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Registry No. 2, 5145-42-6; 4, 56054-64-9; 5, 34670-79-6; 6, 85004-79-1; 7, 6939-12-4; 8a, 85004-80-4; 8b, 85004-81-5; 9a, 85004-82-6; 9b, 34670-89-8; 10, 85004-83-7; 11a, 85004-84-8; 11b, 85004-85-9; 12a, 85004-86-0; 12b, 85004-87-1; 13, 85004-88-2; 14, 85004-89-3; 15, 85004-90-6; 16, 85004-91-7; 18, 34670-86-5; diazomethane, 334-88-3; ethylenimine, 151-56-4; acetaldoxime, 107-29-9.

Bleomycin Analogues. Phenylthiazole Models of the Bithiazole Moiety of Bleomycin A_2

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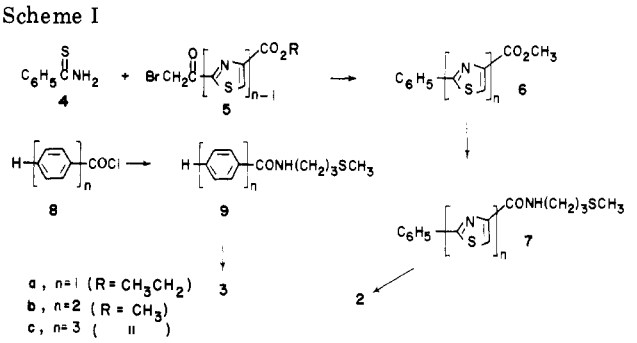
Amides of 2-phenylthiazole-4-carboxylic acid, 2'-phenyl-2,4'-bithiazole-4-carboxylic acid and 4,4'-biphenylcarboxylic acid containing the (3-aminopropyl)dimethylsulfonium chloride side chain of bleomycin A_2 have been prepared and their binding to poly(dA-dT) has been studied by proton nuclear magnetic resonance spectroscopy. Both the phenylthiazole and phenylbithiazole derivatives exhibit high-field shifts for their hydrogens in the presence of the polynucleotide which are considerably larger than those observed in the analogous completely heterocyclic systems studied previously (Sakai, T. T.; Riordan, J. M.; Glickson, J. D. *Biochemistry* **1982**, *21*, 805). The intercalative nature of the binding of these analogues was further indicated by viscometric measurements using calf thymus DNA. The data show that a phenyl ring allows the aromatic systems to interact with the base pairs of the polynucleotide to a greater extent than a thiazole ring in the same position. Possible models for the interaction of these derivatives with DNA are considered. The hydrogens of the biphenyl derivative show an interaction that is substantially less than those observed in the heterocycle-containing systems, suggesting that the ring system is oriented improperly or that the ring system is nonplanar. The analogous phenyl (benzoyl) compound does not bind, showing the requirement for an extended aromatic system for intercalation. The utility of these observations for the design of possible synthetic analogues of the bleomycins is discussed. None of the derivatives exhibited toxicity when tested against L1210 leukemia cells in culture.

Previous studies from this laboratory[1] have shown that the DNA binding region of the antitumor antibiotic bleomycin A_2 resides in the cationic terminal dipeptide of the molecule (Figure 1). This association of the drug with DNA is believed to be a requisite for subsequent (or simultaneous) reactions that degrade the nucleic acid, a process that appears to be responsible for the biological activity of the drug.[2] It has also been demonstrated that the interaction of analogues of this cationic terminus with poly(dA-dT) can be modified by the choice of substituents on the aromatic bithiazole systems.[3] In those studies, amides of 2,4'-bithiazole-4-carboxylic acid (**1b**) and of 2,4':2',4''-terthiazole-4-carboxylic acid (**1c**) (Figure 2) containing the (3-aminopropyl)dimethylsulfonium chloride side chain of bleomycin A_2 were found to intercalate in the nucleic acid to a greater extent than 2'-alkyl-substituted 2,4'-bithiazole derivatives structurally more like the parent compound. Introduction of the third aromatic ring markedly enhanced the interaction with poly(dA-dT). As a part of ongoing studies on synthetic fragments and analogues of bleomycin A_2, we have sought to prepare derivatives of the DNA binding region that retained the enhanced intercalative properties of derivatives such as **1b** and **1c** but which might be more amenable to chemical modification than the bithiazole system. To this end, several phenyl derivatives of the thiazole systems have been prepared, and the interaction of these analogues with poly(dA-dT) has been studied by proton nuclear magnetic resonance spectroscopy and viscometry.

The desired derivatives were prepared by using precursors described previously for the synthesis of the thiazole derivatives.[1a,3b] Thiobenzamide (**4**) was condensed with ethyl bromopyruvate (**5a**), methyl 2-(2-bromoacetyl)thiazole-4-carboxylate (**5b**), or methyl 2'-(2-bromoacetyl)-2,4'-bithiazole-4-carboxylate (**5c**) in anhydrous *N,N*-dimethylformamide (Scheme I) to form the phenylthiazole (**6a**), phenylbithiazole (**6b**), or phenylterthiazole (**6c**) ester, respectively, in good yield. Aminolysis of the esters with neat 3-(methylthio)propylamine gave the amides in high yield.

Scheme I



For the phenyl and biphenyl derivatives, benzoyl chloride (**8a**) or 4-phenylbenzoyl chloride (**8b**) (prepared from 4,4'-biphenylcarboxylic acid and thionyl chloride) was treated with 3-(methylthio)propylamine in anhydrous dioxane containing triethylamine to give the amides (**9a,b**) in good yield.

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(1) (a) Sakai, T. T.; Riordan, J. M.; Booth, T. E.; Glickson, J. D. *J. Med. Chem.* **1981**, *24*, 279-285. (b) Glickson, J. D.; Pillai, R. P.; Sakai, T. T. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 2967-2971.
(2) See, for example, (a) Burger, R. M.; Peisach, J.; Horwitz, S. B. *Life Sci.*, **1981**, *28*, 715-727. (b) "Bleomycin: Chemical, Biochemical and Biological Aspects"; Hecht, S. M., Ed.; Springer-Verlag: New York, 1979.
(3) (a) Sakai, T. T.; Riordan, J. M.; Glickson, J. D. *Biochemistry* **1982**, *21*, 805-816. (b) Riordan, J. M.; Sakai, T. T. *J. Heterocycl. Chem.* **1981**, *18*, 1213-1221.

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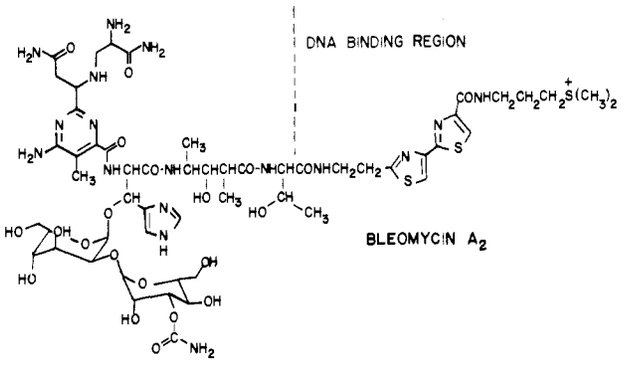

Figure 1. Structure of bleomycin A_2 showing the DNA binding region.

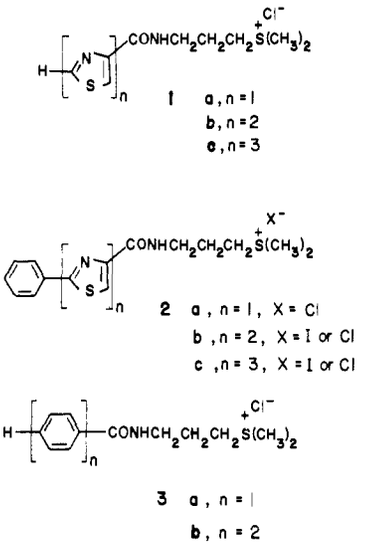

Figure 2. Structures of analogues of the cationic terminus of bleomycin A_2.

Methylation of the thioethers was carried out as described previously[3b] with a 1:1 (v/v) mixture of iodomethane and methanol. Sulfonium iodide **2b** was crystalline, while iodides **2a**, **3a**, and **3b** were amorphous solids that readily discolored upon exposure to air. For combustion analyses, all iodide salts with the exception of **2b** were converted to the respective chloride derivatives, and all studies were carried out with the chloride salts. Sulfonium salt **2c** proved too insoluble in water as either the iodide, chloride, acetate, or nitrate salt to permit studies.

Results

The utility of poly(dA-dT) as a model DNA for NMR studies has been demonstrated by Patel[4] and by previous studies from this laboratory.[1,3a,5] The interaction of the various sulfonium derivatives with the polynucleotide was monitored by the temperature dependence of the resonances of the ligand and nucleic acid separately and in combination. The conditions were chosen so as to maximize the effects of the resonances of the ligands. A nucleic acid phosphate to ligand ratio (molar) of 7.8 was used in all studies. Further increases in this ratio did not increase the perturbations of the ligand resonances. No effort was made to maximize the effects on the nucleic acid resonances. All temperature-induced perturbations were found

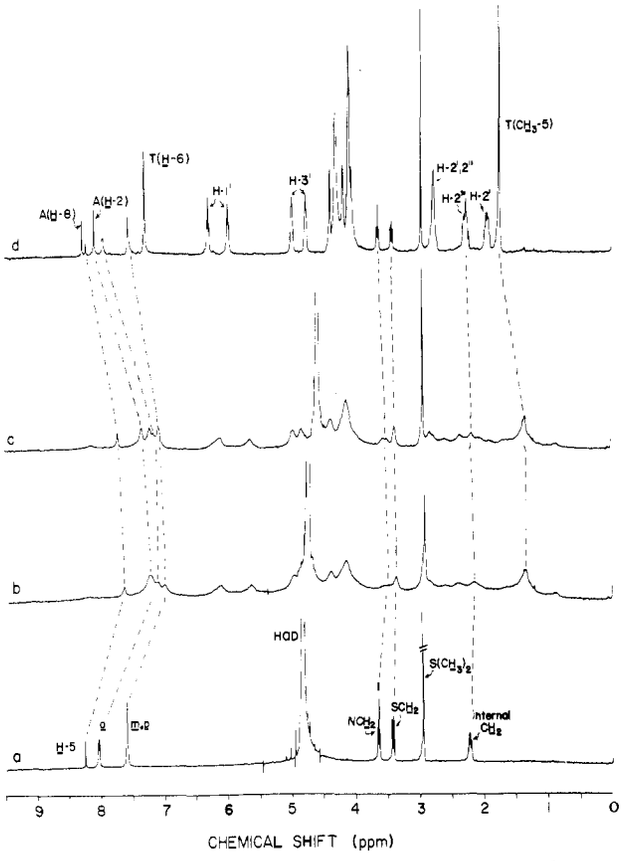

Figure 3. Proton NMR spectra at 400 MHz of compound **2a** (1.2 mM) (a) alone at 30 °C and in the presence of 10 mM poly(dA-dT) at (b) 30, (c) 50, and (d) 75 °C. The buffer is 0.01 M sodium phosphate–0.10 M sodium chloride in D_2O, pH (meter reading) 6.8.

to be fully reversible. Typical spectra are shown in Figure 3 for compound **2a** alone and in the presence of poly(dA-dT) at various temperatures. Resonances of hydrogens on the ligand are broadened greatly, indicating immobilization due to binding to the nucleic acid. In addition, they are shifted to high field, showing that they are experiencing the ring-current effects emanating from the base pairs of the polynucleotide. Such shifts are generally taken to be an indication of the intercalation of the affected hydrogens into the nucleic acid. As the poly(dA-dT) undergoes the helix–coil transition, the high-field shifts are lost, and the resonances of the ligand [and poly(dA-dT)] sharpen markedly, showing that the binding of the ligand requires the duplex structure of the nucleic acid.

The temperature dependence of the aromatic resonances of **2a** and **2b** is shown in Figures 4 and 5, respectively. All of the resonances are shifted to high field in the presence of the nucleic acid but to varying degrees. The thiazole C_5H resonance of **2a** shows a maximum shift of about 0.65 ppm, and the phenyl ortho and meta/para resonances show shifts of 0.83 and 0.62 ppm, respectively (Figure 4), indicating that the various regions of the aromatic systems experience different degrees of shielding arising from insertion into the base-pair region. By comparison, the thiazole C_5 H and $C_{5'}$ H resonances of **2b** exhibit shifts of 0.35 and 0.86 ppm, respectively (Figure 5a), while the phenyl ortho and meta/para resonances shift by 0.70 and 0.41 ppm, respectively (Figure 5b).

Since the phenyl-substituted thiazole derivatives exhibited larger chemical-shift perturbations than the analogous bi- and terthiazole systems, it was reasoned that a biphenyl system might be capable of a similar, if not greater, interaction with poly(dA-dT). Biphenyl **3b**, how-

(4) (a) Patel, D. J. *J. Polym. Sci.* **1978**, *62*, 117–141. (b) Patel, D. J. *Acc. Chem. Res.* **1979**, *12*, 118–125. (c) Patel, D. J.; Canuel, L. L. *Biopolymers* **1977**, *17*, 857–873. (d) Patel, D. J.; Canuel, L. L. *Eur. J. Biochem.* **1978**, *90*, 247–254.

(5) Chen, D. M.; Sakai, T. T.; Glickson, J. D.; Patel, D. J. *Biochem. Biophys. Res. Commun.* **1980**, *92*, 197–205.

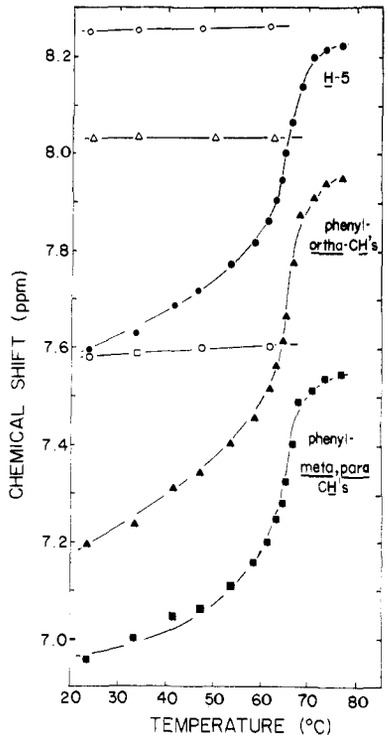

Figure 4. Temperature dependence of the chemical shifts of the C_5 (○, ●), phenyl ortho (△, ▲), and phenyl meta/para hydrogens (□, ■) of compound **2a** (1.2 mM) in the absence (open symbols) and presence (closed symbols) of 10 mM poly(dA-dT). The conditions are described in the legend to Figure 3.

Table I. ^{1}H NMR High-Field Chemical Shifts for Cationic Side-Chain Resonances[a]

	chemical shift, δ			
no.	NCH_2	internal CH_2	CH_2S	$S(CH_3)_2$
2a	0.15	0.075	0.075	0.045
2b	0.06	0.06	0.05	0.01
3b	0.10	0.05	0.04	<0.01

[a] Values are the chemical shift of the resonance in the absence of poly(dA-dT) minus that in the presence of poly(dA-dT) measured at 25 °C.

ever, showed considerably smaller ring-current shifts than the phenylthiazoles, with the maximum shifts of the order of 0.25 ppm (Figure 6). The monophenyl (benzoyl) derivative **3a** did not show any ring-current effects in the presence of poly(dA-dT).

In Table I are shown the chemical-shift perturbations experienced by the cationic side-chain hydrogens of derivatives **2a**, **2b**, and **3b**. The NCH_2 of **2a** shows a shift of 0.16 ppm compared with a shift of 0.06 ppm for the corresponding resonance of **2b**. The other resonances of the side chain are affected in a similar manner in going from **2a** to **2b**. The smaller shifts experienced by the side-chain hydrogens of **2b** indicates that the side chain, as well as the first thiazole ring of **2b**, are farther away from the base-pair region than are the corresponding hydrogens of **2a**.

The side-chain hydrogens of **3b** experience relatively small ring-current shifts. This observation, together with the relatively small shifts for the aromatic hydrogens of this derivative, shows that the interaction with poly(dA-dT) involves only a small degree of intercalation of the molecule.

Ambiguity in the assignment of many of the nucleic acid sugar resonances makes it difficult to attribute ligand-induced perturbations to specific atoms of the polynucleotide; however, the resonances of the bases are fairly easily followed. Phenylthiazole **2a** has minimal effects on the resonances of the nucleic acid. There is a small (~0.15 ppm) low-field shift of the A(H-2) resonance in the premelting region in the presence of this ligand (Figure 7). Such an effect is generally attributed to an increase in the distance between the base pairs of the polynucleotide caused by insertion of an intercalant. This increase in distance results in each base feeling a diminished effect of the neighboring bases. Similarly, there may be a large decrease in ring-current shifts due to separation of the bases, which is largely compensated for by the ring-current effects of the intercalated molecule. There is an increase of 5 °C in the T_m of this resonance; however, there are no residual shifts for the denatured polynucleotide (for temperatures greater than T_m), indicating that there are no stacking interactions between the ligand and the bases of the single-stranded form of the nucleic acid.

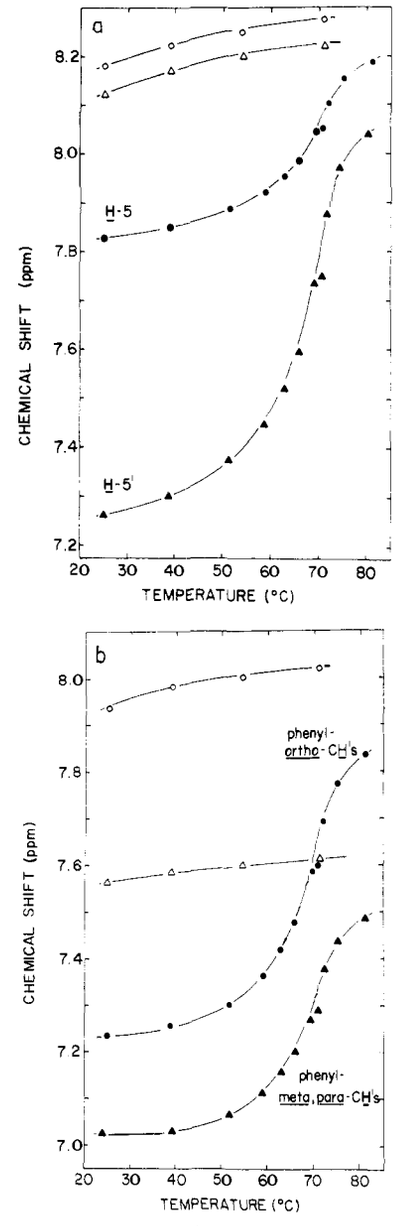

Figure 5. Temperature dependence of the chemical shifts of the (a) C_5 H and $C_{5'}$ H resonances and (b) phenyl ortho and meta/para hydrogens of compound **2b** (1.2 mM) in the absence (open symbols) and presence (closed symbols) of 10 mM poly(dA-dT). The conditions are the same as described in the legend to Figure 3.

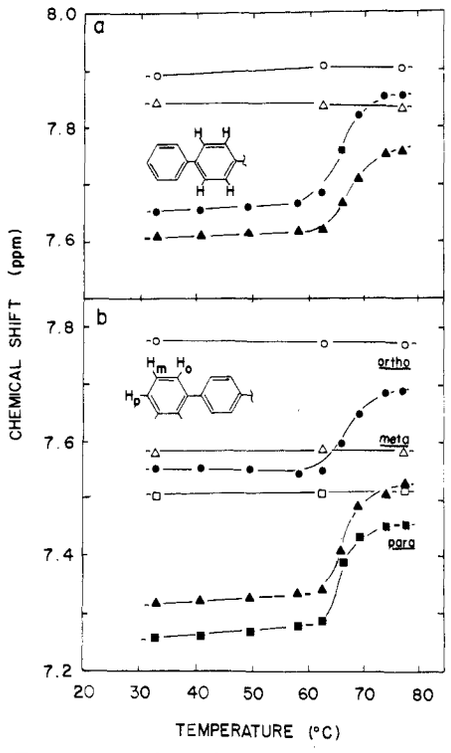

Figure 6. Temperature dependence of the chemical shifts of the aromatic resonances of **3b** (1.2 mM) in the absence (open symbols) and presence (closed symbols) of 10 mM poly(dA-dT). The closed symbols correspond to the same shaped open symbols. The conditions are as described in the legend to Figure 3.

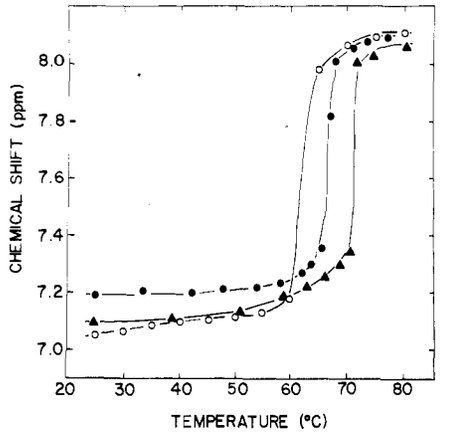

Figure 7. Temperature dependence of the chemical shift of the A(H-2) resonance of poly(dA-dT) (10 mM) alone (○) and in the presence of 1.2 mM concentrations of compound **2a** (●) and **2b** (▲). Compound **3b** causes the same perturbations as compound **2a**. The conditions are as described in the legend to Figure 3.

The binding of phenylbithiazole **2b** causes very little change in the temperature dependence of the A(H-2) resonance in the premelting region compared to the nucleic acid alone (Figure 7), indicating that the ring-current effects of the intercalating system compensate for the loss of high-field shifts due to the separation of the base pairs. Interestingly, the apparent T_m of this resonance in increased by approximately 10 °C, while that of the T(H-6) resonance is increased by about 7 °C. The A(H-2) resonance shows a small (~0.05 ppm) residual shift for the denatured polynucleotide. The T(CH_3-5) resonance is not appreciably affected in its shift or its T_m in the presence of **2b**. The differential behavior of the T_m's of the various resonances of the nucleic acid suggests that there may be stacking interactions that influence the chemical shift of

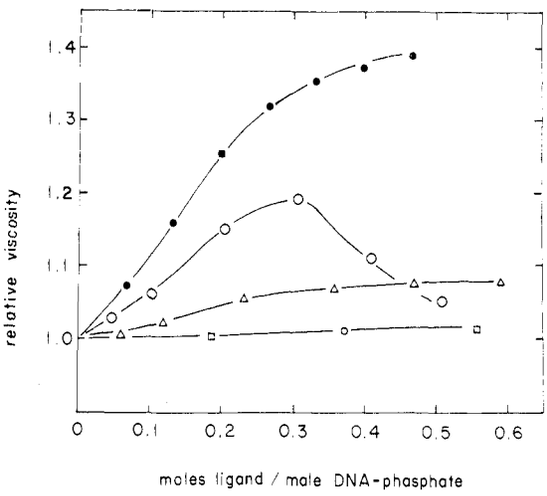

Figure 8. Effects of compounds **2a** (△), **2b** (○), and **3b** (□) on the viscosity of calf thymus DNA (1.2 mM) in 0.01 M sodium phosphate–0.10 M sodium chloride–0.1 mM EDTA (pH 7.0) at 25 °C. The effects of ethidium bromide (●) are shown for comparison.

the resonances in the complex. The H-1′, H-2′, and H-2″ resonances are all perturbed to varying degrees, but the shifts are small (≤0.1 ppm), as are the increases in T_m (≤2 °C).

Biphenyl **3b** affects the A(H-2) resonance in a manner similar to compound **2a** (Figure 7). There is a small low-field shift caused by the presence of the ligand and a 5 °C increase in T_m for this resonance. Small high-field shifts are observed for the T(H-6) and both H-1′ resonances, which are accompanied by a slight (2–3 °C) increase in T_m for these resonances (not shown). The T(CH_3-5) resonance is affected only to the extent of a small (2 °C) increase in its T_m.

To help ascertain whether the high-field shifts of the aromatic hydrogens of the analogues were, in fact, reflecting intercalation, viscometric measurements were performed with calf thymus DNA. Intercalation should be reflected in a lengthening of the DNA helix with an accompanying increase in viscosity of the DNA solution. The data obtained for the phenylthiazole derivatives are shown in Figure 8, together with the curve obtained with the known intercalating agent ethidium bromide under the same conditions. Phenylthiazole **2a** causes a small increase in the viscosity of calf thymus DNA and the increase has reached a maximum beyond an analogue to DNA phosphate ratio of about 0.3. Phenylbithiazole **2b** causes a larger initial effect on the viscosity; however, it also causes precipitation of the nucleic acid, particularly at the higher ratios of analogue to nucleic acid. This precipitation is reflected in a decrease in the viscosity of the solution at values of this ratio greater than about 0.3. Attempts to prevent precipitation by using lower concentrations of nucleic acid and ligand were unsuccessful. Terthiazole **1c** shows an effect similar to that of phenylthiazole **2b** (not shown); however, some precipitation of DNA was evident with this compound also. Biphenyl **3b** shows only a very small effect on the viscosity of DNA.

These data show that the relative effects of these derivatives on DNA viscosity are in line with the degree of interaction with poly(dA-dT) inferred from their chemical-shift perturbations in the NMR studies. Interestingly, under the same conditions, bleomycin A_2 shows no effect on the viscosity of DNA, indicating that the binding of the parent drug is not intercalative in nature, or, if it is, the interaction of the chromophore with the nucleic acid is not strongly intercalative.

Biological Activity. None of the sulfonum derivatives prepared in this study exhibited toxicity toward leukemia L1210 cells in culture at concentrations up to 5 mM. As with the binding studies, phenylterthiazole **2c** was not sufficiently soluble to permit screening.

Discussion

The data presented here show that the type of ring substituted in thiazole systems, as well as the number of rings in such systems, has an effect on the degree of intercalation of these DNA-binding fragments and on the geometry of these interactions. In the bithiazole series studied previously,[3a] both the C_5 H and $C_{5'}$ H resonances of compounds such as **1b** were found to exhibit the same high-field shifts in the presence of poly(dA-dT), although the magnitudes differed from analogue to analogue. This indicates that in such systems both hydrogens experience similar environments upon binding to the nucleic acid. In fact, the relatively small magnitude of these shifts suggests that the binding of these bithiazoles is only slightly greater than that of the parent bleomycin. When the number of rings is increased to give the terthiazole **1c**, the high-field shifts experienced by the aromatic hydrogens is increased substantially.[3a] Interestingly, C_5 H and $C_{5'}$ H still both show the same shift; the effect diminishes markedly for $C_{5''}$ and $C_{2''}$ H. Thus, the first and second rings interact maximally with the nucleic acid in the terthiazole, possibly by overlap with the purine rings in poly(dA-dT). The third (double primed) ring may be placed out into one of the grooves of the nucleic acid so that it feels substantially smaller ring-current effects from the base-pair region. This could occur either by placement of the ring system completely through the base-pair region so that the third ring extends into the groove opposite to that in which binding occurs or by the ring being turned away from the intercalation site. The small effect of the terthiazole on the viscosity of DNA suggests that the latter is the case.

Substitution of a phenyl ring for the "end" thiazole ring in these bi- and terthiazoles has profound effects on the intercalation of these compounds. Phenylthiazole **2a** intercalates well, exhibiting a maximum high-field shift of greater than 0.80 ppm for the *o*-hydrogens of the phenyl ring; the C_5 H and and *m*- and *p*-hydrogens of the phenyl ring show shifts of the order of 0.60 ppm. These effects are considerably larger than those observed in the analogous bithiazole **1b**. With phenylbithiazole **2b**, the $C_{5'}$ H resonance exhibits a maximum high-field shift of about 0.86 ppm, and the phenyl *o*-hydrogen resonance exhibits one of about 0.70 ppm. In this case, the C_5 H and the phenyl *m*- and *p*-hydrogens show the smaller perturbations. Thus, the phenyl ring and the second (primed) thiazole ring of **2b** interact most strongly with the nucleic acid, whereas in terthiazole **1c**, the maximum effect is seen on the first two (thiazole) rings. Comparison of **2a** and **2b** also shows the presence of differences. Therefore, in comparing two- and three-ring systems, different types of rings in analogous positions do not give the same binding geometry; similarly, the same ring in a different position does not provide an analogous binding geometry.

These differential effects may be due, in part, to specific orientations about the interring bonds that serve to place analogous (or homologous) hydrogens in locations of greater or lesser ring-current effects emanating from the nucleic acid bases. It is known that the C_5 H and $C_{5'}$ H atoms of methyl 2′-(2-aminoethyl)-2,4′-bithiazole-4-carboxylate are on opposite sides of the bond joining the two thiazole rings in crystals of this component of bleomycin.[6] This "anti" orientation may not allow both thiazole hydrogens to be offered simultaneously to the nucleic acid upon binding. Such an interaction might be manifested in the two hydrogens experiencing considerably different ring-current effects arising from the nucleic acid. Similarly, the observation of similar shifts for both hydrogens might be indicative of a change in orientation of the rings with respect to one another or of a fortuitous positioning of both hydrogens in regions of similar shift effects.

The differences between the heterocyclic derivatives and the phenyl-containing derivatives may also arise from preferred stacking interactions between, for example, the phenylthiazole ring system and a nucleic acid base. Binding, then, might occur to maximize this stacking. In **2b**, this might force the "first" thiazole ring into a position in the complex in which it feels smaller ring-current effects than the stacked phenylthiazole system. That this might be the case is suggested by the fact that the phenyl hydrogens of **2a** and **2b**, as well as the hydrogens of the thiazole ring *adjacent to* the phenyl rings, experience the largest ring-current effects from the nucleic acid. Furthermore, the A(H-2) resonance of poly(dA-dT) in the presence of **2b** shows an unusually large increase in its T_m, which is not in agreement with the T_m's of the other nucleic acid resonances. This suggests (but in no way proves) that a stacking interaction is occurring that perturbs A(H-2) specifically. Such an effect is not evident for phenylthiazole **2a**.

These differences in binding geometries are further reflected in the chemical shifts of the hydrogens of the cationic side chain. These shifts show that the ring systems that experience the largest ring-current shifts for the C_5 H resonance (i.e., the ring closest to the side chain) also show relatively large effects for the side chain NCH_2 closest to the ring system. This shows that the side chain is pulled in closer to the nucleic acid in those derivatives.

We have been able to determine the crystal structure of the iodide salt of phenylbithiazole **2b** and have found that the two thiazole rings in this molecule also assume an "anti" orientation with respect to one another.[7] Using this structural information, we have modeled the interaction of this compound with self-complementary deoxydinucleoside monophosphates by using energy-minimimzation techniques.[7] This model, which is shown in Figure 9a, is in complete agreement with the NMR data presented here. The phenyl ring extends into the base-pair region; the smallest interaction with the base pairs is evident for the first thiazole ring, with the second (primed) thiazole ring showing an intermediate degree of intercalation. In Figure 9b,c, we have shown possible models for the interaction of compounds **2a** and **1c**, based on the NMR data available. The orientations are in no way unique but serve to illustrate the general features of these interactions. Compound **2a** is shown inserted in the base-pair region slightly more than **2b** to take into account the shifts felt by the two rings, as well as the larger shifts experienced by the side-chain hydrogens of **2a**. Compound **1c** is shown with its third (double primed) thiazole ring extending into the groove to account for the smaller shifts experienced by the hydrogens on this ring. These models show that even those ring systems showing large ring-current shifts are not completely intercalated; i.e., the entire molecule is not inserted into the base-pair region. This is evident

(6) Koyama, G.; Nakamura, H.; Muraoka, Y.; Takita, T.; Maeda, K.; Umezawa, H.; Iitaka, Y. *Tetrahedron Lett.* **1968**, 44, 4635–4638.
(7) Kuroda, R.; Neidle, S.; Riordan, J. M.; Sakai, T. T. *Nucleic Acids Res.* **1982**, *10*, 4753–4763.

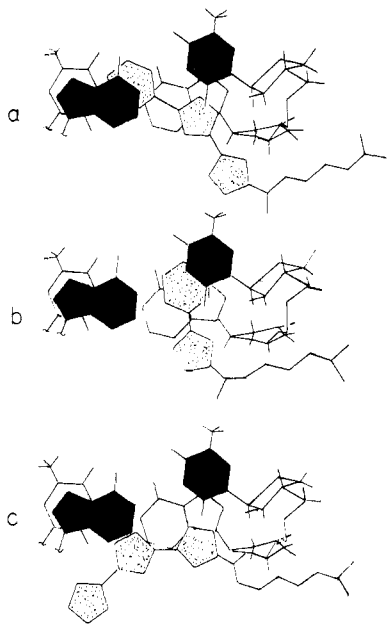

Figure 9. (a) Model for the interaction of compound **2b** with a base-pair region of DNA. The base pairs are shown as the solid figures; the ligand is shown stippled. Only the sugar residues on the right side are depicted. (Adapted from ref 7.) Also shown are possible models for the interaction of (b) compound **2a** and (c) compound **1c**.

in the only modest increase in intrinsic viscosity observed with these derivatives and may reflect the fact that the ionic interaction between the sulfonium side chain and the nucleic acid backbone is "holding back" the planar groups from full intercalation. We are pursuing more detailed modeling studies with these derivatives as X-ray structures become available.

The data on the biphenyl compound show that a two-ring system per se is not sufficient for intercalation. The hydrogens of this compound show shifts considerably smaller than those of **2a** and **2b**. A simple explanation is that the two rings are not coplanar and therefore not able to intercalate. However, it is tempting to attribute this lack of intercalation to an "improper" orientation of the second phenyl ring with respect to the cationic group. The requirement for an ionic interaction may place this second ring in some orientation unfavorable for insertion. It is possible that a *m*-biphenyl derivative might offer its second ring to a nucleic acid in a manner more appropriate for intercalation while maintaining the ionic interaction with the nucleic acid backbone. We are hoping to prepare the properly substituted *m*-biphenyl to examine this possibility.

The inability of the benzoyl derivative to bind is in agreement with the previously reported inability of monothiazoles, such as **1a** (Figure 2), to bind to poly(dA-dT)[3a] and suggests that an extended aromatic system is required for intercalative binding. That this is not due merely to an insufficient distance between the cationic group and the potential intercalating group is shown by the fact that extension of the side chain in the monothiazole series does not permit intercalation (Riordan, J. M.; Sakai, T. T.; unpublished results). Ionic binding by itself apparently does not bring the side chain sufficiently close to the nucleic acid for the side-chain hydrogens to feel ring-current effects. In order for this to happen, intercalative binding must occur, which brings the chain close to the base-pair region.

The lack of biological activity of these derivatives would seem to indicate that binding to DNA per se is not sufficient for toxicity. However, on the basis of the present data, we cannot say if these derivatives are, in fact, able to penetrate cells to be able to bind to DNA. Further studies are in progress.

The results presented here show that the substitution of a thiazole ring by a phenyl ring in bithiazole systems related to bleomycin A_2 can allow greater interaction with DNA than is observed in the purely heterocyclic systems. This suggests the possibility of using substituted phenyl derivatives to prepare simple bleomycin analogues capable of degrading DNA. However, care must be chosen in the placement of these substituents relative to the DNA-binding site. Under conditions that allow intercalation of the phenylthiazoles, the parent bleomycin does not affect DNA viscosity (Figure 8). However, at the same salt and buffer concentrations, we have previously noted[1,5] that the thiazole hydrogens of bleomycin A_2 show maximal high-field shifts at about 55 °C and that these shifts diminish as the temperature is raised (denaturation to the single-stranded polynucleotide) or lowered to near ambient temperature. Binding of the rest of the bleomycin molecule is retained in the latter case, as indicated by the retention of the perturbations of other groups that were perturbed when the thiazoles were intercalated. This has been attributed to a tightening of the nucleic acid structure, which forces the bithiazole system from between base pairs to assume an orientation such that the aromatic hydrogens can no longer feel the base-pair ring currents. This change in orientation may be due to steric effects arising from the bulk of the bleomycin molecule, since this anomalous temperature dependence is lost as the rest of the bleomycin molecule is "clipped off" in the models. This information indicates that although the present model compounds do, in fact, bind more tightly than the parent compound, care must be taken in the introduction of potential reactive groups onto the DNA-binding moieties in a manner such that the binding is not affected deleteriously. By appropriate choice of sites of substitution on phenyl systems and numbers of rings, it may be possible to prepare derivatives having varying affinities and sequence specificities for binding to and degrading DNA.

Experimental Section

Melting points were determined on a Laboratory Devices Mel-Temp apparatus and are uncorrected. Infrared spectra were obtained on a Perkin-Elmer Model 710B spectrophotometer. Proton NMR measurements were made on a Bruker WH-400 spectrometer. Chemical shifts are referenced to internal tetramethylsilane (organic solvents) or sodium 4,4-dimethyl-2,2,3,3-tetradeuterio-4-silapentanoate (aqueous solvents). Thin-layer chromatography was run on precoated silica gel F_{254} plates (0.25-mm thickness, Eastman Kodak, Rochester, NY). All analytical samples were found to be homogeneous upon chromatography with ethyl acetate, ethyl acetate–petroleum ether (bp 35–60 °C) (4:1, v/v) or chloroform–methanol (3:1, v/v) as solvent systems. Preparative thin-layer chromatography was run on silica gel GF_{254} plates (1-mm thickness, Analtech, Newark, DE). Combustion analyses were performed by Atlantic Microlabs, Atlanta, GA.

Poly(dA-dT) (P-L Biochemicals, Milwaukee, WI) was purified by dialysis against 0.1 M sodium phosphate (pH 7.0)–1 M sodium chloride–1 mM EDTA and then distilled water and obtained as a lyophilized solid. Concentrations of poly(dA-dT) were determined by using a molar absorptivity of 6.6×10^3 M^{-1} cm^{-1} per nucleotide residue.[8] Proton NMR measurements involving poly(dA-dT) were carried out in D_2O (99.8 atom % D, Aldrich Chemicals, Milwaukee, WI) containing 0.01 M sodium phosphate–0.10 M sodium chloride, pH (meter reading) 6.8. Spectra were obtained in pulse Fourier transform mode with 5-mm (o.d.)

(8) Inman, R. B.; Baldwin, R. L. *J. Mol. Biol.* **1962**, *5*, 172–184.

spinning sample tubes. Temperatures in the NMR probe were measured by using the separation of ethylene glycol resonances.[9] All spectra were obtained after collecting 512 transients.

For viscosity measurements, calf thymus DNA (Sigma Chemical Co., St. Louis, MO) was prepared by dialysis as described previously.[1a]

Thiobenzamide (4), ethyl bromopyruvate (**5a**), benzoyl chloride (**8a**), 4,4′-biphenylcarboxylic acid, and thionyl chloride were purchased from Aldrich Chemicals and used without further purification. Methyl 2-(2-bromoacetyl)thiazole-4-carboxylate (**5b**) and methyl 2′-(2-bromoacetyl)-2,4′-bithiazole-4-carboxylate (**5c**) were prepared as described previously.[3b] 3-(Methylthio)-propylamine was obtained from Eastman Kodak and used without purification. 3-(Benzamido)propyl methyl sulfide (**9a**) and [3-(benzamido)propyl]dimethylsulfonium chloride (**2a**) were prepared as described by Fujii et al.[10]

Ethyl 2-Phenylthiazole-4-carboxylate (6a). A solution of thiobenzamide (4; 1.51 g, 11.0 mmol) and ethyl bromopyruvate (**5a**; 2.16 g, 11.1 mmol) in 10 mL of anhydrous *N,N*-dimethylformamide was stirred at room temperature for 15 h, after which time solvent was removed in vacuo. The residue after evaporation was suspended in 50 mL of dichloromethane, and the mixture was washed with saturated sodium bicarbonate (50 mL) and then with water (50 mL). The organic fraction was dried over anhydrous magnesium sulfate and then evaporated to give an oil. Crystallization of the oil from a mixture of ethanol and toluene gave 1.06 g (41%) of **6a** as colorless crystals: mp 47–48 °C (lit.[11] mp 44–46 °C); IR (Nujol) 1725 (ester C=O) cm^{-1}; NMR ($CDCl_3$) δ 8.15 (s, 1, H-5), 8.09 (m, 2, phenyl ortho CH's), 7.46 (m, 3, phenyl meta and para CH's), 4.45 (q, 2, CH_2), 1.43 (t, 3, CH_3).

Methyl 2′-Phenyl-2,4′-bithiazole-4-carboxylate (6b). A solution of 4 (374 mg, 2.0 mmol) and methyl 2-(2-bromoacetyl)thiazole-4-carboxylate[3b] (**5b**; 477 mg, 1.8 mmol) in 2.5 mL of *N,N*-dimethylformamide was stirred at room temperature for 15 h. The crystalline product that formed during the course of the reaction was filtered and washed with cold ethyl acetate, giving 485 mg (89%) of **6b**: mp 175–176 °C (lit.[10] mp 177–178 °C); IR (Nujol) 1720 (ester C=O) cm^{-1}; NMR ($CDCl_3$) δ 8.23 (s, 1, H-5), 8.15 (s, 1, H-5′), 8.02 (m, 2, phenyl ortho CH's), 7.48 (m, 3, phenyl meta and para CH's), 3.99 (s, 3, CH_3).

Methyl 2″-Phenyl-2,4′:2′,4″-terthiazole-4-carboxylate (6c). A solution of 4 (203 mg, 1.48 mmol) and methyl 2′-(2-bromoacetyl)-2,4′-bithiazole-4-carboxylate[3b] (**5c**; 421 mg, 1.21 mmol) in 6 mL of *N,N*-dimethylformamide was heated at 60 °C for 2 h. The material that crystallized upon cooling of the reaction mixture was filtered, washed with anhydrous ether, and dried in vacuo, giving 328 mg (82%) of **6c**, mp 260–262 °C. A portion was recrystallized from toluene to give an analytical sample: mp 261–262 °C; IR (Nujol) 1730 (ester C=O) cm^{-1}; NMR ($CDCl_3$) δ 8.23 (s, 1, H-5), 8.20 (s, 1, H-5′), 8.08 (s, 1, H-5″), 8.03 (m, 2, phenyl ortho CH's), 7.49 (m, 3, phenyl meta and para CH's), 4.00 (s, 3, CH_3). Anal. ($C_{17}H_{11}N_3O_2S_3$) C, H, N.

Methyl 3-(2-Phenylthiazole-4-carboxamido)propyl Sulfide (7a). A solution of **6a** (209 mg, 0.90 mmol) in 0.5 mL of 3-(methylthio)propylamine was heated at 75 °C for 4.5 h. After cooling, the reaction mixture was poured into 25 mL of water and the pH was adjusted to 2 with concentrated HCl. The acidified mixture was extracted with three 25 mL portions of dichloromethane. The combined extracts were dried with anhydrous magnesium sulfate and evaporated to give an oil. Preparative thin-layer chromatography on silica gel with ethyl acetate–chloroform (1:1, v/v) gave 205 mg (78%) of **7a** as an oil: IR (neat) 3500 (sh) and 3340 (NH), 1665 (amide C=O), 1545 (amide II) cm^{-1}; NMR ($CDCl_3$) δ 8.09 (s, 1, H-5), 7.96 (m, 2, phenyl ortho CH's), 7.63 (s, 1, NH), 7.47 (m, 3, phenyl meta and para CH's), 3.60 (q, 2, NCH_2), 2.62 (t, 2, SCH_2), 2.14 (s, 3, SCH_3), 1.97 (p, 2, $CH_2CH_2CH_2$). Anal. ($C_{14}H_{16}N_2OS_2$) C, H, N.

Methyl 3-(2′-Phenyl-2,4′-bithiazole-4-carboxamido)propyl Sulfide (7b). A solution of **6b** (203 mg, 0.67 mmol) in 2 mL of 3-(methylthio)propylamine was heated at 80 °C for 2 h. The reaction mixture was poured into 100 mL of ice, and the resulting precipitate was filtered and dried in vacuo, giving 243 mg (96%) of **7b**, mp 85–87 °C. Recrystallization of a portion of the material from ethanol–water gave an analytical sample: mp 86.5–88 °C; IR (Nujol) 3590, 3425, 3300 (NH), 1640 (amide C=O), 1550 (amide II) cm^{-1}; NMR ($CDCl_3$) δ 8.15 (s, 1, H-5), 8.03 (m, 3, H-5′ and phenyl ortho CH's), 7.65 (t, 1, NCH_2), 7.50 (m, 3, phenyl meta and para CH's), 3.60 (q, 2, NCH_2), 2.63 (t, 2, SCH_2), 2.14 (s, 3, SCH_3), 1.98 (p, 2, $CH_2CH_2CH_2$). Anal. ($C_{17}H_{17}N_3OS_3$) C, H, N.

Methyl 3-(2″-Phenyl-2,4′:2′,4″-terthiazole-4-carboxamido)propyl Sulfide (7c). A suspension of **6c** (328 mg, 0.85 mmol) in 6 mL of 3-(methylthio)propylamine was heated at 60–70 °C for 6 h. Cooling of the reaction mixture gave a solid, which was filtered and dried in vacuo: yield 228 mg; mp 200–202 °C. The filtrate was poured into 50 mL of water, and the resulting precipitate was collected by filtration and dried, giving an additional 131 mg of **7c**: mp 192–195 °C; total yield 92%. Recrystallization of a portion of the material from ethanol–chloroform gave an analytical sample: mp 200.5–202 °C; IR (Nujol) 3400 (NH), 1660 (amide C=O), 1545 (amide II) cm^{-1}; NMR ($CDCl_3$) δ 8.14 (s, 1, H-5), 8.10 (s, 1, H-5′), 8.04 (m, 2, phenyl ortho CH's), 8.03 (s, 1, H-5″), 7.59 (t, 1, NH), 7.51–7.47 (m, 3, phenyl meta and para CH's), 3.61 (q, 2, NCH_2), 2.64 (t, 2, SCH_2), 2.15 (s, 3, SCH_3), 1.98 (p, 2, $CH_2CH_2CH_2$). Anal. ($C_{20}H_{18}N_4OS_4$) C, H, N.

Methyl 3-(4-Phenylbenzamido)propyl Sulfide (9b). A mixture of 4,4′-biphenylcarboxylic acid (1.0 g, 5 mmol) and 5 mL of thionyl chloride was stirred at reflux for 4 h, after which time excess thionyl chloride was removed in vacuo. The residual crude acid chloride **8b** (1.08 g, 5 mmol) was dissolved in 5 mL of anhydrous dichloromethane containing triethylamine (0.56 g, 5.5 mmol), and the solution was treated with 3-(methylthio)-propylamine (578 mg, 5.5 mmol) at room temperature for 2 h with stirring. The reaction mixture was poured into 50 mL of water and extracted with dichloromethane (3 × 20 mL). The combined organic fractions were washed with 0.1 N HCl (2 × 20 mL) and water (3 × 20 mL) and dried with anhydrous magnesium sulfate. Removal of solvent afforded 1.2 g of crude **9b** (84%). Recrystallization of a portion of the material from ethanol gave an analytical sample: mp 135–137 °C; IR (Nujol) 3330 (NH), 1635 (amide C=O), 1530 (amide II) cm^{-1}; NMR ($CDCl_3$) δ 7.86 (m, 2, H-2, H-6), 7.66 (m, 2, H-3, H-5), 7.61 (m, 2, H-2′, H-6′), 7.47 (m, 2, H-3′, H-5′), 7.39 (m, 1, H-4′), 6.51 (t, 1, NH), 3.61 (q, 2, NCH_2), 2.64 (t, 2, SCH_2), 2.14 (s, 3, SCH_3), 1.97 (p, 2, $CH_2CH_2CH_2$). Anal. ($C_{17}H_{19}NOS$) C, H, N.

General Procedure for the Preparation of Dimethylsulfonium Derivatives (2 and 3). A solution of the methylthio ether (**7** or **9**) (~0.2 mmol) in 0.5 mL of iodomethane–methanol (1:1, v/v) was allowed to stand at room temperature for 10 h. Excess iodomethane and methanol were removed with a stream of nitrogen.

Compound **2a** was obtained as an amorphous yellow solid, which tended to discolor. This material was converted to the corresponding chloride derivative by passage of an aqueous solution of the iodide salt through a column (1 × 10 cm) of Dowex 1X8 (chloride form). Lyophilization of the eluate gave an 86% yield of **2a** as the trihydrate: NMR (D_2O) δ 8.24 (s, 1, H-5), 8.03 (m, 2, phenyl ortho CH's), 7.58 (m, 3, phenyl meta and para CH's), 3.63 (t, 2, NCH_2), 3.41 (t, 2, SCH_2), 2.93 [s, 6, $S(CH_3)_2$], 2.20 (m, 2, $CH_2CH_2CH_2$). Anal. ($C_{15}H_{19}ClN_2OS_2 \cdot 3H_2O$) C, H, N.

Compound **2b** was obtained in 96% yield upon evaporation of the reaction solution. Crystallization of the material from methanol gave an analytical sample: NMR (Me_2SO-d_6) δ 8.70 (t, 1, NH), 8.36 (s, 1, H-5), 8.32 (s, 1, H-5′), 8.03 (m, 2, phenyl ortho CH's), 7.57 (m, 3, phenyl meta and para CH's), 3.46 (q, 2, NCH_2), 3.34 (t, 2, SCH_2), 2.90 [s, 6, $S(CH_3)_2$], 2.02 (p, 2, $CH_2CH_2CH_2$). Anal. ($C_{18}H_{20}IN_3OS_3$) C, H, N.

Derivative **2c** was obtained as a white solid, which is only sparingly soluble in water, methanol, or ethanol: yield 81%; NMR (Me_2SO-d_6) δ 8.73 (t, 1, NH), 8.47 (s, 1, H-5), 8.37 (s, 1, H-5′), 8.35 (s, 1, H-5″), 8.05 (m, 2, phenyl ortho CH's), 7.60 (m, 3, phenyl meta and para CH's), 3.47 (q, 2, NCH_2), 3.34 (t, 2, SCH_2), 2.89 [s, 6, $S(CH_3)_2$], 2.02 (p, 2, $CH_2CH_2CH_2$). Anal. ($C_{21}H_{21}IN_4OS_4 \cdot H_2O$) C, H, N.

Conversion of the iodide salt of **2c** to the corresponding chloride, acetate, or nitrate derivatives by using the appropriate anion form of Dowex 1X8 did not yield a material with improved solubility properties.

(9) VanGeet, A. L. *Anal. Chem.* **1968**, *40*, 2227–2229.
(10) Fujii, A.; Takita, T.; Shimada, N.; Umezawa, H. *J. Antibiot.* **1974**, *27*, 73–77.
(11) Hall, G. E.; Walker, J. *J. Chem. Soc.* **1966**, 1337–1359.

Viscometric Measurements. Viscometry was performed with Cannon-Ubbelohde semimicro dilution viscometer cells (Cannon Instrument Co., State College, PA). The temperature was maintained at 25 ± 0.1 °C by a Cannon constant temperature bath. Calf thymus DNA (0.7–1.5 mM in nucleotide residues) in 0.01 M sodium phosphate–0.10 M sodium chloride–0.1 mM EDTA (pH 7.0) was titrated with successive increments of the desired analogue dissolved in the same buffer. After each addition, the solution was thoroughly mixed, and the viscosity was measured a minimum of 5 times. Each experimental point is the average of these determinations. The relative viscosity (the ratio of the viscosity at any given analogue concentration to the viscosity of that DNA solution prior to the addition of any analogue) was then plotted as a function of the molar ratio of analogue to DNA phosphate.

Cell Culture Experiments. Mouse leukemia L1210 cells were maintained in suspension culture in RPMI 1640 medium supplemented with 10% fetal bovine serum (Calbiochem, LaJolla, CA) and incubated at 37 °C in an atmosphere of 5% carbon dioxide. Compounds were assayed for biological activity based on the effects of the analogues on cell number or on the ability of treated cells to form clones once removed from the presence of the compound.

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Registry No. **2a**, 85318-76-9; **2b** (X = I), 83579-43-5; **2c** (X = I), 85318-77-0; **4**, 2227-79-4; **5a**, 70-23-5; **5b**, 76275-88-2; **5c**, 80337-80-0; **6a**, 59937-01-8; **6b**, 7113-08-8; **6c**, 85318-71-4; **7a**, 85318-72-5; **7b**, 85318-73-6; **7c**, 85318-74-7; **8b**, 14002-51-8; **9b**, 85318-75-8; poly(dA-dT), 26966-61-0; 3-(methylthio)propylamine, 4104-45-4; 4,4'-biphenylcarboxylic acid, 92-92-2.

3'-Amino-2',3'-dideoxyribonucleosides of Some Pyrimidines: Synthesis and Biological Activities

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3'-Amino-2',3'-dideoxyribonucleosides of thymine, uracil, and 5-iodouracil (1–3) were synthesized from the corresponding 2'-deoxyribonucleosides via the *threo*-3'-chloro and the *erythro*-3'-azido derivatives. Corresponding aminonucleosides of 5-bromouracil, 5-chlorouracil, and 5-fluorouracil (4–6) were synthesized enzymatically with 3'-amino-2',3'-dideoxythymidine as the aminopentosyl donor and thymidine phosphorylase (EC 2.4.2.4) as the catalyst. 3'-Amino-2',3'-dideoxycytidine (7) was synthesized by amination of the 3'-azido precursor of 3'-amino-2',3'-dideoxyuridine. The biological activity of 3'-amino-2',3'-dideoxy-5-fluorouridine (6) was notable among this group of aminonucleosides. It had an ED_{50} of 10 μM against adenovirus and was not appreciably cytotoxic to mammalian cells in culture. It also had activity against some Gram-positive bacteria but not against a variety of Gram-negative bacteria. The other aminonucleosides (1–5 and 7) lacked or exhibited weak antiviral and antibacterial activities. The only compounds in this group that were appreciably toxic to mammalian cells in culture were the thymidine and deoxycytidine analogues (1 and 7).

Only a few 3'-amino-2',3'-dideoxyribonucleosides of pyrimidines have been reported. The first such nucleosides synthesized contained the thymine, 5-methylcytosine, and *N*-butyl-5-methylcytosine moieties.[1] 3'-Amino-2',3'-dideoxythymidine (1) was found to have appreciable antitumor activity but only weak antiherpes virus activity.[1c,2] Later, the corresponding nucleoside of 5-(2-bromovinyl)-uracil was reported to have appreciable activity against herpes simplex virus (type I).[3] Recently, the uracil (2) and cytosine (7) congeners were reported to have considerable antitumor activity.[4]

In this study, the 3'-amino-2',3'-dideoxyribonucleosides of the 5-halogenouracils, as well as that of thymine, uracil, and cytosine, were synthesized. The ability of these aminonucleosides to inhibit the growth of mammalian cells and a variety of viruses and bacteria in vitro was assessed.

Results and Discussion

Chemistry. The synthetic routes for the 3'-amino-2',3'-dideoxyribonucleosides listed in Table I are summarized in Scheme I. The abbreviated names provided in Table I are used below. 3'-NH_2-dThd (1) and 3'-NH_2-dUrd (2) were prepared from the corresponding 2'-deoxyribonucleosides. The initial step was tritylation of the 5'-hydroxy groups by reaction with trityl chloride in pyridine at 100 °C.[5] Next, chlorination in the 3'-position was achieved by reaction with triphenylphosphine and carbon tetrachloride in dimethylacetamide.[6] After the 5'-hydroxy group was deblocked by treatment with 80% acetic acid, the 1-(3-chloro-2,3-dideoxy-β-D-*threo*-pentofuranosyl) derivatives were obtained. Their *threo* configuration was confirmed by NMR (dd for $H_{1'}$).[6,7] These *threo*-3'-chloro derivatives were converted to the corresponding *erythro*-3'-azido derivatives by displacement with lithium azide.

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(1) (a) N. Miller and J. J. Fox, *J. Org. Chem.*, **29**, 1772 (1964). (b) J. P. Horwitz, J. Chua, and M. Noel, *J. Org. Chem.*, **29**, 2076 (1964). (c) T.-S. Lin and W. H. Prusoff, *J. Med. Chem.*, **21**, 109 (1978).
(2) (a) Y.-C. Cheng and W. H. Prusoff, *Biochemistry*, **13**, 1179 (1974). (b) P. H. Fischer, T.-S. Lin, and W. H. Prusoff, *Biochem. Pharmacol.*, **28**, 991 (1979).
(3) R. Busson, L. Colla, H. Vanderhaeghe, and E. DeClercq, *Nucleic Acid Symp. Ser.*, **9**, 49 (1981).
(4) T.-S. Lin and W. R. Mancini in "Abstracts of Paper", 184th National Meeting of the American Chemical Society, Kansas City, MO, Sept 1982, American Chemical Society, Washington, DC, 1982, Abstr MEDI 31.
(5) W. W. Zorbach and R. S. Tipson, Eds., *Synth. Proced. Nucleic Acid Chem.*, 1, 321 (1968).
(6) J. P. H. Verheyden and J. G. Moffatt, *J. Org. Chem.*, **37**, 2289 (1972).
(7) G. A. Freeman, L. M. Beacham III, and A. Ragouzeos, *Synth. Commun.*, **10**, 685 (1980).

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