Synthesis and Biological Evaluation of Dihydromotuporamine Derivatives in Cells Containing Active Polyamine Transporters

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Dihydromotuporamine C (**4**) and its 4,4-triamine analogue (**5**) were synthesized in good yield using ring-closing metathesis (RCM) methods. Comparison of their biological activities (K_i determinations in L1210 cells and IC_{50} determinations in L1210, CHO, and CHO-MG cells) revealed that the motuporamine derivatives do not use the polyamine transporter (PAT) for cellular entry. Bioevaluation of a N^1-(anthracen-9-ylmethyl)-N^1-(ethyl)homospermidine control (**7**) revealed that the presence of a N^1 tertiary amine center imparted a significant reduction in the PAT affinity of the polyamine conjugate and abolished its PAT-targeting selectivity.

Introduction

The nonselective delivery of drugs to both targeted tumor cells and healthy cells is a major shortcoming of current chemotherapies. Enhanced cell targeting during drug delivery could diminish nonspecific toxicities by reducing uptake by healthy cells. Using existing cellular transporters for drug delivery provides opportunities for molecular recognition events to assist in the cell targeting process.

Polyamines are essential cellular growth factors.[1] Tumor cells have been shown to contain elevated polyamine levels and have active polyamine transport systems to import exogenous polyamines.[1] Because of the enhanced cellular need for these amine growth factors and an activated transport system for their import, one can selectively deliver polyamine−drug conjugates to particular cell types.[1−8] The structural tolerances accommodated by the polyamine transporter (PAT) allow for the import of non-native polyamine conjugates.[2−6]

Prior work in our laboratory involved evaluation of the L1210 (murine leukemia cells) cytotoxicity and PAT affinity of conjugates containing either branched[2,3] or linear polyamine motifs[4−6] attached to an anthracene nucleus.[2,3,9] Moreover, cytotoxicity comparisons of Chinese hamster ovary (CHO) cells, which have high PAT activity, and a mutant line CHO-MG, which has no PAT activity, were used to identify conjugates that use the PAT for cellular entry.[4] Indeed, certain linear triamine motifs were identified as excellent vector systems for the PAT.[4,5] In particular, the homospermidine conjugate **1b** (e.g., a 4,4-triamine) had 150-fold higher cytotoxicity in CHO cells than in the mutant cell line CHO-MG.[4,5,10,11] In addition, a direct correlation was found between cytotoxicity and the ability of the polyamine conjugates to use the PAT for cellular entry. This strategy was further illustrated when **1b** was shown to be 10−30 times more selective in killing B16 melanoma cells over Mel A (normal melanocyte) cells.[12] Therefore, the 4,4-triamine motif was found to impart excellent PAT selectivity in this earlier anthracenylmethyl series.

Further evaluation of a series of N^1-substituted homospermidine derivatives demonstrated that large N^1-substituents (e.g., pyrenylmethyl, conjugate **2**) could be accommodated by the PAT. However, even with the "optimal" 4,4-triamine "message" present, there was a significant decrease in PAT uptake of polyamine conjugates when the N^1 tether length was increased from methylene **1b** to ethylene **1c** to propylene **1d** (Figure 1).[12] A new triamine-PAT model was presented that summarized the structure−activity findings and indicated the presence of an adjacent hydrophobic pocket near the polyamine binding site.[12] Molecular modeling studies using the **1b**−**d** series estimated that this pocket was >100 Å^3 in volume. In summary, prior work had demonstrated that relatively large hydrophobic motifs (with short tethers) could be shuttled into cells as long as the proper polyamine message was present.

A recent report by Andersen et al. described the bioactivity of the motuporamines, which are natural polyamines isolated from *Xestospongia exigua*, a sea sponge collected from the outer reef of Motupore Island, Papua New Guinea.[13] The native motuporamines each contain a large hydrophobic heterocycle appended to a polyamine motif and have some similarity to compounds **1a**−**d**. Indeed, motuporamine C (**3**) and dihydromotuporamine C (**4**) each contain a 15-membered macrocycle annealed to a 3,3-triamine motif and demonstrated high cytotoxicity against MDA-231 breast carcinoma cells and good anti-invasive properties with tumor cells.[13] Of key interest to us was the finding that the carbazole derivative **6** also had good anti-invasive properties. Because of the similar molecular motifs present in **1**−**6**, it was possible that they may have similar biological modes of action.

The motuporamines naturally contain the 3,3-triamine (norspermidine) sequence, a sequence we found to not be PAT-selective in our earlier screens. Because

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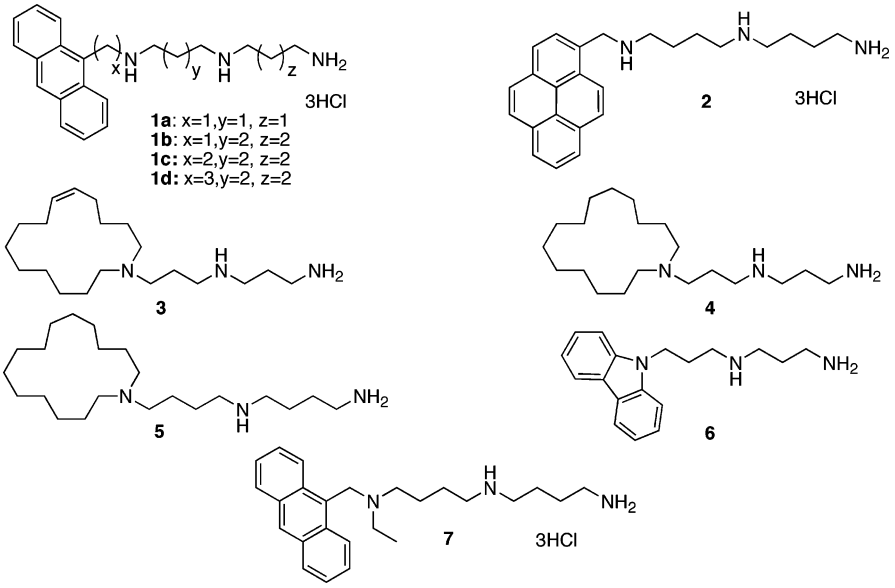


Figure 1. Polyamine architectures **1**−**7**.

these materials had not previously been screened for their PAT selectivity, we embarked upon their total synthesis in order to evaluate their PAT-targeting capabilities. By design, our synthetic plan was modular and allowed for introduction of both native (3,3-triamine) and non-native (4,4-triamine) polyamine sequences.

Our goal was simple. If the motuporamines used the PAT for cellular entry, then it was possible that our earlier structure−activity results could be applied to this new system to improve its efficacy and delivery profile. Dihydromotuporamine C (**4**) was chosen for alteration because it was the most efficacious of the Andersen series.[13a] This report describes both the synthesis and biological evaluation of dihydromotuporamine C and new analogues.

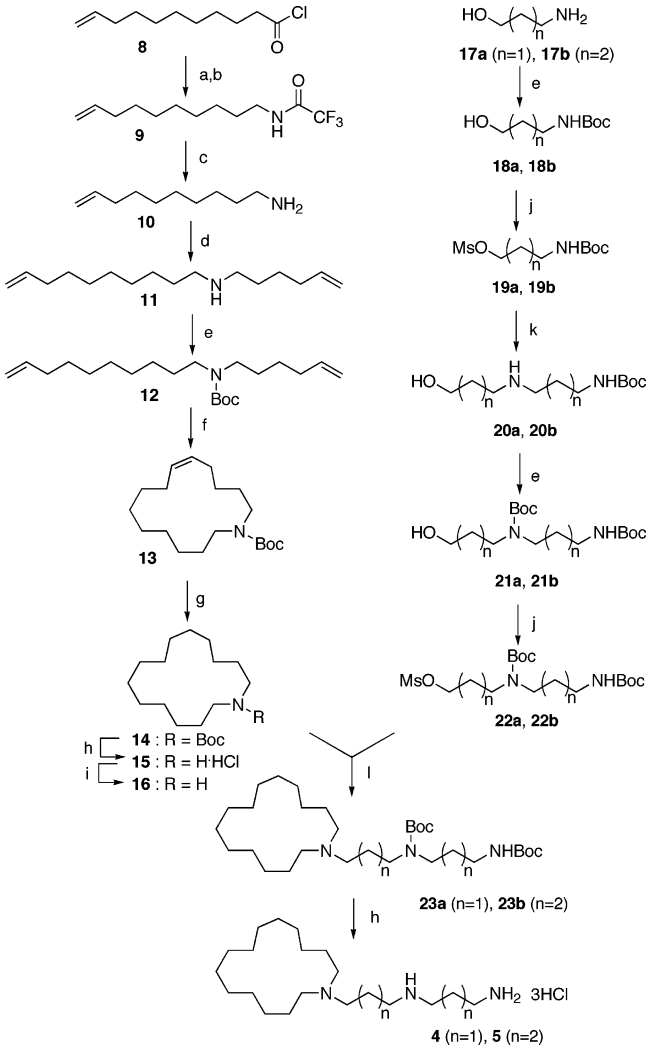
Results and Discussion

Synthesis. New conjugate **5** was synthesized in order to probe whether homospermidine (proven best for PAT in our earlier studies)[2−6] could enhance the PAT-mediated delivery of the motuporamine scaffold. For comparison, dihydromotuporamine C (**4**) was also synthesized. As the motuporamine scaffolds, **3**−**5**, contain a tertiary nitrogen at the N^1 position, and an *N*-ethyl control, **7**, was also synthesized for comparison.

Compounds **4** and **5** were synthesized from commercially available 10-undecenoyl chloride. As shown in Scheme 1, trifluoroacetamide **9** was synthesized from 10-undecenoyl chloride using a modified Curtius rearrangement,[14] which gave the primary amine **10**. Secondary amine **11** was synthesized via alkylation of **10** with 6-hexenyl mesylate, **24**. An unsuccessful attempt was made to cyclize the free secondary amine **11** by ring closing metathesis (RCM) with Grubb's catalyst.[15] Since the presence of the amine may compete for the metal reagent, we first Boc-protected (Boc: *tert*-butyloxycarbonyl) **11** to give the carbamate **12**, which was successfully cyclized by RCM to give alkene **13**.

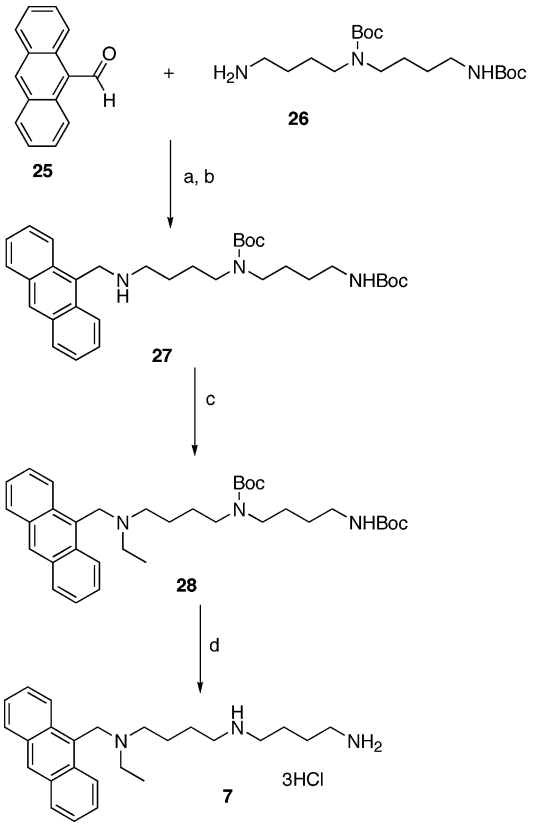
Andersen reported that the presence of the endocyclic alkene (a mixture of cis and trans isomers) resulted in reduced bioactivity.[13] Therefore, the aliphatic macro-

Scheme 1[a]



[a] Reagents: (a) NaN_3, TBAB; (b) TFA; (c) $NaOH/H_2O/EtOH$; (d) methanesulfonic acid hex-5-enyl ester (**24**)/CH_3CN/NEt_3; (e)10% NEt_3/MeOH/di-*tert*-butyl dicarbonate; (f) Grubb's catalyst/CH_2Cl_2; (g) $EtOH/H_2$/Pd−C; (h) EtOH/4 N HCl; (i) aqueous Na_2CO_3/CH_2Cl_2; (j) $MsCl/NEt_3/CH_2Cl_2$; (k) 4-amino-1-butanol, CH_3CN; (l) CH_3CN/NEt_3.

Scheme 2[a]



[a] Reagents: (a) 25% MeOH/CH_2Cl_2; (b) 50% MeOH/CH_2Cl_2, $NaBH_4$; (c) K_2CO_3, CH_3CN, C_2H_5Br; (d) 4 N HCl/ EtOH.

cyclic amine **16** was generated via hydrogenation of alkene **13** followed by removal of the Boc protecting group with 4 N HCl and neutralization of the resultant HCl salt with a Na_2CO_3 solution.

An earlier amino-alcohol strategy[16,17] was employed to access the mesylates **22a** and **22b**. Boc protection of the respective amino alcohols **17a** and **17b** gave carbamates **18a** and **18b**, which were then O-sulfonylated to give the respective mesylates **19a** and **19b**. Mesylate displacement with the respective amino alcohols gave the secondary amines **20a** and **20b**, which were then *N*-Boc-protected to give **21a** and **21b** and finally mesylated in successive steps to form **22a** and **22b**. Cyclic amine **16** was then alkylated with **22a** and **22b**, respectively, to form tertiary amines **23a** and **23b**. These tertiary amines provided the respective compounds **4** and **5** upon treatment with 4 N HCl.

Compound **7** was synthesized from the commercially available aldehyde **25**. The di-Boc-protected amine **26** was synthesized as reported earlier.[12] As shown in Scheme 2, reductive amination of aldehyde **25** with amine **26** gave compound **27**, which was alkylated with C_2H_5Br to give the *N*-ethyl derivative **28**. Boc removal with 4 N HCl provided compound **7**.

Biological Evaluation. Once synthesized, the conjugates (**1a**, **1b**, **4**, **5**, and **7**) were screened for cytotoxicity in L1210, CHO, and CHO-MG cells. L1210 (mouse leukemia) cells were selected to enable comparisons with the published IC_{50} and K_i values for a variety of related polyamine substrates.[2–6] Chinese hamster ovary (CHO) cells were chosen along with a mutant cell line (CHO-MG) in order to comment on selective transport via the PAT.[4–6,9] The results are shown in Table 1.

L1210 K_i and IC_{50} Studies. The K_i values in Table 1 were determined for [^{14}C]spermidine uptake and reflect the affinity of the polyamine derivative for the polyamine transporter on the cell surface. The IC_{50} values listed in Table 1 represent the concentration of the polyamine conjugate required to reduce the relative cell growth by 50%. With both parameters, one can determine whether high affinity for the transporter (e.g., low K_i value) translated into high cytotoxicity (e.g., low IC_{50} value).

A low K_i value means that the compound has a very high affinity for binding to the PAT. A priori one may have expected that conjugates with high PAT affinity would be transported efficiently into the cell by this transporter. However, prior results[12] showed that the K_i values were of minimal value in predicting the cytotoxicity and transport of these systems. Indeed, PAT substrates with very low K_i values ($\ll 1\ \mu$M) actually inhibited their own import. In the series of anthracene−triamine conjugates studied, substrates with moderate K_i values ($\sim 2\ \mu$M) were efficiently transported into cells via PAT.[4] In summary, the K_i values provided insight into how alterations of polyamine structure influence the affinity of the polyamine conjugate for the PAT system.

Earlier studies[5] showed that as the distance between the two nitrogens of the polyamine sequence was changed from three (**1a**, $K_i = 33.4\ \mu$M) to four (**1b**, $K_i = 1.8\ \mu$M) methylene units, the K_i values decreased. As shown in Table 1, this trend indicated a higher PAT affinity for **1b**, which contained the homospermidine motif. This same trend was observed in the dihydromotuporamine derivatives **4** ($K_i = 9.9\ \mu$M) and **5** ($K_i = 6.2\ \mu$M), albeit to a lesser degree. In short, introduction of the 4,4-triamine sequence increased the binding affinity of the substrate for the PAT.

Inspection of **1b** and **5** indicated that the presence of a tertiary amine at the N^1 position may reduce the derivative's PAT affinity. Therefore, the tertiary amine **7** was synthesized (Scheme 2) as a control. Rewardingly, the K_i value significantly increased ($\sim$13-fold) when the nitrogen at the N^1 position was converted to a tertiary center (e.g., K_i values: **1b**, 1.8 μM; **7**, 24.4 μM). These trends suggested that structural changes in the N^1-substitution pattern could also significantly alter the PAT affinity of the anthracene conjugates.

An interesting trend was apparent in the L1210 IC_{50} values of the compounds **1a**, **1b**, **4**, **5**, and **7** (Table 1). As the nitrogen at the N^1 position was changed from a secondary nitrogen (e.g., **1a** and **1b**) to a tertiary nitrogen (**4**, **5**, and **7**), the IC_{50} values also increased (**1a**, 1.8 μM; **1b**, 0.3 μM; **4**, 3.0 μM; **5**, 18.5 μM; **7**, 22.2 μM). Indeed, N-ethylation of the 2° amine in **1b** to give 3° amine **7** resulted in a 74-fold increase in L1210 IC_{50} value! Therefore, the degree of N^1-alkylation has significant effect on cytotoxicity. Further experiments in CHO cells revealed that these cytotoxicity differences were related to the ability of these materials to use the PAT for cellular entry.

CHO and CHO-MG Studies. Chinese hamster ovary (CHO) cells were chosen along with a mutant cell line (CHO-MG) in order to comment on how the synthetic conjugates gain access to cells.[4–6,9] The CHO-MG cell line is polyamine-transport-deficient and was isolated

Table 1. Biological Evaluation of Polyamine Derivatives in L1210, CHO, and CHO-MG Cells[a]

compd (tether)	L1210 IC_{50}, μM	L1210 K_i, μM	ref	CHO-MG IC_{50}, μM	CHO IC_{50}, μM	IC_{50} ratio[b]
1a: Antmethyl (3,3)	1.8 ± 0.4	33.4 ± 2.6	4	3.4 ± 0.5	1.9 ± 0.4	1.8
1b: Antmethyl(4,4)	0.30 ± 0.04	1.8 ± 0.1	4	66.7 ± 4.1	0.45 ± 0.10	148
4: dihydroMotu (3,3)	3.0 ± 0.5	9.9 ± 0.5		10.0 ± 2.6	10.5 ± 1.6	1
5: dihydroMotu (4,4)	18.5 ± 2.9	6.2 ± 0.5		28.2 ± 5.6	30.0 ± 4.1	1
7: *N*-ethyl-*N*-Antmethyl (4,4)	22.2 ± 1.2	24.4 ± 1.5		21.9 ± 0.9	22.2 ± 0.7	1

[a] Definitions: column 1, Ant = anthracen-9-yl; dihydroMotu = dihydromotuporamine; ref in column 4 denotes the reference number in which the data was originally reported. A blank in the ref column denotes new data. Cells were incubated for 48 h with the respective conjugate. [b] The ratio denotes the (CHO-MG/CHO) IC_{50} ratio, a measure of PAT selectivity.

after selection for growth resistance to methylglyoxalbis-(guanylhydrazone), MGBG ($CH_3C[=N-NHC(=NH)-NH_2]CH[=N-NHC(=NH)NH_2]$), using a single-step selection after mutagenesis with ethyl methanesulfonate.[10,11]

For the purposes of this study, the CHO-MG cell line represents cells with no PAT activity and provided a model for alternative modes of entry or action, which are independent of PAT. These alternative modes of entry include passive diffusion or utilization of another transporter. The alternative modes of action may also include interactions on the outer surface of the plasma membrane or other membrane receptor interactions.

In contrast, the parent CHO cell line represents a cell type with high PAT activity.[10,11] Therefore, highly selective PAT ligands should give high (CHO-MG/CHO) IC_{50} ratios.

As reported earlier,[12] dramatic differences in CHO and CHO-MG cytotoxicity (Table 1) were observed with **1b** (CHO-MG/CHO IC_{50} ratio: 148). Indeed, the CHO-MG/CHO IC_{50} ratios in Table 1 suggested that PAT targeting is significantly influenced by the degree of substitution at the N^1 position in the polyamine motif. For example, N^1-anthracenylmethylhomospermidine **1b**, which contains a secondary nitrogen at the N^1 position, was nearly 50 times more cytotoxic (CHO IC_{50} of **1b** is 0.45 μM) than compound **7**, which contains a tertiary nitrogen at the N^1 position (CHO IC_{50} of **7** is 22.2 μM). Moreover, there was complete loss of PAT selectivity with the 3°-amine-containing derivative **7** (CHO-MG/CHO ratio =1).

The fact that a lower IC_{50} value was observed for dihydromotuporamine derivative **4** (which contains the norspermidine vector) than for compound **5** (which contains the homospermidine vector) cannot be explained by their differential PAT selectivity (because both had CHO-MG/CHO IC_{50} ratios of 1 in Table 1). Instead, this observation may be due to the intrinsic toxicity of **4** over **5** or may result from a differential uptake via a non-PAT transport system, which is unknown at this time.

The CHO findings with **1a** and **4** were as expected because each derivative contained the norspermidine motif (i.e., a 3,3-triamine), which was shown to have minimal PAT selectivity in earlier studies.[2−6] However, the fact that both **5** and **7**, each containing the "PAT-optimized" 4,4-triamine motif, were not PAT-selective was very interesting and warranted further study.

Further comparisons of CHO-MG/CHO ratios in Table 1 revealed that in general compounds that contain the N^1 tertiary substitution pattern (**4**, **5**, and **7**) were not selective for PAT and gave similar cytotoxicities in both the CHO and CHO-MG cell lines. This observation explains why the motuporamine adducts, which contain N^1 tertiary amines, do not follow the same trend observed with the earlier anthracene series. This dramatic N^1-substitution effect has direct bearing on the future design of polyamine conjugates and implies that the attachment of "drug cargoes" via an N^1 secondary amine linker is required for PAT-mediated delivery.

The N^1 tertiary amine center also increases the steric crowding near the N^1-ammonium ion.[18] This in turn may shield the positive charge (on N^1) from docking to its complementary anionic site on the PAT. Increasing the N^1-substituent size has been shown to correlate with higher K_i values in a related dialkylated tetraamine series.[7c] Indeed, lower PAT affinity is observed in Table 1 as a 13-fold higher K_i value for **7** vs **1b**.

Beyond the steric factors imparted by the degree of N^1-substitution, there are also conformational preferences associated with the presence of a N^1 tertiary amine center, which may influence PAT recognition. Indeed, delineating the conformational preferences of **1b**, **4**, **6**, and **7** will be the subject of a future molecular modeling report on these systems.

Conclusions

Two dihydromotuporamine and anthracene−polyamine derivatives were synthesized and evaluated for their ability to target the polyamine transporter in two CHO cell lines (via CHO-MG/CHO IC_{50} ratios). Both K_i and IC_{50} values were also determined in L1210 cells for comparison to earlier conjugates. The presence of a tertiary amine at the N^1 position blocked the ability of both the dihydromotuporamines (**4** and **5**) and the N^1-ethyl-N^1-(anthracenylmethyl)polyamine (**7**) to utilize the PAT for cellular entry.

The fact that N-ethylation (**7**, K_i = 24.4 μM) causes a 13-fold increase in the K_i value of **1b** (K_i = 1.8 μM) suggests a reduced affinity for PAT by the tertiary amine adduct. Moreover, the dramatic difference in CHO-MG/CHO IC_{50} ratios for **1b** (IC_{50} ratio of 148) and **7** (IC_{50} ratio of 1) indicated a complete loss of PAT selectivity (and targeting) upon formation of the N^1 tertiary center in **7**. In conclusion, the identification of the structural tolerances accommodated by the PAT (e.g., N^1 2° amines) will greatly accelerate the discovery of new PAT-selective motifs.

Experimental Section

Materials. Silica gel (32−63 μm) and chemical reagents were purchased from commercial sources and used without further purification. All solvents were distilled prior to use. For reactions with Grubb's Catalyst, CH_2Cl_2 was dried over

P_2O_5 and distilled under an Ar atmosphere. All other reactions were carried out under an N_2 atmosphere. ^1H and ^{13}C spectra were recorded at 300 or 75 MHz, respectively. TLC solvent systems are listed as volume percents, and NH_4OH refers to concentrated aqueous NH_4OH. All tested compounds provided satisfactory elemental analyses.

Biological Studies. Murine leukemia cells (L1210), CHO, and CHO-MG cells were grown in RPMI medium supplemented with 10% fetal calf serum, glutamine (2 mM), penicillin (100 U/mL), and streptomycin (50 μg/mL). L-Proline (2 μg/mL) was added to the culture medium for CHO-MG cells. Cells were grown at 37 °C under a humidified 5% CO_2 atmosphere. Aminoguanidine (2 mM) was added to the culture medium to prevent oxidation of the drugs by the enzyme (bovine serum amine oxidase) present in calf serum. Trypan blue staining was used to determine cell viability before the initiation of a cytotoxicity experiment. L1210 cells in early log-phase to mid-log-phase were used.

IC_{50} Determinations. Cell growth was assayed in sterile 96-well microtiter plates (Becton-Dickinson, Oxnard, CA). L1210 cells were seeded at 6×10^4 cells/mL of medium (100 μL/well). CHO and CHO-MG cells were plated at 1×10^4 cells/mL. Drug solutions (10 μL/well) of appropriate concentration were added at the time of seeding for L1210 cells and after an overnight incubation for the other cells. After exposure to the drug for 48 h, cell growth was determined by measuring formazan formation from 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium using a Titertek Multiskan MCC/340 microplate reader for absorbance (540 nm) measurements.[19]

K_i Procedure. The ability of the conjugates to interact with the polyamine transport system was determined by measuring competition by the conjugates against radiolabeled spermidine uptake in L1210 cells. This procedure was used to obtain the data listed in Table 1. Initially, the K_m value of spermidine transport was determined as previously described.[20]

The ability of conjugates to compete for [^{14}C]spermidine uptake was determined in L1210 cells by a 10 min uptake assay in the presence of increasing concentrations of competitor, using 1 μM [^{14}C]spermidine as substrate. K_i values for inhibition of spermidine uptake were determined using the Cheng−Prusoff equation[21] from the IC_{50} value derived by iterative curve fitting of the sigmoidal equation describing the velocity of spermidine uptake in the presence of the respective competitor.[22,23] L1210 cells were grown and maintained according to established procedures[24] and were washed twice in HBSS prior to the transport assay.

N^1-[3-(Azacyclopentadec-1-yl)propyl]propane-1,3-diamine Hydrochloride Salt (4). A solution of Boc-protected **23a** (70 mg, 133 mmol) was dissolved in absolute ethanol (2.28 mL) and stirred at 0 °C for 10 min. A 4 N HCl solution (3.64 mL) was added to the reaction mixture dropwise and stirred at 0 °C for 20 min and then at room temperature overnight. The solution was concentrated in vacuo to give **4** as a white solid in 99% yield: ^1H NMR (CD_3OD) δ 3.13−3.34 (m, 10H, NCH_2), 3.09 (t, 2H, NCH_2), 2.23 (br q, 2H, CH_2), 2.13 (br q, 2H, CH_2), 1.77 (br q, 4H, CH_2), 1.36−1.58 (m, 12H, CH_2); ^{13}C NMR (CD_3OD) 53.5, 53.3, 46.3, 38.2, 27.9, 27.6, 27.5, 25.8, 25.6, 23.2, 22.8; HRMS (FAB) calcd for $C_{20}H_{43}N_3$ (M + H − 3HCl)$^+$ 326.3535, found 326.3551.

N^1-[4-(Azacyclopentadec-1-yl)butyl]butane-1,4-diamine Hydrochloride Salt (5). Compound **5** was prepared in 99% yield by the same procedure as stated for **4** by using **23b** as starting material. **5:** ^1H NMR (CD_3OD) δ 3.20 (m, 6H, NCH_2), 3.11 (m, 4H, NCH_2), 3.01 (t, 2H, NCH_2), 1.68−1.98 (m, 6H, CH_2), 1.28−1.51 (m, 20H, CH_2); ^{13}C NMR (CD_3OD) 55.6, 53.3, 48.4, 40.2, 27.9, 27.6, 27.5, 25.7, 24.5, 24.4, 23.1, 22.9; HRMS (FAB) calcd for $C_{22}H_{47}N_3$·3HCl (M + H − 3HCl)$^+$ 354.3848, found 354.3848.

***N*-(4-Aminobutyl)-*N'*-anthracen-9-ylmethyl-*N'*-ethylbutane-1,4-diamine Hydrochloride Salt (7).** A solution of Boc-protected **28** (140 mg, 0.26 mmol) was dissolved in absolute ethanol (2.28 mL) and stirred at 0 °C for 10 min. A 4 N HCl solution (3.64 mL) was added to the reaction mixture dropwise and stirred at 0 °C for 20 min and then at room temperature overnight. The solution was concentrated in vacuo to give **7** as a yellow solid in 93% yield: ^1H NMR (D_2O) δ 8.76 (s, 1H), 8.23 (m, 4H), 7.78 (m, 2H), 7.68 (m, 2H), 5.33 (s, 2H), 3.43 (m, 2H), 3.19 (m, 2H), 3.04 (t, 2H), 3.00 (t, 2H), 2.83 (t, 2H), 1.74 (m, 6H), 1.47 (m, 5H); ^{13}C NMR (CD_3OD) δ 133.0, 132.9, 132.6, 130.9, 129.4, 126.7, 124.3, 121.4, 53.4, 51.0, 40.1, 25.8, 24.7, 24.5, 22.3, 9.7; HRMS (FAB) calcd for $C_{25}H_{35}N_3$·3HCl (M − 3HCl)$^+$ 377.2831, found 377.2831.

Dec-9-enylamine (10). A solution of 10-undecenyl chloride (**8**, 5 g, 25 mmol) and TBAB (200 mg, 0.62 mmol) in CH_2Cl_2 (60 mL) was cooled to 0 °C. Sodium azide (1.93 g, 30 mmol) was dissolved in water (10 mL) and added to the reaction mixture, which was subsequently stirred at 0 °C for 3 h. The mixture was placed in a separatory funnel and washed with water (3 × 40 mL). The organic layer was separated and dried with anhydrous $MgSO_4$ for 24 h. Continuous evolution of N_2 (as very small bubbles) was observed during this period. The reaction mixture was filtered, and TFA (2.75 mL, 37 mmol) was added dropwise to the filtrate, which was subsequently refluxed overnight. The cooled reaction mixture was washed with saturated $NaHCO_3$, dried with anhydrous Na_2SO_4, filtered, and concentrated. TLC ($CHCl_3$/hexane (1:1)) showed one major spot (R_f = 0.5), which was isolated by column chromatography and provided the trifluoroacetamide **9** as a colorless oil in 63% yield: R_f = 0.5 (50% $CHCl_3$/hexane); ^1H NMR ($CDCl_3$) δ 6.90 (br s, 1H, NH), 5.71−5.87 (m, 1H, CH), 4.86−5.04 (m, 2H, CH_2), 3.33 (q, 2H, NCH_2), 2.03 (q, 2H, CH_2), 1.58 (q, 2H, CH_2), 1.20−1.44 (m, 10H, CH_2); ^{13}C NMR ($CDCl_3$) δ 157.7, 139.5, 116.2, 114.2, 40.9, 34.2, 29.4, 29.2, 29.1, 29.00, 28.98, 27.0.

Trifluoroacetamide **9** (100 mg, 0.398 mmol) was dissolved in ethanol (2 mL). Aqueous sodium hydroxide (0.2 N, 4 mL) was added, and the reaction solution was stirred overnight. TLC showed the complete conversion of **9**. Ethanol was evaporated, and the product was extracted with CH_2Cl_2 (2 × 20 mL). The combined organic layers were dried with anhydrous Na_2SO_4, filtered, and concentrated to give **10** as a pale-yellow oil in 97% yield: R_f = 0.4, MeOH/$CHCl_3$/NH_4OH (10:89:1); ^1H NMR ($CDCl_3$) δ 5.72−5.88 (m, 1H, CH), 4.86−5.04 (m, 2H, CH_2), 2.67 (t, 2H, NCH_2), 2.03 (q, 2H, CH_2), 1.20−1.50 (m, 12H, CH_2); ^{13}C NMR ($CDCl_3$) δ 139.5, 114.2, 42.2, 34.23, 34.17, 29.84, 29.82, 29.5, 29.3, 27.1.

Dec-9-enylhex-5-enylamine (11). Methanesulfonic acid hex-5-enyl ester **24** (178 mg, 1 mmol) and 10-decenylamine (465 mg, 3 mmol) were dissolved in acetonitrile (10 mL) and stirred at 75 °C overnight under an N_2 atmosphere. After TLC confirmed the disappearance of the mesylate, the solution was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (20 mL) and washed three times with aqueous sodium carbonate. The organic layer was separated, dried with anhydrous anhydrous Na_2SO_4, filtered, and concentrated under vacuum. Flash column chromatography of the residue gave **11** as a yellow oil in 80% yield: R_f = 0.35, MeOH/$CHCl_3$/NH_4OH (0.5:9.5:1 drop); ^1H NMR ($CDCl_3$) δ 5.71−5.88 (m, 2H, CH), 4.86−5.04 (m, 4H, CH_2), 2.60 (m, 4H, NCH_2), 2.05 (q, 4H, CH_2), 1.22−1.60 (m, 16H, CH_2); ^{13}C NMR ($CDCl_3$) δ 139.3, 138.9, 114.6, 114.3, 50.4, 50.2, 34.2, 34.0, 30.4, 30.0, 29.9, 29.8, 29.4, 29.3, 27.8, 27.04; HRMS (FAB) calcd for $C_{16}H_{32}N$ (M + H)$^+$ 238.2535, found 238.2540.

Dec-9-enylhex-5-enylcarbamic Acid *tert*-Butyl Ester (12). A solution of the secondary amine **11** (210 mg, 0.886 mmol) in triethylamine/MeOH (1:7 v/v, 25 mL) was stirred at 0 °C for 10 min. A solution of di-*tert*-butyl dicarbonate (289 mg, 1.33 mmol) in MeOH (10 mL) was added dropwise over 10 min. The mixture was stirred for 1 h. The temperature was then allowed to gradually rise to room temperature, and the mixture was stirred overnight. The mixture was then concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 and washed with deionized water. The organic layer was separated, dried with anhydrous Na_2SO_4, filtered, and concentrated. The residue **12** could be either

purified by flash column chromatography on silica gel or used in the next step without further purification: yield 95%; R_f = 0.5, 50% $CHCl_3$/hexane; ^1H NMR ($CDCl_3$) δ 5.71−5.88 (m, 2H, CH), 4.86−5.04 (m, 4H, CH_2), 3.02−3.27 (bm, 4H, NCH_2), 2.05 (q, 4H, CH_2), 1.34−1.58 (m, 16H, CH_2), 1.30 (br s, 9H, CH_3); ^{13}C NMR ($CDCl_3$) δ 156.0, 139.4, 138.8, 114.7, 114.3, 79.1, 47.3, 47.1, 34.2, 33.8, 29.8, 29.7, 29.4, 29.3, 28.8, 27.2, 26.5; HRMS (FAB) calcd for $C_{21}H_{40}NO_2$ $(M + H)^+$ 338.3059, found 338.3085.

Azacyclopentadec-6-ene-1-carboxylic Acid *tert*-Butyl Ester (13). Separate solutions of **12** (600 mg, 1.78 mmol) and Grubb's catalyst (48 mg, 0.060 mmol, 3.37 mol %) each in dry CH_2Cl_2 (75 mL) were simultaneously added dropwise to a refluxing solution of dry CH_2Cl_2 (550 mL) over a period of 6 h under an Ar atmosphere. After the addition was complete, the reaction mixture was allowed to reflux for 12 h. After the consumption of **12** was checked by ^1H NMR ($CDCl_3$), the solvent was removed and the residue was purified by column chromatography (50% $CHCl_3$/hexane). The pale-yellow oil **13** was isolated in 72% yield (mixture of *E* and *Z* isomers): R_f = 0.5, 50% $CHCl_3$/hexane; ^1H NMR ($CDCl_3$) δ 5.30 (m, 2H, CH), 3.10−3.23 (m, 4H, NCH_2), 2.07 (m, 4H, CH_2), 1.22−1.61 (m, 25H, 8 × CH_2, 3 × CH_3); ^{13}C NMR ($CDCl_3$) δ 154.4, 131.68, 131.65, 131.9, 130.8, 79.1, 49.6, 49.5, 47.9, 32.3, 32.21, 31.5, 31.3, 29.98, 28.97, 28.8 (3C), 28.6, 28.5, 28.4, 28.0, 27.7, 27.6, 27.2, 27.1, 26.9, 26.7, 26.4; HRMS (FAB) calcd for $C_{19}H_{36}NO_2$ $(M + H)^+$ 310.2746, found 310.274.

Azacyclopentadecane-1-carboxylic Acid *tert*-Butyl Ester (14). Pd on charcoal (20 wt %, 74 mg) was added to a stirring solution of alkene **13** (354 mg, 1.14 mmol) in ethanol (5 mL). The flask was briefly evacuated, and H_2 gas was admitted via a balloon. The reaction mixture was allowed to stir overnight under a H_2 atmosphere at room temperature. The black suspension was then filtered through a layer of Celite and washed with MeOH. The combined filtrates were evaporated. Compound **14** was obtained as a pale-yellow viscous oil in 99% yield: ^1H NMR ($CDCl_3$) δ 3.18 (m, 4H, NCH_2), 1.58 (m, 4H, CH_2), 1.21−1.49 (m, 29H, 10 × CH_2, 3× CH_3); ^{13}C NMR ($CDCl_3$) δ 156.0, 79.7, 48.3, 28.4, 28.0, 27.4, 26.5, 26.1, 26.0, 25.1.

Azacyclopentadecane Hydrochloride Salt (15). A solution of Boc-protected azacyclopentadecamine **14** (352 mg, 1.13 mmol) was dissolved in absolute ethanol (12 mL) and stirred at 0 °C for 10 min. A 4 N HCl (18 mL) solution was added dropwise, and stirring was continued at 0 °C for 20 min and then at room temperature overnight. The solution was concentrated in vacuo to give **15** as a white solid in 99% yield: ^1H NMR ($CDCl_3$) δ 3.06 (t, 4H, NCH_2), 1.74 (q, 4H, CH_2), 1.40−1.52 (m, 20H, CH_2); ^{13}C NMR ($CDCl_3$) δ 44.7, 26.8, 26.52, 26.48, 25.0, 23.67; HRMS (FAB) calcd for $C_{14}H_{29}N{\cdot}HCl$ $(M - Cl)^+$ 212.2378, found 212.2372.

(3-Hydroxypropyl)carbamic Acid *tert*-Butyl Ester (18a). A solution of 3-aminopropanol (**17a**, 5 g, 66 mmol) in triethylamine/MeOH (1:7 v/v, 150 mL) was stirred at 0 °C for 10 min. A solution of di-*tert*-butyl dicarbonate (21.8 g, 99 mmol) in MeOH (50 mL) was added dropwise over 10 min. The mixture was stirred for 1 h under a N_2 atmosphere. The temperature was allowed to gradually rise to room temperature, and the solution was stirred overnight. The solution was evaporated under reduced pressure, and the residue was dissolved in CH_2Cl_2 and washed with deionized water. The organic layer was separated, dried over anhydrous Na_2SO_4, filtered, and concentrated to give a clear oil **18a** (90%) that was used in the next step without further purification: R_f = 0.4 (40% acetone/hexane); ^1H NMR ($CDCl_3$) δ 5.20 (br s, 1H, NH), 3.62 (m, 2H, OCH_2), 3.11 (m, 2H, NCH_2), 1.64 (t, 2H, CH_2), 1.41 (s, 9H, CH_3).

(4-Hydroxybutyl)carbamic Acid *tert*-Butyl Ester (18b). Compound **18b** was prepared by the same procedure as for **18a** using 4-aminobutanol (**17b**). **18b**: yield 90%; R_f = 0.4 (40% acetone/hexane); ^1H NMR ($CDCl_3$) δ 4.96 (bs, 1H, NH), 3.62 (m, 2H, OCH_2), 3.11 (m, 2H, NCH_2), 2.49 (m, 1H, OH), 1.55 (m, 4H, CH_2), 1.41 (s, 9H, CH_3).

Methanesulfonic Acid 3-*tert*-Butoxycarbonylaminopropyl Ester (19a). To a solution of the alcohol **18a** (9.91 g, 57 mmol) and triethylamine (39.1 mL, 280 mmol) in CH_2Cl_2 (130 mL) at 0 °C, methanesulfonyl chloride (32.42 g, 28 mmol) was added dropwise over 30 min under a N_2 atmosphere. The reaction mixture was stirred at 0 °C for 1 h and was slowly warmed to room temperature and stirred overnight under N_2. The reaction mixture was then cooled to 0 °C, and a 4 M NaOH solution (50 mL) was added slowly with vigorous stirring. The organic phase was separated and washed with water (2 × 70 mL). The organic phase was separated, dried over anhydrous Na_2SO_4, filtered, and concentrated to give the product **19a** as a clear oil (95%) that was used in the next step without further purification: R_f = 0.5 (40% acetone/hexane); ^1H NMR ($CDCl_3$) δ 5.06 (br s, 1H, NH), 4.27 (t, 2H, OCH_2), 3.21 (m, 2H, NCH_2), 3.01 (s, 3H, CH_3), 1.88 (quin, 2H, CH_2), 1.43 (s, 9H, CH_3).

Methanesulfonic Acid 4-*tert*-Butoxycarbonylaminobutyl Ester (19b). Compound **19b** was prepared by the same procedure as for **19a** using **18b** as the starting alcohol. **19b**: yield 95% R_f = 0.5 (40% acetone/hexane); ^1H NMR ($CDCl_3$) δ 4.85 (br s, 1H, NH), 4.27 (t, 2H, OCH_2), 3.16 (m, 2H, NCH_2), 3.01 (s, 3H, CH_3), 1.78 (quin, 2H, CH_2), 1.59 (quin, 2H, CH_2), 1.43 (s, 9H, CH_3).

[3-(3-Hydroxypropylamino)propyl]carbamic Acid *tert*-Butyl Ester (20a). The mesylate **19a** (3.26 g, 13 mmol) and 3-aminopropanol (3.87 g, 52 mmol) were dissolved in acetonitrile (20 mL). The mixture was then stirred at 75 °C under a N_2 atmosphere overnight. After the confirmation of the disappearance of the mesylate by TLC, the solution was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (20 mL) and washed three times with aqueous sodium carbonate. The organic layer was separated, dried with anhydrous Na_2SO_4, filtered, and concentrated under vacuum. Flash column chromatography of the residue gave **20a** as a light-yellow oil: yield 68%; R_f = 0.35 (1:10:89 NH_4OH/MeOH/$CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.05 (br t, 1H, NH), 3.77 (t, 2H, OCH_2), 3.16 (q, 2H, $BocNCH_2$), 2.83 (t, 2H, NCH_2), 2.67 (t, 2H, NCH_2), 1.69 (quin, 4H, CH_2), 1.43 (s, 9H, CH_3).

[4-(4-Hydroxybutylamino)butyl]carbamic Acid *tert*-Butyl Ester (20b). Compound **20b** was prepared in 65% yield by the same procedure as for **20a** using 4-aminobutanol and **19b** as the starting materials. **20b**: R_f = 0.35 (1:10:89 NH_4OH/MeOH/$CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.05 (br s, 1H, NHCO), 3.54 (t, 2H, OCH_2), 3.07 (m, 2H, $BocNCH_2$), 2.61 (m, 4H, NCH_2), 1.64 (quin, 4H, CH_2), 1.55 (quin, 4H, CH_2), 1.42 (s, 9H, CH_3).

(3-*tert*-Butoxycarbonylaminopropyl)-(3-hydroxypropyl)carbamic Acid *tert*-Butyl Ester (21a). A solution of **20a** (1.27 g, 5.5 mmol) in triethylamine/MeOH (1:7 v/v, 150 mL) was stirred at 0 °C for 10 min. A solution of di-*tert*-butyl dicarbonate (1.79 g, 8.2 mmol) in MeOH (50 mL) was added dropwise over 10 min. The mixture was stirred for 1 h at 0 °C. The temperature was then allowed to gradually rise to room temperature, and the solution was stirred overnight under an N_2 atmosphere. The solution was then evaporated under reduced pressure, and the residue was dissolved in CH_2Cl_2 and washed with deionized water several times. The organic layer was separated, dried with anhydrous Na_2SO_4, filtered, and concentrated to give **21a** as a colorless oil (90%) that was used in the next step without further purification: R_f = 0.7 (1:10:89 NH_4OH/MeOH/$CHCl_3$); ^1H NMR (CD_3OD) δ 3.55 (t, 2H, OCH_2), 3.28 (t, 2H, NCH_2), 3.24 (t, 2H, NCH_2), 3.04 (q, 2H, NCH_2), 1.62−1.81 (m, 4H, CH_2), 1.44 (s, 9H, CH_3), 1.42 (s, 9H, CH_3).

(4-*tert*-Butoxycarbonylaminobutyl)-(4-hydroxybutyl)carbamic Acid *tert*-Butyl Ester (21b). Compound **21b** was prepared in 92% yield by the same procedure as for **21a** using **20b** as the starting alcohol. **21b**: R_f = 0.7 (1:10:89 NH_4OH/MeOH/$CHCl_3$); ^1H NMR ($CDCl_3$) δ 4.67 (m, 1H, NH), 3.59 (t, 2H, OCH_2), 2.98−3.22 (m, 6H, NCH_2), 1.30−1.52 (m, 26H, 4 × CH_2, 6 × CH_3).

Methanesulfonic Acid 3-[*tert*-Butoxycarbonyl-(3-*tert*-butoxycarbonylaminopropyl)amino]propyl Ester (22a).

Methanesulfonyl chloride (862 mg, 7.52 mmol) was added dropwise to a solution of the di-Boc alcohol **21a** (500 mg, 1.51 mmol) and triethylamine (1.04 mL, 7.52 mmol) in CH_2Cl_2 (40 mL) at 0 °C under a N_2 atmosphere. The reaction mixture was stirred at 0 °C for 1 h and allowed to slowly warm to room temperature and stirred overnight under a N_2 atmosphere. The reaction mixture was then cooled to 0 °C, and a 4 M NaOH solution (50 mL) was added slowly with vigorous stirring. The organic phase was separated and washed with water (2 × 70 mL). The organic phase was separated, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to give the product **22a** as a pale-yellow oil (90%) that was used in the next step without further purification. $R_f = 0.6$ (10% acetone/$CHCl_3$); ^{1}H NMR (CD_3OD) δ 4.59 (bs, 1H, NH), 4.24 (t, 2H, OCH_2), 3.23 (t, 2H, NCH_2), 3.12 (q, 2H, NCH_2), 3.07 (s, 3H, CH_3), 3.02 (t, 2H, NCH_2) 1.91 (q, 2H, CH_2), 1.68 (q, 2H, CH_2), 1.46 (s, 9H, CH_3), 1.43 (s, 9H, CH_3).

Methanesulfonic Acid 4-[*tert*-Butoxycarbonyl-(4-*tert*-butoxycarbonylaminobutyl)amino]butyl Ester (22b). Compound **22b** was prepared in 90% yield by the same procedure as for **22a** using **21b** as starting alcohol. **22b**: $R_f = 0.6$ (10% acetone/$CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 4.59 (br s, 1H, NH), 4.24 (t, 2H, OCH_2), 3.15 (m, 6H, $BocNCH_2$), 3.04 (s, 3H, CH_3), 1.74 (q, 2H, CH_2), 1.62 (q, 2H, CH_2), 1.35−1.58 (m, 22H, 2 × CH_2, 6 × CH_3).

[3-(Azacyclopentadec-1-yl)propyl]-(3-*tert*-butoxycarbonylaminopropyl)carbamic Acid *tert*-Butyl Ester (23a). A saturated sodium carbonate solution (20 mL) was added to a vigorously stirred solution of **15** (332 mg, 1.34 mmol) in CH_2Cl_2 (20 mL). The organic layer was separated and was washed twice with saturated sodium carbonate. The combined organic layers were dried with anhydrous Na_2SO_4, filtered, and concentrated to give the free amine **16** as a pale-yellow semisolid in 98% yield: ^{1}H NMR ($CDCl_3$) δ 2.65 (t, 4H, NCH_2), 1.52 (q, 4H, CH_2), 1.21−1.44 (m, 20H, CH_2); ^{13}C NMR ($CDCl_3$) δ 48.2, 28.7, 27.3, 27.1, 27.0, 26.9, 25.7.

Compound **22a** (481 mg, 1.17 mmol) was dissolved in acetonitrile (5 mL) and added to a stirring solution of the free amine **16** (272 mg, 1.29 mmol) and triethylamine (130 mg, 1.29 mmol) in CH_2Cl_2 (2.5 mL). The mixture was then stirred at 75 °C overnight under a N_2 atmosphere. TLC showed traces of starting material **22a** even after 48 h, but the solution was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (20 mL) and washed three times with aqueous sodium carbonate. The organic layer was separated, dried with anhydrous Na_2SO_4, filtered, and concentrated under vacuum. Flash column chromatography of the residue gave **23a** as a yellow oil (30%): $R_f = 0.43$, MeOH/$CHCl_3$ (4:96); ^{1}H NMR ($CDCl_3$) δ 3.00−3.33 (m, 6H, $BocNCH_2$), 2.48 (br s, 6H, NCH_2), 1.68 (m, 4H, CH_2), 1.20−1.55 (m, 42H, 12 × CH_2, 6 × CH_3); ^{13}C NMR ($CDCl_3$) δ 162.6, 156.1, 79.9, 54.0, 53.1, 28.7, 27.4, 27.0, 26.8, 26.5, 26.2; HRMS (FAB) calcd for $C_{30}H_{60}N_3O_4$ $(M + H)^+$ 526.4584, found 526.4602.

[4-(Azacyclopentadec-1-yl)butyl]-(4-*tert*-butoxycarbonylaminobutyl)carbamic Acid *tert*-Butyl Ester (23b). Compound **23b** was prepared in 35% yield by the same procedure as for **23a** by using **22b. 23b**: $R_f = 0.41$, 4% MeOH/$CHCl_3$; ^{1}H NMR ($CDCl_3$) δ 3.18−3.27 (br t, 6H, $BocNCH_2$), 2.38 (t, 6H, NCH_2), 1.22−1.61 (m, 50H, 16 × CH_2, 9 × CH_3); ^{13}C NMR ($CDCl_3$) δ 156.02, 155.98, 79.9, 55.4, 54.0, 46.8, 40.5, 28.74, 28.66, 27.7, 27.4, 27.0, 26.8, 26.5, 26.1; HRMS (FAB) calcd for $C_{32}H_{64}N_3O_4$ $(M + H)^+$ 554.4897, found 554.4884.

Methanesulfonic Acid Hex-5-enyl Ester (24). Methanesulfonyl chloride (2.43 mL, 32 mmol) was added slowly to a solution of 6-hexenol (800 mg, 7.9 mmol) in CH_2Cl_2 (50 mL) containing triethylamine (TEA, 4.42 mL, 32 mmol) at 0 °C. After 1 h, the reaction mixture was allowed to stir for 24 h at room temperature. The mixture was cooled in an ice bath, and 50% aqueous NaOH solution (20 mL) was added slowly. The reaction mixture was stirred for 10 min. The organic layer was separated and washed with deionized water. The organic layer was again separated, dried with anhydrous Na_2SO_4, filtered, and concentrated. The crude mesylate **24** was used in the next step without further purification: yield 98%; $R_f = 0.50$, $CHCl_3$/hexane (1:1); ^{1}H NMR ($CDCl_3$) δ 5.68−5.88 (m, 1H, CH), 4.97−5.04 (m, 2H, CH_2), 4.23 (t, 2H, OCH_2), 3.03 (s, 3H, CH_3), 2.10 (q, 2H, CH_2), 1.78 (q, 2H, CH_2), 1.56 (q, 2H, CH_2).

{4-[(Anthracen-9-ylmethyl)amino]butyl}-(4-*tert*-butoxycarbonylaminobutyl)carbamic Acid *tert*-Butyl Ester (27). To a stirred solution of **26** (0.366 g, 1 mmol) in 25% MeOH/CH_2Cl_2 (10 mL), was added a solution of 9-anthraldehyde **25** (0.175 g, 0.85 mmol) in 25% MeOH/CH_2Cl_2 (10 mL) under N_2. The mixture was stirred at room temperature overnight until the imine formation was complete (monitored by NMR). The solvent was removed in vacuo, the solid residue was dissolved in 50% MeOH/CH_2Cl_2 (20 mL), and the solution was cooled to 0 °C. $NaBH_4$ (2.55 mmol) was added in small portions to the solution, and the mixture was stirred at room temperature overnight. The solvent was removed in vacuo, and the solid residue was dissolved in CH_2Cl_2 (30 mL) and washed with 10% aqueous Na_2CO_3 solution (3 × 30 mL). The CH_2Cl_2 layer was dried over anhydrous Na_2SO_4, filtered, and removed in vacuo to give an oily residue. The oil was purified by flash column chromatography (5% MeOH/$CHCl_3$) to yield the product **27** as a pale-yellow thick oil (0.38 g, 85%): $R_f = 0.3$ (5% MeOH/$CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.34 (s, 1H), 8.29 (d, 2H), 7.94 (m, 2H), 7.51 (m, 2H), 7.42 (m, 2H), 4.67 (s, 2H), 3.00−3.205 (m, 6H), 2.86 (t, 2H), 1.32−1.70 (m, 26H); ^{13}C NMR ($CDCl_3$) δ 155.9, 155.4, 131.7, 131.4, 130.1, 129.1, 127.1, 126.0, 124.8, 124.0, 79.1, 53.5, 50.3, 46.9, 46.6, 45.8, 40.3, 28.6, 28.5, 27.5. HRMS (FAB) *m/z* calcd for $C_{33}H_{47}N_3O_4$ $(M)^+$ 549.3572, found 549.3572.

[4-(Anthracen-9-ylmethylethylamino)butyl]-(4-*tert*-butoxycarbonylaminobutyl)carbamic Acid *tert*-Butyl Ester (28). Bromoethane (134 mg, 1.2 mmol) was dissolved in anhydrous acetonitrile and was added to the stirring mixture of compound **27** (225 mg, 0.41 mmol) and anhydrous K_2CO_3 (170 mg, 1.23 mmol). The mixture was then stirred at 75 °C under a N_2 atmosphere overnight. After the confirmation of the disappearance of the **27** by TLC, the solution was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (20 mL) and washed three times with aqueous sodium carbonate. The organic layer was separated, dried with anhydrous Na_2SO_4, filtered, and concentrated under vacuum. Flash column chromatography of the residue gave **28** as a light-yellow oil: yield 71%; $R_f = 0.35$ (5% MeOH/$CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 8.52 (d, 2H), 8.38 (s, 1H), 7.94 (d, 2H), 7.47 (m, 4H), 4.50 (s, 2H), 2.77−3.20 (m, 6H), 2.64 (m, 2H), 2.51 (m, 2H), 1.20−1.62 (m, 26H), 1.16 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 156.0, 155.5, 131.5, 131.4, 130.0, 129.0, 127.3, 125.4, 125.3, 124.8, 79.1, 52.6, 50.9, 47.6, 47.0, 46.6, 40.5, 28.7, 27.5, 25.8, 24.6, 12.2; HRMS (FAB) *m/z* calcd for $C_{35}H_{51}N_3O_4$ $(M)^+$ 577.3880, found 577.3880.

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Supporting Information Available: Elemental analyses for compounds **4**, **5**, **7**, **11**−**13**, **23a**, **23b**, **27**, and **28** and the ^{1}H and ^{13}C spectra of compounds **4**, **5**, **7** and **15**. This material is available free of charge via the Internet at http://pubs.acs.org.

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