Supporting Information

Guanosine-Based Hydrogel as a Supramolecular Scaffold for Template-Assisted Macrocyclization

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1.0 General Information:

All starting materials were obtained from commercial suppliers and used as received. All experiments were carried out under an inert atmosphere of argon in flame-dried apparatus and the reaction mixture was magnetically stirred. Wherever needed solvents were dried using standard procedures reported in D. D. Perrin, W. L. F. Armarego, Purification of Laboratory Chemicals, Pergamon Press, Oxford, 3rdedn, 1988. Products were purified by flash chromatography on silica gel (100–200 mesh, Merck). Unless otherwise stated, yields refer to analytical pure samples. NMR spectra were recorded in $CDCl_3$ unless otherwise stated. **1H-NMR** spectra were recorded at 500 MHz using Brüker AVANCE 500 MHz and JEOL 400 MHz instruments at 278 K. Signals are quoted as δ values in ppm. Data are reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, br = broad, m = multiplet), and coupling constants (Hz). **13C-NMR** spectra were recorded on either a JEOL-400 (100 MHz) or a Brüker AVANCE 500 MHz (125 MHz) with complete proton decoupling. Chemical shifts (δ) are reported in ppm downfield from tetramethylsilane with the solvent as the internal reference ($CDCl_3$: δ77.00 ppm). HRMS analyses were performed with Q-TOF YA263 high resolution (Water Corporation) instruments by +ve mode electrospray ionization. All general chemicals were purchased from Sigma-Aldrich.

2.0 Preparation of Hydrogel:

2.1 Preparation of guanosine hydrogel (G-gel):

The G-gel was prepared by mixing guanosine, (10 mg, 0.035 mmol), phenylboronic acid, (4.3 mg, 0.035 mmol, 1.0 equiv.) and KOH (1.4 mg, 0.025 mmol, 0.7 equiv.) in 0.5 mL MilliQ water. The solution was heated to 80 °C until a clear solution was obtained. The resulting solution was allowed to cool to room temperature and left undisturbed for gel formation. A clear and strong gel was formed within 5 min. The gel was found to have pH ~ 8 and was stable through pH~12-pH~7. The gel decomposed beyond this pH range resulting in white precipitation and became a clear sol at pH < 2.

2.2 Preparation of cytidine hydrogel (C-gel):

The C-gel was prepared by mixing cytidine C (15 mg, 0.061 mmol), phenylboronic acid, (0.061 mmol, 1.0 equiv), and silver acetate (4.5 mg, 0.030 mmol, 0.5 equiv) in 0.5 mL aqueous solution. It was then heated at 80 °C to obtain a clear solution. The resulting solution was then allowed to cool down to 30 °C and kept in dark. Gelation was slow and was observed after standing the solution for a period of 2 days at 30 °C and after a period of 5 days when incubated at temperatures lower than 30 °C.

Table S1: Macrocyclization reaction in presence of different components of the G-gel.

Hydrogel composition (in aqueous solution)	**Observation**
Guanosine + alkyne + azide	No reaction occurred (NR)
$PhB(OH)_2$ + alkyne + azide	NR
KOH + alkyne + azide	NR
Guanosine + $PhB(OH)_2$ + alkyne + azide	NR
Guanosine + KOH + alkyne + azide	NR
$PhB(OH)_2$ + KOH + alkyne + azide	NR
Guanosine + $PhB(OH)_2$ + KOH + alkyne + azide	Formation of macrocycle
Cytidine + silver acetate + $PhB(OH)_2$ + alkyne + azide	No formation of macrocycle

3. In-situ click reaction in nucleic acid hydrogels:

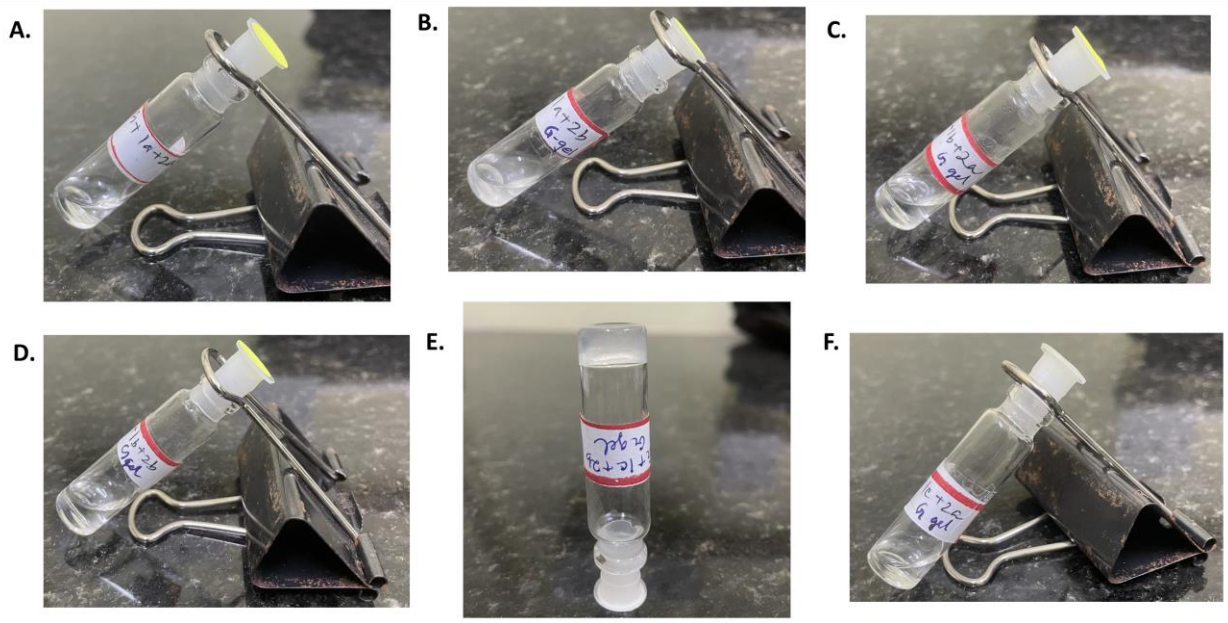


Figure S1. G-gel after incubation with A) **1a+2a**; B) **1a+2b**; C) **1b+2a**; D) **1b+2b;** E) **1c+2b**; F) **1c+2a** after 5 days.

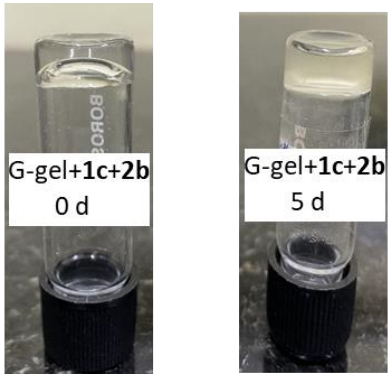


Figure S2. Formation of G-gel after incubation with **1c** and **2b** at 0 day (within ~ 1h) and after 5 days.

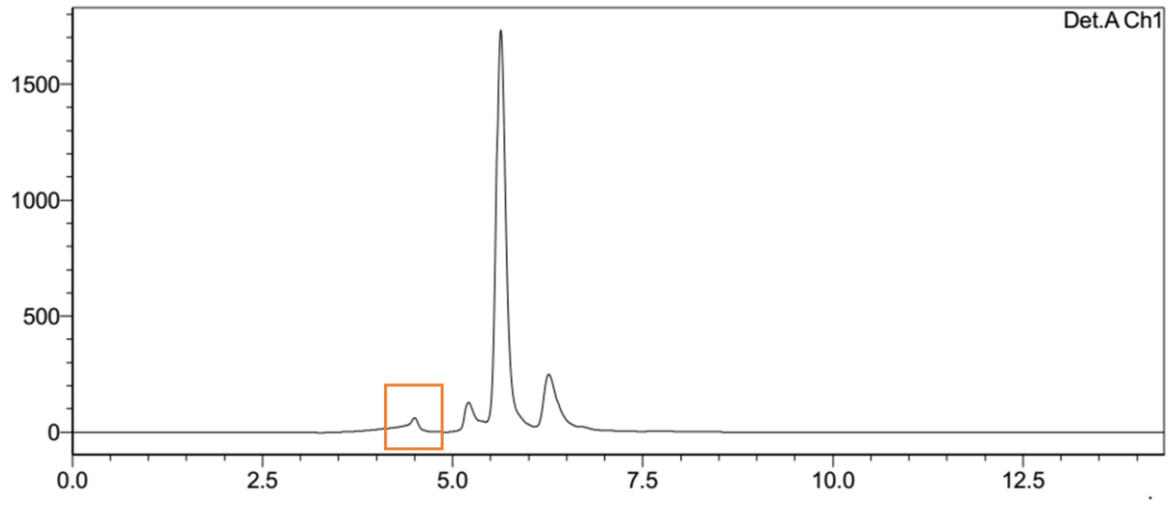


Figure S3. HPLC of azide and alkyne treated G-gel extract reveals formation of macrocycle **M1**.

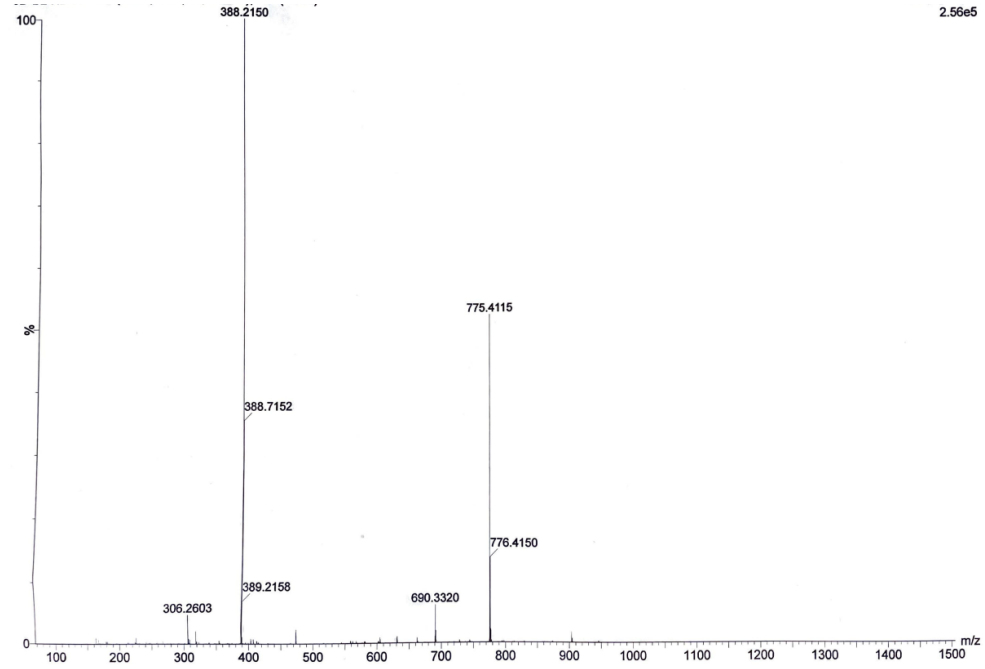


Figure S4. ESI-MS analysis of purified **M1** from gel extract.

Click reaction in cytidine gel (C-gel):

When the C-gel is incubated with **1c** and **2b**, the clear translucent gel becomes black opaque. The ESI-MS analysis of the gel extract reveals no peak corresponding to **M1** at m/z ~ 774.

Figure S5. Image of cytidine gel incubated with alkyne (**1c**) and azide (**2b**).

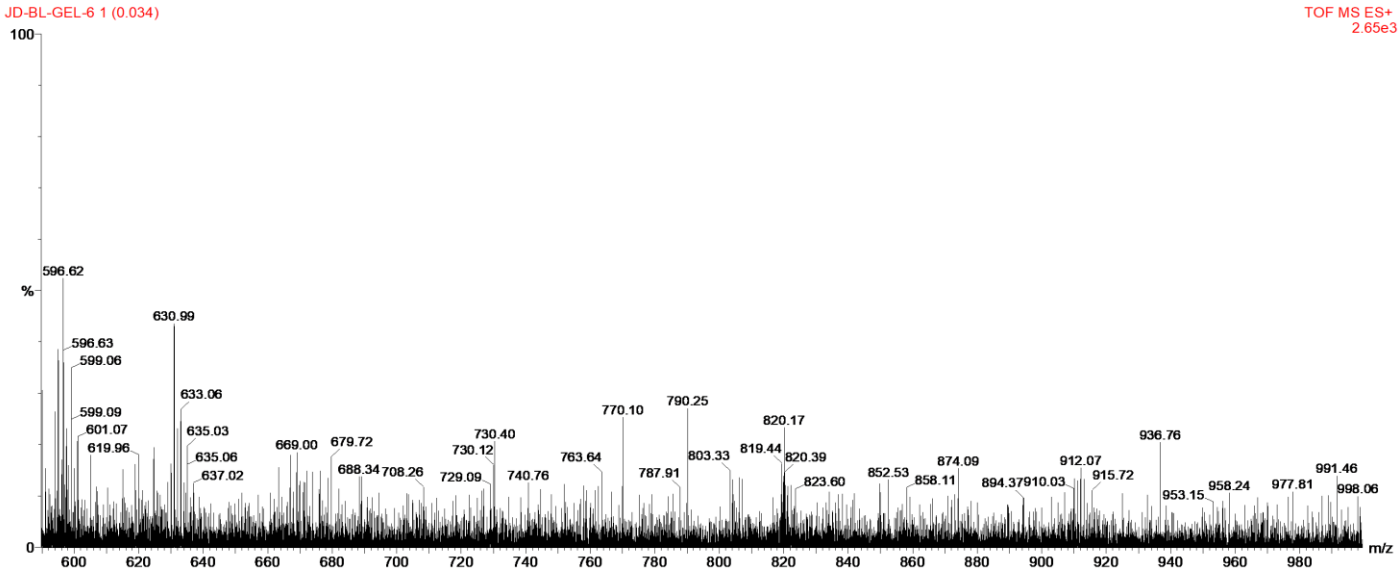
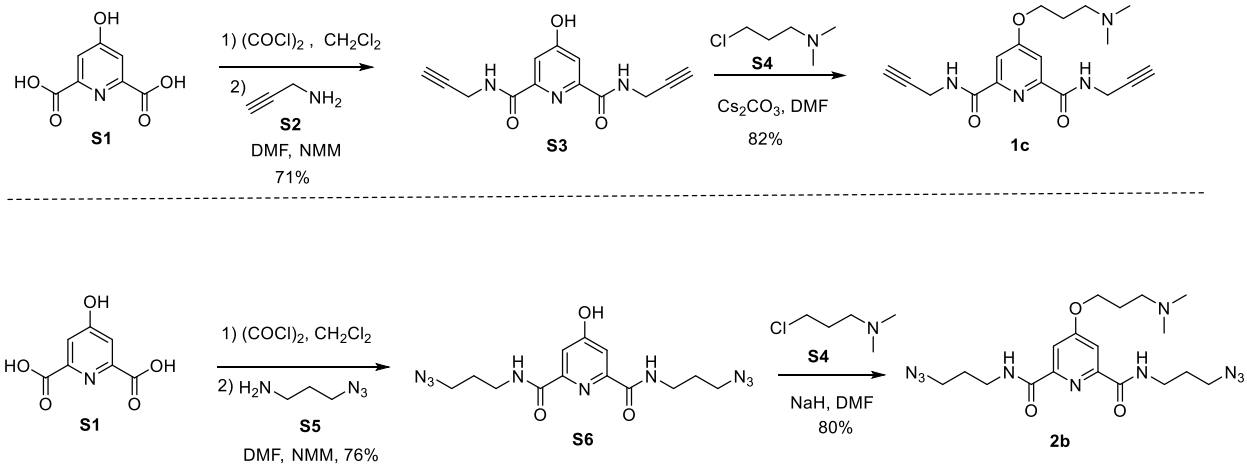


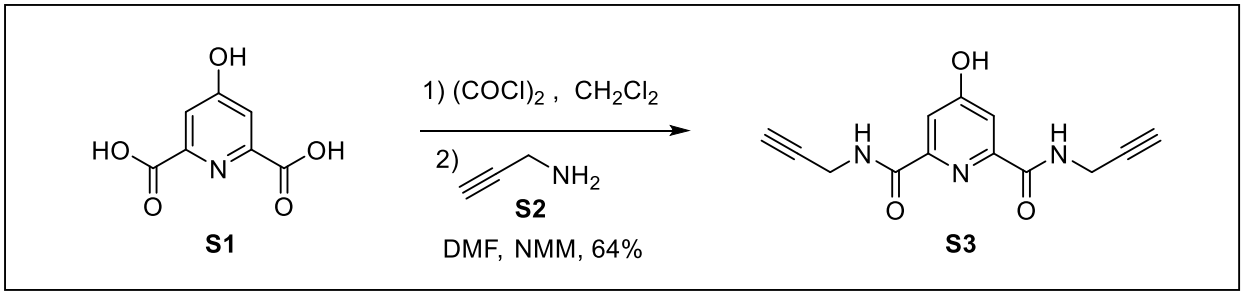
Figure S6. The ESI-MS spectra of the cytidine gel incubated with alkyne (**1c**) and azide (**2b**).

4. Synthesis of chemical compounds:

Synthesis of bisalkyne 1c and bisazide 2b building blocks:



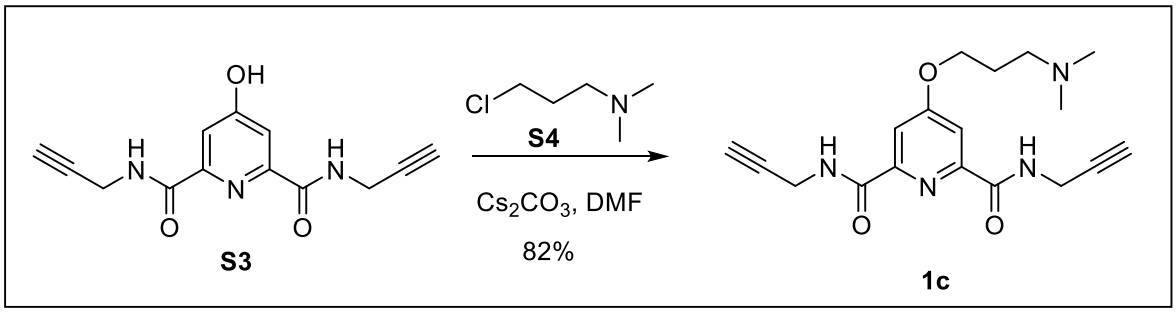
Scheme S1. Synthesis of alkyne and azide building blocks for the macrocycles.



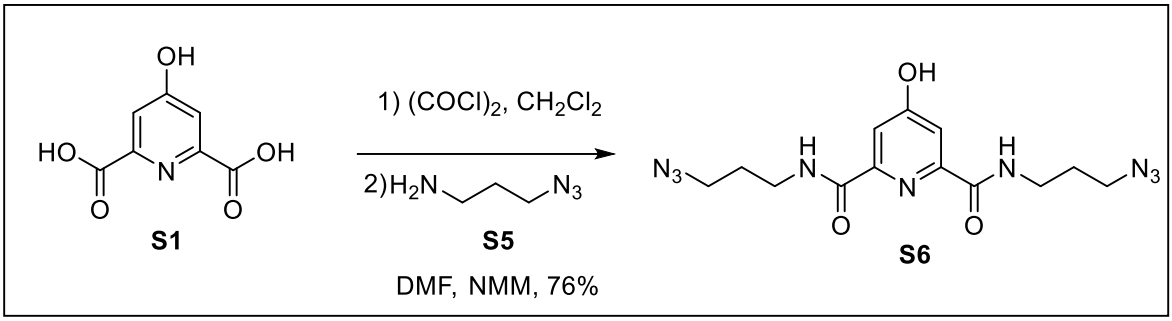
Synthesis of bisalkyne S3: Oxalyl chloride (15.0 mL) was added drop wise to chelidamic acid **S1** (300.0 mg, 1.63 mmol, 1.0 eqv) in dichloromethane at 0 °C and the mixture was stirred at room temperature for 4 h. The reaction mixture was concentrated and dried under vacuum to provide the crude acid dichloride, which was dissolved in DMF (10.0 mL) and cooled to 0 °C. NMM (1.5 mL, 13.7 mmol, 8.4 eqv) and propargylamine **S2** (0.35 mL, 5.5 mmol, 4 eqv) were added under an argon atmosphere at 0 °C and the resulting mixture was stirred at room temperature for 12h. The solvent was concentrated under vacuum and the residue was purified by column chromatography using MeOH-CH_2Cl_2 (4:96) to give the compound **S3** (184 mg, 71%) as a white solid. **1H-NMR** (400 MHz, DMSO-d_6); 9.69 (2H, t, *J* = 7.2), 7.54 (2H, d, *J* = 1.7), 4.16–4.14 (4H, m), 3.21 (2H, t, *J* = 2.1 Hz); **13C-NMR** (125 MHz, DMSO-d_6): 166.6, 163.0, 150.3,

111.6, 81.1, 73.2, 28.1; HRMS (ESI) calcd for $C_{13}H_{11}N_3NaO_3$ $[M+Na]^+$: 280.0698; Found: 280.0692.

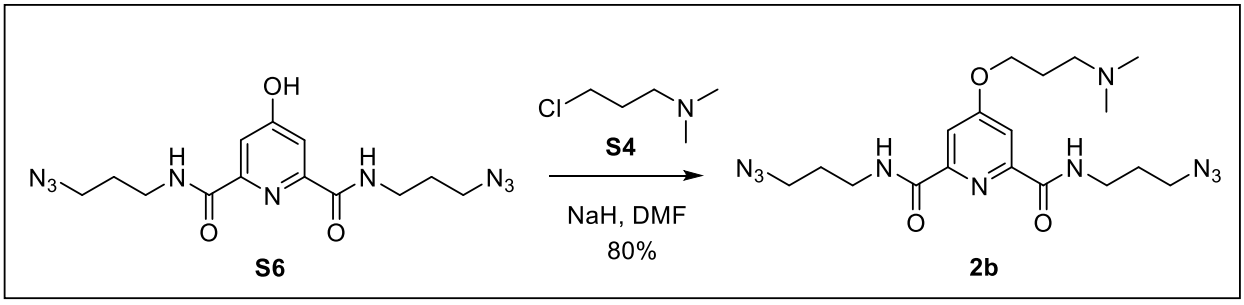
Synthesis of bisalkyne 1c: Cs_2CO_3 (570 mg, 1.75 mmol, 3 eqv) was added to a solution of **S3** (150.0 mg, 0.583 mmol, 1.0 eqv) in dry DMF (10 mL) and stirred for 30 min at rt under argon atmosphere. Then 3-dimethylamino-1-propyl chloride hydrochloride **S4** (202.4 mg, 1.28 mmol, 2.2 eqv) was added and stirred at 70 °C for overnight. The reaction mixture was concentrated to dryness and purified by flash chromatography using MeOH-CH_2Cl_2 (6:94) to obtain the desired product **1c** as a colorless solid (164 mg, 82%). **1H-NMR (**500 MHz, CD_3OD); 7.76 (2H, s), 4.25-4.21 (2H, m), 4.22 (4H, d, *J* = 2.5), 2.99 (2H, t, *J* =7.8), 2.64 (6H, s), 2.63-2.62 (2H, t, *J* = 2.5), 2.20- 2.19 (2H, m); **13C-NMR (**125 MHz, CD_3OD): 169.2, 165.4, 152.0, 112.1, 80.5, 72.2, 68.2, 56.9, 45.4, 29.5, 27.6; HRMS (ESI) calcd for $C_{18}H_{22}N_4NaO_3$: 365.1590; Found: 365.1594.



Synthesis of bisazide compound S6: Oxalyl chloride (10.0 mL) was added drop wise to a solution of chelidamic acid **S1** (300.0 mg, 1.63 mmol, 1.0 eqv.) in CH_2Cl_2 at 0 °C and the resulting mixture was stirred at 80 °C for 4h. The reaction mixture was concentrated and dried under vacuum to provide the crude acid chloride, which was dissolved in DMF (10.0 mL) and cooled to 0 °C. NMM (1.5 mL, 13.7 mmol, 8.4 equiv.) and 3-azidopropylamine **S5** (570.5 mg, 3.82 mmol, 5.7 eqv.) were added under an argon atmosphere at 0 °C and the mixture was further stirred at room temperature for 12 h. The solvent was removed in vacuum and the residue was purified by column chromatography using MeOH-CH_2Cl_2 (4:96) to give the bisazide **S6** as a white solid (364 mg, 76%). **^{1}H NMR** (500 MHz, Chloroform-*d*); δ 8.32 (s, 2H), 7.85 (s, 2H), 3.57 (4H, q, *J* = 6.2 Hz), 3.46 (4H, t, *J* = 6.3 Hz), 1.96 – 1.91 (4H, m); **^{13}C NMR** (125 MHz, Chloroform-*d*) δ 167.2, 164.4, 150.3, 113.0, 49.8, 37.8, 28.7; HRMS (ESI) calcd for $C_{13}H_{17}N_9NaO_3$: 370.1352; Found 370.1345.

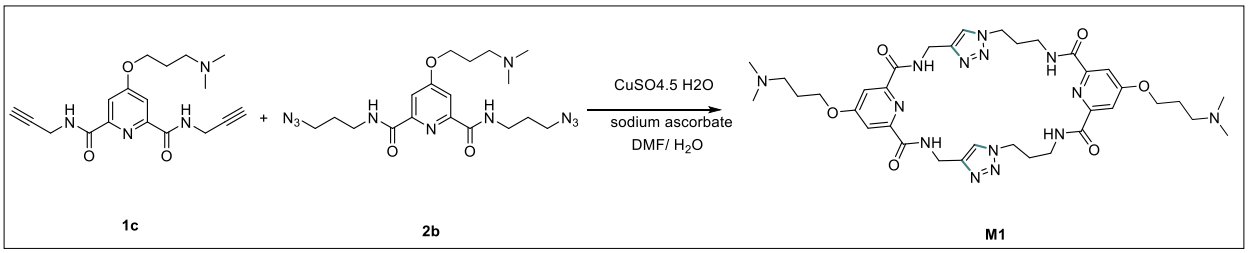


Synthesis of bisazide 2a: To a solution of **S6** (200.0 mg, 0.57mmol, 1 eqv) in dry DMF (10 mL) was added sodium hydride (27.6 mg, 1.15mmol, 2 eqv) slowly at 0 °C over a period of 15 min. After 30 min, 3-dimethylamino-1-propyl chloride hydrochloride **S4** (183.0 mg, 1.15 mmol, 2 eqv) was added. The mixture was kept another 30 min at 0 °C and then refluxed for 24 h. The reaction mixture was quenched with water and extracted with CH_2Cl_2 (3 x 15 mL). The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuum to afford desired product **2c** as a brown solid (198 mg, 80%). **1H-NMR** (500 MHz, $CDCl_3$); 8.36 (2H, t, *J* = 5.9), 7.81 (2H, s), 4.19 (2H, t, *J* = 6.4), 3.53 (4H, q, *J* = 7.6), 3.40 (4H, t, *J*= 8.1), 3.26 (2H, s), 2.87 (6H, s), 2.38 (2H, s), 1.93 (4H, m); **13C-NMR** (125 MHz, $CDCl_3$); 168.0, 163.9, 150.7, 111.2, 67.2, 55.9, 49.7, 45.4, 37.5, 28.8, 27.0; HRMS (ESI) calcd for $C_{18}H_{29}N_{10}O_3$: 433.2424; Found 433.2432.



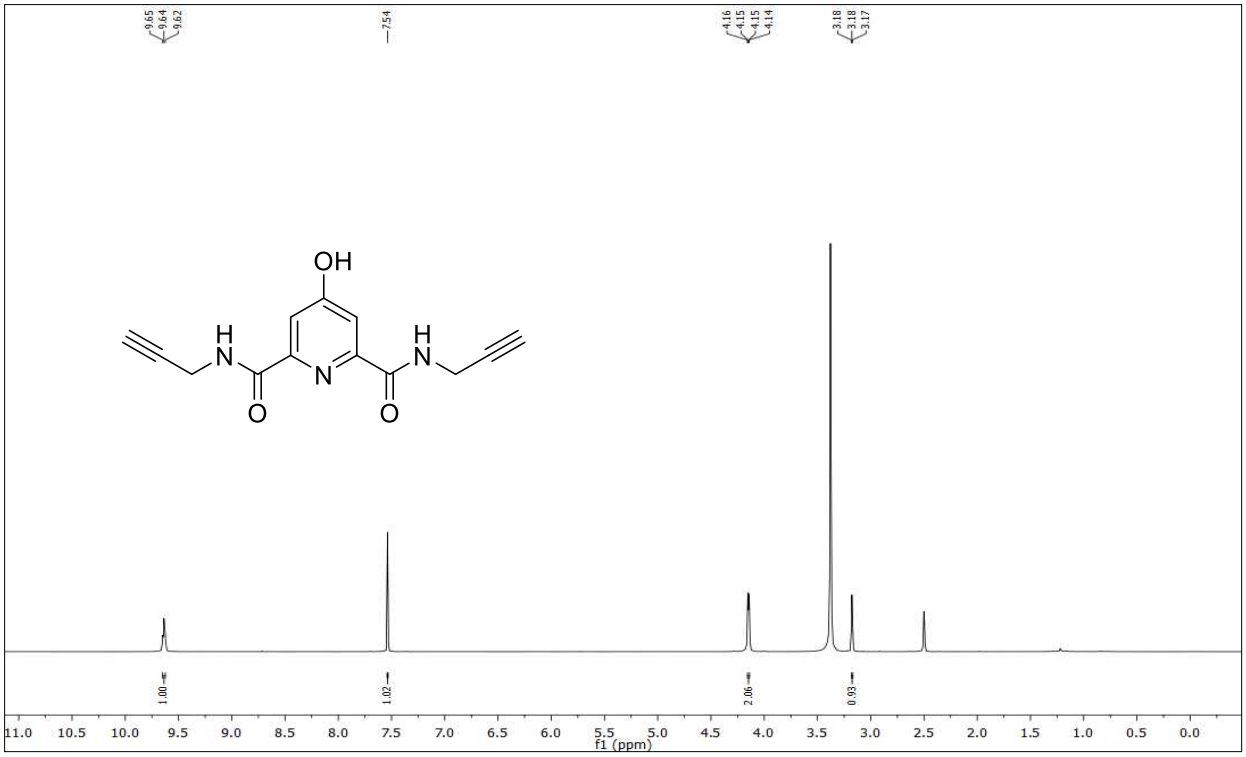
Synthesis of macrocycle: A mixture of sodium ascorbate (27 mg, 0.10 mmol, 0.5 equiv) and $CuSO_4.5H_2O$ (4.16 mg, 0.021mmol, 0.1 equiv) was taken in (4:1) DMF:H_2O (500 mL). A solution of bisalkyne **1c** (75.0 mg, 0.21 mmol, 1.0 equiv) and bisazide **2b**(90.7 mg, 0.21mmol, 1 equiv) in 1:1 DMF:H_2O (100 mL) was slowly added to the above mixture over a period of 24 h. The mixture was further stirred for another 12 h at room temperature. The reaction mixture was then concentrated under vacuum and the residue was purified by column chromatography using MeOH:CH_2Cl_2 (18:82) to give the corresponding product (92.1 mg, 70%) as a white solid. **^{1}H**

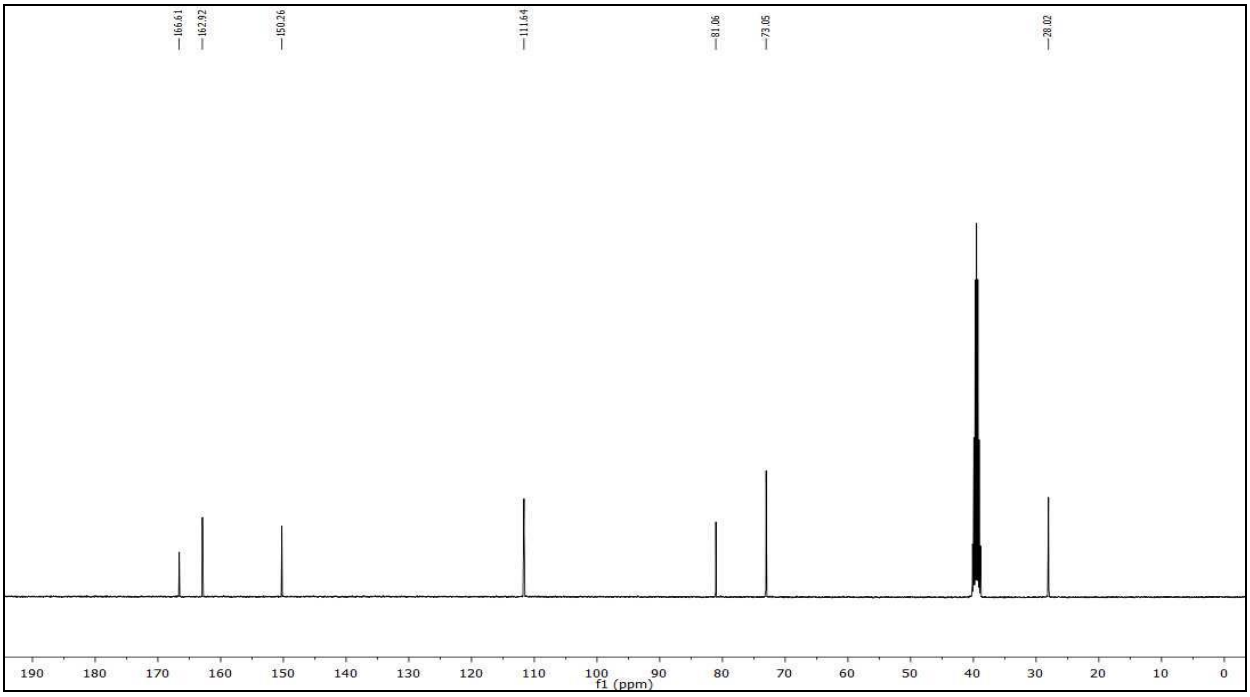
NMR (400 MHz, DMSO-d_6); δ 9.80 (2H, t, *J* = 6.3 Hz), 9.23 (2H, t, *J* = 6.0 Hz), 8.02 (2H, s,), 7.71 (2H, s), 7.59 (2H, s), 4.63 (4H, d, *J* = 6.2 Hz), 4.43 (4H, t, *J* = 6.1 Hz), 4.23 (4H, dd, *J* = 6.6 Hz), 3.29 (4H, dd, *J* = 5.7 Hz), 2.39 (4H, t, *J* = 5.4 Hz), 2.16 (12H, s), 2.05-2.00 (4H, m), 1.89 (4H, q, *J* = 6.9 Hz); **^{13}C NMR** (100 MHz, DMSO-d_6); δ 167.2, 167.1, 162.9, 162.9, 150.6, 145.0, 123.2, 110.5, 109.9, 66.9, 66.8, 55.2, 47.2, 45.0, 44.9, 36.6, 34.4, 30.1, 26.2, 26.2; HRMS (ESI) calcd for $C_{36}H_{51}N_{14}O_6$: 775.4116; Found 775.4113.



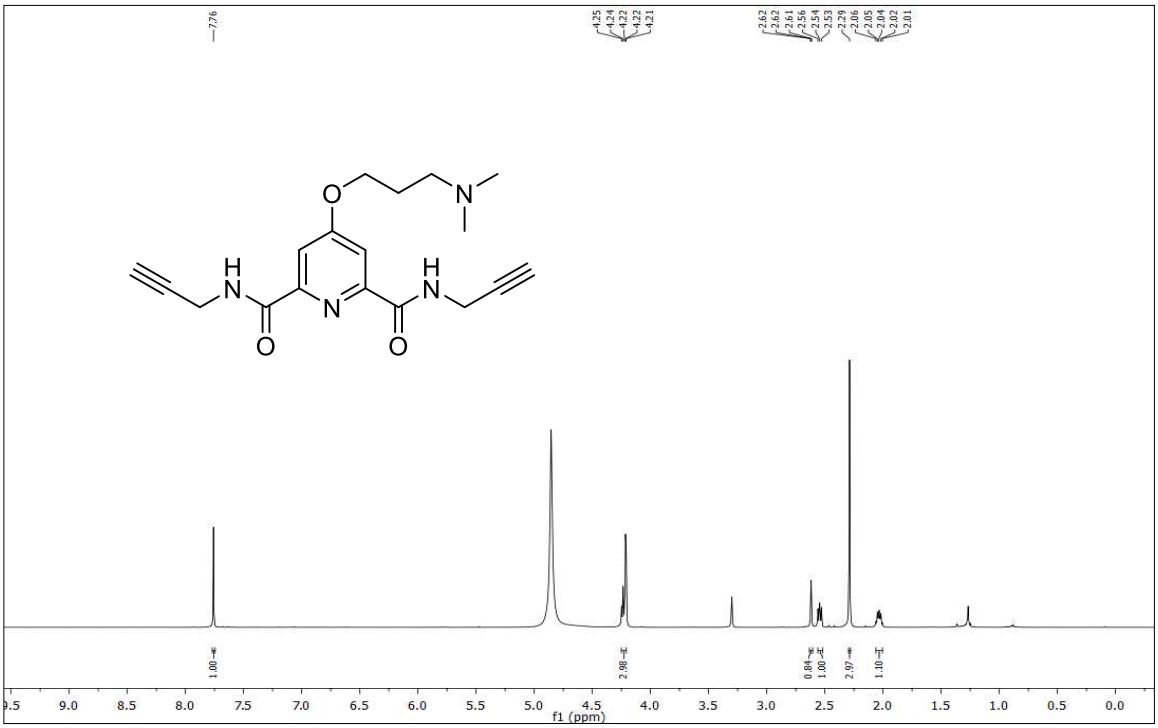
5. ^{1}H NMR and ^{13}C NMR spectra of compounds: ^{1}H NMR (400 MHz, DMSO-d6):

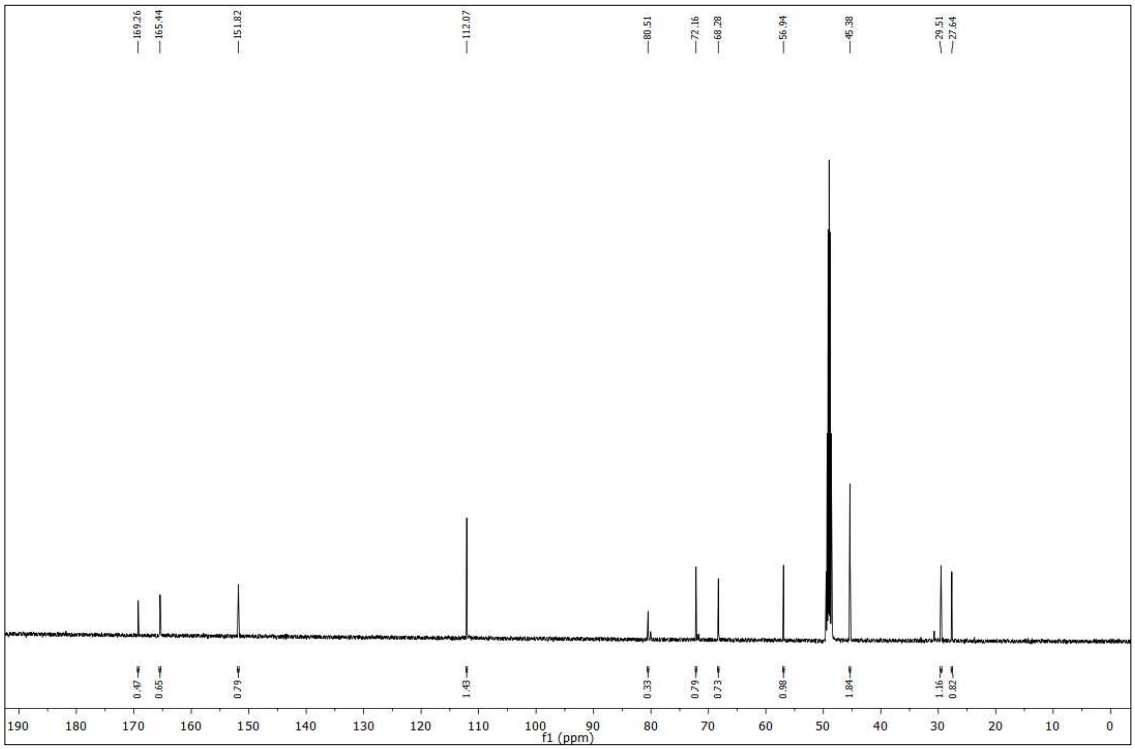
^{1}H- and ^{13}C-NMR of Compound S3:



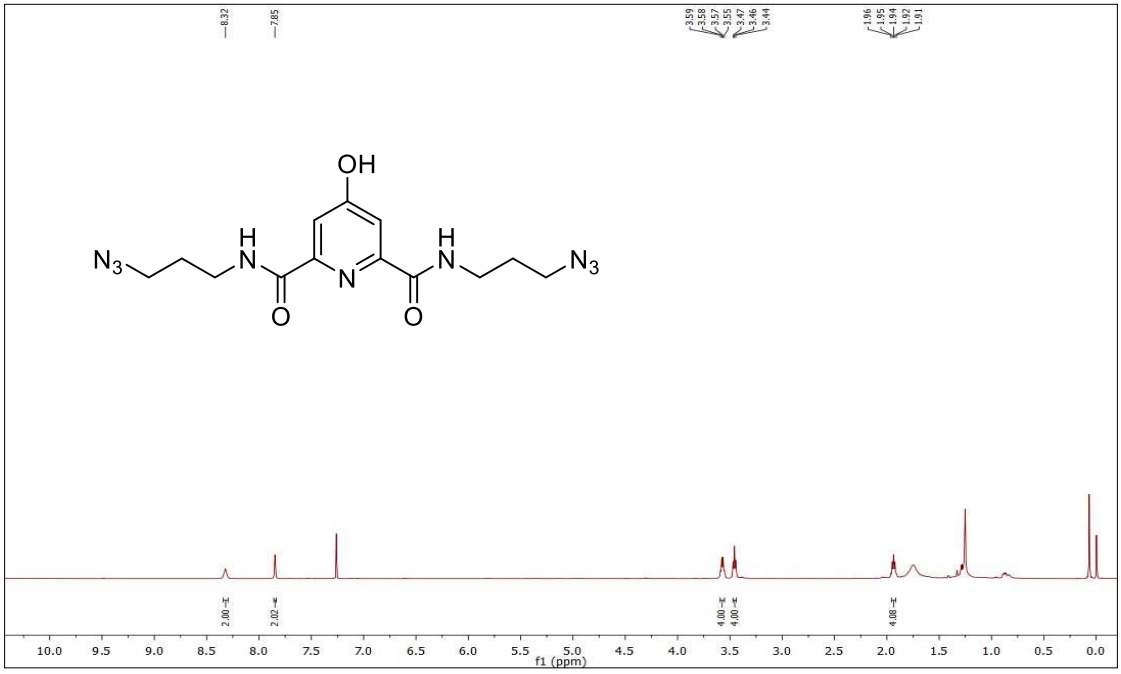


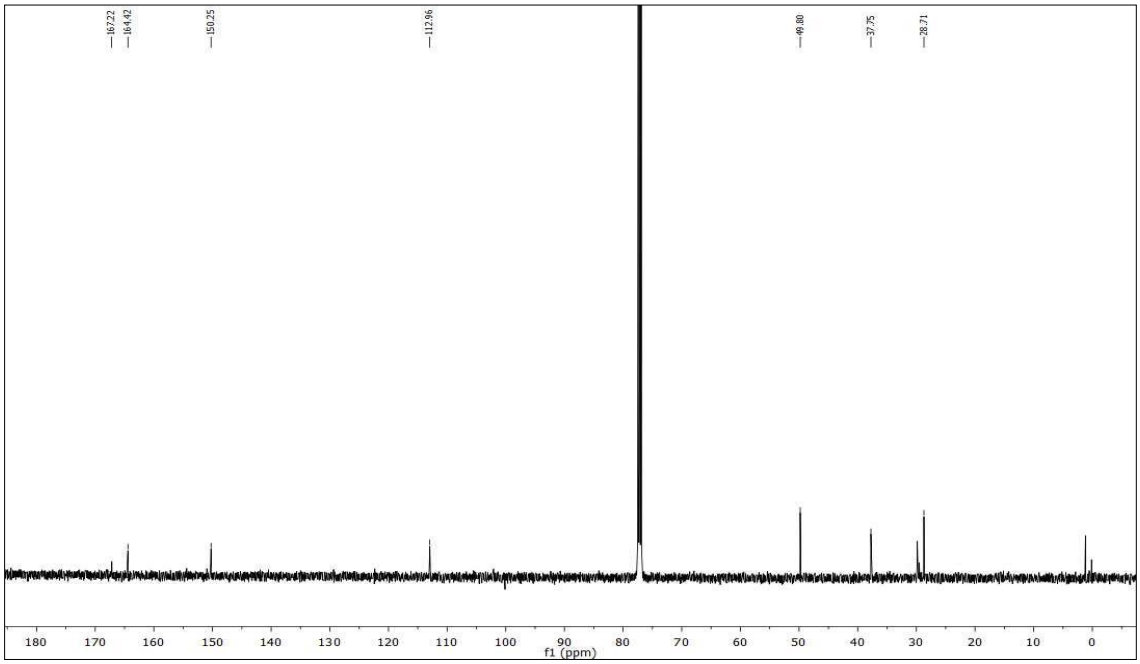
^{1}H- and ^{13}C-NMR of Compound 1c:



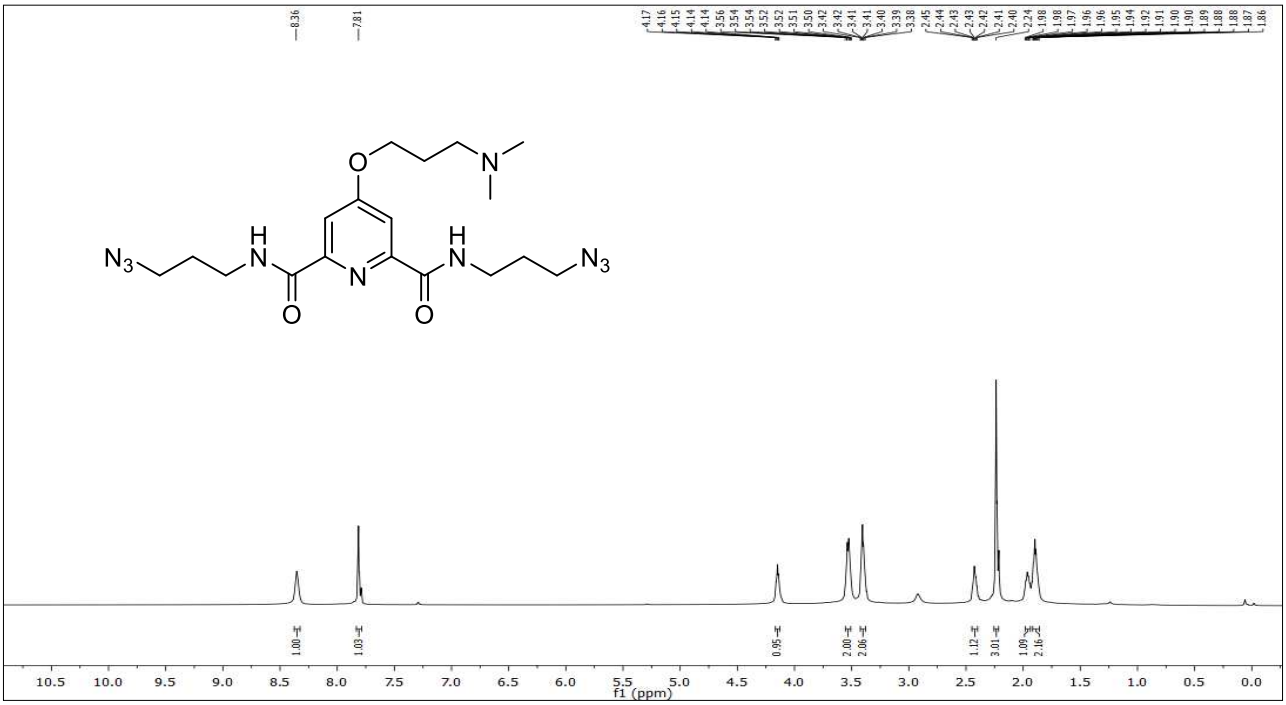


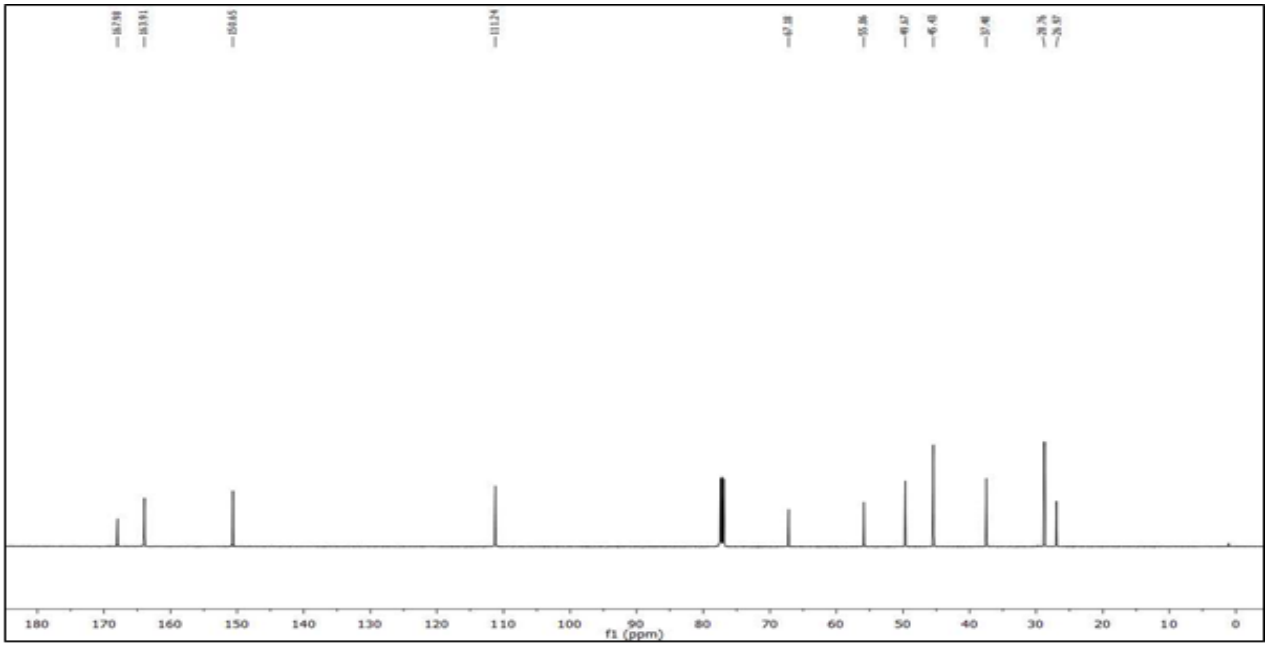
^{1}H- and ^{13}C-NMR of Compound S6:



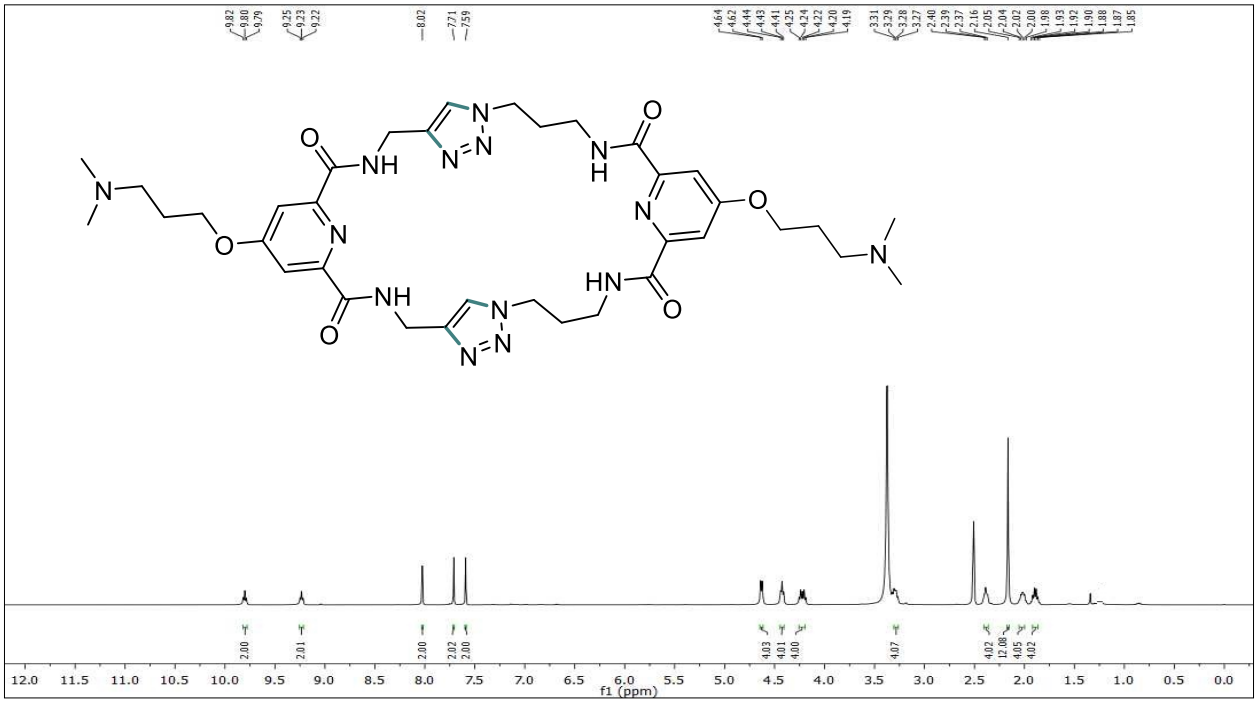


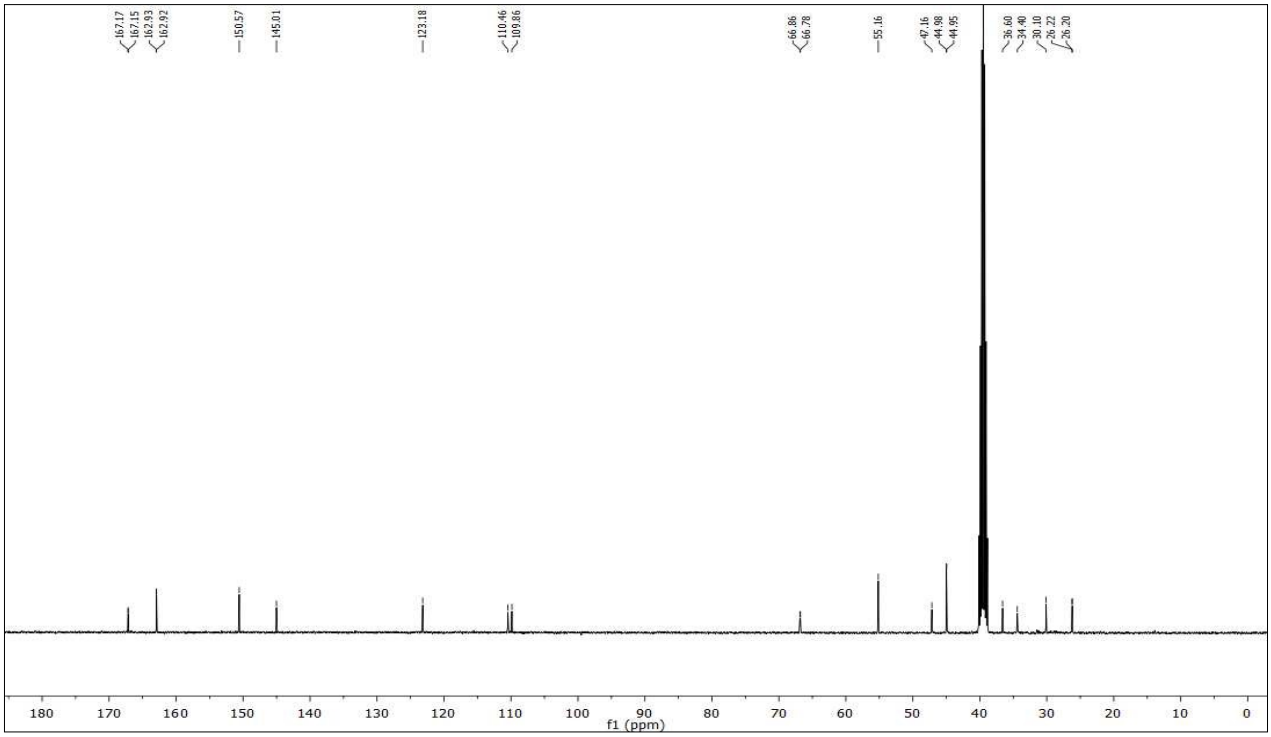
^{1}H- and ^{13}C-NMR of Compound 2b:





^{1}H, ^{13}C-NMR of macrocycle M1:





6. Powder X-ray Diffraction (PXRD) study: Powder X- ray Diffraction (PXRD) experiment was carried out with a dried thin film of the gel on a glass slide using an X'Pert PRO X-ray Powder Diffractometer (PANalytical, Netherlands made) from an angle range of 20° to 35° C.

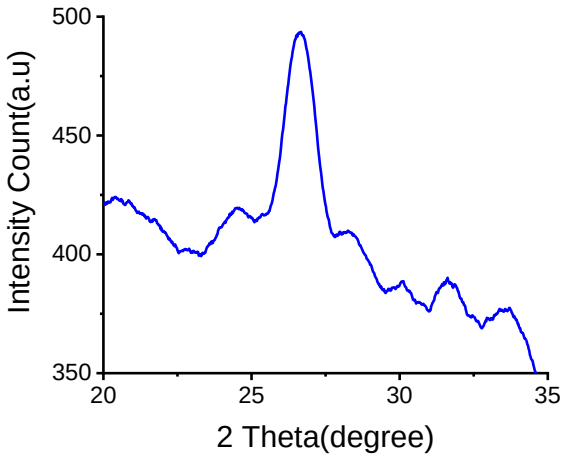


Figure S7. PXRD pattern of G-gel.

7. DSC analysis of the hydrogel:

The G-gel was heated in oven to remove the water content to obtain a xerogel (15 mg), which was subsequently used for the DSC Study. All heating and cooling steps were carried out at 10 °C/min. DSC was carried out with required amount of the hydrogel which in the range of−10 to 140°C using LVC pans in a PerkinElmer made Diamond DSC machine at a scan rate of 10°C min−1.

The Differential Scanning Calorimetry experiments of the hydrogel samples imbibed with the fragments were carried out after an incubation period of 5 days.

8. TEM and AFM analysis:

For the TEM experiment, 5 mg of the G-gel was diluted with 1 mL of MilliQ water to obtain a transparent dispersion and then 10 µL of the sample was drop casted on the carbon coated copper grid and allowed to adsorb for 5 mins on the grid. The excess solution was removed by slanting the grid over lint free wipers. The sample was dried overnight in desiccator and it was analyzed using ultra high-resolution field emission gun transmission electron microscopy (UHR-FEG-TEM), manufactured by JEOL 2100 KeV at 200 KeV.

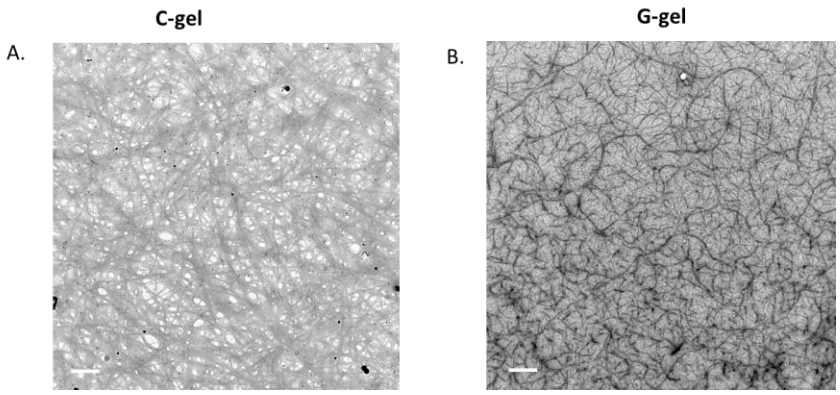


Figure S8.TEM image of A) C-gel and B) G-gel; (Scale bar- 0.2 µm)

The AFM was carried out on a NT-MDT in semi-contact mode. In order to obtain a transparent dispersion 1 mg of the G-gel was diluted with 1 mL of MilliQ water. 10 µL of aqueous hydrogel dispersion was drop casted onto the freshly cleaved mica (Agar Supplies) and allowed to dry in a desiccator for 16 h prior to imaging. The images were analyzed in Gatan and WSxM 5.0 software respectively.

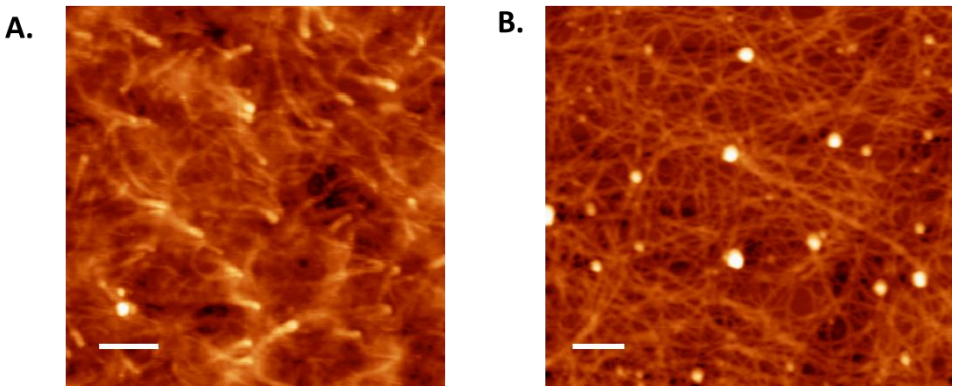


Figure S9. AFM image of A) G-gel and B) C-gel; (Scale bar-0.4 µm)

9. Rheological measurements

For the rheological experiments, a compact rheometer, Anton Paar MCR 102 was used. A parallel plate (PP 25) was used as the measuring system. In order to confirm the gel nature of the compound, the viscoelastic properties in terms of amplitude sweep tests were performed at a constant temperature of 298 K. For all the experiments a constant 10 rad/s angular frequency (ω) was maintained within the range of 0.01% to 100% shear strain. The rheological studies of the

G4 gel imbibed with alkyne and azide fragment and macrocycle were obtained after incubation for a period of 5 days.

Sample	% Strain for G′, G″ crossover	% Strain corresponding to LVR
Macrocycle (M1)	27.7	0.14
Alkyne(1c) +Azide(2b)	9.62	0.8
Alkyne 1c	3.76	0.35
Azide 2b	2.17	0.42

Table S2. Rheological analysis of G-gel imbibed with **M1**, **1c+2b**, **1c** and **2b**.

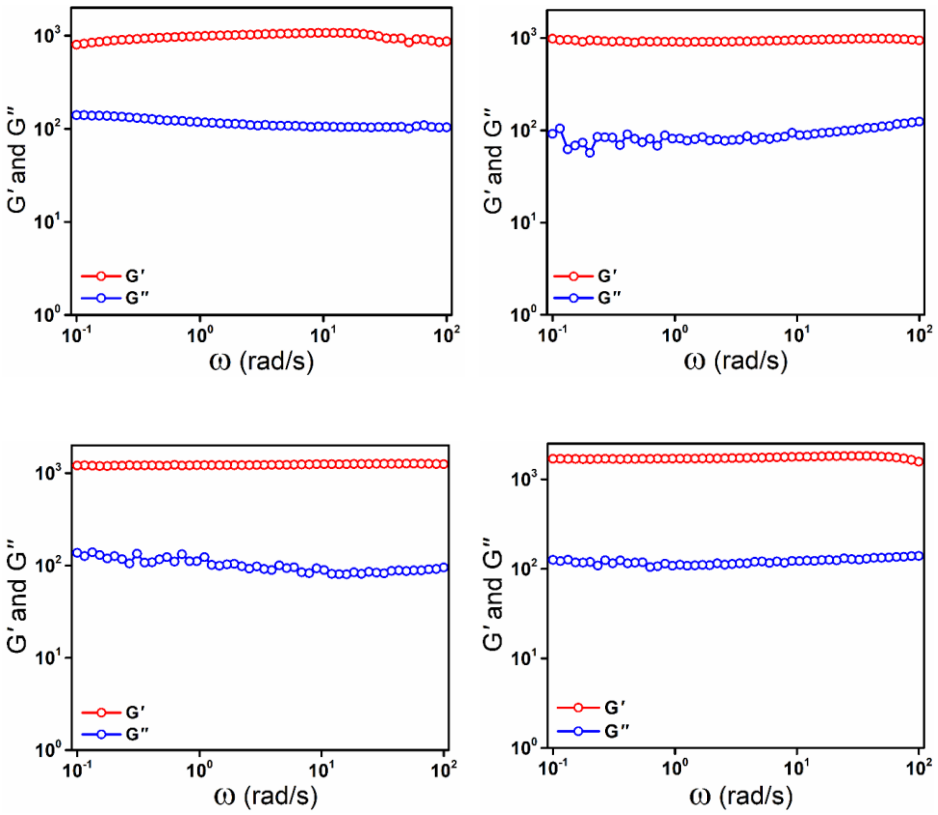


Figure S10. Frequency sweep experiments of the different combinations of hydrogel and the compounds - i) G-gel+**1c**, ii) G-gel+**2b**, iii) G-gel+**1c**+**2b**, and iv) G-gel + **M1**.

10. Complementary size of M1 with G-quartet

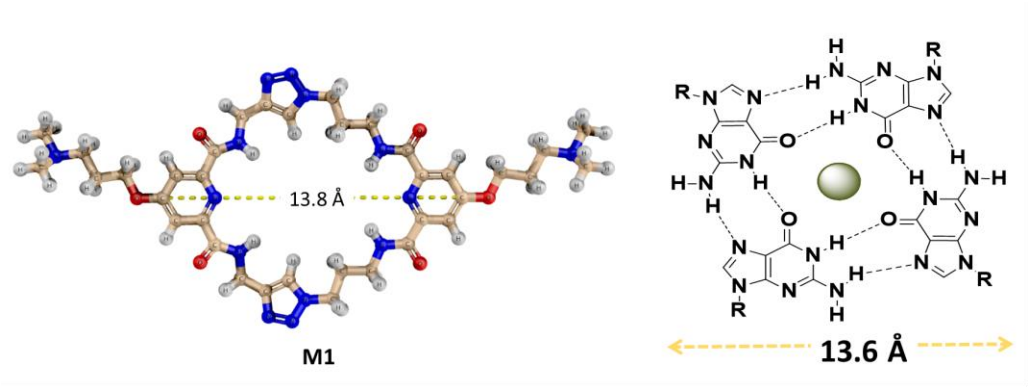


Figure S11. Complementary structural geometry of **M1** with G-quartet.

11. NMR titration of *c-MYC* and M1:

The *cMYC* DNA was purchased from Eurofins MWG Operon in HPSF purity grade and further purified via HPLC. The titration was performed with a DNA concentration of 100 µM in 25 mM Tris•HCl buffer (pH = 7.4) with 100 mM KCl in 5% d_6-DMSO/95% H_2O. Ligand stock solution in 100% d_6-DMSO was added directly to the NMR tube during the titration. 2,2-dimethyl-2-silapentene-5-sulphonate (DSS) was used as an internal reference. Excitation sculpting[1] or jump-return[2] was used for water suppression. All spectra were recorded on a 600 MHz NMR-spectrometer equipped with CryoProbe Prodigy TCI $^{1}H/^{19}F$ [^{13}C, ^{15}N] Z-GRD.

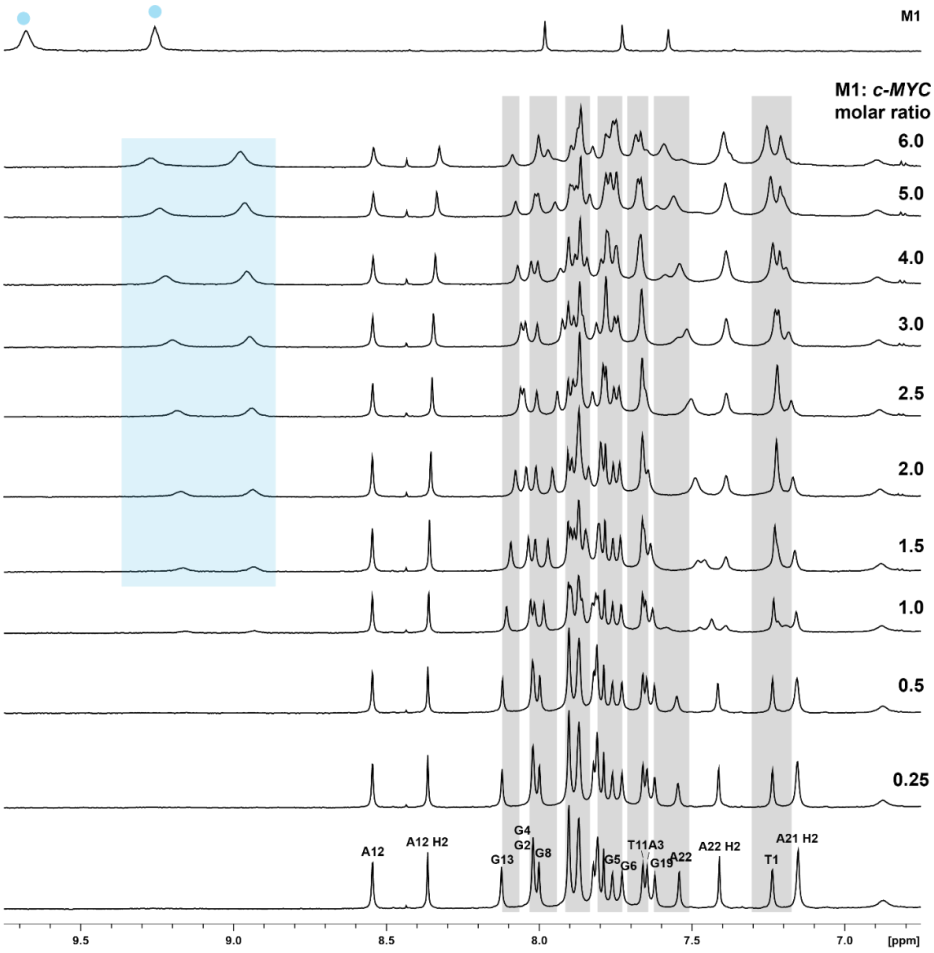


Figure S12. Aromatic region of 1D ^{1}H NMR spectrum of the c-MYC22 G-quadruplex DNA with increasing [**M1**]:[DNA] ratio. The spectra were recorded at 298 K, 600 MHz. Experimental conditions: 100 µM DNA in 25 mM Tris•HCl (pH 7.4) buffer containing 100 mM KCl in 5% d_6-DMSO/95% H_2O. Grey areas mark the shifted aromatic resonances of *c-MYC*, ligand signals are highlighted with blue.

1. T.-L. Hwang and A. Shaka, *Journal of Magnetic Resonance, Series A*, 1995, **112**, 275-279.
2. A. Ambrus, D. Chen, J. Dai, R. A. Jones and D. Yang, *Biochemistry*, 2005, **44**, 2048-2058.