Section 1A.1

Introduction

An important aspect of development of various anticancer drugs is to target the DNA causing its destruction by oxidative pathway.[1] There are various mechanisms by which DNA can be cleaved amongst which the radical mediated abstraction of hydrogen from the sugar moiety followed by oxidative cleavage[2] as well as alkylation of DNA bases[3] cover a wide range of molecules. The enediyne natural products and the N-mustards are representative examples of each of these classes.

Apart from these, the bispropargyl sulphones have recently emerged as a new class of DNA-cleaving agents.[4] They exert their activity *via* isomerisation to the allenic sulfone which subsequently follows either a Maxam-Gilbert type cleavage[5] or an oxidative cleavage through Garratt-Braverman rearrangement.[6, 7] The reactivity of bispropargyl sulfone is observed only under alkaline pH when it gets isomerised to the corresponding allene form.[8] Recently, from our laboratory, we have reported a novel photochemical way to enhance the DNA-cleaving efficiency of bispropargyl cyclic sulfones. The process involves the photoisomerisation of the thermally stable *E*-form (**1A.001**) to the *Z*-form (**1A.002**) which, by virtue of higher chemical reactivity towards isomerization into bis allenic sulphone, brings about greater extent of DNA cleavage.[9]

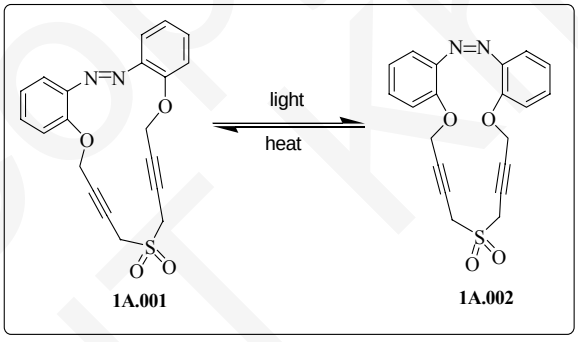


Figure 1A.01

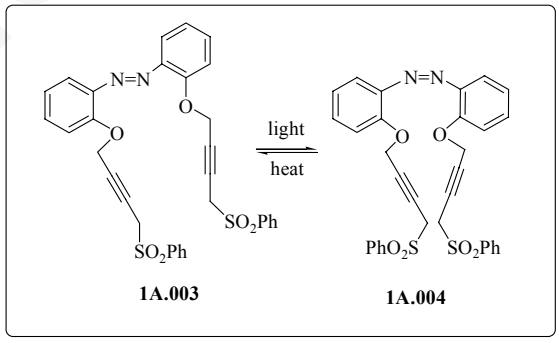


Figure 1A.02

In the present dissertation, the design, synthesis and reactivity of novel azobenzene based bispropargyl bissulfone (**1A.003 & 1A.004**) are described and its reactivity (chemical, photochemical and biological) is compared with the reactivity of the cyclic bispropargyl

sulfone, the chemistry of which is already established from our laboratory. The rationale behind having an azobenzene based template is also highlighted.

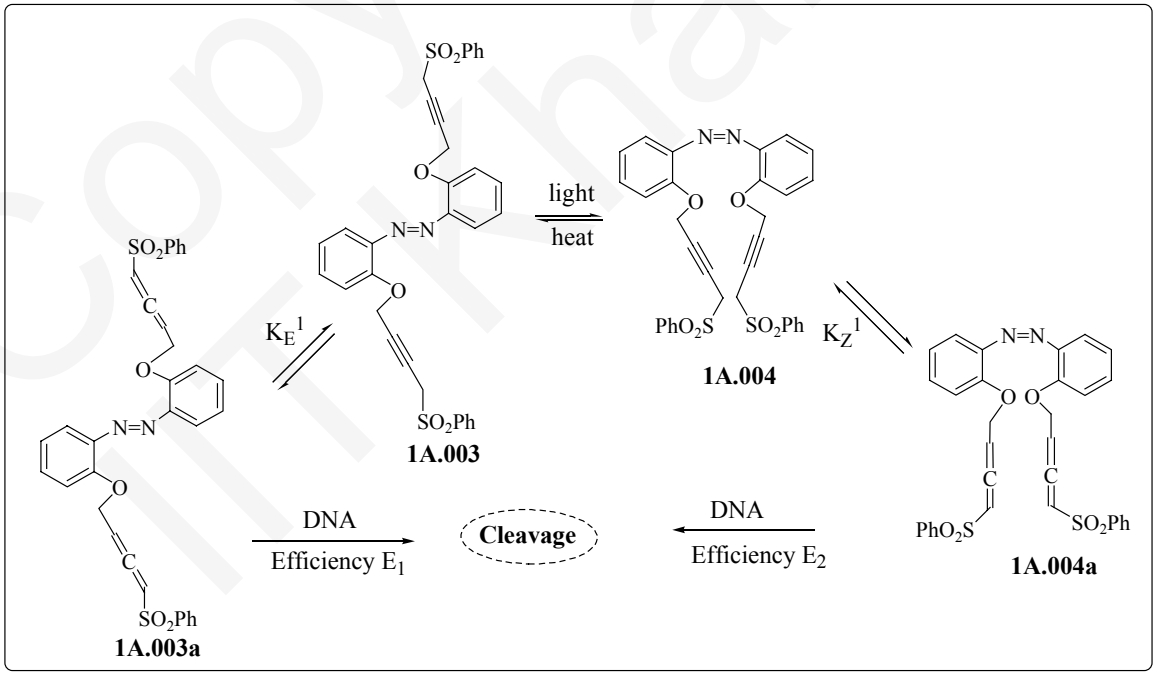
Section 1A.2

Objective

As the cyclic bispropargyl sulfones (**1A.001 & 1A.002**) showed novel chemical and biological properties, we were tempted to check the chemical as well as biological properties of the acyclic analogues (**1A.003 & 1A.004**). In particular we would like to address the following points:

a) The extent of isomerisation from bispropargyl to bisallenic form in the acyclic analogue (for both the trans and the cis forms).
b) The chemical stability of the bisallene in cis isomer of the acyclic analogue.
c) The rate of thermal reisomerisation of the azo system from cis to trans form and the comparison of rates between the cyclic and the acyclic sulfones.
d) The efficiency of DNA cleavage and comparison with that of the cyclic analogue.

The above objectives can be summarized in **Scheme 1A.01**



Scheme 1A.01

To fulfil our above objectives, we designed the sulfones **1A.003** and **1A.005** (**1A.005** was chosen as another reference for acyclic propargylic sulfone)**.**

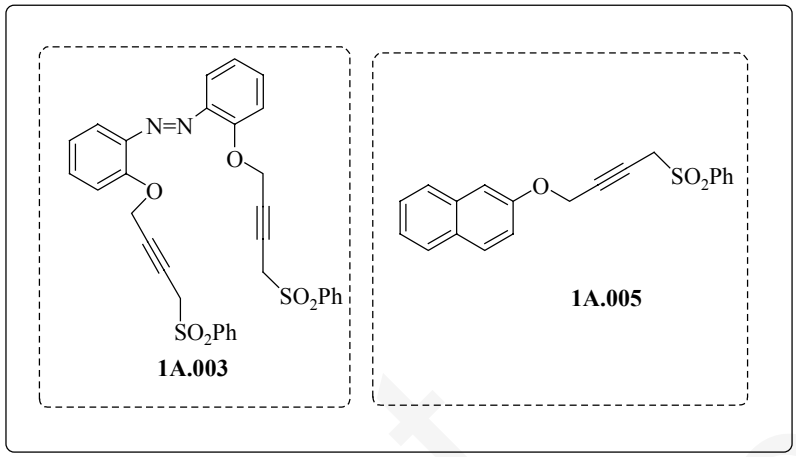


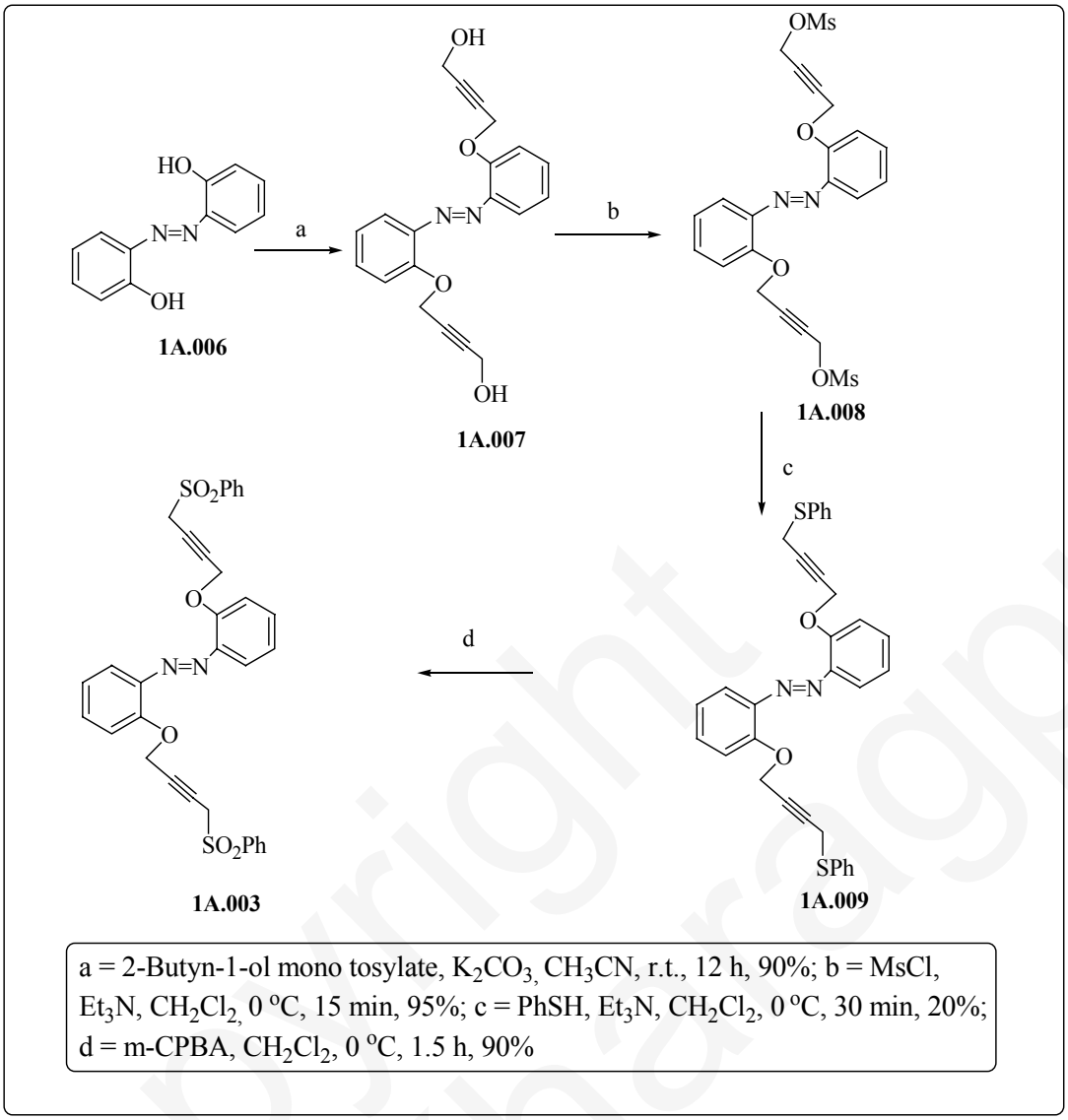
Figure 1A.03: The target acyclic sulfones

Section 1A.3

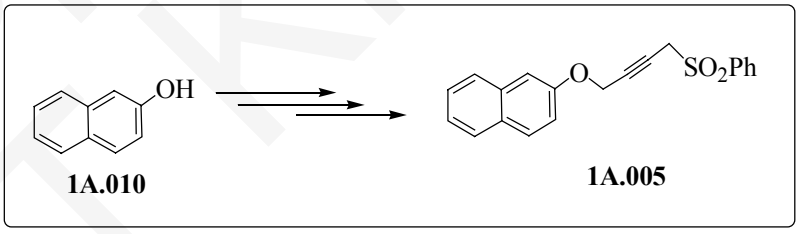
Synthesis

The starting material in case of **1A.003** is the commercially available 2, 2'–bishydroxy azobenzene (**1A.006**). This was doubly alkylated with butyn-1, 4-diol mono tosylate in presence of K_2CO_3 and DMF. The resulting diol (**1A.007**), isolated in 90% yield, was converted to the bismesylate (**1A.008**) (95% yield) followed by nucleophilic displacement by thiophenol (PhSH) in presence of triethylamine giving bissulphide (**1A.009**) in 20% yield. The sulfide upon oxidation with m-CPBA at 0 ^oC gave the desired sulfone (**1A.003**) which was isolated as orange solid after column purification in 90% yield (**Scheme 1A.02**). The formation of the sulfone (**1A.003**) was confirmed by the appearance of two 4H multiplets in the ^1H NMR spectrum at **δ 4.91** and **δ 3.99**, as compared to 4H singlets at **δ 4.83** and **δ 3.56** in the sulfide **1A.009.**

The other target compound **1A.005** was sythesised in a similar fashion starting from β-napthol as described in **Scheme 1A.02** as an oily liquid in 60% yield. It was also characterized by spectral data.



Scheme 1A.02: Synthesis of sulfone **1A.003**



Scheme 1A.03: Synthesis of sulfone **1A.005**

Section 1A.4

Spectral Characterization

The structures of both the sulfones **1A.003** and **1A.005** were in agreement with NMR and mass spectral data.

The methylene hydrogens in the bissulfide (**1A.009**) appeared as two separate singlets at **δ 4.83** and **δ 3.56**. Upon oxidation the methylene protons are shifted downfield, now appearing at **δ 4.91** and **δ 3.99** and as multiplets. All the proton assignments are shown in **Figure 1A.04**.

The structures of the other sulfone (**1A.005**) and the corresponding sulfide (**1A.011**) were similarly confirmed by NMR and mass spectroscopic data. The proton assignments are shown in **Figure 1A.05**.

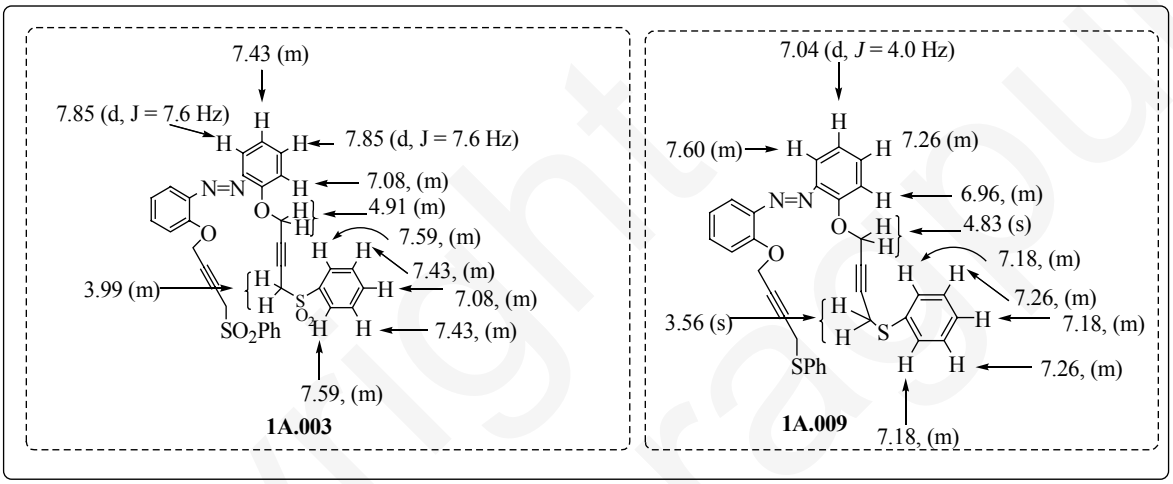


Figure 1A.04

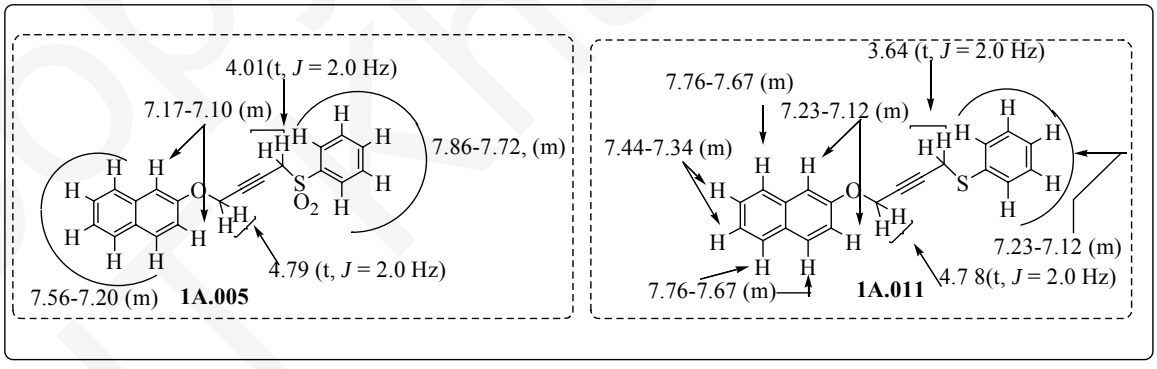


Figure 1A.05

Final confirmation about the structures came from ESI mass spectrum which showed the MH^+ peaks at m/z 599 and 337 for **1A.003** and **1A.005**, respectively.

It is to be noted that the sulfone **1A.003** when isolated by column chromatography, exists in the most stable trans (*E*) configuration. This is deduced from the following:

i) Strong UV absorption maxima at λ_{max} 360 nm characteristic of the π-π * transition.[10]

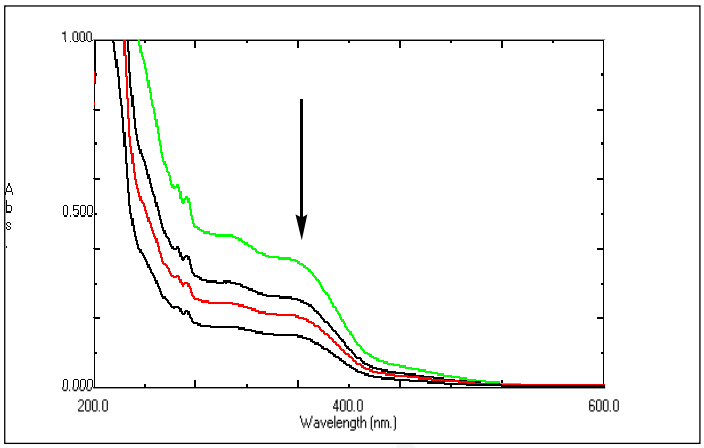


Figure 1A.06: UV absorption spectra

ii) The appearance of the aromatic protons at downfield region **δ** 7.85-7.43 indicating planarity of the aromatic rings in the azo benzene moiety which is achievable only in the trans form.

Section 1A.5

Isomerization Study

After recording the UV spectra of **1A.003**, we proceeded with the photoisomerization study. The wave length for the π-π * transition (354 nm) was selected as the irradiation wave length. Thus, the DCM solution of the compound (in stable *E* form) was taken and was irradiated with a high pressure Hg-lamp for 5 h. The colour of the solution was initially orange, which upon irradiation became yellow. The solvent was removed under cold condition and the ^{1}H NMR was recorded. It produced a photostationary state rendering a 3:1 mixture of *Z* and *E*-isomers with the *Z*-isomer showing a new λ_{max} at 410 nm. In the ^{1}H NMR spectrum, new peaks arrived at **δ** 6.6 (2, 2'-aromatic **H**s) and **δ** 4.6 (-OC**H**$_2$) besides signals for aromatic hydrogens which were assigned to the *Z*-isomer (**Figure 1A.07**). The kinetics of thermal reisomerization was followed by monitoring the ^{1}H-NMR at different time points at a temperature of 25 ^{o}C. Kinetic study showed the first order rate constant of 0.050 h^{-1} and $t_{1/2}$ of 14 h (**Figure 1A.08**). Interestingly, the thermal reisomerization was significantly slower as compared to the cyclic sulfone **1A.002** where strain as well as π-π repulsion[11] obviously playing major roles in speeding up the

reisomerization. The NMR solution upon heating at 60 °C for 1.5 hr showed complete conversion of the *Z*-isomer to the *E*-isomer.

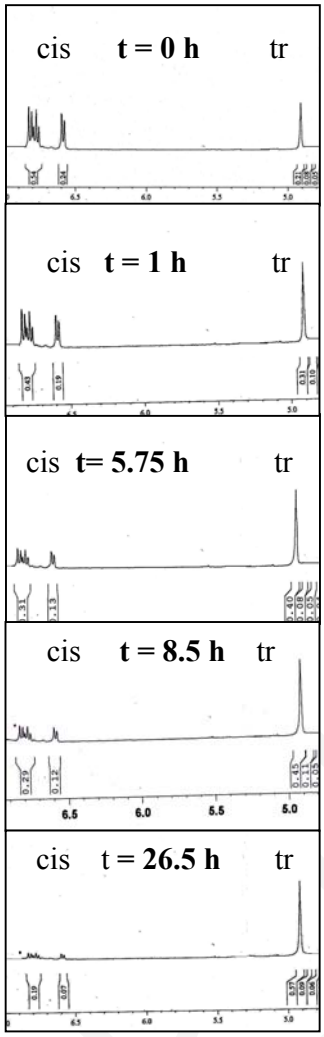
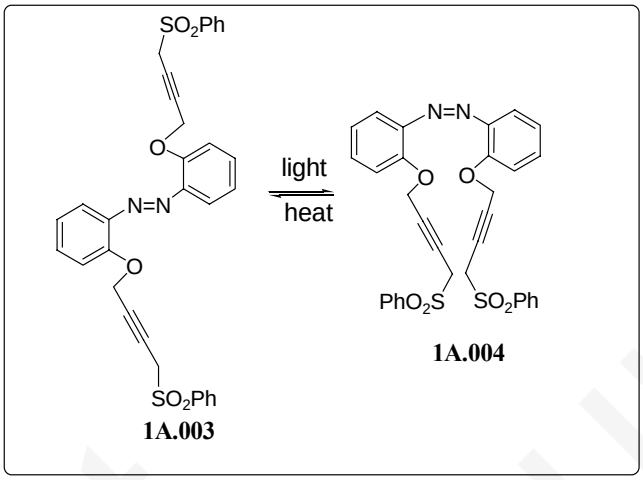


Figure 1A.07



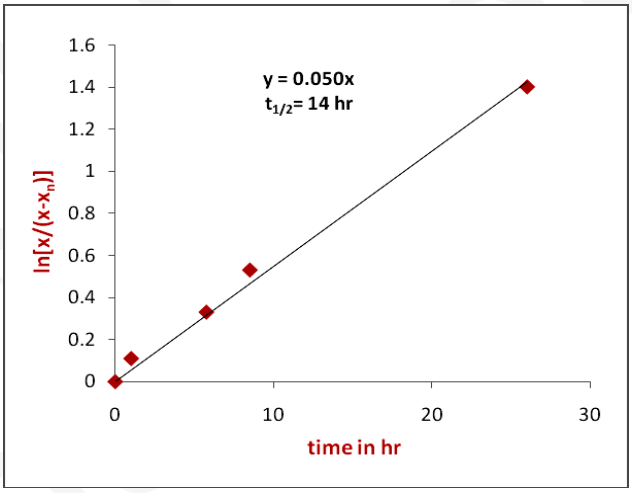


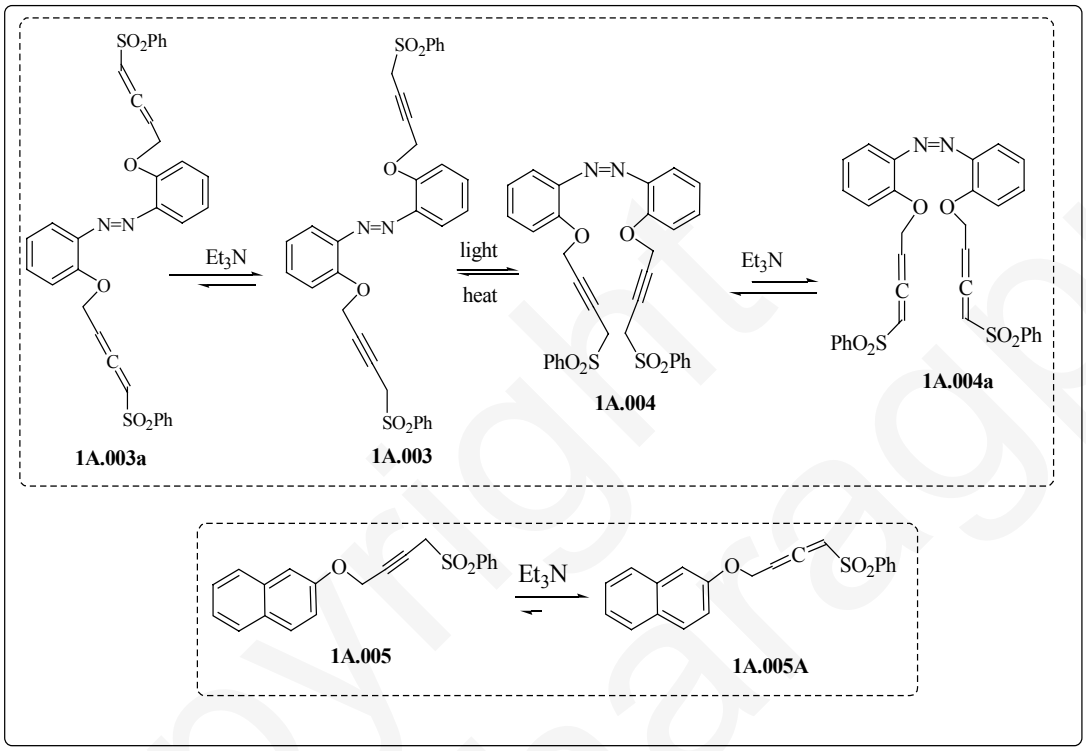
Figure 1A.08

Section 1A.6

Chemical Reactivity under Basic Conditions

The chemical reactivity of the sulfone **1A.003** under basic condition was evaluated by studying the kinetics of isomerisation from propargyl to allene. For this, a $CDCl_3$ solution of the compound **1A.003** was taken and triethylamine (3.0 eq) at 25 °C was added and the progress of the reaction was followed by monitoring the ^1H NMR profile. Within 10 min, the equilibrium was achieved between the bispropargyl and bisallenic bissulfone; although the equilibrium favoured the bispropargyl system (ratio of propargyl to allene was 6:1). Similar result was observed in case of the *Z*-sulfone **1A.004**. The formation of

allene moiety was indicated by the appearance of new peaks at the olefin region at **δ** 6.28 and **δ** 6.09. The ratio of bispropargyl to bisallene did not alter even after keeping the solution for 12 h at 25 ^{o}C. This result is in sharp contrast with that of the monopropargyl sulfone **1A.005**. In this case though the equilibrium was reached within 15 min, however, the allene **1A.005a** was favoured under equilibrium condition (ratio of allene to propargyl was 2:1).



Scheme 1A.04

In case of isomerization of sulfone **1A.003** or **1A.004**, the formation of bis allene was confirmed by trapping with methanol[12] to produce the dimethoxy derivative detected by mass spectrometry, m/z 631 (MH^+). Peak corresponding to the monoallene could not be observed. The ^{1}H NMR spectrum is also consistent with the bisallene. Similarity of the two propargylic arms may be the reason for the failure to observe the monoallene which is immediately converted to bisallene. Regarding the inversion of allene-propargyl ratio for **1A.003/1A.003a** or **1A.004/1A.004a** pair as compared to the **1A.005/1A.005a** pair, it is possible that the steric strain as imposed by the peri-hydrogens (**Figure 1A.09**) to the methylene is less in the propargyl form than that in the allene form for **1A.003/1A.003a** or **1A.004/1A.004a** pair. For **1A.005/1A.005a** pair, the greater conformational flexibility

allows the formation of more stable allene form in excess. The aryl-O bond length is possibly larger in the propargyl form because of higher electronegativity of sp-carbon. The NMR profiles are shown in **Figure 1A.10** and **Figure 1A.12.**

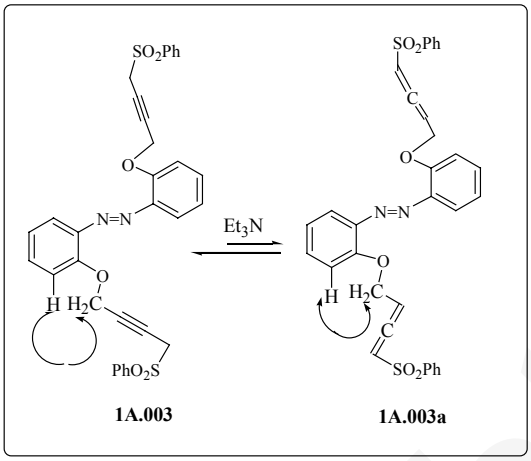


Figure 1A.09

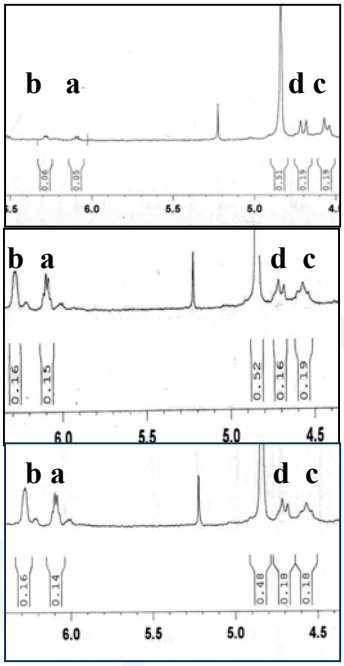


Figure 1A.10

In Fgure 1A.09:
a,b : peaks for bisallene
c,d : peaks for bispropargyl

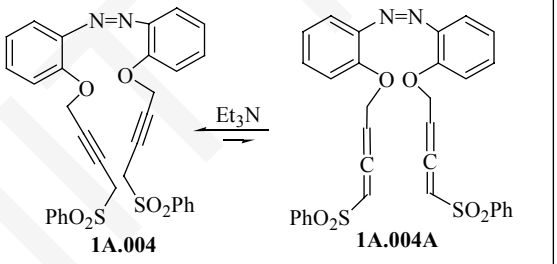


Figure 1A.11

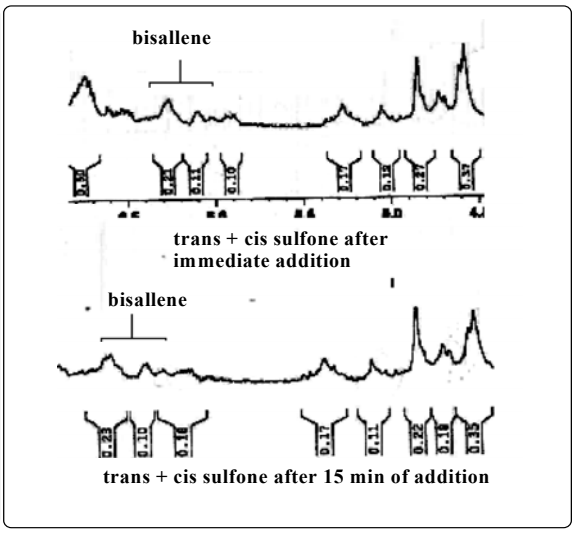


Figure 1A.12

Section 1A.7

DNA Cleavage Experiments

The efficiency of DNA-cleavage of the bissulfones was then studied. We expected lower extent of cleavage as the cleavage can take place only via alkylation, cleavage via Garratt-Braverman rearrangement is not possible in this case though in case of cyclic sulfone both the mechanisms are possible. The experiments were carried out at 37 ^{o}C using pBR 322 supercoiled plasmid DNA at pH 8.5. Although the better thermal stability of the *Z*-isomer **1A.004** in comparison to the corresponding cyclic analogue **1A.002**, helped us to carry out longer incubation for DNA-cleavage study, but the lower extent of allene formation reduced the efficiency of DNA-cleavage. As a matter of fact, both the *E*-isomer **1A.003** and the 3:1 mixture of of *Z*- and *E*- isomers (**1A.004** and **1A.003**) are both poor DNA-cleaving agent[13]. The mixture however showed ~20% better cleavage compared to the **1A.003**. This is probably because of better binding capacity of **1A.004** (*Z*-isomer) than **1A.003** (*E*-isomer) due to greater complimentarily in shape[14] which is evident from the 3D view (**Figure 1A.13**).

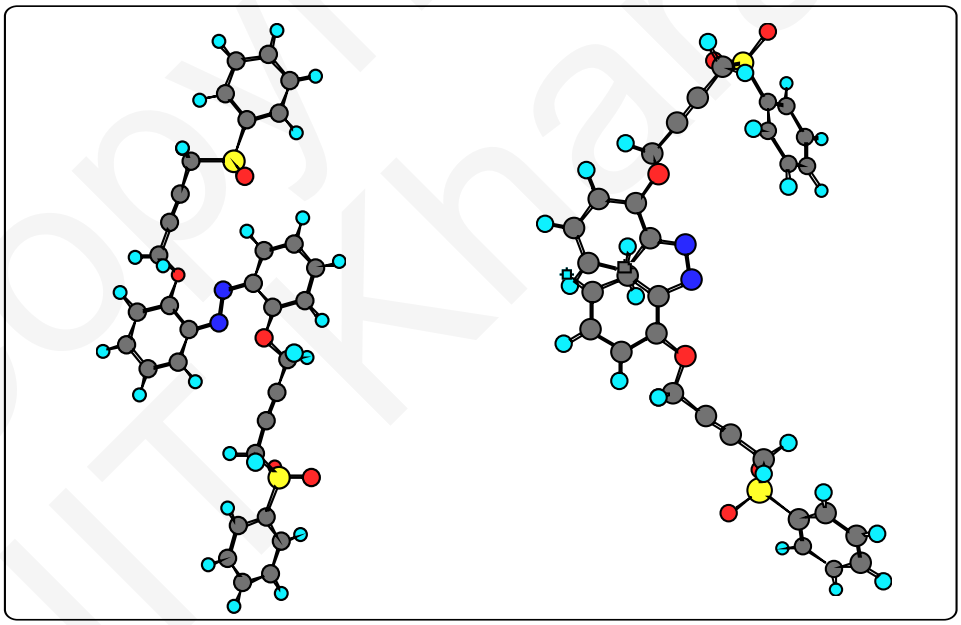


Figure 1A.13

3D view of compound 1A.003 and 1A.004

The monopropargyl sulfone **1A.005** also showed slightly higher cleavage activity as compared to sulfone **1A.003**. This is because of greater extent of allene formation in the latter case. However both the acyclic sulfones were much inferior as compared to the cyclic one in terms of DNA-cleavage.

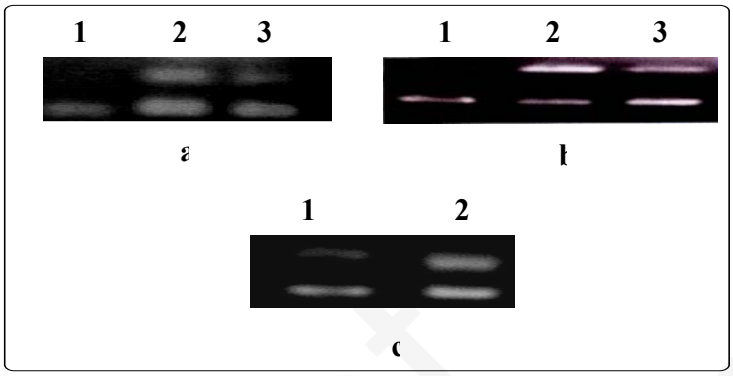


Figure 1A.14

Figure 1A.14: (**a**) DNA cleavage experiment of compounds **1A.003** and **1A.004** after 2.5 h incubation at 37 ^{o}C; **lane 1**: control DNA in TAE buffer (pH 8.5, 7 µL) + CH_3CN (10 µL); **lane 2**: DNA in TAE buffer (pH 8.5, 7 µL) + ***Z***-sulfone **1A.004** (0.02 mM) in CH_3CN (5 µL); **lane 3**: DNA in TAE buffer (pH 8.5, 7 µL) + ***E***-sulfone **1A.003** (0.02 mM) in CH_3CN (5 µL); (**b**) DNA cleavage experiment of compounds **1A.002** & **1A.001** after 1.5 h incubation at 37 ^{o}C; **lane 1**: control DNA in TAE buffer (pH 8.5, 7 µL) + CH_3CN (10 µL); **lane 2**: DNA in TAE buffer (pH 8.5, 7 µL) + ***Z***-sulfone **1A.002** (0.02 mM) in CH_3CN (5 µL); **lane 3**: DNA in TAE buffer (pH 8.5, 7 µL) + ***E***-sulfone **1A.001** (0.02 mM) in CH_3CN (5 µL); (**c**) DNA cleavage experiment of compound **1A.005** after 2.5 h incubation at 37 ^{o}C **lane 1**: control DNA in TAE buffer (pH 8.5, 7 µL) + CH_3CN (10 µL); **lane 2**: DNA in TAE buffer (pH 8.5, 7 µL) + sulfone **1A.005** (0.02 mM) in CH_3CN (5 µL)

Compound No.	**1A.001**	**1A.002**	**1A.003**	**1A.004**	**1A.005**
% cleavage	**27**	**39**	**40**	**70**	**42**

Table 1A.01: Determination of % of DNA cleavage from Densitometry

Section 1A.8

Conclusion

a) We have successfully synthesized acyclic azo bispropargyl bissulfone.

b) The *Z*-isomer of the acylic sulfone is more thermally stable than that of the cyclic analogue.

c) The DNA-cleavage ability of the acyclic analogue is less compared to the cyclic analogue.

d) The presence of having a sulfone in a cyclic network is very important in order to develop highly efficient DNA-cleaving agent.

Section 1A.9

Experimental

Section 1A.9.1

General Experimental

Melting points (m.p.) were recorded on a Toshniwal hot-coil stage melting point apparatus and were uncorrected. Among the spectra, ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a 200 MHz (Bruker) and 400 MHz (Bruker) spectrometer. ^{2}H-chloroform was used as solvent unless otherwise mentioned. The peak at δ 7.26 for residual $CHCl_3$ was taken as a reference. Chemical shifts were expressed in δ unit and ^{1}H-^{1}H coupling constants in Hz. IR spectra were recorded on Perkin-Elmer 883 using KBr pellet for solids and $CHCl_3$ as a solvent or neat for liquids. The characteristic peaks were expressed in cm^{-1}. Mass spectra were obtained from CRF-IIT, Kharagpur.

All the dry solvents used for reactions were purified according to the standard protocols. Benzene and tetrahydrofuran (THF) were distilled from sodium/ benzophenone under inert atmosphere. Chloroform and dichloroform (DCM) were dried over phosphorus pentoxide (P_2O_5) or calcium hydride (CaH_2). Ethanol and methanol were dried over calcium oxide (CaO) and then over magnesium turnings. Acetonitrile, triethylamine (Et_3N) and n-butylamine were distilled from calcium hydride. Potassium carbonate (K_2CO_3) was dried by heating followed by vacuum drying. Acetone was distilled from iodine/potassium permanganate under dry argon/nitrogen atmosphere. All the solvents for chromatography (column and preparative layer) were distilled prior to use. In most of the column chromatographic purifications, ethyl acetate (EA/EtOAc) and petroleum ether (PE) of boiling range 60-80^{o}C were used as eluents. Columns were prepared with silica gel (Si-gel, 60-120 mesh, SRL).

The phrase “usual work-up” or worked up in “usual manner” refers to the washing of the organic phase with water, brine, drying over anhydrous sodium sulphate, filtration and evaporation under reduced pressure.

Section 1A.9.2

Gel Electrophoresis of DNA

Preparation of Electrophoresis Buffer

Tris-(hydroxymethyl)-aminomethane (6.05 gm) and glacial acetic acid (CH_3COOH) (1.43 mL) were dissolved in 25 mL H_2O. 2.5 mL of an EDTA solution (which was made by dissolving 1.86 g EDTA in 10 mL 1 (N) NaOH) was also added to the above solution. From this stock solution, 2 mL was taken and diluted 50 times with H_2O to make the TAE electrophoresis buffer.

Preparation of 1.1% Agarose Gel

Agarose (1.1 gm) was suspended in 100 mL of electrophoresis buffer. The mixture was heated to ~90 ^oC till the solution became transparent. It was cooled to ~50 ^oC and aqueous ethidium bromide solution (10 µL, 0.5 mg/mL) was added to it. Then it was slowly poured on to the gel container.

Control DNA solution

10 µL pBR322 DNA-solution of concentration 250 µg / mL was mixed with 10 µL of 40% sucrose solution and 5 µL of 0.25% bromophenol blue solution.

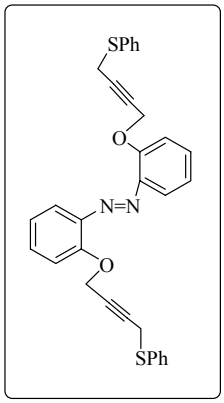
Sample solution

Sample (0.02-0.025 mmol) was first dissolved in CH_3CN (250 µL) of solvent. From this 15 µL was taken and mixed with 15 µL DNA solution (250 µg/mL).The resulting solution was incubated at 37 ^oC for 24 h. The solution was then mixed with 40% sucrose (20 µL) and 0.25% bromophenol blue (5 µL) solution. From the above mixture 20 µL solution was loaded on 1.1% neutral agarose gel and was subjected to electrophoresis in a horizontal slab gel apparatus using 1 x TAE as the buffer. During the 1 h experiment, 75 V was applied by following the convention 1-5 V/cm where cm represents the distance between the electrodes. DNA cleavage was indicated by the formation of relaxed circular DNA (Form II). The bands were photographed using the GELDOC photographic system. The gel documentation was carried with UVP-GELDOC, Cambridge UK. Concentration

of each band was determined by densitometry using image processing software (Kodak 1D version V.3.6.3).

Section 1A.9.3

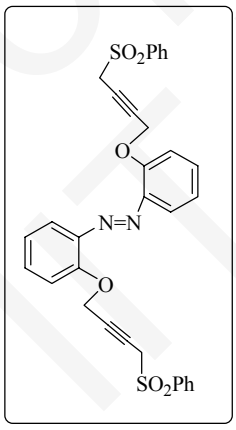
General procedure for the synthesis of the compounds and their spectral data



Bis-[2-(4-phenylsulfanyl-but-2-enyloxy)-phenyl]-diazene (1A.009)

To a solution of mesylate (**1A.008**) (450 mg, 0.89 mmol) in dry DCM (15 mL), Et_3N (2.67 mmol) was added followed by the addition of thiophenol (2.67 mmol) at 0 ^oC. The reaction was allowed to stir at r.t. for 30 min at 0 ^oC. It was then worked up in usual manner. The title compound was isolated by column chromatography (PE: EA = 7:1); **State:** Orange solid; **Yield**: 20%; **m.p.** 120-124 ^oC; **ν_{max} (KBr, cm^{-1})** 2081, 1630, 776; **δ_H (400MHz)** 7.60 (2H, m, aromatic-**H**), 7.26 (6H, m, aromatic-**H**), 7.18 (6H, m, aromatic-**H**), 7.04 (2H, d, *J* = 4.0 Hz, aromatic-**H**), 6.96 (2H, t, *J* = 7.6 Hz, aromatic-**H**), 4.83 (4H, s, -OC**H**$_2$C≡C), 3.56 (4H, s, -C≡C-C**H**$_2$SPh); **δ_C (100 MHz)** 155.0, 143.4, 132.0, 130.0, 128.9, 128.8, 126.8, 121.8, 117.6, 115.4, 83.9, 78.1, 57.8, 22.8; **Mass (ES$^+$):** *m/z* 535 (MH^+).

Bis-[2-(4-phenylsulfonyl-but-2-enyloxy)-phenyl]-diazene (1A.003)

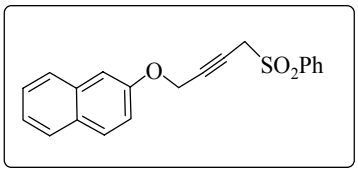


Bissulfide (**1A.009**) (225 mg, 0.42 mmol) was dissolved in 15 mL dry DCM keeping the temperature at 0^o C, then 6 eq (2.52 mmol) of m-CPBA was added and the reaction was allowed to stir for 1.5 h. It was partitioned between DCM and water and the organic layer was washed with saturated aq. solution of $NaHCO_3$, Na_2SO_3, Na_2CO_3 and finally with brine. It was dried over Na_2SO_4, evaporated, and then was subjected to column chromatography to get pure product using PE: EA = 1:1 as eluent; **State:** Orange solid; **Yield**: 90%; **m.p.** 128-132 ^oC; **ν_{max} (KBr, cm^{-1})** 2345,1638, 770; **δ_H (400MHz)** 7.85 (4H, d, *J* = 7.6 Hz, aromatic-**H**), 7.59 (4H, m, aromatic-**H**), 7.43 (6H, m, aromatic-**H**), 7.08 (4H, m, aromatic-**H**), 4.91 (4H, m, -OC**H**$_2$C≡C), 3.99 (4H, m, -C≡C-C**H**$_2$SO$_2$Ph); **δ_C (100**

MHz) 134.3, 134.2, 132.2, 129.2, 129.1, 128.7, 128.6, 122.1, 117.6, 115.2, 82.6, 75.6, 57.4, 42.6; **Mass (ES^+):** *m/z* 599 (MH^+).

2-(4-Benzenesulphonyl-but-2-ynyloxy)-napthalyne (1A.005)

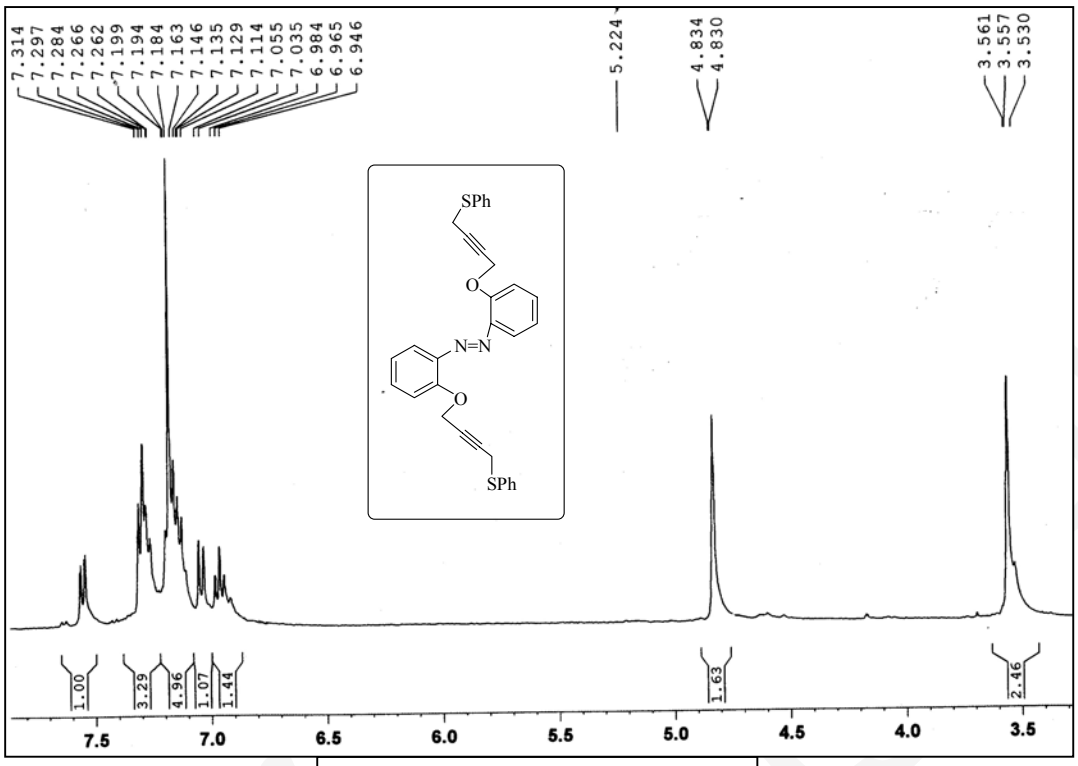
This was synthesized from Napthalen-2-ol (Beta-napthol) following same reaction pathway as described in **Scheme 1**: **State:** Oily liquid; **Yield**: 60%; ν_{max} **($CHCl_3$, cm^{-1})** 2339, 1642, 782; δ_H **(200MHz)** 7.86-7.72 (5H, m, aromatic-**H**), 7.50-7.26 (5H, m, aromatic-**H**), 7.17-7.10 (2H, m, aromatic-**H**), 4.79 (2H, t, *J* = 2.0 Hz, -OC**H_2**C≡C), 4.01 (2H, t, *J* = 2.0 Hz, -C≡C-C**H_2**SO_2Ph); δ_C **(50 MHz)** 157.7, 138.5, 134.6, 133.5, 129.5, 129.4, 129.3, 127.7, 126.8, 126.5, 126.4, 123.7, 118.8, 105.8, 82.3, 79.4, 59.5, 42.2; **Mass (ES^+):** m/z 337 (MH^+).



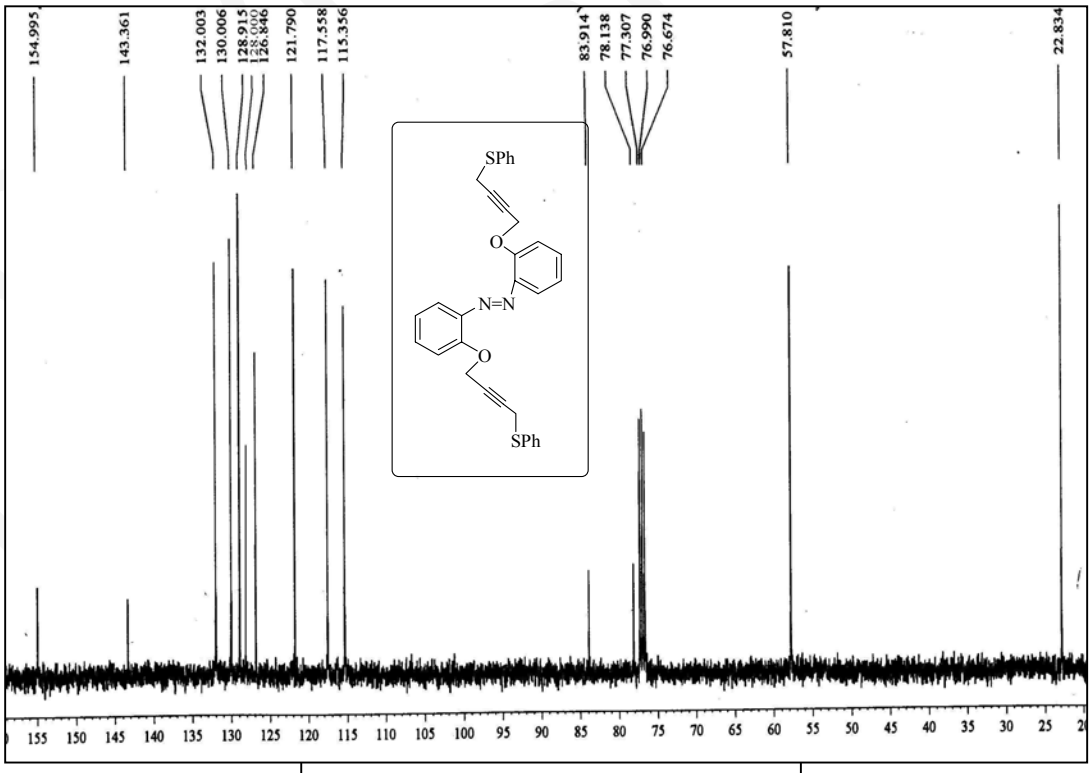
The synthetic procedure and the spectral data of other compounds (**1A.007** and **1A.008**) of this chapter were already reported from our laboratory.

Section 1A.10

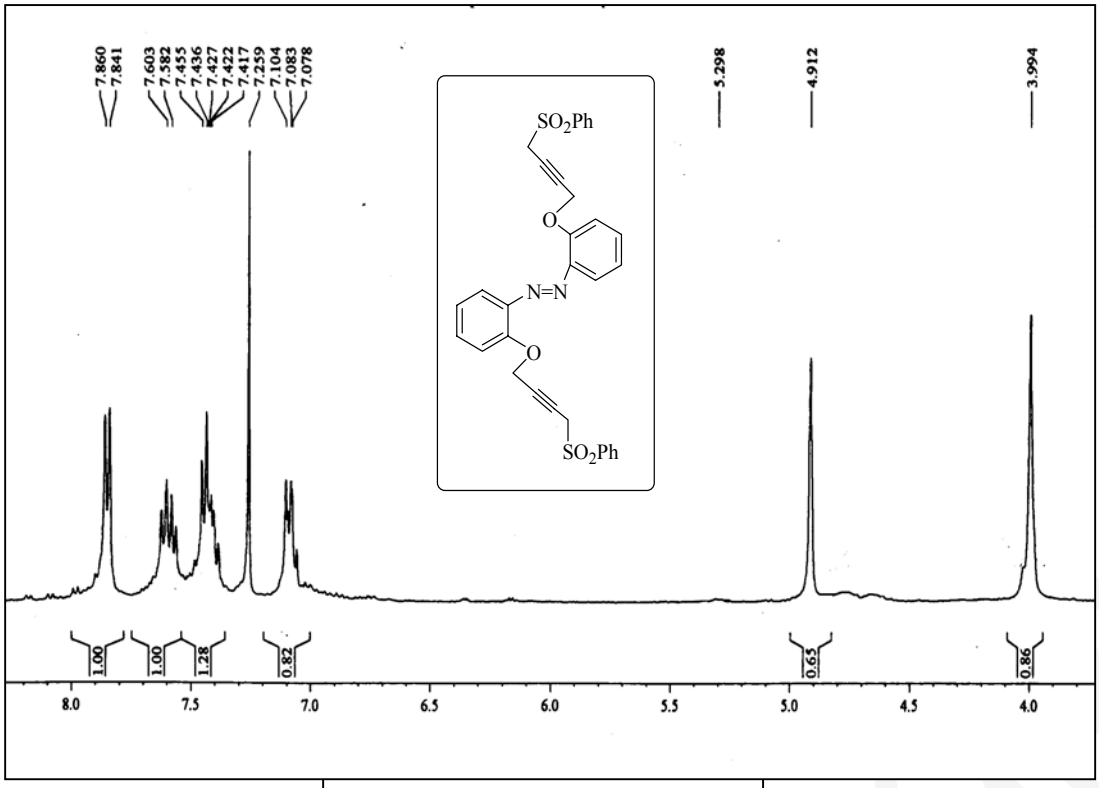
Selected NMR Spectra



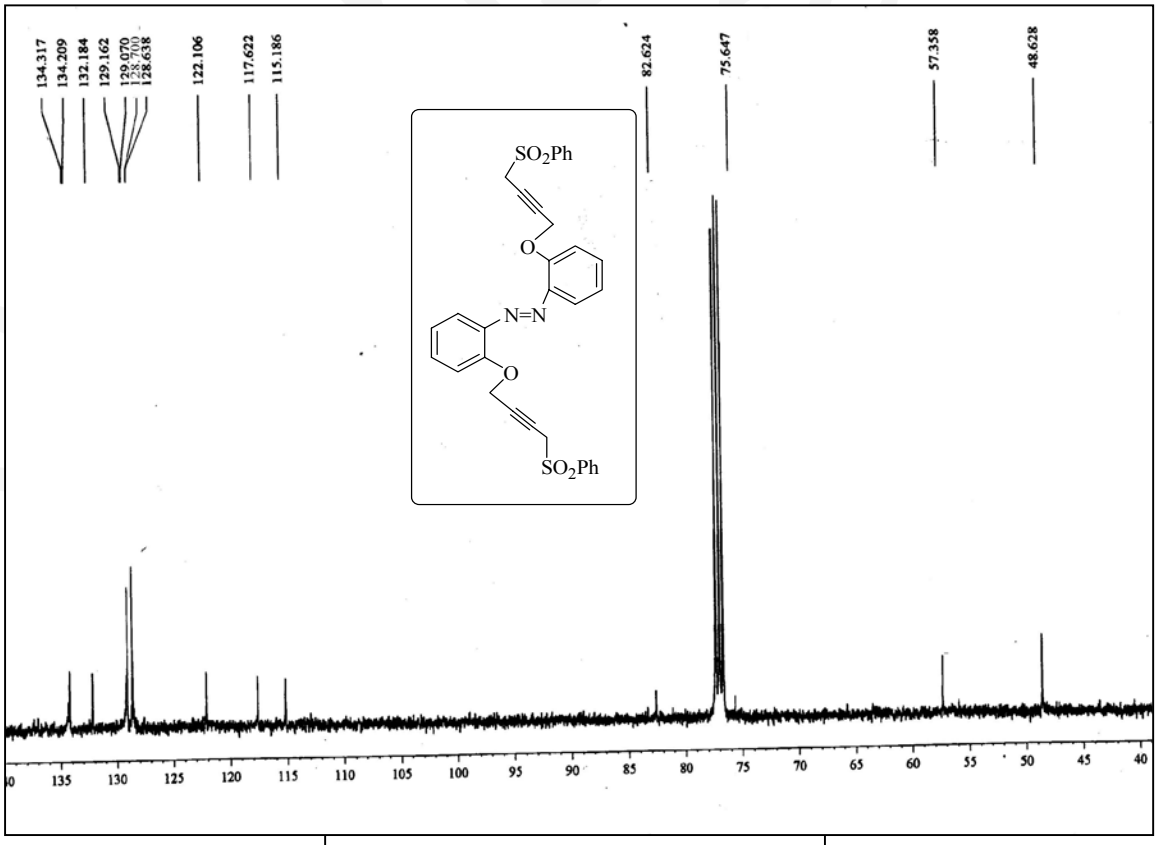
^{1}H NMR of compound 1A.009



^{13}C NMR of compound 1A.009



^{1}H NMR of compound 1A.003



^{13}C NMR of compound 1A.003

Section 1A.11
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13. 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.
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