a femoral artery was also catheterized for repetitive blood sampling. The drugs were injected as a bolus 40–50 min after the injection of pentobarbital. The temperature of the animals was monitored throughout and automatically maintained at 36–37 °C. Before the injection of a compound, a blood sample was taken to determine the basal blood glucose level. The blood was collected in 60-μL microhematocrit capillary tubes and centrifuged. The plasma was directly analyzed for glucose with a Beckman glucose analyzer. Samples were taken after 2, 5, 10, 20, 30, 40, and 50 min. Each data point is the mean of four to six animals.

Control experiments (basal), to determine the level of blood glucose throughout the experiment, were performed in exactly the same manner. But only a buffer bolus (0.5 mL, NaCl 145 mM, Na_2CO_3 2 mM, BSA 0.25%) without glucagon or analogue was injected.

In the antagonist studies, both glucagon (1 μg/kg) and analogue were injected at time 0 min, dissolved in the same 0.5 mL buffer (Figure 4).

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Amplification of Bleomycin-Mediated Degradation of DNA

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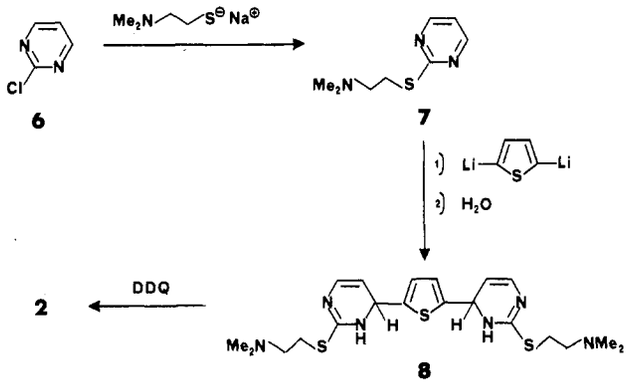
Three simple and independent tests have been introduced for studying the effect of DNA intercalating compounds on the bleomycin-mediated digestion of DNA in vitro. These methods are based on (i) hyperchromic changes of DNA solution, (ii) changes in viscosity of DNA solution, and (iii) HPLC quantitative analysis of the four bases released from digested DNA. All three tests give comparable results. However, the viscometric method is technically the simplest and at the same time the most sensitive. The amplification of the bleomycin-mediated degradation of DNA by three unfused heteropolyaromatic intercalator molecules, namely *N*-[2″-(dimethylamino)ethyl]-4-thien-2′-ylpyrimidin-2-amine (**1N**), *N*,*N*-dimethyl-2-[(4′-thien-2″-ylpyrimidin-2′-yl)thio]ethylamine (**1S**), and newly synthesized 2,5-bis[2′-[[2″-(dimethylamino)ethyl]thio]pyrimidin-4′-yl]thiophene (**2**) correlates well with the respective DNA binding constants for these compounds and is concentration dependent. The amplification activity of these compounds increases with increasing concentrations. The strongly binding compound **2** is the best amplifier of bleomycin in vitro found so far. Fused heteropolyaromatic systems, like ethidium bromide, are modest amplifiers of bleomycin at low concentrations but strongly inhibit the bleomycin chemistry at high concentrations.

The anticancer drug bleomycin is a family of enzyme-like molecules that bind with and cause degradation of DNA in the presence of ferrous ion and molecular oxygen[1] (Figure 1). The drug continues to interest oncologists because of its remarkable lack of bone marrow toxicity and preferential accumulation in cancerous cells. Because of pulmonary toxicity, bleomycin is used clinically in low doses, mainly in synergistic combinations with other anticancer agents, e.g., cisplatin, mytomycin, methotrexate, or doxurubicin.[2] Another approach to improving bleomycin chemotherapy is to find compounds (amplifiers) that alone do not have significant activity or toxicity but that enhance the activity of the drug. Amplifiers of the cytotoxic effect of bleomycin may, in general, (i) increase the intracellular level of the drug,[3] (ii) inhibit the DNA repair and/or synthesis,[4] or (iii) bind with DNA and by so doing stimulate directly drug-induced DNA fragmentation.[5] Modification of the bleomycin-mediated digestion of DNA by ethidium bromide and similar fused-ring intercalator molecules was noticed a decade ago.[5c] In general, enhancement[5c] of the digestion rate for low concentrations and inhibition[5c,6] for high concentrations of the intercalator were observed. More recent studies with mouse L1210 leukemia cells have suggested that combination chemotherapy of bleomycin with low levels of DNA intercalating agents may represent a novel and rational approach to the treatment of cancer.[5a] Recently we have also reported that

Scheme I



two unfused-ring compounds, **1N** and **1S**, intercalate with DNA and their binding constants correlate well with the

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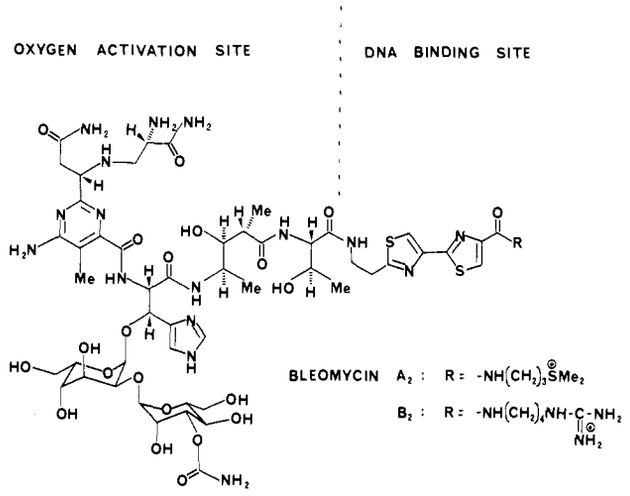
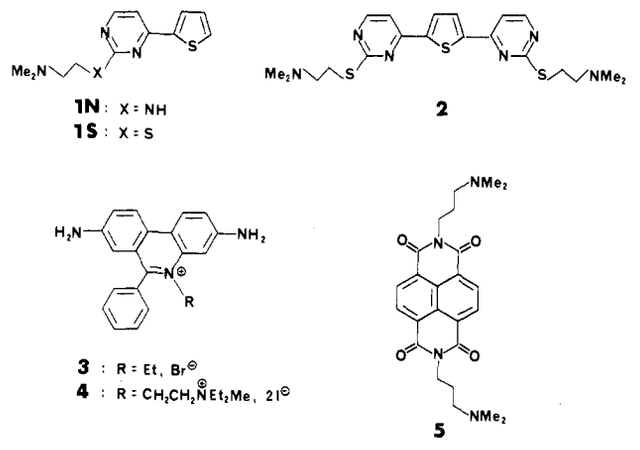


Figure 1. Structures of selected bleomycins. Other bleomycins also differ in the structure of the cationic side chain R.

respective biological activities as amplifiers of phleomycin,[7] an anticancer drug closely related to bleomycin.

In the present research, we have examined the action of bleomycin on DNA in the presence of these two compounds and their tricyclic analogue **2**. The effects of **1–2** are also compared to those of fused-ring, classical intercalators **3–5**.



Results

Synthesis of 2. 2,5-Bis[2′-[[2″-(dimethylamino)-ethyl]thio]pyrimidin-4′-yl]thiophene (**2**) was prepared according to Scheme I. Reaction of 2-chloropyrimidine (**6**) with sodium 2-(dimethylamino)ethylthiolate furnished *N*,*N*-dimethyl-2-(pyrimidin-2′-ylthio)ethylamine (**7**). Subsequent coupling of compound **7** (2 equiv) with 2,5-dilithiothiophene produced a bis-dihydropyrimidine derivative **8**, which, without isolation, was dehydrogenated with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) to give **2**.

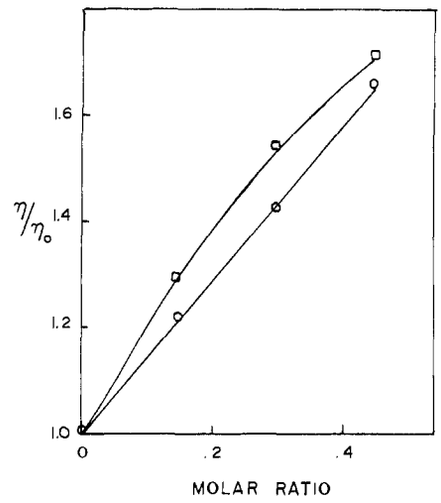


Figure 2. Viscometric titrations of sonicated calf thymus DNA with 2 (□) and 3 (○). The reduced specific viscosity ratio (ζ/ζ_0) is plotted as a function of the molar ratio of **2** and **3** to DNA base pairs. The titrations were conducted in PIPES 00 buffer at 28 °C.

DNA-Binding Studies. Binding characteristics of compounds **1**[7] and **3–5**[8,9] with DNA have been reported previously. All these compounds intercalate with DNA. A newly synthesized tricyclic compound **2** causes significant increases in viscosity in a titration of DNA solution (Figure 2). These changes are larger than those caused by ethidium bromide under the same conditions and, therefore, strongly suggest that the heteroaromatic systems of **2** bind with DNA through intercalation as well. This suggestion is further supported by the following proton NMR and spectrophotometric data for the complex of **2** with DNA. There are large upfield shifts ($\Delta\delta$ = −0.61, −0.62, and −0.37 ± 0.01 ppm for H3, H5′, and H6′, respectively, at a 0.3 ratio of **2**/DNA base pairs at 65 °C) and line broadening of the aromatic protons of **2**. The effect on the NCH_3 protons is smaller and in the opposite direction ($\Delta\delta$ = +0.16 ± 0.01 ppm). At 35 °C and a 0.5 ratio of compound to base pair, the DNA imino proton upfield chemical shift changes are −0.34 ± 0.02 and −0.52 ± 0.02 ppm for the AT and GC base pairs, respectively. We have previously shown that shifts of this type are characteristic of ring current effects of the base pairs on the intercalator and of the intercalator on base pair imino protons in an intercalator complex.[10]

In the region above 300 nm, where interference by DNA absorption is not a problem, **2** has an absorption peak at 359 nm (ϵ 33 850 M^{-1} cm^{-1}). Addition of DNA to **2** causes a shift of the absorption maximum to longer wavelengths and a decrease in extinction coefficient. In the titration of **2** with DNA, there is an isosbestic point at 383 nm. Beer's law plots in concentrated DNA solutions (~100 fold excess of DNA to **2**) were used to calculate the bound extinction coefficient of 16 800 M^{-1} cm^{-1} for **2** at the wavelength of maximum absorbance for the free compound. The spectrophotometric data were used to obtain a DNA equilibrium binding constant of approximately 13

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Table I. Activity of Compounds 1–5 on the Bleomycin-Mediated Digestion of Calf Thymus DNA[a]

compd	DNA binding constant K, M^{-1}	relative rate of DNA digestion as measured by: relative flow time decreases of DNA solution:[b] $(t_0 - t_2)/(t_0 - t_1)$ A[d]	B[e]	release of DNA bases[c] A[d]	B[e]
		1.0		1.0	
1N	4 400[f]	1.3	1.6	1.2	2.3
1S	18 700[f]	1.5	1.7	1.9	3.4
2	13 000 000	4.2	4.3	10.0	11.2
3	900 000[g]	1.8	0.1	2.1	0.5
4	10 000 000[g]	1.2	0.03	2.5	0.2
5	>1 000 000[h]	1.5	0.03	1.5	0.2

[a] See the Experimental Section for concentrations and general conditions. [b] The flow times t_0, t_1, and t_2 are for DNA alone, DNA with activated bleomycin, and DNA with activated bleomycin plus compounds 1–5, respectively. [c] Measured by HPLC. Total integrated area of peaks corresponding to the four bases released from DNA in the absence of compounds 1–5 was taken as 1.0. [d] One intercalator molecule per three base pairs DNA. [e] Three molecules per one base pair DNA for **1N**, **1S**, and **3**–**5**; one molecule per two base pairs DNA for **2** (higher concentrations caused precipitation of DNA). [f] Taken from ref 7. [g] Extrapolated from results in ref 8. [h] Extrapolated from results in ref 9.

$\times 10^6$ M^{-1} for **2** under the conditions of this work. The binding constants for **1**–**5** are given in Table I.

Bleomycin-Induced Hyperchromicity of DNA. Activated bleomycin initially produces widely separated breaks in the DNA strands that have little effect on the DNA UV absorption spectrum. As the number of breaks increase and become more closely spaced, single-stranded regions and short low-melting double-helical pieces of DNA are produced, which result in a cooperative increase in absorption until the double helix is completely dissociated.[11] Increases in UV absorption at 260 nm were, thus, used in this work to monitor the reaction of bleomycin with DNA. This simple, but direct, test was also used to evaluate effects of compounds **1**–**5** on the bleomycin-mediated DNA cleavage.

The effect of bleomycin on DNA in the presence of ferrous ion and a thiol compound is shown in Figure 3. As expected, a cooperative increase in absorption is observed with increasing destablization and base pair unstacking as a function of the number of cleavage points in the helix. The effects of three unfused intercalators, **1N**, **1S**, and **2** (weak, intermediate, and strong binding with DNA, respectively), and ethidium bromide (**3**)—a fused, strongly binding intercalator—on the bleomycin-induced degradation of DNA are also shown in Figure 3. As can be seen in Figure 3, there is a dramatic difference between the effects of unfused compounds **1**–**2** and ethidium bromide (**3**). Low concentrations of **1N** and **1S** cause a negligible initial acceleration of the bleomycin reaction (up to 30 min), but the changes that follow this initial period are substantial and correlate well with the respective DNA-binding constants. Under the same conditions the effect of compound **2** is even more dramatic. The initial hyperchromic changes of the DNA solution increase with increasing concentrations of unfused compounds **1**–**2**. This correlation is exemplified in Figure 3 by the effects caused by two different concentrations of **1S**. On the other hand, low concentrations of ethidium bromide cause a slight

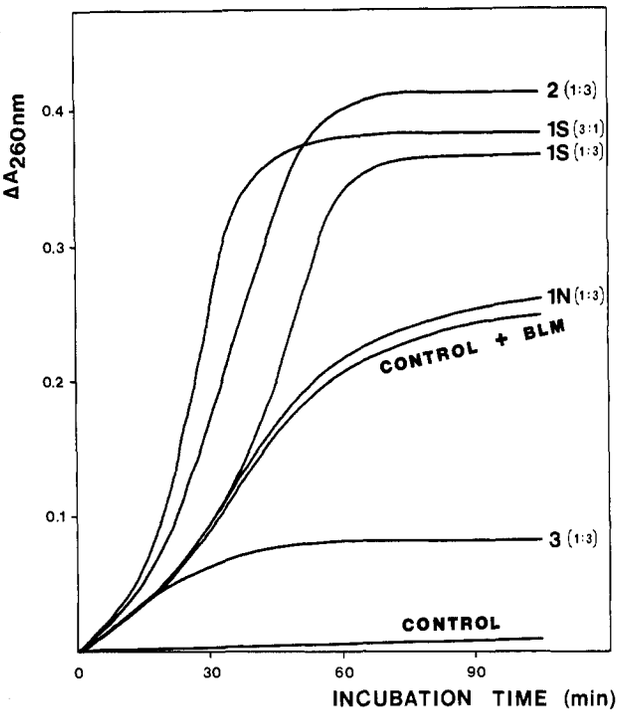

Figure 3. Hyperchromic changes caused by the action of activated bleomycin on calf thymus DNA in the presence of various heteropolyaromatic compounds (DNA intercalators) indicated at the respective curves. The ratios of the intercalator molecules to the number of DNA base pairs are shown in parentheses. A curve marked "CONTROL" shows absorption changes for a solution containing DNA, ferrous ion, and dithiothreitol (iron-reducing agent) but without bleomycin and the heteropolyaromatic intercalator. The hyperchromic changes, obtained in the presence of unfused compounds **1N**, **1S**, or **2** and activated bleomycin, are greater than the respective thermally induced increases in UV absorption of the DNA solutions obtained in the presence of **1N**, **1S**, or **2** but without bleomycin, under the same concentration conditions. See the Experimental Section for concentrations and general conditions.

enhancement of the initial hyperchromic changes (too small to be noticed in the scale of Figure 3) that is followed by a strong inhibitory effect. Higher concentrations of ethidium bromide than those indicated in Figure 3 result only in inhibition of the bleomycin action on DNA. The bleomycin-mediated cleavage of DNA, as measured by hyperchromic changes, is almost completely inhibited at a ratio of one molecule of ethidium bromide per two base pairs of DNA. Fused-ring compounds **4** and **5** cause similar effects (data not shown for the sake of clarity of Figure 3).

In a control experiment, UV absorption of a solution containing only DNA, one of the compounds **1**–**2**, and dithiothreitol (without bleomycin) was monitored as a function of incubation time. The same concentrations of the solution components, as in the standard experiments, were used. Constant absorption readings were obtained for up to 2 h of incubation, and these indicated that DNA was stable under these conditions. Addition of ferrous ion to this solution resulted in slight increases in UV absorption. The same increases (Figure 3) were obtained for a solution containing DNA, ferrous ion and dithiothreitol (without compounds **1**–**2** and bleomycin), which indicated again that compounds **1**–**2** do not cause degradation of DNA.

Viscometric Studies of DNA Digestion. Bleomycin-mediated digestion of DNA, as discussed above, also results in decreases in the viscosity of DNA solutions. The initial breaks produced in DNA cause little overall base

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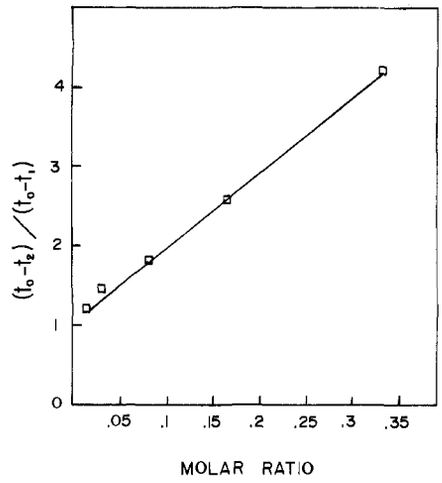

Figure 4. Effect of **2** on the relative flow time decreases, $(t_0 - t_2)/t_0 - t_1)$, of DNA solution. The flow times t_0, t_1 and t_2 are for DNA solution alone, DNA with activated bleomycin, and DNA with activated bleomycin plus compound **2**. The relative flow time decreases are plotted as a function of the molar ratio of **2** to DNA base pairs. See the Experimental Section for additional data.

pair unstacking and have little effect on UV absorption, but create regions in DNA with significantly enhanced flexibility. This results in large decreases in solution viscosity that are quite dependent on the effective length of the DNA molecules. We have found that the viscosity changes or viscometric flow times can serve as a basis for a very sensitive test to evaluate the effects of the additives **1–5** on the reaction of bleomycin with DNA. Since the most dramatic changes are observed for the initial stages of the DNA degradation, this test is complementary to the hyperchromicity studies (relatively insensitive for short reaction times). The initial relative rates of the DNA digestion in the absence and presence of compounds **1–5**, as measured by flow time decreases, are presented in Table I. A ratio of 1.0 indicates no effect, while values above 1.0 indicate amplification, and values below 1.0 indicate inhibition of bleomycin cleavage of DNA. At a ratio of one intercalator molecule per three DNA base pairs (column A), compounds **1–5** are all amplifiers. At higher concentrations (column B), compounds **1–2** show even stronger activity, whereas dramatically lower relative reaction rates are observed in the presence of fused compounds **3–5**. DNA binding constants for **1–2** correlate well with the respective initial viscosity decreases, but no such correlation exists for fused-ring intercalators **3–5**. Tricyclic compound **2** is the best amplifier of bleomycin in vitro found so far. Its activity decreases monotonically with decreasing concentration, as measured by the sensitive viscometric method, and is noticeable even at the ratio of one molecule per 60 DNA base pairs (the first experimental point in Figure 4).

HPLC of DNA Degradation Products. The products of DNA strand scission by Fe(II)–bleomycin include oligonucleotides, four possible base propenals, and four possible bases with ratios dependent on the concentration of molecular oxygen.[1] Such a mixture of products is difficult to quantitate. In addition, dithiothreitol, an iron reducing agent used in this work, is known to react with base propenals,[1c] thus making the quantitation of the bleomycin reaction with high-molecular-weight heterogeneous DNA even more difficult. In this work, in order to overcome the effect of oxygen concentration on the product distribution, relatively low concentrations of DNA and bleomycin and a buffer solution equilibrated with molecular oxygen were used. Thus, the reacting solutions contained a relatively constant level of oxygen. We have found that under such conditions the HPLC analysis of free bases is a convenient way to monitor the bleomycin-mediated degradation of DNA. A simple isocratic elution with 0.1 M ammonium trifluoroacetate results in an efficient separation of free bases from other DNA degradation products. Selected, typical chromatograms taken in the presence of **2** (chart A), without an intercalator additive (chart B), and in the presence of ethidium bromide (chart C) are shown in Figure 5. All four bases are observed, but cytosine and thymine constitute more than 90% of the total amount of these products under the experimental conditions employed. Quantitative results of this method that include the effects of compounds **1–5** on the bleomycin-mediated digestion of DNA are also summarized in Table I. As can be seen from Figure 5 and Table I, the results of the HPLC test agree well with those of the hyperchromicity and viscosity studies. Thus the DNA binding constants for unfused-ring amplifiers correlate well with the initial relative rates of the release of DNA bases and, again, these results show that compound **2** is the most efficient amplifier of bleomycin. In agreement with the previous data, high concentrations of fused-ring intercalators **3–5** cause inhibition of the release of free bases from DNA.

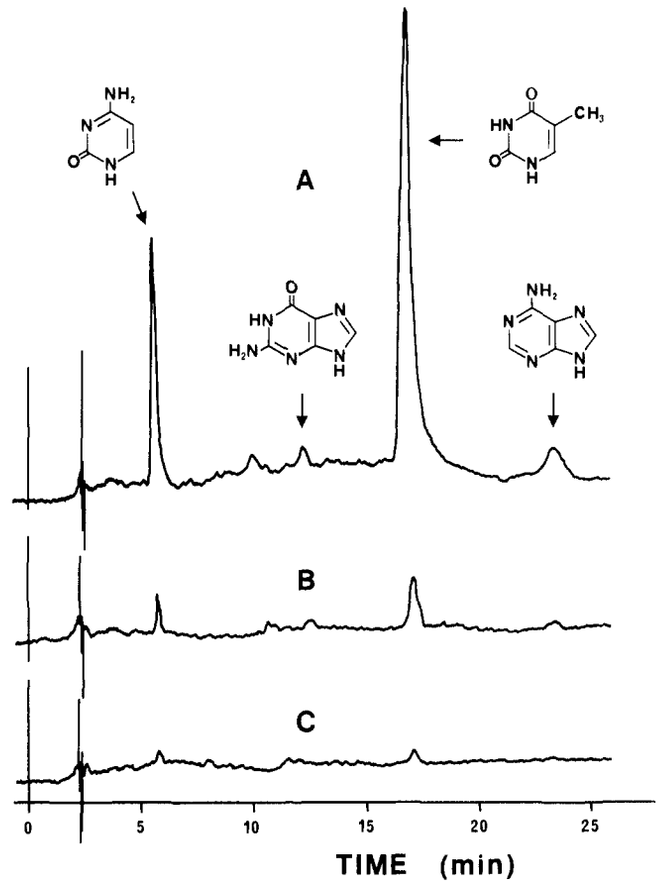

Figure 5. HPLC analyses of bleomycin-treated DNA: A, in the presence of **2** (a 1/3 ratio of **2** per DNA base pairs); B, without an intercalator additive; C, in the presence of **3** (a 3/1 ratio of **3** per DNA base pairs). See the Experimental Section for concentrations and general conditions.

Discussion

Chromatographic analysis of radioactive products from ^3H-, ^{14}C-, or ^{32}P-labeled DNA was used previously to evaluate the effects of intercalator molecules on the bleomycin-mediated cleavage of DNA.[5,12] In the present

research we have introduced three independent and much simpler methods that are suitable for a preliminary and routine screening of DNA binding compounds as potential amplifiers of bleomycin. All three tests give comparable results, but they differ in sensitivity. The viscometric method is technically the simplest and at the same time the most sensitive for high-molecular-weight DNA samples. Substantial changes in viscosity of the DNA solution are observed at the digestion level of DNA that corresponds to the detection limit of bases released from DNA, as measured by the HPLC method (approximately 10^{-11} M). The UV method is the least sensitive for short reaction times for reasons mentioned previously in this work. Therefore, the viscometric test is especially suited for the evaluation of bleomycin amplifiers in vitro at very low levels of DNA digestion and using low, biologically relevant concentrations of DNA, bleomycin, and amplifier.

There are several important findings from our studies of the effect of DNA intercalating molecules on the bleomycin-mediated digestion of DNA. First of all, the unperturbed DNA double helix is clearly not the best target for bleomycin. Intercalating molecules cause unwinding of the DNA helix and conformational changes in its sugar portions, known sites of attack by bleomycin-induced free radicals.[1] These conformational changes apparently facilitate the subsequent chemical reaction. *Unfused* ring systems **1** and **2** are better amplifiers of bleomycin than rigid, planar intercalators **3–5** because the former compounds apparently cause more effective conformational perturbations of the DNA tertiary structure. This may be due to the torsional conformational freedom in the unfused polyaromatic molecules. It can be expected that the dynamics of the unfused systems cause more extensive structural changes and/or breathing motions of the DNA base pairs in the intercalation complex than fused systems of similar molecular area. This effect is then transmitted to the sugar moieties and/or enhances bleomycin interations. In full agreement with this hypothesis, unfused compound **2** causes more extensive lengthening of DNA than ethidium bromide under the same conditions (Figure 2) and, at the same time, is a better amplifier.

This hypothesis alone, however, cannot explain the experimental facts that DNA saturated with **1–2** is an excellent target for bleomycin while compounds **3–5** exhibit a strong inhibitory effect on DNA cleavage under similar conditions. Compound **2**, which is the best amplifier of bleomycin in vitro found so far, also exhibits the greatest amplification activity at high concentration. Its DNA binding constant (Table I) far exceeds that of bleomycin,[13] and is also the greatest of all binding constants for compounds tested in this work. Since interaction of bleomycin with DNA is the necessary condition for the efficient digestion of DNA,[1,14] it appears that DNA saturated with **2** still can bind bleomycin. In contrast, we believe that fused molecules **3–5** compete with bleomycin for DNA binding sites. While at low concentrations of **3–5** there are sites on DNA that can accept bleomycin molecules, the binding of bleomycin becomes impossible when DNA is saturated with strongly bound compounds **3–5**. This is in agreement with the findings that interactions of intercalator molecules **3–5** with DNA are neither base sequence selective nor, apparently, groove specific.[8,9] Bleomycin binds preferentially at the purine–pyrimidine sequences through intercalation or partial intercalation of its bithiazole portion and electrostatic interaction between the cationic side chain and the DNA anionic backbone[1,15] (Figure 1). It has been suggested that these interactions take place stereospecifically in the minor groove of DNA.[1a] The lack of inhibition of bleomycin by high concentrations of amplifiers **1–2**, and especially by strongly binding compound **2**, can be explained by the stereoselective, amplifier–DNA interaction, which takes place in the major groove of DNA only. Such interaction is not expected to hinder the formation of the bleomycin–DNA complex in the minor groove of DNA. This working hypothesis is strongly supported by the recent reports[12] on the effect of various C-terminal bithiazole analogues of bleomycin on the DNA degradation by bleomycin. These compounds mimic closely the DNA-binding portion of the bleomycin molecule and, accordingly, exhibit similar selectivity in binding with DNA. They block the binding of bleomycin to DNA, and are strong inhibitors of DNA degradation by bleomycin.

Relatively little work has been done on intercalation of unfused polyaromatic ring systems with DNA.[15,16] Clearly, additional studies are needed to better understand differences in DNA intercalation phenomena between different unfused-ring systems. An understanding of this problem undoubtedly will result in design of better amplifiers of bleomycin.

So far we have shown that 4-(2-thienyl)pyrimidines with amino side chains attached are good bleomycin amplifiers. For **2** and similar unfused intercalators, the amino (cationic in H_2O) side chain is an important part of the molecular system. The cationic substituent not only increases solubility of the molecule but also increases the DNA binding constant through electrostatic interaction with the anionic DNA backbone. The latter feature often helps to obtain an intercalation complex for aromatics which without the cationic group bind very weakly with DNA.[16] The cationic side chain also increases the binding constant of a fused intercalator system, as shown in Table I for a pair of compounds **3** and **4**. Nevertheless, both compounds exhibit similar effects on the bleomycin-mediated cleavage of DNA.

Experimental Section

N-[2″-(Dimethylamino)ethyl]-4-thien-2′-ylpyrimidin-2-amine (**1N**),[7,17] *N*,*N*-dimethyl-2-[(4′-thien-2″-ylpyrimidin-2′-yl)thio]-ethylamine (**1S**),[7,17] and 2,7-bis[2′-(dimethylamino)ethyl]-1,2,3,6,7,8-hexahydro-1,3,6,8-tetraoxo-2,7-diazapyrene (**5**)[18] were prepared and purified as reported. Ethidium bromide (**3**) and propidium iodide (**4**) were obtained from Sigma and Calbiochem, respectively, and purified as previously discussed.[19] Bleomycin was a mixture of bleomycin sulfates obtained from Sigma. Calf thymus DNA was sonicated for NMR experiments, phenol ex-

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tracted, dialyzed, and characterized as previously described.[8] PIPES 00 buffer (0.01 M PIPES without EDTA, pH 7) was used in all experiments with bleomycin.

***N,N*-Dimethyl-2-(pyrimidin-2′-ylthio)ethylamine (7).** Metallic sodium (1.19 g, 52 mmol) was reacted with anhydrous ethanol (100 mL) and the resultant solution of sodium ethoxide was treated with 2-(dimethylamino)ethanethiol hydrochloride (3.68 g, 26 mmol). The mixture was stirred for 10 min at 25 °C, then treated with 2-chloropyrimidine (2.84 g, 24.8 mmol), and stirred for 10 h at 80 °C. The ethanol was evaporated on a rotary evaporator and the residue was extracted with ether (3 × 50 mL). Removal of the ether on a rotary evaporator was followed by Kugelrohr distillation (80 °C (0.1 mmHg)) of the oily residue to give **7** (4.1 g, 90%): ^{1}H NMR ($CDCl_3/Me_4Si$) δ 2.30 (s, 6 H, NCH_3), 2.4–2.8 (m, 2 H, NCH_2), 3.1–3.4 (m, 2 H, SCH_2), 6.90 (t, J = 4.5 Hz, 1 H, H 5′), 8.45 (d, J = 4.5 Hz, 2 H, H4′ and H6′). Anal. ($C_8H_{13}N_3S$) C, H, N.

2,5-Bis[2′-[[2″-(dimethylamino)ethyl]thio]pyrimidin-4′-yl]thiophene (2). A solution of *n*-butyllithium in hexane (1.8 M, 1.64 mL, 2.94 mmol) was added dropwise to a stirred solution of 2,5-dibromothiophene (356 mg, 1.47 mmol) in dry ether (50 mL) at −40 °C under a nitrogen atmosphere. After the addition was complete, stirring was continued for an additional 20 min, and the resultant solution of 2,5-dilithiothiophene was treated dropwise at −40 °C with a solution of **7** (600 mg, 3.27 mmol) in ether (10 mL). The mixture was stirred at −40 °C for 30 min and then at 23 °C for 30 min, quenched with water (1 mL), and treated with a solution of 2,3-dichloro-5,6-dicyanobenzoquinone (742 mg, 3.27 mmol) in tetrahydrofuran (10 mL). The mixture was refluxed for 30 min and then stirred with an aqueous solution of NaOH (10%, 25 mL). The organic layer was separated, dried (Na_2SO_4), and concentrated. Chromatography on silica gel (hexanes/CH_2Cl_2/Et_3N, 7/2/1) afforded **2** as an oil (165 mg, 25%): ^{1}H NMR ($CDCl_3/Me_4Si$) δ 2.36 (s, 12 H, NCH_3), 2.45–2.90 (m, 4 H, NCH_2), 3.20–3.50 (m, 4 H, SCH_2), 7.16 (d, J = 5 Hz, 2 H, H5′), 7.67 (s, 2 H, H3 and H4), 8.41 (d, J = 5 Hz, 2 H, H6′).

A hot solution of **2** (165 mg) in ethanol (10 mL) was treated with hydrobromic acid (24%, 1 mL). The mixture was refluxed briefly and cooled to precipitate a dihydrobromide of **2**: mp 311–312 °C. Anal. ($C_{20}H_{26}N_6S_3 \cdot 2HBr$) C, H, N.

Spectrophotometric Measurements. Both Cary 17D and Cary 219 spectrophotometers were used for UV–visible spectral measurements. Cell holders were thermostated by using Haake A81 circulating water baths. Temperature measurements were made by using either YSI 42 SC or Electromedics IT 610 temperature monitors. Before each experiment, a thermistor probe was inserted into the buffer, the temperature was monitored until the buffer had equilibrated at the desired temperature, and the probe was removed. Wavelength scans and absorbance measurements were made in quartz cells from 1- to 5-cm lengths. Extinction coefficients of both free and intercalated compound **2** were determined at the point of optimum spectral change.

For determination of binding isotherms, absorbance values of **2** in the presence of DNA samples were entered into a real-time interactive computer program that converted these values into ν (moles of **2** bound per mole of DNA base pairs) and free ligand concentrations with the free and bound extinction coefficients for the compound. Only binding data in the fraction bound range between 0.2 and 0.8 were used. Binding data outside of this range are subject to large errors as a result of experimental error in determining the free and bound extinction coefficients. A complete binding isotherm can be obtained by varying the initial DNA concentration. Upon termination of the binding experiment, the computer calculated a first-order approximation of the K and n values (where K is the equilibrium constant and n is the number of base pairs per binding site) and then plotted the data along with the theroretical curve from the site-exclusion model of McGhee and von Hippel.[20] The computer then calculated nonlinear least-squares best fit values for K and n and presented a graphical display of the results in the form of a Scatchard plot.

NMR. Proton (400 MHz) NMR spectra were obtained on a Varian VXR-400 spectrometer. The effect of **2** on DNA ^{1}H imino protons and chemical shift changes of protons of **2** on addition of DNA were measured as previously described.[7,21]

DNA–Bleomycin Reactions: General Conditions. Stock solutions (37 °C) were added in the order given below to PIPES 00 buffer to reach the final volume of 1.5 mL and the following final concentrations: calf thymus DNA 2.6×10^{-4} M (concentration of nucleotide equivalents); compounds **1–5**, 4.4×10^{-5} M or 4.4×10^{-4} M (ratios at 1/3 or 3/1 of molecules per DNA base pairs, respetively); $FeSO_4$, 7.6×10^{-6} M; dithiothreitol, 5×10^{-4} M; bleomycin, 3×10^{-6} M. The resultant solution was incubated at 37 °C.

Hyperchromicity Studies. The reactant solution was prepared in a UV cell of a Cary 17D spectrophotometer thermostated as described above. The absorbance of the solution was continuously monitored at 260 nm after initiation of the reaction until the absorbance change with time became insignificant.

Viscometric Studies. All viscosity experiments were conducted in Cannon-Ubbelohde series 75 semi-micro dilution viscometers which were maintained at constant temperature in a Cannon Model M-1 water bath. Solutions were added to the viscometer with modified Hamilton syringes that had extensions for inserting the syringe all the way to the bottom sample resevoir of the viscometer. Viscometric titrations were performed as previously described by adding alliquots of a concentrated stock solution of the desired compound to a DNA solution in the viscometer.[22] DNA digestion experiments were conducted in the same viscometers. The decrease in flow time for a DNA solution in the presence of activated bleomycin was measured and compared to the flow time decrease caused by bleomycin in the presence of **1–5**. After mixing, the flow times were immediately determined and are reported at an average time of 1 min after initiation of DNA digestion. Comparisons at longer times gave similar results.

HPLC Analyses. The standard reaction mixture was incubated at 37 °C for 30 min and then analyzed promptly by HPLC on a Rainin Microsorb C_{18} column (5 μm, 4.6 × 250 mm). Elution was with 0.1 M CF_3COONH_4 at a rate of 1 mL/min. Determination of DNA bases was with an LDC/Milton Roy Spetromonitor D UV detector operating at 254 nm and coupled with a Shimadzu C-R3A Chromatopac Data Processor (electronic integration of peak area). The products were identified by co-injection with original samples of DNA bases and by comparison of their UV spectra with those of the authentic samples. The total amount of the four DNA bases released from DNA after 30 min of incubation in the presence of activated bleomycin and **2** (Table I and Figure 5) corresponds to 40% of the maximum amount measured after 12 h of incubation.

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