

***In situ* Formation of Transcriptional Modulators by Non-canonical DNA i-Motifs**

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1.0 General information. All solvents and reagents were purified by standard techniques reported in Armarego, W. L. F., Chai, C. L. L., Purification of Laboratory Chemicals, 5th edition, Elsevier, 2003; or used as supplied from commercial sources (Sigma-Aldrich Corporation® unless stated otherwise). All reactions were generally carried out under inert atmosphere unless otherwise noted. TLC was performed on Merck Kieselgel 60 F254 plates, and spots were visualized under UV light. Products were purified by flash chromatography on silica gel (100-200 mesh, Merck). ¹H and ¹³C NMR spectra were recorded on either Bruker ADVANCE 500 (500 MHz and 125 MHz), or JEOL 400 (400 MHz and 100 MHz) instruments using deuterated solvents as detailed and at ambient probe temperature (300 K). Chemical shifts are reported in parts per million (ppm) and are referred to the residual solvent peak. The following notations are used: singlet (s); doublet (d); triplet (t); quartet (q); multiplet (m); broad (br). Coupling constants are quoted in Hertz and are denoted as *J*. Mass spectra were recorded on a Micromass® Q-ToF (ESI) spectrometer.

2.0 Synthesis of Au@Fe₃O₄ nanoparticles. The Fe₃O₄ nanoparticles were prepared by mixing FeCl₃ and FeCl₂ in a 1:2 molar ratio in basic solutions. 1 gm of FeCl₃.6H₂O and 0.4 gm of FeCl₂.4H₂O were dissolved in 100 mL milliQ water and stirred continuously. Then 25 % NH₄OH solution were added slowly to the mixture to make the solution pH 9.0. The reaction mixture was then stirred for 30 min at room temperature. After reaching to pH 9, precipitation was observed and the precipitate was isolated by magnetic decantation and washed with milliQ water. The precipitate was then dissolved in HClO₄ (50 ml, 2 M) and then collected by centrifugation. The nanoparticles were then resuspended in 50 ml of milliQ water.

Next, an aqueous solution of HAuCl₄ (2.26 ml, 2.0 mg ml⁻¹) was mixed with 15.75 ml deionized water and then heated to boiling. Then, 0.75 ml of Fe₃O₄ nanoparticle solution was added into the reaction mixture followed by the addition of sodium citrate (0.75 ml, 80 mM) with continuous stirring. The colour of the solution was gradually changed from brown to burgundy. The reaction mixture was heated to 85 °C under stirring for 5 min. After cooling, the solution was sonicated for 5 min and then Au@Fe₃O₄ NPs were collected using a magnet, washed three times and redispersed in milliQ water.

3.0 Synthesis of DNA coated Au@Fe₃O₄ NPs: The buffers and solutions were degassed under N₂ stream to avoid oxidative dimerization of thiolated DNA sequences into disulfide dimers. Gold coated magnetic nanoparticles functionalized with thiolated DNA sequences were synthesized by mixing an aqueous dispersion of Au@Fe₃O₄ nanoparticles (50 μL) with 50 μL of 5'-thiol-capped DNA sequence (concentration 25 μM, preannealed). The *c-MYC* and *BCL2* G-quadruplex sequences and the thiolated duplex DNA sequence were attached to the nanoparticles in 100 mM Tris.KCl buffer, pH 7.4 and thiolated *c-MYC* and *BCL2* i-motif sequences were linked to the nanoparticles in 10 mM sodium cacodylate buffer, pH 5.5. After incubation for 16 h with intermittent stirring, the nanoparticles were separated using external magnet and washed with the corresponding buffer solutions. The volume of the nanoparticle solution was then made up to 100 μL with the respective buffer solution. The HPLC purified thiol-capped DNA sequences were supplied by Eurofins Genomics. The sequences of the DNAs are as follows:

c-MYC C₄: 5'-[ThiC6]TCCCCACCTTCCCCACCCTCCCCACCCTCCCCA

BCL2 C₄: 5'-[ThiC6]CAGCCCCGCTCCCGCCCCCTTCCTCCCGCGCCCGCCCCT

c-MYC G₄: 5'-[ThiC6]TGGGGAGGGTGGGGAGGGTGGGGGAAGG

BCL2 G₄: 5'-[ThiC6]AGGGGCGGGCGCGGGAGGAAGGGGGCGGGAGCGGGGCTG

dsDNA: 5'-[ThiC6]CAAAAATTTTTGCAAAAATTTTTG

The concentration of DNA grafted on Au@Fe₃O₄ NPs was determined by comparing the absorbance maxima of the UV-Vis spectra of *c-MYC* C₄-DNA (25 μM) and the residual solution collected after magnetic separation of NPs from the DNA solution. The approx. DNA concentration of DNA linked Au@Fe₃O₄ NPs was calculated to be ~ 20 μM.

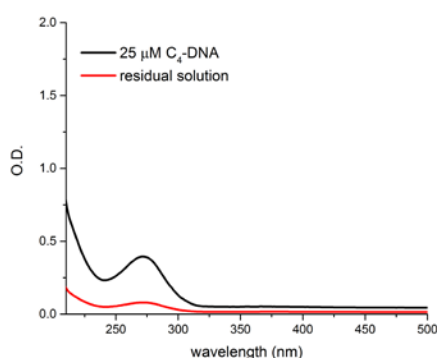
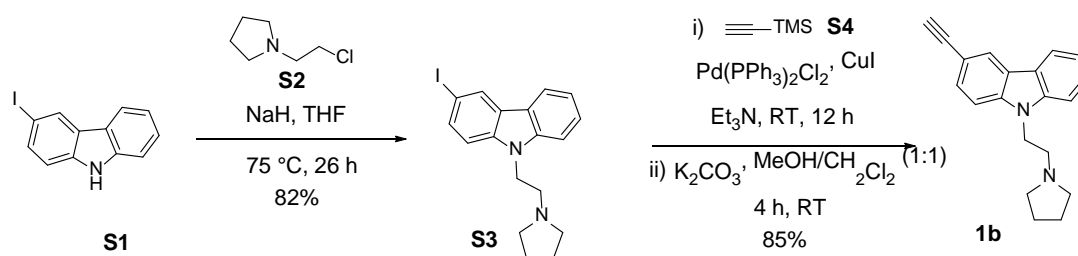


Figure S1. UV-Vis spectra of C₄-DNA (25 μM) and residual solution in 10 mM sodium cacodylate buffer, pH 5.5.

4.0 Preparation of alkynes 1b-d.

(a) Preparation of alkyne 1b:

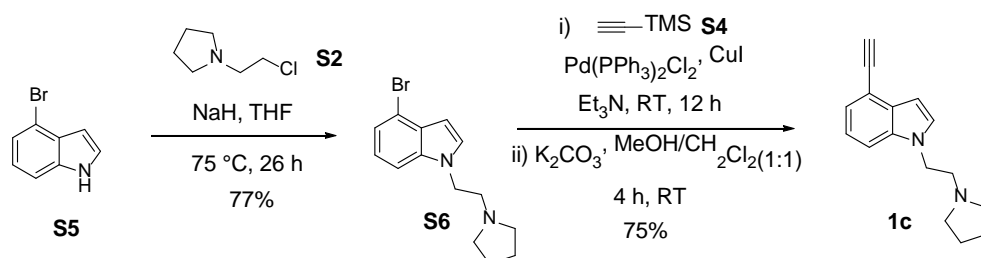


A mixture of 3-iodo-9H-carbazole **S1** (500 mg, 1.71 mmol) and NaH (164.2 mg, 6.84 mmol) in 20 mL THF was stirred at room temperature for 2 h under nitrogen atmosphere. In another flask, 1-(2-chloroethyl)pyrrolidine hydrochloride **S2** (873 mg, 5.13 mmol) and NaOH (1.5 g) were diluted in 5 mL water and cooled to room temperature, then the upper layer of this solution was added drop-wise to the above mixture. The reaction mixture was refluxed for additional 24 h. After the removal of the solvent under reduced pressure, the residue was purified by column chromatography on silica gel using CH₂Cl₂/methanol (20:1) as eluent. Re-crystallization from ethanol gave the compound **S3** (547.20 mg, 82%) as a yellow liquid. ¹H NMR (500 MHz, CDCl₃): 8.38 (s, 1H), 8.00 (d, 1H, *J* = 7.6), 7.71 (d, 1H, *J* = 7.6), 7.47-7.45 (m, 3H), 7.25-7.22 (m, 1H), 4.57 (t, 2H, *J* = 7.6), 3.01 (t, 2H, *J* = 6.7), 2.76 (m, 4H), 1.87 (m, 4H). ¹³C NMR (400 MHz, CDCl₃): 140.2, 139.5, 134.0, 129.5, 122.9, 120.7, 119.1, 110.8, 108.7, 81.6, 54.5, 53.7, 41.9, 23.6. HRMS (ESI) calculated for [C₁₈H₂₀IN₂] 391.0671, Found 391.0665.

To a solution of **S3** (250 mg, 0.641 mmol) in Et₃N (5 mL) PdCl₂(PPh₃)₂ (45 mg, 0.064 mmol), and CuI (24.4 mg, 0.13 mmol) were added at room temperature. The mixture was stirred for 30 min and then TMS-acetylene **S4** (0.18 mL, 1.28 mmol) was added drop wise. The resulting mixture was stirred under an argon atmosphere for 12 h. The solvents were concentrated, washed with brine, and dried over anhydrous Na₂SO₄. The crude product was purified by column chromatography and the product was stirred with 5 equivalent K₂CO₃ in methanol-CH₂Cl₂ solution under an argon atmosphere for 4 h. The solvents were concentrated, washed with brine, and dried over anhydrous Na₂SO₄. The crude product was purified by column chromatography to obtain alkyne **1b** (157.13 mg, 85%). ¹H NMR (500 MHz, CDCl₃): 8.25 (s, 1H), 8.06 (d, 1H, *J* = 7.6), 7.59 (d, 1H, *J* = 8.4), 7.49-7.44 (m, 2H), 7.38 (d, 1H, *J* = 8.4), 7.26 (t, 1H, *J* = 6.7),

4.48 (t, 2H, $J = 7.6$), 3.07 (s, 1H), 2.98 (t, 2H, $J = 7.5$), 2.66 (m, 4H), 1.85-1.82 (m, 4H). ^{13}C NMR (400 MHz, CDCl_3): 140.8, 140.3, 129.9, 126.5, 125.9, 124.8, 120.7, 120.5, 119.8, 119.2, 109.0, 108.7, 85.1, 75.3, 54.6, 54.2, 42.6, 23.7. HRMS (ESI) calculated for $[\text{C}_{20}\text{H}_{21}\text{N}_2]$ 289.1705, Found 289.1695.

(b) Preparation of alkyne 1c:

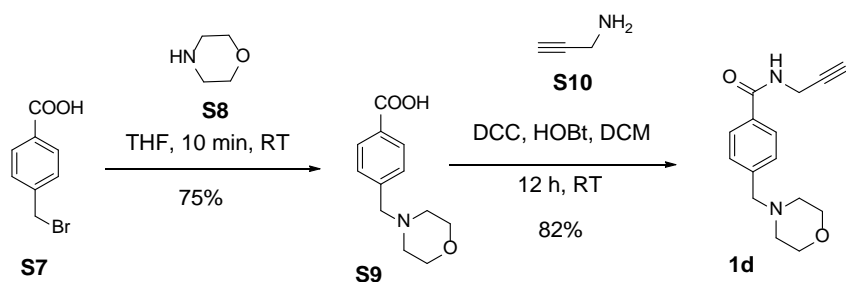


A mixture of 4-bromo indole **S5** (335.2 mg, 1.71 mmol) and NaH (164.2 mg, 6.84 mmol) in 20 mL THF was stirred at room temperature for 2 h under nitrogen atmosphere. In another flask, 1-(2-chloroethyl) pyrrolidine hydrochloride **S2** (872.5 mg, 5.13 mmol) and NaOH (1.5 g) were diluted in 5 mL water and cooled to room temperature, then the upper layer of this solution was added drop-wise to the above mixture. The reaction mixture was refluxed for an additional 24 h. After the removal of the solvent under reduced pressure, the residue was purified by column chromatography on silica gel using CH_2Cl_2 /methanol (20:1) as eluent. Re-crystallization from ethanol gave the compound **S6** (386 mg, 77%) as a yellow liquid. ^1H NMR (500 MHz, CDCl_3): 7.31 (d, 1H, $J = 7.5$), 7.27 (d, 1H, $J = 8.4$), 7.20 (m, 1H), 7.07-7.04 (m, 1H), 6.54 (m, 1H), 4.27 (t, 2H, $J = 7.6$), 2.89 (t, 2H, $J = 7.6$), 2.56 (s, 4H), 1.79 (s, 4H). ^{13}C NMR (125 MHz, CDCl_3): 136.5, 129.4, 128.7, 122.5, 122.4, 115.1, 108.7, 101.9, 55.7, 54.5, 46.3, 23.7. HRMS (ESI) calculated for $[\text{C}_{14}\text{H}_{18}\text{BrN}_2]$ 293.0653, Found 293.0646.

To a solution of **S6** (187.9 mg, 0.641 mmol) in Et_3N (5 mL) added $\text{PdCl}_2(\text{PPh}_3)_2$ (45 mg, 0.064 mmol), and CuI (24.4 mg, 0.13 mmol) at room temperature. The mixture was stirred for 30 min and then TMS-acetylene **S4** (0.18 mL, 1.28 mmol) was added drop wise. The resulting mixture was stirred under an argon atmosphere for 12 h. The solvents were concentrated, washed with brine, and dried over anhydrous Na_2SO_4 . The crude product was purified by column chromatography and the product was stirred with 5

equivalent K_2CO_3 in methanol- CH_2Cl_2 solution under an argon atmosphere for 4 h. The solvents were concentrated, washed with brine, and dried over anhydrous Na_2SO_4 . The crude product was purified by column chromatography to produce alkyne **1c** (114.6 mg, 75%). 1H NMR (400 MHz, $CDCl_3$): 7.31 (d, 1H, $J = 8.5$), 7.27 (d, 1H, $J = 8.5$), 7.20 (d, 1H, $J = 3.8$), 7.08-7.04 (m, 1H), 6.54 (d, 1H, $J = 3.8$), 4.26 (t, 2H, $J = 7.3$), 3.26 (s, 1H), 2.88 (t, 2H, $J = 7.3$), 2.56 (s, 4H), 1.78 (s, 4H). ^{13}C NMR (125 MHz, $CDCl_3$): 136.5, 132.3, 128.6, 122.5, 122.4, 115.1, 108.7, 101.8, 79.3, 55.9, 54.5, 46.3, 23.7. HRMS (ESI) calculated for $[C_{16}H_{19}N_2]$ 239.1470, Found 239.1465.

(c) Preparation of alkyne **1d**:

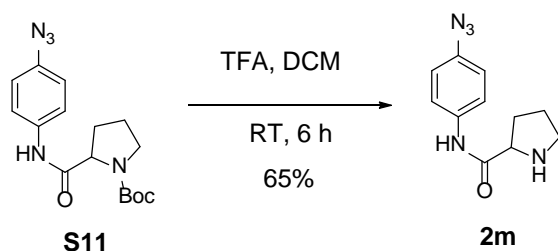


Compound **S9** (prepared by following the protocol described by Qiu *et. al.*¹) (1.0 g, 4.52 mmol) was added to the solution of DCC (1.1 g, 5.42 mmol) and HOBT (732 mg, 5.42 mmol) in CH_2Cl_2 (10 mL). The mixture was stirred for 5 minutes at room temperature. Then propargyl amine **S10** (0.3 mL, 5.42 mmol) was added to the solution. After completion of the reaction, the reaction mixture was quenched by addition of $NaHCO_3$ solution. The mixture was extracted with CH_2Cl_2 (3 x 5 mL) and the combined organic layer was washed with water and brine, dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel ($CH_2Cl_2/MeOH$, 20:1) to give the compound **1d** (957 mg, 82 % yield) as colorless solid. 1H NMR (500 MHz, $CDCl_3$): 7.76 (d, 2H, $J = 8.4$), 7.47 (d, 2H, $J = 8.4$), 4.25-4.23 (m, 2H), 4.08-4.06 (m, 2H), 3.48-3.46 (m, 4H), 2.30 (s, 1H), 1.94-1.92 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): 166.4, 143.1, 136.9, 130.1, 127.7, 81.6, 79.4, 76.9 (merged with $CDCl_3$ peak), 72.2, 49.3, 30.0. HRMS (ESI) calculated for $[C_{15}H_{19}N_2O_2]$ 259.1447, Found 259.1435.

¹ Qiu, J.; Xu, B.; Huang, Z.; Pan, W.; Cao, P.; Liu, C.; Hao, X.; Song, B.; Liang, G. *Bioorganic Med. Chem.* **2011**, *19*, 5352.

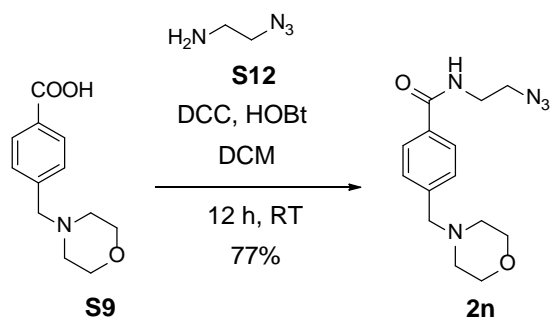
5.0 Preparation of azides.

(a) Preparation of **2m**:



To a slurry of **S11** (1.00 g, 3.0 mmol) in CH_2Cl_2 (6 mL) was added 2.2 equiv of TFA. The resulting solution was stirred at ambient temperature for 6 h. The solvent was evaporated in vacuo to obtain the azide **2m** (451 mg, 65%) as a yellow liquid. ^1H NMR (400 MHz, CDCl_3): 7.42 (d, 2H, $J = 8.5$), 7.31 (d, 2H, $J = 6.7$), 3.74 (s, 1H), 2.97 (s, 1H), 2.88 (s, 1H), 2.10 (s, 1H), 1.91 (s, 1H), 1.66-1.64 (m, 2H). ^{13}C NMR (100 MHz, CDCl_3): 173.5, 137.0, 131.6, 120.9, 116.2, 60.9, 49.9, 30.6, 26.2. HRMS (ESI) calculated for $[\text{C}_{11}\text{H}_{14}\text{N}_5\text{O}]$ 232.1198, Found 232.1186.

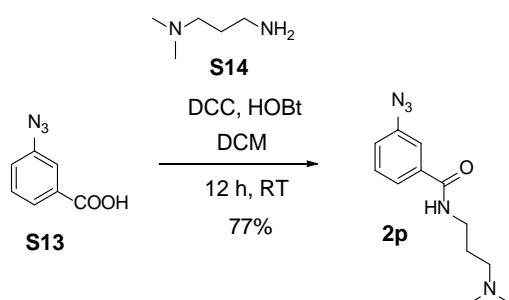
(b) Preparation of **2n**:



Compound **S9** (1.0 g, 4.52 mmol) was added to a solution of DCC (1.1 g, 5.42 mmol) and HOBT (732 mg, 5.42 mmol) in CH_2Cl_2 (10 mL). The mixture was stirred for 5 minutes at room temperature. Then compound **S12** (0.47 g, 5.42 mmol) was added to the solution. After completion of the reaction, the reaction mixture was quenched by addition of NaHCO_3 solution. The mixture was extracted with CH_2Cl_2 (3 X 5 mL) and the organic layer was washed with water and brine, dried over MgSO_4 and the solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel

(CH₂Cl₂/MeOH, 20:1) to give the compound **2n** (1 g, 77 % yield) as colorless solid. ¹H NMR (400 MHz, DMSO d⁶): 8.90 (s, 1H), 7.98 (d, 2H, *J* = 10), 7.74 (d, 2H, *J* = 10), 3.72 (s, 2H), 3.69-3.6 (m, 4H), 3.50 (2H, merged with DMSO water peak), 2.65 (s, 4H), 1.78-1.74 (m, 2H). ¹³C NMR (125 MHz, DMSO d⁶): 166.3, 142.6, 136.4, 129.9, 126.9, 66.1, 61.9, 52.9, 47.2, 33.0, 25.2. HRMS (ESI) calculated for [C₁₄H₂₀N₅O₂] 290.1617, Found 290.1605.

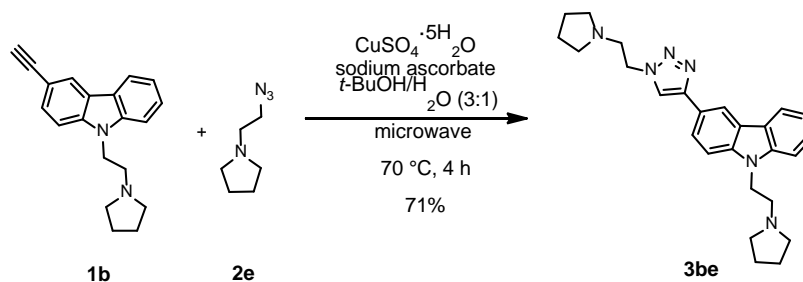
(c) Preparation of 2p:



A mixture of 3-azido benzoic acid **S13** (500 mg, 3.1 mmol) and HOBT (612.5 mg, 4.0 mmol) was dissolved in 10 mL CH₂Cl₂. After cooling to 0 °C, DCC (825.32 mg, 4.0 mmol) and 3-(dimethylamino)propylamine **S14** (0.51 mL, 2.8 mmol) were added and the mixture was stirred overnight at room temperature. CH₂Cl₂ was evaporated in vacuo. The crude product was purified by column chromatography (CH₂Cl₂:100% then CH₂Cl₂/MeOH (20:1) to give the azide **2p** (590 mg, 77%) as a colorless liquid. ¹H NMR (400 MHz, CDCl₃): 8.70 (s, 1H), 7.52 (d, 1H, *J* = 7.4), 7.44 (s, 1H), 7.37-7.33 (m, 1H), 7.08-7.06 (m, 1H), 3.53-3.49 (m, 2H), 2.49 (t, 2H, *J* = 6.0), 2.28 (s, 6H), 1.75 (t, 2H, *J* = 5.4). ¹³C NMR (100 MHz, CDCl₃): 165.2, 139.6, 136.2, 130.0, 123.9, 121.7, 117.7, 64.1, 45.5, 37.6, 32.3. HRMS (ESI) calculated for [C₁₂H₁₈N₅O] 248.1511, Found 248.1495.

6.0 Preparation of triazole products.

Preparation of 3be:



Alkyne **1b** (50 mg, 0.173 mmol) was dissolved in a 1:2 mixture of $t\text{-BuOH}/\text{H}_2\text{O}$ (4 mL). Copper (II) sulphate pentahydrate (4.3 mg, 0.017 mmol) and sodium ascorbate (3.4 mg, 0.017 mmol) were added and the solution was stirred for 10 min. The corresponding azide **2e** (36.4 mg, 0.26 mmol) was added and the mixture was then heated for 4 h at 70 °C under microwave irradiation. After cooling to room temperature, the reaction mixture was concentrated. The crude product was purified by flash column chromatography (from CH_2Cl_2 (100%) to $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (10:1) to $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_4\text{OH}$ (10:1:0.5) to give the compound **3be** as a yellow liquid (52.6 mg, 71%). ^1H NMR (500 MHz, CDCl_3): 8.57 (s, 1H), 8.12 (d, 1H, $J = 8.0$), 7.91 (d, 2H, $J = 8.0$), 7.48-7.43 (m, 3H), 7.24 (t, 1H, $J = 8.0$), 4.54 (t, 2H, $J = 6.5$), 4.48 (t, 2H, $J = 8.0$), 3.03 (t, 2H, $J = 8.0$), 2.92 (t, 2H, $J = 8.0$), 2.65 (s, 4H), 2.59 (s, 4H), 1.82 (s, 4H), 1.80 (s, 4H). ^{13}C NMR (100 MHz, CDCl_3): 148.8, 140.8, 140.3, 126.2, 124.0, 123.4, 120.7, 119.6, 119.4, 117.9, 109.0, 108.9, 55.7, 54.6, 54.3, 54.1, 49.5, 42.5, 23.7, 23.6. HRMS (ESI) calculated for $[\text{C}_{26}\text{H}_{33}\text{N}_6]$ 429.2767, Found 429.2764.

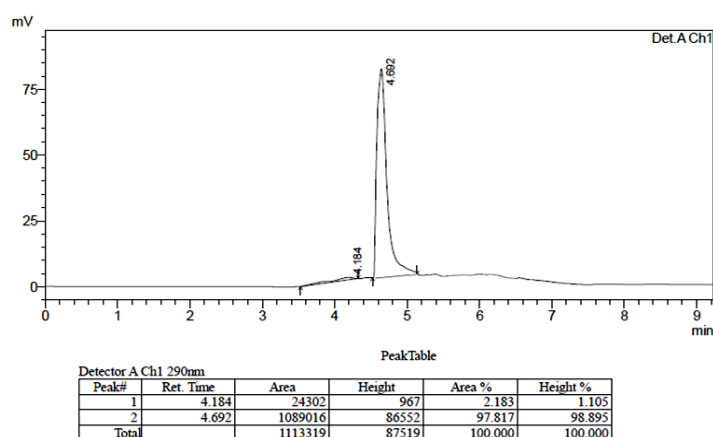
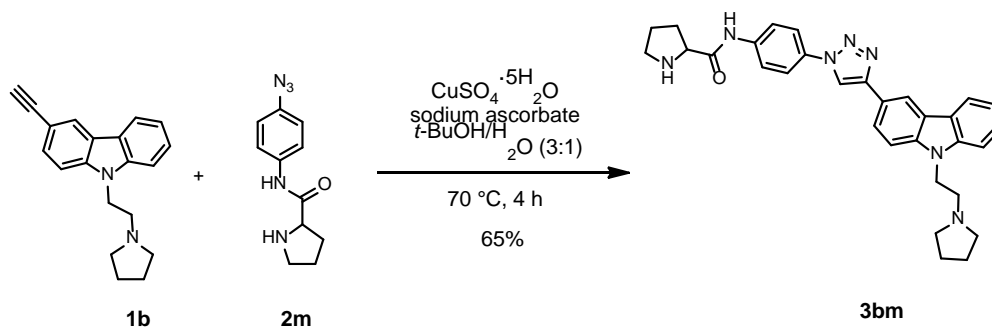


Figure S2. HPLC trace of triazole product **3be**.

Preparation of 3bm:



Alkyne **1b** (50 mg, 0.173 mmol) was dissolved in a 1:2 mixture of *t*-BuOH/H₂O (4 mL). Copper (II) sulphate pentahydrate (4.3 mg, 0.017 mmol) and sodium ascorbate (3.4 mg, 0.017 mmol) were added and the solution was stirred for 10 min. The corresponding azide **2m** (60.1 mg, 0.26 mmol) was added and the mixture was then heated for 4 h at 70 °C under microwave irradiation. After cooling to room temperature, the reaction mixture was concentrated. The crude product was purified by flash column chromatography (from CH₂Cl₂ (100%) to CH₂Cl₂/MeOH (10:1) to CH₂Cl₂/MeOH/NH₄OH (10:1:0.5) to give the compound **3bm** as a yellow liquid (90 mg, 65%). ¹H NMR (400 MHz, DMSO d₆): 10.11 (sbr, 1H), 9.42 (sbr, 1H), 9.27 (s, 1H), 8.75 (s, 1H), 8.23 (d, 1H, *J* = 7.3), 8.08 (d, 1H, *J* = 8.3), 7.98 (d, 2H, *J* = 8.8), 7.84 (d, 2H, *J* = 8.3), 7.73 (d, 1H, *J* = 8.3), 7.54 (t, 1H, *J* = 7.8), 7.30 (t, 1H, *J* = 7.3), 4.78 (t, 2H, *J* = 6.8), 4.42-4.37 (m, 1H), 3.66-3.61 (m, 4H), 3.19-3.11 (m, 2H), 2.05-1.95 (m, 4H), 1.90-1.85 (m, 2H), 1.23-1.21 (m, 4H). ¹³C NMR (100 MHz, DMSO d₆): 167.2, 148.2, 140.1, 139.5, 138.2, 132.7, 126.4, 123.7, 122.9, 122.4, 121.9, 120.7, 120.5, 119.8, 118.6, 117.3, 109.8, 109.6, 59.8, 53.7, 51.3, 45.9, 29.5, 28.9, 23.5, 22.7. HRMS (ESI) calculated for [C₃₁H₃₄N₇O] 520.2825, Found 520.2814.

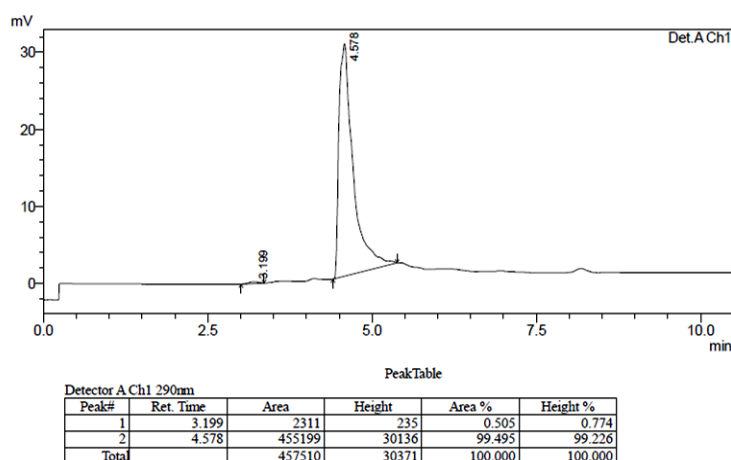
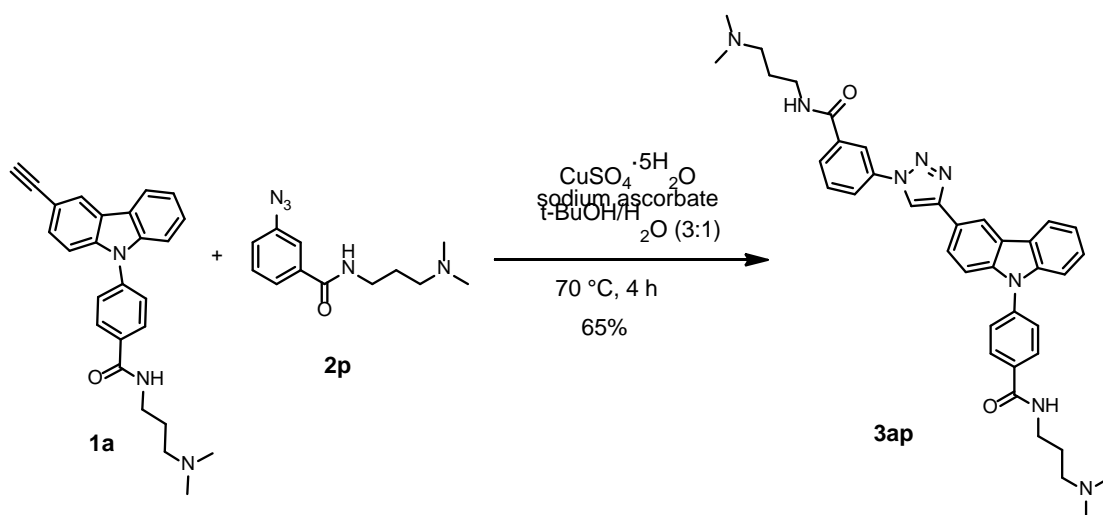


Figure S3. HPLC trace of triazole product **3bm**.

Preparation of 3ap:



Alkyne **1a** (68.4 mg, 0.17 mmol) was dissolved in a 1:2 mixture of $t\text{-BuOH}/\text{H}_2\text{O}$ (4 mL). Copper (II) sulphate pentahydrate (4.3 mg, 0.017 mmol) and sodium ascorbate (3.42 mg, 0.0173 mmol) were added and the solution was stirred for 10 min. The corresponding azide **2p** (64.30 mg, 0.26 mmol) was added and the mixture was then heated for 4 h at $70\text{ }^\circ\text{C}$ under microwave irradiation. After cooling to room temperature, the reaction mixture was concentrated. The crude product was purified by flash column chromatography (from CH_2Cl_2 (100%) to $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (10:1) to $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_4\text{OH}$ (10:1:0.5) to give the compound **3ap** as a yellow solid (72.28 mg, 65%). ^1H NMR (500 MHz, CDCl_3): 9.22 (s, 1H), 8.74 (s, 1H), 8.72 (s, 1H), 8.37 (s, 1H), 8.32 (s, 1H), 8.21 (d, 1H, $J = 8.0$), 8.04 (d, 2H, $J = 8.4$), 8.00 (d, 1H, $J = 8.4$), 7.93 (d, 1H, $J = 8.4$), 7.86 (d, 1H, $J = 8.4$), 7.67 (d, 2H, $J = 8.0$), 7.61 (d, 1H, $J = 8.0$), 7.51 (d, 1H, $J = 8.4$), 7.45 (d, 2H, $J = 8.4$), 7.37-7.31 (m, 1H), 3.67-3.60 (m, 4H), 2.59-2.54 (m, 4H), 2.38 (s, 6H), 2.35 (s, 6H), 1.85-1.79 (m, 4H). ^{13}C NMR (100 MHz, CDCl_3): 166.4, 165.1, 149.5, 141.2, 140.7, 136.8, 133.9, 130.1, 128.8, 126.8, 126.7, 124.3, 123.8, 122.6, 120.9, 118.9, 118.1, 117.0, 110.3, 110.1, 59.8, 59.7, 45.7, 45.6, 41.4, 41.1, 25.3, 24.8. HRMS (ESI) calculated for $[\text{C}_{38}\text{H}_{43}\text{N}_8\text{O}_2]$ 643.3509, Found 643.3509.

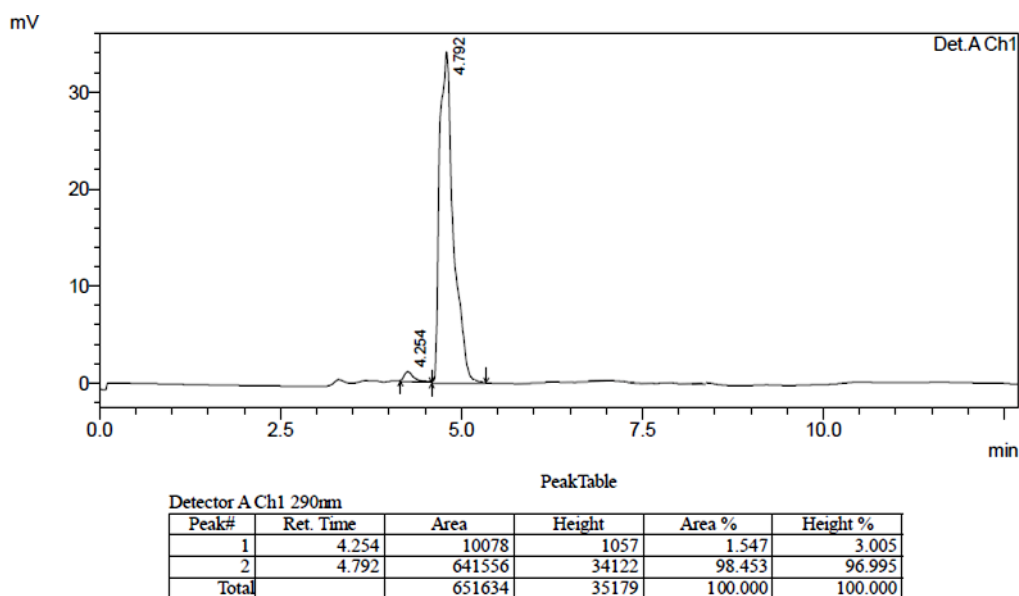


Figure S4. HPLC trace of triazole product **3ap**.

7.0 General procedure for DNA•Au@Fe₃O₄ templated cycloaddition. To a suspension of DNA nano-temple (10 μ L), alkynes **1a-d**, individually and azides **2a-q** were added and the volume was made upto 50 μ L with corresponding buffer (for G₄ DNA and dsDNA, the buffer is 100 mM Tris.KCl buffer, pH 7.4 and for i-motif (C₄) DNA, the buffer is 10 mM sodium cacodylate buffer, pH 5.5). Final concentrations of alkynes and azides were as follows: alkyne (1 μ M) and azide (4 μ M), each and DNA concentration is approximately 4-5 μ M. The reaction mixture containing the nano-temple and azide-alkyne building blocks was continuously shaken at room temperature for 4 days. After 4 days of incubation, the DNA nano-templates were separated from the reaction mixture using a magnet and the nano-templates were then washed thrice with the respective buffer to remove the unreacted starting materials. Subsequently, the nano-temple was dispersed in 50 μ L of corresponding buffer solution and the dispersion was then heated for 5 min at 65 $^{\circ}$ C in the presence of 1 M LiCl. The nano-temple was instantly separated by magnetic decantation and the supernatant was analysed by HPLC. The HPLC fractions corresponding to different peaks were identified by ESI-MS spectroscopy. The HPLC analysis was performed using 5.0 μ m ODS2 reverse phase column (4.6 \times 250 mm) using 290 nm detection wavelength. Flow rate was 0.5 ml min⁻¹ CH₃CN/H₂O (90:10) in 0.1% TFA over 20 min.

8.0 Monitoring DNA•Au@Fe₃O₄-mediated in situ click reaction by HPLC analysis.

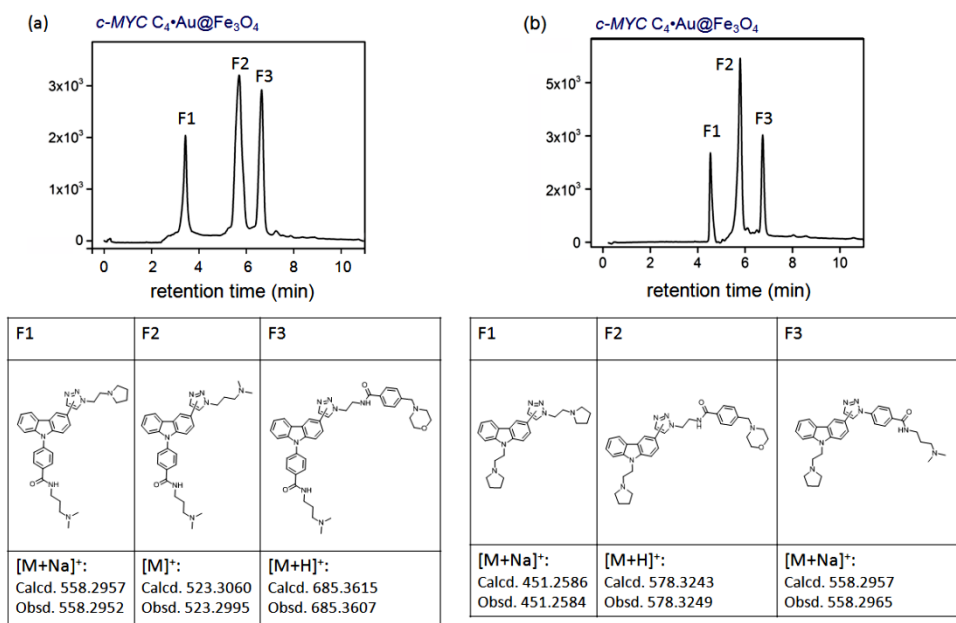


Figure S5a-b. HPLC chromatograms and observed mass of the products obtained with *c-MYC* C₄•Au@Fe₃O₄ from (a) alkyne **1a** and (b) alkyne **1b**.

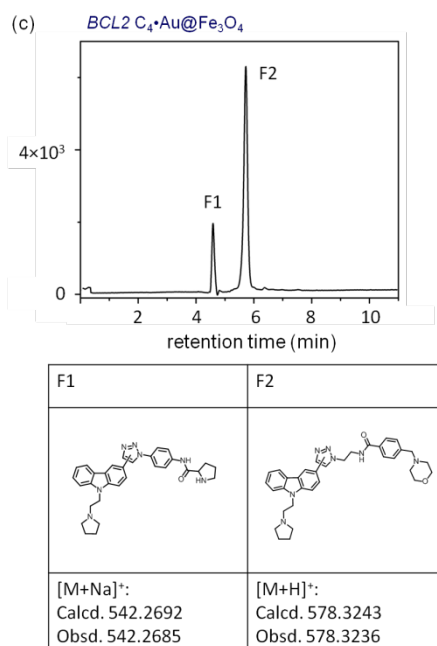


Figure S5c. HPLC chromatograms and observed mass of the products obtained with *BCL2* C₄•Au@Fe₃O₄ from alkyne **1b**.

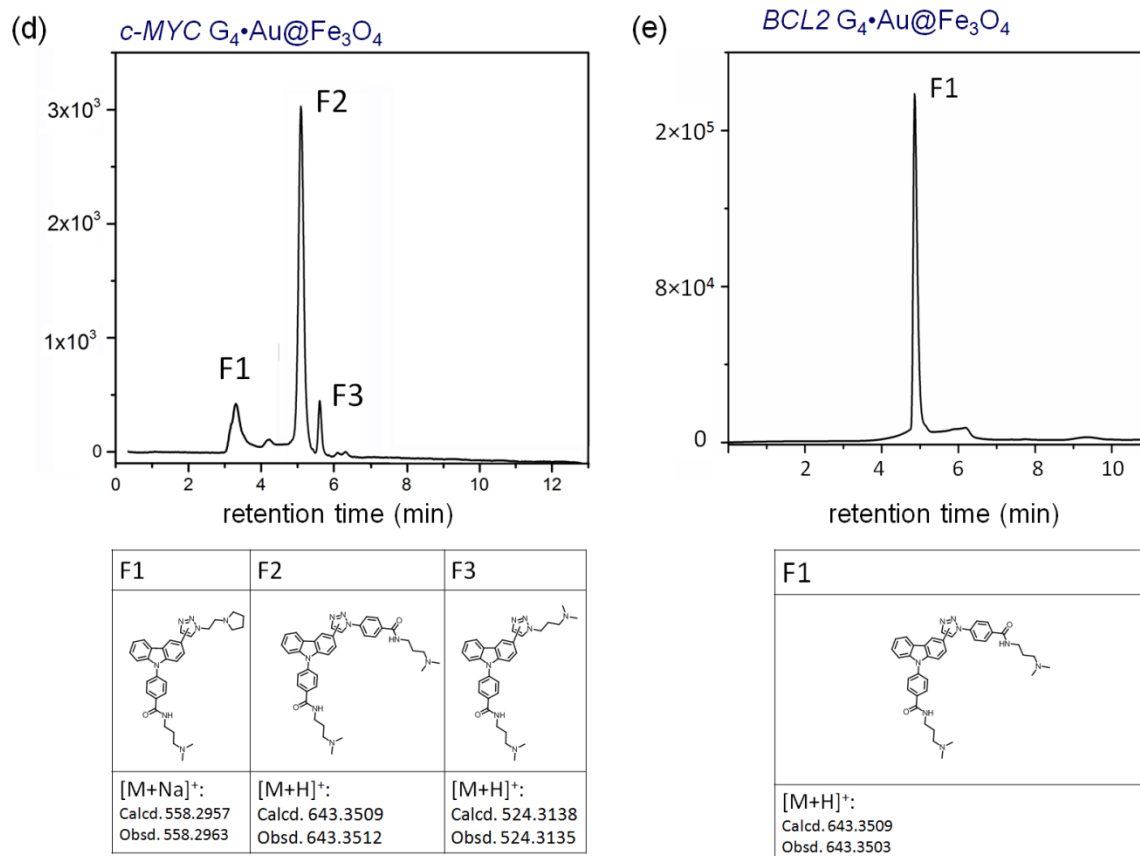


Figure S5d-e. HPLC chromatograms and observed mass of the products obtained with *c-MYC* G₄•Au@Fe₃O₄ and *BCL2* G₄•Au@Fe₃O₄ from alkyne **1a**.

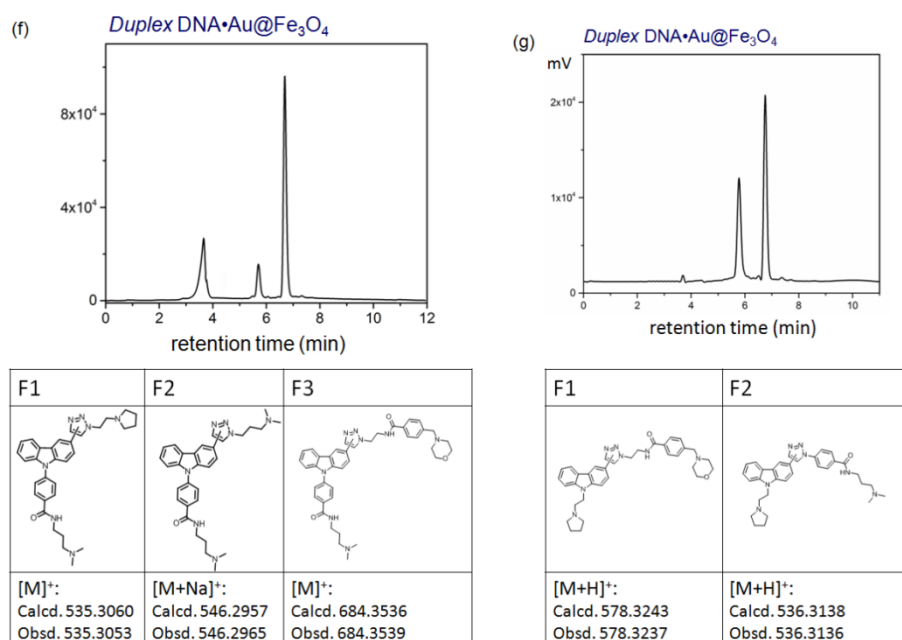


Figure S5f-g. HPLC chromatograms and observed masses of the products obtained with duplex DNA•Au@Fe₃O₄ from alkyne **1a** and alkyne **1b**.

9.0 In situ click experiments with control dsDNA nanotemplates.

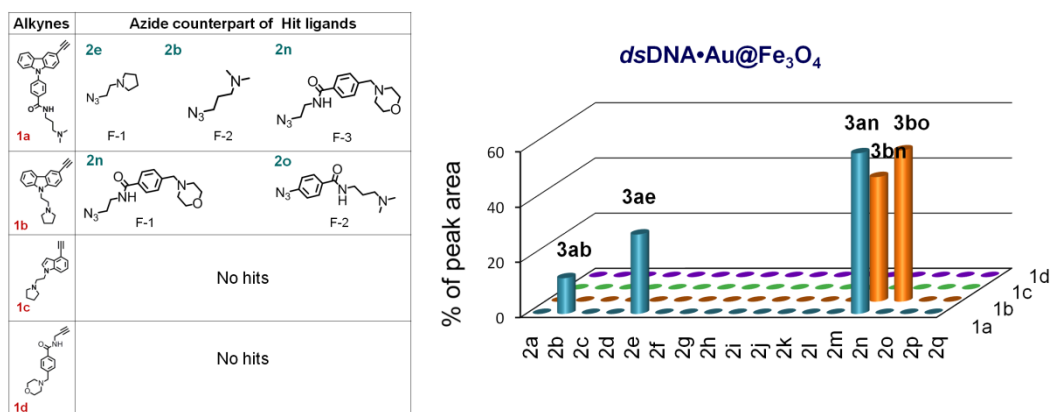


Figure S6. *In situ* click experiments with control dsDNA nanotemplates. The hit compounds formed in TGS using *dsDNA*•Au@Fe₃O₄ nanotemplates as determined by HPLC/MS analysis.

10.0 Determination of the regiochemistry of the triazole products (3be, 3bm, 3ao and 3ap). The regiochemistry of the triazole compounds (**3be**, **3bm**, **3ao** and **3ap**), generated by DNA•Au@Fe₃O₄ was determined by comparing the HPLC traces of the templated cycloaddition (*in situ* reaction) with the typical thermal and Cu-(I) catalyzed reactions between the corresponding alkyne and azide.

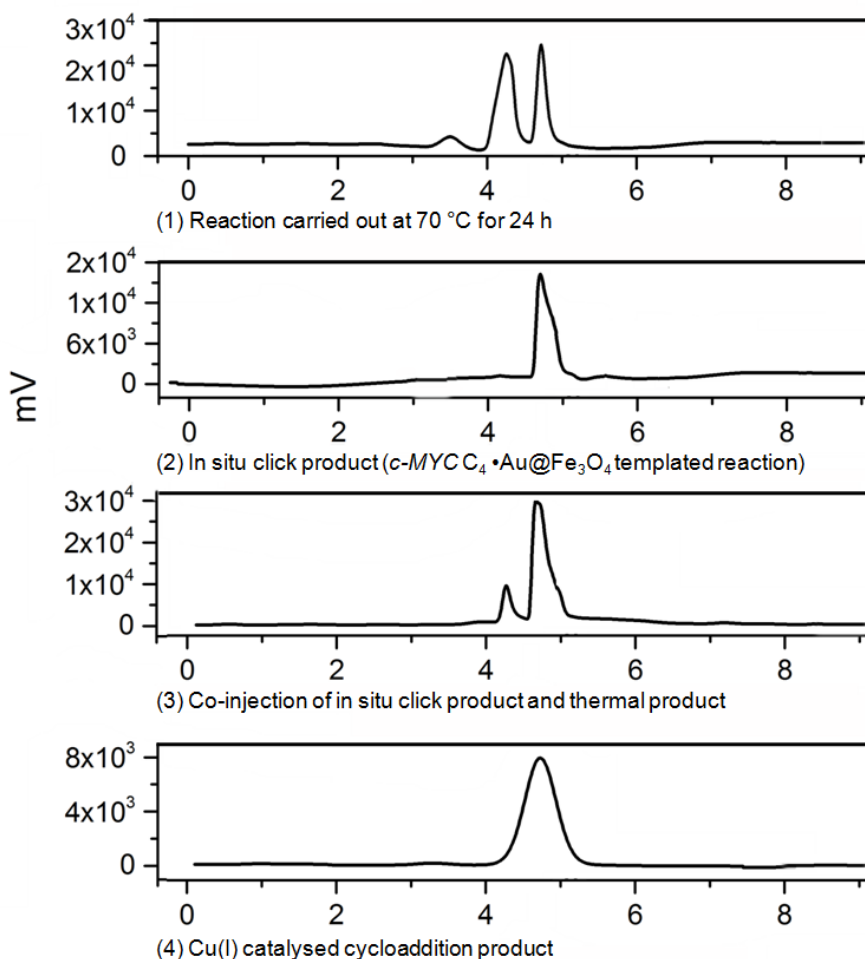


Figure S7. HPLC traces of the products obtained in the 1, 3-dipolar cycloaddition between alkyne **1b** and azide **2e** obtained under different conditions.

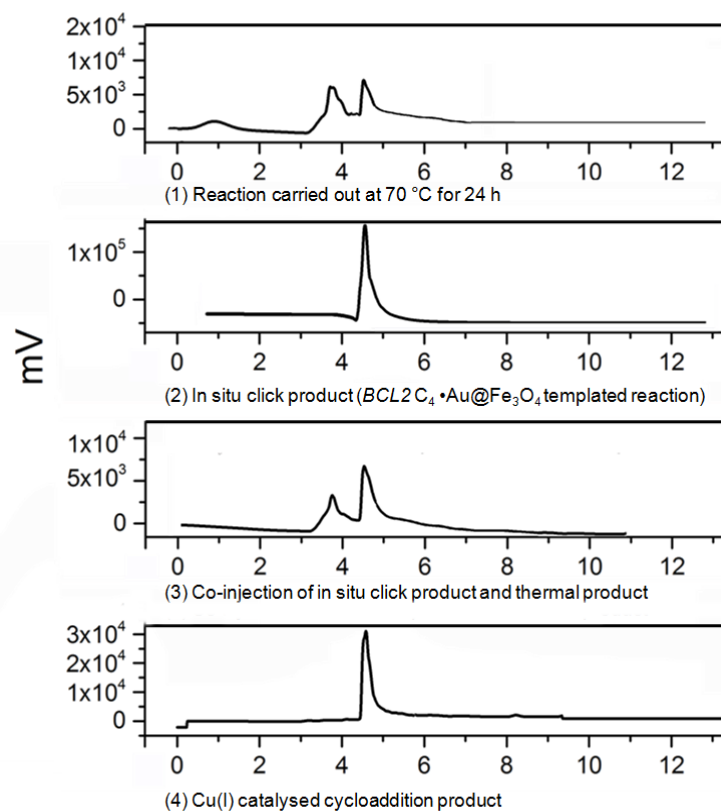


Figure S8. HPLC traces of the products obtained in the 1, 3-dipolar cycloaddition between alkyne **1b** and azide **2m** obtained under different conditions.

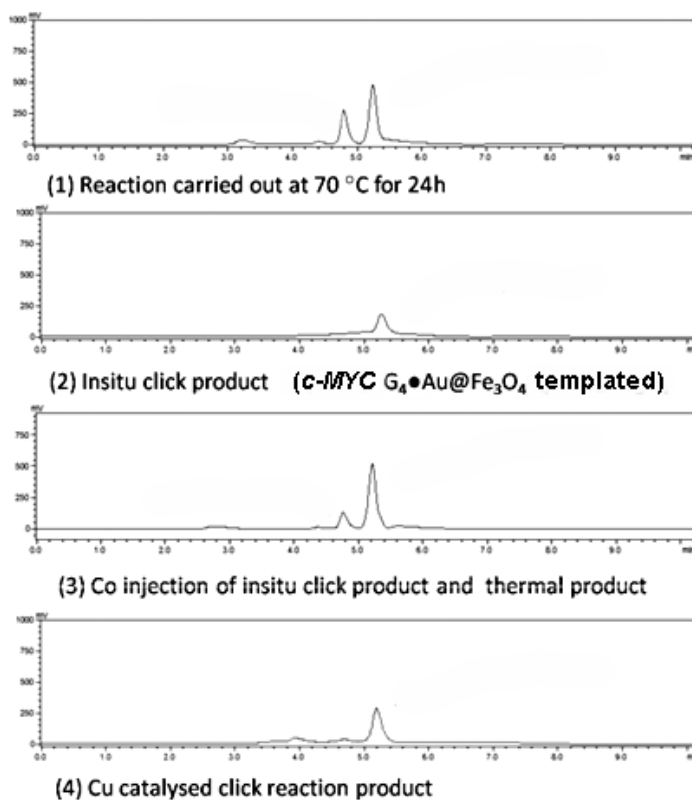


Figure S9. HPLC traces of the products obtained in the 1, 3-dipolar cycloaddition between alkyne **1a** and azide **2o** obtained under different conditions.

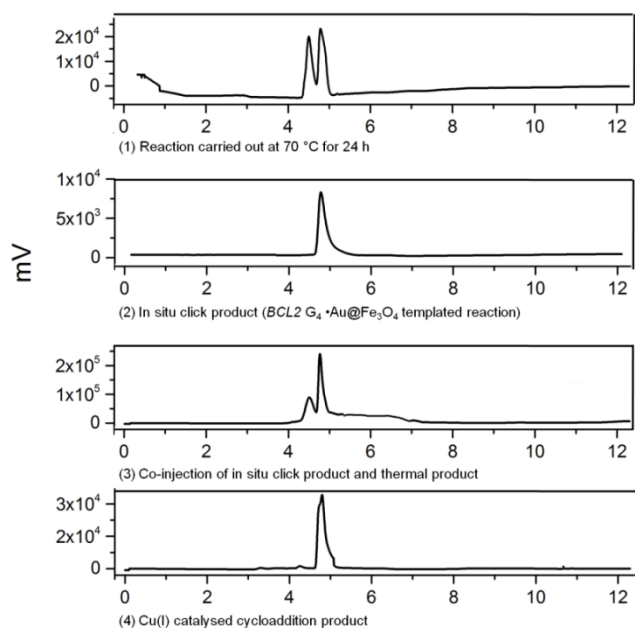


Figure S10. HPLC traces of the products obtained in the 1, 3-dipolar cycloaddition between alkyne **1a** and azide **2p** obtained under different conditions.

11.0 Relative yield of triazole leads.

The yields of lead *anti*-triazoles were determined by performing time dependent cycloaddition of corresponding alkyne (**1a** and **1b**) and azide (**2e**, **2m**, **2o** and **2p**) fragments in the presence of DNA nanotemplates. Alkyne **1b** reacted with azide **2e** in the presence of *c-MYC* i-motif nanotemplate to form *anti-3be* in 48% yield. Similarly, lead *anti-3bm* (from **1b** and **2m** by *BCL2* i-motif), *anti-3ao* (from **1a** and **2o** by *c-MYC* G-quadruplex) and *anti-3ap* (from **1a** and **2p** by *BCL2* quadruplex) were obtained in 60%, 44% and 56% relative yields, respectively (Figure 8 and Figure S6-S9).

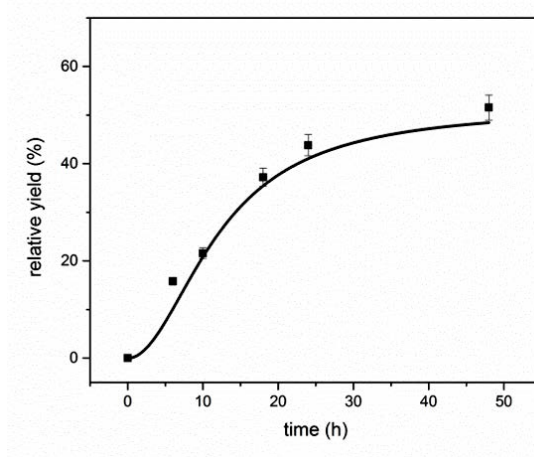


Figure S11. Relative yield of **3be** from *c-MYC* C₄•Au@Fe₃O₄ templated reaction with alkyne **1b** and azide **2e**.

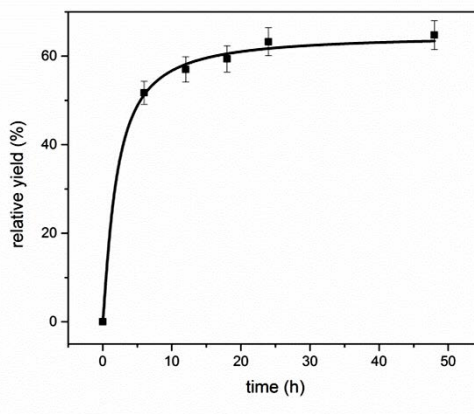


Figure S12. Relative yield of **3bm** from *BCL2* C₄•Au@Fe₃O₄ templated reaction with alkyne **1b** and azide **2m**.

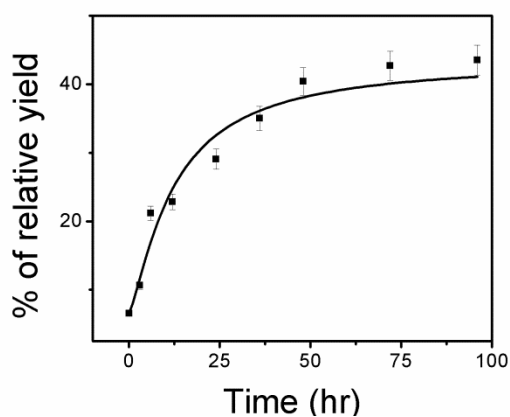


Figure S13. Relative yield of **3ao** from *c-MYC* G₄•Au@Fe₃O₄ templated reaction with alkyne **1a** and azide **2o**.

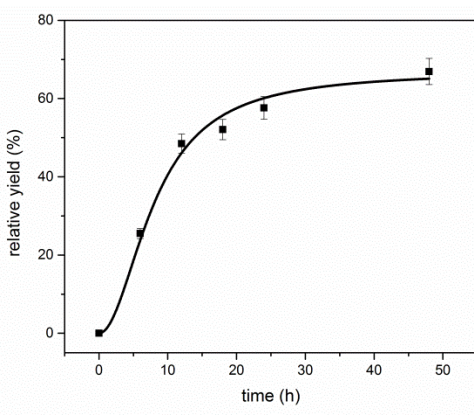


Figure S14. Relative yield of **3ap** from *BCL2* G₄•Au@Fe₃O₄ templated reaction with alkyne **1a** and azide **2p**.

12.0 FRET melting analysis. FRET melting experiments were performed using 0.2 μM concentration of 5'-FAM and 3'-TAMRA labelled DNA sequences. FRET melting experiments with G-quadruplex DNA and Duplex DNA were carried out in 60 mM potassium cacodylate buffer (pH 7.4) and the FRET melting assay with i-motif DNA sequences were carried out in 10 mM sodium cacodylate buffer (pH 5.5). The DNA sequences were annealed at a concentration of 400 nM by heating at 95 $^{\circ}\text{C}$ for 5 min followed by gradual cooling to room temperature at a controlled rate of 0.1 $^{\circ}\text{C min}^{-1}$ and then kept at 4 $^{\circ}\text{C}$ for overnight. The assay was carried out on a real-time PCR apparatus (Light Cycler 480 II System) by incubating the dual labelled DNA oligonucleotides (200 nM) with 1 μM of each triazole derivatives (**3be**, **3bm**, **3ao** and **3ap**) separately for 1 h. Fluorescence measurements were taken with an excitation wavelength of 483 nm and a detection wavelength of 533 nm at intervals of 1 $^{\circ}\text{C}$ over the range of 37–95 $^{\circ}\text{C}$. Melting temperatures were calculated using Origin Pro 8 data software. Dual fluorescently labelled DNA oligonucleotides used in these experiments are:

c-MYC C₄ ($T_m = 47.2$ $^{\circ}\text{C}$): 5'-FAM-TCCCCACCTTCCCCACCCTCCCCACCCTCCCCA-TAMRA-3'

BCL2 C₄ ($T_m = 44.0$ $^{\circ}\text{C}$): 5'-FAM-CAGCCCCGCTCCCGCCCCCTTCCTCCCGCGCCCGCCCCT-TAMRA-3'

c-MYC G₄ ($T_m = 68.3$ $^{\circ}\text{C}$): 5'-FAM-TGGGGAGGGTGGGGAGGGTGGGGAAGG-TAMRA-3'

BCL2 G₄ ($T_m = 72.1$ $^{\circ}\text{C}$): 5'-FAM-AGGGGCGGGCGGGAGGAAGGGGGCGGGAGCGGGGCTG-TAMRA-3'

dsDNA ($T_m = 63.4$ $^{\circ}\text{C}$): 5'-FAM-CAAAAATTTTTGCAAAAATTTTTG-TAMRA-3'

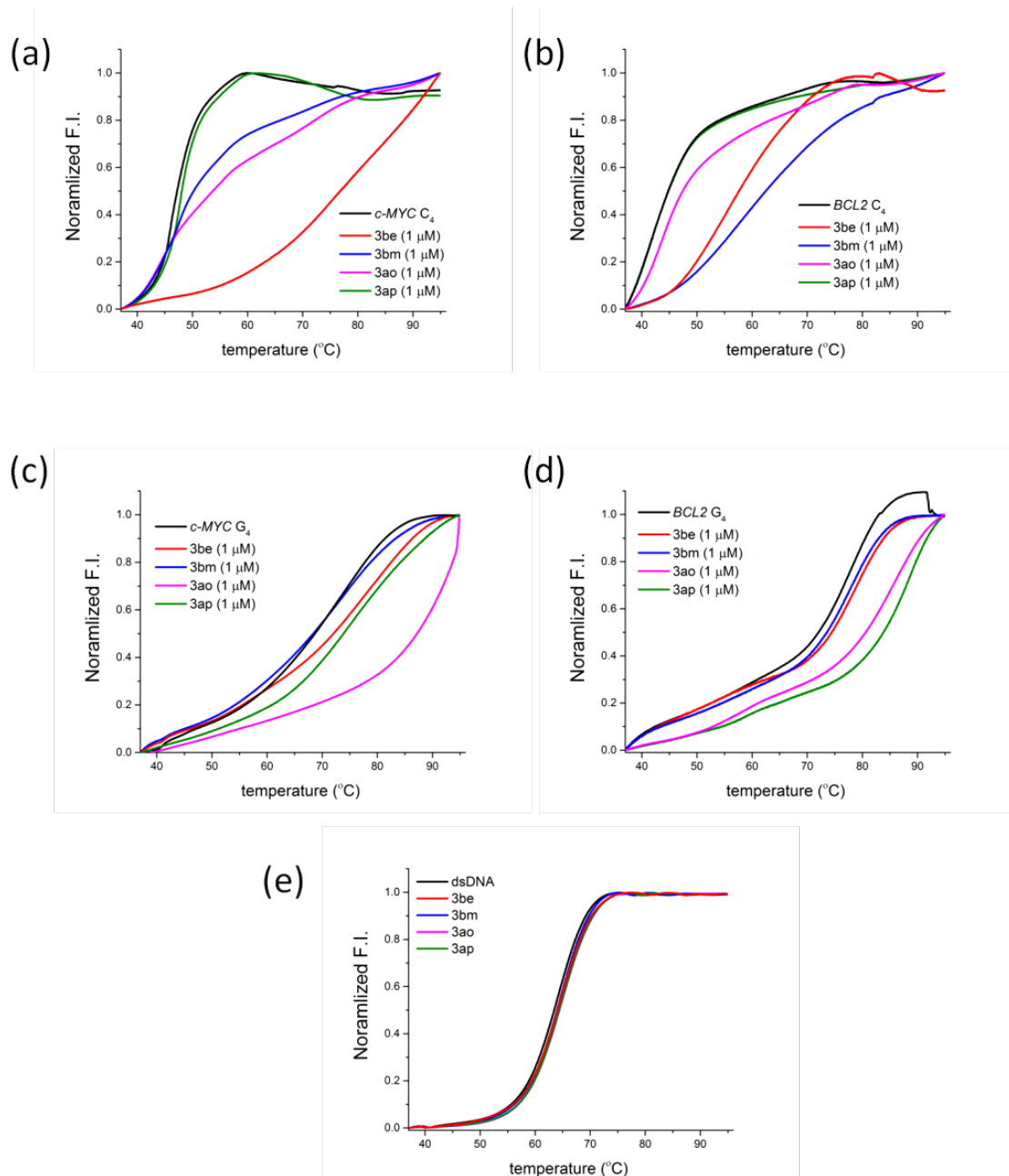


Figure S15. FRET melting curves of 200 nM 5'-FAM and 3'-TAMRA labelled (a) *c-MYC* i-motif (C₄), (b) *BCL2* i-motif (C₄), (c) *c-MYC* G-quadruplex (G₄), (d) *BCL2* G-quadruplex (G₄) and (e) dsDNA with 1 μ M **3be**, **3bm**, **3ao** and **3ap**. FRET melting experiments with G-quadruplex DNA and dsDNA were carried out in 60 mM potassium cacodylate buffer (pH 7.4) and the FRET melting assay with i-motif DNA sequences were carried out in 10 mM sodium cacodylate buffer (pH 5.5).

13.0 Fluorimetric spectroscopic titration. The fluorescence spectra were recorded on a Horiba Jobin Yvon Fluoromax 3 instrument at 25 °C in a 10 mm path-length quartz cuvette with filtered buffers. For G-quadruplex and Duplex DNA 100 mM Tris.KCl buffer (pH 7.4) was used and for i-motif DNA 10 mM sodium cacodylated (pH 5.5) buffer was utilized. Fluorescence titrations were performed with successive addition of the DNA solution into the 1 μ M ligand solution.

In this experiment, following DNA sequences were utilised:

c-MYC C₄: 5'-TCCCCACCTTCCCCACCCTCCCCACCCTCCCCA-3'

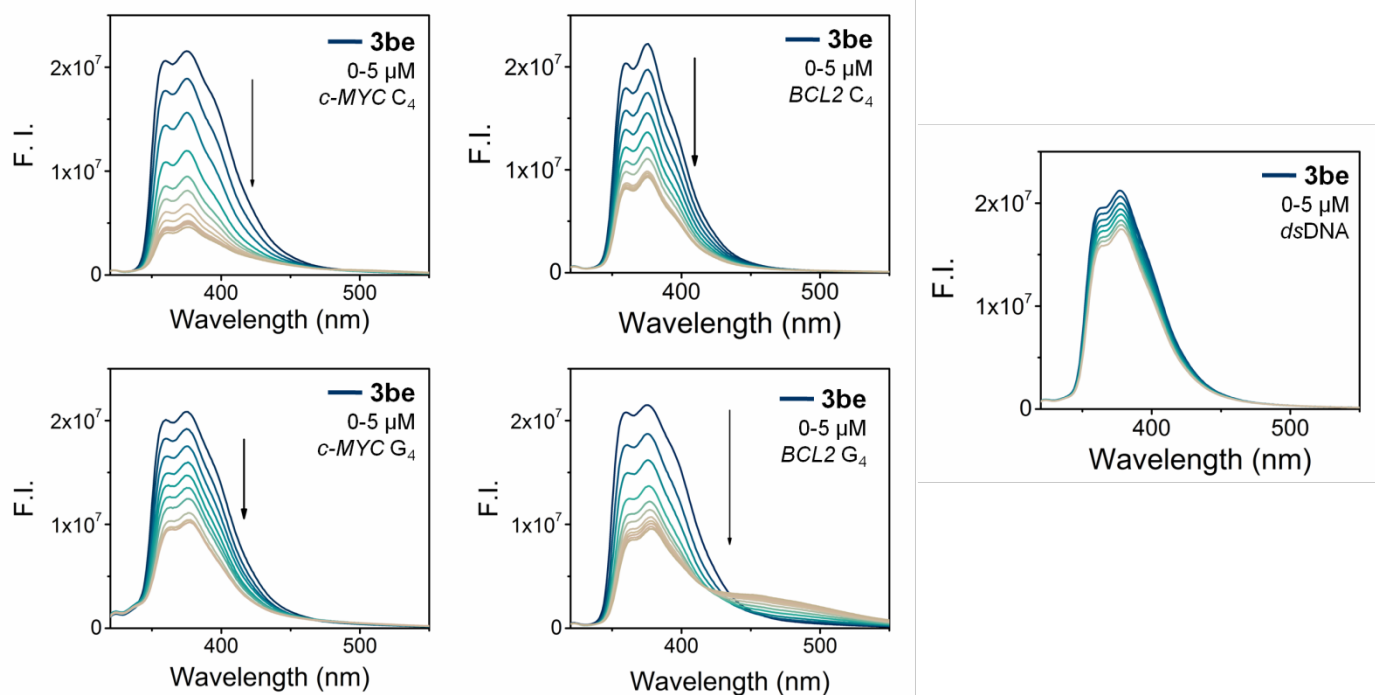
BCL2 C₄: 5'-CAGCCCCGCTCCCGCCCCCTTCCTCCCGCGCCCGCCCCT-3'

c-MYC G₄: 5'-TGGGGAGGGTGGGGAGGGTGGGGGAAGG-3'

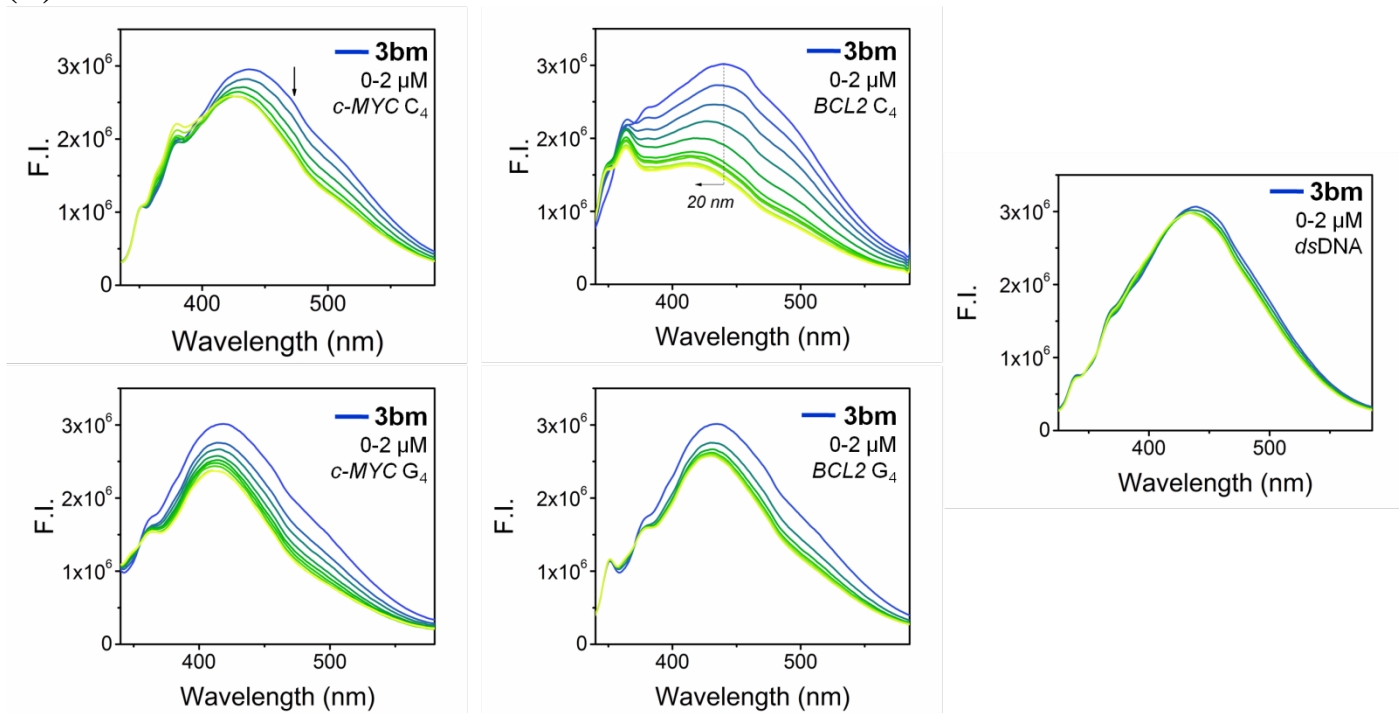
BCL2 G₄: 5'-AGGGGCGGGCGCGGGAGGAAGGGGGCGGGAGCGGGGCTG-3'

dsDNA: 5'-CAAAAATTTTTGCAAAAATTTTTG-3'

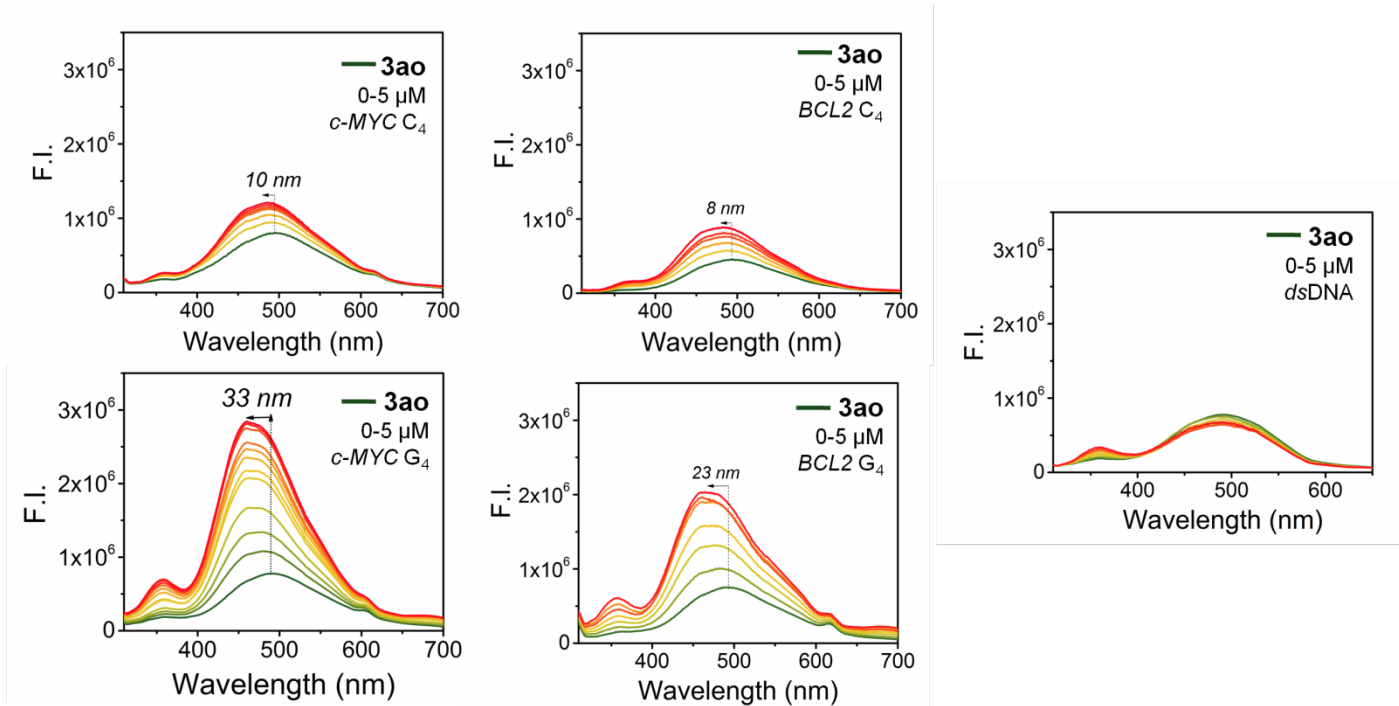
(a)



(b)



(c)



(d)

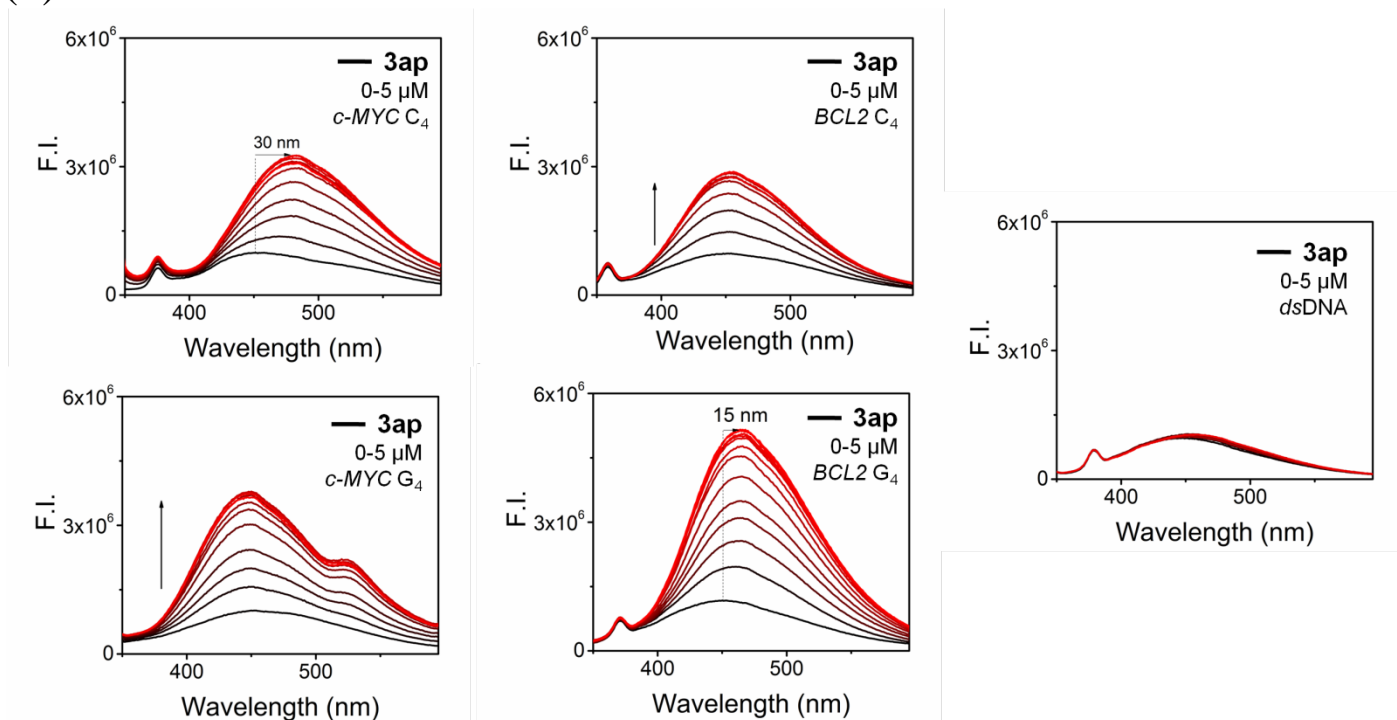


Figure S16. Fluorescence titrations of 1 μM triazole lead compounds [(a) **3be**, (b) **3bm**, (c) **3ao** and (d) **3ap**] with incremental addition of *c-MYC* C₄, *BCL2* C₄ in 10 mM Na-cacodylate buffer, pH 5.5 and with *c-MYC* G₄, *BCL2* G₄ and dsDNA in 100 mM Tris.KCL buffer, pH 7.4.

14.0 Competitive binding assay.

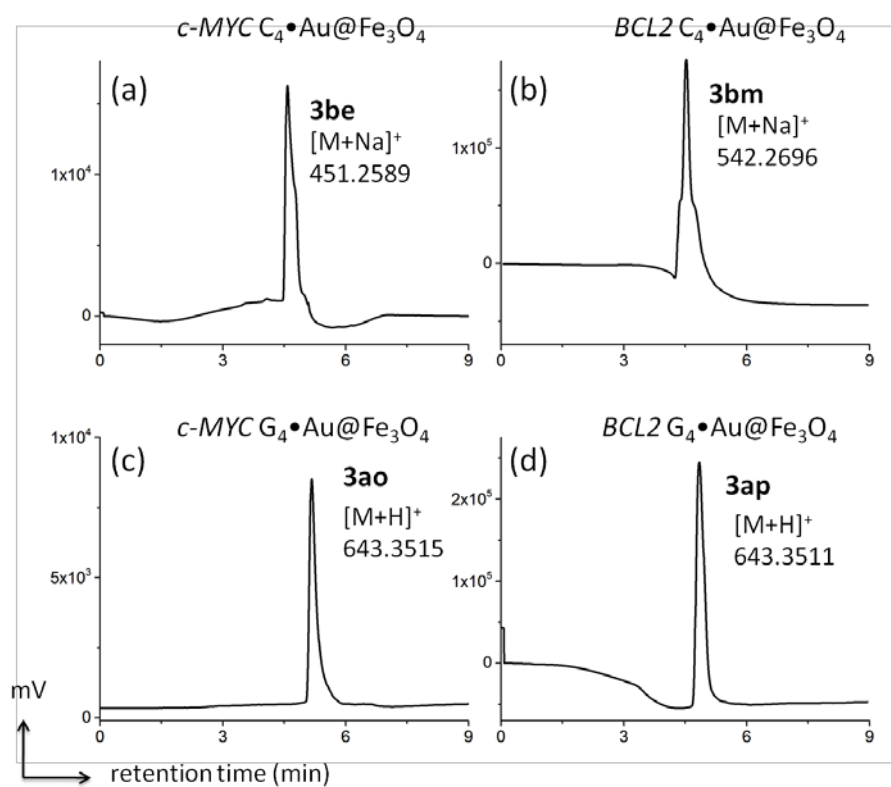


Figure S17. Triazoles, obtained in competition experiments performed with *c*-MYC and *BCL2* i-motif and G-quadruplex DNA nanotemplates were analysed using HPLC and mass.

15.0 1D ^1H NMR titration. The DNA oligos were purchased from Eurofins MWG Operon in HPSF purity grade and further purified with HPLC. During the titration the G₄ DNA oligos were provided as a 100 μM solution in 25 mM Tris·HCl buffer (pH 7.4) with 100 mM KCl in 5% d₆-DMSO/95% H₂O. The i-motif oligos were provided as a 100 μM solution in 10 mM sodium cacodylate buffer (pH 5.5) in 5% d₆-DMSO/95% H₂O. Small amounts of the ligand stock solution in 100% d₆-DMSO were added directly into the NMR tube (8.2 % d₆-DMSO at the end of the titration). 2,2-dimethyl-2-silapentane-5-sulphonate (DSS) was used as internal reference. Gradient-assisted excitation sculpting pulse sequences² or jump-return-Echo³ was used for water suppression.

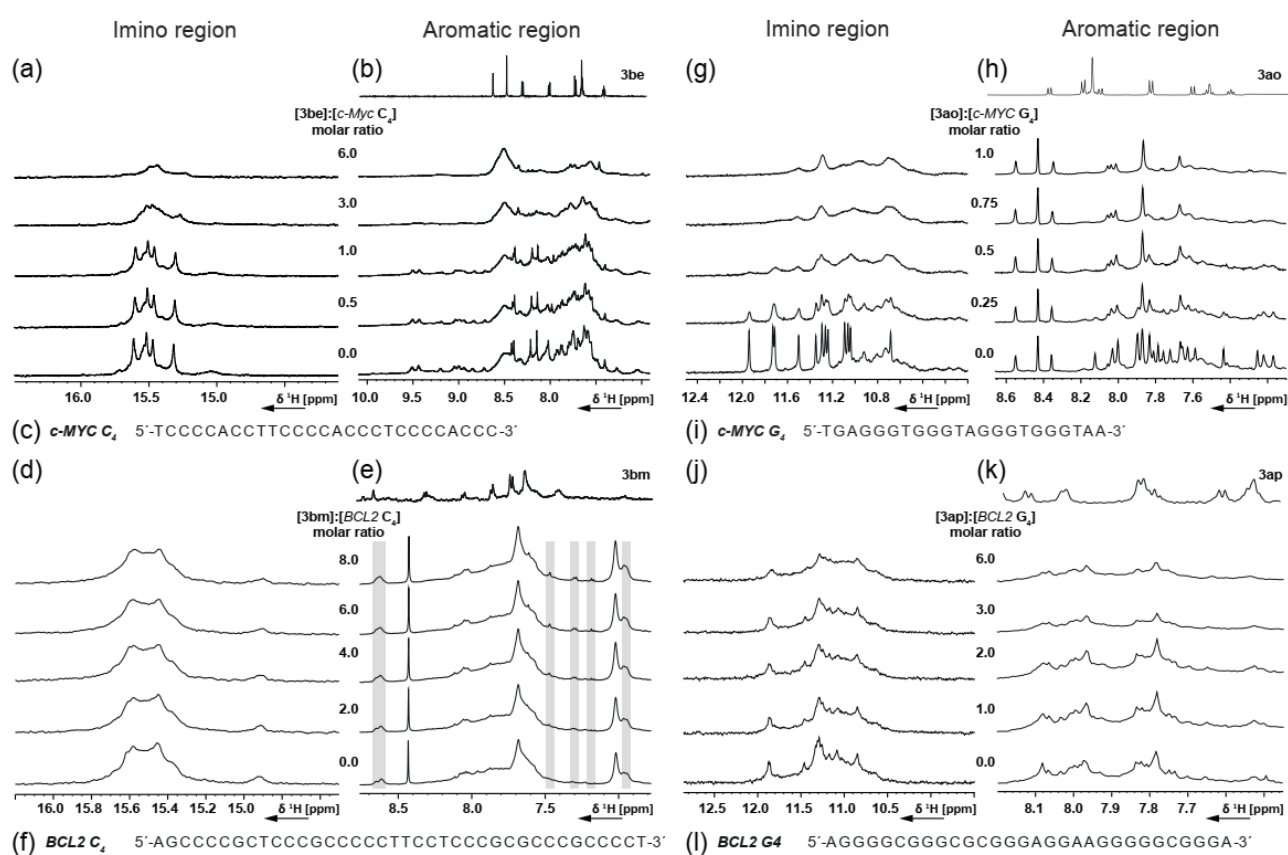


Figure S18. (a) Imino and (b) aromatic region of 1D ^1H NMR spectrum of the *c-MYC* i-motif with increasing [3be]:[*c-MYC* i-motif] ratio at pH 5.5 and the aromatic region of 3be alone. (c) Sequence of *c-MYC* i-motif used for titration. (d) The imino and (e) aromatic proton regions of the 1D ^1H NMR spectra of *BCL2* i-motif DNA with different equivalents (0-8) of 3bm and 3bm in absence of DNA at pH 5.5. (f) *BCL2* i-motif sequence used in the NMR titration. (g) The imino and (h) aromatic proton regions of the 1D ^1H NMR spectra of *c-MYC* quadruplex DNA with different equivalents (0-1) of 3ao at pH 7.4 and 3ao in buffer alone. (i) The sequence of *c-MYC* quadruplex DNA used in NMR titration. Panel (j) and (k) show the 1D ^1H

² T. L. Hwang, A. J. Shaka, *J. Magn. Res.* **1995**, *112*, 275.

³ V. Sklenár, A. Bax, *J. Magn. Res.* **1987**, *74*, 469.

NMR spectrum of the imino and aromatic region of *BCL2* G-quadruplex DNA with increasing [**3ap**]:[DNA] ratio and the aromatic region of **3ap** in absence of DNA. (l) Sequence of *BCL2* quadruplex DNA used in NMR titration. Experimental conditions: Titrations with i-motif DNA were performed in 10 mM sodium cacodylate (pH 5.5) buffer in 5% d^6 -DMSO/95% H₂O while G-quadruplex DNA titrations were performed in 25 mM Tris·HCl (pH 7.4) buffer containing 100 mM KCl in 5% d^6 -DMSO/95% H₂O. The spectra were recorded at 298 K, 600 MHz with 100 μ M DNA concentration. The 1D ¹H NMR ligand spectra were recorded at 500 μ M ligand concentration in the respective titration buffer at 298 K, 600 MHz.

16.0 Circular dichroism spectroscopy. CD spectra were recorded on a JASCO J-815 spectrophotometer using a 1 mm path-length quartz cuvette. Aliquots of ligand were added in increments to pre-annealed G-quadruplex (100 mM Tris.KCl buffer, pH 7.4) and i-motif (10 mM sodium cacodylate buffer, pH 5.5) DNA. The DNA concentration used was 10 μ M. The CD spectra represented an average of three scans and were smoothed and zero corrected. Final analysis of the data was carried out using Origin 8.0.

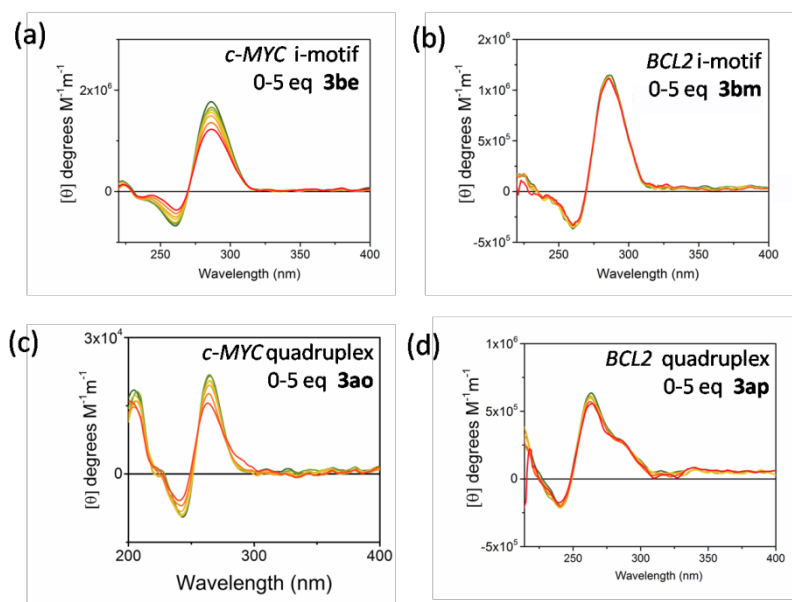


Figure S19. CD spectra of (a) *c-MYC* C₄ DNA (10 μ M) in 10 mM Na-cacodylate buffer (pH 5.5) titrated with 0-5 eq of **3be**, b) *BCL2* C₄ DNA (10 μ M) 10 mM Na-cacodylate buffer (pH 5.5) titrated with 0-5 eq of **3bm**, c) *c-MYC* G₄ DNA (10 μ M) in 100 mM Tris.KCl buffer (pH 7.4) titrated with 0-5 eq of **3ao**, (d) *BCL2* G₄ DNA (10 μ M) in 100 mM Tris.KCl buffer (pH 7.4) titrated with 0-5 eq of **3ap**.

17.0 XTT cell viability assay. Cells were grown at a density of 10^4 - 10^5 cells/well in 100 μ L of culture medium and treated with increasing concentrations of the ligands and incubated for 24 h. Control cells were treated with 0.05% DMSO. Viability experiments were performed in triplicate on 96-well plates with designated doses. The XTT/PMS reagent was prepared by mixing 4 mg of XTT in 4 mL of culture medium followed by the addition of 10 μ L of 10 mM PMS solution (in PBS). 25 μ L of this freshly prepared reagent mixture was then directly added to each well after 24 h ligand treatment in 100 μ L of culture media and incubated for 2 h at 37 $^{\circ}$ C. The absorbance of XTT formazan was read at 450 nm on Multiskan FC microplate spectrophotometer (Thermo Scientific). Percentage cell viability was calculated by using the following equation (2):

$$\% \text{ of cell viability} = \frac{\text{O.D. of treated cells}}{\text{O.D. of control cells}} \times 100 \text{ (Equation 2)}$$

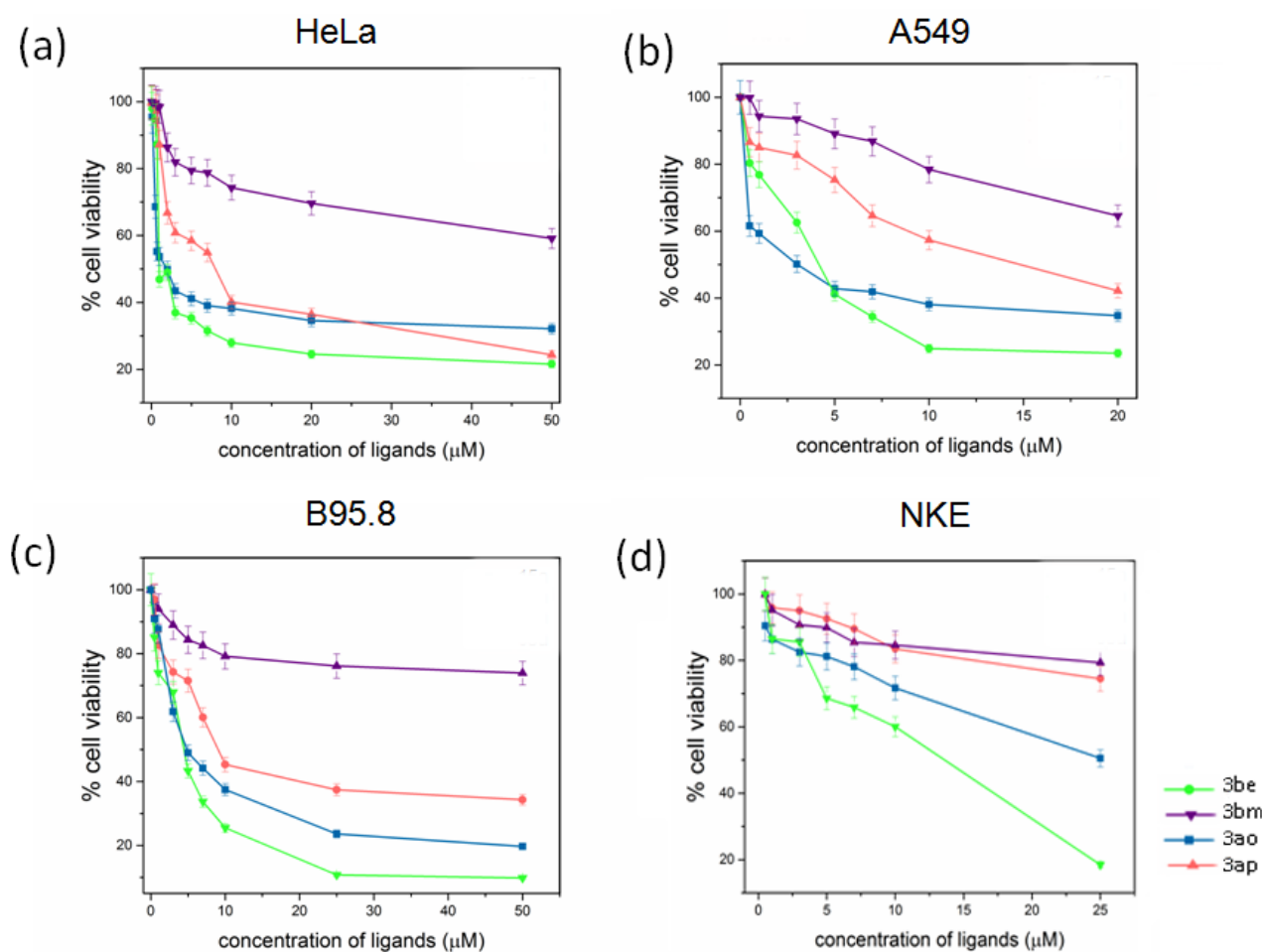


Figure S20. XTT cell viability assay showing IC₅₀ values of **3ao**, **3be**, **3bm** and **3ap** over 24 h in different cancer cell lines (HeLa, A549 and B95.8) and normal cell line NKE.

18.0 Confocal Microscopy of HeLa cells treated with lead ligands.

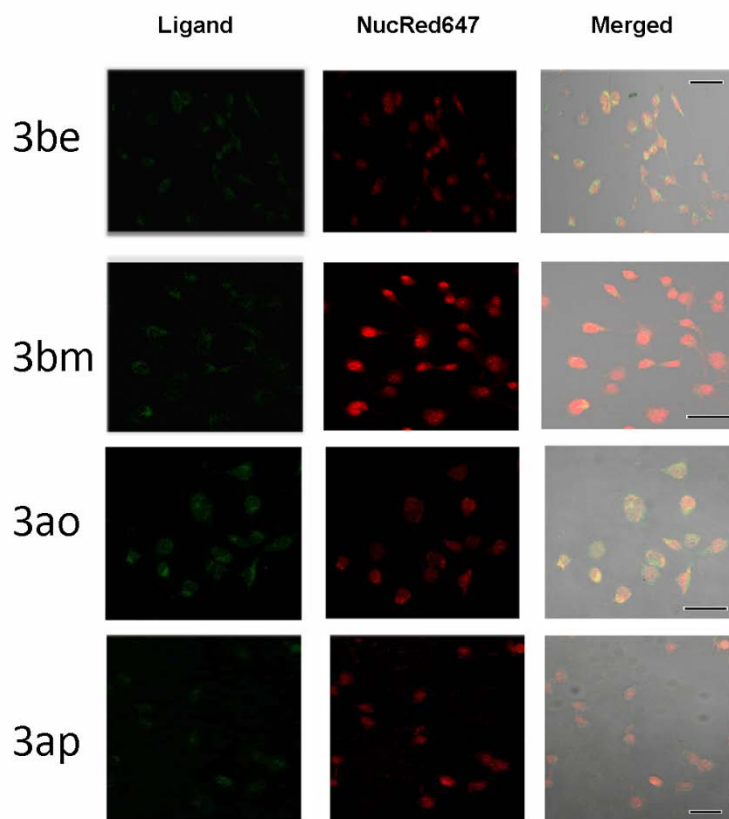


Figure S21. Confocal imaging of HeLa cells treated with triazole leads at $\frac{1}{2}$ IC₅₀ dose for 24 h. The nucleus was counterstained with NucRed Live647 ReadyProbe Reagent, incubation time 15 min. Scale bar 20 μ m.

19.0 FACS analysis for apoptosis detection. HeLa cells were seeded at a density of 1×10^6 cells/mL in each well of a six-well plate and allowed to grow overnight. Cells were treated with the ligands for 24 h at 37 °C. After treatment, cells were trypsinized, repeatedly washed with cold PBS and centrifuged at 1800 rpm for 5 min to collect the cell pellet and the supernatants were discarded. Cells were then resuspended in 350 μ L of $1 \times$ Annexin-V binding buffer (0.01 M HEPES, pH 7.4, 0.14 M NaCl, 2.5 mM CaCl_2) and treated with 5 μ L Annexin V-FITC and 2 μ L propidium iodide (1 mg/mL). After incubation for 5 min on ice, each sample was analyzed immediately using fluorescence-activated cell sorting (FACS) analysis (BD Biosciences, Mountain View, CA, USA). Approximately 10,000 cells were detected for each sample.

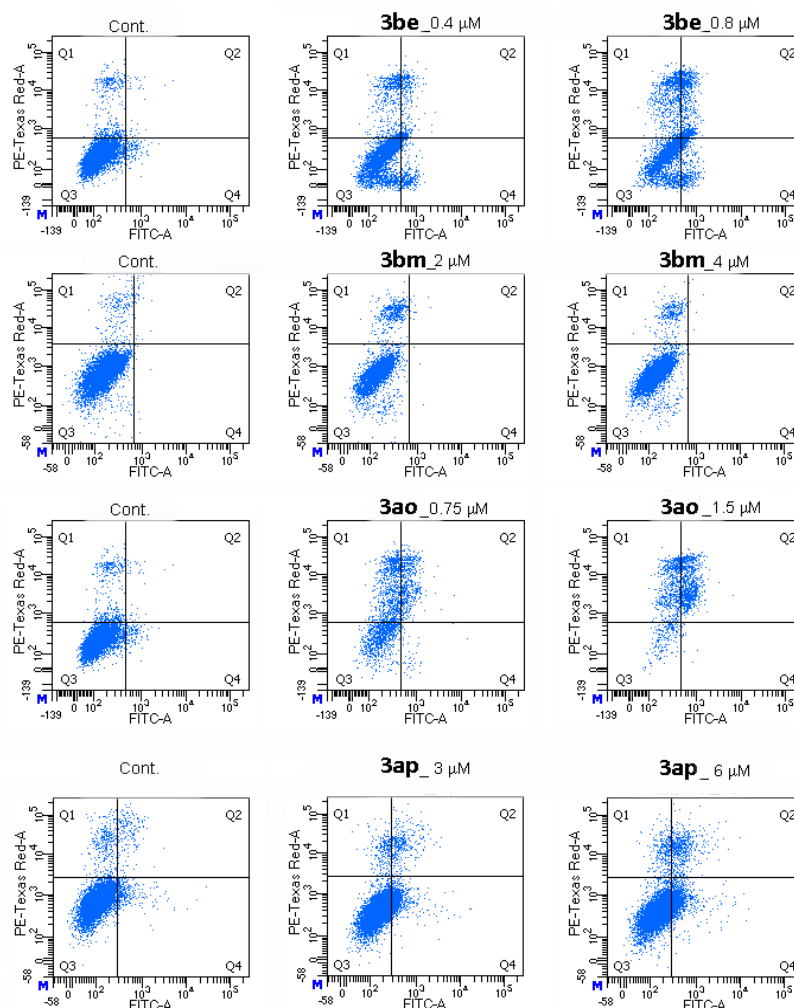


Figure S22. Flow cytometric analysis of FITC-Annexin V/PI stained control and treated cells after 24 h treatment with **3ao**, **3be**, **3bm** and **3ap**.

20.0 Real-time PCR. Total RNA was isolated after 24 h ligand treatment using TRIzol reagent (Invitrogen, Life Technologies) according to the manufacturer's instructions. RNA was quantified, and 1 µg of RNA was used for cDNA preparation using Verso cDNA synthesis kit. The relative transcript expression level for genes was measured by quantitative real-time PCR using SYBR Green-based method. ΔC_t values were calculated by the difference in threshold cycles (C_t) between test and control samples. *18s rRNA* gene was used as an internal control for normalizing the cDNA concentration of each sample. Primers used for monitoring the gene expressions are as follows;

c-MYC forward: 5'- CTGCGACGAGGAGGAGGACT-3'

c-MYC reverse: 5'- GGCAGCAGCTCGAATTTCTT-3'

BCL2 forward: 5'- ACAACATCGCCCTGTGGATGAC-3'

BCL2 reverse: 5'- TTGTTTGGGGCAGGCATGTT-3'

18s rRNA forward: 5'-GATTCCGTGGGTGGTGGTGC-3'

18s rRNA reverse: 5'-AAGAAGTTGGGGGACGCCGA-3'

For amplification of *c-MYC* and *BCL2* gene, the samples were subjected to pre-incubation at 95 °C for 10 min and then 40 cycles of 95°C (15 sec) and 60°C (60 sec).

21.0 Western Blot. Cells were collected after 24 h post-treatment with ligands, washed with ice cold PBS twice, and lysed with cold cell lysis buffer (20 mM Tris, 100 mM NaCl, 1 mM EDTA in 0.5% Triton X-100) for 30 min on ice, and debris was removed by centrifugation at 12000g for 15 min. Aliquots of the supernatants were used for protein determination by Folin-Lowry method. Equal amounts of protein (70 µg) were subjected to 12 % SDS/PAGE, and after gel electrophoresis, the proteins were electrophoretically transferred onto nitrocellulose membranes. Then, membranes were blocked with 4 % BSA for 2 h and then incubated overnight at 4 °C with different primary antibodies. The membranes were washed three times with 1x TBST and then incubated for 2 h with HRP linked respective secondary antibodies. Blots were washed thrice with 1x TBST and then once with 1x TBS and bands were acquired by the addition of TMB or NBD/BCIP substrate. Relative band intensities were determined by using ImageJ software.

Primary antibodies used:

Anti c-MYC antibody - Rabbit origin (Invitrogen)

Anti BCL2 antibody - Rabbit origin (SIGMA Aldrich)

Anti GAPDH antibody - Mouse origin (Invitrogen)

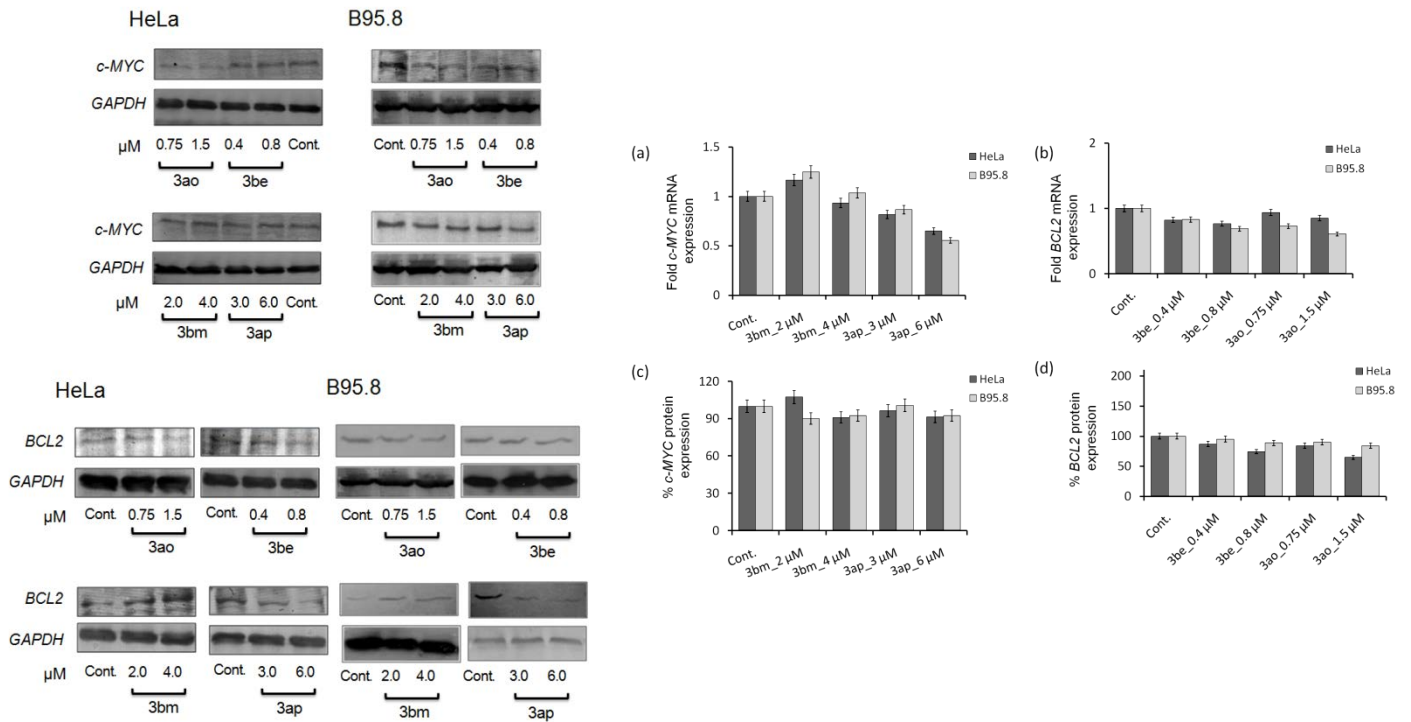


Figure S23. Western Blot analysis of *c-MYC* and *BCL2* expression in HeLa and B95.8 cell lines after treatment with leads 3be, 3bm, 3ao and 3ap at the indicated concentrations for 24 h.

22.0 Dual Luciferase Reporter Assay. HeLa cells were grown for overnight at 37 °C, 5% CO₂. 1 µg of c-MYC Del4 plasmid construct (Addgene plasmid #16604-Del4)⁴, BCL2 promoter construct (Addgene plasmid #15381-LB322)⁵ and non G.C rich promoter construct were transfected separately into HeLa cells using 2 µl Lipofectamine 2000 (Invitrogen). A Renilla luciferase plasmid, pRL-TK (0.5 µg) was co-transfected with each construct for normalization. Transfection was performed in 1% FBS supplemented DMEM medium, which was replaced by complete media after a 8 h incubation at 37 °C, 5% CO₂. The cells were then treated with ligands and after 36 h incubation, RNAs were isolated from cells and reverse transcribed into cDNA. Firefly and renilla luciferase expressions were measured from the cDNA by qRT-PCR using SYBR Green-based method. The primers used are as follows;

Renilla F 5'-GGAATTATAATGCTTATCTACGTGC-3'
 Renilla R 5'-CTTGCGAAAAATGAAGACCTTTTAC-3'
 Firefly F 5'-CTCACTGAGACTACATCAGC-3'
 Firefly R 5'-TCCAGATCCACAACCTTCGC-3'

qRT-PCR program: i) 95 °C for 10 min

ii) (95 °C for 15 sec, 60 °C for 15 sec and 72 °C for 60 sec) X 40 cycles

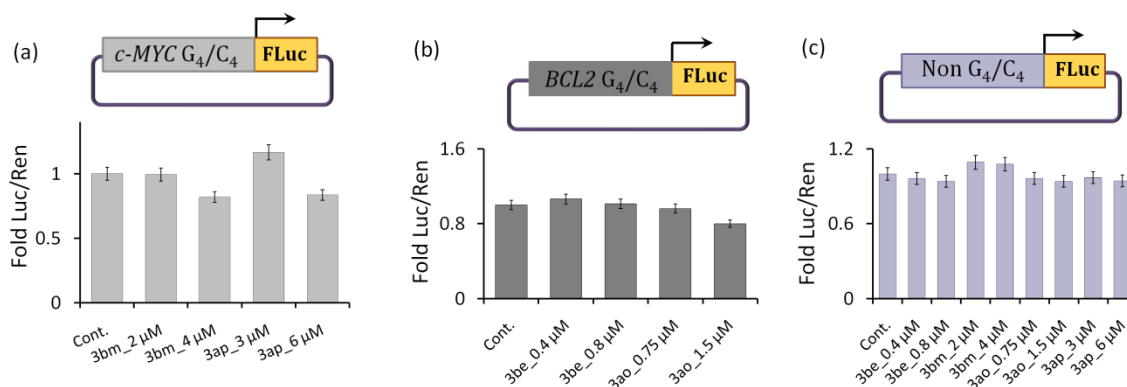


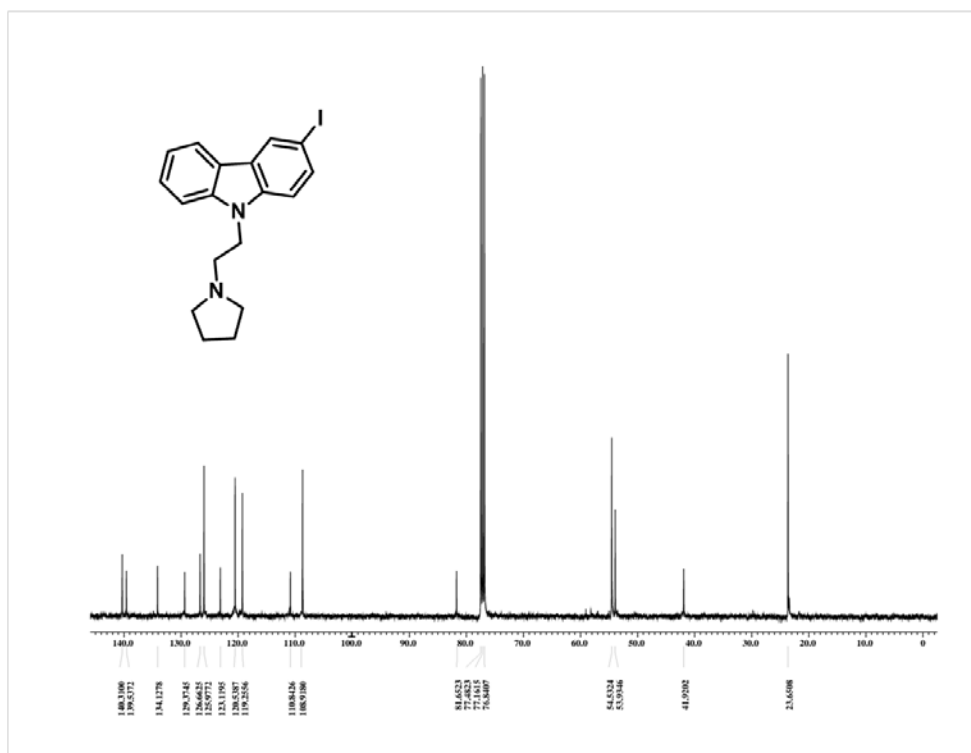
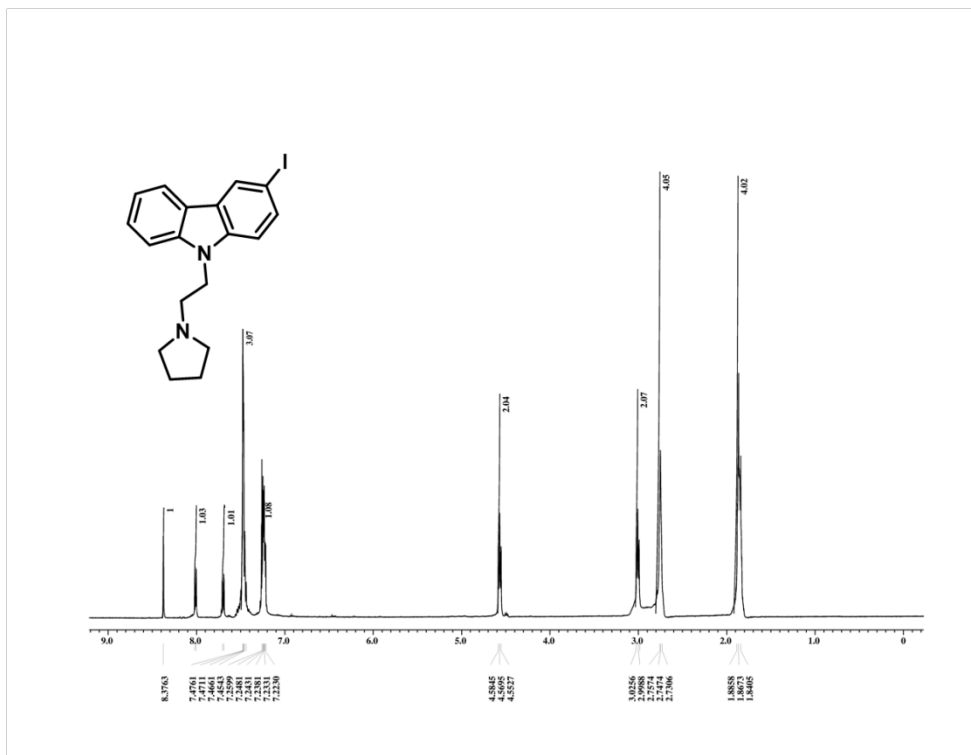
Figure S24. *In vitro* dual luciferase reporter assay for evaluating the effect of **3be**, **3bm**, **3ao** and **3ap** on promoter activity of *c-MYC*, *BCL2* and non G.C rich control promoter constructs after 36 h post-transfection treatment. Experiments were performed in triplicates. *, p< 0.05.

⁴T. C. He, A. B. Sparks, C. Rago, H. Hermeking, L. Zawel, L. T. da Costa, P. J. Morin, B. Vogelstein, K. W. Kinzler, *Science* **1998**, *281*, 1509-1512.

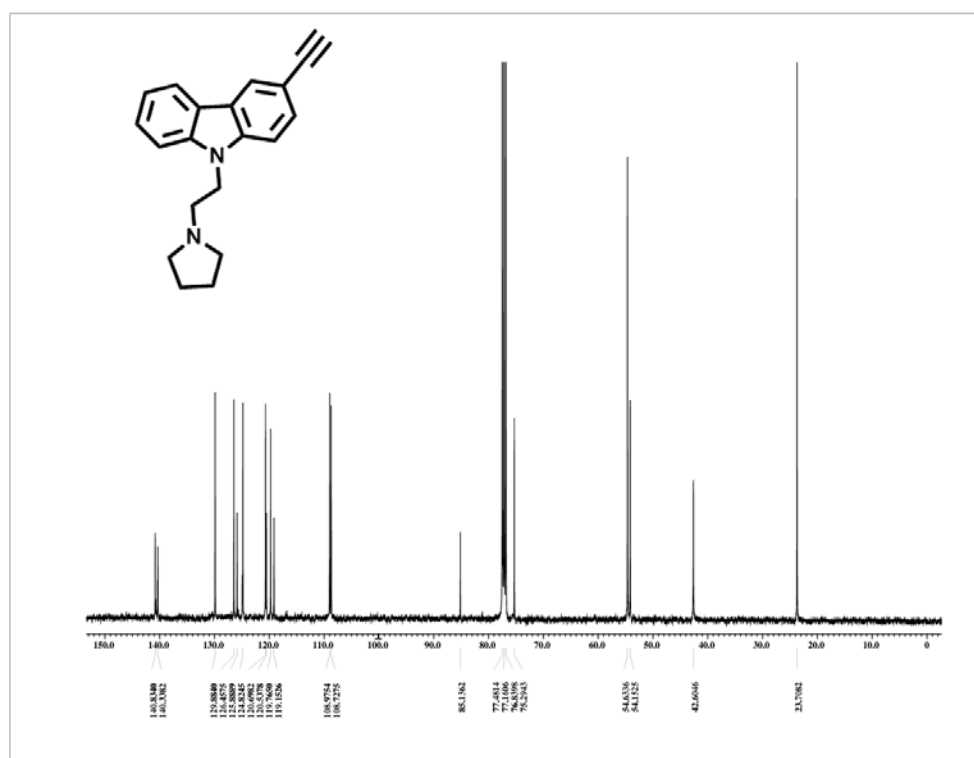
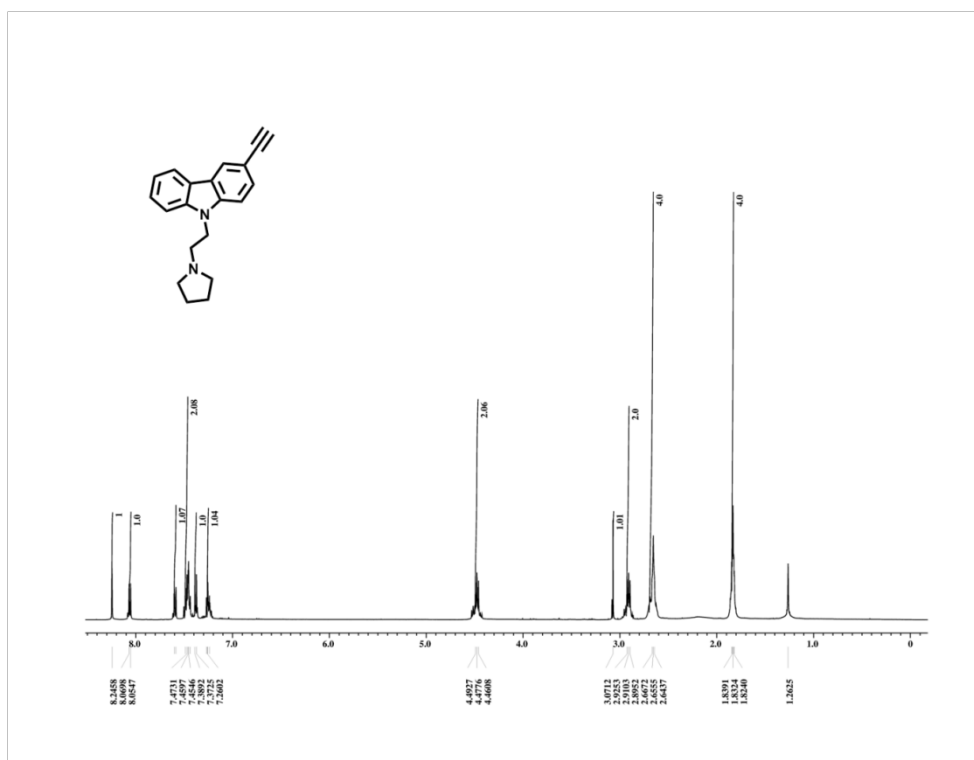
⁵C. A. Heckman, J. W. Mehew, G. G. Ying, M. Introna, J. Golay, L. M. Boxer, *J. Biol. Chem.* **2000**, *275*, 6499-6508.

23.0 NMR spectra of compounds.

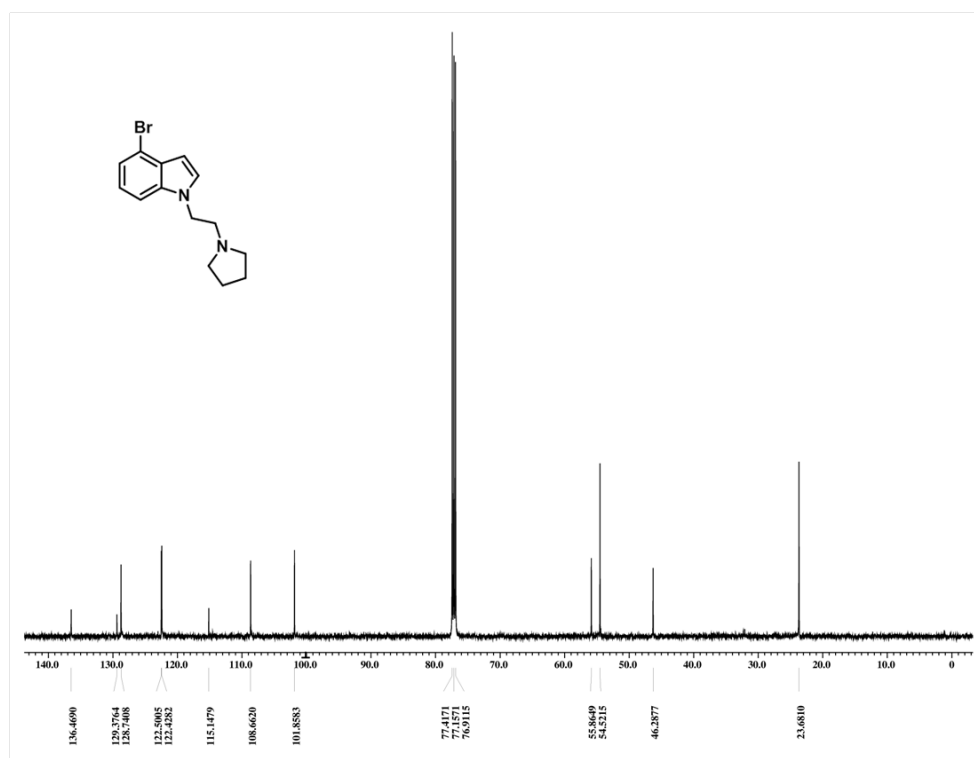
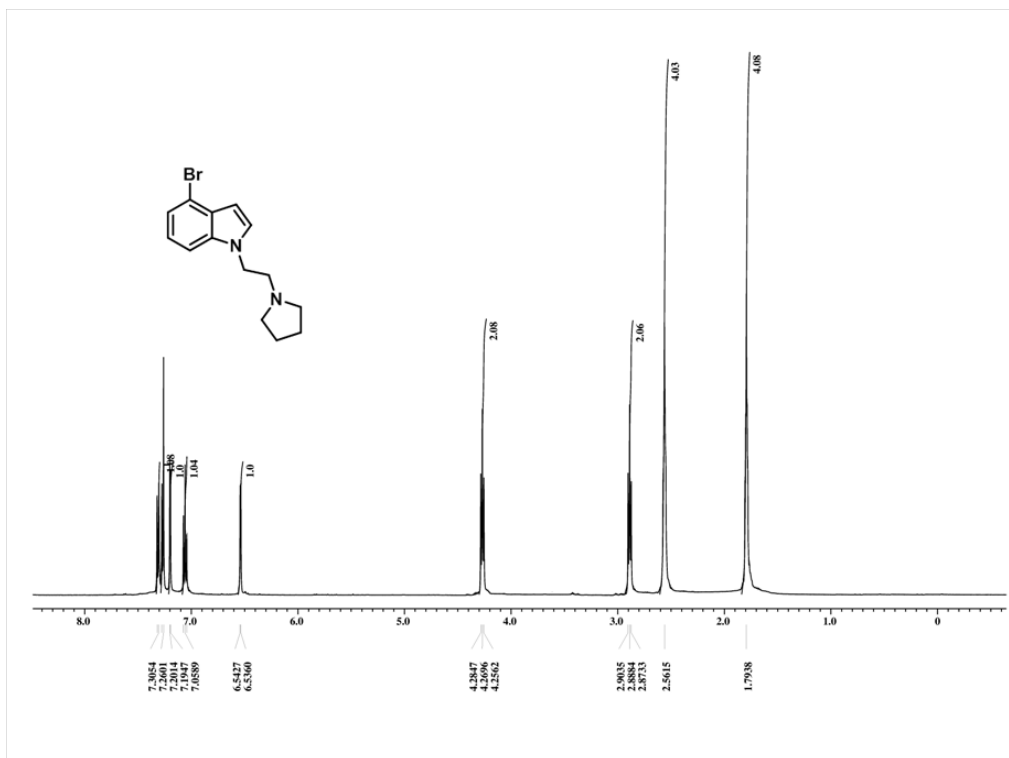
^1H and ^{13}C NMR of S3:



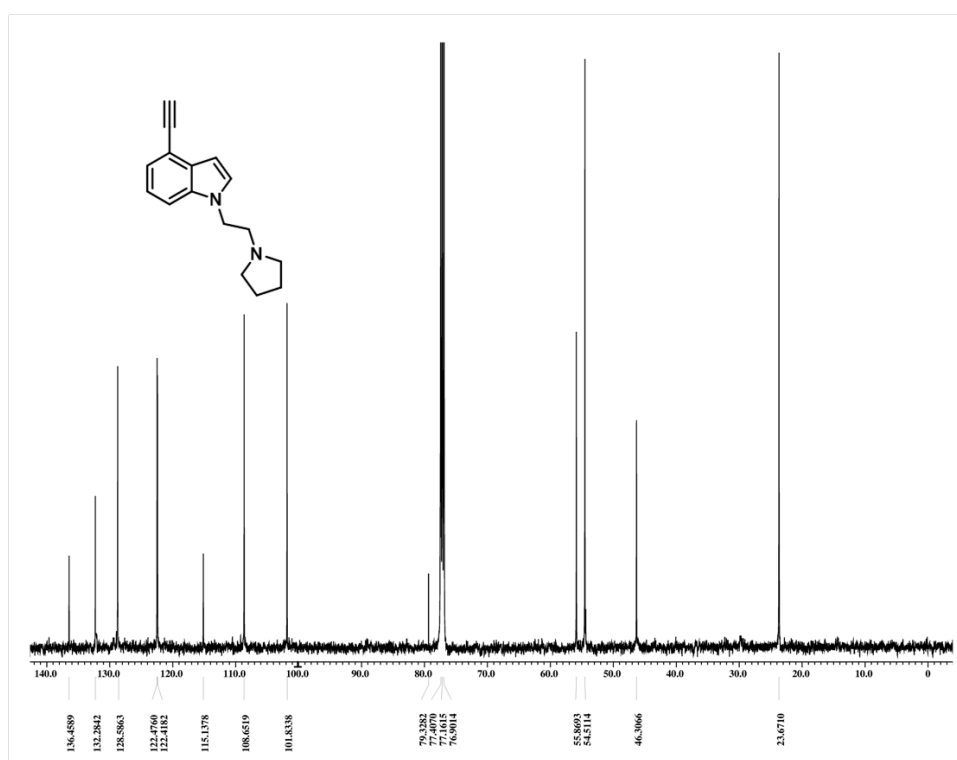
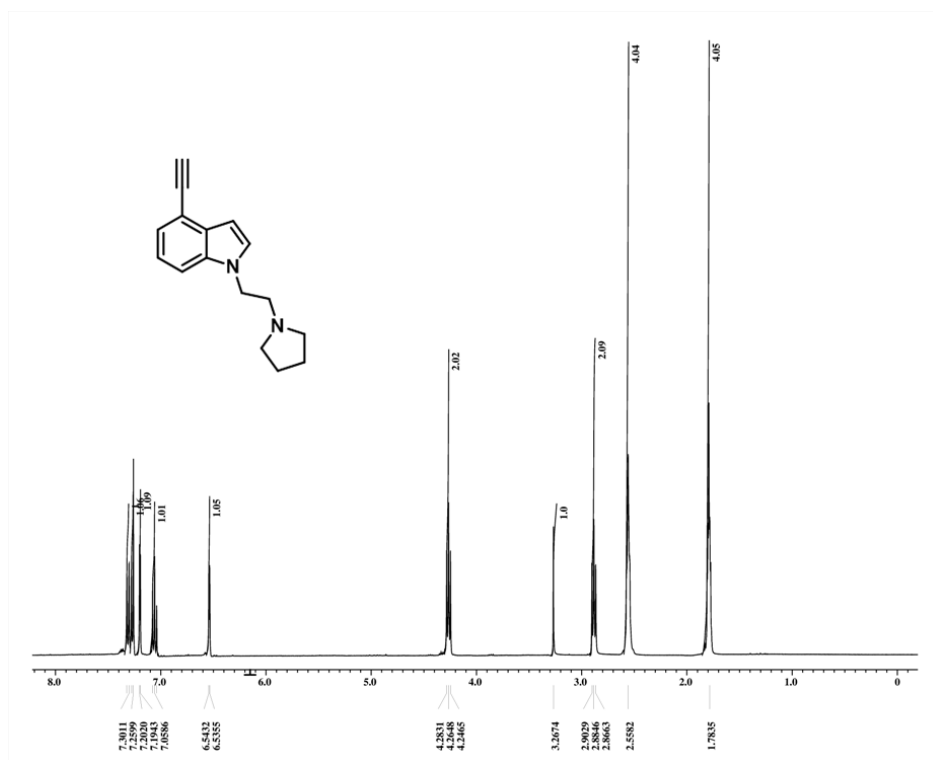
^1H NMR and ^{13}C NMR of 1b:



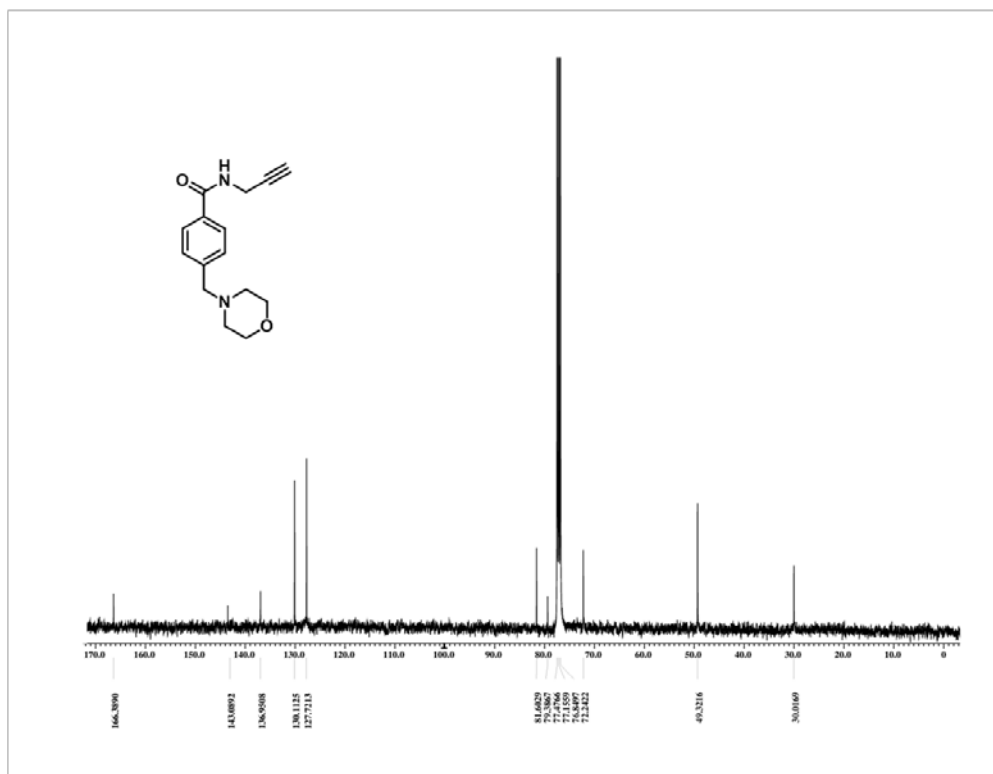
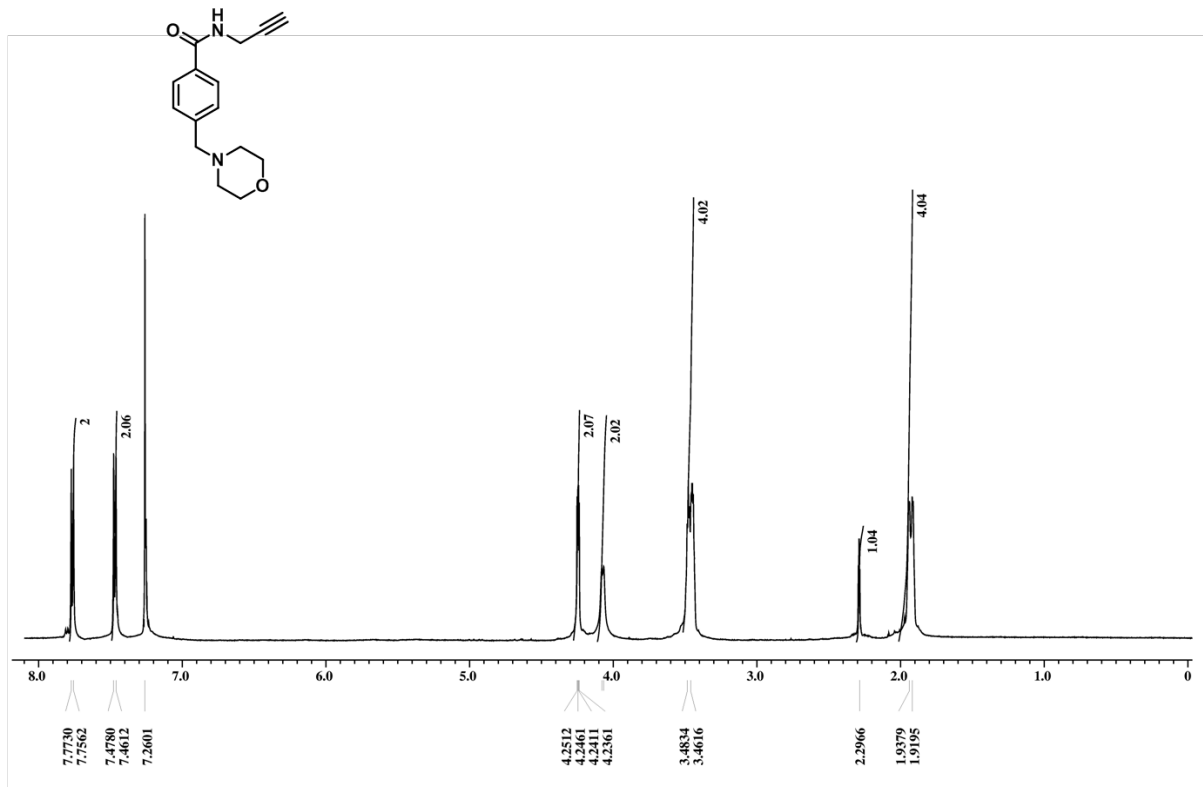
^1H NMR and ^{13}C NMR of S6:



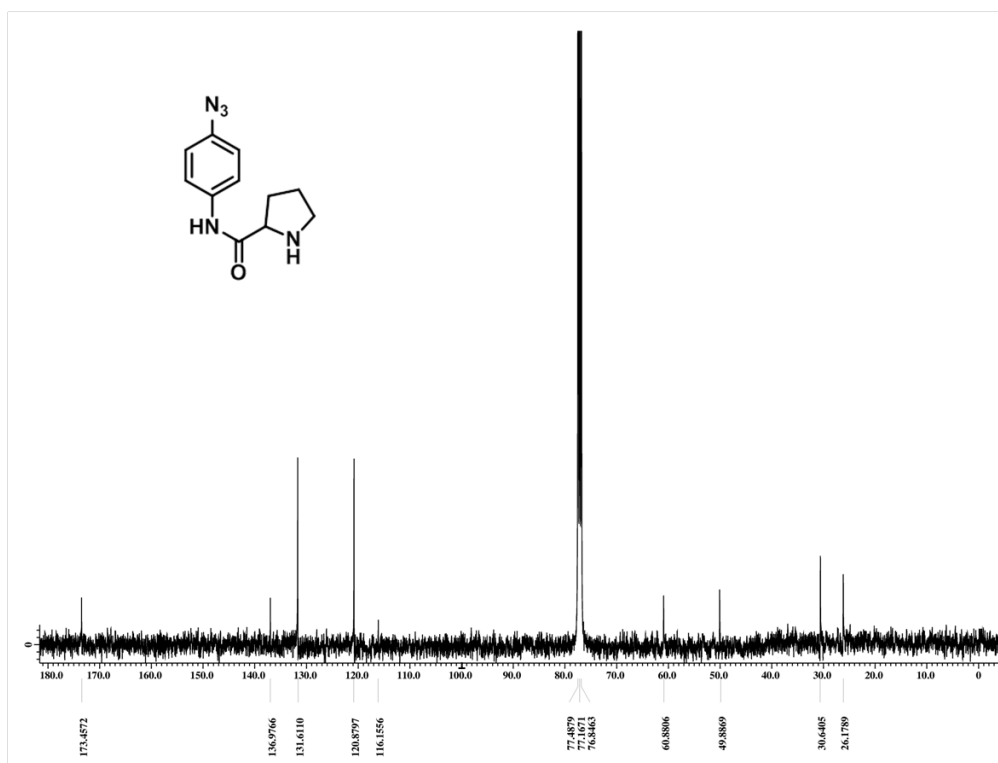
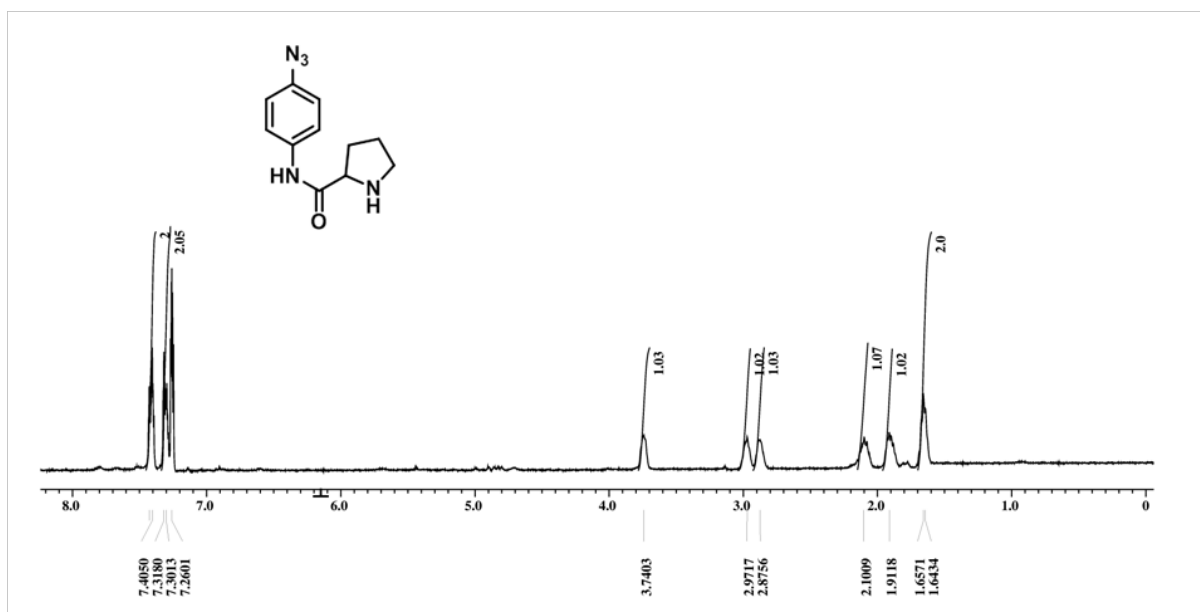
^1H NMR and ^{13}C NMR of 1c:



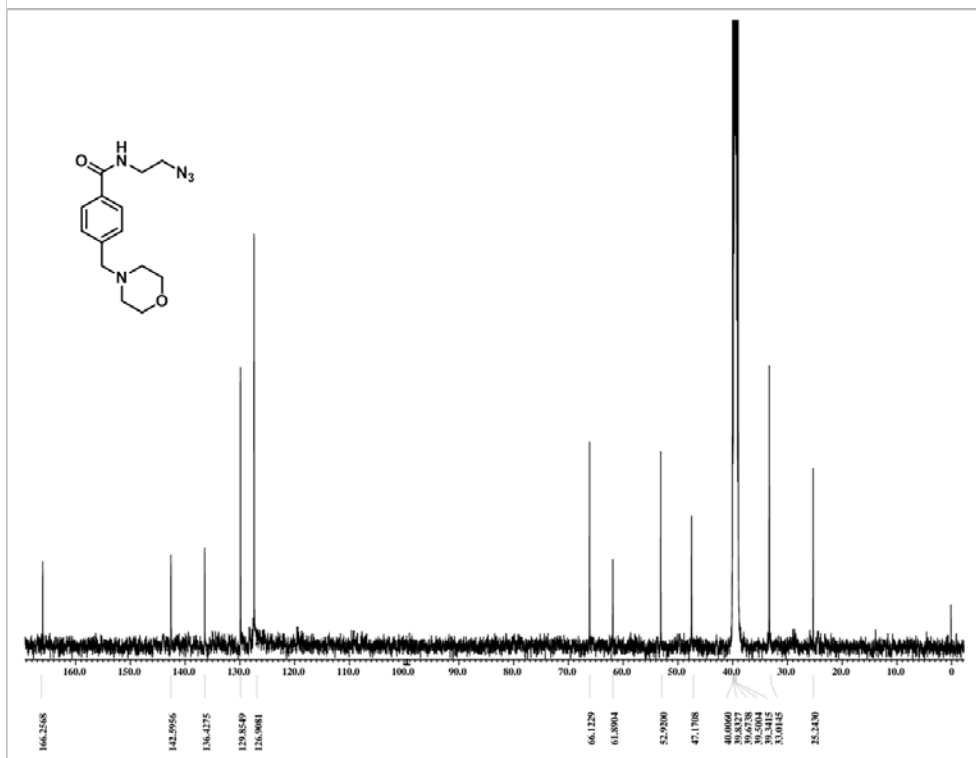
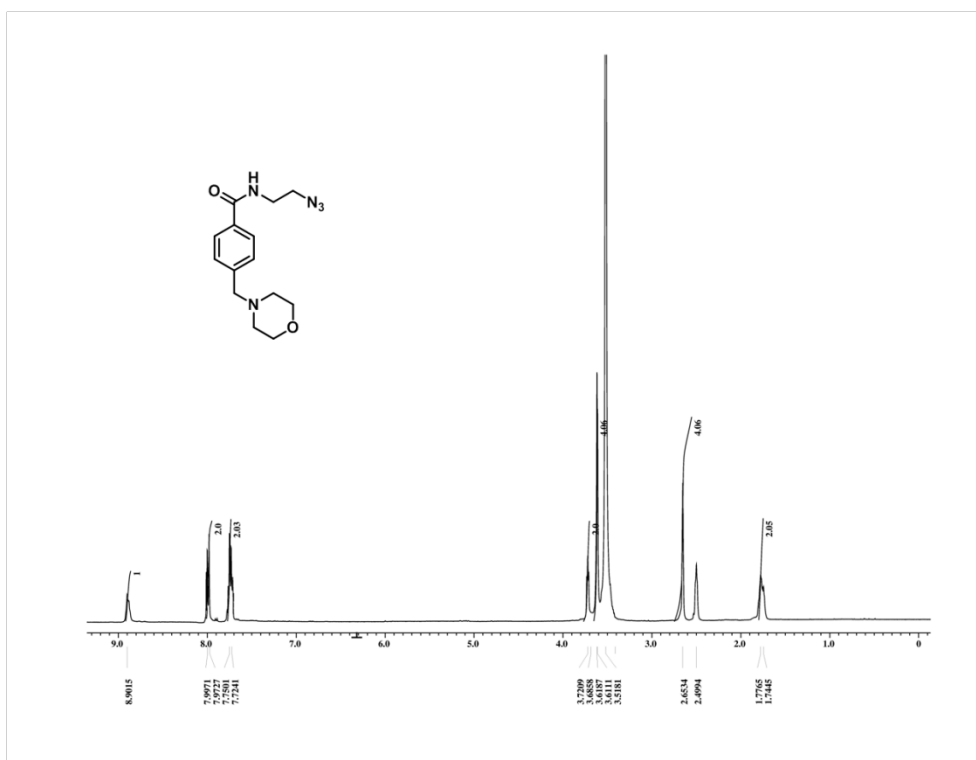
^1H and ^{13}C NMR of 1d:



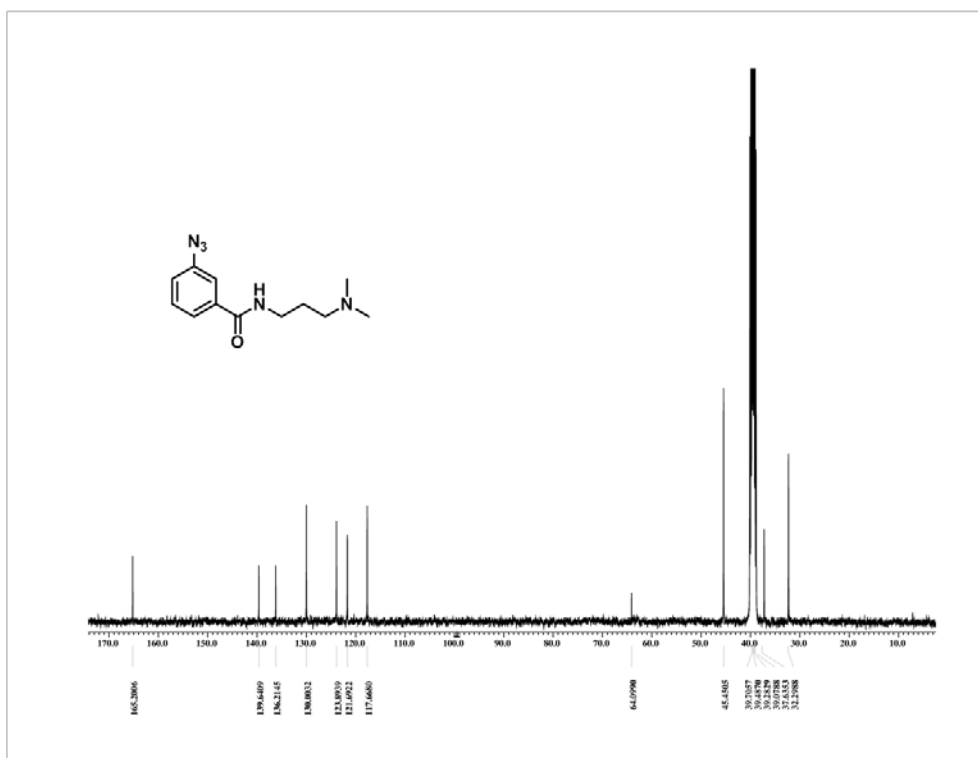
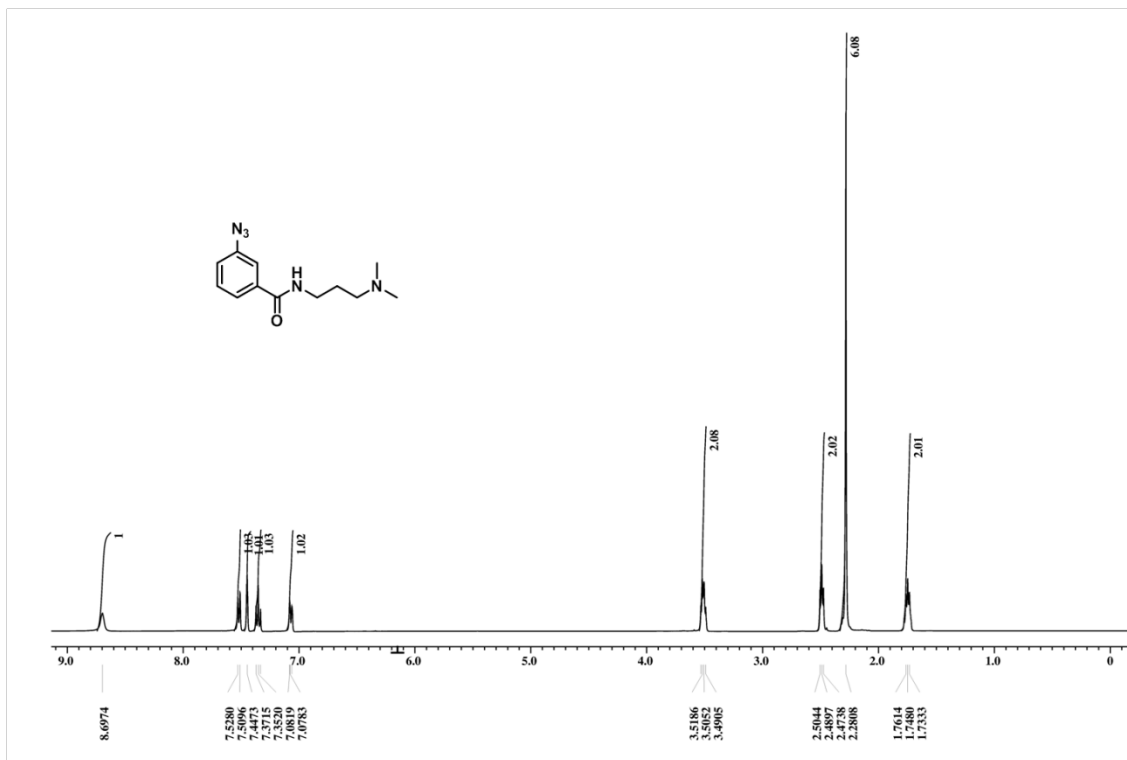
^1H and ^{13}C NMR of 2m:



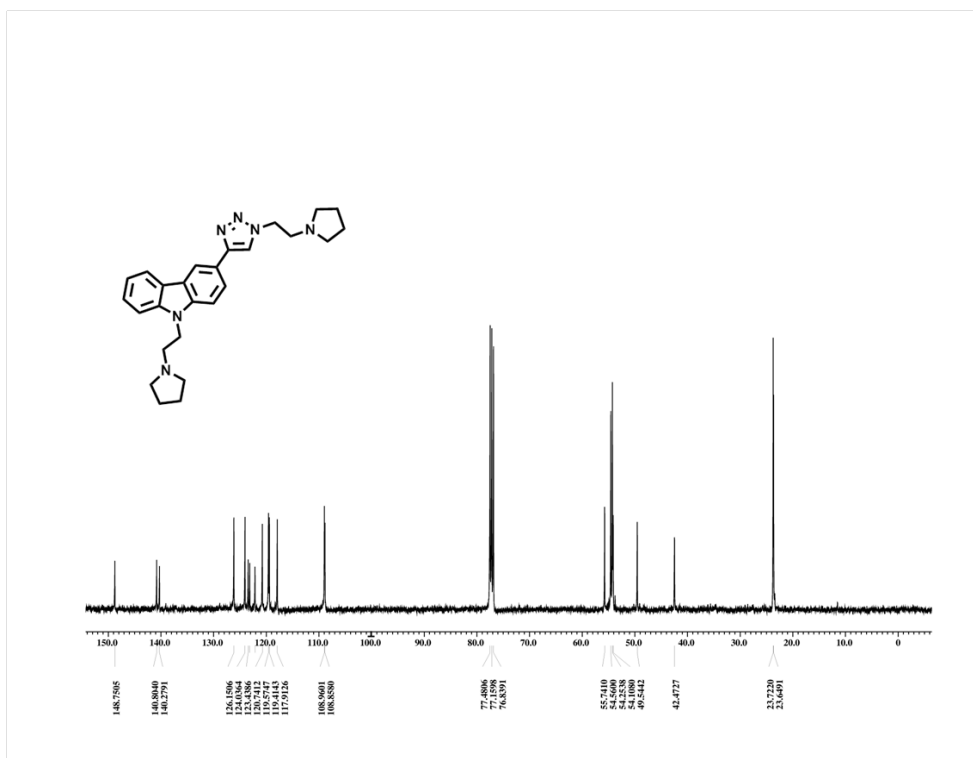
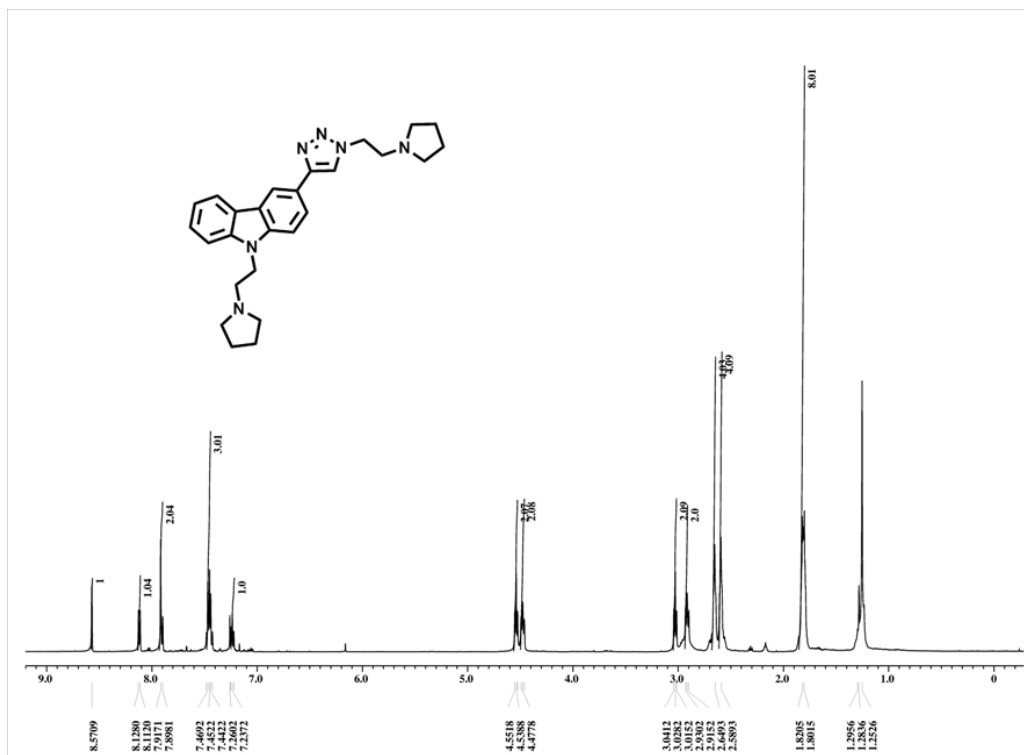
^1H and ^{13}C NMR of 2n:



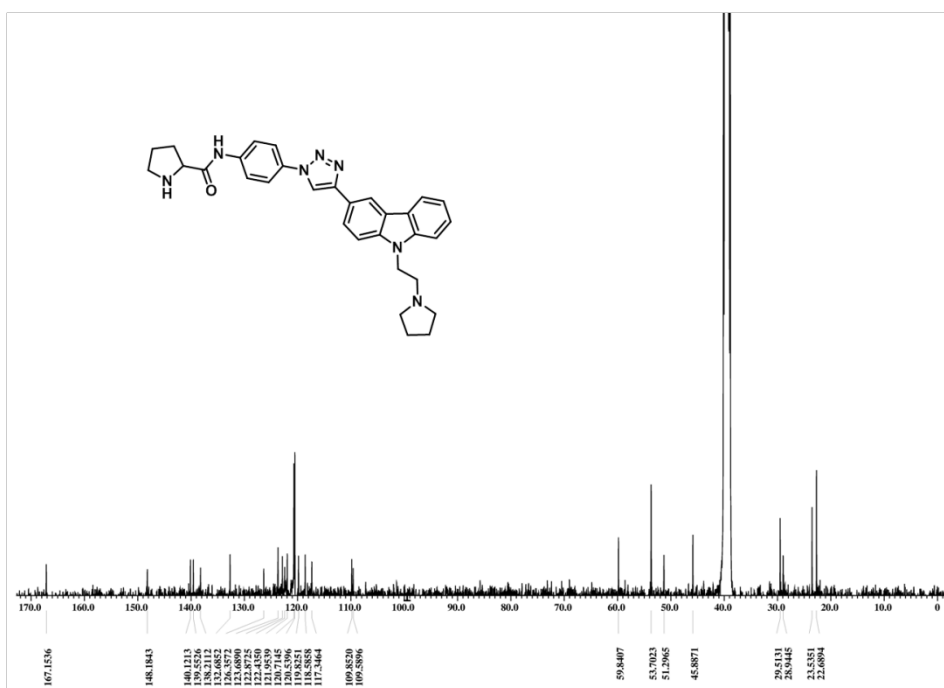
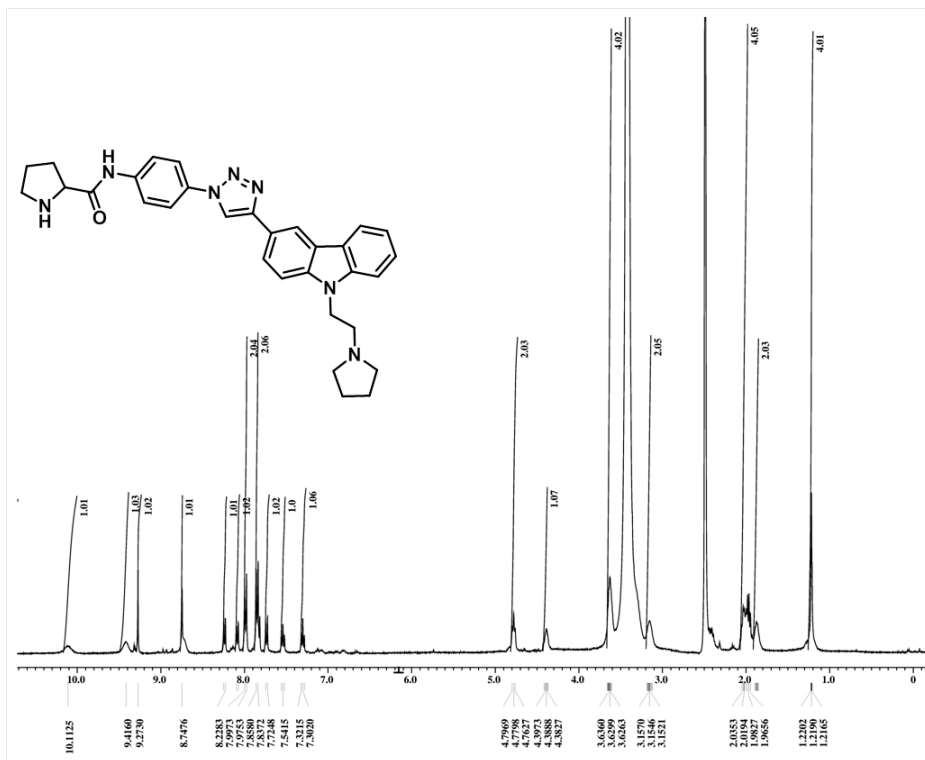
¹H NMR and ¹³C NMR of 2p:



^1H NMR and ^{13}C NMR of 3be:



^1H NMR and ^{13}C NMR of 3bm:



^1H NMR and ^{13}C NMR of 3ap:

