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Synthesis and DNA Binding Properties of C3-, C12-, and C24-Substituted Amino-Steroids Derived from Bile Acids

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Abstract—Seven new amino- and guanidino-substituted steroids have been synthesized from bile acid precursors, either deoxycholic acid or lithocholic acid. Their DNA binding properties have been examined using an ethidium displacement assay, through studies of hyperchromicity and thermal denaturation, and by circular dichroism. Comparison is made to simple aliphatic polyamines such as putrescine, 1,12-diaminododecane, spermidine, and spermine.

Introduction

The biogenic polyamines spermine and spermidine are present in millimolar concentrations in the cell where they serve a variety of roles related primarily to their nucleic acid binding abilities.[3] Polyammonium compounds make use of both hydrogen bonding as well as favorable electrostatic interactions between their polycations and the polyanionic sugar-phosphate backbone of DNA or RNA. The 3–4 carbon spacing between ammonium groups in spermine and spermidine permits nearly full protonation at pH 7 and provides a relatively high positive charge density in these small molecules.[4] As the length of the carbon chain between ammonium groups increases in aliphatic polyamines, hydrophobic contributions to binding begin to play a significant role.[5] Curiously, nature has taken advantage of this design in the form of steroidal diamine natural products. Figure 1 shows five members of a small class of steroidal diamines isolated from plant sources.[6] It is interesting to note that these compounds, all with antibiotic activity, share common structural features—one of the ammonium groups is in the 3β position and the other is typically appended at C20 although it is directly bonded to C17 in the case of dipyrandium. The ammonium groups in these natural products come in all types: primary, secondary, tertiary and quaternary. Primary ammonium groups provide more hydrogen bonding opportunities with their receptor targets while quaternary ammoniums are advantageous in providing a permanent positive charge that is not subject to fluctuations in pH. In small molecule studies, there are conflicting results about the magnitude of the effect of peralkylation of ammonium groups on nucleic acid binding ability, although generally peralkylation diminishes the strength of DNA binding.[5,7] Since it is thought that DNA is the likely target of the steroidal diamine natural products, it is of interest to study a family of structurally related, simple analogues for their DNA binding ability.[8]

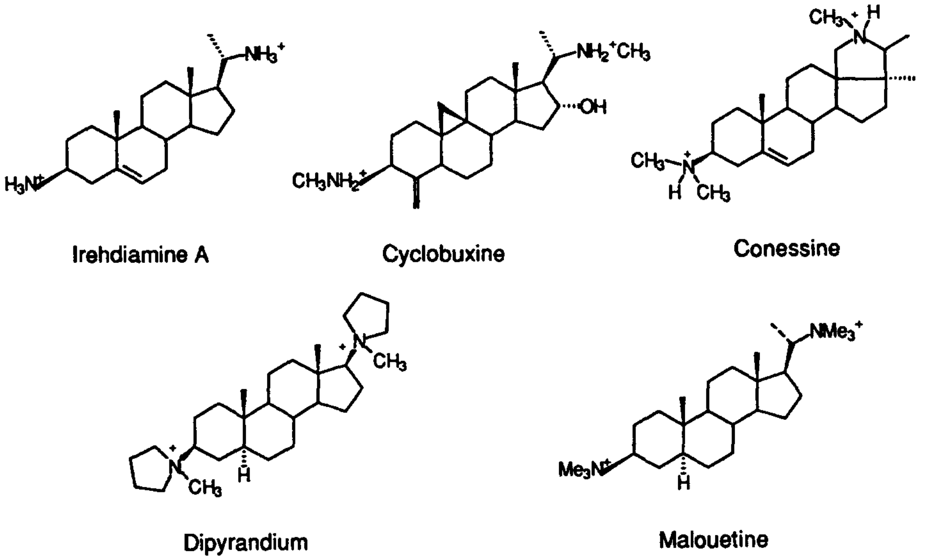


Figure 1. Naturally occurring steroidal diamines.

Bile acids are a ready source of highly functionalized steroids that allow conversion of hydroxyl groups at C3, C7, and C12 as well as the carboxylate group at C24 to ammonium ions. Thus, we began a course of study in which the number of ammonium groups, their point of attachment to the steroid and their stereochemistry could be varied in a systematic way to probe the DNA-binding ability of polyaminosteroids.[9] The common bile acids, cholic acid and its analogues, differ from the steroidal diamine natural products in possessing a *cis*-A,B ring fusion, the 5β-cholane structure. Although one study[8a] shows that this stereochemistry reduces the DNA binding ability, these compounds were nevertheless a very attractive starting point. Thus, the compounds reported in this first series are derived from either lithocholic acid or deoxycholic acid, and include diamines **1**–**4**, triamine **5**, amino-guanidine **6**, and a tetraamine, **7**.

Results and Discussion

Synthesis

Aminosteroids **1**–**6** were prepared by first reducing the appropriate bile acid to the C24 alcohol followed by conversion of the hydroxyl groups to amines via either azide formation or oxime formation. The routes chosen to each target compound were based on the differential reactivity of the sites due to steric encumbrance: C24>C3>C12. The synthesis of tetraamine **7**, a steroid dimer, began with amide coupling of two lithocholic acid molecules with putrescine followed by introduction of the C3 ammonium groups. All compounds were ultimately converted to hydrochloride salts for use in DNA binding studies.

The synthesis of diamine **1**, outlined in Scheme 1, began with triol **12** obtained by reduction of deoxycholic acid.[10] The C24 hydroxyl group is the most reactive toward silylating agents, and thus the *tert*-butyldiphenylsilyl ether at C24 alone (**13**) can be prepared in 90% yield.[11] Davis and Orchard have reported a method of introduction of nitrogen substituents at C12 of the 5β-cholane framework through use of carefully optimized conditions of NaN_3/HMPA, PPh_3 and $(CF_3CO)_2O$ leading to the 12β-trifluoroacetamide.[12] In our hands, diol **13** failed to give the 3β,12β-diamine after a 13-day reaction with NaN_3 in HMPA followed by Staudinger reduction.[13] We turned instead to methods involving the 3,12-diketone as an intermediate. Oxidation of **13** with pyridinium dichromate (PDC) afforded the 3,12-diketo-steroid **14** in 89% isolated yield. The TBDPS protecting group was then removed with $n\text{-}Bu_4N^+F^-$ to provide corresponding alcohol **15** in 89% yield. Attempts to convert this 3,12-diketone directly to a diamine via reductive amination (NH_4OAc, $NaCNBH_3$)[14] gave unacceptably low yields, and so an alternative route was found in formation of the 3,12-dioxime **16**, prepared with hydroxylamine hydrochloride as a precipitate in 90% yield. Dioxime **16** was then reduced with sodium/*n*-propanol, and the resulting diamine was chromatographically purified. After conversion to the HCl salt, the 3α,12α-diammonium compound **1** was obtained in 62% yield from **16**. NMR analysis was consistent with the assignment of the α configuration at both C3 and C12.

Scheme 2 shows the synthetic route to **2**, the 3β,12α-epimer of **1**. In this case, the C3 and C12 hydroxyl groups had to be manipulated separately. One can take advantage of the higher reactivity of the C3 position (indeed, C12 is neopentyl-like and very inert!) in a Mitsunobu type reaction of azide. In this case, use of the bis-pyridine adduct of zinc azide,[15] PPh_3, and diethylazodicarboxylate (DEAD) led to smooth inversion of configuration at C3 to yield the mono-azide **17** in 87% yield. Oxidation at C12 to give azido-ketone

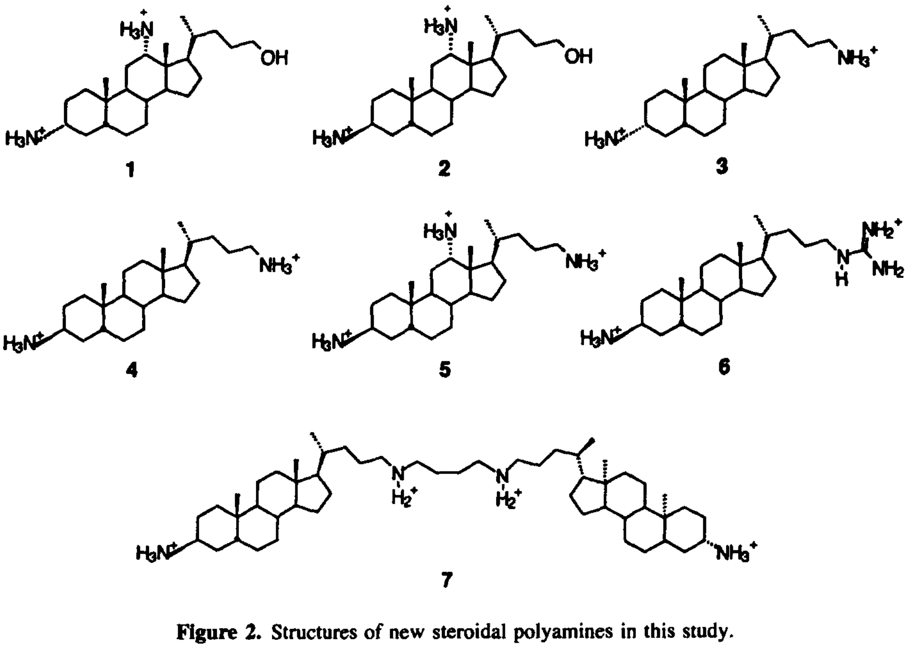
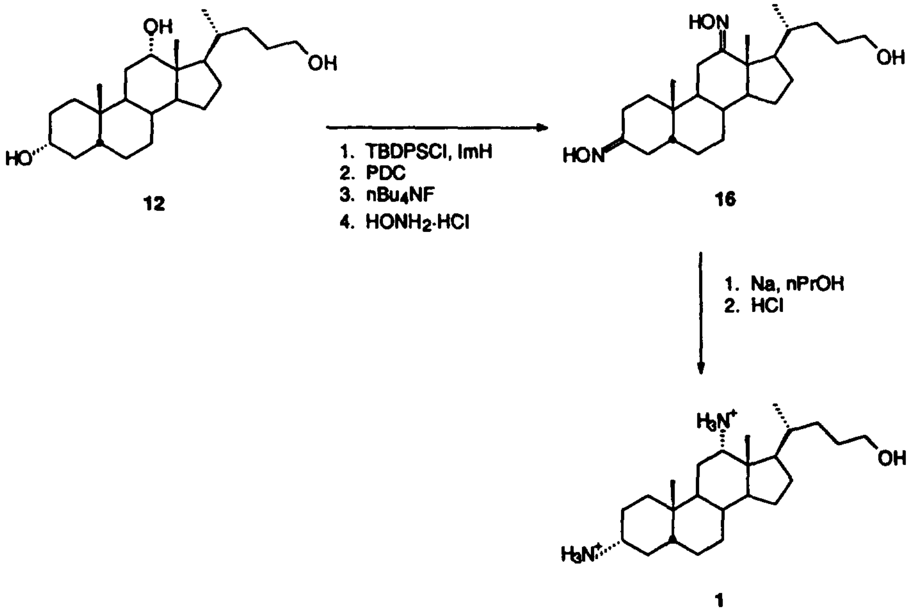


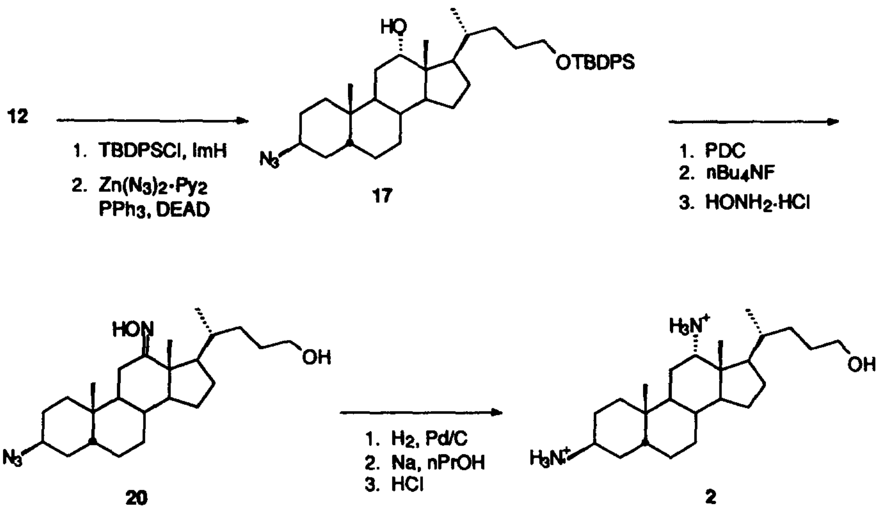
Figure 2. Structures of new steroidal polyamines in this study.



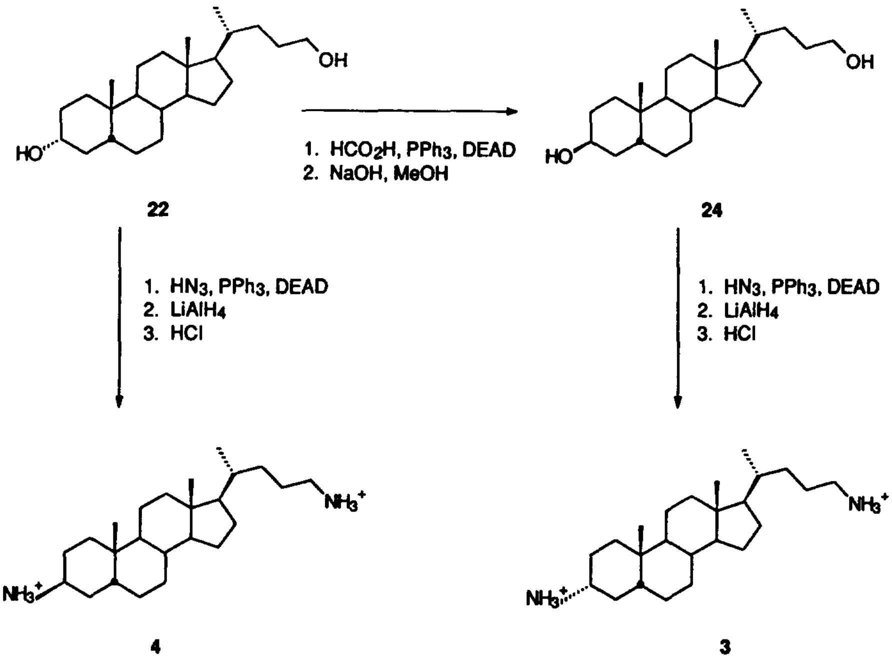
Scheme 1.

18 was accomplished as before (PDC, 90% yield), removal of the silyl protecting group with *n*-$Bu_4N^+F^-$ provided the free C24 alcohol **19** (84%), and the oxime could be prepared at C12 (compound **20**) in 88% yield. Attempts to reduce both the azido group and the oxime in one step using either $LiAlH_4$ or Na/*n*PrOH failed to completely reduce both groups at the same time. In a two-step reduction, the azido group of **20** was converted to the corresponding amine (**21**) by catalytic hydrogenation (83%), and the oxime was reduced by reaction with Na/*n*PrOH. After chromatographic purification and treatment with HCl, the diammonium compound **2** was obtained (55% yield from **21**). Again the stereochemical assignment of the 12α-amino group was consistent both with the NMR data as well as literature reports on related compounds.[16]

Parallel routes were followed to prepare the two C3 epimers of 5β-cholane-3,24-diamine, **3** and **4**, as shown in Scheme 3. Lithocholic acid was the starting material for these target molecules, and it was first reduced to the C24 alcohol, **22**, following a procedure analogous to that for preparation of **12**. While substitution reactions are difficult at C12, they are straightforward at C3 and C24. Thus, Mitsunobu reactions were employed simultaneously at these centers. In order to prepare the 3α-epimer, **3**, the configuration at C3 needed to be inverted. This was accomplished by conversion to the corresponding diformate ester **23** followed by hydroxide basic hydrolysis giving the 3β,24-diol **24** in 75% yield. Compounds **3** and **4** were then prepared from **24** and **22** respectively by treatment with hydrazoic acid, PPh_3, and DEAD to give the corresponding diazides, **25** and **26**. These underwent $LiAlH_4$ reduction providing the diamines **27** and **28** which subsequently could be converted to the HCl salts **3** and **4**.



Scheme 2.

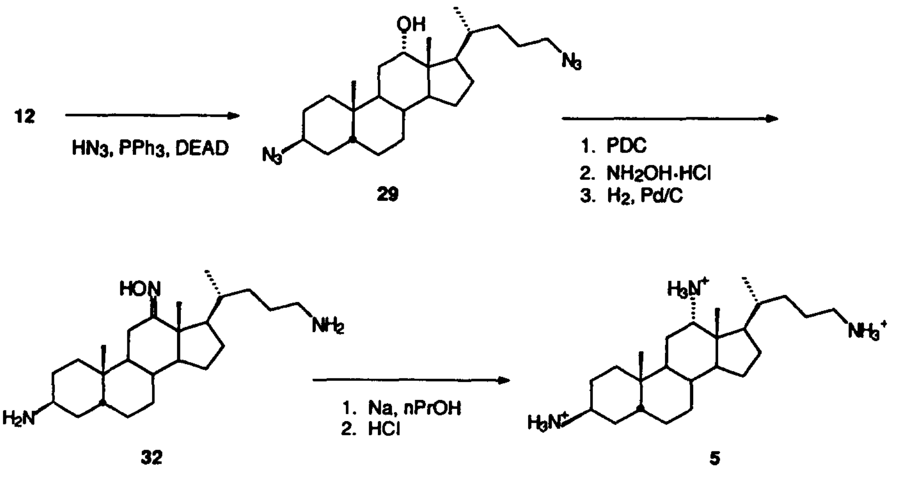


Scheme 3.

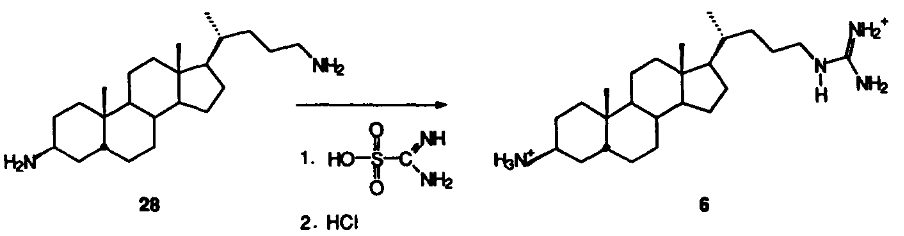
The knowledge gained from the successful syntheses of the diamines 1–4 enabled us to devise a solid strategy for the preparation of the steroidal triamine **5** as shown in Scheme 4. When triol **12** was subjected to the Mitsunobu reaction using hydrazoic acid, only the hydroxyl groups at C3 and C24 underwent substitution to give diazide **29** in 72% yield. Oxidation at C12 with PDC (**30**, 83%), conversion to the hydroxylamine (**31**, 88%), and catalytic hydrogenation provided the diamino-oxime **32** in 81% yield. Finally, reduction with Na/*n*PrOH and conversion to the HCl salt gave the triammonium compound **5** in 61% yield.

In order to compare the efficacy of guanidinium groups versus ammonium groups in DNA binding, compound **6** was prepared in 81% yield by reaction of the free diamine **28** with aminoiminomethanesulfonic acid (AIMSA)[17] as shown in Scheme 5. It is interesting to note that even under forcing conditions, the 3-amino substituent was unreactive with AIMSA as well as other common guanylating agents.[18] Thus, we were unable to prepare a 3,24-diguanidinosteroid for comparison to the mono-amino, mono-guanidino steroid.

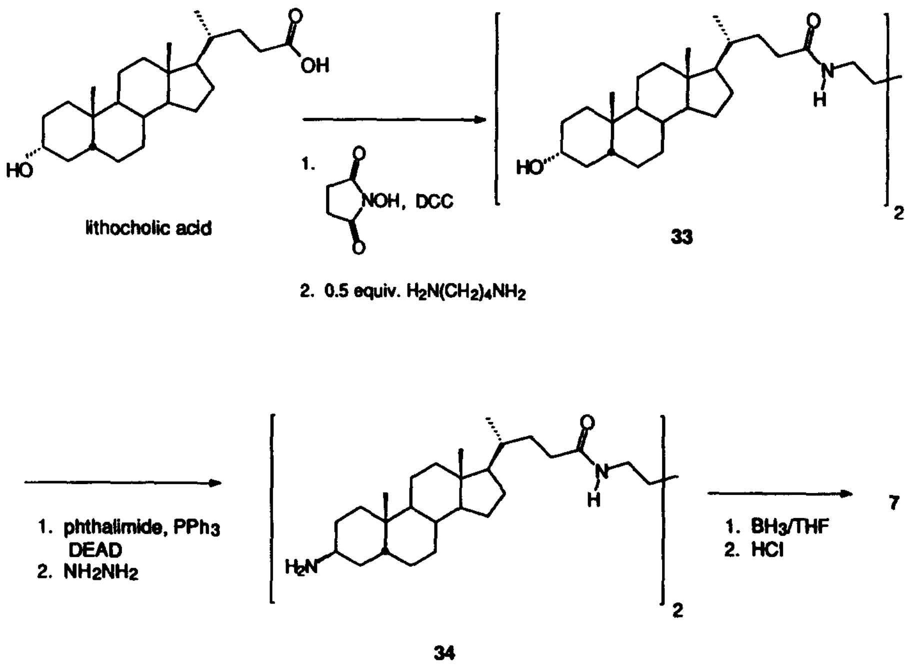
Finally, we sought a steroidal tetraamine that could be easily prepared in order to compare the role of hydrophobicity in such a compound to the naturally occurring tetraamine spermine. Our laboratory[19] and others[20] have prepared steroid dimers and higher oligomers for molecular recognition studies, and a certain amount of success has been gained using amide coupling techniques. Thus, tetraamine **7** (Scheme 6) was obtained by first coupling 2 equiv. lithocholic acid with one equiv. 1,4-diaminobutane (putrescine) via the *N*-hydroxysuccinimide ester to give diamide **33** in 77% yield. The 3β-amino group was introduced using again a



Scheme 4.



Scheme 5.



Scheme 6.

Mitsunobu-type reaction employing phthalimide as the nucleophile, since the low solubility of the diamide precluded more typical procedures. Subsequent treatment with hydrazine provided the 3β,3β'-diamine **34** (44%). A BH_3/THF reduction and HCl treatment then led to formation of the tetraammonium steroid dimer **7** in 60% yield.

Ethidium displacement studies

The lack of a chromophore in steroidal diamines eliminates direct determination of DNA binding constants by equilibrium dialysis and spectrophotometric analysis. One method commonly employed to study the nucleic acid binding behavior of polyammonium compounds involves a fluorescence assay in which the displacement of ethidium bound to DNA is monitored.[21] Such a study leads to determination of C_{50} values which are defined as the concentration of polyamine leading to a 50% reduction in the fluorescence intensity of DNA saturated with ethidium. C_{50} values do not directly give a binding constant because the mode and stoichiometry of binding of ethidium, an aromatic intercalator, and aliphatic steroidal polyamines are certainly different. However, this study does offer qualitative comparisons of DNA binding ability. Studies were conducted under 0.01 SHE buffer conditions (8 mM NaCl, 2 mM HEPES, 0.05 mM EDTA) at pH 7, 25 °C, and the C_{50} values so obtained for steroidal polyamines **1**–**7** along with those of some simple aliphatic polyamines are listed in Table 1.

In the series of simple polyamines, putrescine, spermidine, and spermine, the successive addition of an ammonium group at the end of a 3–4 carbon chain has the effect of increasing the DNA affinity (i.e. lowering the C_{50}) of the polyamine by a factor of 30–60. For a series of linear aliphatic compounds ranging from diamines to hexaamines, Stewart and Gray found a roughly linear correlation between the total charge on the molecule at pH 7 and the log of the C_{50} value.[5b] On the other hand, hydrophobicity also plays a major role in DNA binding as seen for 1,12-diaminododecane, **9**, which shows an unusually low C_{50} value[5b] (compare entries 8 and 9, Table 1). Both of these effects, total positive charge and hydrophobicity, are important in steroidal polyamine binding, but surprisingly, stereochemistry is sometimes a key factor. For example, the 3-amino epimers **1** and **2** (Table 1, entries 1 and 2) differ in C_{50} values by an order of magnitude. On the other hand, this effect is not observed for epimers **3** and **4** which show essentially identical binding to DNA. The fact that the 3,24-diamines

Table 1. Polyamine binding to poly[d(AT)], calf thymus DNA, and poly[d(GC)]

		C_{50}[a] (μM)			T_m/r[b]	% H[c]
Entry	polyamine	poly[d(AT)]	CT-DNA	poly[d(GC)]	poly[d(AT)]	poly[d(AT)]
1	3α,12α-diamine (**1**)	280	290	380	3	0
2	3β,12α-diamine (**2**)	34	29	42	10	0
3	3α,24-diamine (**3**)	8	11	18	45	7
4	3β,24-diamine (**4**)	10	16	24	48	9
5	3β,12α,24-triamine (**5**)	10	14	17	28	0
6	3β-amine,24-guanidine (**6**)	2.5	4.7	6.0	59	17
7	3β,3β',24,24'-tetraamine (**7**)	0.16	0.24	0.18	52[d]	—
8	putrescine (**8**) $H_3N^+(CH_2)_4NH_3^+$		(1700[e])		4	0
9	1,12-diaminododecane (**9**) $H_3N^+(CH_2)_{12}NH_3^+$		(300[f])		17	0
10	spermidine (**10**) $H_3N^+(CH_2)_4NH_2^+(CH_2)_3NH_3^+$		(27[e])		20	0
11	spermine (**11**) $H_3N^+(CH_2)_3NH_2^+(CH_2)_4NH_2^+(CH_2)_3NH_3^+$	2.7 (2.8[e])	1.0 (1.6[e], 1.2[f])	1.1 (1.2[e])	65	0

[a] Polyamine concentration necessary to displace 50% of DNA-bound ethidium. Values in parentheses were determined in other laboratories. [b] Slope of a linear plot of T_m vs r, $r = 0$ to 0.3. [c] % hyperchromicity. See experimental section for details. [d] Determined for $r = 0$ to 0.15 because of the low solubility of **7**. [e] Reference 5. [f] Reference 7.

3 and **4** bind more strongly than the 3,12-diamines **1** and **2** again points to the role of hydrophobicity. Surprisingly, triamine **5** (Table 1, entry 5) binds no more strongly than any of the diamines. In this case, it appears that the DNA affinity that might be gained from introduction of the third ammonium group is compensated by a loss in hydrophobicity when an ammonium group at C12 interrupts the hydrophobic steroid skeleton. Substitution of an ammonium group for a guanidinium group (compound **4** versus **6**) results in an approximately 4-fold increase in DNA affinity. The strongest binding steroidal polyamine of the series is **7**, a structure that maintains large hydrophobic regions as well as carrying a +4 charge.

Another feature observed in steroidal polyamine–DNA binding is a consistent trend showing a preference (two to three-fold in most cases) for AT versus GC sequences. No particular sequence specificity was anticipated, although this trend would be consistent with minor groove binding since AT tracts are narrower and typically exhibit stronger hydrophobic contacts with bound drugs than GC repeat sequences.[22] A similar preference has been observed for the natural products irehdiamine A[23] and cyclobuxine[24] as well as other androstane derivatives.[8c]

Thermal denaturation

In order to gain further insight into the mode of DNA binding, studies of thermal denaturation of poly[d(AT)] were performed in the presence of steroids **1–7**, and the effects were compared to those of the simple polyamines **8–11**. Poly[d(AT)] was chosen because the temperature at which it undergoes a helix to coil transition (T_m) is conveniently studied at an ionic strength close to that used in the determination of C_{50} values. That is, a T_m of 46 °C is observed in 0.025 SHE buffer conditions in the absence of added polyamine. Either stabilization (an increase in T_m) or destabilization (a decrease in T_m) of the helical duplex can then be observed in the presence of polycations. Incremental addition of polyamines **1–11** led to an initial stabilization of the duplex, seen as an increase in the T_m, for all compounds, although the magnitude of the effect varied substantially. Some of the results are shown in Figure 3 in which the T_m is plotted as a function of the ratio of polyamine:nucleotide concentrations, r. Results for all compounds are listed in Table 1 as T_m/r values which correspond to the amount of helix stabilization per aliquot of polyamine for low values of r (0–0.3); essentially this corresponds to the initial slopes of the curves in Figure 3. The trends in the T_m/r values generally follow those of the C_{50}'s with one notable exception; steroidal triamine **5** shows less of a T_m/r effect than its diamine analogue **4** (compare entries 4 and 5 in Table 1) and indeed is more similar to spermidine, whereas the C_{50} values of **4** and **5** are identical.

Additional insight is gained from examination of a more extensive range of r values up to a [polyamine]:[nucleotide] ratio of 0.9. The simple polyamines such as putrescine **8**, and spermidine **10**, approach a maximum value of T_m and level off with increasing r. Steroidal triamine **5** behaves similarly. On the other hand, steroids that appear to bind strongly, such as **4** and **6** initially stabilize and then destabilize the helical duplex. This may point to an alternative binding mode for the hydrophobic polycations. The destabilization effect is very pronounced for the guanidinium-containing steroid **6**, clearly observable for the diamine **4**, but not very evident for the hydrophobic linear dication **9**.[25] Interestingly, the bell-shaped T_m versus r curve is also observed for many of the natural product steroidal diamines.[6a,23,24,26] Unfortunately, we were unable to complete the study of tetraamine **7** due to its low water solubility.

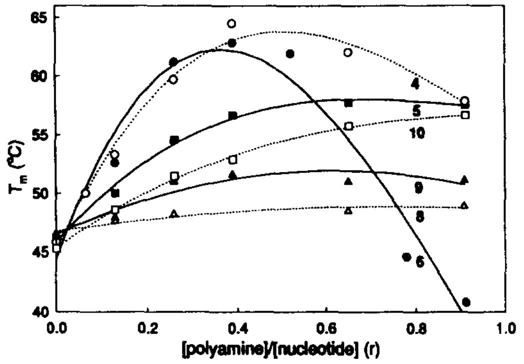


Figure 3. Effect of polyamine binding on the T_m of poly[d(AT)]. Compound 4 –O–; compound 5 -■-; compound 6 -●- ; compound 8 -Δ–; compound 9 -▲-; compound 10 –□–.

Hyperchromicity

The absorbance of poly[d(AT)] at 260 nm was also monitored as a function of added polyamine ($r = 0.5$) to evaluate the possibility of interruptions in base stacking manifested as an increase in absorbance or hyperchromicity. None of the linear aliphatic polyamines and few of the steroidal polyamines had any effect (Table 1). However, those compounds displaying bell-shaped behavior in the T_m versus r plots did lead to hyperchromicity, and again the steroidal guanidinium compound **6** showed the most dramatic effect (Table 1, entry 6). Hyperchromicity is often associated with phenomena such as intercalation which disrupts the normal base stacking interactions.[26]

Circular dichroism

In order to clarify the DNA binding interactions observed by the steroidal polyamines circular dichroism (CD) studies were conducted with poly[d(AT)]. In Figure 4, changes in the CD spectrum of poly[d(AT)] at various concentrations of compound **6** is depicted. For the steroidal diamines **4** and **6**, enhancement in the intensity of the positive band centered at 267 nm ($[\Theta]_{267}$) in conjunction with a slight red shift of this band to 272 nm was observed at low r values. In contrast, at high r values ($r > 0.4$ for **4** and $r > 0.2$ for **6**) a decrease in the $[\Theta]_{267}$ and $[\Theta]_{250}$ bands was seen. A complete titration of the poly[d(AT)] with increasing concentrations of compounds **4** or **6** revealed a biphasic effect on the $[\Theta]_{272}$ CD band (Fig. 5) analogous to the biphasic dependence of T_m measurements on steroid concentration (Fig. 3). The observed CD spectral changes are similar to results obtained for the steroids irehdiamine A and 3β,17β-bis(dimethylamino)-(5α)-androstane.[6a,27] The increase in the positive CD band has been previously attributed to conformational changes in the DNA helix resulting from electrostatic, H-bonding, and hydrophobic interactions with the steroidal polyamine.[8c,27] Furthermore, the decrease in both the positive and the negative CD band upon addition of high concentrations of steroid was suggested to be a result of either an extensive destabilization of the DNA helical structure via H-bonding interactions with random coils or formation of steroidal micelles on the helix surface.[8c,27]

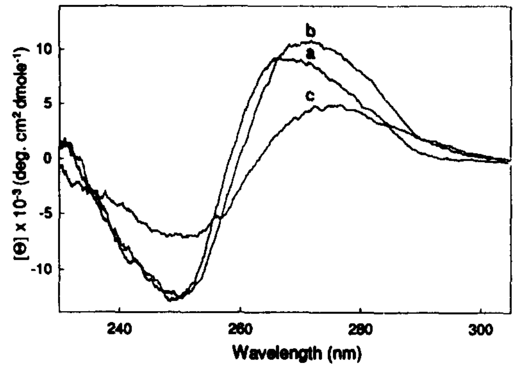


Figure 4. CD spectra of poly[d(AT)] in the presence of different concentrations of compound **6**. (a) [polyamine]/[nucleotide] (r) = 0; (b) $r = 0.22$, (c) $r = 0.88$.

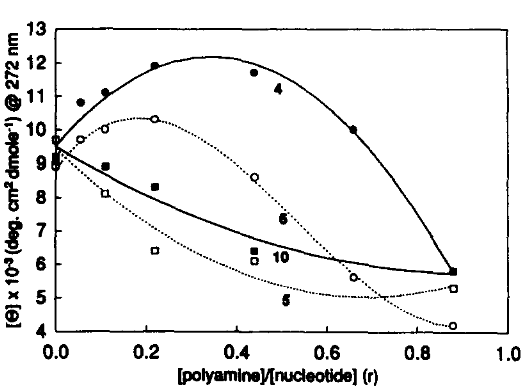
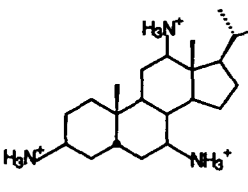


Figure 5. Effect of polyamine binding on the circular dichroism of poly[d(AT)]. Compound 4 -●-; compound 5 -□–; compound 6 -O–; compound 10 -■-.

In sharp contrast to results obtained for **4** and **6**, the steroidal triamine **5** induces only a decrease in the $[\Theta]_{267}$ band of poly[d(AT)] with saturation occurring at high r values (Fig. 5). This result is atypical for steroidal polyamines but it appears to be similar to the CD spectral changes induced by spermidine, **10** (Fig. 5). The decrease in the $[\Theta]_{267}$ band cannot be attributed to destabilization of the DNA helix since T_m measurements (Fig. 3) indicate a stabilization of the DNA upon addition of **5**. Additionally, no hyperchromicity of poly[d(AT)] was observed (Table 1), which would be expected if destabilization of the helical structure was occurring. Such CD spectral changes are consistent with an increase in the DNA winding angle resulting in a transformation from a B DNA structure towards a C-like conformation.[28] The effect of **5** on DNA is similar to those previously observed for monovalent cations and linear aliphatic polyamines.[29]

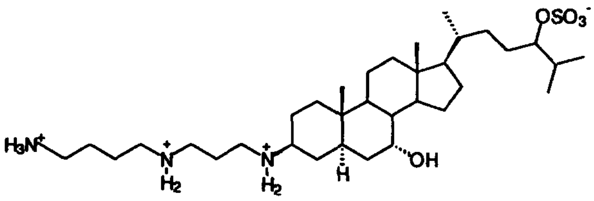
Conclusions

Bile acids such as lithocholic and deoxycholic acids provide a convenient framework for the construction of new aminosteroids. High-yielding reactions were found for the conversion of the α-hydroxyls to either β-amino groups via Mitsunobu reactions or to α-amino groups via reduction of an oxime. In DNA binding studies, four structural features appear to affect the strength and type of DNA interaction: stereochemistry of the appended ammonium group, type of cation (ammonium versus guanidinium), total charge on the molecule, and the presence of large hydrophobic regions. In one example, the 3,12-diamines **1** and **2**, the relative stereochemistry at C3 accounted for a 10-fold difference in DNA binding ability as measured by C_{50} values, while in another example (**3** versus **4**), the two epimers displayed very similar DNA binding behavior according to C_{50}, T_m and hyperchromicity studies. Hence, little can be predicted regarding stereochemistry at this point. The comparison of diamine **4** with amino-guanidine **6** showed a consistent preference in all studies for the guanidinium group as a DNA binding unit. This is perhaps not surprising considering the frequency with which nature chooses arginine residues as binding groups in DNA–protein complexes. On the other hand, the fact that triamine **5** shows no improvement in DNA binding over diamines **3** and **4** was unexpected, and points to the role of hydrophobicity. Most of the strongly binding steroids, **3**, **4**, **6** and **7**, retain a large hydrophobic steroid group with ionic appendages at both extremes, C3 and C24. Tetraamine **7** shows higher DNA affinity than spermine, which must be due to a hydrophobic component of binding. However, it should be noted that hydrophobic binding might be primarily due to low water solubility rather than to any particular attraction to the DNA helix. Indeed, low water solubility prevented a thorough study of compound **7**. Taken together, these results suggest that charge alone is not the major determinant of DNA binding for steroidal polyamines. For example, we would predict that a steroidal tetraamine derived from a single cholic acid unit such as **35** would exhibit considerably weaker binding than the hydrophobic tetraamine **7** and might more resemble spermine in its properties.



35

How do these synthetic steroidal polyamines compare to their natural product counterparts? Unfortunately, no C_{50} measurements have been reported for the natural products, but a wealth of other information exists in the form of T_m, CD, hyperchromicity, and NMR studies. In general, the synthetic steroids bearing only C3 and C24 cations most closely resembled the natural product diamines, many of which show hyperchromicity, and biphasic behavior in both T_m and CD studies. The biphasic behavior is suggestive of an initial stabilization of the helix via a conformational change followed by denaturation. While the exact nature of the DNA conformational changes induced by **4** and **6** (at low steroid concentrations) is not known, it is interesting to note that the observed increase in the positive CD band and the increase in T_m values are reminiscent of the results obtained for DNA intercalators, most notably ethidium bromide.[30] Since steroids are thick, non-planar molecules, it seems unlikely that **4** and **6** interact with DNA through an intercalative process as we understand it for ethidium; however, they may induce the unwinding and unstacking of DNA by formation of kinks in the helical structure. Sobell *et al.* have proposed that irehdiamine A interacts with DNA by the partial insertion of the ligand between base pairs inducing a kink in the DNA helix.[31] The DNA kink is believed to be stabilized by hydrophobic interactions between the steroid and the nucleobases. Subsequent ^1H NMR,[26] hydrodynamic and electric dichroism,[32] topological,[33] and molecular modeling studies[34] have supported this formation of DNA kinks by the naturally occurring steroidal diamines. The data presented here, particularly with respect to compounds **4** and **6**, suggest that some of the synthetic steroidal polyamines behave similarly.



36

Overall, the DNA binding data of the new steroidal polyamines indicate that these molecules fall into two general classes, those that behave as hydrophobic polycations and give biphasic behavior in binding studies (e.g. compounds **4** and **6**), and those that act similarly to spermine and spermidine (e.g. compound **5**). In this context, it would be particularly interesting to learn about the DNA binding behavior of steroids with single polyamine appendages rather than compounds with monoamines substituted at multiple sites. The recently isolated natural product squalamine (**36**)[35] which bears both a large hydrophobic steroid framework as well as a C3-appended spermidine group represents an intriguing example of such a molecule.

Experimental

Materials and instrumentation

Solvents were ACS reagent grade or HPLC grade from Fisher Scientific (Springfield, NJ) and were used as received. Organic chemicals and common reagents were purchased from either Aldrich Chemical Company (Milwaukee, WI) or Sigma Chemical Company (St Louis, MO), and were used as obtained without further purification unless noted. The deuterated solvents $CDCl_3$ (99.8 atom%), CD_3OD (99.5 atom%), DMSO-d_6 (99.9 atom%) and D_2O (99.8 atom%) were obtained from Cambridge Isotope Laboratory (Woburn, MA).

Calf thymus DNA Type I and poly[d(GC)] were purchased from Sigma; poly[d(AT)] was purchased from Pharmacia. All reactions were conducted under an atmosphere of dry nitrogen.

Melting points were measured with a Thomas-Hoover apparatus and are uncorrected. Infrared (IR) spectra were recorded using a Perkin–Elmer 1600 Series FT spectrophotometer with samples run as neat, liquid solutions in methylene chloride, or KBr disks. Absorption maxima are designated in cm^{-1}. Ultraviolet/visible (UV) spectra were obtained using a Perkin–Elmer Lambda 5 spectrometer. Fluorescence spectra were measured on a Perkin–Elmer LS-5B Luminescence spectrometer. Circular dichroism spectra were recorded on a Jasco-20A spectropolarimeter at room temperature. Positive ion fast atom bombardment (FAB) spectra were acquired using 3-nitrobenzyl alcohol as the matrix solvent on a Kratos MS 890/DS 90 mass spectrometer system using xenon atoms as ion source at 7 kV. The mass range was calibrated with cesium iodide in the positive ion mode. Elemental analyses were performed by Atlantic Microlab (Norcross, GA). NMR spectra were obtained on General Electric QE-300 (^{1}H-NMR, 300.15 MHz) (^{13}C NMR, 75.48 MHz) spectrometer. All NMR samples were prepared in $CDCl_3$ or CD_3OD. Chemical shifts (δ) are expressed as parts per million (ppm) relative to $\delta(CD_3Cl) = 7.26$ ppm or $\delta(CD_3OD) = 3.30$ ppm for ^{1}H NMR or $\delta(CD_3Cl) = 77.00$ ppm or $\delta(CD_3OD) = 49.00$ ppm for ^{13}C NMR. When peak multiplicities are reported, the following abbreviations are used: *s*, singlet; *d*, doublet; *t*, triplet; *m*, multiplet; *br*, broadened.

24-(3α,12α-Dihydroxy-5β-cholanyl) t-butyldiphenylsilyl *ether (TBDPS) (13).* Compound (**13**) was prepared by Sharts' method.[11] A solution of 3α,12α-dihydroxy-5β-cholan-24-ol (**12**)[10] (0.337g, 0.89 mmol) and imidazole (0.136g, 2 mmol) were dissolved in 4.5 mL of DMF and 0.30 mL (0.32g, 1.16 mmol) of TBDPSCl were added dropwise. After stirring at room temperature for 2 h, the mixture was diluted with diethyl ether (40 mL) and poured into 40 mL of 1 N HCl. The organic layer was separated and the aqueous layer extracted with diethyl ether (2 × 25 mL). The ether layers were combined and washed with 1 N HCl (1 × 75 mL), water (2 × 75 mL) and brine (2 × 75 mL). The organic layer was dried ($MgSO_4$), filtered, and evaporated under reduced pressure to provide a yellow oil. Flash chromatography of the residue over silica gel using ethyl acetate:hexane (1:2) as eluent furnished the silyl ether (**13**) 0.49 g (90%) as a pure white solid. Mp 68–70 °C; lit. 69–70 °C; ^{1}H NMR ($CDCl_3$): δ 7.65 (4H, *m*, ArH), δ 7.39 (6H, *m*, ArH), δ 3.98 (1H, C_{12}-H), δ 3.62 (3H, *m*, C_3-H + C_{24}-H_2), δ 1.04 (9H, *s*, *t*-butyl), δ 0.92 (6H, *t*, C_{21}-H_3 + C_{19}-H_3), δ 0.66 (3H, *s*, C_{18}-H_3); ^{13}C NMR ($CDCl_3$): δ 135.54, 134.14, 129.45 and 127.52 (aromatic carbon), δ 73.24 (C_{12}), δ 71.80 (C_3), δ 64.40 (C_{24}).

24-(3,12-Diketo-5β-cholanyl) TBDPS ether (14). Diol (13) (4.94 g, 8 mmol) was dissolved in anhydrous CH_2Cl_2 (80 mL). Powdered 4 Å molecular sieves (1.2 g) and pyridinium dichromate (18.06 g, 48 mmol) were then added and the mixture was stirred at room temperature overnight. The mixture was diluted with anhydrous diethyl ether (80 mL), and filtered. After removal of solvent under reduced pressure, purification was accomplished by the use of flash column chromatography using ethyl acetate:hexane (1:5) as eluent to yield the diketone (**14**) as a colorless solid (4.35 g, 89%). Mp 58–60 °C; Anal. calcd for $C_{40}H_{56}O_3Si$: C, 78.38; H, 9.21. Found: C, 78.15; H, 9.28; MS (FAB, *m*-nitrobenzyl alcohol): m/z 613 (M^+ + H), 555, 535, 339. IR (KBr) υ_{max} 3070, 3048, 2930, 2859, 1707, 1472, 1460, 1446, 1427, 1384, 1111, 1025, 1008, 823 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.65 (4H, *m*, ArH), δ 7.40 (6H, *m*, ArH), δ 3.63 (2H, *t*, *J* = 6.3 Hz, C_{24}-H_2), δ 1.04 (9H, *s*, *t*-butyl), δ 0.82 (3H, *d*, *J* = 6.3 Hz, C_{21}-H_3); ^{13}C NMR ($CDCl_3$): δ 213.97, 211.82 (C_3 and C_{12}), δ 135.45, 134.06, 129.36 and 127.45 (aromatic carbon), δ 64.28 (C_{24}).

3,12-Diketo-5β-cholan-24-ol (15). To silyl ether (**14**) (4.67g, 7.62 mmol) in anhydrous THF (100 mL) was added Bu_4NF (1.0 M in THF; 15.24 mmol, 15.24 mL). After 18 h, the mixture was poured into H_2O (100 mL) and extracted with Et_2O (3 × 100 mL). The combined organic layers were washed with brine (100 mL), dried ($MgSO_4$), and evaporated. The residue was chromatographed on silica gel (1:2, ethyl acetate:chloroform) to afford the alcohol (**15**) (2.53g, 89%). Mp 113–114 °C; Anal. calcd for $C_{24}H_{38}O_3$: C, 76.96; H, 10.23. Found: C, 76.88; H, 10.27; MS (EI): m/z 374 (M^+), 341, 287, 260, 247; IR (KBr) υ_{max} 3396, 2942, 2865, 1702, 1459, 1446, 1383, 1268, 1024 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 3.57 (2H, *t*, *J* = 6.3 Hz, C_{24}-H_2), δ 1.05 (6H, *d*, C_{18}-H_3 + C_{19}-H_3)), δ 0.82 (3H, *d*, *J* = 6.3 Hz, C_{21}-H_3); ^{13}C NMR ($CDCl_3$): δ 214.10, 211.99 (C_3 and C_{12}), δ 63.25 (C_{24}).

3,12-Dioxime-5β-cholan-24-ol (16). A solution of the diketone (**15**) (2.32 g, 6.2 mmol), $NH_2OH{\cdot}HCl$ (1.25 g, 17.98 mmol) and sodium acetate (2.44 g, 29.76 mmol) in 70 mL of 95% ethanol was heated to reflux for 24 h. The ethanol was removed *in vacuo*, rendered sufficiently acidic with 1 N HCl to dissolve all solid material, and made alkaline with aqueous sodium carbonate. The precipitate that formed was collected by filtration and washed with water: the mother liquor and washings were extracted with chloroform (3 × 100 mL), and the solvent was removed *in vacuo*. The combined crops (2.25 g, 90%) were suitable without further purification. Mp 151–152 °C; Anal. calcd for $C_{24}H_{40}N_2O_3{\cdot}H_2O$: C, 68.21; H, 10.02; N, 6.63. Found: C, 67.98; H, 9.67; N, 6.41; MS (FAB, *m*-nitrobenzyl alcohol): m/z 405 (M^+ + H), 387, 369; IR (KBr) υ_{max} 3329, 2930, 2865, 1654, 1447, 1383, 1277, 1054, 1004, 985, 938, 920 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 3.60 (2H, *t*, *J* = 6.3 Hz, C_{24}-H_2), δ 1.03 (3H, *s*, C_{18}-H_3), δ 0.82 (6H, *br*, C_{19}-H_3 + C_{21}-H_3); ^{13}C NMR ($CDCl_3$): δ 165.87, 160.51 (C_3 and C_{12}), δ 63.41 (C_{24}).

3α,12α-Diamino-5β-cholan-24-ol dihydrochloride (1). Sodium (4 g) was added portionwise during 4 h to a solution of the oxime (**16**) (0.4 g, 1 mmol) in boiling propan-1-ol (80 mL) under an atmosphere of nitrogen, and the mixture was kept at reflux overnight. The cooled mixture was poured into brine, and the aqueous layer was extracted with ether. The combined organic extract was evaporated to dryness under reduced pressure, and the residue was dissolved in ether, washed with brine, and dried ($MgSO_4$). After removal of the solvent under reduced pressure, flash column chromatography of the residue over silica gel using CH_3OH:$CHCl_3$:28% NH_4OH (14:14:1) as eluent provided diamine as a pure white solid. Mp 119–120 °C; Anal. calcd for $C_{24}H_{44}N_2O{\cdot}H_2O$: C, 73.05; H, 11.75; N, 7.10. Found: C, 73.38; H, 11.51; N, 7.10; MS (FAB, *m*-nitrobenzyl alcohol): m/z 377 (M^+ + H), 360, 344, 289; IR (KBr) υ_{max} 3370, 2937, 2864, 1570, 1449, 1383 cm^{-1}; ^1H NMR (CD_3OD): δ 3.51 (2H, *br*, C_{24}-H_2), δ 3.13 (1H, *br*, C_{12}-H), δ 2.71 (1H, *br*, C_3-H), δ 1.00 (3H, *d*, *J* = 5.7 Hz, C_{21}-H_3), δ 0.94 (C_{19}-H_3), δ 0.77 (C_{18}-H_3); ^{13}C NMR (CD_3OD): δ 63.22 (C_{24}), δ 55.42 (C_{12}), δ 52.10 (C_3), δ 23.39 (C_{19}), δ 17.71 (C_{21}), δ 13.94 (C_{18}).

A 1.0 M HCl ethereal solution (1.06 mL) was added to the solution of diamine (100 mg, 0.26 mmol) in chloroform (5 mL) and methanol (0.5 mL). The mixture was concentrated under reduced pressure and dry ether was added. The precipitated salt was recrystallized from methanol–ether to give the dihydrochloride salt (**1**) (62%, two steps overall). Mp > 250 °C; Anal. calcd for $C_{24}H_{44}N_2O{\cdot}2HCl{\cdot}3H_2O$: C, 57.24; H, 10.41; N, 5.56; Cl, 14.08. Found: C, 57.59; H, 9.92; N, 5.61; Cl, 14.24; MS (FAB, *m*-nitrobenzyl alcohol): m/z 377 (M^+-2HCl + H), 307, 289; IR (KBr) υ_{max} 3414, 2926, 2874, 1525, 1449, 1382, 1055, 1013 cm^{-1}; ^1H NMR (CD_3OD): δ 3.67 (1H, *br*, C_{12}-H), δ 3.52 (2H, *t*, *J* = 6.3 Hz , C_{24}-H_2), δ 3.14 (1H, *br*, C_3-H), δ 1.03 (3H, *d*, *J* = 5.7 Hz, C_{21}-H_3), δ 0.99 (3H, *s*, C_{19}-H_3), δ 0.89 (3H, *s*, C_{18}-H_3) ^{13}C NMR (CD_3OD): δ 63.28 (C_{24}), δ 57.49 (C_{12}), δ 52.32 (C_3), δ 22.75 (C_{19}), δ 17.37 (C_{21}), δ 13.42 (C_{18}).

24-(3β-Azido-12α-hydroxy-5β-cholanyl) TBDPS ether (17). Zinc azide–bis(pyridine) complex ($Zn(N_3)_2{\cdot}2Py$, 46 mg, 0.15 mmol) was prepared from zinc nitrate, sodium azide, and pyridine according to the procedure of Rollin and Viaud.[15] The zinc azide complex was added to a mixture of compound (**13**) (123 mg, 0.2 mmol) and triphenylphosphine (105 mg, 0.4 mmol) in anhydrous toluene (3 mL). To this stirred mixture at room temperature, diethylazodicarboxylate (DEAD) (0.079 mL, 0.4 mmol) was added dropwise, causing a slightly exothermic reaction. After stirring overnight, the heterogeneous mixture was filtered through a Celite pad, concentrated *in vacuo*, and purified by flash chromatography (CH_2Cl_2) to afford the pure mono-azide (**17**) 106 mg as a syrup (yield 87%). Anal. calcd for $C_{40}H_{59}N_3Si$: C, 74.83; H, 9.26; N, 6.55. Found: C, 74.79; H, 9.31; N, 6.48; MS (FAB, *m*-nitrobenzyl alcohol): m/z 642 (M^+ + H), 596, 555, 535; IR (CH_2Cl_2 solution) υ_{max} 2940, 2861, 2102, 1449, 1428, 1111 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.59 (4H, *m*, ArH), δ 7.34 (6H, *m*, ArH), δ 3.96 (1H, C_{12}-H), δ 3.91 (1H, C_3-H), δ 3.63 (2H, *t*, C_{24}-H_2), δ 1.07 (9H, *s*, *t*-butyl), δ 0.97 (6H, C_{21}-H_3 + C_{19}-H_3), δ 0.70 (3H, *s*, C_{18}-H_3); ^{13}C NMR ($CDCl_3$): δ 135.54, 134.19, 129.40 and 127.50 (aromatic carbon), δ 73.22 (C_{12}), δ 64.40 (C_{24}), δ 58.76 (C_3).

24-(3β-Azido-12-keto-5β-cholanyl) TBDPS ether (18). The mono-hydroxyl compound (**17**) (436 mg, 0.68 mmol) was dissolved in CH_2Cl_2 (30 mL). Powdered 4 Å molecular sieves (100 mg) and pyridinium dichromate (2.5 g, 6.65 mmol) were added, and the mixture was stirred overnight at room temperature. The mixture was then diluted with anhydrous diethyl ether (50 mL) and filtered. Purification was accomplished by the use of flash column chromatography using ethyl acetate: hexane (1:18) as eluent to yield the azido-keto compound (**18**) as a colorless syrup (391 mg, 90%). Anal. calcd for $C_{40}H_{57}N_3O_2Si$: C, 75.07; H, 8.98; N, 6.57. Found: C, 75.02; H, 9.04; N, 6.53; MS (FAB, *m*-nitrobenzyl alcohol): m/z 640 (M^++H), 612, 582 (M^+-*t*-C_4H_9), 562; IR (CH_2Cl_2 solution) υ_{max} 2934, 2862, 2104, 1700, 1428, 1270, 1110 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.70 (4H, *m*, ArH), δ 7.40 (6H, *m*, ArH), δ 3.94 (1H, C_3-H), δ 3.67 (2H, *t*, C_{24}-H_2), δ 1.06 (12H, *t*-butyl + C_{19}-H_3), δ 1.02 (3H, *t*, C_{18}-H_3), δ 0.85 (3H, *d*, C_{21}-H_3); ^{13}C NMR ($CDCl_3$): δ 214.84 (C_{12}), δ 135.51, 134.29, 129.41 and 127.51 (aromatic carbons), δ 64.54 (C_{24}), δ 58.85 (C_3), δ 58.37 (C_{13}), δ 57.57 (C_{11}).

3β-Azido-12-keto-5β-cholan-24-ol (19). To the silyl ether (**18**) (3.15 g, 4.93 mmol) in anhydrous THF (65 mL) was added Bu_4NF (1.0 M in THF; 9.86 mmol, 9.86 mL). After 18 h, the mixture was poured into H_2O (200 mL) and extracted with Et_2O (3 × 200 mL). The combined organic layers were washed with brine (200 mL), dried ($MgSO_4$), and concentrated. The residue was chromatographed on silica gel (1:6, ethyl acetate: chloroform) to afford the free alcohol (**19**) (1.65 g, 84%). Mp 125–126 °C; Anal. calcd for $C_{24}H_{39}N_3O_2$: C, 71.78; H, 9.79; N, 10.46. Found: C, 71.75; H, 9.85; N, 10.44; MS (FAB, *m*-nitrobenzyl alcohol): m/z 402 (M^+ + H), 384, 374, 356; IR (KBr) υ_{max} 3489, 3258, 2940, 2917, 2873, 2859, 2102, 1697, 1447, 1257, 1019 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.92 (1H, *br*, C_3-H), δ 3.57 (2H, *t*, *J* = 6.3 Hz, C_{24}-H_2), δ 1.01 (6H, *d*, C_{18}-H_3 + C_{19}-H_3), δ 0.82 (3H, *d*, *J* = 6.6 Hz, C_{21}-H_3); ^{13}C NMR ($CDCl_3$): δ 215.02 (C_{12}), δ 63.29 (C_{24}), δ 58.27 (C_3), δ 23.08 (C_{18}), δ 18.87 (C_{19}), δ 11.59 (C_{21}).

3β-Azido-12-oxime-5β-cholan-24-ol (20). A solution of the keto compound (**19**) (1.65 g, 4.11 mmol), $NH_2OH{\cdot}HCl$ (0.42 g, 6 mmol) and sodium acetate (0.81 g, 9.88 mmol) in 30 mL of 95% ethanol was heated to reflux for 24 h. The ethanol was removed *in vacuo*, rendered sufficiently acidic with 1 N HCl to dissolve all solid material, and made alkaline with aqueous sodium carbonate. The precipitate that formed was collected by filtration and washed with water, the mother liquor and washings were extracted with chloroform (3 × 10 mL), and the solvent was removed *in vacuo*. The combined crops (1.50 g, 88%) were used without further purification. Mp 187–188 °C; Anal. calcd for $C_{24}H_{40}N_4O_2$: C, 69.19; H, 9.68; N, 13.45. Found: C, 68.95; H, 9.70; N, 13.34; MS (FAB, *m*-nitrobenzyl alcohol): m/z 417 (M^+ + H), 399, 371, 289; IR (KBr)

υ_{max} 3406, 2940, 2868, 2104, 1628, 1446, 1329, 1311, 1260, 1051, 1019, 935, 902, 856 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 3.92 (1H, *br*, C_3-H), δ 3.59 (2H, *t*, *J* = 6.3 Hz, C_{24}-H_2) , δ 1.01 (3H, *s*, C_{18}-H_3), δ 0.92 (6H, *m*, C_{19}-H_3 + C_{21}-H_3); ^{13}C NMR ($CDCl_3$): δ 166.08 (C_{12}), δ 63.46 (C_{24}), δ 58.54 (C_3).

3β-Amino-12-oxime-5β-cholan-24-ol (21). The azido-oxime (**20**) (1.1 g, 2.64 mmol) was dissolved in absolute ethanol (50 mL), and 250 mg of 10% Pd/C was added to the mixture. After hydrogenation for 5 h at 45 psi, the catalyst was filtered and washed several times with methanol. After removing the solvent *in vacuo*, the residue was chromatographed on silica gel (50:50:1, CH_3OH:$CHCl_3$:28% NH_4OH) to afford the amino-oxime compound (**21**) (0.85g, 83%). Mp 188–200 °C; Anal. calcd for $C_{24}H_{42}N_2O_2$: C, 73.80; H, 10.84; N, 7.17. Found: C, 73.68; H, 10.89; N, 7.11; MS (FAB, *m*-nitrobenzyl alcohol): m/z 391 (M^+ + H), 373, 356. IR (KBr) υ_{max} 3368, 3345, 3279, 3165, 2935, 1634, 1587, 1449, 1376, 1350, 1275, 1256, 1060, 1047, 1028, 1012, 976, 940, 922, 867, 813 cm^{-1}; ^{1}H NMR (CD_3OD/1% $CDCl_3$): δ 3.50 (2H, *t*, *J* = 6.3 Hz, C_{24}-H_2), δ 3.14 (1H, *br*, C_3-H), δ 1.05 (3H, *s*, C_{18}-H_3), δ 0.95 (3H, *d*, *J* = 6.6 Hz, C_{21}-H_3), δ 0.91 (3H, *s*, C_{19}-H_3); ^{13}C NMR (CD_3OD/1% $CDCl_3$): δ 166.08 (C_{12}), δ 63.59 (C_{24}), δ 47.26 (C_3), δ 23.76 (C_{18}), δ 20.34 (C_{21}), δ 12.74 (C_{19}).

3β,12α-Diamino-5β-cholan-24-ol dihydrochloride (2). Sodium (1.8 g) was added portionwise during 4 h to a solution of the oxime (**21**) (0.5 g, 1.28 mmol) in boiling propan-1-ol (50 mL) under an atmosphere of nitrogen overnight. The cooled mixture was poured into brine, and the aqueous layer was extracted with ether. The combined organic extract was evaporated to dryness under reduced pressure and the residue was dissolved in ether, washed with brine, and dried ($MgSO_4$). After removal of the solvent under reduced pressure, flash column chromatography of the residue over silica gel using CH_3OH:$CHCl_3$:28% NH_4OH (14:14:1) as eluent provided diamine as a pure white solid. Mp 122–124 °C; Anal. calcd for $C_{24}H_{44}N_2O{\cdot}HCl{\cdot}2H_2O$: C, 64.18; H, 11.00; N, 6.24. Found: C, 63.82; H, 10.55; N, 6.12; MS (FAB, *m*-nitrobenzyl alcohol): m/z 377 (M^+ + H), 360, 343, 289; IR (KBr) υ_{max} 3352, 3247, 2934, 2866, 1601, 1518, 1444, 1384, 1060, 1016, 976 cm^{-1}; ^{1}H NMR (CD_3OD): δ 3.50 (2H, *br*, C_{24}-H_2), δ 3.41 (1H, *br*, C_{12}-H), δ 3.14 (1H, *br*, C_3-H), δ 1.03 (6H, *m*, C_{21}-H_3 + C_{19}-H_3), δ 0.84 (C_{18}-H_3); ^{13}C NMR (CD_3OD): δ 63.39 (C_{24}), δ 56.53 (C_{12}), δ 47.26 (C_3), δ 23.27 (C_{21}), δ 17.69 (C_{18}), δ 13.84 (C_{19}).

A 1.0 M HCl ethereal solution (2.6 mL) was added to the solution of diamine (245 mg, 0.65 mmol) in chloroform (10 mL) and methanol (1 mL). The mixture was concentrated under reduced pressure and dry ether was added. The precipitated salt was recrystallized from methanol–ether to give the dihydrochloride salt (**2**) (55% two steps overall). Mp > 250 °C; Anal. calcd for $C_{24}H_{46}N_2OCl_2{\cdot}2H_2O$: C, 59.37; H, 10.38; N, 5.77; Cl 14.60. Found: C, 59.15; H, 9.92; N, 5.77; Cl, 13.92; MS (FAB, *m*-nitrobenzyl alcohol): m/z 377 (M^+-2HCl + H), 360, 307; IR (KBr) υ_{max} 3404, 2942, 2868, 1508, 1448, 1384 cm^{-1}; ^{1}H NMR (CD_3OD): δ 3.68 (1H, *br*, C_{12}-H), δ 3.54 (1H, *br*, C_3-H), δ 3.50 (2H, *t*, *J* = 6.3 Hz, C_{24}-H_2), δ 1.02 (6H, *m*, C_{21}-H_3 + C_{19}-H_3), δ 0.89 (C_{18}-H_3); ^{13}C NMR (CD_3OD): δ 63.28 (C_{24}), δ 57.32 (C_{12}), δ 49.20 (C_3), δ 22.98 (C_{21}), δ 17.38 (C_{18}), δ 13.44 (C_{19}).

3α-Hydroxy-5β-cholan-24-ol (22). The diol (**22**) was prepared in 94% yield from lithocholic acid by following the procedure described for the preparation of compound (**12**).[10] Mp 176–177 °C; Anal. calcd for $C_{24}H_{42}O_2$: C, 79.50; H, 11.67. Found: C, 78.74; H, 11.63; MS (FAB, *m*-nitrobenzyl alcohol): m/z 345 (M^+-OH), 327, 289, 257, 215. IR (KBr) υ_{max} 3325, 2925, 2861, 1448, 1383, 1068, 1053, 1016 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 3.61 (3H, *m*, C_3-H and C_{24}-H_2), δ 0.92 (6H, *m*, C_{21}-H_3 + C_{19}-H_3), δ 0.65 (3H, *s*, C_{18}-H_3); ^{13}C NMR ($CDCl_3$): δ 71.93 (C_3), δ 63.62 (C_{24}).

5β-Cholan-3β,24-diol bisformate ester (23). To a solution of the diol (**22**) (1.82 g, 5 mmol) and triphenyl phosphine (5.25 g, 20 mmol) in 60 mL of THF stirred under nitrogen in an ice bath was slowly added formic acid (0.92 g, 20 mmol) and diethyl azodicarboxylate (3.32 mL, 20 mmol) in THF (20 mL). The ice bath was removed, and the mixture was stirred overnight at room temperature. After the THF was evaporated in vacuum, 30 mL of 1:1 mixture of hexane:ether was then added to the residue. Triphenylphosphine oxide and diethyl hydrazine dicarboxylate were filtered off and washed with hexane:ether (1:1, 15 mL). After removal of the solvents, the residue was chromatographed on silica gel eluting with $CHCl_3$:hexane (1:1.5) yielding (**23**) as a pure solid (1.67 g, 80%). Mp 80–81 °C; Anal. calcd for $C_{26}H_{42}O_4$: C, 74.60; H, 10.11. Found: C, 74.54; H, 10.13; MS (FAB, *m*-nitrobenzyl alcohol): m/z 419 (M + H)$^+$, 373 (M^+-OCHO), 327; IR (KBr) υ_{max} 2926, 2864, 1728, 1709, 1469, 1449, 1383, 1194 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 8.05, 8.04 (2H, *s*, –OCHO), δ 5.21 (1H, *br*, C_3-H), δ 4.12 (2H, *m*, C_{24}-H_2), δ 0.96 (3H, *s*, C_{19}-H_3), δ 0.91 (3H, *d*, *J* = 6.6 Hz, C_{21}-H_3), δ 0.64 (3H, *s*, C_{18}-H_3); ^{13}C NMR ($CDCl_3$): δ 161.05, 160.68 (–OCHO), δ 70.95 (C_3), δ 64.51 (C_{24}).

3β-Hydroxy-5β-cholan-24-ol (24). The diformyloxy compound (**23**) (1.6 g, 3.82 mmol) was heated to reflux for 2 h with 5% methanolic sodium hydroxide (25 mL). The reaction mixture was cooled and water added whereupon the dialcohol (**24**) crystallized. The crystals were filtered, washed and dried. Recrystallization from acetone gave pure diol compound (**24**) (1.30 g, 94%). Mp 153–154 °C; Anal. calcd for $C_{24}H_{42}O_2{\cdot}0.5\ H_2O$: C, 77.57; H, 11.66. Found: C, 78.00; H, 11.42; MS (EI): m/z 362 (M^+), 329, 305, 248, 233, 215; IR (KBr) υ_{max} 3332, 2937, 2863, 1470, 1446, 1375, 1056, 1030, 1016 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 4.10 (1H, *br*, C_3-H), δ 3.60 (2H, *m*, C_{24}-H_2), δ 0.95 (3H, *s*, C_{19}-H_3), δ 0.91 (3H, *d*, *J* = 6.3 Hz, C_{21}-H_3), δ 0.64 (3H, *s*, C_{18}-H_3); ^{13}C NMR ($CDCl_3$): δ 67.15 (C_3), δ 63.58 (C_{24}).

5β-Cholane-3α,24-diazide (25). To a solution of the diol (**24**) (1 g, 2.76 mmol) and triphenyl phosphine (2.89 g, 11.03 mmol) in 24 mL of benzene and 16 mL of THF stirred under nitrogen in an ice bath. Hydrazoic acid (11.03 mmol, 7.21 mL of a 1.53 N solution of HN_3 in $CHCl_3$)[4] and diethyl azodicarboxylate (1.83 mL, 11.03 mmol) were slowly added. The ice bath was removed, and the mixture was stirred overnight at room temperature. Benzene and THF were evaporated under vacuum, and 30 mL of a 1:1 mixture of hexane:ether was added to the residue. Triphenylphosphine oxide and diethyl hydrazine dicarboxylate were filtered off and washed with hexane:ether (1:1, 15 mL). After removal of the solvents, the residue was chromatographed on silica gel eluting with $CHCl_3$:hexane (1:8) to afford (**25**) as a pure oil (1.07 g, 94%). Anal. calcd for $C_{24}H_{40}N_6$: C, 69.86; H, 9.77; N, 20.37. Found: C, 69.85; H, 9.83; N, 20.28; MS (FAB, *m*-nitrobenzyl alcohol): m/z 385 (M^+-N_2 + H), 369, 355, 342, 300; IR (neat) υ_{max} 2935, 2865, 2088, 1449, 1377, 1363, 1256 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.21 (3H, *m*, C_3-H + C_{24}-H_2), δ 0.92 (3H, *s*, C_{19}-H_3), δ 0.90 (3H, *d*, *J* = 6.3 Hz, C_{21}-H_3), δ 0.63 (3H, *s*, C_{18}-H_3); ^{13}C NMR ($CDCl_3$): δ 61.09 (C_3), δ 51.78 (C_{24}).

5β-Cholane-3α,24-diamine (27) and dihydrochloride (3). A solution of the diazide (**25**) (0.85 g, 2.06 mmol) in dry THF (25 mL) was slowly added to a stirred solution of $LiAlH_4$ (0.33 g, 8.24 mmol) in dry THF (50 mL) at 0 °C. After the addition the reaction was heated at reflux for 16 h and then cooled, and 0.33 mL of water was carefully added, followed by 0.33 mL of 15% KOH solution and then another 0.99 mL of water. The resultant white precipitate was removed by filtration and thoroughly washed with ether. After removal of the solvents, flash column chromatography of the residue over silica gel using CH_3OH:$CHCl_3$:28% NH_4OH (13:13:1) as eluent provided diamine (**27**) as a pure solid. Mp 154–156 °C; MS (FAB, *m*-nitrobenzyl alcohol): m/z 361 (M + H)$^+$, 344; IR (KBr) υ_{max} 3281, 2930, 2861, 1577, 1471, 1378, 1326 cm^{-1}; ^1H NMR (CD_3OD): δ 2.68 (1H, *br*, C_3-H), δ 2.61 (2H, *m*, C_{24}-H_2), δ 0.94 (6H, *br*, C_{19}-H_3 + C_{21}-H_3), δ 0.68 (3H, *s*, C_{18}-H_3); ^{13}C NMR (CD_3OD): δ 52.38 (C_3), δ 42.97 (C_{24}), δ 24.06 (C_{19}), δ 19.24 (C_{21}), δ 12.54 (C_{18}).

A 1.0 M HCl ethereal solution (1.02 mL) was added to the solution of diamine (**27**) (96 mg, 1.61 mmol) in chloroform (5 mL) and methanol (0.5 mL). The mixture was concentrated under reduced pressure and dry ether was added. The precipitated salt was recrystallized from methanol–ether to give the dihydrochloride salt (**3**) (31% two steps overall). Mp > 250 °C; Anal. calcd for $C_{24}H_{46}N_2Cl_2$: C, 66.49; H, 10.69; N, 6.46; Cl, 16.35. Found: C, 66.22; H, 10.70; N, 6.41; Cl, 16.43; MS (FAB, *m*-nitrobenzyl alcohol): m/z 361 (M^+-2HCl + H), 344, 307; IR (KBr) υ_{max} 3446, 2936, 1605, 1490, 1448, 1383 cm^{-1}; ^1H NMR (CD_3OD): δ 3.12 (1H, *br*, C_3-H), δ 2.87 (2H, *br*, C_{24}-H_2), δ 1.00 (6H, *br*, C_{19}-H_3 + C_{21}-H_3), δ 0.70 (3H, *s*, C_{18}-H_3); ^{13}C NMR (CD_3OD): δ 52.42 (C_3), δ 41.40 (C_{24}), δ 23.66 (C_{19}), δ 19.03 (C_{21}), δ 12.47 (C_{18}).

5β-Cholane-3β,24-diazide (26). A solution of diol **22** (0.72 g, 2.0 mmol) and Ph_3P (1.59 g, 6 mmol) in 15 mL of benzene and 10 mL of THF was stirred under nitrogen in an ice bath. Hydrazoic acid (8 mmol, 6.7 mL of a 1.19 N solution of HN_3 in $CHCl_3$)[36] and diethyl azodicarboxylate (1.0 mL, 6 mmol) were slowly added separately. The ice bath was removed and the mixture was stirred overnight at room temperature. The benzene and THF were evaporated under vacuum, and 30 mL of a 1:1 mixture of hexane:ether was added to the residue. Triphenylphosphine oxide and diethyl hydrazine dicarboxylate were filtered off and washed with hexane:ether (1:1, 15 mL). After removal of the solvents, the residue was chromatographed on silica gel eluting with $CHCl_3$:hexane (1:8). The product was isolated as a pure oil (0.66 g, 80%). Anal. calcd for $C_{24}H_{40}N_6$: C, 69.86; H, 9.77; N, 20.37. Found: C, 69.87; H, 9.84; N, 20.25. MS (FAB, *m*-nitrobenzyl alcohol): m/z 385 (M^+-N_2 + H), 355, 342, 289; IR (neat) υ_{max} 2934, 2863, 2096, 1448, 1377, 1315, 1284, 1268 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.95 (1H, *br*, C_3-H) δ 3.22 (2H, *m*, C_{24}-H_2), δ 0.92 (6H, *m*, C_{21}-H_3 + C_{19}-H_3), δ 0.65 (3H, *s*, C_{18}-H_3); ^{13}C NMR ($CDCl_3$): δ 58.79 (C_3), δ 51.96 (C_{24}).

5β-Cholane-3β,24-diamine (28) and dihydrochloride (4). A solution of the diazide (**26**) (1.55 g, 3.75 mmol) in dry THF (100 mL) was slowly added to a stirred solution of $LiAlH_4$ (0.57 g, 15 mmol) in dry THF (100 mL) at 0 °C. After the addition the reaction was heated at reflux for 16 h and then cooled. Water (0.57 mL) was carefully added, followed by 0.57 mL of 15% KOH solution, and then another 1.71 mL of water. The resulting white precipitate was removed by filtration and thoroughly washed with ether. After removal of the solvents, flash column chromatography of the residue over silica gel using CH_3OH:$CHCl_3$:28% NH_4OH (20:20:1) as eluent provided diamine (**28**) as a pure solid. Mp 145–147 °C; MS (FAB, *m*-nitrobenzyl alcohol): m/z 361 (M^+ + H), 344, 289; IR (KBr) υ_{max} 3384, 2936, 2863, 1560, 1449, 1384, 1328 cm^{-1}; ^1H NMR (CD_3OD): δ 3.26 (1H, *br*, C_3-H), δ 2.64 (2H, *m*, C_{24}-H_2), δ 0.97 (3H, *s*, C_{19}-H_3), δ 0.92 (3H, *d*, *J* = 6.3 Hz, C_{21}-H_3), δ 0.65 (3H, *s*, C_{18}-H_3); ^{13}C NMR (CD_3OD): δ 47.45 (C_3), δ 42.26 (C_{24}), δ 24.23 (C_{19}), δ 19.05 (C_{21}), δ 12.49 (C_{18}).

A 1.0 M HCl ethereal solution (6.46 mL) was added to the solution of diamine (**28**) (582 mg, 1.61 mmol) in chloroform (10 mL) and methanol (1 mL). The mixture was concentrated under reduced pressure and dry ether was added. The precipitated salt was recrystallized from methanol–ether to give the dihydrochloride salt (**4**) (90% two steps overall). Mp > 250 °C; Anal. calcd for $C_{24}H_{46}N_2Cl_2$·$2.5H_2O$: C, 60.23; H, 10.74; N, 5.85. Found: C, 60.50; H, 10.50; N, 5.80; MS (FAB, *m*-nitrobenzyl alcohol): m/z 361 (M^+-2HCl + H), 344, 307, 289; IR (KBr) υ_{max} 3423, 2940, 1611, 1502, 1446, 1383 cm^{-1}; ^1H NMR (CD_3OD): δ 3.61 (1H, *br*, C_3-H), δ 2.90 (2H, *m*, C_{24}-H_2), δ 1.04 (3H, *s*, C_{19}-H_3), δ 0.95 (3H, *d*, *J* = 6.3 Hz, C_{21}-H_3), δ 0.67 (3H, *s*, C_{18}-H_3); ^{13}C NMR (CD_3OD): δ 49.47 (C_3), δ 41.14 (C_{24}), δ 23.76 (C_{19}), δ 18.90 (C_{21}), δ 12.35 (C_{18}).

3β,24-Diazido-5β-cholan-12α-ol (29). A solution of triol **12** (4.54 g, 12 mmol) and triphenyl phosphine (7.55 g, 28.8 mmol) in 60 mL of benzene and 36 mL of THF was stirred under nitrogen at room temperature. Hydrazoic acid (28.8 mmol, 22.2 mL of a 1.30 N solution of HN_3 in $CHCl_3$)[36] and diethyl azodicarboxylate (4.8 mL, 28.8 mmol) was slowly added. The mixture was stirred overnight at room temperature. The benzene and THF were evaporated in vacuum, and 100 mL of a 1:1 mixture of $CHCl_3$/*n*-hexane was then added to the residue. Triphenylphosphine oxide and diethyl hydrazine dicarboxylate were filtered off. After removal of the solvents, the residue was chromatographed on silica gel eluting with $CHCl_3$. The product (**29**) was isolated as an oil (3.71 g, 72%). Anal. calcd for $C_{24}H_{40}N_6O$: C, 67.25; H, 9.41; N, 19.61. Found: C, 67.10; H, 9.43; N, 19.56; MS (FAB, *m*-nitrobenzyl alcohol): m/z 429 (M^+ + H), 401 (M^+-N_2 + H), 383, 355, 340; IR (neat) υ_{max} 3498, 2935, 2092, 1447, 1378, 1348, 1254, 1031 cm^{-1}; ^1H NMR ($CDCl_3$): δ 4.00 (1H, *br*, C_{12}-H), δ 3.94 (1H, *br*, C_3-H), δ 3.23 (2H, *m*, C_{24}-H_2), δ 0.97 (3H, *d*, *J* = 6.3 Hz, C_{21}-H_3), δ 0.93 (3H, *s*, C_{19}-H_3), δ 0.67 (3H, *s*, C_{18}-H_3); ^{13}C NMR ($CDCl_3$): δ 73.16 (C_{12}), δ 58.71 (C_3), δ 51.88 (C_{24}).

3β,24-Diazido-5β-cholan-12-one (30). The mono-alcohol **29** (3.69 g, 8.61 mmol) was dissolved in CH_2Cl_2 (32 mL). Powdered 4 Å molecular sieves (500 mg) and pyridinium dichromate (12.9 g, 34.44 mmol) were then added, and the mixture was stirred at room temperature for 24 h. The mixture was then diluted with anhydrous diethyl ether (50 mL), filtered and the filtrate concentrated to afford the product. The last traces of chromium species were removed by filtering an ethereal solution through a small silica gel column. The pure diazido-keto compound (**30**) was obtained by flash chromatography as a colorless syrupy solid (3.05 g, 83%) using ethyl acetate:hexane (2.5:1). Mp 88–89 °C; Anal. calcd for $C_{24}H_{38}N_6O$: C, 67.57; H, 8.98; N, 19.70. Found: C, 67.62; H, 9.01; N, 19.65; MS (FAB, *m*-nitrobenzyl alcohol): m/z 427 (M^++ H), 399, 356, 214; IR (KBr) υ_{max} 2930, 2874, 2096, 1698, 1458, 1448, 1318, 1254, 1018 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.92 (1H, *br*, C_3-H), δ 3.22 (2H, *t*, *J* = 6.6 Hz, C_{24}-H_2), δ 1.03 (3H, *s*, C_{19}-H_3), δ 1.01 (3H, *s*, C_{18}-H_3), δ 0.84 (3H, *d*, *J* = 6.6 Hz, C_{21}-H_3); ^{13}C NMR ($CDCl_3$): δ 214.51 (C_{12}), δ 58.79 (C_3), δ 51.88 (C_{24}).

3β,24-Diazido-5β-cholan-12-oxime (31). A solution of the ketone **30** (3.00 g, 7.03 mmol), 0.73 g of $NH_2OH{\cdot}HCl$ (10.55 mmol) and 1.38 g sodium acetate (16.87 mmol) in 35 mL of 95% ethanol was refluxed overnight. The ethanol was removed *in vacuo*, and the residue was rendered sufficiently acidic with 1 N HCl to dissolve all solid material, and then made alkaline with aqueous sodium carbonate. The precipitate that formed was collected by filtration and washed with water. The mother liquor was extracted with chloroform (3 × 10 mL), and the solvent was removed *in vacuo*. The combined crops (1.50 g, 88%) were sufficient for use without further purification. Mp 52–54 °C; Anal. calcd for $C_{24}H_{39}N_7O$: C, 65.27; H, 8.90; N, 22.20. Found: C, 65.19; H, 8.95; N, 22.18; MS (FAB, *m*-nitrobenzyl alcohol): m/z 442 (M^+ + H), 399, 368, 307, 289; IR (KBr) υ_{max} 3327, 2933, 2867, 2095, 1654, 1448, 1383, 1316, 1254, 944, 917 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.93 (1H, *br*, C_3-H), δ 3.25 (2H, *m*, C_{24}-H_2), δ 1.03 (3H, *s*, C_{19}-H_3), δ 0.95 (6H, *m*, C_{18}-H_3 + C_{21}-H_3); ^{13}C NMR ($CDCl_3$): δ 166.45 (C_{12}), δ 59.53 (C_3), δ 51.91 (C_{24}).

3β,24-Diamino-5β-cholan-12-oxime (32). The diazido-oxime compound (**31**) (2.25 g, 5.10 mmol) was dissolved in absolute ethanol (30 mL) and 500 mg of 10% Pd/C was added to the mixture. After hydrogenation for 12 h at 45 psi, the catalyst was filtered and washed with methanol several times. After removing the solvent *in vacuo*, the residue was chromatographed on silica gel (8:8:1, CH_3OH:CH_2Cl_2:28% NH_4OH) to afford the diamino-oxime compound (**32**) (1.61 g, 81%). Mp 153–155 °C; Anal. calcd for $C_{24}H_{43}N_2O{\cdot}0.7H_2O$: C, 71.67; H, 11.13; N, 10.45. Found: C, 71.65; H, 10.99; N, 10.45; MS (FAB, *m*-nitrobenzyl alcohol): m/z 390 (M^+ + H), 372, 355 289; IR (KBr) υ_{max} 3360, 2930, 2862, 1637, 1588, 1448, 1383, 941, 921 cm^{-1}; ^1H NMR (CD_3OD): δ 3.16 (1H, *br*, C_3-H), δ 2.62 (2H, *m*, C_{24}-H_2), δ 1.06 (3H, *s*, C_{19}-H_3), δ 0.97 (3H, *d*, *J* = 6.6 Hz, C_{21}-H_3), δ 0.91 (3H, *s*, C_{18}-H_3); ^{13}C NMR (CD_3OD): δ 165.91 (C_{12}), δ 47.45 (C_3), δ 43.03 (C_{24}).

5β-Cholane-3β,12α,24-triamine trihydrochloride (5). Sodium (5.8 g) was added portionwise during 4 h to a solution of the oxime (**32**) (1.45 g, 3.72 mmol) in boiling propan-1-ol (70 mL) under an atmosphere of nitrogen overnight. The cooled mixture was made slightly acidic with 1 N HCl. The propanol was distilled off *in vacuo*. The residue was dissolved in 5 N NaOH solution, and the alkaline solution was extracted with $CHCl_3$ (3 × 100 mL). The combined organic extract was evaporated to dryness under reduced pressure. Flash chromatography of the residue over silica gel using CH_3OH:$CHCl_3$:28% NH_4OH (7:7:1) as eluent, provided the triamine as a white noncrystalline solid. ^1H NMR (CD_3OD): δ 3.25 (1H, *br*, C_3-H), δ 3.13 (1H, *br*, C_{12}-H), δ 2.62–2.66 (2H, *m*, C_{24}-H_2), δ 1.00 (3H, *d*, *J* = 6.6 Hz, C_{21}-H_3), δ 0.98 (3H, *s*, C_{19}-H_3), δ 0.77 (3H, *s*, C_{18}-H_3); ^{13}C NMR (CD_3OD): δ 55.73 (C_{12}), δ 47.95 (C_3), δ 42.71 (C_{24}), δ 23.87 (C_{19}), δ 17.94 (C_{21}), δ 14.20 (C_{18}).

A 1.0 M HCl ethereal solution (8.04 mL) was added to the solution of the triamine (500 mg, 1.34 mmol) in methanol (10 mL). The mixture was concentrated under reduced pressure, and dry ether was added. The precipitated salt was recrystallized from methanol–ether to give the trihydrochloride salt (**5**) (61% two steps overall). Mp > 250 °C; Anal. calcd for $C_{24}H_{48}N_3Cl_3{\cdot}2.5H_2O$: C, 54.38; H, 10.08; N, 7.89; Cl, 20.07. Found: C, 54.40; H, 10.00; N, 7.89; Cl, 20.10; MS (FAB, *m*-nitrobenzyl alcohol): m/z 376 (M^+-2HCl + H), 342, 289; IR (KBr) υ_{max} 3432, 2956, 1602, 1518, 1455, 1386, 1013 cm^{-1}; ^1H NMR (CD_3OD): δ 3.69 (1H, *br*, C_{12}-H), δ 3.61 (1H, *br*, C_3-H), δ 2.86–2.92 (2H, *m*, C_{24}-H_2), δ 1.07–1.05 (6H, *t*, C_{21}-H_3 + C_{19}-H_3), δ 0.90 (3H, *s*, C_{18}-H_3); ^{13}C NMR (CD_3OD): δ 57.37 (C_{12}), δ 49.17 (C_3), δ 41.20 (C_{24}), δ 22.98 (C_{19}), δ 17.31 (C_{21}), δ 13.44 (C_{18}).

3β-Amino-24-guanidino-5β-cholane dihydrochloride (6). Aminoiminomethanesulfonic acid (0.1 g, 0.8 mmol) prepared by the literature method[17] was added to the 3β,24-diamine (**28**) (0.144 g, 0.4 mmol) in 8 mL of methanol at room temperature and refluxed for 12 h. The cooled mixture was made slightly acidic with 1 N HCl and the methanol was distilled off *in vacuo*. The residue was dissolved in 3 N aqueous NaOH, and the precipitate was isolated by filtration. ^{1}H NMR (CD_3OD/1%$CDCl_3$): δ 3.13 (1H, *br*, C_3-H), δ 3.03 (2H, *m*, C_{24}-H_2), δ 0.96 (3H, *s*, C_{19}-H_3), δ 0.93 (3H, *J* = 6.3 Hz, C_{19}-H_3), δ 0.65 (3H, *s*, C_{18}-H_3); ^{13}C NMR (CD_3OD/1%$CDCl_3$): δ 158.65 (HNC(NH)NH_2) δ 47.02 (C_3).

A 1.0 M HCl ethereal solution (1.28 mL) was added to the solution of the monoguanidine (170 mg, 0.32 mmol) in chloroform (5 mL) and methanol (5 mL). The mixture was concentrated under reduced pressure and dry ether was added. The precipitated salt was recrystallized from methanol–ether to give the dihydrochloride salt (**6**) (81% two steps overall). Mp > 250 °C; Anal. calcd for $C_{25}H_{48}N_4Cl_2 \cdot 1.5H_2O$: C, 59.74; H, 10.23; N, 11.15; Cl, 14.11. Found: C, 60.16; H, 10.19; N, 11.45; Cl, 14.20; MS (FAB, *m*-nitrobenzyl alcohol): m/z 402 (M^+-2HCl + H), 386, 289, 202; IR (KBr) υ_{max} 3336, 3160, 2935, 2862, 1654, 1508, 1449, 1383 cm^{-1}; ^{1}H NMR (CD_3OD/1%CD_3Cl): δ 3.57 (1H, *br*, C_3-H), δ 3.136 (2H, *m*, C_{24}-H_2), δ 1.02 (3H, *s*, C_{19}-H_3), δ 0.95 (3H, *d*, *J* = 6.3 Hz, C_{21}-H_3), δ 0.69 (3H, *s*, C_{18}-H_3); ^{13}C NMR (CD_3OD/1%CD_3Cl): δ 158.51 (HNC(NH_2)NH_2), δ 49.28 (C_3), δ 42.87 (C_{24}), δ 23.82 (C_{19}), δ 19.07 (C_{21}), δ 12.49 (C_{18}).

N,N'-*Bis(3α-hydroxy-5β-cholan-24-yl)-1,4-diaminobutane (33).* Lithocholic acid (5.44 g, 15 mmol) was dissolved in a solution of 120 mL of dry tetrahydrofuran (THF) and 30 mL of dry acetonitrile, and 1.78 g (15 mmol) of *N*-hydroxysuccinimide was added. To the resulting homogeneous solution was added dropwise 3.44 g (16.5 mmol) of dicyclohexylcarbodiimide (DCC) in 30 mL of dry THF at 10–15 °C. The mixture was stirred at room temperature for 4 h, and the precipitate of *N,N'*-dicyclohexylurea was removed by filtration. Chloroform (450 mL) was added to the filtrate, and the solution was washed with 10% sodium carbonate (2 × 150 mL) and with water (2 × 150 mL) and dried over sodium sulfate. Chloroform was removed *in vacuo*, and the white solid was recrystallized from a 1:1 mixture of chloroform and *n*-hexane to obtain the succinimide ester (5.92 g, 86%). ^{1}H NMR ($CDCl_3$): δ 3.65–3.56 (1H, *m*, C_3-H), δ 2.83 (4H, *s*, succimide ester), δ 2.70–2.46 (2H, *m*, C_{23}-H_2), δ 0.94 (3H, *d*, *J* = 6.0 Hz, C_{21}-H_3), δ 0.91 (3H, *s*, C_{19}-H_3), δ 0.65 (3H, *s*, C_{18}-H_3); ^{13}C NMR ($CDCl_3$): δ 169.07 (carbonyl), δ 71.86 (C_3).

The succinimide ester of lithocholic acid thus obtained (5.86 g, 12.75 mmol) was dissolved in 150 mL of dry DMF and added dropwise at 0 °C to 30 mL of dry DMF which contained 0.65 mL (6.375 mmol) 1,4-diaminobutane. Stirring was continued for 13 h at room temperature. The solvent was removed *in vacuo*, and flash column chromatography of the residue over silica gel using CH_3OH:$CHCl_3$ (1:13) as eluent provided the amide dimer (**33**) as a pure solid (4.5 g, 90%). Mp 202–204 °C; Anal. calcd for $C_{52}H_{88}N_2O_4 \cdot 0.5H_2O$: C, 76.70; H, 11.02; N, 3.44. Found: C, 76.82; H, 11.06; N, 3.42; MS (FAB, *m*-nitrobenzyl alcohol): m/z 805 (M^++H), 772, 501, 447; IR (KBr) υ_{max} 3324, 2928, 2851, 1656, 1626, 1570, 1448, 1310, 1243 cm^{-1}; ^{1}H NMR (1:1, CD_3OD:$CDCl_3$): δ 3.50–3.38 (2H, *m*, C_3-H), δ 3.04 (4H, *br*, CONH-CH_2), δ 0.80–0.78 (12H, *t*, C_{21}-H_3 + C_{19}-H_3), δ 0.50 (6H, *s*, C_{18}-H_3); ^{13}C NMR (1:1 CD_3OD:$CDCl_3$): δ 174.88 (C_{24}), δ 71.19 (C_3).

N,N'-*Bis(3β-amino-5β-cholan-24-yl)-1,4-diaminobutane (34).* A solution of the amide dimer (**33**) (2.32 g, 2.98 mmol), phthalimide (1.22 g, 8.14 mmol), and triphenylphosphine (2.08 g, 7.92 mmol) in DMF (50 mL) was treated with diethyl azodicarboxylate (1.29 mL, 7.80 mmol). The reaction was stirred at room temperature under nitrogen. After 24 h, solvent was removed, and the residue was purified by a silica gel column with CH_3OH:$CHCl_3$ (1:25) as eluant to give a mixture of 3α-NPht amide dimer and some inseparable impurities. ^{1}H NMR ($CDCl_3$): δ 7.77–7.67 (8H, *m*, aromatic C-H), δ 4.40 (2H, *br*, C_3-H), δ 3.27 (4H, *br*, CONH-CH_2), δ 1.01 (6H, *s*, C_{19}-H_3), δ 0.92 (6H, *d*, *J* = 6.3 Hz, C_{21}-H_3), δ 0.64 (6H, *s*, C_{18}-H_3); ^{13}C NMR ($CDCl_3$): δ 173.87 (C_{24}), δ 168.73 (carbonyl of phthalimide), δ 133.67, 132.20 and 122.87 (aromatic), δ 77.20 (C_3).

A mixture of the NPht amide dimer and hydrazine hydrate ($N_2H_4 \cdot H_2O$, 3.00 mL, 59.6 mmol) in ethanol (50 mL) was heated to reflux for 4 h. After being cooled, the resulting phthalhydrazide was removed. The filtrate was concentrated *in vacuo*, and the residue was purified by column chromatography on silica gel with CH_3OH:CH_2Cl_2:28% NH_4OH (12:12:1) as eluant to obtain pure 3α-amino amide dimer (**34**) [1.02 g, 44% from (**30**)]. Mp 195–197 °C; Anal. calcd for $C_{52}H_{90}N_4O_2 \cdot 2H_2O$: C, 74.41; H, 11.29; N, 6.68. Found: C, 74.38; H, 11.15; N, 6.52; MS (FAB, *m*-nitrobenzyl alcohol): m/z 803 (M^+ + H), 429; IR (KBr) υ_{max} 3282, 2934, 2863, 1658, 1563, 1443, 1381 cm^{-1}; ^{1}H NMR (CD_3OD + 1%$CDCl_3$): δ 3.18 (6H, *br*, C_3-H + CONHCH_2), δ 0.96 (6H, *s*, C_{19}-H_3), δ 0.92 (6H, *d*, *J* = 6.6 Hz, C_{21}-H_3), δ 0.64 (6H, *s*, C_{18}-H_3); ^{13}C NMR (CD_3OD/1%$CDCl_3$): δ 174.73 (C_{24}), δ 45.77 (C_3), δ 23.20 (C_{19}), δ 17.66 (C_{21}), δ 11.41 (C_{18}).

N,N-*Bis(3β-amino-5β-cholan-24-yl)1,4-diaminobutane tetrahydrochloride (7).* A 1M THF solution of borane (32 mL) was added slowly to a mixture of diamide **34** (312 mg, 0.4 mmol) in THF (10 mL). The reaction mixture was heated at reflux for 8 h and then cooled and carefully hydrolyzed with 6 N HCl (32 mL). The bulk of the THF was distilled away, and the resulting aqueous mixture heated at reflux for 4 h. The mixture was cooled, basified by sodium hydroxide pellets, and then extracted with $CHCl_3$ (4 × 30 mL). The combined organic extracts were dried ($MgSO_4$) and concentrated to a residue that was chromatographed on silica gel

[$CH_3OH:CH_2Cl_2$:28% NH_4OH (10:10:1)] to give the desired tetraamine. Mp 134–136 °C; MS (FAB, *m*-nitrobenzyl alcohol): m/z 775 (M^+ + H), 415, 223; IR (KBr) υ_{max} 3422, 2934, 2861, 1560, 1448, 1384 cm^{-1}; ^{1}H NMR (CD_3OD/1%$CDCl_3$): δ 3.15 (2H, *br*, C_3-H), δ 2.58–2.51 (8H, *m*, C_{24}-H_2 + C_{25}-H_2), δ 0.94 (6H, *s*, C_{19}-H_3), δ 0.91 (6H, *d*, *J* = 6.3 Hz, C_{21}-H_3), δ 0.64 (6H, *s*, C_{18}-H_3); ^{13}C NMR (CD_3OD/1%$CDCl_3$): δ 47.31 (C_3), δ 51.06, 50.26 (C_{24} + C_{25}), δ 24.46 (C_{19}), δ 19.30 (C_{21}), δ 12.66 (C_{18}).

A 1.0 M HCl ethereal solution (0.86 mL) was added to the solution of the tetraamine (80 mg, 0.11 mmol) in chloroform (5 mL) and methanol (0.5 mL). The mixture was concentrated under reduced pressure and dry ether was added. The precipitated salt was recrystallized from methanol–ether to give the tetra-hydrochloride salt (7) (60% two steps overall). Mp > 250 °C; Anal. calcd for $C_{52}H_{98}N_4Cl_4{\cdot}5H_2O$: C, 61.76; H, 10.76; N, 5.54. Found: C, 62.02; H, 10.58; N, 5.44; MS (FAB, *m*-nitrobenzyl alcohol): m/z 775 (M^+-4HCl + H), 415, 214; IR (KBr) υ_{max} 3384, 2938, 2860, 1617, 1508, 1456, 1383 cm^{-1}; ^{1}H NMR (CD_3OD/1%$CDCl_3$): δ 3.56 (2H, *br*, C_3-H), δ 3.04–2.92 (8H, *m*, C_{24}-H_2 + C_{25}-H_2), δ 1.02 (6H, *s*, C_{19}-H_3), δ 0.96 (6H, *d*, *J* = 6.3 Hz, C_{21}-H_3), δ 0.68 (6H, *s*, C_{18}-H_3); ^{13}C NMR (CD_3OD/1%$CDCl_3$): δ 48.72 (C_3), δ 49.30, 47.96 (C_{24} + C_{25}), δ 23.81 (C_{19}), δ 18.98 (C_{21}), δ 12.50 (C_{18}).

General procedure for fluorometric determination of C_{50} values. Excitation of the buffer solution (3 mL in 1 cm path length glass cuvette) was accomplished by using a high-pressure mercury lamp at 547 nm. Fluorescence emission was measured at 595 nm. Ethidium bromide was first dissolved in the 0.01 SHE buffer solution (2 mM HEPES, 8 mM NaCl, and 0.05 mM EDTA, pH 7.0, 25 °C) to provide [Ethidium] = 1.26 μM. A solution of calf thymus DNA, poly[d(AT)], or poly d(GC) was added to 3 mL of the ethidium solution so that the fluorescence reading of the ethidium–DNA solution increased from 13 to 100 and the final concentration of DNA (base pair) was 1.31 μM.[5,21] The steroidal polyamines in SHE buffer solution were added in microliter portions from microsyringe (Rainin Co.) through a fine plastic catheter (USA/Scientific) and the fluorescence reading recorded after each addition. The C_{50} value was measured as the concentration of steroidal polyamines required to reduce the fluorescence of the DNA–ethidium complex to 50% (scale reading 56.5). The measurement had an experimental error of 10%.

T_m *Measurements.* Melting temperatures of solutions containing 0.025 SHE buffer (23 mM NaCl, 2 mM HEPES, 0.05 mM EDTA, pH 7.0), 23 μM of poly[d(AT)] (per nucleotide) and varying concentrations of polyamine (1.5 μM–21 μM) were measured by recording the absorbance at 260 nm as a function of temperature. The DNA/polyamine solutions were incubated at 20 °C for 45 min subsequent to T_m determination. T_m measurements were initiated at 20 °C and the temperature was raised in 5 °C steps in the range of 20 °C to 90 °C. The temperature was allowed to stabilize for 5 min at each step prior to recording the absorbance reading. The T_m value was then determined by taking the temperature at the midpoint between the baselines of a plot of absorbance at 260 nm versus temperature.

Hyperchromicity measurements. The hyperchromicity of solutions containing 0.025 SHE buffer (23 mM NaCl, 2 mM HEPES, 0.05 mM EDTA, pH 7.0), 23 μM of poly[d(AT)] (per nucleotide) and 11.5 μM of the desired polyamine were measured at 260 nm at 20 °C. Hyperchromicity is defined as the per cent increase in the absorbance of poly[d(AT)] at 260 nm after the addition of polyamine. The DNA/polyamine solutions were incubated at 20 °C for 45 min prior to absorbance determination.

Circular dichroism studies. Circular dichroism (CD) measurements of solutions containing 0.025 SHE buffer (23 mM NaCl, 2 mM HEPES and 0.05 mM EDTA), 36 μM of poly[d(AT)] (per nucleotide) and varying concentrations of polyamine (2.0 μM – 32 μM). The molar ellipticity [Θ] was calculated from the observed ellipticity as previously described in the literature.[37]

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References and Notes

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