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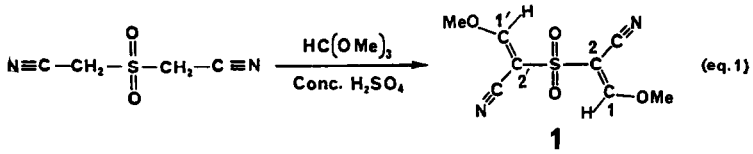
A NOVEL BIFUNCTIONAL REAGENT: 2,2'-BIS(METHOXYMETHYLENE)-2,2'-SULFONYLDIACETONITRILE

Ramachandra S. Hosmane* and Craig M. Bertha

Laboratory for Chemical Dynamics, Department of Chemistry,
University of Maryland Baltimore County
Baltimore, Maryland 21228

Preparation, properties and reactions of the title reagent are reported. The reagent is potentially useful for cross-linking proteins and/or nucleic acids.

In continuation of our program aimed at exploring novel reagents for use in organic/bioorganic syntheses,[1] we have set out to design bifunctional organic reagents. These constitute an important class of organic compounds in view of their potential to cross-link macrobiomolecules; cross-linking agents have long played pivotal roles in investigations of structure and function of proteins[2] and nucleic acids.[3] Natural/modified natural cross-linkers such as mitomycins,[3a] psoraleins,[3b] and anthramycins[3c] have been the subjects of extensive study in nucleic acid chemistry, whereas synthetic homo- or heterobifunctional cross-linking reagents containing e.g. aldehyde, ester, imide, azide or imidate groups have dominated the protein field.[2] However, use of these functional groups has drawbacks. Aldehydes readily form Schiff bases with amine nucleophiles, but the reaction is often reversible, requiring a reduction[2m] step to stabilize the product. Ester and imide groups, on the other hand, frequently show low reactivity under physiological conditions. Azide couplings are photo-induced[2r] and the imidates are hydrolyzed easily.[1g] We report here the synthesis, structure, properties, and reactions of a novel homobifunctional reagent, 2,2'-bis(methoxymethylene)-2,2'-sulfonyldiacetonitrile (1), which contains the hitherto unexploited enol ether functionality for cross-linking. The reagent is easy and inexpensive to prepare on a large scale, is stable in storage, and is highly reactive toward amine nucleophiles of both proteins and nucleic acids. Furthermore, the product bis-enamines are remarkably resistant to hydrolysis, and thus do not require reduction after cross-link formation.



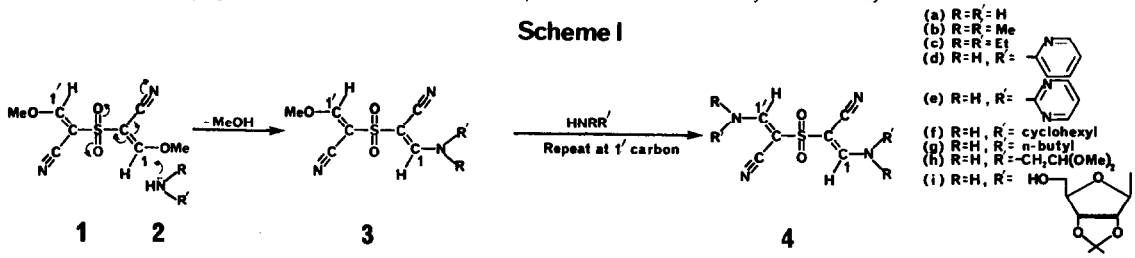
1

The reagent **1** can be prepared (eq. 1) by heating at reflux a mixture of 2,2'-sulfonyldiacetonitrile[4] (20 g, 0.14 mol), trimethyl orthoformate (450 mL), dry acetonitrile (300 mL) and conc. H_2SO_4 (1.6 mL) under N_2 for 22 hours. The clear yellow solution, after evaporation to dryness at ≤40 °C leaves a solid residue which, upon trituration with a mixture of ethyl acetate/ether (1:1, 100 mL), followed by recrystallization from xylenes, provides pure **1** (45-50% yield, m.p. 207-209 °C) which can be stored indefinitely in a refrigerator with adequate protection from moisture. The compound was characterized by elemental microanalyses (C,H,N)[5] coupled with spectral data: ^{1}H NMR (Me_2SO-d_6) δ 4.23 (s, 6H, two OCH_3), 8.37 (s, 2H, two CH); IR (KBr) 3030 (=CH), 2250 (C≡N), 1590 (C=C), 1370, 1170 (SO_2)

cm^{-1}; UV (MeOH) λ_{max} 248 nm. A single crystal X-ray analysis of 1[6] revealed an E-configuration for each half of the molecule on either side of the sulfonyl moiety, while the conformational relationship of each half with respect to the other was anti, as depicted. The X-ray data also suggested considerable electron delocalization extending over the five atom chains from the ether oxygens to the nitriles or the sulfone.

Reagent 1 is a bis-enol ether of two electrophilic cyanoacetaldehyde molecules joined at C2 and C2' by an additionally highly activating sulfone function. It should, therefore, be prone to a facile conjugate addition-elimination process initiated by a variety

Scheme I



1 2 3 4

of nucleophiles. Thus, attack at C1 by amine nucleophiles (e.g. adenine 6-NH_2 in nucleic acids or lysine ϵ-NH_2 in proteins) followed by elimination of a molecule of methanol will produce enamine 3 (Scheme I). The latter, upon reaction with a second molecule of amine, would produce the desired bis-enamine 4. Initially, we studied the reaction of 1 with two equivalents each of nine amine nucleophiles (2), including primary, secondary, ribosyl, and heterocyclic amines at room temperature using acetonitrile or methanol as solvent. The reaction was practically instantaneous in most cases. The product bis-enamines (4a-4i)(Table I) were fully characterized by ^1H NMR, IR, mass spectral data, and combustion analyses (C,H,N & S).[5] Notable in the ^1H NMR spectra of 4d and 4e was a significant downfield shift of the olefinic CH (1,1') (δ ≈9.0) relative to that in non-heterocyclic enamines (δ ≈7.6). The observed anomaly--too large to be attributed to inductive and mesomeric effects--can be reconciled by considerations of magnetic anisotropy of the heterocyclic ring. Additionally, the (E,E)-configurational and anti-conformational geometry found in 1[6] was also evident in the crystal structure of 4b.[7]

TABLE I: Reaction Products of 1 with Amine Nucleophiles

Compound No.	m.p.(°C)	Yield(%)	C=CH Absorption in ^1H NMR (DMSO-d_6) δ
4a	229(dec)	93	7.61(dd, J=16.0 & 8.5 Hz)
4b	188	61	7.54(s)
4c	139	92	7.54(s)
4d	>300	87	8.98(d, J=11.6 Hz)
4e	247(dec)	34	8.91(br)
4f	239-240	99	7.68(d, J=14.7 Hz)
4g	145-146	99	7.69(d, J=15.0 Hz)
4h	106-112	78	7.69(d, J=14.6 Hz)
4i	foam	36	7.94(br) (β-anomer)

These encouraging results prompted us to react 1 analogously with a few nucleic acid

bases/nucleosides and amino acid derivatives. Representative molecules from each category included adenine/adenosine (a purine base), cytosine/cytidine (a pyrimidine), and esters[8] of amino acids glycine, serine and lysine. Our results are summarized in Scheme II and Table II. While lysine (Me-ester) can attack 1 from either the α- or ε-NH_2 function, only one adduct (8) was isolated. The structure was assigned by comparison of its C=CH absorption in ^1H NMR (δ 7.70, see Table II) with that of the bis-n-butyl derivative 4g (δ 7.69), which significantly

Scheme II

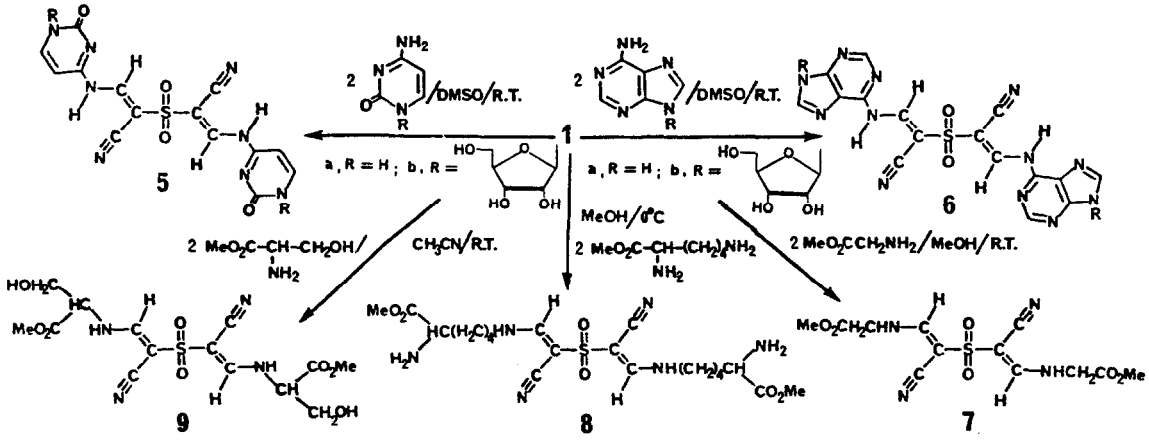


TABLE II: Reaction Products of 1 with Nucl. Acid Bases and Esters of Amino Acids

Compound No.	m.p.(°C)	Yield(%)	C=CH Absorption in ^1H NMR (DMSO-d_6) δ
5a	>300	85	8.75(s)
5b	185(dec)	58	8.78(s)
6a	>290	89	9.58(s)
6b	170(dec)	60	9.56(s)
7	197-199(dec)	56	7.83(d, J=14.4 Hz)
8	syrup	43	7.70(br)
9	glass	53	7.86(d, J=13.0 Hz)

differed from the corresponding signal in 7 (7.80) or 9 (7.86). These values are consistent with the inductive effects of α-CO_2Me/NH_2 groups, which are farther from the C1 and C1' methine in 8 than in 7 or 9.

The stabilities of 1 and its product bis-enamines in aqueous medium are critical for suitability of the reagent for application to biological systems. To explore this, we studied the reactivity of 1 and 4c with water. While 1 was stable for up to four minutes in a threefold molar excess of water at room temperature, 4c was unchanged when treated with even a thousandfold molar excess of water for several days. These results are encouraging in view of the instantaneous reaction of 1 with amines. Indeed, stirring of 1 in aqueous acetonitrile for five minutes, followed by reaction with diethylamine, afforded 4c in 82% yield.

Finally, the diagonal distance between C1 and C1' in 1 is crucial in effecting site-directed inter/intrastrand or inter/intrasubunit cross-links in nucleic acids or proteins. This distance, calculated from the two sets of fractional coordinates and unit-cell

measurements in the X-ray analysis of 1[6] averaged 4.98 Å. The computed[9] interstrand distance between two diagonally opposed guanine residues in a synthetic DNA double helix ranges from 3.3-3.9 Å. Likewise, the estimated average diagonal distance between α_1,α_2- or β_1,β_2-lysine residues lining the periphery of the DPG (2,3-diphosphoglycerate) pocket of hemoglobin ranges from 5-7 Å.[2j] Thus, 1 possesses the necessary physical and chemical features to potentially effect cross-links in double-helical DNA and/or hemoglobins. Indeed, our preliminary experiments with 1 on hemoglobins show the formation of cross-links.[10] In light of the current epidemic of AIDS and the heightened interest[2c] in hemoglobin cross-linking in search of an efficacious blood substitute for emergency transfusions, reagent 1 carries a timely significance.

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8. Esters of amino acids were employed for simplicity of work-up.
9. Interstrand diagonal G-NH_2<--->NH_2-G distance was computed by Dr. Robert Pearlman of the College of Pharmacy, University of Texas at Austin; his assistance is gratefully acknowledged.
10. Preliminary experiments were performed on bovine and human hemoglobins by Profs. E. Bucci and C. Fronticelli of the University of Maryland School of Medicine, Baltimore, MD. Their assistance in this regard is gratefully acknowledged.

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