The Effect of Polyamine Homologation on the Transport and Cytotoxicity Properties of Polyamine−(DNA-Intercalator) Conjugates

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An efficient five-step synthetic method was developed to access a homologous series of spermidine−acridine and spermidine−anthracene conjugates. The derivatives were comprised of a spermidine fragment covalently tethered at its N4 position to either an acridine or anthracene nucleus via an aliphatic chain (e.g., spermidine−[aliphatic tether]−acridine). The distance separating the spermidine and aromatic nucleus was altered by using different tethers comprised of four or five methylene units, respectively. These ligands (**2**−**5**) were shown to inhibit human DNA topoisomerase-II (TOPO-II) activity at 10 μM. Enzymatic activity was assessed as the ability to unknot (decatenate) and cleave kinetoplast DNA (kDNA). Polyamine conjugation did not disrupt the ability of the acridine−spermidine conjugates **2** and **3** to inhibit TOPO-II activity as compared with the 9-aminoacridine and 9-(*N*-butyl)aminoacridine controls (at 10 μM). In general, the acridine derivatives (**2** and **3**) showed higher TOPO-II inhibitory activity than their anthracene counterparts (**4** and **5**). However, this trend was reversed in a whole cell assay with L1210 (murine leukemia) cells, wherein the anthracene analogues were more potent than their acridine counterparts. In this regard the qualitative enzyme-based assay did not predict the trends in the corresponding IC_{50} values. Within either series insertion of an additional methylene unit did not significantly alter activity. While the appended spermidine unit did not disrupt TOPO II inhibition by the tethered DNA intercalator, it did provide an alternative mode of entry into the cell as demonstrated by spermidine protection assays. These results were compared with a spermine-intercalator analogue. Of all the conjugates tested the N^4-(4-(9-aminoacridinyl)butyl)spermine hexahydrochloride (conjugate **16**)resulted in the highest degree of L1210 cell rescue upon cotreatment of the cells with exogenous spermidine. It was concluded that the monoalkylated spermine motif present in **16** holds promise as a better vector than its N4 monoalkylated spermidine counterpart.

Introduction

In eukaryotic cells, DNA is segmentally unfolded within the nucleus by enzymes called topoisomerases.[1] Topoisomerase type I (TOPO-I) and type II (TOPO-II) represent two classes of the known mammalian DNA topoisomerases. To untwist densely packed DNA, these enzymes generate transient breaks within the DNA strands, allow for topological changes to occur and then reseal the break.[1] TOPO-I and TOPO-II act by creating temporary single-strand and double-strand breaks in DNA, respectively. Presently, two equilibrating complexes ("cleavable" and "noncleavable") have been shown to exist between topoisomerase II and DNA.[2] The equilibrium is normally shifted toward the "noncleavable" complex as the "cleavable" form results in permanent DNA strand breaks.[3,4] The "noncleavable" complex allows for the cleaved strands to untwist and to be reannealed, which in turn allows for the local separation of complementary polynucleotide strands and ultimately the generation of relaxed supercoils (both of which are important events for DNA replication and RNA transcription).

An anti-cancer strategy suggested by Nelson et al.[5] involves perturbing this equilibrium toward the "cleavable" complex, an event that results in permanent DNA strand breaks and cell death. This strategy was realized with amsacrine (AMSA, **1**), a drug currently used in the treatment of acute nonlymphocytic leukemia (see Figure 1).[2] In particular, TOPO-II has been identified as the primary target of **1** *in vitro*,[4] and AMSA has been shown to give similar DNA strand breaks in both cultured mammalian cells and isolated nuclei.[3,4] Nelson and others have suggested that AMSA shifts the aforementioned equilibrium toward the "cleavable" complex by forming a ternary complex of AMSA/topoisomerase II/ and DNA.[5] This AMSA-stabilized complex is the putative cause of cytotoxicity of this weak DNA intercalator.[5,6] Other molecules, which stimulate the formation of the "cleavable" complex, would be of clear value in terms of

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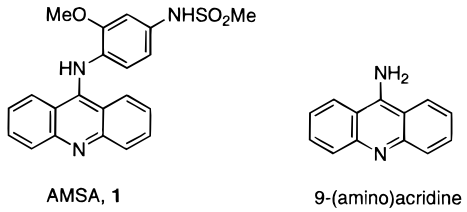

Figure 1. Amsacrine (AMSA) and 9-(amino)acridine.

developing new chemotherapeutics and further elucidating the specific mechanisms involved in the DNA cleaving process.[5]

It seemed possible that structural modifications of **1** would further enhance the TOPO-II inhibitory activity and the anti-proliferative properties of AMSA. In designing new ligands, we recognized that the intercalative association of **1** with DNA was likely attributed to the 9-aminoacridine component of AMSA (Figure 1). Indeed, many linear tricyclic systems are widely accepted as efficient DNA intercalators.[7,8] Mere intercalation of these ligand types, however, is not sufficient for anti-neoplastic activity as shown in a series of substituted acridines.[8] After an extensive investigation, Denny and co-workers found that both DNA intercalation and an appropriately placed side chain appeared to be an absolute requirement for antitumor activity with the acridine systems studied.[8]

We were interested in whether a DNA intercalator with an appended polyamine side chain such as spermidine (SPD, $H_2N(CH_2)_3NH(CH_2)_4NH_2$) or spermine (SPM, $H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$) would show altered transport behavior as the conjugate may interact with the polyamine transporter (PAT) present in cancer cells lines.[9] Such an interaction was expected to provide an alternative mode of entry into the cell and possibly provide a more selective chemotherapeutic. Indeed, such polyamine−DNA intercalator conjugates may act as dual vector systems, which target the DNA of rapidly dividing cell types.[9] The design of conjugates **2**−**5** (structures shown in Figure 2) is predicated upon the well-known affinity of polyamines for DNA and the established DNA binding modes of acridines[5,9] and anthracenes.[10] Furthermore, DNA intercalation models predict that a multitude of anionic site charges are available for binding of additional basic functions beyond those present in the intercalator framework.[11] Since polyamines exist as polycations *in vivo*, the conjugates (e.g., **2**) which have an appended polycationic tail may bind to these available sites thereby increasing the binding affinity and potency of the intercalator. Therefore, the combination of these agents (polyamines and DNA intercalators) were expected to provide new DNA targeting vectors, which significantly disrupt TOPO-II activity. This report outlines the synthesis and biological evaluation of several conjugates of this type.

In this regard, there has been recent interest in tethering polyamines to existing cancer drugs and bio-

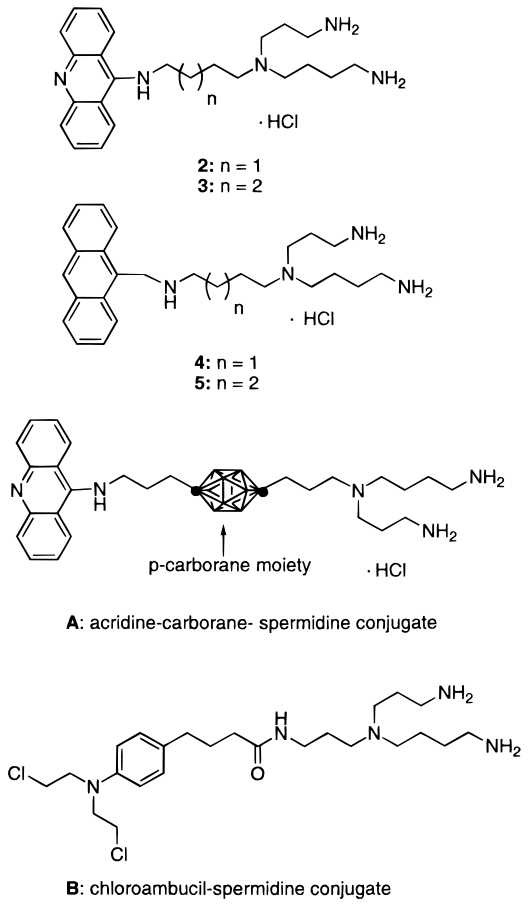

Figure 2. Acridine- and anthracene-based conjugates **2**−**5**, Sjöberg's conjugate **A**, and Cohen's conjugate **B**.

active agents to augment their activity and specificity.[9,12−19] Earlier investigations have shown that one can utilize polyamine conjugation to convey cytotoxic drugs (e.g., Figure 2: **A** and **B**) into rapidly growing cells.[9,12,13] During our biological evaluation of conjugates **2**−**5** Sjöberg and co-workers published their synthesis of conjugate **A**, a potential candidate for boron neutron capture therapy (BNCT).[9] In another example, Cohen and others found that a chloroambucil−spermidine conjugate **B** was 10 000-fold more effective in forming intrastrand cross-links with naked DNA. *In vivo* studies with plasmacytoma cells in mice showed that the polyamine conjugate was 4-fold more potent in inhibiting tumor growth than the unconjugated chloroambucil. They concluded that the conjugate **B** utilized the polyamine uptake apparatus as evidenced by its low K_i (vs spermidine) and the fact that exogenous

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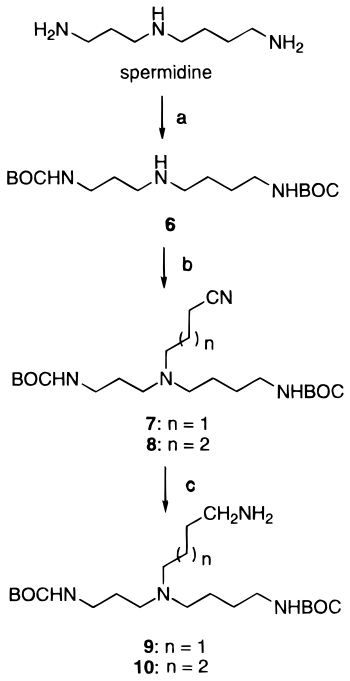
Scheme 1[a]



[a] Key: (a) BOC-ON, THF, 0 °C; (b) Br-$CH_2(CH_2)_nCH_2CN$, refluxing acetonitrile, solid K_2CO_3; (c) H_2 gas (75 psi), NH_4OH, Raney Ni (50% slurry), absolute EtOH saturated with NH_3 gas.

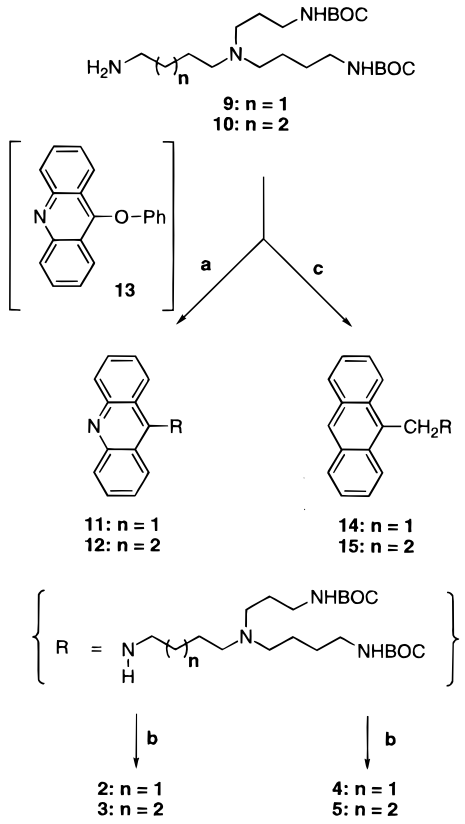
spermidine reduced the toxicity of the conjugate.[12,13] The present study also demonstrated that polyamine conjugates can also function as vectors,[19] which assist covalently attached components into cells.

Results and Discussion

Synthesis. The synthesis of each conjugate involves a series of protection and deprotection steps necessary for the synthesis of substituted polyamines and acridines.[11,20−25] In each case, spermidine was conjugated at the N4 position via an aliphatic carbon tether to either an acridine or anthracene nucleus. This central attachment was predicated upon the findings by Porter in 1982, wherein spermidine could be extensively derivatized at the central N4 position and still be taken up by the polyamine transporter.[23] The N4-alkylation step was designed to maintain the basicity of the N-4 nitrogen, which was also shown to be critical to uptake.[12,13,23]

Each conjugate (**2**−**5**) required the generation of a functionalized spermidine fragment with selectively protected amino groups. As shown in Scheme 1, the synthesis began with the selective acylation of the primary amino groups of spermidine with 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile (BOC-ON) to give the known N^1,N^8-bis(*tert*-butoxycarbonyl)spermidine **6** in 79% yield.[9,24,25] N^4-Alkylation of **6** with either 4-bromobutyronitrile or 5-bromovaleronitrile gave the corresponding nitriles **7** and **8** in 92% and 86% yield, respectively. Raney nickel reduction of **7** and **8** in the presence of ammonia under a high pressure of H_2 gave the respective amines **9** (40%) and **10** (74%).

Scheme 2[a]



[a] Key: (a) 9-chloroacridine, molten phenol, 50 °C; (b) 4 N HCl; (c) 9-anthraldehyde, 4 Å molecular sieves followed by $NaBH_4$.

The syntheses of the acridine conjugates **2** and **3** are shown in Scheme 2. The respective amines **9** and **10** were coupled to 9-chloroacridine using an excess of molten phenol to give **11** and **12** in 81% and 78% yield, respectively. The mechanism of the coupling reaction was shown to go through a 9-phenoxyacridine intermediate **13**.[9,26a,27] This finding was confirmed by isolating **13** and demonstrating its facile reaction with amine **9** to give the acridine derivative **11**.[26b] The BOC protecting groups on the spermidine−acridine conjugates **11** and **12** were then deprotected using 4 N HCl to give the respective HCl salts (**2** and **3**) in 79% yield for each conjugate.

In addition to the acridine derivatives, two anthracene−spermidine conjugates (**4** and **5**) were synthesized. As shown in Scheme 2, the respective amines **9** and **10** were condensed with 9-anthraldehyde and after imine reduction with $NaBH_4$ afforded the tethered amine adducts **14** (66%) and **15** (68%), respectively. The BOC protecting groups of **14** and **15** were then deprotected using 4 N HCl to give the HCl salt of the desired conjugates **4** (91%) and **5** (93%), respectively.

For comparison purposes, the monoalkylated spermine (SPM) derivative **16** (Scheme 3) and 9-(*N*-butylamino)-acridine **17** (Figure 3) were also synthesized. Using

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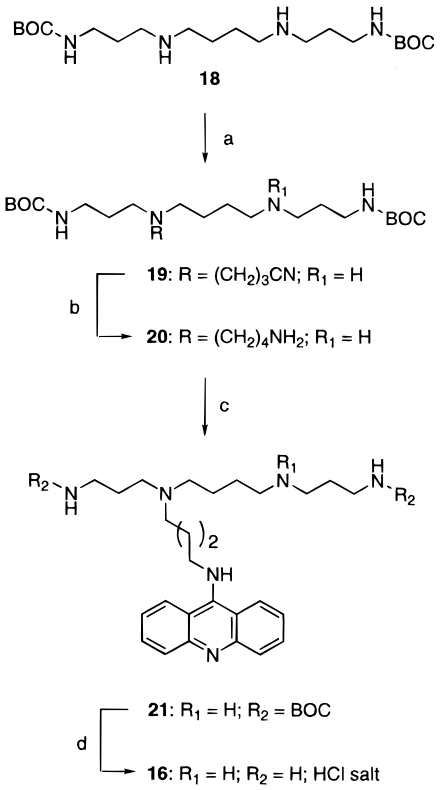
Scheme 3[a]



[a] Key: (a) 1 equiv of 4-bromobutyronitrile, K_2CO_3, refluxing CH_3CN; (b) Raney Ni, concentrated NH_4OH, H_2 (75 psi); (c) 9-chloroacridine, molten phenol, 100 °C; (d) 4 N HCl, 1,4-dioxane.

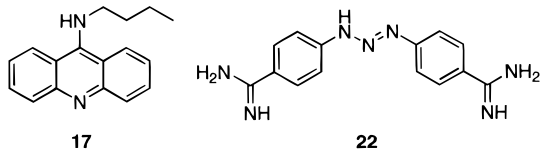


Figure 3. Structures of 9-(*N*-butyl)aminoacridine **17** and berenil **22**.

similar methods, the bis BOC-protected spermine derivative **18**[28] was monoalkylated to its corresponding nitrile **19** (73%), which was then reduced to amine **20** (95%) and coupled with 9-phenoxyacridine to give acridine **21** (70%), which was then deprotected to give conjugate **16** as its HCl salt (72%). Compound **17** was synthesized from 9-chloroacridine, phenol, and *N*-butylamine in 72% yield.

Biological Evaluation. The spermidine conjugates **2**−**5** were evaluated for their ability to inhibit human DNA topoisomerase II activity. Enzymatic activity was assessed as the ability to unknot (decatenate) and cleave *Crithidia fasciculata* kinetoplast DNA (kDNA).[29−31] A simple electrophoretic assay[32] allowed for the visualization of the reaction products (see the Experimental Section). Due to its high molecular weight, catenated

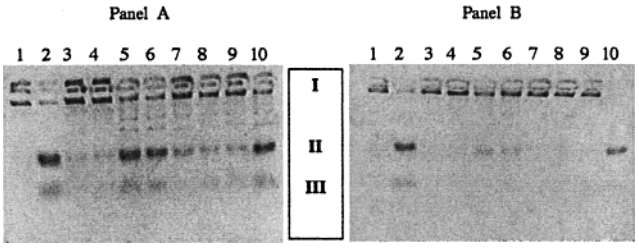


Figure 4. Topoisomerase II inhibition studies. Decatenation assay results are displayed as a negative image of the electrophoresis gel. All lanes contain 0.5 μg of kDNA and 4.0 units of human TOPO-II, with the exception of Lane 1. The respective drugs and conjugates were evaluated at 10 μM (panel A) and 20 μM (panel B). Lane 1: negative control (kDNA only). Lane 2: positive control (TOPO-II). Lane 3: acridine conjugate **2**. Lane 4: acridine conjugate **3**. Lane 5: anthracene conjugate **4**. Lane 6: anthracene conjugate **5**. Lane 7: berenil. Lane 8: 9-aminoacridine **23**. Lane 9: 9-(*N*-butylamino)acridine **17**. Lane 10: spermidine. Forms of DNA are denoted as Roman numerals: (intact kDNA (**I**, top); nicked circular DNA **II**, middle); and relaxed circular DNA (**III**, bottom).

DNA (kDNA) has limited migratory abilities and remains near the top well (form **I**), but the lower molecular weight decatenated forms [i.e., nicked-circular (form **II**) and relaxed DNA (form **III**)] migrate further down the gel. A potent TOPO-II inhibitor is expected to completely abrogate the decatenation process and to leave (after electrophoresis) significant amounts of kDNA remaining in the well.

Decatenation reactions were carried out in the presence of the conjugates **2**−**5** (at 10 and 20 μM, respectively). For comparison, reactions were also carried out with equivalent concentrations of berenil **22** (a.k.a. diminazene aceturate, a known inhibitor of TOPO-II),[33,34] 9-aminoacridine **23**, 9-(*N*-butylamino)acridine **17**, and spermidine (SPD), respectively (see structures in Figures 1 and 3). The results of these TOPO-II experiments are shown in Figure 4 (10 μM, panel A; 20 μM, panel B). As shown in Figure 4, conjugates **2**−**5** (lanes 3−6) clearly interfere with the (TOPO II-mediated) decatentation of kDNA in a concentration dependent manner. Independent of concentration (i.e., 10 or 20 μM), the acridine conjugates **2** and **3** exhibit significantly greater inhibitory activity than the respective anthracene derivatives **4** and **5**. This difference is most readily observed by the significant amount of kDNA remaining in the well in Figure 4 (panel A, lanes 3 and 4 versus lanes 5 and 6). Another apparent difference is the ladder of bands (i.e., partial kDNA networks) observed between forms **I** and **II**. These bands are indicative of diminished TOPO-II activity, wherein only partial decatenation activity takes place. Complete inhibition of TOPO-II activity should result in the absence of these ladder bands (as all the kDNA remains intact). Notably, the amount of these partial kDNA networks is significantly reduced upon increasing the concentration of the anthracene conjugates to 20 μM (see panel B, lanes 5 and 6). The enhanced activity of the acridine conjugates (versus the anthracene derivatives) is consistent with the higher binding constants which have been observed with acridines and DNA.[10,18,35,36]

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Table 1. IC_{50} Values (μM)

compd	IC_{50} (47 h)	IC_{50} (94 h)
2 (SPD C4 acridine)	75	55
3 (SPD C5 acridine)	>100	>100
4 (SPD C4 anthracene)	13	6
5 (SPD C5 anthracene)	7	5
16 (SPM mono C4 acridine)	23	17
17 9-(*N*-butylamino)acridine	2	2

Equally important results were obtained by carrying out the decatenation reactions in the presence of the minor-groove binder berenil **22** (lane 7) and the known DNA intercalator, 9-aminoacridine **23** (lane 8).[35] Other controls included derivative **17** (lane 9), which represents a molecular fragment of conjugate **2** without the polyamine moiety, and spermidine alone (lane 10).

Evaluation of these compounds provided both a verification of inhibition, as well as, insight into the contributions that each molecular fragment (i.e., intercalator and polyamine) had to the observed inhibitory activity. Examination of both gels reveals that the acridine derivatives (**2**, **3**, **17**, and **23**) possess similar potency as the berenil control **22**. These results are consistent with the avid binding characteristics observed with these altered ligand types with model oligonucleotides *in vitro*[10,36,37a] and the ability of other acridines to inhibit the DNA decatenation activity of TOPO-II.[37b] Incremental changes in tether length (**2** vs **3** and **4** vs **5**) did not seem to influence the efficacy of either system. Since the conjugates **2** and **3** were virtually equivalent to the acridine controls (lanes 8 and 9) and spermidine (lane 10) had little effect on decatentation, one can conclude that the appended polyamine did not disrupt the ability of the intercalator component to inhibit TOPO-II activity.

L1210 Studies. L1210 (murine leukemia) cells were challenged with various concentrations of the conjugates (**2**−**5** and **16**) and their respective IC_{50} values at 47 and 94 h are listed in Table 1. Even though the nonpolyamine-containing 9-(*N*-butyl)aminoacridine control was the most potent derivative tested, conjugation of a polyamine component onto the 9-aminoacridine architecture clearly modulates its bioactivity (Table 1: **2** vs 9-(*N*-butyl)aminoacridine). Surprisingly, even though **2** and **3** were more potent inhibitors of TOPO-II in the above gel assay, **4** and **5** were more efficacious in the whole cell-based assay. This reverse trend implies that either (a) TOPO-II inhibition is not the mechanism of action for these conjugates or (b) that gaining access to the cell (and eventually the nucleus) is more important than TOPO-II inhibition alone or (c) that a balance is needed between transport into the cell and TOPO-II inhibitory ability. Interestingly, conversion of the appended polyamine component into the longer spermine backbone increased the potency of **2** as shown by the lower IC_{50} of the mono-alkylated spermine derivative **16**. The reported mean IC_{50} value for AMSA **1** is 0.26 μM for six human cancer lines with varying levels of TOPO-II α and β isoforms.[37b] As both **1**[37b] and the acridines tested (**2** and **3**) are potent inhibitors of TOPO-II (by gel assay), the cytotoxicity difference is not likely due to TOPO-II inhibition, but the drug's ability to enter the cell. To relate potency to uptake by the polyamine transporter, we conducted several spermidine protection experiments.

Analysis of Polyamine Conjugate Transport. Are the polyamine−DNA intercalator conjugates recognized and transported by the polyamine uptake apparatus of L1210 cells? As mentioned previously, the observation by Cohen et al.[13] that a chloroambucil−spermidine conjugate **B** (Figure 2) has a low K_i value in competition experiments involving radiolabeled spermidine suggested the possibility that some of the conjugates may also be recognized by the polyamine transporter (PAT).

Several polyamine derivatives were examined. Two controls, dimethylspermidine (DMSPD) and diethylspermidine (DESPD) have been shown to gain entry into the cell via the PAT.[38] Both of these derivatives are toxic to L1210 cells, and kinetic assays have revealed that these SPD analogues inhibited PAT-mediated transport of SPD via a competitive mechanism.[38] In terms of their respective affinity for PAT, the K_i for inhibition of SPD uptake for DMSPD and DESPD has been reported to be 5 and 19 μM, respectively.[38] Based on these K_i values, the approximate K_m for DMSPD and DESPD transport via the PAT is 2.5 and 9.5 μM, respectively.[38] The cellular response to DMSPD and DESPD is death, which results from polyamine depletion.[38] Since the mode of SPD inhibition is competitive, and an excess of SPD is present in the system, it is expected that transport of both DMSPD and DESPD will be reduced by more than 90%, which in turn will significantly reduce cell death. Therefore, inhibition of PAT-mediated transport of DMSPD and DESPD will be observed as a decrease in cell death.

In general, the spermidine (SPD) protection assays were performed to determine whether uptake of selected polyamine analogues is mediated, in part or in whole, by the polyamine transport apparatus (PAT). To answer this question, competition assays were performed in the absence and presence of SPD. Figure 6 below illustrates the competitive uptake scenario probed by these experiments. To maximize the protective effects of spermidine (SPD), an excess of SPD (25 μM) was used during these experiments. Using a $25K_m$ excess of SPD ensures that SPD is transported into the cell at nearly V_{max}, and hence, provides a high level of competition with the selected polyamine derivatives for the PAT protein.

The results of experiments using DMSPD and DESPD are shown in Figure 5, panels 1 and 2, respectively. The graphs clearly indicate that over a 48 h period, the selected assay format provides sufficient sensitivity to reveal changes in cell viability. The low concentration range of DESPD and DMSPD used in these experiments was intended to produce a low-level of toxicity and, hence, amplify any observed SPD-mediated protection.

Examination of data obtained with DMSPD reveals a significant reduction of cell death in the presence of SPD. Specifically, at the lowest concentration of DMSPD (0.6 μM), the presence of SPD decreases the observed response by 97%. A 77% decrease in response was observed at the highest concentration of DMSPD (11 μM). This level of protection is consistent with a competitive mechanism. More significantly, the response can be predicted using eq 1. When 0.6 and 2.5 μM are substituted for [DMSPD] and K_m, respectively, and 25 and 2.2 μM are

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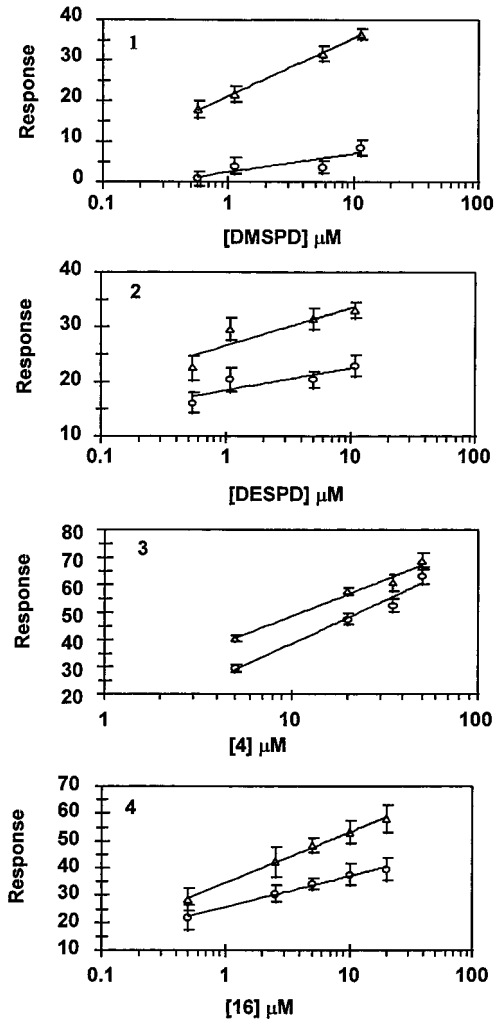

Figure 5. Spermidine protection experiments. See Methods section for description of experimental conditions. No spermidine (triangles), 25 μM spermidine (circles). A decreased response to added drug in the presence of spermidine is consistent with reduced drug uptake. Data shown represent the average of two independent trials, each performed in triplicate.

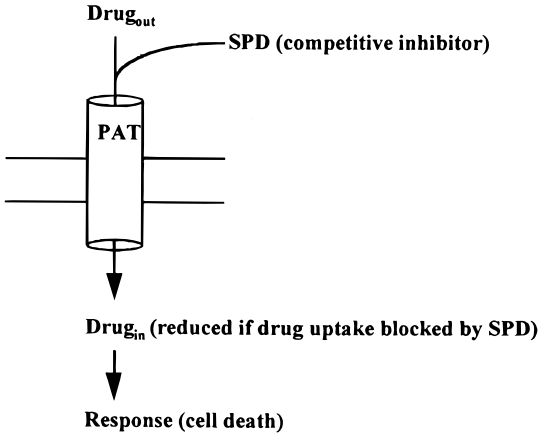

Figure 6. Competitive scenario for polyamine uptake.

substituted for [SPD] and K_i, respectively, the relative rate of transport in the presence of SPD, V_o/V_{max}, is calculated to be 0.02 or 98% inhibited (e.g., a 97% decreased response was observed). The predicted V_o/V_{max} for 11 μM DMSPD is 0.25 or 75% inhibited (e.g.,77% decrease was observed). These values are in excellent agreement with the observed level of SPD-mediated protection. These results also support the assumption that PAT-mediated uptake is coupled to the toxicity of these substrates.

$$\frac{V_o}{V_{max}} = \frac{[\text{DMSPD}]}{K_m\left(1 + \frac{[\text{SPD}]}{K_i}\right) + [\text{DMSPD}]} \quad (1)$$

Equation 1 was also used to predict the level of inhibition that would be expected when DESPD was present in the culture medium. According to the calculations, at both 0.6 and 11 μM DESPD, the relative rate of transport would have been inhibited by more than 90%. The results of this set of SPD protection experiments reveal a significantly lower degree of protection (Figure 5, panel 2). The decreased sensitivity to SPD can be better understood by inclusion of an alternative transport process, and perhaps even an additional transport protein. Analogous behavior has been observed using *in vitro* model systems designed to study the effect of uptake blockers on cellular responses to agonists.[39−41]

A reduced sensitivity to added spermidine was also observed with **2** (not shown) and **4** (panel 3, Figure 5). There are several possible interpretations of these results. The first is that **4** exhibits an even greater affinity for the transport apparatus than either DMSPD, DESPD, or SPD. The second possibility, and the most reasonable, is that **4** displays a low affinity for PAT and a second process is responsible for transport of this analogue. In this regard, the spermidine-based ligands (e.g., **2** and **4**) may not represent the optimum vector architecture. However, experiments with the spermine (SPM) derivative **16** and L1210 cells revealed that *significant* cell rescue (panel 4, Figure 5) was observed upon addition of exogenous spermidine (25 μM). These observations are compelling as they suggest that conjugates such as **16** (which has an IC_{50} comparable to **4**) may be using the polyamine transport apparatus to gain entry into L1210 cells. Moreover, these preliminary observations support spermidine's antagonistic character in these studies and support the premise that certain conjugates can compete with spermidine for the transporter while others are excluded due to their structure.[42] In summary, the selectivity of the PAT protein can be used to deliver specific architectures into cells.

Conclusions

In terms of vector design, the ability to modulate the delivery characteristics of drug conjugates and to ultimately direct them to specific cell types are mutually inclusive goals. Our goal was not to generate a highly toxic agent with little inherent selectivity (e.g., **17**). Instead, we sought to better understand the role of

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(42) It should be noted that other protection experiments with a bisalkylated spermine derivative (Scheme 3, **16a:** compound **16** where $R_1 = -(CH_2)_4NH$-acridine and $R_2 = H$) showed essentially no spermidine protection, unpublished work.

polyamine conjugation in terms of drug delivery. This report demonstrated not only the sensitivity of the polyamine transporter to pendant ligands (like acridine and anthracene) but also suggests a possible strategy for using this pathway to gain entry into cells.

Lengthening of the polyamine tail of **2** by an additional aminopropyl group directly enhanced the potency of the altered conjugate (see **16**). Although further work is needed, these results suggest that of the series studied thus far the monoalkylated tetraamine motif (**16**, R = H) may be the best vector to access the transporter. Intuitively, conjugates which more closely resemble the parent polyamines are more likely to interact with and be recognized by the transporter. These findings suggest that superior vectors (with even higher affinities for PAT) may be accessed by either extending the appended polyamine further from the polyaromatic core or by further aminoalkylation of the parent polyamine chain. Our future studies will not only focus on this insight, but will also round off the systematic study of tether length we have begun to establish. In summary, these results suggest that the appended polyamine is a "value-added" fragment, which can be used to tailor the delivery and potency of polyamine-containing conjugates.

Experimental Section

Materials. Silica gel (36−63) mesh 60 Å was purchased from Selecto Scientific (Norcross, GA). Chemical reagents were purchased either from the ACROS Chemical Co. or the Sigma Chemical Co. and used without further purification. Agarose was purchased from the Bio-Rad Co. ^{1}H NMR spectra were recorded at 200 MHz (unless otherwise indicated). TLC solvent systems are based on volume % and NH_4OH refers to concentrated aqueous NH_4OH. The human type II topoisomerase (p170 form) and kinetoplast DNA were purchased from Topogen, Inc. (Columbus, OH). All biologically evaluated conjugates gave satisfactory elemental analyses prior to testing. Elemental analyses were performed by Atlantic Microlabs (Norcross, GA).

TOPO-II Assay. The protocol for assaying topoisomerase II activity is well established.[29−31] Briefly, decatenation assays were performed in buffer A containing 50 mM Tris-HCl (pH 8.0), 120 mM KCl, 10 mM $MgCl_2$, 30 μg/mL bovine serum albumin, 0.5 mM dithiothreitol, and 0.5 mM ATP. Reaction mixtures contained 0.5 μg of catenated kinetoplast DNA, four units of topoiosomerase II, and the appropriate polyamine conjugate **2**−**5** at 10 μM (Figure 4, panel A) (or at 20 μM for panel B results). Controls were run in parallel and contained no inhibitor and no enzyme. Berenil, 9-aminoacridine, and 9-(*N*-butyl)aminoacridine were also evaluated at 10 μM (and 20 μM). Reaction mixtures were incubated at 37 °C for 30 min. Decatenation reactions were quenched with 20 mM Na_2EDTA, and 100 μg/mL proteinase K. Reaction products were separated by electrophoresis at 2.5 V/cm through a 1.5% agarose gel. The gel contained 0.5 μg/mL ethidium bromide and was submerged in buffer B [1X TAE buffer: 40 mM Tris-HCl, 25 mM sodium acetate, and 1 mM EDTA (pH 8.5)]. The reaction products were visualized under ultraviolet light.

IC_{50} Determinations and Methods for Transport-Related Studies. Murine leukemia cells (L1210) were obtained from the American Type culture collection (ATCC). All reagents and materials used specifically for the growth and maintenance of this cell line were obtained from Sigma Chemical Co. and Fisher Scientific, Inc. Cells were grown and maintained in RPMI-1640 medium, supplemented with 10% horse serum and 1% antibiotic/antimycotic cocktail (100 units of penicillin, 100 μg of streptomycin, and 0.25 μg of amphotericin B). Cells were grown at 37 °C in a 5% CO_2 atmosphere. For these studies, cells were plated out into sterile 96-well microtiter plates and grown for the specified period of time in the absence and presence of increasing concentrations of drug. A positive control containing methotrexate was included in all trials. Cell viability was determined using a colorimetric assay based on the NAD(P)-dependent production of Formazan.[43,44] Trypan blue staining was used to determine cell viability before the initiation of the cytotoxicity and transport experiments. Typically, samples contained less than 5% trypan blue positive cells (dead). In these experiments, L1210 cells in early to mid log-phase were used.

Spermidine Protection Assays. L1210 cells were seeded into 96-well plates, grown in medium containing the polyamine oxidase inhibitor, aminoguanidine (250 μM), and increasing concentrations of drug, in the presence and absence of a saturating concentration of spermidine (25 μM, approximate $K_m = 1.0\ \mu$M). Cells were grown for 48 h. Relative cell viability was determined using the colorimetric MTS/PMS assay.[43,44] After a 2 h incubation, the absorbance at 490 nm was recorded using a Wallac Victor^2 plate reader operating in absorbance mode. The relative viability was determined using the following ratio:

$$100 \times [(A_{490\ \text{cells + drug}} - A_{490\ \text{drug blank}})/(A_{490\ \text{cells only}} - A_{490\ \text{blank}})]$$

***N*^{1},*N*^{8}-Dimethylspermidine Trihydrochloride (DMSPD) and *N*^{1},*N*^{8}-Diethylspermidine Trihydrochloride (DESPD).** The dimethyl and diethyl SPD derivatives were synthesized by the methods of Bergeron[38] in 65% and 63% yield from spermidine, respectively. **DMSPD**: ^{1}H NMR (D_2O) δ 3.12−2.90 (m, 8H), 2.66 (s, 3H), 2.65 (s, 3H), 2.12−1.95 (m, 2H), 1.75−1.68 (m, 4H); ^{13}C NMR (D_2O): δ 51.1, 49.9, 48.6, 47.3, 35.6, 35.5, 25.6, 25.5, 25.4. Anal. Calcd for $C_9H_{26}N_3Cl_3$: C, 38.24; H, 9.27; N, 14.86. Found: C, 38.29; H, 9.36; N, 14.90. **DESPD**: ^{1}H NMR (D_2O) δ 3.19−3.04 (m, 12H), 2.18−2.06 (m, 2H), 1.81−1.73 (m, 4H), 1.34−1.24 (m, 6H). Anal. Calcd for $C_{11}H_{30}N_3Cl_3$: C, 42.52; H, 9.73; N, 13.52. Found: C, 42.37; H, 9.73; N, 13.42.

***N*^{4}-(4-(9-Aminoacridinyl)butyl)spermidine Tetrahydrochloride (2).** The butyl adduct (**11**) (0.27 g, 0.42 mmol) was dissolved in 1,4-dioxane (5 mL) and stirred at 0 °C. HCl (4 N, 14 mL) was added. The reaction mixture was stirred under a nitrogen atmosphere for 4 h at room temperature. The disappearance of **11** was monitored by TLC (0.5% NH_4OH/CH_3OH), and the reaction was complete after 4 h. The volatiles were removed under reduced pressure to give **2** as a yellow oil (0.213 g, 79%). An analytically pure sample was obtained by recrystallization from (25% absolute ethyl alcohol/diethyl ether) to give a yellow semisolid, which was protected against light (with aluminum foil) and stored under nitrogen at 0 °C. **2**: $R_f = 0.3$, 12% NH_4OH/CH_3OH; ^{1}H NMR (CD_3OD) δ 8.59 (d, 2H), 7.90 (m, 4H), 7.55 (t, 2H), 4.25 (t, 2H), 3.35 (m, 6H), 3.06 (m, 4H), 2.39−1.70 (m, 10H); high-resolution FAB MS *m*/*z* found 394.2965 (MH^+), $C_{24}H_{35}N_5O_4$ requires 394.2970. Anal. Calcd for $C_{24}H_{35}N_5O_4 \cdot 4HCl/4.5H_2O$: C, 46.46; H, 7.80; N, 11.28. Found: C, 46.34; H, 7.42; N, 11.31.

***N*^{4}-(5-(9-Aminoacridinyl)pentyl)-spermidine Tetrahydrochloride (3).** The pentylamine adduct **12** (0.373 g, 0.56 mmol) was dissolved in 1,4-dioxane (6 mL) at 0 °C. HCl (4 N, 18 mL) was added. The reaction mixture was stirred under a nitrogen atmosphere for 4 h at room temperature. The disappearance of **12** was monitored by TLC (1% NH_4OH/CH_3OH) and was complete after 4 h at room temperature. The volatiles were removed under reduced pressure to give **3** as a yellow oil (0.29 g, 79%). An analytically pure sample of **3** was obtained by recrystallization (from 25% absolute ethyl alcohol/diethyl ether) to give a yellow semisolid, which was protected against light (with aluminum foil) and stored under nitrogen at 0 °C. **3**: $R_f = 0.4$, 12% NH_3/CH_3OH; ^{1}H NMR (CD_3OD) δ 8.60 (d, 2H), 7.90 (m, 4H), 7.60 (t, 2H), 4.21 (t, 2H), 3.30 (m, 6H), 3.05

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(m, 4H), 2.30−1.50 (m, 12H); Anal. Calcd for $C_{25}H_{37}N_5 \cdot 4HCl/4H_2O$: C, 48.01; H, 7.89; N, 11.20. Found: C, 48.33; H, 7.64; N, 11.31.

***N*⁴-(4-(9-Aminomethylanthracenyl)butyl)spermidine Tetrahydrochloride (4).** The anthracene derivative **14** (0.30 g, 0.49 mmol) was dissolved in 1,4-dioxane (5 mL) at 0 °C, and 4 N HCl (14 mL) was added. The solution was stirred under a nitrogen atmosphere for 4 h at room temperature. The complete consumption of **14** was monitored by TLC ($R_f = 0.3$, 10% CH_3OH/EtOAc). The volatiles were removed under vacuum to give a pale yellow oil (0.28 g, 91%). Upon standing, a pale yellow semisolid **4** was obtained which was protected against light (with aluminum foil) and stored under a nitrogen atmosphere at 0 °C. **4**: $R_f = 0.3$, 8% NH_4OH/CH_3OH; ^{1}H NMR (CD_3OD) δ 8.60 (s, 1H), 8.42 (d, 2H), 8.09 (d, 2H), 7.62 (m, 4H), 5.28 (s, 2H), 3.52−3.00 (m, 12H), 2.30 (m, 4H), 2.05−1.79 (m, 6H). Anal. Calcd for $C_{26}H_{38}N_4 \cdot 4HCl/4.4H_2O$: C, 49.43; H, 8.10; N, 8.87. Found: C, 49.10; H, 7.70; N, 8.71.

***N*⁴-(5-(9-Aminomethylanthracenyl)pentyl)spermidine Tetrahydrochloride (5).** The anthracene adduct **15** (0.620 g, 1 mmol) was dissolved in 1,4-dioxane (5 mL) at 0 °C, and 4 N HCl (14 mL) was added. The reaction mixture was stirred under nitrogen atmosphere for 4 h at room temperature. The complete consumption of **15** was monitored by TLC (R_f = 0.3, 10% CH_3OH/EtOAc). The volatiles were removed under reduced pressure to give a pale yellow oil (0.60 g, 93%). Upon standing, a pale yellow semisolid **5** was obtained which was protected against light (with aluminum foil) and stored under a nitrogen atmosphere at 0 °C. **5**: $R_f = 0.36$, 8% NH_4OH/CH_3OH;^{1}H NMR (CD_3OD) δ 8.70 (s, 1H), 8.46 (d, 2H), 8.16 (d, 2H), 7.63 (m, 4H), 5.35 (s, 2H), 3.46−2.99 (m, 12H), 2.22 (m, 4H), 2.05−1.50 (m, 8H); High-resolution FAB MS *m*/*z* found 421.3312 (MH^+), $C_{27}H_{40}N_4$ requires 421.3331. Anal. Calcd for $C_{27}H_{40}N_4 \cdot 4HCl/4.2H_2O$: C, 50.50; H, 8.23; N, 8.73. Found: C, 50.35; H, 7.87; N, 8.64.

***N*¹,*N*⁸-Bis(*tert*-butoxycarbonyl)spermidine (6).** Spermidine (2.22 g, 15.3 mmol) was dissolved in 45 mL of THF and stirred for 20 min at 0 °C. A solution of 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile) (BOC-ON, 7.53 g, 30.5 mmol) dissolved in 85 mL of THF was added dropwise with constant stirring. The reaction was carried out under a nitrogen atmosphere. After the addition was complete, the reaction was further stirred for 1 h at 0 °C. The disappearance of spermidine was monitored by TLC ($R_f = 0.35$, 3% NH_4OH/CH_3OH). The volatiles were removed under reduced pressure to give a residue, which was redissolved in CH_2Cl_2 and washed with aqueous Na_2CO_3. The organic layer was separated and dried over anhydrous Na_2SO_4, filtered, concentrated and subjected to flash chromatography (1% NH_4OH/CH_3OH) to give derivative **6** (4.41 g, 79%). An analytically pure sample was obtained by recrystallization (from 25% diethyl ether, 1:1 EtOAc/ hexane). **6**: $R_f = 0.35$, 1% NH_4OH/CH_3OH; ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.20 (br s, 1H, NH), 4.82 (br s, 1H, NH), 3.19 (m, 4H), 2.61 (m, 4H), 1.62 (m, 2H), 1.54 (m, 4H), 1.42 (s, 18H). Anal. Calcd for $C_{17}H_{35}N_3O_4$: C, 59.10; H, 10.21; N, 12.16. Found: C, 59.18; H, 10.25; N, 12.18.

***N*⁴-(3-Cyanopropyl)-*N*¹,*N*⁸-bis(*tert*-butoxycarbonyl)spermidine (7).** Protected spermidine **6** (2.5 g, 7.2 mmol) and 4-bromobutyronitrile (1.4 g, 9.0 mmol) were dissolved in 15 mL of acetonitrile, and solid K_2CO_3 (0.87 g, 9.0 mmol) was added. The vigorously stirred mixture was heated to reflux. The disappearance of **6** was monitored by TLC ($R_f = 0.35$, 1% NH_4OH/CH_3OH). The reaction was complete in 4 h. The volatiles were removed under reduced pressure to give a residue, which was redissolved in CH_2Cl_2 and washed with aqueous Na_2CO_3 (pH = 10). The organic phase was separated, dried over anhydrous Na_2SO_4, filtered, concentrated, and subjected to flash chromatography (20% hexane/EtOAc) to remove the impurities followed by 10% CH_3OH/EtOAc to elute the desired nitrile **7** (1.7 g, 92%). **7**: $R_f = 0.39$, 10% CH_3OH/EtOAc; ^{1}H NMR ($CDCl_3$) δ 5.19 (br s, 1H), 4.82 (br s, 1H), 3.14 (m, 4H), 2.49 (m, 2H), 2.41 (m, 6H), 1.77 (m, 2H), 1.62 (m, 2H), 1.56−1.38 (m, 22H, includes signal at 1.44 (s, 18H)); high-resolution FAB MS *m*/*z* found 413.3130 (MH^+), $C_{21}H_{41}N_4O_4$ requires 413.3128.

***N*⁴-(4-Cyanobutyl)-*N*¹,*N*⁸-bis(*tert*-butoxycarbonyl)spermidine (8).** Protected spermidine **6** (1.5 g, 4.3 mmol) and 5-bromovaleronitrile (1.05 g, 6.5 mmol) were dissolved in 15 mL of acetonitrile, and solid K_2CO_3 (0.51 g, 5.2 mmol) was added. The vigorously stirred mixture was heated to reflux. The disappearance of **6** was monitored by TLC ($R_f = 0.35$, 1% NH_4OH/CH_3OH). The reaction was complete in 4 h. The volatiles were removed under reduced pressure to give a residue, which was redissolved in CH_2Cl_2 and washed with aqueous Na_2CO_3 (pH = 10). The organic phase was separated, dried over anhydrous Na_2SO_4, filtered, concentrated and subjected to flash chromatography (100% EtOAc) to remove the impurities followed by 20% CH_3OH/EtOAc to elute the desired nitrile **8** (1.58 g: 86%). **8**: R_f = 0.39, 20% CH_3OH/EtOAc; ^{1}H NMR ($CDCl_3$) δ 5.25 (br s, 1H), 4.75 (br s, 1H), 3.12 (m, 4H), 2.49 (m, 6H), 2.40 (m, 2H), 1.65 (m, 4H), 1.50 (m, 6H), 1.43 (s, 18H). Anal. Calcd for $C_{22}H_{42}N_4O_4$: C, 61.94; H, 9.92; N, 13.13. Found: C, 62.29; H, 9.61; N, 13.48.

***N*⁴-(4-Aminobutyl)-*N*¹,*N*⁸-bis(*tert*-butoxycarbonyl)spermidine (9).** Nitrile **7** (2.0 g, 4.80 mmol) was dissolved in absolute EtOH and transferred to a Parr shaker bottle. Raney nickel (50% slurry: 1.6 gm) and 3.4 mL of concentrated NH_4OH were added to the solution. NH_3 gas was then passed through the solution at 0° C for 15 min. The reaction vessel was quickly attached to the Parr shaker and was partially evacuated. H_2 gas was introduced at a pressure of 75 psi. The mixture was shaken and the disappearance of **7** was monitored by TLC (10% CH_3OH/EtOAc). After the reaction was complete (78 h) the catalyst was filtered off and washed using hot absolute EtOH. The filtrate was concentrated under reduced pressure and the residue was dissolved in CH_2Cl_2 and washed with aqueous Na_2CO_3 (pH = 10). The organic layer was separated, dried over anhydrous Na_2SO_4, filtered, concentrated and subjected to flash chromatography using 3% NH_4OH/ CH_3OH to give the desired amine **9** (0.80 g: 40%). Note: when this reaction was repeated on a smaller scale the yield was 80% (126 mg of **9**). **9**: $R_f = 0.4$, 3% NH_4OH/CH_3OH; ^{1}H NMR ($CDCl_3$) δ 5.56 (br s, 1H), 5.00 (br s, 1H), 3.13 (m, 4H), 2.84 (br s, 2H), 2.74 (br s, 2H), 2.42 (m, 6H), 1.62 (t, 2H), 1.55−1.40 (m, 26H, includes a signal at 1.48 (s, 18H)); ^{13}C NMR ($CDCl_3$) δ 156.34, 79.15, 79.02, 54.03, 53.75, 52.66, 41.88, 40.61, 40.05, 31.10, 28.68, 28.65, 28.16, 26.91, 24.59, 24.42. Anal. Calcd for $C_{21}H_{44}N_4O_4$: C, 60.54; H, 10.64; N, 13.45. Found: C, 60.36; H, 10.67; N, 13.45.

***N*⁴-(5-Aminopentyl)-*N*¹,*N*⁸-bis(*tert*-butoxycarbonyl)spermidine (10).** Nitrile **8** (1.2 g, 2.80 mmol) was dissolved in absolute EtOH and transferred to a Parr shaker bottle. Raney nickel (50% slurry: 1.6 gm) and 3.4 mL of concentrated NH_4OH were added to the solution. NH_3 gas was then passed through the solution at 0 °C for 15 min. The reaction vessel was attached to the Parr shaker and was partially evacuated. H_2 gas was introduced at a pressure of 75 psi. The disappearance of **8** was monitored by TLC (20% CH_3OH/EtOAc). After the reaction was complete (78 h) the catalyst was filtered off and washed with hot absolute EtOH. The filtrate was concentrated under reduced pressure and the residue was dissolved in CH_2Cl_2 and washed with aqueous Na_2CO_3 (pH = 10). The organic phase was separated, dried over anhydrous Na_2SO_4, filtered, concentrated and subjected to flash chromatography using 1% NH_4OH/ CH_3OH to elute the impurities followed by 2.5% NH_4OH/ CH_3OH to elute the desired amine **10** (0.90 g: 74%). **10**: $R_f = 0.39$, 2.5% NH_4OH/CH_3OH; ^{1}H NMR ($CDCl_3$) δ 5.56 (br s, 1H), 5.01 (br s, 1H), 3.13 (m, 4H), 2.70 (m, 2H), 2.40 (m, 6H), 1.60 (m, 12H), 1.45 (s, 18H). Anal. Calcd for $C_{22}H_{46}N_4O_4$: C, 60.85; H, 10.77; N, 12.90. Found: C, 60.81; H, 10.64; N, 12.78.

***N*⁴-(4-(9-Aminoacridinyl)butyl)-*N*¹,*N*⁸-bis(*tert*-butoxycarbonyl)spermidine (11).** 9-Chloroacridine (0.175 g, 0.80 mmol) and phenol (0.771 g, 8.0 mmol) were heated to 50 °C with constant stirring under a nitrogen atmosphere. The amine **9** (0.340 g, 0.80 mmol) was predissolved in molten phenol. The temperature of the phenol/amine **9** mixture was maintained at 45 °C to keep phenol in the molten state. The molten mixture was then added to the acridine mixture dropwise and the reaction heated to 110 °C and stirred for 15

min. The reaction was monitored by TLC (R_f = 0.3, 20% EtOAc/hexane) and (3% NH_4OH/CH_3OH) for the complete consumption of 9-chloroacridine and amine **9**, respectively. After the reaction was complete the mixture was subjected to flash chromatography using (100% CH_3OH) to elute impurities followed by 0.5% NH_4OH/CH_3OH to give the acridine derivative **11** (0.39 g: 80%). **11**: R_f = 0.4, 0.5% NH_4OH/CH_3OH; ^{1}H NMR (CD_3OD) δ 8.10 (m, 4H), 7.65 (t, 2H), 7.35 (t, 2H), 3.80 (t, 2H), 3.10 (m, 4H), 2.35 (m, 6H), 1.58 (m, 10H), 1.40 (s, 18H); high-resolution FAB MS *m/z* found 594.3998 (MH^+), $C_{34}H_{51}N_5O_4$ requires 594.3998. Anal. Calcd for $C_{34}H_{51}N_5O_4 \cdot 1.5\ H_2O$: C, 65.78; H, 8.76; N, 11.28; Found: C, 65.54; H, 8.64; N, 11.00.

***N*^{4}-(5-(9-Aminoacridinyl)pentyl)-*N*^{1},*N*^{8}-bis(*tert*-butoxycarbonyl)spermidine (12).** 9-Chloroacridine (0.175 g, 0.82 mmol) and phenol (0.770 g, 8.2 mmol) were heated to 50 °C with constant stirring under a nitrogen atmosphere. The pentylamine derivative **10** (0.350 g, 0.82 mmol) was predissolved in molten phenol. The temperature of the phenol/amine **10** mixture was maintained at 45 °C to keep phenol in the molten state. The molten mixture was then added to the acridine mixture dropwise and the reaction was heated to 110 °C and stirred for 15 min. The reaction was monitored by TLC (R_f = 0.3, 20% EtOAc/hexane) and (2.5% NH_4OH/CH_3OH) for the complete consumption of 9-chloroacridine and amine **10**, respectively. After the reaction was complete, the mixture was subjected to flash chromatography using 100% CH_3OH to elute impurities followed by 1% NH_4OH /CH_3OH to give the acridine derivative **12** (0.37 g: 78%). **12**: (R_f = 0.4, 1% NH_4OH/CH_3OH); ^{1}H NMR ($CDCl_3$) δ 8.10 (m, 4H), 7.65 (t, 2H), 7.32 (t, 2H), 5.31 (br s, 1H), 4.85 (br s, 1H), 3.81 (t, 2H), 3.10 (m, 4H), 2.35 (m, 6H), 1.80 (m, 2H), 1.55 (m, 10H), 1.41 (s, 18H). Anal. Calcd for $C_{35}H_{53}N_5O_4 \cdot 1.5\ H_2O$: C, 66.22; H, 8.89; N, 11.03. Found: C, 65.89; H, 9.12; N, 11.34.

9-Phenoxyacridine (13). 9-Chloroacridine (0.5 g, 2.3 mmol) and phenol (2.25 g, 23 mmol) were heated to 100 °C with constant stirring for 15 min under a nitrogen atmosphere. The crude mixture was recrystallized using 25% EtOAc/hexane to give **13** (4.62 g: 72%):[9,26] ^{1}H NMR ($CDCl_3$) δ 9.10 (d, 2H), 8.25 (d, 2H), 8.10 (t, 2H), 7.69 (m, 2H), 7.40 (m, 2H), 7.20 (m, 1H), 6.89 (d, 2H); high-resolution FAB MS *m/z* found 272.1073 (MH^+), $C_{19}H_{13}N_1O_1$ requires 272.1075.

***N*^{4}-(4-(9-Methylaminoanthracenyl)butyl)-*N*^{1},*N*^{8}-bis(*tert*-butoxycarbonyl)spermidine (14).** 9-Anthraldehyde (0.204 g, 0.99 mmol) was dissolved in 20% dry MeOH/CH_2Cl_2 (1.5 mL), and the amine **9** (0.456 g, 1.09 mmol) (dissolved in 1 mL of the same solvent mixture) was added dropwise. After the mixture was stirred for 6 h at room temperature under a nitrogen atmosphere, activated 4 Å molecular sieves were added and the mixture was stirred for an additional 24 h. The consumption of 9-anthraldehyde was monitored by TLC (R_f = 0.3, 14% EtOAc/hexane). After 30 h the reaction mixture was filtered and the volatiles were removed under reduced pressure to give a bright yellow powder (0.65 g) that was used without further purification. The powder was dissolved in 50% CH_2Cl_2/MeOH (2 mL), and the solution was cooled to 0 °C. $NaBH_4$ (0.165 g, 0.44 mmol) was added and the solution was stirred at 0 °C for 3 h and then at room temperature for 1 h. The volatiles were removed under vacuum to give a pale yellow residue. The residue was then dissolved in CH_2Cl_2 and washed with water. The organic layer was separated, dried over anhydrous Na_2SO_4, filtered and concentrated to give a pale yellow crude mixture. The mixture was then subjected to flash chromatography using 100% EtOAc to elute the impurities followed by 10% CH_3OH/EtOAc to give the desired adduct **14** (0.4 g: 66%). **14**: R_f = 0.3, 10% CH_3OH/EtOAc; ^{1}H NMR ($CDCl_3$) δ 8.35 (m, 3H), 8.00 (d, 2H), 7.50 (m, 4H), 5.42 (br s, 1H), 4.91 (br s, 1H), 4.72 (s, 2H), 3.06 (m, 4H), 2.86 (t, 2H), 2.35 (m, 6H), 1.60−1.20 (m, 28H, this chemical shift range includes the signal at 1.40 (s, 18H)). Anal. Calcd for $C_{36}H_{54}N_4O_4 \cdot 0.5\ H_2O$: C, 70.21; H, 9.00; N, 9.10. Found: C, 70.21; H, 9.00; N, 9.10.

***N*^{5}-(5-(9-Methylaminoanthracenyl)pentyl)-*N*^{1},*N*^{8}-bis-(*tert*-butoxycarbonyl)spermidine (15).** 9-Anthraldehyde (0.305 g, 1.47 mmol) was dissolved in 20% dry MeOH/CH_2Cl_2 (1.5 mL), and amine **10** (0.7 g, 1.63 mmol, dissolved in the same solvent mixture, 2 mL) was added dropwise. After the mixture was stirred for 6 h at room temperature under a nitrogen atmosphere, activated 4 Å molecular sieves were added and the mixture was stirred for an additional 24 h. The consumption of 9-anthraldehyde was monitored by TLC (R_f = 0.3, 14% EtOAc/hexane). After 30 h the reaction mixture was filtered and the volatiles were removed under reduced pressure to give a bright yellow powder that was used without further purification. The powder was dissolved in 50% CH_2Cl_2/MeOH (3 mL), and the mixture was cooled to 0 °C. $NaBH_4$ (0.165 g, 0.44 mmol) was added, and the solution was stirred at 0 °C for 3 h and at room temperature for 1 h. The volatiles were removed under reduced pressure to give a pale yellow residue. The residue was then dissolved in CH_2Cl_2 and washed with water. The organic layer was then separated, dried over anhydrous Na_2SO_4, filtered, and concentrated to give a pale yellow crude mixture. The residue was then subjected to flash chromatography using 100% EtOAc to elute the impurities followed by 10% CH_3OH/EtOAc to give the anthracene adduct **15** (0.62 g: 68%). **15**: R_f = 0.3, 10% CH_3OH/EtOAc; ^{1}H NMR ($CDCl_3$) δ 8.35 (m, 3H), 8.01 (d, 2H), 7.50 (m, 4H), 5.60 (s, 1H), 5.2 (s, 1H), 4.71 (s, 2H), 3.10 (m, 4H), 2.85 (t, 2H), 2.38 (m, 6H), 1.70−1.20 (m, 30H, this chemical shift range includes the signal at 1.42 (s, 18H)); high-resolution FAB MS *m/z* found 621.4364 (MH^+), $C_{37}H_{56}N_4O_4$ requires 621.4379. Anal. Calcd for $C_{37}H_{56}N_4O_4 \cdot 1.2\ H_2O$: C, 69.17; H, 9.16; N, 8.72. Found: C, 69.22; H, 8.80; N, 8.33.

***N*^{4}-(4-(9-Aminoacridinyl)butyl)spermine Hexahydrochloride 16.** The acridine derivative **21** (0.12 g, 0.18 mmol) was dissolved in dioxane (1.5 mL) at 0 °C followed by the addition of 4N HCl (1.5 mL). The mixture was stirred at room temperature. The disappearance of the starting material was monitored by TLC (R_f = 0.24, 2% NH_4OH/CH_3OH). After 4 h, the mixture was concentrated under reduced pressure and the product was recrystallized with CH_3OH and Et_2O to give the hygroscopic salt **16** (84 mg, 72%). **16**: ^{1}H NMR (CD_3OD) δ 8.50 (d, 2H), 7.90 (t, 2H), 7.75 (d, 2H), 7.50 (t, 2H), 4.20 (broad m, 2H), 3.38 (broad m, 6H), 3.20 (broad m, 8H), 2.40−1.80 (broad m, 12H). Anal. Calcd for $C_{27}H_{47}N_6Cl_5 \cdot 3.5\ H_2O$: C, 46.59; H, 7.82; N, 12.08. Found: C, 46.22; H, 7.45; N, 11.95.

9-*N*-Butylaminoacridine 17. 9-Chloroacridine (0.5 g, 2.3 mmol) and phenol (2.26 g, 10 mmol) were heated to 50 °C with constant stirring under a nitrogen atmosphere. Butylamine (0.168 g, 2.3 mmol) was predissolved in molten phenol. The molten mixture was then added to the acridine mixture dropwise and the reaction heated to 110 °C and stirred for 15 min. The reaction was monitored by TLC (R_f = 0.3, 20% EtOAc/hexane), which revealed complete consumption of 9-chloroacridine. After the reaction was complete, the mixture was subjected to flash chromatography eluting with 20% MeOH/EtOAc (R_f = 0.4) to give the *N*-butylamino derivative **17** (0.41 g, 72%). **17**: ^{1}H NMR (CD_3OD) δ 8.50 (d, 2H), 7.98 (m, 2H), 7.80 (d, 2H), 7.57 (m, 2H), 4.15 (t, 2H), 1.98 (m, 2H), 1.51 (m, 2H), 1.01 (t, 3H); HRMS *m/z* calcd for $C_{17}H_{19}N_2$ (MH^+) 251.1545, found 251.1549. TLC (with both UV and I_2 detection) revealed one spot. The ^{1}H NMR spectrum of **17** (in CD_3OD) is provided as additional proof of purity for 9-(*N*-butylamino)-acridine **17** (see the Supporting Information).

***N*^{1},*N*^{12}-Bis(*tert*-butoxycarbonyl)spermine 18.** Spermine (9.87 g, 49 mmol) was dissolved in THF (150 mL) and cooled to 0 °C. BOC-ON (24.04 g, 98 mmol) in THF (300 mL) was added dropwise, and the reaction mixture was stirred for 2 h at 0 °C, resulting in the complete conversion of spermine as mentioned by TLC (R_f = 0.4, 40% NH_4OH/CH_3OH). The reaction mixture was then warmed to room temperature and partitioned between a saturated aqueous Na_2CO_3 solution and dichloromethane. The organic layer was separated, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to afford the crude product. The crude product was subjected to flash chromatography on silica gel and eluted with 5% NH_4OH/CH_3OH to give di-BOC amine **18** (14.67 g, 75%).[28] **18**: R_f = 0.30 in 5% NH_4OH/CH_3OH; ^{1}H NMR ($CDCl_3$) δ 5.19 (broad s, 2H), 3.20 (q, 4H), 2.62 (m, 8H), 1.62−1.25 (m, 26H, this chemical shift range includes the signal at 1.40 (s, 18H)).

Anal. Calcd for $C_{20}H_{42}N_4O_4$: C, 59.67; H, 10.52; N, 13.92. Found: C, 59.37; H, 10.31; N, 13.74.

***N*⁴-(3-Cyanopropyl)-*N*¹,*N*¹²-bis(*tert*-butoxycarbonyl)-spermine 19.** Amine **18** (4.23 g, 10.5 mmol) and 4-bromobutyronitrile (1.55 g, 10.5 mmol) were dissolved in acetonitrile (10 mL). Potassium carbonate (1.74 g, 12.6 mmol) was then added. The mixture was refluxed with constant stirring. TLC (15% NH_4OH/CH_3OH, $R_f = 0.45$) was used to monitor the consumption of the starting material. After 24 h the reaction mixture was concentrated, redissolved in dichloromethane and washed with aqueous Na_2CO_3. The organic layer was separated, dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude residue was subjected to flash chromatography and eluted with 1% NH_4OH/CH_3OH to give **19** (2.54 g, 73% after the recovery of unreacted starting material). **19**: $R_f = 0.20$ in 1% NH_4OH/CH_3OH; ^1H NMR ($CDCl_3$) δ 5.40 (broad s, 2H), 3.15 (m, 4H), 2.85−2.30 (broad m, 12H), 1.82−1.15 (m, 28H, this chemical shift range includes the signal at 1.40 (s, 18H)). Anal. Calcd for $C_{24}H_{47}N_5O_4 \cdot 0.5\ H_2O$: C, 60.22; H, 10.11; N, 14.63. Found: C, 60.35; H, 9.92; N, 14.41.

***N*⁴-(4-Aminobutyl)-*N*¹,*N*¹²-bis(*tert*-butoxycarbonyl)spermine 20.** Nitrile **19** (2.54 g, 5.4 mmol) was dissolved in absolute EtOH in a Parr shaker bottle. Concentrated NH_4OH (5 mL) was added to the solution followed by the addition of Raney nickel catalyst (50% slurry, 3.50 g). NH_3 gas was then passed through the solution at 0 °C for 20 min. The reaction mixture was placed in a Parr shaker, briefly evacuated, and H_2 gas introduced at a pressure of 75 psi. The disappearance of starting material was monitored by TLC (1% NH_4OH/CH_3OH, $R_f = 0.20$). The reaction took 78 h to complete. The suspension was filtered and the recovered catalyst was washed with absolute EtOH. The filtrate was concentrated and redissolved in CH_2Cl_2. The CH_2Cl_2 solution was washed with aqueous Na_2CO_3 (pH = 10), separated, dried over anhydrous Na_2SO_4, filtered and concentrated. The product was purified by flash chromatography and eluted with 16% NH_4OH/CH_3OH to give the desired amine **20** (2.4 g, 95%). **20**: $R_f = 0.25$ in 10% NH_4OH/CH_3OH; ^1H NMR ($CDCl_3$) δ 5.60 (broad s, 1H), 5.20 (broad s, 1H), 3.20 (m, 4H), 2.69 (broad m, 4H), 2.40 (broad m, 8H), 1.80−1.35 (broad m, 30H, this chemical shift range includes the signal at 1.43 (s, 18H)); HRMS *m*/*z* calcd for $C_{24}H_{51}N_5O_4$ (M + H) 474.4019, found M + H 474.4001. Anal. Calcd for $C_{24}H_{51}N_5O_4 \cdot 0.7\ H_2O$: C, 59.28; H, 10.86; N, 14.40. Found: C, 58.99; H, 10.43; N, 14.32.

***N*⁴-(4-(9-Aminoacridinyl)butyl)-*N*¹,*N*¹²-bis(*tert*-butoxycarbonyl)spermine 21.** 9-Chloroacridine (0.06 g, 0.26 mmol) and phenol (0.20 g, 2.09 mmol) were heated to 50 °C for 20 min. Amine **20** (0.15 g, 0.32 mmol) dissolved in hot phenol was added to the stirred mixture. The reaction mixture was heated to 100 °C. The disappearance of starting amine was monitored by TLC (10% NH_4OH/CH_3OH, $R_f = 0.20$). After 20 min, the reaction mixture was concentrated, redissolved in CH_2Cl_2 and washed with aqueous Na_2CO_3 (pH = 10). The organic layer was separated, dried over anhydrous Na_2SO_4, filtered, concentrated, and purified by flash chromatography (eluting with 2% NH_4OH/CH_3OH) to give the acridine derivative **21** (0.12 g, 70%). **21**: $R_f = 0.24$ in 2% NH_4OH/CH_3OH; ^1H NMR ($CDCl_3$ + 25% CD_3OD) δ 8.40 (d, 2H), 7.92 (d, 2H), 7.80 (t, 2H), 7.45 (t, 2H), 4.05 (t, 2H), 3.12 (m, 4H), 2.78 (m, 4H), 2.49 (broad m, 6H), 2.01−1.30 (broad m, 30H, this chemical shift range includes a signal at δ 1.40 (s, 18H)); HRMS *m*/*z* calcd for $C_{37}H_{59}N_6O_4$ (M + H) 651.4598, found M + H 651.4561.

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Supporting Information Available: An ^1H NMR spectrum of **17** in CD_3OD is provided as additional proof of purity. This material is available free of charge via the Internet at http://pubs.acs.org.

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