tioned media was returned to the cultures. Viability was assessed the next day with the vital stains fluorescein diacetate and propidium iodide.[49] At least 100 cells in randomly chosen fields were counted per culture to determine percent viability. Each compound was tested at $n = 3$ with cultures prepared on different days.

Modified LDL Studies. Noninduced resident peritoneal macrophage (MPM) cells were isolated from BALB/c mice as described by Schultz and co-workers,[50] resuspended in Dulbecco's modified Eagle's medium (DMEM) at a concentration of (2–5) $\times 10^6$ cells/mL, and stored in ice until needed. The macrophage cell counts were estimated using a hemacytometer.

Degradation of [^{125}I]lipoprotein (BTI, MA) was measured according to the modified methods of Henriksen et al.[51] and Drevon et al.[52] Aliquots of freshly prepared MPM were incubated with [^{125}I]lipoprotein (1–5 μg/mL) in triplicate at 37 °C for 4 h in DMEM (1.5 mL) containing 10% FCS and gentamicin (50 μg/mL) in polystyrene tubes.[20] The degradation was terminated by adding bovine serum albumin (final concentration, 10 mg/mL) and TCA (final concentration, 10%). After centrifugation, the supernatant was filtered through a 0.45-μm Millipore (type HAWP) filter. $AgNO_3$ (final concentration, 1.5%) was added to precipitate free iodide, and the radioactivity of the supernatant was measured in a Micromedic System gamma spectrometer. Specific degradation was defined as the total radioactivity in the medium minus the radioactivity not inhibited by 200 μg/mL Ac-LDL or Cu^{2+}-oxidized LDL. The specific degradation of [^{125}I]Ac-LDL was typically about 95% at an [^{125}I]Ac-LDL concentration of 1.5 μg/mL. The level of significance between groups was calculated using Student's t test.

Cu^{2+}-catalyzed oxidative LDL modification was performed in the following manner: radiolabeled or unlabeled LDL (100–300 μg/mL) in the presence or absence of test agent was incubated with Cu^{2+} (5 μM) in HBSS-Tris HCl medium (pH 8.3) in polystyrene tubes at 37 °C for 20 h. After incubation, aliquots of unlabeled samples were used immediately for an estimate of LDL oxidation by measuring its ability to competitively inhibit scavenger receptor mediated degradation of [^{125}I]Ac-LDL by MPM. Aliquots of ^{125}I-radiolabeled LDL, on the other hand, were first diluted and the degree of oxidation assessed by direct MPM scavenger receptor mediated degradation as described above.

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Registry No. 1, 50-53-3; 2, 92-84-2; 3, 1771-18-2; 4, 24316-35-6; 5, 137966-36-0; 6, 137966-38-2; 7, 137966-39-3; 8, 138008-49-8; 9, 137966-40-6; 10, 1927-44-2; 11, 137966-41-7; 12, 137966-42-8; 13, 137966-43-9; 14, 137966-44-0; 15, 137966-45-1; 16, 137966-46-2; 17, 137966-47-3; 18, 6631-94-3; 19, 137966-48-4; 19 alcohol derivative, 137966-49-5; 19 acetate derivative, 137966-50-8; 20, 137966-51-9; 21, 137966-52-0; 22, 137966-53-1; 22 bromide derivative, 137966-54-2; 23, 137966-55-3; 24, 137966-56-4; ethyl 2-(10*H*-phenothiazin-2-yloxy)acetate, 137966-57-5.

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α-Methyl Polyamines: Metabolically Stable Spermidine and Spermine Mimics Capable of Supporting Growth in Cells Depleted of Polyamines

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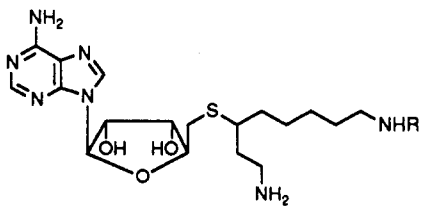
In order to assess the tolerance of the target enzyme spermine synthase for α-substituents on the aminopropyl moiety of the substrate spermidine, 1-methylspermidine (MeSpd, **2**) was synthesized. It was determined that MeSpd is a poor substrate for spermine synthase and is not a substrate for spermidine N^1-acetyltransferase, suggesting that α-methylated polyamines might be metabolically stable and therefore useful tools for studying polyamine effects in intact cells. On the basis of initial cellular results with **2**, 1-methylspermine (MeSpm, **3**) and 1,12-dimethylspermine (Me_2Spm, **4**) were also synthesized. When added to cells (L1210, SV-3T3, or HT29) depleted of both putrescine and spermidine by prior treatment with α-(difluoromethyl)ornithine (DFMO), these α-methylated polyamines were able to restore cell growth to that observed in the absence of DFMO. In accord with the enzyme data noted above, metabolic studies indicated a slow conversion of **2** to **3**, but no metabolism of **4** in these cells. It was concluded from these results that the α-methylated polyamines are able to substitute for the natural polyamines spermidine and spermine in critical biochemical processes which involve polyamines for continued cell growth. In accord with the hypothesis, preliminary data indicate that MeSpd and Me_2Spm are as effective as spermidine and spermine, respectively, in promoting the conversion of B-DNA to Z-DNA.

The polyamines putrescine, spermidine, and spermine are synthesized and degraded via a series of enzyme-catalyzed reactions which are now well-established[1] (Scheme I). We have described the synthesis[2,3] and biological activity[4,5] of two highly specific multisubstrate adduct inhibitors[6] of the aminopropyltransferases. These compounds, *S*-adenosyl-1,8-diamino-3-mercaptooctane (AdoDATO, **1a**) and *S*-adenosyl-1,12-diamino-3-mercapto-9-azadodecane (AdoDATAD, **1b**), are very potent inhibitors of spermidine synthase and spermine synthase, respectively. In the course of investigating the biological activity of these inhibitors, we observed that AdoDATAD was



1a: R = H
b: R = $(CH_2)_3NH_2$

† Pennsylvania State University.

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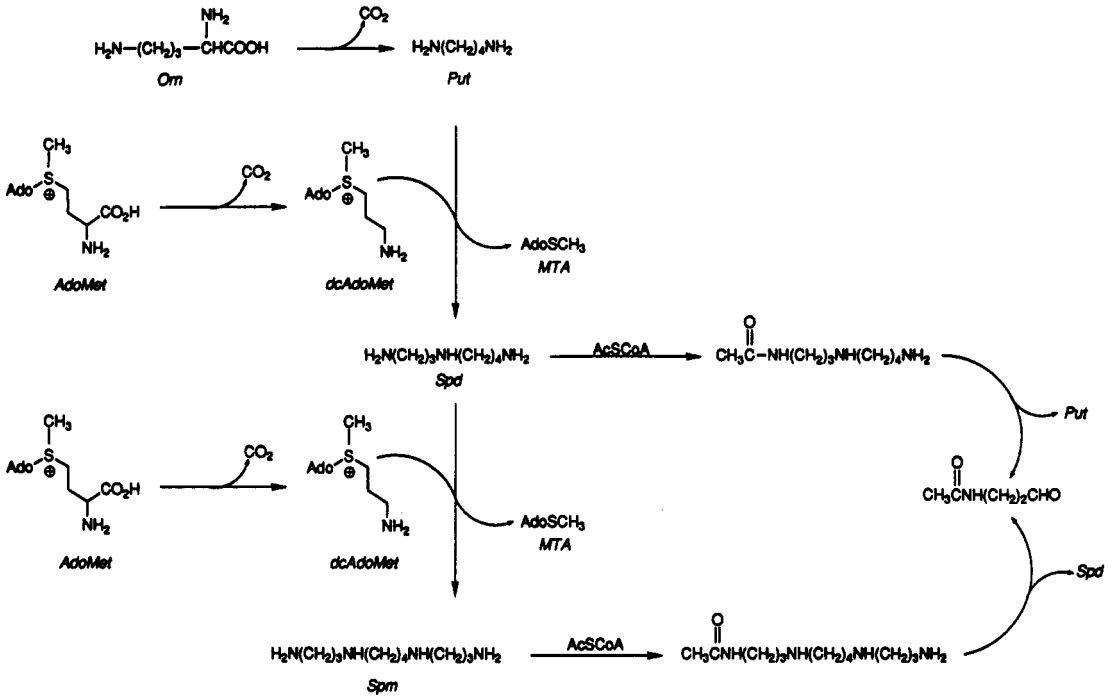
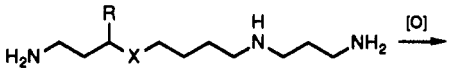
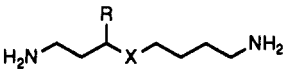
Scheme I



metabolized by serum amine oxidases, apparently to AdoDATO and the toxic metabolites acrolein and H_2O_2 (eq 1).[5] This is a well-known serum-mediated reaction



a: R = S-Ado, X = CH_2 (Ado-DATAD, **1b**)
b: R = H, X = NH (spermine)



a: R = S-Ado, X = CH_2 (AdoDATO, **1a**)
b: R = H, X = NH (spermidine)

$$+ CH_2{=}CHCHO + NH_3 + H_2O_2 \quad (1)$$

of the natural polyamines, e.g., spermine and spermidine, and is thought to be responsible for polyamine toxicity in cultured cells.[7] Although this type of metabolism can be prevented by coadministration of the amine oxidase inhibitor aminoguanidine,[5] we sought to prevent the AdoDATAD metabolism by alteration of the parent drug molecule. On the basis of numerous examples in the literature,[8] we focused our attention on α-methyl substituents as a way to prevent enzyme-catalyzed oxidative deamination. In order to assess the tolerance of the target enzyme spermine synthase for α-methyl substituents, we first synthesized 1-methylspermidine (**2**). Our reasoning was that if spermine synthase could catalyze the aminopropylation reaction of the α-methyl substituted substrate **2**, the corresponding multisubstrate adduct α-methyl-AdoDATAD should be an effective inhibitor of spermine synthase but not susceptible to serum amine oxidase-mediated metabolism. In this paper we report the synthesis of **2** and show that it is substrate, albeit a poor one, for spermine synthase, but it is also a very poor substrate for the other major enzyme of intracellular spermidine metabolism, spermidine/spermine N^1-acetyltransferase (SSAT, EC 2.3.1). As a result, we have synthesized two additional α-methyl polyamines, 1-methylspermine (**3**) and 1,12-dimethylspermine (**4**), in order to evaluate the use of metabolically stable analogues of the natural polyamines spermidine and spermine as probes of intracellular function of these biosynthetic cations.

Chemistry

Our strategy for synthesizing the α-methyl substituted polyamines **2–4** involved a disconnection across one of the internal carbon–nitrogen bonds. This bond might then be formed as an amide with an appropriately substituted β-amino acid and a suitably blocked putrescine derivative. Once this bond is formed, simple reduction of the amide to the secondary amine after deblocking of the terminal nitrogens should give the desired polyamine. This synthetic route is outlined in Scheme II.

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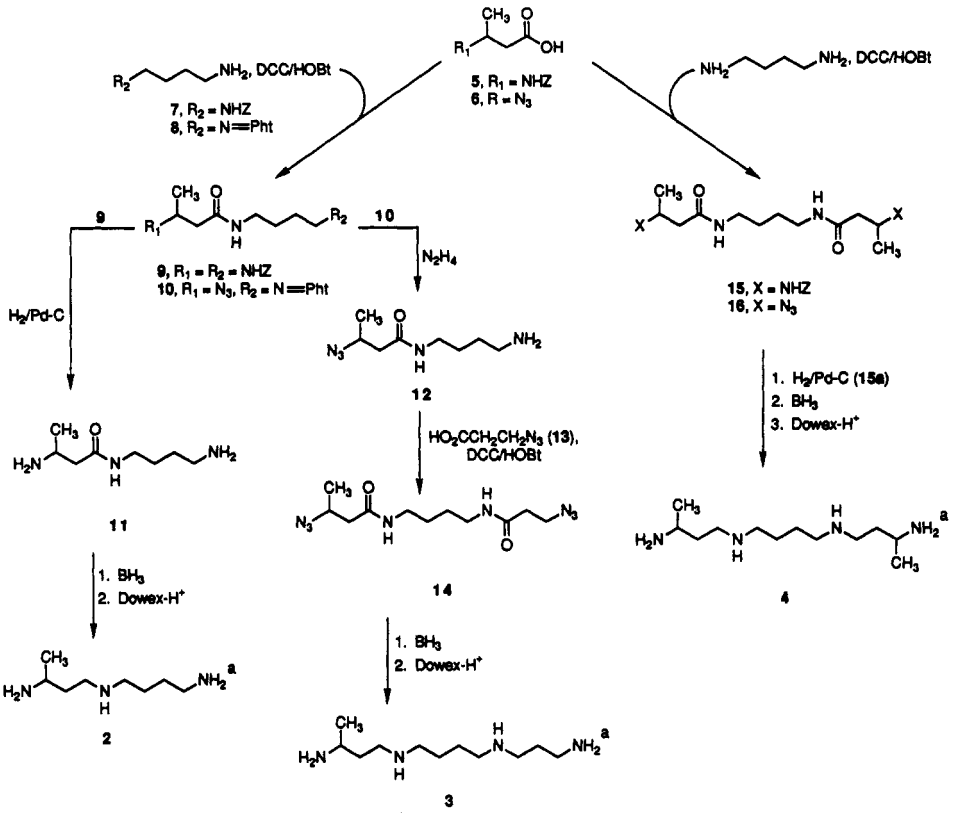
Scheme II[a]



[a] Isolated as HCl salt.

Commercially available D,L-3-aminobutyric acid was acylated with benzyl chloroformate (ZCl) to give D,L-3-(Z-amino)butyric acid **5**, a suitable precursor for coupling (DCC/HOBt) with N^4-Z-1,4-diaminobutane (**7**). Coupling of **5** and **7** gave amide **9** in moderate yield. Unfortunately, compound **9** proved to be especially insoluble in most organic solvents, thus complicating the purification of this material from the reaction byproduct DCU. Reduction of the amide functionality of **9** directly in BH_3·THF was impractical due to the poor solubility of **9** in THF. However, prior catalytic hydrogenation of the terminal carbobenzyloxy protecting groups to give the diamine compound **11** proved feasible since **11** was much more soluble in the reduction milieu. Conversion of **11** to 1-methylspermidine (**2**) using BH_3·THF was then accomplished in moderate yield.

In order to synthesize compounds **3** and **4** using this methodology, we wanted to address the solubility problems incurred with compound **9**. It was envisioned that the extended semirigid structure of **9** containing a relatively polar carbobenzyloxy group led to its poor solubility in THF. Replacement of the terminal Z-protected amines with the less polar azide functionality might lend greater solubility to these intermediates. It would also be expedient to be able to simultaneously reduce both the amide and azide moieties in order to liberate the tri- and tetraamines in one step, thus simplifying the synthesis. Conversion of crotonic acid to 3-azidobutyric acid (**6**) was accomplished in moderate yield. Compound **6** was then condensed with amine **8** to produce amide **10**. The phthalimido protecting group of **10** was then removed and the free amine **12** was condensed with 3-azidopropionic acid (**13**) to form diazido diamide **14**. In accord with our expectations, compound **14** was freely soluble in THF and could be reduced directly with BH_3·THF to polyamine **3**.

Similarly, the bis-Z (**15**) and bis-azido (**16**) versions of a symmetrical diamide were produced. Compound **15**, as expected, had the same solubility problems as found with compound **9**. For reduction, the same approach was taken; i.e., first removing the Z protecting groups via hydrogenation to produce an intermediate diamine which was then reduced to compound **4**. Compound **16**, however, was freely soluble in THF and could be reduced to compound **4** directly with BH_3·THF.

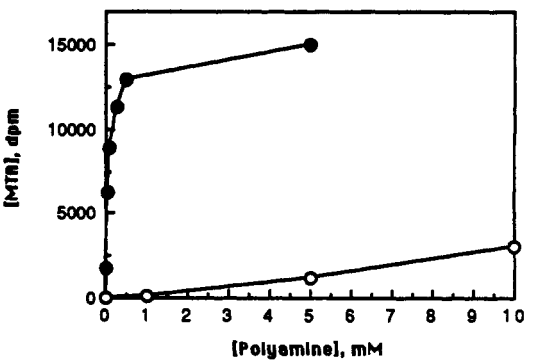


Figure 1. Activity of spermidine (●) and 1-methylspermidine (○) as substrates for spermine synthase. MTA = 5′-Deoxy-5′-(methylthio)adenosine.

Biological Results and Discussion

As shown in Figure 1, 1-methylspermidine (MeSpd, **2**) is a poor substrate for spermine synthase isolated from rat brain. Although this was disappointing in terms of our desire to incorporate an α-methyl substituent into Ado-DATAD as discussed in the introduction, we decided to investigate the substrate properties of MeSpd (**2**) with the other major enzyme of spermidine metabolism, SSAT. At a concentration of 1 mM, MeSpd was acetylated at only 2% of the rate of the parent polyamine, spermidine. Taken together, these results suggested that **2** might be sufficiently stable metabolically to be useful as a probe of polyamine function in vitro. Initial experiments showed

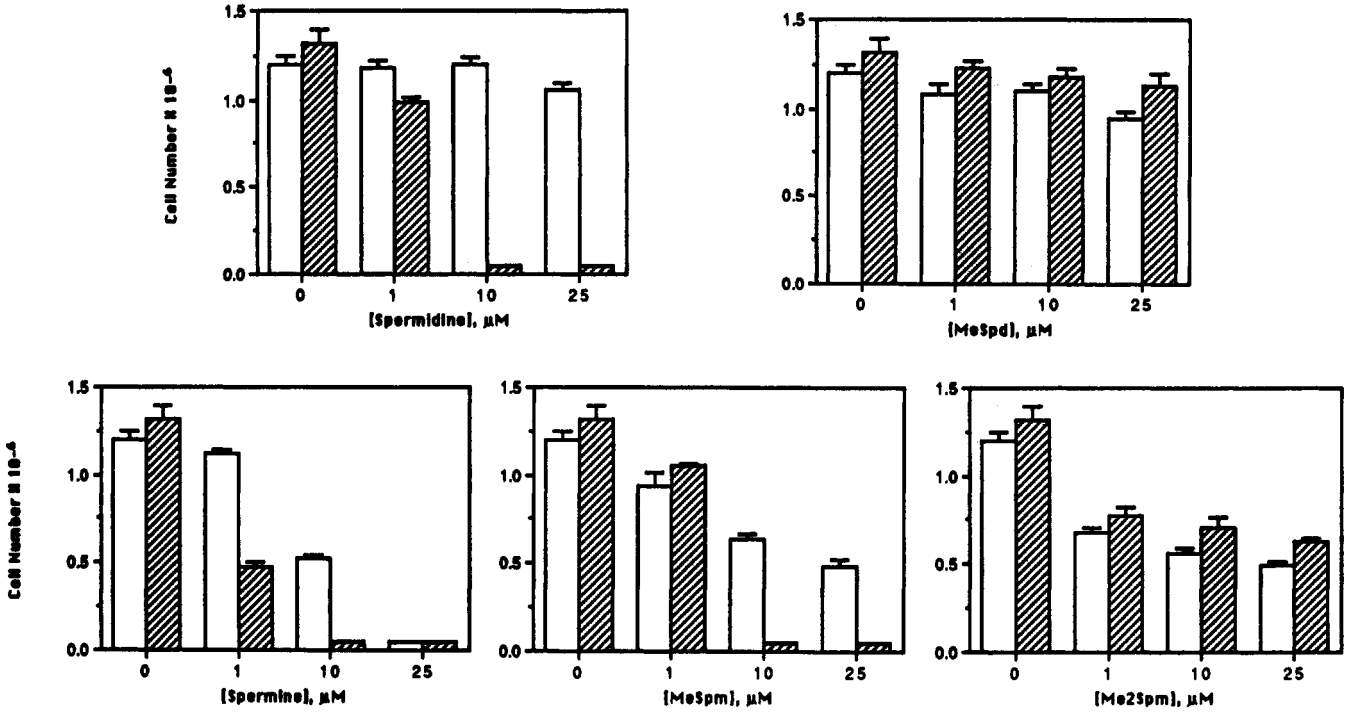


Figure 2. Toxicity of spermidine, spermine, and α-methyl derivatives 2–4 in L1210 cells grown in the presence (open bars) or absence (hashed bars) of the amine oxidase inhibitor aminoguanidine (1 mM).

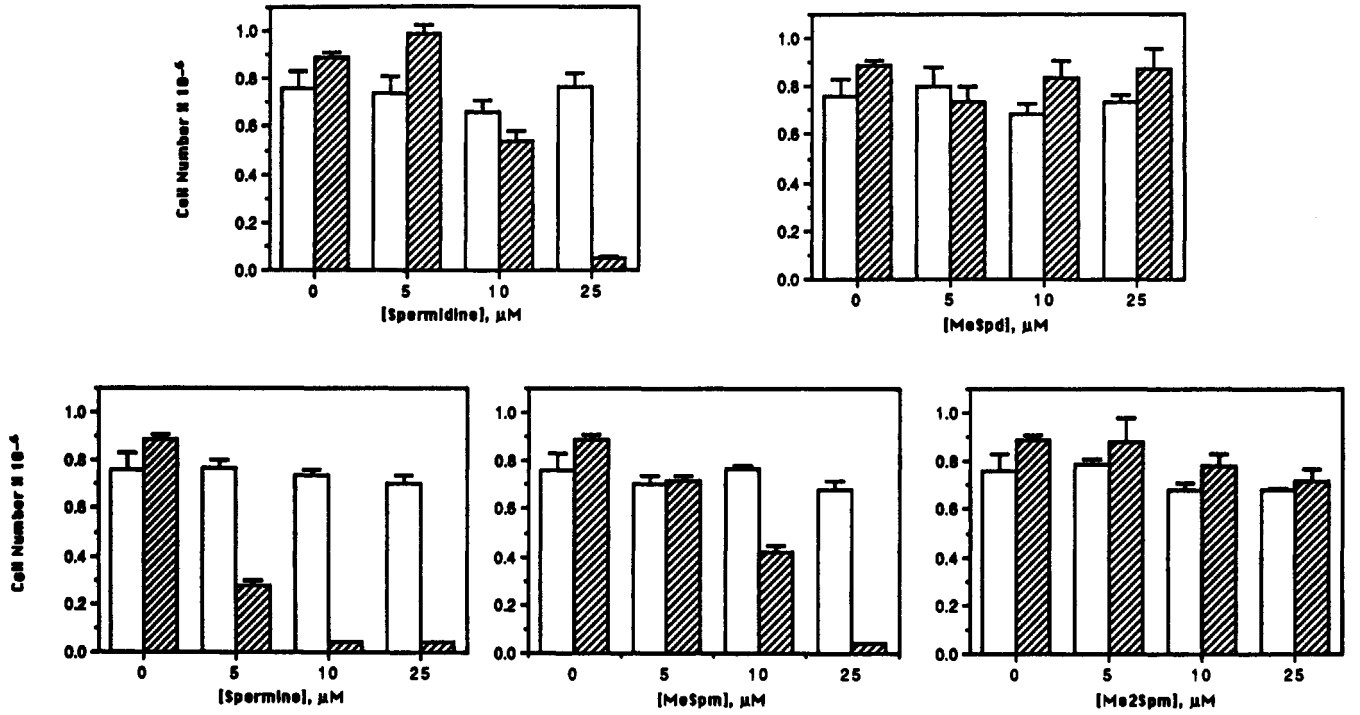


Figure 3. Toxicity of spermidine, spermine, and α-methyl derivatives 2–4 in HT29 cells grown in the presence (open bars) or absence (hashed bars) of the amine oxidase inhibitor aminoguanidine (1 mM).

that 2 is capable of rescuing cells from the cytostatic effects of α-(difluoromethyl)ornithine (DFMO) (vide infra), thus indicating that 2 is capable of mimicking the growth-stimulating effects of the natural polyamine spermidine.

The initial observations on the oxidative metabolism of AdoDATAD were made in L1210 cells.[5] Since this same type of oxidative metabolism is thought to be responsible for the observed toxicity of spermine (eq 1) and spermidine in a variety of cells,[7] we studied the effects of spermidine and MeSpd (2) on cell growth using L1210 (Figure 2) and HT29 (Figure 3) cells. The most striking observation in these experiments was the lack of toxicity of MeSpd to these cells even in the absence of the serum amine oxidase inhibitor aminoguanidine. This is strong support for the synthetic rationale; i.e., α-methyl substitution on AdoDATAD or spermidine should render these compounds resistant to oxidative metabolism.

It is also noteworthy that it was only necessary to add a methyl group at C-1 in order to completely prevent the toxicity of spermidine toward cells in culture. This addition would not be expected to affect oxidation at the other end of the molecule (C-8) and indicates that the toxic metabolites produced by oxidases in the medium (or possibly in the cells) are formed only by oxidation of the aminopropyl end of the molecule and not by attack on the nitrogen at the C-8 position. This supports the concept

Table I. Polyamine Levels (nmol/mg Protein) in DFMO-Treated L1210 Cells Exposed to α-Methyl Polyamines[a]

addition	Put	Spd	MeSpd	Spm	MeSpm	Me_2Spm
none (t = 0 h)	[b]	1.0 ± 0.2		13.4 ± 1.3		
none	[b]	[b]		7.3 ± 1.0		
10 μM Spd	0.8 ± 0.2	38.0 ± 2.4		16.3 ± 1.7		
10 μM MeSpd (2)	[b]	[b]	67.2 ± 6.6	1.1 ± 0.1	7.8 ± 0.8	
10 μM Spm	[b]	6.4 ± 0.6		35.6 ± 3.5		
10 μM MeSpm	[b]	[b]	[b]	1.7 ± 0.5	63.4 ± 2.3	
10 μM Me_2Spm	[b]	0.2 ± 0.1	[b]	3.2 ± 0.3		61.9 ± 2.1
no DFMO[c]	5.8 ± 0.5	41.0 ± 2.0		9.1 ± 0.9		

[a] Unless otherwise noted, all cultures were exposed to 5 mM DFMO for 72 h prior to the start of the experiment and throughout the remaining time period of 72 h. Cultures were refed at 72 h. Standard deviation is based on results from at least four separate experiments. [b] Below detection limits of 0.2 nmol/mg protein. [c] Not treated with DFMO. Total time in culture = 72 h.

Table II. Polyamine Levels (nmol/mg Protein) in DFMO-Treated HT29 Cells Exposed to α-Methyl Polyamines[a]

addition	Put	Spd	MeSpd	Spm	MeSpm	Me_2Spm
			Experiment A			
none (t = 0 h)	[b]	0.3 ± 0.1		5.4 ± 0.9		
none	[b]	0.7 ± 0.1		3.8 ± 0.8		
10 μM Spd	[b]	23.6 ± 0.8		10.3 ± 0.5		
10 μM MeSpd (2)	[b]	[b]	13.3 ± 0.5	1.1 ± 0.1	7.8 ± 0.4	
25 μM Spd	[b]	25.1 ± 2.8		9.0 ± 0.7		
25 μM MeSpd (2)	[b]	[b]	16.8 ± 2.3	1.2 ± 0.1	8.6 ± 0.8	
10 μM Spm	[b]	7.9 ± 1.3		21.5 ± 4.3		
10 μM MeSpm (3)	[b]	0.4 ± 0.2	0.2 ± 0.04	0.7 ± 0.03	21.3 ± 0.3	
15 μM Spm	[b]	4.8 ± 0.2		21.7 ± 1.5		
25 μM MeSpm (3)	[b]	0.3 ± 0.04	[b]	0.8 ± 0.04	24.2 ● 1.2	
no DFMO[c]	4.4 ± 0.4	9.8 ± 1.5		10.5 ± 3.0		
			Experiment B			
none (t = 0 h)	[b]	1.0 ± 0.2		7.1 ± 1.1		
none	[b]	0.4 ± 0.1		4.4 ± 1.2		
10 μM Spm	[b]	4.4 ± 0.4		21.5 ± 1.2		
10 μM Me_2Spm (4)	[b]	[b]	[b]	0.9 ± 0.1		18.2 ± 1.1
25 μM Me_2Spm (4)	[b]	[b]	[b]	0.7 ± 0.2		17.3 ± 3.1
no DFMO[c]	5.9 ± 0.4	13.4 ± 0.4		17.8 ± 1.0		

[a] Unless otherwise noted, all cultures were exposed to 5 mM DFMO for 120 h prior to the start of the experiment and throughout the remaining time period of 72 h (experiment A) or 96 h (experiment B). Cultures were refed at 96 h. Standard deviation is based on results from at least four separate experiments. [b] Below detection limits of 0.2 nmol/mg protein. [c] Not treated with DFMO. Total time in culture = 96 h.

that the major toxic products are derived from the action of the bovine serum oxidase which is reported to attack only the aminopropyl end of spermidine.[9] The results are also in agreement with early studies in which the decomposition of the oxidation products produced by this enzyme to yield acrolein was shown to be a major factor in the toxicity of polyamines.[7,10] However, the direct toxicity of the aldehydes themselves or other degradation products as suggested by Smith et al.[11] cannot be ruled out.

On the basis of these results, the synthesis of the analogous spermine derivative 1,12-dimethylspermine (Me_2Spm, 4) was carried out as described above. In addition, the unsymmetrical spermine derivative 1-methylspermine (MeSpm, 3), a possible metabolite of MeSpd (2), was prepared. When assayed as substrates for SSAT, spermine and the methylated derivatives 3 and 4 were acetylated at 41%, 20%, and <2% of the rate of spermidine, respectively. Thus, not unexpectedly at this point, spermine analogue 4, with both aminopropyl groups containing an α-methyl substituent, is not a substrate for this acetyltransferase whereas analogue 3, which still contains an unsubstituted aminopropyl group, is a substrate. Shown in Figures 2 and 3 are toxicity data for spermine, MeSpm (3), and Me_2Spm (4). As was the case with spermidine vs MeSpd (2) discussed above, the most striking observation in these experiments was the lack of toxicity of Me_2Spm, especially to the HT29 cells, even in the absence of aminoguanidine. Although there is less complete protection in the case of the L1210 cells, comparable effects were observed with or without aminoguanidine. As in the case of spermidine derivative 2, these data indicate that α-methyl substitution at both aminopropyl groups of spermine leads to a spermine analogue which is resistant to oxidative deamination (eq 1). Monomethyl spermine derivative MeSpm (3) was somewhat less sensitive than the parent polyamine spermine to oxidative metabolism. Toxic metabolites were produced (Figures 2 and 3), with L1210 cells once again being more sensitive than HT29 cells to 3, especially in the absence of aminoguanidine.

In order to test whether compounds 2–4 could replace their natural polyamine equivalents in supporting cell growth, experiments were set up in which mammalian cells were cultured in the presence of DFMO, an inhibitor of endogenous polyamine synthesis. It is well-known that the addition of this compound to cell cultures leads to a depletion of intracellular polyamines and a cessation of cell growth which can be reversed by the addition of either spermidine or spermine.[12] Therefore, attempts were made to reverse the growth inhibition produced by DFMO by the addition of MeSpd, MeSpm, and Me_2Spm (Figures

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Table III. Polyamine Levels (nmol/mg Protein) in DFMO-Treated SV3T3 Cells Exposed to α-Methyl Polyamines[a]

addition	Put	Spd	MeSpd	Spm	MeSpm	Me_2Spm
			Experiment A			
none (t = 0 h)	0.5 ± 0.02	0.3 ± 0.1		9.3 ± 1.0		
none	*b*	*b*		13.5 ± 1.0		
1 μM Spd	*b*	*b*		11.5 ± 0.9		
1 μM MeSpd	*b*	*b*	11.8 ± 1.6	3.8 ± 0.5	2.4 ± 0.4	
10 μM Spd	*b*	8.7 ± 1.9		9.5 ± 2.3		
10 μM MeSpd (2)	*b*	*b*	26.8 ± 2.5	0.4 ± 0.2	4.2 ± 0.4	
25 μM MeSpd (2)	*b*	*b*	31.2 ± 4.9	0.3 ± 0.1	4.4 ± 0.6	
1 μM Spm	*b*	*b*		14.3 ± 0.8		
1 μM MeSpm (3)	*b*	*b*	0.6 ± 0.2	3.5 ± 0.6	11.8 ± 2.2	
10 μM Spm	*b*	2.6 ± 0.5		20.6 ● 6.2		
10 μM MeSpm (3)	*b*	*b*	2.7 ± 1.0	0.3 ± 0.2	33.7 ± 2.8	
25 μM MeSpm (3)	*b*	*b*	0.4 ± 0.1	0.8 ± 0.04	33.1 ± 5.3	
			Experiment B			
none (t = 0 h)	*b*	*b*		7.8 ± 0.4		
none	*b*	*b*		7.7 ± 0.9		
1 μM Me_2Spm (4)	*b*	*b*		7.8 ± 0.9		4.2 ± 0.7
10 μM Spm	*b*	11.5		24.5		
10 μM Me_2Spm (4)	*b*	*b*	1.2 ± 0.2	3.3 ± 0.4		29.4 ± 1.1
25 μM Spm	*b*	3.1		31.5		
25 μM Me_2Spm (4)	*b*	*b*	*b*	1.0 ± 0.1		24.8 ± 1.2
no DFMO[c]	0.9 ± 0.3	26.4 ± 1.6		7.1 ± 1.0		

[a] Unless otherwise noted, all cultures wre exposed to 5 mM DFMO for 72 h prior to the start of the experiment and throughout the remaining time period of 72 h. Standard deviation is based on results from at least four separate experiments. Where no standard deviation is given, the experiment was run only in duplicate. [b] Below detection limits of 0.05 nmol/mg protein (experiment A) or 0.2 nmol/mg protein (experiment B). [c] Not treated with DFMO. Total time in culture = 72 h.

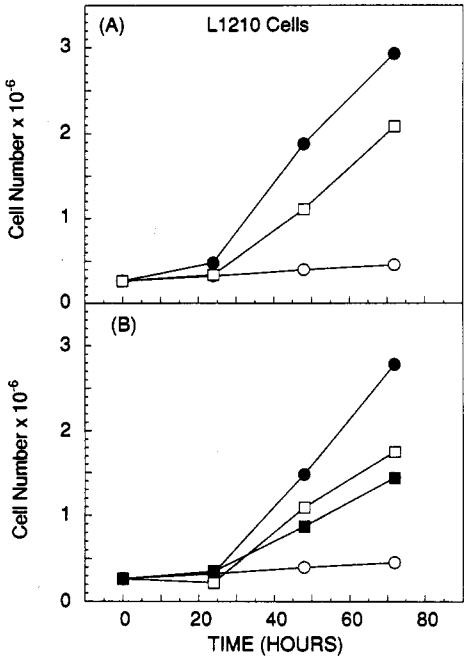

Figure 4. Rescue of DFMO-treated L1210 cells by spermidine (panel A, ●) or spermine (panel A, □) vs α-methyl derivatives **2** (panel B, ●), **3** (panel B, □), and **4** (panel B, ■). All polyamines were present at a concentration of 10 μM and cells were cultured in the presence of 1 mM aminoguanidine. In both panels, cells not rescued with added polyamines show little or no growth (○).

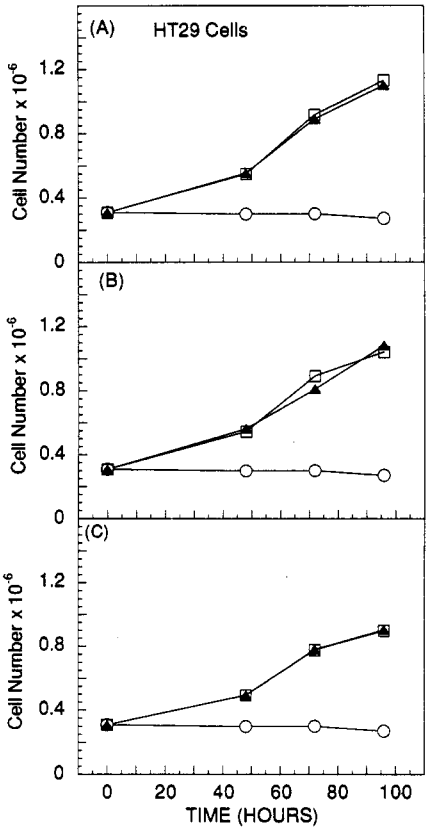

Figure 5. Rescue of DFMO-treated HT29 cells by spermine (panel A) vs α-methyl derivatives **2** (panel B) or **4** (panel C) at concentrations of 0 (○), 10 (▲), and 25 μM (□). Cells were cultured in the presence of 1 mM aminoguanidine. In a separate experiment, cells treated with 10 or 25 μM spermidine showed growth curves superimposable with those shown in panel A.

4–6). As shown in Figure 4, addition of 10 μM MeSpd was as effective as spermidine itself in supporting the growth of mouse L1210 cells and MeSpm was as active as spermine in this respect. Me_2Spm was able to stimulate the growth of cells exposed to DFMO but was slightly less active than spermine itself. Similar results were obtained with human colon carcinoma HT29 cells (Figure 5) and with virally transformed mouse fibroblasts, SV-3T3 cells (Figure 6). Dose–response studies with these cells showed that 10 μM concentrations were needed to give a maximal response and that spermine and the methyl-substituted spermines did not differ in this respect.

The polyamine levels present in cells exposed to DFMO and then treated with compounds **2–4** to stimulate growth are shown in Tables I–III. As expected, the exposure of L1210 cells to DFMO produced a complete loss of putrescine and spermidine with a small decline in spermine. Addition of 10 μM MeSpd to the culture medium led to

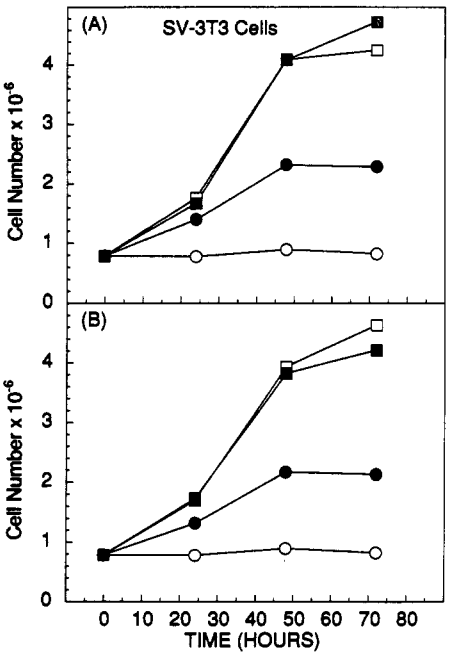

Figure 6. Rescue of DFMO-treated SV3T3 cells by spermine (panel A) vs α-methyl derivative 4 (panel B) at concentrations of 0 (○), 1 (●), 10 (□) and 25 μM (■). Cells were cultured in the presence of 1 mM aminoguanidine.

a substantial accumulation of this compound and to the presence in the cells of a smaller amount of MeSpm (Table I). This level of MeSpm confirms the results obtained with the isolated spermine synthase enzyme (Figure 1), showing that MeSpd is a weak substrate for this enzyme. When MeSpm or Me_2Spm was added to the cultures, they were accumulated to high levels in the L1210 cells, but there was no evidence for significant metabolism of these compounds.

The polyamine content of HT29 cells exposed to DFMO and the methylated polyamines also showed that the compounds were accumulated in the cells and that MeSpd was converted into MeSpm (Table II). Untreated HT29 cells have a higher ratio of spermine to spermidine than L1210 cells, which presumably reflects the larger amount of spermine synthase in these cells. In agreement with this, the extent of conversion of MeSpd into MeSpm was much greater in the HT29 cells. A small amount of MeSpd was found in the HT29 cells treated with 10 μM MeSpm. This is consistent with a very weak ability of MeSpm to serve as substrate for the acetylase/oxidase pathway of converting spermine. However, this amounts to less than 1% conversion as compared to more than 35% conversion of spermine into spermidine in the HT29 cells treated with spermine itself (Table II). No metabolism of Me_2Spm was detected.

The content of polyamines found in SV-3T3 cells treated in the same way was consistent with the findings in L1210 and HT29 cells. There was a clear indication of some conversion of MeSpd into MeSpm, a very small conversion of MeSpm into MeSpd, and essentially no metabolism of Me_2Spm (Table III).

In all cases there was a major decrease in the cellular spermine content when MeSpd, MeSpm, or Me_2Spm was added to the DFMO-treated cultures (Tables I–III). This decline is clearly due to the fact that the cells treated in this way are able to grow but are unable to continually synthesize spermine in new cells. The inability to form spermine is likely to be due predominantly to the lack of putrescine, and hence spermidine synthesis, owing to the presence of DFMO, but a minor contributory factor may be the presence of the methylated derivatives which could affect spermine synthase activity.

It can be seen from the results in Tables I and III that MeSpd, MeSpm, and Me_2Spm are accumulated in the L1210 cells and the SV-3T3 cells to much higher levels than spermidine or spermine themselves. It is known that polyamines are transported into cells by an active transport system, that this system will also transport polyamine derivatives, and that it is regulated by the internal polyamine content.[13,14] These results indicate that the methylated derivatives are likely to be good substrates for this system but are less effective than their normal counterparts in repressing the transport activity when present at high levels in the cells. These properties, combined with their relative lack of metabolism, suggest that the methylated compounds will be very useful as model substrates for the further study of this transport system. Interestingly, the HT29 cells, which are known to have a much lower activity of the polyamine transport system when compared to rodent cell line,[15] did not show a greater accumulation of the methylated polyamines over the natural compounds.

Conclusion

Substitution of a methyl group α to one or both terminal nitrogen atoms on the aminopropyl end of spermidine or spermine has proven effective in preventing amine oxidase-mediated metabolism of compounds **2**–**4**. This is in accord with results obtained with the ODC inhibitor (2*R*,5*R*)-6-heptyne-2,5-diamine (*R*,*R*-MAP)[16] and N^1,N^8-bis(3-aminobutyl)-1,8-octanediamine,[17] both developed by Merrell-Dow. Although different amine oxidases may be involved, this structural modification seems to be quite general in preventing oxidative metabolism of polyamine analogues. Cellular toxicity associated with the oxidative degradation of the natural polyamines spermidine and spermine is therefore markedly diminished with α-methyl polyamines, especially **2** and **4**. Dimethylation at the carbon α to the terminal nitrogen atoms has been effective in blocking this metabolism in a series of polyamine analogues synthesized by Nagarajan and Ganem.[18,19] Similar to what was found in the present work, the *gem*-dimethyl derivatives analogous to **2** and **4**, 1,1-dimethylspermidine and 1,1,12,12-tetramethylspermine, respectively, were

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found to be poor substrates for spermine synthase and SSAT. Although the *gem*-dimethyl compounds were effective in facilitating growth of DFMO-treated SV3T3 cells, they were not as effective as the natural polyamine spermidine; no data were presented which would allow a comparison with spermine.[19] In contrast, compounds **2–4** are nearly as effective as either spermidine or spermine (Figures 4–6) in rescuing cells from the cytostatic effects of DFMO. The *gem*-dimethyl compounds do not have any deleterious effects on the growth of SV3T3 cells and accumulate intracellularly when added in the presence of aminoguanidine.[19] A major difference in the present work and the previous work with *gem*-dimethyl analogues is the finding that compounds such as **2–4** can be added to cells in the absence of the amine oxidase inhibitor aminoguanidine. Chemically, it seems reasonable to speculate that this also may be possible with the *gem*-dimethyl analogues. The ability to add metabolically stable analogues of spermidine and spermine to cells without the toxicity seen with the natural products should be of great value in future biochemical and pharmacological studies.

The development of new polyamine analogues traditionally has been focused upon maximizing antimetabolite activity while limiting the analogue's ability to substitute for the natural polyamines. N^1,N^8-Diethylspermidine and N^1,N^{12}-diethylspermine are two representative polyamine antimetabolites which have been found to stop cell growth by inducing SSAT activity and/or lowering ODC and AdoMet DC activities.[20] In either case, a reduction of intracellular spermidine is observed. However, both N^1,N^8-diethylspermidine and N^1,N^{12}-diethylspermine are thought to substitute partially for the natural polyamines.[20d] In the present work, the α-methylated polyamines **2–4** appear to substitute very effectively for the natural polyamines in DFMO rescue experiments using the three cell lines investigated so far.

Because the α-methylated polyamines substitute so completely and effectively for the natural polyamines in cellular rescue experiments, one might imagine that they may substitute in many more specific polyamine functions. Because these analogues are not further metabolized, they may represent a useful tool for kinetic investigations into polyamine uptake and transport across cellular membranes. In addition, the study of intracellular receptor interactions such as those involving the NMDA receptor,[21] hypusine biosynthesis,[22] and polyamine–DNA interactions[23] might be facilitated by the use of these new analogues. In this regard, it is interesting to note that MeSpd (**2**) and Me_2Spm (**4**) are effective in stimulating the B–Z transition in poly(dG-MedC) at concentrations in the 1–20 μM range, similar to that observed with the natural polyamines spermidine and spermine (H. Basu and L. Marton, personal communication). These data suggest new directions for development of additional analogue modifications, e.g., synthesis of specific stereoisomers of polyamines **2** and **4**. Further studies to examine possible stereospecificity in these interactions are in progress.

Experimental Section

^{1}H NMR and ^{13}C NMR spectra were obtained using Bruker WM 270, AM 300, or WM 360 spectrometers with chemical shifts referenced to tetramethylsilane internal standard. ^{1}H NMR spectra recorded in deuterium oxide, unless otherwise indicated, were referenced to tetramethylsilane external standard in $CDCl_3$. ^{13}C NMR spectra in D_2O were referenced to the $CDCl_3$ contained in an external standard. All melting points were obtained using a Mel-Temp apparatus and are uncorrected. IR spectra were recorded on a Nicolet FTIR spectrometer. High-resolution mass spectra were obtained using a VG 70-250S mass spectrometer. Elemental analyses were acquired from Atlantic Microlabs (Atlanta, GA). Analytical thin-layer chromatography was done on either silica gel plates (EM Science, 5554-7) with 254-nm fluorescent indicator or Eastman cellulose plates with fluorescent indicator. Flash column chromatography refers to the method described by Still et al. using silica gel 60.[24] Solvents were purchased in their anhydrous form and used directly or dried by distillation from sodium benzophenone ketyl (tetrahydrofuran), calcium hydride (dichloromethane), or barium oxide (DMF). Unless otherwise indicated, all chemicals were used directly from the supplier without further purification. All experiments were carried out in oven-dried or flame-dried glassware and reaction solutions were magnetically stirred. Reactions involving air- or moisture-sensitive material were carried out under a positive pressure of dry nitrogen. D,L-3-[(Carbobenzyloxy)amino]butyric acid (**5**)[25] was prepared as previously described.

D,L-3-Azidobutyric Acid (6). To solid white crotonic acid (6.46 g, 75 mmol) was added 20 mL of glacial acetic acid. With stirring, sodium azide (19.5 g, 300 mmol) in 40 mL of H_2O was slowly added. The reaction was stirred for 1 h at room temperature followed by 24 h at reflux temperature. After 24 h, the reaction mixture was extracted with $CHCl_3$ (4 × 100 mL), dried ($MgSO_4$), filtered, and evaporated under high vacuum to give 4.96 g of a yellow oil. Distillation at reduced pressure (bp 72–75 °C, 0.3 mmHg) (lit.,[26] *R* isomer, bp 105–110 °C, 0.1 Torr) gave 4.64 g (48%) of **6** as a clear and colorless liquid: ^{1}H NMR ($CDCl_3$) δ 11.42 (s, 1 H), 4.02–3.91 (m, 1 H), 2.60–2.45 (m, 2 H), 1.35–1.34 (d, 3 H); ^{13}C NMR ($CDCl_3$) δ 176.84, 53.84, 40.73, 19.24; FTIR (neat, cm^{-1}) 2980, 2650, 2116, 1722, 1413, 1237, 934. Anal. ($C_4H_7N_3O_2$) C, H, N.

***N*-[4-[(Carbobenzyloxy)amino]butyl]phthalimide.** *N*-(4-Aminobutyl)phthalimide hydrochloride (**8**, 583 mg, 2.29 mmol) was added to 15 mL of dry DMF with *N*-methylmorpholine (0.554 mL, 5.95 mmol). The off-yellow solution was stirred for 1 h to dissolve all the starting materials. The solution was then cooled to 0 °C and benzyl chloroformate (0.457 mL, 2.24 mmol) was added via syringe. The ice bath was removed after 10 min and the reaction was stirred at room temperature overnight. After 14 h the DMF was removed in vacuo to give a dry residue. The residue was then taken up in 15 mL of $CHCl_3$ and washed with 1 N HCl, 1 N $NaHCO_3$, and H_2O, dried ($MgSO_4$), filtered, and evaporated in vacuo to give 751 mg of a yellow solid. Crystal-

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lization from EtOH gave 617 mg (76%) of the desired unsymmetrically protected 1,4-diaminobutane as a white crystalline solid: mp 107.5–109.5 °C; ^{1}H NMR ($CDCl_3$) δ 7.84–7.81 (m, 2 H), 7.74–7.68 (m, 2 H), 7.34 (s, 5 H), 5.08 (s, 2 H), 4.83 (s, 1 H), 3.72–3.68 (t, 2 H), 3.27–3.21 (q, 2 H), 1.74–1.66 (m, 2 H), 1.60–1.53 (m, 2 H); FTIR (Nujol, cm^{-1}) 3311, 2931, 2854, 2727, 1771, 1701, 1546, 1462, 1384, 1272, 1166, 1054, 745, 723. Anal. ($C_{20}H_{20}N_2O_4$) C, H, N.

N^1-(Carbobenzyloxy)-1,4-diaminobutane (7). *N*-[4-[(Carbobenzyloxy)amino]butyl]phthalimide (255 mg, 0.724 mmol) was placed in a 35-mL round-bottom flask along with 8 mL of absolute ethanol. The flask was then cooled to 0 °C. Hydrazine monohydrate (112 mg, 2.24 mmol) was slowly added via syringe into the cooled solution. The reaction mixture was stirred for 30 min while the ice was allowed to melt. The ice bath was then removed and the reaction was stirred at room temperature for 15 min. The solution was then slowly brought to reflux temperature with formation of a grey precipitate. Upon cooling the reaction solution a white precipitate formed. Ethanol was then carefully removed in vacuo to give an off-white residue. Water (20 mL) was added to this residue and was stirred for several hours. The aqueous mixture was then acidified to pH 4–5 (glacial acetic acid) with formation of white phthalhydrazide precipitate. The phthalhydrazide precipitate was then removed by filtration and the filtrate was brought to pH 13 with 4 N NaOH. The aqueous mixture was then extracted with $CHCl_3$ (3 × 30 mL), and the organic extract dried ($MgSO_4$) and evaporated in vacuo to give 129 mg (79%) of **7** as a yellow oil which solidified on standing. A small portion of this residue (43 mg) was dissolved in absolute ethanol (5 mL) and was crystallized as the HCl salt by bubbling dry HCl gas through the mixture: mp 189.5–195 °C (lit.[27] mp 195–197 °C); ^{1}H NMR ($CDCl_3$, free base) δ 7.34 (s, 5 H), 5.29 (s, 1 H), 5.08 (s, 2 H), 3.21–3.16 (m, 2 H), 2.70–2.67 (t, 2 H), 1.55–1.42 (m, 4 H), 1.19 (s, 2 H); ^{13}C NMR ($CDCl_3$, free base) δ 156.37, 136.67, 128.33, 127.87, 66.37, 41.62, 40.81, 30.67, 27.28; FTIR (neat, free base, cm^{-1}) 3332, 3035, 2936, 2866, 1712, 1539, 1456, 1258, 1138, 1026, 914, 745, 699.

***N*-(4-Azidobutyl)phthalimide.** *N*-(4-Bromobutyl)phthalimide (1.50 g, 5.3 mmol) was added together with sodium azide (1.05 g, 16.2 mmol) into a flask containing 6 mL of DMF. Using an oil bath, the reaction temperature was maintained at 57 °C and stirring was continued overnight. After 16 h the reaction flask was allowed to cool and then the reaction contents were poured into 30 mL of ice water. A white precipitate formed immediately. This mixture was stirred on ice for 25 min and then the white solid was collected by filtration, giving 1.18 g (91%) of the desired unsymmetrically protected 1,4-diaminobutane: mp 40.5–42.0 °C; ^{1}H NMR ($CDCl_3$) δ 7.87–7.71 (m, 4 H), 3.75–3.70 (t, 2 H), 3.36–3.31 (t, 2 H), 1.81–1.56 (m, 4 H); ^{13}C NMR ($CDCl_3$) δ 168.32, 133.94, 132.08, 123.23, 50.89, 37.26, 26.23, 25.81; FTIR (Nujol, cm^{-1}) 3466, 3065, 2945, 2868, 2516, 2102, 1771, 1722, 1616, 1047, 723.

***N*-(4-Aminobutyl)phthalimide Hydrochloride (8).** To a thick-walled 250-mL hydrogenation bottle was added *N*-(4-azidobutyl)phthalimide (1.1 g, 4.51 mmol), along with 100 mg of $Pd(OH)_2$/carbon catalyst, 5 mL of 1 N HCl, and 22 mL of reagent-grade methanol. The mixture was then hydrogenated at 32 psi hydrogen pressure for 36 h. The catalyst was filtered out through a 45-μm Millipore filter with 1 in. of Celite powder on top. The solvent was then removed in vacuo to give 1.15 g of a yellow oil which solidified on standing. Crystallization from MeOH/Et_2O gave 810 mg (70%) of **8** as light yellow flakes: mp 204–206.5 °C; ^{1}H NMR (D_2O, ref to residual HOD peak) δ 7.67 (m, 4 H), 3.56–3.51 (t, 2 H), 2.93–2.87 (t, 2 H), 1.59–1.56 (m, 4 H); FTIR (Nujol, cm^{-1}) 3402, 2868, 1778, 1722, 1616, 1574, 1462, 1377, 1068, 723. Anal. ($C_{12}H_{14}N_2O_2$·HCl) C, H, N.

N^1-(Carbobenzyloxy)-N^4-[3-[(carbobenzyloxy)amino]butyryl]-1,4-diaminobutane (9). White solid *N*-(carbobenzyloxy)-1,4-diaminobutane hydrochloride (**7**, 107 mg, 0.413 mmol) and D,L-3-Z-aminobutyric acid (**5**, 98 mg, 0.413 mmol) were combined together in a 25-mL two-neck round-bottom flask along with DCC (102 mg, 0.496 mmol), *N*-methylmorpholine (63 mg, 0.62 mmol), and hydroxybenzotriazole (56 mg, 0.413 mmol). The flask was cooled to 0 °C. Two milliliter of dry DMF was then added via syringe. The ice bath was removed after 15 min and the reaction was stirred for 36 h with the color changing from clear yellow to cloudy yellow. Precipitated DCU was removed by filtration and the resulting filtrate was evaporated in vacuo to give a white solid. The solid residue was triturated with $CHCl_3$ to give **9** with some residual DCU contaminants. Crystallization from methanol gave 82 mg (45%) of **9** as a white microcrystalline solid: mp 168–172 °C; ^{1}H NMR (DMSO-d_6) δ 7.79 (s, 1 H), 7.31 (s, 10 H), 7.19 (s, 1 H), 7.13 (s, 1 H), 5.00 (s, 4 H), 3.88–3.84 (m, 1 H), 2.99–2.97 (m, 4 H), 2.31–2.09 (m, 2 H), 1.04–1.02 (s, 4 H), 1.03 (d, 3 H); ^{13}C NMR (DMSO-d_6) δ 169.72, 156.05, 155.09, 137.24, 128.25, 127.63, 65.04, 44.23, 42.32, 39.96 (under DMSO peak), 38.07, 26.81, 26.35, 20.23; FTIR (KBr, cm^{-1}) 3456, 3303, 3070, 2951, 2871, 1689, 1635, 1540, 1455, 1310, 1260, 1200, 1146, 1112, 1071, 781, 746, 721, 696; HRMS (EI, 70 eV) calcd for $C_{24}H_{31}N_3O_5$ (MH^+) 441.2264, found 441.2275.

N^1-Phthaloyl-N^4-(3-azidobutyryl)-1,4-diaminobutane (10). D,L-3-Azidobutyric acid (**6**, 507 mg, 3.9 mmol) and N^1-phthaloyl-1,4-diaminobutane hydrochloride (**8**, 1 g, 3.9 mmol) were combined with DCC (972 mg, 4.71 mmol), HOBT (584 mg, 4.3 mmol), NMM (874 mg, 8.6 mmol), and DMF (15 mL) in a 100-mL round-bottom flask and was stirred at room temperature for 2.5 days. The precipitated DCU was removed by filtration and the DMF was removed from the resulting filtrate by short-path distillation (0.1 mmHg, 25 °C). The resulting residue was stirred with EtOAc for 30 min and then the additional DCU precipitate was collected by filtration. The remaining orange filtrate was washed with 3 × 30 mL of 1 N HCl, 3 × 30 mL of 1 N $NaHCO_3$, and 2 × 30 mL of water. The organic layer was dried ($MgSO_4$), filtered, and evaporated to give 1.2 g of yellow solid. This solid was crystallized from EtOAc to give 785 mg (61%) of **10** as a light yellow crystalline solid: mp 118.5–120.5 °C; ^{1}H NMR ($CDCl_3$) δ 7.83–7.80 (m, 2 H), 7.70–7.68 (m, 2 H), 5.80 (s, 1 H), 4.01–3.96 (m, 1 H), 3.71–3.67 (t, 2 H), 3.33–3.28 (m, 2 H), 1.75–1.67 (m, 2 H), 1.59–1.51 (m, 2 H), 1.30–1.28 (d, 3 H); ^{13}C NMR ($CDCl_3$) δ 169.59, 168.45, 133.98, 132.17, 123.26, 54.88, 43.38, 39.06, 37.44, 26.65, 26.08, 19.44. Anal. ($C_{16}H_{19}N_5O_3$) C, H, N.

N^1-(3-Aminobutyryl)-1,4-diaminobutane (11). N^1-Z-N^4-(3-Z-butyryl)-1,4-diaminobutane (**9**, 502 mg, 1.13 mmol) was partially dissolved in 15 mL of reagent-grade methanol in a 250-mL hydrogenation bottle. Palladium catalyst (62 mg, 10% on carbon) was then carefully added to the solution. The resulting mixture was gently slurried and then placed under 40 psi hydrogen pressure with mechanical shaking for 24 h. Hydrogen pressure was then released and the resulting solution was filtered through a 0.45-μm Zetapore membrane with 1 in. Celite powder on top. Evaporation of the methanol in vacuo gave a white, sticky residue. Trituration of the residue with water resulted in extraction of the desired product; contaminating DCU was removed by filtration. Lyophilization of the resulting aqueous filtrate gave 165 mg (97%) of **11** as a clear glassy hygroscopic solid which was sufficiently pure for conversion to **2**: ^{1}H NMR (D_2O) δ 3.19–3.11 (m, 1 H), 3.07–3.02 (t, 2 H), 2.77–2.72 (t, 2 H), 2.17–2.14 (m, 2 H), 1.49–1.32 (m, 4 H), 0.95–0.93 (d, 3 H); ^{13}C NMR (D_2O, HCl salt) δ 171.88, 45.34, 39.41, 39.13, 38.80, 25.50, 24.37, 17.82.

1-Methylspermidine Trihydrochloride (2). N^1-(3-Aminobutyryl)-1,4-diaminobutane (**11**, 124 mg, 0.72 mmol) was added to a 25-mL round-bottom flask in methanol solution which was subsequently dried in vacuo to an oily residue. Dry THF (10 mL) was then added. BH_3·THF complex (1 M, 6.1 mL, 6.1 mmol) was added to the reaction flask via syringe followed by heating at reflux temperature for 48 h, during which time the starting material dissolved off the sides of the reaction vessel. After 48 h the reaction was cooled to ambient temperature and stirred for 24 h. The reaction was then quenched with a minimum volume of 6 N HCl and allowed to stand at ambient temperature for 3 days. Following removal of THF in vacuo, the crude reaction solution was then poured directly onto a Dowex 50W-X8 (H^+ form) (25-mL resin bed) cation-exchange column, the column was washed with distilled H_2O, 1 N HCl, and 2.3 N HCl, and **2** finally eluted with 3.3 N HCl. The acidic mixture was evaporated in vacuo, and the resulting solid was resuspended in distilled water and lyophilized to give a glassy solid. This material was impure by ^{1}H NMR analysis, so the free base was isolated by dissolving this solid in

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water, adding 4 N NaOH to pH 14, and extracting with $CHCl_3$ (3 × 10 mL). The HCl salt was then reisolated by dissolving the free base in 1 N HCl and lyophilizing to give 69 mg (36%) of **2** as a glassy, hygroscopic, colorless solid: mp (HCl salt) 283 °C dec; ^1H NMR ($CDCl_3$, free base) δ 3.02–2.96 (m, 1 H), 2.73–2.59 (m, 6 H), 1.57–1.42 (m, 11 H), 1.08–1.04 (d, 3 H); ^{13}C NMR ($CDCl_3$, free base) δ 49.78, 47.32, 45.58, 41.89, 39.86, 31.37, 27.28, 24.32; HRMS (EI, 70 eV) calcd for $C_8H_{21}N_3$ (MH^+) 160.1814, found 160.1809. Anal. ($C_8H_{21}N_3$·3HCl) C, H, N.

N^1-(3-Azidobutyryl)-1,4-diaminobutane (12). N^1-Phthaloyl-N^4-(3-azidobutyryl)-1,4-diaminobutane (**10**, 500 mg, 1.52 mmol) was added to a two-neck 100-mL round bottom flask and dissolved in 15 mL of reagent-grade methanol. A reflux condenser was positioned on the flask and hydrazine (295 mg, 5.89 mmol) was added via syringe. The reaction mixture was heated to reflux for 6 h followed by room temperature stirring overnight. Methanol was then removed in vacuo and the resulting white residue was dissolved in 20 mL of 4 N NH_4OH. The aqueous mixture was then extracted with 4 × 20 mL of chloroform. The resulting organic layer was dried ($MgSO_4$), filtered, and evaporated in vacuo to give 250 mg (83%) of **12** as a clear, sticky, and colorless oil which was sufficiently pure for conversion to **14**: ^1H NMR ($CDCl_3$, free base) δ 6.35 (s, 1 H), 4.03–3.96 (m, 1 H), 3.28–3.21 (m, 2 H), 2.73–2.68 (t, 2 H), 2.27–2.25 (d, 2 H), 1.56–1.44 (m, 4 H), 1.34 (s, 2 H), 1.30–1.27 (d, 3 H); ^{13}C NMR (MeOD-d_4, HCl salt) δ 172.75, 56.28, 43.59, 40.37, 39.55, 27.24, 25.81, 19.68; FTIR (Nujol, free base, cm^{-1}) 3302, 3188, 2905, 2117, 1652, 1558, 1461, 1378, 975, 724.

3-Azidopropionic Acid (13). Acrylic acid (5.40 g, 75 mmol) was added to a 250-mL three-neck round-bottom flask with water (20 mL) and sodium azide (19.5 g, 300 mmol). Acetic acid (glacial) (20 mL) was added and the resulting yellow mixture was heated to approximately 80 °C for 2 days followed by 1 day at room temperature. The black reaction mixture was transferred to a 250-mL separatory funnel and extracted with CH_2Cl_2 (5 × 100 mL). The combined organic layers were dried ($MgSO_4$), filtered, and evaporated in vacuo to give a light amber oil. Excess acetic acid was removed via short-path distillation (0.5 mmHg, 25 °C) to give 3.2 g of light amber oil. This oil was purified again by short-path distillation (bp 73–75 °C, 0.4 mmHg) (lit.[28] bp 74–75 °C, 0.45 mmHg) to give 1.67 g (19%) of **13** as a clear and colorless liquid: ^1H NMR ($CDCl_3$) δ 9.15 (s, 1 H), 3.59–3.55 (t, 2 H), 2.65–2.60 (t, 2 H).

N^1-(3-Azidobutyryl)-N^4-(4-azidopropionyl)-1,4-diaminobutane (14). 3-Azidopropionic acid (**13**, 139 mg, 1.20 mmol) was combined with N^1-(3-azidobutyryl)-1,4-diaminobutane (**12**, 240 mg, 1.20 mmol) along with DCC (298 mg, 1.44 mmol), HOBt (179 mg, 1.32 mmol), NMM (146 mg, 1.44 mmol), and DMF (10 mL) in a 100-mL round-bottom flask. The resulting solution was stirred at room temperature for 2.5 days. Precipitated DCU was removed via vacuum filtration and the resulting filtrate was vacuum distilled to remove excess DMF (25–45 °C at 0.1 mmHg) and give a yellow residue. This residue was transferred to a separatory funnel using chloroform and was washed with 3 × 20 mL of 1 N HCl, 3 × 20 mL of 1 N $NaHCO_3$, and 2 × 20 mL of H_2O. The resulting organic layer was dried ($MgSO_4$), filtered, and evaporated in vacuo to give 200 mg of yellow solid. Flash column chromatography on silica gel (100% CH_2Cl_2 to 10% MeOH in CH_2Cl_2; I_2 detection) gave 101 mg of DCU-contaminated product. Eluting a second column with 1%, then 3%, and finally 10% MeOH in CH_2Cl_2 gave 52 mg (15%) of **14** as a white solid: ^1H NMR ($CDCl_3$) δ 6.35 (s, 2 H), 4.08–3.97 (m, 1 H), 3.66–3.60 (m, 2 H), 3.34–3.28 (m, 4 H), 2.46–2.42 (t, 2 H), 2.34–2.31 (m, 2 H), 1.59–1.55 (m, 4 H), 1.33–1.31 (d, 3 H); ^{13}C NMR ($CDCl_3$) δ 170.07, 169.89, 54.91, 53.35, 47.48, 43.28, 39.15, 39.08, 35.85, 26.78, 26.67, 19.47; HRMS (CI, NH_3) calcd for $C_{11}H_{20}N_8O_2$ (M^+) 297.1787, found 297.1786.

1-Methylspermine Tetrahydrochloride (3). N^1-(3-Azidobutyryl)-N^4-(4-azidopropionyl)-1,4-diaminobutane (**14**, 50 mg, 0.169 mmol) was added to a 25-mL round-bottom flask fitted with a reflux condenser. THF (5 mL) was added via syringe, dissolving all the solid. BH_3·THF solution (1.0 M, 2.02 mL, 2 mmol) was slowly added and the resulting mixture was heated at reflux temperature for 24 h. After this time, the mixture was stirred at room temperature for 6 h. An ice bath was then used to cool the reaction mixture to 0 °C, followed by slow addition of 1.5 mL of 6 N HCl. This mixture was then heated at reflux temperature for 6 h followed by room temperature stirring overnight. THF was removed in vacuo followed by dilution of the resulting aqueous layer to 9 mL of total volume with distilled H_2O. This mixture was then applied to a Dowex 50W-X8 (H^+ form) cation-exchange column (25-mL volume of resin). Step gradient elution with distilled H_2O, 1 N HCl, 2.3 N HCl, 3.3 N HCl, and finally 4.3 N HCl resulted in product elution at 4.3 N HCl. The fractions containing the desired product were pooled and excess HCl was removed in vacuo followed by lyophilization to give 54 mg of yellow solid. This material proved impure by ^1H NMR analysis, so it was dissolved in 1 mL of water, and the solution was made basic (pH 14) with solid NaOH. Extraction with 3 × 5 mL $CHCl_3$ and evaporation gave 19 mg of free base. This oil was then taken up in 1 N HCl and lyophilized to give 26 mg (42%) of the 4-HCl salt (**3**) as a white solid: mp 247 °C dec; ^1H NMR (D_2O) δ 3.54–3.48 (m, 1 H), 3.22–3.10 (m, 10 H), 2.20–1.92 (m, 4 H), 1.83–1.78 (m, 4 H), 1.37–1.35 (d, 3 H); ^{13}C NMR (D_2O) δ 47.11, 45.44, 44.65, 44.03, 36.70, 30.50, 23.82, 22.85, 17.34; HRMS (DCI, NH_3) calcd for $C_{11}H_{28}N_4$ (M^+) 217.2392, found 217.2405.

N^1,N^4-Bis[3-[(carbobenzyloxy)amino]butyryl]-1,4-diaminobutane (15). D,L-3-Z-aminobutyric acid (**5**, 592 mg, 2.5 mmol), putrescine (100 mg, 1.13 mmol), DCC (516 mg, 2.5 mmol), and HOBt (372 mg, 2.75 mmol) were combined in a 100-mL round-bottom flask and 20 mL of THF was added to effect solution. After cooling to 0 °C, *N*-methylmorpholine (253 mg, 2.5 mmol) was added via syringe. The resulting milky white slurry was stirred at room temperature for 48 h with formation of a yellow-white precipitate. The desired product and DCU appeared to be precipitates in the reaction mixture and were isolated via filtration using a Büchner funnel with THF washes to remove residual reactants. The resulting white powder (1 g) was dissolved in boiling methanol and allowed to cool to room temperature slowly and then refrigerated overnight. White microcrystalline **15** (365 mg, 64%) was isolated by vacuum filtration: mp 213.5–215 °C; ^1H NMR (DMSO-d_6) δ 7.80–7.79 (m, 2 H), 7.42–7.27 (m, 10 H), 7.15 (m, 1 H), 5.05 (s, 4 H), 3.86–3.83 (m, 2 H), 3.08–2.98 (s, 4 H), 2.29–2.06 (m, 4 H), 1.44–1.34 (s, 4 H), 1.02–1.00 (d, 6 H); HRMS (FAB^+) calcd for $C_{12}H_{36}N_4O_6$ (M^+) 527.2870, found 527.2868. Anal. ($C_{12}H_{36}N_4O_6$) C, H, N.

N^1,N^4-Bis(3-azidobutyryl)-1,4-diaminobutane (16). 3-Azidobutyric acid (**6**, 1 g, 7.74 mmol) and putrescine (310 mg, 3.52 mmol) were combined with DCC (1.60 g, 7.74 mmol), HOBt (523 mg, 3.87 mmol), NMM (783 mg, 7.74 mmol), and THF (10 mL) in a 100-mL round-bottom flask and the resulting solution was stirred at room temperature for 3 days. Precipitated DCU was removed by filtration and the resulting filtrate was dried in vacuo to give a yellow-orange solid. This solid was dissolved in a minimum volume of ethyl acetate with subsequent filtration of additional DCU precipitate. The filtrate was washed with 3 × 20 mL of 1 N HCl, 3 × 20 mL of 1 N $NaHCO_3$, 3 × 20 mL of H_2O, and 1 × 20 mL of brine solution. The resulting organic layer was dried ($MgSO_4$), filtered, and evaporated in vacuo to give a yellow solid (718 mg). Flash chromatography on silica gel (100% $CHCl_3$ to 10% MeOH in $CHCl_3$) gave 331 mg (30%) of **16** as a white solid: mp 83–84.5 °C; ^1H NMR ($CDCl_3$) δ 6.06 (s, 2 H), 4.08–3.97 (m, 2 H), 3.38–3.24 (m, 4 H), 2.33–2.30 (m, 4 H), 1.59–1.55 (m, 4 H), 1.33–1.31 (d, 6 H); ^{13}C NMR δ 169.79, 54.94, 43.39, 39.11, 26.79, 19.51. Anal. ($C_{12}H_{22}N_8O_2$) C, H, N.

1,12-Dimethylspermine Tetrahydrochloride (4). Method A. N^1,N^4-Bis[3-[(carbobenzyloxy)amino]butyryl]-1,4-diaminobutane (**15**, 852 mg, 1.6 mmol) was placed into a 250-mL hydrogenation bottle along with 100 mg of PtO_2 catalyst and 15 mL of methanol. The resulting slurry was hydrogenated at 40 psi hydrogen pressure for 24 h. The catalyst was then removed via filtration through 1 in. Celite powder on top of a 0.45-μm Millipore filter. The methanol was then removed in vacuo to give 480 mg of sticky oil. After drying under vacuum for 24 h, 5 mg of oil was removed and the remaining material (475 mg, 1.84 mmol) was mixed with BH_3·THF (1 M, 27.6 mL, 27.6 mmol). The resulting mixture was initially cooled to 0 °C and then brought to reflux

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temperature for 36 h. The reaction was then cooled to 0 °C and 30 mL of 6 N HCl was slowly added. The ice bath was removed and the mixture was stirred at room temperature for 4 h. THF was removed in vacuo and the resulting aqueous layer was basified to pH 14 with solid KOH. The solution, which appeared to be saturated with KOH, was extracted with 5 × 30 mL of chloroform, dried ($MgSO_4$), filtered, and evaporated in vacuo to give 143 mg of yellow oil. This oil was dissolved in 5 mL of water, and 6 N HCl was added to pH 6. This solution was applied to a Dowex 50W-X8 (H^+ form) resin. Step gradient elution with distilled H_2O, 1 N HCl, 2.3 N HCl, 3.3 N HCl, and finally 4.3 N HCl resulted in product eluting at 4.3 N HCl to give 172 mg (28%) of **4** as a yellow solid after lyophilization: mp 180 °C br, dec; ^1H NMR (D_2O) δ 3.32–3.28 (m, 2 H), 3.02–2.94 (m, 8 H), 1.98–1.89 (m, 2 H), 1.84–1.75 (m, 2 H), 1.61 (br s, 4 H), 1.16–1.15 (d, 6 H); ^{13}C NMR (D_2O) δ 47.40, 45.86, 44.30, 30.76, 23.10, 17.70; HRMS (CI, NH_3) calcd for $C_{12}H_{30}N_4$ (M^+) 231.2549, found 231.2541.

Method B. N^1,N^4-Bis(3-azidobutyryl)-1,4-diaminobutane (**16**, 239 mg, 0.765 mmol) was added to a 25-mL round-bottom flask followed by attachment of a reflux condenser. THF (5 mL) was added via syringe, dissolving all the solid. BH_3·THF solution (1.0 M, 9.18 mL, 9.18 mmol) was slowly added and the resulting mixture was brought to reflux temperature for 24 h. After this time, the mixture was stirred at room temperature for 6 h. An ice bath was used to cool the reaction mixture to 0 °C, followed by slow addition of 1.5 mL of 6 N HCl. This mixture was then heated to reflux for 6 h followed by room temperature stirring overnight. THF was removed in vacuo followed by dilution of the resulting aqueous layer to 9 mL of total volume. This mixture was then applied to a Dowex 50W-X8 (H^+ form) cation-exchange column (100 mL volume of resin). Step gradient elution with distilled H_2O, 1 N HCl, 2.3 N HCl, 3.3 N HCl, and finally 4.3 N HCl resulted in product elution at 4.3 N HCl. The fractions containing the desired product were pooled and excess HCl was removed in vacuo followed by lyophilization to give 85 mg of yellow solid. This material proved impure by ^1H NMR analysis, so it was dissolved in 1 mL of water and was made basic (pH 14) with solid NaOH. Extraction with 3 × 5 mL of $CHCl_3$ and evaporation gave 73 mg of free base. This oil was then taken up in 1 N HCl and lyophilized to give 109 mg (38%) of the 4HCl salt (**4**) as a white solid: mp 250 °C dec; ^1H NMR (D_2O) δ 3.54–3.48 (m, 2 H), 3.23–3.15 (m, 8 H), 2.2–2.09 (m, 2 H), 2.06–1.96 (m, 2 H), 1.82–1.79 (m, 4 H), 1.37–1.35 (d, 6 H); ^{13}C NMR (D_2O) δ 47.10, 45.45, 44.02, 30.49, 22.85, 17.34; HRMS (DCI, NH_3) calcd for $C_{12}H_{30}N_4$ (M^+) 231.2549, found 231.2549. Anal. ($C_{12}H_{30}N_4$·4HCl) C, H, N.

Enzyme Assays and Cell Culture. Methods for the preparation and assay of spermine synthase and for the determination of growth and polyamine content of SV-3T3 cells are described by Pegg et al.[29] Preparation and assay of human SSAT[30] and cell culture experiments using L1210 cells[5] or HT29 cells[31] were carried out as previously described. Metabolism and uptake of compounds **2–4** were studied using HPLC analysis of cellular extracts[29] prepared at various times from cells exposed to these compounds as described in the text and legends. The retention time (t_R, min) during a typical experiment were as follows: spermidine (33.9), MeSpd (34.5), spermine (40.0), MeSpm (40.5), and Me_2Spm (40.9).

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Registry No. *dl*-**2**, 137946-02-2; *dl*-**2**·3HCl, 137945-92-7; *dl*-**3**, 137946-01-1; *dl*-**3**·HCl, 137945-93-8; **4**, 137946-03-3; **4**·4 HCl, 137945-94-9; *dl*-**5**, 2835-82-7; *dl*-**6**, 138051-81-7; **7**, 62146-62-7; **8**·HCl, 35517-18-1; *dl*-**9**, 137945-95-0; *dl*-**10**, 137945-96-1; *dl*-**11**, 137945-97-2; *dl*-**12**, 137964-65-9; **13**, 18523-47-2; *dl*-**14**, 137945-98-3; **15**, 137945-99-4; **16**, 137946-00-0; SSAT, EC2.3, 64885-84-3; spermidine, 124-20-9; spermine, 71-44-3; putrescine, 110-60-1; crotonic acid, 3724-65-0; *N*-[4-[(carbobenzyloxy)amino]butyl]-phthalimide, 66917-07-5; *N*-(4-azidobutyl)phthalimide, 66917-06-4; *N*-(4-bromobutyl)phthalimide, 5394-18-3; acrylic acid, 79-10-7.

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Binding of Phenylalkylamine Derivatives at 5-HT1C and 5-HT2 Serotonin Receptors: Evidence for a Lack of Selectivity

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Certain phenylalkylamine derivatives have been considered to bind selectively at 5-HT2 serotonin receptors. It is now recognized that the most widely used derivatives, i.e., 1-(2,5-dimethoxy-4-X-phenyl)-2-aminopropanes where X = Me (DOM), Br (DOB), and I (DOI) (**1–3**, respectively) also bind at the more recently identified population of serotonin 5-HT1C receptors. The purpose of the present investigation was to determine whether simple phenylalkylamines bind selectively at one population of receptors over the other. An examination of 34 derivatives reveals (i) similar structure–affinity relationships and (ii) a significant correlation (r = >0.9, n = 25) between 5-HT1C and 5-HT2 affinity. None of the compounds included in the present study displayed more than a 10-fold selectivity for one population of these receptors over the other; the results suggest that these compounds (including the widely used 5-HT2 agonists DOB and DOI) are 5-HT1C/5-HT2 agents.

The 5-HT2 population of serotonin (5-hydroxytryptamine) receptors has been implicated in cardiovascular function, muscle contraction, depression, anxiety, psychoses, and hallucinogenic activity (see refs 1–3 for recent reviews). Much of the impetus for clinical research in this area is directly related to the discovery of the "selective"

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