Experimental Section

Chemical Methods. Melting points were determined in open glass capillaries with a Büchi-Tottoli melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 1420 spectrophotometer. ^1H NMR spectra were determined on a Perkin-Elmer R 32 spectrometer with Me_4Si as an internal standard. All spectral data for the products were consistent with the assigned structures. Microanalyses were performed by the Department of Organic Chemistry, University of Frankfurt, FRG, and were correct within ±0.4% of the theoretical values. TLC was performed on Merck silica gel plates 60 F 254 in toluene/chloroform/acetone (10:6:4). Spots were visualized under 254-nm illumination or with Dragendorff spraying reagent.

General Procedure for the Preparation of Carbamates 3–10. To a stirred solution of 15 mmol of the corresponding alcohol in 40 mL of anhydrous Et_2O was added dropwise a solution of 16 mmol of the substituted phenyl isocyanate in 25 mL of anhydrous Et_2O. The mixture was stirred for 24 h at room temperature. The insoluble solid (*N,N'*-bis(4- or 3-chlorophenyl)urea) was removed by filtration and the Et_2O removed by rotary evaporation. The residue was dissolved in dilute HCl and was extracted with Et_2O (3 × 30 mL). The aqueous layer was made alkaline with saturated sodium carbonate and extracted with Et_2O (3 × 30 mL). The extract was dried (Na_2SO_4), and the solvent was evaporated to yield yellow or colorless oils, which solidified upon standing. The products were used without further purification to form the desired tertiary and quaternary salts (Table I) in the usual manner; IR (KBr/Nujol) ν_{max} 1720–1740 (C=O), 1595–1612 (C=C), 1215–1230 (C-O-C); ^1H NMR (CD_3OD) δ 6.77–7.93 (m, 4 H, aromatic), 5.8–6.2 (m, 1 H, C=CH), 4.4–4.7 (s, 2 H, OCH_2), 2.7–2.97 (s, 3 H, NCH_3), 3.15–3.25 (s, 6 H, $^+N(CH_3)_2$).

***N*-Methyl-3-(hydroxymethyl)-3-pyrroline (11).** To a stirred suspension of 0.26 g (6.8 mmol) of $LiAlH_4$ in 26 mL of anhydrous THF, cooled at −45 °C, was added dropwise 1.7 g (12 mmol) of *N*-methyl-3-pyrroline-3-carboxylic acid methyl ester[18] in 17 mL of anhydrous THF over a period of 0.5 h. The mixture was stirred at −40 °C for 3 h. The excess of $LiAlH_4$ was carefully decomposed by dropwise treatment with acetone/H_2O (1:1). After addition of 20 mL of ether, the white solid was removed by suction filtration and was washed with acetone. The solvent was removed by rotary evaporation, and the oily residue was purified by vacuum distillation to yield 0.74 g (54%) of a colorless oil; bp 55–57 °C (0.1–0.2 mmHg); mp (HBr/acetone) 95 °C; IR (KBr) ν_{max} 3050 (OH); ^1H NMR ($CDCl_3$) δ 5.65 (m, 1 H, C=CH), 4.15 (s, 2 H, CH_2O), 3.48–3.67 (m, 4 H, CH_2NCH_2), 2.47 (s, 3 H, NCH_3). Anal. (picrate) ($C_{16}H_{20}N_4O_9$) C, H, N.

Pharmacology. Male Wistar rats weighing ca. 200–300 g were anesthetized with pentobarbitone sodium (60 mg/kg ip). The left jugular vein was cannulated for the administration of drugs. Arterial blood pressure was measured from the cannulated right common carotid artery. After catheterization of the trachea, the rats were pithed with a steel rod and artificial respiration with room air was provided (1 mL/100 g of body weight, 60 strokes/min). Body temperature was carefully monitored with a rectal thermometer and was maintained at 37 ± 1 °C by an overhead heating lamp. All drugs were dissolved in 0.9% saline and given iv.

Acknowledgment. We thank Dr. R. Hammer (Boehringer Ingelheim KG) and Dr. W. L. Nelson (University of Washington) for the generous gift of McN-A-343 (1) and its *trans*-dihydro analogue (**2a**), respectively, and M. Wagner for excellent secretarial assistance.

Molecular Basis for Anticancer Drug Amplification: Interaction of Phleomycin Amplifiers with DNA

L. Strekowski,*† S. Chandrasekaran,† Yueh-Hwa Wang,† W. Daniel Edwards,‡ and W. David Wilson*†

Department of Chemistry and Laboratory for Microbial and Biochemical Sciences, Georgia State University, Atlanta, Georgia 30303-3083, and Department of Chemistry, University of New Hampshire, Durham, New Hampshire 03824.
Received November 15, 1985

The interaction of two phleomycin amplifiers, *N,N*-dimethyl-2-[[4'-(thien-2''-yl)pyrimidin-2'-yl]thio]ethylamine (**1S**, high activity) and *N*-[2''-(dimethylamino)ethyl]-4-(thien-2'-yl)pyrimidin-2-amine (**1N**, low activity) with DNA has been evaluated. The visible absorption bands of both compounds shift to longer wavelengths, and both exhibit hypochromicity on titration with DNA. The effects for **1S** at low concentration are significantly greater than for **1N**. **1S** increases the DNA Tm by 2.5 °C while **1N** causes only a 1.0 °C increase under the same conditions. Spectrophotometric binding analysis of the interaction of **1S** and **1N** with calf thymus DNA indicates that **1S** binds over 4 times more strongly to this DNA than **1N**. Both compounds increase DNA viscosity, cause downfield shifts in DNA ^{31}P NMR spectra, and shift the DNA imino base pair protons upfield, conclusively demonstrating that they bind to DNA by intercalation. Signals for the aromatic protons of **1S** and **1N** are shifted upfield on addition of DNA as expected for intercalation. The shifts for all aromatic protons are similar on **1S** and on **1N**, indicating that both the pyrimidine and thiophene are inserted between the DNA base pairs in the complex. NOE experiments demonstrate that the compounds are in the s-cis conformation both free in solution and in the DNA intercalation complex. Semiempirical INDO/S calculations indicate greater polarization of the π-electron system of **1S** than **1N**. This greater polarization may account for the stronger interaction of **1S** with DNA base pairs than **1N**. The interaction of these compounds with DNA is strongly correlated with their biological amplification activity.

One method of enhancing the activity of currently available anticancer drugs is to find compounds that alone may have no activity but that amplify the action of the drugs. Considerable biological data are available on the activity amplification of phleomycin (PLM) and bleomycin (BLM) by a variety of compounds (amplifiers);[1] however, little is known about the mechanism(s) of amplification in these or any other systems. The best amplifiers of PLM and BLM are composed of at least two fused or unfused conjugated aromatic rings, and they are cationic or can acquire a positive charge by protonation of a nitrogen. As a rule, an anionic or potential anionic center in the molecule decreases activity. Since these features affect the

† Georgia State University.
‡ University of New Hampshire.

(1) (a) For a review see: Brown, D. J.; Grigg, G. W. *Med. Res. Rev.* 1982, 2, 193. (b) Aliano, A. N.; Allen, T. E.; Brown, D. J.; Cowden, W. B.; Grigg, G. W.; Kavulak, D.; Lan, S.-B. *Aust. J. Chem.* 1984, 37, 2385. (c) Allen, T. E.; Brown, D. J.; Cowden, W. B.; Grigg, G. W.; Hart, N. K.; Lamberton, J. A.; Lane, A. *J. Antibiot.* 1984, 37, 376 and references therein.

0022-2623/86/1829-1311$01.50/0 © 1986 American Chemical Society

interaction of an aromatic molecule with DNA in the same fashion, it has been strongly suggested that binding of the amplifier molecule to DNA is essential for the PLM and BLM amplification.[2] We have chosen the PLM amplifier system as a model to study the molecular basis of amplification because of the wealth of biological data available and because PLM is thought to act at the DNA level in vivo.[1] Currently the interaction of anticancer drugs with and on DNA offers one of the most promising routes for the rational design of such drugs.

The work reported here is the first attempt to characterize and quantitate the amplifier–DNA interaction and to determine whether the strength of interaction correlates with the biological activity (amplification of PLM activity). The two compounds tested, **1S** and **1N** (structure in Figure 5), are closely related in structure, but **1S** is highly active in standard test systems and in vivo[1c] while **1N** has only marginal activity.

Experimental Section

N,N-Dimethyl-2-[[4′-(thien-2″-yl)pyrimidin-2′-yl]thio]ethylamine (**1S**) and N-[2″-(dimethylamino)ethyl]-4-(thien-2′-yl)pyrimidin-2-amine (**1N**) were prepared as previously reported.[3] The free bases were purified by silica gel column chromatography using benzene/triethylamine (9:1) as an eluent and transformed into hydrobromides,[3] and the salts were crystallized three times with ethanol before use. Treatment of the salts with dilute NaOH solution followed by extraction with diethyl ether gave free bases that were found to be pure by TLC using analytical silica gel and alumina plates, and benzene/triethylamine (9:1), benzene/triethylamine/ethanol (8:1:1), or hexanes/triethylamine/ethanol (8:1:1) as eluents.

Buffers. PIPES 00 buffer, 0.01 M PIPES, 10^{-3} M EDTA, pH 7 was used unless otherwise indicated.

DNA. Calf thymus DNA was sonicated, phenol extracted, dialyzed, and characterized as previously described.[4]

Viscosity. Viscometric titrations were conducted as previously described[5] in PIPES 00 buffer with DNA sonicated to approximately 500 base pairs.

Spectrophotometric Measurements. Scans and extinction coefficients for free and bound compounds, as well as equilibrium parameters, were determined as previously described.[6]

Thermal Melting. DNA and complexes with **1S** and **1N** at a ratio of 0.3 mol of compound to DNA phosphate were heated in a Cary 219 spectrophotometer with a five-sample automatic cell changer. Temperature was increased 0.5 °C/min by a Haake water bath and PG20 temperature programmer.

NMR. Measurements of the effect of **1S** and **1N** on DNA ^{1}H imino protons and ^{31}P NMR signals were made as previously described.[6] Chemical shift changes of protons of **1S** and **1N** on addition of DNA were measured. Proton (270-MHz) NMR spectra were obtained on a JEOL GX 270 spectrometer under the following conditions: typically 2000 scans; 2.15-s pulse repetition rate; 0.1-Hz line broadening; 16K data points; TSP reference; 4000-Hz spectral width; 100% D_2O/phosphate buffer containing 15 mM NaH_2PO_4, 0.1 mM EDTA, 0.1 M NaCl; 5 mM **1N** and **1S**; temperature 60 °C; 0.8-mL sample volume in a 5-mm NMR tube. Proton nuclear Overhauser difference spectra were obtained under the following conditions: 10-s saturation time; 21-μs pulse width (corresponding to 90° pulse); 4-Hz line broadening; 16K data points; 4000-Hz spectral width; 128 scans; D_2O containing TSP as reference; 15-mM **1N** and **1S**; temperature 60 °C; 0.8-mL sample volume in a 5-mm NMR tube. The high temperature was used to obtain monomer ligand at NMR concentrations and to obtain fast exchange between free and bound compound. Spectrophotometric measurements indicate that the DNA Tm under these conditions is >75 °C and the DNA is, thus, in the native state in the NMR experiments.

Calculations. The π-electronic structures for compounds **1S** and **1N** were computed by using the intermediate neglect of differential overlap (INDO/S) method and parameters reported by Ridley and Zerner.[7] The nearest-neighbor interatomic distances and the nearest-neighbor bond angles were those of structurally related derivatives.[8] The computations were performed for the planar s-cis conformations shown on Figure 4.

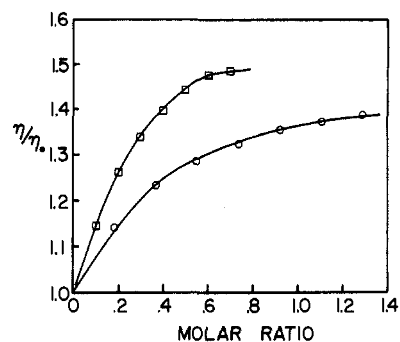


Figure 1. Viscometric titrations of sonicated calf thymus DNA with **1S** (□) and **1N** (○). The reduced specific viscosity ratio (η/η_0) is plotted as a function of the molar ratio of **1S** or **1N** to DNA base pairs. The titrations were conducted in PIPES 00 buffer at 28 °C.

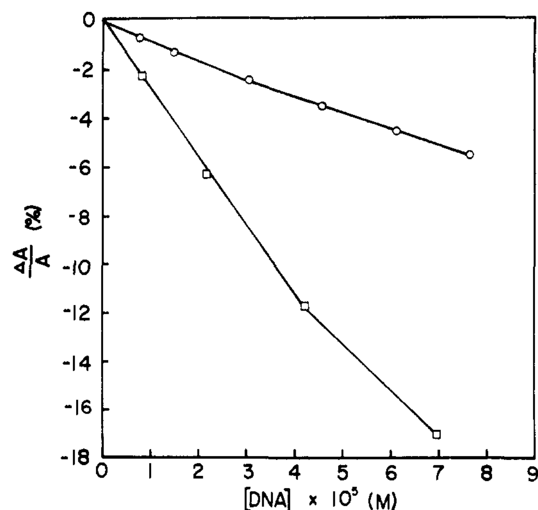


Figure 2. Spectrophotometric results for the titration of **1S** (□) and **1N** (○) with calf thymus DNA. The change in absorbance divided by the initial absorbance in percent is plotted vs. the DNA concentration. The titrations were conducted at 25 °C in PIPES 00 buffer. The initial absorbance of **1S** was 0.772 and of **1N** was 0.784, both at their peak wavelengths.

Results

Viscometric Titrations. As can be seen in Figure 1, both **1S** and **1N** cause increases in DNA viscosity in a

(2) Grigg, G. W.; Gero, A. V.; Sasse, W. H.; Sleigh, M. J. *Nucleic Acids Res.* **1984**, *12*, 9083.
(3) Brown, D. J.; Cowden, W. B.; Strekowski, L. *Aust. J. Chem.* **1982**, *35*, 1209.
(4) Wilson, W. D.; Krishnamoorthy, C. R.; Wang, Y.-H.; Smith, J. C. *Biopolymers* **1985**, *24*, 1941.
(5) Jones, R. L.; Lanier, A. C.; Keel, R. A.; Wilson, W. D. *Nucleic Acids Res.* **1980**, *8*, 1613.
(6) Wilson, W. D.; Wang, Y.-H.; Kusuma, S.; Chandrasekaran, S.; Yang, N. C.; Boykin, D. W. *J. Am. Chem. Soc.* **1985**, *107*, 4989.
(7) Ridley, J.; Zerner, M. C. *Theor. Chim. Acta* **1973**, *32*, 111.
(8) (a) Scheinbeim, J.; Schempp, E. *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.* **1976**, *B32*, 607. (b) Giuseppetti, G.; Tadini, C. *Cryst. Struct. Commun.* **1977**, *6*, 263. (c) Champaigne, E.; Folting, K.; Huffman, J. C.; Selby, T. P. *J. Heterocycl. Chem.* **1981**, *18*, 575. (d) Furberg, S.; Grogaard, J.; Smedsrud, B. *Acta Chem. Scand., Ser. B* **1979**, *B33*, 715. (e) Hawley, D. M.; Ferguson, G. *J. Chem. Soc. B* **1971**, 843. (f) Koyama, G.; Nakamura, H.; Muraoka, Y.; Takita, T.; Maeda, K.; Umezama, H. *Tetrahedron Lett.* **1968**, 4635. (g) Phillips, T.; Bryan, F. R. *Acta Crystallogr., Sect. A: Cryst. Phys., Diffr., Theor. Gen. Crystallogr.* **1969**, *A25*, Part S3, 200.

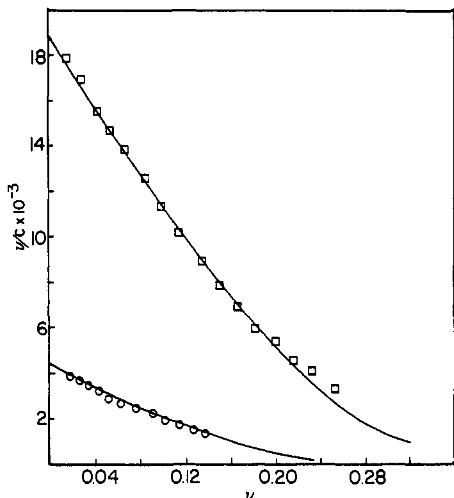


Figure 3. Scatchard plot for binding of **1S** (□) and **1N** (○) to calf thymus DNA in PIPES 00 buffer at 25 °C. The points in the figure are experimental results, and the solid lines are nonlinear least-squares best-fit results using the site-exclusion model as discussed in the text.

titration of DNA by the compounds. **1S** causes a slightly larger increase in viscosity than **1N** and has a steeper slope in the viscometric titration.

Thermal Melting. In a denaturation experiment with calf thymus DNA, a complex of 1S with DNA at a ratio of 0.3 mol of **1S** to DNA phosphate caused a 2.5 °C increase in the Tm. Under the same conditions, **1N** caused only a 1.0 °C increase in the DNA Tm.

Spectrophotometric Results. In the region above 300 nm, where interference by DNA absorption is not a problem, **1S** has an absorption peak at 324 nm (ϵ 14600 M^{-1} cm^{-1}) and **1N** has a peak at 333 nm (ϵ 10800 M^{-1} cm^{-1}). Addition of DNA to both compounds causes a shift of the absorption maximum to longer wavelengths and a decrease in extinction coefficient. In the titration with DNA, **1S** has an isosbestic point at 341 nm and **1N** at 364 nm. As can be seen in Figure 2, the absorbance decreases caused by a specific DNA concentration were much larger for **1S** than for **1N**. Addition of large amounts of DNA or Beer's law plots in concentrated DNA solutions were used to calculate bound extinction coefficients of 7500 M^{-1} cm^{-1} for **1S** and 6530 M^{-1} cm^{-1} for **1N** at the wavelengths of maximum absorbance for the free compounds. Titrations of **1S** and **1N** into DNA samples of intermediate concentration, such that a range of bound compound is obtained, can be used to generate binding isotherms by using the free and bound extinction coefficients.[4,6] Examples of these types of experiments, in a Scatchard plot for visualization, are shown for **1S** and **1N** in Figure 3. The solid lines in Figure 3 are nonlinear least-squares best-fit values using the site exclusion model of McGhee and von Hippel[9]

$$\nu/c = K[1 - n\nu][(1 - n\nu)/[1 - (n - 1)\nu]]^{n-1}$$

where ν is the moles of **1S** or **1N** bound per DNA base pair, c is the free concentration of **1S** or **1N**, K is the binding equilibrium constant, and n is the number of base pairs per binding site.[4,6] The equilibrium constants obtained by using this method to fit the results in Figure 3 are 18700 M^{-1} for **1S** and 4400 M^{-1} for **1N** binding to calf thymus DNA under these conditions.

NMR. Both **1S** and **1N** cause significant downfield shifts in DNA ^{31}P NMR signals and upfield shifts in the

(9) McGhee, J. D.; von Hippel, P. H. *J. Mol. Biol.* **1974**, *86*, 469.

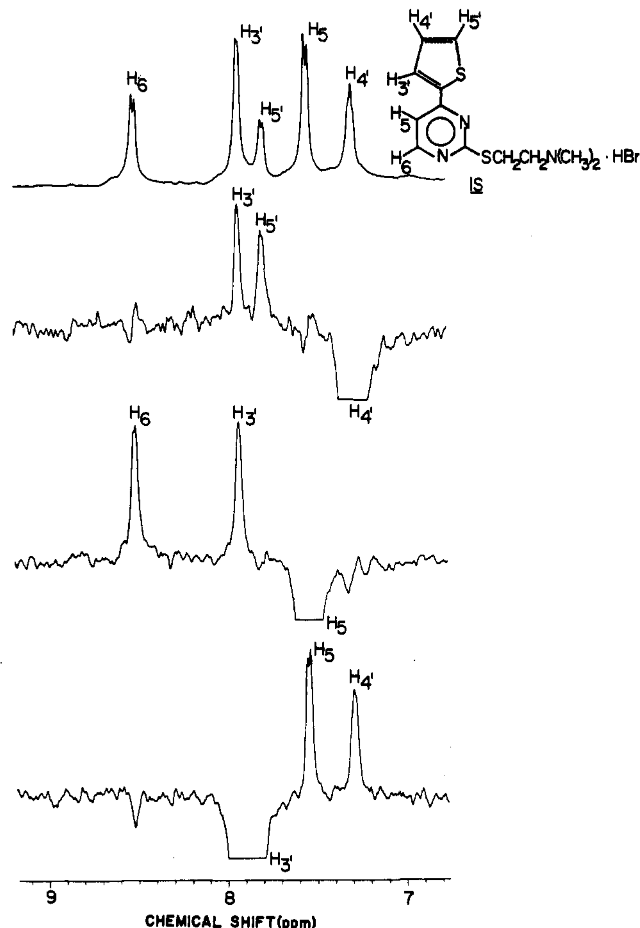


Figure 4. 270-MHz proton NMR NOE difference spectra of **1S** (15 mM) in D_2O at 60 °C. Protons $H_{3'}$, H_5, and $H_{4'}$ were irradiated with a saturation time of 10 s. Spectra were recorded after 128 scans and an accumulation time of 56 min.

Watson–Crick base pair imino hydrogen-bonded protons in NMR titrations. At a 0.5 ratio of compound to base pair, the downfield ^{31}P shifts of DNA are approximately 0.3 ppm for both **1S** and **1N**. The DNA imino proton upfield chemical shift changes are approximately 0.2 ppm for both compounds.

The ^{31}P and imino proton NMR experiments focus specifically on DNA as the drugs are added, but it is also possible to look at protons on the drug on addition of DNA. The first step in this process is to assign critical protons for observation as DNA is added. Assignments of the protons of **1S** and **1N** were made by using coupling patterns, chemical shifts, and 2D COSY experiments,[10] and assignments for all aromatic and the $N(CH_3)_2$ protons are given in Table I. To determine the relative orientation of the nonfused thiophene and pyrimidine rings in **1S** and **1N**, we have conducted both 1D and 2D NOE experiments[10] on the free compounds. As can be seen in Figure 4, using **1S** as an example, irradiation of H-5 of the pyrimidine ring in both **1S** and **1N** results in a strong NOE at H-6 of the same ring and a strong NOE at H-3′ of the thiophene ring. In the same way irradiation of H-3′ of the thiophene ring in both compounds results in strong NOE's to H-4′ of the same ring and an even stronger NOE to H-5 of the pyrimidine. The long-wavelength (>300 nm) absorption band obtained with both compounds indicates that the pyrimidine and thiophene rings must be essen-

(10) Bax, A. *Two-Dimensional NMR in Liquids*; Delft University: Delft, Holland, 1982. Sanders, J. K. M.; Mersh, J. D. *Prog. NMR Spectrosc.* **1982**, *15*, 353 and references therein.

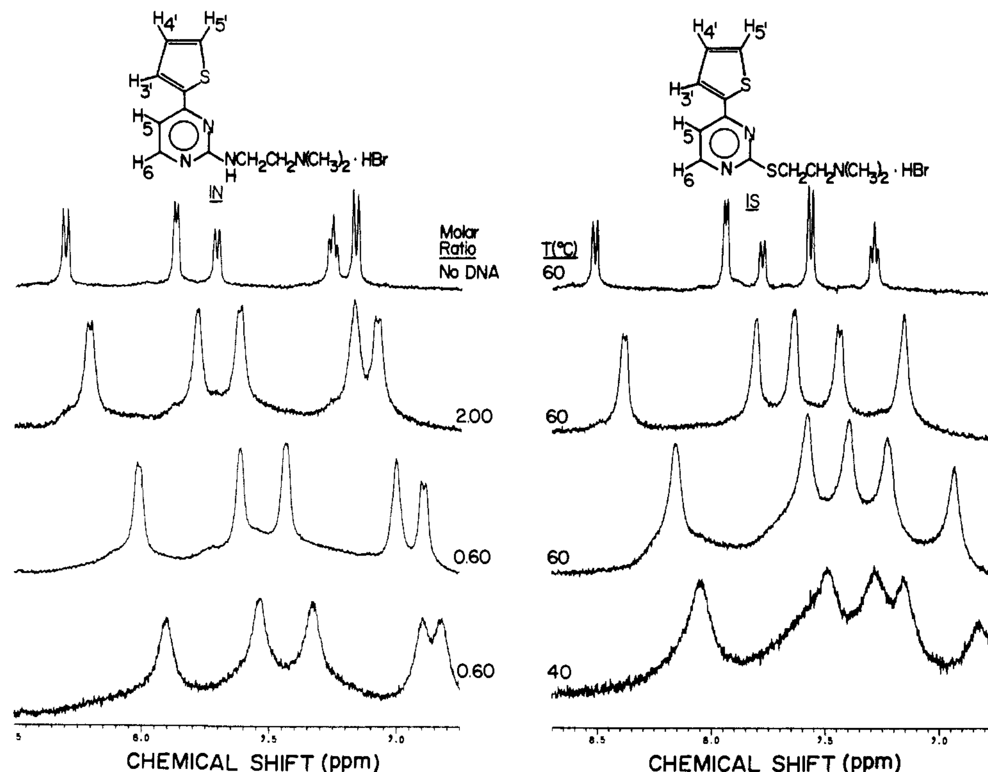


Figure 5. 270-MHz ^{1}H NMR spectra of the intercalation complexes of **1N** (left) and **1S** (right) with calf thymus DNA as a function of ratio (**1N** or **1S** to DNA base pairs) and temperature. The samples (0.8 mL in volume) were 5 mM in **1N** or **1S** in D_2O and contained 0.015 M phosphate, 0.1 mM EDTA, 0.1 M NaCl (pH 7.00), and TSP as reference. Spectra were obtained on a JEOL GX-270 NMR spectrometer under the following conditions: 16K data points; 4000-Hz spectral width; 2.5-s pulse repetition rate; 2000 scans; 0.1-Hz line broadening.

Table I. Proton Chemical Shifts of **1N** and **1S** as a Function of Added DNA at 60 °C

ratio[a]	H_6	$H_{3'}$	$H_{5'}$	$H_{4'}$	H_5	$N(CH_3)_2$
			Compound **1N**[b]			
no DNA	8.33	7.89	7.73	7.28	7.19	2.98
10.00	8.30	7.87	7.71	7.26	7.16	2.97
2.00	8.21	7.78	7.62	7.17	7.08	2.97
1.20	8.14	7.73	7.56	7.11	7.01	2.96
0.60	8.01	7.62	7.43	7.00	6.89	2.95
0.30	7.86	7.48	7.29	6.87	6.75	2.93
0.15	7.63	7.29	7.07	6.67	6.56	2.92
total shift[c]	–0.70	–0.60	–0.66	–0.61	–0.63	–0.06
			Compound **1S**[b]			
no DNA	8.52	7.95	7.78	7.29	7.57	2.96
10.00	8.49	7.92	7.76	7.27	7.55	2.95
2.00	8.38	7.81	7.64	7.16	7.43	2.94
1.20	8.31	7.73	7.56	7.08	7.36	2.94
0.60	8.15	7.58	7.40	6.93	7.23	2.92
0.30	7.94	7.38	7.18	6.73	7.03	2.89
0.15	7.74	7.19	6.97	6.55	6.86	2.82
total shift[c]	–0.78	–0.76	–0.81	–0.74	–0.71	–0.14

[a] Ratio of the molarity of **1S** or **1N** to the DNA concentration in base pair molarity. [b] Proton assignments were made by standard 1D and 2D methods. The numbering system is shown in Figure 4. [c] The shift difference (ppm) between the compound free in solution and with an excess of DNA (ratio 0.15) is given.

tially planar, and the NOE values presented above indicate that both of the compounds must be in the s-cis conformation shown in Figure 5.[11] We have titrated **1S** and **1N** with sonicated calf thymus DNA to determine how the aromatic protons shift under the influence of the ring current of DNA base pairs. Results at two ratios and two temperatures are shown in Figure 5, and results for additional ratios and for the side-chain *N*-methyl protons are included in Table I. There are large upfield shifts and line broadening of the aromatic protons but little effect on the *N*-CH_3 protons. In both compounds the aromatic proton shifts are quite similar for protons on the pyrimidine and thiophene rings (–0.76 ± 0.05 for **1S** and –0.66 ± 0.04 for **1N** at the 0.15 ratio). A 1D NOE experiment[10] on these intercalation complexes gave a strong NOE between the 5 and 3′ protons, demonstrating that the compounds remain in the s-cis orientation when bound to DNA.

Calculations. We have carried out semiempirical INDO/S computations on the electronic structure of **1S** and **1N**. Both compounds have the π-electron system polarized positively with respect to the hetero substituent that constitutes the negative end of the dipole. However, this polarization is greater for **1S** than **1N**, as reflected by the Mulliken charge distributions and dipole moments (7.16 D for **1S** and 3.50 D for **1N**).

Discussion

The increases in DNA viscosity, downfield shifts of DNA ^{31}P signals, and upfield shift of hydrogen-bonded base pair imino protons indicate unequivocally that both **1S** and **1N** bind to DNA by intercalation.[5,6] Few nonfused ring systems are known to bind to DNA by intercalation but the long-wavelength absorption bands of **1S** and **1N** as well as neighboring ring current effects in their proton NMR spectra strongly suggest that these compounds have a planar arrangement of the nonfused pyrimidine and thiophene rings in solution. This conclusion is supported by the finding that inter-ring NOE's (H-5 to H-3′) are as strong as or even stronger than intra-ring NOE's (H-5 to H-6 and H-3′ to H-4′) as shown in Figure 4. The NOE experiment also establishes that **1S** and **1N** are in the s-cis conformation as shown in Figure 5 and remain in that

(11) Similar compounds adopt a planar s-cis form in the crystalline state: Kuroda, R.; Neidle, S.; Riordan, J. M.; Sakai, T. *Nucleic Acids Res.* **1982**, *10*, 4753 and references therein.

conformation when bound to DNA.

The large upfield shifts of the aromatic protons of **1S** and **1N** also agree with a model of intercalation of the nonfused rings with DNA. The small shift of the N-CH_3 (and other side chain) protons indicates that the cationic substituent, as expected, is not intercalated. Similar experiments with other intercalators have shown large differences in the aromatic ring proton chemical shifts that depend on the overlap of the intercalator and the DNA base pairs.[12] The similarity of the shifts for all protons on **1S** and all protons on **1N** indicates that both the thiophene and pyrimidine rings are intercalated in the complex and that both have very similar overlap with the DNA base pairs.[12] The slightly smaller shifts for **1N** are probably due primarily to the weaker binding of **1N** than **1S** to DNA.

An interesting result of this study is that even though **1S** and **1N** have similar conformations and both bind to DNA by intercalation, their strength of interaction with DNA is significantly different. The increase in DNA Tm is larger for **1S** than for **1N** and the equilibrium constant for **1S** binding to DNA more than 4 times the binding constant for **1N** with DNA under the same conditions. In a standard biological test with phleomycin, **1S** shows significantly higher activity than **1N**.[1] Since our NMR studies, using ligand and DNA ^1H and ^{31}P signals, have shown that the complexes of **1S** and **1N** with DNA base pairs are structurally similar, this difference in interaction strength was at first quite surprising. However, this difference can be explained, assuming that charge-transfer forces are important for the intercalation complexes of **1S** and **1N** with DNA. Thus, compound **1S** has the lower π-electron density, as reflected by INDO/S calculations, and it is expected to form the stronger complexes in which DNA bases act as electron donors. A similar interaction model, in which charge-transfer forces are believed to be important, has been employed to describe binding of nucleosides to riboflavin.[13] In addition, the dipole moment of **1S** is larger than for **1N**, but the dipole moments have the same relative orientation in the molecular systems. This would serve to orient the molecules in a similar manner with respect to the dipole moment of the DNA base pairs, as observed, but would give a stronger interaction of **1S** with the base pairs than **1N**, also as observed.

In summary, all of these experimental findings conclusively demonstrate that **1S** and **1N** bind to DNA by intercalation with their rings in an s-cis conformation. In agreement with the biological activity of the two compounds, the interaction of **1S** with DNA is significantly stronger than for **1N**, and these results strongly suggest that phleomycin amplification by these compounds may involve an interaction of the amplifier and phleomycin with DNA. More detailed studies of these compounds are in progress.

Acknowledgment. This work was supported by the American Cancer Society, by NIH Grant GM 30267 (W.D.W.), and by Grant 85-34 from the Milheim Foundation for Cancer Research (L.S.).

(12) Chandrasekaran, S.; Kusuma, S.; Boykin, D. W.; Wilson, W. D. *Magn. Res. Chem.*, in press.

(13) Ts'o, P. O. P. In *Basic Principles in Nucleic Acid Chemistry*; Ts'o, P. O. P., Ed.; Academic: New York, 1974; pp 526–562.

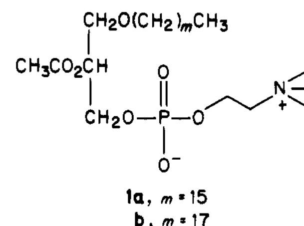
Analogues of Platelet Activating Factor. 5.[1] Multiple Oxygen Substitution of the Alkoxy Chain

A. Wissner,* C. A. Kohler, and B. M. Goldstein

Metabolic Disease Research and Cardiovascular Biological Research Sections, Medical Research Division, American Cyanamid Company, Lederle Laboratories, Pearl River, New York 10965. *Received November 18, 1985*

Racemic analogues of platelet activating factor (PAF) in which the alkoxy chain ($R = -O(CH_2)_{15}CH_3$) has been replaced with a chain containing multiple ether linkages near the end (**17**, $R = -O(CH_2)_8O(CH_2)_2O(CH_2)_2OCH_3$) or at the beginning (**18**, $R = -O(CH_2)_2O(CH_2)_2O(CH_2)_9CH_3$) of the chain have been prepared. Both compounds exhibit reduced hypotensive and platelet aggregation responses compared to racemic C_{16}-PAF (**1a**). This reduction in the biological activities is more apparent when the new oxygen atoms are located near the end of the chain. This substitution of additional oxygen atoms into the alkoxy chain does not result in any dramatic gains in selectivity of the biological responses.

Platelet activating factor (PAF) is an alkyl ether phospholipid of structure **1** composed primarily of the C_{16} and C_{18} homologues. It is a potent activator of various inflammatory cells such as platelets, neutrophils, and basophils and is one of the important mediators of anaphylaxis and inflammation. PAF is also a potent hypotensive agent.[2]



Over the last few years a number of groups have reported the preparation and biological activity of PAF

(1) For the previous publication in the series see: Wissner, A.; Sum, P.-E.; Schaub, R. E.; Kohler, C. A.; Goldstein, B. M. *J. Med. Chem.* 1985, *29*, 328.

 © 1986 American Chemical Society