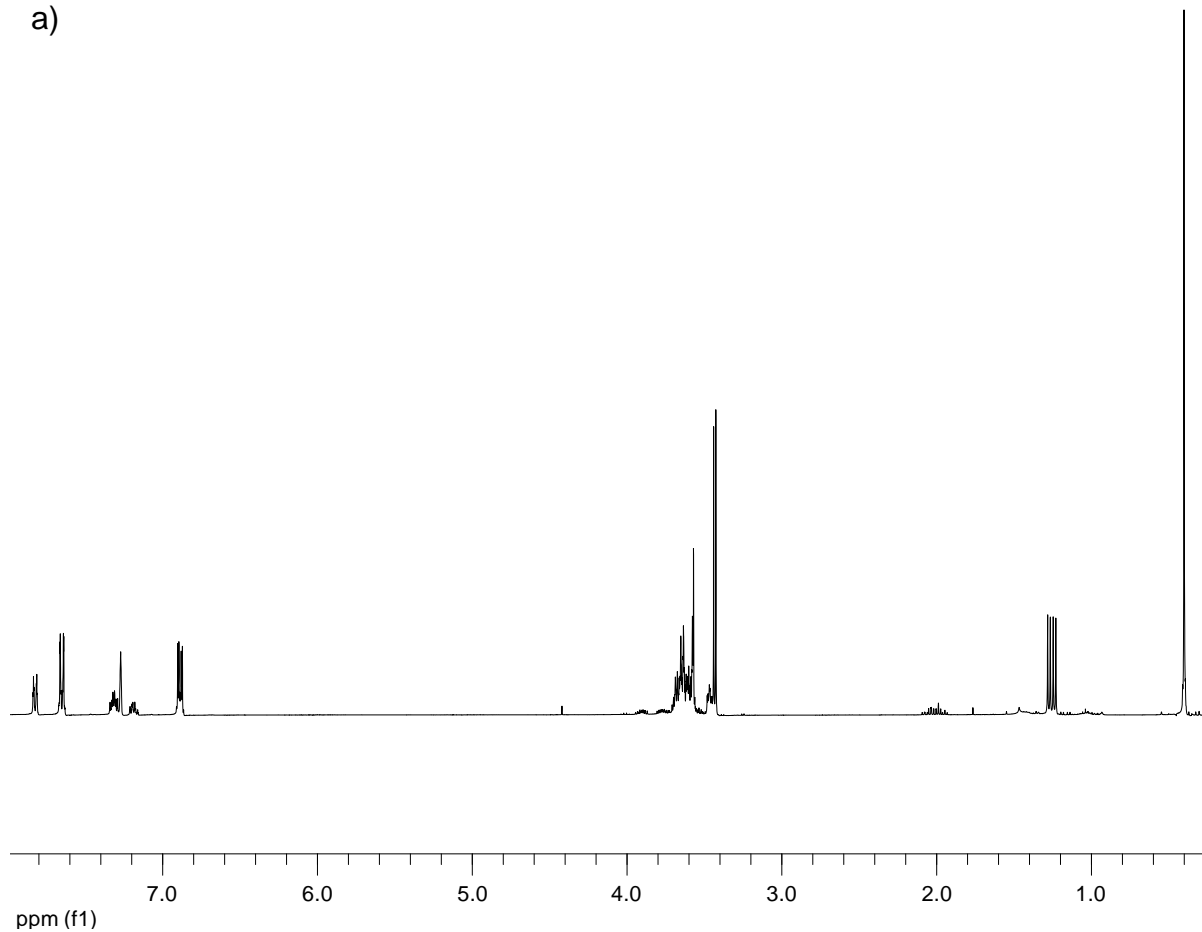


5 - Appendix

a)



Spectrum Title:

ajp53-1-8-8 f15-22

Frequency (MHz):

(f1) 400.132

Original Points Count:

(f1) 16384

Actual Points Count:

(f1) 32768

Acquisition Time (sec):

(f1) 2.5559

Spectral Width (ppm):

(f1) 16.020

Pulse Program:

ZG30

Temperature:

300

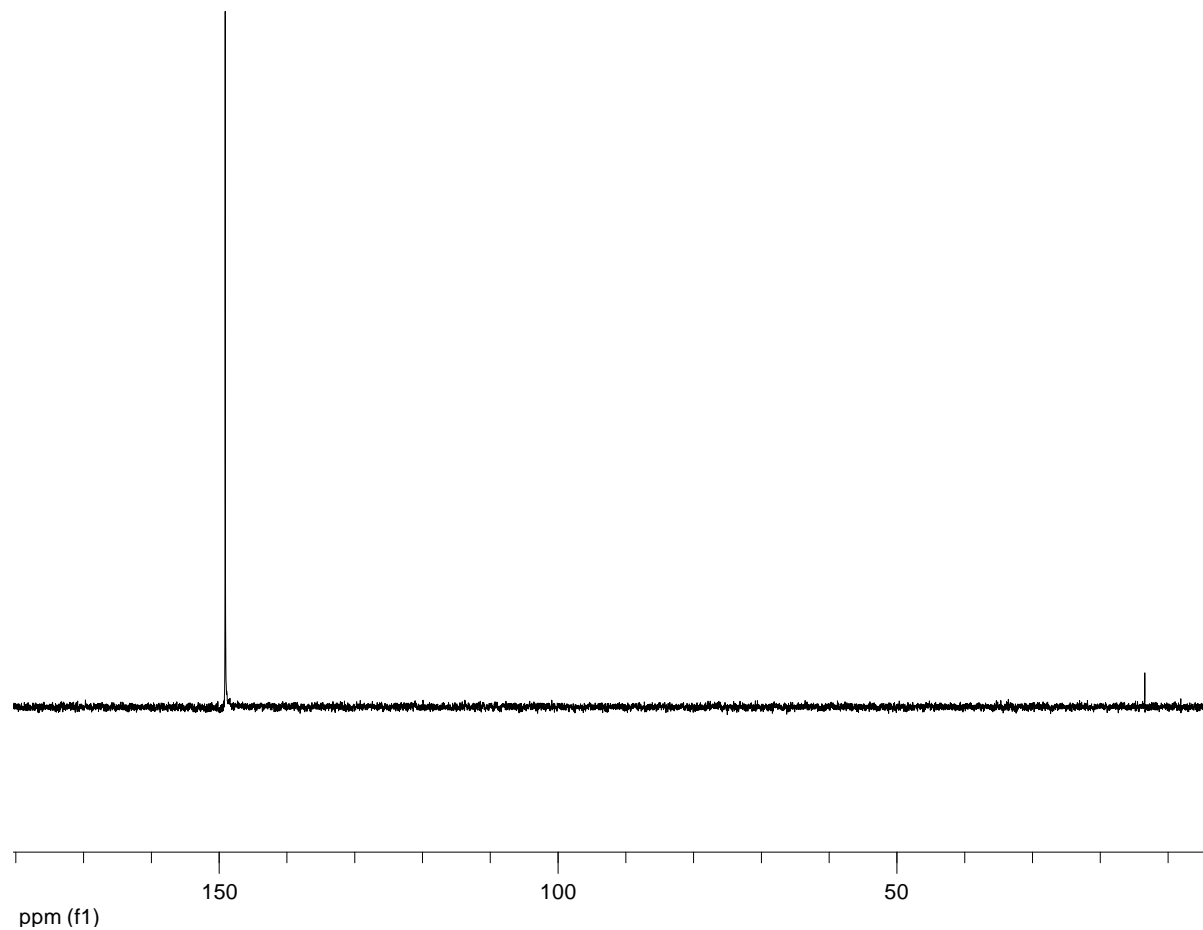
Number of Scans:

16

Acq. Date:

Thu Nov 13 12:12:42 AM

b)



Spectrum Title:
ajp53-1-8-8 f15-22

Frequency (MHz):
(f1) 161.988

Original Points Count:
(f1) 8192

Actual Points Count:
(f1) 16384

Acquisition Time (sec):
(f1) 0.2015

Spectral Width (ppm):
(f1) 250.947

Pulse Program:
ZGIG30

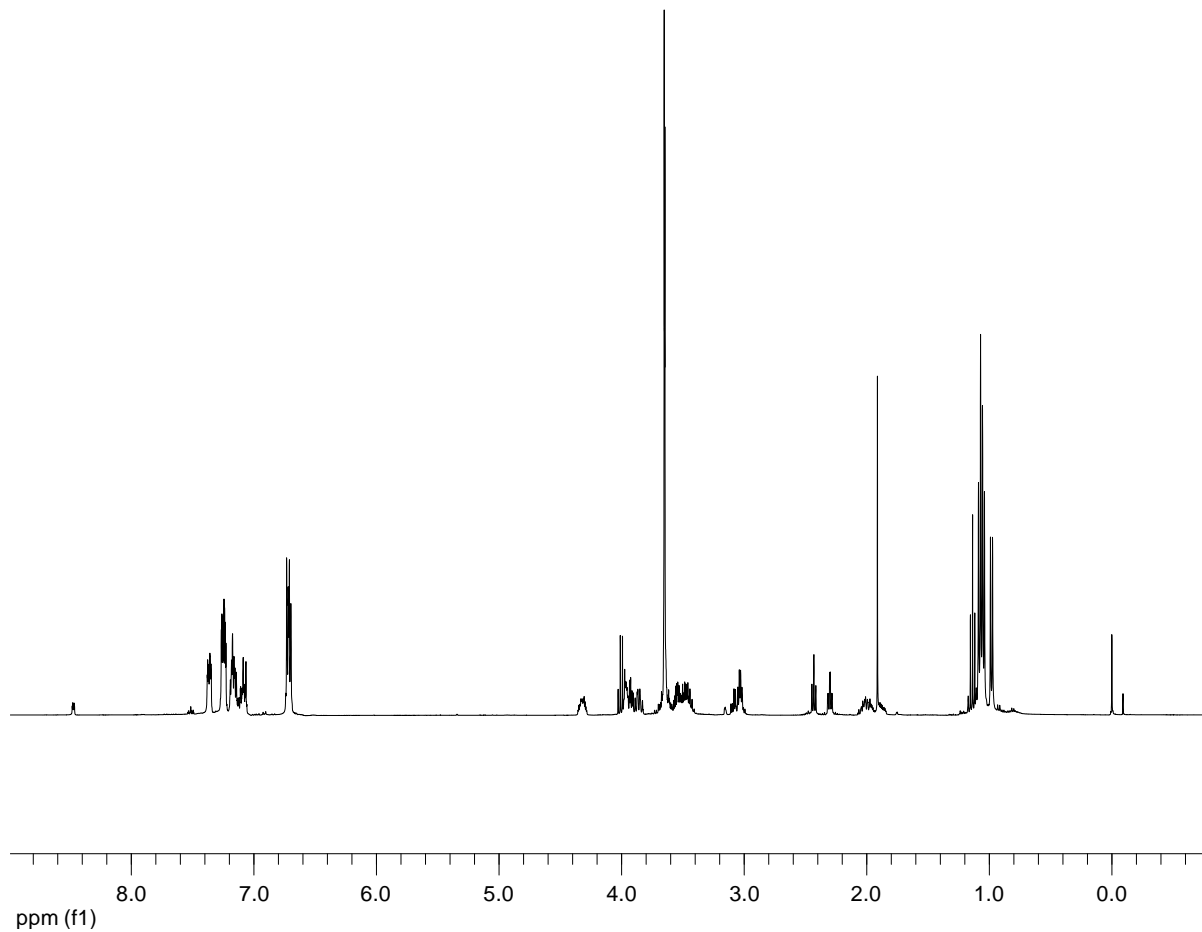
Temperature:
300

Number of Scans:
128

Acq. Date:
Thu Nov 13 12:16:02 AM

Figure 5.1. NMR spectra for compound 2 where a) ^1H and b) ^{31}P .

a)



Spectrum Title:
ajp53-2-78-phosphoramidite

Frequency (MHz):
(f1) 400.132

Original Points Count:
(f1) 16384

Actual Points Count:
(f1) 32768

Acquisition Time (sec):
(f1) 2.5559

Spectral Width (ppm):
(f1) 16.020

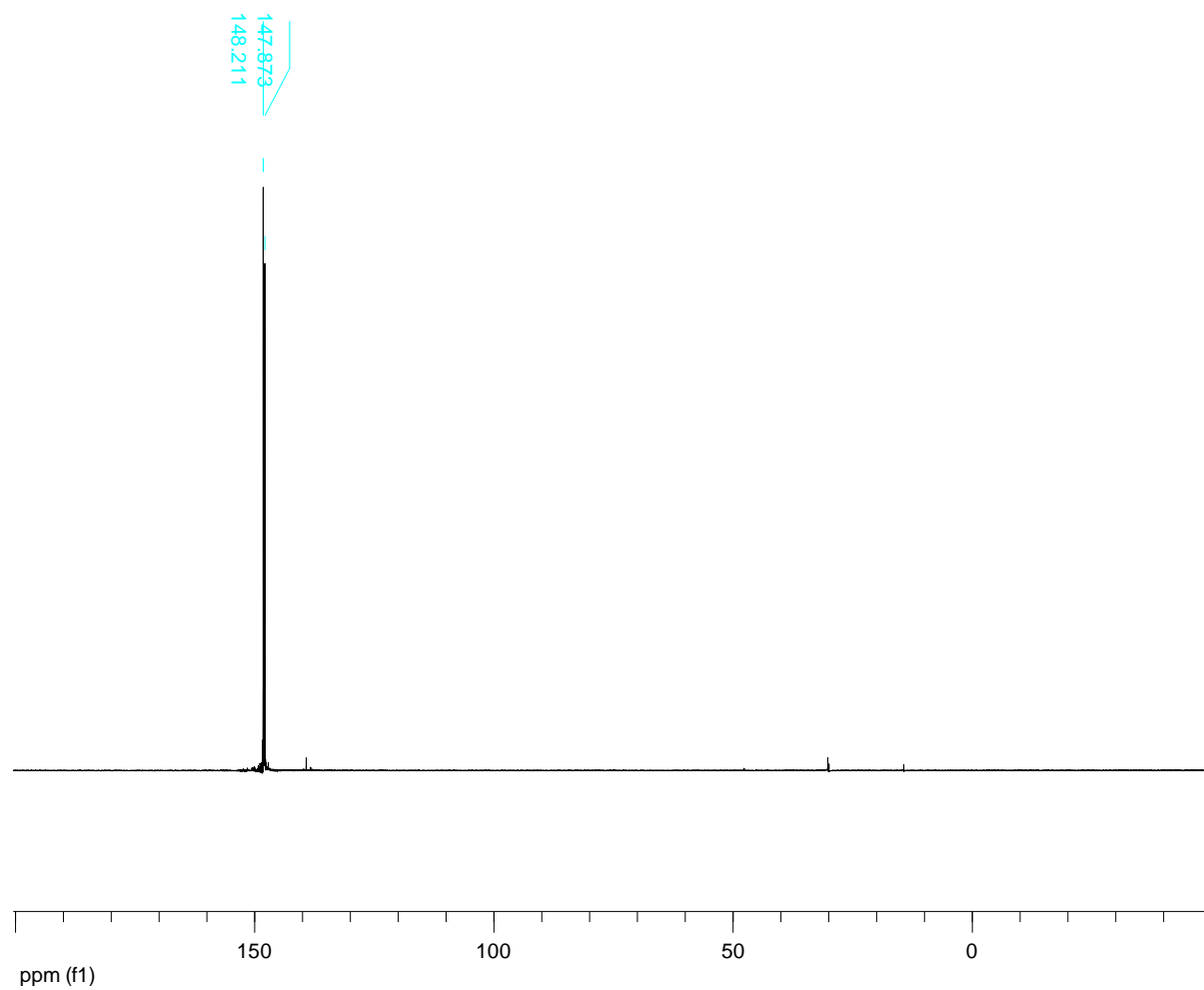
Pulse Program:
ZG30

Temperature:
300

Number of Scans:
16

Acq. Date:
Thu Dec 09 05:40:45 PM

b)



Spectrum Title:
ajp53-2-78-phosphoramidite

Frequency (MHz):

(f1) 161.988

Original Points Count:

(f1) 8192

Actual Points Count:

(f1) 16384

Acquisition Time (sec):

(f1) 0.2015

Spectral Width (ppm):

(f1) 250.947

Pulse Program:

ZGIG30

Temperature:

300

Number of Scans:

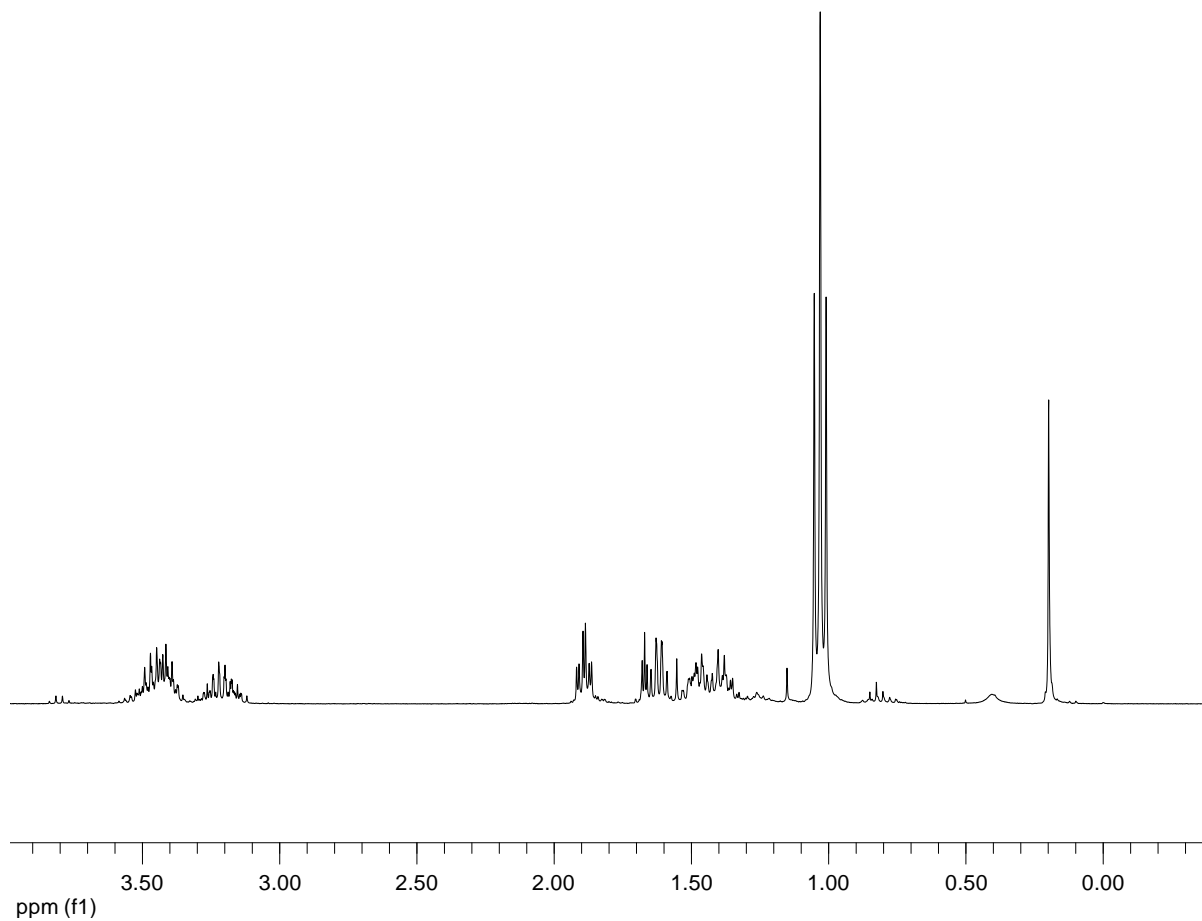
128

Acq. Date:

Thu Dec 09 05:44:11 PM

Figure 5.2. NMR spectra for compound 8 where a) ^1H and b) ^{31}P .

a)



Spectrum Title:

ajp53-alkyne-sm

Frequency (MHz):

(f1) 299.902

Original Points Count:

(f1) 16384

Actual Points Count:

(f1) 32768

Acquisition Time (sec):

(f1) 3.4210

Spectral Width (ppm):

(f1) 15.969

Pulse Program:

ZG30

Temperature:

300

Number of Scans:

64

Acq. Date:

Tue Jun 01 06:31:13 PM

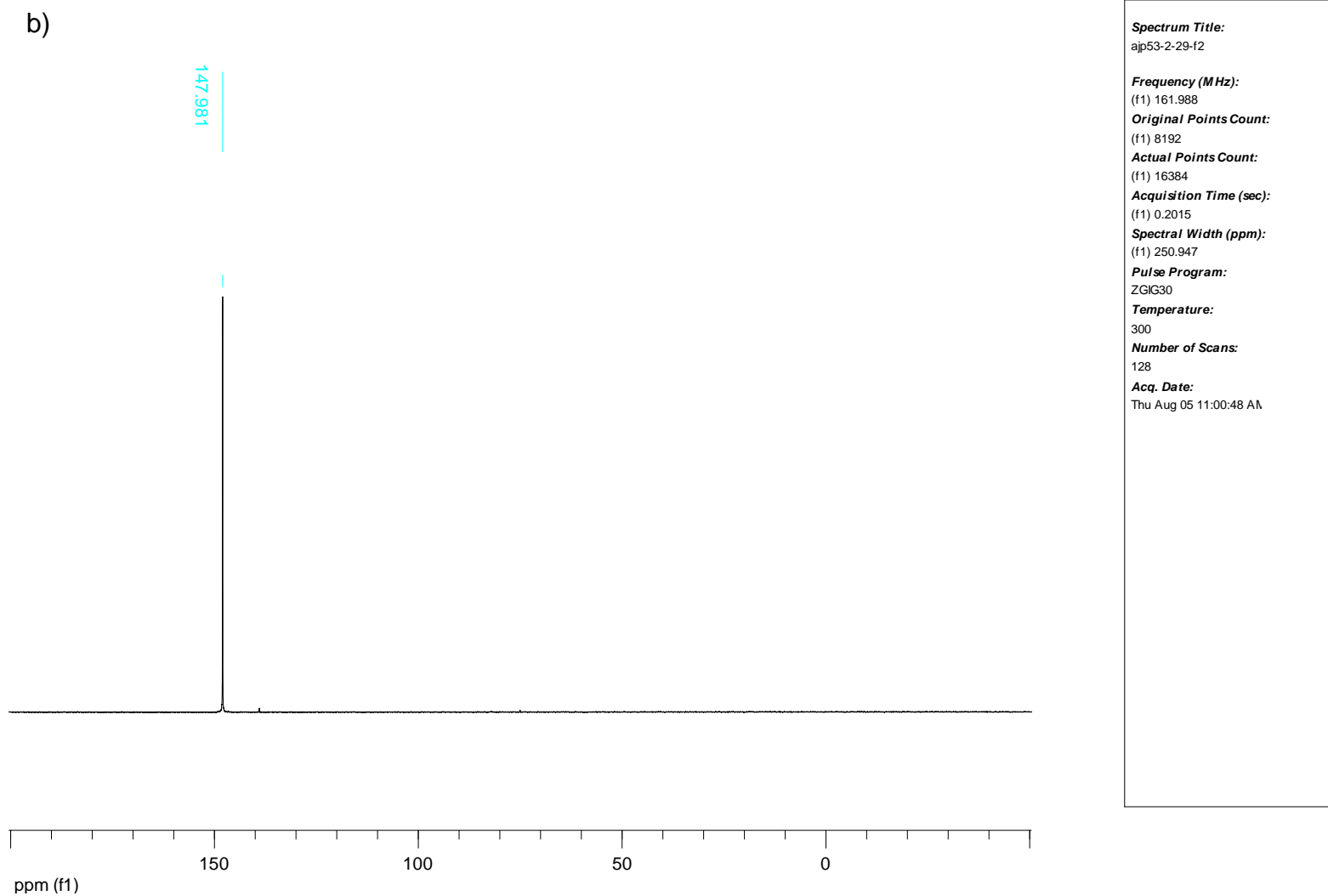
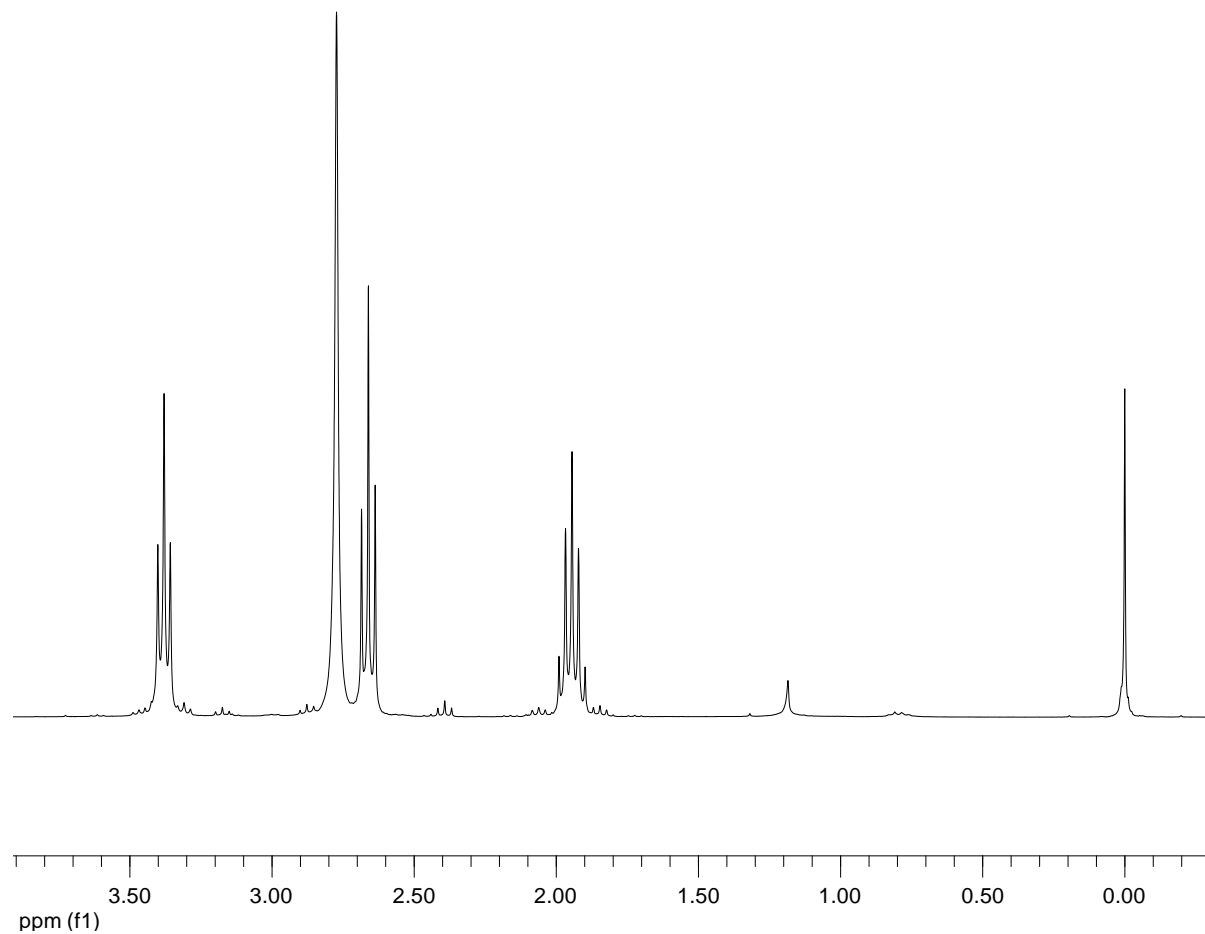


Figure 5.3. NMR spectra for compound 3 where a) ^1H and b) ^{31}P .



Spectrum Title:
ajp53-1-52-2 checking material

Frequency (MHz):
(f1) 299.902

Original Points Count:
(f1) 16384

Actual Points Count:
(f1) 32768

Acquisition Time (sec):
(f1) 3.4210

Spectral Width (ppm):
(f1) 15.969

Pulse Program:
ZG30

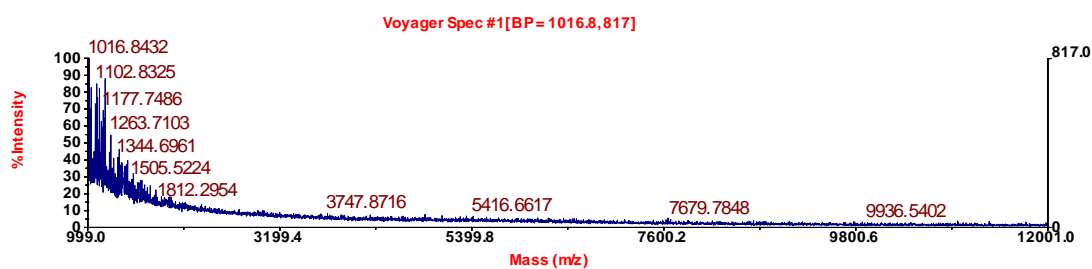
Temperature:
300

Number of Scans:
64

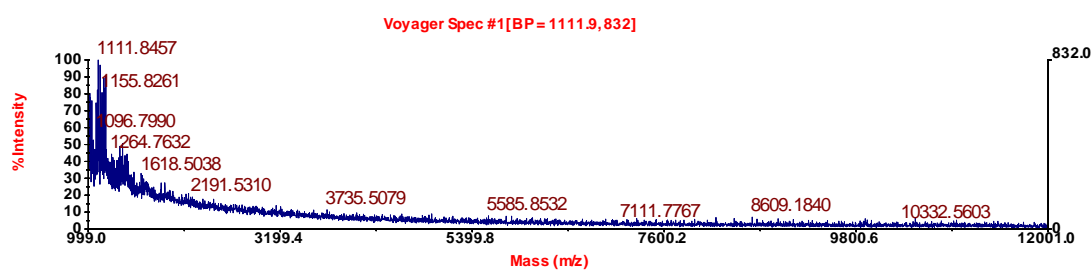
Acq. Date:
Mon Sep 28 02:49:15 PM

Figure 5.4. ^1H NMR spectra for compound 4.

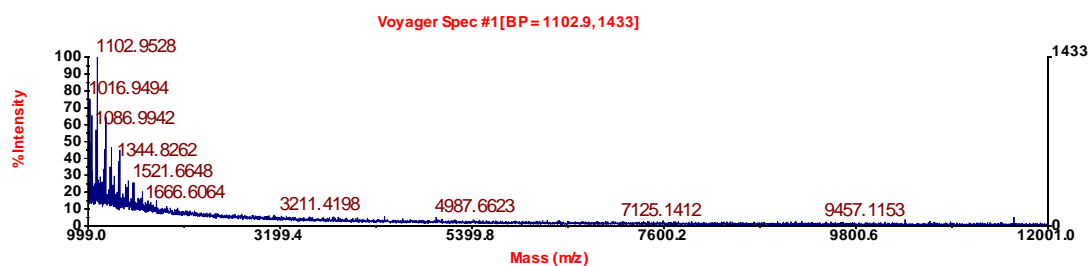
a)



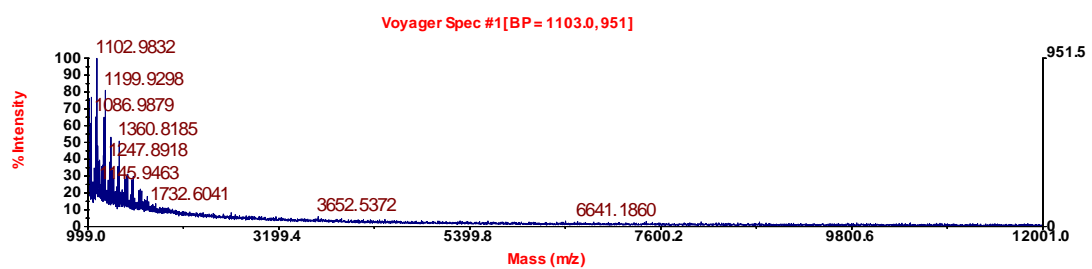
b)



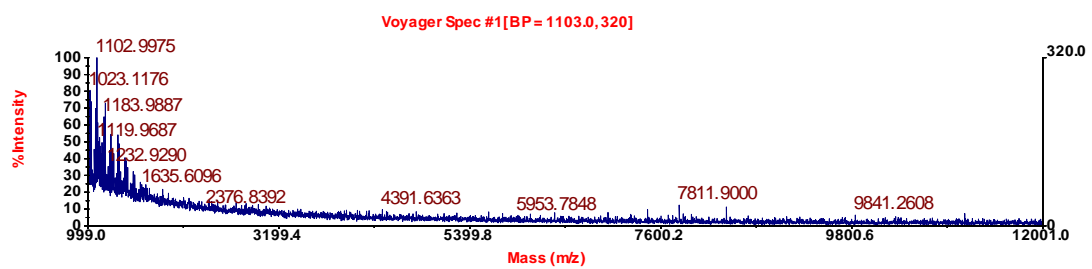
c)



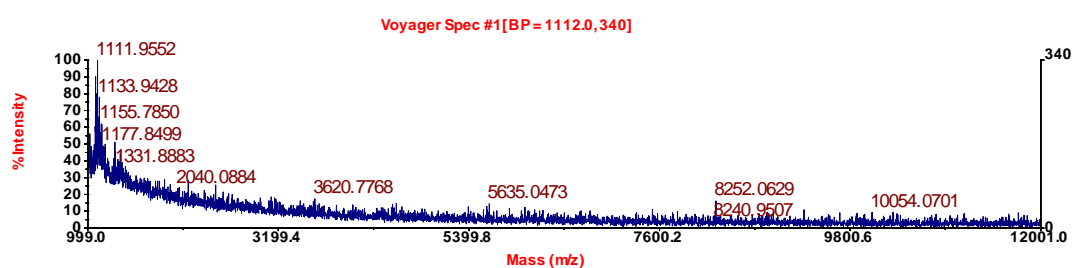
d)



e)



f)



g)

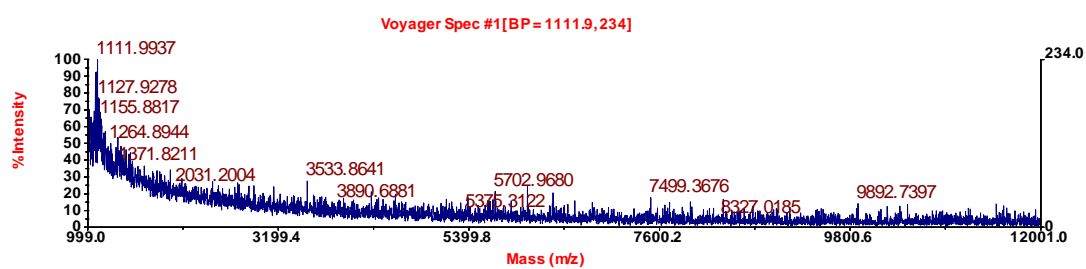


Figure 5.5. MADLI-TOF samples run in negative mode after attempted purification by RP-HPLC for RNA1(a-d) and RNA1_A (e-g). Expected mass of RNA1 at $[M+H] = 7952.15$ and RNA1_A at $[M+H] = 8131.29$

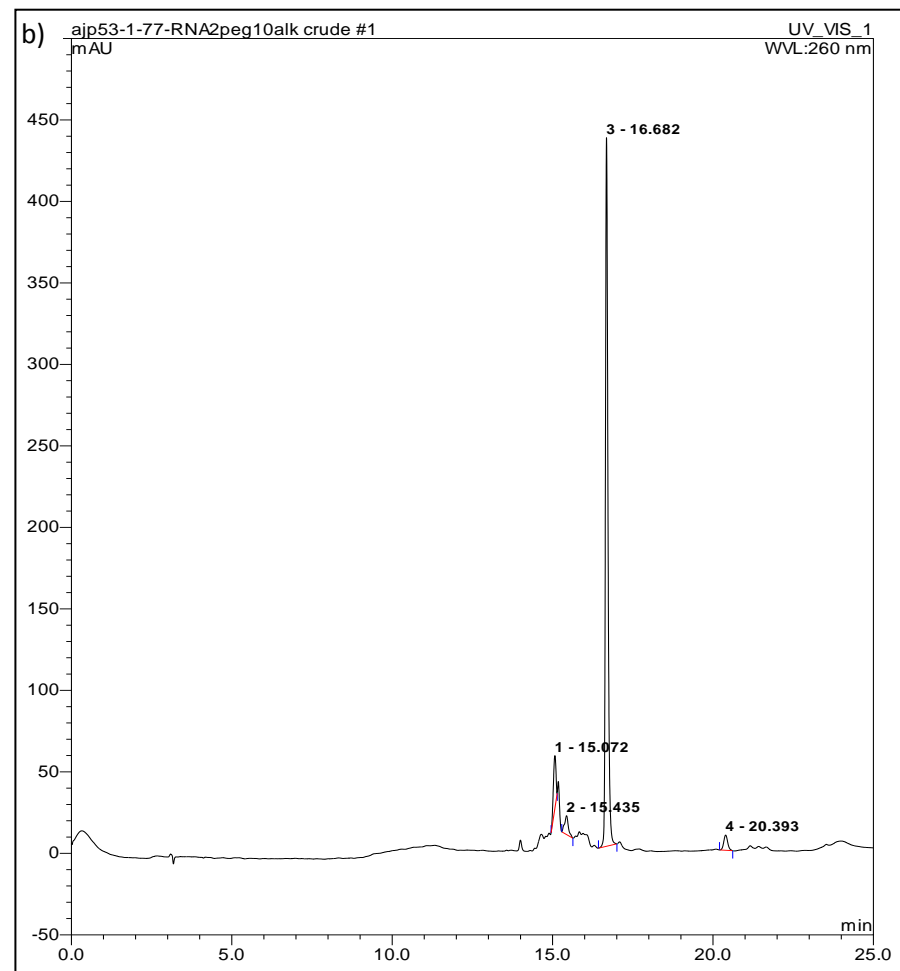
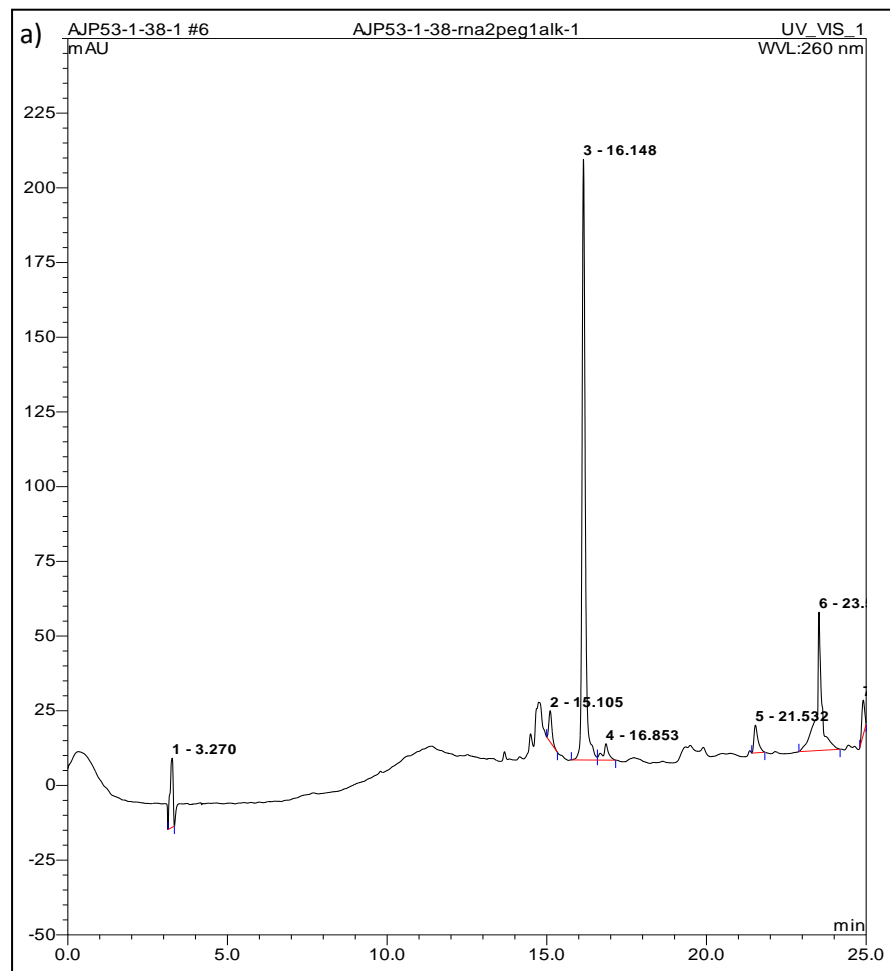


Figure 5.6. Crude RP-HPLC traces for a) RNA2_B and b) RNA2_C. RP-HPLC was carried out using a standard protocol with 5% - 60% gradients.

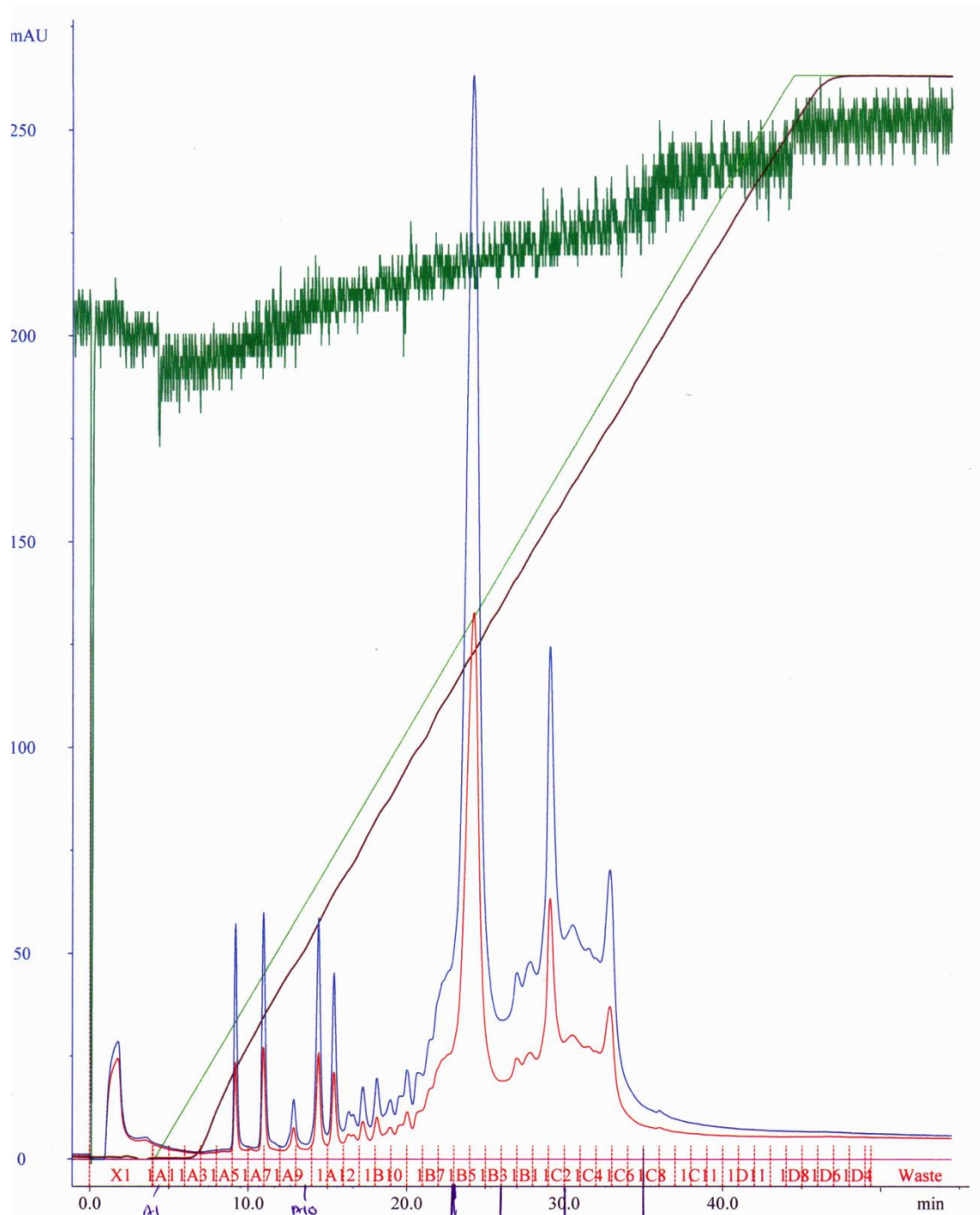
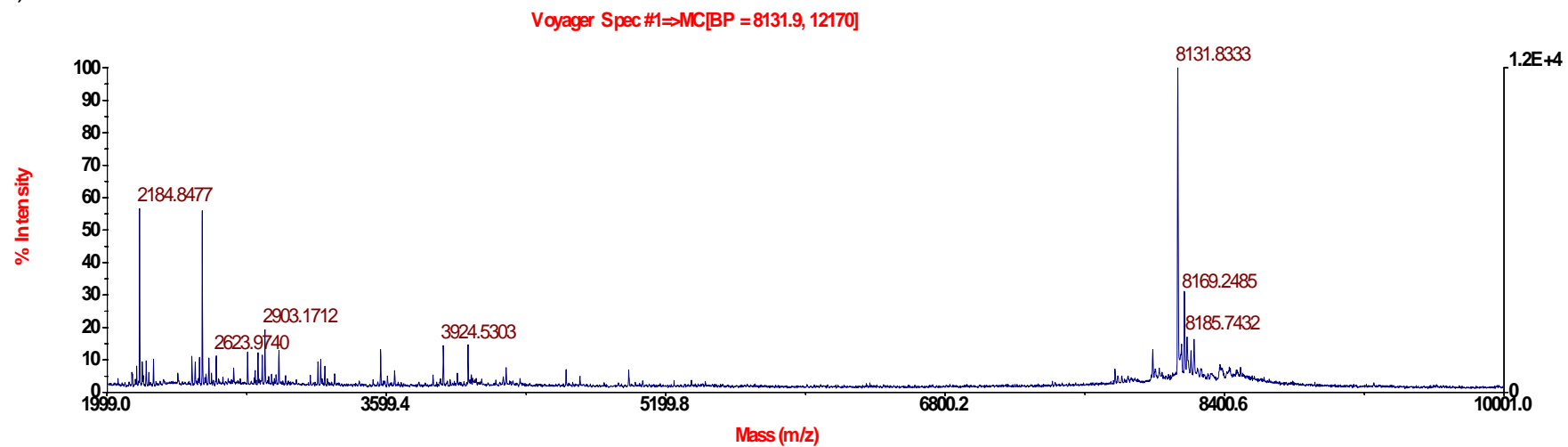
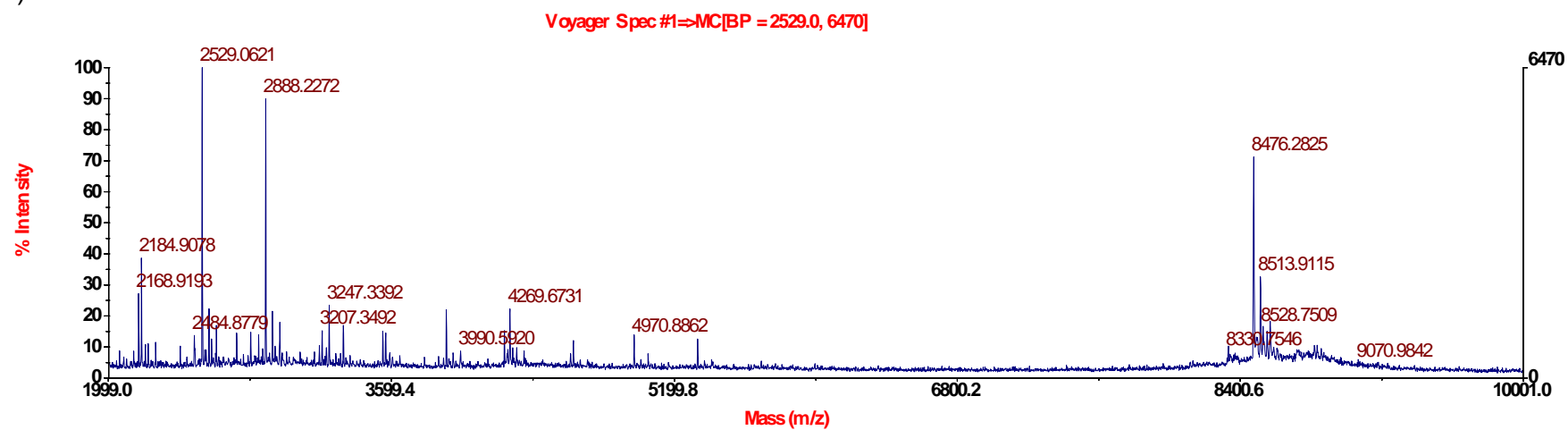


Figure 5.7. Ion exchange trace for RNA1_C purification running a gradient from 0% to 100% over 40 minutes. Where buffer A is 20mM NaCl with 10mM Hepes and buffer B is 1M NaCl with 10mM Hepes. The blue line indicates absorption at 260nm, the red line absorption at 280nm and the light green line the gradient..

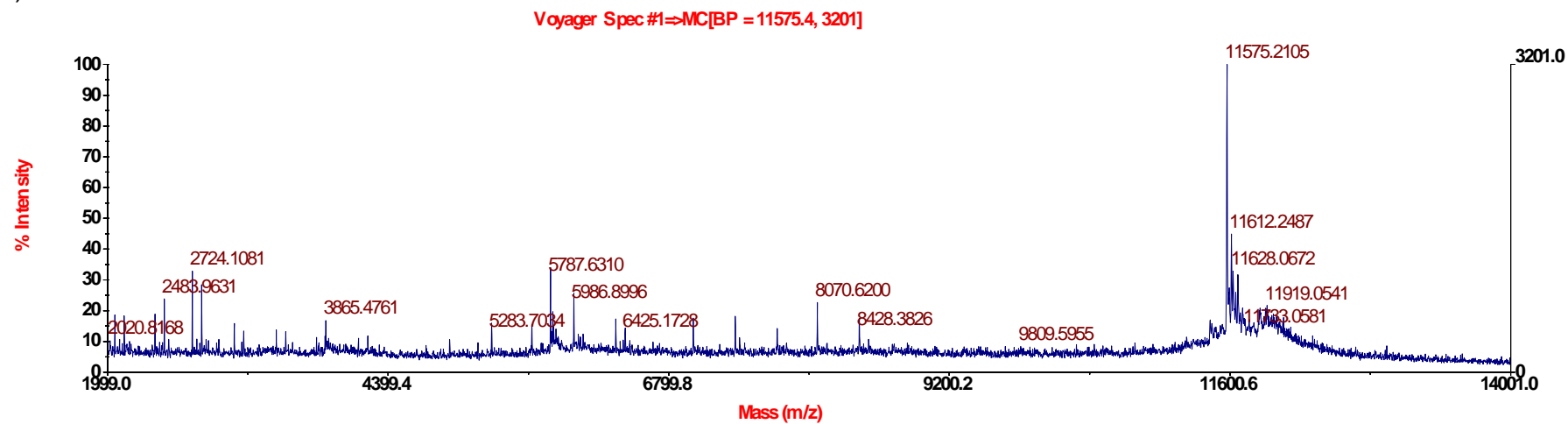
a)



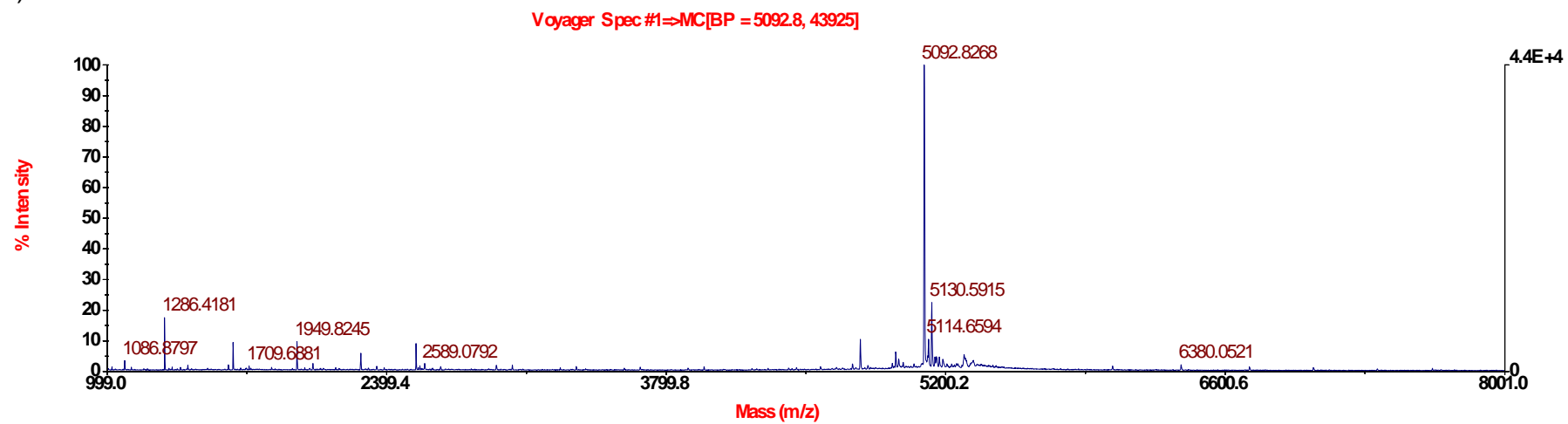
b)



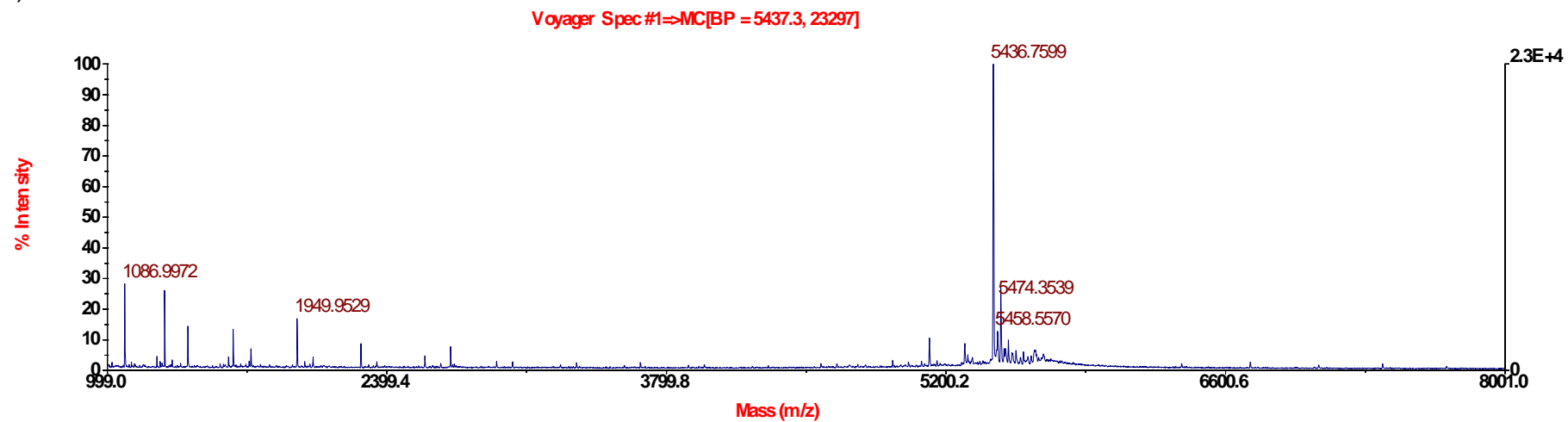
c)



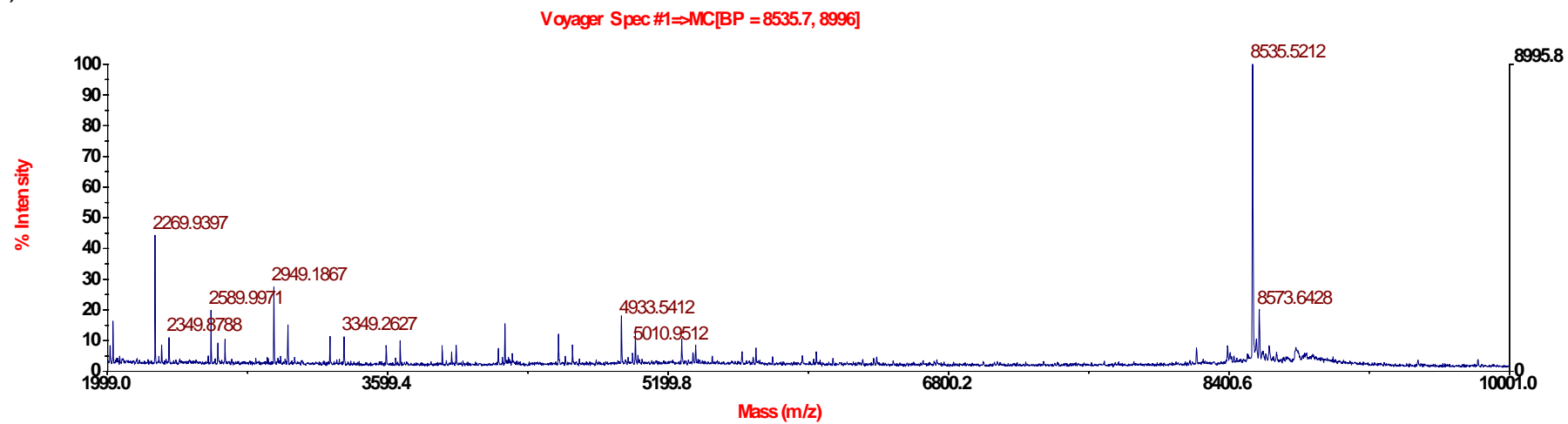
d)



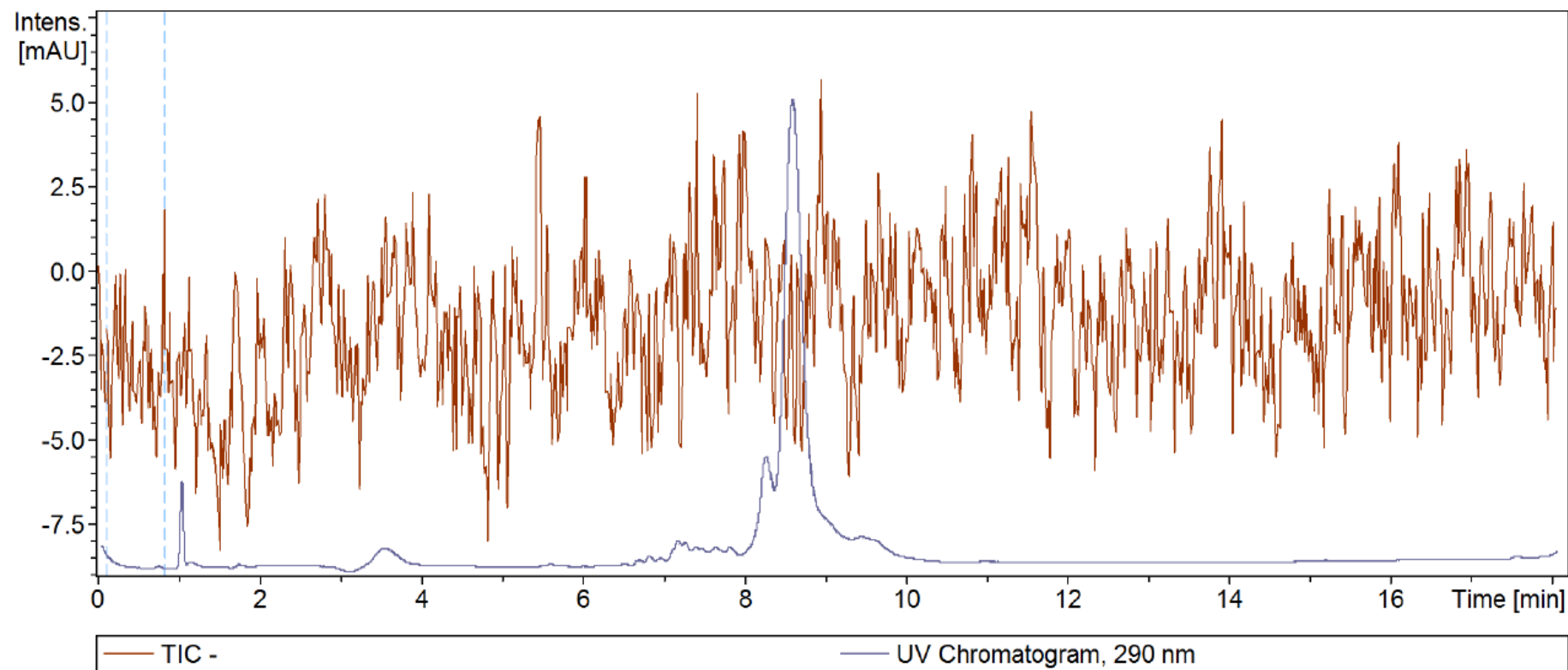
e)



f)



g)



h)

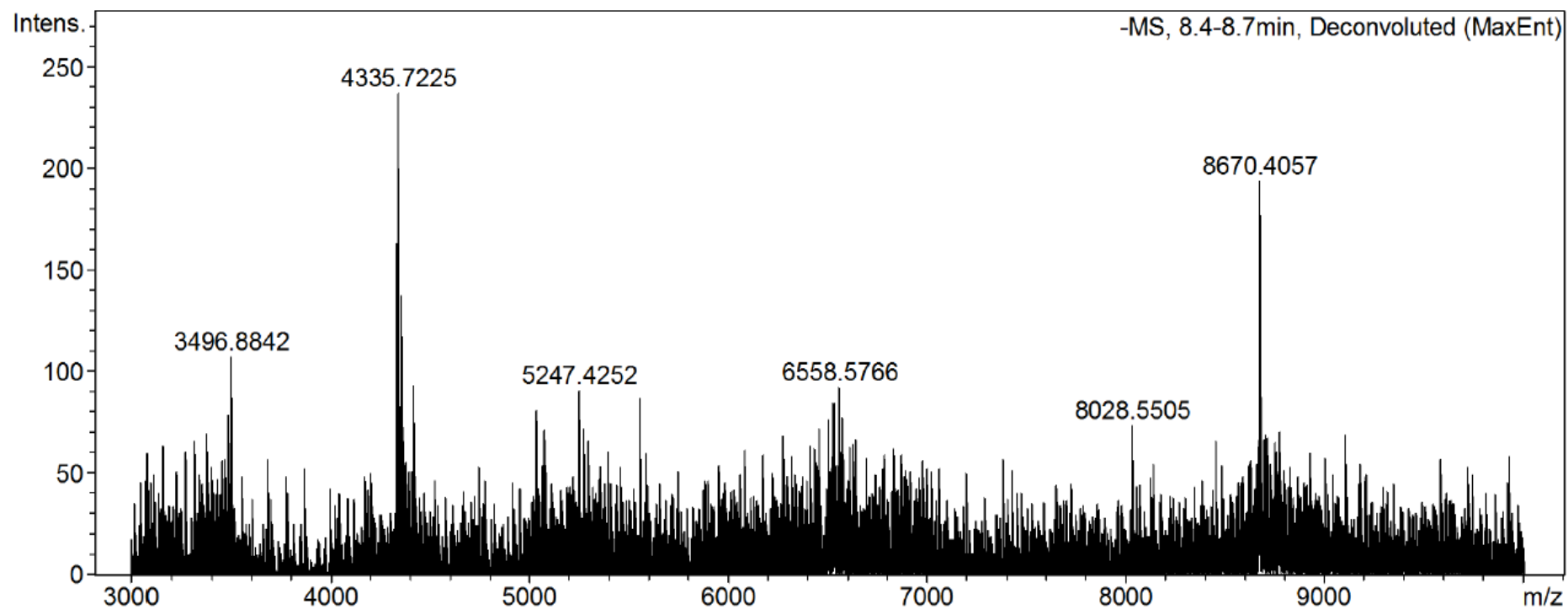
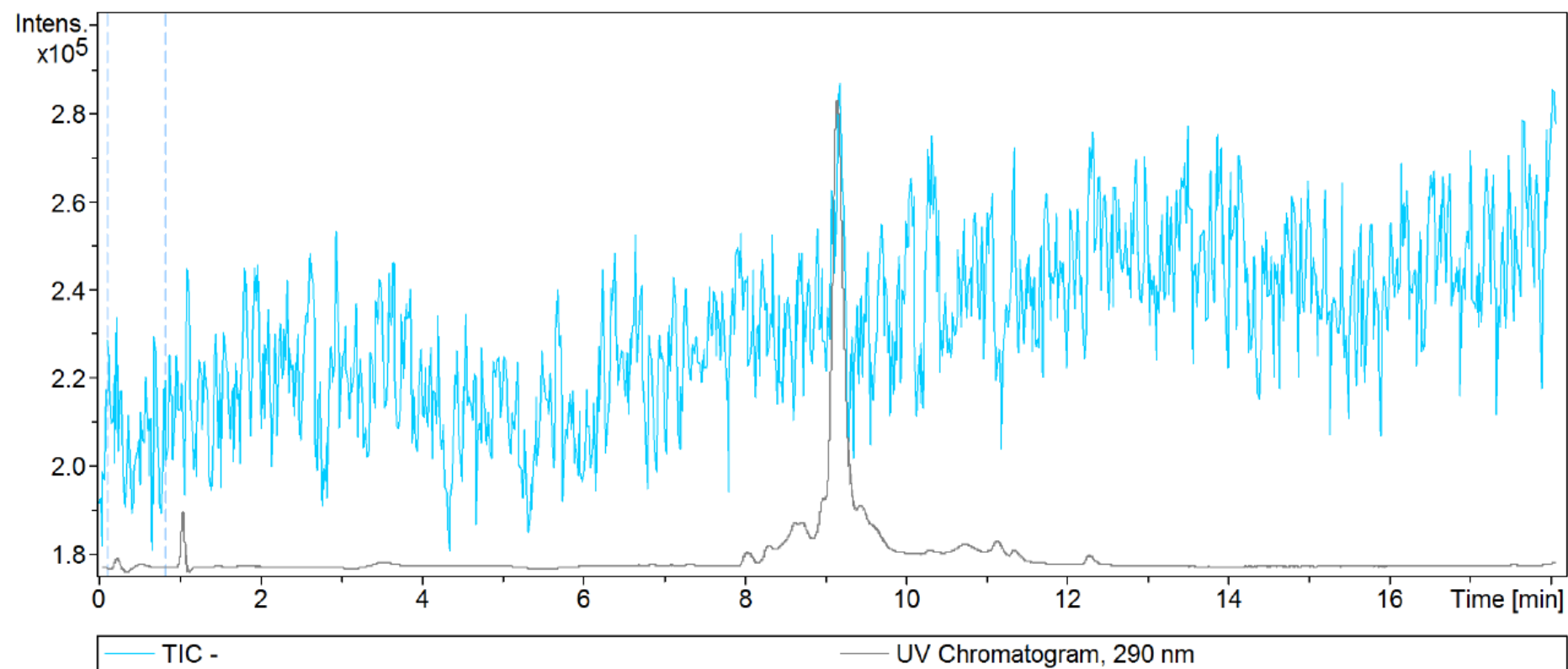
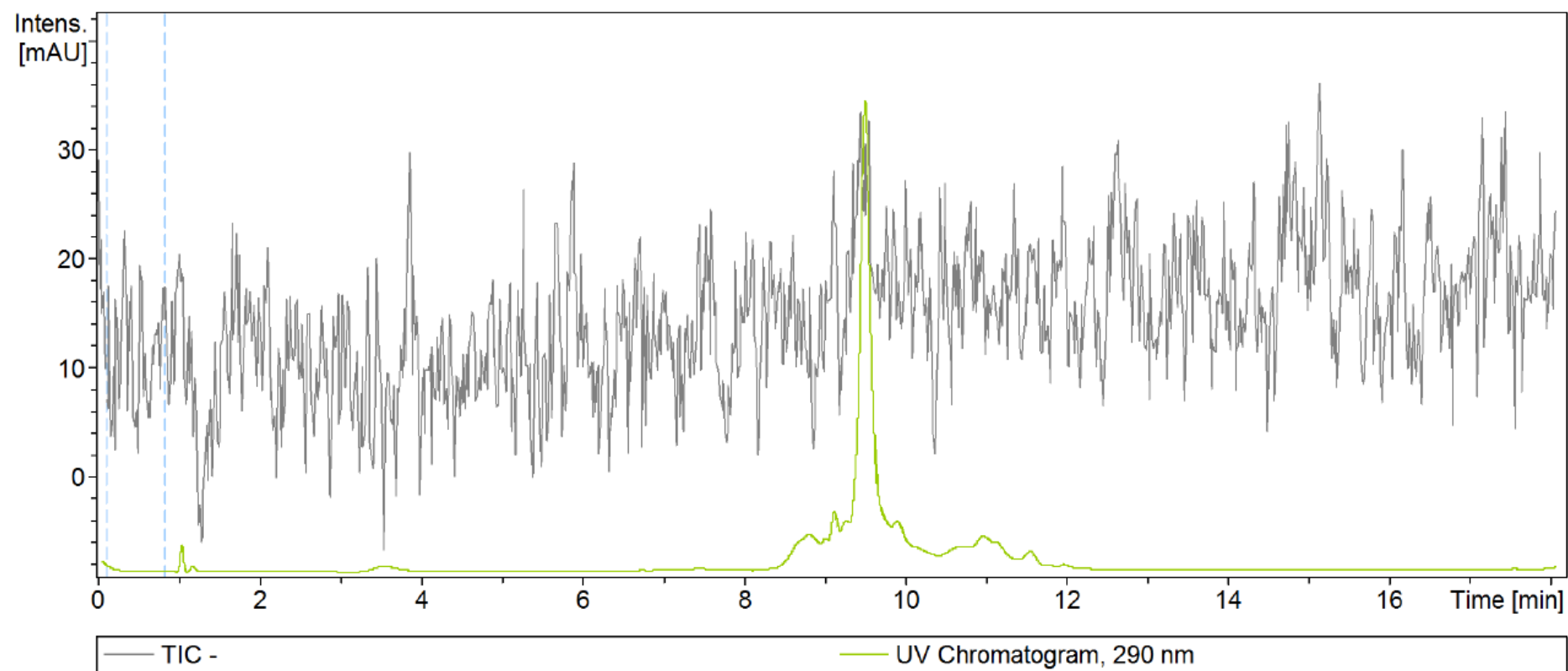


Figure 5.8. **a-f)** MADLI-TOF samples run in negative mode after attempted purification for RNA1_A (a), RNA1_C (b), RNA1_E (c), RNA2_A (d), RNA2_B (e) and RNA2_c (f). **g-h)** HPLC MS for RNA1_G after desalting.

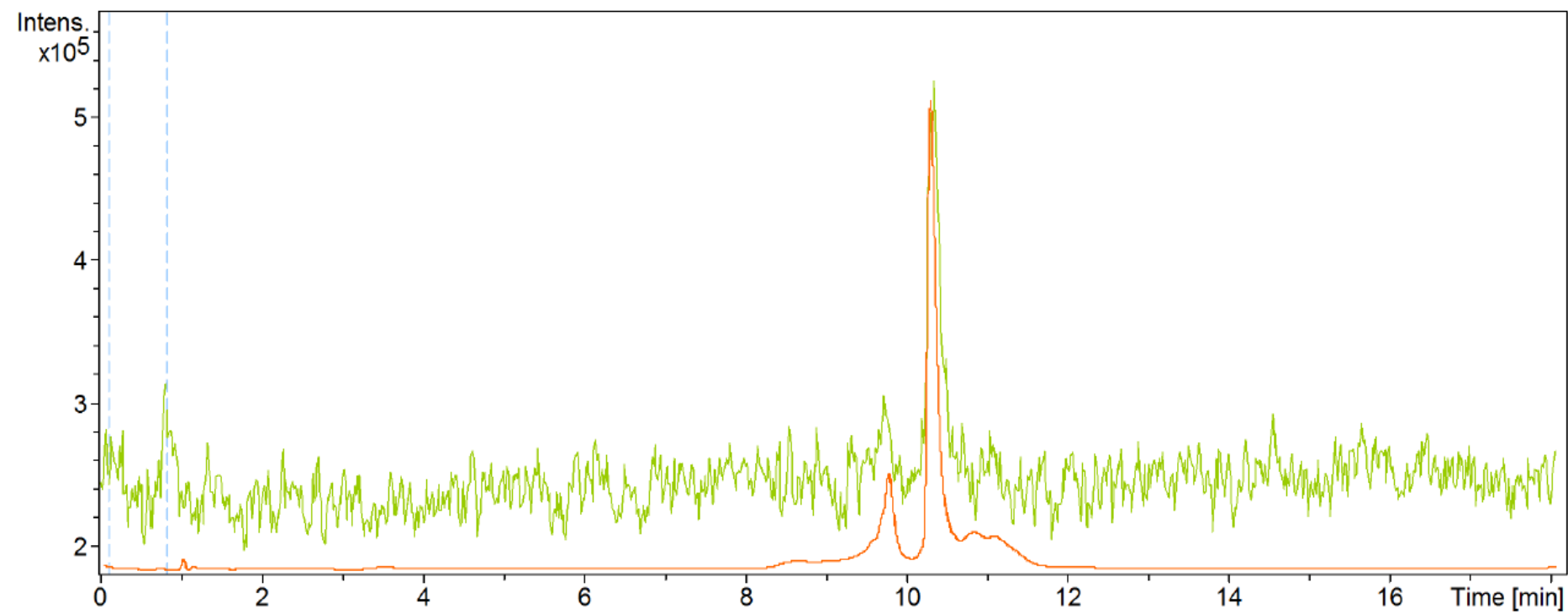
a)



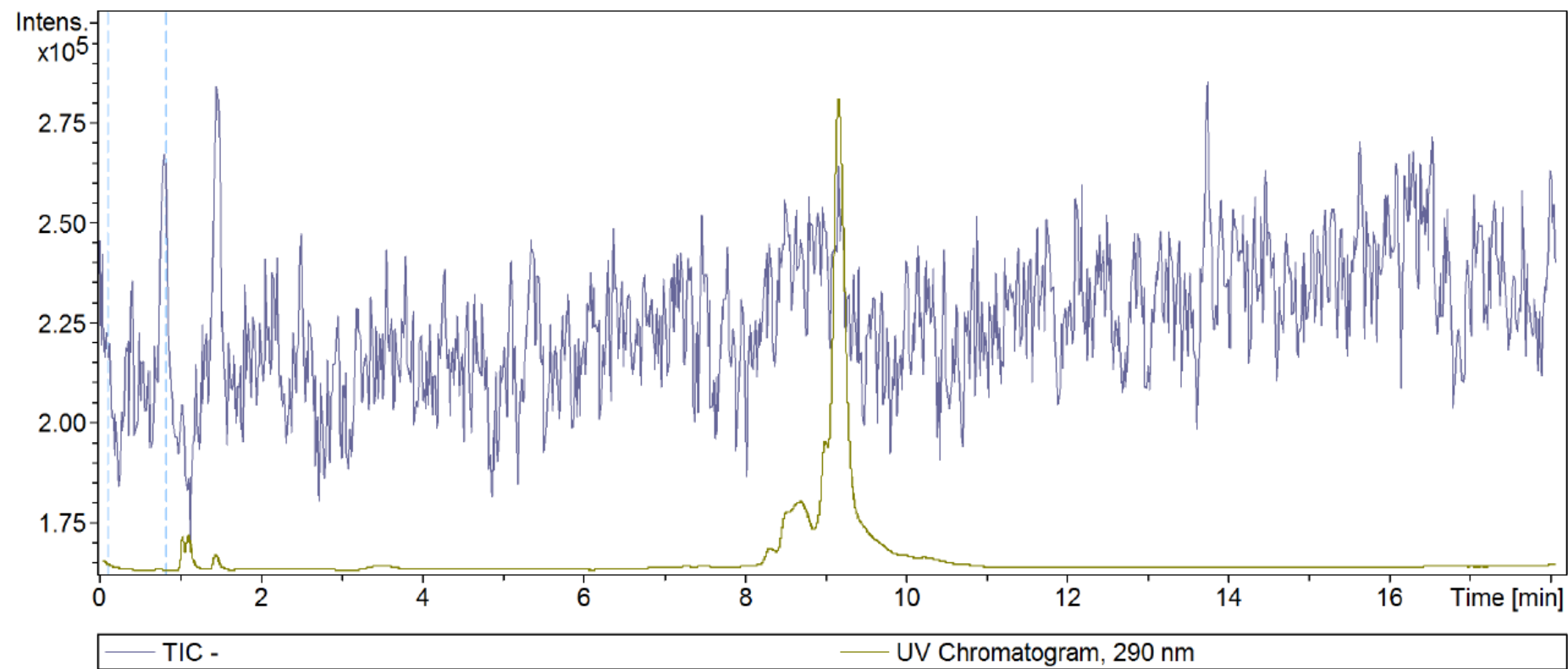
b)



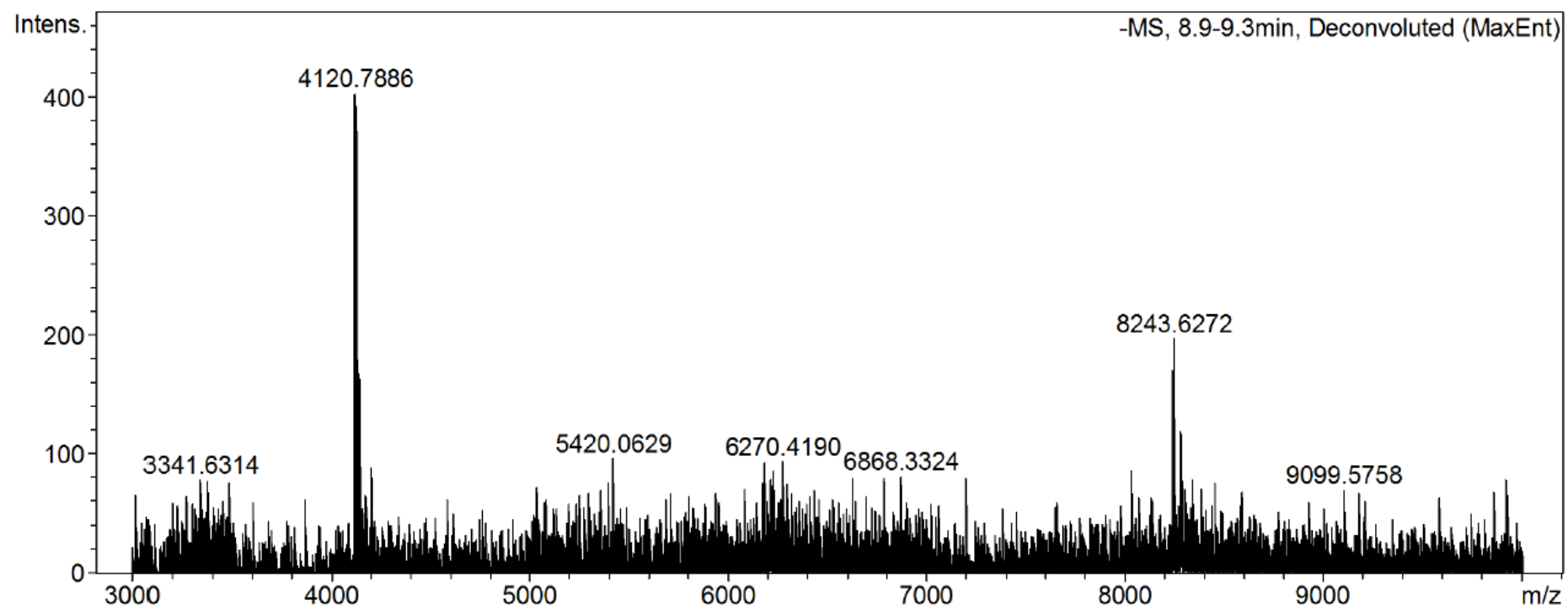
c)



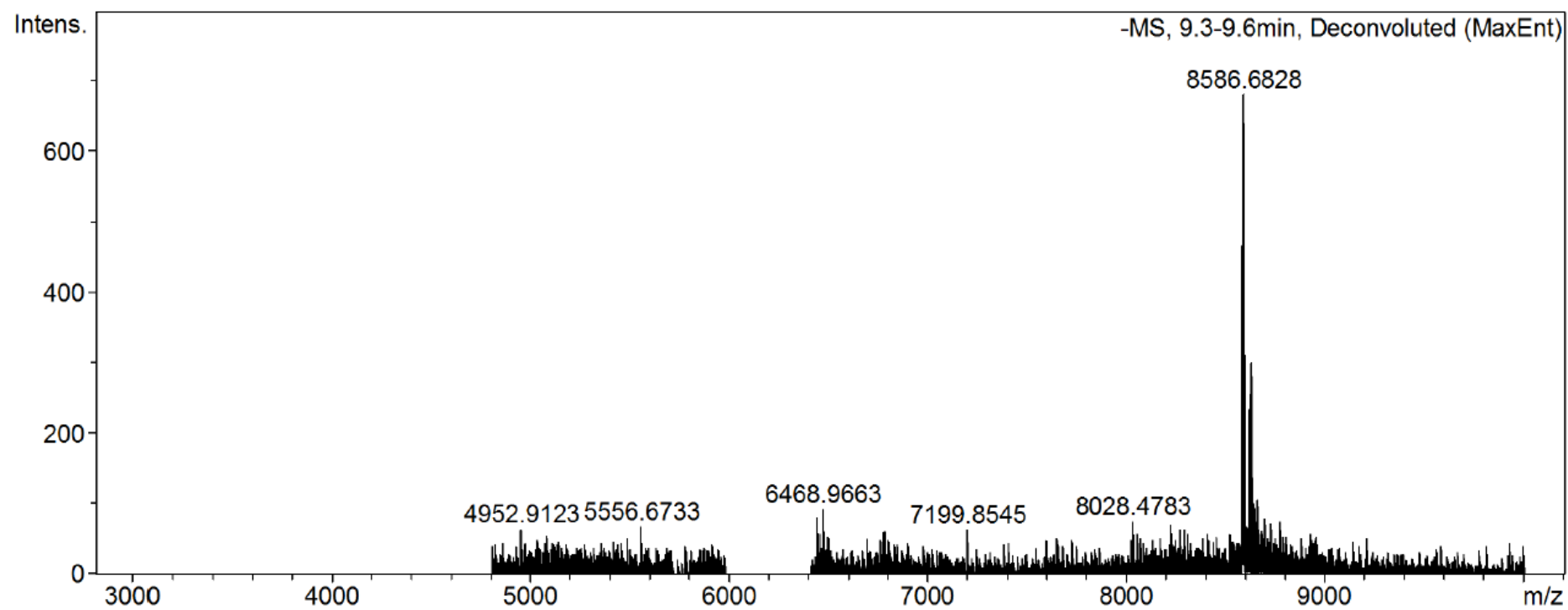
d)



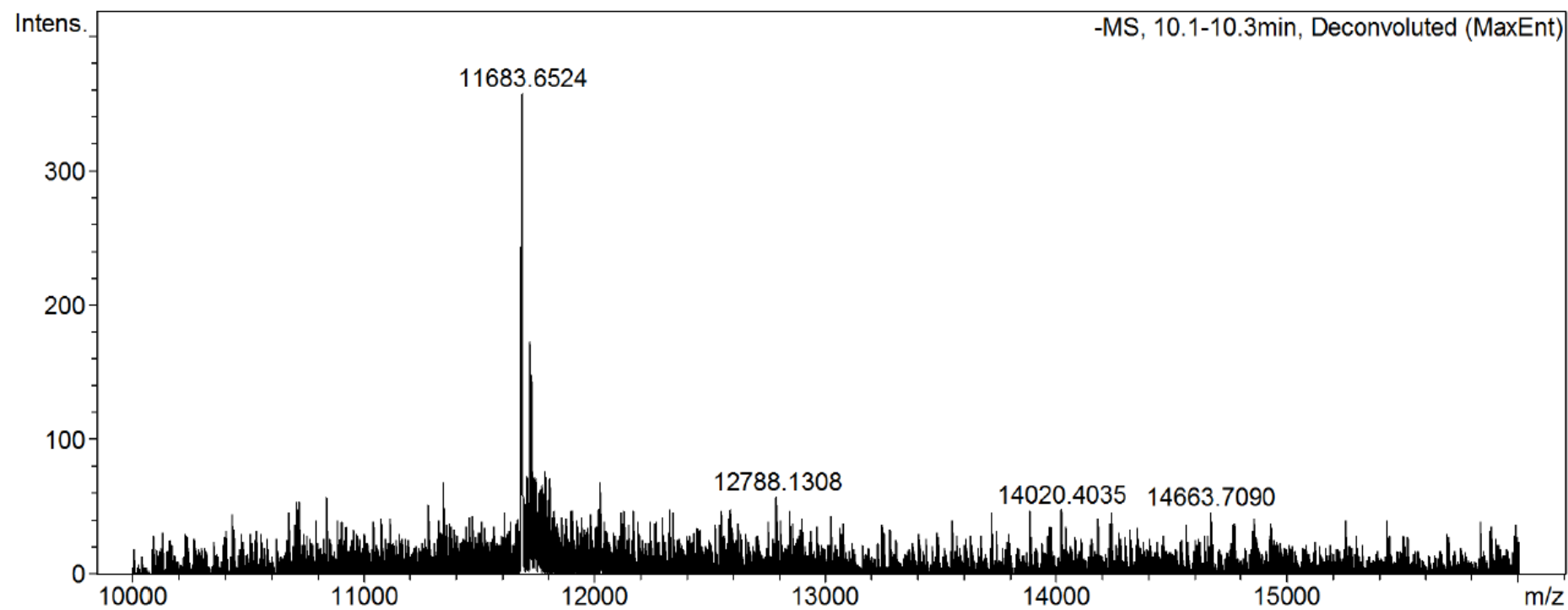
e)



f)



g)



h)

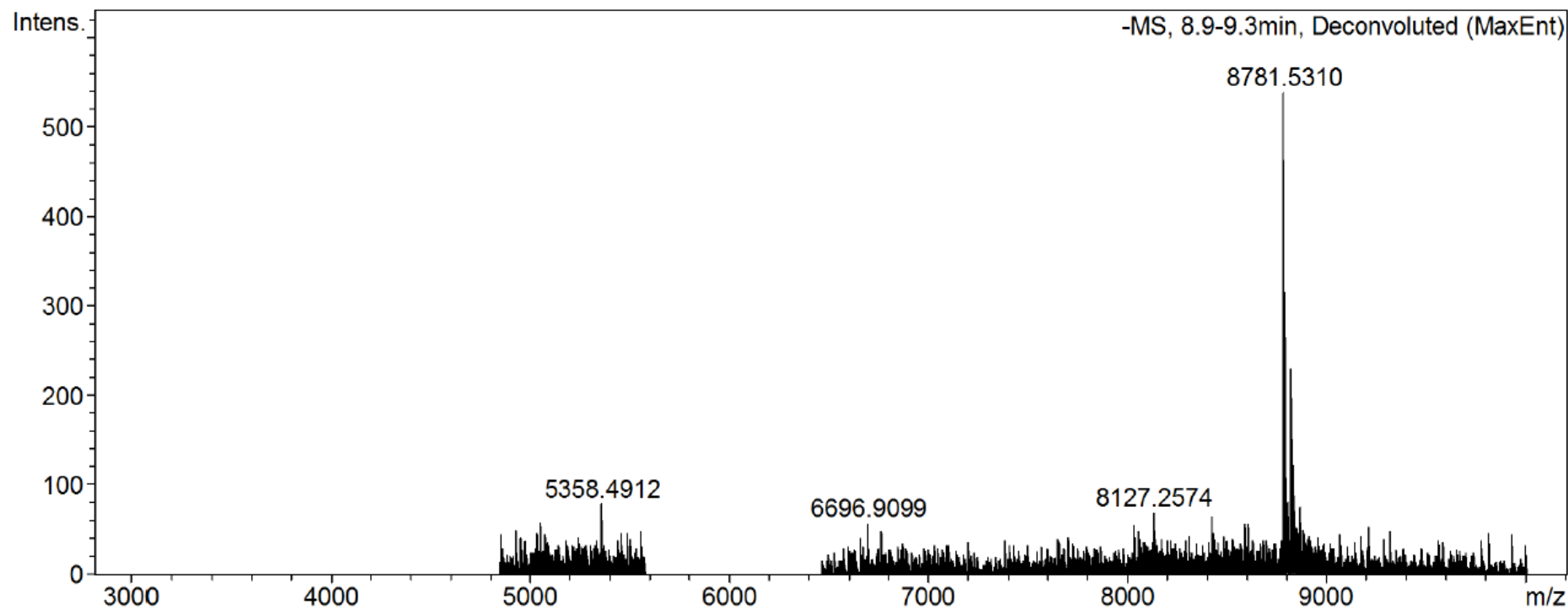


Figure 5.9. HPLC MS traces for the reaction of compound 4 with RNA1_A, RNA1_C, RNA1_E and RNA1_G. Where traces **a-d** are the HPLC traces and **e-h** are the MS data of the HPLC peaks for RNA1_B, RNA1_D, RNA1_F and RNA1_H, respectively.

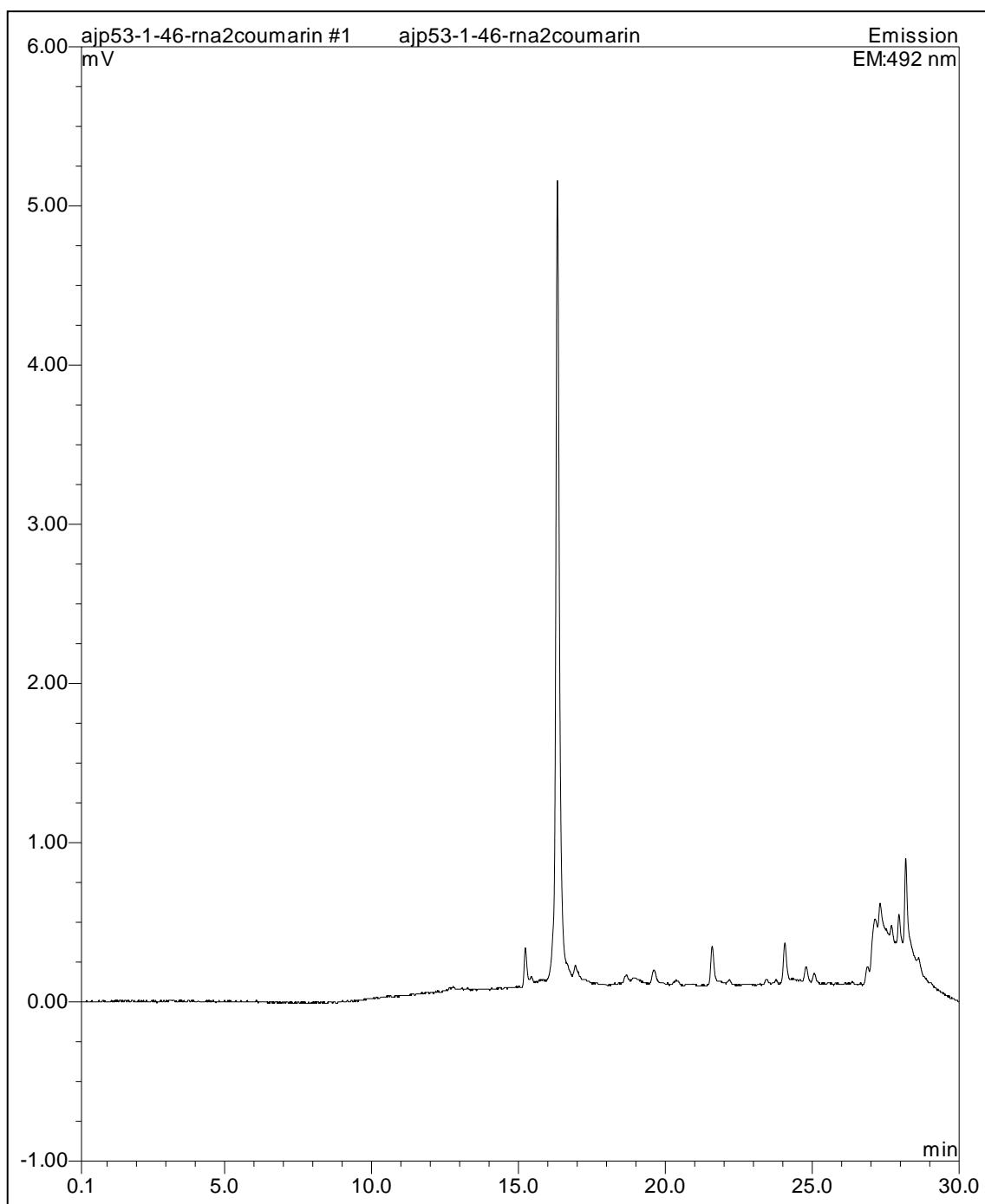


Figure 5.10. Crude RP-HPLC traces for RNA2_A reacting with coumarin azide.

RP-HPLC was carried out using a standard protocol with 5% - 60% gradient.

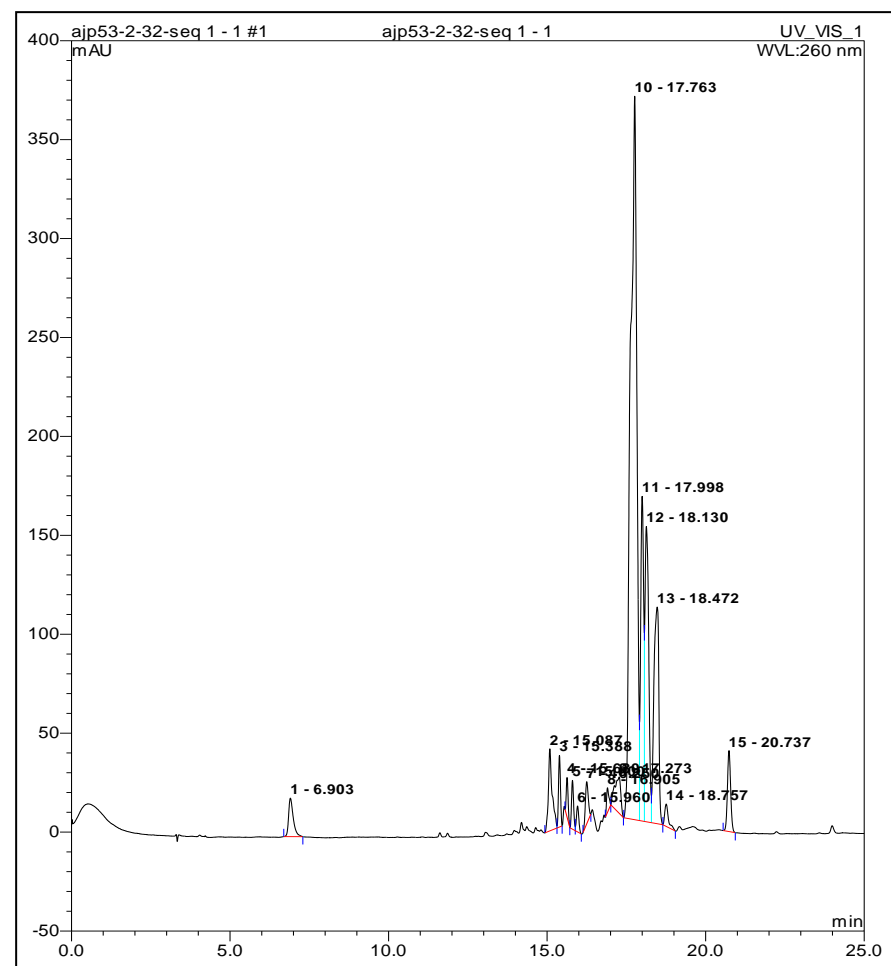
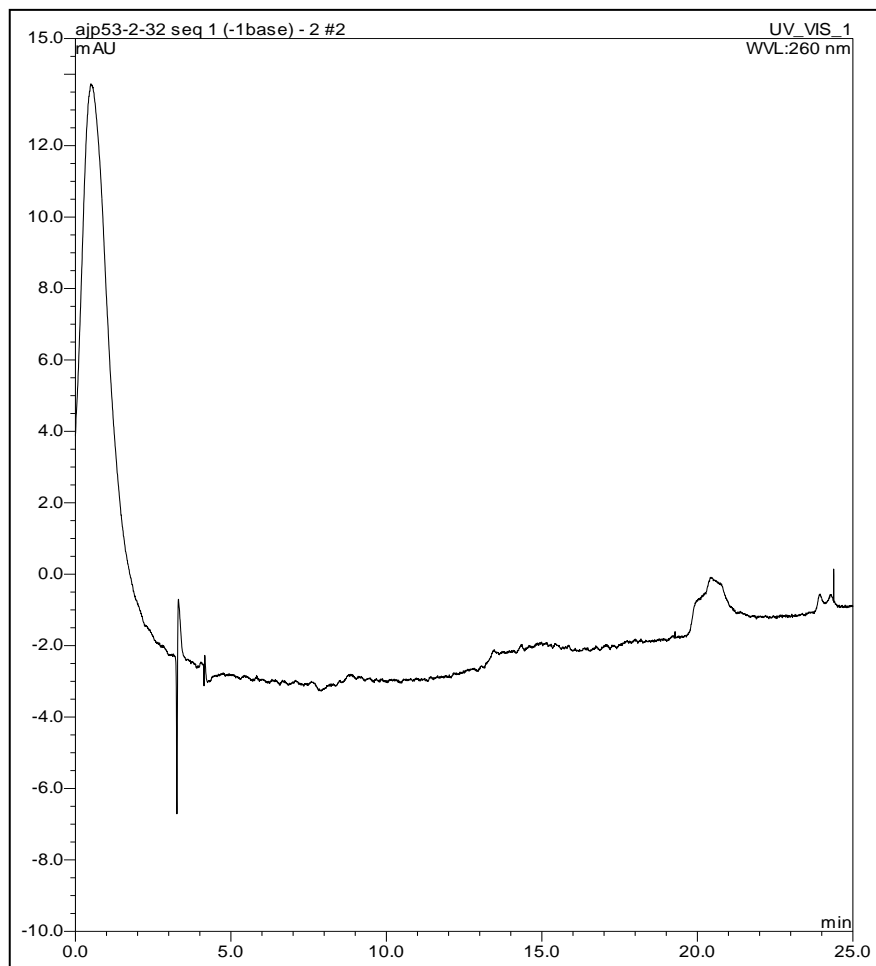
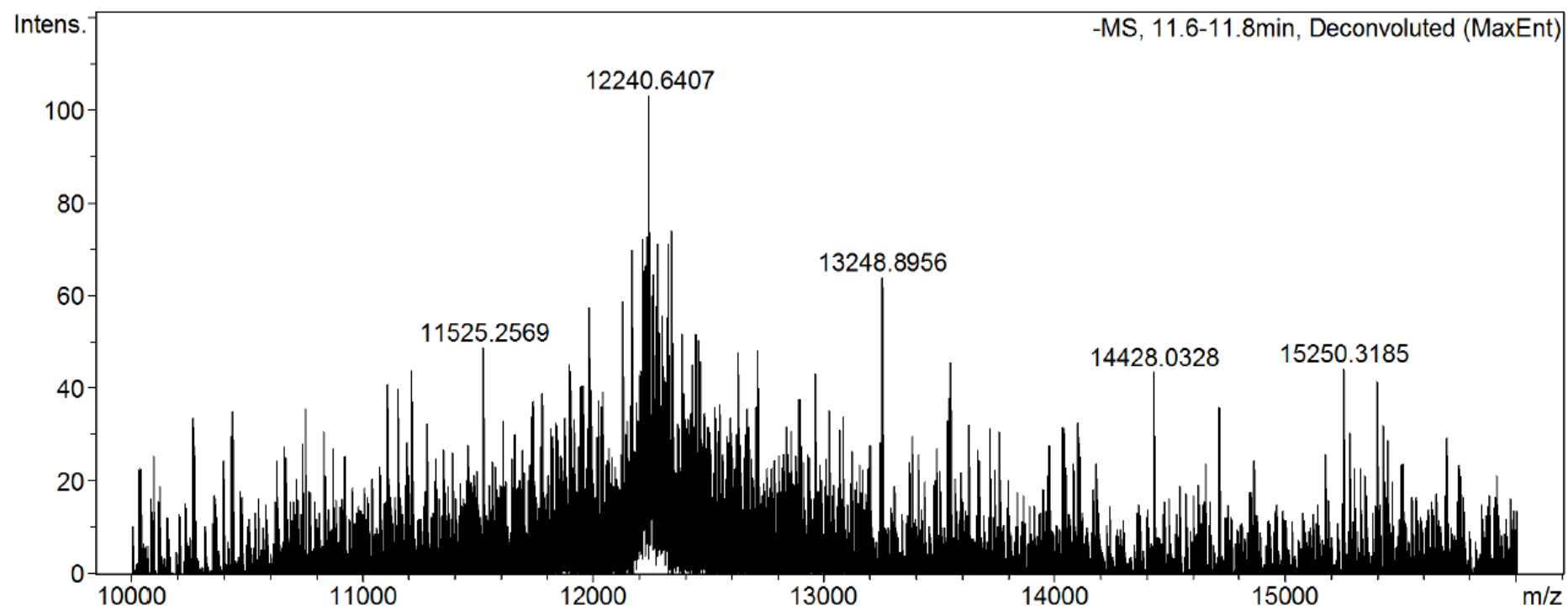


Figure 5.11. Crude RP-HPLC traces of pS DNA1 (a) and pS DNA2 (b). RP-HPLC was carried out using a standard protocol with 5% - 60% gradient.

a)



b)

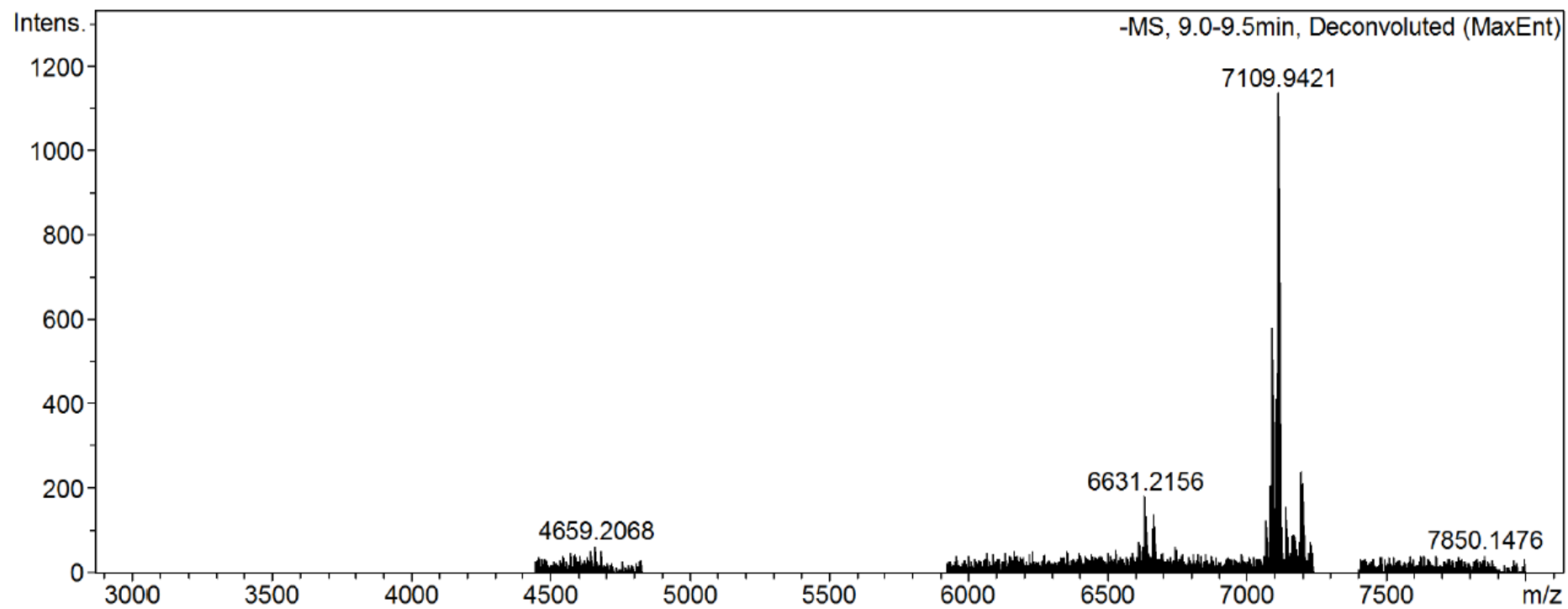
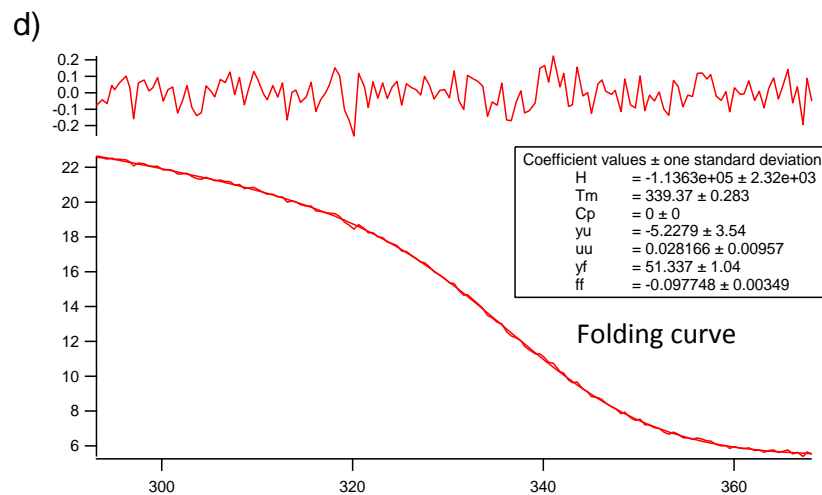
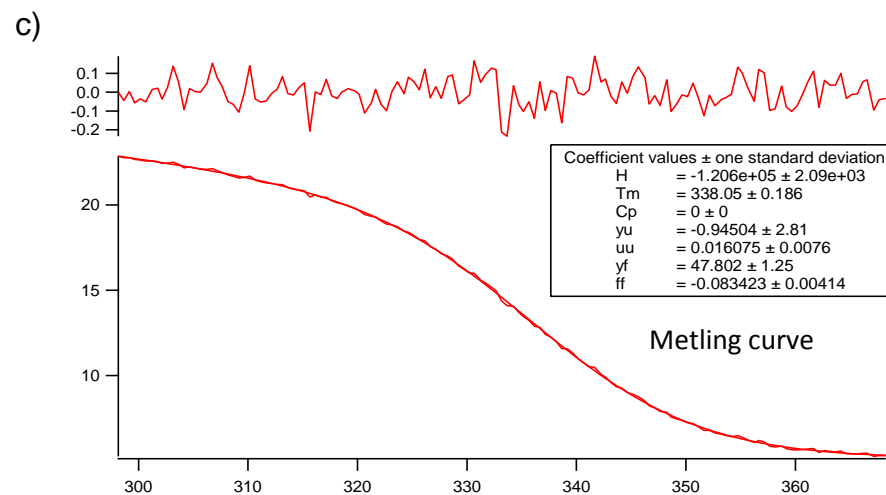
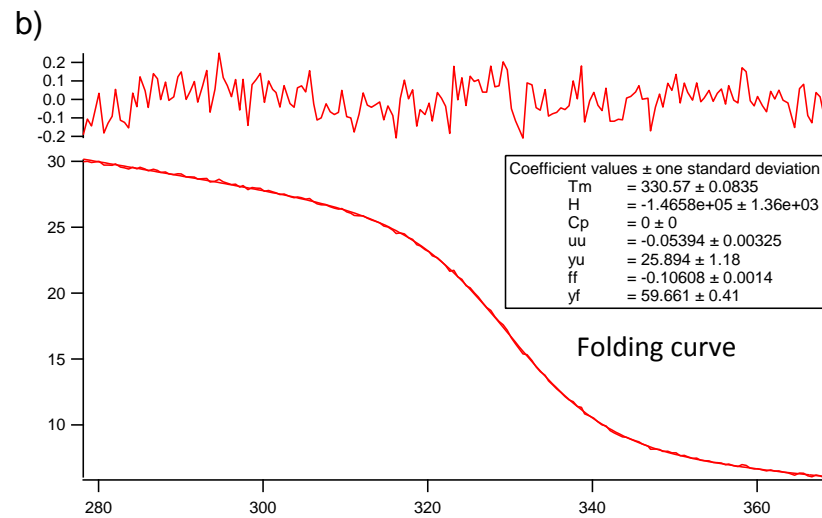
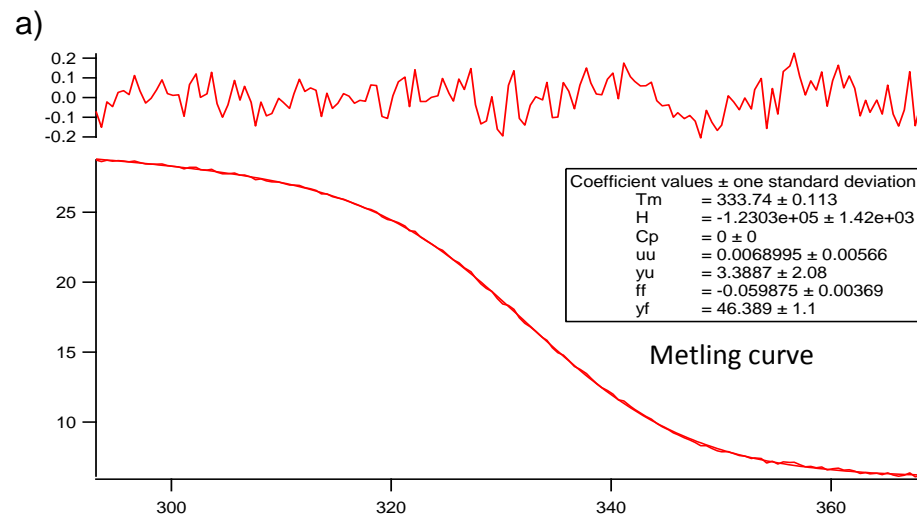
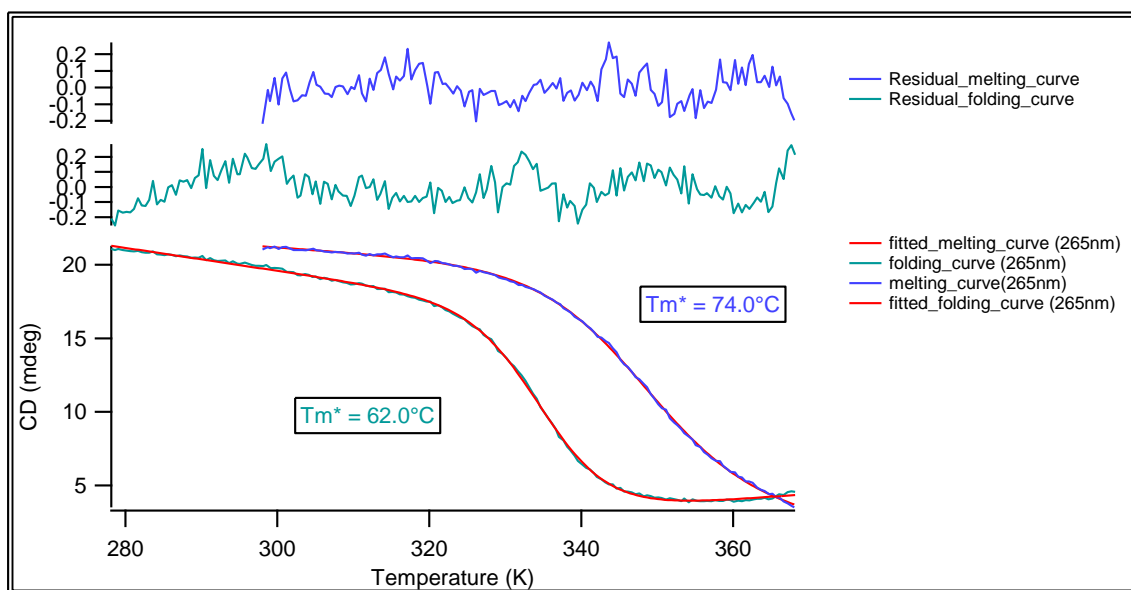


Figure 5.12. Example MS data for RNA1_E (a) and RNA2_A (b).



e)



f)

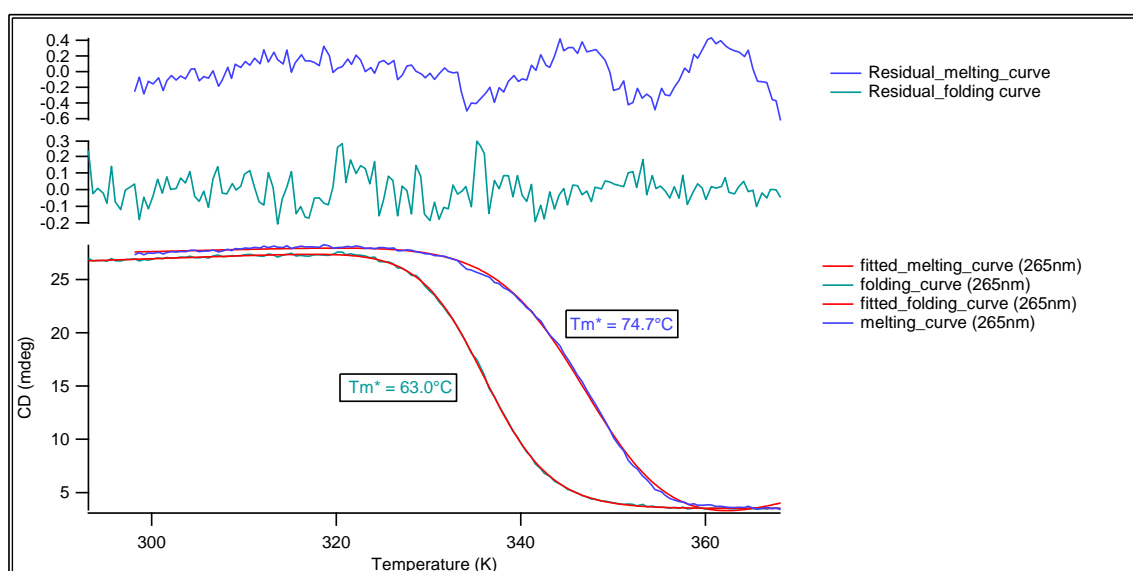


Figure 5.13. Fitted CD spectra for RNA1 (a-b), pS RNA12 (c-d), RNA18 (e) and RNA19 (f). CD data was measured at 265nm for 4 μ M concentrations in KCl 100mM, potassium phosphate (KP) 10mM, pH 7 in water.

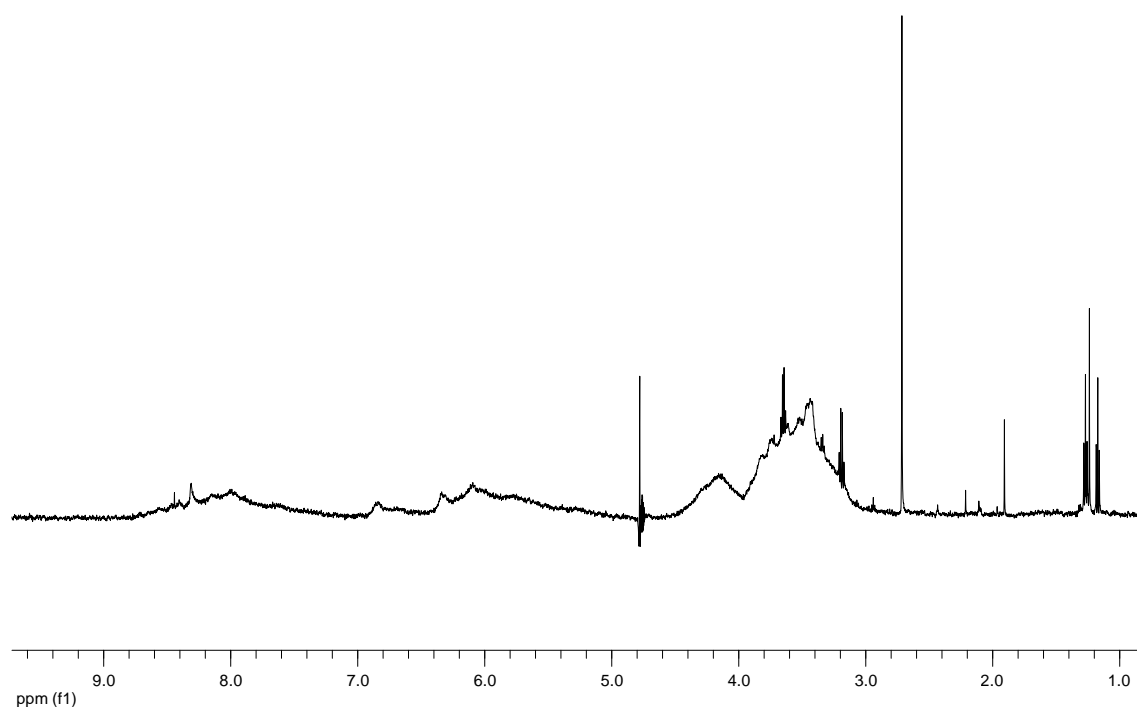


Figure 5.14. NMR spectra for pS RNA12. NMR experiments were carried out at 25°C in KCl 100mM, potassium phosphate (KP) 10mM, pH 7 in water.

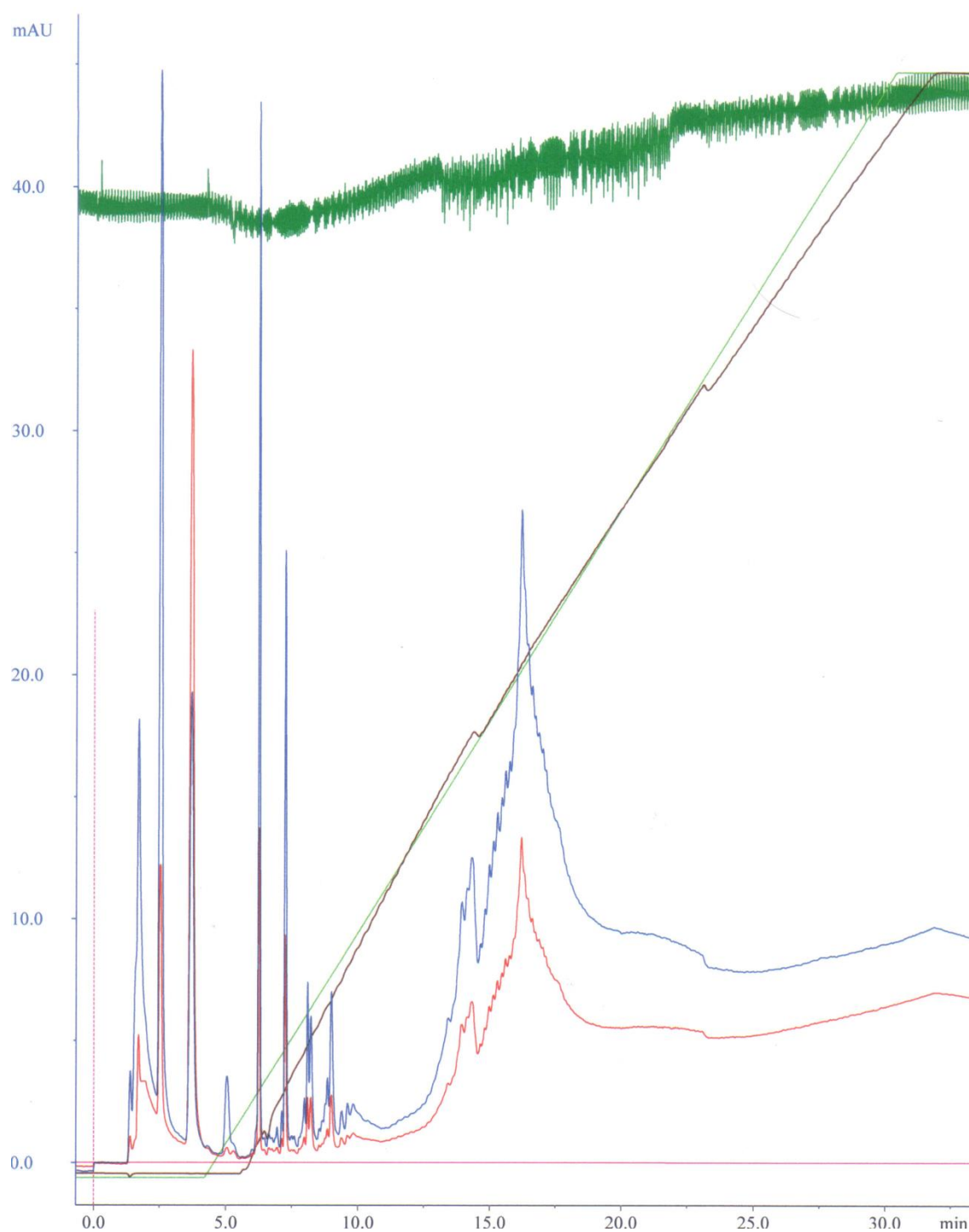


Figure 5.15. Ion exchange trace for RNA6 purification running a gradient from 0% to 100% over 40 minutes. Where buffer A is 20mM NaCl with 10mM Hepes and buffer B is 1M NaCl with 10mM Hepes. The blue line indicates absorption at 260nm, the red line absorption at 280nm and the light green line the gradient..

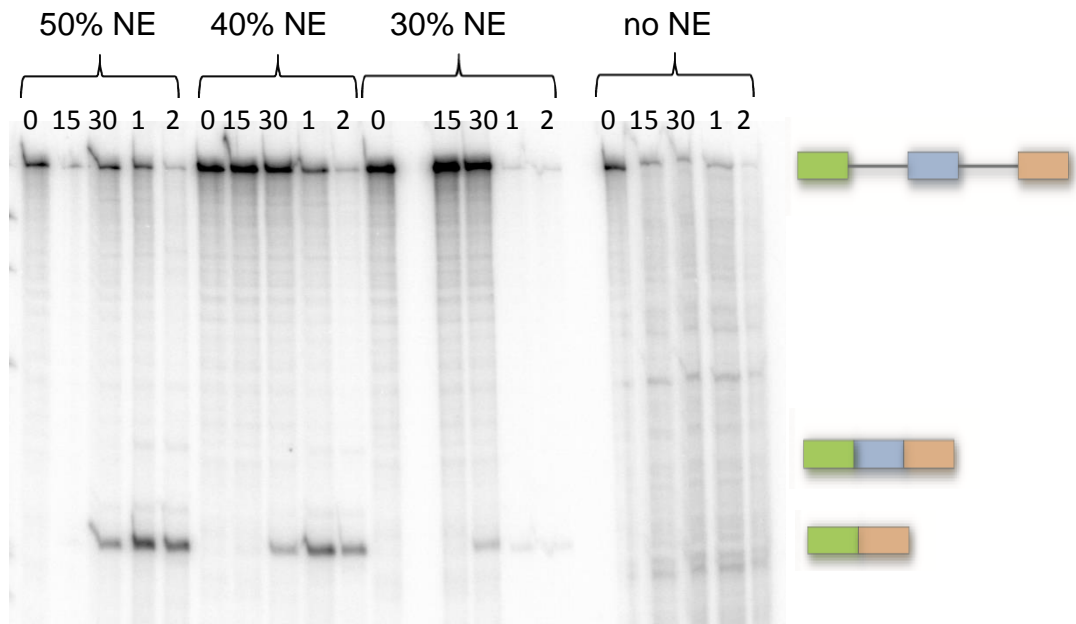


Figure 5.16 Time points 0, 15, 30, 1 and 2 hours taken during an SMN2 splicing in the presence of 50%, 40%, 30% and no NE as shown on the gel.

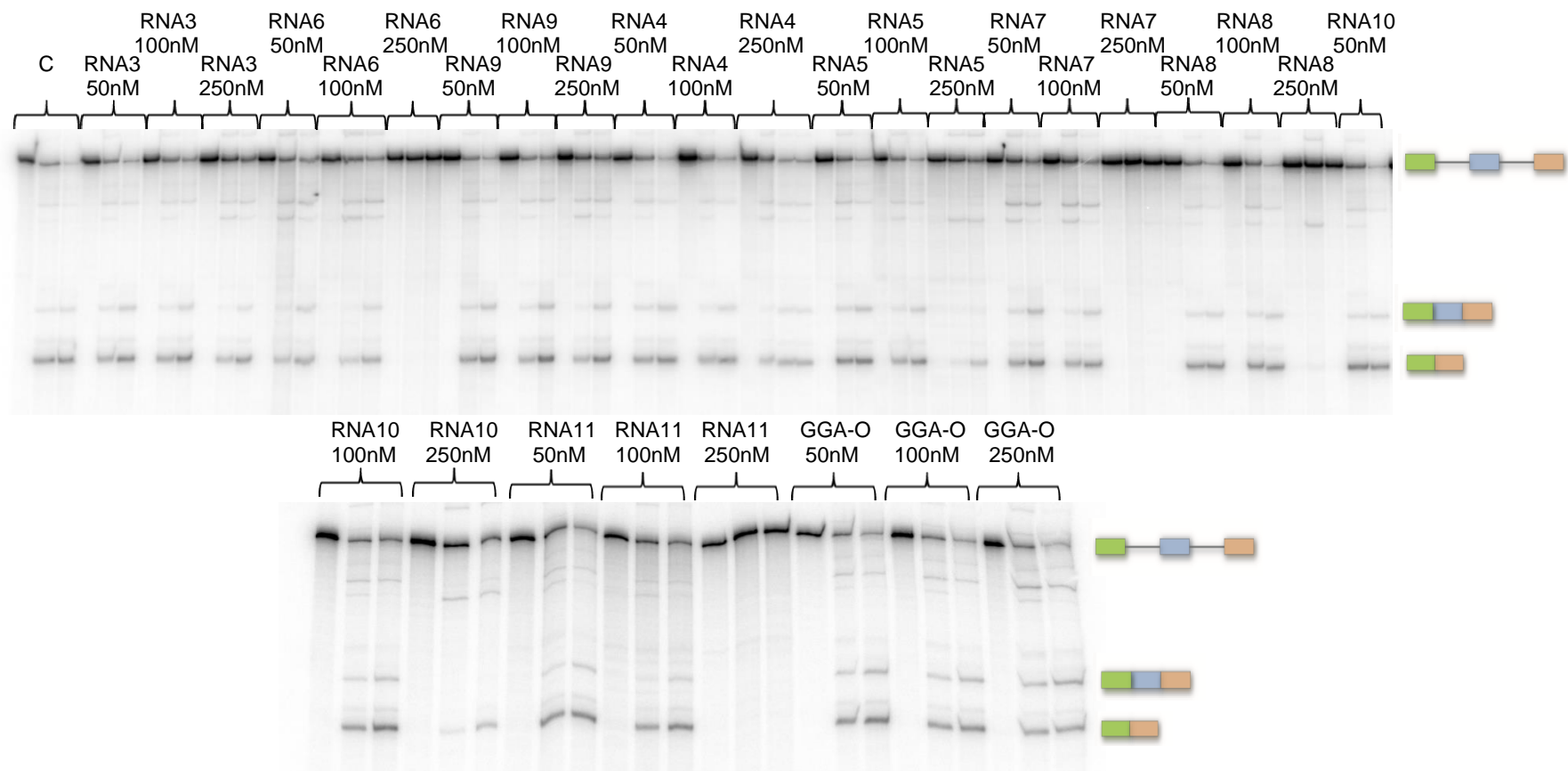


Figure 5.17 Assay in triplicate of splicing of SMN2 exon 7 in the presence of RNAs 3-11 and GGA-O. Splicing reactions were incubated for 2 hours and products separated by denaturing gel electrophoresis. The ONs used are shown above the lanes. (a & b) at 50, 100 and 250nM.

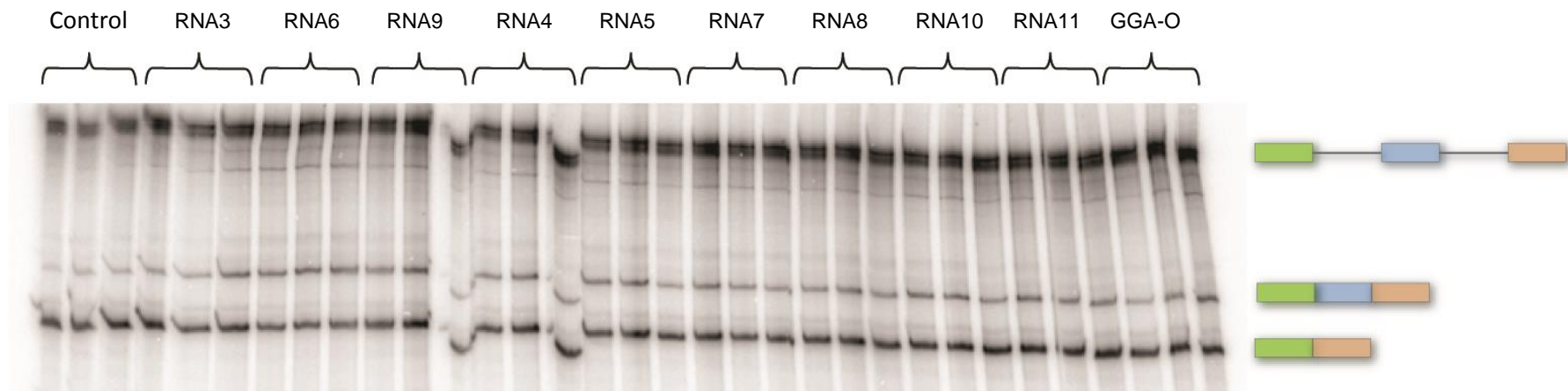


Figure 5.18 Assay in triplicate of splicing of SMN2 exon 7 in the presence of RNAs 3-11 and GGA-O. Splicing reactions were incubated for 2 hours and products separated by denaturing gel electrophoresis. The ONs used are shown above the lanes at 50nM.

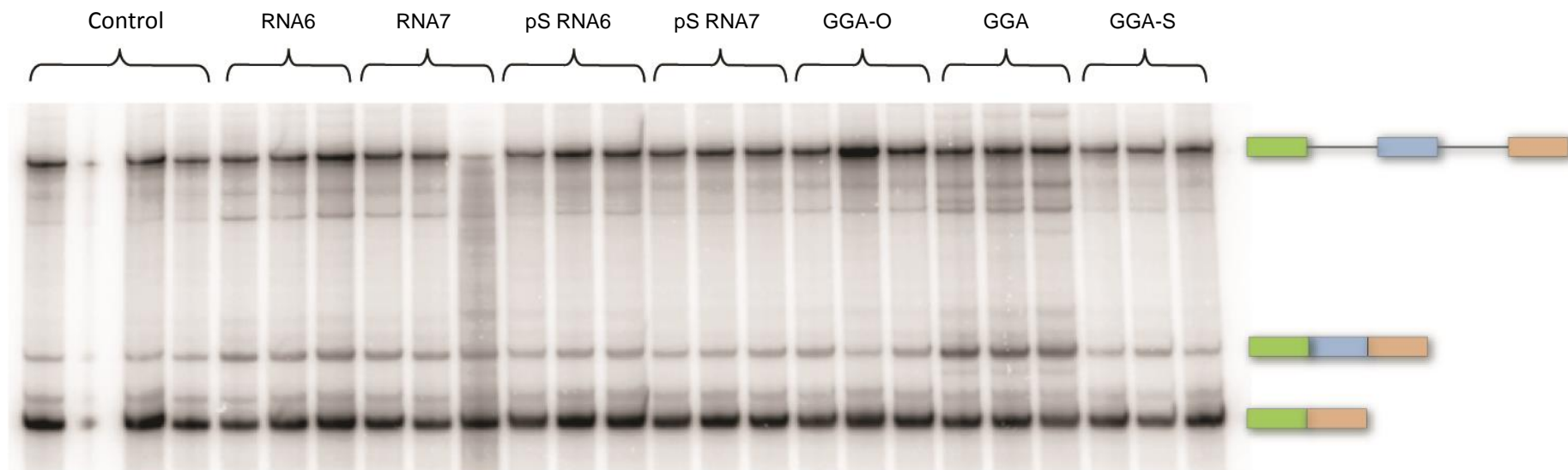


Figure 5.19 Assay in triplicate of splicing of SMN2 exon 7 in the presence of RNA6, RNA7, pS RNA6, pS RNA7, GGA-O, GGA and GGA-S. Splicing reactions were incubated for 2 hours and products separated by denaturing gel electrophoresis. The ONs used are shown above the lanes at 100nM.

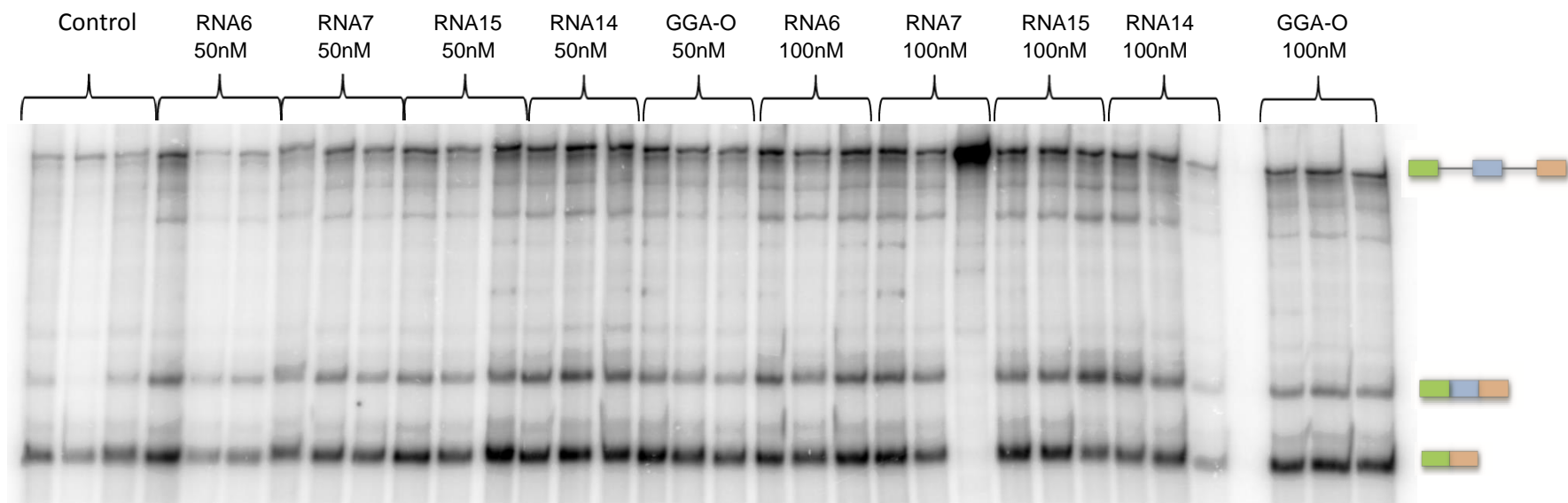


Figure 5.20 Assay in triplicate of splicing of SMN2 exon 7 in the presence of RNA6, RNA7, RNA14, RNA15 and GGA-O. Splicing reactions were incubated for 2 hours and products separated by denaturing gel electrophoresis. The ONs used are shown above the lanes at 50 and 100nM.

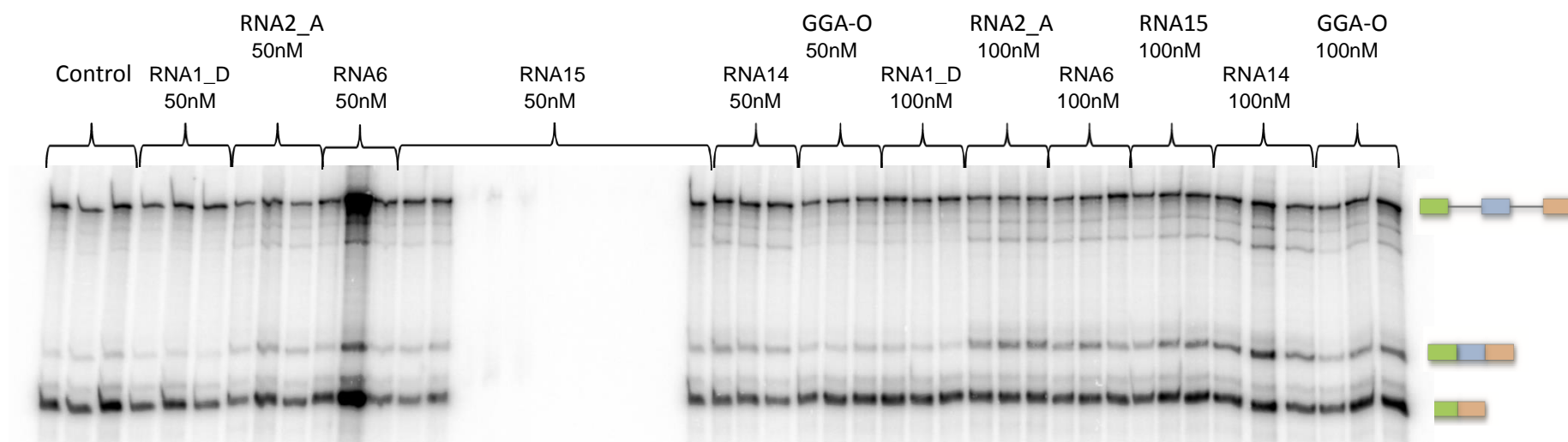


Figure 5.21 Assay in triplicate of splicing of SMN2 exon 7 in the presence of RNA1_D, RNA2_A, RNA6, RNA14, RNA15 and GGA-O. Splicing reactions were incubated for 2 hours and products separated by denaturing gel electrophoresis. The ONs used are shown above the lanes at 50 and 100nM.

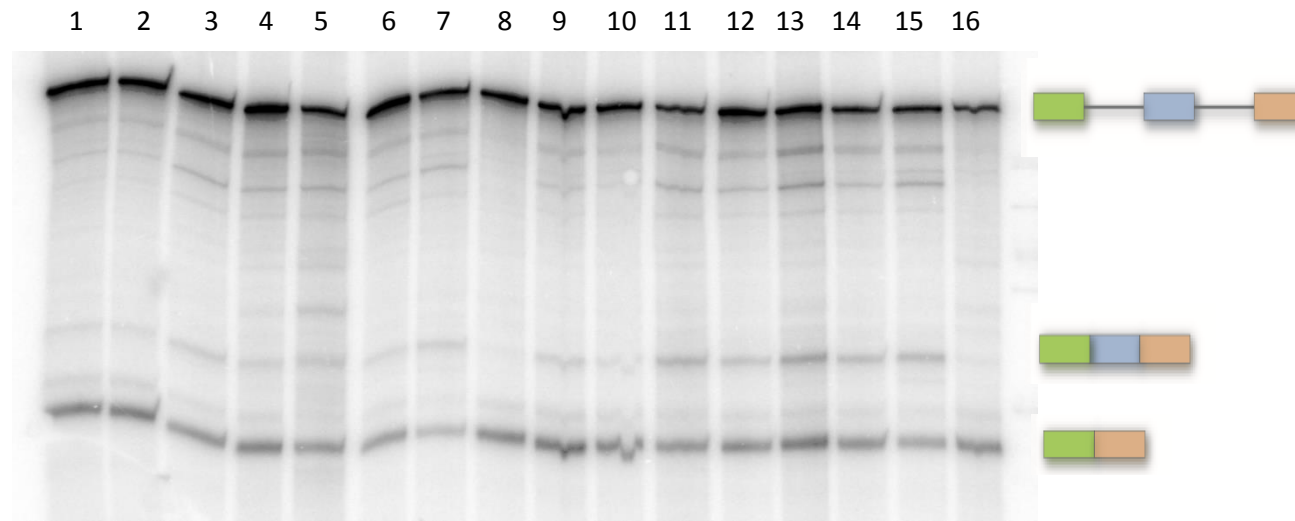


Figure 5.22 Assay in triplicate of splicing of SMN2 exon 7 in the presence of RNA2_A, 2'OMe NT, RNA6, RNA14, RNA15, GGA-O and GGA. Splicing reactions were incubated for 2 hours and products separated by denaturing gel electrophoresis. Lanes 1-8 are splicing reactions carried out with 3.2mM MgCl₂ with lanes 9-16 carried out with 2mM MgCl₂. Lanes 1 & 9) water control. Lanes 2 & 10) RNA2_A at 200nM. Lanes 3 & 11) 2'OMe NT at 200nM. Lanes 4 & 12) RNA6 at 200nM. Lanes 5 & 13) RNA15 at 200nM. Lanes 6 & 14) RNA14 at 200nM. Lanes 7 & 15) GGA-O at 200nM. Lanes 8 & 16) GGA at 200nM.

. AAATTAATAC GACTCACTAT A**GGGCTGCTG GTTGTCTACC CATGGACCCA**
TTTAATTATG CTGAGTGATA TCCCGACGAC CAACAGATGG GTACCTGGGT

51. **GAGGTTCTTC GAGTCCTTTG GGGACCTGTC CTCTGCAAAT GCTGTTATGA**
CTCCAAGAAG CTCAGGAAAC CCCTGGACAG GAGACGTTTA CGACAATACT

101. **ACAATCCTAA GGTGAAGGCT CATGGCAAGA AGGTGCTGGC TGCCTTCAGT**
TGTTAGGATT CCACTTCCGA GTACCGTTCT TCCACGACCG ACGGAAGTCA

151. **GAGGGTCTGA GTCACCTGGA CAACCTCAAA GGCACCTTTG CTAAGCTGAG**
CTCCCAGACT CAGTGGACCT GTTGGAGTTT CCGTGGAAAC GATTCTGACTC

201. **TGAACTGCAC TGTGACAAGC TGCACGTGGA TCCTGAGAAC TTCAGG**GTGA****
ACTTGACGTG ACACTGTTCG ACGTGCACCT AGGACTCTTG AAGTCCCCT

251. **GTTTGGGGAC CCTTGATTGT TCTTTCTTTT TCGCTATTGT AAAATTCATG**
CAAACCCCTG GGAACATAA AGAAAGAAAA AGCGATAACA TTTTAAGTAC

301. **TTATATGGTC GACAGACTAT CAACTTAATT TCTGATCATA TTTTGTTGAA**
AATATACCAG CTGTCTGATA GTTGAATTAA AGACTAGTAT AAAACAACAA

351. **TAAAATAAGT AAAATGTCTT GTGAAACAAA ATGCTTTTTA ACATCCATAT**
ATTTTATTCA TTTTACAGAA CACTTTGTTT TACGAAAAAT TGTAGGTATA

401. **AAAGCTATCT ATATATAGCT ATCTATATCT ATATAGCTAT TTTTTTAAAC**
TTTCGATAGA TATATATCGA TAGATATAGA TATATCGATA AAAAAAATTG

451. **TTCTTTTATT TTCCTTACAG GGTTTTAGAC AAAATCAAAA AGAAGGAAGG**
AAGGAAATAA AAGGAATGTC CCAAAATCTG TTTTAGTTTT TCTTCCTTCC

501. **TGCTCACATT CCTTAAATCA GGAGTAAGTC TGCCAGCATT ATGAAAGTGA**
ACGAGTGTA AAGGAATTAGT CCTCATTCAG ACGGTCGTAA TACTTTCACT

551. ATCTTACTTT TGTAAACTT TATGGTTTGT GGAAAACAAA TGTTTTTGAA
TAGAATGAAA ACATTTTGAA ATACCAAACA CCTTTTGTTT ACAAAAACTT

601. CATTTAAAAA GTTCAGATGT TAGAAAGTTG AAAGGTTAAT GTAAAACAAT
GTAAATTTT CAAGTCTACA ATCTTTCAAC TTTCCAATTA CATTTTGTTA

651. CAATATTAAA GAATTTTGAT GCCAAAATA TTAGATAAAA GGTTAATCTA
GTTATAATTT CTAAAACTA CGGTTTTGAT AATCTATTTT CCAATTAGAT

701. CATCCCTACT AGAATTCTCA TACTTAACTG GTTGGTTGTG TGGAAGAAAC
GTAGGGATGA TCTTAAGAGT ATGAATTGAC CAACCAACAC ACCTTCTTTG

751. ATACTTTCAC AATAAAGAGC TTTAGGATAT GATGCCATTT TATATCACGT
TATGAAAGTG TTATTTCTCG AAATCCTATA CTACGGTAAA ATATAGTGCA

801. CGACTCTGCT AACCATGTCA TGCCTTCTTC TTTTCCTAC AGCTCCTGGG
GCTGAGACGA TTGGTACAGT ACGGAAGAAG AAAAAGGATG TCGAGGACCC

851. CAACCGTGCTG GGTATTGGGC TGGCTCAACA ATTTTGGCAGGTAAGTT
GTTGGCACGAC CCATAACCCG ACCGAGTTGT TAAAACCGTCCATTCAA

Figure 5.23. Primary sequence of the SMN2 mini gene used in all transcriptions. The SMN2 mini gene consists of intron 6, exon 7 and intron 7 of SMN ligated in the middle of intron 2 of a β globin two exon construct (introns highlighted in orange and exons highlighted in blue).

1. GGGCTGCTG GTTGTCTACC CATGGACCCA GAGGTTCTTC GAGTCCTTTG
 51. GGGACCTGTC CTCTGCAAAT GCTGTTATGA ACAATCCTAA GGTGAAGGCT
 101. CATGGCAAGA AGGTGCTGGC TGCCTTCAGT GAGGGTCTGA GTCACCTGGA
 151. CAACCTCAAA GGCACCTTTG CTAAGCTGAG TGAAGTGCAC TGTGACAAGC
 201. TGCACGTGGA TCCTGAGAAC TTCAGGgtga gtttggggac ccttgattgt
 251. tctttctttt tcgctattgt aaaattcatg ttatatggtc gacagactat
 301. caacttaatt tctgatcata tttgttgaa taaaataagt aaaatgtctt
 351. gtgaaacaaa atgcttttta acatccatat aaagctatct atatatagct
 401. atctatatct atatagctat ttttttaac ttcctttatt ttccttacag
 451. GGTTTTAGAC AAAATCAAAA AGAAGGAAGG TGCTCACATT CCTTAAATCA
 501. GGAgtagtc tgccagcatt atgaaagtga atcttacttt tgtaaaactt
 551. tatggtttgt ggaaaacaaa tgtttttgaa catttaaaaa gttcagatgt
 601. tagaaagtg aaaggttaat gtaaaacaat caatattaaa gaattttgat
 651. gccaaaacta ttagataaaa ggtaaatcta catccctact agaattctca
 701. tacttaactg gttggttg tggaagaaac atacttcac aataaagagc
 751. tttaggatat gatgccattt tatatcacgt cgactctgct aaccatgtca
 801. tgccttcttc ttttctac agCTCCTGGG CAACCGTGCTG GGTATTGGGC
 851. TGGCTCAACA ATTTTGGCAGGTAAGTT

Figure 5.24. Shows the SMN2 RNA sequence after transcription. Where lower case letters represent introns and capitals letter represent exons. Letters in green indicate β globin sequences and letters in red indicate SMN2 sequences.