the parent and hydroxylated analogues was seen with spermidine/spermine N^1-acetyltransferase (SSAT) upregulation in the DENSPM series. The hydroxylated analogues, especially (*R*,*R*)-$(HO)_2$-DENSPM, were much less effective at upregulation than the parent DENSPM. Finally, a comparison of the toxicity of (*R*,*R*)-$(HO)_2$DENSPM with that of DENSPM at subchronic doses revealed that the neurological effects seen with DENSPM were now absent.

Introduction

It is now clear that *N*-alkylated polyamines exhibit antineoplastic activity against a number of murine and human tumor lines both in vitro and in vivo.[1,2] The currently ongoing phase II clinical trials with diethylnorspermine has further underscored interest in these molecules. Although it is certainly well-established that these polyamine analogues utilize the polyamine transport apparatus for incorporation into cells,[3,4] deplete polyamine pools,[5] drastically reduce the level of ornithine decarboxylase (ODC)[6,7] and *S*-adenosylmethionine decarboxylase (AdoMetDC) activities,[8] and in some cases upregulate spermidine/spermine N^1-acetyltransferase (SSAT),[9−12] the precise mechanism by which they induce death in tumor cells still remains somewhat of a mystery.[13] A systematic structure−activity study has shown that very small structural alterations in these polyamine analogues can cause pronounced changes in their biological activity at both the cellular and whole animal level.[5] This is exemplified by the differences in how various analogues compete for transport, as well as how they impact on key polyamine biosynthetic enzymes. For example, while the K_i values of DESPM and DEHSPM for the polyamine transport system are within error of each other, the DENSPM value is over 10 times as great.[14] Although the tetraamines N^1,N^{12}-diethylspermine (DESPM), N^1,N^{11}-diethylnorspermine (DENSPM), and N^1,N^{14}-diethylhomospermine (DEHSPM) suppress ODC and AdoMetDC to about the same level at equimolar concentrations, the impact of both DESPM and DEHSPM on cell growth is much faster than that observed for DENSPM. At a cellular level, the most profound differences between the three analogues are related to their ability to stimulate SSAT.[10−12] The tetraamine DENSPM upregulates SSAT by 15-fold in L1210 murine leukemia cells, while DESPM and DEHSPM stimulate SSAT by 4.6- and 1.4-fold, respectively.[10] The SSAT stimulation is even more notable in other cell lines.[15,16]

At the whole animal level, small structural alterations also cause a significant difference in pharmacologic behavior. DEHSPM is a potent antidiarrheal, while DENSPM has little antitransit activity.[17] DEHSPM also decreases blood pressure in animals, while neither DENSPM[18] nor DESPM possesses this property. In whole animals, all three analogues are first metabolized by *N*-deethylation to the corresponding tetraamine.[14,19,20]

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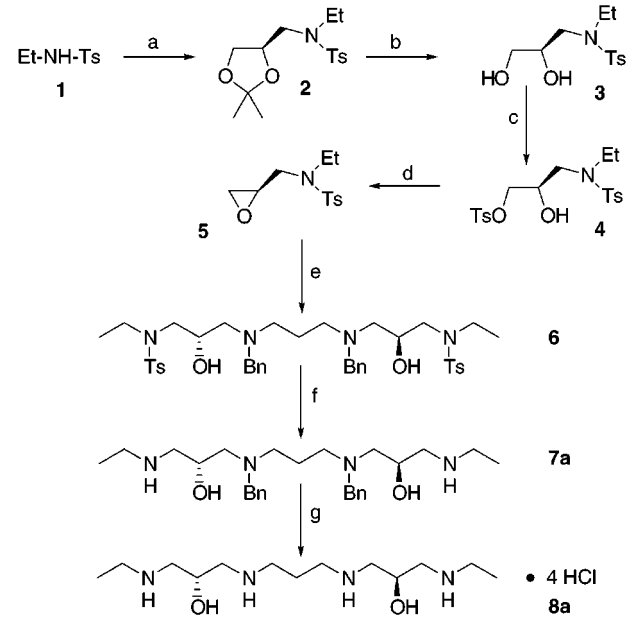
Scheme 1. Synthesis of (*R*,*R*)-(HO)$_2$DENSPM **(8a)** Using Tosylamide **1**[a]



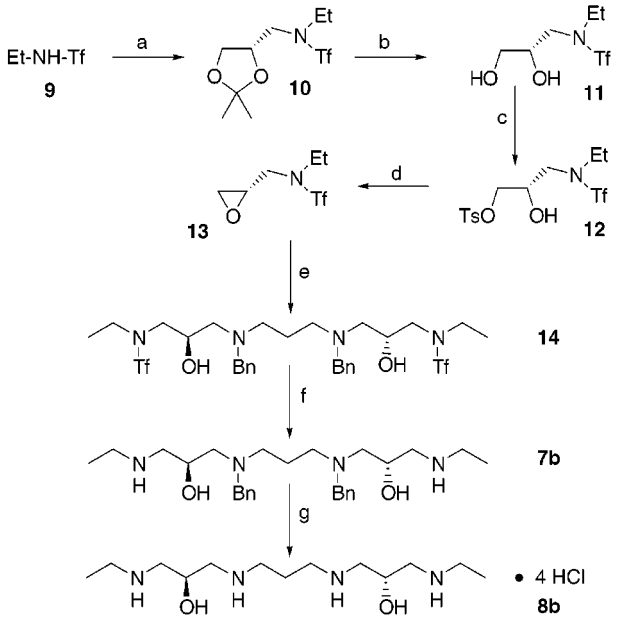
[a] Reagents: (a) (*S*)-(−)-4-(chloromethyl)-2,2-dimethyl-1,3-dioxolane, NaH, NaI, 15-crown-5, DMF, 80 °C, 44%; (b) 1 N HCl, acetone, 95 °C, 93%; (c) TsCl, pyridine, 67%; (d) K_2CO_3, MeOH, 92%; (e) *N*,*N*′-dibenzyl-1,3-diaminopropane, EtOH, 78 °C, 84%; (f) Na/naphthalene, DME, −78 °C, 50%; (g) H_2, Pd−C, HCl, EtOH, 82%.

Spermine and norspermine are then processed by deaminopropylation[14,19] through the polyamine metabolic network. However, because of the presence of the aminobutyl fragments in homospermine,[20] it is not terminally *N*-acylated and cannot be further catabolized. Thus in animals it remains unmetabolized for a protracted period of time. These differences in susceptibility to metabolic degradation correlate to the toxicity profiles: DENSPM is the least toxic,[19] DEHSPM the most.[20] While DENSPM's chronic dose-limiting toxicity is gastrointestinal, its subacute toxicity is neurological. When treated with 200 mg/kg ip for 6 days, a very high dose, animals developed severe neurological signs including pronounced ataxia, intention tremors, and motor dysfunction. This dose, with allometric scaling, is nearly 10 times higher than that given to patients. While it seemed this would be an unlikely problem in the clinic, it nevertheless represented an easily observable side effect to help evaluate how structural alterations in the polyamine backbone impact toxicity. Thus, this paper focuses on methods for the synthesis of chiral bis(hydroxy)polyamine analogues and the effect of this hydroxylation on the compounds' activity against L1210 cells in cell culture and on animal toxicity.

Synthesis

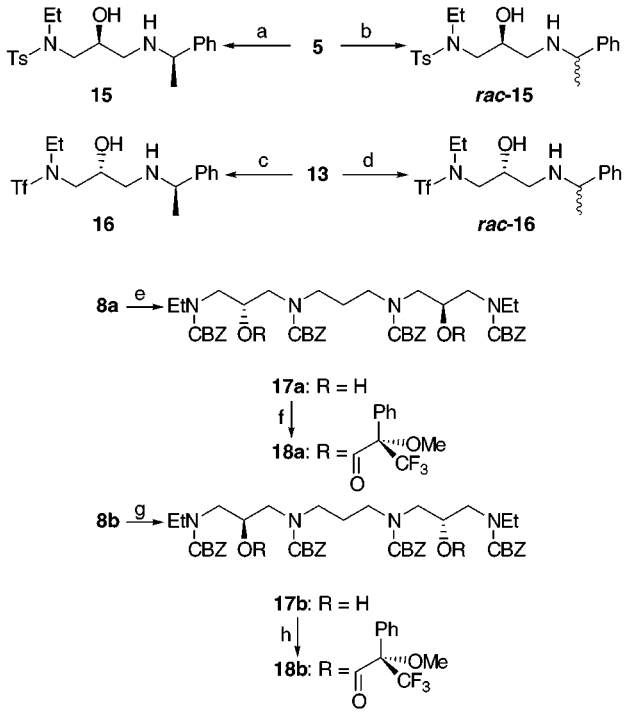
Four different hydroxylated tetraamines were synthesized: (*R*,*R*)- and (*S*,*S*)-N^1,N^{11}-diethyl-2,10-dihydroxynorspermine [(HO)$_2$DENSPM, **8a** in Scheme 1 and **8b** in Scheme 2, respectively] and (*S*,*S*)- and (*R*,*R*)-N^1,N^{14}-diethyl-3,12-dihydroxyhomospermine [(HO)$_2$DEHSPM, **29a** in Scheme 5 and **29b** in Scheme 6, respectively]. The key step in both (HO)$_2$DENSPM cases is the addition of *N*,*N*′-dibenzyl-1,3-diaminopropane[21] to 2 equiv of an appropriately substituted epoxide (**5** in Scheme 1 and **13** in Scheme 2). The synthetic sequence for the preparation of (*R*,*R*)-(HO)$_2$DENSPM (**8a**) began with the reaction of tosylamide **1** with (*S*)-(−)-4-(chloromethyl)-2,2-dimethyl-1,3-dioxolane to produce **2** in

Scheme 2. Synthesis of (*S*,*S*)-(HO)$_2$DENSPM **(8b)** Using Triflamide **9**[a]



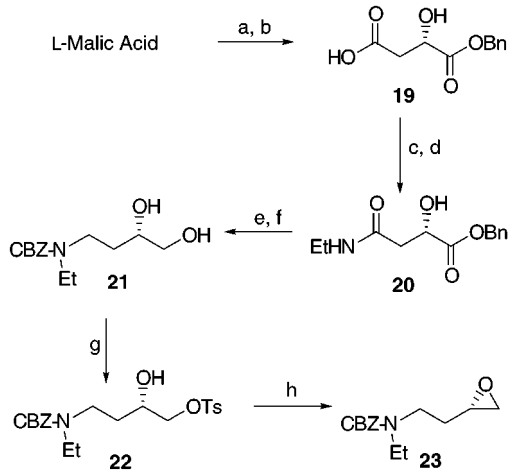
[a] Reagents: (a) (*S*)-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol, PPh_3, diisopropyl azodicarboxylate, 82%; (b) 1 N HCl, acetone, 95 °C, 87%; (c) TsCl, pyridine, 68%; (d) K_2CO_3, MeOH, 68%; (e) *N*,*N*′-dibenzyl-1,3-diaminopropane, EtOH, 78 °C, 80%; (f) $LiAlH_4$, THF, 58%; (g) H_2, Pd−C, HCl, 68%.

Scheme 3. Check of Conservation of Optical Purity for Both (HO)$_2$DENSPM Syntheses[a]



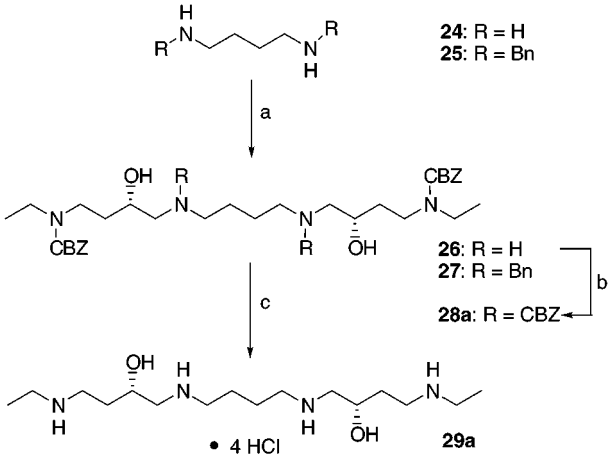
[a] Reagents: (a) (*R*)-(+)-1-phenylethylamine, EtOH, 78 °C, 47%; (b) *rac*-1-phenylethylamine, EtOH, 78 °C, 77%; (c) (*R*)-(+)-1-phenylethylamine, EtOH, 78 °C, 79%; (d) *rac*-1-phenylethylamine, EtOH, 78 °C, 81%; (e) *N*-(benzyloxycarbonyloxy)succinimide, $KHCO_3$, Et_2O(aq), 54%; (f) (*R*)-(−)-Mosher's acid chloride, pyridine, 100%; (g) e, 72%; (h) f, 94%.

Scheme 4. Synthesis of Epoxide **23**[a]



[a] Reagents: (a,b) TFAA,[34] then BnOH,[33] 100%; (c,d) *N*-hydroxysuccinimide, DCC, then ethylamine in THF, 64%; (e,f) BH_3−THF, then *N*-(benzyloxycarbonyloxy)succinimide, 33%; (g) TsCl, pyridine, 72%; (h) K_2CO_3, MeOH, 86%.

Scheme 5. Synthesis of (*S*,*S*)-$(HO)_2$DEHSPM (**29a**)[a]

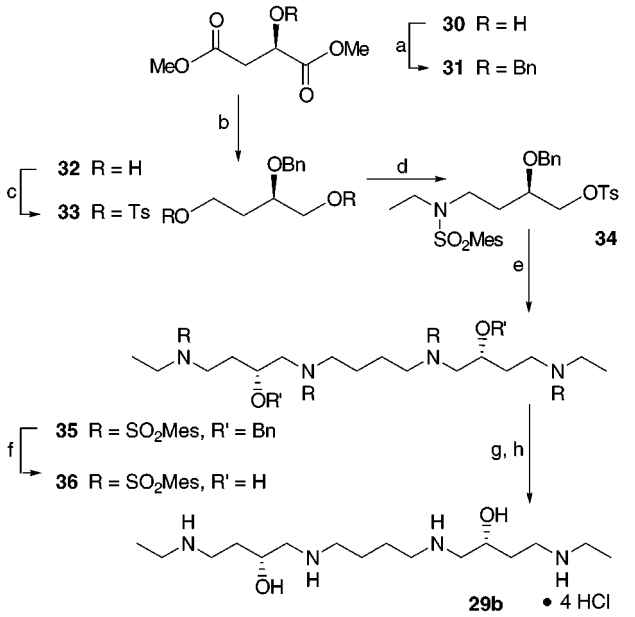


[a] Reagents: (a) **23**, EtOH; (b) benzyl chloroformate, NEt_3, $CHCl_3$, 22%; (c) H_2, Pd−C, HCl, 85%.

44% yield. Deprotection to the 1,2-diol **3** and selective monotosylation[22] to **4** followed by base-induced ring closure furnished epoxide **5** in good yield (57% over three steps). The epoxide **5** was then coupled with *N*,*N*′-dibenzyl-1,3-diaminopropane to give the fully protected tetraamine **6** (84% yield). Removal of the *p*-toluenesulfonyl groups of **6** was accomplished in 50% yield with sodium naphthalenide,[23] providing **7a**. Finally, hydrogenolytic debenzylation furnished (*R*,*R*)-$(HO)_2$DENSPM (**8a**) as its crystalline tetrahydrochloride salt (82% yield).

Alkylation of the ethylamino group with the chiral three-carbon segment was accomplished by a Mitsunobu coupling[24−26] in the initial step of the synthesis of (*S*,*S*)-$(HO)_2$DENSPM (**8b**) (Scheme 2). Specifically, reacting *N*-ethyltriflamide (**9**)[25] with (*S*)-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol gave the dioxolane **10** in 82% yield (Scheme 2).[27] The masking group of the external nitrogens of the tetraamine was changed from *p*-toluenesulfonyl to trifluoromethanesulfonyl in order to facilitate alkylation under Mitsunobu conditions. The steps leading to **13**, analogous to steps b−d in Scheme 1, proceeded in good yields (40% overall). Ring opening of

Scheme 6. Synthesis of (*R*,*R*)-$(HO)_2$DEHSPM (**29b**)[a]



[a] Reagents: (a) benzyl 2,2,2-trichloroacetimidate, CF_3SO_3H, 85%; (b) $LiBH_4$, THF, 83%; (c) TsCl, pyridine, 87%; (d) NaH, $EtNHSO_2Mes$, DMF, 77%; (e) NaH, $MesSO_2HN(CH_2)_4NHSO_2Mes$, DMF, 50%; (f) Pd-black, H_2, HOAc, H_2O, 86%; (g) Na/naphthalene, DME; (h) EtOH, HCl, 21%.

epoxide **13** (2 equiv) at the less hindered carbon by *N*,*N*′-dibenzyl-1,3-diaminopropane gave the fully protected tetraamine **14** in 80% yield. An initial attempt to deprotect **14** with sodium naphthalenide[23] gave back only starting material; therefore, stepwise unmasking of the nitrogens was employed. Removal of the trifluoromethanesulfonyl protective groups in **14** was achieved by reaction with lithium aluminum hydride,[28] giving **7b** in 58% yield. Hydrogenolysis of the internal benzyl moieties afforded (*S*,*S*)-$(HO)_2$DENSPM (**8b**) as its tetrahydrochloride salt (68% yield).

The conservation of optical purity in both synthetic sequences was verified in the following manner (Scheme 3). The epoxide **5** was reacted with both racemic and (*R*)-1-phenylethylamine, yielding the amino alcohols ***rac*-15** and **15**, respectively. The ^{1}H NMR spectrum of **15** showed the presence of only one diastereomer upon comparison with the spectrum of ***rac*-15**. Thus we concluded that no racemization occurred up to formation of the epoxide **5**. In the same fashion, amino alcohols ***rac*-16** and **16** were prepared from the triflamide-substituted epoxide **13**. In this case the ^{1}H NMR spectra demonstrated that **16** contained about 2% of the diastereomeric compound (as indicated by the doublet at 2.45 ppm with coupling constant of $J = 6.2$ Hz). Thus, epoxide **13** may contain about 2% of the other enantiomer.

Chiral integrity was again addressed at the end of both syntheses (Schemes 1 and 2). The tetraamines **8a** and **8b** were derivatized to **17a** and **17b**, respectively, by benzyloxycarbonyl groups (CBZs) at their nitrogens and converted to their respective diastereomeric Mosher's ester[29] derivatives **18a** and **18b** (Scheme 3). Upon evaluation of the ^{1}H and ^{19}F NMR spectra, the best differentiation between **18a** and **18b** was observed in the ^{19}F NMR spectra at 45 °C in 12% $CDCl_3/CD_3OD$ ($\Delta\delta \approx 20$ Hz).[30] The spectrum of **18a** exhibited <2% of the

signal corresponding to **18b**. The NMR of **18b** gave a single peak, in which a similarly minor signal matching that of **18a** could be obscured.[31] Thus, both procedures had successfully produced enantiomeric $(HO)_2$DENSPM compounds with only minor racemization. A further indication for the conservation of optical purity throughout both syntheses is that the absolute values of optical rotation for the enantiomeric pairs **7a**/**7b, 8a**/**8b,** and **17a**/**17b**, respectively, are very similar.

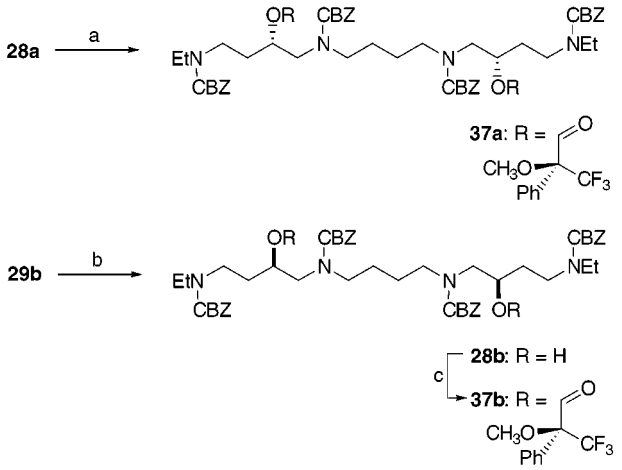
A synthesis of (*R*,*R*)-$(HO)_2$DEHSPM has already been published from our laboratories.[17] Two chiral 4-amino-2-hydroxybutyl groups were attached to *N*,*N*′-dibenzylputrescine employing (*S*)-(+)-epichlorohydrin, followed by displacement of the chloride by cyanide. The latter step of this route does not easily lend itself to scale-up as racemization may occur if the reaction conditions are not carefully controlled. The mechanism for this racemization may involve formation of an azetidinium intermediate in which ring opening by cyanide anion would lead to epimerization.[32]

A similar chiral epoxide coupling approach was used to access N^1,N^{14}-diethyl-(3*S*,12*S*)-dihydroxyhomospermine [(*S*,*S*)-$(HO)_2$DEHSPM, **29a** in Scheme 5]. The synthesis began with the conversion of L-malic acid to its α-monobenzyl ester **19** (Scheme 4).[33−35] Activation of **19** as its *N*-hydroxysuccinimide ester and reaction of this intermediate with ethylamine yielded ethylamide **20** (64%). The reduction of **20** with borane−THF complex followed by protection of the secondary amino group with CBZ gave **21** in 33% yield. Selective tosylation to **22** and base-promoted ring closure to epoxide **23** completed the production of this synthon (62% yield over two steps).

The reaction of epoxide **23** with putrescine (**24**) and its *N*,*N*′-dibenzylated derivative **25** was investigated (Scheme 5), and it was found that: (a) both diamines **24** and **25** react with **23** only in protic solvents, i.e., alcohols; (b) primary diamine **24** reacts faster than the more hindered analogue **25**; and (c) benzyl derivative **27** reacts to some degree with the alcohol solvent at the carbobenzyloxy group. For the sake of brevity, we describe only the optimal conditions: reaction of **24** with 2 equiv of **23** at 55 °C in ethanol, followed by protection of the amino groups with benzyl chloroformate, gave **28a** in 22% yield over two steps. Hydrogenolysis of all four protecting groups of **28a** produced target tetraamine **29a** as its crystalline tetrahydrochloride salt in 85% yield.

The synthesis of (*R*,*R*)-N^1,N^{14}-diethyl-3,12-dihydroxyhomospermine tetrahydrochloride (**29b**, Scheme 6) was undertaken to explore an alternative method of accessing enantiomerically pure 3,12-$(HO)_2$DEHSPM compounds with the goal of improving the overall yield. This route (Scheme 6) began with benzylation of the commercially available diester **30** to give the benzyl ether **31**,[36] followed by reduction using $LiBH_4$ to the diol **32**.[37] Our yields for these literature preparations were comparable (85% and 83%, respectively) to those previously reported.[36,37] The ditosylate intermediate **33** was readily obtained by derivatization of the diol **32** with *p*-toluenesulfonyl chloride in pyridine (87% yield).[38,39] Alkylation at the less hindered site of ditosylate **33** was a key step in the synthesis of the macrocyclic dihydroxamate siderophore alcaligin in these laboratories.[39] Selective reaction of **33** at C-4 with the *N*-ethylmesitylenesulfonamide anion to provide synthon **34** in 77% yield was similarly pivotal to this synthesis. Monotosylate **34** contains a protected ethylamine, the protected chiral hydroxyl group, and a tosylate leaving group at C-1. Treatment of 2 equiv of **34** with *N*,*N*′-bis(mesitylenesulfonyl)-1,4-butanediamine[40] (NaH/DMF) resulted in fully protected intermediate **35** in 50% yield.

Scheme 7. Check of Conservation of Optical Purity for Both $(HO)_2$DEHSPM Syntheses[a]



[a] Reagents: (a) (*R*)-(−)-Mosher's acid chloride, pyridine, 90%; (b) *N*-(benzyloxycarbonyloxy)succinimide, Et_2O(aq), 64%; (c) a, 92%.

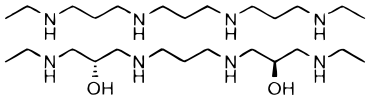
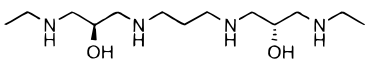
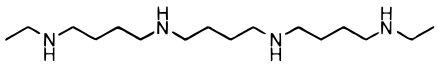
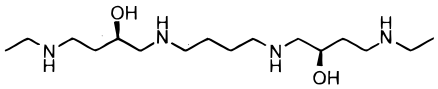
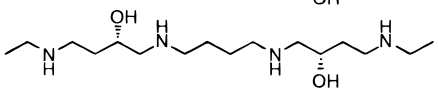
Attempts to remove all of the protecting groups of diether tetrasulfonamide **35** using an alkali metal in liquid ammonia did not efficiently generate polyamine **29b**; thus, a two-step unmasking scheme was employed. Cleavage of the benzyl ether protecting groups by catalytic reduction with palladium black[41] gave the diol tetrasulfonamide **36** (86% yield). Finally, conversion of the four mesitylenesulfonamides to the amines with sodium naphthalenide[23] and acidification gave the target tetrahydrochloride salt **29b** in somewhat low yield (21%).

The stereochemical integrity of the reaction sequences to the $(HO)_2$DEHSPMs was verified (Scheme 7). The precursor **28a** was reacted with (*R*)-(−)-Mosher's acid chloride[29] to give Mosher's ester derivative **37a** in 90% yield. In addition, the final polyamine **29b** was first protected as the tetra-CBZ derivative **28b** (64% yield) and subsequently converted to the diastereomeric Mosher's ester **37b** (92% yield). At 45 °C, the ^{19}F resonance for **37a** was observed at −71.57 ppm and the signal for **37b** appeared, clearly separated, at −71.80 ppm ($\Delta\delta \approx 65$ Hz). The spectra of **37a** and **37b** were essentially free of resonances indicative of the other diastereomer.[31] Additionally, the specific optical rotations for the enantiomeric compound pairs **28a**/**28b** and **29a**/**29b** gave nearly identical absolute values. Thus, both routes produced enantiomerically pure $(HO)_2$DEHSPM compounds within the limits of detection.

Biological Evaluations

The in vitro biological properties of the hydroxylated polyamine analogues will be presented in three data sets: the 48-h and 96-h IC_{50} values against L1210 cells and the corresponding K_i values for the polyamine transport apparatus (Table 1); the effect on polyamine pools (Table 2); and the impact on ornithine decarboxy-

Table 1. Structures, Abbreviations, L1210 Growth Inhibition, and Transport

Structure	Abbreviation	IC_{50} (μM)[a] 48 h	96 h	K_i (μM)[b]
	DENSPM	20	2.0	17
	(*R*,*R*)-$(HO)_2$DENSPM	3.0	2.0	68
	(*S*,*S*)-$(HO)_2$DENSPM	1.0	0.6	43
	DEHSPM	0.2	0.07	1.4
	(*R*,*R*)-$(HO)_2$DEHSPM	40	0.08	1.7
	(*S*,*S*)-$(HO)_2$DEHSPM	2.0	0.06	2.4

[a] 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 represent the mean of at least two or three experiments with a variation typically less than 10%.

Table 2. Impact of Polyamine Analogues on Polyamine Pools

compound	concn (μM)	PUT[a]	SPD[a]	SPM[a]	analogue[b]
DENSPM	10	30	14	31	1.59
	100	0	6	30	2.44
(*R*,*R*)-$(HO)_2$DENSPM	3	9	14	63	1.88
	15	5	9	54	1.99
(*S*,*S*)-$(HO)_2$DENSPM	1	66	46	94	1.18
	5	5	7	56	2.02
DEHSPM	0.15	97	91	110	0.21
	0.75	49	40	101	1.89
	10	3	1	57	3.65
(*R*,*R*)-$(HO)_2$DEHSPM	40	5	19	107	2.41
	200	4	13	104	2.43
(*S*,*S*)-$(HO)_2$DEHSPM	2	3	11	62	2.82
	10	3	4	53	3.40

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

lase, *S*-adenosylmethionine decarboxylase, and spermidine/spermine N^1-acetyltransferase activities (Table 3). All tables include not only data on (*R*,*R*)- and (*S*,*S*)-$(HO)_2$DENSPM and (*R*,*R*)- and (*S*,*S*)-$(HO)_2$DEHSPM but also data on DENSPM and DEHSPM for comparison. Finally, a sample of (*R*,*R*)-$(HO)_2$DENSPM was evaluated for neurological toxicity in mice.

IC_{50} Studies. Unlike DENSPM, both (*R*,*R*)- and (*S*,*S*)-$(HO)_2$DENSPM had similar low 48-h and 96-h IC_{50} values and were thus much more active than the parent drug at 48 h (Table 1).

The scenario for DEHSPM and its hydroxylated analogues (*R*,*R*)- and (*S*,*S*)-$(HO)_2$DEHSPM was quite the opposite. At 48 h the parent DEHSPM was much more active than either of the hydroxylated analogues. Furthermore there was a large difference between the (*R*,*R*)- and (*S*,*S*)-$(HO)_2$DEHSPM 48-h IC_{50} values, although this difference disappeared at 96 h. This difference between the 48-h and 96-h IC_{50} is particularly large (500-fold) with the (*R*,*R*)-isomer.

Table 3. Effect of Polyamine Analogues 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 (10)[b]	42 (31)	1471
(*R*,*R*)-$(HO)_2$DENSPM	35 (8)	59 (68)	177
(*S*,*S*)-$(HO)_2$DENSPM	61 (6)	51 (58)	816
DEHSPM	7 (3)	41 (49)	110
(*R*,*R*)-$(HO)_2$DEHSPM	55 (46)	68 (72)	102
(*S*,*S*)-$(HO)_2$DEHSPM	17 (16)	52 (59)	114

[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 reduced ODC to 6.7 ± 2.6% of untreated control; 1 μM DEHSPM reduced AdoMetDC to 40.7 ± 6.2% of untreated control; and 2 μM DENSPM increased SSAT levels to 1471 ± 120% 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] Experiments assessing the impact of increasing the analogue concentration to 10 μM on ODC and AdoMetDC activity were also performed on cells treated for 4 and 6 h, respectively. These results are indicated in parentheses in the table. Data shown represent the mean of at least three experiments and have variances consistent with those suggested by the positive control data presented above.

***K_i* Studies.** The ability of the hydroxylated analogues to compete with radiolabeled SPD for uptake was evaluated. Both (*R*,*R*)- and (*S*,*S*)-$(HO)_2$DENSPM had substantially higher K_i values, 68 and 43 μM, respectively, in comparison to that of DENSPM, 17 μM (Table 1). This was not the case with the hydroxylated homospermines (*R*,*R*)- and (*S*,*S*)-$(HO)_2$DEHSPM, which had K_i values of 1.7 and 2.4 μM, essentially the same as that of the parent drug DEHSPM (1.4 μM) and thus similar to the spermine K_i of 1.2 μM.

Polyamine Pools. The following guidelines were adopted for studying the impact of the analogues on polyamine pools (Table 2). The measurements were made after a 48-h exposure to the analogue, and at least two different concentrations of analogue were evaluated.

Generally, the effect on polyamine pools was evaluated at the 48-h IC_{50} concentration and at 5 times this number.

At the lower concentrations, the (*S*,*S*)-$(HO)_2$DENSPM differed significantly from the (*R*,*R*)-isomer and the parent DENSPM. (*S*,*S*)-$(HO)_2$DENSPM at 1 μM had a comparatively modest effect on polyamine pools, reducing putrescine to 66%, spermidine to 46%, and spermine to 94% of control. At 3 μM the (*R*,*R*)-enantiomer depleted putrescine, spermidine, and spermine to 9%, 14%, and 63% of control values, respectively. At 100 μM DENSPM, putrescine was depleted to below detectable limits and spermidine was reduced to around 6% of controls, while spermine levels were diminished to 30%. At 15 μM (*R*,*R*)-$(HO)_2$DENSPM, putrescine was reduced to 5% of control, spermidine to 9%, and spermine to 54%. At 5 μM (*S*,*S*)-analogue, native polyamines were reduced to the same extent within experimental error. Finally, at 5 times the IC_{50} both (*R*,*R*)- and (*S*,*S*)-$(HO)_2$DENSPM achieved similar intracellular levels of 2 mM, as did DENSPM itself.

Although DEHSPM has a much lower 48-h IC_{50} value than DENSPM, its impact on the polyamine pools even at 0.75 μM, 5 times the 48-h IC_{50}, is much less significant. Putrescine was reduced to 49% and spermidine to 40%, and spermine was unchanged. However, higher concentrations of DEHSPM (e.g., 10 μM) were shown to reduce the polyamine levels at 48 h to well below this. At 5 times the 48-h IC_{50} value, 200 μM (*R*,*R*)-$(HO)_2$DEHSPM reduced putrescine to 4% of control and spermidine to 13% with essentially no impact on spermine. At 10 μM (*S*,*S*)-$(HO)_2$DEHSPM, again 5 times the 48-h IC_{50} concentration, putrescine and spermidine were reduced to 3% and 4% of control and spermine to 53% of control. At the 48-h IC_{50} concentrations, 40 μM (*R*,*R*)-$(HO)_2$DEHSPM and 2 μM (*S*,*S*)-$(HO)_2$DEHSPM both reduced putrescine to below 5% and spermidine to below 19%. The (*R*,*R*)-isomer had no effect on spermine while the (*S*,*S*)-isomer reduced it to 62% of control. Finally, at 5 times the IC_{50} value the (*S*,*S*)-enantiomer achieved slightly higher concentrations intracellularly than the (*R*,*R*)-isomer, 3.4 mM versus 2.4 mM.

Impact of Analogues on ODC, AdoMetDC, and SSAT. As previously described, there is little correlation between the ODC and AdoMetDC levels and a polyamine analogue's 48-h and 96-h IC_{50} values.[40] At 4 h, 1 μM DENSPM reduced ODC activity to 7% of control, while (*R*,*R*)- and (*S*,*S*)-$(HO)_2$DENSPM lowered ODC to 35% and 61% of control, respectively (Table 3). However, increasing the analogue concentration to 10 μM resulted in a marked decrease in ODC activity, to 8% and 6% of control values, by (*R*,*R*)- and (*S*,*S*)-$(HO)_2$DENSPM, respectively. Diethylhomospermine was as active as DENSPM at 1 μM, lowering ODC to 7% of control. Again, both hydroxylated compounds were less active at 1 μM with a reduction to 55% and 17% of control ODC by (*R*,*R*)- and (*S*,*S*)-$(HO)_2$DEHSPM, respectively. In contrast to the finding with the hydroxylated DENSPM analogues, raising the concentration of the hydroxylated DEHSPM analogues to 10 μM did not result in a dramatic further reduction of ODC activity. Diethylnorspermine, (*R*,*R*)-$(HO)_2$DENSPM, and (*S*,*S*)-$(HO)_2$DENSPM reduced AdoMetDC to 42%, 59%, and 51%, respectively, of control. Diethylhomospermine reduction of AdoMetDC was equally unimpressive, to 41%, with the same or slightly less reduction in activity seen with the (*R*,*R*)- and (*S*,*S*)-$(HO)_2$DEHSPM, 68% and 52%, respectively. Even raising the analogue concentration to 10 μM produced little additional effect on AdoMetDC activity. Finally, while DENSPM had a profound effect on SSAT, increasing it by 1471%, (*S*,*S*)-$(HO)_2$DENSPM elevated it by 816% and (*R*,*R*)-$(HO)_2$DENSPM by only 177%. None of the DEHSPM analogues had any detectable impact on SSAT.

In Vivo Toxicity. Clinically, DENSPM has so far presented with minimal side effects. However, we have shown that at very high subchronic doses, neurological side effects can be induced in mice. When 12 male C-57/Bl/6 mice were treated with DENSPM tetrahydrochloride at a dose of 200 mg/kg/day for 6 days, all of the animals displayed severe neurological signs during the course of treatment. These signs included severe ataxia, intention tremors, and motor dysfunction. By day 3, 2 of the animals died of seizures and by day 6 a total of 10 of the 12 animals had died from neurological sequelae. In a parallel experiment, for 6 days 12 mice were given an equimolar dose (217 mg/kg/day ip) of (*R*,*R*)-$(HO)_2$DENSPM tetrahydrochloride with a lower ee value than the current compound. None of these 12 animals presented with any neurological symptoms at all. None of the animals died, nor was there weight loss or any other adverse effect. When this experiment was repeated with three mice given enantiomerically pure (*R*,*R*)-$(HO)_2$DENSPM, again virtually no signs of toxicity were observed. Thus it seems that hydroxylation of diethylnorspermine, while actually increasing the parent compound's antiproliferative activity in cell culture at 48 h, at the same time markedly decreases the in vivo toxicity. Acute and chronic studies of both (*R*,*R*)- and (*S*,*S*)-$(HO)_2$DEHSPM as well as both (*R*,*R*)- and (*S*,*S*)-$(HO)_2$DENSPM are now underway.

Discussion

Synthetic routes to (*R*,*R*)-$(HO)_2$DENSPM, (*S*,*S*)-$(HO)_2$DENSPM, (*R*,*R*)-$(HO)_2$DEHSPM, and (*S*,*S*)-$(HO)_2$DEHSPM have been successfully executed, and the chiral integrity of the schemes was verified. Four separate synthetic approaches were evaluated, one for each of the hydroxylated analogues. Both routes to $(HO)_2$DENSPM consisted of seven steps and utilized the same central fragment, *N*,*N*′-dibenzyl-1,3-diaminopropane, but different chiral epoxides, one a triflamide and the other a tosylamide. The Mitsunobu coupling of *N*-ethyltriflamide (**9**) with the chiral dioxolane carbinol, leading to the chiral synthon **10**, was more efficient than alkylation of *N*-ethyltosylamide (**1**) with the chloromethyldioxolane. We did observe a minor amount of the (*R*)-enantiomer ($\leq$2%) in the production of (*S*)-triflamide epoxide **13** on the route to (*S*,*S*)-$(HO)_2$DENSPM (**8b**). We do not regard this level of impurity as a significant problem. No such racemization was detected in making tosylamide epoxide **5** in the route to (*R*,*R*)-$(HO)_2$DENSPM. The Mosher's ester derivatives (**18a** and **18b**) of the $(HO)_2$DENSPMs confirmed that the level of racemization was not problematic.

The two synthetic routes leading to the longer hydroxylated tetraamines (*S*,*S*)- and (*R*,*R*)-$(HO)_2$DEHSPM (**29a** and **29b**, respectively) began with the appropriate

malic acid. Its L-enantiomer was transformed into *N*-CBZ-*N*-ethyl-*N*-epoxybutyl synthon **23**, which was reacted with putrescine to assemble the polyamine framework of **29a**. Our alternate route to the enantiomeric $(HO)_2$DEHSPM (**29b**) began with commercially available dimethyl D-malate (**30**), which was converted into the chiral tosylate **34**. Bis-alkylation of *N*,*N*′-bis(mesitylenesulfonyl)-1,4-butanediamine with **34** completed the assembly of the skeleton of (*R*,*R*)-$(HO)_2$DEHSPM (**29b**). Chiral integrity was maintained throughout both routes to the dihydroxylated homospermines, as confirmed by ^{19}F NMR spectral analysis of the appropriate Mosher's ester derivatives **37a** and **37b**.

At a cellular level in vitro, the most notable difference between the polyamine analogues DENSPM and DEHSPM and their hydroxylated counterparts occurred at the onset of growth inhibition and impact on polyamine enzymes. The hydroxylated DENSPM analogues inhibited growth faster than DENSPM, while the hydroxylated DEHSPM analogues were slower in onset than DEHSPM. Under our standard experimental conditions, reduction in ODC activity by all of the hydroxylated analogues was less than that seen with the parent drugs. However, suppression of AdoMetDC was very much like that observed with the parents. The most notable difference in alteration of polyamine enzyme activity was associated with SSAT and the hydroxylated DENSPMs. The upregulation of SSAT induced by (*S*,*S*)-$(HO)_2$DENSPM was 55% of that seen with DENSPM, while (*R*,*R*)-$(HO)_2$DENSPM upregulated SSAT only 12% as effectively as DENSPM. Finally, at the whole animal level in vivo there was a remarkable difference in neurotoxicity between DENSPM and (*R*,*R*)-$(HO)_2$DENSPM. At equimolar doses, the parent DENSPM is uniformly neurotoxic, while the hydroxylated analogue is virtually nontoxic. This suggests that hydroxylation may be a viable approach to altering the toxicity profile of polyamine analogues. However, further long-term chronic toxicity trials are necessary.

Experimental Section

General. Reagents were purchased from the Aldrich, Fluka, or Sigma Chemical Co. and were used without further purification. Fisher Optima-grade solvents were routinely used. DMF and THF were distilled, the latter from sodium and benzophenone. Silica gel 32−60 (40 μm "flash") from Selecto, Inc. (Kennesaw, GA) was used for flash column chromatography. NMR spectra were recorded on a Varian Unity 300 with ^{1}H NMR spectra at 300 MHz, ^{13}C NMR spectra at 75 MHz, and ^{19}F NMR spectra at 282 MHz. Chemical shifts are given in parts per million downfield from an internal tetramethylsilane standard, unless otherwise specified. Coupling constants (*J*) are in Hz. Optical rotations were measured at 589 nm (sodium D line) unless otherwise indicated with a Perkin-Elmer 341 polarimeter; *c* equals grams of compound per 100 mL of solution. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. FAB mass spectra were run in a 3-nitrobenzyl alcohol or glycerol matrix. Elemental analyses were performed by Atlantic Microlabs, Norcross, GA.

***N*-[(4*R*)-2,2-Dimethyl-1,3-dioxolan-4-ylmethyl]-*N*-ethyl-*p*-toluenesulfonamide (2).** A solution of **1** (10.00 g, 50.2 mmol) in DMF (90 mL) was cooled to 0 °C under an Ar atmosphere, and 60% NaH (3.00 g, 75.3 mmol) was carefully added. The reaction mixture was warmed to room temperature, and NaI (375 mg, 2.5 mmol) and 15-crown-5 (550 mg, 495 μL, 2.5 mmol) were added. After heating to 80 °C, (*S*)-(−)-4-(chloromethyl)-2,2-dimethyl-1,3-dioxolane (11.00 g, 9.4 mL, 75.3 mmol) was added dropwise. The reaction mixture was kept at this temperature for 70 h, then cooled to room temperature, and poured onto ice-cold water (500 mL). The aqueous layer was extracted with EtOAc (3 × 150 mL). The combined organic layers were washed with saturated NaCl solution (100 mL), dried over $MgSO_4$, and concentrated in vacuo. The resulting oil was purified by flash chromatography (15% EtOAc/cyclohexane) to give **2** (6.87 g, 44%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.11 (t, 3 H, J = 7.3), 1.33 (s, 3 H), 1.40 (s, 3 H), 2.42 (s, 3 H), 3.10 (dd, 1 H, J = 14.5, 6.2), 3.17−3.47 (m, 3 H), 3.78 (dd, 1 H, J = 8.6, 6.4), 4.09 (dd, 1 H, J = 8.6, 6.2), 4.29 (m, 1 H), 7.30 (d, 2 H, J = 8.1), 7.70 (m, 2 H); HRMS *m*/*z* calcd for $C_{15}H_{24}NO_4S$ 314.1426, found 314.1425; $[\alpha]^{22}_D$ +14.1 (*c* 0.95, $CHCl_3$). Anal. ($C_{15}H_{23}NO_4S$) C, H, N.

***N*-[(2*R*)-2,3-Dihydroxypropyl]-*N*-ethyl-*p*-toluenesulfonamide (3).** Hydrochloric acid (1 N, 40 mL) was added to a solution of **2** (6.60 g, 21.1 mmol) in acetone (20 mL). The reaction mixture was heated to 95 °C for 1 h, then cooled to room temperature, and concentrated in vacuo. Purification by flash chromatography (30% cyclohexane/EtOAc) gave **3** (5.33 g, 93%) as an oil: ^{1}H NMR ($CDCl_3$) δ 1.09 (t, 3 H, J = 7.1), 2.43 (s, 3 H), 3.06 (dd, 1 H, J = 14.5, 7.3), 3.16−3.40 (m, 3 H), 3.53 (m, 2 H), 3.81 (m, 1 H), 7.38 (m, 2 H), 7.72 (m, 2 H); HRMS *m*/*z* calcd for $C_{12}H_{20}NO_4S$ 274.1113, found 274.1114; $[\alpha]^{22}_D$ −1.0 (*c* 0.99, $CHCl_3$).

***N*-Ethyl-*N*-[(2*R*)-2-hydroxy-3-*p*-toluenesulfonatopropyl]-*p*-toluenesulfonamide (4).** A solution of **3** (5.74 g, 21.0 mmol) in anhydrous pyridine (50 mL) was cooled to 0 °C under an Ar atmosphere, and TsCl (4.41 g, 23.1 mmol) was added in portions. The reaction mixture was maintained at 4 °C for 22 h, diluted with Et_2O (500 mL), and extracted with 1 N HCl (4 × 50 mL). The organic phase was dried over $MgSO_4$, concentrated in vacuo, and purified by flash chromatography (5% acetone/$CHCl_3$) to yield **4** (6.00 g, 67%) as a white solid: mp 110−112 °C; ^{1}H NMR (CD_3OD) δ 1.01 (t, 3 H, J = 7.2), 2.42 (s, 3 H), 2.46 (s, 3 H), 2.99 (m, 1 H), 3.05−3.30 (m, 3 H), 3.94 (m, 2 H), 4.09 (m, 1 H), 7.37 (m, 2 H), 7.45 (d, 2 H, J = 8.6), 7.6 (d, 2 H, J = 8.4), 7.81 (d, 2 H, J = 8.4); $[\alpha]^{23}_D$ −10.1 (*c* 1.01, $CHCl_3$). Anal. ($C_{19}H_{25}NO_6S_2$) C, H, N.

***N*-[(2*R*)-2,3-Epoxypropyl]-*N*-ethyl-*p*-toluenesulfonamide (5).** To a solution of **4** (5.89 g, 13.8 mmol) in CH_3OH (100 mL) was added K_2CO_3 (2.02 g, 14.6 mmol). The suspension was stirred at room temperature for 3 h under an Ar atmosphere and then was poured into a mixture of H_2O (150 mL) and CH_2Cl_2 (150 mL). Saturated NaCl solution was added, and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic phase was dried over $MgSO_4$ and concentrated in vacuo. Purification by flash chromatography (20% EtOAc/cyclohexane) gave **5** (3.24 g, 92%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.16 (t, 3 H, J = 7.0), 2.42 (s, 3 H), 2.55 (dd, 1 H, J = 4.6, 2.4), 2.79 (t, 1 H, J = 4.6), 2.97 (dd, 1 H, J = 14.9, 6.4), 3.08 (m, 1 H), 3.26 (m, 1 H), 3.34 (m, 1 H), 3.63 (dd, 1 H, J = 15.2, 3.5), 7.30 (d, 2 H, J = 7.9), 7.70 (m, 2 H); ^{13}C NMR ($CDCl_3$ = 77.0 ppm) δ 13.91, 21.45, 43.89, 45.17, 50.04, 50.90, 127.03, 129.71, 136.84, 143.33; HRMS *m*/*z* calcd for $C_{12}H_{18}NO_3S$ 256.1007, found 256.1047; $[\alpha]^{24}_D$ +21.4 (*c* 0.95, $CHCl_3$). Anal. ($C_{12}H_{17}NO_3S$) C, H, N.

(2*S*,10*S*)-*N*^{1},*N*^{11}-Bis(*p*-toluenesulfonyl)-*N*^{4},*N*^{8}-dibenzyl-*N*^{1},*N*^{11}-diethyl-2,10-dihydroxynorspermine (6). A solution of **5** (3.09 g, 12.1 mmol) and *N*,*N*′-dibenzyl-1,3-diaminopropane (1.54 g, 6.06 mmol) in EtOH (50 mL) was heated to reflux for 19 h under an Ar atmosphere. The solvent was removed under reduced pressure, and the residue was purified by flash chromatography (5% then 10% acetone/$CHCl_3$) to give **6** (3.89 g, 84%) as a colorless, viscous oil: ^{1}H NMR (CD_3OD) δ 1.02 (t, 6 H, J = 7.0), 1.70 (m, 2 H), 2.40 (s, 6 H), 2.42 (m, 4 H), 2.48 (m, 4 H), 2.76 (dd, 2 H, J = 14.7, 8.1), 3.20 (m, 2 H), 3.26 (m, 2 H), 3.36 (dd, 2 H, J = 14.7, 3.5), 3.46 (d, 2 H, J = 13.4), 3.63 (d, 2 H, J = 13.4), 3.84 (m, 2 H), 7.12−7.30 (m, 10 H), 7.34 (d, 4 H, J = 7.9), 7.67 (m, 4 H); ^{13}C NMR ($CDCl_3$ = 77.0 ppm) δ 13.89, 21.46, 24.36, 44.61, 51.75, 52.12, 57.69, 58.88, 66.97, 127.13, 127.25, 128.41, 129.06, 129.63, 136.77, 138.33, 143.15;

HRMS *m/z* calcd for $C_{41}H_{57}N_4O_6S_2$ 765.3720, found 765.3721; $[\alpha]^{24}_D$ −27.8 (*c* 1.06, $CHCl_3$).

(2*R*,10*R*)-N^4,N^8-Dibenzyl-N^1,N^{11}-diethyl-2,10-dihydroxynorspermine (7a). Anhydrous 1,2-dimethoxyethane (DME; 75 mL) was added dropwise to a mixture of Na (2.46 g, 107 mmol) and naphthalene (16.88 g, 132 mmol)[23] under an Ar atmosphere. The reaction mixture was stirred for 2 h at room temperature to give a dark green solution. A portion of this solution was added dropwise to **6** (3.56 g, 4.65 mmol) in DME (150 mL) at −78 °C until a green color persisted. The reaction mixture was quenched with saturated $NaHCO_3$ (8 mL) and warmed to room temperature. Solid K_2CO_3 (50 g) was added, and the mixture was stirred for 2 h. The solids were filtered off and washed with Et_2O (3 × 100 mL). The filtrate was concentrated in vacuo and purified by flash chromatography (CH_3OH; 5% then 10% concentrated NH_4OH/CH_3CN) to give **7a** (1.06 g, 50%) as a viscous oil: ^{1}H NMR (CD_3OD) δ 1.09 (t, 6 H, J = 7.3), 1.70 (m, 2 H), 2.33−2.70 (m, 16 H), 3.51 (d, 2 H, J = 13.4), 3.63 (d, 2 H, J = 13.4), 3.79 (m, 2 H), 7.10−7.45 (m, 10 H); HRMS *m/z* calcd for $C_{27}H_{45}N_4O_2$ 457.3543, found 457.3549; $[\alpha]^{25}_D$ −65.8 (*c* 1.12, $CHCl_3$).

(2*R*,10*R*)-N^1,N^{11}-Diethyl-2,10-dihydroxynorspermine Tetrahydrochloride (8a). Hydrochloric acid (1 N, 17.5 mL, 17.5 mmol) and 10% Pd−C (200 mg) were added to **7a** (1.00 g, 2.19 mmol) in EtOH (50 mL). The reaction mixture was stirred under H_2 at 1 atm for 22 h, then filtered through Celite (0.5 cm), and washed with water (50 mL) and EtOH (50 mL). The solvents were removed in vacuo. The residue was taken up in water (15 mL), filtered through a syringe filter (0.2 μm, PVDF), and again concentrated. Recrystallization from aqueous EtOH gave **8a** (761 mg, 82%) as small white crystals: ^{1}H NMR (D_2O, NaTSP) δ 1.32 (t, 6 H, J = 7.2), 2.14 (m, 2 H), 3.03−3.30 (m, 16 H), 4.27 (tt, 2 H, J = 9.6, 3.2); ^{13}C NMR (D_2O; dioxane = 67.19 ppm) δ 10.99, 23.00, 43.92, 45.36, 49.99, 50.72, 63.52; HRMS *m/z* calcd for $C_{13}H_{33}N_4O_2$ 277.2604, found 277.2578; $[\alpha]^{24}_D$ −2.1 (*c* 0.75, 1 N HCl). Anal. ($C_{13}H_{36}Cl_4N_4O_2$) C, H, Cl, N.

***N*-Ethyltrifluoromethanesulfonamide (9).** Trifluoromethanesulfonic anhydride (10 g, 35 mmol) in CH_2Cl_2 (20 mL) was added dropwise over 20 min to ethylamine in THF (2.0 M, 51 mL, 102 mmol) in CH_2Cl_2 (100 mL) at −78 °C under an Ar atmosphere. The reaction mixture was stirred for 2.5 h, warmed to room temperature, and washed with 1 N HCl (2 × 100 mL). The organic phase was dried over $MgSO_4$ and concentrated in vacuo. Distillation (bp 60 °C/ca. 2 mmHg) [lit. bp 95−97 °C/0.3 mmHg][25] gave **9** (3.35 g, 54%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.29 (t, 3 H, J = 7.3), 3.38 (m, 2 H), 4.76 (m br, 1 H); HRMS (EI) *m/z* calcd for $C_3H_6F_3NO_2S$ 177.0071, found 177.0028.

***N*-[(4*S*)-2,2-Dimethyl-1,3-dioxolan-4-ylmethyl]-*N*-ethyltrifluoromethanesulfonamide (10).** (*S*)-(+)-2,2-Dimethyl-1,3-dioxolane-4-methanol (1.47 mL, 1.57 g, 11.9 mmol) and triphenylphosphine (3.75 g, 14.3 mmol) were added to **9** (2.11 g, 11.9 mmol) in THF (50 mL) under an Ar atmosphere. Diisopropyl azodicarboxylate (2.81 mL, 2.89 g, 14.3 mmol) was added dropwise at room temperature, and the reaction mixture was stirred for 4.5 h. The solvent was removed in vacuo, and the crude product was purified by flash chromatography (15% EtOAc/cyclohexane) to give **10** (2.83 g, 82%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.28 (t, 3 H, J = 7.3), 1.35 (s, 3 H), 1.42 (s, 3 H), 3.33 (m, 1 H), 3.57−3.65 (m, 3 H), 3.66 (dd, 1 H, J = 8.6, 6.4), 4.11 (dd, 1 H, J = 8.6, 6.4), 4.31 (m, 1 H); HRMS *m/z* calcd for $C_9H_{17}F_3NO_4S$ 292.0830, found 292.0853; $[\alpha]^{24}_D$ −23.1 (*c* 1.02, $CHCl_3$). Anal. ($C_9H_{16}F_3NO_4S$) C, H, N.

***N*-[(2*S*)-2,3-Dihydroxypropyl]-*N*-ethyltrifluoromethanesulfonamide (11).** A solution of **10** (2.81 g, 9.65 mmol) in acetone (20 mL) and 1 N HCl (40 mL) was heated to reflux for 1 h. The solvent was removed in vacuo, and the residue was purified by flash chromatography (60% EtOAc/cyclohexane) to give **11** (2.12 g, 87%) as an oil: ^{1}H NMR (CD_3OD) δ 1.27 (t, 3 H, J = 7.0), 3.20−3.40 (m, 1 H), 3.46−3.74 (m, 5 H), 3.85 (m, 1 H); HRMS *m/z* calcd for $C_6H_{13}F_3NO_4S$ 252.0517, found 252.0558; $[\alpha]^{24}_D$ −6.0 (*c* 1.00, $CHCl_3$). Anal. ($C_6H_{12}F_3NO_4S$) C, H, N.

***N*-Ethyl-*N*-[(2*S*)-2-hydroxy-3-*p*-toluenesulfonatopropyl]trifluoromethanesulfonamide (12).** *p*-Tosyl chloride (1.90 g, 9.98 mmol) was introduced in portions to **11** (2.28 g, 9.08 mmol) in pyridine (20 mL) at 0 °C under an Ar atmosphere. The reaction mixture was stirred for 5.5 h, diluted with Et_2O (200 mL), and extracted with 1 N HCl (4 × 50 mL). The organic layer was dried over $MgSO_4$, concentrated in vacuo, and purified by flash chromatography (10%, 20%, then 50% EtOAc/cyclohexane) to furnish **12** (2.50 g, 68%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.26 (t, 3 H, J = 7.1), 2.47 (s, 3 H), 2.55 (d, 1 H, J = 4.8), 3.28−3.64 (m, 4 H), 3.96−4.17 (m, 3 H), 7.38 (m, 2 H), 7.80 (m, 2 H); HRMS *m/z* calcd for $C_{13}H_{19}F_3NO_6S_2$ 406.0606, found 406.0603; $[\alpha]^{25}_D$ −5.0 (*c* 1.04, $CHCl_3$). Anal. ($C_{13}H_{18}F_3NO_6S_2$) C, H, N.

***N*-[(2*S*)-2,3-Epoxypropyl]-*N*-ethyltrifluoromethanesulfonamide (13).** According to the method described for compound **5**, **12** was reacted with K_2CO_3 (915 mg, 6.62 mmol) in CH_3OH (20 mL). Purification by flash chromatography (10% EtOAc/cyclohexane) generated **13** (949 mg, 68%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.30 (t, 3 H, J = 7.1), 2.58 (dd, 1 H, J = 4.5, 2.2), 2.86 (t, 1 H, J = 4.5), 3.08 (br m, 1 H), 3.15 (m, 1 H), 3.59 (m, 2 H), 3.92 (m, 1 H); $[\alpha]^{24}_D$ −35.0 (*c* 0.98, $CHCl_3$). Anal. ($C_6H_{10}F_3NO_2S$) C, H, N.

(2*R*,10*R*)-Bis(trifluoromethanesulfonyl)-N^4,N^8-dibenzyl-N^1,N^{11}-diethyl-2,10-dihydroxy-N^1,N^{11}-norspermine (14). Compound **13** (823 mg, 3.53 mmol) was reacted with *N*,*N*′-dibenzyl-1,3-diaminopropane (449 mg, 1.76 mmol) by the method used to synthesize **6**. Purification by flash chromatography (10% acetone/$CHCl_3$) provided **14** (1.02 g, 80%) as a colorless, viscous oil: ^{1}H NMR (CD_3OD) δ 1.19 (t, 6 H, J = 7.1), 1.69 (quint, 2 H, J = 7.2), 2.40 (d, 4 H, J = 6.6), 2.49 (m, 4 H), 3.00 (br m, 2 H), 3.45 (d, 2 H, J = 13.1), 3.65 (d, 2 H, J = 13.1), 3.40−3.73 (m, 6 H), 3.85 (m, 2 H), 7.12−7.43 (m, 10 H); HRMS *m/z* calcd for $C_{29}H_{43}F_6N_4O_6S_2$ 721.2528, found 721.2519; $[\alpha]^{24}_D$ +31.0 (*c* 1.05, $CHCl_3$). Anal. ($C_{29}H_{42}F_6N_4O_6S_2$) C, H, N.

(2*S*,10*S*)-N^4,N^8-Dibenzyl-N^1,N^{11}-diethyl-2,10-dihydroxynorspermine (7b). A solution of $LiAlH_4$ (1.0 M in THF, 3.72 mL, 3.72 mmol) was carefully added to **14** (448 mg, 0.62 mmol) in THF (30 mL) at room temperature under an Ar atmosphere. The reaction mixture was heated to reflux for 19 h, cooled to room temperature, and carefully hydrolyzed with H_2O (435 μL), 15% NaOH (435 μL), and H_2O (1.3 mL). The solids were filtered and washed with Et_2O (2 × 5 mL). The filtrate was concentrated in vacuo and was purified by flash chromatography (5% then 10% concentrated NH_4OH/CH_3CN). The product was dissolved in CH_3OH (5 mL), filtered through a syringe filter (0.2 mm, PVDF), and concentrated in vacuo to give **7b** (165 mg, 58%) as a viscous oil: ^{1}H NMR (CD_3OD) δ 1.10 (t, 6 H, J = 7.1), 1.70 (m, 2 H), 2.34−2.70 (m, 16 H), 3.52 (d, 2 H, J = 13.4), 3.62 (d, 2 H, J = 13.4), 3.79 (m, 2 H), 7.10−7.45 (m, 10 H); HRMS *m/z* calcd for $C_{27}H_{45}N_4O_2$ 457.3543, found 457.3547; $[\alpha]^{26}_D$ +62.1 (*c* 1.10, $CHCl_3$).

(2*S*,10*S*)-N^1,N^{11}-Diethyl-2,10-dihydroxynorspermine Tetrahydrochloride (8b). Compound **7b** (162 mg, 0.35 mmol) in 1 N HCl (2.84 mL) and EtOH (10 mL) was stirred under H_2 (1 atm) as described for the synthesis of **8a**. Recrystallization from aqueous EtOH gave **8b** (101 mg, 68%) as small white crystals: ^{1}H NMR (D_2O, NaTSP) δ 1.32 (t, 6 H, J = 7.3), 2.13 (m, 2 H), 3.03−3.28 (m, 16 H), 4.27 (tt, 2 H, J = 9.6, 3.2); ^{13}C NMR (D_2O; dioxane = 67.19 ppm) δ 11.02, 23.00, 43.93, 45.37, 49.99, 50.71, 63.50; HRMS *m/z* calcd for $C_{13}H_{33}N_4O_2$ 277.2604, found 277.2601; $[\alpha]^{24}_D$ +1.8 (*c* 0.77, 1 N HCl). Anal. ($C_{13}H_{36}Cl_4N_4O_2$) C, H, Cl, N.

***N*-Ethyl-*N*-[(2*S*)-2-hydroxy-3-[(1*R*)-1-phenylethylamino]propyl]-*p*-toluenesulfonamide (15).** A solution of **5** (23 mg, 91 μmol) and (*R*)-(+)-1-phenylethylamine (22 mg, 23 μL, 183 μmol) in EtOH (5 mL) was heated to reflux for 17 h under an Ar atmosphere. Solvent was removed, and the crude product was purified by flash chromatography (50% EtOAc/cyclohexane, then EtOAc) to give **15** (16 mg, 47%) as a colorless oil: ^{1}H NMR (CD_3OD) δ 1.01 (t, 3 H, J = 7.2), 1.37 (d, 3 H, J = 6.7), 2.42 (s, 3 H), 2.43 (dd, 1 H, J = 12.3, 7.7), 2.54 (dd, 1 H, J = 12.3, 4.4),

3.03 (dd, 1 H, J = 14.5, 6.8), 3.11−3.28 (m, 3 H), 3.76 (q, 1 H, J = 6.7), 3.83 (m, 1 H), 7.15−7.35 (m, 5 H), 7.36 (m, 2 H), 7.67 (m, 2 H); HRMS m/z calcd for $C_{20}H_{29}N_2O_3S$ 377.1899, found 377.1932; $[\alpha]^{24}_D$ +28.7 (c 0.78, $CHCl_3$).

***N*-Ethyl-*N*-[(2*S*)-2-hydroxy-3-(1-phenylethylamino)propyl]-*p*-toluenesulfonamide (*rac*-15).** A solution of **5** (19 mg, 76 μmol) and (±)-1-phenylethylamine (18 mg, 152 μmol) in EtOH (5 mL) was heated to reflux for 17 h under an Ar atmosphere. The reaction mixture was concentrated in vacuo, and the crude product was purified by flash chromatography (33% EtOAc/cyclohexane, then EtOAc) to yield ***rac*-15** (22 mg, 77%) as a colorless oil: ^{1}H NMR (CD_3OD) δ 1.01 (t, 3 H, J = 7.2), 1.03 (t, 3 H, J = 7.3), 1.37 (d, 6 H, J = 6.7), 2.33 (dd, 1 H, J = 12.3, 8.4), 2.42 (s, 6 H), 2.43 (dd, 1 H, J = 12.3, 7.7), 2.54 (dd, 1 H, J = 12.3, 4.4), 2.64 (dd, 1 H, J = 12.3, 3.7), 2.96−3.30 (m, 8 H), 3.76 (q, 2 H, J = 6.7), 3.83 (m, 1 H), 3.90 (m, 1 H), 7.17−7.34 (m, 10 H), 7.38 (m, 4 H), 7.67 (m, 4 H).

***N*-Ethyl-*N*-[(2*R*)-2-hydroxy-3-[(1*R*)-1-phenylethylamino]propyl]trifluoromethanesulfonamide (16).** By the procedure used for the preparation of **15**, **13** (19 mg, 82.3 μmol) was reacted with (*R*)-(+)-1-phenylethylamine (20 mg, 165 μmol) and purified by flash chromatography (5% CH_3OH/$CHCl_3$) to give **16** (23 mg, 79%) as a colorless oil: ^{1}H NMR (CD_3OD) δ 1.22 (t, 3 H, J = 7.1), 1.37 (d, 3 H, J = 6.8), 2.34 (dd, 1 H, J = 12.3, 8.1), 2.56 (dd, 1 H, J = 12.3, 4.0), 3.28−3.44 (m, 2 H), 3.57 (m, 1 H), 3.76 (q, 1 H, J = 6.6), 3.92 (m, 1 H), 7.15−7.35 (m, 5 H); HRMS m/z calcd for $C_{14}H_{22}F_3N_2O_3S$ 355.1303, found 355.1303; $[\alpha]^{24}_D$ +31.0 (c 1.10, $CHCl_3$).

***N*-Ethyl-*N*-[(2*R*)-2-hydroxy-3-(1-phenylethylamino)propyl]trifluoromethanesulfonamide (*rac*-16).** Compound **13** (23 mg, 101 μmol) was reacted with (±)-1-phenylethylamine (24 mg, 202 μmol) according to the procedure of ***rac*-15** and was purified by flash chromatography (5% $CH_3OH/CHCl_3$) to give ***rac*-16** (29 mg, 81%) as a colorless oil: ^{1}H NMR (CD_3OD) δ 1.21 (t, 3 H, J = 7.3), 1.22 (t, 3 H, J = 7.1), 1.37 (d, 3 H, J = 6.8), 1.37 (d, 3 H, J = 6.8), 2.34 (dd, 1 H, J = 12.3, 8.1), 2.45 (d, 2 H, J = 6.2), 2.56 (dd, 1 H, J = 12.3, 4.0), 3.15−3.65 (m, 4 H), 3.75 (q, 1 H, J = 6.6), 3.76 (q, 1 H, J = 6.6), 3.85 (m, 1 H), 3.92 (m, 1 H), 7.15−7.35 (m, 10 H).

(2*R*,10*R*)-*N*^{1},*N*^{11}-Diethyl-2,10-dihydroxy-*N*^{1},*N*^{4},*N*^{8},*N*^{11}-tetrakis(benzyloxycarbonyl)norspermine (17a). Potassium bicarbonate (197 mg, 1.97 mmol) and *N*-(benzyloxycarbonyloxy)succinimide (112 mg, 0.45 mmol) were added to a mixture of **8a** (32 mg, 75 μmol) in H_2O (5 mL) and Et_2O (5 mL) at 0 °C, and the reaction mixture was stirred at room temperature for 4 h. Water (20 mL) was added, and the layers were separated. The aqueous layer was extracted with Et_2O (3 × 30 mL). The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. The crude product was purified by flash chromatography (50% then 60% EtOAc/cyclohexane) to give **17a** (33 mg, 54%) as a colorless oil: ^{1}H NMR (CD_3OD) δ 0.92−1.22 (m, 6 H), 1.55−1.95 (m, 2 H), 2.80−3.60 (m, 16 H), 3.85−4.10 (m, 2 H), 4.90−5.25 (m, 8 H), 7.10−7.50 (m, 20 H); HRMS m/z calcd for $C_{45}H_{57}N_4O_{10}$ 813.4075, found 813.4064; $[\alpha]^{24}_D$ −5.1 (c 1.00, 1 N HCl).

(2*R*,10*R*)-2,10-Bis[(*S*)-α-methoxy-α-(trifluoromethyl)phenylacetoxy]-*N*^{1},*N*^{11}-diethyl-*N*^{1},*N*^{4},*N*^{8},*N*^{11}-tetrakis(benzyloxycarbonyl)norspermine (18a). The reaction was carried out in an oven-dried 5- × 175-mm NMR tube, fitted with a rubber septum, under an Ar atmosphere. The reagents were injected via syringe in the following order: anhydrous pyridine (300 μL), (*R*)-(−)-Mosher's acid chloride (24 μL, 108 μmol), CCl_4 (200 μL), and then a solution of **17a** (23 mg, 28 μmol) in CCl_4 (500 μL). The reaction mixture was shaken and allowed to stand at room temperature for 18 h. $CHCl_3$ (20 mL) was added, and the solution was washed with saturated $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash chromatography (25% EtOAc/cyclohexane) to give **18a** (35 mg, 100%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 45 °C) δ 1.02 (m, 6 H), 1.50 (m, 2 H), 2.75 (m, 2 H), 3.44 (s, 3 H), 2.85−3.70 (m, 14 H), 4.80−5.20 (m, 8 H), 5.43 (m, 2 H), 7.20−7.48 (m, 30 H); ^{19}F NMR ($CDCl_3$, $CFCl_3$, 45 °C) δ −71.75; ^{19}F NMR (12% $CDCl_3$/CD_3OD, $CFCl_3$, 45 °C) δ −71.20; HRMS m/z calcd for $C_{65}H_{71}F_6N_4O_{14}$ 1245.4871, found 1245.4818; $[\alpha]^{26}_D$ −27.2 (c 1.68, $CHCl_3$).

(2*S*,10*S*)-*N*^{1},*N*^{11}-Diethyl-2,10-dihydroxy-*N*^{1},*N*^{4},*N*^{8},*N*^{11}-tetrakis(benzyloxycarbonyl)norspermine (17b). According to the procedure described for the preparation of **17a**, **8b** (37 mg, 89 μmol) was reacted with $KHCO_3$ (233 mg, 2.32 mmol) and *N*-(benzyloxycarbonyloxy)succinimide (132 mg, 0.53 mmol). Purification of the crude product by flash chromatography (10%, then 25% acetone/$CHCl_3$) gave **17b** (52 mg, 72%) as a colorless oil: ^{1}H NMR (CD_3OD) δ 0.95−1.20 (m, 6 H), 1.75 (m, 2 H), 2.80−3.60 (m, 16 H), 3.96 (m, 2 H), 4.95−5.20 (m, 8 H), 7.10−7.50 (m, 20 H); HRMS m/z calcd for $C_{45}H_{57}N_4O_{10}$ 813.4075, found 813.4083; $[\alpha]^{27}_D$ +4.7 (c 1.01, 1 N HCl).

(2*S*,10*S*)-2,10-Bis[(*S*)-α-methoxy-α-(trifluoromethyl)phenylacetoxy]-*N*^{1},*N*^{11}-diethyl-*N*^{1},*N*^{4},*N*^{8},*N*^{11}-tetrakis(benzyloxycarbonyl)norspermine (18b). According to the procedure given for the synthesis of **18a**, **17b** (51 mg, 69 μmol) was reacted with (*R*)-(−)-Mosher's acid chloride (38 μL, 176 μmol) to furnish **18b** (65 mg, 94%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 45 °C) δ 0.96 (m, 6 H), 1.55−1.75 (m, 2 H), 3.41 (s, 3 H), 2.85−3.65 (m, 16 H), 4.90−5.20 (m, 8 H), 5.43 (m, 2 H), 7.22−7.48 (m, 30 H); ^{19}F NMR ($CDCl_3$, $CFCl_3$, 45 °C) δ −71.81; ^{19}F NMR (12% $CDCl_3$/CD_3OD, $CFCl_3$, 45 °C) δ −71.27; HRMS m/z calcd for $C_{65}H_{71}F_6N_4O_{14}$ 1245.4871, found 1245.4852; $[\alpha]^{26}_D$ −13.6 (c 1.64, $CHCl_3$). Anal. ($C_{65}H_{70}F_6N_4O_{14}$) C, H, N.

Benzyl (2*S*)-4-(Ethylamino)-2-hydroxy-4-oxobutanoate (20). *N*-Hydroxysuccinimide (6.83 g, 59.4 mmol) and DCC (12.25 g, 59.4 mmol) were added to **19** (12.10 g, 54.0 mmol) in CH_2Cl_2 (300 mL) under an Ar atmosphere at 0 °C. The ice bath was removed after 30 min, and the reaction mixture was stirred at room temperature for an additional 4 h. Solids were filtered and washed with CH_2Cl_2, and the solvent was removed in vacuo. The residue was dissolved in THF (100 mL) and cooled to 0 °C, and ethylamine in THF (2.0 M, 27 mL, 54 mmol) was added dropwise. The reaction mixture was stirred for 2 h at 0 °C and then concentrated to dryness. The residue was dissolved in EtOAc (250 mL) and extracted with saturated $NaHCO_3$ (50 mL) and brine (50 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. Flash chromatography (25% hexane/EtOAc) afforded **20** (8.72 g, 64%) as a white solid: mp 64−66 °C; ^{1}H NMR ($CDCl_3$) δ 1.12 (t, 3 H, J = 7.3), 2.61 (dd, 1 H, J = 15.2, 6.8), 2.72 (dd, 1 H, J = 15.4, 4.0), 3.27 (m, 2 H), 3.73 (d, 1 H, J = 4.2), 4.53 (m, 1 H), 5.21 (d, 1 H, J = 12.1), 5.26 (d, 1 H, J = 12.1), 5.76 (br m, 1 H), 7.32−7.42 (m, 5 H); $[\alpha]^{22}_D$ −23.4 (c 1.14, CH_3OH). Anal. ($C_{13}H_{17}NO_4$) C, H, N.

(2*S*)-4-[*N*-(Benzyloxycarbonyl)ethylamino]-1,2-butanediol (21). A solution of BH_3 in THF (1 M, 160 mL, 160 mmol) was added dropwise to **20** (8.50 g, 33.8 mmol) in THF (150 mL) at room temperature under an Ar atmosphere. After 17 h, 4 N HCl was slowly added until the pH reached 4. The reaction mixture was stirred at room temperature for 4 h, then concentrated, and neutralized with 1 N NaOH; Et_2O (200 mL) was added. *N*-(Benzyloxycarbonyloxy)succinimide (9.26 g, 37.2 mmol) and $NaHCO_3$ (2.84 g, 33.8 mmol) were added at 0 °C, and the mixture was stirred at room temperature for 16 h. The layers were separated, and the aqueous layer was further extracted with EtOAc (3 × 100 mL). The organic layers were washed with $NaHCO_3$ (50 mL), dried over Na_2SO_4, and solvent was removed in vacuo. Flash chromatography (5% CH_3OH/$CHCl_3$) gave **21** (3.00 g, 33%) as a colorless oil: ^{1}H NMR (CD_3OD) δ 1.13 (t, 3 H, J = 7.0), 1.56 (m, 1 H), 1.80 (m, 1 H), 3.30−3.55 (m, 6 H), 3.57 (m, 1 H), 5.11 (s, 2 H), 7.25−7.48 (m, 5 H); ^{13}C NMR (CD_3OD) δ 13.53, 14.17, 33.07, 33.65, 43.25, 43.60, 44.95, 45.31, 67.32, 68.13, 70.96, 128.83, 129.05, 129.53, 138.25, 157.95; HRMS m/z calcd for $C_{14}H_{22}NO_4$ 268.1549, found 268.1542; $[\alpha]^{24}_D$ −3.8 (c 1.03, $CHCl_3$). Anal. ($C_{14}H_{21}NO_4$) C, H, N.

(2*S*)-4-[*N*-(Benzyloxycarbonyl)ethylamino]-1-*p*-toluenesulfonato-2-butanol (22). *p*-TsCl (2.20 g, 11.5 mmol) was added to a solution of **21** (2.80 g, 10.5 mmol) in CH_2Cl_2 (20 mL) and pyridine (20 mL) at 0 °C under an Ar atmosphere. The reaction mixture was stirred for 1 h, then kept at 5 °C for

16 h, and stirred again at 0 °C for 1.5 h. The solution was diluted with H_2O (100 mL) and extracted with $CHCl_3$ (3 × 100 mL). The combined organic layers were washed with 0.5 N HCl (100 mL) and H_2O (100 mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash chromatography (40% EtOAc/hexane) to give **22** (3.16 g, 72%) as a colorless oil: ^{1}H NMR (CD_3OD) δ 1.09 (t, 3 H, J = 7.0), 1.54 (m, 1 H), 1.69 (m, 1 H), 2.45 (s, 3 H), 3.20−3.40 (m, 4 H), 3.70 (m, 1 H), 3.90 (m, 2 H), 5.10 (s, 2 H), 7.24−7.40 (m, 5 H), 7.42 (d, 2 H, J = 8.1), 7.78 (m, 2 H); HRMS *m/z* calcd for $C_{21}H_{28}NO_6S$ 422.1637, found 422.1664; $[\alpha]^{22}_D$ −0.8 (*c* 0.98, $CHCl_3$). Anal. ($C_{21}H_{27}NO_6S$) C, H, N.

(3*S*)-*N*-(Benzyloxycarbonyl)-*N*-ethyl-3,4-epoxybutylamine (23). Potassium Bicarbonate (1.13 g, 8.18 mmol) in CH_3OH (100 mL) was added to **22** (3.13 g, 7.44 mmol). The mixture was stirred at room temperature under an Ar atmosphere for 100 min, then poured into a mixture of H_2O (100 mL) and CH_2Cl_2 (100 mL). The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic extracts were dried over Na_2SO_4, and the solvent was removed in vacuo. Flash chromatography (33% EtOAc/hexane) afforded **23** (1.59 g, 86%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.14 (t, 3 H, J = 6.8), 1.78 (m, 1 H), 1.86 (m, 1 H), 2.45 (m, 1 H), 2.72 (m, 1 H), 2.92 (m, 1 H), 3.24−3.55 (m, 4 H), 5.14 (s, 2 H), 7.25−7.44 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 13.25, 13.87, 31.56, 32.07, 42.12, 42.46, 43.52, 44.28, 46.89, 50.14, 66.89, 127.77, 127.86, 128.41, 136.85, 155.94; HRMS *m/z* calcd for $C_{14}H_{20}NO_3$ 250.1443, found 250.1442; $[\alpha]^{23}_D$ −11.6 (*c* 0.97, $CHCl_3$). Anal. ($C_{14}H_{19}NO_3$) C, H, N.

(3*S*,12*S*)-N^1,N^{14}-Diethyl-3,12-dihydroxy-N^1,N^5,N^{10},N^{14}-tetrakis(benzyloxycarbonyl)homospermine (28a). A solution of **23** (1.18 g, 4.33 mmol) and **24** (209 mg, 2.37 mmol) was heated in EtOH (100 mL) at 55 °C for 3 days under an Ar atmosphere. The solvent was removed under reduced pressure to give **26**, which was dissolved in $CHCl_3$ (100 mL) and cooled to 0 °C, followed by addition of benzyl chloroformate (1.01 g, 5.93 mmol) and triethylamine (959 mg, 9.48 mmol). The ice bath was removed after 5 min, and the reaction mixture was stirred at room temperature for 2 h and diluted with $CHCl_3$ (100 mL), which was washed with 1 N HCl (20 mL), H_2O (20 mL), and brine (20 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. Purification by flash chromatography (33%, then 25% hexane/EtOAc, then 10% $CH_3OH/CHCl_3$) gave **28a** (453 mg, 22%) as a colorless oil: ^{1}H NMR (CD_3OD) δ 1.10 (m, 6 H), 1.35−1.82 (m, 8 H), 2.90−3.55 (m, 16 H), 3.74 (m, 2 H), 5.09 (s, 8 H), 7.22−7.42 (m, 20 H); HRMS *m/z* calcd for $C_{18}H_{63}N_4O_{10}$ 855.4544, found 855.4500; $[\alpha]^{21}_D$ +3.3 (*c* 0.97, CH_3OH).

(3*S*,12*S*)-N^1,N^{14}-Diethyl-3,12-dihydroxyhomospermine Tetrahydrochloride (29a). Hydrochloric acid (1 N, 4.0 mL) and 10% Pd−C (50 mg) were introduced into **28a** (427 mg, 0.50 mmol) in EtOH (40 mL). The reaction mixture was stirred under H_2 (1 atm) for 20 h, filtered through Celite, and concentrated in vacuo. The residue was taken up in H_2O (3 × 5 mL), filtered through a syringe filter (0.2 μm), and concentrated in vacuo. Recrystallization from aqueous EtOH gave **29a** (196 mg, 85%) as white crystals: ^{1}H NMR (D_2O, NaTSP) δ 1.30 (t, 6 H, J = 7.3), 1.74−2.04 (m, 8 H), 2.98−3.32 (m, 16 H), 4.05 (tt, 2 H, J = 9.6, 3.3); ^{13}C NMR (D_2O, 1,4-dioxane = 67.19 ppm) δ 11.14, 23.25, 31.00, 43.63, 44.39, 47.58, 52.70, 65.22; HRMS *m/z* calcd for $C_{16}H_{39}N_4O_2$ 319.3073, found 319.3073; $[\alpha]^{23}_D$ +8.7 (*c* 1.05, 1 N HCl). Anal. ($C_{16}H_{42}Cl_4N_4O_2$) C, H, Cl, N.

Dimethyl (2*R*)-2-(Benzyloxy)succinate (31). Compound **31** was prepared by benzylation of (*R*)-dimethyl malate with benzyl 2,2,2-trichloroacetimidate in 85% yield using a literature method:[36] $[\alpha]^{24}_D$ +70.7 (lit. $[\alpha]^{23}_D$ +70)[36] (*c* 1.0, $CHCl_3$).

(2*R*)-2-(Benzyloxy)-1,4-butanediol (32). Compound **32** was made in 83% yield using a literature procedure[37] by reduction of **31** modified with the use of $LiBH_4$: $[\alpha]^{23}_D$ +15 (lit. $[\alpha]^{23}_D$ +15)[37] (*c* 1.0, $CHCl_3$).

(2*R*)-2-(Benzyloxy)-*O*,*O*′-ditosyl-1,4-butanediol (33). Compound **33** was made in 87% yield by treatment of **32** with *p*-TsCl in pyridine by a known method:[38] mp 86−87 °C (lit. ***ent*-33**)[39] mp 93 °C; ^{1}H NMR ($CDCl_3$) δ 1.72−1.91 (m, 2 H), 2.42 (s, 3 H), 2.44 (s, 3 H), 3.69−3.77 (m, 1 H), 3.94−4.19 (m, 4 H), 4.30 (d, 1 H, J = 11.4), 4.49 (d, 1 H, J = 11.4), 7.12−7.18 (m, 2 H), 7.25−7.36 (m, 7 H), 7.72−7.79 (m, 4 H); $[\alpha]^{24}_{546}$ +34.1 (lit. ***ent*-33** $[\alpha]^{25}_{546}$ −34.9)[39] (*c* 0.64, $CHCl_3$).

(2*R*)-2-(Benzyloxy)-4-[*N*-(mesitylenesulfonyl)ethylamino]-*O*-tosyl-1-butanol (34). Sodium hydride (60%, 1.5 g, 37 mmol) was added to *N*-ethylmesitylenesulfonamide[42] (6.5 g, 29 mmol) in DMF (20 mL) at 0 °C under a N_2 atmosphere, and the mixture was stirred for 0.5 h. A solution of **33** (18.7 g, 37.1 mmol) in DMF (100 mL) was added to the reaction mixture, followed by stirring for 16 h at room temperature. The mixture was cooled in an ice bath, cautiously quenched with H_2O (10 mL), concentrated in vacuo, and extracted with EtOAc (150 mL). The extract was washed with H_2O (150 mL) and brine (150 mL) and then dried over Na_2SO_4. Solvent removal in vacuo followed by flash chromatography (25% EtOAc/cyclohexane) gave **34** (19.8 g, 77%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.01 (t, 3 H, J = 7.1), 1.69 (m, 2 H), 2.27 (s, 3 H), 2.44 (s, 3 H), 2.55 (s, 6 H), 3.11−3.32 (m, 4 H), 3.51−3.59 (m, 1 H), 3.94 (d, 1 H, J = 4.8), 3.96 (d, 1 H, J = 4.8), 4.37 (d, 1 H, J = 11.4), 4.41 (d, 1 H, J = 11.4), 6.90 (s, 2 H), 7.17−7.21 (m, 2 H), 7.28−7.34 (m. 5 H), 7.73−7.78 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 12.79, 20.87, 21.60, 22.69, 29.61, 40.33, 41.38, 70.60, 71.99, 74.06, 127.67, 127.74, 127.87, 128.30, 129.87, 131.90, 132.70, 133.19, 137.65, 140.03, 142.34, 144.92; HRMS calcd for $C_{29}H_{38}NO_6S_2$ 560.2141 (M + 1), found 560.2124; $[\alpha]^{23}_D$ +14 (*c* 1.00, CH_3OH). Anal. ($C_{29}H_{37}NO_6S_2$) C, H, N.

(3*R*,12*R*)-Dibenzyloxy-N^1,N^{14}-diethyl-N^1,N^5,N^{10},N^{14}-tetrakis(mesitylenesulfonyl)homospermine (35). Sodium hydride (60%, 1.37 g, 33.9 mmol) was added to *N*,*N*′-bis-(mesitylenesulfonyl)-1,4-butanediamine[40] (5.1 g, 11 mmol) in DMF (50 mL) at 0 °C under a N_2 atmosphere, and the mixture was stirred for 0.5 h. Sodium iodide (107 mg, 0.70 mmol) and **34** (13.3 g, 23.6 mmol) in DMF (100 mL) were added, followed by stirring at 60 °C for 16 h. The mixture was cooled in an ice bath, cautiously quenched with H_2O (20 mL), concentrated in vacuo, and extracted with EtOAc (200 mL). The extract was washed with 1% $NaHSO_3$ (150 mL), H_2O (2 × 150 mL), and brine (150 mL), then dried over Na_2SO_4. Solvent removal in vacuo followed by flash chromatography (1:2:7 MeOAc:petroleum ether:toluene) gave **35** (7.0 g, 50%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.98 (t, 6 H, J = 7.2) 1.46−1.65 (m, 8 H), 2.26 (s, 6 H), 2.28 (s, 6 H), 2.54 (s, 12 H), 2.55 (s, 12 H), 3.04−3.18 (m, 16 H), 3.28−3.36 (m, 2 H), 4.24 (d, 2 H, J = 11.4), 4.32 (d, 2 H, J = 11.4), 6.90 (d, 8 H, J = 5.7), 7.18−7.34 (m, 10 H); HRMS calcd for $C_{66}H_{91}N_4O_{10}S_4$ 1227.5618 (M + 1), found 1227.5701; $[\alpha]^{23}_D$ +0.8 (*c* 1.00, CH_3OH).

(3*R*,12*R*)-N^1,N^{14}-Diethyl-3,12-dihydroxy-N^1,N^5,N^{10},N^{14}-tetrakis(mesitylenesulfonyl)homospermine (36). A solution of **35** (9.33 g, 7.60 mmol) in HOAc (300 mL) and H_2O (15 mL) was divided into two about equal portions. Palladium black was added to each (1.03 and 1.07 g), and the mixtures were shaken under 1 atm H_2 at room temperature (3.5 and 4 h, respectively).[41] The mixtures were filtered through Celite; solids were washed with HOAc (3 × 50 mL). Combined washings and filtrates were concentrated in vacuo; the resulting oil was purified by flash chromatography (50% hexane/EtOAc) to give **36** (6.87 g, 86%) as a white foam: mp 135−137 °C; ^{1}H NMR ($CDCl_3$) δ 0.98 (t, 6 H, J = 7.5), 1.33−1.54 (m, 8 H), 2.29 (s, 6 H), 2.30 (s, 6 H), 2.57 (s, 24 H), 3.02−3.32 (m, 14 H), 3.36−3.48 (m, 2 H), 3.69−3.81 (br m, 2 H), 6.94 (s, 4 H), 6.95 (s, 4 H); HRMS calcd for $C_{52}H_{79}N_4O_{10}S_4$ 1047.4679 (M + 1), found 1047.4710; $[\alpha]^{26}_D$ −2.4 (*c* 0.97, $CHCl_3$). Anal. ($C_{52}H_{78}N_4O_{10}S_4$) C, H, N.

(3*R*,12*R*)-N^1,N^{14}-Diethyl-3,12-dihydroxyhomospermine Tetrahydrochloride (29b). A solution of **36** in DME (80 mL) was cooled to −78 °C under a N_2 atmosphere and titrated with a solution of sodium naphthalenide in DME [freshly prepared by stirring Na (3.00 g, 130 mmol) and naphthalene (19.50 g, 143 mmol) in DME (80 mL) under N_2 for 2.5 h at room temperature].[23] Upon reaching a dark green endpoint, the mixture was quenched with saturated $NaHCO_3$ (25 mL) and allowed to warm to room temperature. Potassium carbonate (45 g) was added, and the mixture was stirred

overnight under N_2. Diethyl ether (150 mL) was added, the mixture was filtered, and solids were washed with Et_2O (3 × 50 mL). The combined filtrate and washings were concentrated in vacuo, and 1 N HCl (130 mL) was added. The mixture was acidified to pH 1 with concentrated HCl, then filtered; solvents were removed in vacuo. The residue was suspended in EtOH (160 mL) and centrifuged; the brown supernatant was decanted. The remaining solids were dissolved in 2 N NaOH (15 mL) and extracted with Et_2O (10 × 100 mL). The combined extracts were dried over Na_2SO_4 and filtered, and solvents were removed in vacuo. The product was dissolved in EtOH (40 mL) and acidified to pH 1 with 1 N HCl. Solvent removal in vacuo gave 0.35 g of **29b** (21%) as white plates: mp > 280 °C; ^{1}H NMR (D_2O, NaTSP) δ 1.29 (t, 6 H, J = 7.4), 1.75−2.03 (m, 8 H), 3.02−3.38 (m, 16 H), 4.05 (tt, 2 H, J = 9.7, 3.2); ^{13}C NMR (D_2O, 1,4-dioxane = 67.19 ppm) δ 11.16, 23.24, 31.00, 43.64, 44.41, 47.57, 52.69, 65.22; HRMS calcd for $C_{16}H_{39}N_4O_2$ 319.3073 (M + 1), found 319.3102; $[\alpha]^{25}_D$ −8.6 (*c* 1.00, 1 N HCl). Anal. ($C_{16}H_{42}Cl_4N_4O_2$) C, H, N.

(3*S*,12*S*)-3,12-Bis[(*S*)-α-methoxy-α-(trifluoromethyl)-phenylacetoxy]-*N*^{1},*N*^{14}-diethyl-*N*^{1},*N*^{5},*N*^{10},*N*^{14}-tetrakis(benzyloxycarbonyl)homospermine (37a). Compound **28a** (20 mg, 23 μmol) was reacted with (*R*)-(−)-Mosher's acid chloride (14 μL, 73 μmol) using the procedure of **18a**. Flash chromatography (33% EtOAc/hexane) furnished **37a** (27 mg, 90%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 45 °C) δ 1.02 (t, 6 H, J = 7.0), 1.32 (m, 4 H), 1.82 (m, 4 H), 2.90−3.54 (m, 16 H), 3.43 (s, 6 H), 5.09 (s, 8 H), 5.23 (m, 2 H), 7.20−7.60 (m, 30 H); ^{19}F NMR ($CDCl_3$, $CFCl_3$ as internal standard, 45 °C) δ −71.57; HRMS *m*/*z* calcd for $C_{68}H_{77}F_6N_4O_{14}$ 1287.5340, found 1287.5319; $[\alpha]^{22}_D$ −7.7 (*c* 1.35, $CHCl_3$).

(3*R*,12*R*)-*N*^{1},*N*^{14}-Diethyl-3,12-dihydroxy-*N*^{1},*N*^{5},*N*^{10},*N*^{14}-tetrakis(benzyloxycarbonyl)homospermine (28b). A sample of **29b** (50 mg, 108 μmol) was reacted with *N*-(benzyloxycarbonyloxy)succinimide (162 mg, 0.65 mmol) utilizing the procedure of **17a**. Purification by flash chromatography (25% hexane/EtOAc) gave **28b** (59 mg, 64%) as a colorless oil: ^{1}H NMR (CD_3OD) δ 1.10 (m, 6 H), 1.35−1.82 (m, 8 H), 2.94−3.50 (m, 16 H), 3.74 (m, 2 H), 5.09 (s, 8 H), 7.22−7.38 (m, 20 H); HRMS *m*/*z* calcd for $C_{48}H_{63}N_4O_{10}$ 855.4544, found 855.4513; $[\alpha]^{25}_D$ −3.2 (*c* 0.95, CH_3OH).

(3*R*,12*R*)-3,12-Bis[(*S*)-α-methoxy-α-(trifluoromethyl)-phenylacetoxy]-*N*^{1},*N*^{14}-diethyl-*N*^{1},*N*^{5},*N*^{10},*N*^{14}-tetrakis(benzyloxycarbonyl)homospermine (37b). Compound **28b** (36 mg, 42 μmol) was reacted with (*R*)-(−)-Mosher's acid chloride (30 μL, 161 μmol) as in the preparation of **18a**. Flash chromatography (33% EtOAc/hexane) gave **37b** (50 mg, 92%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 45 °C) δ 1.05 (t, 6 H, J = 6.6), 1.12−1.36 (m, 4 H), 1.72−1.98 (m, 4 H), 2.70−2.88 (m, 2 H), 2.90−3.09 (m, 2 H), 3.11−3.42 (m, 12 H), 3.48 (s, 6 H), 4.97−5.08 (m, 4 H), 5.11 (s, 2 H), 5.17−5.33 (m, 4 H), 7.23−7.52 (m, 30 H); ^{19}F NMR ($CDCl_3$, $CFCl_3$ as internal standard, 45 °C) δ −71.80; HRMS *m*/*z* calcd for $C_{68}H_{77}F_6N_4O_{14}$ 1287.5340, found 1287.5384; $[\alpha]^{25}_D$ −22.4 (*c* 1.33, $CHCl_3$).

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 while in logarithmic growth (0.5−1.0 × 10^5 cells/mL) with the polyamine derivatives dissolved in sterile water and filtered through a 0.2-μm syringe filter. Following a 48-h period, cells were reseeded and incubated for an additional 48 h.

After the indicated time periods, 200 μL of cell suspension was removed from the flask for counting. Cell number was determined by electronic particle counting (model Z_F coulter counter, Coulter Electronics, Hialeah, FL) and confirmed periodically with hemocytometer measurements.

The percentage of control growth was determined as follows:

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

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

Polyamine Pool Analysis. While in logarithmic growth, cells were treated with the polyamine derivatives. At the end of the treatment period, cell suspensions were sampled, washed twice in ice-cold, incomplete medium, and pelleted for extraction using 0.6 N perchloric acid,[40] then freeze-fractured in liquid nitrogen/hot water three times. Each supernatant was frozen at −20 °C until analysis of polyamine content by HPLC.[19]

Uptake Determinations. The polyamine derivatives were studied for their ability to compete with [^{3}H]SPD for uptake into L1210 leukemia cells in vitro.[5,40] Cell suspensions were incubated in 1 mL of culture medium containing 1, 2, 4, 6, 8, and 10 μM radiolabeled SPD alone or in the presence of 10, 25, and 50 μM polyamine analogue for 20 min at 37 °C. At the end of the incubation period, tubes were centrifuged at 1200 rpm for 5 min at 0−4 °C. The pellet was washed twice with 5 mL of cold RPMI-1640 containing 1 mM SPD, digested in 200 μL of 1 N NaOH at 60 °C for 1 h, and neutralized with 500 μL 1 N HCl. The material was transferred to a vial for scintillation counting. Lineweaver−Burke plots indicated a simple competitive inhibition with respect to SPD.

Enzyme Assays. ODC and AdoMetDC activities were determined according to the procedures of Seely and Pegg[43] and Pegg and Pösö,[44] 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.[11] Cells treated with DENSPM were positive controls.

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