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Development of Polyamine Transport Ligands with Improved Metabolic Stability and Selectivity against Specific Human Cancers

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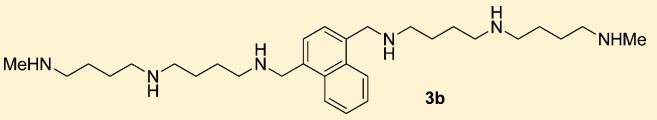
Supporting Information

ABSTRACT: Polyamine homeostasis is critical for life and is accomplished via a balance of polyamine biosynthesis, degradation, and transport. Rapidly dividing cancer cells have been shown to have high polyamine transport activity compared to normal cells, likely due to their high requirement for polyamine metabolites. The polyamine transport system (PTS) is a therapeutically relevant target, as it can provide selective drug delivery to cancer cells. This report describes the synthesis and biological evaluation of multimeric polyamine derivatives as efficient PTS ligands. Arylmethyl-polyamine derivatives were synthesized to address two important concerns in PTS drug design: (a) PTS selectivity and (b) stability to amine oxidases. N^1,$N^{1\prime}$-[Naphthalene-1,4-diylbis(methylene)]bis{N^4-[4-(methylamino)-butyl]}butane-1,4-diamine}, **3b**, was found to have an optimal balance between these parameters and demonstrated excellent targeting of melanoma (e.g., MALME-3M) and breast cancer cells (e.g., T47D) over other cancer cell lines. These results provide a method to selectively target cancers via their intrinsic need for polyamine metabolites.



INTRODUCTION

Polyamines are low molecular weight aliphatic amines and are essential metabolites for cell growth.[1] Tumor cells have been shown to contain elevated polyamine levels and have active polyamine transport systems to import exogenous polyamines.[1a] This special characteristic of cancer cells allows for cell-selective drug delivery of polyamine−drug conjugates to particular cell types.[1,2] The polyamine transport system (PTS) is an important target, as many cancer cells need to import polyamines in order to sustain their growth rate and for cell survival.

Although the PTS has been recognized as an important target for cell-selective drug delivery, this system is still poorly understood.[1d] Without a defined target, most of the advances in this field have been empirically derived from homologous series of polyamine derivatives. In this regard, the design factors are based upon lessons learned from incremental changes made along the polyamine backbone. Previous work has shown that the number of methylene spacer units, the size of the N^1 substituent, and the degree of N^1 substitution all influence PTS-mediated delivery.[2,3,4a] Initially, it was found that an anthryl−homospermidine conjugate, **1a** (Figure 1), had 150-fold higher cytotoxicity in PTS-active CHO cells[2c] than in the mutant cell line CHO-MG, which was PTS-deficient.[4b]

The toxicity of **1a** was shown to be directly related to the uptake of **1a** in each respective cell line.[2c] In this regard, the relative toxicity of the compound in these two CHO cell lines could be used to rank the ability of the compound to use the polyamine transport system. For example, compounds that did not use the PTS for cell entry would give CHO-MG/CHO IC_{50} ratios near 1, whereas highly selective PTS targeting compounds would give high IC_{50} values in CHO-MG and low IC_{50} values in CHO, that is, CHO-MG/CHO IC_{50} ratios $\gg$1.

After demonstration of the high selectivity of **1a** for targeting the PTS, another polyamine "message" was added to the aryl platform in an effort to enhance the PTS selectivity.[5] Rewardingly, these new disubstituted systems (Figure 1, **2a**−**4a**) showed a dramatic increase in PTS selectivity (e.g., CHO-MG/CHO IC_{50} ratios for **1a**, 150; **2a**, >2222).[5] Efforts to further improve the selectivity via a 1,3,5-trisubstituted benzene derivative **5a** revealed that the trisubstituted design was actually a potent polyamine transport inhibitor (PTI).[5] In sum, disubstituted designs were more optimal for gaining access to cells via the PTS. While this was a definite advance, other factors needed to be addressed in terms of further optimizing PTS targeting.

One implied limitation of polyamine-based compounds is their potential metabolism by polyamine oxidase (PAO). PAO activity dramatically reduces the PTS selectivity of polyamine-containing derivatives in culture via compound degradation. This degradation is inhibited when aminoguanidine (AG, a known inhibitor of PAO) is added to the growth medium.[6] PAO is known to be present in fetal bovine serum as well as in

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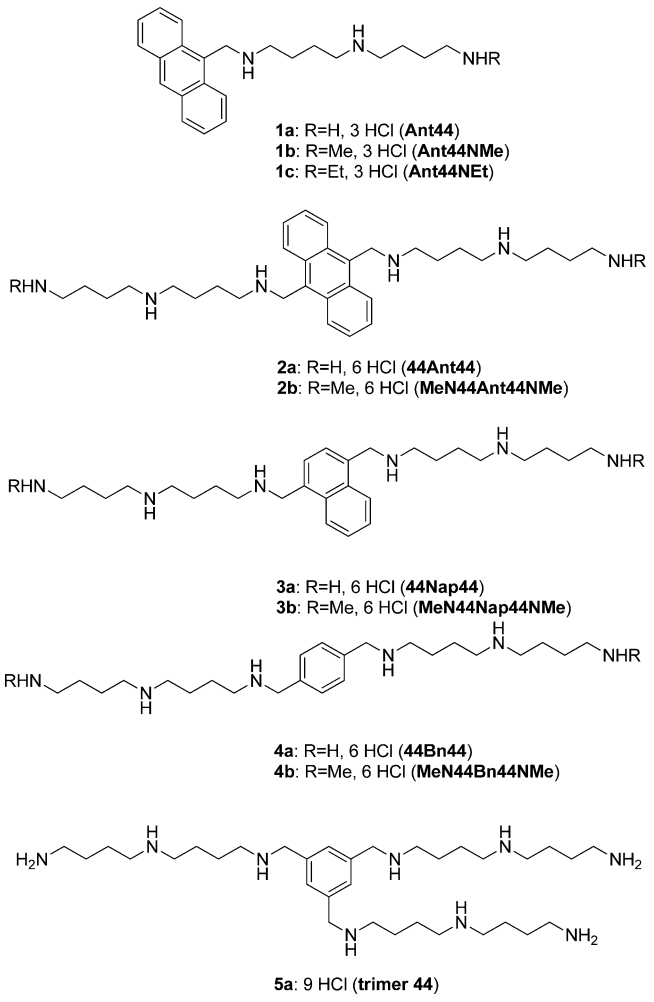

Figure 1. Structures of compounds **1**−**5a**.

human blood.[7] Since PAO targets primary amines, it also metabolizes polyamine-based derivatives containing primary amines. In terms of compound design, the primary amine can be protected by N-methylation to avoid the metabolic and degradative effects of PAO activity.[8] For example, compound **1a**, which lacks a terminal N-methyl group, showed profound loss in PTS selectivity in the absence of AG (CHO-MG/CHO IC_{50} ratio with AG, 150; without AG, 4).[8] The N-methylated derivative of **1a**, compound **1b**, was shown to retain its PTS selectivity in the absence of AG (CHO-MG/CHO IC_{50} ratio of 12.3 with AG and 11.5 without AG).[8] This protective feature came at the cost of PTS selectivity, however, as **1b** had 10-fold lower PTS selectivity than **1a**.

In terms of N-alkyl substituents, the N-methyl substituent was superior to the related N-ethyl derivative, **1c**. Specifically, **1c** gave a CHO-MG/CHO IC_{50} ratio of 5.9 with AG and 3.9 without AG.[8] Therefore, we elected to pursue the N-methyl derivatives because the methyl group introduced a minor steric constraint to the terminal amine group (a feature found to be key for PTS targeting), introduced the desired stability to amine oxidases (as seen in the AG experiments), and had provided enhanced PTS targeting (i.e., higher CHO-MG/CHO IC_{50} ratios) over the related N-ethyl derivative in the related monosubstituted anthryl−polyamine series.[8]

Prior work in our group sought to optimize the polyamine sequence, number of polyamine substituents, aryl core, and metabolic stability of PTS-targeting compounds (Figure 2).[2c,5,8] In an effort to further optimize our design, we investigated whether N-methylation technology could be combined with the disubstituted design to generate superior PTS ligands, which were more metabolically stable (Figure 2). Indeed, compounds **2b**, **3b**, and **4b** were generated with the expectation that they would show enhanced metabolic stability over their non-methylated counterparts (**2a**, **3a**, and **4a**) while also maintaining high PTS selectivity in the absence of AG. This report describes our successful implementation of this strategy and our findings with these remarkable new PTS-targeting compounds.

RESULTS AND DISCUSSION

Synthesis. Multiple synthetic routes were explored to generate compounds **2b**, **3b**, and **4b** in high purity; however, each was problematic. Our early methods attempted to build the disubstituted targets **2b**, **3b**, and **4b** by elaborating the polyamine architecture stepwise from the aryl core. While simple in principle, this approach led to unexpected purification difficulties due to incomplete reactions. The lesson learned from these endeavors was that it was important to (1) N-methylate at the beginning of the synthesis (not at the end) and (2) preassemble the N-methylpolyamine construct as a selectively Boc-protected motif and then anneal the entire polyamine component to the aryl core in one step to avoid purification issues. In this regard, it was determined that the key intermediate in generating **2b**, **3b**, and **4b** was the selectively protected polyamine **10**. As shown in Scheme 1, protected amine **10** was generated stepwise from **5b**.

The synthesis of compounds **2b**, **3b**, and **4b** began with the methylation of commercially available amino alcohol **5b**. This was accomplished through an acylation reaction with ethyl formate and reduction of the resulting formamide to **6** by use of $LiAlH_4$ in THF.[9a] This N-methylated amine was then Boc-protected to generate **7** in 84% yield, followed by subsequent O-mesylation to afford mesylate **8**.[9b] Mesylate **8** was then reacted with excess putrescine (5 equiv) in refluxing acetonitrile to afford **9** in 90% yield. This proved to be very valuable, as the entire N-methyl homospermidine chain was generated prior to attachment to the aryl core via reductive amination. To address future purification issues, **9** was converted into the desired Boc-protected polyamine **10**.

The conversion of **9** to **10** was based upon prior work in our lab where selectively protected triamines could be separated from their respective condensation products with arylaldehydes.[2g] This method takes advantage of the distinct polarity differences between primary and secondary amines.[10] In order to use this approach, a method was needed to selectively introduce a Boc group onto the secondary amine of **9** in the presence of a primary amine. Previous work has shown that the chemoselectivity of imine formation with salicylaldehyde occurs with primary amines exclusively in the presence of secondary amines.[11] Therefore, the primary amine of **9** was sequestered as an imine, and the secondary amine was selectively Boc-protected. Subsequent imine hydrolysis then generated the desired primary amine, **10**. Note: the imine hydrolysis step in 0.1 M HCl/EtOH on a larger scale surprisingly led to considerable Boc deprotection and reduced yields. An alternative acid-free route described by Khomutov et al.[11] involving $MeONH_2$ and imine−oxime exchange was later found to be superior for this particular hydrolysis step. Nevertheless, by the former approach, polyamine **10** was

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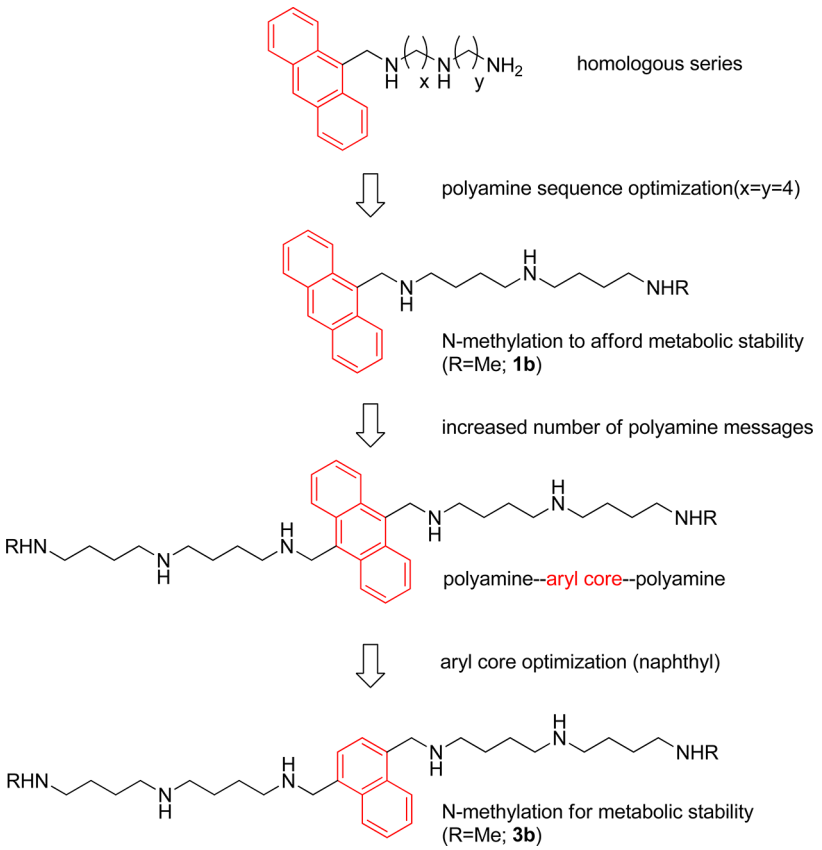
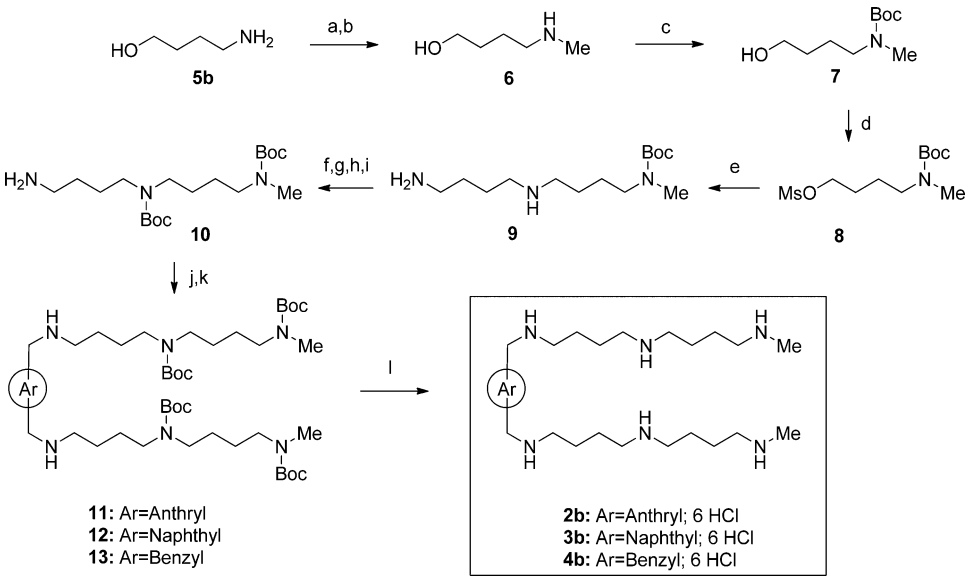


Figure 2. Evolution of PTS targeting compound design.[2c,5,8]

Scheme 1[a].



[a]Reagents: (a) Ethyl formate, EtOH; (b) $LiAlH_4$, THF; (c) di-*tert*-butyl dicarbonate, 10% TEA/MeOH; (d) MsCl, TEA, CH_2Cl_2; (e) putrescine (5 equiv), K_2CO_3, CH_3CN; (f) salicylaldehyde, Na_2SO_4, 25% $MeOH/CH_2Cl_2$; (g) di-*tert*-butyl dicarbonate, MeOH; (h) 0.1 M HCl, EtOH; (i) aqueous Na_2CO_3; (j) aryldialdehyde, 25% $MeOH/CH_2Cl_2$; (k) $NaBH_4$, 50% $MeOH/CH_2Cl_2$; (l) 4 M HCl, EtOH.

synthesized with excellent regioselective control in 47% overall yield from **9**.

As shown in Scheme 1, the desired architectures were generated by reacting polyamine **10** with the appropriate aryldialdehyde via reductive amination. Compounds **11**−**13** were successfully generated and purified (55−74% yield). The methodology developed to generate **10** proved to be invaluable, as the purifications of **11**−**13** were straightforward by column chromatography (see Experimental Section). Finally, with compounds **11**−**13** in hand, each was treated with 4 M HCl to obtain the respective final compounds **2b**, **3b**, and **4b** in near-quantitative yields.

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Table 1. Biological Evaluation of Polyamine Derivatives (1−4) in CHO and CHO-MG* cells in the Presence and Absence of Aminoguanidine[a]

	with AG			without AG		
compd	CHO-MG* IC_{50} (μM)	CHO IC_{50} (μM)	IC_{50} ratio[b]	CHO-MG* IC_{50} (μM)	CHO IC_{50} (μM)	IC_{50} ratio[b]
1a (Ant44)	13.7 (±1.3)	0.32 (±0.01)	43	2.2 (±0.1)	1.5 (±0.02)	1.4
1b (Ant44NMe)	10.7 (±1.2)	2.8 (±0.2)	**3.8**	11.3 (±2.2)	2.1 (±0.06)	**5.4**
2a (44Ant44)	>100	0.028 (±0.001)	>3571	8.4 (±0.7)	4.0 (±0.3)	2.1
2b (MeN44Ant44NMe)	>100	0.083 (±0.004)	**>1204**	>100	0.084 (±0.002)	**>1190**
3a (44Nap44)	>100	0.022 (±0.002)	>4545	52.1 (±7.5)	5.5 (±0.5)	9.5
3b (MeN44Nap44NMe)	>100	0.044 (±0.002)	**>2272**	>100	0.039 (±0.001)	**>2564**
4a (44Bn44)	19.6 (±0.8)	0.027 (±0.001)	727	56.5 (±3.5)	10.3 (±0.9)	5.5
4b (MeN44Bn44NMe)	51.5 (±2.6)	0.030 (±0.001)	**1715**	54.0 (±2.4)	0.041 (±0.002)	**1316**

[a]Cells were incubated for 48 h at 37 °C with the respective conjugate. Where indicated, AG (1 mM) was incubated with cells for 24 h prior to compound addition. All experiments were done in triplicate. Note: a negative control, anthryl−putrescine conjugate (not shown, see ref 3b), gave a CHO-MG/CHO IC_{50} value of 1, indicating a lack of PTS-targeting in CHO.[3b] Ratios for the N-methyl derivatives (**1b**, **2b**, **3b**, and **4b**) are highlighted in boldface type to illustrate their retention of selectivity in the absence of AG. [b]CHO-MG*/CHO IC_{50} ratio, a measure of PTS selectivity.

CHO and CHO-MG* Studies. CHO cells were chosen along with a mutant cell line (CHO-MG*) to investigate how the synthetic conjugates gain access to cells.[2c−e,12] The CHO-MG* cell line is polyamine transport-deficient and represents a model for alternative modes of entry (other than PTS), including passive diffusion or use of another transporter. The CHO cell line, on the other hand, represents cells with high polyamine transport activity.[4a,c] A comparison of toxicity in these two CHO cell lines provided a screen that would detect selective use of the polyamine transport system. High utilization of the PTS by the polyamine compounds would be very toxic to CHO cells. However, reduced toxicity would be expected in CHO-MG* cells.[2c−e,12] Ultimately, a CHO-MG*/CHO IC_{50} ratio was determined for each compound, where a high ratio would be achieved for highly PTS selective compounds. The results for this PTS screen are listed in Table 1.

The CHO-MG* line was derived from the original CHO-MG line obtained from Flintoff.[4a,13] This cell line proved to be very useful in screening compounds for their PTS selectivity. For example, the respective CHO-MG/CHO IC_{50} ratios for previously synthesized polyamine−anthryl conjugates **1a** and **4a** were 148[3b] and 677,[5] whereas the CHO-MG*/CHO IC_{50} ratios of **1a** and **4a** were 43 and 727, respectively. Compounds **2a** and **3a** also served as controls and gave similar findings with CHO-MG* as published earlier with CHO-MG.[5] Specifically, we confirmed that the IC_{50} values of **2a** and **3a** were >100 μM in both CHO-MG and CHO-MG* cells. Given the similar results and trends obtained with the CHO-MG* cell line with these four control compounds and the fact that the original stocks of CHO-MG were no longer available from their original source, CHO-MG* cells were used in our PTS screen. Rewardingly, we observed 1000-fold differences in toxicity with the new compounds (Table 1) in the CHO-MG* and CHO cell lines.

Several trends are evident in Table 1. First, a dramatic increase in PTS selectivity was observed when a second polyamine message was attached to the cytotoxic (aryl) core (e.g., CHO-MG*/CHO IC_{50} ratio of **2a** >3571 vs CHO-MG*/CHO IC_{50} ratio of **1a** 43). This was expected, as an increase in the number of appended polyamine messages had been previously shown to increase the compound's ability to gain entry via the PTS.[5] Second, in the aryl series (**2a**, **3a**, and **4a**), the anthryl and naphthyl cores seemed to outperform the benzene core, **4a**. Third, cell culture experiments were routinely run in the presence of aminoguanidine (AG, 1 mM), a known inhibitor of PAO, to avoid degradation of the polyamine derivative.[6] Experiments performed in the absence of aminoguanidine (AG) showed a dramatic decrease in PTS selectivity (e.g., CHO-MG*/CHO IC_{50} ratio with AG for **2a** >3571, CHO-MG*/CHO IC_{50} ratio without AG for **2a** 2.1). However, the related N-methyl derivative **2b** showed that metabolic stability could be achieved via N-methylation (CHO-MG*/CHO IC_{50} ratio with AG for **2b** >1204, CHO-MG*/CHO IC_{50} ratio without AG for **2b** >1190). This retention of PTS selectivity in the absence of AG additive was also seen with **3b** and **4b**.

By design, compounds **2b**, **3b**, and **4b** incorporated the best features of the disubstituted platforms (enhanced PTS targeting) and the N-methylation strategy (metabolic stability). Rewardingly, all the N-methylated disubstituted systems (**2b**, **3b**, and **4b**) *retained their high PTS selectivity in the absence of AG additive* (Table 1). With these important caveats addressed, the targeting ability of these compounds was then evaluated in human and mouse cancer cell lines.

MALME-3 and MALME-3M Studies. Compounds **2**−**4** were sent to the National Cancer Institute (NCI) to evaluate their performance in 60 human cancer cell lines at 10 μM. The results for compounds **1**−**4** are provided in the Supporting Information. It is important to note that this screen is performed in medium containing fetal bovine serum in the absence of AG. Thus, the screen was performed in the presence of amine oxidases, which can degrade nonmethylated compounds like **3a** (as well as **1a**, **2a**, and **4a**). The NCI screen showed that **3a** (and presumably its metabolites) had mixed outcomes across a wide range of different cancer cell lines. For example, **3a** (10 μM) was toxic to SW-620, T-47D, and K-562 cells and stimulatory to NCI-H460, HCT-15, MCF-7, and ACHN cells (Supporting Information, Figure S5). While this finding was interesting in its own right, it likely is an artifact of compound degradation due to the absence of the polyamine oxidase inhibitor AG.

An even more interesting finding was the remarkable selectivity observed with compounds **2b** and **3b** in specific cancer cell lines. For example, the more stable motif **3b** showed no stimulatory effects and was very toxic to melanoma cell lines such as MALME-3M and UACC-257 as well as to the human breast cancer line T-47D (Supporting Information, Figure S6).

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The exquisite sensitivity of the MALME-3M cell line was reconfirmed by the NCI through a five-dose assay where a dose-dependent response was observed (data not shown). Armed with this insight, we purchased the MALME-3M (melanoma) cell line and its noncancerous counterpart MALME-3 from ATCC and evaluated them in our laboratory. These matched cell lines were originally acquired from the same patient and provided a convenient method to assess PTS selectivity for the human melanoma line (MALME-3M) over the noncancerous primary cell line (MALME-3). Separate experiments in MALME-3M confirmed the expected enhanced stability of the N-methylated systems **1b**, **2b**, **3b**, and **4b** in the absence of AG, further validating the earlier findings in the CHO lines. In order to compare the methylated and nonmethylated systems, however, both MALME-3 and MALME-3M were evaluated in the presence of AG, and the results are shown in Table 2.

Table 2. Biological Evaluation of Polyamine Derivatives (1−4) in MALME-3 and MALME-3M cells in the Presence of Aminoguanidine[a]

compd	MALME-3 IC_{50} (μM)	MALME-3M IC_{50} (μM)	IC_{50} ratio[b]
1a (Ant44)	0.83 (±0.03)	0.27 (±0.01)	3.1
1b (Ant44NMe)	0.62 (±0.01)	0.45 (±0.01)	**1.4**
2a (44Ant44)	0.69 (±0.02)	0.017 (±0.001)	41
2b (MeN44Ant44NMe)	1.00 (±0.01)	0.062 (±0.002)	**16**
3a (44Nap44)	1.27 (±0.09)	0.018 (±0.001)	71
3b (MeN44Nap44NMe)	0.82 (±0.06)	0.014 (±0.001)	**59**
4a (44Bn44)	0.09 (±0.01)	0.005 (±0.0002)	18
4b (MeN44Bn44NMe)	0.020 (±0.001)	0.010 (±0.0001)	**2**

[a]Cells were incubated for 96 h at 37 °C with the respective conjugate. AG (1 mM) was determined to be nontoxic and was incubated with MALME-3M and MALME-3 cells for 24 h prior to compound addition. This facilitated comparisons between the compounds tested. All experiments were done in triplicate in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. The IC_{50} ratios observed with the preferred N-methyl derivatives appear in boldface type. [b]MALME-3/MALME-3M IC_{50} ratio, a measure of selectivity.

A 96-h viability assay was developed to accommodate the slow growth rate of MALME-3 (doubling time ~96 h) when using the medium described in Table 2. Note: MALME-3M had a doubling time ~48 h. By growing both cell lines in the same medium over the same time period in the presence of AG (1 mM), direct comparisons could be made.

With this assay in place, compounds **1**−**4** showed high cytotoxicity (IC_{50} < 500 nM) in MALME-3M, with the disubstituted platforms (**2**−**4**) being the most potent in this cell line (Table 2). It was also observed that the nonmethylated derivatives (**1a**, **2a**, **3a**, and **4a**) all showed enhanced selectivity over their methylated counterparts in the presence of AG. We noted that **3a** demonstrated significant toxicity to MALME-3M (IC_{50} = 18 nM) in our experiments that was not observed in the original NCI screen of **3a** at 10 μM. We speculate that this loss in potency of **3a** at NCI was due to the absence of AG in their screen. Compound **3b** gave a high MALME-3/MALME-3M ratio of 59 (Table 2), and control experiments in MALME-3M revealed that compound **3b** retained its potency in the presence and absence of AG.

The aryl core of each compound also demonstrated a significant effect as the benzene-containing compounds (**4a** and **4b**) proved to be the most toxic, while the toxicities for the anthryl (**2a** and **2b**) and naphthyl (**3a** and **3b**) derivatives were very similar (Table 2). As anticipated from our CHO studies, the monosubstituted compounds **1a** and **1b** were significantly less toxic in MALME-3M and exhibited relatively low selectivity for targeting the MALME-3M cell line over MALME-3. This was reflected in their low MALME-3/MALME-3M IC_{50} ratios of 3.1 and 1.4, respectively (Table 2). The enhanced selectivity observed with the disubstituted series (especially **2** and **3**) is consistent with these agents being PTS-selective agents with increased preferential uptake by the MALME-3M line over its matched MALME-3 line.

Despite the enhanced toxicity of **4a** and **4b** in MALME-3M, the benzene-based derivatives demonstrated the poorest selectivity for the MALME-3M cell line over the MALME-3 cell line in the disubstituted series. This was reflected in their MALME-3/MALME-3M IC_{50} ratios of 18 and 2, respectively (Table 2). This result is consistent with findings observed in CHO/CHO-MG* (Table 1), where **4a** and **4b** also exhibited lower PTS selectivity. Their enhanced toxicity, and modest selectivity may be attributed to the xylyl core being more prone to metabolism and being degraded into toxic metabolites.[3b] Indeed, prior work showed that the related monosubstituted benzyl-homospermidine motif was converted to free homospermidine, whereas the naphthyl and anthryl derivatives were dramatically less prone to degradation.[3b]

Regardless of the rationale for the performance of **4a** and **4b**, in the present studies the anthryl and naphthyl cores (**2** and **3**) demonstrated increased selectivity for MALME-3M over MALME-3. This finding was attributed to enhanced uptake of these compounds through the PTS of cancer cells (i.e., MALME-3M) compared to their reduced uptake into noncancerous cells (i.e., MALME-3).

Murine Cell Line Studies. Prior work with compound **1a** indicated the sensitivity of the B16 mouse melanoma cell line to aryl−polyamine conjugates.[17] Since compound **3b** was highly selective in targeting specific human cancer lines, we surveyed its toxicity in several murine cancer cell lines in the absence of AG (Table 3). These included CT26.CL25 (murine colorectal adenocarcinoma), Pan02 (murine pancreatic adenocarcinoma), 4T1 (murine mammary gland adenocarcinoma), ID8 (murine ovarian adenocarcinoma), and B16F10 (murine melanoma) cells. As shown in Table 3, compound **3b** provided IC_{50} values ranging from 30 to 131 nM in the murine cell lines tested after

Table 3. IC_{50} Values (72 h) of Compound 3b in Murine Cancer Cell Lines[a]

murine cell line	IC_{50} (μM)
CT26.CL25	0.0295 (±0.0003)
ID8	0.0464 (±0.004)
4T1	0.0576 (±0.0026)
B16F10	0.0747 (±0.0001)
Pan02	0.1308 (±0.003)

[a]Tumor cells were plated at 5000 cells/well in a 96-well dish in medium not containing aminoguanidine. Increasing concentrations of compound **3b** (MeN44Nap44NMe) were added to the culture medium 24 h after plating. Cells were harvested after 72 h of treatment with **3b**. Cell growth was determined by measuring the ability of viable cells to convert hydroxyethyldisulfide to mercaptoethanol by use of the CellCountEZ cell survival assay kit (Rockland Immunochemicals, Inc., Gilbertsville, PA).[16] All experiments were performed in triplicate.

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a 72 h incubation period. Rewardingly, the availability of both human and mouse cell lines that show sensitivity to **3b** provide important in vivo tools to further evaluate this compound in murine models of human cancers.

The hypothesis that the observed toxicities in human and mouse cell lines were attributed to uptake through the PTS was further tested in a series of spermidine rescue experiments.

Spermidine Rescue Experiments. Spermidine (Spd) is a native polyamine that has high affinity for the PTS and is a cellular growth factor. In an effort to determine whether the cytotoxicities observed with compounds **2**−**4** were polyamine-transport-related, cytotoxicity measurements were performed in the presence of increasing doses of spermidine (Spd) in MALME-3M and MALME-3 cells. It was expected that, at high Spd concentrations, Spd would outcompete compounds **2**−**4** for cellular entry via the PTS and rescue the cells from the toxic effects of the PTS-targeting compound. Rewardingly, high doses of exogenous Spd were able to significantly rescue (back to ∼90% viability) MALME-3M and MALME-3 cells challenged with an IC_{50} dose of compounds **2** and **3** in the presence of AG. These results supported the premise that these compounds gain access to these cells primarily via their PTS. Examples of the spermidine rescue experiment outcomes with **3a** and **3b** are shown in Figure 3, and the results found with compounds **2a,b** and **4a,b** are shown in the Supporting Information.

As shown in Figure 3, both **3a** and **3b**, when dosed near their respective IC_{50} value in MALME-3M and MALME-3 cells, could be rescued back to ≥90% viability with the addition of exogenous spermidine.

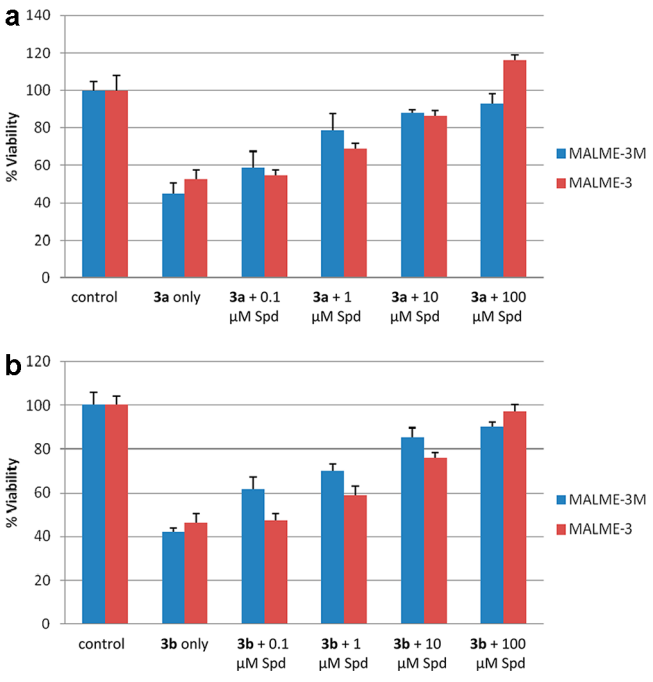

Figure 3. Ability of Spd to rescue MALME-3M and MALME-3 cells treated with (a) compound **3a** (44Nap44) and (b) compound **3b** (MeN44Nap44NMe). Cells were incubated for 96 h at 37 °C with **3a** at 0.02 μM (MALME-3M) and 1 μM (MALME-3) or with **3b** at 0.02 μM (MALME-3M) and 0.8 μM (MALME-3). AG (1 mM) was determined to be nontoxic and was incubated with cells for 24 h prior to drug addition. Control represents untreated cells. All experiments were done in triplicate. Note: Spd (100 μM) was nontoxic to both cell lines.

In contrast, when exogenous Spd (100 μM) was added to MALME-3M cells treated with **4a** and **4b**, a lower level of rescue (∼70% viability) was observed (Supporting Information). We speculate that compounds **4a** and **4b** gain intracellular access via both PTS and non-PTS related pathway(s) in this cell line because the viability of MALME-3M cells was only partially rescued by the PTS ligand spermidine. Compounds like **2** and **3**, which showed high selectivity in the CHO and CHO-MG* screen and selective targeting of MALME-3M cells, showed consistent rescue by exogenous spermidine. In contrast, **4a** and **4b**, which did not demonstrate the same degree of selectivity in the CHO and CHO-MG* screen or selective MALME-3M targeting over MALME-3, showed a lower degree of rescue with exogenous spermidine (100 μM).

We also noted that exogenous spermidine provided significant cell rescue in the related murine cell lines. As seen with the human MALME lines above, exogenous spermidine (100 μM), when co-dosed with **3b** (up to 5 μM **3b**) in each of these murine cell lines, provided significant cell rescue from the cytotoxic effects of **3b** in vitro (see Supporting Information).

We rationalized these collective findings as being due to the relative propensities of these compounds to use the polyamine transport system for cell entry. Compounds that are able to enter cells selectively via the PTS should give high CHO-MG*/CHO IC_{50} ratios. Cells treated with these PTS-targeting compounds should be rescued by the presence of exogenous spermidine. Compounds **2** and **3** clearly exhibited these properties in our assays, which is consistent with their ability to target the PTS of these cells.

It is also interesting to note that while Spd was able to rescue both MALME-3M and MALME-3 from the toxic effects of **2**−**4**, a large excess of Spd was necessary. Typically, 100 μM Spd was needed to obtain significant rescue. Since 50% relative cell viability could be obtained at very low doses of compounds **2**−**4** (i.e., 0.01−0.06 μM for MALME-3M and 0.02−1 μM for MALME-3), we noted that 100 μM Spd represented a very large molar excess compared to the dosed compound. Indeed, high levels of Spd were necessary to outcompete these compounds. These observations may be explained by the significant differences in K_i values. For example, in radiolabeled spermidine competition experiments in murine leukemia L1210 cells, the K_i values for compounds **2a** (K_i = 0.39 μM), **3a** (K_i = 0.17 μM), and **4a** (K_i = 0.52 μM) were all significantly lower than that for Spd (K_m = 2.46 μM).[2d,5] These findings suggest that the disubstituted compounds have a significantly higher affinity for the PTS as compared to Spd, which would explain the large molar excess of Spd necessary to outcompete **2**−**4** for the PTS.

As shown in Tables 1 and 2, in general the N-methyl derivatives had lower toxicity in CHO and lower selectivity in targeting MALME-3M cells than their corresponding non-methylated derivatives when AG was present. These results are consistent with prior work in our lab, which demonstrated that N-methylation can result in a lower affinity for the PTS as well as lower toxicity. For example, compound **1a** gave a L1210 K_i value of 1.8 μM and L1210 IC_{50} of 0.30 μM, whereas **1b** gave a K_i value of 8.2 μM and L1210 IC_{50} of 0.40 μM.[8] These results also reaffirm the need to design compounds that balance the compound's PTS-binding affinity, stability to amine oxidase, and toxicity in order to optimize selective delivery via the PTS.

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Of all the systems we have studied to date, compound **3b** provides the best balance of these properties.

While **1**−**4** target cell types with active polyamine transport systems, it is not clear as to how these disubstituted compounds actually kill cells once imported. We note that the monosubstituted derivative **1a** was shown to induce apoptosis in B16 cells and IL-3-dependent FL5.12A pro B cells as well as HL-60 cells.[17,18] Compound **1a** was also shown to induce mitochondrial membrane potential loss and cytochrome *c* release and induced apoptosis in human hepatoma BEL-7402 cells via a caspase-dependent pathway.[19] N-Alkylated polyamines have also been shown to increase spermidine/spermine N^1-acetyltransferase (SSAT) activity in certain cell types. For example, Porter and co-workers[14] showed that treating MALME-3M cells with dialkylated tetraamines (e.g., N^1,N^{11}-diethylnorspermine) led to a dramatic increase in SSAT activity followed by decreased levels of native polyamine pools and cell death. SSAT induction, however, was most significant with aminopropyl-containing substrates, which are absent in our systems. Nevertheless, future studies will explore whether these new PTS-targeting agents act via the SSAT induction pathway[14b] or other mechanisms (e.g., topoisomerase II inhibition[2a,b] or mitochondrial membrane destabilization[19]) to sensitize MALME-3M cells to cell death.

CONCLUSIONS

Studies in CHO and CHO-MG* illustrated that a disubstituted aryl design coupled with terminal N-methylation of the appended polyamine chains provided selective PTS agents with improved stability to amine oxidases. These results also demonstrated that the aryl core plays a key role, with anthryl and naphthyl cores being the most PTS-selective of the series tested. These trends were also observed in the MALME-3M/MALME-3 screen, where anthryl and naphthyl systems exhibited higher selectivity toward the MALME-3M melanoma line. The fact that exogenous spermidine provided significant rescue to MALME-3M cells treated with compounds **2a**, **2b**, **3a**, or **3b** further supported the compounds' utilization of the PTS for cell entry. This work was also extended to include several murine cancer cell lines, where the PTS-targeting compounds showed the same sensitivity to exogenous spermidine.

Most significantly, we have now demonstrated PTS-mediated cell-selective delivery in a human melanoma line, MALME-3M, over its normal matched control line, MALME-3. This approach, which is based upon the intrinsic need of cancers for polyamine growth factors, provides a new strategy to target cancers with high polyamine transport activity.[20] The observed cell selectivity and metabolic stability of **3b** are significant findings that could have direct applications in identifying and treating tumors with high polyamine transport activity. Future work will evaluate this PTS-targeting technology in the appropriate in vivo models.

EXPERIMENTAL SECTION

Materials. Silica gel 32−63 μm and chemical reagents were purchased from commercial sources and used without further purification. All solvents were distilled prior to use. All reactions were carried out under an N_2 atmosphere. ^{1}H and ^{13}C spectra were recorded at 400 or 75 MHz, respectively. Thin-layer chromatography (TLC) solvent systems are listed as volume percentages, and NH_4OH refers to concentrated aqueous ammonium hydroxide. All tested compounds (**1**−**4**) provided satisfactory elemental analyses and were ≥95% pure; these include compounds **2b**−**4b** from this report. Compounds **1a**, **1b**, **2a**, **3a**, **4a**, and **5a** were synthesized previously.[2c,5,8]

Biological Studies. CHO, CHO-MG*, MALME-3M, and MALME-3 cells were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Note: the medium must contain L-proline (≥2 μg/mL) for proper growth of the CHO-MG* cells. All cells were grown at 37 °C under a humidified 5% CO_2 atmosphere. When appropriate, aminoguanidine (1 mM) was added to the growth medium to prevent oxidation of the compounds by the enzyme (bovine serum amine oxidase) present in the serum. Cells in early to mid-log phase were used. CHO-MG* cells were derived from a thawed pellet of CHO-MG cells[13] left at room temperature for 24 h. This time period did not adversely affect the polyamine transport properties of the CHO-MG* cells, as determined by separate control experiments with four PTS compounds. The CHO-MG* cell line is available upon request from the corresponding author.

IC_{50} Determinations. Cell growth was assayed in sterile 96-well microtiter plates (Costar 3599, Corning, NY). CHO and CHO-MG* cells were plated at 10 000 cells/mL. MALME-3M and MALME-3 cells were plated at 5000 cells/mL. The respective cell suspension (90 μL, containing 1 mM aminoguanidine) was added to each well. Solutions at the appropriate concentration of each compound (10 μL/well) were added after an overnight incubation of each CHO cell line (90 μL cell suspension). After exposure to the compound for 48 h (CHO and CHO-MG*) or 96 h (MALME-3M and MALME-3), cell growth was determined by measuring formazan formation from 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfenyl)-2*H*-tetrazolium, inner salt (MTS, 20 μL) by use of a SynergyMx Biotek microplate reader for absorbance (490 nM) measurements.[15] CT26.CL25 (murine colorectal adenocarcinoma), Pan02 (murine pancreatic adenocarcinoma), 4T1 (murine mammary gland adenocarcinoma), ID8 (murine ovarian adenocarcinoma), and B16F10 (murine melanoma) cells were plated at 5000 cells/well in a 96-well microtiter plate and treated with the respective solutions of each compound for 72 h, beginning 24 h after the cells were plated. All murine in vitro tumor cell experiments contained 1 mM aminoguanidine and were performed in the presence and absence of spermidine (100 μM; see Supporting Information). Cell growth of murine tumor cell lines was determined by the ability of viable cells to convert hydroxyethyldisulfide to mercaptoethanol via the CellCount-EZ cell survival assay kit (Rockland Immunochemicals, Inc., Gilbertsville, PA).[16] All cell-based experiments were run in triplicate.

Syntheses. *N^1,$N^{1\prime}$-[Anthracene-9,10-diylbis(methylene)]bis{N^4-[4-(methylamino)butyl]butane-1,4-diamine}, **2b**.* Boc-protected compound **11** (271 mg, 0.28 mmol) was first dissolved in EtOH (20 mL) and stirred at 0 °C for 10 min. A 4 M HCl solution (12 mL, 48 mmol) was then added dropwise, and the mixture was stirred for 30 min at 0 °C. The temperature was then allowed to rise to room temperature and stirring continued under N_2 for 4 h. The volatiles were then removed in vacuo to produce **2b** as its HCl salt (orange solid, 215 mg, 93%). ^{1}H NMR (D_2O) δ 8.33 (dd, 4H), 7.82 (dd, 4H), 5.18 (s, 4H), 3.38 (t, 4H), 3.16 (12H), 2.76 (s, 6H), 1.84 (m, 16H); ^{13}C NMR (D_2O) δ 185.5, 129.9, 127.7, 125.1, 124.0, 48.2, 47.5, 46.9, 46.8, 42.9, 32.7, 22.9, 22.8, 22.7, 22.6. HRMS (FAB) *m*/*z* calcd for $C_{34}H_{56}N_6$ $(M + H)^+$ 549.4566; found 549.4639.

*N^1,$N^{1\prime}$-[Naphthalene-1,4-diylbis(methylene)]bis{N^4-[4-(methylamino)butyl]butane-1,4-diamine}, **3b**.* Compound **3b** was generated as its HCl salt by the procedure described for compound **2b**, except that **12** was used as the starting material. Yellow solid (98%); ^{1}H NMR (D_2O) δ 8.25 (dd, *J* = 6.3, 3.2 Hz, 2 H), 7.84 (dd, *J* = 6.3, 3.2 Hz, 2H), 7.76 (s, 2H), 4.84 (s, 4H), 3.31 (t, *J* = 8.1 Hz, 4H), 3.13 (m, 12H), 2.75 (s, 6H), 1.84 (m, 16H); ^{13}C NMR (D_2O) δ 134.3, 132.2, 131.5, 130.9, 126.8, 51.2, 51.0, 50.2, 49.9, 35.7, 25.9, 25.8, 25.6. HRMS (FAB) *m*/*z* calcd for $C_{30}H_{54}N_6$ $(M + H)^+$ 499.4410; found 499.4479.

*N^1,$N^{1\prime}$-[1,4-Phenylenebis(methylene)]bis{N^4-[4-(methylamino)-butyl]butane-1,4-diamine}, **4b**.* Compound **4b** was generated as its HCl salt by the procedure described for compound **2b**, except that **13** was used as the starting material. White solid (99%); ^{1}H NMR (D_2O) δ 7.61 (s, 4H), 4.28 (s, 4H), 3.21−3.12 (t, 16H), 2.73 (s, 6H), 1.78

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(m, 16H); ^{13}C NMR (D_2O) δ 135.0, 133.4, 53.5, 51.1, 49.7, 49.4, 35.6, 25.7, 25.62, 25.58, 25.5. HRMS (FAB) m/z calcd for $C_{26}H_{52}N_6$ $(M + H)^+$ 449.4253; found 449.4326.

4-Methylaminobutan-1-ol, ***6***.[9a] To a stirred solution of 4-aminobutanol **5b** (4.31 g, 48.4 mmol) in EtOH (50 mL) was added ethylformate (5.86 mL, 75.5 mmol), and the mixture was stirred at reflux for 18 h under N_2. The solution was evaporated under reduced pressure, and the crude formamide was used in the next step without further purification. The reaction mixture was then dissolved in THF (25 mL) and added to a suspension of $LiAlH_4$ (5.50 g, 145 mmol) in THF (50 mL) dropwise under a drying tube while stirring. The reaction mixture was brought to reflux and monitored by TLC (20% EtOH/80% $CHCl_3$) and ^{1}H NMR ($CDCl_3$). After 2 h, the starting material was consumed, and H_2O (4.16 mL) was added to the cooled reaction mixture, followed by 4 M NaOH (4.16 mL) and H_2O (12.5 mL) with vigorous stirring. The precipitate was then removed by filtration, and the filtrate was concentrated in vacuo. The residue was redissolved in $CHCl_3$, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to give **6** as a colorless oil (2.76 g, 56%). ^{1}H NMR ($CDCl_3$) δ 3.74 (br s, 2H), 3.57 (t, 2H), 2.62 (t, 2H), 2.43 (s, 3H), 1.50−1.75 (m, 4H), which matched literature values.[9a]

(4-Hydroxybutyl)methylcarbamic Acid tert-Butyl Ester, ***7***.[9b,21] A solution of **6** (2.76 g, 26.8 mmol) in TEA/MeOH (1:7 v/v, 100 mL) was stirred at 0 °C for 10 min. A solution of di-*tert*-butyl dicarbonate (8.76 g, 40.2 mmol) in MeOH (20 mL) was added dropwise over 10 min. The mixture was stirred for 1 h under N_2 atmosphere. The temperature was allowed to gradually rise to room temperature, and the solution was stirred overnight. The solution was evaporated under reduced pressure, and the residue was dissolved in CH_2Cl_2 and washed with deionized water. The organic layer was separated, dried over anhydrous Na_2SO_4, filtered, and concentrated to give a colorless oil **7** that was used in the next step without further purification (4.54 g, 84%). ^{1}H NMR ($CDCl_3$) δ 3.66 (t, 2H), 3.23 (t, 2H), 2.83 (s, 3H), 1.78 (br s, 1H), 1.51−1.60 (m, 4H), 1.44 (s, 9H), which matched literature values.[21]

Methanesulfonic Acid 4-(tert-Butoxycarbonylmethylamino)butyl Ester, ***8***.[9b] To a solution of the alcohol **7** (4.54 g, 22.24 mmol) and TEA (15.5 mL, 111 mmol) in CH_2Cl_2 (60 mL) at 0 °C was added methanesulfonyl chloride (12.73 g, 111 mmol) dropwise over 30 min under a N_2 atmosphere. The reaction mixture was stirred at 0 °C for 1 h and was slowly warmed to room temperature and stirred overnight under N_2. The reaction mixture was then cooled to 0 °C, and a 1 M NaOH solution (500 mL) was added slowly with vigorous stirring. The organic phase was separated and washed with deionized water. The organic phase was again separated, dried over anhydrous Na_2SO_4, filtered, and concentrated to give the product **8** as a colorless oil that was used in the next step without further purification (6.03 g, 96%). ^{1}H NMR ($CDCl_3$) δ 4.25 (t, 2H), 3.28 (t, 2H), 3.02 (s, 3H), 2.84 (s, 3H), 1.78 (m, 2H), 1.66 (m, 2H), 1.44 (s, 9H), which matched literature values.[9b]

[4-(4-Aminobutylamino)butyl]methylcarbamic Acid tert-Butyl Ester, ***9***. Putrescine (9.45 g, 107 mmol, 5 equiv) was dissolved in acetonitrile (200 mL) with K_2CO_3 (14.79 g, 107 mmol) and the mixture was stirred under N_2. Mesylate **8** (6.03 g, 21.4 mmol) dissolved in acetonitrile (60 mL) was added dropwise over 30 min with stirring under N_2. After 30 min, the reaction mixture was brought to reflux and stirred overnight. The reaction mixture was then cooled, solid K_2CO_3 was filtered off, and the filtrate was concentrated in vacuo. The residue was redissolved in CH_2Cl_2 (200 mL) and washed six times with saturated aqueous Na_2CO_3 to remove the unreacted putrescine. The organic layer was then dried over anhydrous Na_2SO_4, filtered, and concentrated to give the product **9** as a clear oil that was used in the next step without further purification [yield 5.31 g (90% yield of crude mixture)]. ^{1}H NMR ($CDCl_3$) δ 3.21 (t, 2H), 2.84 (s, 3H), 2.71 (t, 2H), 2.62 (t, 4H), 1.40−1.60 (m, 17H). Note: this same displacement chemistry has been performed with an amino alcohol.[9b]

tert-Butyl N-(4-Aminobutyl)-N-[4-(tert-butoxycarbonylmethylamino)butyl]carbamate, ***10***. To a stirred solution of **9** (5.54 g, 20.3 mmol) and anhydrous Na_2SO_4 (23 g, 160 mmol) in 25% MeOH/CH_2Cl_2 (500 mL) at room temperature was added salicylaldehyde (2.47 g, 20.3 mmol) dropwise over 5 min, and the reaction was allowed to stir for 1 h. After ^{1}H NMR ($CDCl_3$) showed complete conversion to the imine, the reaction was cooled to 0 °C and di-*tert*-butyl dicarbonate (4.42 g, 20.3 mmol) was added as a solid. The reaction was then stirred for 45 min at room temperature. The volatiles were then removed under reduced pressure and the residue was redissolved in absolute EtOH (400 mL) and cooled to 0 °C. A 1 M HCl (30 mL) solution was added dropwise, and then the reaction was warmed to room temperature and allowed to stir for 2 h. After hydrolysis of the imine was complete, the volatiles were removed under reduced pressure. The residue was then redissolved in CH_2Cl_2 and washed three times with saturated aqueous Na_2CO_3 to generate the free base. The free base was then purified by column chromatography, R_f = 0.28 (1% NH_4OH/10% MeOH/89% CH_2Cl_2), to give **10** as a pale yellow oil (3.55 g, 47%). The lower-than-expected yield here was likely due to the surprising partial removal of Boc groups during the 2 h acid hydrolysis step. Note: an alternative hydrolysis procedure under acid-free conditions was later found to give higher yields in this type of transformation.[11] ^{1}H NMR ($CDCl_3$) δ 3.18 (t, 6H), 2.82 (s, 3H), 2.71 (t, 2H), 1.25−1.65 (m, 26H); ^{13}C NMR ($CDCl_3$) δ 155.8, 155.5, 79.3, 79.2, 48.6, 48.0, 49.6, 41.0, 40.7, 34.1, 28.6, 28.4, 28.0, 25.8, 25.2, 24.8.

Di-tert-butyl {[{[Anthracene-9,10-diylbis(methylene)]bis(azanediyl)}bis(butane-4,1-diyl)]bis{4-(tert-butoxycarbonylmethylamino)butyl}carbamate, ***11***. To a stirred solution of amine **10** (600 mg, 1.61 mmol) in 25% MeOH/CH_2Cl_2 (25 mL) was added a solution of anthracene-9,10-dicarboxaldehyde (172 mg, 0.73 mmol) in 25% MeOH/CH_2Cl_2 (20 mL). The reaction was then stirred at room temperature under N_2 overnight. After complete imine formation was determined by ^{1}H NMR, the solvents were then removed in vacuo and the residue was redissolved in 50% MeOH/CH_2Cl_2 (25 mL). The solution was then cooled to 0 °C, followed by addition of $NaBH_4$ (166 mg, 4.38 mmol) in small portions, and the mixture was stirred at room temperature for 2 h. After the reduction was complete, the solvents were removed in vacuo, the residue was redissolved in CH_2Cl_2, and the organic layer was washed three times with aqueous Na_2CO_3, separated, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to give **11** as a yellow oil (505 mg, 73%). R_f = 0.28 (6% MeOH/0.5% NH_4OH/93.5% CH_2Cl_2). ^{1}H NMR ($CDCl_3$) δ 8.37 (dd, 4H), 7.53 (dd, 4H), 4.70 (s, 4H), 3.19 (t, 12H), 2.89 (t, 4H), 2.81 (s, 6H), 1.57−1.44 (m, 52H); ^{13}C NMR ($CDCl_3$) δ 155.8, 155.6, 132.1, 130.1, 125.7, 124.9, 79.1, 50.4, 46.9, 46.0, 34.1, 28.5, 27.5. HRMS (FAB) m/z calcd for $C_{54}H_{88}N_6O_8$ $(M + H)^+$ 949.6664; found 949.6736.

Di-tert-butyl [{[{[Naphthalene-1,4-diylbis(methylene)]bis(azanediyl)}bis(butane-4,1-diyl)]bis[(tert-butoxycarbonyl)azanediyl]}bis(butane-4,1-diyl)]bis(methylcarbamate), ***12***. Compound **12** was generated by the procedure described for compound **11**, except that naphthalene-1,4-dicarboxaldehyde was used. Yellow oil (55%), R_f = 0.29 (10% MeOH/0.5% NH_4OH/89.5% CH_2Cl_2). ^{1}H NMR ($CDCl_3$) δ 8.21 (dd, 2H), 7.52 (dd, 2H), 7.71 (s, 2H), 4.22 (s, 4H), 3.19 (t, 12H), 2.81 (s, 3H), 2.75 (t, 4H), 1.31−1.62 (m, 52H); ^{13}C NMR ($CDCl_3$) δ 155.8, 155.6, 135.5, 132.2, 125.8, 125.6, 124.4, 79.2, 79.1, 51.8, 49.8, 46.9, 34.1, 28.5, 27.5. HRMS (FAB) m/z calcd for $C_{50}H_{86}N_6O_8$ $(M + H)^+$ 899.6507; found 899.6601.

Di-tert-butyl [{[1,4-Phenylenebis(methylene)]bis(azanediyl)}bis(butane-4,1-diyl)]-bis{[4-(tert-butoxycarbonylmethylamino)butyl]-carbamate), ***13***. Compound **13** was generated by the procedure described for compound **11**, except that benzene-1,4-dicarboxaldehyde was used. Yellow oil (74%), R_f = 0.25 (7% MeOH/0.5% NH_4OH/92.5% CH_2Cl_2). ^{1}H NMR ($CDCl_3$) δ 7.27 (s, 4H), 3.76 (s, 4H), 3.20 (t, 12H), 2.83 (s, 6H), 2.64 (t, 4H), 1.53−1.45 (m, 52H); ^{13}C NMR ($CDCl_3$) δ 155.7, 155.5, 138.9, 128.1, 79.0, 53.6, 49.0, 46.9, 34.0, 28.4, 27.3. HRMS (FAB) m/z calcd for $C_{46}H_{84}N_6O_8$ $(M + H)^+$ 849.6351; found 849.6423.

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■ ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR spectra for compounds **2b**, **3b**, **4b**, **6**−**9** (^{1}H NMR only), and **10**−**13**; one table with elemental analyses for compounds **2b**, **3b**, **4b**, and **11**−**13**; and 13 figures showing NCI 60 cell screen reports for compounds **1**−**4** at 10 μM, spermidine rescue experiments in MALME-3M and MALME-3 with **2** and **4**, and cytotoxicity curves of **3b** in murine tumor cell types (PanO2, 4T1, ID8, and B16F10) with and without 100 μM spermidine. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

PTS, polyamine transport system; CHO, Chinese hamster ovary; CHO-MG, Chinese hamster ovary cells which are defective in polyamine transport; PTI, polyamine transport inhibitor; AG, aminoguanidine; Boc, *tert*-butyloxycarbonyl; NCI, National Cancer Institute; Spd, spermidine; THF, tetrahydrofuran; TEA, triethylamine

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