Reagents for Bioorganic Synthesis. 4. A Novel Bifunctional Cross-Linking Reagent. Preparation, Structure, Properties, and Reactions of 2,2'-Sulfonylbis[3-methoxy-(*E*,*E*)-2-propenenitrile][1]

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Received November 14, 1989

The reagent 2,2'-sulfonylbis[3-methoxy-(*E*,*E*)-2-propenenitrile] (1) was prepared by the reaction of 2,2'-sulfonyldiacetonitrile with excess trimethyl orthoformate, catalyzed by concentrated sulfuric acid. Configurational and conformational features of 1 were elucidated by singel-crystal X-ray analysis. The length of the cross-linking bridge in 1 was 4.98 ± 0.1 Å. The reagent was highly reactive at room temperature with a variety of primary, secondary, and heterocyclic amines, as well as with amino acid esters of glycine, lysine, and serine, and with nucleic acid bases and nucleosides adenine/adenosine and cytosine/cytidine. The bis-enamine products (4–11) were isolated in good to excellent yields. The structure, properties, and reactions of the bis-enamines were also investigated. Employing diethylamine, the rate of formation of bis-enamine was assessed and contrasted with the rate of hydrolysis of 1. Reagent 1 possesses adequate aqueous stability and as such qualifies as a potential biological cross-linking agent. The bis-enamine products 4 were highly resistant to hydrolysis but underwent facile amine exchange reactions with other primary and secondary amines. Thus, they too may be viable candidates for cross-linking. Finally, the use of 1 to cross-link hemoglobin subunits has been demonstrated.

Introduction

We have set out to design bifunctional organic reagents as part of a broad program aimed at exploring novel reagents for organic/bioorganic syntheses.[2] Bifunctional reagents have potentially useful applications in biological systems, the anticipated principal use being for cross-linking proteins and/or nucleic acids. Cross-linking reagents have long played major roles in investigations of structure and function of both proteins[3] and nucleic acids.[4] Psoralens, a family of natural photochemical cross-linkers that form covalent bonds between interstrand pyrimidine residues of double helical DNA and RNA,[4e,f] have been extensively employed to probe static and dynamic structural features and functional properties of nucleic acids.[4b] Investigations of bifunctional reagents have gained added stimulus in light of the recent isolation and characterization of a covalently cross-linked adduct between the antitumor antibiotic mitomycin C and interstrand guanine residues of DNA.[4a] This finding, while providing direct proof for the long-suspected mode of action of mitomycin C,[4g] has spurred synthetic maneuvers in other antitumor antibiotics, e.g. anthramycins,[4c,h] so as to enhance their biological activity as well as to lend support for the postulated biochemical mechanisms of action.[5] In the field of proteins, synthetic homo- or heterobifunctional cross-linking reagents containing reactive functionalities such as aldehyde, ester, imide, azide, or imidate have been the subjects of extensive study.[3] However, there are some drawbacks associated with the use of these functional groups. Whereas aldehydes readily form Schiff bases with amine nucleophiles, the reaction is often reversible, requiring further reduction[3m] to stabilize the product. Ester and imide functions, on the other hand, frequently suffer from low reactivity under physiological conditions. Azide

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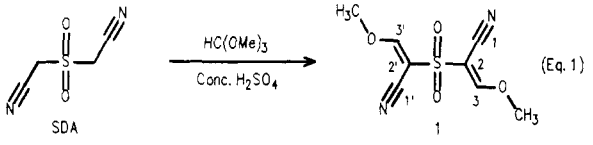
couplings require photoactivation,[3r] and imidates are highly prone to hydrolysis.[2g] We report here the preparation, properties, structure, and reactions of a novel homobifunctional reagent, 2,2′-sulfonylbis[3-methoxy-(*E*,*E*)-2-propenenitrile] (1) which uses the hitherto unexploited enol–ether group for cross-linking. The reagent, with its one-step synthesis, is easy to prepare on a large scale, inexpensive, stable in storage for long periods, and highly reactive toward amine nucleophiles present in both nucleic acids and proteins. Furthermore, the product bis-enamines are remarkably resistant to hydrolysis, requiring no reduction after the formation of cross-links. We have assessed the aqueous stability of 1 by comparison of its rate of hydrolysis with the rate of reaction with a representative amine. Finally, we have recently demonstrated[1b] the biomacromolecular cross-linking ability of 1, using oxy- and deoxyhemoglobins, as an example.

Results and Discussion

(a) Synthesis, Structure, and Properties of 1. Reagent 1 was prepared by the reaction of 2,2′-sulfonyldiacetonitrile[6] with excess trimethyl orthoformate, catalyzed by concentrated sulfuric acid (eq 1). Obtained in

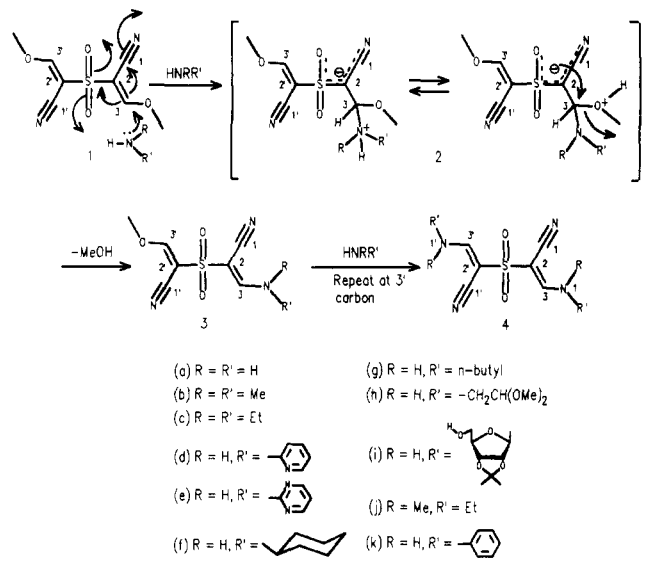


45–50% yield, 1 is a colorless, crystalline solid which is indefinitely stable when stored with proper protection from moisture. It is a bis-enol methyl ether of two acetaldehyde molecules connected to each other at the 2-carbon atom by a highly activating sulfonyl group. Each C2 is joined to an additional electron-withdrawing nitrile group. Therefore, 1 would be susceptibile to a facile conjugate addition–elimination process at its C3,C3′ junctions, initiated by a variety of nucleophiles. A single-crystal X-ray analysis of 1[7] confirmed the presence of considerable electron delocalization over the five-atom chains extending from the ethereal oxygens to the nitriles and the sulfone. The X-ray data also revealed an *E* configuration for each half molecule on either side of the sulfonyl moiety, while the conformational relationship of each half with respect to the other was anti, as depicted. Furthermore, the distance between C3 and C3′, the two potential cross-linking sites, was 4.98 ± 0.1 Å as computed from the two sets of fractional coordinates and unit-cell measurements.

The study of reactivity of 1 toward amine nucleophiles is important in view of the contemplated applications of the reagent to cross-link nucleic acids and/or proteins. The target amines in nucleic acids are the extranuclear NH_2's of adenosine, guanosine, or cytidine, whereas in proteins they are the ϵ-NH_2 of lysine or, if the N-terminus happens to be at the cross-linking site, the α-NH_2. In any event, the conjugate addition of an amine at the highly electrophilic C3 of 1 will produce an energetically favored, resonance-delocalized species 2 (Scheme I). The intermediate 2, upon elimination of a molecule of methanol, would yield the enamine 3. The latter, after reaction with a second molecule of the same amine at the C3′ junction, would yield bis-enamine 4.

(b) Reaction of 1 with Common Amine Nucleophiles. Initially, we studied the reaction of 1 with 11

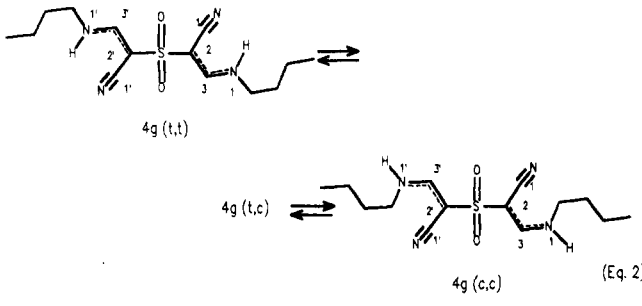
Scheme I



simple amines, including representative primary, secondary, ribosyl, and heterocyclic amines (Scheme I). The reaction was carried out at room temperature, employing 2 or more equiv of amine with acetonitrile or methanol as a solvent. Reaction was practically instantaneous in most cases, yielding product bis-enamines (**4a–k**). Compound **4b** was also obtained by the reaction of 2,2′-sulfonyldiacetonitrile with dimethylformamide dimethyl acetal.

(c) Structure and Properties of Bis-enamine Products. Single-crystal X-ray analysis of **4c** (Figure 1a) exhibited a delocalized structure analogous to that of 1[7] described above. The *E*,*E* configurational and anti conformational geometry were also present in **4c**. The observed bond angles, C3(3′)–N1(1′)–R(R′) ≃ 120°, and the bond lengths, C3(3′)–N1(1′) ≃ 1.3 Å, were consistent with sp^2-hybridized enamine nitrogens. Furthermore, the torsional angles defining the positions of the CH_2 groups in the N–Et side chains with respect to the C3(3′)–C2(2′) double bond were close to either 180° or 0°, indicative of delocalization of the lone pair of electrons of the amine nitrogens into the π systems. The existence of restricted rotation around the C3(3′)–N1(1′) bond of the bis-enamines was also reflected in the ^1H NMR spectrum of **4b**, which showed two distinct signals for the methyl groups in the δ 3.2 region. Increasing the sample temperature resulted in gradual coalescence of the methyl signals, and at 60 °C, a single methyl absorption integrating for 12 protons was observed.

In view of restricted rotation about the C3(3′)–N1(1′) bond, three geometrical isomers of 4 are possible in cases where either primary amines or secondary amines with nonequivalent R, R′ groups are involved in the reaction with 1. For example, the reaction of 1 with butylamine would yield **4g** (trans,trans), **4g** (cis,cis), and **4g** (trans,cis) isomers (eq 2). (Please note that the cis–trans nomen-



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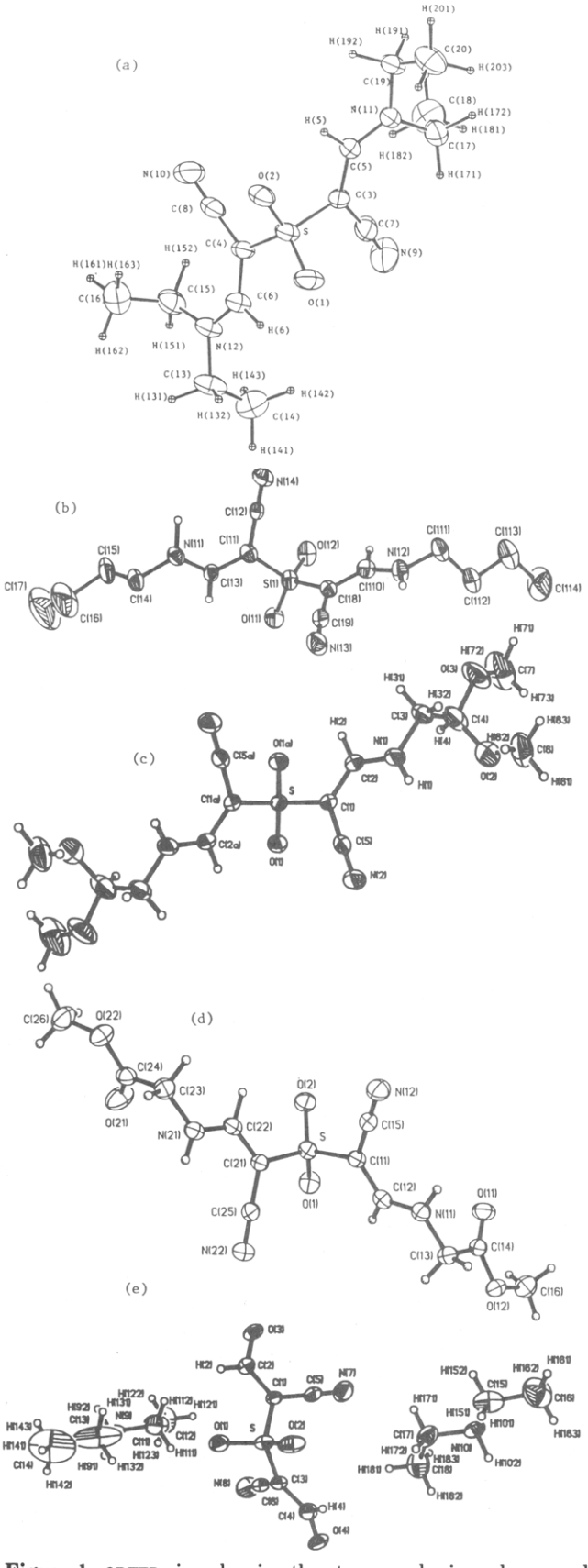


Figure 1. ORTEP view showing the atom numbering scheme and thermal ellipsoids at the 30% probability level of (a) **4c**, (b) **4g**, (c) **4h**, (d) **5**, and (e) **13**.

clature is employed here to distinguish the geometry about the C3(3′)–N1(1′) pseudo double bond from the one about the C3(3′)–C2(2′) double bond described above.) However, only the trans,trans isomers (t,t) were observed in the

Table I. Calculated Values for Trans,Trans (t,t), Cis,Trans (c,t), and Cis,Cis (c,c) Isomer Ratios for Compounds 4f, 4g, 4h, 4j, 5, 6, and 7

	isomer		
compd	t,t, %	c,t, %	c,c, %
4f	56	38	6
4g	59	36	5
4h	69	28	3
4j	41	46	13
5	52	40	8
6	60	35	5
7	64	32	4

Table II. Forward Rates of Interconversion of 4g (Trans) and 4g (Cis) Isomers as a Function of Temperature

temp, °C	k_f, s^{-1}	temp, °C	k_f, s^{-1}
53.6	0.94	82.3	12
58.8	2.1	87.2	16
63.3	3.5	92.0	21
68.0	5.1	96.8	28
72.8	6.7	101.5	38
77.5	9.4	106.4	57

crystal structure of both **4g** and **4h** (Figure 1, parts b and c). Nevertheless, the ^{1}H NMR spectrum of **4g** in deuterated dimethyl sulfoxide exhibited signals corresponding to two distinct sets of resonances in a relative ratio of 10:3. Similar observations were made for **4f** (3:1), **4h** (5:1), and **4j** (9:5). The lack of a third set of resonances is presumably due to the large distance between protons of each half of the molecule such that the magnetic environments of the halves are unaffected by each other. Thus, it is not possible to distinguish the c,t isomer from a mixture of c,c + t,t isomers. Performing a simple calculation[8] allows determination of the actual ratio of the three isomers, provided an assumption is made that the isomerization for half of the molecule is independent of the other half. The calculated isomer ratios for **4f–h** and **4j** are listed in Table I. Apparently, the t,t isomer is predominant also in solution, followed by the t,c isomer, except in the case of **4j**.

We studied the kinetics for the trans–cis isomerization about the conformationally restricted C3(3′)–N1(1′) bond in **4g** at ambient as well as at higher temperatures. To this end, we utilized the technique of ^{1}H NMR lineshape analysis.[10] The exchange constant τ is related to the

(8) The ^{1}H NMR data for bis-enamines **4f–h,j**, and **5–7** provide the trans:cis ratio R for the C1(1′)–N1(1′) bonds. However, because isomerization can take place about both the C1–N1 and C1′–N1′ bonds within one molecule, three isomers would be present in equilibrium with each other:

$$(t,t) \overset{K'}{\rightleftharpoons} (t,c') + (c,t') \overset{K''}{\rightleftharpoons} (c,c)$$

The t:c ratio provided by the ^{1}H NMR data is for (t) $\overset{K}{\rightleftharpoons}$ (c). If an assumption is made that the isomerization of each half of the molecule is independent of the other, then the relationship between K, K', and K'' can be determined. The forward isomerization of (t,t) can take place by isomerization of either of the bonds. However, the reverse process from (t,c′) or (c,t′) back to (t,t) can only take place by isomerization of the c bond and thus, $K' = 2K$. For the second equilibrium, the production of (c,c) can take place by isomerization of the t bond, but the reverse can proceed by isomerization of either of the bonds in (c,c) and therefore, $K'' = {}^1/_2K$. It follows that $K' = 4K''$.[9] It is not possible to distinguish (t,c′) from (c,t′) for the bis-enamines due to the center of symmetry about the sulfur atom in each, so let (t,c) = (t,c′) + (c,t′). The relationship between K' and K'' yields: (t,c)/(t,t) = 4(c,c)/(t,c) (eq 1). If we let the concentration of each species be in concentration units of mole fractions, then (t,t) + (t,c) + (c,c) = 1 (eq 2). The equation that describes the ratio R that is obtained from the ^{1}H NMR data is: [2(t,t) + (t,c)]/[2(c,c) + (t,c)] = Σt bonds/Σc bonds = R (eq 3). Having three equations, and three unknowns, the mole fractions for (t,t), (t,c), and (c,c) can be solved as a function of R: (t,t) = $1 - 2/(R + 1) + [1/(R + 1)^2]$; (t,c) = $2/(R + 1) - [2/(R + 1)^2]$; and (c,c) = $1/(R + 1)^2$.

(9) For a similar explanation based on the ionization of diacids, see: Gould, E. S. *Mechanism and Structure in Organic Chemistry*; Holt, Rinehart, and Wilson: New York, 1959; pp 201–202.

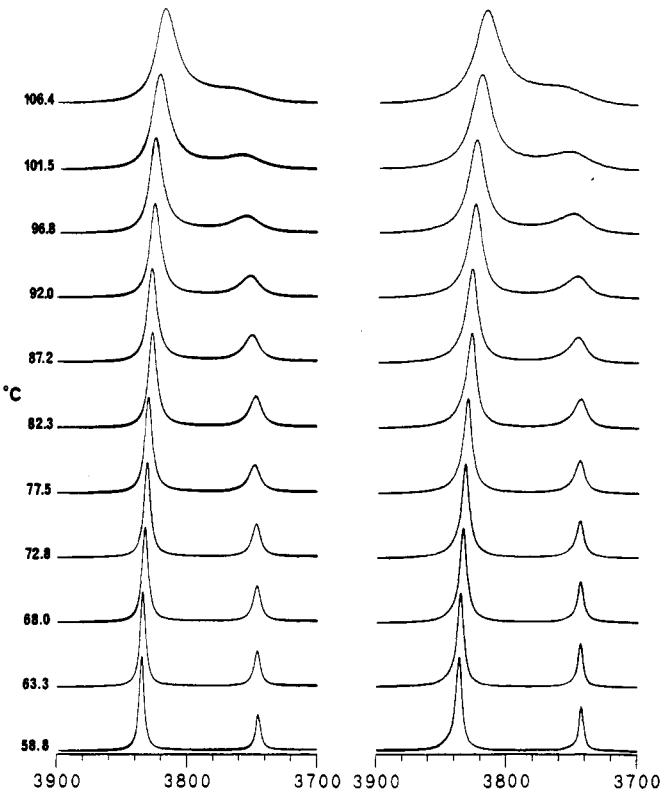


Figure 2. Calculated (left) and observed proton NMR spectra of **4g** for the methine hydrogens as a function of temperature; scale is in hertz.

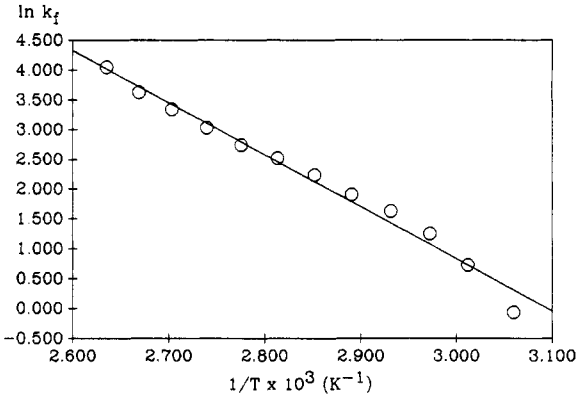
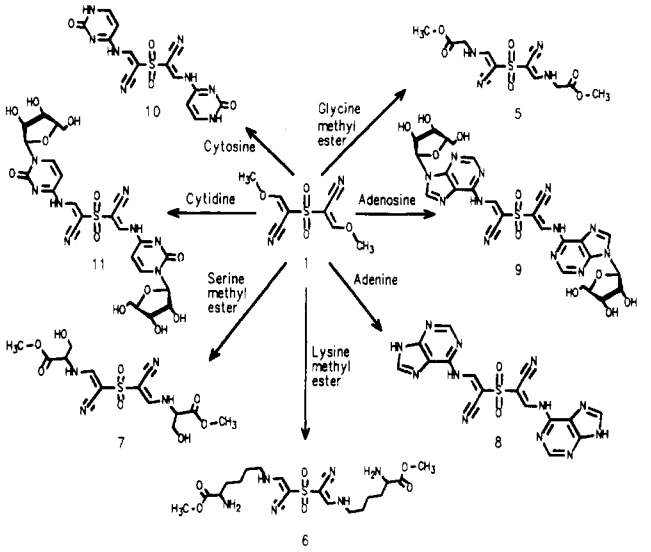


Figure 3. Arrhenius plot for isomerization of **4g** (trans).

relative isomer populations P by the equation, $\tau = P_{4g(c)}/k_f = P_{4g(t)}/k_r$, where k_f and k_r are the rates of forward and reverse reactions, respectively.[10a,b] The average values for the parameter P were computed from the peak area integrations of the observed 500-MHz ^{1}H NMR spectral signals for the =CH groups in the two isomers in the stopped-exchange region ($t \leq 44$ °C) and the extrapolated region (t = 45–120 °C). The best value for τ was derived by computer simulation of the spectral lineshapes of the said methine protons for various trial and error values of τ until the simulated and the experimental values became virtually identical. The generated lineshape analyses over

Scheme II



a temperature range, 60–110 °C, are depicted in Figure 2; the corresponding forward rate constants ($k_f = P_{4g(c)}/\tau$) are listed in Table II. The forward rate of interconversion of the isomers at 25 °C was computed by extrapolation of an Arrhenius plot of ln k_f versus $1/T$ (Figure 3) to give a value of k_f = 0.1 s^{-1}. We also calculated the energy of activation for the forward isomerization process by linear least-square analyses of the data obtained from the above Arrhenius plot. The E_a for the process was found to be $\simeq$ 17 kcal/mol (73 kJ/mol). The high E_a value corroborates the existence of considerable rotational barrier about the C3(3′)–N1(1′) bond of **4g**, as discussed earlier.

The ^{1}H NMR spectrum of **4i** was complicated by the presence of α- and β-anomers for each of the cis,cis, cis,-trans, and trans,trans geometrical orientations. A 500-MHz ^{1}H NMR spectrum of **4i** revealed several distinct signals in the region, δ 7.8–8.5, corresponding to the olefinic 3(3′)-CH. The two anomers could be distinguished based upon the anticipated[11] downfield signal for the anomeric proton of the α-anomer relative to that of the β-anomer. Also, the anomeric coupling constant for the α-anomer was higher (5.5 Hz) than that for the β-anomer (4.0 Hz), as expected.[11] Furthermore, a large chemical shift difference ($\Delta\delta$ = 0.16) was observed between the two methyl groups of the 2′,3′-isopropylidene function of the β anomer, as precedented.[12]

A single product was formed in the reactions of **1** with 2-aminopyridine (**4d**), 2-aminopyrimidine (**4e**), or aniline (**4k**). The olefinic 3(3′)-CH in **4d**, **4e**, or **4k** was considerably downfield (δ 8.1–9.0) relative to that in nonaromatic enamines **4a–c** and **4f–h** ($\delta \simeq$ 7.6). Equally dramatic was the appearance of the enamine NH's at δ 11.0, 11.6, and 12.2, respectively, for **4k**, **4d**, and **4e**, as opposed to δ <9.0 for the nonaromatic enamine NH's. This marked difference in chemical shifts of CH or NH between aromatic/heteroaromatic and nonaromatic enamines is obviously too large to account for based solely on the inductive and

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mesomeric effects of the aromatic/heteroaromatic rings. The anomaly can, however, be reconciled by considerations of the magnetic anisotropy of the phenyl, pyridine, or the pyrimidine ring. Whereas the enamine NH can experience the effect of aromatic/heteroaromatic magnetic anisotropy either in the cis or the trans orientation, such an effect on the olefinic CH would be minimum, if any, in the cis geometry. Therefore, the only isomer obtained in the reactions of 1 with 2-aminopyridine (**4d**), 2-aminopyrimidine (**4e**), or aniline (**4k**) must bear a trans,trans configuration.

(d) Reaction of 1 with Amino Acid Esters and Nucleic Acid Bases/Nucleosides. The above results prompted us to investigate the reactions of **1** with amino acids and nucleic acid bases/nucleosides. Representative amino acids included glycine, lysine, and serine, while adenine/adenosine and cytosine/cytidine represented, respectively, purine and pyrimidine nucleic acid bases/nucleosides (Scheme II). The products from reactions of **1** with the esters[13a] of glycine (i.e. **5**), lysine (**6**), and serine (**7**), performed at room temperature in methanol or acetonitrile, each exhibited two sets of ^{1}H NMR resonances analogous to the ones described above for **4f**, **4g**, and **4h**. The t,t, t,c, and c,c isomer ratios are calculated as before and collected in Table I. Once again, only the trans,trans orientation was prevalent in the solid state as revealed by a single-crystal X-ray analysis of **5** (Figure 1d). While the ambident lysine methyl ester can attack **1** with either the α- or the ϵ-NH_2 end, only one regioisomer was isolated. The structure **6** was assigned for this product by comparison of the ^{1}H NMR signal for =CH absorption in either the major trans isomer (δ 7.70) or the minor cis isomer (δ 7.50) with that of the analogous bis-*n*-butyl adduct **4g** (trans δ 7.69 and cis δ 7.47), which differed significantly from the corresponding signal in **5** (δ 7.83 and 7.76, respectively) or in **7** (δ 7.86 and 7.60, respectively). These values are consistent with the inductive effect of the α-CO_2Me group, which is farther from the C3 and C3′ methine in **6** than in **5** or **7**.

The ^{1}H NMR spectra of products **8–11**, formed by reaction of **1** with adenine, adenosine, cytosine, and cytidine, respectively, at room temperature using dimethyl sulfoxide as solvent, exhibited similar features as the heterocyclic adducts, **4d** and **4e**, mentioned above. The point of attachment of adenine/adenosine to C-3(3′) of **1** was assigned as N^6 based upon (a) the broad 3(3′)-CH singlet in the ^{1}H NMR spectrum of **8** or **9** which became a sharp singlet upon exchange with D_2O, and (b) the UV spectrum of **8** or **9** ($\lambda_{max} \simeq 341$ nm) which pointed to the presence of extended conjugation that could not be attributed to either a 1-substituted adenine/adenosine ($\lambda_{max} \simeq 258$ nm)[14] or an unconjugated enamine, e.g. **4b** ($\lambda_{max} = 285$ nm). The magnetic anisotropy of the heterocyclic rings was evident in the chemical shifts of the olefinic =CH absorptions, which ranged from $\delta \simeq 8.75$ (in cytosine/cytidine) to $\delta \simeq 9.6$ (in adenine/adenosine). Again, only one isomer could be detected, whose spectral characteristics were consistent with the *E,E* configuration for each half molecule, an anti conformational relationship of each half with respect to the other, and the trans,trans geometry around the rotationally restricted enamine C–N bond.

(e) Aqueous Stability of Reagent 1 and Its Products. A critical characteristic of a cross-linking reagent suitable for application in biological systems is adequate stability in aqueous media. It is equally important that the cross-linked product be stable to hydrolysis. In this regard, a study of reactions of **1** and its bis-enamines **4** with water was in order. While the TLC of a mixture of **1** and a 3-fold molar excess of water revealed mostly the unreacted **1** for up to 4 min, compound **4c** was resistant to hydrolysis even in a 1000-fold molar excess of water for several days. These results were encouraging in view of the high reactivity of **1** toward amine nucleophiles, which would potentially offset the reagent's brief aqueous stability, and because the high stability of the product necessitates no further reduction after the cross-link formation unlike in the case of bis-aldehydic reagents.[3m] Nevertheless, if **1** were to be a biological cross-linking agent, it must withstand a predominantly aqueous environment which is much more than a mere 3-fold excess of water! Thus, it was crucial to evaluate and contrast the rate of reaction of **1** with amines in water at various pH with that of hydrolysis of **1** under the same or similar experimental conditions. The hydrolysis of **1** was monitored at 25.0 °C (μ = 0.1, NaCl) at pH values in the range of 3.9–7.6 by the observance of the decay in the UV absorbance at 246 nm. Acetate buffer (pH 3.9–5.6) and phosphate buffer (pH 5.8–7.6) were used for the pH range. The rate was independent of buffer concentration with acetate in the range 0.02–0.15 M and with phosphate in the range 0.008–0.085 M; thus an average value was taken for each pH studied. At pHs above 7.6 and below 3.9 the UV absorbance decays to a finite value above 0, indicating the formation of unknown UV absorbing intermediate(s). At pH 9.2 the UV absorbance increases, probably due to the formation of an intermediate with an ϵ value larger than that of **1**. For the pH range studied the rate constant for the hydrolysis qualitatively remains the same. The rate for the pH range studied ranged from 0.08 to 0.13 (average 0.1) s^{-1}. Due to the inability to obtain good data fittings, the rates of hydrolysis are only semiquantitative (Figure 4). There are two reasons for this: (1) at pHs at the extremes of the range, the formation of UV absorbing intermediates begins to occur and (2) the presence of two reactive sites in the molecule C3 and C3′ results in the simultaneous decay occurring at two different rates. The latter problem is thought to be due to different reactivities of C3(C3)′ in an unreacted molecule and a half-hydrolyzed molecule.

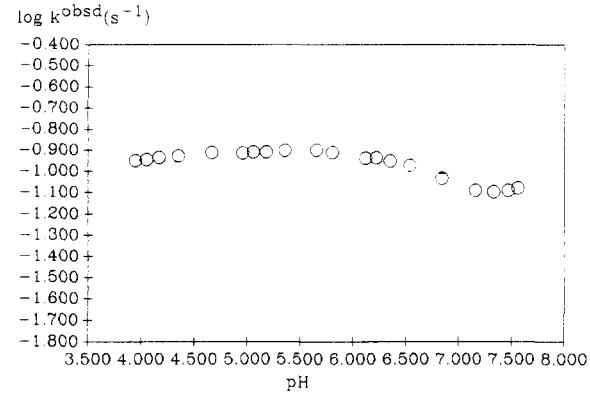


Figure 4. Plot of log k^{obsd} vs pH for reagent 1.

The reaction of **1** with diethylamine at 25.0 °C (μ = 0.1, NaCl) was carried out a pH 7.4 in phosphate buffer (0.013 M) and was monitored by observing the UV absorbance at 285 nm, which corresponds to the formation of **4c**. The data fitting was poor, which is probably due to the presence of two reactive sites in **1**, both C3 and C3′. Thus the rate of formation of the intermediate enol–ether–enamine most

(13) (a) Esters of amino acids were employed for simplicity of workup. (b) Prager, B.; Jacobson, P. *Beilsteins Handbuch Der Organischen Chemie*, 4th ed.; Julius Springer: Berlin, Germany, 1922; vol. 4, p 340.

(14) Lister, J. H., *Fused Pyrimidines, Part II: Purines*; Brown, D. J., Ed.; Wiley-Interscience: New York, 1971; pp 489–490.

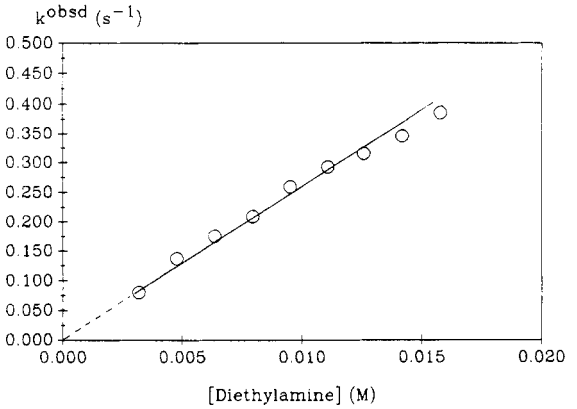


Figure 5. Rate of reaction of 1 with diethylamine vs diethylamine concentration at pH = 7.40.

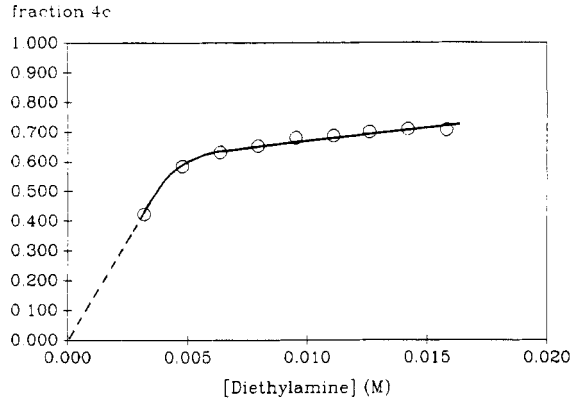
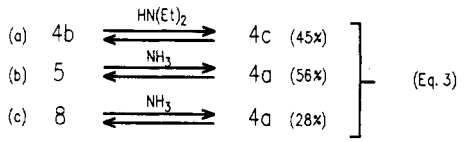


Figure 6. Fraction of 1 partitioning to 4c vs diethylamine concentration.

likely proceeds at a different rate than the formation of the bis-enamine 4c from this intermediate. By varying the concentration of the diethylamine, it was found that the rate is linearly dependent on its concentration (Figure 5). If the extinction coefficient for 4c in the above buffer (4.3×10^4 cm^{-1} M^{-1}) is utilized as well as the absorbance at the infinity point for each reaction, it is possible to obtain a rough idea of the fraction of 1 that partitions to the product 4c in aqueous solution (Figure 6). Thus, about 50% of 1 reacts with diethylamine at a diethylamine concentration of 0.004 M. From Figure 4, the average hydrolysis rate is 0.1 s^{-1}. The concentration of diethylamine that provides a corresponding rate of formation of enamine is about 0.004 M (Figure 5), which is the same as the concentration needed to provide a 50% reaction of 1 with diethylamine (Figure 6). Taking into consideration the large concentration of water (55.5 M) in comparison with that the amine (0.004 M), it can be concluded that the rate of reaction of 1 with diethylamine, and possibly with other amines as well, in aqueous medium is significantly faster than the rate of hydrolysis.

(f) Reactivity of 1 and the Bis-enamines. For the thorough assessment of the candidacy of 1 for a biological cross-linking reagent, a few more key questions need to be answered: (1) How electrophilic is reagent 1? Would it react with amides? If it does, the use of 1 as a cross-linking reagent for proteins would be limited, given the amide backbone of proteins. (2) What are the chemical properties of bis-enamines 4? Will they react, as do regular enamines, with electrophiles from their β-carbons? Would they undergo exchange reactions with other amines? As they exhibit greater aqueous stability, could they be better choices as cross-linking reagents than 1? (3) What are the products of hydrolysis of 1 and 4 in neutral, basic, and acidic pH? The answer to question 1 was a pleasing "no" for amides when 1 failed to react with acetamide in refluxing acetonitrile for 8 h. While 1 did react with aniline to give **4k** (vide supra) in excellent yield, it failed to react with the less basic and more bulky diphenylamine even at reflux for 20 h. Seeking an answer for question 2, we reacted **4b** with several common electrophiles, e.g. $POCl_3$, CH_3I, CH_3COCl, $ClCH_2CHO$, or $ClCH_2C{\equiv}N$. No reaction could be detected with any of the electrophiles even at elevated temperatures. Similarly, an attempted reaction of **4a** with either glyoxal or chloroacetaldehyde failed to show any product formation. Therefore, 4 does not behave as a nucleophile. The implied electrophilic nature, corroborated with the implied partial positive charge on the enamine nitrogens as discussed above, prompted us to probe the amine exchangeability in 4. Indeed, compound **4b**, upon incubation with excess diethylamine in acetonitrile for several days, produced **4c** in 45% yield (eq 3a).

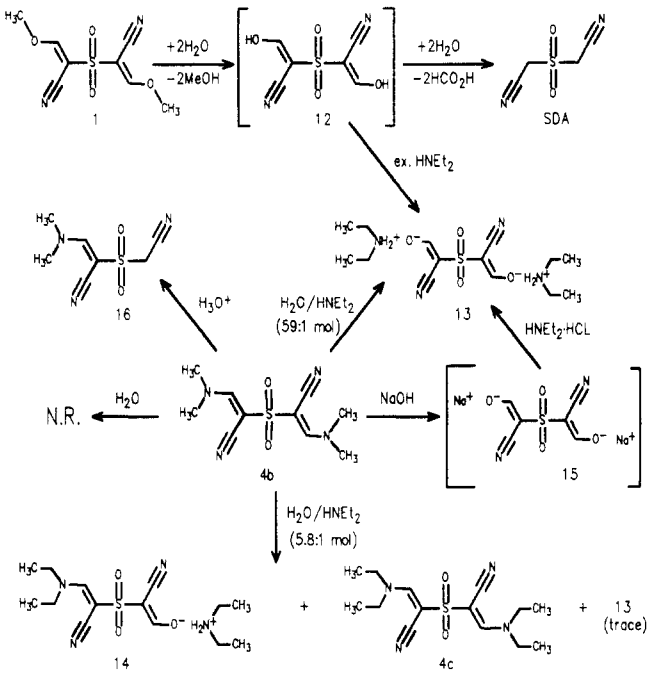


The reaction of **5** with ammonia to produce **4a** proceeded much faster and with a higher yield (56%) (eq 3b). The latter reaction is noteworthy as the reactant **5** possesses, in addition to the electrophilic 3(3′)-CH junctions, two ester carbonyls as potential targets for nucleophilic attack by ammonia. However, under the ambient reaction temperature employed, we did not detect the formation of the corresponding amides. Interestingly, compound **8** also underwent amine exchange with ammonia to give **4a** in 28% yield (eq 3c). Thus, the bis-enamines can potentially undergo amine exchange reactions with amino acids (proteins) and heterocyclic bases (nucleic acids), and so, they are also viable candidates as biological cross-linking reagents; albeit less reactive, they have the advantage of being more stable than 1. In this context, while the acyclic products such as **4a–c** may be preferred for the cross-linking of proteins, enamines such as **4d,e,k**, **8**, or **10**, which are equipped with potentially intercalatable aromatic/heteroaromatic rings, may be more appropriate for nucleic acids.

To answer question 3 above concerning hydrolysis, the reaction of 1 in 5 M aqueous acetonitrile at room temperature was carefully monitored by TLC [silica gel, $CHCl_3$–MeOH (5:1)] at regular intervals. Within a few minutes, two new—intensely UV-absorbing and lower R_f—spots were detected, which gradually started fading away. All attempts to isolate these intermediates only afforded the UV-transparent but I_2-stainable 2,2′-sulfonyldiacetonitrile (SDA), which was also found to be the final product of hydrolysis. Consistent with the mechanism proposed for the reaction of 1 with amines (see Scheme I), one of the speculated intermediates was the bis-enol (**12**) (Scheme III). Intermediate **12** was ultimately trapped as its bis-diethylammonium salt (**13**) and its structure was confirmed by single-crystal X-ray analysis (Figure 1e).

The bis-enamines 4, which exhibit considerable aqueous stability, were further scrutinized for hydrolysis under basic and acidic reaction conditions. When **4b** was treated with a mixture of water and diethylamine in various ratios of molar concentrations at room temperature, the product formed was one or a combination of **13**, **14**, and **4c** (Scheme III). Since water alone failed to react with **4b** even after a ~7-day period, the formation of **13** could only be ex-

Scheme III



plained by invoking a nucleophilic attack by the stronger hydroxide ion, present in small amounts in the equilibrium mixture of water and diethylamine, or by general base catalysis. Indeed, a separate reaction of **4b** with aqueous NaOH readily gave **15**, which was conveniently trapped as **13** by treatment with diethylammonium hydrochloride. The acid hydrolysis of **4b**, on the other hand, was less facile. While **4b** resisted hydrolysis in 0.4 M sulfuric acid at ambient temperature, the reaction occurred upon heating to produce **16** as the major product. Finally, as an example of a cross-linked nucleic acid, the hydrolysis of bis-enamine **11** was explored with 2 N HCl at room temperature (eq 4). The reaction mixture, as monitored

$$\mathbf{11} \xrightarrow{H_3O^+} \text{Cytidine} + \text{SDA} + HCO_2H \quad \text{(Eq. 4)}$$

by ^{1}H NMR spectroscopy, indicated gradual formation of cytidine and formic acid. This conclusion was corroborated by the observation of peak enhancement with external addition of authentic products. These species, along with SDA, were positively identified in the residue left after evaporation of the reaction mixture. The isolated products are consistent with the intermediacy of the bis-enol intermediate **12** in the acidic hydrolysis of **11** and possibly in that of bis-enamines **4** as well.

(g) Reaction of 1 with Hemoglobins. We have recently demonstrated[1b] that cell-free hemoglobins can be cross-linked with **1** under both oxygenated and deoxygenated conditions. While isoelectric focusing (IEF) revealed the presence of modified hemoglobins, sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) confirmed the formation of covalently cross-linked products corresponding to dimers of hemoglobin subunits at molecular weights of 29–30 ± 4 kDa. Nevertheless, the presence of many modified hemoglobins in IEF pointed to the reagent's lack of specificity. The design of a more targeted reagent that has increased affinity for the 2,3-diphosphoglycerate (2,3-DPG) pocket of hemoglobin—an anionic "sink" surrounded by positively charged lysine residues[15]—may involve replacement of the neutral nitrile groups of **1** with the negatively charged carboxylate groups,[16] and such an endeavor is currently in progress.

(15) Zubay, G. *Biochemistry*; Addison-Wesley Publishing Co., Inc.: London, 1986; pp 111–117.

Conclusions

We have synthesized a novel, highly electrophilic bifunctional cross-linking reagent which is very reactive with the building blocks of both proteins (amino acids) and nucleic acids (heterocyclic bases/nucleosides). We have also explored its structure, properties, and reactions to assess its suitability as a biological cross-linking agent. The reagent is easy to synthesize, is stable in storage, and is capable of reacting in a predominantly aqueous environment. We have demonstrated the potential utility of **1** in cross-linking proteins, using hemoglobins as an example. Furthermore, we have shown that the bis-enamine products **4** may also be viable candidates for cross-linking in view of their ready amine exchangeability and high aqueous stability. The reagents **1** and **4** may be useful to cross-link other proteins, nucleic acids, or proteins with nucleic acids. We are currently investigating all these possibilities while also making structural modifications in these reagents to suit the cross-linking requirements of specific biological molecules in question.

Experimental Section

^{1}H and ^{13}C NMR spectra were recorded at 80 or 500 and 125 MHz, respectively, using DMSO-d_6 unless otherwise indicated. Apparent multiplicity is designated by the abbreviation, ap = apparent). The multiplicity of ^{13}C NMR peaks is based on ^{1}H decoupled off-resonance spectra. Electron impact (EI) mass spectra were recorded at 70 eV. Melting points are uncorrected. X-ray crystal structure analyses were performed at the Department of Chemistry and Electrical Engineering, Southern Methodist University, Dallas, TX. Lineshape measurements for dynamic ^{1}H NMR studies of **4g** were made at 500.11 MHz, equipped with a variable-temperature unit capable of 0.1 °C precision and which was calibrated for high-temperature studies utilizing ethylene glycol as a standard. The ^{1}H NMR chemical shifts were referred to Me_4Si as an internal standard. Dry solvents were prepared as follows: methanol was distilled from CaH_2 and was stored over molecular sieves; acetonitrile was distilled from CaH_2, followed by distillation from P_2O_5 and storage over molecular sieves; DMF and DMSO were distilled at reduced pressure from CaH_2 and were subsequently stored over molecular sieves. All yields reported are for dried compounds that require no further purification for use in other reactions.

2,2′-Sulfonylbis[3-methoxy-(*E*,*E*)-2-propenenitrile] (1). A solution of 2,2′-sulfonyldiacetonitrile[6] (19.8 g, 137 mmol), trimethyl orthoformate (450 mL, 4.11 mol), dry acetonitrile (300 mL), and concentrated sulfuric acid (1.6 mL) was heated at reflux under N_2 for 22 h. The reaction mixture was cooled and evaporated to dryness at ≤40 °C, which resulted in an orange residue. The latter, upon trituration with a mixture of ethyl acetate/ether (1:1, 100 mL), followed by recrystallization from xylenes, provided pure **1** (14–15.6 g, 45–50%): mp 207–209 °C; ^{1}H NMR δ 8.37 (s, 2 H), 4.24 (s, 6 H); ^{13}C NMR δ 176.27 (d), 110.46 (s), 94.76 (s), 65.63 (q); IR (KBr) 3030 (=CH), 2250 (CN), 1590 (C=C, 1370, 1170 (SO_2) cm^{-1}; UV (MeOH) λ_{max} 248 nm.

Anal. Calcd for $C_8H_8N_2O_4S$: C, 42.10, H, 3.53; N, 12.27. Found: C, 42.43; H, 3.67; N, 12.05.

General Procedure for the Preparation of the Bis-enamines (4). A mixture of **1** (1.0 g, 4.39 mmol) and the appropriate amine (2–20-fold molar equiv) in dry methanol (**4a–c,f,g**) or dry acetonitrile (**4d,e,h–k**) (5–10 mL of solvent/mmol of **1**) was stirred at room temperature under anhydrous conditions (N_2 or $CaCl_2/CaSO_4$ guard tube) for 15 min to 24 h, depending upon completion of the reaction as monitored by TLC. The reaction

(16) (a) *Advances in Blood Substitute Research*; Bolin, R. B., Beyer, R. P., Nemo, G. J., Eds.; Alan R. Liss, Inc.: New York, 1983. (b) Miller, I. F. Synthetic Blood Substitutes: Where Are We and Where Do We Go from Here? In *CRC Crit. Rev. Bioeng.* **1978**, 149–177.

solution was evaporated to dryness on a rotary evaporator, and the residue obtained was purified either by recrystallization from appropriate solvent(s), by flash chromatography, or by centrifugal thin-layer chromatography on a Chromatotron using silica gel. The purification method employed, percentage yield, melting point, and spectral data for compounds **4a–k** are listed below.

2,2′-Sulfonylbis[3-amino-(*E*,*E*)-2-propenenitrile] (4a). Method A. Reaction of 1 with NH_3 via the General Procedure. Purified by trituration of the solid residue with ethyl acetate/petroleum ether (1:1), followed by filtration, drying, and recrystallization from ethanol/water (10:1) with petroleum ether added after cooling: tiny white crystals; 93%; mp 229 °C dec; ^{1}H NMR δ 8.41 (d, 2 H, J = 16 Hz, ex. with D_2O), 8.31 (d, 2 H, J = 8.5 Hz, ex. with D_2O), 7.61 (dd, 2 H, J = 16 Hz, J = 8.5 Hz); ^{13}C NMR δ 155.66 (d), 114.01 (s), 82.95 (s); IR (KBr) 3430, 3395, 3340, 3270, 3230 (NH_2), 3075 (=C—H), 2200 (CN), 1660 (C=C) cm^{-1}; mass spectrum, m/e 198 (M^+, 89), 131 (70); UV (MeOH) λ_{max} 275 nm.

Anal. Calcd for $C_6H_6N_4O_2S$: C, 36.36; H, 3.05; N, 28.27; S, 16.18. Found: C, 36.28; H, 3.07; N, 28.19; S, 16.12.

Method B. Reaction of 5 with NH_3. Ammonia gas was bubbled through a solution of **5** (210 mg, 0.61 mmol) in dry acetonitrile (20 mL) for 5 min. The homogeneous solution was stirred at room temperature for 2 h and then carefully evaporated to dryness on a rotary evaporator to yield an orange residue. The majority of the residue was dissolved in a mixture of methanol–acetone (1:2) and was filtered to remove insoluble material. This solution was loaded onto a dry Chromatotron plate made up of silica gel (Kieselgel 60 GF_{254}, thickness = 2 mm), which was subsequently eluted with a mixture of chloroform–acetone (3:2). Appropriate fractions were pooled and evaporated to afford **4a** as a solid (68 mg, 56%). The ^{1}H NMR and IR spectra for the product were identical with those of **4a** prepared by method A.

Method C. Reaction of 8 with NH_3. The procedure employed was similar to that described in method B, except that dry DMF was the solvent used in place of acetonitrile and that the reaction mixture was stirred for 6 days, replenishing NH_3 once a day. The eluting solvent system used for Chromatotron separation was chloroform–methanol (6:1). The spectral data of the product, isolated in 28% yield, are superimposable with those of **4a** obtained by method A or B above.

2,2′-Sulfonylbis[3-(dimethylamino)-(*E*,*E*)-2-propenenitrile] (4b). Method A. Reaction of 1 with Dimethylamine via the General Procedure. Purified by recrystallization from 2-propanol–water (10:1): 75%; colorless crystals; mp 188 °C; ^{1}H NMR δ 7.57 (s, 2 H), 3.23 (s, 6 H), 3.20 (s, 6 H); ^{13}C NMR δ 154.39 (d), 115.99 (s), 80.72 (s), 46.93 (q), 38.16 (q); IR (KBr) 3030 (=C—H), 2185 (CN), 1625 (C=C), 1362, 1128 (SO_2) cm^{-1}; mass spectrum, m/e 254 (M^+, 3.4), 95 (100); UV (MeOH) λ_{max} 285 nm (ε 42 300), (pH 13.7) 285 (32 200), (pH 0.2) 286 (37,300).

Anal. Calcd for $C_{10}H_{14}N_4O_2S$: C, 47.23; H, 5.55; N, 22.03; S, 12.61. Found: C, 47.31; H, 5.59; N, 21.97; S, 12.54.

Method B. Reaction of 2,2′-Sulfonyldiacetonitrile (SDA) with Dimethylformamide Dimethyl Acetal (DMF DMA). A solution of SDA (6.0 g, 41.62 mmol), DMF DMA (25 mL, 188 mmol), and trifluoroacetic acid (0.05 mL) in dry acetonitrile (150 mL) was stirred at room temperature for 30 min, followed by heating at reflux for 1^3/$_4$ h. The reaction solution was cooled and evaporated to dryness on a rotary evaporator to leave an off-white solid. This solid was recrystallized from 2-propanol–water (260 mL:20 mL) and gave white crystals (7.628 g, 72%) whose TLC and ^{1}H NMR properties are identical with those of **4b** obtained by method A.

2,2′-Sulfonylbis[3-(diethylamino)-(*E*,*E*)-2-propenenitrile] (4c). Method A. Reaction of 1 with Diethylamine via the General Procedure. Purified by recrystallization from hot ethanol: 92%; colorless crystals; mp 141–142 °C; ^{1}H NMR δ 7.55 (s, 2 H), 3.54 (m, 8 H), 1.18 (ap t, 12 H); ^{13}C NMR δ 152.38 (d), 115.59 (s), 80.36 (s), 52.13 (t), 42.97 (t), 13.66 (m); IR (KBr) 3025 (=C—H), 2980, 2940 (C—H), 2193 (CN), 1613 (C=C) cm^{-1}; UV (MeOH) λ_{max} 287 nm (ε 46 200), (pH 12.3) 287 (43 300), (pH 0.6) 287 (43 800).

Anal. Calcd for $C_{14}H_{22}N_4O_2S$: C, 54.17; H, 7.14; N, 18.05; S, 10.33. Found: C, 54.02; H, 7.16; N, 17.98; S, 10.27.

Method B. Reaction of 4b with Diethylamine. A homogeneous solution of **4b** (540 mg, 2.12 mmol) and diethylamine (25 mL, 0.24 mmol) in dry acetonitrile (25 mL) was stirred for 17 days at room temperature. The reaction solution was evaporated to dryness on a rotary evaporator to yield an orange-brown solid. This solid was dissolved in acetone, and the insoluble impurities were filtered in vacuo. The filtrate was loaded on a dry 4-mm thick silica gel (kieselgel 60 GF_{254}) Chromatotron plate. The loaded band was allowed to dry and the plate was eluted with chloroform/petroleum ether (1:1) for a few minutes, followed by elution with chloroform only. The large UV-absorbing band was collected by pooling of the appropriate fractions and evaporation to dryness on a rotary evaporator, affording **4c** (296 mg, 45%). The ^{1}H NMR spectrum of this compound is identical with that of **4c** prepared by method A above.

2,2′-Sulfonylbis[3-(pyrid-2-ylamino)-(*E*,*E*)-2-propenenitrile] (4d). Reaction of 1 with 2-Aminopyridine via the General Procedure. Purified by recrystallization from ethanol: 87%; mp >300 °C; ^{1}H NMR δ 11.61 (s, 2 H, ex. with D_2O), 8.98 (s, 2 H), 8.41 (d, 2 H, J = 4.5 Hz), 7.87 (t, 2 H, J = 8.0 Hz), 7.30 (d, 2 H, J = 8.5 Hz), 7.23 (dd, 2 H, J = 5.0 Hz, J = 7.5 Hz); IR (KBr) 3330 (NH), 3065 (=C—H), 2205 (CN), 1625 (C=C) cm^{-1}; UV (MeOH) λ_{max} 333 nm, sh 308, 265 (weak).

Anal. Calcd for $C_{16}H_{12}O_2N_6S$: C, 54.54; H, 3.43; N, 23.85; S, 9.10. Found: C, 54.46; H, 3.46; N, 23.79; S, 9.15.

2,2′-Sulfonylbis[3-(pyrimidin-2-ylamino)-(*E*,*E*)-2-propenenitrile] (4e). Reaction of 1 with 2-Aminopyrimidine via the General Procedure. Purified by dissolving in hot DMF/water (2:1), followed by cooling, and precipitation with cold water: 75%; powder; mp 247 °C (dec); ^{1}H NMR δ 12.23 (s, 2 H, ex. with D_2O), 8.91 (s, 2 H), 8.76 (d, 4 H, J = 5.0 Hz), 7.34 (t, 2 H, J = 5.0 Hz); ^{13}C NMR δ 159.10 (d), 156.49 (s), 148.54 (d), 117.96 (d), 111.93 (s), 89.13 (s); IR (KBr) 3346 (NH), 3077 (=C—H), 2205 (CN), 1630 (C=C) cm^{-1}; UV (MeOH) λ_{max} 317 nm.

Anal. Calcd for $C_{14}H_{10}N_8O_2S$: C, 47.45; H, 2.84; N, 31.62; S, 9.05. Found: C, 47.45; H, 2.87; N, 31.55; S, 8.99.

2,2′-Sulfonylbis[3-(cyclohexylamino)-(*E*,*E*)-2-propenenitrile] (4f). Reaction of 1 with Cyclohexylamine via the General Procedure. Purified by trituration with ether, followed by recrystallization from methanol: 99%; fluffy colorless crystals; mp 239–240 °C. In DMSO-d_6 solution, two isomers were detected in a 3:1 ratio as evidenced by ^{1}H NMR data: (major isomer) δ 8.81 (br, 2 H, ex. with D_2O), 7.69 (s, 2 H), 3.33 (m, 2 H), 1.0–1.8 (m, 20 H); ^{1}H NMR (minor isomer) δ 8.66 (br d, 2 H, J = 7.0 Hz, ex. with D_2O), 7.36 (d, 2 H, J = 6.0 Hz), 3.73 (m, 2 H), 1.0–1.9 (m, 20 H); IR (KBr) 3300, 3226 (NH's), 3035 (=C—H), 2932, 2855 (C—H), 2197 (CN), 1620 (C=C) cm^{-1}; mass spectrum, m/e 362 (M^+, 21), 213 (18); UV (MeOH) λ_{max} 284 nm (ε 39 400), (pH 12.4) 282 (27 600), (pH 0.6) 284 (40 700).

Anal. Calcd for $C_{18}H_{26}N_4O_2S$: C, 59.64; H, 7.23; N, 15.45; S, 8.84. Found: C, 59.56; H, 7.27; N, 15.42; S, 8.81.

2,2′-Sulfonylbis[3-(*n*-butylamino)-(*E*,*E*)-2-propenenitrile] (4g). Reaction of 1 with *n*-Butylamine via the General Procedure. Purified by recrystallization from EtOH–H_2O: 99%; colorless crystals; mp 149–150 °C. In DMSO-d_6 solution, two isomers were detected in a 10:3 ratio as evidenced by ^{1}H NMR data: (major isomer) δ 8.76 (br, 2 H, ex. with D_2O), 7.72 (s, 2 H), 3.30 (t, 4 H, J = 6.5 Hz), 1.48 (quintet, 4 H, J = 7.1 Hz), 1.27 (sextet, 4 H, J = 7.2 Hz), 0.88 (m, 6 H); ^{1}H NMR (minor isomer) δ 8.64 (br, 2 H, ex. with D_2O), 7.47 (s, 2 H), 3.43 (t, 4 H, J = 6.5 Hz), 1.55 (quintet, 4 H, J = 7.2 Hz), 1.34 (sextet, 4 H, J = 7.5 Hz), 0.90 (m, 6 H); ^{13}C NMR δ 156.47 (d), 114.53 (s), 80.92 (s), 48.57 (t), 31.98 (t), 18.84 (t), 13.45 (q); IR (KBr) 3290, 3233 (NH's), 3035 (=C—H), 2960, 2930, 2870 (C—H), 2195 (CN), 1630 (C=C) cm^{-1}; UV (MeOH) λ_{max} 282 nm (ε 37 900), (pH 12.3) 278 (26 900), (pH 0.8) 282 (39 600).

Anal. Calcd for $C_{14}H_{22}N_4O_2S$: C, 54.17; H, 7.14; N, 18.05; S, 10.33. Found: C, 54.23; H, 7.18; N, 18.02; S, 10.28.

2,2′-Sulfonylbis[3-[(2,2-dimethoxyethyl)amino]-(*E*,*E*)-2-propenenitrile] (4h). Reaction of 1 with Aminoacetaldehyde Dimethyl Acetal via the General Procedure. Purified by flash chromatography on silica gel (40–63 μm), using $CHCl_3$–acetone (9:1) as an eluting solvent system: 78%; off-white crystals upon trituration with ether; mp 104–106 °C. In DMSO-d_6 solution, the presence of two isomers were detected in a 5:1 ratio by ^{1}H NMR data: (major isomer) δ 8.6–8.9 (br m, 2 H, ex. with D_2O), 7.69 (d, 2 H, J = 14.6 Hz), 4.38 (t, 2 H, J = 5.0 Hz), 3.2–3.5 (m, 16 H); ^{1}H NMR (minor isomer) δ 8.6–8.9 (br m, 2 H, ex. with D_2O),

7.52 (d, 2 H, J = 8.4 Hz), 4.57 (t, 2 H, J = 5 Hz), 3.60 (m, 4 H), 3.35 (m, 12 H); IR (KBr) 3295, 3235 (NHs), 3045 (=C—H), 2210 (CN), 1635 (C=C) cm^{-1}; UV (MeOH) λ_{max} 283.5 nm.

Anal. Calcd for $C_{14}H_{22}N_4O_6S$: C, 44.91; H, 5.92; N, 14.96; S, 8.56. Found: C, 44.86; H, 5.94; N, 14.91; S, 8.63.

2,2′-Sulfonylbis[3-[(2,3-*O*-isopropylidene-D-ribofuranosyl)amino]-(*E*,*E*)-2-propenenitrile] (4i). Reaction of 1 with 2,3-*O*-Isopropylidene-D-ribofuranosylamine via the General Procedure. Purified by flash chromatography on silica gel (40–63 μm), employing $CHCl_3$–acetone (9:1) as an eluting solvent: 36%; off-white foam; ^1H NMR δ 8.8–9.5 (br m, NH, ex. with D_2O), 8.45 (s, =CH), 8.1 (br m, NH, ex. with D_2O), 8.05 (s, =CH), 7.95 (s, =CH), 7.75 (s, =CH), 6.25 (d, J = 5.5 Hz, anomeric CH), 5.45 (d, J = 4.0 Hz, anomeric CH), 4.2–5.2 (m, ribose CH's + OH), 4.1 (br s, ribose CH_2), 3.5 (br s, ribose CH_2), 1.44 (s, CH_3), 1.28 (s, CH_3); IR (KBr) 3700–3100 (br), 2200 (CN), 1615 (C=C) cm^{-1}.

Anal. Calcd for $C_{22}H_{30}N_4O_{10}S$: C, 48.70; H, 5.57; N, 10.30; S, 5.91. Found: C, 48.48; H, 5.67; N, 10.16; S, 5.91.

2,2′-Sulfonylbis[3-(*N*-ethyl-*N*-methylamino)-(*E*,*E*)-2-propenenitrile] (4j). Reaction of 1 with *N*-Ethylmethylamine via the General Procedure. Purified by chromatography on a Chromatotron plate of silica gel (kieselgel 60 GF_{254}, 2 mm thick), using $CHCl_3$–acetone (9:1) as an eluting solvent system, followed by recrystallization from ethanol: 84%; fine colorless crystals; mp 130–132 °C. In DMSO-d_6 solution, the presence of two isomers in a 9:5 ratio was detected by ^1H NMR data: (major isomer) δ 7.60 (s, 2 H), 3.50 (q, 4 H, J = 7.0 Hz), 3.20 (s, 6 H), 1.16 (t, 6 H, J = 7.2 Hz); ^1H NMR (minor isomer) δ 7.55 (s, 2 H), 3.56 (q, 4 H, J = 7.2 Hz), 3.24 (s, 6 H), 1.19 (t, 6 H, J = 7.2 Hz); ^{13}C NMR (major isomer) δ 153.47 (d), 115.95 (s), 80.91 (s), 54.42 (t), 35.92 (q), 13.52 (q); ^{13}C NMR (minor isomer) δ 153.36 (d), 115.63 (s), 80.08 (s), 45.14 (t), 44.45 (q), 12.12 (q); IR (KBr) 2192 (CN), 1612 (C=C), 1363, 1132 (SO_2) cm^{-1}; mass spectrum, m/e 282 (M^+, 36).

Anal. Calcd for $C_{12}H_{18}N_4O_2S$: C, 51.04; H, 6.42; N, 19.84; S, 11.36. Found: C, 51.08; H, 6.43; N, 19.79; S, 11.29.

2,2′-Sulfonylbis[3-anilino-(*E*,*E*)-2-propenenitrile] (4k). Reaction of 1 with Aniline via the General Procedure. Purified by recrystallization from acetonitrile: 82%; white crystals; mp 261–263 °C; ^1H NMR δ 10.96 (br d, 2 H, J = 12.3 Hz, ex. with D_2O), 8.13 (m, 2 H, becomes s with D_2O), 7.5–7.1 (m, 10 H); IR (KBr) 2200 (C≡N), 1630, 1587 (C=C); UV (MeOH) λ_{max} 332, 284, 218.5 nm.

Anal. Calcd for $C_{18}H_{14}N_4O_2S$: C, 61.70; H, 4.03; N, 15.99; S, 9.15. Found: C, 61.68; H, 4.06; N, 15.94; S, 9.17.

General Procedure for the Reaction of 1 with Amino Acid Esters. A homogeneous solution of 1 in dry methanol or acetonitrile and an appropriate amino acid methyl ester (2–3 molar equiv), freshly liberated from the corresponding HCl salt with Ag_2O/MeOH,[13b] was stirred at room temperature under N_2 atmosphere for 70 min to 3 h, depending upon completion of the reaction as detected by TLC. The reaction mixture was evaporated to dryness, and the residue was purified by either flash chromatography on silica gel (40–63 μm), using $CHCl_3$–acetone (6:1) at 3–4 psi, or by reverse-phase flash chromatography on a C_{18} column, using MeOH–H_2O (1:1/2:1) as the eluting solvent system. The purified material was recrystallized, where applicable, from the appropriate solvent(s). Method of purification, percentage yields, melting points, and spectral data for compounds 5–7 are listed below.

2,2′-Sulfonylbis[3-[[(methoxycarbonyl)methyl]amino]-(*E*,*E*)-2-propenenitrile] (5). Reaction of 1 with Glycine Methyl Ester via the General Procedure. Purified by flash chromatography, followed by recrystallization from acetone–ligroin: 56%; mp 197–199 °C. In DMSO-d_6 solution, the presence of two isomers of 5, in a 13:5 ratio, was detected by ^1H NMR data: (major isomer) δ 8.6–9.0 (br, 2 H, ex. with D_2O), 7.83 (d, 2 H, J = 14.4 Hz), 4.22 (d, 4 H, J = 5.4 Hz), 3.69 (s, 6 H); ^1H NMR (minor isomer) δ 8.6–9.0 (br, 2 H, ex. with D_2O), 7.76 (d, 2 H, J = 13.6 Hz), 4.36 (d, 4 H, J = 5.3 Hz), 3.71 (s, 6 H); ^{13}C NMR (major isomer) δ 169.72 (s), 158.20 (d), 113.83 (s), 82.68 (s), 52.11 (q), 48.77 (t); IR (KBr) 3300, 3245 (NH's), 2205 (CN), 1755 (CO_2Me), 1635 (C=C) cm^{-1}; UV (MeOH) λ_{max} 280.5 nm.

Anal. Calcd for $C_{12}H_{14}N_4O_6S$: C, 42.10; H, 4.12; N, 16.37; S, 9.36. Found: C, 42.15; H, 4.16; N, 16.35; S, 9.31.

2,2′-Sulfonylbis[3-[[5-amino-5-(methoxycarbonyl)pent-1-yl]amino]-(*E*,*E*)-2-propenenitrile] (6). Reaction of 1 with Lysine Methyl Ester via the General Procedure. Purified by reverse flash chromatography: light brown glass; 36%. In DMSO-d_6 solution, the presence of two isomers with a 7:2 ratio were detected by ^1H NMR: (major isomer) δ 8.7 (br, 2 H, ex. with D_2O), 7.73 (s, 2 H), 4.1 (br, 4 H, ex. with D_2O), 3.63 (s, 6 H), 3.5–3.1 (m, 6 H), 1.9–1.1 (m, 12 H); (minor isomer) δ 8.7 (br, 2 H, ex. with D_2O), 7.50 (s, 2 H), 4.1 (br, 4 H, ex. with D_2O), 3.70 (s, 6 H), 3.5–3.1 (m, 6 H), 1.9–1.1 (m, 12 H); IR (KBr) 2195 (CN), 1735 (CO_2Me), 1625 (C=C) cm^{-1}.

Anal. Calcd for $C_{20}H_{32}N_6O_6S$: C, 49.57; H, 6.66; N, 17.34; S, 6.62. Found: C, 49.18; H, 6.37; N, 17.77; S, 6.85.

2,2′-Sulfonylbis[3-[[2-hydroxy-1-(methoxycarbonyl)-ethyl]amino]-(*E*,*E*)-2-propenenitrile] (7). Reaction of 1 with Serine Methyl Ester via the General Procedure. Purified by flash chromatography: 53%; foam or glass. In DMSO-d_6 solution, the presence of two isomers of 7, in a 4:1 ratio, was detected by ^1H NMR data: (major isomer) δ 8.76 (br, 2 H, ex. with D_2O), 7.86 (d, 2 H, J = 13 Hz), 5.23 (t, 2 H, J = 5.2 Hz, ex. with D_2O), 4.48 (m, 2 H), 3.77 (m, 4 H), 3.69 (s, 6 H); ^1H NMR (minor isomer) δ 8.91 (br, 2 H, ex. with D_2O), 7.60 (d, 2 H, J = 8.0 Hz), 5.44 (t, 2 H, ex. with D_2O), 4.73 (m, 2 H), 3.86 (m, 4 H), 3.72 (s, 6H); IR (KBr) 2200 (CN), 1740 (CO_2Me), 1623 (C=C) cm^{-1}.

Anal. Calcd for $C_{14}H_{18}N_4O_8S$: C, 41.79; H, 4.51; N, 13.92; S, 7.97. Found: C, 41.71; H, 4.55; N, 13.86; S, 7.89.

General Procedure for the Reaction of 1 with Nucleic Acid Bases and Nucleosides. A homogeneous solution of 1 and dry nucleic acid base/nucleoside (2 molar equiv) in dry DMSO (4–5 mL/mmol of base or 2–3 mL/mmol of nucleoside used) was stirred at room temperature for 19–24 h. Enough distilled water (nucleic acid bases) or 2-propanol (nucleosides) was added to the reaction solution to cause complete precipitation. The solid that separated was filtered in vacuo using a fine porous sintered-glass funnel and was dried in a drying pistol overnight. Further purification was effected by dissolving the compound in DMF followed by reprecipitating it with either H_2O (bases) or 2-propanol (nucleosides). Percentage yields, melting points, and spectral data for compounds 8–11 are listed below.

2,2′-Sulfonylbis[3-(9*H*-purin-6-ylamino)-(*E*,*E*)-2-propenenitrile] (8). Reaction of 1 with adenine via the general procedure: yellow solid; 89%; mp >290 °C; ^1H NMR δ 13.7 (br, 2 H, ex. with D_2O), 9.58 (br s, 2 H, sharp with D_2O), 8.67 (s, 2 H), 8.60 (s, 2 H); IR (KBr) 2215 (CN), 1645 (C=C) cm^{-1}; UV (MeOH–H_2O, 9:1) λ_{max} 341.5 nm (ε 44 600), (pH 12.5) 345.5, 383; (pH 1) 278, 332.

Anal. Calcd for $C_{16}H_{10}N_{12}O_2S \cdot 1^1/_2H_2O$: C, 41.65; H, 2.84; N, 36.43; S, 6.95. Found: C, 41.66; H, 2.88; N, 36.34; S, 6.99.

2,2′-Sulfonylbis[3-[(9-β-D-ribofuranosyl-9*H*-purin-6-yl)-amino]-(*E*,*E*)-2-propenenitrile] (9). Reaction of 1 with adenosine via the general procedure: yellow solid; 60%; 170 °C dec; ^1H NMR δ 12.6 (br, 2 H, ex. with D_2O), 9.56 (br s, 2 H, sharp with D_2O), 8.87 (s, 2 H), 8.73 (s, 2 H), 6.05 (d, 2 H, J = 5.3 Hz), 4.75 (br, 6 H, ex. with D_2O), 4.61 (t, 2 H, J = 5.0 Hz), 4.21 (t, 2 H, J = 4.1 Hz), 4.00 (m, 2 H, J = 3.6 Hz), 3.66 (m, 4 H); ^{13}C NMR δ 152.30, 151.49, 148.49, 143.97, 121.97, 112.07, 91.44, 87.93, 85.78, 73.98, 70.24, 62.07, 61.19; IR (KBr) 3065 (=C—H), 2212 (CN), 1635 (C=C) cm^{-1}; UV (MeOH) λ_{max} 341 nm, (pH 11.2) 253.5, 345, 392, (pH 0.1) 259, 339.5.

Anal. Calcd for $C_{26}H_{26}N_{12}O_{10}S \cdot H_2O$: C, 43.58; H, 3.94; N, 23.45; S, 4.47. Found: C, 43.37; H, 3.98; N, 23.33; S, 4.41.

2,2′-Sulfonylbis[3-[(2-oxo-1*H*-pyrimidin-4-yl)amino]-(*E*,*E*)-2-propenenitrile] (10). Reaction of 1 with cytosine via the general procedure: yellow solid; 85%; mp >300 °C; ^1H NMR δ 11.75 (br, 2 H, ex. with D_2O), 8.75 (br s, 2 H, sharp with D_2O), 7.88 (d, 2 H, J = 6.8 Hz), 6.29 (d, 2 H, J = 6.8 Hz); IR (KBr) 2215 (CN) cm^{-1}.

Anal. Calcd for $C_{14}H_{10}N_8O_4S \cdot 1^1/_4H_2O$: C, 41.13; H, 3.08; N, 27.40; S, 7.84. Found: C, 41.20; H, 3.08; N, 27.30; S, 7.79.

2,2′-Sulfonylbis[3-[(1-β-D-ribofuranosyl-2-oxo-1*H*-pyrimidin-4-yl)amino]-(*E*,*E*)-2-propenenitrile] (11). Reaction of 1 with cytidine via the general procedure: yellow solid; 58%; mp 185 °C dec; ^1H NMR δ 11.8 (br, 2 H, ex. with D_2O), 8.77 (br s, 2 H, sharp with D_2O), 8.50 (d, 2 H, J = 7.3 Hz), 6.41 (d, 2 H, J = 7.2 Hz), 5.78 (d, 2 H, J = 1.4 Hz), 5.7–4.4 (br, 6 H, ex.

with D_2O), 3.96 (s, 6 H), 3.70 (m, 4 H); IR (KBr) 3085 (=C—H), 2210 (CN) cm^{-1}.

Anal. Calcd for $C_{24}H_{26}N_8O_{12}S \cdot 1^1/_4H_2O$: C, 42.83; H, 4.27; N, 16.65; S, 4.76. Found: C, 43.17; H, 4.31; N, 16.24; S, 4.74.

Bis(diethylammonium salt) of 2,2′-Sulfonylbis[3-oxido-(*E*,*E*)-2-propenenitrile] (13). Method A. Preparation from 4b. A solution of **4b** (546 mg, 2.145 mmol), and diethylamine (3 mL, 29 mmol) in distilled H_2O (30 mL, 1.7 mol) was stirred at room temperature for 4 days. The reaction solution was evaporated to dryness on the rotary evaporator, yielding a crystalline solid mixed with a yellow viscous liquid. Toluene was added several times and the mixture was repeatedly evaporated to dryness. The residue was triturated with petroleum ether/acetonitrile, and the resultant white crystalline solid was collected by filtration. A second crop of crystals was obtained from the filtrate after it was placed in a freezer overnight. The total yield was 401 mg (54%). The compound can be recrystallized from hot acetonitrile–hexane into colorless crystals: mp 139–141 °C; ^1H NMR δ 8.70 (s, 2 H), 8.3 (br, 2 H, ex. with D_2O), 3.3 (br, 2 H, ex. with D_2O), 2.94 (q, 8 H, J = 7.3 Hz), 1.16 (t, 12 H, J = 7.2 Hz); ^{13}C NMR δ 178.97 (d), 119.53 (s), 87.21 (d), 41.37 (t), 10.98 (q); IR (KBr) 2190 (CN) cm^{-1}; UV (MeOH) λ_{max} 262 nm.

Anal. Calcd for $C_{14}H_{26}N_4O_4S$: C, 48.54; H, 7.56; N, 16.17. Found: C, 48.52; H, 7.60; N, 16.16.

Method B. Preparation from 1. A homogeneous solution of **1** (209 mg, 0.92 mmol) and distilled H_2O (2 mL, 111 mmol) in acetonitrile (5 mL) was stirred at room temperature for 90 min. After this period, diethylamine (3 mL, 29 mmol) was added to the reaction solution, and this homogeneous solution was stirred for an additional 42 h at room temperature. The reaction solution was evaporated to dryness on a rotary evaporator, followed by repeated coevaporations with toluene, yielding a crystalline solid suspended in a viscous yellow liquid. Trituration with petroleum ether–acetonitrile and filtration afforded **13** (167 mg, 52%). The melting point and ^1H NMR data for this compound were identical with those of **13** obtained by method A.

Method C. Preparation from 4b. A solution of **4b** (366 mg, 1.44 mmol) in 0.38 M NaOH (10.4 mL, 4 mmol) was stirred for 24 h. TLC no longer revealed any starting material, but only a slow-moving UV absorbing spot (R_f = 0.09 in $CHCl_3$–MeOH, 2:1), with several faint impurities. No change in TLC was apparent even after stirring the solution for a week. A solution of diethylamine hydrochloride (3.29 g, 30 mmol) in 10 mL of water was added. After 1 h the solution was evaporated to dryness on a rotary evaporator with small additions of toluene to azeotropically remove any remaining water. The yellow residue was triturated with MeOH, and the crystalline NaCl which separated was filtered out. The methanolic filtrate was evaporated to a small volume, and 12 mL of $CHCl_3$ was added. This was loaded onto a dry SiO_2 Chromatotron plate (2 mm thickness, kieselgel 60 GF_{254}). The plate was eluted with a mixture of $CHCl_3$–MeOH in a step gradient of increasing polarity: 9:1, 5:1, 4:1, 2:1. The appropriate fractions containing the compound of interest (R_f = 0.12 in $CHCl_3$–MeOH, 2:1) were combined and were evaporated to dryness on a rotary evaporator. Trituration of the residue with CH_3CN–petroleum ether yielded a white crystalline solid 257.3 mg (52%): mp 138–140 °C. The IR spectrum of the material was identical with that prepared from methods A and B above.

Diethylammonium Salt of 3′-(Diethylamino)-3-oxido-2,2′-sulfonyl-(*E*,*E*)-2,2′-dipropenenitrile (14). Reaction of 4b with Aqueous Diethylamine. A solution of **4b** (700 mg, 2.75 mmol) and diethylamine (35 mL, 0.34 mol) in distilled water (35 mL, 1.94 mol) was stirred at room temperature for 90 h. TLC (SiO_2) revealed the presence of **4c**, **13**, and a new UV-absorbing compound with an intermediate R_f. The reaction solution was evaporated to dryness on the rotary evaporator, followed by coevaporation with toluene. The resulting orange gum was dissolved in acetone (40 mL) and was evaporated onto flash silica gel (40–63 μm, 6.0 g). Chloroform was added, and the resulting slurry was loaded onto a column packed with flash silica gel (40–63 μm, 60 g) in chloroform. The column was eluted with chloroform–methanol (9:1) at 3–4 psi. The first UV-absorbing compound was obtained, after evaporation of the appropriate pooled fractions on the rotary evaporator, as a yellow solid, which was recrystallized from ethanol, yielding colorless crystals of **4c** (64 mg, 8%). The ^1H NMR spectrum of the latter solid matched that of **4c** prepared from **1** (see above). A second, slower moving, UV-absorbing compound **14** was obtained after evaporation of the appropriate pooled fractions on the rotary evaporator as an orange gum which solidified after several hours in the refrigerator (310 mg, 41%). The amount of **13** present was too small to be isolated. Compound **14** can be recrystallized from cold acetone–ether, yielding small cubic crystals: mp 99–100 °C; ^1H NMR δ 8.81 (s, 1 H), 8.15 (br, 2 H, ex. wtih D_2O), 7.42 (s, 1 H), 3.45 (q, 4 H, J = 7.2 Hz), 2.92 (q, 4 H, J = 7.2 Hz), 1.15 (t, 12 H, J = 7.2 Hz); IR (KBr) 2180 (CN), 1612 (C=C) cm^{-1}.

Anal. Calcd for $C_{14}H_{24}N_4O_3S$: C, 51.20; H, 7.37; N, 17.06; S, 9.76. Found: C, 51.31; H, 7.43; N, 17.05; S, 9.68.

(*E*)-2-[(Cyanomethyl)sulfonyl]-3-(diethylamino)-2-propenenitrile (16). Acidic Hydrolysis of 4b. A solution of **4b** (2.0 g, 7.87 mmol) and concentrated H_2SO_4 (2.5 mL, 46.9 mmol) in distilled H_2O (125 mL) was heated to a gentle reflux for 40 min. The reaction solution was cooled in an ice bath and was carefully neutralized with solid Na_2CO_3. A white crystalline solid, which came out of solution, was collected by filtration and washed with ether. The crystalline solid was recrystallized from water (30 mL) (883 mg, 56%): mp 120–122 °C; ^1H NMR δ 7.65 (s, 1 H), 4.92 (s, 2 H, ex. with D_2O), 3.28 (s, 6 H); IR (KBr) 3038 (=C—H), 2990, 2940 (C—H), 2255, (CH_2CN), 2200 (CN), 1640 (C=C) cm^{-1}; mass spectrum, m/e 199 (M^+, 37), 159 (100), 95 (69).

Anal. Calcd for $C_7H_9N_3O_2S$: C, 42.20; H, 4.55; N, 21.09; S, 16.09. Found: C, 42.29; H, 4.57; N, 21.03; S, 16.02.

Cross-Linking Studies of 1 with Deoxy- and Oxyhemoglobins (HbA_o). Please refer to ref 1b for experimental details.

Procedure for Lineshape Analysis of 4g. The ^1H NMR lineshapes were simulated by utilizing the GEMXCH program of the General Electric GN-Series software. The spectral parameters used for the simulation included the frequencies of the two sites. Actual frequencies (Hz) were input except for $t \geq 92.0$ °C where deviation of a plot of frequencies vs temperature from linearity required that extrapolated values be used. The average of the line widths from the region t = 29.6–53.6 °C were used for each simulation. The populations obtained from peak area integrations were linear until 44.0 °C, and extrapolation was necessary after this point. The averages of the values up to t = 44.0 °C and the extrapolated values above were the initial populations used ($P_{4g(t)}/P_{4g(c)}$ = 0.69/0.31). The exchange constant τ was varied until the simulated lineshapes gave the lowest possible root mean square (RMS) deviation from the experimental spectra. The compound **4g** was studied in DMSO-d_6 with a drop of D_2O added in order to allow exchange of the NH protons adjacent to the vinylic protons, thereby causing the latter protons to appear as singlets rather than doublets. The two types of vinylic protons appeared as sharp singlets in the stopped-exchange region near room temperature. The temperature ranged from 29.6 to 120.7 °C (at $\simeq$5 °C increments). The temperature range utilized in determining the parameters was greater than the temperature range of the exchange region lineshape simulations.

The more populated site had a chemical shift which varied linearly with temperature in the stopped-exchange region whereas the less populated site had a chemical shift independent of temperature in the stopped-exchange region.

In order to calculate the rate constant for the forward isomerization it was necessary to use the equation $\tau = P_{4g(c)}/k_f = P_{4g(t)}/k_r$ where k_f and k_r are the rates of the forward and reverse reactions, respectively. $P_{4g(c)}$ and $P_{4g(t)}$ are the relative populations of the cis and trans isomers, respectively. The average τ values for the four sets of data and $P_{4g(c)}$ = 0.31 were used to calculate the k_f's. Once the rate constant for the forward reaction was obtained, an Arrhenius plot or a plot of ln k_f versus $1/T$(K) provided a means of estimating the activation barrier for the forward reaction. The slope of the plot is $-E_a/R$ where R is the gas constant and E_a is the activation barrier. The rate of the forward reaction of 25 °C was determined by extrapolation of the Arrhenius plot back to $1/T = 1/298$.

The experimental, extrapolated, and simulated data employed in lineshape analysis of compound **4g** are given as supplementary material.

Kinetic Procedure. The hydrolysis of **1** was monitored at 246 nm in a Gilford Response spectrophotometer. Immediately before use, **1** (11–13 mg, 0.048–0.057 mmol) was dissolved in 5.00

mL of freshly distilled methanol. With the use of a hollow glass stirring rod that was bent up at one end, 30 μL of the solution of 1 was added to a cuvette containing temperature-equilibrated buffer solution. For all reactions, the spectrophotometer was equipped with a hole in the sample compartment directly above the cuvette. The hole could be closed with a removable rubber stopper. The stopper was removed and 1 was added to the cuvette with stirring as quickly as possible utilizing the glass rod, and the stopper was replaced. With practice, this procedure can be completed in a few seconds.

The data for hydrolysis of 1 were analyzed by fitting the points to an exponential curve using a nonlinear least-squares computer program. Varying the buffer concentration in each case had little or no effect on the hydrolysis of 1 so the average of all values for each pH was used in the pH vs rate of hydrolysis profile. The pH values of the solutions did not change when measured before and after a run. The pH values used for the hydrolysis profile were the initial buffer pHs.

The reaction of 1 with diethylamine was monitored at 285 nm. Varying amounts of a solution of diethylamine in methanol (0.965 M) were added to the temperature-equilibrated buffer. To these solutions, 12.5 μL of a methanolic solution of 1 (0.0102 M) was quickly added using the glass rod through the hole in the sample compartment. Each run was done in temperature-equilibrated buffer. The data were analyzed by fitting the points to an exponential curve using a nonlinear least-squares computer program. The reaction of 1 with diethylamine in aqueous solution was first-order with respect to the concentration of the diethylamine with stable infinity points. The absorbance after one minute for each run (∞) was used to calculate the fraction of 1 that was partitioning to the product **4c** as opposed to being hydrolyzed.

Single-Crystal X-ray Analyses of Compounds 4c,g,h, 5, and 13. Suitable crystals were grown through slow crystallization from the appropriate solvents (see pertinent experimental procedures above). Data were collected on either a Syntex $P2_1$ four-circle diffractometer or an automatic Nicolet R_{3m}/V diffractometer at room temperature using graphite monochromated Mo Kα radiation. The unit cell dimensions were obtained by a least-squares fit of 15 centered reflections in the range of $10° < 2\theta < 25°$. Intensity data were collected by using a $\theta/2\theta$ scan type in the range of $3° < 2\theta < 45°$. Three standard reflections monitored after every 100 reflections did not show any significant change in intensity during data collection. Intensities were corrected for decay and Lorentz polarization effects but not for absorption. The structure was solved, and all non-hydrogen atoms were found by using results of SHELXTL-PLUS.[17] After several cycles of refinements using SHELX76,[18] the positions of hydrogen atoms were located on a difference Fourier map, except for methyl hydrogens, which were calculated. Hydrogen atoms were included in the final refinement with isotropic thermal parameters. Refinement proceeded to converge by minimizing the function $\Sigma w(|F_o| - |F_c|)^2$, where the weight, w, is $\sigma(F)^{-2}$. The discrepancy indices $R = \Sigma||F_o| - |F_c||/\Sigma w|F_o|$, $R_w = [\Sigma w(|F_o| - |F_c|)^2/\Sigma(|F_o|)^2]^{1/2}$, and $w = 1/\sigma^2(F_o) + k(F_o)^2$ are presented below.

Crystallographic Data. A. Compound 4c: $C_{12}H_{22}N_4O_2S$; $M_r = 310.41$; P_1; $a = 9.474$ (3), $b = 10.638$ (3), $c = 8.477$ (4) Å; $\alpha = 93.89$ (3)°; $\beta = 90.74$ (3)°; $\gamma = 91.37$ (3)°; $V = 852.0$ (5) Å^3; $Z = 2$; $D_x = 1.21$ g cm^{-3}; Mo Kα = 0.71069 Å; $\mu = 0.19$ mm^{-1}. Final $R = 0.042$ for 2243 reflections.

B. Compound 4g: $C_{14}H_{22}N_4O_2S$; $M_r = 310.41$; P_1; $a = 11.50$ (2), $b = 12.63$ (3), $c = 14.24$ (4) Å; $\alpha = 96.9$ (2)°; $\beta = 108.5$ (2)°; $\gamma = 111.1$ (2)°; $V = 1765$ (8) Å^3; Mo Kα = 0.71073 Å; $\mu = 0.84$ mm^{-1}. Final $R = 0.077$ for 3909 reflections.

C. Compound 4h: $C_{14}H_{22}N_4O_6S$; $M_r = 374.46$; monoclinic, $C2/c$; $a = 17.51$ (1), $b = 11.901$ (8), $c = 11.721$ (7) Å; $\beta = 123.84$ (4)°; $V = 2029$ (2) Å^3; $Z = 4$; $D_x = 1.23$ g cm^{-3}; Mo Kα = 0.71069 Å; $\mu = 1.84$ cm^{-1}; $F(000) = 792$; $T = 295$ K. Final $R = 0.049$ for 1373 observed reflections.

D. Compound 5: $C_{12}H_{14}O_6N_4S$; $M_r = 342.33$; $P2_1/c$; $a = 11.993$ (6), $b = 10.005$ (5), $c = 14.22$ (1) Å; $\beta = 105.36$ (6)°; $V = 1636$ (2) Å^3; $Z = 4$, $D_x = 1.39$ g cm^{-3}; Mo Kα = 0.71069 Å; $\mu = 2.21$ mm^{-1}. Final $R = 0.048$ for 2886 reflections.

E. Compound 13: $C_{14}H_{26}N_4O_4S$; $M_r = 346.50$; monoclinic, $P2_1/c$; $a = 10.300$ (6), $b = 12.893$ (9), $c = 15.155$ (9) Å; $\beta = 107.17$ (4)°; $V = 1918$ (2) Å^3; $Z = 4$; $D_x = 1.20$ g cm^{-3}; Mo Kα = 0.71069 Å; $\mu = 1.95$ cm^{-1}; $F(000) = 800$; $T = 295$ K. Final $R = 0.071$ for 2171 observed reflections.

Acknowledgment. This investigation was supported by grants from the American Heart Association, Maryland Affiliate, Inc. (R.S.H.), National Institutes of Health (R.S.H.; no. CA 36154), National Science Foundation (N.S.H.; no. CHE-88-00328), and the Robert A. Welch Foundation (N.S.H.; N-1016). We are grateful to our colleagues at UMBC for helpful suggestions and comments. We are especially thankful to Drs. R. Pollack and D. Whalen for advice on kinetic measurements and calculation of isomer ratios, respectively, to Dr. M. Summers on ^{1}H NMR lineshape analysis, and to Dr. M. Hulce for carefully reading and correcting stylistic errors in the manuscript.

Supplementary Material Available: Tables of bond lengths, bond angles, torsional angles, and positional parameters for compounds **4c,g,h**, **5**, and **13** and tables of experimental, extrapolated, and simulated data employed in the lineshape analyses of compound **4g** (29 pages). Ordering information is given on any current masthead page.

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