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

Phenanthroline polyazamacrocycles as G-quadruplex DNA binders

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Synthesis

[16]phenN_4: Yield 92%. m.p. 208–209ºC. ^1H NMR (600 MHz, D_2O): δ (ppm) 1.45 (m, 4H, $NCH_2CH_2C\mathit{H}_2$), 1.81 (m, 4H, $NCH_2C\mathit{H}_2CH_2$), 3.23 (m, 4H,$NC\mathit{H}_2CH_2CH_2$), 4.66 (s, 4H, phen$C\mathit{H}_2N$), 7.78 (d, 2H, phen), 7.99 (s, 2H, phen) and 8.53 (d, 2H, phen); ^{13}C NMR (150 MHz, D_2O): δ (ppm) 25.46 ($NCH_2CH_2\mathit{C}H_2$), 25.65 ($NCH_2\mathit{C}H_2CH_2$), 47.59 ($N\mathit{C}H_2CH_2CH_2$), 51.64 (phen$\mathit{C}H_2N$), 123.02, 127.07, 129.10, 138.88, 144.12 and 151.37 (phen). HRMS-ESI *m*/*z* calcd for $C_{20}H_{25}N_4$ $[L+H]^+$ 321.2074, found 321.2128.

[32]phen$_2$$N_4$: Yield 70%. m.p. 201–202°C. ^1H NMR (600 MHz, D_2O): δ (ppm) 1.37 (m, 8H, $NCH_2CH_2C\mathit{H}_2$), 1.73 (m, 8H, $NCH_2C\mathit{H}_2CH_2$), 3.16 (m, 8H, $NC\mathit{H}_2CH_2CH_2$), 4.61 (s, 8H, phen$C\mathit{H}_2N$), 7.73 (d, 4H, phen), 7.88 (s, 4H, phen) and 8.45 (d, 4H, phen); ^{13}C NMR (150 MHz, D_2O): δ (ppm) 25.39 ($NCH_2CH_2\mathit{C}H_2$), 25.58 ($NCH_2\mathit{C}H_2CH_2$), 47.68 ($N\mathit{C}H_2CH_2CH_2$), 51.22 (phen$\mathit{C}H_2N$), 123.31, 127.21, 129.01, 139.49 ,144.12 and 151.12 (phen)). HRMS-ESI *m*/*z* calcd for $C_{40}H_{49}N_8$ $[L+H]^+$ 641.4075, found 641.4088.

[30]phen$_2$$N_6$: Yield 40%. m.p. 224–225°C. ^1H NMR (600 MHz, D_2O): δ (ppm) 3.34 (t, 8H, $NCH_2C\mathit{H}_2N$), 3.53 (t, 8H, $NC\mathit{H}_2CH_2N$), 4.65 (s, 8H, phen$C\mathit{H}_2N$), 7.46 (d, 4H, phen), 7.81 (s, 4H, phen), 8.18 (d, 4H, phen); ^{13}C NMR (150 MHz, D_2O): δ (ppm) 43.81 ($NCH_2\mathit{C}H_2N$), 45.45 ($N\mathit{C}H_2CH_2N$), 51.18 (phen$\mathit{C}H_2N$), 67.83 (dioxane), 122.15, 127.33, 128.84, 139.07, 143.44, 151.35 (phen). HRMS-ESI *m*/*z* calcd for $C_{36}H_{43}N_{10}$ $[L+H]^+$ 615.3667, found 615.3708.

[34]phen$_2$$N_6$: Yield: 50%; m.p. 227–228 ºC; ^1H NMR (600 MHz, D_2O, 25 ºC): d=2.31 (m, 8H; $NCH_2CH_2CH_2N$), 3.27 (t, 16H; $NCH_2CH_2CH_2N$), 3.43 (t, 16H; $NCH_2CH_2CH_2N$), 4.71 (s, 8H; phenCH_2N), 7.82 (d, 4H; phen), 8.01 (s, 4H; phen), 8.56 ppm (d, 4H; phen); ^{13}C NMR (150 MHz, D_2O): δ (ppm) 44.26 ($NCH_2CH_2CH_2N$), 44.52 ($NCH_2CH_2CH_2N$), 44.72 ($NCH_2CH_2CH_2N$), 48.99 (methanol), 52.08 (phenCH_2N), 67.97 (dioxane), 123.43, 127.26, 129.32, 139.32, 144.32, 151.09 ppm (phen). HRMS-ESI *m*/*z* calcd for $C_{40}H_{51}N_{10}$ $[L+H]^+$ 671.4293, found 671.4341.

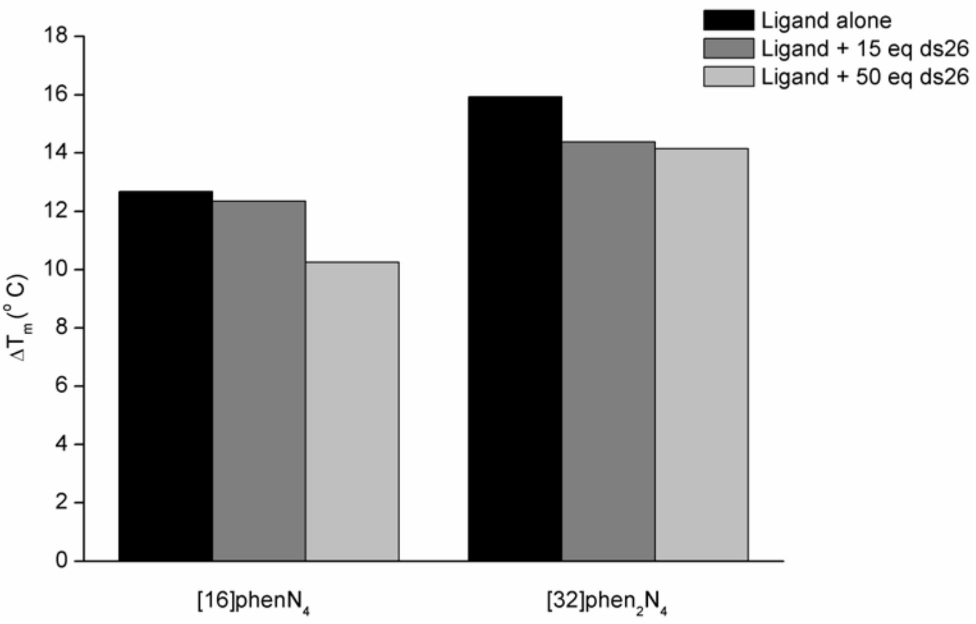


Fig. S1 FRET-melting stabilization by [16]phenN_4 and [32]phen$_2N_4$ (1 μM) of the labelled F21T sequence (0.2 μM) in buffer 10 mM KCl + 90 mM LiCl and in the presence of increasing concentrations of competitor ds26 (0, 15 and 50 equivalents).

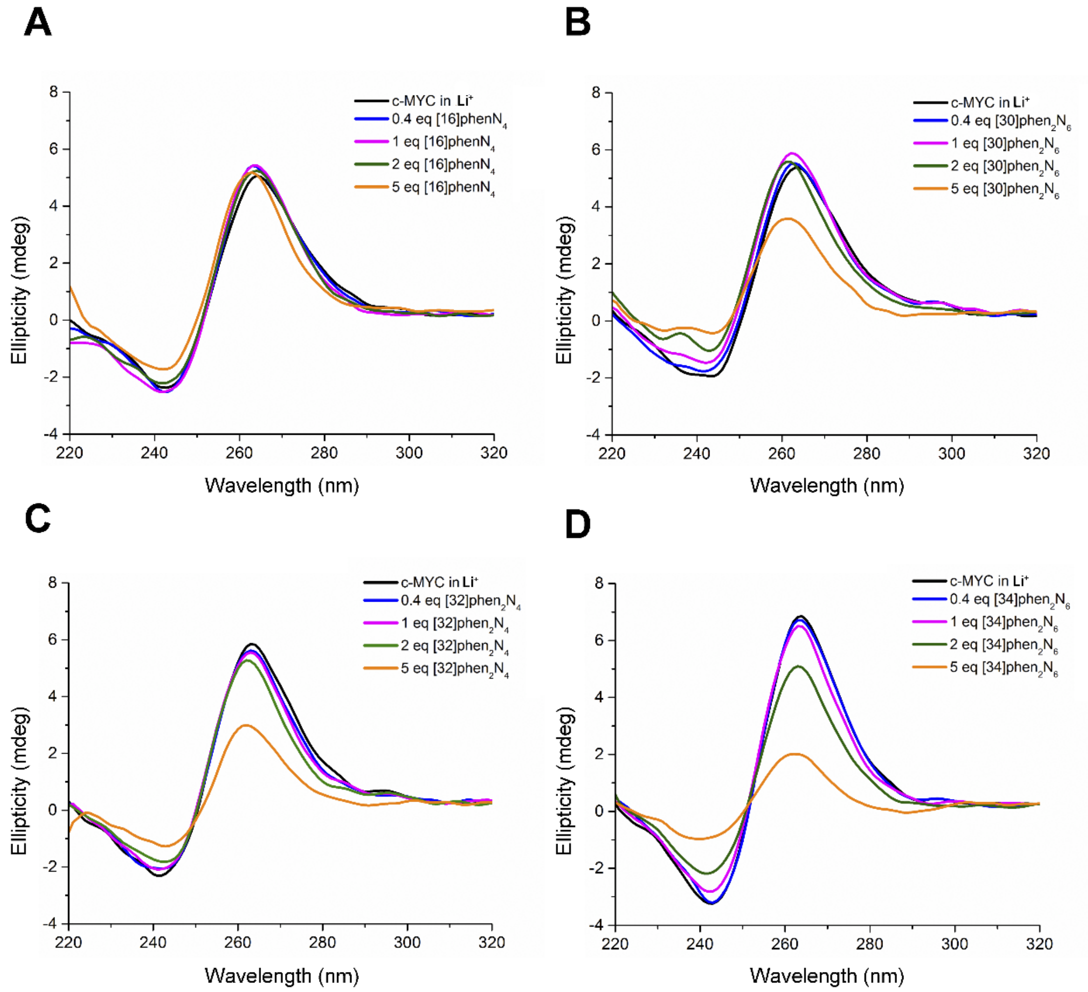


Fig. S2 CD titration spectra of (A) [16]phenN_4, (B) [30]phen$_2N_6$, (C) [32]phen$_2N_4$ and (D) [34]phen$_2N_6$ with c-MYC G4 in 100 mM LiCl. Some of the intermediate spectra were removed for better clarity.

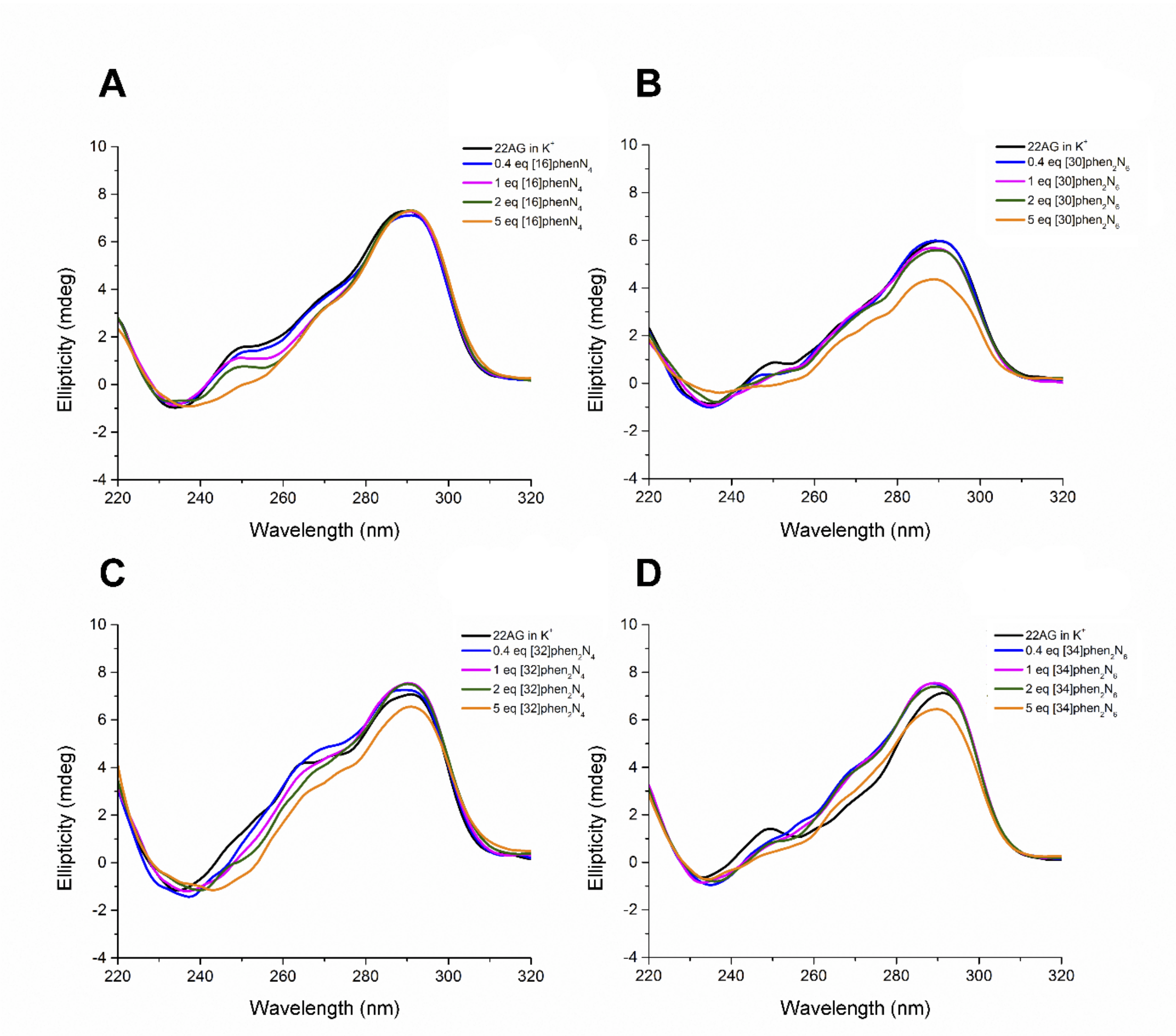


Fig. S3 CD titration spectra of (A) [16]phenN_4, (B) [30]$phen_2N_6$, (C) [32]$phen_2N_4$ and (D) [34]$phen_2N_6$ with 22AG G4 in 10 mM KCl + 90 mM LiCl. Some of the intermediate spectra were removed for better clarity.

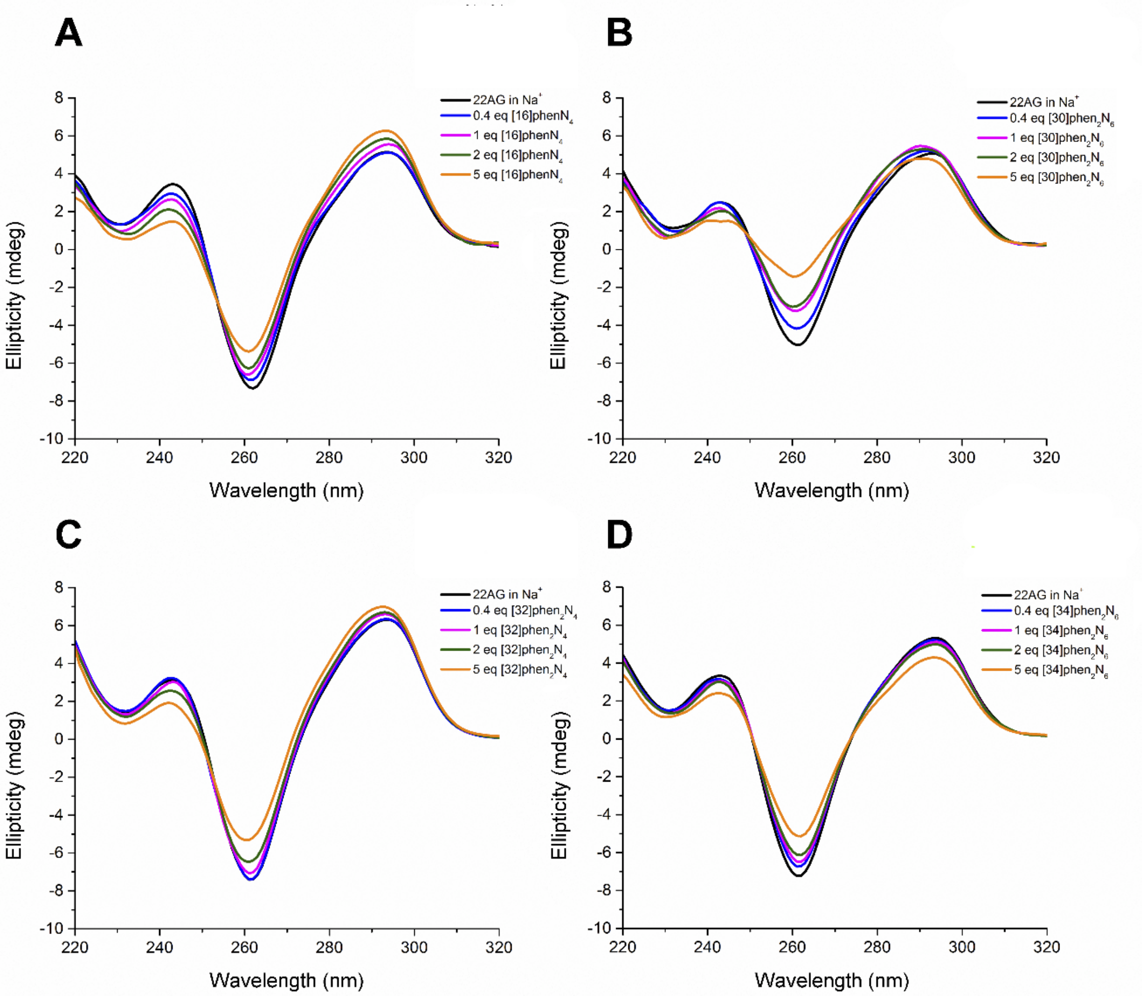


Fig. S4 CD titration spectra of (A) [16]phenN_4, (B) [30]phen$_2N_6$, (C) [32]phen$_2N_4$ and (D) [34]phen$_2N_6$ with 22AG G4 in 100 mM NaCl. Some of the intermediate spectra were removed for better clarity.

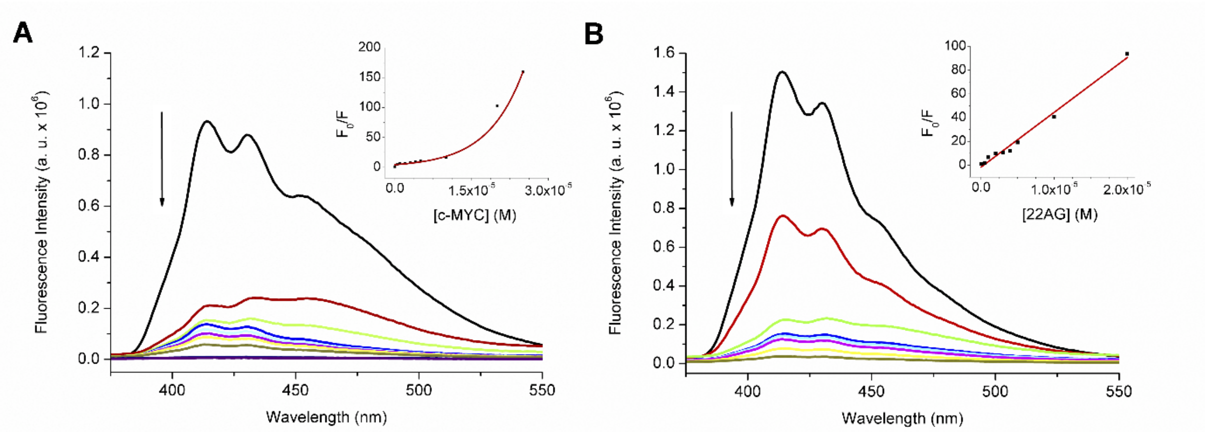


Fig S5 Fluorescence titrations of the ligand [16]phenN_4 with increasing concentration of (A) c-MYC and (B) 22AG G4s [5 μM in 100 mM KCl, pH 7.2, concentrations 0–25 μM]. Insets: Stern-Volmer plots obtained by plotting F_0/F as a function of DNA concentration.

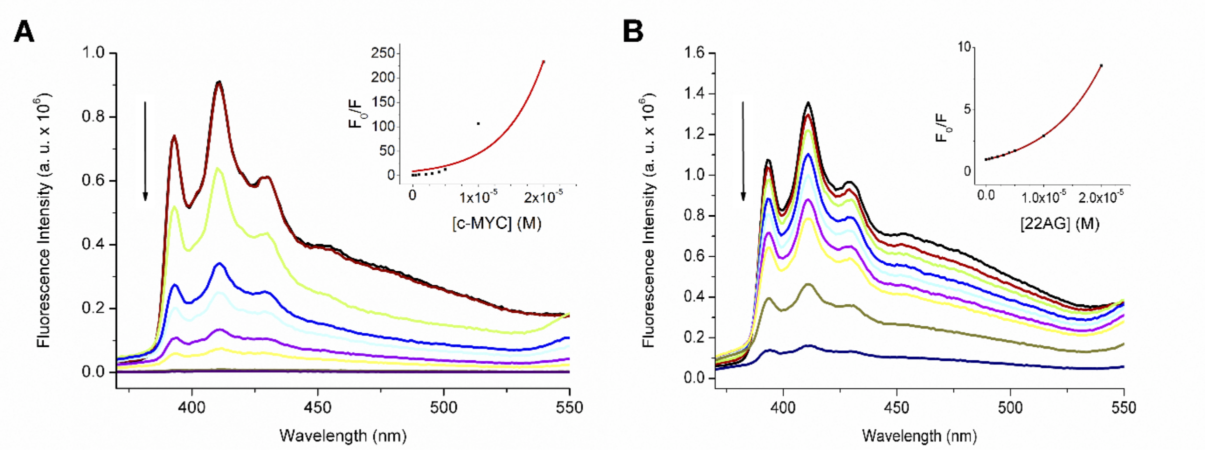


Fig S6 Fluorescence titrations of the ligand [30]phen$_2N_6$ with increasing concentration of (A) c-MYC and (B) 22AG G4s [5 μM in 100 mM KCl, pH 7.2, concentrations 0–25 μM]. Insets: Stern-Volmer plots obtained by plotting F_0/F as a function of DNA concentration.

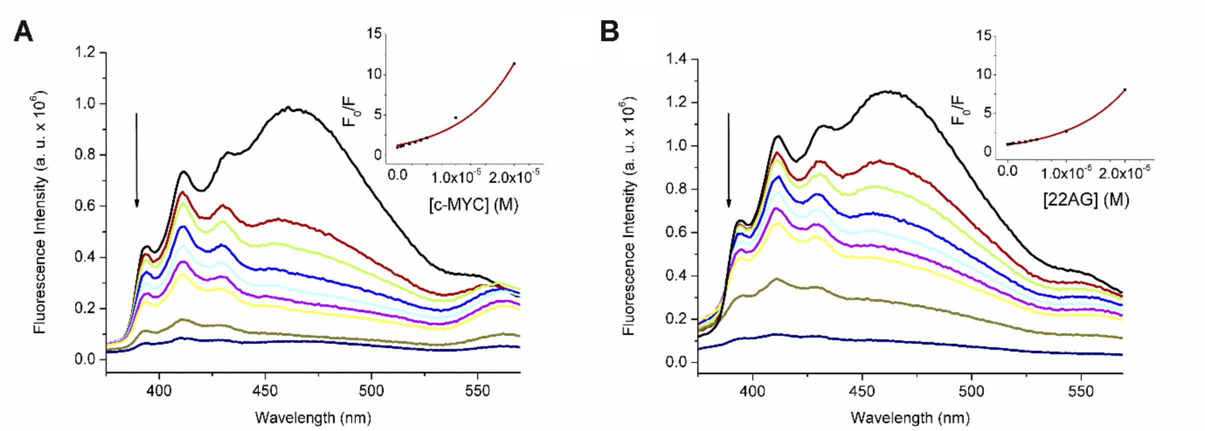


Fig S7 Fluorescence titrations of the ligand [34]phen$_2N_6$ with increasing concentration of (A) c-MYC and (B) 22AG G4s [5 μM in 100 mM KCl, pH 7.2, concentrations 0–25 μM]. Insets: Stern-Volmer plots obtained by plotting F_0/F as a function of DNA concentration.

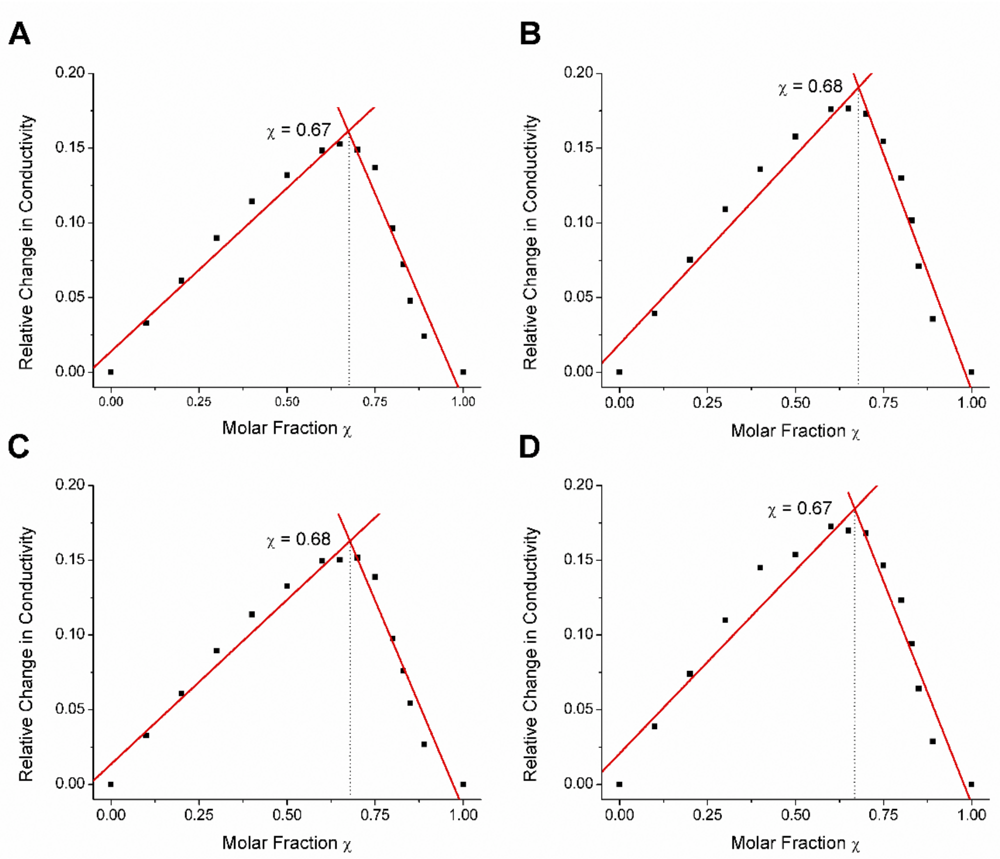
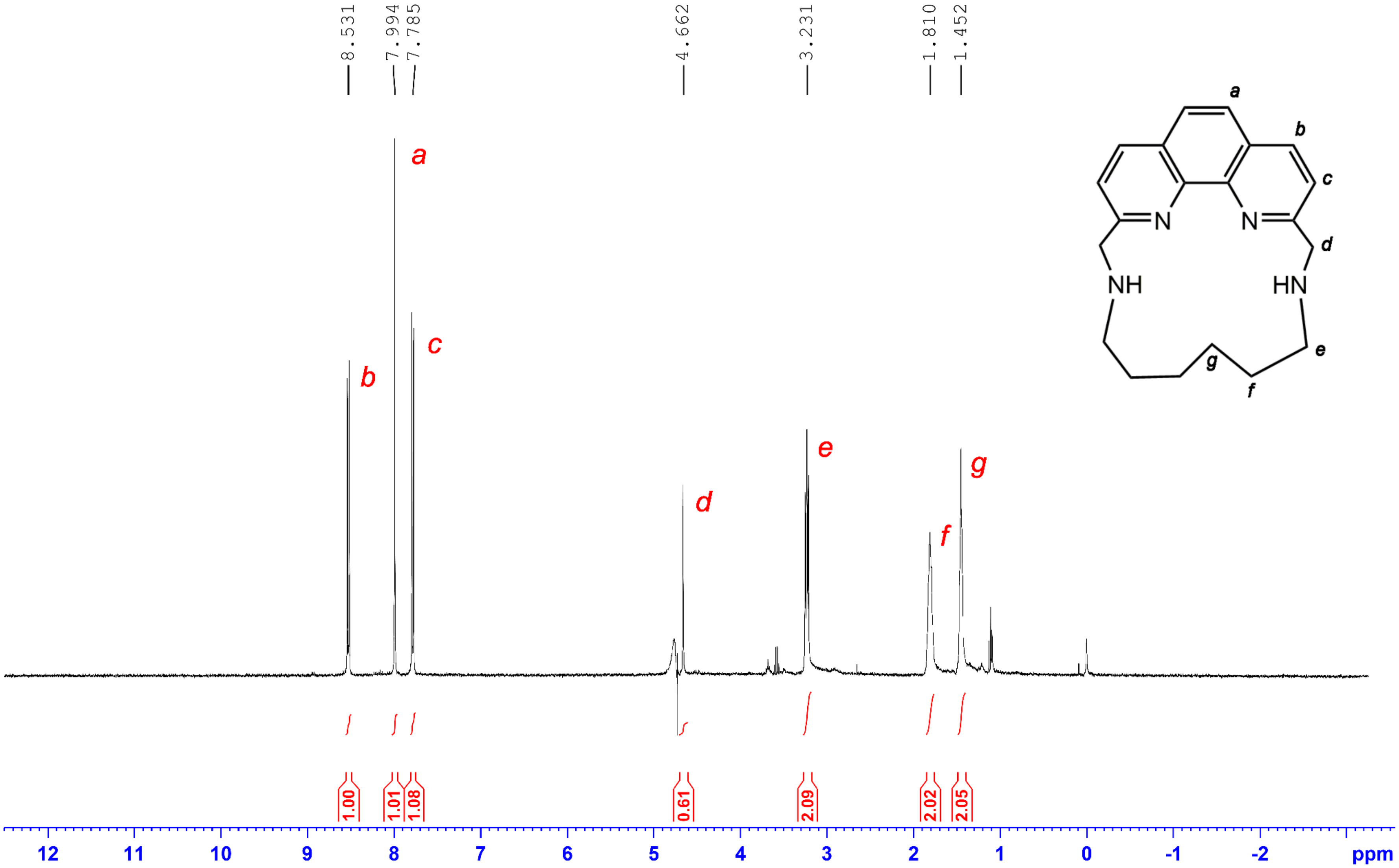
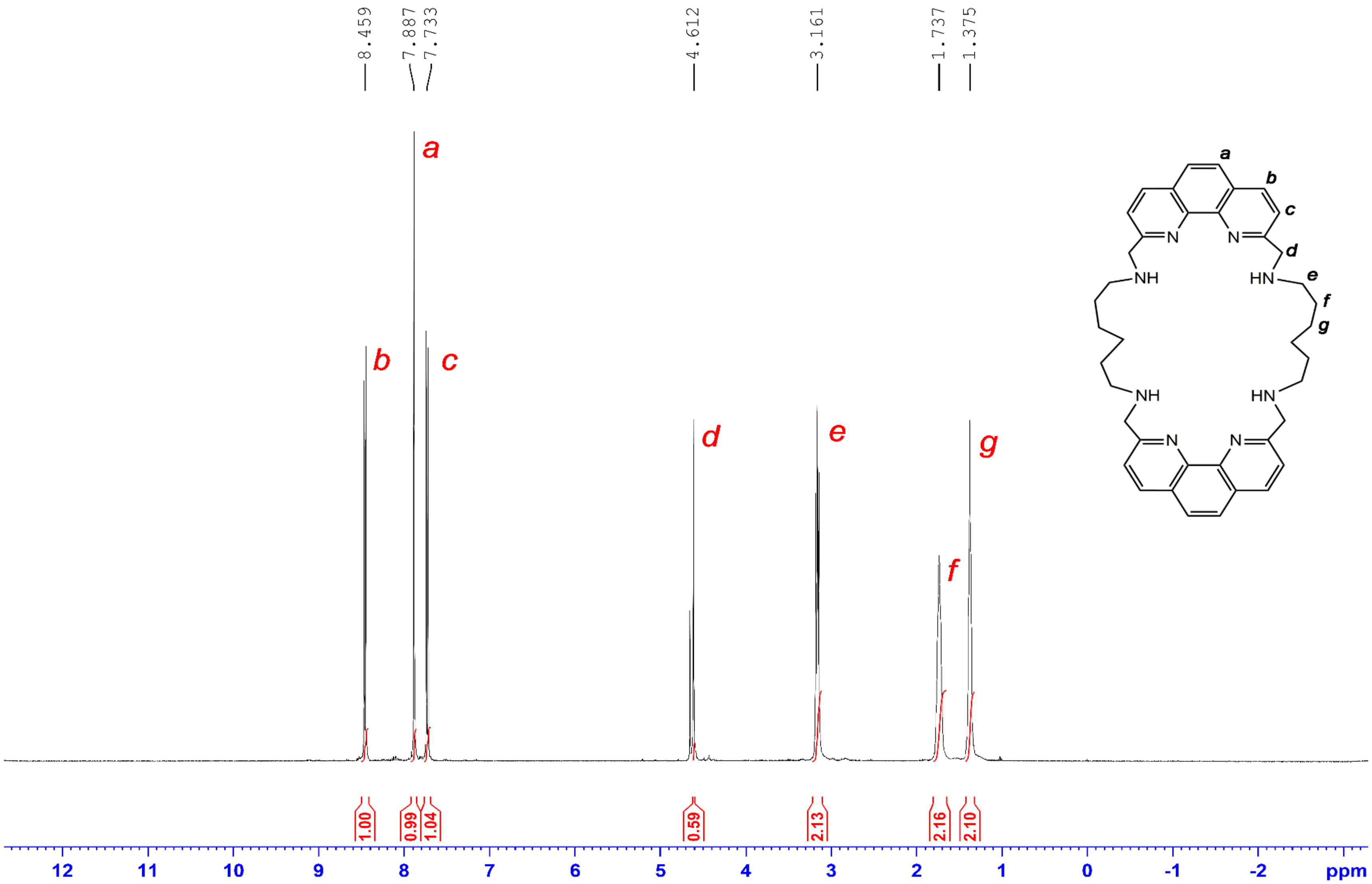


Fig. S8 Job plots resulting from the method of continuous variation analysis for (A) ligand [16]phenN_4 with c-MYC G4, (B) ligand [16]phenN_4 with 22AG G4, (C) [32]phen$_2N_4$ with c-MYC G4 and (D) [32]phen$_2N_4$ with 22AG G4.

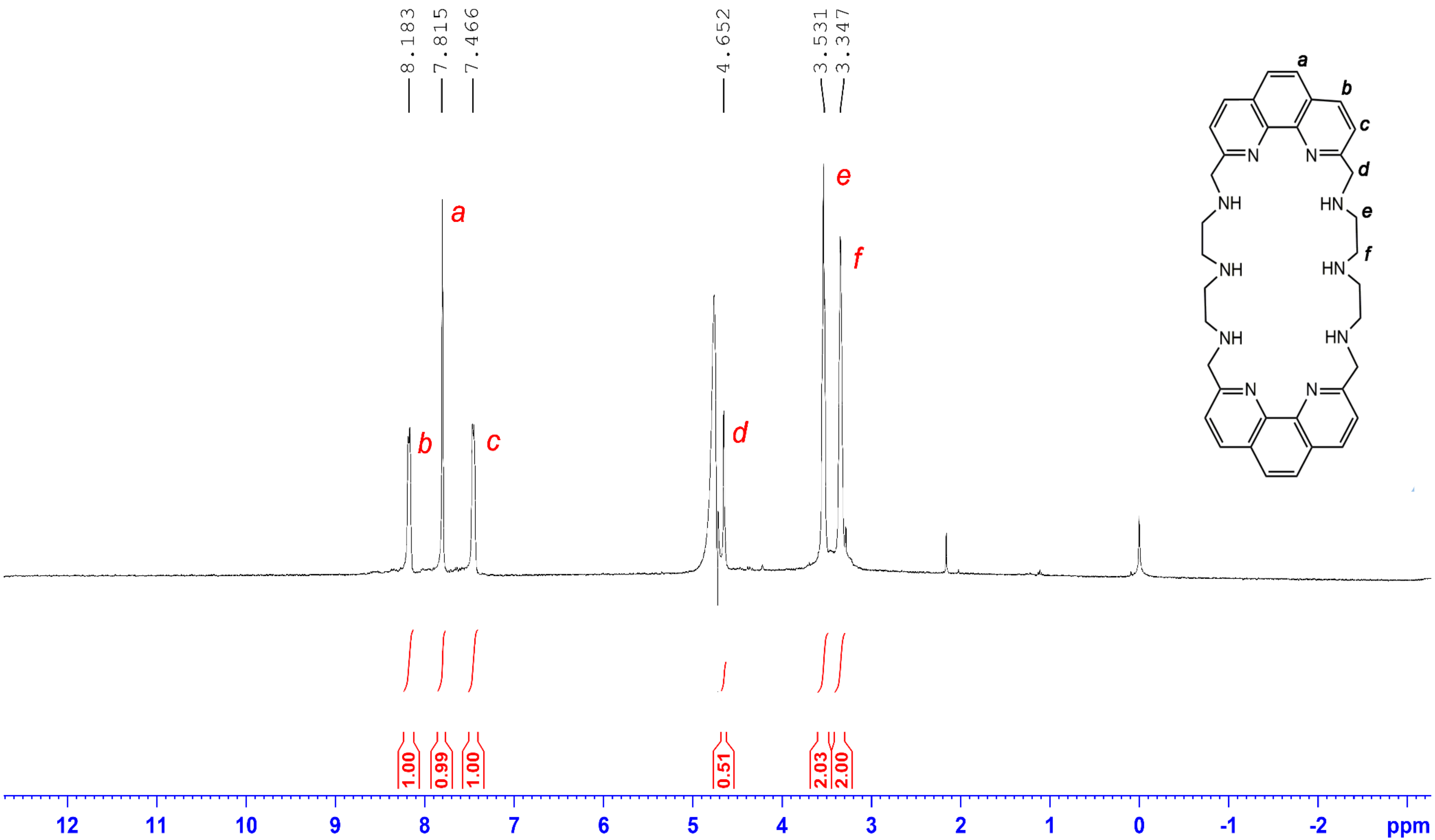
(A)


8.531
7.994
7.785
4.662
3.231
1.810
1.452
a
b
c
d
e
f
g
N
N
NH
HN
1.00
1.01
1.08
0.61
2.09
2.02
2.05
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
ppm

(B)


8.459
7.887
7.733
4.612
3.161
1.737
1.375
a
b
c
d
e
f
g
1.00
0.99
1.04
0.59
2.13
2.16
2.10
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
ppm
N
NH
HN

(C)



8.183
7.815
7.466
4.652
3.531
3.347
a
b
c
d
e
f
1.00
0.99
1.00
0.51
2.03
2.00
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
ppm
N
HN
NH

(D)

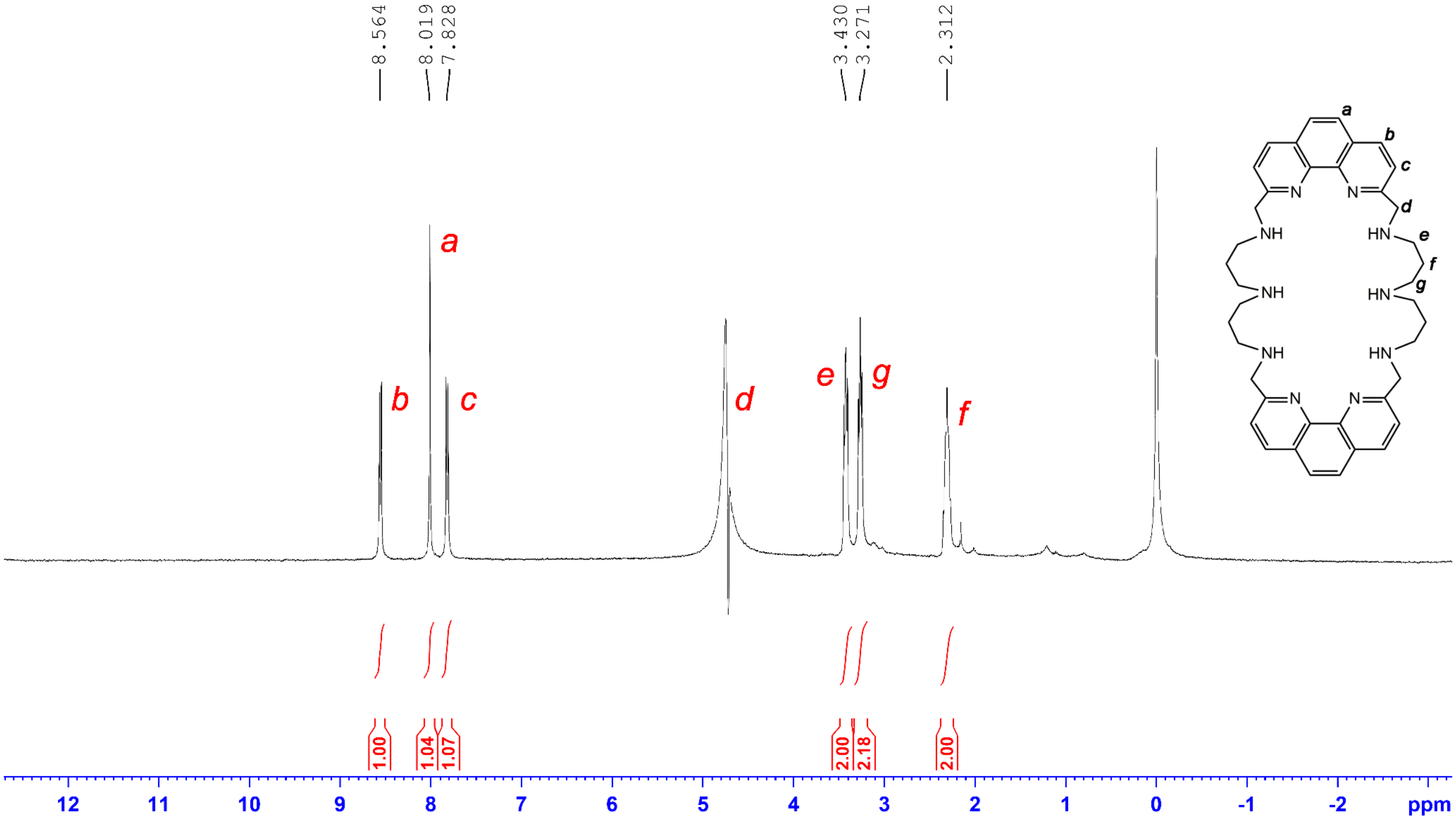
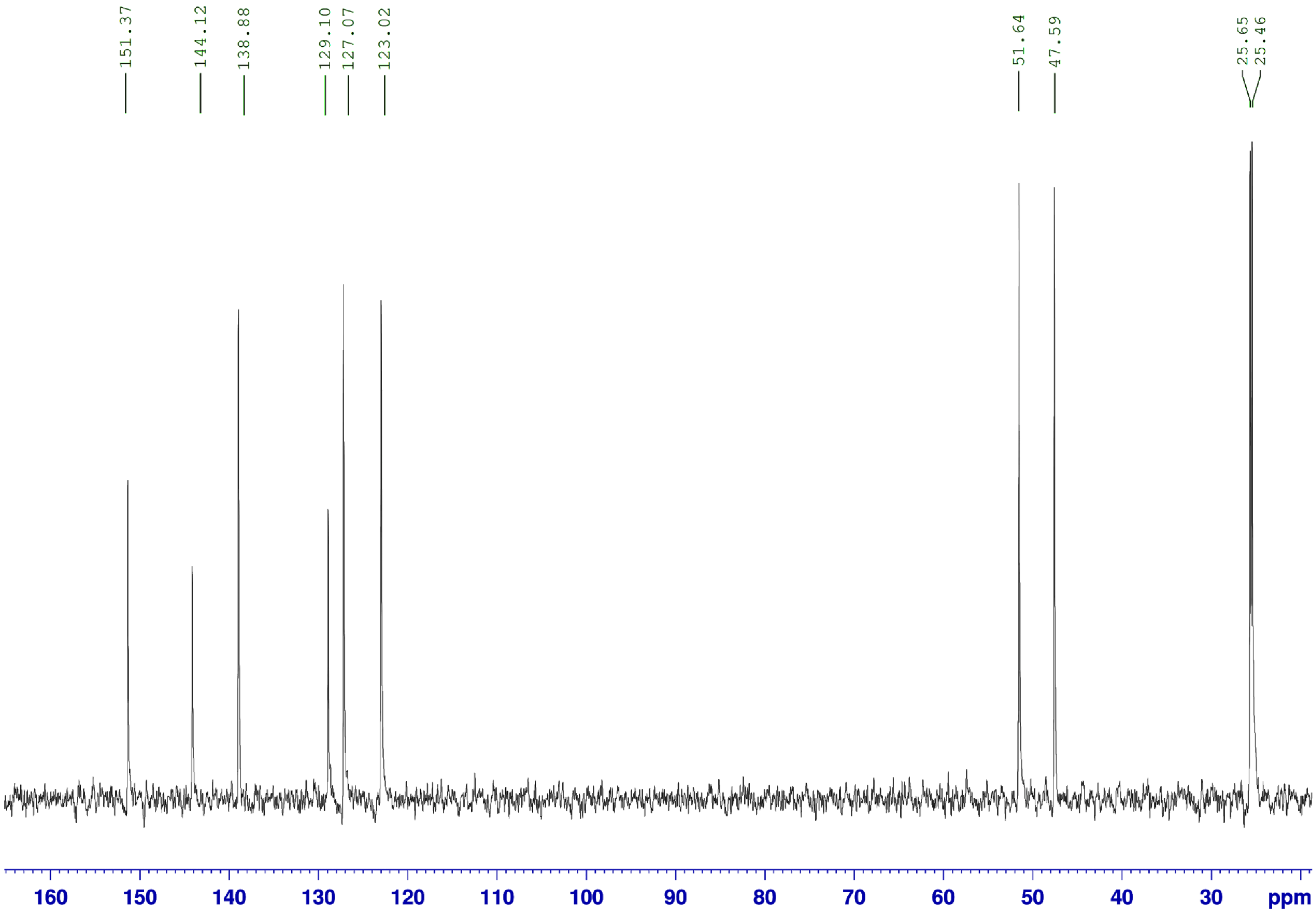
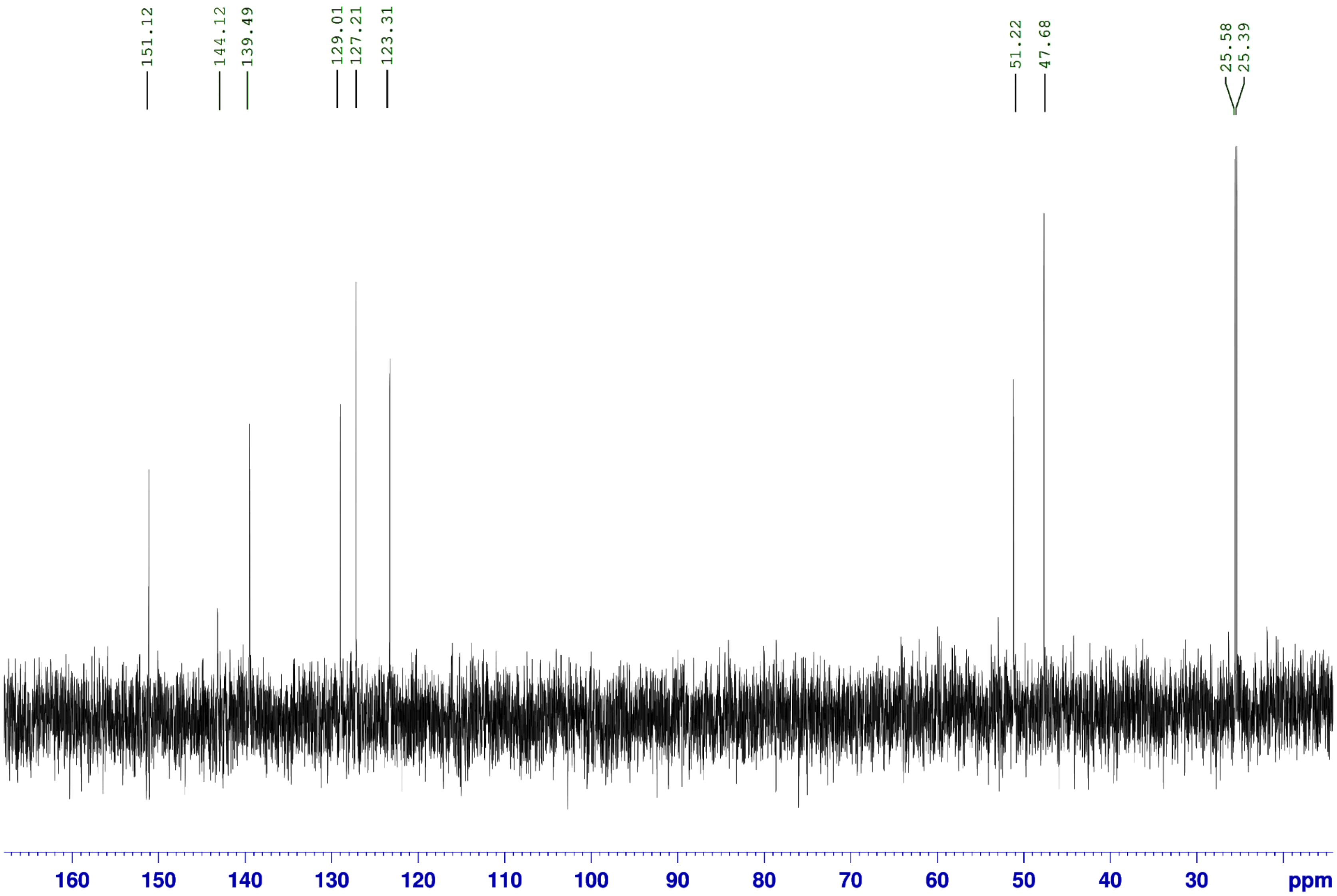


Fig. S9- ^{1}H NMR spectra of the ligands (A) [16]phenN_4, (B) [32]phen$_2$$N_4$ (C) [30]phen$_2$$N_6$ and (D) [34]phen$_2$$N_6$ (protons d are underneath the water signal and could not be assigned).

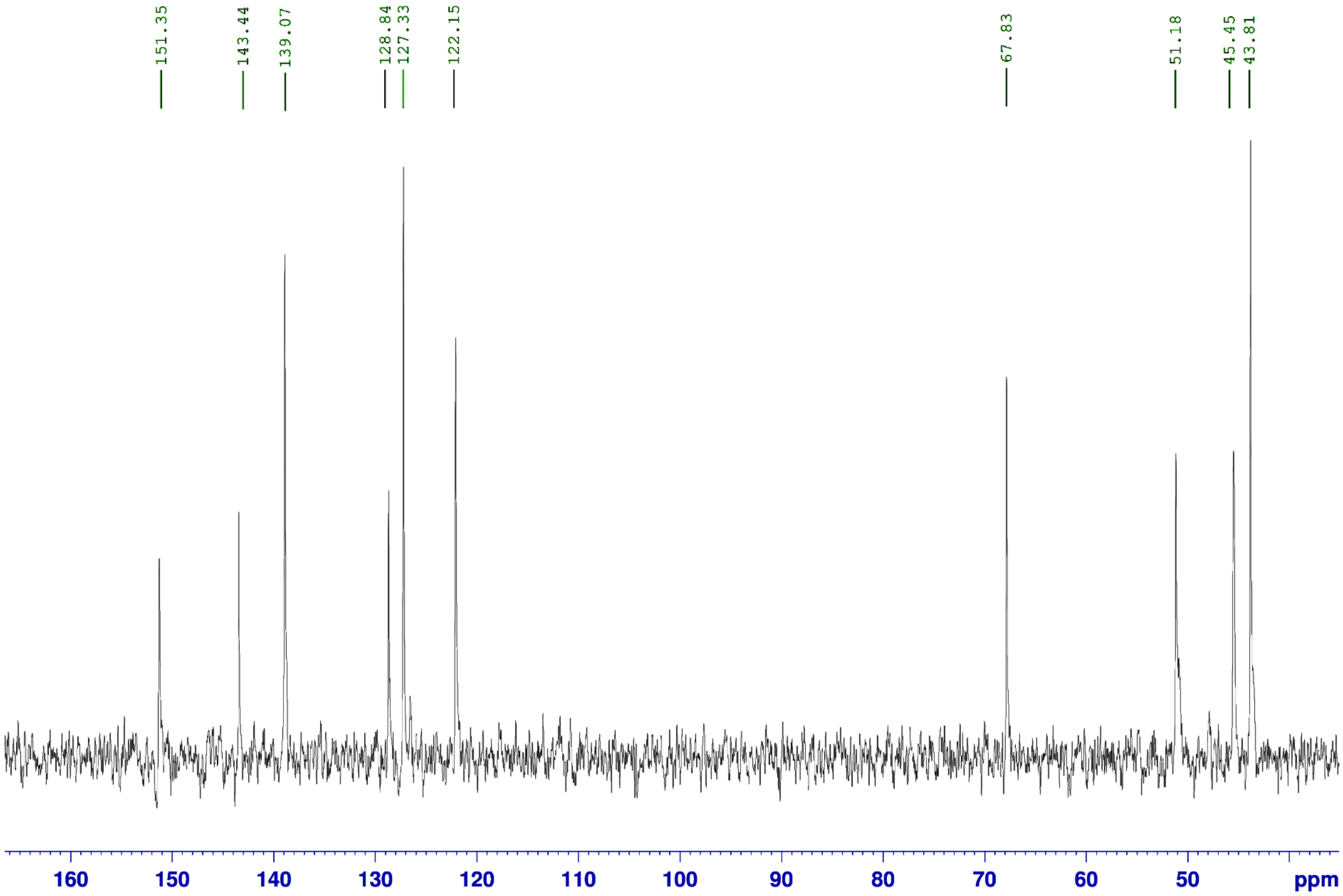
(A)


151.37
144.12
138.88
129.10
127.07
123.02
51.64
47.59
25.65
25.46
160
150
140
130
120
110
100
90
80
70
60
50
40
30
ppm

(B)


151.12
144.12
139.49
129.01
127.21
123.31
51.22
47.68
25.58
25.39
160
150
140
130
120
110
100
90
80
70
60
50
40
30
ppm

(C)


151.35
143.44
139.07
128.84
127.33
122.15
67.83
51.18
45.45
43.81
160
150
140
130
120
110
100
90
80
70
60
50
ppm

(D)

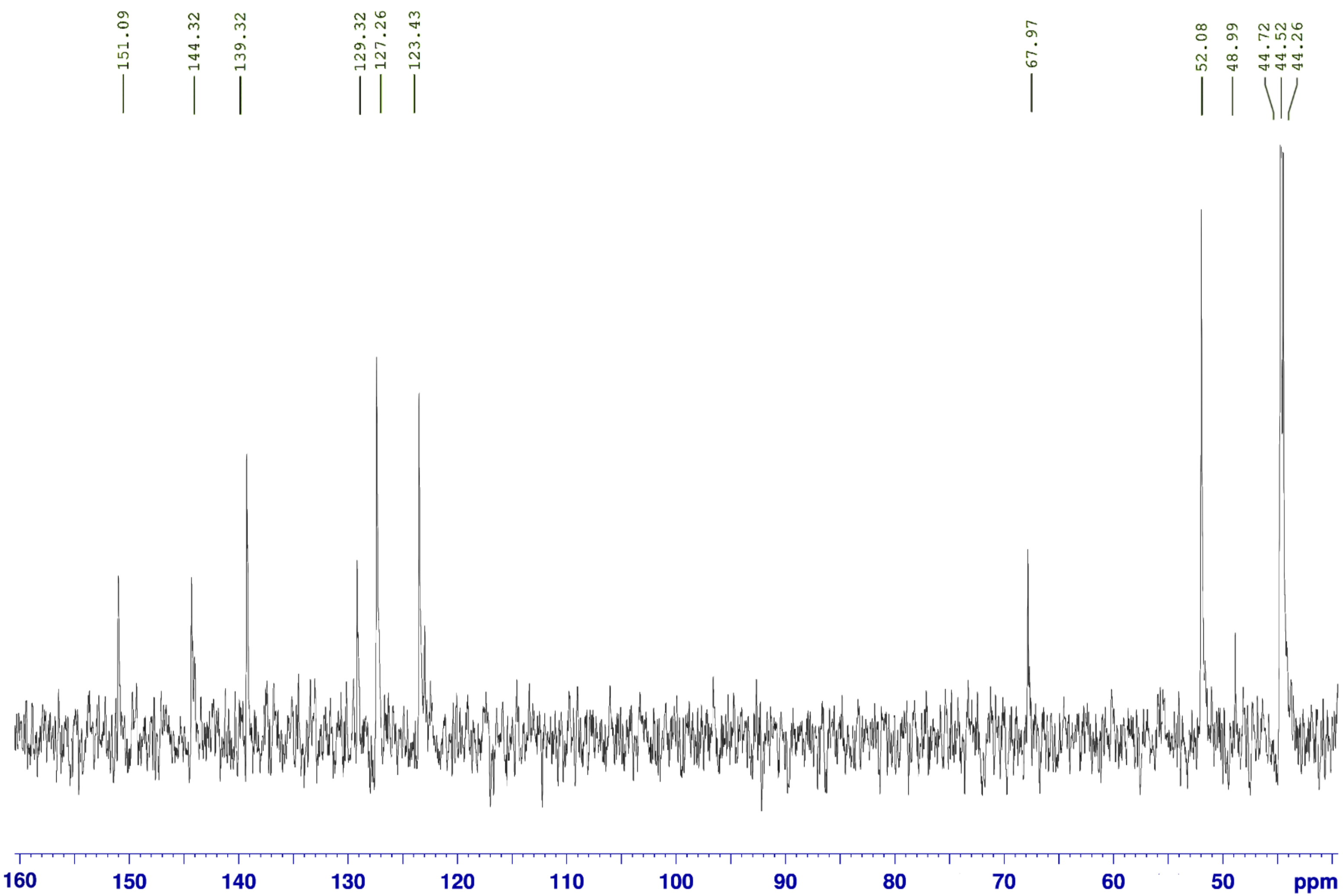


Fig. S10- ^{13}C NMR spectra of the ligands (A) [16]phenN_4, (B) [32]phen$_2N_4$ (C) [30]phen$_2N_6$ and (D) [34]phen$_2N_6$.