# Structural Effects in Novel Steroidal Polyamine−DNA Binding

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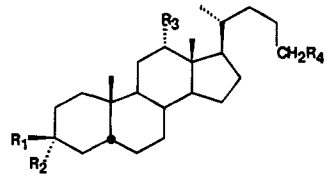
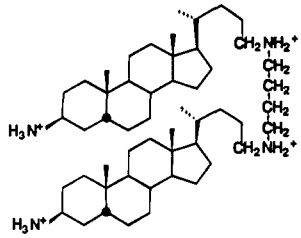
Efforts to understand the interactions of both drugs and toxins with DNA as well as the desire for new methods of DNA manipulation have spurred the design and synthesis of small molecules that bind in specific ways to DNA. The biogenic polyamines (putrescine, spermidine, spermine) bind primarily in the minor groove and are thought to hydrogen bond with donor atoms on the edges of the base pairs rather than associating with the phosphate backbone.[1] Studies of simple aliphatic polyamines[2] suggest that the three- to four-carbon spacing between ammonium groups is nearly ideal for matching the spacing of base pairs along the minor groove ladder while ensuring full protonation of the amines at pH 7. The naturally occurring steroidal diamines such as irehdiamine A, malouetine, dipyrandium, and chonemorphine bearing ammonium groups at C3 and C17 (or the adjacent carbon, C20) of the steroid are amphiphilic in nature, presenting a large hydrophobic group between the cationic extremities.[3] Their biophysical features include unwinding of superhelical DNA, increasing the duplex denaturation temperature, and altering of the UV and CD spectra of DNA while such biochemical functions as aiding in membrane permeability, ion transport, and DNA replication and mutagenesis are also observed.[4] For dipyrandium, binding to DNA is proposed to occur in the minor groove in conjunction with 5′-d(TA) kinks.[5] The steroidal diamines, as well as the recently reported steroidal spermidine, squalamine, isolated from sharks, are of considerable interest as antibiotics.[6]

Along this vein, we have focused upon the design of new synthetic steroidal polyamines in which the number and position of ammonium or guanidinium groups could be varied. Bile acids such as cholic acid and its derivatives offer a convenient framework for construction of such species.[7] Accordingly, we prepared the steroidal diamines **1**−**4**, triamine **5**, amino−guanidinium **6**, and the steroid dimer **7**, a tetraamine, and examined their binding to DNA.




**1** $R_1$ = H, $R_2$ = $NH_3^+$, $R_3$ = $NH_3^+$, $R_4$ = OH
**2** $R_1$ = $NH_3^+$, $R_2$ = H, $R_3$ = $NH_3^+$, $R_4$ = OH
**3** $R_1$ = H, $R_2$ = $NH_3^+$, $R_3$ = H, $R_4$ = $NH_3^+$
**4** $R_1$ = $NH_3^+$, $R_2$ = H, $R_3$ = H, $R_4$ = $NH_3^+$
**5** $R_1$ = $NH_3^+$, $R_2$ = H, $R_3$ = $NH_3^+$, $R_4$ = $NH_3^+$
**6** $R_1$ = $NH_3^+$, $R_2$ = H, $R_3$ = H, $R_4$ = $NHC(NH_2)NH_2^+$




**7**

The polyamines were synthesized from either deoxycholic acid or lithocholic acid, depending on whether or not functionality was desired at C12.[8] Both acids were first reduced to the corresponding C24 alcohols using $BH_3$/THF.[9] Both the 3α-OH and the 12α-OH groups could be converted to α-$NH_2$ groups by oxidation with pyridinium dichromate, formation of the oxime, and reduction with Na/*n*-propanol.[10] The 3β-$NH_2$ group was introduced by conversion of the 3α-OH to the corresponding 3β-azide under Mitsunobu conditions ($HN_3$ or $Zn(N_3)_2$, $PPh_3$, DEAD)[11] followed by reduction of the azide with either catalytic hydrogenation or $LiAlH_4$. This latter procedure was also used to introduce the C24 amine, which could be subsequently converted to a guanidinium group using aminoiminomethanesulfonic acid.[12] Finally, the dimeric steroid **7** was synthesized by a 2:1 coupling of lithocholic acid with 1,4-diaminobutane via the *N*-hydroxysuccinimide ester, the Mitsunobu method to introduce the amine at C3, and reduction of the amides with $BH_3$/THF. Subsequently, all of the free steroidal amines were converted to polyammonium salts by treating with ethereal HCl, and the salts were used in DNA binding studies. Variations on these synthetic procedures were necessary, including protection and deprotection at various sites depending upon the desired target molecule.[13] All new compounds were purified by column chromatography and successfully characterized by $^1H$ and $^{13}C$ NMR, IR, FAB-MS, and elemental analysis.

DNA binding studies were carried out using calf thymus DNA (CT-DNA) as well as poly[d(AT)] and poly[d(GC)]. The experimental method employed was an ethidium displacement assay following previously reported literature.[2,14] The $C_{50}$ value was determined as the concentration of the steroidal polyamine leading to a 50% reduction in the fluorescence intensity of bound ethidium (excitation at 547 nm, emission at 595 nm) under 0.01 SHE buffer conditions (8 mM NaCl, 2 mM HEPES, 0.05 mM EDTA, [ethidium] = 1.26 μM, [DNA base pairs] = 1.31 μM, pH 7, 25 °C). Although $C_{50}$ values cannot be directly translated into binding constants because the mode and stoichiometry of binding by ethidium and by steroidal polyamines is certainly not the same, they nevertheless offer a convenient qualitative means of comparing structural effects of the polyamine on DNA binding. As a point of reference, independent studies estimate a dissociation constant, $K_d$, in the range 0.1−1.0 μM for spermine binding to DNA[15] while studies in our laboratory and others place the $C_{50}$ value at about 1 μM.[2a,14b]

A comparison of $C_{50}$ values for polyamines and DNA is provided in Table 1. For the series putrescine−spermidine−

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**Table 1.** Polyamine Binding to Poly[d(AT)], Calf Thymus DNA, and Poly[d(GC)]

| entry | polyamine | $C_{50}$[a] ($\mu$M) poly[d(AT)] | CT-DNA | poly[d(GC)] |
|---|---|---|---|---|
| 1 | putrescine, $H_3N^+(CH_2)_4NH_3^+$ | | (1700[b]) | |
| 2 | spermidine, $H_3N^+(CH_2)_4NH_2^+(CH_2)_3NH_3^+$ | | (27[b]) | |
| 3 | spermine, $H_3N^+(CH_2)_3NH_2^+(CH_2)_4NH_2^+(CH_2)_3NH_3^+$ | 2.7 (2.8[b]) | 1.0 (1.6,[b] 1.2[c]) | 1.1 (1.2[b]) |
| 4 | 3α,12α-diamine **1** | 280 | 290 | 380 |
| 5 | 3β,12α-diamine **2** | 34 | 29 | 42 |
| 6 | 3α,24-diamine **3** | 8 | 11 | 18 |
| 7 | 3β,24-diamine **4** | 10 | 16 | 24 |
| 8 | 3β,12α,24-triamine **5** | 10 | 14 | 17 |
| 9 | 3β-amine 24-guanidine **6** | 2.5 | 4.7 | 6.0 |
| 10 | 3β,3β′,24,24′-tetraamine **7** | 0.16 | 0.24 | 0.18 |

[a] Polyamine concentration necessary to displace 50% of DNA-bound ethidium under the following conditions: [DNA-bp]$_0$ = 1.31 $\mu$M, [ethidium]$_0$ = 1.26 $\mu$M, 0.01 SHE buffer (8 mM NaCl, 2 mM HEPES, 0.05 mM EDTA, pH 7.0). Values reported are the average of at least three independent experiments and are ±10%. [b] Reference 2. [c] Reference 14b.

spermine, it is seen that the successive addition of an ammonium group on a $C_3-C_4$ chain provides a 30−60-fold increase in DNA binding (i.e., a 30−60-fold decrease in $C_{50}$). The new steroidal polyamines show the following binding characteristics: (i) All of the steroidal diamines show substantially stronger DNA binding than the simple diamine putrescine. This is likely due to a substantial hydrophobic contribution to binding. (ii) Diamines **1** and **2** (entries 4 and 5) differ only in the configuration of the ammonium group at C3, yet their binding abilities differ by an order of magnitude. The same sensitivity to stereochemistry at C3 is not observed when the second ammonium group is placed on the flexible hydrocarbon chain at C24 rather than in a rigidly defined site such as at C12. (Compare entries 6 and 7). (iii) Binding of the steroidal triamine **5** (entry 8) to DNA gives $C_{50}$ values that are nearly identical to those of the two related diamines **2** and **4**. One interpretation is that the triamine binds via only two out of three of the ammonium groups. Alternatively, the expected gain in binding energy of a third ammonium group may be compromised by the loss of a large hydrophobic area when C12 is aminated in addition to C3 and C24. (iv) Conversion of the 24-amino group of diamine **4** to a guanidinium group results in a 4-fold enhancement in binding (compare entries 7 and 9). This is likely due to the greater hydrogen-bonding ability of guanidinium groups as demonstrated in a number of protein−DNA complexes in which arginine residues play a major role in DNA recognition.[16] (v) Tetraamine **7** binds to DNA about 2 orders of magnitude better than does the triamine, and nearly an order of magnitude better than does spermine. This again supports the notion that inclusion of large hydrophobic regions such as a steroid nucleus aids substantially in DNA binding.

Additional insight concerning the role of hydrophobicity was gained from thermal denaturation studies of poly[d(AT)] as a function of added polyamine (Figure 1). Spermidine binding to poly[d(AT)] results in stabilization of duplex DNA observed as an increase in $T_m$. This effect saturates at a [polyamine]:[nucleotide] ratio, $r$, of about 0.5. Diamine **4**, however, initially stabilizes the duplex and then destabilizes it at higher $r$ values. The same behavior has been observed for other steroidal diamines, notably irehdiamine A.[17] The guanidinium-appended steroid **6** displayed this curvature even more markedly in the $T_m$ vs $r$ study. In contrast, the steroidal triamine **5** behaved much more like the simple triamine, spermidine, in $T_m$ studies suggesting that the binding mode of **5** may be more like the biogenic amines. Interruption of the hydrophobic region of the steroid by introduction of the 12α-amino group midway along

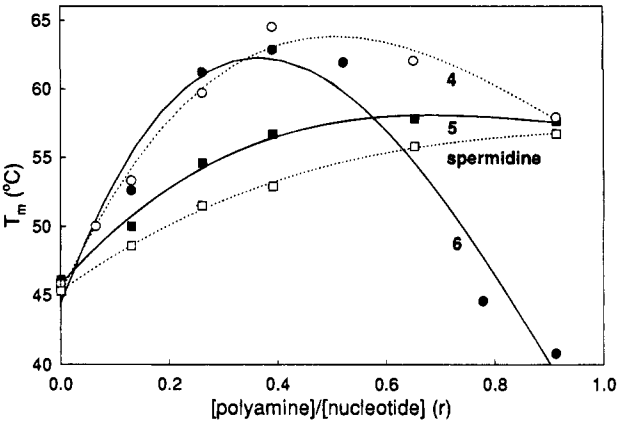


**Figure 1.** Effect of polyamine binding on $T_m$ of poly[d(AT)]. $T_m$ values were measured by monitoring the 260 nm absorbance using 0.025 SHE buffer (23 mM NaCl, 2 mM HEPES, 0.05 mM EDTA, pH 7.0). Error limits are ±1°. Compound **4**, - -○- -; compound **5**, −■−; compound **6**, −●−; spermidine, - -□- -.

the 14-carbon span between C3 and C24 apparently makes **5** resemble spermidine as it binds to DNA. Interestingly, the complementary and opposite effect has already been seen when putrescine binding is compared to that of 1,12-diaminododecane. The chain-lengthened diamine demonstrates higher binding affinity as well as a bell-shaped $T_m$ vs $r$ curve for poly[d(AT)] binding.[17a] Consistent with this analysis, the dications **4** and **6** caused respectively 9% and 17% hyperchromicity of poly[d(AT)] at 260 nm whereas **5** as well as spermine and spermidine showed no change in absorbance.[18] Hyperchromicity is often associated with a disruption in base stacking related to partial intercalation.[4a,17b,c]

In conclusion, this new series of steroidal polyamines demonstrates that DNA binding can be tailored by the stereo- and regiochemistry of appended ammonium groups in addition to the total number of ammoniums and the hydrophobic contribution of the steroid nucleus. At least two different binding modes are observed, one resembling the biogenic amines such as spermidine and the other more characteristic of the natural product steroidal diamines. The bile acid framework offers a convenient entry into such molecules and will now aid in correlation of the structure of steroidal polyamines to their biological activity.

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**Supplementary Material Available:** Synthetic procedures and spectral identification of compounds **1**−**7** (33 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(18) Measured at $r$ = 0.5.