Supporting Information

Trienediynes on a 1, 3, 5-trisubstituted benzene template: A New Approach for Enhancement of Reactivity

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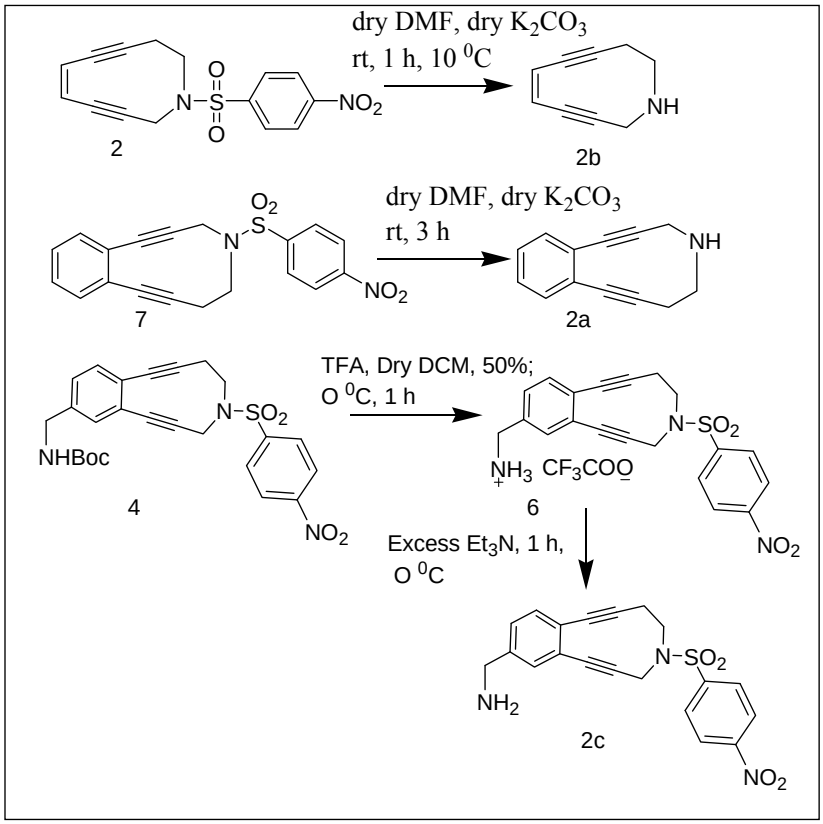
Experimental Section

General Remarks

All the reactions were monitored by TLC using polygram^{R} SILG/UV_{254} precoated (0.25 mm) silica gel TLC plates. Column chromatography was done with silica gel (60-120 or 230-400 mesh). NMR data were obtained with 200 MHz and 400 MHz Bruker NMR instruments. The following abbreviations are used to describe peak patterns where appropriate: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, app. = apparently and b = broad signal. All coupling constants (*J*) are given in Hz. Mass spectra were recorded in ESI+ mode (70 eV).



Synthesis of amine precursor 2a, 2b, 2c

Synthesis of compound **2a**

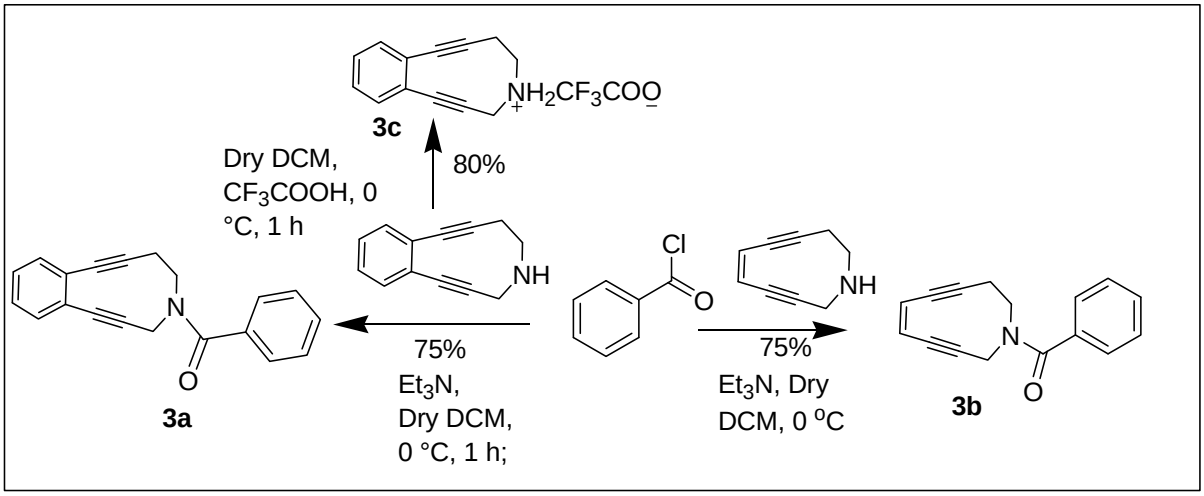
The cyclic sulphonamide 7 (0.110 g, 0.30 mmol) was dissolved in dry DMF (4mL) followed by dry potassium carbonate (0.124 g, 0.90 mmol) and thiophenol (0.036 mL, 0.36 mmol).The reaction mixture was stirred for 1 h at room temperature.The organic layer was extracted with ethyl acetate and concentrated in vacuum using liquid nitrogen, The pure cyclic enediyne amine **2a** was obtained pure by column chromatography as a brown oil using 10% methanol in dichloromethane as the eluent. State: Brown oil; Yield:70%; δ_H (400 MHz, d_6 Acetone): 7.24-7.20 (2H, m), 7.15-7.12 (2H, m), 3.78 (1H, bs), 3.58 (2H, s), 3.18 (2H, t, J=5.4 Hz).

Synthesis of compound **2b**

The cyclic sulphonamide 2 (0.04 g, 0.126 mmol) was dissolved in dry DMF (4 mL) followed by dry potassium carbonate (0.052 g, 0.378 mmol) and thiophenol (0.015 mL, 0.151 mmol).The reaction mixture was stirred for 1 h at 10 °C.The organic layer was extracted with ethyl acetate and concentrated in vacuum using liquid nitrogen, The pure cyclic enediyne amine 2b was obtained purely column chromatography as a brown oil using 10% methanol in dichloromethane as the eluent. State: Brown oil, Yield:75%; ^{1}H-NMR spectrum of the compound 2b was not recorded because the compound was unstable and cyclizes at 17 °C. It was directly taken forward to make the trienediyne **1b.**

Synthesis of compound **2c**

The Boc protected cyclic sulphonamide **4** was dissolved in dry DCM and treated with TFA (10 eq) for 30 min at 0°C and 2 h at 15°C then the whole reaction mixture was evaporated using liquid N_2 in the vacuum pump and washed 3 times with (3×5 mL) dry benzene and the Iintermediate enediyne 6 was finally isolated as the TFA salt. Then the salt is immediately treated with excess Et_3N (10 eq) to provide the free amine 2c for the next step. ^{1}H-NMR spectrum of compound 2c was not recorded because the crude amine **2c** was generated in situ in the reaction medium.



Synthesis of monomeric enediynes

Synthesis of compound **3a/3b**

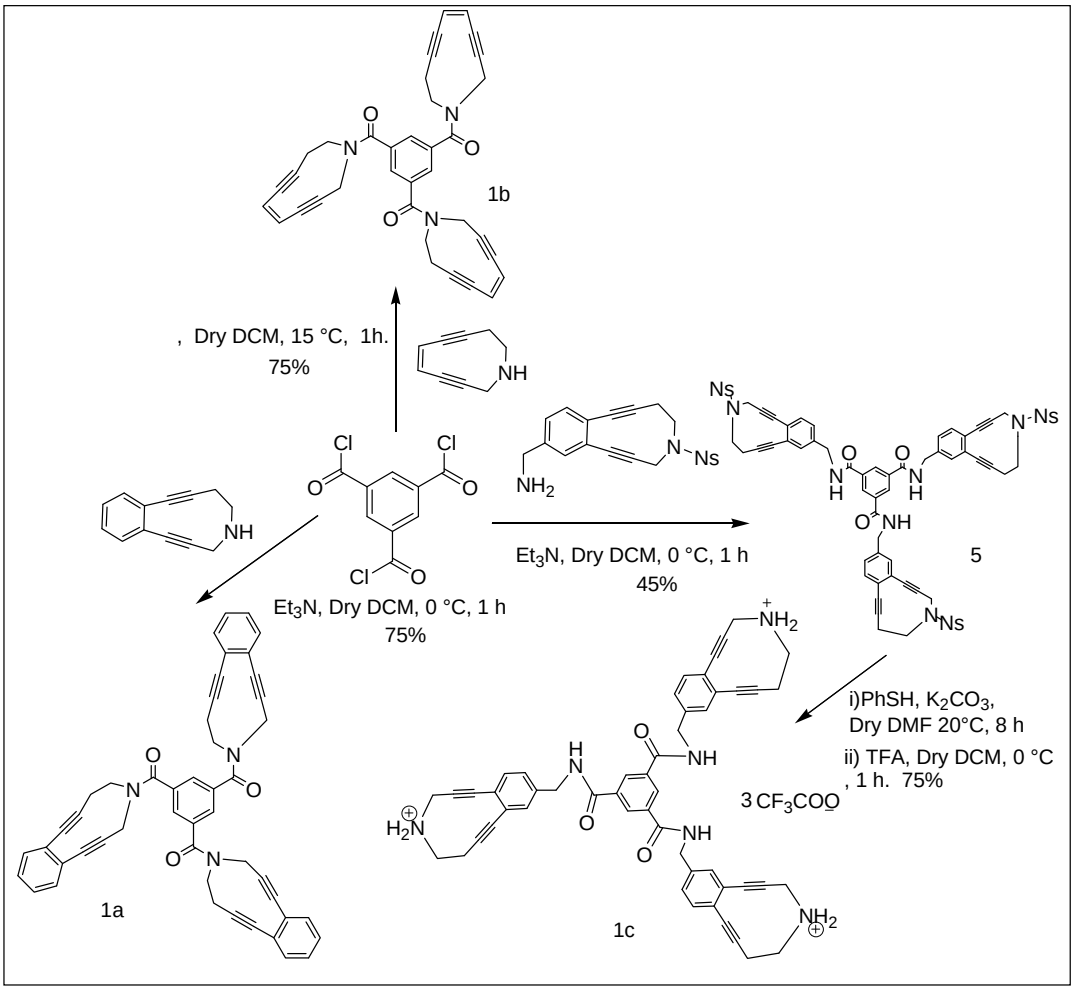
Compound **3a:** To a solution of compound **2a** (50 mg, 0.276 mmol) in dry DCM (15 ml) at 0° C, Benzoyl chloride (25 mg, 0.094mmol) and Et_3N (39 µl, 0.277 mmol) were added to it and the reaction was continued for 30 mins under inert atmosphere. It was then partitioned between DCM and water. The organic layer was washed with brine water, dried over Na_2SO_4 and concentrated in vacuum. The target compound was isolated by column chromatography (Si-gel, PE:EA=2:1) compound **3a** ***State:*** sticky liquid; ***Yield:*** 75%; δ_H (400MHz, $CDCl_3$): 7.62-7.61 (2H, m), 7.41-7.35 (7H, m), 5.28 (2H, s), 4.21 (2H, s), 2.96 (2H, s); δ_C (100MHz, $CDCl_3$): 171.9**,** 135.7, 135.5, 130.3, 129.4, 128.4, 127.6, 127.5, 99.1, 96.1, 93.9, 88.3, 82.6, 50.6, 44.5, 18.9. HRMS (ESI^+) calcd for $C_{20}H_{15}NO$ 285.1155 found 285.1159.

Compound **3b** ***State:*** sticky liquid; ***Yield:*** 75%; δ_H (400MHz, $CDCl_3$): 7.60-7.58 (2H, m), 7.48-7.38 (3H, m), 4.17 (2H, s), 3.89 (2H, s), 2.92 (2H, s); δ_C (100MHz, $CDCl_3$): 171.9**,** 130.3, 128.7, 127.5, 125.4, 122.1, 1036, 97.7, 88.7, 82.3, 50.8, 44.6, 22.9.

Synthesis of compound **3c**

The free amine **2a** was treated with TFA (10 eq) for 30 min at 0°C and 2 h at 15°C then the whole reaction mixture was evaporated using liquid N_2 in the vacuum pump and washed 3 times with (3×5 mL) dry benzene and the target enediyne **3c** was finally isolated as the TFA salt. ***State:*** sticky liquid; ***Yield:*** 67%; δ_H (400 MHz, d^4 MeOH): 7.42-7.32 (4H, m), 4.19 (2H, s), 3.64 (2H, broad s), 2.88 (2H, t, *J*=5); δ_C (100 MHz, d^4 MeOH): 159.5, 128.6, 128.1,

127.8, 126.9, 95.3, 88.9, 87.4, 83.4, 49.3, 39.3, 18.4; HRMS (ESI^+) calcd for $C_{13}H_{12}N$ 182.0971, found 182.0977**.**



Synthesis of trimer enediynes

Synthesis of compound **1a**

To a solution of compound **2a** (50 mg, 0.276 mmol) in dry DCM (15 ml) at 0° C, Mesitoyl chloride (25 mg, 0.094mmol) and Et_3N (39 µl, 0.277 mmol) were added to it and the reaction was continued for 30 mins under inert atmosphere. It was then partitioned between DCM and water. The organic layer was washed with brine water, dried over Na_2SO_4 and concentrated in vacuum. The target compound was isolated by column chromatography (Si-gel, PE:EA=2:1), ***State:*** Solid; δ_H (400 MHz, d^6 Acetone): 7.80 (3H, s), 7.33 (12H, bs), 4.37 (6H, s), 4.35 (6H, bs), 2.87 (6H, bs); δ_C (100 MHz, d^6 Acetone): 170.3, 138.3, 131.0, 127.8, 127.3, 100.1, 95.7, 88.6, 83.3, 51.4, 44.6, 19.3; HRMS (ESI^+) calcd for $[C_{48}H_{33}N_3O_3]H^+$ 700.2600, found 700.2683.

Synthesis of compound **1b**

To a solution of compound **2b** (50 mg, .382 mmol) in dry DCM (15 ml) at 0° C, Mesitoyl chloride (34 mg, 0.127 mmol) and Et_3N (53 µL, .386 mmol) were added to it and the reaction was continued for 30 mins under inert atmosphere. It was then partitioned between DCM and water. The organic layer was washed with brine water, dried over Na_2SO_4 and concentrated in vacuum using liquid N_2. The target compound was isolated by column chromatography (Si-gel, PE:EA=2:1) ***State:*** White solid; ***m.p***. 160°C; ***Yield:*** 64% Solid substance; δ_H (400 MHz, $CDCl_3$): 7.85 (3H, bs), 5.87 (6H, m), 4.15 (6H, s), 3.88 (6H, bs), 2.93 (6H, bs); δ_C (100 MHz, $CDCl_3$): 169.0, 130.1, 128.7, 128.2, 124.5, 122.4, 102.4, 96.4, 87.4, 83.3, 51.3, 42.4, 22.0; HRMS (ESI^+) calcd for $C_{36}H_{27}N_3O_3$ 549.2052, found 549.1736. different fragment of the compound. calcd for $C_9H_3N_3O_3$ 201.0174, found 202.1751, calcd for $C_{18}H_{13}NO$ 291.0891, found 292.1846, calcd for $C_{26}H_{20}N_2O_2$ 392.1525, found 392.2306.

Compound **4** ***State:*** sticky liquid; ***Yield:*** 85%; δ_H (400MHz, $CDCl_3$): 8.26-8.23 (2H, m), 8.11-8.09 (2H, m), 7.26-7.17 (3H, m), 4.88 (1H, bs), 4.33 (2H, s), 4.28 (2H, d, J=5.2 Hz), 3.74 (2H, t, J=5 Hz), 2.77 (2H, t, J=5 Hz), 1.32 (9H, s) ; δ_C (100MHz, $CDCl_3$): 149.9, 145.6, 139.9,129.2, 128.4, 128.3, 127.9, 126.9, 126.8, 124.3, 96.8, 96.7, 92.0, 87.5, 83.6, 79.8, 51.5, 44.1, 42.4, 29.7, 28.4, 27.7, 21.2.

Synthesis of compound **5**

To a solution of compound **2c** (100 mg, 0.089 mmol) in dry DCM (15 mL) at 0° C, Mesitoyl chloride (23 mg, 0.089mmol) and Et_3N (0.1 mL, 0.600 mmol) were added to it and the reaction was continued for 30 mins under inert atmosphere. It was then partitioned between DCM and water. The organic layer was quite viscous and it was concentrated in vacuum and washed with DCM, Hexane and Ether. ***State:*** sticky liquid; ***Yield:*** 55%; δ_H (200 MHz, d^6 DMSO): 9.31-9.27(3H. m), 8.50 (3H, s), 8.37 (6H, d, J=8.6), 8.15 (6H, d, J=8.4), 7.38-7.27 (9H, m), 4.47 (6H, bs), 4.32 (6H, S), 3.50 (6H, bs), 2.77 (6H, bs); δ_C (100MHz, d^6 DMSO): 165.9, 150.4, 143.6, 140.5, 139.9, 135.1, 128.3, 128.0127.9, 127.2, 126.4, 125.1, 99.3, 98.6, 94.9, 94.6, 86.8, 83.1, 51.4, 42.8, 22.2.

Synthesis of compound **1c**

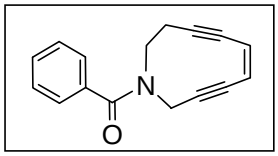
To a solution of compound **5 (**50 mg, 0.036 mmol) in Dry DMF (10 mL), thiophenol (5 µL, 0.055 mmol) and K_2CO_3 (9.936 gm, 0.072 mmol) were added and the mixture was stirred for 8 h at room temperature. After partitioning between water and EtOAc, the organic layer was

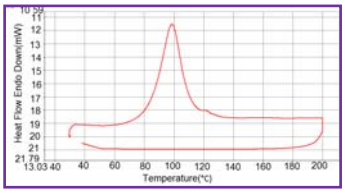
evaporated using liquid N_2 in the vacuum pump and the free amine was isolated by washing with dry MeOH, dry DCM, and dry ether. Then the free amine was treated with TFA (0.02 mL, 0.360 mmol) for 30 m at 0°C, then the whole reaction mixture was evaporated using liquid N_2 in the vacuum pump and washed 3 times with dry benzene and the target enediyne **1c** was finally isolated as the tris-TFA salt. ***State:*** sticky liquid; ***Yield:*** 55%; δ_H (400MHz, d^6 DMSO): 9.36 (3H, s), 7.47-7.34 (9H, m), 4.51 (6H, bs), 4.20 (6H, bs), 3.47 (6H, bs), 2.83 (6H, bs); δ_C (100MHz, d^6 DMSO): 166.0**,** 150.2, 141.2, 139.2, 129.5, 129.4, 128.5, 125.9, 98.8, 90.6, 88.5, 84.1, 55.4, 49.1, 42.9, 19.2.; HRMS (ESI^+) calcd for $[C_{51}H_{42}N_6O_3]H^+$ 787.3397 found 787.3400.

Chemical reactivity: Effect of dendronisation on the kinetics of Bergman cyclization

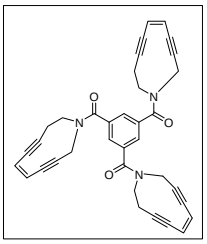
With all substrates in hand, we started to study their chemical behaviour.The thermal stability or in other words chemical reactivity towards Bergman cyclization weas studied by DSC and also by kinetic measurements via ^{1}H-NMR spectroscopy.

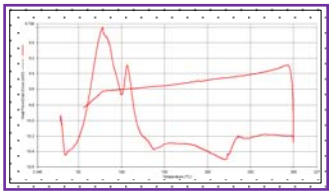
Study of solid phase thermal reactivity by differential scanning calorimetry:



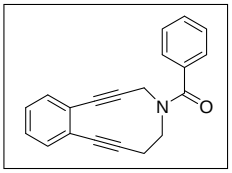


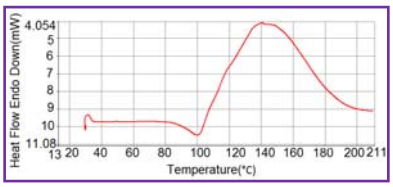
Onset temperature 60°C



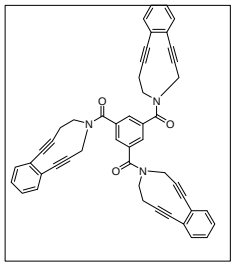


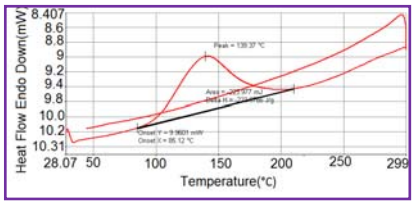
Onset temperature 35°C



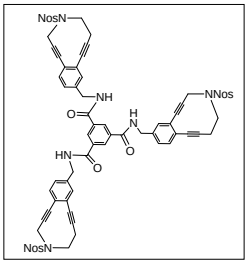


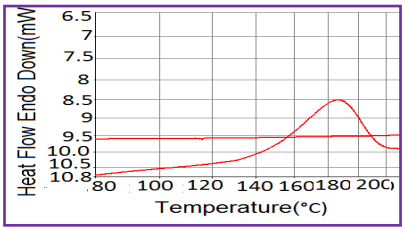
Onset temperature 100°C



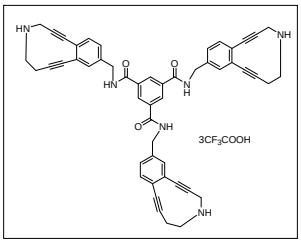


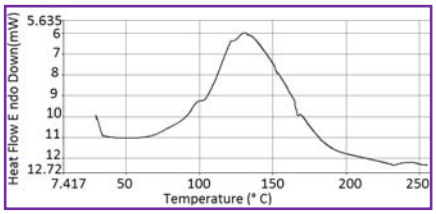
Onset temperature 85°C



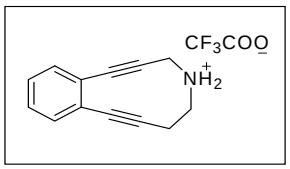


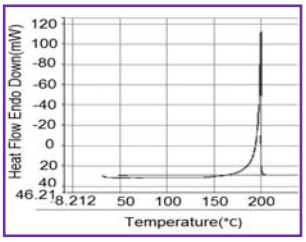
Onset temperature 130°C



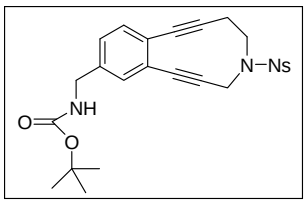


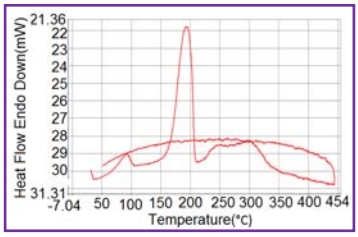
Onset temperature 75°C





Onset temperature 155°C

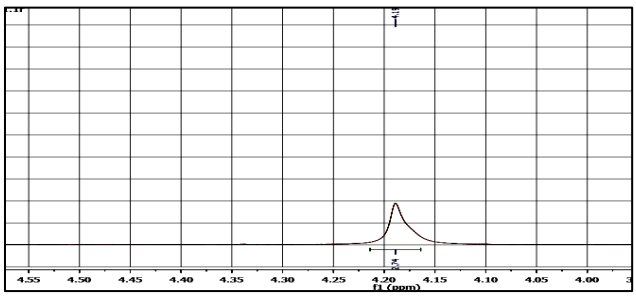




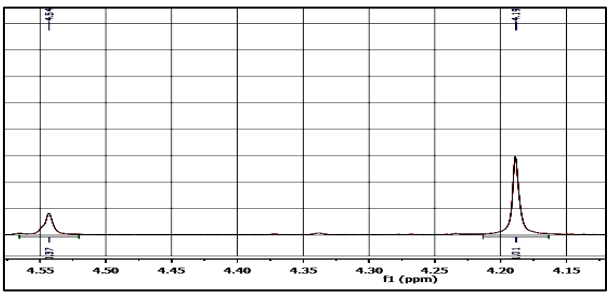
Onset temperature 150°C

Study of solution phase reactivity by NMR kinetics

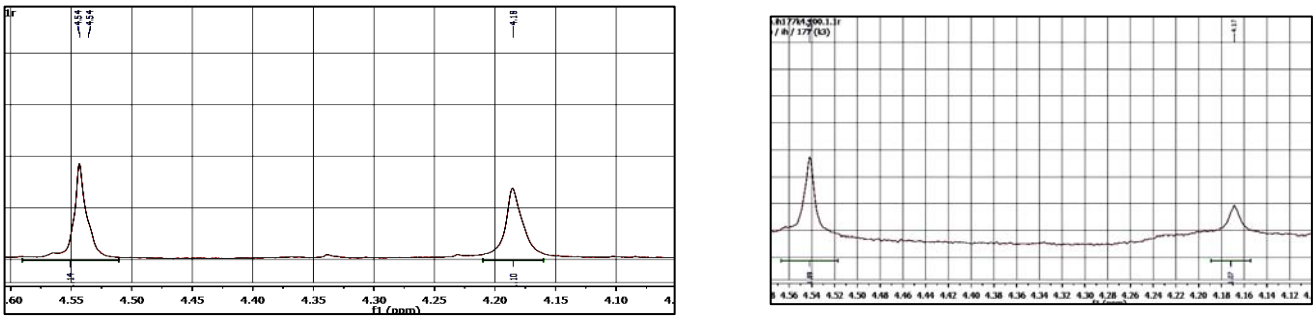
The enediyne **3c** was kept at a constant temperature (75 °C) in d^4- MeOH solution and its ^{1}H-NMR was recorded at different time intervals. New peaks corresponding to the benzisoquinoline derivative began to appear that proved the thermal reactivity corresponds to the Bergman cyclization. In this case, the singlets for the NCH_2 served to follow the kinetics of cyclization. For the isoquinoline derivative, the signal appeared at δ 4.54 while for the reactant **3c** it resonated at δ 4.19.



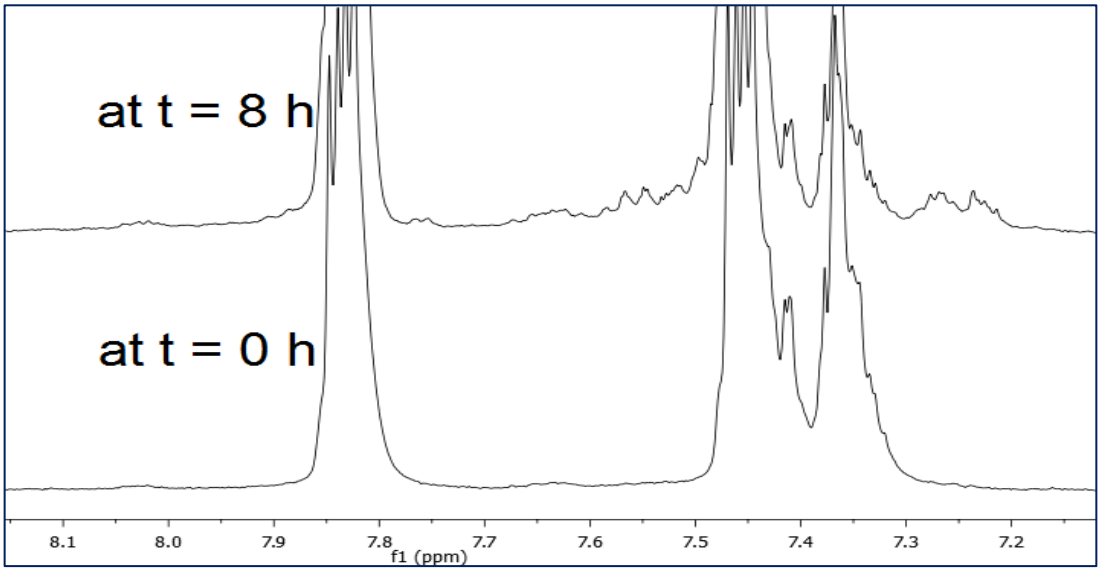
T=0 h



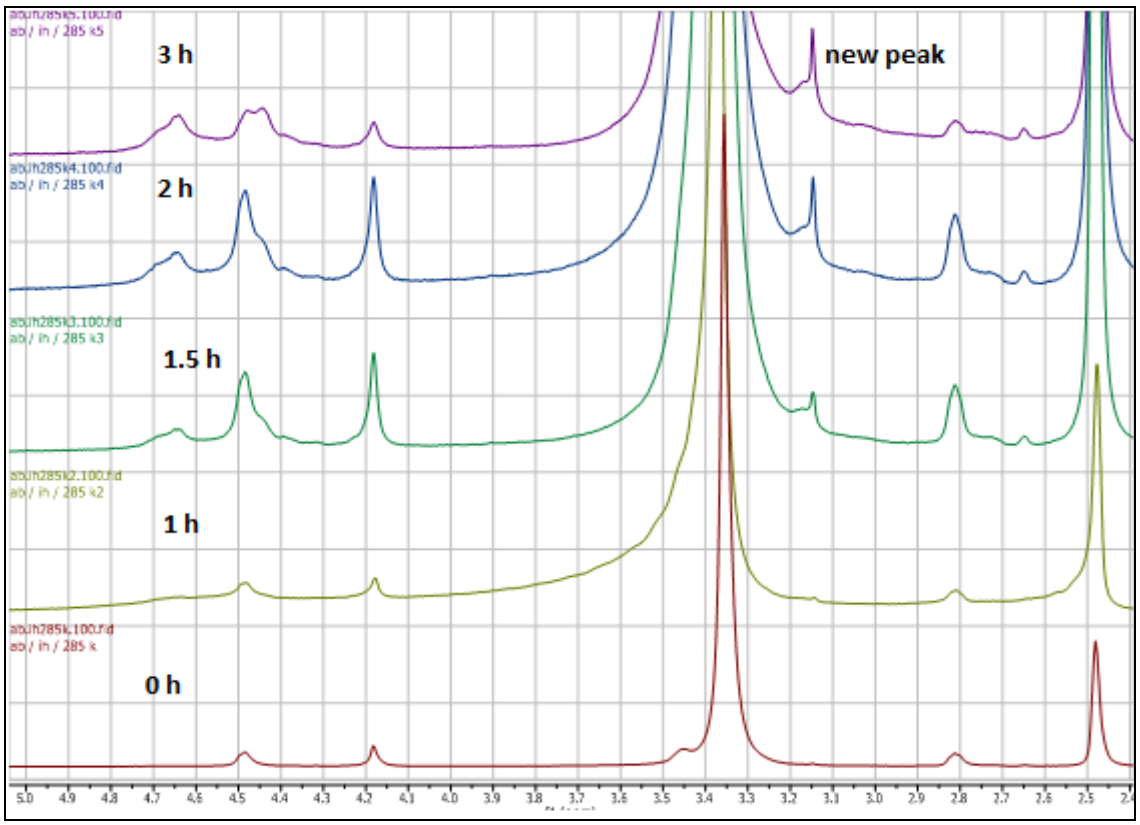
T=4 h



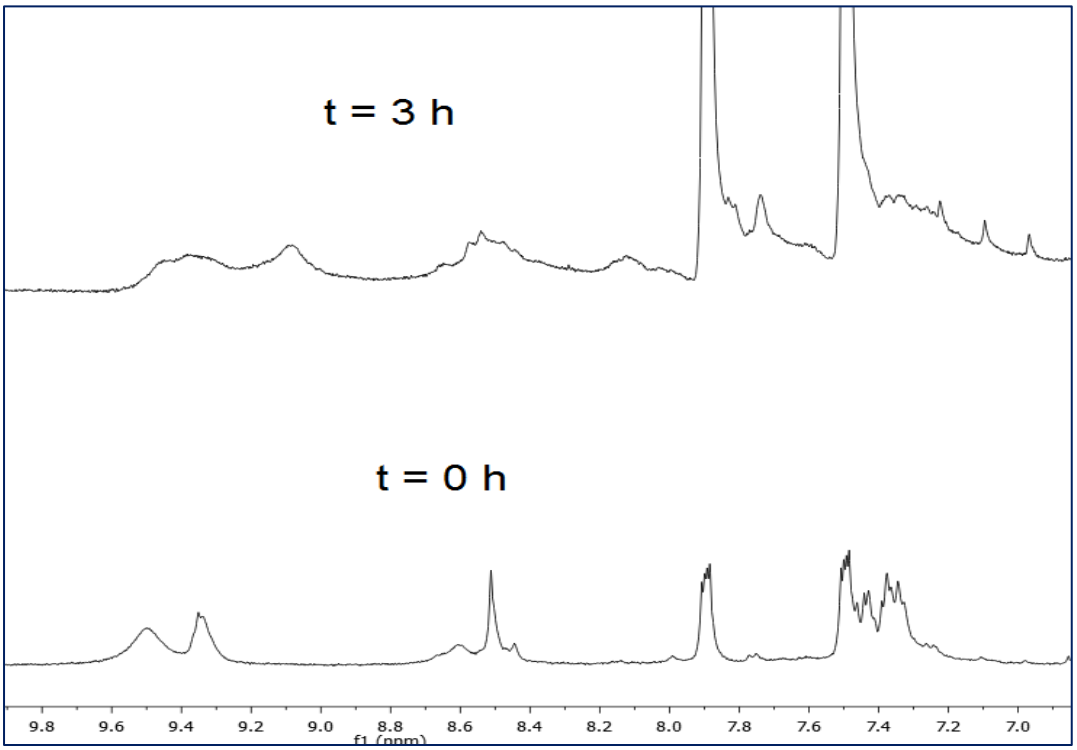
Kinetics of the compound **3c** (Expanded aliphatic region) at 75 °C in d_4- MeOH solution



Kinetics of the compound **3c** (Expanded aromatic region) at 75 °C in d_4- MeOH solution



Kinetics of the compound **1c** (Expanded aliphatic region) at 75 °C in d_6- DMSO solution



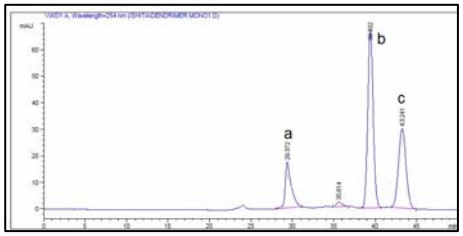
Kinetics of the compound **1c** (Expanded aromatic region) at 75 °C in d_6- DMSO solution

Study of solution phase reactivity by HPLC

The cyclo-aromatization of three ene-diyne units centering a common adaptor complicated the overall situation; an overlapping of signals occured and it was rather difficult to calculate the kinetic parameters on the basis of integration values obtained from such NMR spectra. The matter was finally resolved by high performance liquid chromatographic (HPLC) analysis.The solution phase kinetics was determined by heating a solution of the compounds in a sealed tube at 75 °C in a solution of $CHCl_3$ containing an excess of 1,4 Cyclohexadiene and naphthalene, used as internal standard. 20µL of aliquot from the reaction mixture was injected at regular time intervals for HPLC analysis. The rate of disappearance of starting material followed by concominant appearance of new peaks corresponding to the cycloaromatized products could now be clearly envisoned. From the area under the curve we have calculated the Rate constant (k) and Half life ($t_{1/2}$) of the reactions.

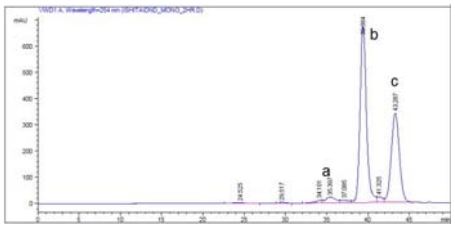
.**HPLC data for compound 3a**

T= 0 **h**



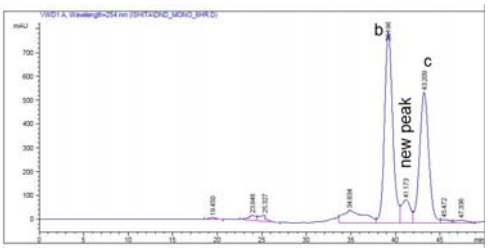
Napthalene: compound **3a** =1.58

T = 2 **h**



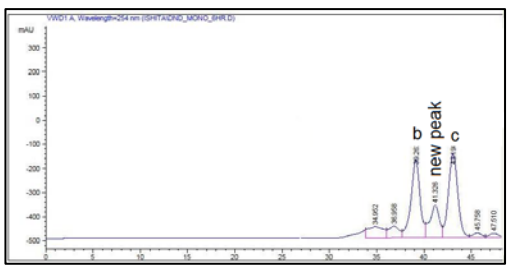
Napthalene: compound **3a** =1.5

T = 6 **h**



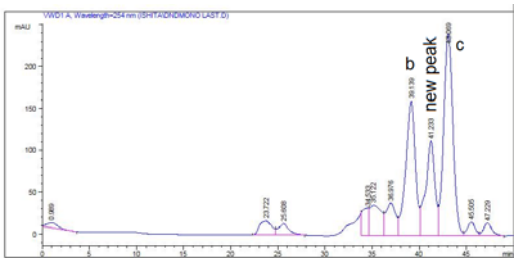
Napthalene: compound **3a** =1.29

T = 14 **h**



Napthalene: compound **3a** =0.90

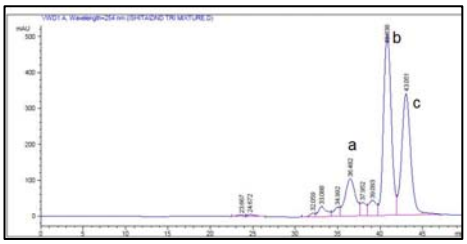
T = 22 **h**



Napthalene: compound **3a** =0.6889

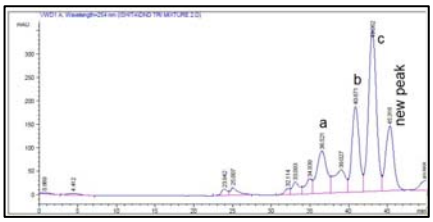
HPLC data for compound 1a

T = 0 **h**



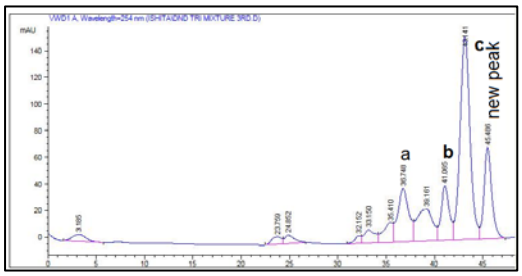
Napthalene: compound **1a** = 1.28

T = 2 **h**



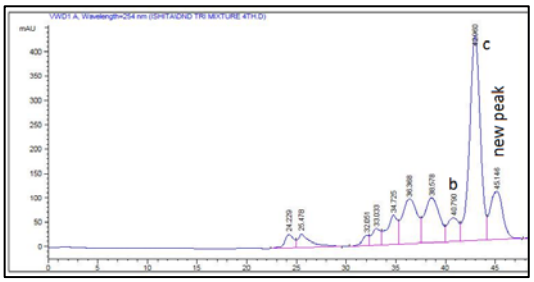
Napthalene: compound **1a** =0.49

T = 4 **h**



Napthalene: compound **1a** = 0.22

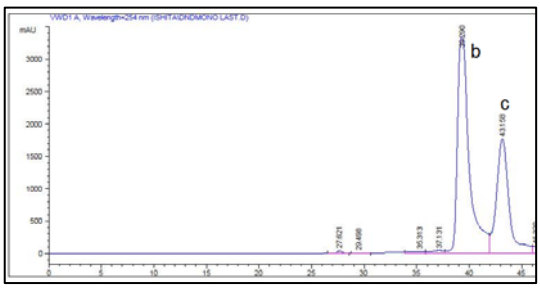
T = 6 **h**



Napthalene: compound **1a** = 0.12

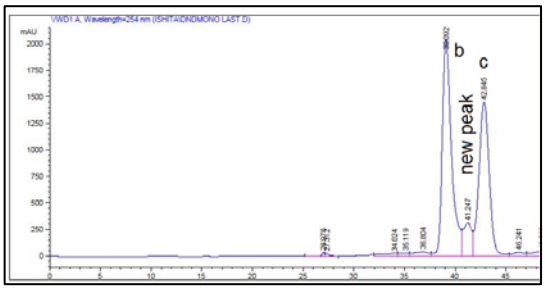
HPLC data for compound 3C

T = 0 **h**



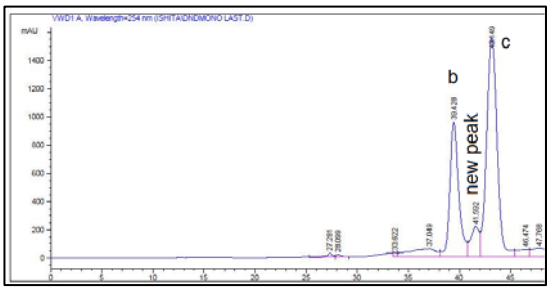
Napthalene: compound **3c** = 1.78

T = 4 **h**



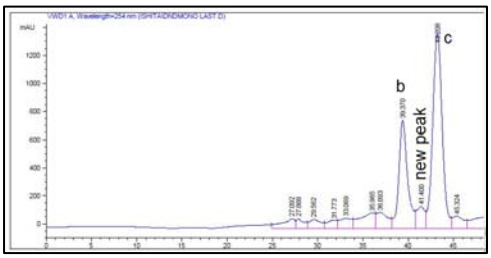
Napthalene: compound **3c** = 1.26

T = 12 **h**



Napthalene: compound **3c** =0.51

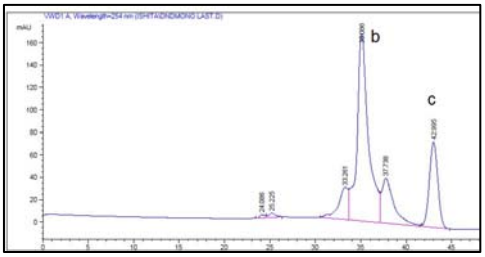
T = 14 **h**



Napthalene: compound **3c** = 0.50

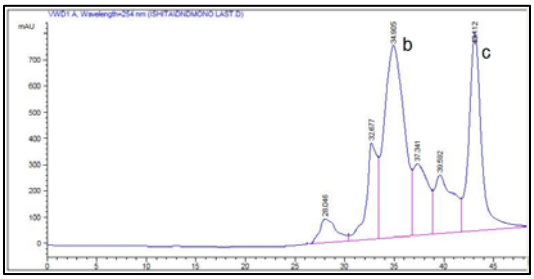
HPLC data for compound 1C

T = 0 **h**



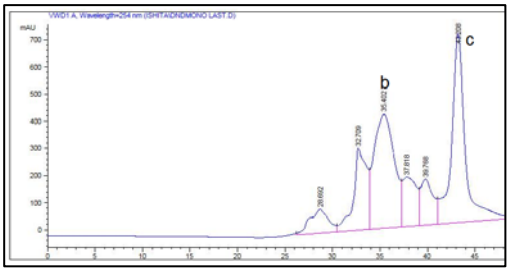
Napthalene: compound 1c =2.96

T = 1 **h**



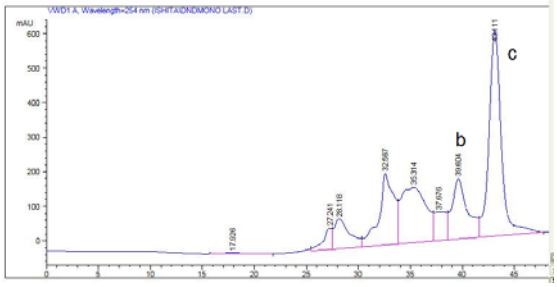
Napthalene: compound **1c = 1.52**

T = 1.5 **h**



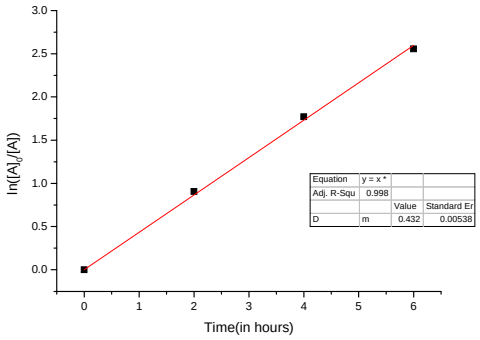
Napthalene: compound **1c** =0.40

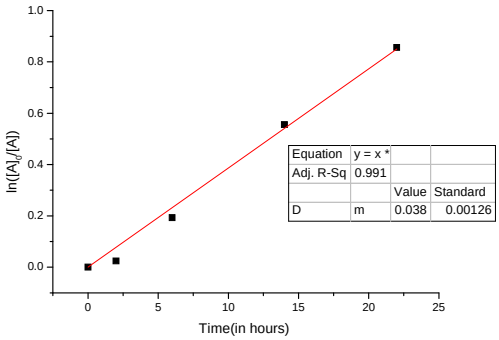
T = 3 **h**



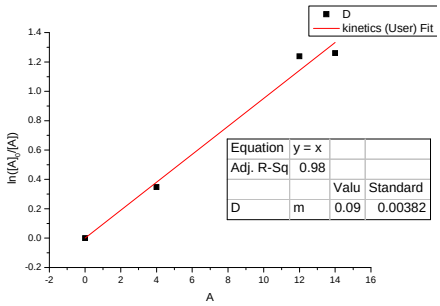
Napthalene: compound **1c** = 0.37

Kinetc plots

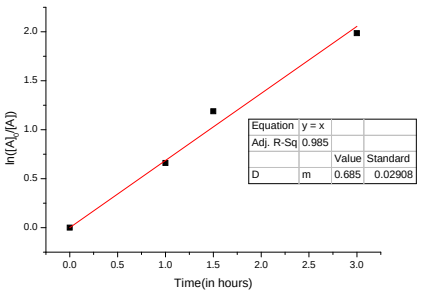




BC kinetic profile of 1a



BC kinetic profile of 3a

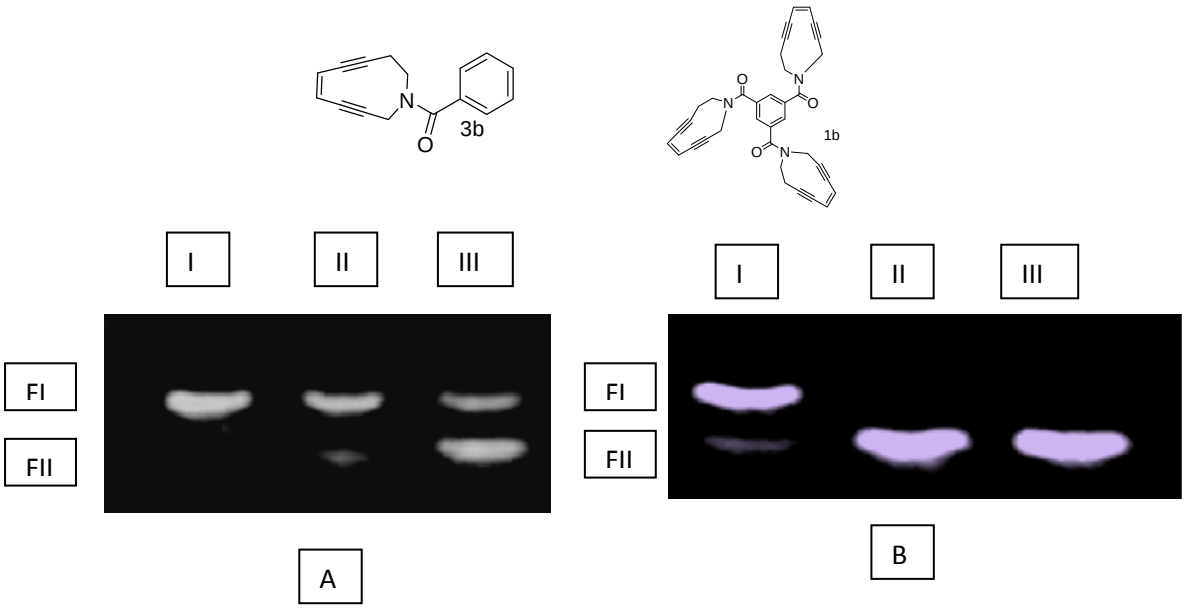


BC kinetic profile of 3c

BC kinetic profile of 1c

Substrate	Rate constant (k)	Half life ($t_{1/2}$)
1a	1.2×10^{-4} Sec^{-1} at 75° C	1.6 h
3a	1.05×10^{-5} Sec^{-1} at 75° C	18.2 h
1c	1.8×10^{-4} Sec^{-1} at 78° C	1.0 h
3c	2.5×10^{-5} Sec^{-1} at 78° C	7.7 h

DNA Cleavage Study with monomer and trimer of the aliphatic analogue



Qualitative Plasmid Relaxation Assays carried out with compounds 3b, 1b(5 µL each from a stock of 20 µM in DMSO) and pBR 322 Plasmid DNA (7 µL from a stock of 0.03 µg/µl at pH 8.0). These were separately mixed with 20 mM phosphate buffer of pH 7.5, incubated at 35° C for 4 h fig A and for 20 h for fig. B ; Lanes I: DNA alone, II: DNA with compound 1b, III: DNA with compound 3b

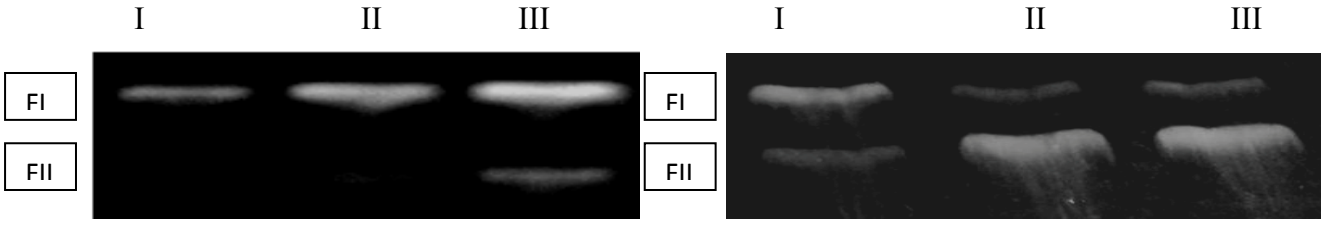
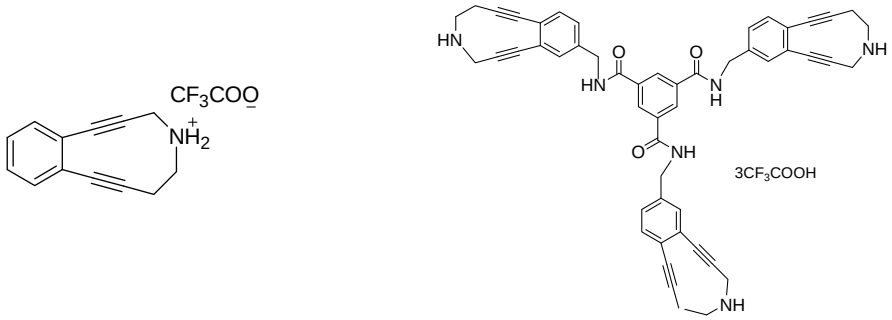


Figure 4: Plasmid Relaxation Assays with compounds **3b** and **1b** (5 µL each from a stock of 20 µM (for **3b**) and 7 µM (for **1b**) in DMSO) and pBR 322 Plasmid DNA (7 µL from a stock of 0.03 µg/µl at pH 8.0). These were separately mixed with 20 mM phosphate buffer of pH 7.5, incubated at 37 ° C for 6 h in **A** and for 24 h in. **B**; Lanes I: DNA alone, II: DNA with **3b**, III: DNA with **1b**.



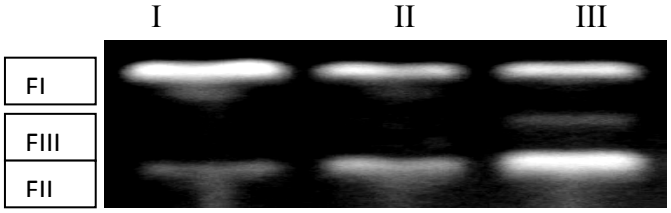
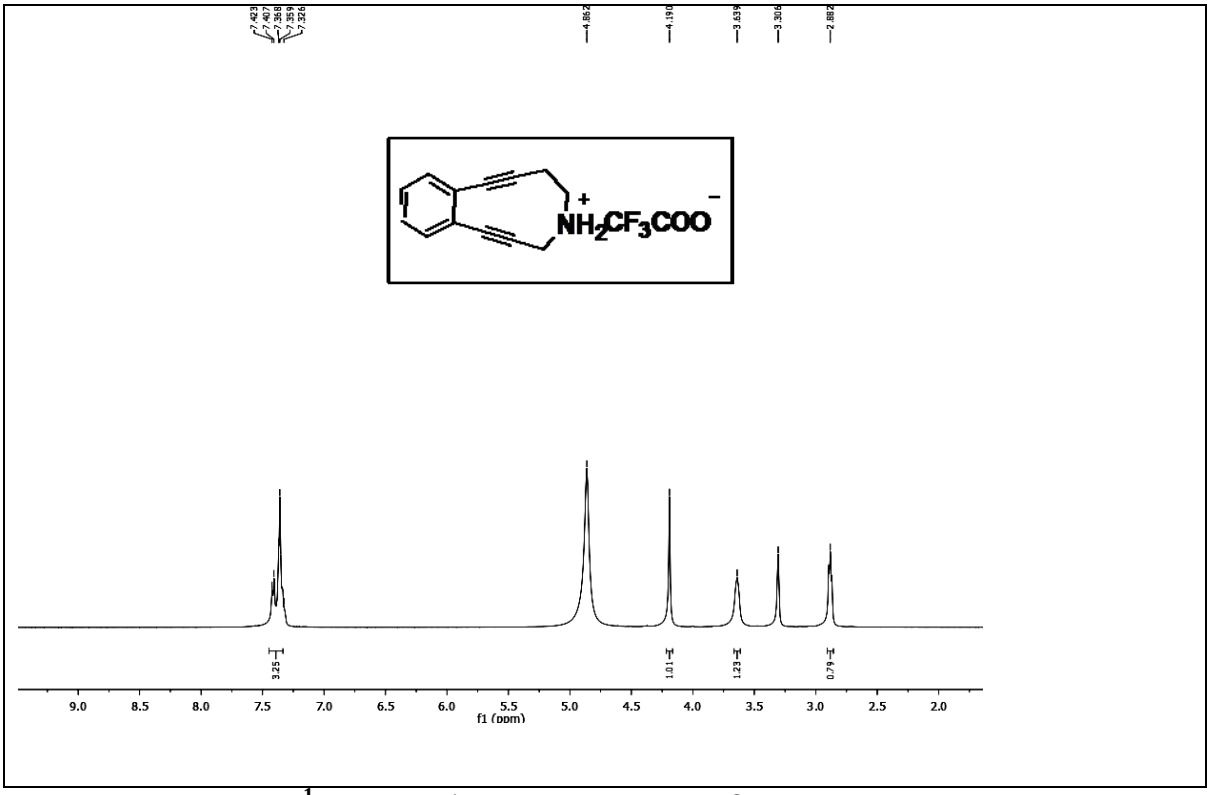
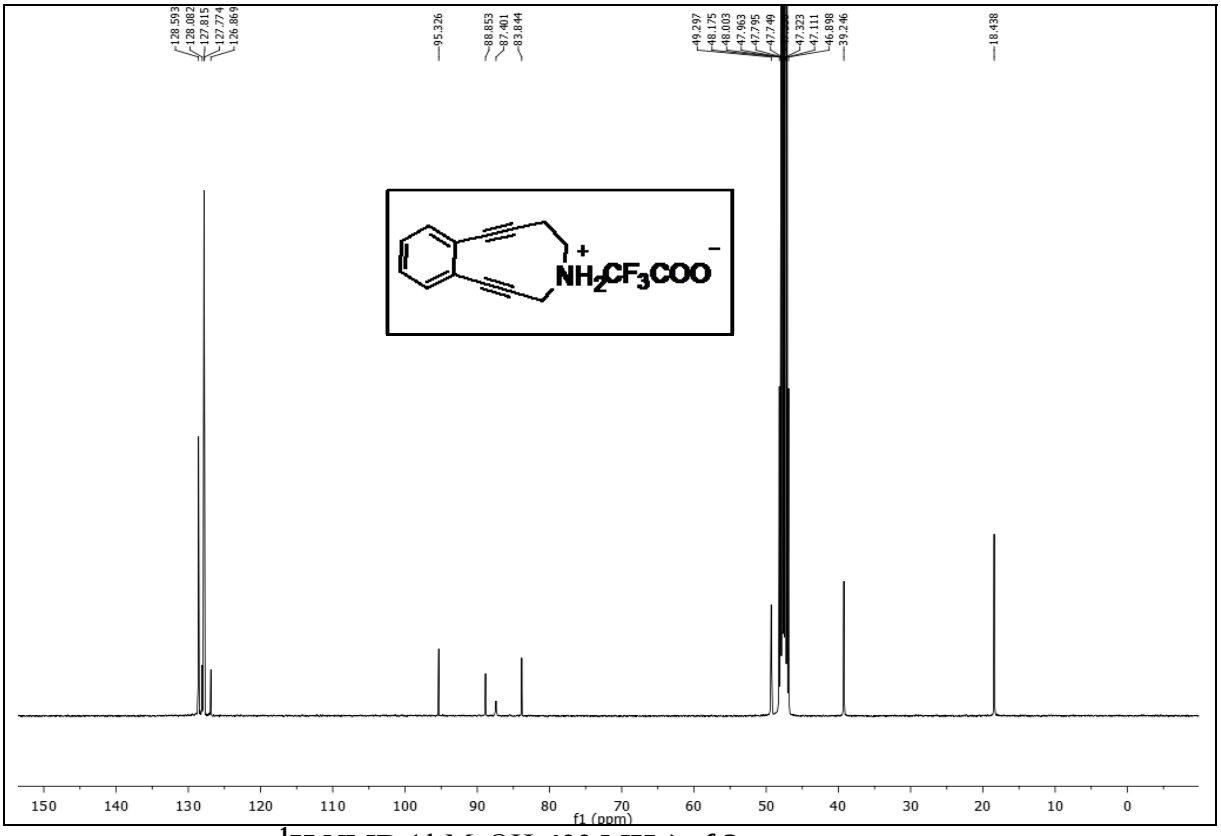


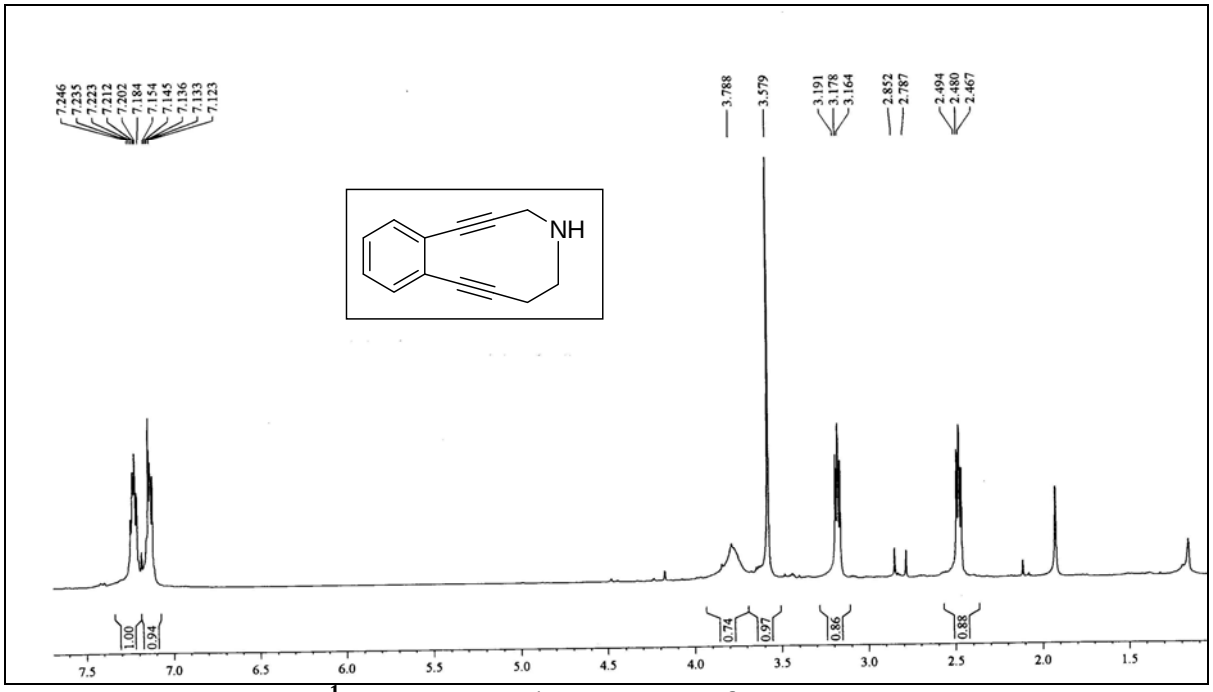
Figure 4: Plasmid Relaxation Assays with compounds **1c** and **3c** (5 µL each from a stock of 30 µM (for 3c) and 10 µM (for 1c) in DMSO) and pBR 322 Plasmid DNA (7 µL from a stock of 0.03 µg/µl at pH 6.5). These were separately mixed with 20 mM phosphate buffer of pH 6.5, incubated at 37 ° C for 30 h in **A** ; Lanes I: DNA alone, II: DNA with **3c**, III: DNA with **1c**.



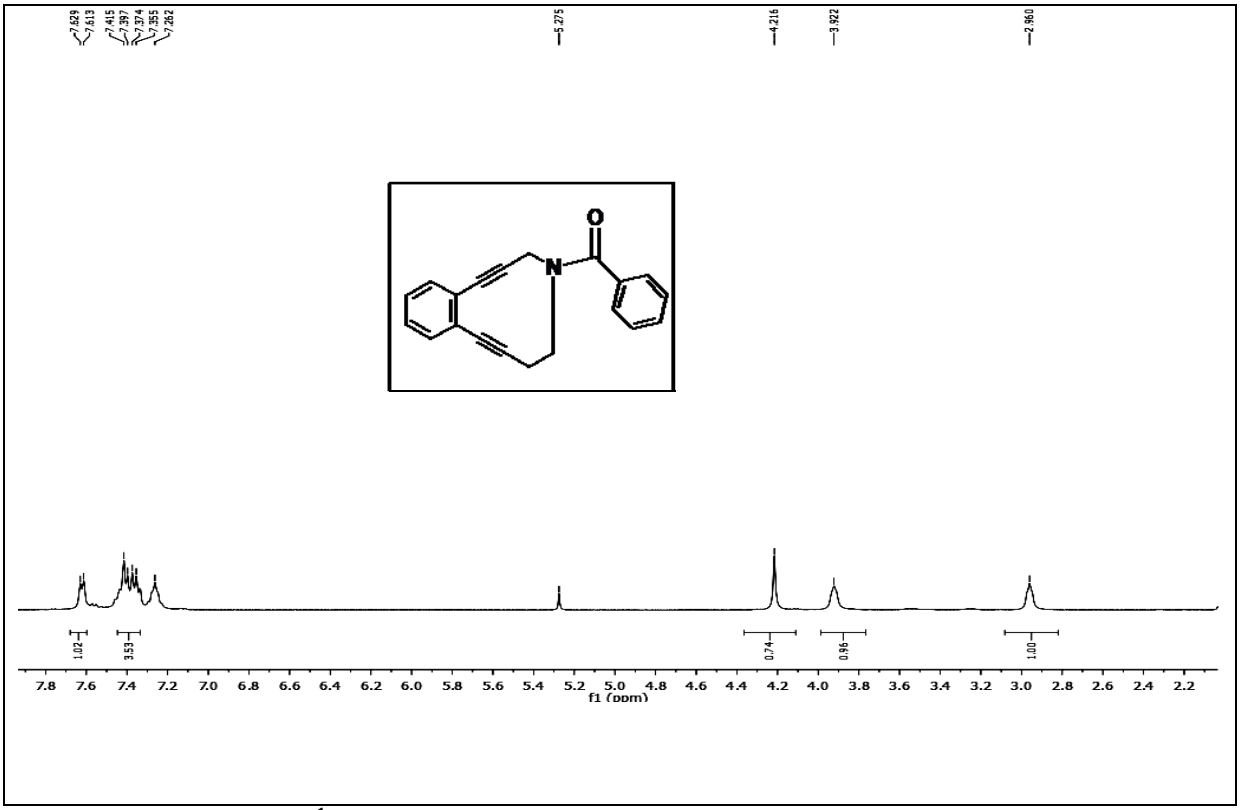
^{1}H NMR (d_4MeOH, **400 MHz) of 3c**



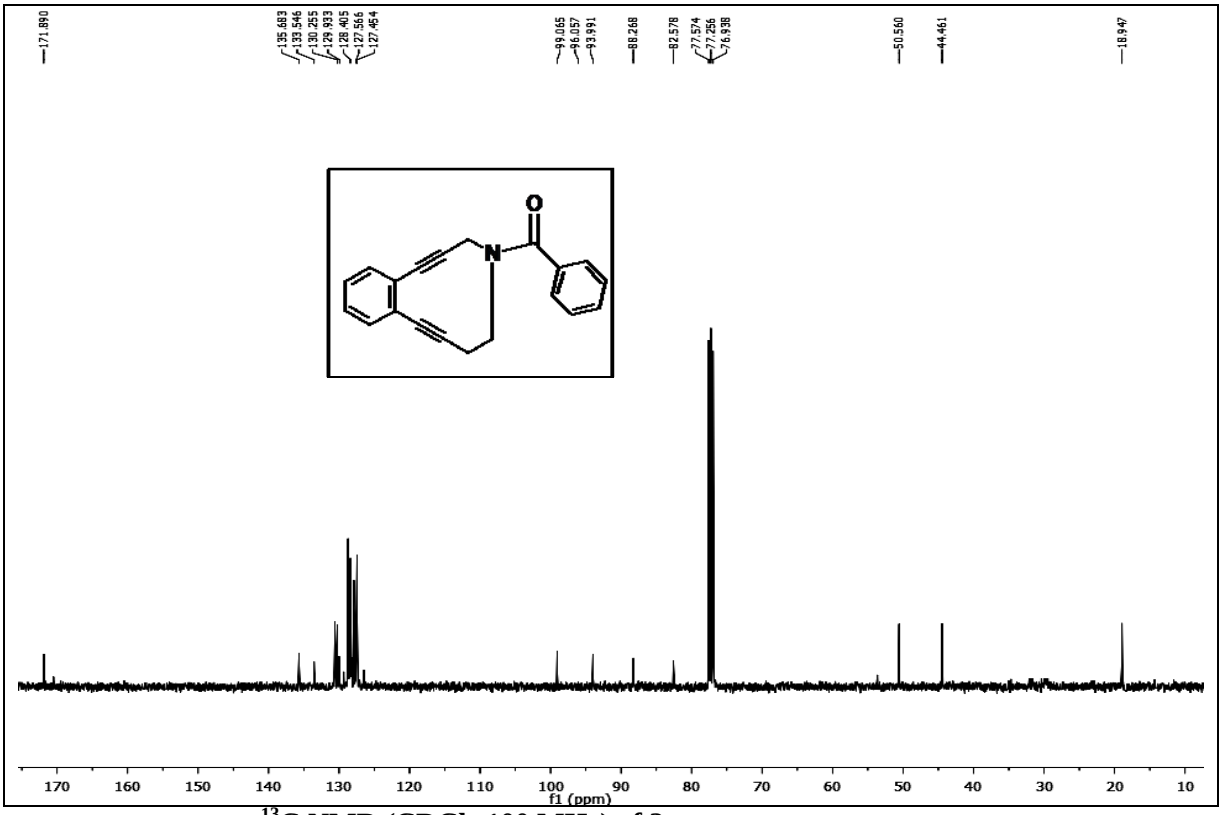
^{1}H NMR (d_4MeOH, **400 MHz) of 3c**



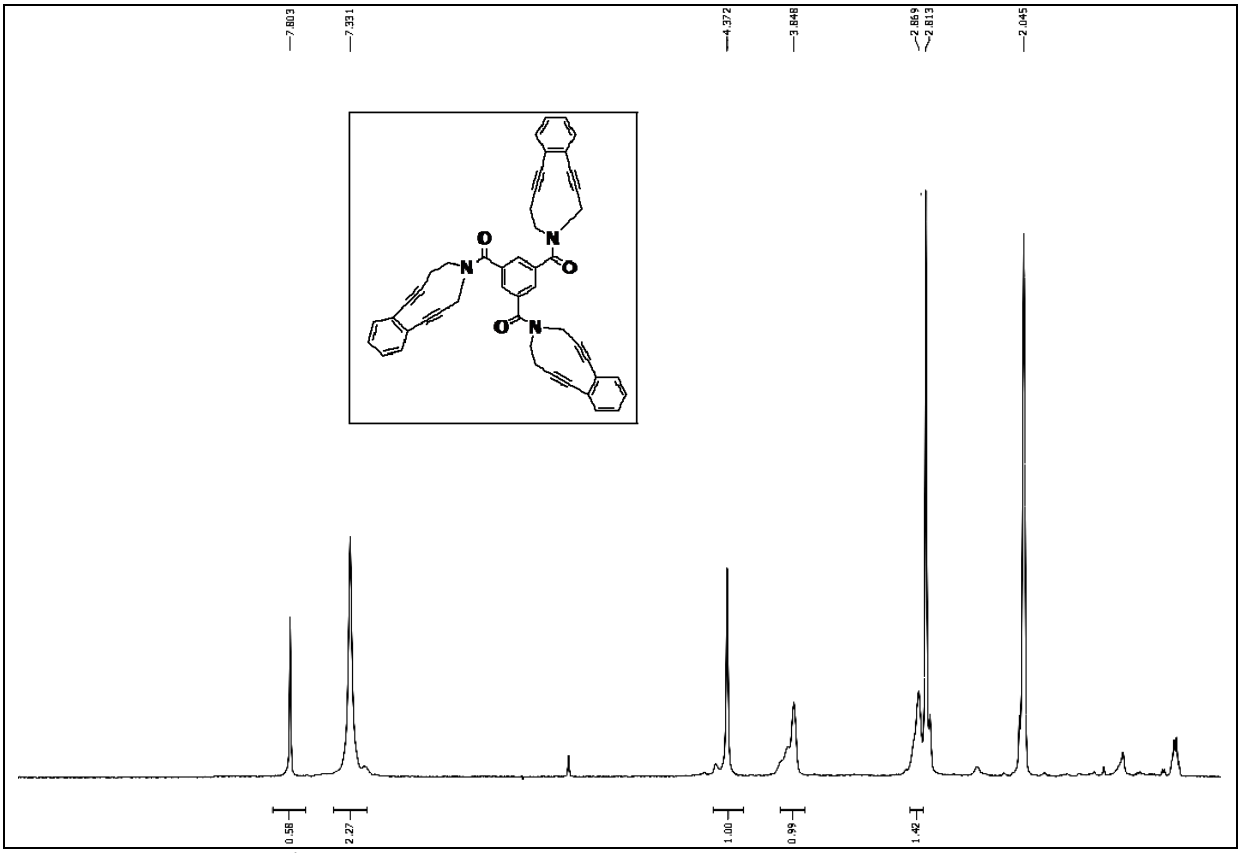
^{1}H NMR ($CDCl_3$**, 200 MHz) of 2a**



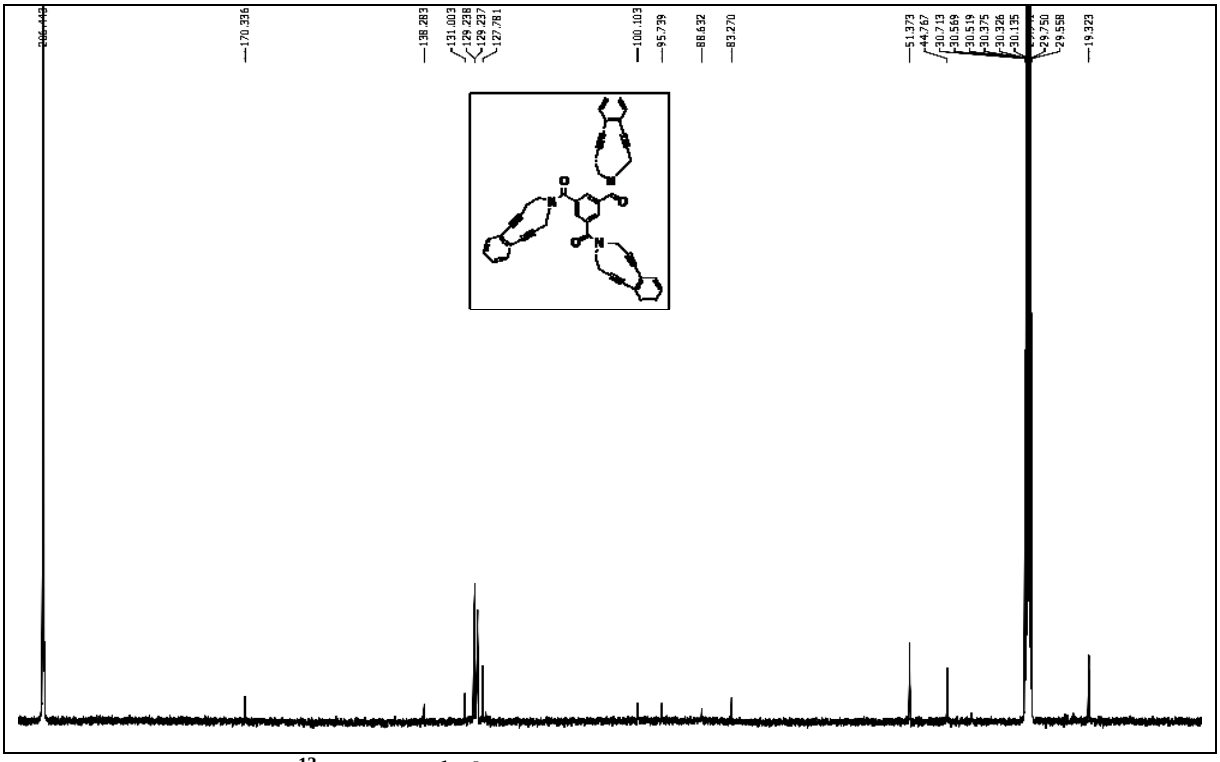
^{1}H NMR ($CDCl_3$, 400 MHz) of 3a



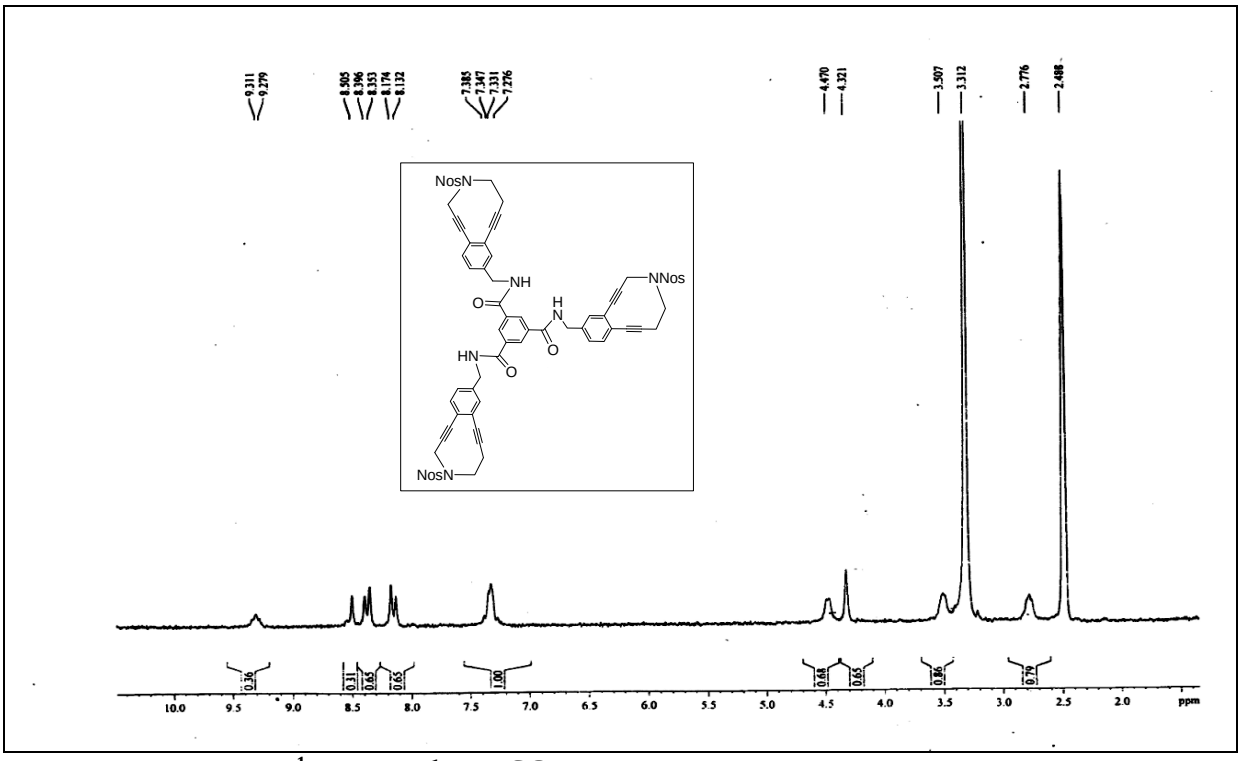
^{13}C NMR ($CDCl_3$, 100 MHz) of 3a



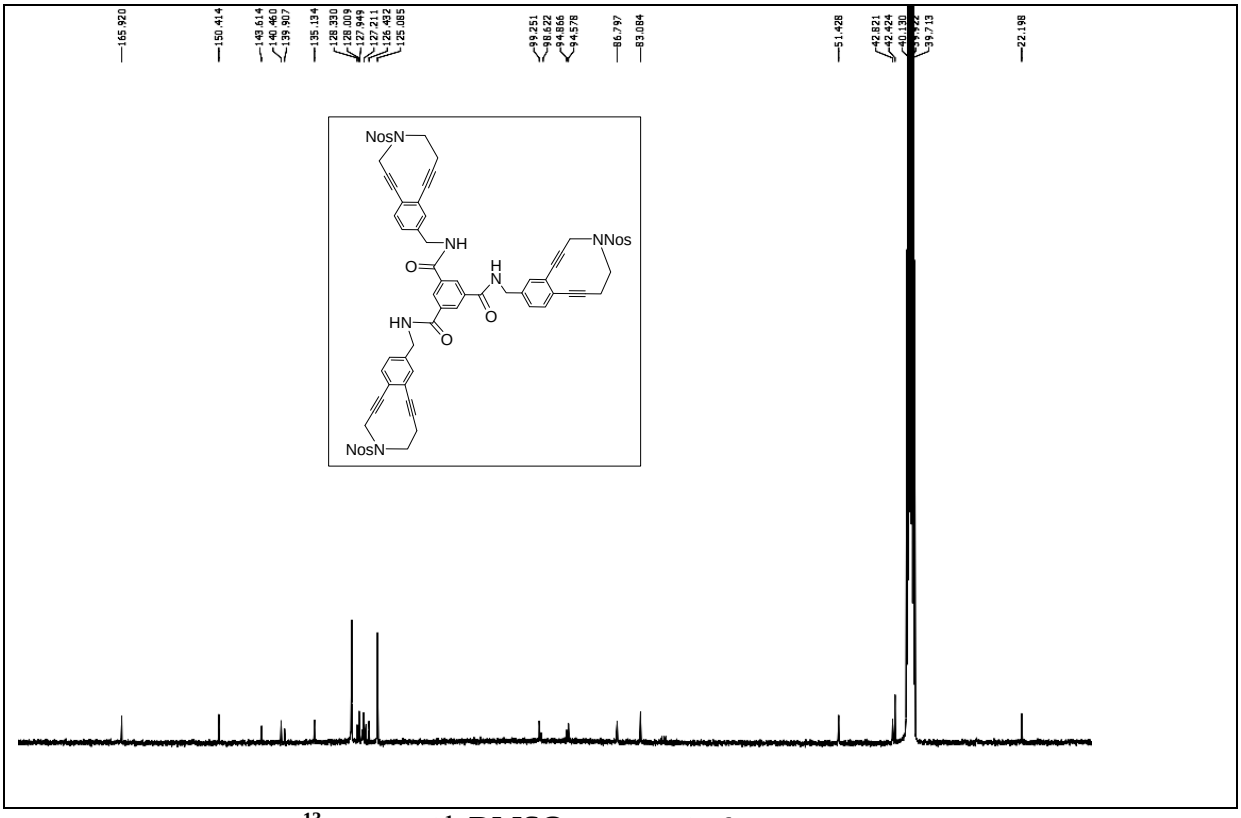
^{1}H NMR (d_6 Acetone, 400 MHz) of 1a



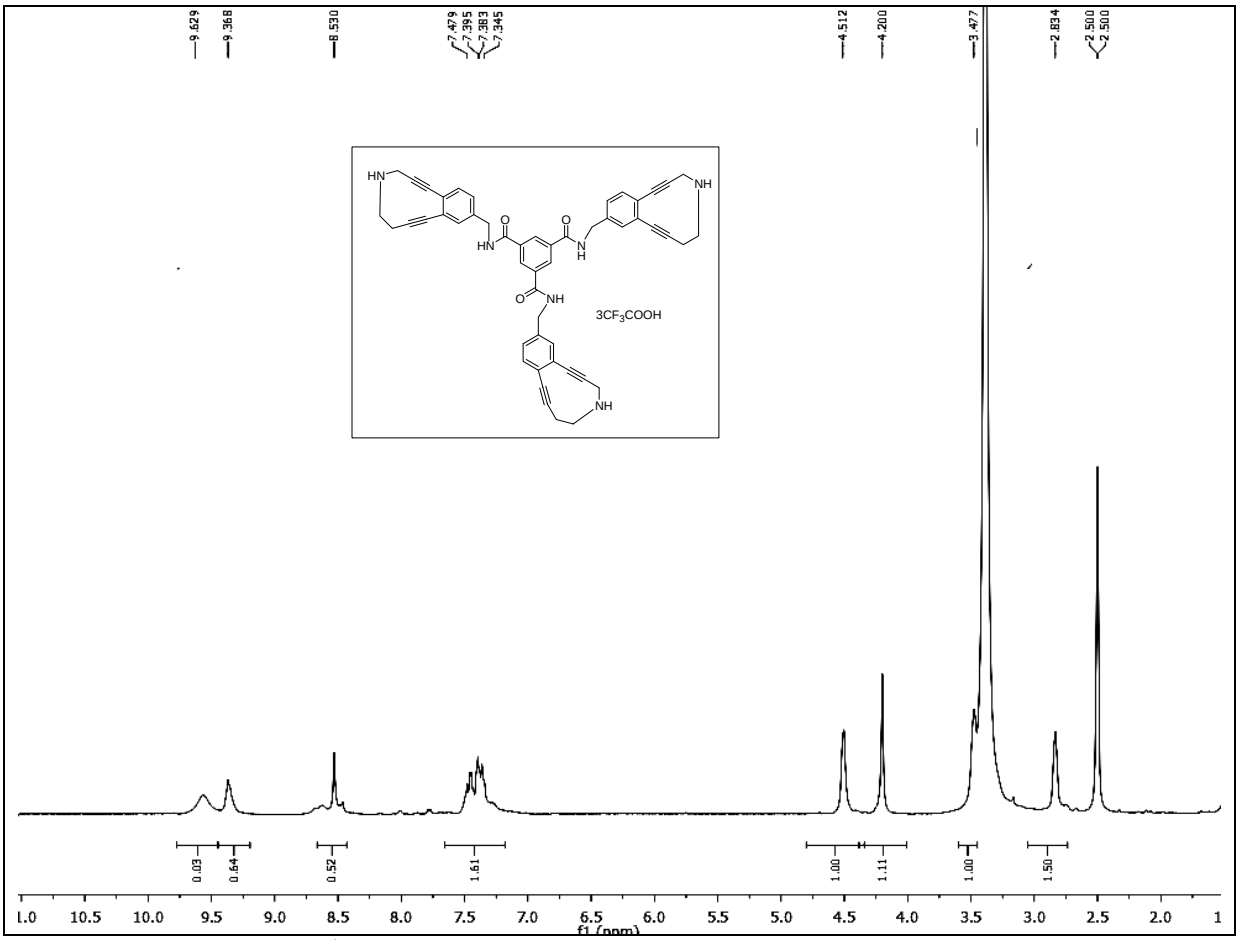
^{13}C NMR (d$_6$ Acetone, 100 MHz) of 1a



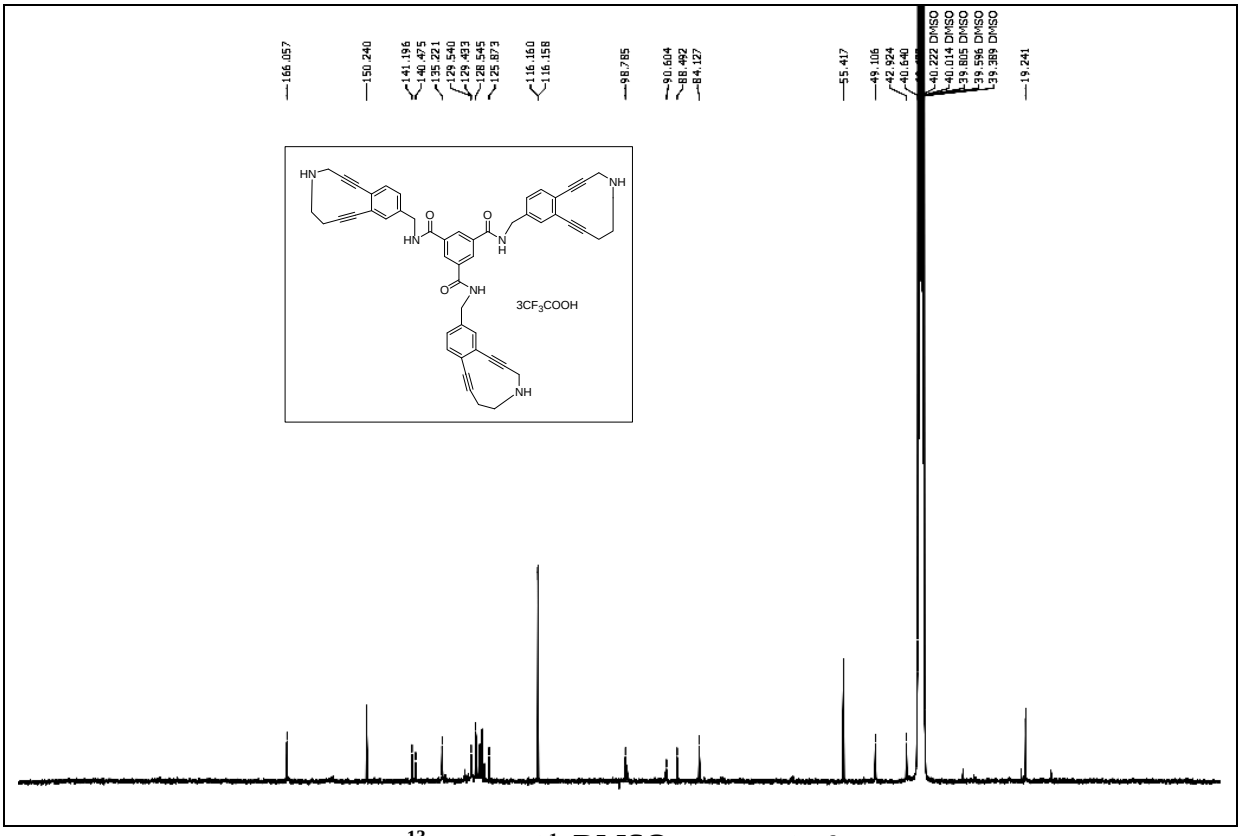
^{1}H NMR (d$_6$ DMSO, 200 MHz) of 5



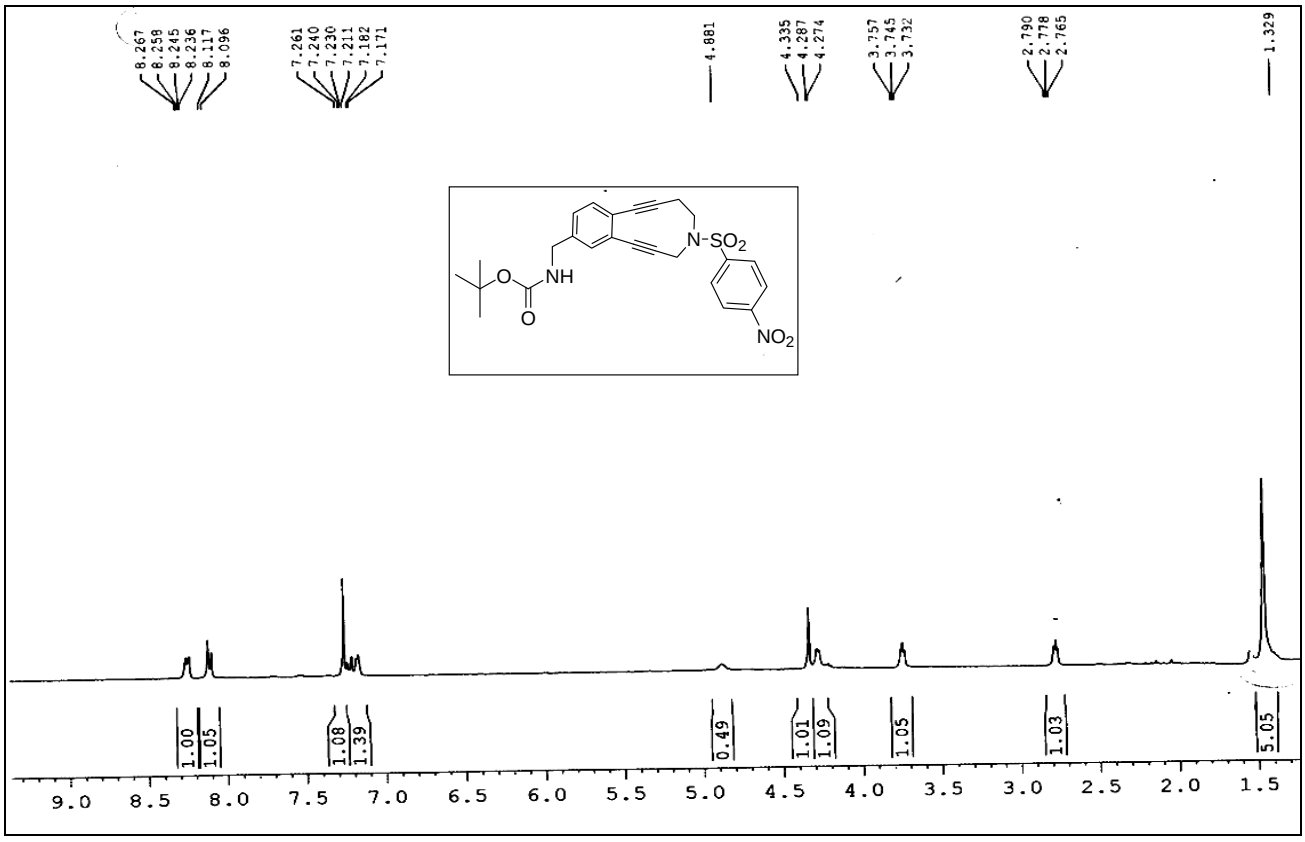
^{13}C NMR (d_6 DMSO, 100 MHz) of 5



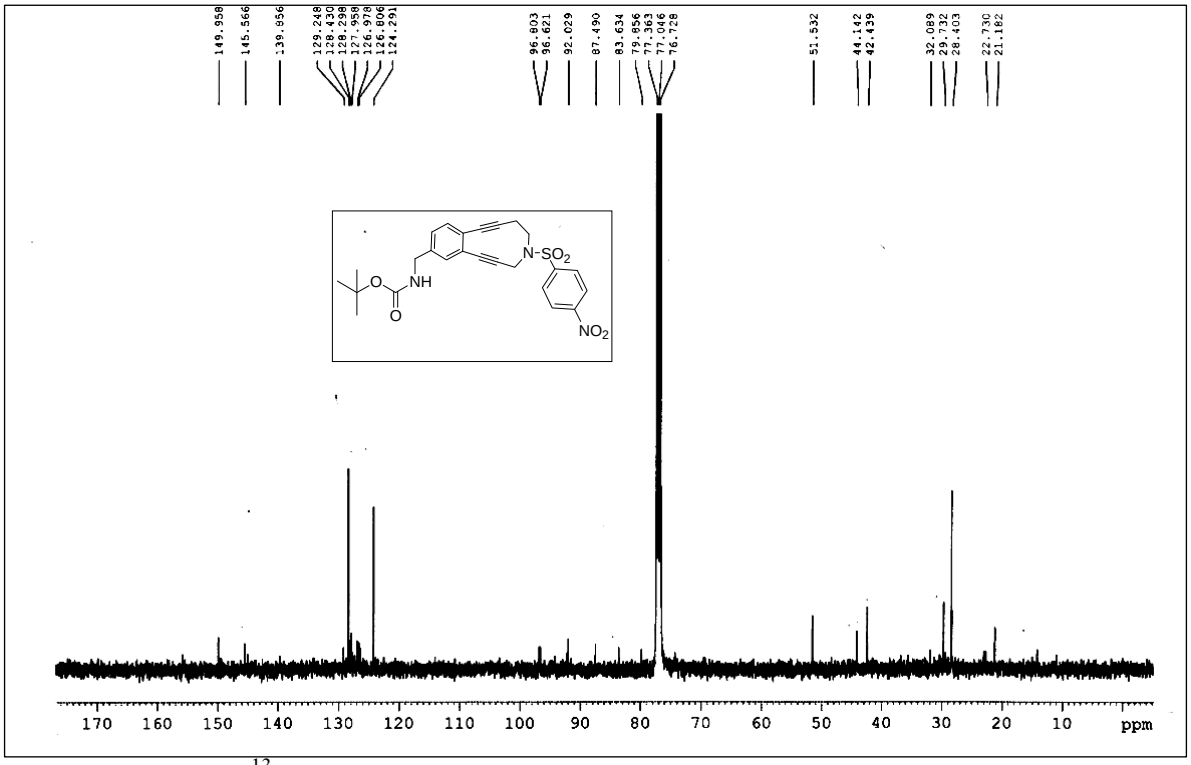
^{1}H NMR (d_6 DMSO, 400 MHz) of 1c



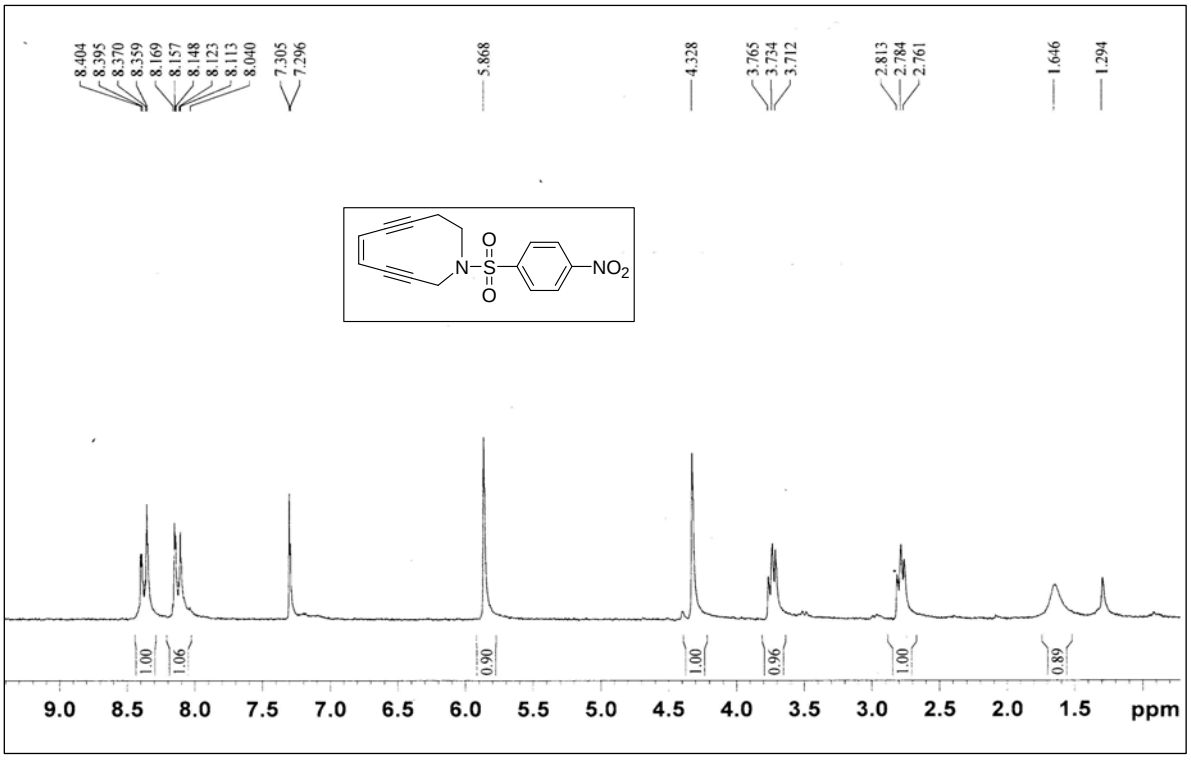
^{13}C NMR (d_6 DMSO, 100 MHz) of 1c



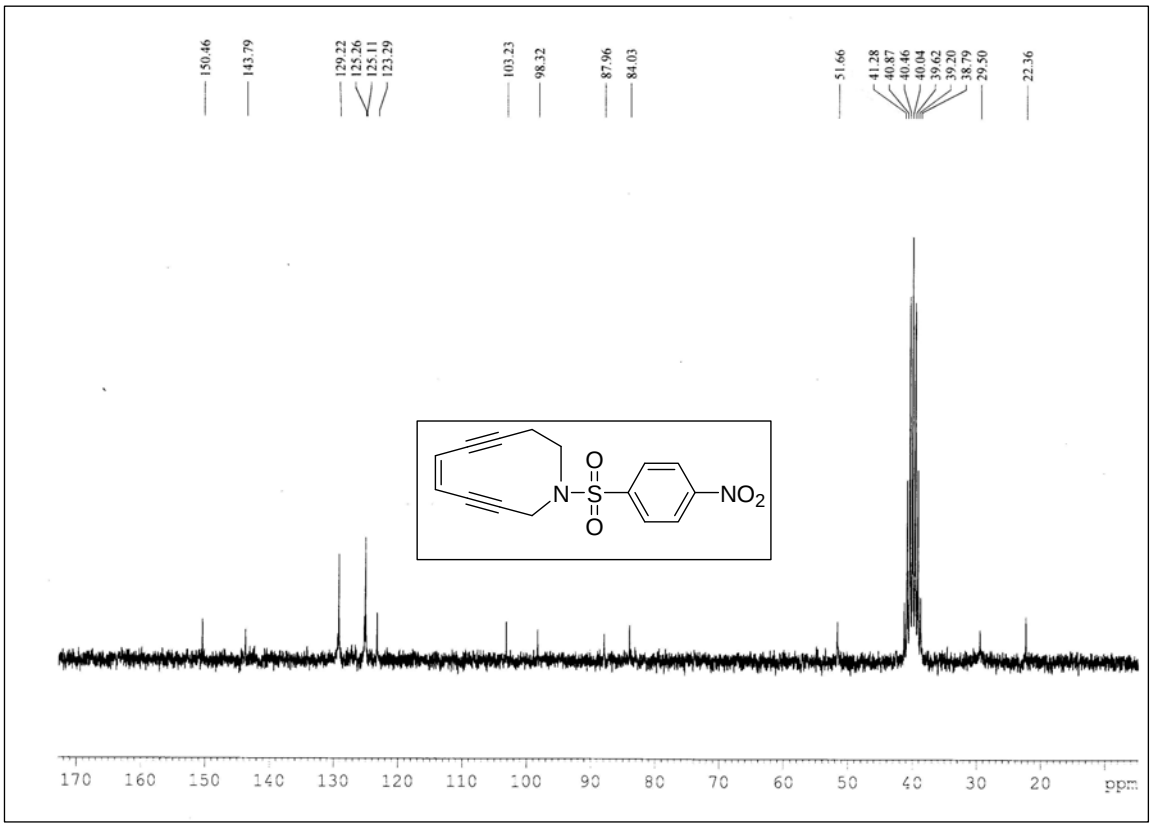
^{1}H NMR ($CDCl_3$, 400 MHz) spectrum of 4



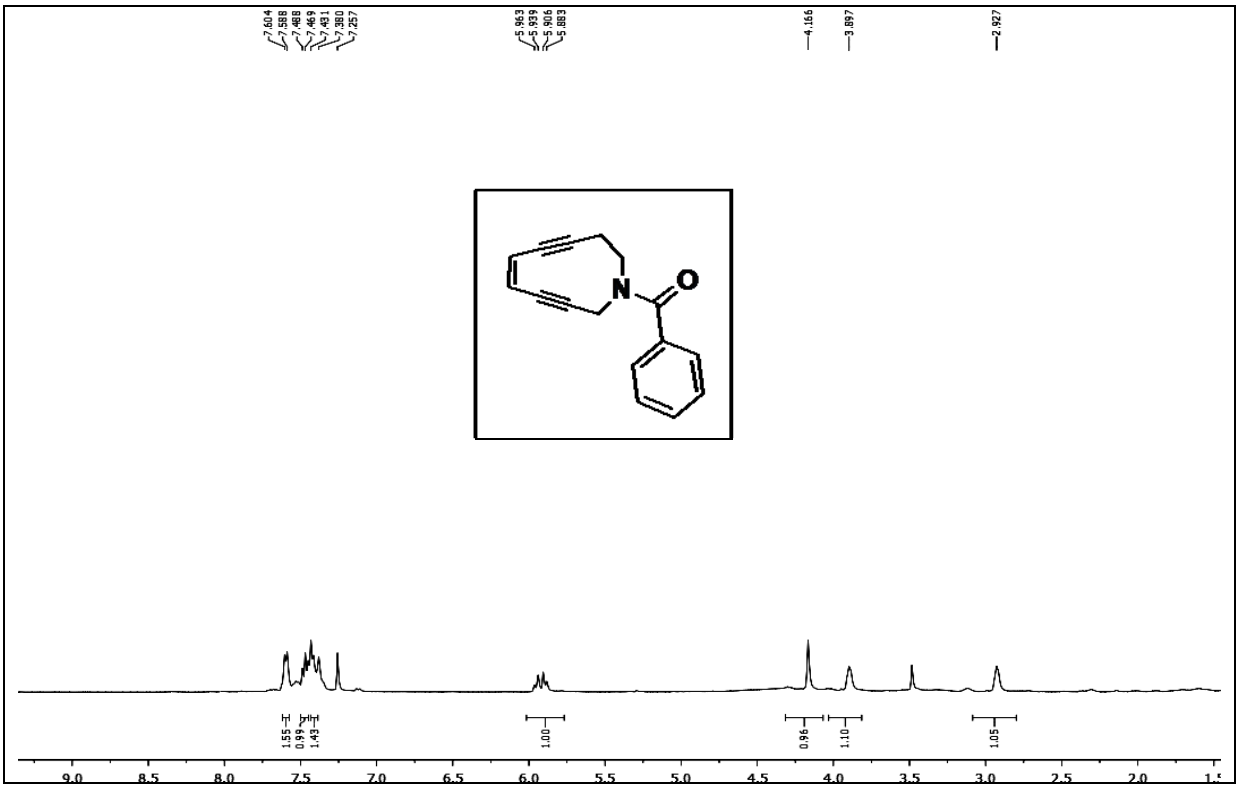
^{13}C NMR ($CDCl_3$, 50 MHz) spectrum of 4



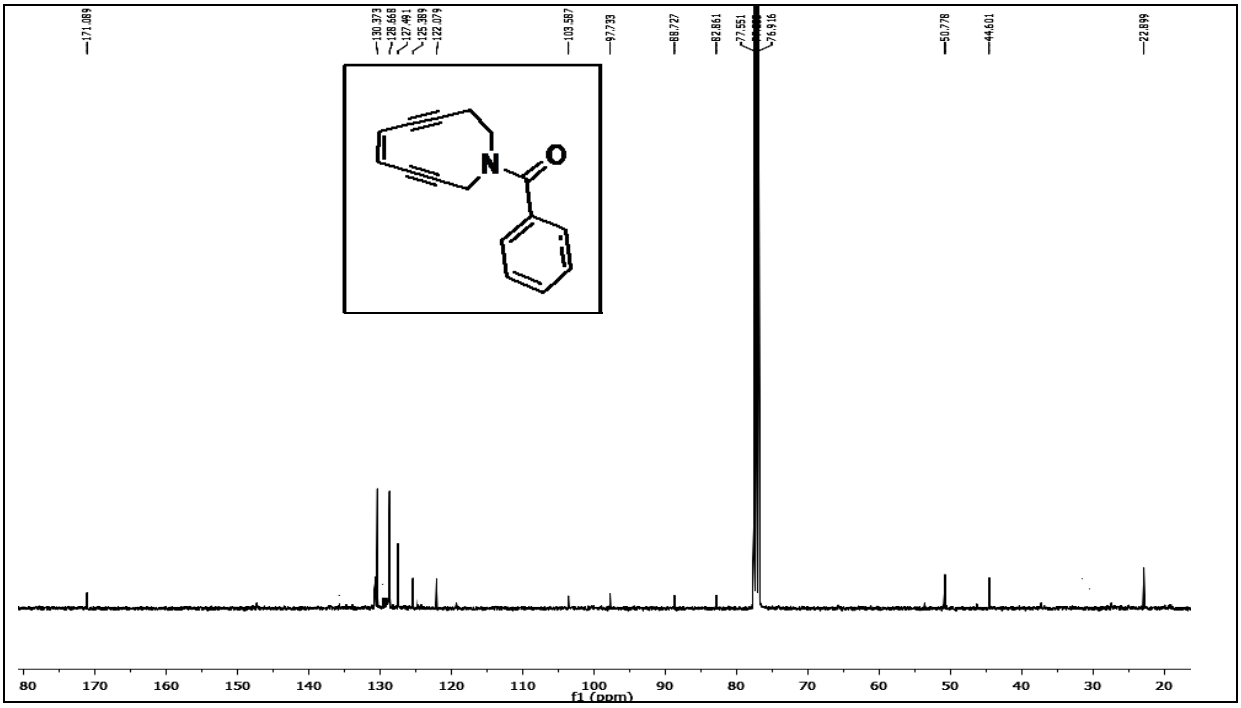
^{1}H NMR ($CDCl_3$, 200 MHz) spectrum of 2



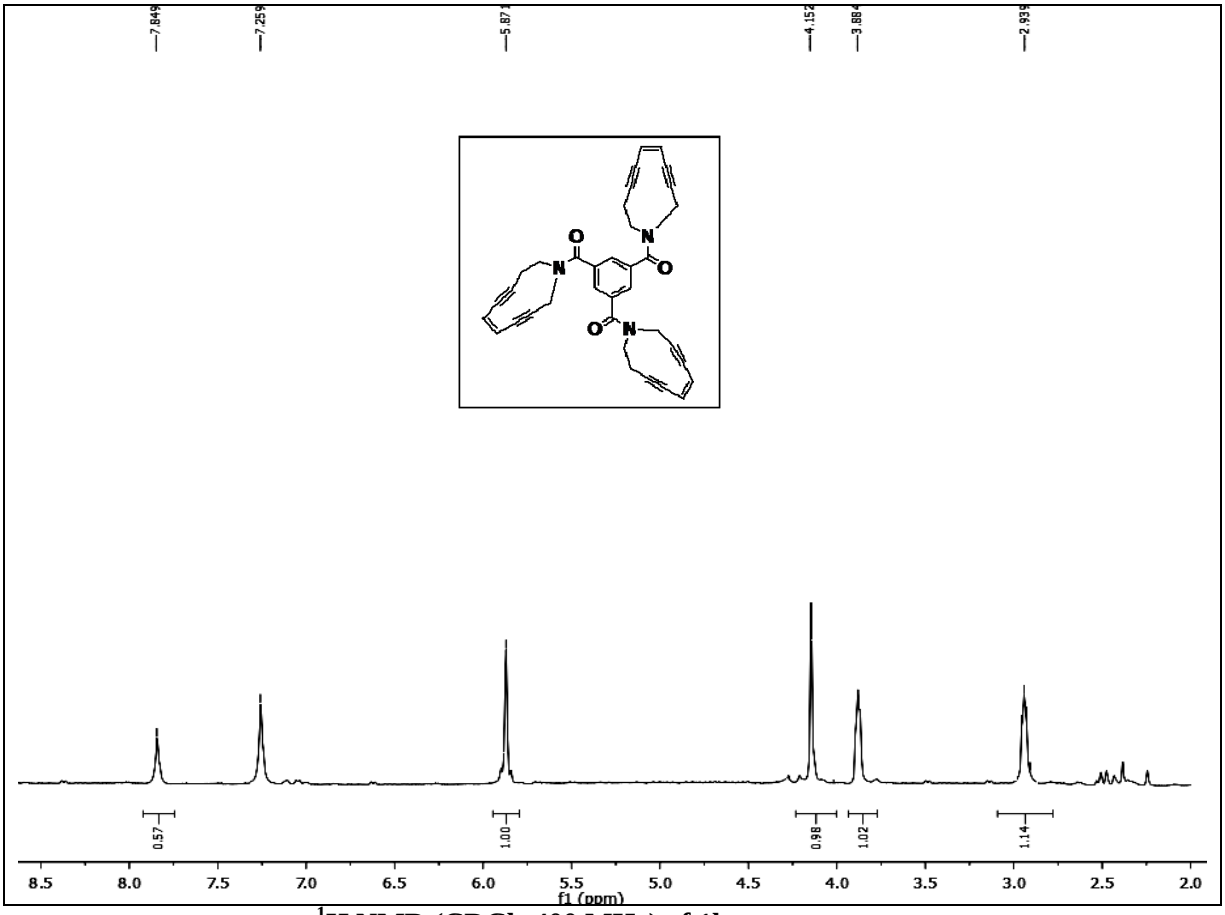
^{1}H NMR ($CDCl_3$**, 50 MHz) of 2**



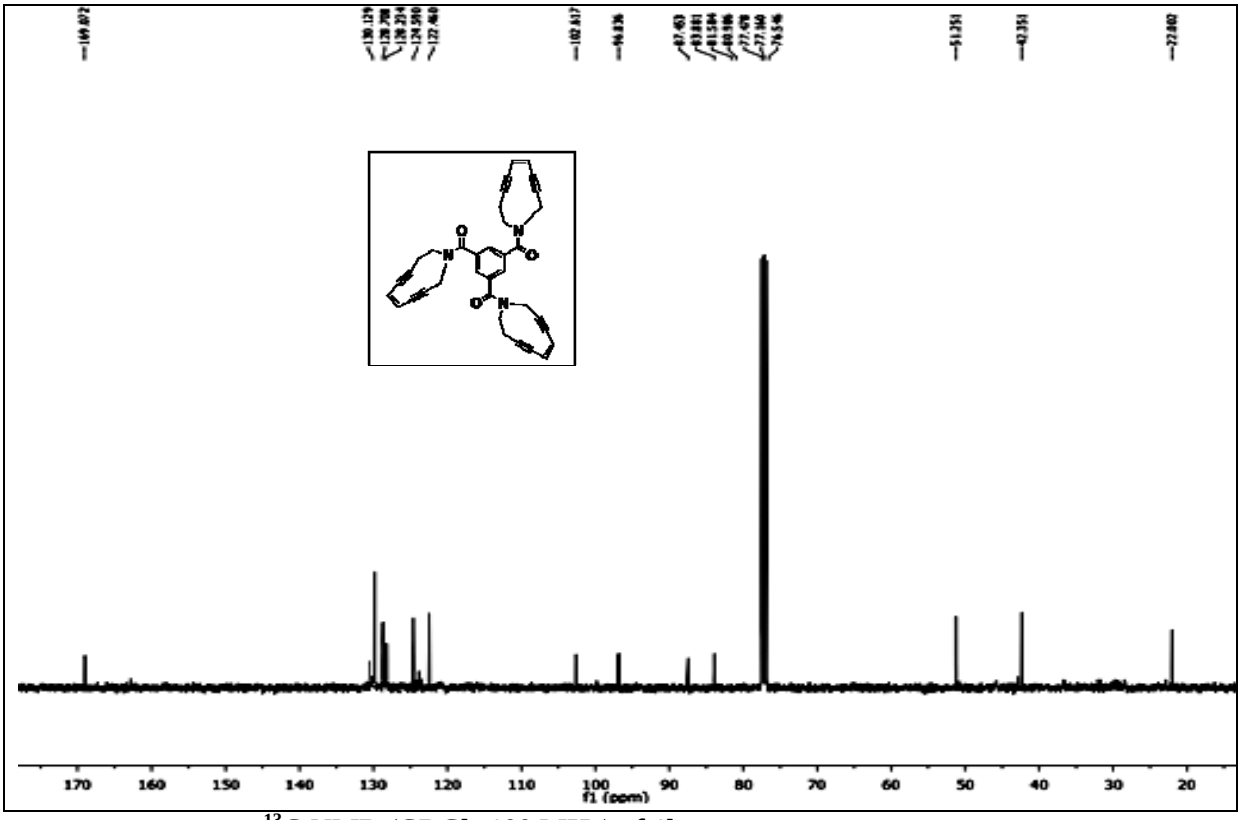
^{1}H NMR ($CDCl_3$**, 400 MHz) of 3b**



^{13}C NMR ($CDCl_3$, 100 MHz) of 3b



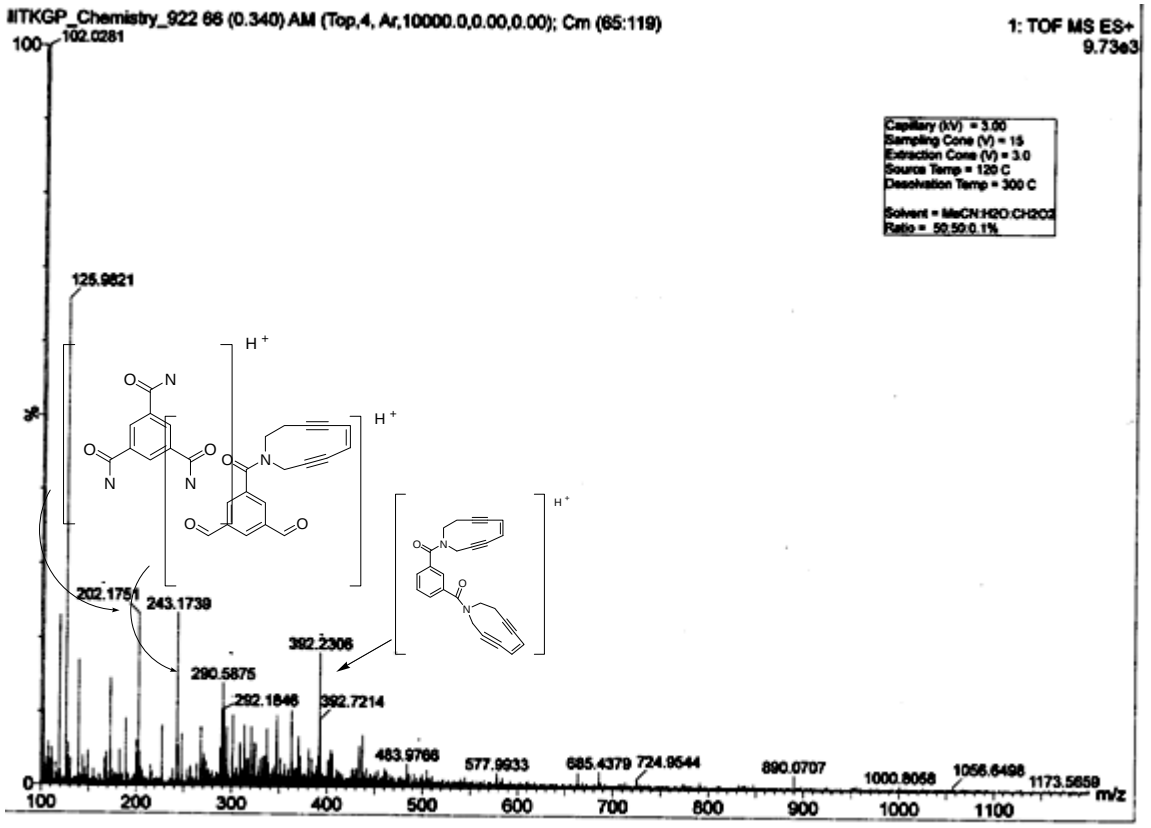
^{1}H NMR ($CDCl_3$, 400 MHz) of 1b

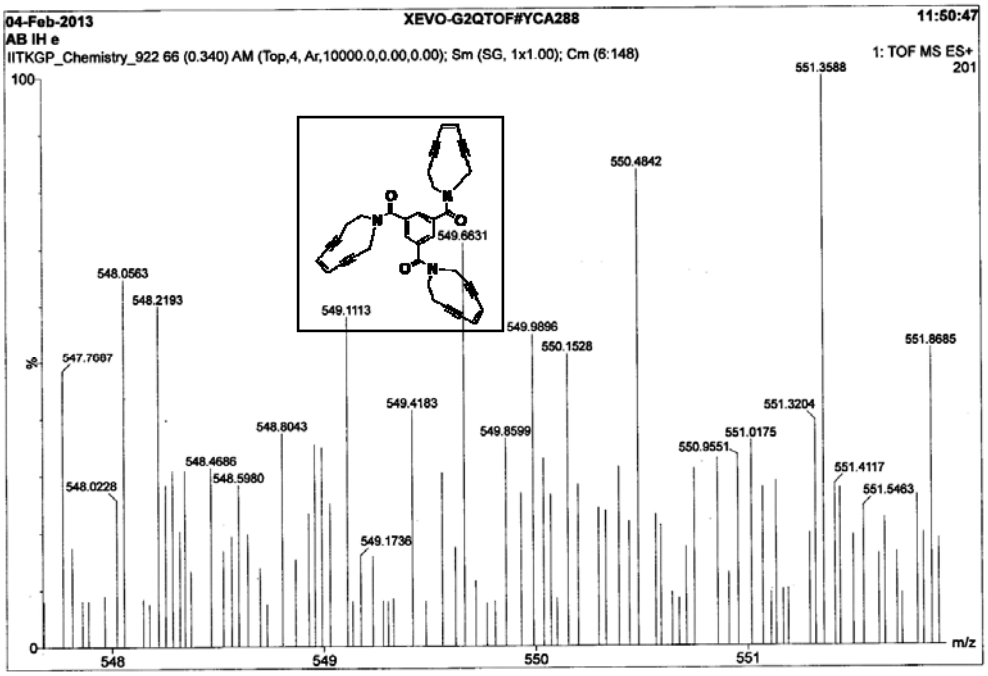


^{13}C NMR ($CDCl_3$, 100 MHz) of 1b

Mass spectrum

Molecular weight=549.2052





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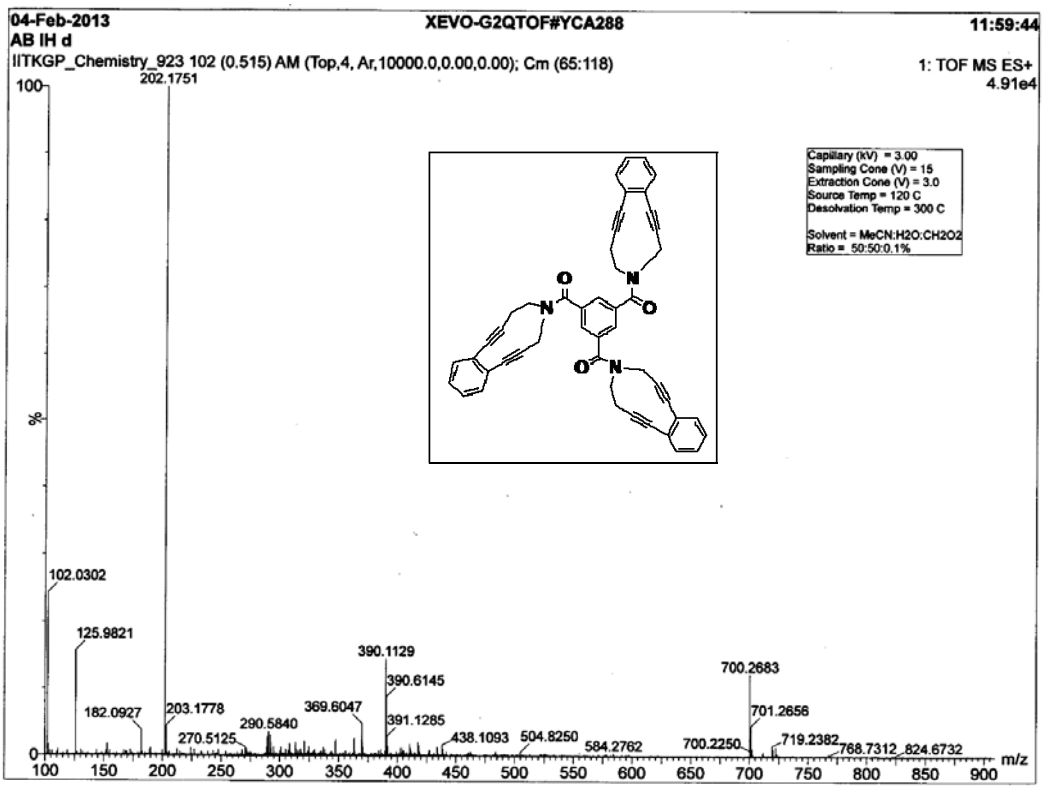

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