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Molecular Basis for Bleomycin Amplification: Conformational and Stereoelectronic Effects in Unfused Amplifiers

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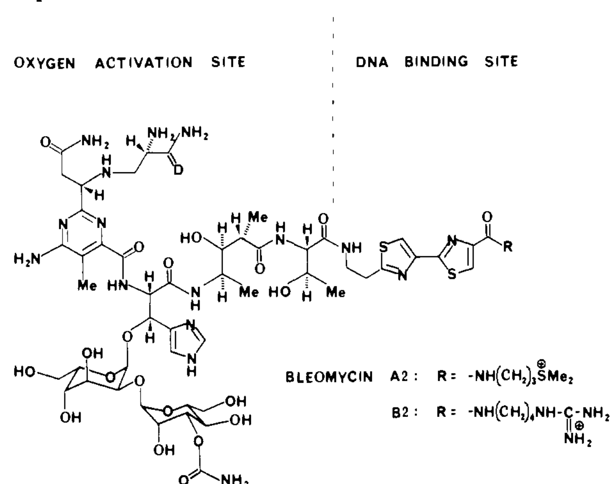
Sixteen unfused heterobiaromatic and biphenyl compounds substituted with an amino side chain (protonated in water) have been tested for (i) binding with DNA and (ii) their effect on the digestion of the DNA double helix by a bleomycin-iron complex. Only the DNA intercalating molecules amplify the digestion of DNA. One 2,2′-bipyridine derivative tested is an inhibitor of the bleomycin reaction because it removes ferrous ion from the bleomycin complex. Polarity of the intercalating unfused biaromatic system is of primary importance for effective binding of the molecule with native DNA and, at the same time, for its amplification activity. The molecules that have the biaromatic system polarized extensively in the direction of the side cationic chain, so that the intercalating sites constitutes a positive part of the dipole, show strong binding with DNA and good amplification activity. For strong intercalative forces that determine the amplification activity, it is important that both the heteroaromatic subsystems of the molecule have positive ends of their dipoles positioned away from the side chain. This work provides general guidelines for synthesis of new highly effective bleomycin amplifiers.

The anticancer and antibiotic properties of bleomycins (Chart I), a family of metal-chelating glycopeptides, are well recognized.[1] It is believed that the ferrous chelate is mainly responsible for the activity of bleomycins in vivo. Many studies have shown that DNA is the target for bleomycins. The ferrous complex is activated in the presence of molecular oxygen, binds with DNA, and causes degradation of the DNA.[2] In addition to synergism,[1] the cytotoxic effect of bleomycins can be potentiated in vitro and in vivo by agents that may not be active alone. The enhancement effect is believed to be due to (i) increasing the intracellular level of the drug,[3] (ii) inhibition of the DNA repair and/or synthesis,[4] (iii) enhancement of the efficiency with which Fe^{2+} is recruited into the drug,[5] (iv) direct action of the potentiating agents on the DNA,[6] and (v) other, less understood mechanisms of action.[7] The activities of phleomycins[7i,8-10] and tallysomycins[8] can also be amplified. The two drugs are structurally related to the bleomycins and are usually described as a family of bleomycin-type drugs.

In our research program on the enhancement of the activity of bleomycin-type drugs, we are interested in developing potentiators that belong to category iv above. The stimulation of bleomycin-induced fragmentation of DNA by DNA-interacting agents was noticed for the first time a decade ago by Bearden.[6a] More recently, Beerman,[6c,d] on the basis of work with ethidium, a classical intercalator, has suggested that the combination of DNA unwinding and strand scission may represent a novel and rational approach to the chemotherapy of cancer. The latter work, however, and recent studies by Grigg,[10a] although conducted on different biological systems, indicate that classical DNA intercalators are poor candidates for future development as the amplifiers. A plot of DNA breakdown as a function of concentration of the intercalator was bell shaped. DNA breakdown was enhanced initially, but above a certain concentration, the proportion of DNA degraded declined, often to zero. On the other hand, conformationally flexible DNA binding dyes gave broad dose-response curves, indicating that the difference between inhibitory concentrations and stimulatory concentrations was greater (often more than 2 orders of magnitude) than it was for ethidium and similar fused-ring DNA intercalators.[10a] Recently, we have shown[6f,10d] that another class of amplifiers, introduced for the first time by Brown,[9]

Chart I. Structures of Bleomycins A2 and B2, the Major Components of Blenoxane



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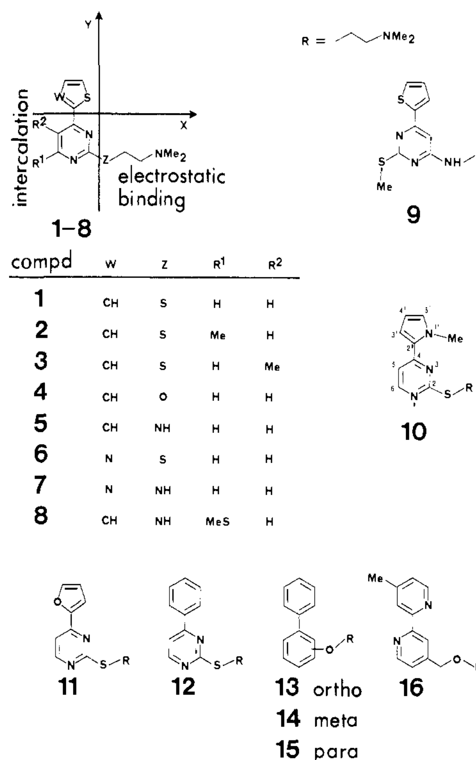
is superior to the classical planar intercalators in catalyzing degradation of DNA by bleomycins. These compounds have a conformationally flexible, unfused polyaromatic system and cationic side chain in the molecule. Our studies indicate that this class of compounds shows promise for development as amplifiers of the bleomycin-type drugs.

There are several objectives in this paper. First, we report the synthesis of a series of simple unfused-ring bicyclic compounds **2–4** and **8–16** (Chart II). They are designed to differ in stereoelectronic features of the bicyclic system and the position of attachment of a side cationic chain. They are close structural analogues of amplifier **1**, which is active in vitro[10f] and in vivo,[10e] and compounds **5–7**, which show mediocre activity.[10f] Compounds **1** and **5–7** have been included in our studies as controls. The most intriguing questions are whether or not structural, stereochemical, and stereoelectronic features of **1–16** play a role in binding with DNA and whether or not the DNA binding characteristics are important for amplification of the activity of bleomycins by these compounds. This work is part of our systematic research efforts directed toward an understanding of the molecular basis for bleomycin amplification.

Results

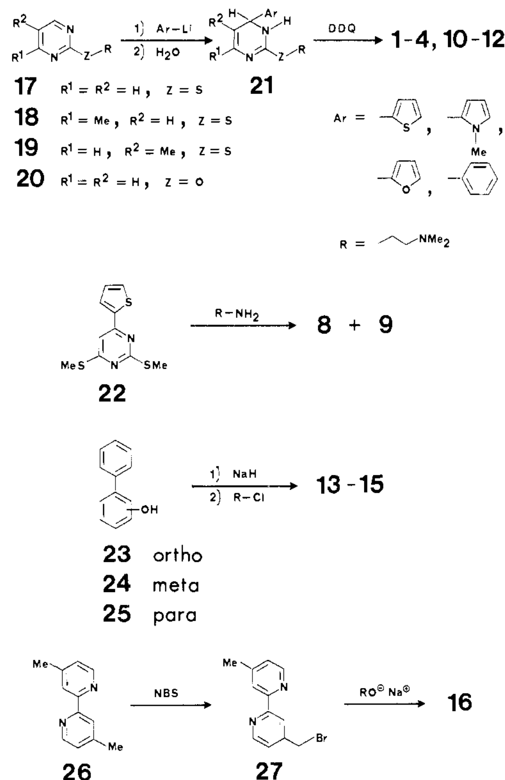
Synthesis. Methods of preparation of compounds **1–4** and **8–16** are given in Chart III. The bicyclic pyrimidine derivatives with a [(dimethylamino)ethyl]thio side chain, **1–3** and **10–12**, and one oxy analogue, **4**, were synthesized through the addition reaction of lithium reagents Ar–Li with suitably substituted pyrimidines **17–20**, followed by aromatization of the resultant dihydropyrimidines **21** with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ). Two amino-substituted isomers **8** and **9** were obtained in a nucleophilic displacement reaction of one methylthio group in compound **22** with N,N-dimethylethylenediamine. A large difference in the chemical shifts for the H5 atom[11]

Chart II. Compounds 1–16 and the Intercalation Model with DNA for Derivatives 1–12 and 14 with a Meta-Substituted Side Amino Chain (Protonated in Water)[a]



[a] Dipole moments (Table II) were computed for the given planar conformations and positioned in the coordinates as shown. The dipole moments for compounds 13, 15, and 16 were computed for the same orientation of the biaromatic systems (parallel to the y axis).

Chart III. Methods of Synthesis of Compounds 1–4 and 10–16



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in the spectra of **8** and **9** permitted the unambiguous structural assignments for the two isomers. Biphenyl amino ethers **13–15** were prepared in the alkylation reaction of the respective phenylphenols **23–25** with 2-(dimethylamino)ethyl chloride. In the preparation of compound **16**, 4,4′-dimethyl-2,2′-bipyridine (**26**) was brominated with *N*-bromosuccinimide, and the resultant monobromo derivative **27** was reacted with sodium 2-(dimethylamino)ethoxide. Some of the compounds discussed here were the subject of a preliminary communication without any synthetic details.[12]

NMR: Mode of Binding. Intercalation of an aromatic compound with DNA induces upfield shifts in the proton NMR spectra of both the intercalating molecule and DNA imino protons, as well as a downfield shift of the DNA ^{31}P signal.[13] Nonintercalative binding modes induce much smaller effects in the spectra of the bound molecule and the DNA. Reasonable line widths in the NMR spectra are obtained for samples with sonicated DNA. Higher temperature increases mobility of both DNA and compound bound with the DNA, which also results in sharper spectra.[13c] The spectra reported in this work were obtained with sonicated DNA at 50 °C. Since other experiments were conducted at 37 °C, the NMR spectra taken at the two temperatures were compared. The respective chemical shift differences were negligible, but the 50 °C spectra were considerably sharper and allowed more accurate analysis of chemical shifts.

Chemical shifts for the protons of pyrimidines **1–12** in the absence of DNA and at a 0.3 ratio of compound to DNA base pair are given in Table I. The assignments were made by using coupling patterns, chemical shifts, and 2D COSY experiments.[14] As can be seen from Table I, large upfield chemical shifts for all the aromatic protons and negligible changes for the absorption of the protons of the side chain are induced by the addition of DNA. These results are in agreement with the DNA intercalation model for compounds **1–12** presented in Chart II. The intercalation of compounds **1–12** with DNA is also supported by changes in DNA ^{31}P NMR and imino proton NMR spectra. At a 0.3 ratio of compound to base pair, the downfield ^{31}P shifts of DNA are 0.2–0.3 ppm, and the DNA imino proton upfield chemical shift changes are approximately 0.2 ppm for all the pyrimidine derivatives **1–12**. At a 0.3 ratio of compound to DNA base pair, the average upfield shifts for aromatic protons of biphenyls **13–15** were only 0.2, 0.4, and 0.25 ppm, respectively. At the same ratio, the DNA imino proton upfield shift changes and DNA ^{31}P downfield shift changes in the presence of **14** were approximately 0.1 ppm. The ortho and para isomers **13** and **15** did not induce any significant changes in the DNA imino proton and ^{31}P NMR spectra. These results suggest that biphenyls **13** and **15** cannot be classified as DNA classical intercalators, while the meta isomer **14** may intercalate *weakly*.

DNA-induced upfield shifts for protons of bipyridine **16** were only 0.02 ppm for H3 and H3′, and in the range of −0.40 to −0.28 for the remaining aromatic protons. No changes in the DNA imino proton NMR spectrum and a downfield shift of only 0.1 ppm in the DNA ^{31}P NMR spectrum in the presence of **16** were observed.

Table I. Proton Chemical Shifts of **2–4** and **6–12** (δ, ppm) in the Absence of DNA (First Lines), at a Molar Ratio of 0.3 of Compound to DNA Base Pair (Second Lines), and Total DNA-Induced Shift (Third Lines)

	proton or substituent at position[b]				
compd[a]	5	6	3′	4′	5′
2	7.41	2.44	7.87	7.27	7.74
	6.78	1.98	7.12	6.55	7.08
	−0.63	−0.46	−0.75	−0.72	−0.66
3	2.46	8.40	7.84	7.32	7.77
	2.00	7.54	7.08	6.48	6.93
	−0.46	−0.86	−0.76	−0.84	−0.84
4	7.57	8.54	7.98	7.29	7.57
	6.78	7.63	7.33	6.68	6.78
	−0.79	−0.91	−0.65	−0.61	−0.79
6	7.79	8.71		8.08	7.90
	7.19	8.04		7.54	7.43
	−0.60	−0.67		−0.54	−0.47
7	7.39	8.50		8.06	7.87
	6.73	7.71		7.42	7.11
	−0.66	−0.79		−0.64	−0.76
8	7.03	2.57	7.82	7.24	7.72
	6.23	2.06	7.07	6.47	6.96
	−0.80	−0.51	−0.75	−0.77	−0.76
9	6.63		7.76	7.23	7.66
	5.86		7.26	6.80	7.22
	−0.77		−0.50	−0.43	−0.44
10	7.42	8.43	7.01	6.32	7.07
	6.60	7.56	6.00	5.44	6.00
	−0.82	−0.87	−1.01	−0.88	−1.07
11	7.52	8.75	7.83	6.74	7.40
	6.89	7.92	7.20	6.10	6.69
	−0.63	−0.83	−0.63	−0.64	−0.71
12[c]	7.70	8.65	7.63	7.63	7.63
	7.02	7.79	6.85	6.85	6.85
	−0.68	−0.86	−0.78	−0.78	−0.78

[a] Spectra of **1** and **5** (DNA-induced shifts in the range −0.60 to −0.41 ppm) have been published.[10d] [b] Protons of NMe_2 of the side chain in **2–4** and **6–12** gave the chemical shifts δ 2.91 ± 0.08 (no DNA) and 2.89 ± 0.05 (with DNA) and the total DNA-induced shifts −0.04 ± 0.06. [c] The respective shifts for H2′(6′) are δ 8.09, 7.44, and −0.65.

Viscometric Titrations. Intercalation of an aromatic compound with DNA results in DNA lengthening and, consequently, in an increase in viscosity of the DNA solution.[13a,b] The viscosity increases with increasing concentrations of the intercalating compound until the DNA binding sites become saturated. Compounds, classified as the intercalators on the basis of the NMR data, gave the following maximum reduced specific viscosity ratios in PIPES 00 buffer (0.019 M [Na^+], pH 7.00) (compound, η/η_0): **1**, 1.66; **2**, 1.78; **3**, 1.85; **4**, 1.38; **5**, 1.40; **6**, 1.61; **7**, 1.36; **8**, 1.70; **9**, 1.37; **10**, 1.64; **11**, 1.40; **12**, 1.50. These changes were smaller but still significant in PIPES 10 buffer (0.12 M [Na^+], pH 7.00). In contrast, the molecules **14** and **16** gave maximum viscosity increases of 1.10 and 1.24, respectively, that were totally eliminated in PIPES 10 buffer. These and the NMR data suggest that compounds **14** and **16** may intercalate only weakly with DNA. Biphenyls **13** and **15** did not affect the DNA viscosity even in PIPES 00 buffer, in agreement with the classification of these compounds as nonintercalators in the classical model.

Stereochemistry. Two major factors responsible for the equilibrium conformation of biaryls in solution are π-electron delocalization energy, which reaches a maximum for coplanar rings, and the interactions between groups ortho to the torsional bond. The equilibrium conformation can be estimated from NMR NOE spectra. Recently, we have shown[12] that bipyridine **16** is s-trans[15] in solution, and

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Table II. Dipole Moments, DNA Equilibrium Binding Constants, and Amplification Activities for Compounds 1–16

compd	dipole moments[a] μ, D			DNA binding constants[b] K, M^{-1}	amplification activities[c] $k_s/k_{s(blank)}$ at	
	μ_x	μ_y	μ_{total}		R = 0.37	R = 3.7
1	+4.54	−4.32	6.27	18700	1.27	3.00
2	+4.90	−3.95	6.29	24800	1.38	3.47
3	+4.95	−4.48	6.68	48000	1.44	3.91
4	+3.32	−3.22	4.62	3400	1.15	1.47
5	+2.86	−1.17	3.09	4400	1.14	1.59
6	+1.71	−3.93	4.28	9250	1.20	2.49
7	+0.02	−0.87	0.87	3900	1.04	1.42
8	+0.62	−1.26	1.40	13700	1.17	1.87
9	−8.03	−1.70	8.21	6800	1.03	1.39
10	+1.82	−4.50	4.85	10100	1.24	2.90
11	+2.81	−1.30	3.10	3500	1.04	1.10
12	+2.24	−3.99	4.58	8600	1.23	2.26
13	−0.86	+2.05	2.22	3000	1.00	1.01
14	−0.54	−1.86	1.94	3000	1.03	1.17
15	−1.88	+0.24	1.90	3000	1.00	1.00
16	+0.10	+1.80	1.80	3000	0.28	0.07

[a] See Chart II for the coordinates and the Experimental Section for methods of computation. The dipole moments are for planar conformations of **1–16** with the torsional bond parallel to the y axis. The ortho biphenyl **13** is apparently nonplanar; however, the dipole moment changes only slightly with the torsional angle between the two phenyl subunits. The component dipole moment is positive if its orientation + → − agrees with the direction of the axis. [b] Determined spectrophotometrically, ±5% for **1–12** and ±1000 M^{-1} for **13–16**. Values for **1** and **5** are taken from ref 10d. [c] R is a molar ratio of compound to DNA base pair. See the Experimental Section for concentrations and general conditions. The rate constant for the degradation of DNA in the presence of bleomycin A2 and in the absence of compounds **1–16** $k_{s(blank)}$ = 0.0207 min^{-1}. Similar amplification activities (±2%) were obtained for the amplification of blenoxane. The correlation coefficients $r \geq 0.995$ and the confidence levels CL ≥ 99% were obtained for all individual constants k_s.

furylpyrimidine **11** is s-trans both free in solution and complexed with DNA. The 4-(2-thienyl)pyrimidine system is s-cis in solution, as shown for compounds **1–5**, **8**, and **9**, and this stereochemistry is retained in the DNA intercalation complexes. These preferential equilibrium conformations are not affected by temperature in the studied range of 35–65 °C. The s-cis stereochemistry of the 4-(2-thienyl)pyrimidine system may be a result of an S···N interatomic attraction or a strong S···H interatomic repulsion in the alternative s-trans conformation. We strongly favor the former alternative because of a short S(thiophene)···N(pyrimidine) contact found for the s-cis form of **3** in the crystal structure.[16] In addition, similar short S···N contacts, indicative of a nonbonded interatomic attraction, have been found in two bithiazole derivatives used as models to study interaction of bleomycin with DNA.[17] The attractive nonbonded interactions of the sulfur atom with other nucleophiles in crystal structures have also been suggested.[18] On the basis of these data, we assume that 4-(2-thiazolyl)pyrimidines **6** and **7** exist in a preferential s-cis conformation.[15] The 4-(1-methylpyrrol-2-yl)pyrimidine **10** also favors the s-cis conformation, as shown by NOE experiments. Thus, irradiation of NMe of the pyrrole in **10** resulted only in a strong NOE at H5′ of the same ring. In the same way, irradiation of H5 of the pyrimidine gave strong NOE's to H6 of the same ring and to H3′ of the pyrrole. Similar spectra were obtained for solutions of **10** in $CDCl_3$ and D_2O, at 25 and 50 °C. NOE experiments on the intercalation complex of **10** at 50 °C demonstrated that compound **10** remains in the s-cis conformation when bound to DNA. The NOE signals were extensively broadened at 37 °C, but the results were also consistent with the preferred s-cis form of **10** complexed with DNA under these conditions. Since all arylpyrimidines **1–12** intercalate with DNA, they exist or can easily attain a conformation with a small torsional angle between the planes of their two aromatic subunits.[19]

Stereoelectronic Effects. The biaryls used in this work, with the exception of biphenyls **13–15** and bipyridine **16**, are combinations of two different aromatic subsystems. Polarization of such molecules can be estimated by proton NMR.[20] Thus, transfer of the electron density from one ring to another results in downfield shifts for the protons of the former ring and upfield shifts for the protons of the aromatic subsystem with the enhanced electron population. The H6 atom of the pyrimidine nucleus is particularly suitable as a probe because its signal (Table I) is not masked by other proton signals. In addition, the H6 is not affected by the ring current of the aromatic substituent at position 4. The chemical shifts for H6 in the alkylthio-substituted arylpyrimidines **1**, **6**, and **10–12** were compared to the chemical shift for H4(6) at δ 8.61 of the reference pyrimidine **17**. The following order of the electron density in the pyrimidine ring was obtained, in which the negative differences (upfield shifts) indicate the enhanced electron density relative to **17**: **10** (−0.18) > **1** (−0.09) > **12** (+0.04) > **6** (+0.10) > **11** (+0.14). These data indicate polarization of the 4-(2-pyrrolyl)pyrimidine system in **10** and the 4-(2-thienyl)pyrimidine system in **1** with the electron-density transfer from the π-excessive heterocycle (pyrrole and thiophene) to the π-deficient pyrimidine. The phenyl substituent exhibits negligible effect while the thiazole of **6** and especially the furan of **11** with a highly

(15) The conformation of a heterobiaryl [s-cis (cisoid) or s-trans (transoid)] is determined by a spatial relationship between two heteroatoms of each ring, nearest to the interring bond. The conformation of a 4-(thiazol-2′-yl)pyrimidine system is determined by the relative orientation of N3 of the pyrimidine and S1′ of the thiazole because S1′ takes precedence over N3′ (IUPAC *Nomenclature of Organic Chemistry*; Pergamon: Oxford, 1979; Section E.).

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(19) The suggested low torsional angle (<8°) for sterically hindered **3** (ref 12) have recently been confirmed by the X-ray diffraction studies (ref 16).

(20) Kauffmann, T. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 1.

electronegative oxygen in the ring are electron-withdrawing groups.

The polarity of the biaryl molecules was also studied by computation of the dipole moments (Table II) for the planar conformations shown in Chart II. In the chosen coordinates, the torsional bond of the arylpyrimidines is parallel to the y axis. For a series of arylpyrimidines with the same substituents, the y component (μ_y) of the total dipole moment indicates the extent of the polarization of the biaryl system. As can be seen from Table II, the y components of the dipole moments are in the order **10** > **1** > **12** > **6** > **11**, the same order that was obtained from the NMR studies.

The effects of the thio, oxy, and amino linkages between the 4-(2-thienyl)pyrimidine system at position 2 and the side cationic chain on the polarity of the molecule can be seen from comparison of the dipole moments for compounds **1**, **4**, and **5** to that of the unsubstituted 4-(2-thienyl)pyrimidine (μ = 4.84 D). As can be seen from Table II, the thio substituent increases polarity of the molecule (**1**, μ = 6.27 D), the oxy linkage has a negligible effect (**4**, μ = 4.62 D), while the amino substituent greatly reduces the polarity of the molecule (**5**, μ = 3.09 D). The thio-substituted 4-(2-thiazolyl)pyrimidine **6** is also more polar than its amino analogue, **7**. These results demonstrate that the thio and amino substituents exhibit strong but opposite polarization effects. This is confirmed through comparison of the dipole moments of two isomers, **8** and **9**. The electron-withdrawing effect of the methylthio group and the electron-donating effect of the amino substituent in **8** (μ = 1.40 D) decrease polarity of the 4-(2-thienyl)pyrimidine system, while the polarity is strongly enhanced in **9** (μ = 8.21 D). It should be noted that compound **9** has a negative end of the dipole vector directed away from the side cationic chain. This is in sharp contrast to other pyrimidine derivatives **1–8** and **10–12**, which are polarized negatively in the direction of side chain. Biphenyl ethers **13–15** are weakly polar molecules with the electron density transferred from the oxygen atom to the aromatic system. This is in agreement with a known electron-donating effect of the alkoxy group.[21]

DNA equilibrium binding constants for compounds **1–16** were measured spectrophotometrically in the region above 300 nm, where interference by DNA absorption is not a problem. The site exclusion model of McGhee and von Hippel[22] was employed to calculate the binding constants from the binding isotherms as described previously.[10d] The DNA binding constants are given in Table II.

In the first approximation the dipole moments for DNA-intercalating compounds **1–8** and **10–12** can qualitatively be correlated with the DNA binding constants for these molecules. Better correlations are found within the series of compounds of a similar molecular size and with the same linkage between the heterobiaryl system and the side chain. Thus, the thio-substituted derivatives can be divided into two series of compounds of the following orders of *both* the dipole moments and the DNA binding constants: **1** > **6** > **11** and **3** > **2** > **10** > **12**. Similarly, the dipole moment and the DNA binding constant for the amino derivative **5** are larger than those for the analogue **7**. Isosteric compounds **8** and **9** have opposite orientations of the molecular dipoles and do not follow these correlations. Biphenyl derivatives **13–15** are weakly polar molecules with the dipole moment vector pointing away from the side chain and have small DNA binding constants.

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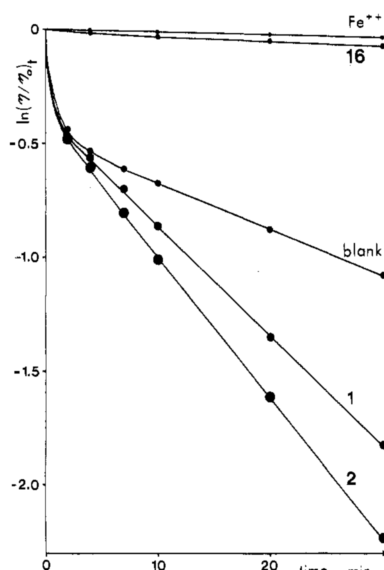

Figure 1. Changes in the reduced specific viscosity of DNA induced by the ferrous chelate of bleomycin in the absence (blank) and presence of compounds **1**, **2**, and **16**, at a ratio of 3.7 mol of compound to mol of DNA base pair. The changes induced by ferrous ion alone (Fe^{2+}) in the absence of bleomycin and heterobiaromatic compounds are also given for comparison. These and analogous plots for other compounds were used to calculate amplification activities given in Table II. See the Experimental Section for concentrations and general conditions.

These data strongly suggest that polarity of the aromatic molecule and orientation of the dipole moment within the aromatic system are major factors responsible for the small molecule–DNA interaction.

For strong intercalative forces it is important that *both* the heteroaromatic subsystems of the molecule have positive ends of their dipoles positioned away from the side chain. This generalization is illustrated through comparison of the binding characteristics of the following pairs of compounds: **1/6**, **1/11**, **1/12**, **11/12**, **5/7**, **8/9**, and taking into account that the oxygen and sulfur atoms are negatively charged in furan and thiophene, respectively, and the nitrogen is a negative end of the dipole in thiazole.[23] Pyrrole is polarized in such a way that the carbon backbone of the molecule constitutes a negative end of the dipole.[23] Therefore, pyrrolylpyrimidine **10** has opposite orientations of the dipoles of the two heteroaromatic subsystems in the molecule. Compound **10** binds weaker with DNA than thienylpyrimidines **1–3**, which have similar orientations of the dipoles for the thiophene and pyrimidine components.

Bleomycin Amplification. Bleomycin-mediated degradation of DNA results in decreases in the viscosity of DNA solution. Previously, we have used the relative viscosity decreases in the absence and presence of amplifiers as a highly sensitive but semiquantitative test for bleomycin amplification.[6f] In this work the viscometric method was further mastered and developed into a quantitative tool for studying the kinetics of the DNA degradation.

In all experiments the molecular ratios of DNA to bleomycin and oxygen to DNA were high. The concentration of ferrous ion remained practically constant throughout the reaction because a relatively high initial concentration of the ferrous ion and dithiothreitol, an iron reducing agent, were used. Under these conditions the

(23) Newkome, G. R.; Paudler, W. W. *Contemporary Heterocyclic Chemistry*; Wiley: New York, 1982; p 14.

DNA viscosity changes in time can be described by the following biphasic eq 1, where η_0 is the initial reduced

$$\left(\frac{\eta}{\eta_0}\right)_t = 0.30\ (\pm 0.07)e^{-k_f t} + 0.71\ (\pm 0.09)e^{-k_s t} \quad (1)$$

specific viscosity for DNA before the addition of bleomycin, η is the reduced specific viscosity for DNA at the reaction (degradation) time t, k_f is the apparent rate constant for the first (fast) process, and k_s is the apparent rate constant for the second (slow) process. In Figure 1 the experimental changes in DNA viscosity induced by bleomycin A2 in the absence (blank) and presence of selected additives **1**, **2**, and **16** are fitted by using eq 1. As can be seen from this figure, the initial observed bleomycin-induced decreases in DNA viscosity are fast, and these are followed by a much slower process. Both fast and slow processes are enhanced in the presence of compound **1**, and even more in the presence of compound **2**. In contrast, bipyridine **16** is an inhibitor of the bleomycin reaction. These effects are concentration dependent, that is, the viscosity is decreasing faster with increasing concentrations of the amplifiers **1** and **2** and are reduced greater with increasing concentration of the inhibitor **16**. Due to the nature of the viscometric measurements the observed rate constants of the fast process bear large errors. The constants k_f of 1.7 ± 0.3, 2.6 ± 0.4, and 2.9 ± 0.4 min^{-1} were estimated for bleomycin alone and in the presence of **1** and **2**, respectively, for the conditions of Figure 1. However, the observed rate constants k_s of the second process could be measured with a high accuracy for all compounds tested in this work. Table II contains the ratios $k_{s(amp)}/k_{s(blank)}$, the amplification activities. Activities larger than one and smaller than one indicate amplification and inhibition, respectively.

It should be emphasized that eq 1 is a simple empirical fitting of the observed experimental results and more work is in progress to interpret the observed rates in terms of a model for the bleomycin-catalyzed degradation of DNA. An interesting finding is that the equation coefficients (0.30 and 0.71) change little with changes in concentration of the amplifiers under the same concentration conditions of DNA and bleomycin. Similar values of the two coefficients were obtained for the reaction of bleomycin with DNA in the absence of an amplifier and in the presence of different amplifiers. However, the reaction rates k_f and k_s are greatly increased in the presence of the amplifier. The fast rate may represent single strand cleavage of DNA at very specific sites.[2] The slower rate may be a combination of double-strand cleavage and single-strand cleavage at less specific sites.

The experiments with blenoxane (mixture consisting mainly of BLM-A2 and BLM-B2) revealed that this drug is amplified as well. Our sample of BLM-A2 showed a slightly higher (by 10%) activity in the absence of an amplifier than the sample of blenoxane of the same concentration (by weight). Nevertheless, both samples were amplified to the same extent after increasing the concentration of blenoxane, so the two solutions showed the same activity in the absence of the amplifier.

Only the intercalating molecules **1–12** and **14** show activity as bleomycin amplifiers. The following orders of activity are obtained for the structurally related compounds: **1** > **6** > **11**, **3** > **2** > **10** > **12**, **5** > **7**, and **8** > **9**. In addition, any compound with a thio linkage between the heterobiaryl system and the side chain is more active than the corresponding amino analogue. The activity orders are exactly the same as the previously discussed orders of the stereoelectronic effects and the DNA binding

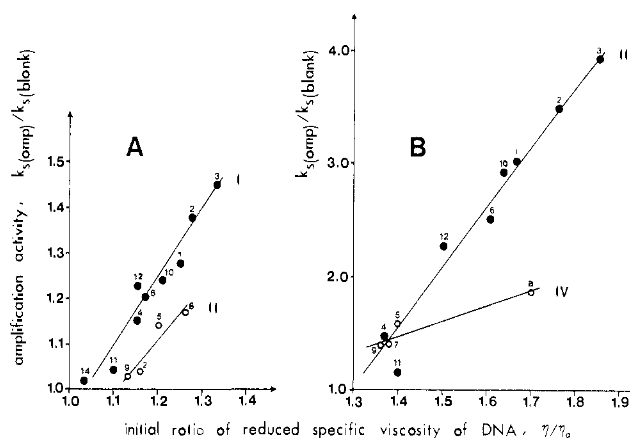

Figure 2. Effects of the initial reduced specific viscosities of DNA in the presence of intercalating compounds 1–12 and 14 (without bleomycin) on the amplification activities, $k_{s(amp)}/k_{s(blank)}$, of these compounds. The plots are given for two molar ratios of compound to DNA base pair, used in the amplification experiments; (A) ratio 0.37 and (B) ratio 3.7. Note different functions: (●), thio- and oxy-substituted compounds; and (○), amino analogues. See the Experimental Section for concentrations and general conditions.

constants within the same series of compounds. These data demonstrate that the activities of bleomycin amplifiers are related to the binding characteristics of the amplifier molecules with DNA. In the same way these qualitative correlations strongly support our hypothesis that the amplifier–DNA complex is a better target for bleomycin than the unperturbed DNA alone. A simple approach to quantitate the amplifier-induced changes on the DNA helix is to measure increases in DNA viscosity upon intercalation of the amplifier molecules in the absence of bleomycin. In Figure 2 the amplification activities are plotted as a function of the initial ratios of reduced specific viscosities of DNA complexed with intercalating compounds. The data are given for two different concentrations of the amplifier used in the amplification tests. As can be seen from Figure 2, the amplification activities of all thio derivatives, **1–3**, **6**, and **10–12**, and the oxy analogue **4** are proportional to the initial reduced specific viscosities of the DNA–amplifier complexes. A similar function is seen for the amino-substituted amplifiers **5**, **7–9**. The latter compounds produce significant elongation of the DNA double helix but, in spite of this, are weak amplifiers. The straight-line equations (I–IV) of Figure 2 are

$$k_{s(amp)}/k_{s(blank)} = -0.678 + 1.594(\eta/\eta_0) \quad \text{(I)}$$

$$n = 9,\ r = 0.959,\ CL = 99.9\%$$

$$k_{s(amp)}/k_{s(blank)} = -0.308 + 1.181(\eta/\eta_0) \quad \text{(II)}$$

$$n = 4,\ r = 0.944,\ CL = 90\%$$

$$k_{s(amp)}/k_{s(blank)} = -6.386 + 5.602(\eta/\eta_0) \quad \text{(III)}$$

$$n = 8,\ r = 0.978,\ CL = 99.9\%$$

$$k_{s(amp)}/k_{s(blank)} = -0.307 + 1.284(\eta/\eta_0) \quad \text{(IV)}$$

$$n = 4,\ r = 0.951,\ CL = 95\%$$

The straight-line functions do not intersect the point of the coordinates corresponding to the native unperturbed DNA double helix ($\eta/\eta_0 = 1$) and lack of amplification ($k_{s(amp)}/k_{s(blank)} = 1$). These data indicate a low limit in the amplifier-induced perturbations of the helix below which the functions are not valid.

Iron Complexation. One of the possible mechanisms for bleomycin amplification may be the amplifier-assisted transfer of ferrous ion to DNA. Accordingly, we have

tested compounds **1–16** for iron complexation. The UV spectra of the active compounds **1–12** taken in the presence of ferrous ion and with the iron solution in a reference cell, were virtually identical with those taken in the absence of iron. In contrast, the spectra of bipyridine **16** (an iron-chelating agent) taken in the absence and presence of iron were significantly different. These results conclusively demonstrate that bleomycin amplifiers **1–12** do not complex ferrous ion.

Discussion

Most of the compounds studied in this work are heteroaryl-substituted pyrimidines. These derivatives exist in one, s-cis or s-trans, major equilibrium conformation in solution with a small torsional angle between the planes of their two aromatic rings. The attractive S···N interaction is apparently responsible for the s-cis form of the 4-(2-thienyl)pyrimidine system, as already discussed. The s-trans conformation of 4-(2-furyl)pyrimidine **11** can be understood in terms of favorable stereoelectronic interactions between electropositive H3′ of the furan with electronegative N3 of the pyrimidine, and electropositive H5 of the pyrimidine with electronegative O1 of the furan. The dipoles of the pyrimidine and furan rings[23] are also expected to interact favorably in the observed s-trans conformation. The suggested small torsional angle for **11** is in agreement with other findings that the preferred oxygen environment is in the plane of the aromatic ring.[24] Apparently, both stereoelectronic and steric factors are responsible for the s-cis form of the 4-(1-methylpyrrol-2-yl)pyrimidine system in **10**. Since pyrimidine and pyrrole have opposite orientations of their dipole moments with respect to their nitrogen atoms,[23] the two dipoles interact favorably in the s-cis conformation of **10**. It can also be expected[25] that steric interactions between groups located ortho to the torsional bond in **10** are less repulsive in the observed s-cis form than in the alternative s-trans form.

Compounds **1–11** intercalate with DNA and their equilibrium conformations in solution are retained in the complexes with DNA. Phenylpyrimidine **12** and biphenyl **14** are also DNA intercalators. These biaryls have a common structural feature, namely a cationic side chain attached at the meta position with respect to the interring bond. The proposed general interaction model of the meta-substituted molecules with DNA is given in Chart II. In this model, an intrinsic conformational freedom of the unfused system may allow the molecule to be accommodated with little steric hindrance between nonplanar DNA base pairs. It is known that the base pairs in DNA are twisted about their long axis (propeller twist),[26] and it seems possible that the torsional twist in **1–12** and **14** may allow bases to obtain a favorable propeller twist in an intercalation complex. Such an interaction is less likely for para-substituted **15**, which does not intercalate with DNA. The torsional twist in ortho biphenyl **13** is apparently too extensive and precludes the intercalation. However, the criterion of the meta substitution is insufficient in itself to ensure the strong intercalation of a cationic bicyclic compound because biphenyl **14** and bipyridine **16** intercalate weakly with DNA.

For the first time we have shown that the stereoelectronic effects in heterobiaryls are of a primary importance for the effective binding of these molecules with DNA. Good heterobicyclic DNA intercalators are polar compounds with the negative end of the dipole pointing out to the cationic side chain of the molecule. The cationic chain interacts electrostatically with the anionic DNA backbone while the *positively* polarized biaromatic system intercalates with DNA base pairs.

The observed differences in the DNA intercalation for compounds of different polarity can be explained, assuming that (partial) charge-transfer forces are important for the intercalation complexes. Thus, compounds that have lower π-electron density at their intercalation sites (Chart II) are also expected to form strong complexes in which DNA bases act as electron donors. A similar intercalation model, in which charge-transfer forces are believed to be important, has been employed to describe binding of nucleosides to riboflavin.[27] In addition, the larger dipole moment of the intercalating molecular system would result in stronger interactions with dipoles of the DNA base pairs, as observed.

There is a parallel behavior between the factors that affect intercalation of biaryls and the enhancement effect induced by these compounds in the bleomycin-mediated degradation of DNA. All data obtained in this work strongly suggest that the enhancement effect is related to the extent of conformational perturbations of the double helix induced by intercalating compounds. Other studies have shown that the bleomycin-mediated cleavage is strongly dependent on the DNA tertiary structure.[28] The conformational factor may also be responsible for the observed parallel behavior between DNA binding constants and amplification activities for DNA intercalating, unfused compounds. It is known that within a series of structurally related compounds a stronger binding with DNA also causes more extensive changes in the helix structure.[29]

There is a puzzling difference, however, between the effects on the bleomycin reaction caused by unfused- and fused-ring intercalators. Low concentrations of the fused-ring compounds such as ethidium or propidium initially increase the rate of the bleomycin-mediated degradation of DNA and then decrease the rate below that for bleomycin alone, as the DNA is more digested. High concentrations of the fused compounds cause only inhibition of DNA degradation; that is, both the digestion rate and the final level of DNA degradation are lower than in the presence of bleomycin alone. In contrast, both the digestion rate and the final level of DNA degradation are enhanced with all concentrations of the unfused amplifiers.[6f] These different effects are observed for the two types of compounds regardless of their DNA equilibrium binding constants. Recently we have reported studies with a tricyclic unfused compound that intercalates strongly with DNA ($K = 13 \times 10^6$ M^{-1}), is a better amplifier than ethidium or propidium at low concentrations (10^{-7}–10^{-5} M) and shows a strong amplification activity under the conditions of complete saturation of DNA binding sites.[6f] To understand the apparent lack of competition between the unfused amplifier and bleomycin molecules for DNA binding sites, we are currently conducting stopped-flow experiments. Thus, the preliminary results indicate a fast dissociation of the unfused amplifier–DNA complexes, while the fused compounds show much slower dynamics. These findings may account for the observed differences

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(25) Ritchie, G. L. D.; Vrbancich, J. *J. Mol. Struct.* **1982**, *78*, 279.
(26) Dickerson, R. E. *J. Mol. Biol.* **1983**, *166*, 419 and references therein.
(27) T'so, P. O. P. In *Basic Principles in Nucleic Acid Chemistry*; T'so, P. O. P., Ed.; Academic: New York, 1974; pp 526–562.
(28) Ueda, K.; Kobayashi, S.; Komano, T. *Nucleic Acids Symp. Ser.* **1985**, *16*, 197.
(29) Jones, R. L.; Lanier, A. C.; Keel, R. A.; Wilson, W. D. *Nucleic Acids Res.* **1980**, *7*, 1613.

in the effects of the two classes of compounds on the bleomycin reaction.

Four compounds of the series discussed in this work, **1** and **5–7**, were tested previously as amplifiers of the phleomycin-mediated inhibition of bacterial growth.[10f] The thio-substituted **1** was highly active while its amino analogue **5** showed marginal activity. The same order of activity was obtained for thio and amino derivatives **6** and **7**, respectively. Several other unfused- and fused-ring compounds with a thio or amino linkage to the cationic side chain were also tested. As a general rule, the thio-substituted derivatives were more active than their amino analogues.[9] Exactly the same result was obtained in this work for a series of thio- and amino-substituted heterobiaryls. As already discussed, there is a parallel behavior between DNA binding characteristics of these compounds and their amplification activities. In addition, amplifier **1** enhanced significantly the activity of phleomycin against Ehrlich's ascites tumor in vivo.[10e] Phleomycin is structurally related to bleomycin, and both drugs cause degradation of DNA by similar mechanisms. These data together with our in vitro results strongly suggest that the in vivo activity of bleomycin may be amplified by the in vitro mechanism, as discussed in this paper.

Experimental Section

Compounds 5–7 and 17. *N*-[2″-(Dimethylamino)ethyl]-4-thien-2′-ylpyrimidin-2-amine hydrobromide (**5·HBr**), *N,N*-dimethyl-2-[4′-thiazol-2″-ylpyrimidin-2′-yl)thio]ethylamine (**6**), *N*-[2″-(dimethylamino)ethyl]-4-thiazol-2′-ylpyrimidin-2-amine (**7**),[10f] and *N,N*-dimethyl-2-(pyrimidin-2′-ylthio)ethylamine (**17**)[6f] were prepared as reported.

***N,N*-Dimethyl-2-[(4′-methylpyrimidin-2′-yl)thio]ethylamine (18).** 2-Chloro-4-methylpyrimidine[30] was reacted with sodium 2-(dimethylamino)ethanethiolate under the conditions for the preparation of the analogue **17**.[6f] Crude **18** was purified by chromatography on silica gel (hexanes/Et_3N/EtOH, 80/19/1) and then by Kugelrohr distillation (130 °C (2 mmHg)) to give a colorless oil (70%): ^{1}H NMR ($CDCl_3$/Me_4Si) δ 2.32 (s, 6 H, NCH_3), 2.43 (s, 3 H, C4′-CH_3), 2.65 (m, 2 H, NCH_2), 3.25 (m, 2 H, SCH_2), 6.76 (d, J = 5 Hz, 1 H, H5′), 8.30 (d, J = 5 Hz, 1 H, H6′). Anal. ($C_9H_{15}N_3S$) C, H.

***N,N*-Dimethyl-2-[(5′-methylpyrimidin-2′-yl)thio]ethylamine (19).** The published procedure[6f] was adopted without modifications for the reaction of 2-chloro-5-methylpyrimidine[31] with sodium 2-(dimethylamino)ethanethiolate. Chromatography and Kugelrohr distillation, as described above, afforded **19** (95%) as an oil: ^{1}H NMR ($CDCl_3$/Me_4Si) δ 2.21 (s, 3 H, C5′-CH_3), 2.32 (s, 6 H, NCH_3), 2.65 (m, 2 H, NCH_2), 3.20 (m, 2 H, SCH_2), 8.29 (s, 2 H, H4′ and H6′). Anal. ($C_9H_{15}N_3S$) C, H.

***N,N*-Dimethyl-2-(pyrimidin-2′-yloxy)ethylamine (20).** Sodium hydride (1.08 g, 45.0 mmol) in dry tetrahydrofuran (100 mL) was treated dropwise with 2-(dimethylamino)ethanol (5 mL, 50 mmol). 2-Chloropyrimidine (5.0 g, 4.37 mmol) was added to the resultant solution of sodium 2-(dimethylamino)ethoxide, and the mixture was stirred at room temperature for 10 h, under a nitrogen atmosphere. The tetrahydrofuran was evaporated on a rotary evaporator and the residue was extracted with hexanes (3 × 50 mL). Removal of the hexanes on a rotary evaporator was followed by Kugelrohr distillation (100 °C (2 mmHg)) of the oily residue to give **20** (6.5 g, 89%) as a colorless oil: ^{1}H NMR ($CDCl_3$/Me_4Si) δ 2.33 (s, 6 H, NCH_3), 2.72 (t, J = 6 Hz, 2 H, NCH_2), 4.43 (t, J = 6 Hz, 2 H, OCH_2), 6.92 (t, J = 5 Hz, 1 H, H5′), 8.47 (d, J = 5 Hz, 2 H, H4′ and H6′). Anal. ($C_8H_{13}N_3O$) C, H.

Syntheses of 1–4 and 10–12: General Procedure. The reactions with organolithium reagents (described below) were conducted in ether freshly distilled from sodium benzophenone ketyl and under an atmosphere of dry, high purity nitrogen. An ether solution of 2-thienyllithium was generated in the reaction of *n*-butyllithium with thiophene as described previously.[10f] The lithiation reactions of furan (2.0 mL, 27 mmol) and 1-methylpyrrole (2.0 mL, 22 mmol) with *n*-butyllithium (2.6 M in hexanes, 1.0 mL, 2.6 mmol) to produce 2-furyllithium and (1-methylpyrrol-2-yl)lithium reagents, respectively, were conducted in ether (25 mL) at −10 °C for 1 h and in the presence of *N,N,N′,N′*-tetramethylethylenediamine (0.5 mL, 3.3 mmol). Phenyllithium was obtained from Aldrich.

A solution of the lithium reagent (2.6 mmol) was cooled to −40 °C, stirred, and treated dropwise with a solution of **17**, **18**, **19**, or **20** (2.5 mmol) in ether (2 mL). The mixture was stirred at −40 °C for 15 min and then at −10 °C for 30 min, quenched with water (1 mL) in tetrahydrofuran (2 mL), and treated with a solution of 2,3-dichloro-5,6-dicyanobenzoquinone (613 mg, 2.7 mmol) in tetrahydrofuran (10 mL). The mixture was stirred at 25 °C for 1 h and then with an aqueous solution of NaOH (10%, 25 mL) for an additional 15 min, and treated with ether (50 mL). The organic layer was separated, washed with the solution of NaOH (3 × 5 mL), dried (Na_2SO_4), and concentrated. Chromatography on silica gel (hexanes/CH_2Cl_2/Et_3N, 7/2/1) afforded an oil. Additional purification was achieved by treatment of the oily product with hydrobromic acid, as described,[6f,10f] followed by crystallization of the resultant hydrobromide from ethanol or a mixture of ethanol and ether. Yields for the compounds prepared by the above procedure; melting points for their hydrobromide salts and UV spectra (pH 7) are given below. Proton NMR spectra are given in Table I.

***N,N*-Dimethyl-2-[(4′-thien-2″-ylpyrimidin-2′-yl)thio]ethylamine hydrobromide (1·HBr):** 65%; mp 208–210 °C (lit.[10f] mp 207–209 °C).

***N,N*-Dimethyl-2-[(6′-methyl-4′-thien-2″-ylpyrimidin-2′-yl)thio]ethylamine hydrobromide (2·HBr):** 70%; mp 199–201 °C; UV $\lambda_{max}^{H_2O}$ 322 nm (ϵ 15 300 M^{-1} cm^{-1}). Anal. ($C_{13}H_{17}N_3S_2$·HBr) C, H, N.

***N,N*-Dimethyl-2-[(5′-methyl-4′-thien-2″-ylpyrimidin-2′-yl)thio]ethylamine hydrobromide (3·HBr):** 82%; mp 174–175.5 °C; UV $\lambda_{max}^{H_2O}$ 327 nm (ϵ 13 150 M^{-1} cm^{-1}). Anal. ($C_{13}H_{17}N_3S_2$·HBr) C, H, N.

***N,N*-Dimethyl-2-[(4′-thien-2″-ylpyrimidin-2′-yl)oxy]ethylamine hydrobromide (4·HBr):** 74%; mp 159.5–160.5 °C; UV $\lambda_{max}^{H_2O}$ 315 nm (ϵ 19 900 M^{-1} cm^{-1}). Anal. ($C_{12}H_{15}N_3OS$·HBr) C, H, N.

***N,N*-Dimethyl-2-[[4′-(1″-methylpyrrol-2″-yl)pyrimidin-2′-yl]thio]ethylamine hydrobromide (10·HBr):** 43%; mp 171–173 °C; UV $\lambda_{max}^{H_2O}$ 335 nm (ϵ 20 740 M^{-1} cm^{-1}). Anal. ($C_{13}H_{18}N_4S$·HBr) C, H, N.

***N,N*-Dimethyl-2-[(4′-furan-2″-ylpyrimidin-2′-yl)thio]ethylamine (11, a free base):** 60%; UV $\lambda_{max}^{H_2O}$ 321 nm (ϵ 17 960 M^{-1} cm^{-1}). Anal. ($C_{12}H_{15}N_3OS$) C, H, N.

***N,N*-Dimethyl-2-[(4′-phenylpyrimidin-2′-yl)thio]ethylamine dihybrobromide (12·2HBr):** 77%; mp 249–251 °C; UV $\lambda_{max}^{H_2O}$ 303 nm (ϵ 14 000 M^{-1} cm^{-1}). Anal. ($C_{14}H_{17}N_3S$·2HBr) C, H, N.

Aminolysis of 2,4-Bis(methylthio)-6-thien-2′-ylpyrimidine (22). A mixture of **22**[32] (504 mg, 2.0 mmol) and *N,N*-dimethylethylenediamine (10 mL, 90 mmol) was heated in a Parr bomb at 165 °C for 8 h. Concentration of the mixture on a rotary evaporator was followed by chromatography on silica gel (hexanes/EtOH/Et_3N, 89/8/3) to furnish, in order of elution, *N*-[2″-(dimethylamino)ethyl]-4-(methylthio)-6-thien-2′-ylpyrimidin-2-amine (**8**) (208 mg, 36%) as an oil and crystalline *N*-[2″-(dimethylamino)ethyl]-2-(methylthio)-6-thien-2′-ylpyrimidin-4-amine (**9**) (254 mg, 44%), mp 71–72 °C (from hexanes). The hydrobromides were obtained by a published procedure[6f,10f] and crystallized from ethanol. **8·HBr**: mp 172–173.5 °C; UV $\lambda_{max}^{H_2O}$ 340 nm (ϵ 14 690 M^{-1} cm^{-1}). **9·HBr**: mp 180–180.5 °C; UV $\lambda_{max}^{H_2O}$ 322 nm (ϵ 11 740 M^{-1} cm^{-1}). Analyses for both isomers ($C_{13}H_{18}N_4S_2$·HBr) C, H, N. Proton NMR spectra for **8** and **9** are given in Table I.

Alkylation of Phenylphenols 23–25: General Procedure. A phenylphenol (**23**, **24**, or **25**, 2.75 g, 16 mmol) was added slowly to a stirred mixture of sodium hydride (0.77 g, 32 mmol) in dry *N,N*-dimethylformamide (100 mL) at 25 °C. 2-(Dimethyl-

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amino)ethyl chloride hydrochloride (2.45 g, 17 mmol) was added in the same manner, and the mixture was stirred at 100 °C for 30 min. Removal of the solvent on a rotary evaporator was followed by addition of water (10 mL) to the residue and extraction with ether (2 × 50 mL). The extract was dried (Na_2SO_4), concentrated to 50 mL, diluted with ethanol (50 mL), and treated with a mixture of hydrobromic acid (48%, 1.9 mL, 17 mmol) and ethanol (5 mL). The precipitate was dissolved in ethanol (50 mL) and crystallized by addition of ether (25 mL). The following compounds were obtained by the above procedure.

***N,N*-Dimethyl-2-(biphenyl-2′-yloxy)ethylamine hydrobromide (13·HBr)**: 66%; mp 110–112 °C; ^{1}H NMR (D_2O/TSP) δ 2.79 (s, 6 H, NCH_3), 3.50 (m, 2 H, NCH_2), 4.36 (m, 2 H, OCH_2), 7.20–7.28 and 7.41–7.60 (2 m, 9 H, aromatic). Anal. ($C_{16}H_{19}$NO·HBr) C, H, N.

***N,N*-Dimethyl-2-(biphenyl-3′-yloxy)ethylamine hydrobromide (14·HBr)**: 51%; mp 140–142 °C; ^{1}H NMR (D_2O/TSP) δ 3.03 (s, 6 H, NCH_3), 3.65 (m, 2 H, NCH_2), 4.50 (m, 2 H, OCH_2), 7.12, 7.35, 7.42, 7.46, 7.52, and 7.58 (6 m, 9 H, aromatic). Anal. ($C_{16}H_{19}$NO·HBr) C, H, N.

***N,N*-Dimethyl-2-(biphenyl-4′-yloxy)ethylamine hydrobromide (15·HBr)**: 70%; mp 188–190 °C; ^{1}H NMR (D_2O/TSP) δ 3.02 (s, 6 H, NCH_3), 3.62 (m, 2 H, NCH_2), 4.49 (m, 2 H, OCH_2), 7.17, 7.43, 7.54, and 7.71 (4 m, 9 H, aromatic). Anal. ($C_{16}H_{19}$NO·HBr) C, H, N.

***N,N*-Dimethyl-2-[[(4″-methyl-2′,2″-bipyridin-4′-yl)methyl]oxy]ethylamine (16)**. A mixture of 4,4′-dimethyl-2,2′-bipyridine (**26**; 1.84 g, 10 mmol), *N*-bromosuccinimide (1.9 g, 10.7 mmol), and benzoyl peroxide (50 mg) in carbon tetrachloride (50 mL) was refluxed for 20 h. The solvent was removed, and the residue containing bromo derivative **27** as a major product was dissolved in *N,N*-dimethylformamide. This solution was added to a solution of sodium 2-(dimethylamino)ethoxide prepared from sodium hydride (0.5 g, 20.8 mmol) and 2-(dimethylamino)ethanol (4 mL, 39.8 mmol) in *N,N*-dimethylformamide (10 mL). The mixture was stirred at 80 °C for 30 min, concentrated on a rotary evaporator to 3 mL, treated with water (20 mL), and extracted with toluene (3 × 25 mL). Chromatography on silica gel (toluene/hexanes/Et_3N, 7/2/1) and then Kugelrohr distillation (150 °C (0.1 mmHg)) afforded analytically pure **16** (0.75 g, 28%) as a colorless oil: ^{1}H NMR (D_2O/TSP) δ 2.30 (s, 6 H, NCH_3), 2.40 (s, 3 H, C4″-CH_3), 2.62 (m, 2 H, NCH_2), 3.65 (m, 2 H, OCH_2), 4.60 (s, 2 H, C4′-CH_2), 7.28 (d, J = 5 Hz, 1 H, H5″), 7.40 (d, J = 5 Hz, 1 H, H5′), 7.71 (s, 1 H, H3″), 7.83 (s, 1 H, H3′), 8.40 (d, J = 5 Hz, 1 H, H6″), 8.51 (d, J = 5 Hz, 1 H, H6′). Anal. ($C_{16}H_{21}N_3O$) C, H, N.

Dipole Moment Computations. All molecular orbital calculations were Hartree–Fock self consistent field (SCF) calculations with an intermediate neglect of differential overlap (INDO) Hamiltonian.[33] Spectroscopic parameters and Mataga–Nishimoto two-electron integrals (INDO/S)[34] were used to calculate the wave function as other parameterization schemes give unrealistic charge distributions. Dipole moments were evaluated by using the dipole length operator in the symmetrically orthogonalized ZDO basis. All dipole integrals were calculated over Slater-type orbitals (STO). One-center charge and polarization terms were included though two-center bond terms were neglected due to ZDO. Geometries were based on idealized bond lengths and bond angles taken from model compounds.[10d]

Buffers. All hydrodynamic and NMR experiments were conducted in buffered solutions at pH 7.00. A standard PIPES 00 buffer contained 0.01 M 1,4-piperazinebis(ethanesulfonic acid) (PIPES) and 0.001 M EDTA. The solution was adjusted to pH 7.00 with NaOH to give total concentration of [Na^+] = 0.019 M. The PIPES 00 buffer without EDTA (pH 7.00) was used in the amplification experiments because EDTA is an inhibitor of the bleomycin reaction. An N00 buffer was used to prepare the stock DNA solution for NMR studies. The N00 buffer contained 0.015 M NaH_2PO_4, 0.0001 M EDTA, and was adjusted to pH 7.00 with NaOH. The NMR spectra were taken in an N10 buffer that contained 0.015 M NaH_2PO_4, 0.0001 M EDTA, and 0.10 M NaCl and was adjusted to pH 7.00 with NaOH to give total concentration of [Na^+] = 0.12 M.

Bleomycin and DNA Samples. Bleomycin A2 and blenoxane (a mixture consisting mainly of BLM-A2 and BLM-B2) were used in the amplification studies. The samples were obtained from the Bristol-Myers Co. through the courtesy of Dr. William T. Bradner. Calf thymus DNA (Worthington Biochemical) was purified of residual proteins, extensively dialyzed in a PIPES 00 buffer, and characterized as previously described.[35] DNA for NMR studies was sonicated to approximately 200 base pairs, phenol- and ether-extracted, ethanol-precipitated, dissolved, and extensively dialyzed in PIPES 00 buffer, followed by N00 buffer as previously described.[35] Concentrations of DNA-P were determined spectrophotometrically at 260 nm with use of an extinction coefficient of 6600 M^{-1} cm^{-1}.

NMR. To prepare the NMR samples of **2–4** and **6–16**, the stock aqueous solution was added to an N10 buffer (0.8 mL). The mixture was repeatedly (at least twice) lyophilized and redissolved in 100% D_2O. In a separate flask a stock solution of DNA in an N00 buffer was repeatedly lyophilized and redissolved in D_2O. The two solutions were combined, and the volume was adjusted with D_2O to reach 0.8 mL. This solution was transferred under a strictly anhydrous atmosphere to a 5-mm NMR tube containing a crystal of TSP as an internal reference. The solutions obtained were 5 mM in **2–4** and **6–16** and 0–17 mM in DNA base pairs. The same procedure was used to prepare a sample of **10** with DNA (8.3 mM in DNA base pairs) for proton nuclear Overhauser experiments. All NMR experiments were conducted on a JEOL GX-270 spectrometer operating at 270 MHz. The conditions for obtaining proton NMR spectra for **2–4** and **6–16** and proton nuclear Overhauser difference spectra for **10**, and the conditions for measurements of the effect of **2–4** and **6–16** on DNA imino protons and ^{31}P NMR signals are given elsewhere.[10d,13b]

Spectrophotometric Measurements. Scans and extinction coefficients, as well as DNA equilibrium binding constants for **2–4** and **6–16**, were determined as previously described.[10d,13b,35]

Viscometric Studies. All viscosity experiments were conducted in Cannon–Ubbelohde series 75 semimicrodilution viscometers, which were maintained at a constant temperature of 37 ± 0.02 °C 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 reservoir of the viscometer. Viscometric titrations were performed as previously described by adding aliquots of a concentrated stock solution of the desired compound to a DNA solution in the viscometer.[6f] 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 **1–16**. The apparent rate constants k_f and k_s (eq 1) were calculated by linear regression as previously described.[36] The correlation coefficients r were evaluated by the Student's t test.

DNA–Bleomycin Reactions: Concentrations. PIPES 00 buffer (without EDTA, pH 7.00) and high molecular weight DNA were used in all experiments with bleomycin. stock solutions (37 °C) were added in the order given below to the PIPES 00 buffer in a viscometer to reach the final volume of 1.5 mL and the following final concentrations: calf thymus DNA, 2.34×10^{-4} M (concentration of nucleotide equivalents); compounds **1–16**, 4.3×10^{-5} M or 4.3×10^{-4} M (ratios of 0.37 or 3.7 of molecules per DNA base pair, respectively); $FeSO_4$, 7.4×10^{-6} M; dithiothreitol, 1.8×10^{-4} M; bleomycin, 1.1×10^{-6} M.

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Synthesis and Testing of Quinone-Based Bis(2,2-dimethyl-1-aziridinyl)phosphinyl Carbamates as Radiation-Potentiating Antitumor Agents

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Two new drug candidates, in which a quinonoid moiety is linked to the reactive bis(2,2-dimethyl-1-aziridinyl)phosphinyl function, have been prepared and tested in vivo for antitumor activity and in vitro as potentiators of the cytotoxic effect of X-irradiation. Without irradiation only moderate effectiveness against leukemia P-388 in mice was exhibited by one of the quinonoid compounds that had sufficient water solubility to be used in the in vivo screening. However, both compounds were shown to potentiate the effect of X-irradiation in vitro by a colony-forming cell culture assay under hypoxic conditions.

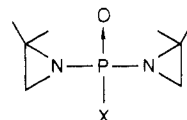
Several quinone compounds have shown useful anticancer activity, the best known of these being the antitumor antibiotics adriamycin, daunorubicin, mitomycin C, and streptonigrin. Other antineoplastic quinones include the naphthoquinone lapachol,[1] Sartorelli's bioreductively activated alkylating quinones (2-(halomethyl)-3-phenyl-1,4-naphthoquinones),[2] Driscoll's series of aziridinyl quinones,[3,4] and certain aminoquinones.[5] Also the *o*-hydroquinones levodopa and dopamine, when activated via oxidation to *o*-quinones by tyrosinase, have been shown to be cytotoxic to human and murine cancer cells, in part due to their ability to inactivate such sulfhydryl-dependent enzymes as DNA polymerase α and reverse transcriptase.[6]

Most of these antineoplastic drugs containing a quinoid moiety appear to exert their major effects via participation in oxidation–reduction reactions, with the active species believed to be the relatively stable semiquinone radical. This species might act directly on DNA[5] or DNA polymerase[6] by transfer of an electron, or by binding to a susceptible group (alkylating activity), or may act by intervening in oxygen-metabolizing processes,[7] promoting the formation of superoxide, hydroxyl, and peroxide radicals.[8]

The quinone antitumor drugs also possess some other characteristics that may contribute to their effectiveness as antineoplastic agents. Because of their planar ring structures, many of the known quinone drugs bind to DNA via intercalation;[7] this binding activity may induce single or double strand breaks in the DNA of carcinoma cells.[5] Moreover, the affinity of these drugs for the DNA helix directs the reactivity of the semiquinone radical toward this target. Many quinones also exhibit good lipid solubility and low levels of ionization, required for penetration of the blood–brain barrier, making them potentially effective agents against tumors of the central nervous system.[4]

Although most of the quinonoid antineoplastic drugs may be transformed to their active semiquinone forms intracellularly via the action of such enzymes as NADPH cytochrome P450 reductase,[9] a second known method of forming the semiquinone form is by irradiation.[10] Thus, the quinones as a group may act as radiation sensitizers, as has been shown in vitro for menaquinone.[10,11] However, the potential radiation sensitizing activity of quinones in vivo has not been so far tested or reported in the literature.

A different class of antineoplastic agents, the multifunctional bis(2,2-dimethyl-1-aziridinyl)phosphinic esters,[12] amides,[13] carbamates,[14] and hydroxycarbamates[15] termed "dual antagonists" (**1**–**3a**), has previously shown the ability



1, X=*O*-alkyl (X=OEt, AB-163)
2, X=NR^1R^2; R^1,R^2=H or alkyl
3, X=$NHCO_2$-alkyl (X=$NHCO_2Et$, AB-132)
3a, X=$ONHCO_2$-alkyl (X=$ONHCO_2Et$, AB-183)

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