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Design, synthesis and reactivity of C_2-symmetric azobenzene-based amino acid-bis(propargyl sulfones)

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ABSTRACT

C_2-Symmetric azobenzene-amino acid linked bis(propargyl sulfones) **1** and **2** containing stable *E* azo moiety have been synthesized. Upon irradiation with long wavelength UV these compounds isomerized to the *Z*-form, whose thermal reisomerization to the *E*-isomer slowed down considerably Under basic pH, the compounds showed DNA cleavage in μmolar concentrations with the *Z*-isomers showing better cleaving efficiency. The difference in cleaving efficiency between the *Z* and the *E*-isomer is more than the corresponding pair of sulfones without amino acid linker.

Bispropargyl sulfones constitute an important class of DNA-cleaving agent.[1] Various modifications have been made on their structures to bring selectivity in their DNA-cleaving action.[2] The mechanism involves isomerisation to the allenic sulfones followed by conjugate addition of a DNA base. This generates a positive charge on the DNA base, resulting in a Maxam–Gilbert type cleavage.[3] An alternative mechanism involving the formation of diradical via Garatt–Braverman rearrangement,[4] has also been proposed. Subsequently, Kerwin reported the synthesis of crown-ether based cyclic bispropargyl sulfone.[5] In alkaline pH, the compound cleaved supercoiled DNA via a Maxam–Gilbert type of alkylation pathway. Dai et al.[6] has reported the synthesis of propargyl sulfones with DNA-intercalating moieties. These compounds showed enhanced DNA-cleaving property with respect to non-intercalating counterpart. Previously[7] we have reported the DNA cleavage exhibited by *E*- and *Z*-isomers of azobenzene-based bispropargyl sulfones. Although, the cleavage efficiency of the *Z*-isomer was higher than the *E*-isomer, the difference was not significant mainly due to the rapid thermal *Z* to *E*-isomerization. Keeping this in mind, we were inspired in synthesizing *E*-azobenzene based sulfones **1** and **2** with amino acid linker. The amino acids are incorporated in order to stabilize[8] the *Z*-isomers **3** and **4** via weak stabilizing interactions like H-bonding, π-stacking etc.[9] The target sulfones are shown in Figure 1. Their chemical, photochemical and DNA cleavage activities were also studied and compared with the sulfones without the amino acid linker. The results are described in this communication.

Docking studies[10] using AutoDock 4.2 were first carried out. The energy minimized docked conformations of *E*- and *Z*-sulfones **1** and **3** are shown in Figure 2. It was observed that the *Z*-isomer **3** closely fits itself in the major groove of DNA (Fig. 3). It docked in such a way that the terminal propargyl sulfone units are directed towards DNA base pairs. It exhibited one hydrogen bond involving oxygen atom of amide group of one side chain to deoxy adenine (DA16) of B-chain with a distance of 2.8 Å. It also showed van der Waal's interaction (hydrophobic) between the benzene ring (sulfone unit) of hydrogen bonded side chain with DT17 of B-chain. The distance between the closest center of aforesaid two units is 3.93 Å. The distance between the midcenter of propargyl unit of one side chain with the nearby deoxy adenine (DA16) residue is 3.93 Å which favors the proposed alkylation reaction due to close proximity of the reaction center. But *E* azo sulfone molecule is loosely bound to the major groove of DNA. It docked in such a way that both terminal propargyl sulfone moeities are directed outwards from DNA base pairs and overall docked energy is comparatively much higher than the *Z*-isomer. It has no hydrogen bonding interactions. The Zazo sulfone molecule also predicted to have lower free energy of binding (−12.15 kcal/mol) than the *E* azo sulfone molecule (−7.53 kcal/mol). In other words, *Z*-azo sulfone molecule is predicted to have the higher binding affinity towards the binding site of DNA as compared to the *E*-isomer.

The synthesis of target sulfones required assembling of three units: azobenzene, amino acids and butyn-1, 4-diol. This involved

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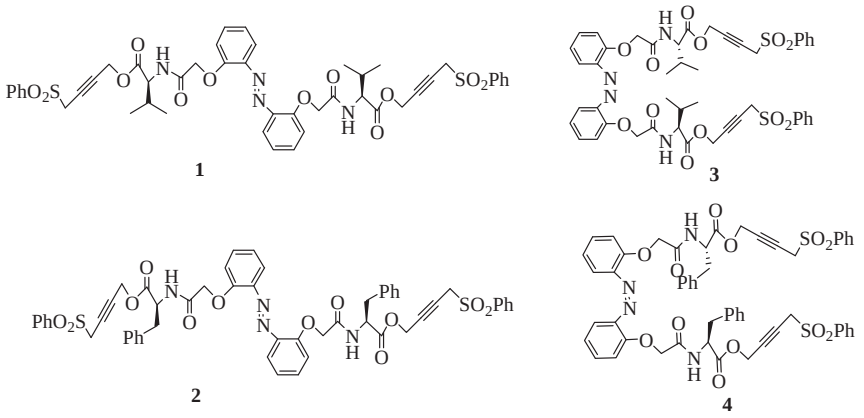


Figure 1. Target sulfones.

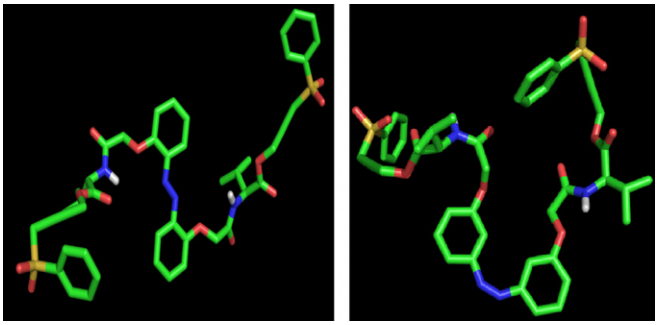


Figure 2. Energy minimized docked conformation.

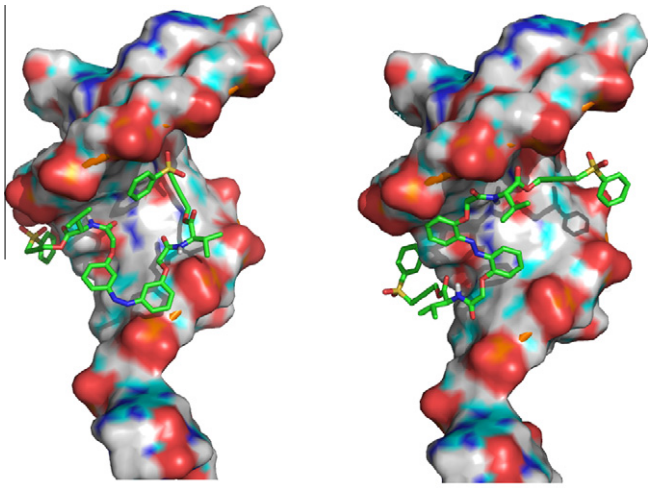


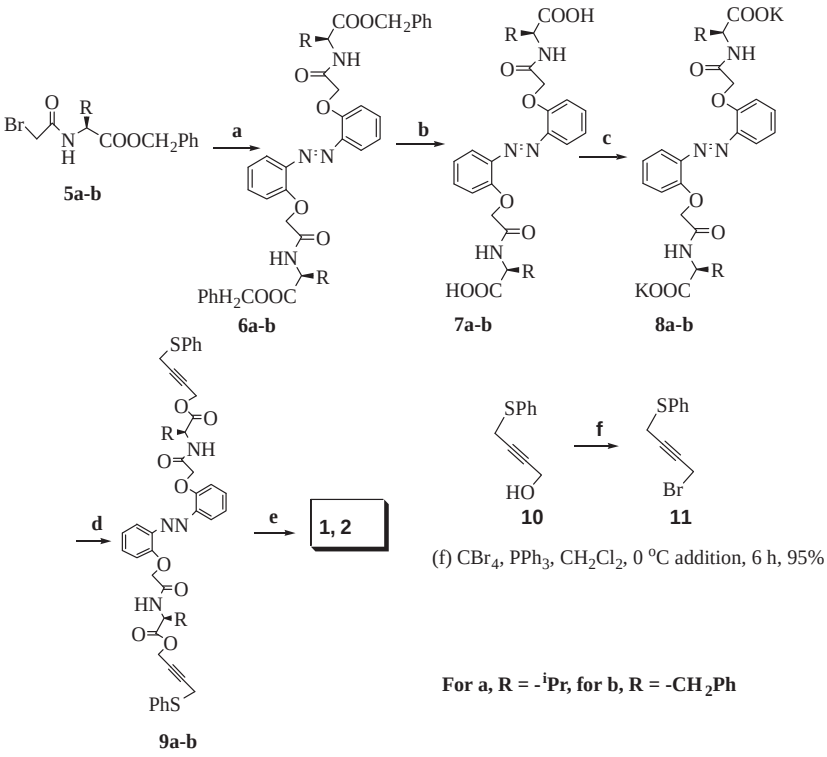
Figure 3. Interaction of DNA–ligand complexes, *Z*-isomer (a) and *E*-isomer (b); here DNA is represented as surface.

initial linking between 2,2′–dihydroxy azobenzene and the bromo-acylated amino acid benzyl ester (**5a–b**). The resulting alkylated products (**6a–b**) upon hydrolysis produced corresponding diacids (**7a–b**). These were converted to the dipotassium salts (**8a–b**) which were then coupled with (4-bromo-but-2-ynyl sulfanyl)-benzene (**11**) derived from 4-phenyl sulfanyl-but-2-yn-1-ol (**10**), to produce **1** and **2**. The synthetic strategy is described in the Scheme 1. All the compounds were characterized by spectral analysis.

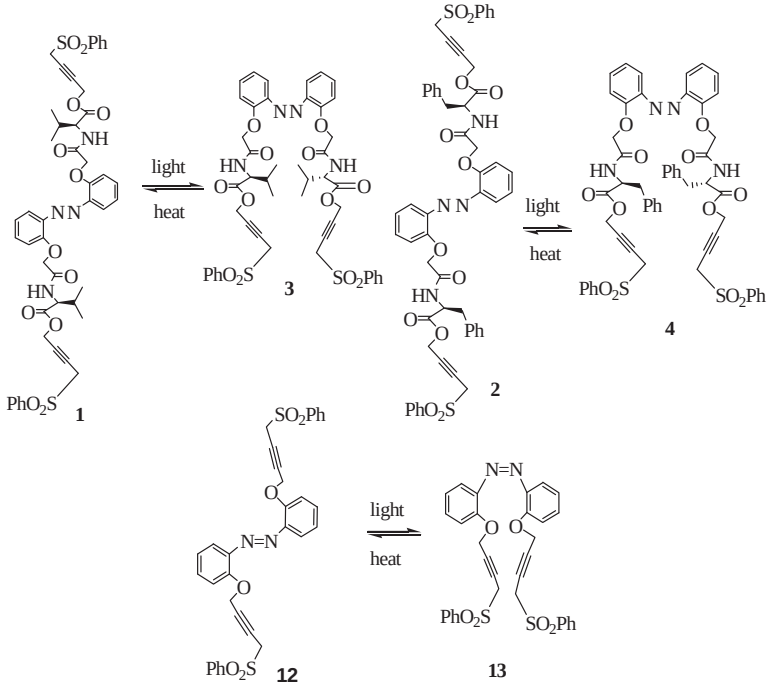
Irradiation of dichloromethane solution of the compound **1** for 3 h under high pressure Hg lamp at 354 nm produced a mixture of *E*- and *Z*-isomers (**1** and **3**) in 1:2 ratio (Scheme 2). Compound **2** produced a photo stationary state of *E*- and *Z*-forms in 1:1.5 ratio under similar condition. The thermal reisomerisation carried out at 30 °C for both the compounds followed first order kinetics. The kinetics was followed by taking ^{1}H NMR at various time points. The half lives for reisomerisation were found to be 72.8 h for compound **3** and 69.2 h for compound **4**. These half lives are much larger than that of bissulfone **13** without amino acid linker.[7] Thus incorporation of amino acids in the bissulfone framework has considerably slowed down the thermal reisomerisation at room temperature.

The chemical reactivity of the sulfones **1** and **2** under basic condition was evaluated by studying the kinetics of isomerisation from propargyl to allene (Scheme 3). For this, a $CDCl_3$ solution of the compound **1** was taken and triethylamine (3 equiv) was added and the progress of reaction was followed by monitoring the ^{1}H NMR profile. New peaks started to appear at δ 6.34 and δ 5.93 which were assigned to the olefinic protons of bisallene **14**.[11] At equilibrium, which was reached after 30 min, the ratio of propargyl to allene was 1:1. This ratio did not alter even after keeping the solution for 12 h at 25 °C. Similar results were observed in case of the corresponding *Z*-isomer **3**. The relative reactivities of the compounds **2** and **4** under basic conditions were also checked. In this case, however, the ratio of propargyl to allene became 1.5:1 at equilibrium. Here again, the extent of allene formation in both *Z* and *E* forms for both the pairs was more as compared to what was observed for the bissulfones **12** and **13**.[7]

DNA-cleaving efficiency of the compounds was then studied. Since we could not isolate the *Z*-isomers in pure form, we had to check the cleavage with pure E and a mixture of *E*- and *Z*-isomers (with excess of *Z*-form). The experiments were carried out at 37 °C using pBR322 supercoiled plasmid DNA at pH 8.5. The greater half lives of the *Z*-isomers (**3** and **4**) allowed us to extend the time of incubation up to 48 h. The gel electrophoresis patterns are shown in Figure 4. Densitometric analysis[12] indicated the extent of cleavage for the compounds (Table 1). From the gel pattern, we can conclude that the mixture containing predominantly the *Z*-form has better cleaving efficiency as compared to the pure *E*-isomer. This is true for both the valine and phenyl alanine based sulfones. Moreover, the difference in the extent of DNA cleavage for *E*- and *Z*-isomers for these amino acid inked azobenzene systems is much more significant as compared to the compounds without amino acid linker **12** and **13** as reported earlier. Incidentally, the same pBR322 DNA was used to study the cleavage potency of **12** and **13**.



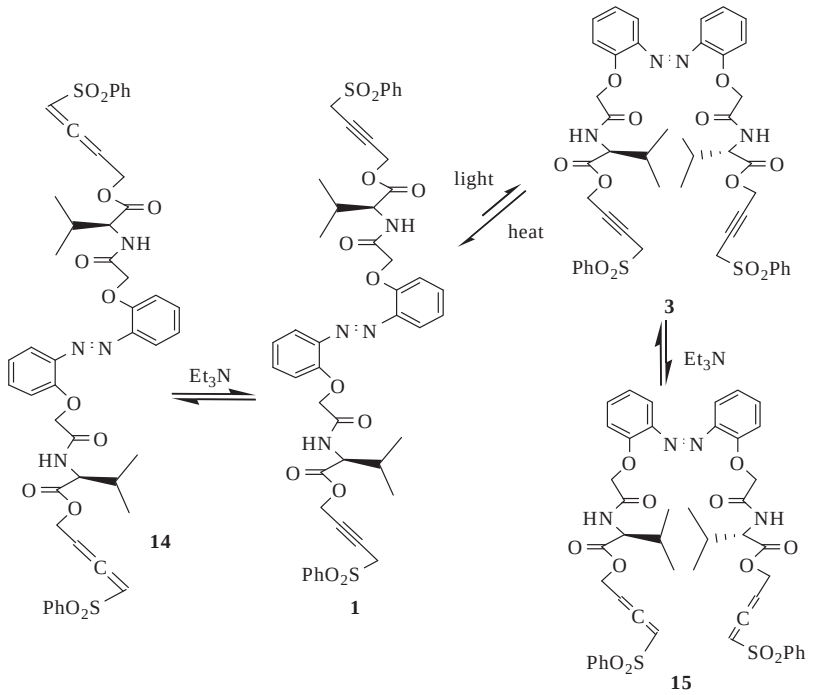
Scheme 1. Synthesis of target sulfones. Reagents and conditions: (a) 2,2′-dihydroxyazobenzene, anhyd Cs_2CO_3, dry CH_3CN, 40–50 °C, 12 h, 50%; (b) LiOH, THF/H_2O, 16–18 h, 60%; (c) $KHCO_3$, MeOH/H_2O, 2 h, 80%; (d) (4-bromo-but-2-ynyl sulfanyl)-benzene, dry DMF, 24 h, 65% (R = –iPr), 60% (R = –CH_2Ph); (e) *m*-CPBA, CH_2Cl_2, 0 °C addition, 1.5 h, 75%.



Scheme 2. Light induced *E*–*Z* isomerization study.

The results are according to our prediction as the design was based on the expected better binding of the *Z*-isomer with ds DNA along with greater half life. DNA-binding studies[13] did indicate one order of magnitude higher binding of the *Z*-isomer (Table 2).

In conclusion, we have successfully synthesized acyclic azo amino acid linked bis(propargyl sulfones). The *Z*-isomers showed higher cleaving efficiency than the corresponding *E*-isomers as was expected from its higher half life, greater extent of allene formation and better DNA binding. Currently, we are engaged



Scheme 3. Base induced isomerization study.

Figure 4. DNA cleavage studies with pBR322: For (A), DNA cleavage experiment of compounds **1** and a mixture containing predominantly *Z*-isomer **3** after 48 h incubation at 37 °C; lane 1: control DNA in TAE buffer (pH 8.5, 7 μL) + CH_3CN (5 μL); lane 2: DNA in TAE buffer (pH 8.5, 7 μL) + *E*-sulfone **1** (0.02 mM) in CH_3CN (5 μL); lane 3: DNA in TAE buffer (pH 8.5, 7 μL) + *Z* and *E*-sulfones **3** and **1** (2:1, 0.02 mM) in CH_3CN (5 μL); For (B), DNA cleavage experiment of compounds **2** and a mixture containing predominantly *Z*-isomer **4** after 48 h incubation at 37 °C; lane 1: control DNA in TAE buffer (pH 8.5, 7 μL) + CH_3CN (5 μL); lane 2: DNA in TAE buffer (pH 8.5, 7 μL) + *E*-sulfone **2** (0.02 mM) in CH_3CN (5 μL); lane 3: DNA in TAE buffer (pH 8.5, 7 μL) + *Z*- and *E*-sulfones **4** and **2** (1.5:1, 0.02 mM) in CH_3CN (5 μL).

Table 1
Extent of DNA-cleavage by densitometry

Compound no	% Cleavage after 48 h of incubation
1	~20
3 + 1 (2:1)	~65
2	~10
4 + 2 (1.5:1)	~45
12	~25
13 + 12 (3:1)	~35

Table 2
DNA–ligand binding constant (the estimated error is within +5%)

Binding constant of compound **1** ($\times 10^4$) M^{-1}	Binding constant of compound **3** + **1** (2:1) ($\times 10^5$) M^{-1}	Binding constant of compound **2** ($\times 10^4$) M^{-1}	Binding constant of compound **4** + **2** (1.5:1) ($\times 10^5$) M^{-1}
11	16	9	15

in the synthesis of water soluble analogs based on similar framework.

Spectral data of selected compounds: IR data taken in KBr pellet and expressed in cm^{-1}, ^1H NMR and ^{13}C NMR were recorded at 400 MHz and 100 MHz, respectively, in $CDCl_3$:

For **9a**: ν_{max} (neat, cm^{-1}) 2078, 1633, 1242, 686; δ_H 7.90 (2H, d, *J* = 7.6 Hz), 7.59 (2H, d, *J* = 9.2 Hz), 7.46–7.38 (6H, m), 7.31–7.26 (4H, m), 7.21 (2H, t, *J* = 7.2 Hz), 7.11–7.05 (4H, m), 4.78–4.59 (10H, m), 3.61 (4H, s, $-CH_2SPh$), 2.21–2.13 (2H, m), 0.90 (6H, d, *J* = 6.8 Hz), 0.81 (6H, d, *J* = 6.8 Hz); δ_C 170.6, 168.0, 155.0, 142.5, 134.8, 132.8, 129.9, 129.0, 126.9, 122.5, 117.6, 114.6, 83.2, 77.4, 68.3, 56.4, 53.0, 31.3, 22.7, 18.9, 17.6; mass (ES^+): *m*/*z* 849 (MH^+); HRMS Calcd for $C_{46}H_{48}N_4O_8S_2 + H^+$ 849.2992 found 849.2997.

For **9b**: ν_{max} ($CHCl_3$, cm^{-1}) 2353, 1217, 1644, 1183, 754; δ_H 7.66–7.60 (4H, m), 7.51 (2H, d, *J* = 3.6 Hz), 7.42–7.41 (6H, m), 7.32–7.20 (6H, m), 7.10–7.01 (12H, m), 5.01–4.47 (10H, m), 3.66 (4H, s), 3.13 (2H, dd, *J* = 8.4, 5.6 Hz, $-CH_2Ph$), 2.99 (2H, q, *J* = 6.8 Hz, $-CH_2Ph$); δ_C 170.2, 167.9, 155.1, 142.5, 135.3, 132.7, 130.1, 129.2, 129.0, 128.5, 128.4, 127.1, 127.0, 122.1, 115.1, 114.1, 83.3, 77.3, 68.9, 53.2, 52.6, 37.8, 22.7; mass (ES^+): *m*/*z* 945 (MH^+); HRMS Calcd for $C_{54}H_{48}N_4O_8S_2 + H^+$ 945.2992 found 945.2998.

For **1**: ν_{max} (neat, cm^{-1}) 2077, 1642, 1139, 749; δ_H 7.94 (4H, d, *J* = 8.0 Hz), 7.81 (2H, d, *J* = 8.0 Hz), 7.67–7.62 (2H, m), 7.56 (6H, t, *J* = 7.6 Hz), 7.47 (2H, t, *J* = 8.0 Hz), 7.10–7.07 (4H, m), 4.79–4.59 (10H, m), 3.97 (4H, s), 2.19–2.11 (2H, m), 0.90 (6H, d, *J* = 6.8 Hz), 0.81 (6H, d, *J* = 6.8 Hz); δ_C 170.5, 168.0, 154.9, 142.5, 137.5, 134.3, 132.9, 129.2, 128.8, 122.5, 117.6, 114.7, 81.3, 74.9, 68.4, 56.4, 52.4, 48.5, 31.2, 18.9, 17.6; mass (ES^+): *m*/*z* 935.42 (MNa^+); HRMS Calcd for $C_{46}H_{48}N_4O_{12}S_2 + H^+$ 913.2788 found 913.2794.

For **2**: ν_{max} (neat, cm^{-1}) 2353, 1642, 1166, 771; δ_H (400 MHz), 7.96 (4H, d, *J* = 6.0 Hz), 7.77–7.53 (10H, m), 7.43 (2H, t, *J* = 7.6 Hz), 7.24–6.92 (14H, m), 4.97–4.37 (10H, m), 3.97 (4H, s), 3.09 (2H, dd, *J* = 12.0, 6.0 Hz), 2.96 (2H, q, *J* = 6.8 Hz); δ_C 170.1, 167.9, 155.1, 142.6, 137.5, 135.2, 134.3, 132.8, 129.3, 129.2, 129.0, 128.8, 127.1, 122.8, 117.7, 115.3, 81.1, 75.1, 68.9, 52.6, 48.5, 37.8, 37.3; mass (ES^+): *m*/*z* 1031.59 (MNa^+); HRMS Calcd for $C_{54}H_{48}N_4O_{12}S_2 + H^+$ 1009.2788 found 1009.2795.

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10. The computer simulated automated docking study was performed using AutoDock4.2. The B-DNA form of DNA decamer d(GGCCAATTGG) (PDB ID: 432D) was used for docking study with *E* azo and *Z* azo sulfone molecule. Water molecules were removed from DNA PDB file and polar hydrogen atoms were added with Gesteiger charges. The coordinates of ligands in its *Z* and *E* configuration were obtained from Dundee PRODRG server.[2] The docking was carried out with Lamarckian Genetic Algorithm (GA). Some key references: Berman, H. M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T. N.; Weissig, H.; Shindyalov, I. N.; Bourne, P. E. *Nucleic Acids Res.* **2000**, *28*, 235; Schuettelkopf, A. W.; Van Aalten, D. M. F. *Acta Crystallogr.* **2004**, *D60*, 1355; Morris, G. M.; Goodsell, D. S.; Huey, R.; Olson, A. J. *J. Comput. Aided Mol. Des.* **1996**, *10*, 293; Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. *J. Comput. Chem.* **1998**, *19*, 1639.
11. The allene will surely be a mixture of diastereomers. However, these could not be distinguished by ^{1}H NMR.
12. The cleavage efficiency was determined by checking the relative UV-absorbance of the bands at 260 nm. The cleavage efficiency was also measured by densitometry using image processing software (Kodak 1D version V.3.6.3) and similar results were obtained. The identity of the bands has been ascertained from the control DNA which has form I as a major band. It is to be noted that the control DNA specimen is usually contaminated with some nicked form (form II). The reason for having alkaline pH 8.5 was to aid the propargyl to allene isomerization.
13. The DNA-binding studies were carried out by absorption titrations which involved addition of a solution of Calf Thymus DNA to a fixed concentration of the probe. A small red shift of the absorption maxima for both the *E*-sulfones (375 nm) and *Z*-sulfones (a shoulder near 438 nm) was observed.