Influence of Polyamine Architecture on the Transport and Topoisomerase II Inhibitory Properties of Polyamine DNA−Intercalator Conjugates

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An efficient five-step synthetic method was developed to access a series of spermine derivatives containing appended acridine, anthracene, and 7-chloroquinoline motifs. The derivatives were composed of a spermine fragment covalently tethered at its N4 and N9 positions to an aromatic nucleus via an aliphatic chain (e.g., **8**: acridine −[C4 aliphatic tether]−spermine−[C4 aliphatic tether]−acridine). The distance separating the spermine and aromatic nuclei was altered via different tethers composed of four or five methylene units. These bis ligands (**8**, **9**, **12**, and **13**) were shown to inhibit human DNA topoisomerase II (topo II) activity at 5 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−spermine conjugates **8** and **9** to inhibit topo II activity as compared with the 9-aminoacridine and 9-(*N*-butyl)aminoacridine controls (at 5 μM). The parent polyamines, spermine (5 μM) and spermidine (10 μM), had little effect on topo II activity. In general, the bis-substituted spermine derivatives (**8**, **9**, **12**, and **13**) were more efficient topo II inhibitors at 5 μM than their monosubstituted spermidine counterparts (**22**−**25**) at 10 μM. Within the bisintercalator spermine series, insertion of an additional methylene unit (i.e., C5 tethers) increased potency 2-fold (**8**, bis-C4-acridine, 47 h IC_{50} = 40 μM; **9**, bis-C5-acridine, IC_{50} = 17 μM). Comparison of the bis- and monoacridine spermine motifs (**8** and **17**) revealed a 4-fold increase in potency for the latter architecture (94 h IC_{50} for **8,** 74 μM; for **17**, 17 μM). In general the bisintercalators (**8**, **9**, **12**, and **13**) behaved as cytostatic agents, while the monosubstituted acridine and anthracene derivatives (**22**−**25**) were cytotoxic. Anthracene-containing conjugates were generally more toxic than their acridine counterparts in an L1210 (murine leukemia) cell assay. Of the conjugates tested the (monointercalator)−spermine motif (e.g., **17**) had the highest affinity for the L1210 polyamine transporter as revealed by spermidine protection experiments.

Introduction

The selective delivery of drugs to targeted cell types is one of the most challenging aspects of modern chemotherapy. Indeed, many of the side effects of current therapeutics result from the nonselective delivery of the toxic chemotherapeutic agent. Ideal drugs should target only the affected cell type. Biosynthesized in humans, spermidine [SPD, $H_2N(CH_2)_3NH(CH_2)_4NH_2$] and spermine [SPM, $H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$] are ubiquitous polyamines, which are important regulators of cell growth and differentiation.[1] In vivo they represent physiological sources of polycations useful in the stabilization of DNA topologies.[2] Many tumor types have been shown to contain elevated polyamine levels and an active polyamine transporter (PAT) for importing exogenous polyamines.[3] These range from neuroblastoma, melanoma, human lymphocytic leukemia, and colonic and lung tumor cell lines to murine L1210 cells.[3] Recognizing the opportunity for selective drug delivery, several anticancer therapies have tried to utilize the PAT to convey cytotoxic and genotoxic agents to rapidly proliferating cells.[4,5]

DNA-intercalating ligands have the capacity to bind tightly but reversibly to DNA by insertion (intercalation) of a flat, aromatic chromophore between the DNA base pairs. Many of the clinically used DNA intercalating agents are potent inhibitors of nucleic acid synthesis, and this was originally considered to be their primary mode of action. For example, amsacrine (AMSA, Figure 1) is a selective inhibitor of DNA synthesis[6] and shows a linear dose−response relationship for inhibition of DNA polymerase I in a cell-free system.[7]

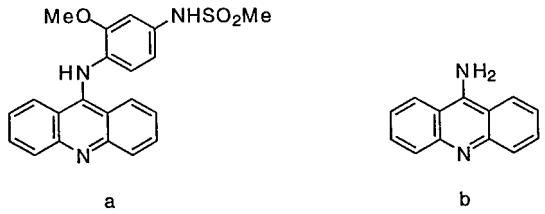

Figure 1. (a) Amsacrine (AMSA), a topoisomerase II inhibitor, and (b) 9-aminoacridine.

Another major biological effect of DNA-intercalating agents is the production of DNA damage. Many intercalating agents cause DNA strand breaks at very low concentrations and usually involve disruption of a topoisomerase enzyme.[8] 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

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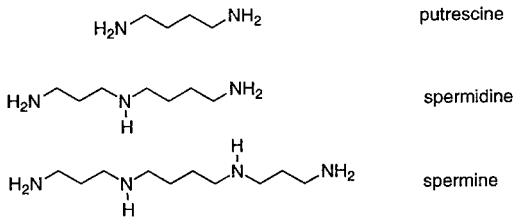


Figure 2. Some naturally occurring polyamines.

to occur, and then reseal the break.[9−12] 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.[10−12] The equilibrium is normally shifted toward the noncleavable complex as the cleavable form results in permanent DNA strand breaks.[10,11] 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.[12] involves perturbing this equilibrium toward the cleavable complex, an event that results in permanent DNA strand breaks and cell death. Molecules that stimulate the formation of the cleavable complex would be of clear value in terms of developing new chemotherapeutics and further elucidating the specific mechanisms involved in the DNA cleaving process.[12] However, mere intercalation of the topoisomerase inhibitor with DNA is not sufficient for antineoplastic activity (as measured in L1210 murine leukemia cells).[4,13] A series of substituted acridines have been evaluated for antineoplastic activity by Denny and co-workers.[13] After an extensive investigation, Denny 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.[13]

Virtually all cells contain substantial amounts of at least one of the polyamines: putrescine, spermidine, or spermine (Figure 2). Polyamines are a requirement for the optimum growth and replication of various cell types and are present in higher concentrations in rapidly proliferating cells.[1,14] The fact that polyamines can be taken up by tissues from the circulation is known, since the metabolism of labeled polyamines has been studied in vivo. Tissues with a high demand for polyamines (prostate, tumors, or normal but rapidly dividing cells) take up exogenous polyamines in increased amounts via a specific uptake system.[3,15] More recently, studies have indicated that polyammonium cations (PACs) have a very high DNA affinity but are loosely bound and can "read" DNA very rapidly because of their otherwise unconstrained motion. These properties make PACs and related polycations ideal for drug delivery when the drug needs to reach specific sites in the DNA.[2] Finally, Porter et al. have shown that a wide variety of N-4 substituents on the spermidine molecule do not disrupt its uptake.[16] Therefore, the internal amine position was selected for the introduction of substituents (with antitumor properties) onto the polyamine backbone.

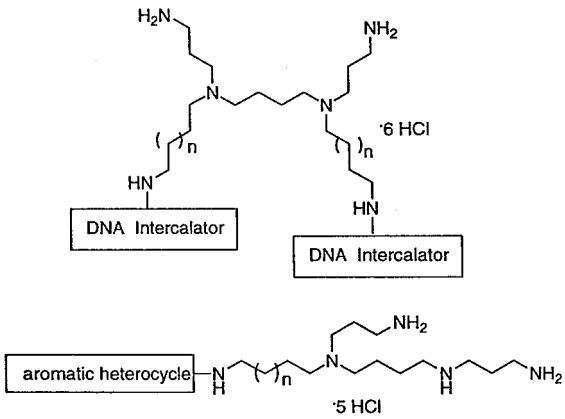


Figure 3. General structures of conjugates predicated upon spermine.

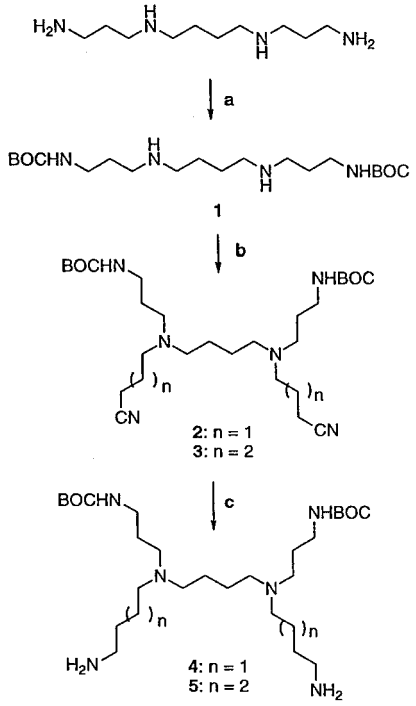
We were interested in whether a DNA intercalator with an appended polyamine side chain such as spermine (SPM) would show enhanced DNA scission activity. The design of the conjugates (shown in Figure 3) is predicated upon the well-known affinity of polyamines for DNA and the established DNA binding modes of the acridine[4,5d] and anthracene nuclei.[17] In 2000 we demonstrated the competitive uptake of spermidine−acridine and spermidine−anthracene conjugates via the polyamine transporter and their in vitro inhibition of topo II.[4] As part of our continuing efforts to define the scope and limitations of PAT for drug delivery, several spermine analogues were designed for comparison to our earlier spermidine models.[4] This paper describes the synthesis and biological evaluation of these SPM−DNA intercalator conjugates.

Results and Discussion

Synthesis. The synthesis of each conjugate involves a series of protection and deprotection steps similar to those outlined by Cain and co-workers for the synthesis of substituted acridines.[18,19] In each case, spermine was conjugated at the N4 (and/or N9) position via an aliphatic carbon tether to an acridine, anthracene, or quinoline nucleus. The quinoline derivative allowed for comparison between bicyclic and tricyclic aromatic nuclei. The central attachment was predicated on 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.[16] The N4,N9-alkylation step was designed to maintain the basicity of the internal nitrogens, which was also shown to be critical to uptake.[16]

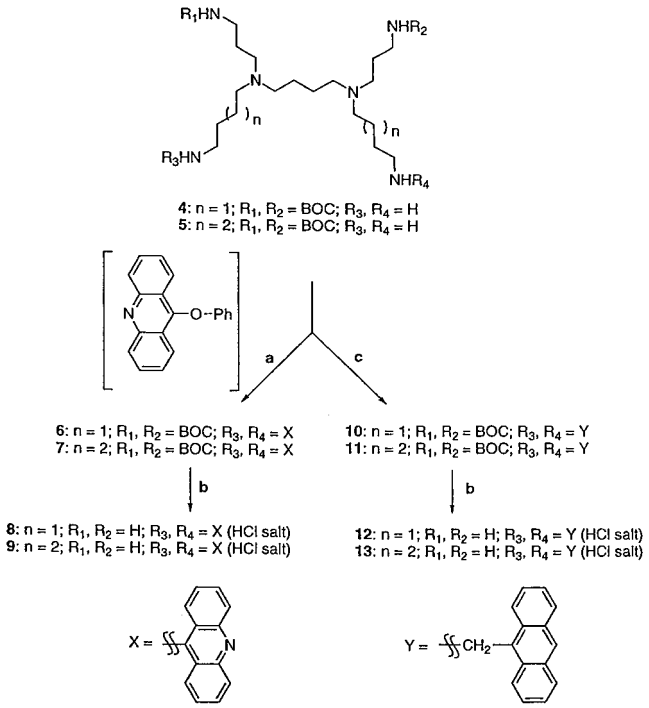
Each conjugate required the generation of a functionalized spermine fragment with selectively protected amino groups. As shown in Scheme 1, the selective acylation of the primary amino groups of spermine with 2-[*t*-(butoxycarbonyl)oxyimino]-2-phenylacetonitrile (BOC−ON) gave N^1,N^{12}-bis(*t*-butoxycarbonyl)spermine **1** in 75% yield.[20,21] N^4,N^9-Alkylation of **1** with either 4-bromobutyronitrile or 5-bromovaleronitrile gave the corresponding nitriles **2** and **3** in 65% and 77% yield, respectively. Raney nickel reduction of **2** and **3** in the presence of ammonia under high pressure (75 psi) of H_2 (g) gave the respective amines **4** (98%) and **5** (97%).

Scheme 1[a]



[a] Reagents: (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.

Scheme 2[a]



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

The syntheses of the bisacridine conjugates **8** and **9** are shown in Scheme 2. The respective amines **4** and **5** were coupled to 9-chloroacridine by use of an excess of molten phenol to give **6** and **7** in 79% and 73% yield, respectively.[4,22] The BOC protecting groups on the spermine−acridine conjugates **6** and **7** were then deprotected with 4 N HCl to give the HCl salts (**8** and **9**) in 60% and 70% yield, respectively.

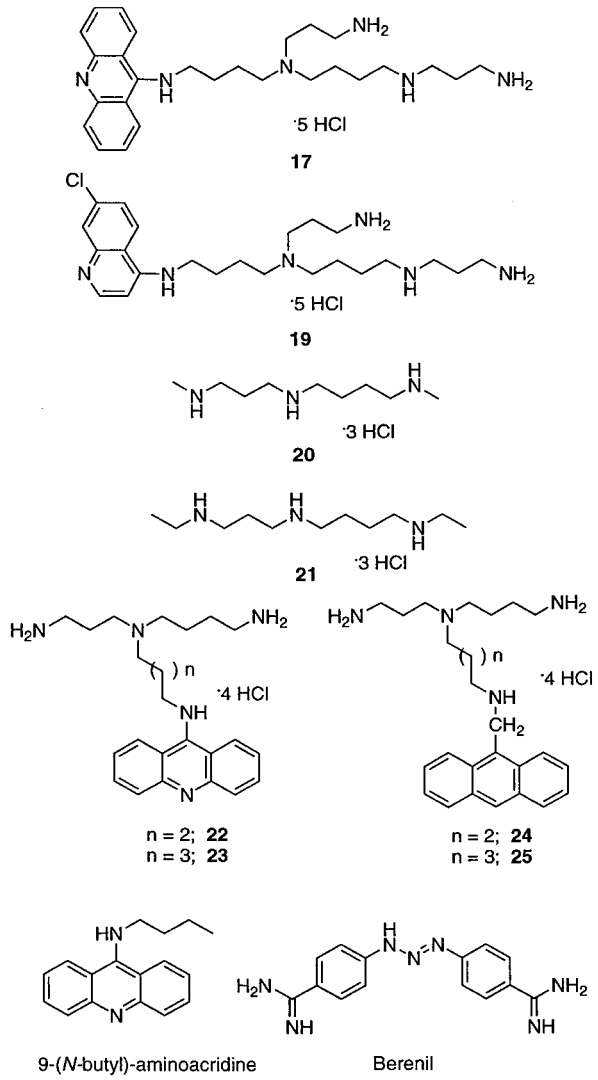
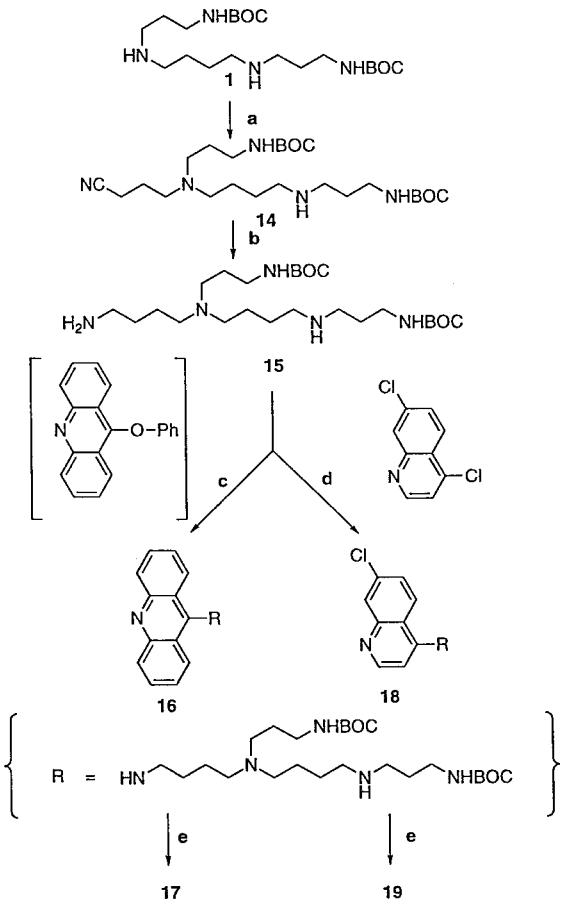


Figure 4. Structures of other amine derivatives tested.

In addition to the acridine derivatives, two anthracene−spermine conjugates (**12** and **13**) were synthesized. As shown in Scheme 2, the respective amines **4** and **5** were condensed with 9-anthraldehyde and, after reduction with $NaBH_4$, afforded the tethered amine adducts **10** (78%) and **11** (75%), respectively.[23] The BOC protecting groups of **10** and **11** were then deprotected with 4 N HCl to give the HCl salt of the desired conjugates **12** (86%) and **13** (80%), respectively.

Two monoacridine− and monoquinoline−spermine conjugates (**17** and **19**, Figure 4) were also synthesized. As shown in Scheme 3, N^4-alkylation of **1** with 1 equiv of 4-bromobutyronitrile gave the corresponding nitrile **14** (73% yield). Raney nickel reduction of **14** in the presence of ammonia and H_2 (g) gave the respective amine **15** (95%). The respective amine **15** was split and condensed either with 9-chloroacridine or 4,7-dichloroquinoline to afford the tethered amine adducts **16** (70%) and **18** (54%), respectively. The BOC protecting groups of **16** and **18** were then deprotected with 4 N HCl to give the HCl salt of the desired conjugates **17** (72%) and **19** (72%), respectively.

Scheme 3[a]



[a] Reagents: (a) Br-$CH_2CH_2CH_2CN$, refluxing acetonitrile, solid K_2CO_3; (b) H_2 gas (75 psi), NH_4OH, Raney Ni (50% slurry), absolute EtOH saturated with NH_3 gas; (c) 9-chloroacridine, molten phenol, 50 °C; (d) 4,7-dichloroquinoline; (e) 4 N HCl, MeOH.

Finally, to compare the activities of these conjugates to known active spermidine analogues, spermidine derivatives (**20** and **21**) were synthesized by the method of Bergeron (65% and 63%, respectively).[24] The related spermidine derivatives (**22**−**25**) were prepared by our published methods and are shown in Figure 4.[4]

Biological Evaluation. There has been recent interest in tethering polyamines to existing cancer drugs and bioactive agents to augment their activity and specificity.[4,5,25−30] Conjugates, which incorporated either spermidine (**22**−**25**)[4,30b] or spermine (**8**, **9**, **12**, and **13**)[30a] were evaluated for their biological activity. Enzymatic activity was assessed as the ability to unknot (decatenate) and cleave *Crithidia fasciculata* kinetoplast DNA (kDNA).[31,32] A simple electrophoretic assay[33] allowed for the visualization of the reaction products (see Experimental Section). Due to its high molecular weight, catenated DNA (kDNA) has limited migratory ability and remains near the top well (form I), but the lower molecular weight decatenated forms [i.e., closed-circular (form II) and linear 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 top well.

Decatenation reactions were carried out in the presence of the bisintercalator conjugates (**8**, **9**, **12**, and **13**) at 5 μM (shown in Figure 5) and monointercalator

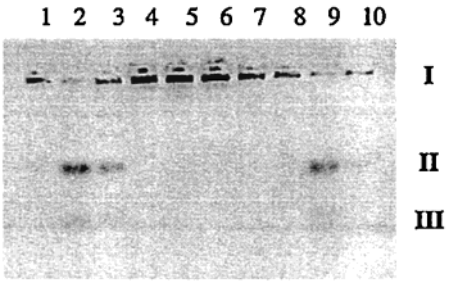


Figure 5. Topoisomerase II inhibition. Decatenation assay results are displayed as a negative image of the electrophoresis gel. All lanes contain 0.5 μg of kDNA and 2.0 units of human topo II, with the exception of lane 1. The respective drugs and conjugates were evaluated at 5 μM. Lane 1, negative control (kDNA only); lane 2, positive control (topo II); lane 3, 9-aminoacridine; lane 4, conjugate **8**; lane 5, conjugate **9**; lane 6, conjugate **12**; lane 7, conjugate **13**; lane 8, Berenil; lane 9, spermine; lane 10, 9-(*N*-butyl)aminoacridine. Forms of DNA are denoted as Roman numerals (I, II, and III, see text).

conjugates **22**−**25** derivatives (shown in Figure 6: 10 μM, panel A; 20 μM, panel B).[4] For comparison, reactions were also carried out with Berenil (Figure 4), a known inhibitor of topo II,[34,35] 9-aminoacridine, 9-(*N*-butylamino)acridine, spermidine, and spermine. At 5 μM, spermine conjugates **8**, **9**, **12**, and **13** (lanes 4−7 in Figure 5) completely inhibit the activity of topo II, resulting in the absence of lower molecular weight DNA bands (i.e., the kDNA remains intact). Spermidine conjugates **22**−**25** (10 μM, lanes 3−6 in Figure 6) clearly interfere with the topo II-mediated decatenation of kDNA but are less efficient inhibitors (as lower molecular weight DNA bands are still generated). This is consistent with the general observation that bisintercalator systems are typically more efficient DNA binding agents than their monointercalator counterparts.[30] The results also parallel the avid binding characteristics observed with these ligands with model oligonucleotides in vitro.[30]

Equally important results were obtained by carrying out the decatenation reactions in the presence of the minor-groove binder Berenil (i.e., diminazene aceturate, lane 8 in Figure 5 and lane 7 in Figure 6) and the known DNA intercalator 9-aminoacridine (lane 3 in Figure 5 and lane 8 in Figure 6).[36] 9-(*N*-Butylamino)acridine (lane 10 in Figure 5 and lane 9 in Figure 6), which represents a molecular fragment of conjugates **8**, **9**, **22,** and **23** without the polyamine moiety, had similar activity as 9-aminoacridine at 5 μM. When tested at 5 μM, the derivatives **8**, **9**, **12**, and **13** possess similar potency as Berenil. Other control reactions, which included unmodified spermine (lane 9 in Figure 5) and unmodified spermidine (lane 10 in Figure 6), had little effect on topo II activity. Evaluation of these compounds provided both verification of inhibition and insight into the contribution that each molecular fragment (i.e., intercalator and polyamine) made to the observed inhibitory activity.

It is important to recognize that the in vitro topo II measurements remove the "transport-into-the-cell" hurdle and measure direct interactions between the conjugates, kDNA, and topo II. By design the polyamine appendage was expected (a) to play a significant role in conjugate uptake and (b) to show dramatic differences both in the concentration of conjugate required to reduce viable cell

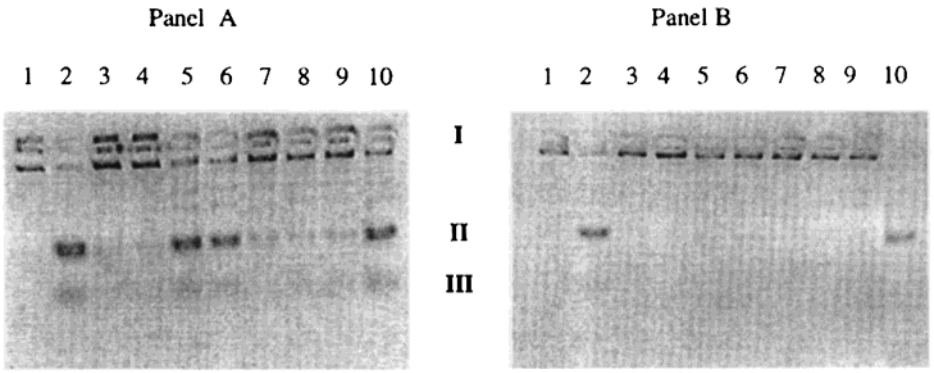


Figure 6. Topoisomerase II inhibition.[4] Decatenation assay results are displayed as a negative image of the electrophoresis gel. All lanes contain 0.5 μg of kDNA and 2.0 units of human topo II, with the exception of lane 1. The respective drugs and conjugates were evaluated at 10 μM (panel A) and 20 μM (panel B). Lane 1, negative control (kDNA only); lane 2, positive control (topo II); lane 3, conjugate **22**; lane 4, conjugate **23**; lane 5, conjugate **24**; lane 6, conjugate **25**; lane 7, Berenil; lane 8, 9-aminoacridine; lane 9, 9-(*N*-butyl)aminoacridine; lane 10, spermidine. Forms of DNA are denoted as Roman numerals (I, II, and III, see text).

Table 1. IC_{50} Values

sample	IC_{50} (μM) 47 h	94 h
8 (SPM bisC4 acridine)	40	74
9 (SPM bisC5 acridine)	17	23
12 (SPM bisC4 anthracene)	44	61
13 (SPM bisC5 anthracene)	18	25
17 (SPM monoC4 acridine)	23	17
19 (SPM monoC4 quinoline)	>100	>100
22 (SPD C4 acridine)	75	55
23 (SPD C5 acridine)	>100	>100
24 (SPD C4 anthracene)	13	6
25 (SPD C5 anthracene)	7	5
9-(*N*-butyl)aminoacridine	2	2

number by 50% (IC_{50}) and in the transport kinetic (K_i) measurements with cancer cell types.

IC_{50} Measurements. A second set of experiments was performed to determine the respective cytotoxicity of **8**, **9**, **12** and **13** toward murine leukemia (L1210) cells grown in tissue culture. The results of these toxicity studies yielded IC_{50} values (Table 1).[37] The reported mean IC_{50} value for the parent, AMSA, is 0.26 μM for six human cancer cell lines with varying levels of topo II α and β isoforms.[38] All tested compounds exhibited concentration dependent toxicity. Time-dependent behavior was also observed. Our goal was not to generate a highly toxic agent with little inherent selectivity [e.g., 9-(*N*-butylamino)acridine, 94 h IC_{50} = 2 μM). Instead, we were interested in how changes in the appended polyamine architecture perturbed the overall efficacy of the conjugate. Interestingly, the bis systems (**8**, **9**, **12**, and **13**) showed lower cytotoxicity at longer time (i.e., the IC_{50} is higher at 94 h than at 47 h). In direct contrast, the positive control with an agent of known toxicity (10 μM, methotrexate) and the monoalkylated spermidine (**22**) and spermine (**17**) derivatives always showed higher toxicity at 94 h than at 47 h (i.e., the IC_{50} value at 94 h is lower than that at 47 h). These observations are consistent with the bisintercalators acting as temporary cytostatic agents. In general, the bis systems containing the C5 tether length (**9** and **13**) had higher activities (i.e., lower IC_{50} values) than the corresponding conjugates containing the C4 tether length (**8** and **12**). This tether trend was also evident in the monosubstituted spermidine−anthracene conjugates (**24** and **25**). For the anthracene conjugates, the spermidine (SPD) derivatives showed higher activity than the related spermine (SPM) derivatives (see Table 1: **12** vs **24** and **13** vs **25**). [Note: 9-(*N*-butylamino)-acridine exhibits the highest activity among all of the derivatives tested, which may be due to multiple modes of activity.] Among the acridine conjugates tested (**8**, **9**, **17**, **22,** and **23**), spermine monoacridine conjugate **17** showed the greatest bioactivity at 94 h (IC_{50} = 17 μM), whereas the related spermidine acridine conjugate (**22**) had a higher IC_{50} (55 μM). In this regard, the spermine framework serves as a better vector than the spermidine motif. These results support our earlier premise[4] that conjugates which more closely resemble the parent polyamines are more likely to be recognized by the polyamine transporter. Spermine monoquinoline conjugate **19** exhibited a much higher IC_{50} value (>100 μM) than any of the other conjugates. This is likely related to the putative mode of action (topo II inhibition).

PAT Studies. The bis systems are potent inhibitors of topo II (by gel assay), and cytostatic agents. In contrast, the spermidine monointercalator systems are less efficient topo II inhibitors and yet are cytotoxic. Therefore, the IC_{50} data cannot be rationalized by the observed topo II inhibitory activity. Instead, one must consider the conjugate's ability to enter the cell. To relate potency to uptake by the polyamine transporter, several spermidine protection experiments were conducted.

Are the polyamine−DNA intercalator conjugates recognized and transported by the polyamine uptake apparatus of L1210 cells? Several polyamine derivatives were examined. Two controls, dimethylspermidine (**20**) and diethylspermidine (**21**) have been shown to gain entry into the cell via the PAT.[4,24] 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.[24] In terms of their respective affinity for PAT, the K_i values for inhibition of SPD uptake for dimethyl **20** and diethyl **21** have been reported to be 5 and 19 μM, respectively.[24] Based on these K_i values, the approximate K_m values for **20** and **21** transport via the PAT are 2.5 and 9.5 μM, respectively.[24] The cellular response to **20** and **21** is death, which results from polyamine depletion.[24] 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 **20** and **21** will be

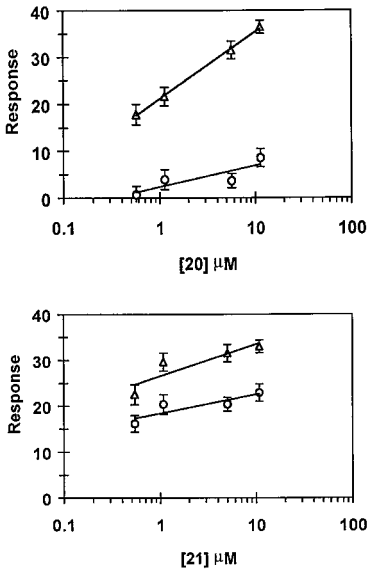


Figure 7. Demonstration of spermidine-mediated protection against dimethylspermidine (**20**, upper panel) and diethylspermidine (**21**, lower panel). See Experimental Section for description of experimental conditions. (△) No spermidine; (○) 25 μM spermidine. Response is equivalent to cell death as determined by the MTS/PMS colorimetric assay. A decreased response (i.e., less cell death) is consistent with reduced drug uptake. Data represent the average of two independent trials, each performed in triplicate.

reduced by more than 90%, which in turn will significantly reduce cell death. Therefore, inhibition of PAT-mediated transport of **20** and **21** by exogenous spermidine will be observed as an increase in cell viability.

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. To maximize the protective effects of spermidine (SPD), an excess of SPD (25 μM) was used during these experiments. Use of 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 with **8**, **12**, **17**, **20**, **21**, and **24** are shown in Figures 7 and 8. The graphs clearly indicate that, over a 48-h period, the selected assay format provides sufficient sensitivity to reveal changes in cell viability. Note: the low concentration range of **20** and **21** used in these experiments was intended to produce a low level of toxicity and, hence, amplify any observed SPD-mediated protection.

A high degree of SPD protection was observed for the terminally alkylated spermidine models **20** and **21** (Figure 7). This is consistent with their low K_i values and high affinity for PAT.[4,24] Moderate SPD protection was observed with the monointercalators **17** and **24** (Figure 8, panels A and B, respectively). Little, if any, SPD protection was observed with the bisintercalators **8** and **12** (Figure 8, panels C and D, respectively).

There are several possible interpretations of these results. The first possibility is that **8** and **12** exhibit an even greater affinity for the transport apparatus than either **20**, **21**, or SPD. This seems unlikely when one

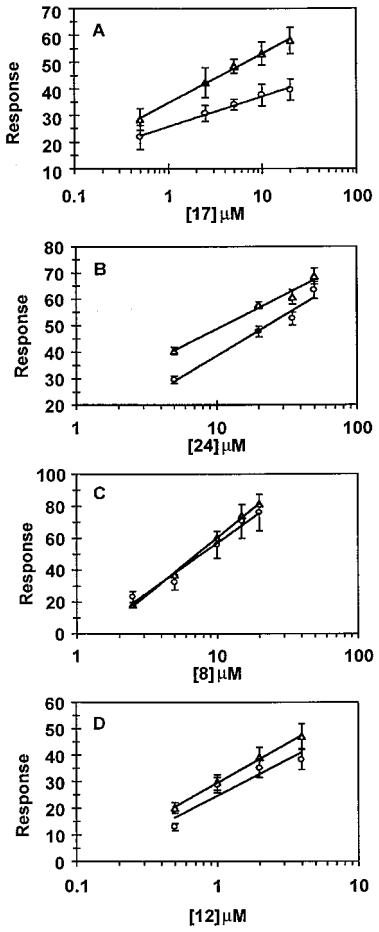


Figure 8. Spermidine protection experiments to assess the ability of different polyamine conjugates to gain entry into the cell via the PAT apparatus. (A) SPM monoC4 acridine (**17**); (B) SPD C4 anthracence (**24**); (C) SPM bisC4 acridine (**8**); (D) SPM bisC4 anthracene (**12**). (△) No spermidine; (○) 25 μM spermidine. Response is equivalent to cell death as determined by the MTS/PMS colorimetric assay. A decreased response (i.e., less cell death) is consistent with reduced drug uptake. Data represent the average of two independent trials, each performed in triplicate.

considers our topo II (and IC_{50}) findings, which suggest that if these analogues were transported efficiently into the cell they would kill it. The second possibility, and the most reasonable, is that **8** and **12** display a low affinity for PAT, and a second process is responsible for transport of these analogues. Analogous behavior has been observed for in vitro model systems designed to study the effect of uptake blockers on cellular responses to agonists.[39] In this regard, the low toxicity observed with the bis ligands (**8** and **12**, Table 1) may be due to their poor transport into the cell by PAT. Therefore, the bisintercalating systems (e.g., **8** and **12**) do not represent optimum vector architectures, which target the PAT.

Spermidine protection experiments with the monointercalators **17** and **24** revealed *significant* cell rescue (Figure 8A,B) upon addition of exogenous spermidine (25 μM). This was especially evident for **17** (Figure 8A). These observations are compelling as they suggest that conjugates such as **17** are using the polyamine transporter (PAT) to gain entry into L1210 cells. Although **17** is transported more readily into the cell, it has a higher 94 h IC_{50} (17 μM) than **24** (6 μM). This finding was rationalized by the observed greater toxicity of

anthracene SPD analogues (Table 1, **24** and **25**) over their acridine counterparts (**22** and **23**). These experiments elegantly delineate the properties required for optimal drug efficacy as a balance between ease of transport and conjugate toxicity. Moreover, our findings support spermidine's antagonistic character in these studies and bolster the premise that certain conjugates can compete with spermidine for the transporter, while others are excluded due to their structure. In summary, the selectivity of the PAT can be used to deliver specific architectures into cancer cells.

Conclusions

We have demonstrated that polyamine homologation can alter the topo II inhibitory activity, the delivery characteristics,[4] cytotoxicity time dependence, and efficacy of the conjugate employed. In terms of vector design, the bis-substituted analogues behaved as temporary cytostatic agents, whereas the monosubstituted polyamine derivatives were cytotoxic. In this regard, longer tether lengths and further aminoalkylation of the polyamine framework may lead to more effective vectors. Future work will continue to illustrate the versatility of polyamines and their ability to function as modulators of drug delivery.

Experimental Section

Materials and Methods. Silica gel 60 (70−230 mesh) was purchased from EM Science, Darmstadt, Germany. All other reagents were purchased from the Acros Chemical Company and used without further purification. ^{1}H NMR spectra were recorded at 200 MHz.

Topo II Assay. The protocol for assaying topoisomerase II activity is well established.[31,32] 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 human topoiosomerase II, and the appropriate polyamine conjugate (**8**, **9**, **12**, or **13**) at 5 μM (Figure 5) and conjugates **22**−**25** (see Figure 6: 10 μM, panel A; 20 μM, panel B). 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 [1× 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 and photographed.

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 by a colorimetric assay based on the NAD(P)H-dependent production of Formazan.[40,41] 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 and 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 $K_m = 1.0$ μM). Cells were grown for 48 h. Relative cell viability was determined by the colorimetric MTS/PMS assay.[40,41] After a 2 h incubation, the absorbance at 490 nm was recorded with a Wallac Victor^2 plate reader operating in absorbance mode. The relative viability was determined from 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*^{12}-Bis(*t*-butoxycarbonyl)spermine (1).**[20,21] 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 monitored 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 *in a vacuum* 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 **1** (14.67 g, 75%). **1**: R_f = 0.30 in 5% NH_4OH/CH_3OH; ^{1}H NMR ($CDCl_3$) δ 5.19 (br s, 2H), 3.20 (q, 4H), 2.60 (m, 8H), 1.60 (m, 8H), 1.40 (s, 18H); Anal. ($C_{20}H_{42}N_4O_4$) C, H, N.

***N*^{4},*N*^{9}-Bis(3-cyanopropyl)-*N*^{1},*N*^{12}-bis(*t*-butoxycarbonyl-)spermine (2).** Amine **1** (4.78 g, 12 mmol) and 4-bromobutyronitrile (8.80 g, 60 mmol) were dissolved in acetonitrile (10 mL). Potassium carbonate (27.2 g, 28 mmol) was then added. The mixture was allowed to reflux 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 10% CH_3OH/EtOAc to give **2** (4.14 g, 65%). **2:** R_f = 0.29 in 10% CH_3OH/EtOAc; ^{1}H NMR ($CDCl_3$) δ 5.10 (br s, 2H), 3.09 (q, 4H), 2.35 (m, 16H), 1.62 (m, 12H), 1.40 (s, 18H); Anal. ($C_{28}H_{52}N_6O_4$) C, H, N.

***N*^{4},*N*^{9}-Bis(4-cyanobutyl)-*N*^{1},*N*^{12}-bis(*t*-butoxycarbonyl)-spermine (3).** Amine **1** (1.20 g, 2.98 mmol) and 5-bromovaleronitrile (1.45 g, 8.95 mmol) were dissolved in acetonitrile (10 mL). Potassium carbonate (2.31 g, 16.8 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 amine. After 24 h the 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 10% CH_3OH/EtOAc to give **3** (1.33 g, 77%). **3:** R_f = 0.30 in 30% $CH_3OH/CHCl_3$; ^{1}H NMR ($CDCl_3$) δ 5.50 (br s, 2H), 3.10 (q, 4H), 2.45 (m, 16H), 1.62 (m, 16H), 1.40 (s, 18H); high-resolution mass spectrum (HRMS) fast atom bombardment (FAB) theory for ($C_{30}H_{56}N_6O_4$) $M + 1$ = 565.4441, found $M + 1$ = 565.4428.

***N*^{4},*N*^{9}-Bis(4-aminobutyl)-*N*^{1},*N*^{12}-bis(*t*-butoxycarbonyl)-spermine (4).** Nitrile **2** (2.69 g, 5 mmol) was dissolved in absolute EtOH in a Parr shaker bottle. Concentrated NH_4OH (4.7 mL) was added to the solution, followed by the addition of Raney nickel catalyst (50% slurry, 3.34 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 and briefly evacuated, and H_2 gas was introduced at a pressure of 75 psi. The disappearance of starting material was monitored by TLC (20% CH_3OH/EtOAc, R_f = 0.40). 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 phase 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 **4** (2.7 g, 98%). **4:** R_f = 0.30 in 16% NH_4OH/CH_3OH; ^{1}H NMR ($CDCl_3$) δ 5.75 (br s, 2H), 3.56 (br s, 4H), 3.12 (br m, 4H), 2.70 (br s, 4H), 2.40 (br m, 12H), 1.60 (br m, 16H), 1.40 (s, 18H); HRMS (FAB) theory for ($C_{28}H_{60}N_6O_4$) $M + 1$ = 545.4754, found $M + 1$ = 545.4763; Anal. ($C_{28}H_{60}N_6O_4 \cdot H_2O$) C, H, N.

***N*^{4},*N*^{9}-Bis(5-aminopentyl)-*N*^{1},*N*^{12}-bis(*t*-butoxycarbonyl-)spermine (5).** Nitrile **3** (1.33 g, 2.35 mmol) was dissolved in absolute EtOH in a Parr shaker bottle. Concentrated NH_4OH (2.4 mL) was added to the solution, followed by the addition of Raney nickel catalyst (50% slurry, 1.67 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 and briefly evacuated, and H_2 gas was introduced at a pressure of 75 psi. The disappearance of starting material was monitored by TLC (30% $CH_3OH/CHCl_3$, R_f = 0.30). 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 phase 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 on silica gel with 16% NH_4OH/CH_3OH to give the desired amine **5** (1.31 g, 97%). **5:** R_f = 0.30 in 20% NH_4OH/CH_3OH; ^{1}H NMR ($CDCl_3$) δ 5.65 (br s, 2H), 3.15 (q, 4H), 2.40 (br m, 12H), 2.05 (br s, 4H), 1.60 (br m, 20H), 1.40 (s, 18H); HRMS (FAB) theory for ($C_{30}H_{64}N_6O_4$) $M + 1$ = 573.5067, found $M + 1$ = 573.5068.

***N*^{4},*N*^{9}-Bis[4-(9-aminoacridinyl)butyl]-*N*^{1},*N*^{12}-bis(*t*-butoxycarbonyl)spermine (6).** 9-Chloroacridine (0.40 g, 2 mmol) and phenol (1.72 g, 18 mmol) were heated to 50 °C for 20 min. Amine **4** (0.50 g, 1 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 (20% NH_4OH/CH_3OH, R_f = 0.32). After the reaction was complete, the mixture was concentrated and 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, purified by flash chromatography, and eluted with 0.5% NH_4OH/CH_3OH to give the desired acridine derivative **6** (0.64 g, 79%). **6:** R_f = 0.40 in 0.5% NH_4OH/CH_3OH; ^{1}H NMR ($CDCl_3$) δ 8.09 (m, 8H), 7.62 (m, 4H), 7.32 (m, 4H), 5.65 (br s, 2H), 5.35 (br s, 1H), 5.25 (br s, 1H), 3.78 (q, 4H), 3.10 (q, 4H), 2.30 (m, 12H), 1.92 (s, 4H), 1.75 (m, 4H), 1.50 (m, 4H), 1.40 (s, 18H); HRMS (FAB) theory for ($C_{54}H_{74}N_8O_4$) $M + 1$ = 899.5911, found $M + 1$ = 899.5938.

***N*^{4},*N*^{9}-Bis[5-(9-aminoacridinyl)pentyl]-*N*^{1},*N*^{12}-bis(*t*-butoxycarbonyl)spermine (7).** 9-Chloroacridine (0.70 g, 3.3 mmol) and phenol (2.58 g, 27 mmol) were heated to 50 °C for 20 min. Amine **5** (0.92 g, 1.6 mmol) dissolved in hot phenol was added to the stirred mixture. The reaction mixture was heated to 100 °C. The disappearance of starting material was monitored by TLC (20% NH_4OH/CH_3OH, R_f = 0.25). After the reaction was complete, the mixture was concentrated, 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, purified by flash chromatography, and eluted with 1% NH_4OH/CH_3OH to give the desired acridine derivative **7** (1.08 g, 73%). **7:** R_f = 0.35 in 1% NH_4OH/CH_3OH; ^{1}H NMR ($CDCl_3$) δ 8.09 (t, 8H), 7.62 (t, 4H), 7.32 (t, 4H), 5.50 (br s, 2H), 3.80 (t, 4H), 3.15 (q, 4H), 2.30 (br m, 12H), 1.75 (br m, 4H), 1.50 (m, 16H), 1.40 (s, 18H); HRMS (FAB) theory for ($C_{56}H_{78}N_8O_4$) $M + 1$ = 927.6224, found $M + 1$ = 927.6214.

***N*^{4},*N*^{9}-Bis[4-(9-aminoacridinyl)butyl]spermine Hexahydrochloride (8).** The acridine derivative **6** (0.64 g, 0.7 mmol) was dissolved in 1,4-dioxane (10 mL) at 0 °C, followed by the addition of 4 N HCl (10 mL). The mixture was stirred for 4 h at room temperature. The disappearance of the starting material was monitored by TLC (R_f = 0.5, 1% NH_4OH/CH_3OH). The mixture was concentrated under reduced pressure and the product was recrystallized with CH_3OH and Et_2O (1:1) to give the hygroscopic salt **8** (0.40 g, 60%). **8**: ^{1}H NMR (D_2O) δ 7.71 (d, 4H), 7.50 (t, 4H), 7.15 (t, 4H), 7.00 (d, 4H), 3.70 (br m, 4H), 3.45 (br m, 12H), 3.24 (t, 4H), 2.30 (br m, 4H), 2.00 (br m, 12H); HRMS (FAB) theory for ($C_{44}H_{58}N_8$) $M + 1$ = 699.4863, found $M + 1$ = 699.4807; Anal. ($C_{44}H_{64}N_8Cl_6 \cdot 3.3H_2O$) C, H, N.

***N*^{4},*N*^{9}-Bis[5-(9-aminoacridinyl)pentyl]spermine Hexahydrochloride (9).** The acridine derivative **9** (0.70 g, 0.76 mmol) was dissolved in dioxane (17 mL) at 0 °C, followed by the addition of 4 N HCl (17 mL). The mixture was stirred for 4 h at room temperature. The disappearance of the starting material was monitored by TLC (R_f = 0.35, 1% NH_4OH/CH_3OH). The mixture was concentrated under reduced pressure and the product was recrystallized with CH_3OH and Et_2O to give the hygroscopic salt **9** (0.38 g, 70%). **9**: ^{1}H NMR (D_2O) δ 7.55 (m, 8H), 7.50 (t, 4H), 7.15 (q, 4H), 7.00 (d, 4H), 3.50 (br m, 4H), 3.35 (br m, 16H), 2.20 (br m, 4H), 1.85 (br m, 12H), 1.50 (br m, 4H); HRMS (FAB) theory for ($C_{46}H_{62}N_8$) $M + 1$ = 727.5176, found $M + 1$ = 727.5192; Anal. ($C_{46}H_{68}N_8Cl_6 \cdot 4H_2O$) C, H, N.

***N*^{4},*N*^{9}-Bis[4-[9-(aminomethyl)anthracenyl]butyl]-*N*^{1},*N*^{12}-bis(t-butoxycarbonyl)spermine (10).** 9-Anthraldehyde (0.43 g, 2 mmol) was dissolved in 25% CH_3OH/CH_2Cl_2 (3 mL). The diamine **4** (0.56 g, 1 mmol) was added to the solution dropwise at room temperature. The mixture was stirred for 24 h. The disappearance of **4** was monitored by TLC (20% NH_4OH/CH_3OH, R_f = 0.32). After the imination was complete, the crude product was concentrated and redissolved in a premixed solvent (50% CH_3OH/CH_2Cl_2) followed by the addition of $NaBH_4$ (0.19 g, 5 mmol). After being stirred for 12 h, the 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, and concentrated. The crude residue was subjected to flash chromatography and eluted with 1% NH_4OH/CH_3OH to furnish the desired anthracene derivative **10** (0.74 g, 78%). **10:** R_f = 0.30 in 1% NH_4OH/CH_3OH; ^{1}H NMR ($CDCl_3$) δ 8.40 (s, 2H), 8.00 (d, 4H), 7.50 (m, 8H), 5.30 (br s, 2H), 4.75 (s, 4H), 3.05 (q, 4H), 2.85 (t, 4H), 2.30 (br m, 12H), 1.60 (m, 16H), 1.40 (s, 18H); HRMS theory for ($C_{58}H_{80}N_6O_4$) $M + 1$ = 925.6319, found $M + 1$ = 925.6322; Anal. ($C_{58}H_{80}N_6O_4$) C, H, N.

***N*^{4},*N*^{9}-Bis[5-[9-(aminomethyl)anthracenyl]pentyl]-*N*^{1},*N*^{12}-bis(t-butoxycarbonyl)spermine (11).** 9-Anthraldehyde (0.36 g, 1.75 mmol) was dissolved in 25% CH_3OH/CH_2Cl_2 (2.5 mL). The diamine **5** (0.50 g, 0.8 mmol) was added to the solution dropwise at room temperature. The mixture was stirred for 24 h. The disappearance of **5** was monitored by TLC (20% NH_4OH/CH_3OH, R_f = 0.25). After the imination was complete, the crude was concentrated and redissolved in a premixed solvent (50% CH_3OH/CH_2Cl_2) followed by the addition of $NaBH_4$ (0.20 g, 5.26 mmol). After being stirred for 12 h, the 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, and concentrated. The crude residue was subjected to flash chromatography and was eluted with 2% NH_4OH/CH_3OH to give the desired anthracene derivative **11** (0.55 g, 75%). **11:** R_f = 0.30 in 2% NH_4OH/CH_3OH; ^{1}H NMR ($CDCl_3$) δ 8.35 (s, 2H), 8.30 (d, 4H), 8.00 (d, 4H), 7.50 (m, 8H), 5.60 (br s, 2H), 4.70 (s, 4H), 3.15 (q, 4H), 2.85 (t, 4H), 2.30 (br m, 12H), 1.60 (br m, 20H), 1.40 (s, 18H); HRMS theory for ($C_{60}H_{84}N_6O_4$) $M + 1$ = 953.6632, found $M + 1$ = 953.6581; Anal. ($C_{60}H_{84}N_6O_4 \cdot 2H_2O$) C, H, N.

***N*^{4},*N*^{9}-Bis[4-[9-(aminomethyl)anthracenyl]butyl]-spermine Hexahydrochloride (12).** The anthracene derivative **10** (0.74 g, 0.8 mmol) was dissolved in dioxane (10 mL) at 0 °C, followed by addition of 4 N HCl (10 mL). The mixture was stirred for 4 h at room temperature. The disappearance of **10** was monitored by TLC (1% NH_4OH/CH_3OH, R_f = 0.30). The solution was concentrated and the resulting solid was recrystallized with ethanol to give **12** as a hygroscopic solid (0.65 g, 86%). **12**: ^{1}H NMR (D_2O) δ 8.36 (s, 2H), 8.15 (d, 4H),

7.95 (d, 4H), 7.67 (t, 4H), 7.55 (t, 4H), 5.02 (s, 4H), 3.30 (br m, 4H), 3.10 (br m, 16H), 2.10 (br m, 4H), 1.80 (br m, 12H); HRMS theory for ($C_{48}H_{64}N_6$) $M + 1 = 725.5271$, found $M + 1 = 725.5274$; Anal. ($C_{48}H_{70}N_6Cl_6 \cdot 2H_2O$) C, H, N.

***N*⁴,*N*⁹-Bis[5-[9-(aminomethyl)anthracenyl]pentyl]-spermine Hexahydrochloride (13).** The anthracene derivative **11** (0.55 g, 0.60 mmol) was dissolved in dioxane (7 mL) at 0 °C, followed by addition of 4 N HCl (7 mL). The mixture was stirred for 4 h at room temperature. The disappearance of **11** was monitored by TLC (2% NH_4OH/CH_3OH, $R_f = 0.30$). The reaction mixture was concentrated and the resulting solid was recrystallized with ethanol to give **13** as a hygroscopic solid (0.36 g, 80%). **13**: ^{1}H NMR (D_2O) δ 8.15 (d, 4H), 7.90 (s, 2H), 7.65 (m, 8H), 7.50 (m, 4H), 4.80 (s, 4H), 3.30 (br m, 20H), 2.30 (br m, 4H), 1.90 (br m, 12H), 1.50 (br m, 4H); HRMS theory for ($C_{50}H_{68}N_6$) $M + 1 = 753.5584$, found $M + 1 = 753.5561$; Anal. ($C_{50}H_{74}N_6Cl_6 \cdot 4H_2O$) C, H, N.

***N*⁴-(3-Cyanopropyl)-*N*¹,*N*¹²-bis(t-butoxycarbonyl)spermine (14).** Amine **1** (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 amine. 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 **14** (2.54 g, 73% after correction for the recovery of unreacted starting material). **14:** $R_f = 0.20$ in 1% NH_4OH/CH_3OH; ^{1}H NMR ($CDCl_3$) δ 5.40 (br s, 2H), 3.15 (m, 4H), 2.50 (br m, 12H), 1.70 (br m, 10H), 1.40 (s, 18H); Anal. ($C_{24}H_{47}N_5O_4 \cdot 0.5H_2O$) C, H, N.

***N*⁴-(4-Aminobutyl)-*N*¹,*N*¹²-bis(*t*-butoxycarbonyl)spermine (15).** Nitrile **14** (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 and briefly evacuated, and H_2 gas was 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 was complete after 78 h. 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 phase 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 **15** (2.4 g, 95%). **15:** $R_f = 0.25$ in 10% NH_4OH/CH_3OH; ^{1}H NMR ($CDCl_3$) δ 5.60 (br s, 1H), 5.20 (br s, 1H), 3.20 (m, 4H), 2.70 (br m, 4H), 2.40 (br m, 8H), 1.60 (br m, 12H), 1.40 (s, 18H); HRMS theory for ($C_{24}H_{51}N_5O_4$) $M + 1 = 474.4019$, found $M + 1 = 474.4001$.

***N*⁴-[4-(9-Aminoacridinyl)butyl]-*N*¹,*N*¹²-bis(*t*-butoxycarbonyl)spermine (16).** 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 **15** (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 the starting amine was monitored by TLC (10% NH_4OH/CH_3OH, $R_f = 0.20$). After 20 min, the reaction mixture was concentrated and 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, purified by flash chromatography, and eluted with 2% NH_4OH/CH_3OH to give the acridine derivative **16** (0.12 g, 70%). **16:** $R_f = 0.24$ in 2% NH_4OH/CH_3OH; ^{1}H NMR ($CDCl_3$ + 25% CD_3OD) δ 8.40 (d, 2H), 7.90 (d, 2H), 7.80 (t, 2H), 7.40 (t, 2H), 4.10 (t, 2H), 3.10 (m, 4H), 2.76 (q, 4H), 2.50 (br m, 6H), 1.80 (br m, 12H), 1.40 (s, 18H); HRMS theory for ($C_{37}H_{59}N_6O_4$) $M + 1 = 651.4598$, found $M + 1 = 651.4561$.

***N*⁴-[4-(9-Aminoacridinyl)butyl]spermine Pentahydrochloride (17).** The acridine derivative **16** (0.12 g, 0.18 mmol) was dissolved in 1,4-dioxane (1.5 mL) at 0 °C, followed by the addition of 4 N 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 **17** (0.084 g, 72%). **17**: ^{1}H NMR (CD_3OD) δ 8.50 (d, 2H), 7.90 (t, 2H), 7.75 (d, 2H), 7.50 (t, 2H), 4.20 (br m, 2H), 3.20 (br m, 4H), 3.20 (br m, 12H), 2.00 (br m, 12H); Anal. ($C_{27}H_{47}N_6Cl_5 \cdot 3.5H_2O$) C, H, N.

***N*⁴-[4-(7-Chloroquinolinyl)butyl]-*N*¹,*N*¹²-bis(*t*-butoxycarbonyl)spermine (18).** 4,7-Dichloroquinoline (0.24 g, 1.26 mmol) and phenol (0.12 g, 1.25 mmol) were heated to 100 °C for 20 min. Amine **15** (0.40 g, 0.84 mmol) dissolved in hot phenol was added to the stirred mixture. The reaction mixture was under 100 °C overnight. The reaction mixture 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, purified by flash chromatography, and eluted with 2% NH_4OH/CH_3OH to give **18** (0.29 g, 54%). **18:** $R_f = 0.20$ in 2% NH_4OH/CH_3OH; ^{1}H NMR (CD_3OD) δ 8.50 (d, 1H), 7.95 (s, 1H), 7.80 (d, 1H), 7.35 (d, 1H), 6.40 (d, 1H), 3.30 (t, 2H), 3.20 (m, 4H), 2.50 (m, 10H), 1.80 (br m, 12H), 1.40 (s, 18H); Anal. ($C_{33}H_{55}O_4N_6Cl \cdot 0.7H_2O$) C, H, N.

***N*⁴-[4-(7-Chloroquinolinyl)butyl]spermine Pentahydrochloride (19).** Quinoline derivative **18** (0.27 g, 0.42 mmol) was dissolved in dioxane (5 mL) at 0 °C, followed by the addition of 4 N HCl (5 mL). The mixture was stirred at room temperature. The disappearance of the starting material was monitored by TLC ($R_f = 0.20$, 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 **19** (0.084 g, 72%). **19:** ^{1}H NMR (D_2O) δ 8.18 (d, 1H), 7.95 (d, 1H), 7.65 (d, 1H), 7.45 (dd, 1H), 6.70 (d, 1H), 3.55 (m, 2H), 3.10 (br m, 14H), 2.05 (br m, 4H), 1.75 (m, 8H); Anal. ($C_{23}H_{44}N_6Cl_6 \cdot 4H_2O$) C, H, N.

***N*¹,*N*⁸-Dimethylspermidine Trihydrochloride (20).** Derivative **20** was synthesized by published methods in 65% overall yield from spermidine.[24] **20**: ^{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 51.1, 49.9, 48.6, 47.3, 35.6, 35.5, 25.6, 25.5, 25.4; Anal. ($C_9H_{26}N_3Cl_3$) C, H, N.

***N*¹,*N*⁸-Diethylspermidine Trihydrochloride (21).** Derivative **21** was synthesized by published methods in 63% overall yield from spermidine.[24] **21**: ^{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. ($C_{11}H_{30}N_3Cl_3$) C, H, N.

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