Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supplementary Information

Selective Recognition of *c-MYC* G-quadruplex DNA Using Prolinamide Derivatives

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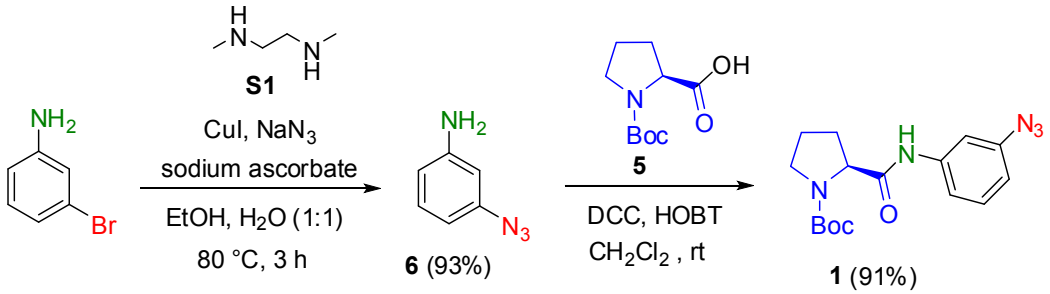
1.0 General Information:

Unless otherwise stated, all reactions were carried out in oven-dried glassware under an inert atmosphere of argon and were monitored by TLC or by ^{1}H NMR as appropriate. All the starting materials were purchased and used without further purification. Normal solvents used in these experiments were reagent grade. All anhydrous solvents were dried by standard techniques reported in Perrin, D. D.; Armarego, W. L. F., Purification of Laboratory Chemicals, 3rd edition, Pergamon Press, Oxford, 1988 and freshly distilled before use or purchased in anhydrous form and used directly. Products were purified by flash chromatography on silica gel (100-200 mesh, Merck) and yields refer to analytical pure samples. ^{1}H NMR spectra were recorded on a 500 MHz using Brüker ADVANCE, and a 400 MHz using JEOL instruments at 278K. ^{13}C NMR spectra were recorded either on a Brüker ADVANCE 500 MHz (125 MHz) or a JEOL-400 MHz (100 MHz) with complete proton decoupling. All signals are reported in ppm with the internal standard chloroform signal at 7.26 ppm (^{1}H) and 77.26 ppm (^{13}C) as a standard. The data is reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad and coupling constant(s) in Hz). Infrared (FTIR) spectra were recorded on a Perkin Elmer spectrophotometer with the KBr disk and KBr plate techniques for solid and liquid samples, ν_{max} cm^{-1}. HRMS analyses were performed with Q-TOF YA263 high resolution (Water Corporation) instruments by +ve mode electrospray ionization.

2.0 Preparation of the ligands: The prolinamide ligands **Pro-1** to **Pro-4** were prepared following our previously reported procedure.[1]

Preparation of azidoprolinamide 1:



Scheme S1: Preprartion of meta azido prolinamide **1**.

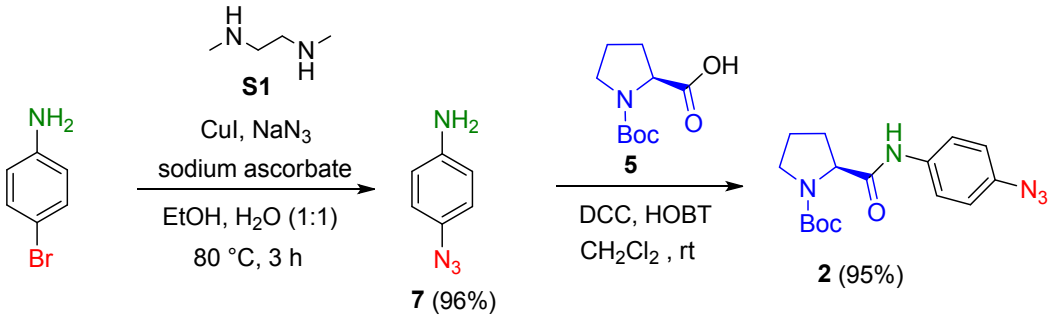
Preparation of 3-azidoaniline 6: Sodium ascorbate (59.6 mg, 0.3mmol, 0.05 equiv), CuI (136.8 mg, 0.69mmol, 0.1 equiv) and ligand **S1** (112 µL, 1.03mmol, 0.15 equiv) were added to a solution of 3-bromoaniline (1.2 g, 6.9 mmol, 1.0 equiv) in EtOH-H_2O (7:3, 30 mL). The resulting mixture was stirred for 15 min at room temperature. Then sodium azide (755 mg, 11.62 mmol, 2.0 equiv) was added under argon atmosphere to the reaction mixture. The reaction mixture was then stirred at reflux for 3 h and then the reaction is cooled and concentrated under reduced pressure. The residue was purified by flash chromatography eluted with hexane-ethyl acetate (95:5) mixture to give the desired azide **6** (869 mg, 93%) as a brown solid. ^{1}H NMR (400 MHz): 7.13 (1H, t, J = 7.9 Hz), 7.48 -7.46 (1H, m), 7.46 – 7.44 (1H, m), 6.32 (1H, t, J = 2.1 Hz), 3.70 (2H, s_{Br}); ^{13}C NMR (100 MHz) 147.7, 140.8, 130.3, 111.6, 108.8, 105.2.

Preparation of azido prolinamide 1: DCC (1.17 g, 5.6 mmol, 1.1 equiv) and HOBT (760 mg, 5.6 mmol, 1.1 equiv) were added to an ice-cold suspension of *N*-Boc proline **5** (1.1 g, 5.1 mmol) in dry CH_2Cl_2 (25 mL) under argon atmosphere and allowed to stir for 45 min. Then a solution of 3-azidoaniline **6** (687 mg, 5.1 mmol, 1.0 equiv) in dry CH_2Cl_2 (25 mL) was added drop-wise to the reaction mixture, and the reaction mixture was allowed to stir for 12 h. Upon

[1] S. Paladhi, J. Das, P. K. Mishra and J. Dash, *Adv. Synth. Catal.,* 2013, **355**, 274.

completion of starting materials (as monitored by TLC), the reaction mixture was filtered through celite, washed with ethyl acetate (50 mL) and concentrated under reduced pressure. The product was then purified by flash chromatography using hexane-ethylacetate (95:5 to 85:15) as eluent to afford the desired product **1** as a yellow solid (1.53 g, 91 %). m.p. = 131-133 °C; ^{1}H NMR (400 MHz): 9.72 (1H, s), 7.36 (1H, s), 7.10 (1H, s), 7.04 (1H, s), 4.49 (1H, s), 3.50- 3.35 (2H, m) 2.41 -1.89 (4H, m), 1.48 (9H , s); ^{13}C NMR (100 MHz): 170.5, 156.0, 140.2, 139.9, 129.6, 115.6, 114.0, 109.8, 80.8, 60.4, 47.1, 28.3, 24.5; FT-IR (KBr): 3341, 3221, 3095, 2941, 2835, 2801, 2561, 2158, 2151, 1751, 1738, 1685, 1631, 1556, 1447, 1221, 1101, 918, 826, 741; HRMS (ESI) calcd for $C_{16}H_{22}N_5O_3$ $(M+H)^+$: 332.1717; found, 332.1721.

Preparation of azidoprolinamide 2:

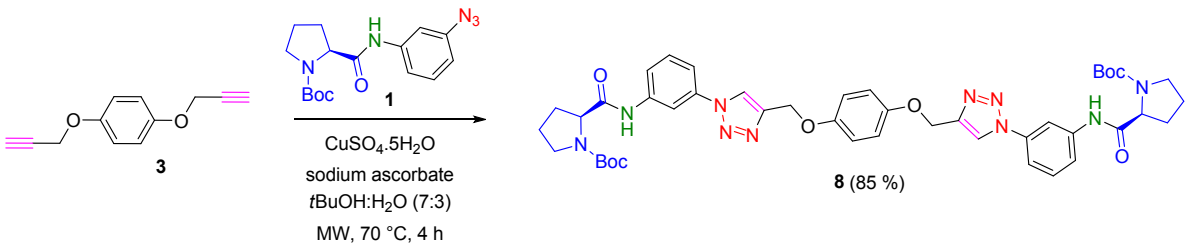


Preparation of 3-azidoaniline 7: Following the similar procedure for the synthesis of azidoaniline **6**, the desired 3-azidoaniline **7** (897 mg, 96%) was obtained from 4-bromoaniline (1.2 g, 6.9 mmol, 1.0 equiv) as a brown solid; ^{1}H NMR (400 MHz) 6.84 (d, 2H, J = 10.7 Hz), 6.67 (d, 2H, J = 10.9 Hz), 3.65 (s_{br}, 2H); ^{13}C NMR (100 MHz) 143.7, 130.0, 119.9, 116.2.

Preparation of azido prolinamide 2: Following the similar procedure for the synthesis of azido prolinamide **1**, the desired azido prolinamide **2** (1.6 g, 95%) obtained from *N*-Boc proline **5** (1.1 g, 5.1 mmol) and 3-azidoaniline **6** (687 mg, 5.1 mmol, 1.0 equiv); ^{1}H NMR (400 MHz): 9.61 (s_{br}, 1H), 7.48 (d, 2H, J = 9.4 Hz), 6.89 (s_{br}, 1H), 4.47 (s_{br}, 1H), 3.45-3.36

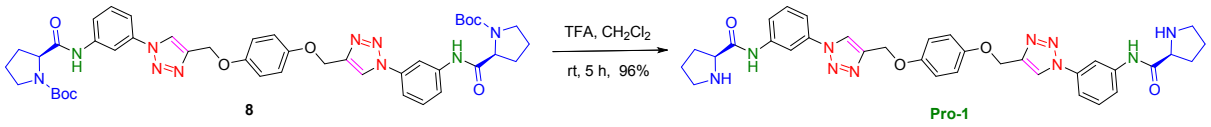
(m, 2H), 2.44 (s_{br}, 1H), 1.99-1.90 (m, 3H), 1.48 (s, 9H); ^{13}C NMR (100 MHz): 170.0, 156.5, 135.5, 134.9, 120.8, 119.2, 80.9, 60.4, 47.3, 28.3, 27.5, 24.5.

Synthesis of compound 8:



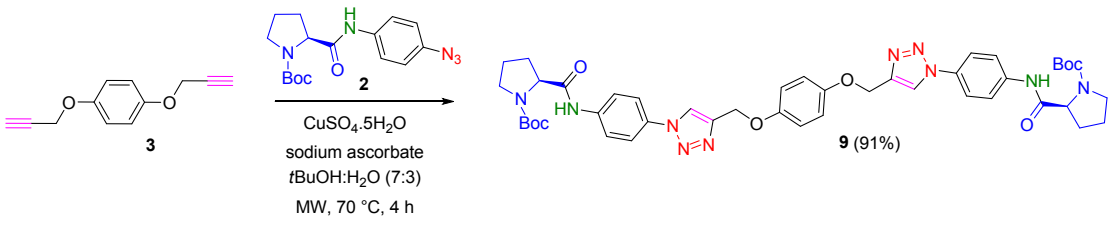
A mixture of 4-bis(prop-2-ynyloxy)benzene **3** (100 mg, 0.55 mmol, 1.0 equiv), sodium ascorbate (21.8 mg, 0.11mmol, 0.2 equiv), $CuSO_4.5H_2O$ (13.8 mg, 0.05 mmol, 0.1 equiv) and azido prolinamide **1** (419 mg, 1.26 mmol, 2.3 equiv) was taken in 5 mL *t*BuOH:H_2O (7:3) in a dried 20 mL microwave vial. The reaction mixture was stirred for 4 h at 70 °C under microwave irradiation and then cooled to room temperature. After removal of solvent under vacuum, the reaction mixture was purified by flash chromatography using hexane-ethyl acetate (50:50 to 30:70) as eluent to obtain **8** (387 mg, 85 %) as a colourless viscous liquid; ^{1}H NMR (400 MHz): 9.90 (s_{br}, 2H), 7.98 (s_{br}, 4H), 7.37 (s_{br}, 6H), 6.95 (s, 4H), 5.20 (s, 4H), 4.53 (s_{br}, 2H), 3.52-3.42 (m, 4H), 2.37 (s_{br}, 2H), 2.10-1.93 (m, 6H), 1.50 (s, 18H); ^{13}C NMR (100 MHz): 170.8, 156.4, 152.8, 144.9, 139.8, 137.1, 130.0, 120.9, 119.4, 116.0, 115.6, 111.3, 80.1, 62.6, 60.6, 47.3, 28.4, 24.5; FT-IR (KBr): 3491, 3310, 3221, 2955, 2991, 2405, 1684, 1633, 1564, 1487, 1396, 1317, 1245, 1143, 1063; HRMS (ESI) calcd for $C_{44}H_{52}N_{10}O_8K$ $(M+K)^+$: 887.3601; found, 887.3603.

Synthesis of compound Pro-1:



TFA (0.15 mL, 1.8 mmol, 6.0 equiv.) was added to a solution of compound **8** (250 mg, 0.3mmol) in dry CH_2Cl_2 (20 mL) and then stirred for 5 h at room temperature. After stirring for 5 h, the reaction mixture was quenched by adding drop-wise a solution of liquid NH_3 (30%) at 0 °C (pH 8-9). The resulting mixture was then extracted with CH_2Cl_2 (3 x 15 mL), dried over Na_2SO_4, and concentrated.The residue was then purified by flash chromatograghy using CH_2Cl_2:MeOH:NH_3 (90:09:01 to 85:13:2) to give **Pro-1** (183 mg, 96 %) as a colorless viscous liquid. ^{1}H NMR (400 MHz, DMSO-d_6): 10.24 (s, 2H), 8.86 (s, 2H), 8.33 (s, 2H), 7.75 (d, 2H, *J* =7.8 Hz), 7.55-7.51 (m, 4H), 7.03 (s, 4H), 5.17 (s, 4H), 3.73 (dd, 2H, *J* = 5.6, 8.8 Hz), 2.91 (t, 4H, *J* = 6.7 Hz), 2.09-1.99 (m, 2H), 1.84-1.74 (m, 2H), 1.69-1.62 (m, 4H), NH protons could not be detected;^{13}C NMR (100 MHz, DMSO-d_6): 174.0, 152.4, 144.1, 139.8, 136.8, 130.2, 122.8, 119.2, 115.8, 114.8, 110.9, 61.5, 60.8, 46.8, 30.5, 25.8; FT-IR (KBr): 3423, 3141, 2971, 2353, 2115, 1691, 1618, 1523, 1434, 1317, 1242, 1153, 1126, 1051;HRMS (ESI) calcd for $C_{34}H_{37}N_{10}O_4$ $(M+H)^+$: 649.2999; found, 649.2994.

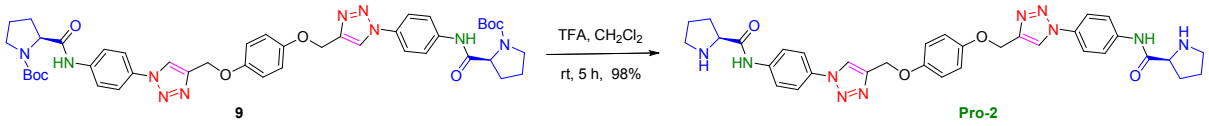
Synthesis of compound 9:



Following the similar procedure for the synthesis of compound **8**, the compound **9** (414 mg, 91 %) was obtained from the reaction between 4-bis(prop-2-ynyloxy)benzene **3** (100 mg, 0.55 mmol, 1.0 equiv) and azido-prolinamide **2** (419 mg, 1.26 mmol, 2.3 equiv); ^{1}H NMR

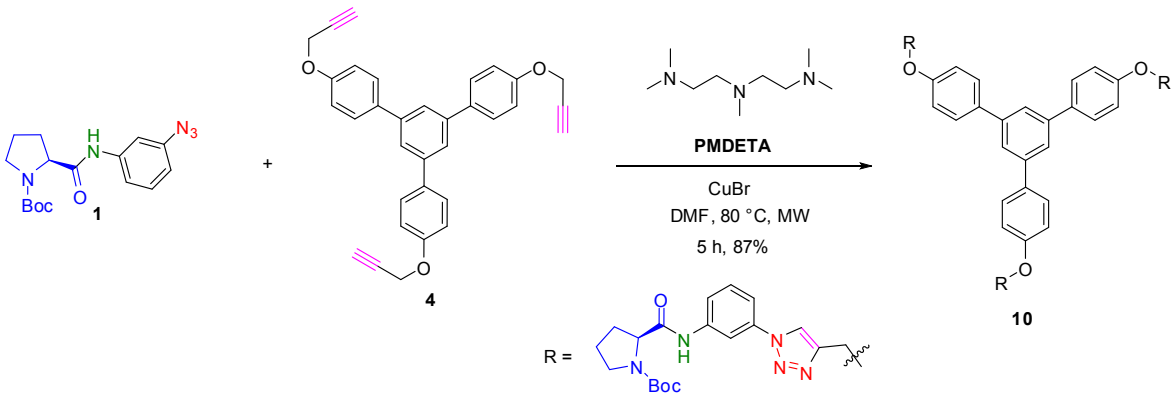
(400 MHz): 9.93 (s, 2H), 7.81 (s, 2H), 7.53 (s, 4H), 7.37 (s, 4H), 6.89 (s, 4H), 5.13 (s, 4H), 4.54 (s, 2H), 3.55-3.54 (m, 4H), 2.23 (s, 2H), 1.96 (s, 4H), 1.91 (s, 2H), 1.49 (s, 18H); ^{13}C NMR (100 MHz): 171.1, 155.7, 152.6, 144.6, 139.2, 131.9, 120.8, 120.0, 115.8, 80.8, 62.3, 60.5, 47.2, 29.0, 28.4, 24.5 (one of the aromatic carbons could not be unambigously detected).

Synthesis of compound Pro-2:



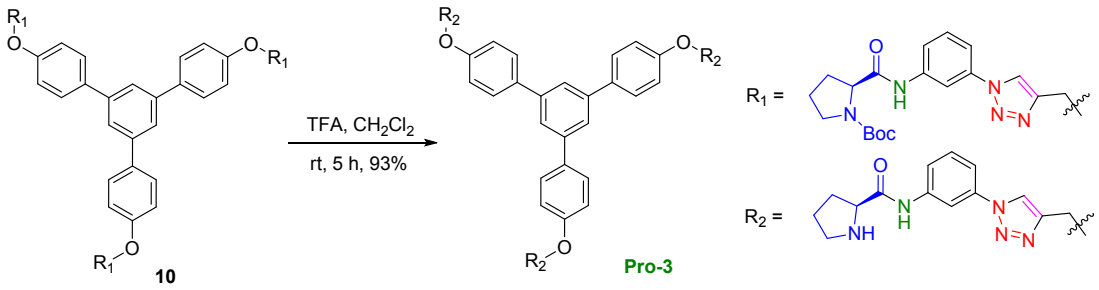
Following the similar procedure for the synthesis of **Pro-1**, the desired ligand **Pro-2** (187 mg, 98 %) was obtained as a colorless solid from compound **9** (250 mg, 0.3 mmol); ^{1}H NMR (400 MHz, DMSO-d_6): 10.20 (s, 2H), 8.85 (s, 2H), 7.89 (d, 4H, J =11.2 Hz), 7.83 (d, 4H, J = 11.2 Hz), 7.03 (s, 4H), 5.16 (s, 4H), 3.73 (s_{br}, 2H), 2.91 (t, 4H, J = 7.5 Hz), 2.07-1.98 (m, 2H), 1.80 (dt, 2H, J = 16.0, 8.1 Hz), 1.68-1.65 (m, 4H), proline NH protons could not be detected; ^{13}C NMR (100 MHz, DMSO-d_6): 173.8, 152.4, 144.0, 138.9, 131.8, 122.6, 120.7, 120.1, 115.7, 61.5, 60.8, 46.8, 30.5, 25.9.

Synthesis of compound 10:



Azido-prolinamide **1** (1.4 g, 2.8 mmol, 4.0 equiv.), CuBr (100 mg, 1.4 mmol, 2.0 equiv) and PMDETA (252 µL, 2.0 equiv) was added to a mixture of the trialkyne **4** (300 mg, 0.7 mmol) in 5 mL dry DMF in a dried 20 mL microwave vial and stirred for 5 h at 80 °C under microwave irradiation. The reaction mixture was concentrated under vacuum and purified by flash chromatography using hexane-ethyl acetate (50:50 to 10:90) as eluent to give **10** (850 mg, 87%) as a colorless viscous liquid; ^{1}H NMR (500 MHz): 9.97 (s_{br}, 3H), 8.01-7.91 (m, 6H), 7.51-7.37 (m, 15H), 7.04 (s_{br}, 18H), 5.16 (s_{br}, 6H), 4.57 (s, 3H), 3.57-3.44 (m, 6H), 2.28-2.09 (m, 6H), 1.94-1.73 (m, 6H), 1.52 (s_{br}, 27H); ^{13}C NMR (125 MHz): 170.9, 162.6, 157.9, 144.5, 141.5, 139.7, 137.1, 134.2, 129.9, 128.4, 123.8, 121.0, 119.3, 115.5, 115.1, 111.0, 81.1, 62.0, 60.7, 47.3, 28.5, 24.4;FT-IR (KBr):3433, 3150, 2967, 2872, 2385, 2114, 1694, 1610, 1531, 1405, 1333, 1242, 1161, 1127, 1037; HRMS (ESI) calcd for $C_{81}H_{88}N_{15}O_{12}$ $(M+H)^{+}$: 1462.6736; found, 1462.6753.

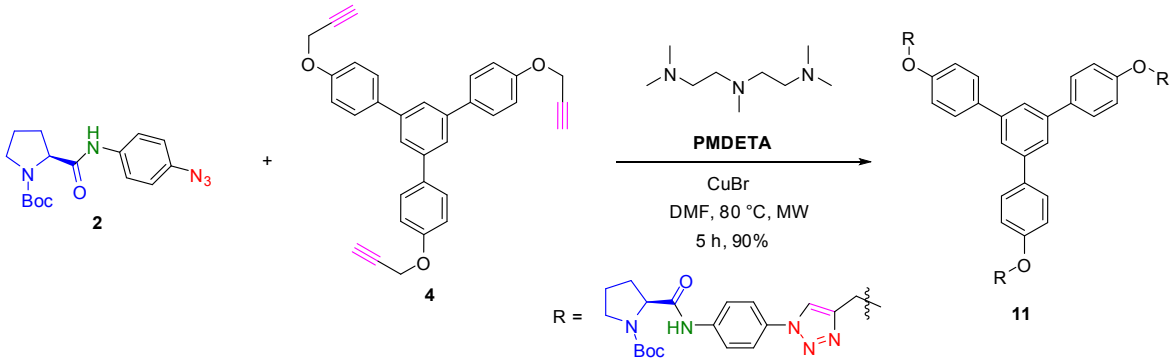
Synthesis of compound Pro-3:



Compound **10** (500 mg, 0.34 mmol) was dissolved in 20 mL of dry CH_2Cl_2 and TFA (0.3 mL, 3.4 mmol, 10.0 equiv.) was added to it. The mixture was stirred for 5 h at room temperature. After consumption of the starting material **10** (monitored by TLC), the reaction mixture was brought to pH 8-9 by adding drop-wise a solution of liquid NH_3 (30%) at 0 °C. Then the reaction mixture was extracted with CH_2Cl_2 (3 x 20 mL), dried in vacuum, purified by flash chromatograghy using CH_2Cl_2:MeOH:NH_3 (90:09:1 to 75:20:5) to give **Pro-3** (377 mg, 93 %) as a colorless solid; m.p. 186-188 °C; ^{1}H NMR (500 MHz, DMSO-d_6): 10.35 (s,

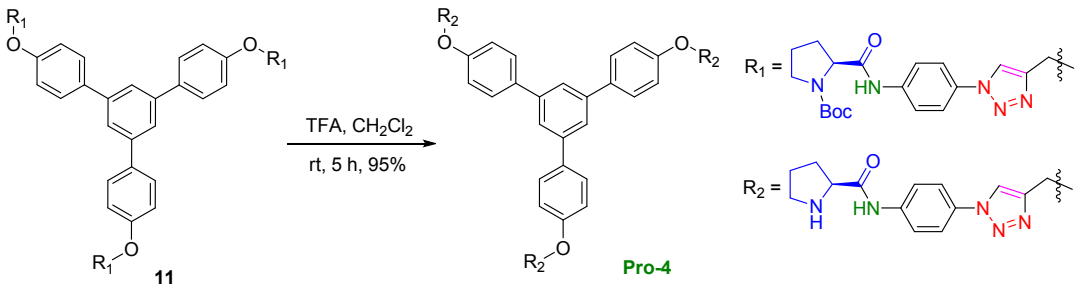
3H), 8.93 (s, 3H), 8.35 (t, 3H, J = 2.5 Hz), 7.82 (d, 6H, J = 8.6 Hz), 7.76-7.74 (m, 6H), 7.59-7.52 (m, 6H), 7.20 (d, 6H, J = 8.7 Hz), 5.32 (s, 6H), 3.82 (dd, 3H, J = 8.5, 5.9 Hz), 2.97-2.95 (m, 6H), 2.12-2.06 (m, 3H), 1.83 (dt, 3H, J = 12.9, 7.0 Hz), 1.70 (td, 6H, J= 13.5, 6.7 Hz), NH protons could not be detected; ^{13}C NMR (125 MHz, DMSO-d_6):172.0, 158.1, 157.9, 144.0, 141.1, 139.8, 136.8, 133.2, 130.3, 128.3, 122.9, 119.3, 116.1, 115.3, 110.9, 61.1, 60.7, 46.6, 30.3, 25.6; FT-IR(KBr): 3367, 2853, 2821, 2396, 2293, 1714, 1622, 1534, 1421, 1407, 1289, 1243, 1178, 1112, 1032; HRMS (ESI) calcd for $C_{66}H_{64}N_{15}O_6$ $(M+H)^+$: 1162.5164; found 1162.5192.

Synthesis of compound 11:



Following the similar procedure for the synthesis of **10**, the desired compound **11** (879 mg, 90%) was obtained from the reaction between azido prolinamide **2** (1.4 g, 2.8 mmol, 4.0 equiv.) and the trialkyne **4** (300 mg, 0.7 mmol); ^{1}H NMR (500 MHz): 10.00 (s_{br}, 3H), 7.78 (s_{br}, 3H), 7.54 (d, 6H, J = 7.2 Hz), 7.47 (s_{br}, 3H), 7.42 (d, 6H, J = 5.1 Hz), 7.32 (s_{br}, 6H), 6.93 (s_{br}, 6H), 5.03 (s_{br}, 6H), 4.58 (s, 3H), 3.62-3.46 (m, 6H), 2.19-2.09 (m, 9H), 1.93 (s_{br}, 3H), 1.53 (s_{br}, 27H); ^{13}C NMR (125 MHz): 171.5, 157.8, 155.3, 144.2, 141.3, 139.3, 134.1, 131.8, 128.3, 123.6, 121.0, 120.8, 119.9, 114.7, 80.7, 61.6, 60.5, 47.3, 28.4, 28.3, 24.5.

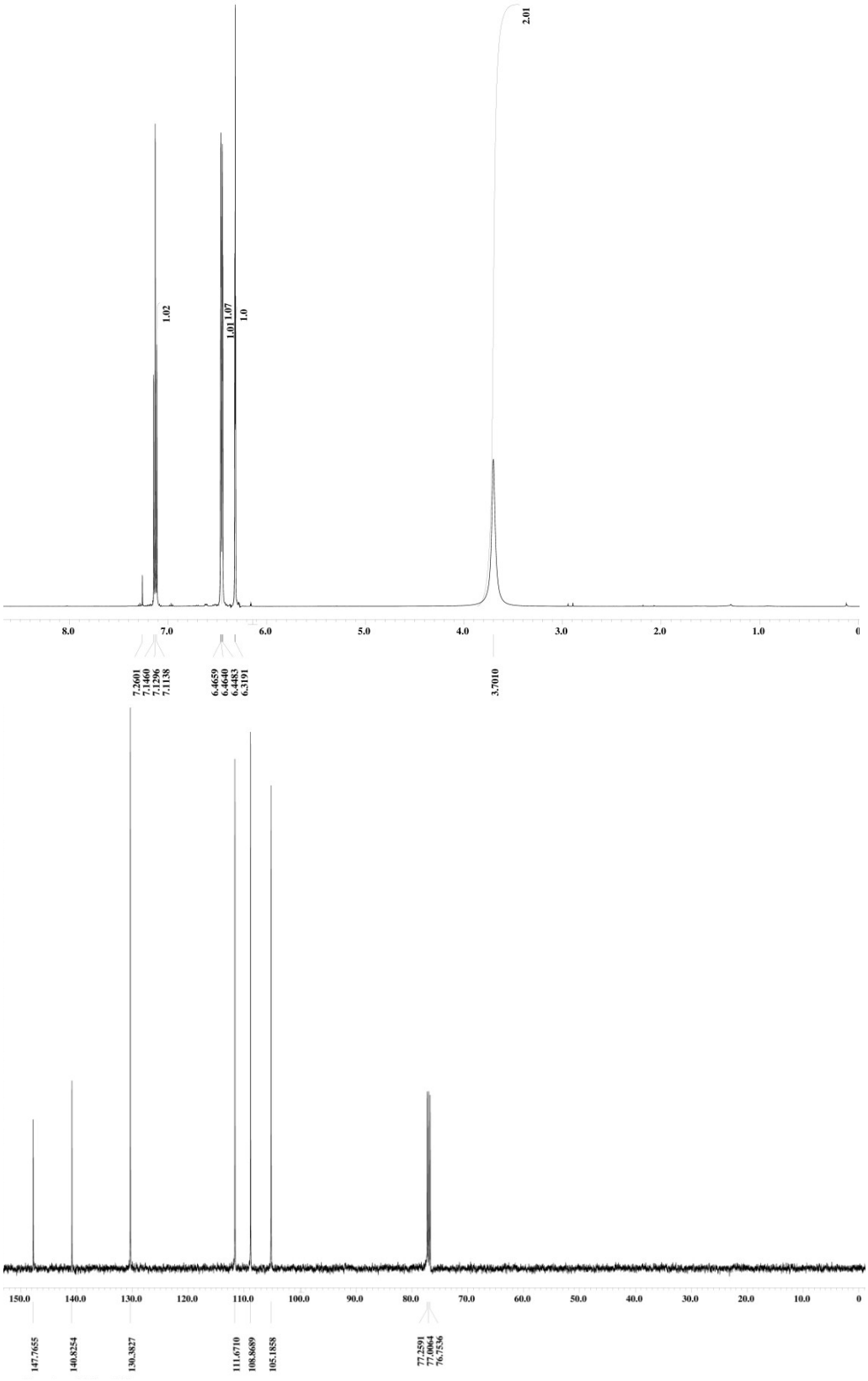
Synthesis of compound Pro-3:



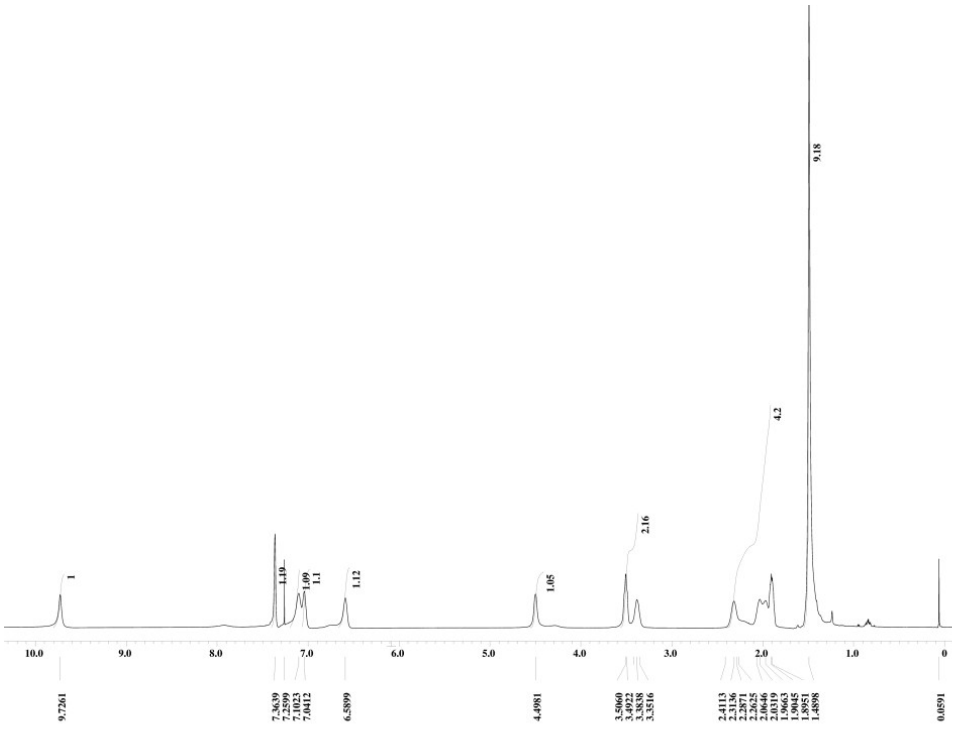
Following the similar procedure for the synthesis of **Pro-3**, the desired ligand **Pro-4** (385 mg, 95 %) was obtained as a colorless solid from compound **11** (500 mg, 0.34 mmol). ^{1}H NMR (500 MHz, DMSO-d_6): 10.20 (s, 3H), 8.92 (s, 3H), 7.90 (d, 6H, J = 9.2 Hz), 7.86 (d, 6H, J = 9.1 Hz), 7.82 (d, 6H, J = 8.8 Hz), 7.76 (s, 3H), 7.20 (d, 6H, J = 8.9 Hz), 5.31 (s, 6H), 3.72 (dd, 3H, J= 8.8, 5.6 Hz), 2.90 (t, 6H, J = 6.7 Hz), 2.06 (ddd, 3H, J= 15.7, 12.5, 7.7 Hz), 1.79 (ddd, 3H, J = 12.7, 12.6, 7.1 Hz), 1.69-1.64 (m, 6H), proline NH protons could not be detected; ^{13}C NMR (125 MHz, DMSO-d_6): 173.9, 157.8, 143.8, 141.0, 138.9, 133.1, 131.8, 128.3, 122.9, 122.6, 120.6, 120.0, 115.2, 61.2, 60.8, 40.0, 30.4, 25.9.

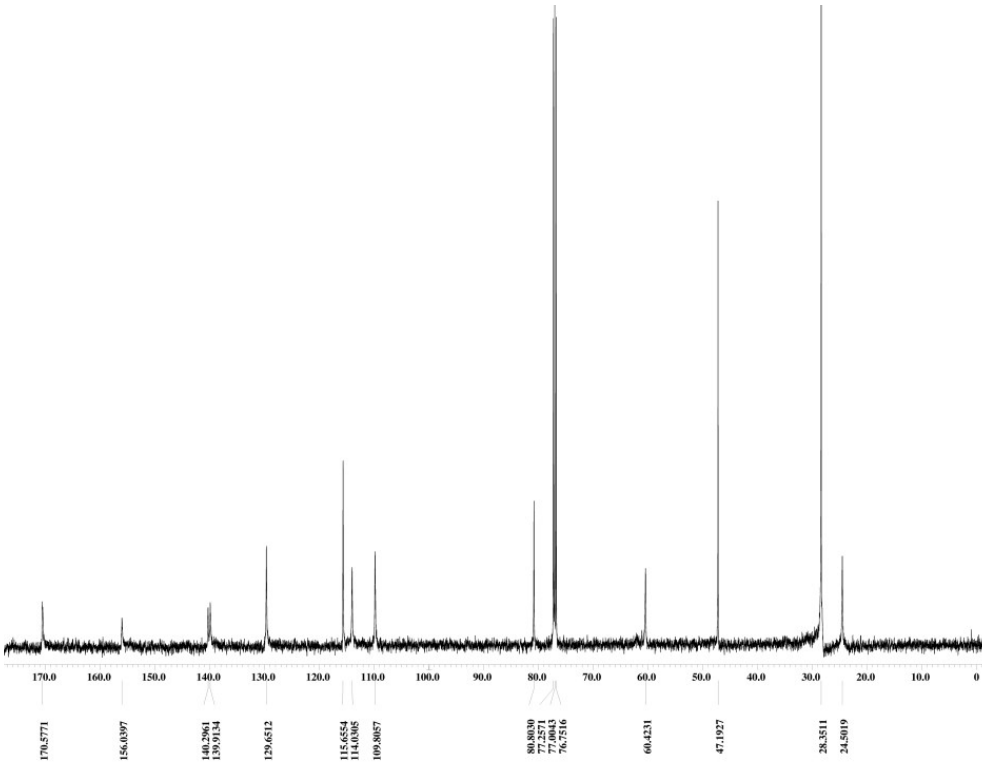
3.0 NMR spectra of all the new compounds

^{1}H and ^{13}C NMR of 3-azidoaniline 6:

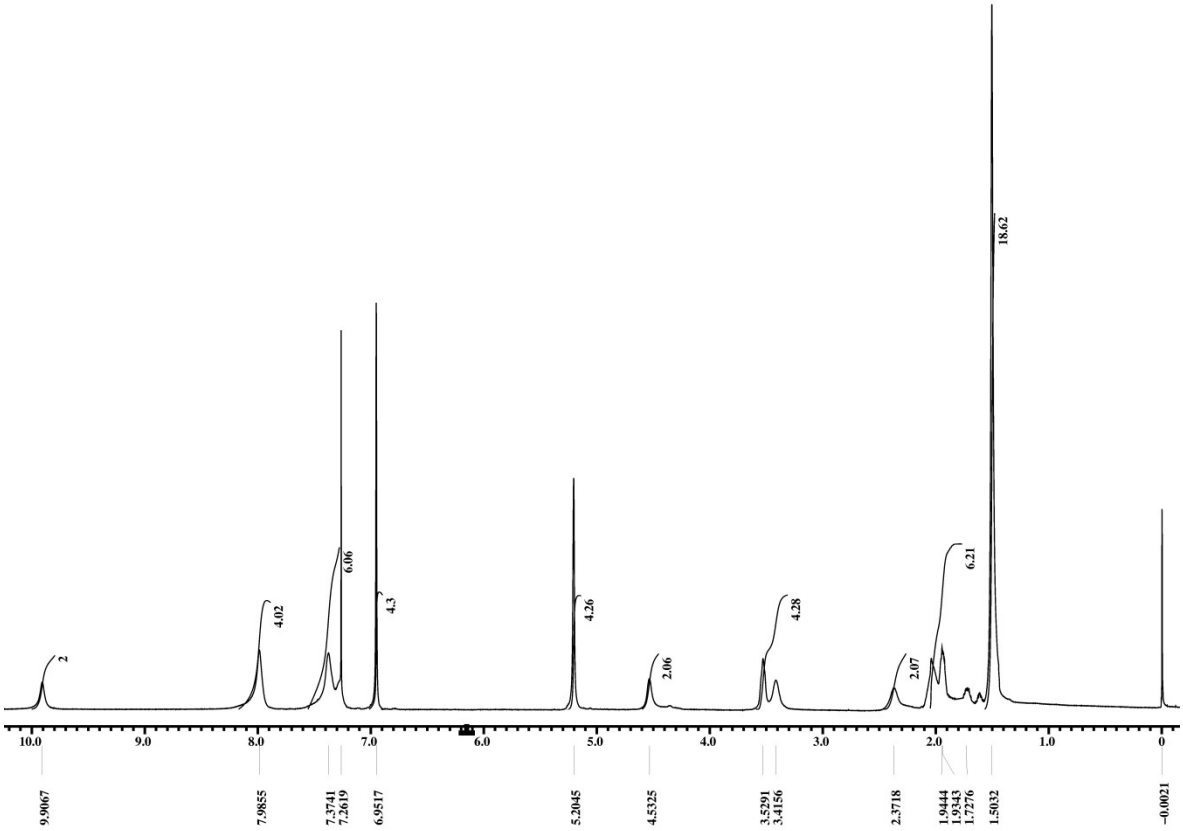


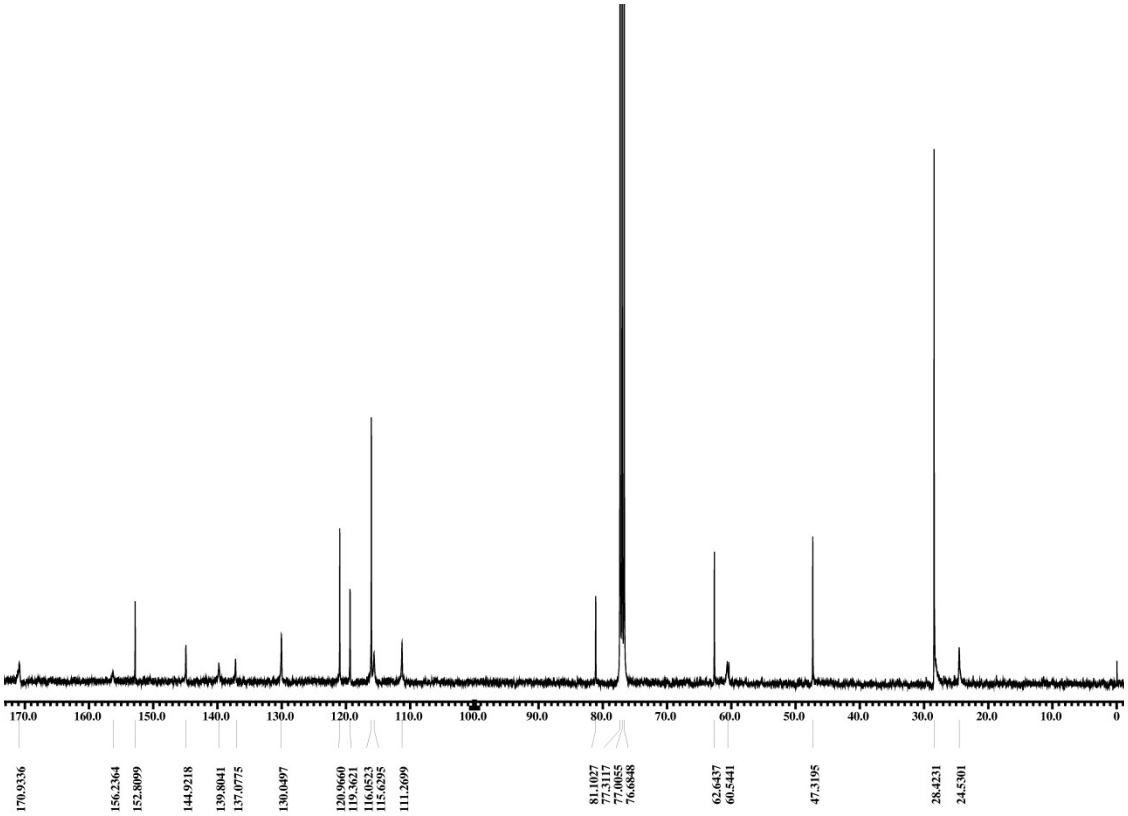
^{1}H and ^{13}C NMR of azido-prolinamide 1:



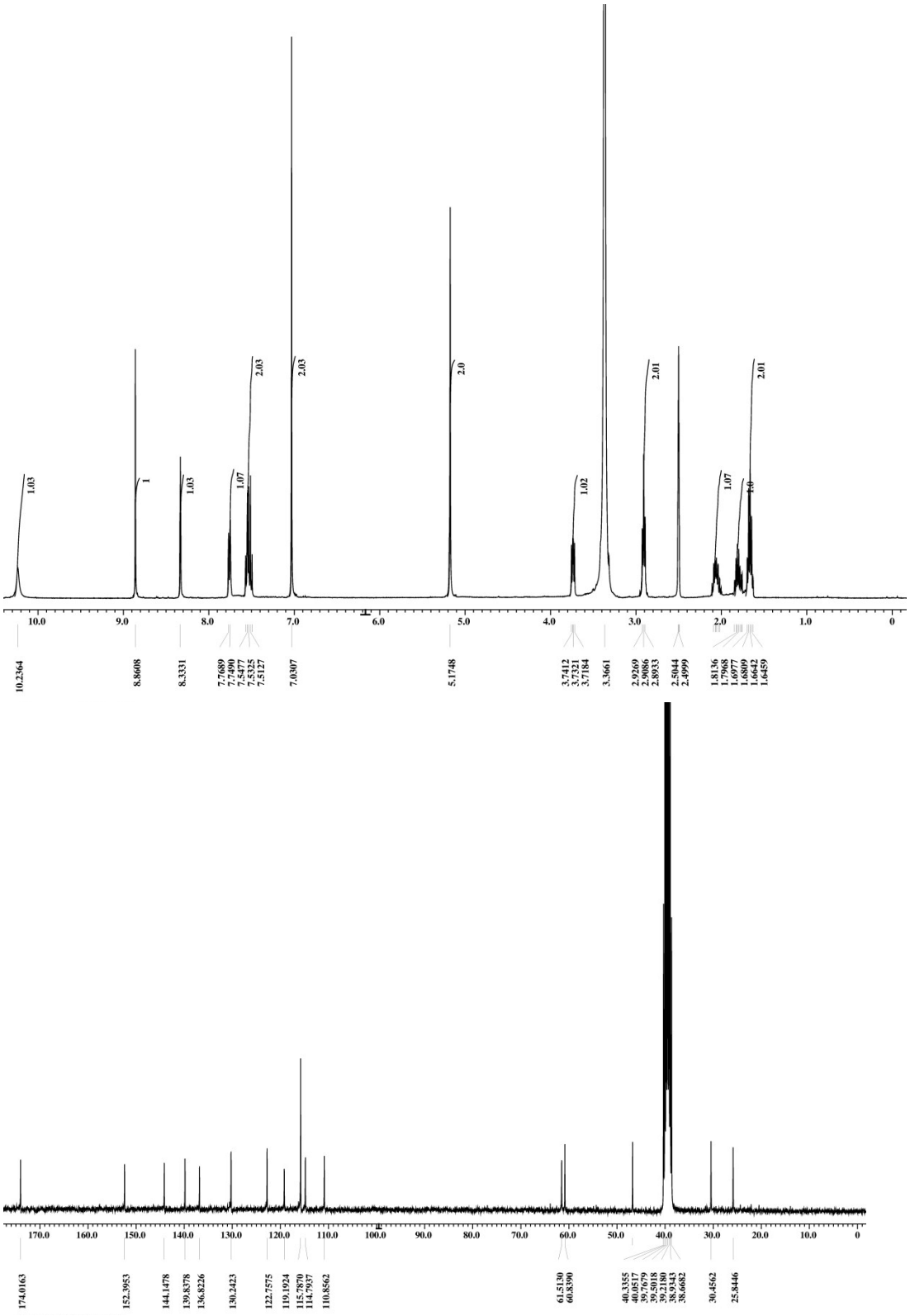


^{1}H and ^{13}C NMR of compound 8:

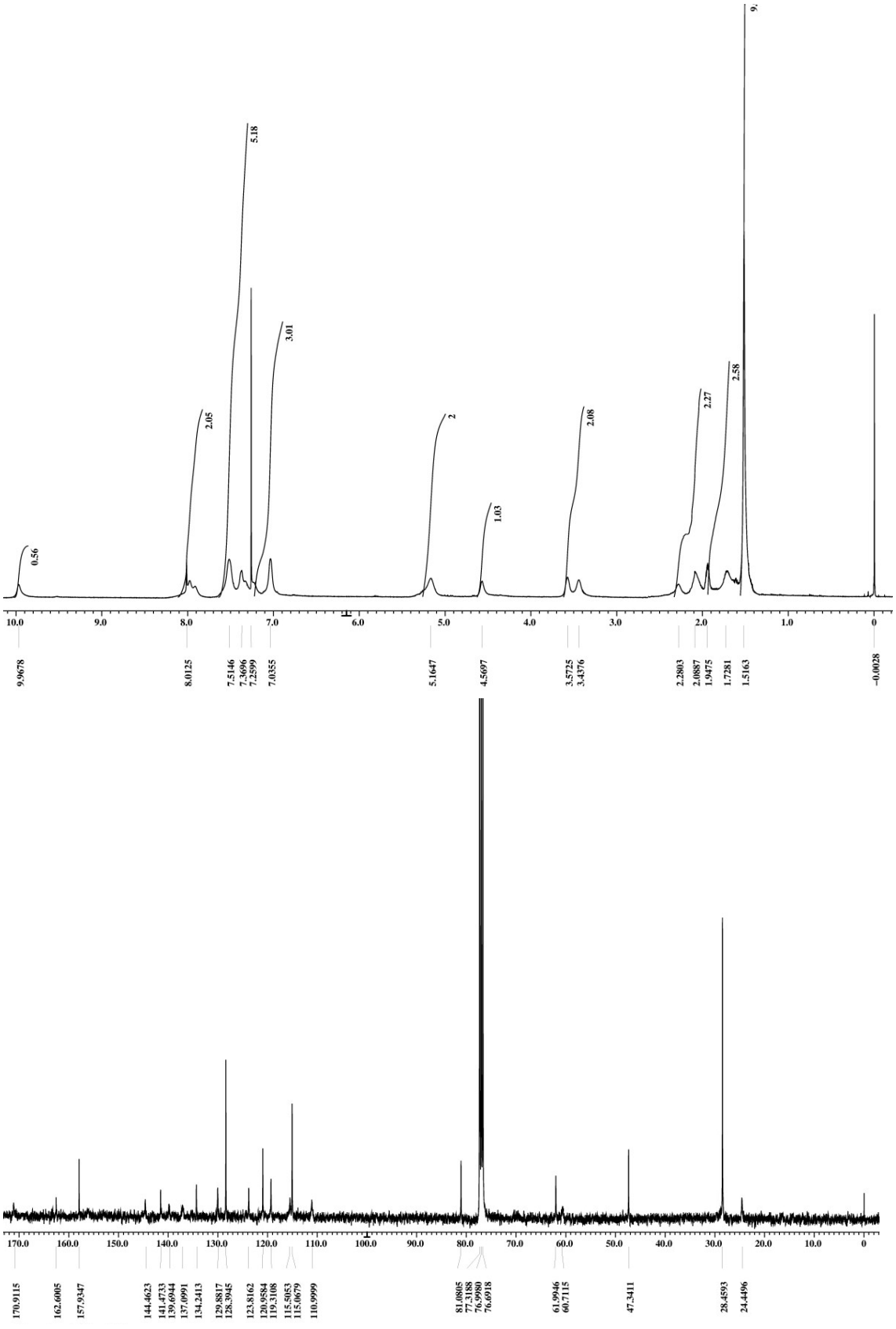




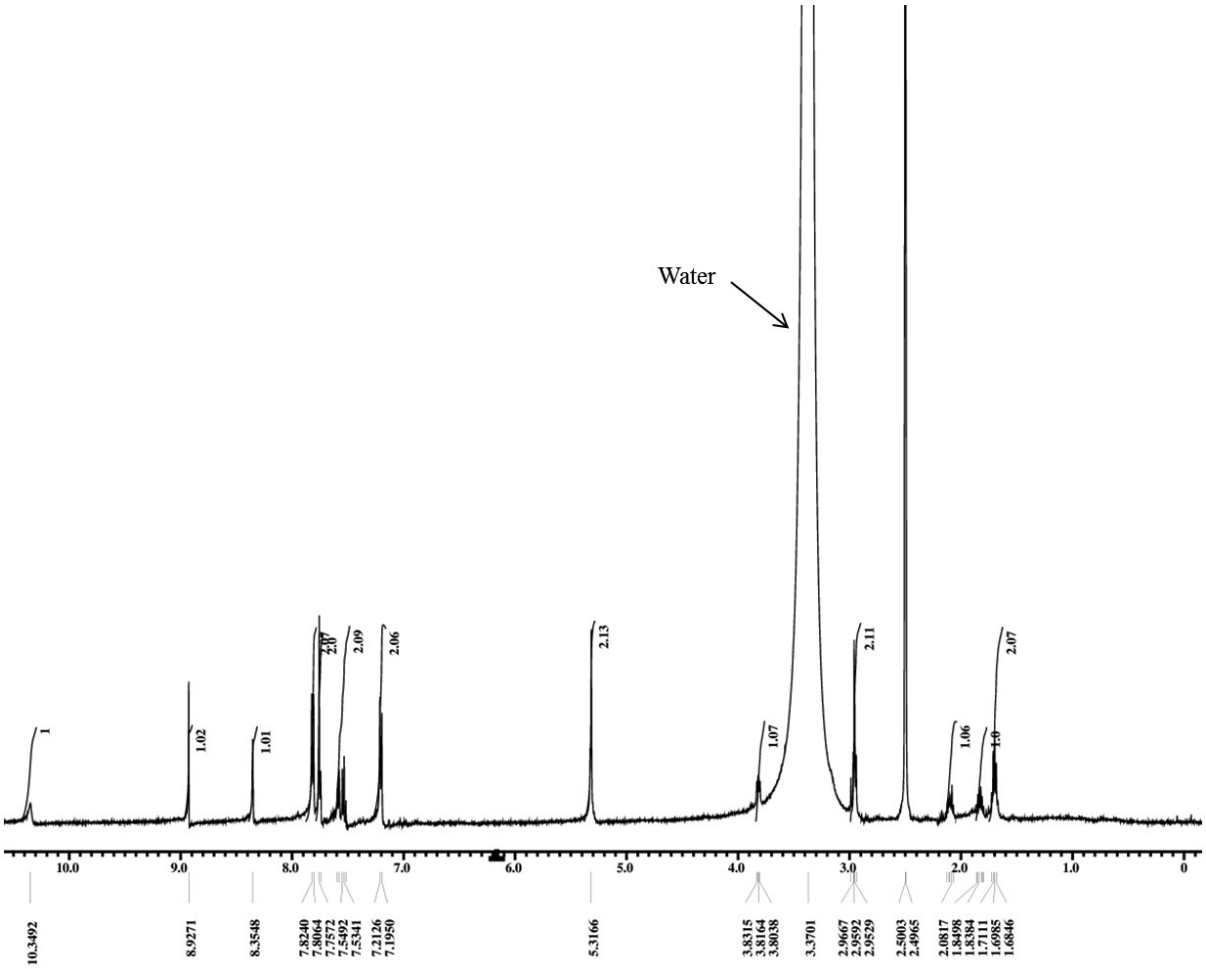
^{1}H and ^{13}C NMR of Pro-1:

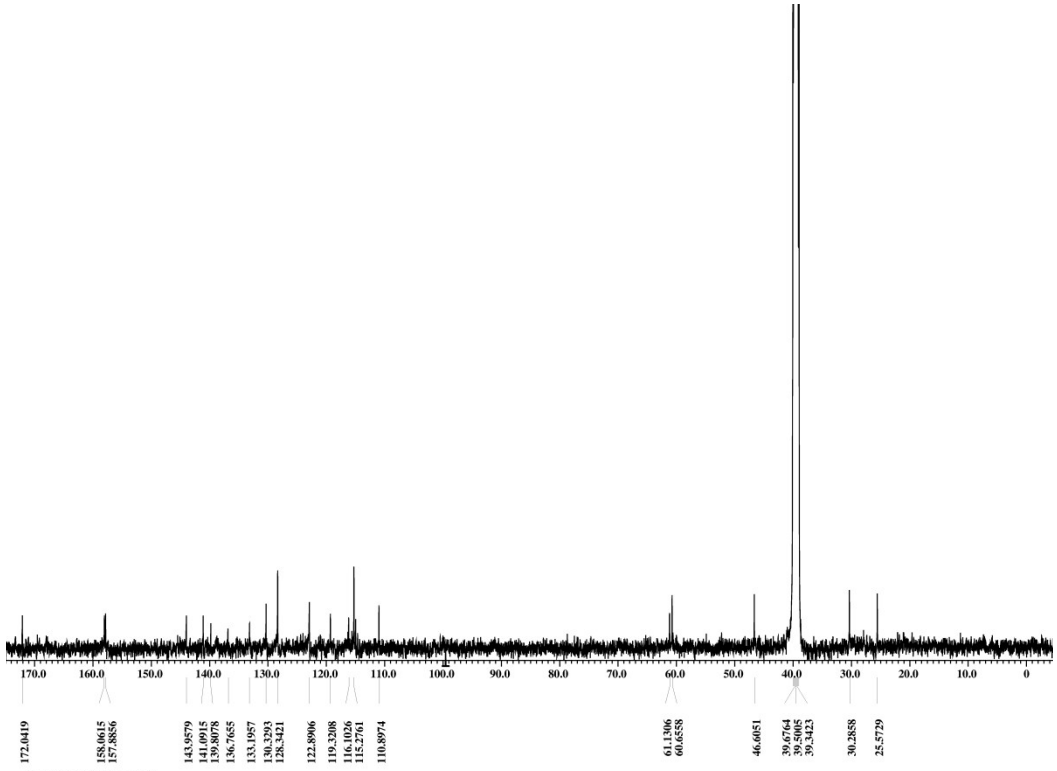


^{1}H and ^{13}C NMR of 10:



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





4.0 Fluorometric analysis

The binding constants were obtained from the gradual decrease of fluorescence intensity of 5′- or 3′-FAM labeled *c-MYC* upon addition of ligands **Pro-3** or **Pro-4** (Figure 3).[2] We monitored the formation of the **Ligand**:*c-MYC* complex. If there are n substantive binding sites of **Ligand** on *c-MYC* , the equilibrium may be written as:

$$n\mathit{Ligand} + \ c\text{ - }MYC \overset{K_a}{\rightarrow} c\text{ - }MYC :(\mathit{Ligand})_n \ \ldots\ldots\ldots\ldots\ldots(S1)$$

so, the binding constant,

$$K_a = {[c\text{ - }MYC :(\mathit{Ligand})_n]}\Big/{[c\text{ - }MYC]\ [\mathit{Ligand}]^n} \ \ldots\ldots\ldots\ldots\ldots\ldots(S2)$$

where [*c-MYC*], [**Ligand**] and [*c-MYC*: (**Ligand**)$_n$] are the concentrations of *c-MYC*, **Ligand** and the *c-MYC*: (**Ligand**)$_n$ complex, respectively. The relative fluorescence intensity is proportional to the concentration of labelled DNA. Thus,

$$[c\text{ - }MYC]/[c\text{ - }MYC]_0 = \ F/F_0 \ \ldots\ldots\ldots\ldots\ldots\ldots(S3)$$

where, [*c-MYC*]$_0$ denotes the total concentration of the free FAM-*c-MYC*, and F and F$_0$ are the fluorescence intensity of FAM-*c-MYC* with and without addition of **Ligand**, respectively.

From equation (S3),

$$\log\left[(F_0 - F)/F\right] = \log K_a + \mathrm{n}\log\left[\mathit{Ligand}\right] \ \ldots\ldots\ldots\ldots\ldots\ldots(S4)$$

The intercept of the linear plot of log [(F$_0$ – F)/F] vs. log [**Ligand**] gives log K$_a$ and the slope gives the value of n (**Figure 3 and Figure S1**). In our case, we find the value of n is close to 1. The K$_a$ was found to be 1.7×10^5 M^{-1} and 2.8×10^5 M^{-1} for **Pro-3** and **Pro-4** respectively.

[2] S. Ghosh, C. Ghosh, S. Nandi and K. Bhattacharyya, *Phys. Chem. Chem. Phys*., 2015, **17**, 8017.

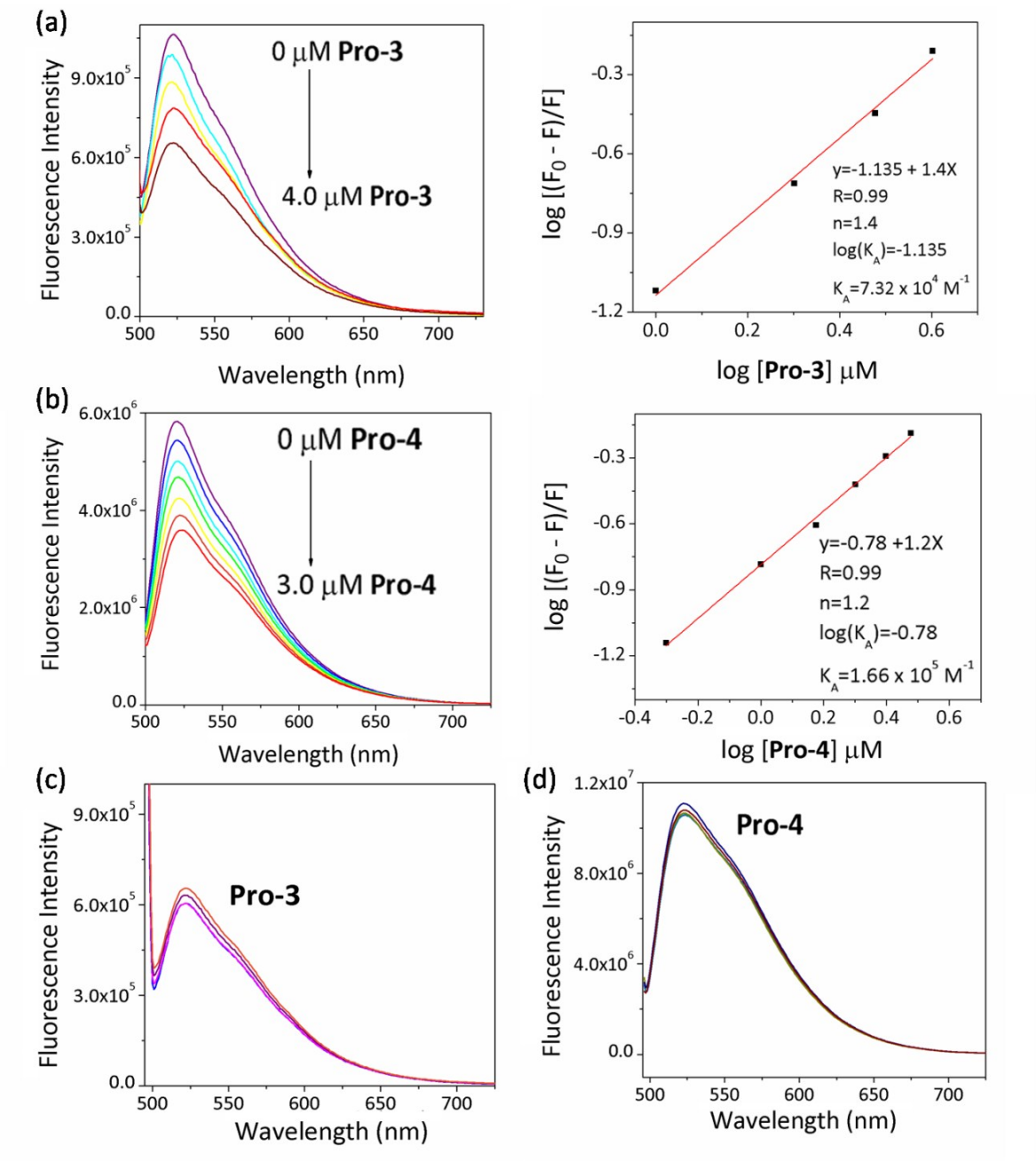


Fig S1. (a) Fluorescence emission spectra of the 3′-FAM labeled *c-MYC* with incremental addition of **Pro-3** (Left). Plot of log [(F_0 – F)/F] as a function of log [**Pro-3**] (Right); (b) Fluorescence emission spectra of the 3′-FAM labeled *c-MYC* with incremental addition of **Pro-4** (Left). Plot of log [(F_0 – F)/F] as a function of log [**Pro-4**] (Right); (c) Fluorescence emission spectra of the 3′-FAM labeled *ds* DNA with incremental addition of **Pro-3**; (d) Fluorescence emission spectra of the 3′-FAM labeled *ds* DNA with incremental addition of **Pro-4.**

5.0 CD spectroscopy

CD spectra are recorded on a JASCO J-815 spectrophotometer by using a 1 mm path length quartz cuvette. Aliquots of **Pro 4** were added stepwise to pre-annealed *c-MYC* ($TGAG_3TG_3TAG_3TG_3TA_2$) quadruplex sequence in Tris•HCl (100 mM) buffer at pH 7.4 containing KCl (100 mM). The DNA concentrations used were 10μM. The CD spectra represent an average of three scans and were smoothed and zero corrected. Final analysis and manipulation of the data was carried out by using Origin 8.0.

Circular dichroism (CD) spectroscopy was used to investigate the effect of **Pro-4** on the conformation of the *c-MYC* G-quadruplex. The CD spectrum of *c-MYC* sequence exhibits a characteristic positive peak at 265 nm and a negative peak at 243 nm for parallel G-quadruplex structure in buffer containing 100 mM KCl and 10 mM Tris·HCl (pH 7.4). Upon addition of 30 μM of ligand **Pro-4**, only a slight increase in ellipticity at 243 nm and a slight decrease in elliptic intensity at 265 nm were observed. This suggests that binding of **Pro-4** does not significantly alter the *c-MYC* parallel G-quadruplex structure (Fig. S1, Supporting Information, ESI).

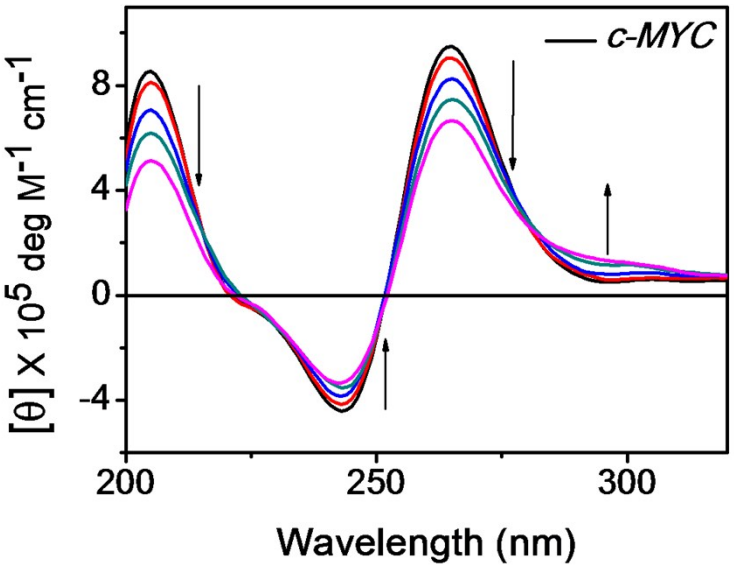


Fig S2. CD spectra of titration experiments of **Pro-4** with 10 μM *c-MYC* G-quadruplex in buffer containing 100 mM KCl and 10 mM tris·HCl at pH 7.4.

6.0 Atomic force microscopy

A mixture of **Pro 4** (10μL, 10μM) and preannealed *c-MYC* quadruplex (10μM) in Tris·HCl (50 mm, pH 7.4) and KCl (100 μM) buffer was incubated for 10 min and then deposited on freshly cleaved mica plates (Agar Supplies) and dried with nitrogen gas for 12 h. The molecules deposited on the mica surfaces were washed with distilled water and then dried carefully under a nitrogen atmosphere prior to imaging. AFM experiments were carried out on an NT-MDT instrument in semicontact mode with a resonance frequency of 120 kHz. The obtained images were analyzed by using the Image Analysis 3.5.0.2060 program (NT-MDT). Under this experimental setup, both the *c-MYC* and the ligand **Pro 4** were independently imaged to give spherical nanoparticles.

7.0 Molecular modeling

Molecular modeling: Molecular docking studies were performed using the Auto-Dock 4.0 program[3] for the quadruplex-ligand interactions. The Protein Data Bank file (PDB ID: 1XAV) [4] was considered as the *c-MYC* quadruplex DNA for docking studies. Gaussian 03 program,[5] B3LYP/6-31+G(d) basis set[6] used for the DFT analysis of **Pro-4** to obtain the

[3] G. M. Morris, D. S. Goodsell, R. S. Halliday, R. Huey, W. E. Hart, R. K. Belew and A. J. Olson, *J. Comput. Chem.,* 1998, **19**, 1639.

[4] A. Ambrus, D. Chen, J. Dai, R. A. Jones and D. Yang, *Biochemistry*, 2005, **44**, 2048.

[5] Gaussian03 program: M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A., Jr. Montgomery, T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez and J. A. Pople, Gaussian 03, revision D.02; Gaussian, Inc.: Wallingford, CT, **2004.**

[6] B3LYP hybrid functional: (a) R. G. Parr, W. Yang, Density Functional Theory of Atoms and Molecules; Oxford University Press: New York, 1989; (b) C. Lee, W. Yang and R. G. Parr, *Phys. Rev. B* 1988, **37**, 785; (c) A. D. Becke, *Phys. Rev. A* 1988, **38**, 3098-3100; (d) A.

energy minimized structures. Next Lamarckian genetic algorithm (LGA) with the default parameters from AutoDock 4.0 program was employed for the docking calculations of the energy minimized structures of **Pro-4**. A maximum of 25 million energy evaluations was applied for the experiment. The results were clustered using a tolerance of 2.0 Å and 10 lowest potential energy structures were collected from the experiment. Chimera 1.6.2 software was used to image docked **Pro-4.***c-MYC* complex structure.

8.0 MTT assay

The human hepatocellular liver carcinoma cells (HepG2) and mouse normal myoblast cells (C2C12) were cultured in DMEM containing high glucose (5.5 mM) supplemented with 10% FBS at pH 7.4. Cells were maintained in tissue culture plates containing 4×10^5 cells/well at 37°C in an atmosphere of 5% carbon dioxide (CO_2)/95% air. The MTT cell proliferation assay determines the ability of living cells to reduce the yellow tetrazole, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to purple formazan crystals by mitochondrial enzymes. For the MTT assay, HepG2 cancer cells were treated with various concentrations of prolinamide derivatives **Pro 3** and **Pro 4** for 24 h. Following incubation with each compound for 24 h, 20 μLof MTT was added (at a concentration of solution 5 mg/mL in phosphate-buffered saline, pH 7.4.) to each well. After incubation for 4 h at 37 °C, the culture medium was removed, and the formazan crystals were dissolved in 200 μL DMSO. Absorbance (A) of formazan dye was measured at 570 nm using a microplate reader. The background absorbance was determined at 690 nm and subtracted from the 570 nm measurement.

The percentage of viable cells was determined by the equation (3):

$$Viablecells(\%) = \frac{A\,of treatedcells}{A\,of untreatedcells} \times 100 \ldots\ldots (3)$$

Following the similar protocol, C2C12 wastreated with various concentrations of **Pro 3** or **Pro 4** and the percentage of viable cells was determined.

D. Becke, *J. Chem. Phys.* 1993, **98**, 5648.

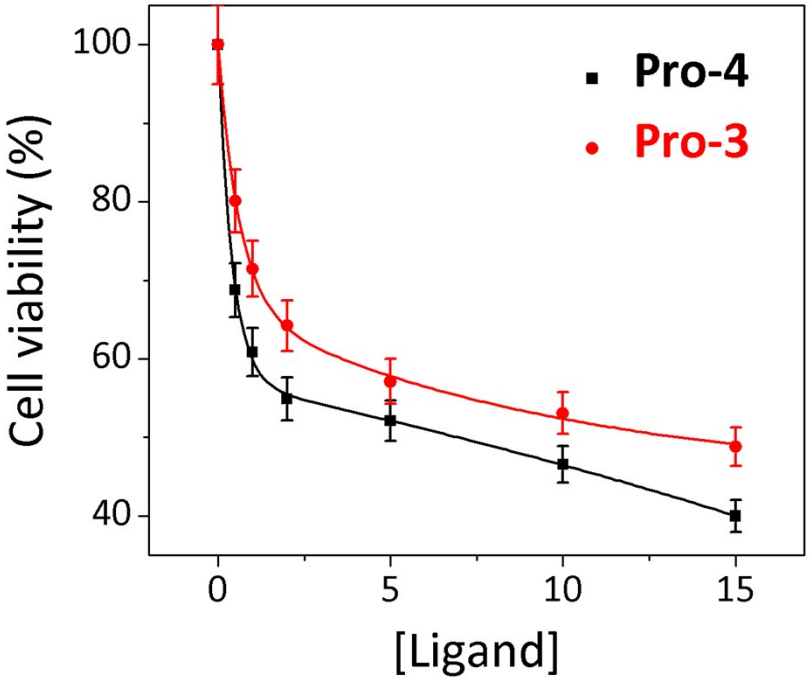


Figure S3. Representative plots for the cell viability (MTT assay) in HepG2 cells with increasing concentration of **Pro-3** and **Pro-4.**