Supporting Information Available

Design, synthesis and DNA/RNA binding studies of nucleic acids comprising stereoregular and acyclic polycarbamate backbone: Polycarbamate Nucleic Acids (PCNA)

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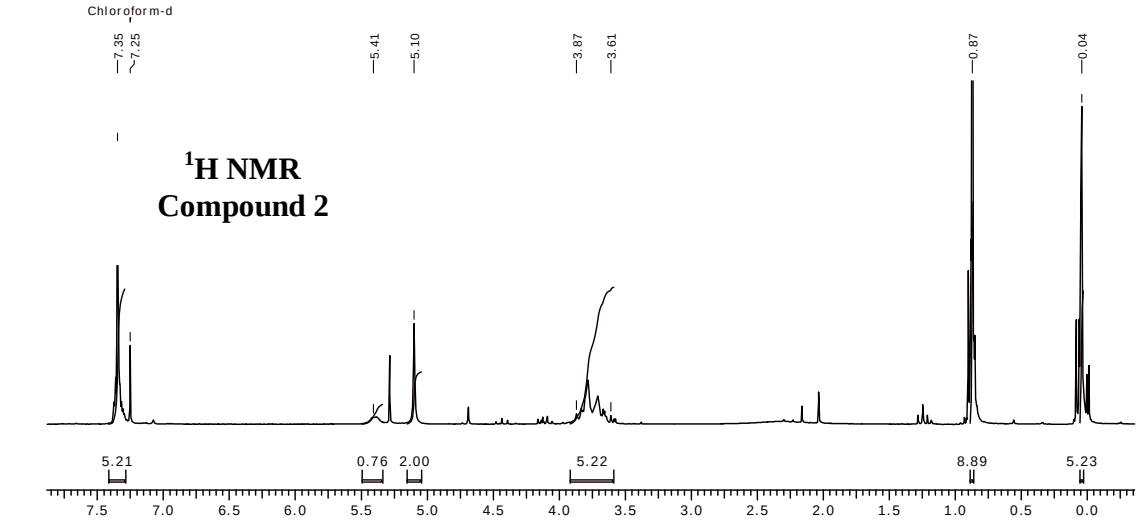
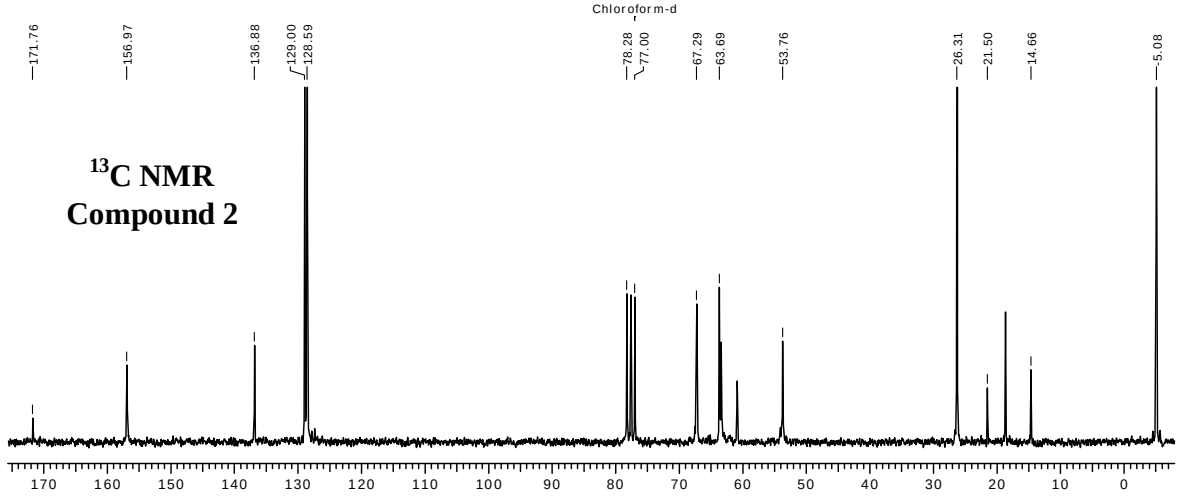
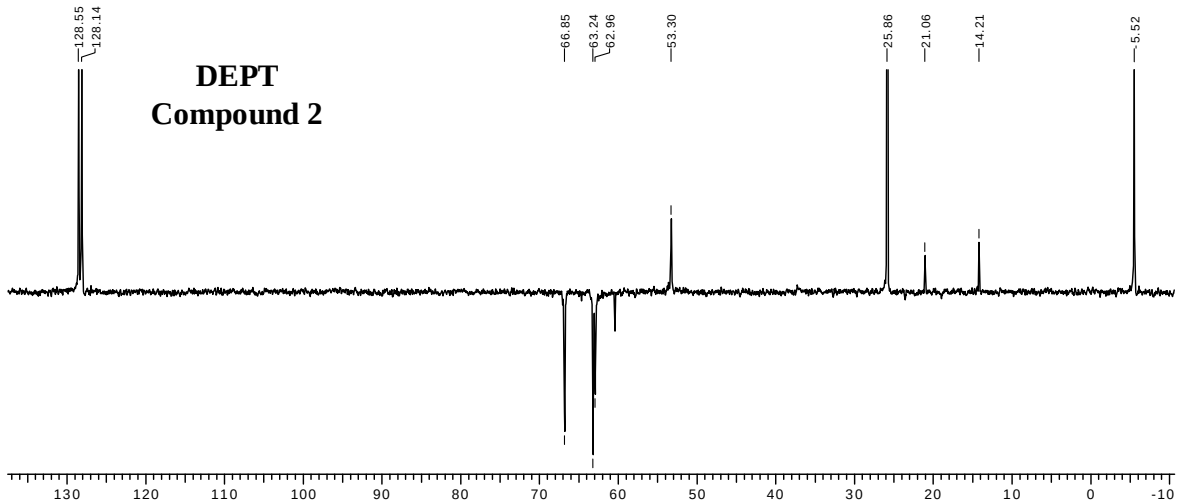
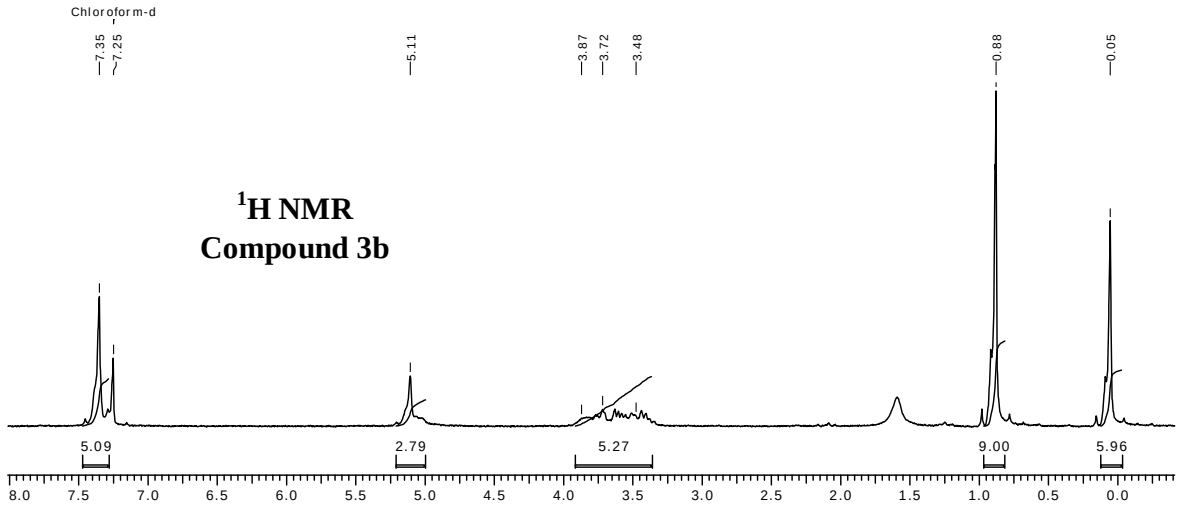
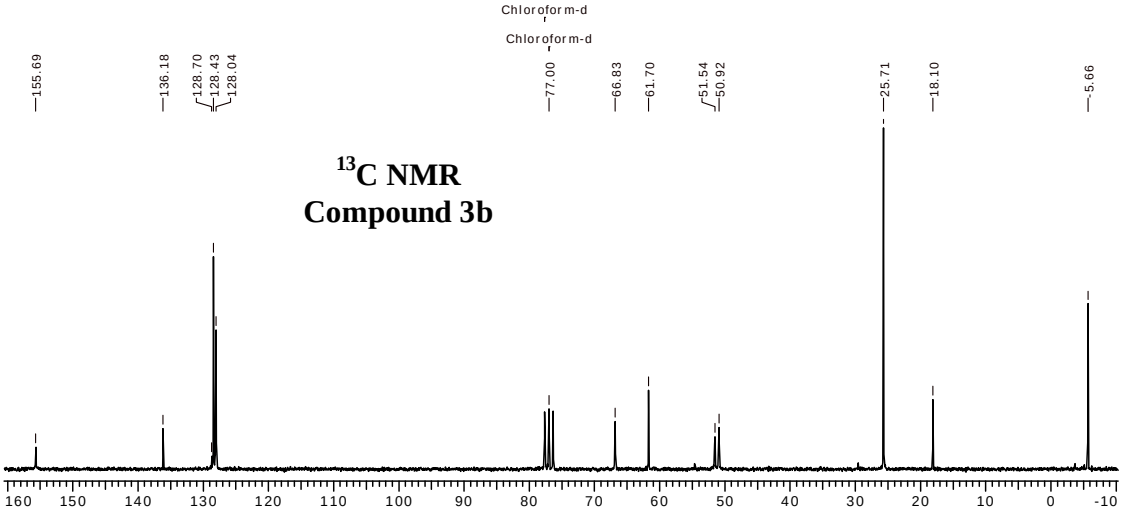

Chloroform-d
7.35
7.25
5.41
5.10
3.87
3.61
0.87
0.04
1H NMR
Compound 2
5.21
0.76
2.00
5.22
8.89
5.23


Chloroform-d
171.76
156.97
136.88
129.00
128.59
78.28
77.00
67.29
63.69
53.76
26.31
21.50
14.66
5.08
13C NMR
Compound 2

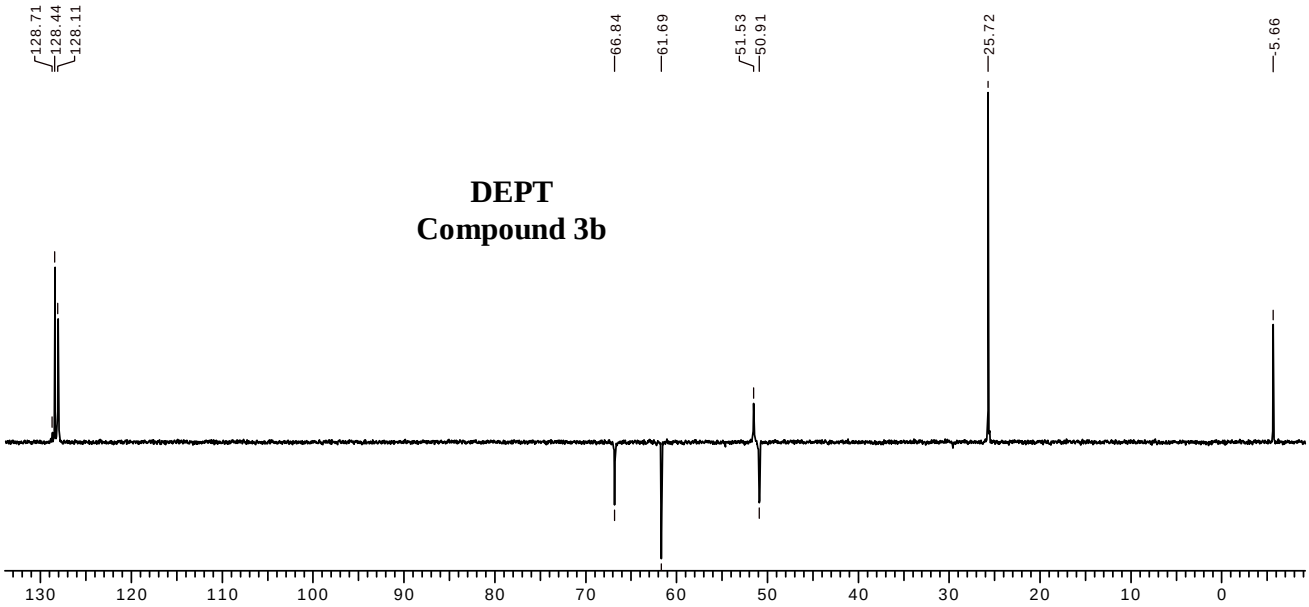

128.55
128.14
66.85
63.24
62.96
53.30
25.86
21.06
14.21
5.52
DEPT
Compound 2



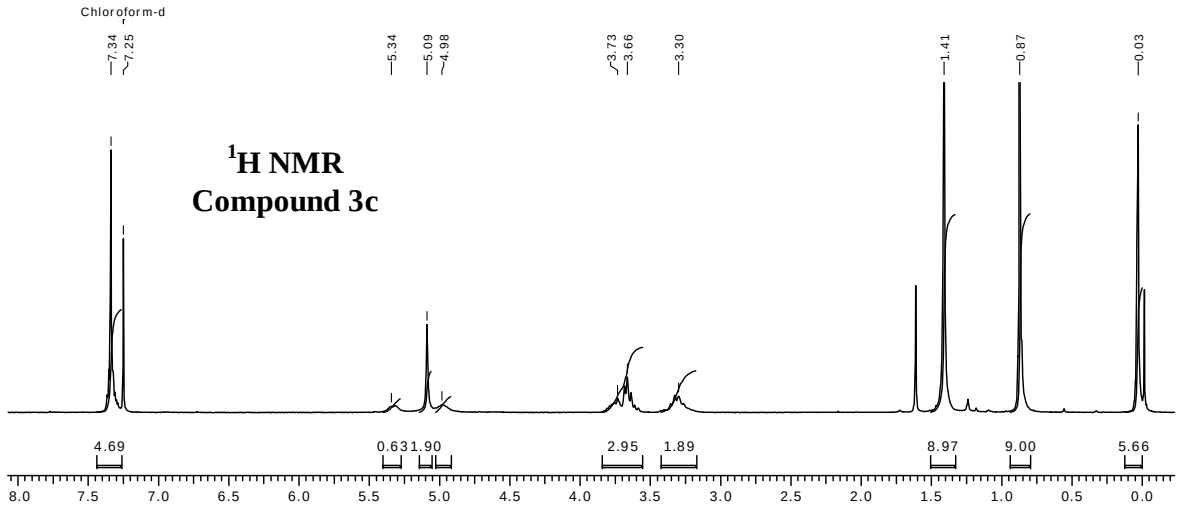
^{1}H NMR
Compound 3b

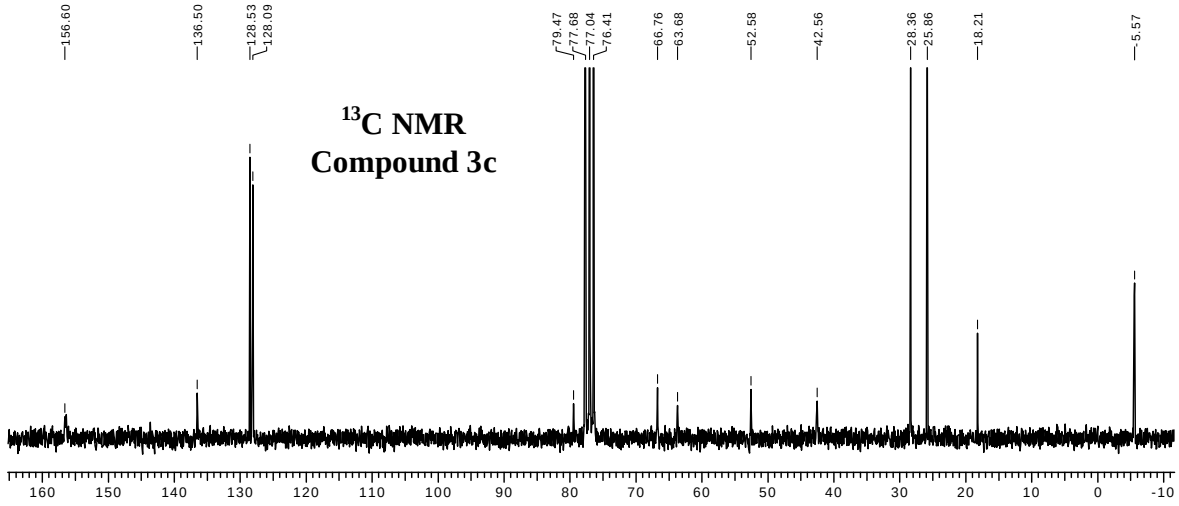


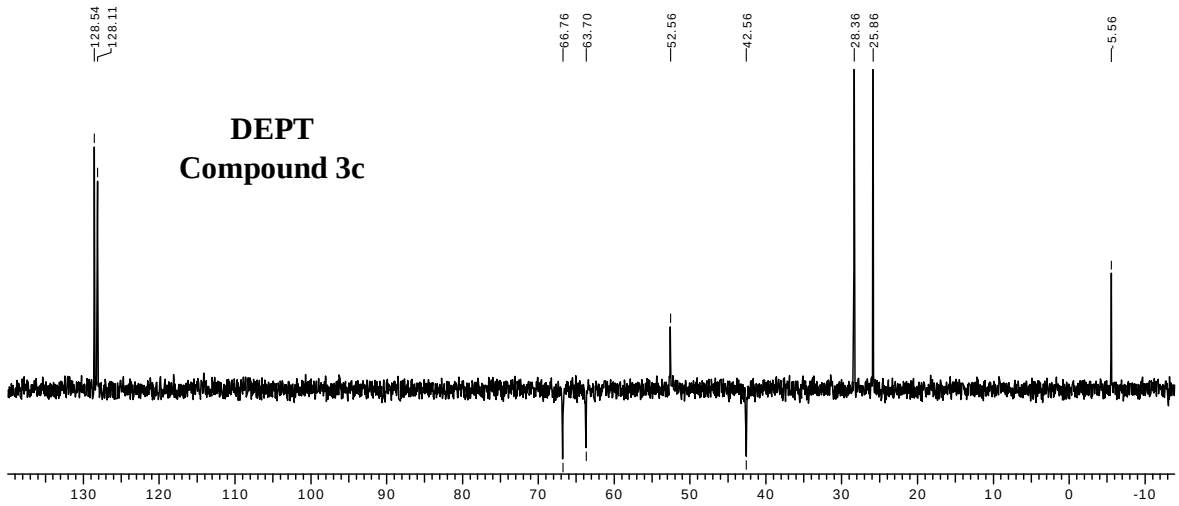
^{13}C NMR
Compound 3b

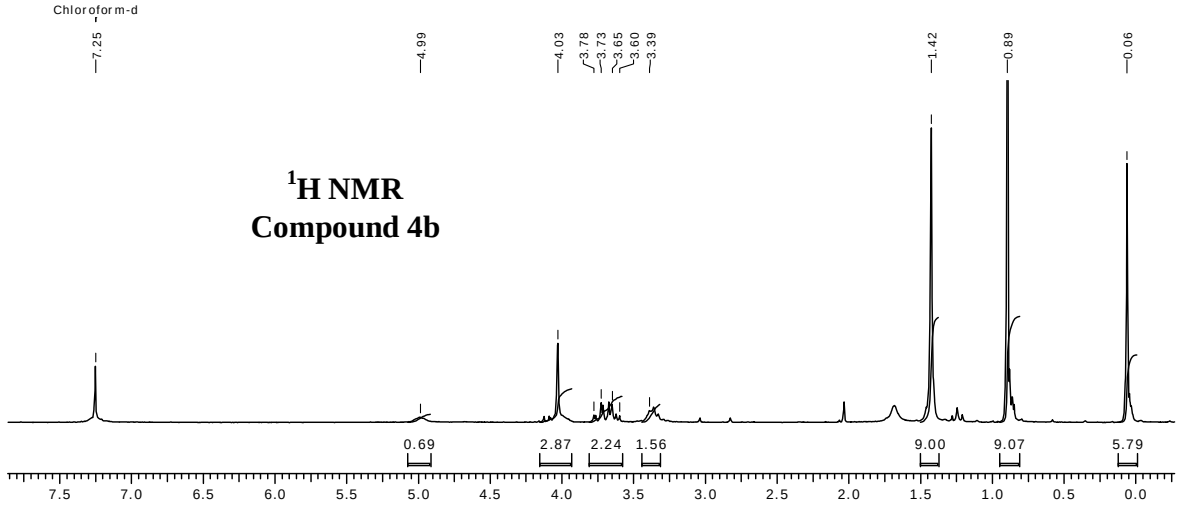


DEPT
Compound 3b

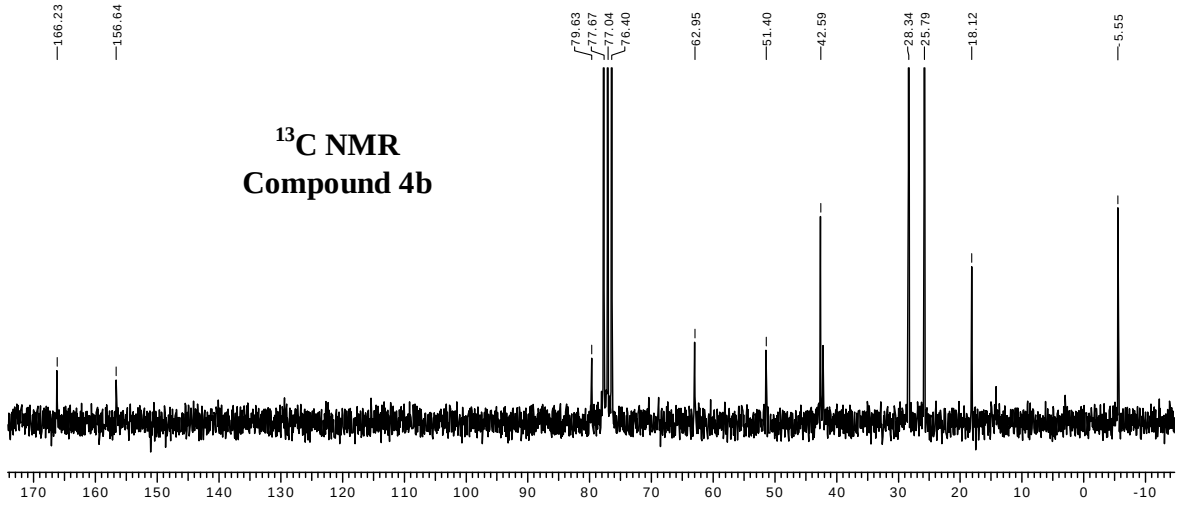

Chloroform-d
7.34
7.25
5.34
5.09
4.98
3.73
3.66
3.30
1.41
0.87
0.03
^{1}H NMR
Compound 3c
4.69
0.63
1.90
2.95
1.89
8.97
9.00
5.66


156.60
136.50
128.53
128.09
79.47
77.68
77.04
76.41
66.76
63.68
52.58
42.56
28.36
25.86
18.21
5.57
^{13}C NMR
Compound 3c

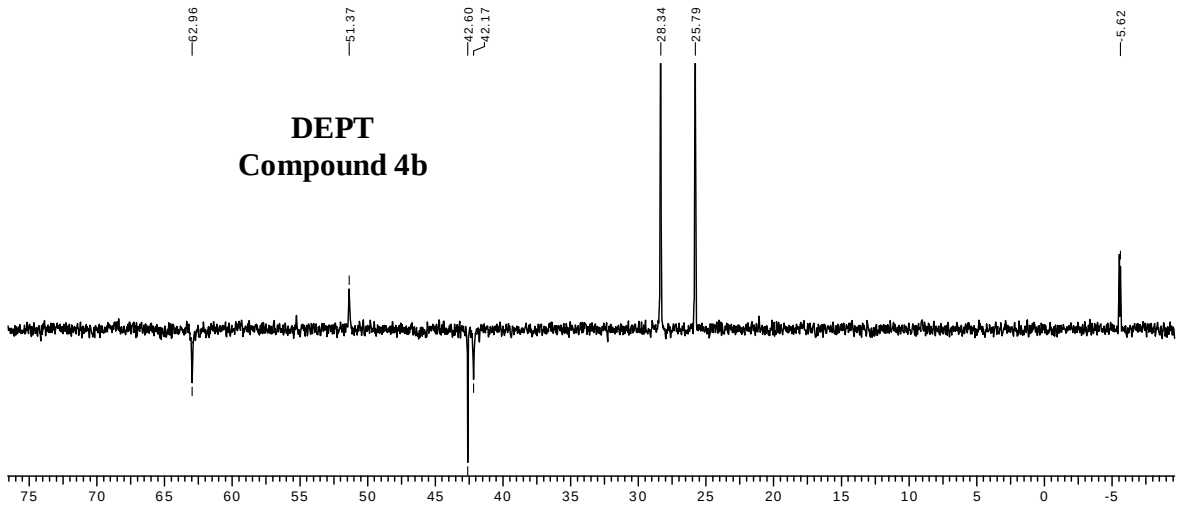

128.54
128.11
66.76
63.70
52.56
42.56
28.36
25.86
5.56
DEPT
Compound 3c



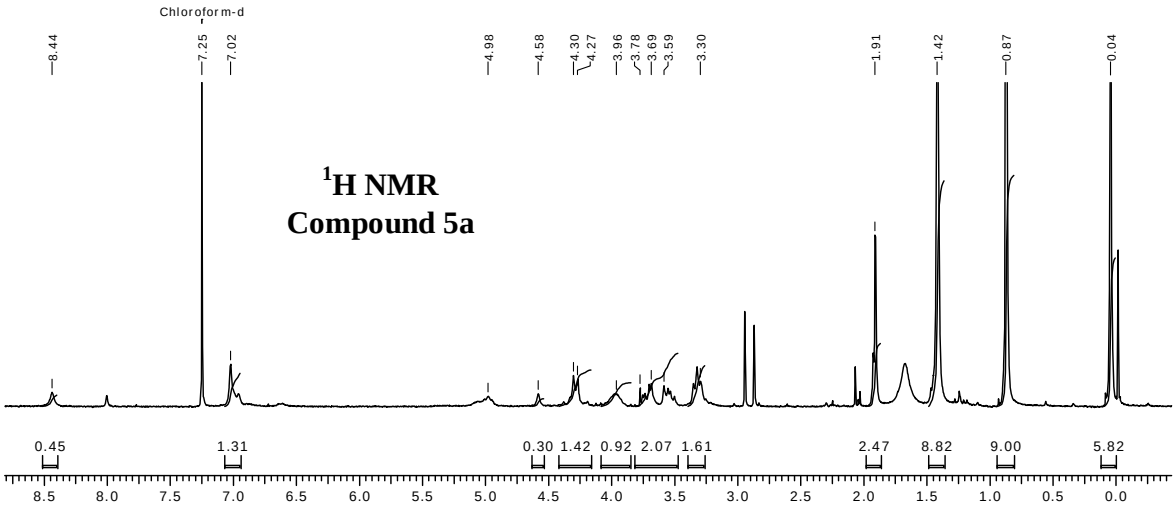
^{1}H NMR
Compound 4b



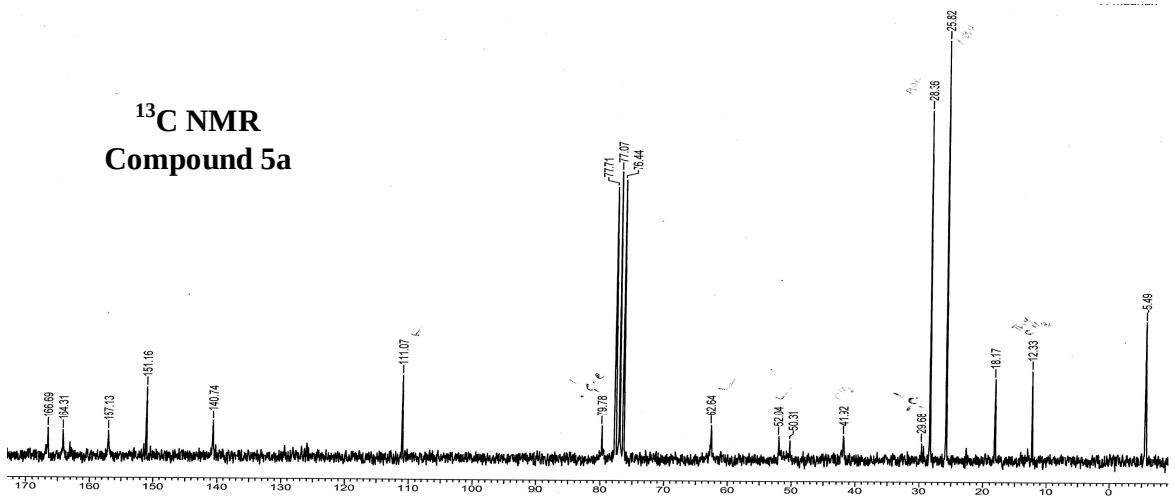
^{13}C NMR
Compound 4b



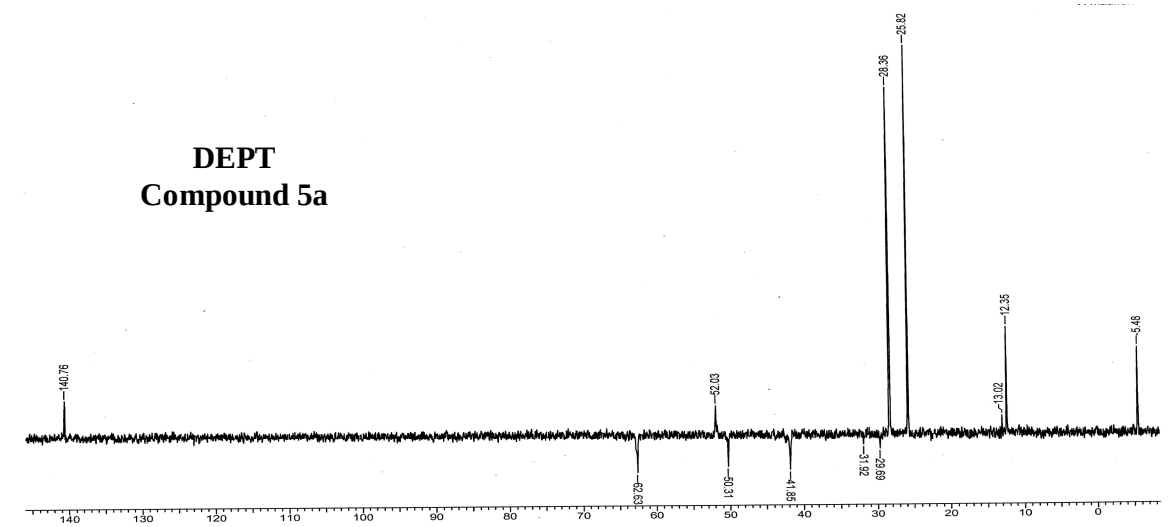
DEPT
Compound 4b



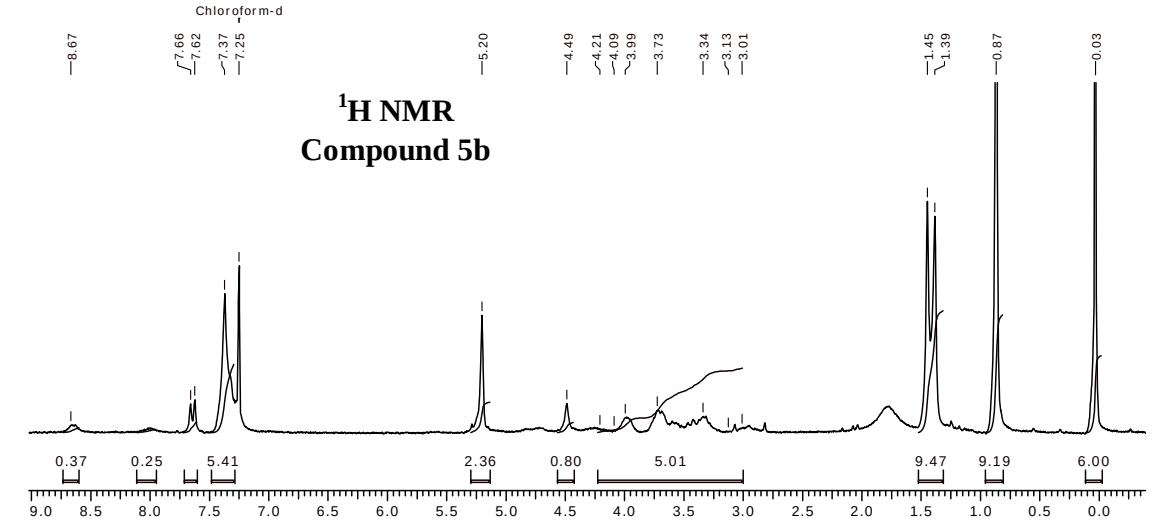
^{1}H NMR
Compound 5a



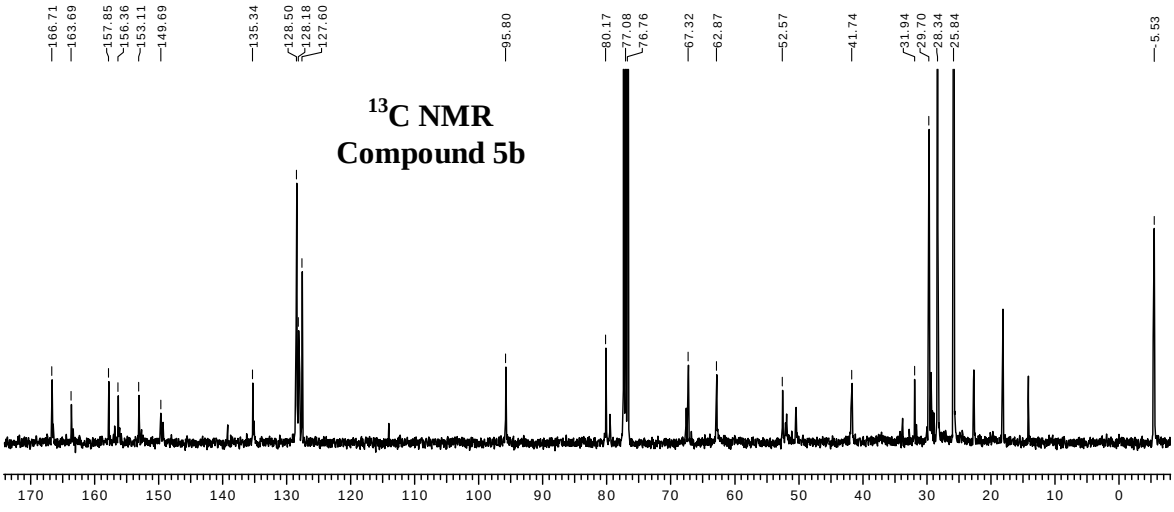
^{13}C NMR
Compound 5a



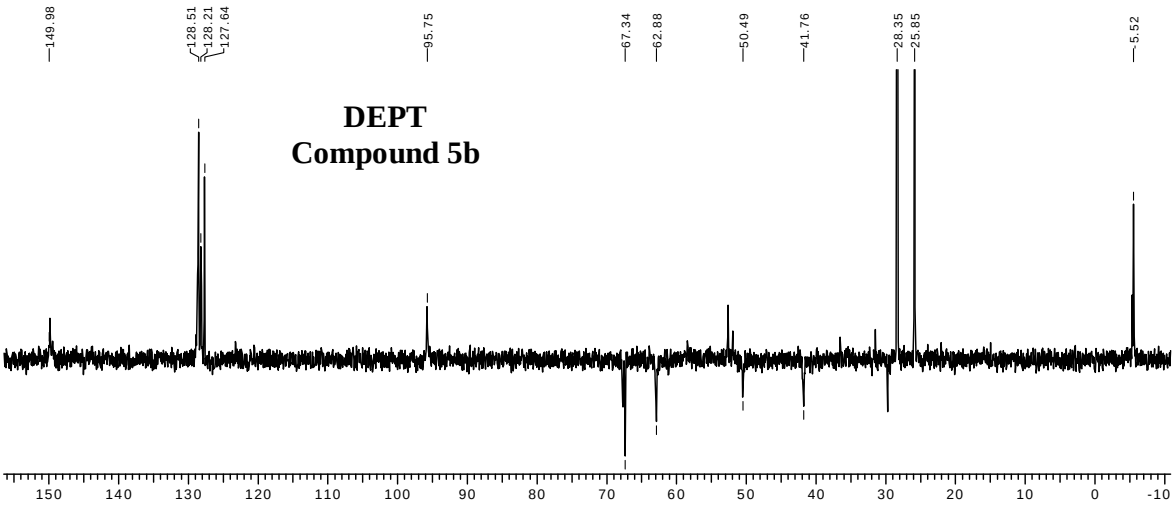
DEPT
Compound 5a



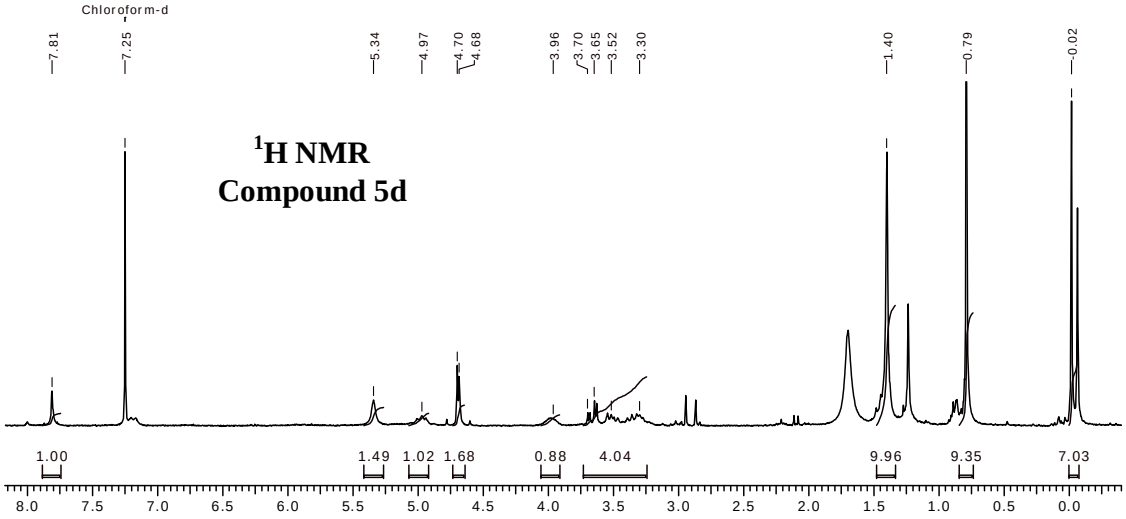
^{1}H NMR
Compound 5b



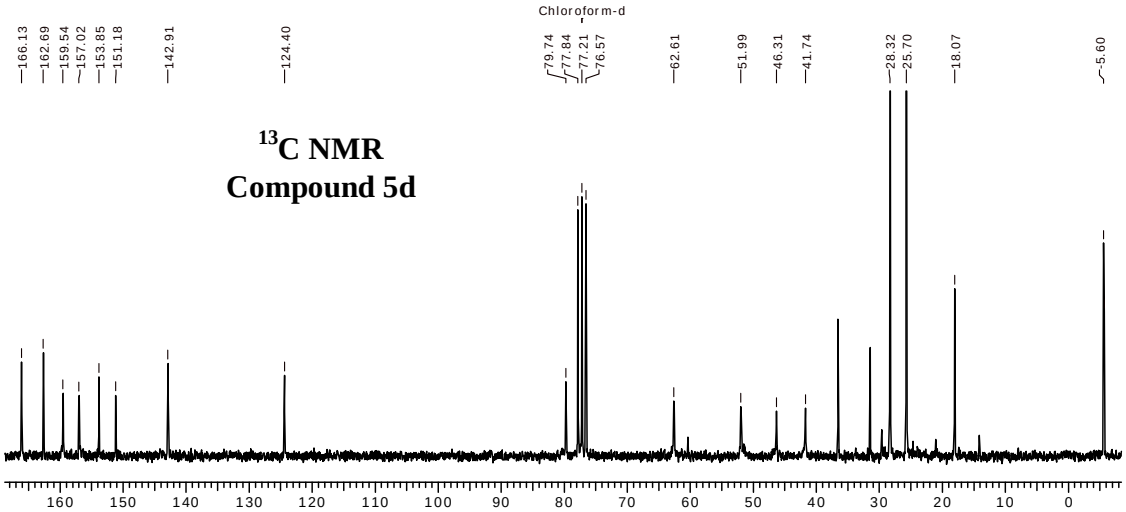
^{13}C NMR
Compound 5b



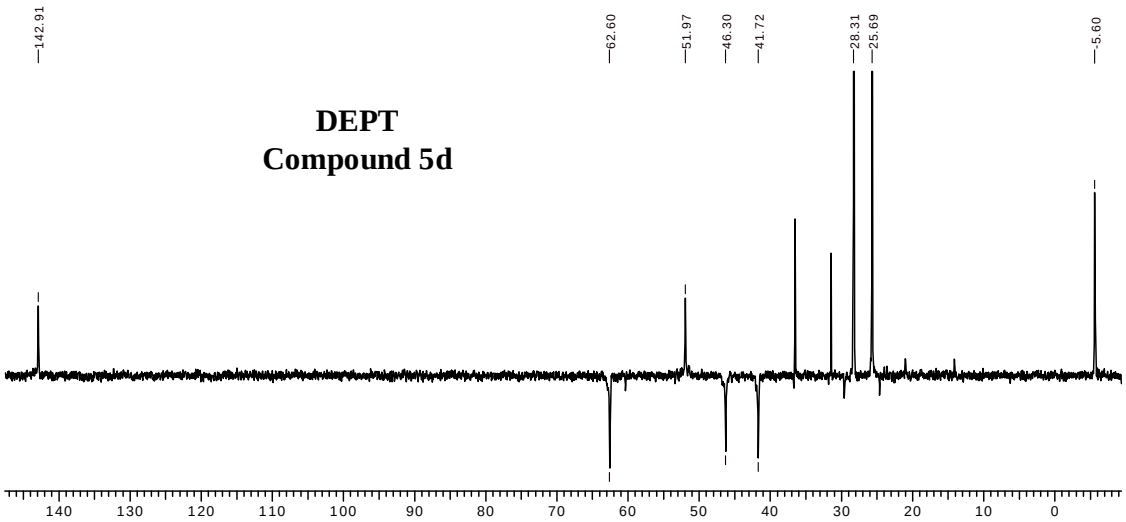
DEPT
Compound 5b



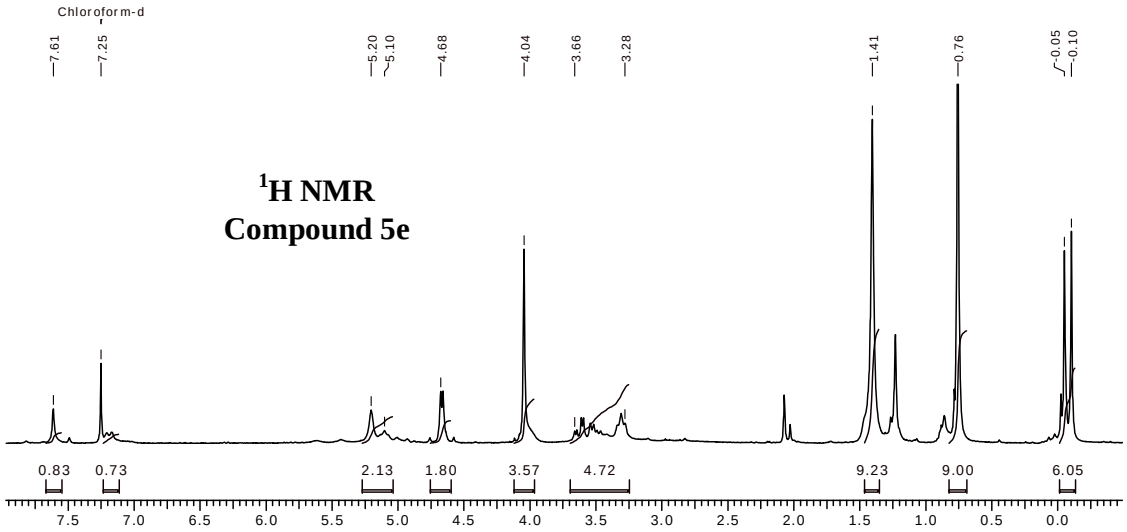
^{1}H NMR
Compound 5d

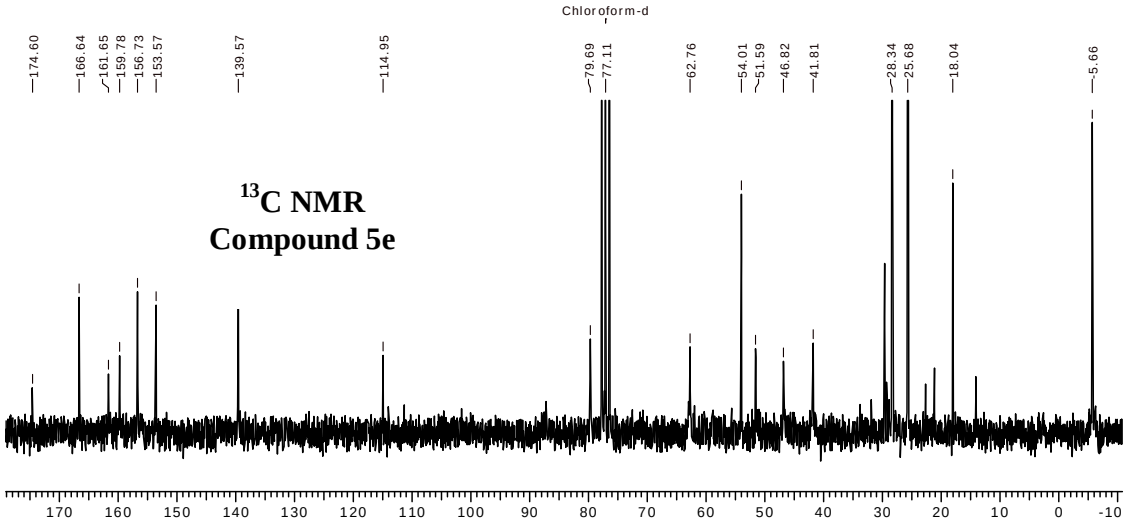


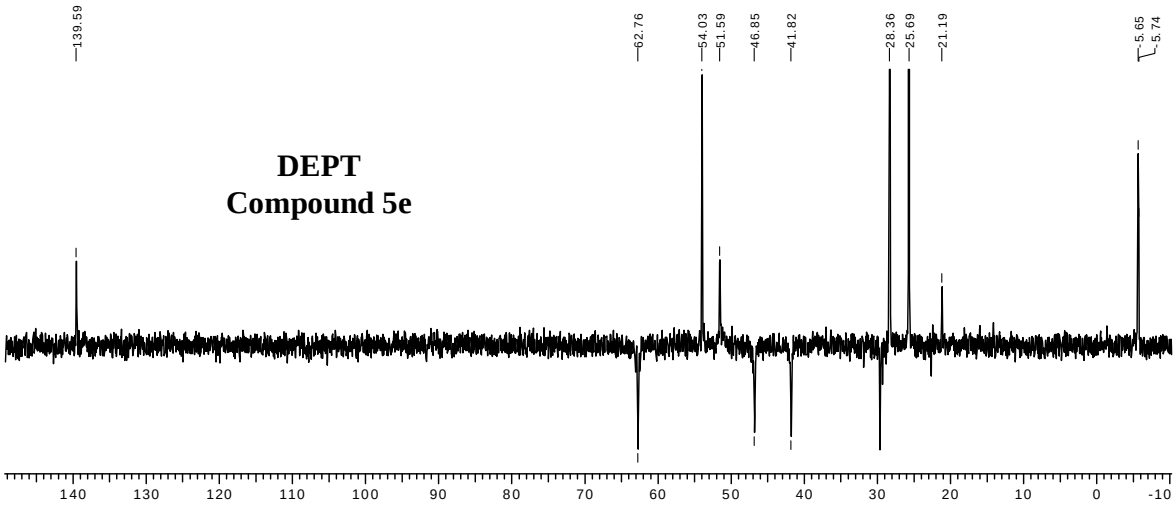
^{13}C NMR
Compound 5d

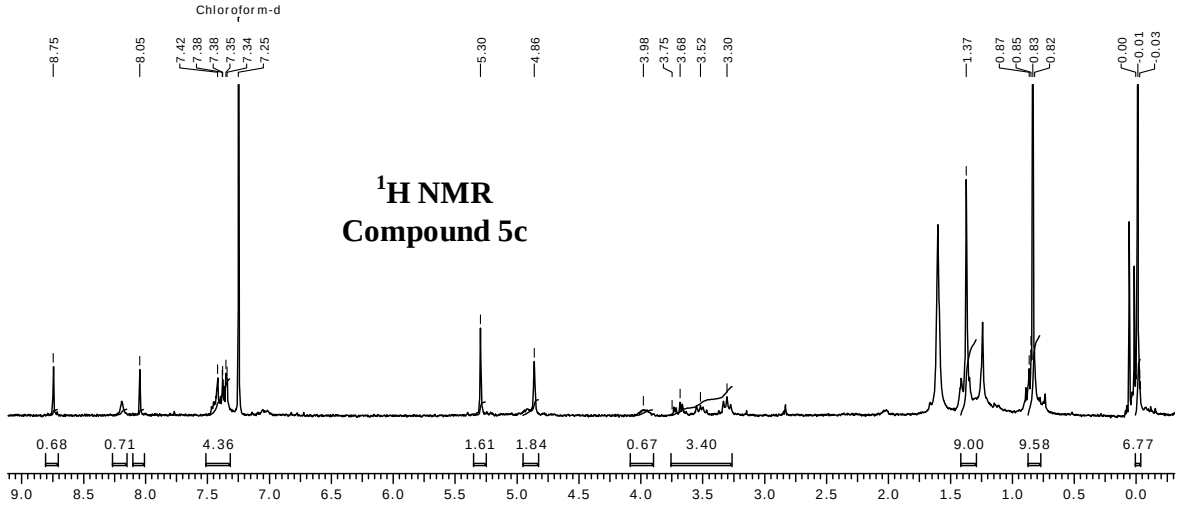


DEPT
Compound 5d

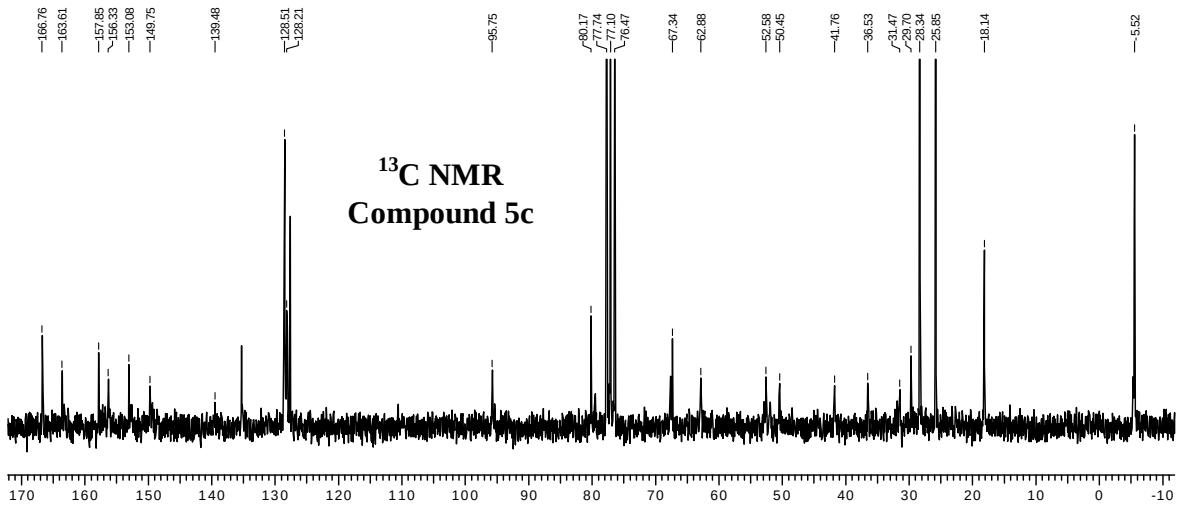

Chloroform-d
7.61
7.25
5.20
5.10
4.68
4.04
3.66
3.28
1.41
0.76
0.05
0.10
^{1}H NMR
Compound 5e
0.83
0.73
2.13
1.80
3.57
4.72
9.23
9.00
6.05


Chloroform-d
174.60
166.64
161.65
159.78
156.73
153.57
139.57
114.95
79.69
77.11
62.76
54.01
51.59
46.82
41.81
28.34
25.68
18.04
5.66
^{13}C NMR
Compound 5e


139.59
62.76
54.03
51.59
46.85
41.82
28.36
25.69
21.19
5.65
5.74
DEPT
Compound 5e



^{1}H NMR
Compound 5c



^{13}C NMR
Compound 5c

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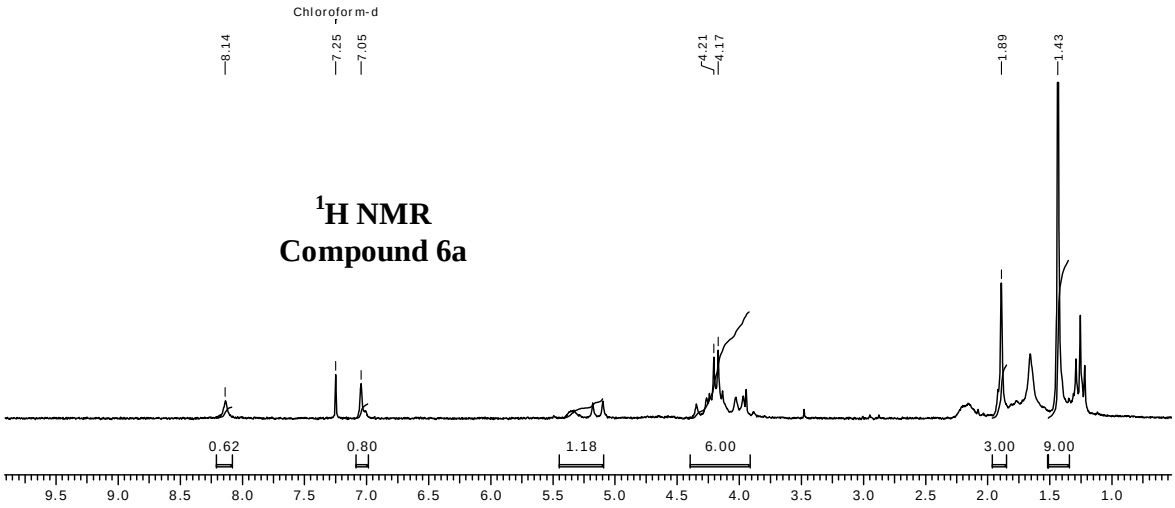
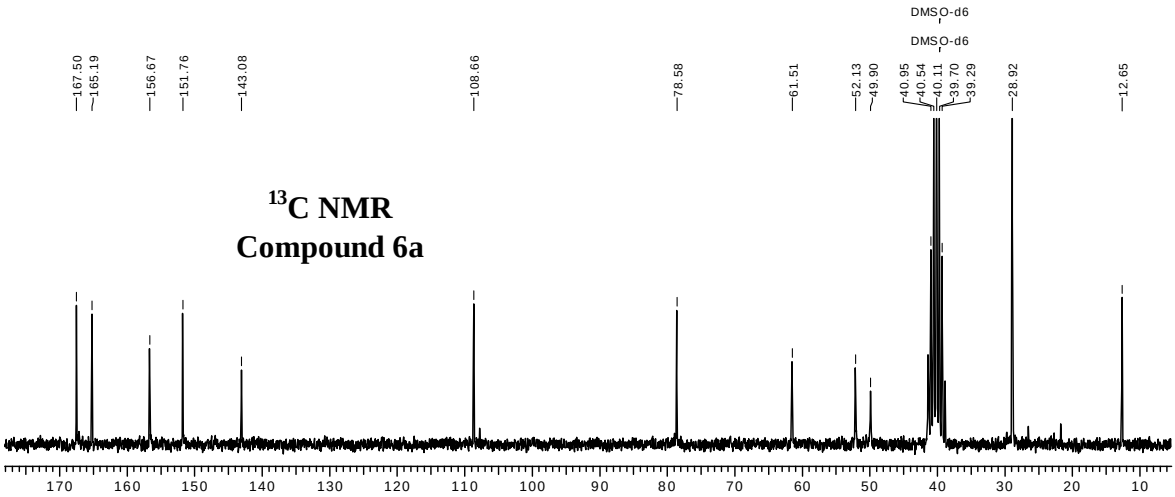
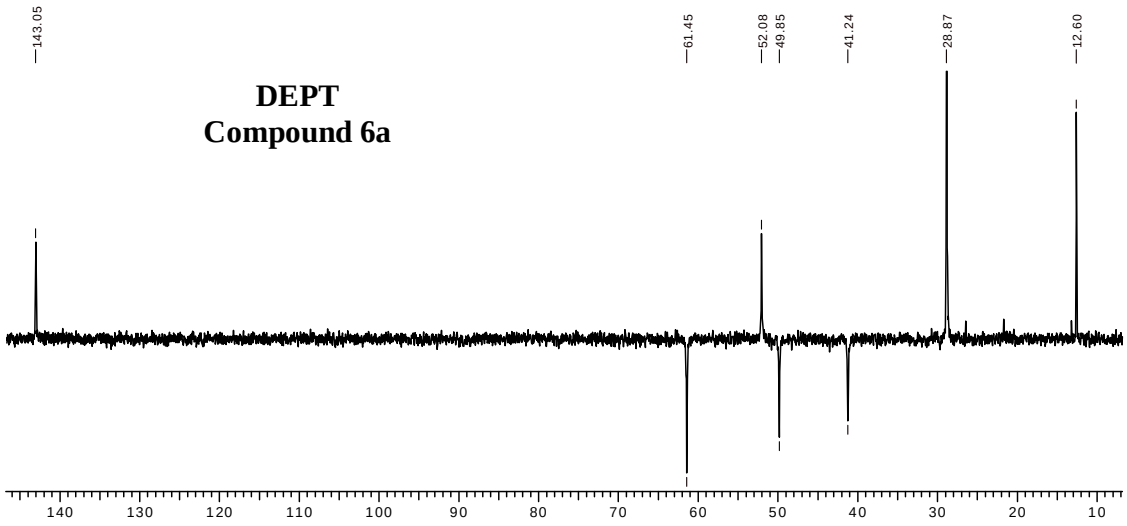


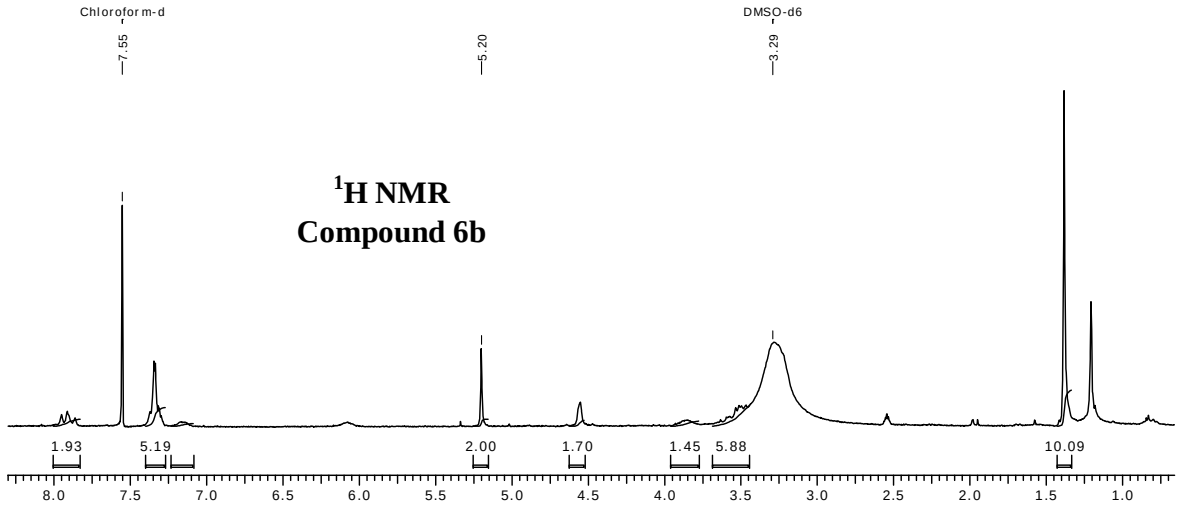
^{1}H NMR
Compound 6a

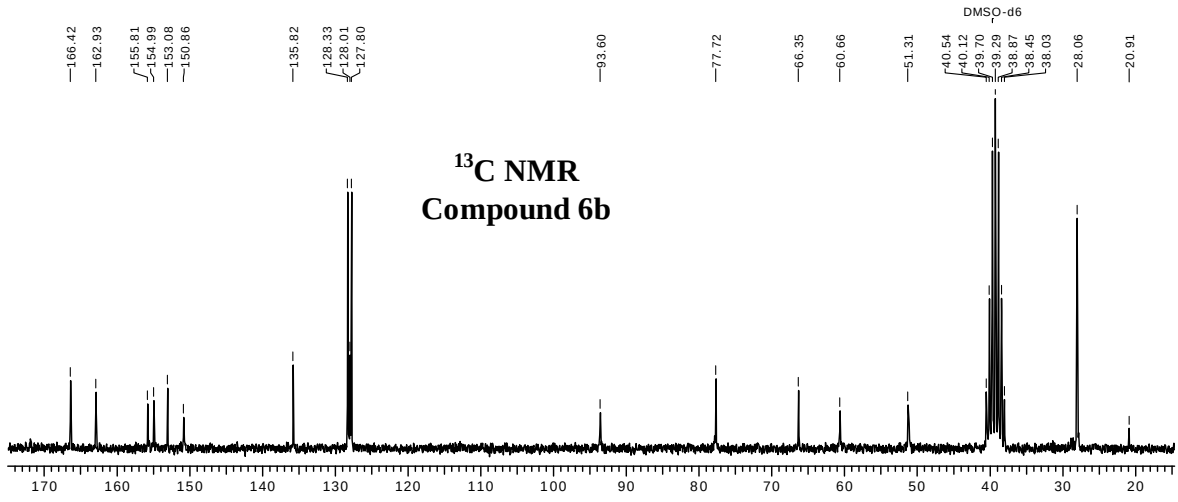


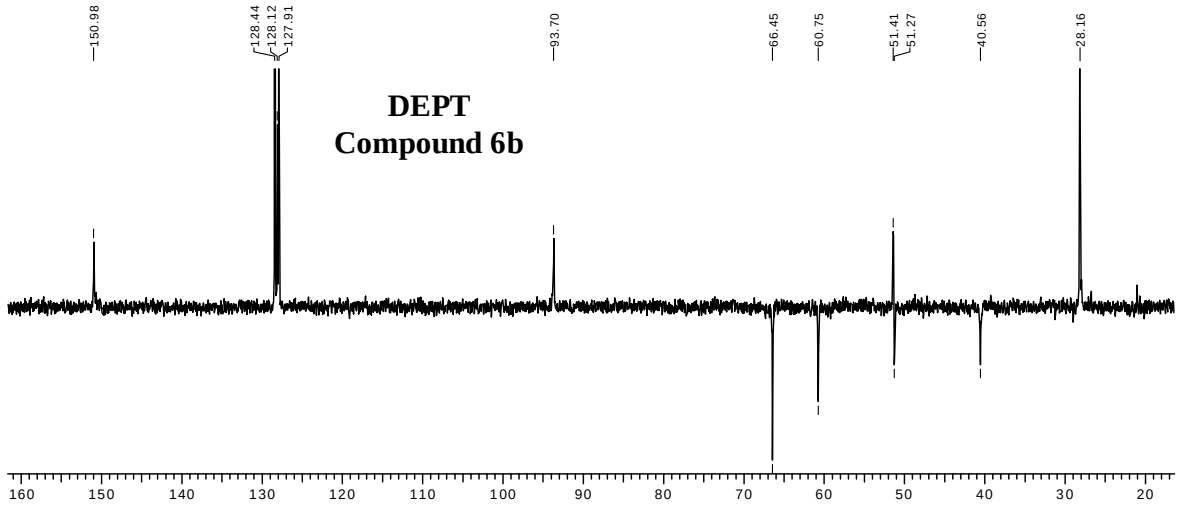
^{13}C NMR
Compound 6a

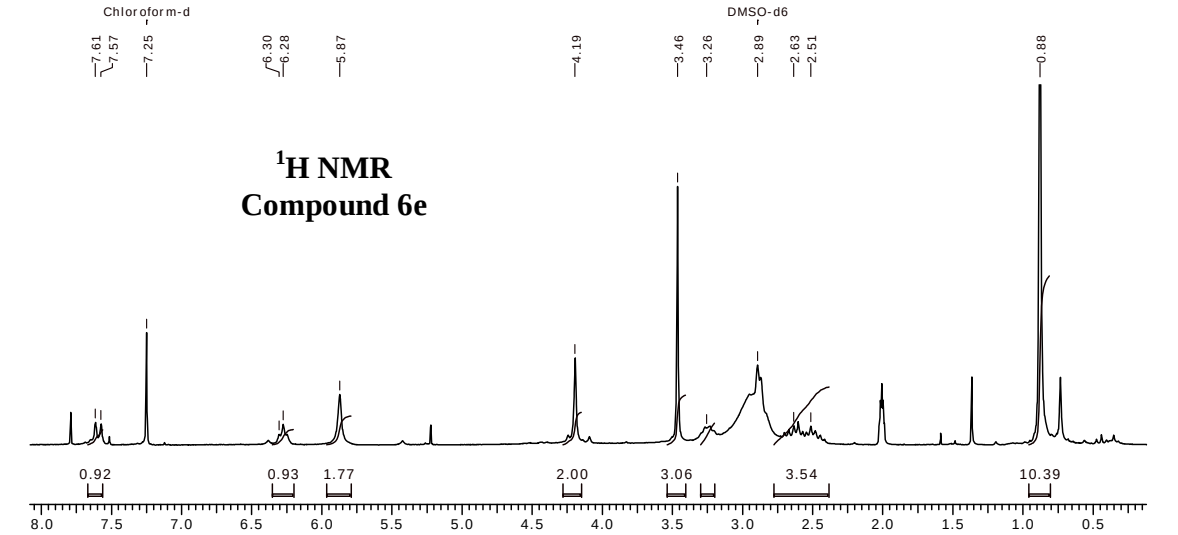


DEPT
Compound 6a

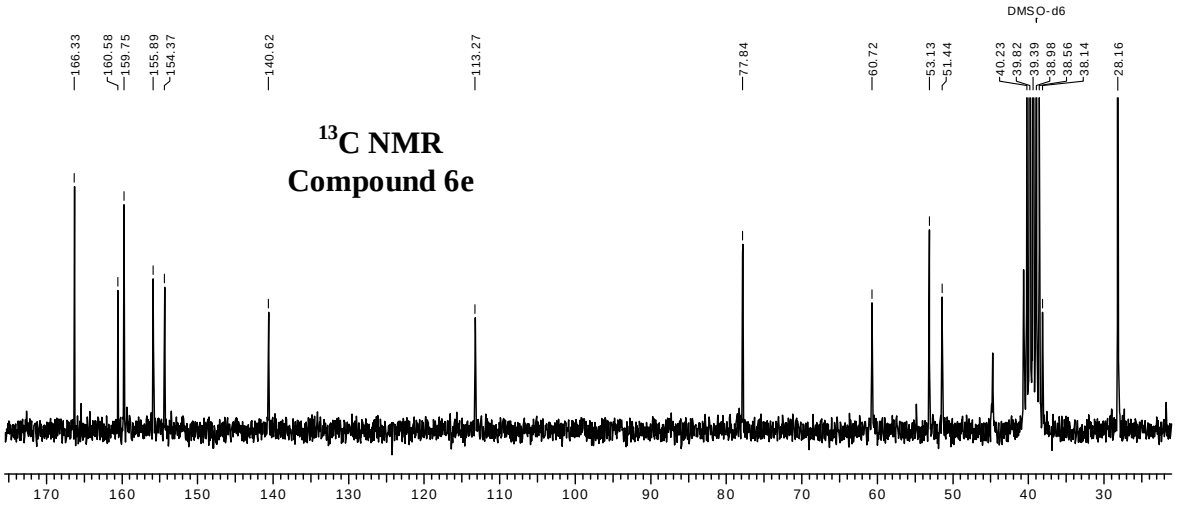

Chloroform-d
7.55
5.20
DMSO-d6
3.29
1H NMR
Compound 6b
1.93
5.19
2.00
1.70
1.45
5.88
10.09
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0


DMSO-d6
166.42
162.93
155.81
154.99
153.08
150.86
135.82
128.33
128.01
127.80
93.60
77.72
66.35
60.66
51.31
40.54
40.12
39.70
39.29
38.87
38.45
38.03
28.06
20.91
13C NMR
Compound 6b
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20

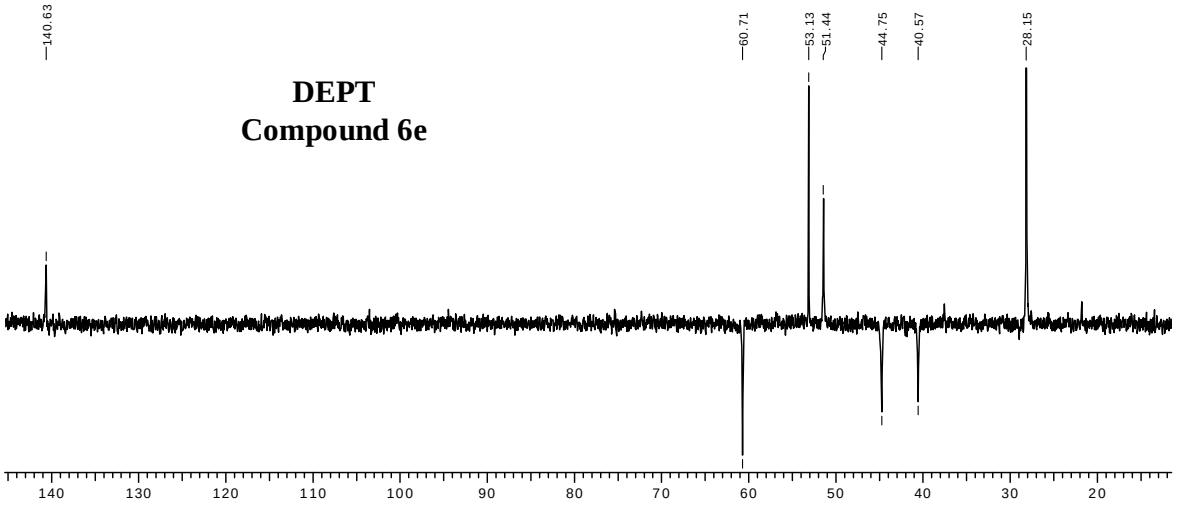

150.98
128.44
128.12
127.91
93.70
66.45
60.75
51.41
51.27
40.56
28.16
DEPT
Compound 6b
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20



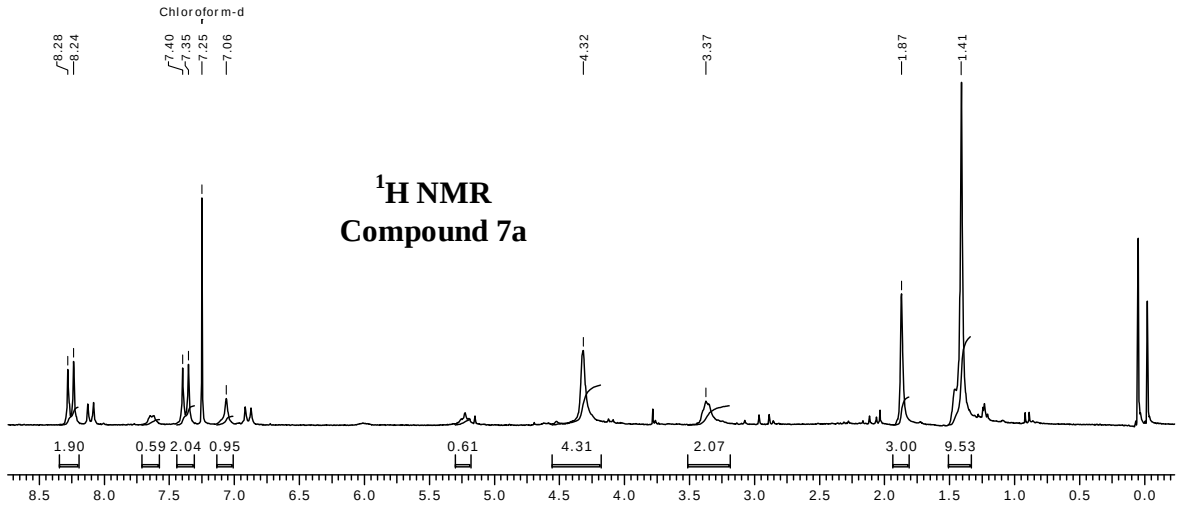
^{1}H NMR
Compound 6e

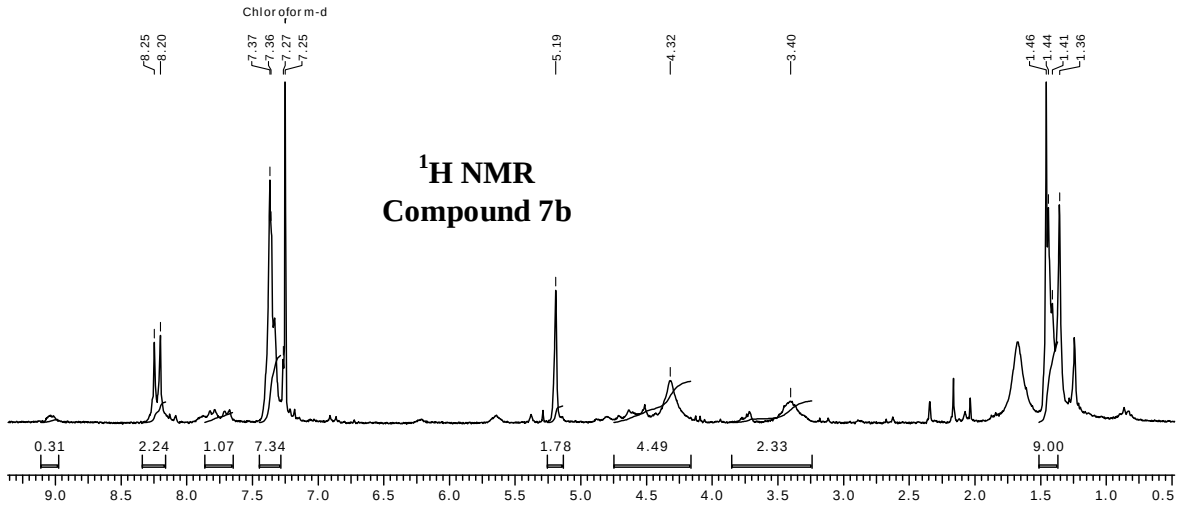


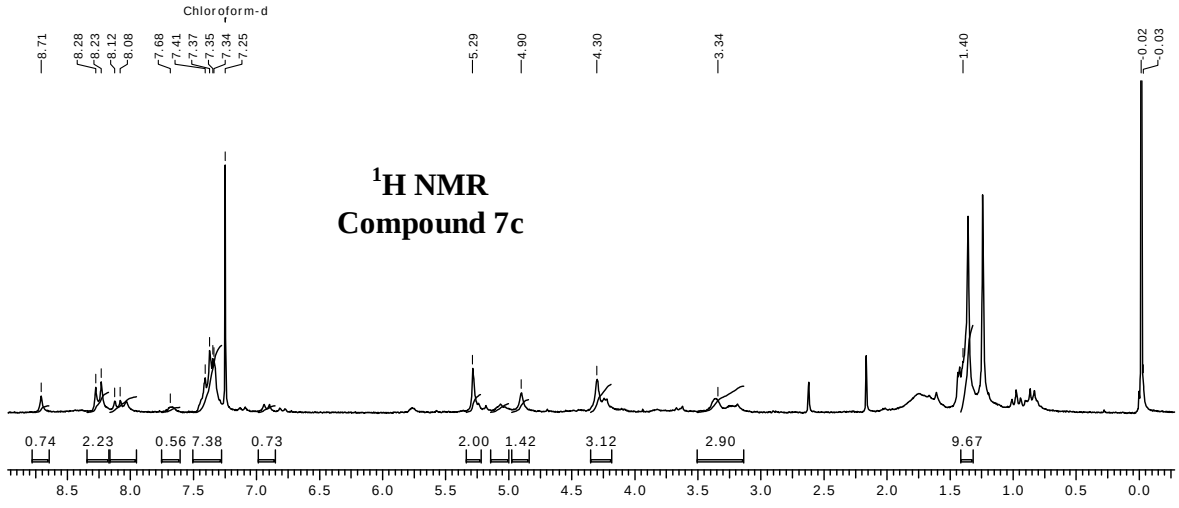
^{13}C NMR
Compound 6e

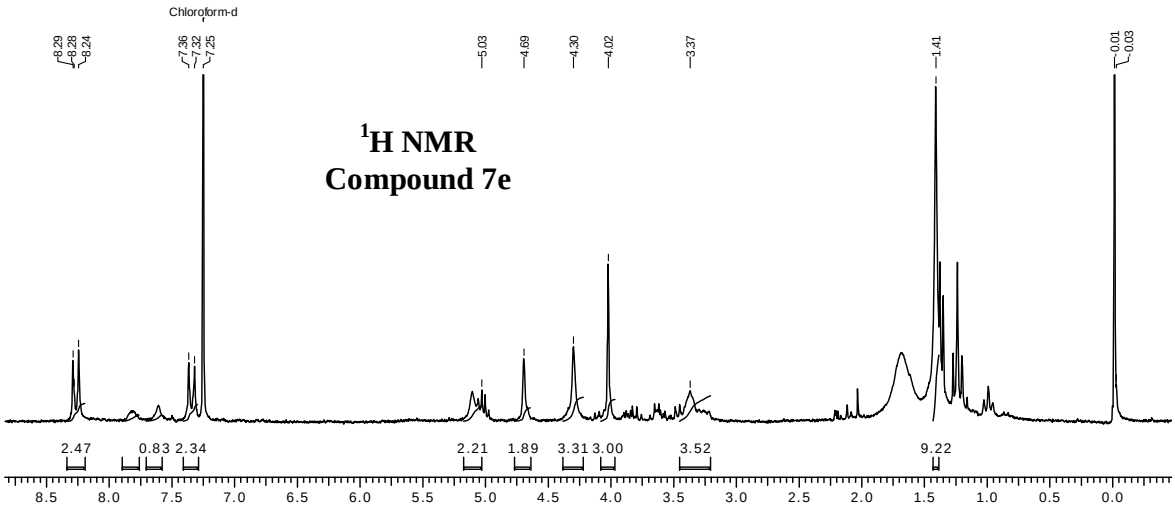


DEPT
Compound 6e

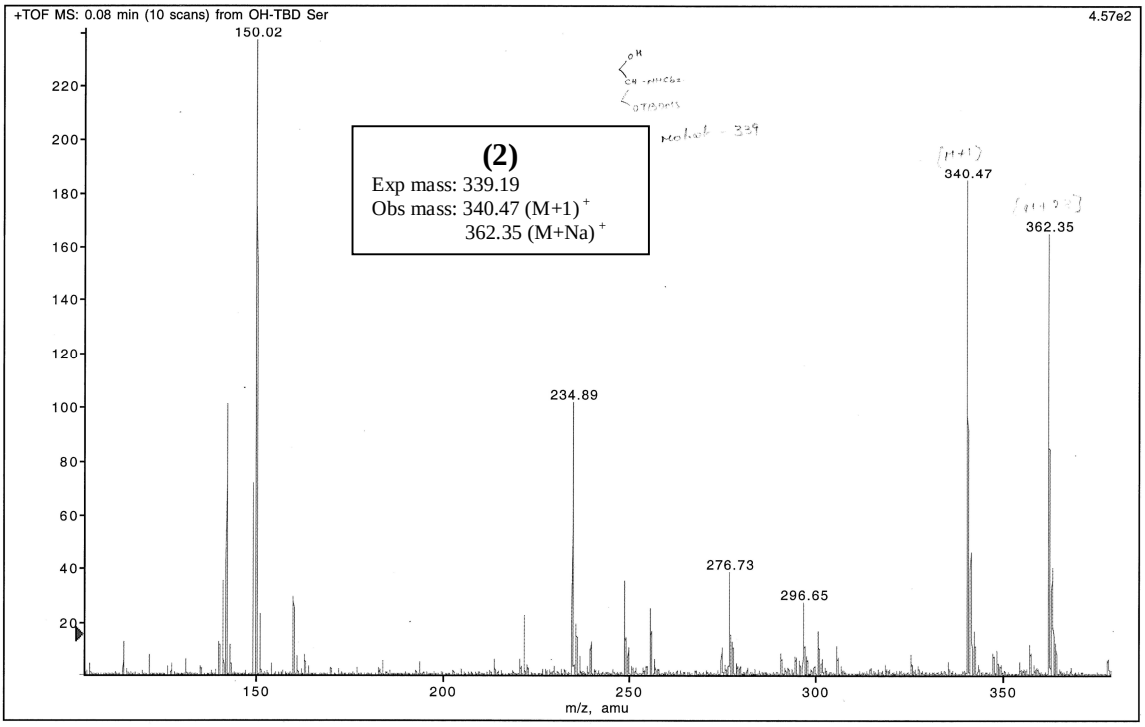

Chloroform-d
8.28
8.24
7.40
7.35
7.25
7.06
4.32
3.37
1.87
1.41
1H NMR
Compound 7a
1.90
0.59
2.04
0.95
0.61
4.31
2.07
3.00
9.53

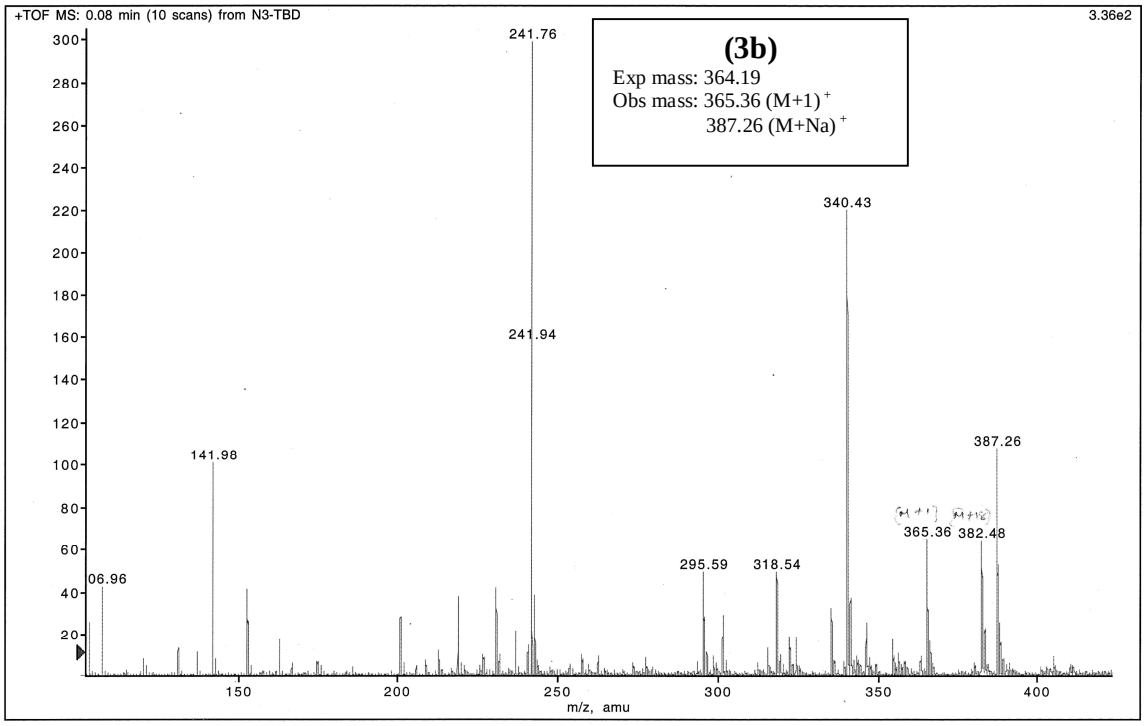

Chloroform-d
8.25
8.20
7.37
7.36
7.27
7.25
5.19
4.32
3.40
1.46
1.44
1.41
1.36
1H NMR
Compound 7b
0.31
2.24
1.07
7.34
1.78
4.49
2.33
9.00

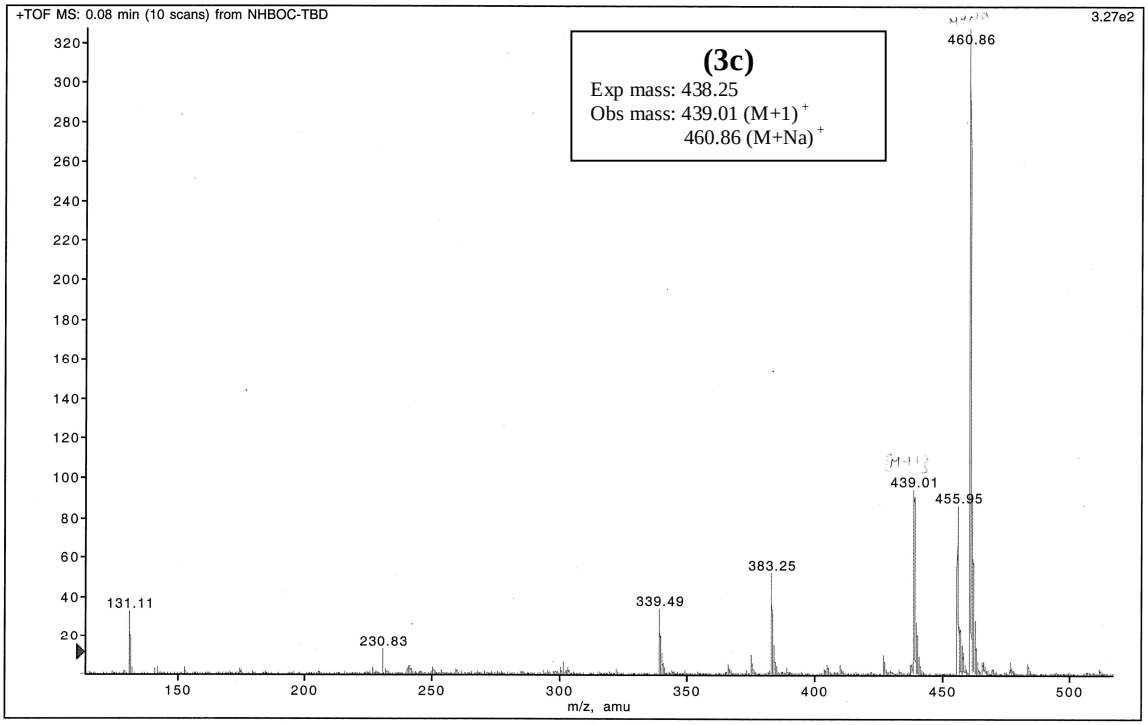

Chloroform-d
8.71
8.28
8.23
8.12
8.08
7.68
7.41
7.37
7.35
7.34
7.25
5.29
4.90
4.30
3.34
1.40
0.02
0.03
1H NMR
Compound 7c
0.74
2.23
0.56
7.38
0.73
2.00
1.42
3.12
2.90
9.67

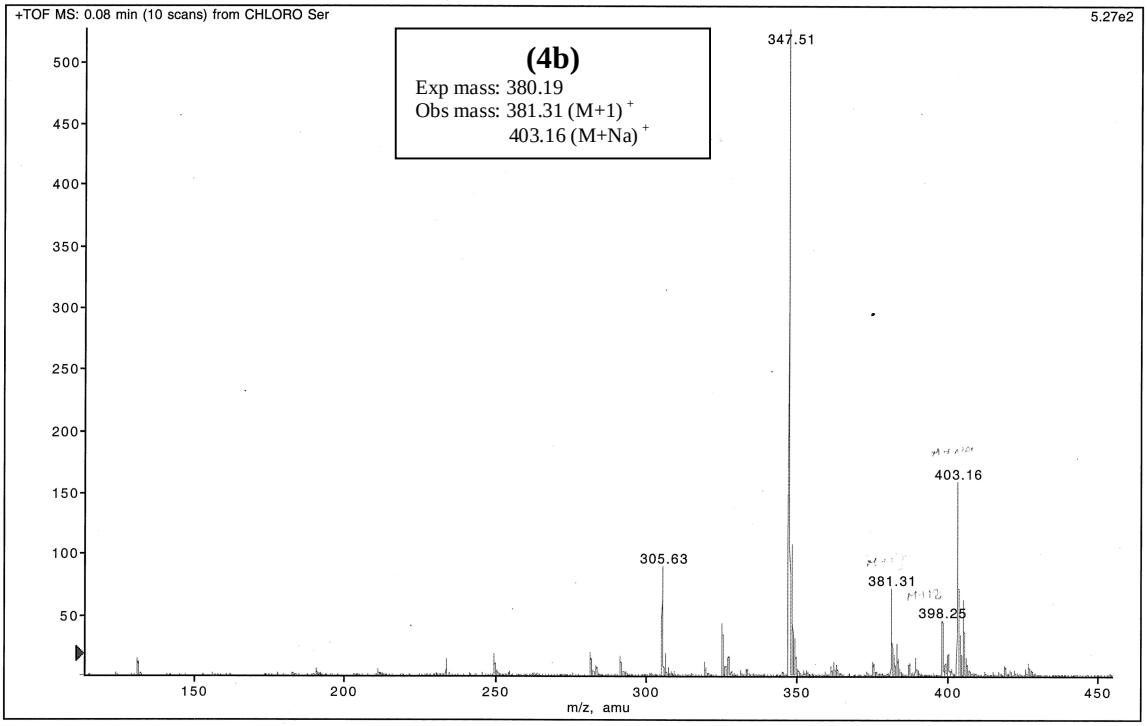


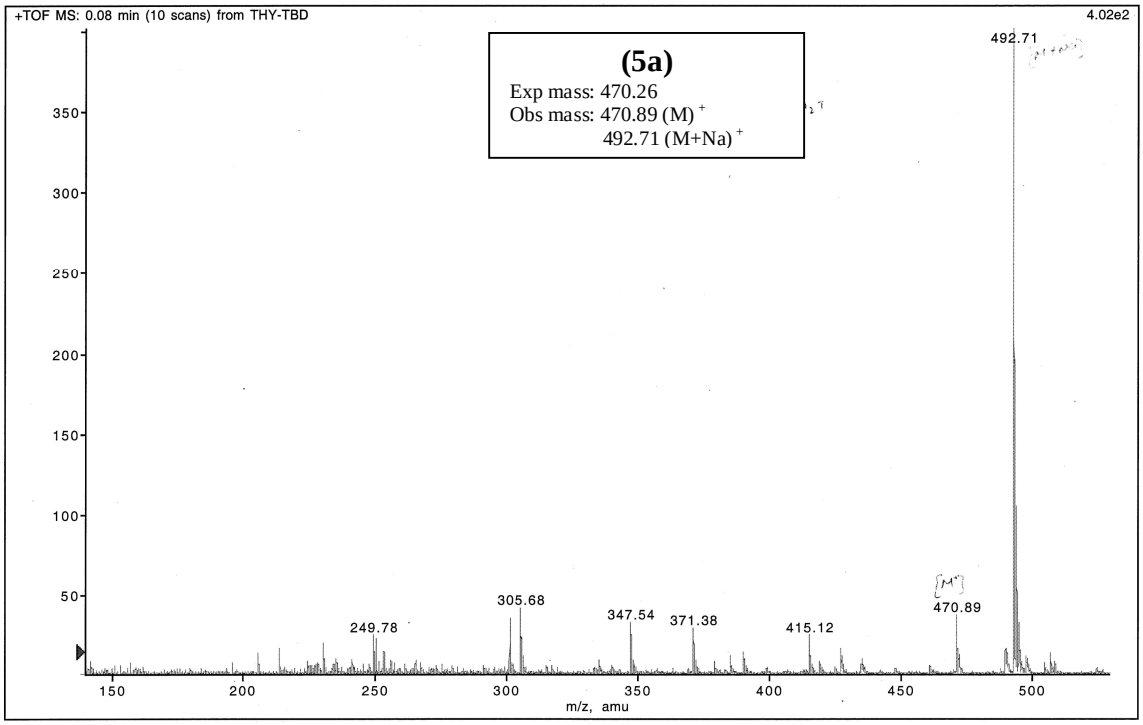
Mass spectra of compounds **2, 3b, 3c, 4a, 4b, 5a, 5b, 5c, 5d, 5e, 6a, 6b, 6c, 6e, 7a, 7b, 7c, 7e**

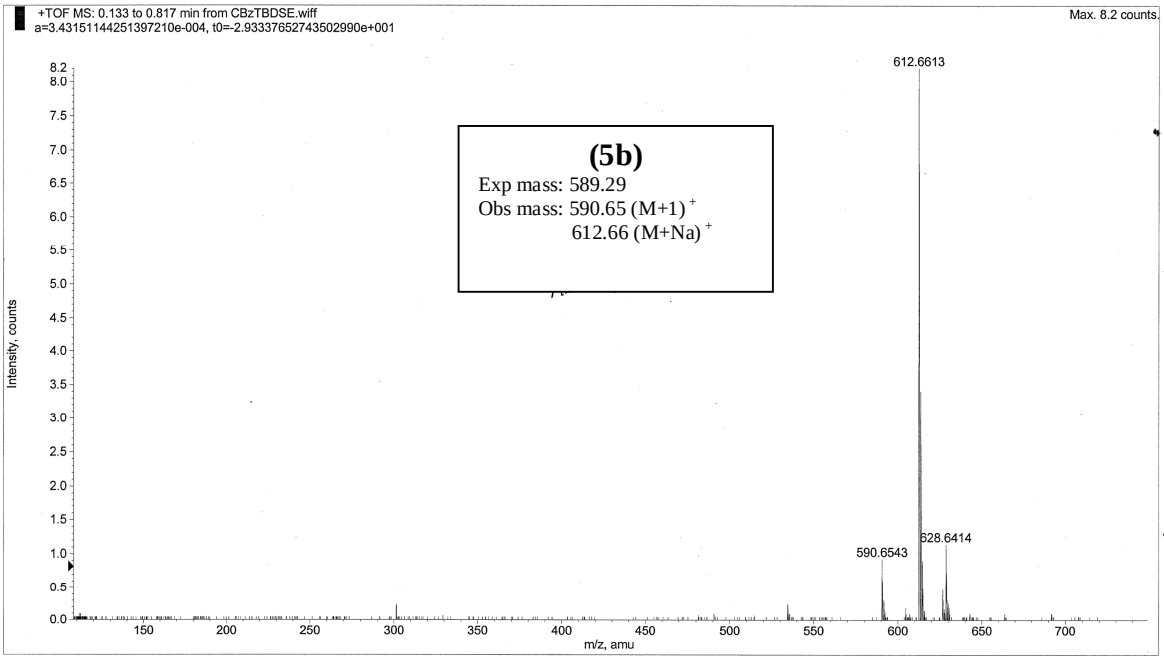


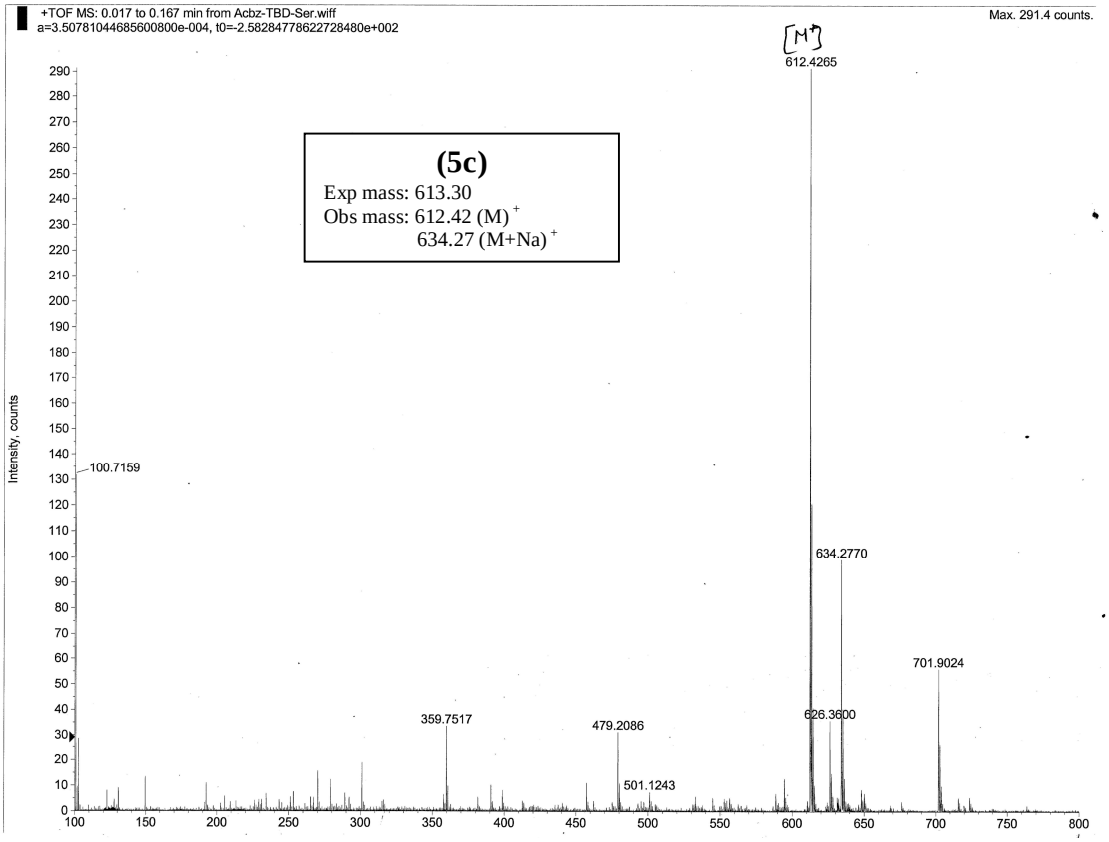

+TOF MS: 0.08 min (10 scans) from N3-TBD
3.36e2
(3b)
Exp mass: 364.19
Obs mass: 365.36 (M+1)+
387.26 (M+Na)+
241.76
340.43
241.94
387.26
141.98
365.36
382.48
295.59
318.54
06.96
m/z, amu

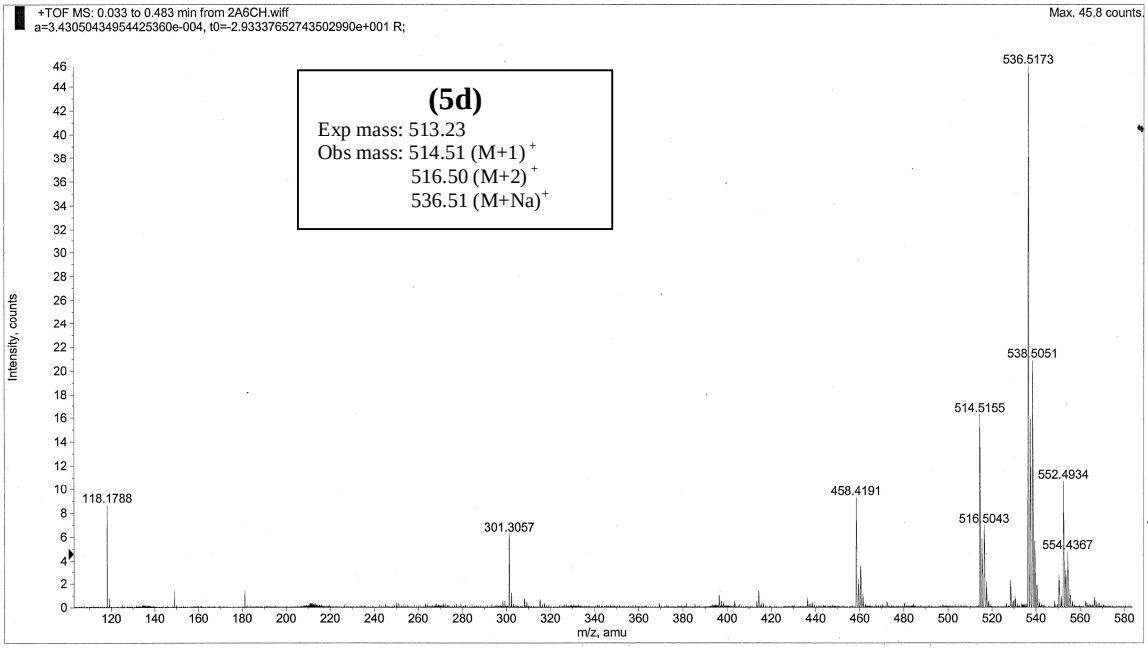

+TOF MS: 0.08 min (10 scans) from NHBOC-TBD
3.27e2
(3c)
Exp mass: 438.25
Obs mass: 439.01 (M+1)+
460.86 (M+Na)+
460.86
439.01
455.95
383.25
131.11
339.49
230.83
m/z, amu

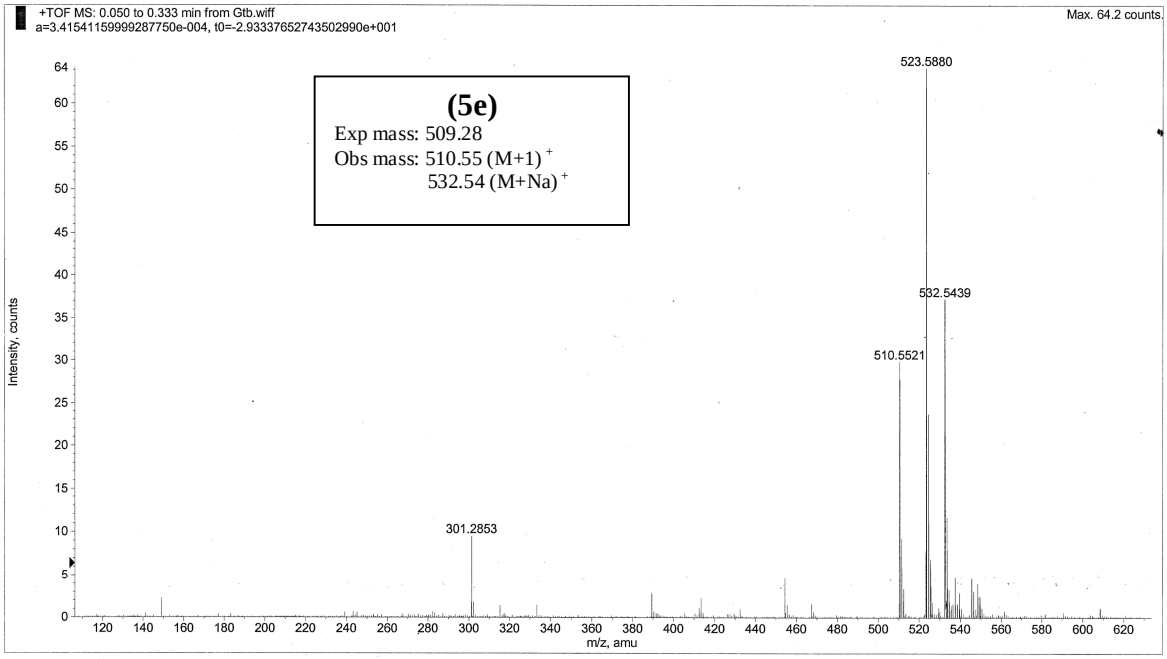

+TOF MS: 0.08 min (10 scans) from CHLORO Ser
5.27e2
(4b)
Exp mass: 380.19
Obs mass: 381.31 (M+1) +
403.16 (M+Na) +
347.51
403.16
305.63
381.31
398.25
m/z, amu

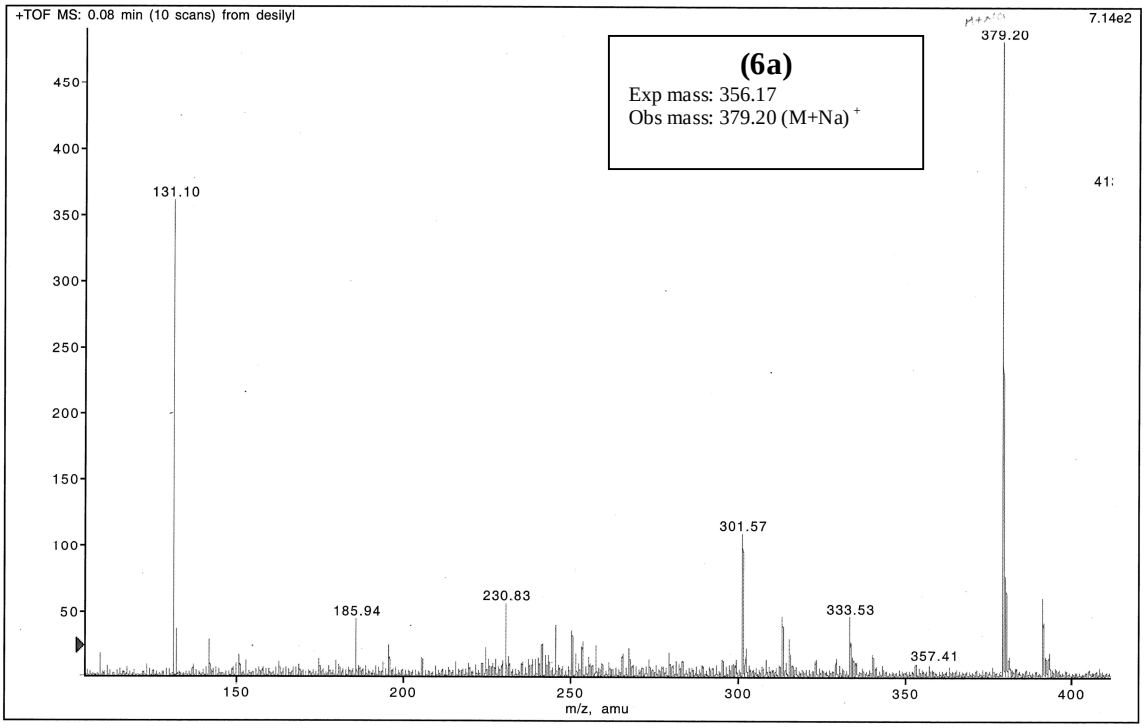

+TOF MS: 0.08 min (10 scans) from THY-TBD
4.02e2
(5a)
Exp mass: 470.26
Obs mass: 470.89 (M) +
492.71 (M+Na) +
492.71
470.89
305.68
347.54
371.38
249.78
415.12
m/z, amu

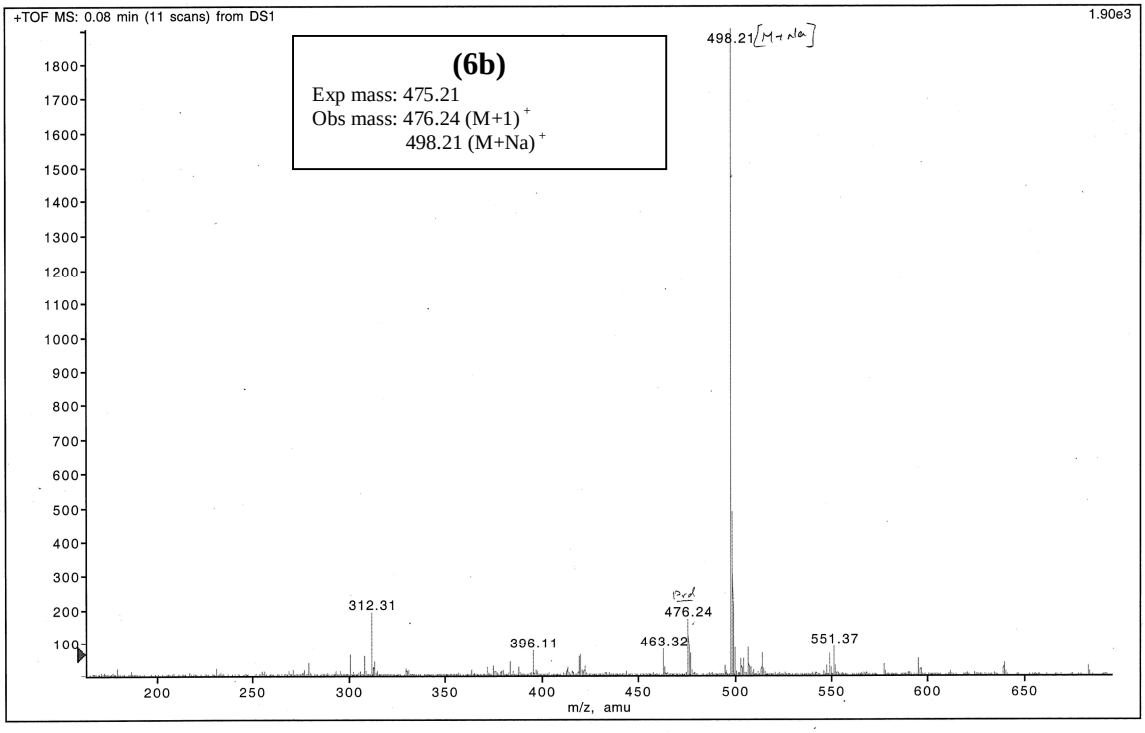

+TOF MS: 0.133 to 0.817 min from CBzTBDSE.wiff
a=3.43151144251397210e-004, t0=-2.93337652743502990e+001
Max. 8.2 counts.
(5b)
Exp mass: 589.29
Obs mass: 590.65 (M+1)+
612.66 (M+Na)+
612.6613
628.6414
590.6543
Intensity, counts
m/z, amu

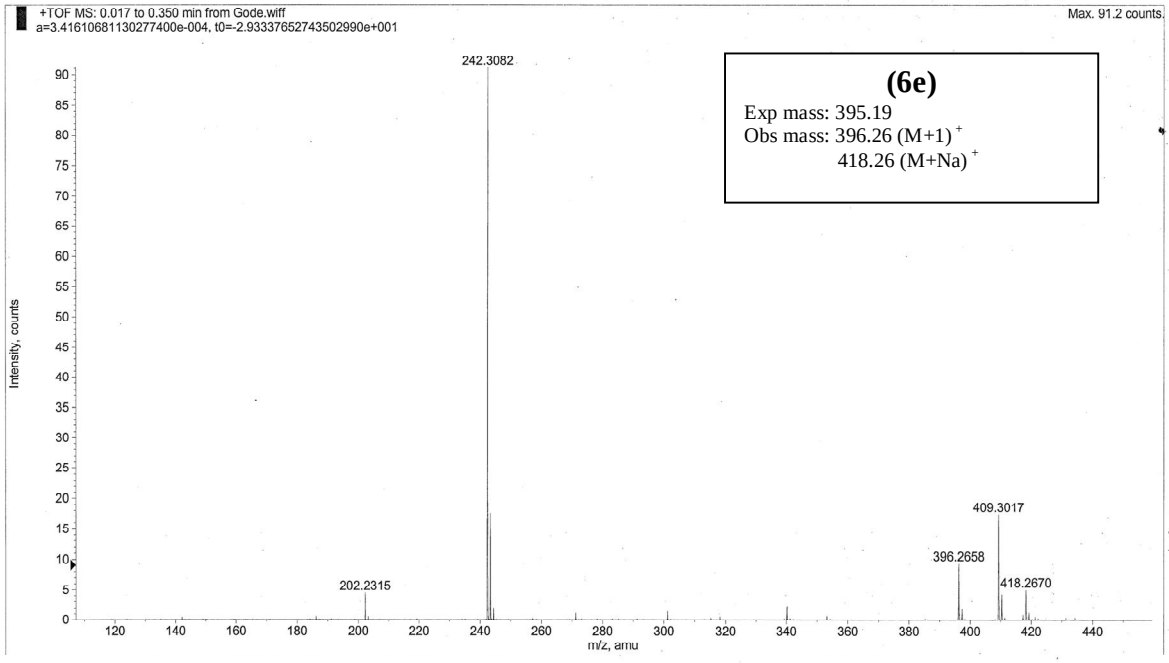

+TOF MS: 0.017 to 0.167 min from Acbz-TBD-Ser.wiff
a=3.50781044685600800e-004, t0=-2.58284778622728480e+002
Max. 291.4 counts.
(5c)
Exp mass: 613.30
Obs mass: 612.42 (M)+
634.27 (M+Na)+
[M+]
612.4265
100.7159
634.2770
701.9024
626.3600
359.7517
479.2086
501.1243
Intensity, counts

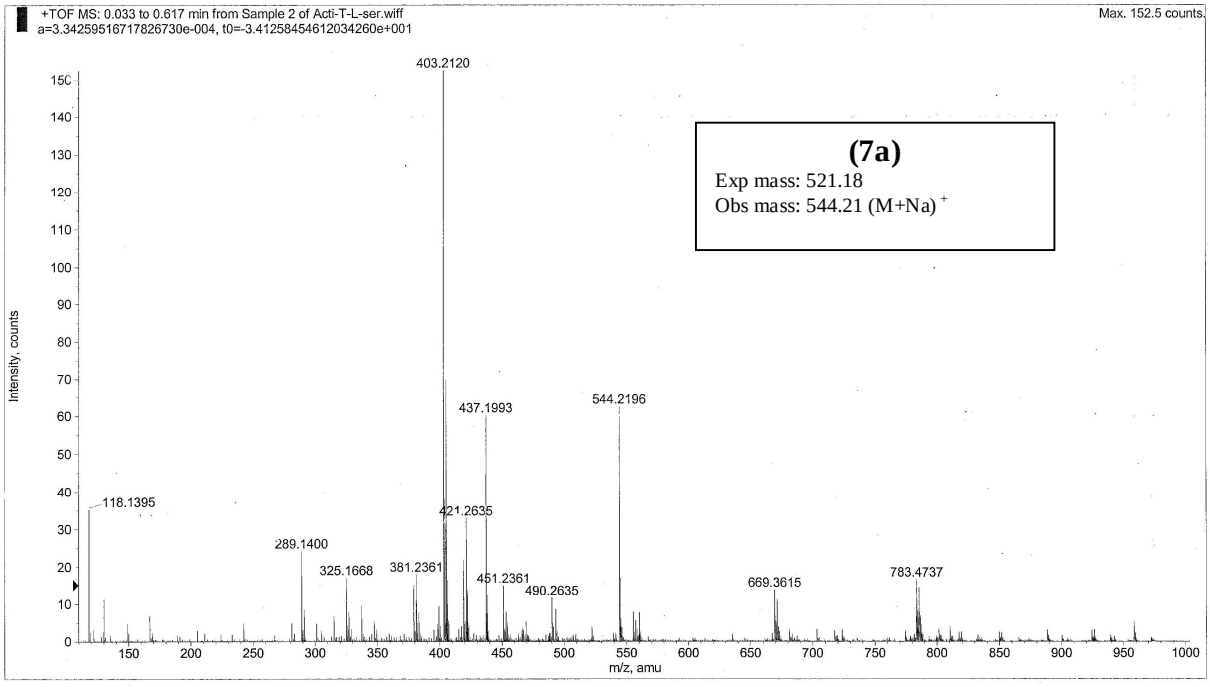

+TOF MS: 0.033 to 0.483 min from 2A6CH.wiff
a=3.43050434954425360e-004, t0=-2.93337652743502990e+001 R;
Max. 45.8 counts.
(5d)
Exp mass: 513.23
Obs mass: 514.51 (M+1)+
516.50 (M+2)+
536.51 (M+Na)+
536.5173
538.5051
514.5155
552.4934
458.4191
516.5043
554.4367
118.1788
301.3057
Intensity, counts
m/z, amu

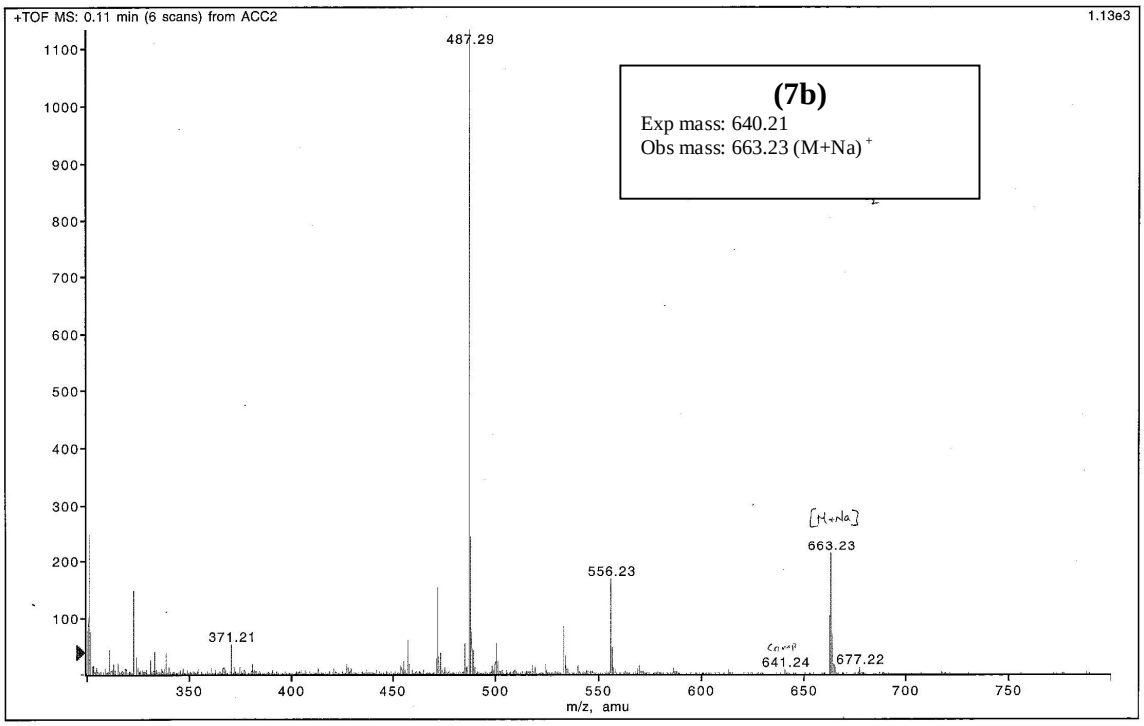

+TOF MS: 0.050 to 0.333 min from Gtb.wiff
a=3.41541159999287750e-004, t0=-2.93337652743502990e+001
Max. 64.2 counts.
(5e)
Exp mass: 509.28
Obs mass: 510.55 (M+1)+
532.54 (M+Na)+
523.5880
532.5439
510.5521
301.2853
Intensity, counts
m/z, amu

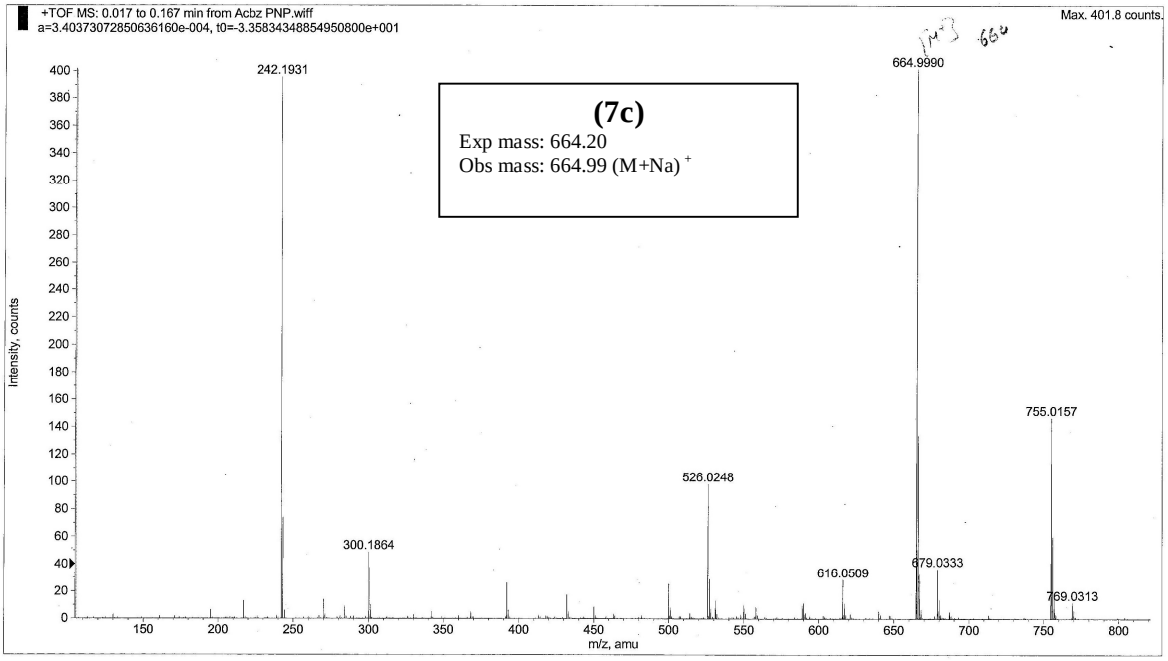

+TOF MS: 0.08 min (10 scans) from desilyl
7.14e2
(6a)
Exp mass: 356.17
Obs mass: 379.20 (M+Na)+
379.20
131.10
301.57
230.83
185.94
333.53
357.41
m/z, amu

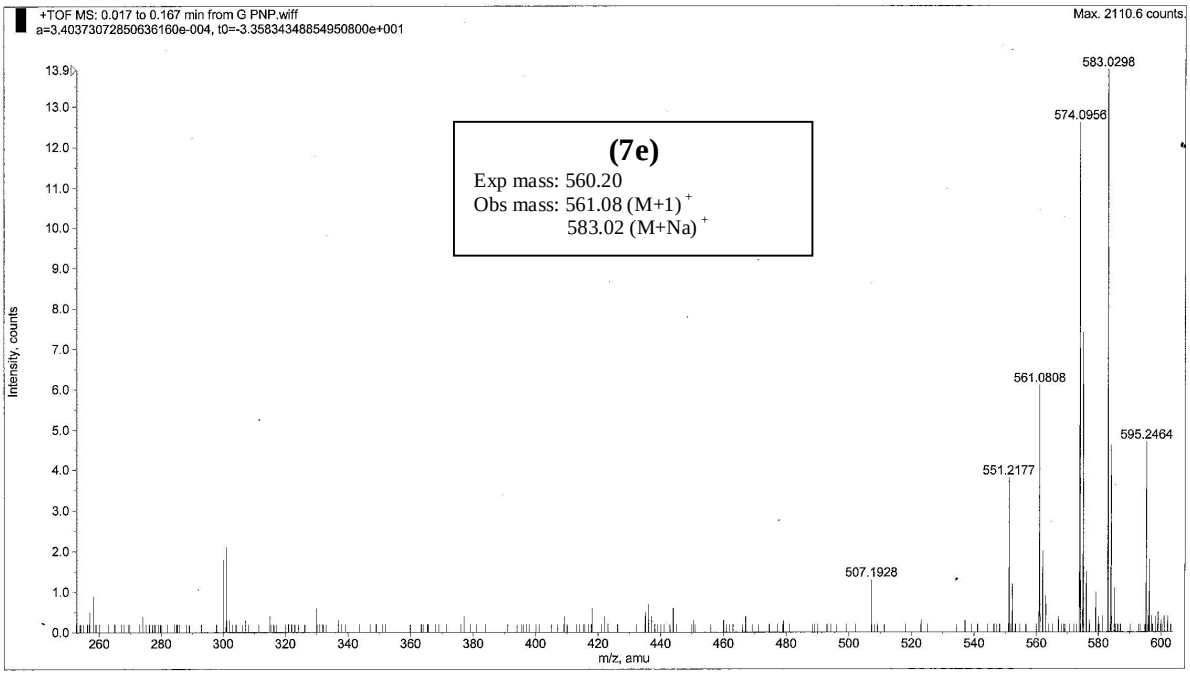

+TOF MS: 0.08 min (11 scans) from DS1
1.90e3
(6b)
Exp mass: 475.21
Obs mass: 476.24 (M+1)+
498.21 (M+Na)+
498.21
312.31
476.24
396.11
463.32
551.37
m/z, amu


+TOF MS: 0.017 to 0.350 min from Gode.wiff
a=3.4161068113027740e-004, t0=-2.9333765274350299e+001
Max. 91.2 counts.
242.3082
409.3017
396.2658
418.2670
202.2315
Intensity, counts
m/z, amu
(6e)
Exp mass: 395.19
Obs mass: 396.26 (M+1) +
418.26 (M+Na) +

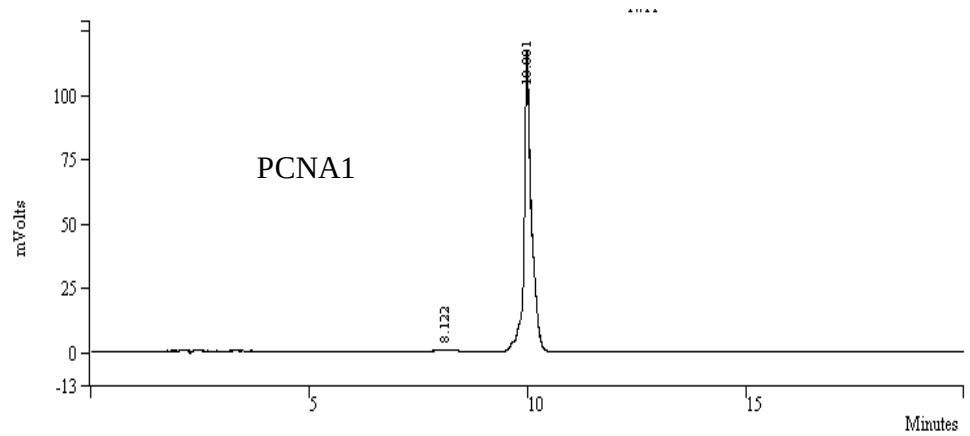

+TOF MS: 0.033 to 0.617 min from Sample 2 of Acti-T-L-ser.wiff
a=3.3425951671782673e-004, t0=-3.4125845461203426e+001
Max. 152.5 counts.
403.2120
544.2196
437.1993
421.2635
118.1395
289.1400
325.1668
381.2361
451.2361
490.2635
669.3615
783.4737
Intensity, counts
m/z, amu
(7a)
Exp mass: 521.18
Obs mass: 544.21 (M+Na) +


+TOF MS: 0.11 min (6 scans) from ACC2
1.13e3
487.29
(7b)
Exp mass: 640.21
Obs mass: 663.23 (M+Na)+
[M+Na]
663.23
556.23
371.21
641.24
677.22
m/z, amu


+TOF MS: 0.017 to 0.167 min from Acbz PNP.wiff
a=3.40373072850636160e-004, t0=-3.35834348854950800e+001
Max. 401.8 counts.
242.1931
664.9990
(7c)
Exp mass: 664.20
Obs mass: 664.99 (M+Na)+
755.0157
526.0248
300.1864
616.0509
679.0333
769.0313
Intensity, counts
m/z, amu

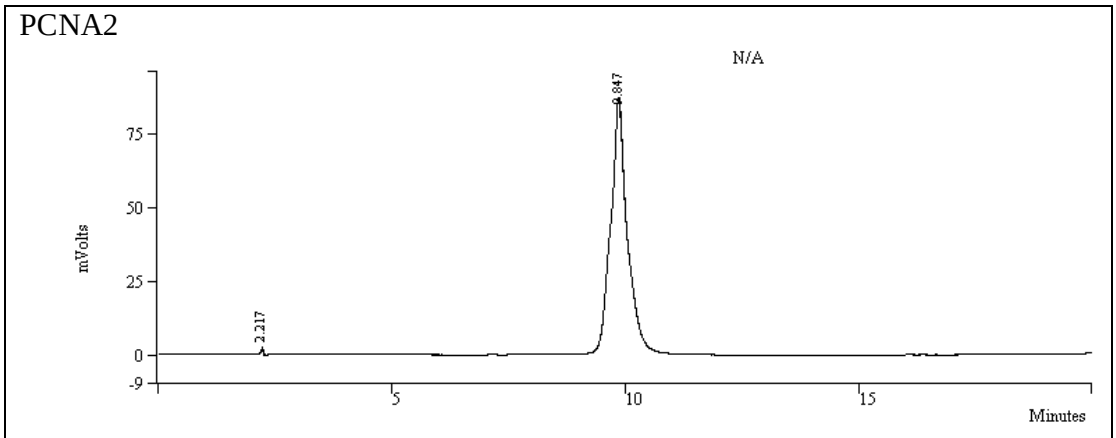

+TOF MS: 0.017 to 0.167 min from G PNP.wiff
a=3.4037307285063616e-004, t0=-3.3583434885495080e+001
Max. 2110.6 counts.
(7e)
Exp mass: 560.20
Obs mass: 561.08 (M+1)+
583.02 (M+Na)+
583.0298
574.0956
561.0808
595.2464
551.2177
507.1928
Intensity, counts
m/z, amu

HPLC profile of PCNA1



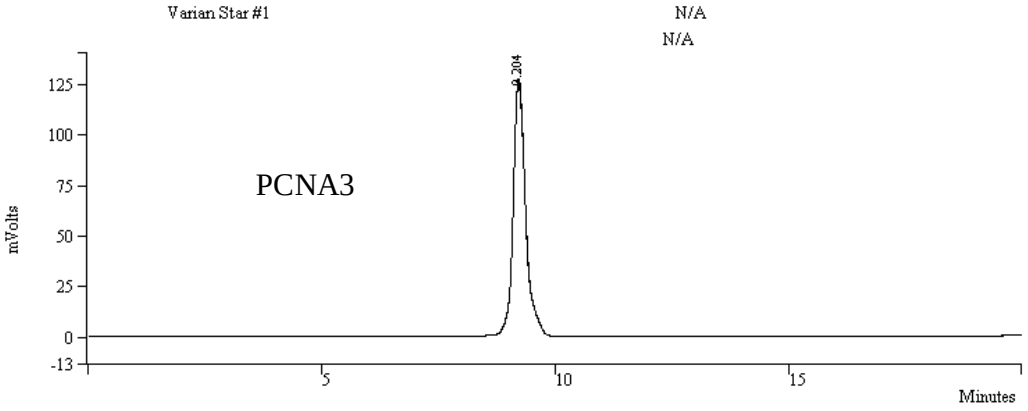
Peak No	Ret. Time (min)	Width 1/2 (sec)	Peak Area (counts)	Result ()
1	8.122	39.3	16995	1.2214
2	10.001	10.4	1374365	98.7785
			1391360	**99.9999**

HPLC profile of PCNA2



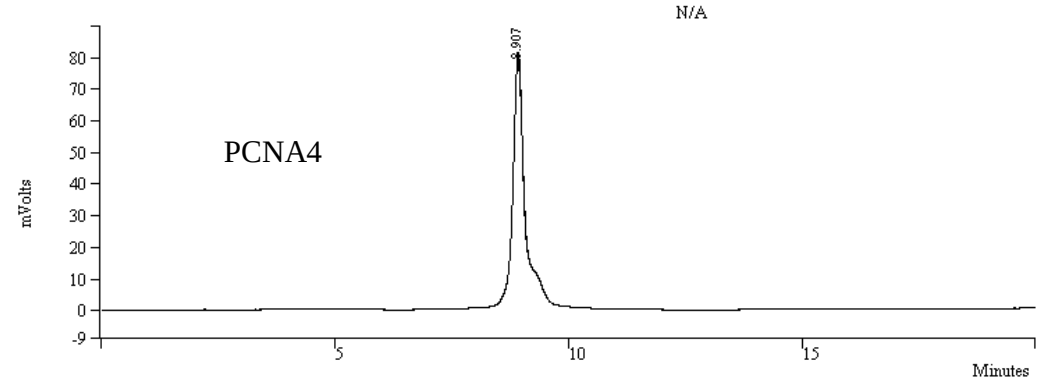
Peak No	Ret. Time (min)	Width 1/2 (sec)	Peak Area (counts)	Result ()
1	2.217	3.4	11573	0.5442
2	9.847	20.0	2114889	99.4558
			2126462	**100.0000**

HPLC profile of PCNA3



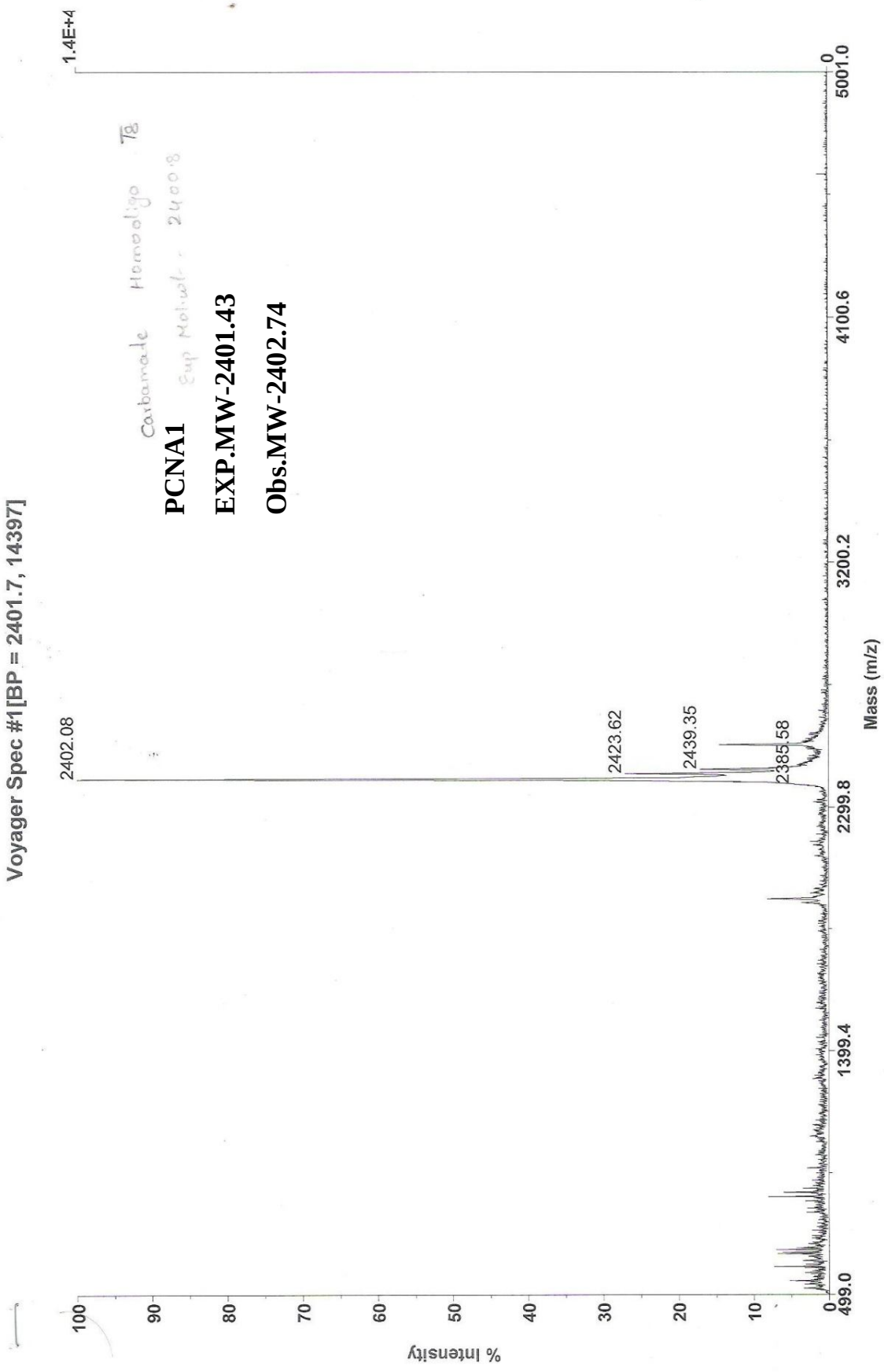
Peak No	Ret. Time (min)	Width 1/2 (sec)	Peak Area (counts)	Result ()
1	9.204	15.8	2398635	100.0000
			2398635	**100.0000**

HPLC of PCNA4

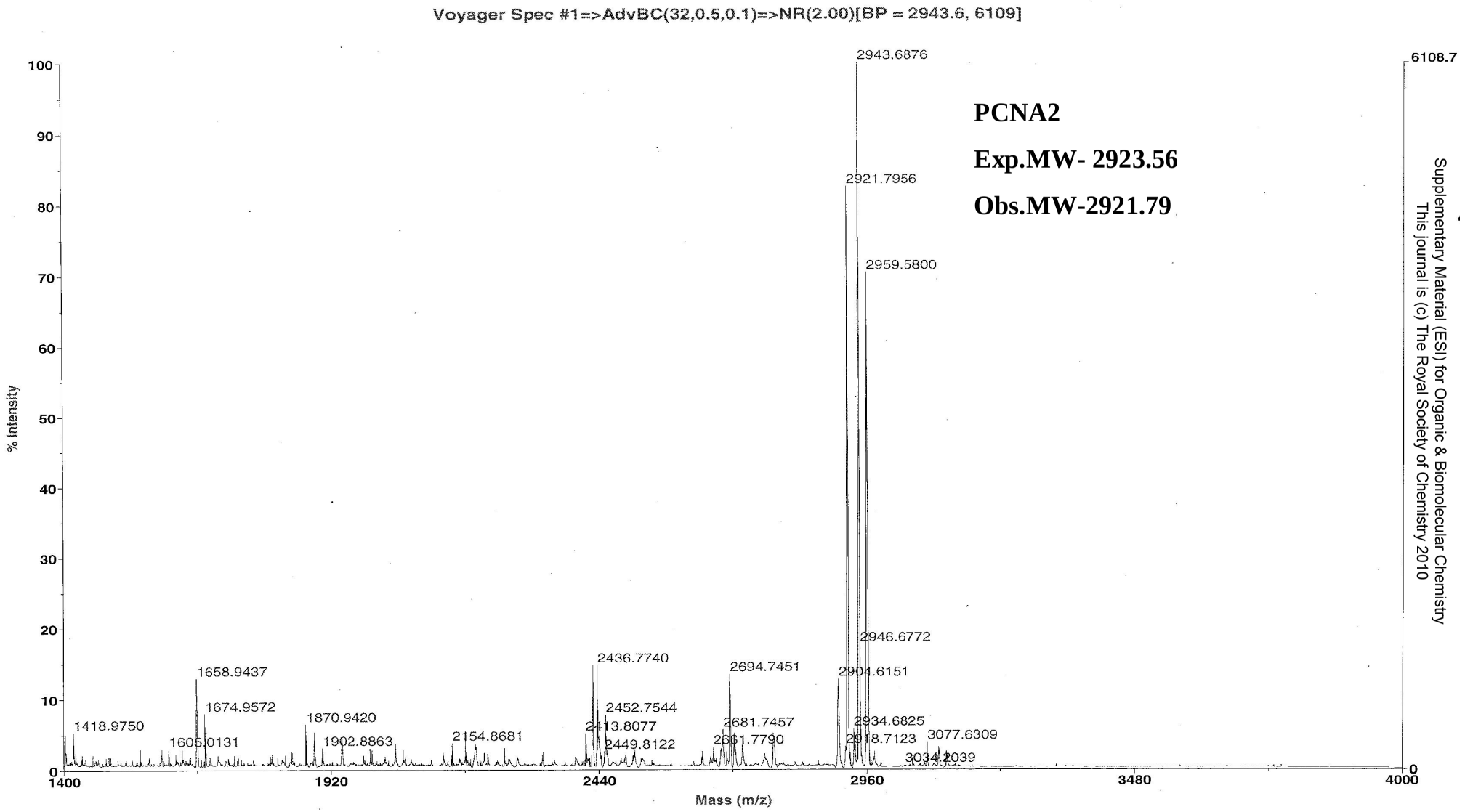


Peak No	Ret. Time (min)	Width 1/2 (sec)	Peak Area (counts)	Result ()
1	8.907	13.4	1525134	100.0000
			1525134	**100.0000**

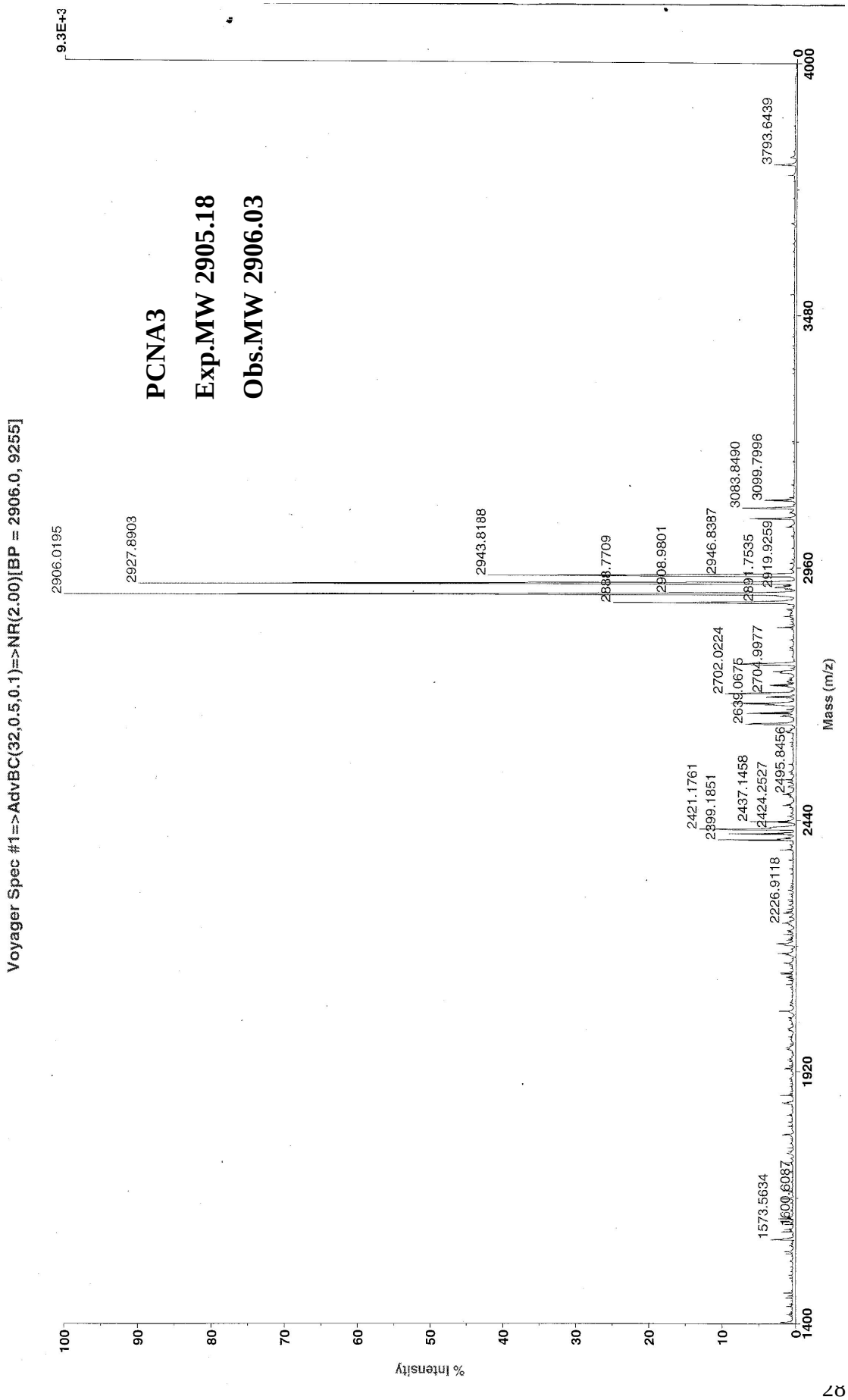
MALDI-TOF of PCNA1



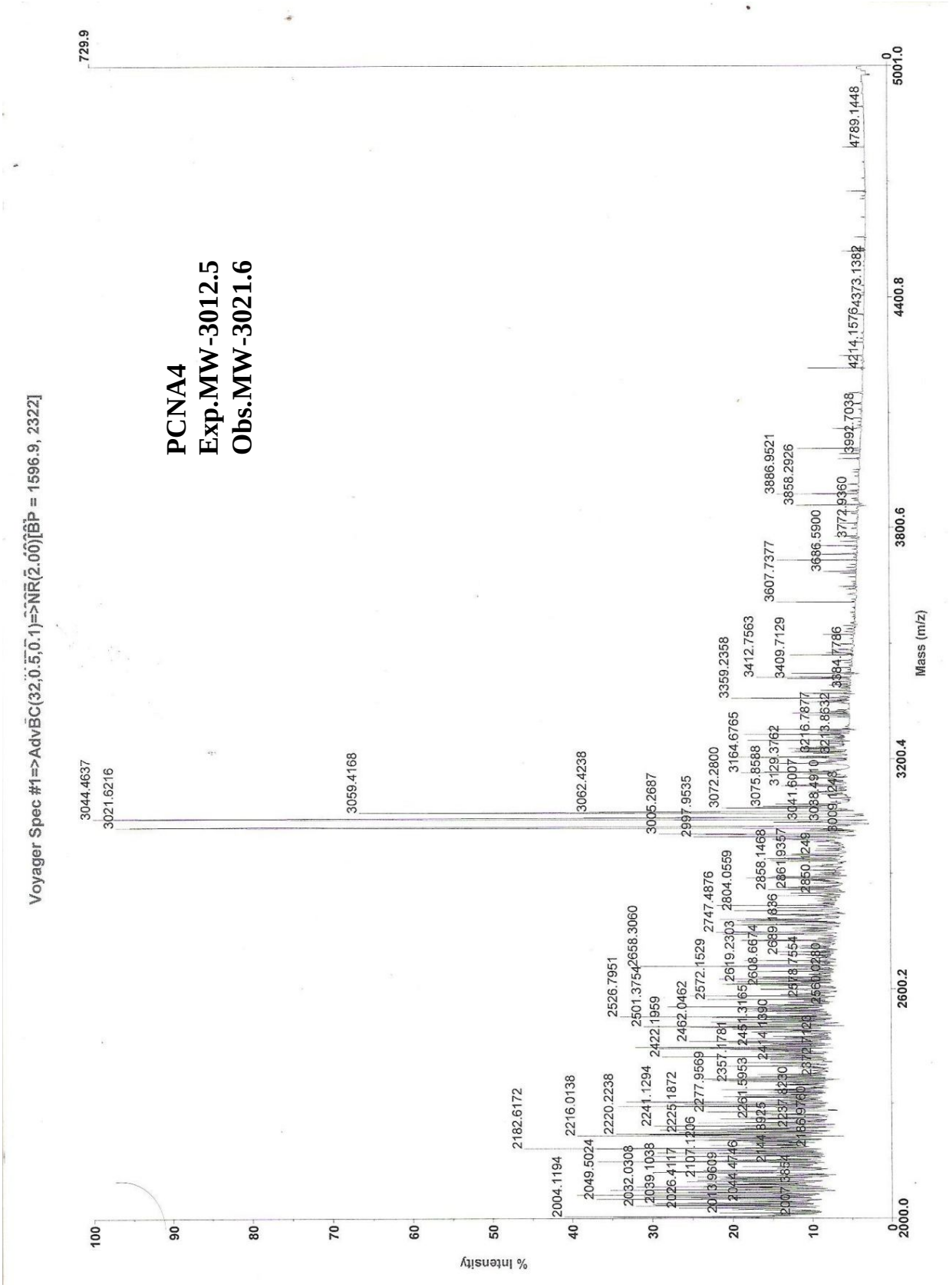
MALDI-TOF of PCNA2



MALDI-TOF of PCNA3PCNA3



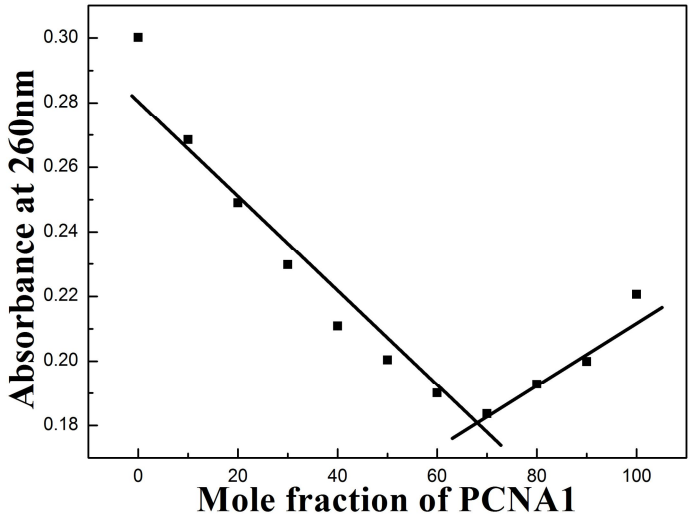
MALDI-TOF of PCNA4



UV Job's plot

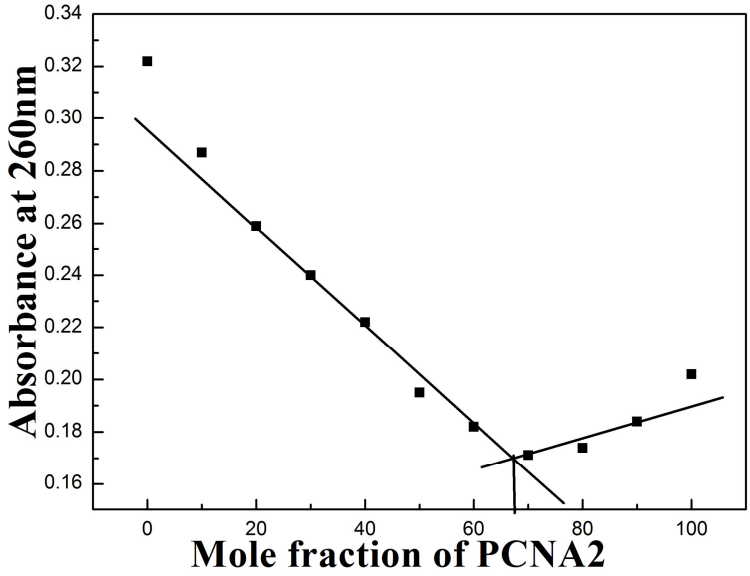
The stoichiometry for PCNA complexation with complementary DNA/RNA sequence was studied by temperature dependent UV-absorbance experiment at 260nm. The PCNA, DNA/RNA are mixed in different molar ratios of 0:100, 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, 90:10, 100:0 in 10 mM Sodium phosphate buffer (pH 7.3), 10 mM NaCl. The samples were annealed by keeping at 90ºC for 2min followed by slow cooling to room temperature. The samples were cooled by keeping at 4º C for overnight. Absorbance was recorded by monitoring at 260nm at 5ºC with Perkin-Elmer *Lambda* 35*UV-VIS* spectrophotometer. The stoichiometry was found to be 2:1 for all the polypyrimidine complexes.

U V Job's plot of PCNA1 with DNA1



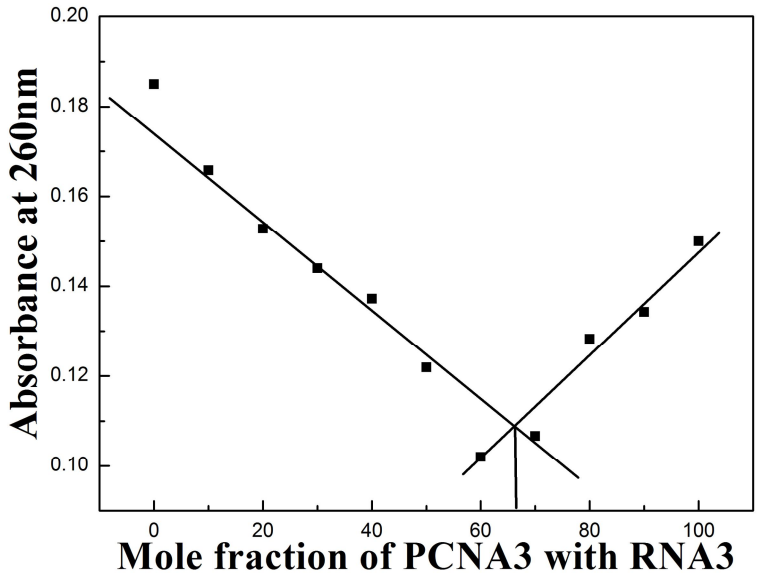
UV- absorbance (at 260nm) of mixtures of PCNA1 and the complementary DNA1 in the relative molar ratios of 0:100, 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, 90:10, 100:0 (Buffer, 10mM Sodium phosphate pH 7.3, 10mM NaCl, 0.1 mM EDTA).

U V Job's plot of PCNA2 with DNA2



UV- absorbance (at 260nm) of mixtures of PCNA2 and the complementary DNA2 in the relative molar ratios of 0:100, 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, 90:10, 100:0 (Buffer, 10mM Sodium phosphate pH 7.3, 10mM NaCl, 0.1 mM EDTA)

U V Job's plot of PCNA3 with RNA3



UV- absorbance (at 260nm) of mixtures of PCNA3 and the complementary RNA3 in the relative molar ratios of 0:100, 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, 90:10, 100:0 (Buffer, 10mM Sodium phosphate pH 7.3, 10mM NaCl, 0.1 mM EDTA

Synthesis of complementary Oligonucleotides

The complementary DNA oligonucleotides were synthesized on Applied Biosystems ABI 3900 High Throughput DNA Synthesizer using standard β-cyanoethyl phospharamidite chemistry. The oligomers were synthesized on polystyrene solid support, followed by ammonia treatment. The oligonucleotides were desalted by gel filtration, their purity was ascertained by RP HPLC on a C18 column to be more than 98% and was used without further purification in the biophysical studies of PCNA. The complementary RNA oligonucleotides were obtained commercially.

List of DNA sequences

DNA1: 5' GC AAA AAA AA CG 3' (complementary to PCNA1)
DNA2: 5' AAG AAA GAG A 3' (complementary (**ap**) to PCNA2)
DNA3: 5' AAG GAA GAA G 3' (complementary (**ap**) to PCNA3)
DNA4: 5' AGT GAT CTA C 3' (complementary (**ap**) to PCNA4)
DNA5: 5' GC AAA **G**AA AA CG 3' (**G:T** mismatch to PCNA1)
DNA6: 5' GC AAA **T**AA AA CG 3' (**T:T** mismatch to PCNA1)
DNA7: 5' AAG A**G**A GAG A 3' (**G:T** mismatch to PCNA2)
DNA8: 5' AAG A**C**A GAG A 3' (**C:T** mismatch to PCNA2)
DNA9: 5' AGA GAA AGA A 3' (complementary (**p**) to PCNA2)
DNA10: 5' AAG G**T**A GAA G 3' (**T:T** mismatch to PCNA3)
DNA11: 5' AGT G**T**T CTA C 3' (**T:T** mismatch to PCNA4)
DNA12: 5' CAT CTA GTG A 3' (complementary (**p**) to PCNA4)
DNA13: 5' GAA GAA GGA A 3' (complementary (**p**) to PCNA3)
RNA1: 5' GC AAA AAA AA CG 3' (complementary to PCNA1)
RNA2: 5' AAG AAA GAG A 3' (complementary (**ap**) to PCNA2)
RNA3: 5' AAG GAA GAA G 3' (complementary (**ap**) to PCNA3)
RNA4: 5' AGU GAU CUA C 3' (complementary (**ap**) to PCNA4)
(**ap)**-antiparallel, (**p)**-parallel

List of DNA sequences used as control for UV-T_m Exp

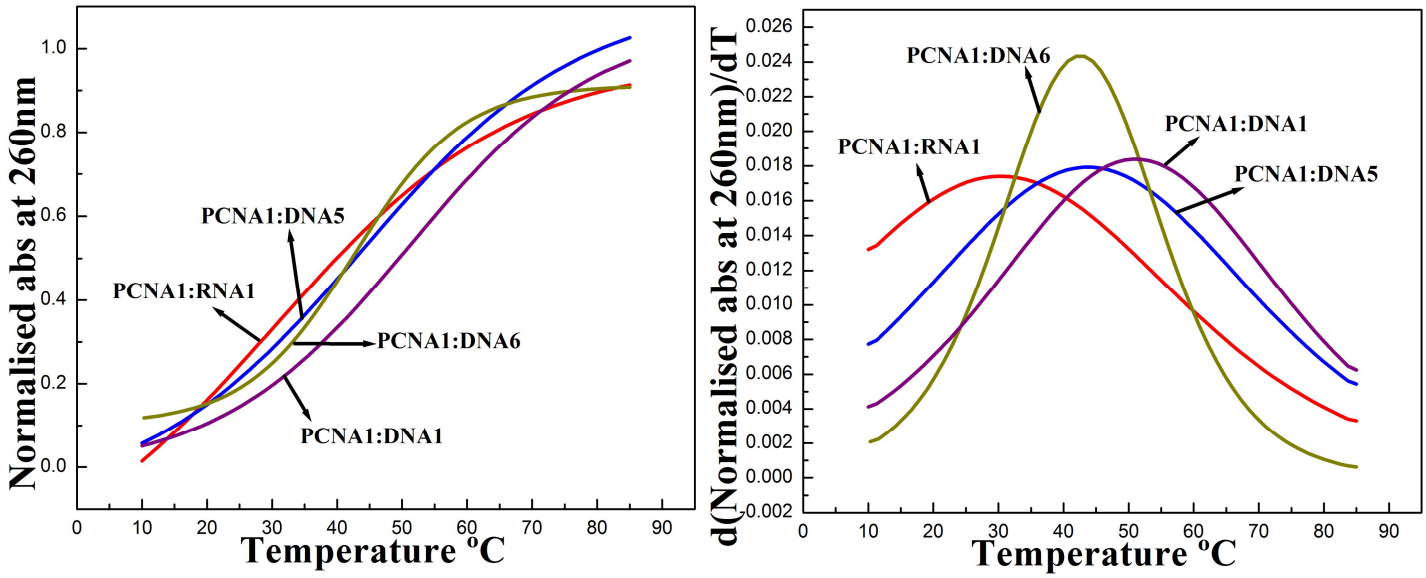
DNA14: 5' TCT CTT TCT T 3'
DNA15: 5' CTT CTT CCT T 3'
DNA16: 5' GTA GAT CAC T 3'

List of PNA sequences used as control for UV-T_m Exp

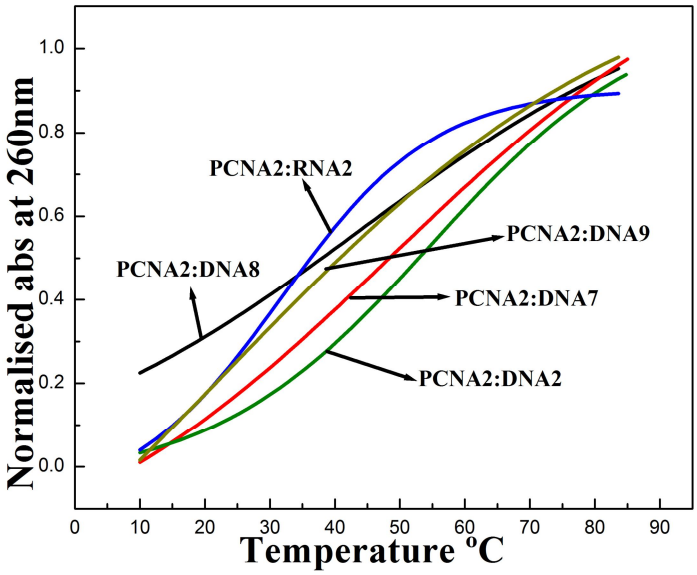
PNA1 = H-*TTTTTTTT*-Lys-NH_2
PNA3 = H-*CTTCTTCCTT*-Lys-NH_2

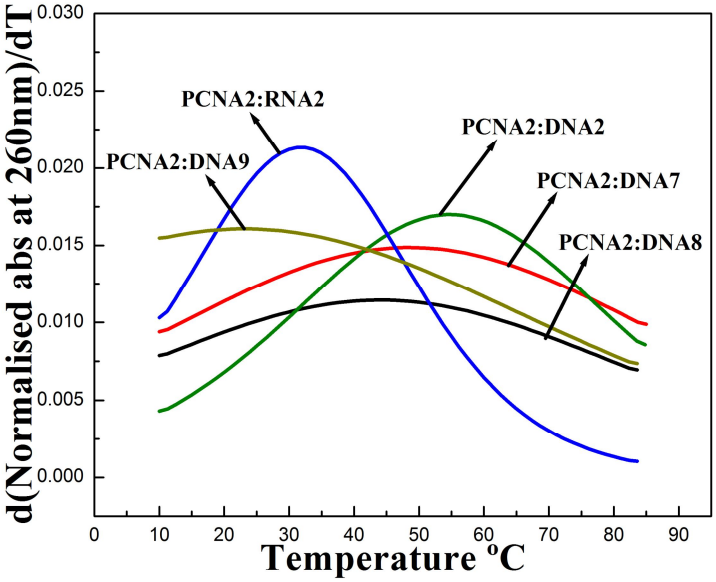
UV-T_m measurement

The complementary DNA and RNA oligomers were synthesized on an Applied Biosystems DNA Synthesizer. The concentration was calculated on the basis of absorbance from the molar extinction coefficients of the corresponding nucleobases. The complexes were prepared in 10mM sodium phosphate buffer, pH 7.3 containing 10mM NaCl , [PCNA] -1μM and were annealed by keeping the samples at 90°C for 2min followed by slow cooling to room temperature. The samples were cooled by keeping at 4°C overnight. Absorbance versus temperature profiles were obtained by monitoring at 260nm with Perkin-Elmer *Lambda* 35 *UV-VIS* spectrophotometer scanning from 5 to 85°C at a ramp rate of 0.5°C per minute. The data were processed using Microcal Origin 5.0 and T_m values derived from the derivative curves.

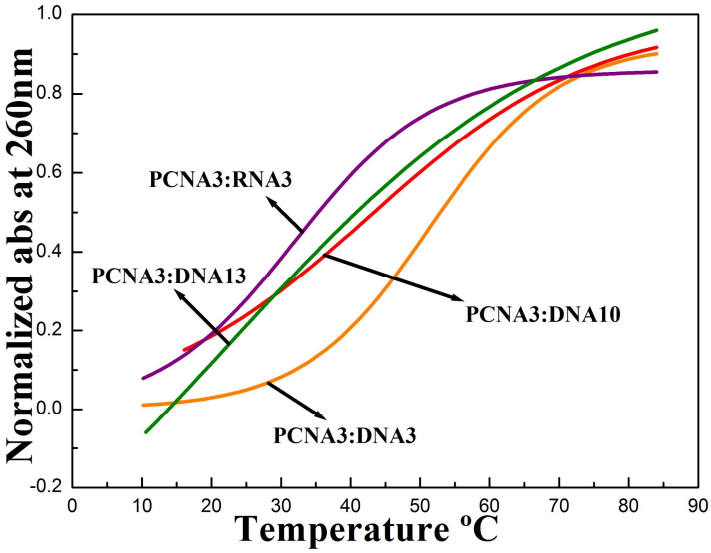


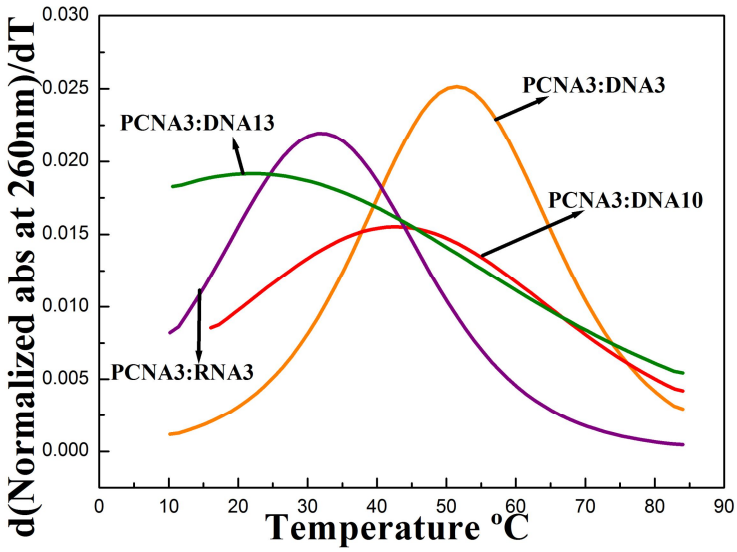
UV-T_m(°C)				
	DNA1	RNA1	DNA5	DNA6
PCNA1	50.8	30.1	43.6	42.4



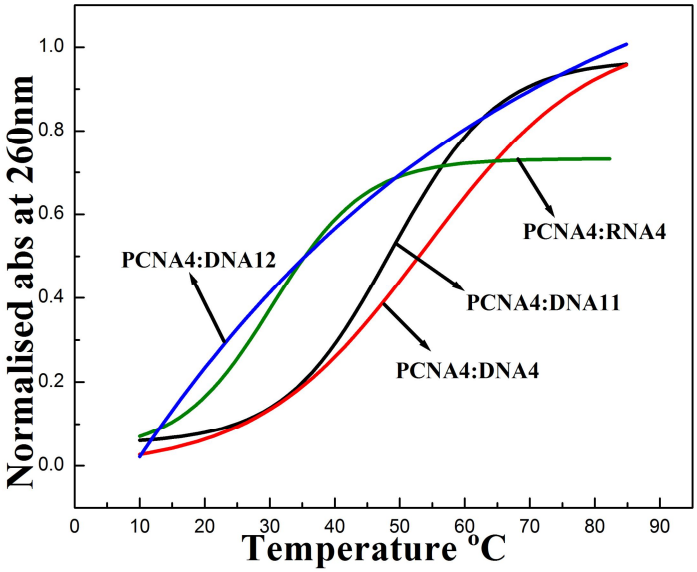


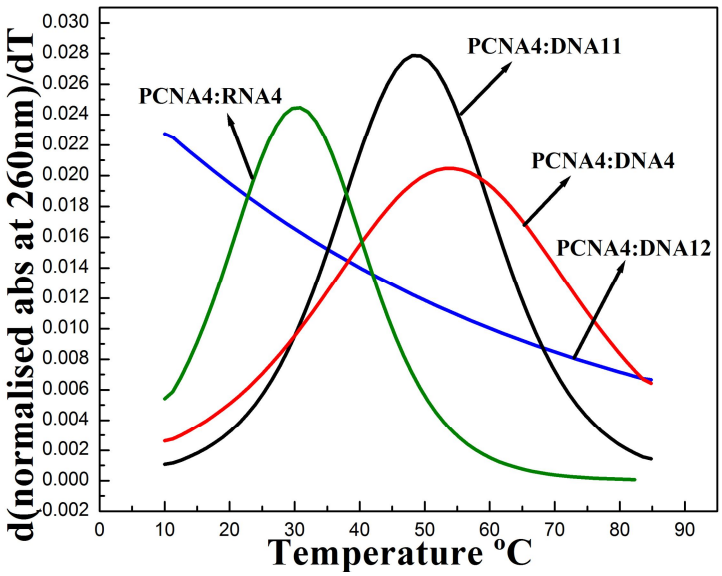
UV-T_m(°C)					
	DNA2	RNA2	DNA7	DNA8	DNA9
PCNA2	54.3	31.6	49.7	45.4	24.2





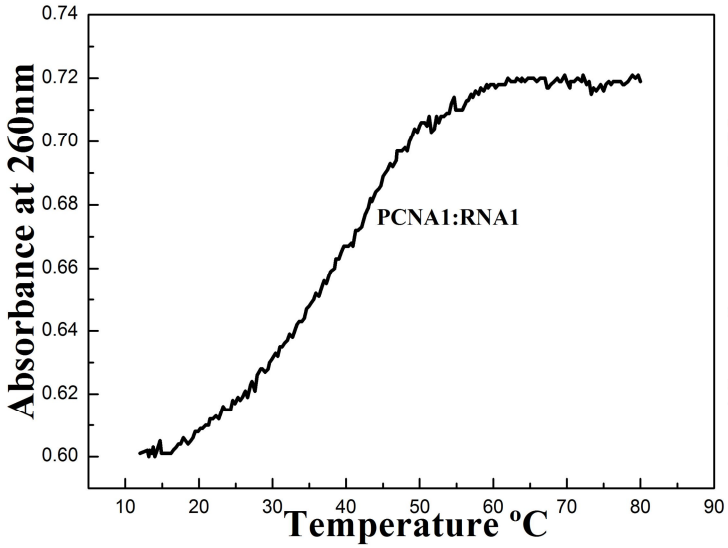
UV-T_m(°C)				
	DNA3	RNA3	DNA10	DNA13
PCNA3	51.4	31.9	43.4	22.01

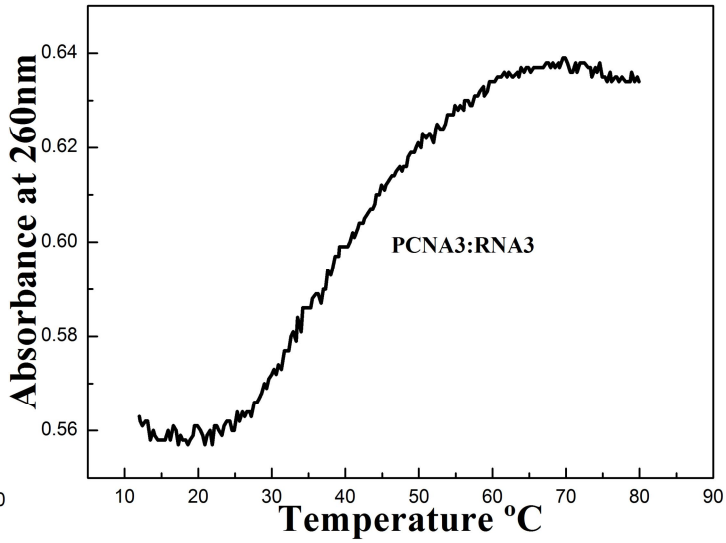




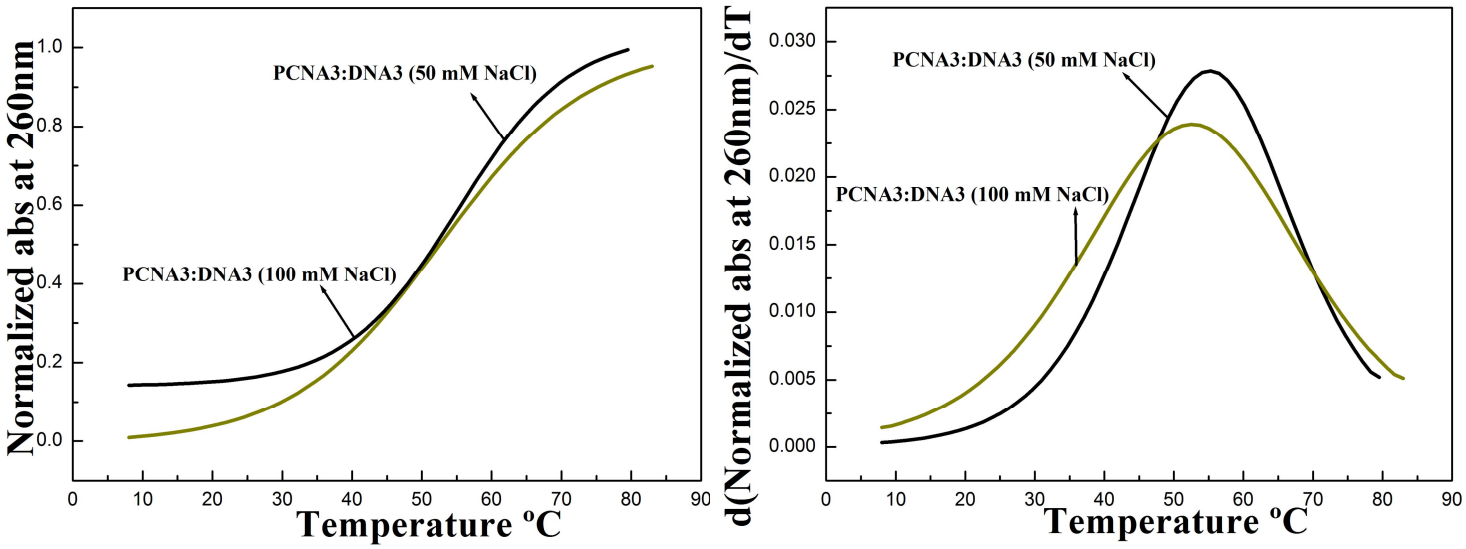
UV-T_m(°C)				
	DNA4	**RNA4**	**DNA11**	**DNA12**
PCNA4	**53.4**	**30.4**	**48.3**	**nd**

Raw data of **PCNA1**, **PCNA3** at higher concentration of oligos [PCNA]-2.5μM with complementary **RNA1, RNA3** showing T_m of **37.7**, **39.4**°C respectively.

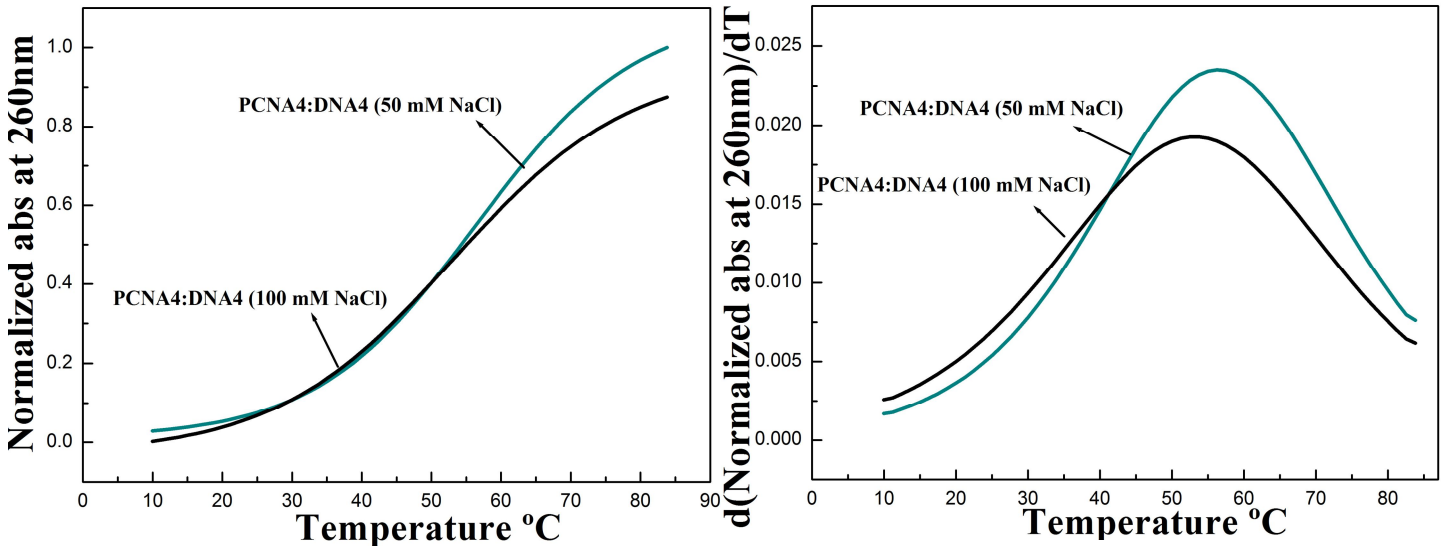




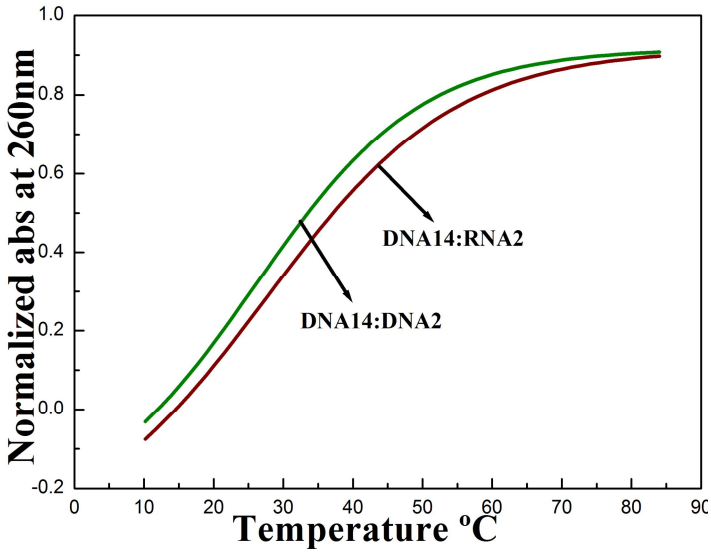
The effect of salt concentration on the UV-T_m of the oligomers was studied by doing the experiment at different salt concentrations.

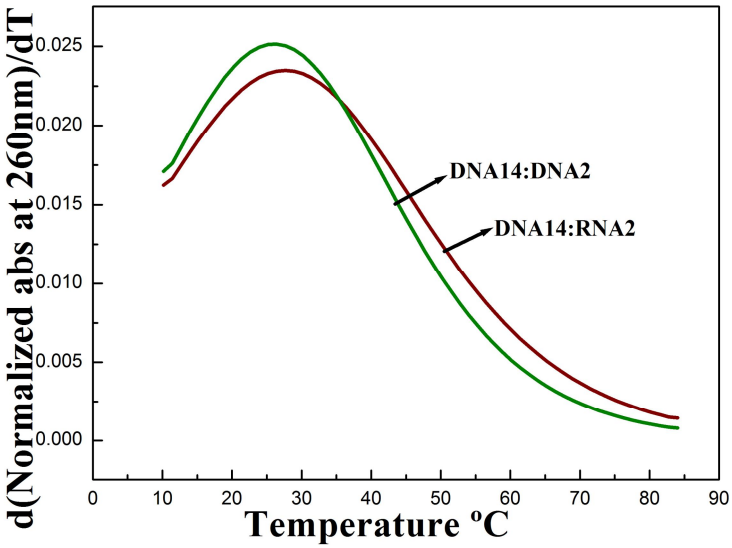


	UV-T_m(°C)	
PCNA3:DNA3	**50 mM NaCl**	**100 mM NaCl**
	55.1	**52.2**

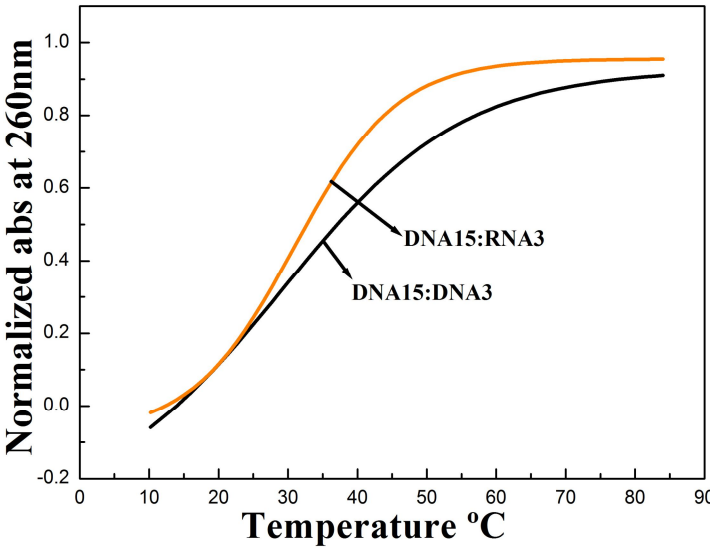


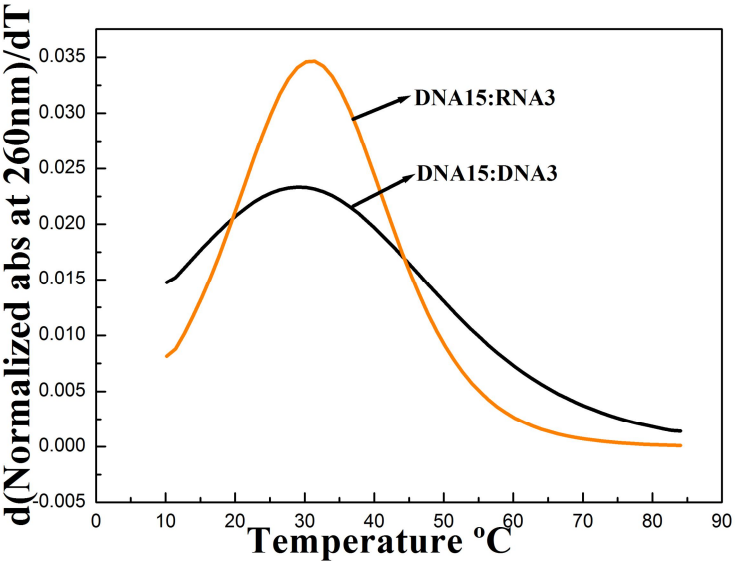
	UV-T_m(°C)	
PCNA4:DNA4	**50 mM NaCl**	**100 mM NaCl**
	56.0	**53.3**



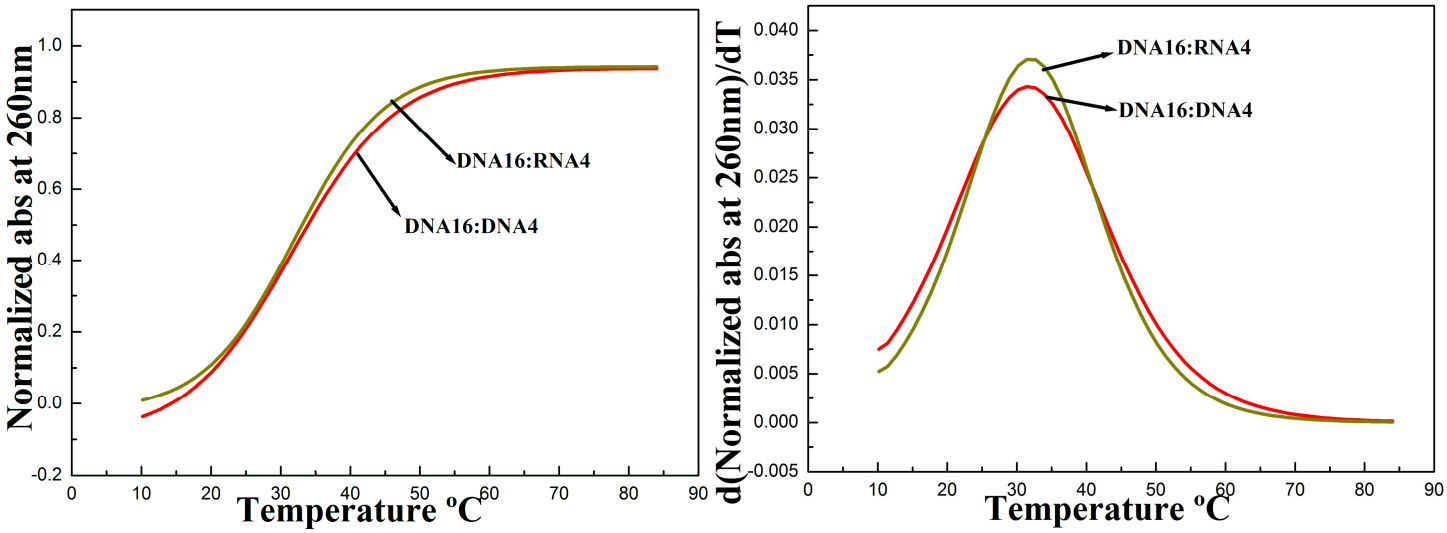


UV-T_m(ºC)		
	DNA2	RNA2
DNA14	26.0	27.6





UV-T_m(ºC)		
	DNA3	RNA3
DNA15	29.1	30.9

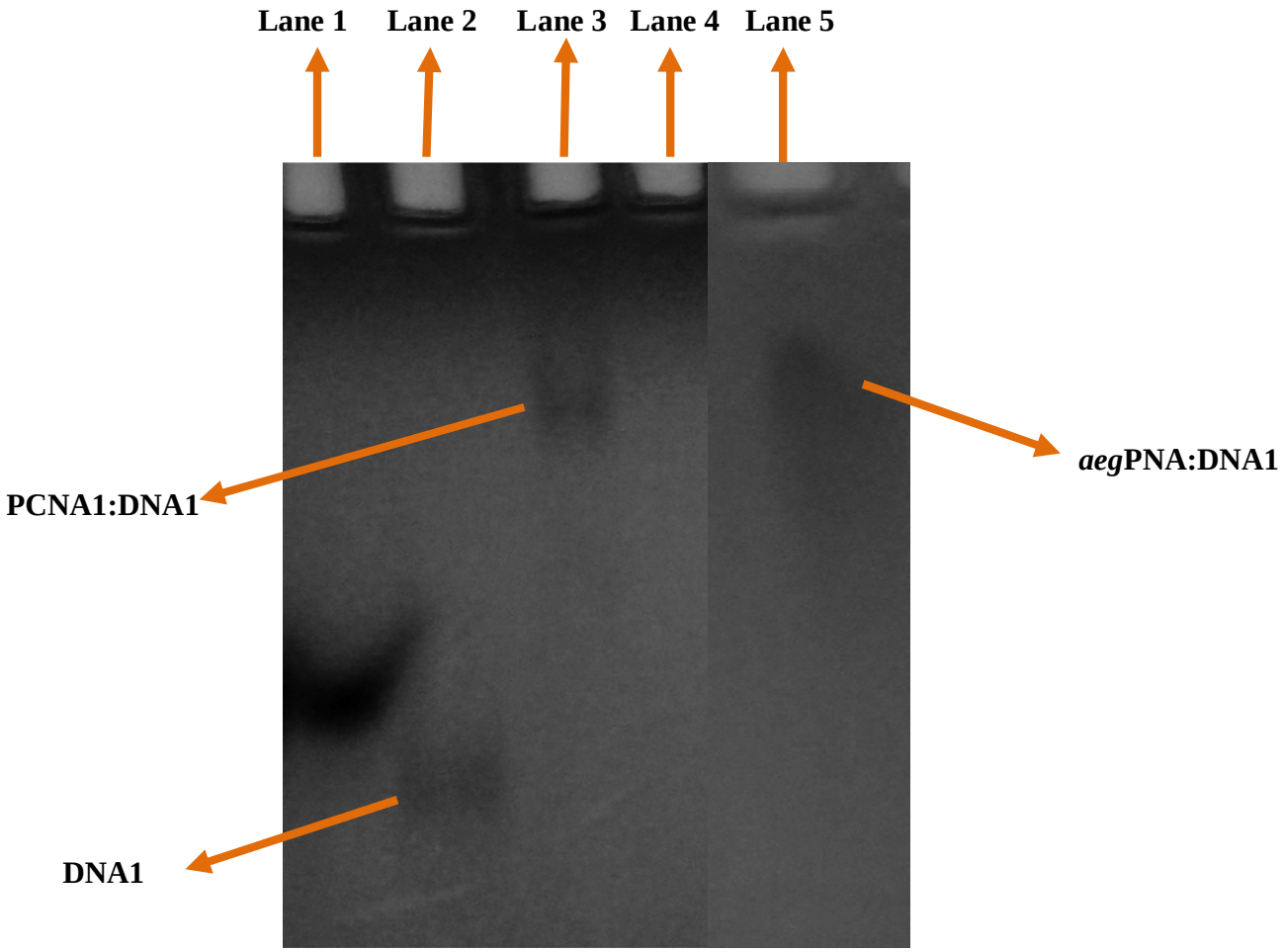


UV-T_m(ºC)		
	DNA4	**RNA4**
DNA16	**31.6**	**32.0**

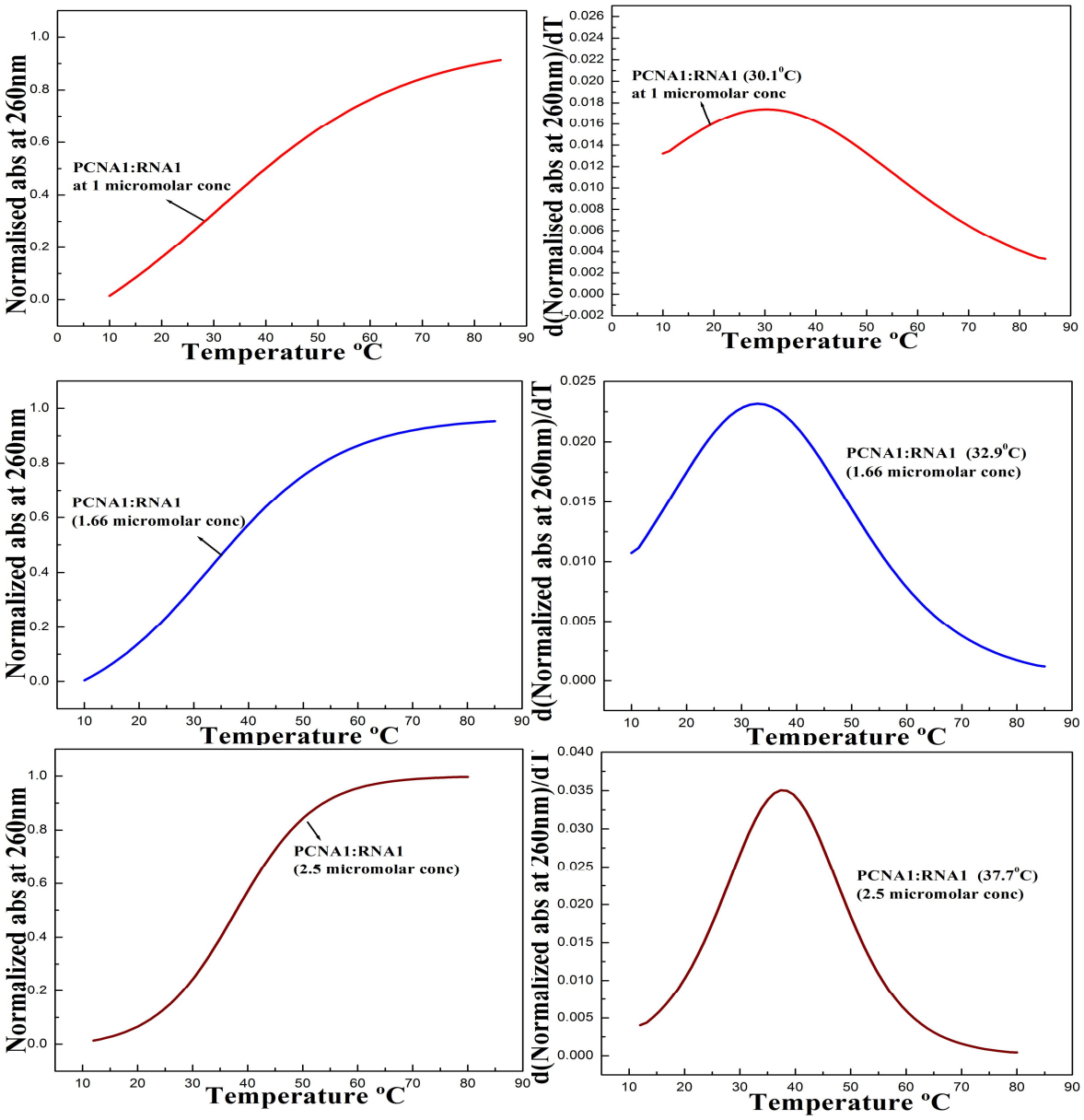
Electrophoretic gel mobility shift assay:

The PCNA1, *aeg*PNA were mixed separately with DNA1 in 2:1 ratio (PCNA1/*aeg*PNA strand, 0.4mM and DNA1, 0.2mM) in water. The samples were lyophilized to dryness and re-suspended in sodium phosphate buffer (10mM, pH 7.3, 10µl) containing EDTA (0.1mM). The samples were annealed by heating to 85ºC for 5 min followed by slow cooling to RT and refrigeration at 4 ºC overnight. To this, 10µl of 40% sucrose in TBE buffer pH 8.0 was added and the sample was loaded on the gel. Bromophenol blue (BPB) was used as the tracer dye separately in an adjacent well. Gel electrophoresis was performed on a 15% non-denaturing polyacrylamide gel (acrylamide:bis-acrylamide, 29:1) at constant power supply of 200V and 10mA, until the BPB migrated to three-

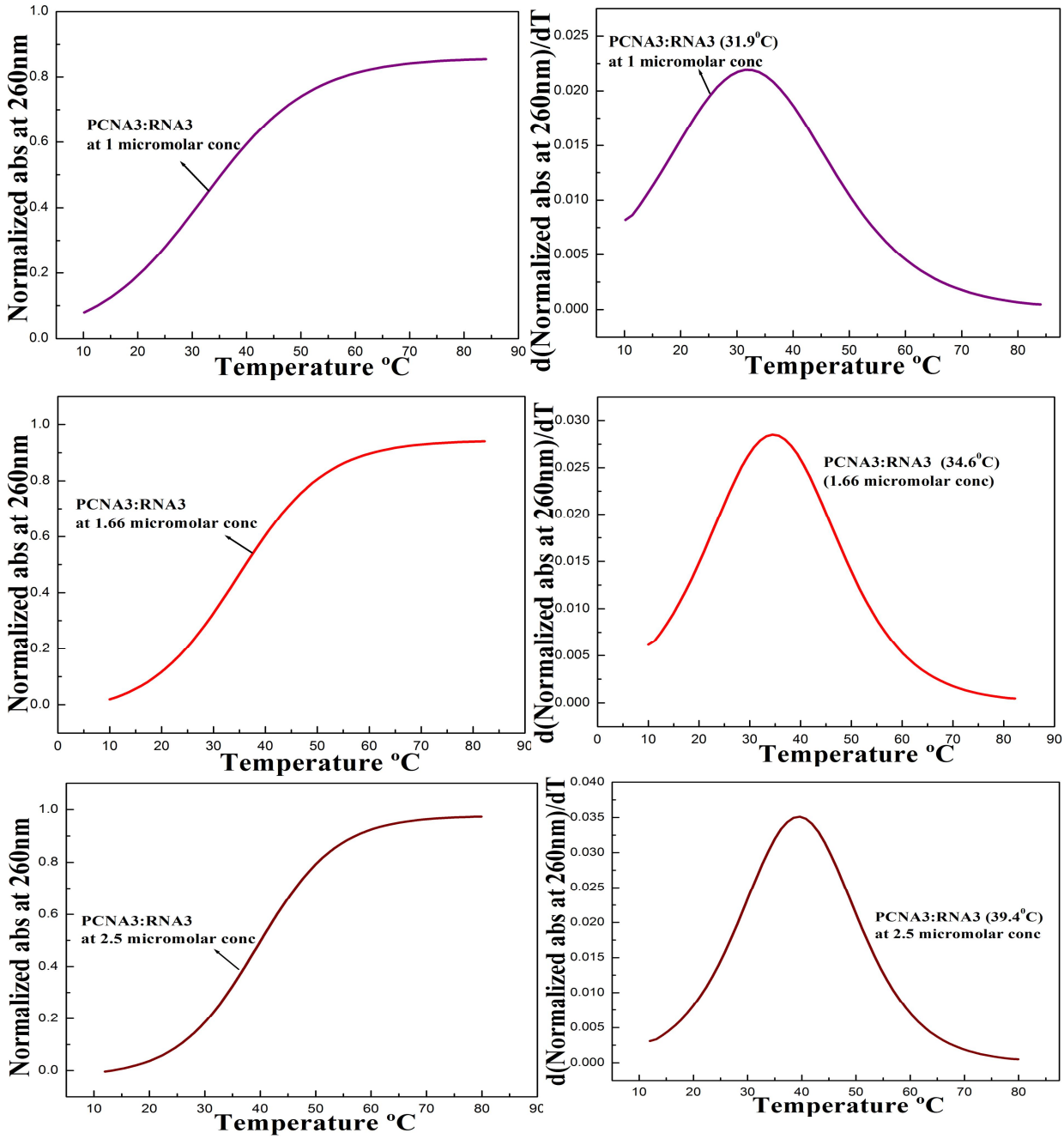
fourth of the gel length. During electrophoresis the temperature was maintained at 10ºC. The spots were visualized through UV shadowing by illuminating the gel placed on a silica gel plate, using UV-light.



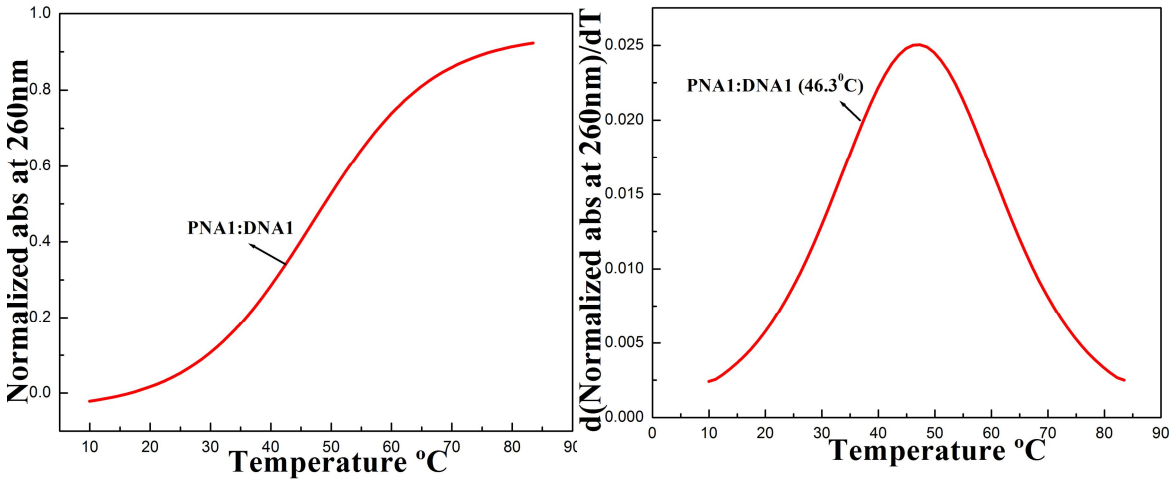
Lane 1 corresponds to Bromophenol blue
Lane 2 corresponds to single stand of DNA1
Lane 3 corresponds to PCNA1:DNA1 complex which is retarded
Lane 4 blank
Lane 5 corresponds to *aeg*PNA: DNA1 (as a control exp)



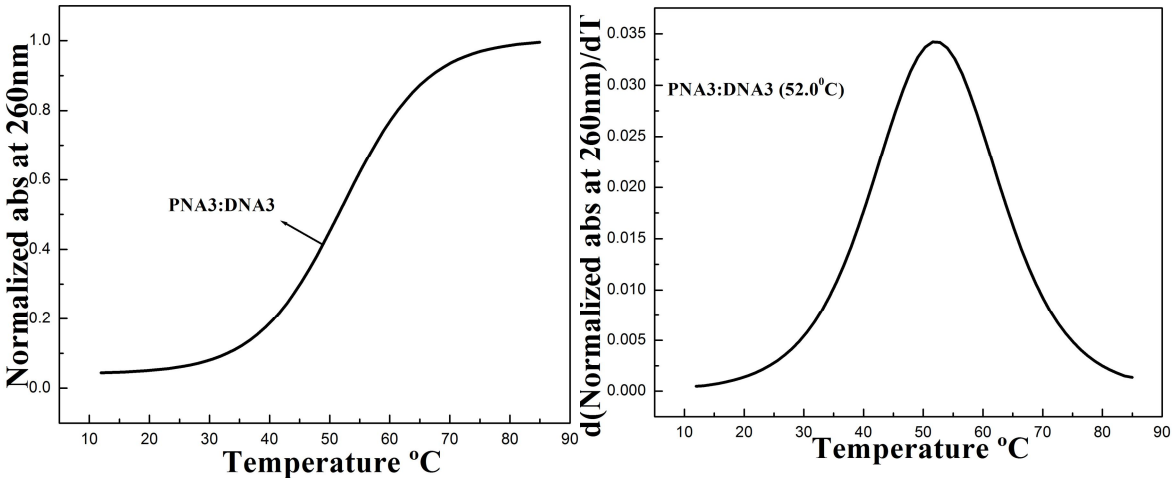
	RNA1		
	1µM	1.66µM	2.5µM
PCNA1	30.1	32.9	37.7



	RNA3		
	1μM	1.66μM	2.5μM
PCNA3	31.9	34.6	39.4



	DNA1
PNA1	46.3



	DNA3
PNA3	52.0