Photoisomerization as a modulator of the DNA-cleaving efficiency of novel azo bispropargyl sulfones

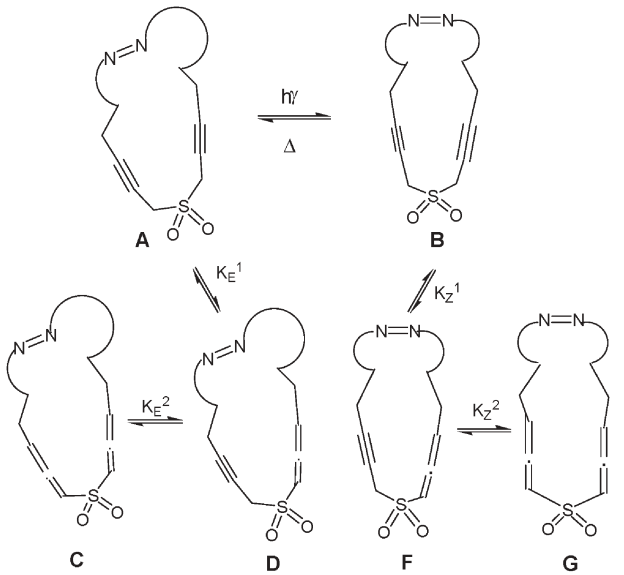
Moumita Kar and Amit Basak*

Received (in Cambridge, UK) 11th May 2006, Accepted 10th July 2006
First published as an Advance Article on the web 3rd August 2006
DOI: 10.1039/b606653d

Novel azobenzene based bispropargyl sulfones have been prepared in the thermally stable *E*-form: irradiation with a high-pressure Hg lamp converted them to the *Z*-isomer which showed higher DNA-cleaving efficiency.

Bispropargyl sulfones have recently emerged as a new class of DNA-cleaving agents.[1] They exert their activity *via* isomerization to the allenic sulfone, which subsequently alkylates the DNA bases, thus allowing a Maxam–Gilbert type cleavage to take place.[2] An alternative pathway, though usually not followed, is the formation of diradicals from the bisallenic sulfone *via* Garratt–Braverman rearrangement[3] and subsequent oxidative DNA damage.[4] It is important to note that diradical formation can take place only if the sulfone gets isomerized to the bisallene, which in many cases may not be possible for stereoelectronic reasons.[5] Since monoallenic sulfones show their DNA cleavage activity *via* an alkylation pathway, they may be inferior in terms of their DNA-cleaving efficiency when compared to the bisallenic sulfones (as the latter can have dual mechanisms for cleavage, namely mono or bis-alkylation, as well as oxidative damage through diradicals). Modulation of the reactivity of bispropargyl sulfones has not drawn much attention, unlike the enediyne counterpart. The only triggering device that is used is the variation of pH, which is kept in the alkaline range.[6a–c] Dai *et al.* has recently exploited photoelectron transfer to enhance the DNA cleaving potency of monopropargyl sulfones.[6d–e] Recently,[7] we have shown how a photochemical *E* to *Z* isomerization can activate azoenediynes towards Bergman cyclization (BC).[8] We were curious to know what happens to the reactivity of azo bispropargyl sulfone systems upon such *E*–*Z* photoisomerization. Our curiosity arises from the fact that the kinetics of BC are dependent upon the distance between the terminal acetylenic carbons which presumably is less in *Z*-azo enediynes. In a similar argument, the reactivity of the *Z*-azo bispropargyl sulfones may be higher, as the allenic carbons (formed by isomerization) should come closer, compared to the corresponding *E*-system, and are thus geared towards Garratt–Braverman cyclization. Thus, we would like to address the following points: does the configuration of the azo moiety (*E* or *Z*) affect the rate of isomerization to the monoallene and subsequently to the bisallene? Does it induce any change in DNA-cleaving efficiency and also any change in the DNA-cleaving mechanism? All these possibilities are summarized in Scheme 1.

In order to have an answer, we synthesized the *E*-azobenzene sulfones **1** and **2** (Fig. 1). These have been successfully photoisomerized to the *Z*-sulfones **3** and **4**. The reactivity of these isomers in presence of a base (triethylamine) as well as their DNA-cleavage ability under alkaline pH revealed interesting results, which are described in this Communication.



Scheme 1 Possible studies involving the azo sulfones.

The starting material for the synthesis of both the sulfones was the commercially available 2,2′-bishydroxy azobenzene (**5**). This was bisalkylated with butyne-1,4-diol mono tosylate (**7**) in the presence of K_2CO_3 and DMF. The resulting diol **8**, isolated in 70% yield, was converted to the dimesylate **9** and the cyclic sulfide **10** was obtained when the dimesylate was treated with Na_2S, preabsorbed in alumina (neutral) in CH_2Cl_2. The sulfide was then oxidized with mCPBA to the sulfone **1** which was isolated as a

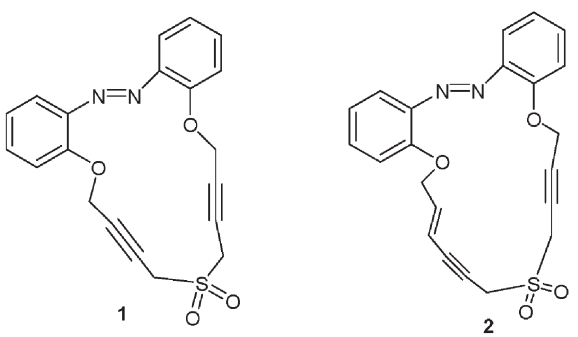
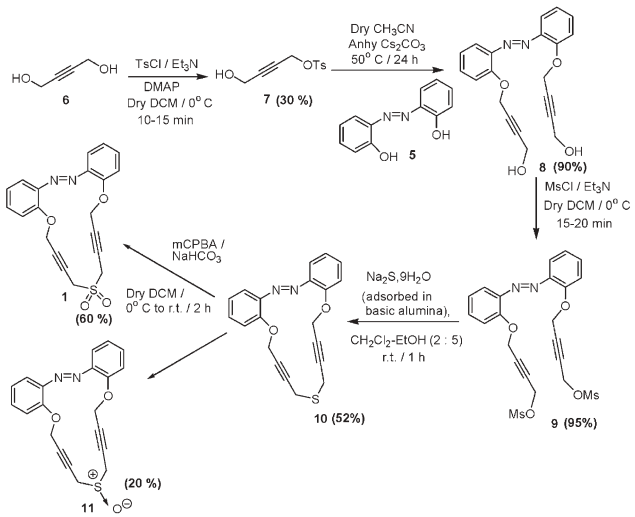
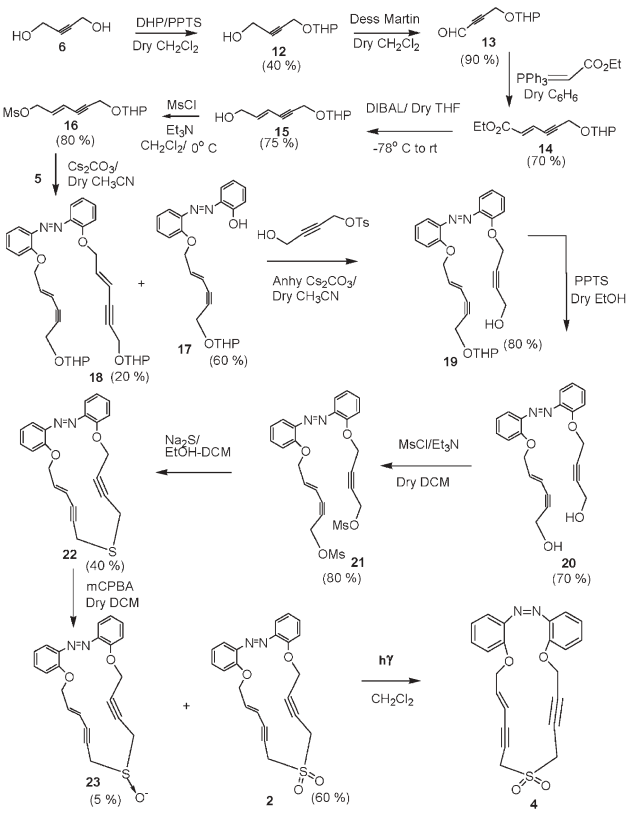


Fig. 1 Azobenzene based bis propargyl sulfones.

Bioorganic Laboratory, Department of Chemistry, Indian Institute of Technology, Kharagpur, India. E-mail: absk@chem.iitkgp.ernet.in; Fax: +91 3222 282252; Tel: +91 3222 283300



Scheme 2 Synthesis of bispropargyl sulfone **1**.

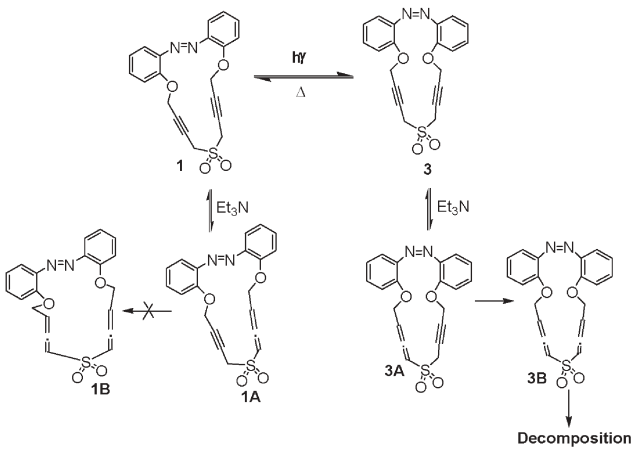


Scheme 3 Synthesis of bispropargyl sulfone **2**.

yellow solid after purification by chromatography (Scheme 2). The formation of the sulfone was confirmed by the appearance of two 4H singlets in the ^{1}H NMR spectrum. The sulfoxide **11** which could also be isolated when the oxidation was carried at 0 °C for a short time gave a typical AB quartet for the methylene attached to sulfur.

For the synthesis of the vinylogous sulfone **2** (Scheme 3), the mesylate **16** was first prepared from partially THP-protected butyne-1,4-diol **12**. The alcohol was oxidized to the aldehyde **13** with Dess Martin reagent[9] and the aldehyde was subjected to Wittig reaction with phosphonium ylide derived from ethyl bromoacetate. The *trans* ester **14** isolated in high yield (90%) was reduced to the alcohol **15** with DIBAL-H. The latter was then converted to the mesylate **16** which was used as the alkylation partner. However, in this case the monoalkylated product **17** was the major product even after prolonged hours of stirring at 50 °C. The expected biscoupled product **18** was isolated in very poor yield (~5%) which could not be processed any further. The mono-coupled product was then converted to the unsymmetrical bis-alkylated product **19** using the butyne-1,4-diol mono tosylate which turned out to be a more reactive system. The resulting diol **20**, obtained after PPTS-deprotection of THP–ether was then converted to the sulfide **22** *via* the mesylate **21**. Oxidation with mCPBA finally afforded the target sulfone **2**. Gratifyingly, the double bond remained intact during the peracid oxidation.

Having successfully prepared the target sulfones,[10] we turned our attention to study the effect of UV-irradiation on these molecules. For this, the sulfone **1** in CH_2Cl_2 (0.005 M) was irradiated with a high pressure Hg lamp for 3 h. ^{1}H-NMR showed the occurrence of isomerization from *E* to *Z* (a ratio of 1 : 3), as confirmed by the appearance of new singlets at δ 4.59 and 3.96. Upon heating at 60 °C for 2 h these signals disappeared, leaving only the signals for the starting *E*-isomer. ^{1}H-NMR based kinetic study at 37 °C showed the first order rate constant for thermal *Z* to *E* isomerization to be 5×10^3 min^{-1}. The other *E*-sulfone **2** behaved similarly when its methanol solution was irradiated for 3 h; in this case the ratio of *E* to *Z* isomer was also 1 : 3. The formation of the *Z*-isomer was indicated by the appearance of three new singlets at δ 4.90, 4.76 and 4.10. The *Z*-isomer can be thermally reisomerized back to the *E* isomer; the rate of thermal reisomerization was, however, much slower ($k = 3 \times 10^2$ min^{-1}) . The chemical reactivity of the sulfones **1** and **3** were then evaluated (Scheme 4). For this, a $CDCl_3$ solution of the sulfone was taken and triethylamine (1.5 eq.) added and the progress of reaction monitored by ^{1}H-NMR. The *E*-sulfone **1** rapidly forms the monoallene, after which there was no further reaction and we ended up with an equilibrium mixture of **1** and its monoallenic counterpart (ratio 1 : 1). The formation of monoallene was confirmed by formation of only the mono methanol adduct (yield ~30%) when the experiment was run in presence of MeOH at 37 °C for 12 h. In the monoallene, the methylene attached to the



Scheme 4 Reactivity of sulfones in the presence of Et_3N.

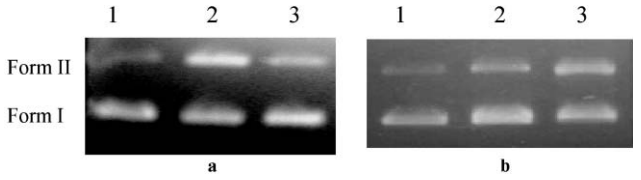


Fig. 2 (a) DNA cleavage experiment of compounds **1** & **3** after 1.5 h incubation at 37 °C; lane 1: control DNA in TAE buffer (pH 8.5, 0.4 μm per bp) (7 μl) + CH_3CN (10 μl); lane 2: DNA in TAE buffer (pH 8.5, 0.4 μm per bp) (7 μl) + *Z*-sulfone (0.02 mM, 2.5 h) in CH_3CN (5 μl); lane 3: DNA in TAE buffer (pH 8.5, 0.4 μm per bp) (7 μl) + *E*-sulfone (0.02 mM, 2.5 h) in CH_3CN (5 μl). (b) DNA cleavage experiment of compounds **2** & **4** after 2.5 h incubation at 20 °C; lane 1: control DNA in TAE buffer (pH 8.5, 0.4 μm per bp) (7 μl) + CH_3CN (10 μl) at; lane 2: DNA in TAE buffer (pH 8.5, 0.4 μm per bp) (7 μl) + *E*-sulfone (0.02 mM, 2.5 h) in CH_3CN (5 μl) at; lane 3: DNA in TAE buffer (pH 8.5, 0.4 μm per bp) (7 μl) + *Z*-sulfone (0.02 mM, 2.5 h) in CH_3CN (5 μl).

sulfone appeared as an AB quartet reflecting their diastereotopic nature due to the chirality of the allene. The *Z*-isomer on the other hand when similarly treated first showed the formation of the monoallene. But within a few minutes (~5 min) the signals corresponding to monoallene disappeared and some unassignable peaks of very small intensity appeared in the olefinic region. Carrying out the reaction in the presence of MeOH (12 h, 37 °C) showed the formation of both mono and bis adduct with MeOH (appearance of peaks in the mass spectra at *m*/*z* 413 and 445). This indicated that the monoallene from the *Z*-isomer got converted into the bis allene which then rapidly decomposed (possibly *via* Garratt–Braverman rearrangement in the absence of MeOH).[11] Thus, there is a distinct change in reactivity between the *E*-and the *Z*-isomer.

Since the *Z*-isomer can isomerize up to the bisallene in the presence of base, it is expected to show higher DNA-cleavage efficiency as it can proceed *via* both an alkylation and an oxidative diradical pathway. DNA-cleaving experiments were thus carried out with the pure *E* isomer and a 1 : 3 mixture of *E* and *Z* isomer (obtained by photoisomerism) at 37 °C using pBR 322 supercoiled plasmid DNA at pH 8.5. The results showed the higher cleavage efficiency[12] for the *Z*-isomer (Fig. 2a) as compared to the *E*-isomer (lane 3). However, the cleavage efficiency finally evened out as more time was allowed. This is because of thermal reisomerization to the *E*-isomer. To slow down this thermal reiomerization process, the experiment was repeated at the lower temperature of 20 °C where the difference in cleavage efficiency became more significant. For the other sulfone, **2**, again the *E*-isomer was found to be less efficient cleaving agent as compared to the *Z*-isomer (Fig. 2b). In this case also, the incubation with DNA was done with the lower temperature of 20 °C to suppress thermal isomerization.

In conclusion, we have successfully developed a novel photo-triggering device involving *E* to *Z* isomerism to modulate the reactivity of azo based bis propargyl sulfones. Currently, we are trying to design molecules for which the thermal reisomerism is slower as compared to those reported herein, by incorporating some strain parameters. Our strategy may find use in the development of anticancer molecules involving photodynamic therapy.[13]

MK is grateful to CSIR, Government of India for a senior research fellowship. AB thanks DST, Government of India for a research grant.

Notes and references

1 K. C. Nicolaou, S. Wendeborn, P. Maligres, K. Isshiki, N. Zein and G. Ellestad, *Angew. Chem., Int. Ed. Engl.*, 1991, **30**, 418.
2 (*a*) A. M. Maxam and W. Gilbert, *Methods Enzymol.*, 1980, **65**, 499; (*b*) A. M. Maxam and W. Gilbert, *Proc. Natl. Acad. Sci. U. S. A.*, 1977, **74**, 560.
3 (*a*) P. J. Garratt and S. B. Neoh, *J. Org. Chem.*, 1979, **44**, 2667; (*b*) Y. S. P. Cheng, P. J. Garratt, S. B. Neoh and V. H. Rumjanek, *Isr. J. Chem.*, 1985, **26**, 101; (*c*) S. Braverman, Y. Duar and D. Segev, *Tetrahedron Lett.*, 1979, 3181.
4 (*a*) Y.-j. Xu, M. S. DeMott, J. T. Hwang, M. M. Greenberg and B. Demple, *DNA Repair*, 2003, **2**, 175; (*b*) A. Liptakova, L. Svorenova, J. Labuda and Z. Durackova, *Conf. Coord. Chem.*, 1995, 407; (*c*) C. Richter, J. W. Park and B. N. Ames, *Proc. Natl. Acad. Sci. U. S. A.*, 1988, **85**, 6465.
5 A. Basak and U. K. Khamrai, *Tetrahedron Lett.*, 1995, **36**, 7913.
6 (*a*) S. M. Kerwin, *Tetrahedron Lett.*, 1994, **35**, 1023; (*b*) W.-M. Dai and K. C. Fong, *Tetrahedron Lett.*, 1995, **36**, 5613; (*c*) W.-M. Dai, K. C. Fong, H. Danjo, S.-i. Nishimoto, M. Solow, W. L. Mak and M. L. Yeung, *Bioorg. Med. Chem. Lett.*, 1996, **6**, 1093; (*d*) K.-i. Haruna, H. Kanezaki, K. Tanabe, W.-M. Dai and S.-i. Nishimoto, *Bioorg. Med. Chem.*, 2006, **14**, 4427; (*e*) K.-i. Haruna, K. Tanabe, A. Ishii, W.-M. Dai, H. Hatta and S.-i. Nishimoto, *Bioorg. Med. Chem. Lett.*, 2003, **11**, 5311.
7 M. Kar, A. Basak and M. Bhattacharya, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 5392.
8 (*a*) R. G. Jones and R. G. Bergman, *J. Am. Chem. Soc.*, 1972, **94**, 660; (*b*) R. G. Bergman, *Acc. Chem. Res.*, 1973, **6**, 25; (*c*) T. P. Lockhart and R. G. Bergman, *J. Am. Chem. Soc.*, 1981, **103**, 4091.
9 D. B. Dess and J. C. Martin, *J. Org. Soc.*, 1983, **48**, 4155.
10 Selected spectral data (all ^1H and ^{13}C NMR were recorded at 200 and 50 MHz, respectively, in $CDCl_3$ unless mentioned otherwise). For **1**: δ_H 7.72 (1H, dd, *J* = 1.5 Hz, 8.5 Hz), 7.45 (2H, dt , *J* = 1.8, 7.5 Hz), 7.21 (4H, m), 4.93 (4H, s), 3.75 (4H, s); δ_C 154, 145, 131.9, 123.4, 119.6, 119, 83, 74, 60.3, 43.3; HRMS calcd for $C_{20}H_{16}N_2O_4S + H^+$ 381.09101 found 381.0874. For **2**: δ_H 7.70 (1H, d, *J* = 7.8 Hz), 7.42 (2H, m), 7.12 (5H, m), 6.30 (1H, d, *J* = 16 Hz), 6.10 (1H, d, *J* = 8.4 Hz), 4.98 (2H, s), 4.65 (2H, d, *J* = 5.0 Hz), 4.03 (4H, s); HRMS calcd for $C_{22}H_{18}N_2O_4S + H^+$ 407.1067 found 407.1096. For **3**: δ_H 7.72 (1H, d, *J* = 8.4 Hz), 7.45 (1H, t, *J* = 7.6 Hz), 7.25 (2H, m), 7.06 (2H, m), 6.84 (2H, d, *J* = 8.24 Hz), 4.59 (4H, s), 3.96 (4H, s); HRMS calcd for $C_{20}H_{16}N_2O_4S + H^+$ 381.0910 found 381.0880. For **4**: δ_H 7.69 (1H, d, *J* = 2 Hz), 7.4 (3H, m), 6.9 (4H, m), 6.31 (1H, dt, *J* = 2 Hz, 16 Hz), 6.11 (1H, d, *J* = 16 Hz), 4.90 (2H, bs), 4.76 (2H, bs), 4.10 (4H, bs); HRMS calcd for $C_{22}H_{18}N_2O_4S + H^+$ 407.1067 found 407.1087.
11 Braverman has recently reported that the rearrangement to the thiophene dioxides is more facile by incorporating additional conjugation to the propargyl system. For the reference see Y. Zafrani, H. E. Gottlieb, M. Sprecher and S. Braverman, *J. Org. Chem.*, 2005, **70**, 10166. This was the rationale for designing sulfone **2**.
12 The cleavage efficiency was approximately 2.5 times for the *Z*-isomer than that for the *E*-isomer. This was determined by checking the relative UV-absorbance of the bands at 280 nm. It is to be noted that the control DNA specimen is usually contaminated with some nicked form (form II).
13 J. Moan and Q. Peng, *Anticancer Res.*, 2003, **23**, 3591.

