Electronic Supplementary Material (ESI) for Molecular BioSystems.

Supporting Information-Part II

How RNase HI (*Escherichia coli*) promoted site-selective hydrolysis works on RNA in duplex with carba-LNA and LNA substituted antisense strands in antisense strategy context

Oleksandr Plashkevych*, Qing Li, and Jyoti Chattopadhyaya*

Chemical Biology Program, Department of Cell and Molecular Biology, Box 581,
Biomedical Center, Uppsala University, SE-751 23 Uppsala, Sweden

*Corresponding Authors: Chemical Biology Program, Department of Cell and Molecular Biology. Biomedical Centre, Uppsala University, SE-75123 Uppsala, Sweden.
Phone: +46-18-4714577, Fax: +46-18-554495, Email: jyoti@boc.uu.se, oleksandr@boc.uu.se

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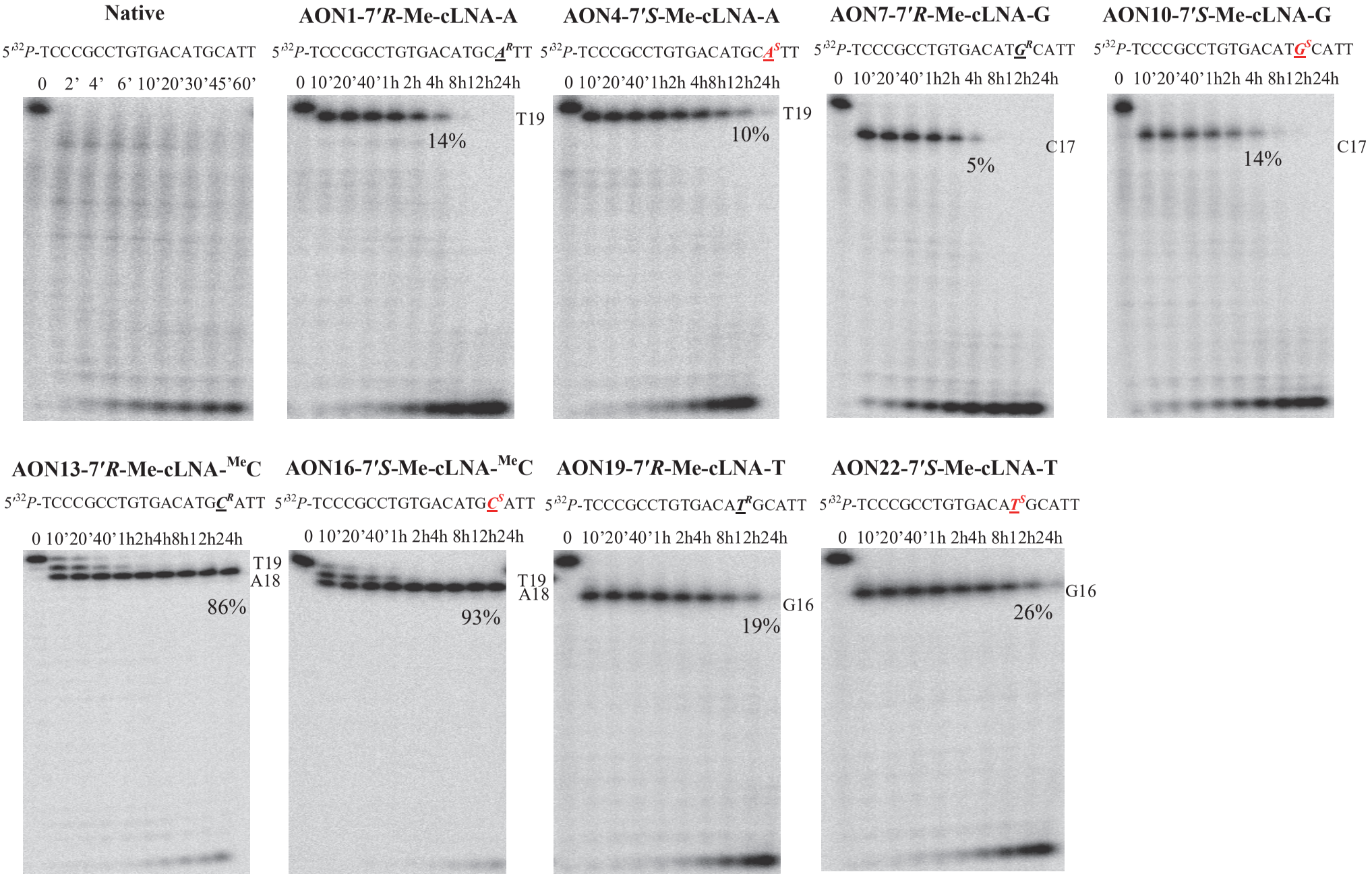


Figure SII.1 (published as Figure 11 in Ref 22) Autoradiograms of 20% denatured PAGE showing SVPDE degradation of 5′-end ^{32}P labeled native AON and modified AON1, 4, 7, 10, 13, 16, 19, 22. The AON% left after 24 hour incubation for each AON is shown below the corresponding band (native - 0%, AON1 - 14%, AON4 - 10%, AON7 - 5%, AON10 - 14%, AON13 - 86%, AON16 - 93%, AON19 - 19%, AON22 - 26%). Digestion conditions: AON 3 μM (5′-end ^{32}P labeled with specific activity 80 000 cpm) in 100 mM Tris-HCl (pH 8.0) and 15 mM $MgCl_2$, 21 °C, total reaction volume 30 μL, SVPDE concentration (6.7 ng/μL)

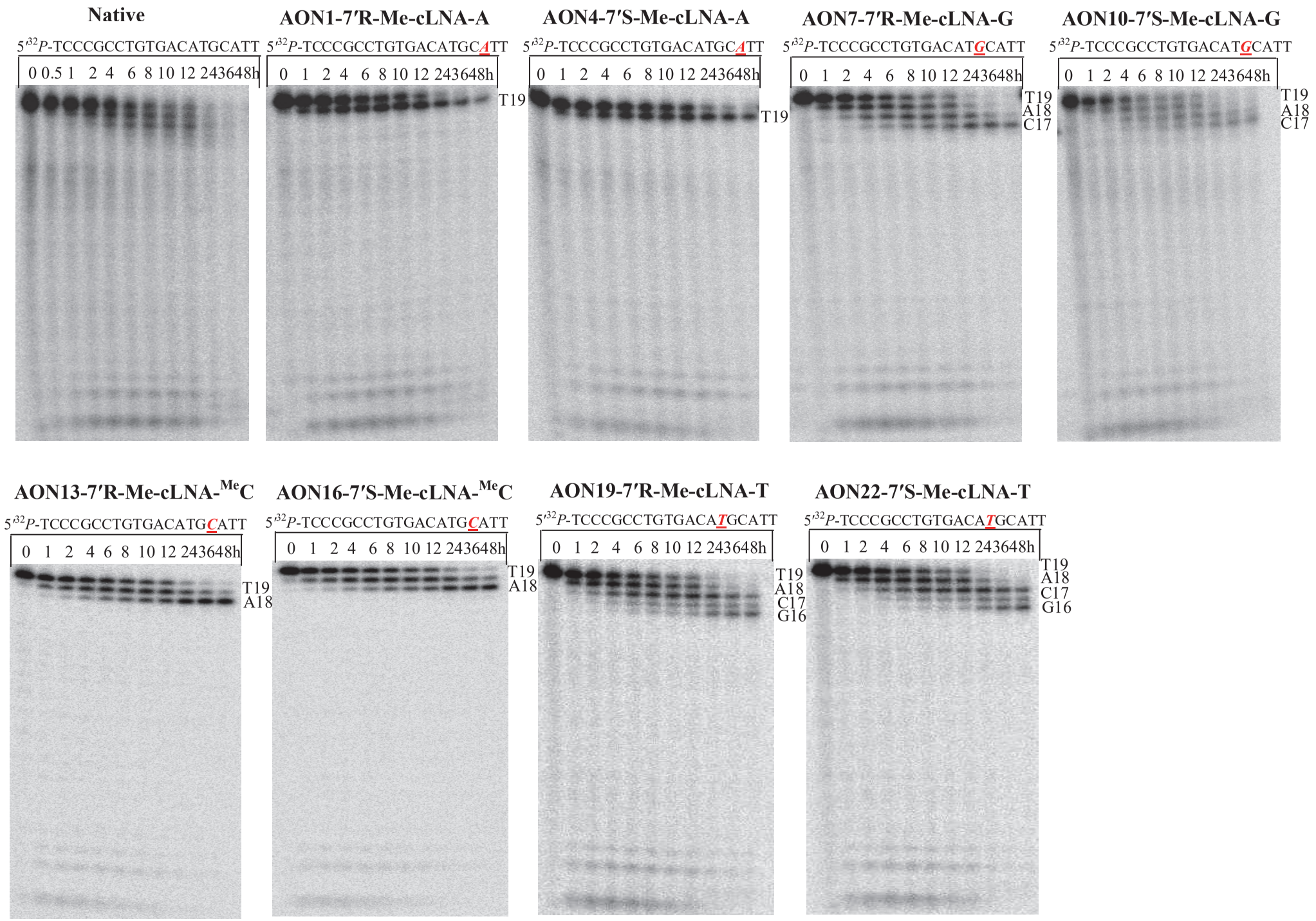


Figure SII.2 Autoradiograms of 20% denatured PAGE showing the stability of 5′-end ^{32}P labeled native AON and modified AON1, 4, 7, 10, 13, 16, 19, 22 in human blood serum.

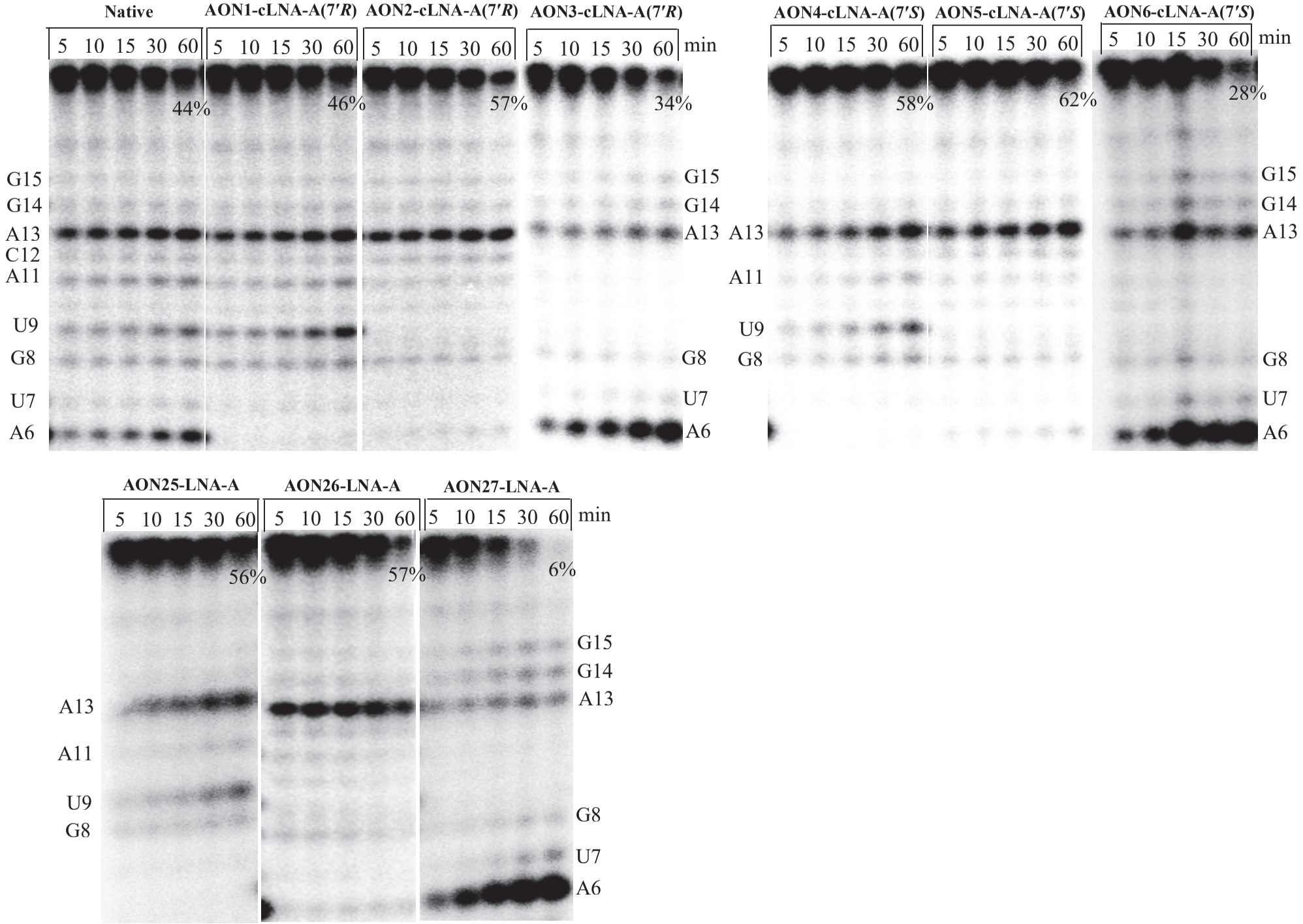


Figure SII.3 Autoradiograms of 20% denaturing PAGE pictures, showing the cleavage kinetics of 5′-^{32}P-labeled target RNA by *E. coli* RNase H1 in the cLNA-A and LNA-A modified AON/RNA hybrids. The intact RNA% left after 1 hour incubation for each AON/RNA is shown below the corresponding band. *Conditions of cleavage reactions*: RNA (0.1 μM) and AONs (2 μM) in buffer containing 20 mM Tris-HCl (pH 8.0), 20 mM KCl, 10 mM $MgCl_2$, and 0.1 mM DTT at 21 °C, 0.08 U of RNase H1, total volume 30 μL.

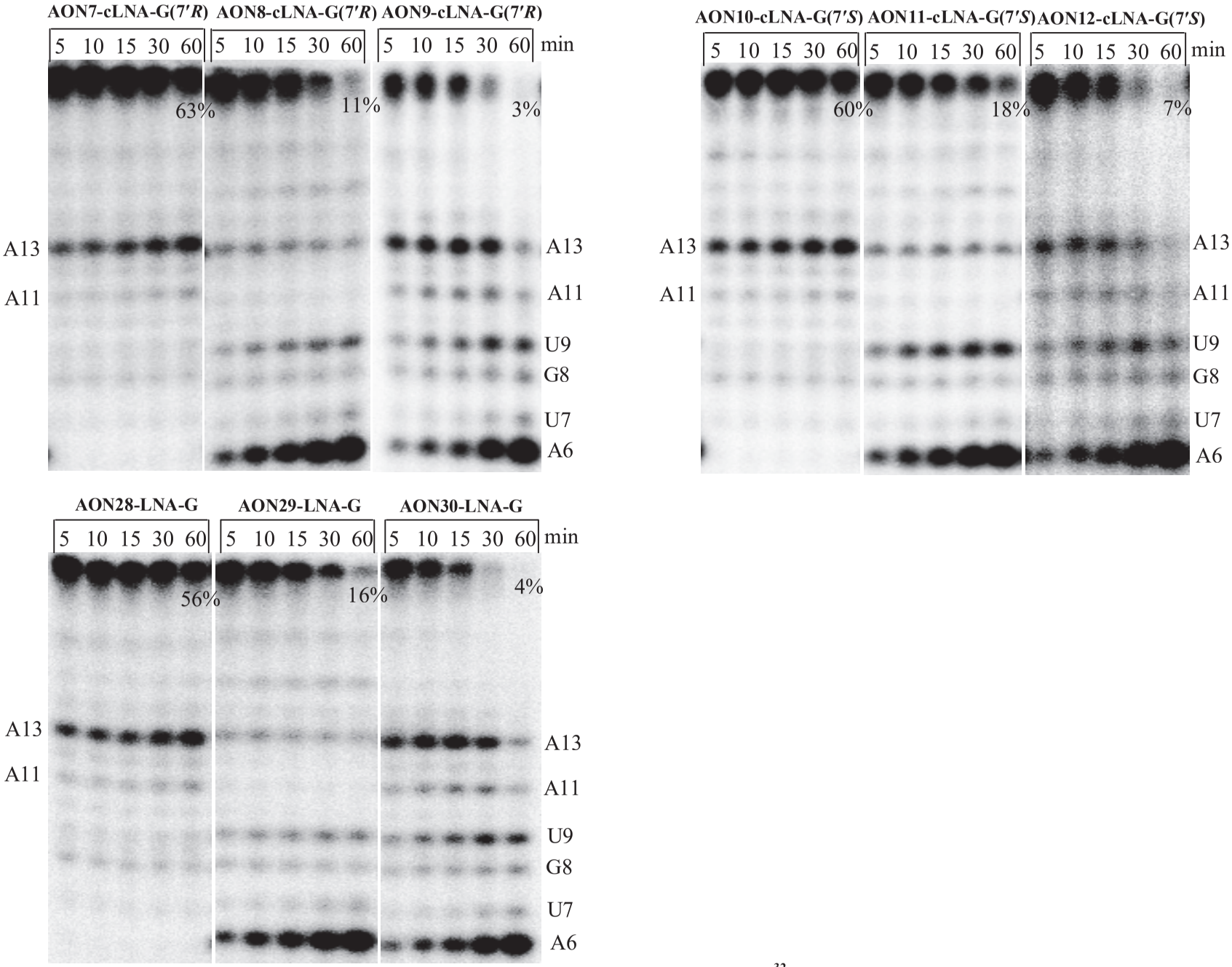


Figure SII.4 Autoradiograms of 20% denaturing PAGE pictures, showing the cleavage kinetics of 5′-^{32}P-labeled target RNA by *E. coli* RNase H1 in the LNA-G and cLNA-G modified AON/RNA hybrids. The intact RNA% left after 1 hour incubation for each AON/RNA is shown below the corresponding band. *Conditions of cleavage reactions*: RNA (0.1 *μM*) and AONs (2 *μM*) in buffer containing 20 mM Tris-HCl (pH 8.0), 20 mM KCl, 10 mM $MgCl_2$, and 0.1 mM DTT at 21 °C, 0.08 U of RNase H1, total volume 30 *μL*.

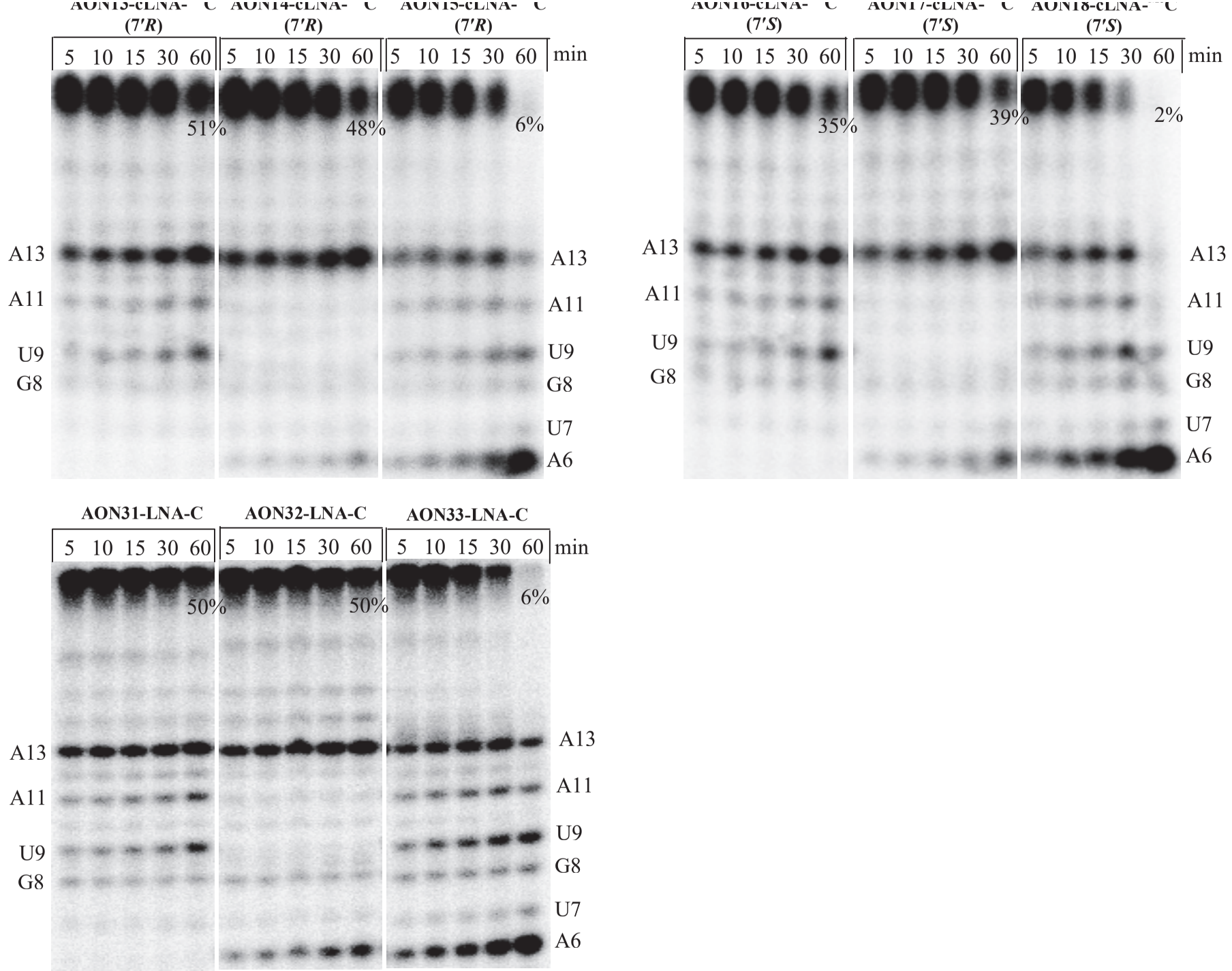


Figure SII.5 Autoradiograms of 20% denaturing PAGE pictures, showing the cleavage kinetics of 5′-^{32}P-labeled target RNA by *E. coli* RNase H1 in the LNA-C and cLNA-^{Me}C modified AON/RNA hybrids. The intact RNA% left after 1 hour incubation for each AON/RNA is shown below the corresponding band. *Conditions of cleavage reactions*: RNA (0.1 μM) and AONs (2 μM) in buffer containing 20 mM Tris-HCl (pH 8.0), 20 mM KCl, 10 mM $MgCl_2$, and 0.1 mM DTT at 21 °C, 0.08 U of RNase H1, total volume 30 μL.

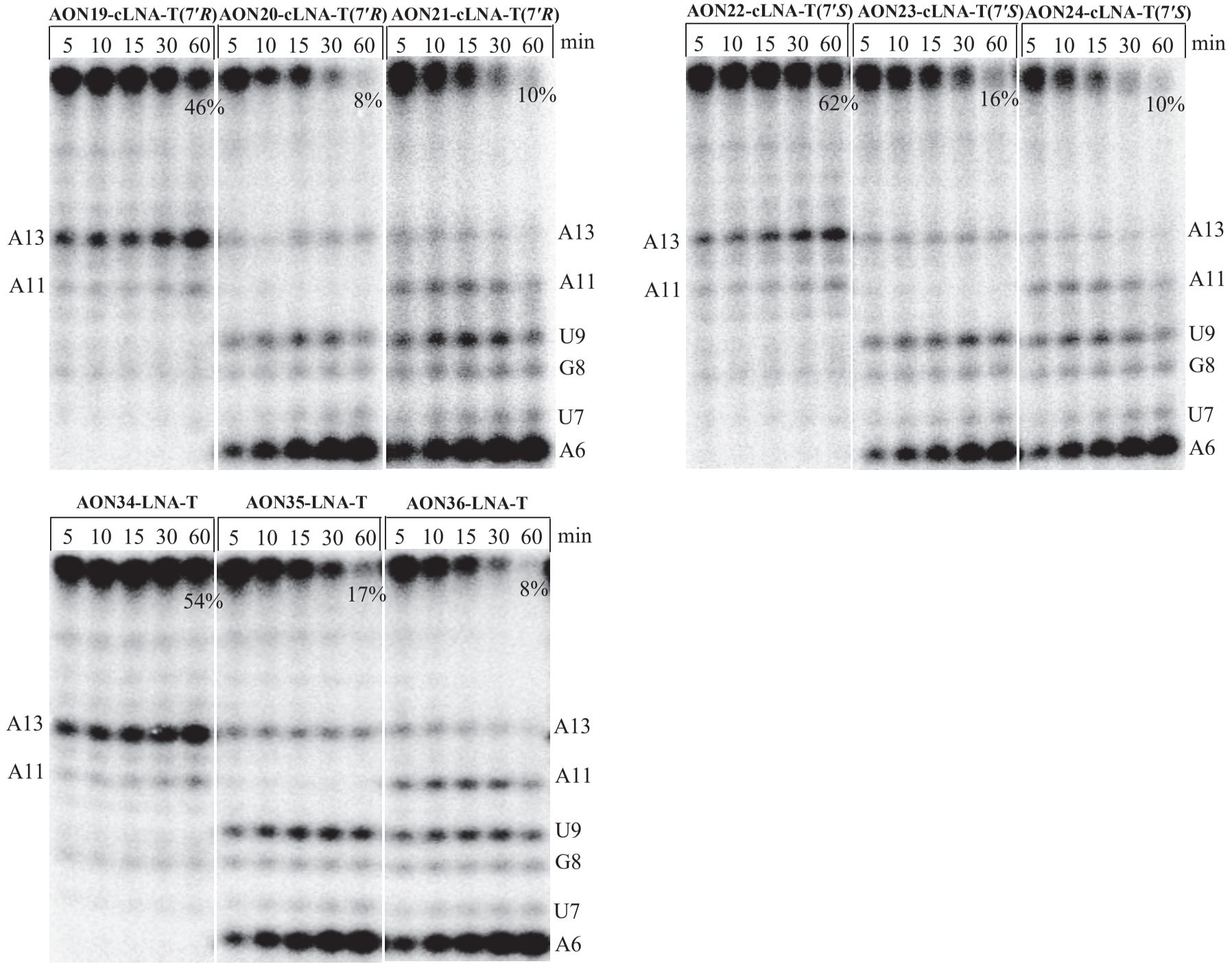


Figure SII.6 Autoradiograms of 20% denaturing PAGE pictures, showing the cleavage kinetics of 5′-^{32}P-labeled target RNA by *E. coli* RNase H1 in the LNA-T and cLNA-T modified AON/RNA hybrids. The intact RNA% left after 1 hour incubation for each AON/RNA is shown below the corresponding band. *Conditions of cleavage reactions*: RNA (0.1 μM) and AONs (2 μM) in buffer containing 20 mM Tris-HCl (pH 8.0), 20 mM KCl, 10 mM $MgCl_2$, and 0.1 mM DTT at 21 °C, 0.08 U of RNase H1, total volume 30 μL.

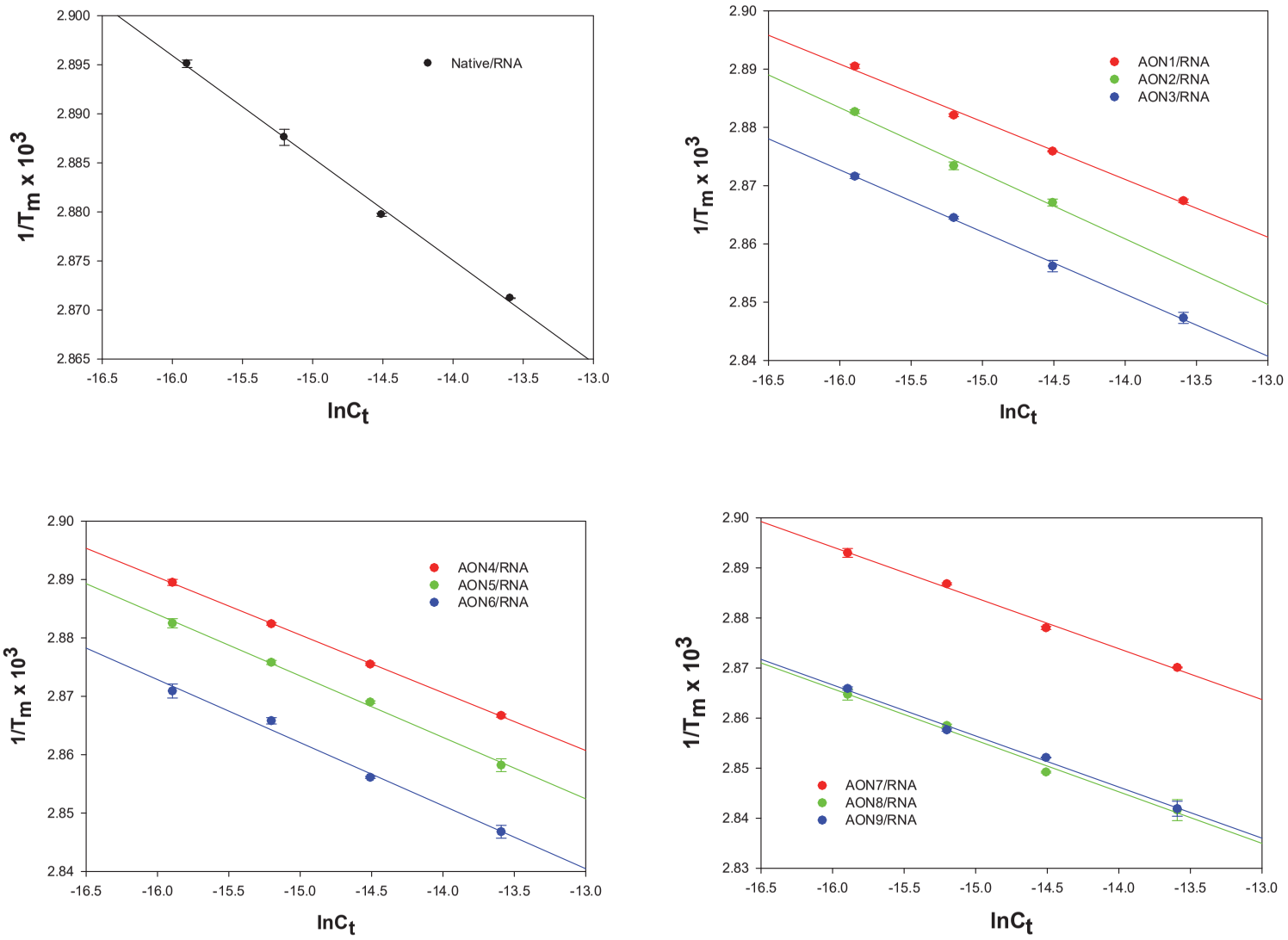


Figure SII.7 Plots of T_m^{-1} vs ln(C_t/4) for native AON/RNA and AONs 1-9/RNA duplexes obtained from concentration-dependent T_m measurement.

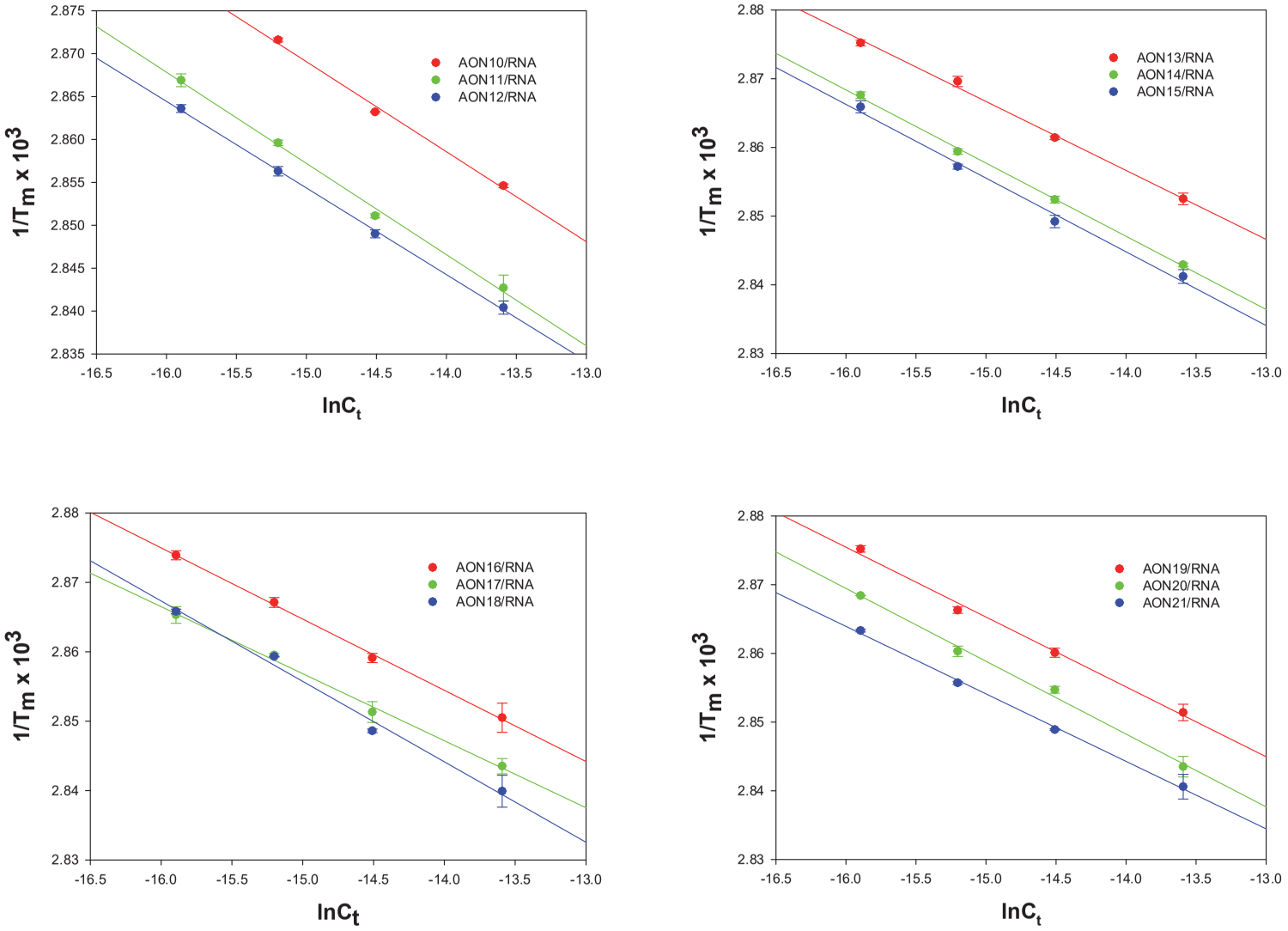


Figure SII.8 Plots of T_m^{-1} vs ln(C_t/4) for AONs 10-21/RNA duplexes obtained from concentration-dependent T_m measurement.

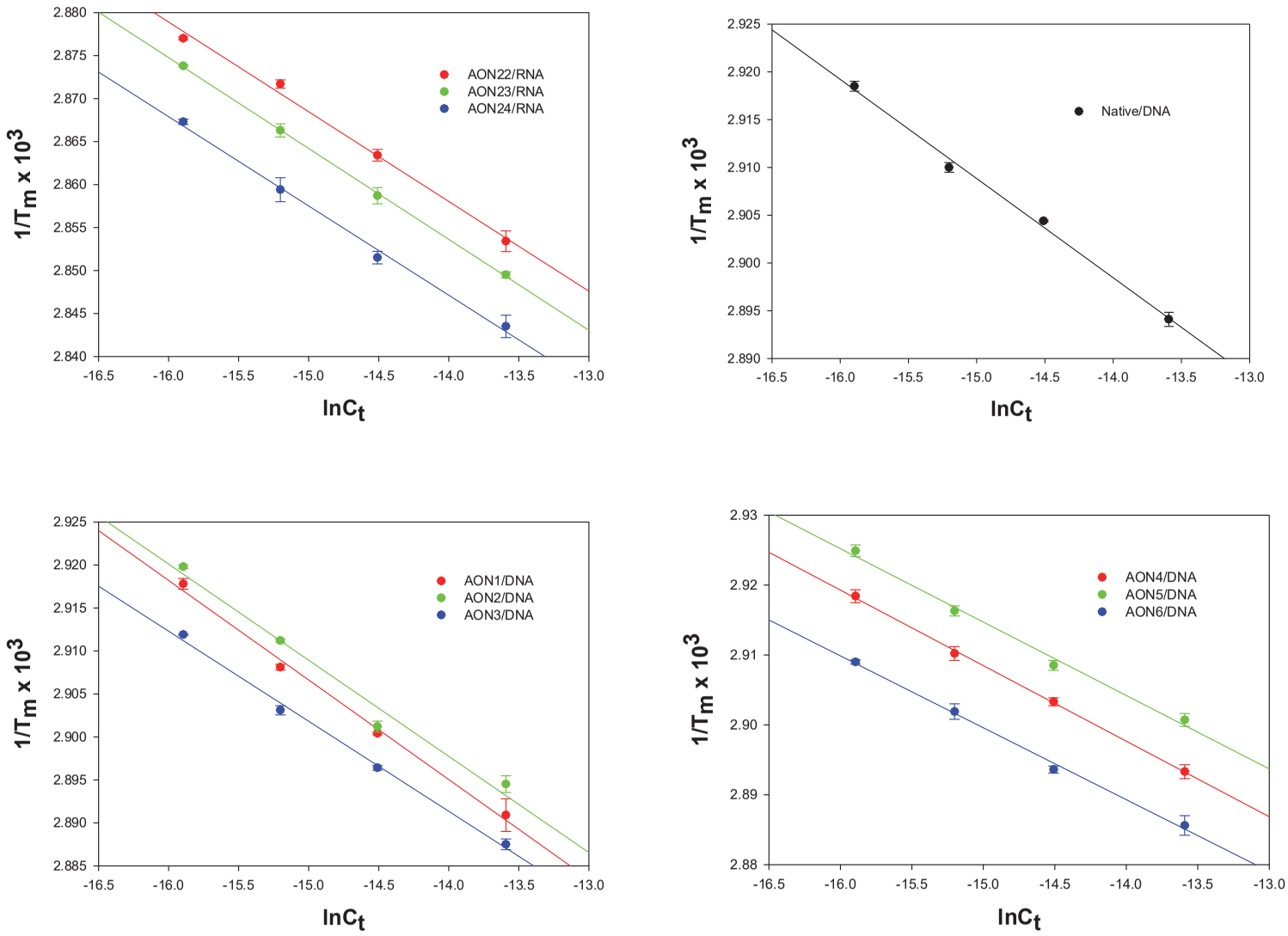


Figure SII.9 Plots of T_m^{-1} vs ln(C_t/4) for AONs 22-24/RNA, native AON/DNA and AONs 1-6/DNA duplexes obtained from concentration-dependent T_m measurement.

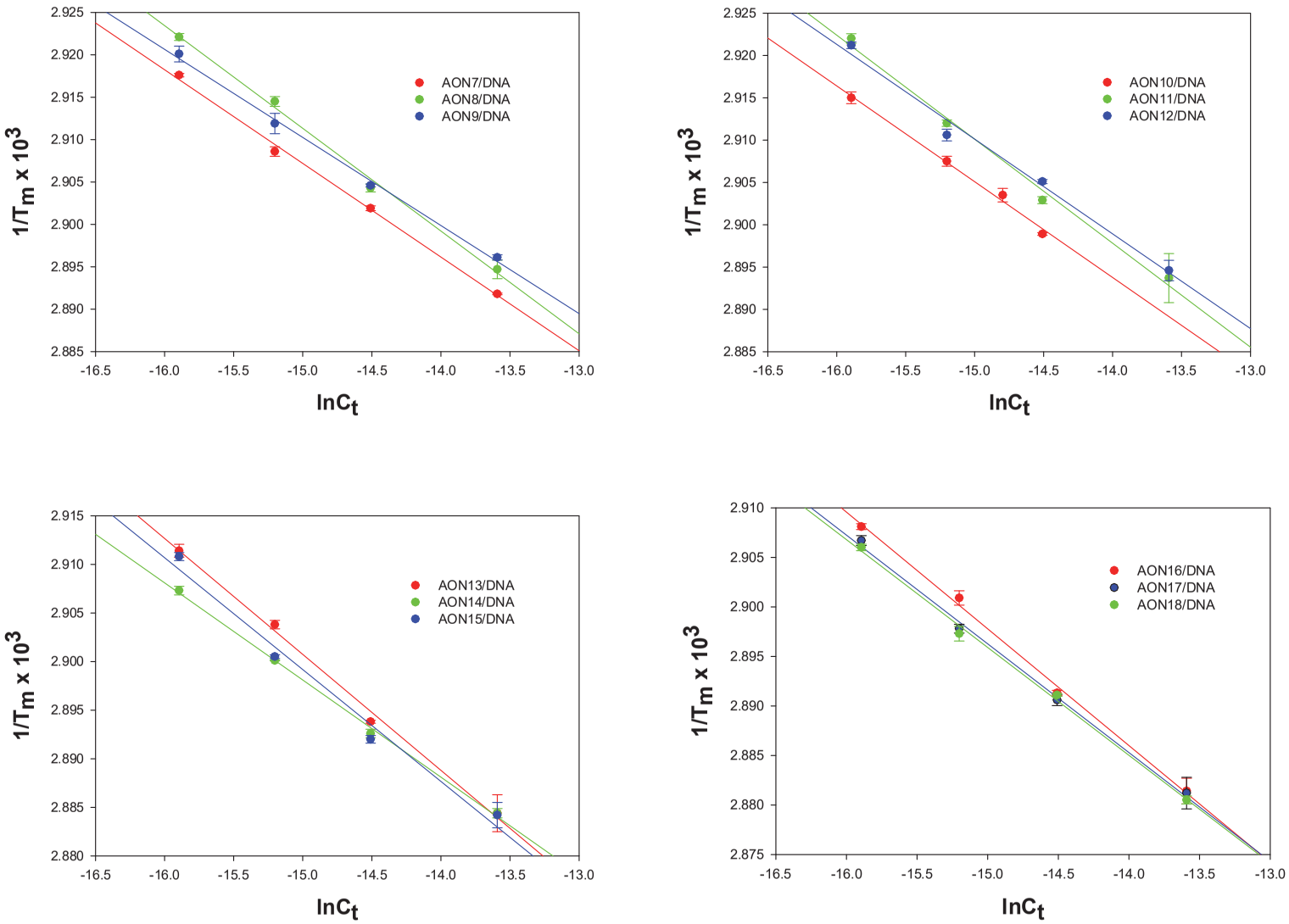


Figure SII.10 Plots of T_m^{-1} vs ln(C_t/4) for AONs 7-18/DNA duplexes obtained from concentration-dependent T_m measurement.

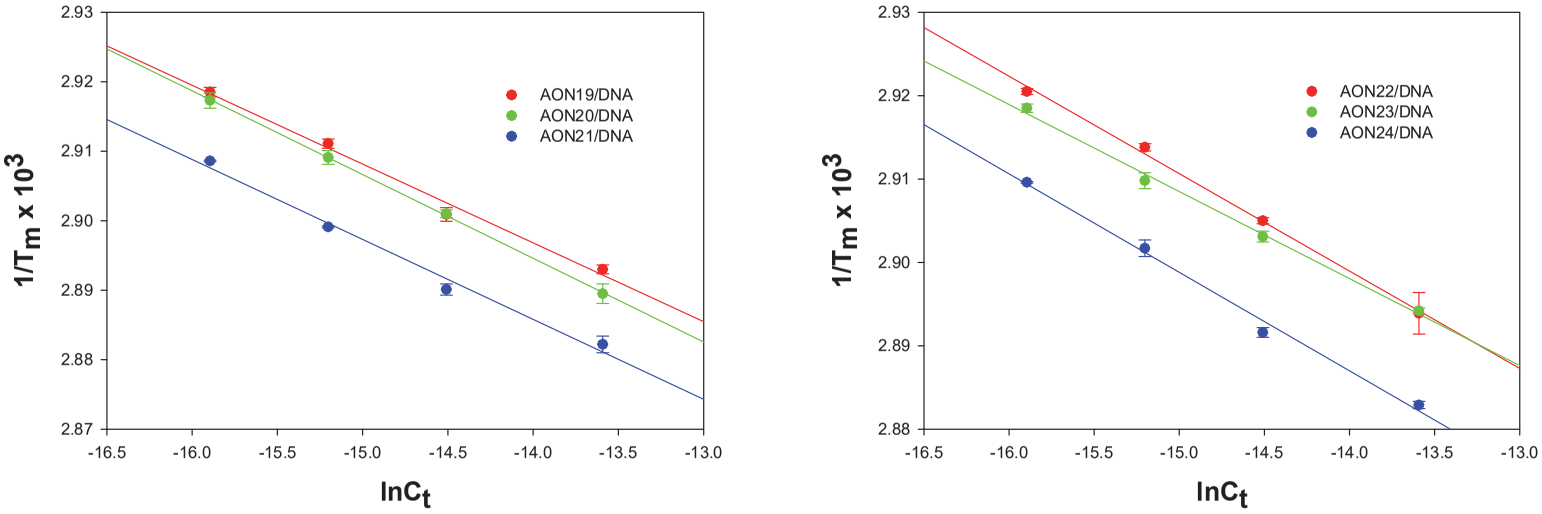
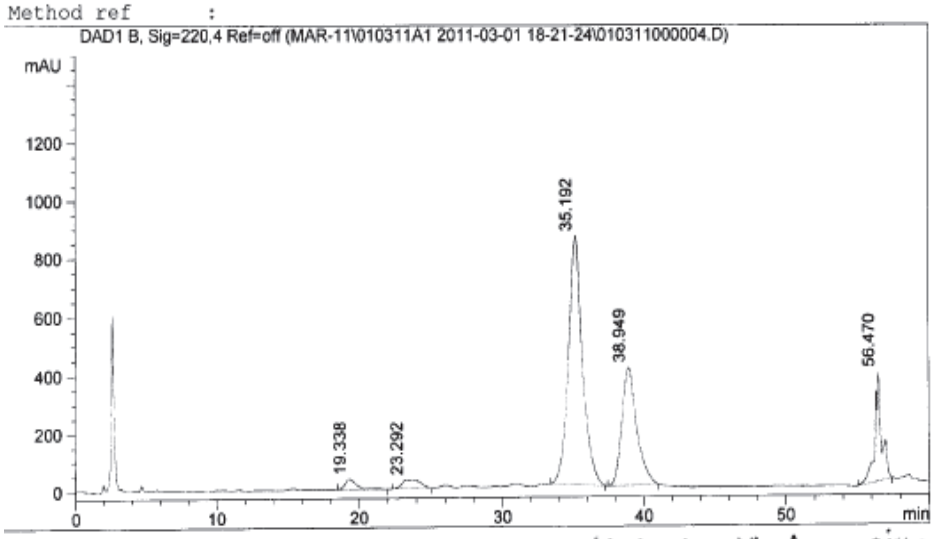


Figure SII.11 Plots of T_m^{-1} vs ln(C_t/4) for AONs 19-24/DNA duplexes obtained from concentration-dependent T_m measurement.

Figure SII.12 HPLC profile of mixtures of 7′*S*-Me-cLNA-A (2b) and cENA-A (2c)

SAMPLE-CR222-111A-5 ANA ->
Column: ZORBAX SB C18 (250X4.6)mm, 5um
Injection date : Tue, 1. Mar. 2011 Location : Vial 51
Sample Name : CR222-111A-5 ANA Inj. No. : 1
Acq Operator : RAMESH Inj. Vol-10 µl
Analysis Method : C:\CHEM32\1\METHODS\CR222 ANA2.M
Last Changed : Tue, 1. Mar. 2011,
Acq. Method : C:\Chem32\1\DATA\MAR-11\010311A1 2011-03-01 18-21-24\ CR222 ANA2.M
Method ref :

Adenine Minor +Endo



DAD1 B, Sig=220,4 Ref=off

Peak #	RT (Min)	Width (Min)	Area	Area %
1	19.338	1.037	2.139e3	2.018
2	23.292	1.414	2.559e3	2.415
3	35.192	1.153	5.914e4	55.820
4	38.949	1.221	2.978e4	28.107
5	56.470	0.547	1.233e4	11.639

*** End of Report***

MPA- 10 mM Ammonium Acetate
MPB- AcN: MeOH (50:50)
Flow - 1.0 ml/min
Gradient -

Time	% B
0.01	70
50	70
51	100
55	100
56	70
60	70

Figure SII.13 HPLC profile of pure 7′*S*-Me-cLNA-A (2b).

CHEMBIOTEK A TCG LIFESCIENCES ENTERPRISE, PUNE,INDIA

```
Sample : CR222-51-111-111-A-5 PK1
Column: Eclipse XDB-C18 Column, 4.6 x 150mm, 5um
Injection date  :Mon, 7. Mar. 2011            Location   :     Vial 5
Sample Name     :CR222-51-111-111-A-5 PK1     Inj. No.   :
Acq Operator    :DIPTEE                       Inj. Vol.  :       4 µl
Analysis Method :C:\CHEM32\2\METHODS\UPLC_GEN_TFA20.M
Last Changed    :Fri, 4. Mar. 2011,
Acq. Method     :C:\Chem32\2\DATA\MAR-11\070311A 2011-03-07 09-22-33\
                 UPLC_GEN_TFA7.M
Method ref      :GS/A0247/09
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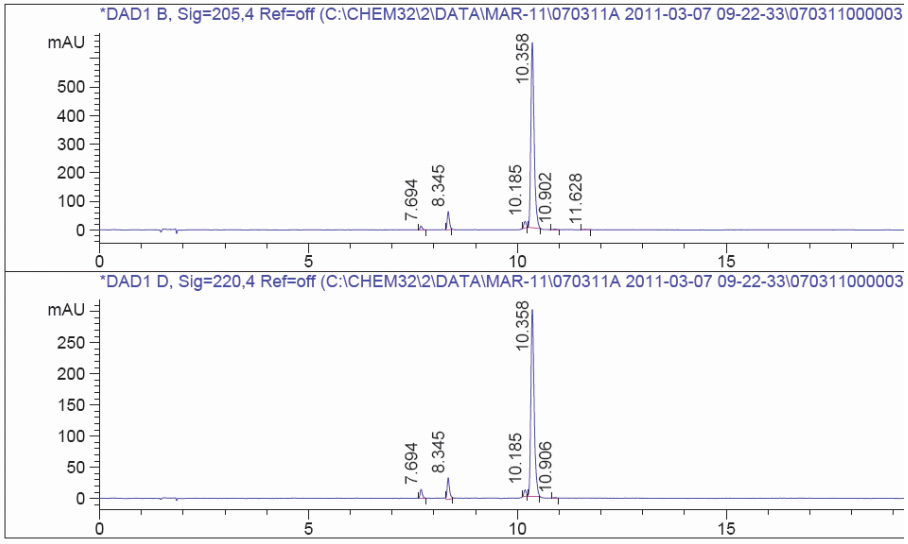
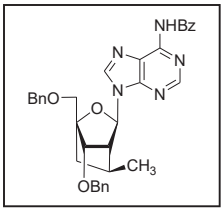




DAD1 B, Sig=205,4 Ref=off

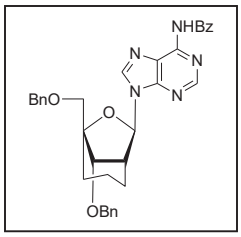
Peak #	RT (Min)	Width (Min)	Area	Area %
1	7.694	0.062	48.779	1.243
2	8.345	0.061	234.943	5.988
3	10.185	0.066	94.091	2.398
4	10.358	0.090	3518.024	89.659
5	10.902	0.105	14.902	0.380
6	11.628	0.135	13.061	0.333

DAD1 D, Sig=220,4 Ref=off

Peak #	RT (Min)	Width (Min)	Area	Area %
1	7.694	0.062	53.867	2.894
2	8.345	0.065	135.641	7.287
3	10.185	0.063	39.575	2.126
4	10.358	0.090	1624.402	87.263
5	10.906	0.096	8.014	0.431

*** End of Report***

Figure SII.14 HPLC profile of pure cENA-A (2c).



CHEMBIOTEK A TCG LIFESCIENCES ENTERPRISE, PUNE,INDIA

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Sample : CR222-51-111-111-A-5 PK2
Column: Eclipse XDB-C18 Column, 4.6 x 150mm, 5um
Injection date  :Mon, 7. Mar. 2011            Location   :      Vial 58
Sample Name     :CR222-51-111-111-A-5 PK2     Inj. No.   :           1
Acq Operator    :DIPTEE                       Inj. Vol.  :         6 µl
Analysis Method :C:\CHEM32\2\METHODS\UPLC_GEN_TFA20.M
Last Changed    :Fri, 4. Mar. 2011,
Acq. Method     :C:\Chem32\2\DATA\MAR-11\070311C 2011-03-07 14-07-15\
                 UPLC_GEN_TFA7.M
Method ref      :GS/A0247/09
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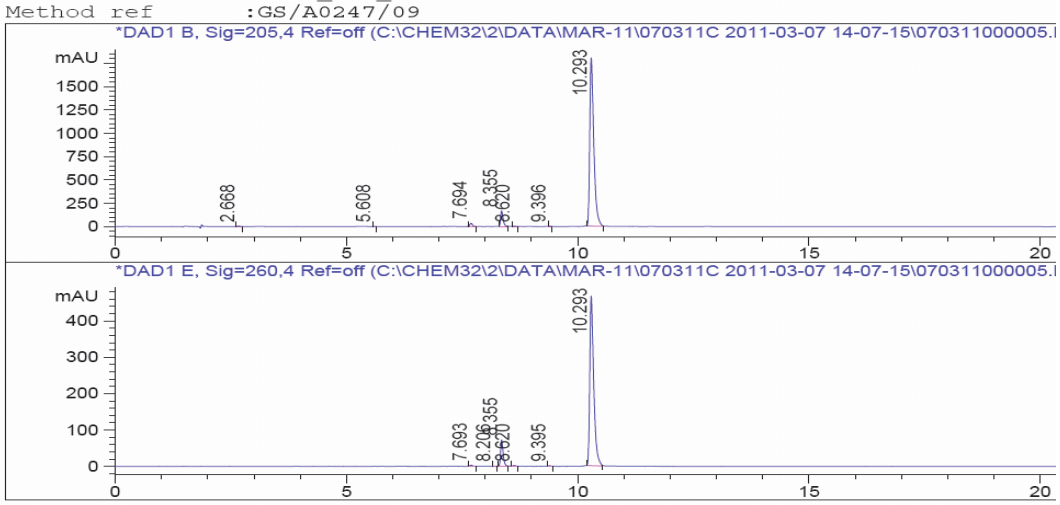


DAD1 B, Sig=205,4 Ref=off

Peak #	RT (Min)	Width (Min)	Area	Area %
1	2.668	0.080	28.073	0.238
2	5.608	0.061	10.871	0.092
3	7.694	0.065	142.602	1.211
4	8.355	0.063	635.803	5.397
5	8.620	0.067	17.323	0.147
6	9.396	0.062	21.487	0.182
7	10.293	0.101	1.092e4	92.732

DAD1 E, Sig=260,4 Ref=off

Peak #	RT (Min)	Width (Min)	Area	Area %
1	7.693	0.065	13.904	0.452
2	8.206	0.062	3.132	0.102
3	8.355	0.064	280.975	9.134
4	8.620	0.073	8.117	0.264
5	9.395	0.081	10.306	0.335
6	10.293	0.098	2759.697	89.713

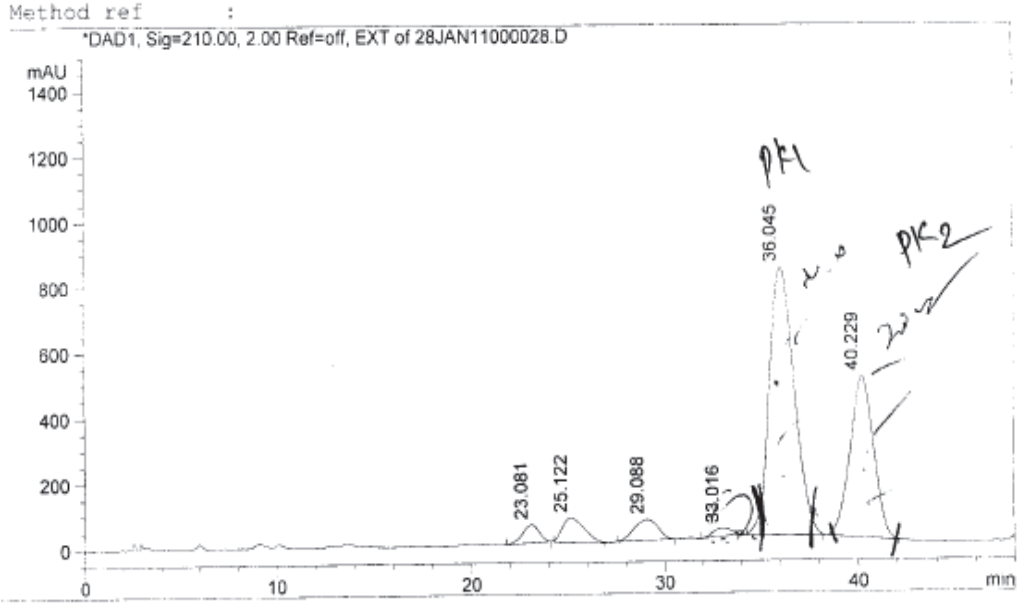
*** End of Report***

Figure SII.15 HPLC profile of mixtures of 7′*S*-Me-cLNA-G (3b) and cENA-G (3c)

CR222-113-27-27-B-1 ->
Column: ZORBAX SB C18 (250X21.2)mm, 7um
Injection date : Fri, 28. Jan. 2011 Location : Vial 51
Sample Name : CR222-113-27-27-B-1 Inj. No. : 0
Acq Operator : RAMESH Inj. Vol. : 500 µl
Analysis Method : C:\CHEM32\1\METHODS\POLAR ANA 2.M
Last Changed : Thu, 27. Jan. 2011,
Acq. Method : C:\CHEM32\1\METHODS\OXAMYL TECH_ISO_85_15 ANA1.M

Guanine Minor + Endo

Method ref :



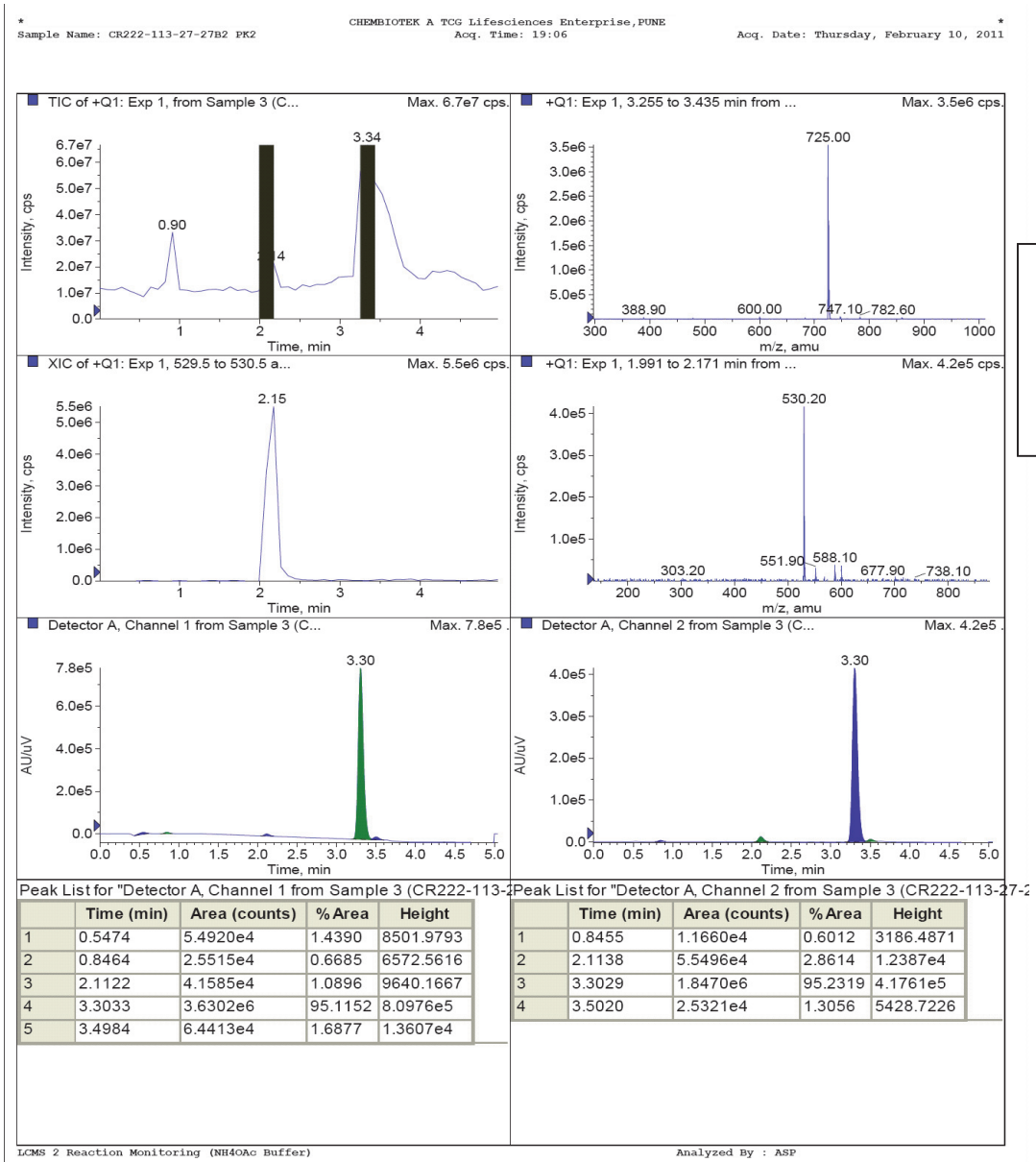
DAD1, Sig=210.00, 2.00 Ref=off, EXT

Peak #	RT (Min)	Width (Min)	Area	Area %
1	23.081	0.991	3.314e3	2.536
2	25.122	1.308	5.879e3	4.498
3	29.088	1.429	5.483e3	4.195
4	33.016	0.735	1.539e3	1.178
5	36.045	1.487	7.310e4	55.935
6	40.229	1.391	4.137e4	31.658

MPA - 10mM AA
MPB - ACN : MeOH (50 : 50)
Flow - 20.0 ml/min
Gradient.
75 % B Isocratic — 50 min

*** End of Report***

Figure SII.16 LC-MS profile of pure 7′*S*-Me-cLNA-G (3b).



	Time (min)	Area (counts)	% Area	Height
1	0.5474	5.4920e4	1.4390	8501.9793
2	0.8464	2.5515e4	0.6685	6572.5616
3	2.1122	4.1585e4	1.0896	9640.1667
4	3.3033	3.6302e6	95.1152	8.0976e5
5	3.4984	6.4413e4	1.6877	1.3607e4

	Time (min)	Area (counts)	% Area	Height
1	0.8455	1.1660e4	0.6012	3186.4871
2	2.1138	5.5496e4	2.8614	1.2387e4
3	3.3029	1.8470e6	95.2319	4.1761e5
4	3.5020	2.5321e4	1.3056	5428.7226

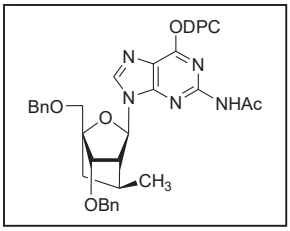
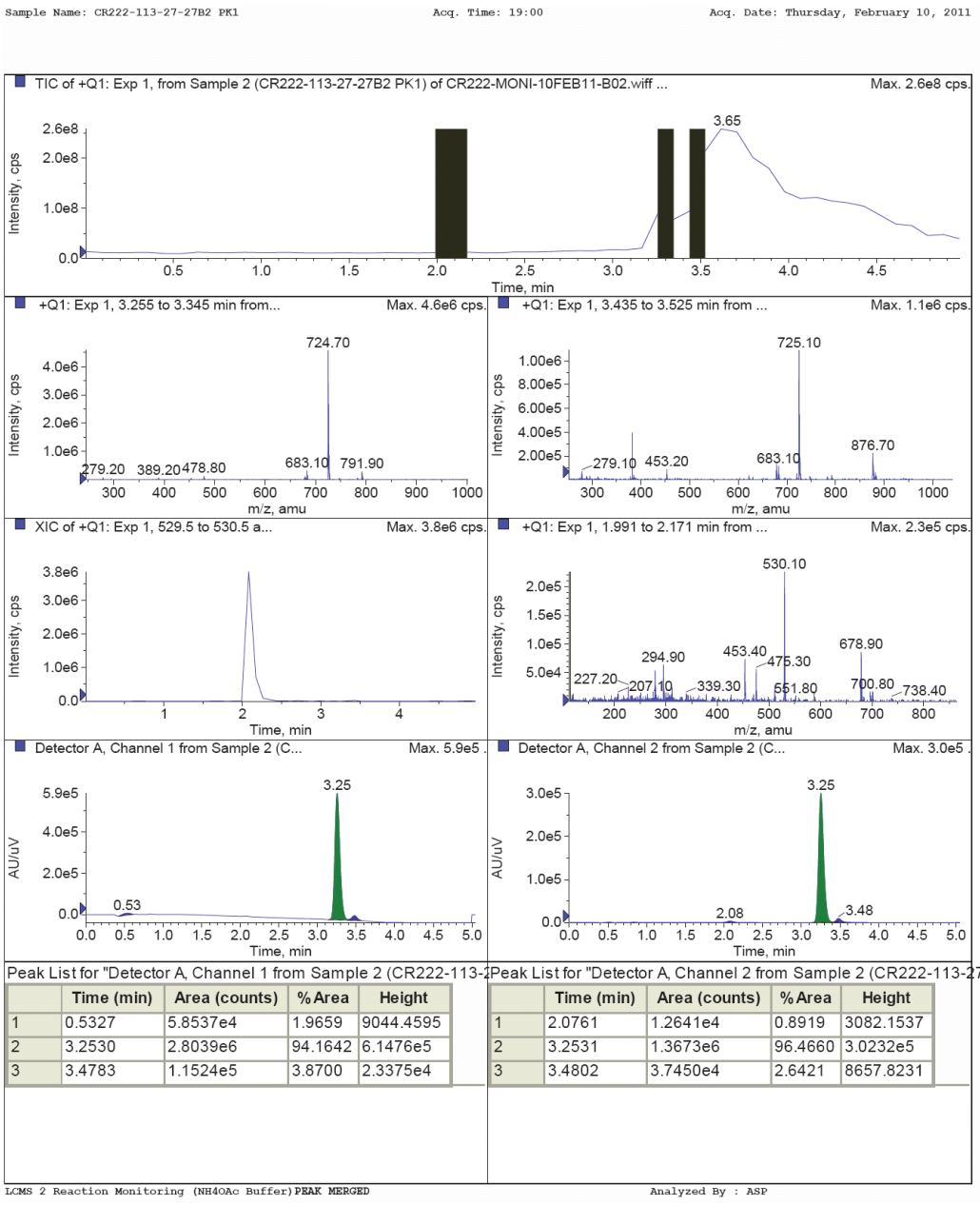


Figure SII.17 LC-MS profile of pure cENA-G (3c).



	Time (min)	Area (counts)	% Area	Height
1	0.5327	5.8537e4	1.9659	9044.4595
2	3.2530	2.8039e6	94.1642	6.1476e5
3	3.4783	1.1524e5	3.8700	2.3375e4

	Time (min)	Area (counts)	% Area	Height
1	2.0761	1.2641e4	0.8919	3082.1537
2	3.2531	1.3673e6	96.4660	3.0232e5
3	3.4802	3.7450e4	2.6421	8657.8231

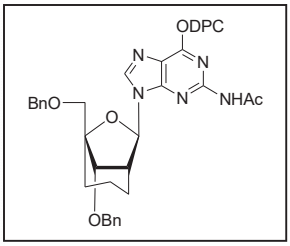


Figure SII.18 HPLC profile of mixtures of 7′*R*-Me-cLNA-^{Me}C (4a), 7′*S*-Me-cLNA-^{Me}C (4b) and cENA-^{Me}C (4c)

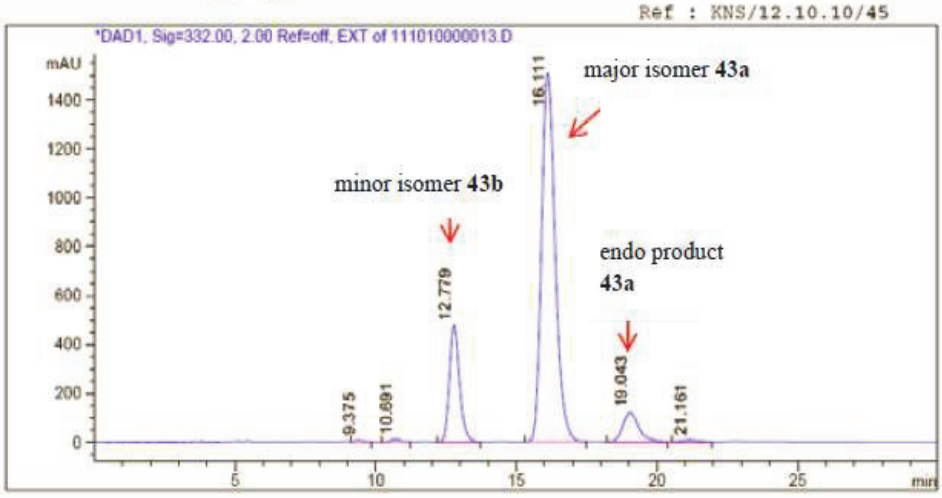
Sample : CR222-124-15-15A1 CHIRAL HPLC

Column: CHIRALPAK-IC (4.6x250) 5µ
ARD/K/7461
Mobile Phase : HEXANE/ETOH : 70/30
Flow rate : 1.0 ml/min
Solubility : MEOH:DCM

Injection Date :Mon, 11. Oct. ->5:34:36 PM Location : Vial 15
Inj. No. : 1
Acq Operator :NARESH
Inj. Vol. : 5 µl
Last Changed : Tue, 12. Oct. 2010, 10:38:43 am

Acq. Method :C:\Chem32\1\DATA\OCT-10\111010 2010-10-11 11-00-56\70 HEXANE-30 ETOH-1.M

Ref : KNS/12.10.10/45



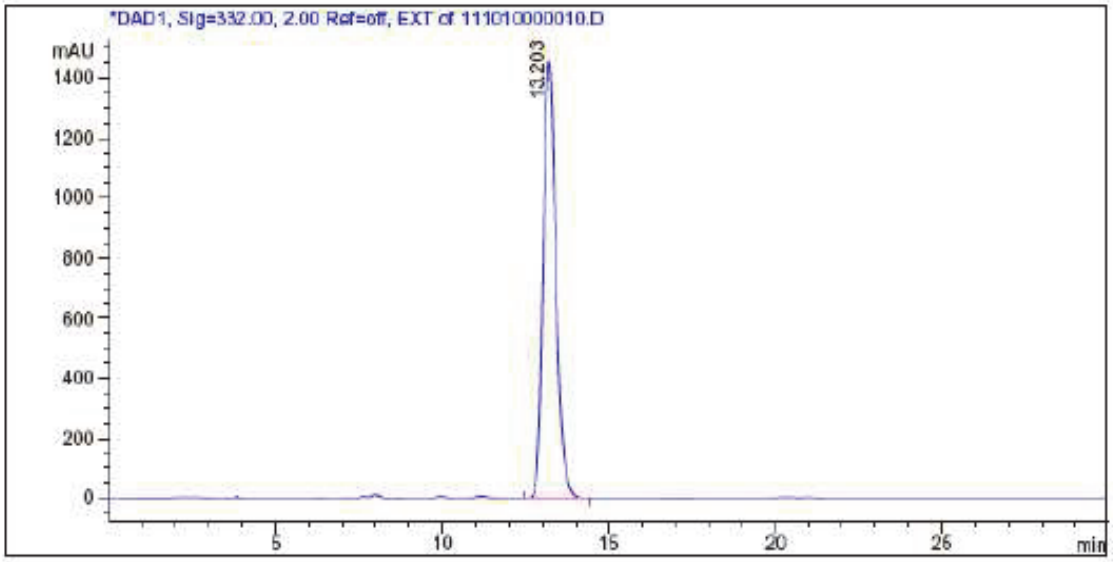
Signal 1: DAD1, Sig=332.00, 2.00 Ref=off, EXT

Peak #	RT [min]	Area	Area %	
1	9.38	104.26	0.15	
2	10.69	232.14	0.34	
3	12.78	11956.79	17.61	43b
4	16.11	50638.02	74.58	43a
5	19.04	4703.85	6.93	43c
6	21.16	263.62	0.39	

*** End of Report ***

Figure SII.19 HPLC profile of pure 7′*R*-Me-cLNA-^{Me}C (4a).

```
Column: CHIRALPAK-IC (4.6x250) 5u
ARD/K/7461
Mobile Phase : HEXANE/ETOH : 70/30
Flow rate : 1.0 ml/min
Solubility : MEOH:DCM
```

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Signal  1: DAD1, Sig=332.00, 2.00 Ref=off, EXT
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Peak #	RT [min]	Area	Area %
1	13.20	39340.28	100.00

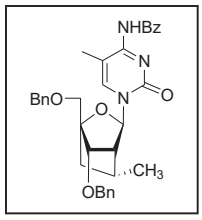
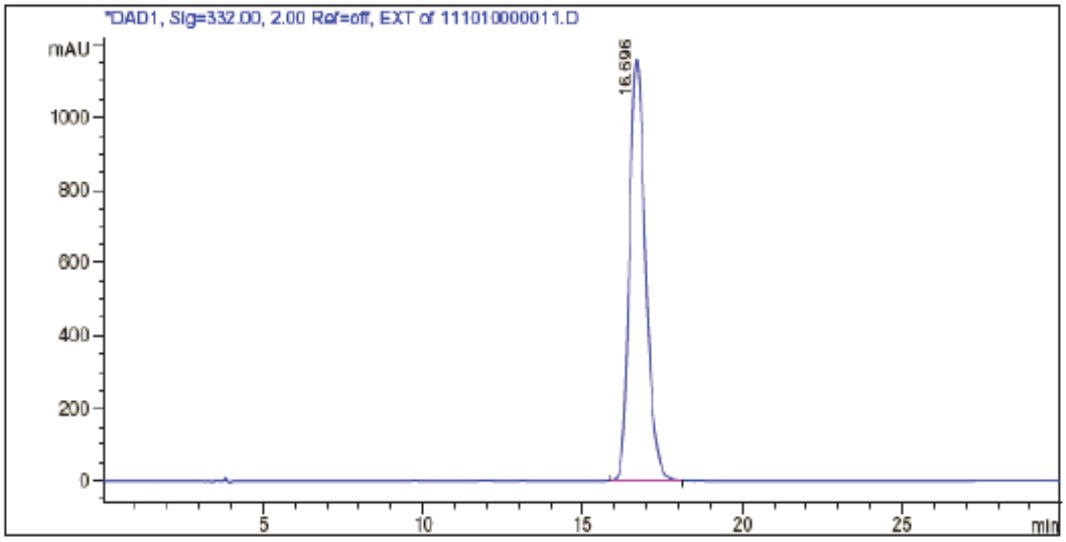


Figure SII.20 HPLC profile of pure 7′*S*-Me-cLNA-^{Me}C (4b).

Column: CHIRALPAK-IC (4.6x250) 5µ
ARD/K/7461
Mobile Phase : HEXANE/ETOH : 70/30
Flow rate : 1.0 ml/min
Solubility : MEOH:DCM



Signal 1: DAD1, Sig=332.00, 2.00 Ref=off, EXT

Peak #	RT [min]	Area	Area %
1	16.70	40555.03	100.00

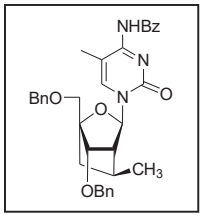
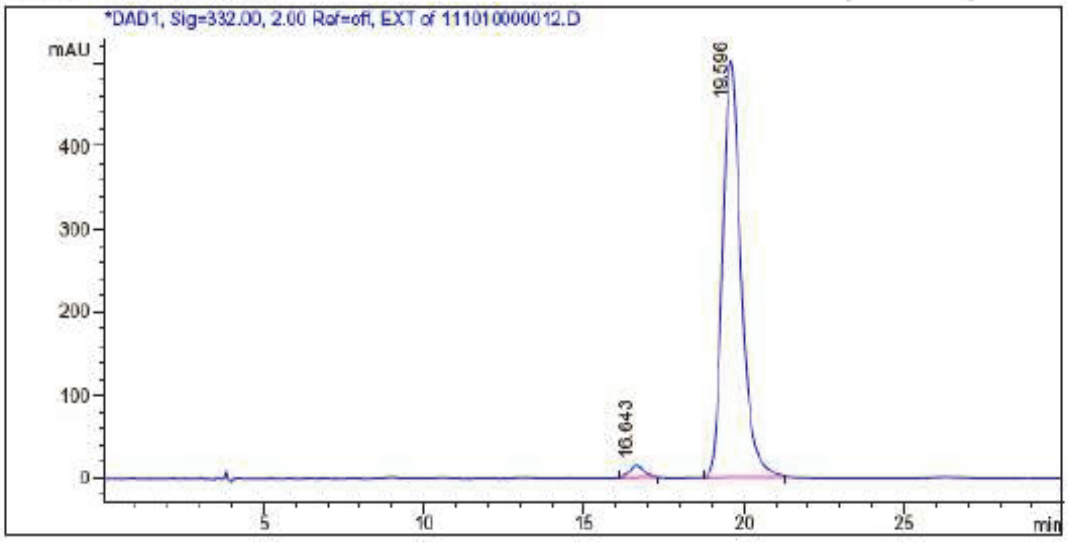


Figure SII.21 HPLC profile of pure cENA-^{Me}C (4c).

Column: CHIRALPAK-IC (4.6x250) 5µ
ARD/K/7461
Mobile Phase : HEXANE/ETOH : 70/30
Flow rate : 1.0 ml/min
Solubility : MEOH:DCM



Signal 1: DAD1, Sig=332.00, 2.00 Ref=off, EXT

Peak #	RT [min]	Area	Area %
1	16.64	443.32	2.15
2	19.60	20186.01	97.85

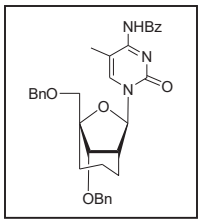


Figure SII.22 HPLC profile of mixtures of 7′*R*-Me-cLNA-T (5a), 7′*S*-Me-cLNA-T (5b) and cENA-T (5c).

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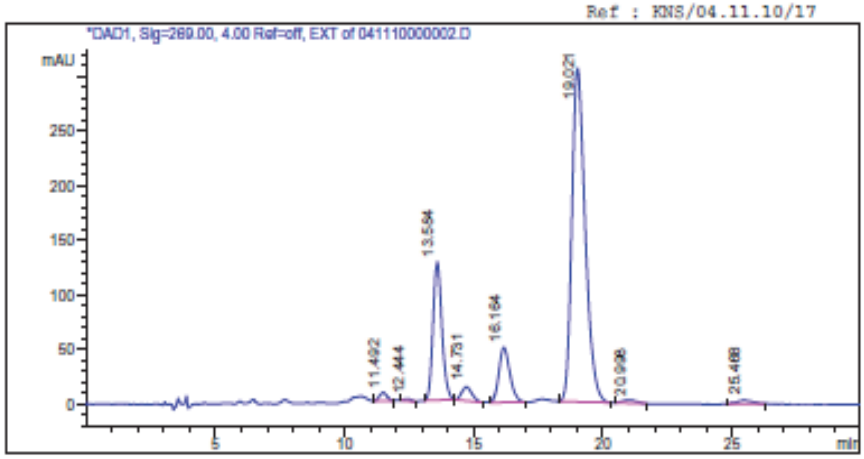
Sample : CR222-119-21-21A CHIRAL HPLC

Column: CHIRALPAK-IC (4.6x250) 5µ
ARD/K/7461
Mobile Phase : HEXANE/ETOH : 70/30.
Flow rate : 1.0 ml/min
Solubility : DCM:MEOH

Injection Date :Thu, 4. Nov. ->11:21:19 AM Location : Vial 43
Inj. No. : 1
Acq Operator :NARESH
Inj. Vol. : 5 µl
Last Changed : Sat, 17. Jul. 2010, 00:42:43 pm

Acq. Method :C:\Chem32\1\DATA\NOV-10\041110 2010-11-04 10-37-16\70 HEXANE-30 ETOH-1.M

Ref : KNS/04.11.10/17



Signal 1: DAD1, Sig=269.00, 4.00 Ref=off, EXT

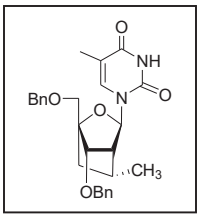
Peak #	RT [min]	Area	Area %
1	11.49	158.33	0.98
2	12.44	37.74	0.23
3	13.58	3040.07	18.74
4	14.73	348.27	2.15
5	16.16	1485.35	9.16
6	19.02	10905.17	67.22
7	21.00	101.91	0.63
8	25.47	145.08	0.89

*** End of Report ***

Minor (7'*S*)

6'-*endo*
Major (7'*R*)

Figure SII.23 HPLC profile of pure 7′*R*-Me-cLNA-T (5a).



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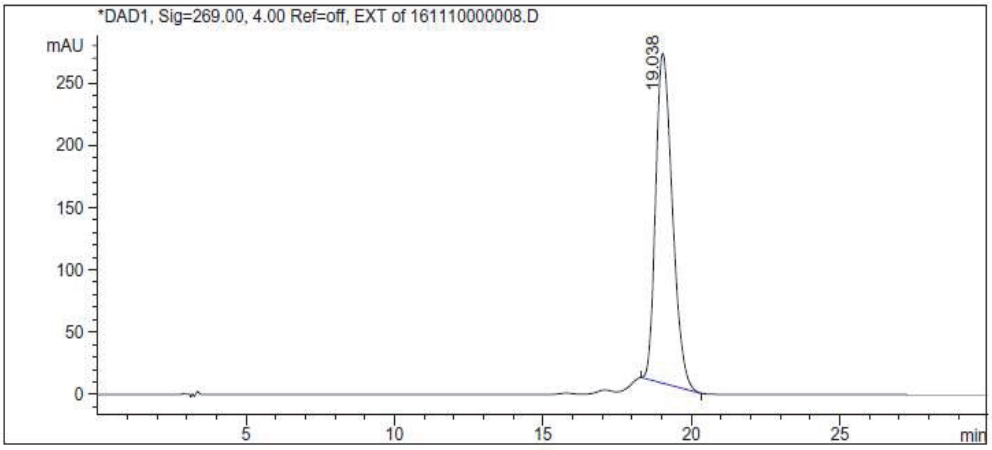
Sample ID : CR222-119-21-21A-PEAK-3

Column : CHIRALPAK-IC (4.6x 250mm) 5µ
ARD/K/7461
Mobile Phase : HEXANE/ETOH : 70/30.
Flow rate : 1.0 ml/min
Solubility : DCM. ->
Injection Date :Tue, 16. ->9:16:15 PM Location : Vial 28
Inj. No. : 1
Acq Operator :NARESH Inj. Vol. : 5 µl

Last Changed :Wed, 17. Nov. 2010, 04:19:26 pm

Acq. Method :C:\Chem32\1\DATA\NOV-10\161110 2010-11-16 16-47-33\70 HEXANE-30 ETOH-1.M

Ref : KNS/18.11.10/17



Signal 1: DAD1, Sig=269.00, 4.00 Ref=off, EXT

Peak #	RT [min]	Area	Area %
1	19.04	10392.84	100.00

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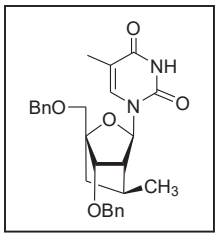
*** End of Report ***

Figure SII.24 HPLC profile of pure 7′*S*-Me-cLNA-T (5b).

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================================================================================
     Sample ID : CR222-119-21-21A-PEAK-1

     Column : CHIRALPAK-IC (4.6x 250mm) 5µ
     ARD/K/7461
     Mobile Phase : HEXANE/ETOH : 70/30.
     Flow rate : 1.0 ml/min
     Solubility : DCM.                                  ->
     Injection Date  :Tue, 16.  ->9:47:47 PM         Location   :    Vial 26
                                                     Inj. No.   :         1
     Acq Operator    :NARESH                         Inj. Vol.  :       8 µl

     Last Changed    :Wed, 17. Nov. 2010,  04:19:26 pm

     Acq. Method     :C:\Chem32\1\DATA\NOV-10\161110 2010-11-16 16-47-33\70 HEXANE-30
                      ETOH-1.M

                                                                Ref : KNS/18.11.10/17
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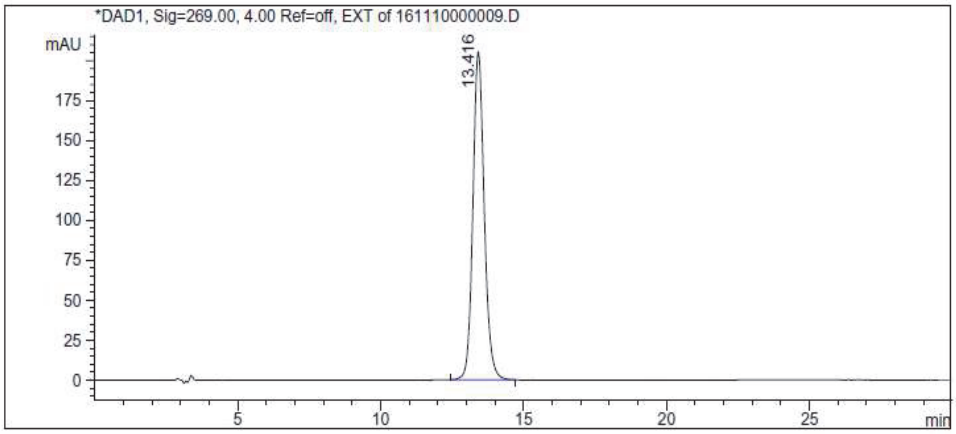


```
Signal  1: DAD1, Sig=269.00, 4.00 Ref=off, EXT

|Peak|   RT    |   Area    | Area % |
| #  |  [min]  |           |        |
|----|---------|-----------|--------|
|   1|    13.42|   5677.62|  100.00|
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                          *** End of Report ***
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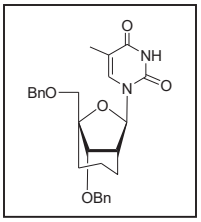
Figure SII.25 HPLC profile of pure cENA-T (5c).



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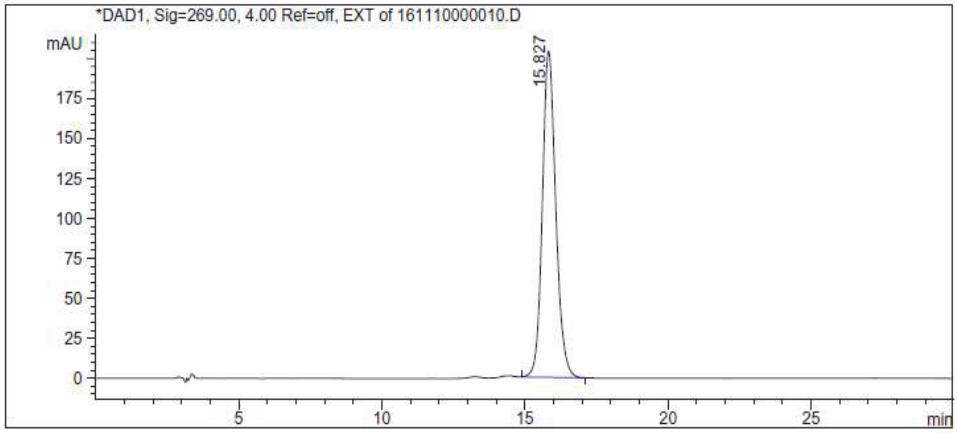
Sample ID : CR222-119-21-21A-PEAK-2

Column : CHIRALPAK-IC (4.6x 250mm) 5µ
ARD/K/7461
Mobile Phase : HEXANE/ETOH : 70/30.
Flow rate : 1.0 ml/min
Solubility : DCM. ->
Injection Date :Tue, 16. ->10:19:21 PM Location : Vial 27
Inj. No. : 1
Acq Operator :NARESH Inj. Vol. : 8 µl

Last Changed :Wed, 17. Nov. 2010, 04:19:26 pm

Acq. Method :C:\Chem32\1\DATA\NOV-10\161110 2010-11-16 16-47-33\70 HEXANE-30 ETOH-1.M

Ref : KNS/18.11.10/17



Signal 1: DAD1, Sig=269.00, 4.00 Ref=off, EXT

Peak #	RT [min]	Area	Area %
1	15.83	6655.12	100.00

*** End of Report ***

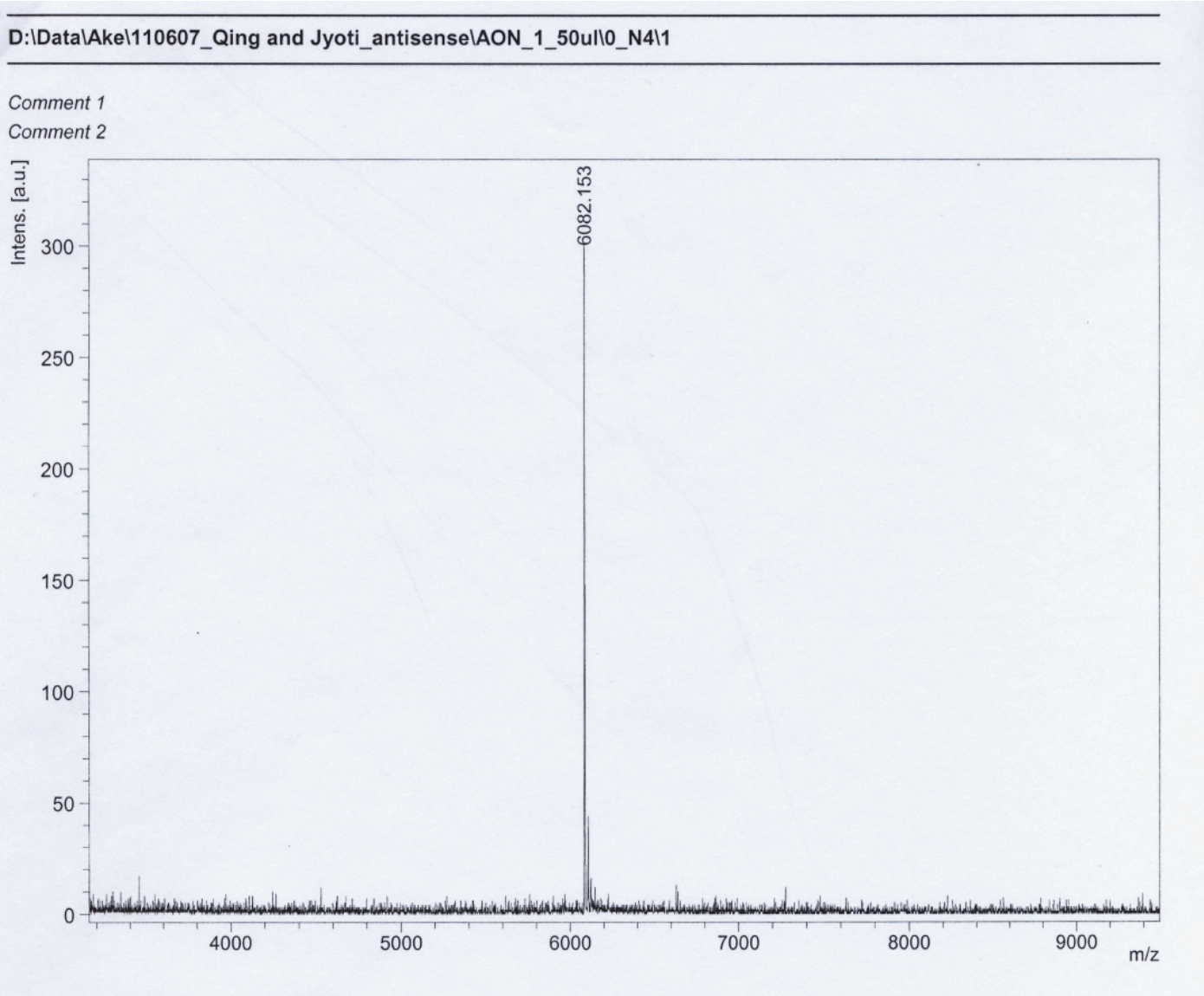


Figure SII.26 MALDI-TOF spectrum of AON1

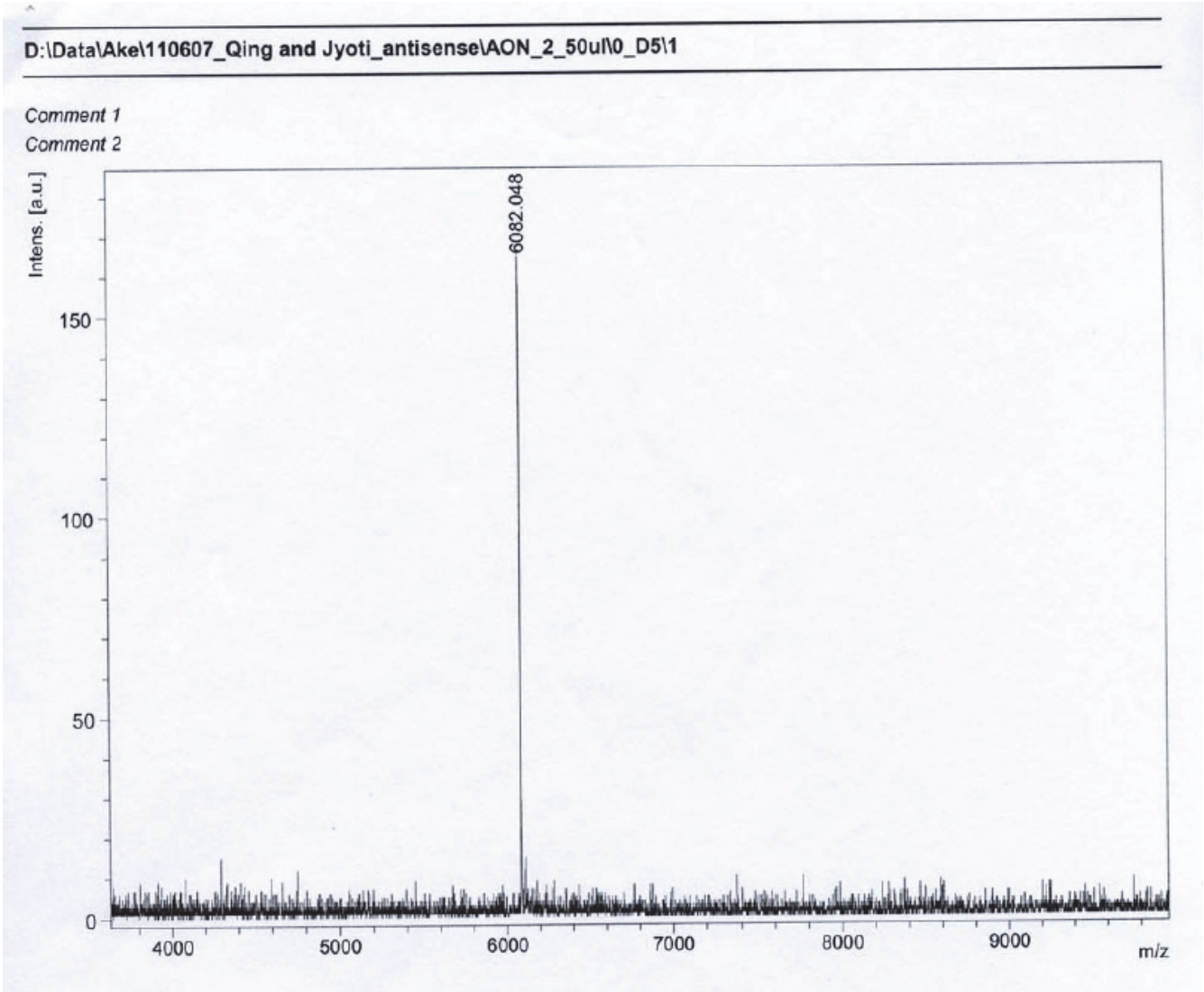


Figure SII.27 MALDI-TOF spectrum of AON2

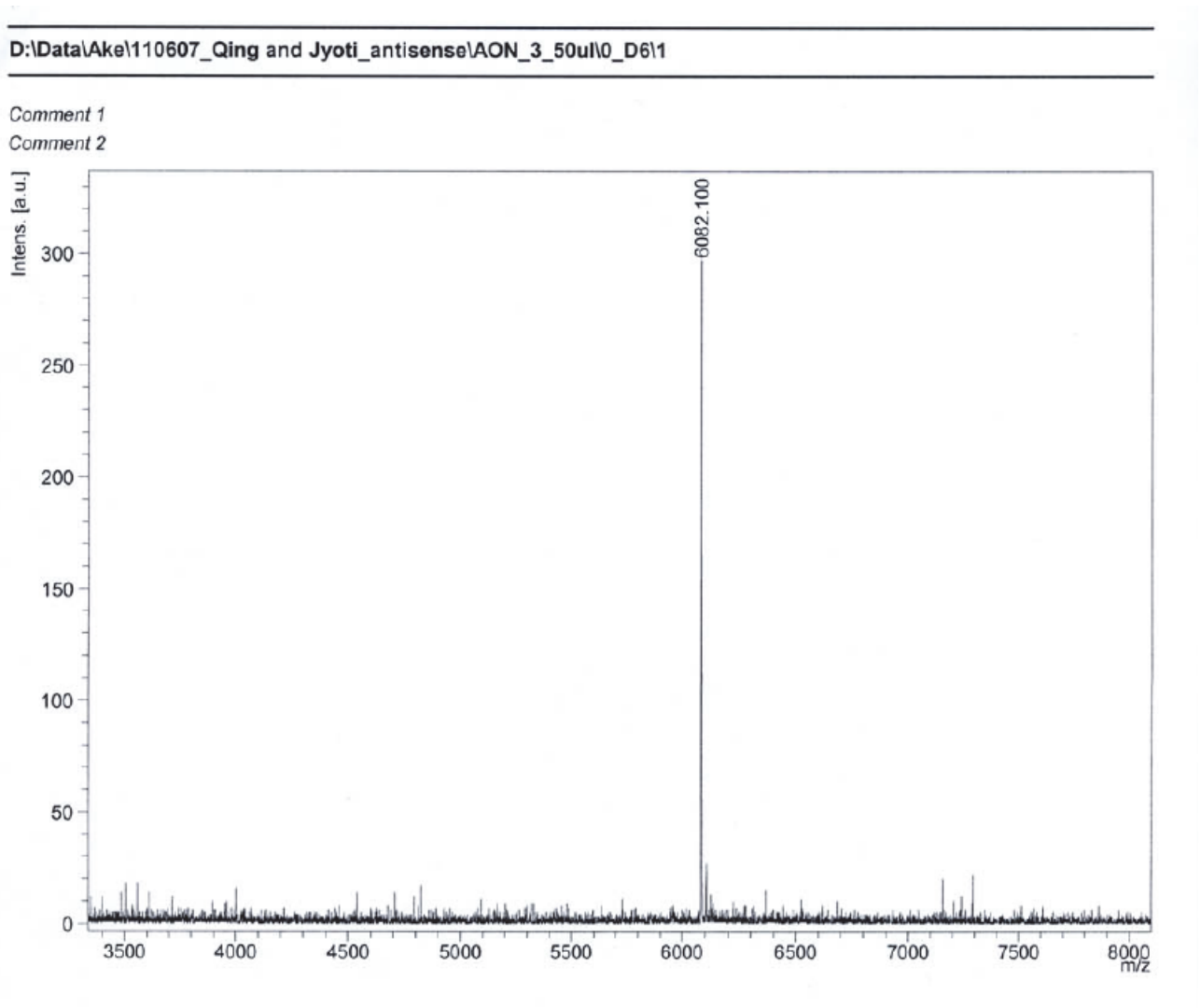


Figure SII.28 MALDI-TOF spectrum of AON3

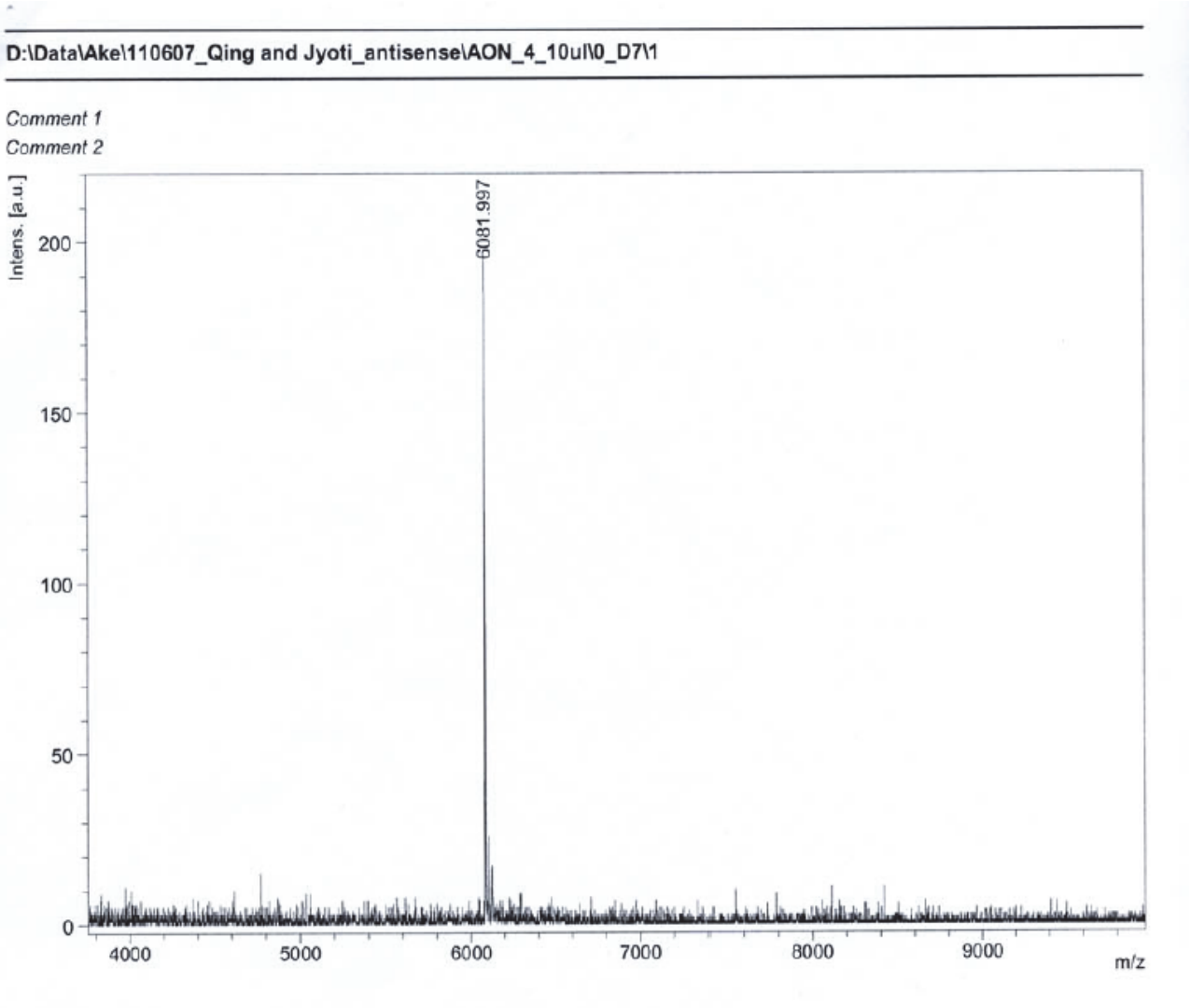


Figure SII.29 MALDI-TOF spectrum of AON4

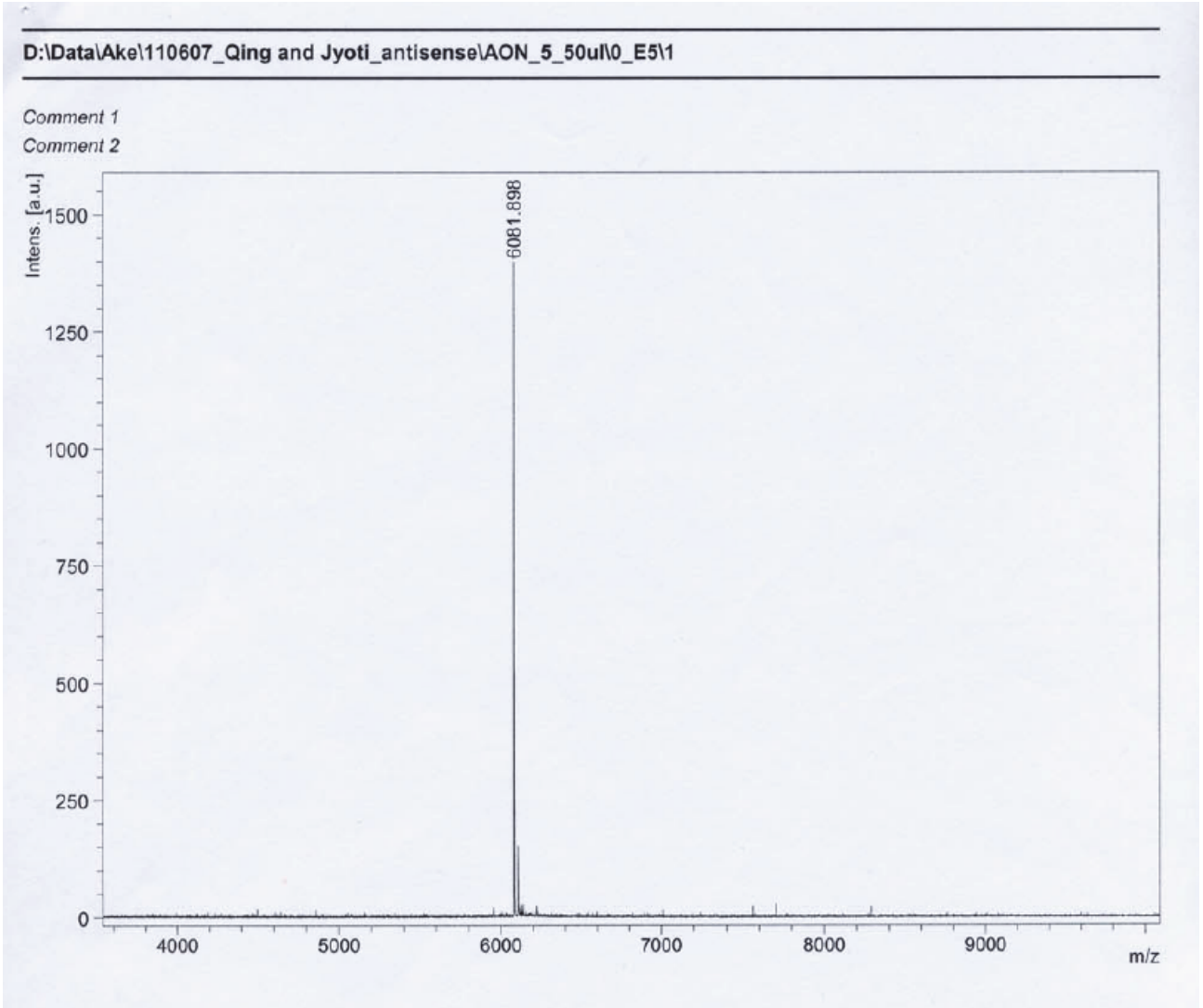


Figure SII.30 MALDI-TOF spectrum of AON5

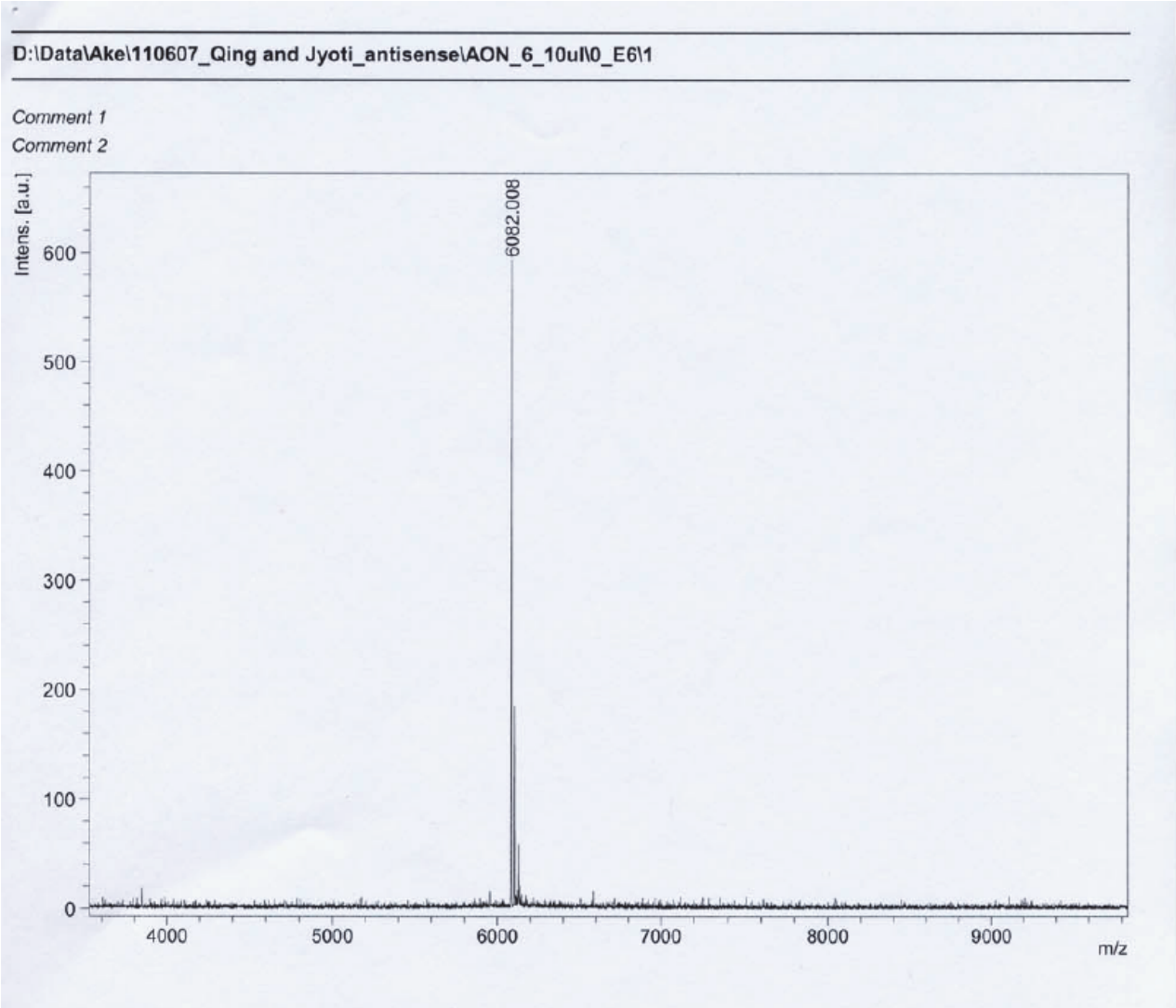


Figure SII.31 MALDI-TOF spectrum of AON6

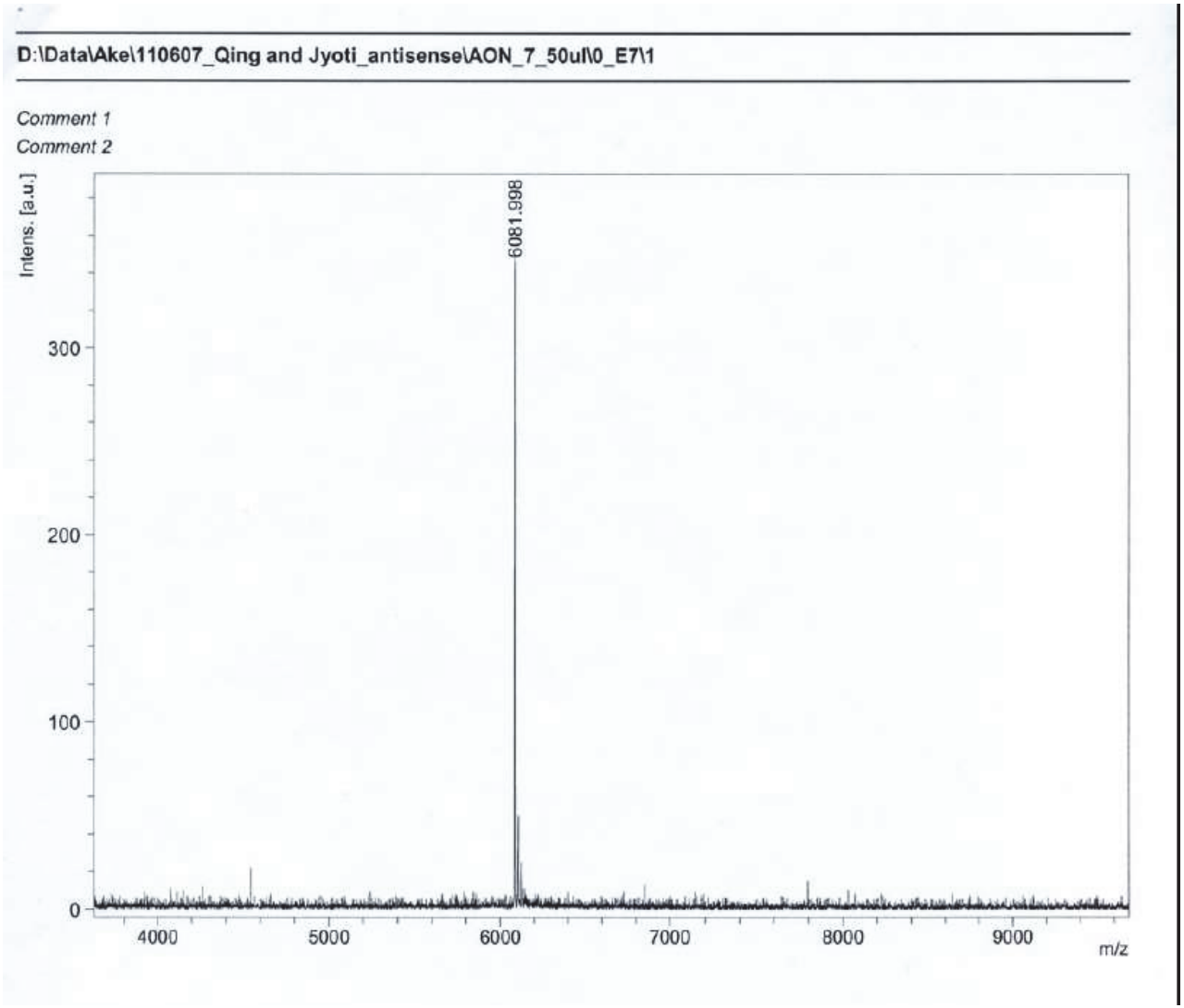


Figure SII.32 MALDI-TOF spectrum of AON7

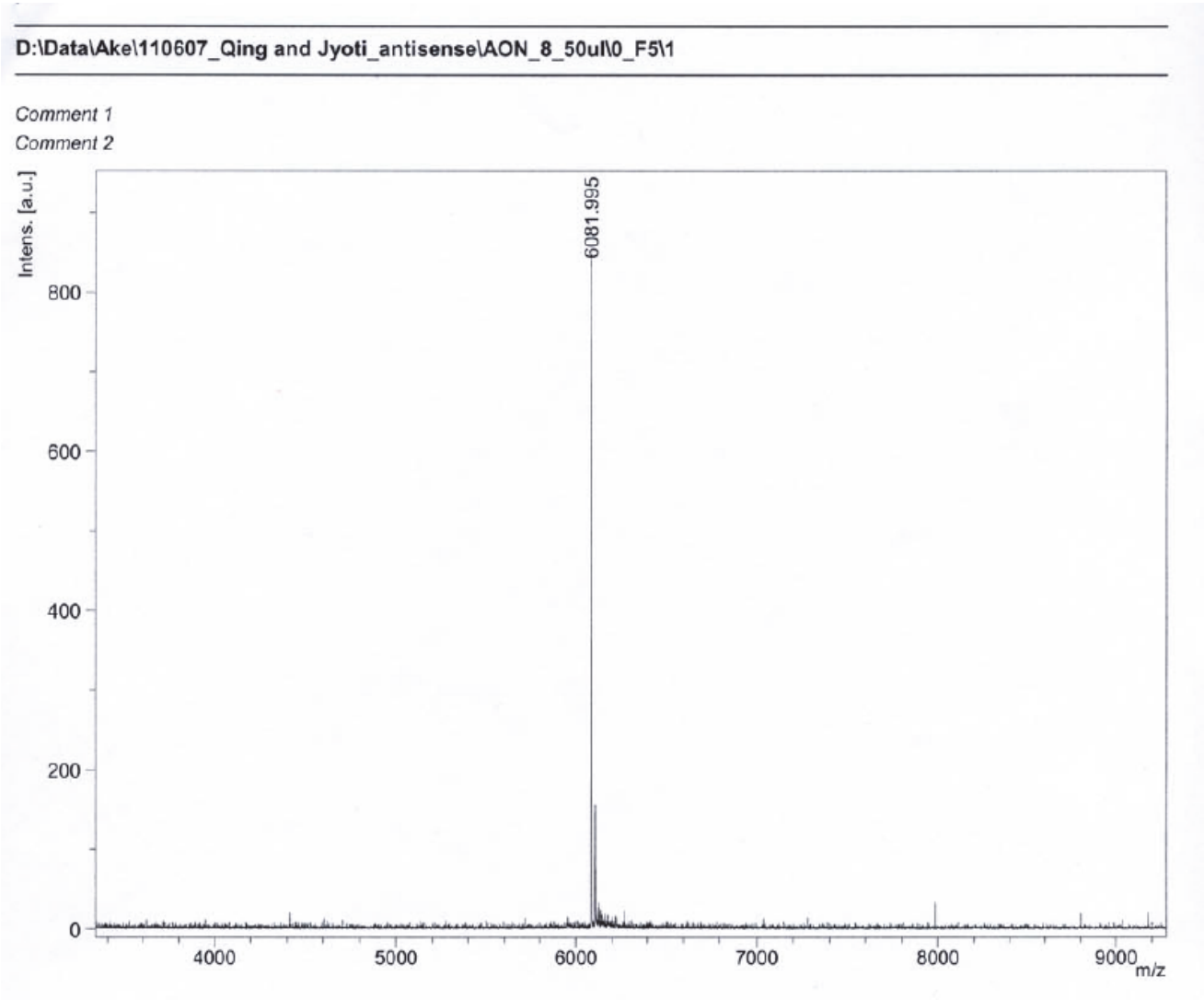


Figure SII.33 MALDI-TOF spectrum of AON8

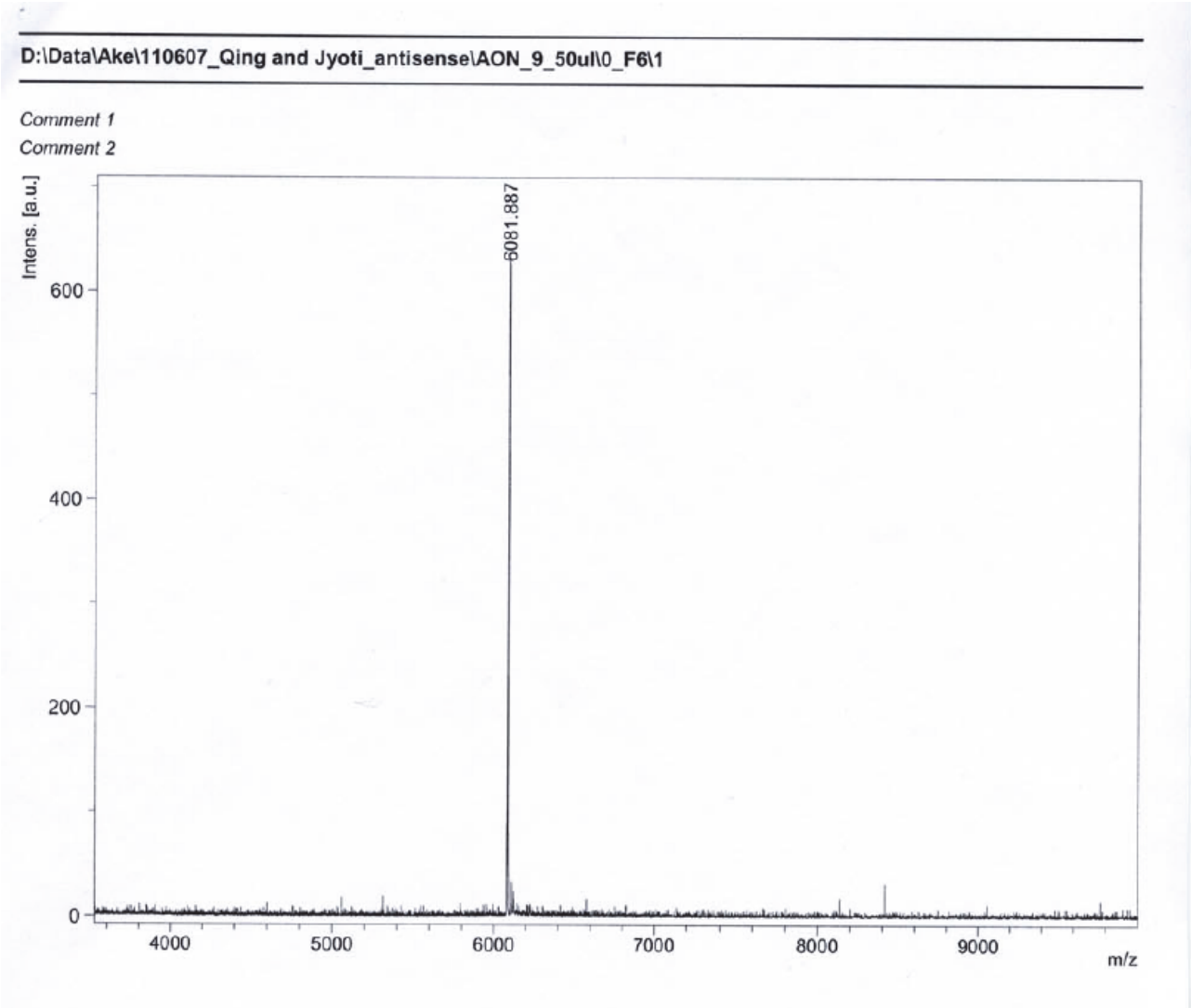


Figure SII.34 MALDI-TOF spectrum of AON9

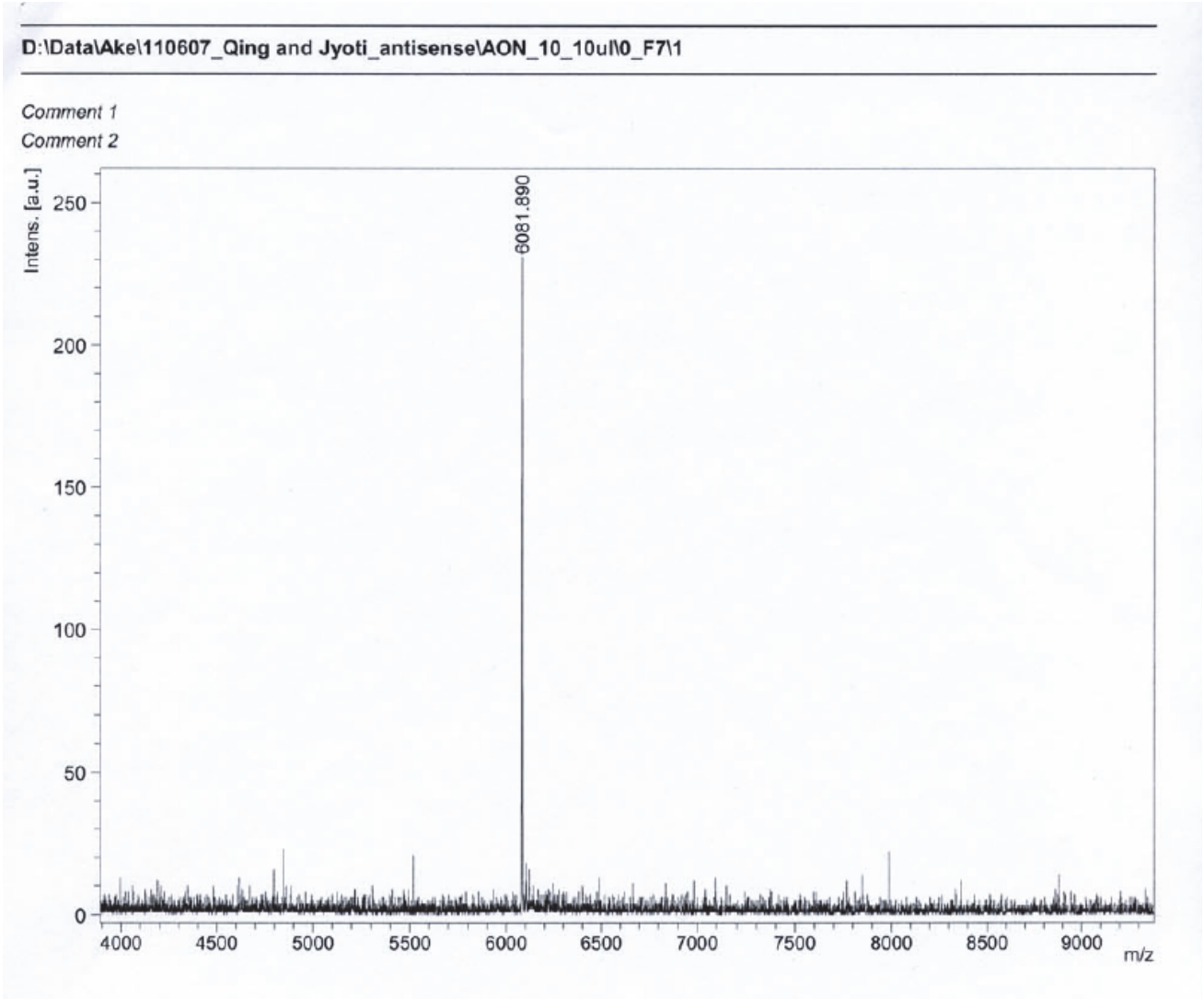


Figure SII.35 MALDI-TOF spectrum of AON10

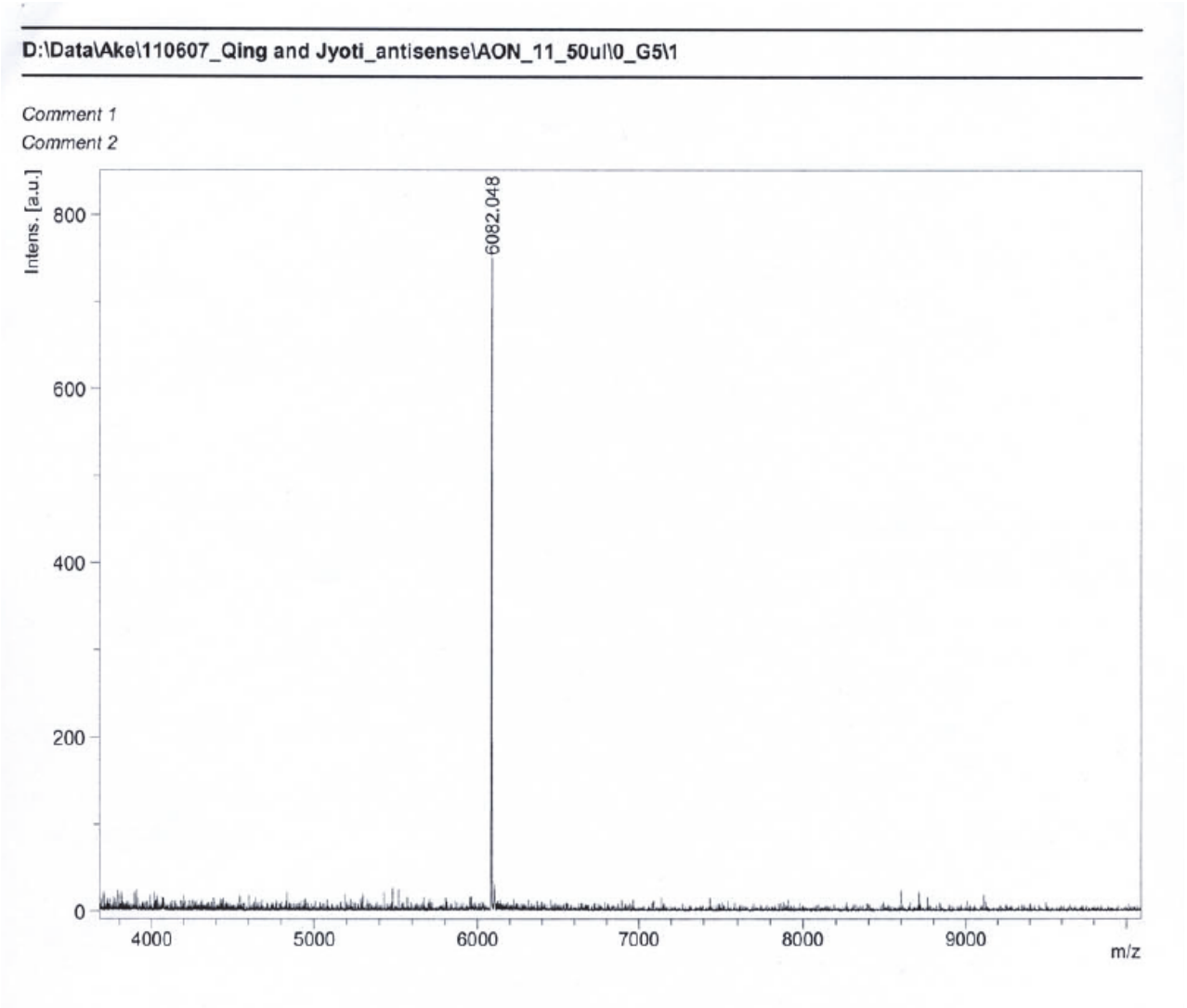


Figure SII.36 MALDI-TOF spectrum of AON11

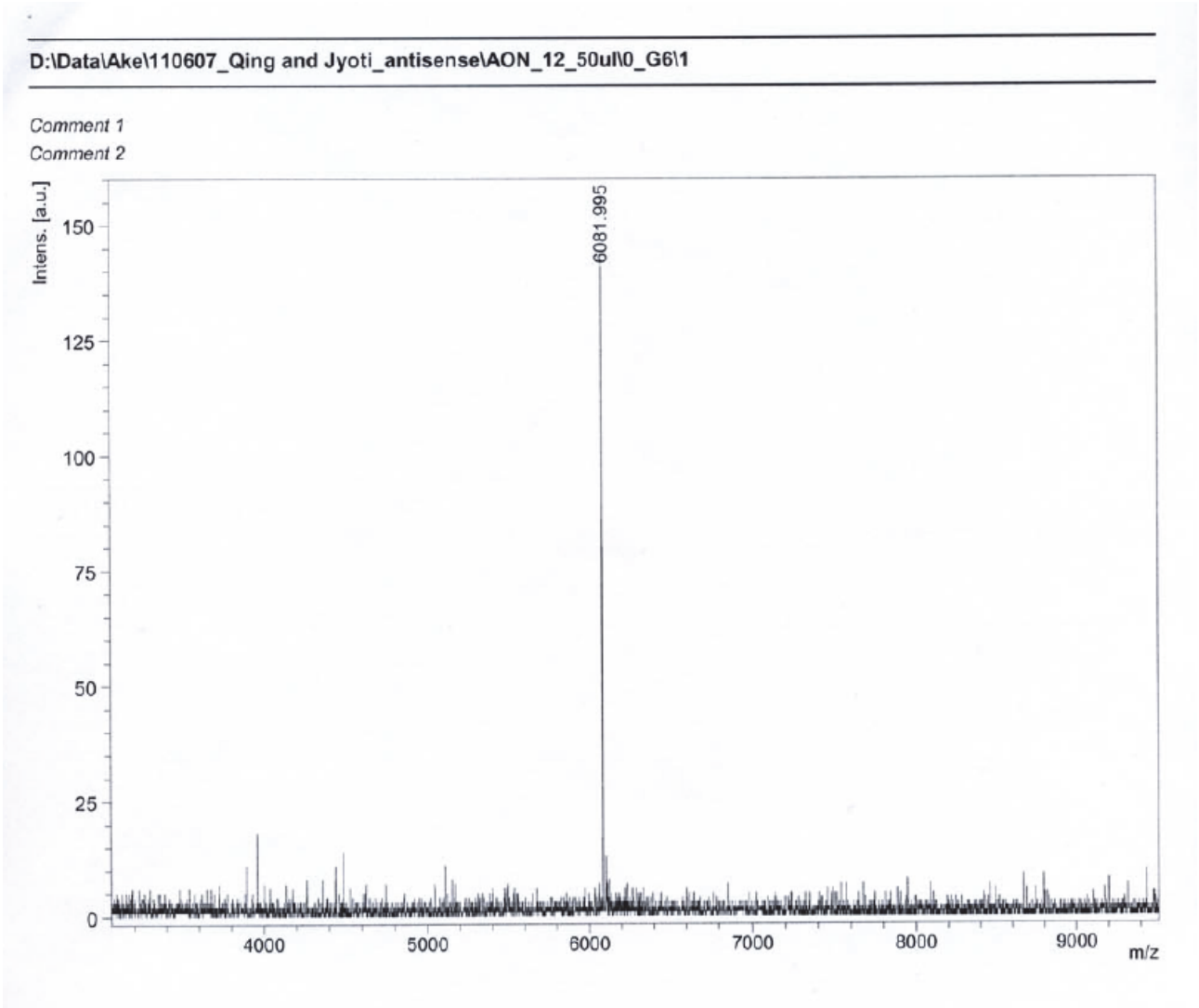


Figure SII.37 MALDI-TOF spectrum of AON12

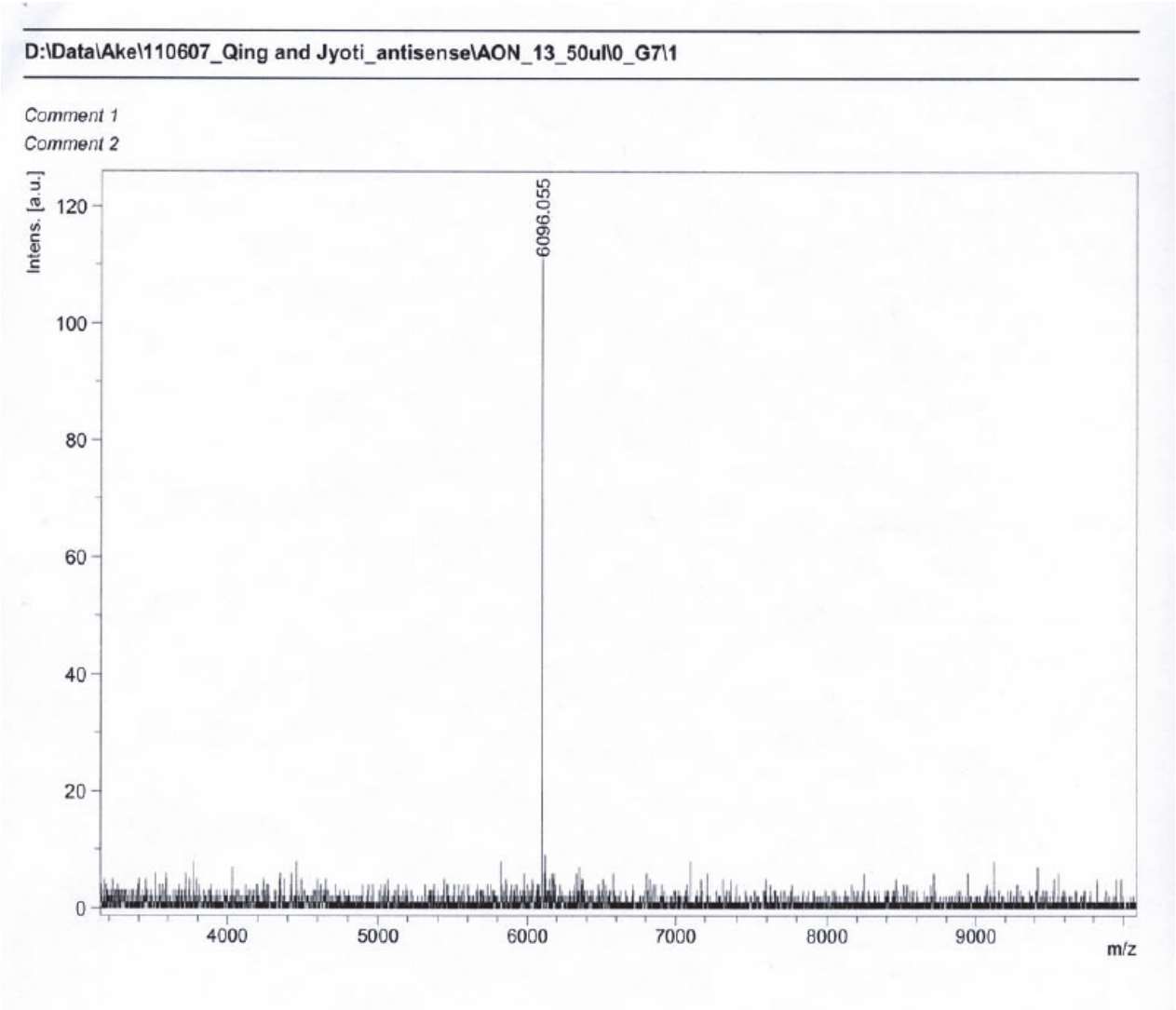


Figure SII.38 MALDI-TOF spectrum of AON13

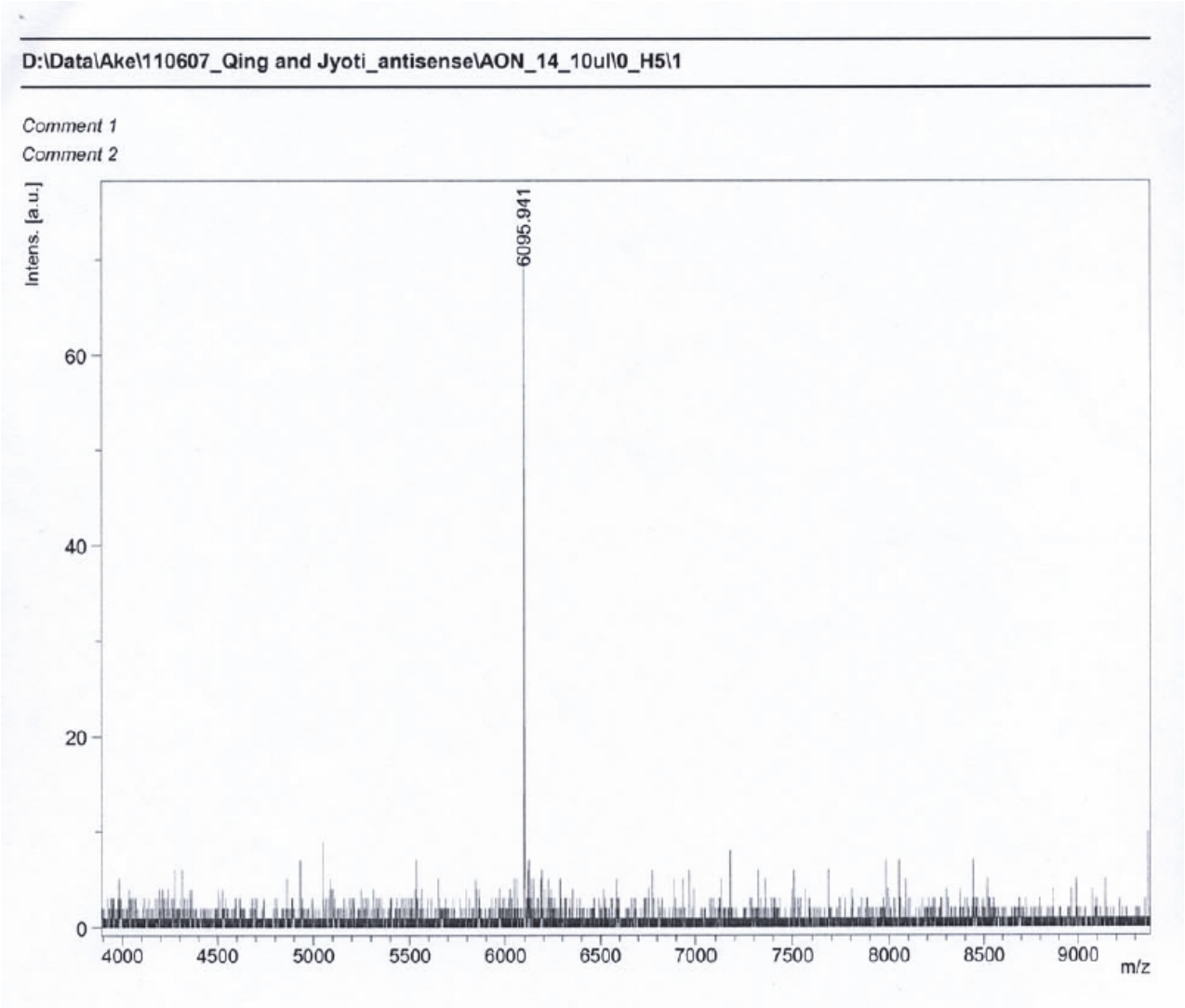


Figure SII.39 MALDI-TOF spectrum of AON14

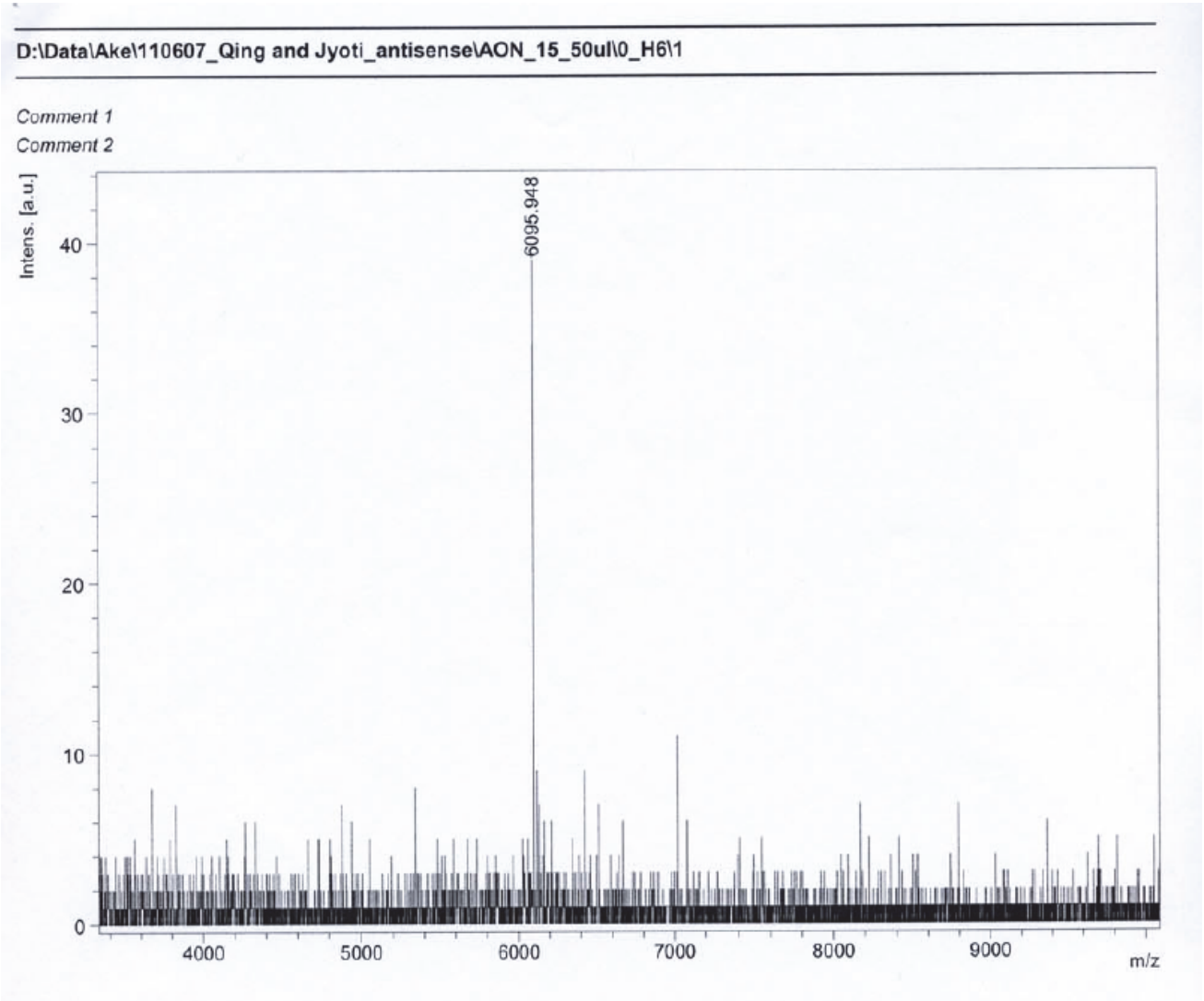


Figure SII.40 MALDI-TOF spectrum of AON15

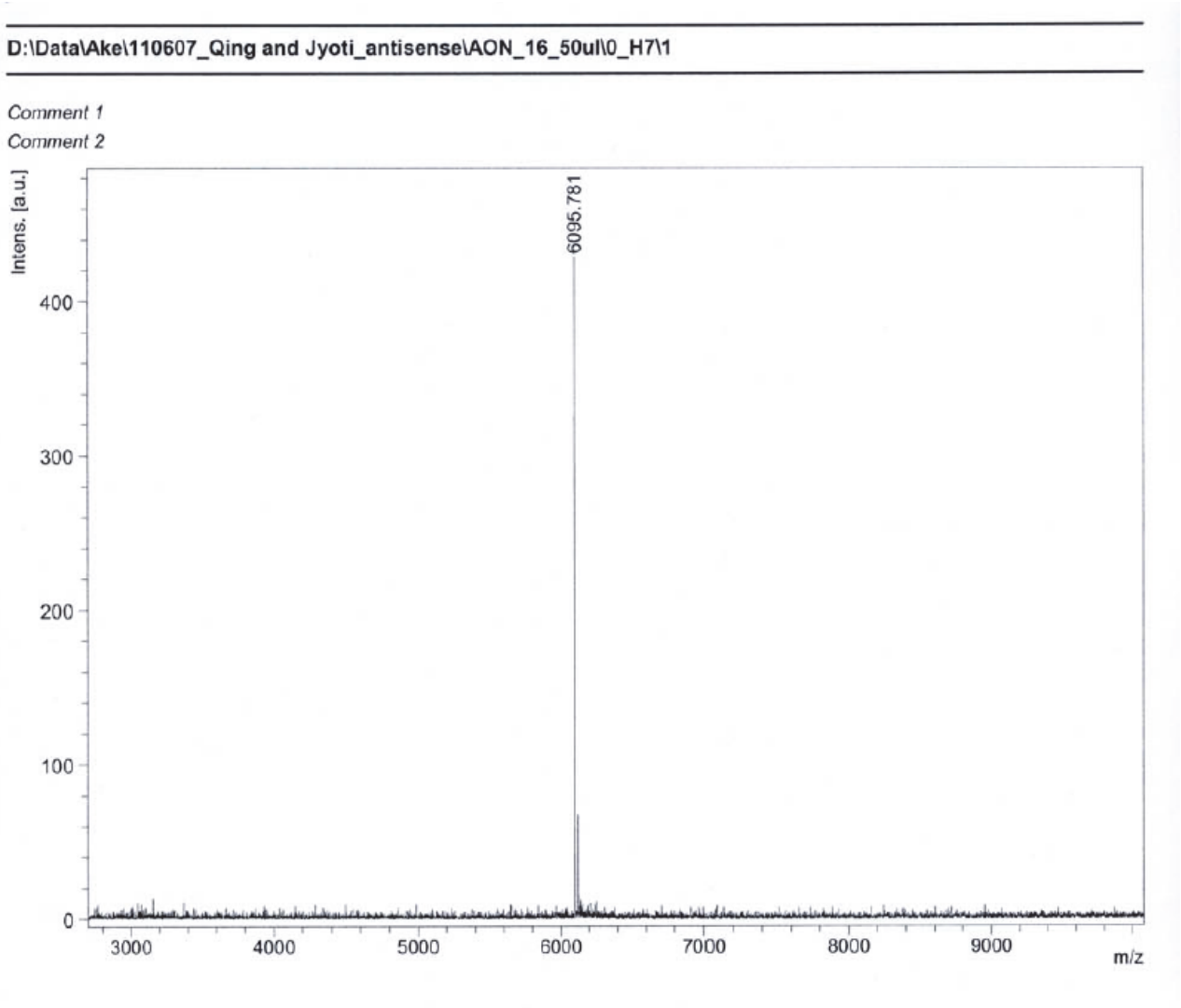


Figure SII.41 MALDI-TOF spectrum of AON16

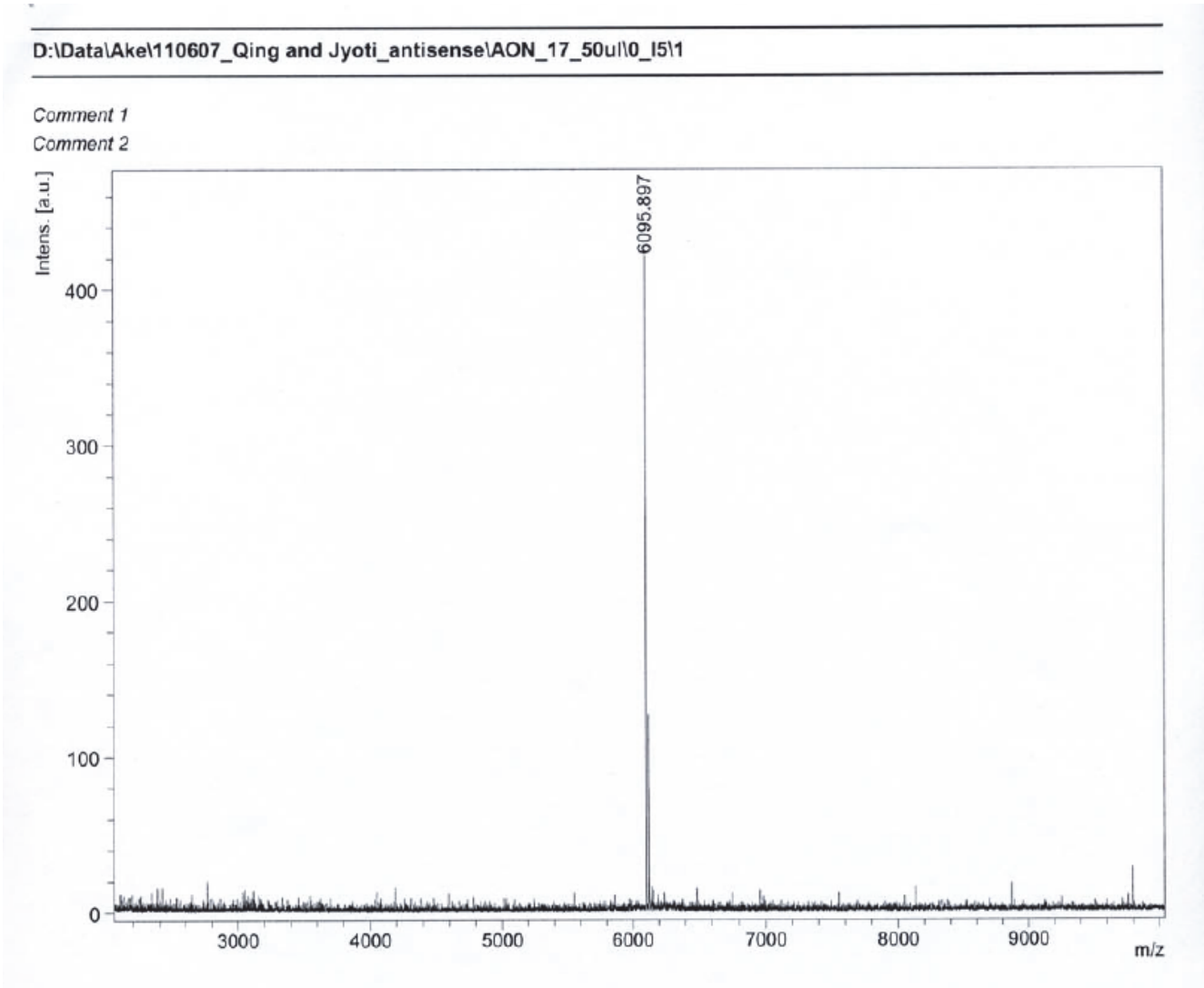


Figure SII.42 MALDI-TOF spectrum of AON17

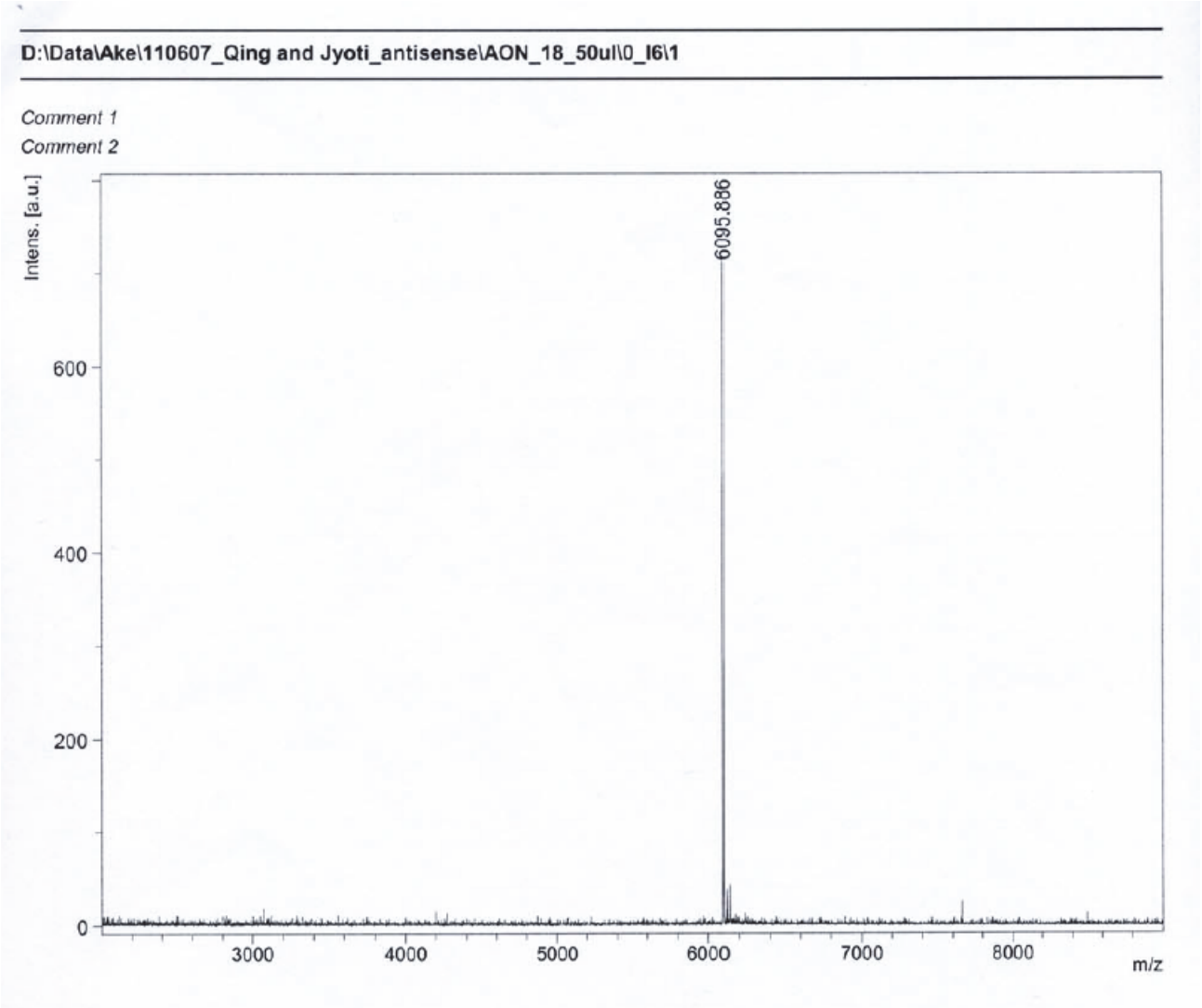


Figure SII.43 MALDI-TOF spectrum of AON18

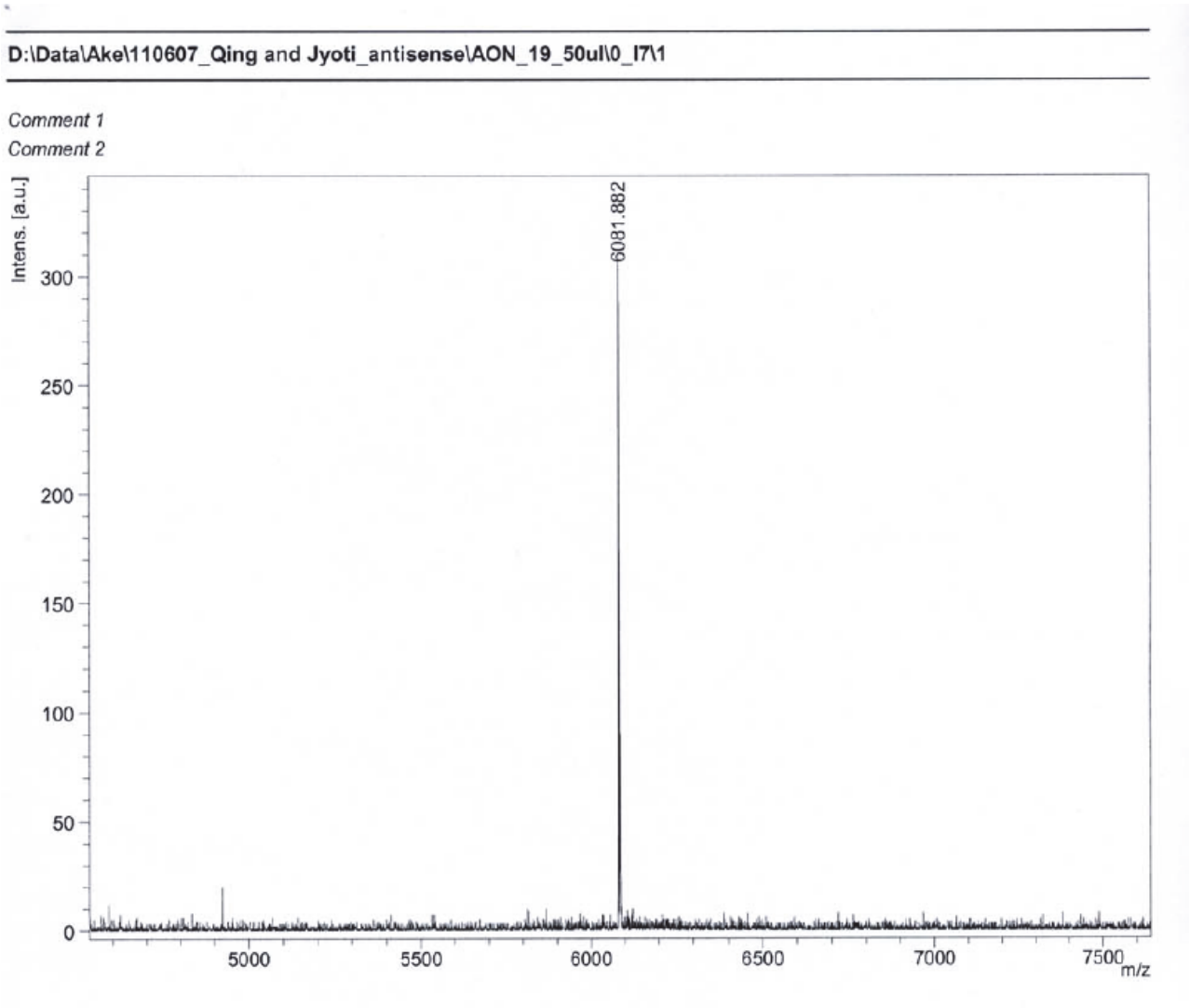


Figure SII.44 MALDI-TOF spectrum of AON19

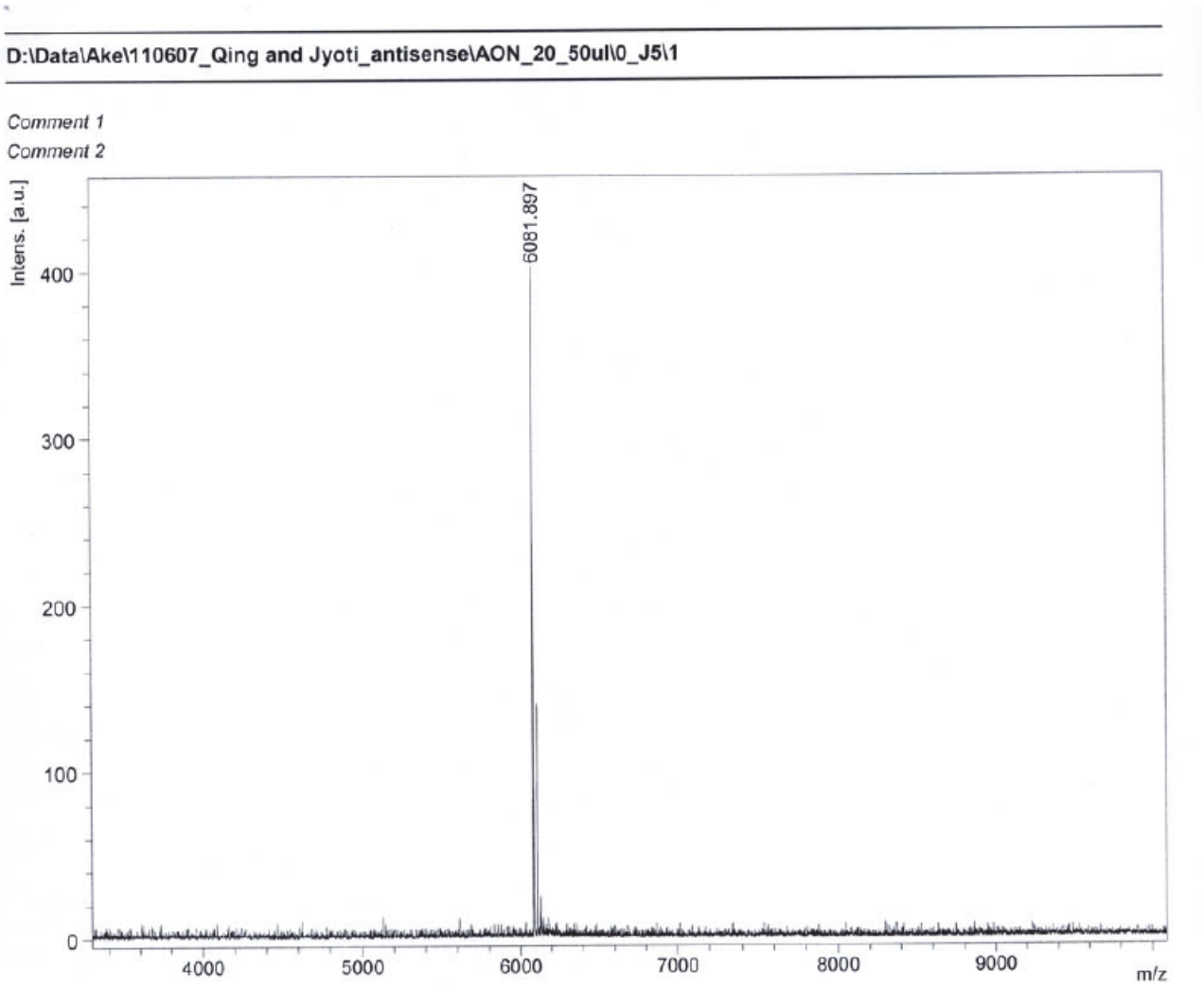


Figure SII.45 MALDI-TOF spectrum of AON20

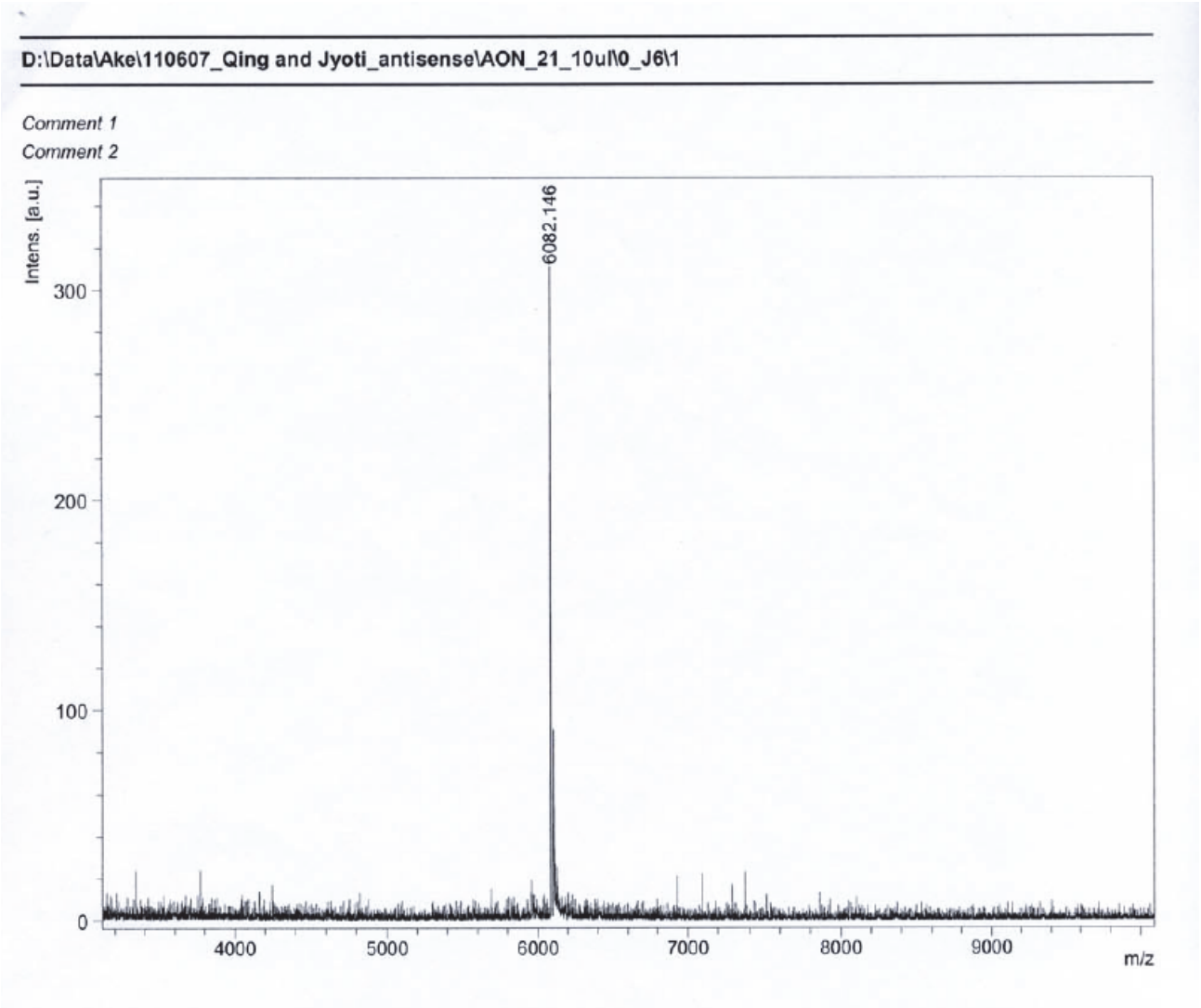


Figure SII.46 MALDI-TOF spectrum of AON21

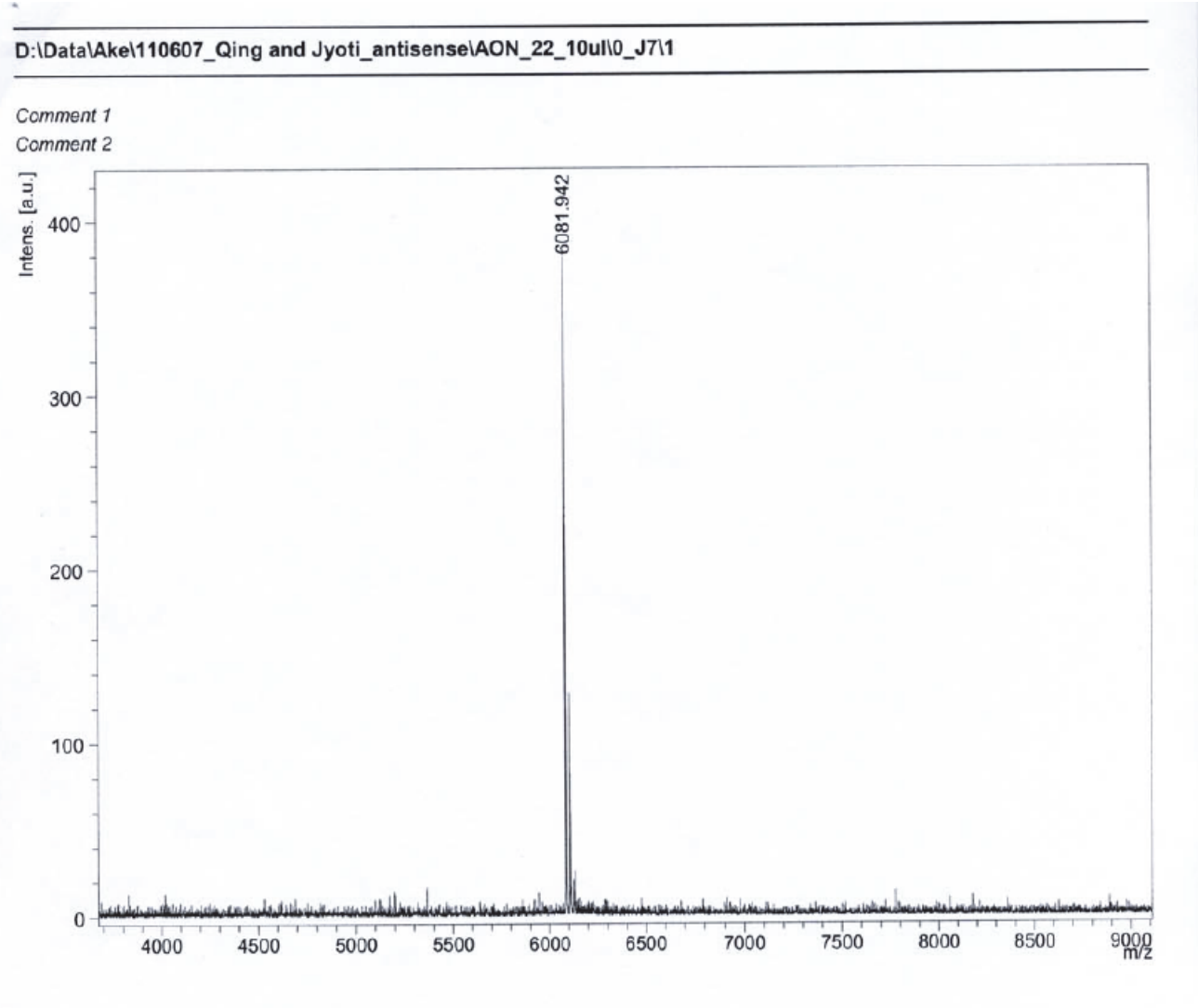


Figure SII.47 MALDI-TOF spectrum of AON22

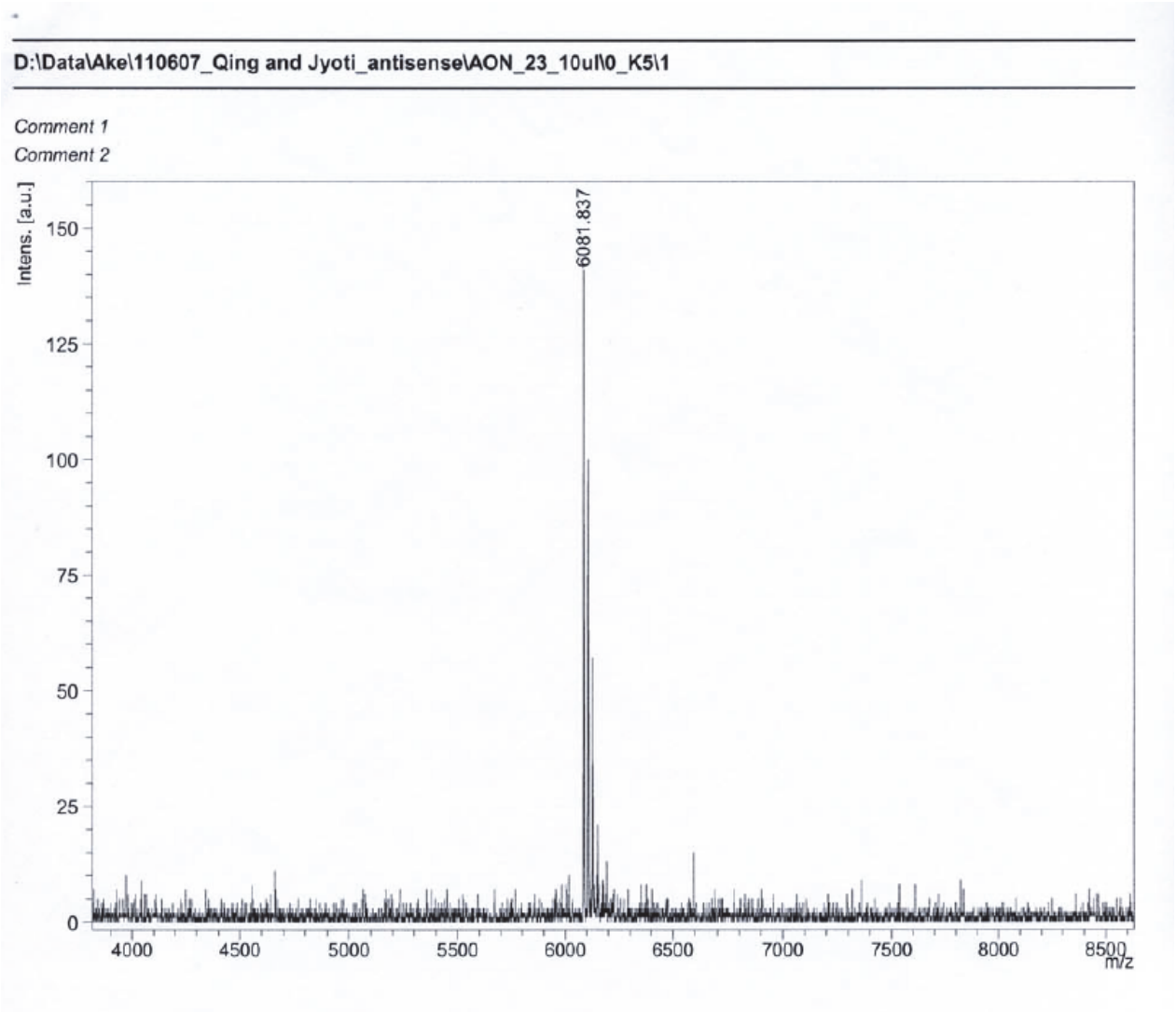


Figure SII.48 MALDI-TOF spectrum of AON23

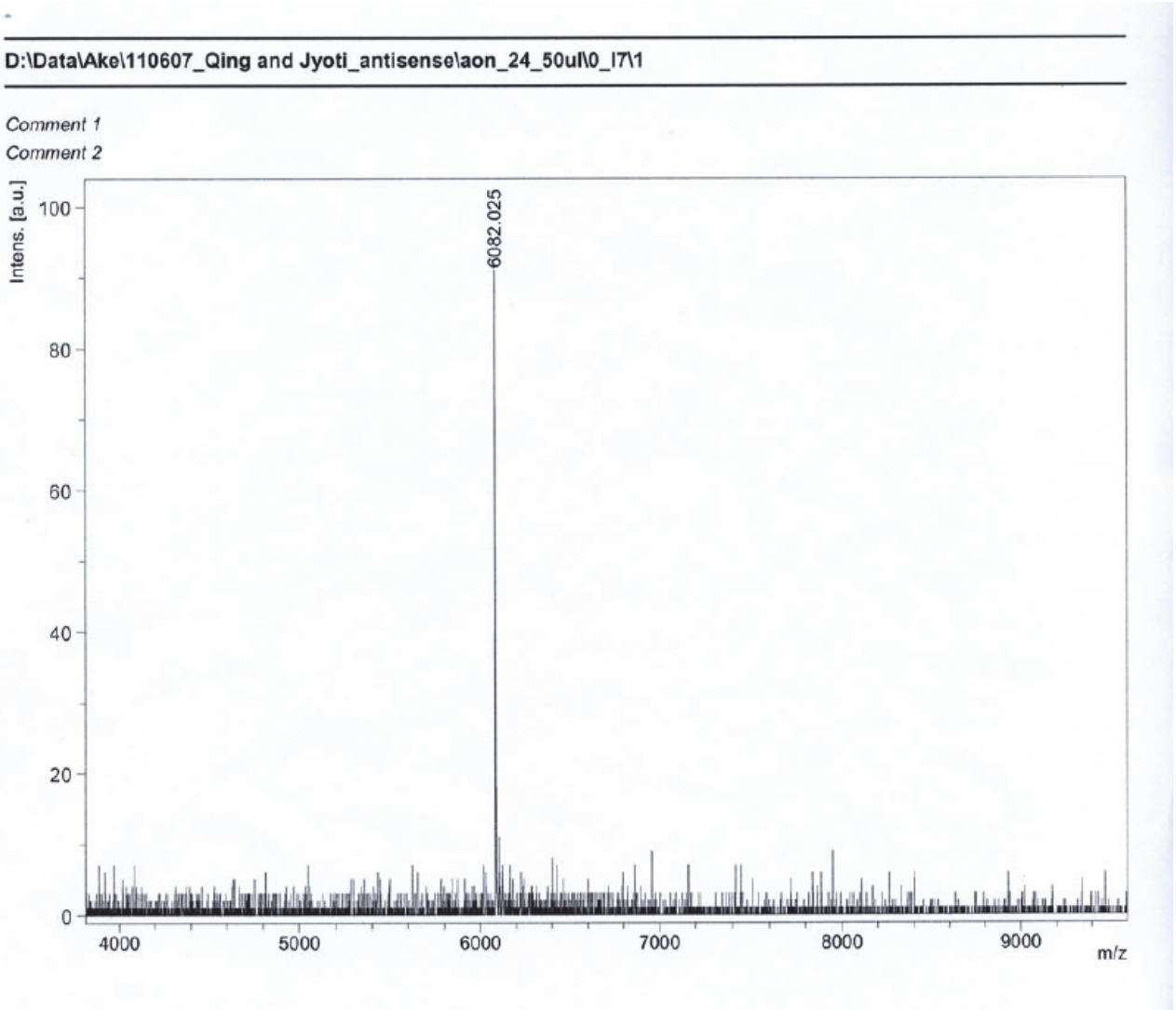


Figure SII.49 MALDI-TOF spectrum of AON24

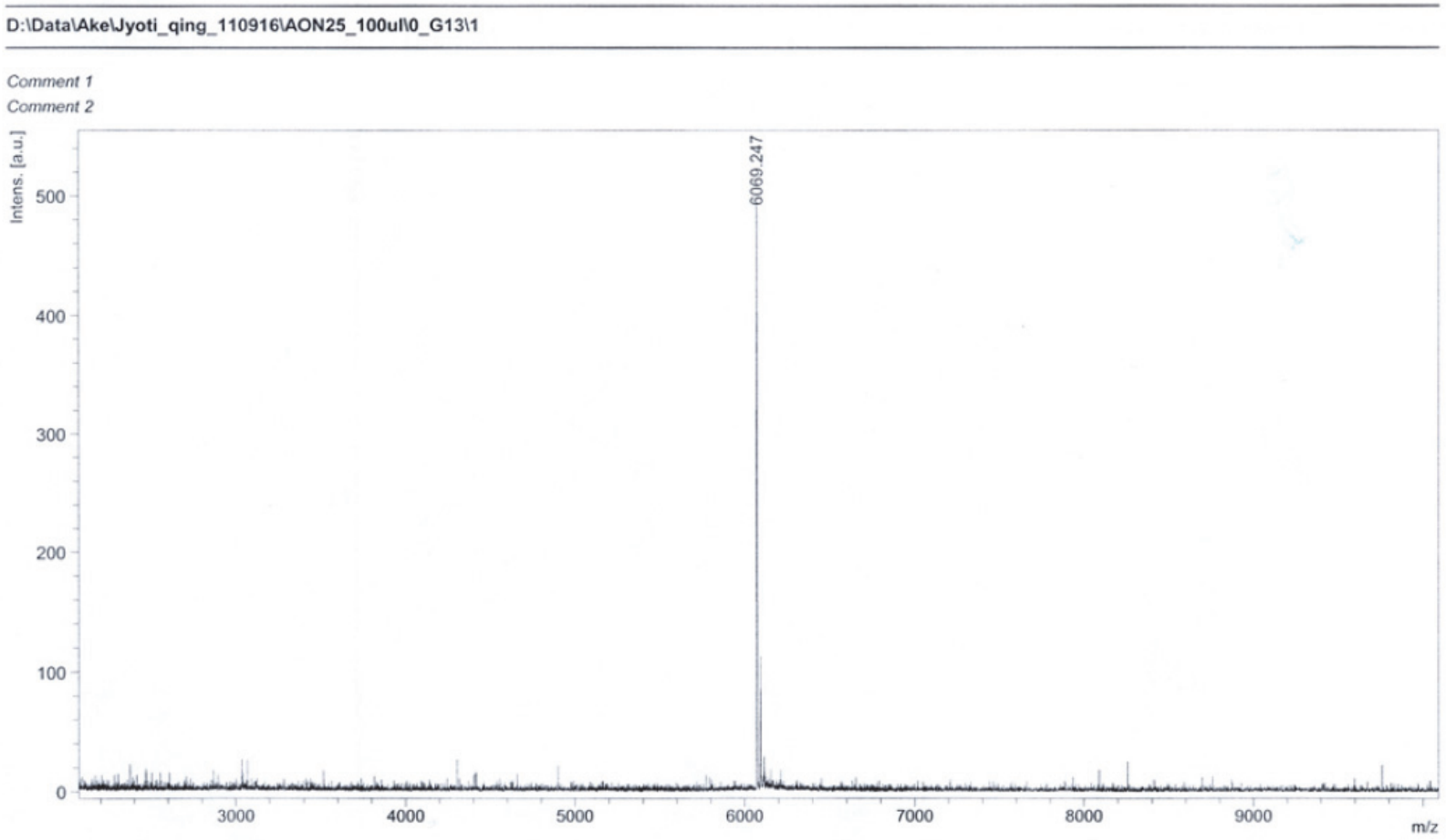


Figure SII.50 MALDI-TOF spectrum of AON28

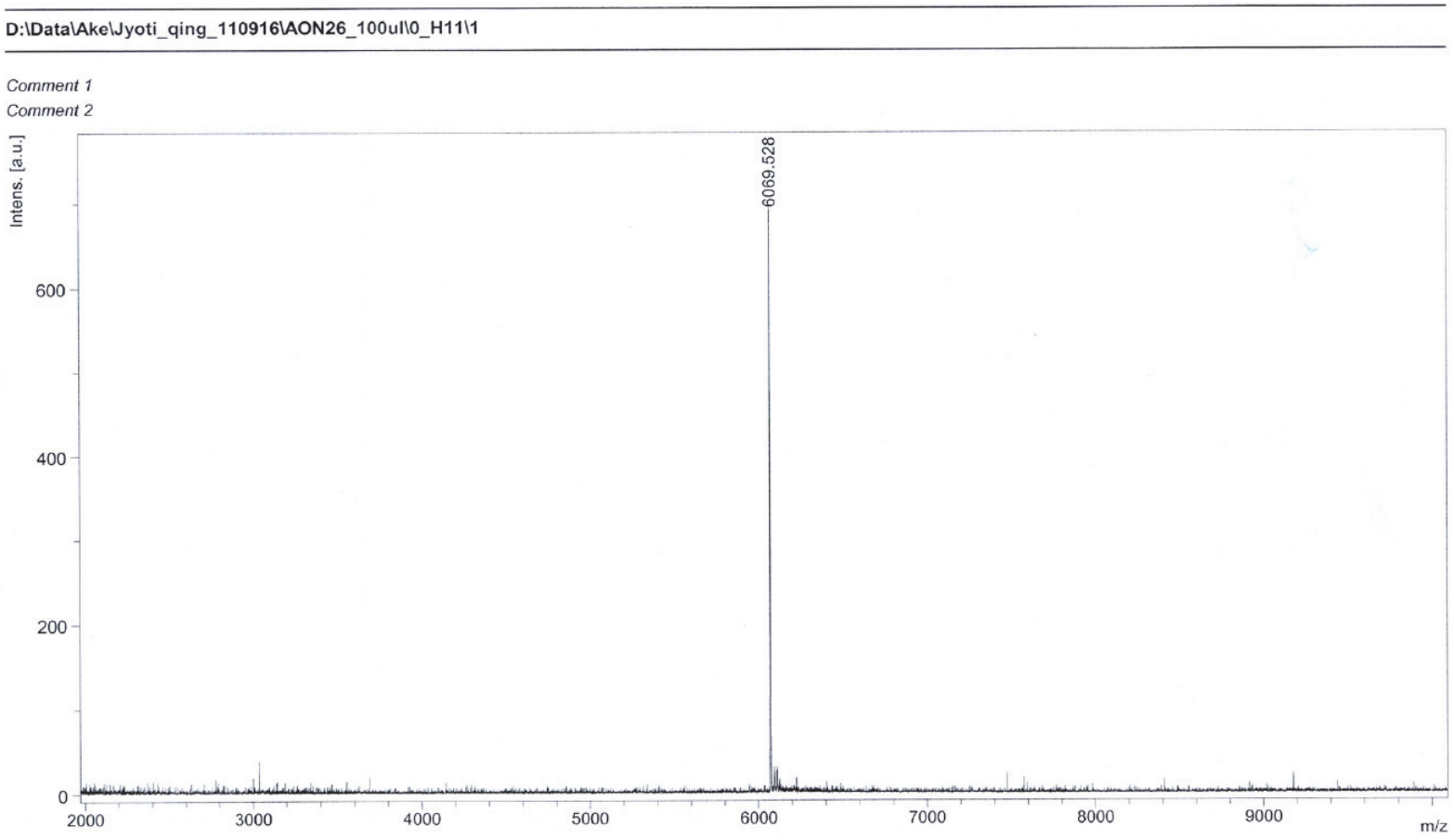


Figure SII.51 MALDI-TOF spectrum of AON29

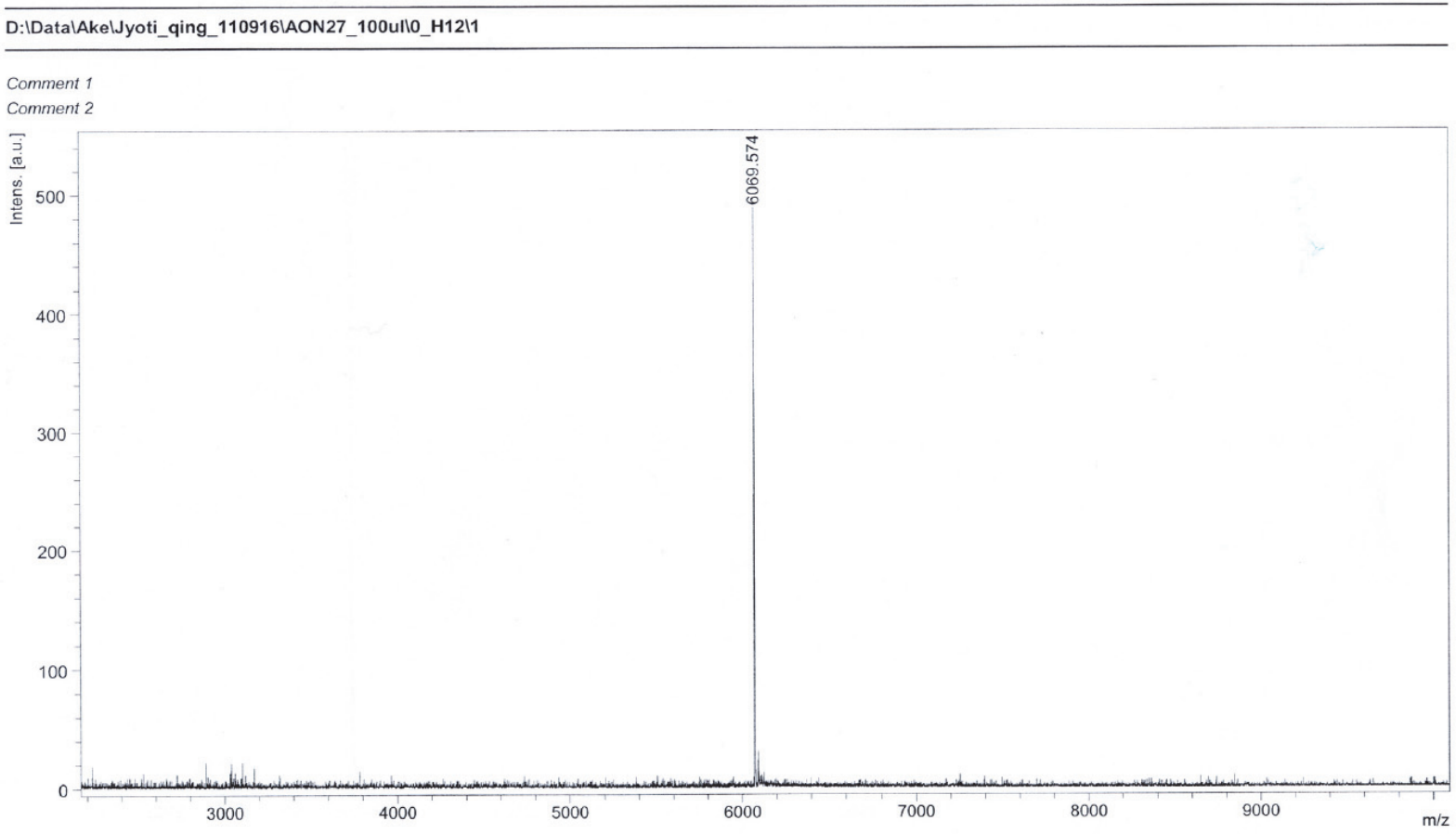


Figure SII.52 MALDI-TOF spectrum of AON30

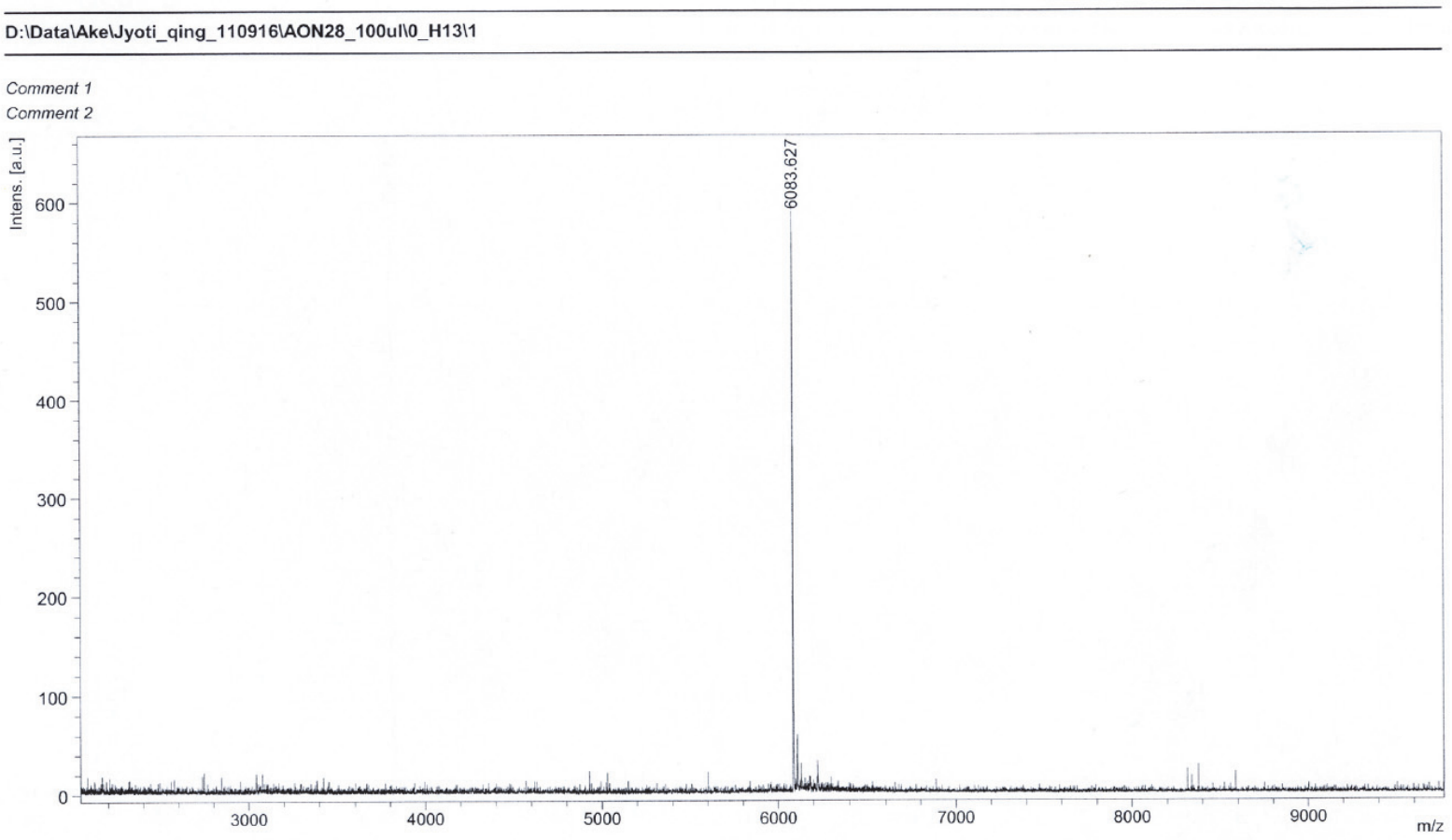


Figure SII.53 MALDI-TOF spectrum of AON31

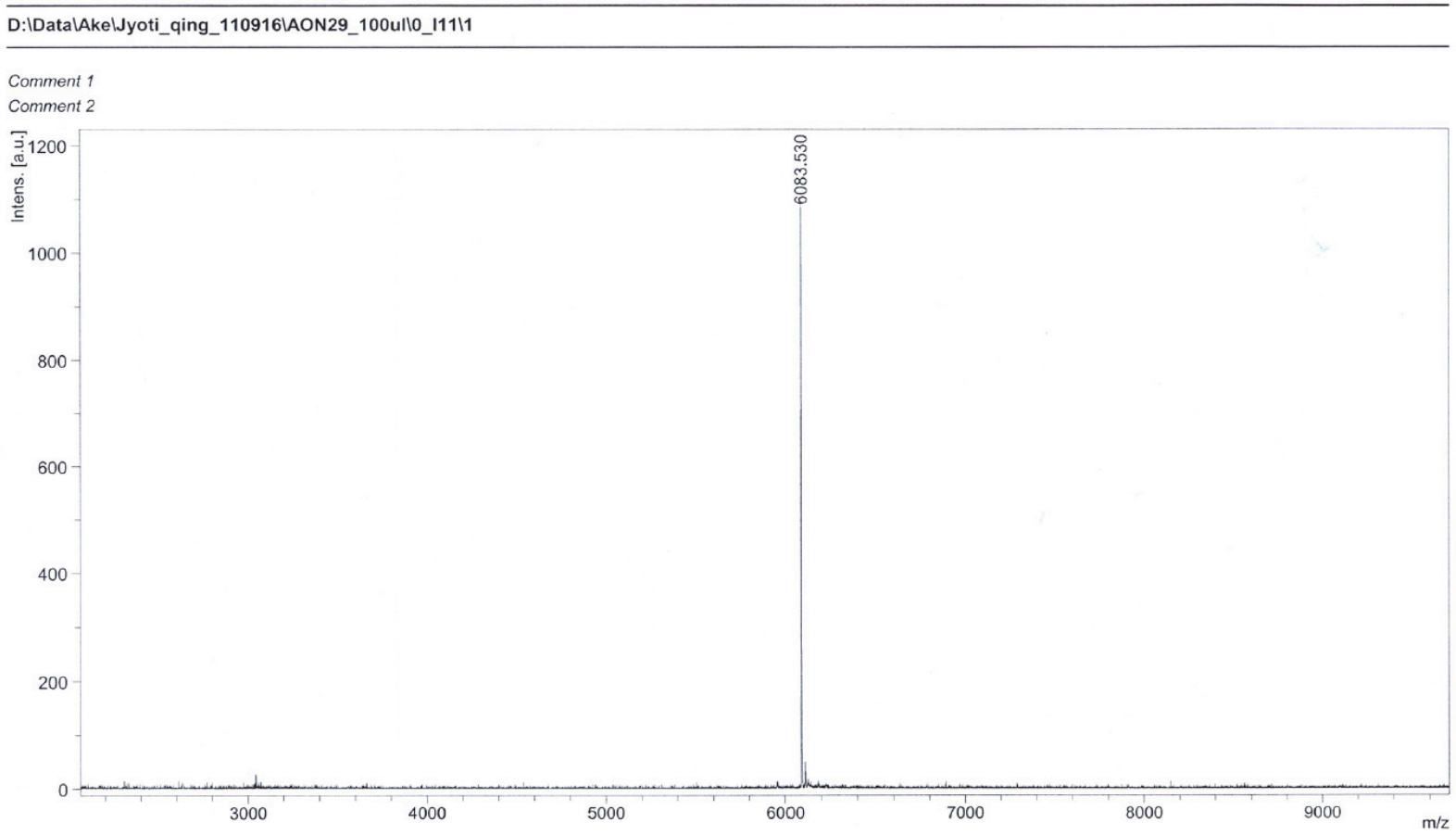


Figure SII.54 MALDI-TOF spectrum of AON32

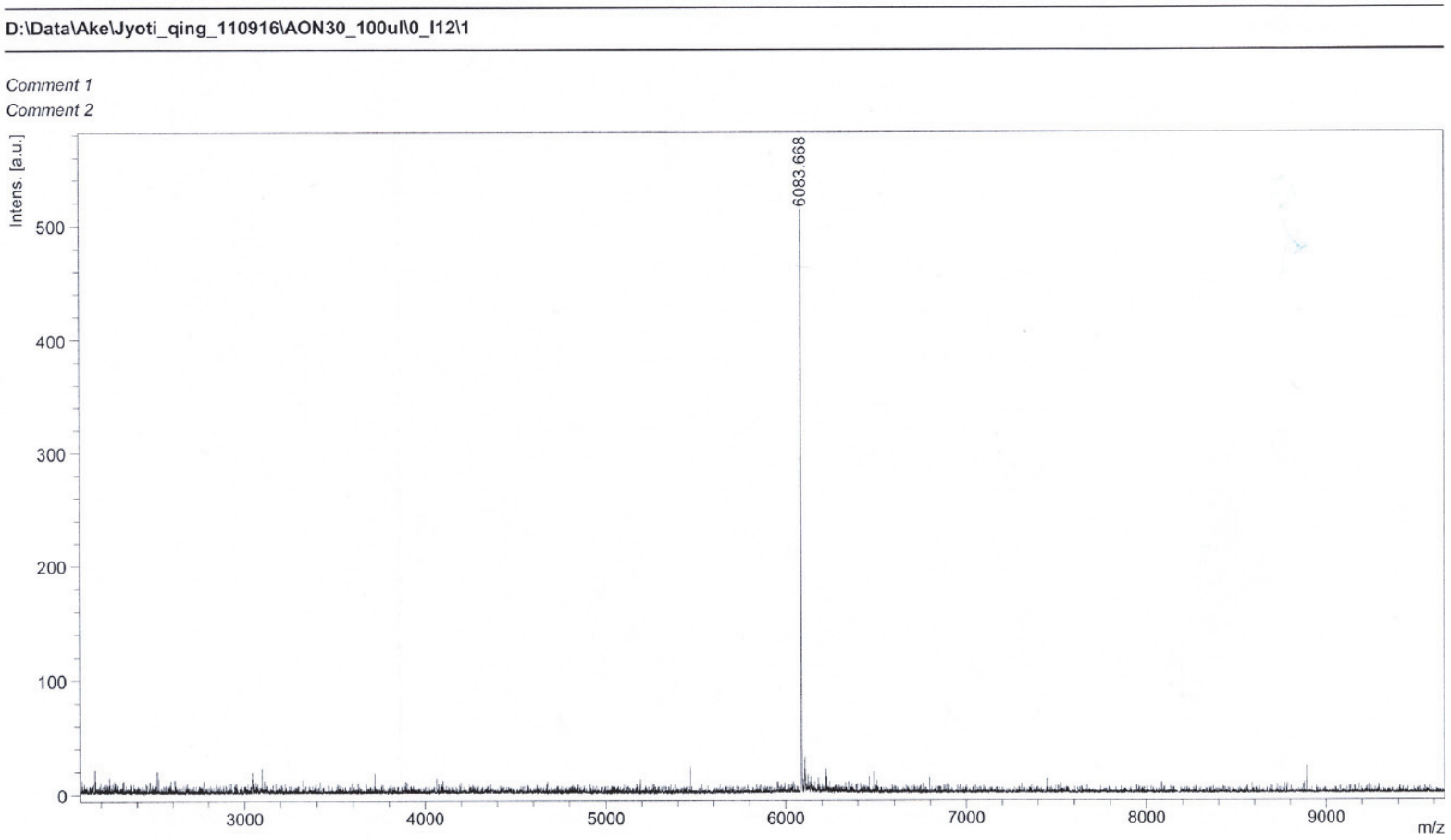


Figure SII.55 MALDI-TOF spectrum of AON33

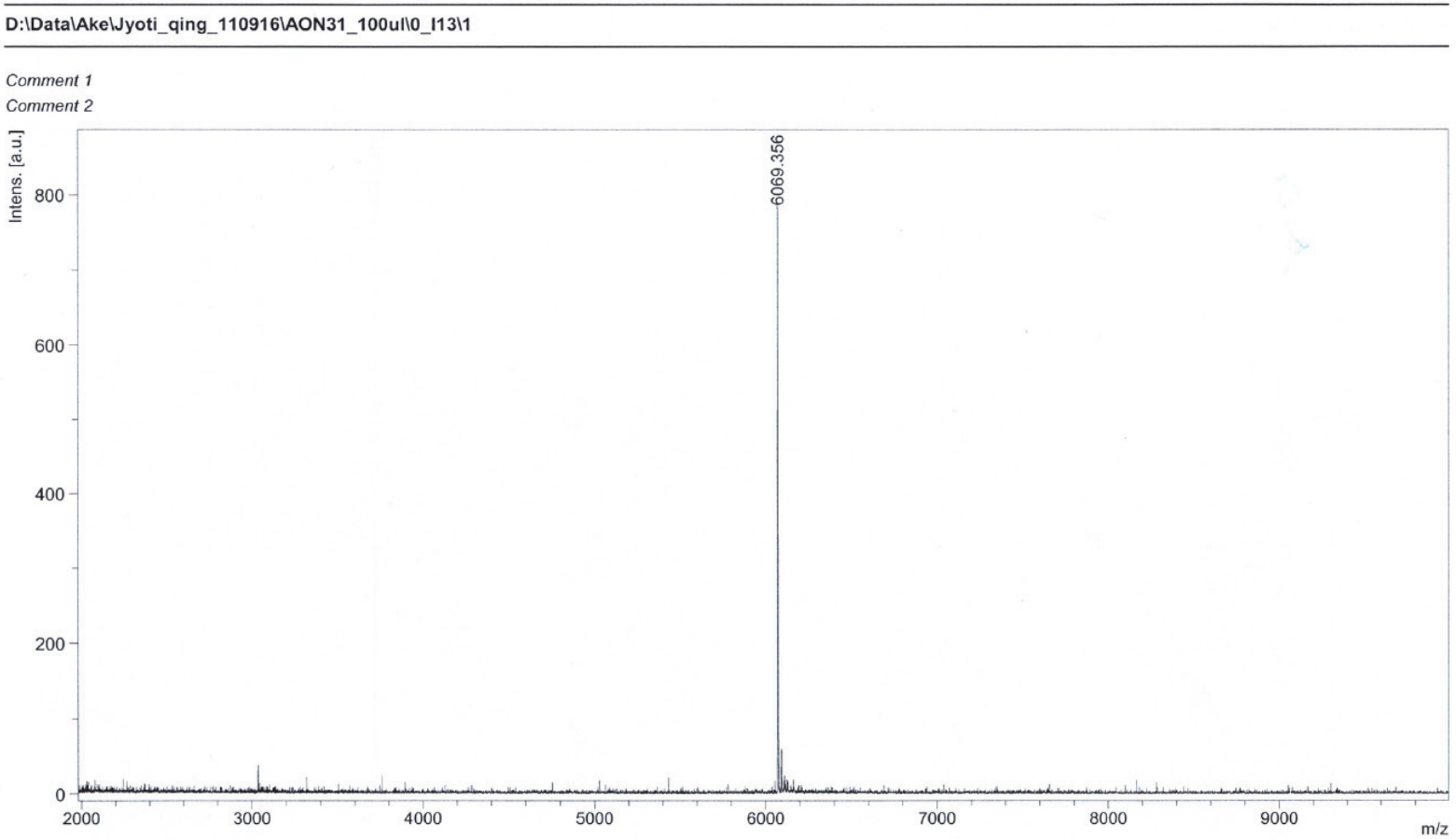


Figure SII.56 MALDI-TOF spectrum of AON34

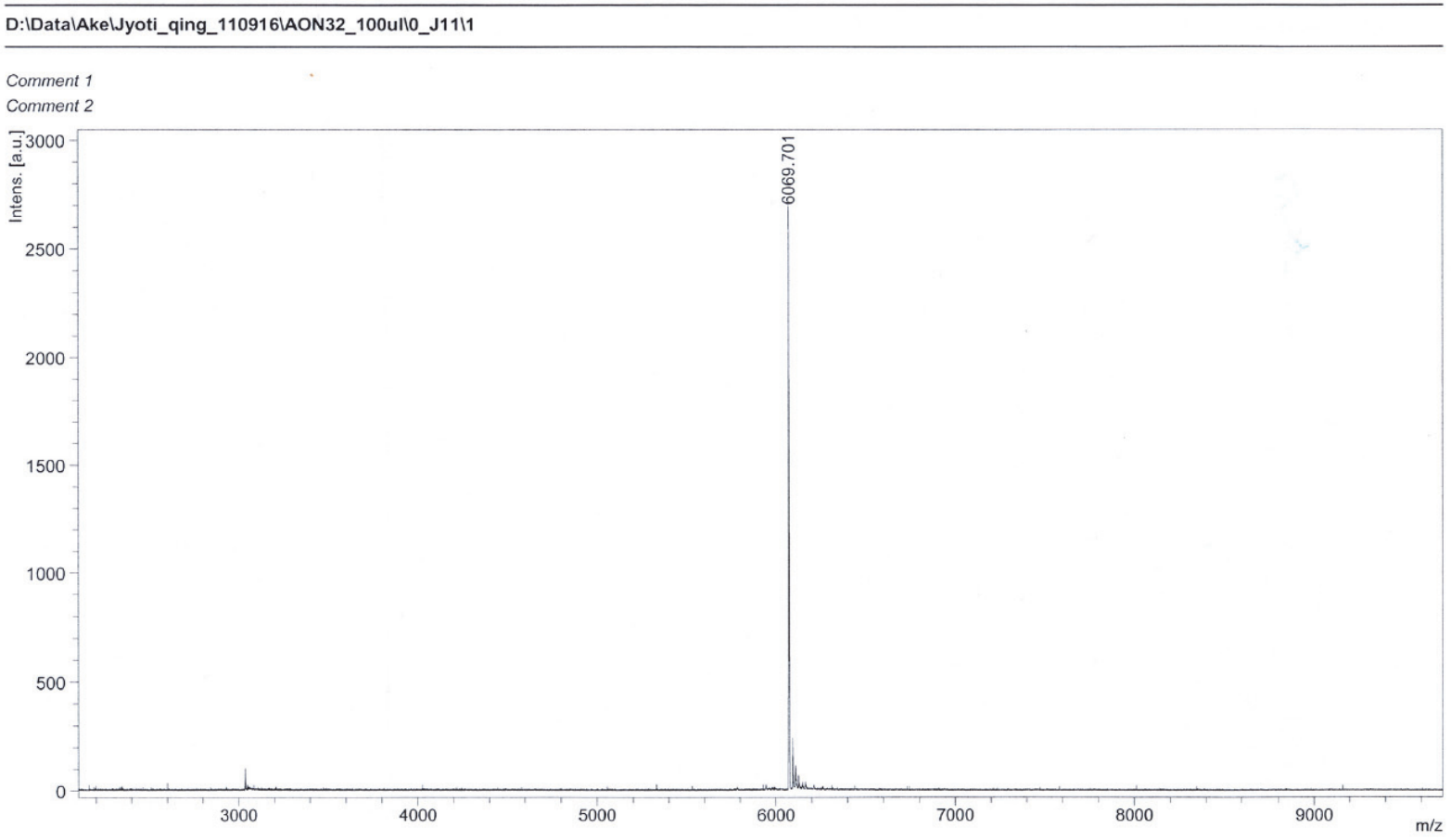


Figure SII.57 MALDI-TOF spectrum of AON35

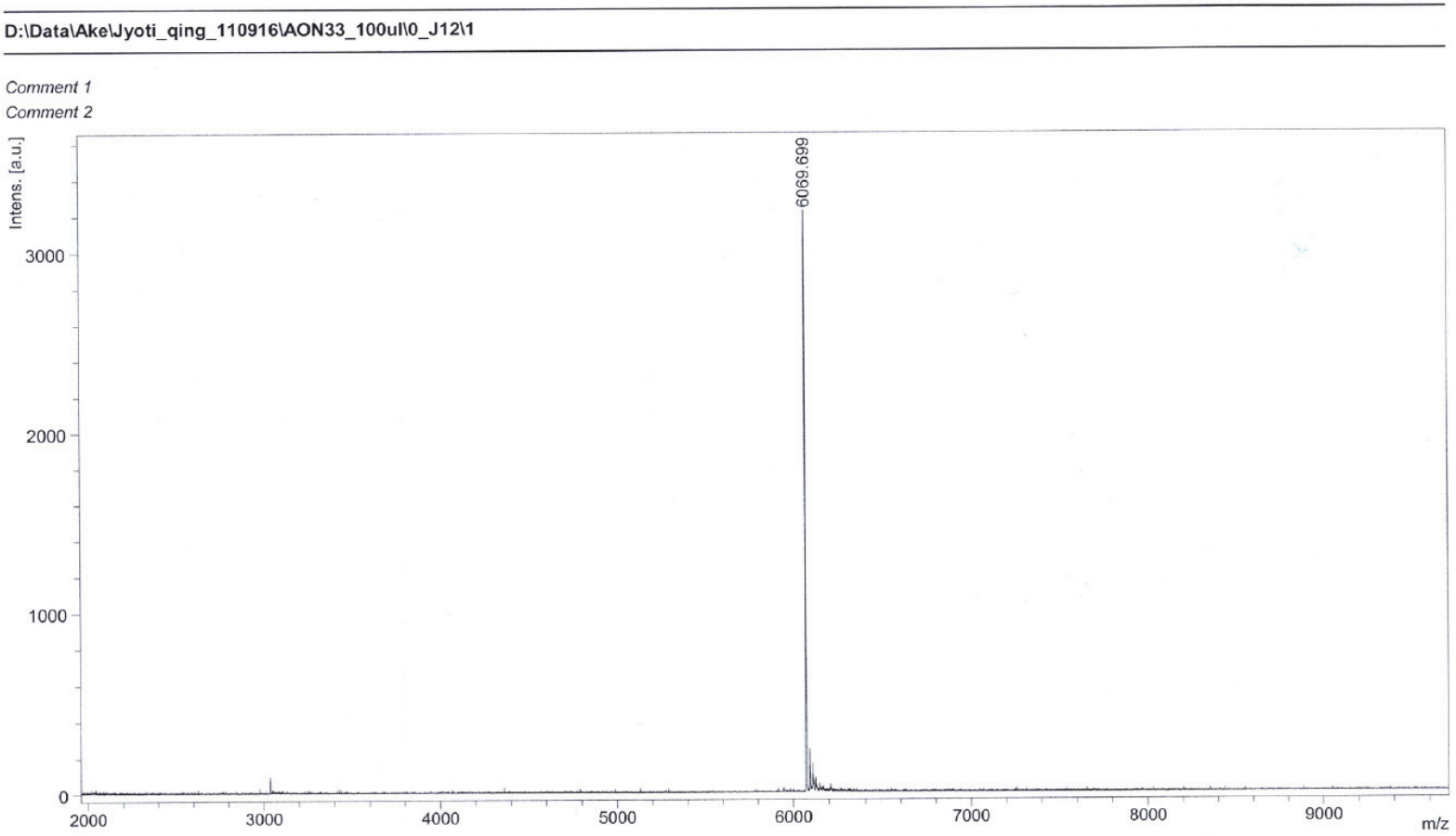


Figure SII.58 MALDI-TOF spectrum of AON36

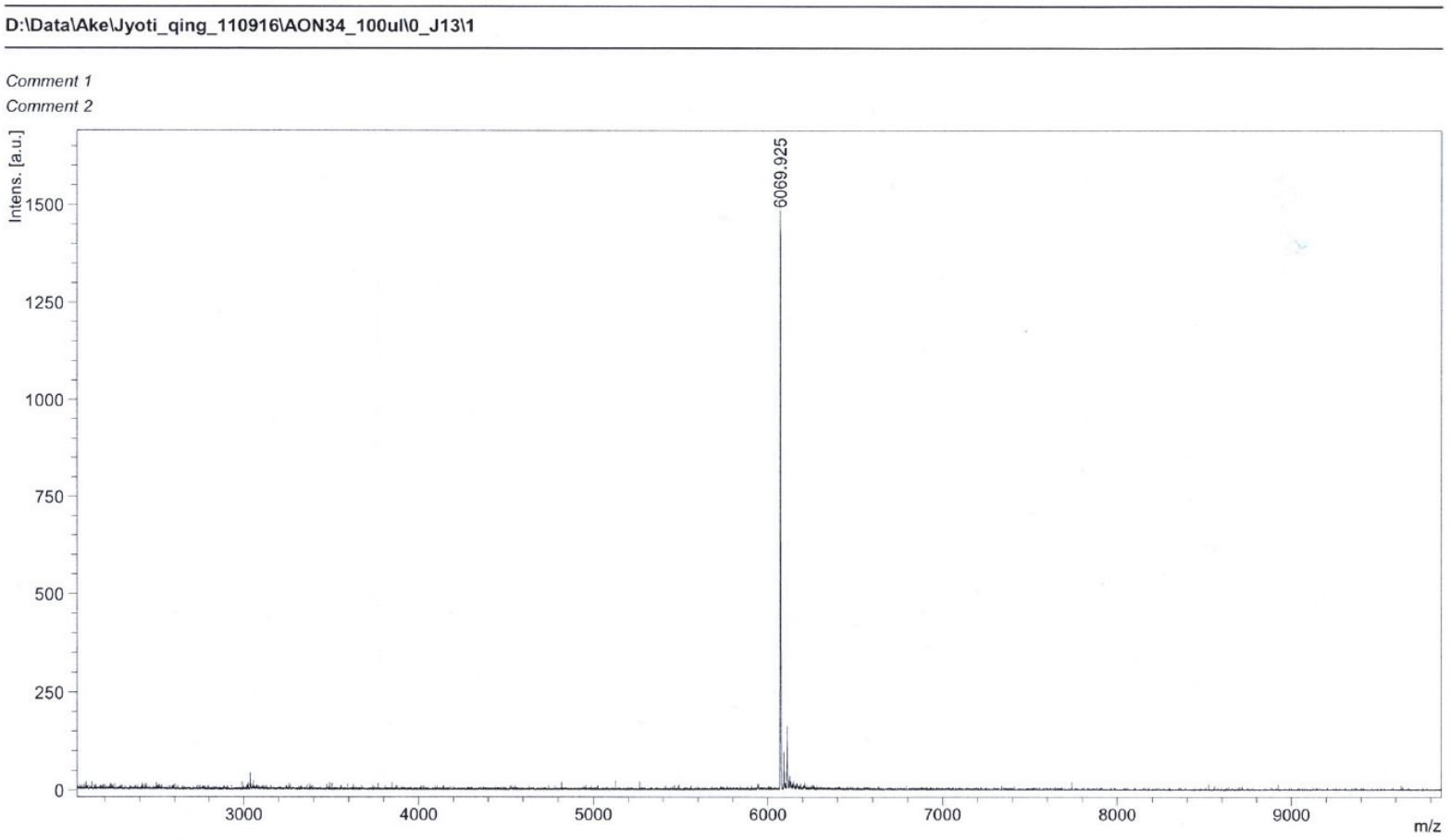


Figure SII.59 MALDI-TOF spectrum of AON25

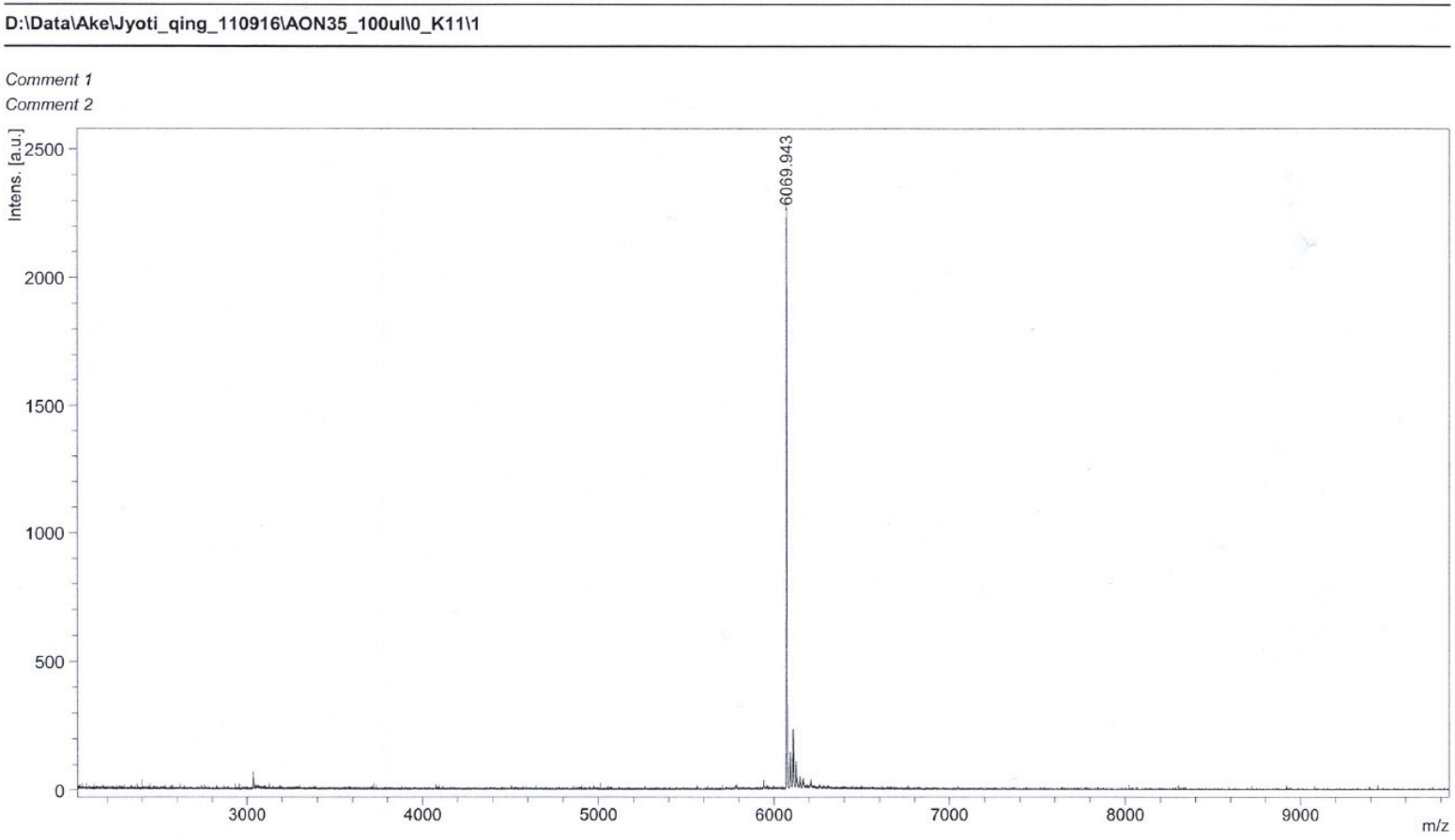


Figure SII.60 MALDI-TOF spectrum of AON26

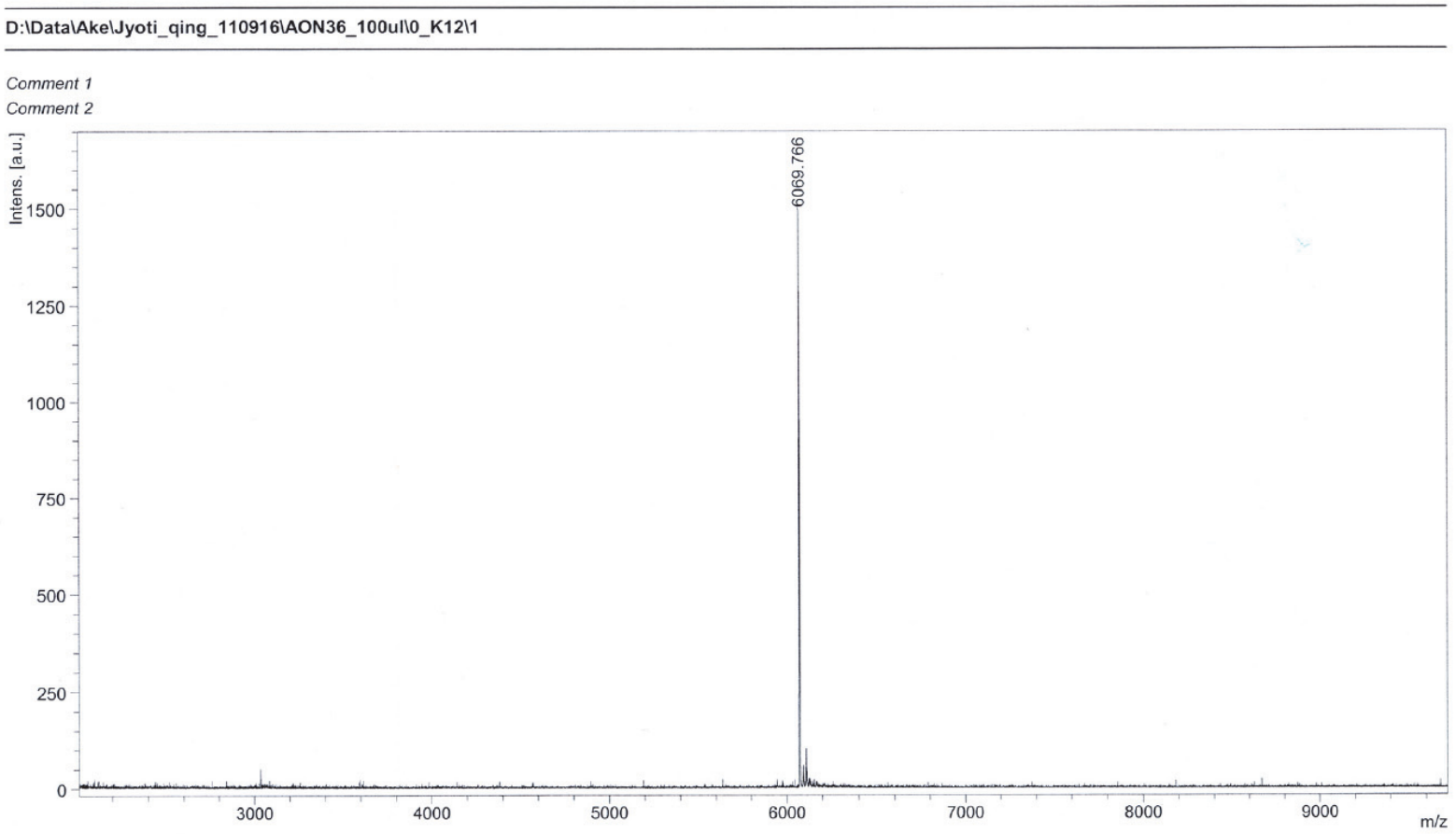


Figure SII.61 MALDI-TOF spectrum of AON27

Table SII.1. Extend of E. coli RNase HI promoted cleavage at 60 minutes of the AON/RNA hybrid duplexes shown as products proportion (%). Cells marked in blue highlight the extend of the cleavage for 6 nt sites (modification protected sequence) from position of modification in 5′-direction of AON. Cells marked in red highlight major changes occurring outside of modification protected sequence.

		Target RNA	5′-r	A	A	U	G	C	A	U	G	U	C
		Position		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
AON sequence	**Modification**	**AON**		**Product proportion (%)**									
3′d (TT ACG TAC AGT GTC CGC CCT)-5′		Native		0.2	0.0	0.3	1.0	1.0	2.3	1.4	12.9	2.2	4.2
3′d (TT **A3**CG TAC AGT GTC CGC CCT)-5′	*7′R*-Me-cLNA-A	AON1		0.6	0.3	**0.5**	**1.3**	**1.4**	**3.0**	**1.2**	**1.7**	2.0	6.2
	7′S-cLNA-A	AON4		0.8	0.6	**0.5**	**1.1**	**1.1**	**2.0**	**1.0**	**1.2**	1.8	5.4
	LNA-A	AON25		1.2	0.3	**0.4**	**1.0**	**2.7**	**1.8**	**1.1**	**2.1**	1.7	4.1
3′d (TT ACG T**A7**C AGT GTC CGC CCT)-5′	*7′R*-cLNA-A	AON2		0.0	0.0	0.0	0.0	0.0	1.0	**0.2**	**2.0**	**1.3**	**2.8**
	7′S-cLNA-A	AON5		0.8	0.6	0.6	0.9	1.1	2.1	**0.9**	**3.0**	**1.7**	**3.6**
	LNA-A	AON26		0.5	0.0	0.1	0.4	0.8	2.2	**0.5**	**4.2**	**1.0**	**3.1**
3′d (TT ACG TAC **A9**GT GTC CGC CCT)-5′	*7′R*-cLNA-A	AON3		1.0	0.6	1.3	0.7	1.0	3.1	2.4	30.4	**3.5**	**2.9**
	7′S-cLNA-A	AON6		1.4	0.7	1.6	0.8	1.0	3.0	2.8	32.7	**4.5**	**3.1**
	LNA-A	AON27		0.2	0.1	1.1	0.4	0.8	1.5	3.3	55.6	**6.2**	**2.4**
3′d (TT AC**G5** TAC AGT GTC CGC CCT)-5′	*7′R*-cLNA-G	AON7		1.5	0.8	0.6	1.2	**1.1**	**2.0**	**0.9**	**2.2**	**1.9**	**3.3**
	7′S-cLNA-G	AON10		0.9	0.4	0.5	1.2	**1.0**	**2.3**	**0.8**	**1.7**	**1.8**	**3.9**
	LNA-G	AON28		1.3	0.6	0.7	1.4	**1.4**	**2.5**	**0.9**	**2.0**	**1.8**	**3.5**
3′d (TT ACG TAC A**G10**T GTC CGC CCT)-5′	*7′R*-cLNA-G	AON8		1.2	0.6	1.4	0.8	1.0	1.9	3.7	45.5	4.7	**3.3**
	7′S-cLNA-G	AON11		0.7	0.4	1.1	0.6	0.8	1.4	3.8	45.0	3.0	**3.1**
	LNA-G	AON29		1.3	0.6	1.7	0.9	1.0	1.8	4.3	45.8	4.5	**3.4**
3′d (TT ACG TAC AGT GTC C**G16**C CCT)-5′	*7′R*-cLNA-G	AON9		0.8	0.5	1.2	1.9	1.7	3.8	3.9	46.8	4.8	5.3
	7′S-cLNA-G	AON12		0.7	0.5	1.4	2.2	2.2	4.1	5.2	61.9	4.7	2.7
	LNA-G	AON30		1.1	0.5	1.4	2.1	2.1	4.3	4.1	49.1	4.4	4.5

Table SII.1. (continue)

		Target RNA	5′-r	A	A	U	G	C	A	U	G	U	C
		Position		1	2	3	4	5	6	7	8	9	10
AON sequence	Modification	AON		Product proportion (%)									
3′d (TT A**C4**G TAC AGT GTC CGC CCT)-5′	*7′R*-cLNA-C	AON13		0.8	0.4	0.3	**0.9**	**0.8**	**1.6**	**0.9**	**0.9**	**1.5**	**3.2**
	7′S-cLNA-C	AON16		1.0	0.6	0.5	**1.1**	**1.1**	**1.8**	**1.2**	**1.3**	**1.9**	**4.2**
	LNA-C	AON31		1.2	0.5	0.5	**1.4**	**1.4**	**2.3**	**1.0**	**1.1**	**1.7**	**3.2**
3′d (TT ACG TA**C8** AGT GTC CGC CCT)-5′	*7′R*-cLNA-C	AON14		0.9	0.4	0.5	0.6	0.7	2.4	1.2	**6.7**	**2.4**	**2.6**
	7′S-cLNA-C	AON17		1.0	0.6	0.7	0.8	0.9	3.7	1.8	**11.0**	**3.2**	**3.4**
	LNA-C	AON32		1.3	0.5	0.7	0.7	0.8	3.5	1.4	**10.9**	**1.8**	**2.9**
3′d (TT ACG TAC AGT GTC **C15**GC CCT)-5′	*7′R*-cLNA-C	AON15		0.7	0.4	0.9	1.6	1.4	2.7	3.4	33.5	4.2	4.9
	7′S-cLNA-C	AON18		1.1	0.6	1.2	2.1	1.8	3.8	4.8	50.4	5.0	5.2
	LNA-C	AON33		0.5	0.4	0.8	2.0	1.8	3.7	3.4	40.5	4.3	4.7
3′d (TT ACG **T6**AC AGT GTC CGC CCT)-5′	*7′R*-cLNA-T	AON19		0.3	0.4	0.5	0.9	1.0	**1.9**	**1.0**	**2.8**	**2.3**	**4.0**
	7′S-cLNA-T	AON22		0.5	0.7	0.4	1.0	1.1	**2.1**	**1.0**	**1.4**	**2.3**	**3.5**
	LNA-T	AON34		0.7	0.2	0.3	0.9	1.0	**1.7**	**0.5**	**1.5**	**1.3**	**3.3**
3′d (TT ACG TAC AG**T11** GTC CGC CCT)-5′	*7′R*-cLNA-T	AON20		0.3	0.5	1.1	0.7	0.9	1.7	4.9	50.1	5.4	4.5
	7′S-cLNA-T	AON23		0.5	0.7	1.1	0.8	1.1	2.7	4.9	39.3	4.9	5.2
	LNA-T	AON35		0.8	0.2	1.0	0.5	0.6	1.3	4.7	43.1	3.5	4.1
3′d (TT ACG TAC AGT G**T13**C CGC CCT)-5′	*7′R*-cLNA-T	AON21		0.8	0.5	1.1	3.3	1.0	2.3	4.9	45.9	5.7	5.3
	7′S-cLNA-T	AON24		0.6	0.7	1.3	3.8	1.4	2.4	5.4	43.2	5.6	4.8
	LNA-T	AON36		0.7	0.2	1.5	4.1	1.3	1.2	4.2	49.5	3.7	4.4

Table SII.1 (continue)

		Target RNA	5′-r	C	A	G	G	C	G	G	G	A	C
		Position		**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
AON sequence	**Modification**	**AON**		**Product proportion (%)**									
3′d (TT ACG TAC AGT GTC CGC CCT)-5′		Native		7.7	1.8	6.0	4.0	17.4	3.3	2.3	3.0	3.7	25.2
3′d (TT **A3**CG TAC AGT GTC CGC CCT)-5′	*7′R*-cLNA-A	AON1		12.5	2.6	5.5	4.3	17.7	3.7	2.9	3.3	3.8	25.4
	7′S-cLNA-A	AON4		8.8	2.6	4.5	4.1	17.0	3.6	2.7	3.2	4.1	33.9
	LNA-A	AON25		10.2	3.9	4.5	4.4	16.4	4.1	2.9	3.1	4.5	29.5
3′d (TT ACG T**A7**C AGT GTC CGC CCT)-5′	*7′R*-cLNA-A	AON2		**2.5**	**1.6**	3.0	5.4	29.8	4.3	3.1	3.4	4.2	35.4
	7′S-cLNA-A	AON5		**2.1**	**2.3**	3.2	5.4	20.5	3.7	3.0	3.6	4.9	36.3
	LNA-A	AON26		**2.2**	**1.7**	3.1	6.1	26.3	5.3	2.9	3.3	4.3	32.2
3′d (TT ACG TAC **A9**GT GTC CGC CCT)-5′	*7′R*-cLNA-A	AON3		**1.8**	**1.4**	**1.9**	**2.7**	**10.7**	4.7	4.1	3.3	3.4	19.1
	7′S-cLNA-A	AON6		**1.8**	**1.4**	**1.7**	**2.4**	**11.5**	4.9	4.1	3.1	3.0	14.5
	LNA-A	AON27		**1.5**	**1.0**	**1.2**	**1.8**	**6.7**	5.2	3.9	2.1	2.1	2.9
3′d (TT AC**G5** TAC AGT GTC CGC CCT)-5′	*7′R*-cLNA-G	AON7		**3.4**	2.6	4.8	4.4	17.5	4.4	3.4	3.9	4.6	35.6
	7′S-cLNA-G	AON10		**3.4**	2.3	5.0	4.7	21.7	4.2	2.7	3.1	3.9	34.5
	LNA-G	AON28		**3.6**	2.1	5.4	4.8	21.6	3.6	2.8	3.2	3.6	33.3
3′d (TT ACG TAC A**G10**T GTC CGC CCT)-5′	*7′R*-cLNA-G	AON8		**7.0**	**1.8**	**1.7**	**1.8**	**4.9**	**2.6**	3.6	3.0	3.0	6.7
	7′S-cLNA-G	AON11		**11.4**	**1.3**	**1.4**	**1.3**	**5.4**	**1.7**	2.3	1.9	1.9	11.5
	LNA-G	AON29		**6.1**	**1.3**	**1.5**	**1.9**	**5.1**	**2.1**	3.2	2.5	2.5	8.5
3′d (TT ACG TAC AGT GTC C**G16**C CCT)-5′	*7′R*-cLNA-G	AON9		9.0	2.2	4.0	2.3	5.2	**1.6**	**1.0**	**0.8**	**0.8**	2.3
	7′S-cLNA-G	AON12		2.5	2.0	2.0	1.7	2.1	**1.3**	**0.8**	**0.6**	**0.5**	0.9
	LNA-G	AON30		8.9	1.6	3.6	1.8	5.2	**1.3**	**0.9**	**0.7**	**0.8**	1.6
3′d (TT A**C4**G TAC AGT GTC CGC CCT)-5′	*7′R*-cLNA-C	AON13		9.0	3.6	6.4	5.2	20.1	4.5	2.9	2.9	3.8	30.2
	7′S-cLNA-C	AON16		11.1	4.3	8.0	6.3	20.3	4.3	2.9	2.7	3.7	21.6
	LNA-C	AON31		8.4	2.2	6.6	3.9	21.4	3.2	2.4	2.9	3.4	31.4

Table SII.1 (continue)

		Target RNA	5′-r	C	A	G	G	C	G	G	G	A	C
		Position		**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
AON sequence	**Modification**	**AON**		**Product proportion (%)**									
3′d (TT ACG TA**C8** AGT GTC CGC CCT)-5′	*7′R*-cLNA-C	AON14		**2.5**	**2.0**	**2.4**	3.8	24.7	6.7	3.6	3.4	3.7	28.7
	7′S-cLNA-C	AON17		**2.1**	**1.8**	**2.4**	5.2	23.9	4.7	3.5	3.2	3.3	22.8
	LNA-C	AON32		**2.3**	**1.4**	**1.8**	2.4	23.6	4.0	3.3	2.8	3.6	30.3
3′d (TT ACG TAC AGT GTC **C15**GC CCT)-5′	*7′R*-cLNA-C	AON15		10.2	3.8	5.9	3.9	**8.4**	**3.0**	**1.7**	**1.6**	**1.9**	5.8
	7′S-cLNA-C	AON18		6.2	2.5	3.1	2.2	**3.3**	**1.7**	**1.0**	**0.9**	**0.9**	2.2
	LNA-C	AON33		11.2	2.0	5.7	2.6	**9.0**	**1.5**	**0.9**	**0.9**	**1.6**	2.5
3′d (TT ACG **T6**AC AGT GTC CGC CCT)-5′	*7′R*-cLNA-T	AON19		**3.6**	3.3	6.9	6.7	22.7	5.3	3.9	3.9	4.7	23.7
	7′S-cLNA-T	AON22		**3.2**	3.4	6.7	6.0	16.8	5.1	4.3	4.7	5.5	30.3
	LNA-T	AON34		**2.0**	1.8	6.0	6.2	24.9	3.6	2.6	3.3	4.2	34.0
3′d (TT ACG TAC AG**T11** GTC CGC CCT)-5′	*7′R*-cLNA-T	AON20		**6.5**	**2.6**	**1.8**	**2.1**	**4.8**	**2.0**	1.9	2.1	1.9	4.3
	7′S-cLNA-T	AON23		**8.6**	**2.3**	**1.9**	**2.4**	**4.8**	**1.8**	2.1	2.9	3.2	8.9
	LNA-T	AON35		**11.1**	**1.4**	**1.5**	**1.9**	**6.2**	**1.4**	1.5	2.4	3.0	10.0
3′d (TT ACG TAC AGT G**T13**C CGC CCT)-5′	*7′R*-cLNA-T	AON21		7.0	3.7	**4.6**	**2.2**	**2.6**	**1.1**	**0.9**	**1.1**	1.6	4.4
	7′S-cLNA-T	AON24		6.4	4.1	**4.9**	**2.5**	**2.8**	**1.5**	**1.2**	**1.3**	2.1	3.9
	LNA-T	AON36		7.6	1.7	**5.4**	**1.6**	**2.9**	**1.1**	**0.8**	**1.0**	1.7	5.5

Table SII.2. Average RNase H cleavage rate ($\mu M\ s^{-1}\ U^{-1}$) during first 60 minutes normalized to 1 unit (U) of enzyme (1 U catalyzes the hydrolysis of 1 nmol of RNA in radiolabeled poly(A)-poly(dT) to acid-soluble material in 20 min at 37°C) of the RNase H promoted cleavage of the AON/RNA.

	Position		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Modification		**5'-r**	**A**	**A**	**U**	**G**	**C**	**A**	**U**	**G**	**U**	**C**
	Native		0.0001	0.0000	0.0001	0.0003	0.0004	0.0008	0.0005	0.0045	0.0008	0.0015
***7'R*-cLNA-A**	**AON1**		0.0002	0.0001	0.0002	0.0005	0.0005	0.0010	0.0004	0.0006	0.0007	0.0021
***7'S*-cLNA-A**	**AON4**		0.0003	0.0002	0.0002	0.0004	0.0004	0.0007	0.0003	0.0004	0.0006	0.0019
LNA-A	**AON25**		0.0004	0.0001	0.0001	0.0004	0.0009	0.0006	0.0004	0.0007	0.0006	0.0014
***7'R*-cLNA-A**	**AON2**		0.0000	0.0000	0.0000	0.0000	0.0000	0.0003	0.0001	0.0007	0.0004	0.0010
***7'S*-cLNA-A**	**AON5**		0.0003	0.0002	0.0002	0.0003	0.0004	0.0007	0.0003	0.0010	0.0006	0.0012
LNA-A	**AON26**		0.0002	0.0000	0.0000	0.0001	0.0003	0.0007	0.0002	0.0014	0.0003	0.0011
***7'R*-cLNA-A**	**AON3**		0.0004	0.0002	0.0004	0.0003	0.0003	0.0011	0.0008	0.0106	0.0012	0.0010
***7'S*-cLNA-A**	**AON6**		0.0005	0.0002	0.0006	0.0003	0.0004	0.0010	0.0010	0.0114	0.0016	0.0011
LNA-A	**AON27**		0.0001	0.0000	0.0004	0.0001	0.0003	0.0005	0.0012	0.0193	0.0021	0.0008
***7'R*-cLNA-G**	**AON7**		0.0005	0.0003	0.0002	0.0004	0.0004	0.0007	0.0003	0.0007	0.0007	0.0012
***7'S*-cLNA-G**	**AON10**		0.0003	0.0002	0.0002	0.0004	0.0004	0.0008	0.0003	0.0006	0.0006	0.0013
LNA-G	**AON28**		0.0004	0.0002	0.0002	0.0005	0.0005	0.0009	0.0003	0.0007	0.0006	0.0012
***7'R*-cLNA-G**	**AON8**		0.0004	0.0002	0.0005	0.0003	0.0003	0.0007	0.0013	0.0158	0.0016	0.0012
***7'S*-cLNA-G**	**AON11**		0.0002	0.0001	0.0004	0.0002	0.0003	0.0005	0.0013	0.0156	0.0011	0.0011
LNA-G	**AON29**		0.0005	0.0002	0.0006	0.0003	0.0003	0.0006	0.0015	0.0159	0.0016	0.0012
***7'R*-cLNA-G**	**AON9**		0.0003	0.0002	0.0004	0.0007	0.0006	0.0013	0.0014	0.0162	0.0017	0.0019
***7'S*-cLNA-G**	**AON12**		0.0002	0.0002	0.0005	0.0008	0.0008	0.0014	0.0018	0.0215	0.0016	0.0009

Table SII.2 (continue)

	Position		1	2	3	4	5	6	7	8	9	10
Modification		**5'-r**	**A**	**A**	**U**	**G**	**C**	**A**	**U**	**G**	**U**	**C**
LNA-G	**AON30**		0.0004	0.0002	0.0005	0.0007	0.0007	0.0015	0.0014	0.0170	0.0015	0.0016
***7'R*-cLNA-C**	**AON13**		0.0003	0.0001	0.0001	0.0003	0.0003	0.0006	0.0003	0.0003	0.0005	0.0011
***7'S*-cLNA-C**	**AON16**		0.0003	0.0002	0.0002	0.0004	0.0004	0.0006	0.0004	0.0005	0.0007	0.0015
LNA-C	**AON31**		0.0004	0.0002	0.0002	0.0005	0.0005	0.0008	0.0003	0.0004	0.0006	0.0011
***7'R*-cLNA-C**	**AON14**		0.0003	0.0001	0.0002	0.0002	0.0003	0.0008	0.0004	0.0023	0.0008	0.0009
***7'S*-cLNA-C**	**AON17**		0.0003	0.0002	0.0002	0.0003	0.0003	0.0013	0.0006	0.0038	0.0011	0.0012
LNA-C	**AON32**		0.0005	0.0002	0.0003	0.0002	0.0003	0.0012	0.0005	0.0038	0.0006	0.0010
***7'R*-cLNA-C**	**AON15**		0.0003	0.0002	0.0003	0.0005	0.0005	0.0009	0.0012	0.0116	0.0015	0.0017
***7'S*-cLNA-C**	**AON18**		0.0004	0.0002	0.0004	0.0007	0.0006	0.0013	0.0017	0.0175	0.0017	0.0018
LNA-C	**AON33**		0.0002	0.0001	0.0003	0.0007	0.0006	0.0013	0.0012	0.0140	0.0015	0.0016
***7'R*-cLNA-T**	**AON19**		0.0001	0.0002	0.0002	0.0003	0.0003	0.0007	0.0003	0.0010	0.0008	0.0014
***7'S*-cLNA-T**	**AON22**		0.0002	0.0002	0.0001	0.0004	0.0004	0.0007	0.0003	0.0005	0.0008	0.0012
LNA-T	**AON34**		0.0003	0.0001	0.0001	0.0003	0.0004	0.0006	0.0002	0.0005	0.0005	0.0011
***7'R*-cLNA-T**	**AON20**		0.0001	0.0002	0.0004	0.0002	0.0003	0.0006	0.0017	0.0174	0.0019	0.0016
***7'S*-cLNA-T**	**AON23**		0.0002	0.0002	0.0004	0.0003	0.0004	0.0010	0.0017	0.0136	0.0017	0.0018
LNA-T	**AON35**		0.0003	0.0001	0.0003	0.0002	0.0002	0.0005	0.0016	0.0149	0.0012	0.0014
***7'R*-cLNA-T**	**AON21**		0.0003	0.0002	0.0004	0.0012	0.0003	0.0008	0.0017	0.0159	0.0020	0.0019
***7'S*-cLNA-T**	**AON24**		0.0002	0.0002	0.0004	0.0013	0.0005	0.0009	0.0019	0.0150	0.0020	0.0017
LNA-T	**AON36**		0.0003	0.0001	0.0005	0.0014	0.0005	0.0004	0.0015	0.0172	0.0013	0.0015

Table SII.2 (continue)

	Position		11	12	13	14	15	16	17	18	19	20
Modification		**5'-r**	**A**	**C**	**A**	**G**	**G**	**C**	**G**	**G**	**G**	**A**
	Native		0.0027	0.0006	0.0021	0.0014	0.0060	0.0012	0.0008	0.0010	0.0013	0.0088
***7'R*-cLNA-A**	**AON1**		0.0043	0.0009	0.0019	0.0015	0.0062	0.0013	0.0010	0.0011	0.0013	0.0088
***7'S*-cLNA-A**	**AON4**		0.0031	0.0009	0.0016	0.0014	0.0059	0.0012	0.0009	0.0011	0.0014	0.0118
LNA-A	**AON25**		0.0036	0.0013	0.0016	0.0015	0.0057	0.0014	0.0010	0.0011	0.0016	0.0103
***7'R*-cLNA-A**	**AON2**		0.0009	0.0006	0.0010	0.0019	0.0104	0.0015	0.0011	0.0012	0.0015	0.0123
***7'S*-cLNA-A**	**AON5**		0.0007	0.0008	0.0011	0.0019	0.0071	0.0013	0.0010	0.0012	0.0017	0.0126
LNA-A	**AON26**		0.0008	0.0006	0.0011	0.0021	0.0091	0.0018	0.0010	0.0012	0.0015	0.0112
***7'R*-cLNA-A**	**AON3**		0.0006	0.0005	0.0006	0.0009	0.0037	0.0016	0.0014	0.0011	0.0012	0.0066
***7'S*-cLNA-A**	**AON6**		0.0006	0.0005	0.0006	0.0008	0.0040	0.0017	0.0014	0.0011	0.0010	0.0050
LNA-A	**AON27**		0.0005	0.0003	0.0004	0.0006	0.0023	0.0018	0.0014	0.0007	0.0007	0.0010
***7'R*-cLNA-G**	**AON7**		0.0012	0.0009	0.0017	0.0015	0.0061	0.0015	0.0012	0.0013	0.0016	0.0124
***7'S*-cLNA-G**	**AON10**		0.0012	0.0008	0.0017	0.0016	0.0075	0.0015	0.0009	0.0011	0.0014	0.0120
LNA-G	**AON28**		0.0012	0.0007	0.0019	0.0017	0.0075	0.0013	0.0010	0.0011	0.0012	0.0116
***7'R*-cLNA-G**	**AON8**		0.0024	0.0006	0.0006	0.0006	0.0017	0.0009	0.0012	0.0010	0.0010	0.0023
***7'S*-cLNA-G**	**AON11**		0.0040	0.0005	0.0005	0.0005	0.0019	0.0006	0.0008	0.0006	0.0007	0.0040
LNA-G	**AON29**		0.0021	0.0005	0.0005	0.0007	0.0018	0.0007	0.0011	0.0009	0.0009	0.0029
***7'R*-cLNA-G**	**AON9**		0.0031	0.0008	0.0014	0.0008	0.0018	0.0006	0.0003	0.0003	0.0003	0.0008
***7'S*-cLNA-G**	**AON12**		0.0009	0.0007	0.0007	0.0006	0.0007	0.0005	0.0003	0.0002	0.0002	0.0003

	Position		11	12	13	14	15	16	17	18	19	20
Modification		**5'-r**	**A**	**C**	**A**	**G**	**G**	**C**	**G**	**G**	**G**	**A**
LNA-G	**AON30**		0.0031	0.0006	0.0013	0.0006	0.0018	0.0005	0.0003	0.0002	0.0003	0.0006
***7'R*-cLNA-C**	**AON13**		0.0031	0.0013	0.0022	0.0018	0.0070	0.0016	0.0010	0.0010	0.0013	0.0105
***7'S*-cLNA-C**	**AON16**		0.0039	0.0015	0.0028	0.0022	0.0071	0.0015	0.0010	0.0010	0.0013	0.0075
LNA-C	**AON31**		0.0029	0.0007	0.0023	0.0014	0.0074	0.0011	0.0008	0.0010	0.0012	0.0109
***7'R*-cLNA-C**	**AON14**		0.0009	0.0007	0.0008	0.0013	0.0086	0.0023	0.0013	0.0012	0.0013	0.0100
***7'S*-cLNA-C**	**AON17**		0.0007	0.0006	0.0008	0.0018	0.0083	0.0016	0.0012	0.0011	0.0011	0.0079
LNA-C	**AON32**		0.0008	0.0005	0.0006	0.0008	0.0082	0.0014	0.0011	0.0010	0.0012	0.0105
***7'R*-cLNA-C**	**AON15**		0.0035	0.0013	0.0020	0.0013	0.0029	0.0010	0.0006	0.0005	0.0007	0.0020
***7'S*-cLNA-C**	**AON18**		0.0021	0.0009	0.0011	0.0008	0.0011	0.0006	0.0004	0.0003	0.0003	0.0007
LNA-C	**AON33**		0.0039	0.0007	0.0020	0.0009	0.0031	0.0005	0.0003	0.0003	0.0005	0.0009
***7'R*-cLNA-T**	**AON19**		0.0012	0.0012	0.0024	0.0023	0.0079	0.0018	0.0014	0.0014	0.0016	0.0082
***7'S*-cLNA-T**	**AON22**		0.0011	0.0012	0.0023	0.0021	0.0058	0.0018	0.0015	0.0016	0.0019	0.0105
LNA-T	**AON34**		0.0007	0.0006	0.0021	0.0021	0.0086	0.0012	0.0009	0.0011	0.0015	0.0118
***7'R*-cLNA-T**	**AON20**		0.0023	0.0009	0.0006	0.0007	0.0017	0.0007	0.0006	0.0007	0.0007	0.0015
***7'S*-cLNA-T**	**AON23**		0.0030	0.0008	0.0007	0.0008	0.0017	0.0006	0.0007	0.0010	0.0011	0.0031
LNA-T	**AON35**		0.0039	0.0005	0.0005	0.0006	0.0021	0.0005	0.0005	0.0008	0.0010	0.0035
***7'R*-cLNA-T**	**AON21**		0.0024	0.0013	0.0016	0.0008	0.0009	0.0004	0.0003	0.0004	0.0006	0.0015
***7'S*-cLNA-T**	**AON24**		0.0022	0.0014	0.0017	0.0009	0.0010	0.0005	0.0004	0.0005	0.0007	0.0013
LNA-T	**AON36**		0.0026	0.0006	0.0019	0.0006	0.0010	0.0004	0.0003	0.0003	0.0006	0.0019

Table SII.3. Cleavage products composition (%) grouped by the sequence context with purine (*Pu*) or pyrimidine (*Py*) nucleotide on the 3′-end as 5′-ApPu-3′ (cleavage sites 5′-A^{1}pA^{2}-3′ and 5′-A^{13}pG^{14}-3′), 5′-ApPy-3′ (5′-A^{2}pU^{3}-3′, 5′-A^{6}pU^{7}-3′ and 5′-A^{11}pC^{12}-3′), 5′-GpPu-3′ (5′-G^{19}pA^{20}-3′, 5′-G^{14}pG^{15}-3′, 5′-G^{17}pG^{18}-3′, and 5′-G^{18}pG^{19}-3′), 5′-GpPy-3′ (5′-G^{8}pU^{9}-3′, 5′-G^{4}pC^{5}-3′ and 5′-G^{15}pC^{16}-3′), 5′-UpPu-3′ (5′-U^{3}pG^{4}-3′ and 5′-U^{7}pG^{8}-3′), 5′-UpPy-3′ (5′-U^{9}pG^{10}-3′) and 5′-CpPu-3′ (5′-C^{5}pA^{6}-3′, 5′-C^{10}pA^{11}-3′, 5′-C^{12}pA^{13}-3′, and 5′-C^{16}pG^{17}-3′) as well as grouped by nucleotide on the 5′-end. Individual cleavage site products are reported in details in Table SII.2 in SI Part II.

AON	Position	Modification	Ap*Pu*, %	Ap*Py*, %	Gp*Pu*, %	Gp*Py*, %	Up*Pu*, %	Up*Py*, %	Cp*Pu*, %	Ap*N*, %	Gp*N*, %	Up*N*, %	Cp*N*, %	Products, %
Native			6	10	13	31	2	2	10	16	44	4	10	75
AON1	A3	7′*R*-cLNA-A	6	16	14	21	2	2	14	22	35	4	14	75
AON4		7′*S*-cLNA-A	5	11	14	19	1	2	13	17	33	3	13	66
AON25		LNA-A	6	12	15	20	2	2	15	18	34	3	15	70
AON2	A7	7′*R*-cLNA-A	3	4	16	32	0	1	9	7	48	1	9	65
AON5		7′*S*-cLNA-A	4	5	17	24	1	2	11	9	41	3	11	64
AON26		LNA-A	4	4	17	31	1	1	11	8	47	2	11	68
AON3	A9	7′*R*-cLNA-A	3	6	13	42	4	3	10	8	55	7	10	81
AON6		7′*S*-cLNA-A	3	5	13	45	4	5	10	9	58	9	10	85
AON27		LNA-A	1	3	10	63	4	6	9	4	73	11	9	97
AON7	G5	7′*R*-cLNA-G	6	6	16	21	1	2	11	13	37	3	11	64
AON10		7′*S*-cLNA-G	6	6	14	25	1	2	11	12	39	3	11	66
AON28		LNA-G	7	7	14	25	2	2	11	13	39	3	11	67
AON8	G10	7′*R*-cLNA-G	3	10	11	51	5	5	9	12	62	10	9	93
AON11		7′*S*-cLNA-G	2	13	7	51	5	3	7	15	58	8	7	88
AON29		LNA-G	3	8	10	52	6	4	8	11	62	10	8	92
AON9	G16	7′*R*-cLNA-G	5	13	5	54	5	5	11	18	59	10	11	98
AON12		7′*S*-cLNA-G	3	7	4	66	7	5	8	10	70	11	8	99
AON30		LNA-G	5	14	4	56	5	4	10	18	61	10	10	98
AON13		7′*R*-cLNA-C	7	11	15	22	1	1	12	18	37	3	12	70

AON16	**C4**	*7′S*-cLNA-C	9	14	16	23	2	2	14	23	38	4	14	78
AON31		LNA-C	8	11	13	24	1	2	10	19	37	3	10	69
AON14	**C8**	*7′R*-cLNA-C	3	5	15	32	2	2	12	9	47	4	12	71
AON17		*7′S*-cLNA-C	3	6	15	36	2	3	11	10	51	6	11	77
AON32		LNA-C	3	6	12	35	2	2	9	9	47	4	9	70
AON15	**C15**	*7′R*-cLNA-C	7	13	9	44	4	4	13	20	53	8	13	94
AON18		*7′S*-cLNA-C	4	11	5	56	6	5	11	15	61	11	11	98
AON33		LNA-C	6	15	6	51	4	4	10	22	57	8	10	97
AON19	**T6**	*7′R*-cLNA-T	7	6	19	26	1	2	14	13	46	4	14	76
AON22		*7′S*-cLNA-T	7	6	21	19	1	2	13	13	40	4	13	70
AON34		LNA-T	7	4	16	27	1	1	10	11	44	2	10	66
AON20	**T11**	*7′R*-cLNA-T	2	9	8	56	6	5	10	11	64	11	10	96
AON23		*7′S*-cLNA-T	2	12	11	45	6	5	10	14	55	11	10	91
AON35		LNA-T	2	13	9	50	6	3	7	15	58	9	7	90
AON21	**T13**	*7′R*-cLNA-T	5	10	6	52	6	6	11	15	58	12	11	96
AON24		*7′S*-cLNA-T	6	10	7	50	7	6	12	15	57	12	12	96
AON36		LNA-T	6	9	5	56	6	4	8	15	62	9	8	95

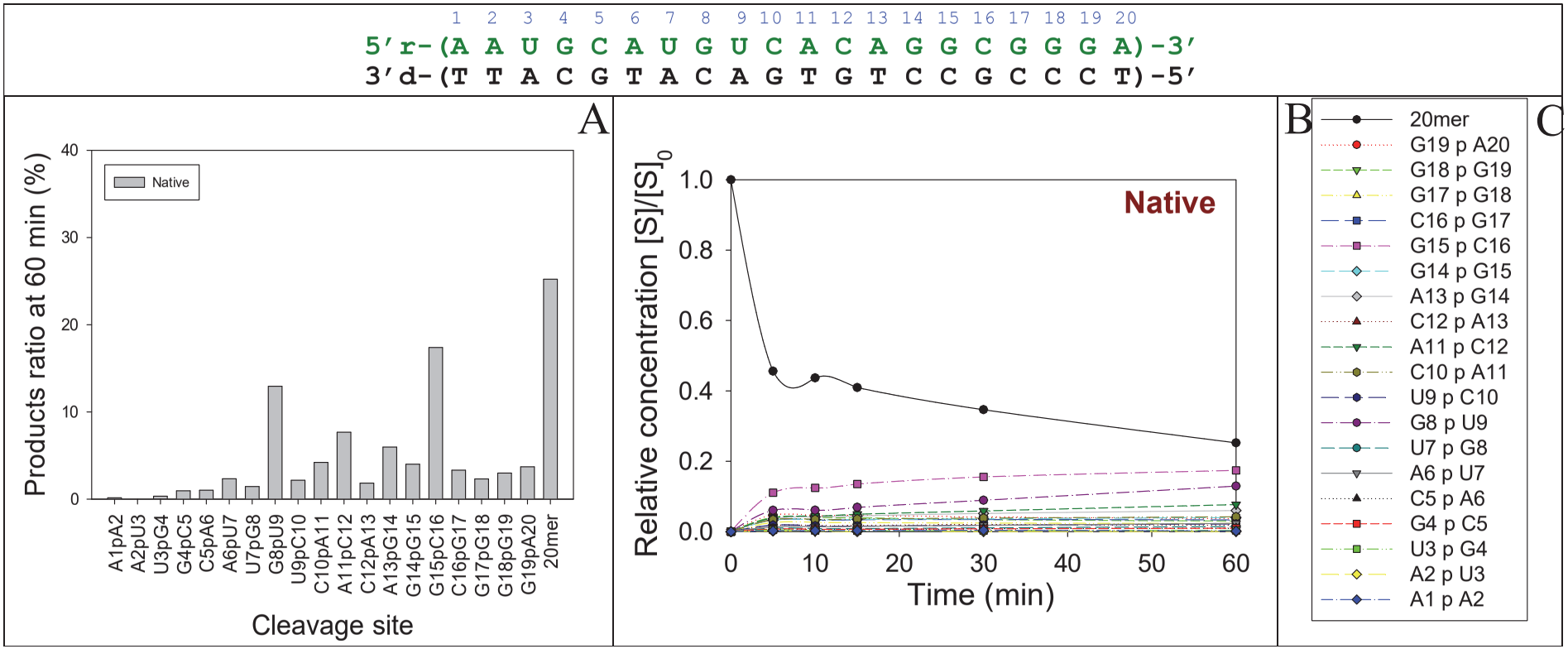


Figure SII.62. *E. coli* RNase H promoted cleavage of the native AON/RNA. **Inset A** shows product ratio (in %) at 60 minutes, **Inset B** is time dependence of the relative concentrations $[S]/[S]_0$ of initial 20mer AON/RNA duplexes and all cleavage products normalized by the initial substrate concentration $[S]_0 = 0.1$ μM. Spline interpolation was used to connect experimental points. **Inset C** is a colour code card to time dependences shown in **Inset B**. **Enzymatic assay conditions**: Target 0.1 μM RNA (specific activity 80 000 cpm) and AON (2 μM) were incubated in a buffer containing 20 mM Tris-HCl (pH 7.5), 20 mM KCl, 10 mM $MgCl_2$, 0.1 mM EDTA, and 0.1 mM DTT at 21 °C in the presence of 0.08 U *E. coli* RNase H.

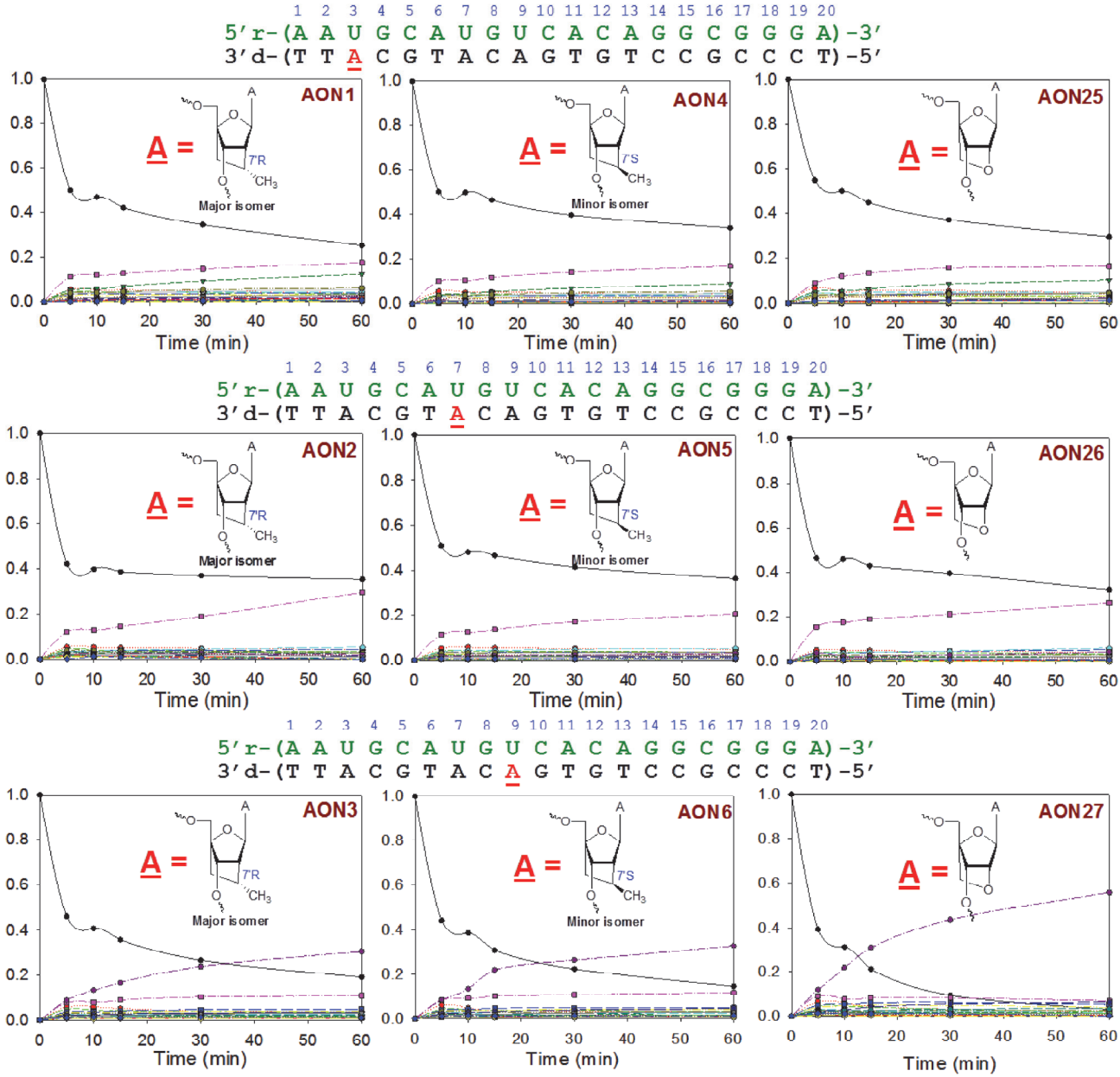


Figure SII.63. *E. coli* RNase H promoted cleavage of AON**1-6,25-27**/RNA heteroduplexes containing modified nucleotides A = *7′R*-Me-cLNA-A (AON**1-3)** or *7′S*-Me-cLNA-A (AON**4-6**) or LNA-A (AON**25-27**) quantified as time dependence of the relative concentrations $[S]/[S]_0$ of initial 20mer AON/RNA duplexes and all cleavage products normalized by the initial substrate concentration $[S]_0$ = 0.1 μM. Corresponding AON sequences, positions and types of modifications are shown. Spline interpolation was used to connect experimental points. Colour code and enzymatic assay conditions are as described in the caption of Figure SII.61.

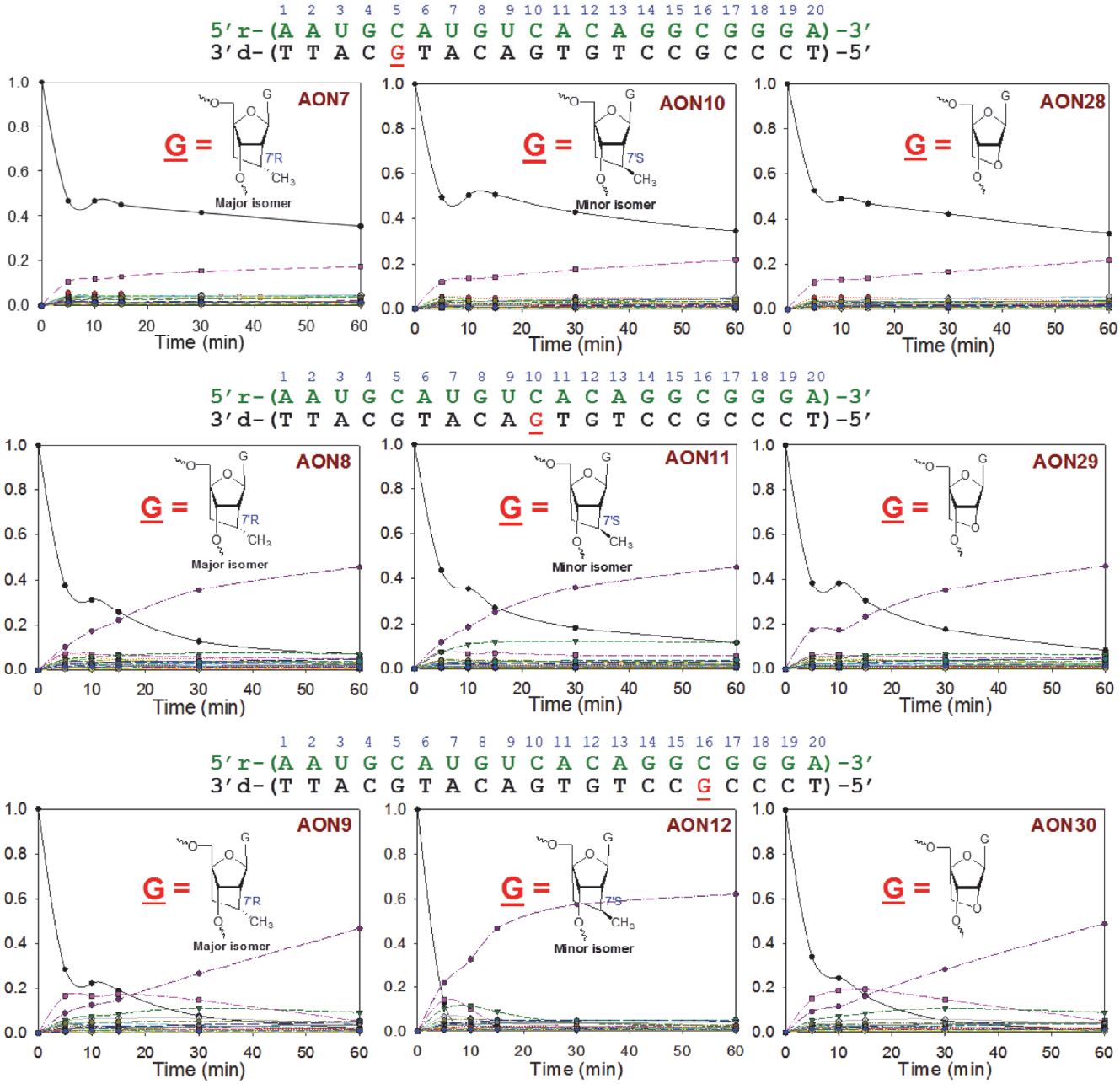


Figure SII.64. *E. coli* RNase H promoted cleavage of AON**7-12,28-30**/RNA heteroduplexes containing modified nucleotides G = *7′R*-Me-cLNA-G (AON**7-9)** or *7′S*-Me-cLNA-G (AON**10-12**) or LNA-G (AON**28-30**) quantified as time dependence of the relative concentrations $[S]/[S]_0$ of initial 20mer AON/RNA duplexes and all cleavage products normalized by the initial substrate concentration $[S]_0$ = 0.1 μM. Corresponding AON sequences, positions and types of modifications are shown. Colour code and enzymatic assay conditions are as described in the caption of Figure SII.61.

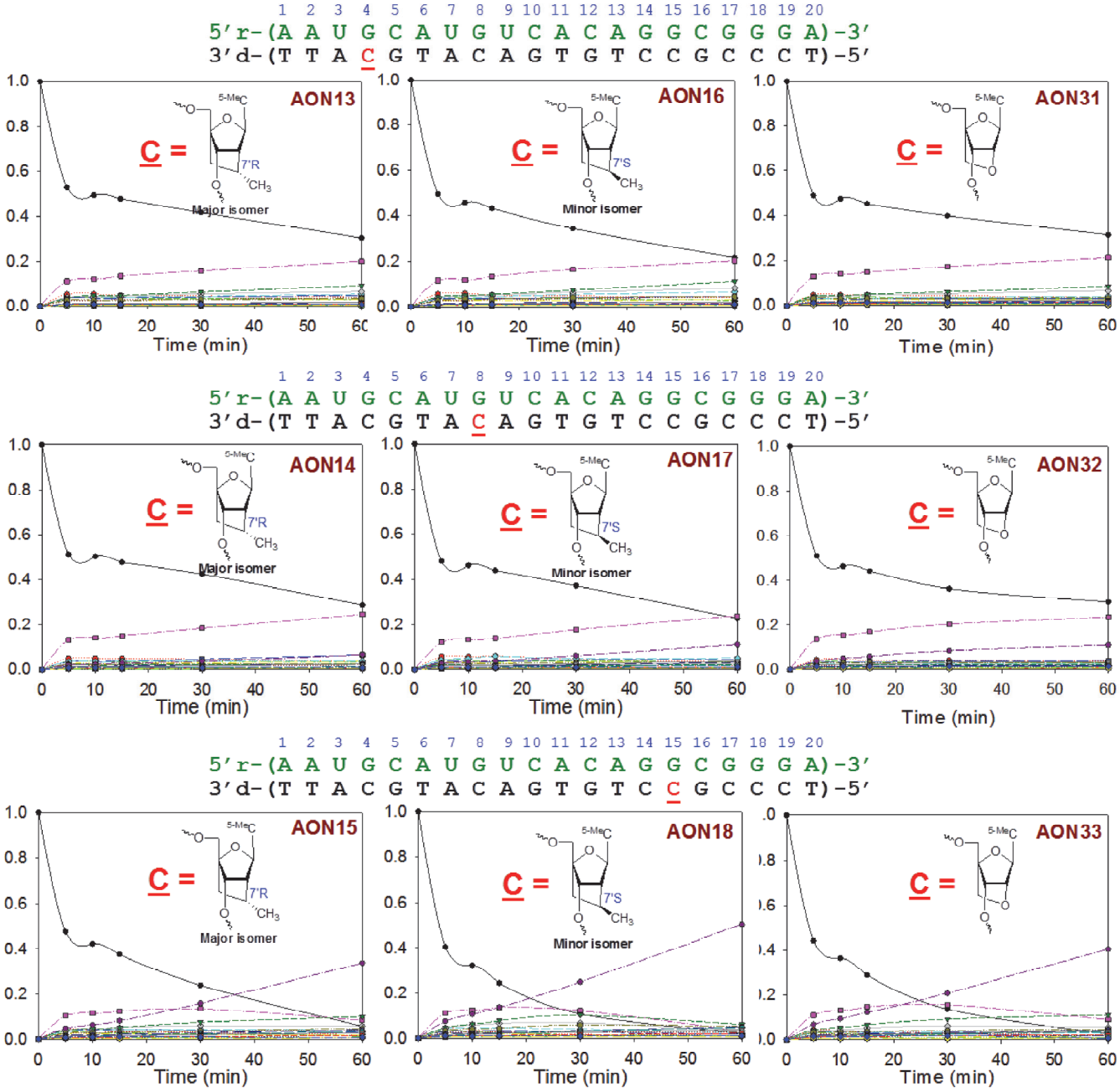


Figure SII.65. *E. coli* RNase H promoted cleavage of AON**13-18,31-33**/RNA heteroduplexes containing modified nucleotides C = *7′R*-Me-cLNA-$^{5\text{-Me}}$C (AON**13-15)** or *7′S*-Me-cLNA-$^{5\text{-Me}}$C (AON**16-18**) or LNA-$^{5\text{-Me}}$C (AON**31-33**) quantified as time dependence of the relative concentrations $[S]/[S]_0$ of initial 20mer AON/RNA duplexes and all cleavage products normalized by the initial substrate concentration $[S]_0 = 0.1$ μM. Corresponding AON sequences, positions and types of modifications are shown. Colour code and enzymatic assay conditions are as described in the caption of Figure SII.61.

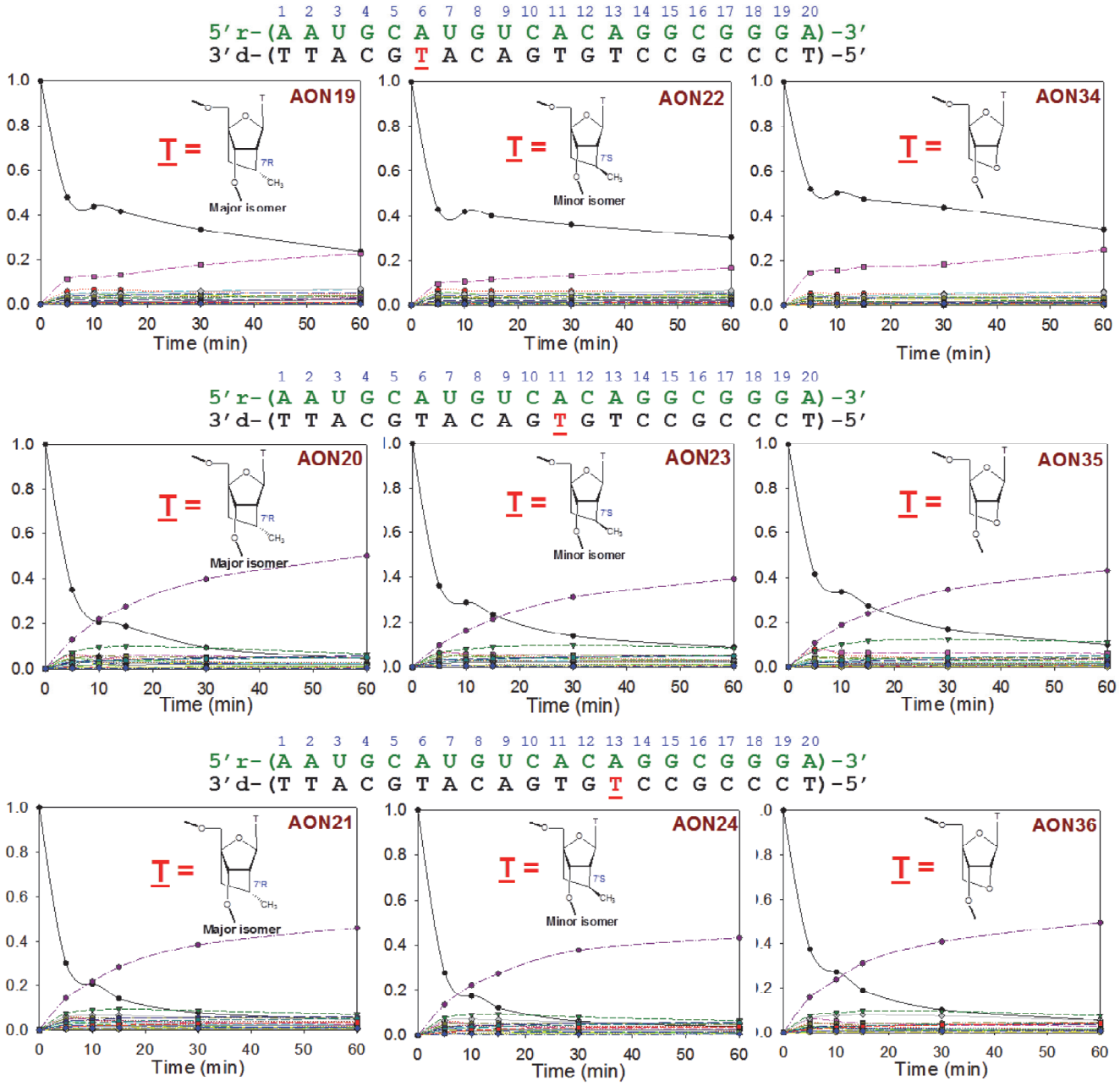


Figure SII.66. *E. coli* RNase H promoted cleavage of AON**19-24,34-35**/RNA heteroduplexes containing modified nucleotides T = *7′R*-Me-cLNA-T (AON**19-21)** or *7′S*-Me-cLNA-T (AON**22-24**) or LNA-T (AON**34-35**) quantified as time dependence of the relative concentrations $[S]/[S]_0$ of initial 20mer AON/RNA duplexes and all cleavage products normalized by the initial substrate concentration $[S]_0$ = 0.1 μM. Corresponding AON sequences, positions and types of modifications are shown. Colour code and enzymatic assay conditions are as described in the caption of Figure SII.61.

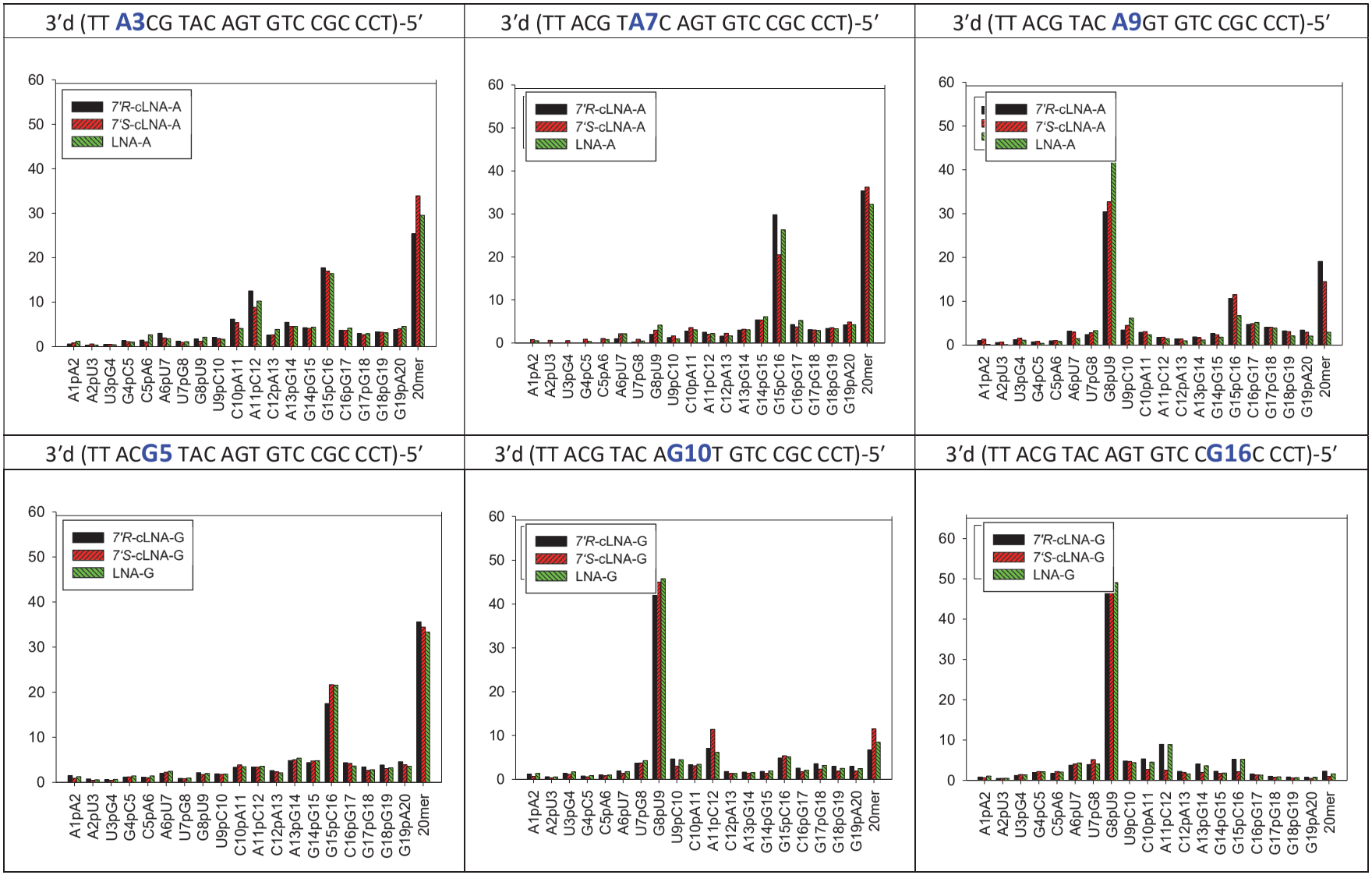


Figure SII.67. Relative concentrations of various products formed due to RNase H promoted degradation of RNA in native and AON**1-36**/RNA hybrid duplexes. The measurement is taken at 60 minutes from the start of the reaction and concentrations are normalized by initial substrate concentration $[S]_0$ = 0.1 μM

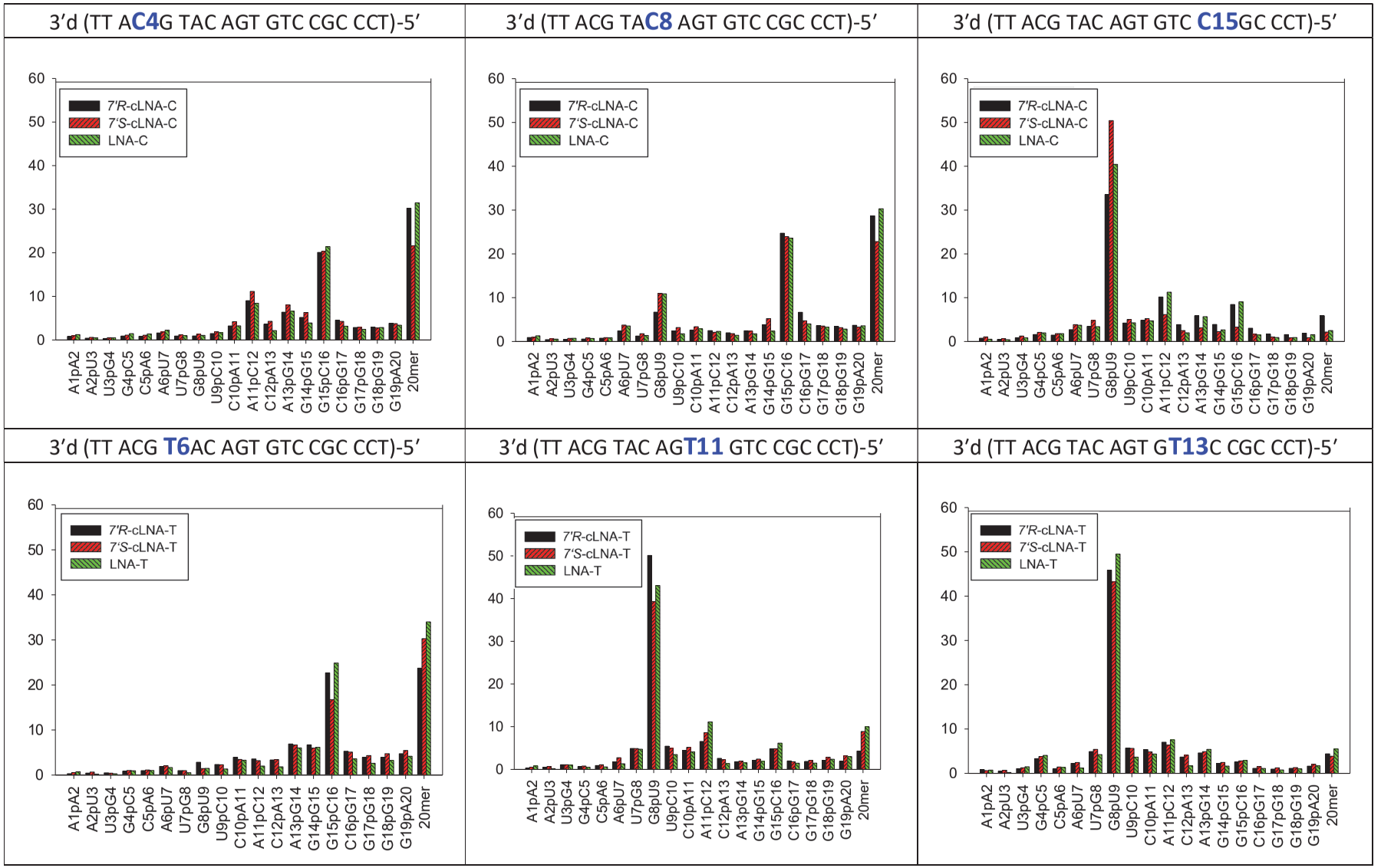


Figure SII.67. (continue)