over each other for all the active isomers. This superimposibility is also shown by comparison of distances between key features of the proposed pharmacophore as shown in Table I. Since there are no low-energy 2-*SS* axial isomers, no 2-*SS* (or *RR*) isomer can superimpose on this pattern. No conformer of the **3** compounds can be matched to the pattern shown in Figures 4 and 5, thus possibly accounting for their activity.

The active conformation of each molecule is such that it allows a hydrogen bond to form between the OH and amine functions. The existence of a hydrogen bond is not necessarily required for activity but helps to stabilize each active molecule in the conformation that contains the proposed pharmacophore. Cheng[16] has also suggested that the axial conformation of compound **2** must be invoked to explain its stereospecific activity and uses mass spectral data to lend support to this conclusion. Our results, based on direct conformational analysis, independently describe the same pharmacophore to account for the stereospecificity of the **2** isomers, the lack of it in the **1** isomers, and the inactivity of the **3** isomers. They further point to the 2-*SS* and 2-*RR* as the inactive forms, a prediction that can be tested by experiment.

Subject to further verification then, our results have led to a description of a pharmacophore for this subclass of antimalarial drugs which can account for their observed behavior and is similar in dimension and character to that proposed by Cheng.

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Synthetic Models of DNA Complexes with Antimalarial Compounds. 2. The Problem of Guanine Specificity in Chloroquine Binding

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Stacking interactions between the aminoquinoline ring of the antimalarial chloroquine and the purine bases have been studied by preparing and examining models in which the quinoline is linked to the base by a trimethylene chain. The degree of stacking of the models which reflects the strength of the interaction was quantitatively determined in water at different temperatures by hypochromism measurement in the uv. Adenine and guanine exhibit equal affinity for the quinoline nucleus as reflected by very close hypochromism values observed for the two models at all temperatures studied.

Chloroquine (**1**),[1] a widely used antimalarial, inhibits several biological functions of plasmodia, the microorganism responsible for the disease, acting at the level of the DNA and RNA polymerases and interfering with protein synthesis.[2] Its mode of action involves binding to the nucleic acids; the influence of this on nucleic acid biosynthesis has been demonstrated in vitro.[3]

The complexation of chloroquine with DNA[4] and with synthetic polynucleotides[5] has been studied. Two types of interaction are involved in complex formation, an electrostatic attraction between the amine group (protonated at physiological pH[6]) of the chloroquine side chain and the phosphate groups of the DNA, and a more specific interaction between the aromatic system of chloroquine and the nucleotide bases.[7] It has been suggested that the protonated aminoquinoline ring is actually intercalated between base pairs in the DNA helix.[8]

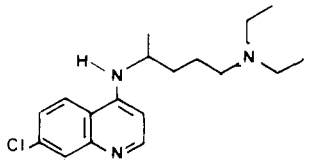
As an approach to the problem of the ring–ring interactions which may take place in the intercalation process, we studied the stacking interactions between the aminoquinoline ring of chloroquine and the nucleotide bases. Models of the form B-C_3-Q (**2**, **3**, and **4**) were prepared, in which the aminoquinoline Q and the base B are joined by a trimethylene chain;[9] such molecules are capable of adopting a folded conformation in which the two aromatic systems interact. The proportion of molecules in the folded conformation will be an indication of the affinity of quinoline for the base. In a previous publication[10] we have thus reported a study of the interaction of aminoquinoline with a purine (adenine) and a pyrimidine (thymine); the extent of interaction with adenine was much greater than that with thymine. This result is in agreement with studies of chloroquine–polynucleotide complexation[4,5] which demonstrated only a weak affinity of the quinoline nucleus for pyrimidine bases. However, the studies of the complexation of chloroquine with polynucleotides did not clearly establish the role of the purine bases, adenine and guanine.

An ambiguity arises from the fact that most of these studies were based on measurements of the perturbation "hypochromicity"[11] in the uv absorption of chloroquine in the complex. The degree of perturbation depends on the nature of the polynucleotide, being stronger for polyG than for polyA, for polydGdC than for polydAdT, and increasing with the (G–C) content in a series of natural DNA's from different sources, while the equilibrium

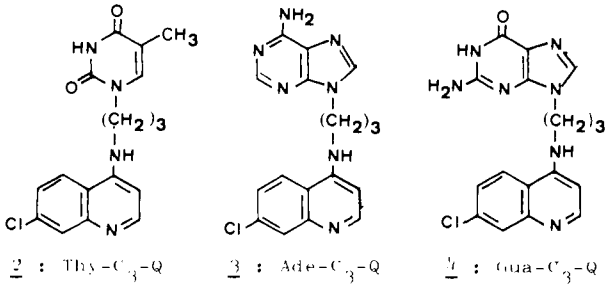
constants are only slightly dependent of the nature of the base.

According to some authors,[5b] the difference observed in the spectrum of chloroquine in the presence of bases is not related to difference in binding; the two bases interact approximately equally with the drug, but guanine exhibits a greater perturbation effect on the chloroquine spectrum as compared with adenine, while, according to others,[5a] the difference reflects a specificity for guanine in the binding. Thus the question remains whether chloroquine shows a specific affinity for guanine or whether for purines in general.

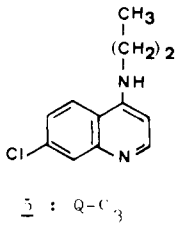
In order to study the ability of monomeric adenine and guanine to complex with the aromatic ring of chloroquine and measure the corresponding perturbations in the uv spectra, we have prepared the model systems **3** and 4 in which the quinoline is linked to adenine and guanine, respectively. The spectroscopic properties of these compounds have been compared with those of the reference substances **5**, **6**, and **7**. We first describe the synthesis of compounds **3**–**7** and then report a study of



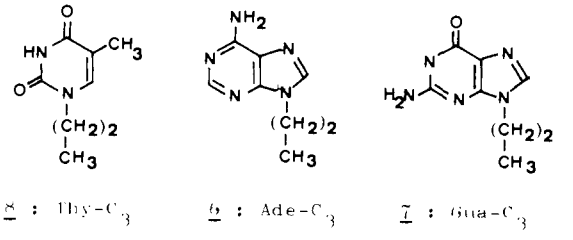
1



2 : Thy-C_3-Q 3 : Ade-C_3-Q 4 : Gua-C_3-Q



5 : Q-C_3

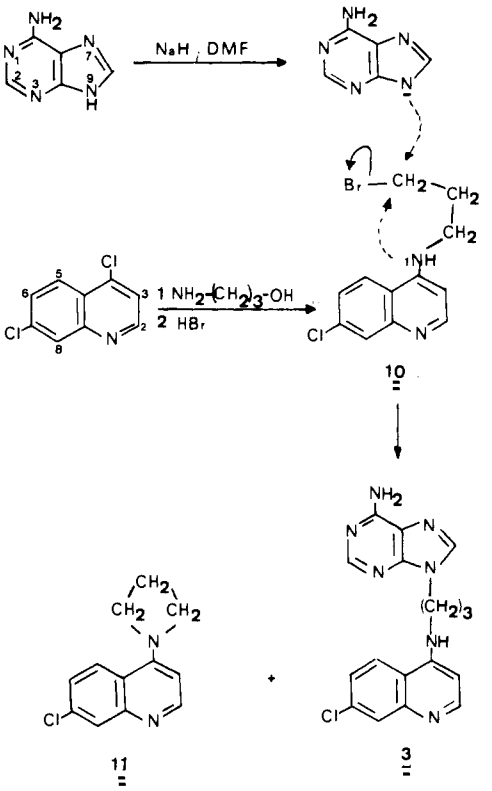


8 : Thy-C_3 6 : Ade-C_3 7 : Gua-C_3

their spectroscopic properties.

Synthesis. Adenine Model, Ade-C_3-Q (3). The nucleophilicity of the anion formed at position 9 of adenine in strongly basic media was used to prepare the 9-alkyl derivatives. The Ade-C_3-Q system could thus be prepared by two different routes. The first one involved bromide displacement from 4-(3-bromopropylamino)-7-chloroquinoline (Q-C_3-Br + Ad^-) (route 1). In the latter, the adenine anion was used to prepare the intermediate 9-(3-aminopropyl)adenine (Ade-C_3NH_2) which was then allowed to react with 4,7-dichloroquinoline (Ade-C_3-NH_2 + Q-Cl) (route 2). A synthesis of the latter type has already been employed by Leonard[12,13] to prepare coenzyme and dinucleotide (Ade-C_3-Ar) models.

Scheme I



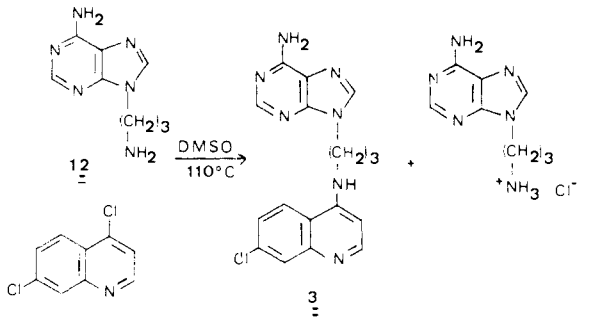
Route 1 (Scheme I). Reaction of excess 3-aminopropanol with 4,7-dichloroquinoline (130 °C, 12 h) gave 4-(3-hydroxypropylamino)-7-chloroquinoline (**9**) in 75% yield. The hydroxyl band appears at 3370 cm^{-1} in the ir. The NMR spectrum shows signals characteristic of the aromatic system: -OH at 4.1 ppm, a triplet at 4.58 ppm due to the hydroxyl-bearing methylene, and a signal at 3.55 ppm due to the methylene group adjacent to the amino function, coupled to the amino proton.

Treatment of **9** with 48% HBr at 120 °C gave 4-(3-bromopropylamino)-7-chloroquinoline hydrobromide (yield 50%) which was converted to the free base **10** (Q-C_3-Br) using an ion-exchange resin. The NMR of the hydrobromide shows a broad signal at 9.5 ppm for the $-NH^+$. The bromomethylene protons and the methylene protons adjacent to the amino group appear together at 3.7 ppm.

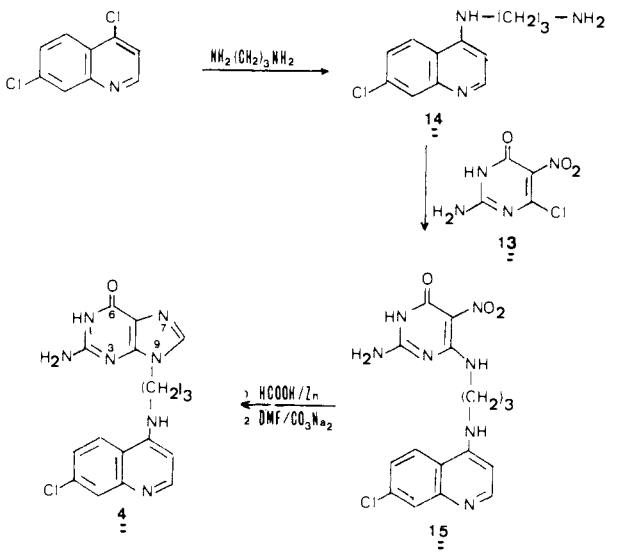
Reaction of adenine with sodium hydride in dimethylformamide at room temperature followed by the addition of the bromo compound **10** gave the desired 4-[3-(aden-9-yl)propylamino]-7-chloroquinoline (**3**, Ade-C_3-Q) in 9% yield. The ir spectrum shows bands characteristic of the adenine (1680 and 1650 cm^{-1}) and 4-aminoquinoline (3300 and 1590 cm^{-1}) systems. The NMR spectrum (Me_2SO) shows signals characteristic of the unprotonated quinoline present in the Q-C_3-X system: two sharps peaks near 8 ppm due to the H-2 and H-8 protons of adenine and the methylene adjacent to the adenine ring appears at 4.26 ppm as in 9-propyladenine.

A second product, isolated in 21% yield, was identified (no NH in the ir spectrum; NMR, four equivalent protons geminal to nitrogen, appearing as a triplet at 4.32 ppm) as the 4-(azetid-1-yl)-7-chloroquinoline (**11**) arising from an intramolecular reaction.[14] It is interesting that a similar intramolecular reaction of the homologue 4-bromobutylaminoquinoline, the precursor of a large number of

Scheme II



Scheme III



chloroquine analogues,[15] has never been observed.

Because of the strong allergenic properties of **10** noted during this work, the second route was developed as an alternative synthesis.

Route 2 (Scheme II). Reaction of 4,7-dichloroquinoline with 9-(3-aminopropyl)adenine[13] in dimethyl sulfoxide at 110 °C for 14 h gave the desired 4-[3-(aden-9-yl)propylamino]-7-chloroquinoline (**3**, Ade-C_3-Q) in 60% yield. The use of dimethyl sulfoxide considerably increased the efficiency of the reaction, compared to hydroxylic solvents (ethoxyethanol, for example) generally used in this series as reaction medium.[16]

Guanine Model, Gua-C_3-Q (4). Direct alkylation of guanine at the 9 position poses more problems than that of adenine. Firstly, prior protection of the 2-amino group is necessary. Secondly, the alkylation results in a mixture of the 7- and 9-derivatives.[17] For these reasons, it is preferable to carry out the alkylation on a precursor of the guanine system. 2-Amino-4-hydroxy-5-nitro-6-chloropyrimidine (**13**)[18] serves this purpose, requiring subsequent amination by the proper 4-(3-aminopropylamino)-7-chloroquinoline (**14**).

The latter compound was obtained by reaction of 4,7-dichloroquinoline with a large excess of 1,3-diaminopropane (Scheme III). The ir spectrum of the hydrochloride of **14** shows absorptions due to a primary amine salt and the quinoline ring system at 3220, 1620, and 1590 cm^{-1}. The NMR shows signals corresponding to an aminoquinoline; the methylene protons adjacent to the primary amine appear at 2.90 ppm.

The chloropyrimidine **13** was then allowed to react with the latter **14** to give intermediate **15**. The reaction of **13** with **14** with methanol as solvent and triethylamine as base gave low yields due to the limited solubility of the pyrimidine. Using dimethyl sulfoxide and diazabicyclooctane at room temperature, the yield was much improved, the product precipitating as it formed. The ir shows bands due to the amino group (3380 cm^{-1}), quinoline and pyrimidine systems (1610 and 1660 cm^{-1}), and the nitro group (1570 cm^{-1}). The NMR (CF_3CO_2D) shows signals characteristic of the quinoline system; the methylene groups adjacent to the quinoline and pyrimidine rings appear as a broad signal at 3.8 ppm. The nitro group was reduced using zinc in formic acid and then, without isolation of the intermediate amine, the formic acid was replaced with dimethylformamide; heating this solution with potassium carbonate brought about cyclization to the imidazole. The product (Gua-C_3-Q) was isolated as the hydrochloride. The ir spectrum shows bands at 3300 (amine) and 1670 and 1610 cm^{-1} (purine and quinoline rings). The NMR (CF_3CO_2D) shows signals typical of protonated quinoline, a sharp signal at 9.03 ppm due to H-8 of the guanine and a multiplet at 4.63 ppm due to the methylene group adjacent to the guanine ring. The uv spectrum in ethanol is the sum of the spectra of the two chromophores. The reference compound, 9-propylguanine (**7**, Gua-C_3), was prepared by a similar method.[12a]

Physical-Chemical Study. Method. The conformational study of the model systems allows a determination of the affinity of the quinoline nucleus for adenine and guanine. Schematically, there are two types of conformation: in one the purine base and the quinoline are sufficiently distant from one another that there is no interaction between them ("free" or "open" form); in the other the two aromatic rings are in contact ("folded" or "stacked" form).

The uv spectrum of the quinoline nucleus and the purine base should be perturbed in the complexed form as it is in the case of the polynucleotide–chloroquine complex,[4] and comparison of the model systems B-C_3-Q with the reference substances B-C_3 and Q-C_3 should allow a measurement of this perturbation. To conform with the conditions used in the study of the real system, water was selected here as the solvent. Because of limited solubility, and to avoid intermolecular interactions, very dilute solutions must be used, conditions suited to uv spectroscopy.

Results

In a preceding publication[10] we have shown that the uv spectrum of the model system Ad-C_3-Q exhibited strong perturbations, being mainly characterized by a strong decrease in the absorption intensity ("hypochromism"); this was interpreted as a proof of the existence of strong interaction between the two chromophores. This hypochromic effect is indeed observed for systems in which the chromophores are stacked one on another. DNA, RNA, synthetic polynucleotides, and dinucleotides all present a hypochromic effect in the uv.[19] According to Tinoco[20] and Rhodes,[21] the hypochromism is due to the electric field created by the electrons of one chromophore acting on the electronic transition moment of the opposite (absorbing) chromophore. The hypochromic effect is defined by $\% H = [1 - f(\text{B-C}_3\text{-Q})/[f(\text{B-C}_3) + f(\text{Q-C}_3)]]100$, where f is the oscillator strength of the transition, i.e., a measure of the intensity of absorption $f = 4.32 \times 10^{-9} \int [\epsilon(\lambda)/\lambda^2] d\lambda$ where ϵ is the molecular extinction coefficient.

Uv spectra of the model compounds **3** (Ade-C_3-Q) and **4** (Gua-C_3-Q) and of the reference compounds **6** (Ade-C_3), **7** (Gua-C_3), and **5** (Q-C_3) were measured, at pH 7, 25 °C, and approximately 10^{-5} M, in water.[22] As seen in Figure 1, the spectra of the two model systems **3** and **4** exhibit a little shift of the absorption bands to longer wavelength

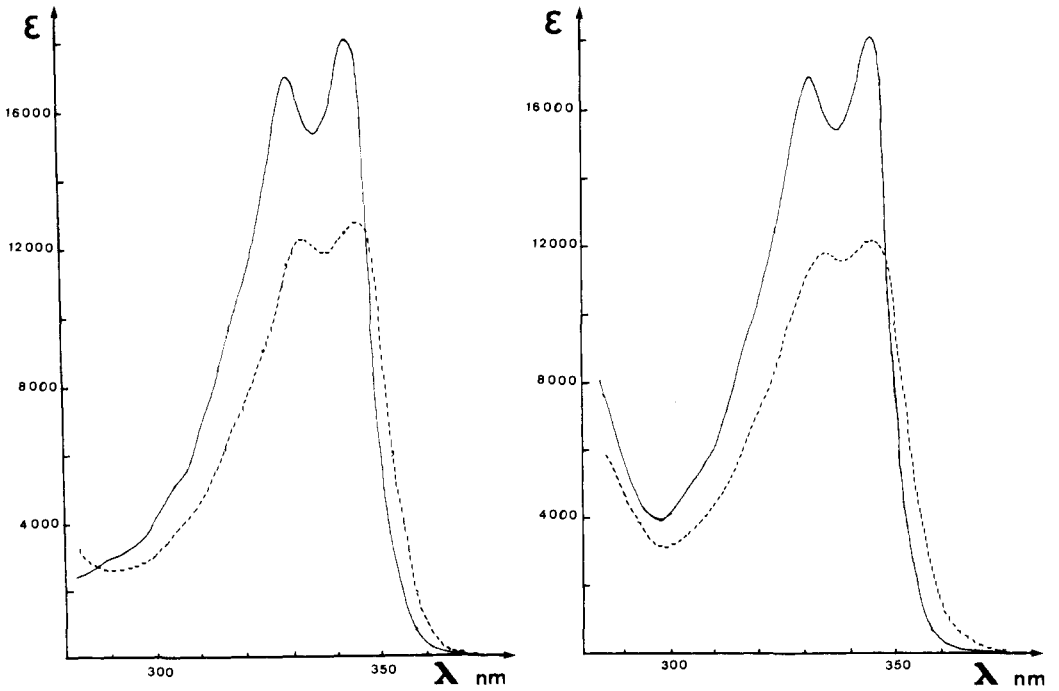


Figure 1. Comparative ultraviolet spectra of model systems (- - -) and reference compounds (—) in water, pH 6.9, 5×10^{-5} M, 25 °C: (left) interaction of adenine-quinoline; (right) interaction of guanine-quinoline.

Table I. Computed Hypochromism Values (% *H*) for the Base–Quinoline Interaction Models, in Water, 20 °C, pH 7, for the 230-300- and 300-380-nm Range

Interaction models	230-300 nm	300-380 nm
Ade-C_3-Q (3)	20 ± 2	25.5 ± 0.5
Gua-C_3-Q (4)	16 ± 2	25.9 ± 0.7
Thy-C_3-Q (2)	5 ± 2	10 ± 1

and a very strong hypochromic effect. A quite similar perturbation is observed for chloroquine in its interaction with DNA.[4e] This means that the molecules exist essentially under a stacked conformation and, hence, prove the affinity of the quinoline nucleus for both purine bases. In addition, it appears that the calculated *H* values are very close for the two models[23] (Table I, *H* = 25.5 and 26%).

Is this result indicative of *equal* affinities of chloroquine for adenine and guanine or, in other words, can hypochromic effects induced by adenine and guanine be compared directly? Very few theoretical studies of the hypochromic effect have been done and do not allow a rigorous answer to this question. Nevertheless, the hypochromic effect has been measured for a large number of biological and analogous systems[12a,b,19] and the conclusions agree well with results obtained from other sources,[24] so that hypochromism can be considered to be directly related to the degree of overlap by stacking of two chromophores and constitutes therefore a semiquantitative measurement of the affinity of two chromophores.

In the systems we have studied, the results seem to show that the quinoline nucleus has equal affinity for guanine and adenine. However, the measurement of the hypochromic effect does not permit the detection of small differences, nor is it clear that the measured values indicate a strong or moderate quinoline–base interaction.

To answer this question it is necessary to analyze the folding–unfolding process for the two models on a more quantitative basis. One convenient approach, although constituting a crude approximation,[25] is to use a two-stage model; the unstacking process is assumed to be a two-state process in which a completely stacked form is in equilibrium with a totally unstacked conformation. This requires the knowledge of the spectroscopic properties of the two forms. While the optical properties of the unstacked form are known from the reference compounds (0% hypochromism), the problem lies in the determination of the hypochromic effect of the totally stacked conformer. One possible approach is to study the B-C_3-Q systems as a function of temperature; if the passage from the open to folded conformation is exothermic, lowering the temperature should increase the latter form. We have measured the hypochromic effect in the region 300–380 nm, where the base does not absorb, for the compounds 3 (Ade-C_3-Q) and 4 (Gua-C_3-Q) as a function of temperature. Measurements were made using sodium phosphate solutions (pH 5.5 at 20 °C) of the compounds at temperatures from −5 to +80 °C.[26] The results are shown in Figures 2 and 3.

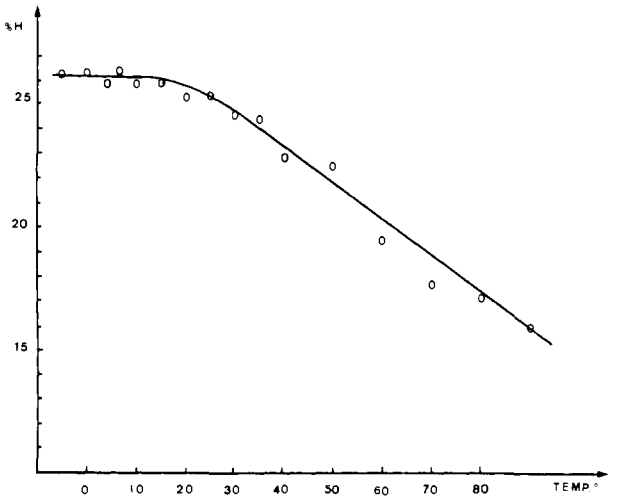


Figure 2. Variation of hypochromism values (% *H*) with temperature for model system 3, Ade-C_3-Q, in water, pH 5.5.

For Ade-C_3-Q, the hypochromic effect is constant from −5 to +15 °C and equal to 26%. This value therefore

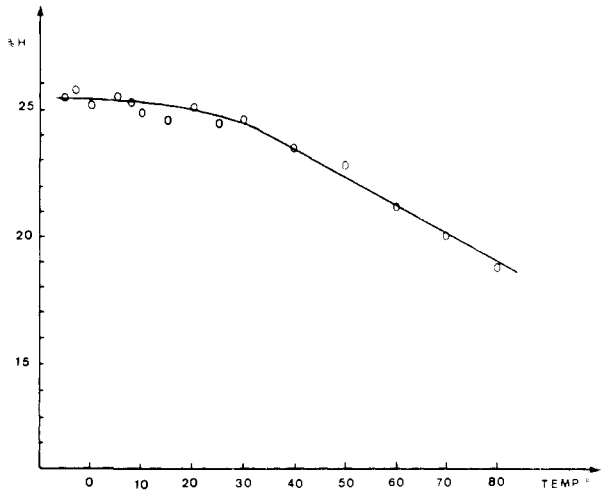


Figure 3. Variation of hypochromism values (% H) with temperature for model system 4, Gua-C_3-Q, in water, pH 5.5.

Table II. Variation of Stacking Interaction with Temperature. Percent of Folded Conformation, Measured for the Three Models, in Water at pH 5.5

	20 °C	40 °C	60 °C
Ade-C_3-Q (3), H_{max} = 26	100 ± 4	88 ± 4	75 ± 4
Gua-C_3-Q (4), H_{max} = 23.5	100 ± 4	92 ± 4	83 ± 4
Thy-C_3-Q (2),[a] $14 \leq H_{max} \leq 26$	40 ≤ % ≤ 80	36 ≤ % ≤ 70	31 ≤ % ≤ 57

[a] The higher value for H_{max} and therefore the lower values for the percent of folded conformation are most probable.

corresponds to H_{max}. After +15 °C, H decreases slowly with increasing temperature; at +90 °C the hypochromic effect (16%) is still strong.

For the case of Gua-C_3-Q, the shape of the curve $H = f(T)$ is virtually identical with an H_{max} = 25.5%, though the temperature at which the compound is no longer 100% in the folded conformation is slightly higher. Here again the decrease of H with temperature is slow.[27] Knowing H_{max}, the equilibrium constant K between open and folded conformations can be found, K = (folded form)/(open form) = $H/(H_{max} - H)$, and the percentage of molecules in the folded conformation at a given temperature is 100 × H/H_{max}. The values found are given in Table II.

At 20 °C, both the adenine and guanine systems are practically 100% in the folded conformation. At higher temperatures the affinity of quinoline seems to be slightly greater for guanine. However, with respect to experimental error the differences are not very significant and the results for the two systems must be considered to be the same. From the equilibrium constants at various temperatures, the enthalpies and entropies changes have been calculated to be $\Delta H°$ = −9 kcal/mol and $\Delta S°$ = −24 eu. The value of $\Delta H°$ is of the same order of magnitude as that found for the interaction of two adenines in a dinucleoside[25b,c] (−10 kcal/mol).

Discussion

We have shown that in the models adenine and guanine exhibit interactions with 4-amino-7-chloroquinoline in neutral aqueous solution, as reflected by the magnitude of the percent hypochromism H.

In the present case where the H values calculated for the two systems at 25 °C are very close, the study of the uv spectra as a function of temperature enables making a comparison on more quantitative bases, due to the fact that a maximum hypochromism value can be reached in both cases at low temperatures.

A first result concerns the strength of the ring–ring interaction; it appears that adenine and guanine complex equally well with quinoline (at least in the geometrical arrangement imposed by the trimethylene chain).

A second conclusion is connected to the relationship between "stacking" and "hypochromism"; adenine and guanine, when stacked with quinoline to the same extent, induce practically the same perturbation on the quinoline spectrum (H = 25% in both cases for 100% stacking). It is to be noted that the constraints which exist in the models impede the relative orientations of the base and quinoline which would give different hypochromic effects, but previous works seem to indicate that differences in the orientation of the chromophores do not lead to drastic changes in the percent hypochromism.[12b]

It is of interest to compare these results with the role of the two bases as defined by biochemical studies on the complexation of chloroquine to nucleic acids. This is the common problem of comparing an exceedingly simplified model to a natural situation. The models have been conceived for the specific study of ring–ring interactions in the absence of all complicating factors which intervene in the stacking of chloroquine in polynucleotides; in the natural binding, such factors as base–base interactions are important, they can create a favorable environment in terms of stereochemistry for quinoline to interact, they create conditions in which the quinoline must compete with the base for interaction; in addition, in the models studied here, the interacting rings are constrained by the trimethylene chain.

The binding of chloroquine to nucleic acids has been most frequently studied by methods comparable to the one outlined here, i.e., by measuring the perturbation of the uv spectrum of chloroquine in the complex.[4,5] The spectral shift and hypochromic effect observed are quite comparable to those described for the present models.

A number of experiments were performed by different authors to study the interaction as a function of base composition.[4e,5a,b] In all cases described (with natural nucleic acids in which the Ade:Gua content is variable, with polyA, polyG, polydAdT, polydGdC), hypochromicity was found to be a function of base composition, increasing with the guanine content of the polymer. From the results of the present study (and with all the limitations of the models as indicated above), it seems that the higher hypochromicity observed for guanine-rich polymers as compared to adenine cannot be attributed to major differences in the intrinsic properties of the purine bases themselves (considered as monomers), neither in terms of differences in their ability to stack with quinoline nor in terms of their spectroscopic properties to induce different hypochromic effects on the quinoline chromophore in a comparable environment.

Experimental Section

All melting points are uncorrected. Infrared spectra were determined on a Perkin-Elmer Model 377 or 237. Uv spectra were obtained using a Cary 15 spectrometer. NMR spectra were recorded on Jeol C 60 H spectrometer using Me_4Si (δ = 0.000) as internal standard. Analyses were performed by the "Service central de microanalyse du Centre National de la Recherche Scientifique".

4-Propylamino-7-chloroquinoline (Q-C_3, 5). A 2.0-g (0.01 mol) portion of 4,7-dichloroquinoline was dissolved in 12.0 g (0.02 mol) of propylamine. The solution was refluxed for 12 h and evaporated in vacuo. Water (100 ml) was added to the residue and the solid was filtered and recrystallized from ethanol–water

Table III. Quantitative Electronic Absorption Data of Model Systems and Reference Compound Q-C_3 (5) in Ethanol and Water

	Ethanol, λ_{max}, nm (ϵ)	Water		
		λ_{max} (0.1 N HCl), nm (ϵ)	λ_{max} (0.05 M phosphate), nm (ϵ)	λ_{max} (0.1 N NaOH), nm (ϵ)
Q-C_3 (5)	330 (11 900), 254 (19 100), 218 (41 700)	343 (18 900), 330 (17 800), 256 (16 800), 220 (35 300)	343 (18 900), 330 (17 600), 256 (16 900), 220 (35 400)	328 (11 300), 254 (18 400), 209 (40 700)
Ade-C_3-Q (3)	327 (12 000), 258 (31 400)	343 (14 500), 332 (13 600), 257 (23 200), 218 (38 300)	345 (12 800), 333 (12 200), 257 (21 100), 218 (35 100)	330 (8800), 255 (23 300)
Thy-C_3-Q (2)	327 (12 300), 258 (25 400)	343 (15 800), 330 (14 600), 257 (20 100)	342 (15 000), 330 (13 800), 256 (19 900), 220 (39 200)	328 (10 700), 254 (20 900)
Gua-C_3-Q (4)	345 (30 600), 332 (16 500), 257 (18 100)	343 (13 600), 332 (12 600), 253 (22 700), 238 (21 600)	345 (12 200), 334 (11 600), 253 (22 200), 238 (21 100)	329 (9800), 255 (26 200)

yielding 1.8 g (80%) of a colorless solid: mp 148–148.5 °C; NMR (Me_2SO-d_6) δ 8.27 (d, 1 H, J = 6 Hz, QC_2H), 8.17 (d, 1 H, J = 9 Hz, QC_5H), 7.70 (d, 1 H, J = 2 Hz, QC_8H), 7.30 (dd, 1 H, J = 2 and 9 Hz, QC_6H), 6.35 (d, 1 H, J = 6 Hz, QC_3H), 3.20 (m, 2 H, $QNHCH_2$), 1.7 (m, 2 H, $QNHCH_2CH_2$), 0.95 (t, 3 H, J = 6 Hz, $QNHCH_2CH_2CH_3$), ir (KBr) 3200, 3060, 2950, 1610, 1580, 1550, 1490, 1470, 1450, 1430, 1370, 1330, 1300, 1270, 1240, 1200, 1150, 1080, 1000, 900, 870, 850, 820, 800, 765 cm^{-1}; uv (EtOH) λ_{max} 218 nm (ϵ 41 000), 253 (19 000), 330 (12 000). Anal. Calcd for $C_{12}H_{13}N_2Cl$: C, 65.31; H, 5.93; N, 12.69; Cl, 16.06. Found: C, 65.47; H, 5.84; N, 12.77; Cl, 16.30.

4-(3-Hydroxypropylamino)-7-chloroquinoline (Q-C_3-OH, 9). A 4.0-g (0.02 mol) portion of 4,7-dichloroquinoline was added to 1.5 g (0.08 mol) of 3-amino-1-hydroxypropane, and the mixture was heated for 12 h to 130 °C. The solution was poured into 50 ml of diethyl ether, resulting in precipitation of a colorless solid, which was recrystallized from ethanol–water yielding 3.5 g (75%) of desired product 9: mp 148–148.5 °C; NMR (Me_2SO-d_6) δ 8.40 (d, 1 H, J = 6 Hz, QC_2H), 8.28 (d, 1 H, J = 9 Hz, QC_5H), 7.41 (dd, 1 H, J = 2 and 9 Hz, QC_6H), 7.29 (d, 1 H, J = 2 Hz, QC_8H), 6.45 (d, 1 H, J = 6 Hz, QC_3H), 4.1 (s, 1 H, –OH), 3.58 (m, 2 H, $QNHCH_2CH_2CH_2OH$), 3.3 (m, 2 H, $QNHCH_2$), 2.85 (m, 2 H, $QNHCH_2CH_2$); ir (KBr) 3370, 3300, 3000, 2900, 2750, 1580, 1520, 1430, 1360, 1340, 1280, 1240, 1140, 1080, 900, 860, 850, 800, 760 cm^{-1}; uv (EtOH) λ_{max} 329 nm (ϵ 11 700), 253 (18 200), 235 (15 700). Anal. Calcd for $C_{12}H_{13}N_2OCl$: C, 60.89; H, 5.53; N, 11.83; Cl, 14.97. Found: C, 60.73; H, 5.50; N, 11.76; Cl, 14.53.

4-(3-Bromopropylamino)-7-chloroquinoline (Q-C_3-Br, 10). A 6.0-g (0.025 mol) portion of 4-(3-hydroxypropylamino)-7-chloroquinoline (9) was dissolved in 18.0 g of 48% hydrobromic acid. After distillation of one-third of the initial volume, the resulting mixture was heated to 120 °C for 3 h and evaporated to dryness, the temperature being lower than 130 °C. The solid was washed with water and recrystallized from ethanol to yield 4.8 g (50%) of the hydrobromide of desired product 10: mp 210–211 °C; NMR (Me_2SO-d_6) δ 9.47 (m, 1 H, QN_1H), 8.63 (d, 1 H, J = 9 Hz, QC_5H), 8.61 (d, 1 H, J = 7 Hz, QC_2H), 8.0 (d, 1 H, J = 2 Hz, QC_8H), 7.66 (dd, 1 H, J = 2 and 9 Hz, QC_6H), 6.92 (d, 1 H, J = 7 Hz, QC_3H), 3.7 (m, 4 H, $QNHCH_2CH_2CH_2Br$), 2.3 (m, 2 H, $QNHCH_2CH_2CH_2Br$).

This hydrobromide was suspended in 250 ml of water and stirred for 24 h with 100 g of dry Dowex (OH^- form) ion-exchange resin. The suspension was eluted through a column containing 100 g of fresh Dowex (OH^- form) with a 50/50 methanol–water mixture. After evaporation of the methanol, the desired product 10 was filtered, yielding 3.6 g (95%): mp 159–160 °C; NMR (Me_2SO-d_6) δ 8.44 (d, 1 H, J = 6 Hz, QC_2H), 8.32 (d, 1 H, J = 9 Hz, QC_5H), 7.8 (d, 1 H, J = 2 Hz, QC_8H), 7.44 (dd, 1 H, J = 9 and 2 Hz, QC_6H), 6.48 (d, 1 H, J = 6 Hz, QC_3H), 3.67 (m, 2 H, $QNHCH_2CH_2CH_2Br$), 3.37 (m, 2 H, $QNHCH_2CH_2CH_2Br$), 2.20 (m, 2 H, $QNHCH_2CH_2CH_2Br$).

Alkylation of Adenine (Route 1). A 0.34-g (0.0071 mol) portion of sodium hydride was suspended in 50 ml of dry dimethylformamide, and 0.8 g (0.006 mol) of adenine was added. The resulting suspension was stirred at ambient temperature for 3 h under nitrogen. To the sodium adenine salt was added, dropwise, for 2 h, 1.8 g (0.006 mol) of 4-(3-bromopropylamino)-7-chloroquinoline (10) in 20 ml of dry dimethylformamide. The resulting mixture was stirred for 24 h at ambient temperature. Solvent was removed in vacuo, leaving a yellow oily residue which was dissolved in ethanol. Ether was added; the precipitate was filtered and recrystallized from methanol to yield 0.2 g (9%) of 3 (Ade-C_3-Q). The filtrate was evaporated giving a solid which was recrystallized from absolute ethanol yielding 0.3 g (21%) of 4-(azetid-1-yl)-7-chloroquinoline (11).

4-[3-(Aden-9-yl)propylamino]-7-chloroquinoline (Ade-C_3-Q, 3): mp 255–256 °C; NMR (Me_2SO-d_6) δ 8.23 (d, 1 H, J = 6 Hz, QC_2H), 8.09 (d, 1 H, J = 9 Hz, QC_5H), 8.03 (s, 1 H, $AdeC_2H$ or $AdeC_8H$), 8.0 (s, 1 H, $AdeC_2H$ or $AdeC_8H$), 7.66 (d, 1 H, J = 2 Hz, QC_8H), 7.25 (dd, 1 H, J = 9 and 2 Hz, QC_6H), 6.86 (m, 2 H, $AdeN_4H$), 6.30 (d, 1 H, J = 6 Hz, QC_3H), 4.26 (m, 2 H, $AdeCH_2$), 3.30 (m, 2 H, $QNHCH_2$), 2.25 (m, 2 H, $QNHCH_2CH_2$–); ir (KBr) 3300, 3140, 1680, 1650, 1610, 1590, 1550, 1490, 1460, 1430, 1380, 1340, 1320, 1250, 1150, 1090, 910, 860, 810 cm^{-1}; uv (EtOH) λ_{max} 327 nm (ϵ 12 000), 258 (31 400). Anal. Calcd for $C_{17}H_{16}N_7Cl$: C, 57.71; H, 4.56; N, 27.7; Cl, 10.02. Found: C, 57.30; H, 4.61; N, 27.8; Cl, 10.11.

4-(Azetid-1-yl)-7-chloroquinoline (11): mp 125.5–126 °C; NMR (Me_2SO-d_6) δ 8.40 (d, 1 H, J = 6 Hz, QC_2H), 7.95 (9 Hz, QC_5H), 7.72 (d, 1 H, J = 2 Hz, QC_8H), 7.34 (dd, 1 H, J = 2 and 9 Hz, QC_6H), 6.20 (d, 1 H, J = 6 Hz, QC_3H), 4.32 [t, 4 H, J = 7 Hz, $QN(CH_2)_2$], 2.40 [m, Q-c-$N(CH_2)_3$]; (KBr) 2860, 1560, 1440, 1290, 1270, 1250, 1240, 1200, 1140, 1070, 935, 920, 880, 850, 790, 760 cm^{-1}; uv (EtOH) λ_{max} 332 nm (ϵ 12 600), 253 (20 500), 218 (34 600). Anal. Calcd for $C_{12}H_{11}N_2Cl$: C, 65.90; H, 5.07; N, 12.81. Found: C, 65.67; H, 5.13; N, 12.98.

4-[3-(Aden-9-yl)propylamino]-7-chloroquinoline (Route 2) (Ade-C_3-Q, 3). To a solution of 2.8 g (0.016 mol) of 9-(3-aminopropyl)adenine (12) in 120 ml of dimethyl sulfoxide was added 1.1 g (0.005 mol) of 4,7-dichloroquinoline. The resulting solution was heated at 110 °C during 14 h. The solvent was then removed in vacuo and 100 ml of distilled water added to the residue. The solid was filtered to yield 1.2 g (62%) of the desired compound 3. This was purified by preparative TLC on silica gel, eluting with benzene–methanol (3:7), followed by crystallization in methanol. The addition to the reaction medium of such bases as triethylamine, potassium carbonate, or diazabicyclooctane leads to no improvement of the yield, side products being formed in larger amounts.

4-(3-Aminopropyl)amino-7-chloroquinoline (Q-C_3-NH_2, 14). A portion of 4.0 g (0.02 mol) of 4,7-dichloroquinoline was added to 7.0 g (0.08 mol) of 1,3-diaminopropane and the mixture warmed at 180 °C during 7 h. The excess of diaminopropane was then evaporated. The solid residue was crystallized from methanol yielding the hydrochloride of the desired product, 14: 2.3 g (43%); mp 260–261 °C; NMR (Me_2SO-d_6) δ 8.38 (d, 1 H, J = 6.0 Hz,

QC_2H), 8.38 (d, 1 H, J = 9.0 Hz, QC_5H), 7.77 (d, 1 H, J = 2.0 Hz, QC_8H), 7.38 (dd, 1 H, J = 9.0 and 2.0 Hz, QC_6H), 6.48 (d, 1 H, J = 6.0 Hz, QC_3H), 3.40 (m, 2 H, QCH_2), 2.90 (m, 2 H, $QCH_2CH_2CH_2CH_2$), 1.95 (m, 2 H, QCH_2CH_2); ir (KBr) 3220, 3060, 2960, 2880, 1620, 1590, 1460, 1440, 1380, 1350, 1290, 1260, 1220, 1170, 1140, 1080, 1030, 900, 865, 850, 810, 765, 640, 575 cm^{-1}. Anal. Calcd for $C_{12}H_{15}N_3Cl_2$: C, 52.76; H, 5.90; N, 15.38; Cl, 25.95. Found: C, 52.76; H, 5.56; N, 15.50; Cl, 25.41.

4-[*N*-(2-Amino-4-hydroxy-5-nitro-6-pyrimidinyl)aminopropyl]amino-7-chloroquinoline (Q-C_3-Pyr, 15). To a solution of 4.7 g (0.02 mol) of 4-(3-aminopropyl)amino-7-chloroquinoline (14) in 50 ml of dimethyl sulfoxide was added 3.8 g (0.02 mol) of 2-amino-4-hydroxy-5-nitro-6-chloropyrimidine (13), followed by 11.2 g (0.1 mol) of diazabicyclooctane. The solution was left at room temperature for 12 h. The crystalline precipitate was filtered and washed successively with water, ethanol, and ether to yield 5.2 g (67%) of desired product 15, which was recrystallized from dimethylformamide: mp >270 °C; NMR (CF_3COOH) δ 11.83 (s, 1 H, $PyrN_3H$), 9.80 (s, 1 H, PyrNH– or QNH–), 8.27 (d, 1 H, J = 7 Hz, QC_2H), 8.05 (d, 1 H, J = 9 Hz, QC_5H), 7.80 (s, 1 H, QC_8H), 7.60 (d, 1 H, J = 9 Hz, QC_6H), 6.73 (d, 1 H, J = 7 Hz, QC_3H), 3.8 (m, 4 H, $QNHCH_2CH_2CH_2Pyr$), 2.4 (m, $QNHCH_2CH_2CH_2Pyr$); ir (KBr) 3380, 3100, 2950, 1660, 1610, 1570, 1555, 1515, 1415, 1325, 1230, 895, 845, 780 cm^{-1}. Anal. Calcd for $C_{16}H_{16}N_7ClO_3$: C, 49.30; H, 4.14; N, 25.15; Cl, 9.09. Found: C, 49.31; H, 4.58; N, 24.16; Cl, 8.57.

4-[3-(Guan-9-yl)propylamino]-7-chloroquinoline (Gua-C_3-Q, 4). To a solution of 3.0 g (8 mmol) of 4-[*N*-(2-amino-4-hydroxy-5-nitro-6-pyrimidinyl)aminopropyl]amino-7-chloroquinoline (15) in formic acid (400 ml) under a dry nitrogen atmosphere was added 20 g of powdered zinc. The resulting mixture was stirred at room temperature for 30 min. Disappearance of the starting material was followed by thin-layer chromatography (on cellulose, eluting with a 15:85 mixture of formic acid and water). Zinc was filtered off under nitrogen atmosphere and the formic acid was removed in vacuo. The resulting oil was dissolved in 400 ml of dimethylformamide previously purged under a stream of deoxygenated nitrogen. The solvent (100 ml) was distilled off to eliminate any trace of formic acid and water. Anhydrous potassium carbonate (5 g) was then added and the mixture was refluxed under nitrogen for 24 h. The solvent was removed in vacuo and the oily residue treated with 100 ml of water. The pH was adjusted to 6 by addition of diluted hydrochloric acid. The resulting powdered precipitate (2 g, 5.8 mmol, 60%) was filtered and identified as the desired product 4. High-purity samples were obtained by a series of purifications implying preparative thin-layer chromatography on silica gel (eluting with methanol containing 1% of ammonia) followed by recrystallization from methanol, solubilization in 0.1 N HCl, precipitation at pH 6, washing of the crystals in water, and recrystallization from methanol: mp 235 °C; NMR (CF_3COOD) δ 9.03 (s, 1 H, $GuaC_8H$), 8.30 (d, 1 H, J = 7 Hz, QC_2H), 8.13 (d, 1 H, J = 9 Hz, QC_5H), 7.86 (s, 1 H, QC_8H), 7.66 (d, 1 H, J = 9 Hz, QC_6H), 6.66 (d, 1 H, J = 7 Hz, QC_3H), 4.63 (m, 2 H, $QCH_2CH_2CH_2G$), 3.90 (m, 2 H, $QCH_2CH_2CH_2$-Gua), 2.55 (m, 2 H, $QCH_2CH_2CH_2$-Gua); ir (KBr) 3400, 3300, 3200, 3100, 2930, 1670, 1610, 1560, 1445, 1350, 1210, 1160, 845, and 780 cm^{-1}; uv (EtOH) λ_{max} 255 nm (ϵ 31 000), 330 (13 200). Anal. Calcd for $C_{17}H_{21}N_7Cl_2O_3$: C, 46.27; H, 4.57; N, 22.22; Cl, 16.06 Found: C, 46.20; H, 4.41; N, 22.16; Cl, 16.10.

Ultraviolet Spectroscopy. For quantitative measurements, about 50 ± 0.1 mg or 5 ± 0.01 mg samples of compound were dissolved in absolute ethanol and then diluted with 0.1 N NaOH, 0.1 N HCl, or the appropriate buffer to give 3–7 × 10^{-5} M solutions having a maximum optical density of 0.8, of known pH and containing 1% ethanol.

Each spectrum was run at least three times and on more than one sample. Values obtained were reproducible within 2%.

Oscillator forces were calculated from optical densities measured every 2.5 nm on a "Wang" calculator programmed using Simpson's rule.

Spectral grade ethanol (Prolabo) and deionized water, distilled under nitrogen, were used. Solutions of potassium dihydrogen phosphate, sodium hydrogen phosphate, potassium hydrogen tartrate, sodium borate, and tris(hydroxymethyl)aminomethane were used as buffers. Uv spectra were recorded on a Cary 15 spectrophotometer (see Table III).

Measurements of Hypochromic Effect as a Function of Temperature. Thermostated cells were used, the temperature of the samples remaining constant within 0.5 °C. To avoid corrections for concentration varying due to temperature differences or to solvent evaporation, the B-C_3-Q and reference compound Q-C_3 were placed simultaneously in thermostated cells, and the spectra were recorded alternately; the hypochromic effect was then obtained from comparison of the two spectra.

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(27) For the interaction model of thymine, the value of the maximum hypochromic effect is not attained.

Nucleosides. 102. Synthesis of Some 3′-Deoxy-3′-Substituted Arabinofuranosylpyrimidine Nucleosides[1]

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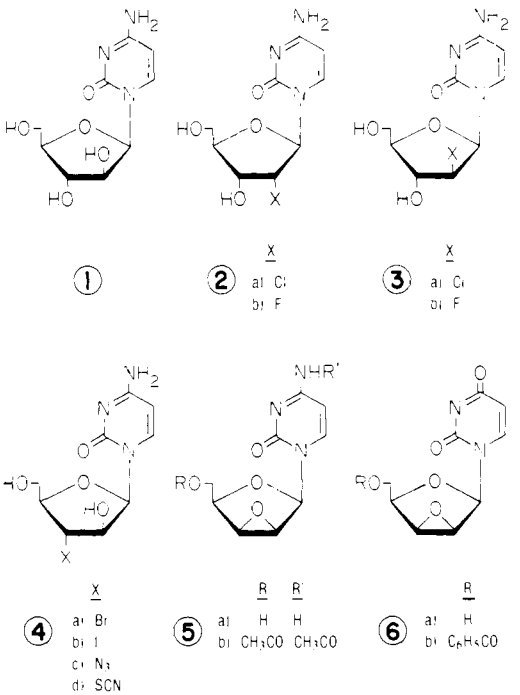
The synthesis of some 3′-deoxy-3′-substituted arabinofuranosylcytosine (**4a–d**) and uracil (**7a–d**, **8a–d**, X = Br, I, N_3, SCN) nucleosides was accomplished by treatment of the requisite 2′,3′-anhydrolyxofuranosylpyrimidine nucleoside (**5**, **6a,b**) with the appropriate ammonium salt in refluxing ethanol. Cleavage of the oxirane ring provided the desired 3′-deoxy-3′-substituted pyrimidine nucleosides (**4a–d**, **7a–d**, and **8a–d**). In vitro screening of compounds **4a–d**, and **7a–d**, with L5178Y cells in culture showed no significant inhibitory properties.

The nucleoside 1-β-D-arabinofuranosylcytosine (1, *ara*-C) is probably the most efficacious drug currently available for the treatment of acute myeoblastic leukemia.[2] Previous reports from this laboratory[3] have described the synthesis of 2′-deoxy-2′-halogeno analogues of cytidine and *ara*-C (compounds **2** and **3**, respectively). Some of these have shown significant activity against leukemic cells in culture.[3b,4] The mechanism(s) of this inhibition, however, has not been ascertained. Nucleosides **2** and **3** may act as analogues of 2′-deoxycytidine or as analogues of *ara*-C, or they may be converted in situ to *ara*-C. Chemically, the conversion of nucleoside types **2** and **3** to *ara*-C is readily accomplished by treatment with base.[3a,5]

Extension of our studies to the synthesis of 3′-deoxy-3′-substituted arabinofuranosylcytosines (4) became of interest as a method of examining the role of the 3′ position as a function of "*ara*-C-like" activity. The simplest method for synthesis of this type of compound seemed to be by cleavage of 2′,3′-anhydrolyxofuranosylcytosine (**5a**).[6] It has been demonstrated previously[7] that nucleophilic attack on 2′,3′-anhydrolyxofuranosyl nucleosides occurs predominantly, if not exclusively, at the 3′ position. A number of methods of cleaving epoxides were investigated using the 2′,3′-anhydrolyxofuranosyluracils **6a** and **6b**. It was found that treatment of such epoxides with ammonium salts proved to be the simplest method. These salts provide a mildly acidic reaction medium which leads to relatively rapid cleavage and allows facile isolation of the products. Thus the 2′,3′-epoxy nucleosides **5a** and **6** were refluxed



in ethanol in the presence of the appropriate ammonium salt until the evolution of ammonia ceased and TLC examination of the reaction mixture showed the absence of