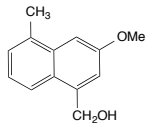
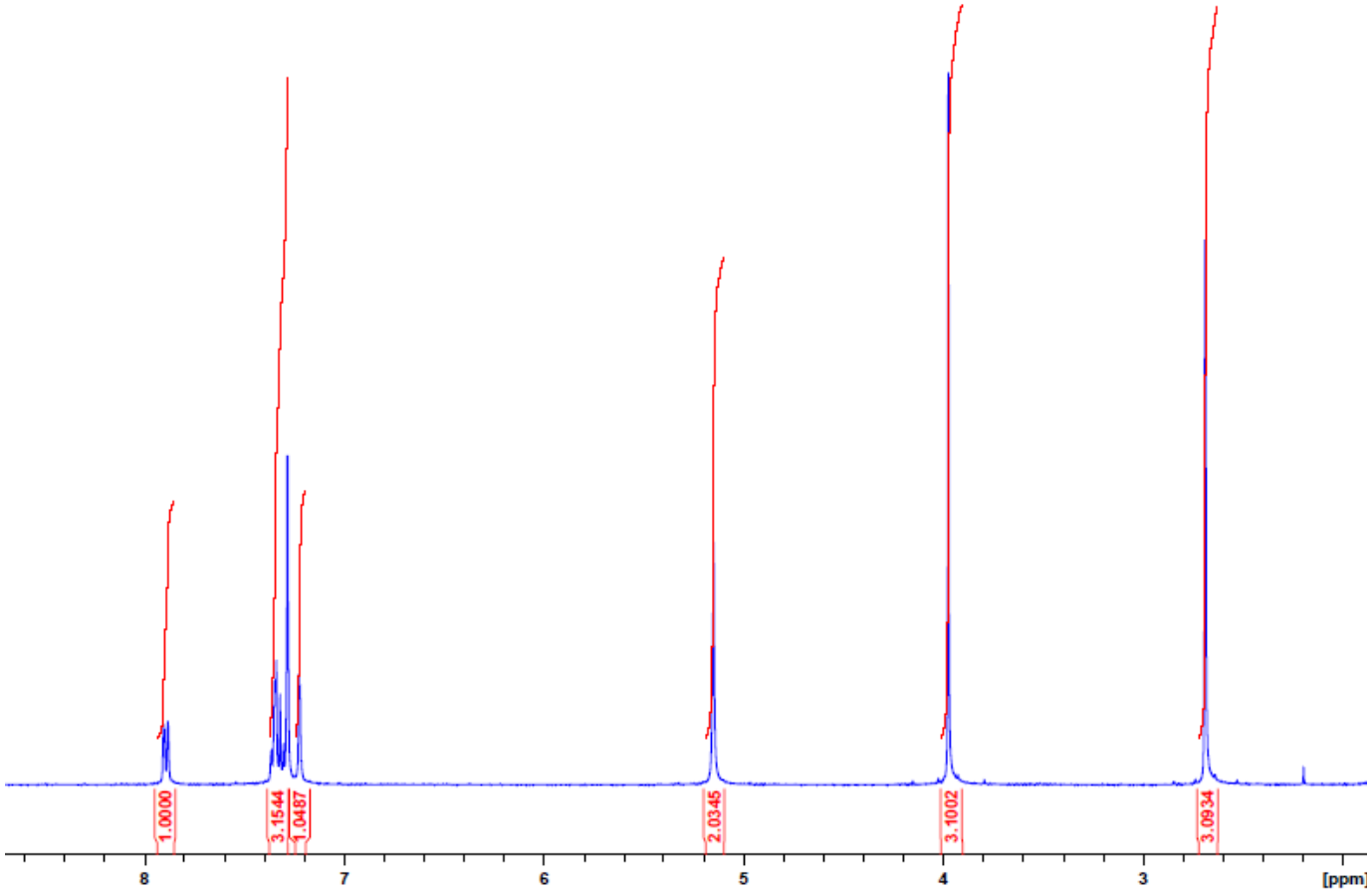
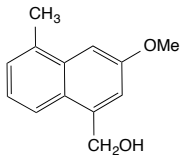


4

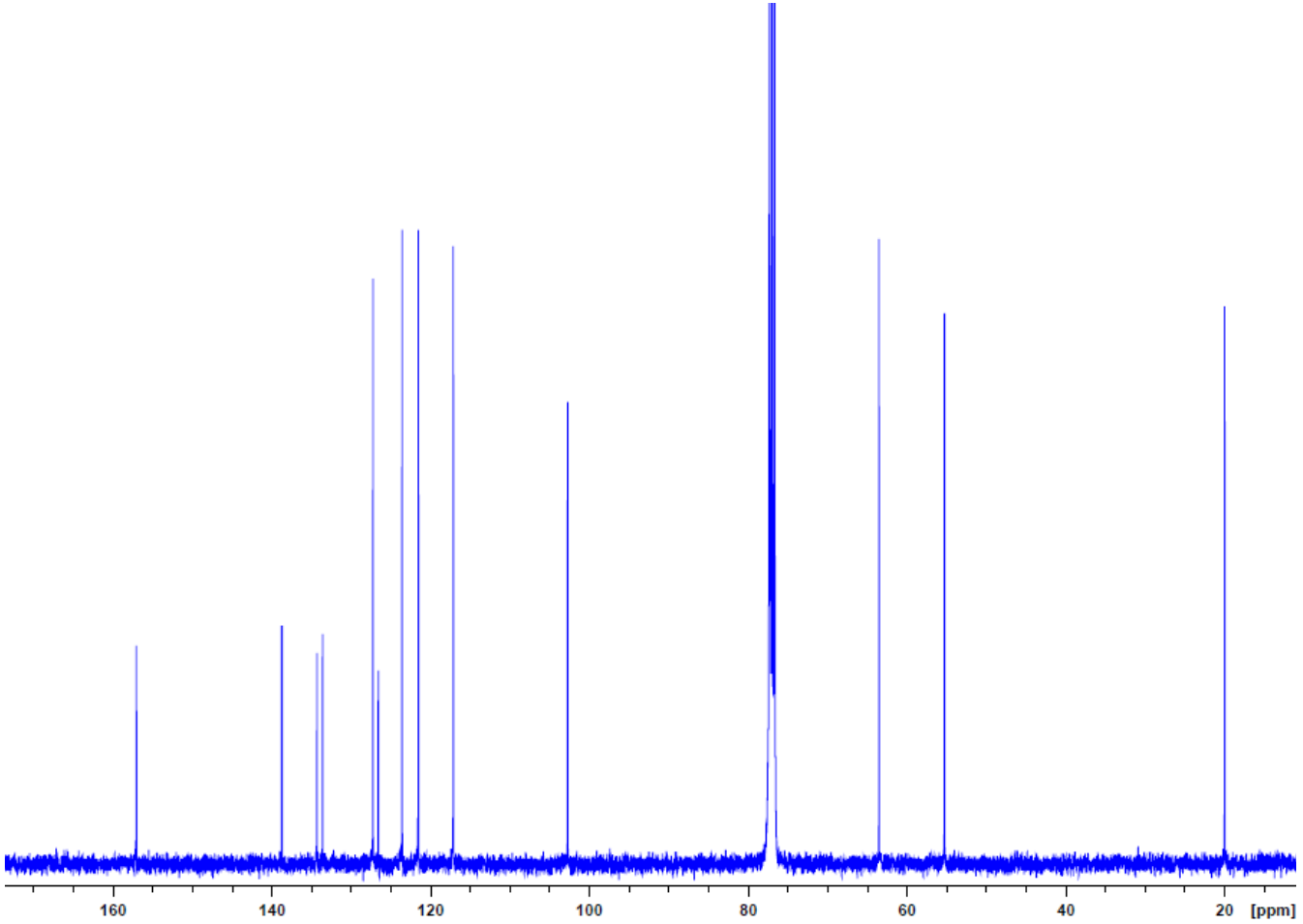
^{1}H NMR (400 MHz, $CDCl_3$)

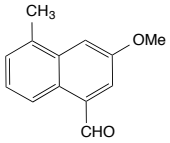




4

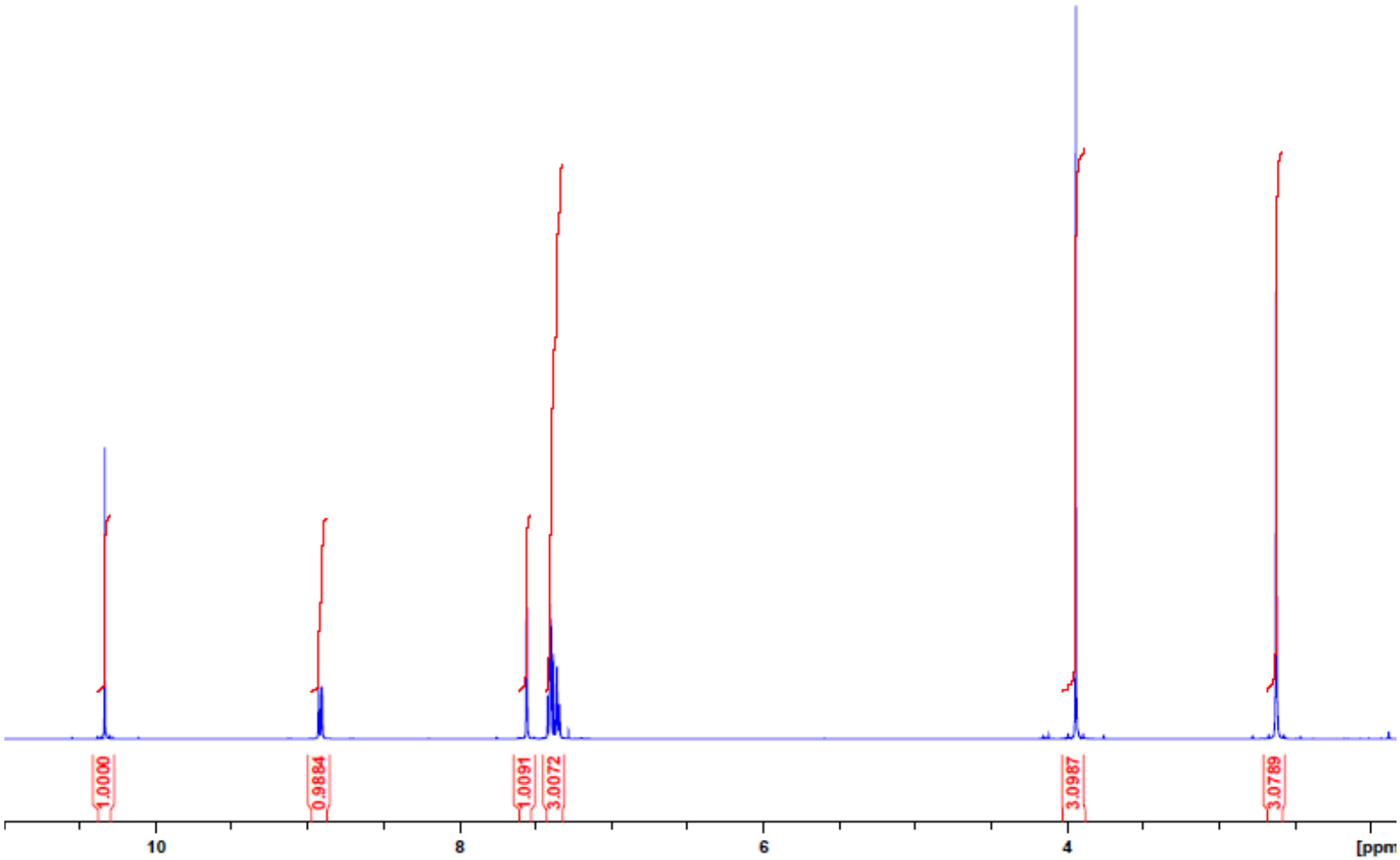
^{13}C NMR (100 MHz, $CDCl_3$)

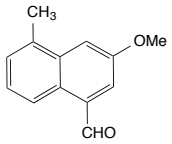




5

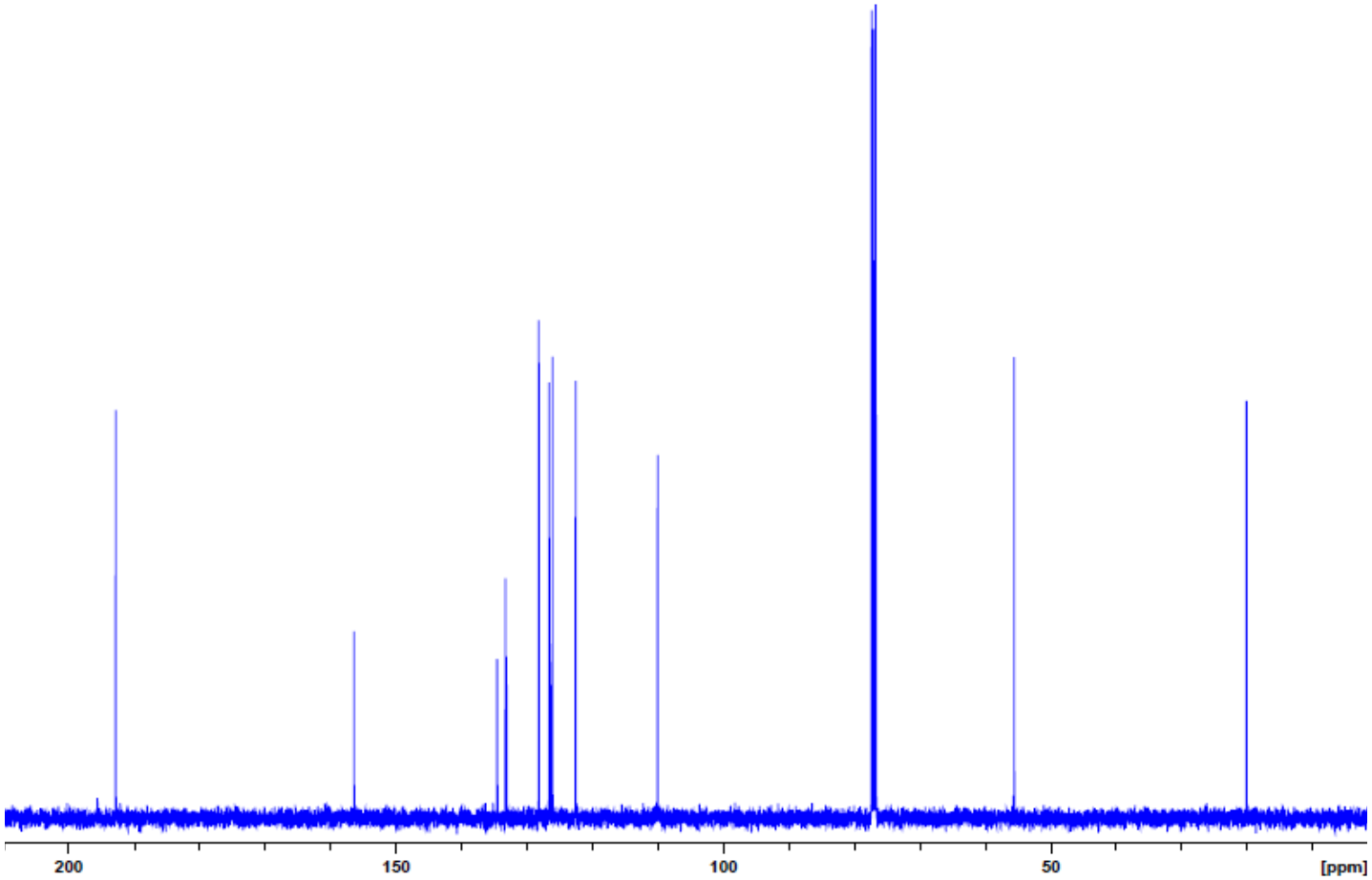
^{1}H NMR (400 MHz, $CDCl_3$)

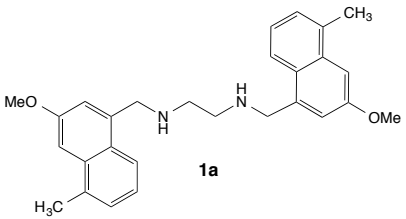




5

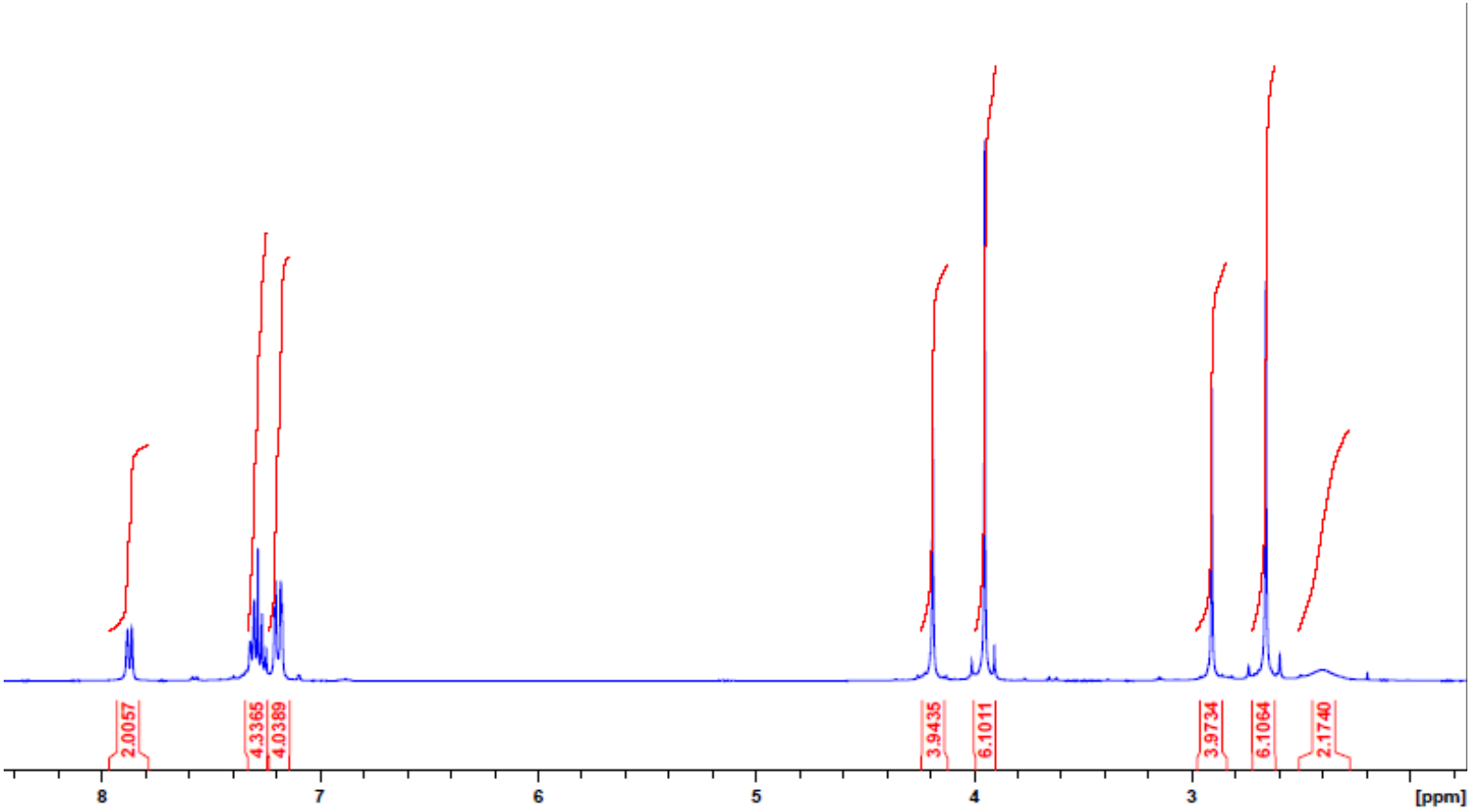
^{13}C NMR (100 MHz, $CDCl_3$)

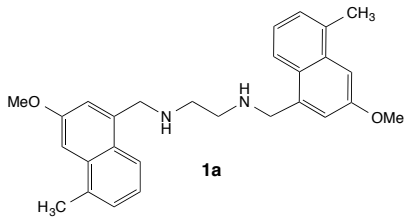




1a

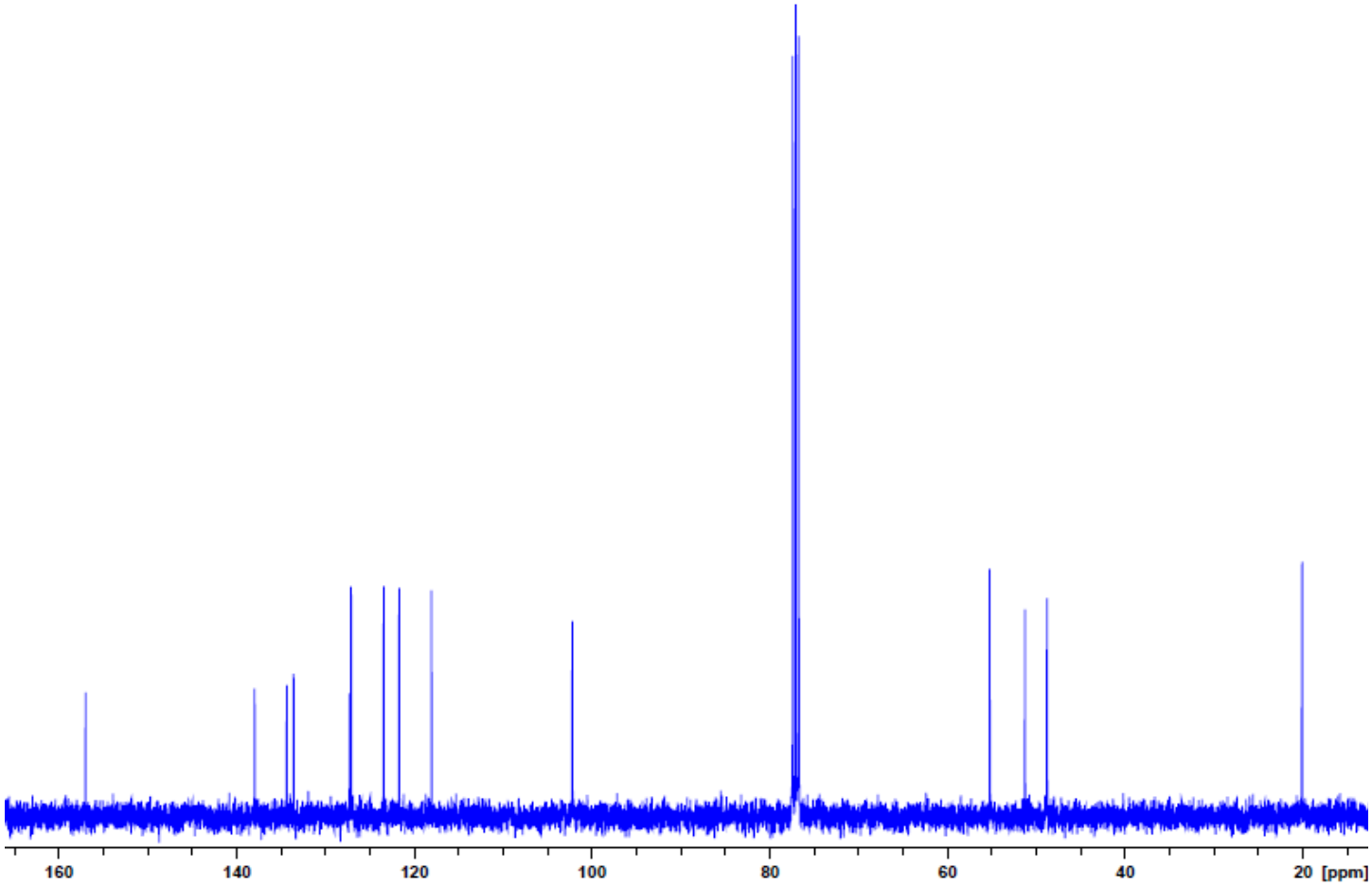
^{1}H NMR (400 MHz, $CDCl_3$)

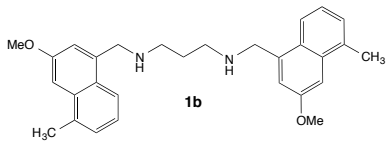




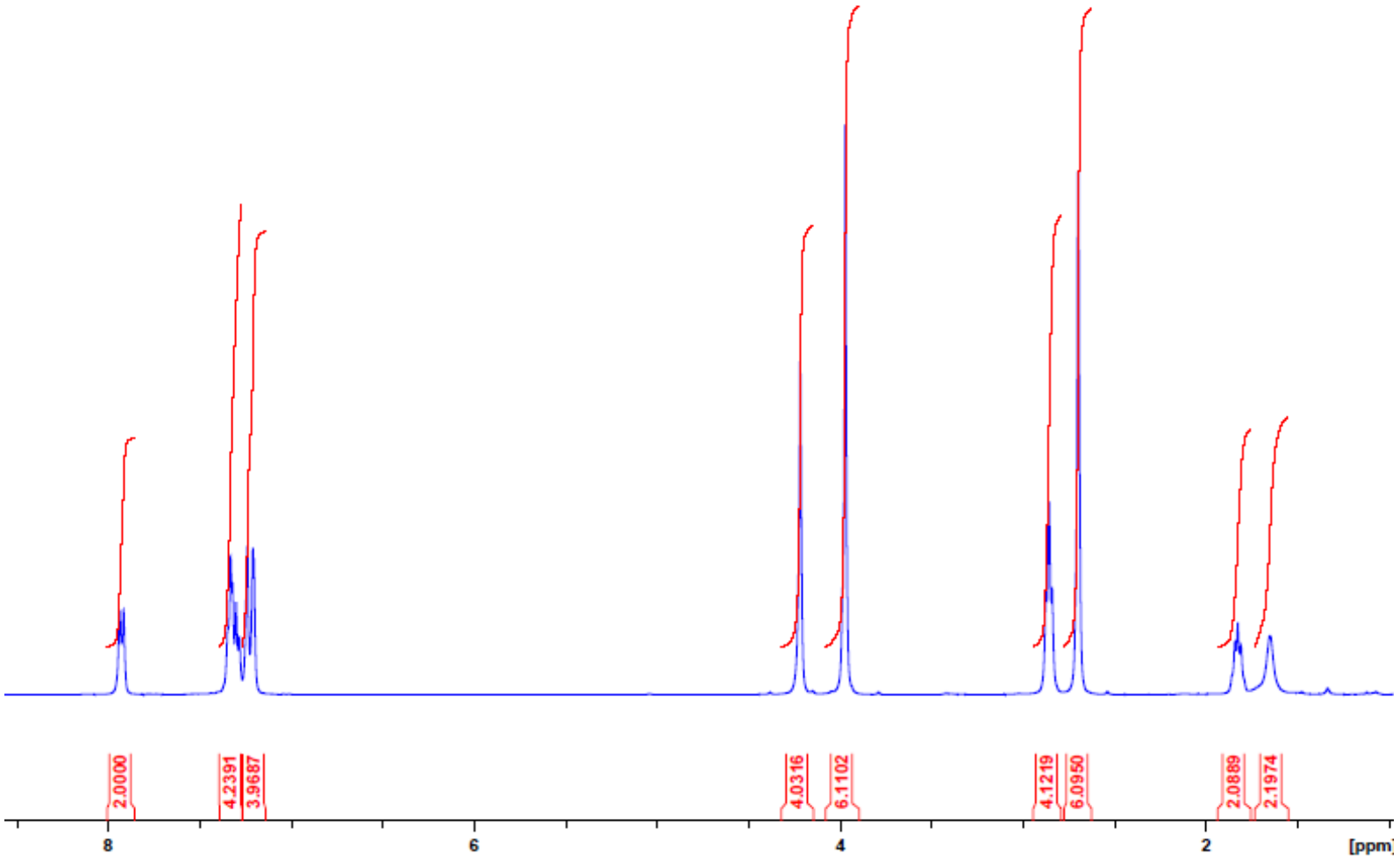
1a

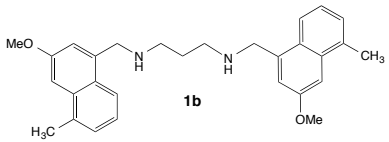
^{13}C NMR (100 MHz, $CDCl_3$)



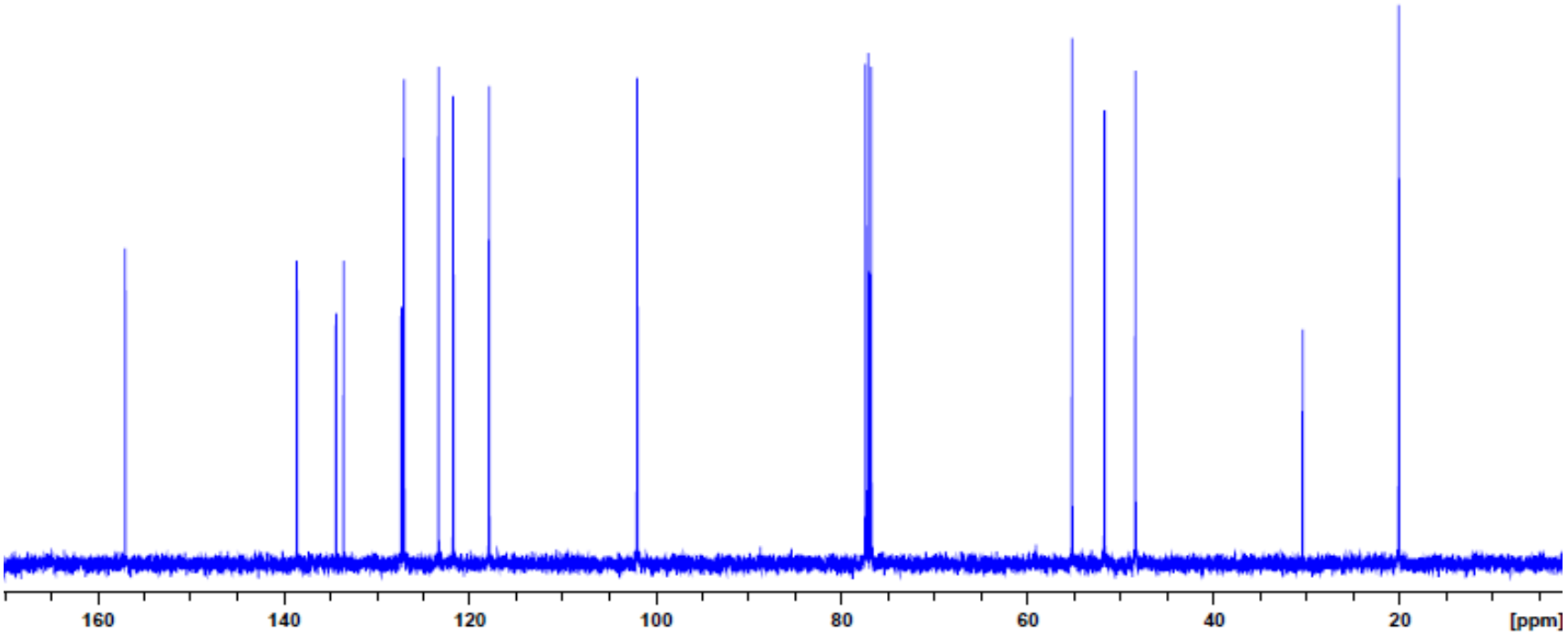


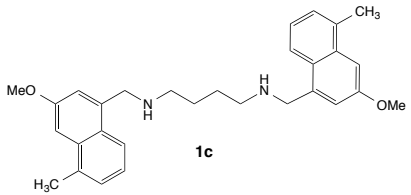
^{1}H NMR (400 MHz, $CDCl_3$)



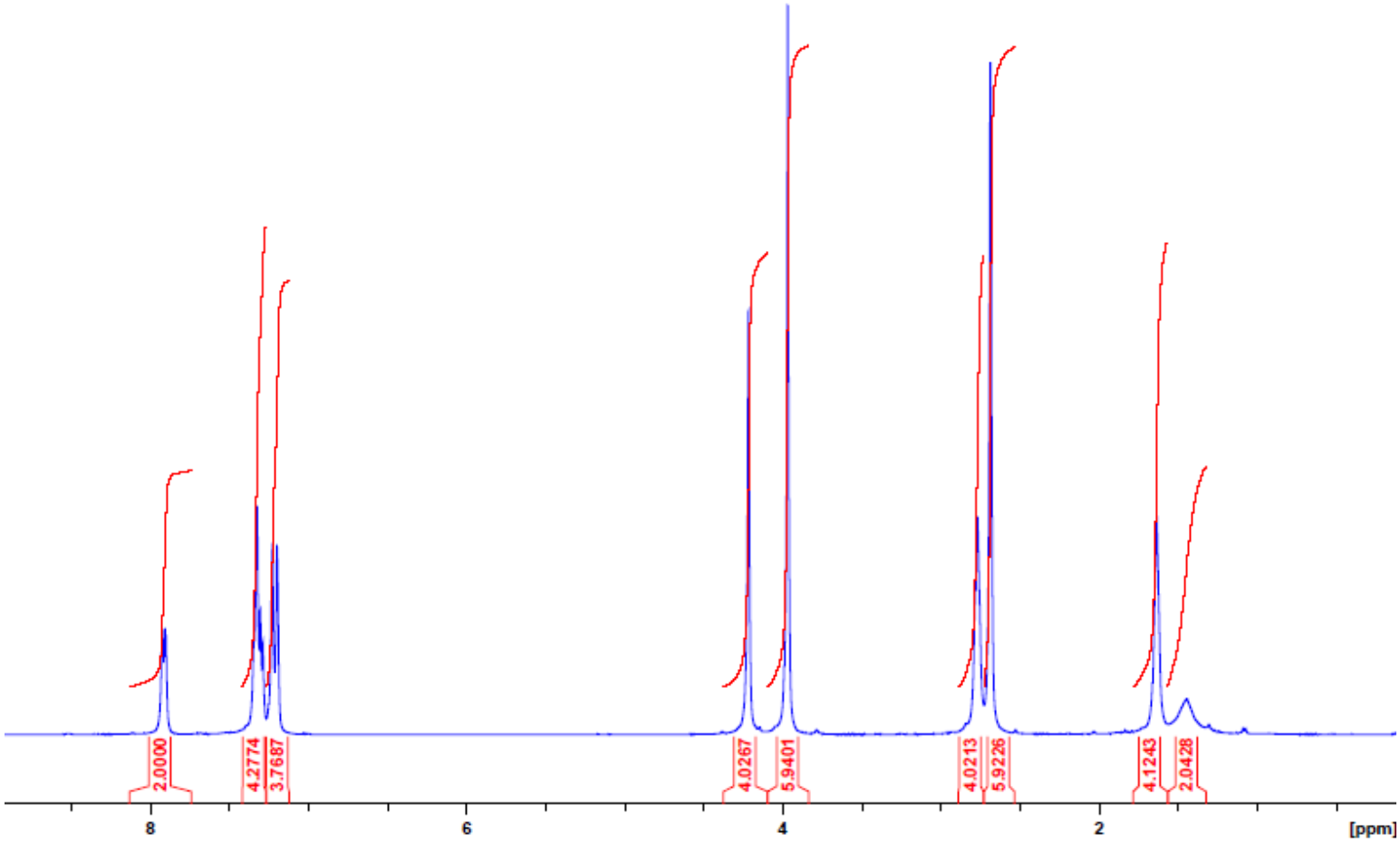


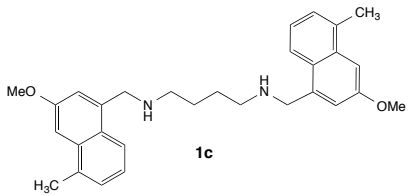
^{13}C NMR (100 MHz, $CDCl_3$)





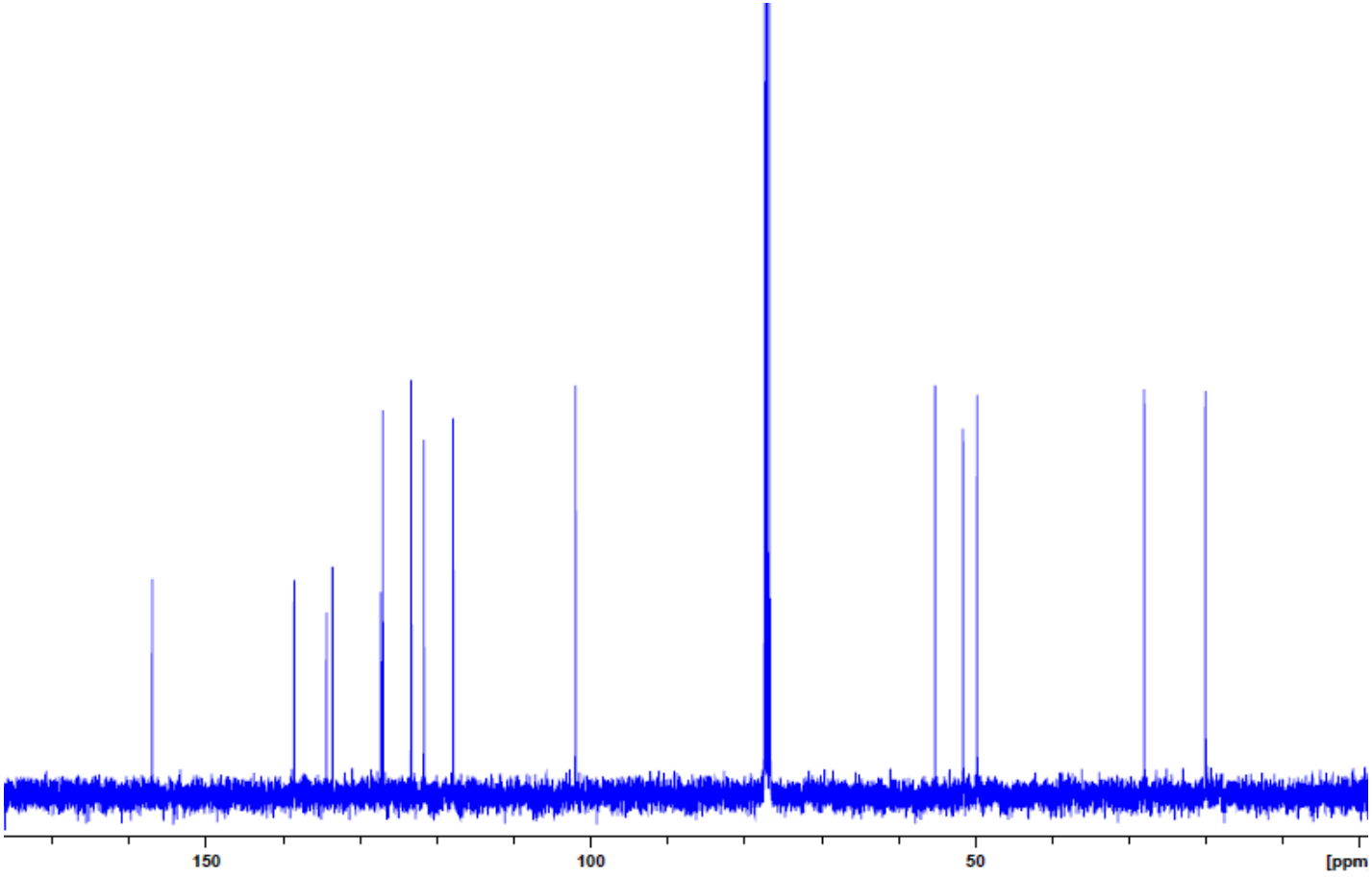
^{1}H NMR (400 MHz, $CDCl_3$)

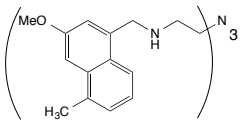




1c

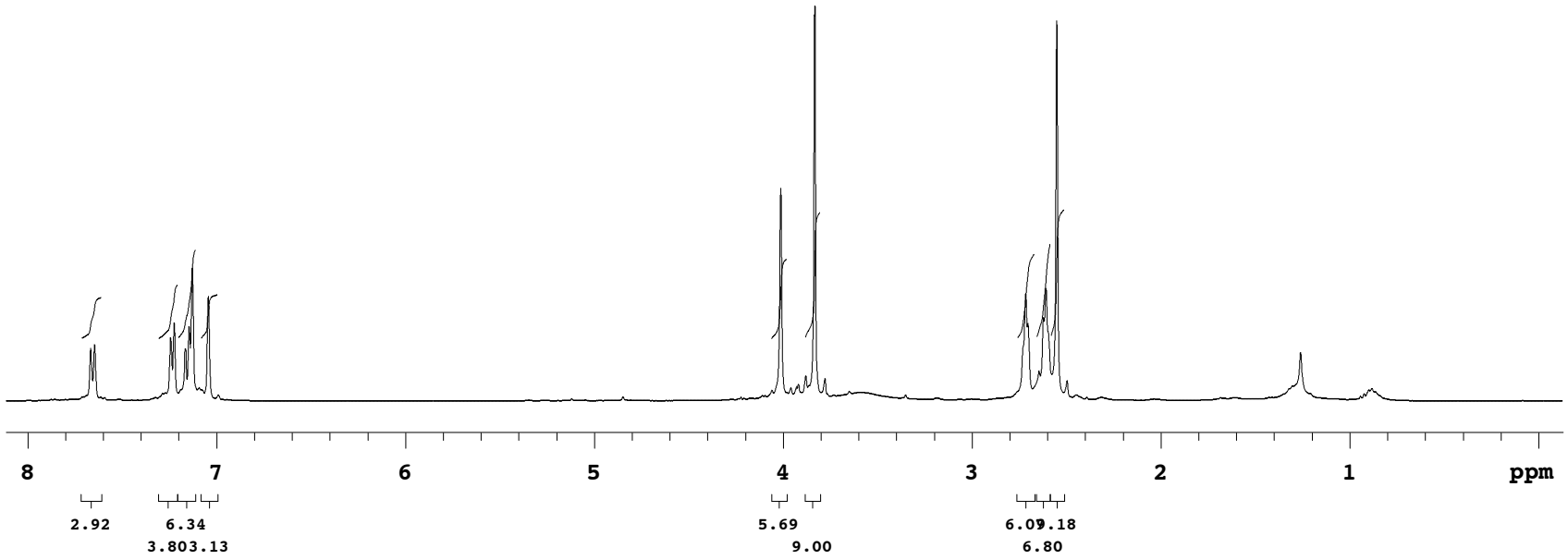
^{13}C NMR (100 MHz, $CDCl_3$)

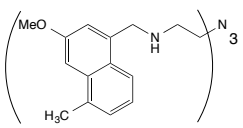




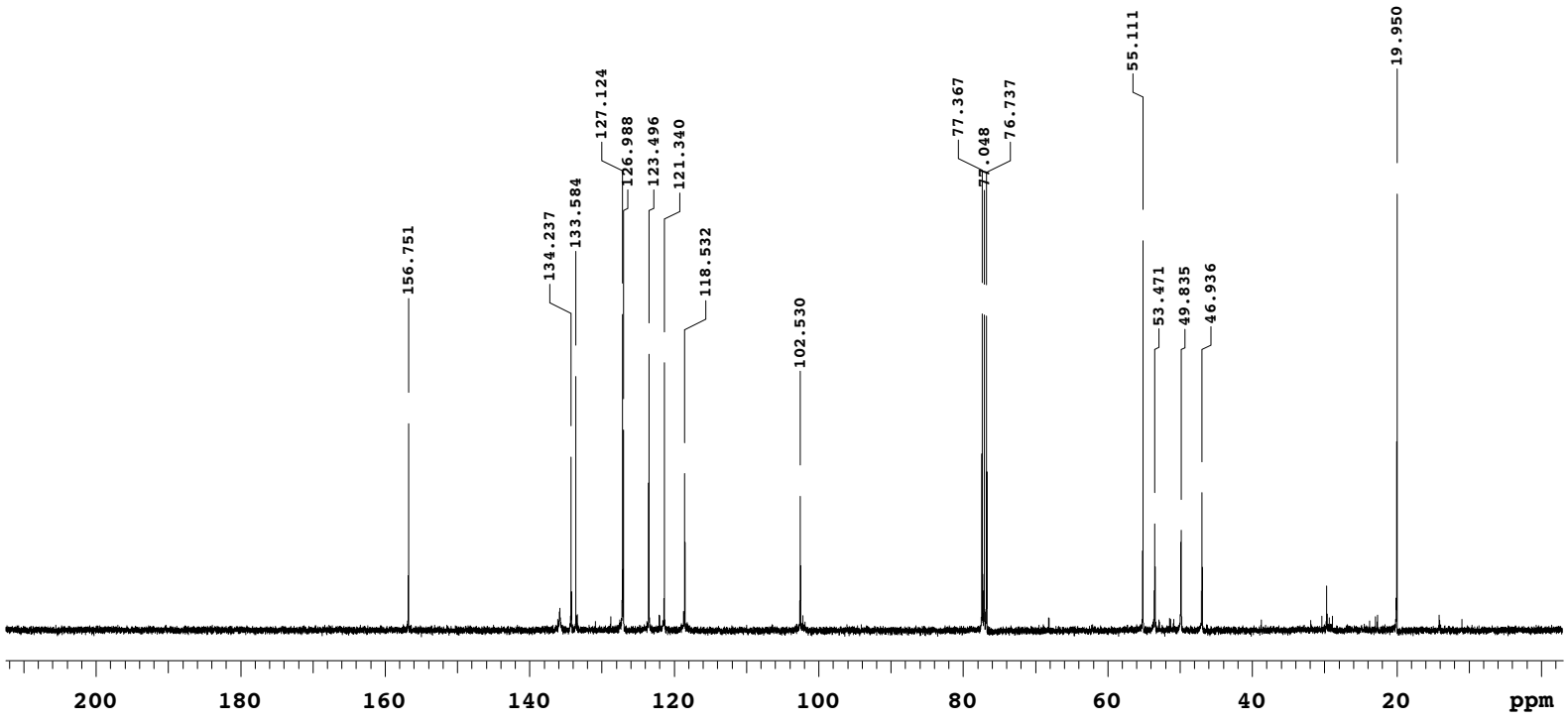
1d

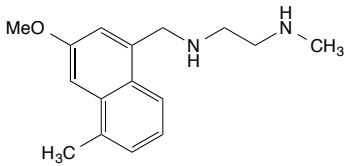
^{1}H NMR (400 MHz, $CDCl_3$)




MeO
N
H
N
3
H3C

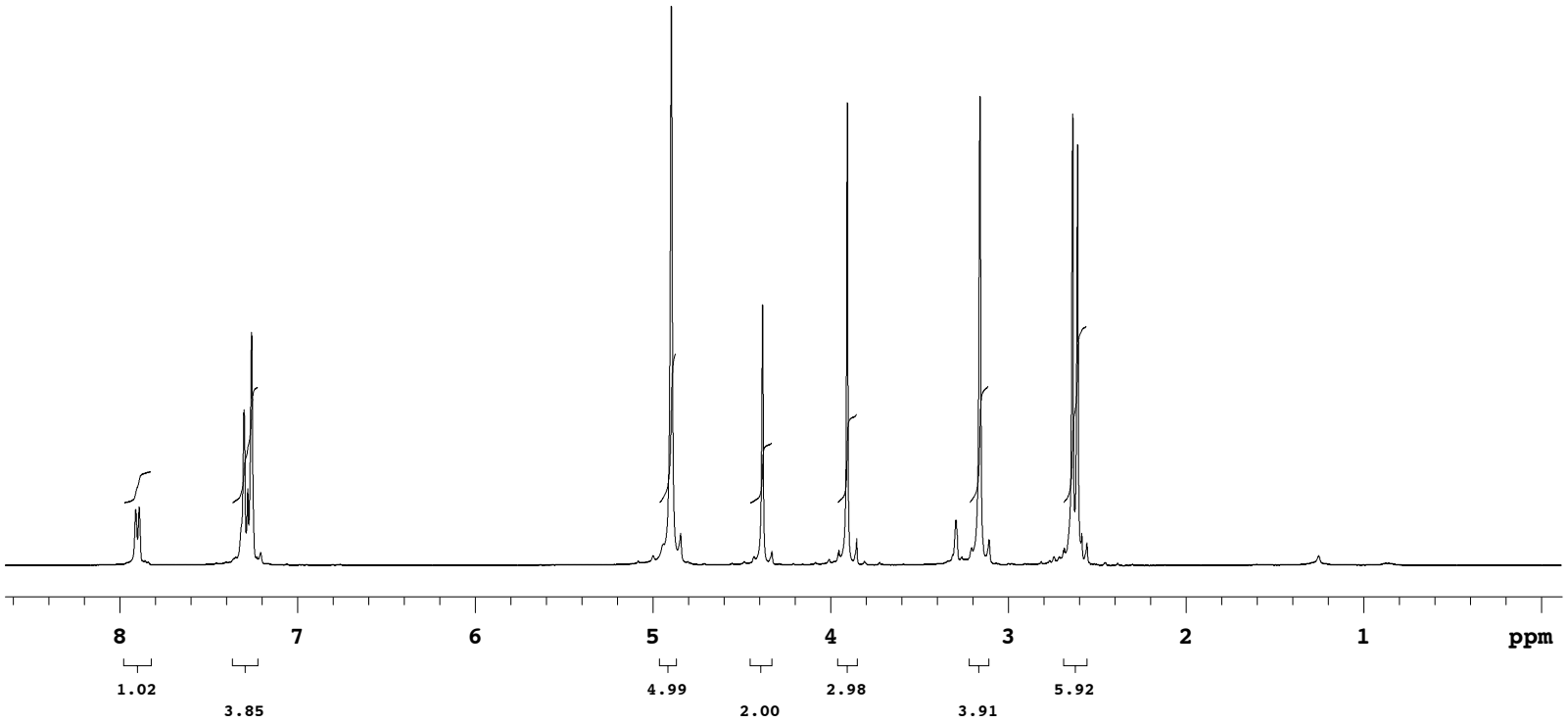
1d

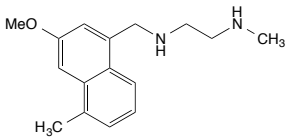

156.751
134.237
133.584
127.124
126.988
123.496
121.340
118.532
102.530
77.367
77.048
76.737
55.111
53.471
49.835
46.936
19.950
200
180
160
140
120
100
80
60
40
20
ppm



2a

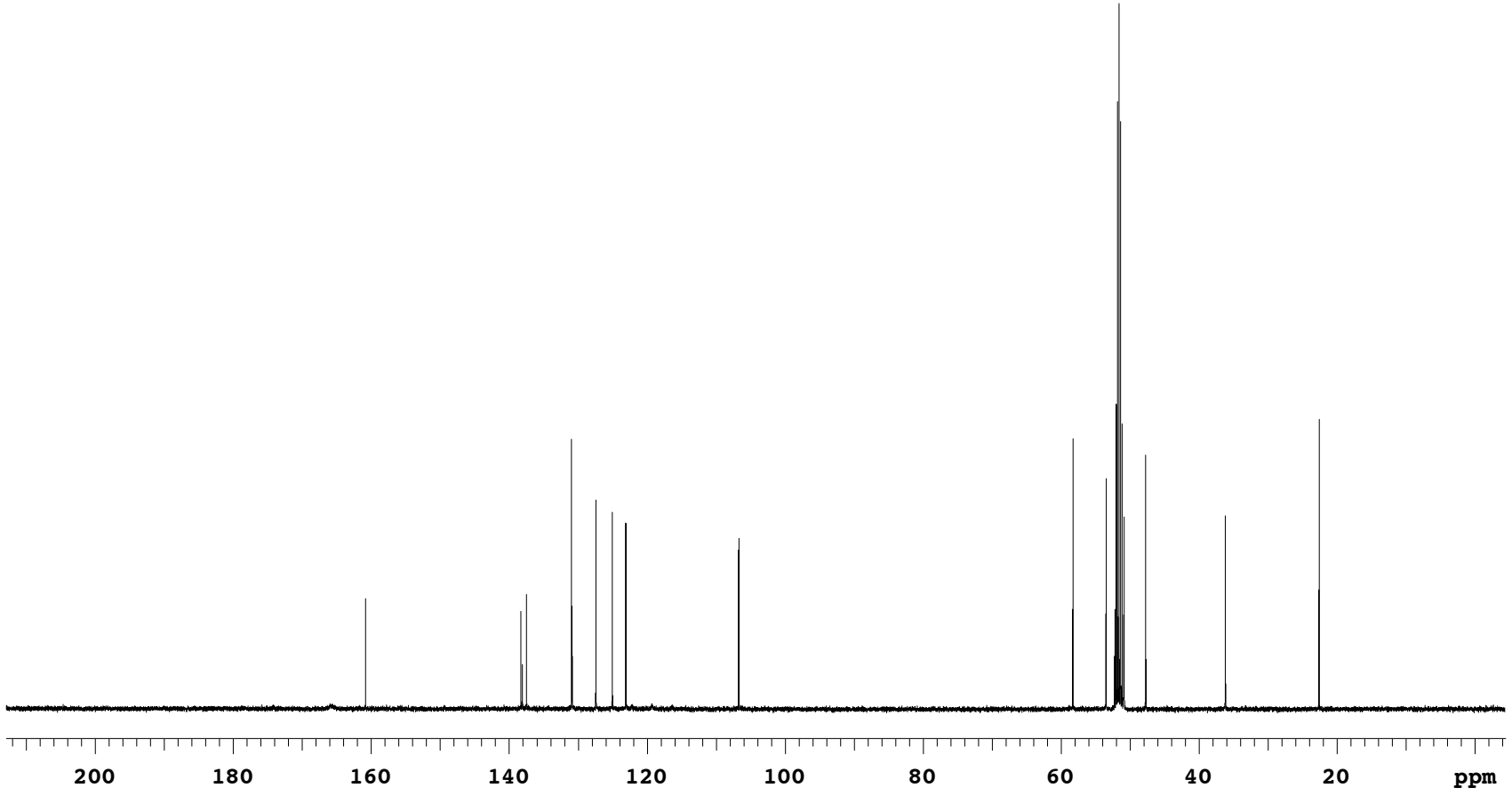
^{1}H NMR (400 MHz, $CDCl_3$)

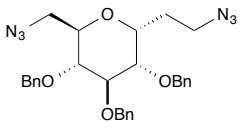




2a

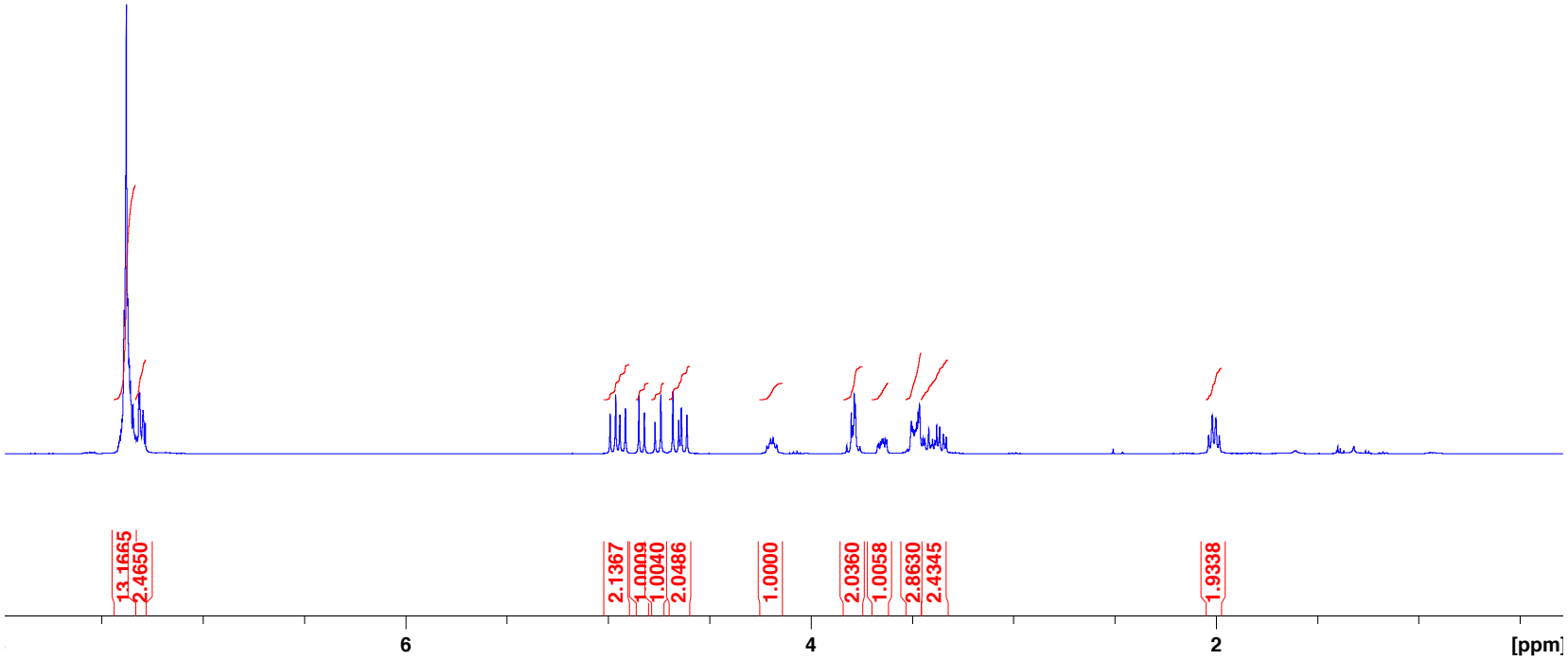
^{13}C NMR (100 MHz, $CDCl_3$)

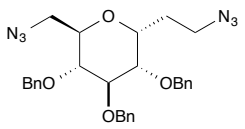




8

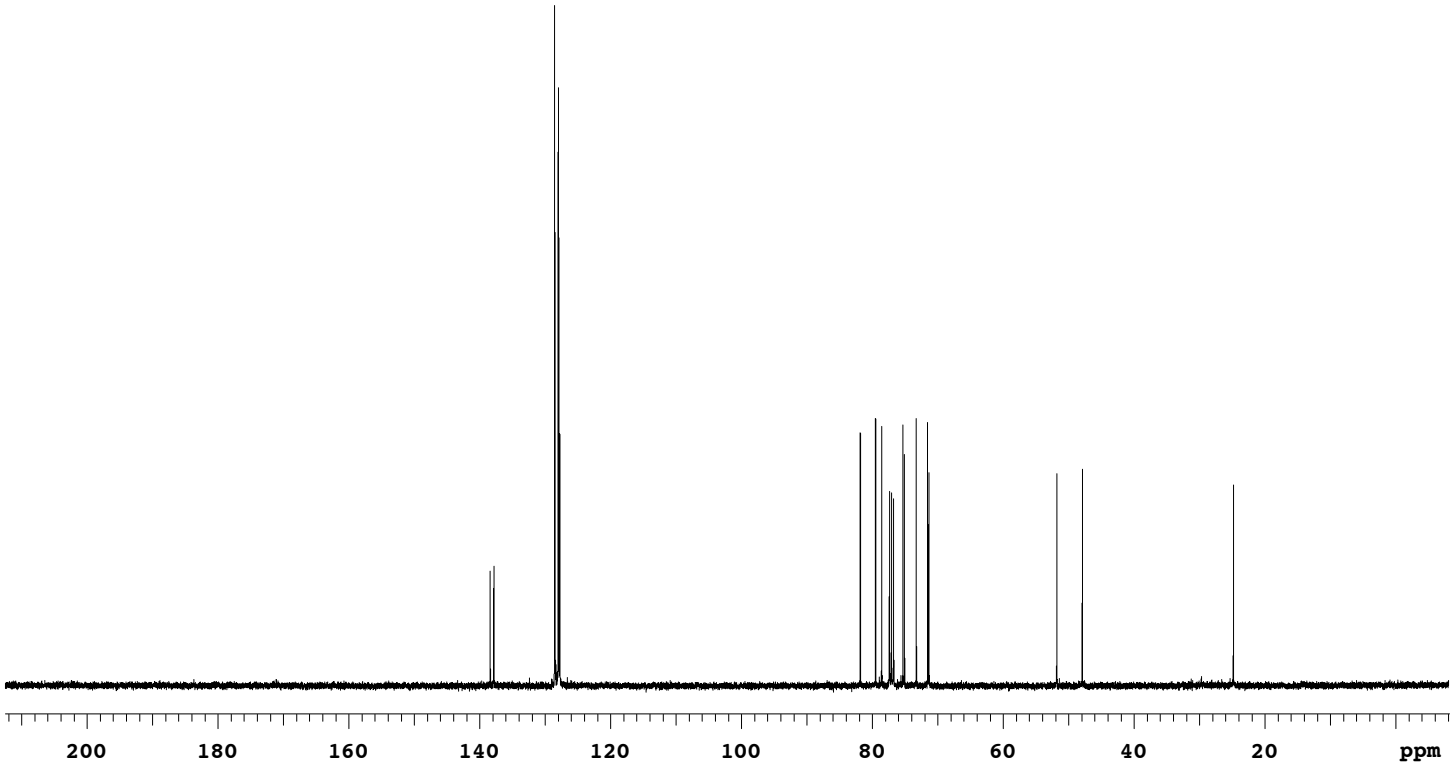
^{1}H NMR (400 MHz, $CDCl_3$)

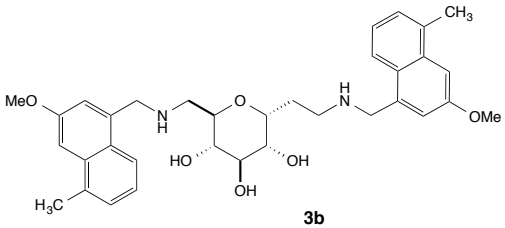




8

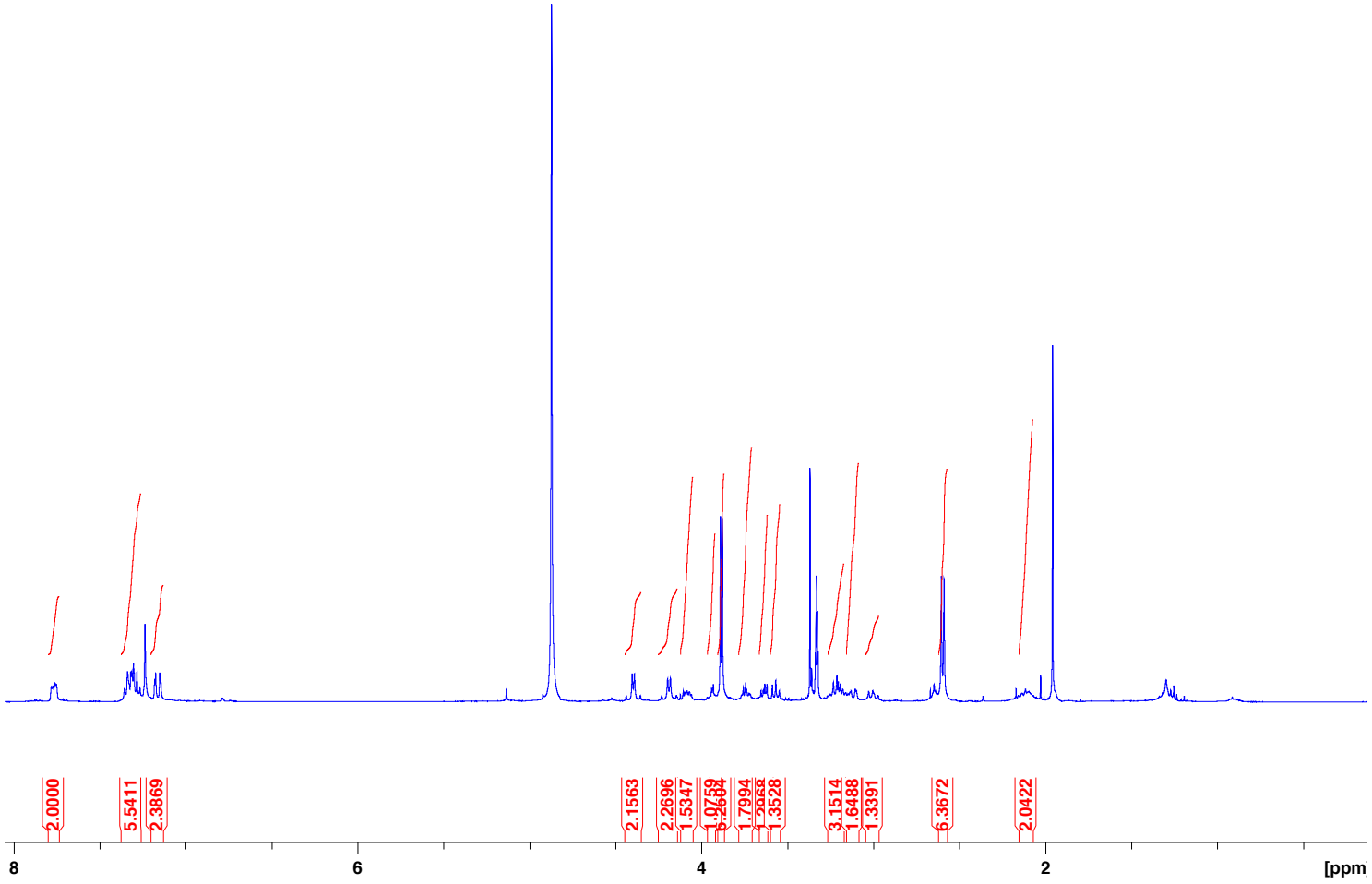
^{13}C NMR (100 MHz, $CDCl_3$)

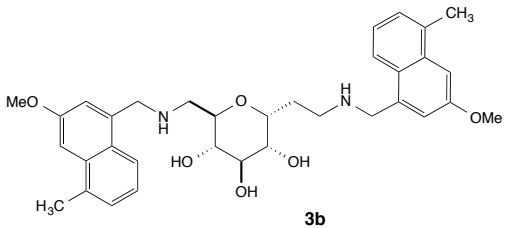




3b

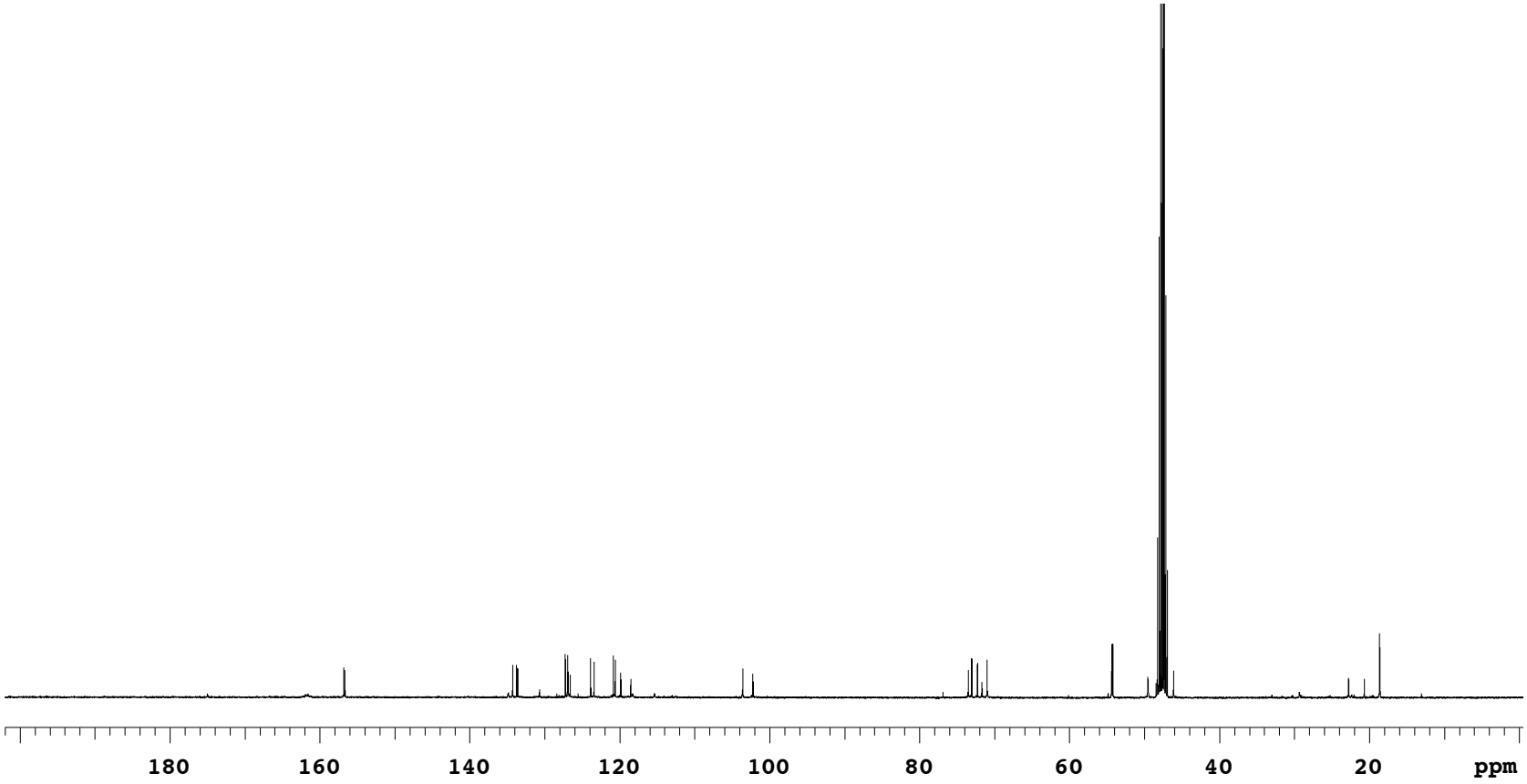
^{1}H NMR (400 MHz, $CDCl_3$)





3b

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



DNA Binding Studies

Materials: CT-DNA was purchased from CALBIOCHEM. Solutions of CT-DNA were prepared in 10mM Tris-EDTA buffer at pH 5.48 (as described in Jenkins: Jenkins, T.C. Optical Absorbance and Fluorescence Techniques for Measuring DNA-Drug Interactions. In *Methods in Molecular Biology, Drug-DNA Interaction Protocols;* Fox, K.R., Ed. Humana: Totowa, 1997; Vol 90, pp.195-217) and gave a 1.83:1 absorbance ratio at 260 nm and 280 nm. DNA and ligand concentrations were determined using 8452A HP Diode Array Spectrophotometer: CT-DNA, e_{260} = 6600 M^{-1} cm^{-1} bp^{-1} (1.82:1 absorbance ratio at 260 nm and 280 nm); poly(dG)•polyd(C), e_{253} = 7400 M^{-1} cm^{-1} bp^{-1}; poly (dA) • poly (dT), e_{260} = 6000 M^{-1} cm^{-1} bp^{-1}; poly(dA-dT)•poly(dA-dT), e_{262} = 6600 M^{-1} cm^{-1} bp^{-1}; Lambda phage DNA: 5 units dissolved in 500 mL buffer provides a solution of 0.76 mM/bp. Methyl Green, e_{631} = 85, 300 M^{-1} cm^{-1}; netropsin, e_{296} 21,500 $M^{-1}cm^{-1}$; Hoechst 33342, e_{340} = 47,000 M^{-1} cm^{-1}; Ethidium Bromide, e_{480} = 5450 M^{-1} cm^{-1}; 1a-1c, 3b, e_{336} = 6089 M^{-1} cm^{-1}; 1d, e_{336} = 9134 M^{-1} cm^{-1}; 2a, e_{336} = 3045 M^{-1} cm^{-1}.

EB competitive experiments

Constant concentrations of CT-DNA (poly(dG)•polyd(C), poly (dA)•poly (dT), poly(dA-dT)•poly(dA-dT), or Lambda phage DNA, 10 μM) and EtBr (10 μM) were titrated with increasing concentrations of the ligands (from 1 mM and 100 μM stock solutions), in the presence or absence of fixed concentrations of NaCl or the competitors methyl green or netropsin. The maximum emission wavelength was 490 nm when the excitation wavelength was 520 nm. Fluorescence titrations were recorded from 520 nm to 692 nm after an equilibration period of 3 min. Ex Slit (nm) = 10.0; Em Slit (nm) = 10.0; Scan Speed (nm/min) = 200.

Viscosity Studies

Viscosity experiments were performed with an Ostwald viscometer in a constant water bath at 23.0 ± 1°C. Solutions of constant DNA concentrations and varying ligand concentrations in Tris-EDTA buffer were incubated for 30 minutes. A digital stopwatch was used to record the flow time. The relative viscosity was calculated as from the following equation:

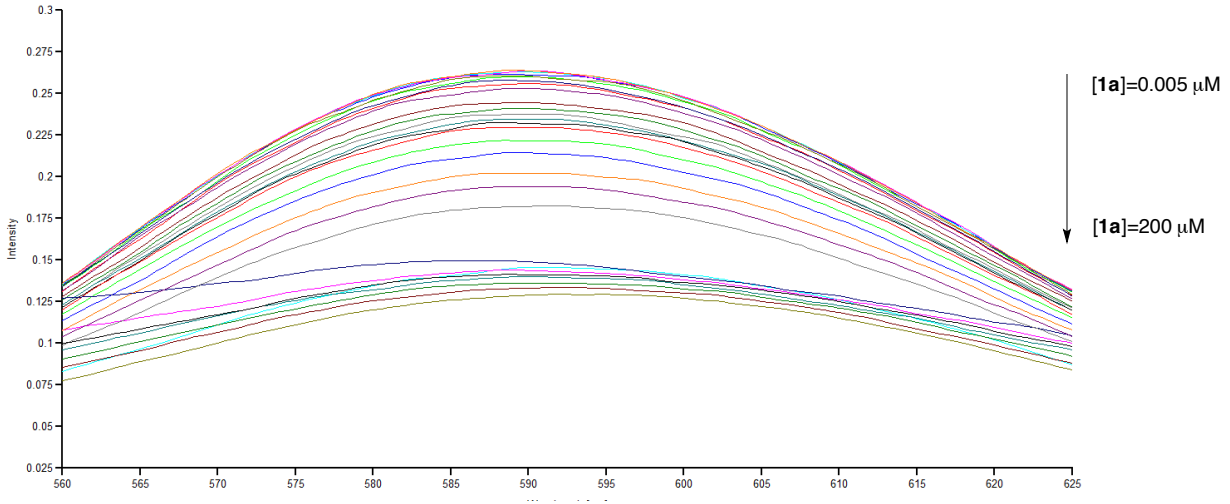
$$\eta = \frac{t - t_0}{t_0}$$

where t_0 and t are the flow time in the absence and presence of the ligand. η is the viscosity in the presence of the ligand and η_0 is the viscosity in the absence of the ligand. The data were graphed as $(\eta/\eta_0)^{1/3}$ vs. [ligand]/[DNA].

Circular Dichroism Studies.

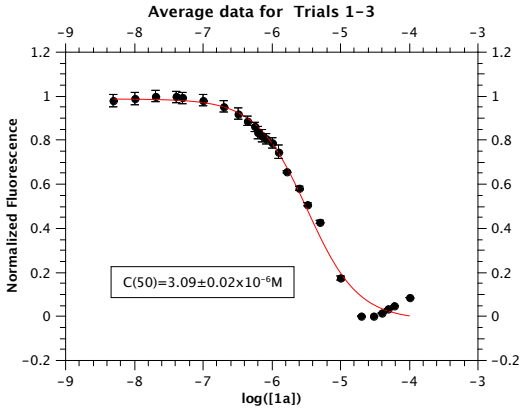
Small aliquots (0.6-5.0 μL) of a concentrated **1d** solution (1 mM) were added to a solution (2 mL, 100 mM KCl, 10 mM SC, 0.5 mM EDTA, pH 6.8) of CT-DNA (80 μM/bp), inverted twice, and incubated for 5 min at 20 °C. The CD spectra were then recorded as an average of three scans from 220 to 310 nm and data recorded in 0.1 nm increments
with an averaging time of 2 s.

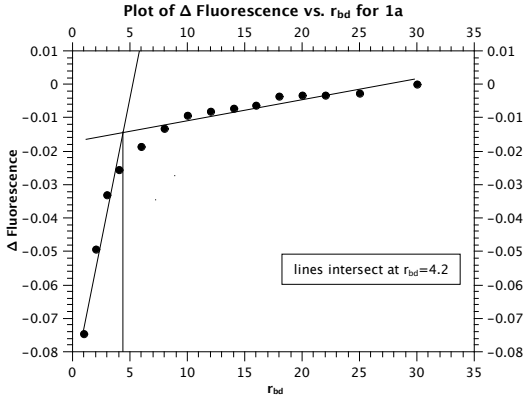
Compound 1a Ethidium displacement studies; K_{app}=2.1x10^{6}M^{-1} x 10/C50

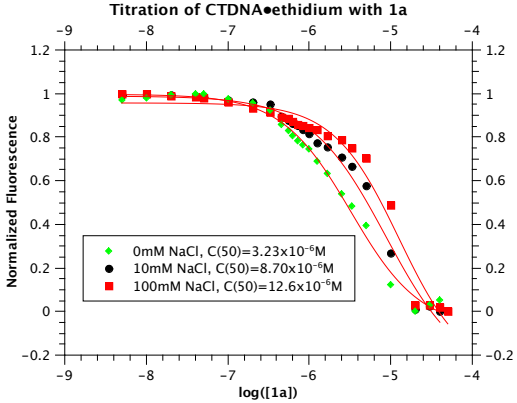


Titration of CT DNA (10 μM) and ethidium (10 μM) with **1a**: 0.005, 0.01, 0.02, 0.04, 0.05, 0.1, 0.2, 0.33, 0.45, 0.56, 0.63, 0.71, 0.83, 1.00, 1.25, 1.67, 2.50, 3.30, 5.0, 10.0, 20.0, 30.0, 40.0, 50.0, 60.0, 100.0, 200.0 μM

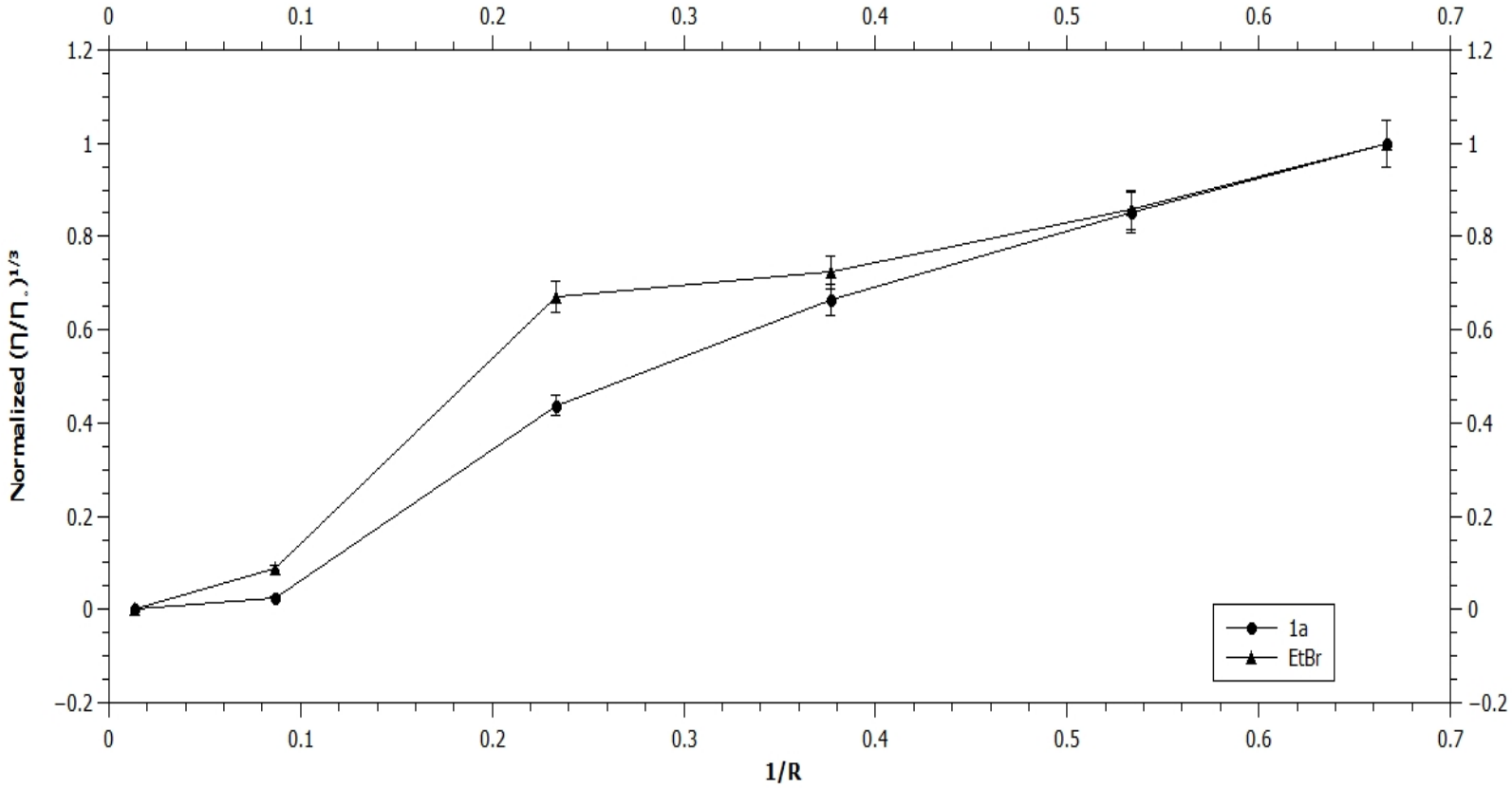
Trial 1: K_{app}= 6.17 x 10^6 M^{-1}; Trial 2: K_{app}= 8.40 x 10^6 M^{-1} ; Trial 3: K_{app}= 7.00 x 10^6 M^{-1}





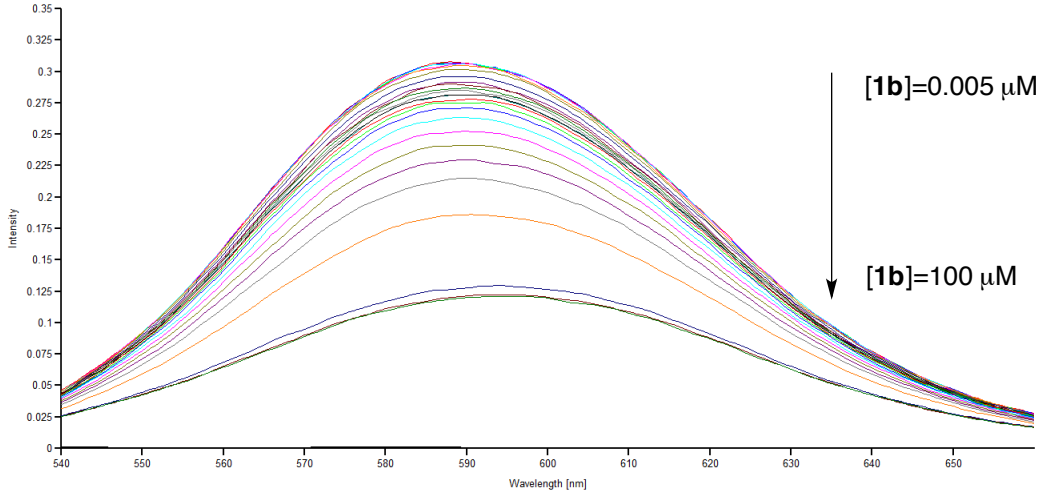


Average **K_{app}=7.19 ± 0.92 x 10^6 M^{-1}**



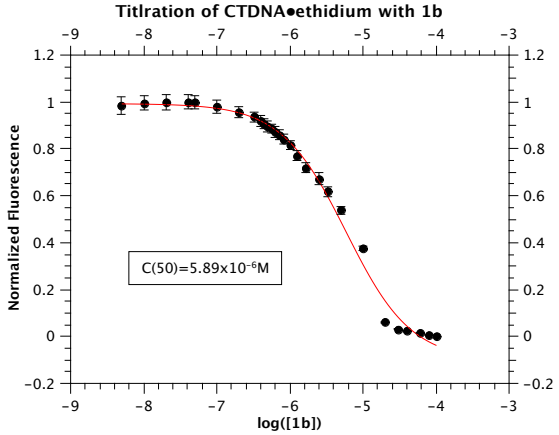
Effect of increasing amount of EtBr (▲) and **1a** (●) on relative viscosity of CT-DNA. EB (▲): [CT-DNA] = 300 μM, [EB]= 4, 26, 70, 113, 160, and 200 μM; **1a** (●): [CT-DNA] = 300 μM, [**1a**]: 8, 52, 140, 226, 320, and 400 μM.

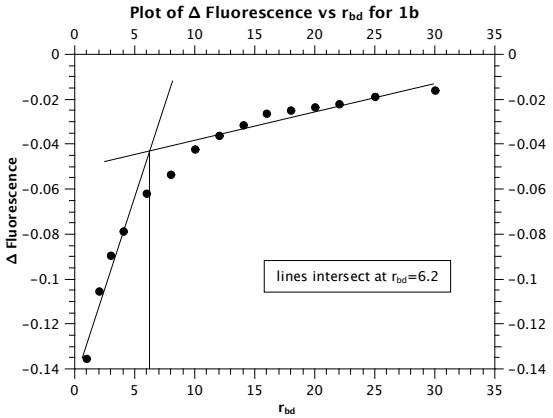
Compound 1b Ethidium displacement studies; K_{app}=2.1x10^{6}M^{-1} x 10/C50



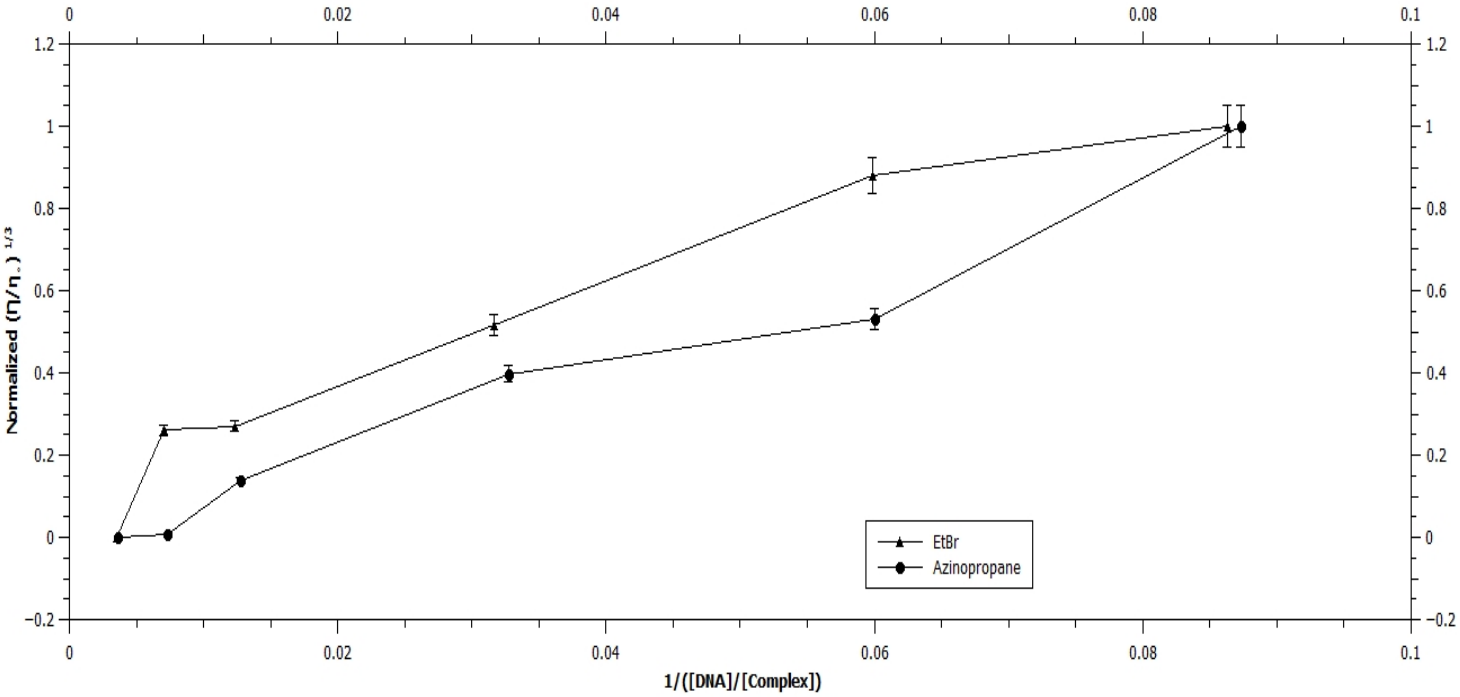
Titration of CT DNA (10 μM) and ethidium (10 μM) with **1b**: 0.005, 0.01, 0.02, 0.04, 0.05, 0.1, 0.2, 0.33, 0.45, 0.56, 0.63, 0.71, 0.83, 1.00, 1.25, 1.67, 2.50, 3.30, 5.0, 10.0, 20.0, 30.0, 40.0, 60.0, 80.0, 100.0 μM

Trial 1: K_{app}=2.35 x 10^{6}M-1 ; Trial 2: K_{app}=3.31 x 10^{6}M^{-1}; Trial 3: K_{app}=3.80 x 10^{6}M^{-1}



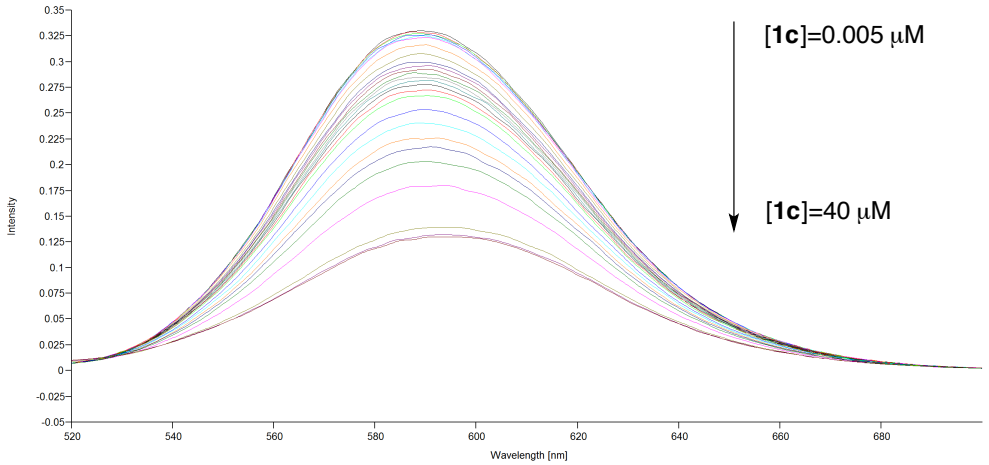


3 trials: Average K_{app}=3.15(±0.60)x10^{6}M^{-1}



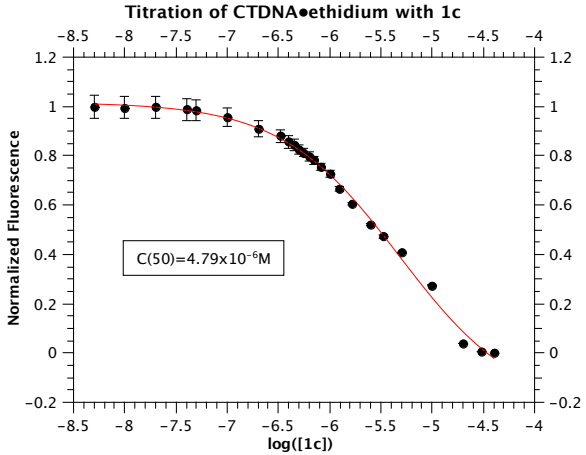
Effect of increasing amount of EtBr (▲) and **1b** (●) on relative viscosity of CT-DNA. EtBr (▲): [CT-DNA] = 301 μM, [EtBr]= 1.06, 2.12, 3.71, 9.54, 18.02, and 25.97 μM; **1b** (●): [CT-DNA] = 298 μM, [**1b**]: 1.08, 2.17, 3.79, 9.76, 17.88, and 26.02 μM

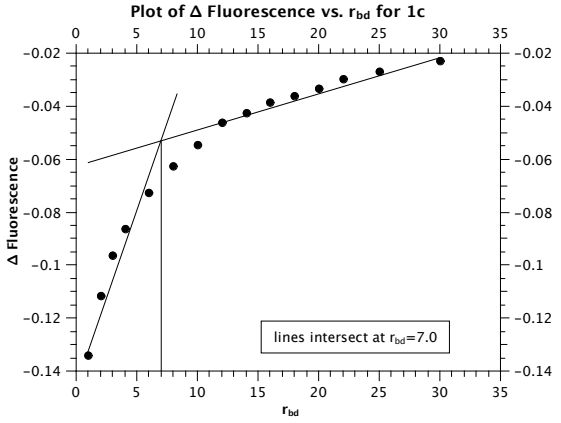
Compound 1c Ethidium displacement studies; K_{app}=2.1x10^{6}M^{-1} x 10/C50



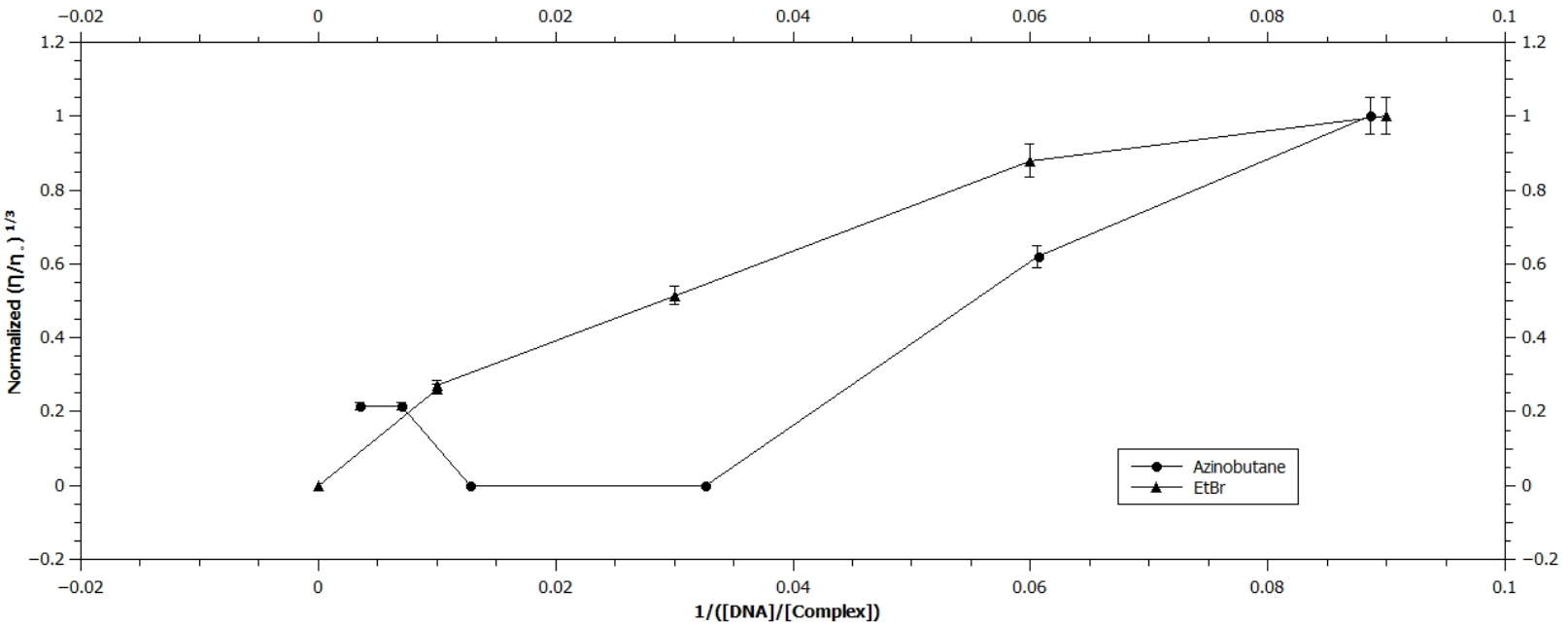
Titration of CT DNA (10 μM) and ethidium (10 μM) with **1c**: 0.005, 0.01, 0.02, 0.04, 0.05, 0.1, 0.2, 0.33, 0.45, 0.56, 0.63, 0.71, 0.83, 1.00, 1.25, 1.67, 2.50, 3.30, 5.0, 10.0, 20.0, 30.0, 40.0 μM

Trial 1: K_{app}=4.64 x 10^{6}M^{-1}; Trial 2: K_{app}=4.71 x 10^{6}M^{-1}; Trial 3: K_{app}=5.65 x10^{6}M^{-1}



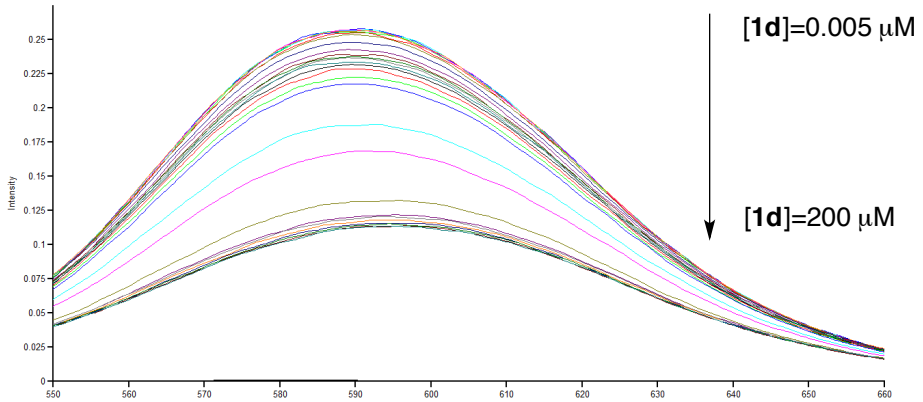


3 trials: **Average:** K_{app}=5.00 (±0.46) x 10^{6}M^{-1}



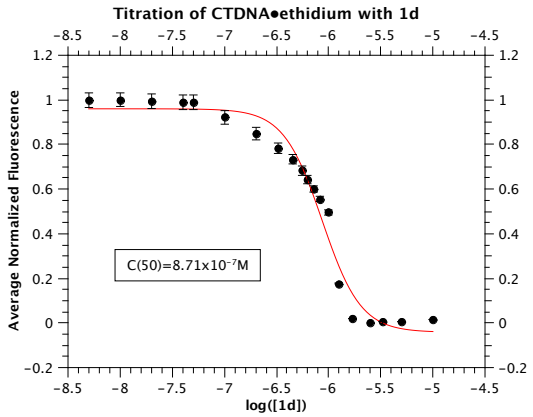
Effect of increasing amount of EtBr (▲) and **1c** (●) on relative viscosity of CT-DNA. EB (▲): [CT-DNA] = 300 μM, [EB]= 4, 26, 70, 113, 160, and 200 μM; **1c** (●): [CT-DNA] = 300 μM, [**1c**]: 1, 2, 4, 10, 18, and 26 μM.

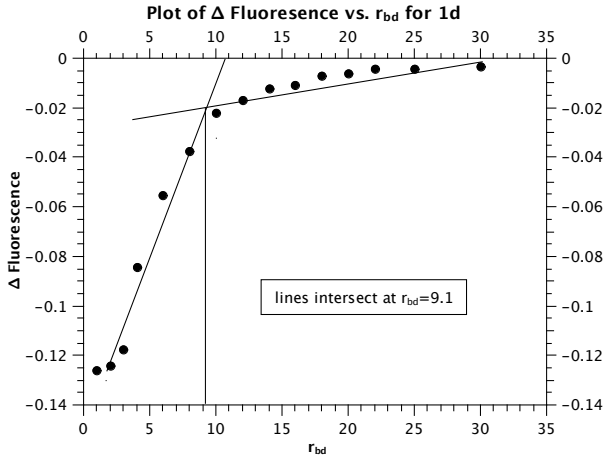
Compound 1d Ethidium displacement studies; K_{app}=2.1x10^{6}M^{-1} x 10/C50



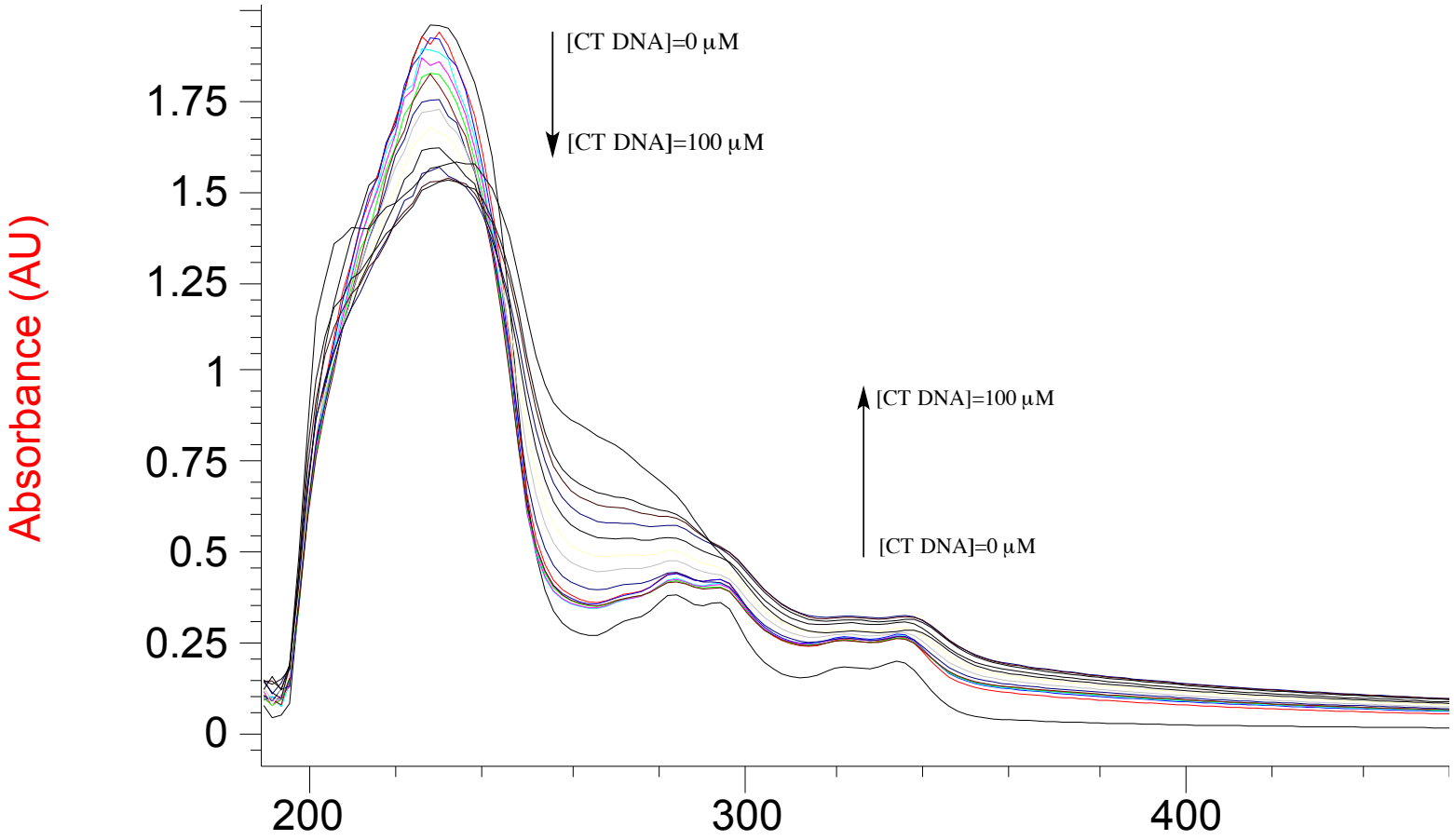
Titration of CT DNA (10 μM) and ethidium (10 μM) with **1d**: 0.005, 0.01, 0.02, 0.04, 0.05, 0.1, 0.2, 0.33, 0.45, 0.56, 0.63, 0.71, 0.83, 1.00, 1.25, 1.67, 2.50, 3.30, 5.0, 10.0, 20.0, 30.0, 40.0, 50.0, 60.0, 100.0, 200.0 μM

Trial 1 K_{app}=26.45 x 10^6 M^{-1}1; Trial 2 K_{app}=24.11 x 10^6 M^{-1}; Trial 3 K_{app}=22.00 x 10^6 M^{-1}

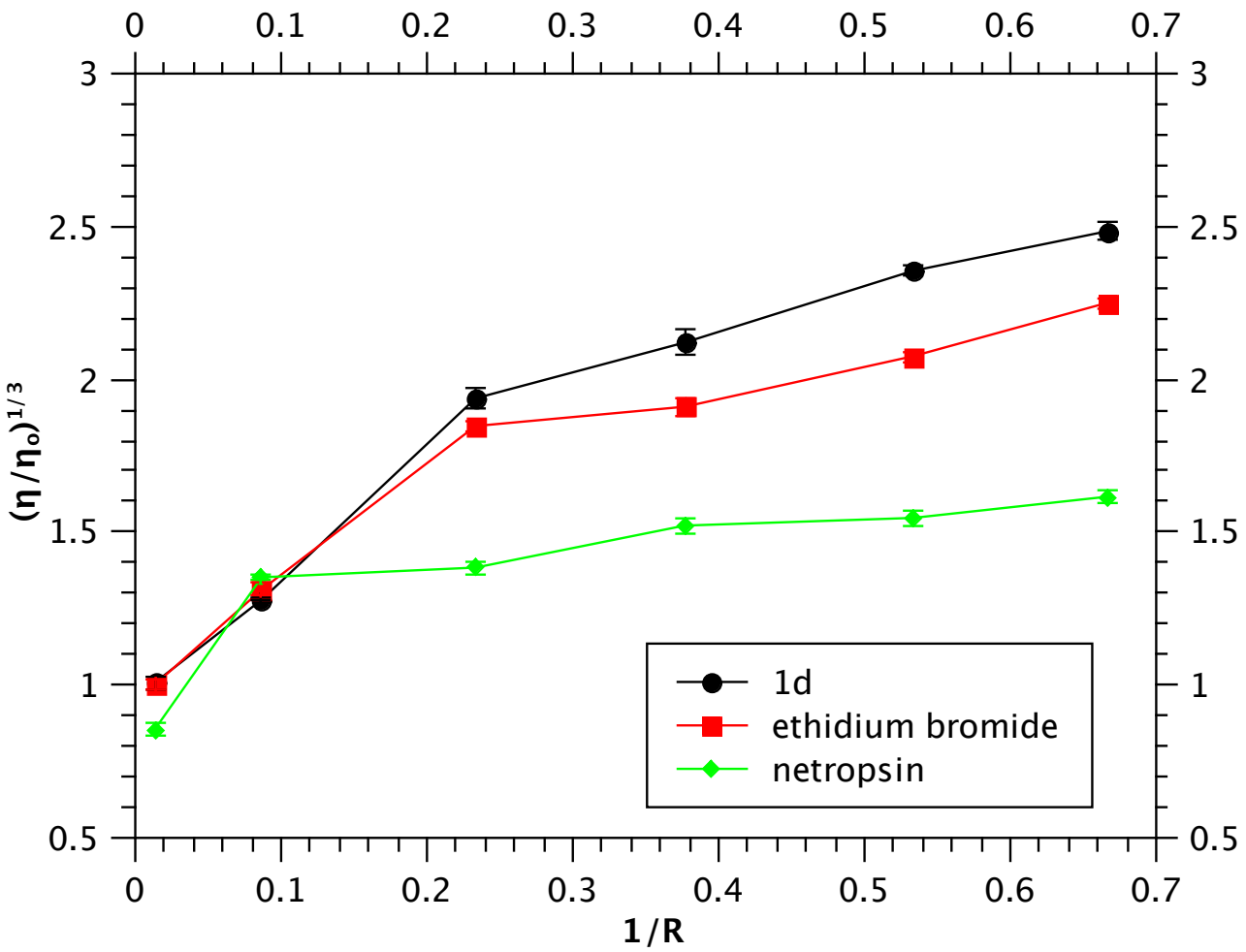




3 trials: **Average *Kapp*=24.18 (± 1.5) x 10^6 M^{-1}**



UV spectra of **1d** (25μM in 10mM Tris-EDTA, pH=5.48) in the presence of varying concentrations of CT-DNA: 0, 0.25, 0.5, 1.0, 2.0, 4.0, 5.0, 10.0, 15.0, 20.0, 25.0, 30.0, 40.0, 50.0, 100 μM.



Effect of increasing amounts of **1d** (●), ethidium bromide (■), and netropsin (♦) on the relative viscosity of CT-DNA. *R*= [DNA(bp)]/[ligand]; **1d** (●): [CT-DNA] = 300 μM, [**1d**]: 1, 2, 4, 10, 18, and 26 μM; ethidium bromide (■): [CT-DNA] = 300 μM, [ethidium bromide]= 4, 26, 70, 113, 160, and 200 μM; netropsin (♦): [CT-DNA] = 300 μM, [netropsin]= 4, 26, 70, 113, 160, and 200 μM.

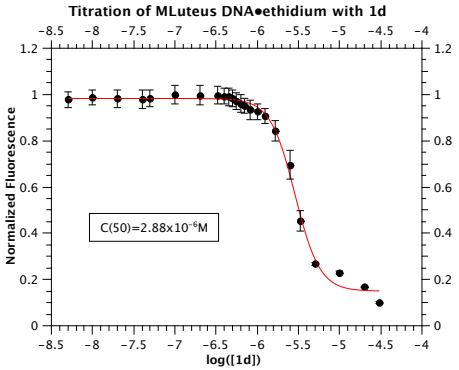
Affinity of **1d** for M. Luteus DNA

Trial 1 K_{app}=7.29x10^{6}M^{-1} ;

Trial 2 K_{app}=9.50x10^{6}M^{-1} ;

Trial 3 K_{app}=6.00x10^{6}M^{-1};

Average K_{app}=7.36(±1.40)x10^{6}M^{-1}



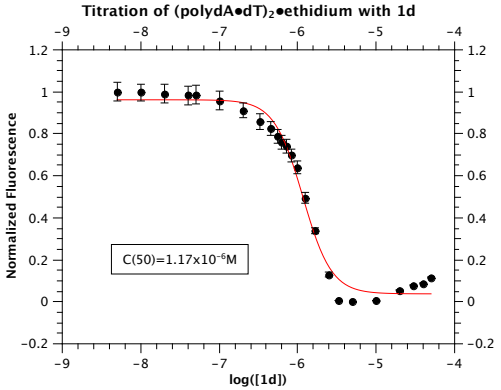
Affinity of **1d** for poly(dA•dT)$_2$

Trial 1 K_{app}=1.71 x10^{7}M^{-1};

Trial 2 K_{app}=2.00 x 10^{7}M^{-1};

Trial 3 K_{app}=1.60 x 10^{7}M^{-1}

Average *K_{app}=17.7±1.5 x 10^{6}M^{-1}*



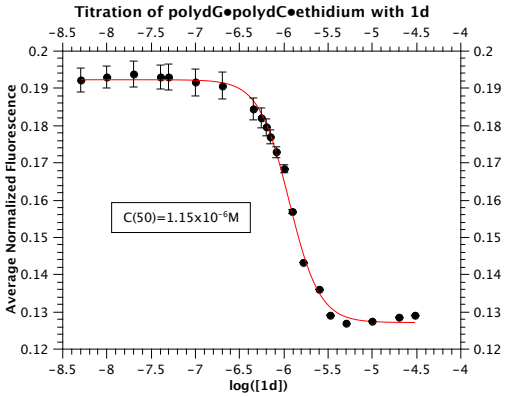
Affinity of **1d** for polydG•polydC

Trial 1: K_{app}=18.9x10^{6}M^{-1}

Trial 2: K_{app}=18.8x10^{6}M^{-1}

Trial 3: K_{app}=17.5x10^{6}M^{-1}

Average *K_{app}=18.4±0.64x10^{6}M^{-1}*



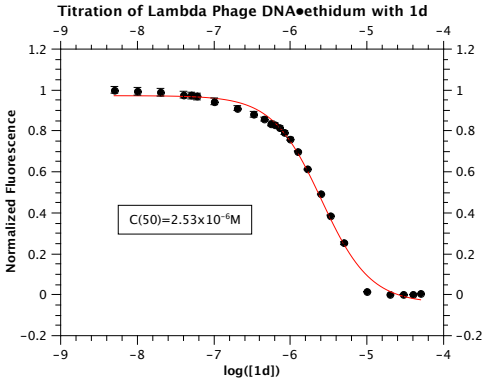
Affinity of **1d** for Lambda Phage DNA

Trial 1: K_{app}=9.05x10^{6}M^{-1}

Trial 2: K_{app}=8.05x10^{6}M^{-1}

Trial 3: K_{app}=6.95x10^{6}M^{-1}

Average *K_{app}=8.02±0.71x10^{6}M^{-1}*



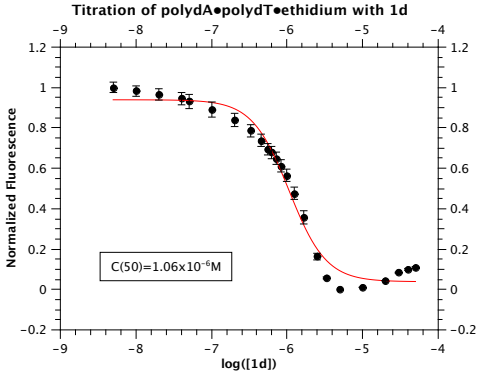
Affinity of **1d** for polydA•polydT

Trial 1: K_{app}=20.2x10^{6}M^{-1}

Trial 2: K_{app}=16.0x10^{6}M^{-1}

Trial 3: K_{app}=23.4x10^{6}M^{-1}

Average *K_{app}=20.0±2.5x10^{6}M^{-1}*



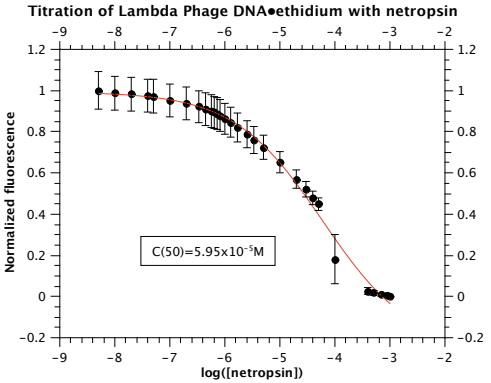
Affinity of netropsin for lambda phage DNA

Trial 1: K_{app}=4.18x10^{5}M^{-1}

Trial 2: K_{app}=3.57x10^{5}M^{-1}

Trial 3: K_{app}=3.63x10^{5}M^{-1}

Average *K_{app}=3.79±0.26x10^{5}M^{-1}*



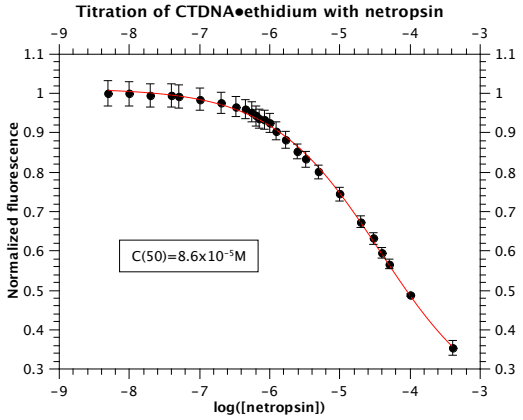
Affinity of netropsin for CT DNA

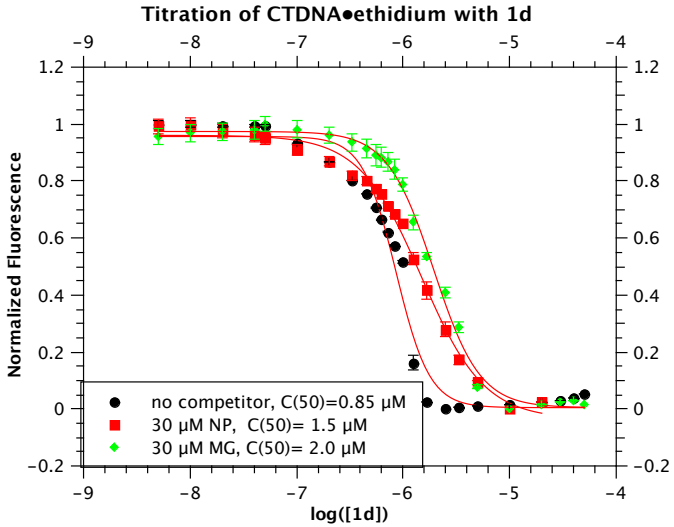
Trial 1: K_{app}=3.30x10^{5}M^{-1}

Trial 2: K_{app}=1.80x10^{5}M^{-1}

Trial 3: K_{app}=1.24x10^{5}M^{-1}

Average K_{app}=*2.11±0.79x10^{5}M^{-1}*





No competitor, K_a=2.47±1.5 x10^7 M^{-1}; 30µM NP, K_a=1.40±1.2 x10^7 M^{-1}; 30µM MG, 1.05±0.9 x 10^{-7} M^{-1}

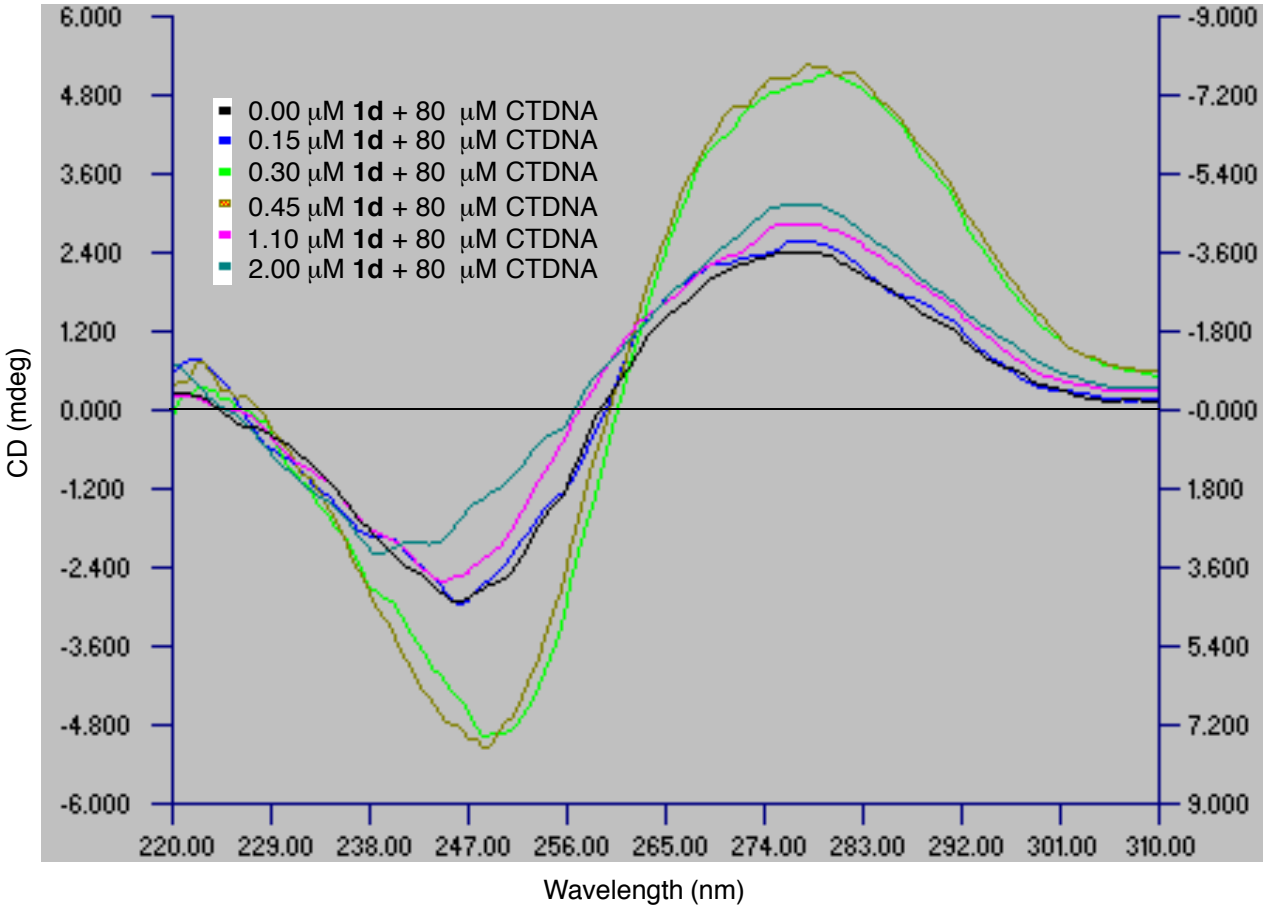
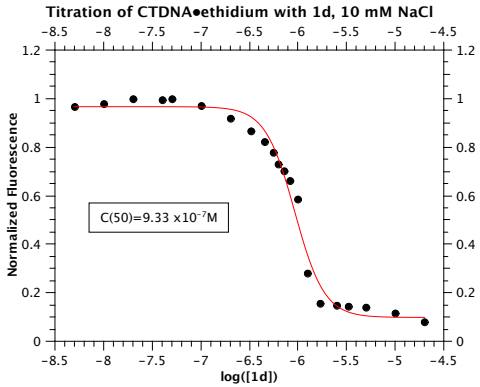
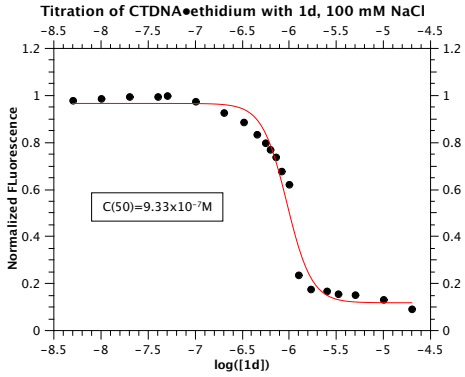


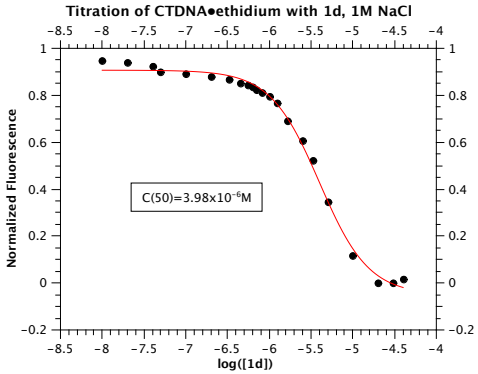
Figure 5. The 220-310 nm region of the CD spectrum of solutions of CT DNA (80 μM) in the absence (black line) and presence of various concentrations of **1d**: blue line, 0.15 μM **1d**; light green line, 0.30 μM **1d**; orange line, 0.45 μM **1d**; red line, 1.1 μM **1d**; dark green line 2.00 μM **1d**



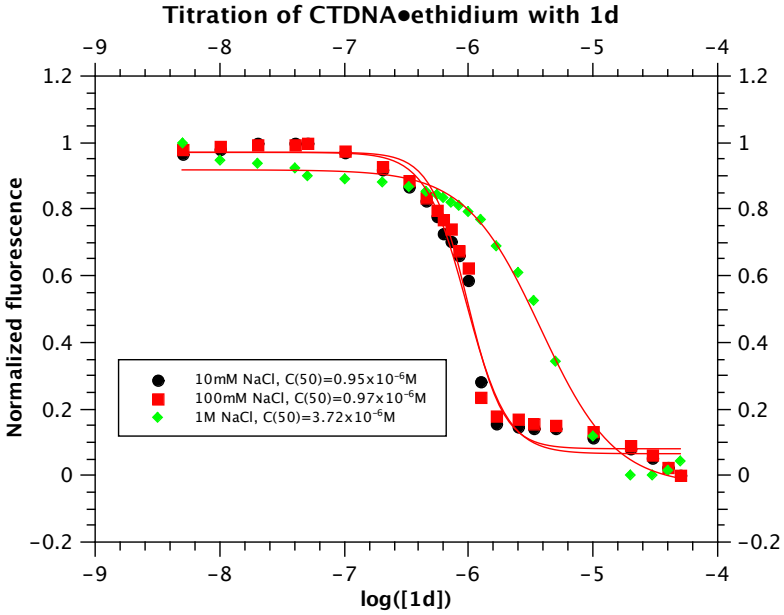
10mM NaCl: K_{app}=**2.25 x 10^{7}M^{-1}**

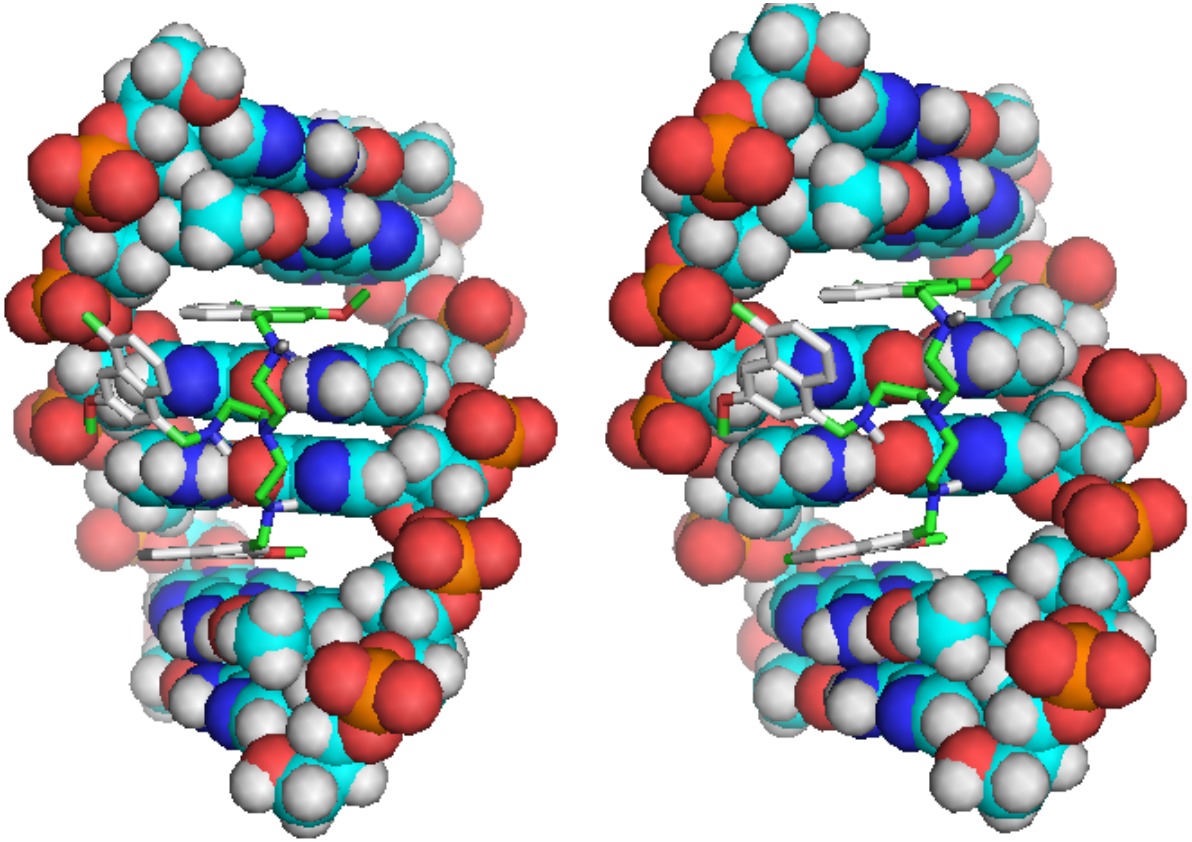


100mM NaCl: K_{app}=**2.25 x 10^{7}M^{-1}**



1M NaCl: K_{app}=**5.30 x 10^{6}M^{-1}**



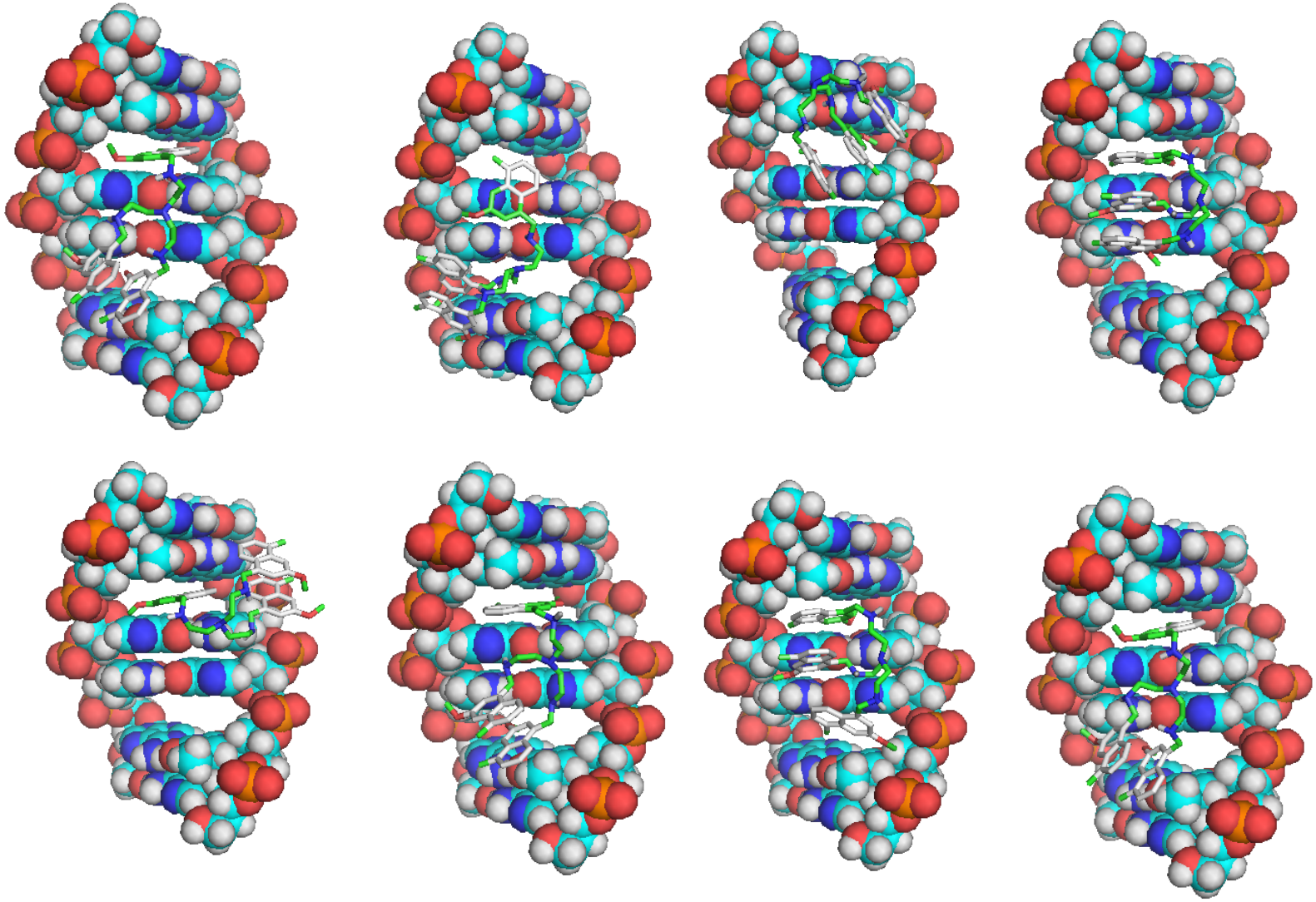


Model for bis-intercalation of ligand 1d in the major groove of DNA sequence 5'-ATGCAT-3',

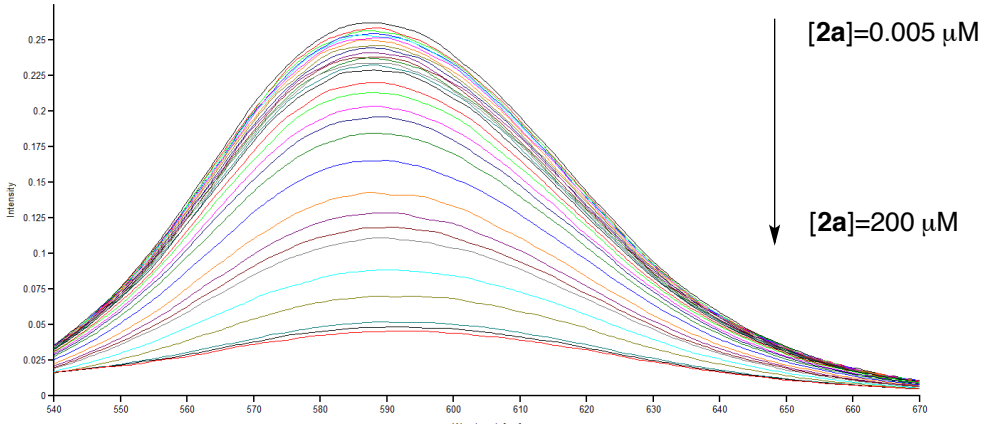
generated by Autodock Vina using DNA (PDB 1x95) and 1d minimized by Spartan 14 for Macintosh

Additional binding modes for the association of 1d with DNA sequence 5’-ATGCAT-3’,

generated by Autodock Vina using DNA (PDB 1x95) and 1d minimized by Spartan 14 for Macintosh

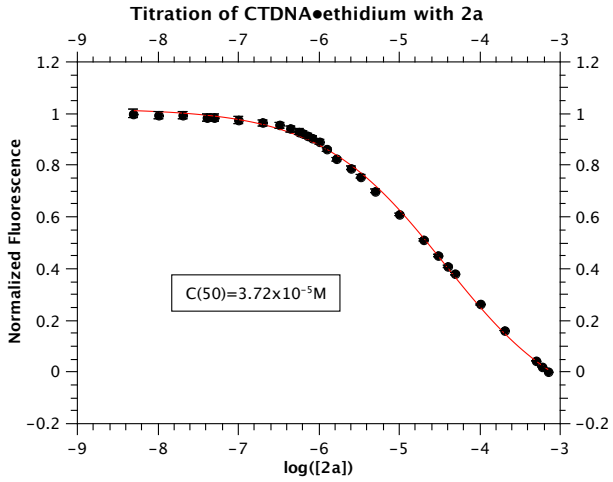


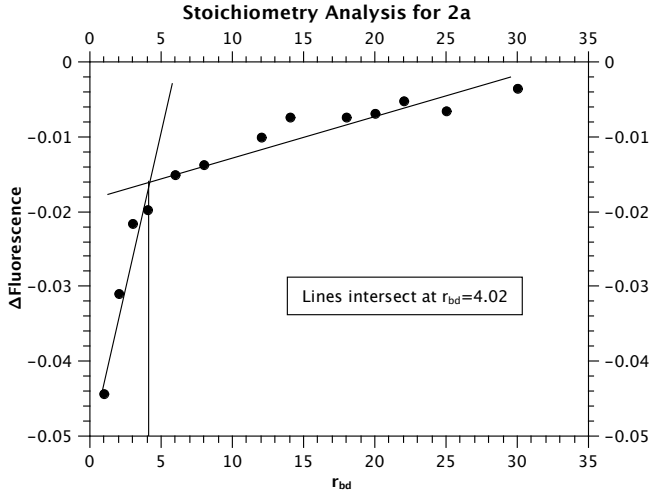
Compound 2a Ethidium displacement studies; K_{app}=2.1x10^{6}M^{-1} x 5/C50



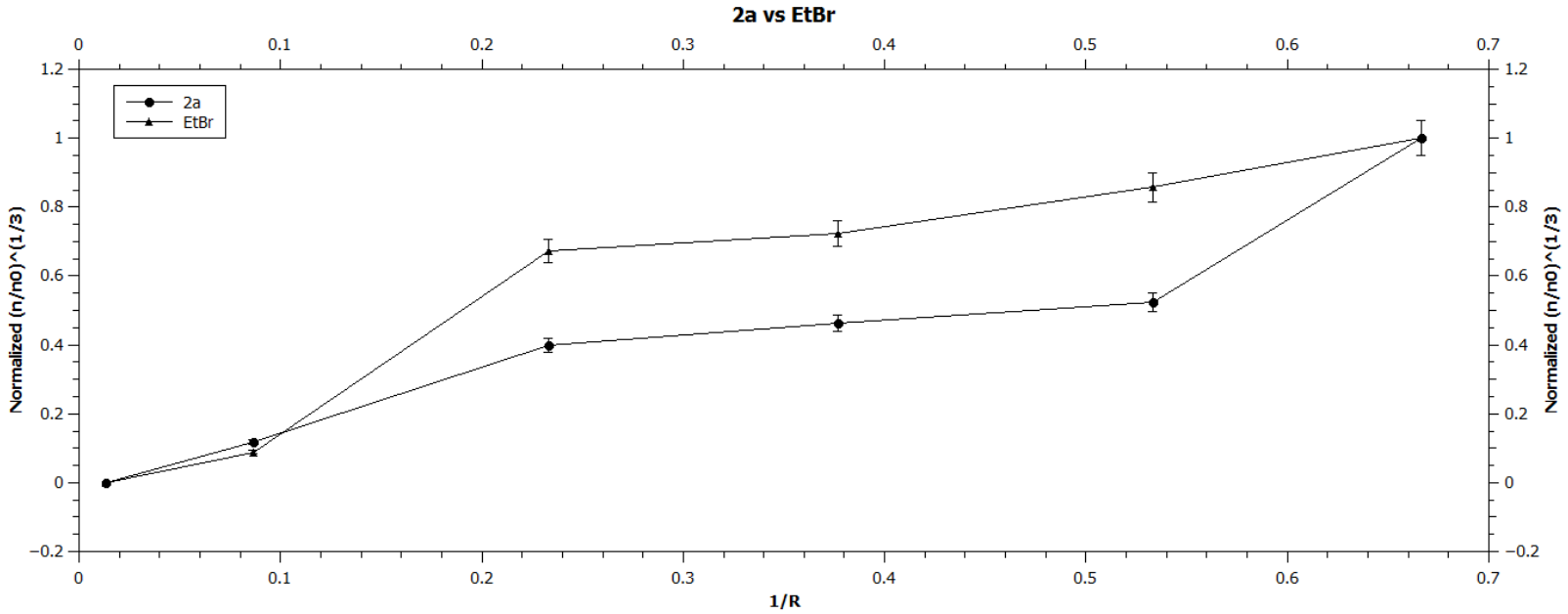
Titration of CT DNA (10 μM) and ethidium (10 μM) with **3b**: 0.005, 0.01, 0.02, 0.04, 0.05, 0.1, 0.2, 0.33, 0.45, 0.56, 0.63, 0.71, 0.83, 1.00, 1.25, 1.67, 2.50, 3.30, 5.0, 10.0, 20.0, 30.0, 40.0, 50.0, 60.0, 100.0, 200.0 μM

Trial 1: K_{app}= 4.36 x10^{5}M^{-1}; Trial 2: K_{app}=5.0x10^{5}M^{-1}; Trial 3: K_{app}= 4.79x10^{5}M^{-1}



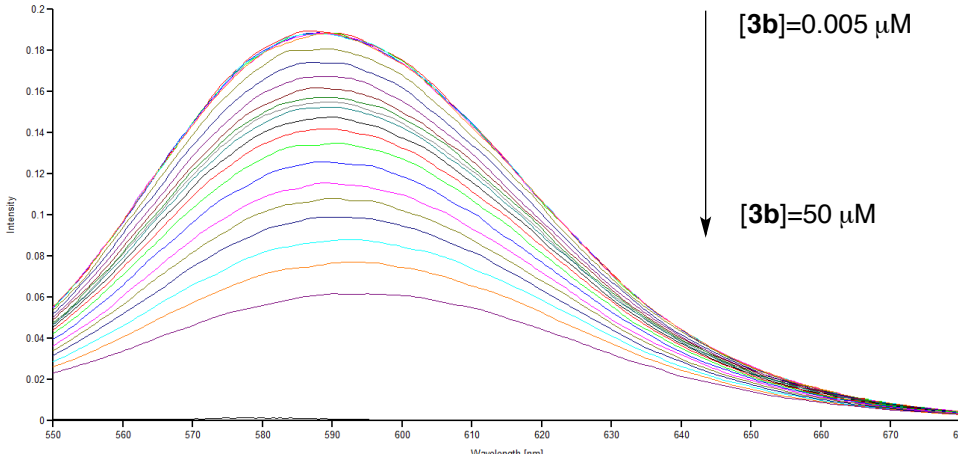


3 trials: **Average K_{app}= 4.72±0.27x10^{5}M^{-1}**



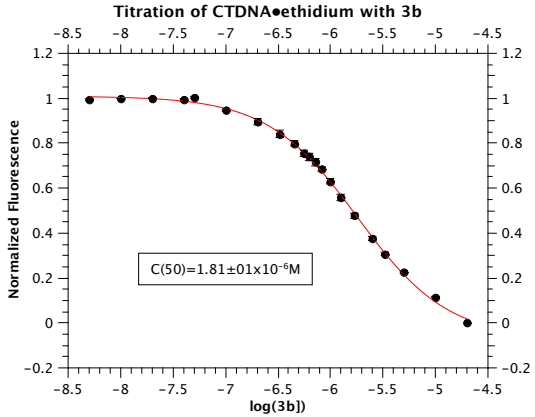
Effect of increasing amount of EtBr (▲) and **2a** (●) on relative viscosity of CT-DNA. EtBr (▲): [CT-DNA] = 300 μM, [EtBr]= 4, 26, 70, 113, 160, and 200 μM; 2a (●): [CT-DNA] = 300 μM, [**2a**]: 1, 2, 4, 10, 18, and 26 μM.

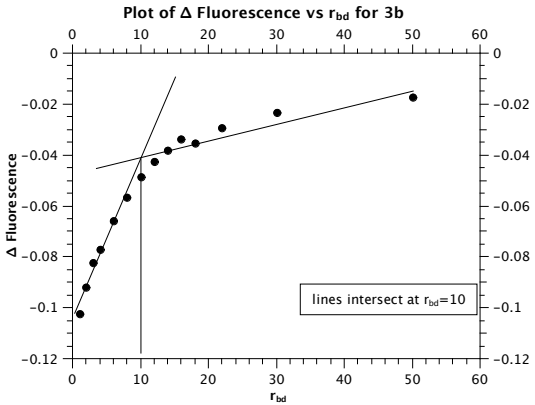
Compound 3b Ethidium displacement studies; K_{app}=2.1x10^{6}M^{-1} x 10/C50



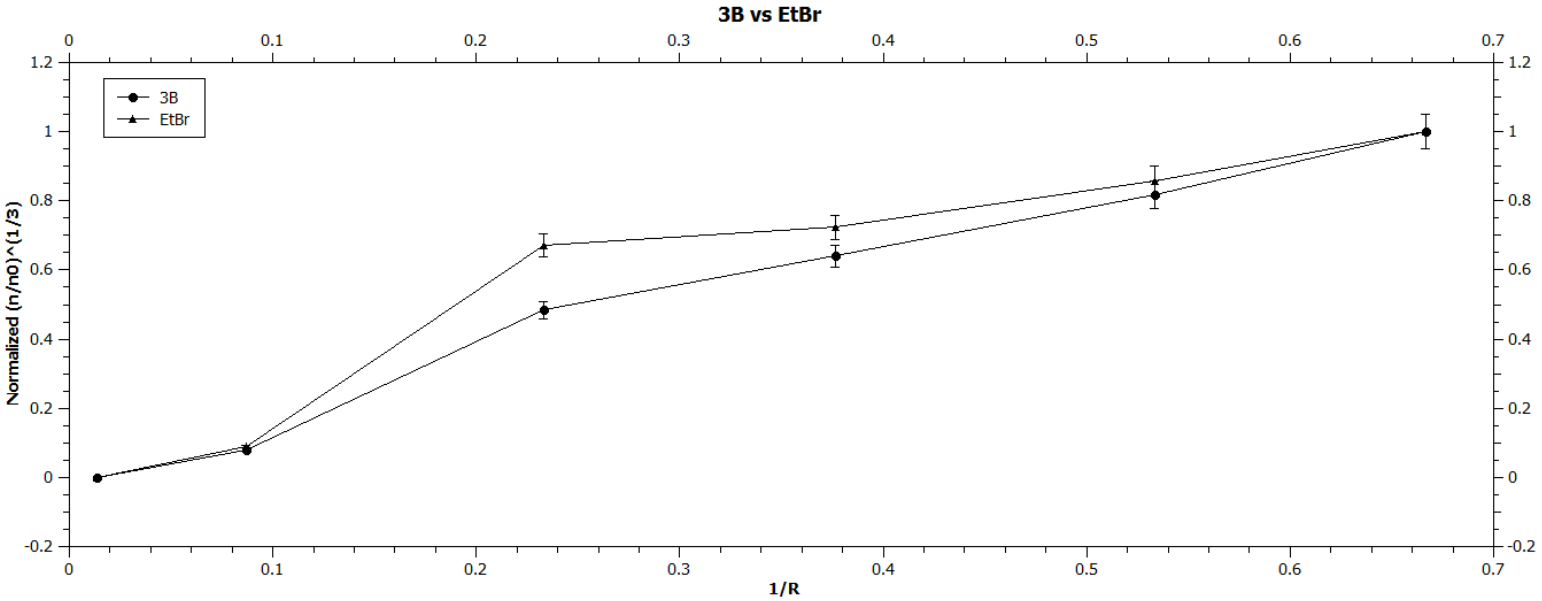
Titration of CT DNA (10 μM) and ethidium (10 μM) with **3b**: 0.005, 0.01, 0.02, 0.04, 0.05, 0.1, 0.2, 0.33, 0.45, 0.56, 0.63, 0.71, 0.83, 1.00, 1.25, 1.67, 2.50, 3.30, 5.0, 10.0, 20.0, 50.0 μM

Trial 1: K_{app}=6.88 x10^{6}M^{-1}; Trial 2: K_{app}=6.99 x10^{6}M^{-1}; Trial 3: K_{app}=5.55 x10^{6}M^{-1}





3 trials: **Average K_{app}=6.47±0.65 x10^{6}M^{-1}**



Effect of increasing amount of EtBr (▲) and **3b** (●) on relative viscosity of CT-DNA. EtBr (▲): [CT-DNA] = 300 μM, [EtBr]= 4, 26, 70, 113, 160, and 200 μM; **3b** (●): [CT-DNA] = 300 μM, [**3b**]: 1, 2, 4, 10, 18, and 26 μM.