Binding to Deoxyribonucleic Acid and Inhibition of Ribonucleic Acid Polymerase by Analogs of Chloroquine†

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A series of chloroquine analogs with polyamine side chains of varying length has been designed and synthesized to examine the nature of the binding of chloroquine-like agents to DNA. The distance between side-chain nitrogens is either 3 or 4 methylene units as is the case for the polyamines spermine and spermidine known to form strong complexes with nucleic acids. The data obtained seem to indicate that binding to DNA is commensurate with the number of basic groups in the side chain. The contribution of the side chain to the total binding energy of the complex was found to be greater than that of the ring based on the elevation of the melting temperature (Tm) of native DNA. However, ring intercalation or some other form of ring interaction appears to be essential for any compound to show significant inhibition of the RNA polymerase reaction since the different polyamines alone do not show any inhibitory effect under identical conditions. In general, there is a correlation between length of the side chain, Tm shifts, and inhibition of the RNA polymerase reaction. However, when a second 7-chloro-4-quinolyl moiety was attached to the polyamine (bisquinolyl derivatives), there was a marked increase in the inhibition of RNA polymerase activity but no increase in Tm. The behavior of a model compound in which the side chain is in an out-of-plane conformation suggests that coplanarity between ring and side chain in 4-alkylaminoquinolines might not be a prerequisite for binding.

The antimalarial drug chloroquine has been shown to interact with DNA[1-3] and to inhibit the ability of DNA to act as a template for DNA replication and for RNA synthesis.[4-6] These effects are thought to result from intercalation of the drug with DNA and may account for part of its antimalarial activity. The intercalation model requires an extension of the DNA helix to allow insertion of the quinoline ring between adjacent base pairs.[4,7,8] Although there is evidence that intercalation of the quinoline ring of chloroquine into DNA can occur, the role of the side chain in the formation of the drug–DNA complex has been poorly understood. Hahn and O'Brien[4,7] suggest that once the quinoline ring has intercalated, the 1,4-diaminopentane side chain of chloroquine falls outside of the contour of the DNA base pairs and interacts ionically with phosphate groups of the complementary strands of DNA across the minor groove of the double helix (Figure 1). In such a model the critical distance between nitrogens in the side chain is 4 methylene units which provides the best fit through spanning of the minor groove. Any departure in either direction from the critical number of 4 resulted in a decrease in antimalarial potency.[4]

In an effort to assess the importance of different polyamine side chains attached to the 7-chloro-4-quinolyl nucleus and to determine whether or not their activity could be explained in terms of an extension of Hahn's model, analogs of the 4-aminoquinoline antimalarial chloroquine were synthesized and studied.[9,10] The changes introduced include variations in length, conformation, and number of basic groups in the side chain. The compounds which were synthesized are listed in Table I.

Some of the polyamine side chains attached to the quinoline ring offer the possibility of interacting more randomly across complementary strands of DNA in a combination of small groove, large groove, and even intermolecular modes of binding, since the distance between the terminal nitrogens has been increased. However, despite such a possibility, the side-chain length specificity observed for unsubstituted polyamines of different lengths in the stabilization of double stranded DNA is most easily explained by the speculation of intramolecular binding across the minor groove.[11-15] Accordingly, spermine, for example, will interact with two adjacent phosphate groups on one DNA strand and then its central portion, 4 methylene units long, will cross the minor groove of DNA to interact with two more adjacent phosphate groups on the other strand.[15]

The importance of the fact that both aminoquinoline side chains and polyamines, regardless of their length, independently seem to show preference toward the minor groove allows one to compare ring *vs.* side-chain interactions in the present series of compounds. Hahn and coworkers report that they were able to show that there was a direct relationship between the antimalarial activity of chloroquine analogs and the changes in Tm produced by the corresponding diamine side chains of different lengths.[4] This observation provides indirect evidence in favor of a bimodal mechanism of action of ring plus side chain.

In view of these findings we were prompted to investigate if it was possible to enhance one mode of attachment (side-chain attachment) without impairing ring intercalation. If Hahn's model is operative, the activity of the compounds should increase just by enhancing the binding ability of the side chain.

Interaction of the 4-aminoquinoline series with calf thymus DNA was examined by elevation of the Tm (the temperature at which 50% hyperchromicity is attained owing to heat denaturation of native DNA), inhibition of DNA function as a template for *Escherichia coli* RNA polymerase *in vitro*, and uv spectra of the drug–DNA complexes. The first measurement gives an indication of the relative importance of the side chain and the quinoline ring in the drug–DNA binding process; the second measurement reveals the extent to which the location of the side chain in the minor groove of DNA may affect the binding of the enzyme to its template or movement of the enzyme along the template; and, thirdly, the uv spectra of free and DNA-bound drugs may serve to compare qualitatively spectral changes with those observed in the spectra of free and DNA-bound chloroquine.[16]

Synthesis. Direct condensation of aliphatic diamines with either 4,7-dichloroquinoline or 4-phenoxy-7-chloroquinoline has been the most common procedure used in the synthesis of 4-alkylaminoquinolines.[17,18] In most cases, the condensation is carried out by using diamines having a tertiary amine function on one end and a primary amine function on the other. In the present series, however, it was desirable to maintain free terminal pri-

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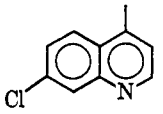
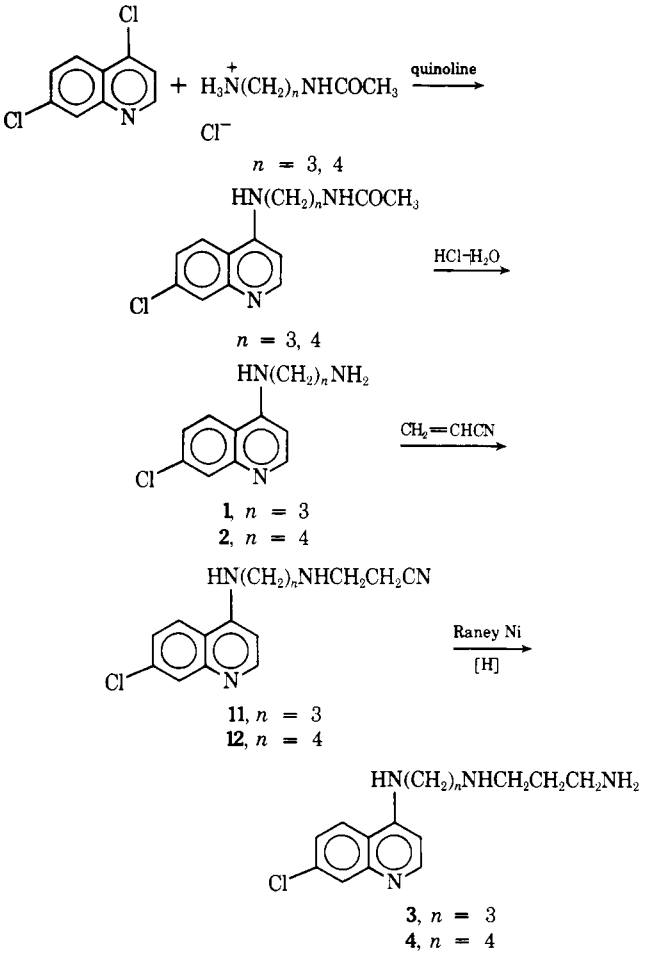
Table I. Chloroquine Analogs

No.	Compound[a]
1	CQ-NH$(CH_2)_3NH_2$
2	CQ-NH$(CH_2)_4NH_2$
3	CQ-NH$(CH_2)_3$NH$(CH_2)_3NH_2$
4	CQ-NH$(CH_2)_4$NH$(CH_2)_3NH_2$
5	CQ-NH$(CH_2)_3$NH$(CH_2)_4$NH$(CH_2)_3NH_2$
6	CQ-NH$(CH_2)_3$NH$(CH_2)_3$NH-CQ
7	CQ-NH$(CH_2)_3$NH$(CH_2)_4$NH$(CH_2)_3$NH-CQ
8	CQ-N$[(CH_2)_3NH_2]_2$

[a]CQ = 

mary amines in all final products owing to the fact that polyamines of this nature react more strongly with DNA.[11,12,19,20] As a consequence of having more than one primary amino nucleophilic site in the starting diamine, the synthetic procedure was expected to lead to a mixture of products. This was circumvented by using the appropriate monoacetylated diamines as starting materials and later removing the acetyl group by acid hydrolysis. Compounds 1–4 were made by use of the reactions indicated in Scheme I. The diamines were selectively acetylated by the method of Tabor, *et al.*,[21] and the products were isolated as hydrochloride salts. As expected, attempted condensation of the monoacetylated diamine hydrochlorides with 4,7-dichloroquinoline at 120–140° did not yield any condensation products. Only in the presence of quinoline as a solvent, the condensation proceeded smoothly giving the desired compounds in yields of 60–80%. Acid hydrolysis of

Scheme I



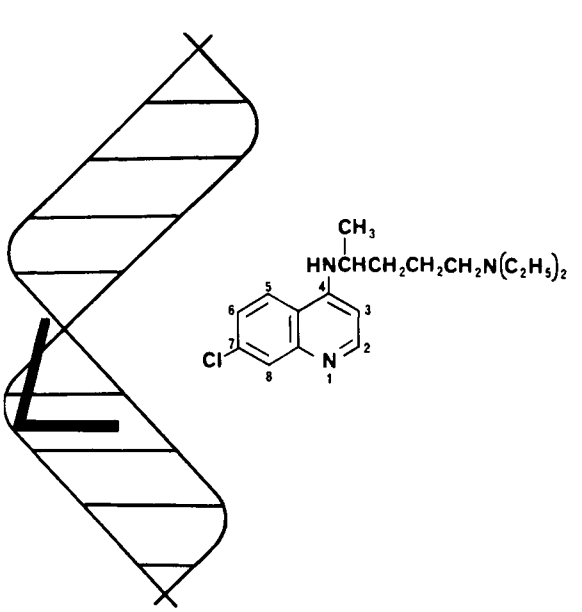


Figure 1. Hypothetical structure of DNA-chloroguine complex and structure of chloroquine. The intercalated quinoline ring is represented by the bar extending into the minor groove.

the condensed products gave the intermediate aminoalkylaminoquinolines 1 and 2 which were isolated as free bases in 60–70% yield. The progressive stepwise lengthening of the side chain was continued by reacting 1 and 2 with acrylonitrile. Catalytic reduction of the corresponding nitriles in Raney nickel with ethanolic ammonia afforded 3 and 4 in 50% yield.

For the spermine analog 5, the stepwise process seemed too long so a direct condensation of spermine and 4,7-dichloroquinoline was attempted. By use of a large excess of the polyamine the desired product 5 was obtained after following a tedious purification procedure. As a consequence, yields of 5 were generally low (40%).

Compounds 6 and 7 were prepared in low yields (16–37%) by reacting a 4,7-dichloroquinoline–polyamine mixture in a 1:1 ratio. In all cases the substitution took place at the termini of the polyamines giving the symmetric molecules 6 and 7 which behaved as high-melting solids. Attempts to prepare the corresponding bisquinolyl derivative of the unsymmetrical polyamine spermidine were unsuccessful and a pure product could not be isolated.

After considering different approaches to the synthesis of the out-of-plane compound 8, it was finally decided to react 4,7-dichloroquinoline with β,β'-iminodipropionitrile, based on the fact that Peck, *et al.*, were able to obtain a low but significant yield of 7-chloro-4-bis(2-hydroxyethyl)-aminoquinoline as a result of the direct condensation of 4-bromo-7-chloroquinoline with diethanolamine in the presence of phenol.[22]

Both reagents were mixed and heated for 1 hr in the presence of phenol at 140°. Following work-up, a crystalline product was isolated in 32% yield. We confirmed that presence of phenol was necessary since in its absence both starting materials were recovered unchanged. However, the product isolated from the reaction in the presence of phenol was not the desired one but instead the monoalkylnitrile 9. Besides spectral evidence that suggested the structure of 9, it was possible to convert it to previously made compound 1 through chemical reduction using $NaBH_4$–Raney nickel.[23] Formation of 9 was envisaged through the intermediacy of the desired bisalkylnitrile 10 *via* an acid-catalyzed rearrangement (Scheme II). Compound 9 is undoubtedly favored by the relief of steric strain which renders the lone pair of the nitrogen again in

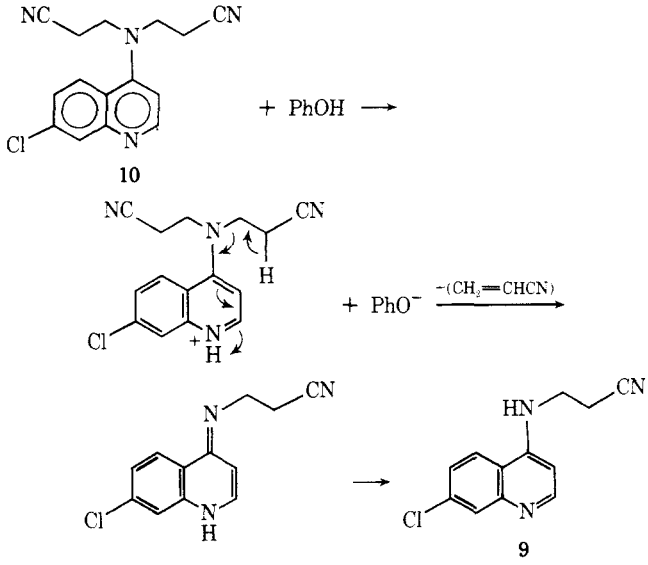
Table II. Melting Temperature (Tm) of Native Calf Thymus DNA in the Presence of Various 7-Chloroquinolyl Derivatives and Their Corresponding Side Chains

Compound	No.	Tm, °C	ΔTm[a]	ΔTm_S	ΔTm_R
None		68	0		
Chloroquine		80.0	12.0		
CQ-$NH(CH_2)_3NH_2$	**1**	80.0	12.0		7.0
$H_2N(CH_2)_3NH_2$		73.0		5.0	
CQ-$NH(CH_2)_4NH_2$	**2**	82.5	14.5		7.5
$H_2N(CH_2)_4NH_2$		75.0		7.0	
CQ-$NH(CH_2)_3NH(CH_2)_3NH_2$	**3**	90.2	22.2		6.8
$H_2N(CH_2)_3NH(CH_2)_3NH_2$		83.4		15.4	
CQ-$NH(CH_2)_4NH(CH_2)_3NH_2$	**4**	91.1	23.1		5.6
$H_2N(CH_2)_4NH(CH_2)_3NH_2$		85.5		17.5	
CQ-$NH(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$	**5**	92.0	24.0		5.0
$H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$		87.0		19.0	

[a] ΔTm is the shift in melting temperature of native DNA produced by the various 7-chloroquinolyl derivatives. ΔTm_S is the shift produced by the polyamine side chains alone. ΔTm_R is the difference between ΔTm and ΔTm_S and represents the contribution of the 7-chloroquinolyl ring to the shift in melting temperature.

a coplanar relationship with the aromatic ring. The intermediacy of **10** in this reaction was proven conclusively by reducing the reaction time to 0.5 hr and isolating pure **10** in 15% yield. Later, it was possible to rearrange **10** to the monoalkylnitrile **9** by acid or base hydrolysis, as expected. Catalytic reduction of **10** using Raney nickel in 10% ethanolic ammonia proceeded smoothly and **8** was isolated and converted to a stable trihydrochloride salt in 75% yield.

Scheme II



Another interesting rearrangement, for which a similar example existed in the literature,[24] took place when **8** was subjected to either basic or acid catalysis to give quantitatively compound **3**. It is probably formed through a possible six-membered transition state.[24]

Biological Results. Evaluation of Melting Temperatures (Tm). The Tm was determined for native calf thymus DNA which had been allowed to react with the 7-chloroquinolyl derivatives or the corresponding diamine side chains (Table II). These heat denaturation studies were performed essentially as described by Hahn, *et al.*,[1,4] for chloroquine. The Tm values obtained using Tris–HCl buffer at pH 7.5[1,4] or potassium phosphate buffer at pH 5.9[3] were virtually the same. This observation agrees with the reported slight effect observed in the uv spectra of DNA-bound chloroquine upon changes of pH in the range of 5.9–9.5.[3] pH-independent Tm values at either 5.9 or 7.5 were also recorded for compound **8** where a difference could have been explained on the basis of a difference in pK_a with respect to the monoalkylaminoquinolines. It is interesting to note, however, that in a similar study, changes in the buffer systems did show a significant difference in the 4-arylaminoquinoline analogs of amodiaquine as reported previously.[25]

The distance between the nitrogens in all the polyamines used is either 3 or 4 methylene units. These are the distances found in the strongest interacting polyamines spermine and spermidine known to form strong complexes with nucleic acids.[15,26-28]

The results show that the compounds stabilize DNA as a function of length and number of basic groups present in the side chain, thus following the same trend observed for the isolated polyamines (Table II).

If according to Hahn's model one considers the overall stability of the drug–DNA complex as the result of a bimodal mechanism of attachment of both the aromatic ring and the side chain, the ΔTm produced by the 7-chloro-4-quinolyl derivatives represents the contributions by the side chain (ΔTm_S) and the aromatic ring (ΔTm_R) (Table II). In this approximation it is assumed that the shifts in Tm result entirely from the process of intercalation and side-chain electrostatic binding across the minor groove.[1,4]

Data from Table II show that when the side chain is increased in size and number of basic groups it becomes the larger contributor to the overall stabilization of the complex. As expected, the effect of the aromatic ring (ΔTm_R) remains relatively constant throughout the series of compounds.

Removal of the side chain, as in 4-amino-7-chloroquinoline, completely eliminates the ability to increase the stability of DNA (Table III) in spite of the fact that the compound actually binds to the biopolymer.[3] Significant activity is only restored when both side chain and ring are covalently linked.

Forced rotation of the side chain in an out-of-plane conformation, which can occur in compound **8** owing to the steric interaction between the amine side chain and the peri C–H constituent, does not decrease the ability to stabilize DNA (ΔTm for compound **8** = 20.0°). Thus, this compound is nearly as active as its isomer **3** and superior to its lower homolog **1**. The results indicate that the sterically rotated molecule can be accommodated well by the receptor site. These findings contrast with the behavior of a reported case in 4-arylaminoquinolines in which deviation from planarity between ring and side chain reduces significantly the molecule's capacity to stabilize DNA.[25]

While it is possible that bisquinolyl derivatives (compounds **6** and **7**) may engage in intermolecular bonding

Table III. Importance of the Covalently Linked Side Chain to Shifts in Tm

Compound	Tm	ΔTm
CQ-NH_2[a]	69.0	1.0
$H_2N(CH_2)_4NH_2$	75.0	7.0
CQ-NH_2 + $H_2N(CH_2)_4NH_2$	73.5	5.5
CQ-$NH(CH_2)_4NH_2$	82.5	14.5

[a] 4-Amino-7-chloroquinoline, prepared according to the procedure of J. C. E. Simpson and P. H. Wright, *J. Chem. Soc.*, 1707 (1948).

between DNA molecules, an increase in Tm of an additional 5–7.5° which would be expected if the second ring intercalated is not observed (ΔTm for **6** = 17.2° and for **7** = 21.5°). Moreover, the Tm is lower than that of the corresponding monoquinolyl analogs **3** and **5** (Table II). With this class of compounds Tm data do not reveal sufficient information as to the nature of the drug–DNA complex, but it can be speculated that one of the rings fails to intercalate or to contribute significantly to the overall stability in any other way.

Inhibition of *E. coli* RNA Polymerase. Interaction of 7-chloro-4-quinolyl analogs with DNA results in inhibition of *in vitro* DNA-dependent RNA synthesis (Table IV). The level of inhibition is rather low for the first members of the series. However, with progressive lengthening and increased binding ability of the side chain, a significant increase in the inhibitory potency is observed. It should be noted that the polyamines by themselves produce a small (5–12%) stimulation of *in vitro* RNA synthesis. Thus, it appears that the presence of the quinoline ring leads to intercalation of a significant number of drug molecules whose side chains block the minor groove of the DNA helix. This would sterically interfere with the approach and binding of the enzyme. Alternatively, inhibition of the local melting of DNA necessary for the process of transcription might be occurring as a result of binding and intercalation of the drug.

The effect of 4-amino-7-chloroquinoline, which lacks a side chain, on the RNA polymerase reaction is very poor (Table IV). This observation complemented with the rest of the data in Table IV reveals that the role of the side chain is also critically important. The almost nonexisting activity of 4-amino-7-chloroquinoline and the rather poor performance of chloroquine as an inhibitor of the RNA polymerase reaction have been increased up to 35 and 4 times, respectively, by the introduction of spermine as side chain (compound **5**, Table IV).

The bisquinolyl compounds proved to be the most effective inhibitors of the RNA polymerase reaction in this series. It appears from the Tm data that only one of the rings is involved in the intercalative process; therefore, the unexpected increase in activity in this class of compounds might be explained on the basis of a steric hindrance to the approach of the enzyme to the DNA surface by the second ring which may project into the minor groove in a trans-like position with respect to the intercalated ring. Another possibility is that the second quinolyl ring interferes with the functioning of the enzyme itself. However, it should be pointed out that the monoquinolyl derivatives do not appear to have a direct inhibitory effect on the enzyme.§

The following points argue in favor of blockade of the minor groove of DNA as the most significant inhibitory action of this series of drugs on the RNA polymerase reaction: (i) increased inhibition of RNA polymerase activity by the bisquinolyl compounds, which cannot be explained by an alteration of the local melting of DNA, since the Tm was actually less than the corresponding monoquinolyl analogs; (ii) dramatic enhancement of the inhibitory potency of 4-amino-7-chloroquinoline by the introduction of the spermine side chain; and (iii) the foregoing argument in relation to the preference of the side chain for the minor groove.

§ Unpublished observation.

Table IV. Relationship of ΔTm to Inhibition of RNA Synthesis by the 7-Chloroquinolyl Derivatives

Compound	ΔTm, °C	Inhibn of RNA polymerase rxn[a] (% decrease from control)
4-Amino-7-chloroquinoline	1	2.20
Chloroquine	12	20
1	12	10
2	14.5	16
3	22.2	47
4	23.1	61
5	24.0	77
6	17.2	98
7	21.5	97
8	20.0	38

[a] RNA polymerase activity was assayed as described in the Experimental Section. The concentration of each drug was 4×10^{-4} *M*. All assays were run in triplicate.

Antimalarial Activity. In the sporozoite-induced chick test,[29] compound **1** was active at 120 mg/kg, **2** was inactive at 120 mg/kg; **3** and **7** were inactive at 160 mg/kg. In the sporozoite-induced mouse test, **2** was active at 320 mg/kg; **3** and **7** were inactive at 320 mg/kg.[30] Sample sizes were inadequate for further tests.

Uv Spectra. The spectra of all mono-*N*-alkyl-substituted quinolines **1–5** show a marked hypochromicity and a bathochromic effect of the order of 8 nm in the region above 280 nm when bound to DNA. Compound **3** (Figure 2) represents a typical example. The bisquinolyl compounds show a smaller bathochromic shift of 5 nm after binding, and the shape of the curve is remarkably similar to that of the mono-N-alkylated quinolines prior to binding (Figure 3). This is in accordance with the previous suggestion that only one ring is intercalated.

It is interesting to point out that the compound 4-amino-7-chloroquinoline showed a significant hypochromicity but absolutely no bathochromic shift after binding to DNA. This suggests that the side chain might be a key factor in the appearance of bathochromic shifts after DNA binding in this series of compounds.

Experimental Section

Melting points were taken in open capillary tubes by use of a Mel-Temp electric block. They are uncorrected. Ir spectra were obtained with a Perkin-Elmer Infracord spectrophotometer. Nmr spectra were obtained with a Varian Associates A-60A spectrometer. Microanalyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich. When indicated, all analytical results were within less than 0.4% of the calculated figures. The melting temperatures of DNA (calf thymus; Worthington) and drug–DNA complexes were determined at 260 nm in 5×10^{-3} *M* Tris-HCl buffer at pH 7.5 by means of a Gilford 2400 recording spectrophotometer equipped with a thermostatically regulated bath. The pH of the buffer was calibrated at room temperature. The Tm for the drug–DNA complexes was determined by the method used by Hahn, *et al.*,[1,4] for the chloroquine–DNA complex. The drugs were used at a concentration of 10^{-5} *M* in solutions containing 20 μg/ml of DNA. Results with some compounds in another buffer[3] were essentially the same. Each experiment was performed simultaneously with four cuvettes with a cell path length of 1 cm. The first cuvette contained buffer, the second one DNA (20 μg/ml), and the third and fourth contained drug plus DNA. Premixing of the drugs with DNA did not cause precipita-

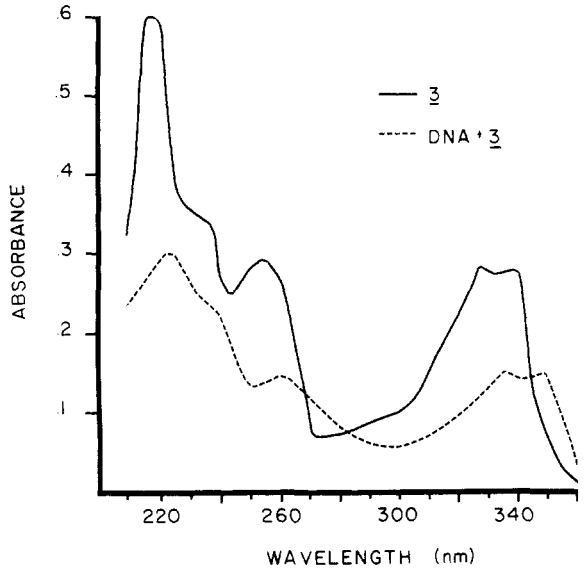


Figure 2. Influence of DNA (40 μg/ml) on the uv spectrum of **3** (2×10^{-5} *M*) in Tris-HCl (pH 7.5).

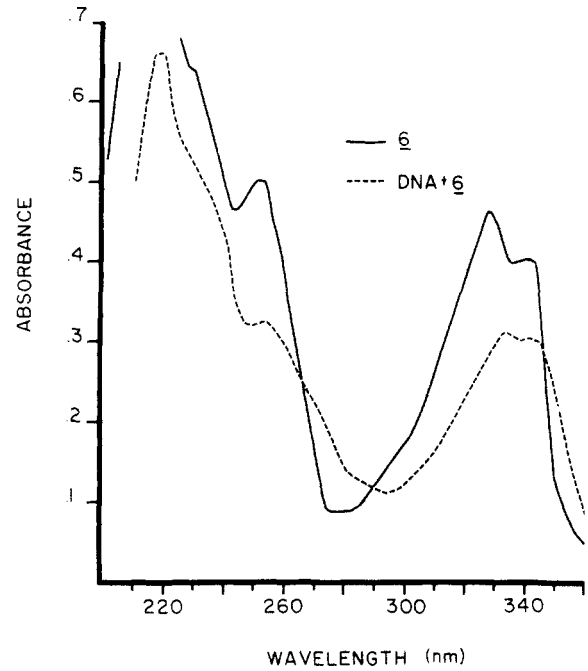


Figure 3. Influence of DNA (40 μg/ml) on the uv spectrum of **6** (2×10^{-5} *M*) in Tris-HCl (pH 7.5).

tion of any kind. The total hyperchromicity for the third and fourth cuvettes after heat denaturation was greater than that for the one containing DNA. In part this may simply reflect the contribution of the drug to the total absorption at 260 nm.

RNA polymerase was prepared from *E. coli* B cells according to the method of Chamberlin and Berg[31] as modified by Richardson.[32] Calf thymus DNA (20 μg) was preincubated with each agent (4×10^{-4} *M*) in 0.4 ml of Tris-HCl (5×10^{-3} *M*, pH 7.5) for 10 min at 37° prior to assay of RNA polymerase activity. The assay mixture (0.4 ml) contained: 28.5 μmol of Tris-HCl (pH 8.0); 42.0 μmol of KCl; 0.84 μmol of dithiothreitol; 0.4 μmol of $MnCl_2$; 100 nmol each of CTP, GTP, and UTP; 25 nmol of ATP; 0.1 μCi of ATP-^{14}C (418 mCi/mmol; New England Nuclear Corp.); 15 μg of polymerase protein; 5 μg of preincubated DNA; and 10^{-4} *M* of each agent to be tested. The assay mixtures were incubated for 10 min at 37°, and the reactions were stopped by the addition of cold 5% TCA. The precipitate was then collected on Millipore filters (0.45-μ pore size), washed three times with 2.5% TCA, dried, and assayed for radioactivity in a Packard Tri-Carb liquid scintillation spectrometer. All assays were run in triplicate. The variation between samples in a given experimental group was routinely less than ±5%.

Ultraviolet spectra were determined by means of a Cary 14 recording spectrophotometer. In each case, the solution to be analyzed contained (unless otherwise indicated) 2.5×10^{-5} *M* drug, 40 μg/ml of DNA, and 5 m*M* of Tris-HCl (pH 7.5).[4] The reference blank contained just DNA in Tris-HCl buffer. The cell path length was 1 cm. Only those bands of the spectra above 280 nm are significant for the observation of any effect in view of the fact that below 280 nm the strong absorption of DNA in both the sample and reference cells results in very wide slit widths, thus indicating that this region of the spectra does not give accurate results.

7-Chloro-4-(3-aminopropylamino)quinoline (1). Method a. Monoacetyl-1,3-diaminopropane was prepared following the method of Tabor, *et al.*, for monoacetyl-1,4-diaminobutane hydrochloride.[21] A mixture of the monoacetylated diamine hydrochloride (37.6 g, 0.189 mol) and 4,7-dichloroquinoline (48.3 g, 0.145 mol) was heated with stirring for 12 hr under nitrogen in the presence of 49 ml of quinoline used as a solvent. The mixture was taken up in hot methanol, treated with an excess of water and concentrated NH_4OH, and extracted with chloroform. Drying over Na_2SO_4 and evaporating the solvent gave a solid which was triturated with acetone and dried. The collected crude solid (43 g, 82%) was immediately hydrolyzed in 625 ml of 6 *N* HCl for 4 hr. The reaction mixture was then made basic with 20% NaOH and extracted with chloroform. After the solution had been dried (Na_2SO_4), the solvent was removed and the oily residue was dissolved in benzene. Precipitation of the product was induced by the addition of petroleum ether. It was further recrystallized from a mixture of benzene and petroleum ether giving 25.5 g (71%) of white crystalline **1**: mp 80–81°; ir (KBr) 3.00 (strong, br, NH_2), 6.20 (medium), 6.30 (strong), 6.50 (medium), 7.50 (medium), 7.75 (medium), 8.75 (medium), 10.75 (medium), 10.95 (medium), 11.40 (medium), 11.60 (medium), 12.40 (medium), 12.95 (medium), and 13.05 μ (medium); nmr ($CDCl_3$) τ 7.50 (m, 2, *J* = 7 Hz, $HNCH_2CH_2CH_2NH_2$), 6.30 (m, 7, $HNCH_2CH_2CH_2NH_2$), 3.15 (d, 1, *J* = 7 Hz, arom), 2,30 (m, 1, arom), 2.08 (d, 1, *J* = 2 Hz, arom), 1.80 (m, 1, arom), and 1.59 (d, 1, *J* = 7 Hz, arom). *Anal.* ($C_{12}H_{14}ClN_3$) C, H, N.

Method b. To a solution of 1 g (0.00434 mol) of **9** in 5 ml of methanol, 0.5 g of Raney nickel catalyst powder (No. 2813, W. R. Grace & Co.) was added and the suspension was vigorously stirred for 10 min. A solution of 0.302 g of $NaBH_4$ in 1.32 ml of 25% NaOH was added dropwise while the temperature was maintained at 50°. After evolution of gas ceased (15 min), the catalyst was filtered off and washed with methanol, and the filtrate was reduced to dryness *in vacuo*. Following treatment with hot benzene, the product was dissolved whereas the starting material remained out of solution. Compound **1** was precipitated by the addition of petroleum ether; it was collected on a filter and dried giving 0.5 g (49%) of **1** identical with the compound obtained previously by method a.

7-Chloro-4-(4-aminobutylamino)quinoline (2). Following a similar experimental procedure as for compound **1** starting with monoacetyl-1,4-diaminobutane hydrochloride[21] and 4,7-dichloroquinoline, compound **2** was obtained in 64% yield. Recrystallization from benzene and petroleum ether gave pure **2** as white crystals: mp 116–118°; ir (KBr) 3.00 (strong, br, NH_2), 6.20 (medium), 6.30 (strong), 6.50 (medium), 7.30 (medium), 7.50 (medium), 8.80 (medium), 11.00 (medium), 11.65 (medium), 12.35 (medium, br), and 13.00 μ (medium). *Anal.* ($C_{13}H_{16}ClN_3$) C, H, N.

7-Chloro-4-[3-(2-cyanoethylamino)propylamino]quinoline (11). To 15.7 g (0.0669 mol) of **1** dissolved in 150 ml of ethanol, 4 g (0.0754 mol) of acrylonitrile was added and the mixture was stirred and heated mildly until the reaction started. Stirring was continued for 18 hr at room temperature and further on a steam batch for 2 more hours. Solvent was removed under reduced pressure and the residue solidified on standing. The compound was recrystallized from benzene giving 17.3 g (90%) of **11**: mp 76–79°; ir (KBr) 3.10 (strong, br, NH), 4.45 (weak, CN), 5.95 (weak), 6.20 (medium), 6.30 (strong), 6.45 (medium), 7.30 (medium), 7.50 (medium), 7.75 (medium), 8.00 (medium), 8.75 (medium), 8.95 (medium), 10.95 (medium), 11.15 (medium), 11.60 (medium), 12.05 (medium, broad), 12.40 (medium), 12.80 (medium), and 12.95 μ (weak); nmr (DMSO-d_6) τ 8.20 (m, 2, *J* = 7 Hz, $HNCH_2CH_2CH_2NH$), 7.00 (m, 10, $HNCH_2CH_2CH_2NH$), 7.00 (m, 10, $HNCH_2CH_2CH_2NHCH_2CH_2CN$), 3.60 (d, 1, *J* = 6 Hz, arom), 2.60 (m, 1, arom), 2.20 (d, 1, *J* = 2 Hz, arom), 1.80 (d, 1, *J* = 9 Hz, arom), and 1.59 (d, 1, *J* = 6 Hz, arom). *Anal.* ($C_{15}H_{17}ClN_4 \cdot H_2O$) C, H, N, H_2O.

7-Chloro-4-[4-(2-cyanoethylamino)butylamino]quinoline

(12). To 6.5 g (0.026 mol) of 2 dissolved in 100 ml of ethanol, 1.5 g (0.0272 mol) of acrylonitrile was added and the reaction carried out as previously. Compound 2 was recrystallized twice from benzene giving 6 g (76%): mp 99–101°; ir (KBr) 3.00 (strong, br, NH), 4.40 (weak, CN), 6.10 (weak), 6.20 (medium), 6.30 (strong), 6.50 (medium), 7.30 (medium), 7.40 (medium), 6.30 (strong), 6.50 (medium), 7.30 (medium), 7.40 (medium), 7.50 (medium), 8.80 (medium), 11.00 (medium), 11.25 (medium), 11.65 (medium), and 12.20 μ (medium). *Anal.* ($C_{16}H_{19}ClN_4$) C, H, N.

7-Chloro-4-[3-(3-aminopropylamino)propylamino]quinoline (3). a. From Catalytic Reduction of 11. W-2 Raney nickel catalyst (1.2 g) was added to a solution of 4 g (0.0136 mol) of 11 in 20 ml of 10% alcoholic ammonia. The mixture was shaken under a pressure of 10 psi until the expected amount of hydrogen was taken up. The catalyst was filtered off, the solvent was removed under reduced pressure, and the oily residue was dissolved in benzene. The compound precipitated upon addition of petroleum ether and cooling. After recrystallization from benzene–petroleum ether, 1.25 g (30%) of 3 was collected: mp 82–83°; ir (KBr) 3.00 (strong, br, NH_2), 6.20 (medium), 6.30 (strong), 6.45 (medium), 6.85 (medium), 7.30 (medium), 7.45 (medium), 8.75 (medium), 8.95 (medium), 10.95 (medium), 11.40 (medium), 11.60 (medium), 12.00 (medium), 12.20 (weak), 12.40 (medium), 12.80 (medium), and 13.00 μ (medium); nmr ($CDCl_3$) τ 8.20 (m, 8, $HNCH_2CH_2CH_2NHCH_2CH_2CH_2NH_2$; D_2O exchange: m, 4, $DNCH_2CH_2CH_2DNCH_2CH_2CH_2ND_2$), 7.32 (t, 2, J = 7 Hz, CH_2NHCH_2), 7.25 (t, 2, J = 7 Hz, CH_2NHCH_2), 7.18 (t, 2, J = 5 Hz, CH_2NH_2), 6.70 (t, 2, J = 6 Hz, CQ-$NHCH_2CH_2CH_2NH_2$), 3.79 (d, 1, J = 5 Hz, arom), 2.80 (m, 1, J = 9 Hz, J' = 2 Hz, arom), 2.38 (d, 1, J = 9 Hz, arom), 2.15 (d, 1, J = 2 Hz, arom), and 1.60 (d, 1, J = 5 Hz, arom). *Anal.* ($C_{15}H_{21}ClN_4$) C, H, N.

b. From Rearrangement of 8. To a solution of 0.5 g of 8 in a few milliliters of a water–ethanol (1:1) mixture was added a few drops of 25% NaOH (enough to make the pH basic to litmus). The solution was refluxed for 24 hr and after cooling and adding more 25% NaOH, the cloudy mixture was extracted with chloroform. The chloroform extracts were dried (Na_2SO_4) and reduced to dryness and the quantitative amount of crude 3 remained as a solid, mp 80°, which possessed an ir spectrum completely superimposable with that of 3 obtained by method a.

7-Chloro-4-[4-(3-aminopropylamino)butylamino]quinoline (4). W-2 Raney nickel catalyst (1.2 g) was added to a solution of 6 g (0.0195 mol) of 12 in 20 ml of 10% alcoholic ammonia. The mixture was shaken at 10 psi of hydrogen until 4 psi were taken up. The catalyst was filtered off, the solvent was removed under reduced pressure, and the oily residue solidified on standing. It was dissolved in chloroform and the solution shaken with water several times to remove impurities responsible for a greenish color. The chloroform layer was dried and evaporated, and the compound was allowed to stand until it solidified. It was washed several times with petroleum ether to give 3 g (49%) of a white solid 4 of satisfactory putity, mp 78–81°. An analytical sample was obtained my microdistillation at a highly reduced pressure: ir (KBr) 3.00 (strong, br, NH_2), 6.20 (medium), 6.30 (strong), 6.50 (medium), 6.75 (medium), 6.90 (medium), 7.30 (medium), 7.50 (medium), 8.80 (medium), 8.90 (weak), 11.05 (medium), 11.40 (weak), 11.70 (medium), 12.20 (medium, br), and 12.90 μ (medium, br); nmr ($CDCl_3$) τ 8.30 (m, 10, $HNCH_2CH_2CH_2CH_2NHCH_2CH_2CH_2NH_2$; D_2O exchange: m, 6, $DNCH_2CH_2CH_2CH_2NDCH_2CH_2CH_2ND_2$), 7.30 (m, 6, $HNCH_2CH_2CH_2CH_2NHCH_2CH_2CH_2NH_2$), 6.80 (t, 2, J = 6 Hz, CQ-$NHCH_2$), 3.70 (d, 1, J = 5 Hz, arom), 2.75 (m, 1, J = 9 Hz, J' = 2 Hz, arom), 2.25 (d, 1, J = 9 Hz, arom), 2.10 (d, 1, J = 2 Hz, arom), and 1.55 (d, 1, J = 5 Hz, arom). *Anal.* ($C_{16}H_{23}ClN_4$) C, H, N.

7-Chloro-4-[3-[4-(3-aminopropylamino)butylamino]propylamino]quinoline (5). Spermine (5 g, 0.0247 mol) was preheated to 120° and 4,7-dichloroquinoline (1 g, 0.005 mol) was added in small portions under vigorous stirring for a period of 0.5 hr. The reaction was continued for 1 hr, cooled immediately after, and treated with a small amount of hot methanol. After the addition of water and 25% NaOH to the methanolic solution, the mixture was extracted with chloroform, the extract was dried, and a residue obtained after removal of chloroform was vacuum distilled to eliminate excess spermine. The semisolid residual product was taken up in hot benzene and precipitated with petroleum ether. This procedure was repeated several times until 0.7 g (38%) of a white solid was obtained: mp 62–64°. Although its ir and nmr spectra are in perfect agreement with the structure, analytical data indicated that the sample was slightly contaminated with spermine: ir (KBr) 3.00 (strong, br, NH_2), 6.20 (medium), 6.35 (strong), 6.50 (medium), 7.35 (medium), 7.55 (medium), 8.80 (medium, br), 11.00 (weak), 11.65 (medium), 12.40 (medium, br), and 12.90 μ (weak, br); nmr ($CDCl_3$) τ 8.35 (m, 8, $HNCH_2CH_2CH_2NHCH_2CH_2CH_2CH_2NHCH_2CH_2CH_2NH_2$), 7.7 (s, 5, $HNCH_2CH_2CH_2NHCH_2CH_2CH_2CH_2NHCH_2CH_2CH_2NHCH_2CH_2CH_2NH_2$), 7.35 (m, 10, $HNCH_2CH_2CH_2NHCH_2CH_2CH_2CH_2NHCH_2CH_2CH_2NH_2$), 6.7 (t, 2, J = 6 Hz, $CQNHCH_2$), 3.80 (d, 1, J = 5 Hz, arom), 2.80 (m, 1, J = 9 Hz, J' = 2 Hz, arom), 2.30 (d, 1, J = 9 Hz, arom), 2.10 (d, 1, J = 2 Hz, arom), and 1.60 (d, 1, J = 5 Hz, arom).

1,4-Bis[3-(7-chloro-4-quinolylamino)propylamino]butane (7). In a nitrogen atmosphere, 4 g (0.02 mol) of 4,7-dichloroquinoline was melted at 100°. Immediately after, 5 g (0.0247 mol) of spermine was added slowly and the temperature was maintained at about 145°. After 2 hr, a solid white mass was formed and the reaction was terminated followed by treatment with hot methanol. The methanolic solution was poured into a chilled 25% NaOH solution. The precipitate which formed was washed with water and dried. The solid was washed further with benzene and finally was recrystallized from acetone giving 4 g (37%) of 7: mp 128–130°; ir (KBr) 2.80–3.00 (strong, br, NH), and 6.06 μ (strong); nmr (CF_3COOH) τ 8.00 (m), 7.68 (m), 6.40 (m), 3.20 (d, 2, J = 6 Hz), and 2.20 (m); calculated ratio of aliphatic/aromatic protons, 2.00; found, 1.54. *Anal.* ($C_{28}H_{34}Cl_2N_6$) C, H, N.

Bis[3-(7-chloro-4-quinolylamino)propyl]imine (6). Starting with 3,3′-iminobis(propylamine), the procedure used for compound 7 was followed yielding 16.7% of 6 which was recrystallized from benzene: mp 190–191°; nmr (CF_3COOH) τ 7.60 (m), 6.60 (m), 6.25 (m), 3.20 (d, 2, J = 6 Hz), and 2.20 (m); calculated ratio of aliphatic/aromatic protons, 1.20; found, 1.18. *Anal.* ($C_{24}H_{25}Cl_2N_5$) H, N; C: calcd, 63.43; found, 62.93.

7-Chloro-4-(2-cyanoethylamino)quinoline (9). A mixture of 5.9 g (0.029 mol) of 4,7-dichloroquinoline, 8.5 g (0.069 mol) of β,β'-iminodipropionitrile, and 4 g of phenol was heated at 140° for 1 hr. The white semisolid mass which formed after cooling was taken up in 1 N acetic acid and extracted with benzene. The acid layer was made basic and extracted with ethyl acetate several times. The ethyl acetate extracts were combined, washed with 0.1 N acetic acid and water, dried (Na_2SO_4), and reduced to dryness. The solid obtained was recrystallized from ethyl acetate giving 2 g (31.5%) of 9: mp 174–175°; ir (KBr) 2.90 (medium, NH), 4.45 (weak, CN), 6.20 (medium), 6.35 (strong), 6.50 (medium), 6.90 (medium), 7.00 (medium), 7.35 (medium), 7.50 (medium), 7.80 (weak), 8.00 (weak), 8.25 (medium), 8.55 (weak), 8.75 (medium), 9.25 (weak), 11.00 (weak, br), 11.30 (weak), 11.50 (weak), 12.25 (medium), and 13.00 μ (weak, br); nmr ($CDCl_3$–CF_3OOH) τ 6.90 (t, 2, J = 7 Hz, CH_2CN), 5.90 (q, 2, J = 6 Hz, $HNCH_2$), 2.90 (d, 1, J = 7 Hz, arom), 2.35 (q, 1, J = 9 Hz, J' = 2 Hz, arom), 2.10 (d, 1, J = 2 Hz, arom), 1.75 (d, 1, J = 9 Hz, arom), and 1.50 (d, 1, J = 7 Hz, arom). *Anal.* ($C_{12}H_{10}ClN_3$) C, H, N.

7-Chloro-4-bis(2-cyanoethyl)aminoquinoline (10). A mixture of 5.9 g (0.029 mol) of 4,7-dichloroquinoline, 8.5 g (0.069 mol) of β,β'-iminodipropionitrile, and 4 g of phenol was heated at 140° for 0.5 hr. The mixture was taken up in 0.1 N acetic acid and extracted with benzene several times. The benzene layer was washed with 25% NaOH and water and dried (Na_2SO_4), and the solid recovered was triturated with ethyl ether. The ether-insoluble material was collected, washed thoroughly with ether, and recrystallized from ethyl acetate–ether to give 1.26 g (15%) of 10: mp 88°; ir (KBr) 4.40 (medium, CN), 6.20 (medium), 6.30 (strong), 6.35 (strong), 6.60 (medium), 7.50 (medium), 7.65 (medium), 7.80 (medium), 7.90 (medium), 8.60 (medium), 9.60 (strong), 11.50 (strong), 11.90 (strong), and 12.00 μ (strong); nmr ($CDCl_3$) τ 7.50 [t, 4, J = 5 Hz, $(CH_2CN)_2$], 6.25 (t, 4, J = 5 Hz, CH_2NCH_2), 2.95 (d, 1, J = 4 Hz, arom), 2.50 (m, 1, J = 7 Hz, J' = 2 Hz, arom), 1.95 (m, 2, arom), and 1.20 (d, 1, J = 4 Hz, arom). *Anal.* ($C_{15}H_{13}ClN_4$) C, H, N.

7-Chloro-4-bis(3-aminopropyl)aminoquinoline (8). A solution of 2.84 g (0.01 mol) of 10 in 10% methanolic ammonia was shaken in a Parr hydrogenator at 20 psi for 8 hr in the presence of 3 g of W-2 Raney nickel. The suspension was filtered and the filtrate reduced to dryness giving 2.2 g (75%) of 8 as a pale yellow oil: ir (neat) 3.00 and 3.10 (medium, NH_2), 3.45 and 3.55 (strong, aliphatic), 6.25 (medium), 6.40 (strong), 6.70 (medium), 7.00 (strong), 7.30 (medium), 7.70 (medium), 8.90 (medium, br), 9.30 (weak, br), 9.50 (weak, br), 11.35 (strong, broad), 12.00 (strong, broad), and 13.00 μ (medium, br); nmr ($CDCl_3$) τ 8.60 [s, 4, $(CH_2NH_2)_2$], 8.25 [q, 4, J = 6 Hz, $(CH_2CH_2CH_2NH_2)_2$], 7.40 [t, 4, J = 6 Hz, $(CH_2CH_2CH_2NH_2)_2$], 6.70 [m, 4, J = 6 Hz, J' = 1 Hz, $(CH_2CH_2CH_2NH_2)_2$], 3.20 (d, 1, J = 7 Hz, arom), 2.60 (m, 1, J = 9 Hz, J' = 2 Hz, arom), 2.00 (m, 2, arom), and 1.70 (m, 1, J' = 2 Hz, arom).

Part of the oil was dissolved in methanolic HCl and the prod-

uct precipitated with ether. The hygroscopic yellow hydrochloride salt was suspended in benzene and the water was removed by azeotropic distillation for 2 hr with a trap. The material was no longer hygroscopic. Recrystallization from methanol–ether gave a white hydrated salt, mp 230–232°. *Anal.* ($C_{25}H_{21}ClN_4 \cdot 3HCl \cdot 0.5H_2O$) C, H, N.

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Potential Inhibitors of *S*-Adenosylmethionine-Dependent Methyltransferases. 1. Modification of the Amino Acid Portion of *S*-Adenosylhomocysteine

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Structural analogs of *S*-adenosyl-L-homocysteine (L-SAH), with modifications of the amino acid portion of the molecule, have been synthesized and their abilities to inhibit catechol *O*-methyltransferase, phenylethanolamine *N*-methyltransferase, histamine *N*-methyltransferase, and hydroxyindole *O*-methyltransferase have been investigated. The data from these inhibition studies have resulted in a delineation of the structural features of SAH which are required for enzymatic binding of this ligand. In general, it was concluded that the terminal amino group, the terminal carboxyl group, and the sulfur atom of the homocysteine portion of SAH are required for maximum binding of SAH by these enzymes. The L configuration of the asymmetric amino acid carbon of SAH is generally required to produce maximum inhibition. The exception appears to be the potent inhibition of histamine *N*-methyltransferase by D-SAH. D-SAH was substantially less effective as an inhibitor of the other enzymes tested. The significance of these data relative to the nature of the SAH binding sites is discussed.

Since the discovery of *S*-adenosyl-L-methionine[1] (SAM),† a great variety of SAM-dependent biological transmethylation reactions have been demonstrated.[2] A general feature of many SAM-dependent methyltransferases is the inhibition produced by the demethylated product, *S*-adenosyl-L-homocysteine (L-SAH). This product inhibition by L-SAH suggests not only a new class of potential inhibitors for methyltransferases but also suggests a possible biological regulatory mechanism. Evidence to support a L-SAH mediated regulatory mechanism was recently reported by Barchas and Deguchi,[3] who observed that L-SAH is a potent inhibitor of several methyltransferases and that a stimulating factor for these enzymes in the rat brain is adenosylhomocysteinase, which degrades L-SAH.

L-SAH has been reported to inhibit the activity of many methylating enzymes, such as catechol *O*-methyltransferase (COMT),[3-5] phenylethanolamine *N*-methyltransferase (PNMT),[3] histamine *N*-methyltransferase (HMT),[6,7] hydroxyindole *O*-methyltransferase (HIOMT),[3,6] tRNA methyltransferase,[8-10] *S*-adenosylmethionine-glycine *N*-methyltransferase[8] and indolethylamine *N*-methyltransferase.[11] Various structural modifications of L-SAH have been carried out in an attempt to elucidate the specificity of the enzymatic binding site for SAH and to develop potential inhibitors of COMT[5,12,13] and tRNA methyltransferase.[9,10,14,15] In an attempt to detect differences in the

†Abbreviations used are: SAM, *S*-adenosyl-L-methionine; L-SAH, *S*-adenosyl-L-homocysteine; D-SAH, *S*-adenosyl-D-homocysteine; SAHO, *S*-adenosyl-L-homocysteine sulfoxide; $SAHO_2$, *S*-adenosyl-L-homocysteine sulfone; COMT, catechol *O*-methyltransferase (E.C. 2.1.1.6); HMT, histamine *N*-methyltransferase (E.C. 2.1.1.8); HIOMT, hydroxyindole *O*-methyltransferase (E.C. 2.1.1.4); PNMT, phenylethanolamine *N*-methyltransferase (E.C. 2.1.1.1); K_{is}, inhibition constant for the slope.