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Synthesis and Characterization of N^1-(4-Toluenesulfonyl)-N^1-(9-anthracenemethyl)triamines

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Abstract: A modular synthetic approach was developed to access triamines with varying tether lengths from commercially available aminoalkanols. Initial *N*-alkylation via reductive amination with anthracene-9-carbaldehyde provided the secondary amines in good yield. Subsequent ditosylation with excess TsCl yielded the respective bis-*N*,*O*-tosylates. The tosylates were reacted with excess putrescine to give the final triamines. X-ray crystallography revealed that the polyamine tail is preferentially oriented over the shielding cone of the anthracene ring.

The naturally occuring linear polyamines **1**−**3** (putrescine, spermidine, and spermine, respectively) have attracted attention because of their unique biological properties and potential as therapeutic templates (Figure 1). Indeed, new polyamine homologues have provided a better understanding of polyamine transport into leukemia cells.[1] Structure−activity relationships have provided important molecular recognition information about the transporter, which can facilitate future drug design. Previous polyamine structural changes included terminal *N*-alkylation, alteration of the methylene tether between nitrogens, and attachment of drug "cargoes" to the polyamine platform.[1−4]

Polyamines exist as polycations in vivo and have a high affinity for biological polyanions such as DNA.[2] In addition, flat polycyclic aromatic systems such as the anthracene and acridine nuclei interact with DNA as minor groove binders or DNA intercalators.[3] Indeed, bioconjugates involving polyamines and these aromatic nuclei (**4** and **5** in Figure 2) have been shown to be efficient topoisomerase II (Topo-II) inhibitors.[4] Interestingly, even though the acridine conjugates **4** were more potent Topo-II inhibitors in vitro, the related anthracene conjugates **5** were more efficient in a whole cell assay involving L1210 murine leukemia cells. Spermidine protection assays suggested that the affinity of these conjugates for the L1210 polyamine transporter could be modulated via alterations in the appended polyamine architecture.[4]

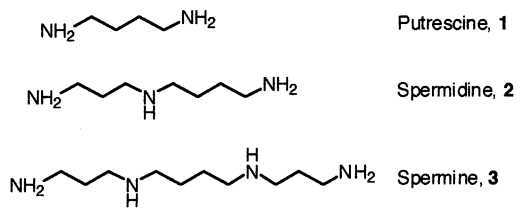

FIGURE 1. The native polyamines.

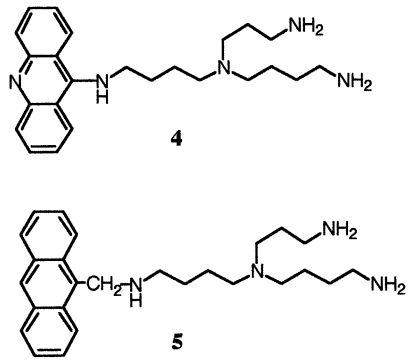

FIGURE 2. Acridine−spermidine (**4**) and anthracene−spermidine (**5**) conjugates.

As part of our continuing investigation of polyamine−anthracene conjugates, we were interested in the size limitations of the transporter. In other words, what were the size constraints of materials with use of the polyamine transport apparatus? How large a drug cargo could be delivered via this transporter? Could a polyamine structural change overcome these constraints? As earlier work pointed to the fact that one could enhance uptake by modifying the polyamine structure, homologous spermidine derivatives were synthesized.

The commercially available amino alcohols were envisioned as versatile synthetic intermediates. Previous reports have shown that successive *N*-alkylation and mesylation steps can be used to construct the linear polyamine chain.[5] In this report, a modular synthetic approach was developed to access polyamine architectures with varying tether distances between the nitrogens and a large *N*-tosyl-*N*-(9-anthracenemethyl) appended "cargo". In this manner, a series of anthracene−polyamine conjugates were prepared for later biological evaluation. In addition to introducing large steric constraints, the tosyl group also imparted interesting long-distance effects on the appended polyamine chain.

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(1) For recent reviews about the synthesis and biological properties of polyamine derivatives see: (a) Casero, R. A., Jr.; Woster, P. M. *J. Med. Chem.* **2001**, *44*, 1−26. (b) Karigiannis, G.; Papaioannou, D. *Eur. J. Org. Chem.* **2000**, *10*, 1841−1863. (c) Kuksa, V.; Buchan, R.; Lin, P. K. T. *Synthesis* **2000**, *9*, 1189−1207. (d) Bergeron, R. J.; Feng, Y.; Weimar, W. R.; McManis, J. S.; Dimova, H.; Porter, C.; Raisler, B.; Phanstiel, O., IV *J. Med. Chem.* **1997**, *40*, 1475−1494. (e) Seiler, N.; Delcros, J.-G.; Moulinoux, J. P. *Int. J. Biochem. Cell Biol.* **1996**, *28* (8), 843−861.

(2) Geall, A. J.; Blagbrough, I. S. *Tetrahedron* **2000**, *56*, 2449−2460.

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(4) (a) Phanstiel, O., IV; Price, H. L.; Wang, L.; Juusola, J.; Kline, M.; Shah, S. M. *J. Org. Chem.* **2000**, *65*, 5590−5599. (b) Wang, L.; Price, H. L.; Juusola, J.; Kline, M.; Phanstiel, O., IV *J. Med. Chem.* **2001**, *44*, 3682−3691.

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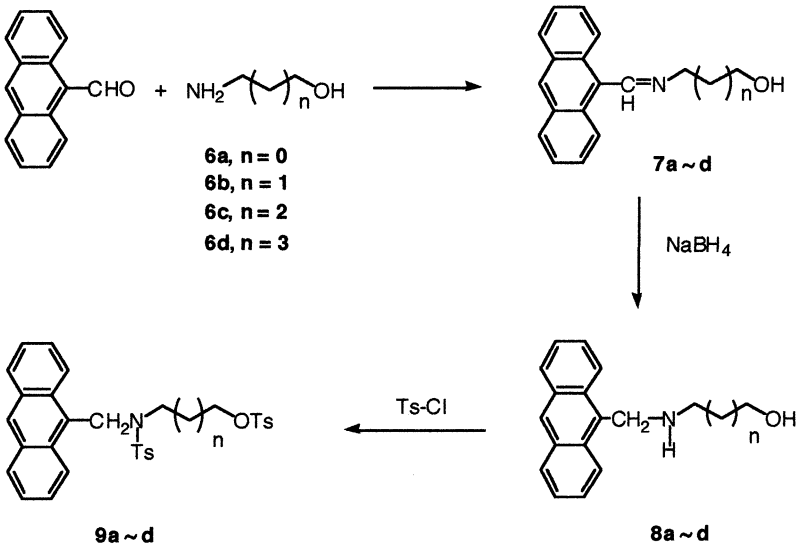
SCHEME 1

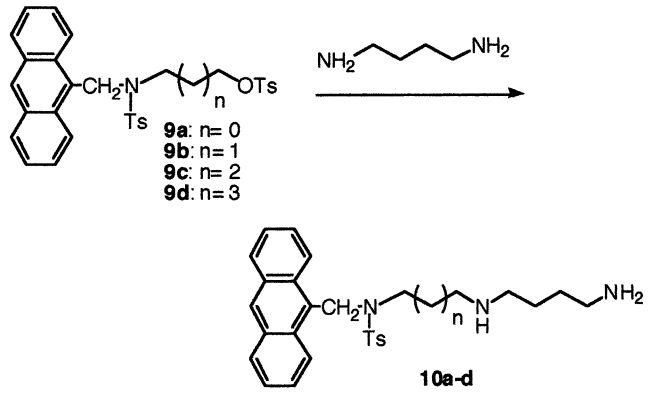


As shown in Scheme 1, the conversion of **6** to **8** was achieved in two steps via in situ generation of the imine **7**. A homologous series of imines (**7a**−**d**) were prepared from different amino alcohols, **6**. The imines were then reduced to **8** with $NaBH_4$ in good yields without further purification. The first step did not require scrupulously dry solvents or molecular sieves as reported for a similar reaction in the literature.[6] Instead, solvent removal by rotary evaporation at 40−50 °C facilitated the forward equilibrium step, and provided yields ranging from 68 to 81% of the 2° amines, **8**.

In the seemingly routine tosylation step, several conditions were tried to increase the product yields and to avoid product decomposition. Upon addition of *p*-toluenesulfonyl chloride (TsCl), the color of the solution became dark even when the reaction was conducted at low temperature (−20 °C). [Note: This discoloration event did not occur if the 2° amine was first protected with a *tert*-butyloxycarbonyl (Boc) group.] After several modifications, the desired bis-*N*,*O*-tosylates (**9a**−**d**) were isolated in good yields and purified prior to their reaction with putrescine. While the primary aliphatic tosylates normally react easily with amines,[5] compound **9** required excess putrescine at elevated temperature (75 °C) and longer time (overnight) to form the target compounds **10** shown in Scheme 2**.** This was likely due to steric factors.

The ^{1}H NMR spectra of the tosylated compounds revealed interesting chemical shifts. Upon tosylation, the "benzylic" CH_2 of the ditosylated compounds (**9**) occurred downfield due to the presence of the strong electron-withdrawing sulfonyl group as expected. Generally speaking, the other CH_2 connected directly to N in **9** is nearly unperturbed because of the countering effects of the new sulfonyl group and the shielding cone associated with the π-system of the aromatic rings.[7] A similar phenomenon was reported for a polyazamacrocycle prepared by Luis et al.[8] The normal trend is that the ^{1}H NMR signal of

SCHEME 2



aliphatic tosylates (RCH_2OTs) appears approximately 0.2 ppm downfield of their alcohol precursors.[7] Throughout the series of **9a**−**d**, the closer the $-CH_2OTs$ group was to the anthracene ring, the further *upfield* its chemical shift appeared. For the series **9a**−**d**, even when the $-CH_2OTs$ group and aromatic rings were separated by six atoms, the chemical shift of the $-CH_2OTs$ group (**9d**: δ 3.35) was still upfield compared with its alcohol precursor (**8d**: δ 3.50). We speculated that the $-CH_2$-OTs group is still under the strong shielding influence of the distal anthracene ring.

The triamine analogues, **10a**−**d**, also exhibited this phenomena. During the conversion from tosylate **9** to triamine **10**, the ^{1}H NMR signals, which belong to the $-CH_2OTs$ group in **9** (δ 3.2−3.4), disappear and new signals appear between δ 1.6 and 2.7. These signals were assigned by ^{1}H−^{1}H COSY experiments and were attributed to the CH_2 N groups associated with the two free amines introduced by the putrescine moiety. In short, certain chemical shift values within the **10** series are significantly upfield of their normal range and mirrored the anthracene proximity effect observed with **9**. By

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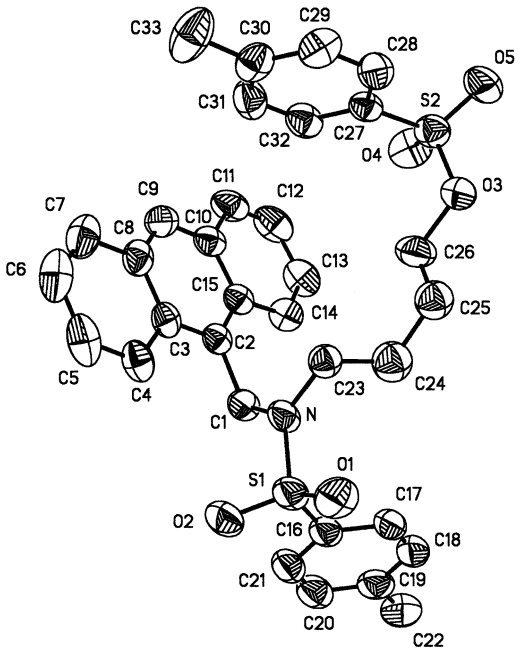

FIGURE 3. X-ray crystal structure of **9c**.

assigning the chemical shifts of the homologous series **9a**−**d** and **10a**−**d**, one can estimate that the shielding cone of the anthracene nucleus effected CH signals, which were 3−9 atoms away from the anthracene core. Before and beyond this six-atom "window", the CH chemical shifts appear near their expected positions. For example, the most distal position from the anthracene core (i.e., the terminal CH_2NH_2 group) varied from δ 2.43 for **10a** to δ 2.70 for **10d**.

To assign this effect to a particular conformation, a single crystal of the ditosylate system, **9c**, was obtained.[9] As shown in Figure 3, the butylene fragment is preferentially oriented toward the anthracene ring system as anticipated. This arrangement places the aliphatic chain over the shielding cone of the anthracene. A priori one may have expected the polyamine tail in **10** to impart this conformational bias due to electronic factors. However, the conformation of **9c** (Figure 3) suggested that the conformational bias of the *N*-tosyl-*N*-anthracenylmethyl group is likely due to steric factors, which require a near anti alignment of the bulky tosyl group and the anthracene ring system. As shown in Figure 3, the *N*-alkyl ligand (N−C23) is oriented toward the anthracene ring via the dihedral angle C2−C1−N−C23 (−30°). In short, the *N*-tosyl-*N*-anthracenylmethyl terminus introduces a conformational constraint on the appended *N*-alkyl chain, which preferentially orients the *N*-alkyl ligand over the anthracene ring system.

In conclusion, an efficient modular method was developed that allows ready access to the polyamines with different tethers between the nitrogen centers. In addition, a novel conformational constraint was introduced via the *N*-tosyl-*N*-anthracenemethyl subunit. Whether this interesting corollary translates to unique biological activity remains to be seen and will be the subject of future work.

Experimental Section

Materials. Silica gel (32−63 μm) was purchased from Scientific Adsorbents Inc. Chemical reagents were purchased from commercial sources and used without further purification. All solvents were distilled prior to use. ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively. TLC solvent systems are based on volume percent and NH_4OH refers to concentrated aqueous NH_4OH. Elemental analyses were performed by Atlantic Microlabs (Norcross, GA). High-resolution mass spectrometry was performed by Dr. David Powell at the University of Florida Mass Spectrometry facility.

General Procedure for the Synthesis of (Anthracen-9-ylmethyl)amino Alcohols, 8a−d. To a stirred solution of amino alcohol (12 mmol) in 10 mL of 25% $MeOH/CH_2Cl_2$ was added a solution of 9-anthraldehyde (10 mmol) in 10 mL of 25% $MeOH/CH_2Cl_2$ under N_2. The mixture was stirred at room temperature overnight, until the imine formation was complete (monitored by TLC). The solvent was evaporated under vacuum to give the crude imine as a bright green solid, which was used for reduction without further purification.

$NaBH_4$ (30 mmol) was added in small portions to the solution of imine in 20 mL of 1:1 $CH_3OH-CH_2Cl_2$ at 0 °C. The mixture was stirred at room temperature overnight, then concentrated under vacuum. The residue was dissolved in 50 mL of CH_2Cl_2 and washed three times with 50 mL of aq Na_2CO_3 (pH 10). The organic layer was separated, dried over anhydrous Na_2SO_4, filtered, and concentrated under vacuum. The residue was purified by flash chromatography on silica gel and NMR data were collected in $CDCl_3$.

General Procedure for the Ditosylation of *N*-(Anthracen-9-ylmethyl)amino Alcohols, 9a−d. The solution of *N*-(anthracen-9-ylmethyl)amino alcohol (5 mmol) in 25 mL of dry pyridine was stirred at −20 °C for 10 min. *p*-Toluenesulfonyl chloride (TsCl, 15 mmol) was added in small portions over an hour period. The mixture was stirred for one additional hour more, and the temperature gradually rose to 0 °C. The reaction flask was placed in a refrigerator (0−5 °C) overnight. The mixture was added into 200 mL of ice−water, and a solid (or viscous liquid) typically precipitated. The solid (or viscous liquid) was washed with deionized water several times. Then the washed solid (or viscous liquid) was recrystallized from CH_2Cl_2−methanol or purified by flash chromatograph on silica gel. Note: The viscous liquids became foamlike solids after pumping. NMR data were collected in $CDCl_3$.

General Procedure for the Preparation of Monotosylated Benzylic Triamines, 10a−d. The ditosylated product **9** (1 mmol) and 1,4-diaminobutane (10 mmol) were dissolved in acetonitrile (10 mL), then stirred at 75 °C under N_2 overnight. After checking the disappearance of ditosylated products by TLC, the solution was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (20 mL) and washed three times with saturated aqueous sodium carbonate. The organic layer was separated, dried over anhydrous sodium sulfate, filtered, and concentrated under vacuum. The residue was purified by flash chromatography on silica gel.

X-ray Crystallography with 9c. The bis-tosylate **9c** (0.2 g) was dissolved in 10 mL 1:1 (v/v) acetone−methanol. The solvent was slowly evaporated in air at room temperature over several days. The single crystal appeared as a pale green prism.

Data were collected at 173 K on a Siemens SMART PLATFORM equipped with A CCD area detector and a graphite monochromator utilizing Mo Kα radiation (λ = 0.71073 Å). Cell parameters were refined with up to 8192 reflections. A full sphere of data (1381 frames) was collected with the ω-scan method (0.3° frame width). The first 50 frames were remeasured at the end of data collection to monitor instrument and crystal stability (maximum correction on *I* was <1%). Absorption corrections by integration were applied based on measured indexed crystal faces.

The structure was solved by the Direct Methods in SHELXTL5, and refined with full-matrix least squares.[9] The non-H

(9) Sheldrick, G. M. *SHELXTL5*: Bruker-AXS: Madison, WI, 2000.

atoms were treated anisotropically, whereas the hydrogen atoms were calculated in ideal positions and were riding on their respective carbon atoms. The chain between N1 and O3 is disordered and was refined in two positions (both of which are oriented toward the anthracene ring). Both conformers are present in the crystal. Their site occupation factors refined to 0.77(1) for the major part (Figure 3) and, consequently, 0.23(1) for the minor part. A total of 385 parameters were refined in the final cycle of refinement using 4824 reflections with $I > 2\sigma(I)$ to yield R_1 and wR_2 of 4.05% and 10.55%, respectively. Refinement was done with F^2.

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Supporting Information Available: Complete characterization data (^{1}H NMR, ^{13}C NMR, mass spectral data, and elemental analyses) for the synthetic intermediates (**8a**−**d** and **9a**−**d**) and products **10a**−**d**, and crystallographic information for **9c**. This material is available free of charge via the Internet at http://pubs.acs.org.

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