Synthetic Elaboration of Native DNA by RASS (SENDR)

Dillon T. Flood,[1⊥] Kyle W. Knouse,[1⊥] Julien C. Vantourout,[1] Seiya Kitamura,[1] Brittany B. Sanchez,[§] Emily J. Sturgell,[§] Jason S. Chen,[§] Dennis W. Wolan,[1] Phil S. Baran,[1*] Philip E. Dawson.[1*]

[1]Department of Chemistry, Scripps Research, 10550 North Torrey Pines Road, La Jolla, California 92037, United States
[§]Automated Synthesis Facility, Scripps Research, 10550 North Torrey Pines Road, La Jolla, CA 92037, United States
[⊥]These authors contributed equally to this work.

Table of Contents

General Experimental

Tetrahydrofuran (THF), *N,N*-dimethylformamide (DMF), and dichloromethane (DCM) and acetonitrile (MeCN) were obtained by passing the previously degassed solvents through an activated alumina column. DMA was purchased from Sigma-Aldrich and used without further drying. (+)-Ψ and (–)-Ψ were obtained from Sigma-Aldrich. DIC (*N,N'*-diisopropylcarbodiimide) was purchased from Oakwood. Deionized water was used in all the reactions, unless otherwise stated. All the other reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. $NaClO_4$ was purchased at the highest commercial grade from Acros Organics. DNA tags was obtained from IDT, Inc., Coralville, IA. Recombinant All enzymes (Shrimp Alkaline Phosphatase (rSAP), ExoIII, T4PNK, and Lambda Exo) was obtained from New England Biolabs, Ipswich, MA. The Cut Smart buffer stock used enzymatic reactions was obtained from New England Biolabs, Ipswich, MA. UltraPure™ Agarose was obtained from Invitrogen, Carlsbad, CA. 50X TAE Buffer (Tris-acetate-EDTA) was obtained from Thermo Fisher Scientific, Waltham, MA. SYBR™ Safe DNA Gel Stain (10,000X) was obtained from Invitrogen, Carlsbad, CA. Gel Loading Dye, Purple (6X), no SDS was obtained from New England Biolabs, Ipswich, MA.

Yields under normal organic conditions refer to chromatographically and spectroscopically (^{1}H, ^{31}P NMR) homogeneous material, unless otherwise stated. TLC was performed using 0.25 mm E. Merck silica plates (60F-254), using short-wave UV light as the visualizing agent, and phosphomolybdic acid or $KMnO_4$ and heat as developing agents. NMR spectra were recorded on Bruker DRX-600, DRX-500, and AMX-400 instruments and are calibrated using residual undeuterated solvent ($CHCl_3$ at 7.26 ppm ^{1}H NMR, 77.16 ppm ^{13}C NMR). The following abbreviations were used to explain multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Column chromatography was performed using E. Merck silica gel (60, particle size 0.043–0.063 mm), and preparative TLC was performed on Merck silica plates (60F-254). High-resolution mass spectra (HRMS) were recorded on Agilent LC/MSD TOF mass spectrometer by electrospray ionization time of flight reflectron experiments. Melting points were recorded on a Fisher-Johns 12-144 melting point apparatus and are uncorrected.

No unexpected or unusually high safety hazards were encountered.

PBS: Phosphate buffered saline—was purchased from commercial and filtered before use. PBS is comprised of NaCl (137 mM), KCl (2.7 mM), Na_2HPO_4 (10 mM), KH_2PO_4 (1.8 mM), at pH 7.4.

Elute Buffer: DNA was eluted from resin using a sodium perchlorate buffer (1 M $NaClO_4$, 40 mM Tris, 20% MeOH, at pH 8.5). This was prepared from $NaClO_4$ (Acros Organics), and filtered after preparation. This buffer could be stored on the bench indefinitely.

HPLC-MS Analysis: Six microliter of the DNA solution was analyzed on a Waters H-Class LC with a Waters BEH C18 column (2.1x55 mm, 1.7 μm, 130 Å) using a gradient of 114 mM HFIP and 14 mM Et_3N in water (A) and methanol (B) (0.3 mL/min, 10-26 %B over 5 minutes) at 60º C. Peak identities were determined by ESI- using the 6- ion.

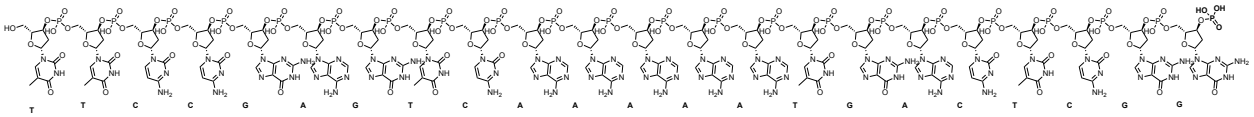
HPLC-TOF High Resolution Analysis: One microliter of the DNA solution was analyzed on a Waters I-Class LC with a Waters BEH C18 column (2.1x55 mm, 1.7 μm, 130 Å) using a gradient of 114 mM HFIP and 14 mM Et_3N in water (A) and methanol (B) (0.3 mL/min, 10-26 %B over 10 minutes) at 60º C. Peak identities were determined by ESI- using the 3- ion.

Deconvolution: data visualization and integration were performed with Mass Lynx V4.1 software.

Conversion determination: the yields of on-DNA products were determined from UV absorbance trace (260 nm) peak area using the equation: Yield%= UV(prod)/UV(total DNA recovered), while ignoring UV coefficient difference for all DNA products and assuming 100% of DNA total recovery. While determining yields, any non-oligo material (as determined by close examination of mass spectra) that absorbed UV 260 nm was subtracted out of the final yield calculation. These peaks were determined to not contain oligo material when a DNA indicative mass spectra was not observed, and are usually attributed to small molecules not removed during the ethanol precipitation. These peaks were generally found at or before 1 minute retention time during analysis.

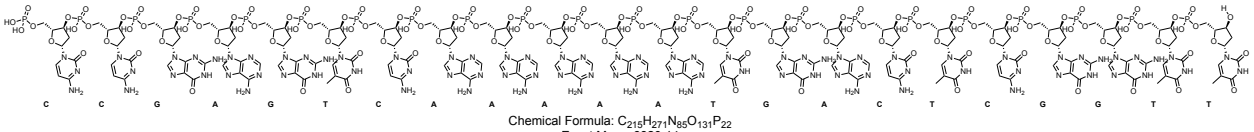
Preparative HPLC: Was performed on the Waters H Class instrument described above using customized gradients for each compound of interest and an automatic divert valve.

DNA starting materials. All DNA starting materials used in this paper and their structures are shown below. DNA was purchased from IDT.



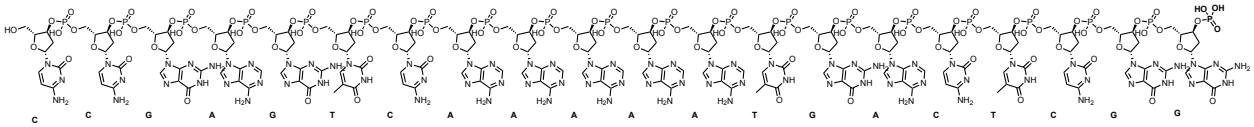
Chemical Formula: $C_{215}H_{271}N_{85}O_{131}P_{22}$
Exact Mass: 6820.14
Molecular Weight: 6823.42

Model DNA (**1**): 5'-TTCCGAGTCAAAAATGACTCGG/3Phos/-3'



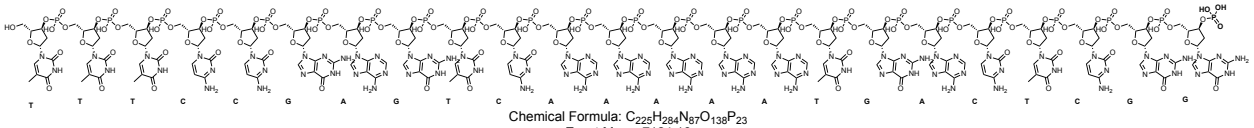
Chemical Formula: $C_{215}H_{271}N_{85}O_{131}P_{22}$
Exact Mass: 6820.14
Molecular Weight: 6823.42

Model DNA (**2**): /5Phos/CCGAGTCAAAAATGACTCGGTT



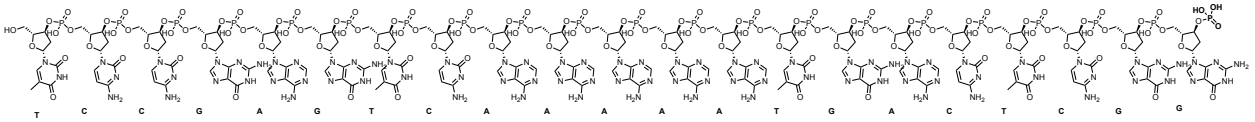
Chemical Formula: $C_{195}H_{245}N_{81}O_{117}P_{20}$
Exact Mass: 6212.05
Molecular Weight: 6215.03

Model DNA (**SI-1**): CCGAGTCAAAAATGACTCGG/3Phos/



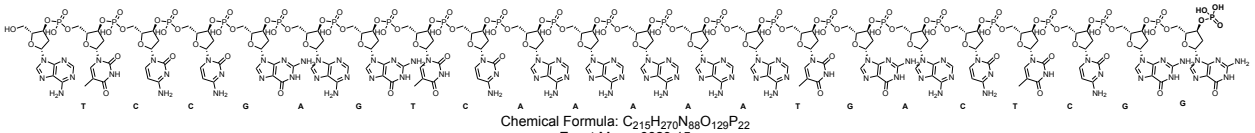
Chemical Formula: $C_{225}H_{284}N_{87}O_{138}P_{23}$
Exact Mass: 7124.18
Molecular Weight: 7127.61

Model DNA (**SI-2**): TTTCCGAGTCAAAAATGACTCGG/3Phos/



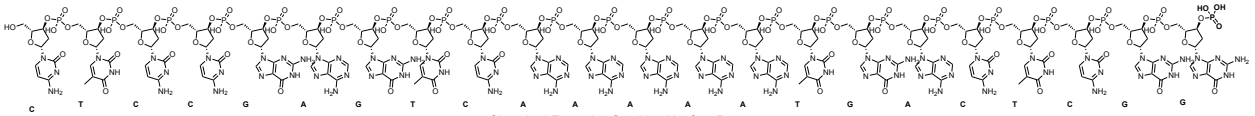
Chemical Formula: $C_{205}H_{258}N_{83}O_{124}P_{21}$
Exact Mass: 6516.09
Molecular Weight: 6519.23

Model DNA (**SI-3**): TCCGAGTCAAAAATGACTCGG/3Phos/



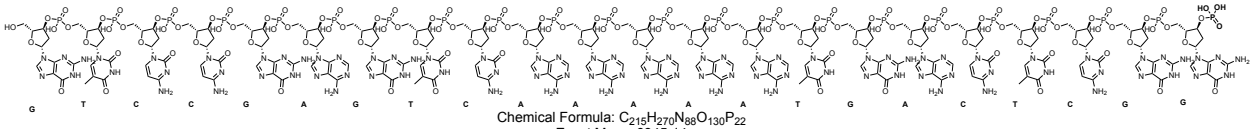
Chemical Formula: $C_{215}H_{270}N_{88}O_{129}P_{22}$
Exact Mass: 6829.15
Molecular Weight: 6832.43

Model DNA (**SI-4**): ATCCGAGTCAAAAATGACTCGG/3Phos/



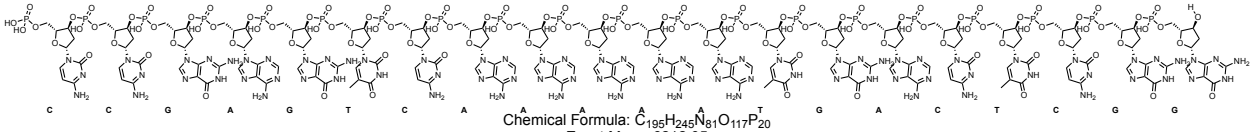
Chemical Formula: $C_{214}H_{270}N_{86}O_{130}P_{22}$
Exact Mass: 6805.14
Molecular Weight: 6808.41

Model DNA (**SI-5**): CTCCGAGTCAAAAATGACTCGG/3Phos/



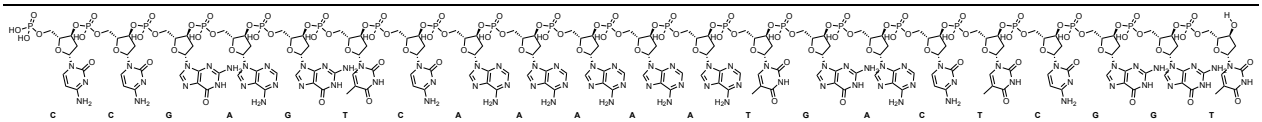
Chemical Formula: $C_{215}H_{270}N_{86}O_{130}P_{22}$
Exact Mass: 6845.14
Molecular Weight: 6848.43

Model DNA (**SI-6**): GTCCGAGTCAAAAATGACTCGG/3Phos/



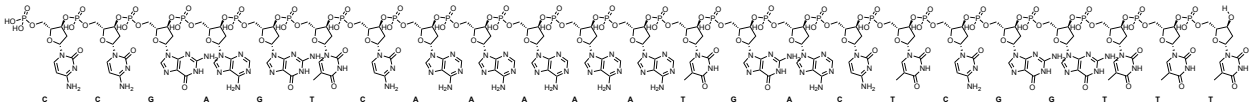
Chemical Formula: $C_{195}H_{245}N_{81}O_{117}P_{20}$
Exact Mass: 6212.05
Molecular Weight: 6215.03

Model DNA (**SI-7**): /5Phos/CCGAGTCAAAAATGACTCGG



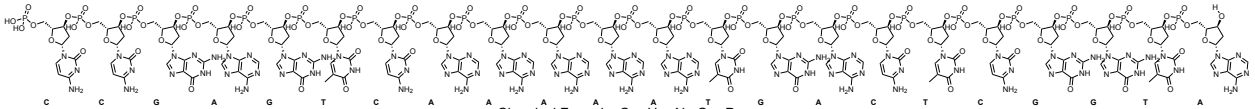
Chemical Formula: $C_{205}H_{258}N_{83}O_{124}P_{21}$
Exact Mass: 6516.09
Molecular Weight: 6519.23

Model DNA (**SI-8**): /5Phos/CCGAGTCAAAAATGACTCGGT



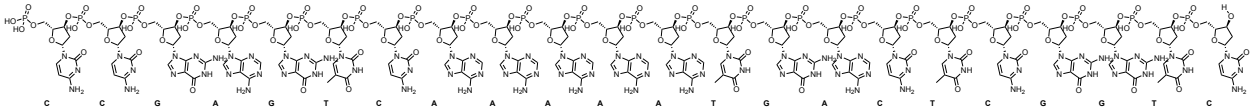
Chemical Formula: $C_{225}H_{284}N_{87}O_{138}P_{23}$
Exact Mass: 7124.18
Molecular Weight: 7127.61

Model DNA (**SI-9**): /5Phos/CCGAGTCAAAAATGACTCGGTTT



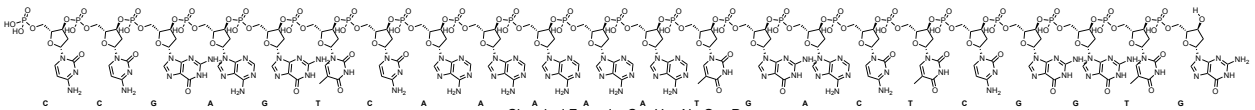
Chemical Formula: $C_{215}H_{270}N_{88}O_{129}P_{22}$
Exact Mass: 6829.15
Molecular Weight: 6832.43

Model DNA (**SI-10**): /5Phos/CCGAGTCAAAAATGACTCGGTA



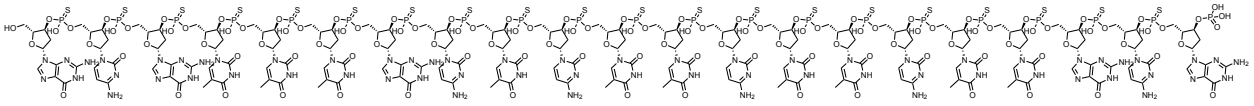
Chemical Formula: $C_{214}H_{270}N_{86}O_{130}P_{22}$
Exact Mass: 6805.14
Molecular Weight: 6808.41

Model DNA (**SI-11**): /5Phos/CCGAGTCAAAAATGACTCGGTC



Chemical Formula: $C_{215}H_{270}N_{88}O_{130}P_{22}$
Exact Mass: 6845.14
Molecular Weight: 6848.43

Model DNA (**SI-12**): /5Phos/CCGAGTCAAAAATGACTCGGTG

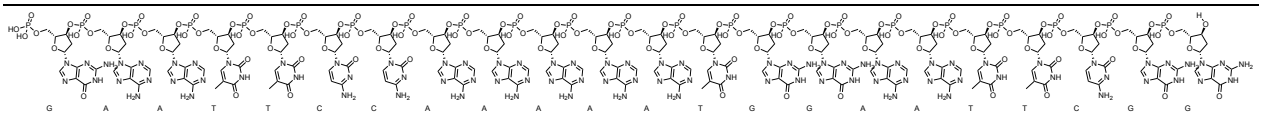


Chemical Formula: $C_{204}H_{264}N_{63}O_{117}P_{21}S_{20}$
Exact Mass: 6757.55
Molecular Weight: 6762.33

Vitravene Analog DNA (**44**): G*C*G*T*T*T*G*C*T*C*T*T*C*T*T*C*T*T*G*C*G/3phos/
* indicates posphorotioate (PS) linkage

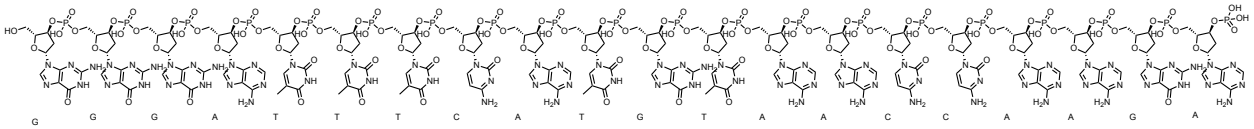
Chemical Formula: $C_{570}H_{713}N_{225}O_{350}P_{58}$
Exact Mass: 18103.97
Molecular Weight: 18112.68

Protein Aptamer DNA (**49**): /5Phos/
ATACCAGCTTATTCAATTAGCAACATGAGGGGGATAGAGGGGGTGGGTTCTCTCGG
CT



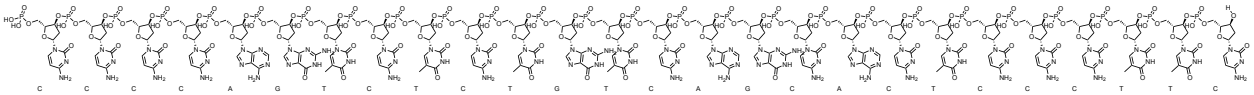
Chemical Formula: $C_{217}H_{271}N_{89}O_{129}P_{22}$
Exact Mass: 6868.16
Molecular Weight: 6871.47

Model DNA (**51**): /5Phos/GAATTCCAAAAATGGAATTCGG



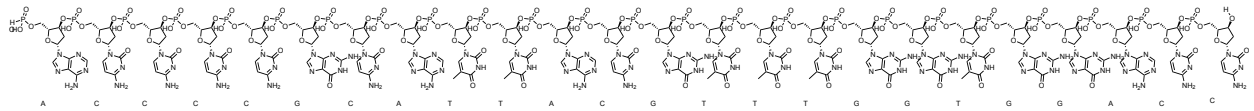
Chemical Formula: $C_{197}H_{247}N_{79}O_{119}P_{20}$
Exact Mass: 6242.05
Molecular Weight: 6245.05

cTegsedi (**54**): GGGATTTCATGTAACCAAGA/3Phos/



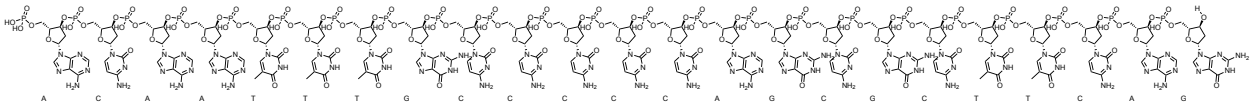
Chemical Formula: $C_{247}H_{321}N_{83}O_{161}P_{26}$
Exact Mass: 7830.27
Molecular Weight: 7834.02

TaqMan Probe Starting Material (**57**): /5Phos/CCCCAGTCTCTGTCAGCACTCCCTTC



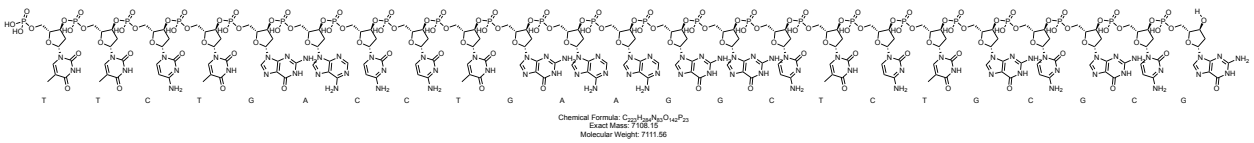
Chemical Formula: $C_{232}H_{296}N_{86}O_{147}P_{24}$
Exact Mass: 7381.20
Molecular Weight: 7384.75

2019-nCov-N1-P Starting Material (**SI-13**): /5Phos/ACCCCGCATTACGTTTGGTGGACC

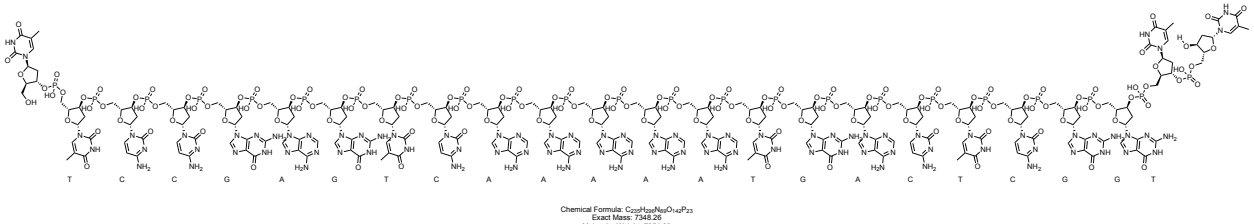


Chemical Formula: $C_{221}H_{283}N_{82}O_{139}P_{23}$
Exact Mass: 7021.16
Molecular Weight: 7024.53

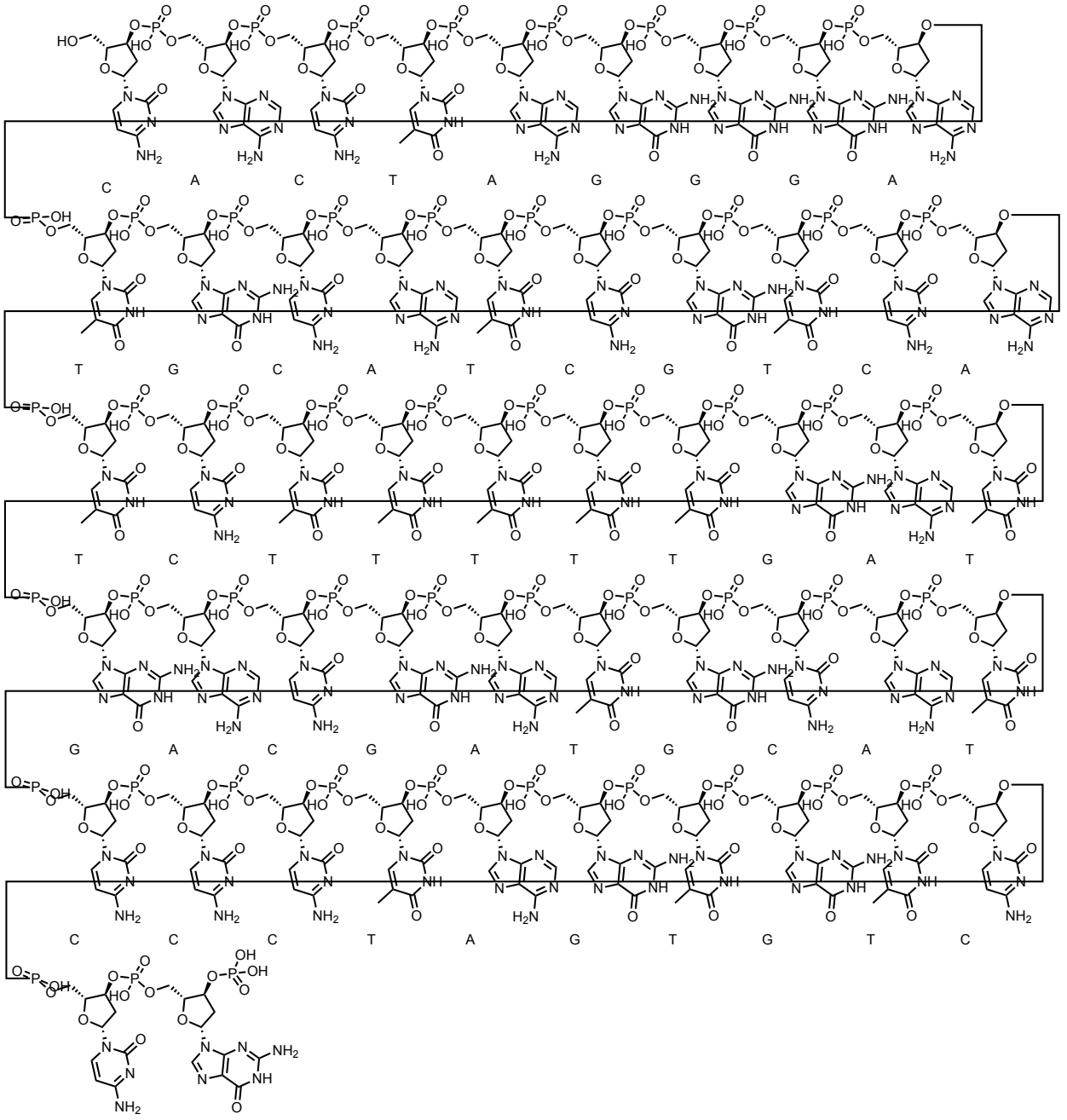
2019-nCov-N2-P Starting Material (**SI-14**): /5Phos/ ACAATTTGCCCCCAGCGCTTCAG



2019-nCov-N3-P Starting Material (**SI-15**): /5Phos/CCCCAGTCTCTGTCAGCACTCCCTTC

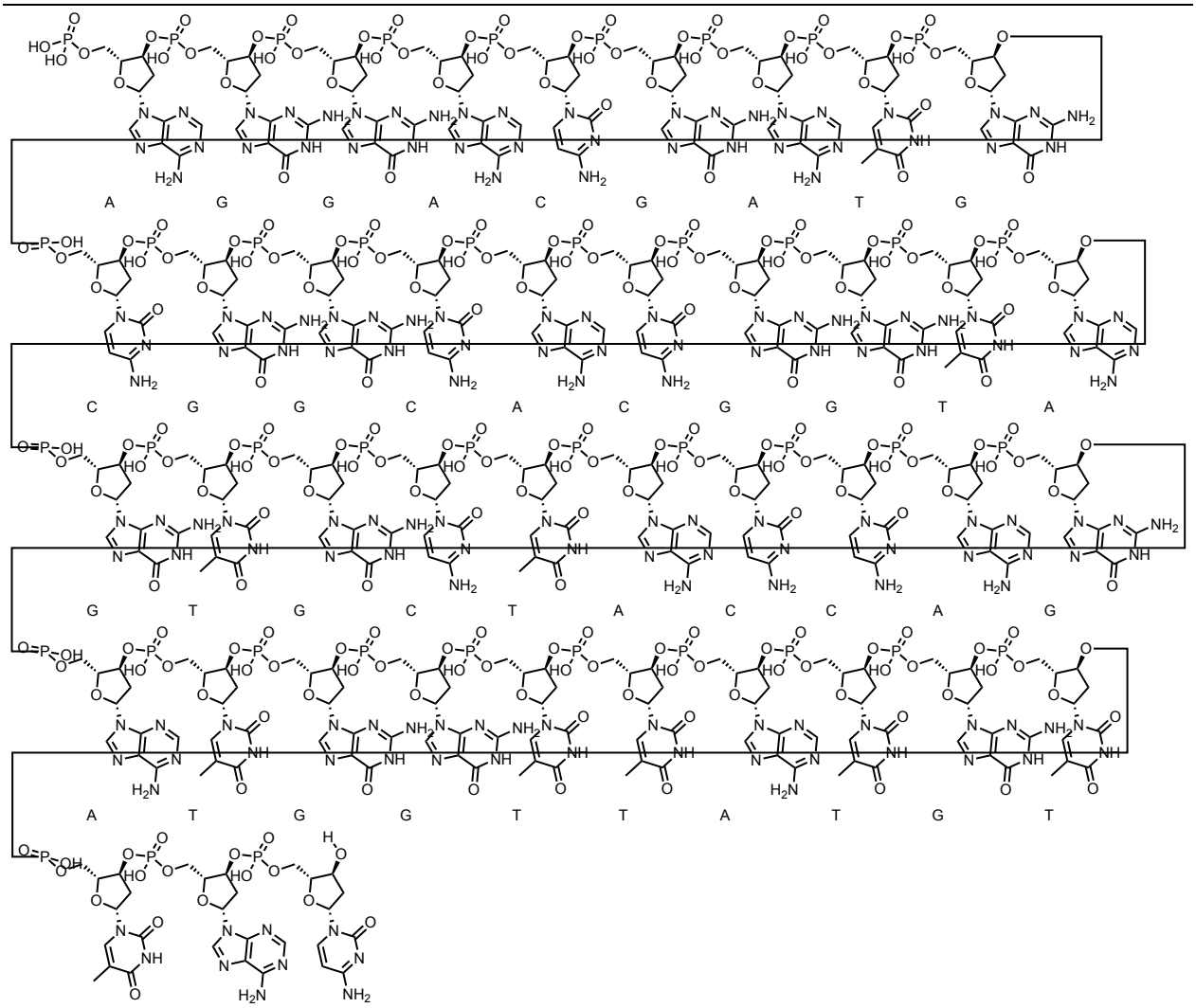


Nonphosphorylated DNA (**SI-16**): CCGAGTCAAAAATGACTCGGTT



Chemical Formula: $C_{497}H_{630}N_{181}O_{313}P_{51}$
Exact Mass: 15719.56
Molecular Weight: 15727.12

High Tm DNA Hairpin (**SI-17**):
CACTAGGGATGCATCGTCATCTTTTTGATGACGATGCATCCCTAGTGTCCG/3Phos/



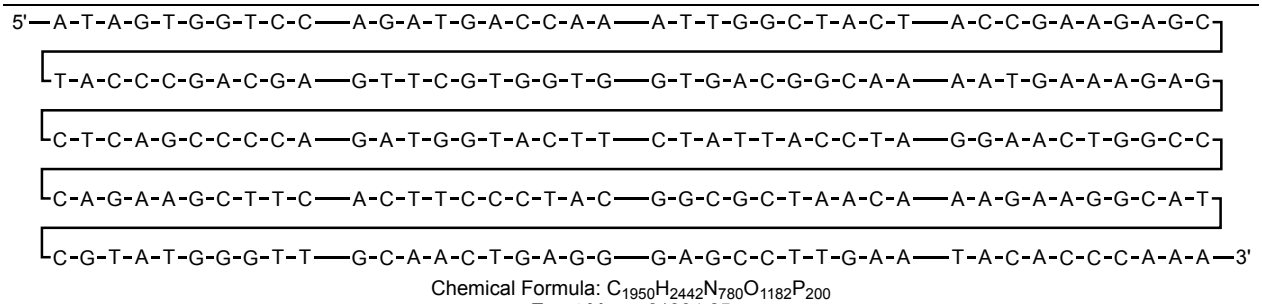
Chemical Formula: $C_{412}H_{516}N_{164}O_{253}P_{42}$
Exact Mass: 13108.15
Molecular Weight: 13114.45

hNE Aptamer (**54**): /5Phos/ aggacgatgcggCACGGTAGTGCTACCAGATGGTTATGTTAC

T/iMe-dC/A +G*+C*+A* T*T*/iMe-dC/* T*A*A* T*A*G* /iMe-dC/*+A*+G* +C/$_3$Phos/

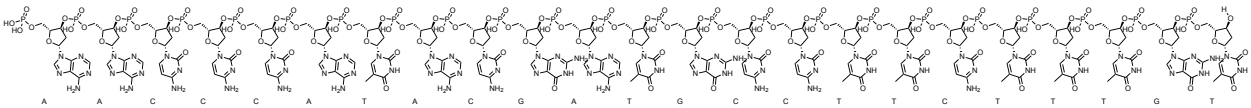
MW: 6300.9

MALAT1 (**48**): T/iMe-dC/A +G*+C*+A* T*T*/iMe-dC/* T*A*A* T*A*G* /iMe-dC/*+A*+G* +C/$_3$Phos/



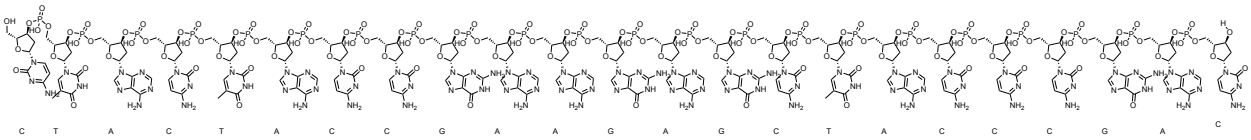
Chemical Formula: $C_{1950}H_{2442}N_{780}O_{1182}P_{200}$
Exact Mass: 61884.25
Molecular Weight: 61914.02

Template Strand **(SI-18):**
/5Phos/atagtggtccagatgaccaaattggctactaccgaagagctacccgacgagttcgtggtggtgacggcaaaatgaaagagctca
gccccagatggtacttctattacctaggaactggcccagaagcttcacttccctacggcgctaacaaagaaggcatcgtatgggttgcaact
gagggagccttgaatacacccaaa



Chemical Formula: $C_{223}H_{286}N_{77}O_{142}P_{23}$
Exact Mass: 7026.15
Molecular Weight: 7029.53

Primer 1 **(SI-19):** /5Phos/aacccatacgatgccttctttgt



Chemical Formula: $C_{221}H_{280}N_{88}O_{132}P_{22}$
Exact Mass: 6959.21
Molecular Weight: 6962.58

Primer 2 (**SI-20)**: ctactaccgaagagctacccgac

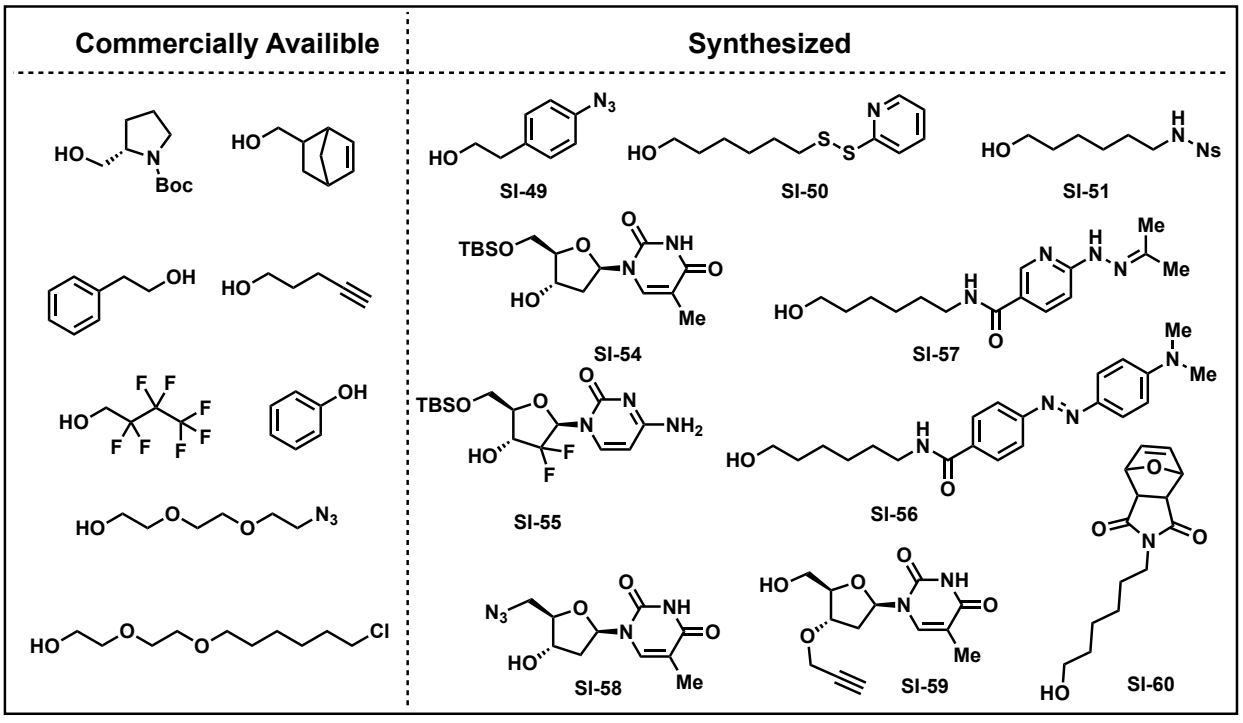
Synthesis and Characterization of Ψ-modules

General Procedure 1 - Synthesis of Ψ-modules

The Ψ-modules were prepared as follow unless otherwise stated. Alcohol (1.0 equiv.) and (–)-Ψ (1.3 equiv.) were dissolved in anhydrous MeCN or DCM (0.1 M) in a flame-dried round-bottom flask. DBU (1.2 equiv.) was added to the reaction mixture while stirring. After 5-10 minutes, the crude reaction mixture was diluted with EtOAc or Et_2O and transferred to a separatory funnel. The organic layer was washed with H_2O, saturated aqueous KH_2PO_4 and brine. After drying over $MgSO_4$ and filtration, the solvent was removed *in vacuo*. The crude product was purified by silica gel column chromatography unless otherwise stated. Protocol adapted from.[1]

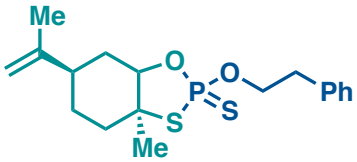
No unexpected or unusually high safety hazards were encountered.

Source of Alcohols used in Synthesis of Ψ-modules



Synthesis of Ψ-1 through Ψ-19

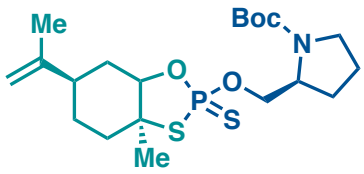
Ψ-1



Prepared according to **General Procedure 1** using **2-phenylethan-1-ol** (2.44 g, 20.0 mmol). Purification using 50% DCM in hexanes afforded **Ψ-1** (6.84 g, 18.6 mmol, 93% yield).

Physical State: Crystaline Solid;
R_f = 0.50 (50% DCM/hexanes);
^{1}H NMR (600 MHz, Chloroform-*d*): δ 7.32 (tt, *J* = 7.8, 1.4 Hz, 2H), 7.29 – 7.22 (m, 3H), 5.01 (q, *J* = 1.4 Hz, 1H), 4.89 – 4.84 (m, 1H), 4.47 – 4.38 (m, 2H), 4.36 (ddd, *J* = 14.2, 10.3, 7.1 Hz, 1H), 3.04 (t, *J* = 7.1 Hz, 2H), 2.60 (d, *J* = 6.2 Hz, 1H), 2.30 – 2.23 (m, 1H), 2.10 (td, *J* = 13.5, 4.3 Hz, 1H), 1.97 (ddt, *J* = 15.0, 4.3, 2.1 Hz, 1H), 1.93 – 1.84 (m, 2H), 1.83 – 1.74 (m, 4H), 1.72 (s, 3H);
^{13}C NMR (151 MHz, Chloroform-*d*): δ 144.61, 136.67, 128.55, 128.05, 126.23, 111.33, 85.20, 68.65, 68.60, 64.76, 38.42, 36.27, 36.22, 33.31, 33.26, 27.34, 27.24, 22.95, 22.27, 21.25;
^{31}P NMR (162 MHz, Chloroform-*d*): δ 101.30;
HRMS (ESI-TOF, m/z): Calcd for $[C_{18}H_{25}O_2PS_2+H]^+$ 369.1112; Found 369.1114.

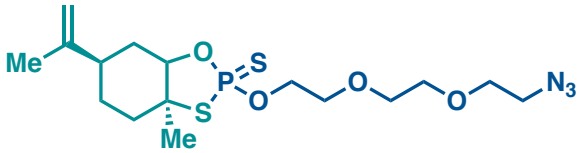
Ψ-2



Prepared according to **General Procedure 1** using ***N*-Boc-L-prolinol** (0.40 g, 2.0 mmol). Purification using a gradient of 50% DCM in hexanes afforded **Ψ-2** (0.431 g, 0.96 mmol, 48% yield).

Physical State: Colorless Oil.
R_f = 0.40 (100% DCM);
^{1}H NMR (600 MHz, Chloroform-*d*): δ 4.99 (q, *J* = 1.5 Hz, 1H), 4.87 (s, 1H), 4.40 (d, *J* = 12.3 Hz, 1H), 4.16 (td, *J* = 9.6, 3.4 Hz, 2H), 3.98 (d, *J* = 63.5 Hz, 1H), 3.46 – 3.23 (m, 2H), 2.62 – 2.51 (m, 1H), 2.28 (dd, *J* = 13.1, 3.7 Hz, 1H), 2.10 (td, *J* = 13.5, 4.2 Hz, 1H), 2.02 – 1.83 (m, 6H), 1.83 – 1.66 (m, 8H), 1.47 (d, *J* = 8.5 Hz, 9H);
^{13}C NMR (151 MHz, Chloroform-*d*): δ 154.66, 154.40, 145.39, 145.20, 111.83, 85.85, 80.03, 79.58, 68.70, 68.65, 68.23, 65.56, 65.37, 56.49, 46.98, 46.82, 39.04, 34.02, 33.96, 28.86, 28.66, 27.82, 27.74, 23.94, 23.57, 23.19, 22.82, 21.82;
^{31}P NMR (162 MHz, Chloroform-*d*): δ 101.67, 101.19;
HRMS (ESI-TOF, m/z): Calcd for $[C_{20}H_{34}NO_4PS_2+H]^+$ 464.1694; Found 464.1689.
.

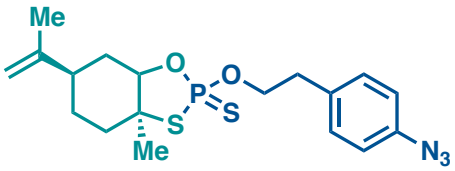
Ψ-3



Prepared according to **General Procedure 1** using **2-(2-(2-azidoethoxy)ethoxy)ethan-1-ol** (0.70 g, 4.0 mmol). Purification using a gradient of 50-100% DCM in hexanes followed by 0-10% EtOAc in DCM afforded **Ψ-3** (0.809 g, 1.92 mmol, 48% yield).

Physical State: Colorless oil;
R_f = 0.70 (100% DCM);
1H NMR (400 MHz, Chloroform-*d*): δ 4.98 (s, 1H), 4.87 (s, 1H), 4.44 (dd, J = 12.8, 3.3 Hz, 1H), 4.41 – 4.32 (m, 1H), 4.32 – 4.20 (m, 1H), 3.80 – 3.60 (m, 9H), 3.39 (t, J = 4.1 Hz, 2H), 2.57 (s, 1H), 2.27 (d, J = 13.6 Hz, 1H), 2.21 – 2.02 (m, 1H), 2.00 – 1.83 (m, 3H), 1.78 (s, 4H), 1.69 (d, J = 2.7 Hz, 3H);
^{13}C NMR (151 MHz, Chloroform-*d*): δ 145.25, 111.86, 85.83, 70.83, 70.79, 70.26, 70.21, 70.17, 67.67, 67.62, 65.20, 50.83, 39.00, 33.86, 33.80, 27.90, 27.79, 23.50, 22.81, 21.80;
^{31}P NMR (162 MHz, Chloroform-*d*): δ 102.15;
HRMS (ESI-TOF, m/z): Calcd for $[C_{16}H_{28}N_3O_4PS_2+H]^+$ 422.1337; Found 422.1327.

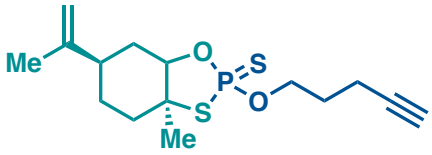
Ψ-4



Prepared according to **General Procedure 1** using **SI-49** (0.326 g, 2.0 mmol). Purification using 15% EtOAc in hexane afforded **Ψ-4** (0.60 g, 1.5 mmol, 75% yield). *Due to the instability of this compound, it was used directly after a crude purification. Crude spectroscopic data could be obtained but not in its pure form.*

Physical State: Amourphous Crystaline Solid;
R_f = 0.56 (15% EtOAc/Hexane);
^{1}H NMR (400 MHz, Chloroform-*d*): δ 7.25 – 7.12 (m, 2H), 7.02 – 6.85 (m, 2H), 4.98 (s, 1H), 4.82 (s, 1H), 4.32 (ddt, J = 17.8, 10.7, 6.6 Hz, 3H), 2.97 (q, J = 5.1, 3.4 Hz, 2H), 2.56 (d, J = 6.4 Hz, 1H), 2.22 (d, J = 13.1 Hz, 1H), 2.05 (ddd, J = 14.2, 10.9, 4.4 Hz, 1H), 1.88 (dtt, J = 18.6, 13.3, 5.3 Hz, 3H), 1.80 – 1.58 (m, 7H).;
^{31}P NMR (162 MHz, Chloroform-*d*): δ 101.32;
HRMS (ESI-TOF, m/z): Calcd for $[C_{18}H_{24}N_3O_2PS_2+H]^+$ 410.1126; Found 410.1135.

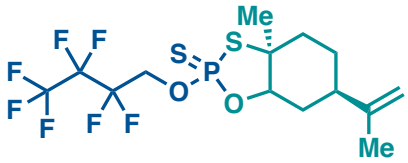
Ψ-5



Prepared according to **General Procedure 1** using **4-pentynol** (1.26 g, 15.0 mmol). Purification using a gradient of 15-20% DCM in hexanes afforded **Ψ-5** (4.56 g, 13.8 mmol, 92%).

Physical State: Colorless Oil;
R_f = 0.46 (50 % DCM in Hexanes);
^{1}H NMR (600 MHz, Chloroform-*d*): δ 5.01 (q, J = 1.5 Hz, 1H), 4.90 – 4.86 (m, 1H), 4.44 (dt, J = 12.7, 3.3 Hz, 1H), 4.32 – 4.25 (m, 1H), 4.27 – 4.20 (m, 1H), 2.58 (t, J = 6.4 Hz, 1H), 2.30 (qd, J = 8.4, 7.7, 2.3 Hz, 3H), 2.11 (td, J = 13.5, 4.3 Hz, 1H), 1.99 – 1.84 (m, 6H), 1.80 – 1.72 (m, 4H), 1.70 (s, 3H);
^{13}C NMR (151 MHz, Chloroform-*d*): δ 144.63, 111.32, 85.22, 82.34, 68.73, 66.76 (d, J = 7.9 Hz), 64.87, 38.44, 33.34 (d, J = 9.0 Hz), 28.54 (d, J = 7.5 Hz), 27.27 (d, J = 15.5 Hz), 22.97, 22.20, 21.24, 14.49;
^{31}P NMR (162 MHz, Chloroform-*d*): δ 101.32;
$[\alpha]_D^{20}$ = -20 (*c* 2.0, $CHCl_3$);
HRMS (ESI-TOF, m/z): Calcd for $[C_{15}H_{23}O_2PS_2+H]^+$ 331.0955; Found 331.0963.

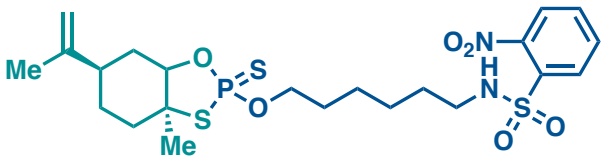
Ψ-6



Prepared according to **General Procedure 1** using **2,2,3,3,4,4,4-heptafluorobutan-1-ol** (0.40 g, 2.0 mmol). Purification using a gradient of 10-20% DCM in hexanes afforded **Ψ-6** (0.249 g, 0.56 mmol, 28% yield).

Physical State: Colorless Oil;
R_f = 0.55 (50% DCM/Hexane);
^{1}H NMR (400 MHz, Chloroform-*d*): δ 5.01 (d, J = 2.3 Hz, 1H), 4.86 (s, 1H), 4.64 (dd, J = 25.1, 12.6 Hz, 1H), 4.57 – 4.44 (m, 2H), 2.60 (s, 1H), 2.31 (d, J = 13.6 Hz, 1H), 2.14 (td, J = 13.3, 4.0 Hz, 1H), 2.04 – 1.65 (m, 10H);
^{13}C NMR (151 MHz, Chloroform-*d*):144.78, 120.53, 118.85, 118.62, 118.40, 116.94, 116.72, 116.50, 115.79, 115.58, 115.53, 115.38, 115.32, 115.04, 114.82, 114.59, 114.08, 114.02, 113.88, 113.82, 113.67, 113.62, 112.37, 112.17, 112.11, 111.96, 110.89, 110.67, 110.41, 109.13, 108.91, 108.68, 108.65, 108.43, 107.37, 107.15, 106.89, 106.67, 86.06, 66.40, 63.13, 63.09, 62.94, 62.90, 62.76, 62.71, 39.01, 33.83, 33.77, 27.75, 27.65, 23.56, 22.54, 21.88;
^{19}F NMR (376 MHz, Chloroform-*d*): δ-81.90 (t, J = 9.2 Hz), -121.62 (dq, J = 11.3, 7.5, 5.7 Hz), -128.39 (d, J = 3.6 Hz);
^{31}P NMR (162 MHz, Chloroform-*d*): δ 103.51;
HRMS (ESI-TOF, m/z): Calcd for $[C_{14}H_{18}F_7O_2PS_2+H]^+$ 447.0452; Found 447.0458.

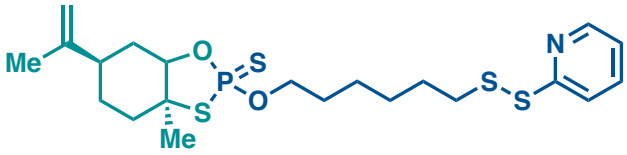
Ψ-7



Prepared according to **General Procedure 1** using **SI-51** (0.90 g, 3.0 mmol). Purification using 25% EtOAc in hexanes afforded **Ψ-7** (1.0 g, 1.82 mmol, 61% yield).

Physical State: colorless solid;
R_f = 0.28 (30% EtOAc/Hexane);
^{1}H NMR (400 MHz, Chloroform-*d*): 8.17 – 8.11 (m, 1H), 7.90 – 7.84 (m, 1H), 7.80 – 7.72 (m, 2H), 5.25 (s, 1H), 4.99 (s, 1H), 4.86 (s, 1H), 4.45 – 4.37 (m, 1H), 4.13 – 4.10 (m, 2H), 3.10 (q, J = 6.8 Hz, 2H), 2.58 (s, 1H), 2.27 (d, J = 13.5 Hz, 1H), 2.15 – 2.05 (m, 1H), 1.90 (td, J = 21.4, 18.7, 14.3 Hz, 4H), 1.78 (s, 3H), 1.69 (d, J = 2.0 Hz, 3H), 1.62 (d, J = 6.5 Hz, 2H), 1.53 (d, J = 7.0 Hz, 2H), 1.33 (s, 4H);
^{13}C NMR (151 MHz, Chloroform-*d*):148.22, 145.25, 133.84, 133.73, 132.95, 131.21, 125.54, 111.83, 85.79, 68.72, 68.67, 65.41, 43.85, 39.00, 33.92, 33.86, 29.99, 29.95, 29.58, 27.91, 27.80, 26.08, 25.13, 23.52, 22.79, 21.80;
^{31}P NMR (162 MHz, Chloroform-*d*): δ 100.65;
HRMS (ESI-TOF, m/z): Calcd for $[C_{22}H_{33}N_2O_6PS_3+H]^+$ 549.1317; Found 549.1326.

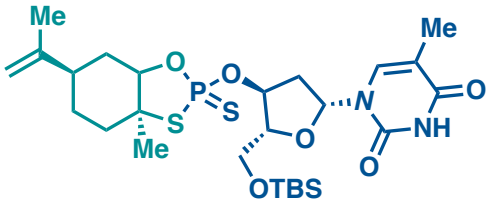
Ψ-8



Prepared according to **General Procedure 1** using **SI-50** (1.35 g, 4.67 mmol). Purification using 100% DCM afforded **Ψ-8** (1.54 g, 3.15 mmol, 68% yield).

Physical State: Light Yellow Oil;
R_f = 0.50 (100% DCM);
^{1}H NMR (600 MHz, Chloroform-*d*): δ 8.45 (ddd, J = 4.9, 1.9, 0.9 Hz, 1H), 7.71 (dt, J = 8.1, 1.1 Hz, 1H), 7.64 (td, J = 7.7, 1.8 Hz, 1H), 7.08 (ddd, J = 7.4, 4.8, 1.1 Hz, 1H), 4.98 (q, J = 1.4 Hz, 1H), 4.87 – 4.84 (m, 1H), 4.42 (dt, J = 12.8, 3.3 Hz, 1H), 4.19 – 4.07 (m, 2H), 2.79 (t, J = 7.3 Hz, 2H), 2.57 (s, 1H), 2.27 (ddt, J = 15.0, 3.2, 1.7 Hz, 1H), 2.09 (td, J = 13.5, 4.2 Hz, 1H), 1.98 – 1.91 (m, 1H), 1.91 – 1.82 (m, 2H), 1.79 – 1.73 (m, 4H), 1.73 – 1.63 (m, 7H), 1.42 (pd, J = 7.1, 3.0 Hz, 2H), 1.41 – 1.31 (m, 2H);
^{13}C NMR (151 MHz, Chloroform-*d*): δ 160.67, 149.73, 145.22, 137.10, 120.67, 119.72, 111.86, 85.71, 68.86 (d, J = 8.2 Hz), 65.35, 38.96 (d, J = 14.8 Hz), 33.90 (d, J = 8.8 Hz), 30.06 (d, J = 6.8 Hz), 28.86, 28.07, 27.91, 27.81, 25.30, 23.54, 22.81, 21.80;
^{31}P NMR (162 MHz, Chloroform-*d*): δ 101.01;
HRMS (ESI-TOF, m/z): Calcd for $[C_{21}H_{32}NO_2PS_4+H]^+$ 490.1132; Found 490.1140.

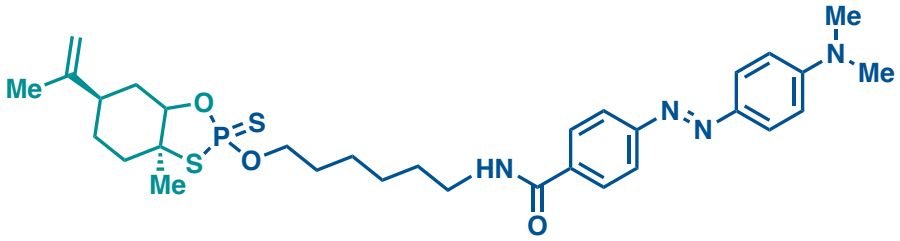
Ψ-9



Prepared according to **General Procedure 1** using **SI-54** (1.0 g, 4.67 mmol). Purification using a gradient of 20-30% EtOAc in hexanes afforded **Ψ-9** (0.80 g, 1.3 mmol, 28% yield).

Physical State: White foam;
R_f = 0.52 (50% EtOAc/hexanes)
^{1}H NMR (400 MHz, Chloroform-*d*): δ 8.41 (s, 1H), 7.54 (s, 1H), 6.39 (dd, J = 8.9, 5.5 Hz, 1H), 5.34 (dd, J = 11.7, 5.7 Hz, 1H), 5.07 (s, 1H), 4.92 (s, 1H), 4.47 (d, J = 12.7 Hz, 1H), 4.26 (s, 1H), 3.97 – 3.85 (m, 2H), 2.59 (s, 1H), 2.49 (dd, J = 13.8, 5.5 Hz, 1H), 2.33 (d, J = 13.5 Hz, 1H), 2.23 – 2.07 (m, 2H), 2.04 – 1.86 (m, 6H), 1.80 (s, 3H), 1.77 – 1.60 (m, 4H), 0.92 (t, J = 2.7 Hz, 9H), 0.13 (t, J = 2.8 Hz, 6H);
^{13}C NMR (151 MHz, Chloroform-*d*): δ 163.73, 150.33, 144.77, 135.24, 112.34, 111.34, 86.26, 86.22, 86.17, 86.04, 84.78, 79.88, 79.83, 66.05, 63.55, 39.72, 39.69, 38.96, 33.81, 33.74, 28.32, 27.90, 27.80, 26.09, 23.50, 22.77, 21.89, 18.48, 12.62, -5.23, -5.25, -5.42;
^{31}P NMR (162 MHz, Chloroform-*d*): δ 101.18.
HRMS (ESI-TOF, m/z): Calcd for $[C_{26}H_{43}N_2O_6PS_2Si+H]^+$ 603.2148; Found 603.2147.

Ψ-10



Prepared according to **General Procedure 1** using **SI-56** (0.368 g, 1.0 mmol). Purification using a gradient of 20-50% EtOAc in hexanes afforded **Ψ-10** (0.382 g, 0.62 mmol, 62% yield).

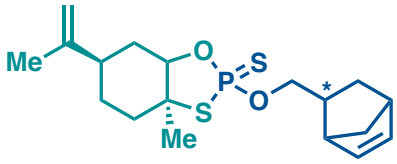
Physical State: dark orange foam;
R_f = 0.58 (50% EtOAc/Hexane);
^{1}H NMR (400 MHz, Chloroform-*d*): δ 8.06 – 7.68 (m, 6H), 6.76 (d, J = 8.6 Hz, 2H), 6.19 (s, 1H), 5.00 (s, 1H), 4.87 (s, 1H), 4.44 (d, J = 12.9 Hz, 1H), 4.17 (t, J = 7.9 Hz, 2H), 3.48 (d, J = 6.9 Hz, 3H), 3.11 (p, J = 1.9 Hz, 6H), 2.57 (s, 1H), 2.29 (s, 1H), 2.16 – 2.03 (m, 1H), 1.92 (dd, J = 25.4, 14.3 Hz, 2H), 1.71 (dd, J = 32.2, 18.3 Hz, 10H), 1.45 (s, 5H);
^{13}C NMR (151 MHz, Chloroform-*d*): δ 167.17, 154.94, 152.98, 145.23, 143.73, 134.92, 127.90, 125.67, 122.34, 111.89, 111.73, 85.78, 68.90, 68.85, 65.38, 40.50, 40.13, 39.02, 33.93, 33.88, 30.11, 30.07, 29.73, 27.93, 27.82, 26.58, 25.39, 23.54, 22.81, 21.81;
^{31}P NMR (162 MHz, Chloroform-*d*): δ 101.78;
HRMS (ESI-TOF, m/z): Calcd for $[C_{31}H_{43}N_4O_3PS_2+H]^+$ 615.2592; Found 615.2593.

Ψ-11

Prepared according to **General Procedure 1** using **SI-57** (0.292 g, 1.0 mmol). Purification using a gradient of 75-100% EtOAc in hexanes afforded **Ψ-11** (0.35 g, 0.65 mmol, 65% yield).

Physical State: Amourphous solid;
R_f = 0.32 (100% EtOAc);
^{1}H NMR (400 MHz, Chloroform-*d*): δ 8.52 (s, 1H), 7.94 (d, J = 9.0 Hz, 1H), 7.17 (d, J = 8.9 Hz, 1H), 6.24 (s, 1H), 4.97 (s, 1H), 4.84 (s, 1H), 4.40 (d, J = 12.8 Hz, 1H), 4.13 – 4.09 (m, 2H), 3.40 (q, J = 7.0 Hz, 2H), 2.55 (s, 1H), 2.25 (d, J = 13.6 Hz, 1H), 2.04 (s, 3H), 1.87 (d, J = 15.8 Hz, 6H), 1.71 (d, J = 34.1 Hz, 8H), 1.59 (s, 2H), 1.39 (d, J = 6.5 Hz, 4H) ;
^{13}C NMR (151 MHz, Chloroform-*d*): δ 165.64, 158.20, 152.28, 148.76, 147.96, 146.17, 145.22, 137.73, 135.23, 123.65, 121.76, 111.88, 107.05, 85.78, 68.90, 68.84, 65.38, 39.97, 39.02, 33.93, 33.87, 30.09, 30.05, 29.73, 27.92, 27.82, 26.54, 25.48, 25.35, 23.54, 22.81, 21.81, 16.36;
^{31}P NMR (162 MHz, Chloroform-*d*): δ 101.18;
HRMS (ESI-TOF, m/z): Calcd for $[C_{25}H_{39}N_4O_3PS_2+H]^+$ 539.2279; Found 539.2280.

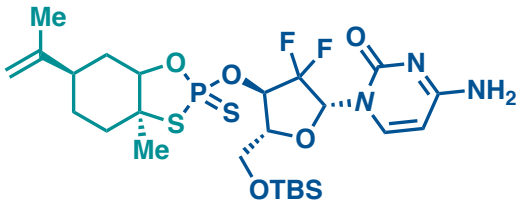
Ψ-12



Prepared according to **General Procedure 1** using **5-Norbornene-2-methanol** (1.24 g, 10 mmol) with the following modifications : The crude reaction was diluted with hexanes (50 mL) then passed through a plug of SiO_2 and concentrated to dryness, the solvent was swapped to methanol and upon standing overnight the product crystalized. Isolation by filtration to afford **Ψ-12** (1.56g, 4.2 mmol, 42% yield) as a mixture of endo and exo isomers.

Physical State: Crystaline solid;
^{1}H NMR (400 MHz, Chloroform-*d*): δ 6.16 (dt, J = 6.2, 3.2 Hz, 2H), 6.09 (s, 2H), 6.02 – 5.96 (m, 2H), 5.01 (s, 3H), 4.89 (s, 3H), 4.52 – 4.42 (m, 3H), 4.05 – 3.91 (m, 1H), 3.88 (d, J = 7.4 Hz, 1H), 3.72 (dt, J = 35.2, 11.4 Hz, 2H), 2.90 (d, J = 8.7 Hz, 2H), 2.83 (s, 2H), 2.74 (s, 1H), 2.59 (s, 3H), 2.47 (s, 2H), 2.29 (d, J = 13.4 Hz, 3H), 2.15 (s, 1H), 2.11 (d, J = 13.5 Hz, 2H), 1.98 (s, 1H), 1.96 – 1.83 (m, 7H), 1.54 (s, 3H), 1.46 (d, J = 8.5 Hz, 2H), 1.33 (d, J = 9.2 Hz, 2H), 1.26 (d, J = 8.3 Hz, 3H), 1.18 (d, J = 15.4 Hz, 1H), 0.54 (t, J = 12.2 Hz, 2H).
^{13}C NMR (151 MHz, $CDCl_3$): δ 144.67, 144.65, 144.64, 144.61, 137.32, 137.16, 136.64, 136.59, 135.73, 135.68, 131.75, 131.63, 111.28, 85.02, 84.99, 84.96, 84.92, 72.36, 72.30, 72.25, 72.19, 71.65, 71.60, 71.54, 64.83, 64.81, 64.77, 64.74, 48.85, 44.54, 44.50, 43.36, 43.31, 43.09, 42.97, 41.85, 41.81, 41.19, 41.15, 38.99, 38.95, 38.88, 38.84, 38.82, 38.77, 38.72, 38.48, 38.46, 33.40, 33.39, 33.36, 33.34, 33.33, 33.31, 28.90, 28.26, 28.16, 27.34, 27.32, 27.31, 27.24, 27.22, 27.20, 22.99, 22.19, 22.17, 21.26, 21.24.
^{31}P NMR (162 MHz, Acetone): δ 101.15, 100.93, 100.64, 100.55;
HRMS (ESI-TOF, m/z): Calcd for $[C_{18}H_{27}O_2PS_2+H]^+$ 371.1268; Found 371.1273.

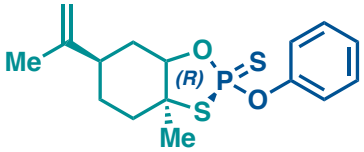
Ψ-13



Prepared according to **General Procedure 1** using **SI-55** (0.377 g, 1.0 mmol). Purification using a gradient of 70-100% EtOAc in hexanes afforded **Ψ-13** (0.35 g, 0.56 mmol, 56% yield).

Physical State: White foam;
R_f = 0.54 (100% EtOAc);
^{1}H NMR (600 MHz, Chloroform-*d*): δ 7.71 (d, J = 7.6 Hz, 1H), 6.40 (t, J = 7.8 Hz, 1H), 5.81 (d, J = 7.6 Hz, 1H), 5.32 (dtd, J = 13.9, 10.2, 7.4 Hz, 1H), 5.02 (s, 1H), 4.86 (d, J = 1.9 Hz, 1H), 4.53 (dt, J = 12.8, 3.5 Hz, 1H), 4.11 (dd, J = 7.3, 2.3 Hz, 1H), 3.98 (dd, J = 11.9, 2.2 Hz, 1H), 3.86 (dd, J = 11.9, 2.2 Hz, 1H), 2.57 (d, J = 6.0 Hz, 1H), 2.30 (dd, J = 13.6, 3.0 Hz, 1H), 2.14 (td, J = 13.5, 4.2 Hz, 1H), 1.97 (ddt, J = 17.1, 4.4, 2.0 Hz, 1H), 1.90 (dt, J = 13.2, 3.4 Hz, 1H), 1.84 (td, J = 13.0, 5.6 Hz, 1H), 1.71 (d, J = 47.0 Hz, 7H), 0.92 (s, 9H), 0.12 (d, J = 11.8 Hz, 6H);
^{13}C NMR (151 MHz, Chloroform-*d*): δ 166.38, 164.46, 154.10, 143.92, 140.83, 122.42, 120.69, 118.95, 111.60, 95.04, 85.28, 83.80, 83.59, 83.38, 79.55, 79.52, 79.49, 71.97, 71.92, 71.81, 71.76, 71.65, 71.60, 66.44, 59.60, 38.35, 33.16, 33.10, 27.12, 27.02, 25.44, 22.95, 21.97, 21.44, 17.87, -5.76, -5.97;
^{19}F NMR (376 MHz, Chloroform-*d*): δ -115.77;
^{31}P NMR (162 MHz, Chloroform-*d*): δ 103.96;
HRMS (ESI-TOF, m/z): Calcd for $[C_{25}H_{40}F_2N_3O_5PS_2Si+H]^+$ 624.1963; Found 624.1965.

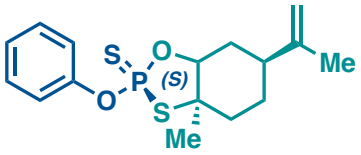
Ψ-(*R*P)-14



Prepared according to **General Procedure 1** using **phenol** (0.94 g, 10 mmol) with the following modifications: After the standard workup, the crude reaction was solvent swapped to methanol (50 mL) and upon standing for several hours the product crystalized. Isolation by filtration afforded **Ψ-(*R*P)-14** (2.21 g, 6.5 mmol, 65% yield).

Physical State: crystalline colorless solid;
^{1}H NMR (600 MHz, Chloroform-*d*): δ 7.34 (t, *J* = 7.9 Hz, 2H), 7.20 (td, *J* = 7.4, 1.4 Hz, 1H), 7.20 – 7.13 (m, 2H), 4.90 (q, *J* = 1.5 Hz, 1H), 4.76 (d, *J* = 1.9 Hz, 1H), 4.37 (dt, *J* = 12.8, 3.3 Hz, 1H), 2.56 (t, *J* = 6.2 Hz, 1H), 2.32 – 2.25 (m, 1H), 2.05 (td, *J* = 13.7, 13.0, 4.7 Hz, 1H), 1.96 – 1.84 (m, 3H), 1.78 – 1.69 (m, 7H);
^{13}C NMR (151 MHz, Chloroform-*d*): δ 150.82, 150.74, 144.75, 129.64, 129.63, 125.67, 125.66, 121.71, 121.68, 111.86, 86.57, 86.55, 65.80, 38.85, 33.83, 33.78, 27.74, 27.64, 23.41, 22.68, 21.87;
^{31}P NMR (162 MHz, Chloroform-*d*): δ 96.40 ;
HRMS (ESI-TOF, m/z): Calcd for $[C_{16}H_{21}O_2PS_2+H]^+$ 341.0799; Found 341.0800.

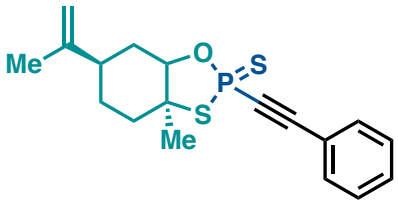
Ψ-(*S*P)-14



Prepared according to **General Procedure 1** using **phenol** (0.94 g, 10 mmol) with the following modifications: Using (+)-Ψ (CAS Number : 2245335-71-9), after the standard workup, the crude reaction was solvent swapped to methanol (50 mL) and upon standing for several hours the product crystalized. Isolation by filtration afforded **Ψ-(*S*P)-14** (2.38 g, 7.0 mmol, 70% yield).

The product had identical characterization data to Ψ-(*R*P)-14.

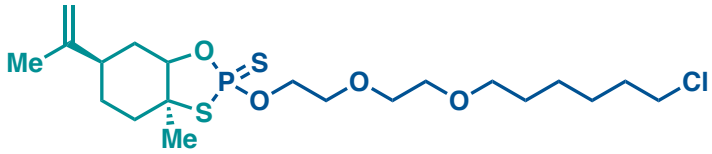
Ψ-15



To a solution of (–)-Ψ (0.447 g, 1.0 mmol, 1 equiv.) in THF (10 mL) was added phenylethynylmagnesium bromide **(**2.0 mL, 2.0 mmol, 2.0 equiv., 1.0 m solution, THF). After stirring for 2 hours the reaction was diluted with 1 :1 Et_2O/hexanes (50 mL) and washed consecutively with water, KH_2PO_4 (saturated aqueous) and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated to a yellow oil. Purification in 10% Et_2O in hexanes afforded **Ψ-15** (0.25 g, 0.72 mmol, 72% yield).

Physical State: yellow amorphous solid;
R_f = 0.4 (10% Et_2O/hexanes);
^{1}H NMR (600 MHz, Chloroform-*d*): δ 7.54 (dd, *J* = 7.1, 2.0 Hz, 2H), 7.44 (td, *J* = 7.4, 1.9 Hz, 1H), 7.37 (td, *J* = 7.8, 2.0 Hz, 2H), 5.02 – 4.97 (m, 1H), 4.91 (s, 1H), 4.56 (ddt, *J* = 12.8, 6.1, 2.8 Hz, 1H), 2.61 (d, *J* = 6.4 Hz, 1H), 2.41 – 2.35 (m, 1H), 2.19 – 2.08 (m, 1H), 1.97 (td, *J* = 11.0, 9.3, 3.4 Hz, 3H), 1.83 – 1.75 (m, 1H), 1.80 (s, 3H), 1.71 (s, 1H);
^{13}C NMR (151 MHz, Chloroform-*d*): δ 145.29, 132.59, 132.57, 130.84, 128.64, 119.88, 119.85, 111.85, 101.85, 101.60, 86.73, 86.62, 85.33, 64.71, 38.98, 33.99, 33.93, 28.36, 28.26, 23.63, 22.84, 22.54;
^{31}P NMR (162 MHz, Chloroform-*d*): δ 61.01;
HRMS (ESI-TOF, m/z): Calcd for $[C_{18}H_{21}OPS_2+H]^+$ 349.0850; Found 349.0844.

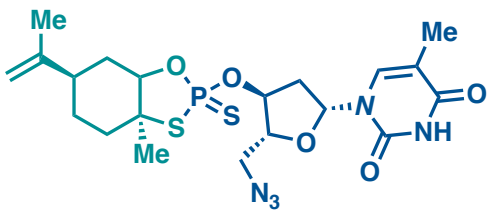
Ψ-16



Prepared according to **General Procedure 1** using 2-(2-((6-chlorohexyl)oxy)ethoxy)ethan-1-ol (0.224 g, 1.00 mmol). Purified in 10-20% EtOAc in hexanes (0.146 g, 0.31 mmol, 31% yield).

Physical State: Yellow Oil
R_f = 0.33 (15% EtOAc/Hexanes)
^{1}H NMR (600 MHz, Chloroform-*d*): δ 4.97 (q, *J* = 1.5 Hz, 1H), 4.85 (d, *J* = 2.0 Hz, 1H), 4.43 (dt, *J* = 12.7, 3.2 Hz, 1H), 4.33 (dddd, *J* = 13.3, 11.4, 6.2, 4.0 Hz, 1H), 4.25 (dddd, *J* = 12.6, 11.4, 5.5, 3.9 Hz, 1H), 3.76 – 3.66 (m, 2H), 3.67 – 3.58 (m, 2H), 3.56 (dd, *J* = 5.5, 4.2 Hz, 2H), 3.52 (t, *J* = 6.7 Hz, 2H), 3.45 (t, *J* = 6.7 Hz, 2H), 2.59 – 2.54 (m, 1H), 2.26 (ddq, *J* = 13.5, 3.4, 1.7 Hz, 1H), 2.08 (td, *J* = 13.5, 4.3 Hz, 1H), 1.93 (ddt, *J* = 14.9, 4.4, 2.2 Hz, 1H), 1.92 – 1.82 (m, 2H), 1.80 – 1.71 (m, 6H), 1.68 (s, 3H), 1.62 – 1.52 (m, 2H), 1.48 – 1.40 (m, 2H), 1.42 – 1.32 (m, 2H);
^{13}C NMR (151 MHz, Chloroform-*d*): δ 145.18, 111.85, 85.76, 71.38, 70.72, 70.19, 70.09, 70.05, 67.63, 67.58, 65.18, 45.17, 38.97, 33.83, 33.77, 32.63, 29.56, 27.86, 27.76, 26.79, 25.52, 23.47, 22.78, 21.78;
^{31}P NMR (162 MHz, Chloroform-*d*): δ 102.07;
HRMS (ESI-TOF, m/z): Calcd for $[C_{20}H_{36}ClO_4PS_2+H]^+$ 471.1559; Found 471.1553

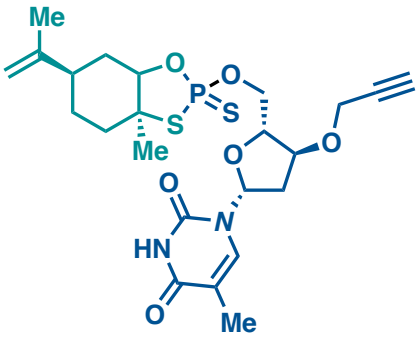
Ψ-17



Prepared according to **General Procedure 1** using **SI-58** (1.4 g, 5.24 mmol). Purified in 30-50% EtOAc in hexanes (2.3 g, 4.48 mmol, 85% yield).

Physical State: white foam;
R_f = 0.66 (60% EtOAc/Hexanes);
^{1}H NMR (600 MHz, Chloroform-*d*): δ 8.34 (s, 1H), 7.36 (q, *J* = 1.3 Hz, 1H), 6.35 (dd, *J* = 8.6, 5.7 Hz, 1H), 5.26 (ddt, *J* = 11.5, 6.7, 2.4 Hz, 1H), 5.06 (q, *J* = 1.4 Hz, 1H), 4.92 – 4.88 (m, 1H), 4.45 (dt, *J* = 12.7, 3.2 Hz, 1H), 4.26 (q, *J* = 3.0 Hz, 1H), 3.80 – 3.70 (m, 2H), 2.60 (t, *J* = 6.1 Hz, 1H), 2.49 (ddd, *J* = 14.2, 5.7, 2.1 Hz, 1H), 2.35 – 2.23 (m, 2H), 2.13 (td, *J* = 13.5, 4.2 Hz, 1H), 1.98 – 1.85 (m, 6H), 1.80 (s, 3H), 1.80 – 1.72 (m, 1H), 1.70 (s, 3H);
^{13}C NMR (151 MHz, Chloroform-*d*): δ 162.71, 149.64, 144.28, 134.36, 111.67, 111.36, 85.74, 83.88, 82.59, 82.55, 77.86, 77.81, 65.68, 51.75, 38.36, 38.16, 38.13, 33.27, 33.21, 27.31, 27.21, 22.93, 22.20, 21.32, 12.26;
^{31}P NMR (162 MHz, Chloroform-*d*): δ 102.22 ;
HRMS (ESI-TOF, m/z): Calcd for $[C_{20}H_{28}N_5O_5PS_2+H]^+$ 514.1348; Found 514.1350

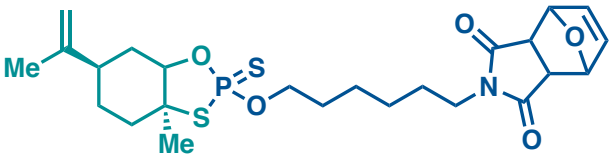
Ψ-18



Prepared according to **General Procedure 1** using **SI-59** (1.4 g, 5.00 mmol). Purified in 50% EtOAc in hexanes (2.13 g, 4.05 mmol, 83% yield).

Physical State: white foam;
R_f = 0.27 (50% EtOAc/Hexanes);
^{1}H NMR (600 MHz, Chloroform-*d*): δ 8.30 (s, 1H), 7.44 (q, *J* = 1.2 Hz, 1H), 6.30 (dd, *J* = 8.4, 5.7 Hz, 1H), 4.97 (q, *J* = 1.5 Hz, 1H), 4.83 (dd, *J* = 2.1, 1.1 Hz, 1H), 4.51 (ddd, *J* = 12.5, 11.5, 3.4 Hz, 1H), 4.43 (ddd, *J* = 12.8, 3.6, 2.5 Hz, 1H), 4.40 – 4.30 (m, 2H), 4.28 – 4.23 (m, 1H), 4.23 – 4.18 (m, 1H), 4.21 – 4.09 (m, 1H), 2.60 (s, 1H), 2.51 – 2.44 (m, 2H), 2.28 (ddt, *J* = 13.4, 3.2, 1.7 Hz, 1H), 2.08 (td, *J* = 13.4, 4.2 Hz, 1H), 2.04 (s, 1H), 2.01 – 1.93 (m, 5H), 1.95 – 1.90 (m, 1H), 1.92 – 1.85 (m, 1H), 1.82 – 1.72 (m, 7H);
^{13}C NMR (151 MHz, Chloroform-*d*): δ 163.60, 150.04, 144.52, 134.86, 111.34, 110.95, 85.99, 84.42, 82.32, 82.27, 78.52, 78.01, 75.10, 67.08, 67.02, 65.50, 56.49, 38.34, 36.93, 33.31, 33.26, 27.27, 27.17, 22.85, 22.14, 21.23, 12.31;
^{31}P NMR (162 MHz, Chloroform-*d*): δ 102.69;
HRMS (ESI-TOF, m/z): Calcd for $[C_{23}H_{31}N_2O_6PS_2+H]^+$ 527.1439; Found 527.1431

Ψ-19

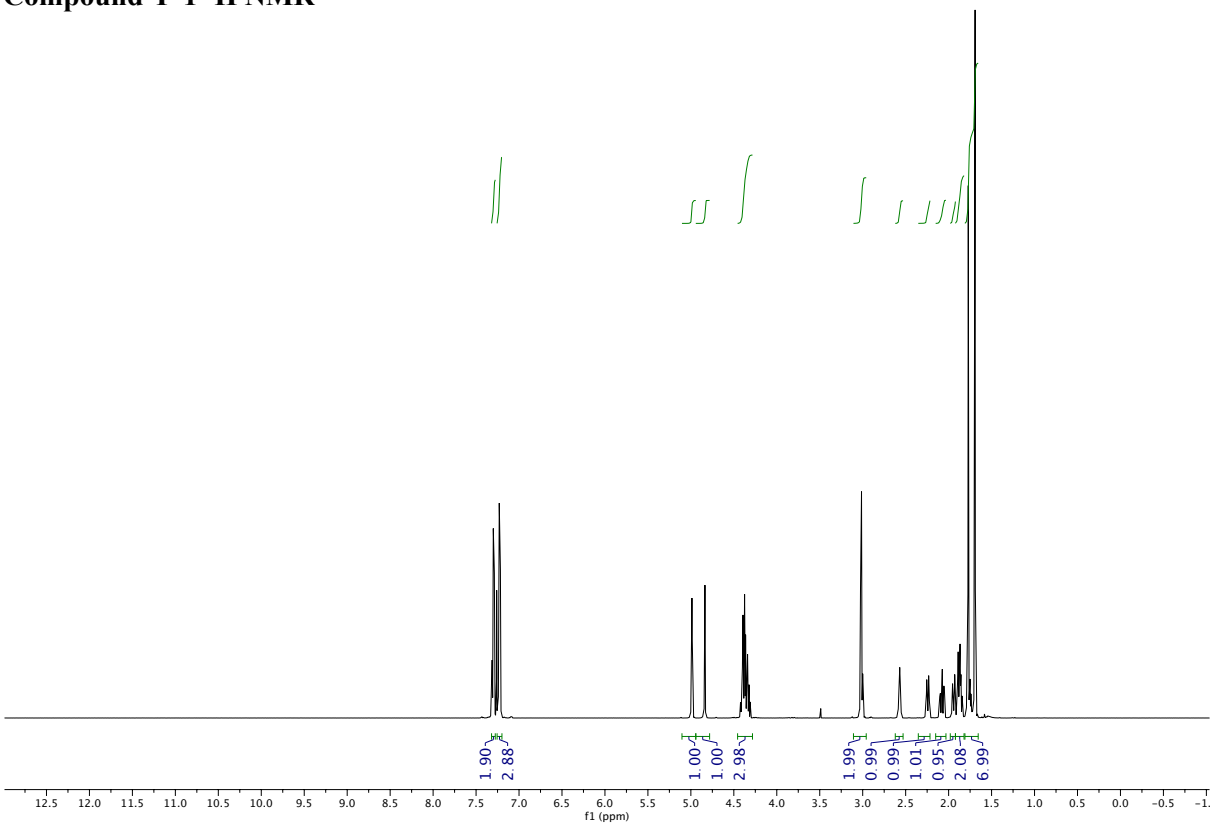


Prepared according to **General Procedure 1** using **SI-60** (0.60 g, 2.26 mmol). Purified in 50% EtOAc in hexanes (0.769 g, 1.50 mmol, 66% yield).

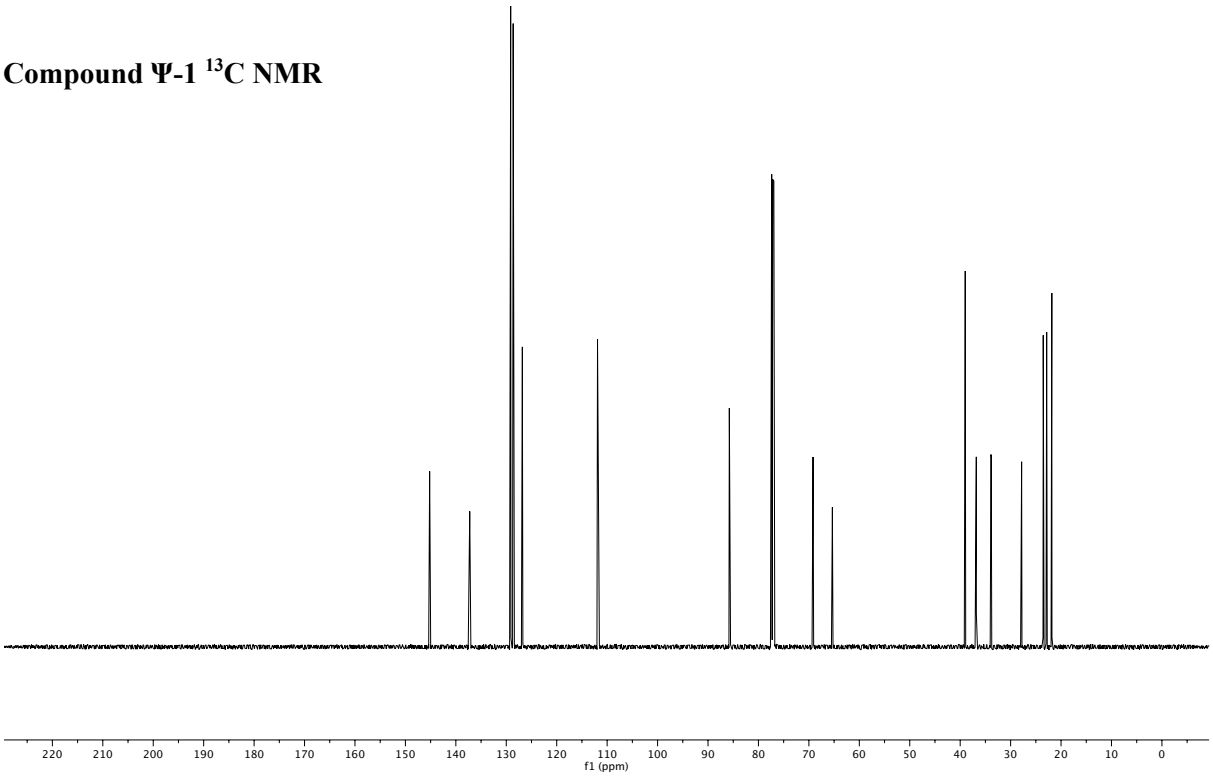
Physical State: yellow oil;
R_f = 0.42 (50% EtOAc/hexanes);
^{1}H NMR (600 MHz, Chloroform-*d*): δ 6.50 (s, 2H), 5.25 (s, 2H), 4.99 (s, 1H), 4.90 – 4.82 (m, 1H), 4.41 (dt, *J* = 12.8, 3.3 Hz, 1H), 4.12 (m, 2H), 3.46 (t, *J* = 7.3 Hz, 2H), 2.83 (s, 2H), 2.60 – 2.53 (m, 1H), 2.27 (m, 1H), 2.09 (td, *J* = 13.5, 4.3 Hz, 1H), 1.94 (m, 1H), 1.90 – 1.81 (m, 2H), 1.80 – 1.70 (m, 4H), 1.70 – 1.61 (m, 5H), 1.55 (m, 2H), 1.40 – 1.33 (m, 2H), 1.29 (m, 2H);
^{13}C NMR (151 MHz, Chloroform-*d*): δ 176.4, 145.2, 136.7, 111.9, 85.7, 81.0, 68.9, 68.8, 65.3, 47.5, 39.0, 38.9, 33.9, 33.9, 30.0, 30.0, 27.9, 27.8, 27.6, 26.3, 25.2, 23.5, 22.8, 21.8;
^{31}P NMR (162 MHz, Chloroform-*d*): δ 101.03;
HRMS (ESI-TOF, m/z): Calcd for $[C_{24}H_{34}NO_5PS_2+H]^+$ 512.1694; Found 512.1694

NMR Spectra

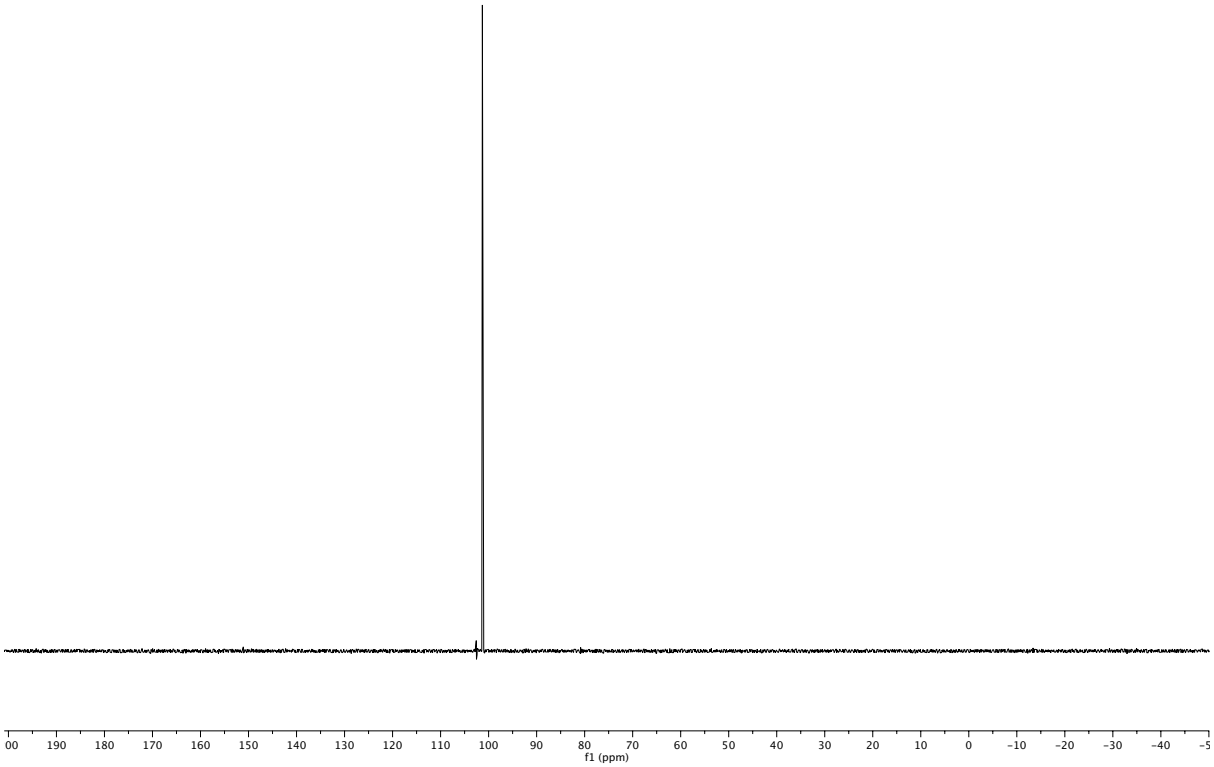
Compound Ψ-1 ^{1}H NMR



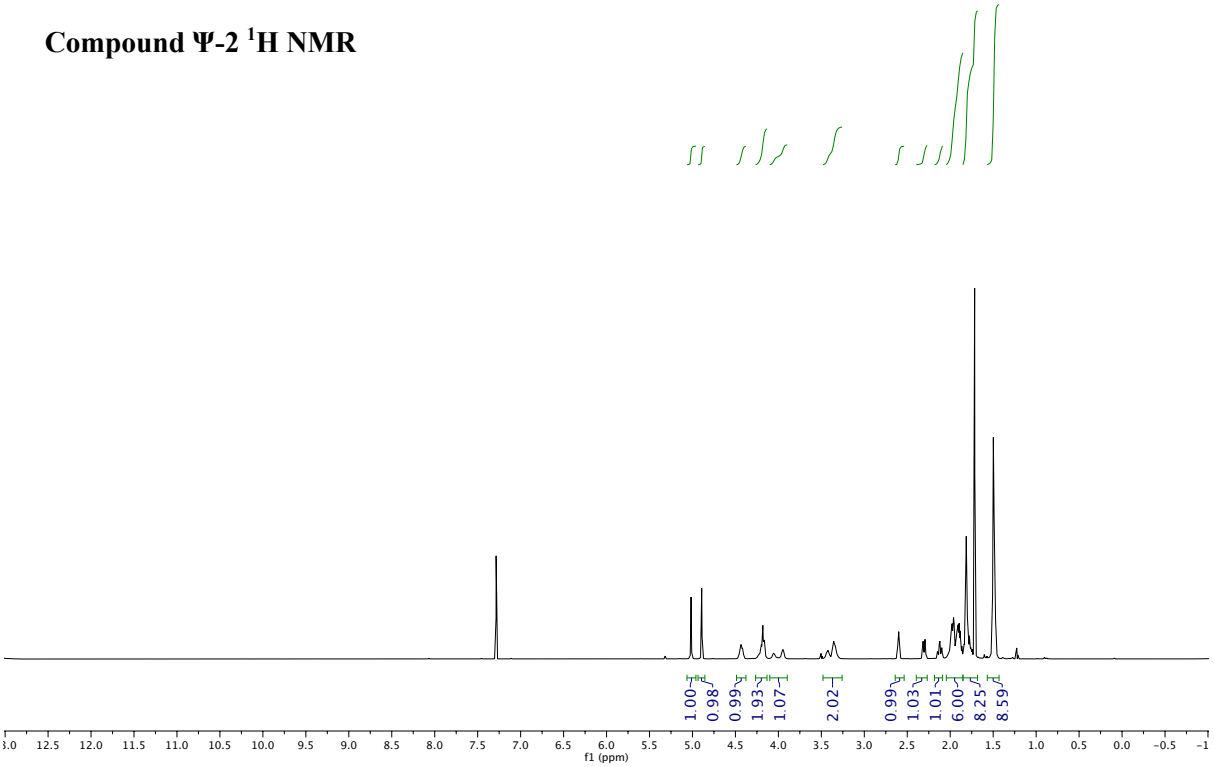
Compound Ψ-1 ^{13}C NMR



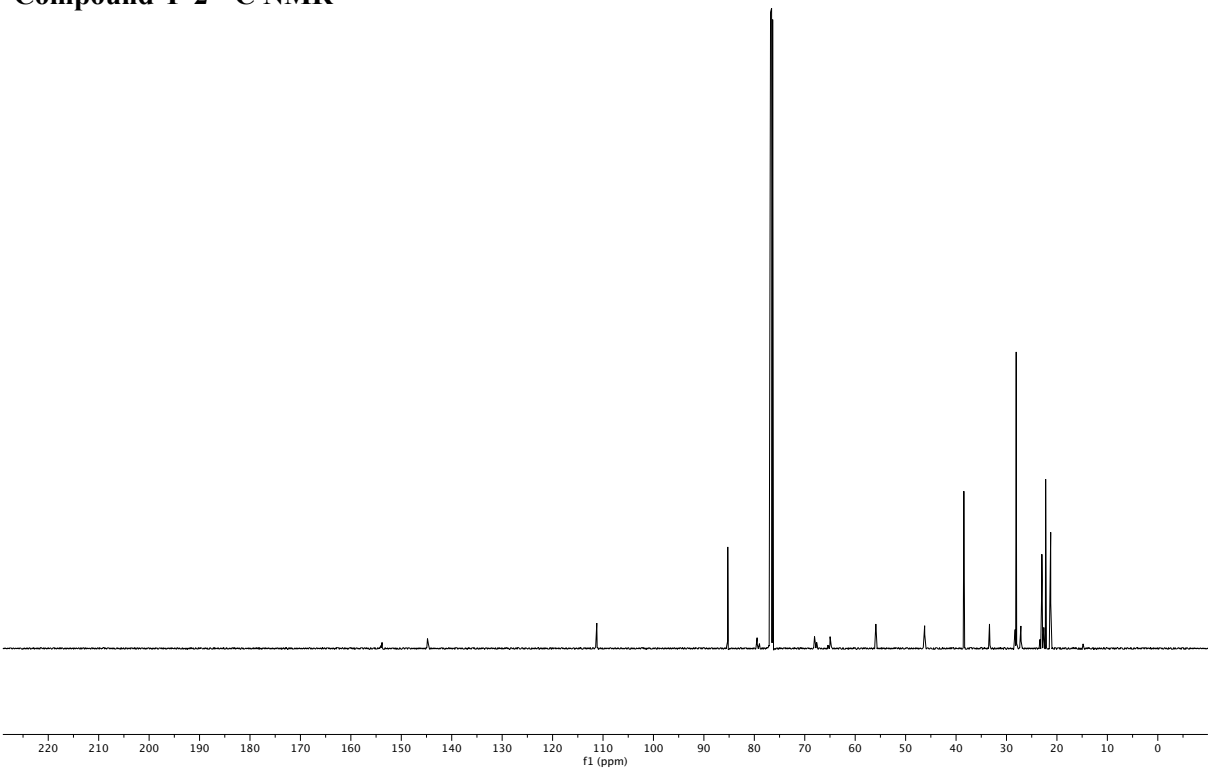
Compound Ψ-1 ^{31}P NMR



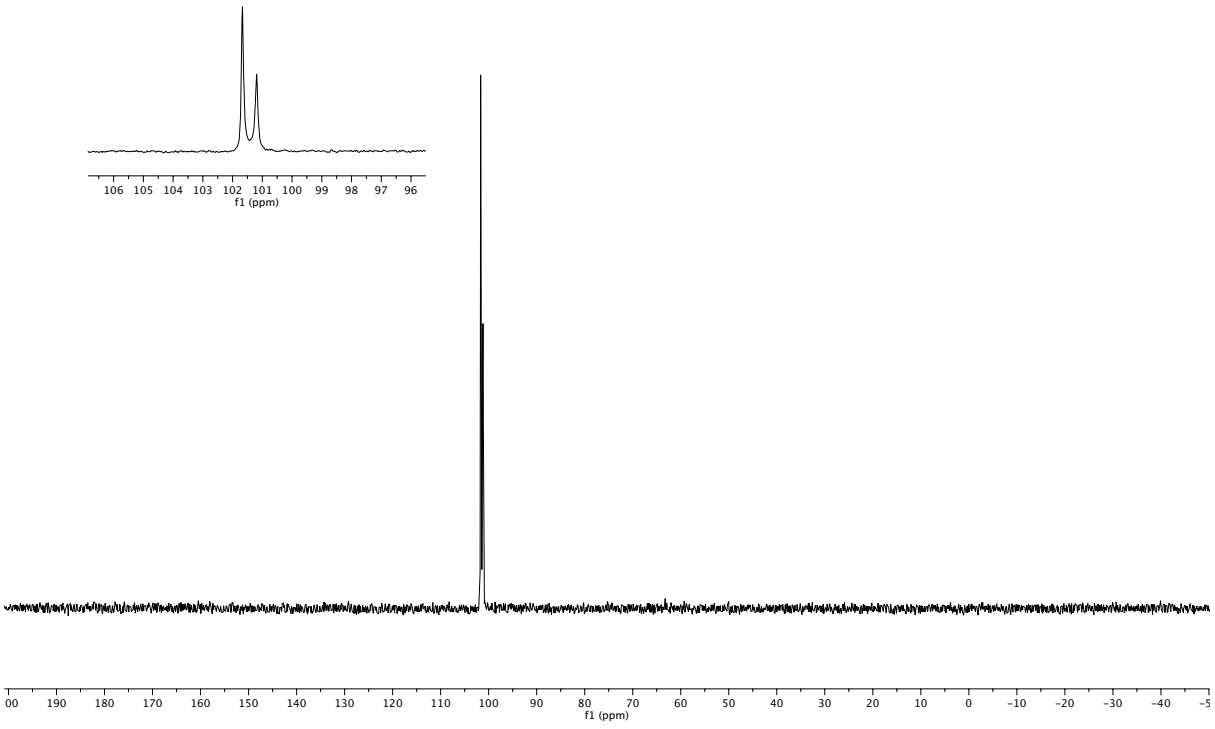
Compound Ψ-2 ^{1}H NMR



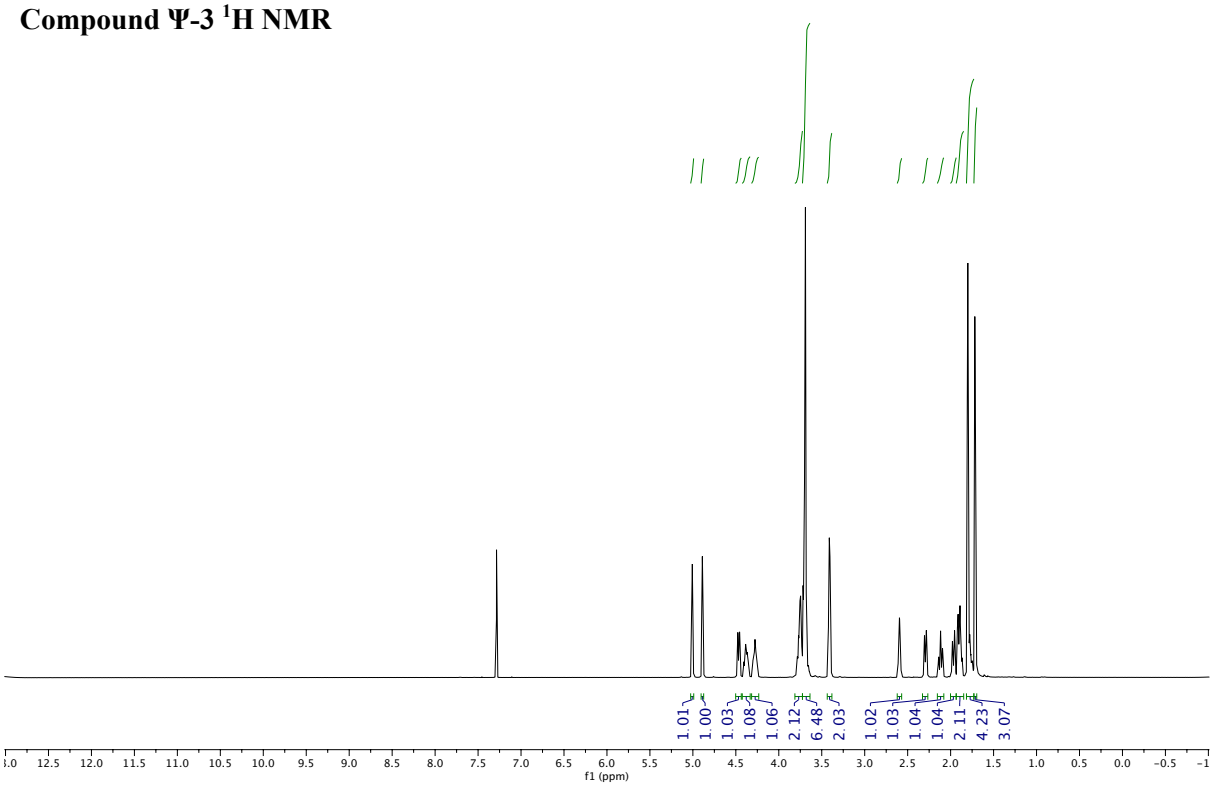
Compound Ψ-2 ^{13}C NMR



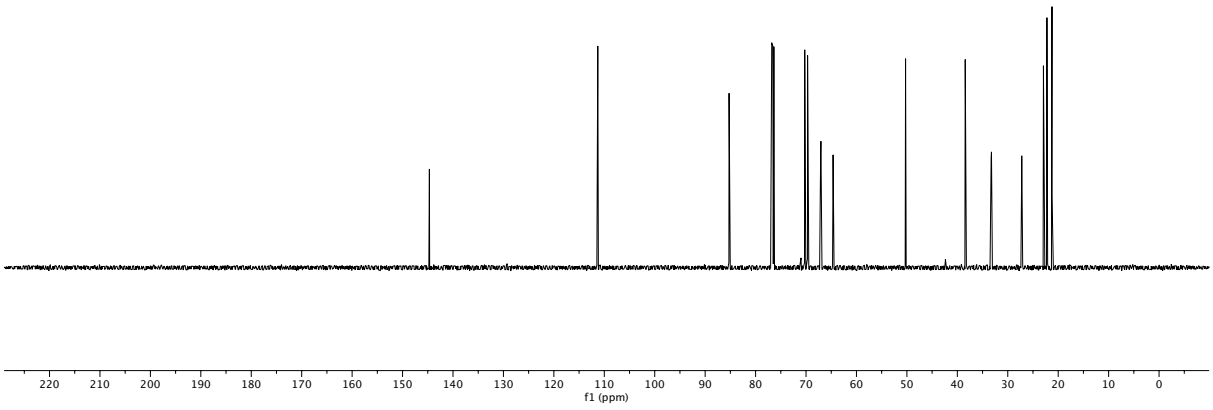
Compound Ψ-2 ^{31}P NMR



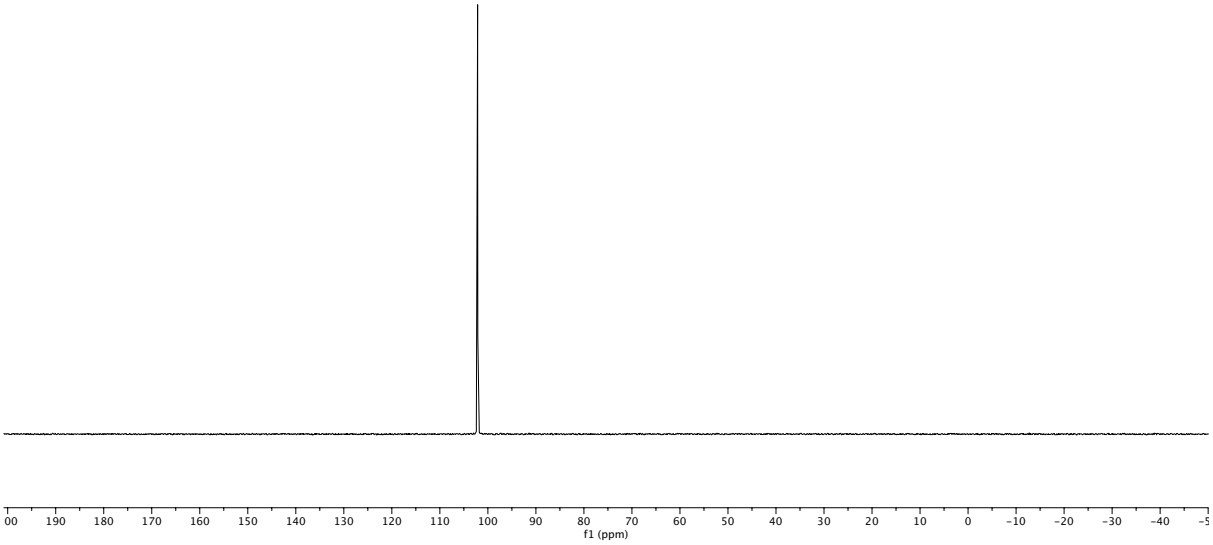
Compound Ψ-3 ^{1}H NMR



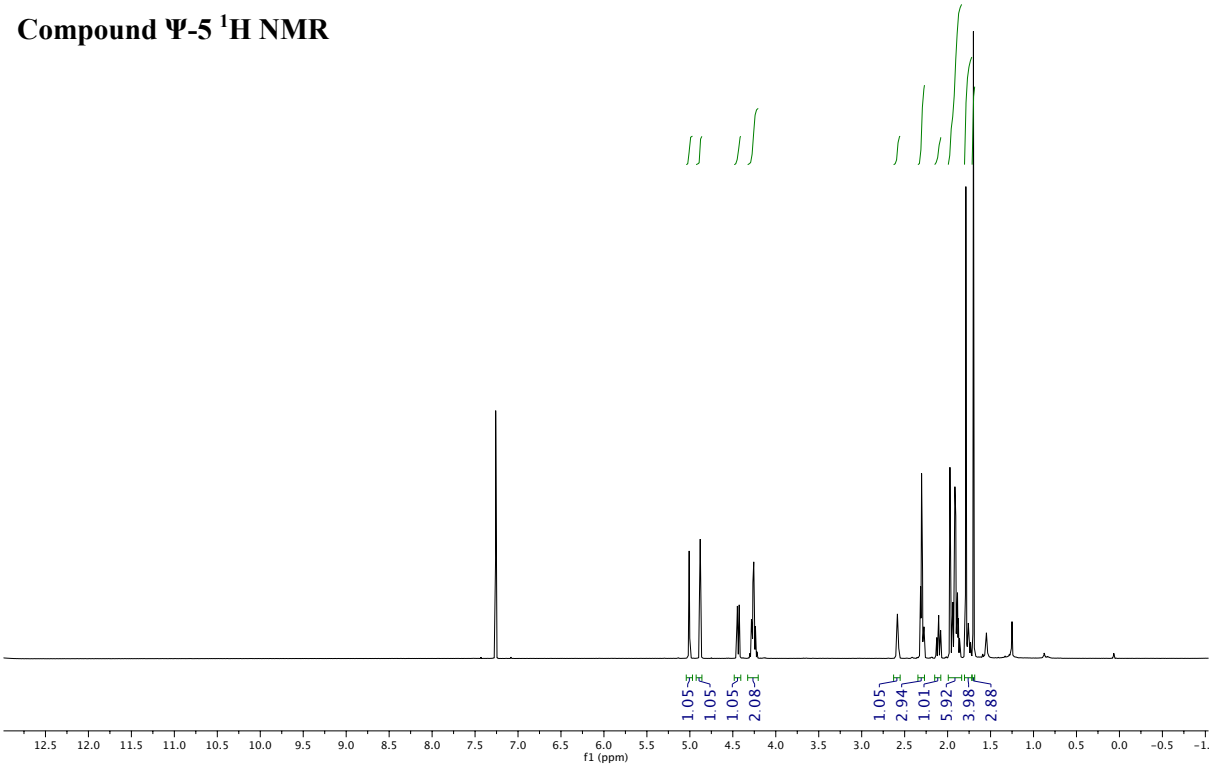
Compound Ψ-3 ^{13}C NMR



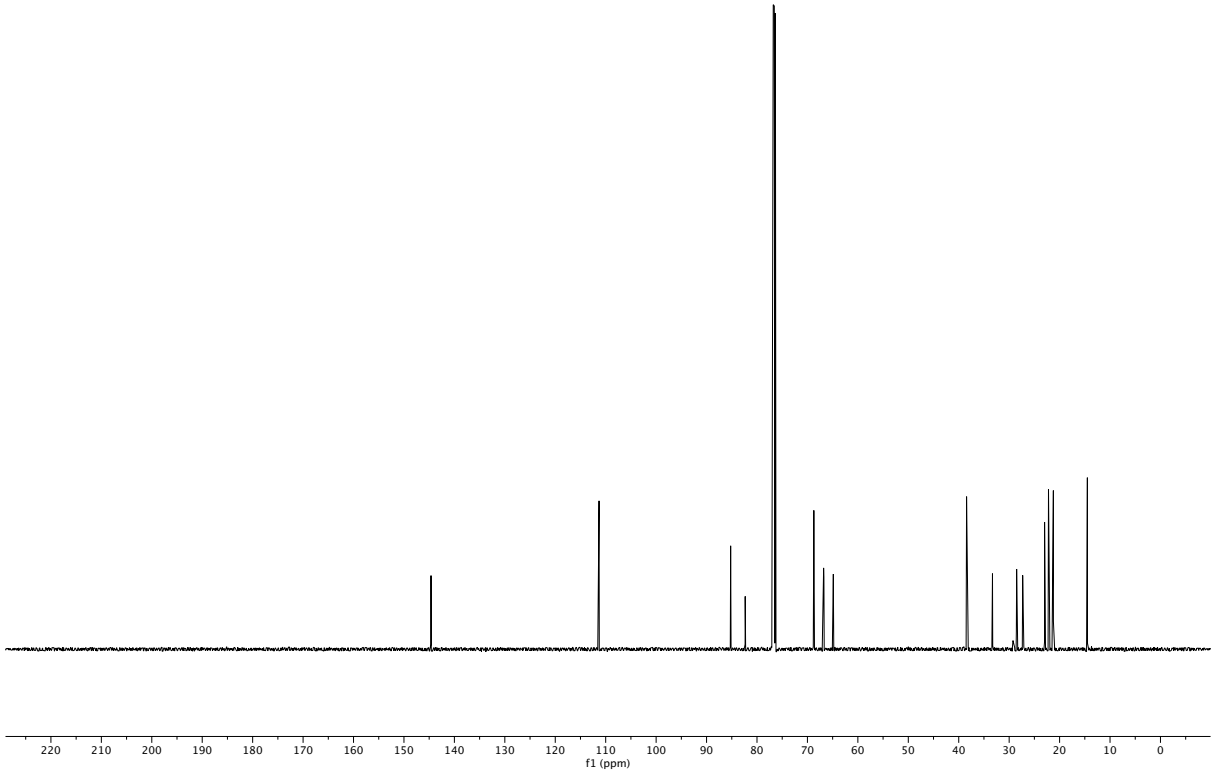
Compound Ψ-3 ^{31}P NMR



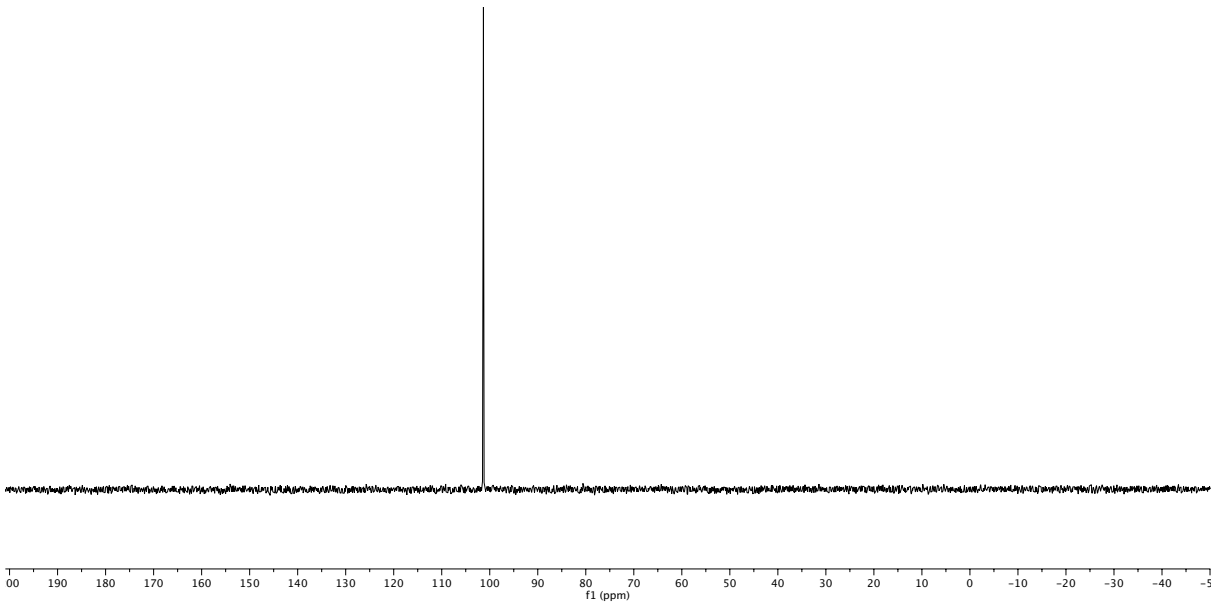
Compound Ψ-5 ^{1}H NMR



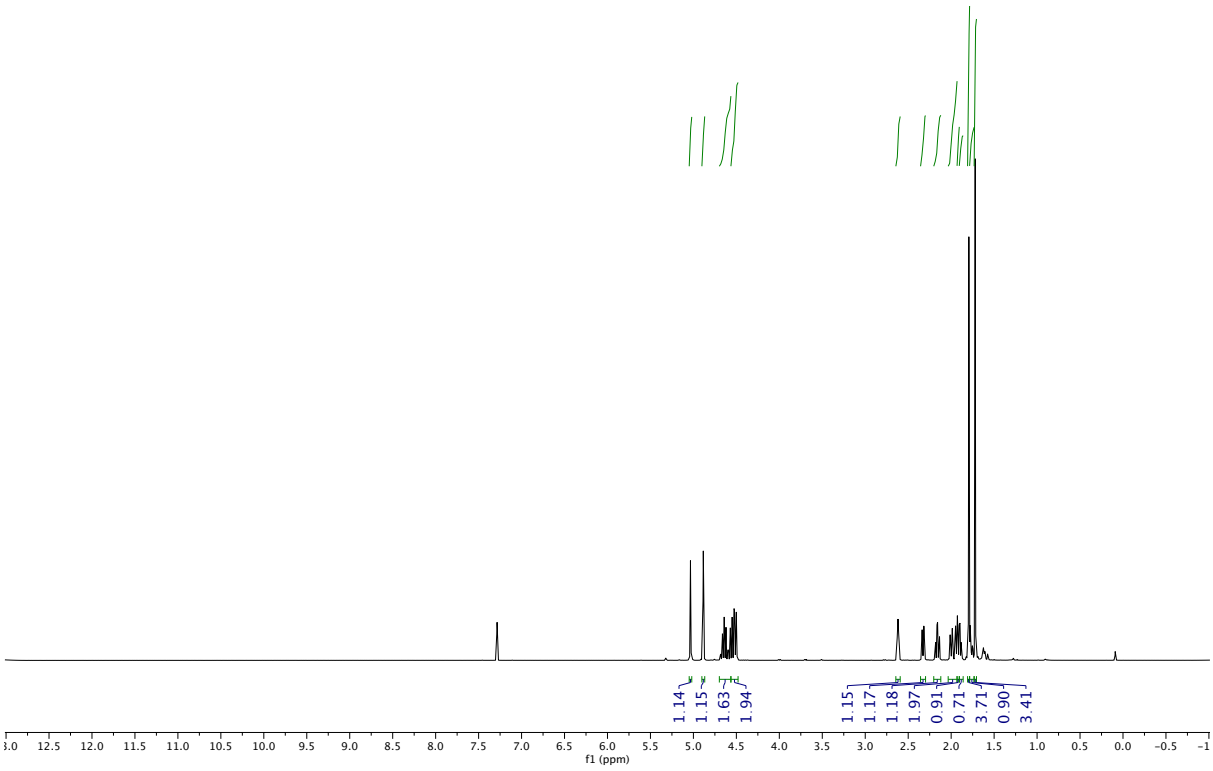
Compound Ψ-5 ^{13}C NMR



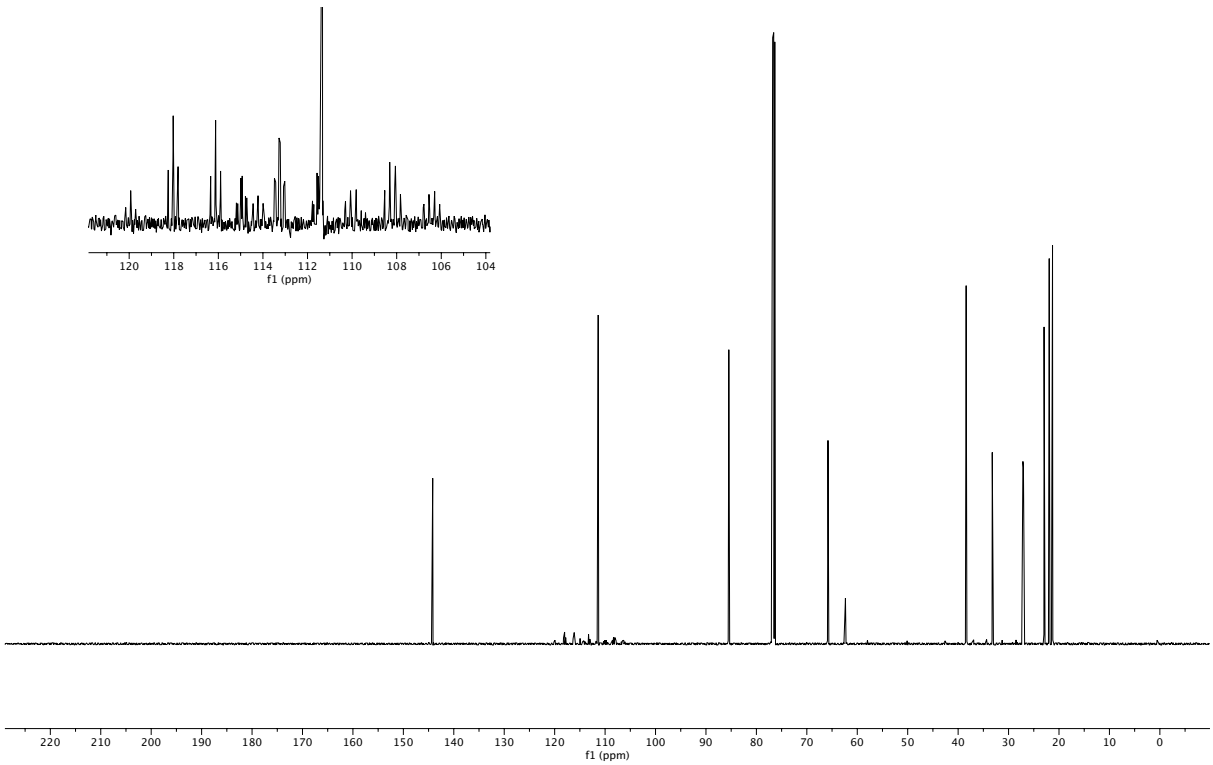
Compound Ψ-5 ^{31}P NMR



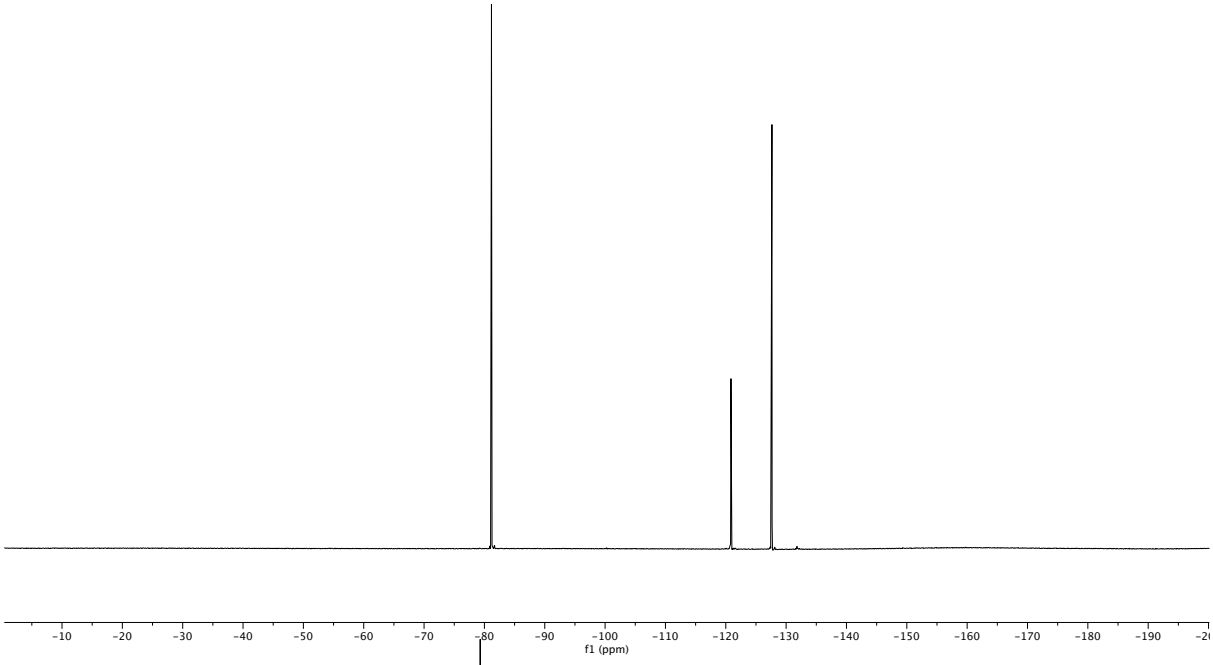
Compound Ψ-6 ^{1}H NMR



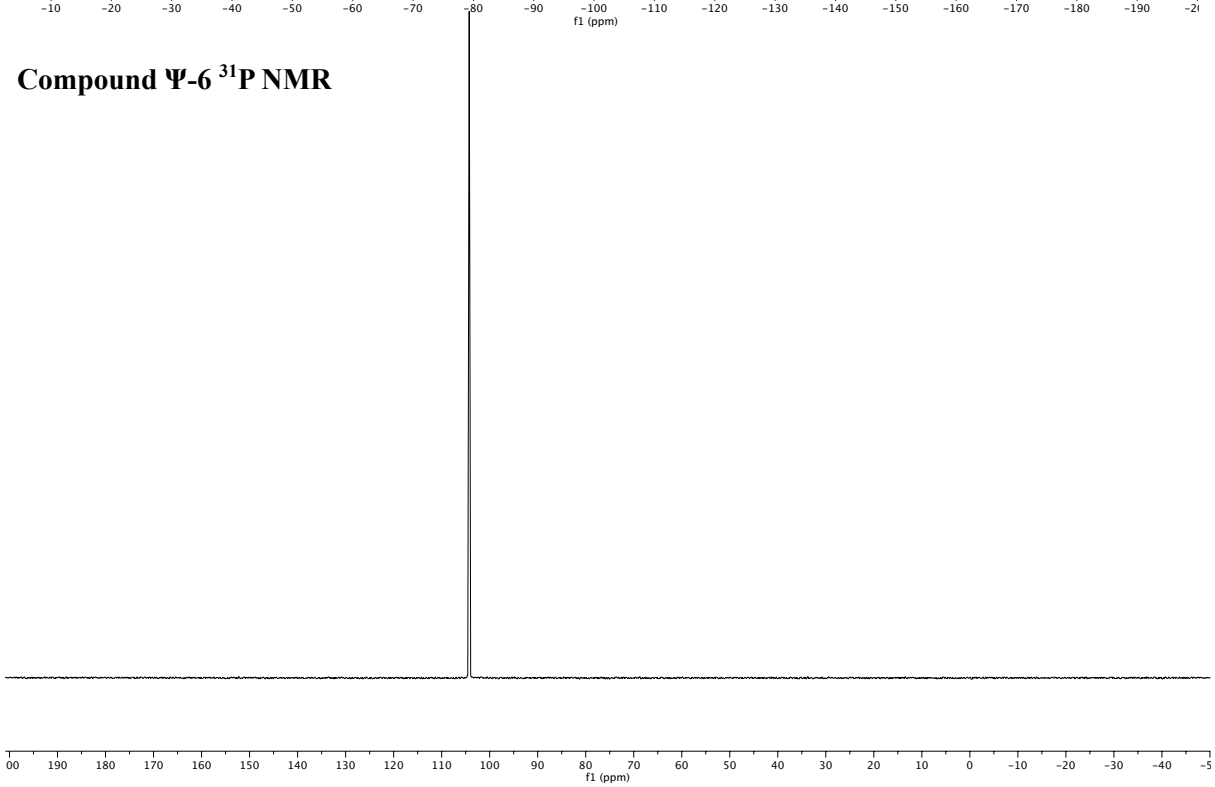
Compound Ψ-6 ^{13}C NMR



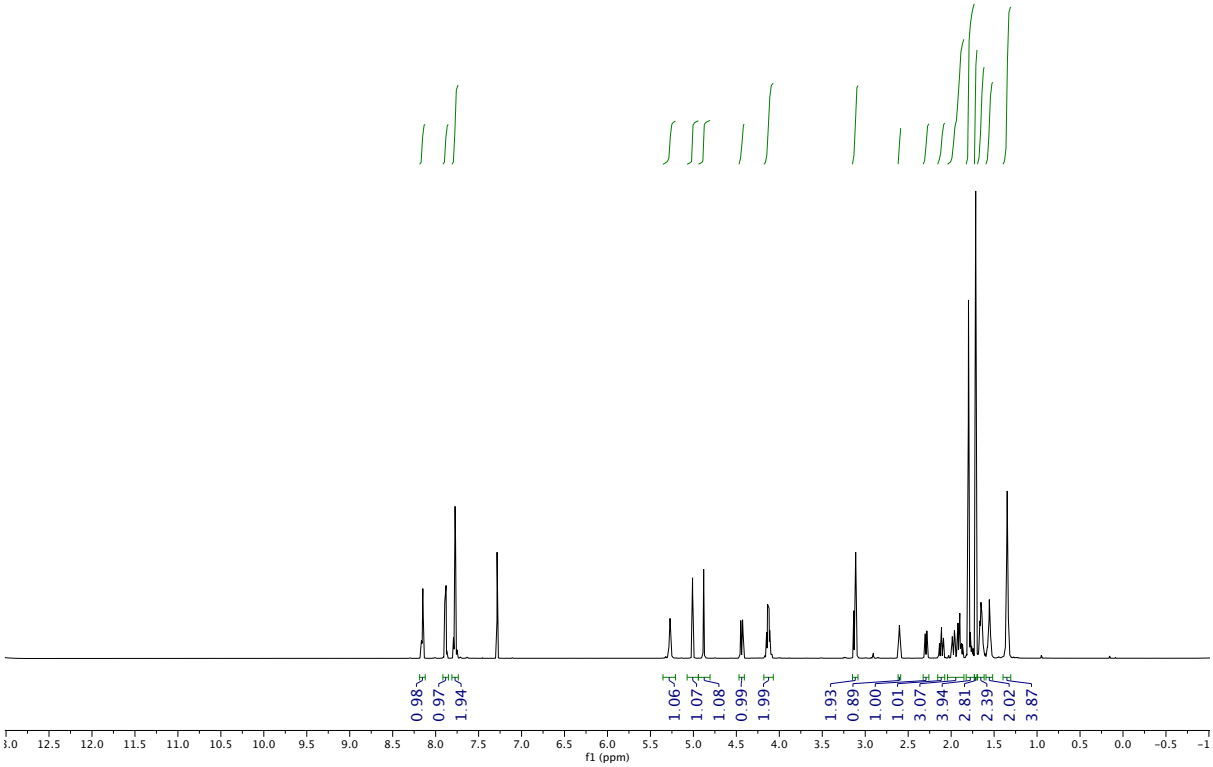
Compound Ψ-6 ^{19}F NMR



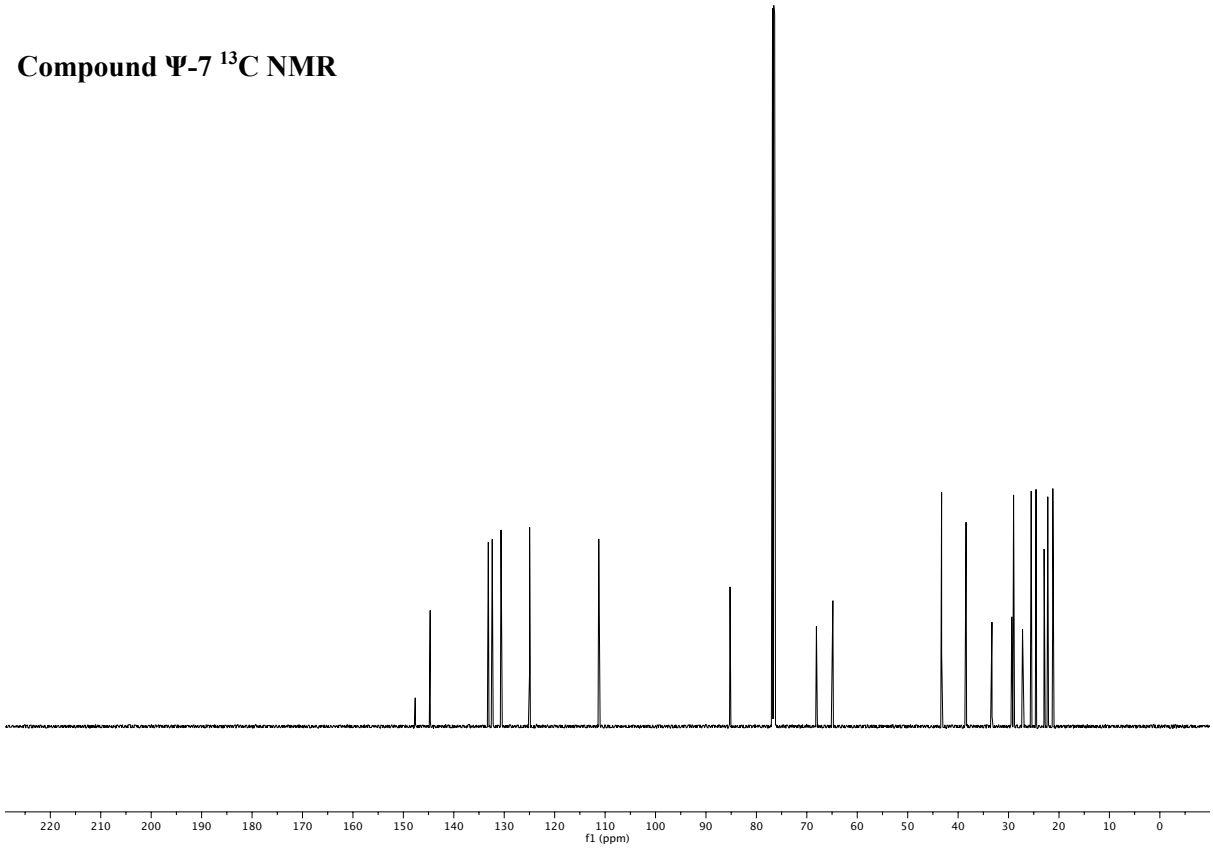
Compound Ψ-6 ^{31}P NMR



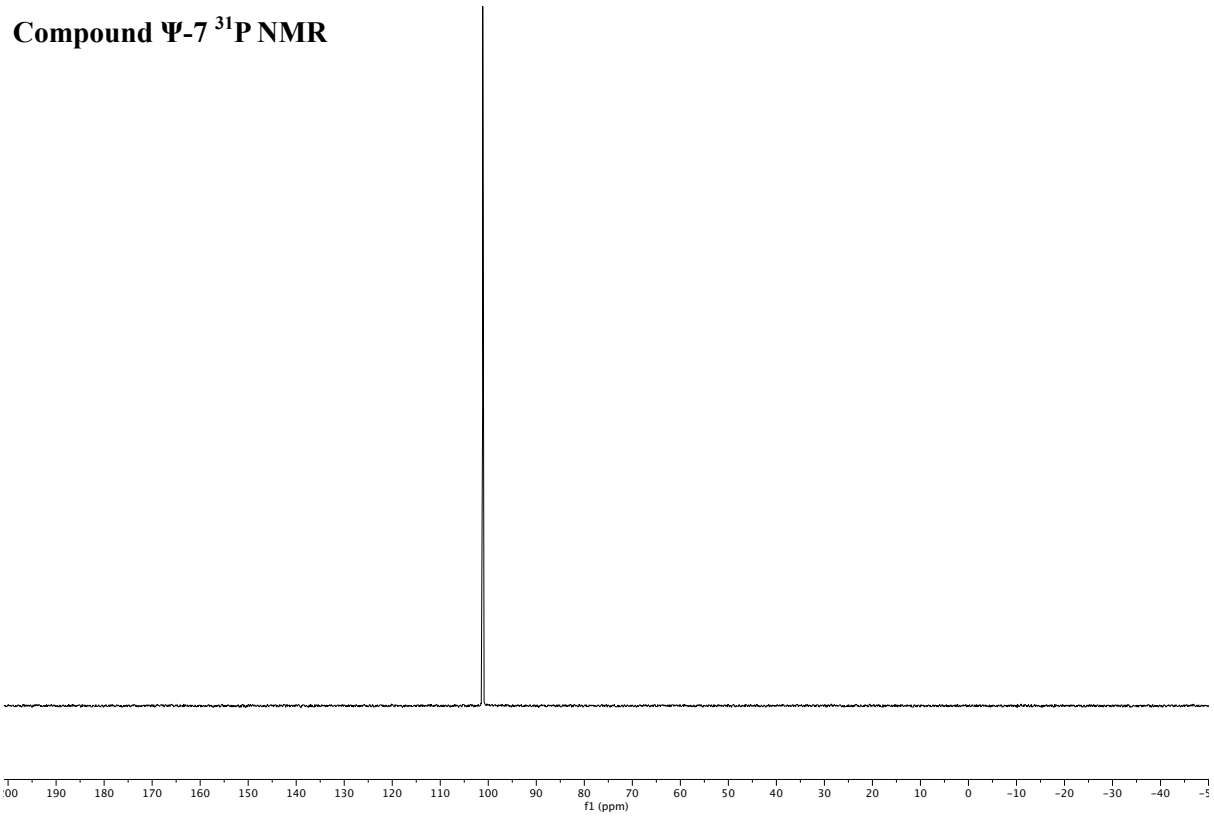
Compound Ψ-7 ^{1}H NMR



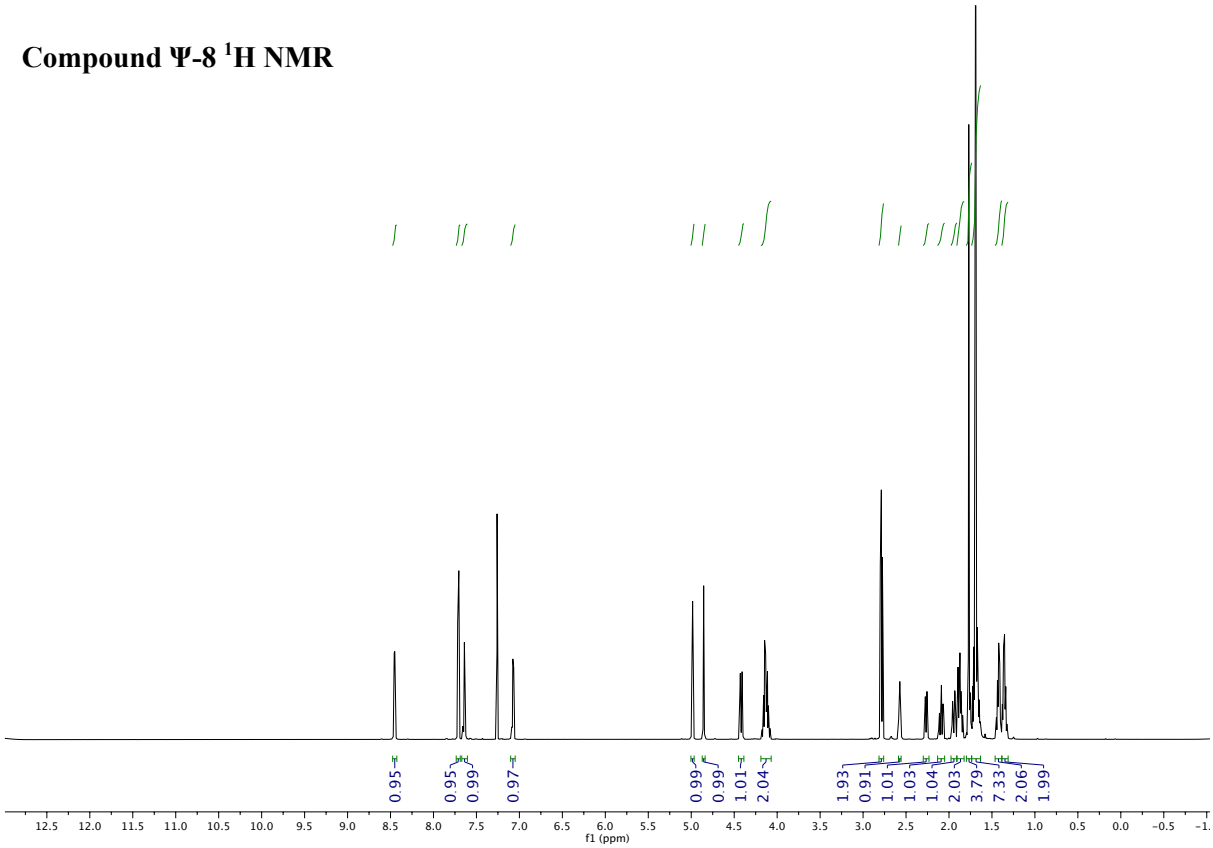
Compound Ψ-7 ^{13}C NMR



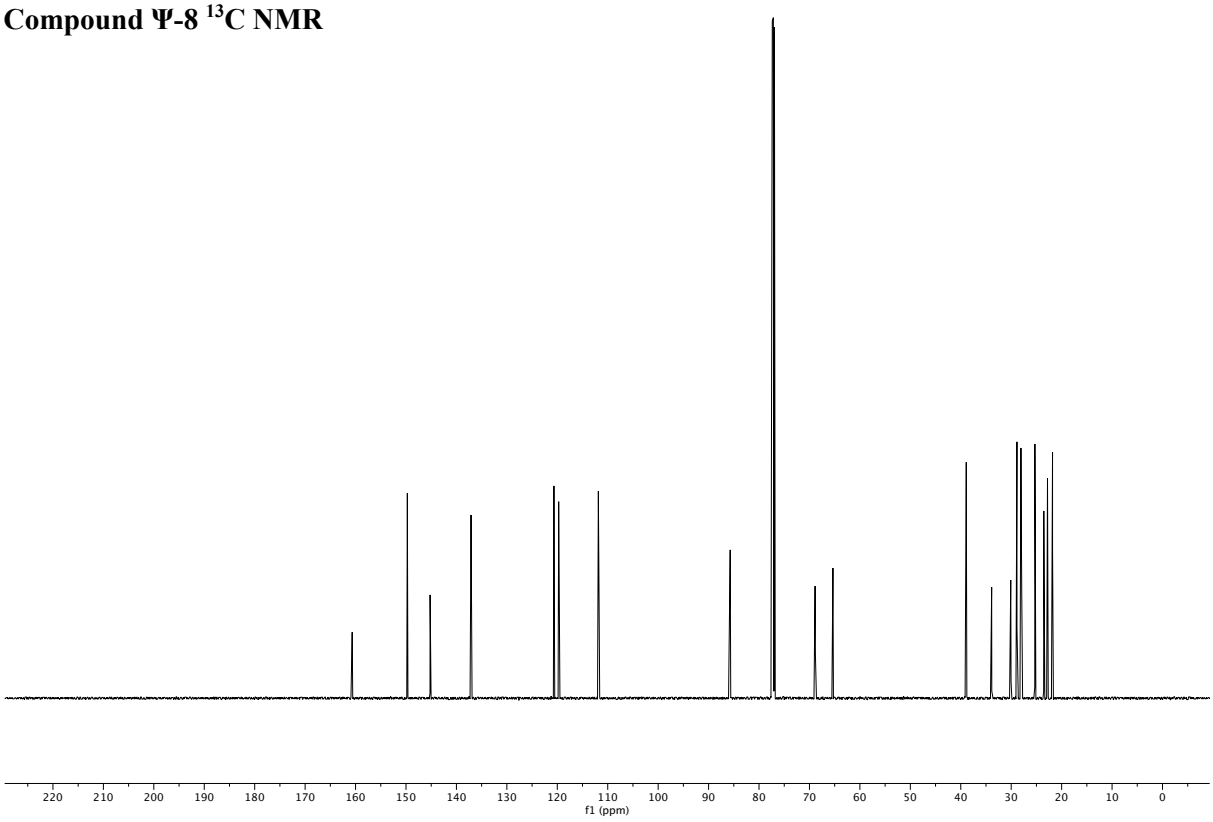
Compound Ψ-7 ^{31}P NMR



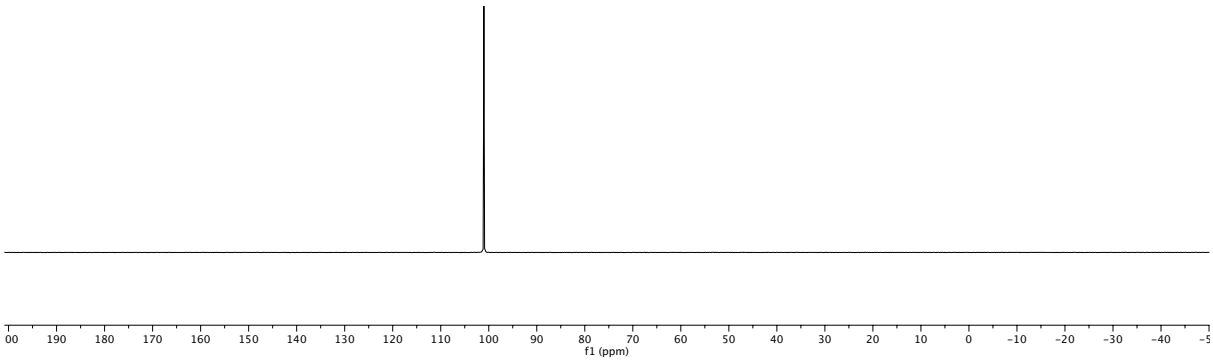
Compound Ψ-8 ^{1}H NMR



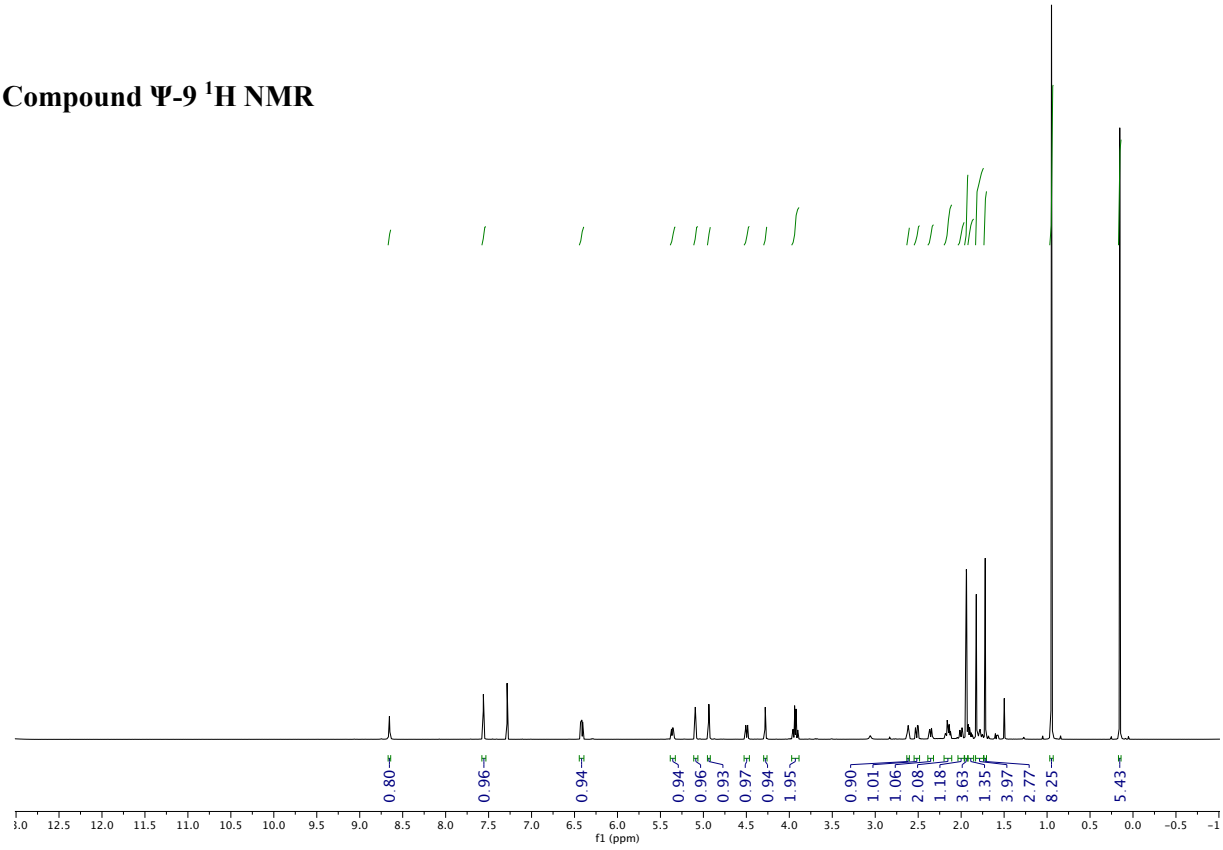
Compound Ψ-8 ^{13}C NMR



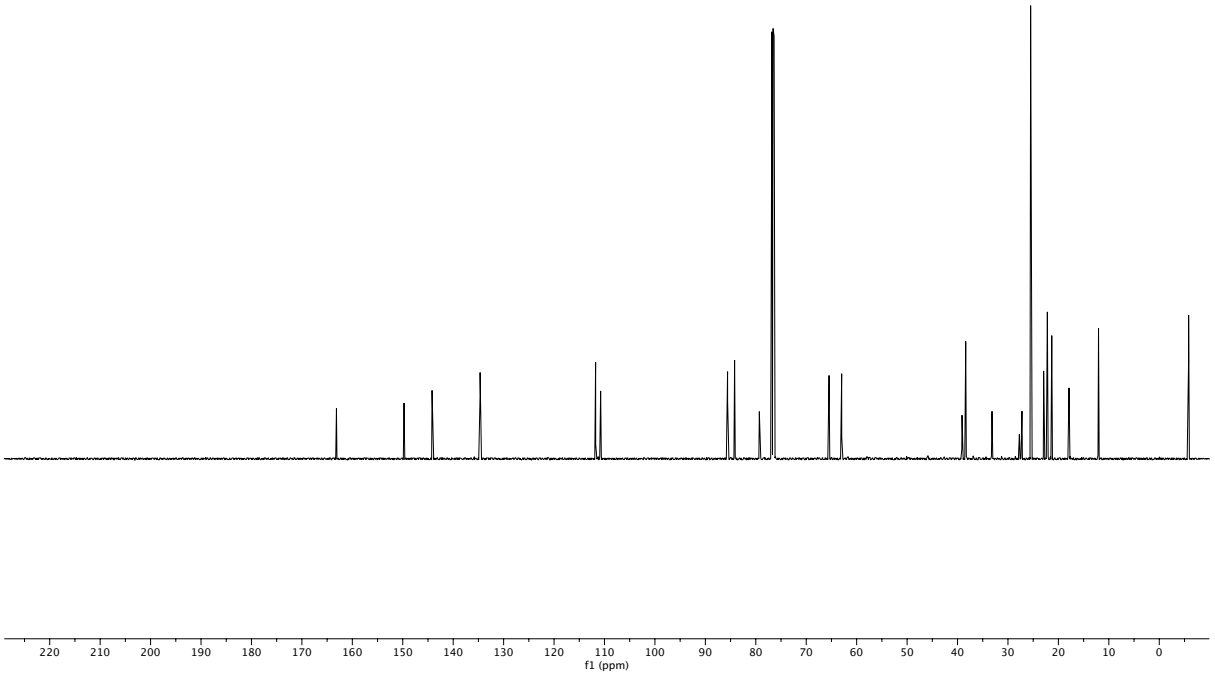
Compound Ψ-8 ^{31}P NMR



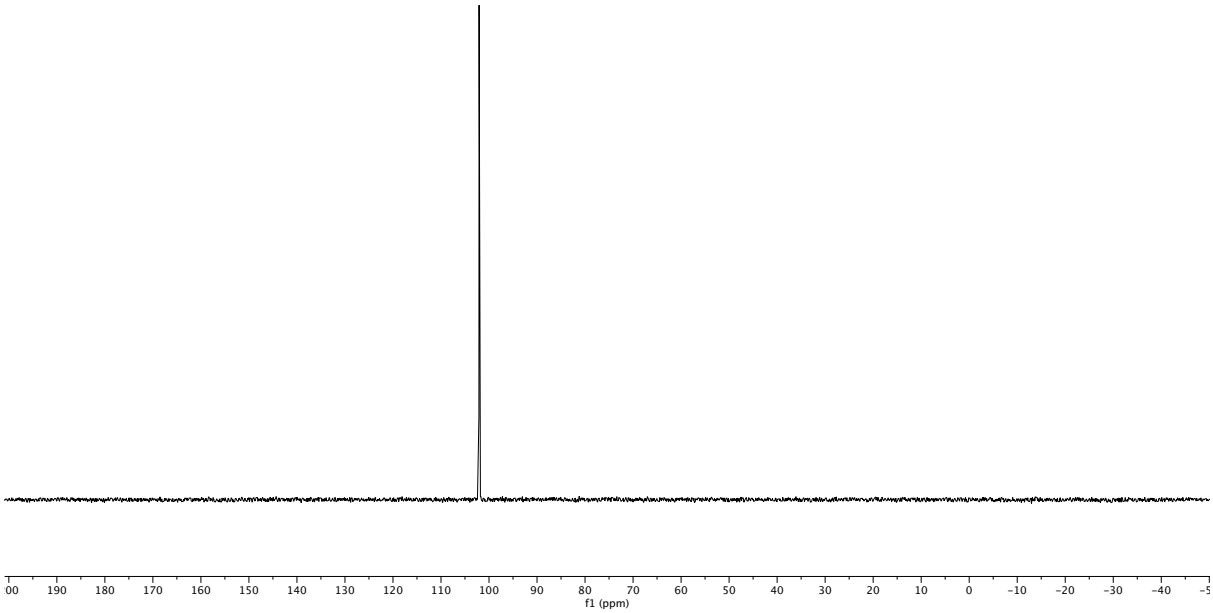
Compound Ψ-9 ^{1}H NMR



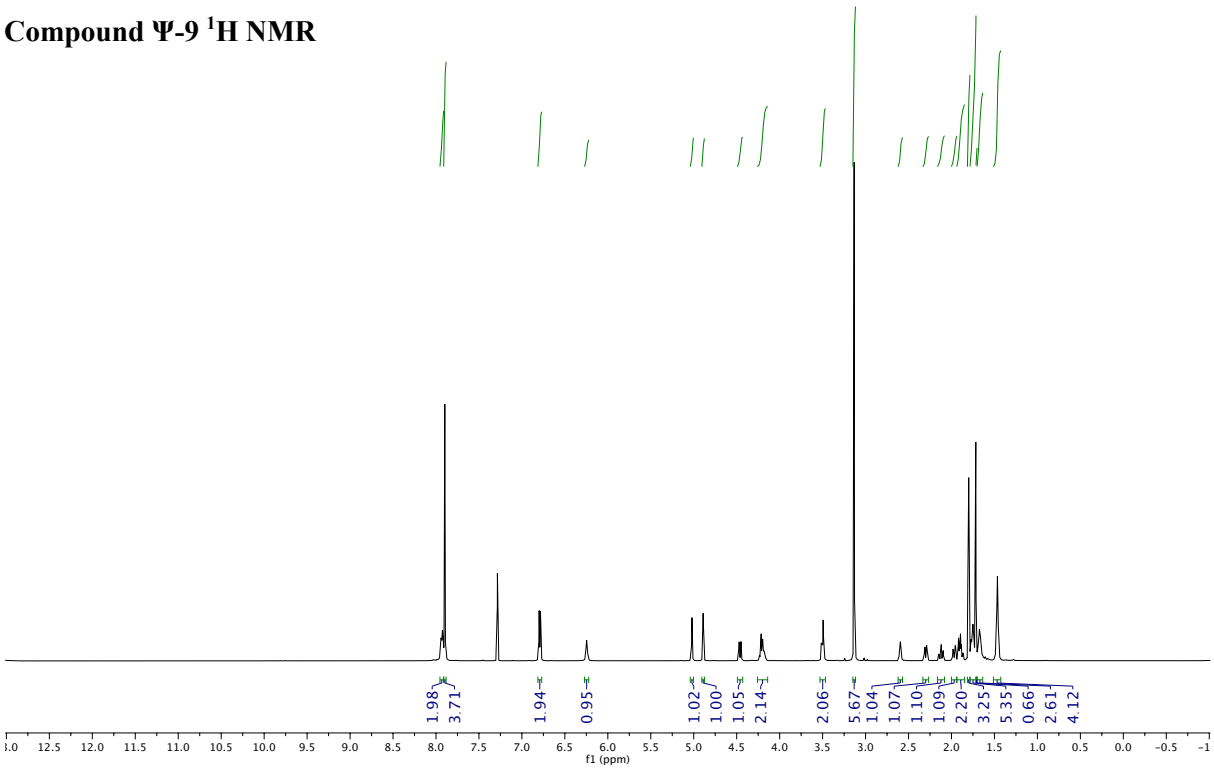
Compound Ψ-9 ^{13}C NMR



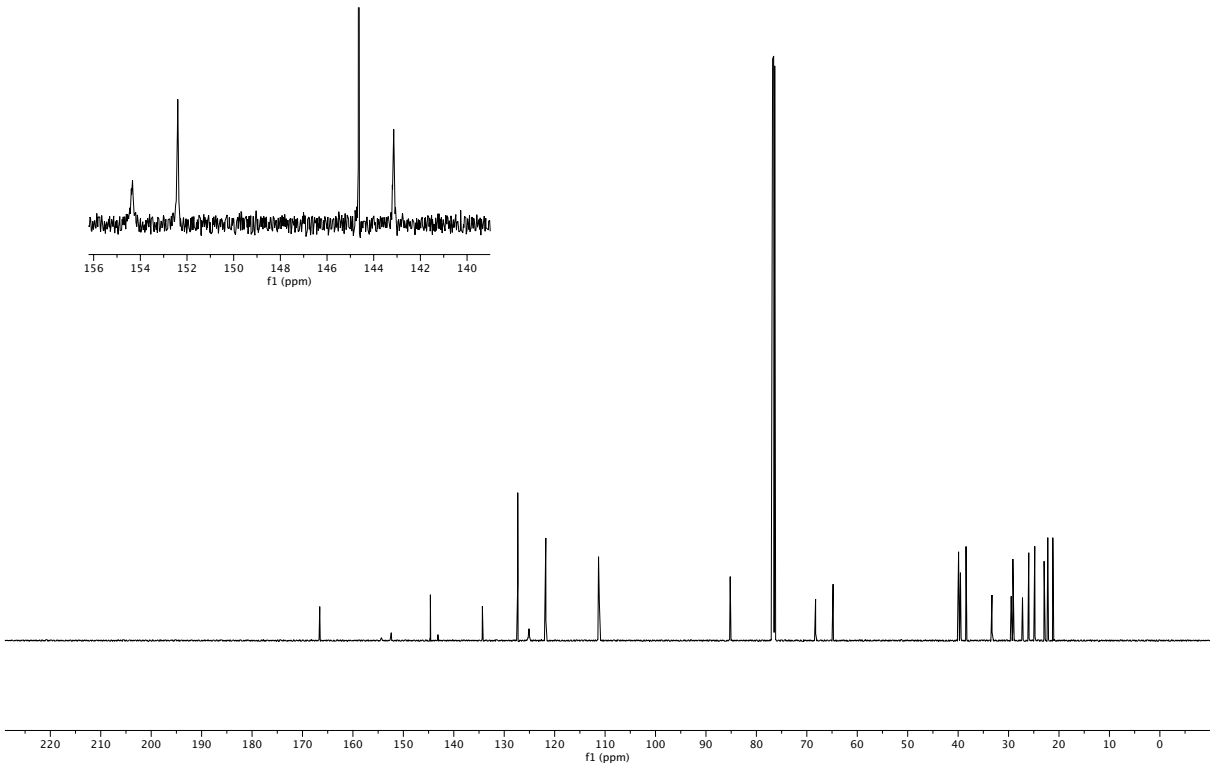
Compound Ψ-9 ^{31}P NMR



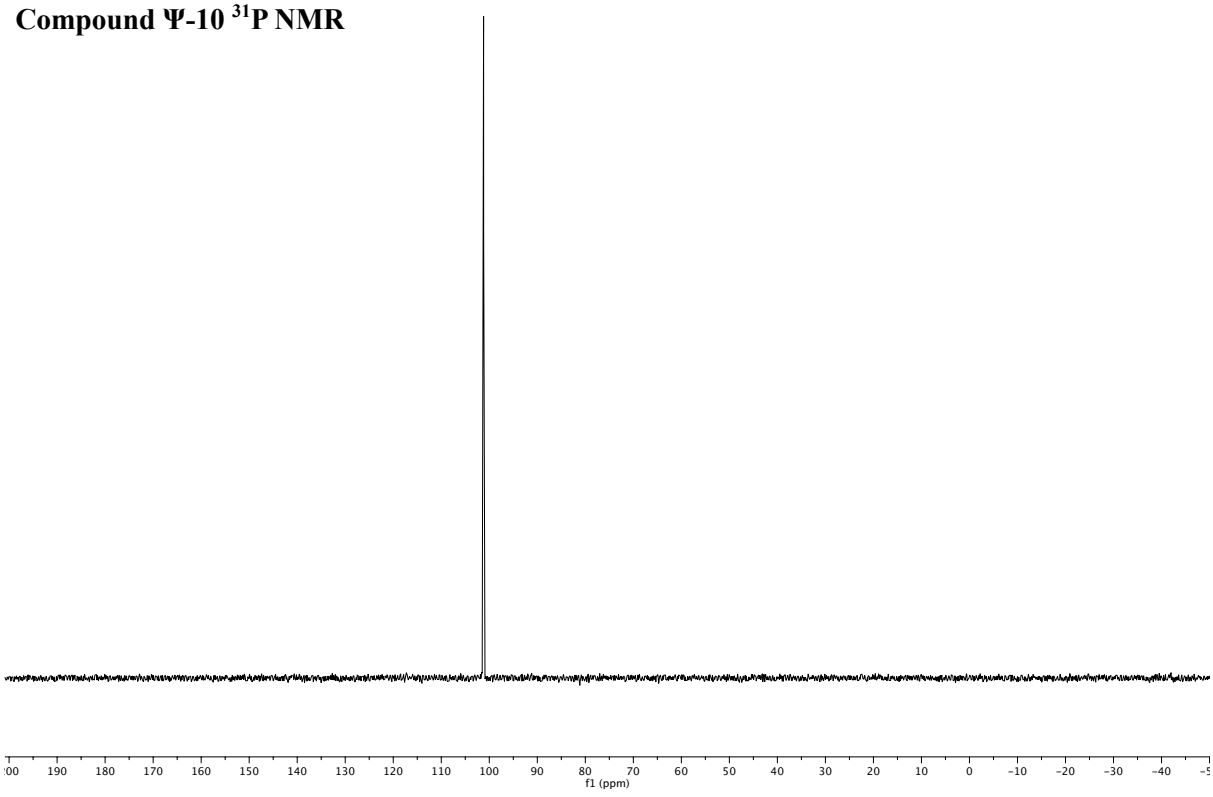
Compound Ψ-9 ^{1}H NMR



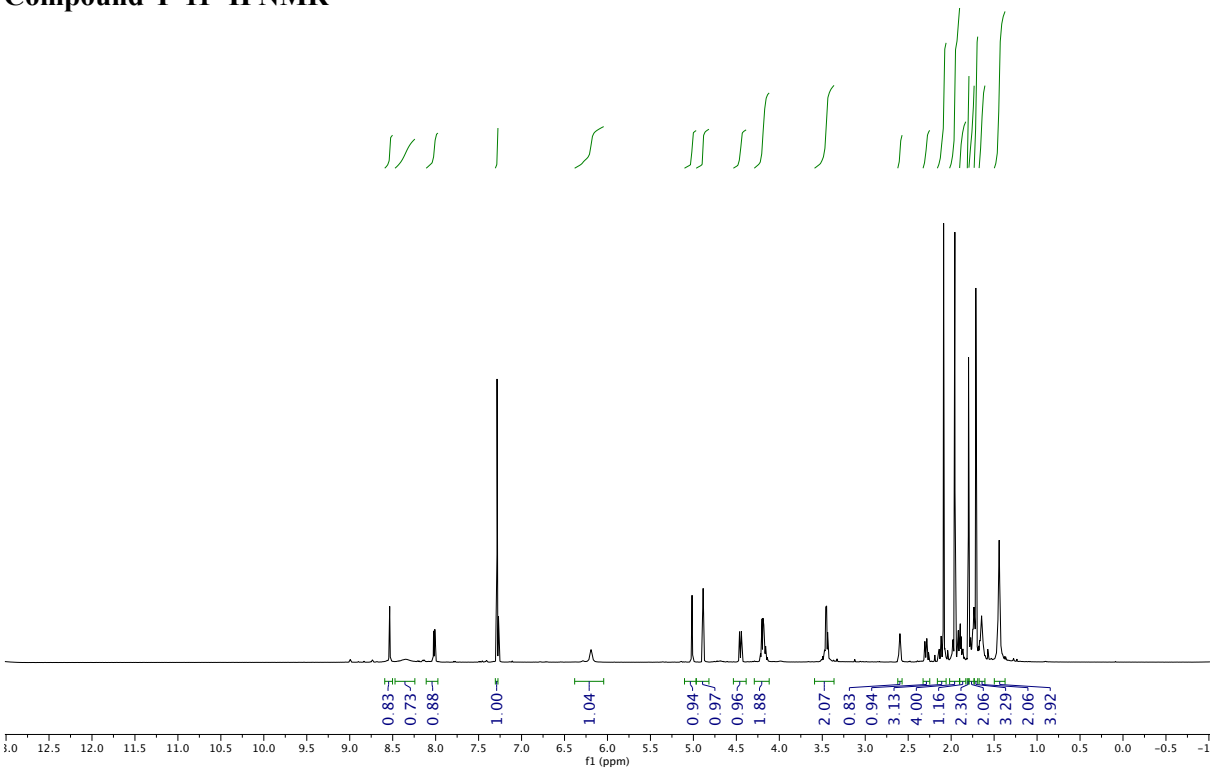
Compound Ψ-10 ^{13}C NMR



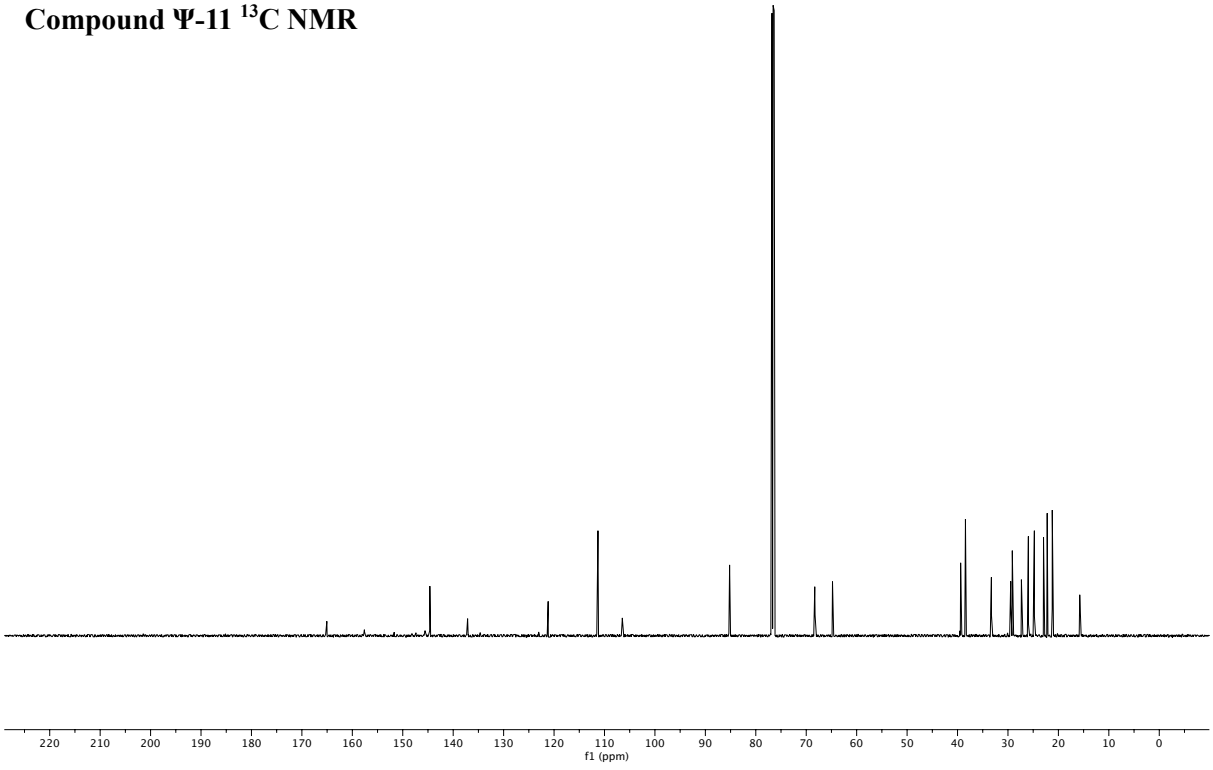
Compound Ψ-10 ^{31}P NMR



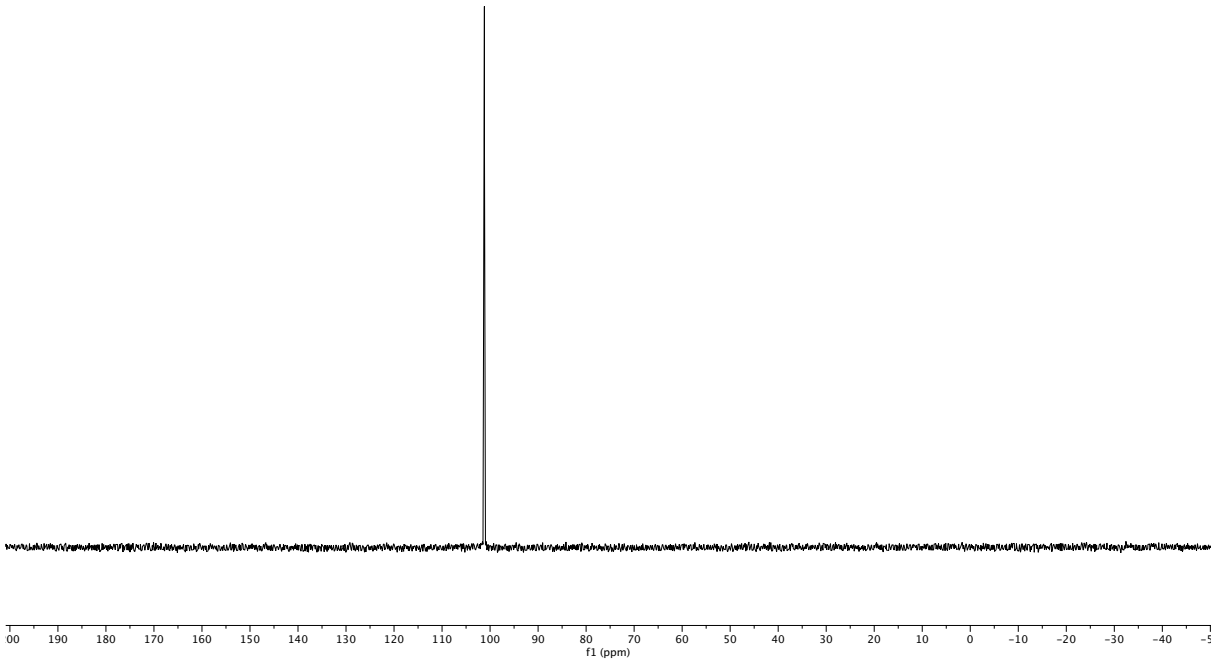
Compound Ψ-11 ^{1}H NMR



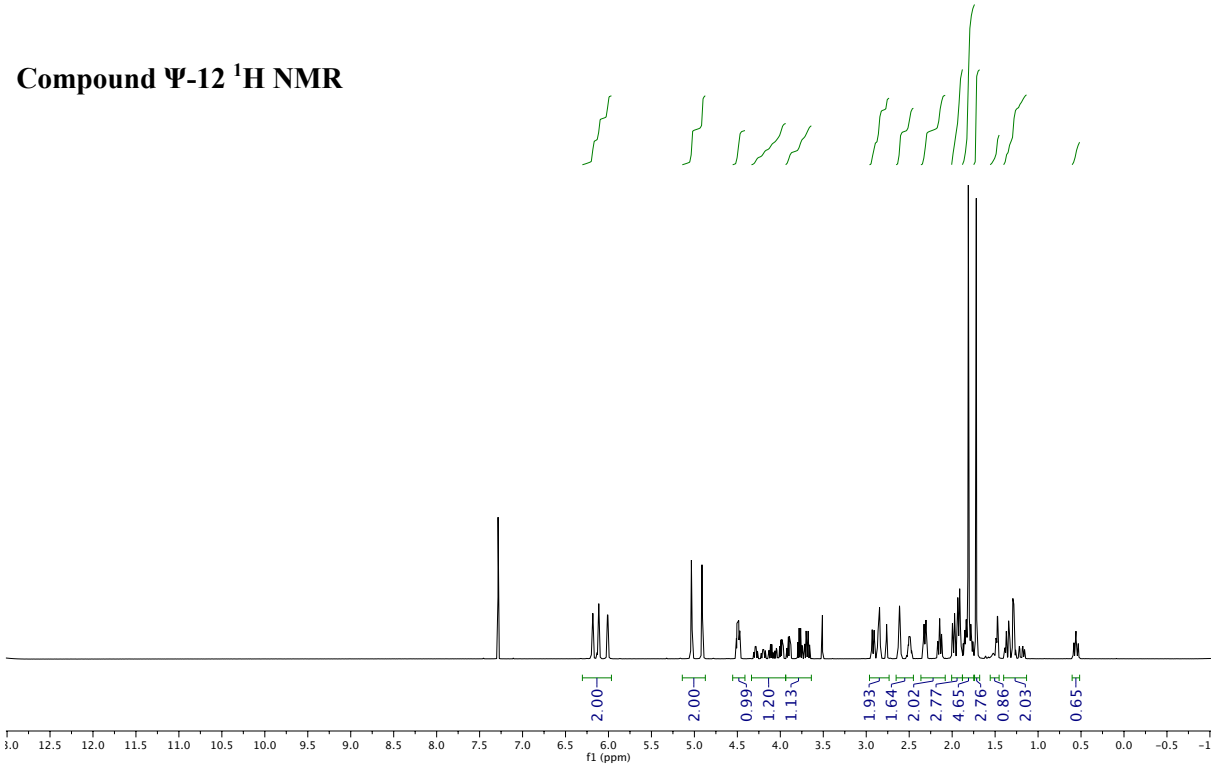
Compound Ψ-11 ^{13}C NMR



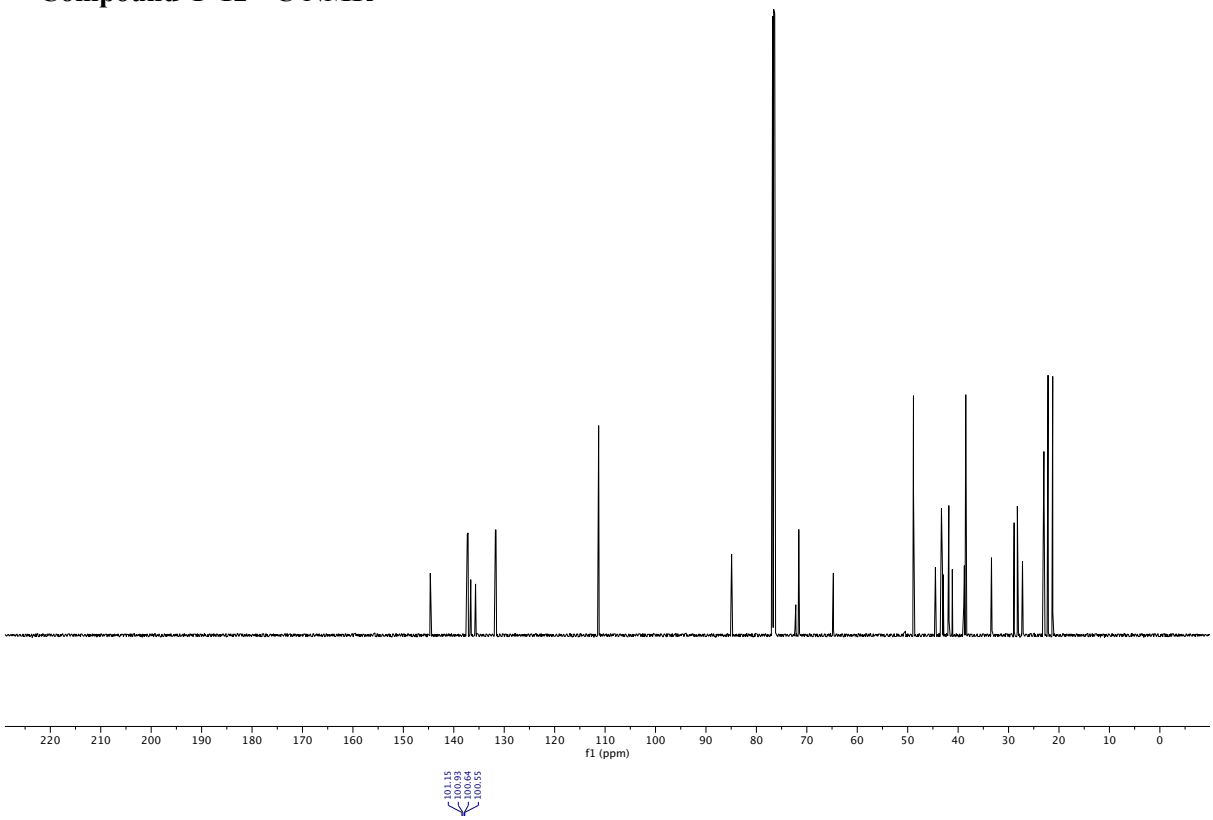
Compound Ψ-11 ^{31}P NMR



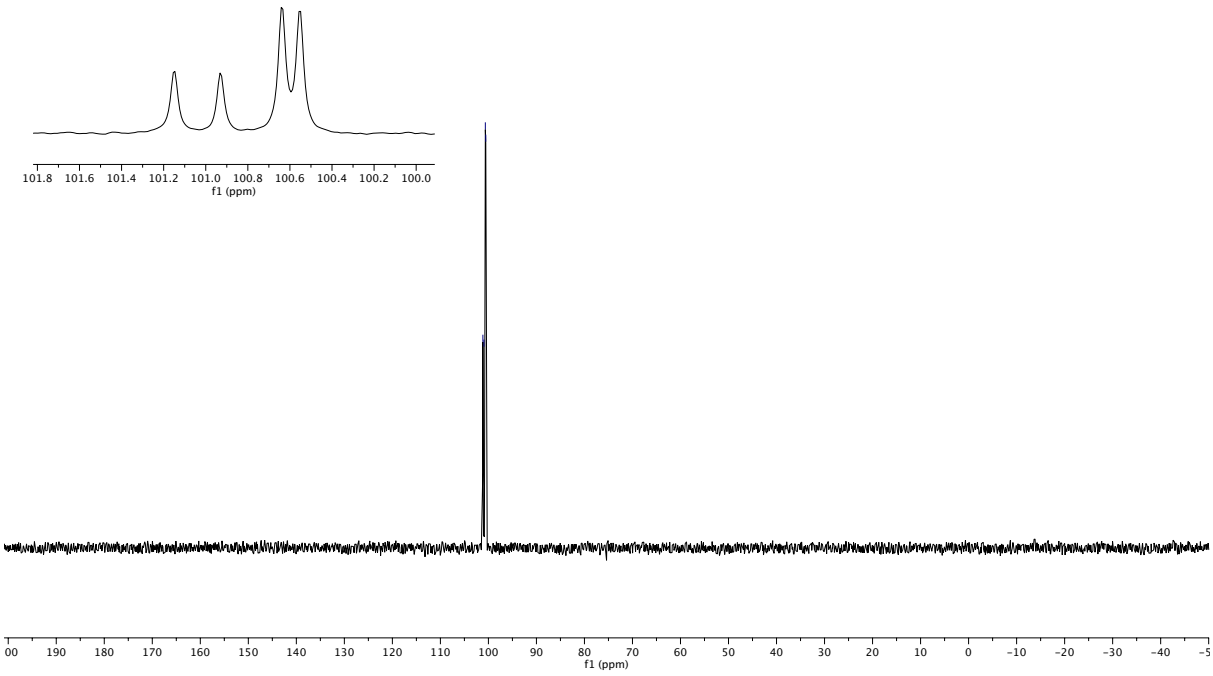
Compound Ψ-12 ^{1}H NMR



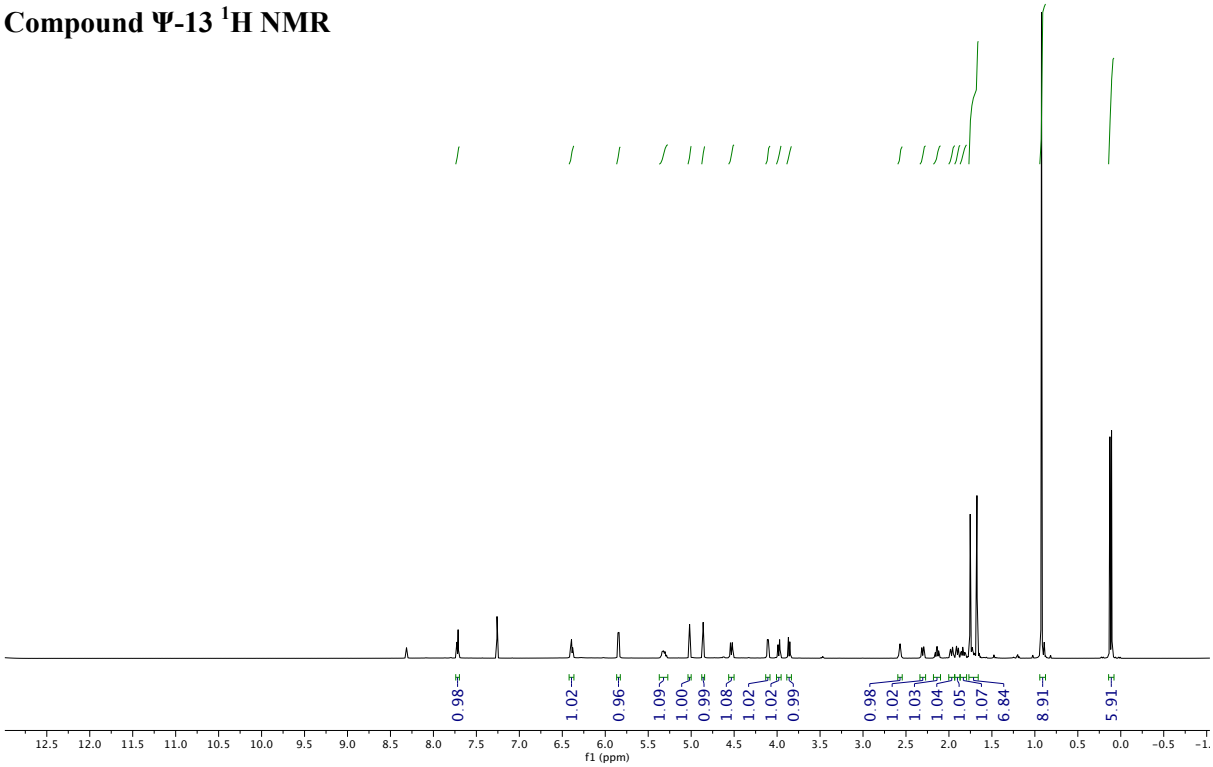
Compound Ψ-12 ^{13}C NMR



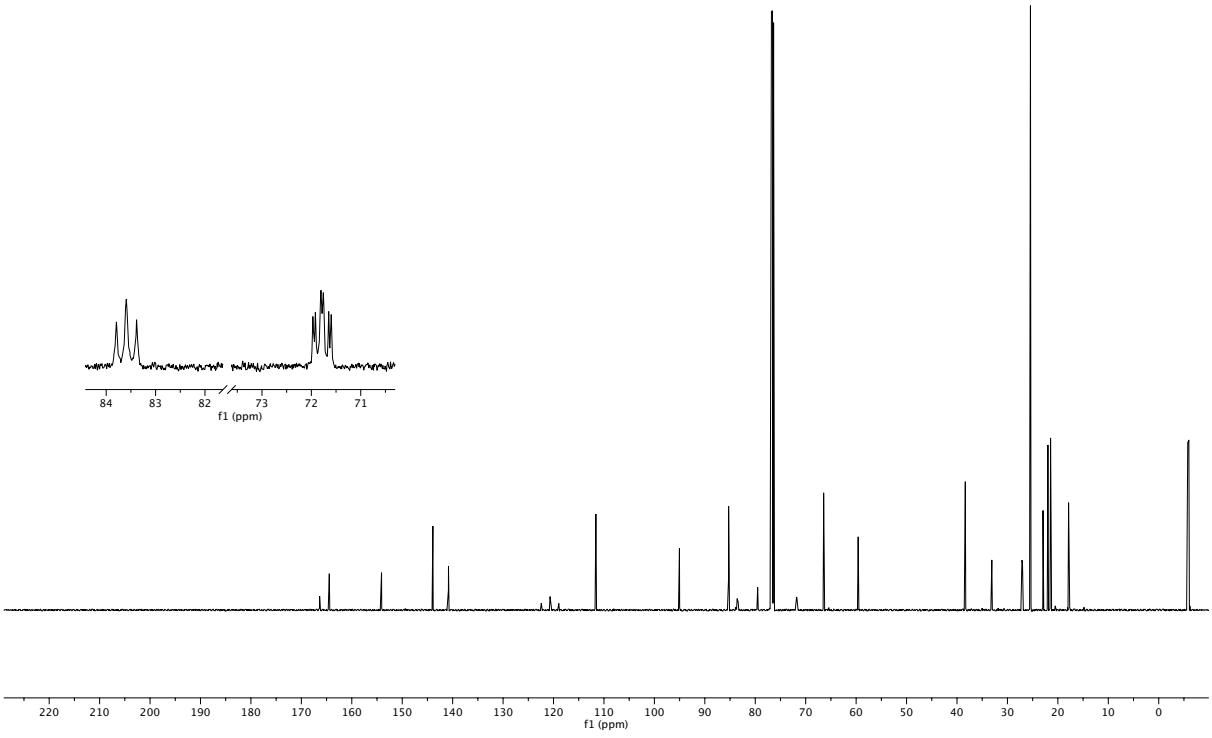
Compound Ψ-12 ^{31}P NMR



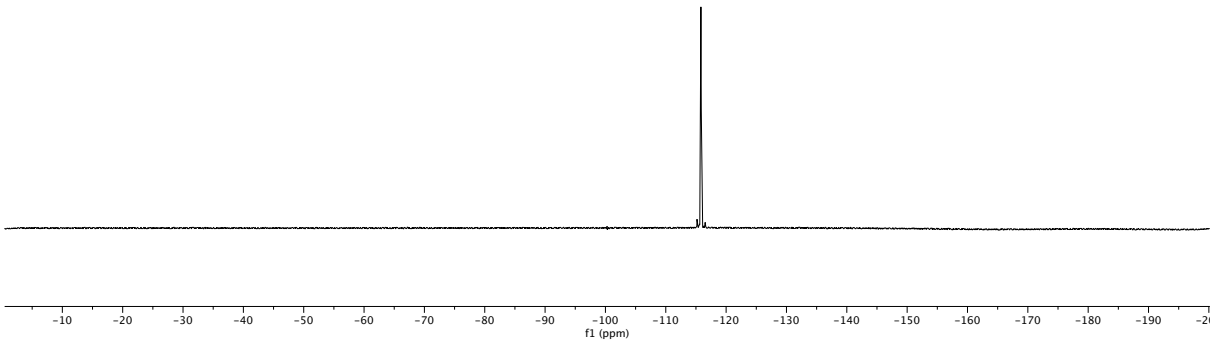
Compound Ψ-13 ^{1}H NMR



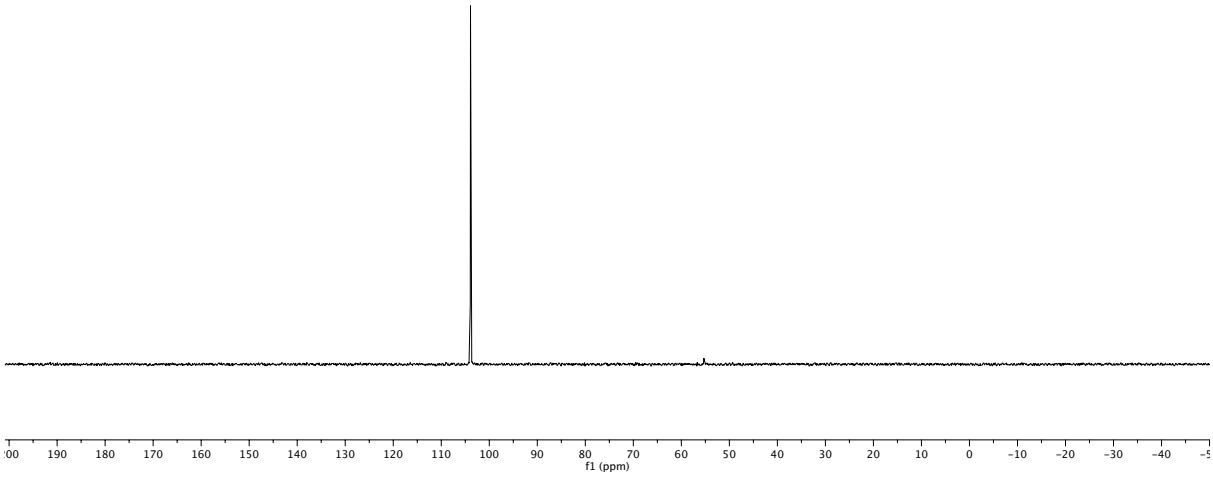
Compound Ψ-13 ^{13}C NMR



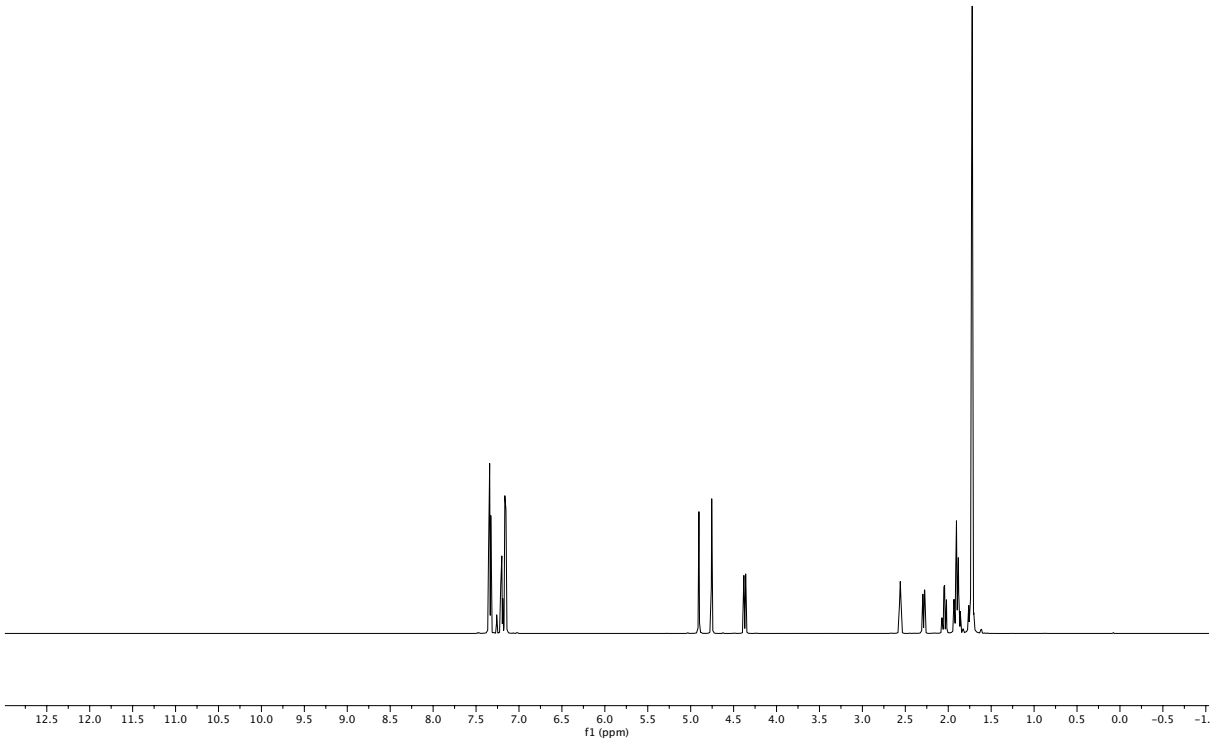
Compound Ψ-13 ^{19}F NMR



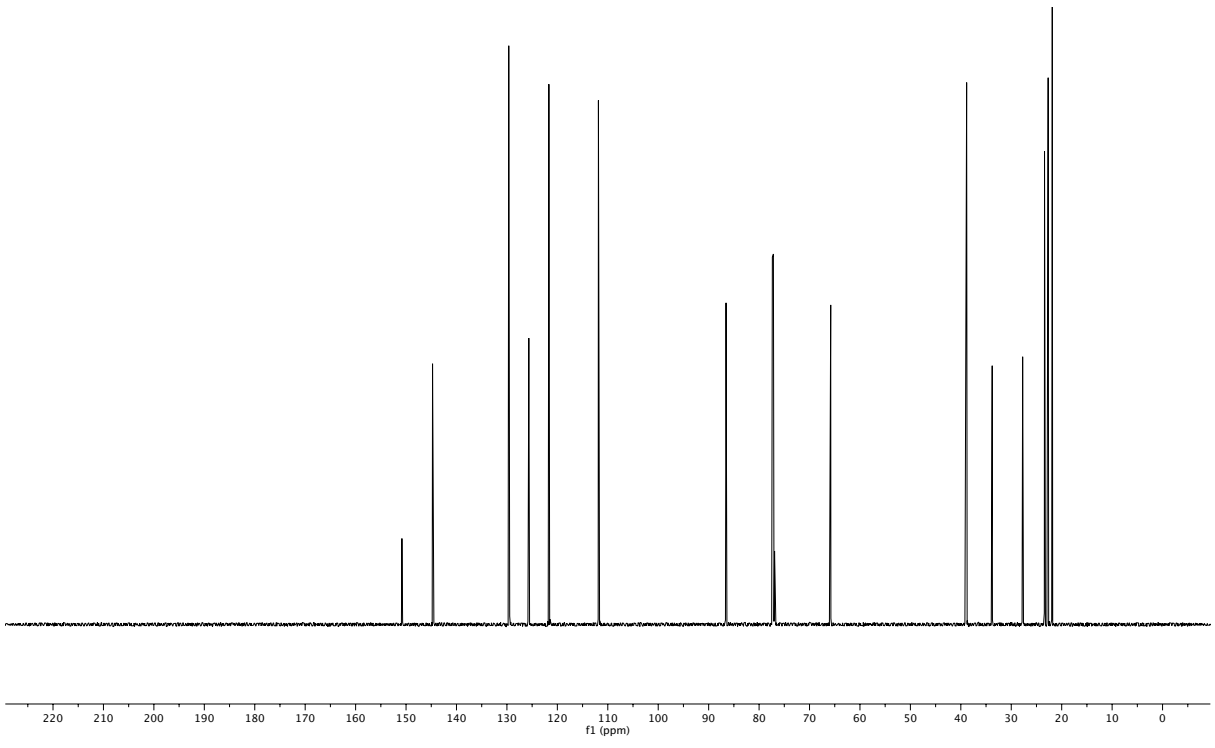
Compound Ψ-13 ^{31}P NMR



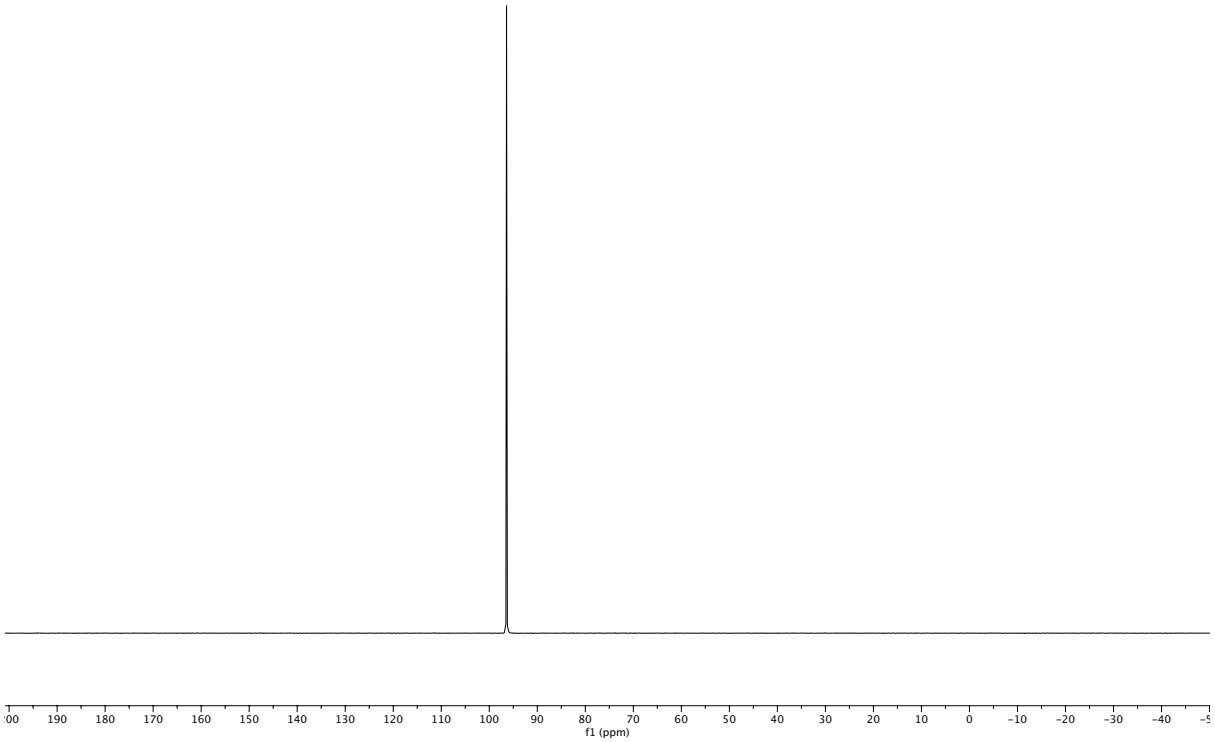
Compound Ψ-(*R*p)-14 ^{1}H NMR



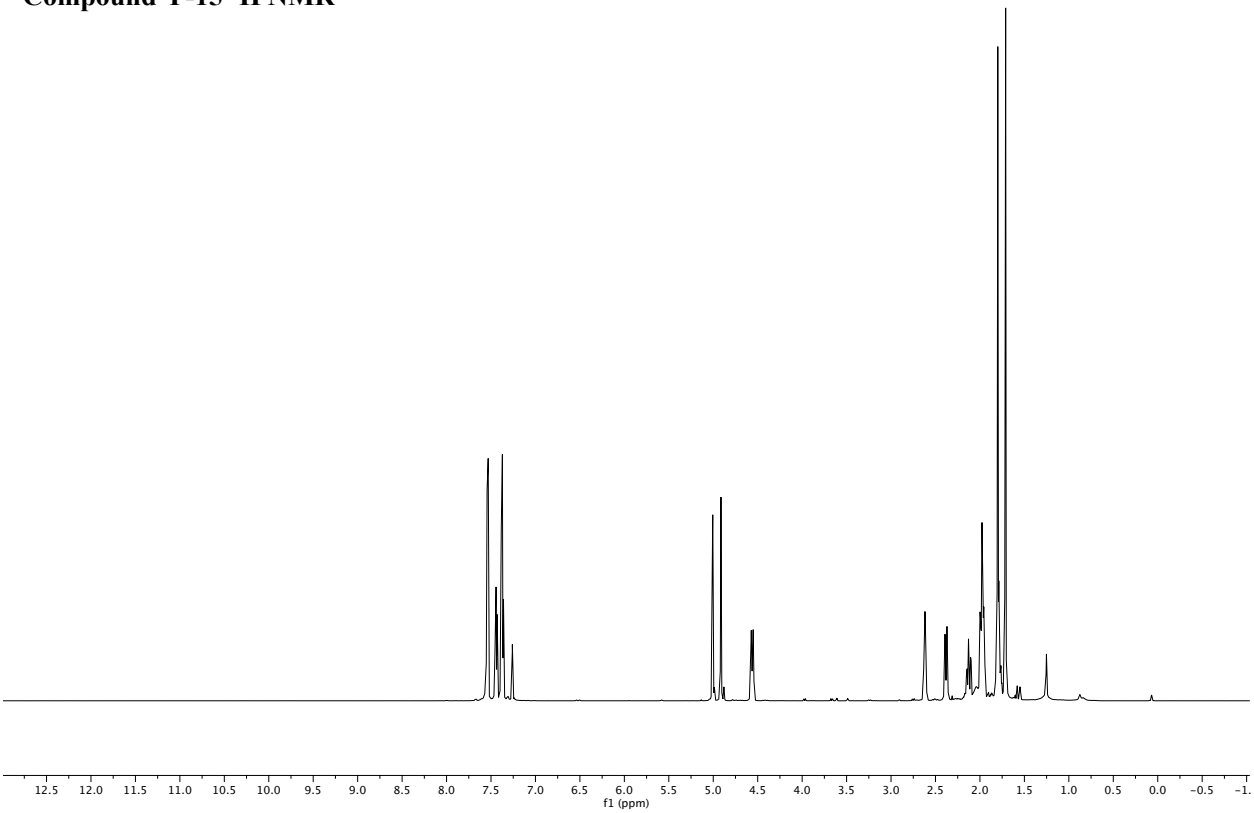
Compound Ψ-(*R*p)-14 ^{13}C NMR



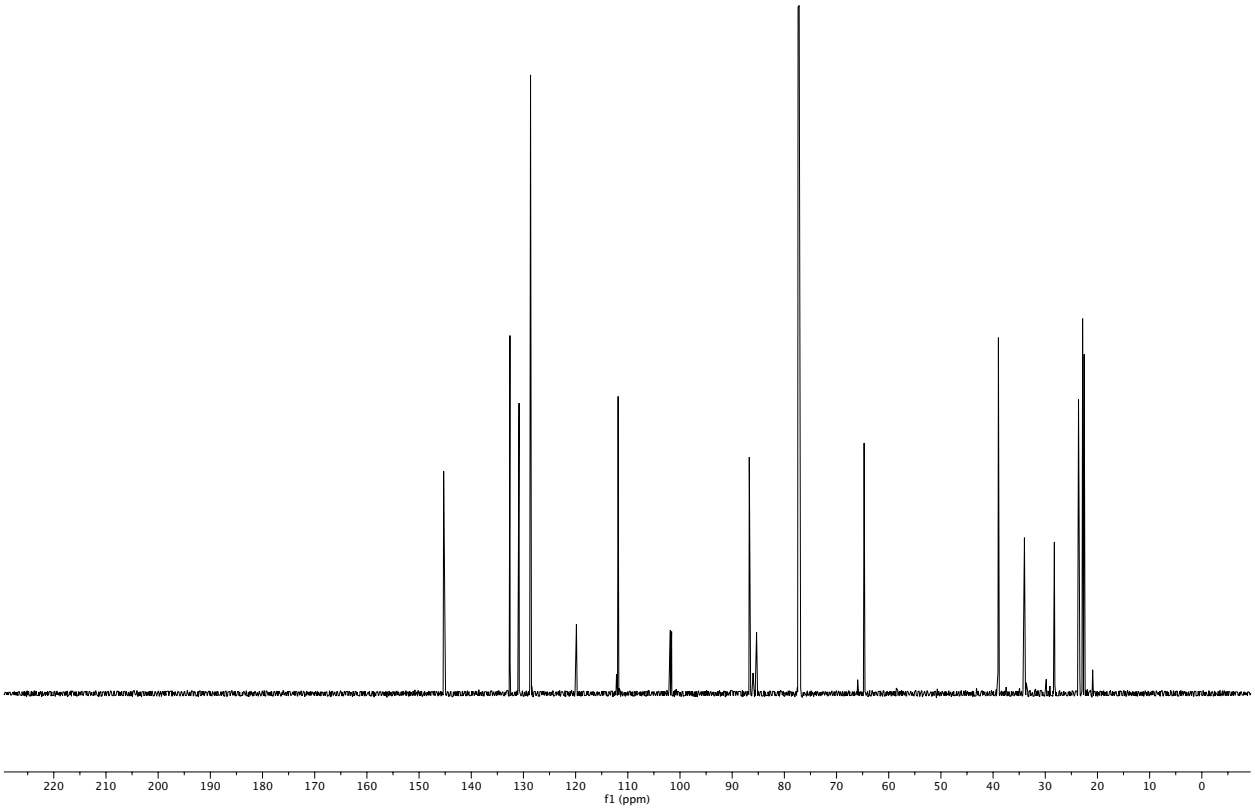
Compound Ψ-(*R*p)-14 ^{31}P NMR



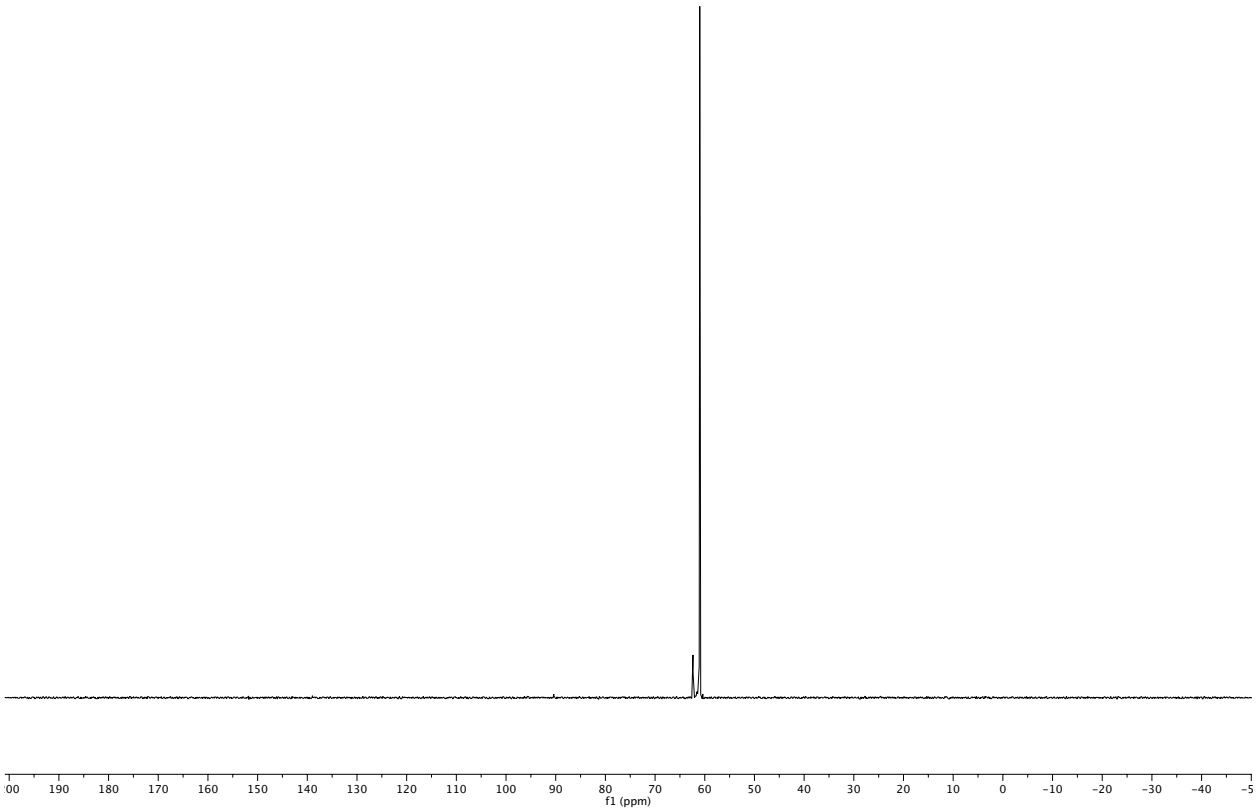
Compound Ψ-15 ^{1}H NMR



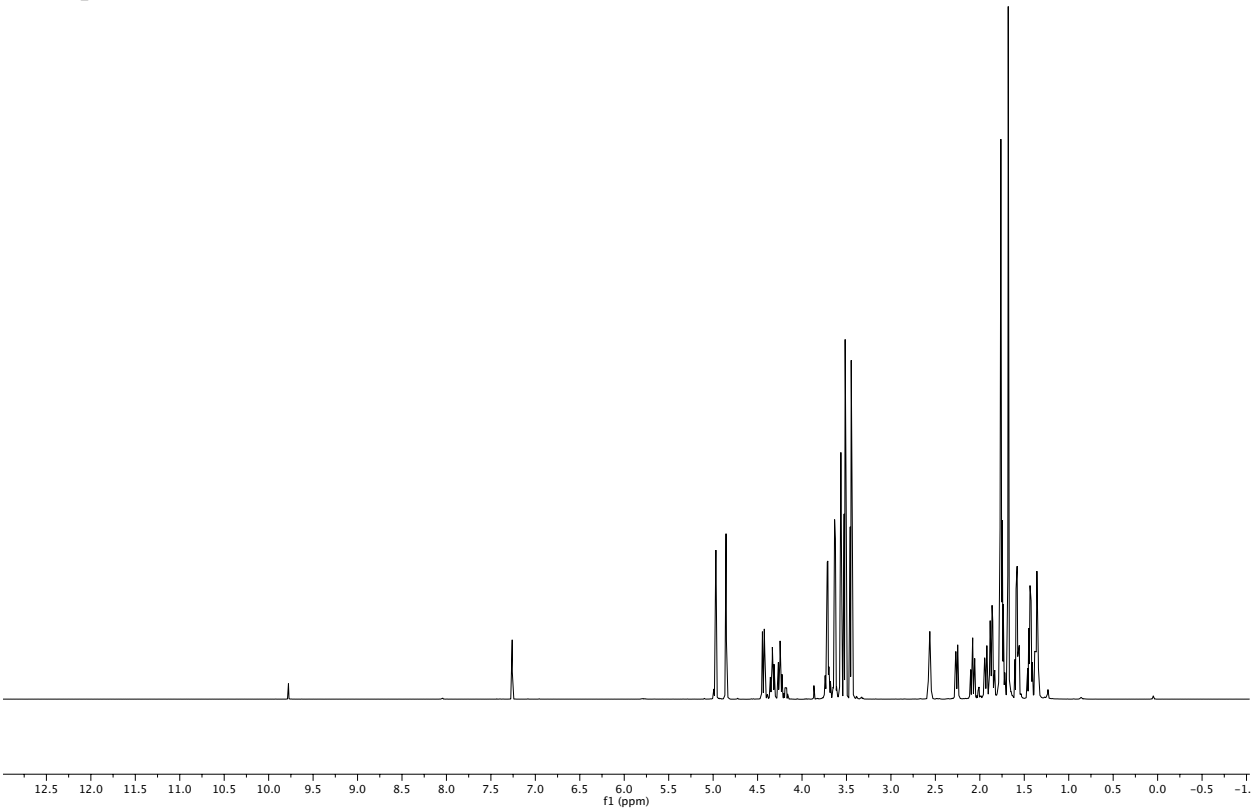
Compound Ψ-15 ^{13}C NMR



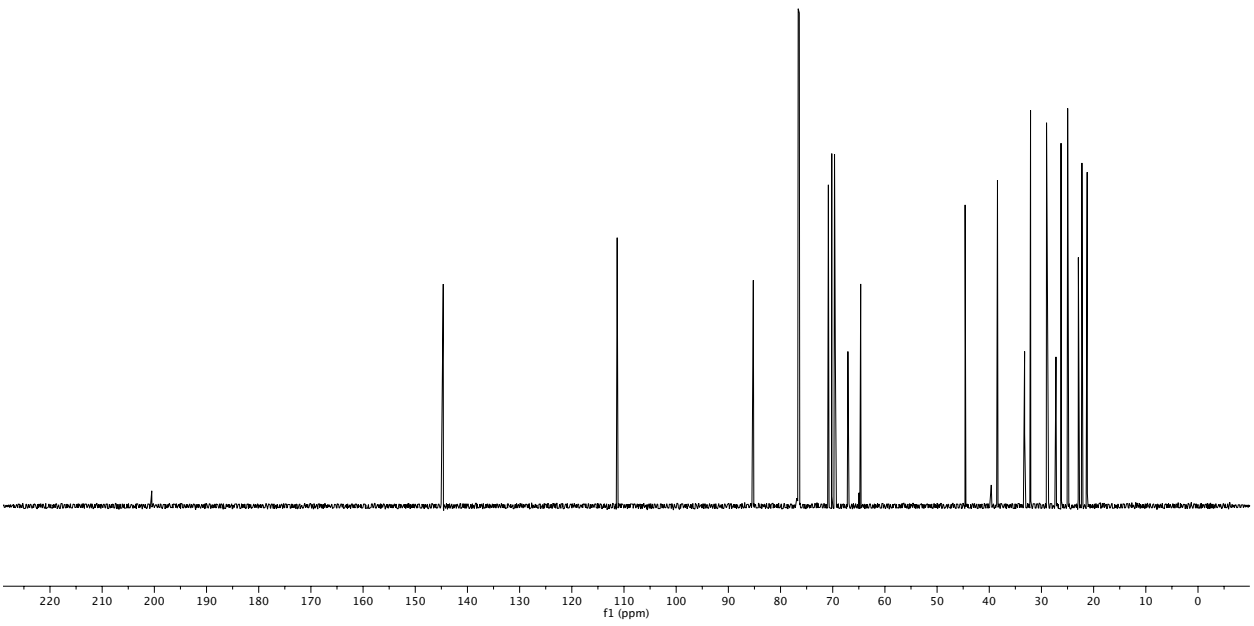
Compound Ψ-15 ^{31}P NMR



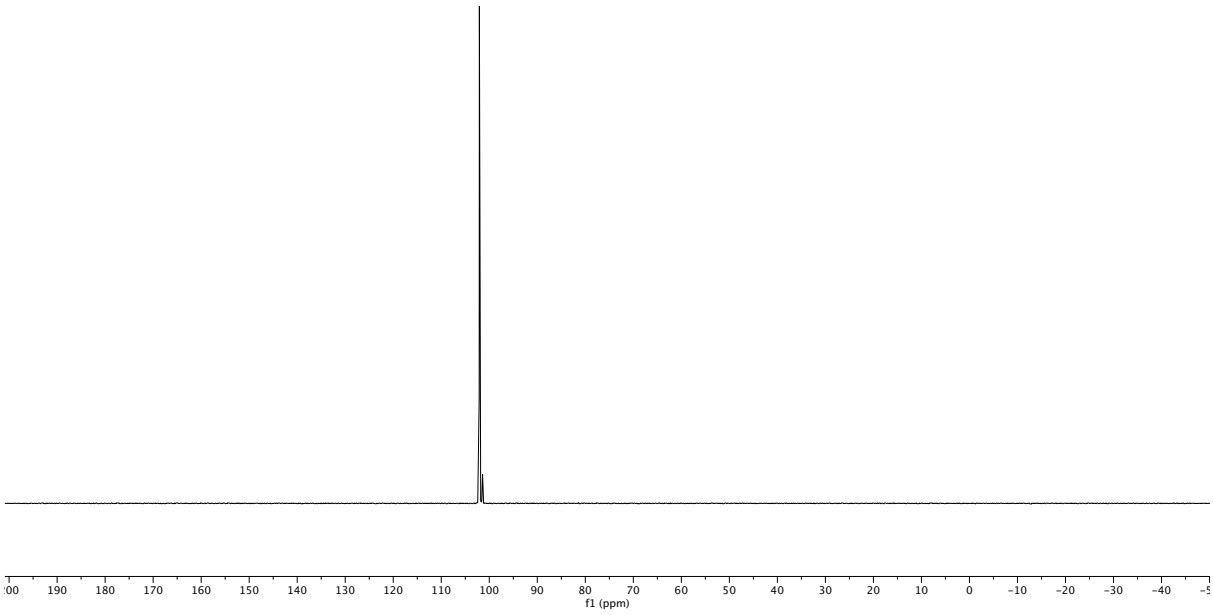
Compound Ψ-16 ^{1}H NMR



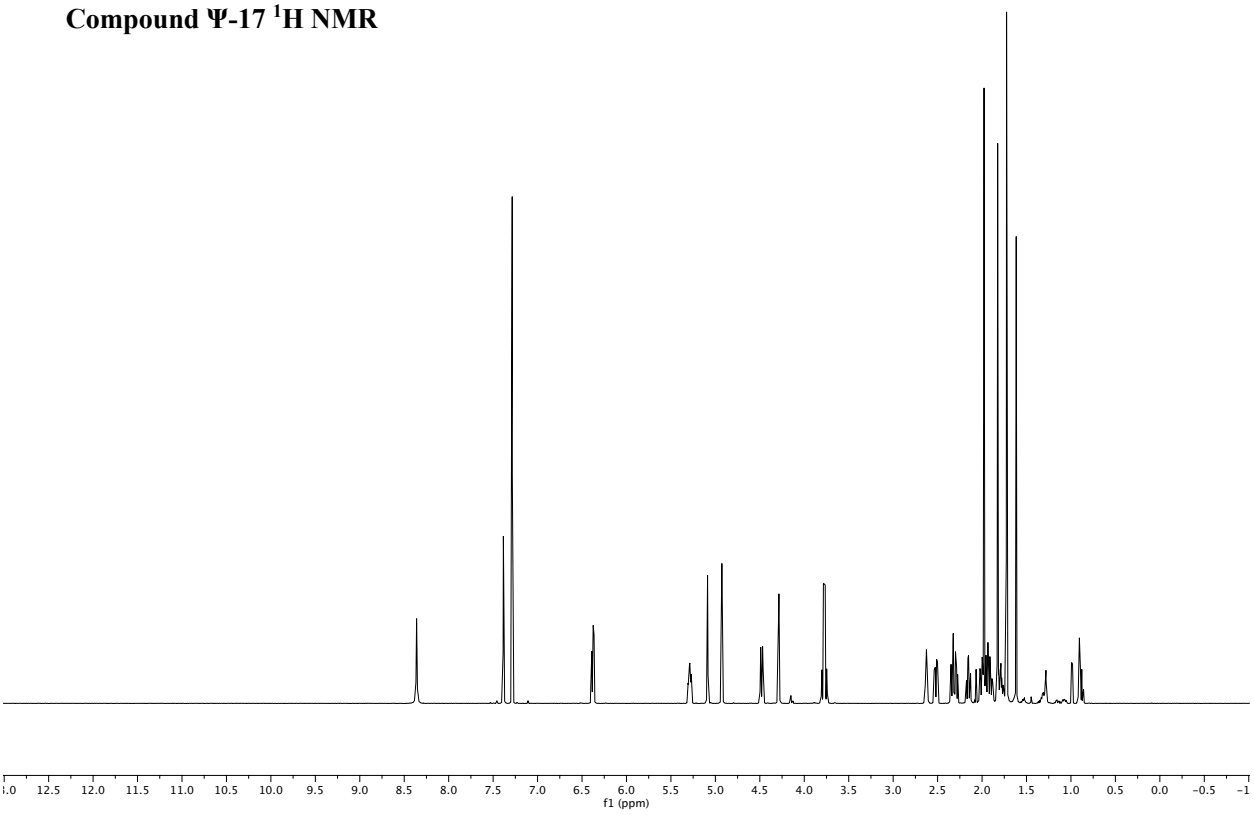
Compound Ψ-16 ^{13}C NMR



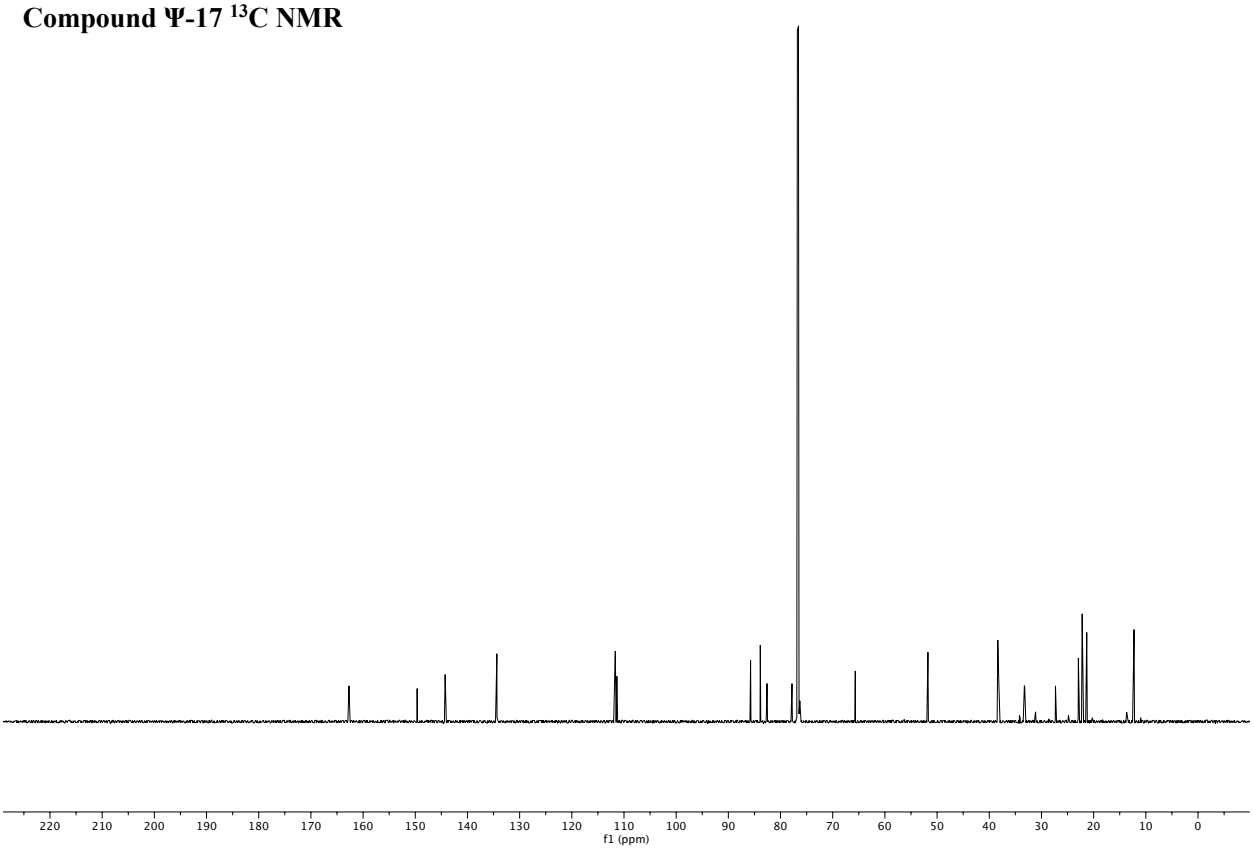
Compound Ψ-16 ^{31}P NMR



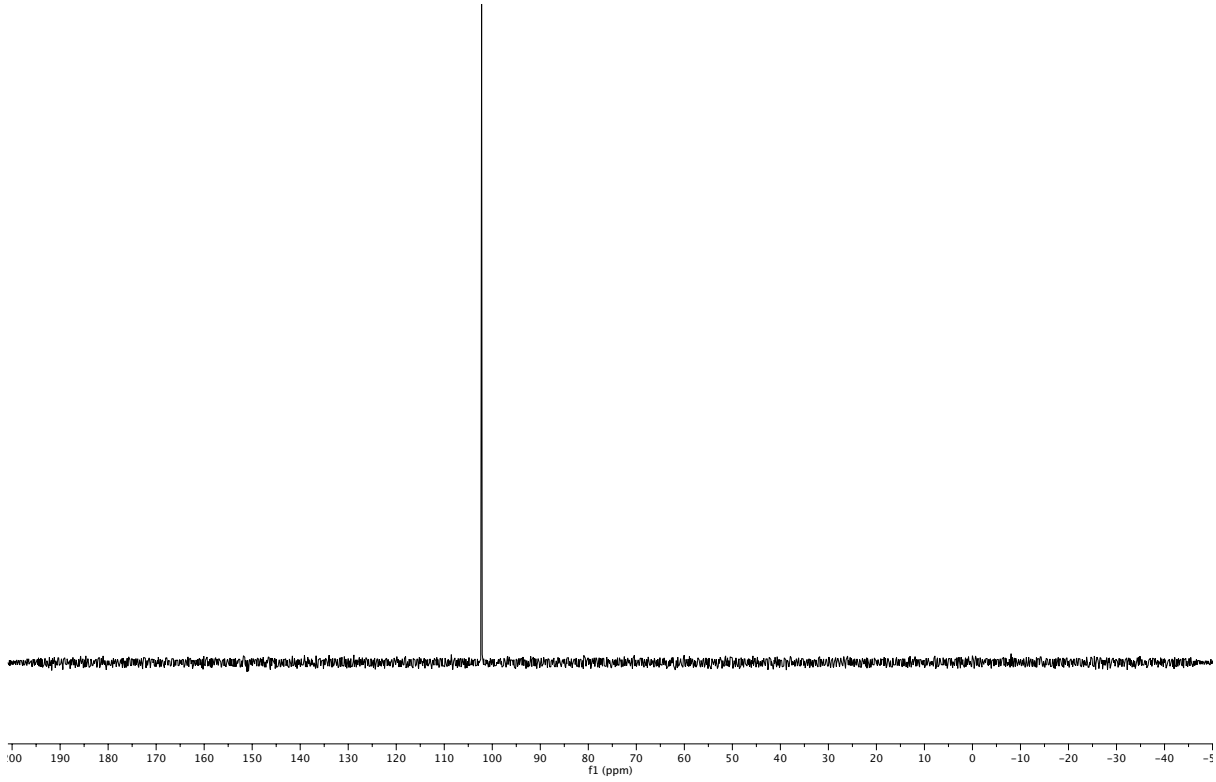
Compound Ψ-17 ^{1}H NMR



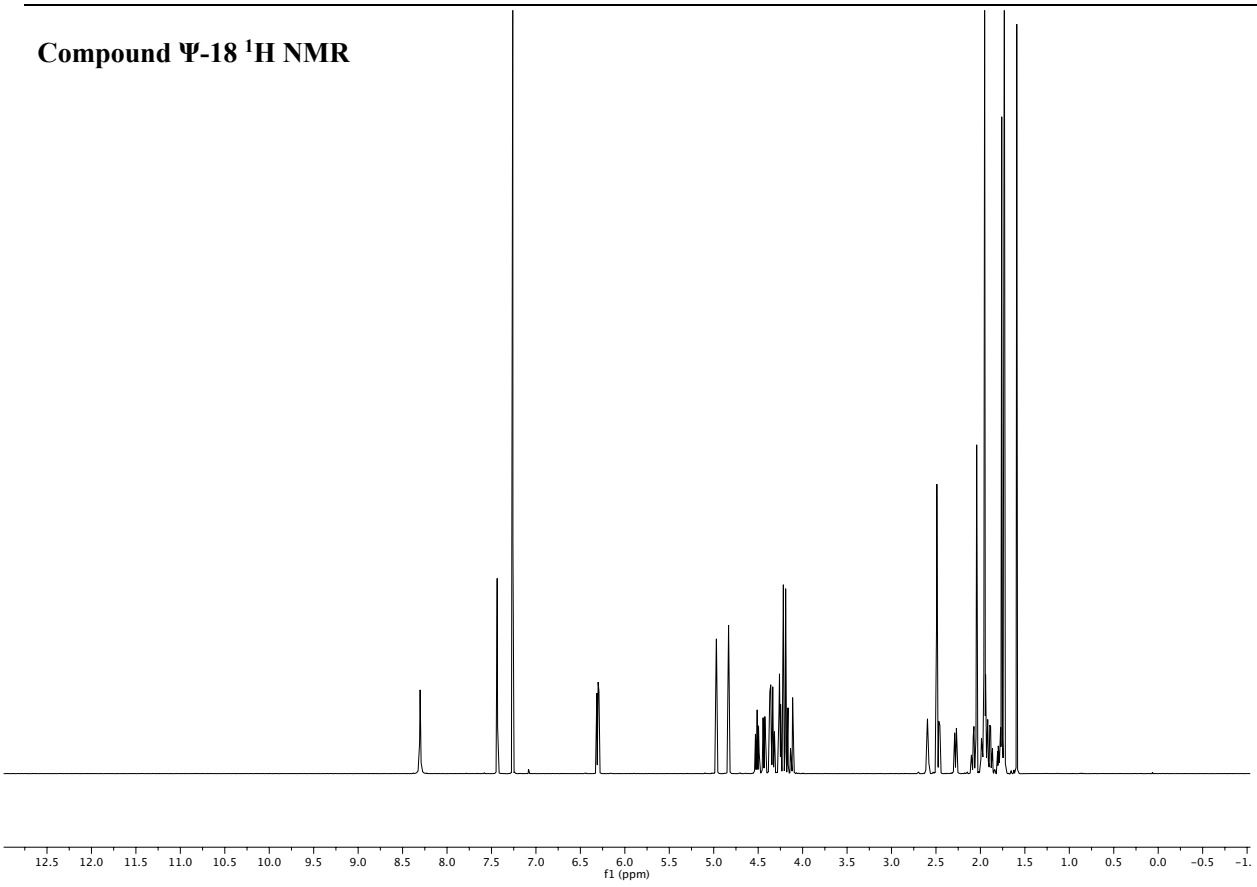
Compound Ψ-17 ^{13}C NMR



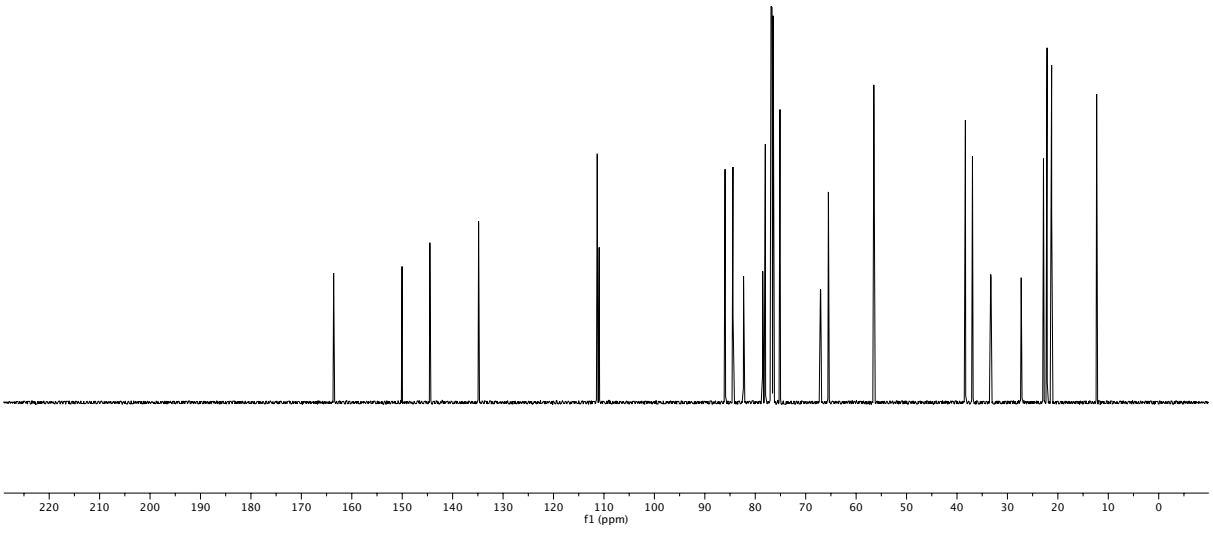
Compound Ψ-17 ^{31}P NMR



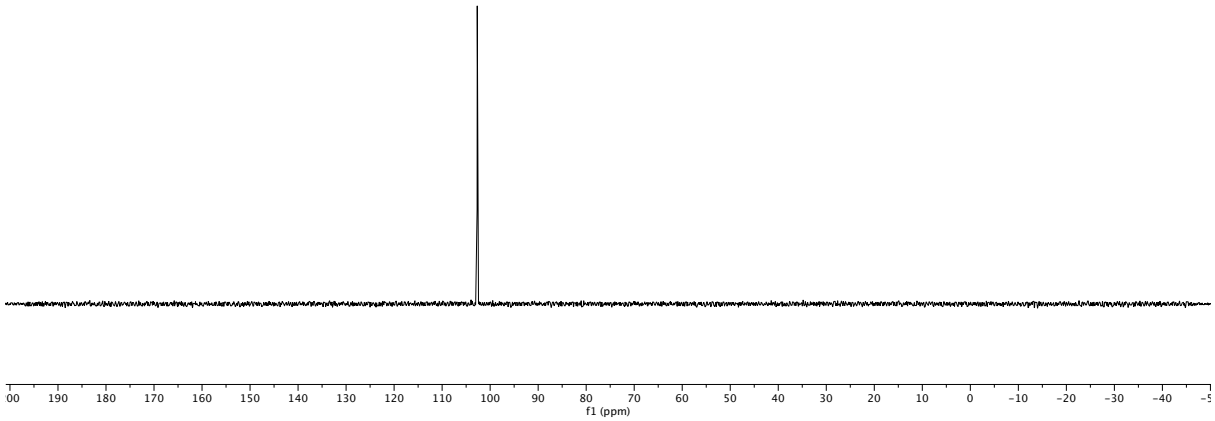
Compound Ψ-18 ^{1}H NMR



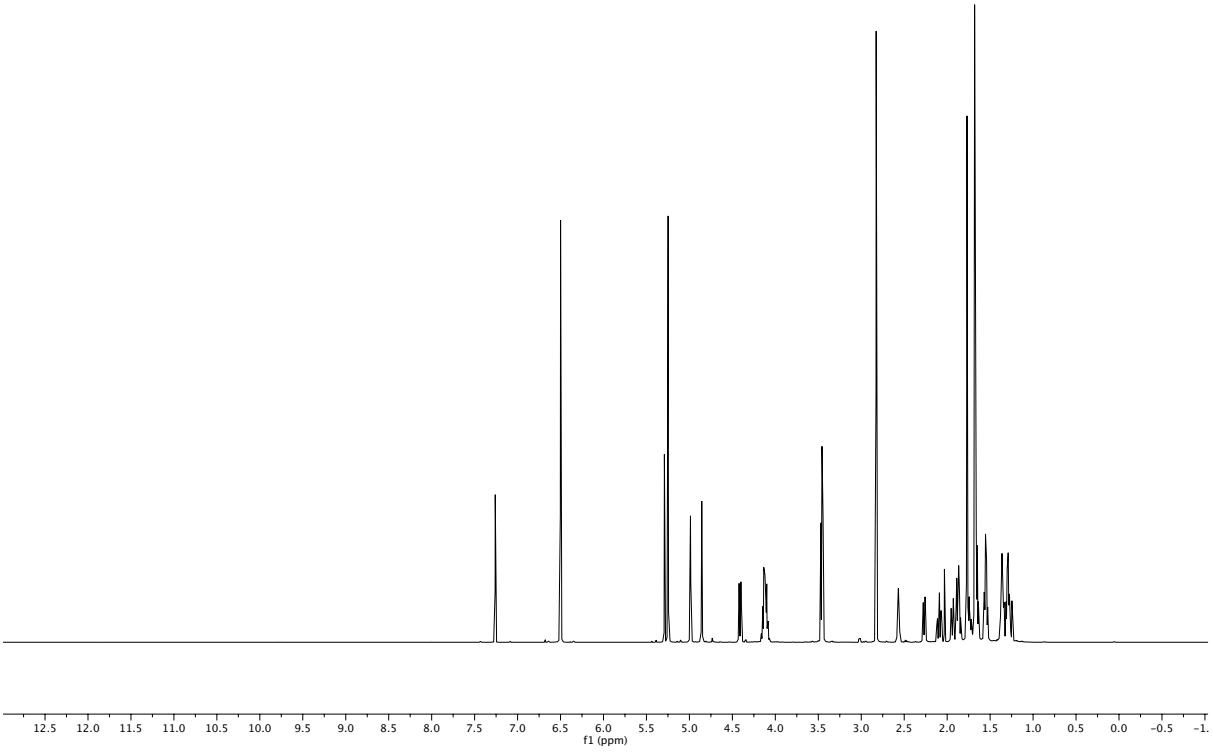
Compound Ψ-18 ^{13}C NMR



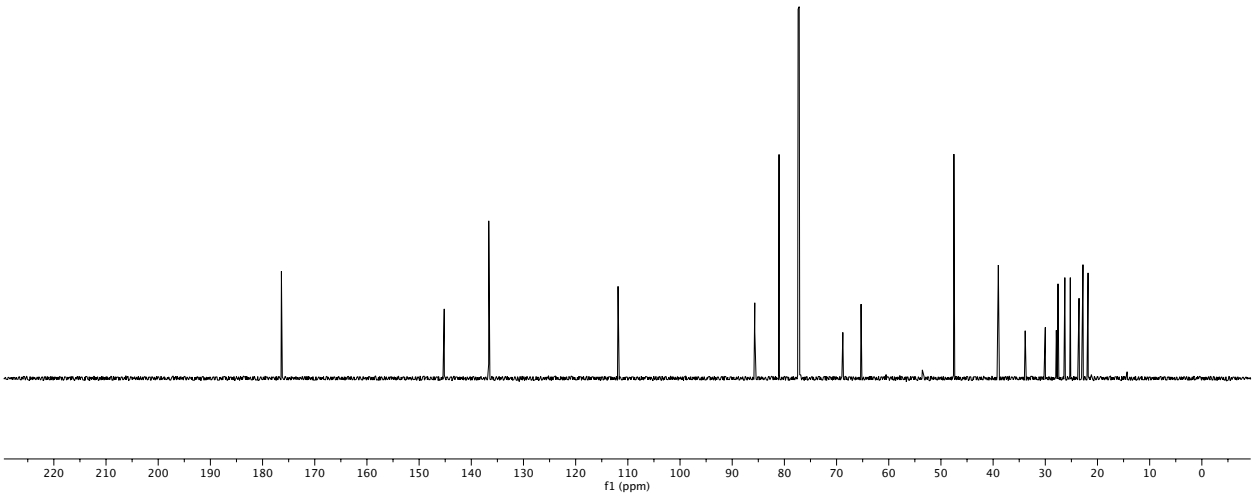
Compound Ψ-18 ^{31}P NMR



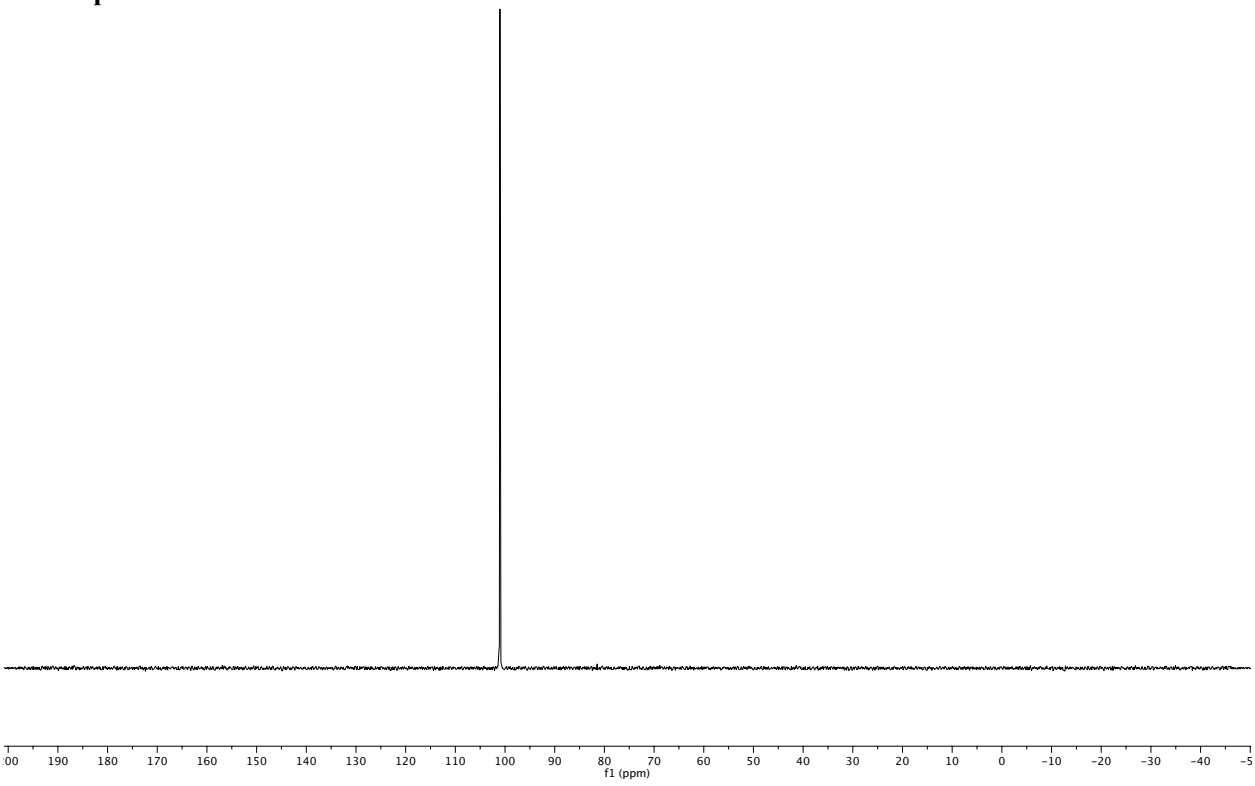
Compound Ψ-19 ^{1}H NMR



Compound Ψ-19 ^{13}C NMR



Compound Ψ-19 ^{31}P NMR



SENDR Protocol

General Procedure 2: SENDR in Microcentrifuge Tubes

Resin Preparation and DNA Loading

1. Cut the tip off of a 1 mL pipette tip and transfer resin (100 µL resin equilibrate in 1:1 PBS:MeCN, ~25 mg dry resin) with an adjustable volume pipette—Phenomenex Strata-XAL—into a 2 mL microcentrifuge tube.
2. Wash Resin with 500-1000 µL PBS.
    a. Spin down the resin slurry and aspirate the resin bed (by sucking off the supernatant with a glass pipet).
3. Add DNA (up to 50 nmol) in 100-500 µL PBS, vortex and incubate at room temp for 5-15 min.

Resin Washing and Drying

4. Wash loaded resin twice with DMA (500 µL)
5. Wash loaded resin three times with dry THF (500 µL)
6. Dry the resin for >2h in a speed vac or stuff a ball of paper in the top (so no resin can escape) and dry under vacuum for >2 h.
7. Resin Washing and Drying

Running the Reaction

8. Dissolve Ψ-Module in dry MeCN (150 mM, 250 µM), and add it to the dry resin tube.
9. Add DBU (450 mM total, 18 µL) to the reaction tube.
10. Vortex for 30 seconds and incubate for 60 minutes at room temperature or 37 ºC.

Working up Reaction

11. Aspirate reaction mixture and discard.
12. Wash reaction with MeCN or DMA (500 µL)
13. Wash with PBS or 1:1 PBS:MeCN (500 µL)

Elute DNA

14. Add 300 uL Elute Buffer (1 M $NaClO_4$, 20% MeOH, 40 mM tris, pH 8.5) to resin bed.
15. Vortex for 30 seconds and agitate for 5-10 minutes.
16. Carefully collect the elution butter with micropipette without sucking up any resin.
17. Desalt with your preferred method.

Note: Strata-XAL if designed for large analytes. Strata-XAL is a 100 µm particle a with 300 Å pore size.

Note: “Washing the resin” refers to adding 500 to 1000 µL of solvent to the resin, vortexing (30 sec), spinning the resin down, and removing the supernatant with a pipette.

Note: Dry acetonitrile should be used for best reaction conversion.

Note: The elute buffer which contains 1 M $NaClO_4$, 20% MeOH, 40 mM tris, pH 8.5 should be accessed with high quality NaClO, Acros Orgaincs is our preferred supplier (part number AC197122500)

Troubleshooting and Frequently Asked Questions

1. When I try to pellet my resin, it is too flocculant and is still in solution after taking out of the centrifuge.
Allow the resin tube to sit for 5-10 minutes undisturbed to allow the resin to settle to the bottom of the tube.

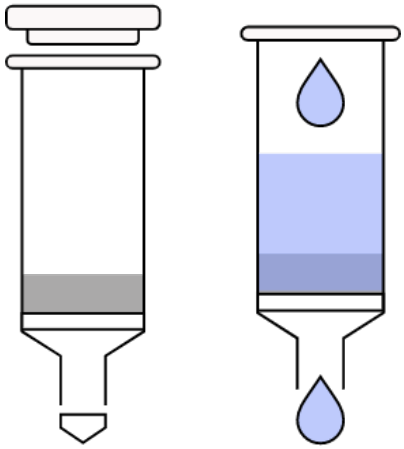
2. How do I remove the supernatant with a pipette?
Gently suck off the supernatant while trying to leave the resin bed undisturbed. Keeping the pipette tip a few millimeters off the top of the resin bed usually helps with this, also allowing a small amount of liquid to remain on the top of the resin bed is alright.

3. My Ψ-Module is not dissolving, what do I do?
To get the Ψ-Module dissolved you may need to sonicate the solution for up to 10 minutes. Usually this is sufficient to dissolve all of the PSI-module. If this does not work, transfer heterogeneous PSI-module solution to the reaction solution and add the DBU. Usually upon addition of DBU the rest of the PSI-module will dissolve.

4. My reaction resulting in low conversions, what should I do.
Try to heat the reaction to 37 ºC.

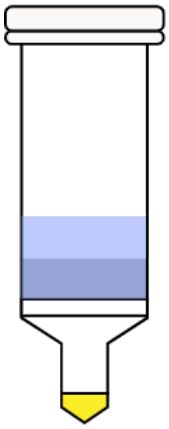
General Procedure 3: SENDR in Fritted Cartridges (Kit Format)

Resin Preparation



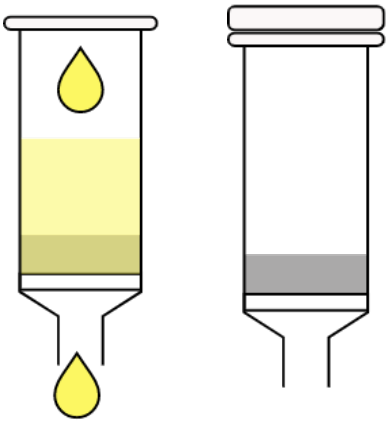
1. Break off bottom of preloaded (with 100 µL Strata-XAL resin in 1:1 PBS:MeCN) Biorad column. Or transfer equilibrated resin (100 µL) to the column as above.
2. Allow packing solution to flow through into waste.
3. Add PBS (500 µL) and allow to flow through into waste.

DNA Loading:



4. Cap the bottom spout and add DNA (up to 50 nmol in 100-500 µL PBS) to column.
5. Cap the top, vortex and agitate for 5-15 min.

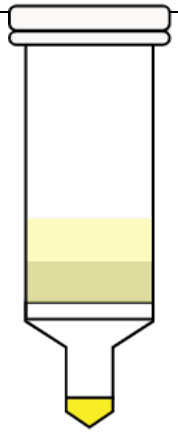
Resin Washing and Drying



6. Wash loaded resin twice with DMA (500 µL) and allow to flow to waste.
7. Wash loaded resin three times with dry THF (500 µL) and allow to flow to waste.
8. Replace the top cap but *not the bottom* and dry the resin for >2h in under vacuum.

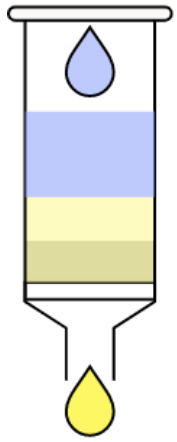
Running the Reaction

9. Add a NEW, CLEAN, and DRY cap to the bottom of the column.
10. Dissolve Ψ-Module in dry MeCN (300 mM, 125 µL) or add the supplied PSI-Module solution to dry resin tube.
11. Add DBU in dry MeCN (300 mM, 125 µL) or add the supplied DBU solution to resin tube.
12. Replace both caps, vortex for 30 seconds, and incubate for 60 minutes at 37 ºC.



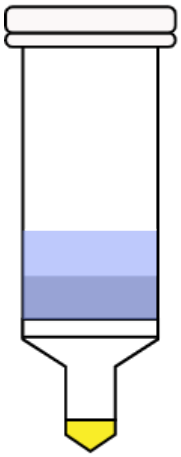
Working up Reaction

13. Remove both caps and allow the reaction mixture to drain to waste.
14. Wash reaction with MeCN or DMA (500 µL) allow to drain to waste
15. Wash with PBS or 1:1 PBS:MeCN (500 µL) allow to drain to waste



Elute DNA

16. Replace bottom cap. Add 300 uL Elute Buffer (1 M $NaClO_4$, 20% MeOH, 40 mM tris, pH 8.5) to resin bed and then replace top cap.
17. Vortex for 30 seconds and agitate for 5-10minutes.
18. Remove both caps and allow elute buffer to drain into a collection tube.
19. Desalt with your preferred method.



Note: Allow flow throughs to occur under gravity not under vacuum.

Note: Agitating the resin can be done with an end over end tumbler or an orbital shaker.

Note: Drying the resin under vacuum can be performed using a lyophilizer, high vac, or speed vac (if the columns fit).

Troubleshooting and Frequently Asked Questions

1. The storage solution in the column takes a long time to drain, what do I do?
Sometimes, an air bubble forms at the bottom of the column and surface tension prevents the solution from flowing through. Put gentle pressure on top of the column using a gloved finger. Once you see a single drop drip out of the bottom of the column, allow the rest of the solution flow through by gravity.

2. When I add the PBS to wash the resin, it takes a long time to drip out?
See above.

3. Why do I need a dry bottom cap while running the reaction?
Any water remaining on the cap might quench the Ψ-Module. Using a new, clean, dry cap ensures that no water enters the reaction mixture.

4. How do I get my DNA out of the elute buffer?
Almost any standard DNA desalting method will work. We tend to use ethanol precipitations. Some prefer other methods that are faster but require different DNA consumable kits. In our hands both silica-based desalting columns and gel filtration worked for desalting of modified DNA.

Optimization of SENDR protocol and conditions

Optimization Procedure

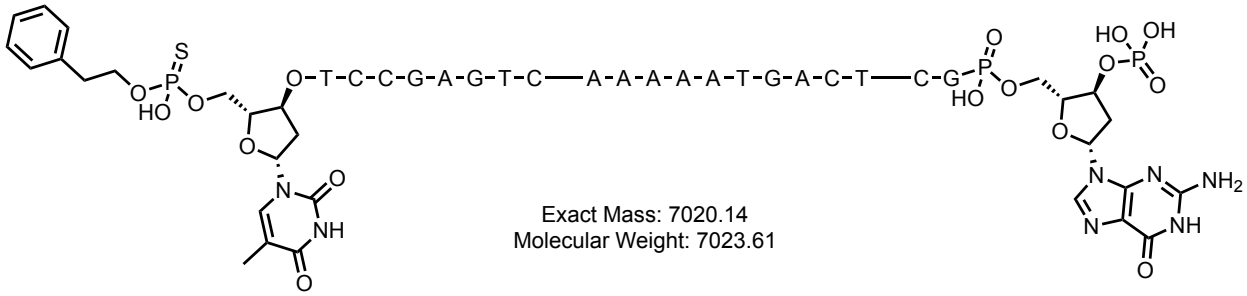
SENDR was optimized by performing reactions in a microcentrifuge tube as described in *General Procedure 2* with various reaction conditions (as described in the main text and Figure 2B). Briefly, 5 nmol of DNA **1** or **2** was loaded onto 100 µL of equilibrated resin. This loaded resin was washed with DMA (500 µL twice) and dry THF (500 µL three times) and dried under vacuum. In the case in which stringent drying was not performed (Figure 2B, Entry 8) the loaded resin was simply washed with acetonitrile (500 µL three times).

Ψ-1 in MeCN (varying concentration, 250 µL) was added to the loaded and dried resin,. Then DBU (various concentration) was added to the reaction mixture. The reaction tube was vortexed for 30 seconds and incubated at room temperature for various amounts of time. The reactions were worked up by aspirating and discarding the reaction solution, and washing the resin bed with MeCN (500 µL) and 1:1 MeCN:PBS (500 µL). Elute buffer (300 µL) was added to the resin bed and the tube was agitated by orbital shaker for 5-10 minutes. The DNA containing elution buffer was collected by carefully pipetting the supernatant. DNA was isolate *via* ethanol precipitation. Ethanol precipitation was performed by adding 10% v/v of a NaCl solution (30 µL, 5M) to the elute buffer and three volumes of cold ethanol (-20 ºC) were added to the tube (~1000µL) and incubated for 18 hours at -20 ºC. The tube was then centrifuged at 13,000 rpm for 15 minutes to pellet the DNA, and the supernatant ethanol was decanted off. The tubes were dried *via* speed vacuum, the DNA was dissolved and HPLC-MS analysis was performed.

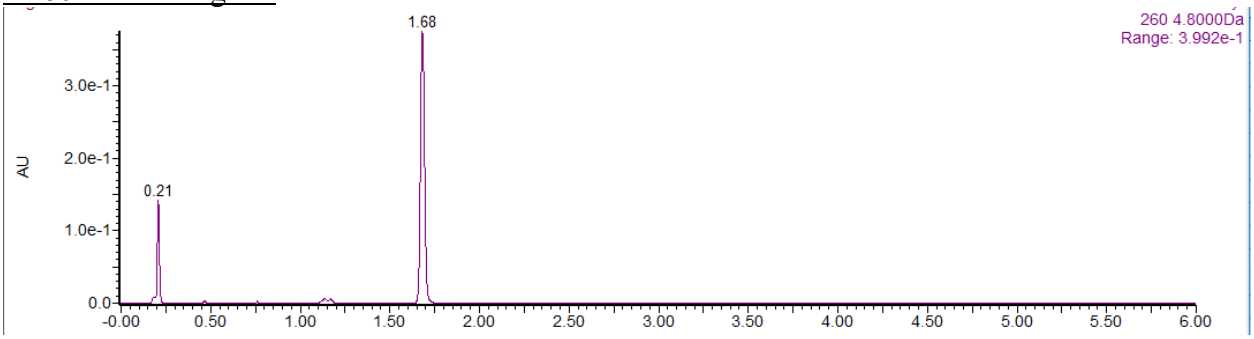
Characterization of SENDR Optimization

LCMS Characterization of 3

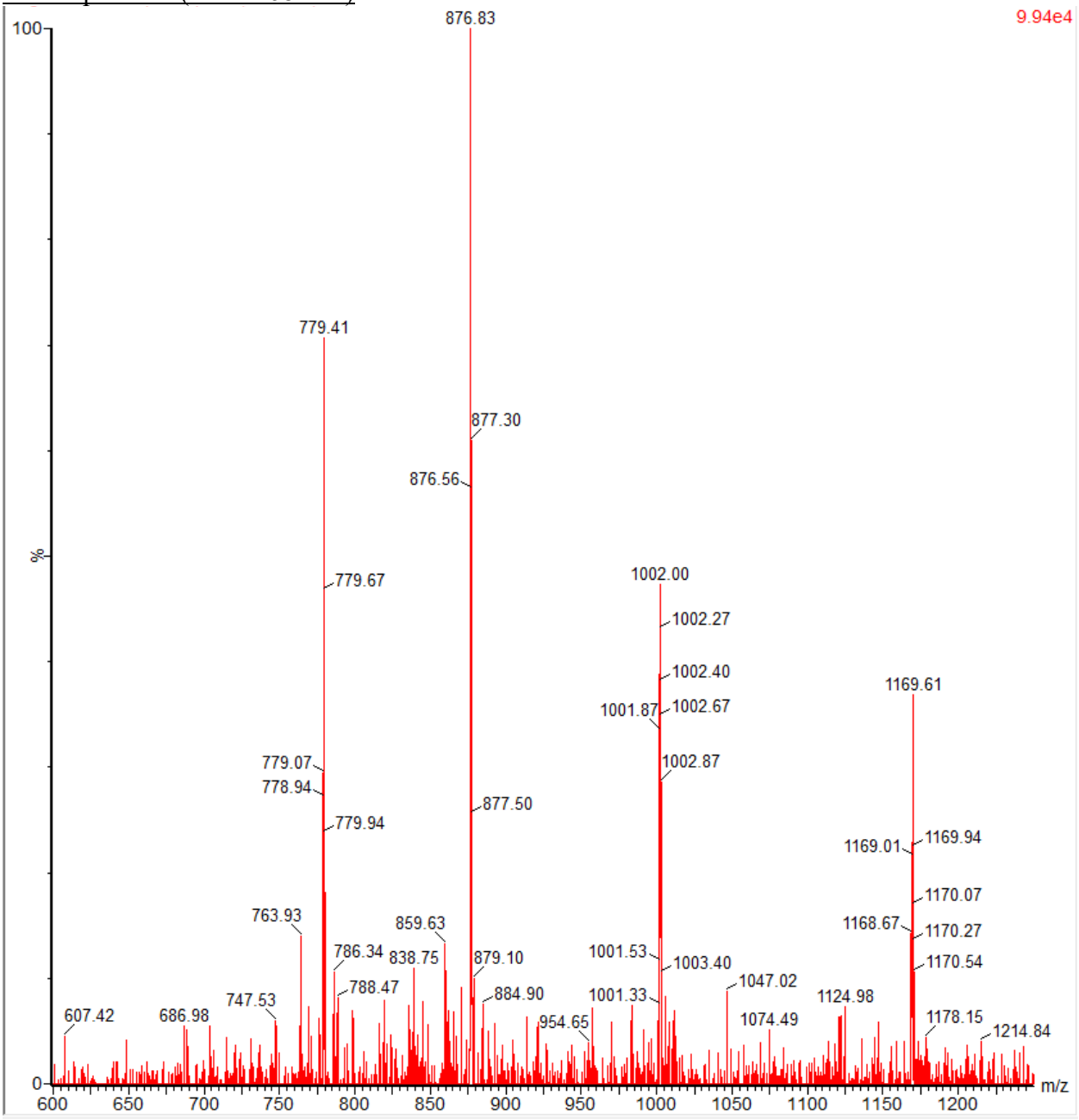
Produced from **1** by General Procedure **2:**
Conversion (A260): 91%
Expected Mass: 7023.61
Expected (M-6)/6 Ion: 1169.60
Found Deconvoluted Mass: 7024.00
Found (M-6)/6 Ion: 1169.61



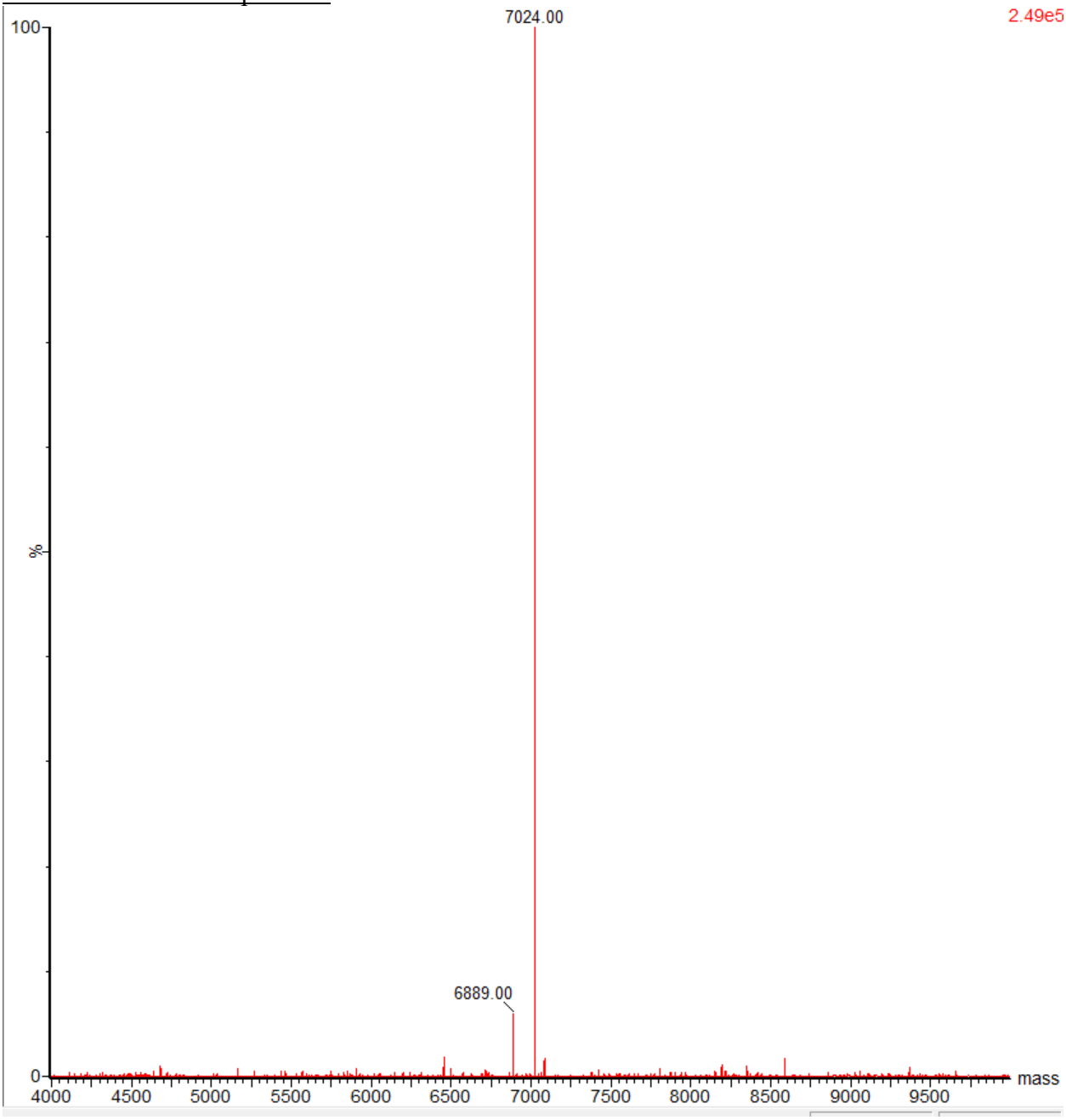
A260 Chromatogram



Mass Spectrum (RT= 1.68 min)

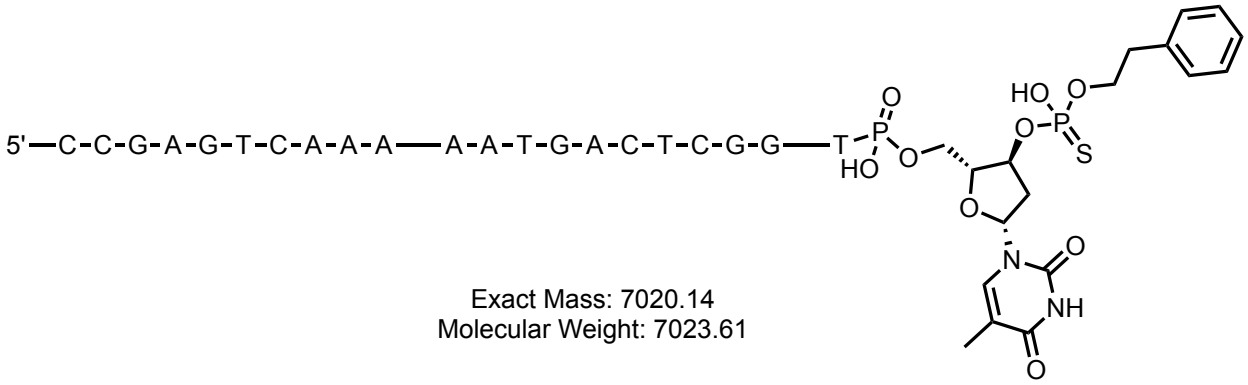


Deconvoluted Mass Spectrum



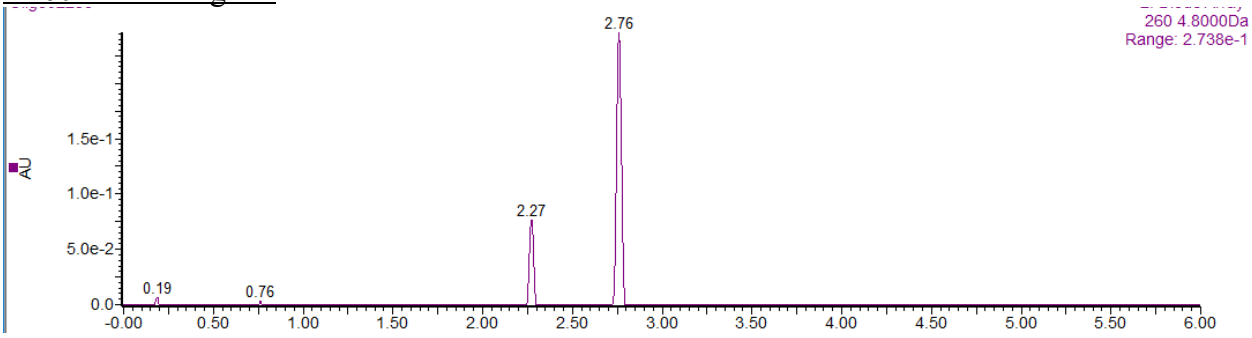
LCMS Characterization of 4

Produced from **2** by General Procedure **2:**
Conversion (A260): 72%
Expected Mass: 7023.61
Expected (M-6)/6 Ion: 1169.60
Found Deconvoluted Mass: 7024.00
Found (M-6)/6 Ion: 1169.01

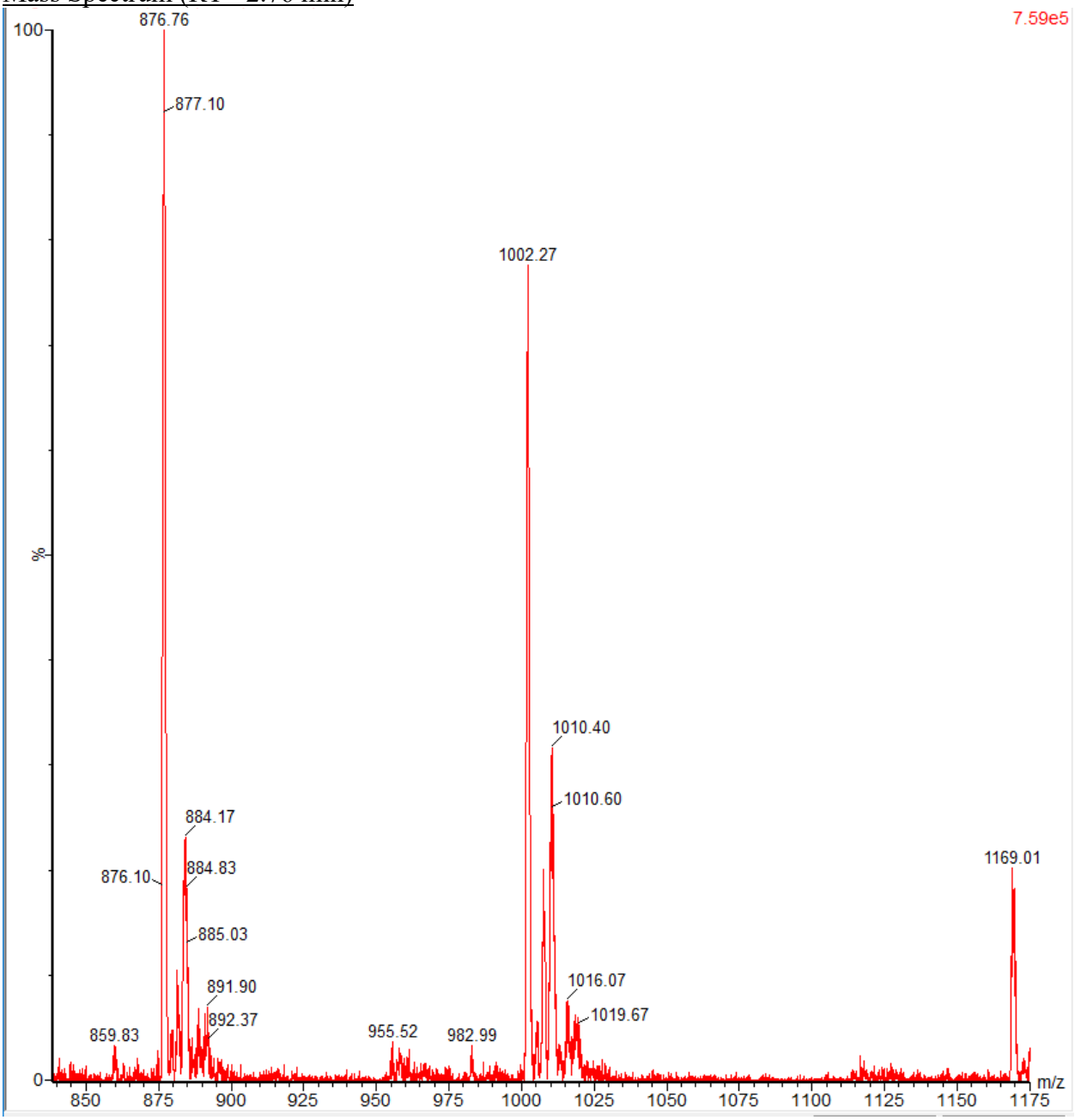


Exact Mass: 7020.14
Molecular Weight: 7023.61

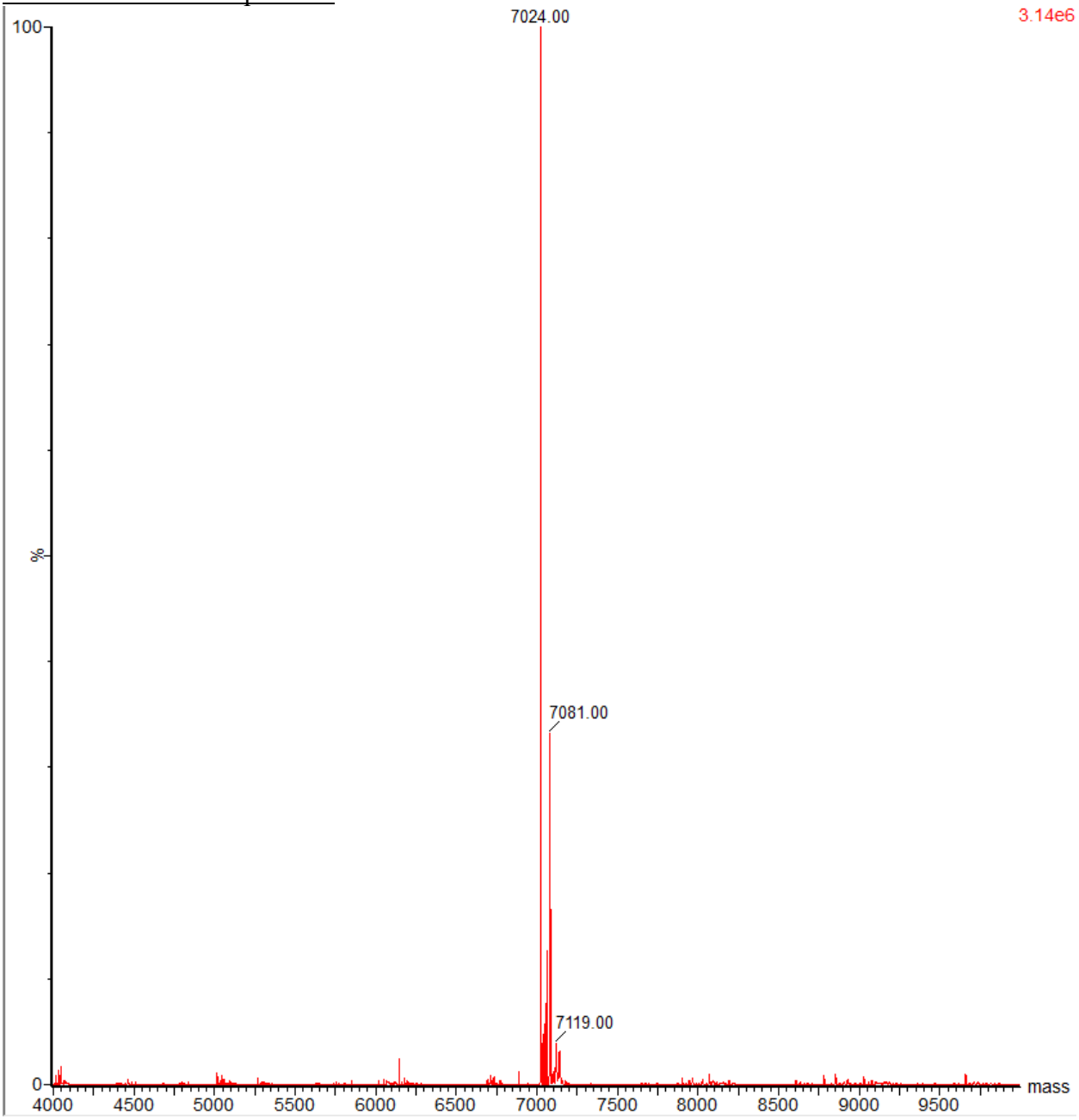
A260 Chromatogram



Mass Spectrum (RT= 2.76 min)

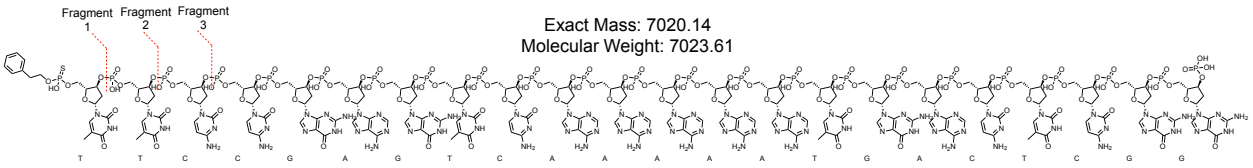


Deconvoluted Mass Spectrum



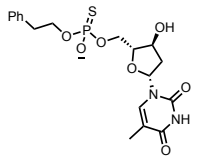
Process for Mass Fragmentation of 3

A crude solution of **3** in water (100 μM) was injected on the Waters I-Class ToF for peak identification. The MS cone voltage was then increased from 5 mV to 30 mV and the same sample was reinjected to fragment the modified oligonucleotide. Diagnostic mass fragments were then extracted, and their presence confirmed in the total ion current. They are plotted below along with total ion current and UV absorbance. UV absorbance is slightly out of alignment because there is a non-zero-time difference between when the compound passes through the UV detector and when it enters the mass spec. This is because of the plumbing between the two modules.



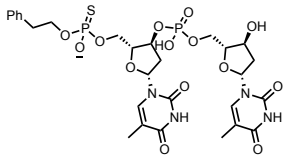
Exact Mass: 7020.14
Molecular Weight: 7023.61

Fragment 1



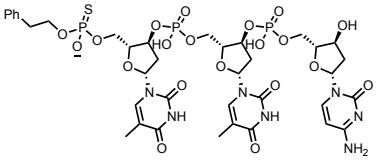
Chemical Formula: $C_{18}H_{22}N_2O_7PS^-$
Exact Mass: 441.09
Molecular Weight: 441.42

Fragment 2

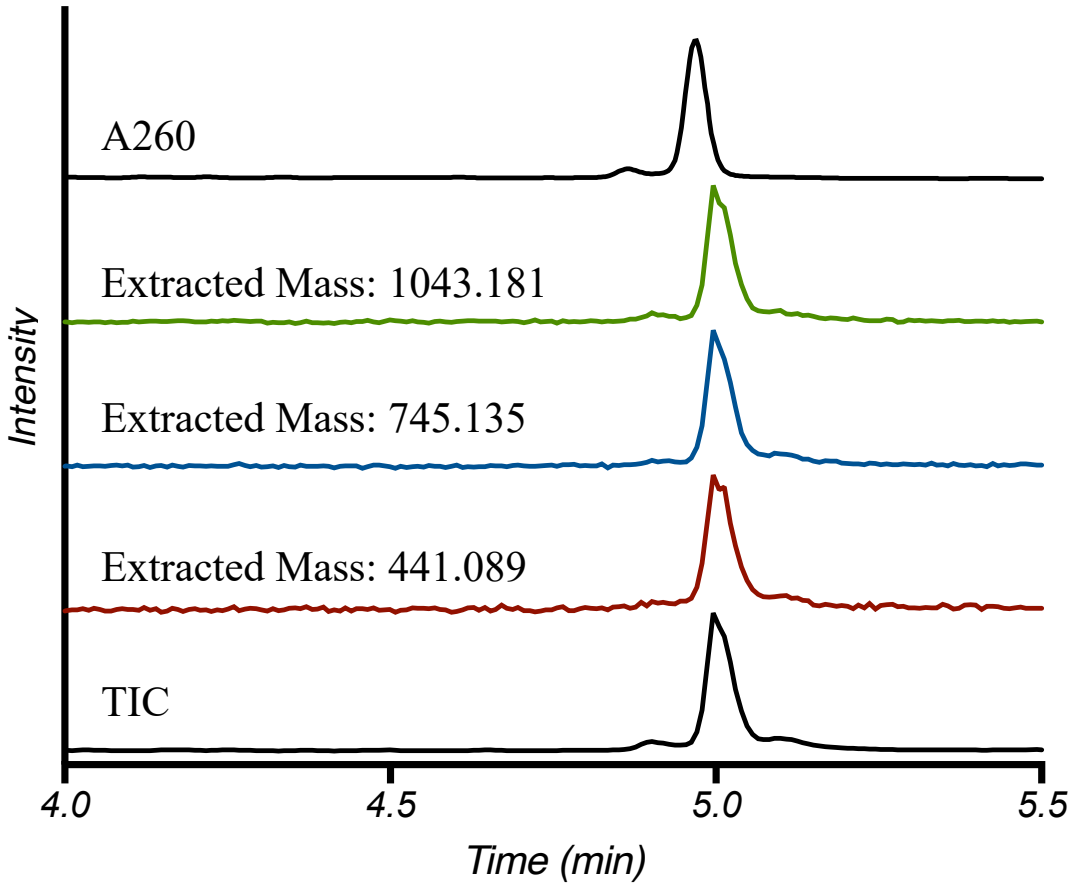


Chemical Formula: $C_{28}H_{35}N_4O_{14}P_2S^-$
Exact Mass: 745.14
Molecular Weight: 745.61

Fragment 3



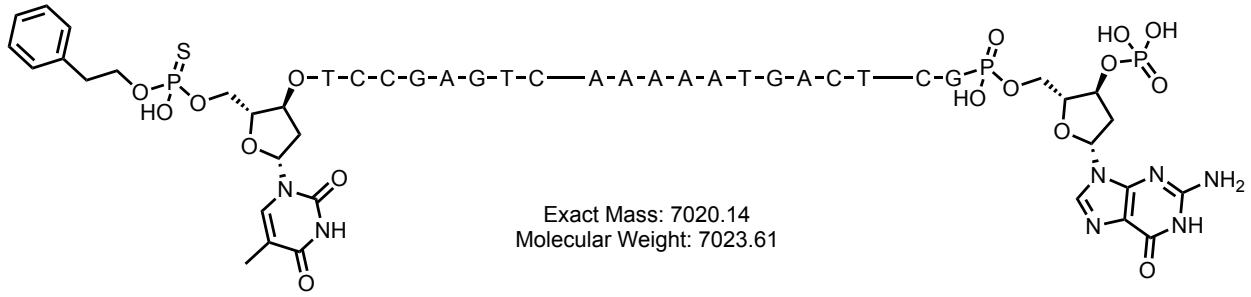
Chemical Formula: $C_{37}H_{47}N_7O_{20}P_3S^-$
Exact Mass: 1034.18
Molecular Weight: 1034.79



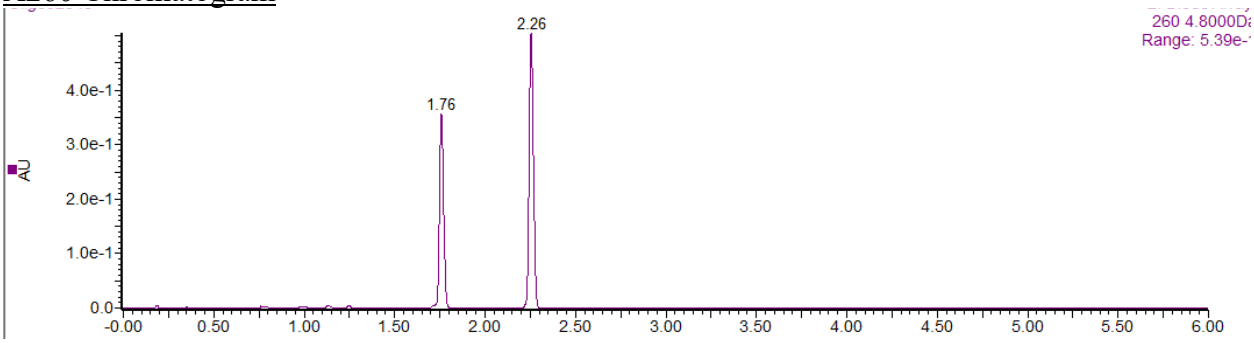
Analysis of Optimization Reactions

Entry 1: 5'

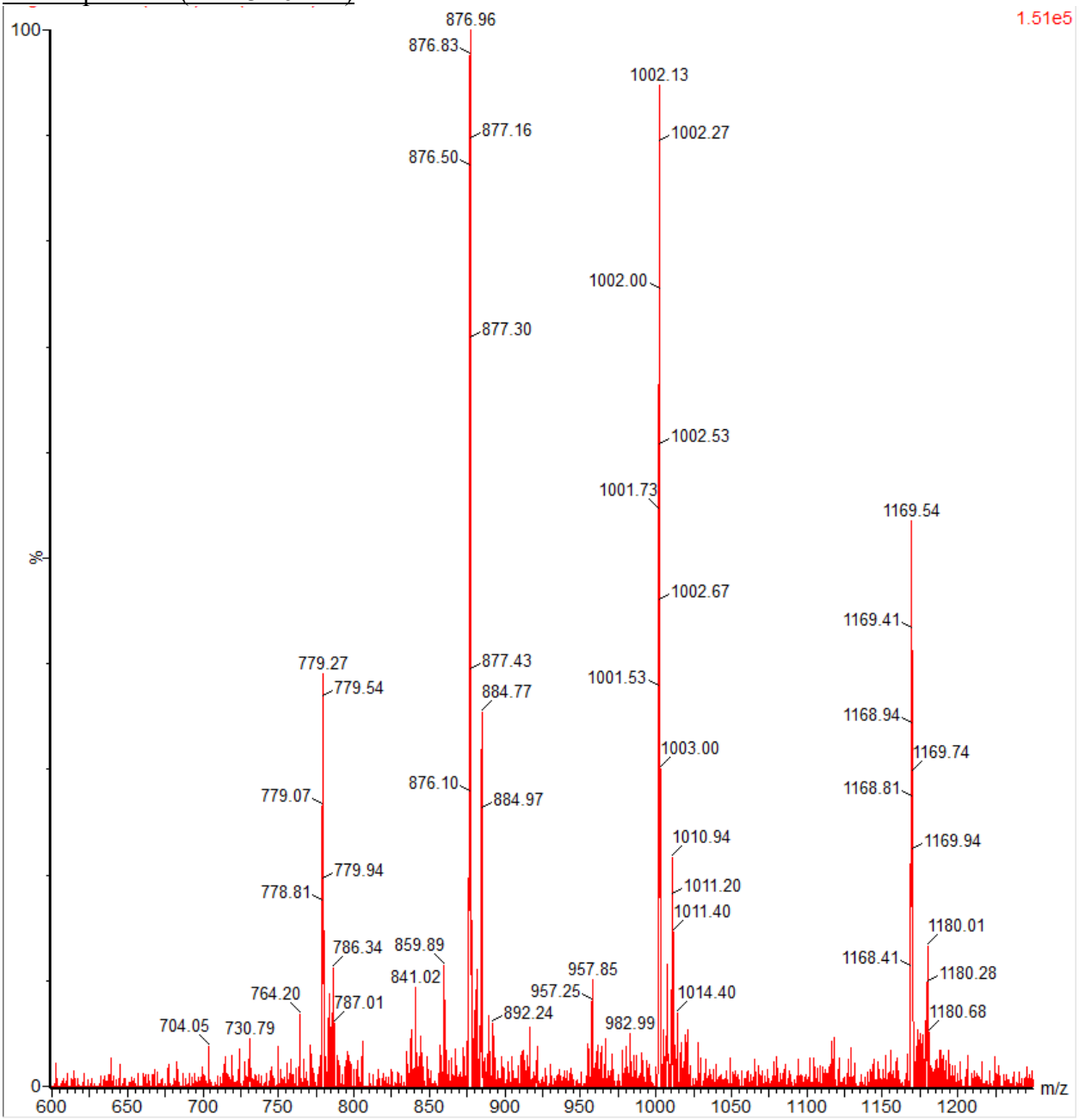
LCMS Characterization of **3**
Produced from **1** by General Procedure **3:**
Conversion (A260): 56%
Expected Mass: 7023.61
Expected (M-6)/6 Ion: 1169.60
Found Deconvoluted Mass: 7024.00
Found (M-6)/6 Ion: 1169.54



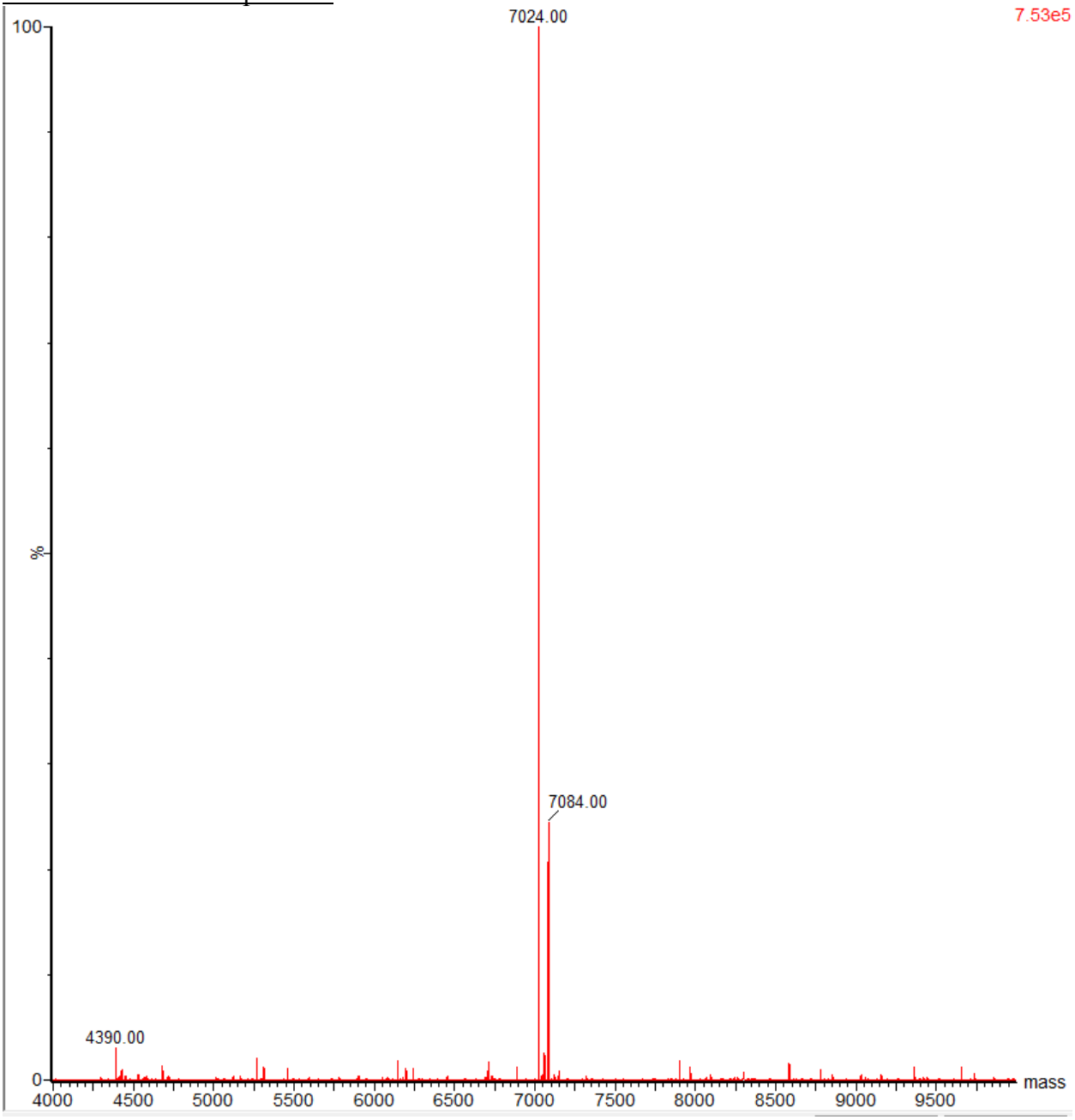
A260 Chromatogram



Mass Spectrum (RT= 3.26 min)

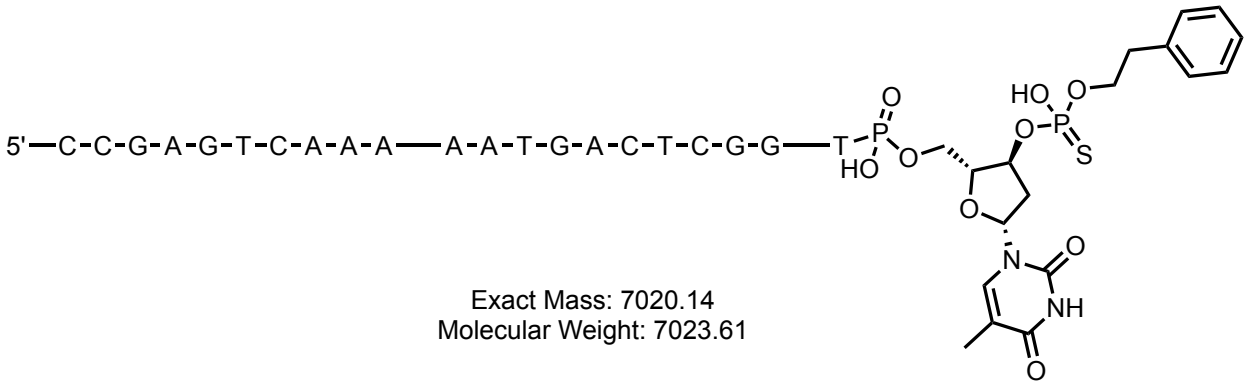


Deconvoluted Mass Spectrum



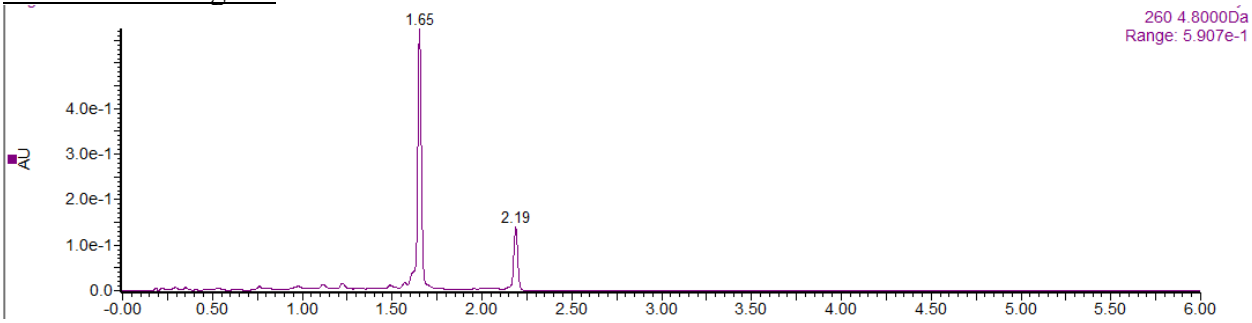
Entry 1: 3'

LCMS Characterization of **4**
Produced from **2** by General Procedure **2:**
Conversion (A260): 17%
Expected Mass: 7023.61
Expected (M-6)/6 Ion: 1169.60
Found Deconvoluted Mass: 7023.00
Found (M-6)/6 Ion: 1169.21

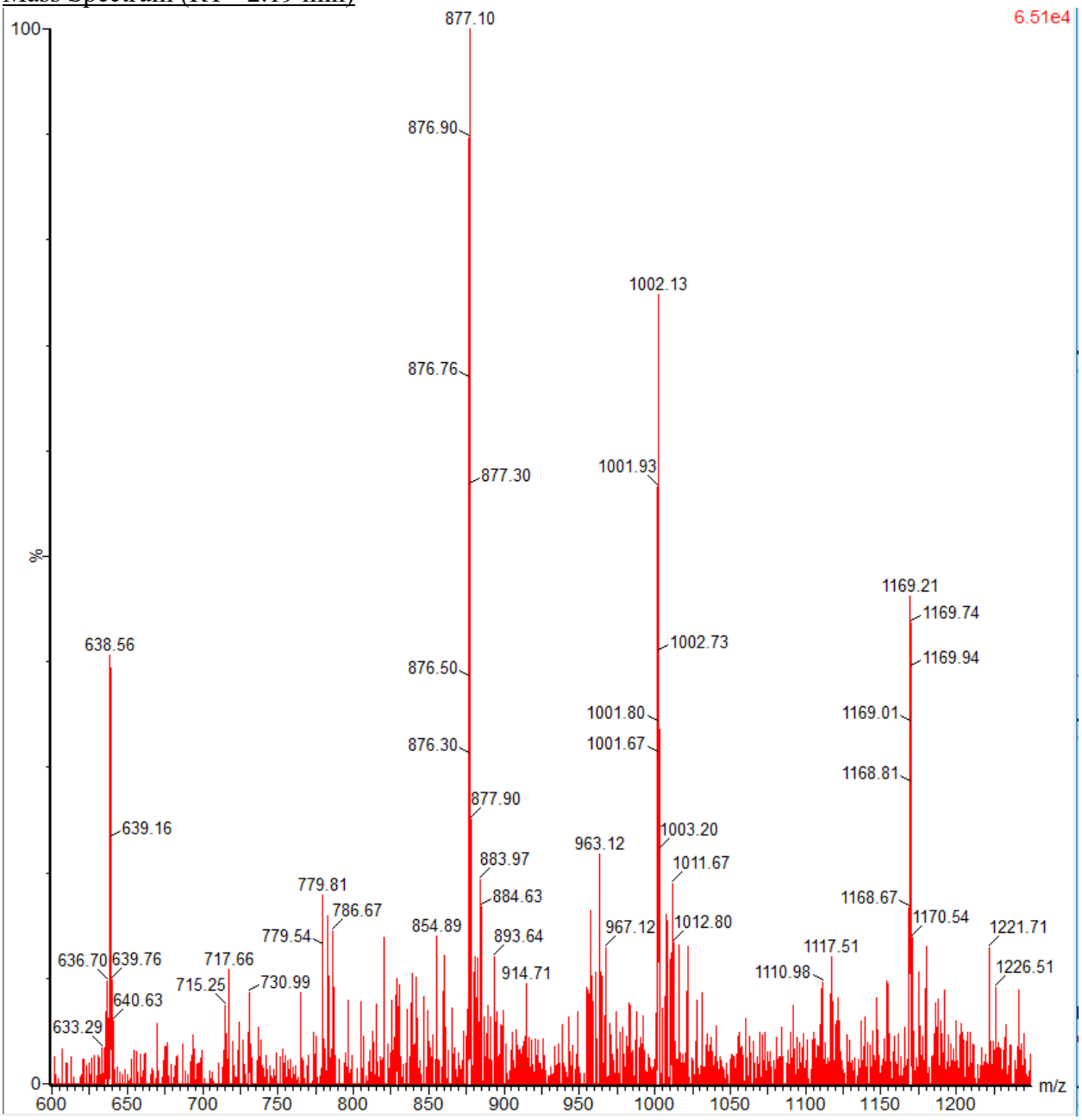


Exact Mass: 7020.14
Molecular Weight: 7023.61

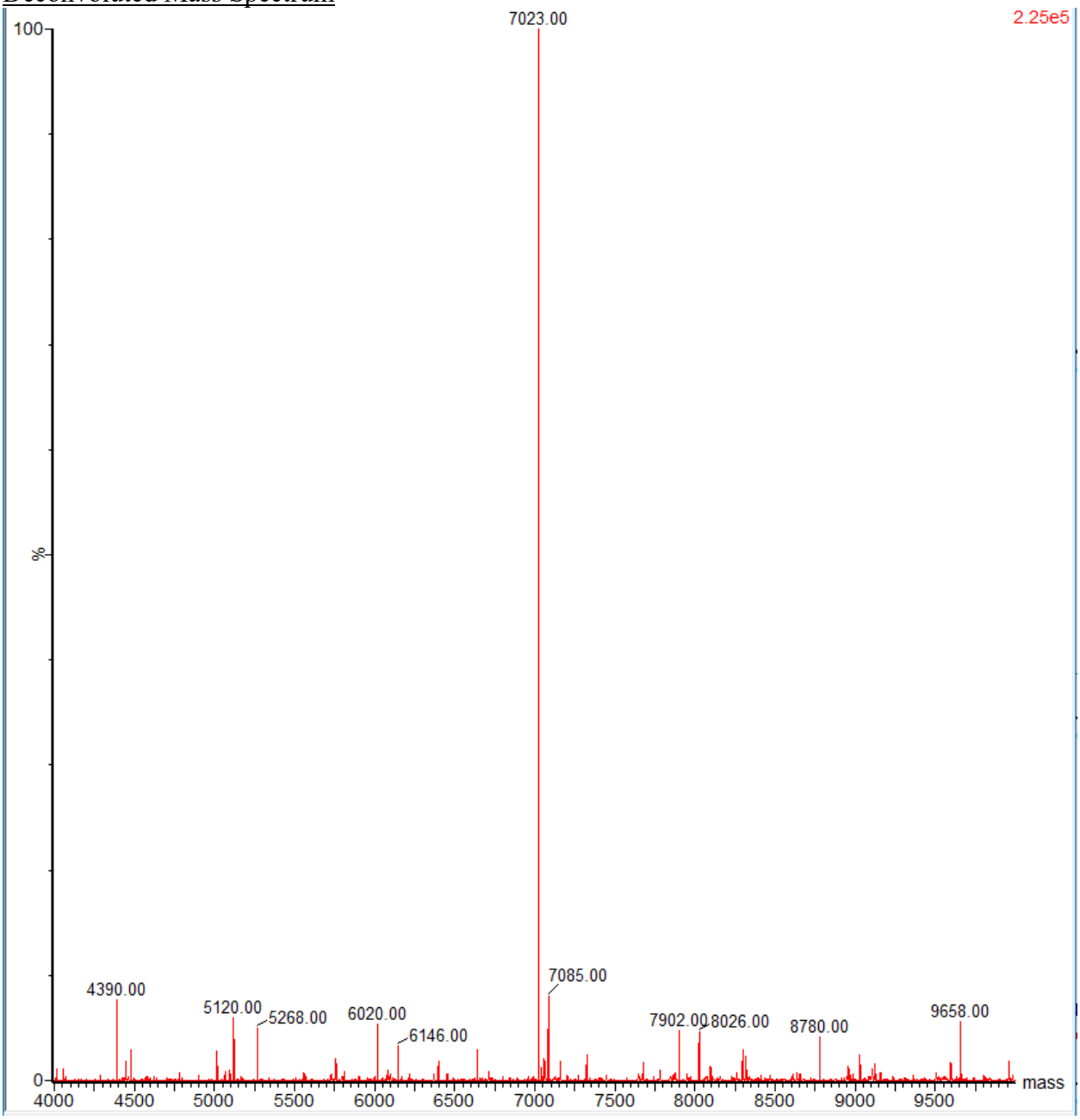
A260 Chromatogram



Mass Spectrum (RT= 2.19 min)

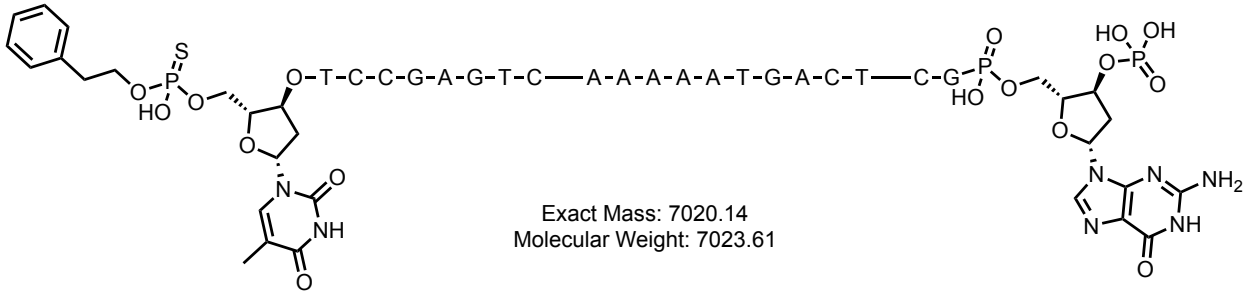


Deconvoluted Mass Spectrum

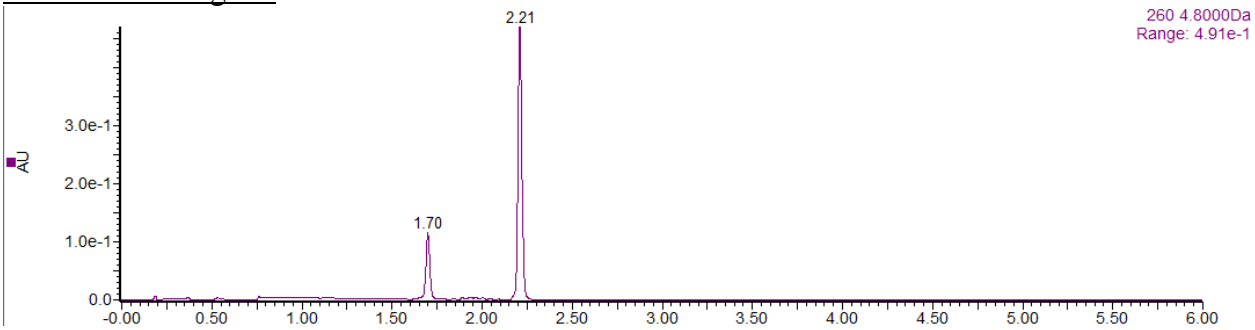


Entry 2: 5'

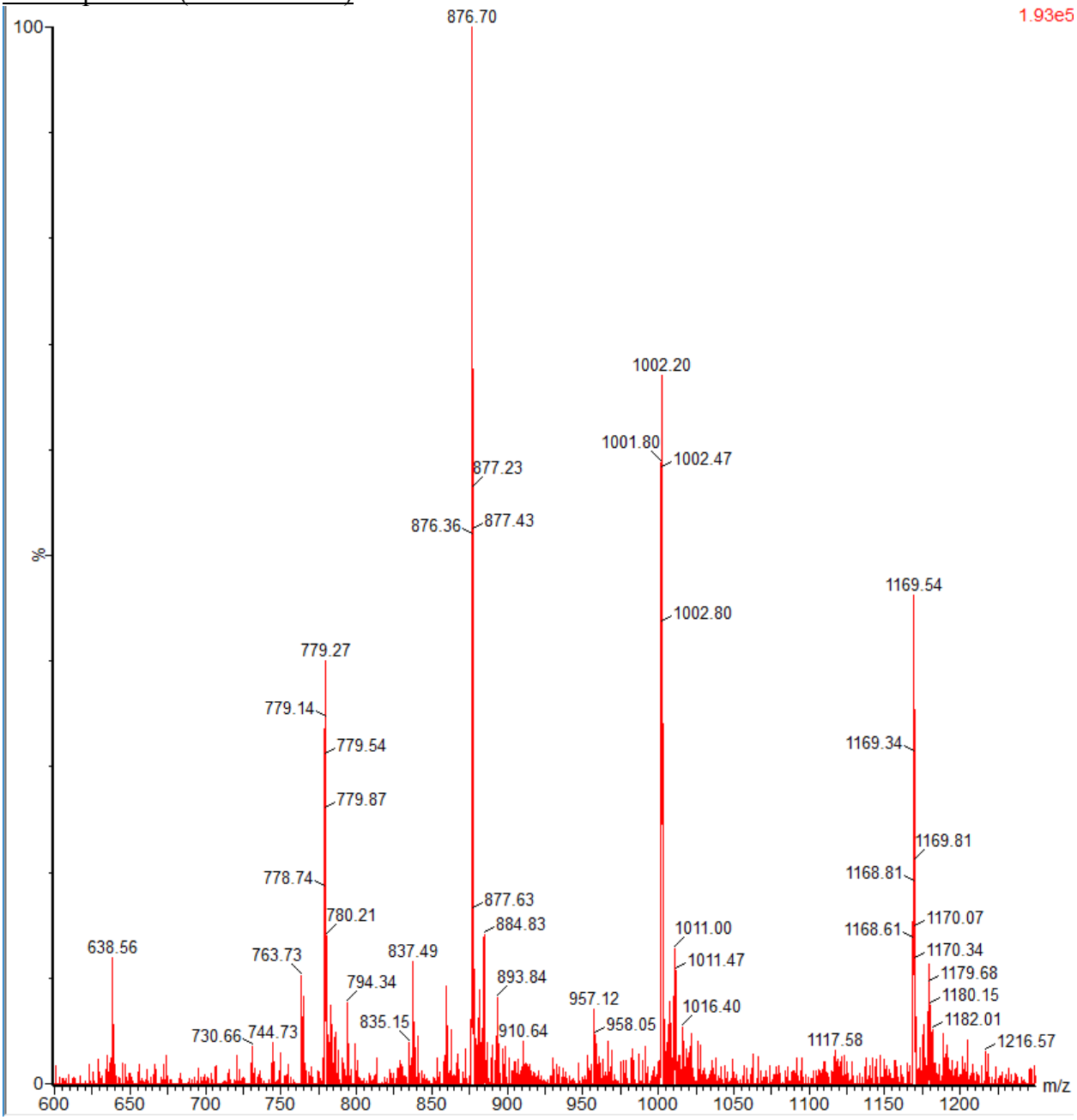
LCMS Characterization of **3**
Produced from **1** by General Procedure **2:**
Conversion (A260): 72%
Expected Mass: 7023.61
Expected (M-6)/6 Ion: 1169.60
Found Deconvoluted Mass: 7023.00
Found (M-6)/6 Ion: 1169.54



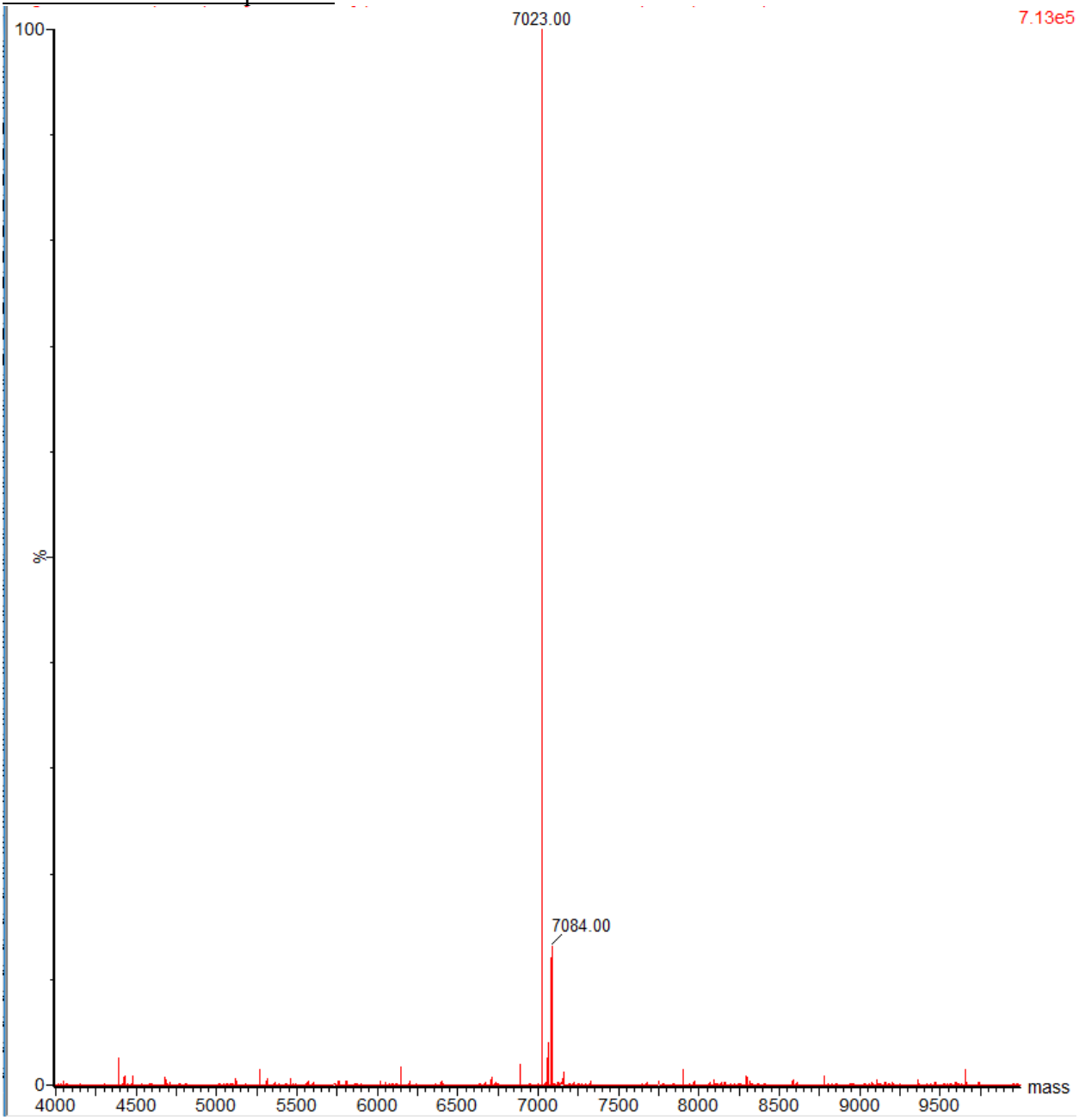
A260 Chromatogram



Mass Spectrum (RT= 2.21 min)

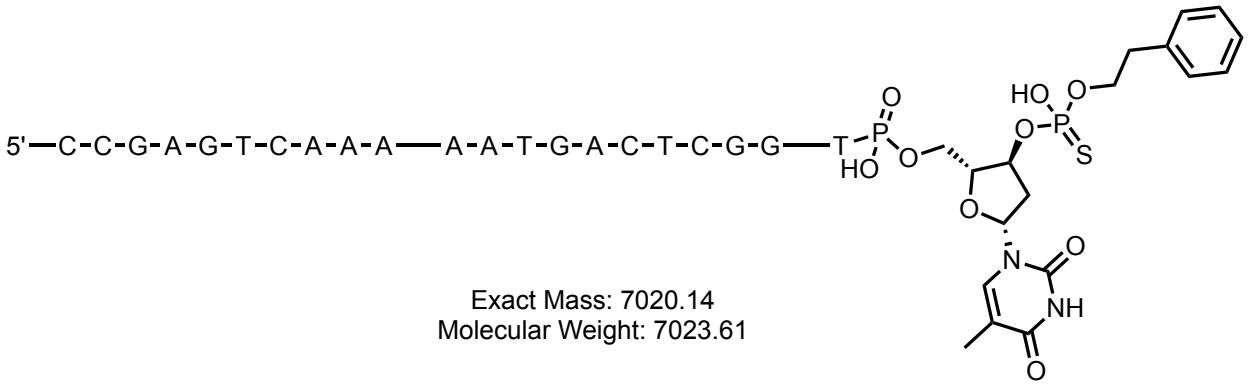


Deconvoluted Mass Spectrum

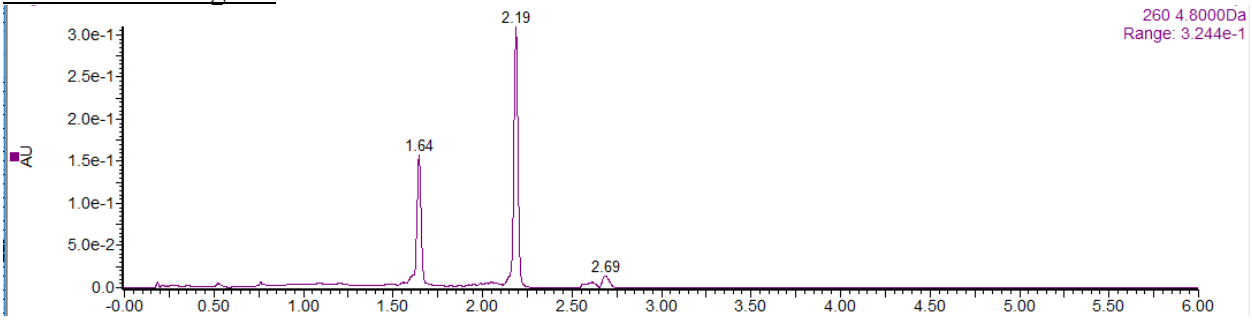


Entry 2: 3'

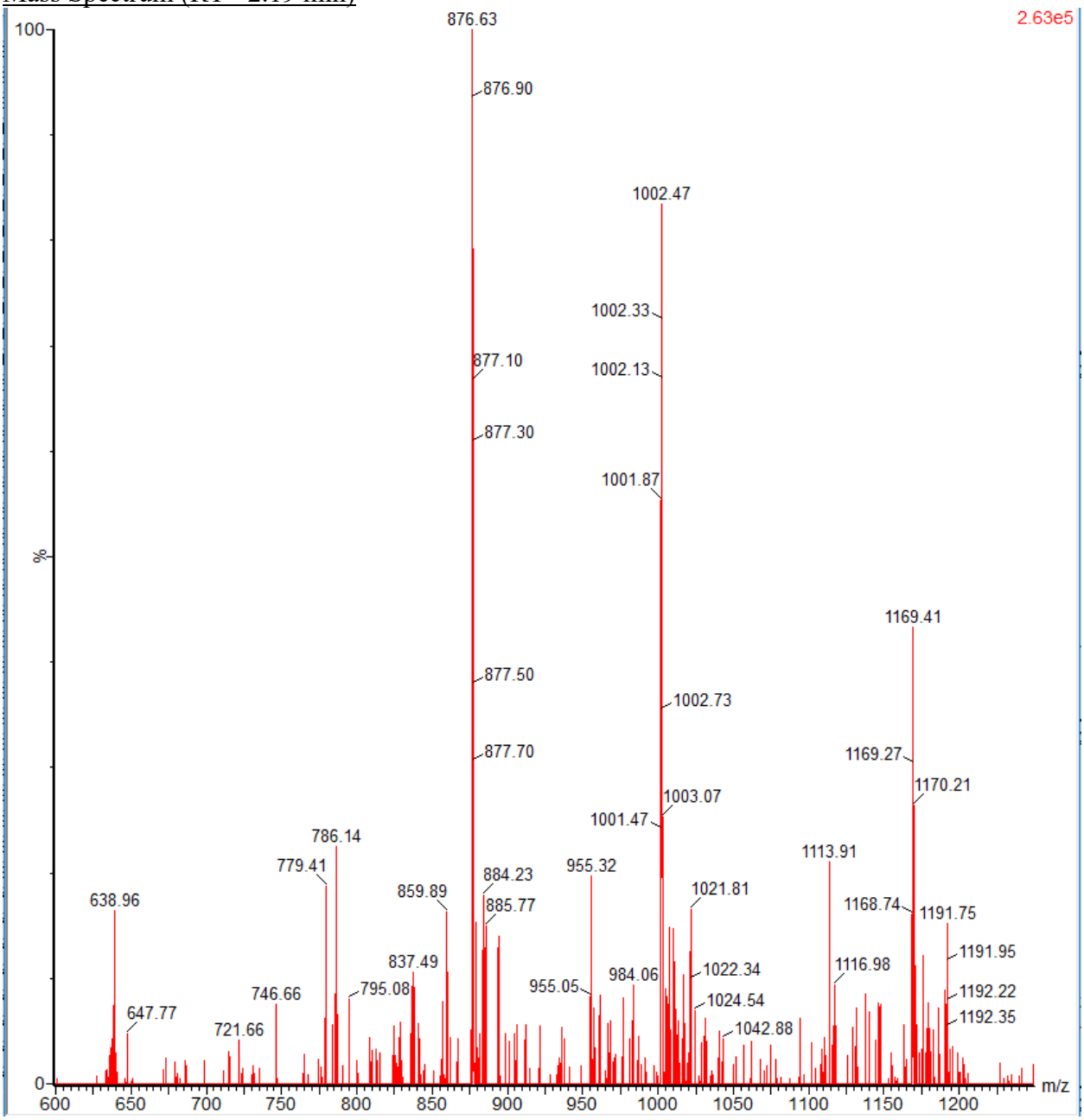
LCMS Characterization of **4**
Produced from **2** by General Procedure **2:**
Conversion (A260): 51%
Expected Mass: 7023.61
Expected (M-6)/6 Ion: 1169.60
Found Deconvoluted Mass: 7024.00
Found (M-6)/6 Ion: 1169.41



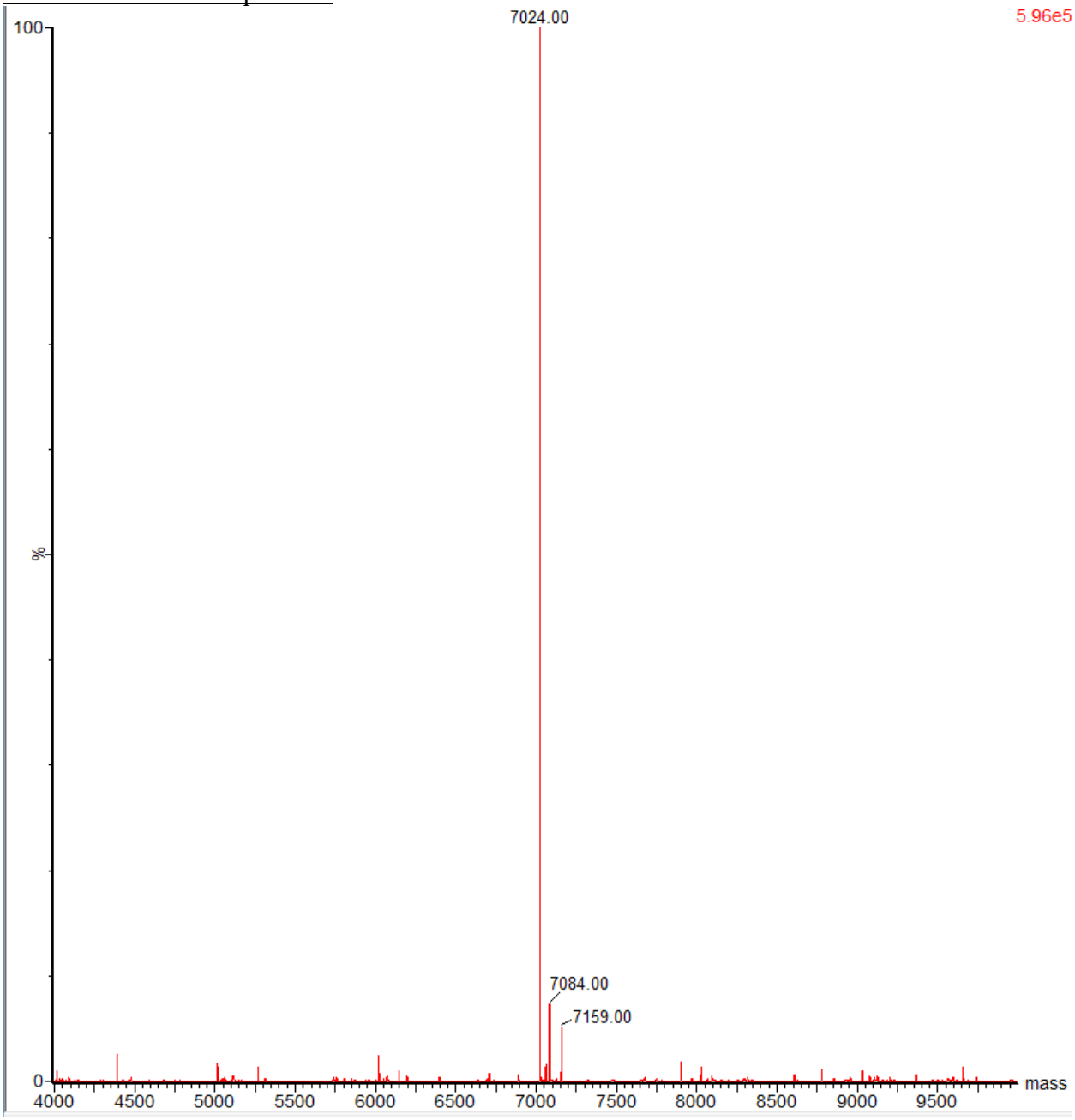
A260 Chromatogram



Mass Spectrum (RT= 2.19 min)

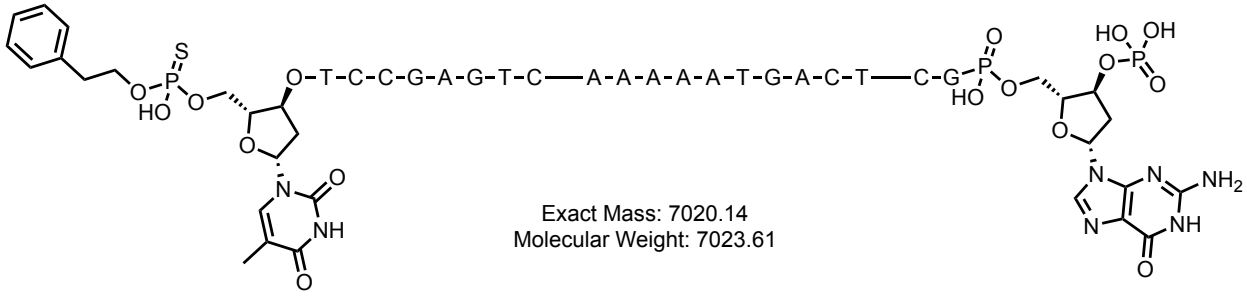


Deconvoluted Mass Spectrum

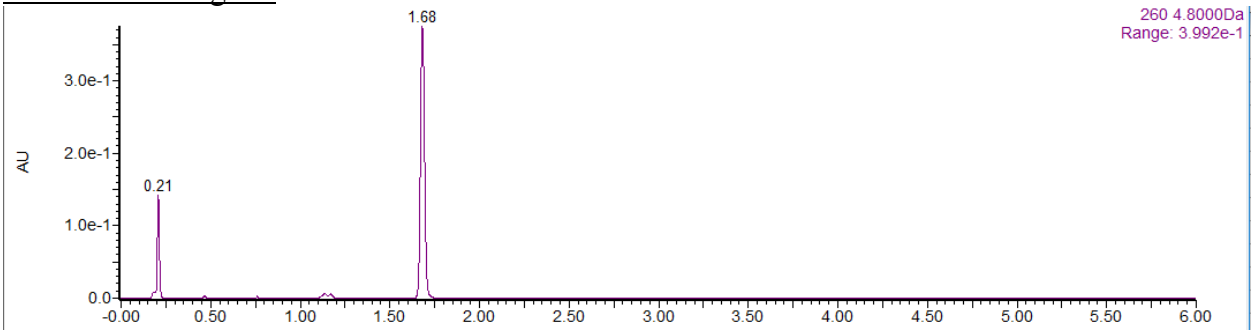


Entry 3: 5'

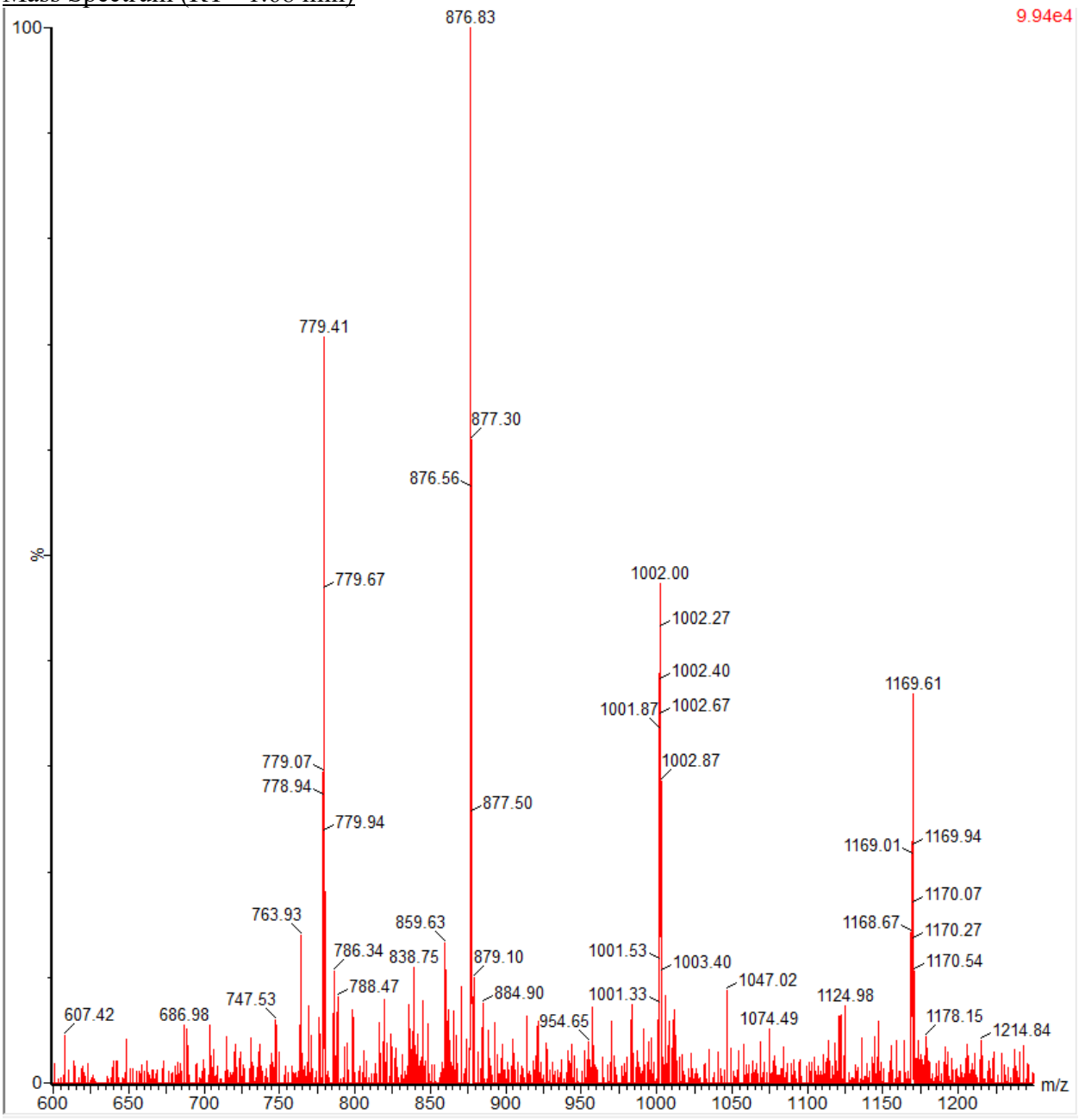
LCMS Characterization of **3**
Produced from **1** by General Procedure **2:**
Conversion (A260): 91%
Expected Mass: 7023.61
Expected (M-6)/6 Ion: 1169.60
Found Deconvoluted Mass: 7024.00
Found (M-6)/6 Ion: 1169.61



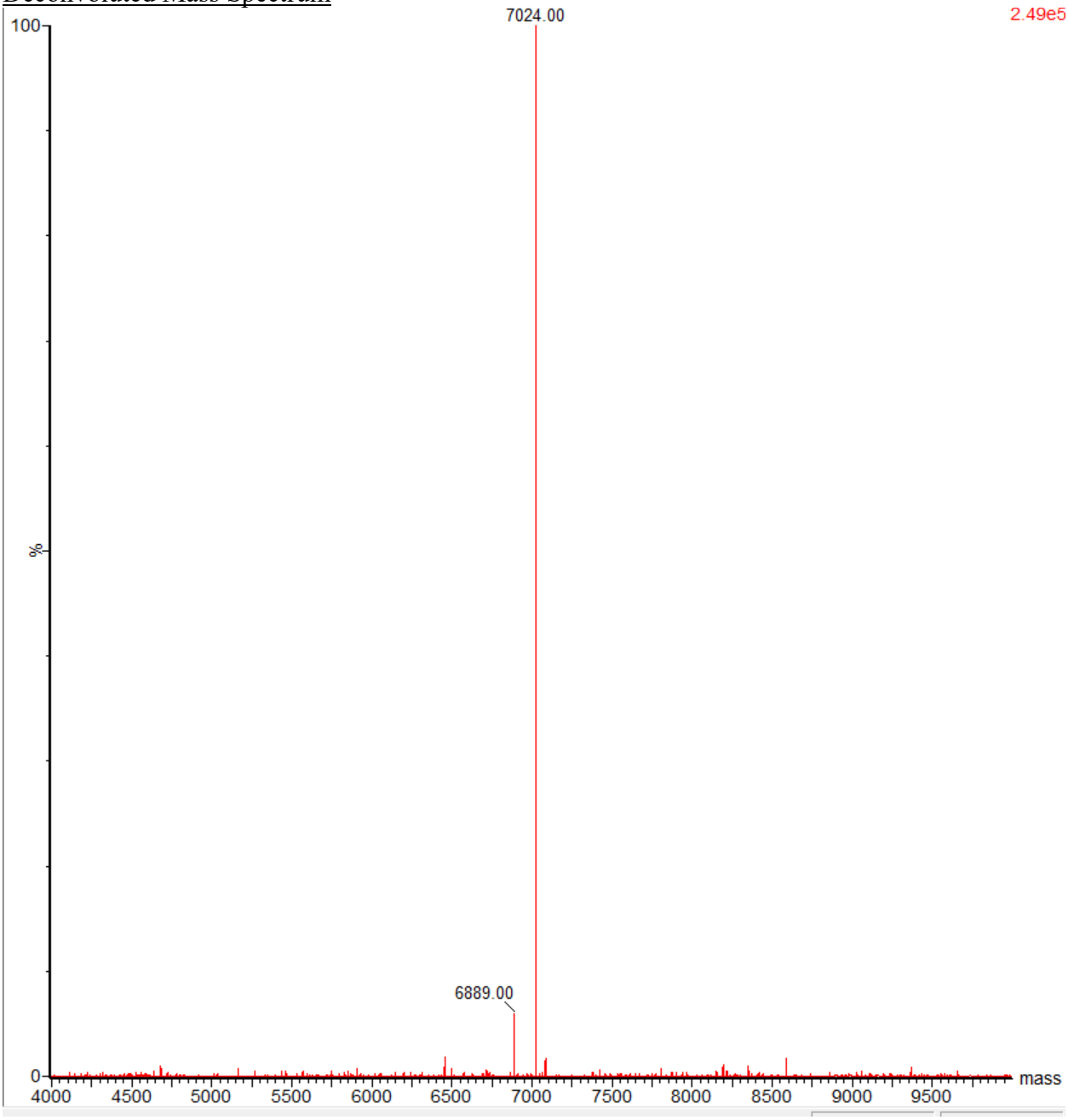
A260 Chromatogram



Mass Spectrum (RT= 1.68 min)

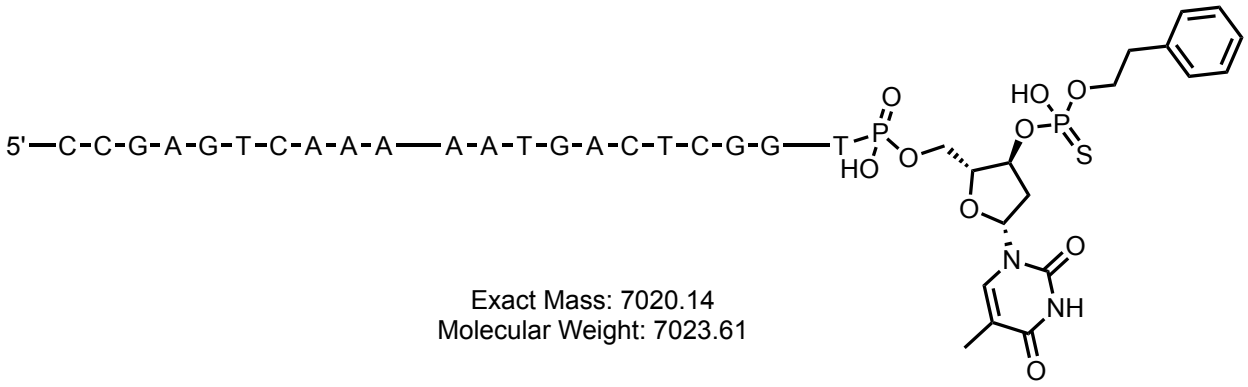


Deconvoluted Mass Spectrum



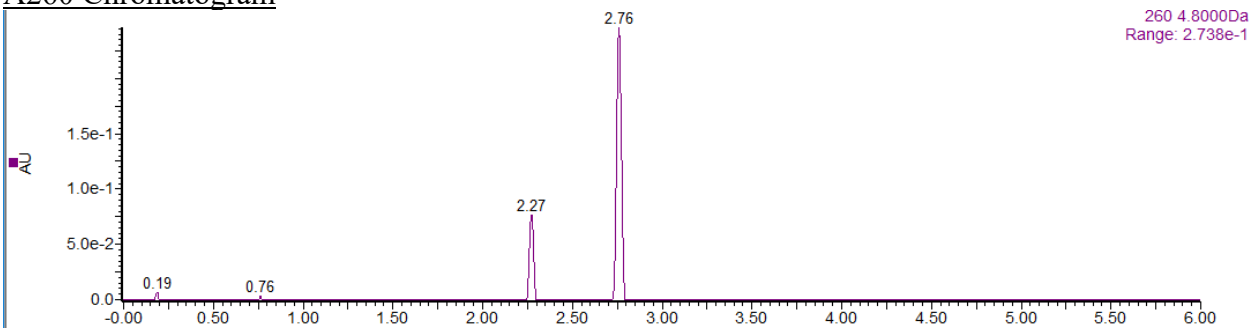
Entry 3: 3'

LCMS Characterization of **4**
Produced from **2** by General Procedure **2:**
Conversion (A260): 72%
Expected Mass: 7023.61
Expected (M-6)/6 Ion: 1169.60
Found Deconvoluted Mass: 7024.00
Found (M-6)/6 Ion: 1169.01

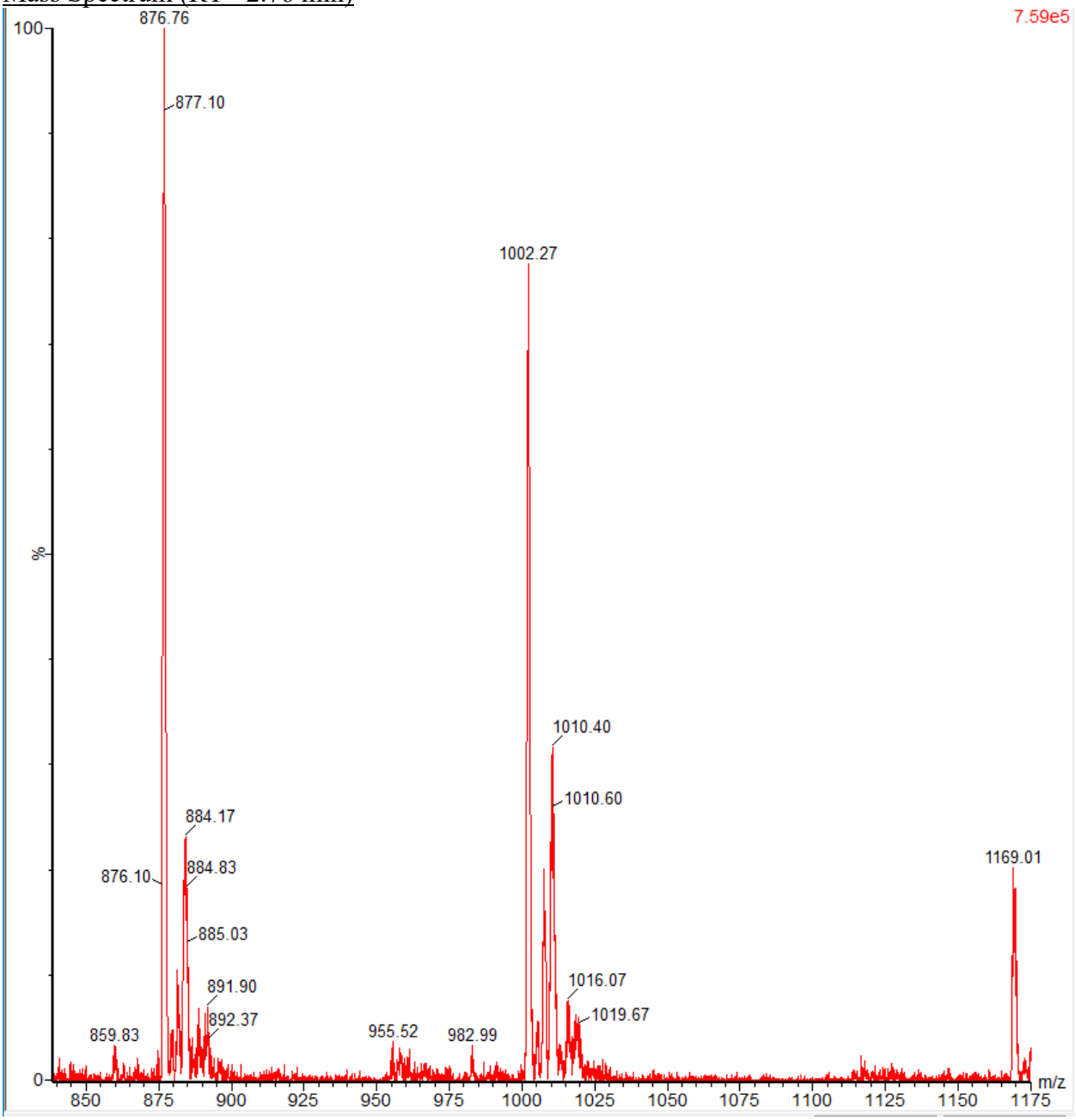


Exact Mass: 7020.14
Molecular Weight: 7023.61

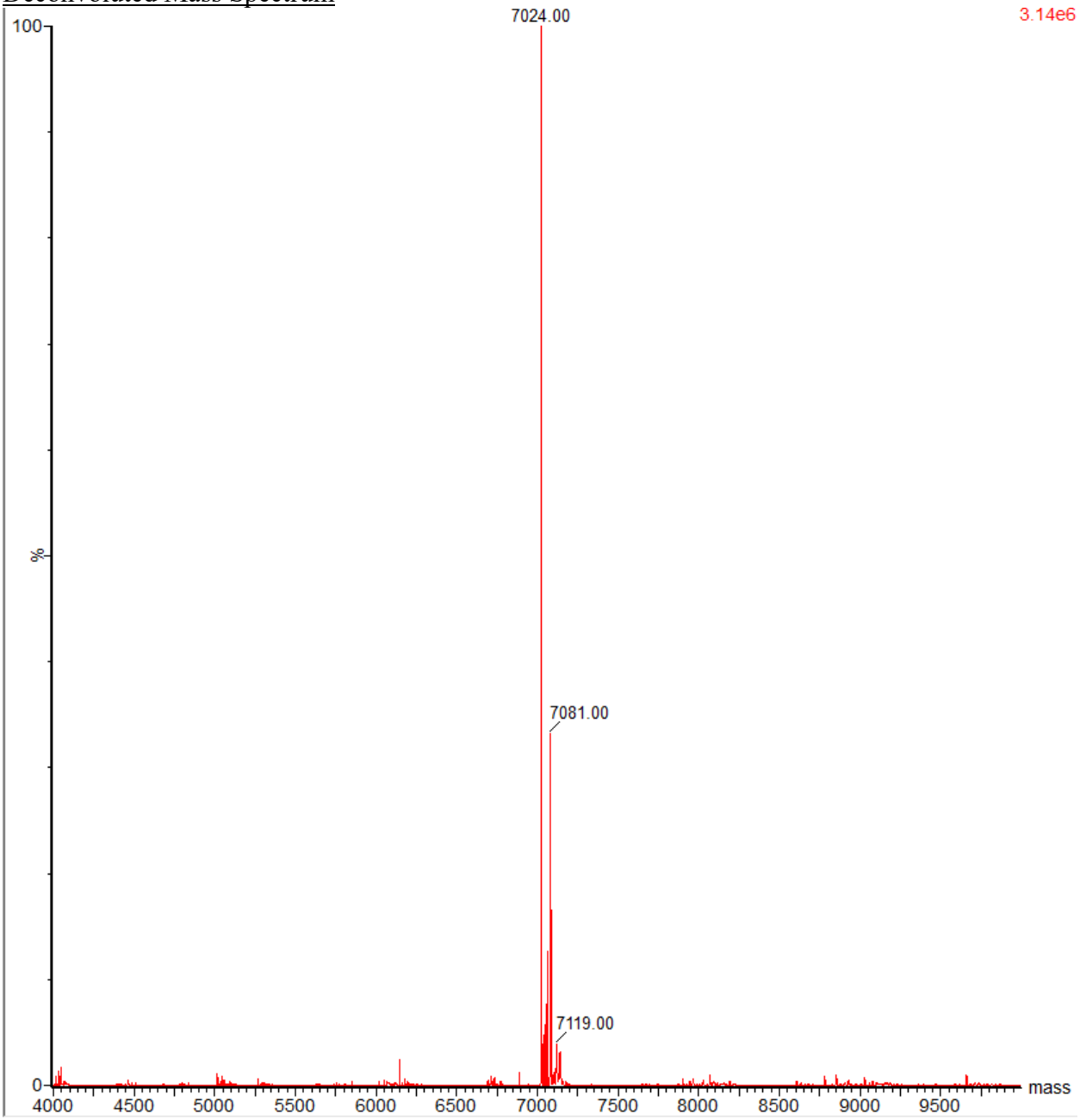
A260 Chromatogram



Mass Spectrum (RT= 2.76 min)

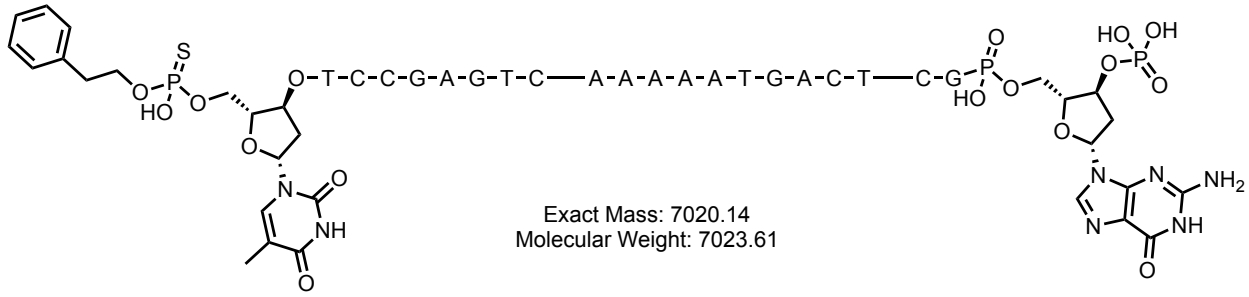


Deconvoluted Mass Spectrum

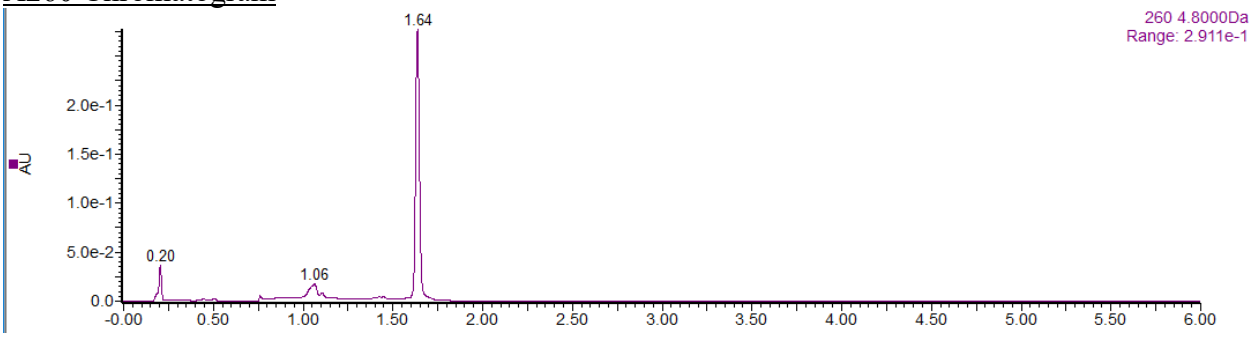


Entry 4: 5'

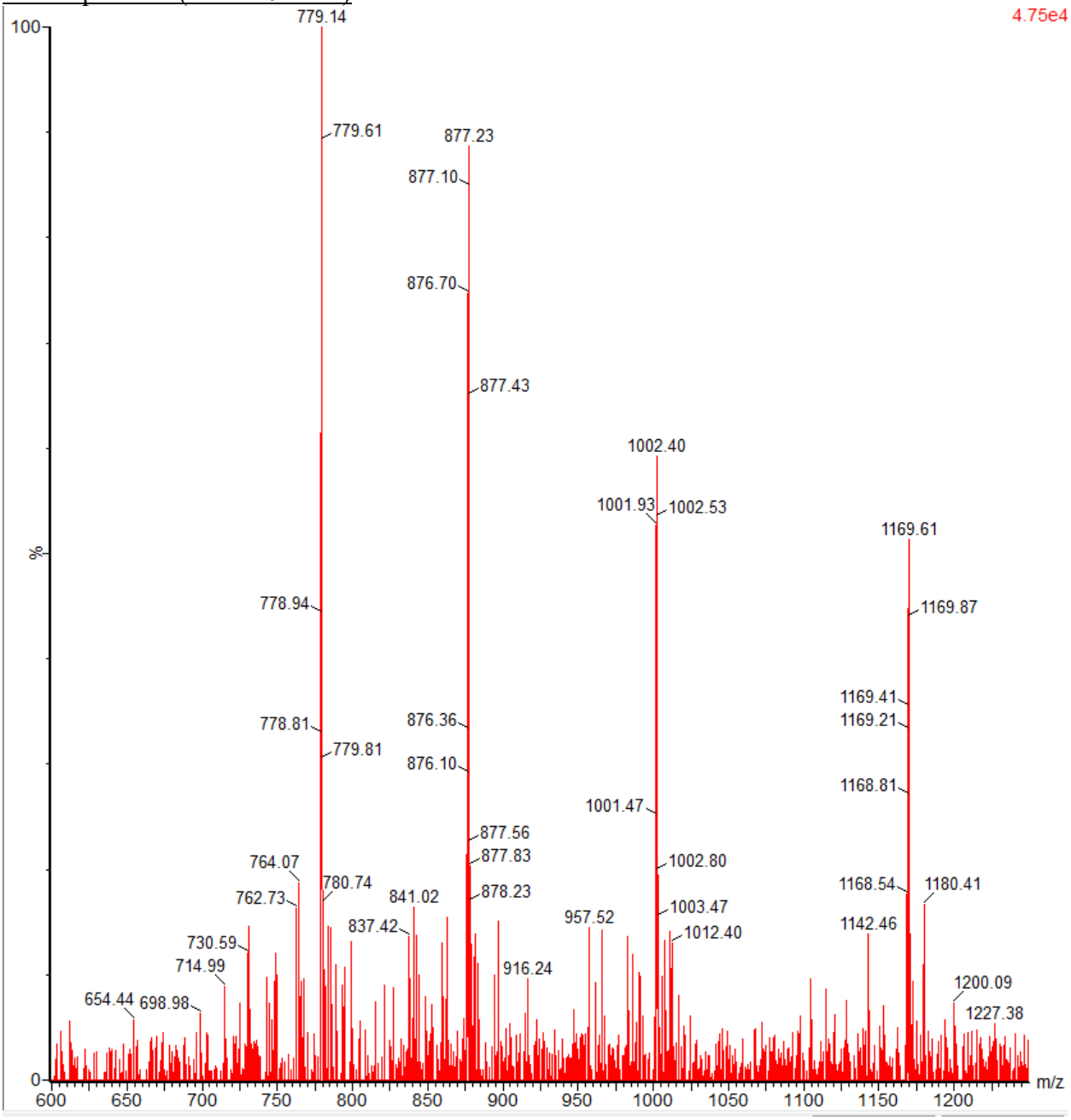
LCMS Characterization of **3**
Produced from **1** by General Procedure **2:**
Conversion (A260): 84%
Expected Mass: 7023.61
Expected (M-6)/6 Ion: 1169.60
Found Deconvoluted Mass: 7023.00
Found (M-6)/6 Ion: 1169.61



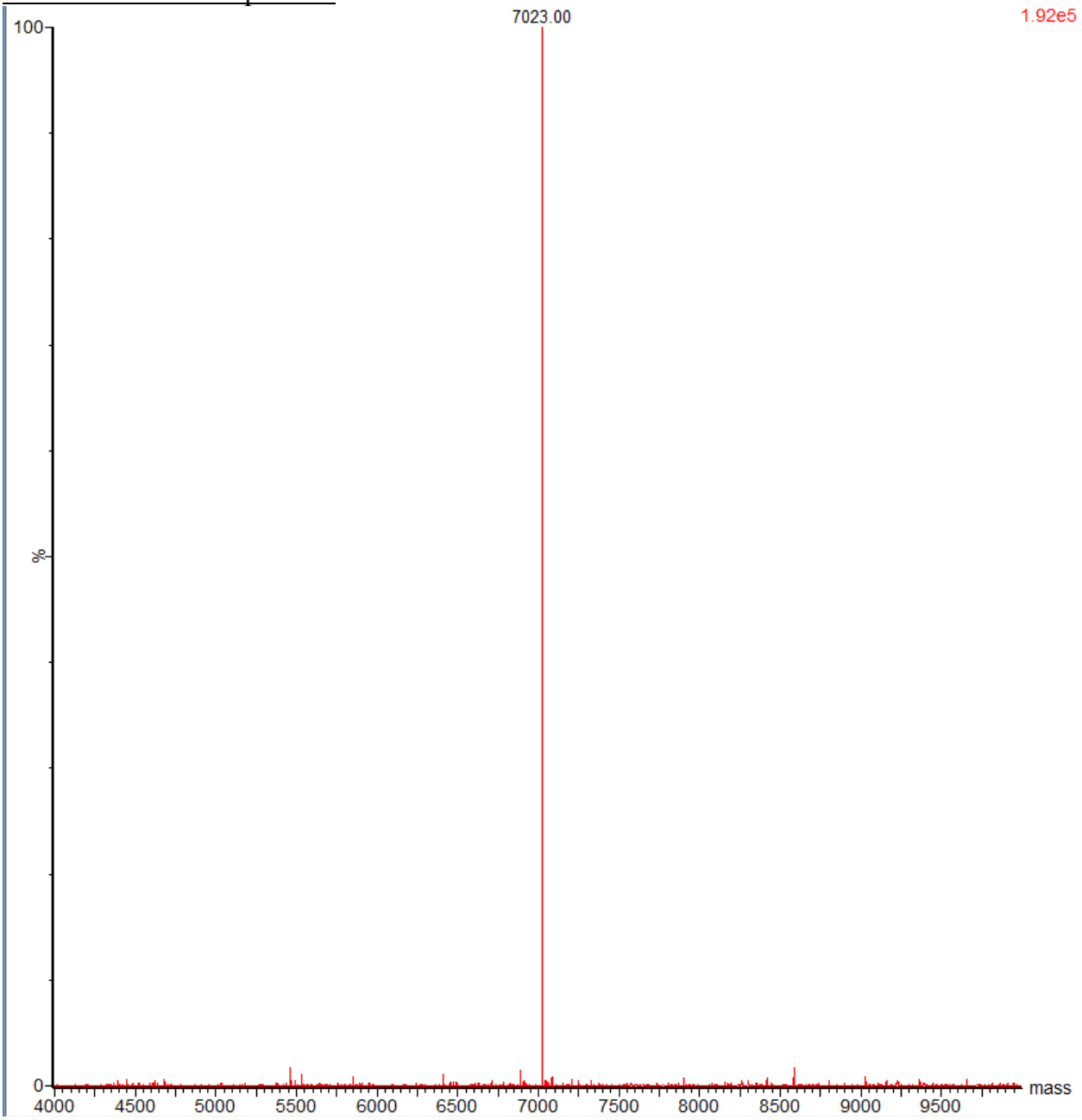
A260 Chromatogram



Mass Spectrum (RT= 1.64 min)

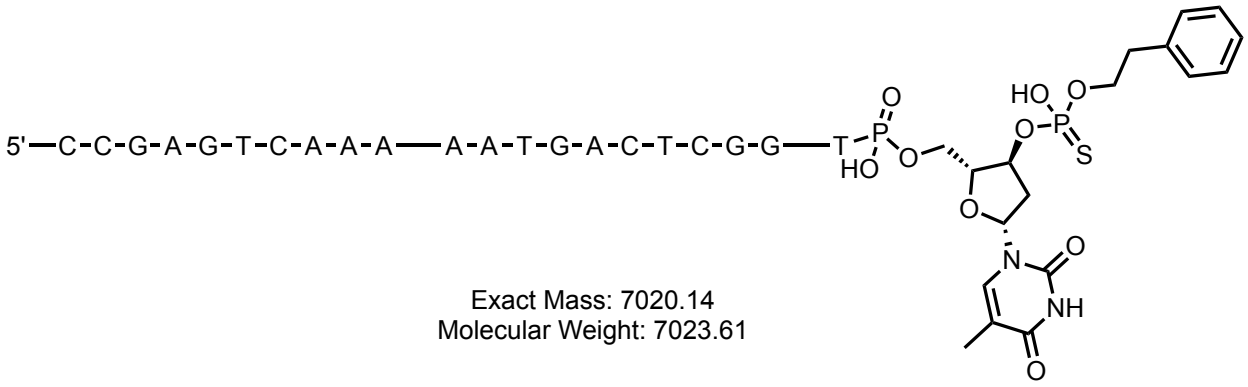


Deconvoluted Mass Spectrum



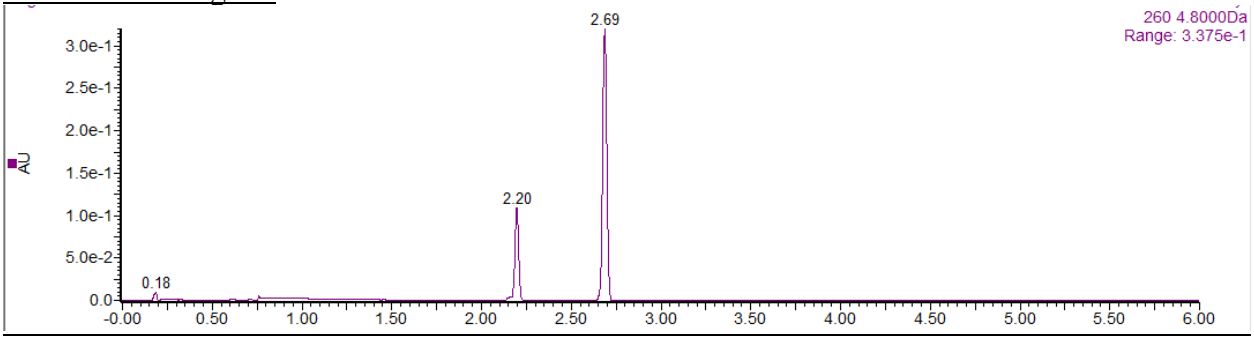
Entry 4: 3'

LCMS Characterization of **4**
Produced from **2** by General Procedure **2:**
Conversion (A260): 62%
Expected Mass: 7023.61
Expected (M-6)/6 Ion: 1169.60
Found Deconvoluted Mass: 7023.00
Found (M-6)/6 Ion: 1169.54

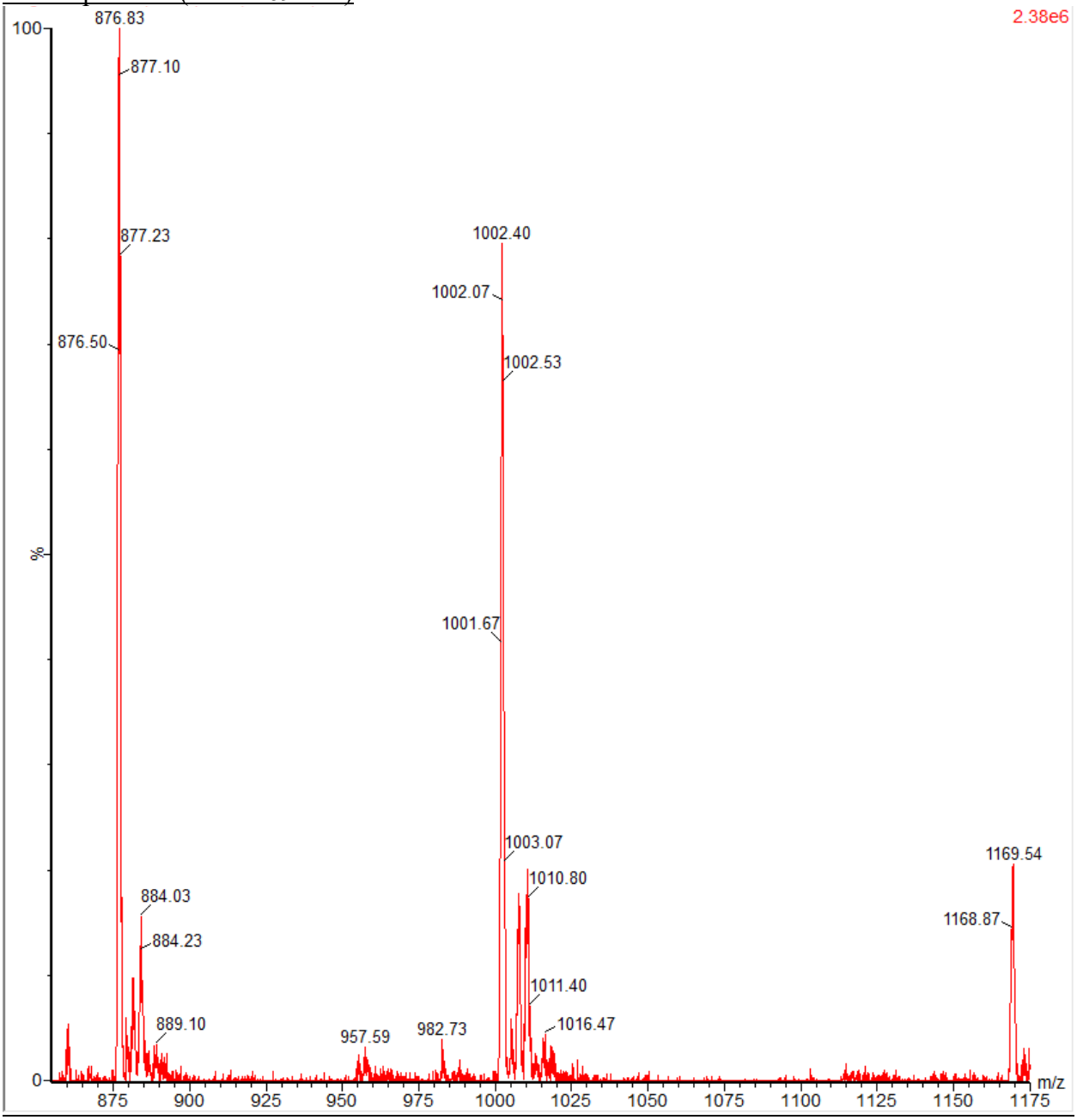


Exact Mass: 7020.14
Molecular Weight: 7023.61

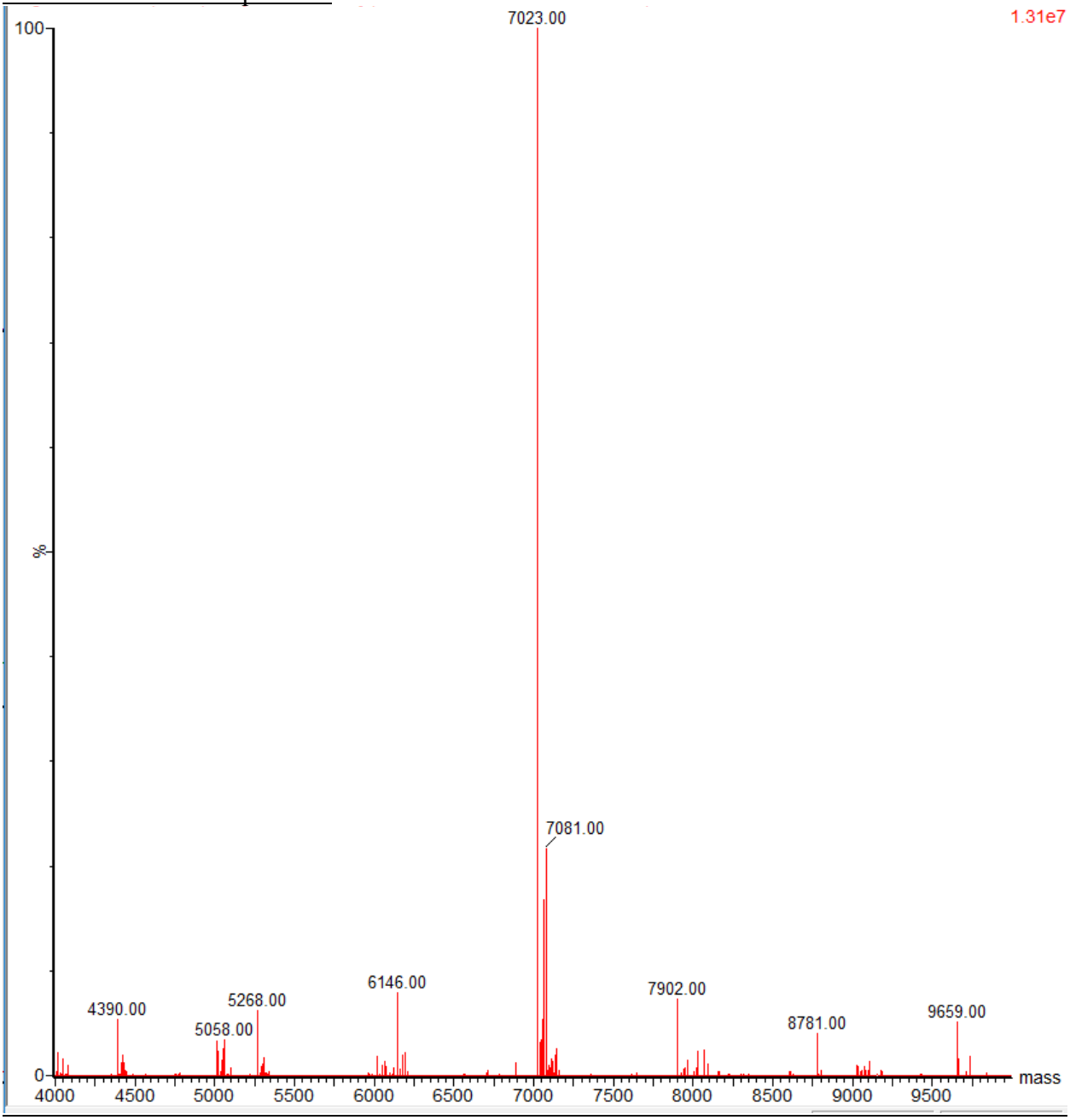
A260 Chromatogram



Mass Spectrum (RT= 2.69 min)

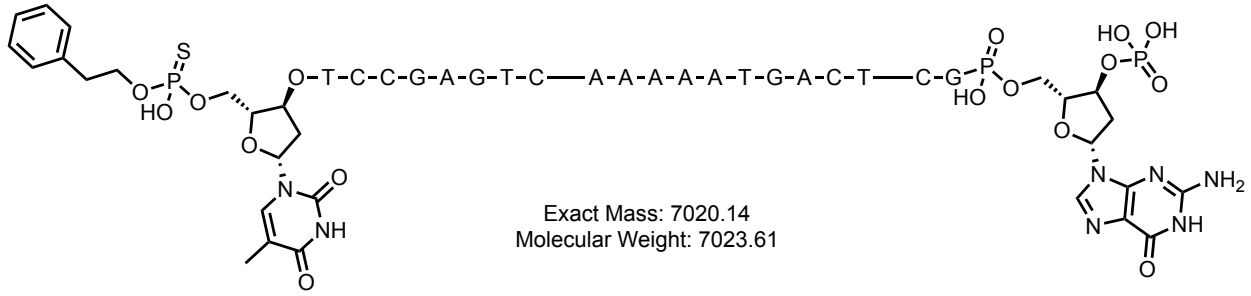


Deconvoluted Mass Spectrum

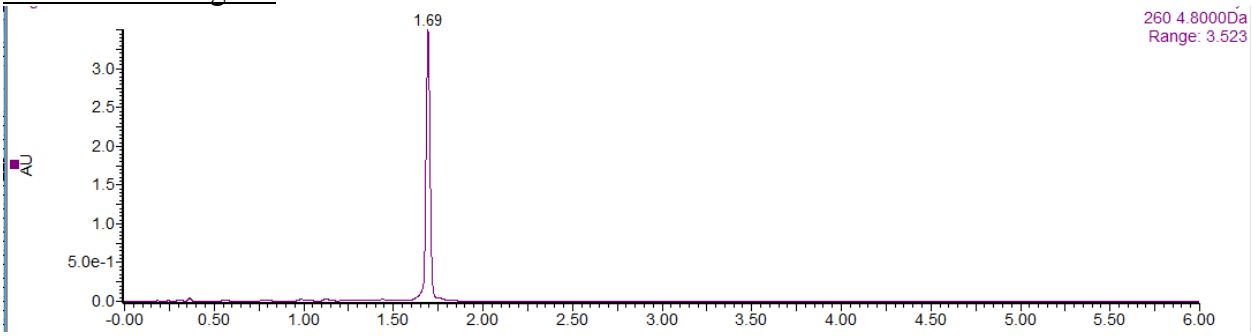


Entry 5: 5'

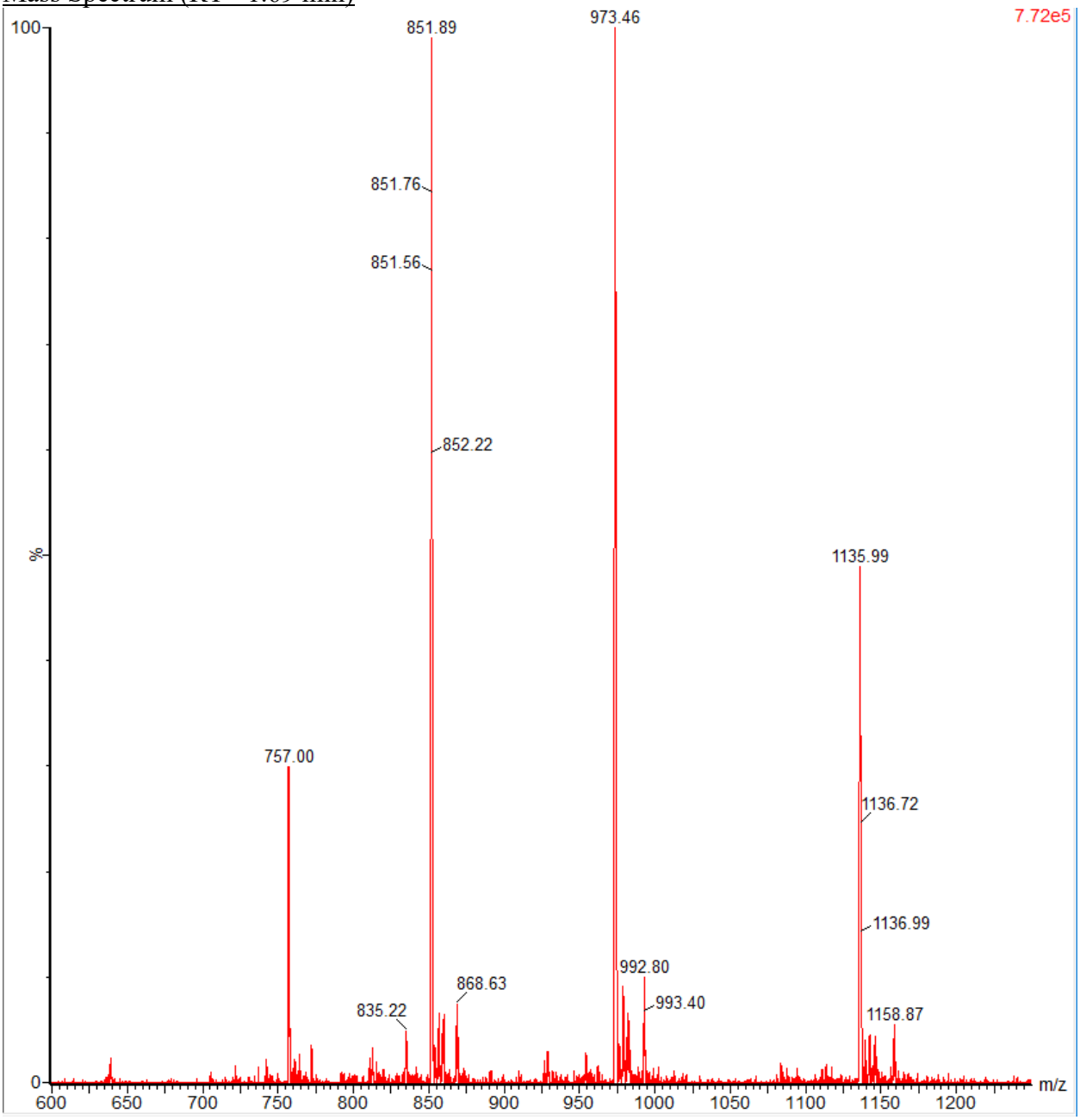
LCMS Characterization of **3**
Produced from **1** by General Procedure **2:**
Conversion (A260): 0%
Expected Mass: 7023.61
Expected (M-6)/6 Ion: 1169.60
Found Deconvoluted Mass: 6824.00 (Starting material **1**)
Found (M-6)/6 Ion: 1135.99 (Starting material **1)**



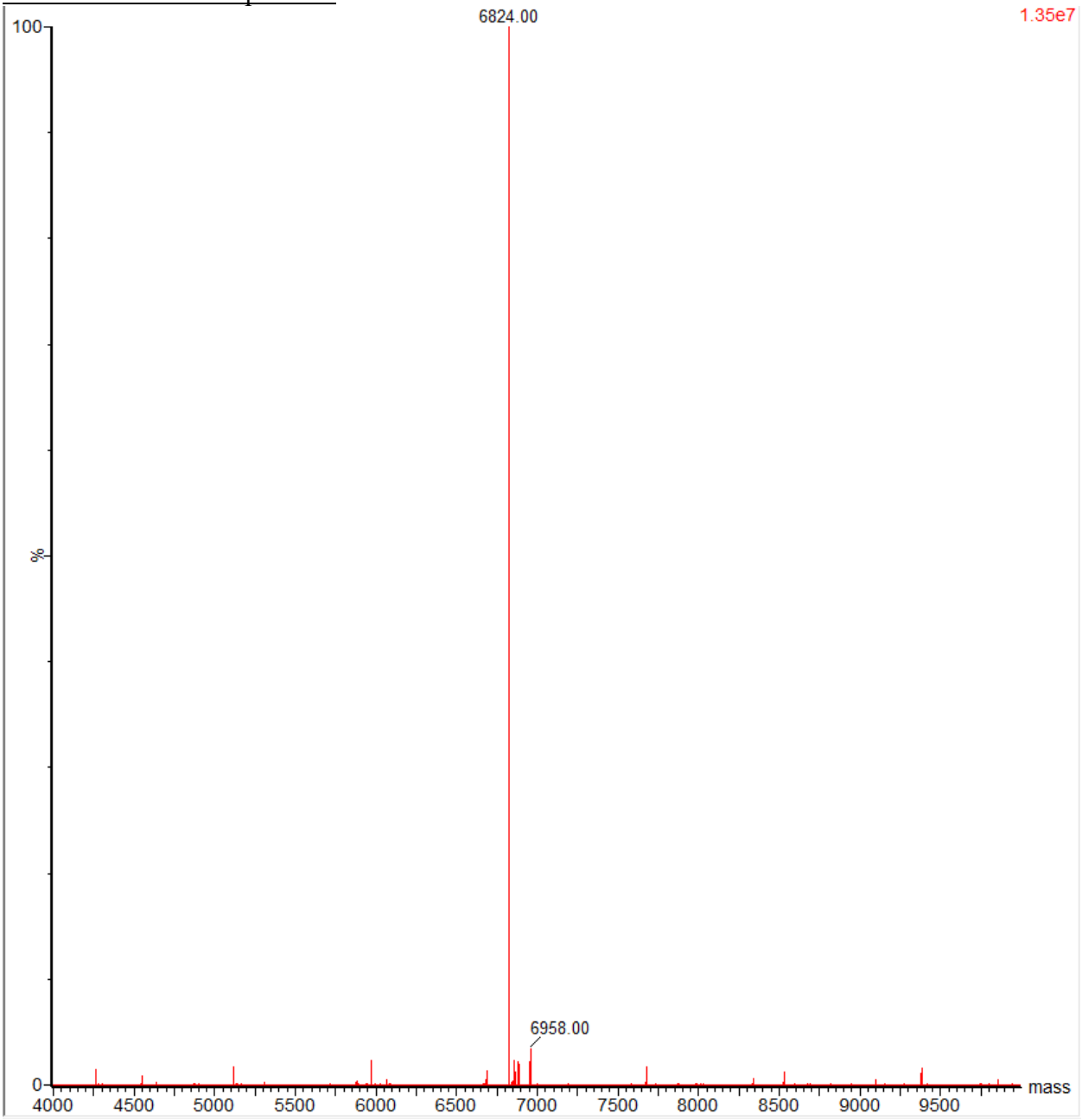
A260 Chromatogram



Mass Spectrum (RT= 1.69 min)

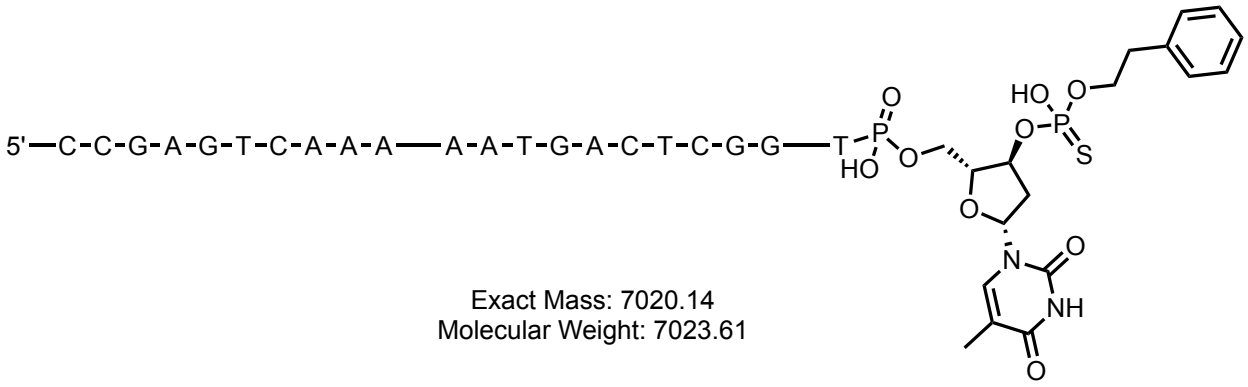


Deconvoluted Mass Spectrum



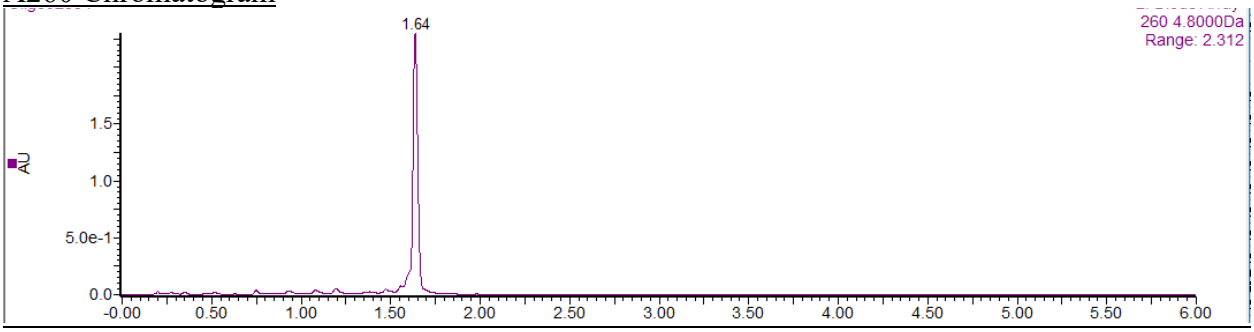
Entry 5: 3'

LCMS Characterization of **4**
Produced from **2** by General Procedure **2:**
Conversion (A260): 0%
Expected Mass: 7023.61
Expected (M-6)/6 Ion: 1169.60
Found Deconvoluted Mass: 6824.00 (Starting material **2**)
Found (M-6)/6 Ion: 1136.12(Starting material **2**)

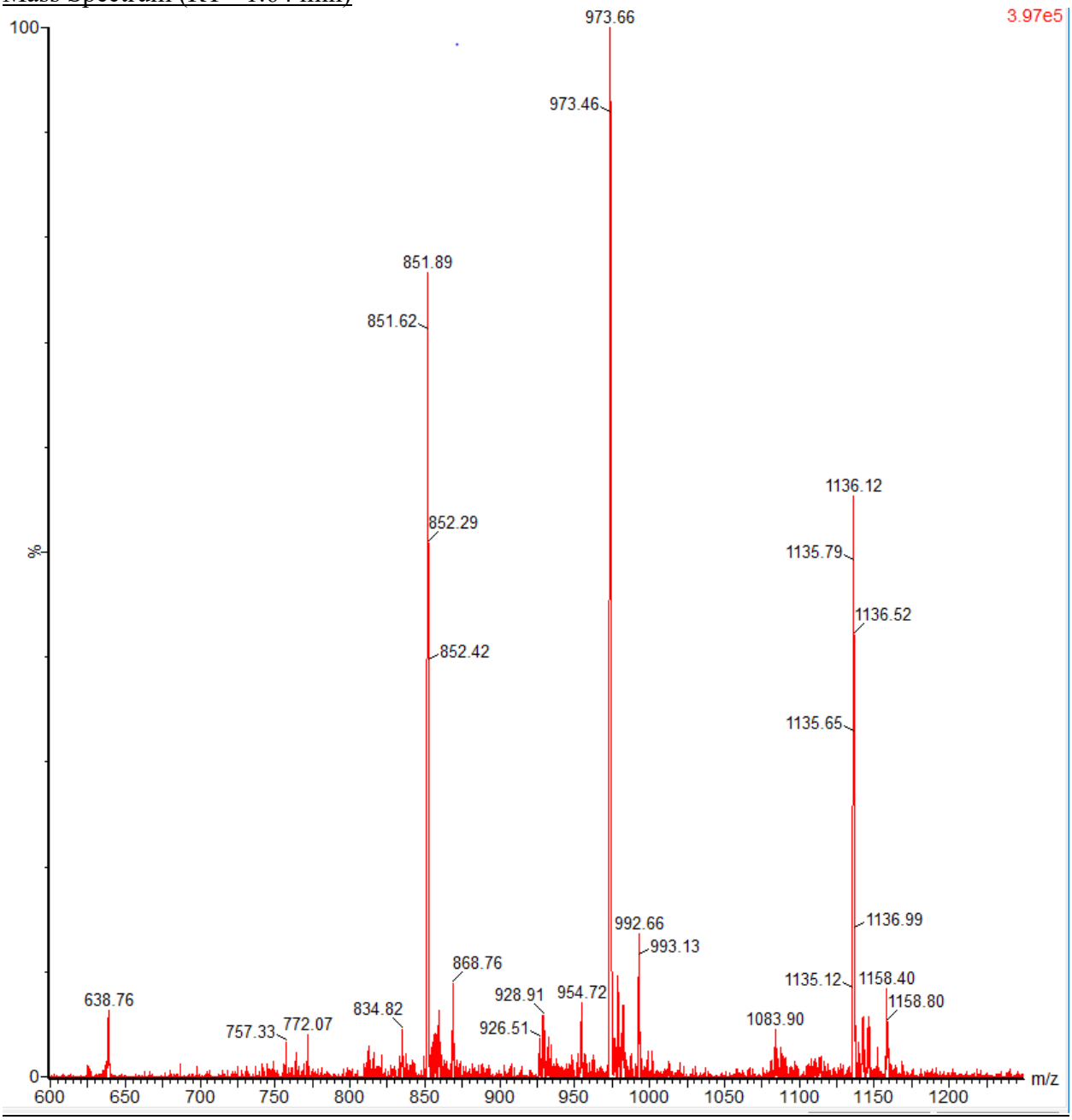


Exact Mass: 7020.14
Molecular Weight: 7023.61

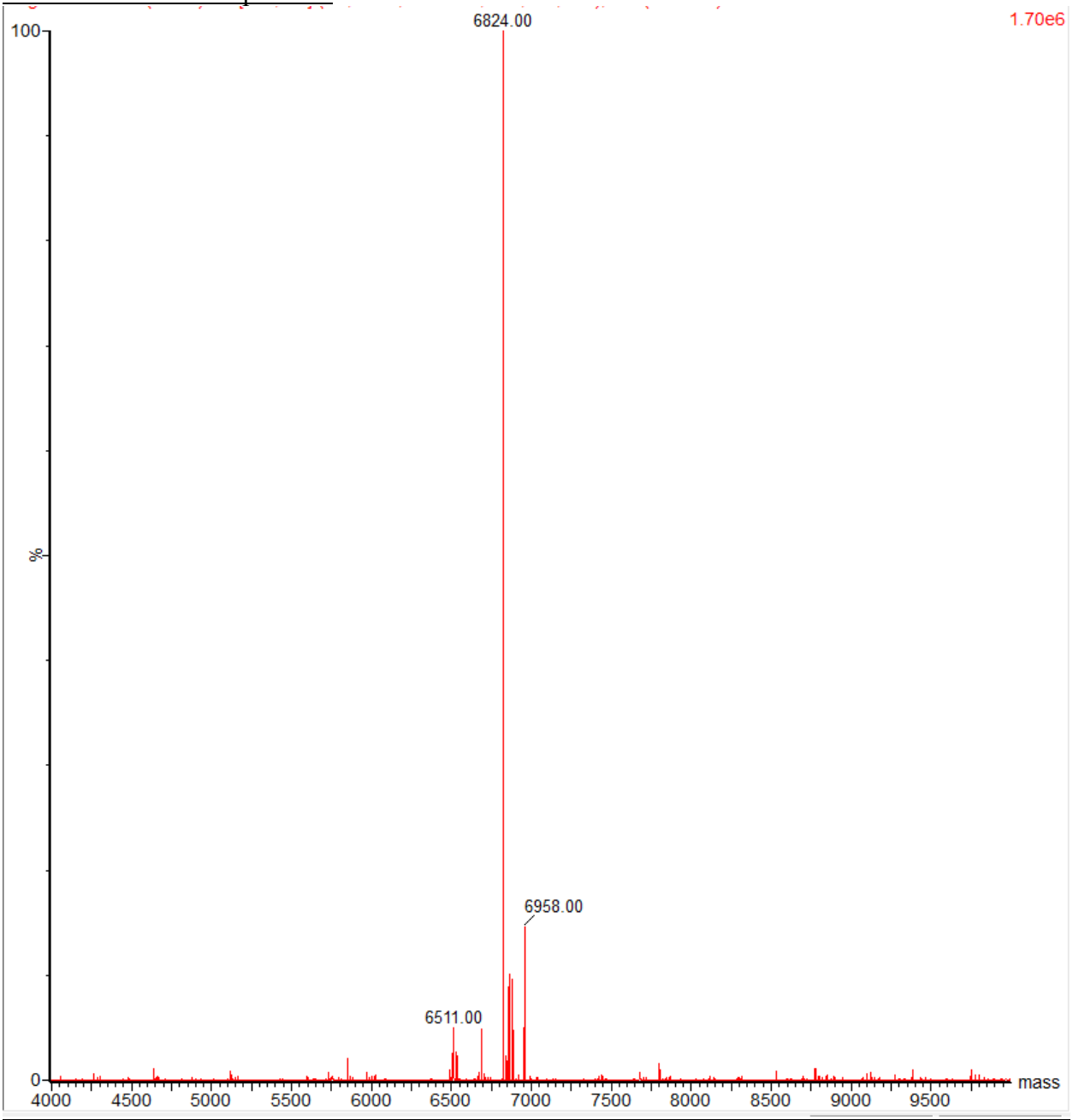
A260 Chromatogram



Mass Spectrum (RT= 1.64 min)

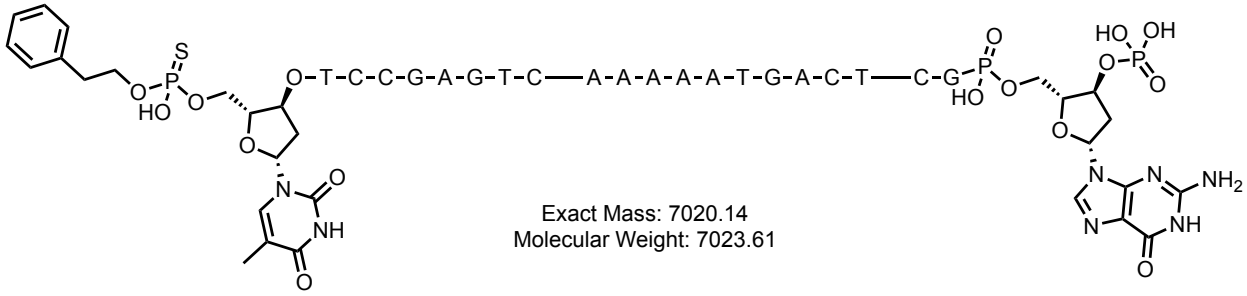


Deconvoluted Mass Spectrum

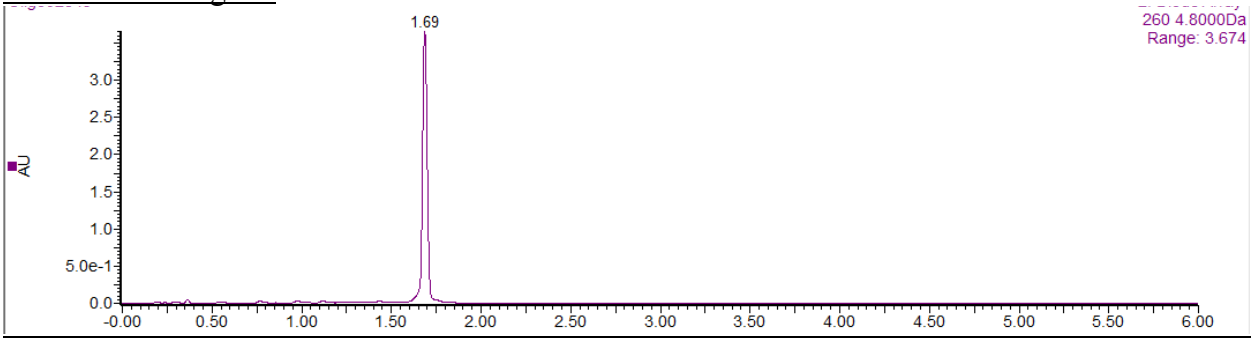


Entry 6: 5'

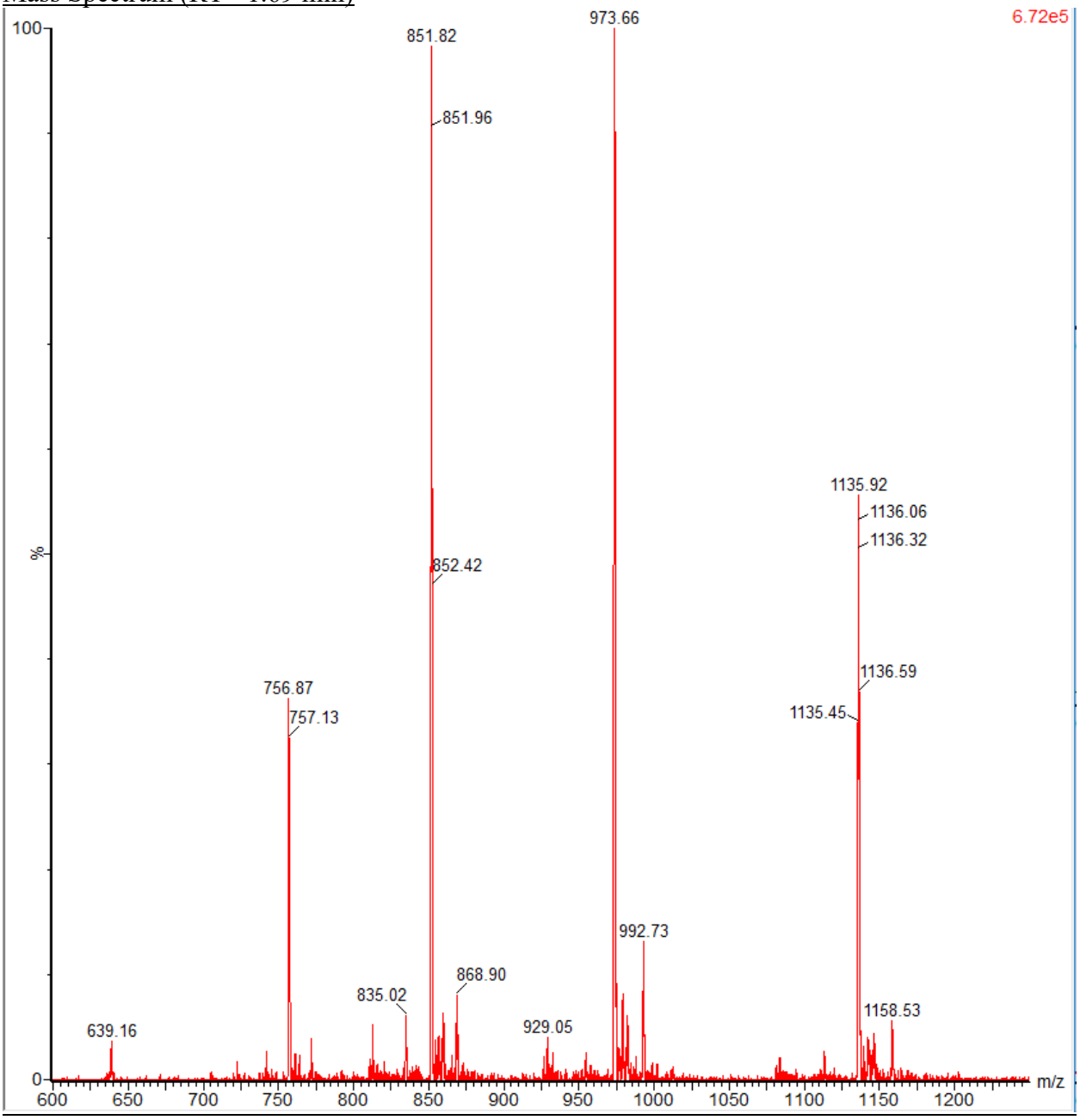
LCMS Characterization of **3**
Produced from **1** by General Procedure **2:**
Conversion (A260): 0%
Expected Mass: 7023.61
Expected (M-6)/6 Ion: 1169.60
Found Deconvoluted Mass: 6823.00 (Starting material **1**)
Found (M-6)/6 Ion: 1135.92 (Starting material **1**)



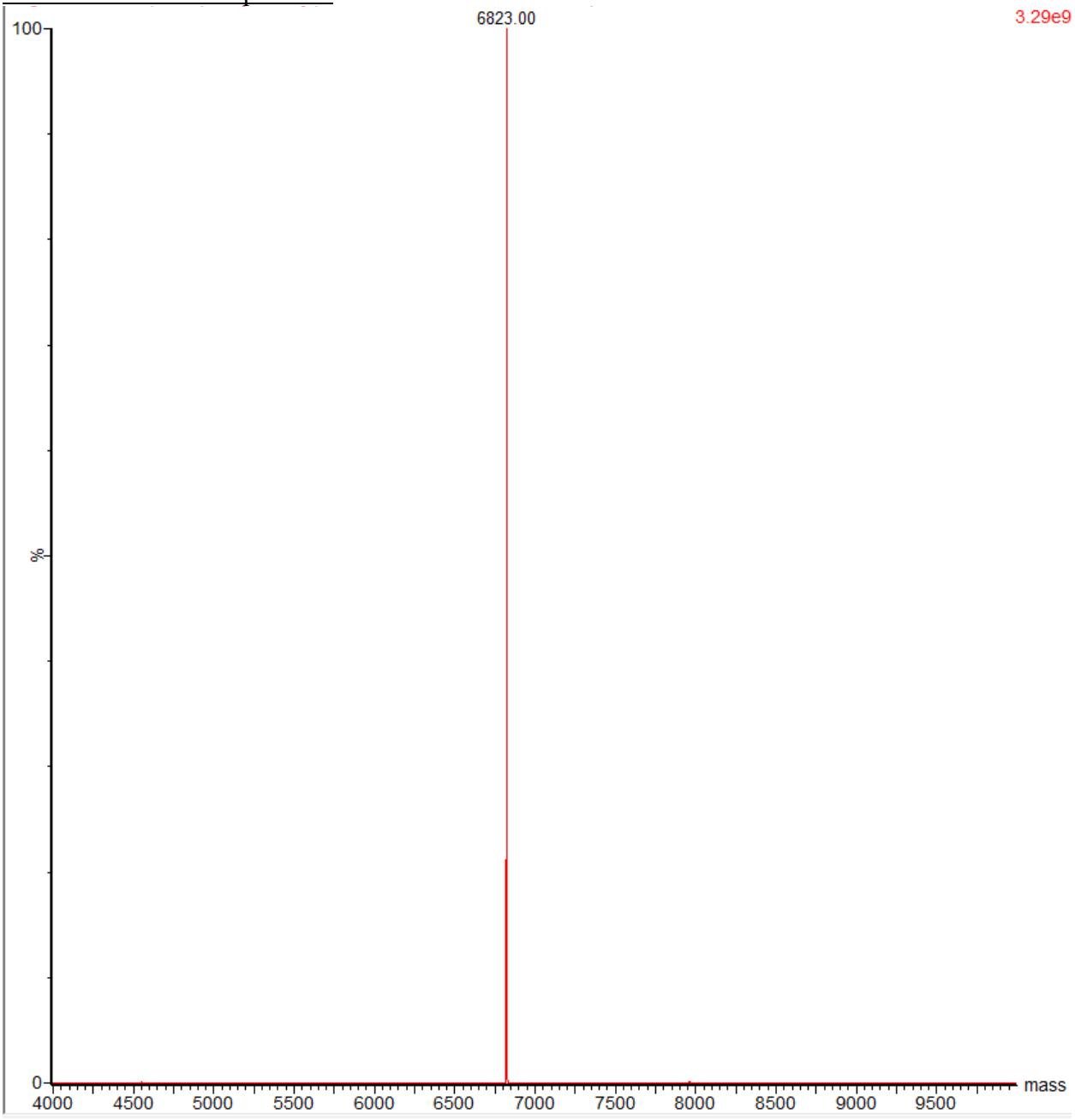
A260 Chromatogram



Mass Spectrum (RT= 1.69 min)

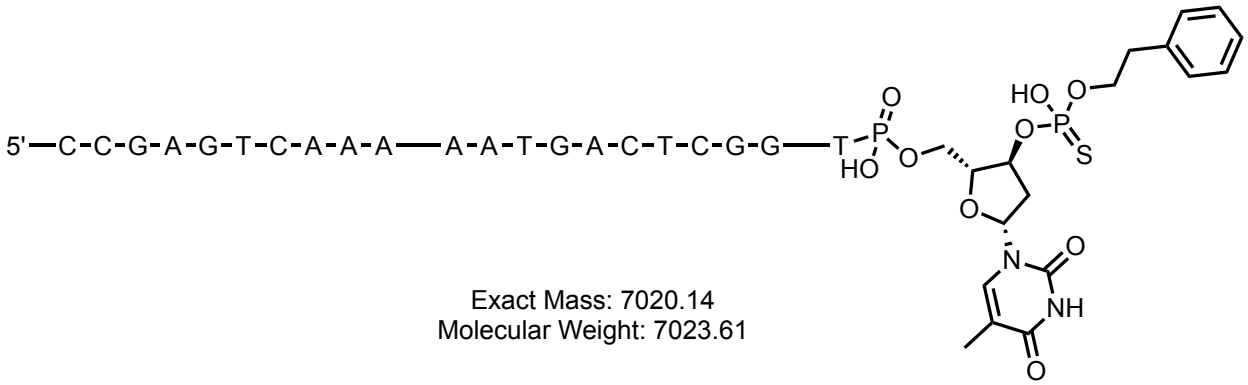


Deconvoluted Mass Spectrum



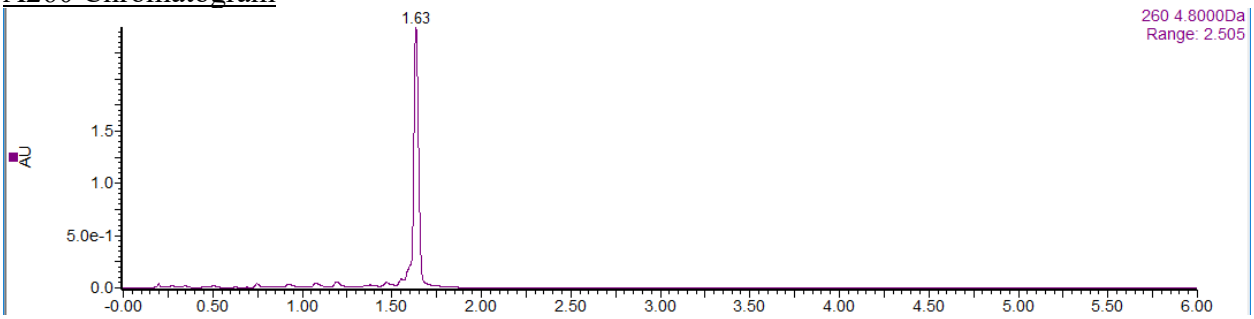
Entry 6: 3'

LCMS Characterization of **4**
Produced from **2** by General Procedure **2:**
Conversion (A260): 0%
Expected Mass: 7023.61
Expected (M-6)/6 Ion: 1169.60
Found Deconvoluted Mass: 6823 (Starting material **2**)
Found (M-6)/6 Ion: 1135.85 (Starting material **2**)

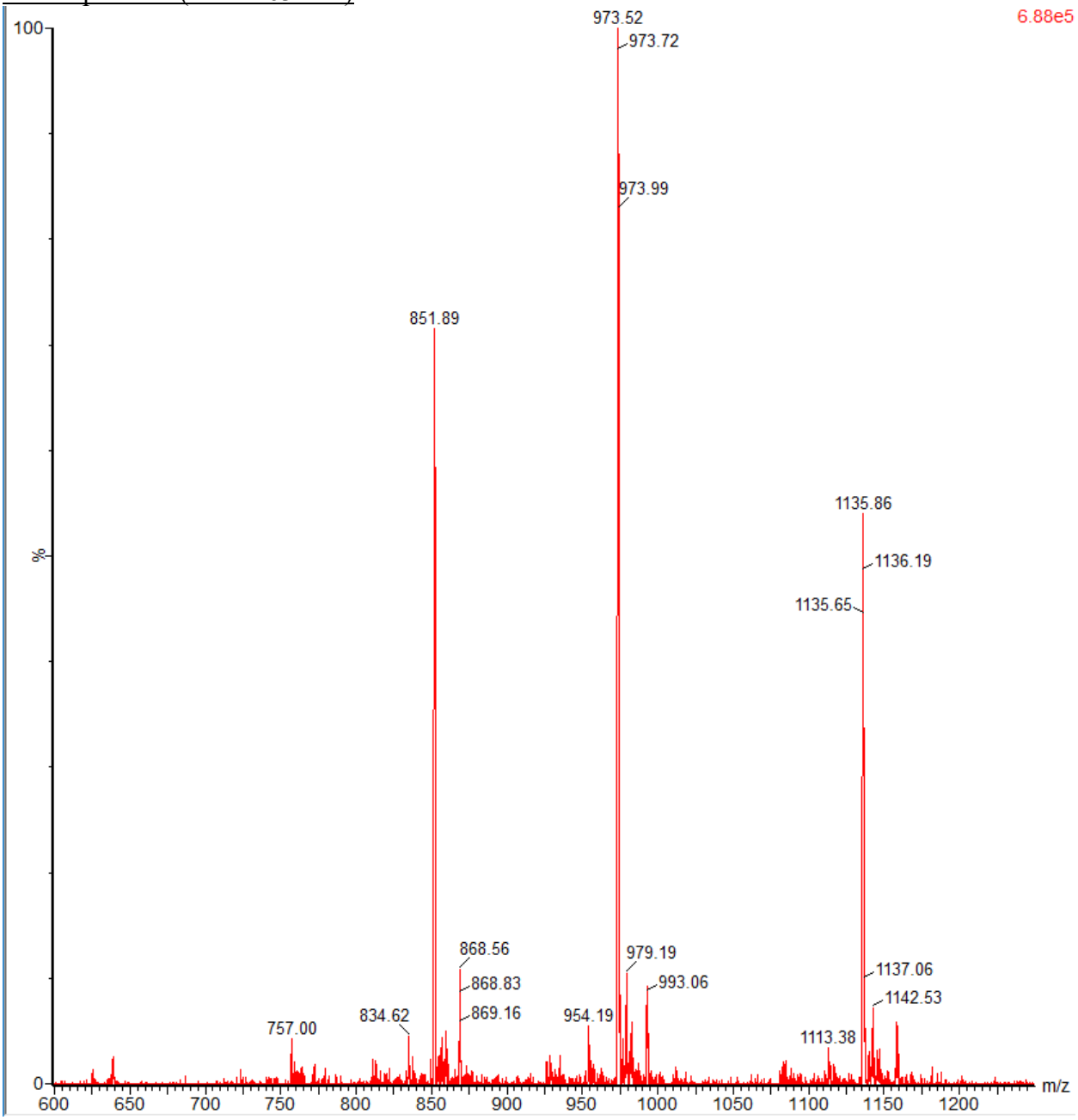


Exact Mass: 7020.14
Molecular Weight: 7023.61

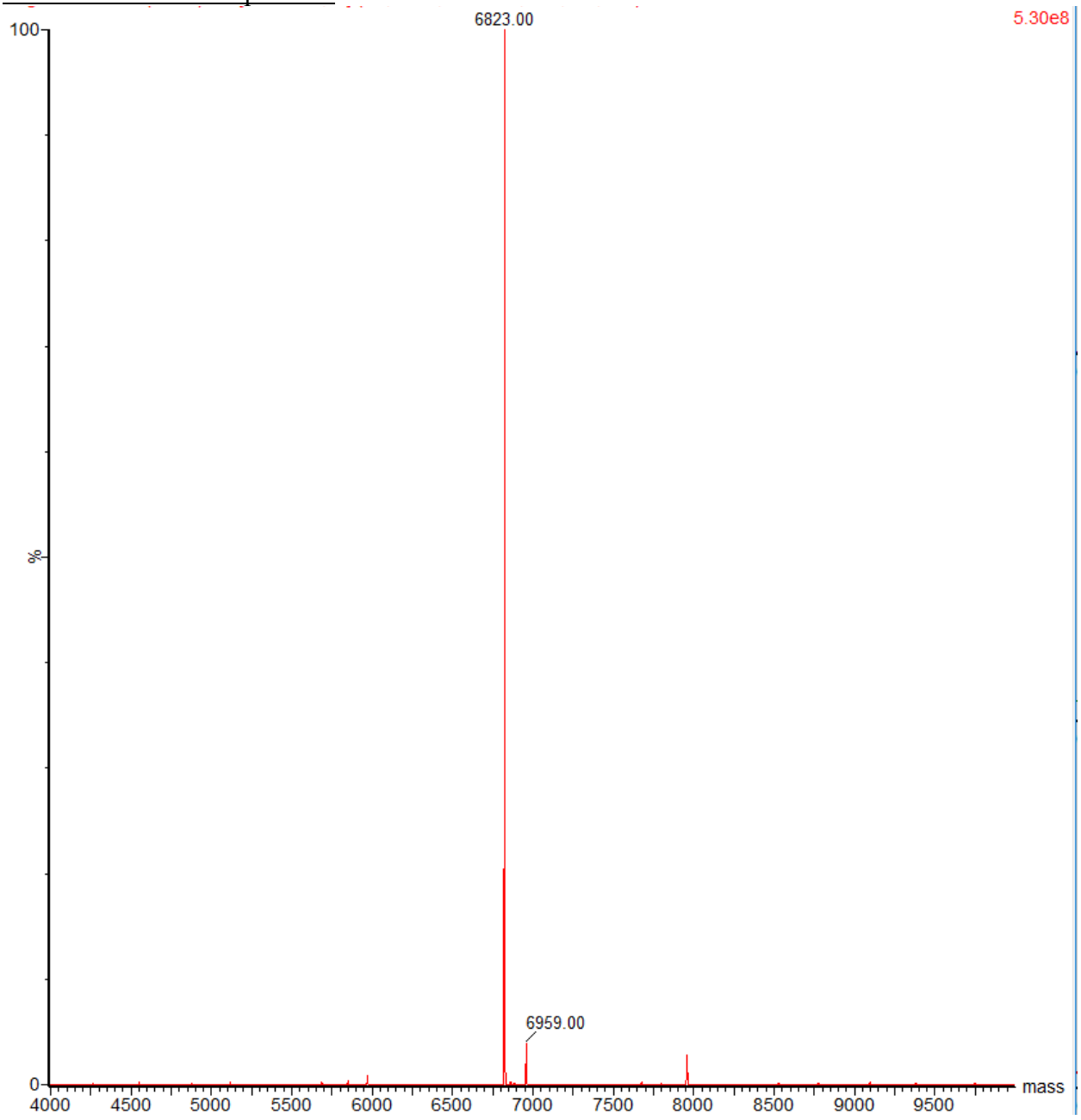
A260 Chromatogram



Mass Spectrum (RT= 1.63 min)

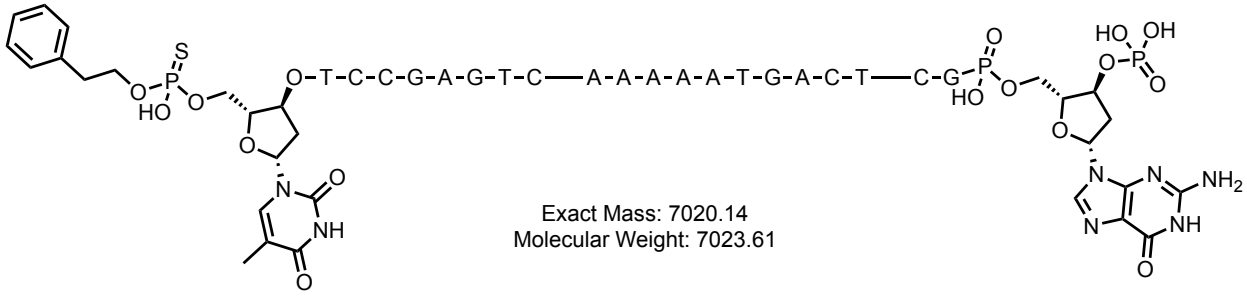


Deconvoluted Mass Spectrum

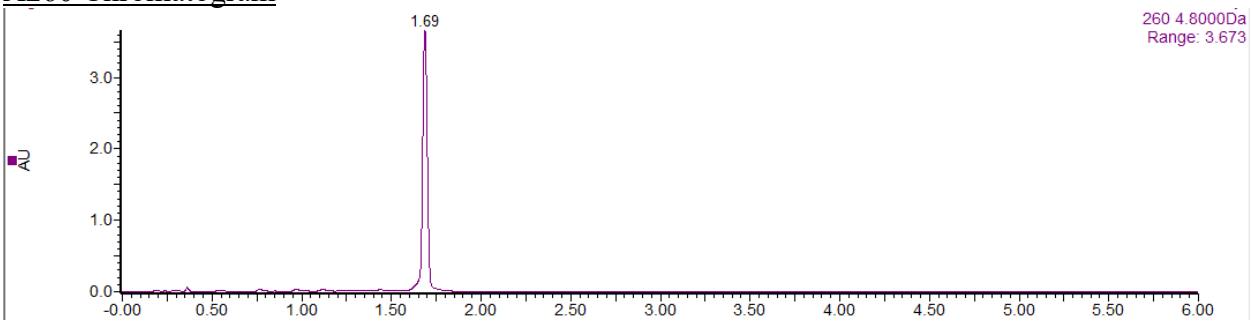


Entry 7: 5'

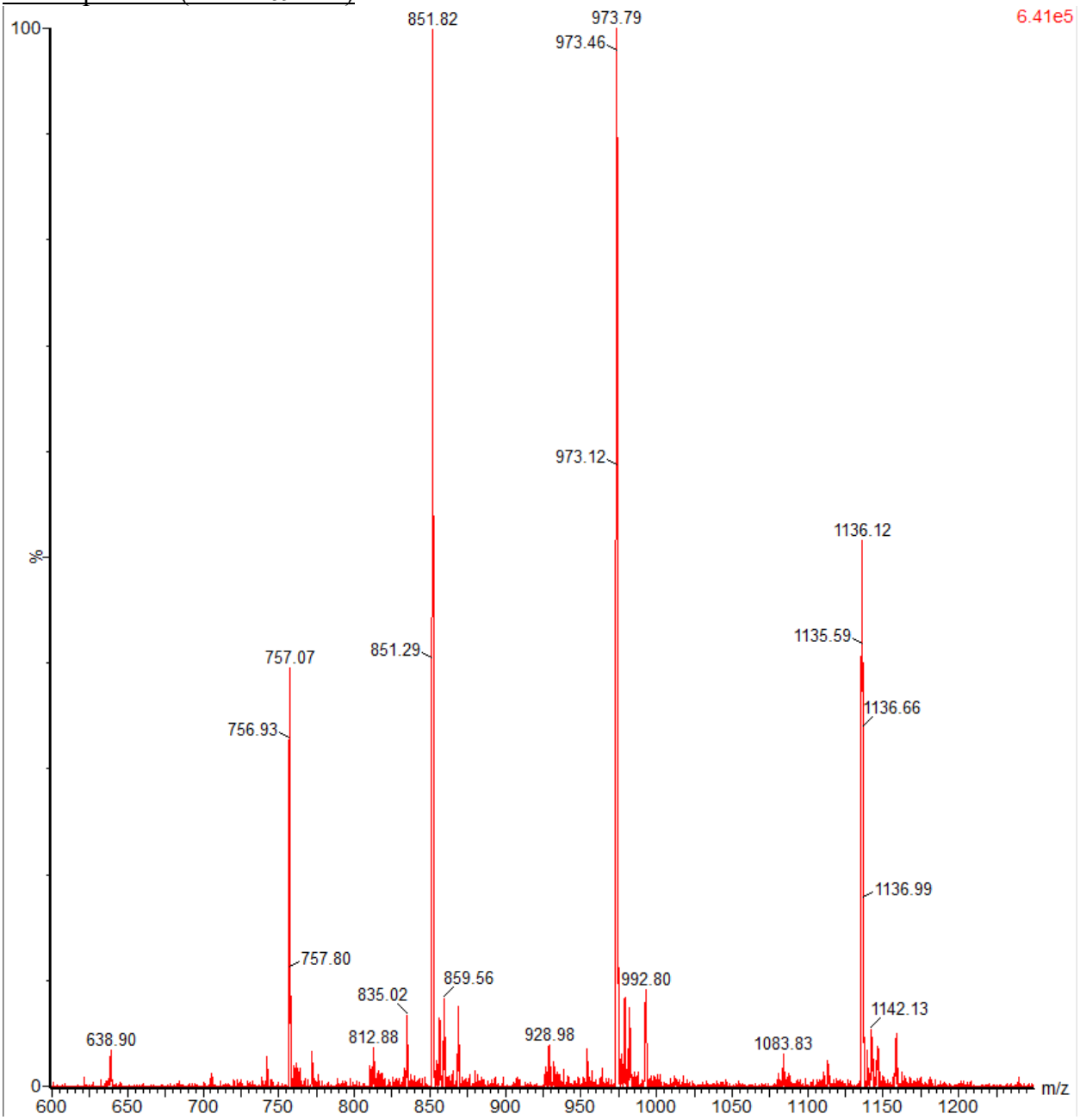
LCMS Characterization of **3**
Produced from **1** by General Procedure **2:**
Conversion (A260): 0%
Expected Mass: 7023.61
Expected (M-6)/6 Ion: 1169.60
Found Deconvoluted Mass: 6823.00 (Starting material **1**)
Found (M-6)/6 Ion: 1136.12



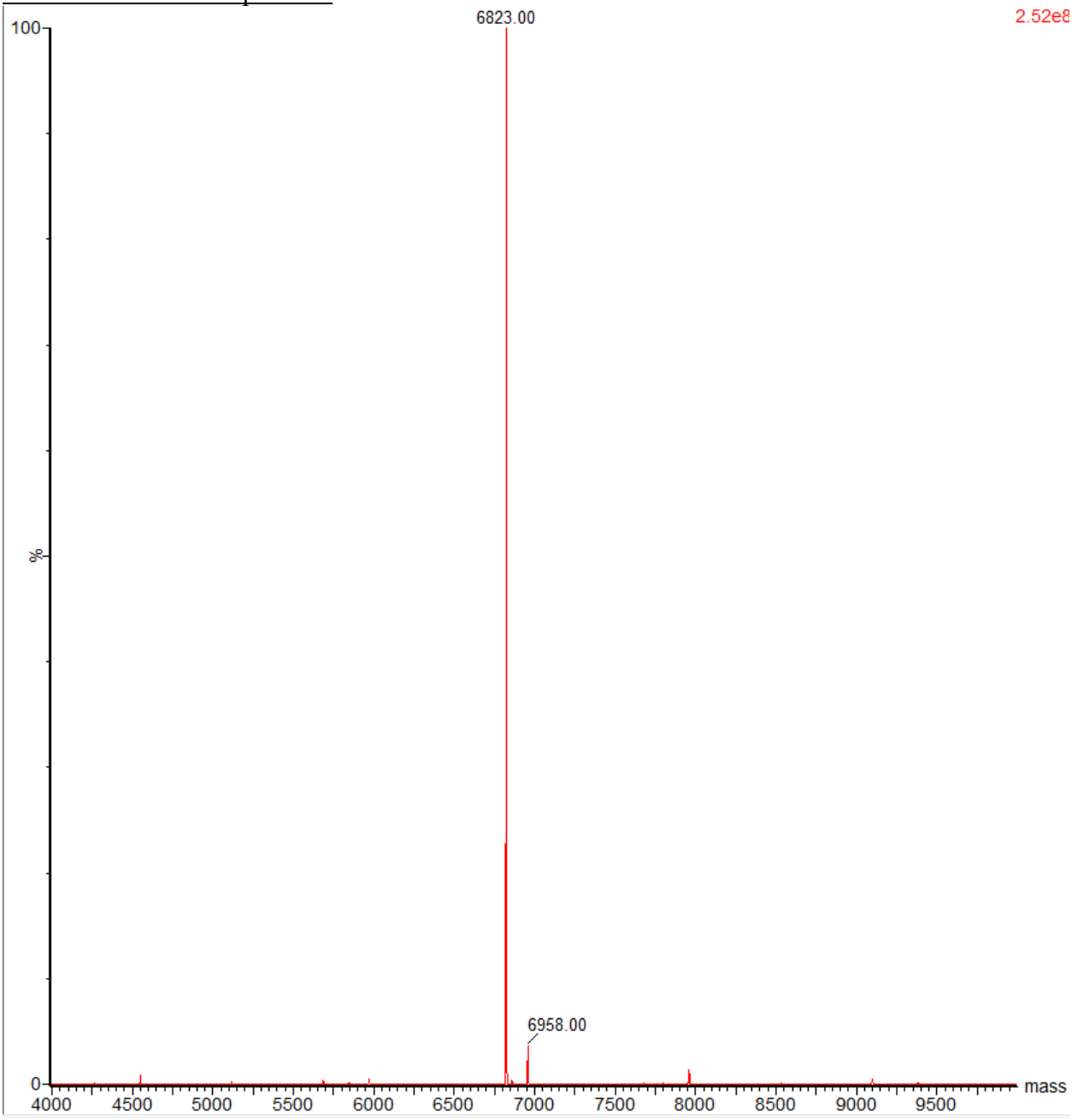
A260 Chromatogram



Mass Spectrum (RT= 1.69 min)

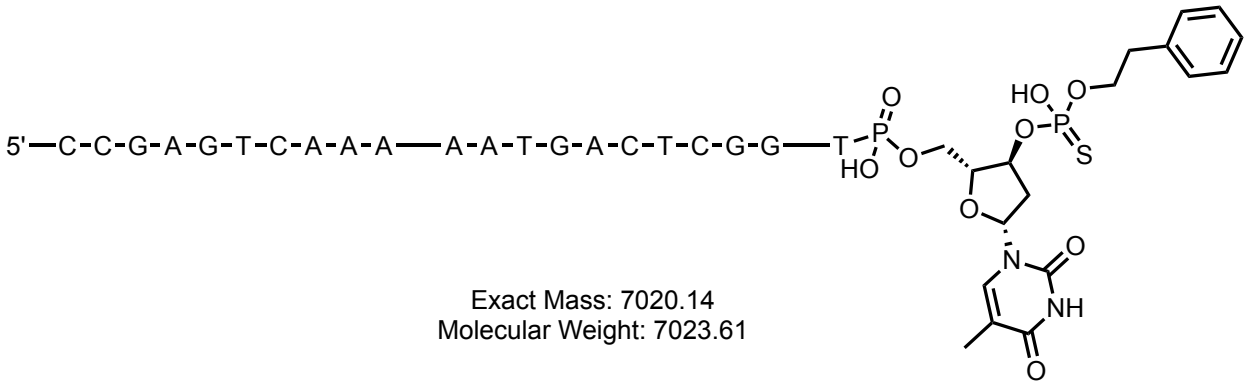


Deconvoluted Mass Spectrum



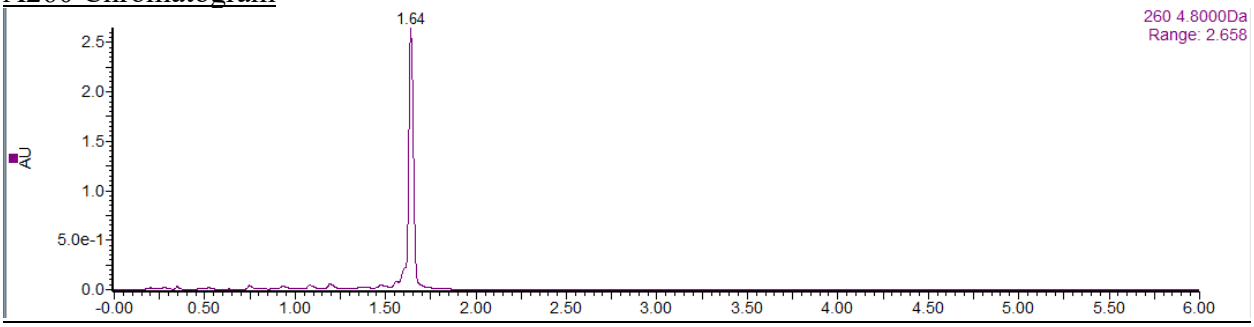
Entry 7: 3'

LCMS Characterization of **4**
Produced from **2** by General Procedure **2:**
Conversion (A260): 56%
Expected Mass: 7023.61
Expected (M-6)/6 Ion: 1169.60
Found Deconvoluted Mass: 6823.00 (Starting material **2**)
Found (M-6)/6 Ion: 1136.26 (Starting material **2**)

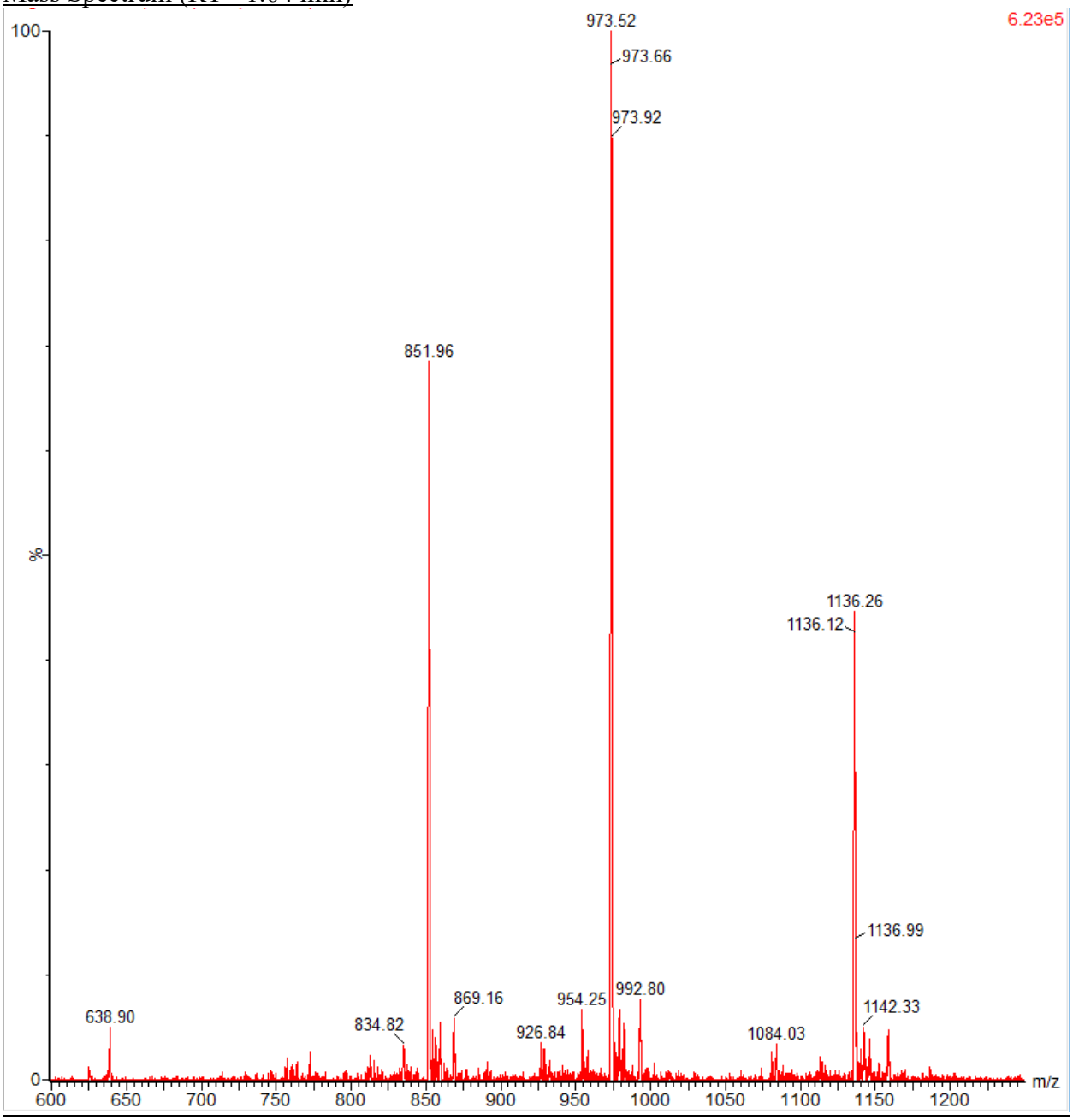


Exact Mass: 7020.14
Molecular Weight: 7023.61

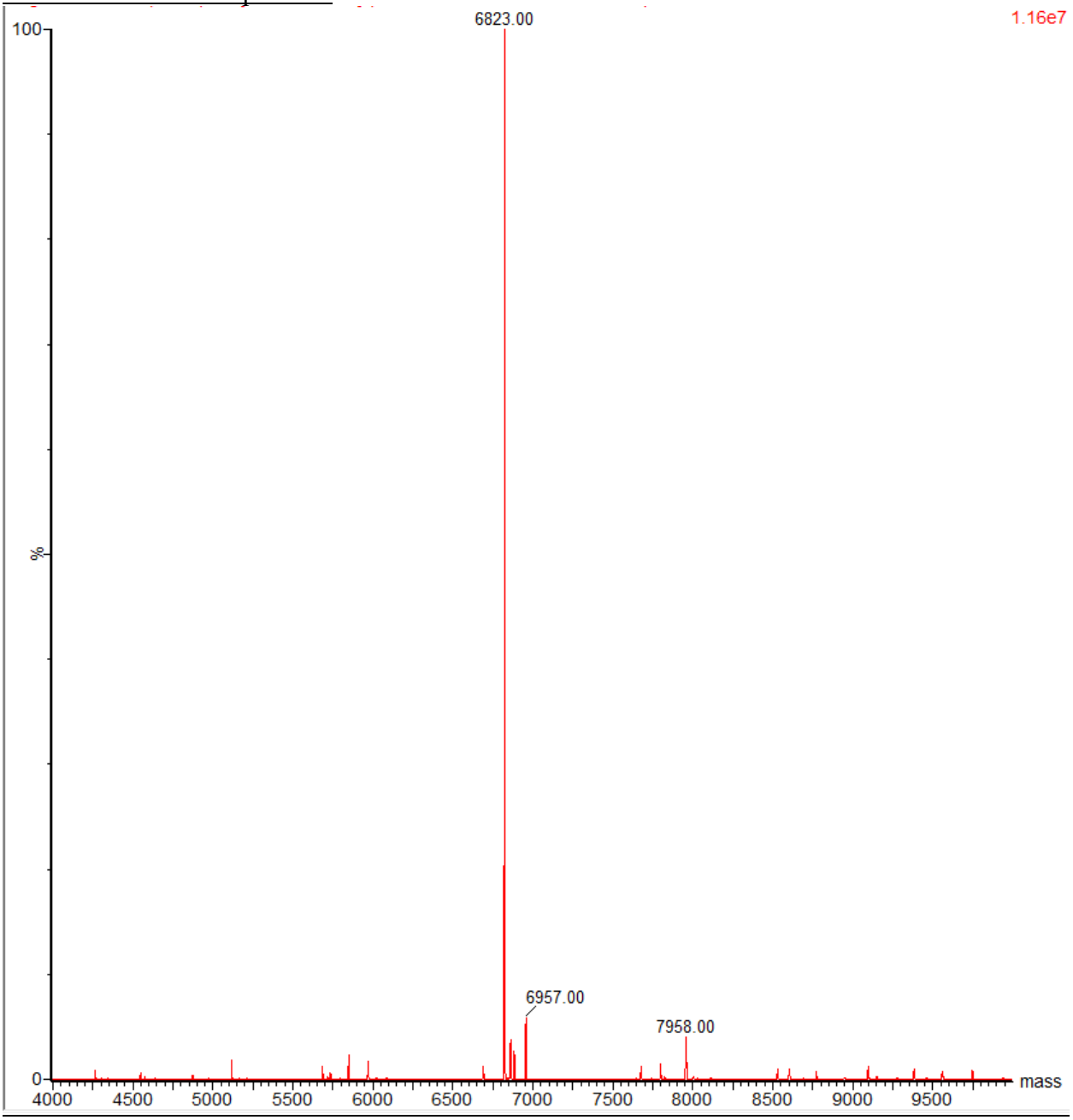
A260 Chromatogram



Mass Spectrum (RT= 1.64 min)

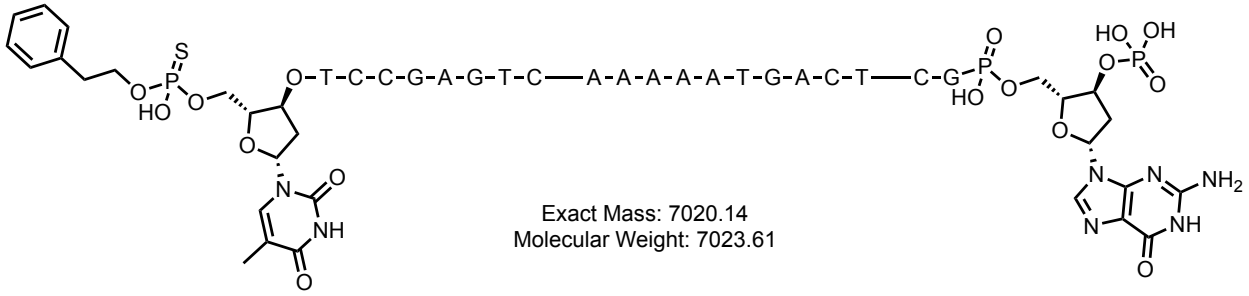


Deconvoluted Mass Spectrum

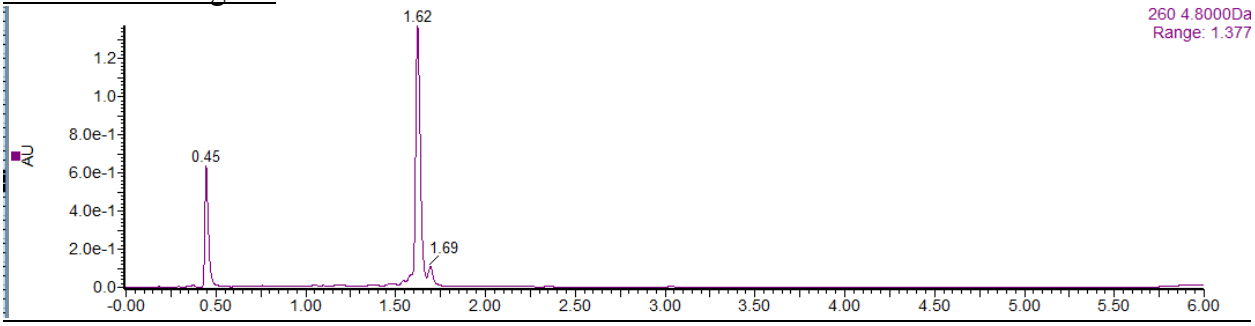


Entry 8: 5'

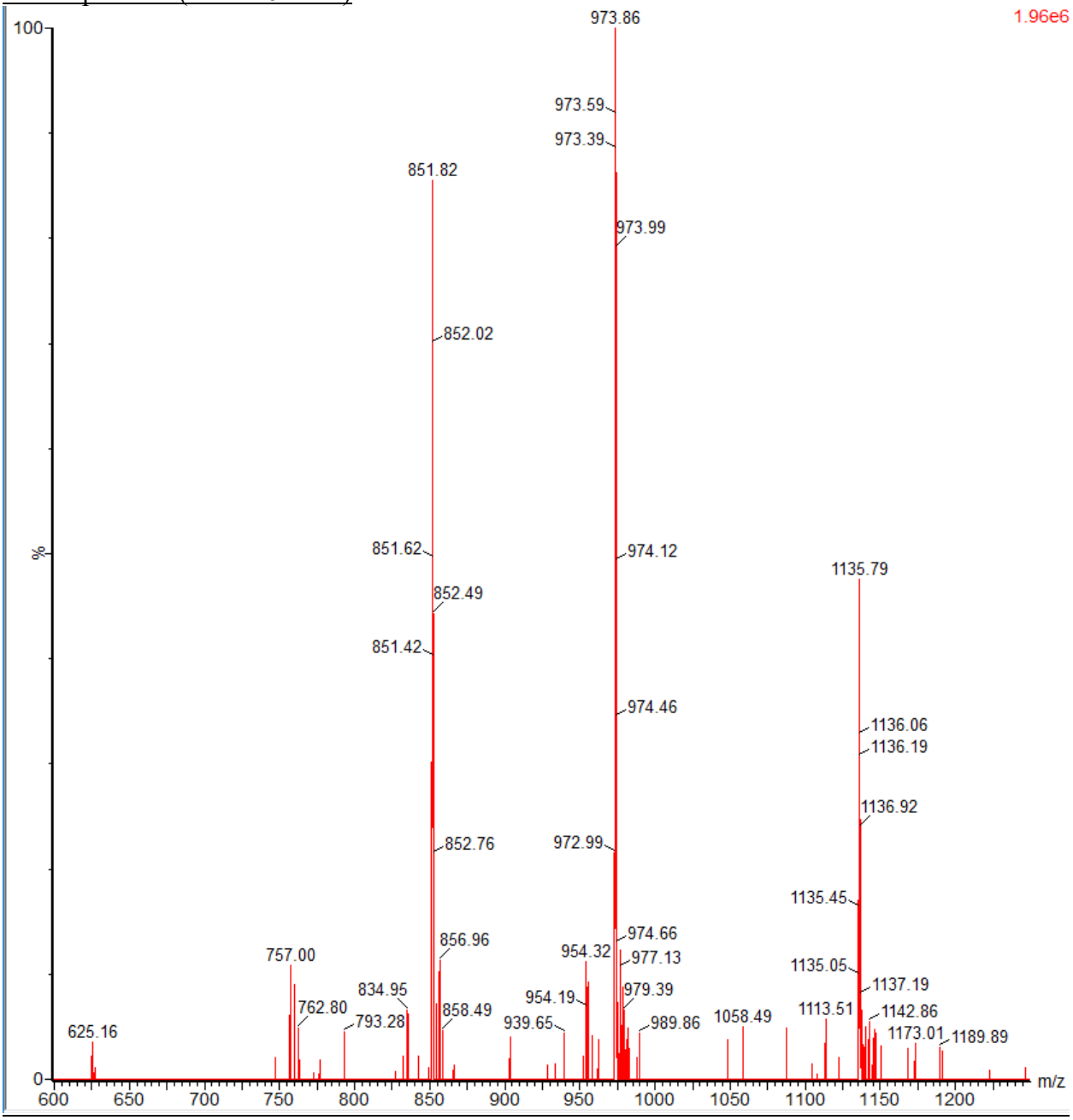
LCMS Characterization of **3**
Produced from **1** by General Procedure **2:**
Conversion (A260): 0%
Expected Mass: 7023.61
Expected (M-6)/6 Ion: 1169.60
Found Deconvoluted Mass: 6824.00 (Starting material **1**)
Found (M-6)/6 Ion: 1135.79 (Starting material **1**)



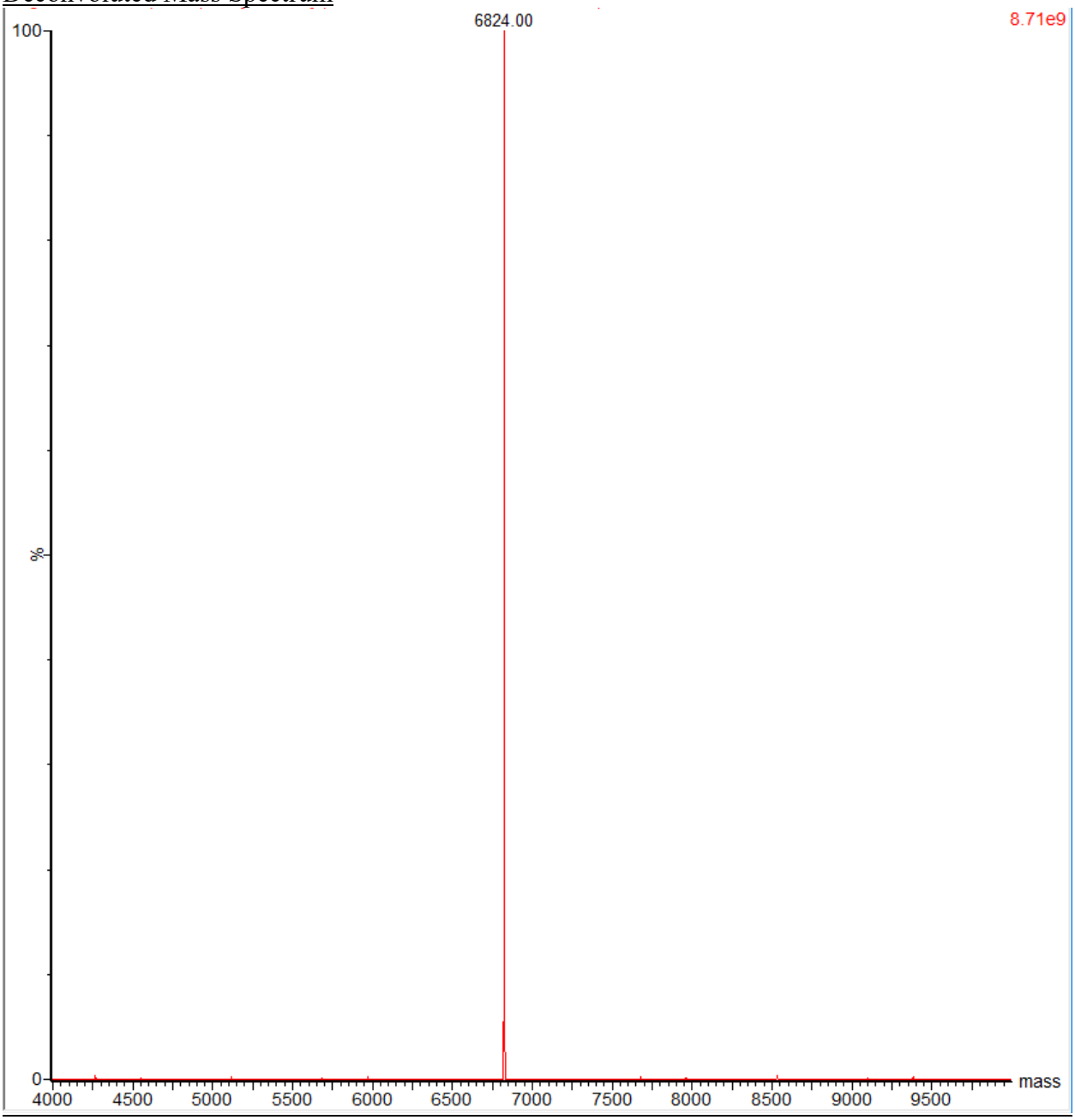
A260 Chromatogram



Mass Spectrum (RT= 1.62 min)

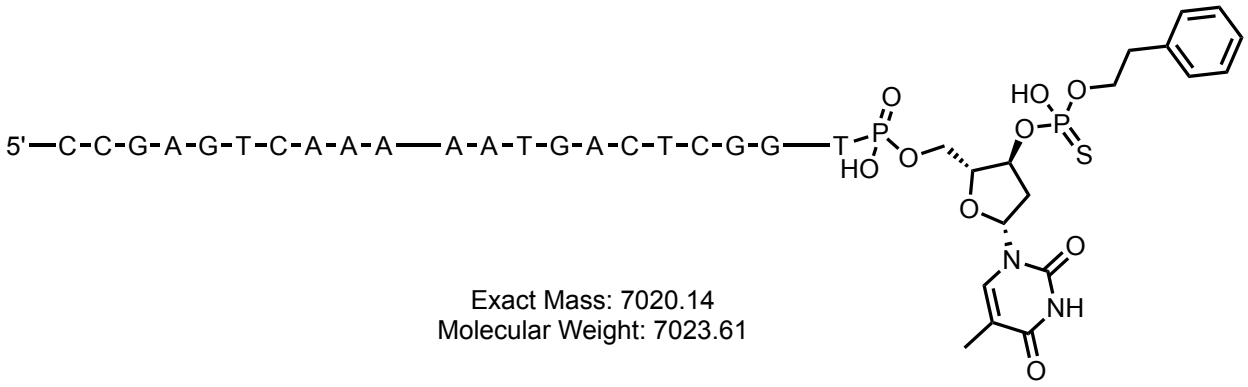


Deconvoluted Mass Spectrum



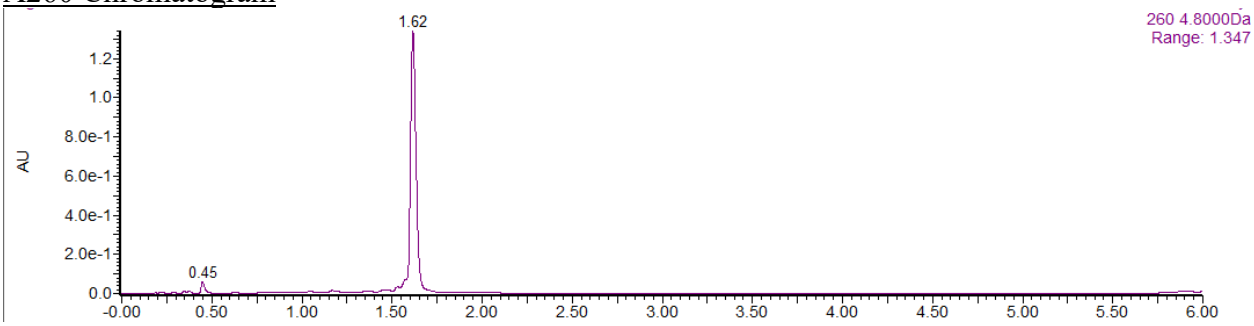
Entry 8: 3'

LCMS Characterization of **4**
Produced from **2** by General Procedure **2:**
Conversion (A260): 0%
Expected Mass: 7023.61
Expected (M-6)/6 Ion: 1169.60
Found Deconvoluted Mass: 6824.00 (Starting material **2**)
Found (M-6)/6 Ion: 1136.32 (Starting material **2**)

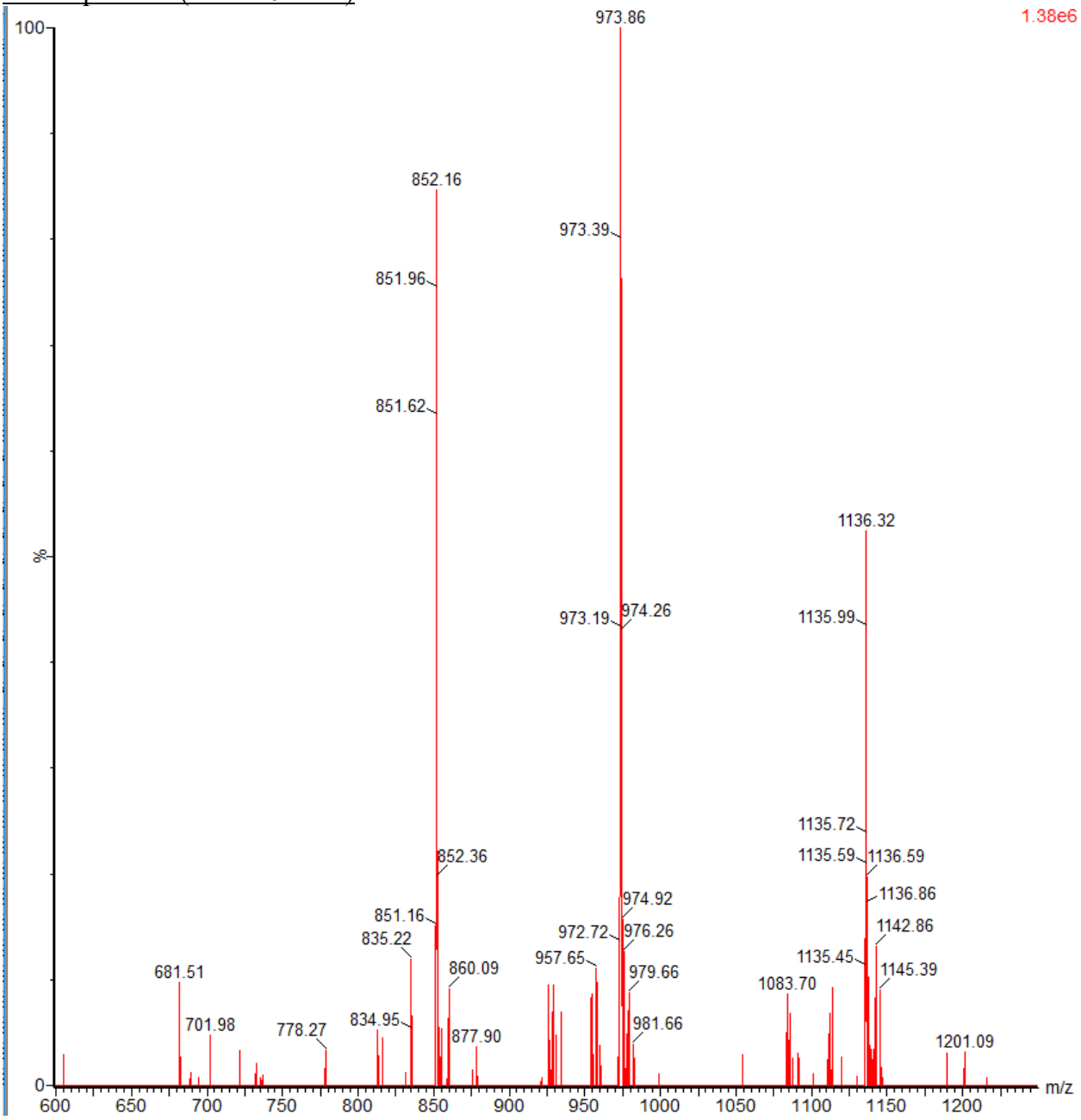


Exact Mass: 7020.14
Molecular Weight: 7023.61

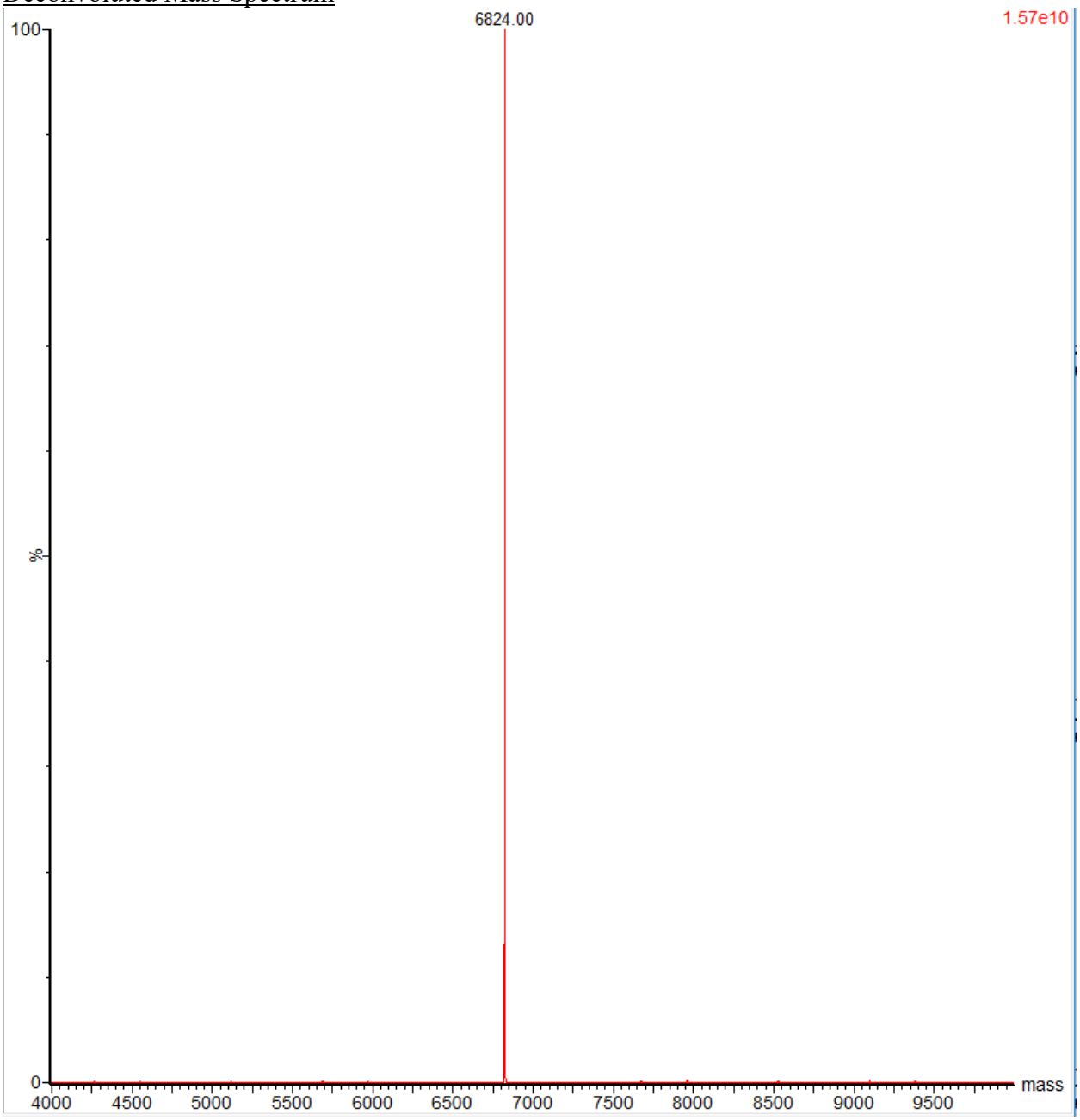
A260 Chromatogram



Mass Spectrum (RT= 1.62 min)

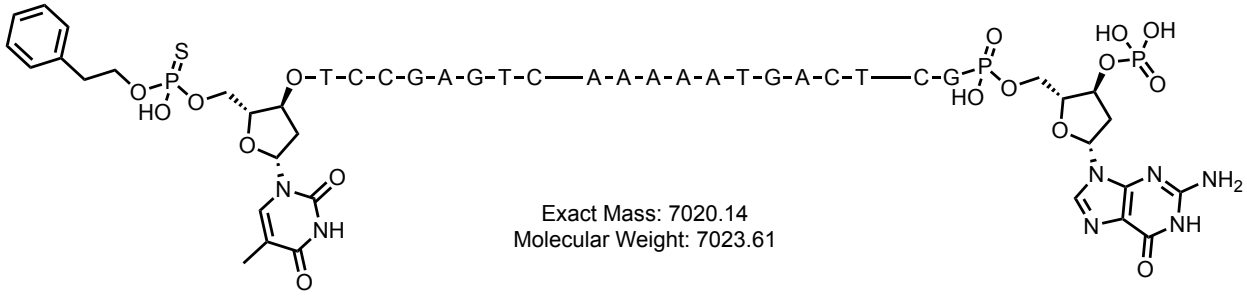


Deconvoluted Mass Spectrum

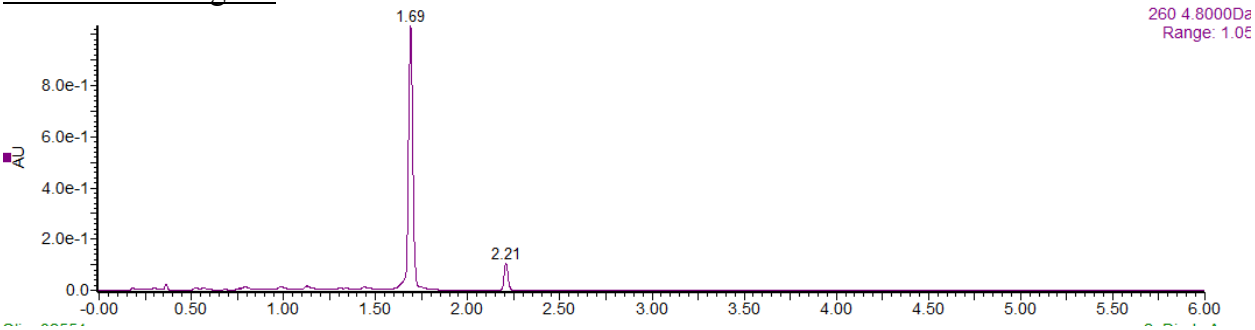


Entry 9: 5'

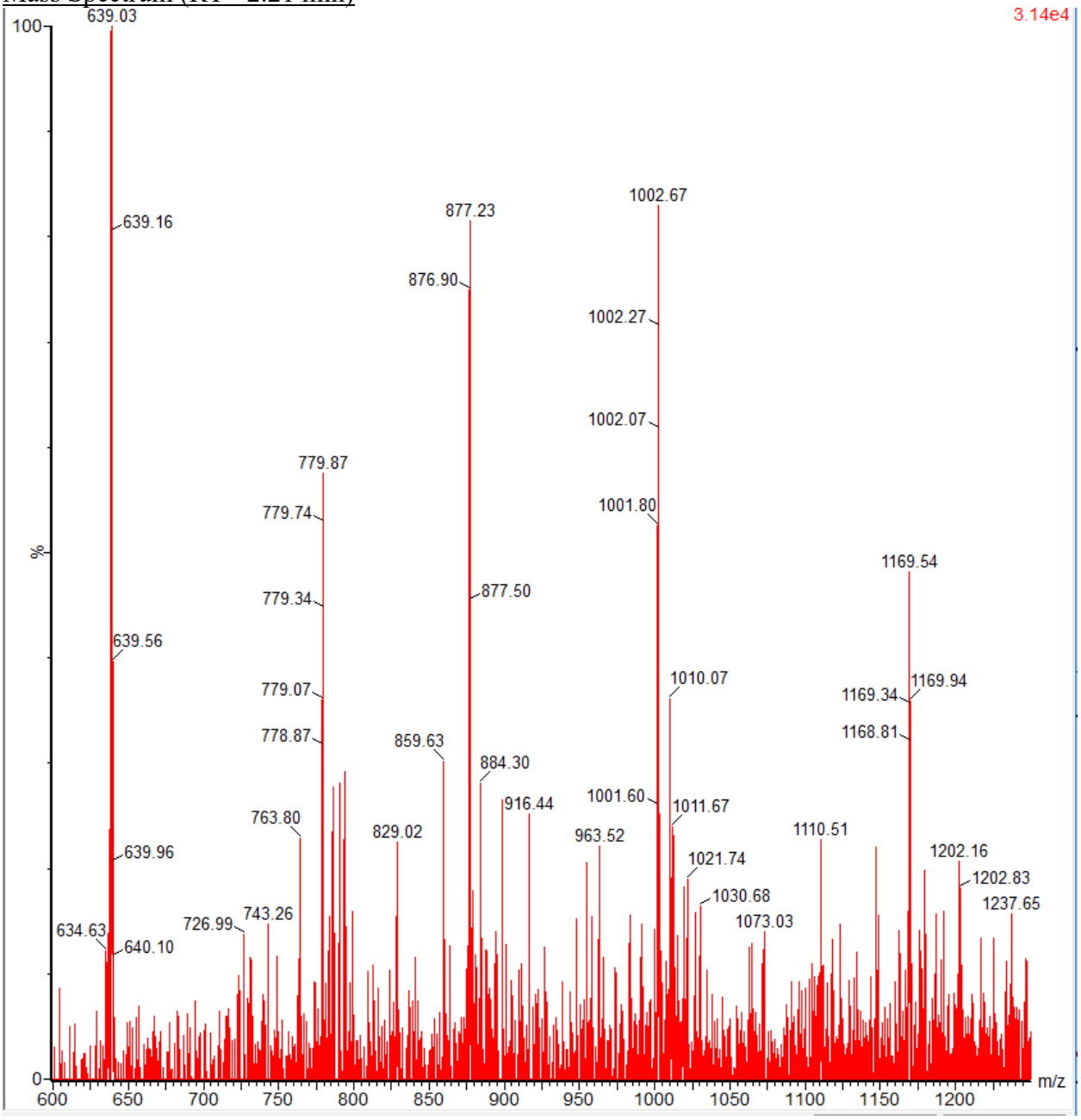
LCMS Characterization of **3**
Produced from **1** by General Procedure **2:**
Conversion (A260): <10%
Expected Mass: 7023.61
Expected (M-6)/6 Ion: 1169.60
Found Deconvoluted Mass: 7024.00
Found (M-6)/6 Ion: 1169.54



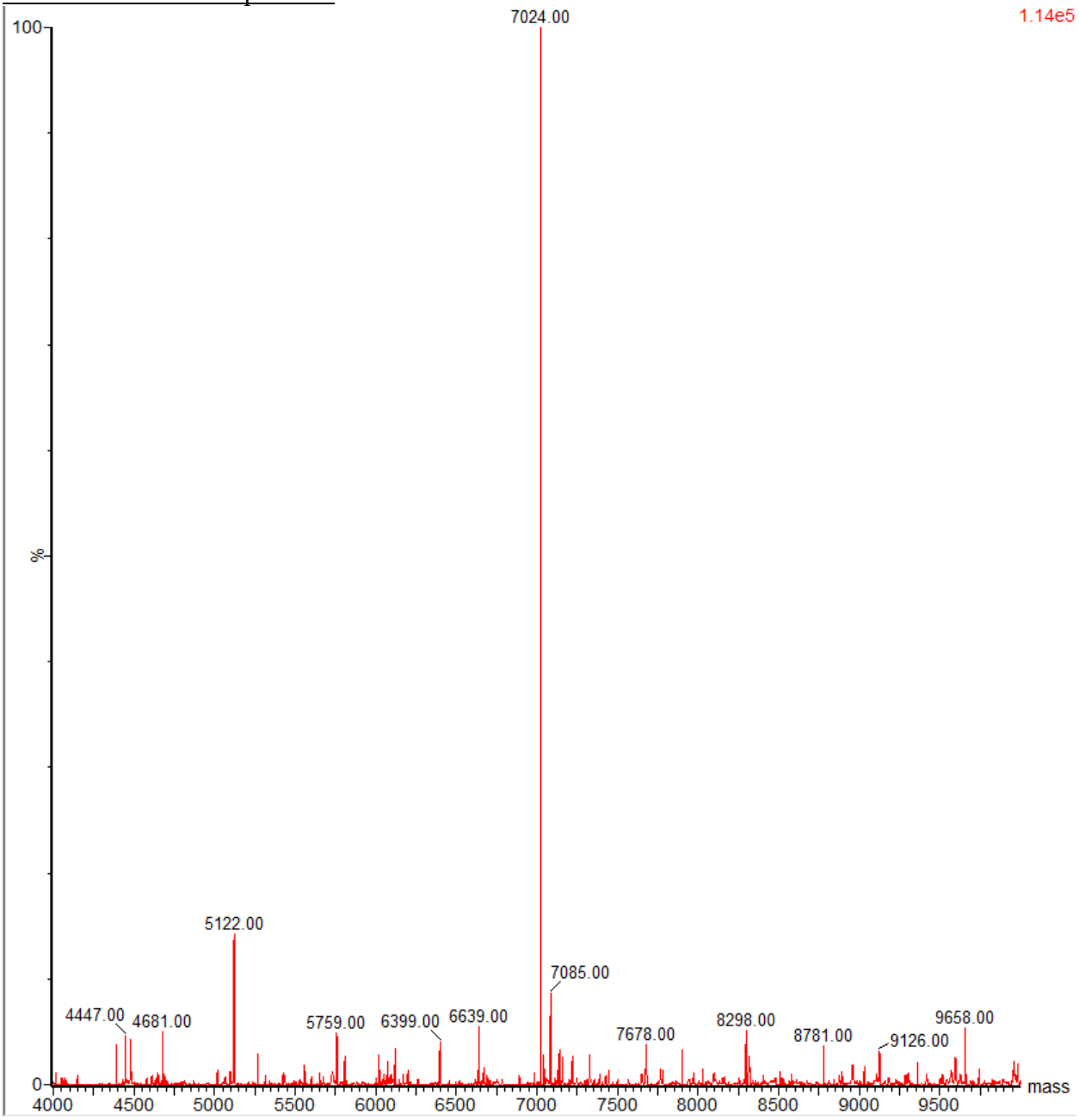
A260 Chromatogram



Mass Spectrum (RT= 2.21 min)

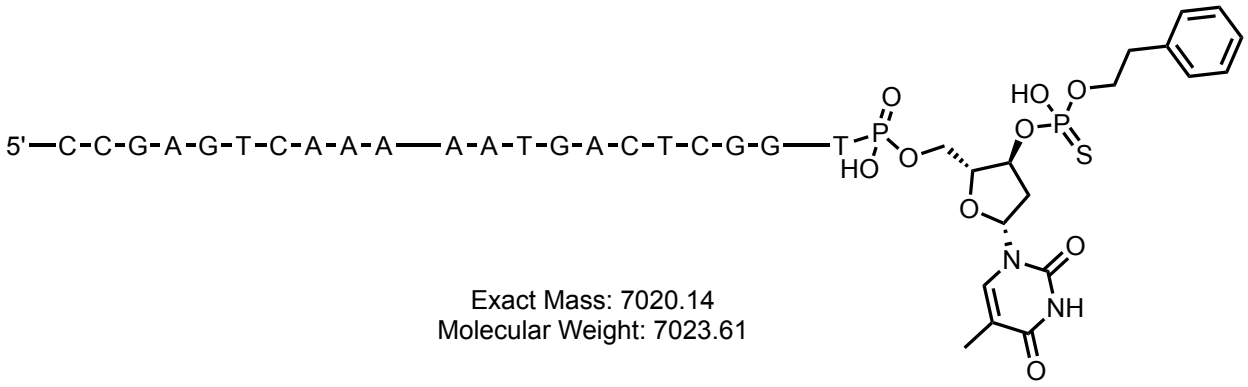


Deconvoluted Mass Spectrum



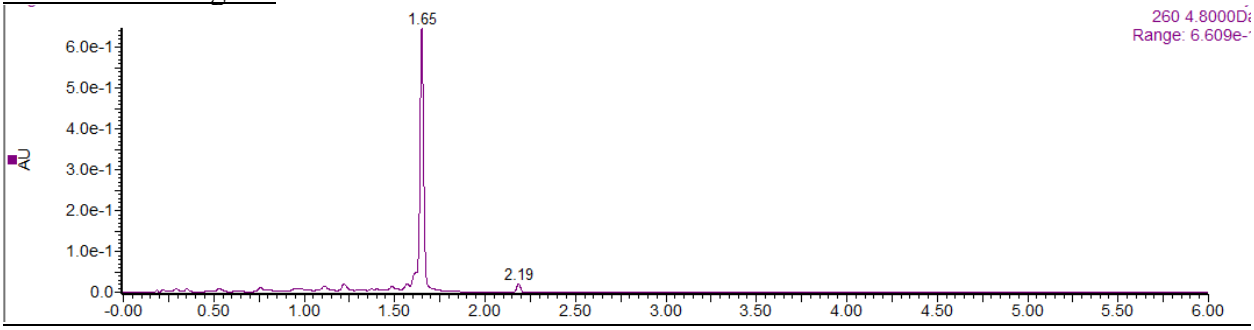
Entry 9: 3'

LCMS Characterization of **4**
Produced from **2** by General Procedure **2:**
Conversion (A260): <10%
Expected Mass: 7023.61
Expected (M-6)/6 Ion: 1169.60
Found Deconvoluted Mass: 7024.00
Found (M-6)/6 Ion: 1169.54

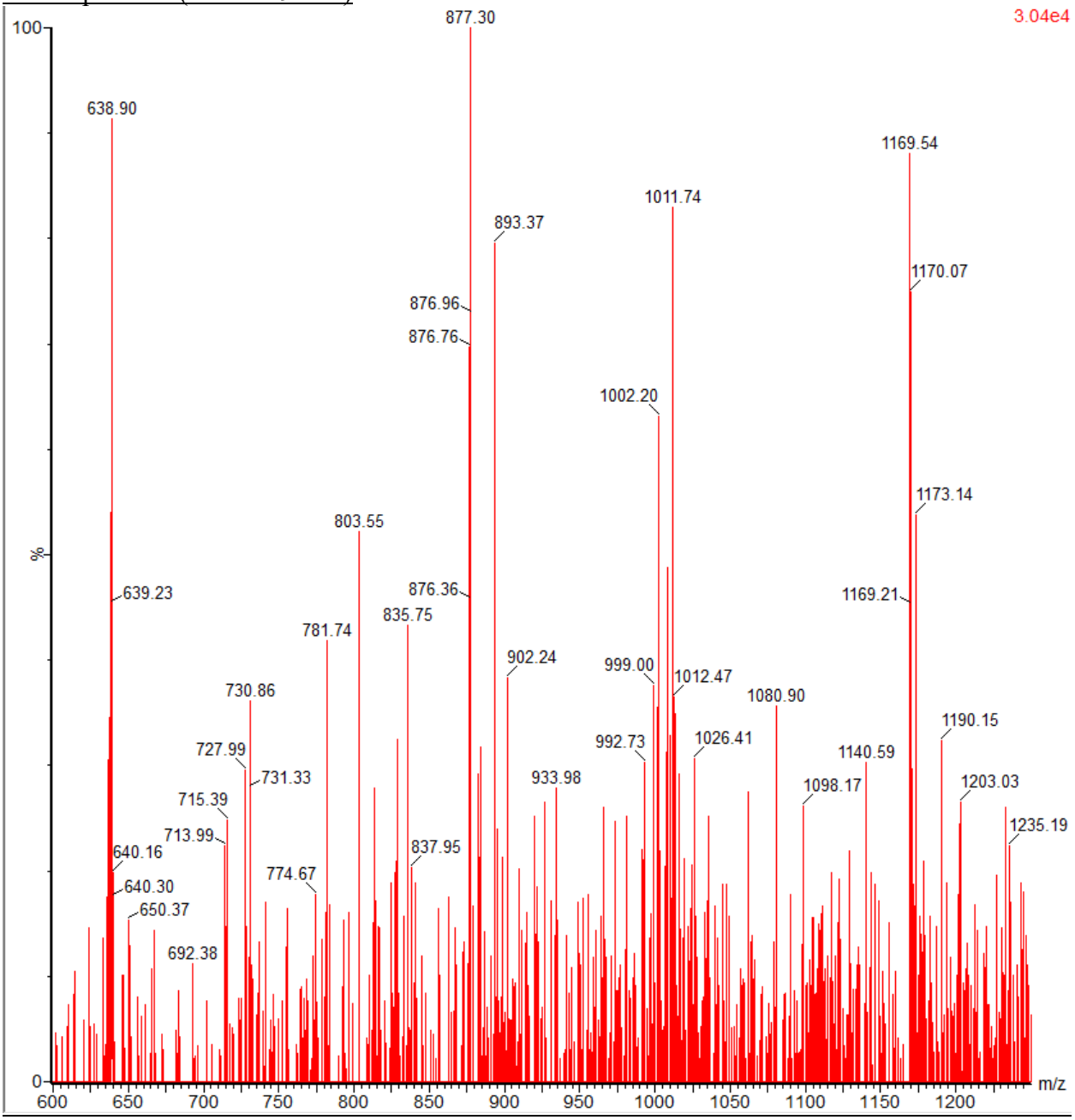


Exact Mass: 7020.14
Molecular Weight: 7023.61

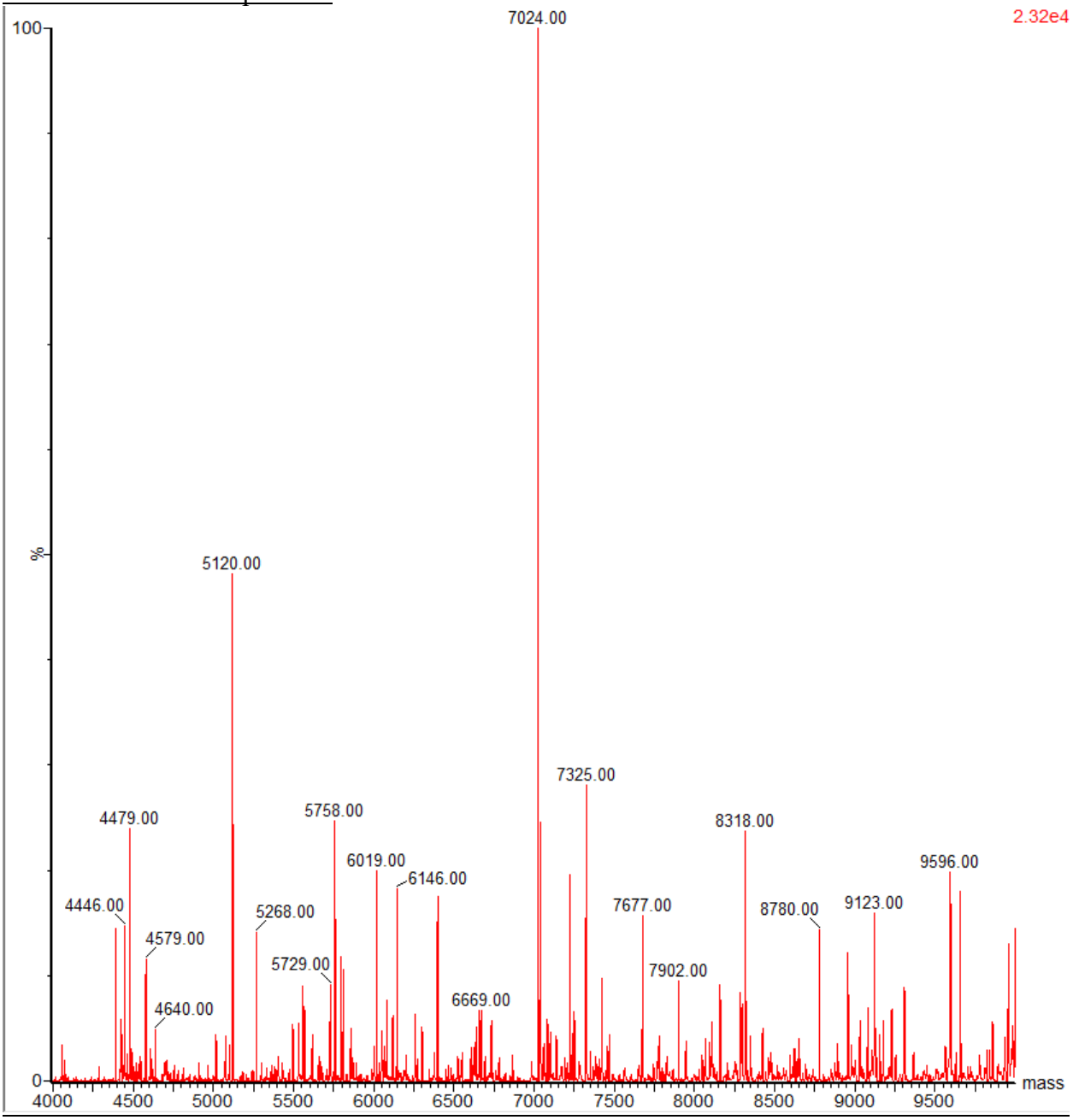
A260 Chromatogram



Mass Spectrum (RT= 2.19 min)

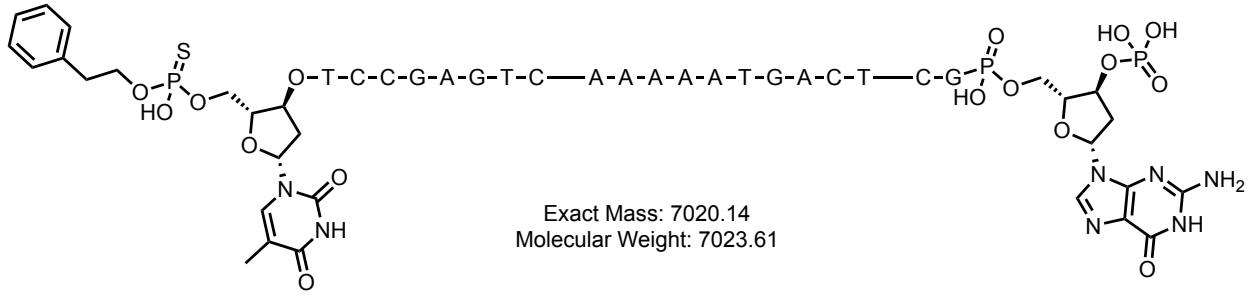


Deconvoluted Mass Spectrum

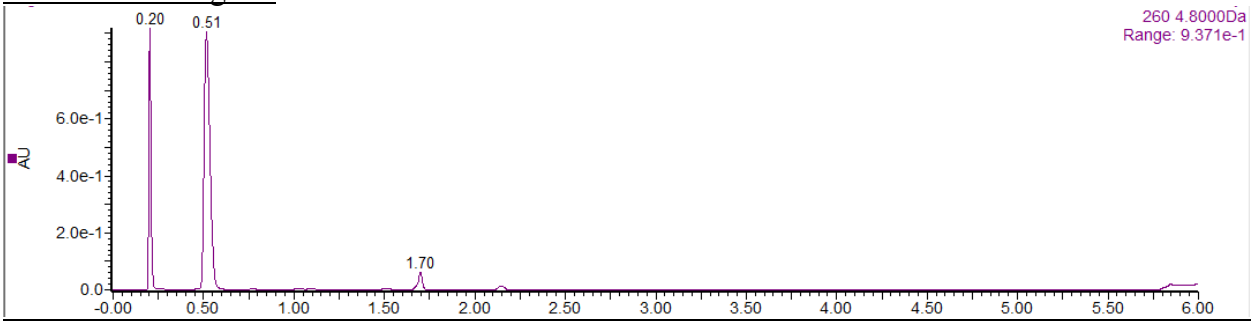


Entry 10: 5'

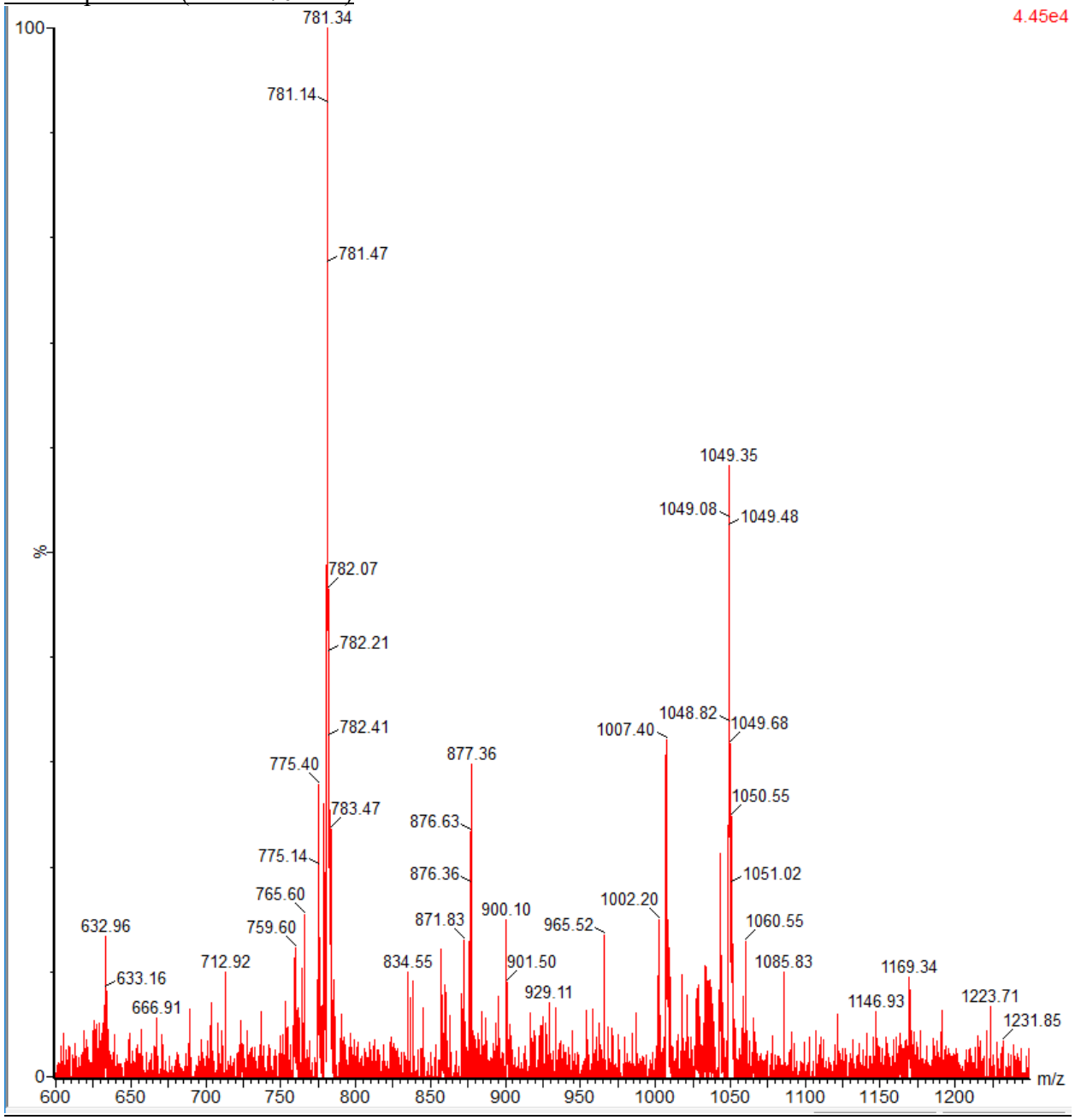
LCMS Characterization of **3**
Produced from **1** by General Procedure **2:**
Conversion (A260): trace
Expected Mass: 7023.61
Expected (M-6)/6 Ion: 1169.60
Found Deconvoluted Mass: 7023.00
Found (M-6)/6 Ion: 1169.34



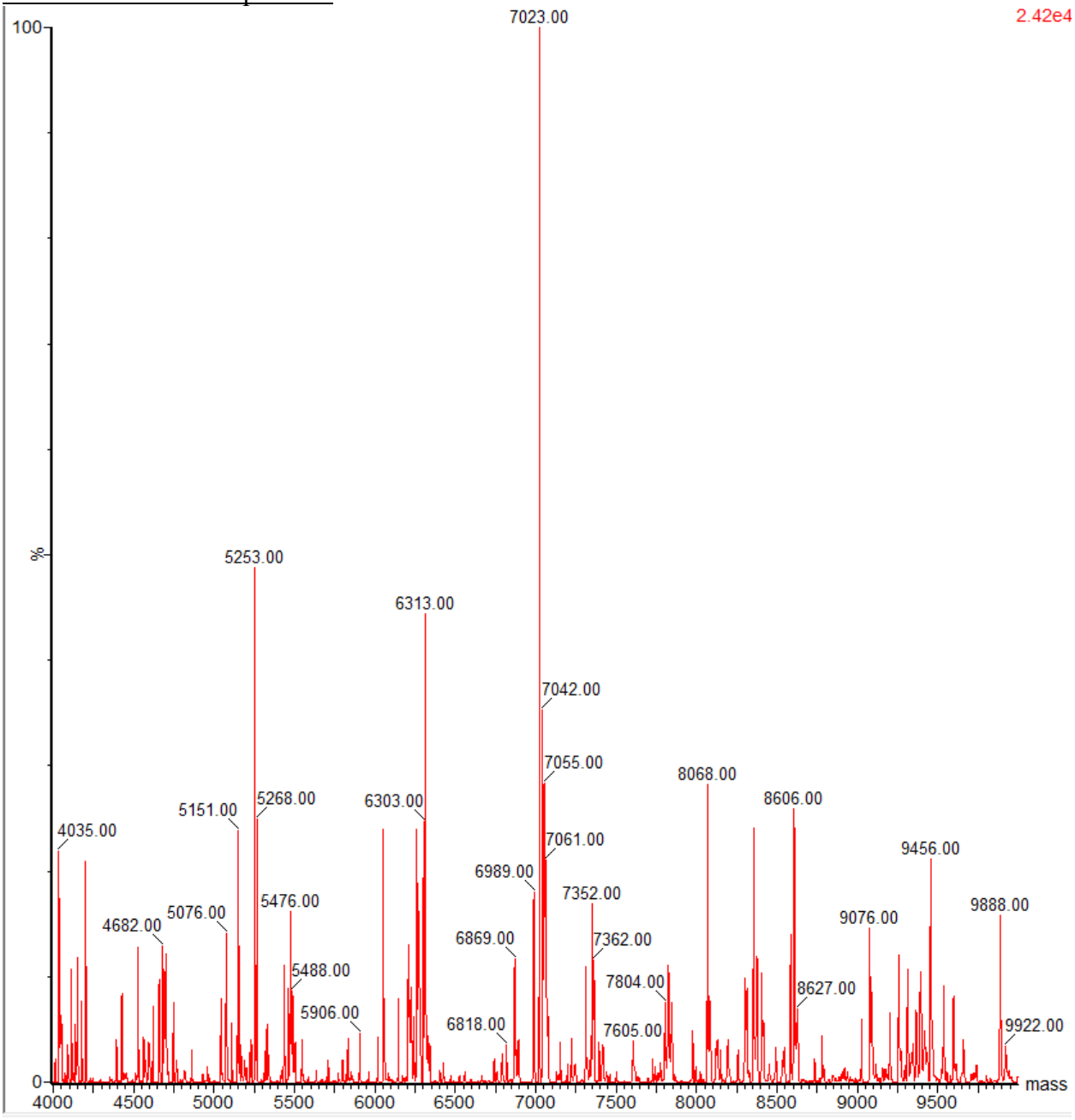
A260 Chromatogram



Mass Spectrum (RT= 1.70 min)

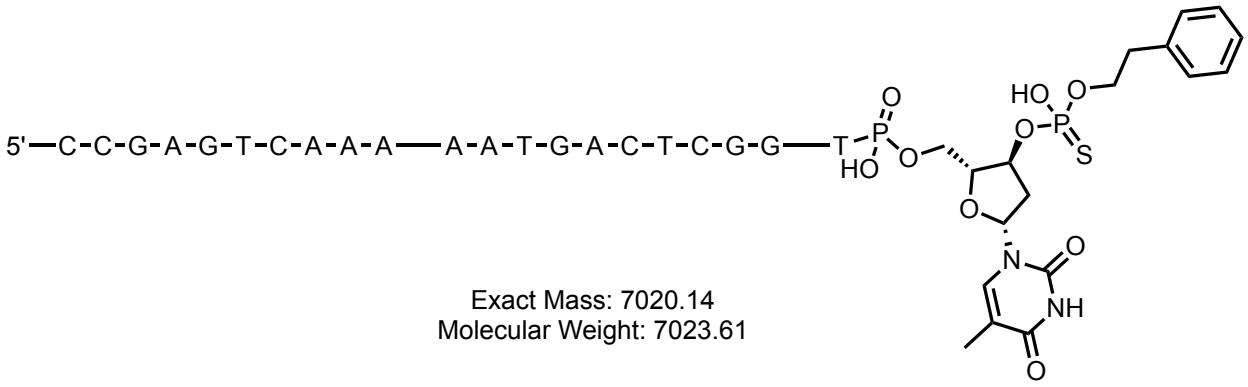


Deconvoluted Mass Spectrum



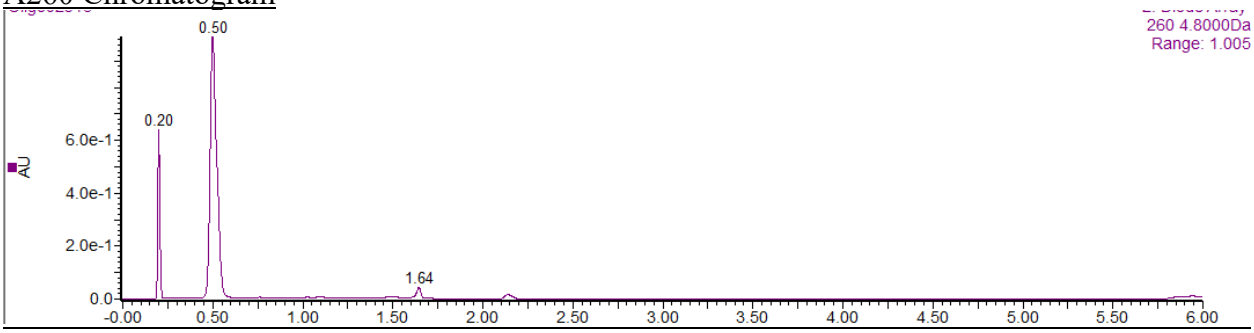
Entry 10: 3'

LCMS Characterization of **4**
Produced from **2** by General Procedure **2:**
Conversion (A260): trace
Expected Mass: 7023.61
Expected (M-6)/6 Ion: 1169.60
Found Deconvoluted Mass: 70234.00
Found (M-6)/6 Ion: 1169.27

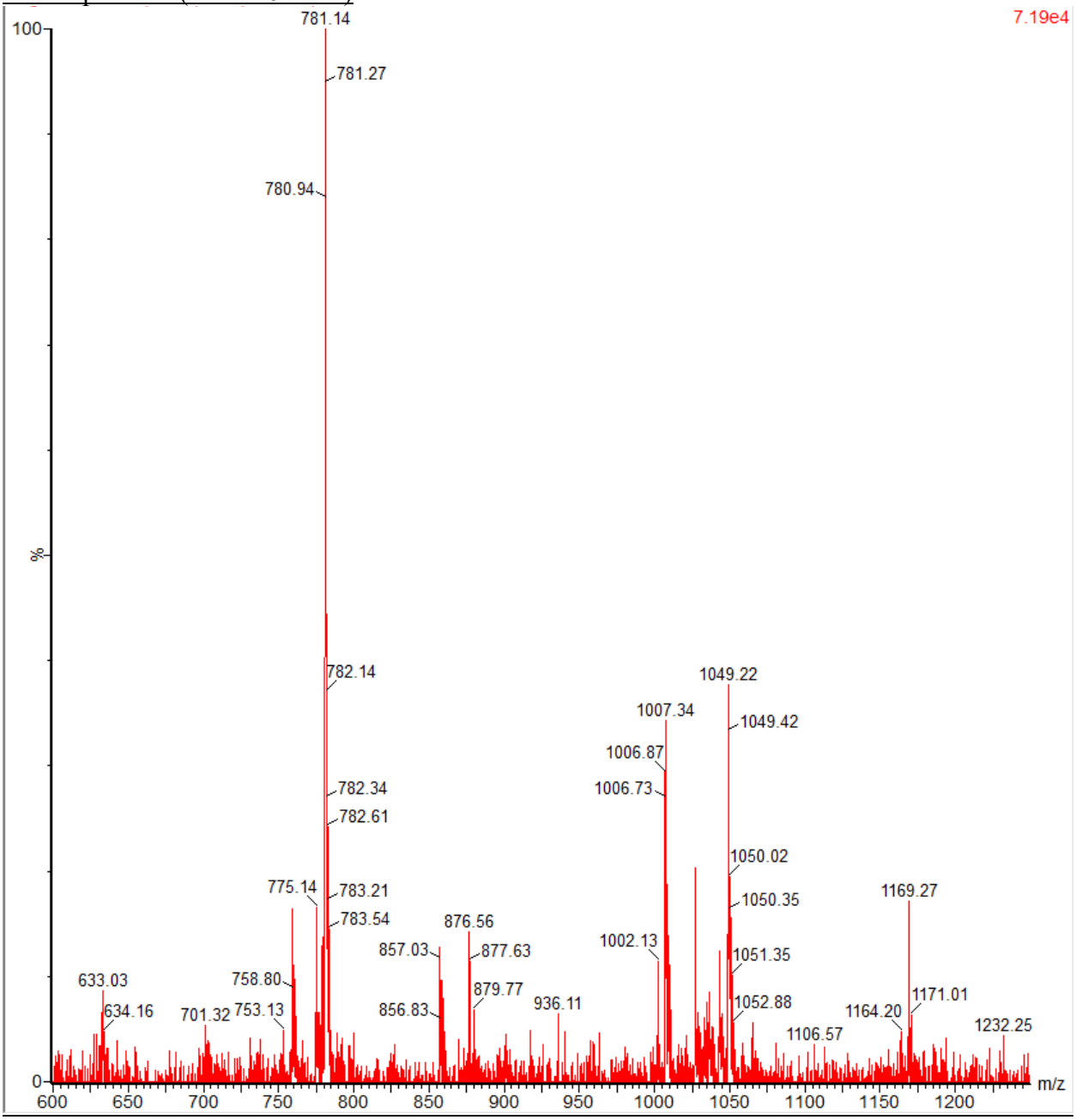


Exact Mass: 7020.14
Molecular Weight: 7023.61

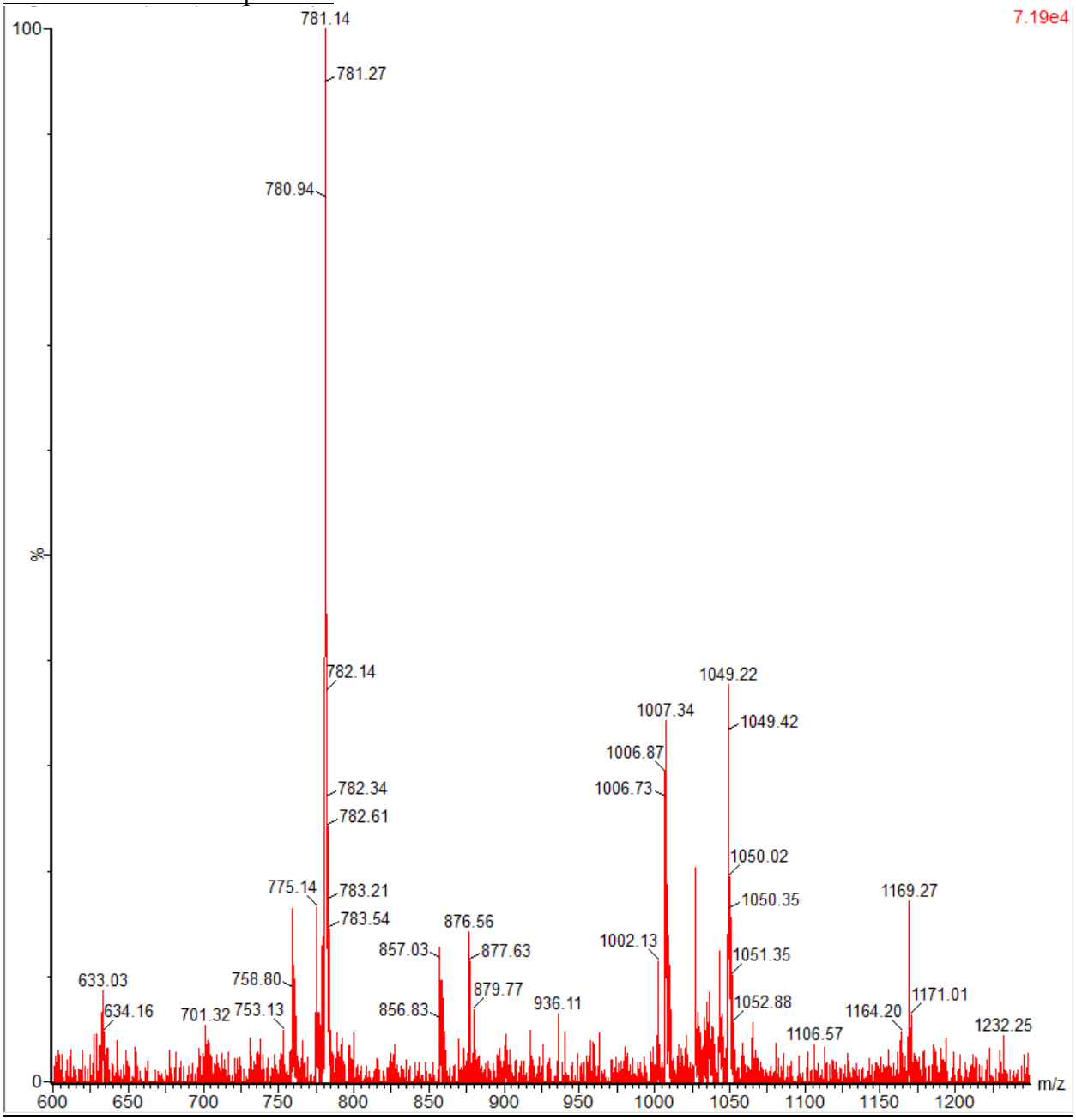
A260 Chromatogram



Mass Spectrum (RT= 1.64 min)



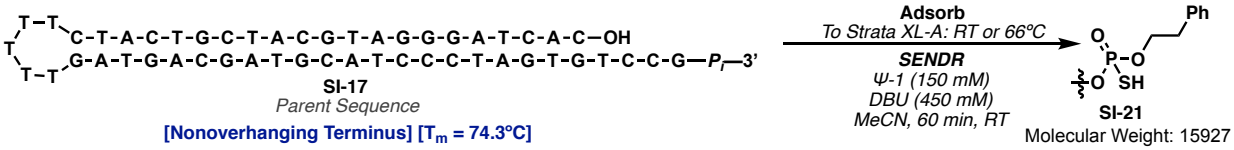
Deconvoluted Mass Spectrum



SENDR on High Tm Hairpin

Hairpin oligo **SI-17** was incubated for 10 minutes at either RT or 66ºC in 100 μL PBS. This solution was pipetted directly onto washed support (50 μL) and quickly vortexed. The reactions were allowed to cool to room temperature before washing and drying was performed according to general procedure 2. SENDR derivatization was performed under standard conditions.

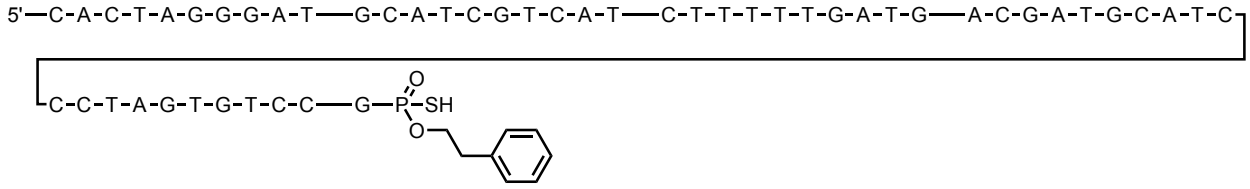
Lableing of High Tm Oligo



Optimization

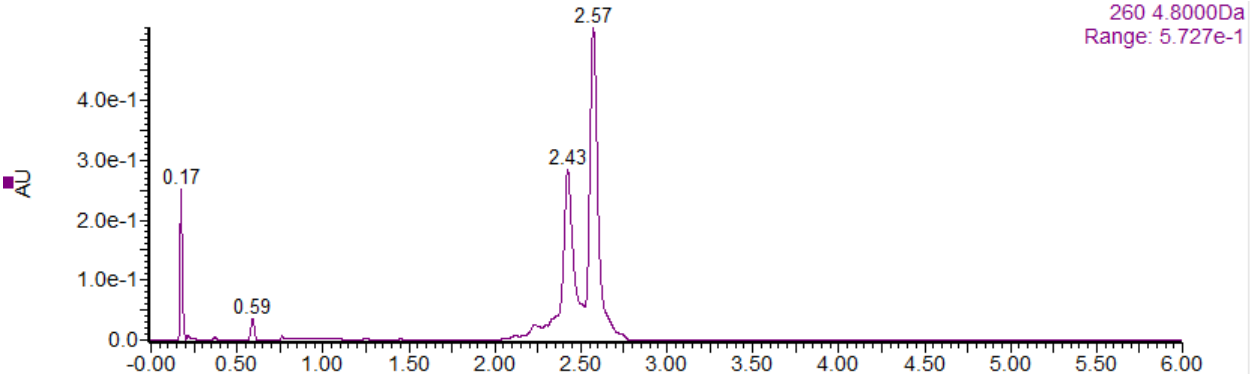
Entry	Conditions	Conversion (%)
1	Adsorb at RT	63
2	Adsorb at 66ºC	70

Entry 1:
LCMS Characterization of **SI-21**
Produced from **SI-17:**
Conversion (A260): 63%
Retention Time: 2.29 min
Expected Mass: 15927.31
Found Deconvoluted Mass: 15930

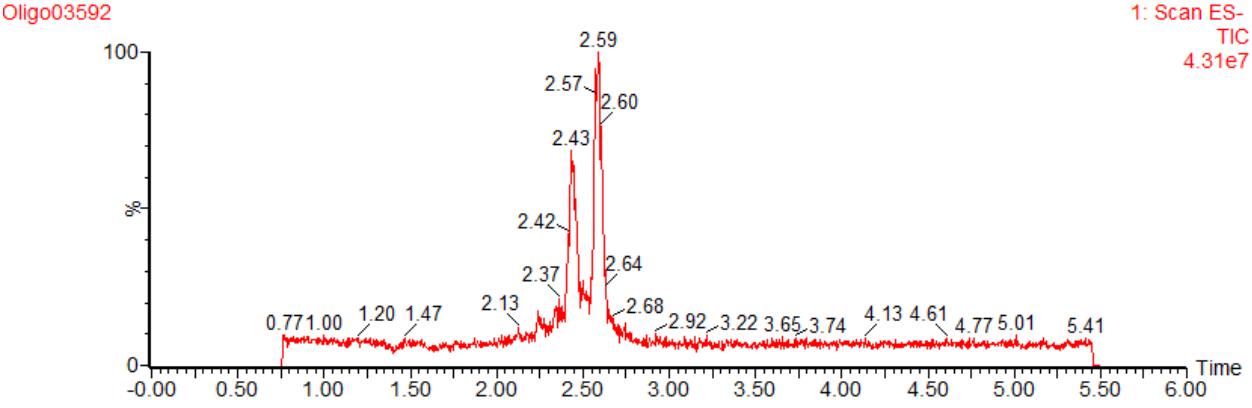


Molecular Weight: 15927.31

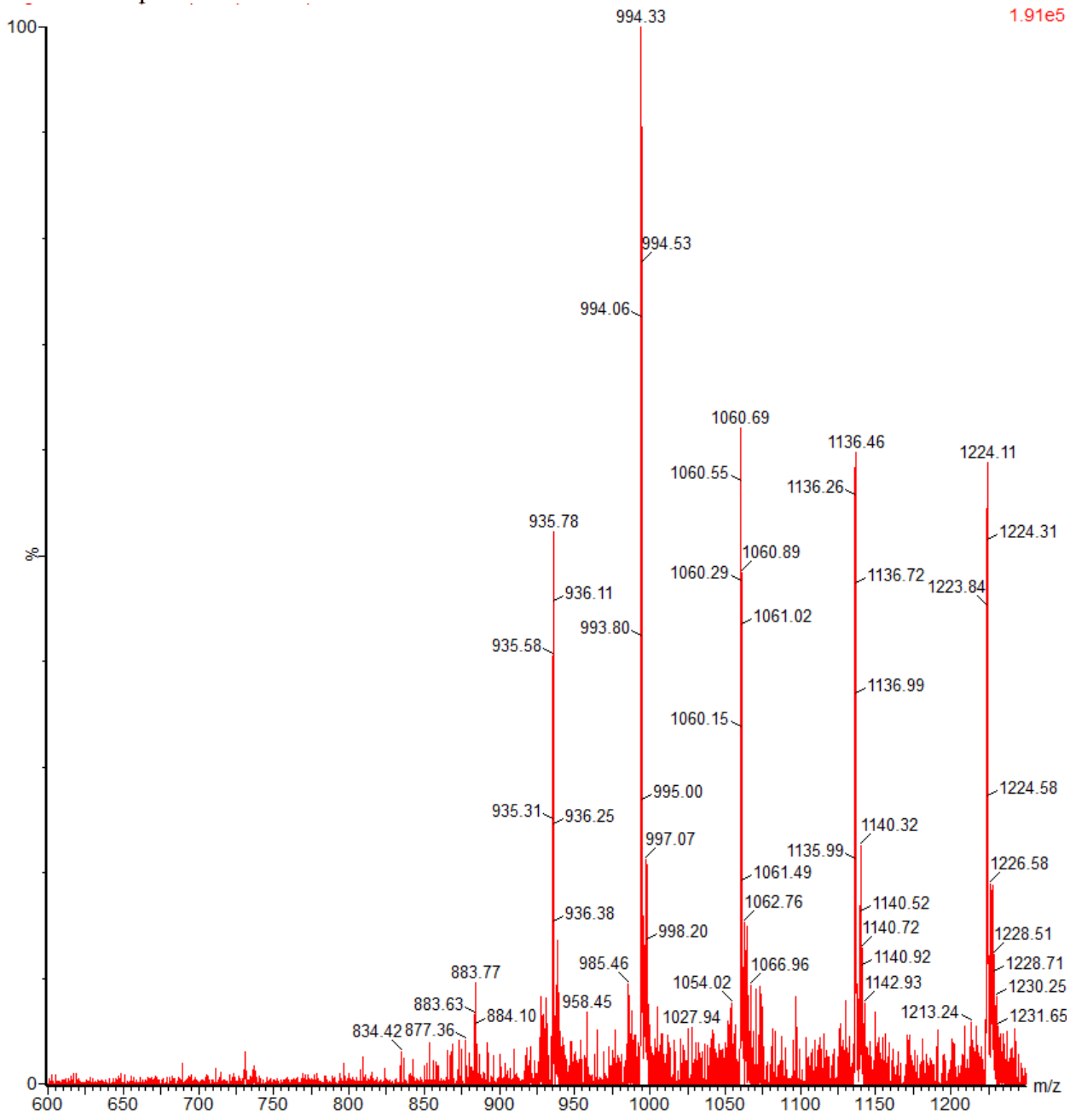
A260



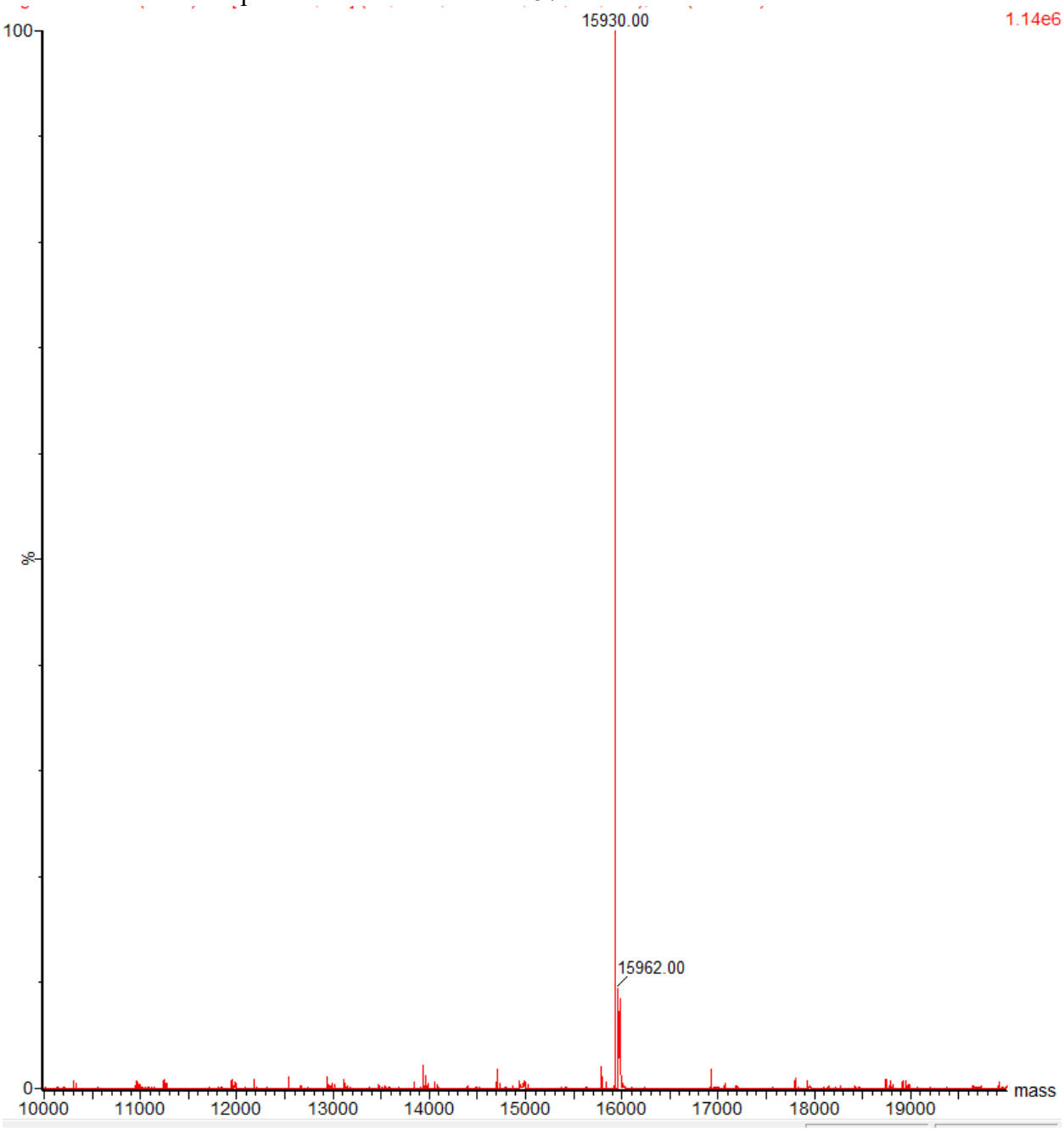
TIC



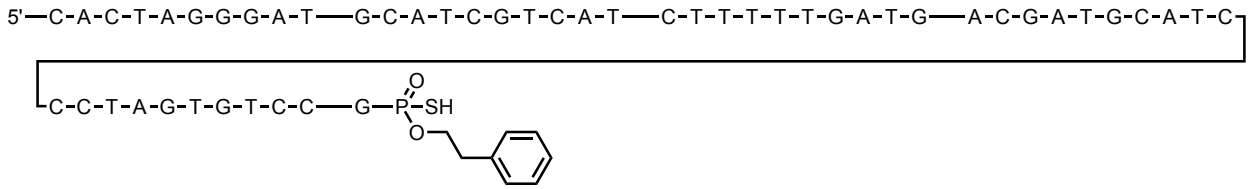
Raw Mass Spectrum Retention Time 2.57 minutes



Deconvoluted Mass Spectrum Retention Time 2.57 Minutes

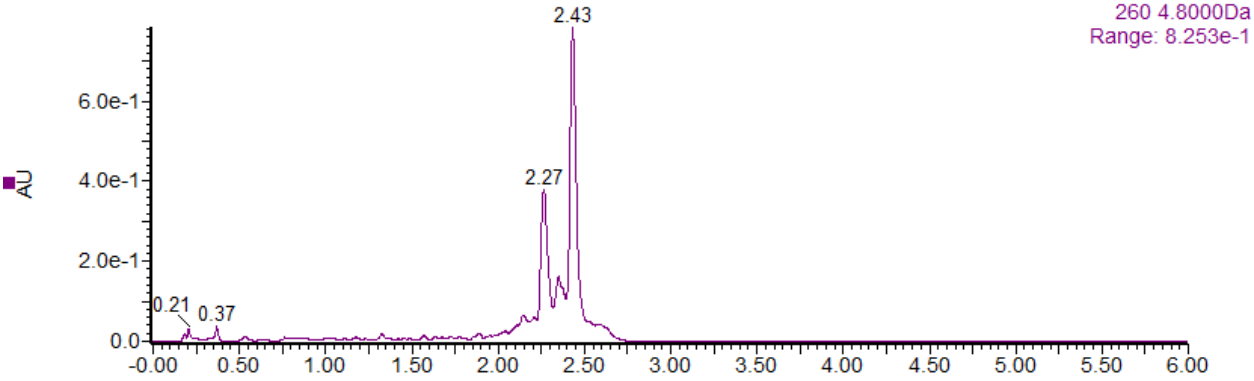


Entry 2:
LCMS Characterization of **SI-21**
Produced from **SI-17:**
Conversion (A260): 70%
Retention Time: 2.29 min
Expected Mass: 15927.31
Found Deconvoluted Mass: 15928

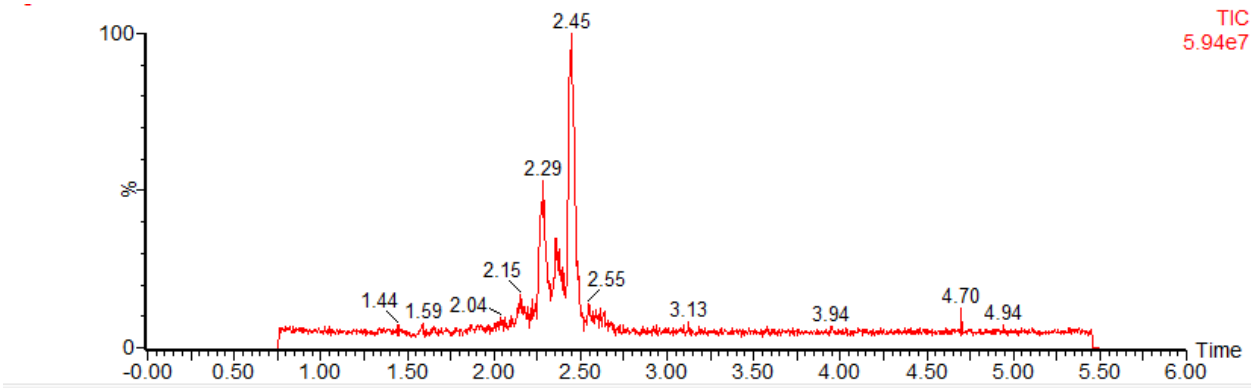


Molecular Weight: 15927.31

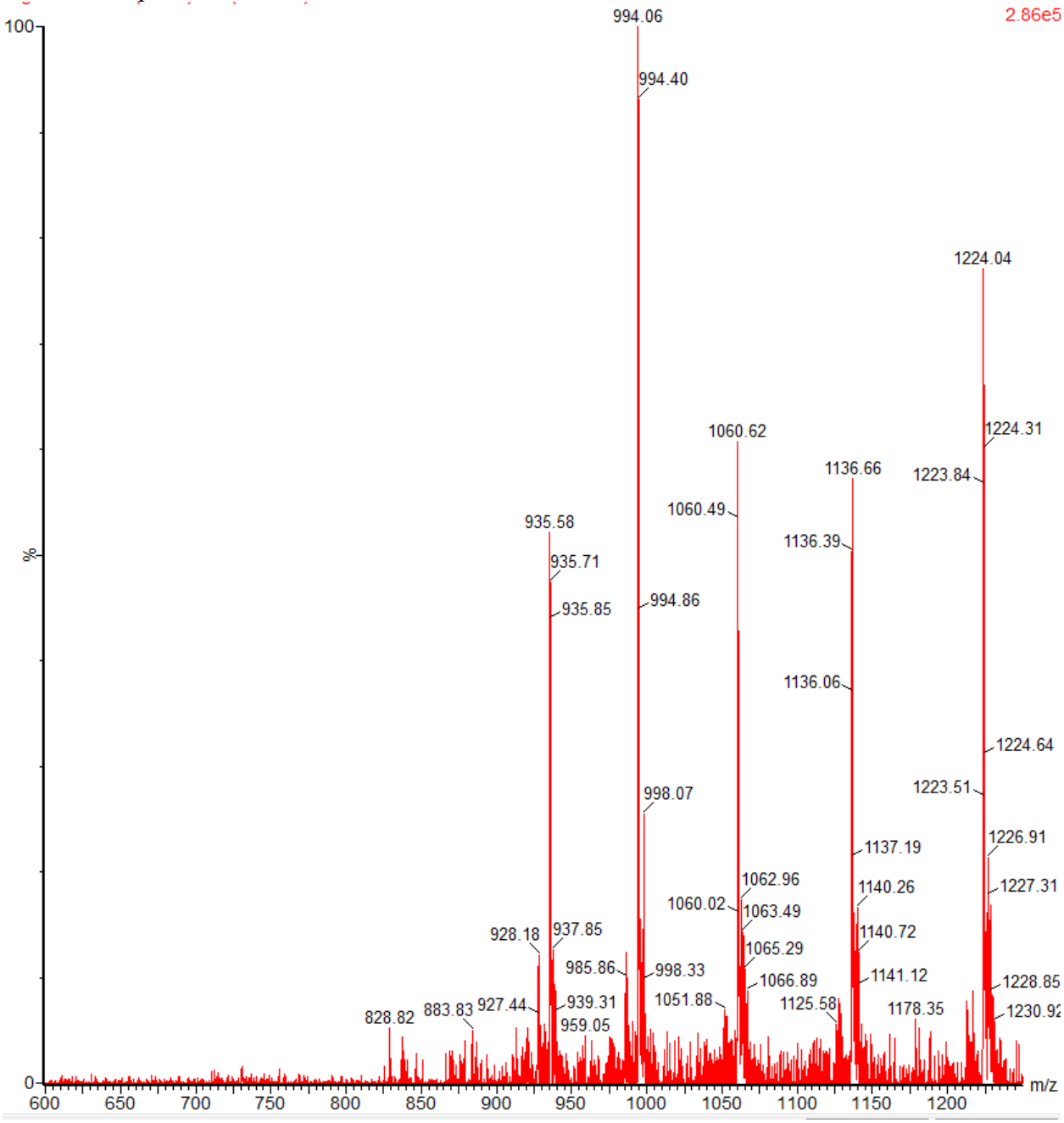
A260



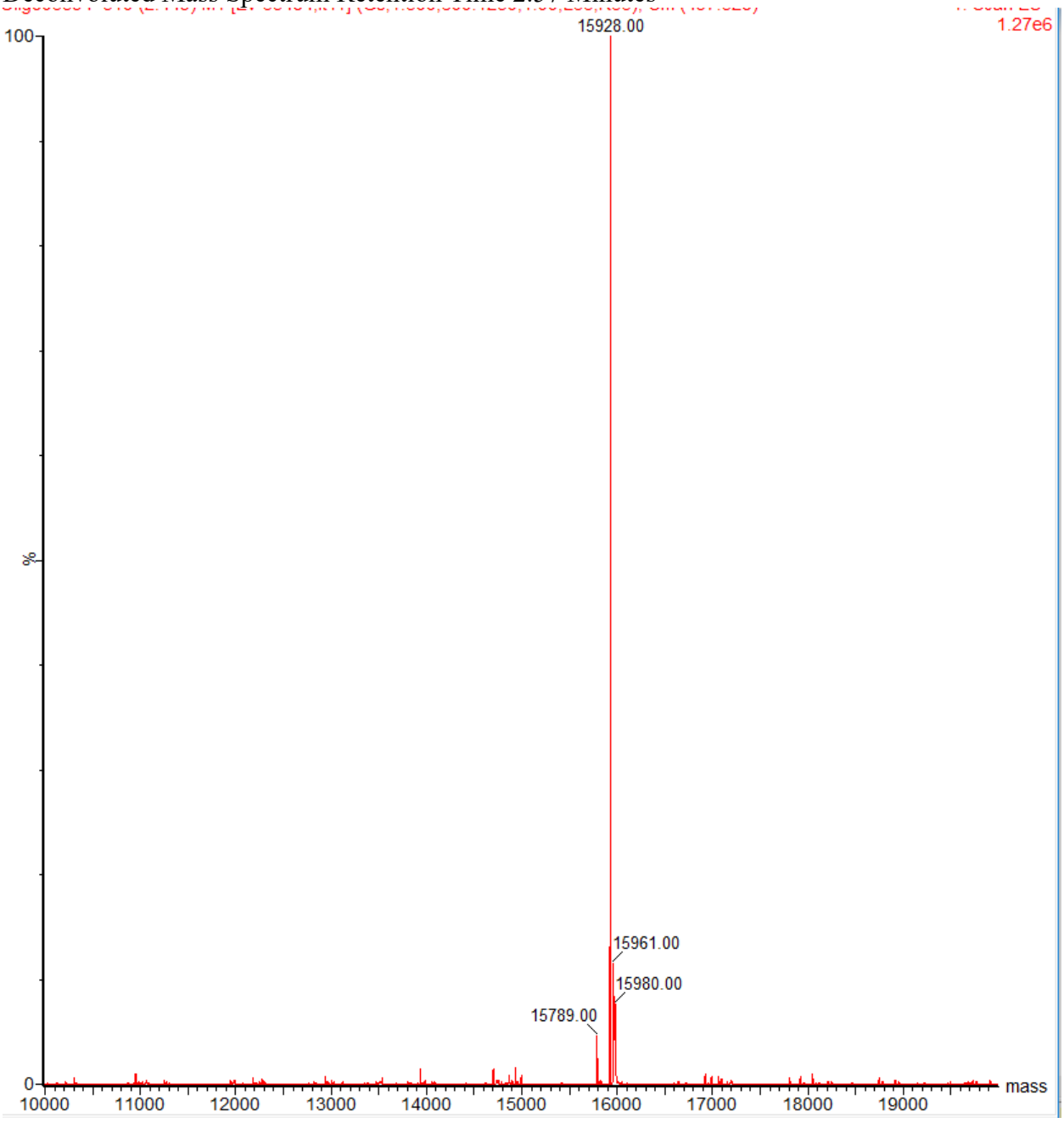
TIC



Raw Mass Spectrum Retention Time 2.57 minutes



Deconvoluted Mass Spectrum Retention Time 2.57 Minutes

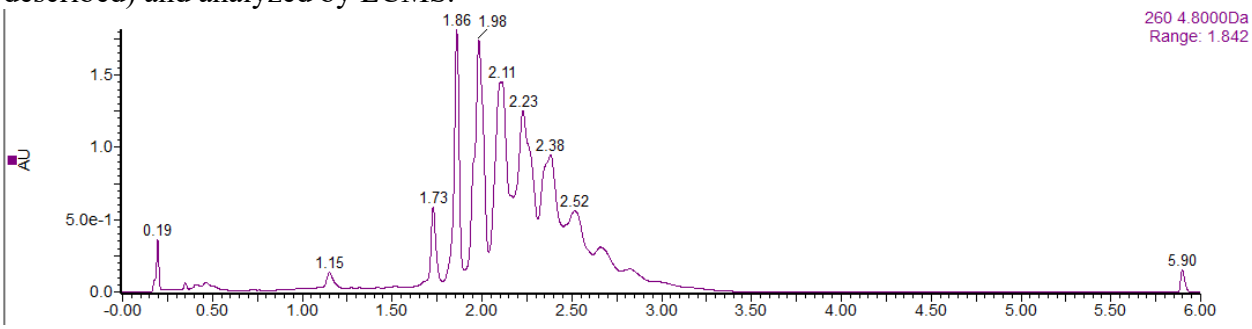


Alcohol Selective Reactions: An Investigation

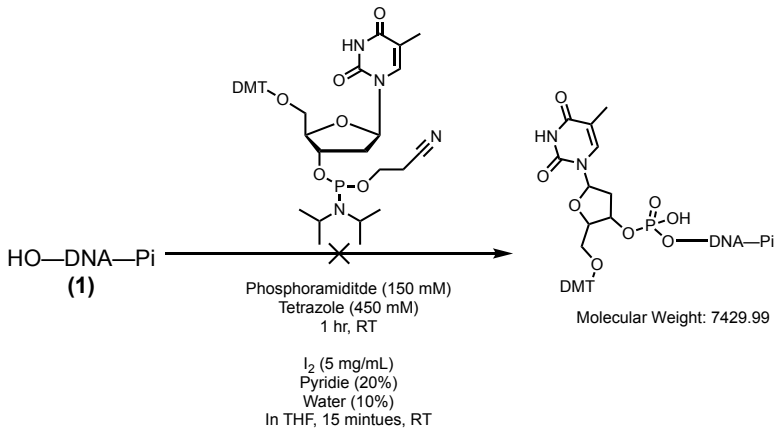
Phosphoramidate Formation

EDC (5 mM)
Phenethylamine (5 mM)
Imidazole (10 mM)
MES buffer (50 mM) pH 6.2
RT, 60 minutes

DNA **2** was dissolved (90 µM) in MES buffer (50 mM) at pH 6.2 containing phenethylaime (5 mM) and imidazole (20 mM). to this solution was added EDC (10 µL) from a stock solution in DMA (50 mM). This reaction was quickly vortexed and allowed to incubate at room temperature for 60 minutes. After 60 minutes the DNA was isolated by ethanol precipitation (previously described) and analyzed by LCMS.

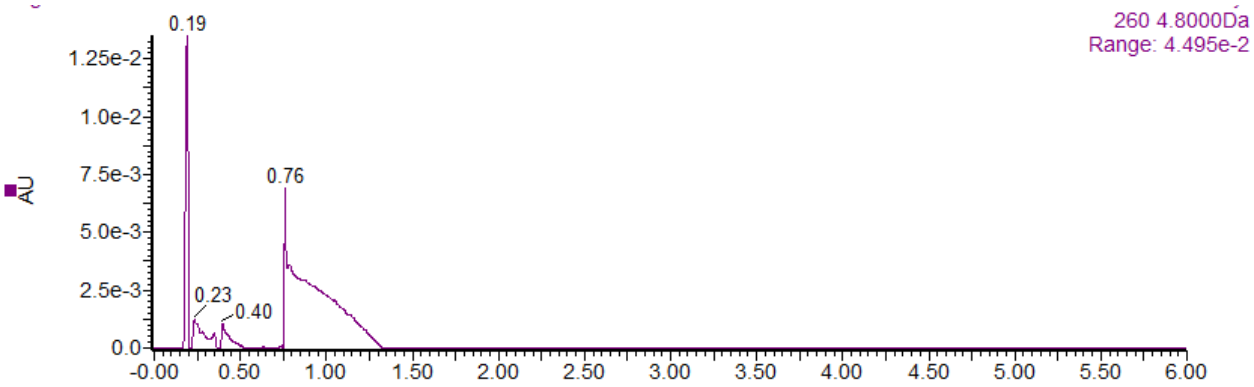


Phosphoramidite Reaction

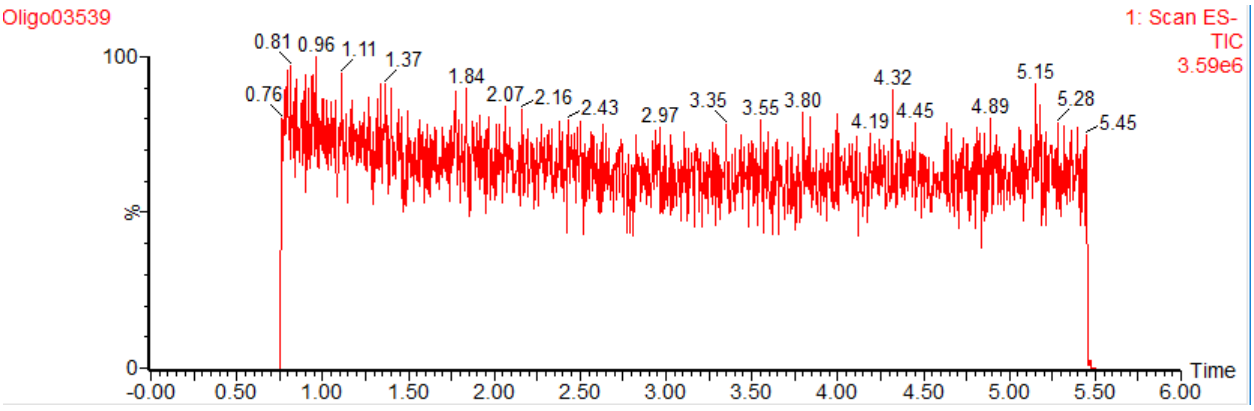


DNA **1** was loaded onto the support according to **General Procedure 2** and dried accordingly. Phorphoramitide (150 mM) and tetrazole (450 mM) were added in dry MeCN and the reaction was allowed to incubate at room temperature for 60 minutes. After 60 minutes the resi was washed once with dry MeCN (500 µL) and oxidation solution was added (iodine, pyridine, water in THF). This was reaction incubated for 15 minutes and then the resin bed washed with MeCN and PBS. The DNA was eluted and precipitated as previously described and finally analyzed by LCMS.

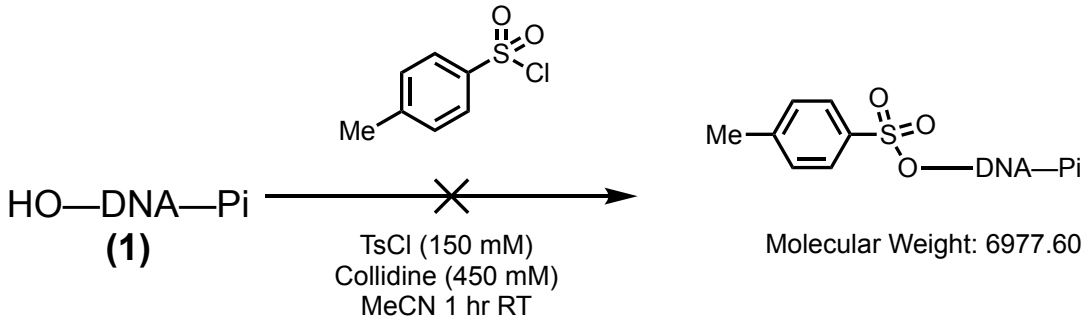
A260



TIC

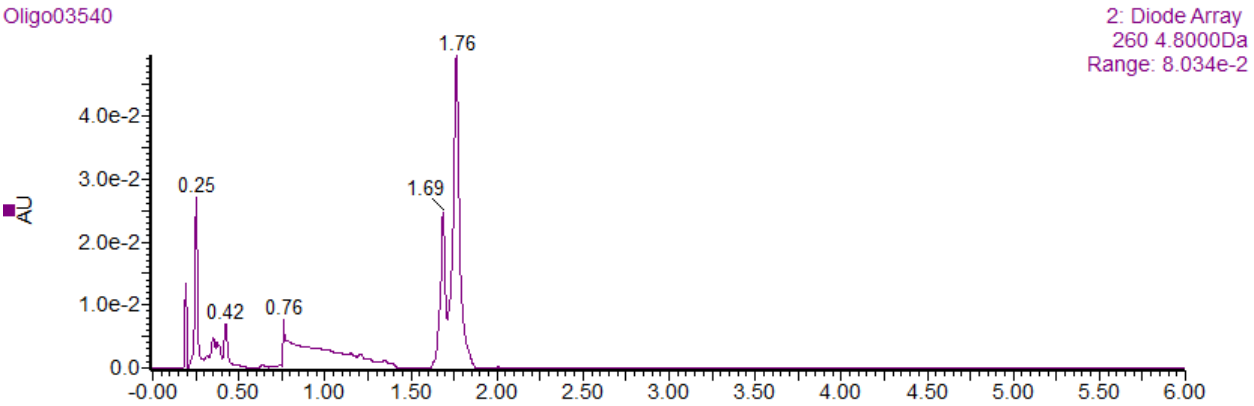


Tosylation reaction

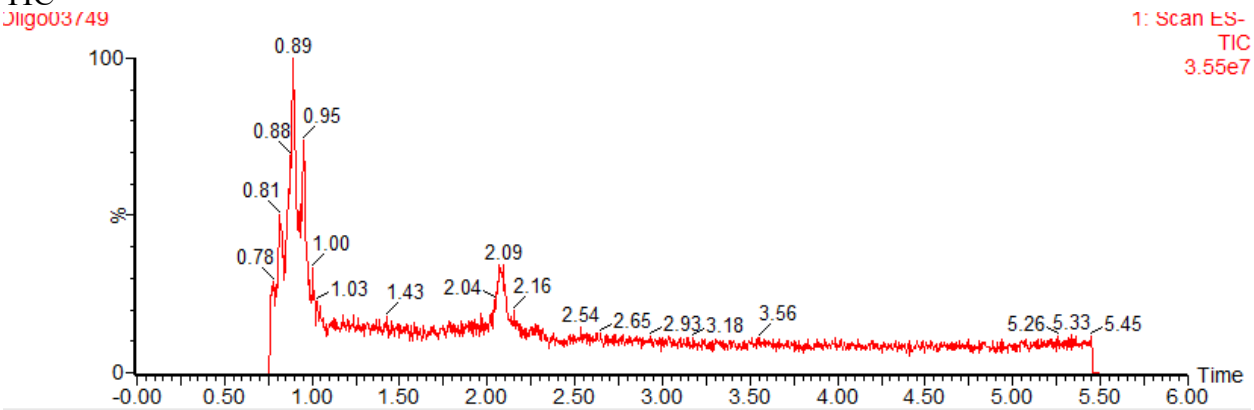


DNA **1** was loaded onto the support according to **General Procedure 2** and dried accordingly. Tosyl Chloride (150 mM) and Collinide (450 mM) were added in dry MeCN and the reaction was allowed to incubate at room temperature for 60 minutes. After 60 minutes the resin was washed once with dry MeCN (500 µL). The DNA was eluted and precipitated as previously described and finally analyzed by LCMS.

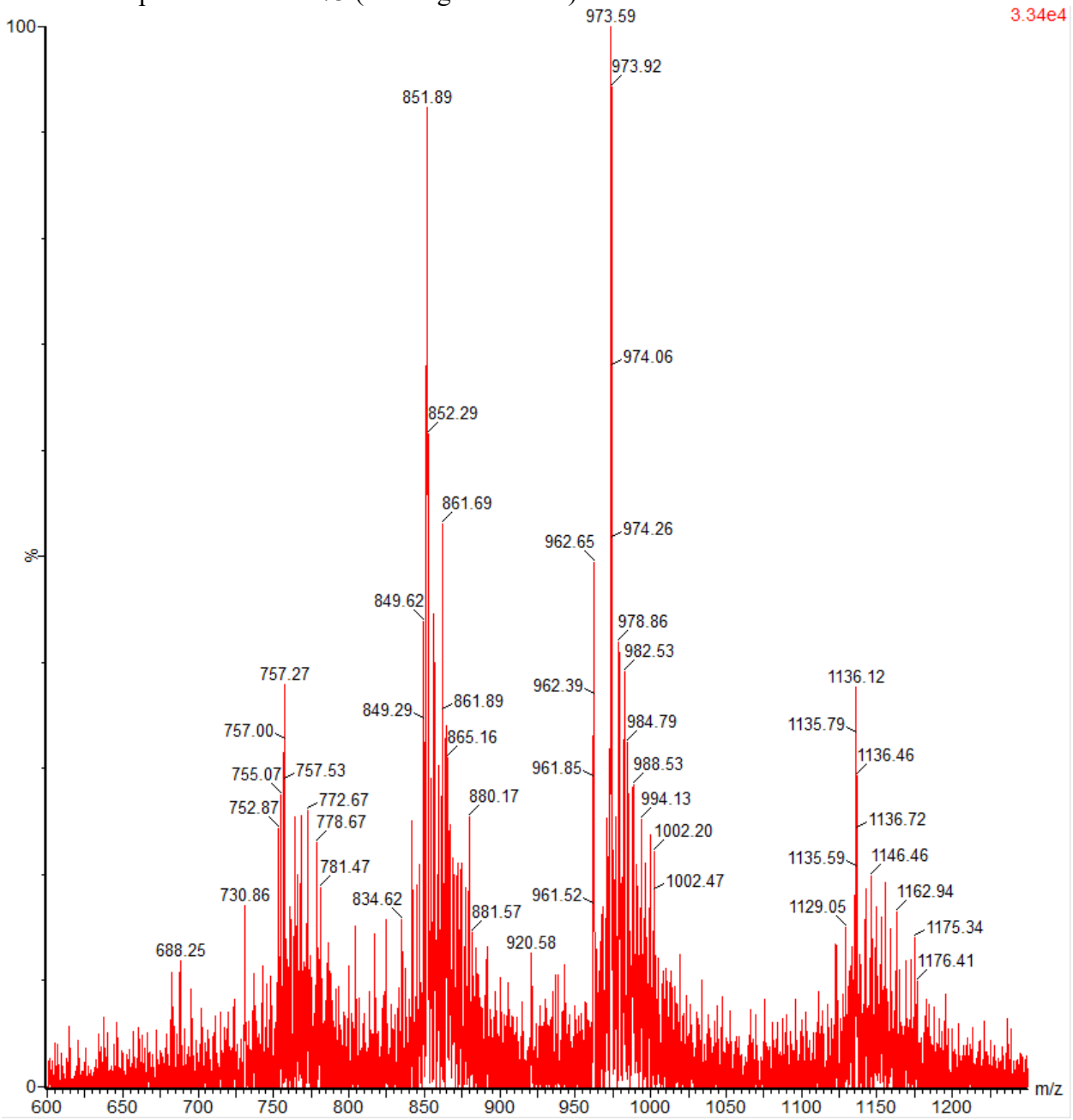
A260



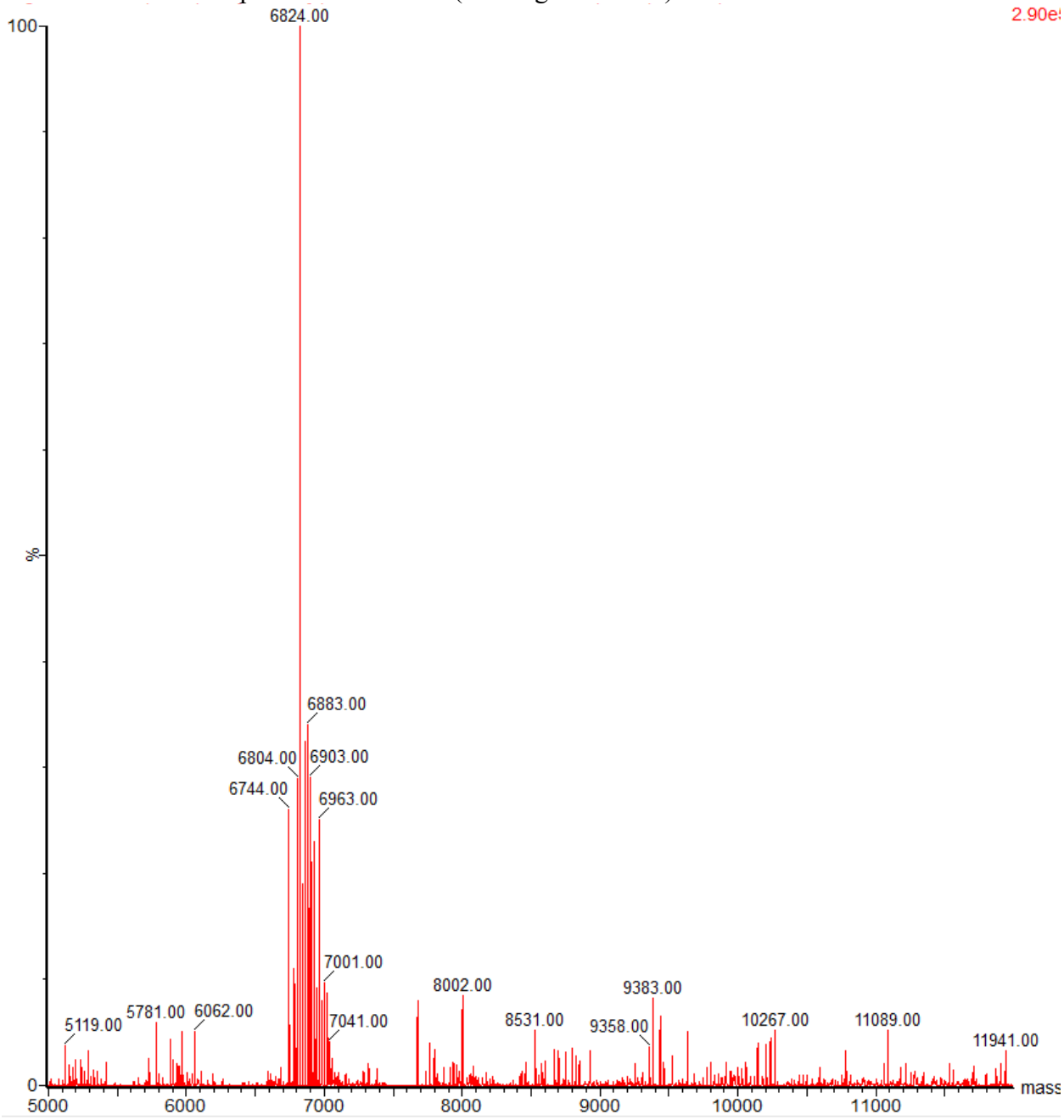
TIC



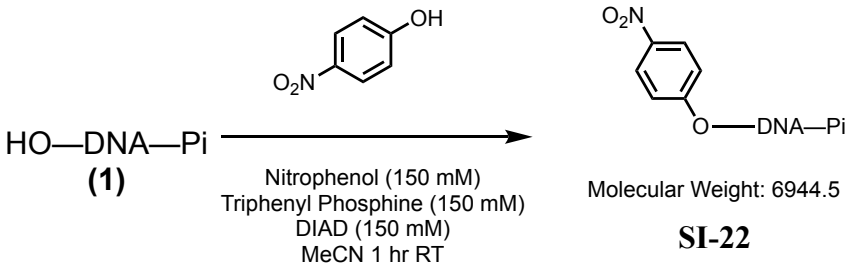
Raw Mass Spectrum RT = 1.73 (Starting material **1**)



Deconvoluted Mass Spectrum RT = 1.73 (Starting material **1**)



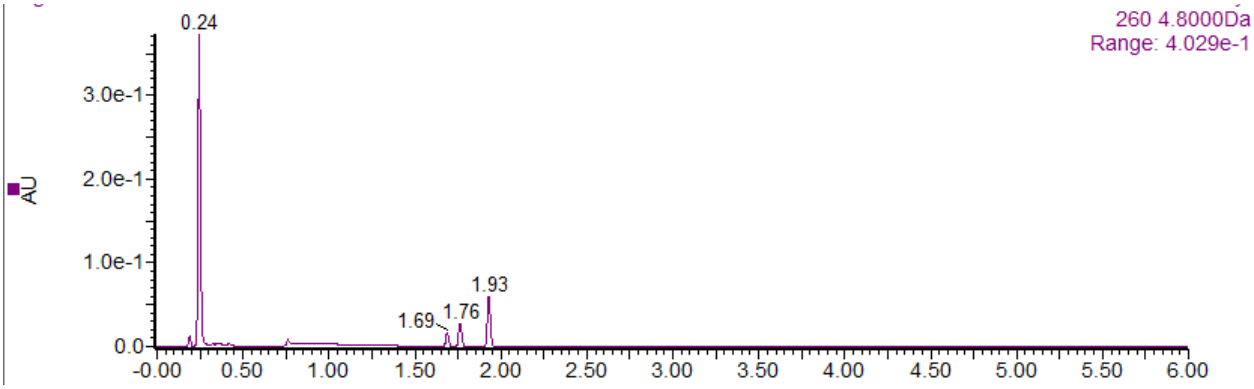
Mitsunobu Reaction



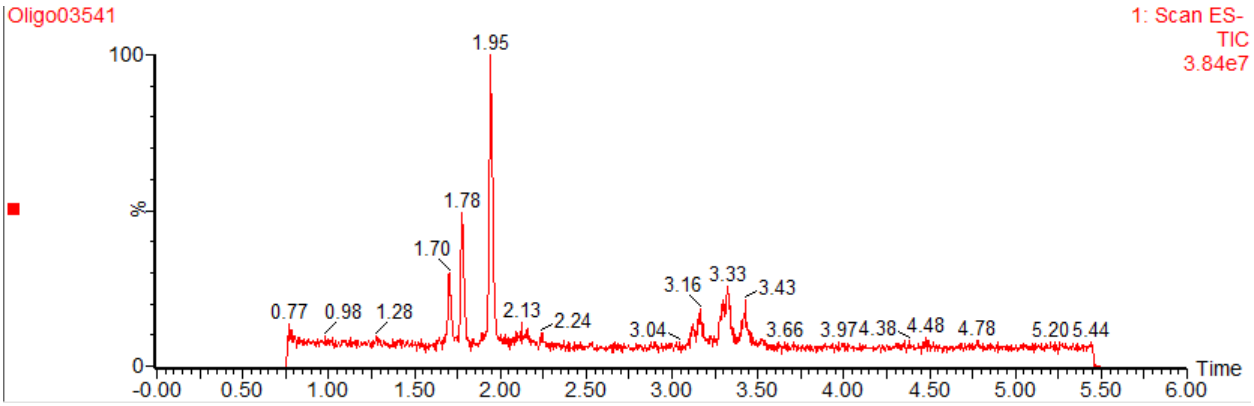
SI-22

DNA **1** was loaded onto the support according to **General Procedure 2** and dried accordingly. Nirtopheol (150 mM) and Triphenylphosphine (150 mM) were added in dry MeCN. DIAD (150 mM) was then spiked in and the reaction was allowed to incubate at room temperature for 60 minutes. After 60 minutes the resin was washed once with dry MeCN (500 µL). The DNA was eluted and precipitated as previously described and finally analyzed by LCMS. Low DNA recovery was observed by UV Vis absorbance (nanodrop).

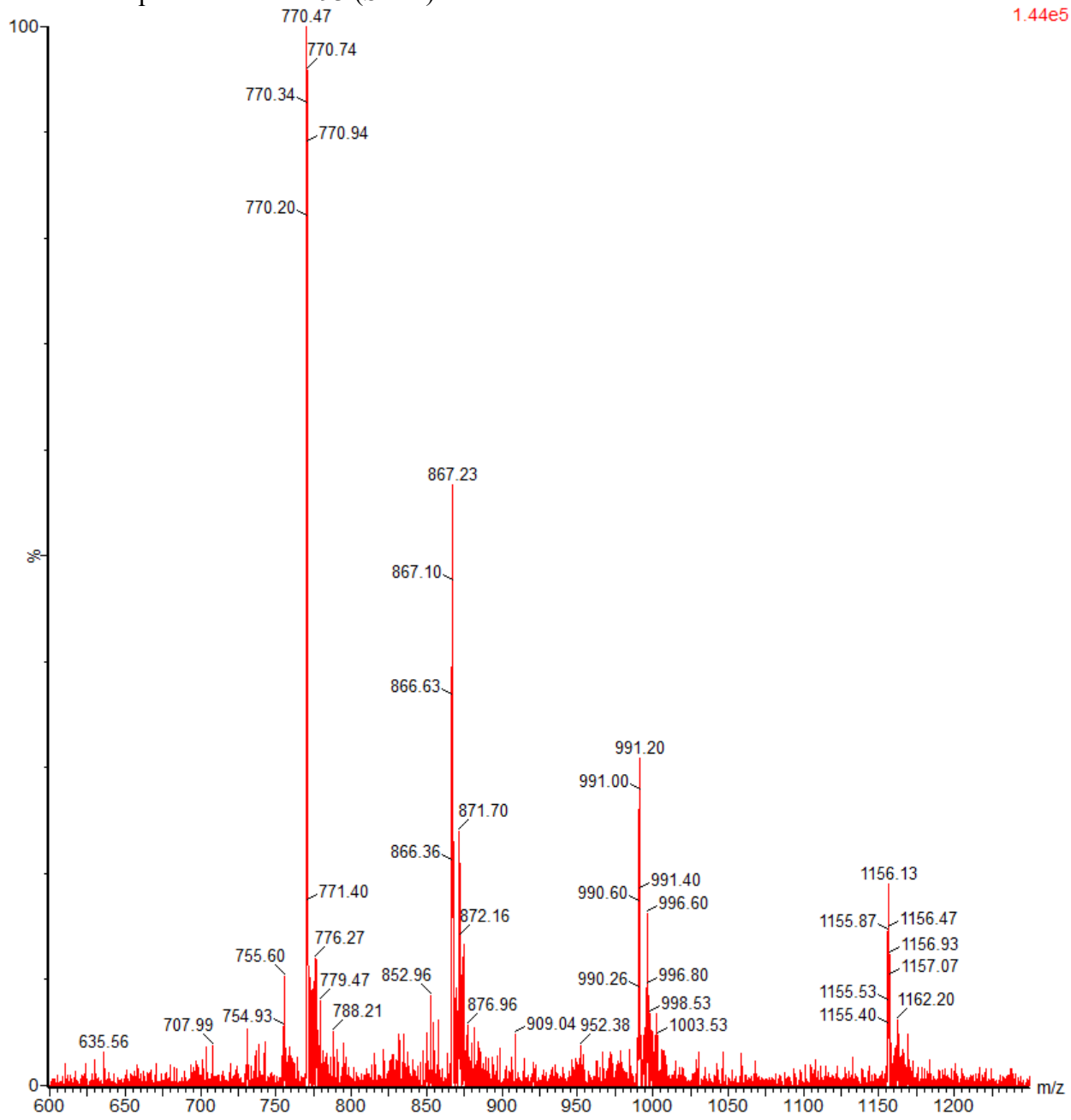
A260



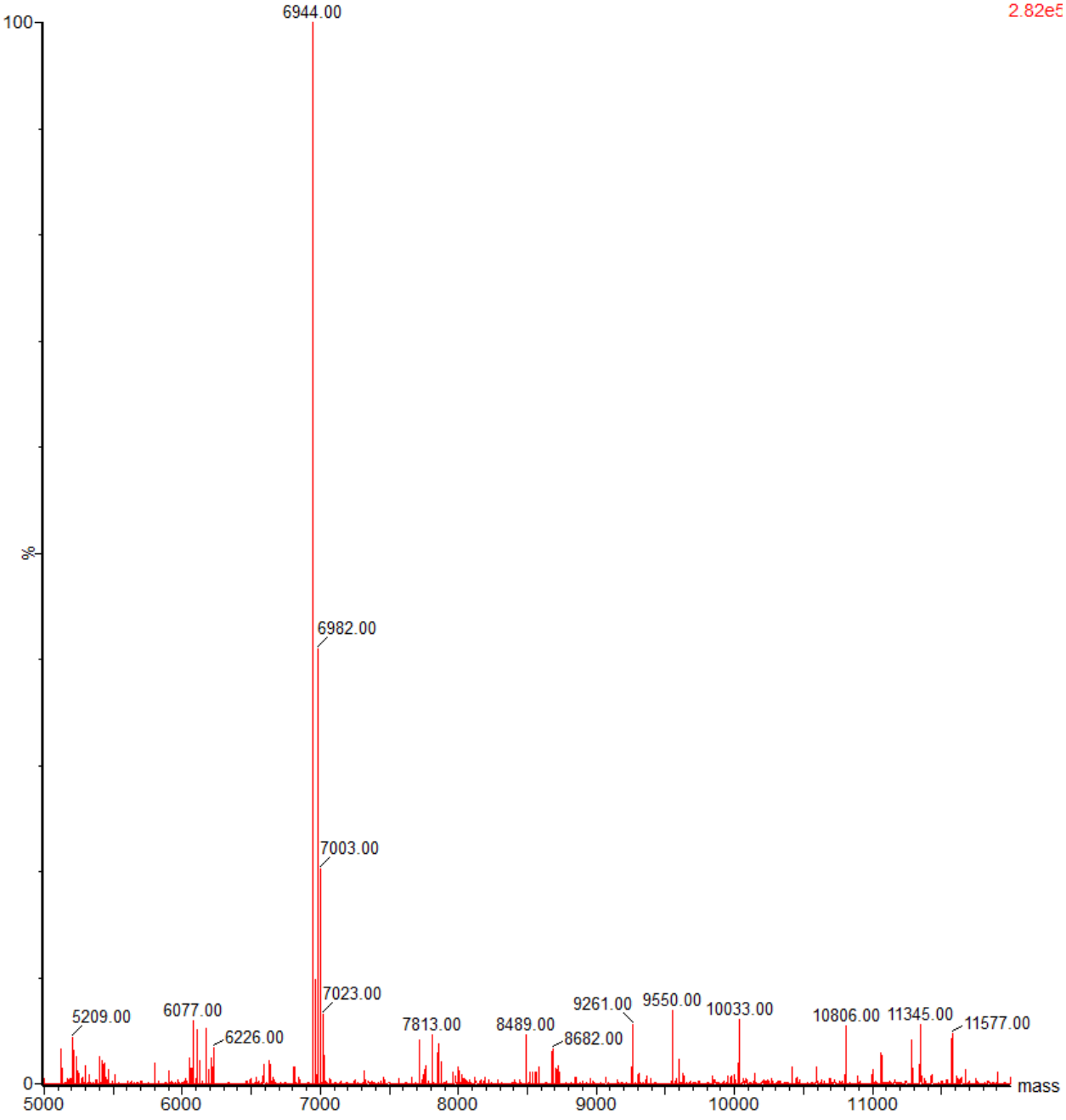
TIC



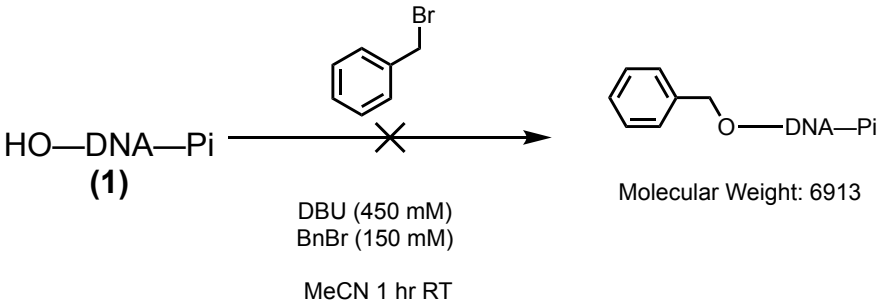
Raw Mass Spectrum RT = 1.93 (**SI-22**)



Deconvoluted Mass Spectrum RT = 1.93 (**SI-22**)

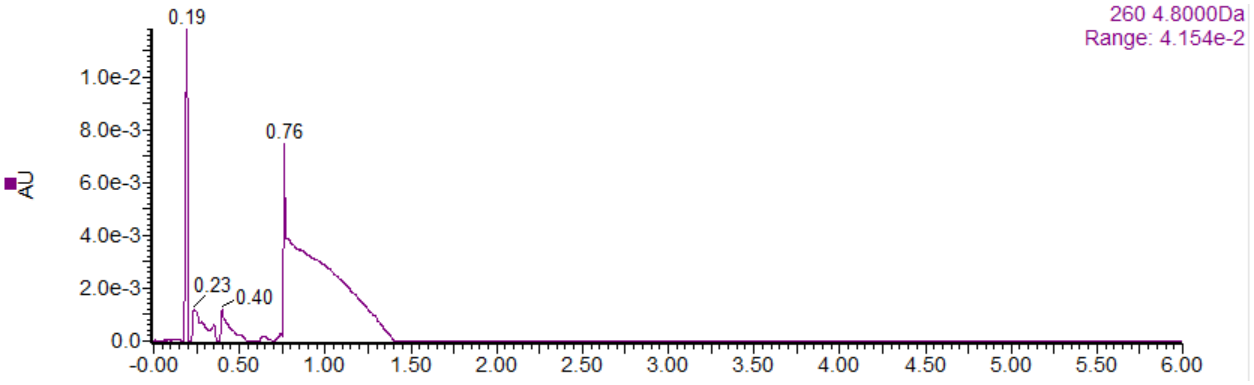


Williamson Ether Synthesis

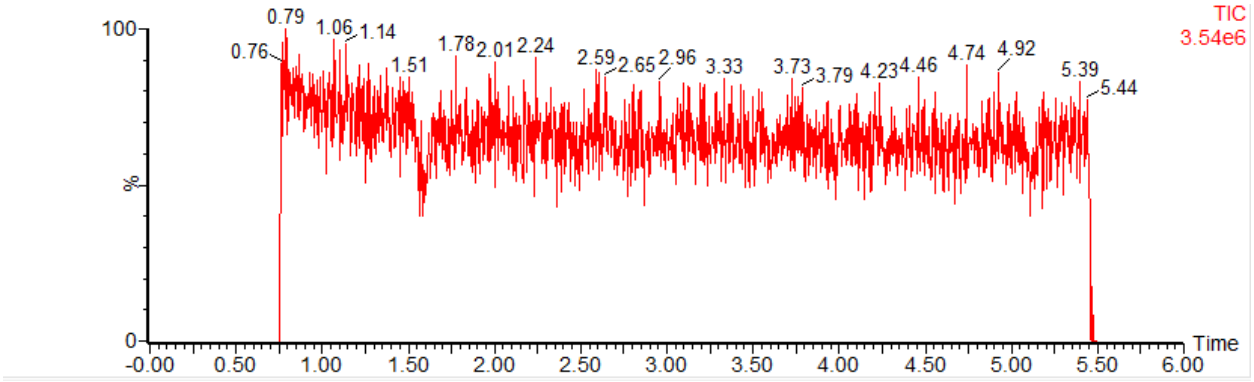


DNA **1** was loaded onto the support according to **General Procedure 2** and dried accordingly. DBU (450 mM) was added in dry MeCN and this was added to the resin from 5 minutes. after 5 minutes benzyl bromide (150 mM) was then spiked in and the reaction was allowed to incubate at room temperature for 60 minutes. After 60 minutes the resin was washed once with dry MeCN (500 µL). The DNA was eluted and precipitated as previously described and finally analyzed by LCMS.

A260



TIC



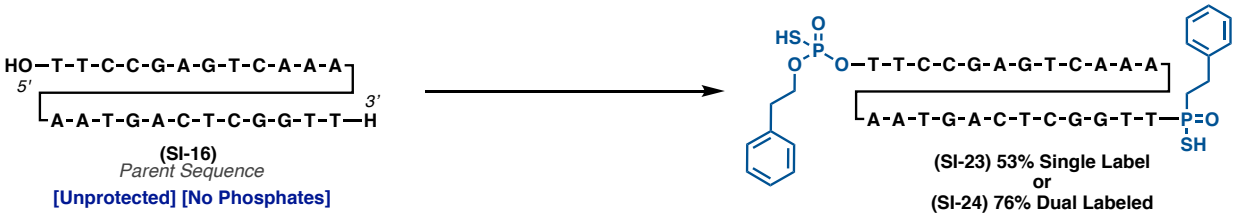
Labeling of DNA Without Phosphate Blocking Groups

General Protocol and Optimization

SENDR on non-phosphate DNA (**SI-16)** was optimized by performing reactions in a microcentrifuge tube as described in *General Procedure 2* with various reaction conditions (as described in the main text and Figure 2B). Briefly, 5 nmol of DNA **(SI-16)** was loaded onto 100 μL of equilibrated resin. This loaded resin was washed with DMA (500 μL twice) and dry THF (500 μL three times) and dried under vacuum. In the case in which stringent drying was not performed (Figure 2B, Entry 8) the loaded resin was simply washed with acetonitrile (500 μL three times).

Ψ-1 in MeCN (varying concentration, 250 μL) was added to the loaded and dried resin. Then DBU (various concentration) was added to the reaction mixture. The reaction tube was vortexed for 30 seconds and incubated at room temperature for various amounts of time. The reactions were worked up by aspirating and discarding the reaction solution, and washing the resin bed with MeCN (500 μL) and 1:1 MeCN:PBS (500 μL). Elute buffer (300 μL) was added to the resin bed and the tube was agitated by orbital shaker for 5-10 minutes. The DNA containing elution buffer was collected by carefully pipetting the supernatant. DNA was isolate *via* ethanol precipitation. Ethanol precipitation was performed by adding 10% v/v of a NaCl solution (30 μL, 5M) to the elute buffer and three volumes of cold ethanol (-20 ºC) were added to the tube (~1000μL) and incubated for 18 hours at -20 ºC. The tube was then centrifuged at 13,000 rpm for 15 minutes to pellet the DNA, and the supernatant ethanol was decanted off. The tubes were dried *via* speed vacuum, the DNA was dissolved and HPLC-MS analysis was performed.

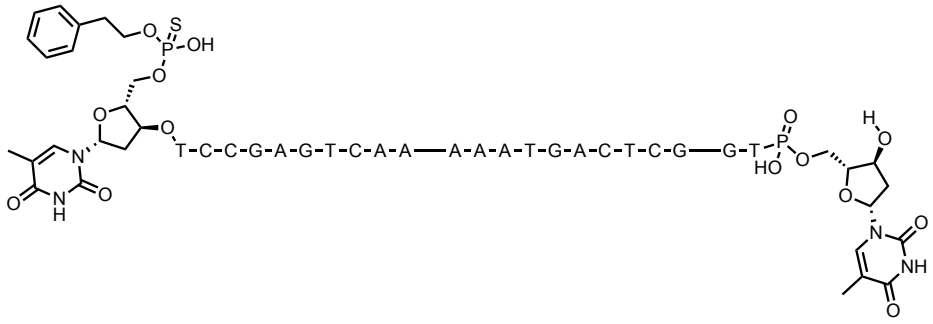
A. Selective Labeling of Non-Phosphorylated DNA



B. SENDR: Optimization of the Coupling Step

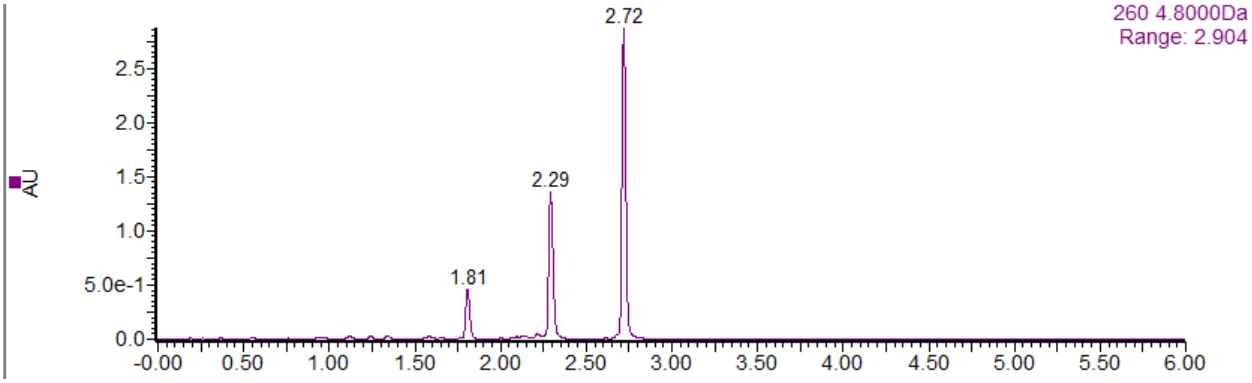
Entry	Conditions	(SI-23) Single Label (%)	(SI-24) Dual Label (%)
1	150 mM Ψ-1, 450 mM DBU, 60 min	30	61
2	150 mM Ψ-1, 450 mM DBU, 30 min	33	51
3	150 mM Ψ-1, 450 mM DBU, 60 min, 37ºC	24	76
4	75 mM Ψ-1, 225 mM DBU, 60 min	53	<5
5	75 mM Ψ-1, 225 mM DBU, 30 min x 2	53	21
6	75 mM Ψ-1, 225 mM DBU, 60 min x 2	50	25
7	75 mM Ψ-1, 225 mM DBU, 120 min	48	17

Entry 1
LCMS Characterization of **Single Label SI-23**
Produced from **SI-16** by General Procedure **3:**
Conversion (A260): 30%
Retention Time: 2.29 min
Expected Mass: 7552.02
Expected (M-8)/8 Ion: 943.0
Found Deconvoluted Mass: 7552.00
Found (M-6)/6 Ion: 942.78

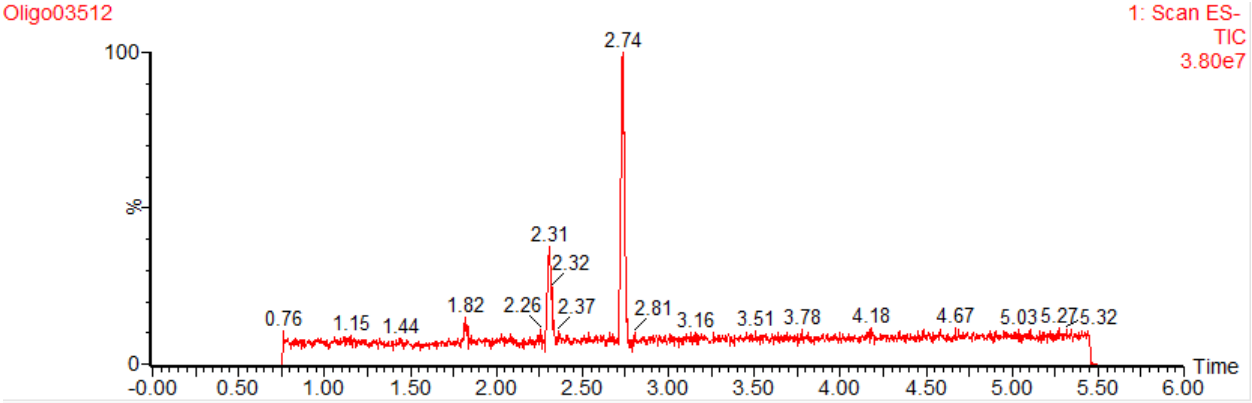


Molecular Weight: 7552.02

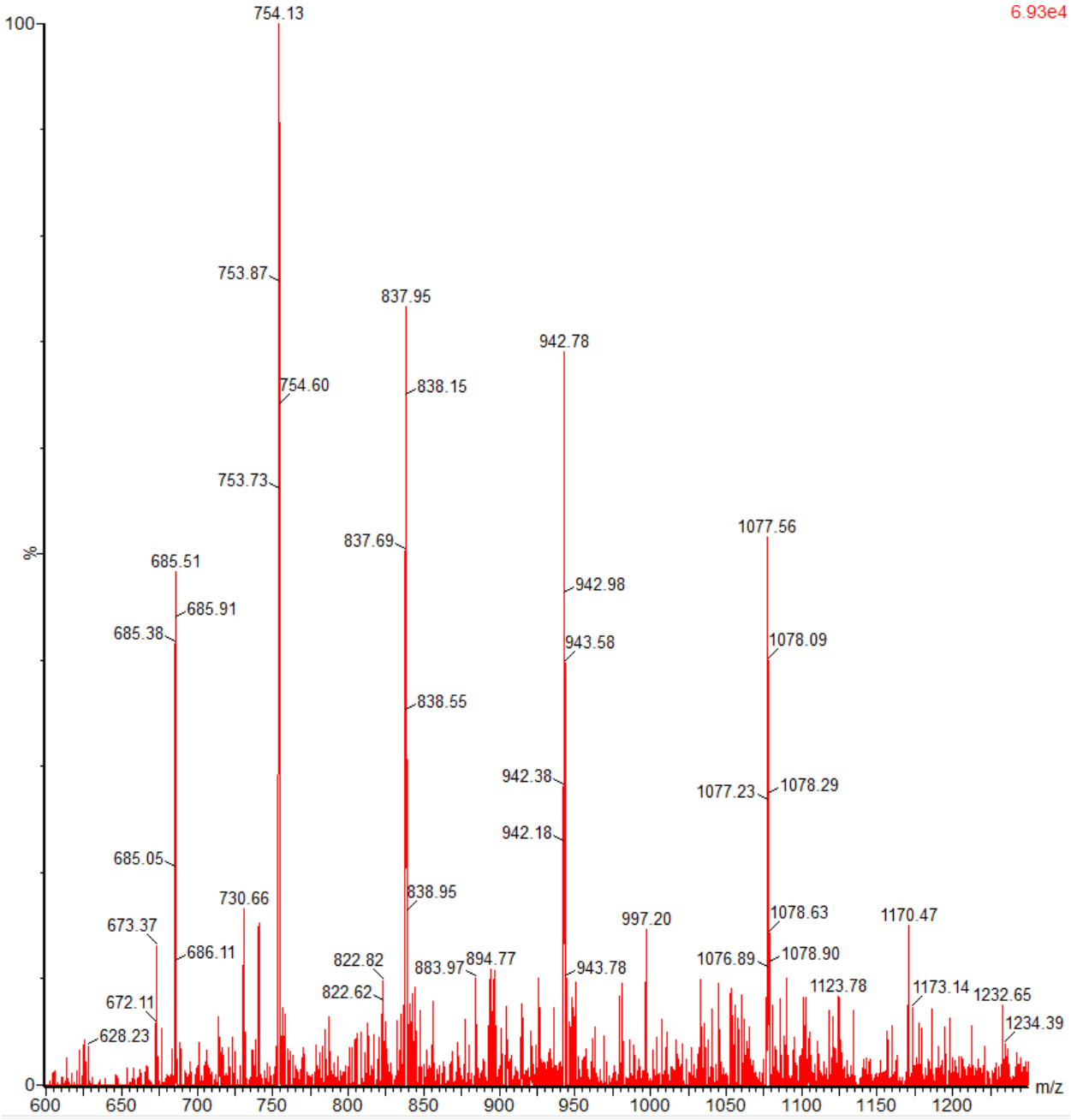
A260



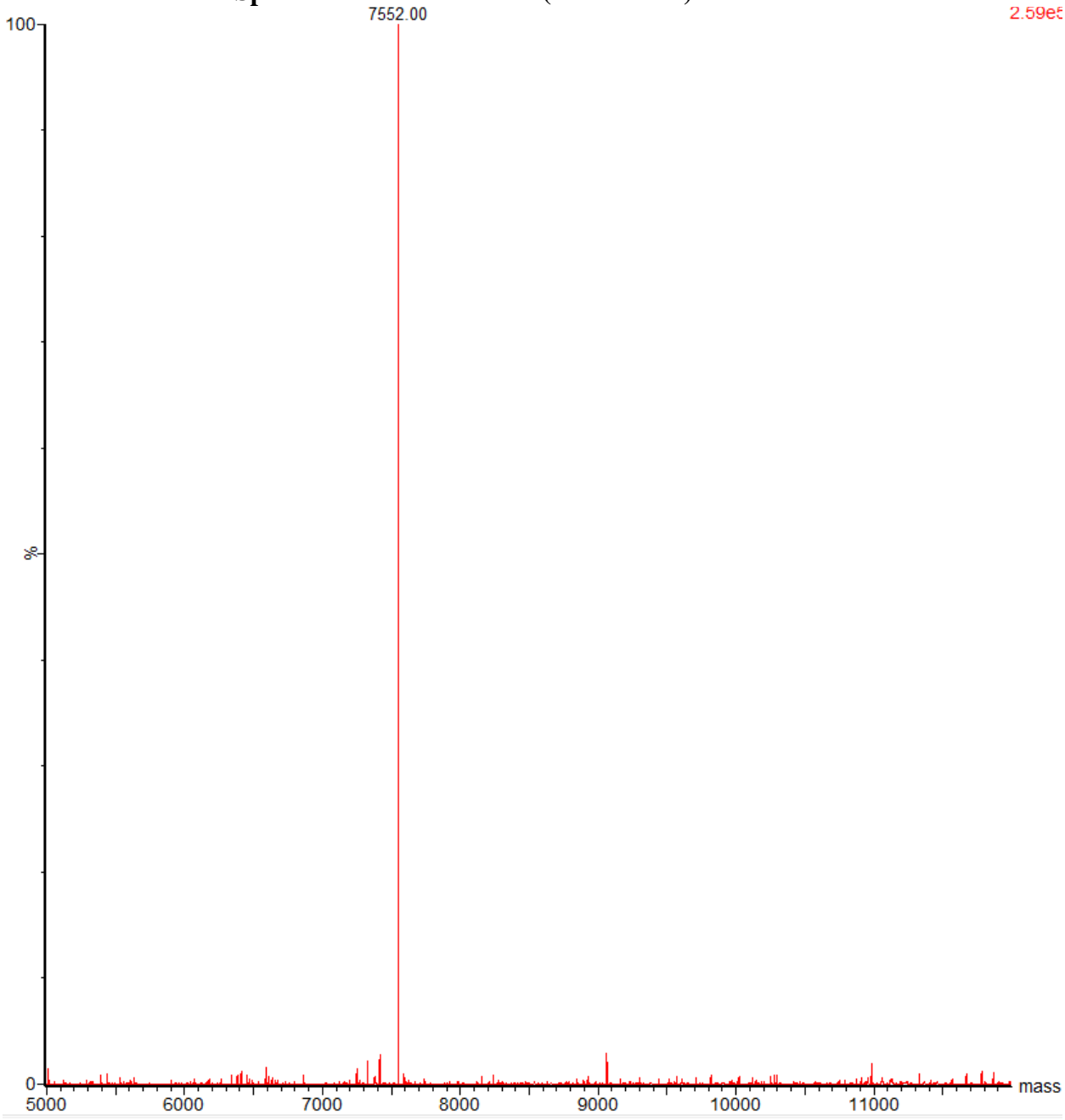
TIC



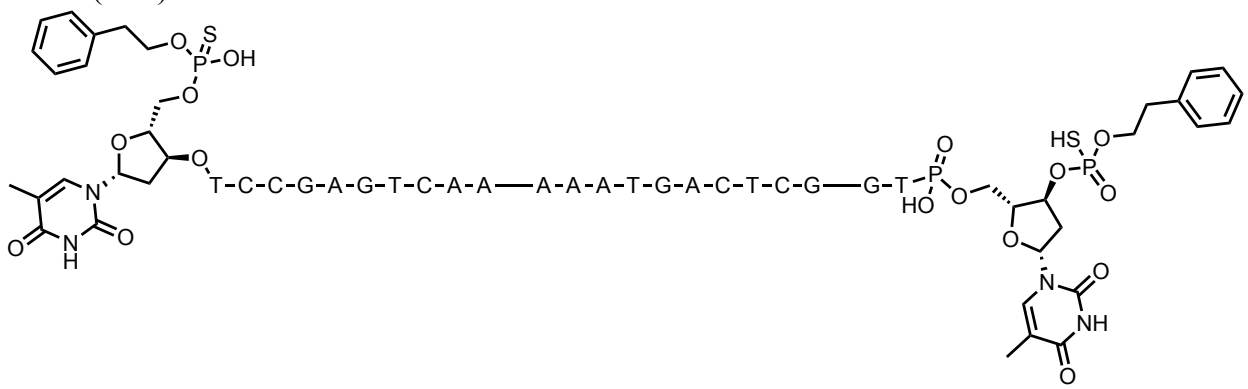
Raw Mass Spectrum Retention Time (2.3 minutes)



Deconvoluted Mass Spectrum Retention Time (2.3 minutes)

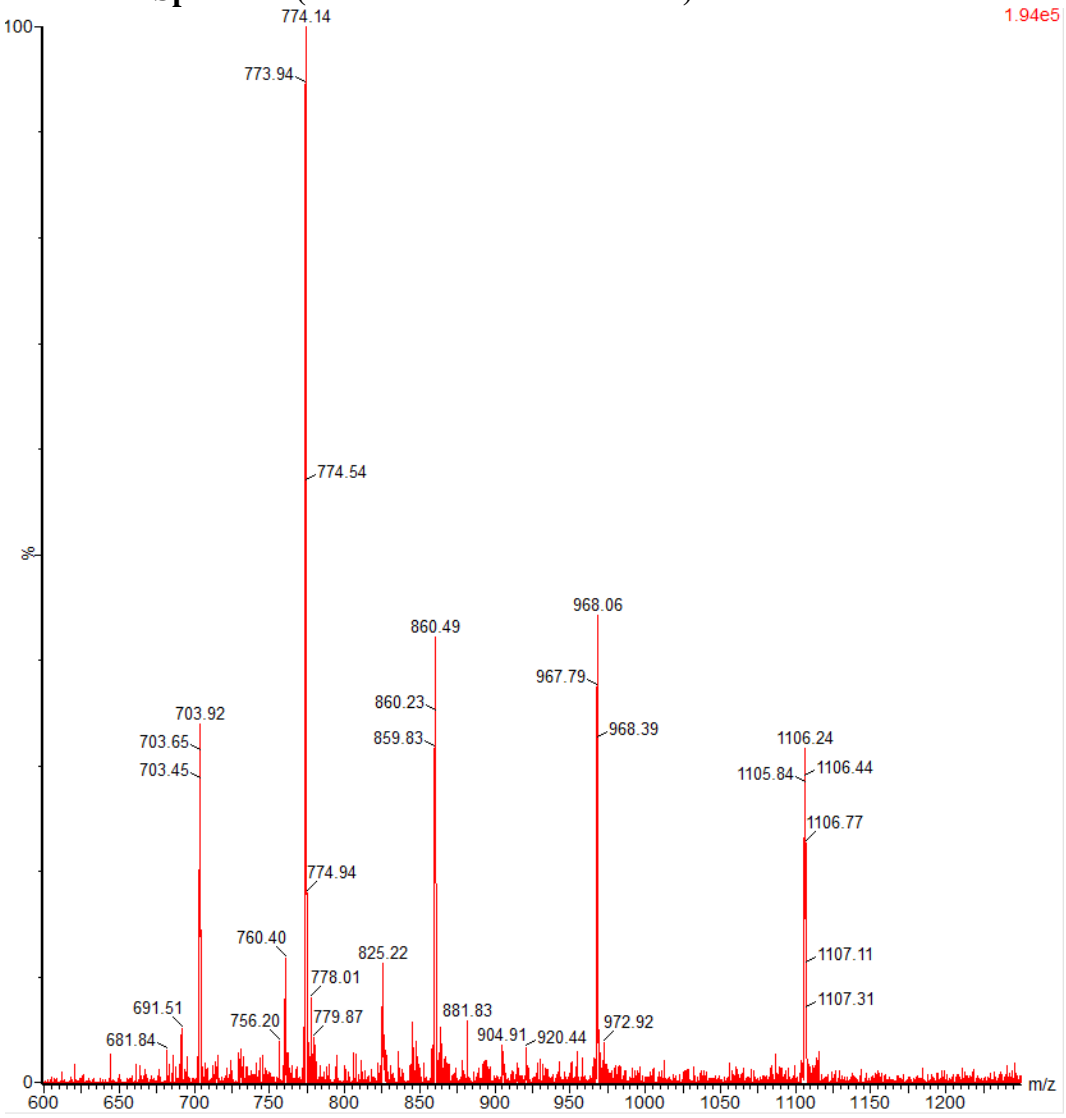


LCMS Characterization of **Double Label SI-24**
Produced from **SI-16** by General Procedure **3:**
Conversion (A260): 61%
Retention Time: 2.72 min
Expected Mass: 7752.2
Expected (M-8)/8 Ion: 968.02
Found Deconvoluted Mass: 7552.00
Found (M-6)/6 Ion: 968.06

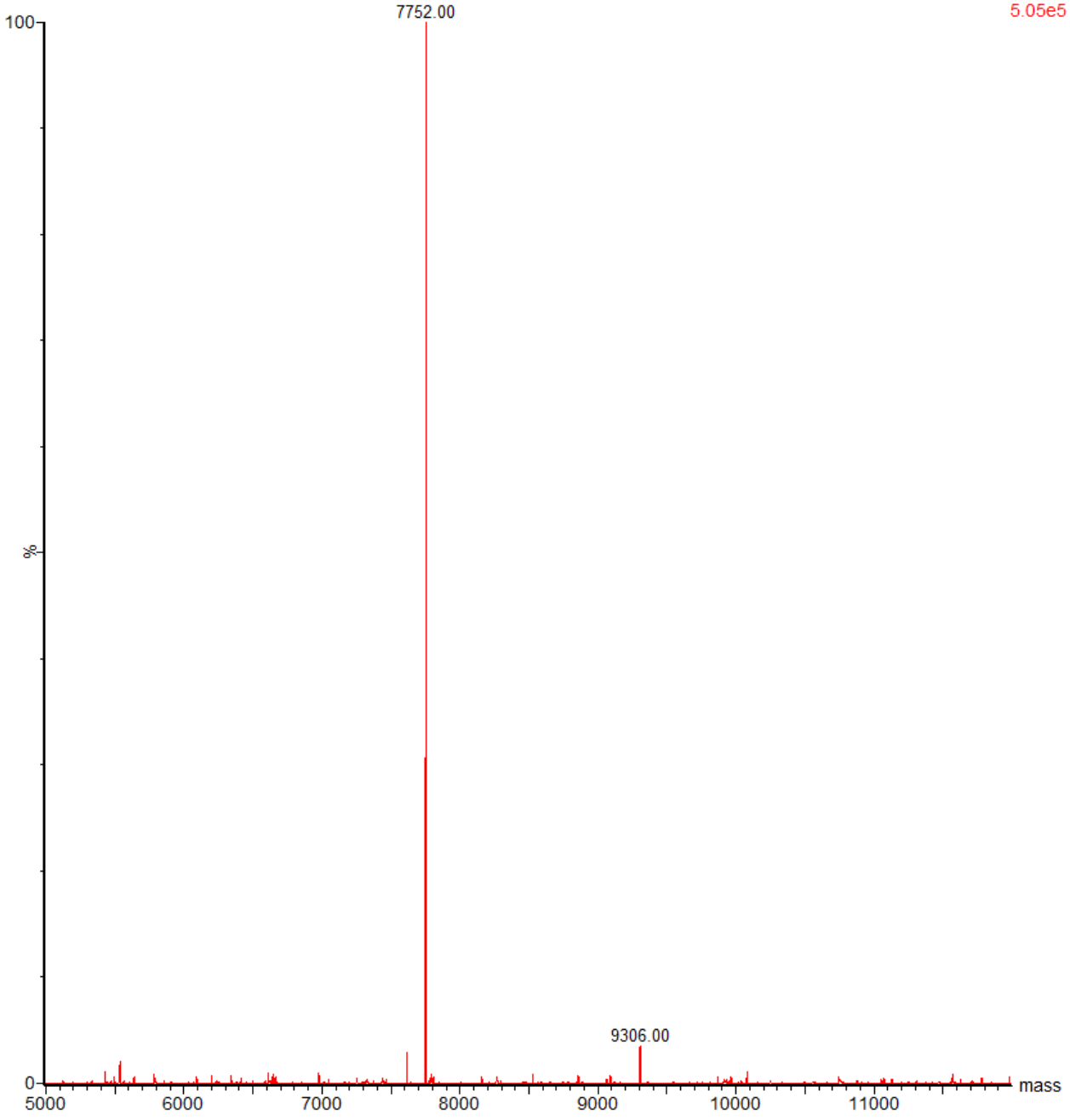


Molecular Weight: 7752.21

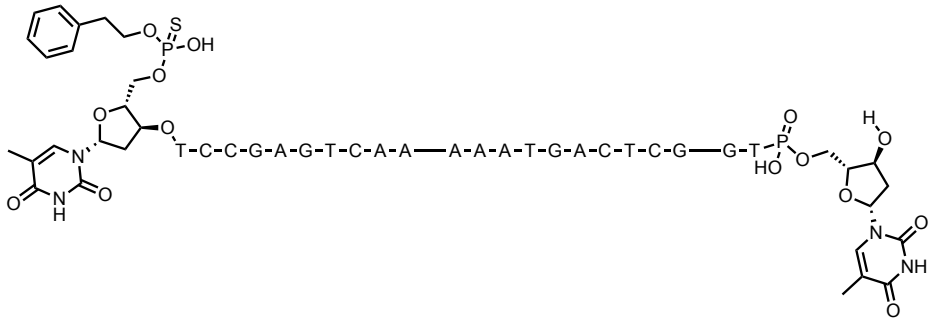
Raw Mass Spectrum (Retention Time 2.7 minutes)



Deconvoluted Mass Spectrum (Retention Time 2.7 minutes)

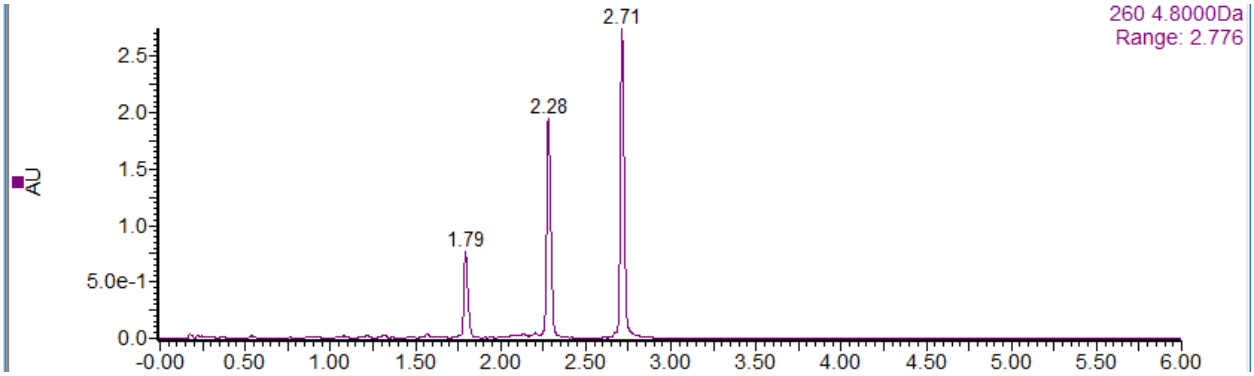


Entry 2
LCMS Characterization of **Single Label SI-23**
Produced from **SI-16** by General Procedure **3:**
Conversion (A260): 33%
Retention Time: 2.29 min
Expected Mass: 7552.02
Expected (M-8)/8 Ion: 943.0
Found Deconvoluted Mass: 7551.00
Found (M-6)/6 Ion: 942.92

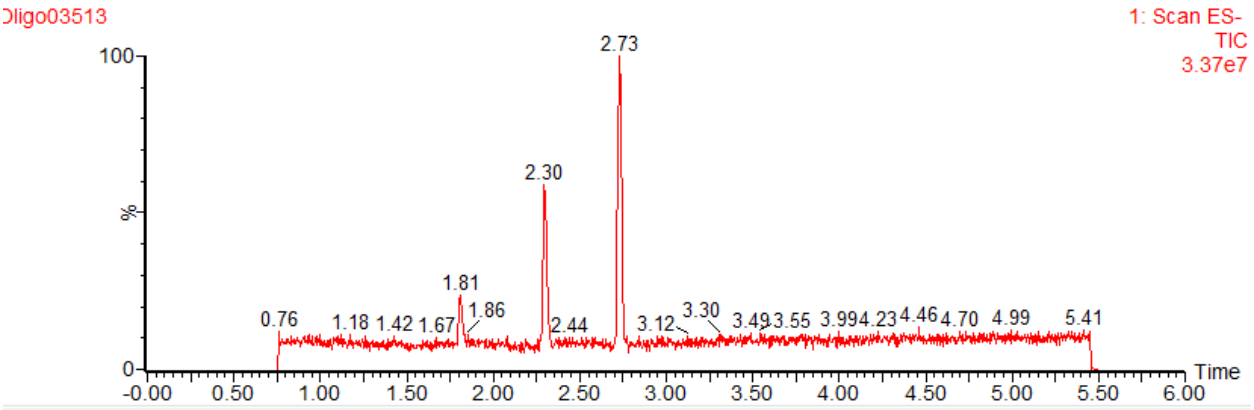


Molecular Weight: 7552.02

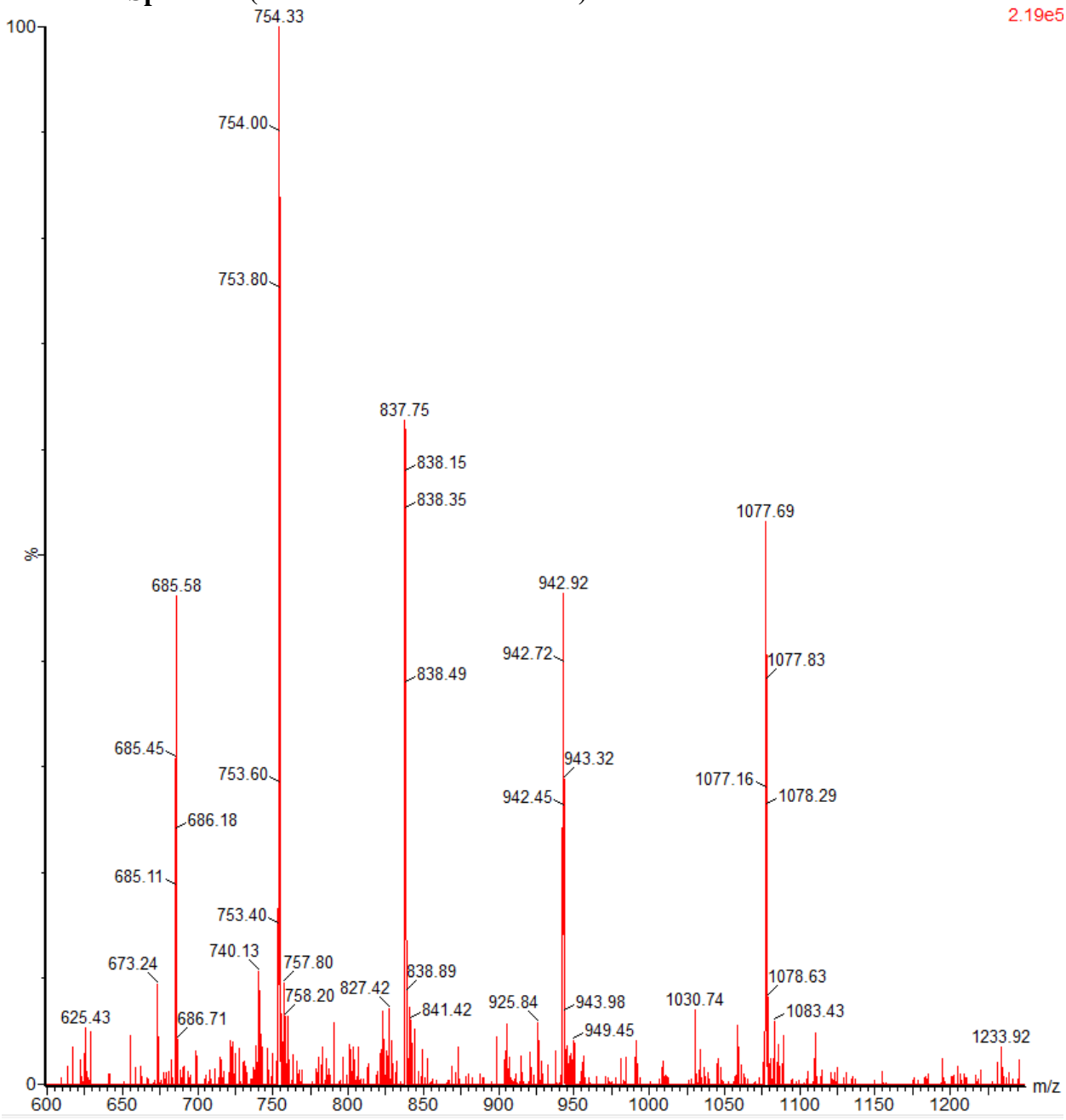
A260



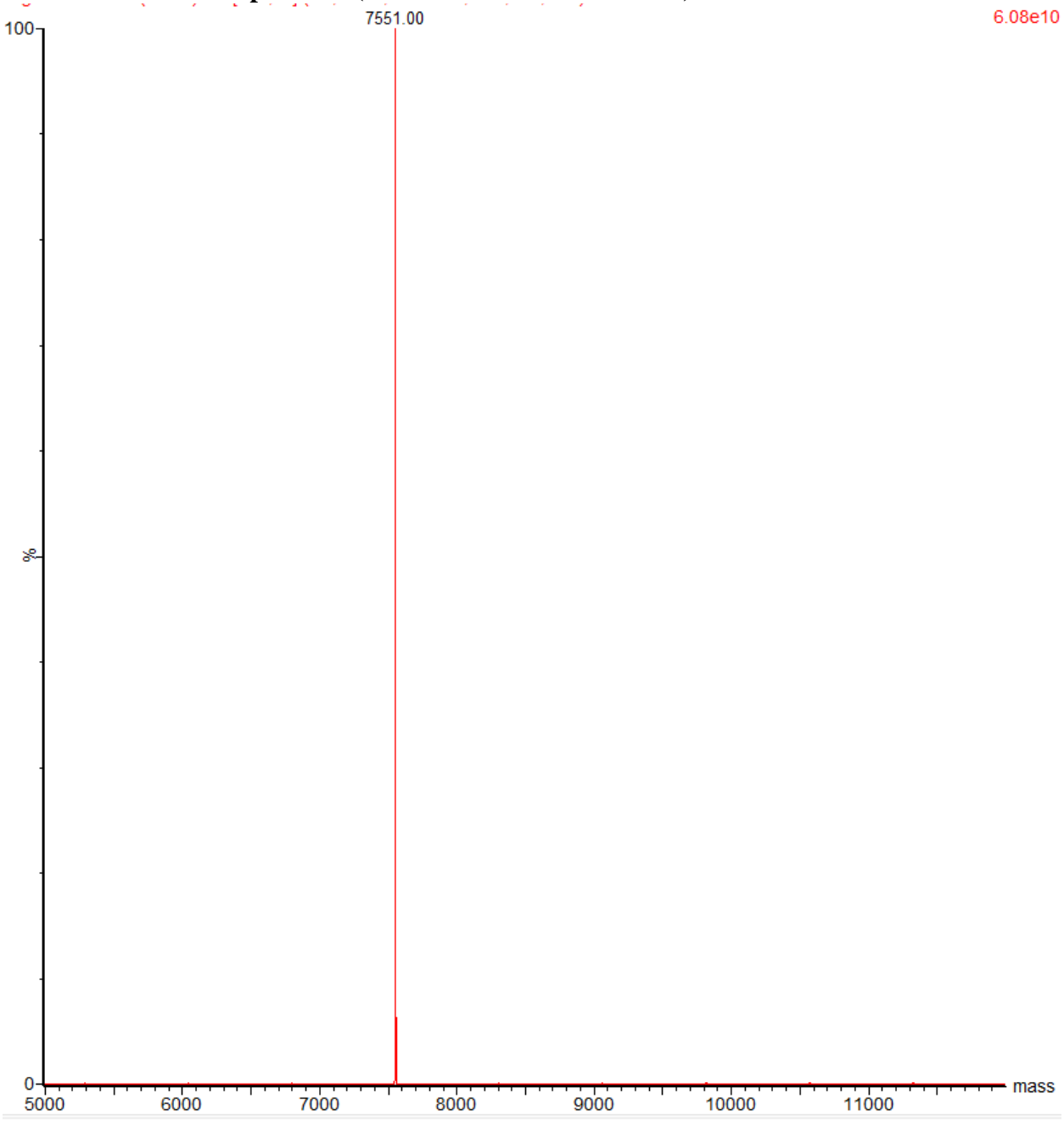
TIC



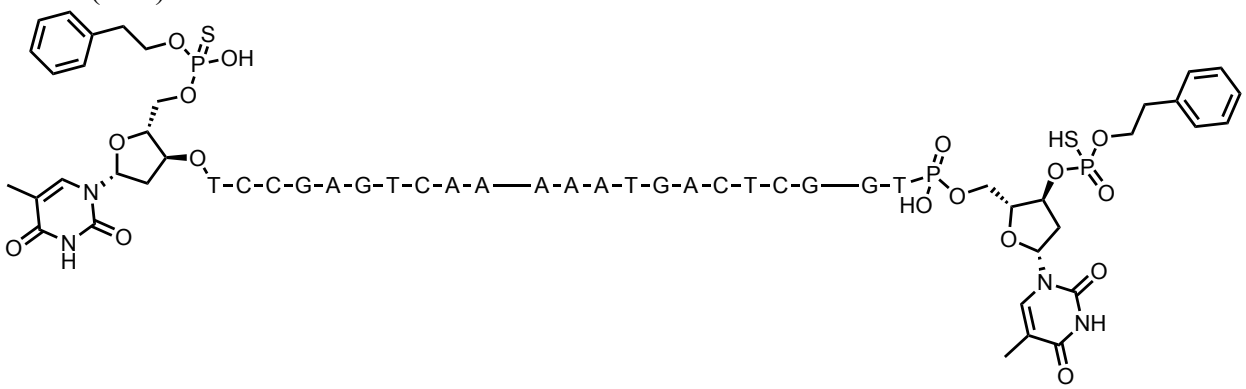
Raw Mass Spectrum (Retention Time 2.3 minutes)



Deconvoluted Mass Spectrum (Retention Time 2.3 minutes)

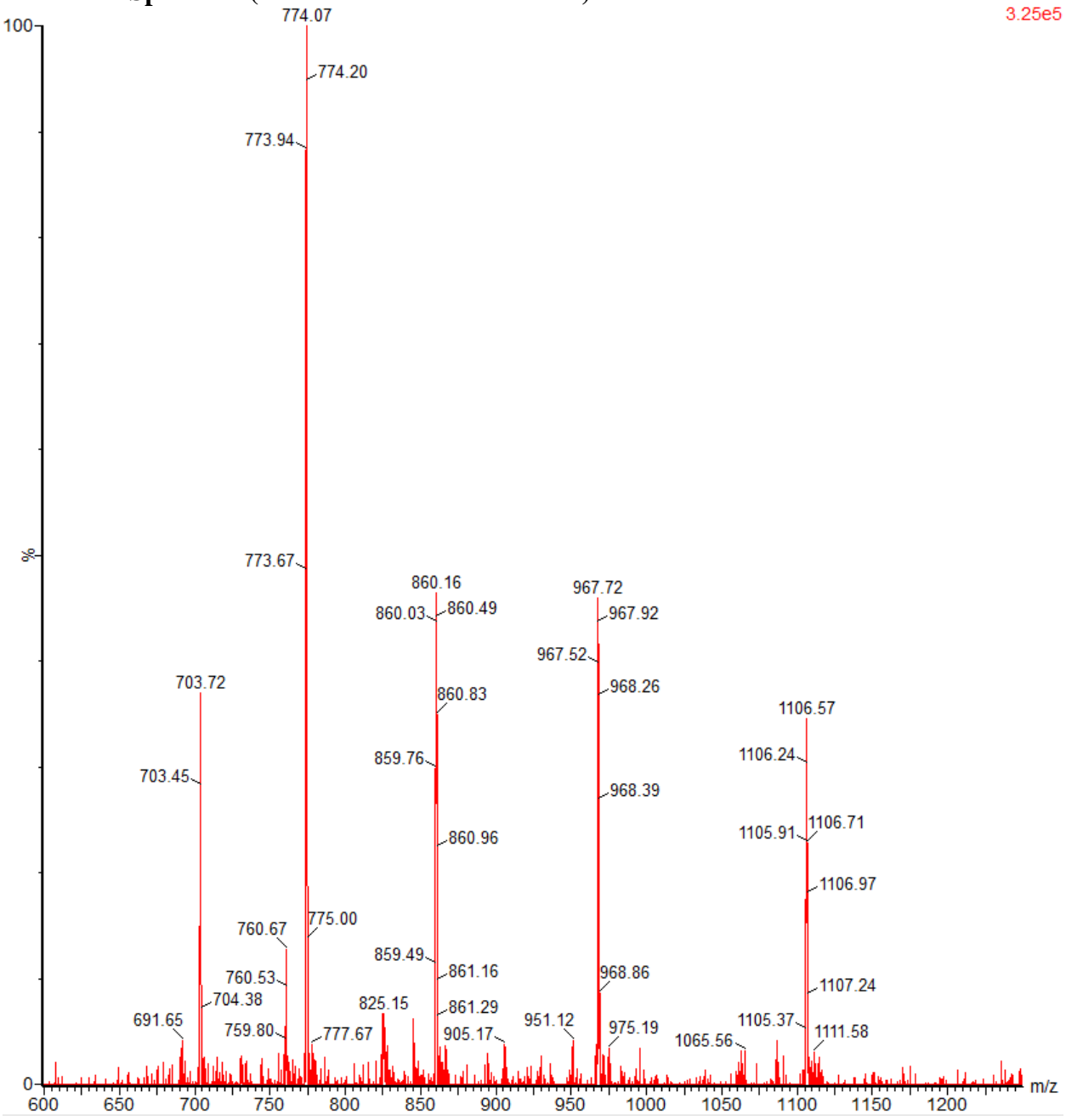


LCMS Characterization of **Double Lable SI-24**
Produced from **SI-16** by General Procedure **3:**
Conversion (A260): 51%
Retention Time: 2.72 min
Expected Mass: 7752.2
Expected (M-8)/8 Ion: 968.02
Found Deconvoluted Mass: 7553.00
Found (M-6)/6 Ion: 967.72

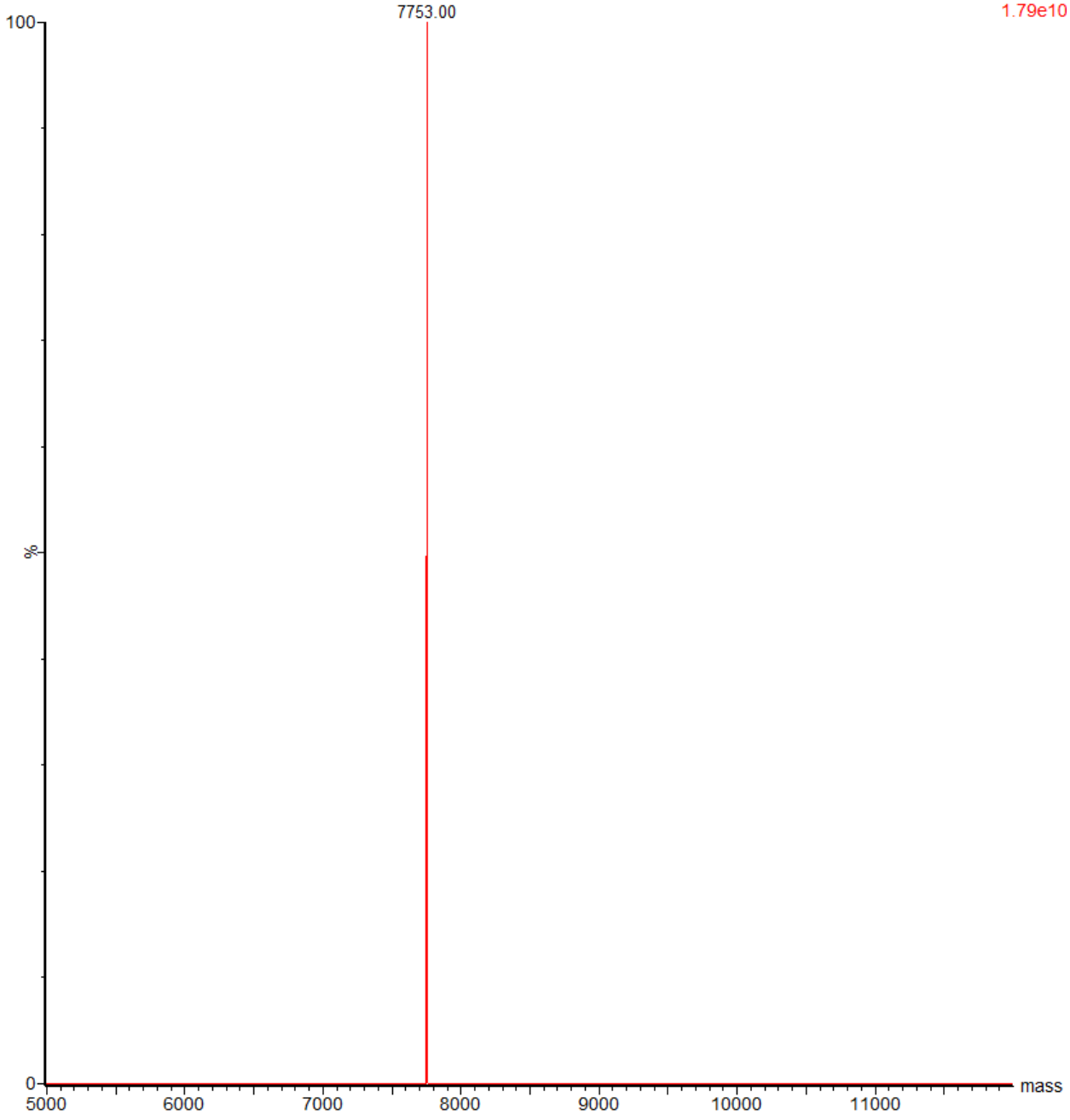


Molecular Weight: 7752.21

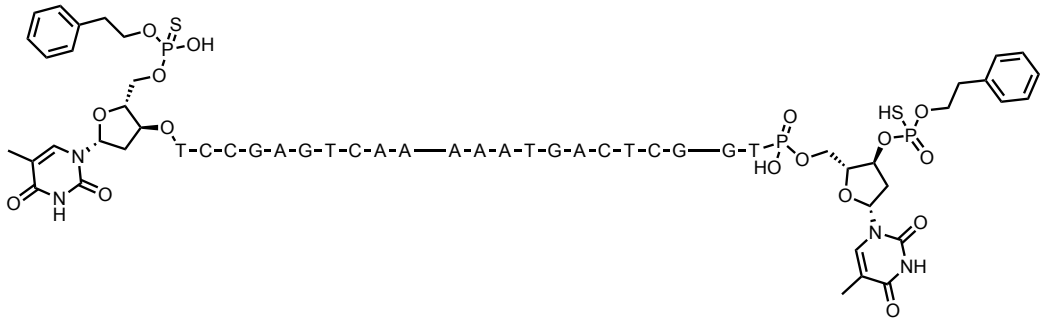
Raw Mass Spectrum (Retention Time 2.7 Minutes)



Deconvoluted Mass Spectrum (Retention Time 2.7 Minutes)

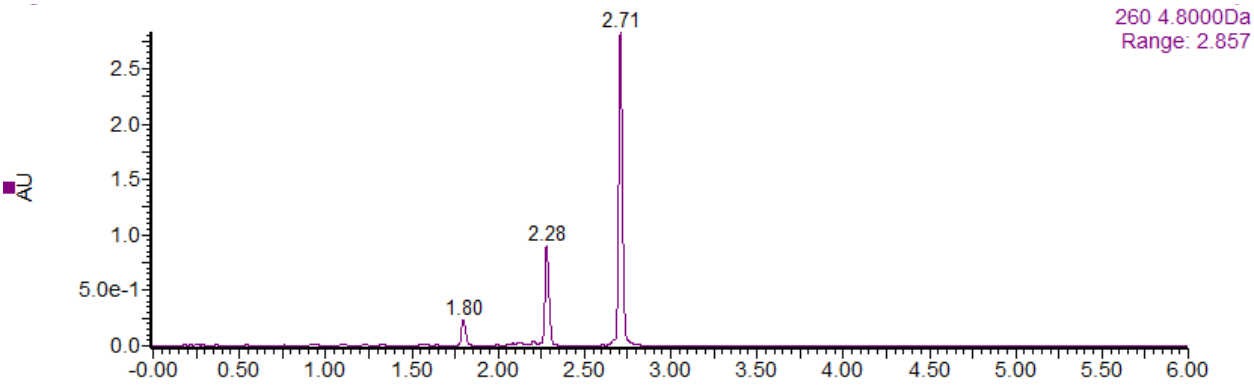


LCMS Characterization of **Double Label SI-24**
Produced from **SI-16** by General Procedure **3:**
Conversion (A260): 76%
Retention Time: 2.71 min
Expected Mass: 7752.2
Expected (M-8)/8 Ion: 968.02
Found Deconvoluted Mass: 7552.00
Found (M-6)/6 Ion: 967.9

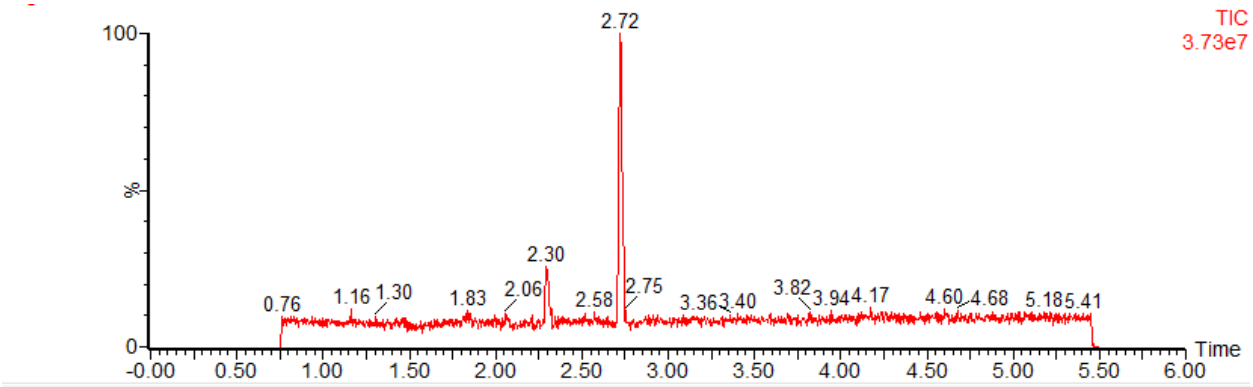


Molecular Weight: 7752.21

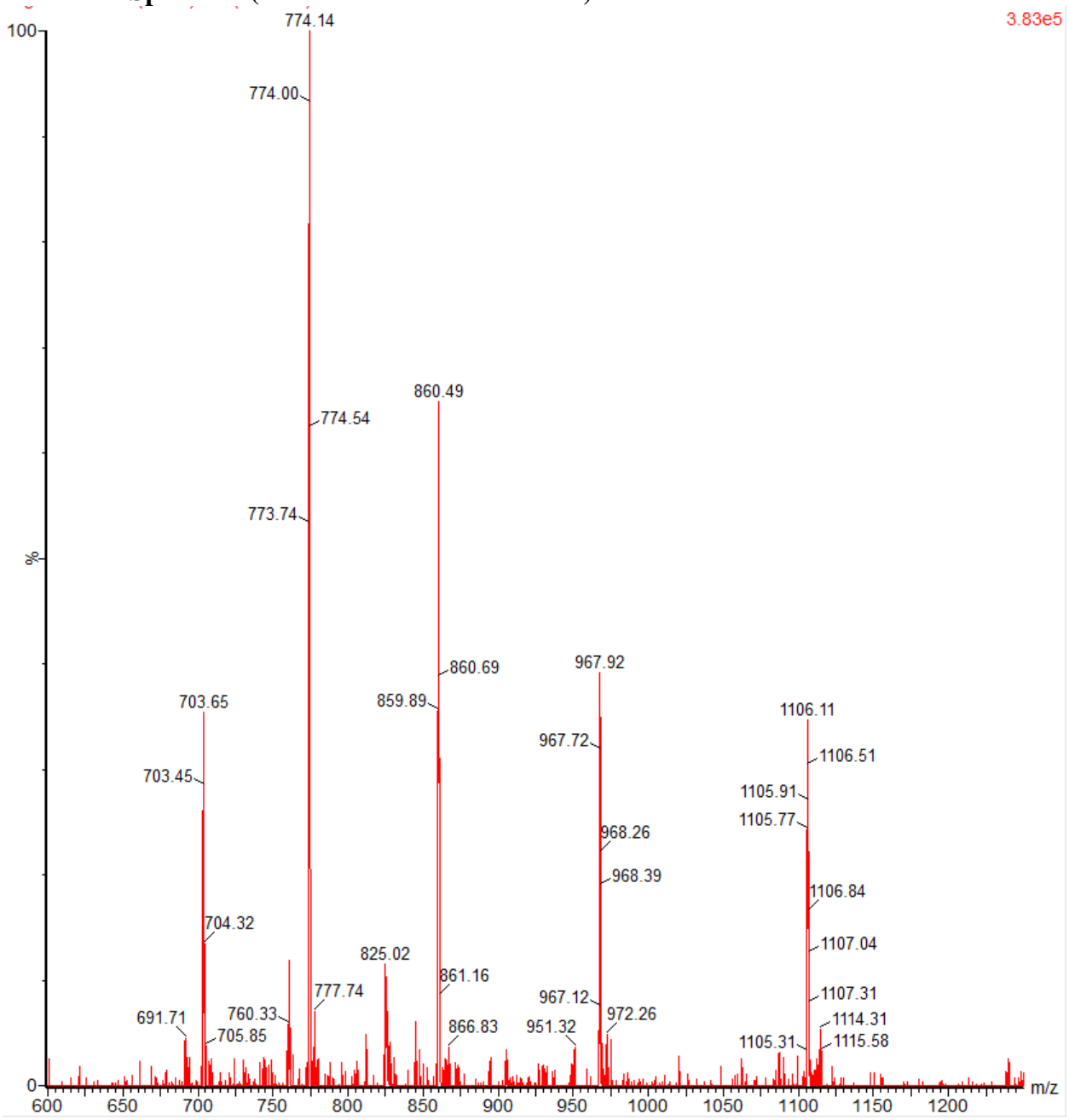
A260



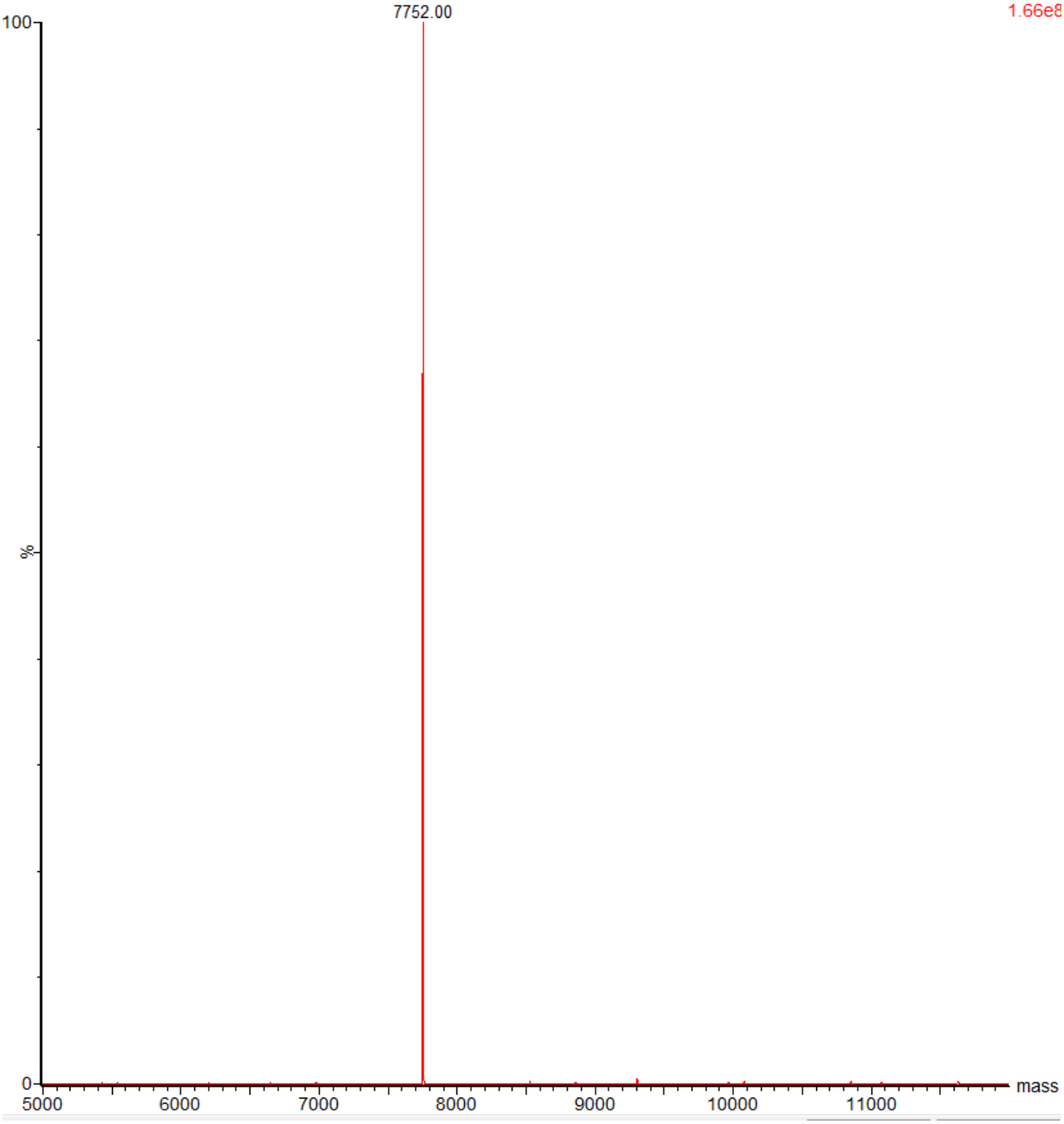
TIC



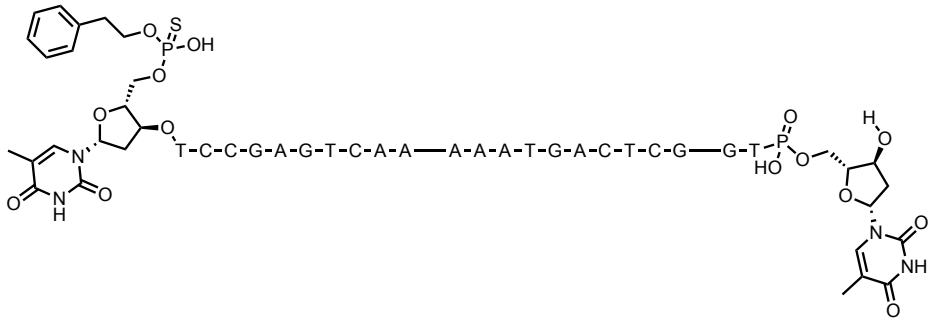
Raw Mass Spectrum (Retention Time 2.7 Minutes)



Deconvoluted Mass Spectrum (Retention Time 2.7 Minutes)

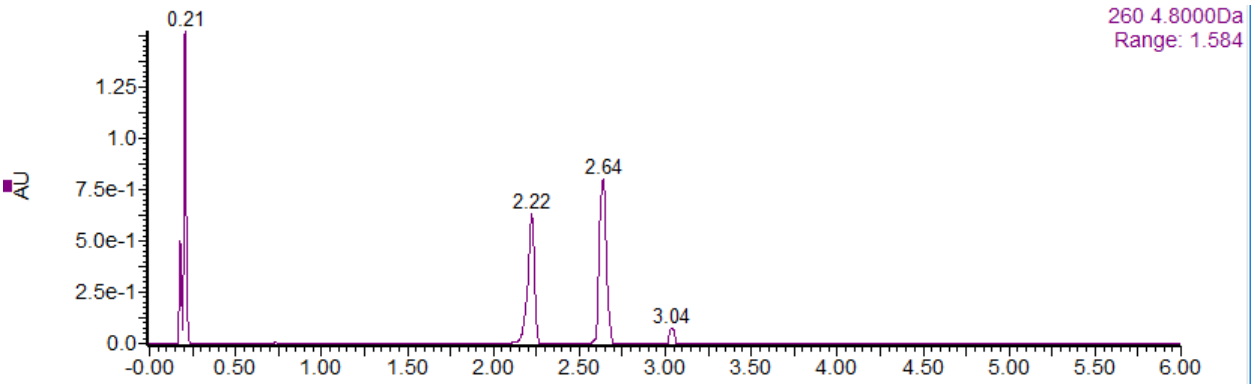


Entry 4
LCMS Characterization of **Single Label SI-23**
Produced from **SI-16** by General Procedure **3:**
Conversion (A260): 53%
Retention Time: 2.29 min
Expected Mass: 7552.02
Expected (M-8)/8 Ion: 943.0
Found Deconvoluted Mass: 7552.00
Found (M-6)/6 Ion: 942.92

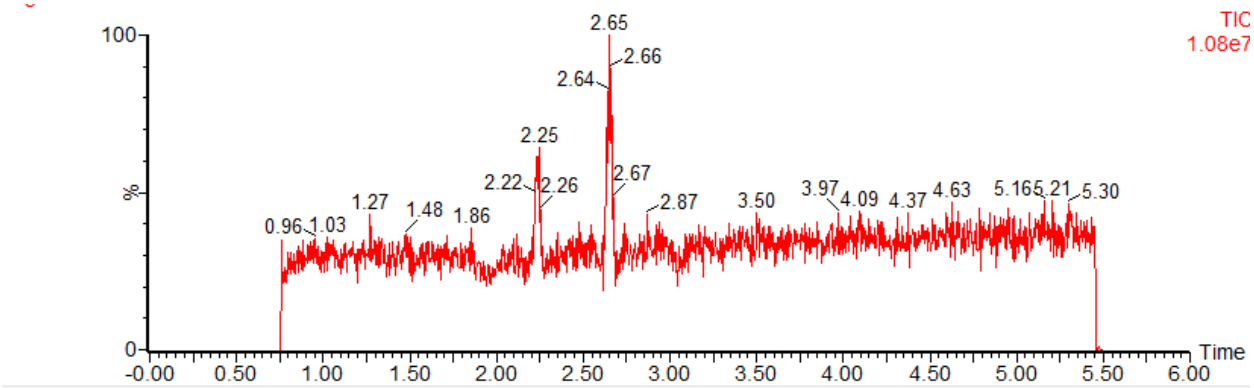


Molecular Weight: 7552.02

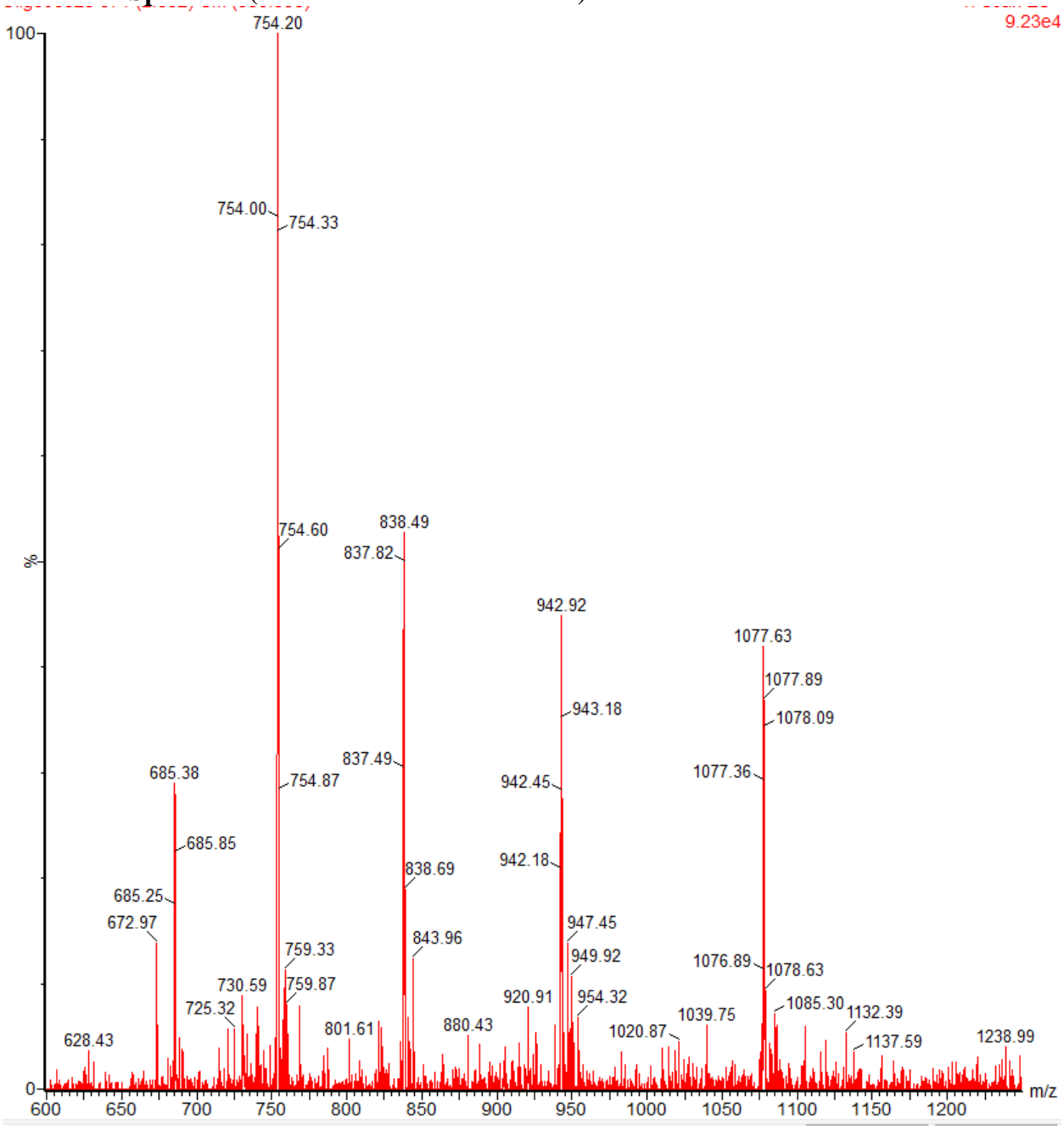
A260



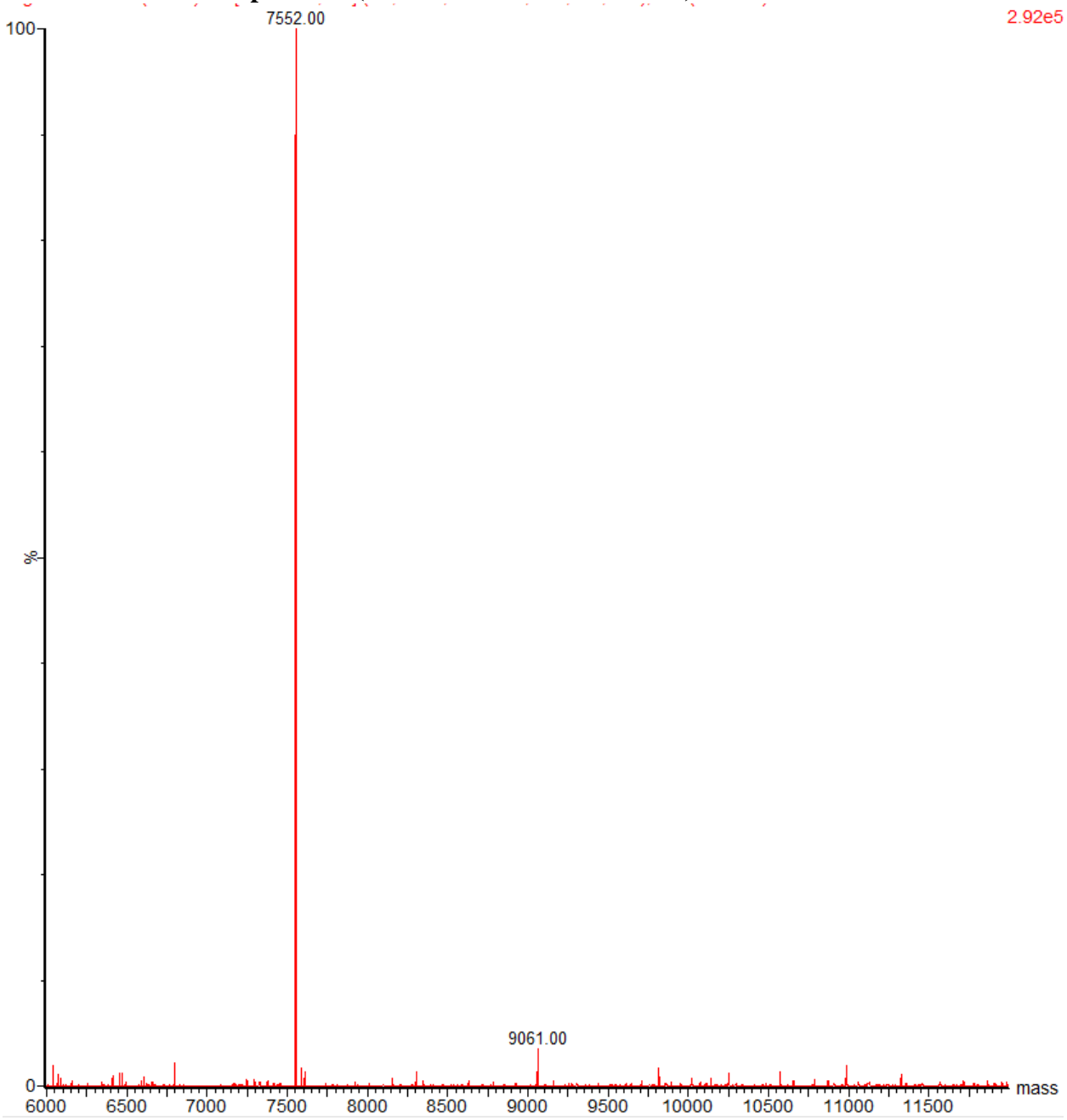
TIC



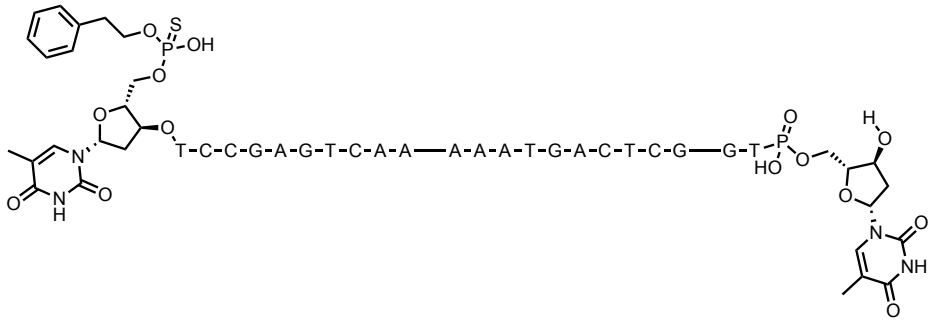
Raw Mass Spectrum (Retention Time 2.3 minutes)



Deconvoluted Mass Spectrum (Retention Time 2.3 minutes)

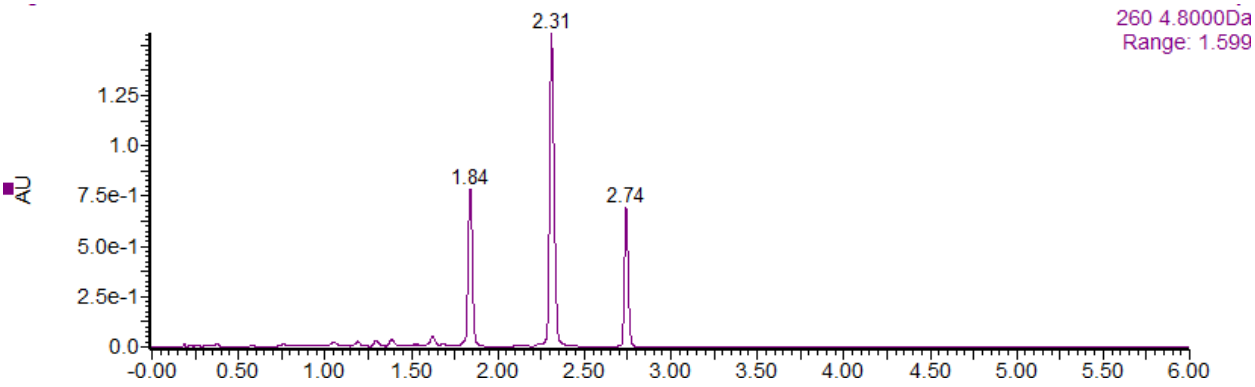


Entry 5
LCMS Characterization of **Single Label SI-23**
Produced from **SI-16** by General Procedure **3:**
Conversion (A260): 53%
Retention Time: 2.29 min
Expected Mass: 7552.02
Expected (M-8)/8 Ion: 943.0
Found Deconvoluted Mass: 7552.00
Found (M-6)/6 Ion: 942.78

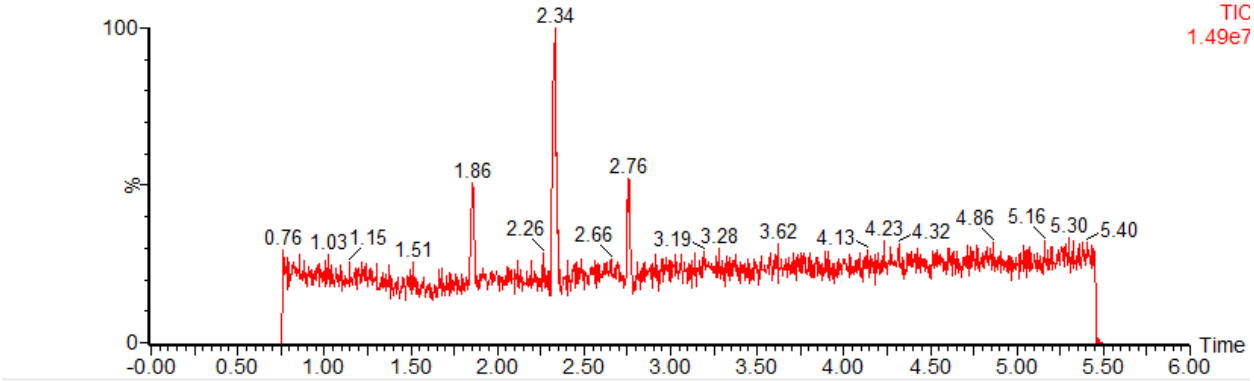


Molecular Weight: 7552.02

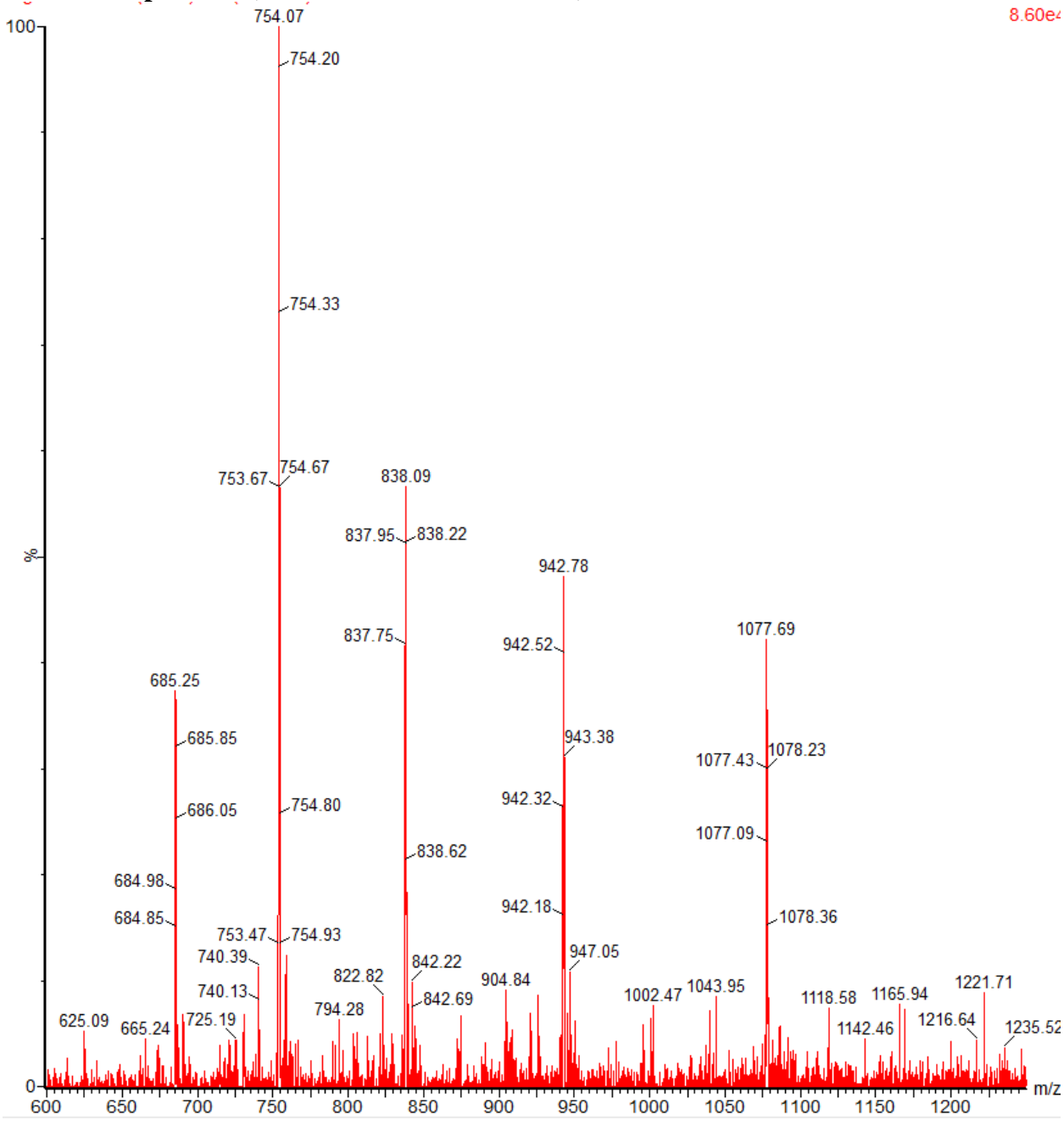
A260



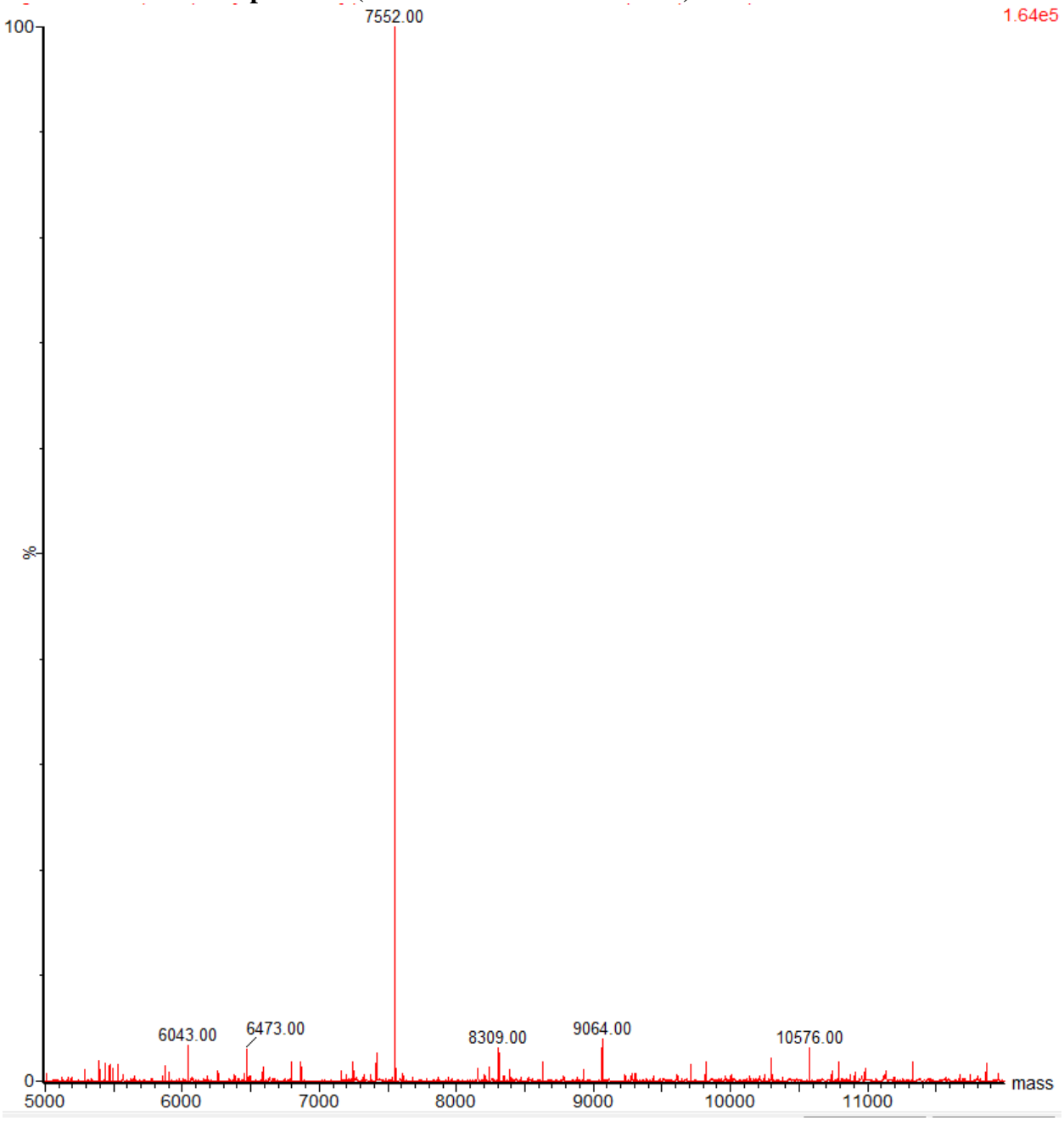
TIC



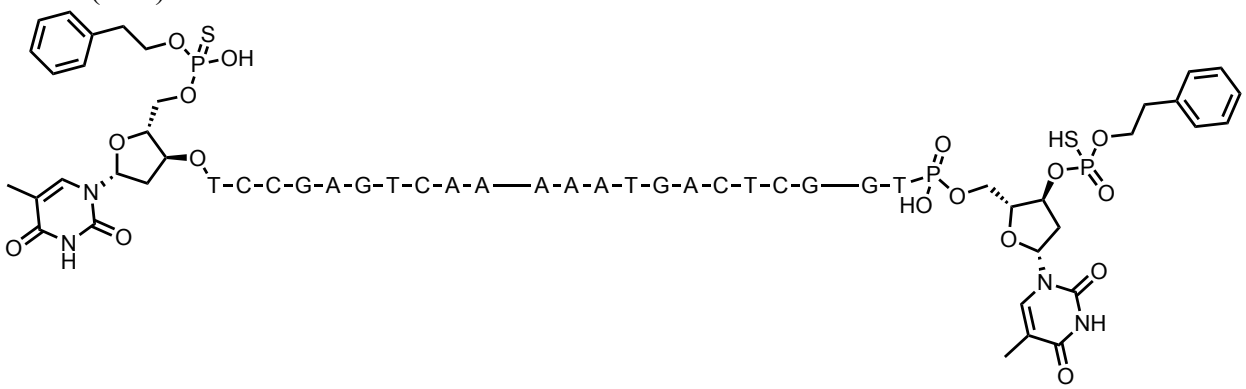
Raw Mass Spectrum (Retention Time 2.3 minutes)



Deconvoluted Mass Spectrum (Retention Time 2.3 minutes)

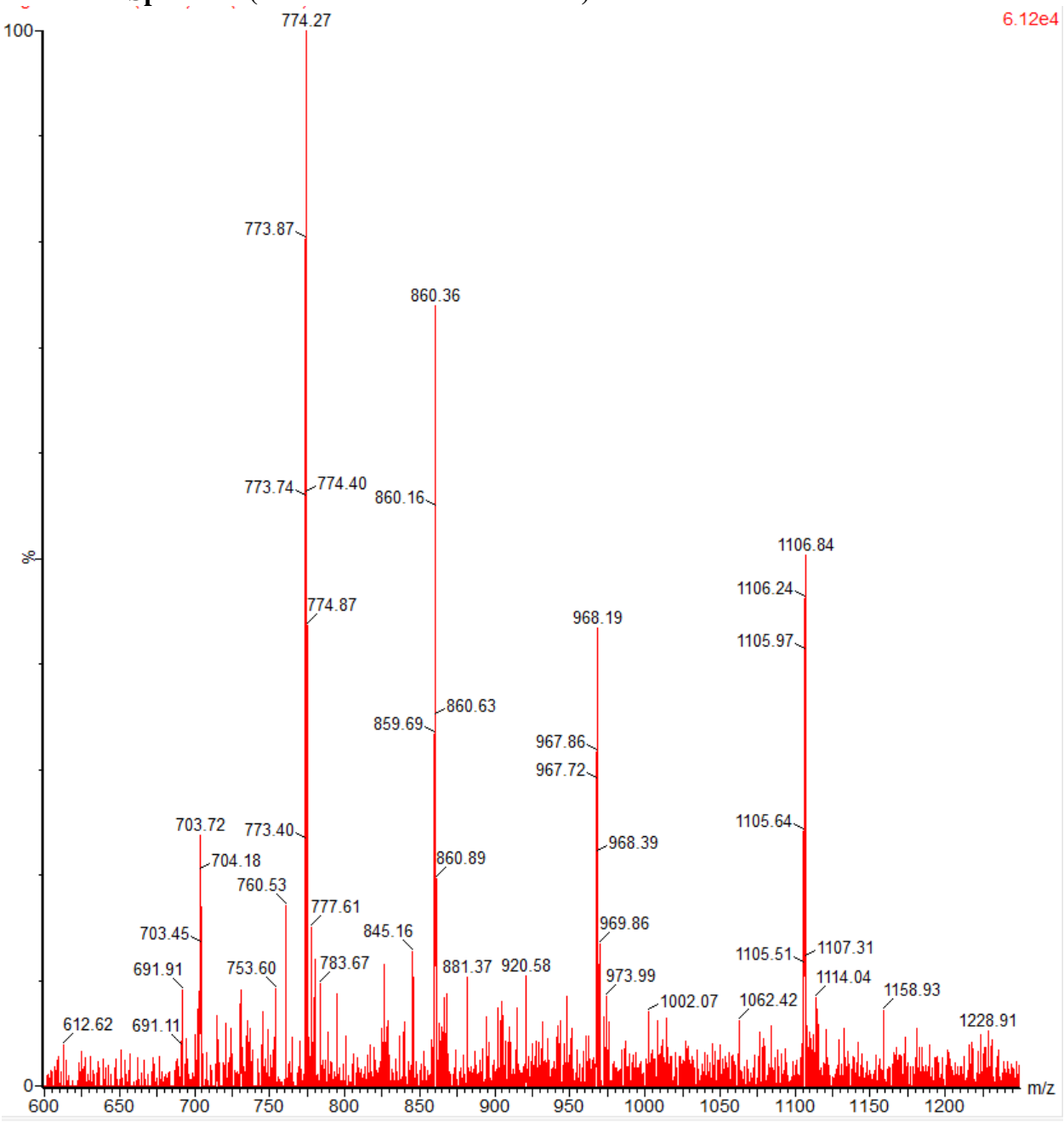


LCMS Characterization of **Double Label SI-24**
Produced from **SI-16** by General Procedure **3:**
Conversion (A260): 21%
Retention Time: 2.72 min
Expected Mass: 7752.2
Expected (M-8)/8 Ion: 968.02
Found Deconvoluted Mass: 7552.00
Found (M-6)/6 Ion: 968.19

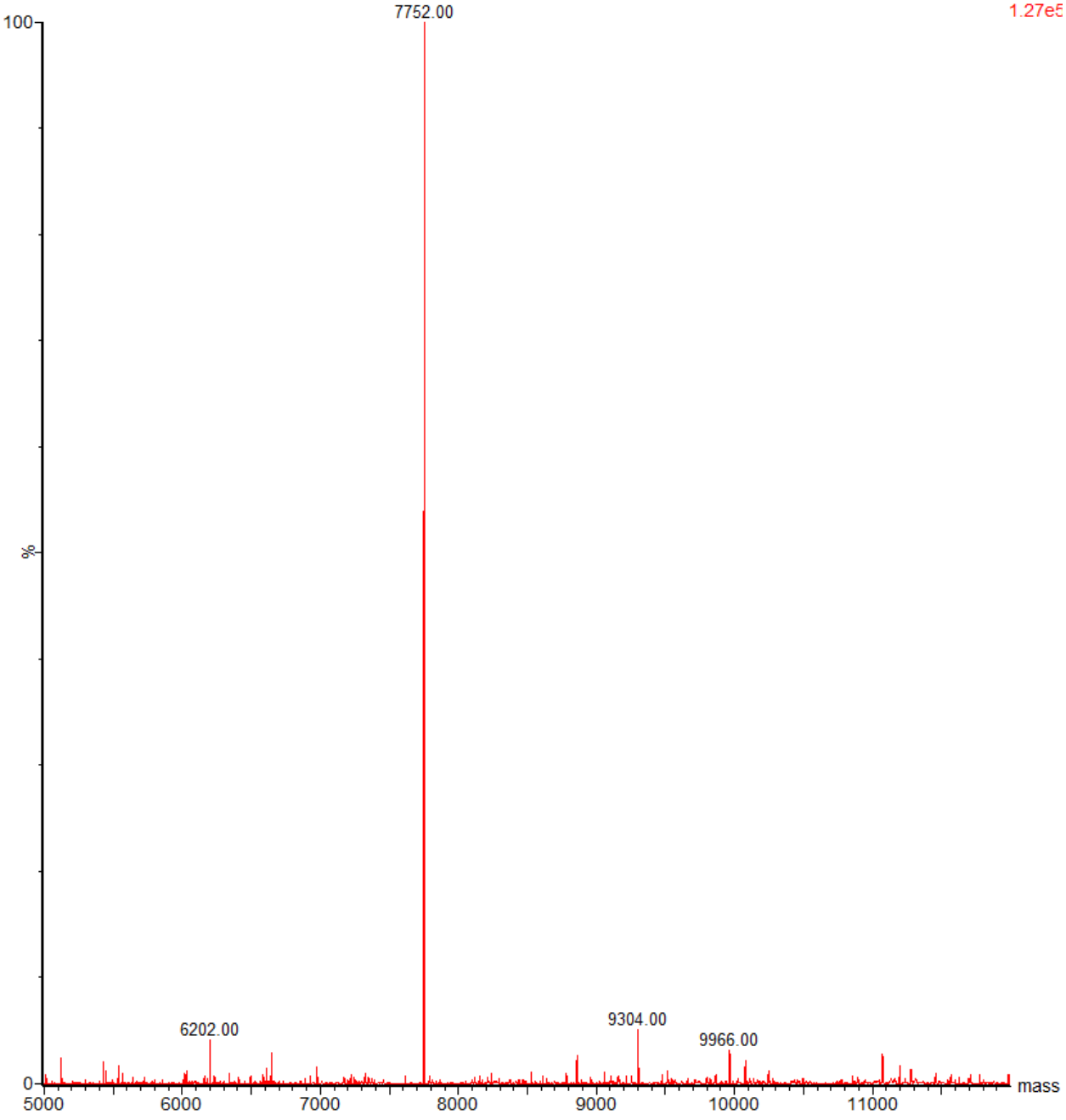


Molecular Weight: 7752.21

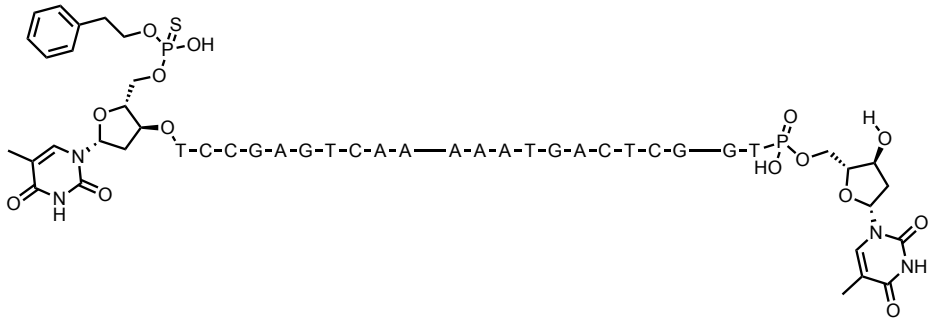
Raw Mass Spectrum (Retention Time 2.7 Minutes)



Deconvoluted Mass Spectrum (Retention Time 2.7 Minutes)

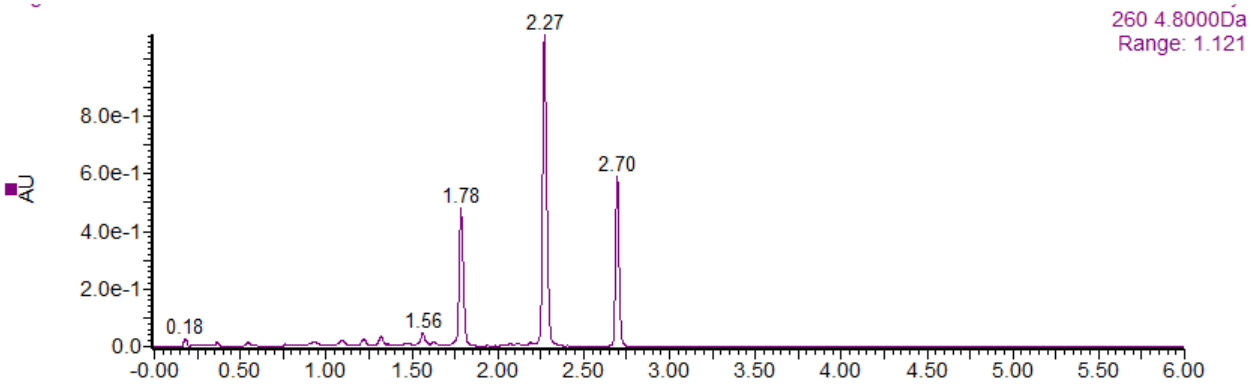


Entry 6
LCMS Characterization of **Single Label SI-23**
Produced from **SI-16** by General Procedure **3:**
Conversion (A260): 50%
Retention Time: 2.29 min
Expected Mass: 7552.02
Expected (M-8)/8 Ion: 943.0
Found Deconvoluted Mass: 7551.00
Found (M-6)/6 Ion: 942.92

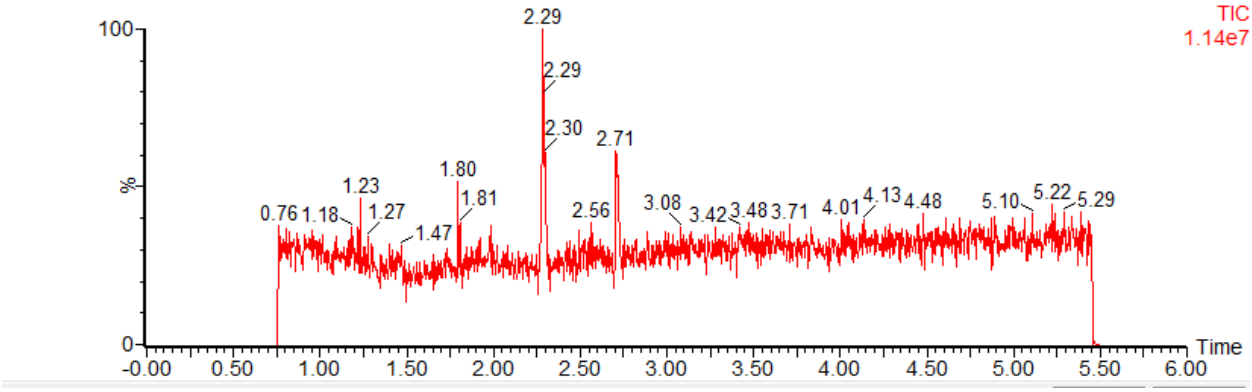


Molecular Weight: 7552.02

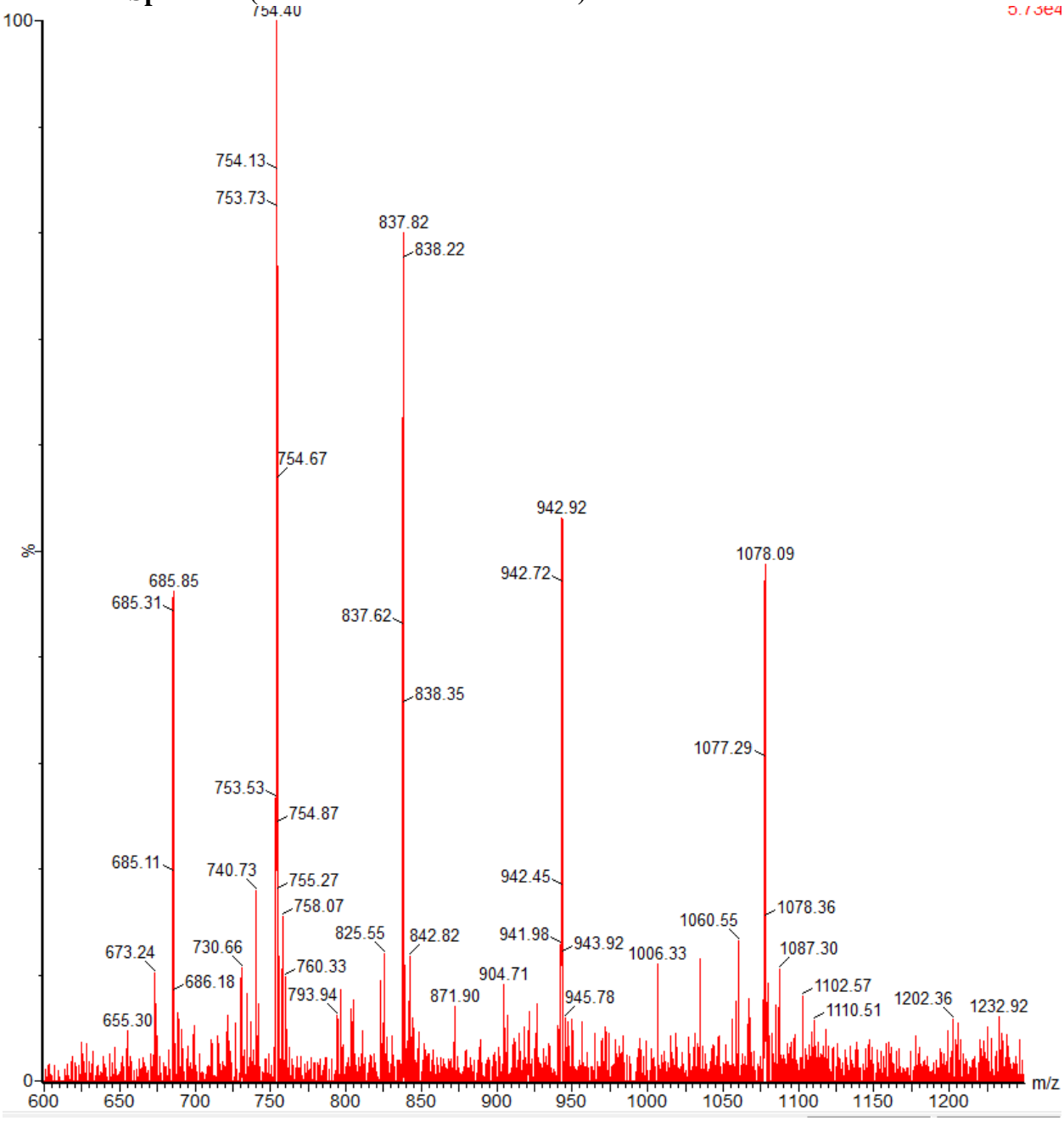
A260



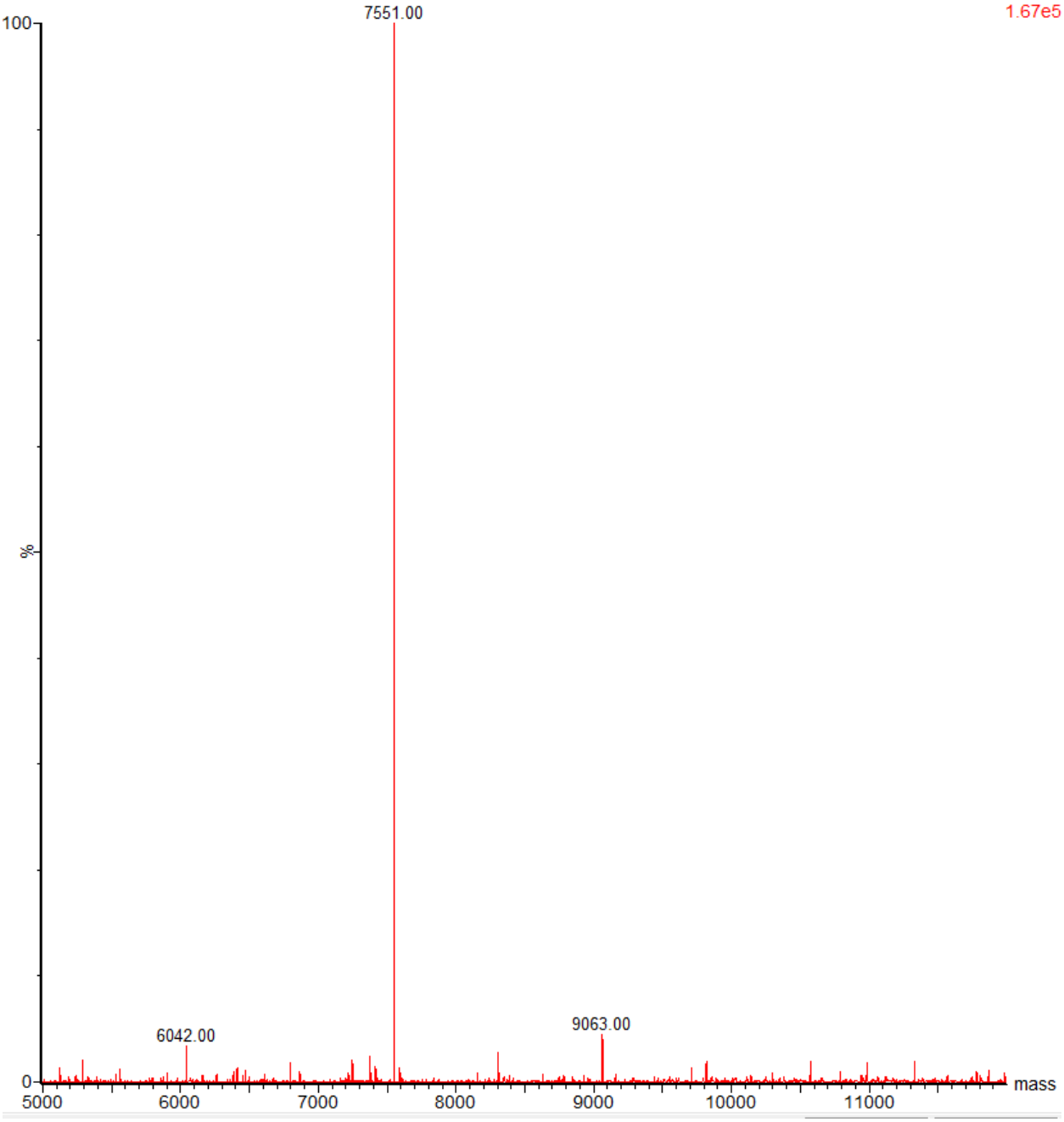
TIC



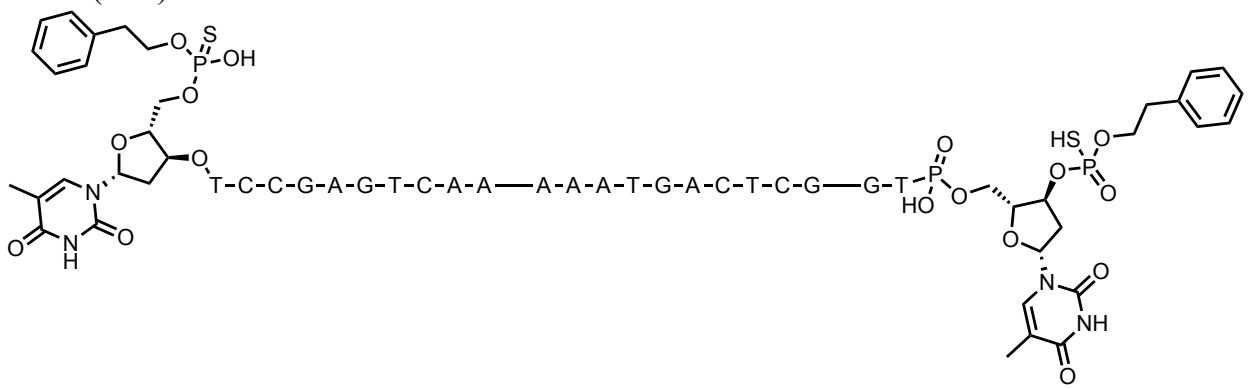
Raw Mass Spectrum (Retention Time 2.3 minutes)



Deconvoluted Mass Spectrum (Retention Time 2.3 minutes)

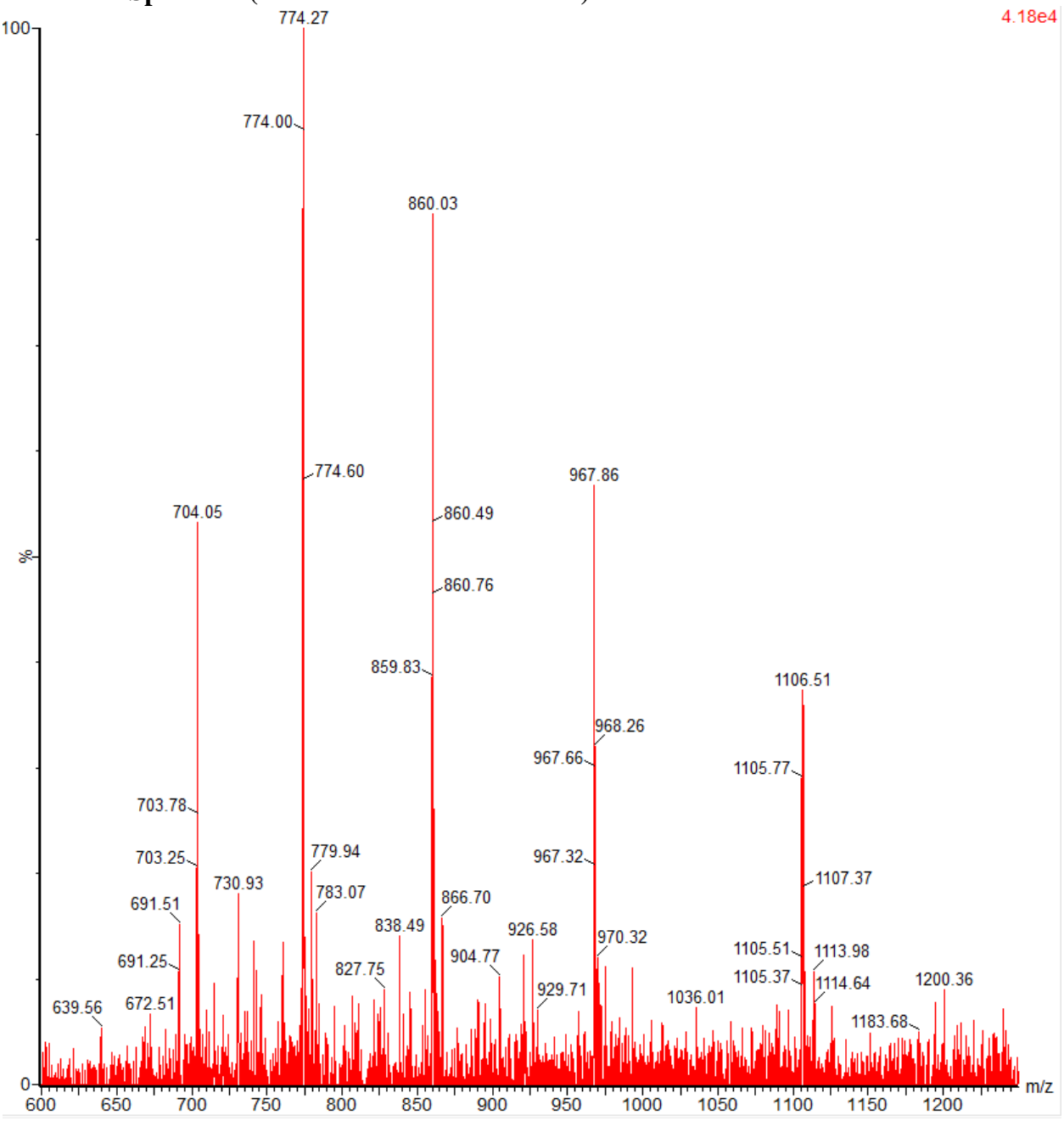


LCMS Characterization of **Double Label SI-24**
Produced from **SI-16** by General Procedure **3:**
Conversion (A260): 25%
Retention Time: 2.72 min
Expected Mass: 7752.2
Expected (M-8)/8 Ion: 968.02
Found Deconvoluted Mass: 7552.00
Found (M-6)/6 Ion: 967.86

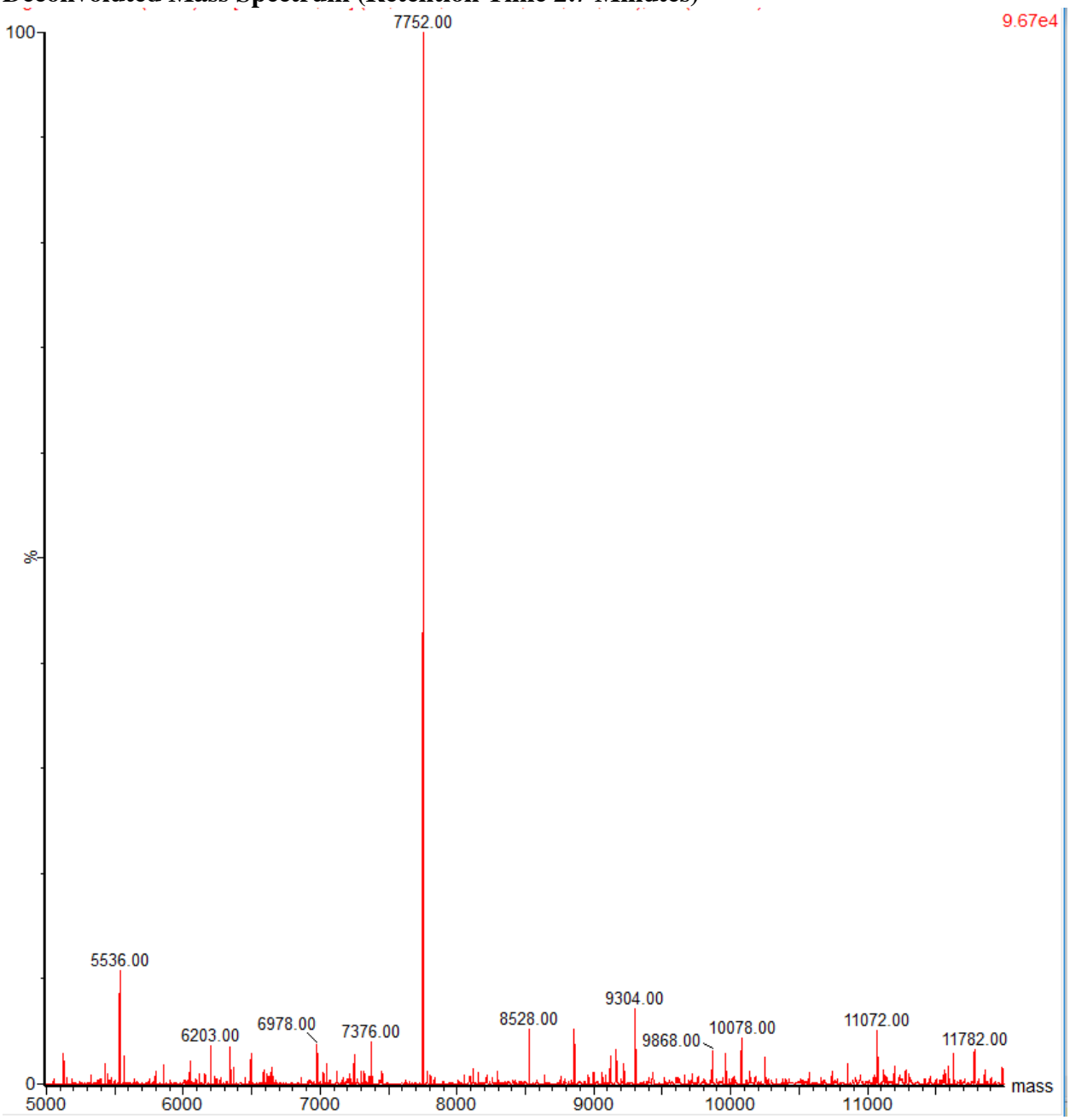


Molecular Weight: 7752.21

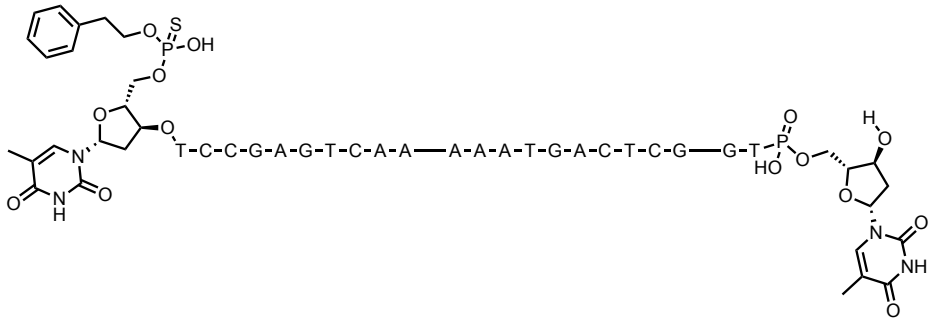
Raw Mass Spectrum (Retention Time 2.7 Minutes)



Deconvoluted Mass Spectrum (Retention Time 2.7 Minutes)

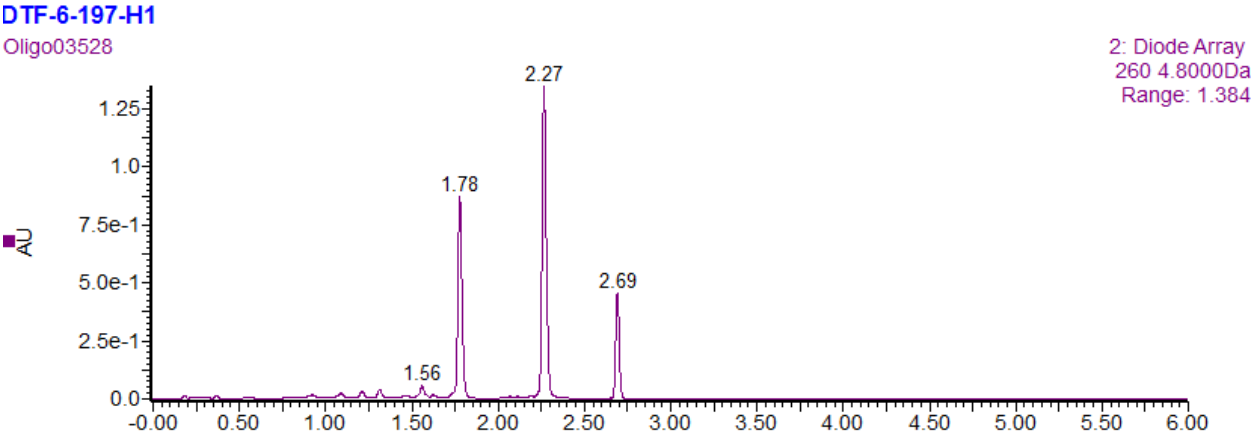


Entry 7
LCMS Characterization of **Single Label SI-23**
Produced from **SI-16** by General Procedure **3:**
Conversion (A260): 33%
Retention Time: 2.29 min
Expected Mass: 7552.02
Expected (M-8)/8 Ion: 943.0
Found Deconvoluted Mass: 7552.00
Found (M-6)/6 Ion: 943.18

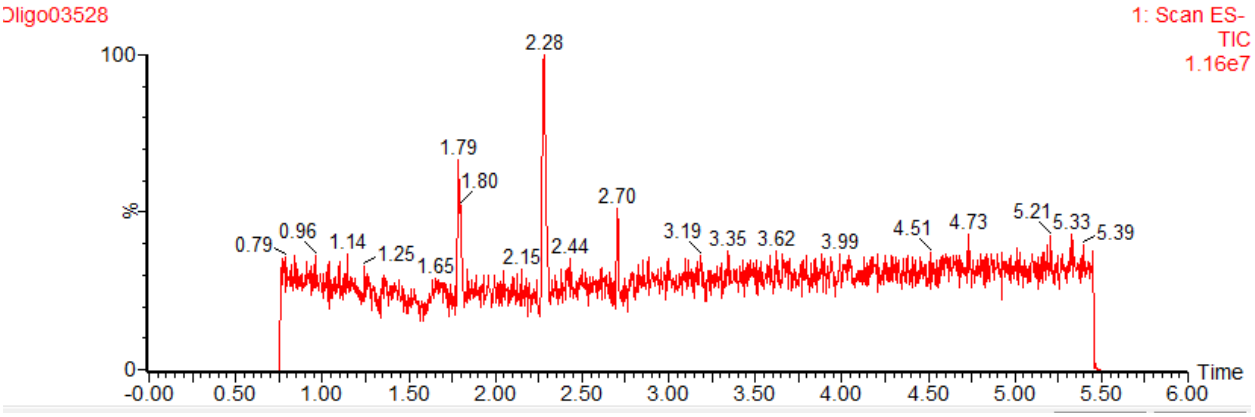


Molecular Weight: 7552.02

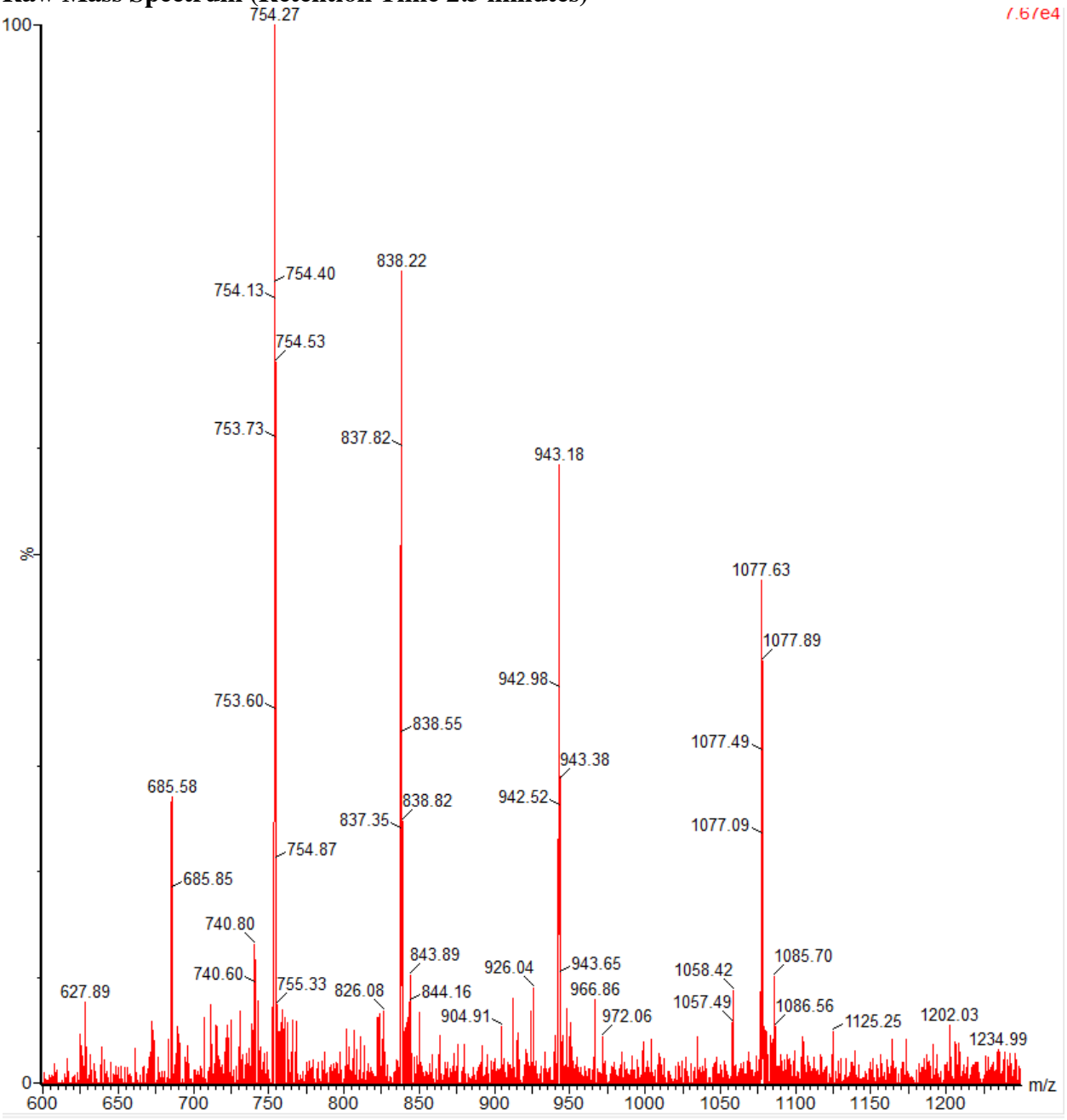
A260



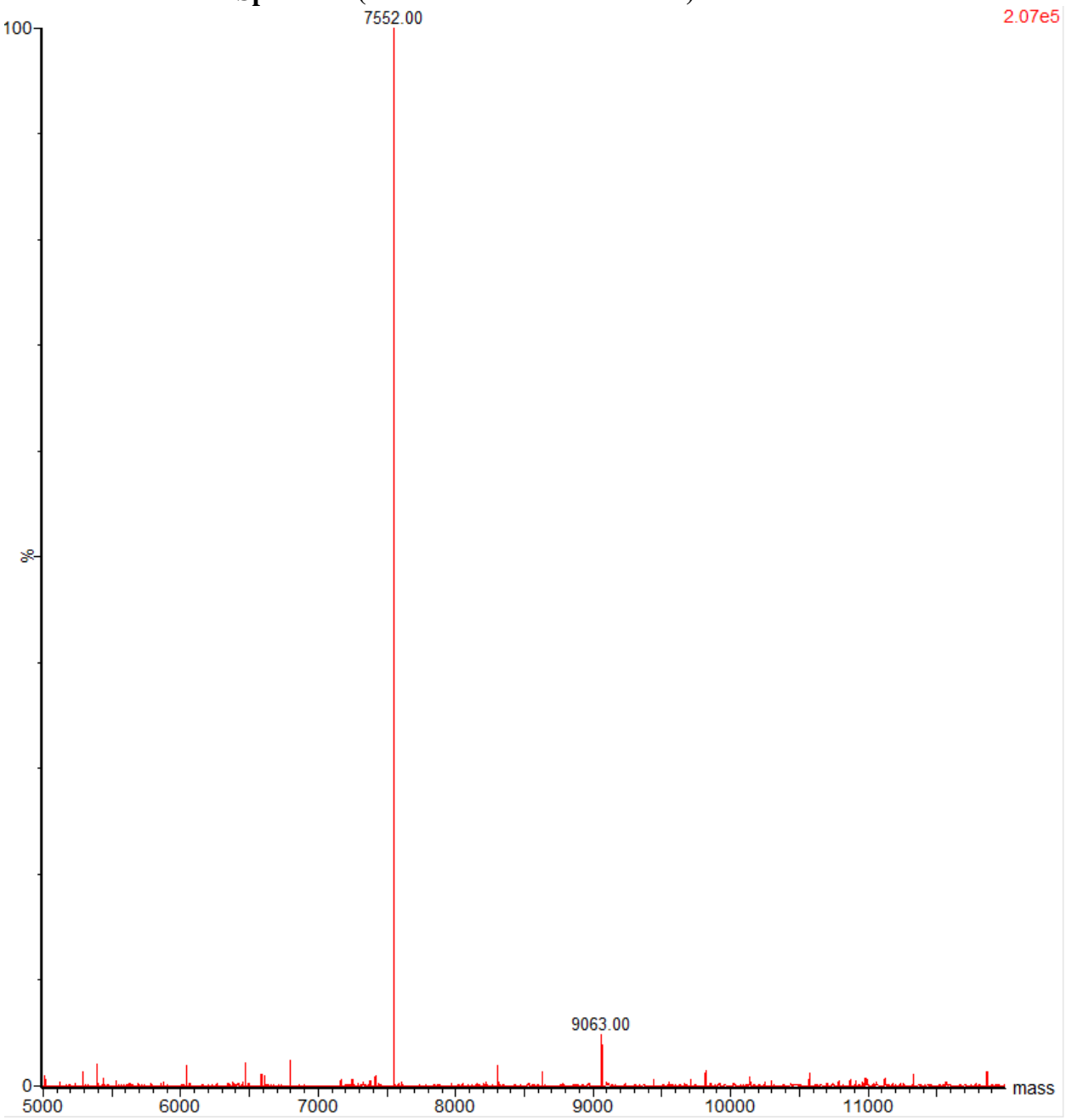
TIC



Raw Mass Spectrum (Retention Time 2.3 minutes)



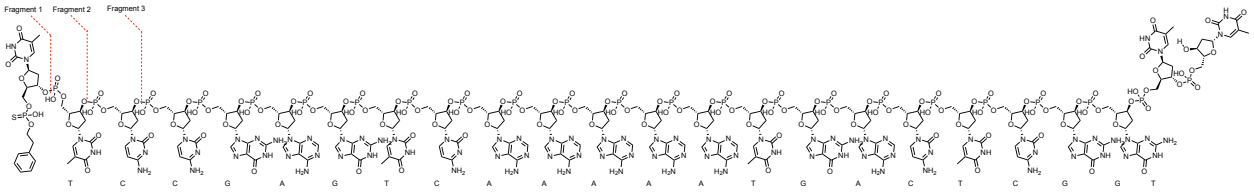
Deconvoluted Mass Spectrum (Retention Time 2.3 minutes)



Structure Confirmation of SI-23

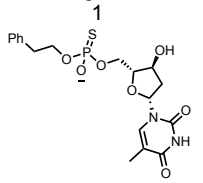
A crude solution of **SI-23** in water (100 μM) was injected on the Waters I-Class ToF for peak identification. The MS cone voltage was then increased from 5 mV to 30 mV and the same sample was reinjected to fragment the modified oligonucleotide. Diagnostic mass fragments were then extracted, and their presence confirmed in the total ion current. They are plotted below.

Mass Fragmentation of SI-23



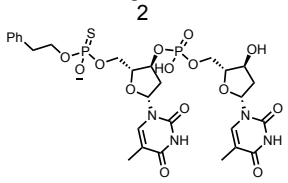
Exact Mass: 7548.27
Molecular Weight: 7552.02

Fragment 1



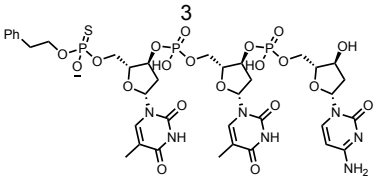
Chemical Formula: $C_{18}H_{22}N_2O_7PS^-$
Exact Mass: 441.09
Molecular Weight: 441.42

Fragment 2

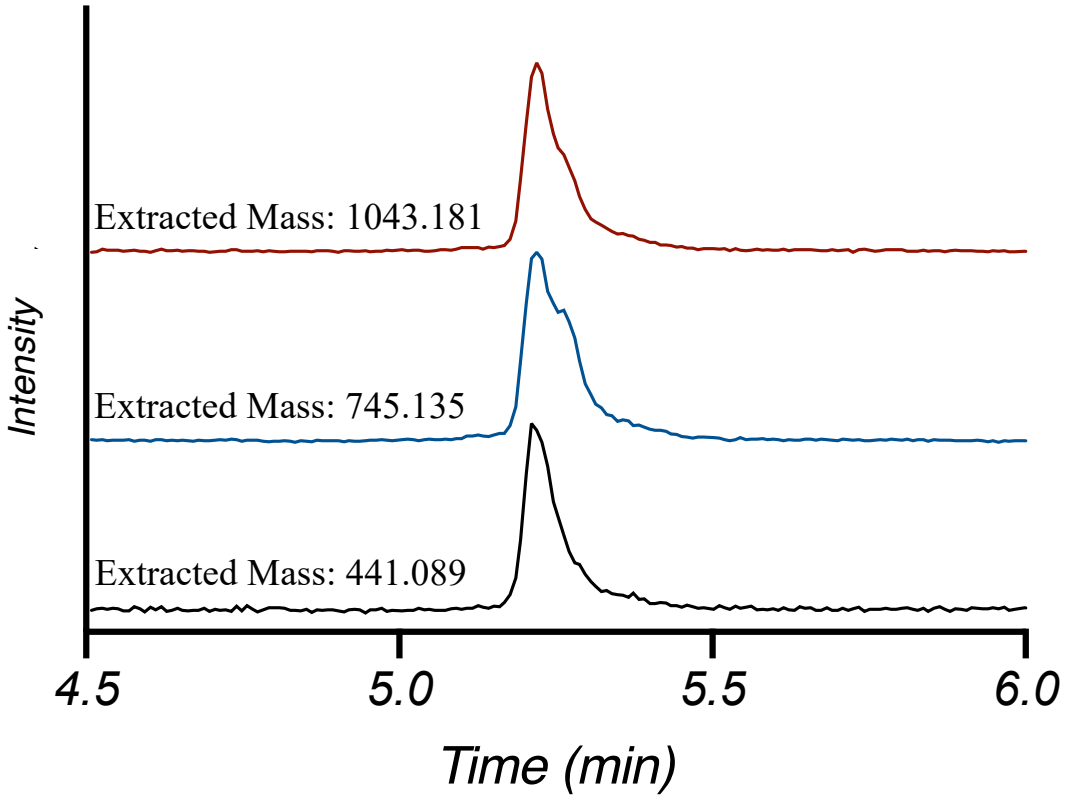


Chemical Formula: $C_{28}H_{35}N_4O_{14}P_2S^-$
Exact Mass: 745.14
Molecular Weight: 745.61

Fragment 3



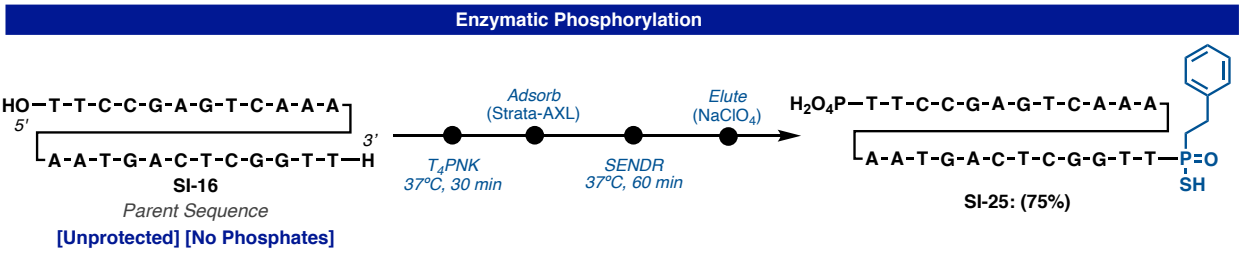
Chemical Formula: $C_{37}H_{47}N_7O_{20}P_3S^-$
Exact Mass: 1034.18
Molecular Weight: 1034.79



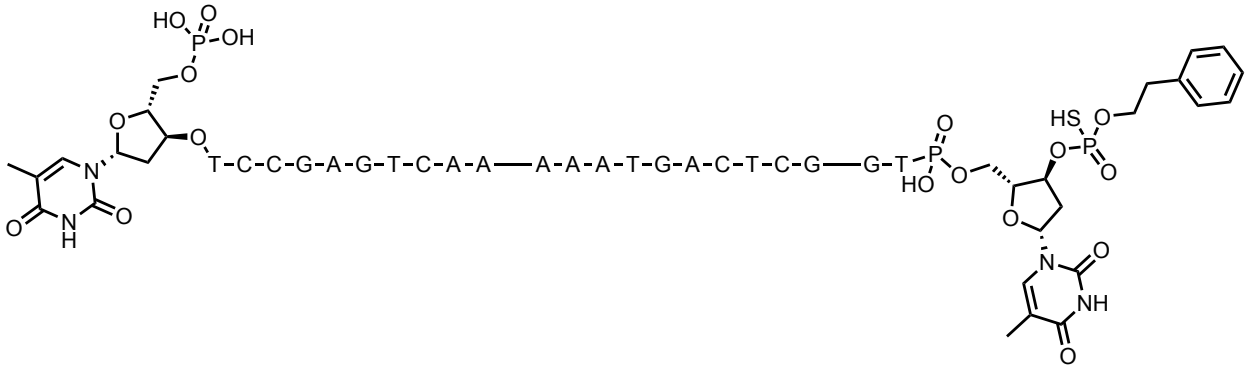
T4PNK Phopshorylation then SENDR

Protocol

Nonphosphorylated DNA **SI-16** was phosphorylated at the 5' end by T4PNK. Briefly, 5 nMol DNA was added to a PCR tube and contiaining CutSmart® (1X), 1 mM ATP, and 5 mM DTT (from 5x stocks). T4PNK was added (2 uL) and the reaction volume was brought to 50 μL total. The reaction tube was allowed to incubate for 30 minutes at 37ºC. After 30 minutes the reaction mixture was added directly to the Strata XL-A support in PBS (500 uL) and vortexed. The support was dried according to General Procedure 2, and standard SENDR conditions were applied.

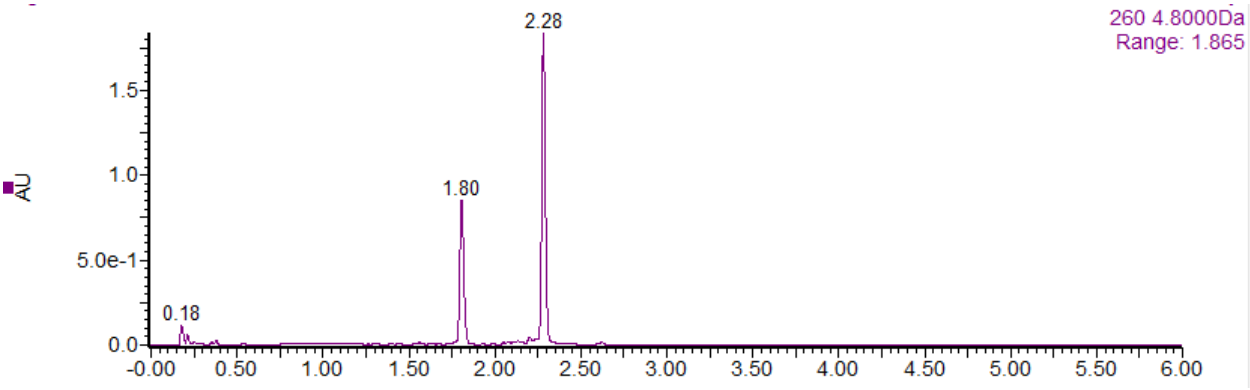


LCMS Characterization of **SI-25**
Produced from **SI-16:**
Conversion (A260): 75%
Retention Time: 2.28 min
Expected Mass: 7632.00
Expected (M-8)/8 Ion: 953.0
Found Deconvoluted Mass: 7632.00
Found (M-6)/6 Ion: 952.78

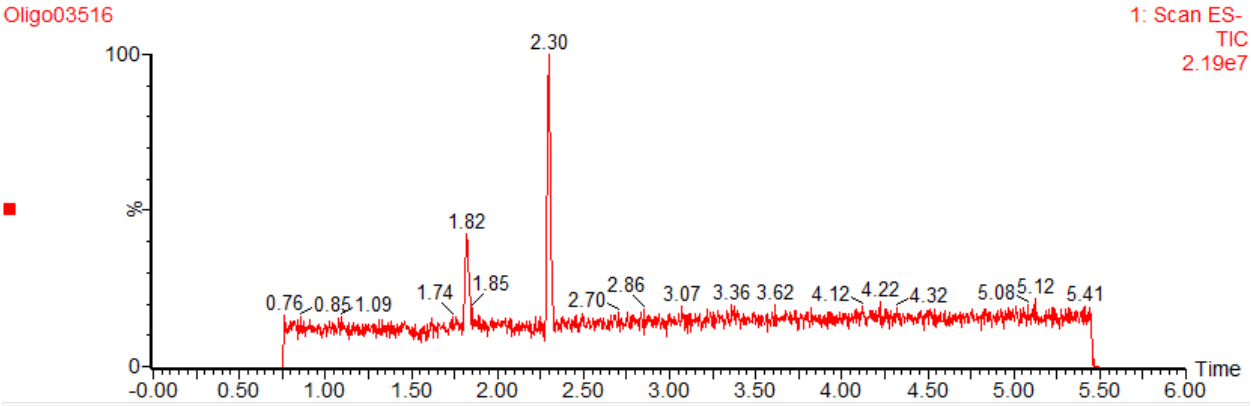


Molecular Weight: 7632.00

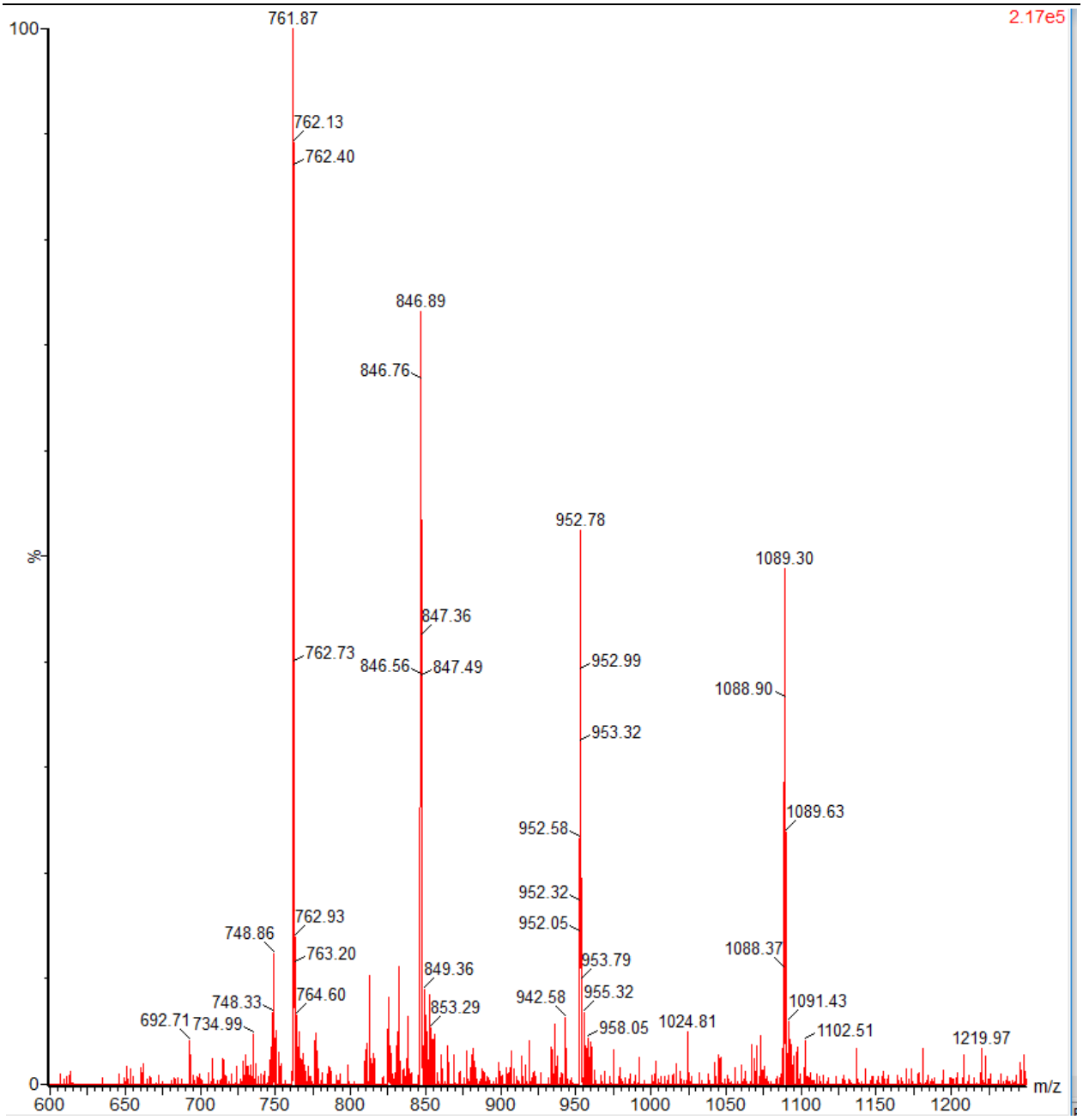
A260



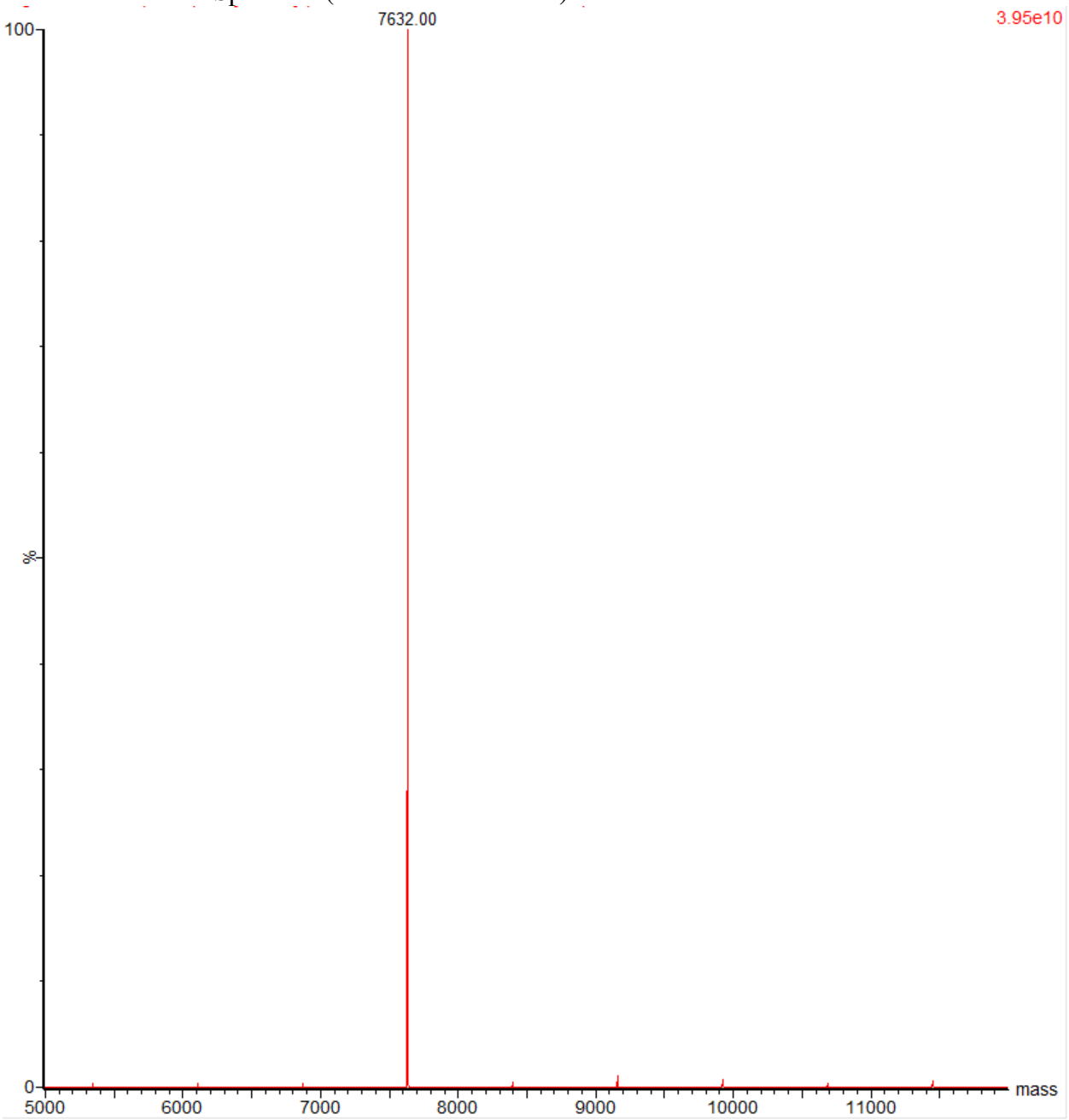
TIC



Raw Mass Spectrum (Retention Time 2.30)


2.17e5
100
%
0
761.87
762.13
762.40
846.89
846.76
952.78
1089.30
847.36
762.73
846.56
847.49
952.99
1088.90
953.32
1089.63
952.58
952.32
952.05
762.93
748.86
763.20
849.36
953.79
1088.37
748.33
764.60
853.29
955.32
942.58
1091.43
692.71
734.99
958.05
1024.81
1102.51
1219.97
m/z
600
650
700
750
800
850
900
950
1000
1050
1100
1150
1200

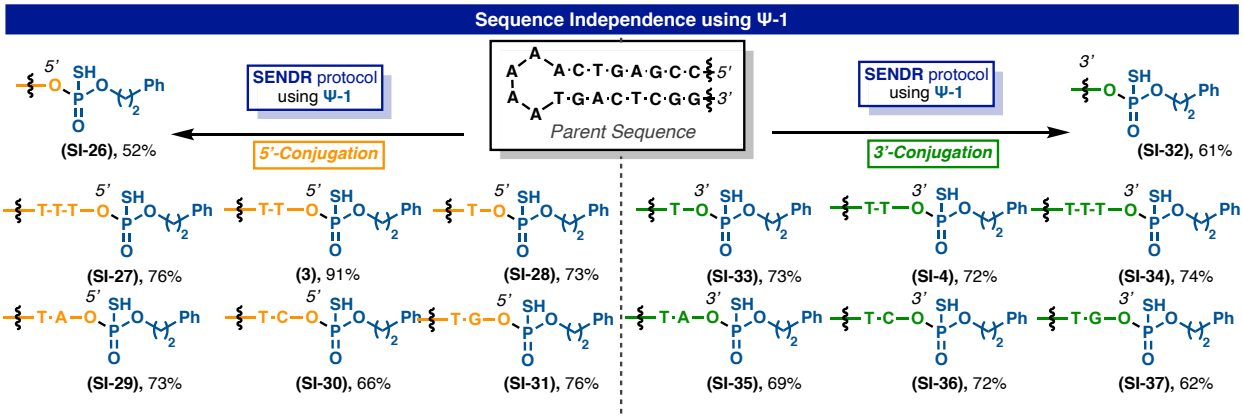
Deconvulted Mass Spectrum (Retention Time 2.30)



Sequence Independence

Procedure

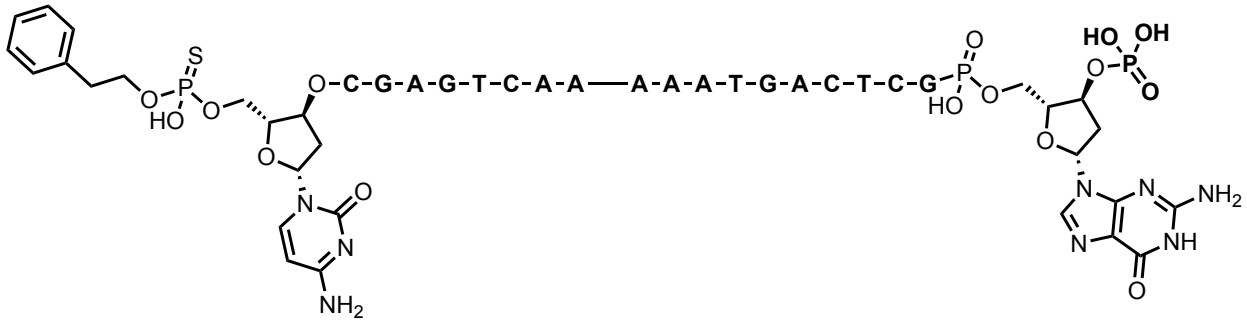
SENDR was optimized by performing reactions in microcentrifuge tube as described in *General Procedure 2.* Briefly, 5 nmol of DNA **1** or **2** was loaded onto 100 µL of equilibrated resin. This loaded resin was washed with DMA (500 µL twice) and dry THF (500 µL three times) and dried under vacuum.
Ψ-1 in MeCN (150 mM, 250 µL) was added to the loaded and dried resin. Then DBU (450 mM, 18 µL) was added to the reaction mixture. The reaction tube was vortexed for 30 seconds and incubated at room temperature for various amounts of time. The reactions were worked up by aspirating and discarding the reaction solution, and washing the resin bed with MeCN (500 µL) and 1:1 MeCN:PBS (500 µL). Elute buffer (300 µL) was added to the resin bed and the tube was agitated by orbital shaker for 5-10 minutes. The DNA containing elution buffer was collected by carefully pipetting the supernatant. DNA was isolated *via* ethanol precipitation. Ethanol precipitation was performed by adding 10% v/v of a NaCl solution (30 µL, 5M) to the elute buffer and three volumes of cold ethanol (-20 ºC) were added to the tube (~1000µL) and incubated for 18 hours at -20 ºC. The tube was then centrifuged at 13,000 rpm for 15 minutes to pellet the DNA, and the extra ethanol was decanted off. The tubes were dried *via* speed vacuum and the DNA was dissolved and HPLC-MS analysis was performed.



Characterization of Sequence Independence

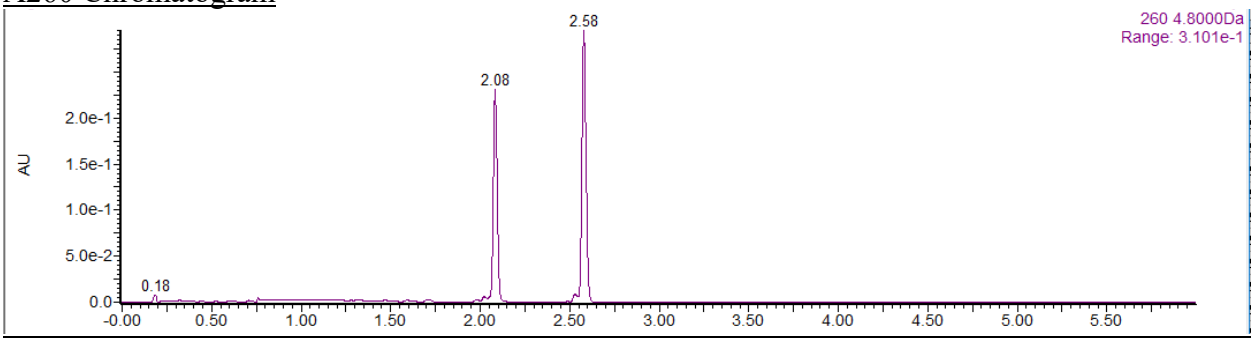
LCMS Characterization of SI-26

Produced from **SI-1** by General Procedure **2:**
Yield (A260): 52%
Expected Mass: 6415.22
Expected (M-6)/6 Ion: 1068.2
Found Deconvoluted Mass: 6415.00
Found (M-6)/6 Ion: 1068.16

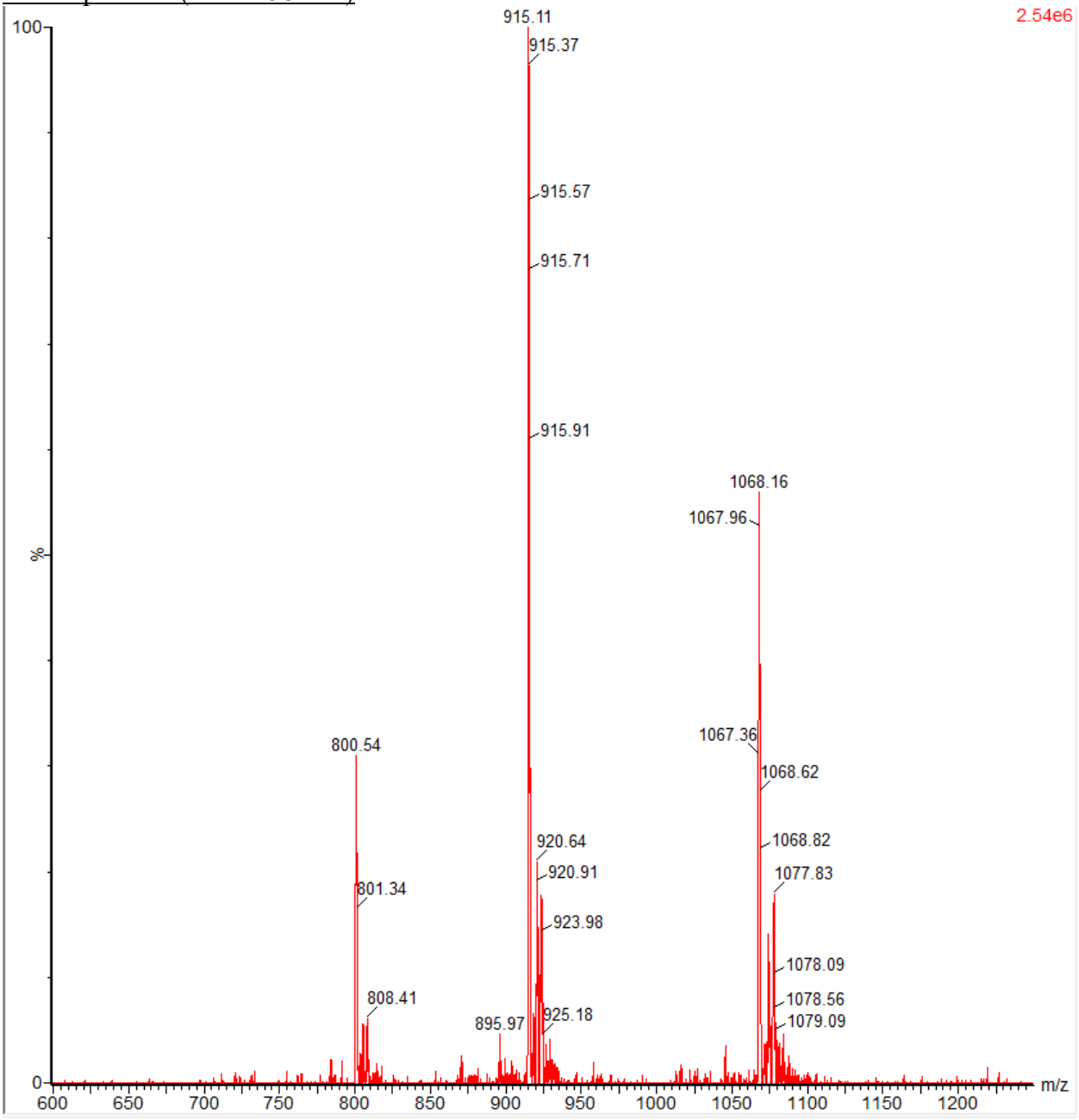


Exact Mass: 6412.05
Molecular Weight: 6415.22

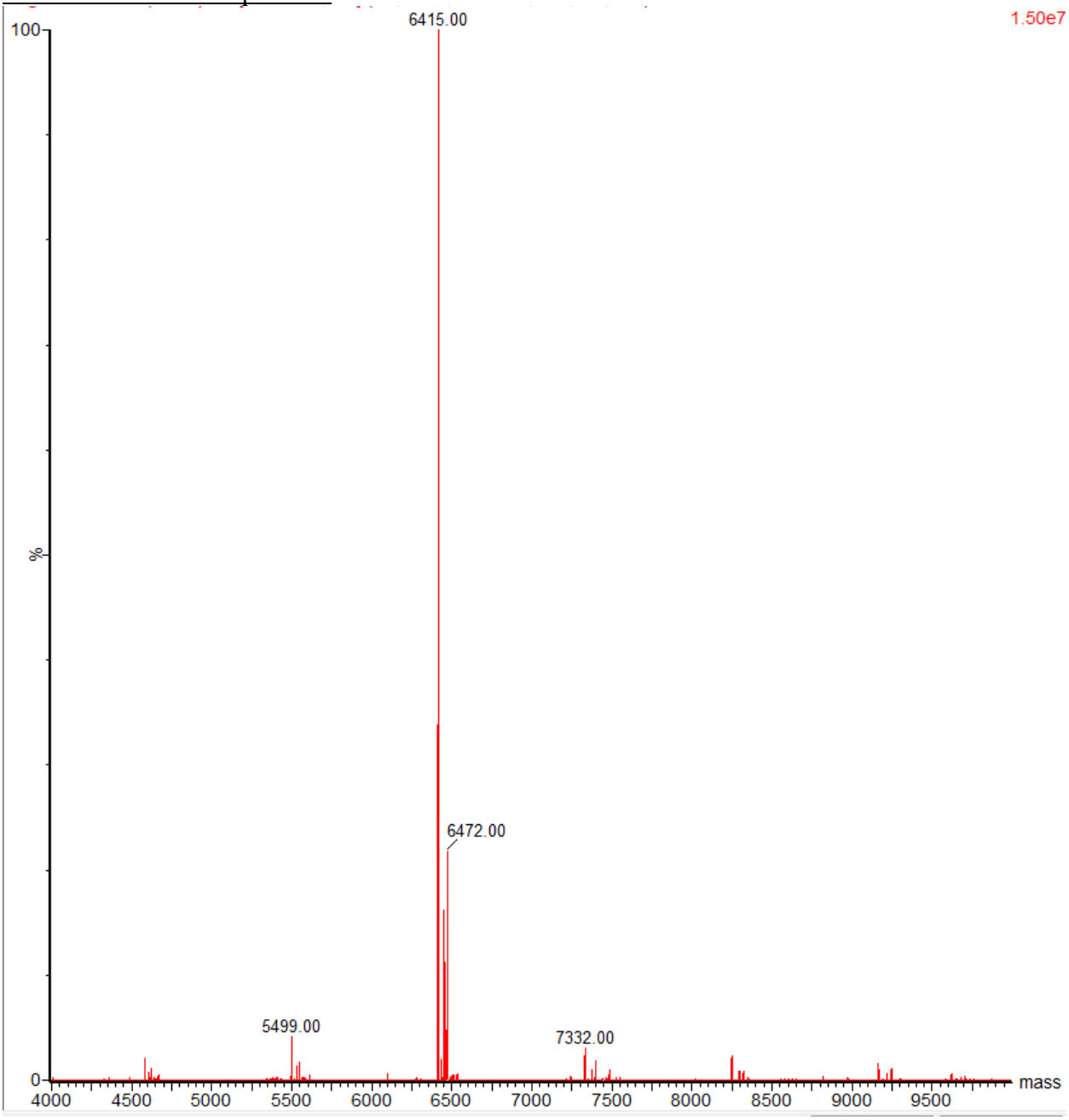
A260 Chromatogram



Mass Spectrum (RT= 2.58 min)

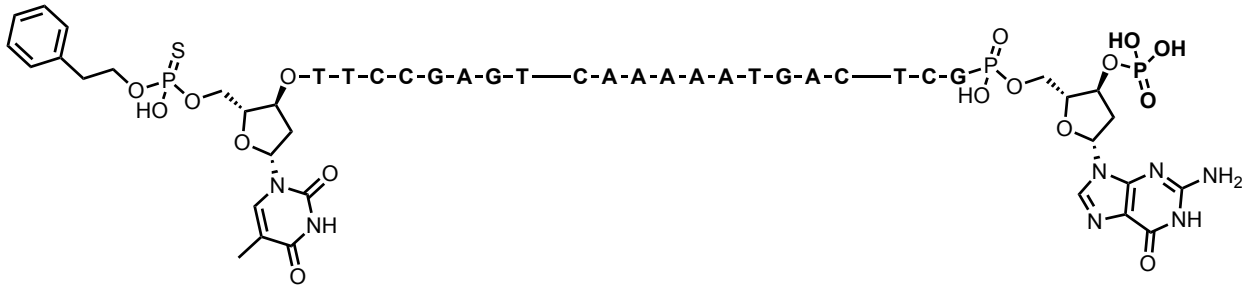


Deconvoluted Mass Spectrum



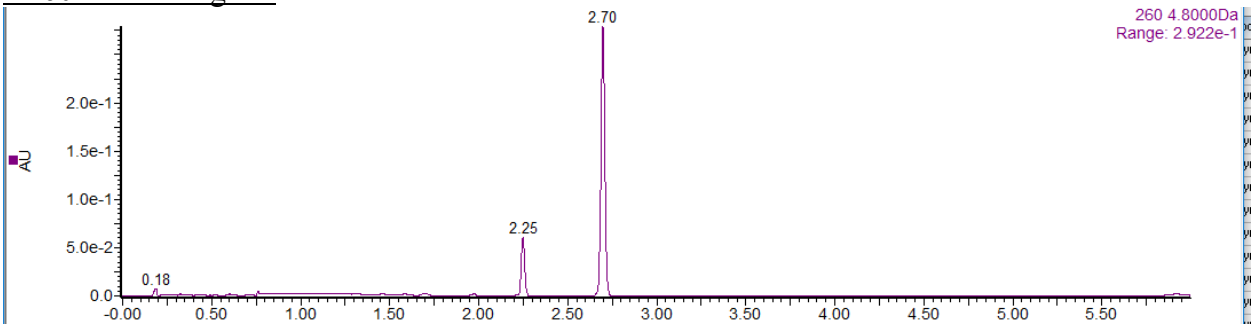
LCMS Characterization of SI-27

Produced from **SI-2** by General Procedure **2:**
Yield (A260): 76%
Expected Mass: 7327.81
Expected (M-6)/6 Ion: 1220.2
Found Deconvoluted Mass: 7328.00
Found (M-6)/6 Ion: 1220.11

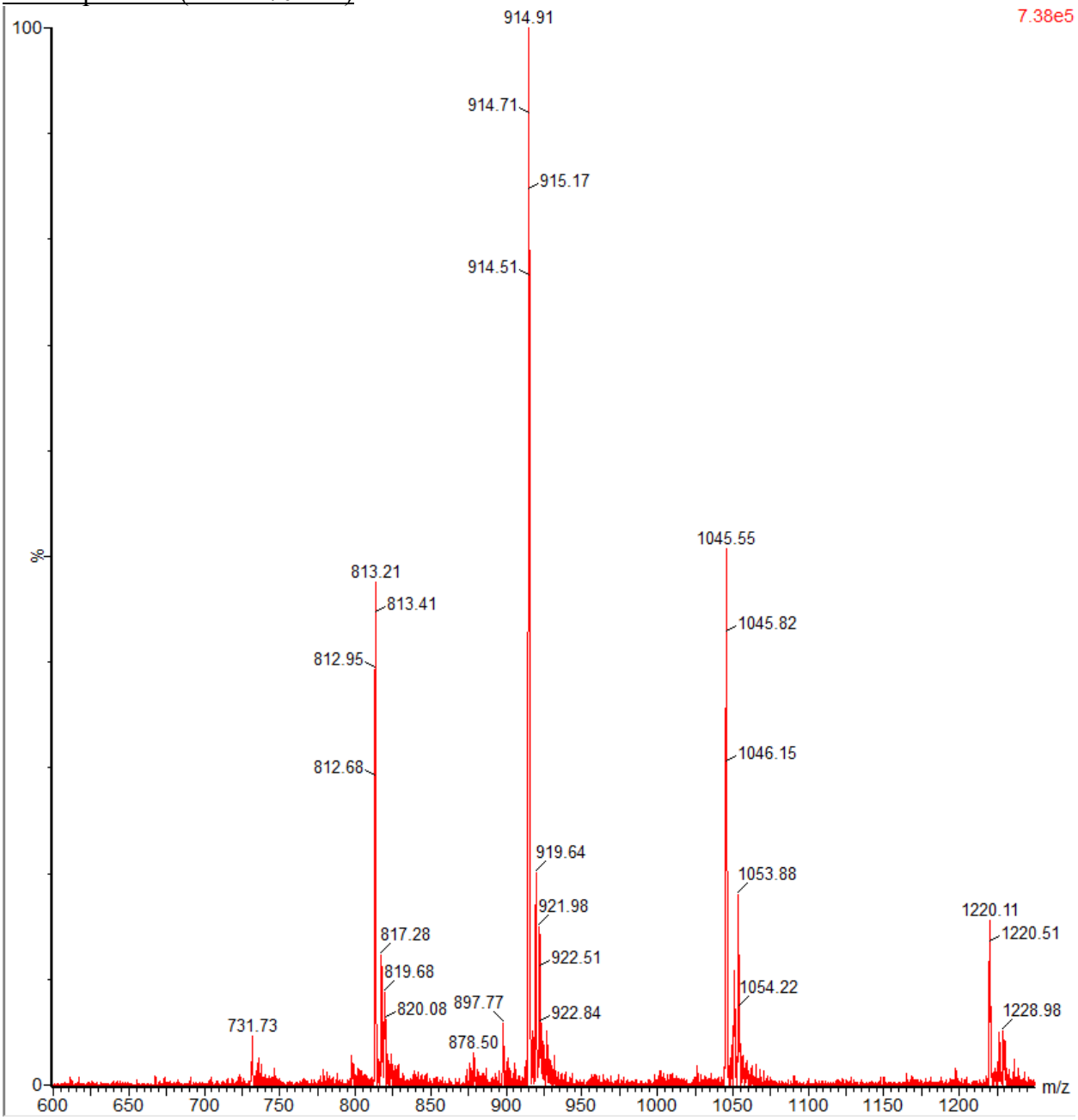


Exact Mass: 7324.19
Molecular Weight: 7327.81

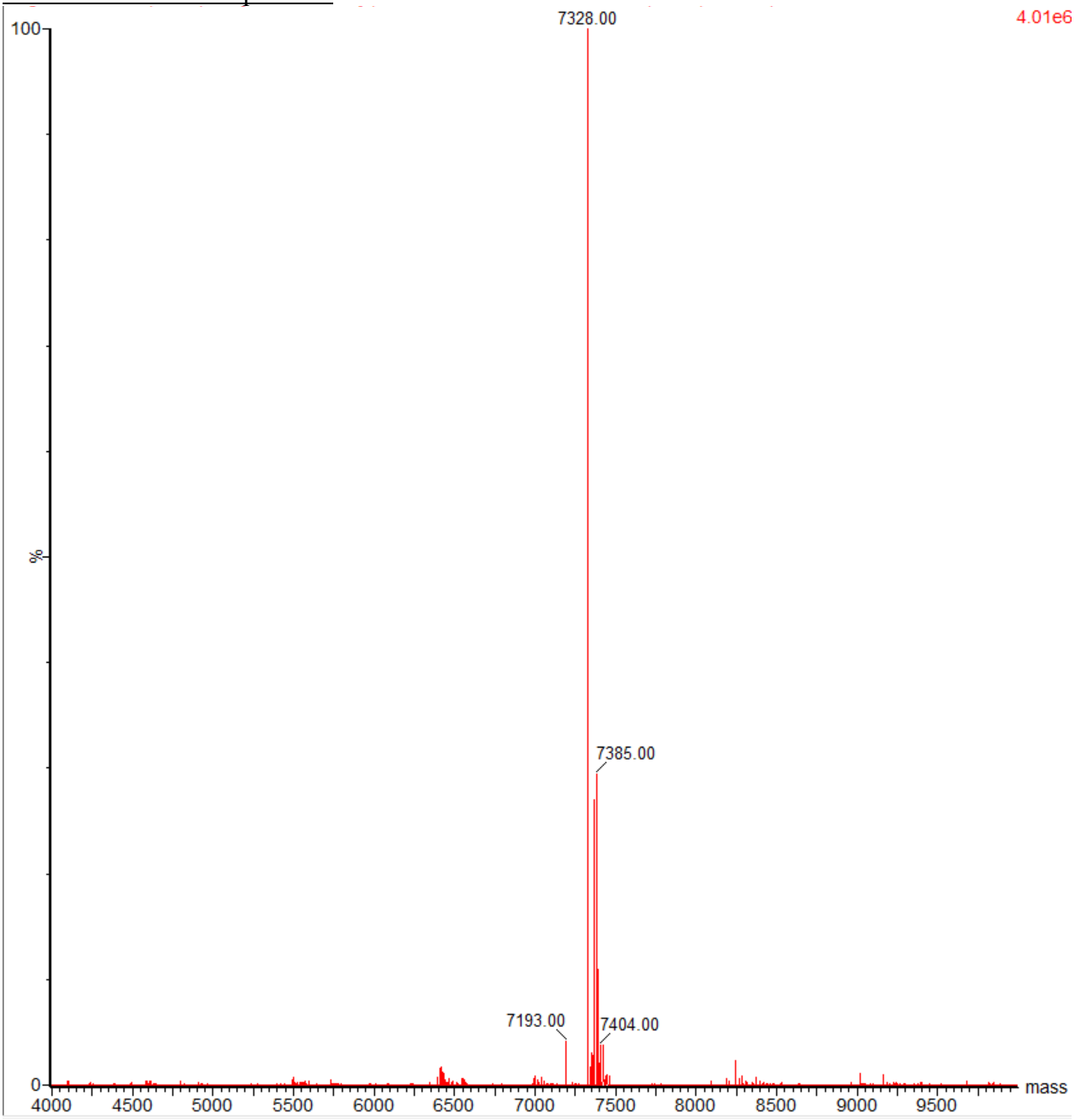
A260 Chromatogram



Mass Spectrum (RT= 2.70 min)

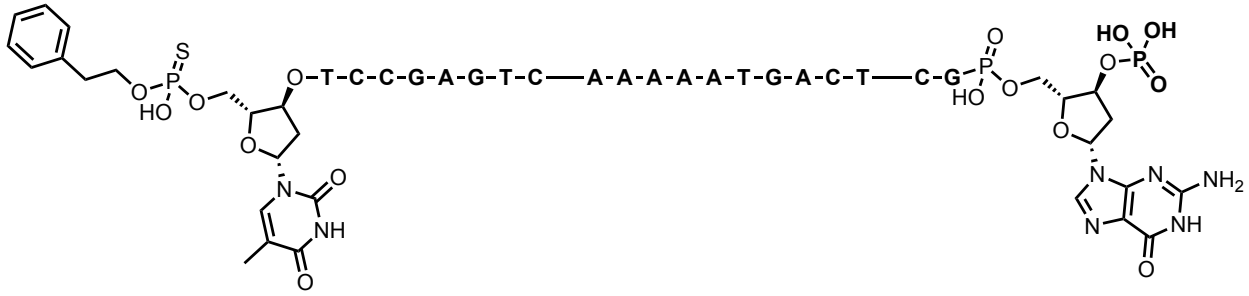


Deconvoluted Mass Spectrum



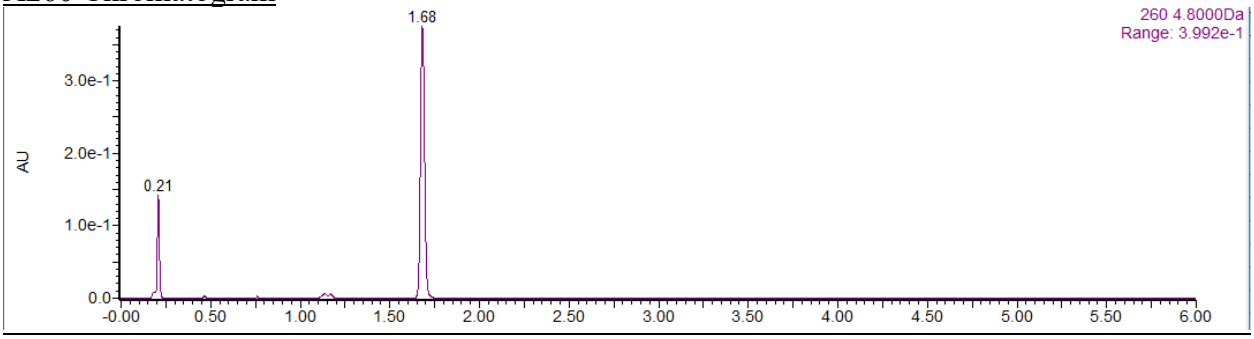
LCMS Characterization of 3

Produced from **1** by General Procedure **2:**
Yield (A260): 91%
Expected Mass: 7023.61
Expected (M-6)/6 Ion: 1169.60
Found Deconvoluted Mass: 7024.00
Found (M-6)/6 Ion: 1169.61

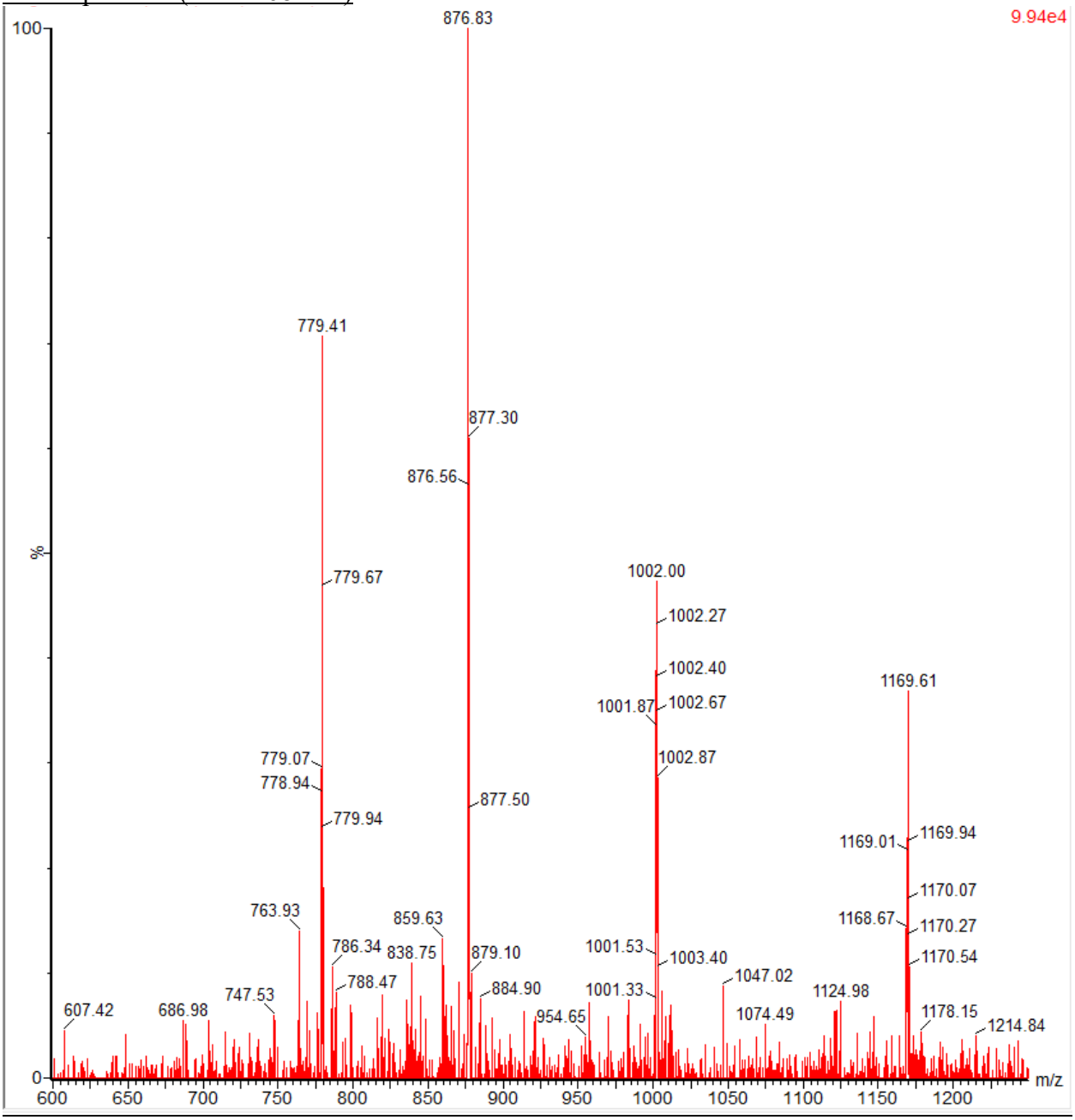


Exact Mass: 7020.14
Molecular Weight: 7023.61

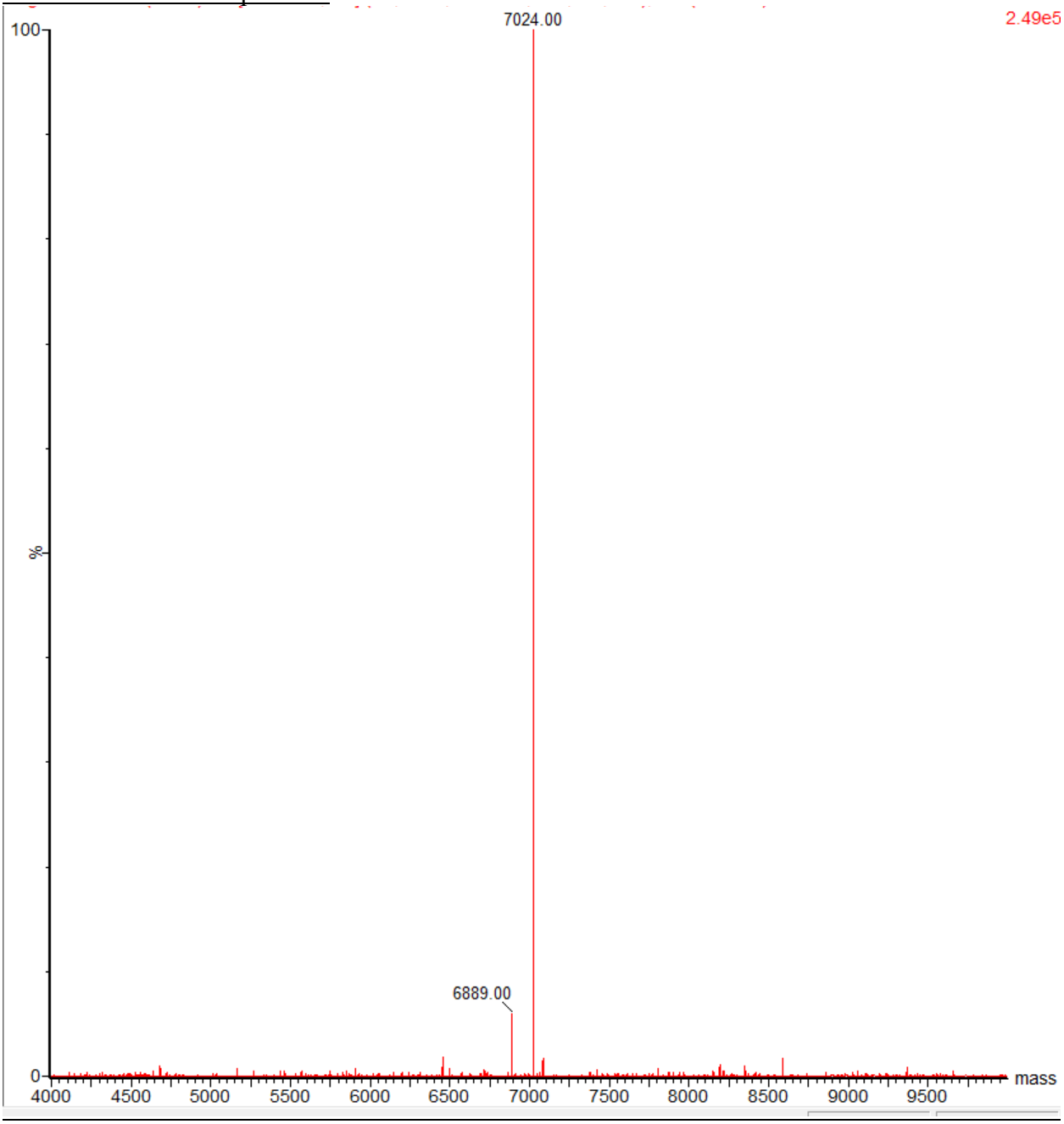
A260 Chromatogram



Mass Spectrum (RT= 1.68 min)

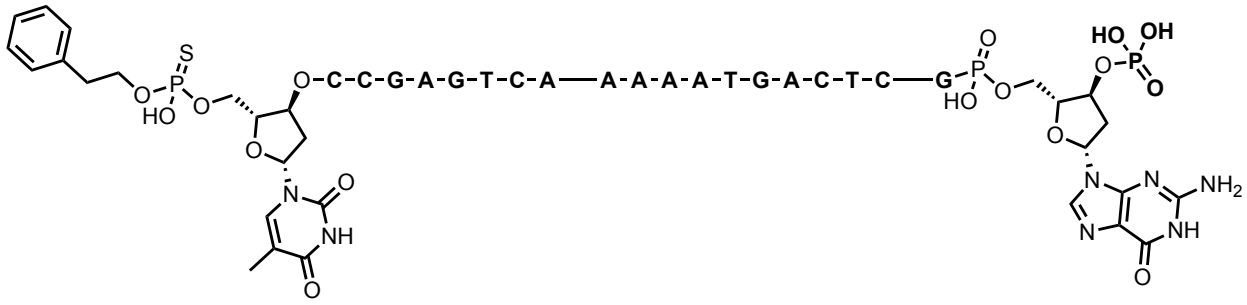


Deconvoluted Mass Spectrum



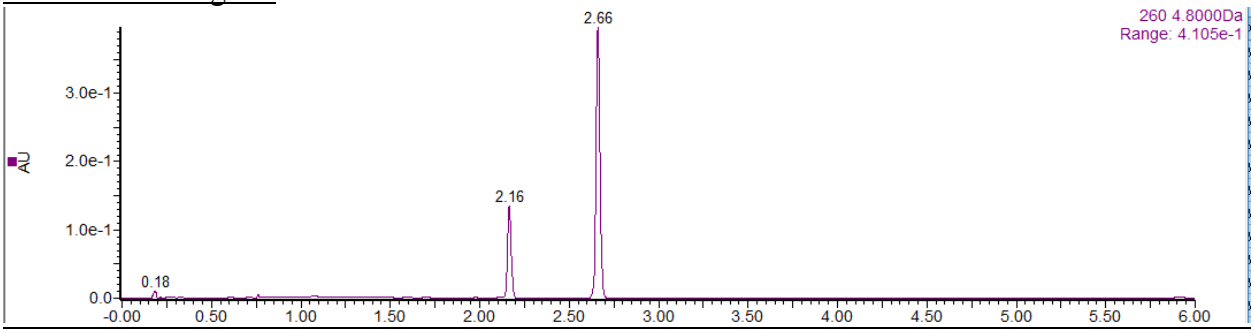
LCMS Characterization of SI-28

Produced from **SI-3** by General Procedure **2:**
Yield (A260): 73%
Expected Mass: 6719.42
Expected (M-8)/8 Ion: 838.93
Found Deconvoluted Mass: 6720.00
Found (M-8)/8 Ion: 838.95

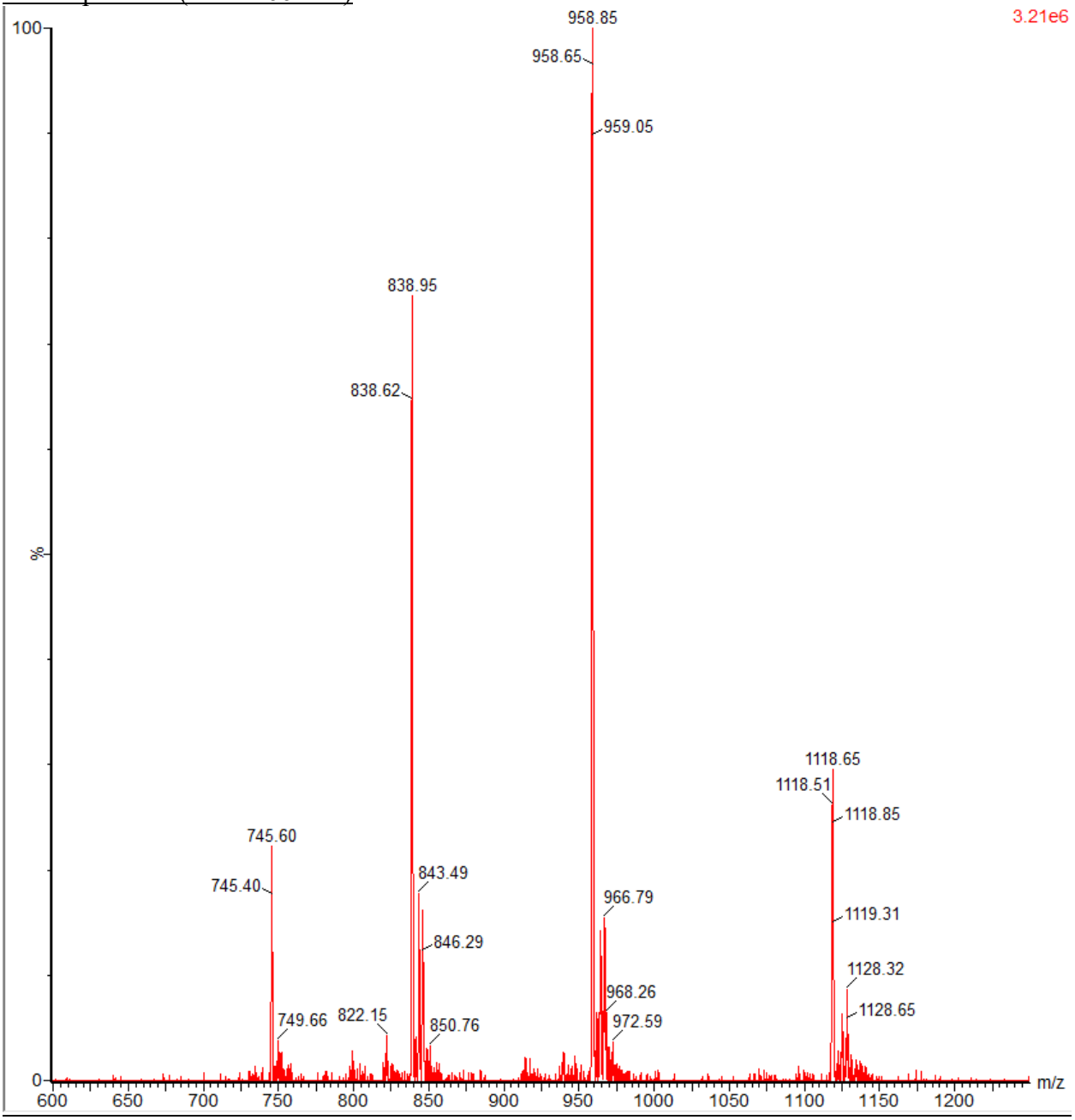


Exact Mass: 6716.10
Molecular Weight: 6719.42

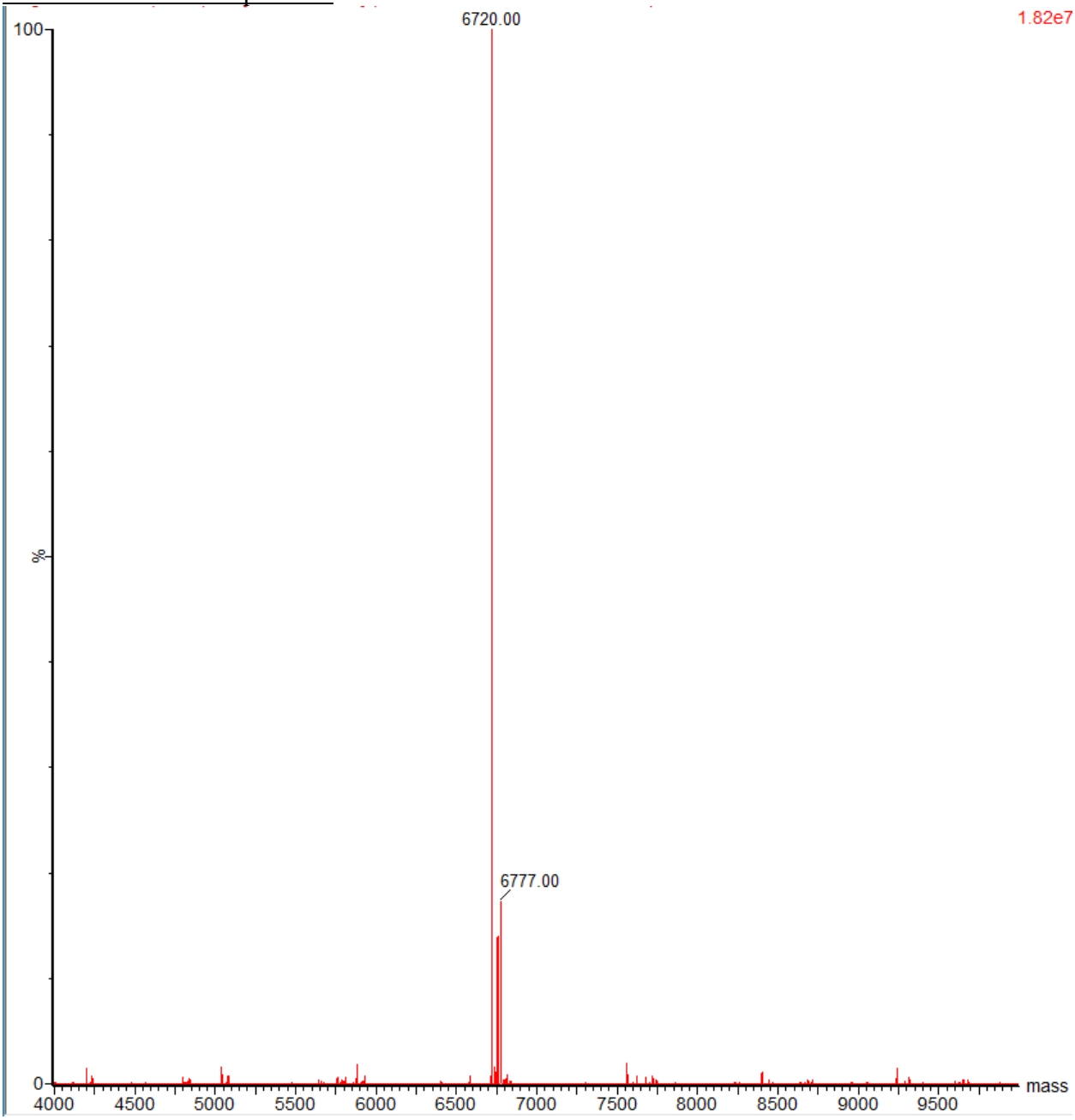
A260 Chromatogram



Mass Spectrum (RT= 2.66 min)

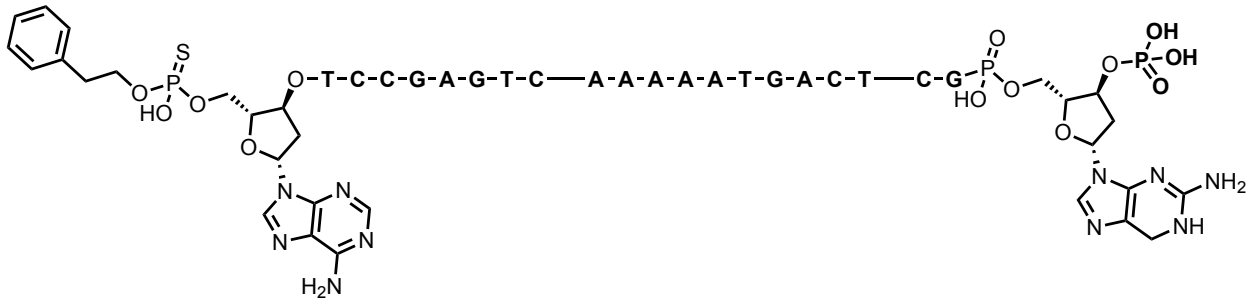


Deconvoluted Mass Spectrum



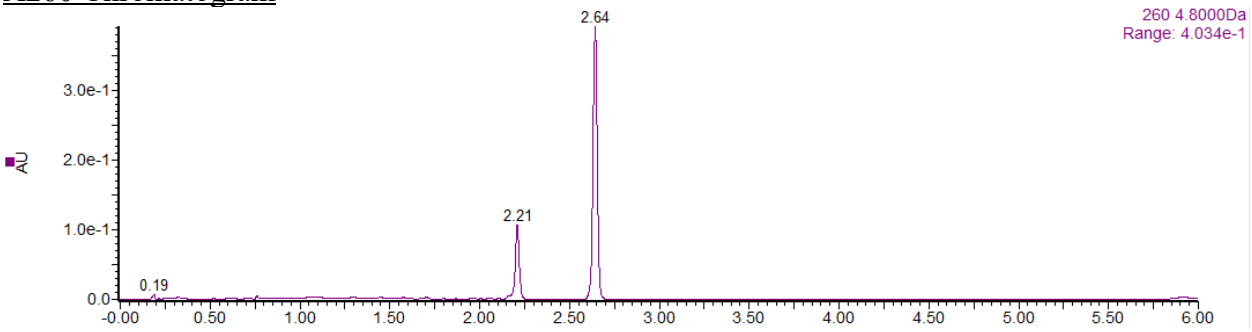
LCMS Characterization of SI-29

Produced from **SI-4** by General Procedure **2:**
Yield (A260): 76%
Expected Mass: 6732.63
Expected (M-6)/6 Ion: 1171.105
Found Deconvoluted Mass: 6733.00
Found (M-6)/6 Ion: 1171.07

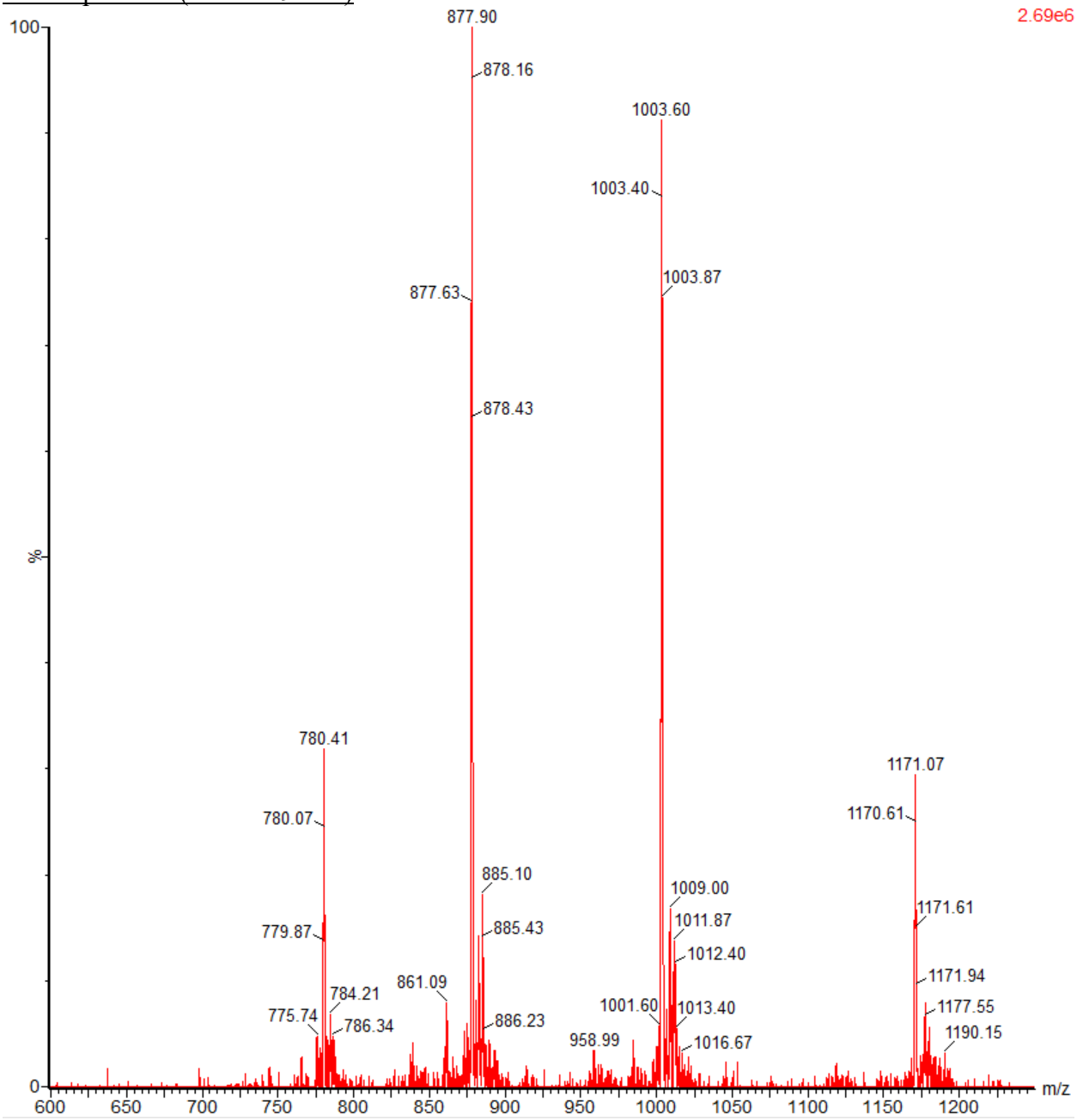


Exact Mass: 7029.16
Molecular Weight: 7032.63

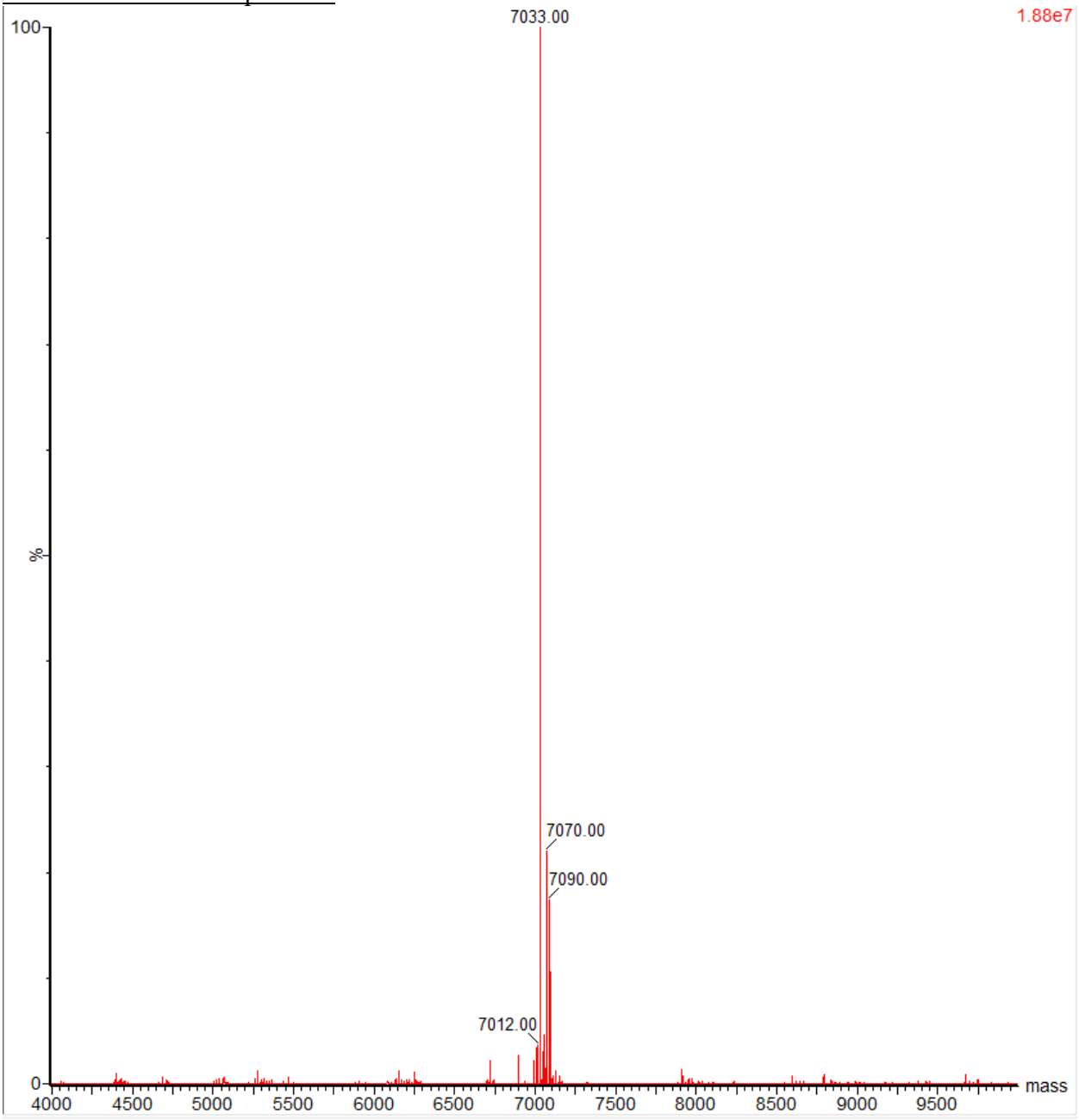
A260 Chromatogram



Mass Spectrum (RT= 2.19 min)

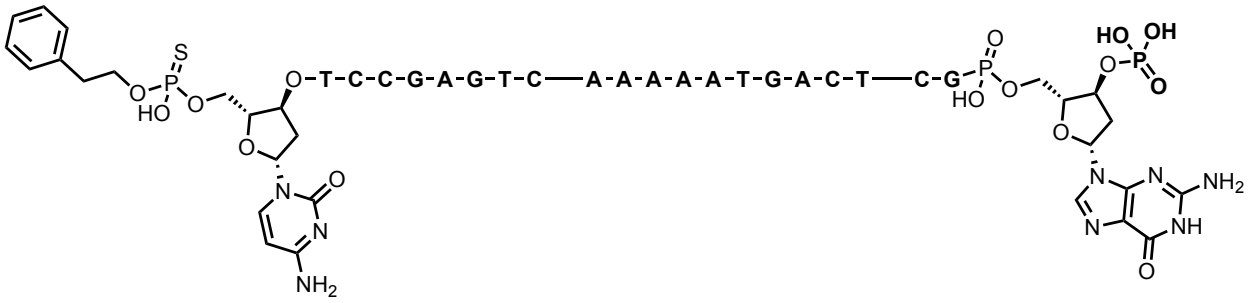


Deconvoluted Mass Spectrum



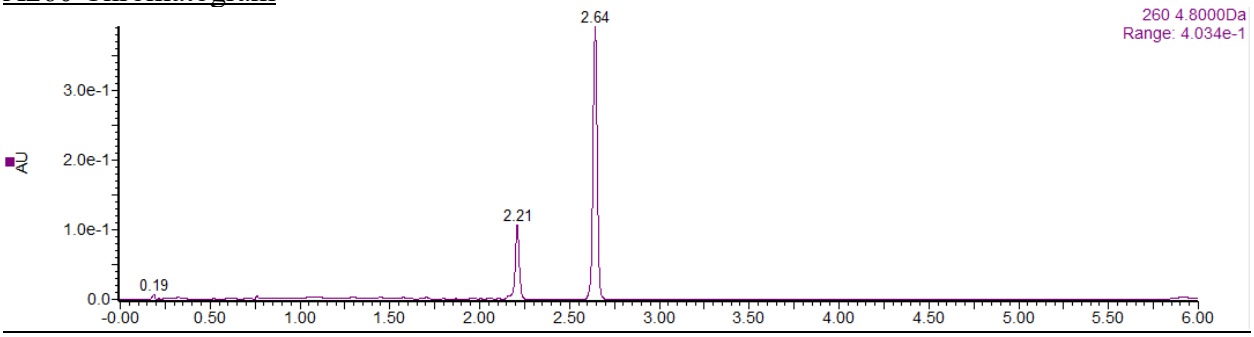
LCMS Characterization of SI-30

Produced from **SI-5** by General Procedure **2:**
Yield (A260): 66%
Expected Mass: 7008.6
Expected (M-6)/6 Ion: 1167.1
Found Deconvoluted Mass: 7008.00
Found (M-6)/6 Ion: 1166.7

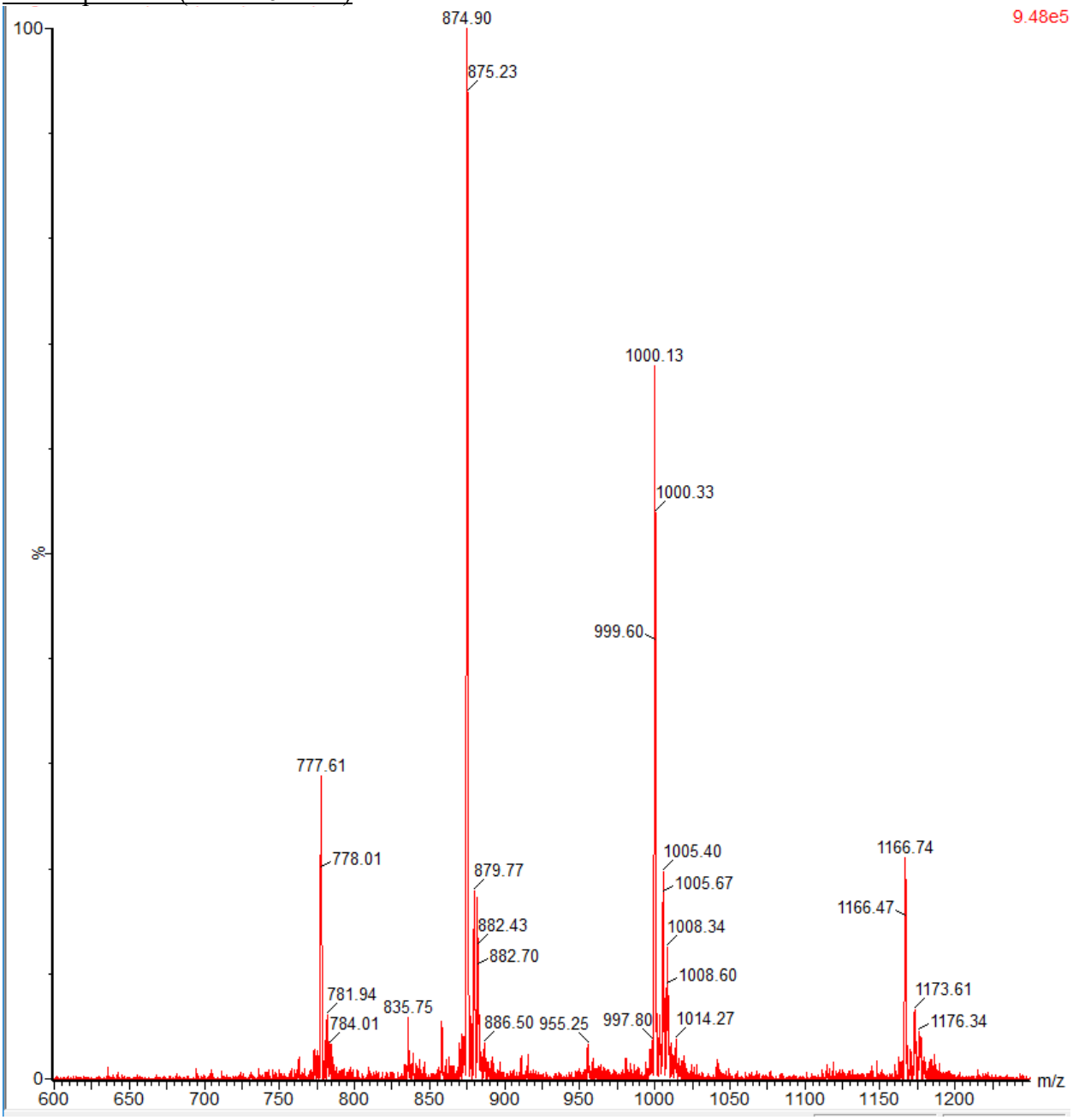


Exact Mass: 7005.14
Molecular Weight: 7008.60

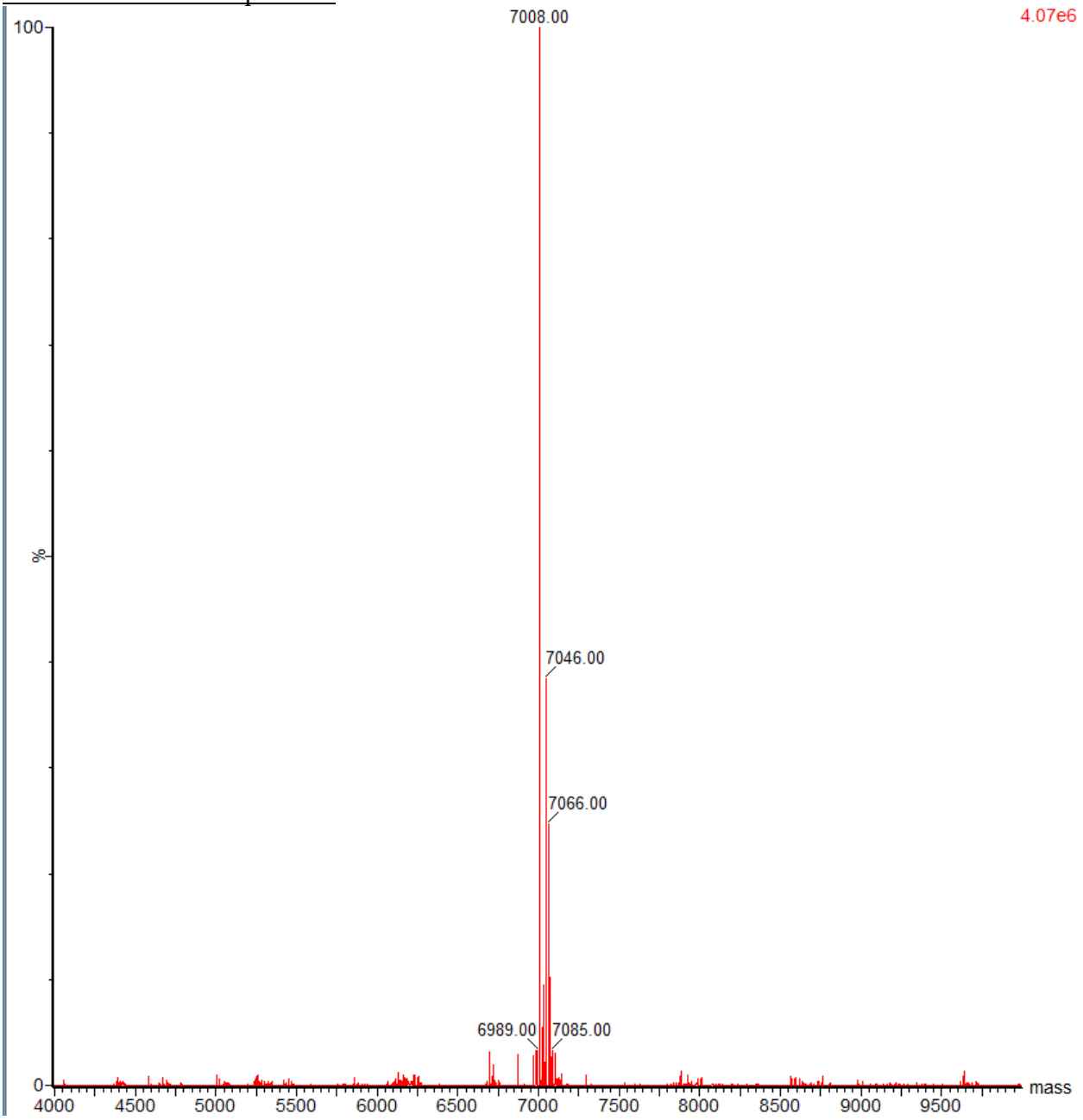
A260 Chromatogram



Mass Spectrum (RT= 2.64 min)

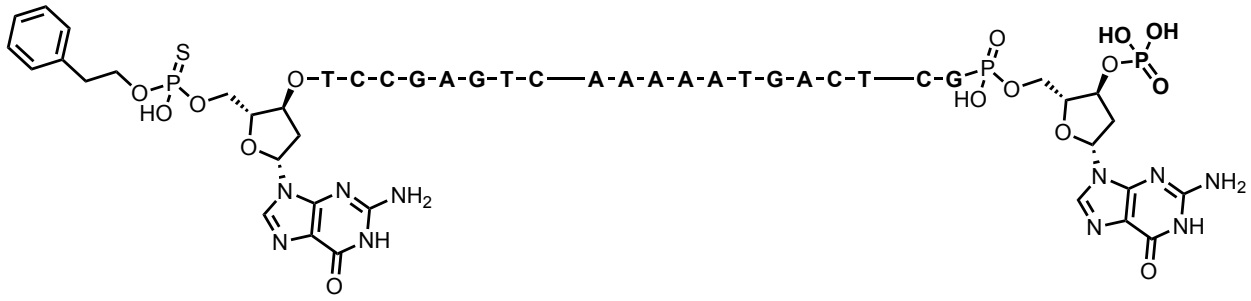


Deconvoluted Mass Spectrum



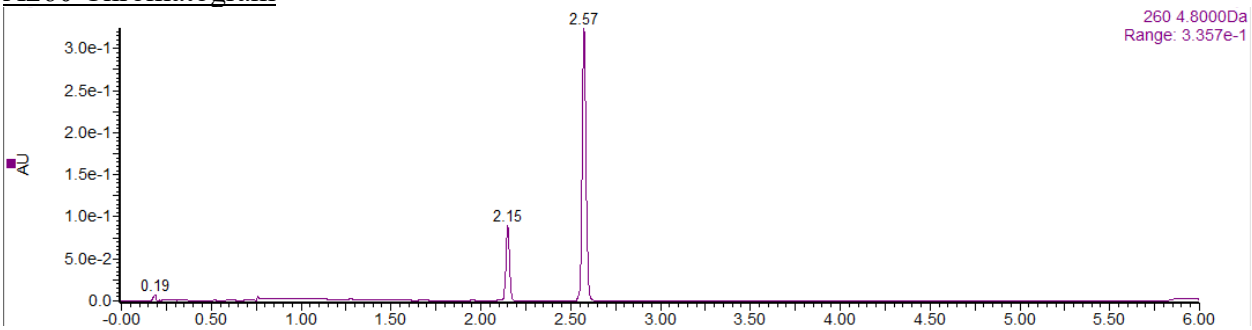
LCMS Characterization of SI-31

Produced from **SI-6** by General Procedure **2:**
Yield (A260): 76%
Expected Mass: 7048.63
Expected (M-6)/6 Ion: 1173.77
Found Deconvoluted Mass: 7049.00
Found (M-6)/6 Ion: 1173.61

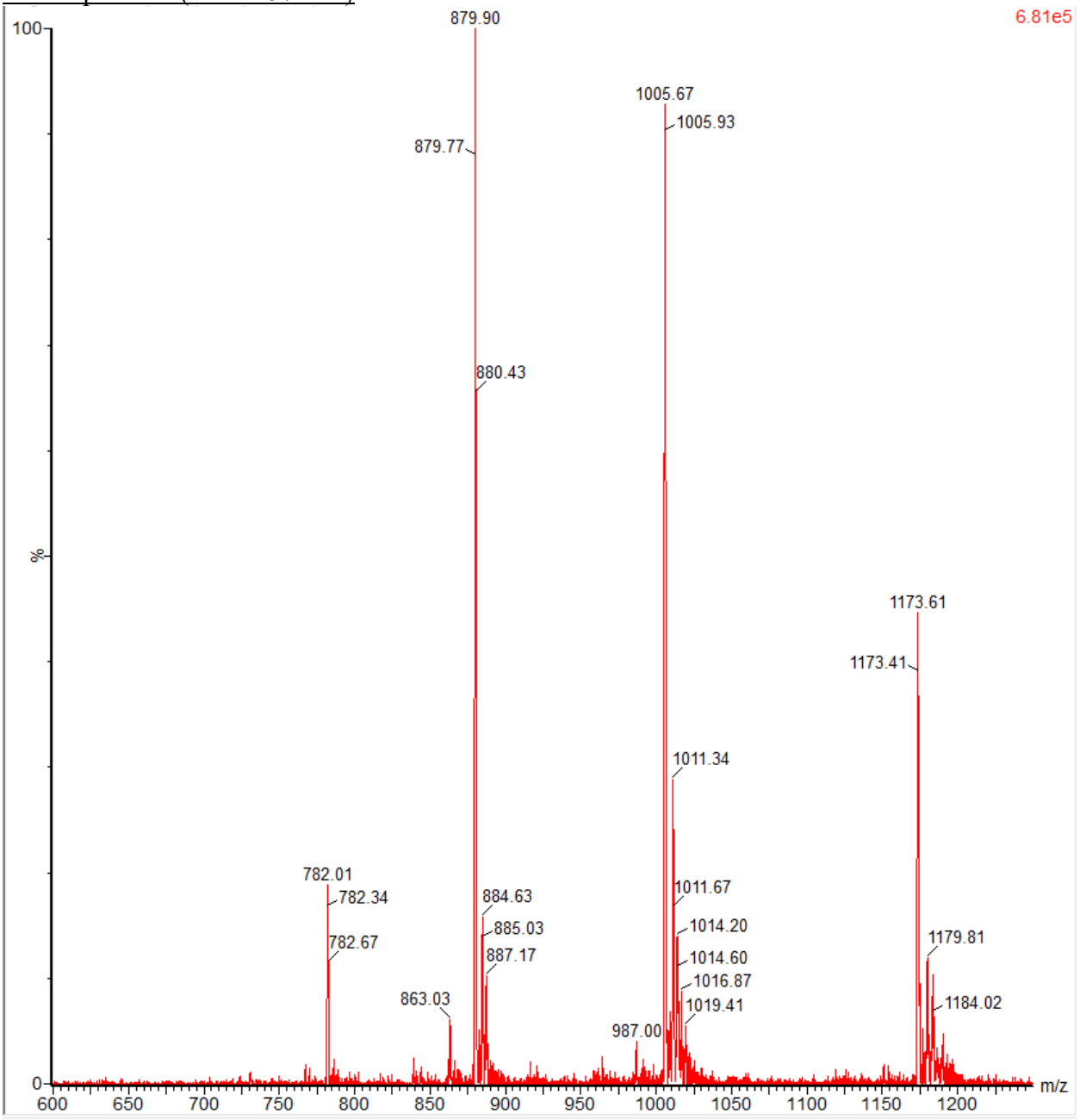


Exact Mass: 7045.15
Molecular Weight: 7048.63

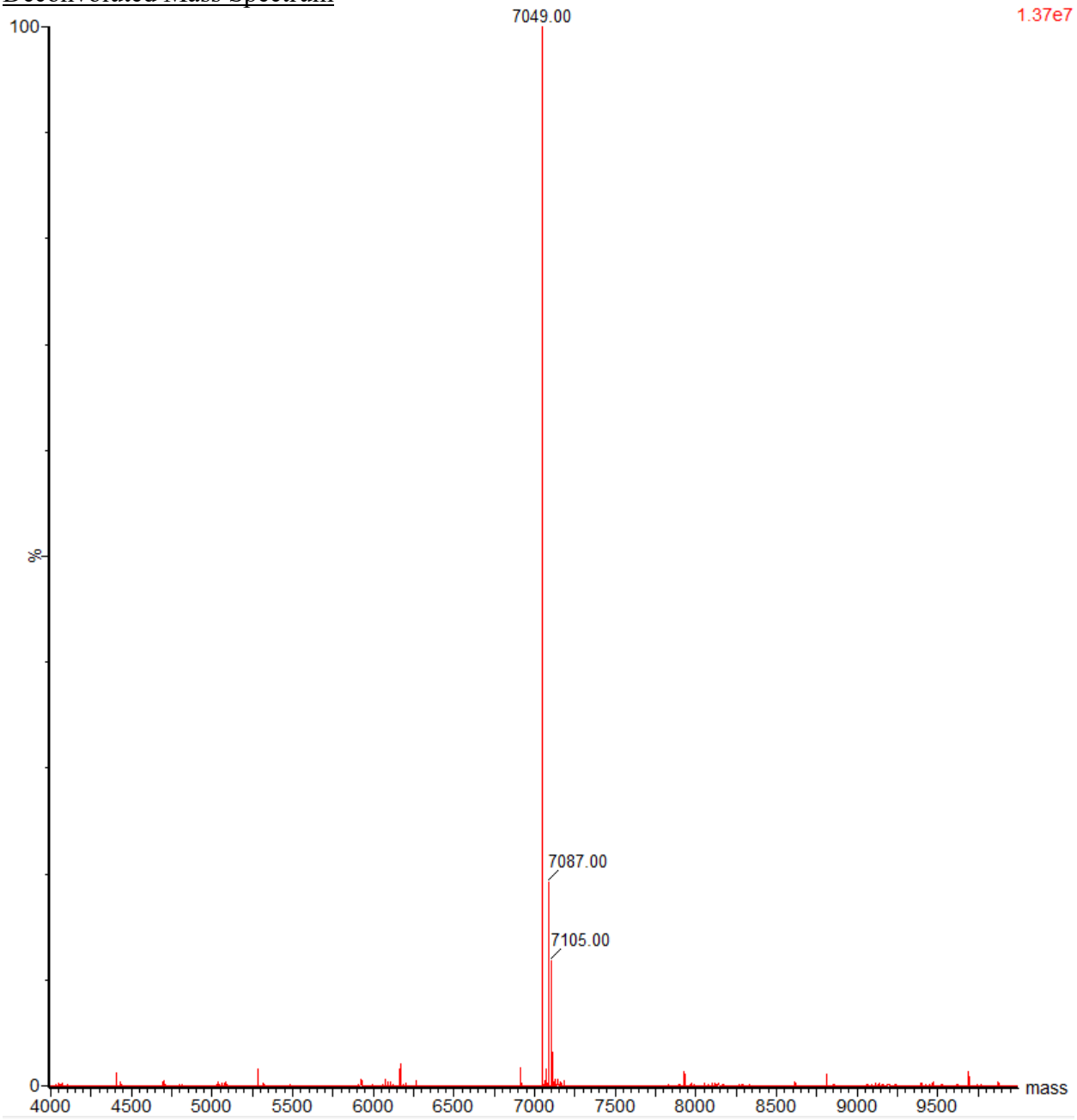
A260 Chromatogram



Mass Spectrum (RT= 2.57 min)

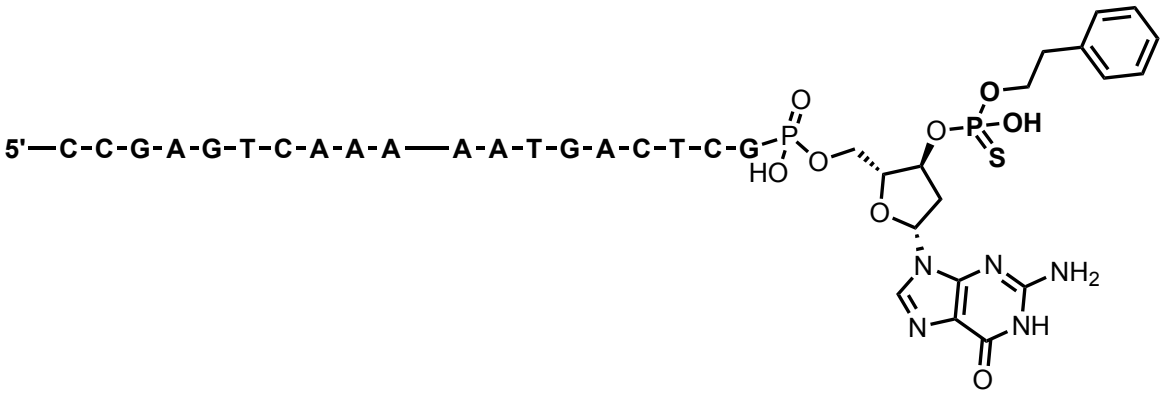


Deconvoluted Mass Spectrum



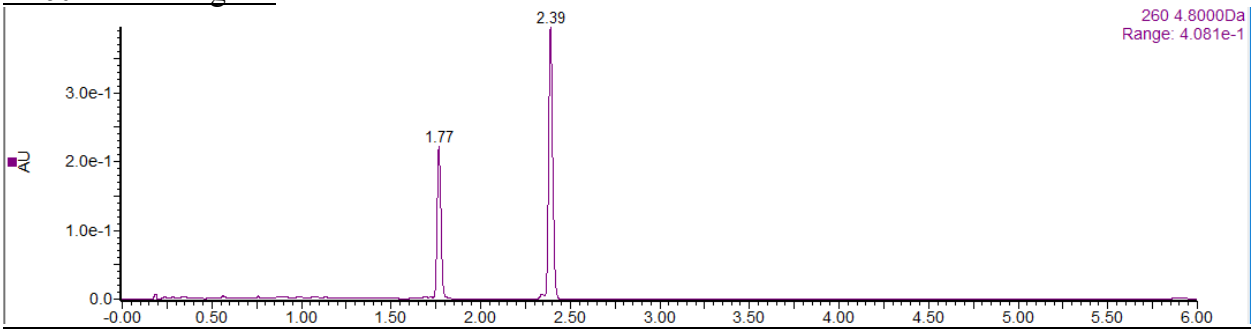
LCMS Characterization of SI-32

Produced from **SI-7** by General Procedure **2:**
Yield (A260): 61%
Expected Mass: 6415.22
Expected (M-6)/6 Ion: 1068.20
Found Deconvoluted Mass: 6145.00
Found (M-6)/6 Ion: 1067.82

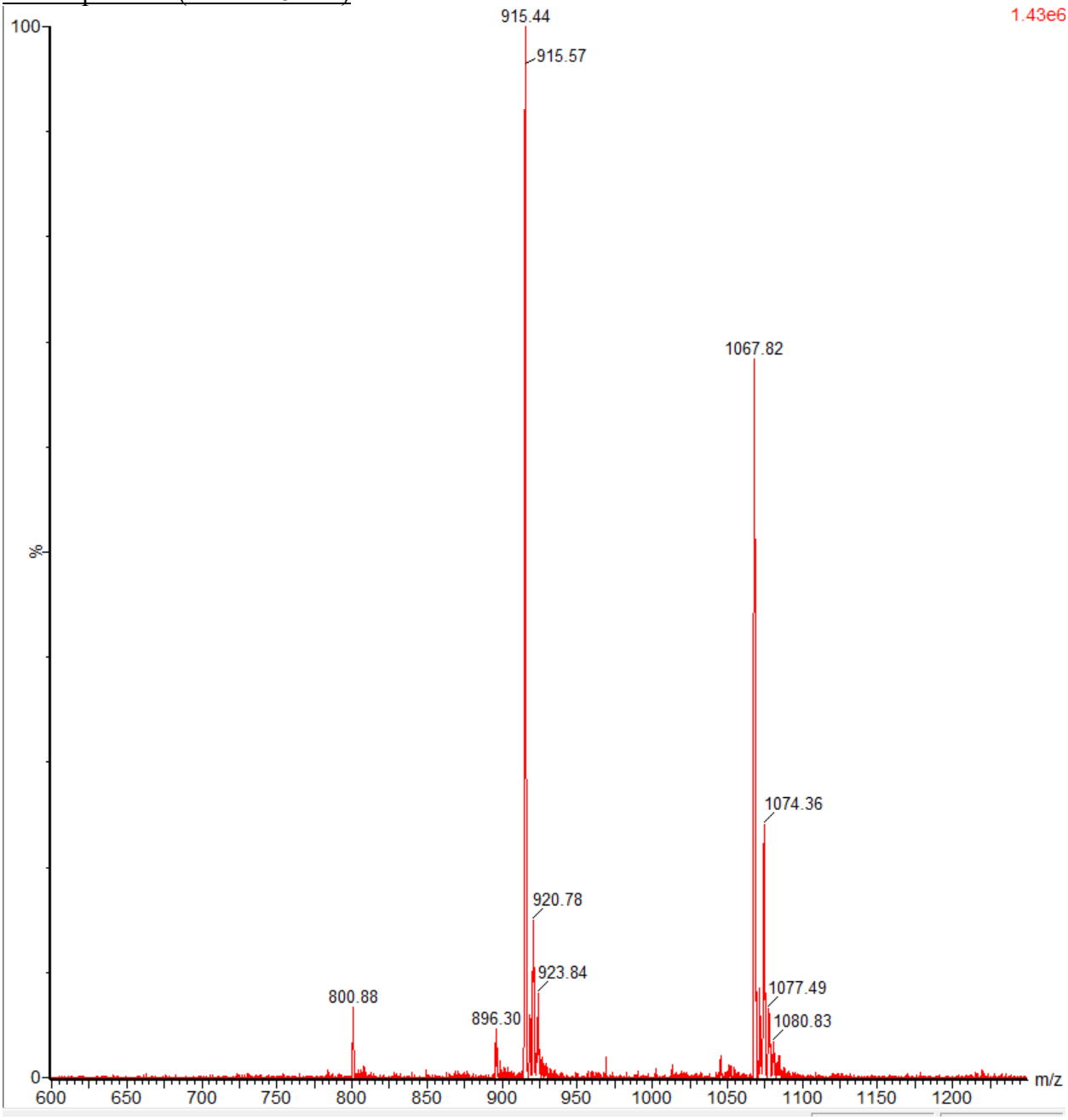


Exact Mass: 6412.05
Molecular Weight: 6415.22

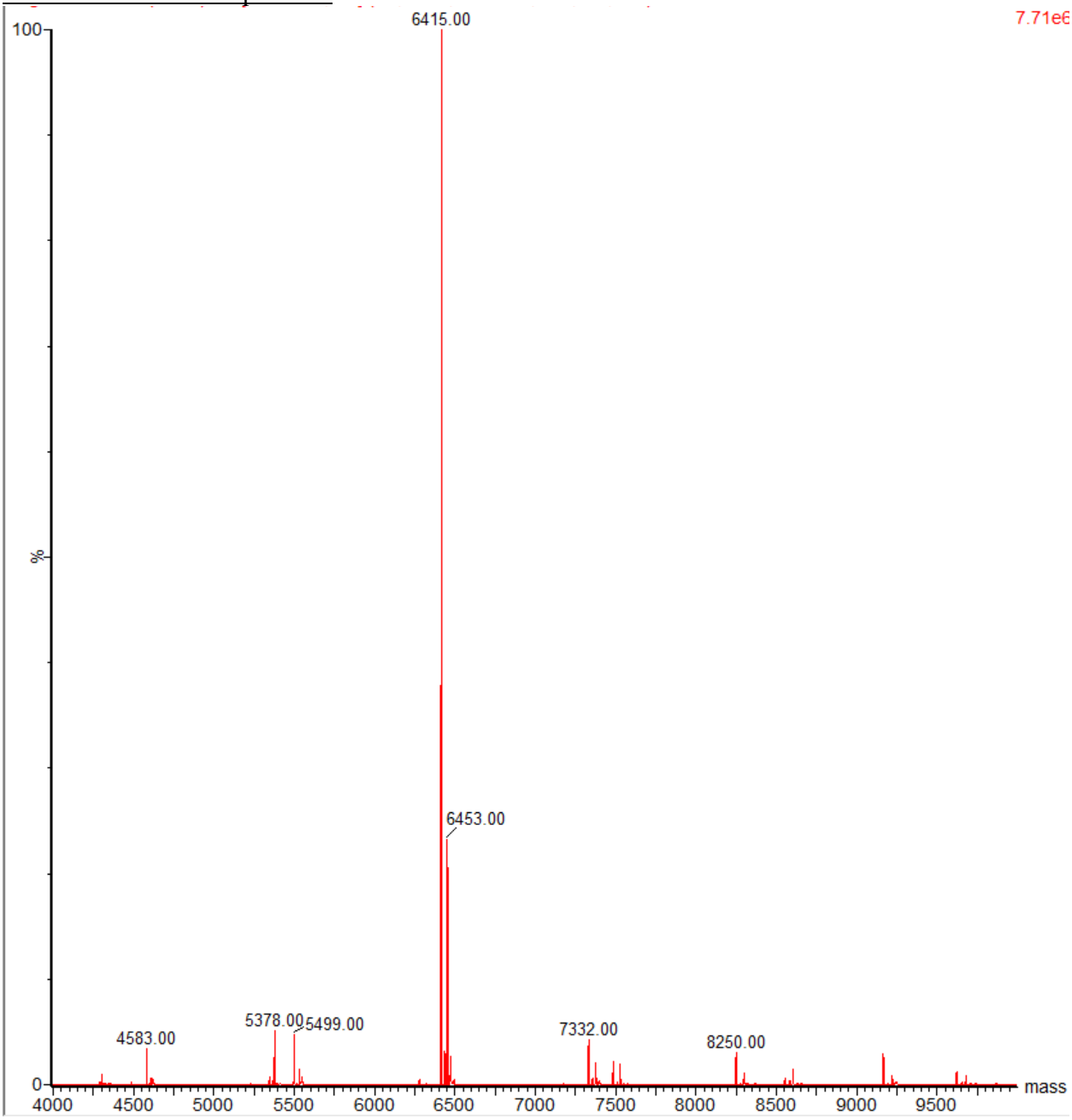
A260 Chromatogram



Mass Spectrum (RT= 2.18 min)

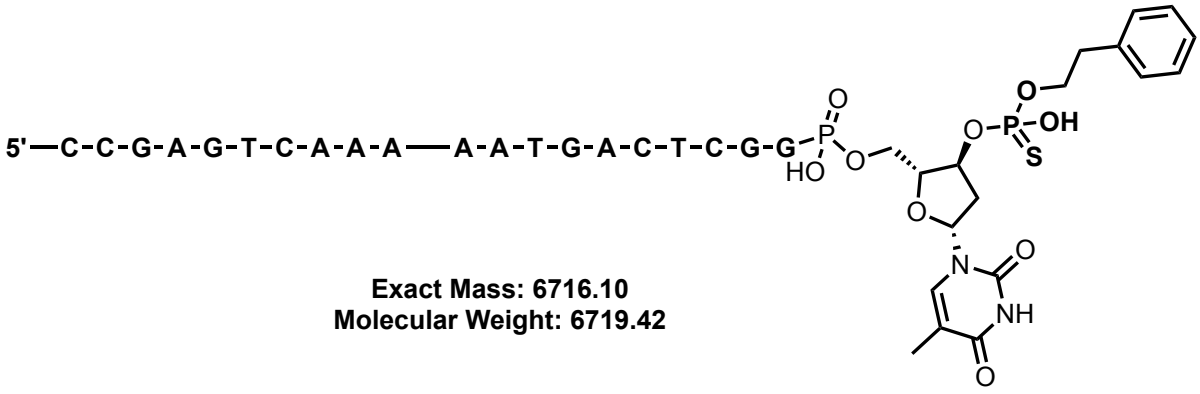


Deconvoluted Mass Spectrum



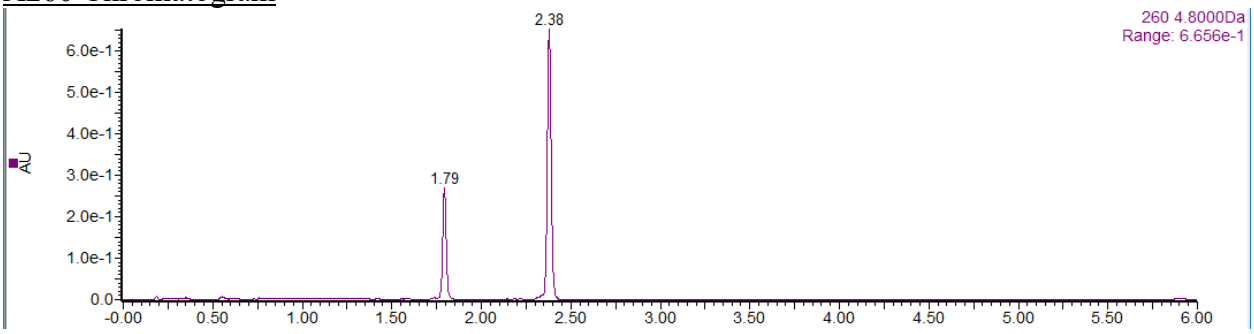
LCMS Characterization of SI-33

Produced from **SI-8** by General Procedure **2:**
Yield (A260): 73%
Expected Mass: 6719.42
Expected (M-6)/6 Ion: 1118.9
Found Deconvoluted Mass: 6719.00
Found (M-6)/6 Ion: 1118.45

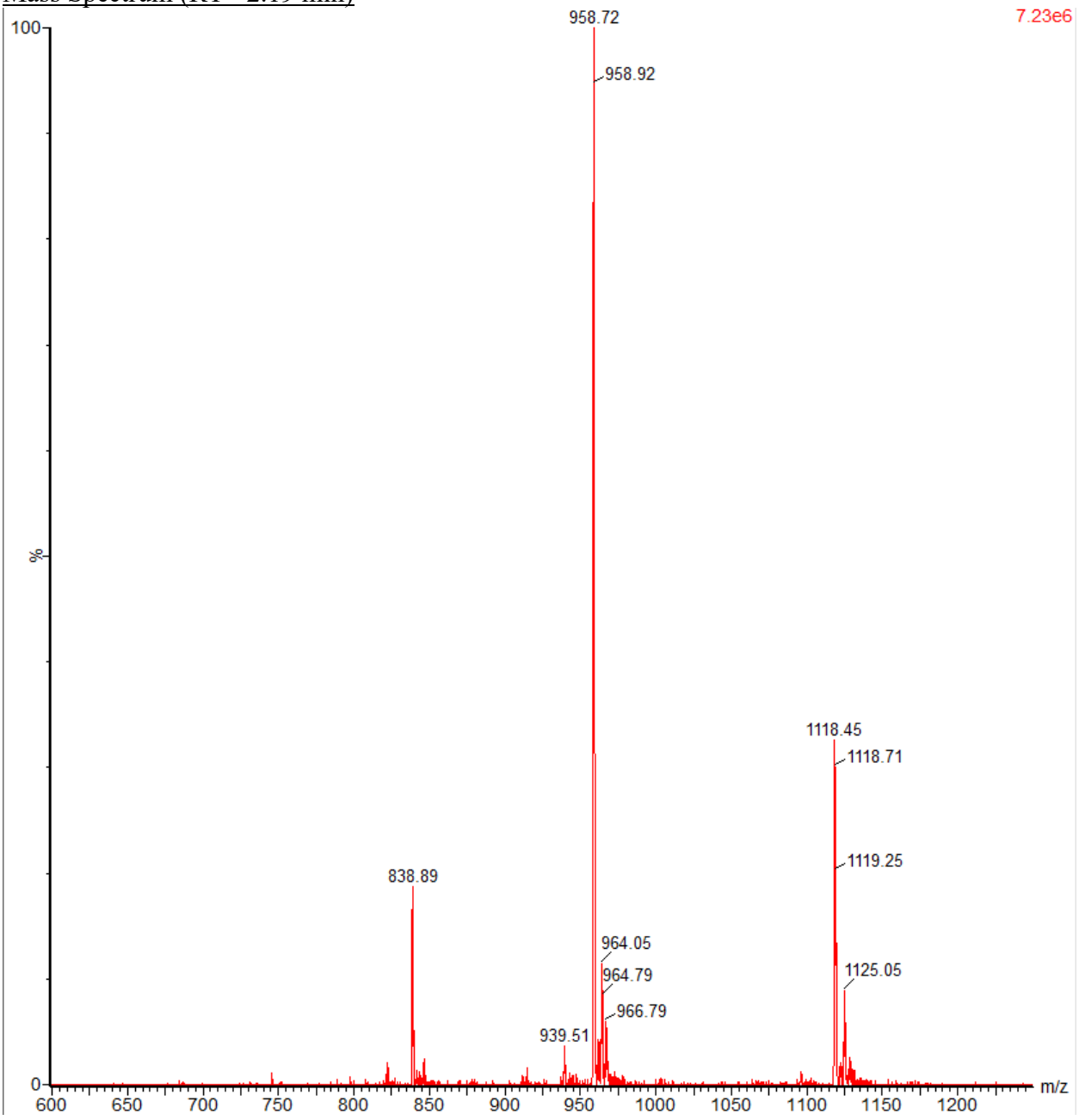


Exact Mass: 6716.10
Molecular Weight: 6719.42

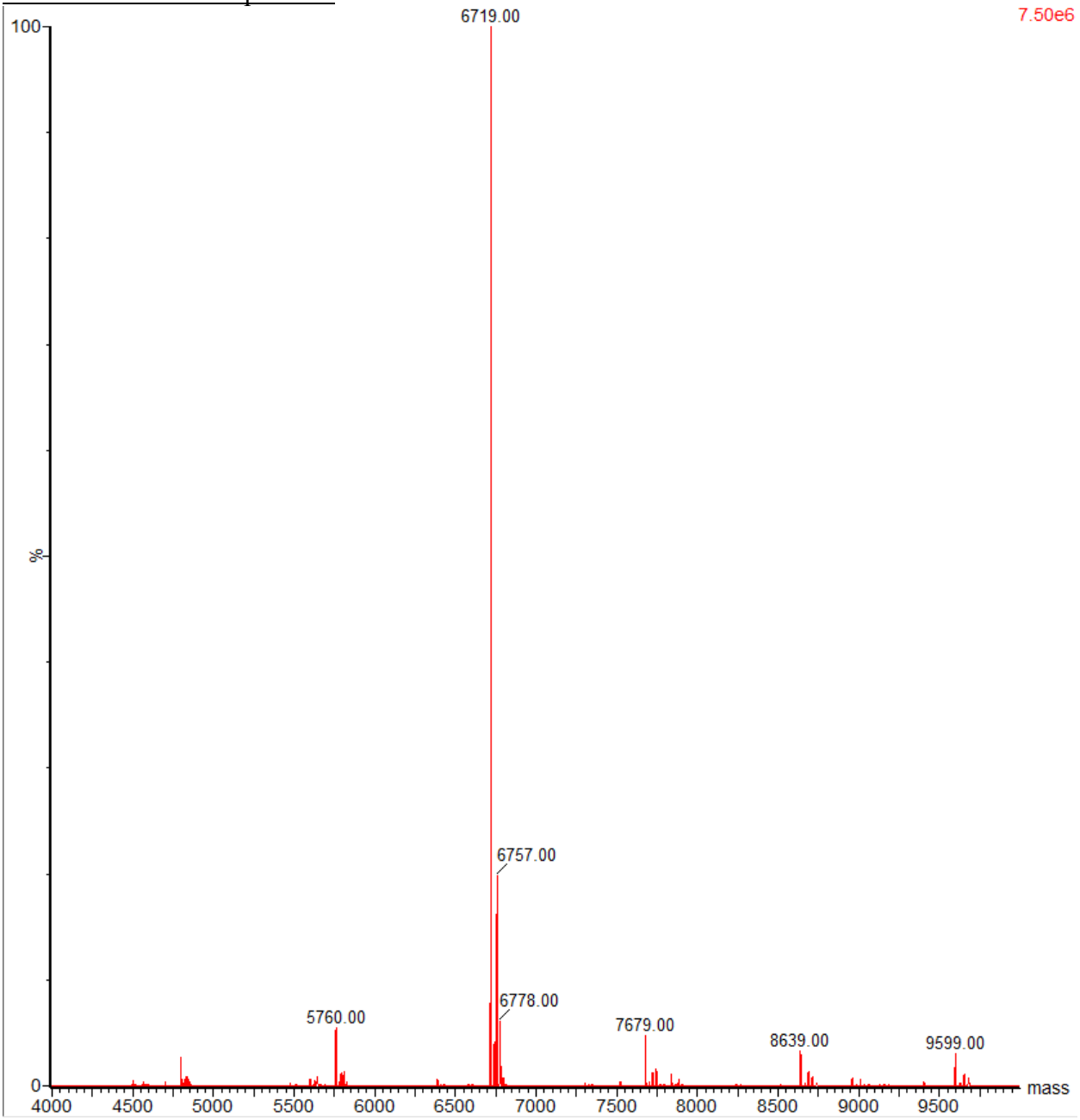
A260 Chromatogram



Mass Spectrum (RT= 2.19 min)

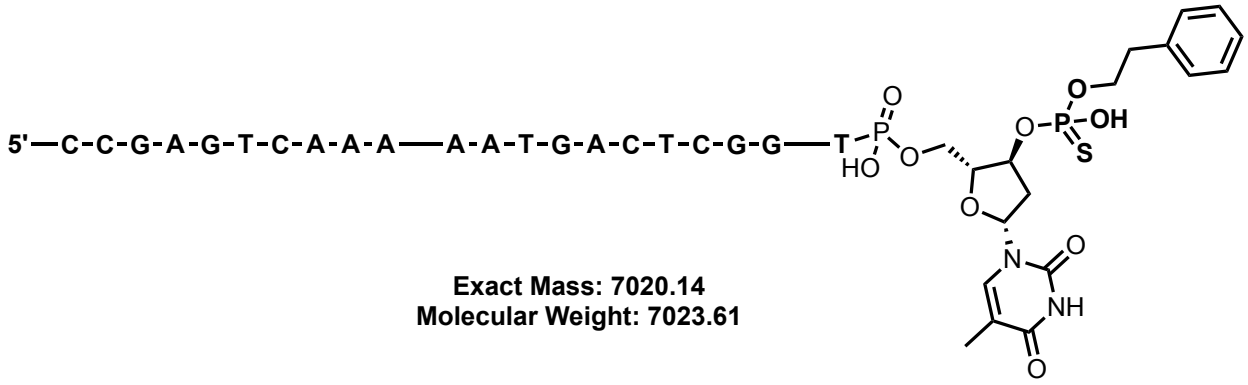


Deconvoluted Mass Spectrum



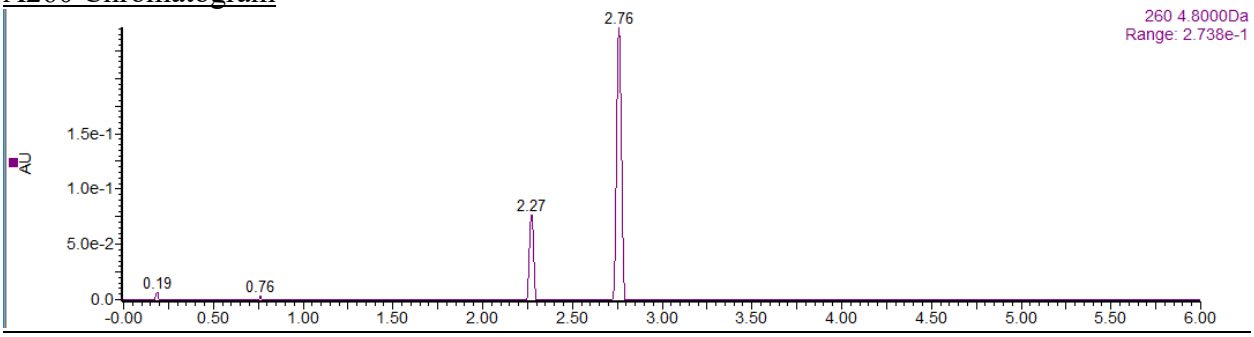
LCMS Characterization of 4

Produced from **2** by General Procedure **1:**
Yield (A260): 72%
Expected Mass: 7023.61
Expected (M-6)/6 Ion: 1169.6
Found Deconvoluted Mass: 7023.00
Found (M-6)/6 Ion: 1169.54

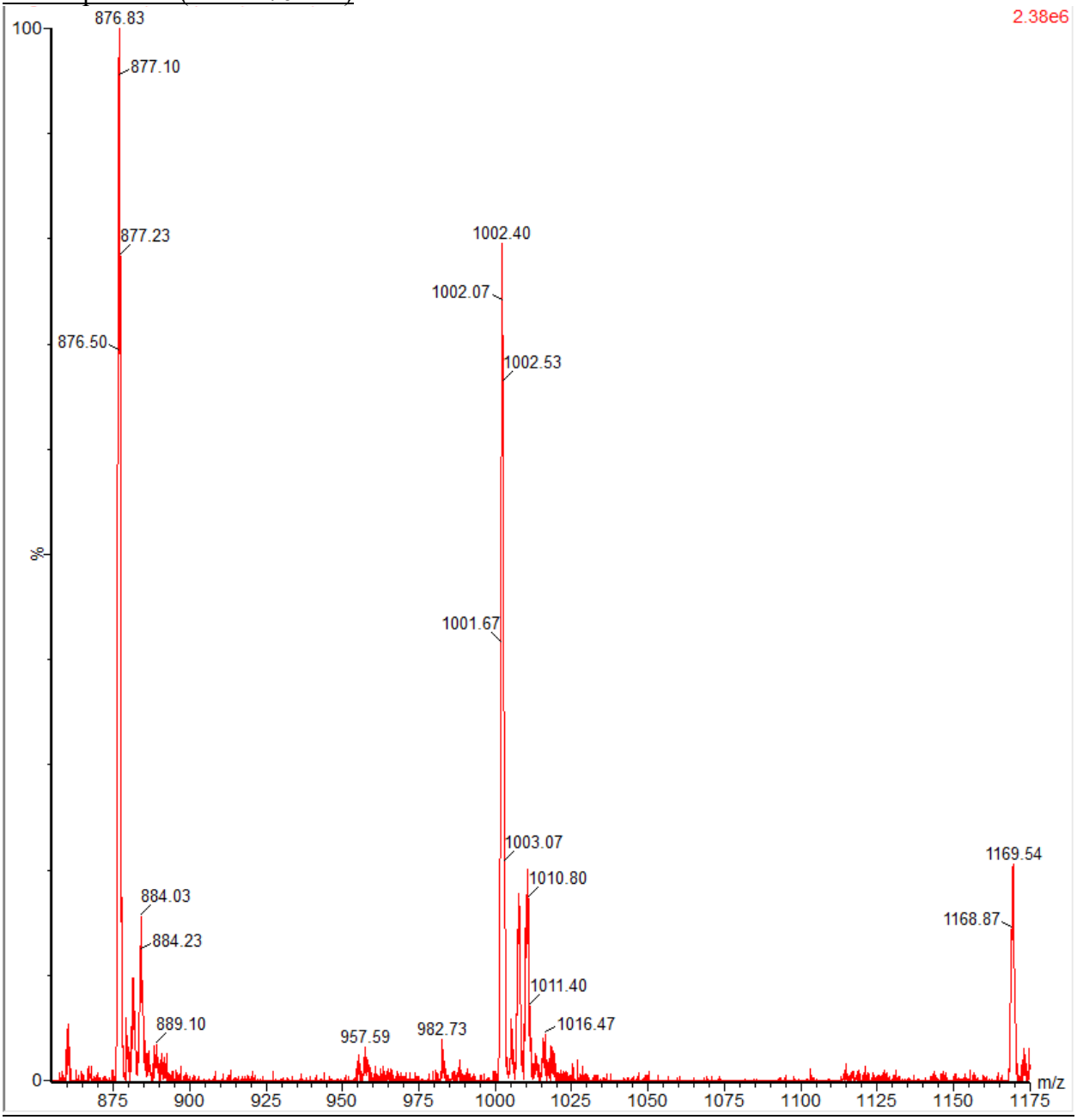


Exact Mass: 7020.14
Molecular Weight: 7023.61

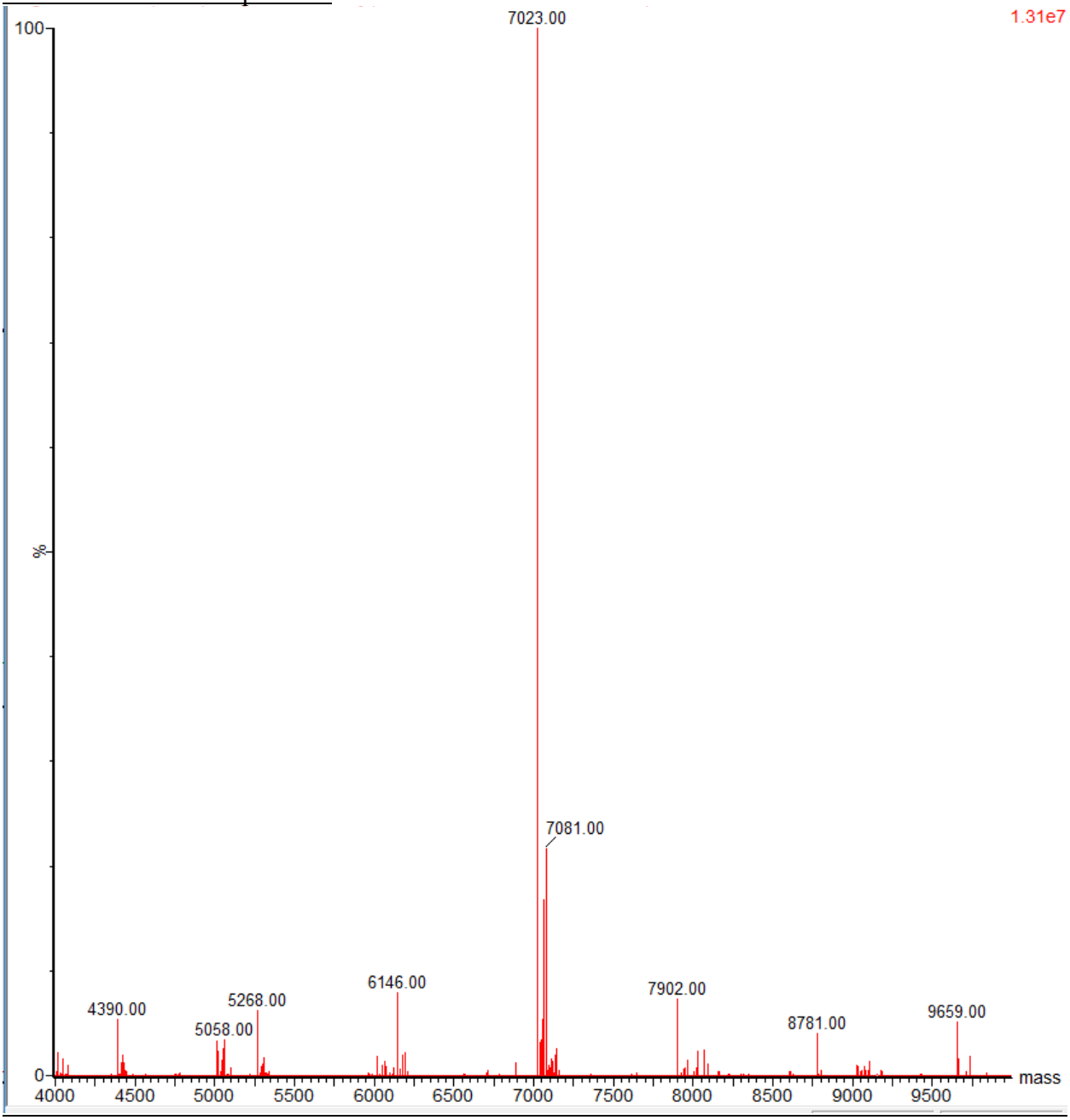
A260 Chromatogram



Mass Spectrum (RT= 2.76 min)

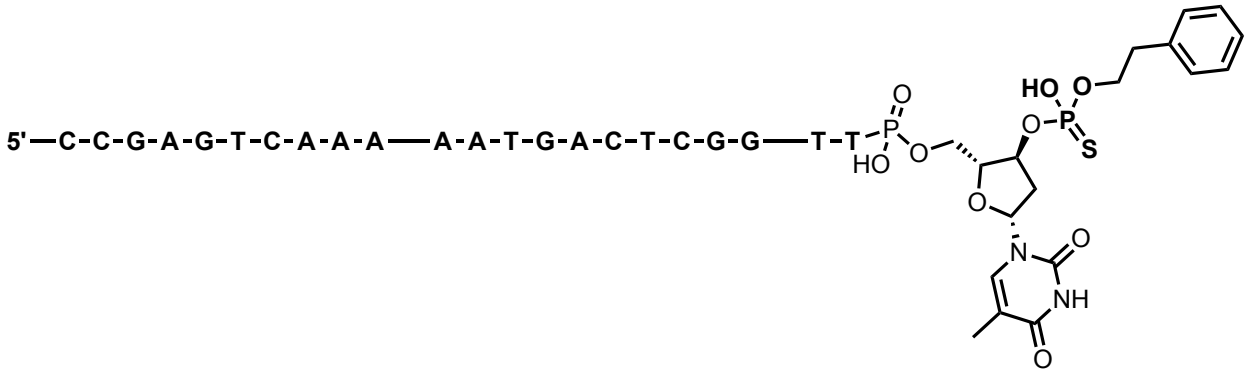


Deconvoluted Mass Spectrum



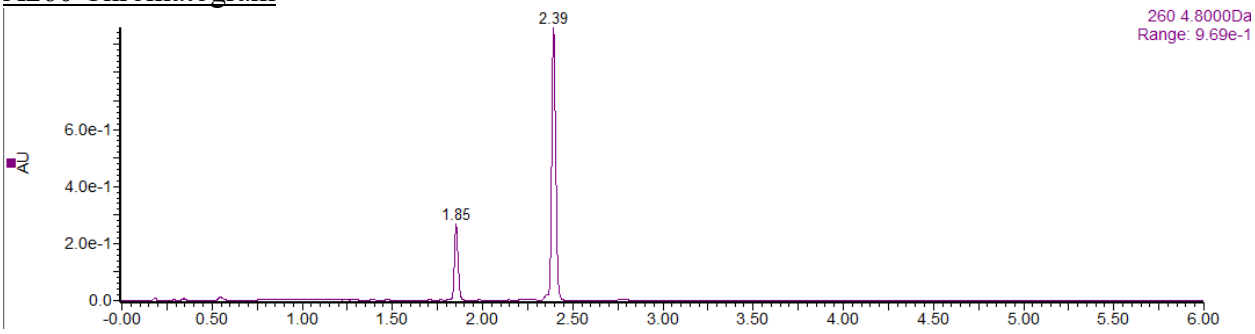
LCMS Characterization of SI-34

Produced from **SI-8** by General Procedure **2:**
Yield (A260): 74%
Expected Mass: 7327.81
Expected (M-6)/6 Ion: 1220.3
Found Deconvoluted Mass: 7328.00
Found (M-6)/6 Ion: 1219.84

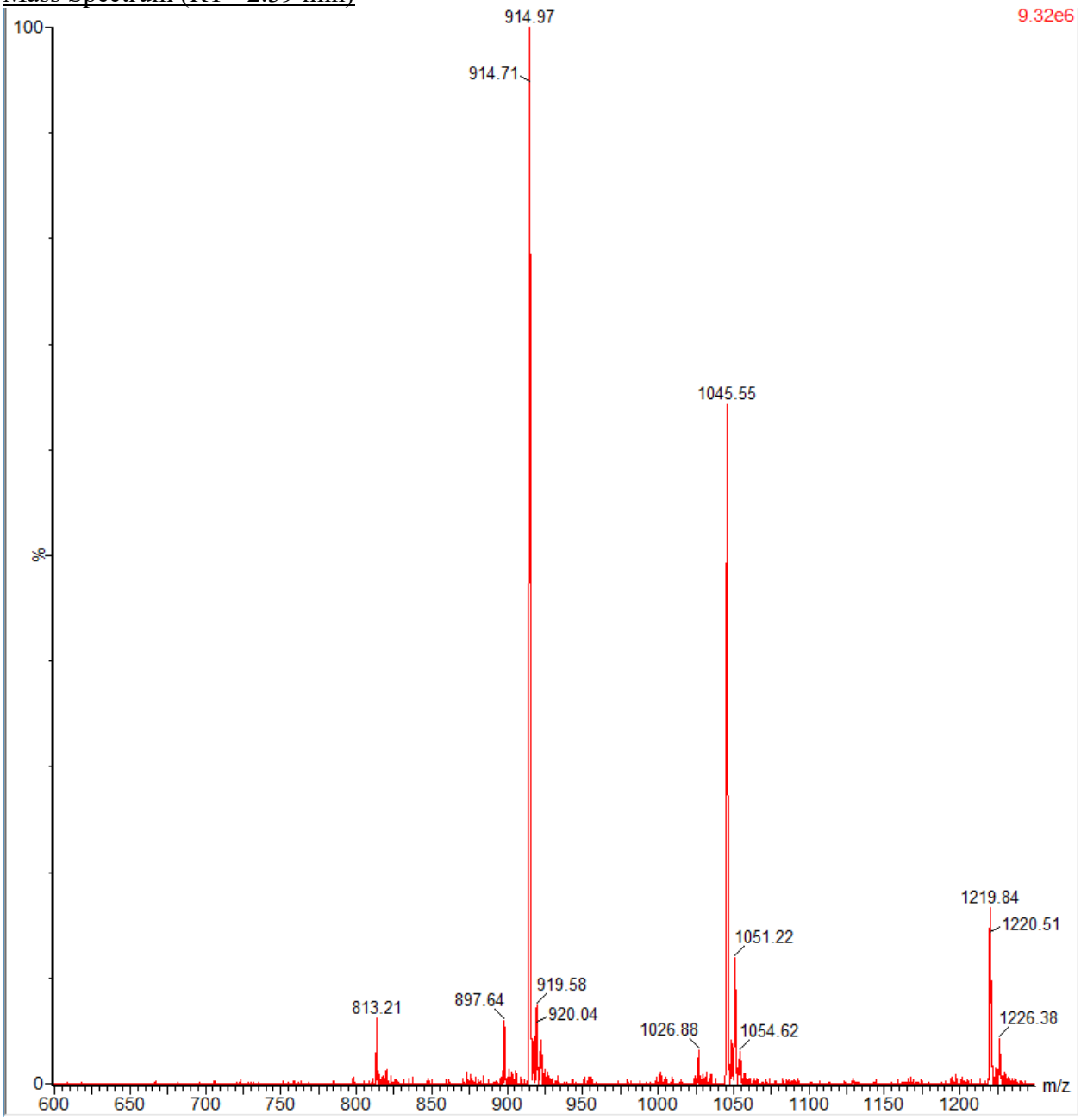


Exact Mass: 7324.19
Molecular Weight: 7327.81

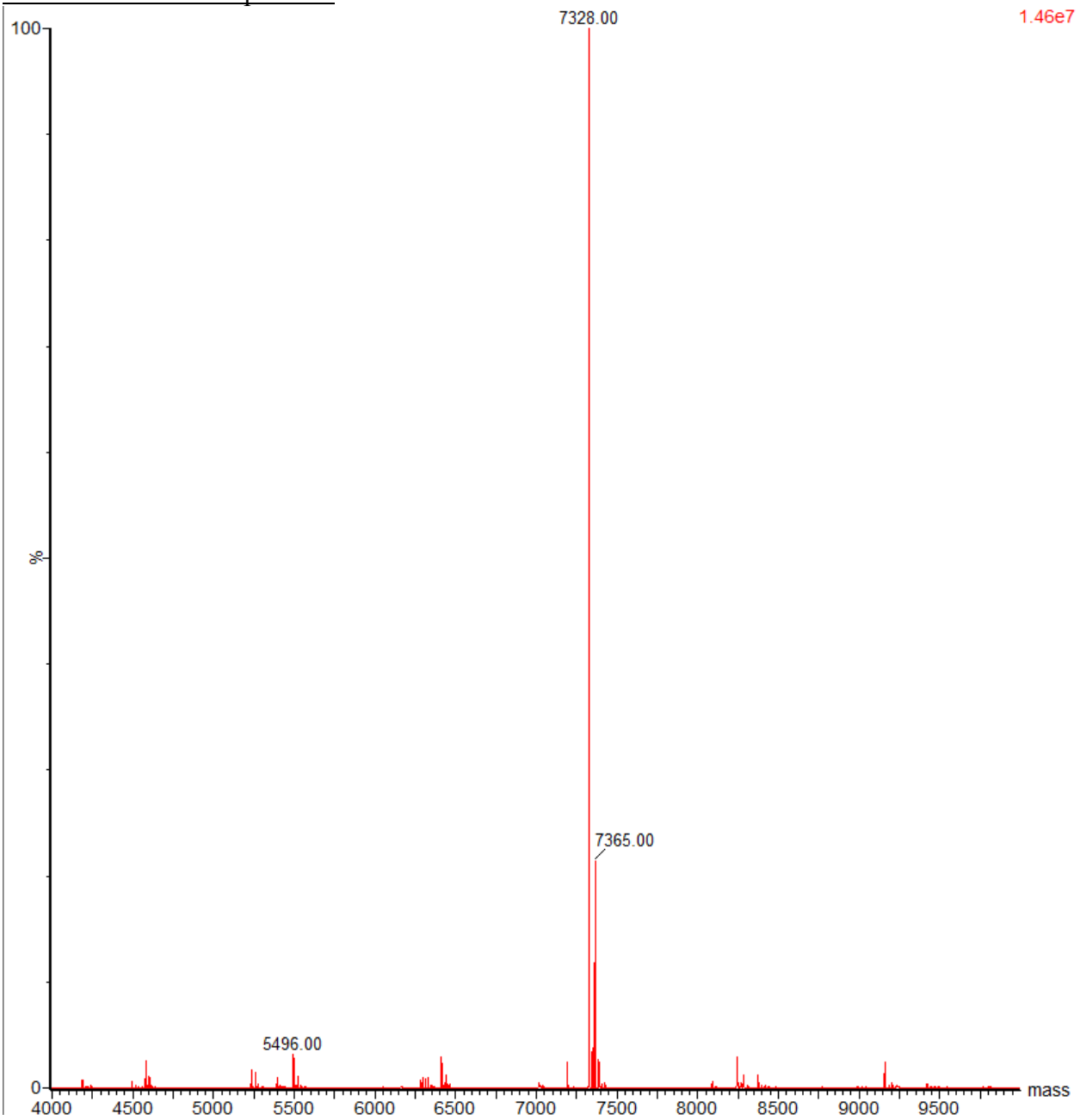
A260 Chromatogram



Mass Spectrum (RT= 2.39 min)

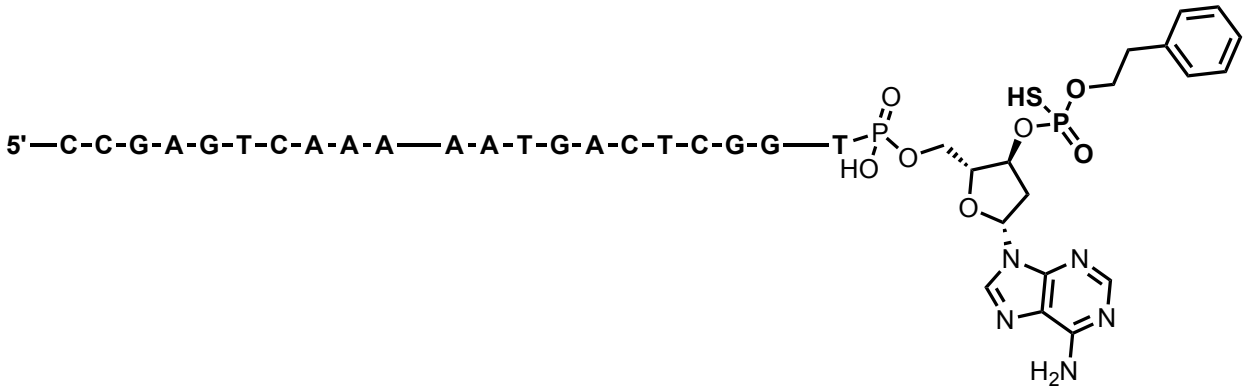


Deconvoluted Mass Spectrum



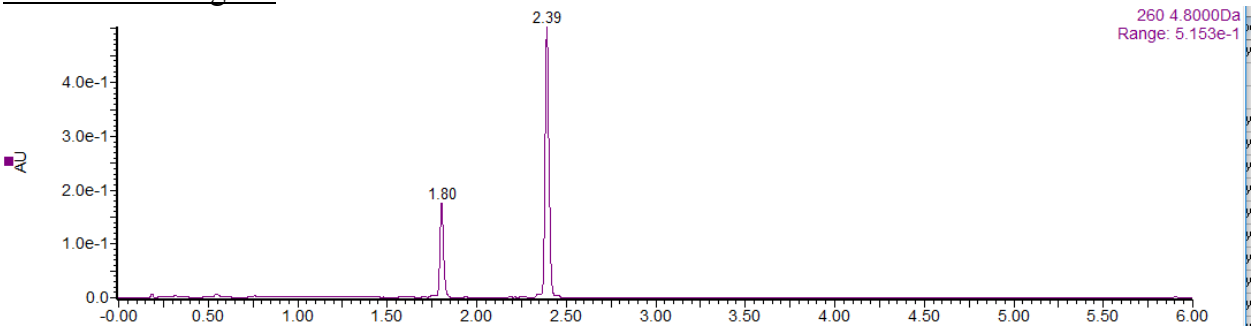
LCMS Characterization of SI-35

Produced from **SI-10** by General Procedure **2:**
Yield (A260): 69%
Expected Mass: 7032.63
Expected (M-6)/6 Ion: 1171.1
Found Deconvoluted Mass: 7032.00
Found (M-6)/6 Ion: 1170.94

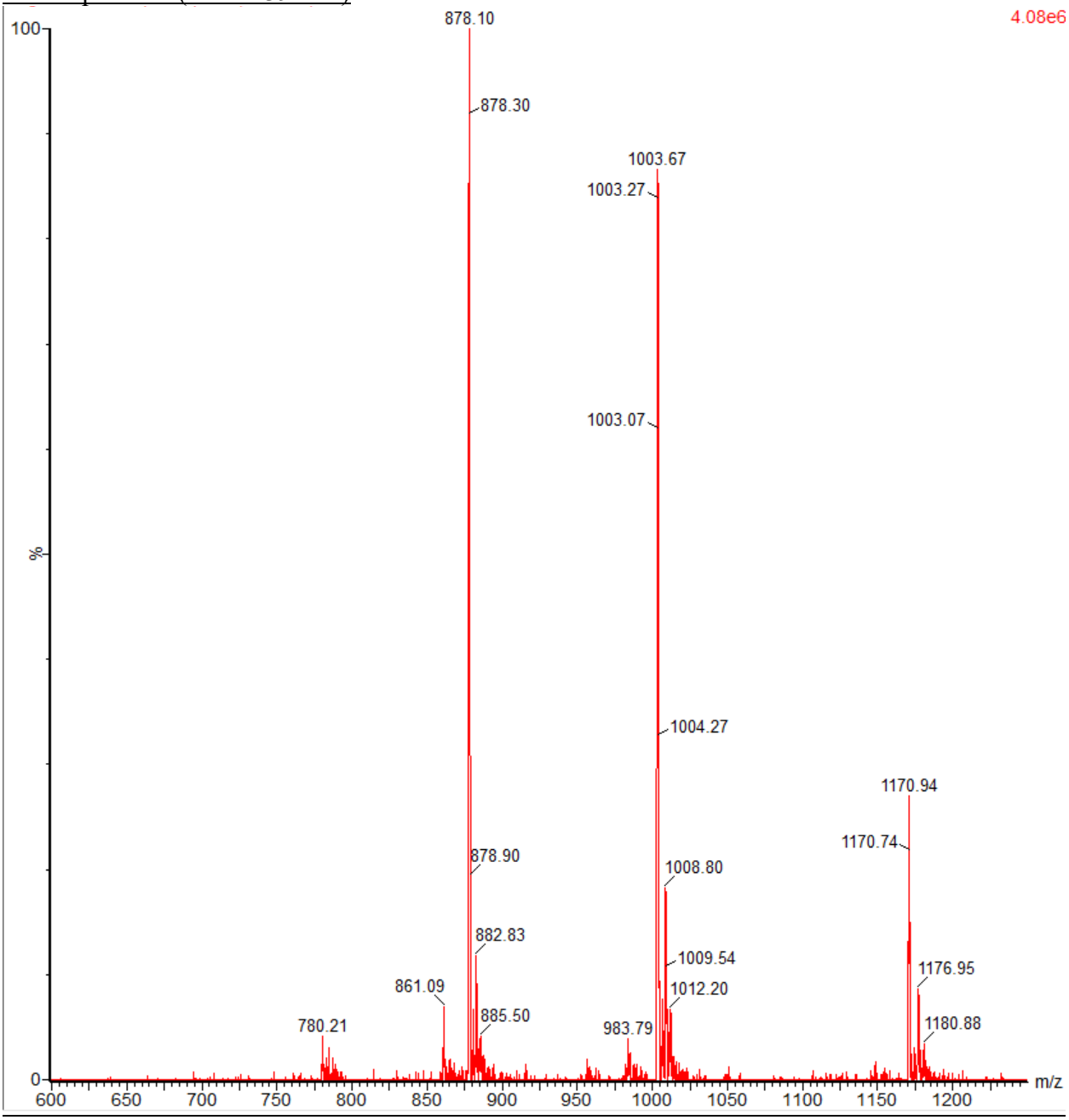


Exact Mass: 7029.16
Molecular Weight: 7032.63

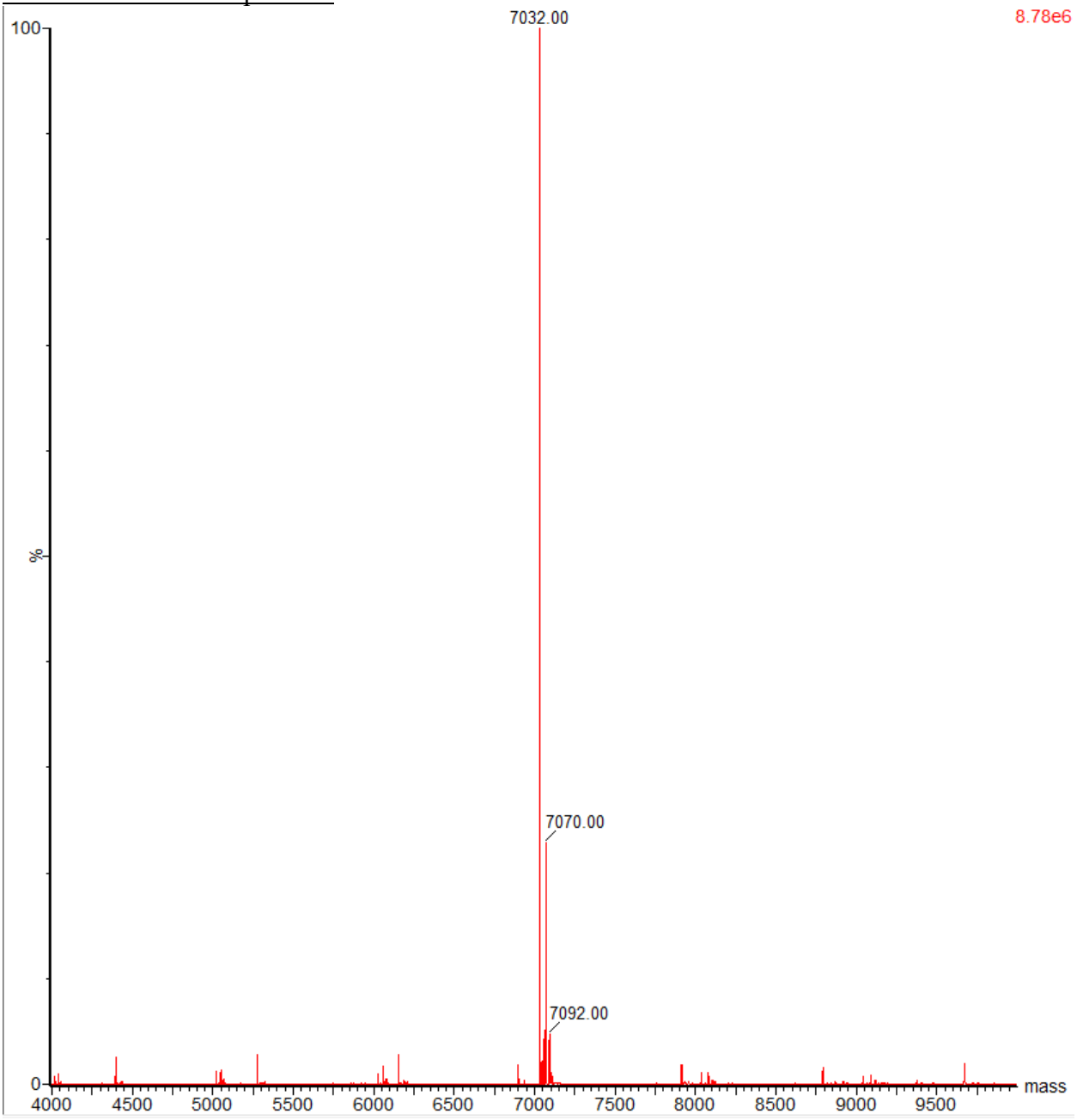
A260 Chromatogram



Mass Spectrum (RT= 2.39 min)

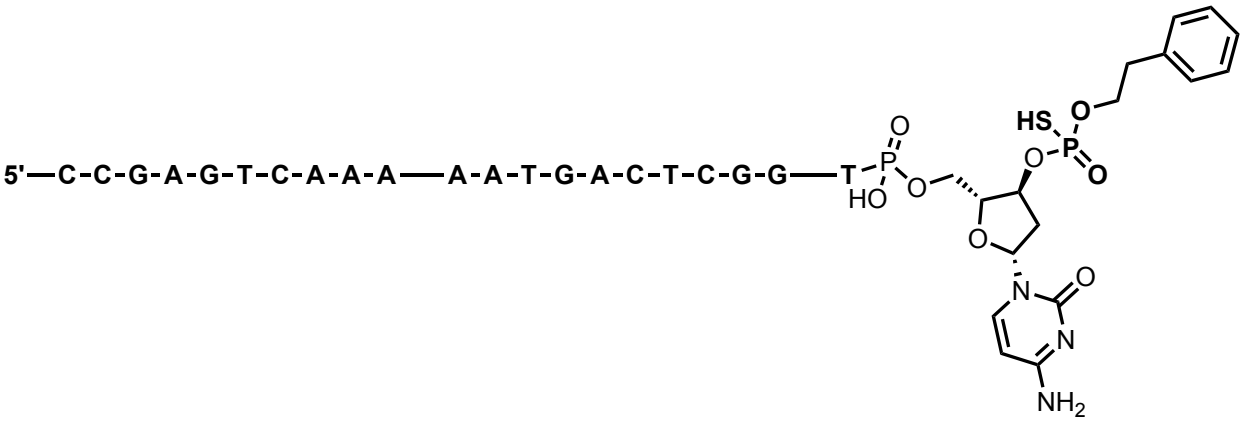


Deconvoluted Mass Spectrum



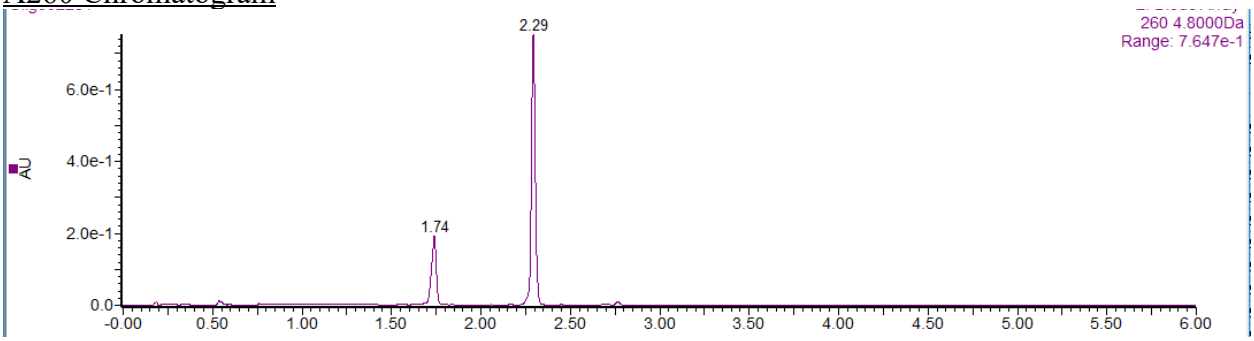
LCMS Characterization of SI-36

Produced from **SI-11** by General Procedure **2:**
Yield (A260): 72%
Expected Mass: 7008.6
Expected (M-6)/6 Ion: 1167.1
Found Deconvoluted Mass: 7008.00
Found (M-6)/6 Ion: 1166.94

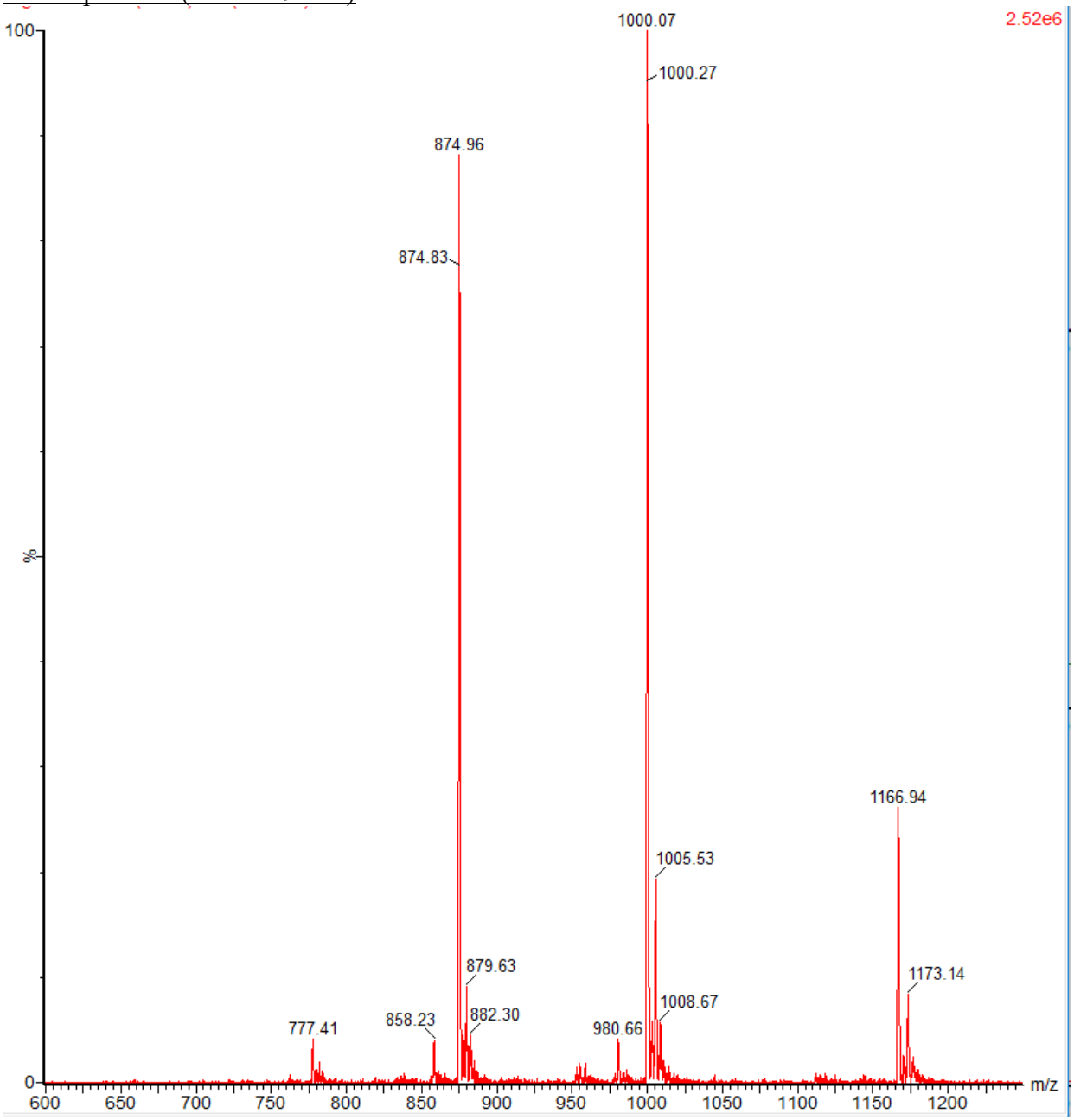


Exact Mass: 7005.14
Molecular Weight: 7008.60

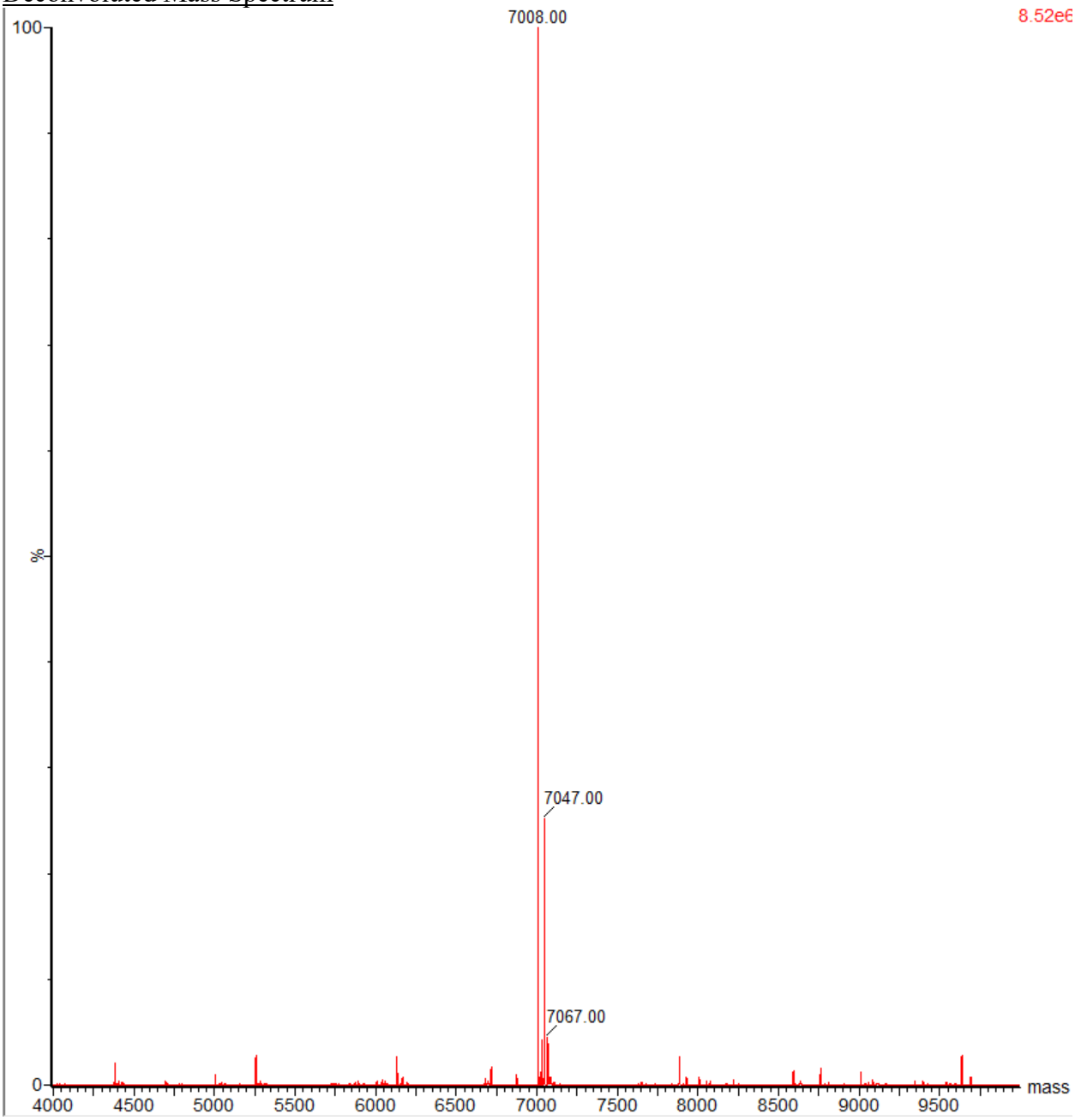
A260 Chromatogram



Mass Spectrum (RT= 2.29 min)

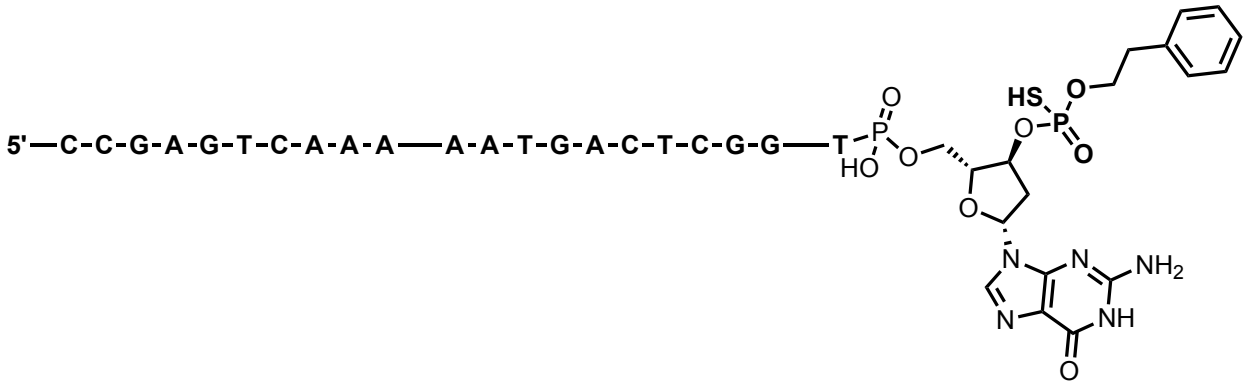


Deconvoluted Mass Spectrum



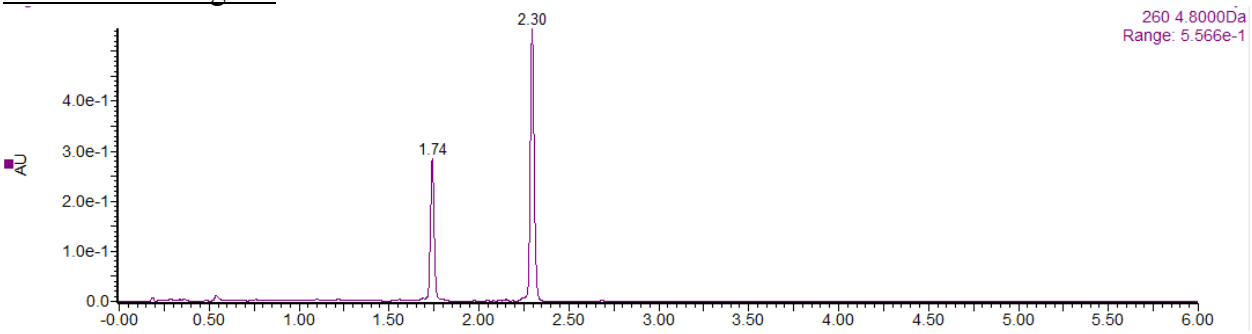
LCMS Characterization of SI-37

Produced from **SI-12** by General Procedure **2:**
Yield (A260): 62%
Expected Mass: 7048.63
Expected (M-6)/6 Ion: 1173.8
Found Deconvoluted Mass: 7049.00
Found (M-6)/6 Ion: 1173.74

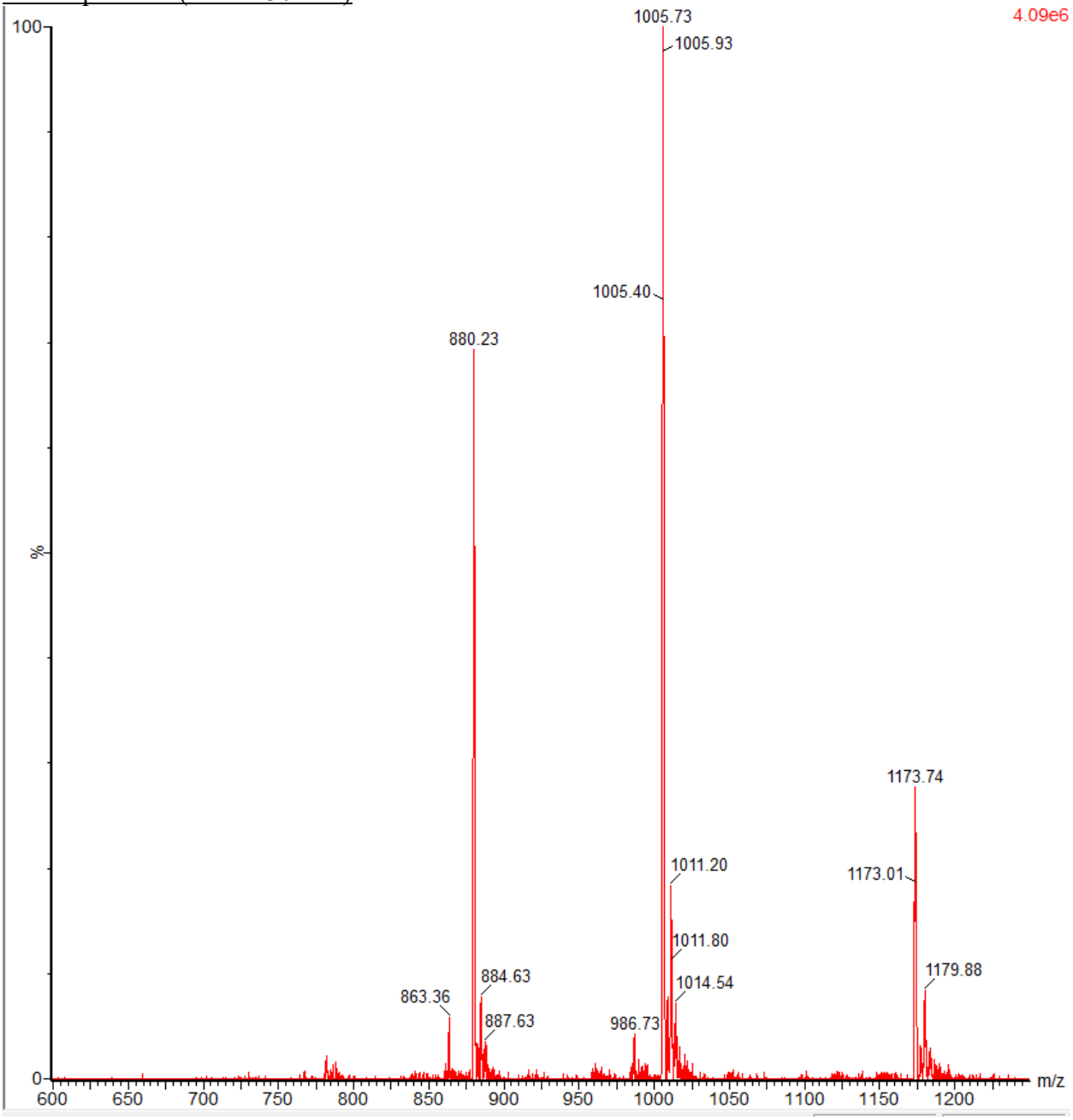


Exact Mass: 7045.15
Molecular Weight: 7048.63

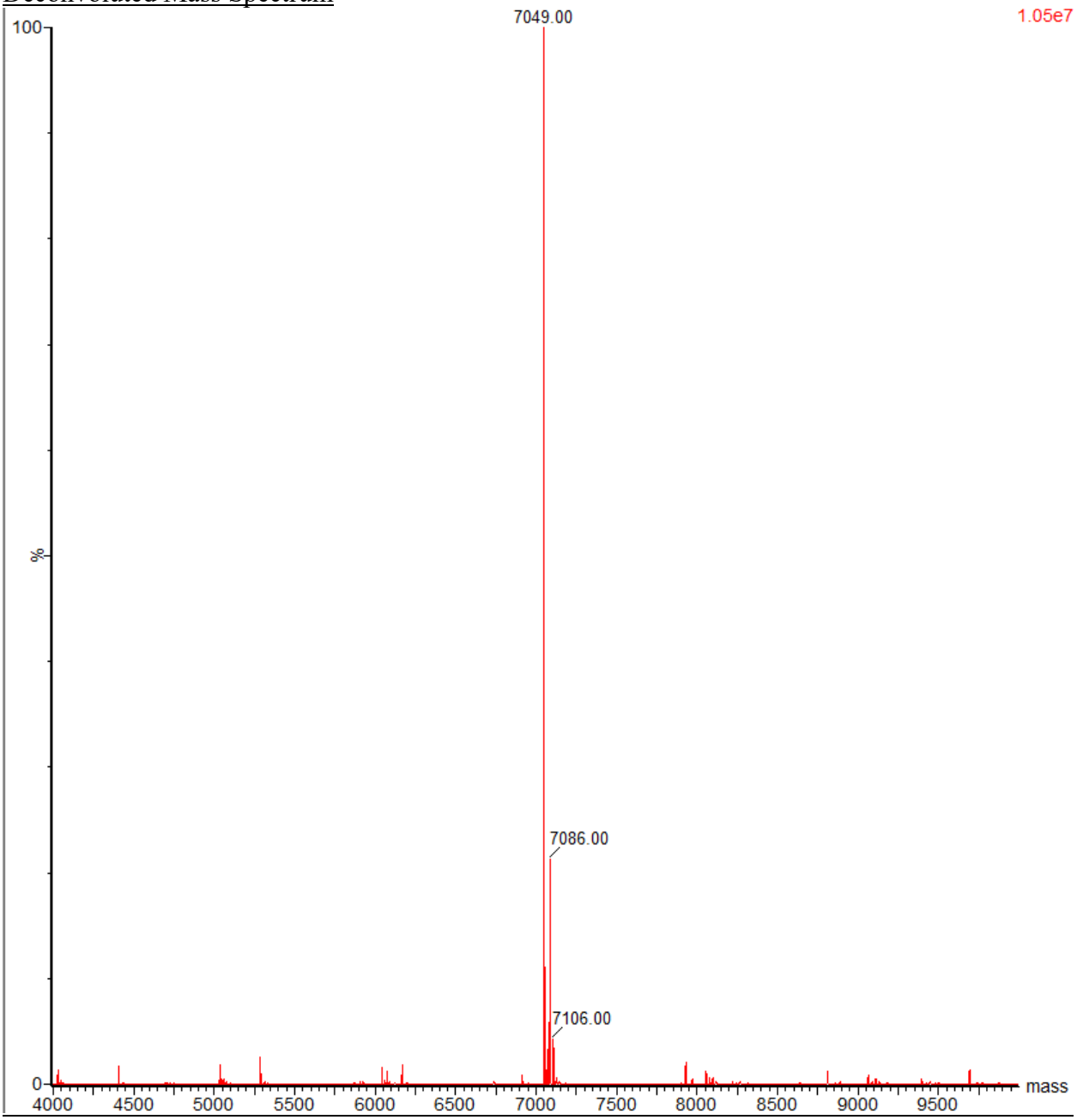
A260 Chromatogram



Mass Spectrum (RT= 2.30 min)



Deconvoluted Mass Spectrum



Substrate scope:

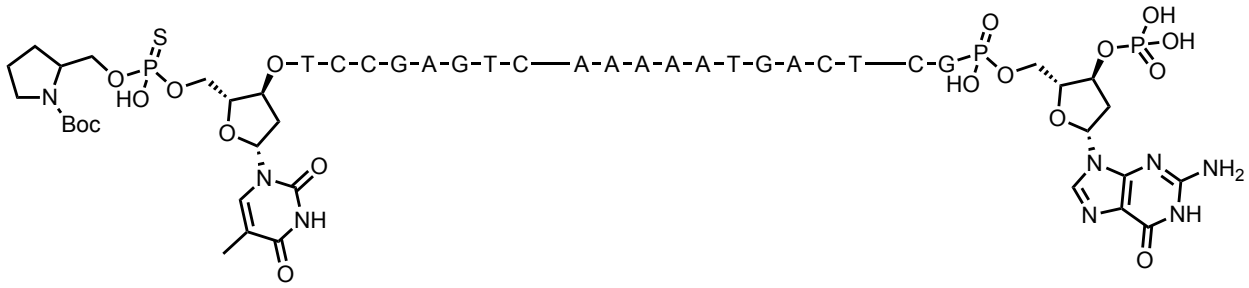
Procedure

SENDR substrate scope was analyzed by performing reactions in microcentrifuge tube as described in *General Procedure 2.* Briefly, 5 nmol of DNA **1** or **2** was loaded onto 100 µL of equilibrated resin. This loaded resin was washed with DMA (500 µL twice) and dry THF (500 µL three times) and dried under vacuum.
Ψ -module in MeCN (150 mM, 250 µL) was added to the loaded and dried resin. Then DBU (450 mM, 18 µL) was added to the reaction mixture. The reaction tube was vortexed for 30 seconds and incubated at room temperature or 37ºC for 60 minutes. The reactions were worked up by aspirating and discarding the reaction solution, and washing the resin bed with MeCN (500 µL) and 1:1 MeCN:PBS (500 µL). Elute buffer (300 µL) was added to the resin bed and the tube was agitated by orbital shaker for 5-10 minutes. The DNA containing elution buffer was collected by carefully pipetting the supernatant. DNA was isolate *via* ethanol precipitation. Ethanol precipitation was performed by adding 10% v/v of a NaCl solution (30 µL, 5M) to the elute buffer and three volumes of cold ethanol (-20 ºC) were added to the tube (~1000µL) and incubated for 18 hours at -20 ºC. The tube was then centrifuged at 13,000 rpm for 15 minutes to pellet the DNA, and the extra ethanol was decanted off. The tubes were dried *via* speed vacuum, the DNA was dissolved, and HPLC-MS analysis was performed.

Characterization of Substrate Scope

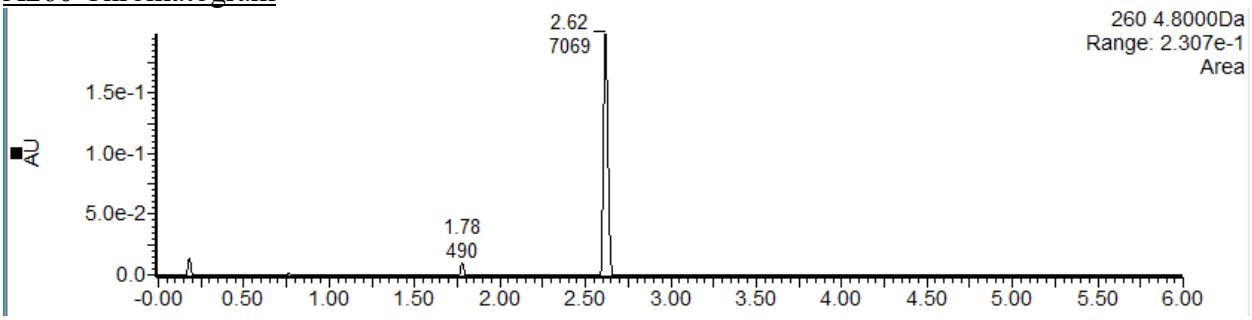
LCMS Characterization of 5

Produced from **1** by General Procedure **2:**
Using **Ψ-2**
Yield (A260): 94%
Expected Mass: 7102.72
Expected (M-6)/6 Ion: 1182.20
Found Deconvoluted Mass: 7102.0
Found (M-6)/6 Ion: 1182.15

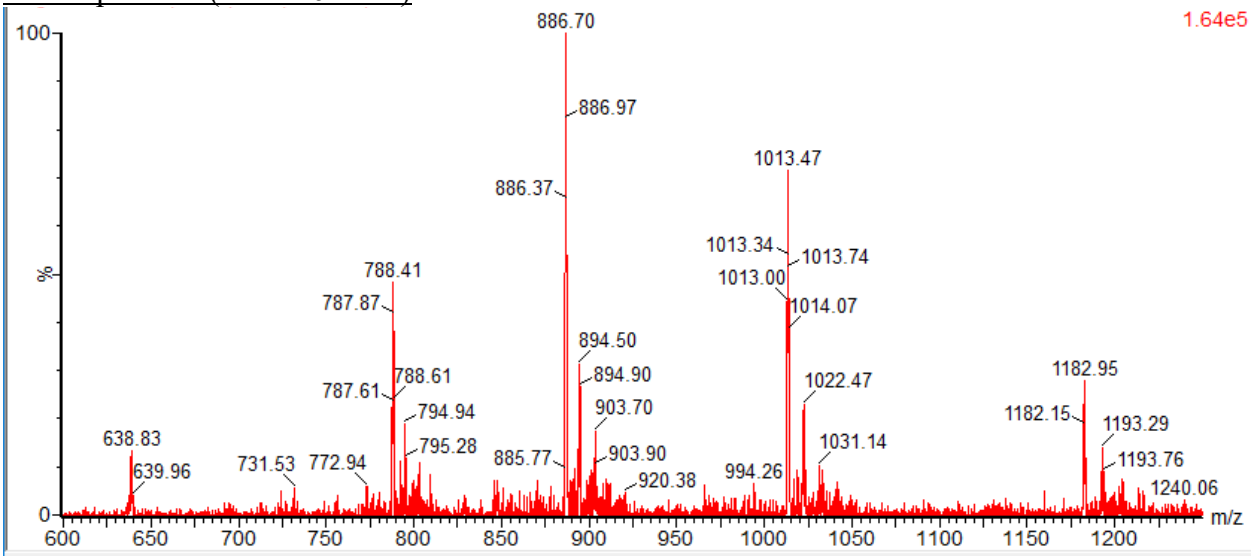


Exact Mass: 7099.21
Molecular Weight: 7102.71

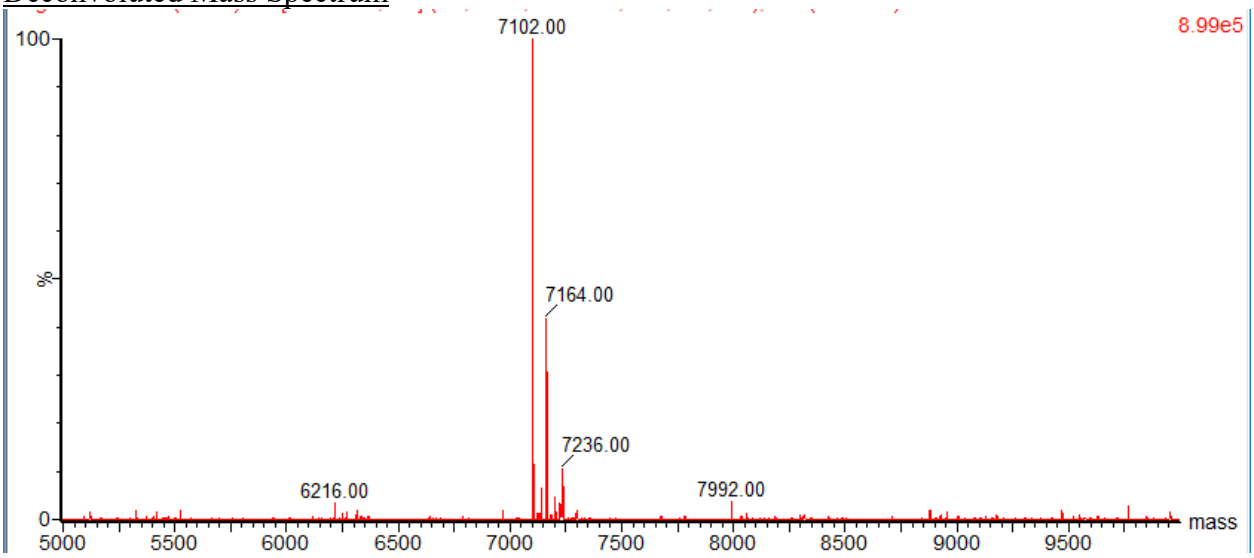
A260 Chromatogram



Mass Spectrum (RT= 2.62 min)

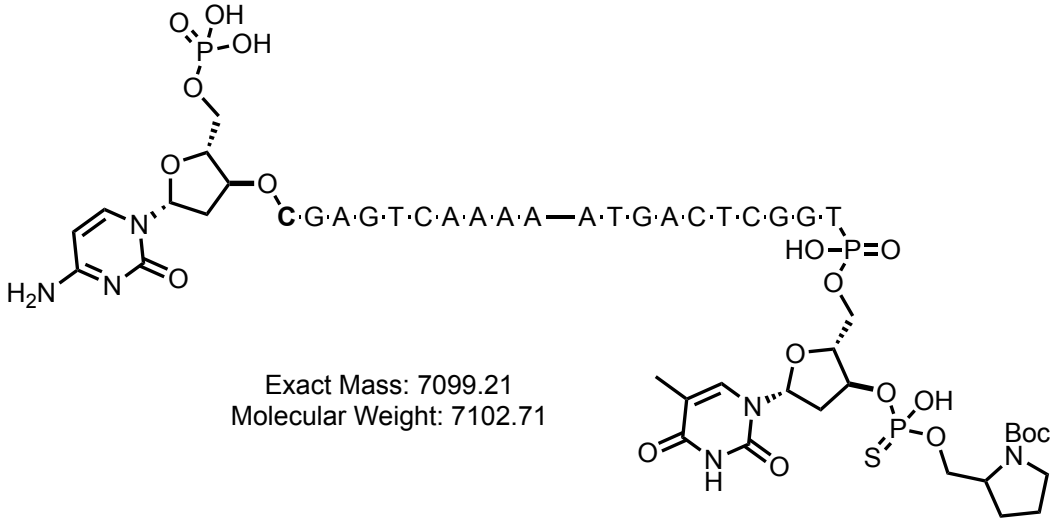


Deconvoluted Mass Spectrum

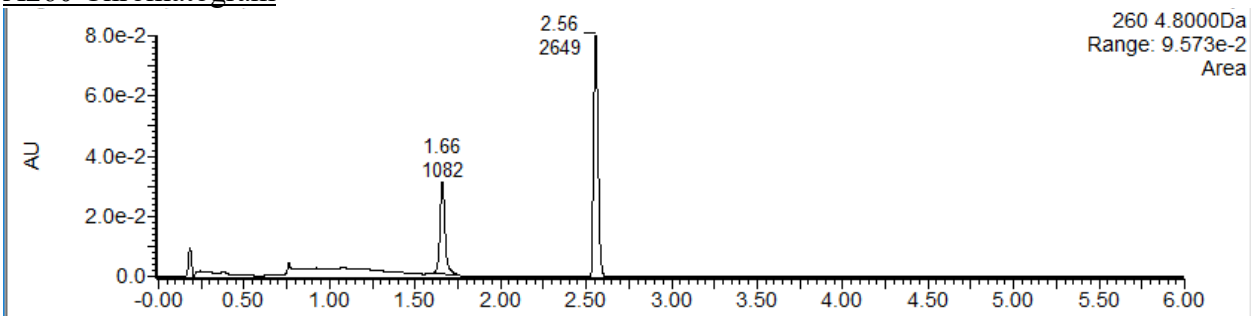


LCMS Characterization of 6

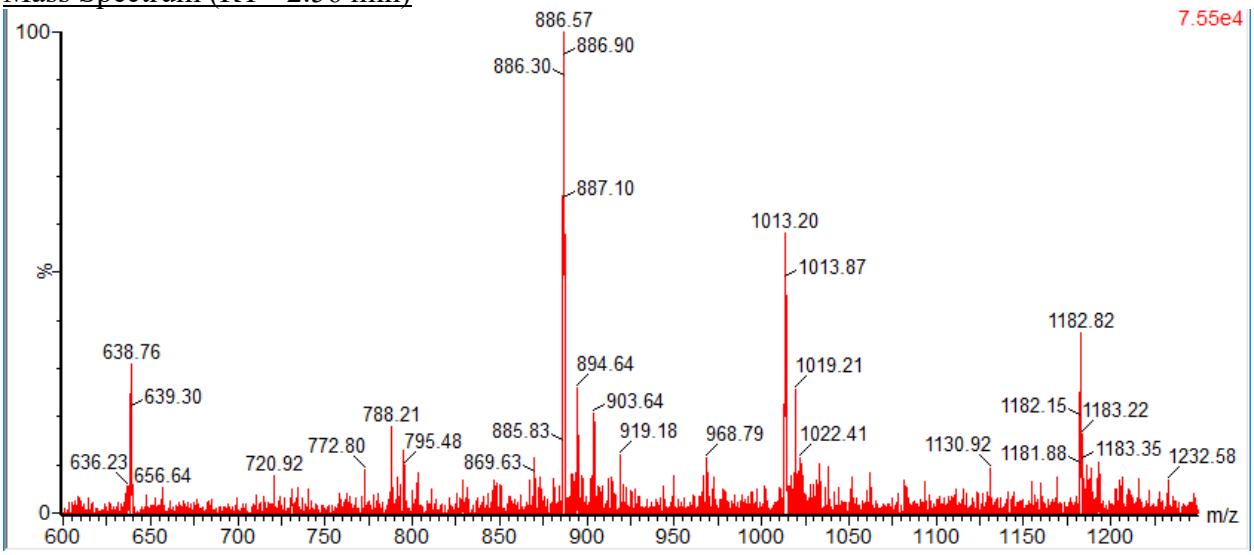
Produced from **2** by General Procedure **2:**
Using **Ψ-2**
Yield (A260): 74%
Expected Mass: 7102.71
Expected (M-6)/6 Ion: 1182.8
Found Deconvoluted Mass: 7102.0
Found (M-6)/6 Ion: 1182.69



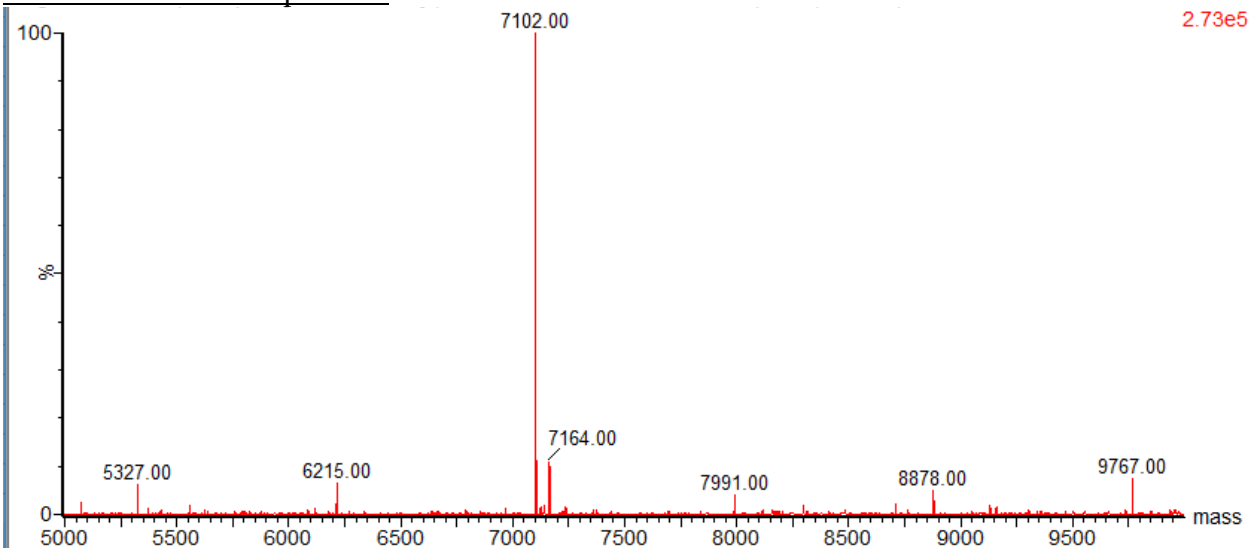
A260 Chromatogram



Mass Spectrum (RT= 2.56 min)

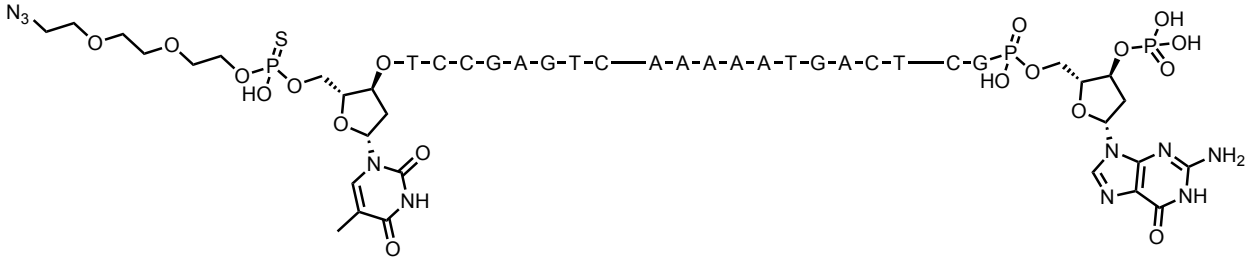


Deconvoluted Mass Spectrum



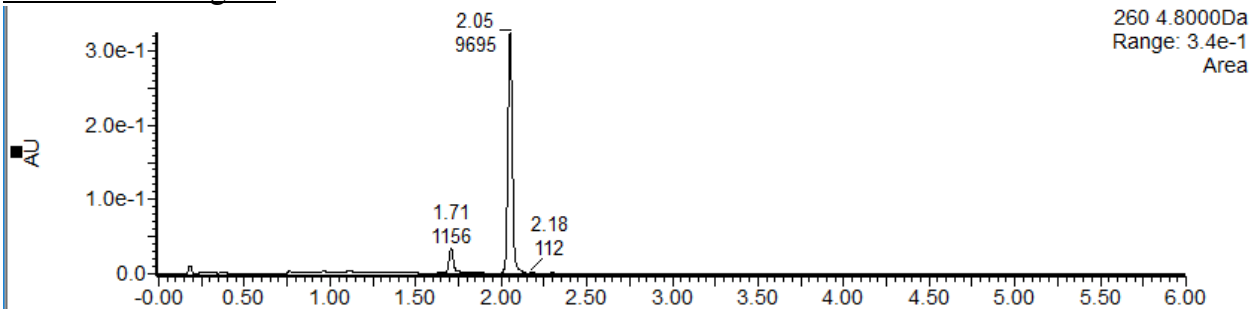
LCMS Characterization of 7

Produced from **1** by General Procedure **2:**
Using **Ψ-3**
Yield (A260): 88%
Expected Mass: 7076.63
Expected (M-6)/6 Ion: 1177.86
Found Deconvoluted Mass: 7075.00
Found (M-6)/6 Ion: 1178.01

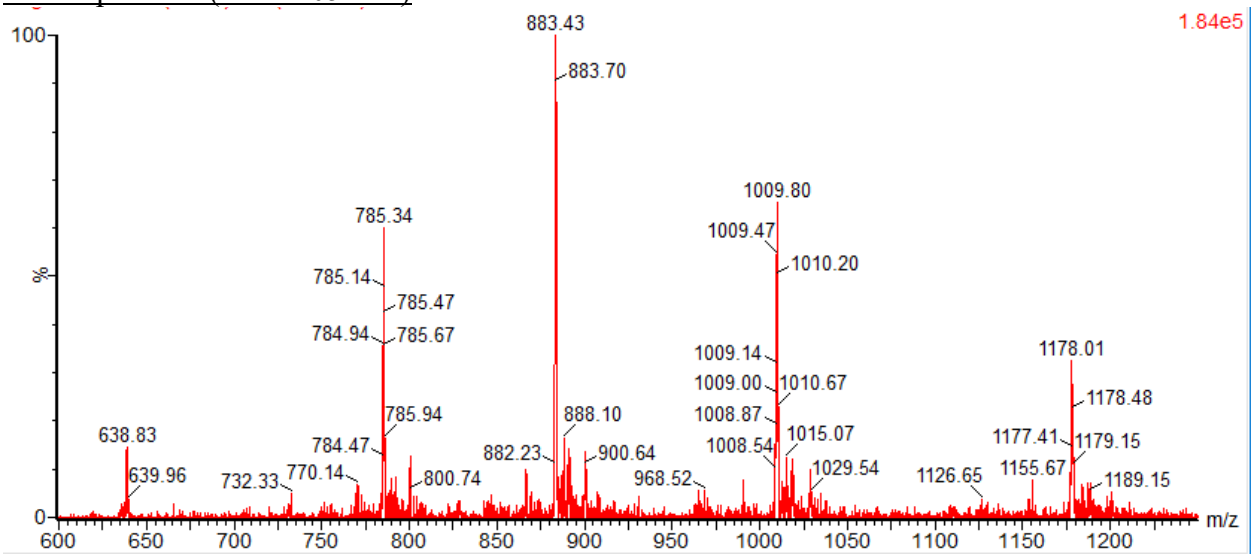


Exact Mass: 7073.17
Molecular Weight: 7076.63

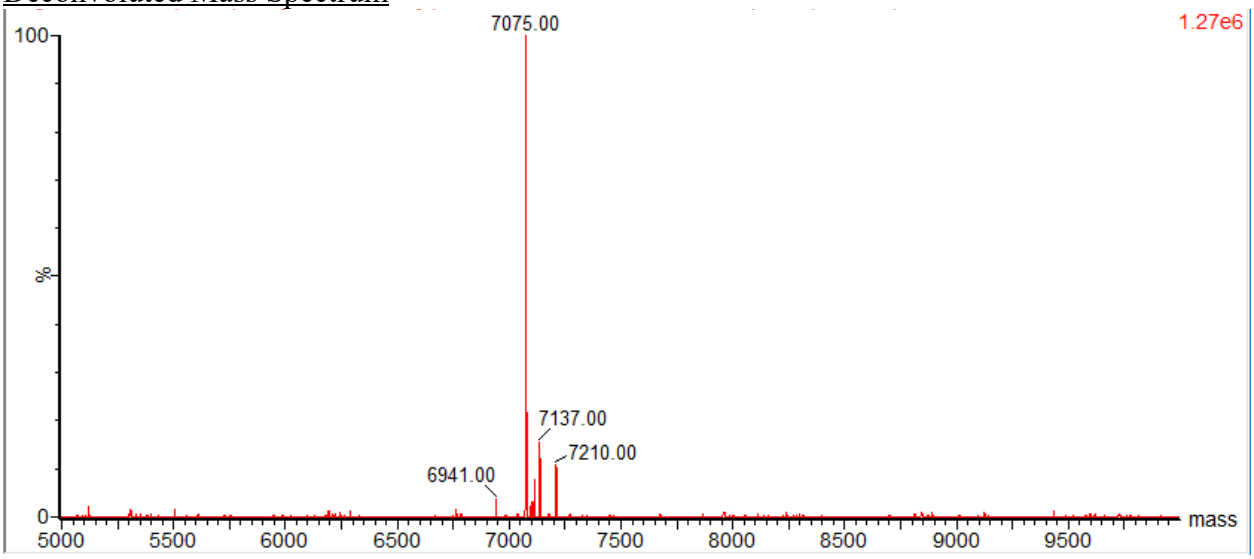
A260 Chromatogram



Mass Spectrum (RT= 2.05 min)

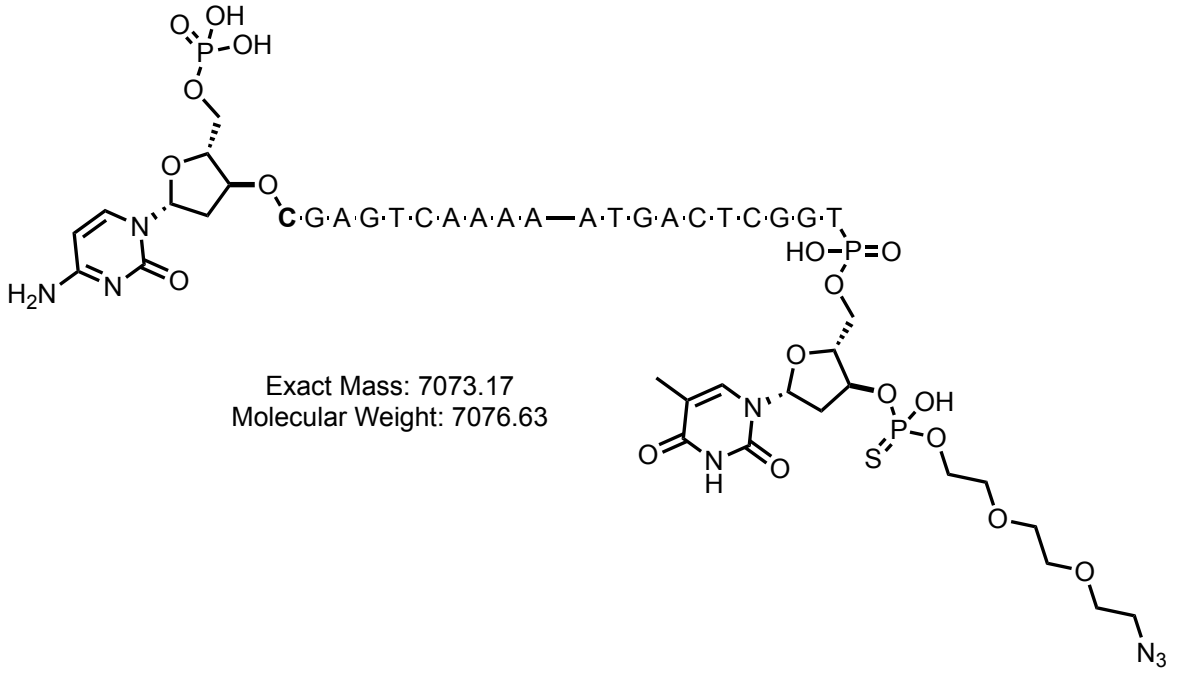


Deconvoluted Mass Spectrum

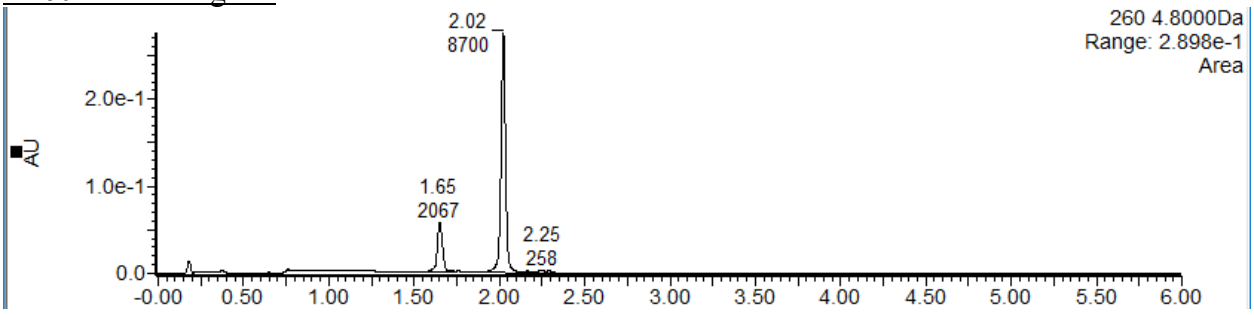


LCMS Characterization of 8

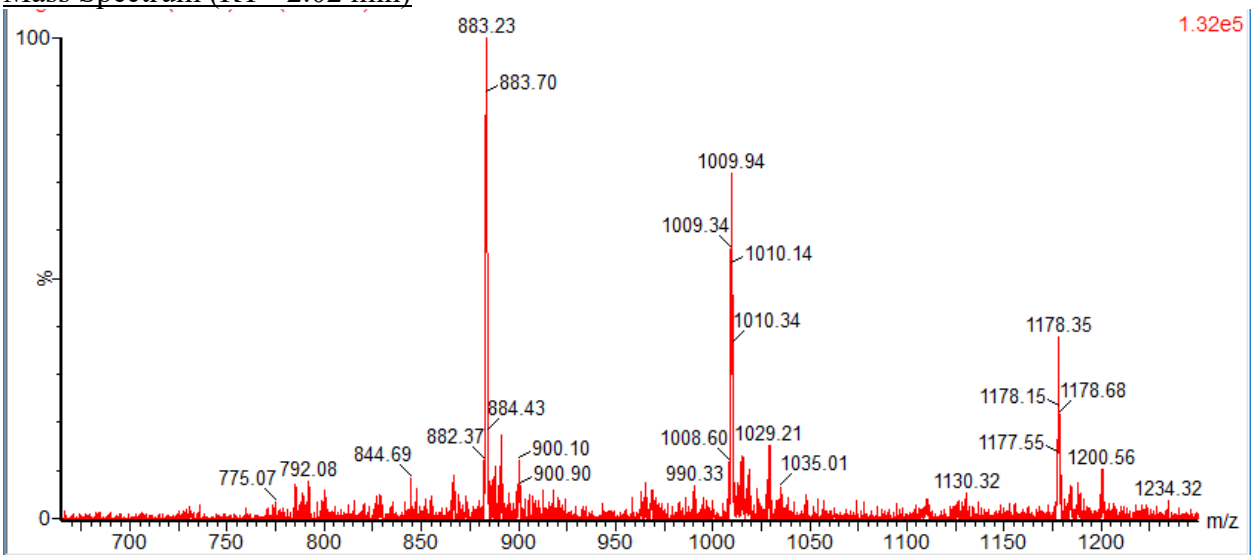
Produced from **2** by General Procedure **2:**
Using **Ψ-3**
Yield (A260): 74%
Expected Mass: 7076.63
Expected (M-6)/6 Ion: 1177.86
Found Deconvoluted Mass: 7076.0
Found (M-6)/6 Ion: 1177.55



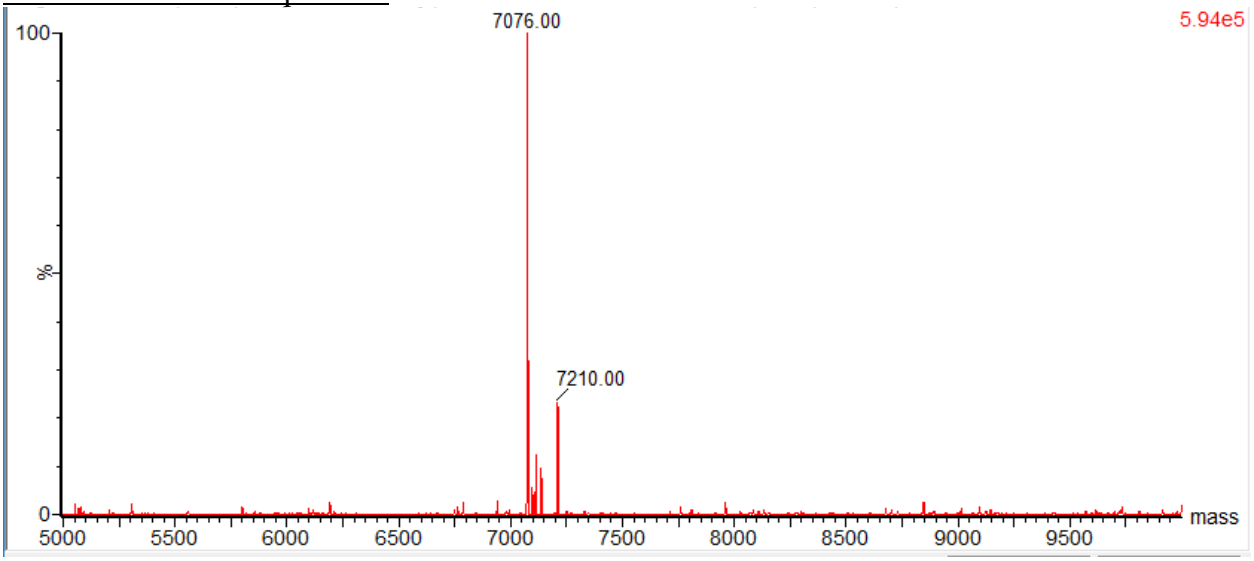
A260 Chromatogram



Mass Spectrum (RT= 2.02 min)

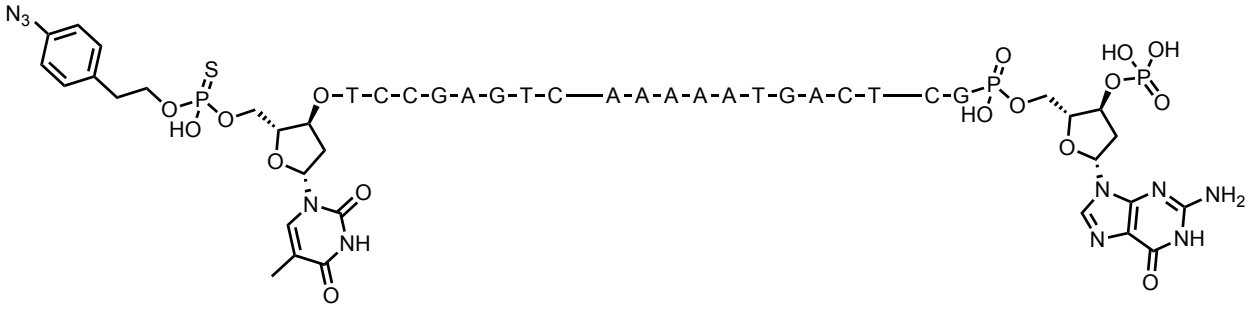


Deconvoluted Mass Spectrum



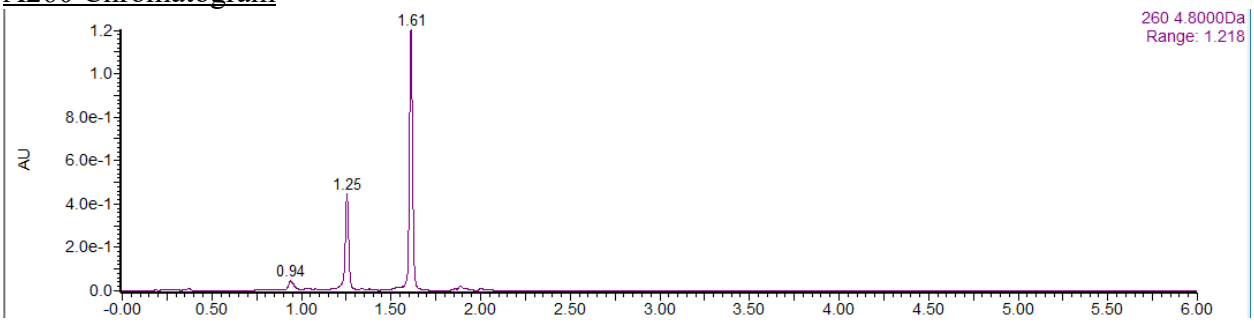
LCMS Characterization of 9

Produced from **1** by General Procedure **2:**
Using **Ψ-4**
Yield (A260): 78%
Expected Mass: 7064.62
Expected (M-6)/6 Ion: 1175.85
Found Deconvoluted Mass: 7064.00
Found (M-6)/6 Ion: 1176.14

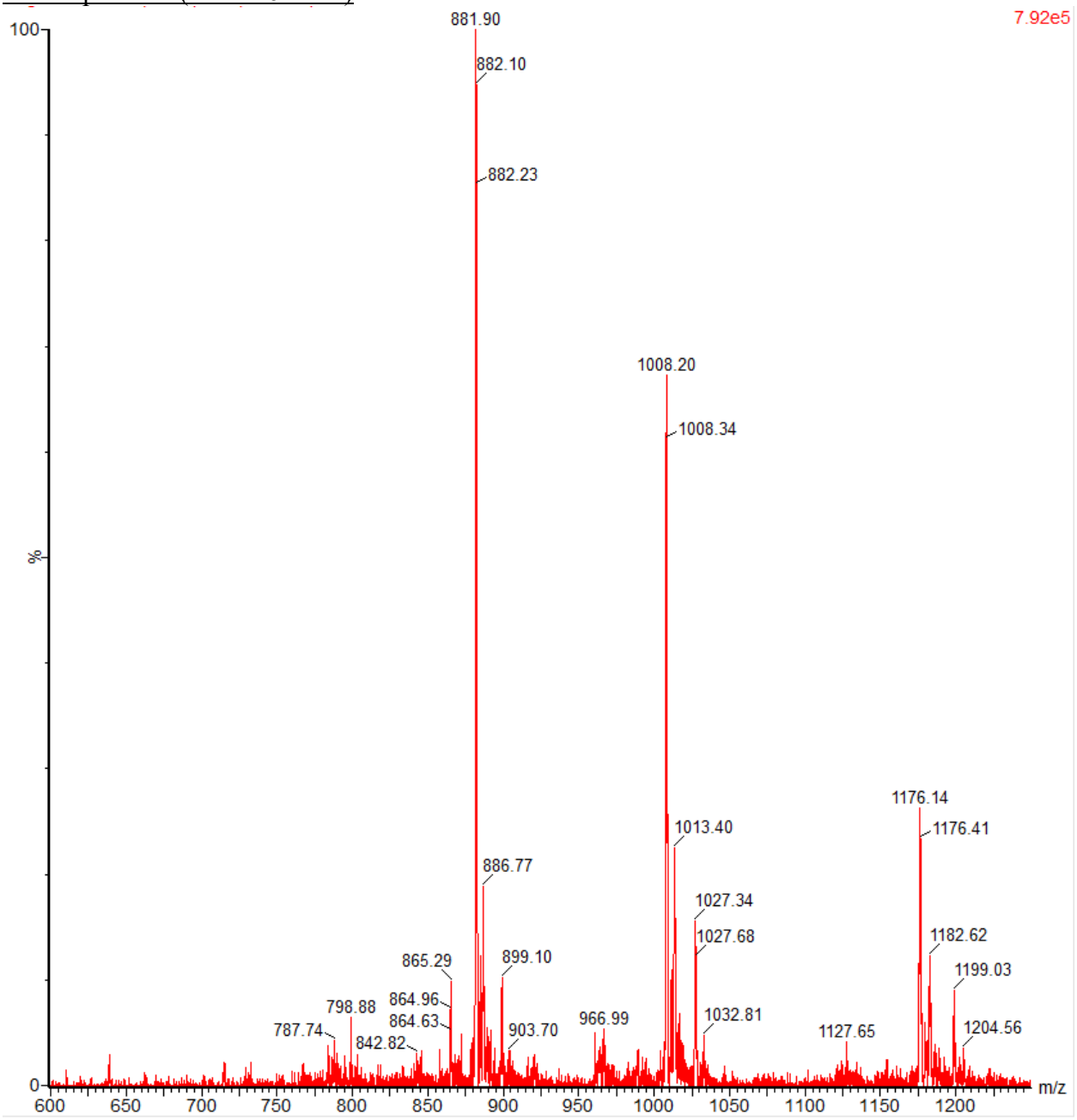


Exact Mass: 7061.15
Molecular Weight: 7064.62

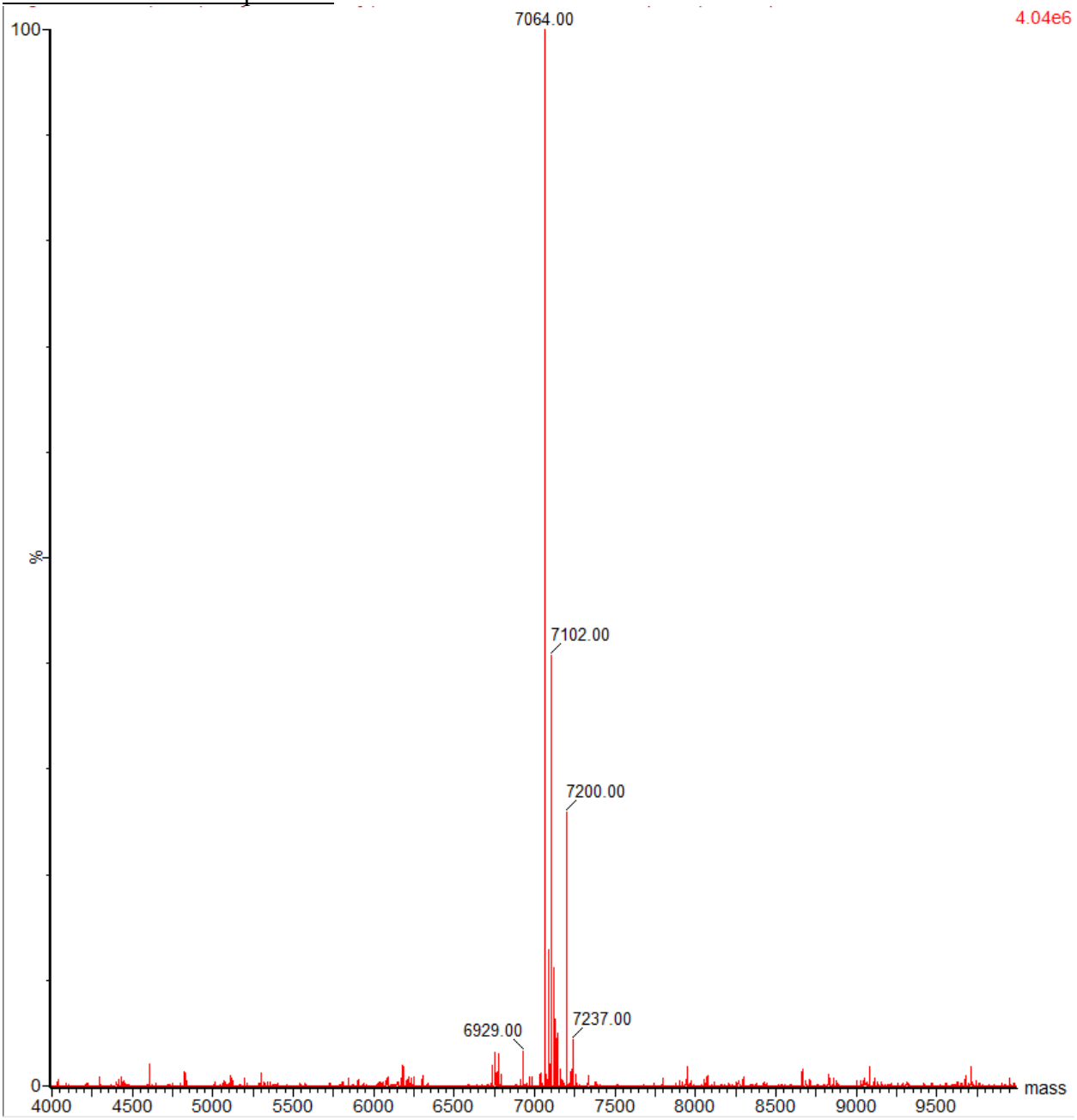
A260 Chromatogram



Mass Spectrum (RT= 1.61 min)

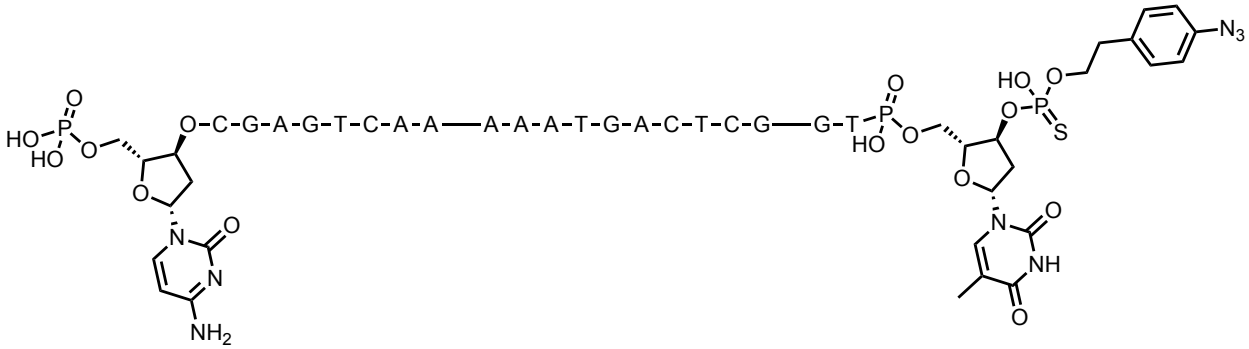


Deconvoluted Mass Spectrum



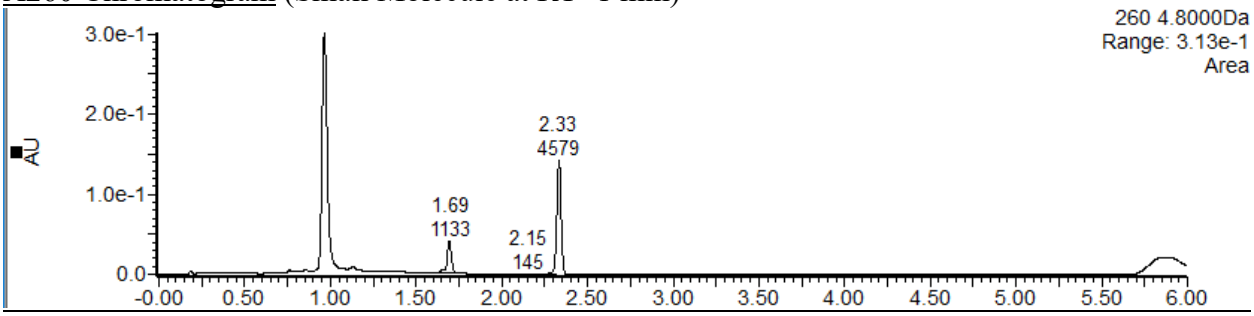
LCMS Characterization of 10

Produced from **2** by General Procedure **2:**
Using **Ψ-4**
Yield (A260): 78%
Expected Mass: 7064.62
Expected (M-6)/6 Ion: 1175.85
Found Deconvoluted Mass: 7065.00
Found (M-6)/6 Ion: 1175.75

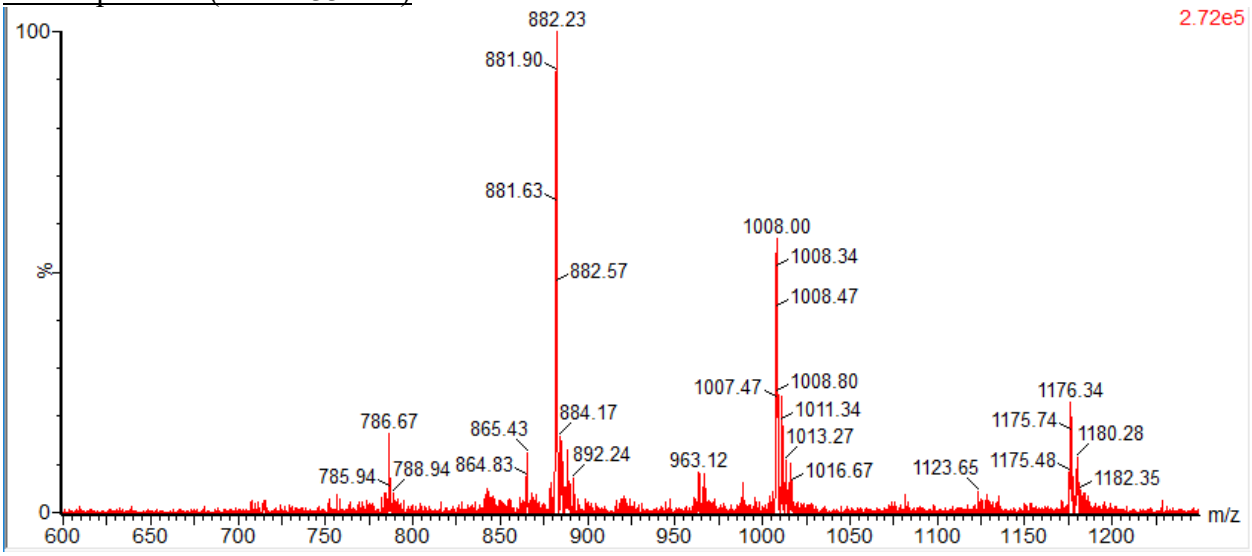


Exact Mass: 7061.15
Molecular Weight: 7064.62

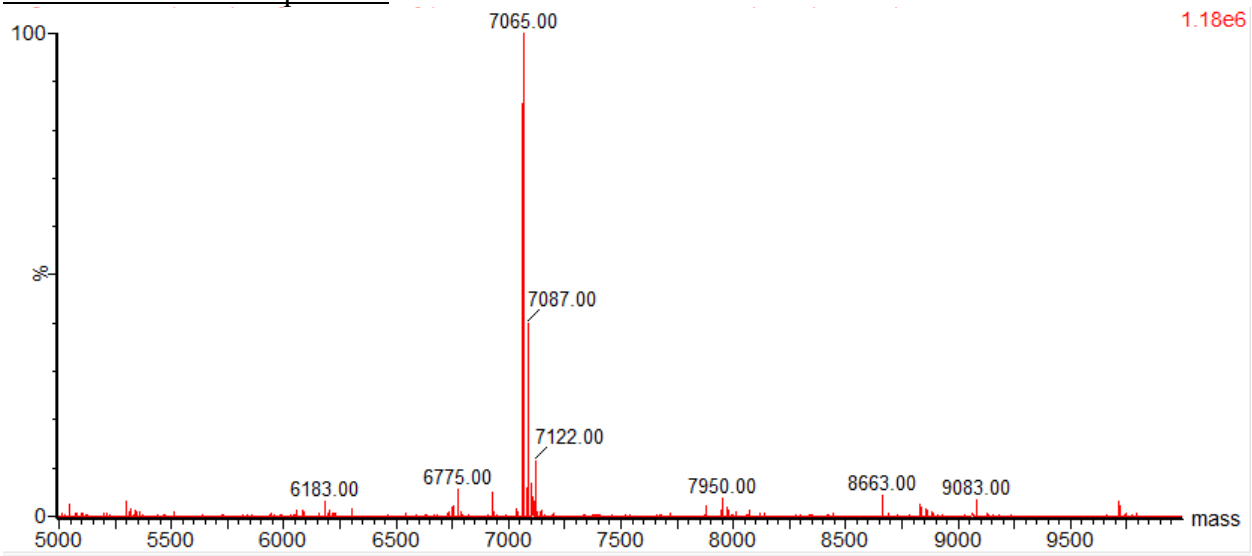
A260 Chromatogram (Small Molecule at RT=1 min)



Mass Spectrum (RT= 2.33 min)

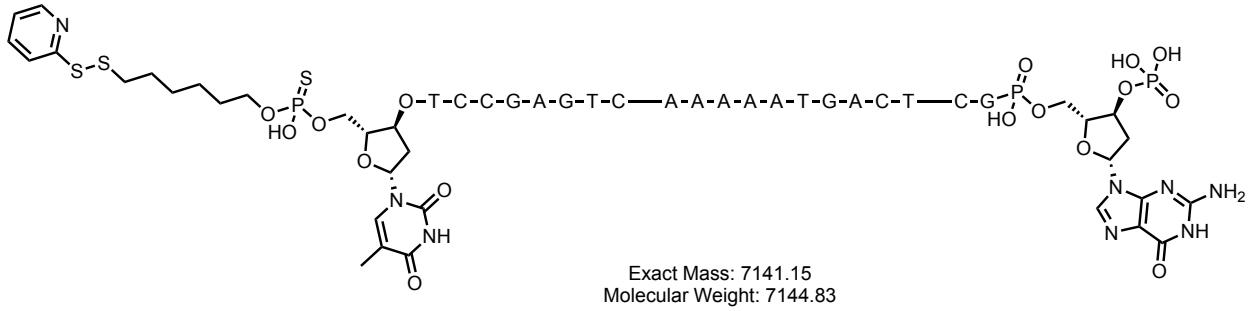


Deconvoluted Mass Spectrum

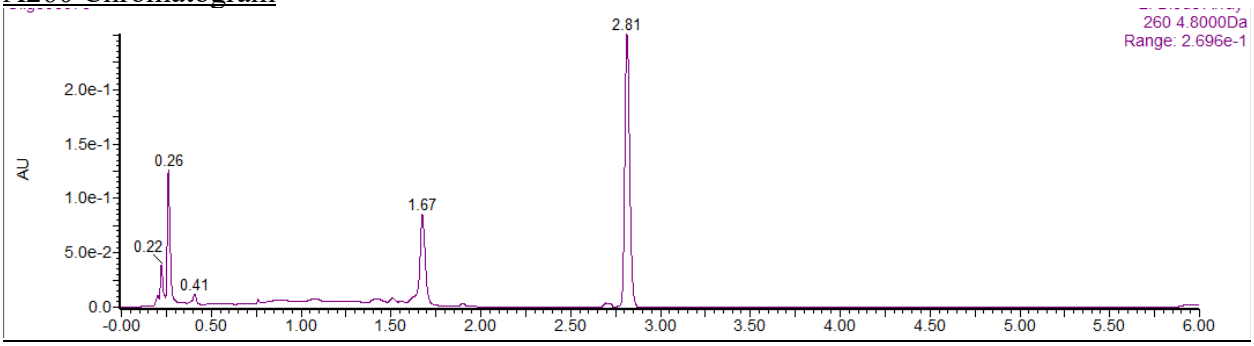


LCMS Characterization of 11

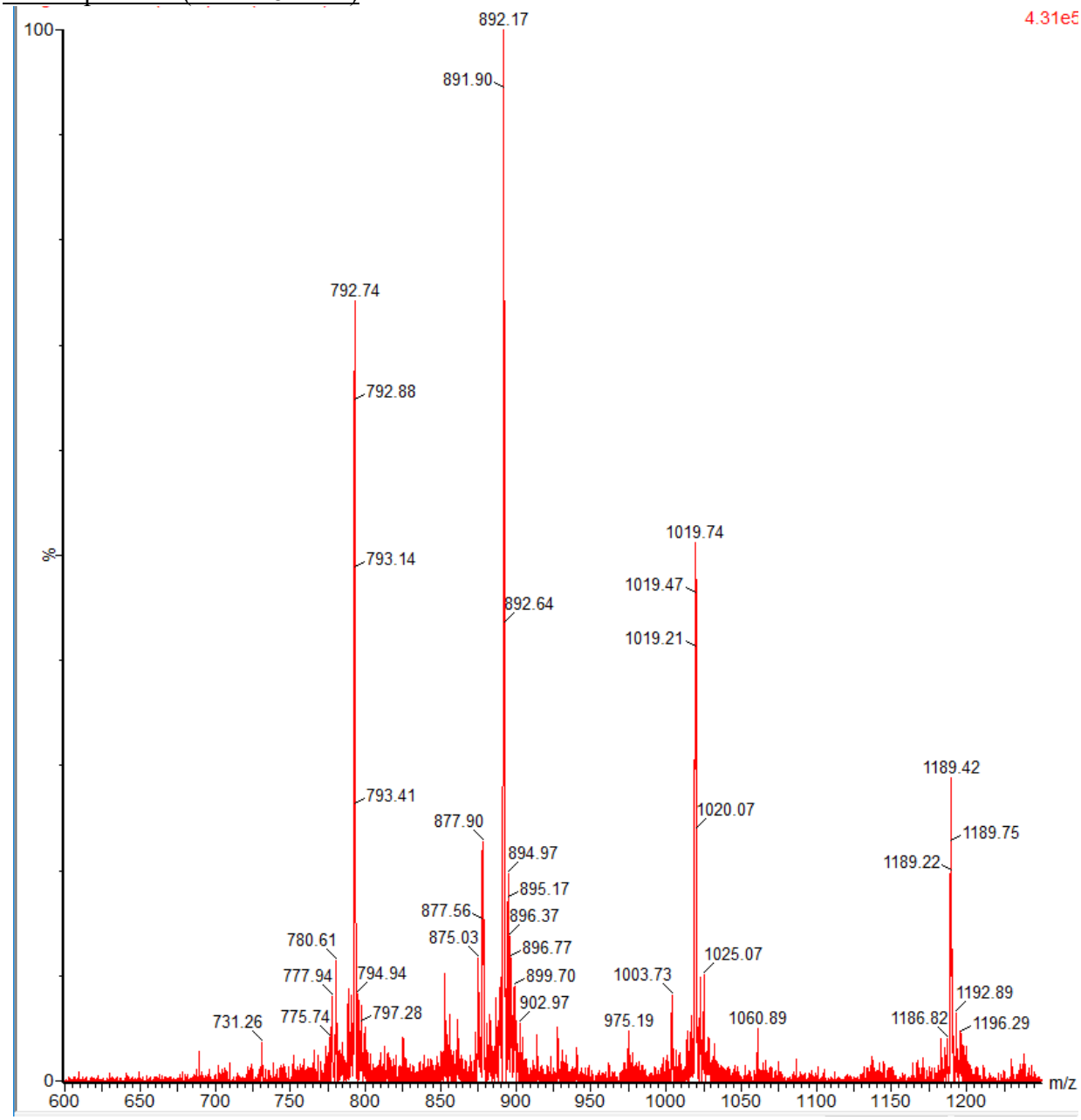
Produced from **1** by General Procedure **2** (Reaction run at 45ºC)**:**
Using **Ψ-8**
Yield (A260): 76%
Expected Mass: 7144.83
Expected (M-6)/6 Ion: 1189.8
Found Deconvoluted Mass: 7144.00
Found (M-6)/6 Ion: 1189.42



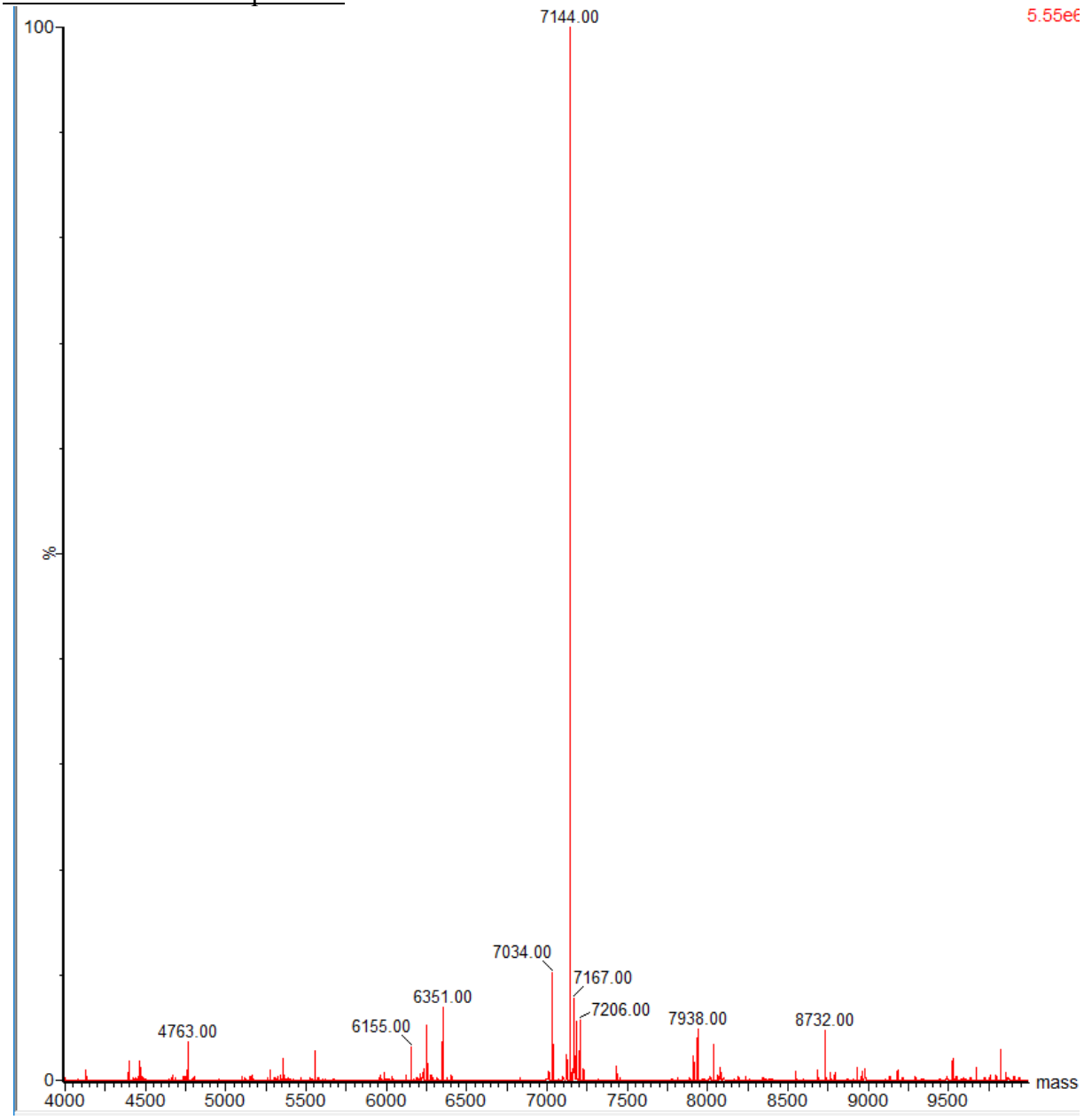
A260 Chromatogram



Mass Spectrum (RT= 2.81 min)

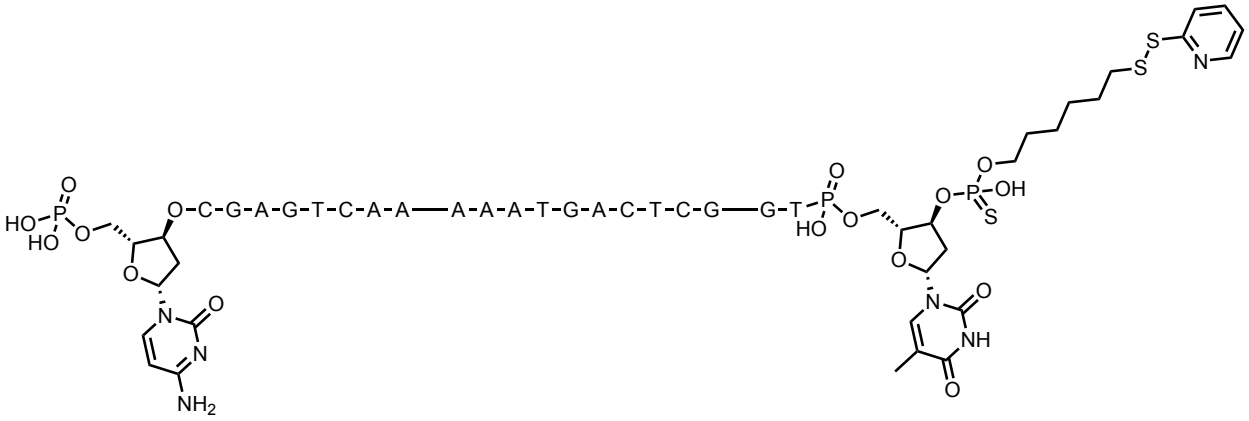


Deconvoluted Mass Spectrum



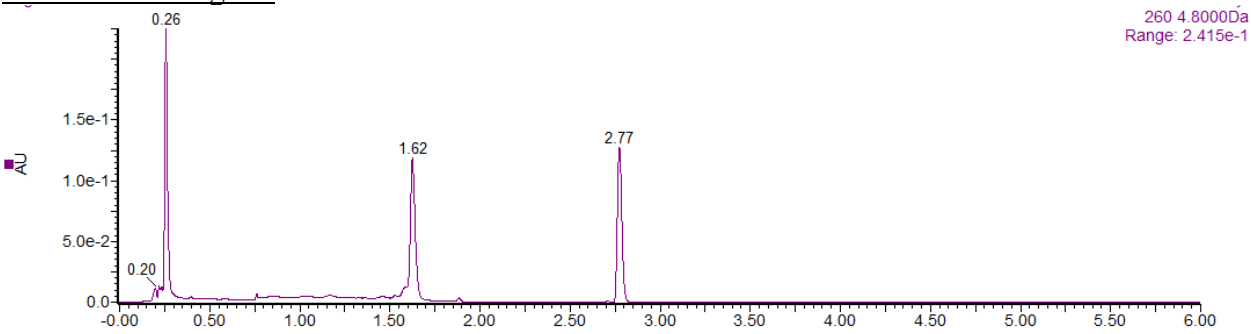
LCMS Characterization of 12

Produced from **2** by General Procedure **2** (reaction run at 45ºC)**:**
Using **Ψ-8**
Yield (A260): 62%
Expected Mass: 7144.83
Expected (M-6)/6 Ion: 1189.8
Found Deconvoluted Mass: 7144.0
Found (M-6)/6 Ion: 1189.4

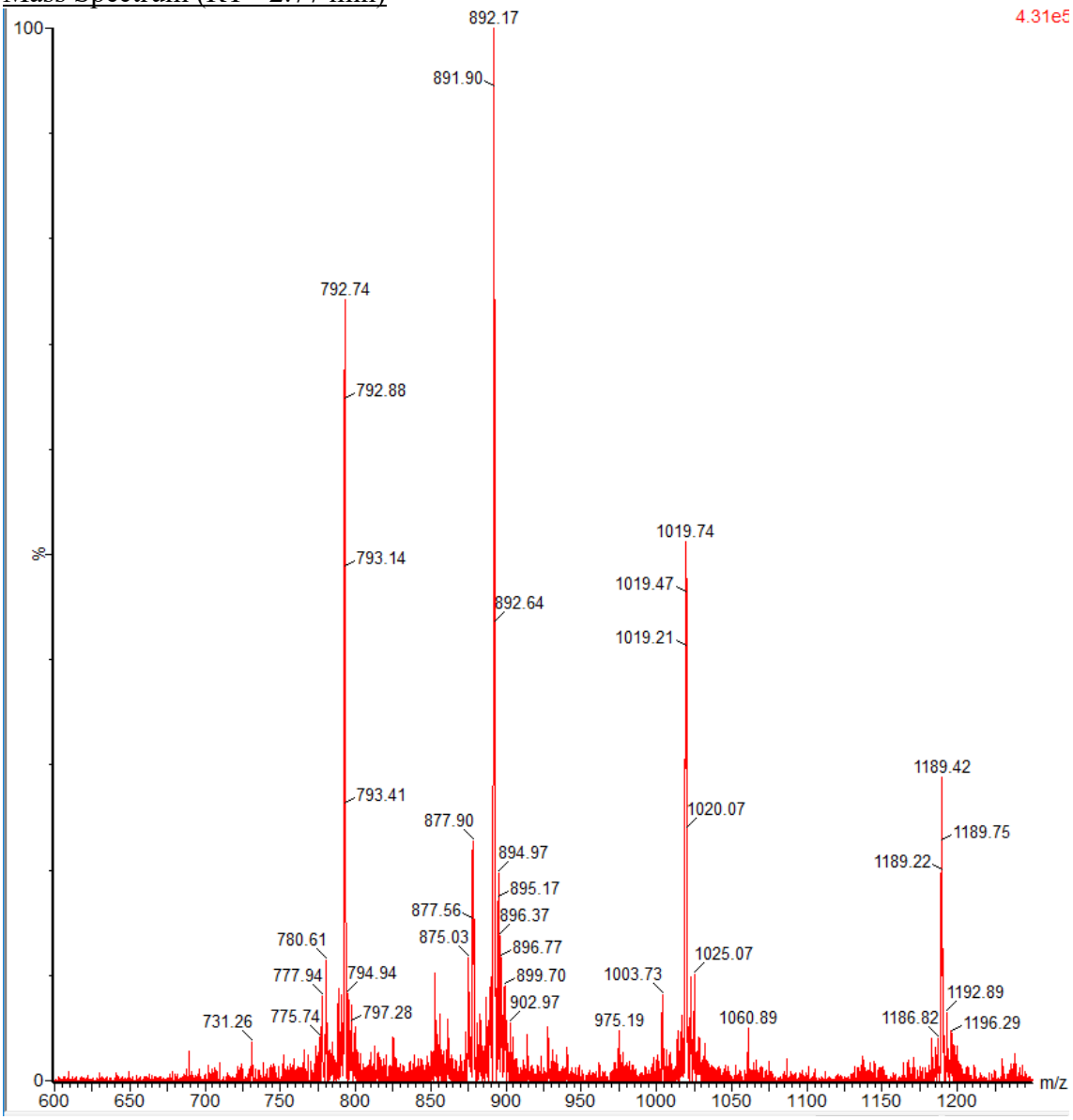


Exact Mass: 7141.15
Molecular Weight: 7144.83

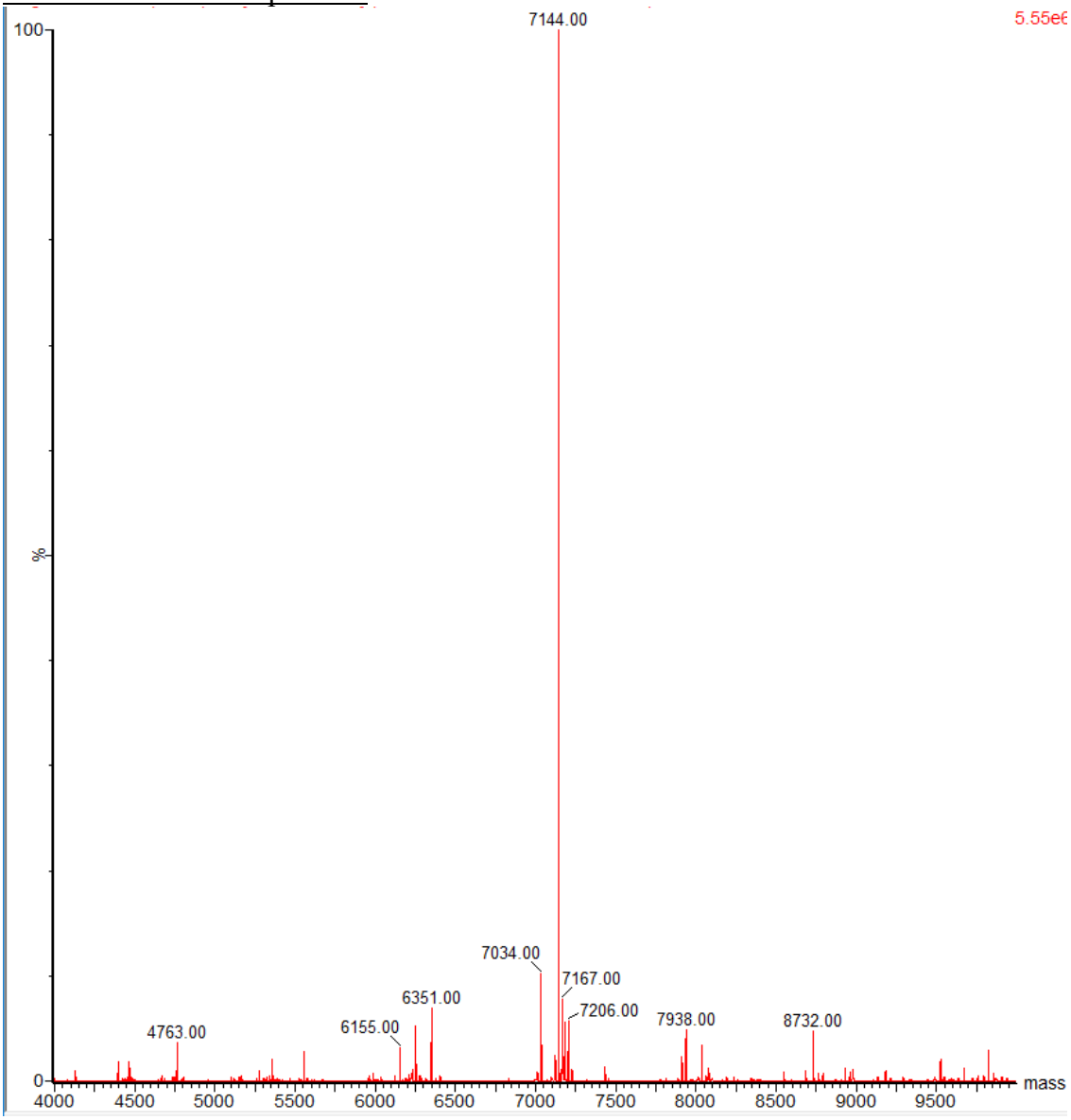
A260 Chromatogram



Mass Spectrum (RT= 2.77 min)

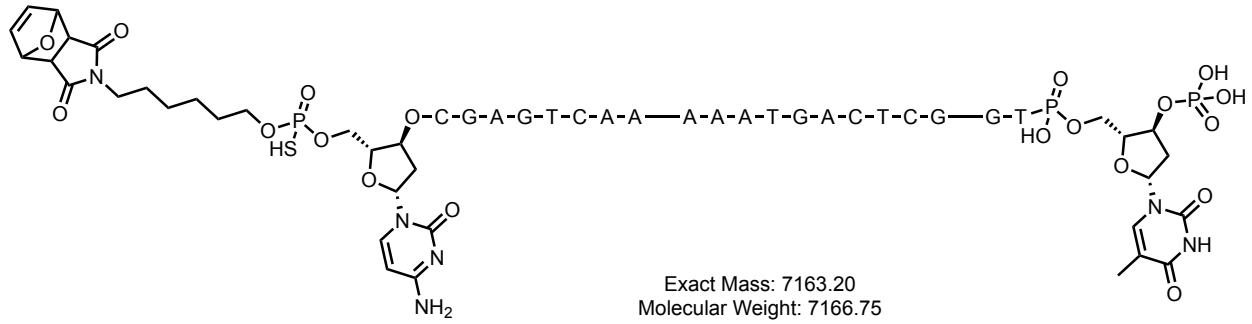


Deconvoluted Mass Spectrum

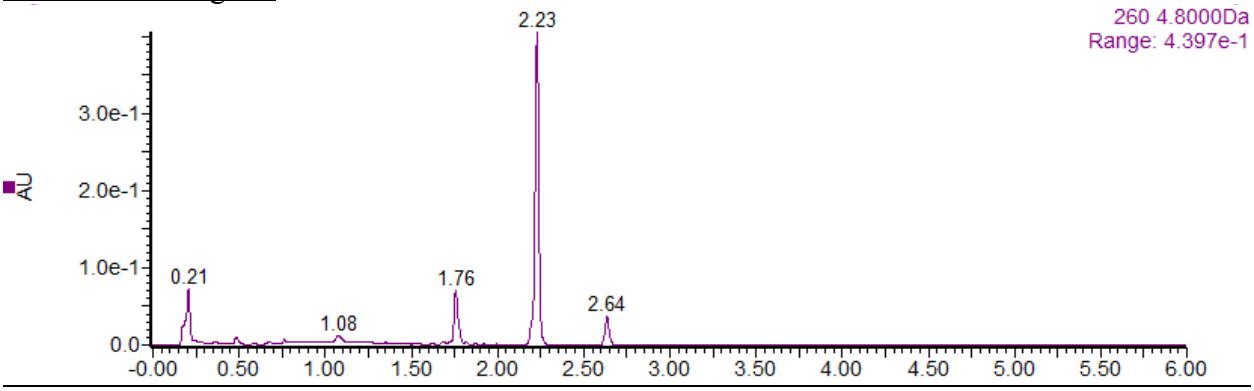


LCMS Characterization of 13

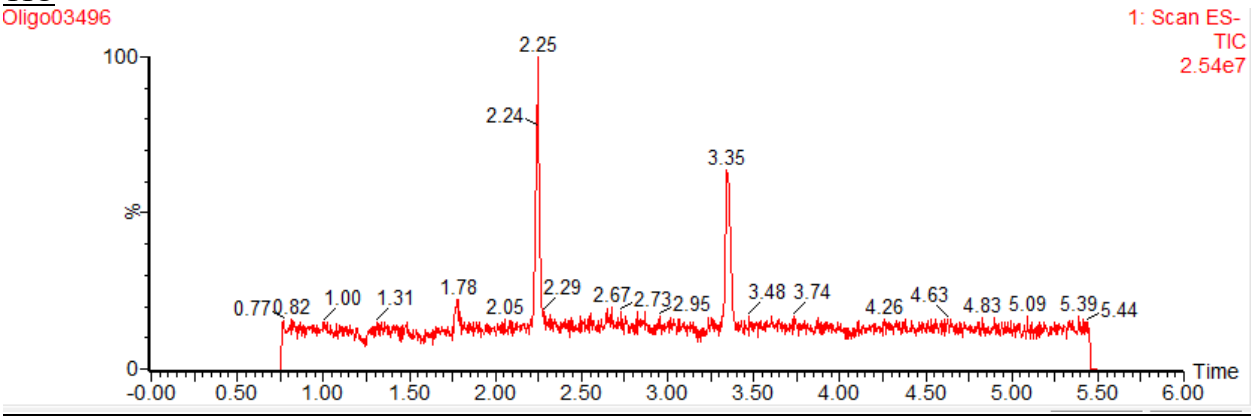
Using **Ψ-19**
Produced from **1** by General Procedure **2** (Reaction Run at 37ºC)**:**
Yield (A260): 84%
Expected Mass: 7166.76
Expected (M-6)/6 Ion: 1193.5
Found Deconvoluted Mass: 7166.00
Found (M-6)/6 Ion: 1193.42



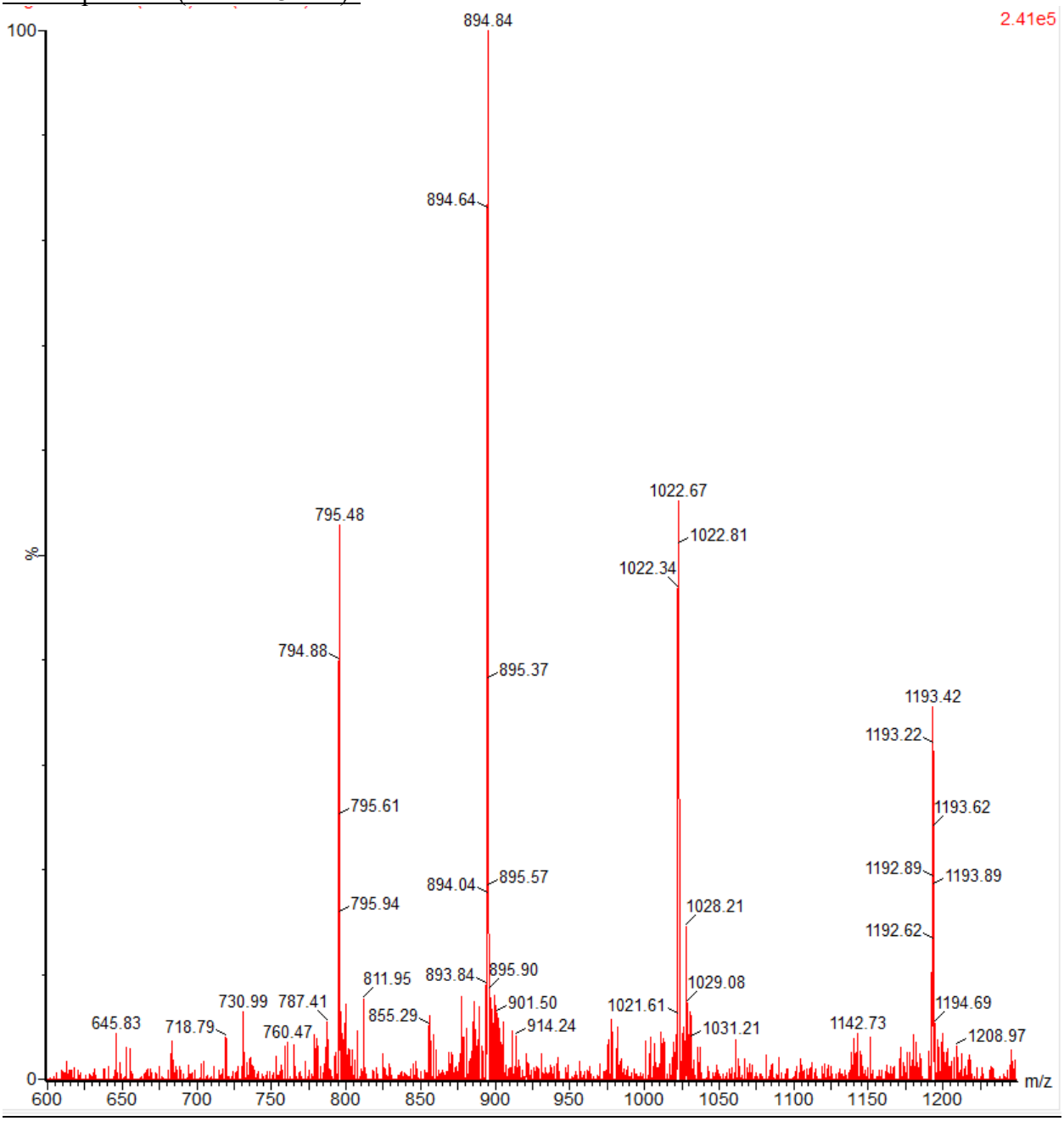
A260 Chromatogram



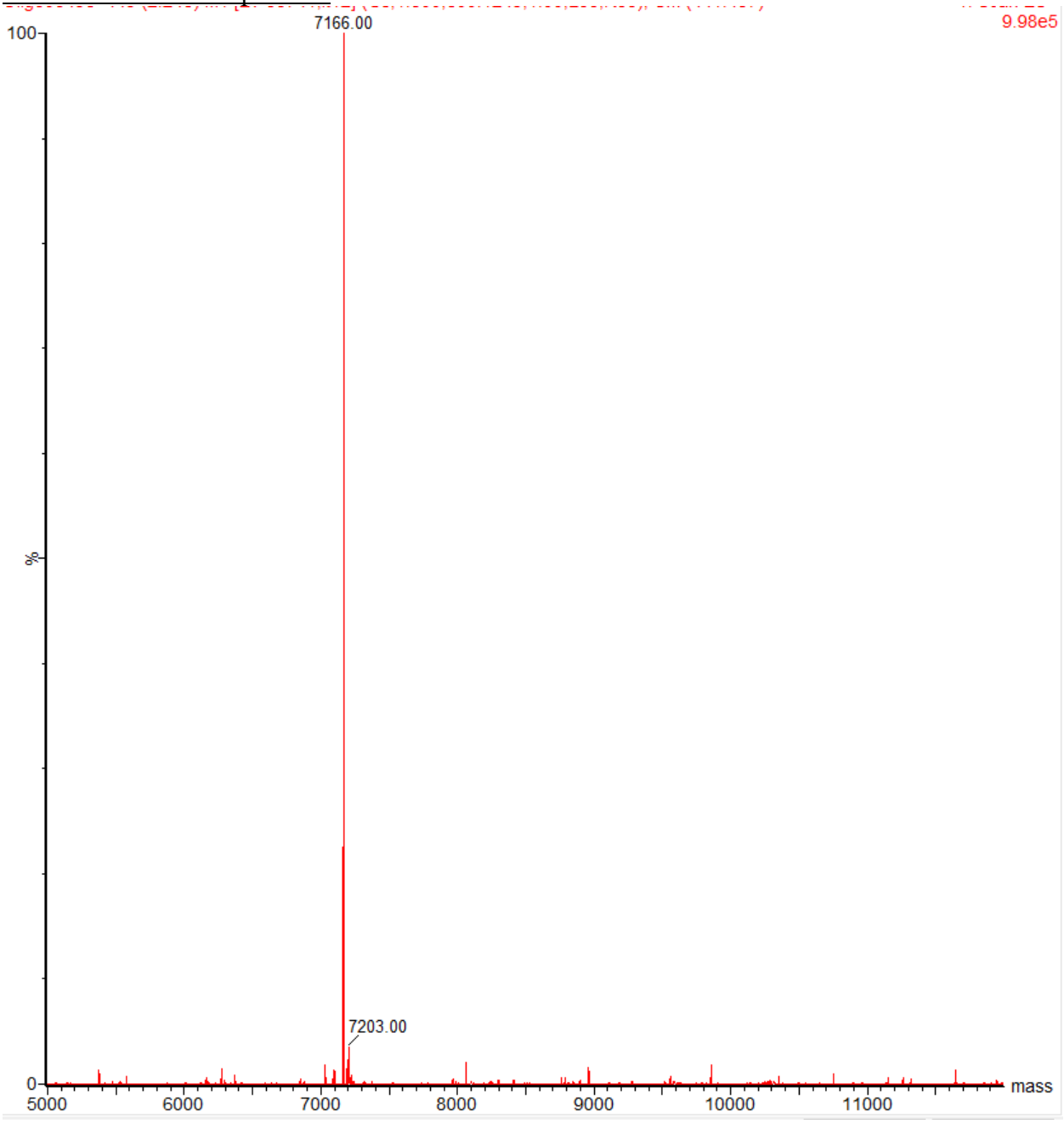
TIC



Mass Spectrum (RT= 2.23 min)

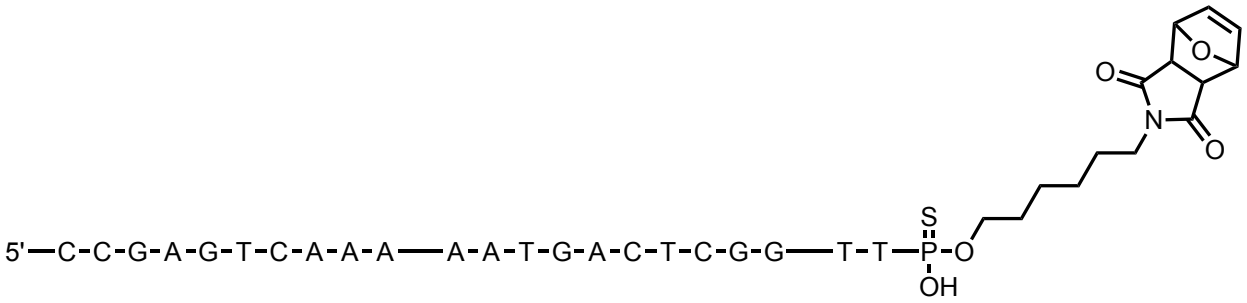


Deconvoluted Mass Spectrum



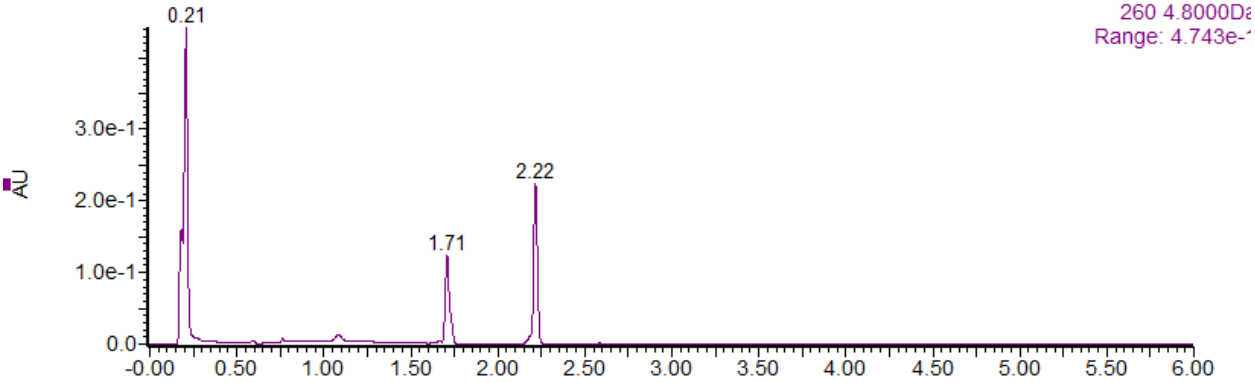
LCMS Characterization of 14

Using **Ψ-19**
Produced from **2** by General Procedure **2** (Reaction Run at 37ºC)**:**
Yield (A260): 62%
Expected Mass: 7166.76
Expected (M-6)/6 Ion: 1193.5
Found Deconvoluted Mass: 7166.0
Found (M-6)/6 Ion: 1193.82

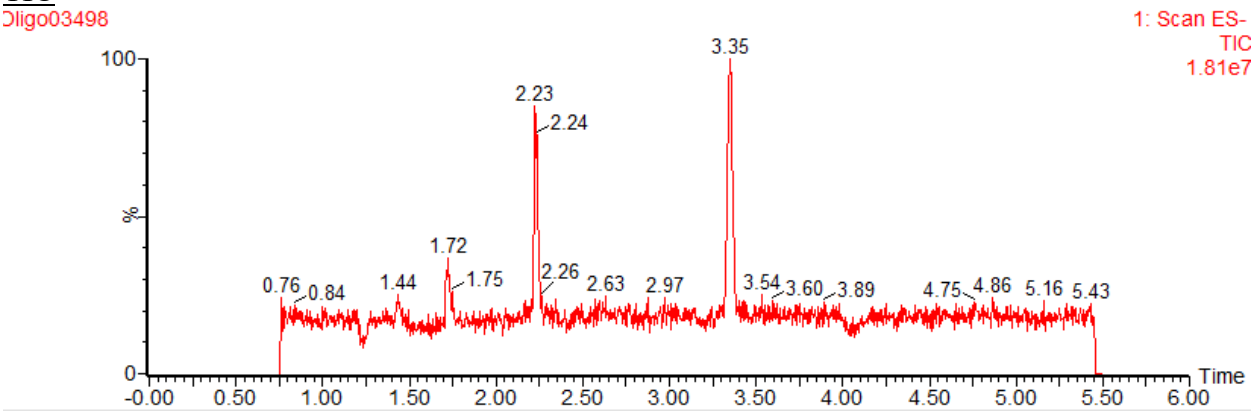


Molecular Weight: 7166.75

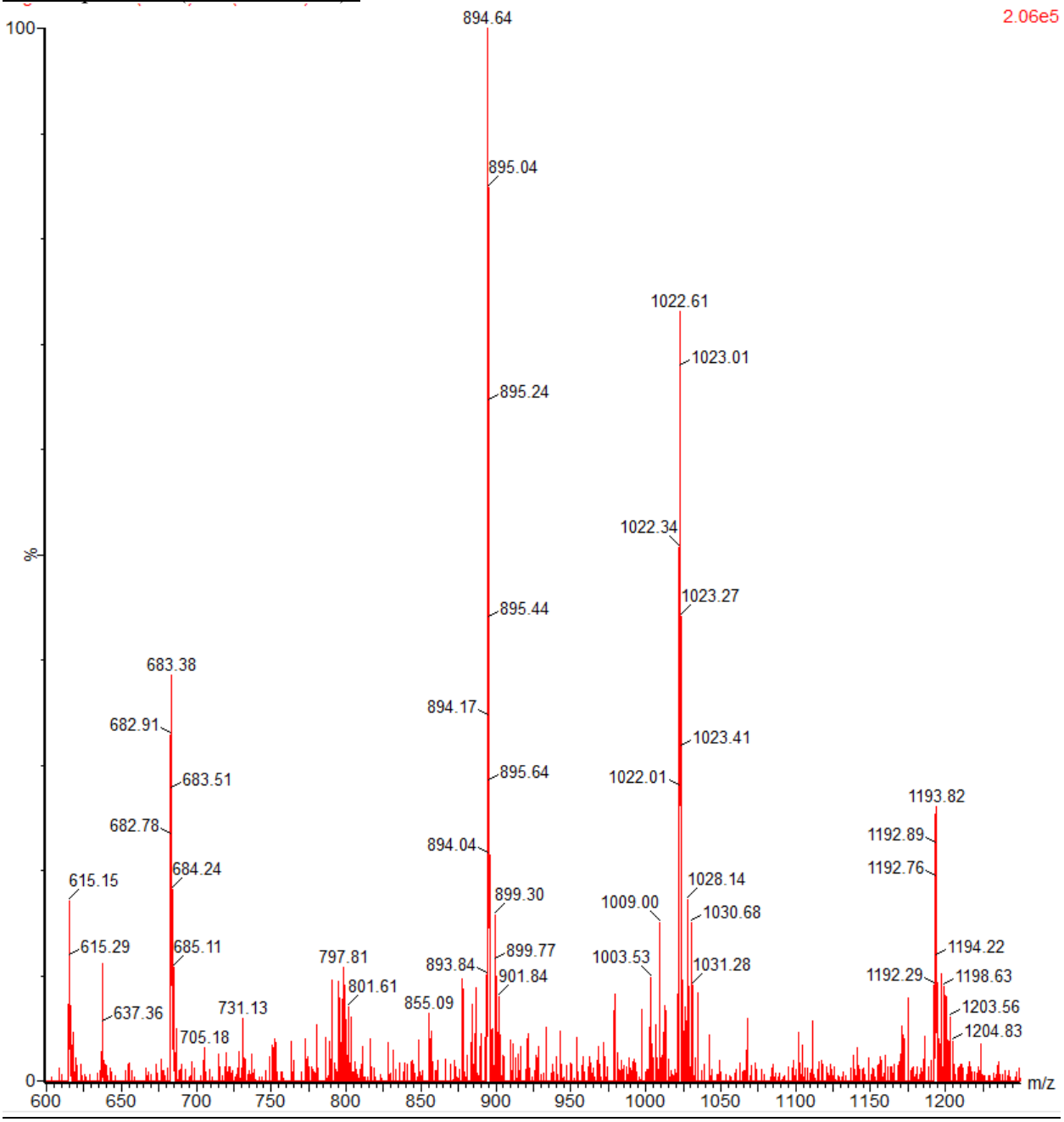
A260 Chromatogram



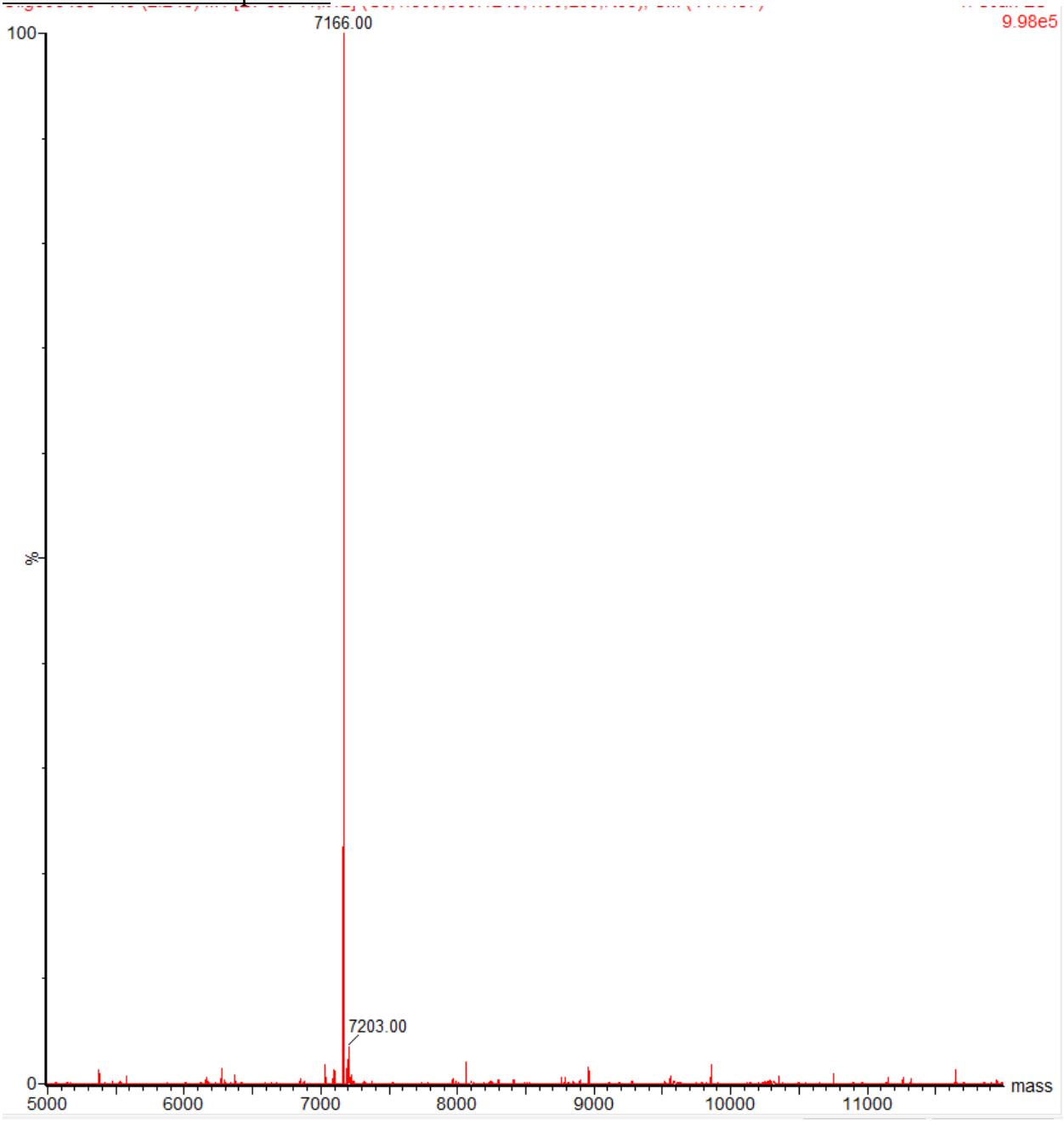
TIC



Mass Spectrum (RT= 2.22 min)

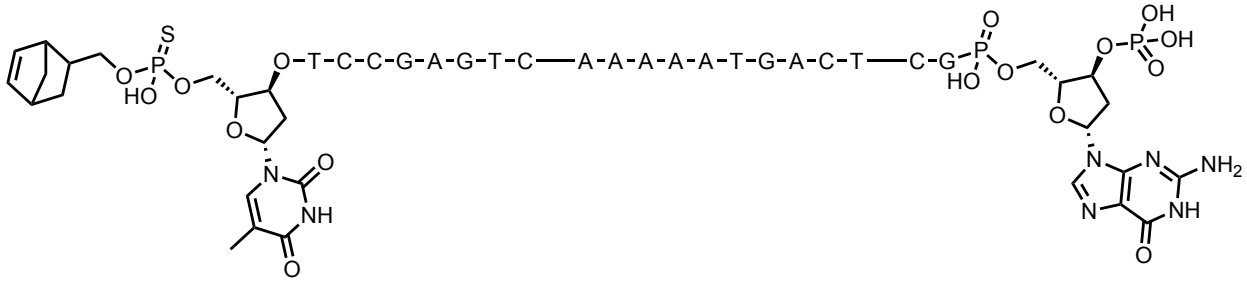


Deconvoluted Mass Spectrum



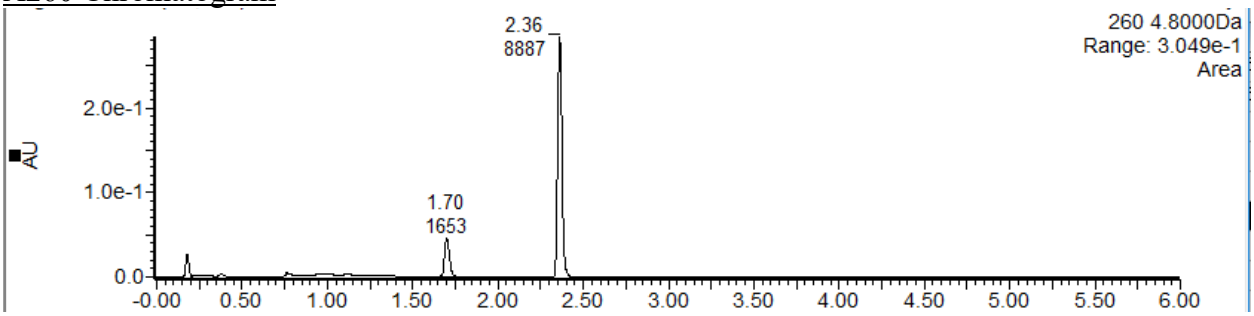
LCMS Characterization of 15

Produced from **1** by General Procedure **2:**
Using **Ψ-12**
Yield (A260): 81%
Expected Mass: 7025.6
Expected (M-6)/6 Ion: 1169.36
Found Deconvoluted Mass: 7026.0
Found (M-6)/6 Ion: 1169.41

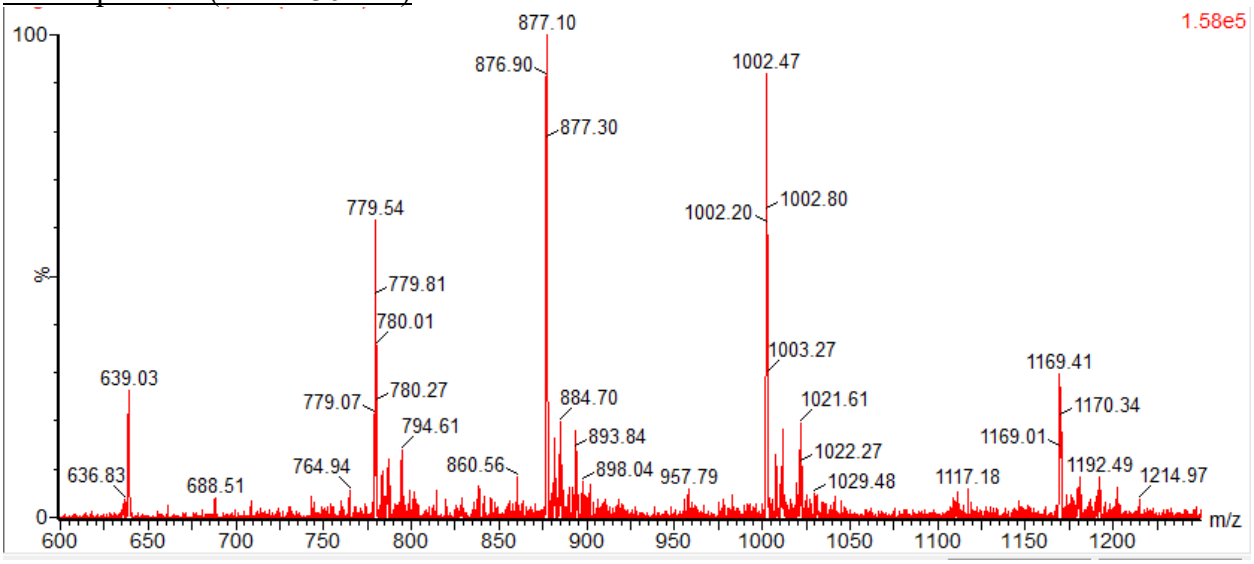


Exact Mass: 7022.16
Molecular Weight: 7025.63

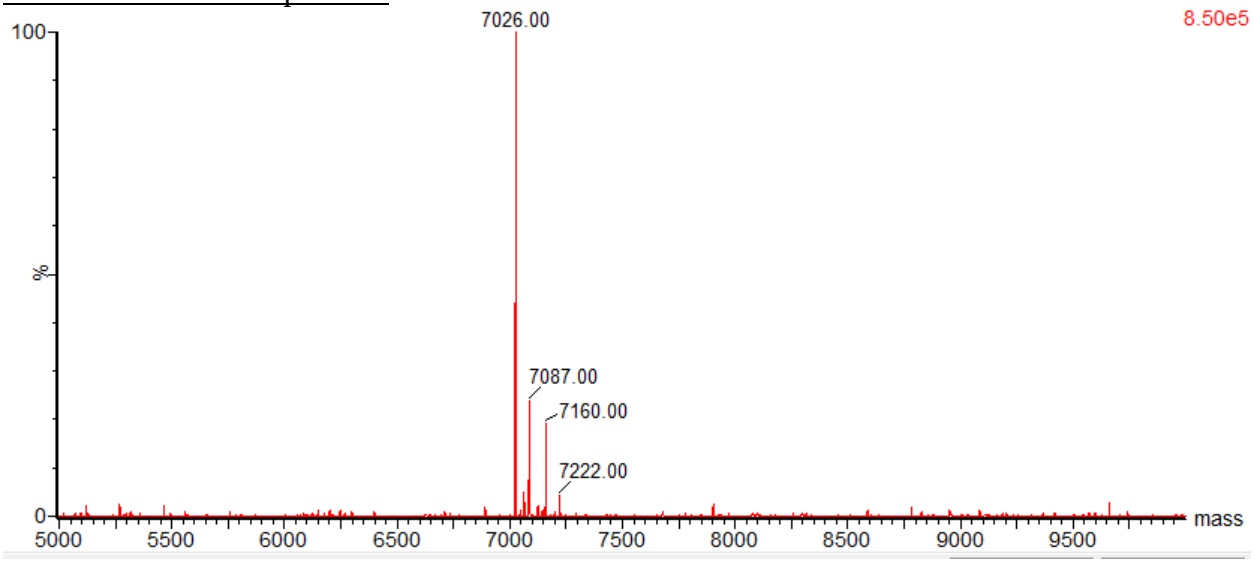
A260 Chromatogram



Mass Spectrum (RT= 2.36 min)

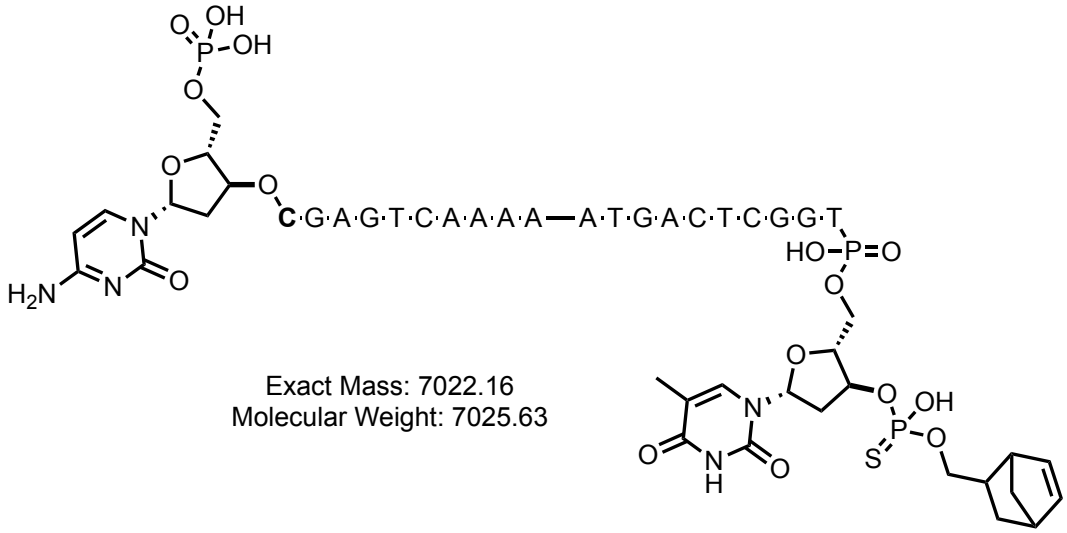


Deconvoluted Mass Spectrum

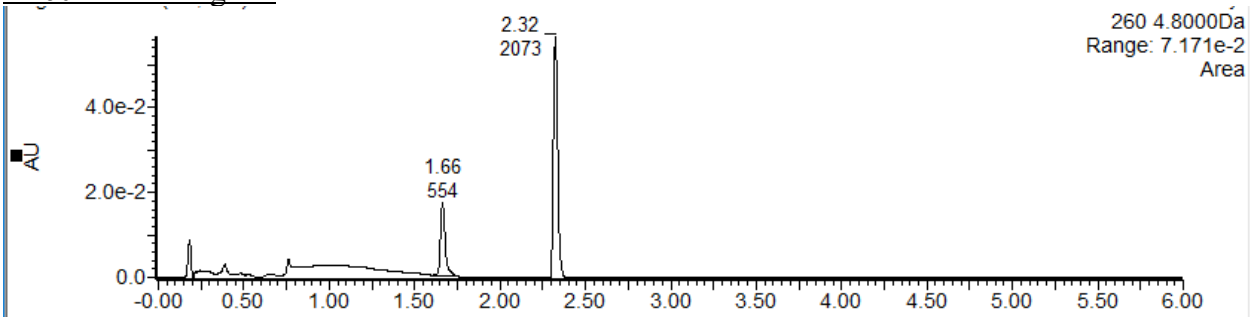


LCMS Characterization of 16

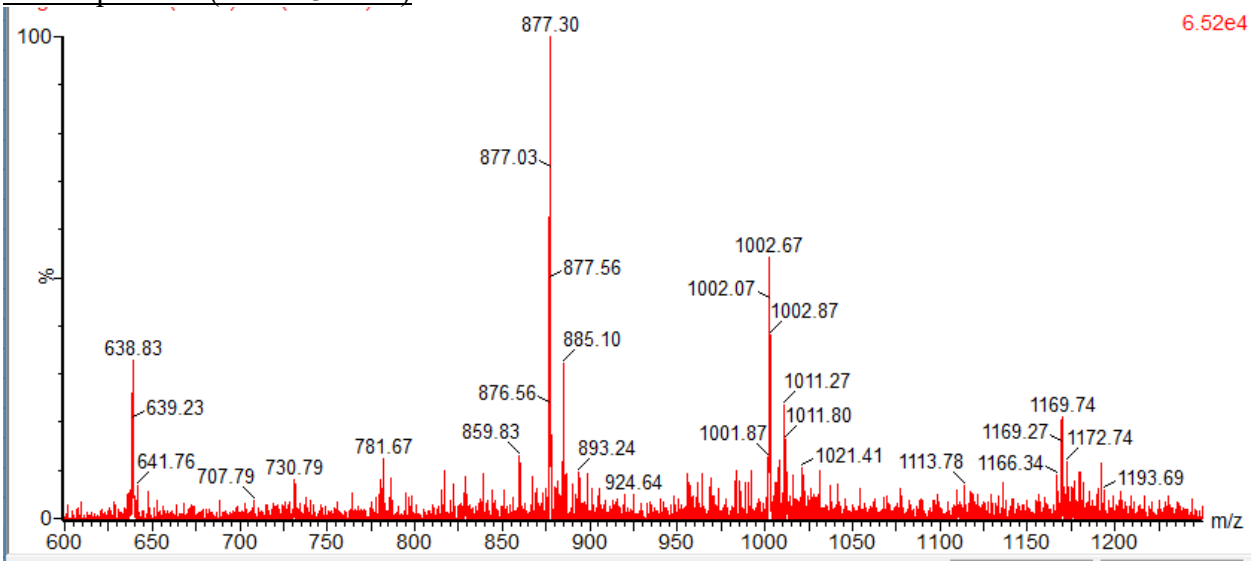
Produced from **2** by General Procedure **2:**
Using **Ψ-12**
Yield (A260): 68%
Expected Mass: 7025.63
Expected (M-6)/6 Ion: 1169.94
Found Deconvoluted Mass: 7025.0
Found (M-6)/6 Ion: 1169.74



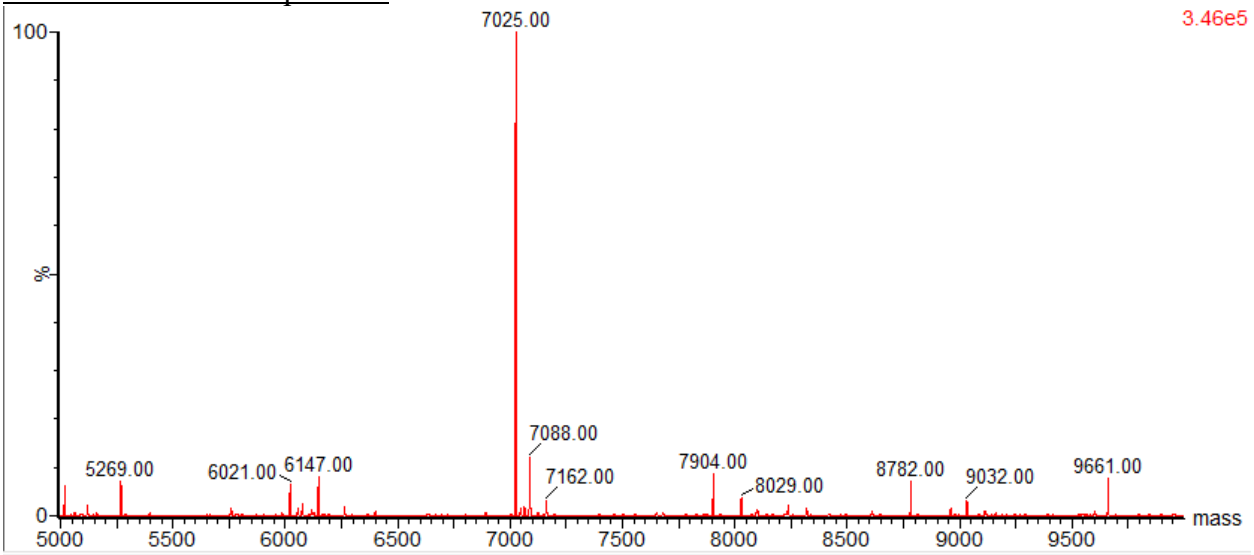
A260 Chromatogram



Mass Spectrum (RT= 2.32 min)

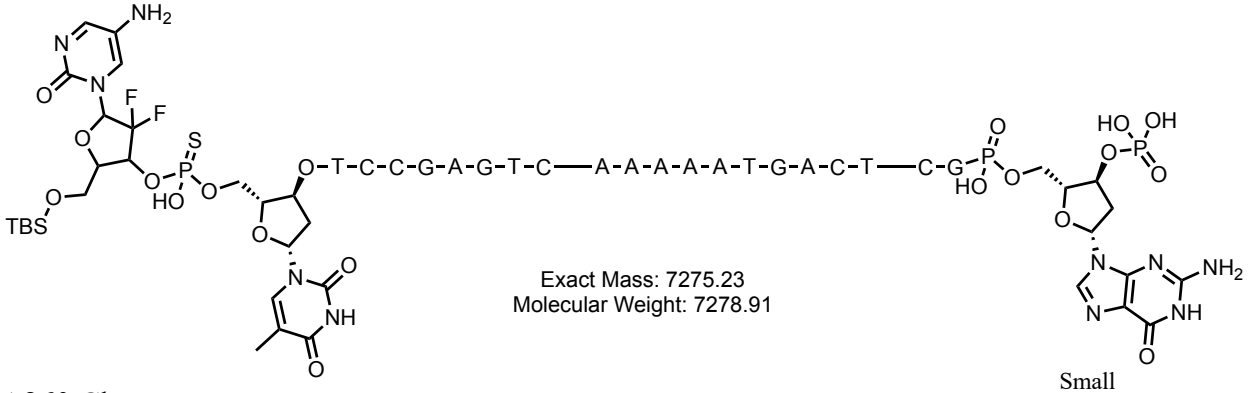


Deconvoluted Mass Spectrum

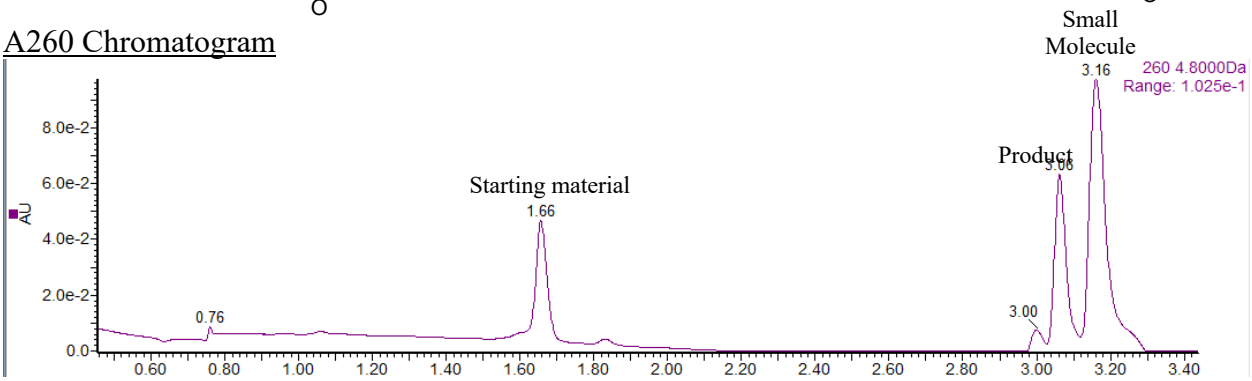


LCMS Characterization of 17

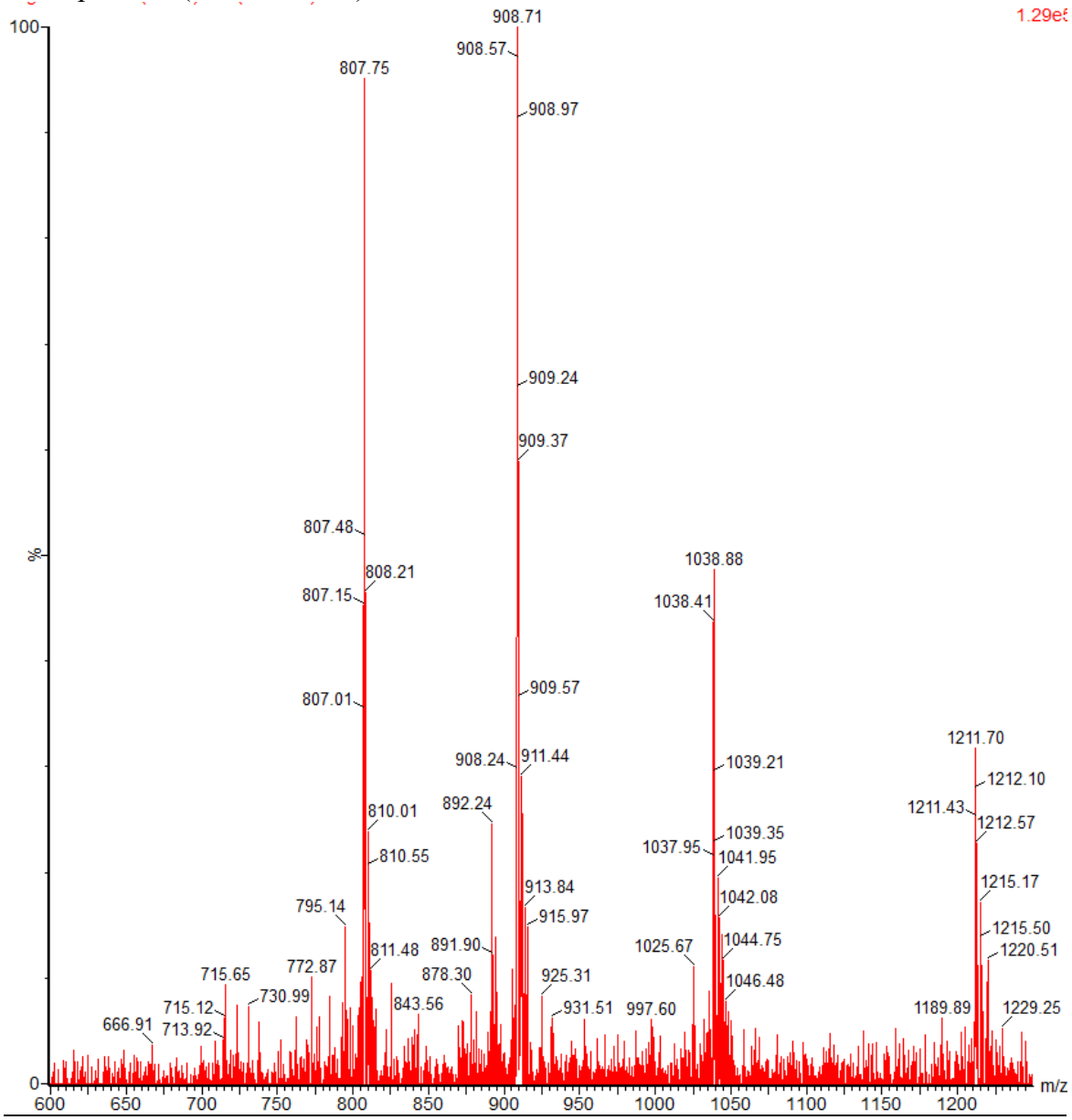
Produced from **1** by General Procedure **2:**
Using **Ψ-13**
Yield (A260): 51%
Expected Mass: 7278.91
Expected (M-6)/6 Ion: 1211.5
Found Deconvoluted Mass: 7279.0
Found (M-6)/6 Ion: 1211.7



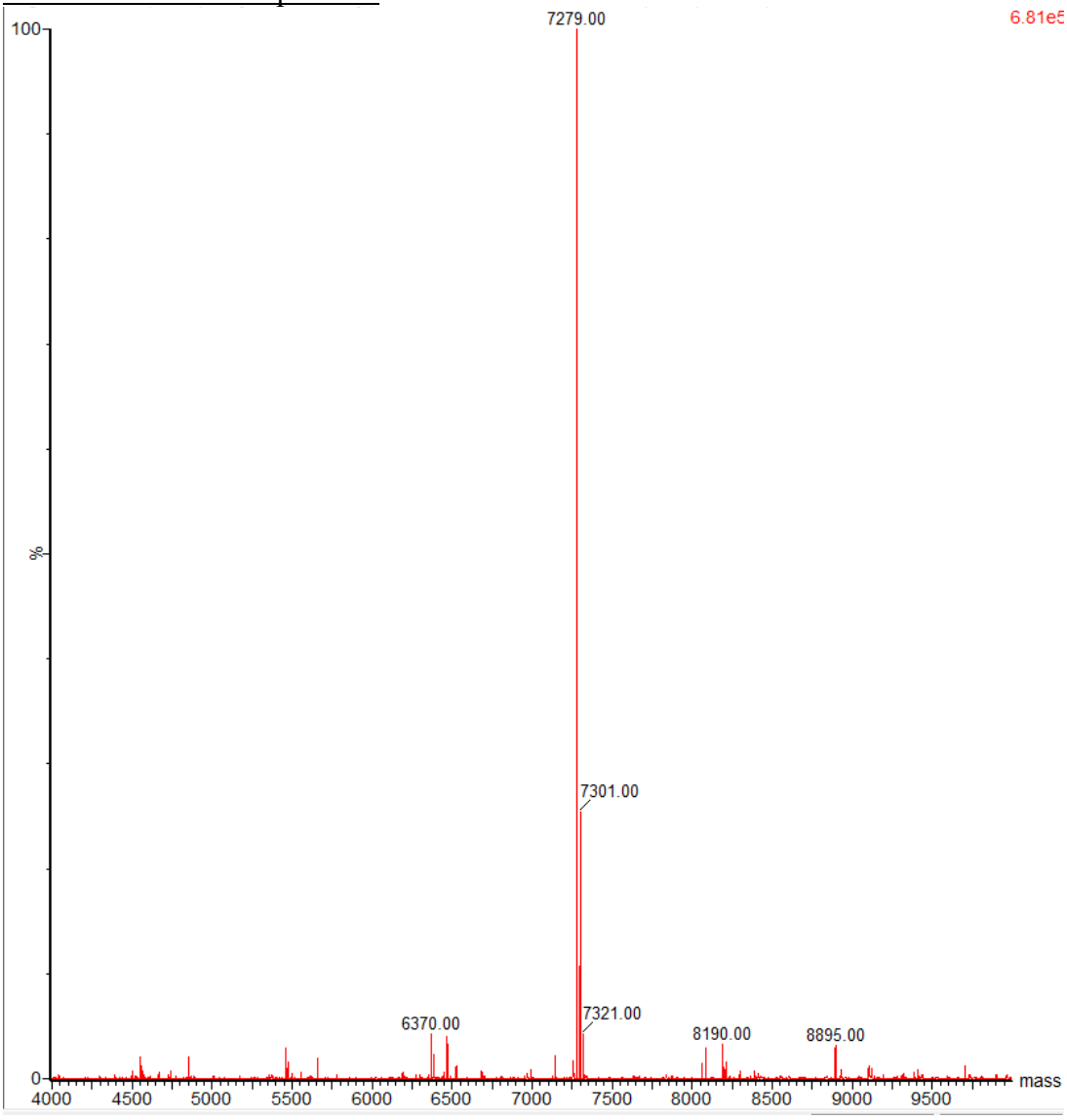
A260 Chromatogram



Mass Spectrum (RT= 3.06 min)

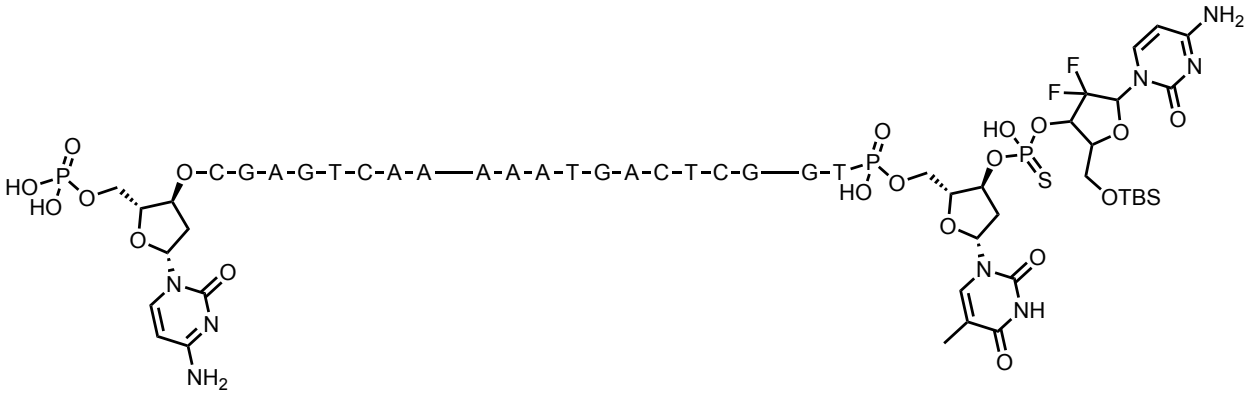


Deconvoluted Mass Spectrum



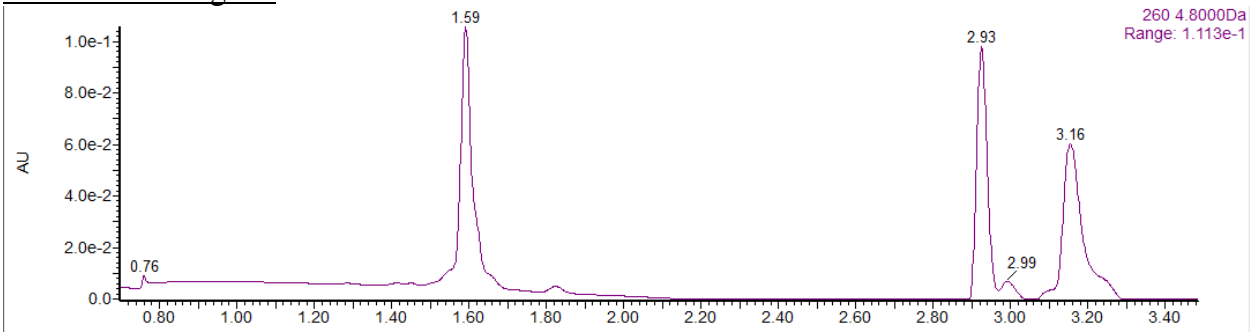
LCMS Characterization of 18

Produced from **2** by General Procedure **2:**
Using **Ψ-13**
Yield (A260): 45%
Expected Mass: 7278.23
Expected (M-6)/6 Ion: 1211.5
Found Deconvoluted Mass: 7279.00
Found (M-6)/6 Ion: 1211.77

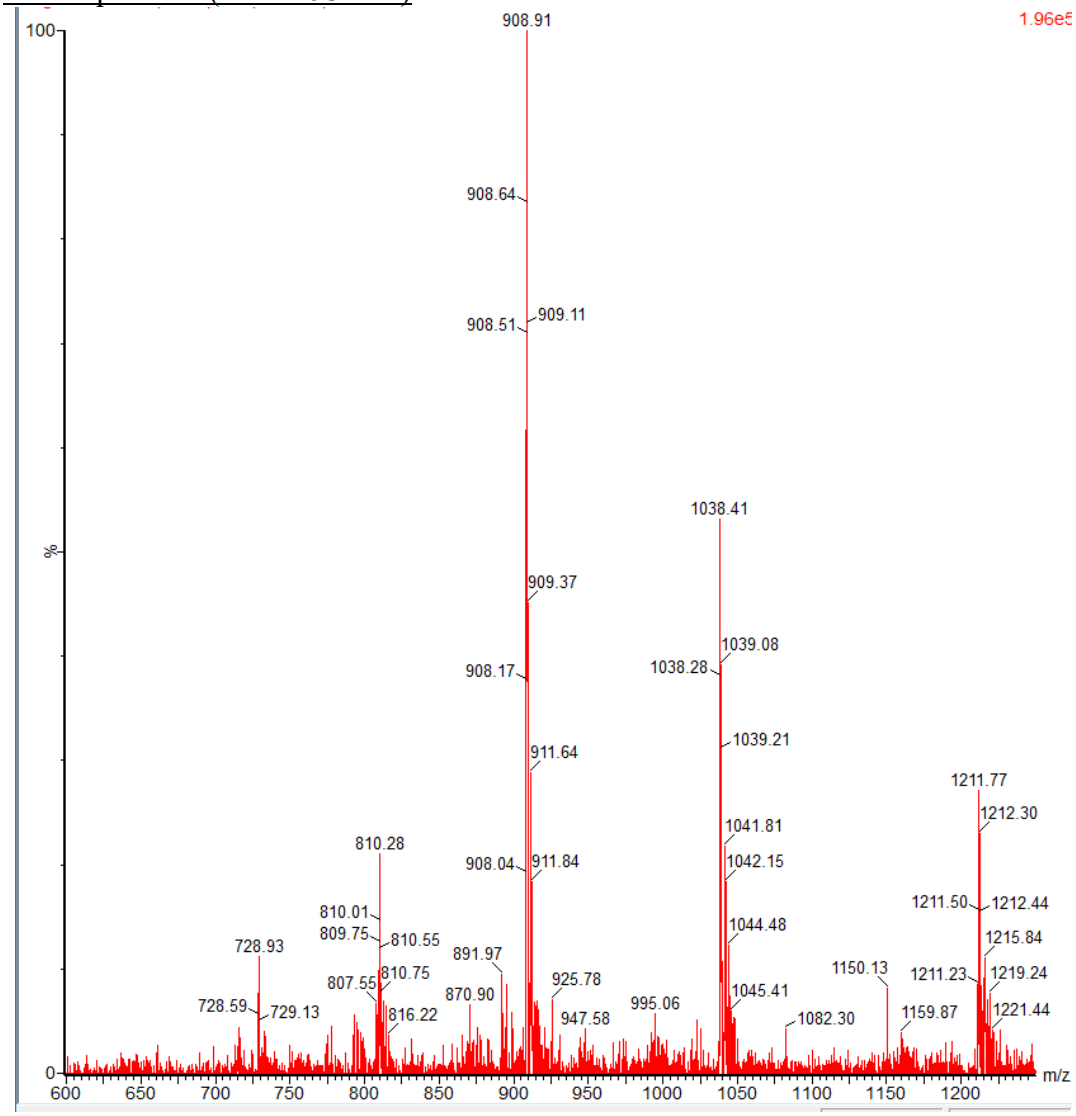


Exact Mass: 7275.23
Molecular Weight: 7278.91

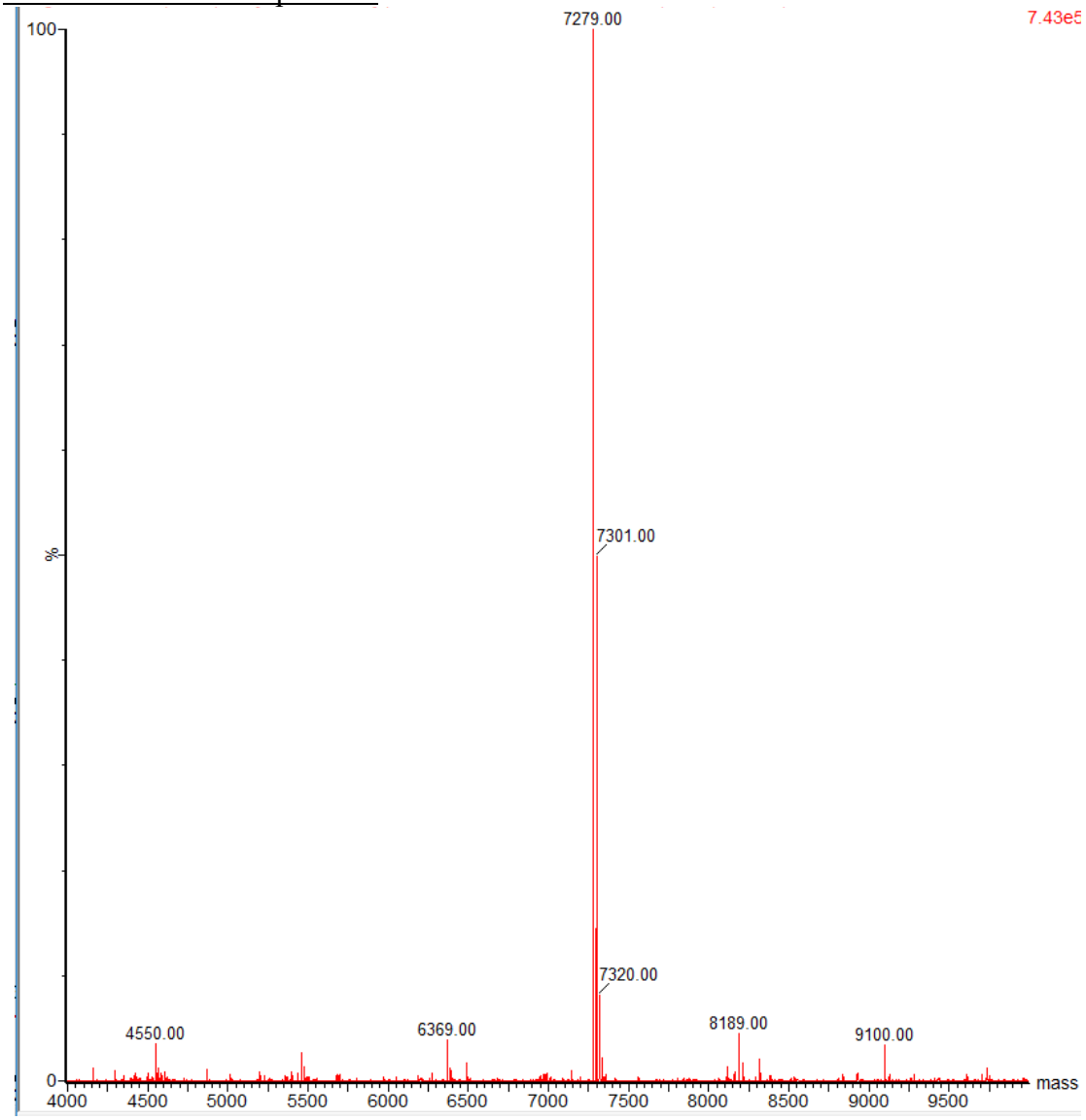
A260 Chromatogram



Mass Spectrum (RT= 2.93 min)

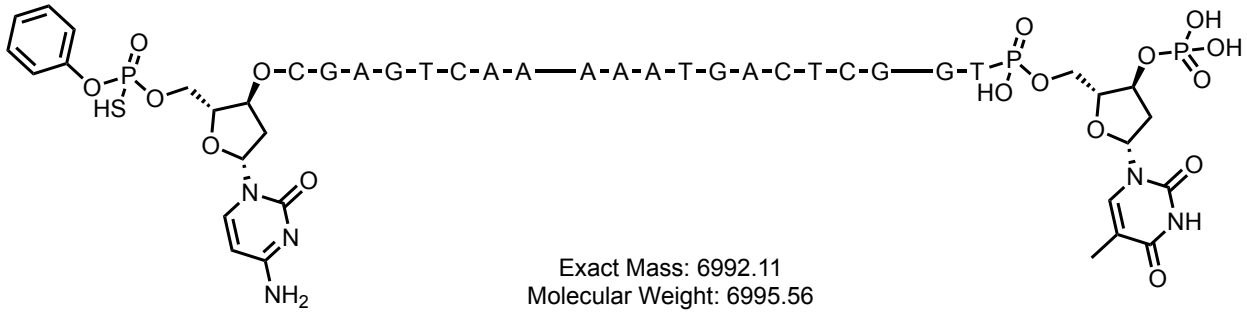


Deconvoluted Mass Spectrum



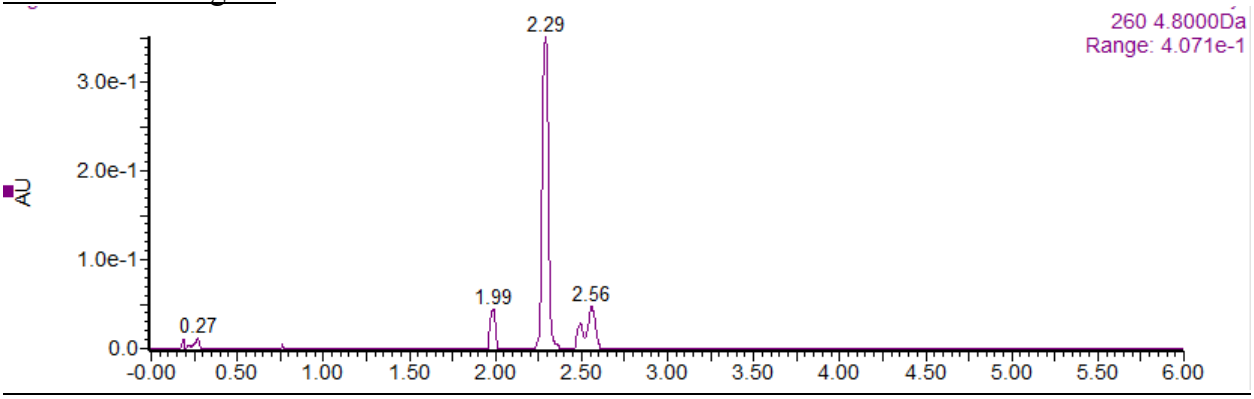
LCMS Characterization of 19

Using **Ψ-14**
Produced from **1** by General Procedure **2 (**Reaction run with 75 mM **Ψ-14** and 225 mM DBU**):**
Yield (A260): 68%
Expected Mass: 6995.11
Expected (M-6)/6 Ion: 1164.9
Found Deconvoluted Mass: 6995.0
Found (M-6)/6 Ion: 1165.0

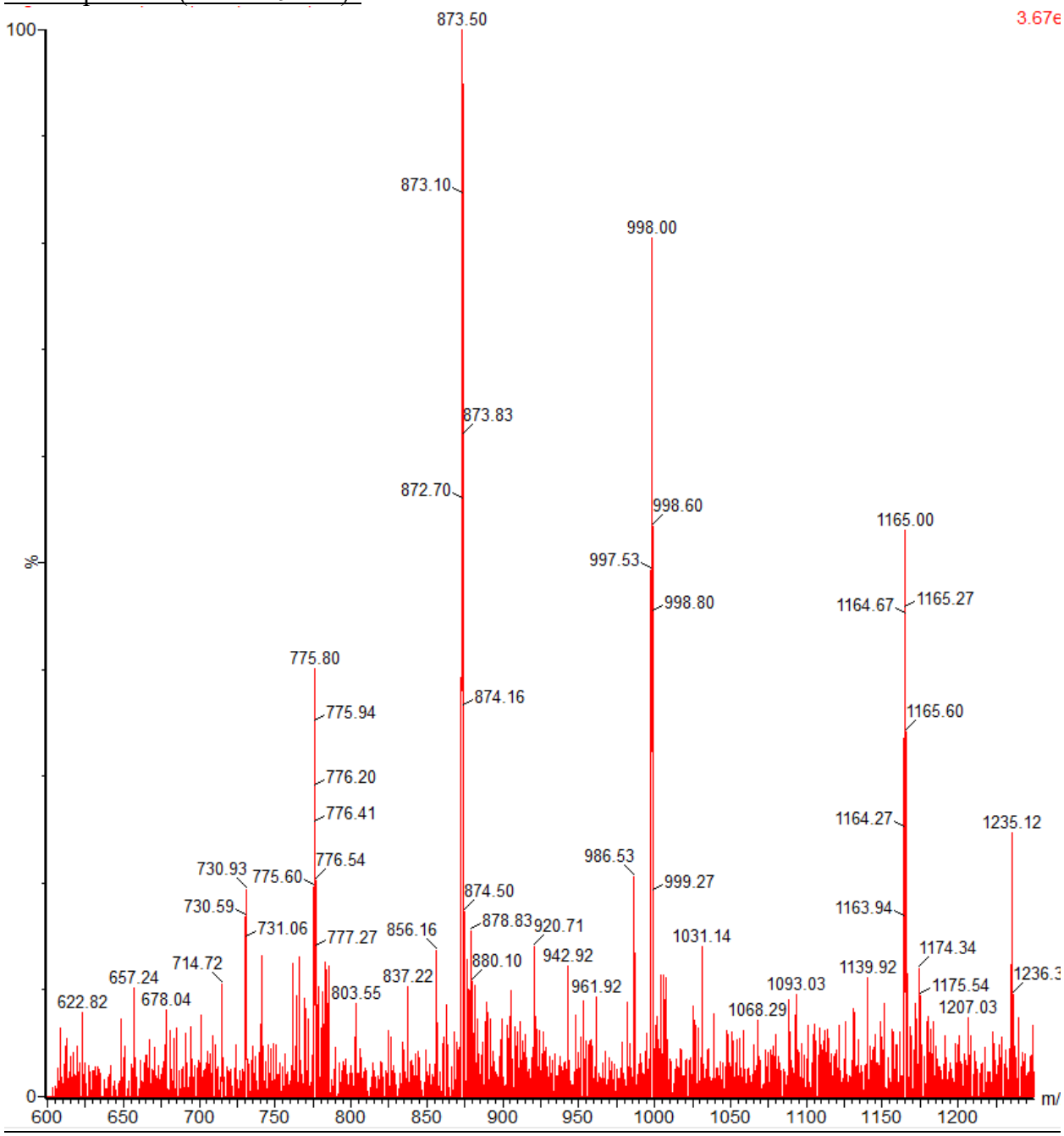


Exact Mass: 6992.11
Molecular Weight: 6995.56

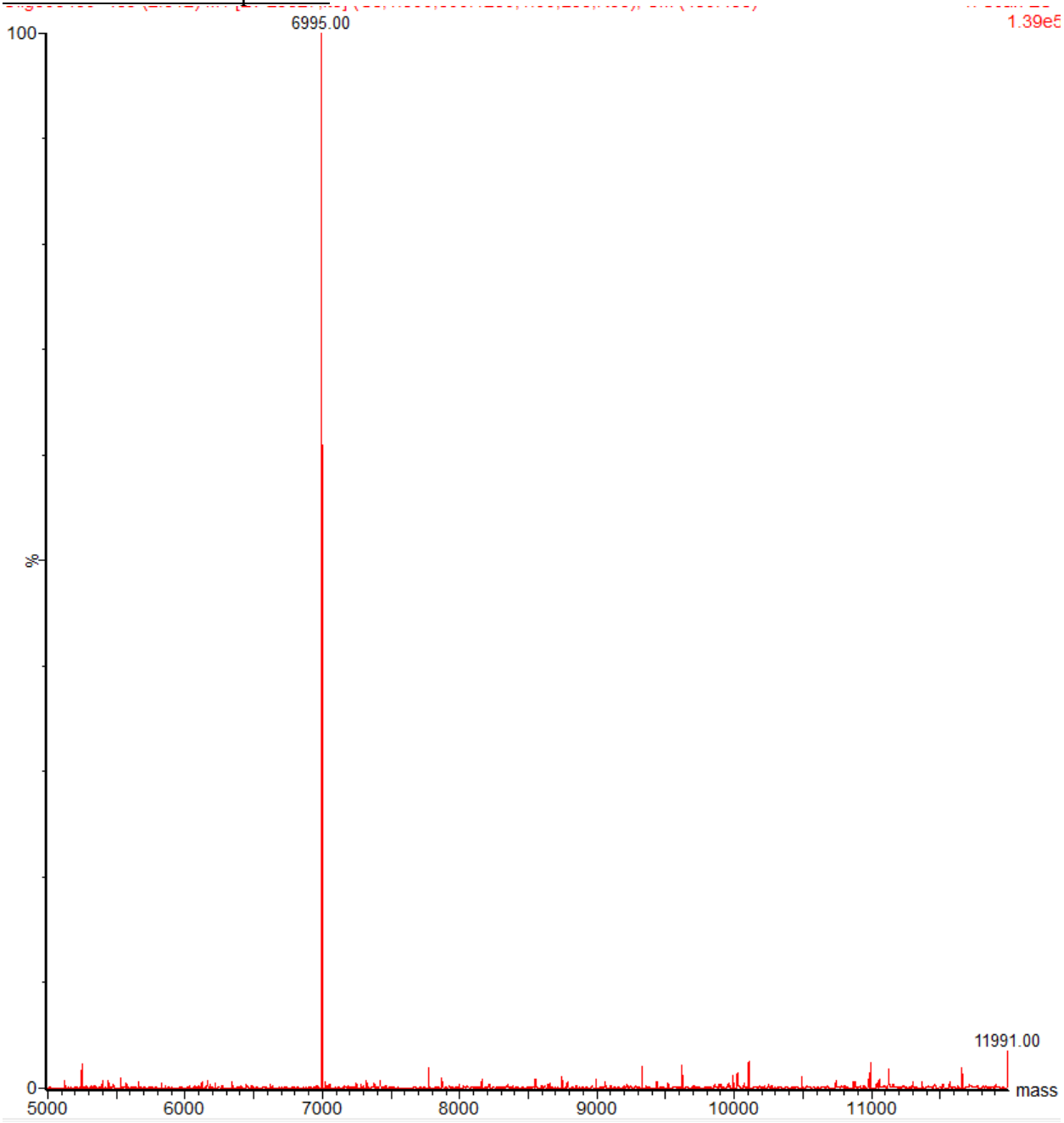
A260 Chromatogram



Mass Spectrum (RT= 2.29 min)

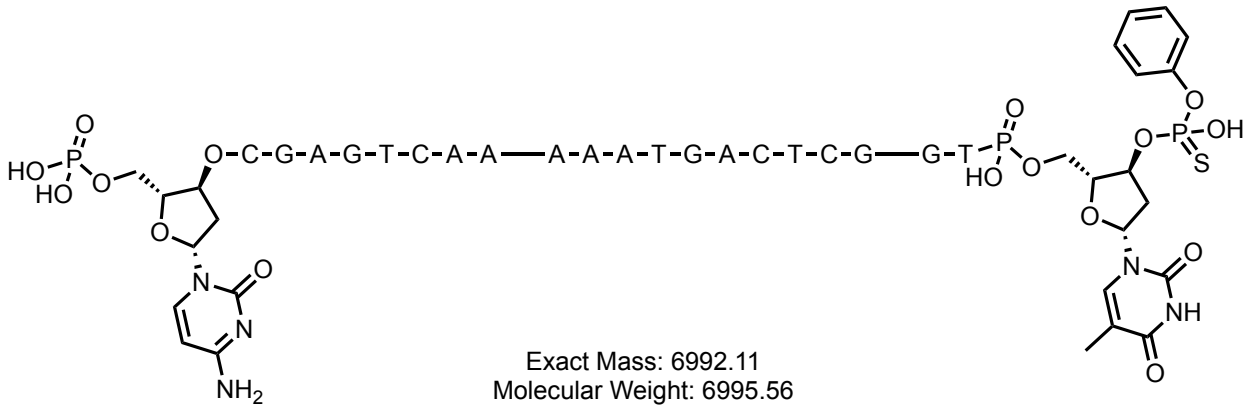


Deconvoluted Mass Spectrum

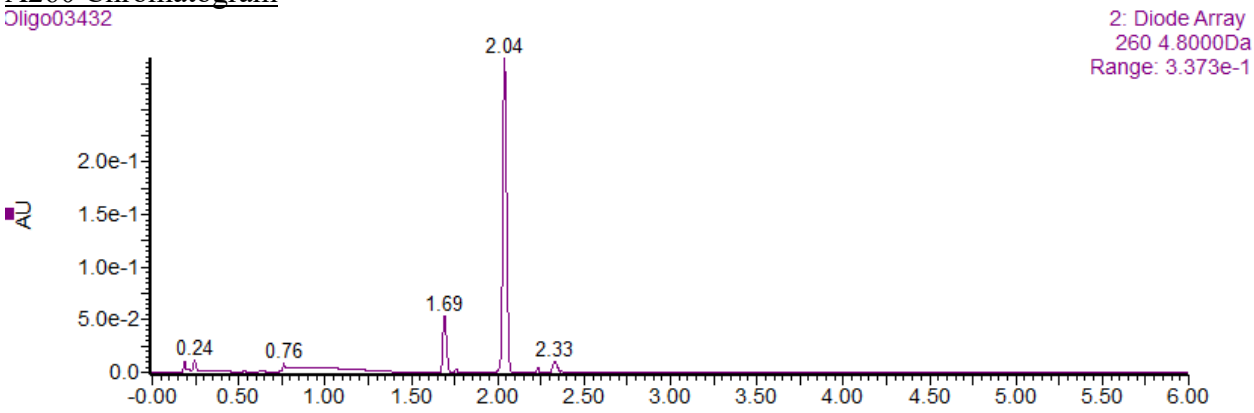


LCMS Characterization of 20

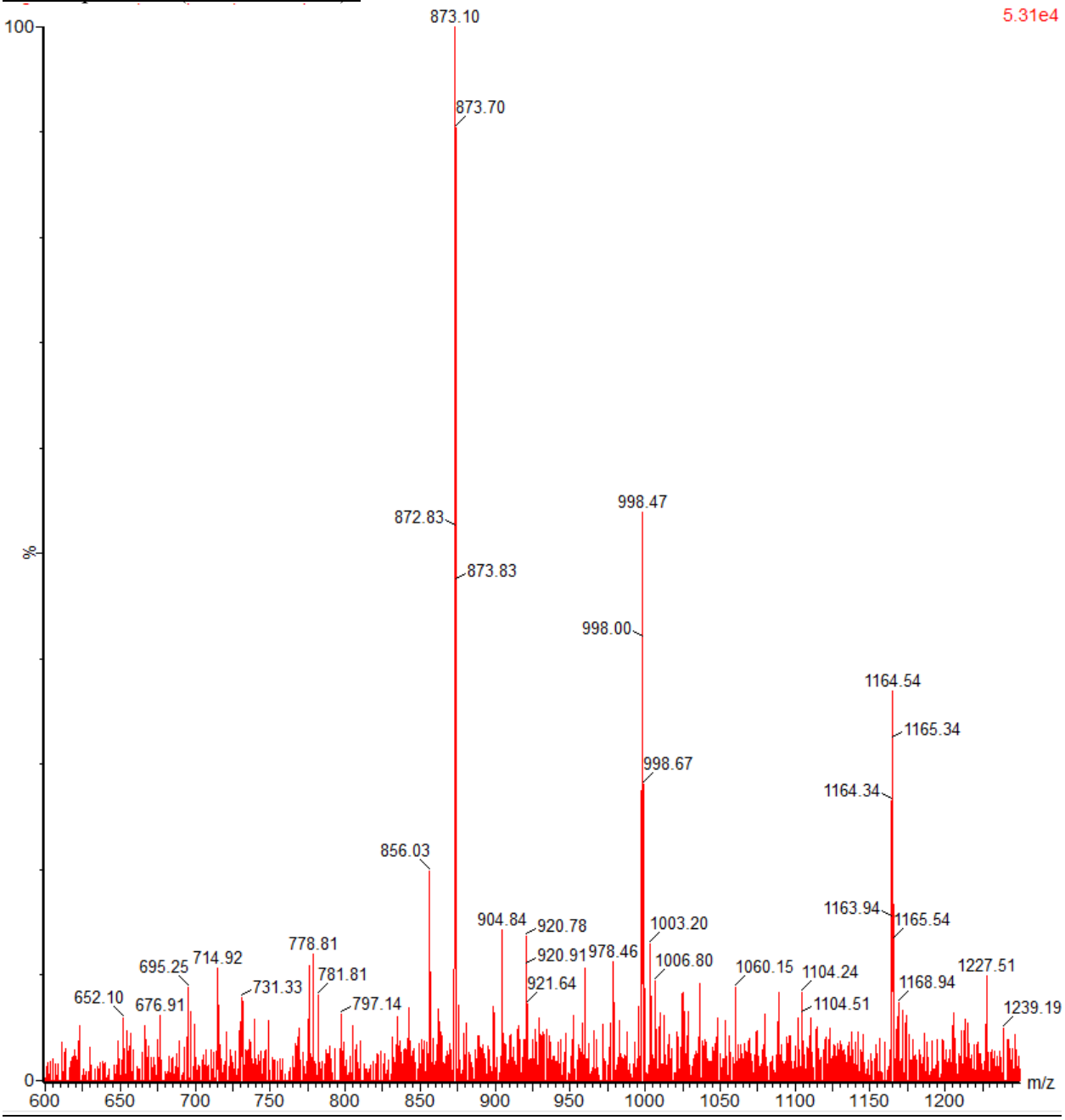
Using **Ψ-14**
Produced from **2** by General Procedure **2 (**Reaction run with 75 mM **Ψ-14** and 225 mM DBU**):**
Yield (A260): 76%
Expected Mass: 6995.11
Expected (M-6)/6 Ion: 1164.9
Found Deconvoluted Mass: 6995.0
Found (M-6)/6 Ion: 1164.54



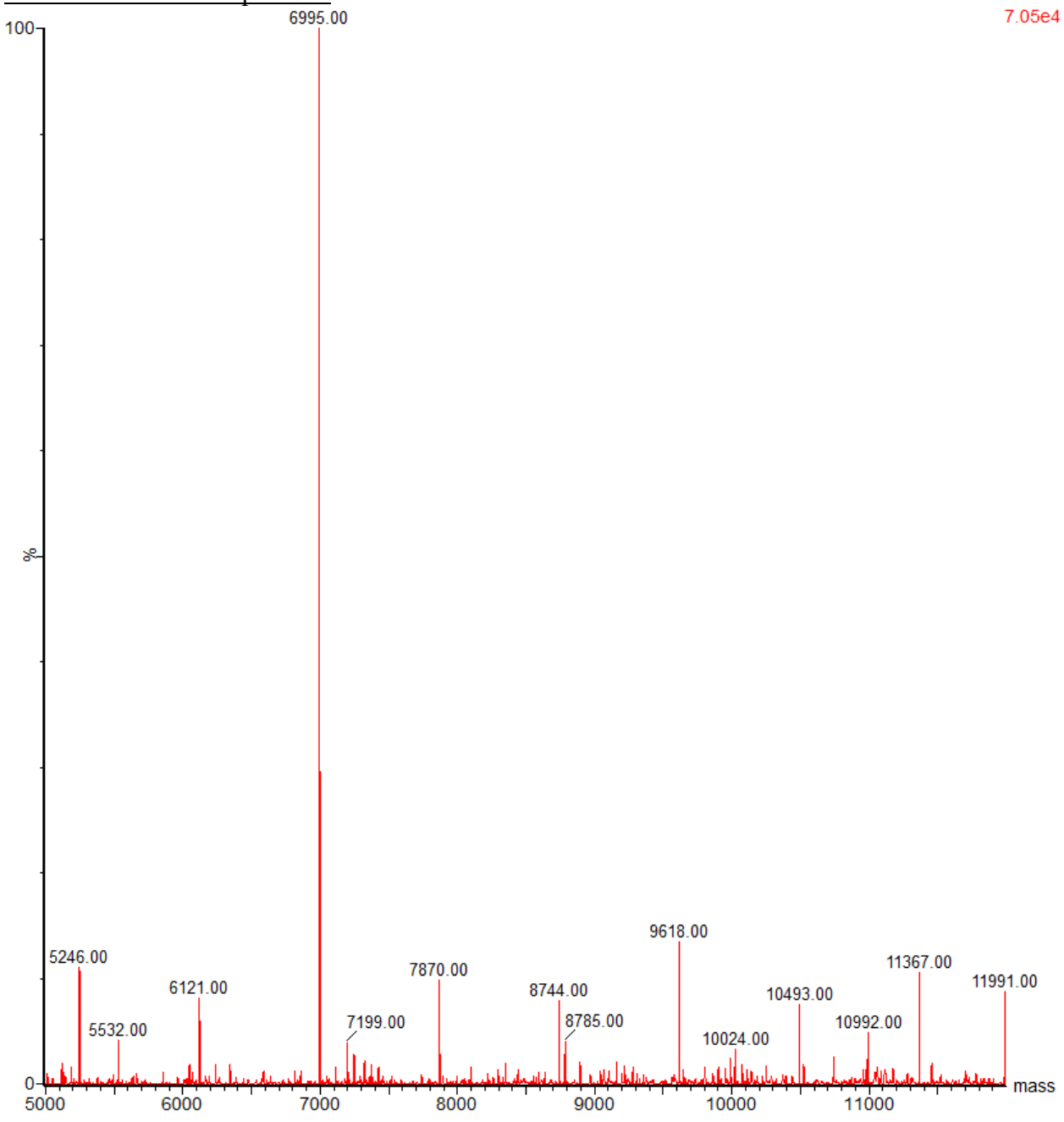
A260 Chromatogram



Mass Spectrum (RT= 2.04 min)

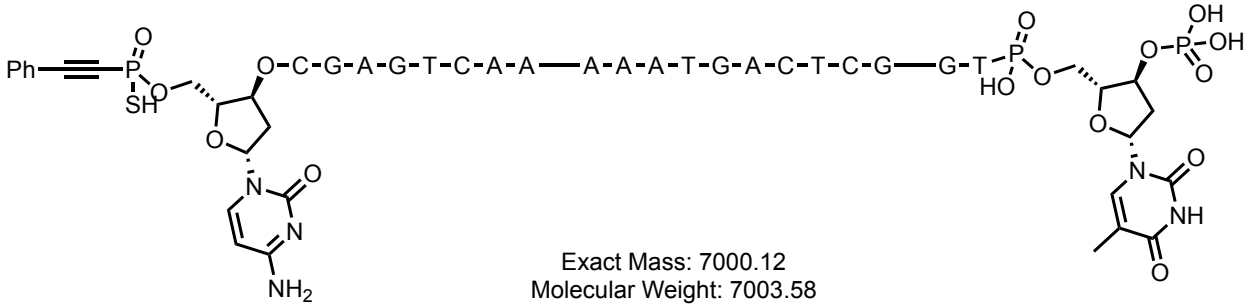


Deconvoluted Mass Spectrum



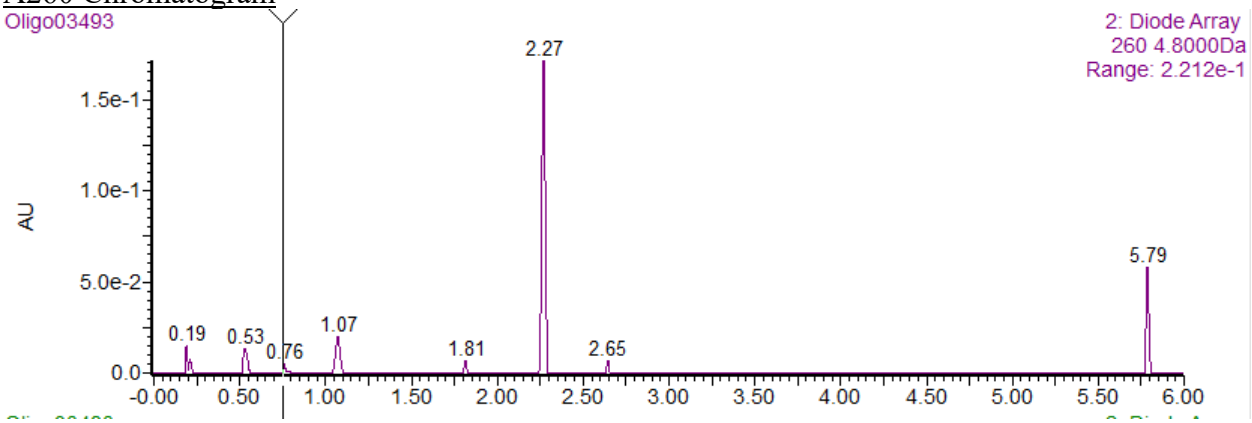
LCMS Characterization of 21

Using **Ψ-15**
Produced from **1** by General Procedure **2 (**Reaction run with 75 mM **Ψ-15** and 225 mM DBU**):**
Yield (A260): 77%
Expected Mass: 7003.6
Expected (M-6)/6 Ion: 1166.3
Found Deconvoluted Mass: 7004.0
Found (M-6)/6 Ion: 1166.2

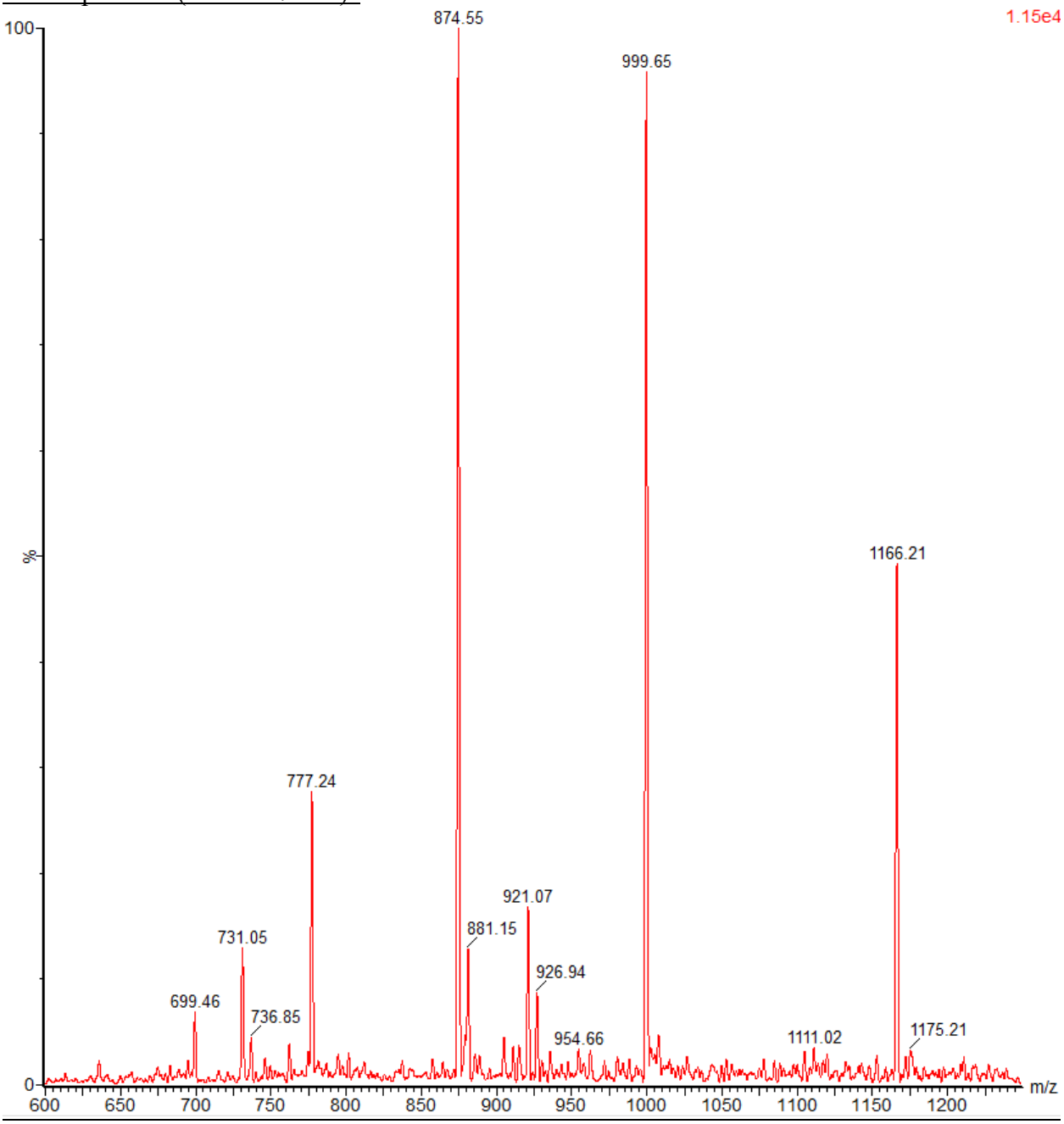


Exact Mass: 7000.12
Molecular Weight: 7003.58

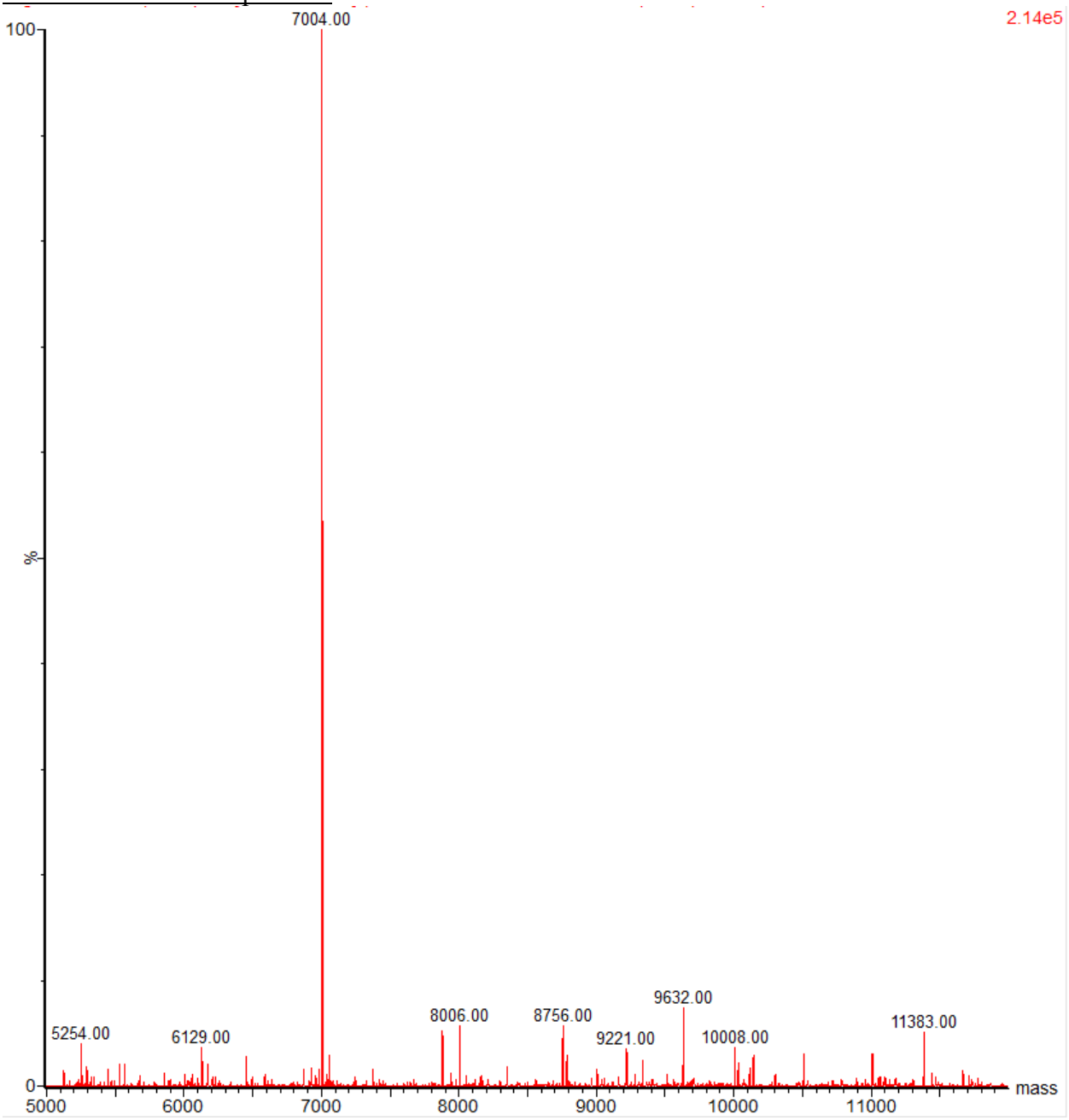
A260 Chromatogram



Mass Spectrum (RT= 2.27 min)

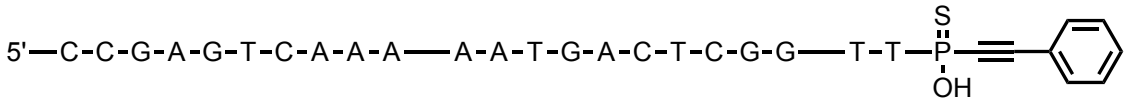


Deconvoluted Mass Spectrum



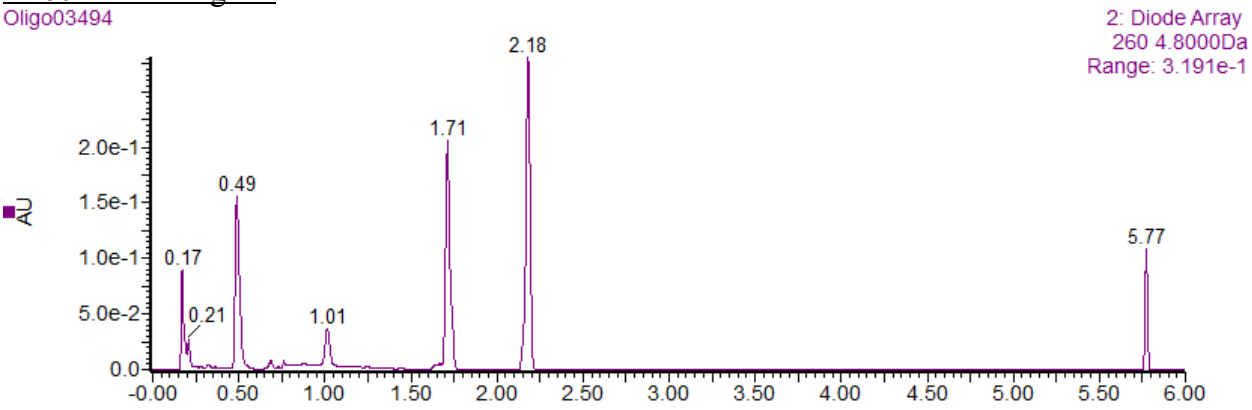
LCMS Characterization of 22

Using **Ψ-15**
Produced from **1** by General Procedure **2** (Reaction run with 75 mM **Ψ-15** and 225 mM DBU**):**
Yield (A260): 56%
Expected Mass: 7003.6
Expected (M-6)/6 Ion: 1166.3
Found Deconvoluted Mass: 7003.0
Found (M-6)/6 Ion: 1166.2

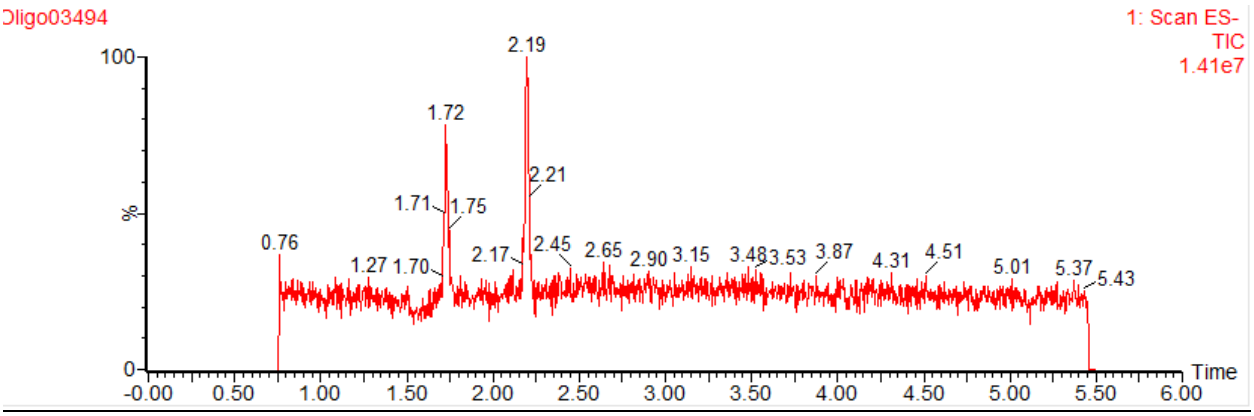


Molecular Weight: 7003.58

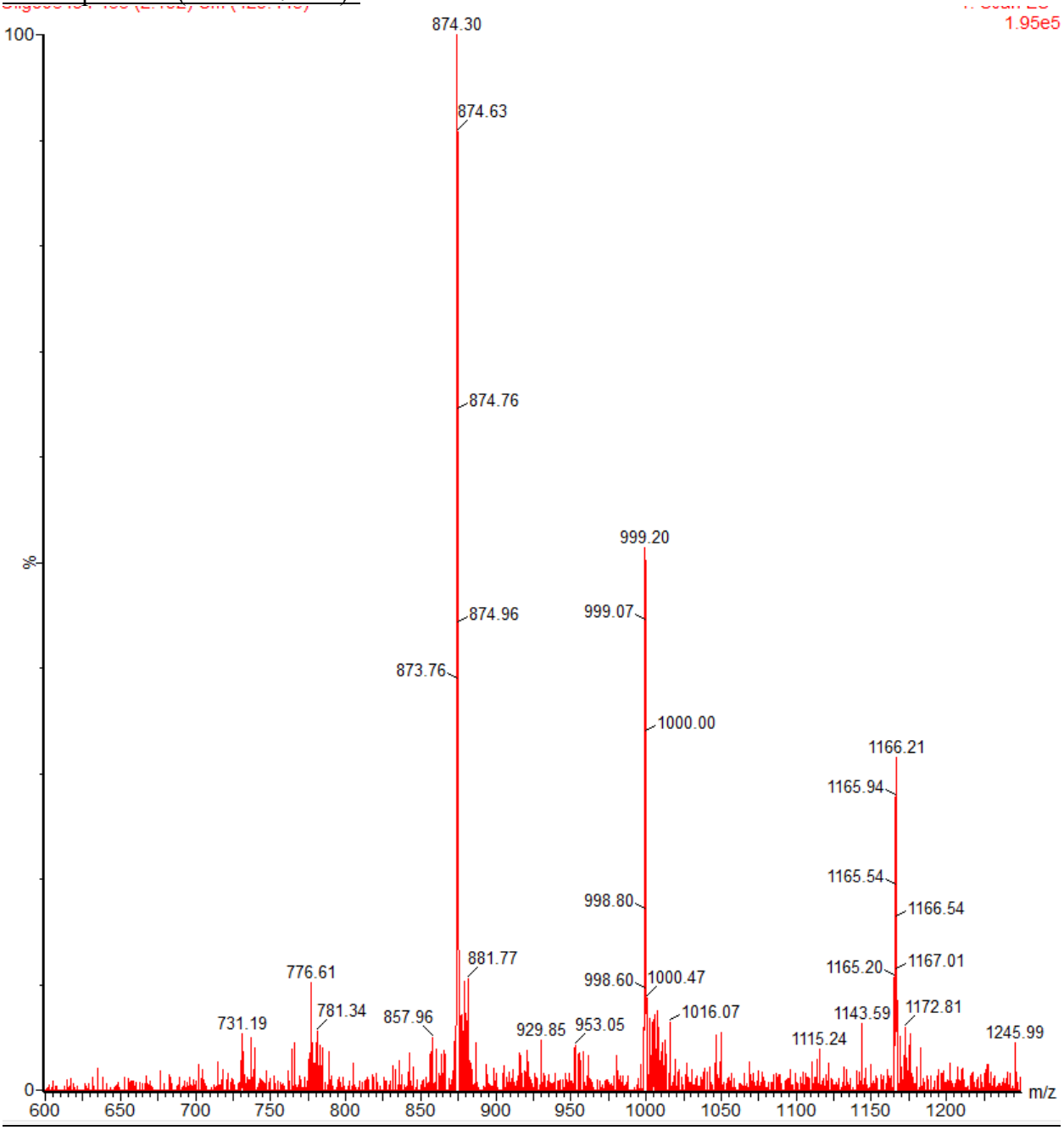
A260 Chromatogram



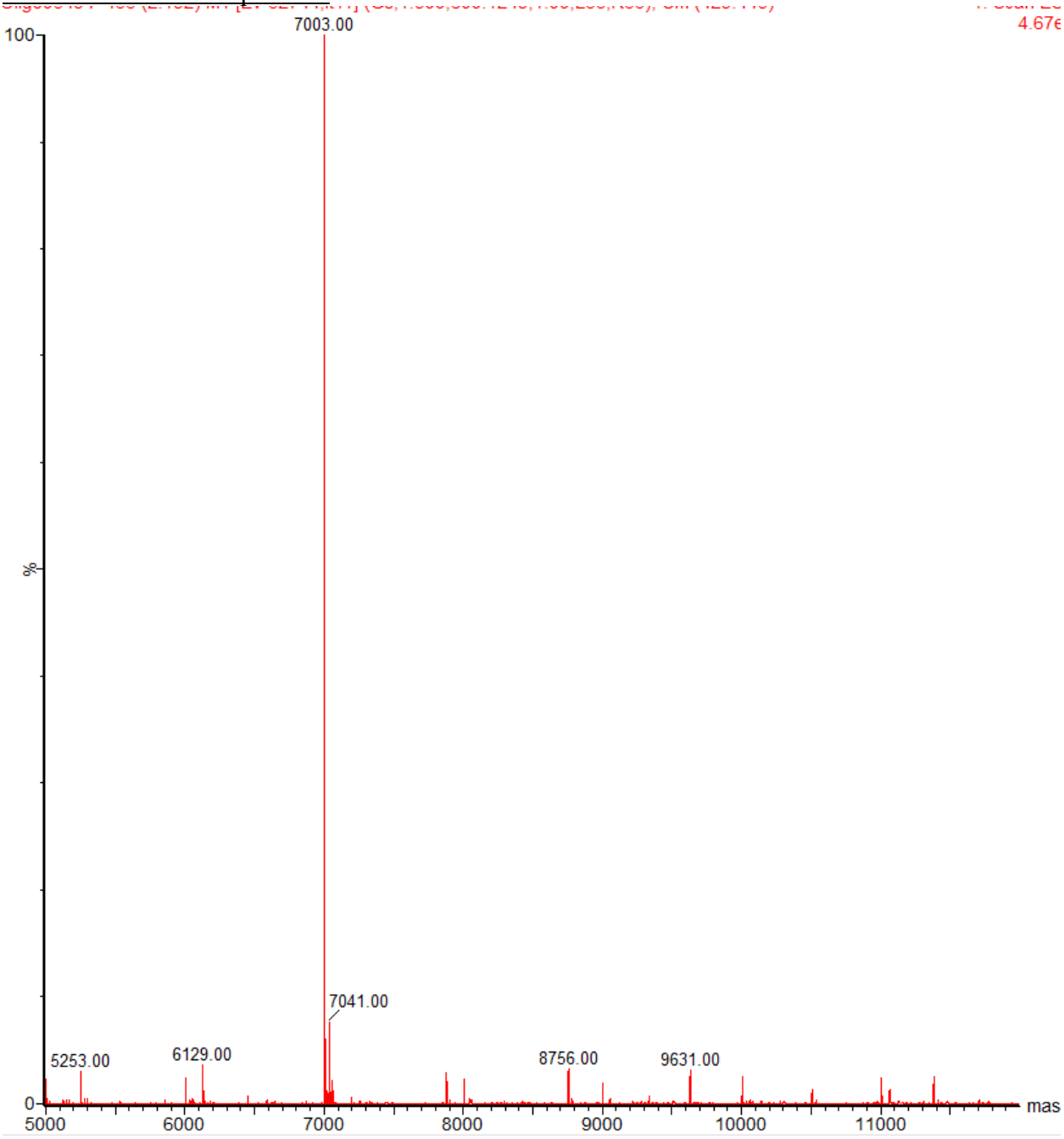
TIC



Mass Spectrum (RT= 2.27 min)

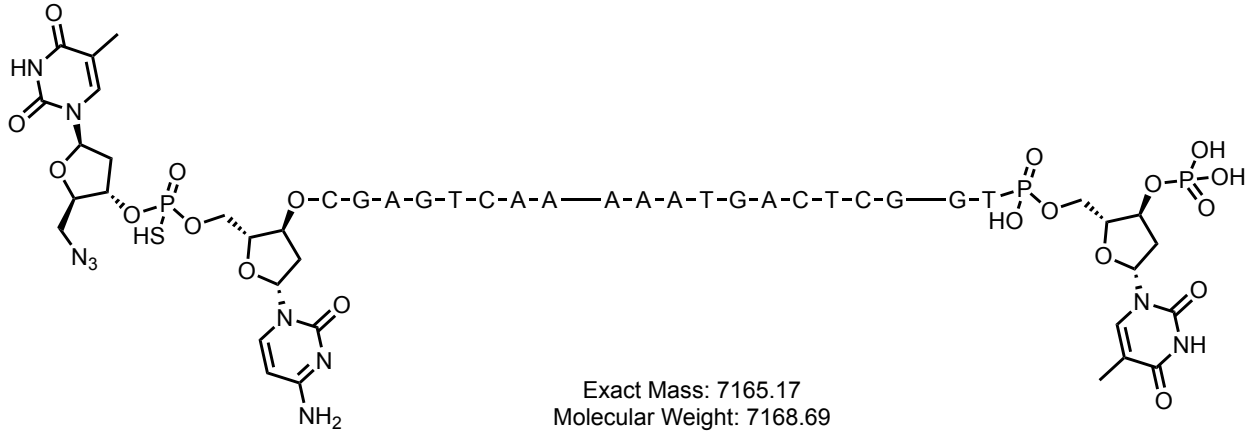


Deconvoluted Mass Spectrum

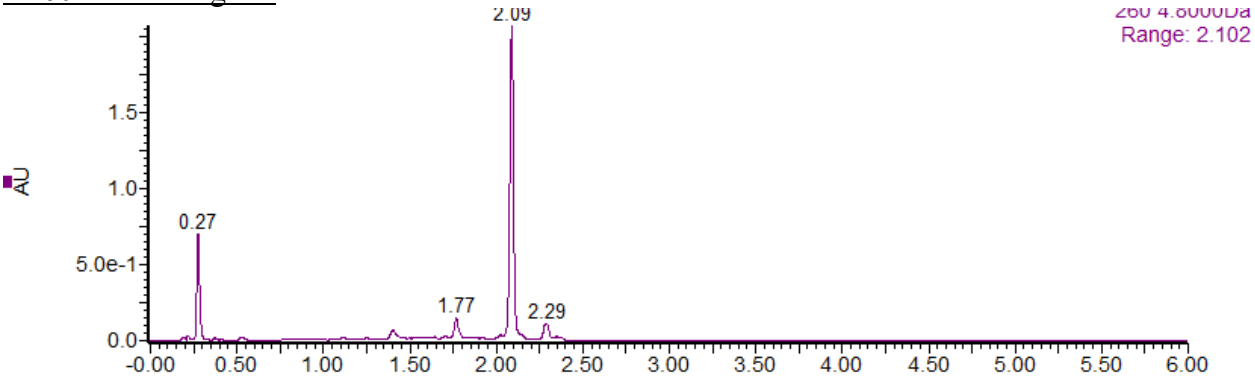


LCMS Characterization of 23

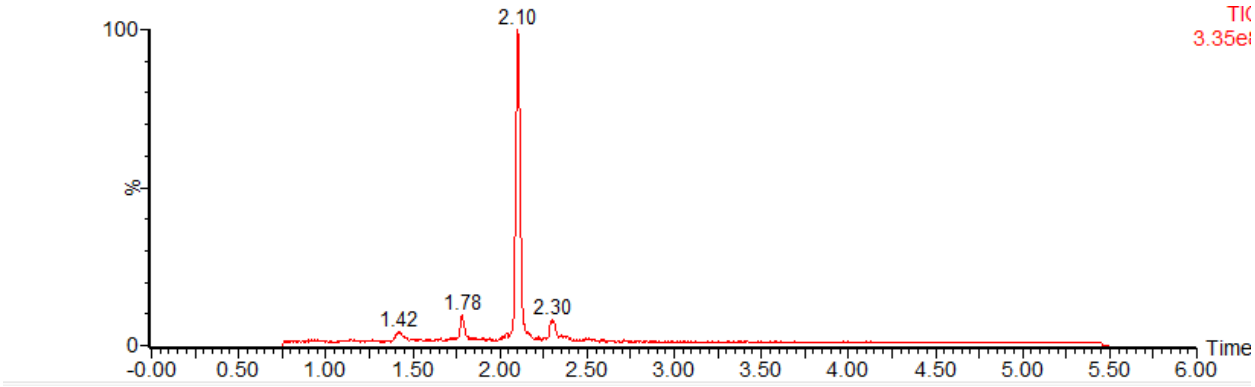
Using **Ψ-17**
Produced from **1** by General Procedure **2** (Reaction run at 37ºC):
Yield (A260): 91%
Expected Mass: 7168.69
Expected (M-6)/6 Ion: 1193.8
Found Deconvoluted Mass: 7169.00
Found (M-6)/6 Ion: 1193.69



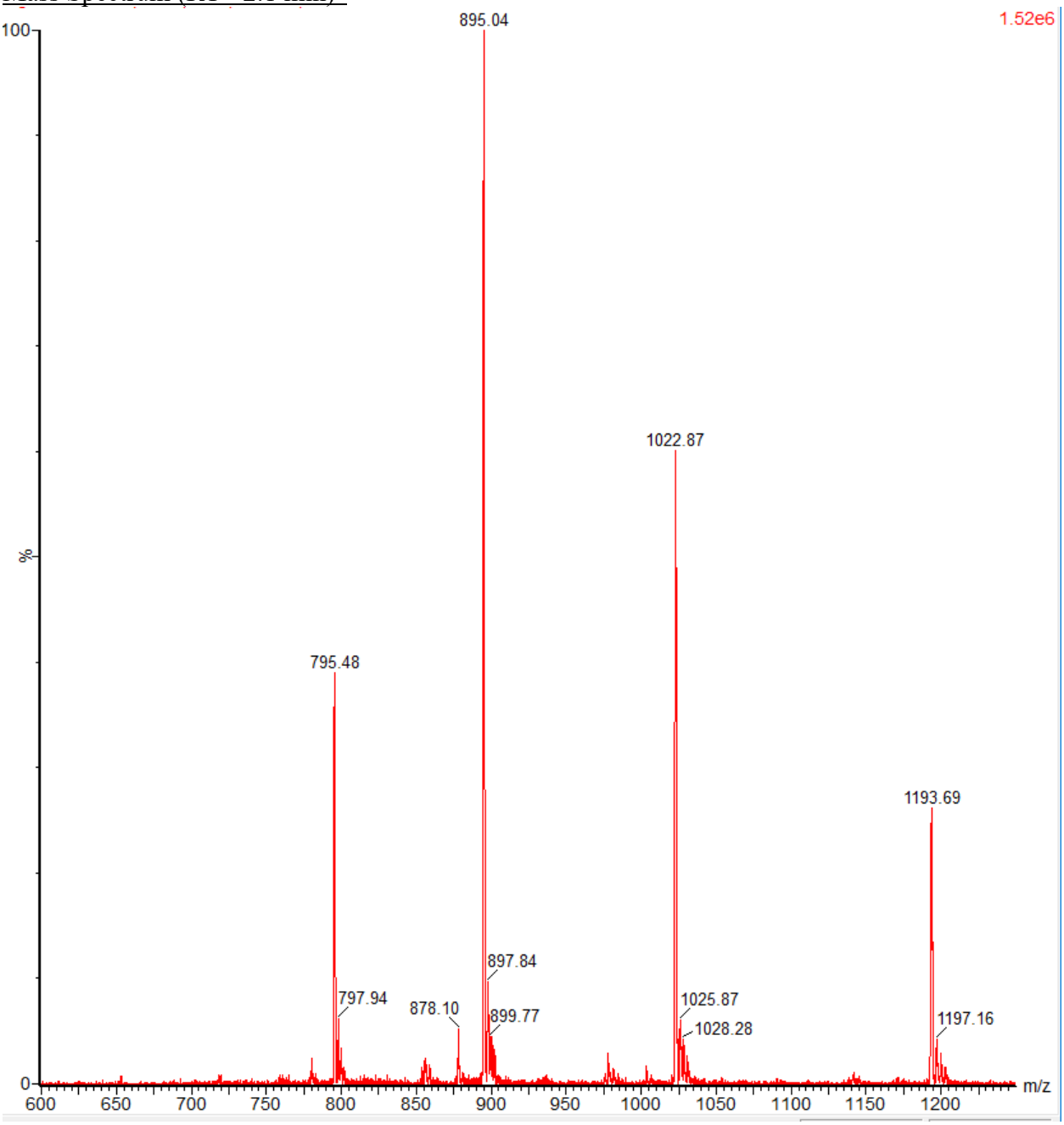
A260 Chromatogram



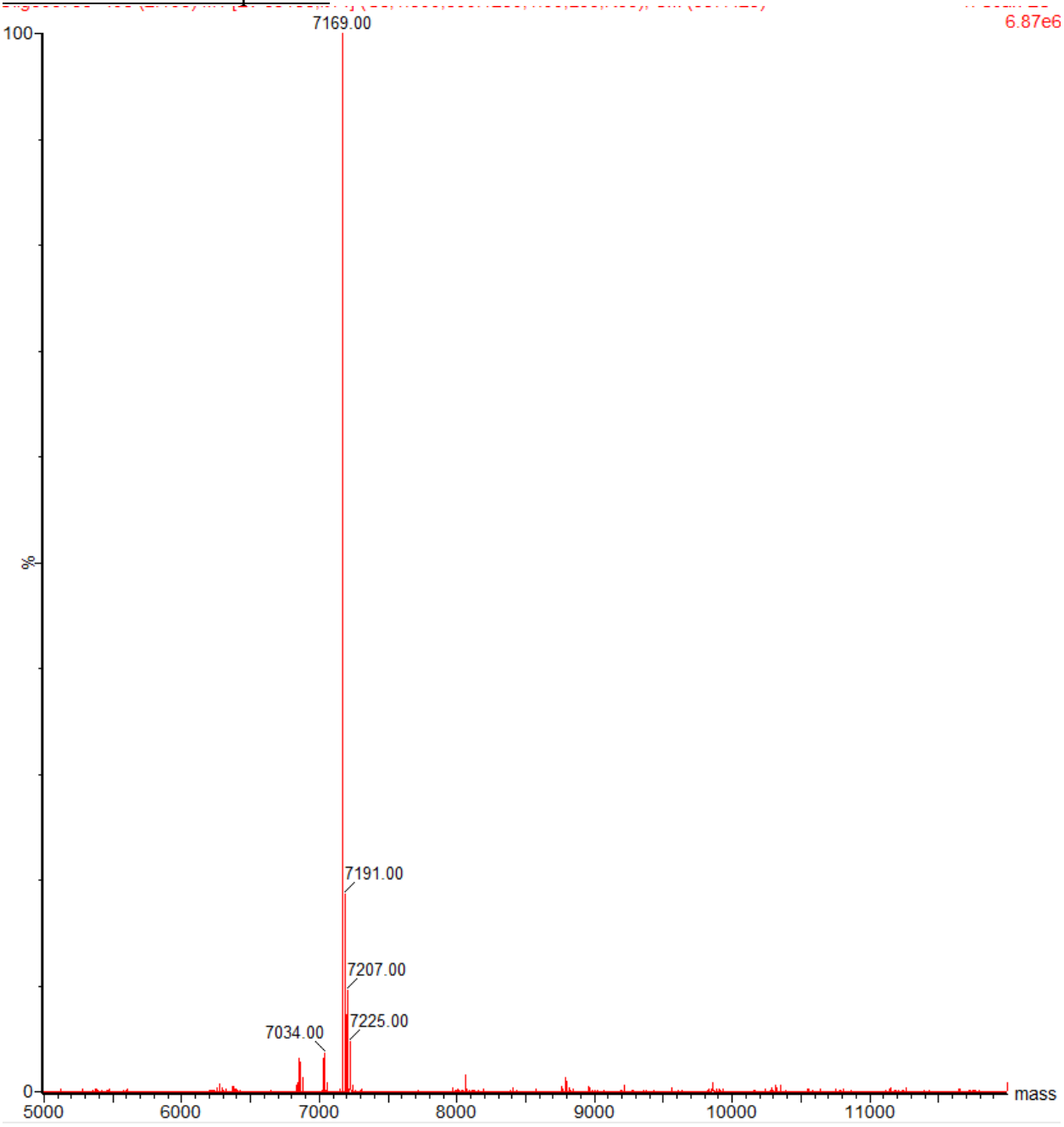
TIC



Mass Spectrum (RT= 2.1 min)

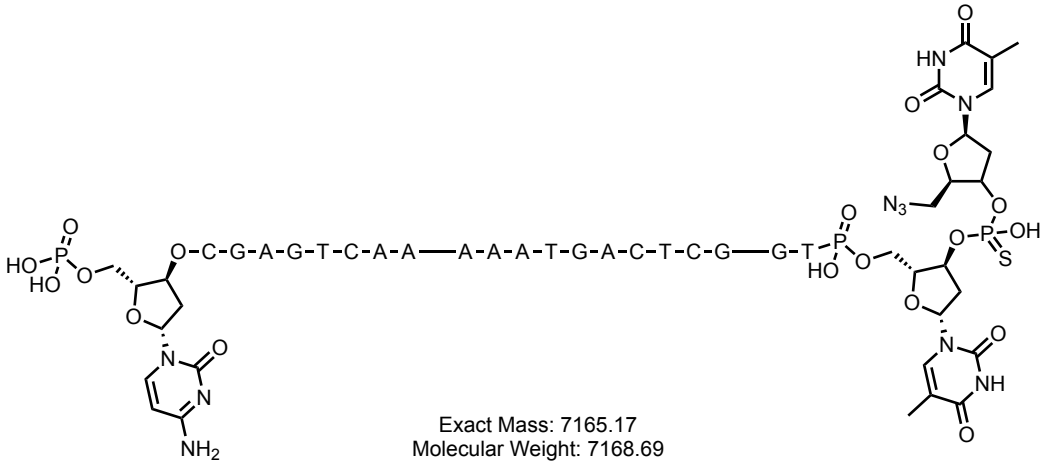


Deconvoluted Mass Spectrum

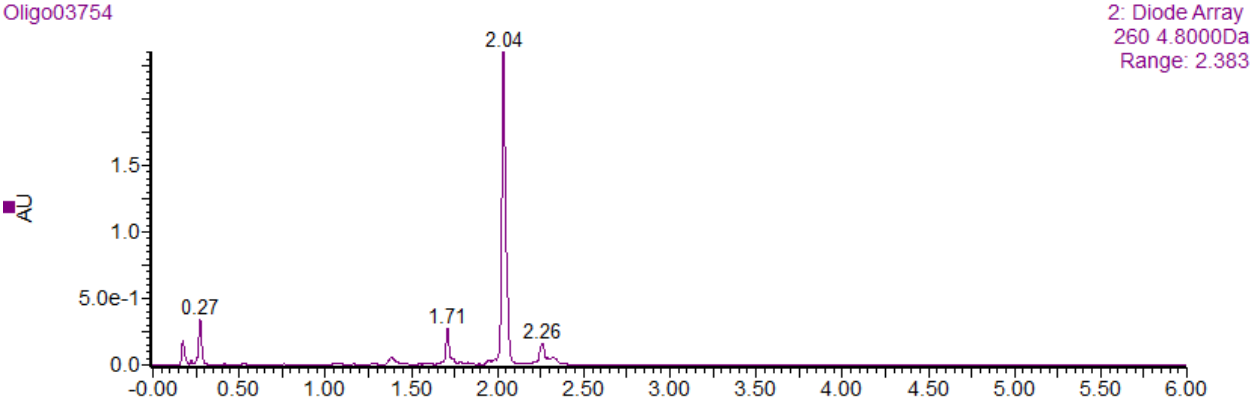


LCMS Characterization of 24

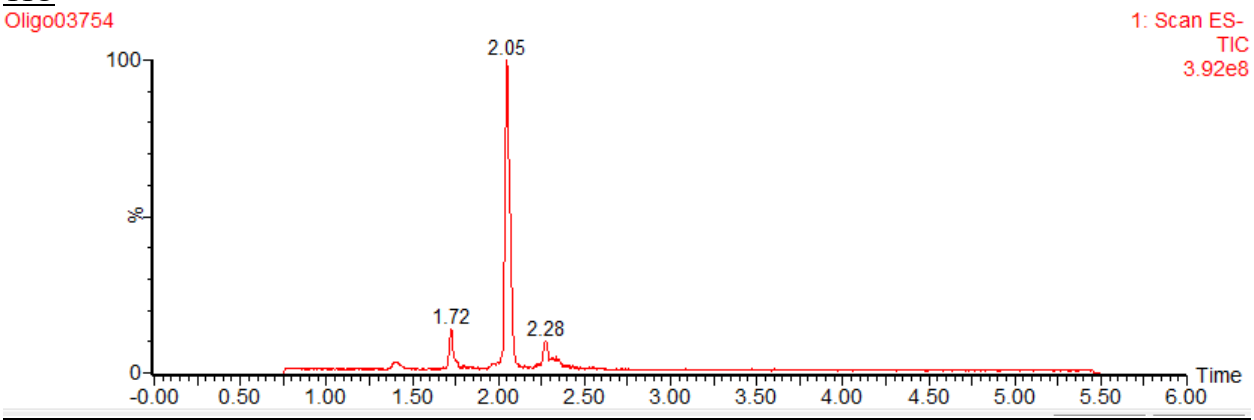
Using **Ψ-17**
Produced from **2** by General Procedure **2** (reaction run with 200 mM **Ψ-17** at 50ºC)
Yield (A260): 89%
Expected Mass: 7168.69
Expected (M-6)/6 Ion: 1193.8
Found Deconvoluted Mass: 7169.00
Found (M-6)/6 Ion: 1193.56



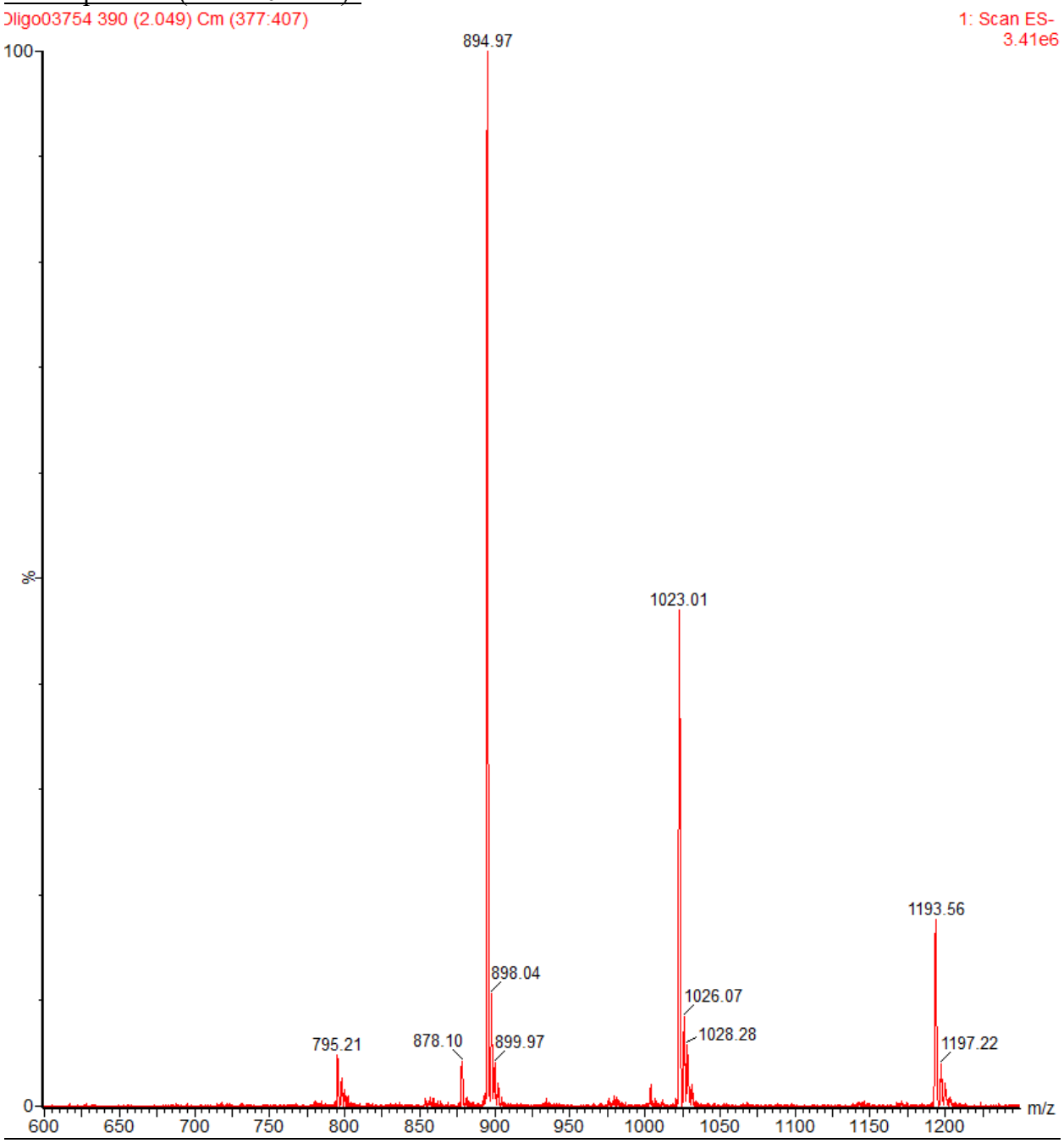
A260 Chromatogram



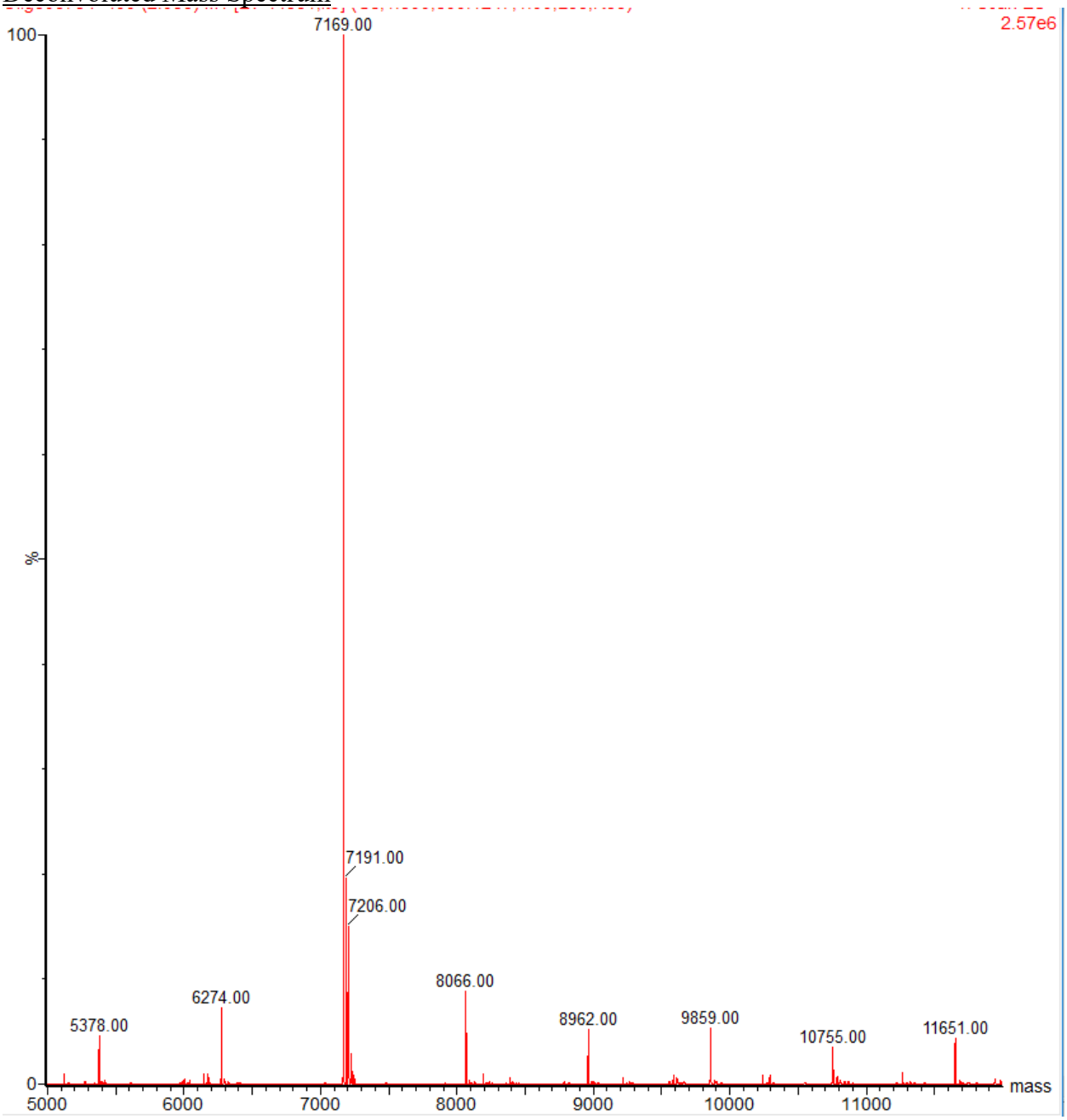
TIC



Mass Spectrum (RT= 2.04 min)

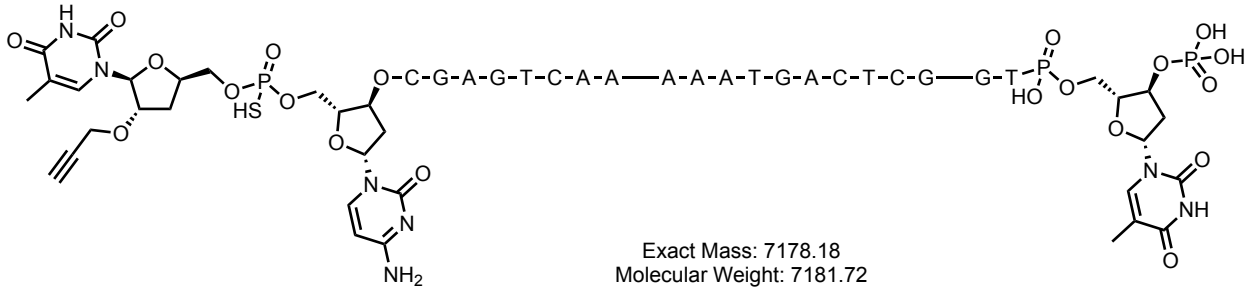


Deconvoluted Mass Spectrum

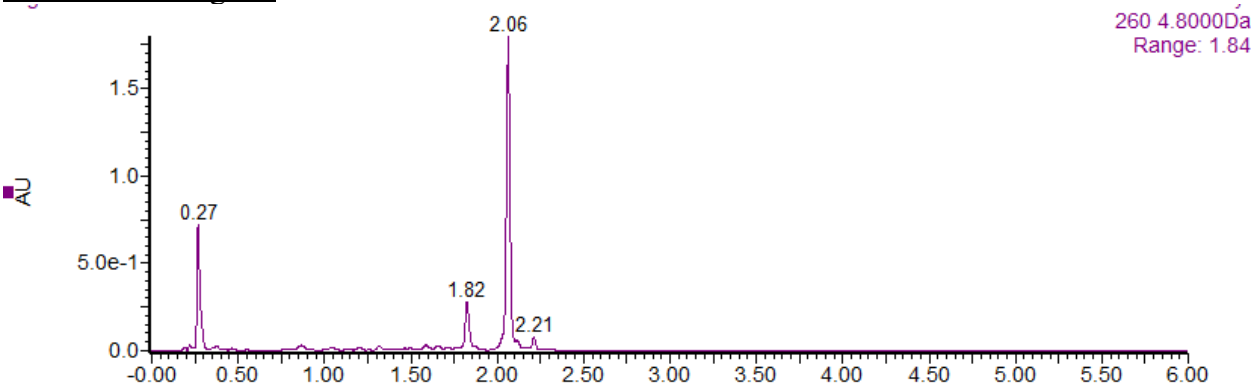


LCMS Characterization of 23

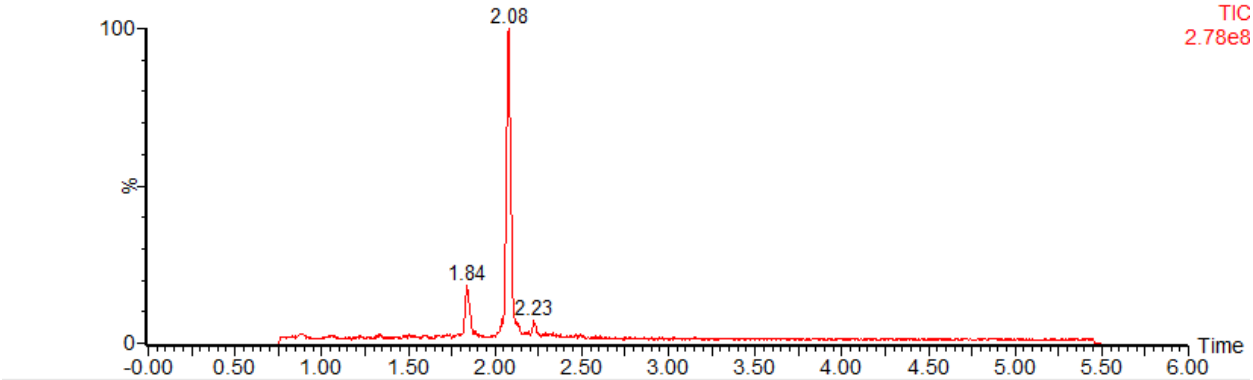
Using **Ψ-18**
Produced from **1** by General Procedure **2** (Reaction run at 37ºC):
Yield (A260): 85%
Expected Mass: 7181.72
Expected (M-6)/6 Ion: 1195.6
Found Deconvoluted Mass: 7182.0
Found (M-6)/6 Ion: 1195.69



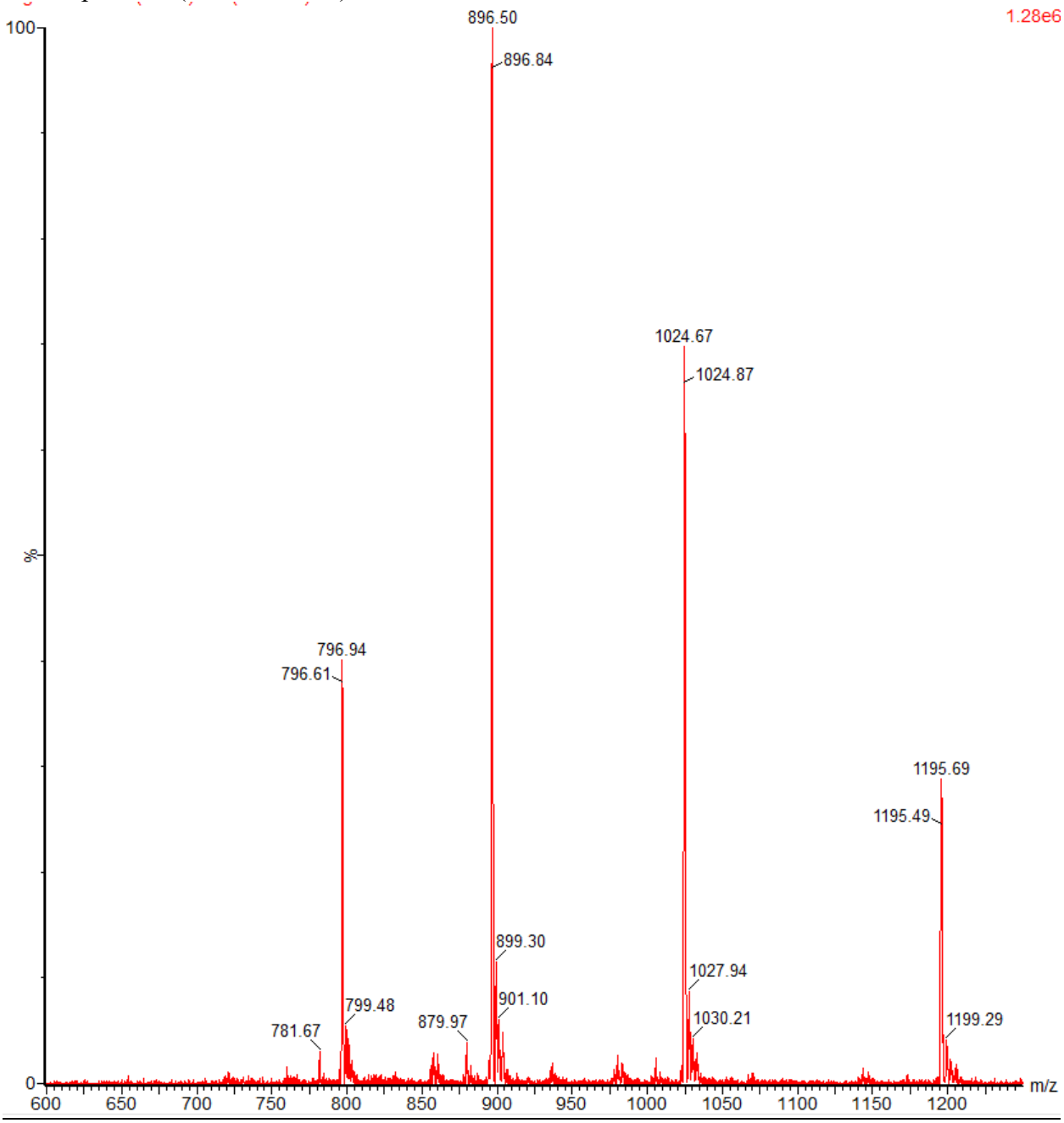
A260 Chromatogram



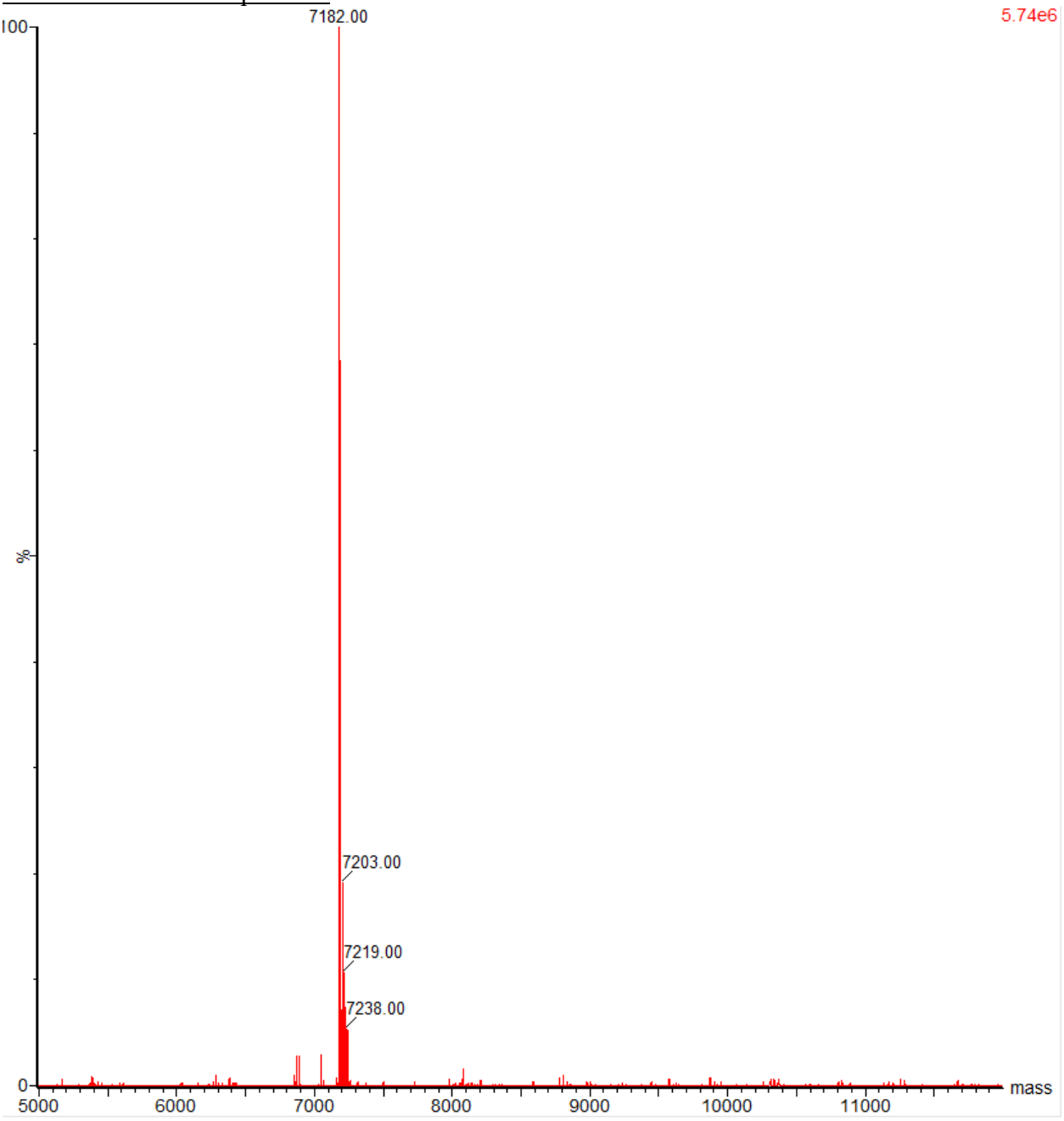
TIC



Mass Spectrum (RT= 2.06 min)

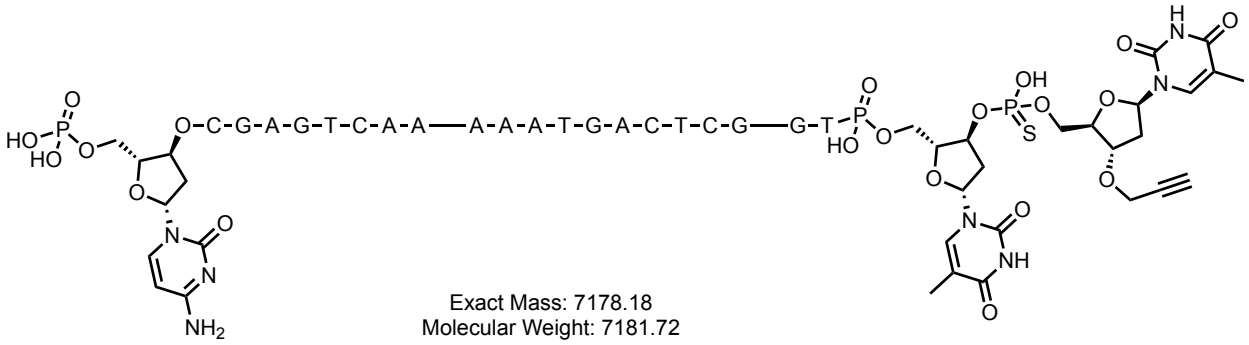


Deconvoluted Mass Spectrum

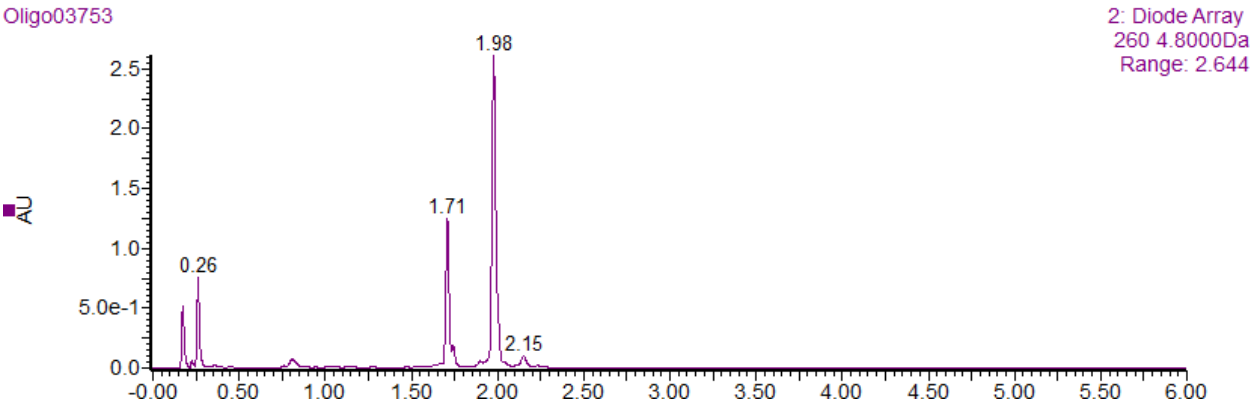


LCMS Characterization of 26

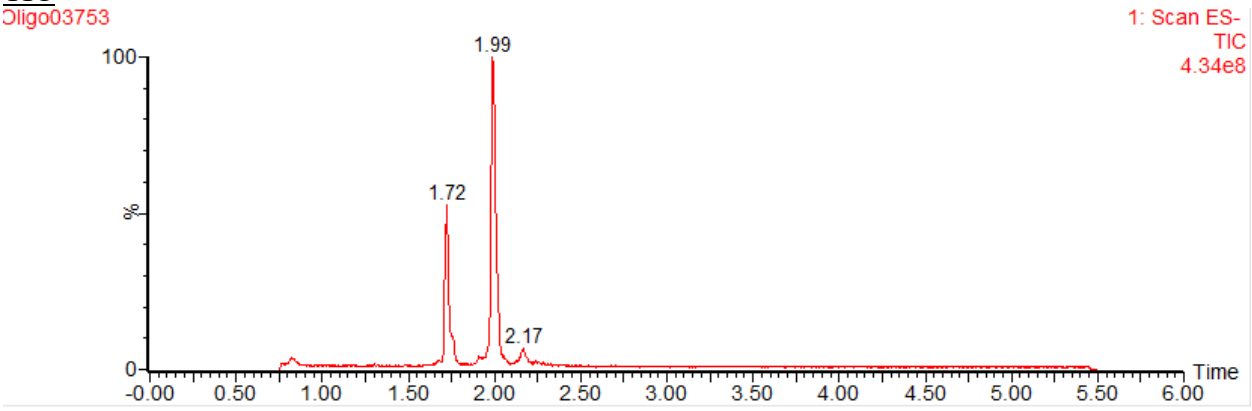
Using **Ψ-18**
Produced from **1** by General Procedure **2** (Reaction run with **Ψ-18** (200 mM) at 50ºC):
Yield (A260): 69%
Expected Mass: 7181.72
Expected (M-6)/6 Ion: 1195.6
Found Deconvoluted Mass: 7182.0
Found (M-6)/6 Ion: 1195.69



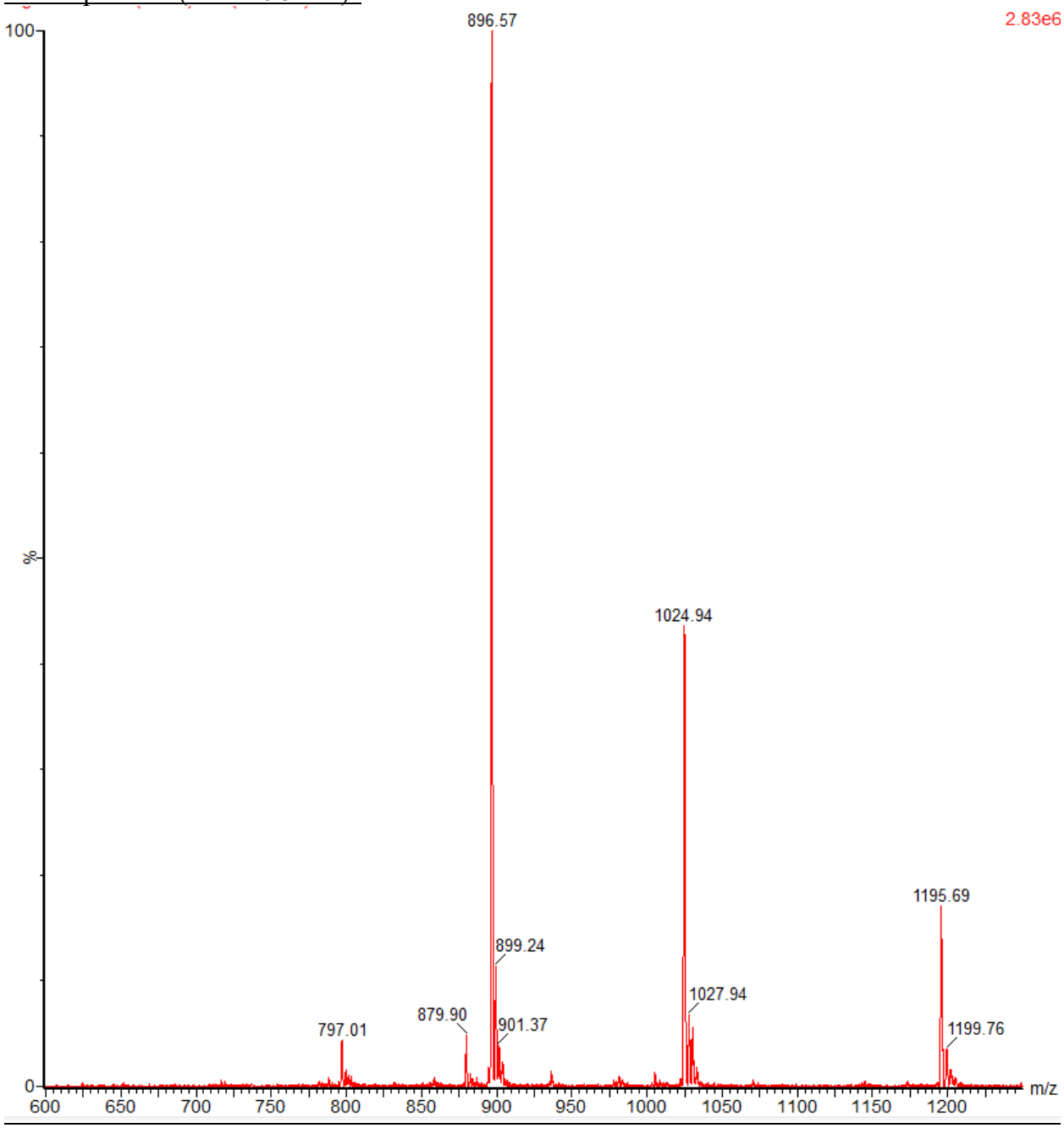
A260 Chromatogram



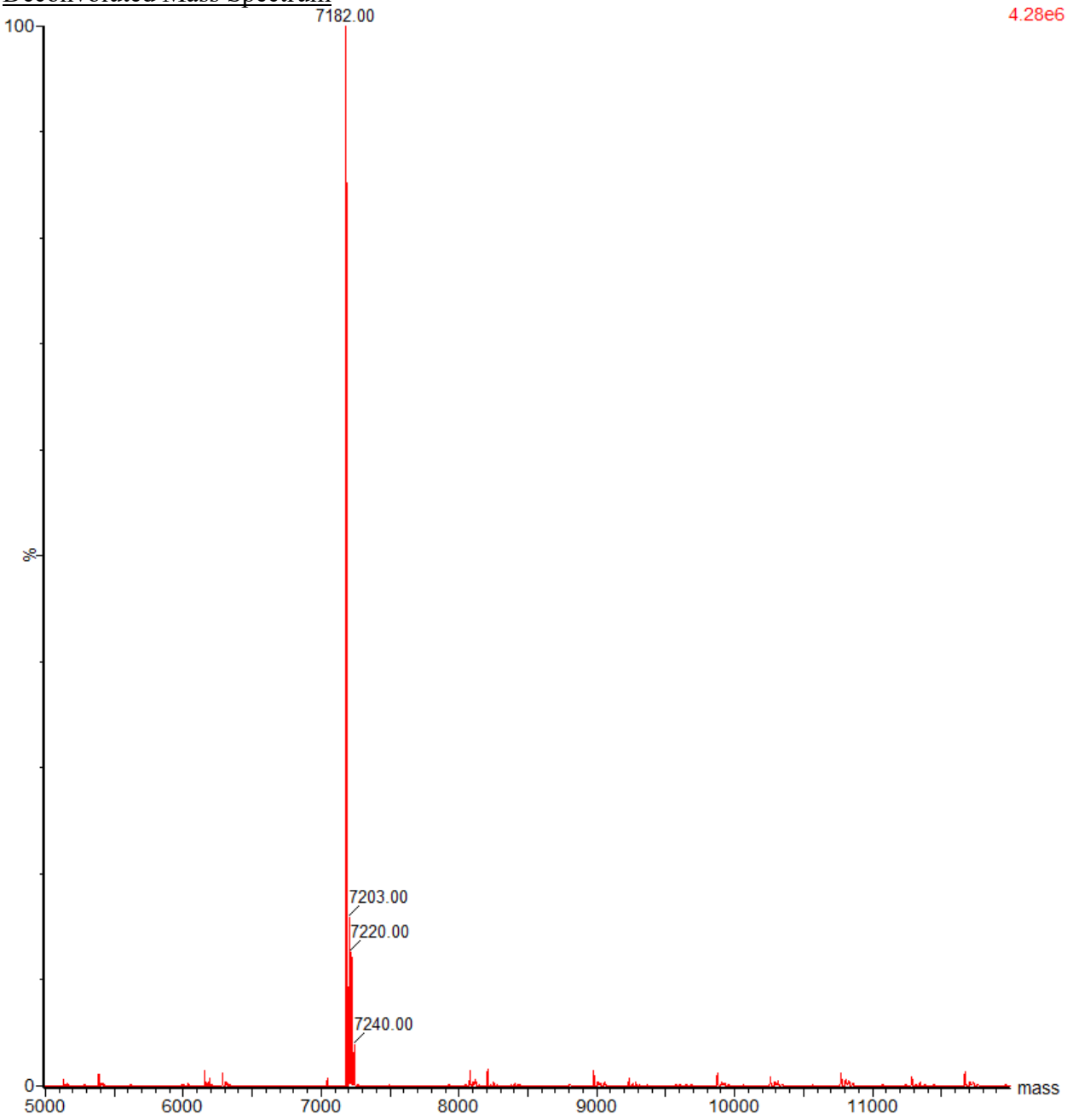
TIC



Mass Spectrum (RT= 1.98 min)

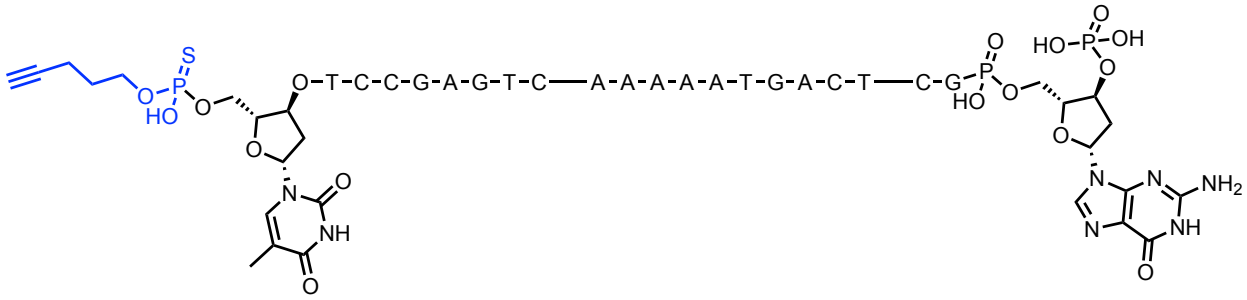


Deconvoluted Mass Spectrum



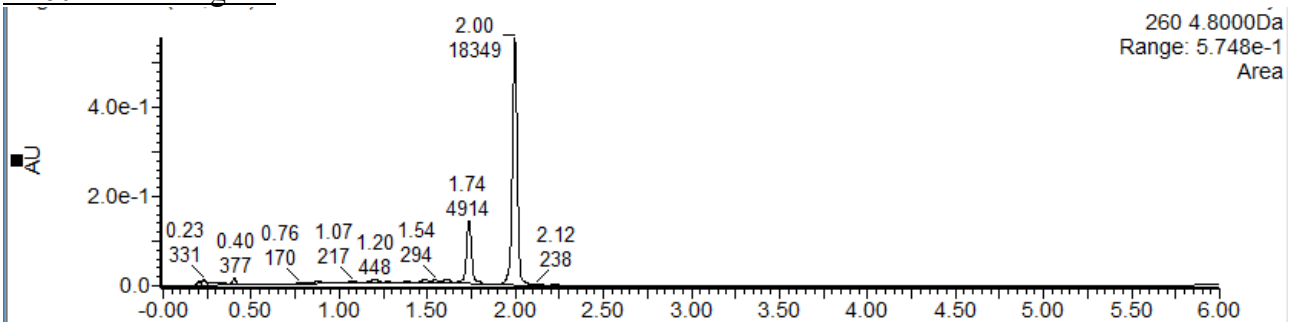
LCMS Characterization of 27

Using **Ψ-5**
Produced from **1** by General Procedure **2:**
Yield (A260): 79%
Expected Mass: 6985.563
Expected (M-6)/6 Ion: 1163.26
Found Deconvoluted Mass: 6986.00
Found (M-6)/6 Ion: 1163.01

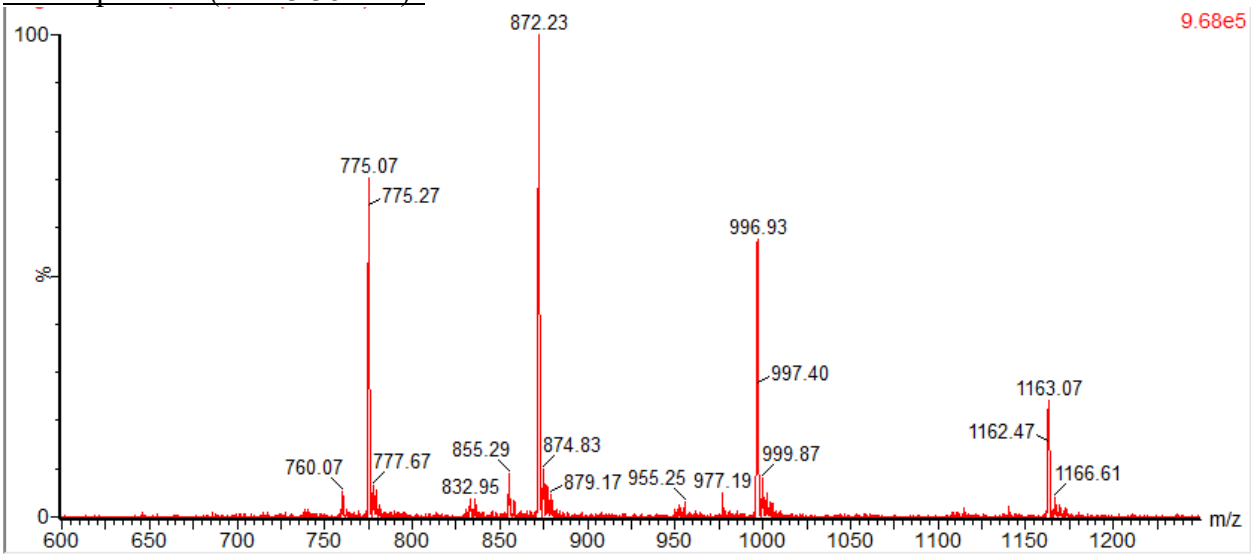


Exact Mass: 6982.129
Molecular Weight: 6985.563

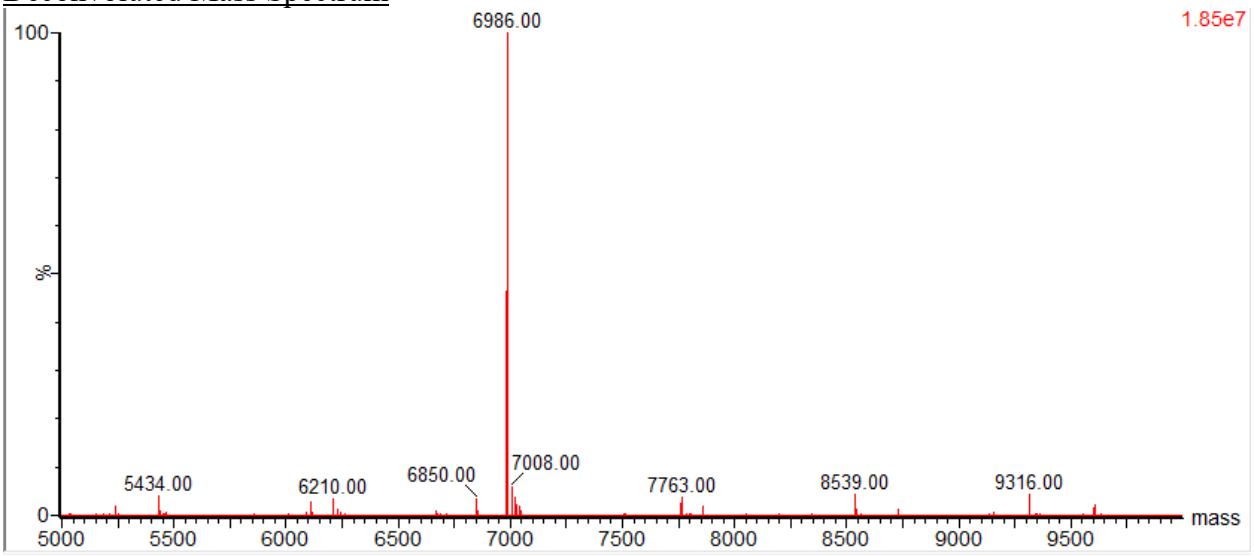
A260 Chromatogram



Mass Spectrum (RT= 3.38 min)

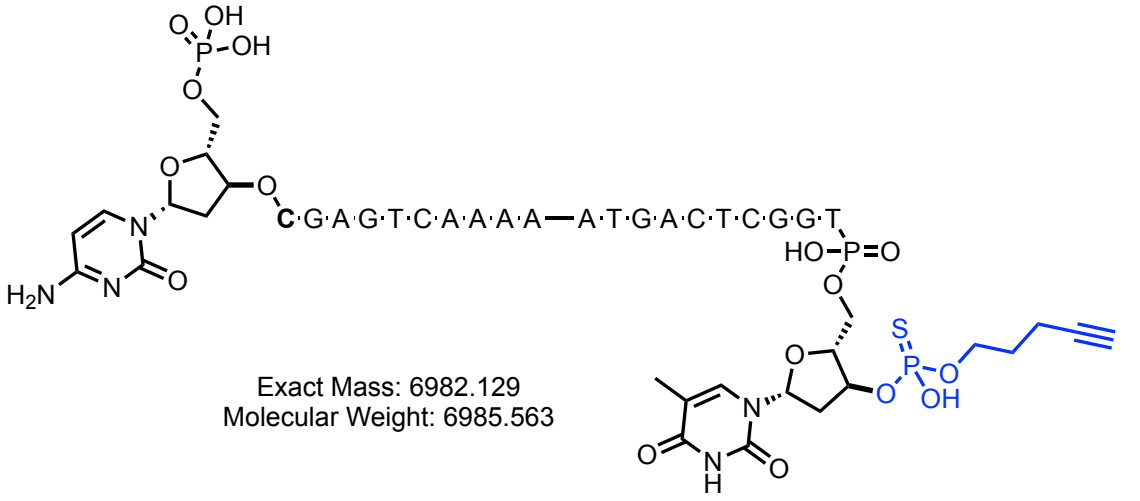


Deconvoluted Mass Spectrum

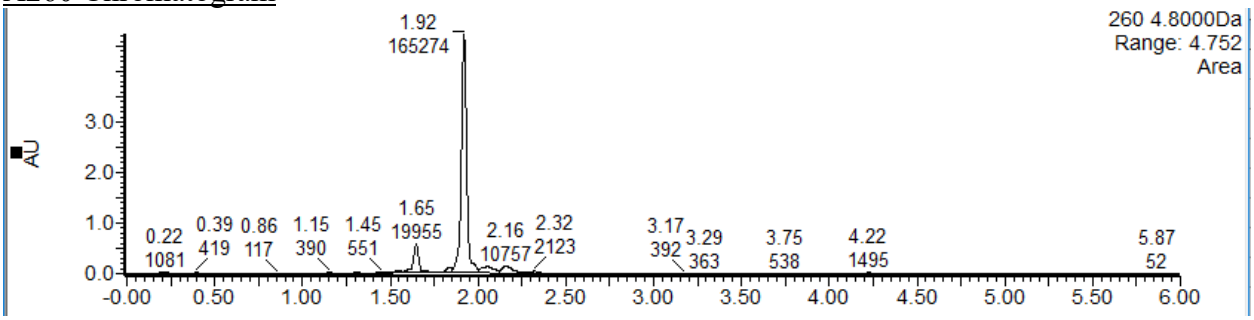


LCMS Characterization of 28

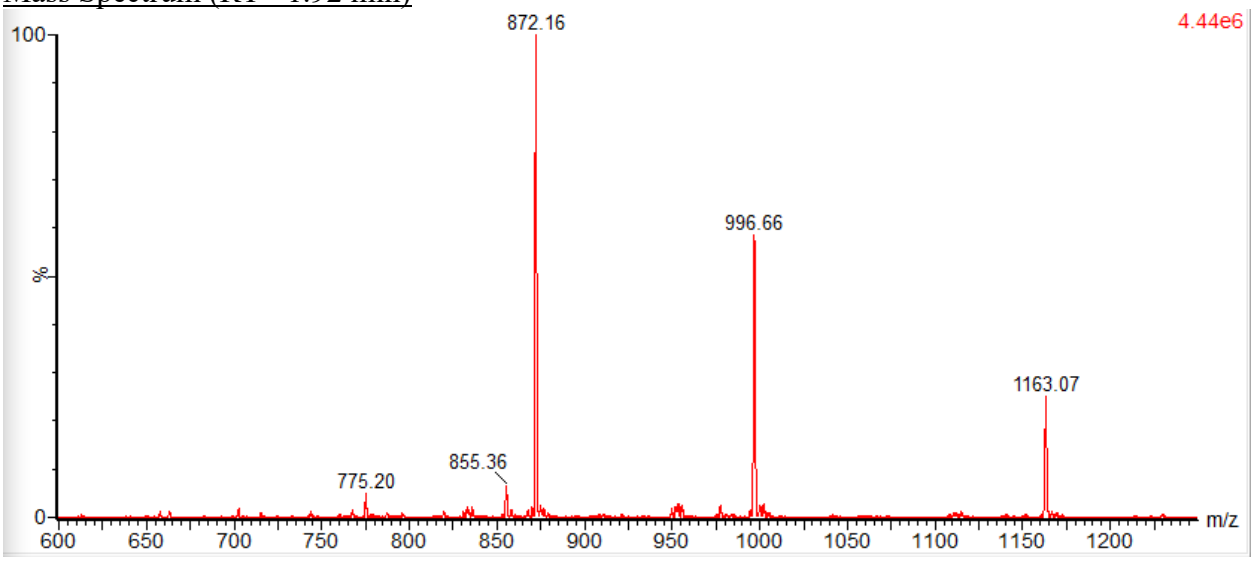
Produced from **2** by General Procedure **2:**
Using **Ψ-5**
Yield (A260): 84%
Expected Mass: 6985.53
Expected (M-6)/6 Ion: 1163.255
Found Deconvoluted Mass: 6986.00
Found (M-6)/6 Ion: 1163.07



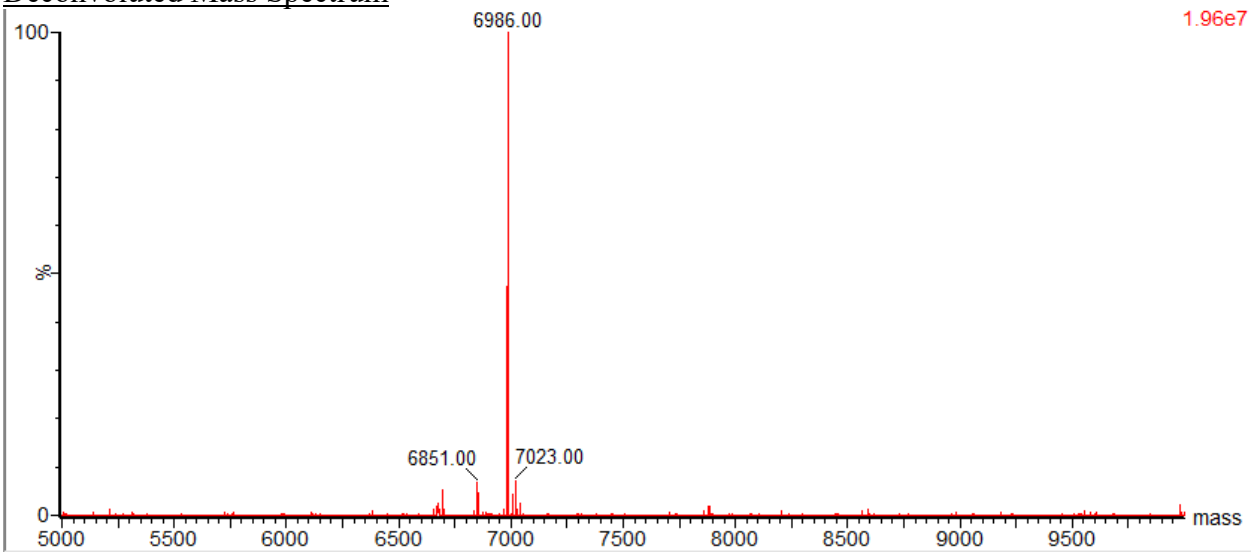
A260 Chromatogram



Mass Spectrum (RT= 1.92 min)

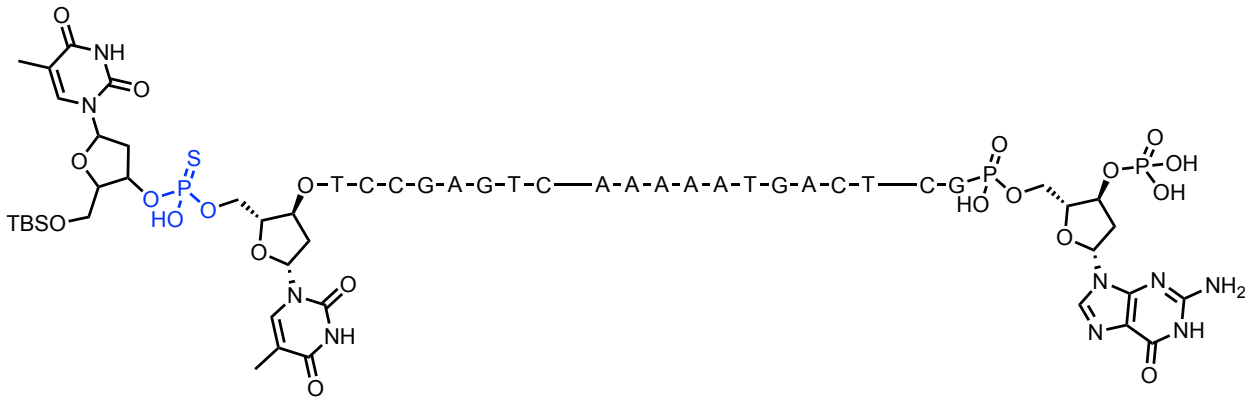


Deconvoluted Mass Spectrum



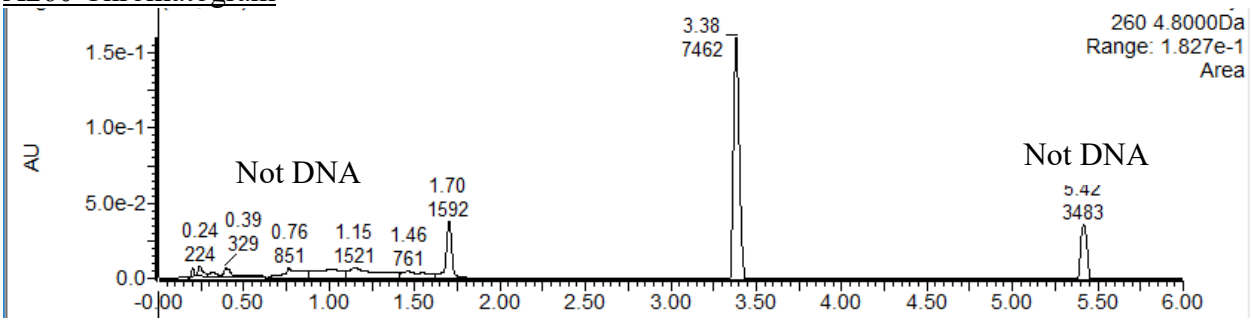
LCMS Characterization of 29

Produced from **1** by General Procedure **2:**
Using **Ψ-9**
Yield (A260): 85%
Expected Mass: 7257.939
Expected (M-6)/6 Ion: 1208.65
Found Deconvoluted Mass: 7258.00
Found (M-6)/6 Ion: 1208.23

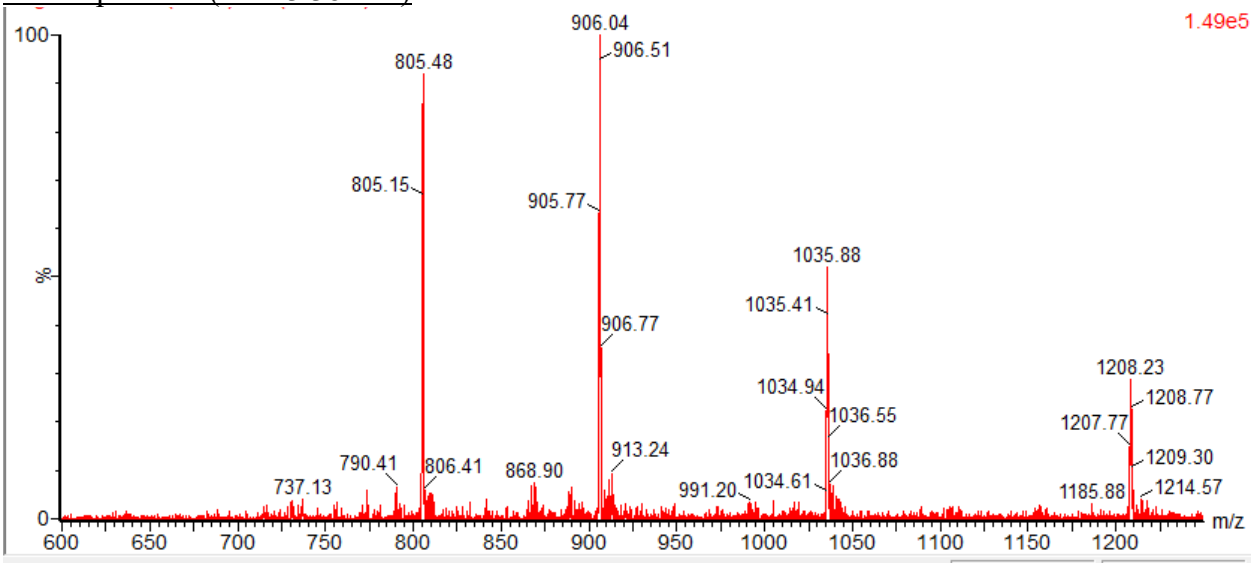


Exact Mass: 7254.248
Molecular Weight: 7257.939

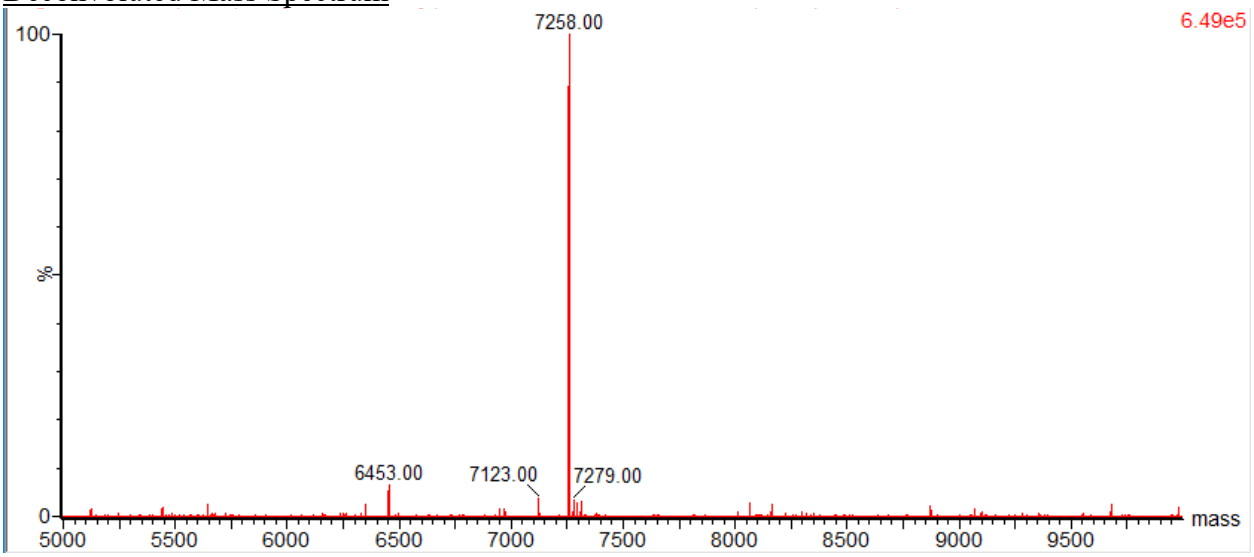
A260 Chromatogram



Mass Spectrum (RT= 3.38 min)

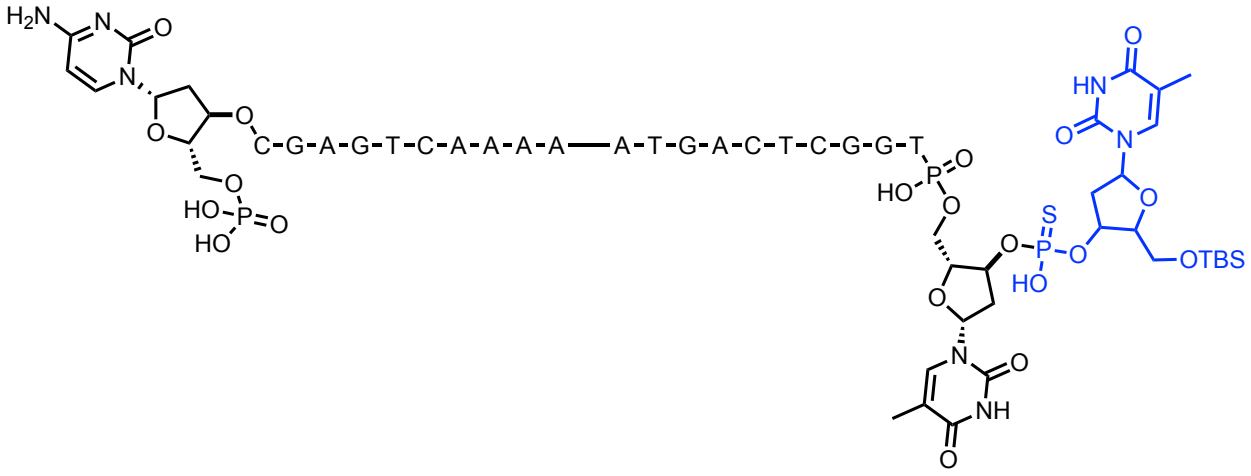


Deconvoluted Mass Spectrum



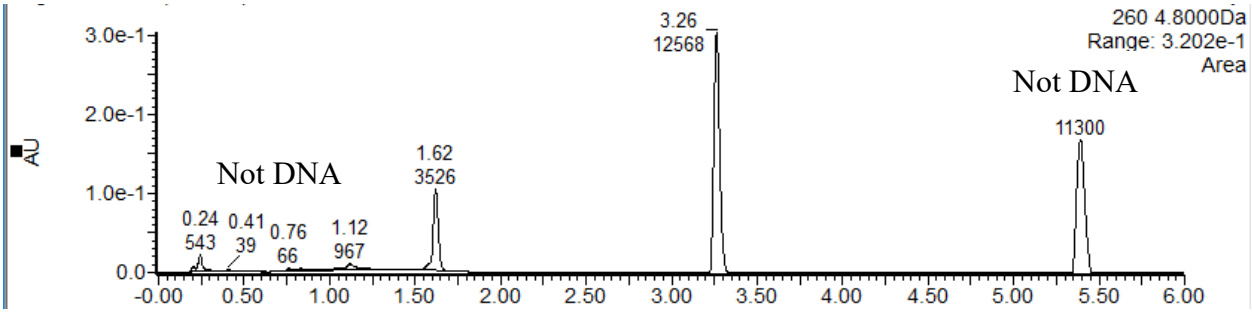
LCMS Characterization of 30

Produced from **2** by General Procedure **2:**
Using **Ψ-9**
Yield (A260): 78%
Expected Mass: 7257.939
Expected (M-6)/6 Ion: 1208.662
Found Deconvoluted Mass: 7258.00
Found (M-6)/6 Ion: 1208.57

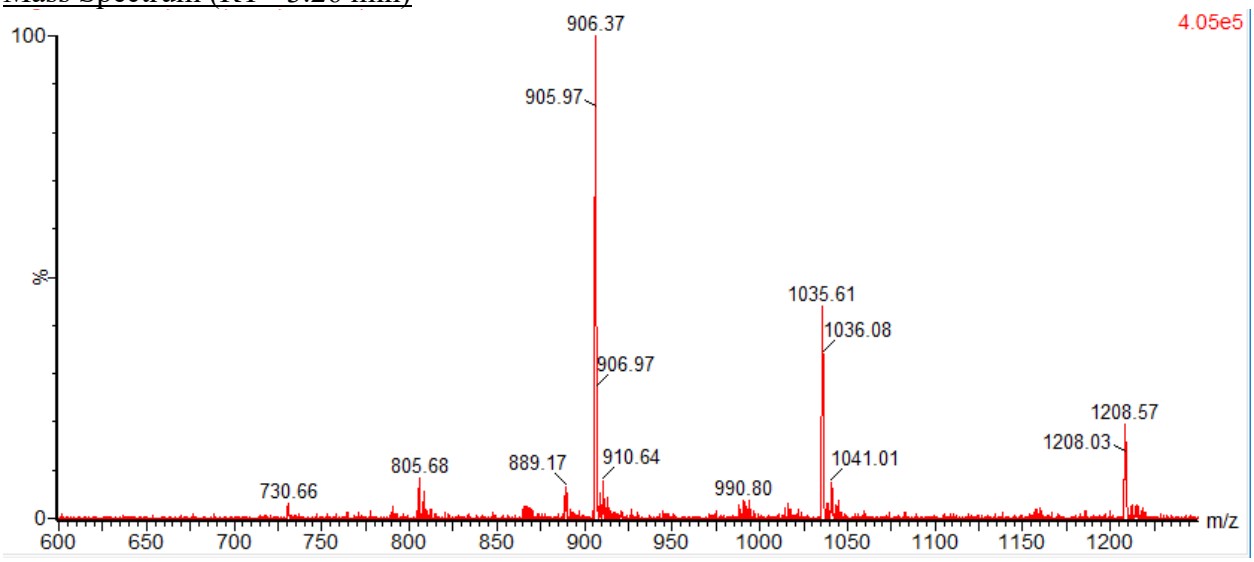


Exact Mass: 7254.248
Molecular Weight: 7257.939

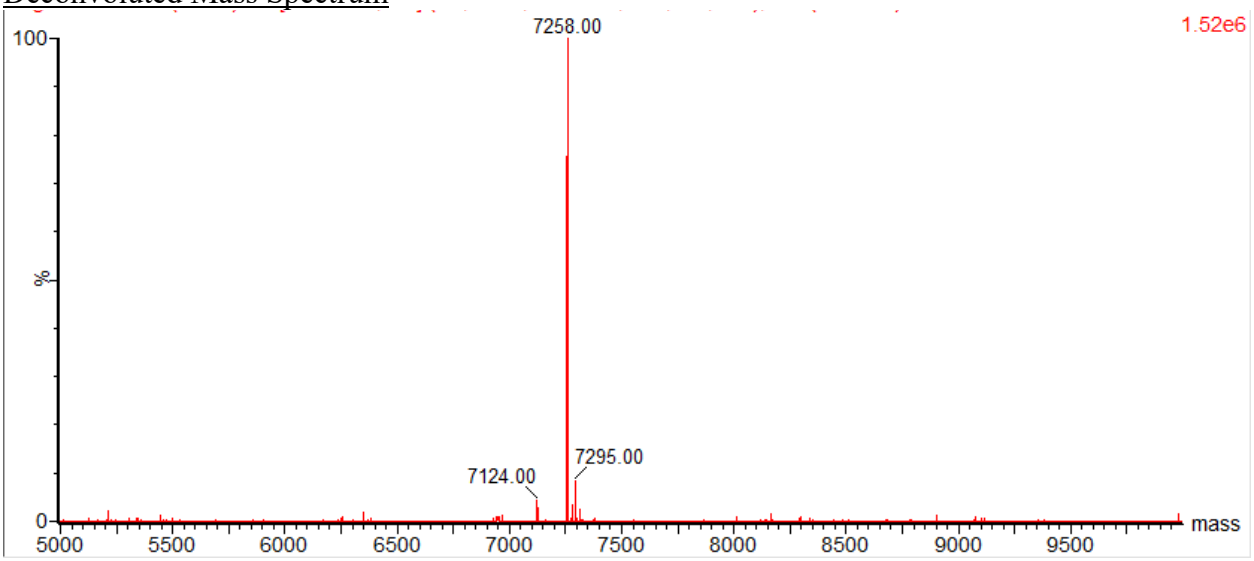
A260 Chromatogram



Mass Spectrum (RT= 3.26 min)

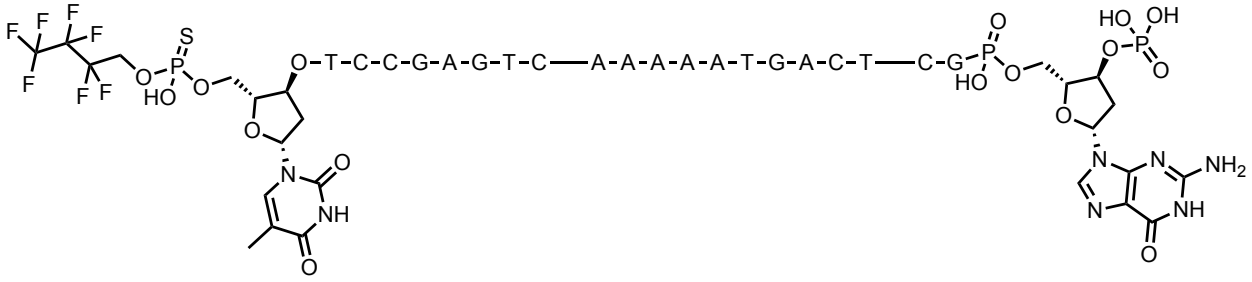


Deconvoluted Mass Spectrum



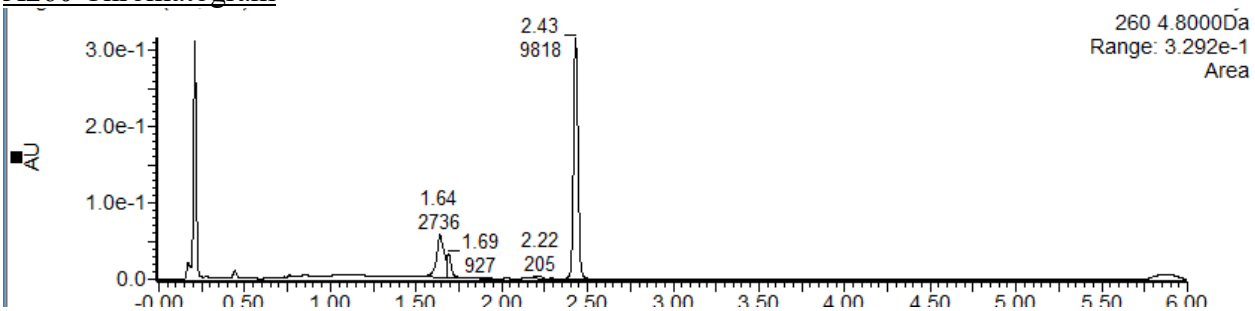
LCMS Characterization of 31

Produced from **1** by General Procedure **2:**
Using **Ψ-6**
Yield (A260): 75%
Expected Mass: 7101.50
Expected (M-6)/6 Ion: 1182.6
Found Deconvoluted Mass: 7102.00
Found (M-6)/6 Ion: 1182.21

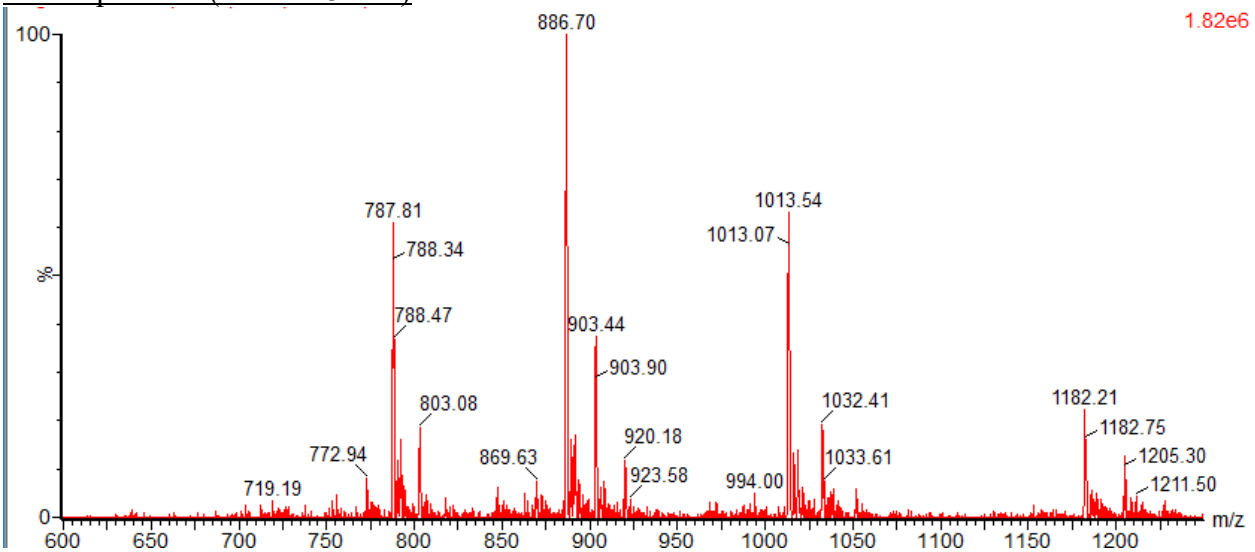


Exact Mass: 7098.08
Molecular Weight: 7101.50

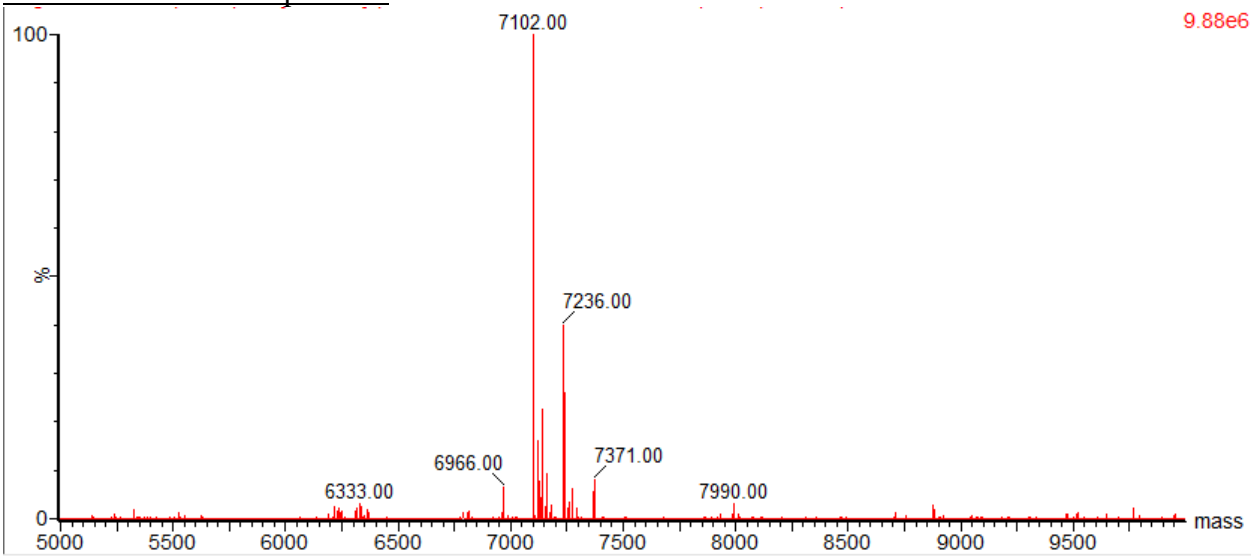
A260 Chromatogram



Mass Spectrum (RT= 2.43 min)

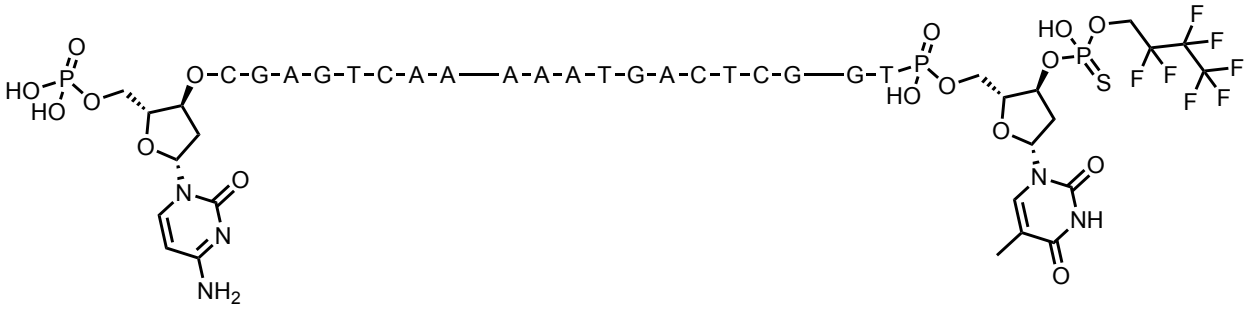


Deconvoluted Mass Spectrum



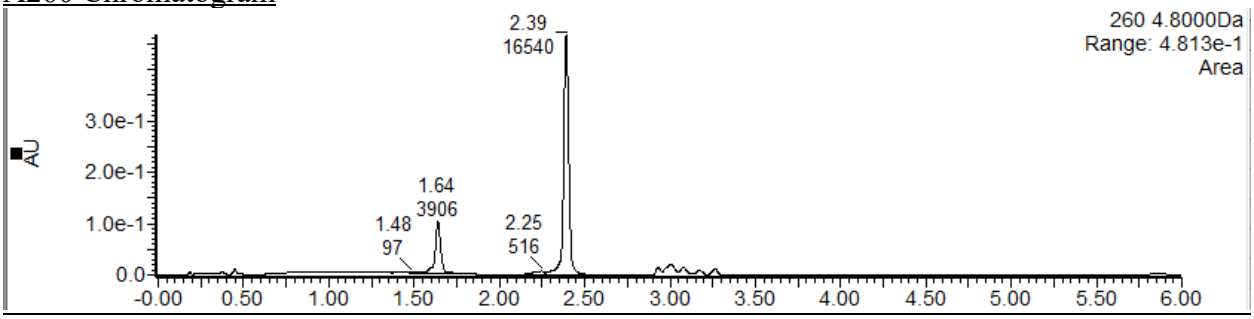
LCMS Characterization of 32

Produced from **2** by General Procedure **2:**
Using **Ψ-6**
Yield (A260): 74%
Expected Mass: 7101.5
Expected (M-6)/6 Ion: 1182.01
Found Deconvoluted Mass: 7102.00
Found (M-6)/6 Ion: 1182.2

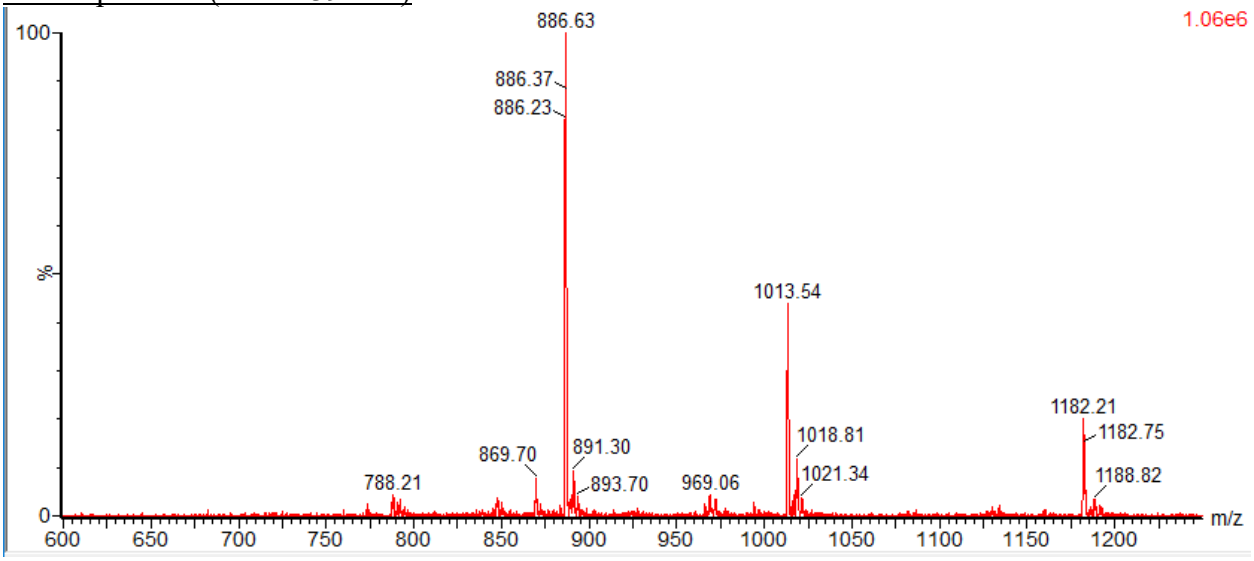


Exact Mass: 7098.08
Molecular Weight: 7101.50

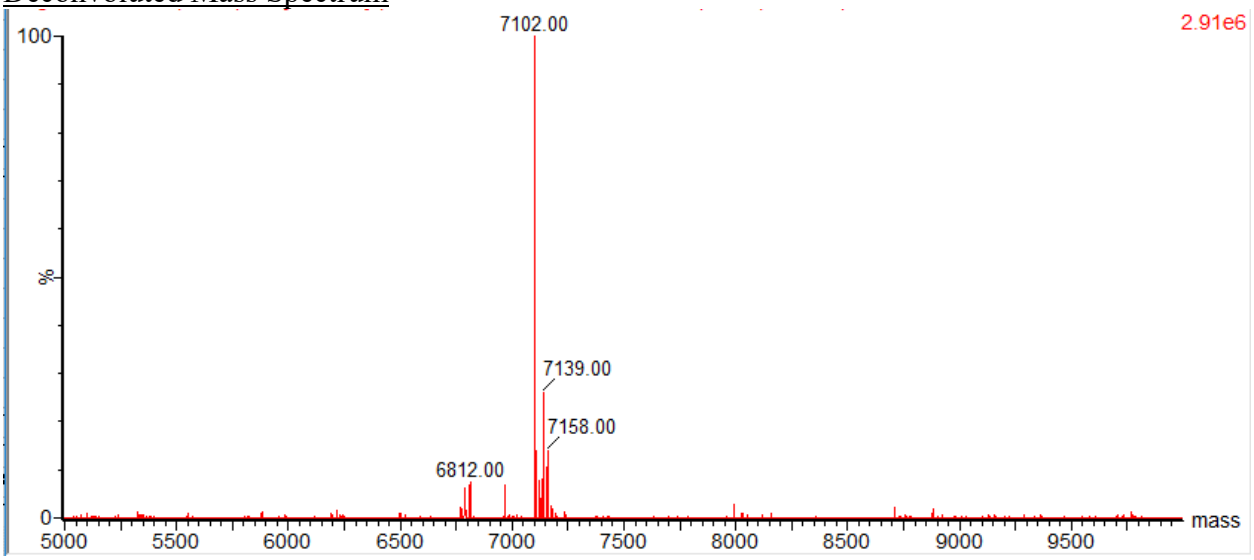
A260 Chromatogram



Mass Spectrum (RT= 2.39 min)

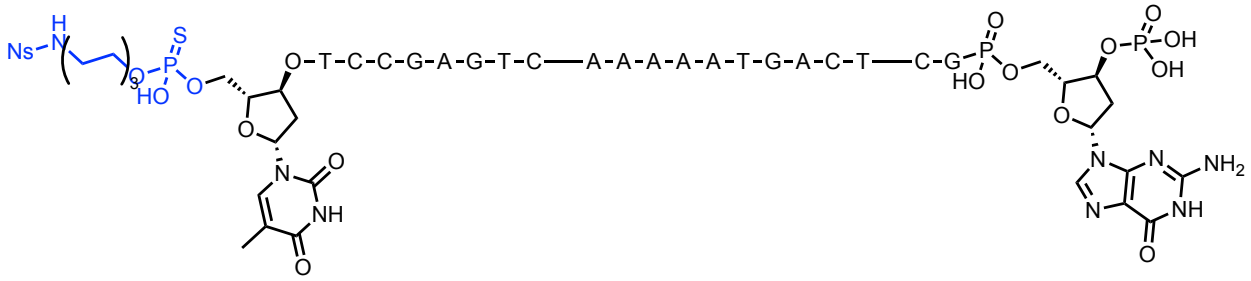


Deconvoluted Mass Spectrum



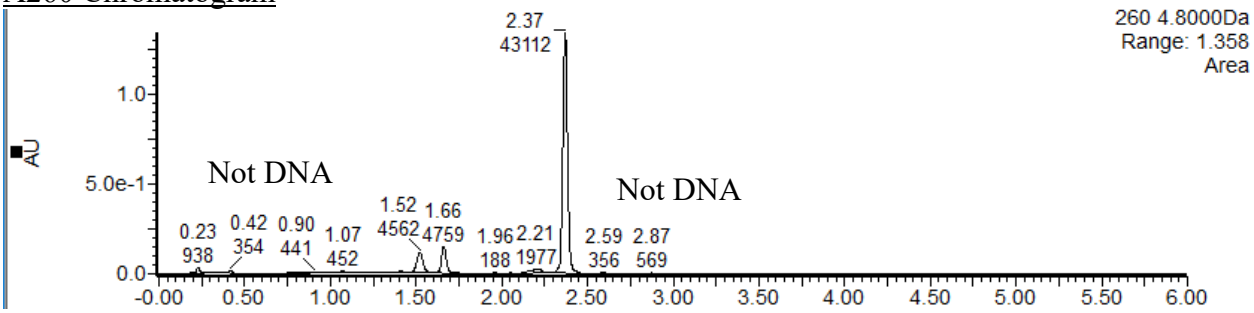
LCMS Characterization of 33

Produced from **1** by General Procedure **2:**
Using **Ψ-7**
Yield (A260): 90%
Expected Mass: 7203.790
Expected (M-6)/6 Ion: 1199.63
Found Deconvoluted Mass: 7204.00
Found (M-6)/6 Ion: 1199.23

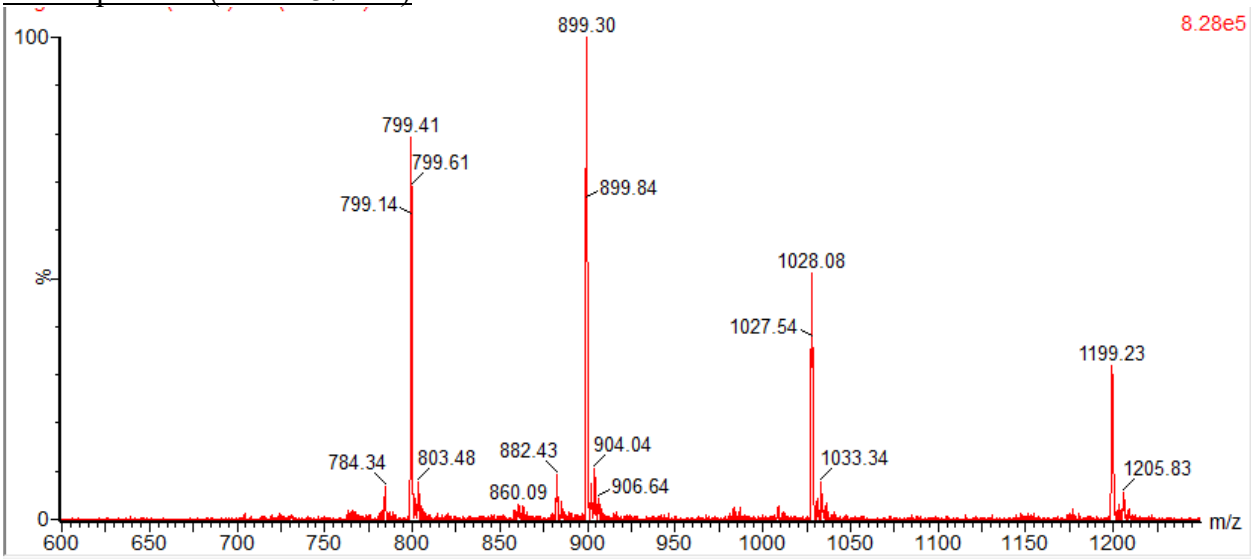


T Exact Mass: 7200.165 G
Molecular Weight: 7203.790

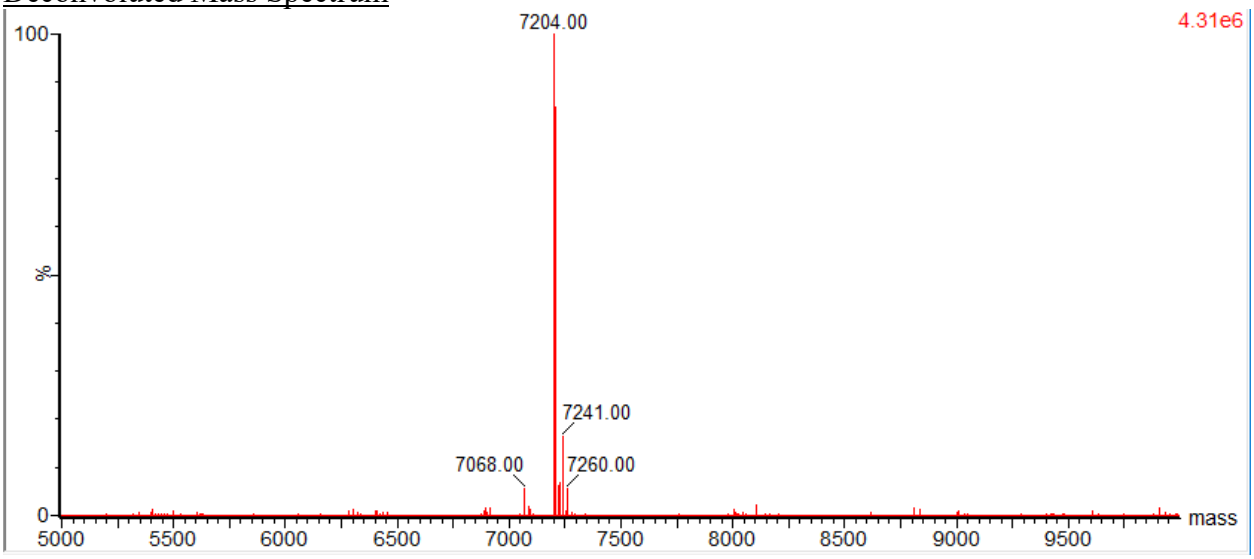
A260 Chromatogram



Mass Spectrum (RT= 2.37 min)

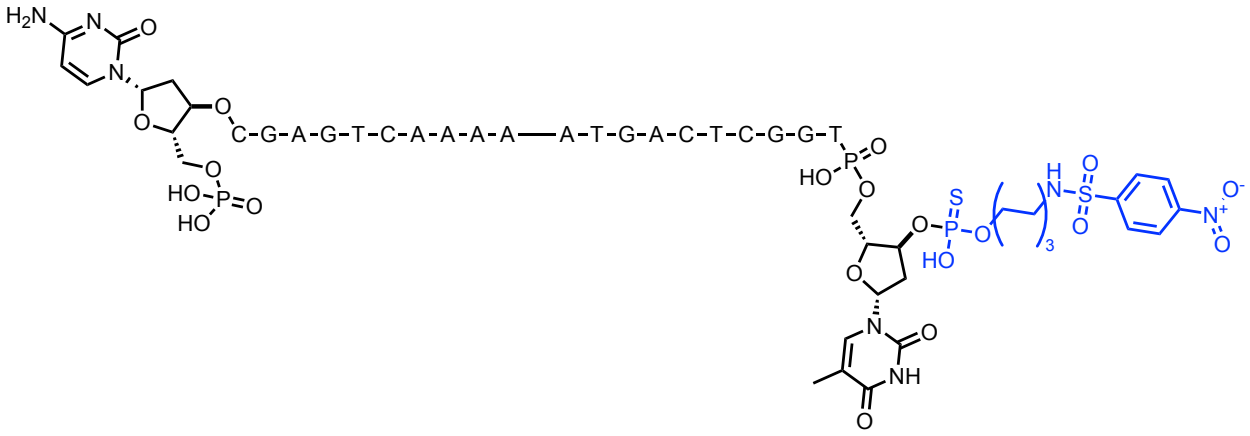


Deconvoluted Mass Spectrum



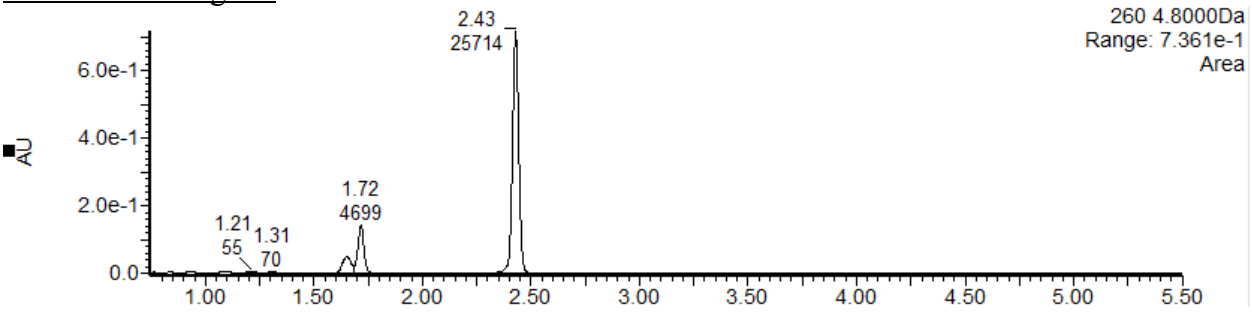
LCMS Characterization of 34

Produced from **2** by General Procedure **1:**
Using **Ψ-7**
Yield (A260): 84%
Expected Mass: 7203.79
Expected (M-6)/6 Ion: 1199.63
Found Deconvoluted Mass:7204.00
Found (M-6)/6 Ion:199.29

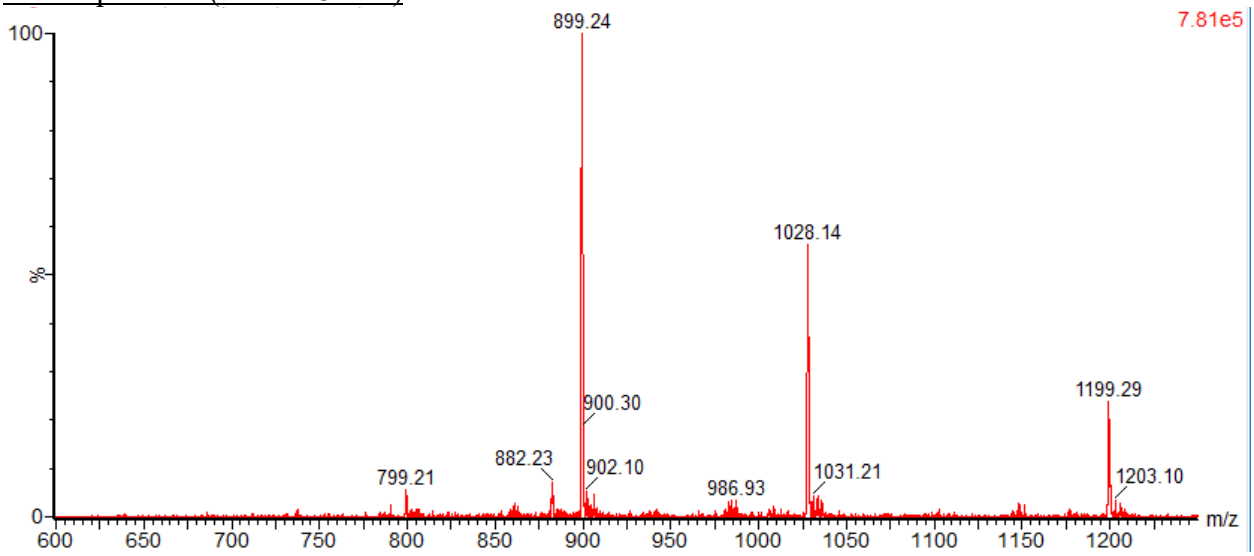


Exact Mass: 7200.165
Molecular Weight: 7203.790

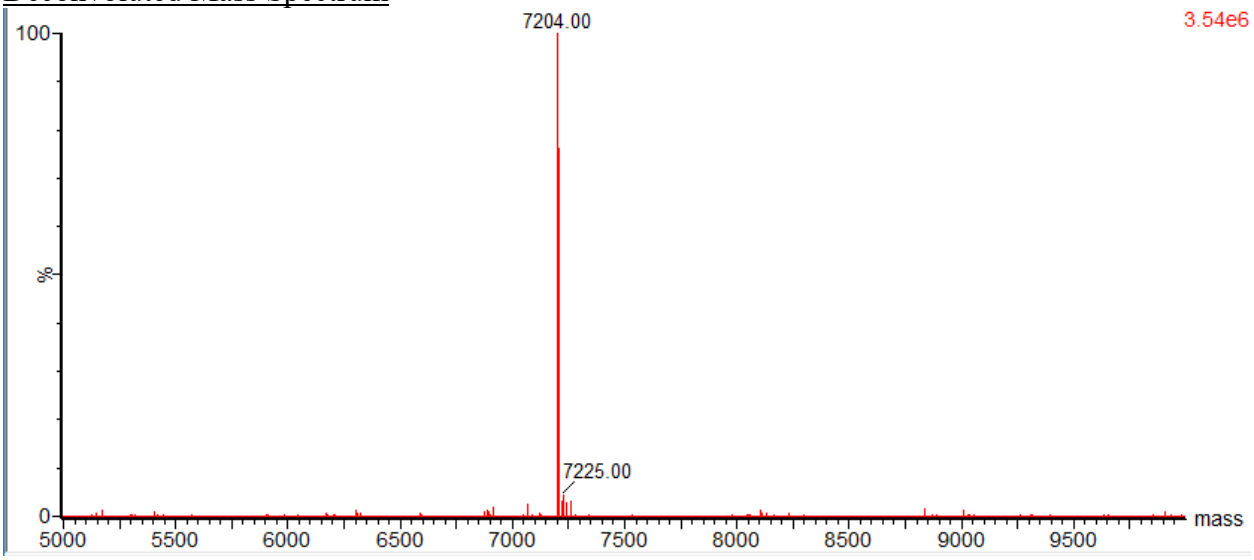
A260 Chromatogram



Mass Spectrum (RT=2.43 min)

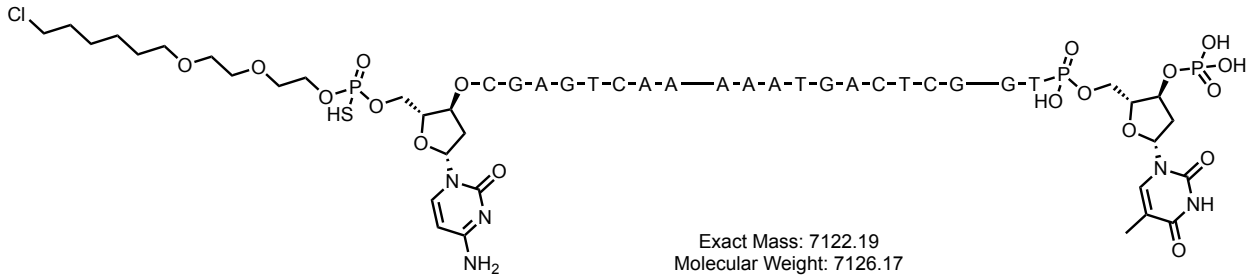


Deconvoluted Mass Spectrum

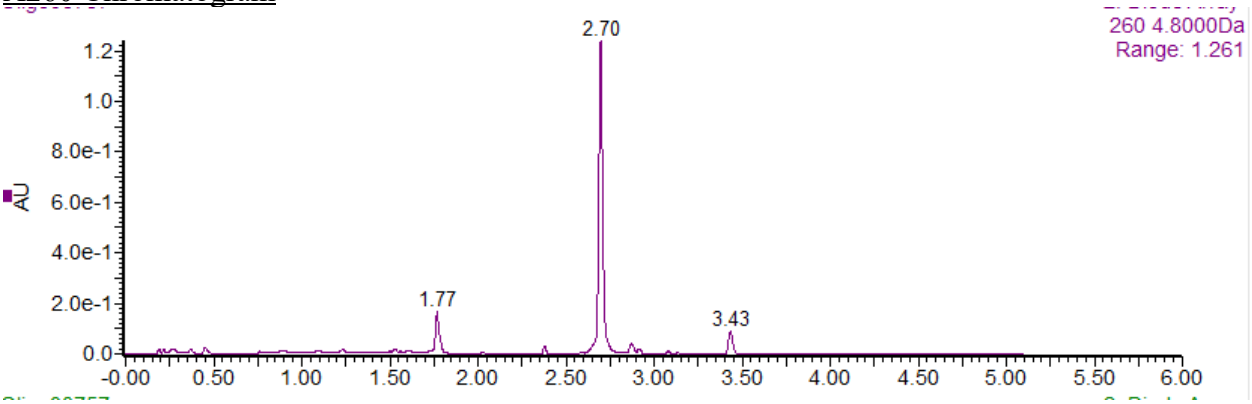


LCMS Characterization of 35

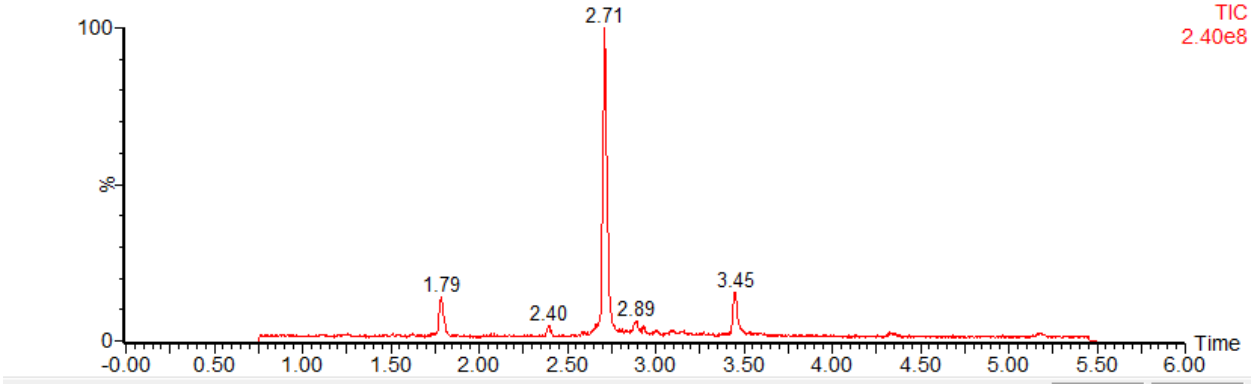
Using **Ψ-16**
Produced from **1** by General Procedure **2:**
Yield (A260): 88%
Expected Mass: 7126.17
Expected (M-6)/6 Ion: 1186.03
Found Deconvoluted Mass: 7126.00
Found (M-6)/6 Ion: 1186.28



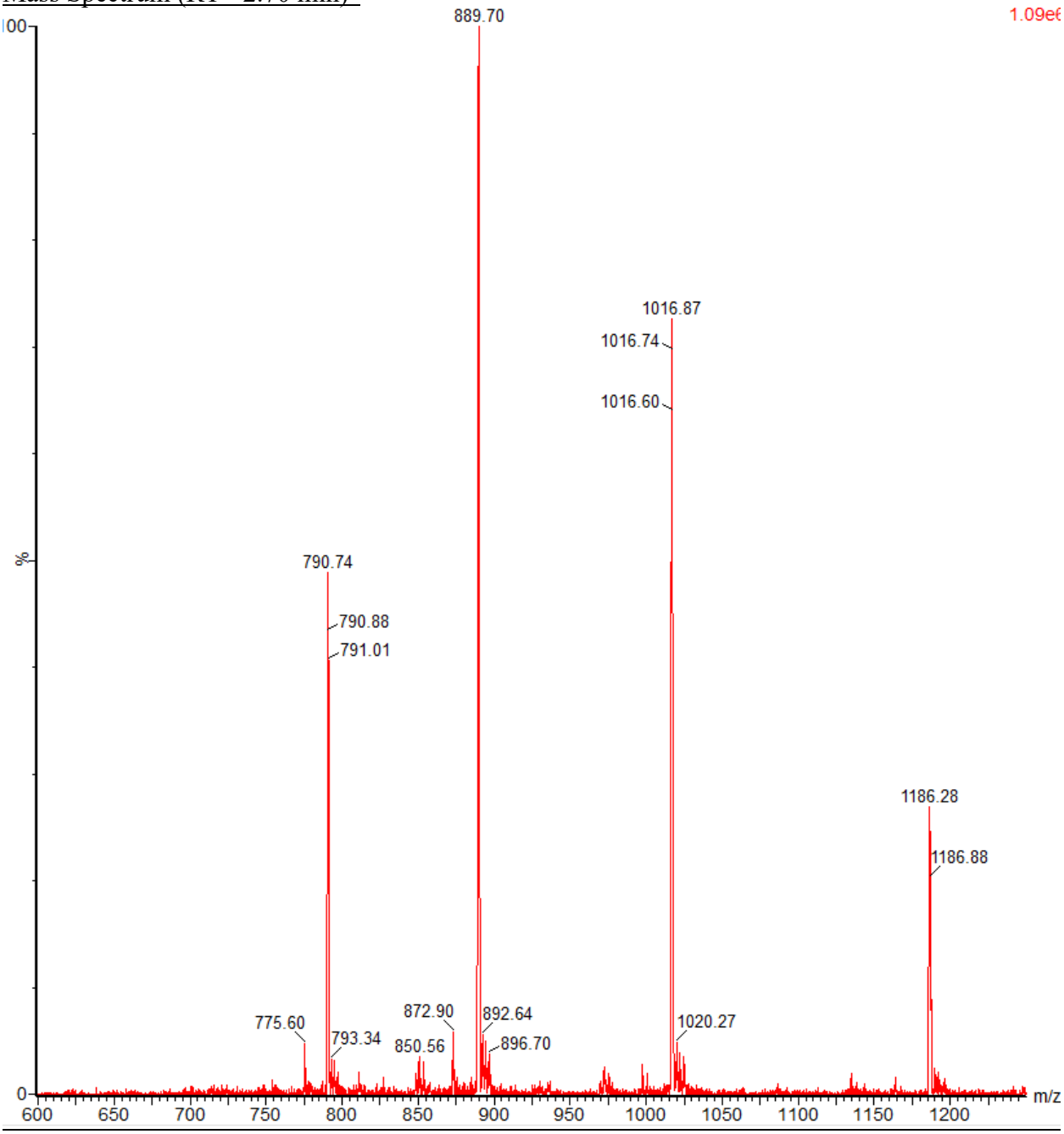
A260 Chromatogram



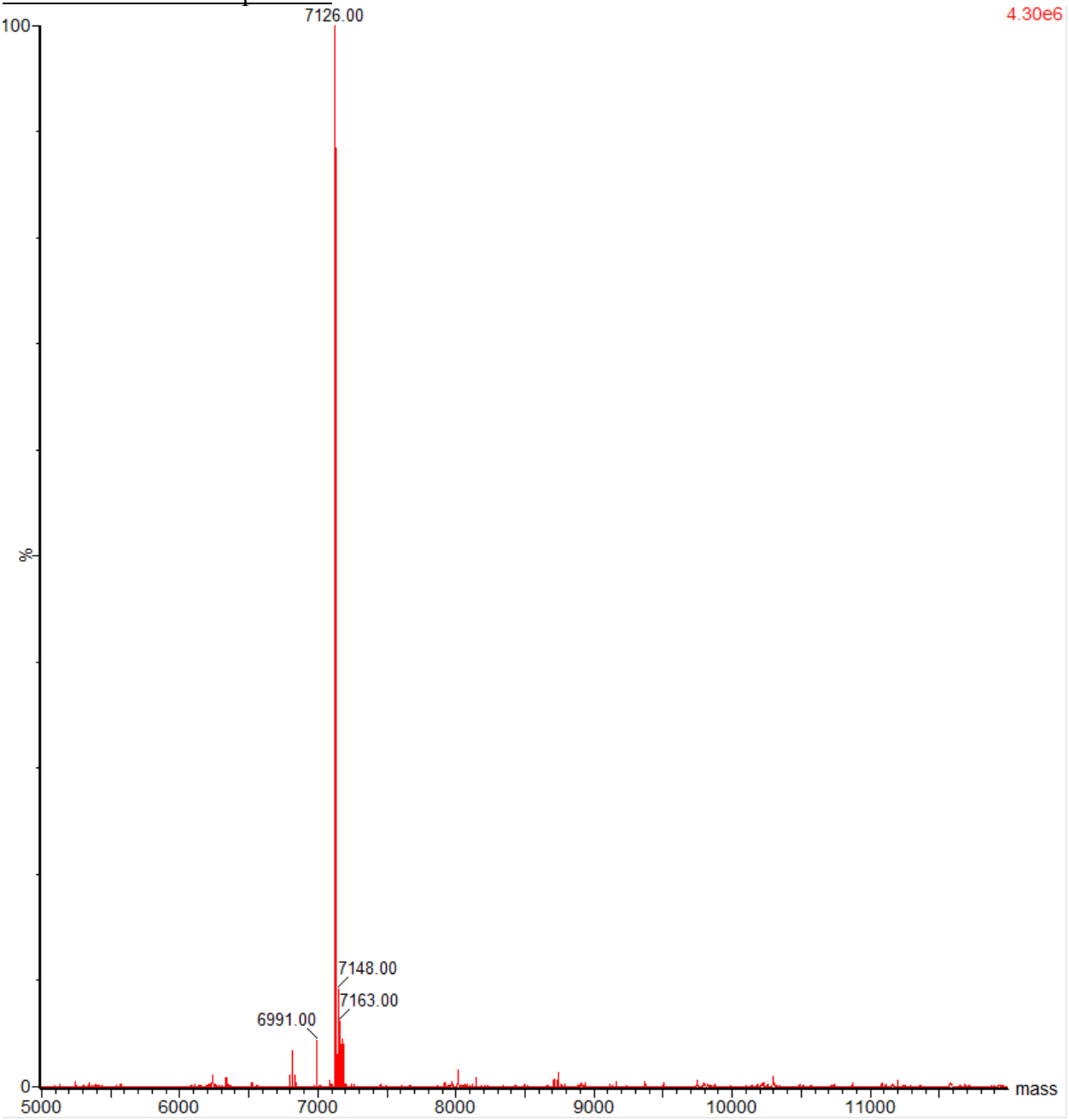
TIC



Mass Spectrum (RT= 2.70 min)

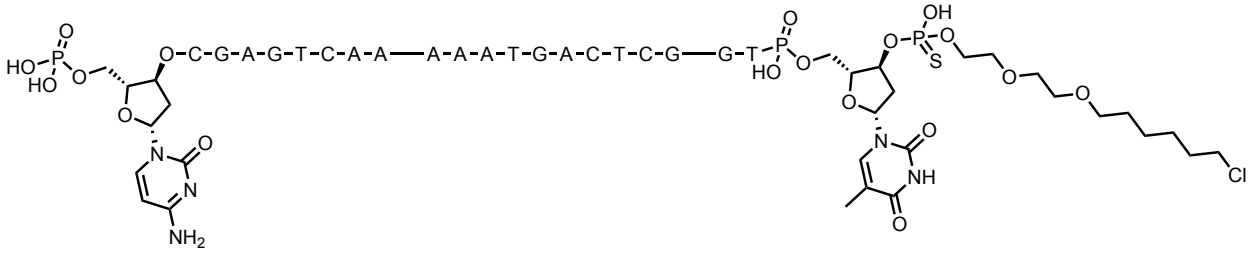


Deconvoluted Mass Spectrum

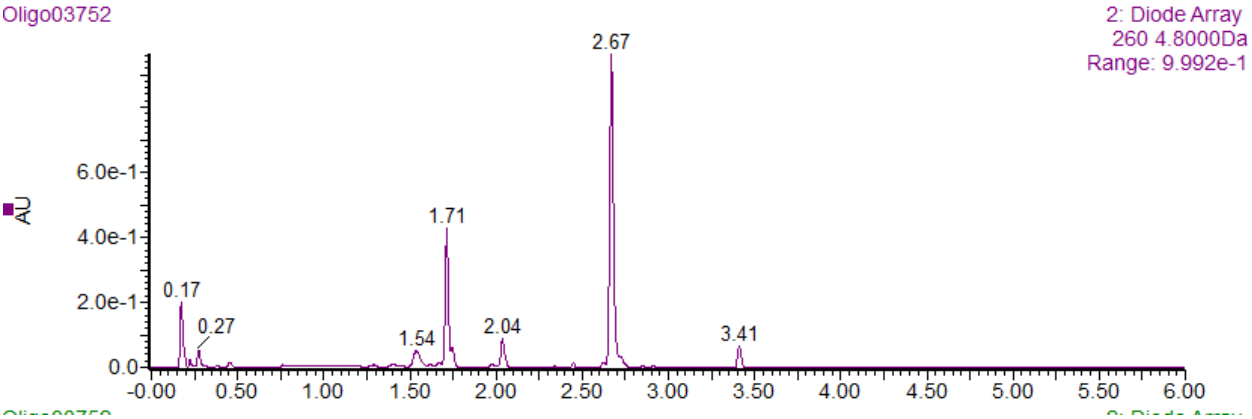


LCMS Characterization of 35

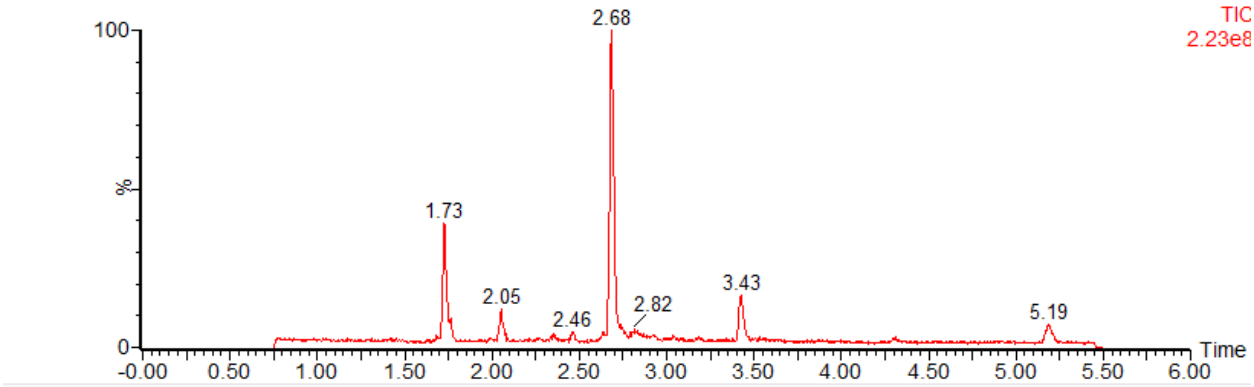
Using **Ψ-16**
Produced from **2** by General Procedure **2:**
Yield (A260): 64%
Expected Mass: 7126.17
Expected (M-6)/6 Ion: 1186.03
Found Deconvoluted Mass: 7126.0
Found (M-6)/6 Ion: 1186.42



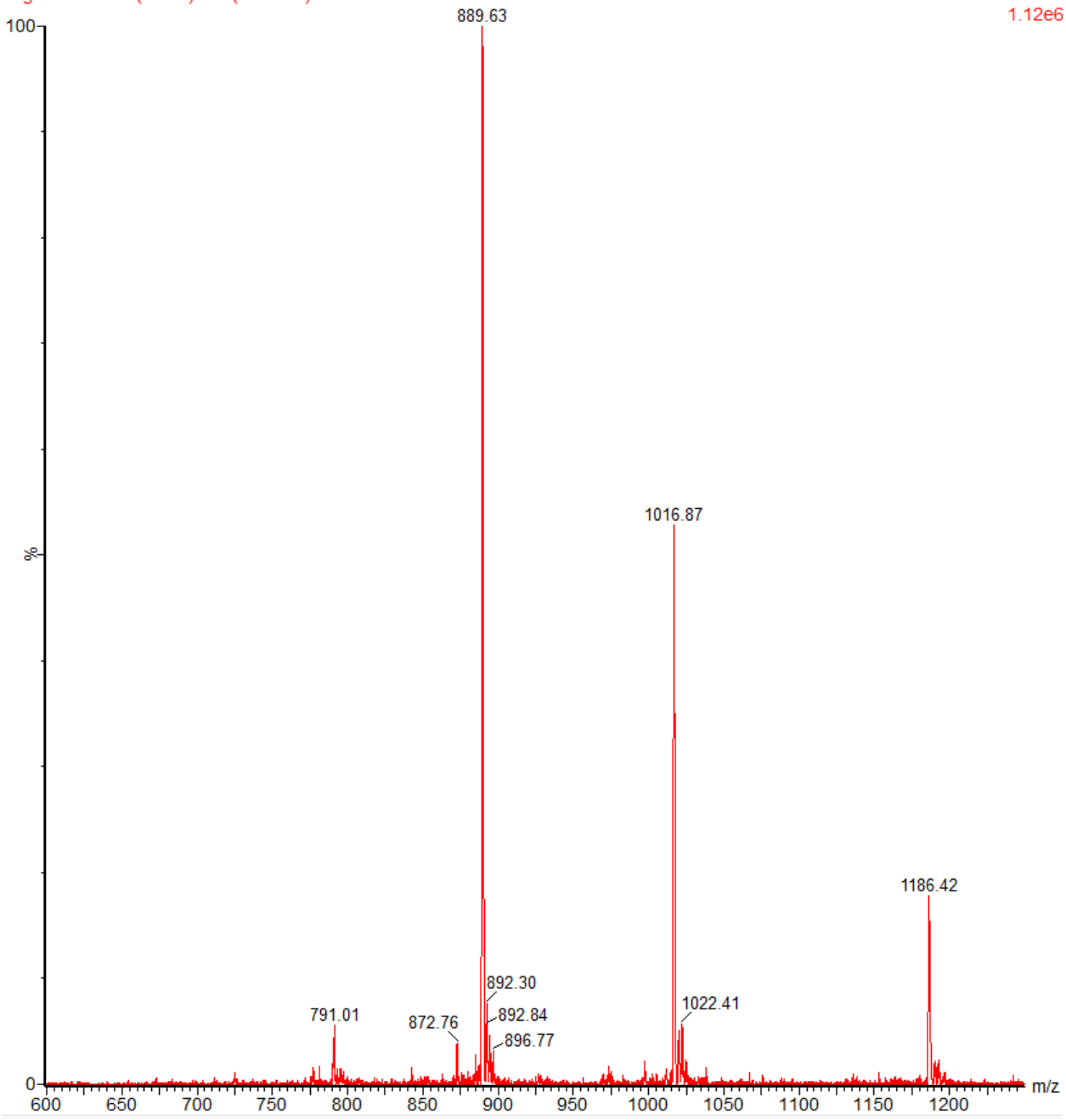
A260 Chromatogram



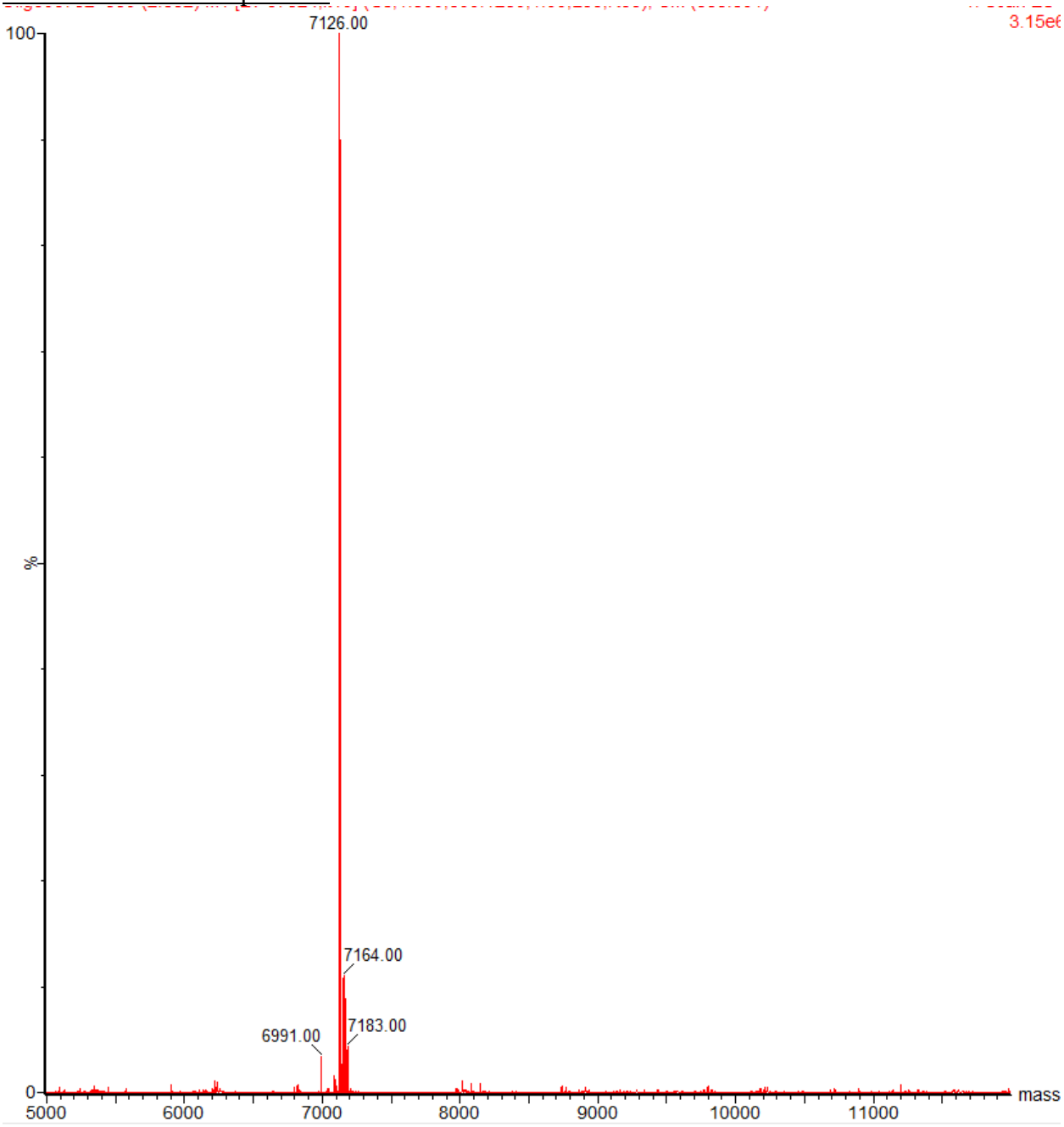
TIC



Mass Spectrum (RT= 2.27 min)

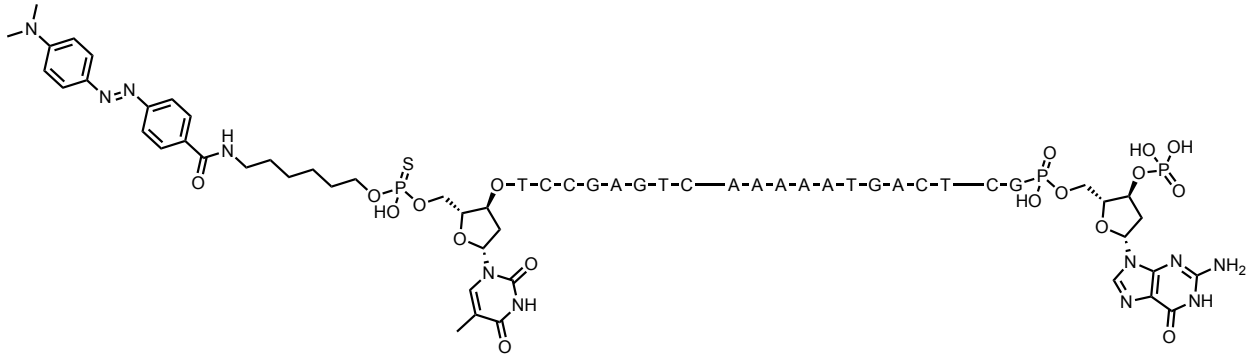


Deconvoluted Mass Spectrum

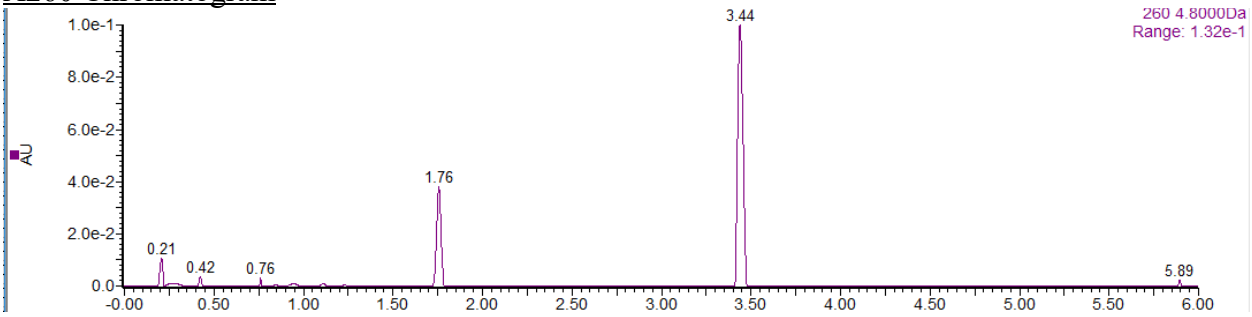


LCMS Characterization of 37

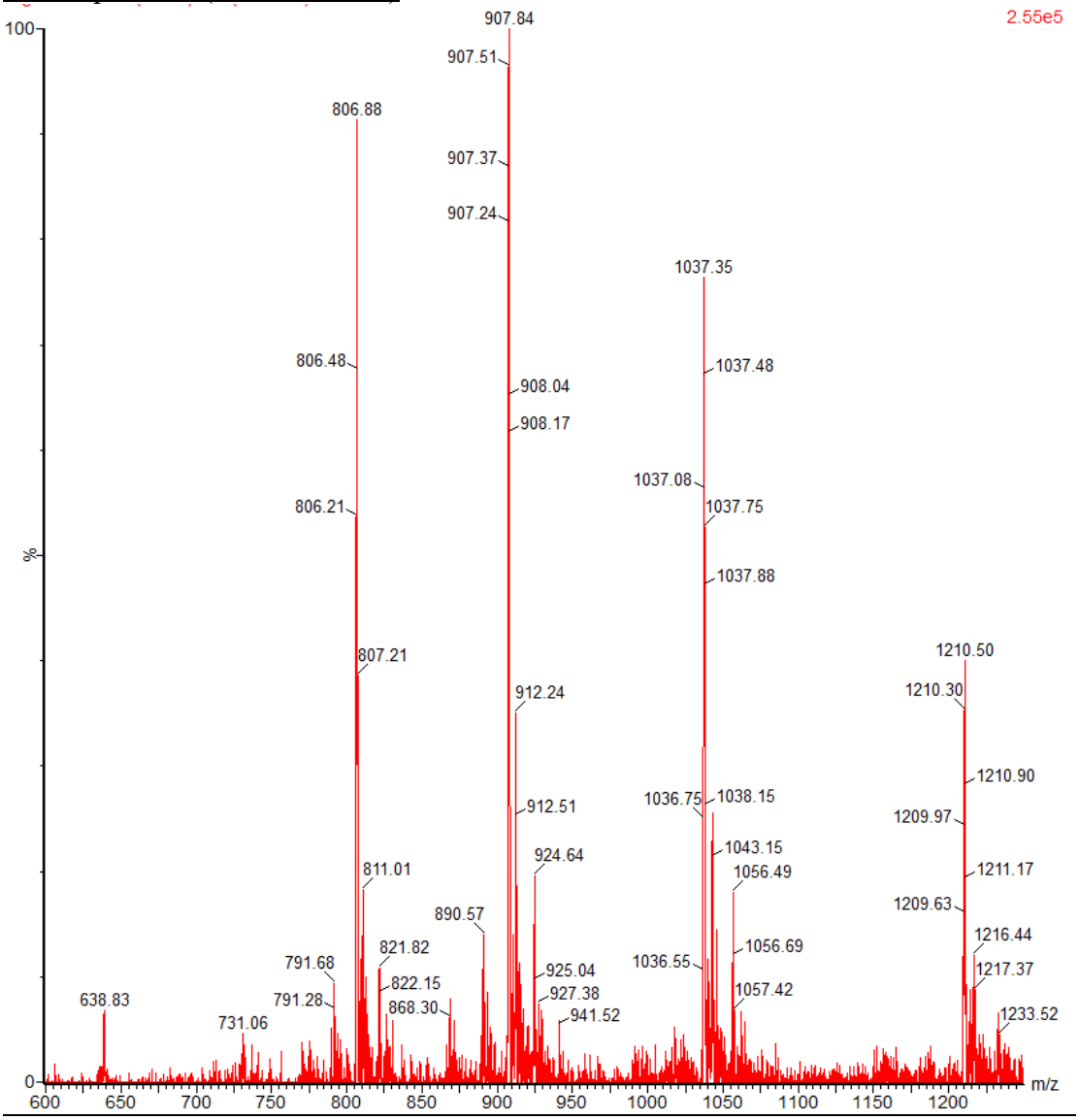
Produced from **1** by General Procedure **2:**
Using **Ψ-10**
Yield (A260): 76%
Expected Mass: 7269.9
Expected (M-6)/6 Ion: 1210.65
Found Deconvoluted Mass: 7270.0
Found (M-6)/6 Ion: 1210.5



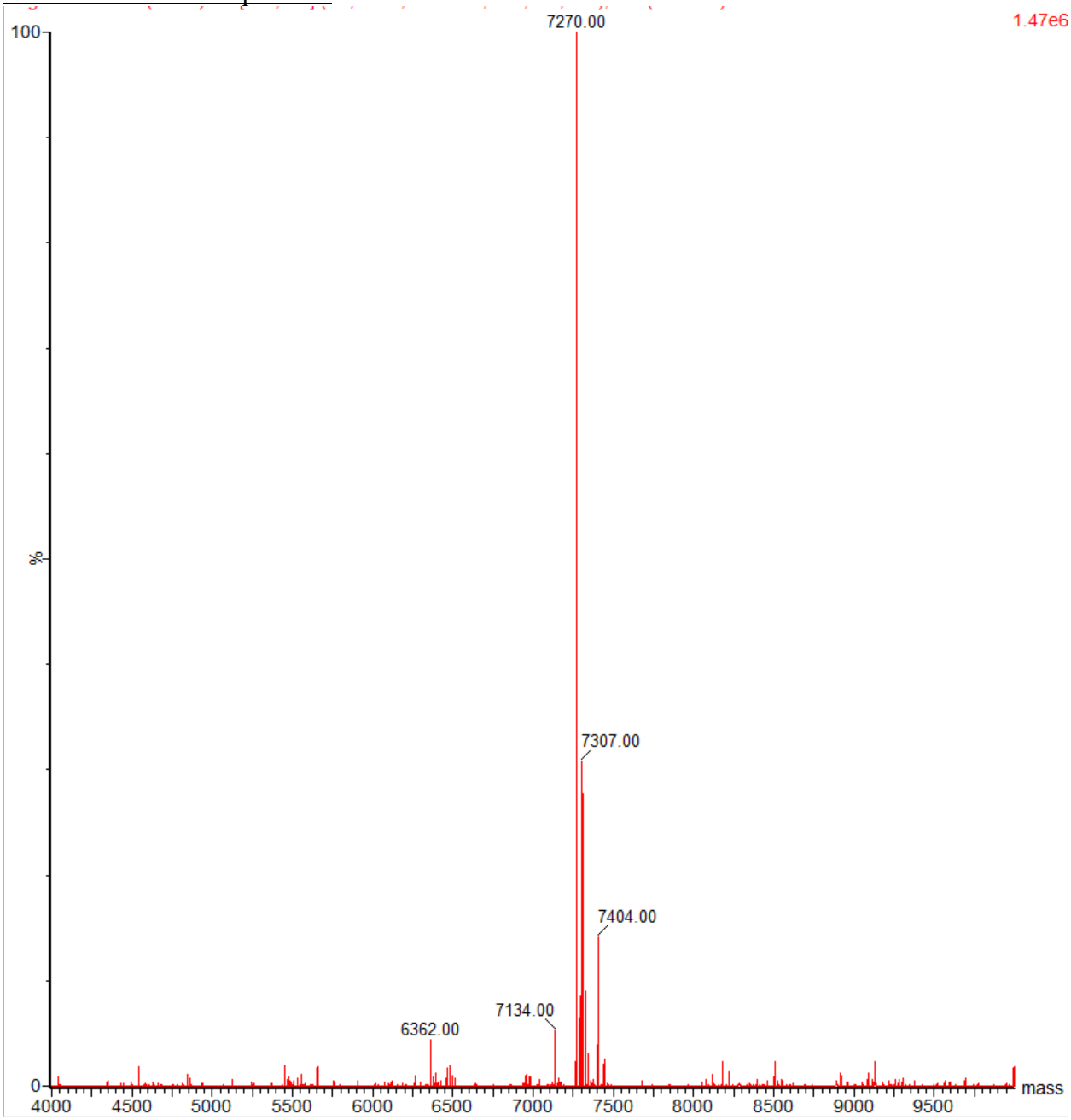
A260 Chromatogram



Mass Spectrum (RT= 3.44 min)

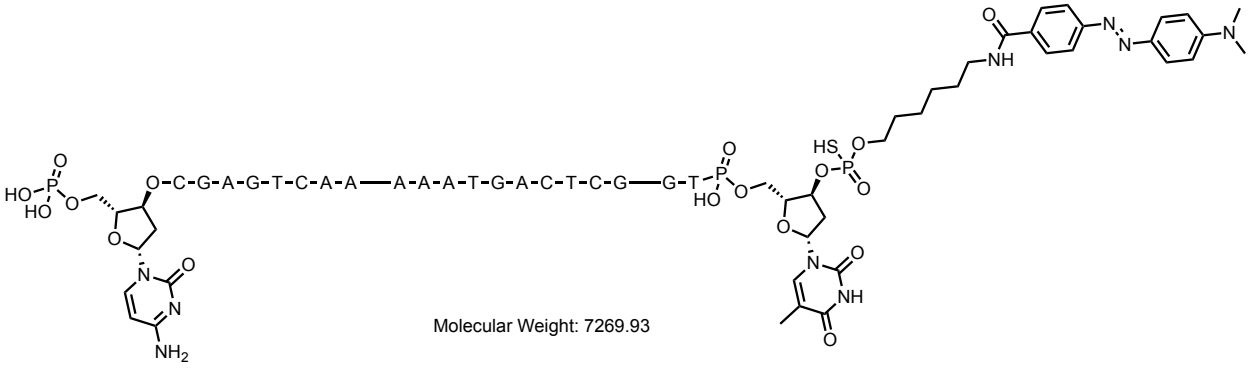


Deconvoluted Mass Spectrum

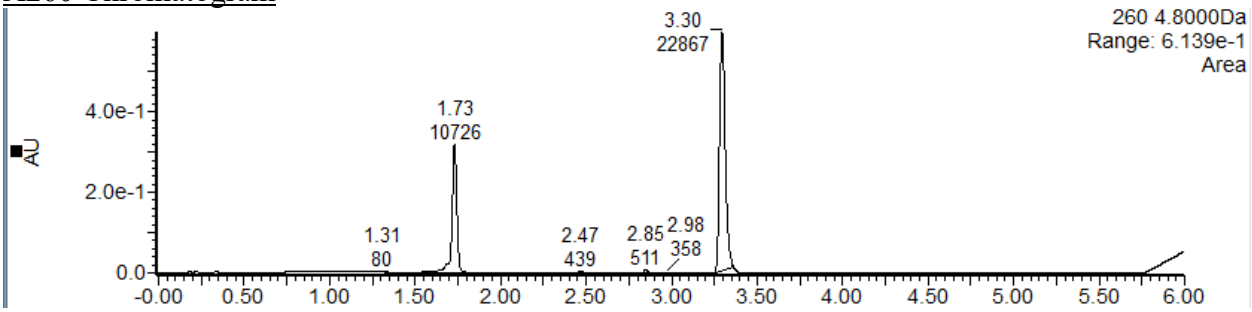


LCMS Characterization of 38

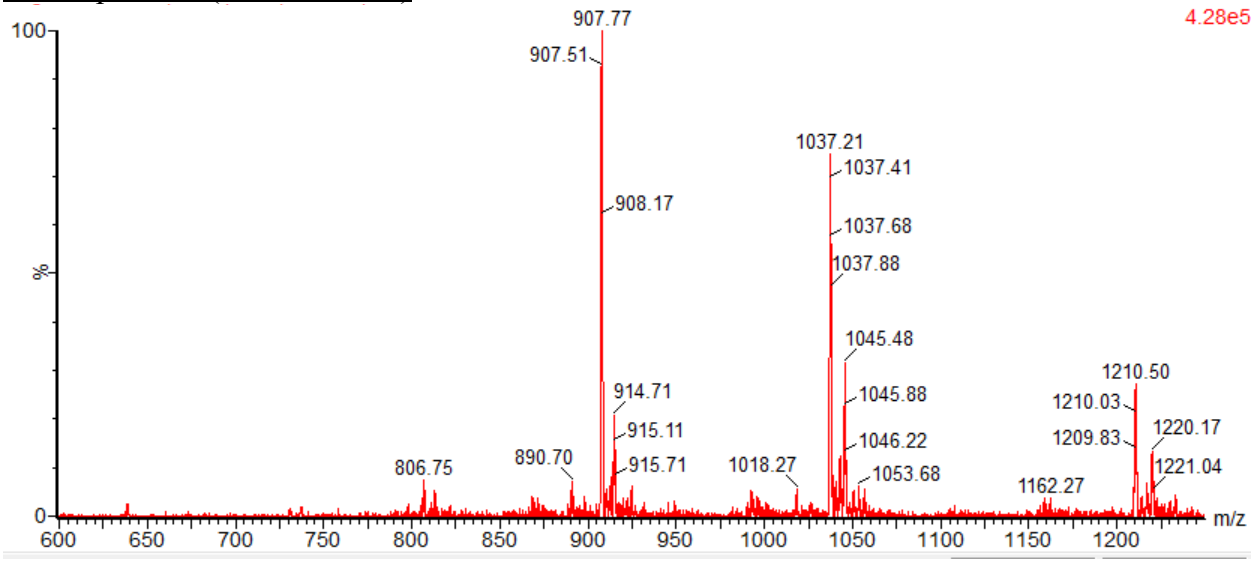
Produced from **2** by General Procedure **2:**
Using **Ψ-10**
Yield (A260): 68%
Expected Mass: 7269.9
Expected (M-6)/6 Ion: 1210.65
Found Deconvoluted Mass: 7270.0
Found (M-6)/6 Ion: 1210.5



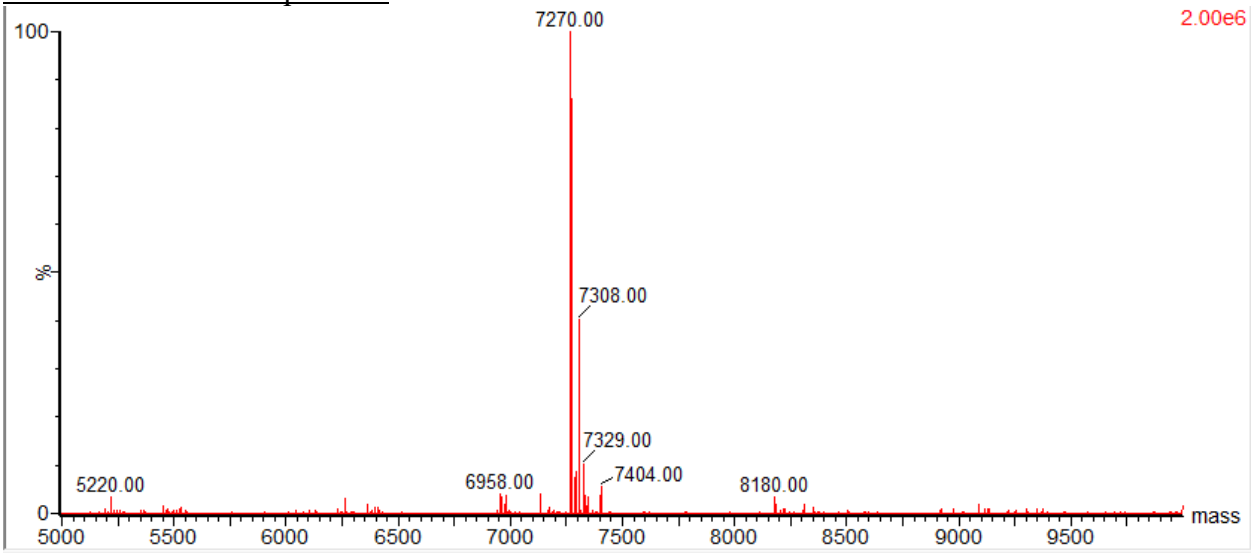
A260 Chromatogram



Mass Spectrum (RT= 3.30 min)

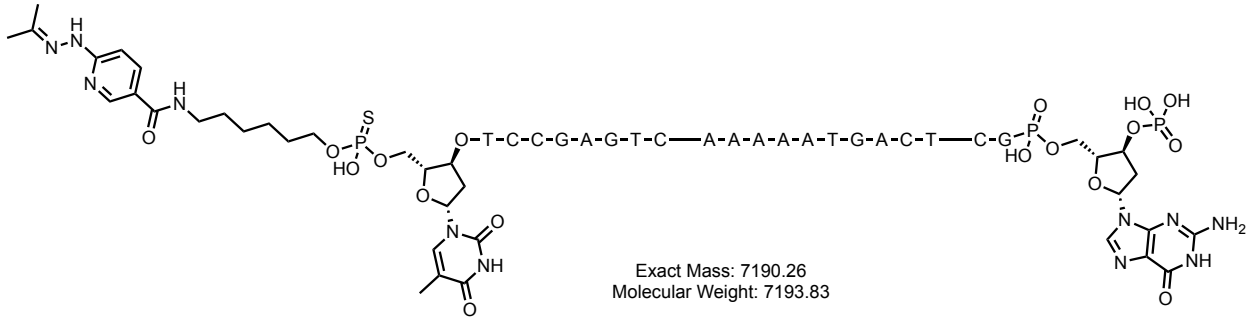


Deconvoluted Mass Spectrum

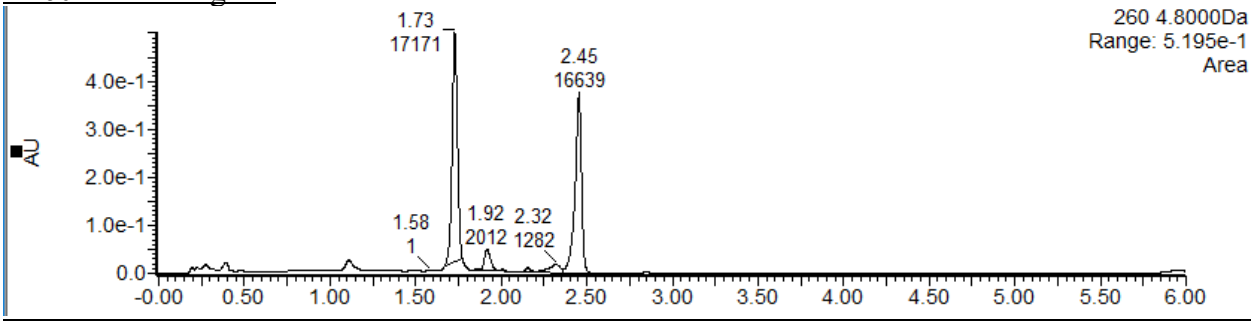


LCMS Characterization of 39

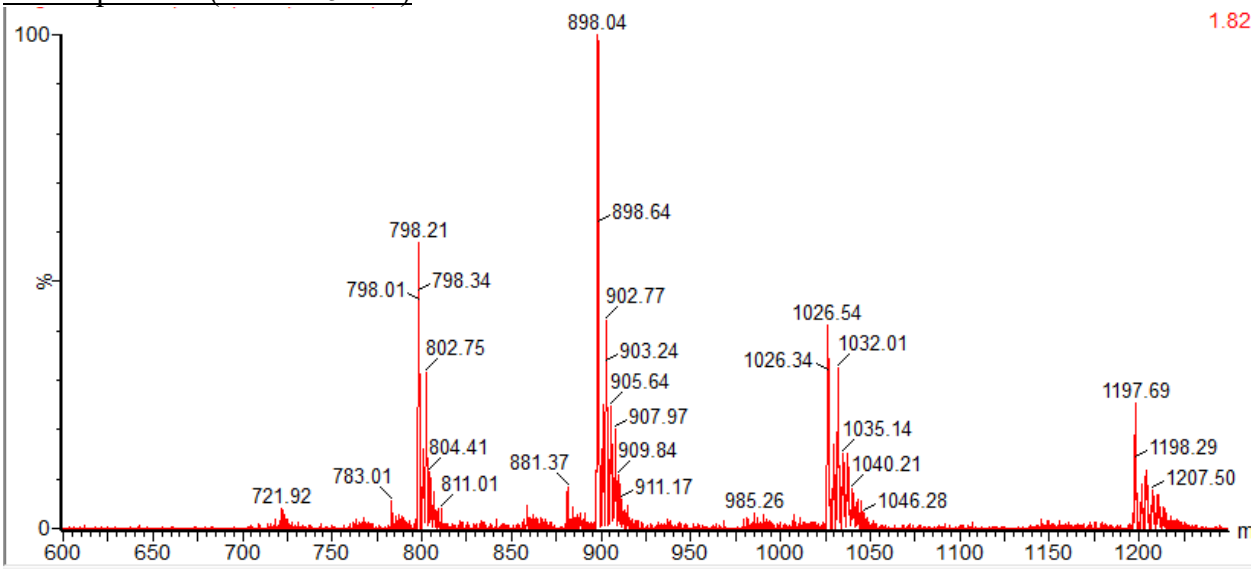
Produced from **1** by General Procedure **2:**
Using **Ψ-11**
Yield (A260): 55%
Expected Mass: 7193.83
Expected (M-6)/6 Ion: 1197.97
Found Deconvoluted Mass: 1197.69
Found (M-6)/6 Ion: 7194 and 7232.00 (potassium adduct)



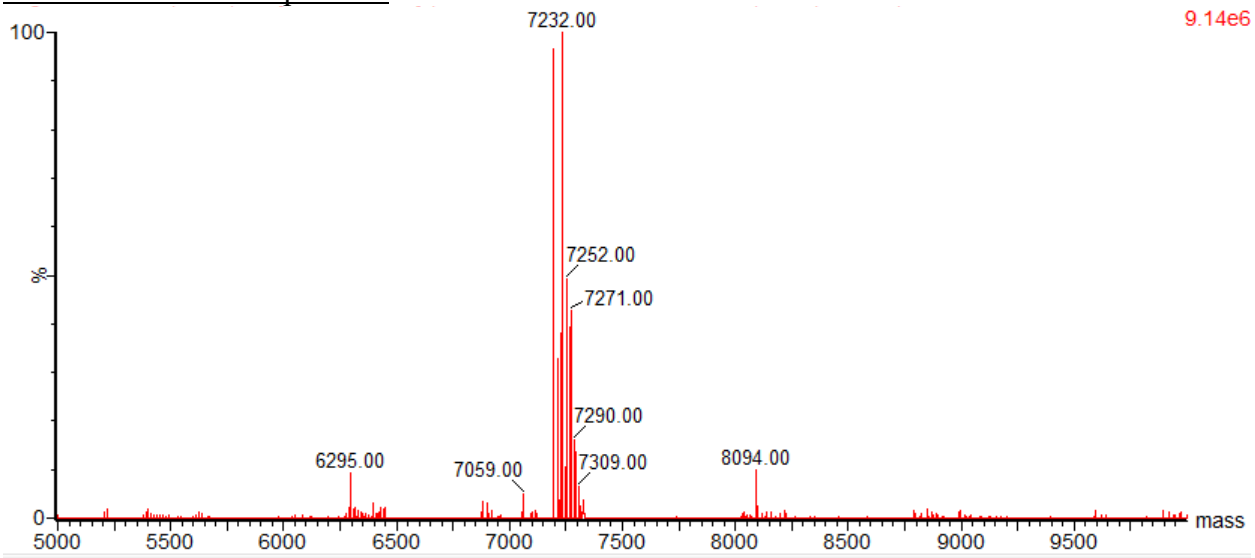
A260 Chromatogram



Mass Spectrum (RT= 2.45 min)

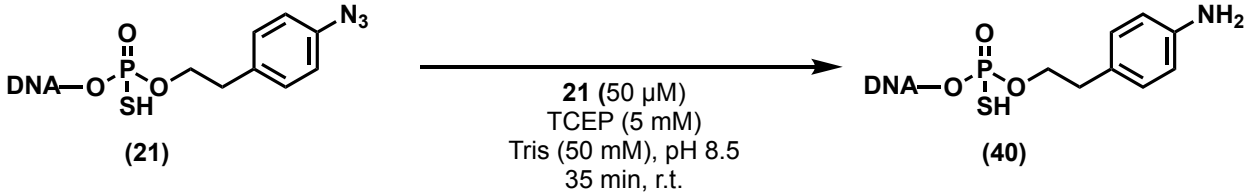


Deconvoluted Mass Spectrum



Secondary Manipulations:

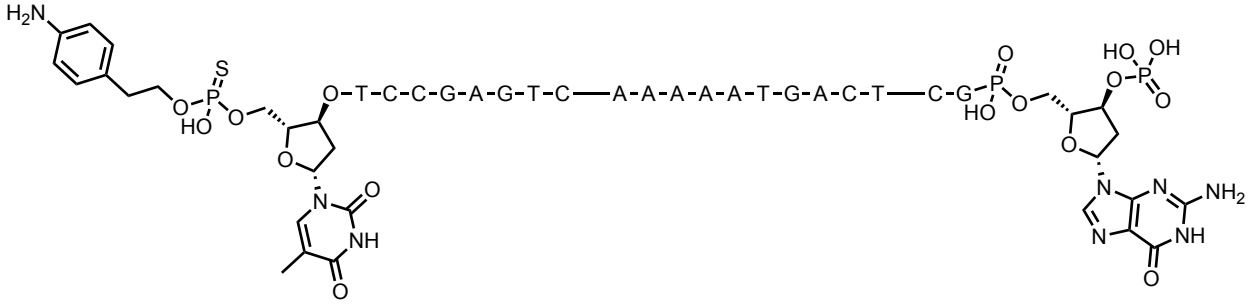
Azide Reduction (40):



Compound **21** (5 nmol) was synthesized by *General Procedure 2* and isolated crude after ethanol precipitation. Solid **21** was dissolved (100 μL) in tris buffer (50 mM) containing TCEP (5 mM) at pH 8.5. This solution was vortexed and transferred to an HPLC vial. This reaction was injected onto the H-Class HPLC at different timepoints. The reaction was found to be complete after 35 minutes at room temperature. Reduction product **40** was characterized below.

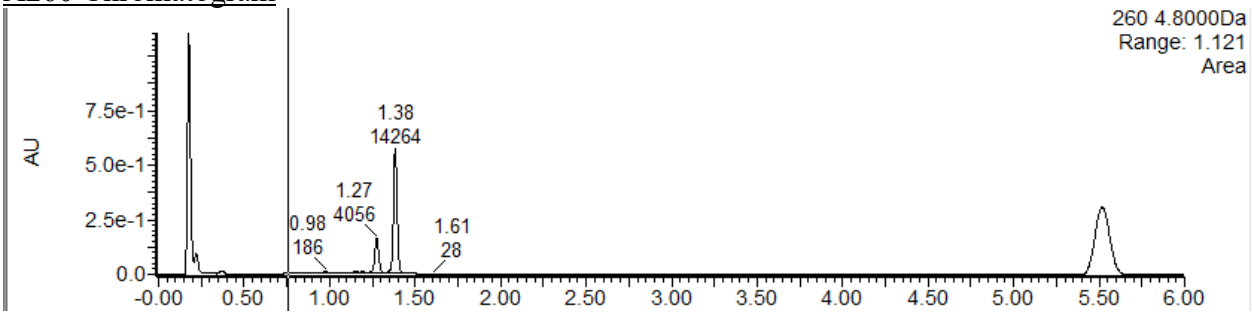
LCMS Characterization of 40

Produced from **21**
Yield (A260): 78% (total yield including **1**)
Expected Mass: 7038.63
Expected (M-6)/6 Ion: 1171.5
Found Deconvoluted Mass: 7039.0
Found (M-6)/6 Ion: 1171.7

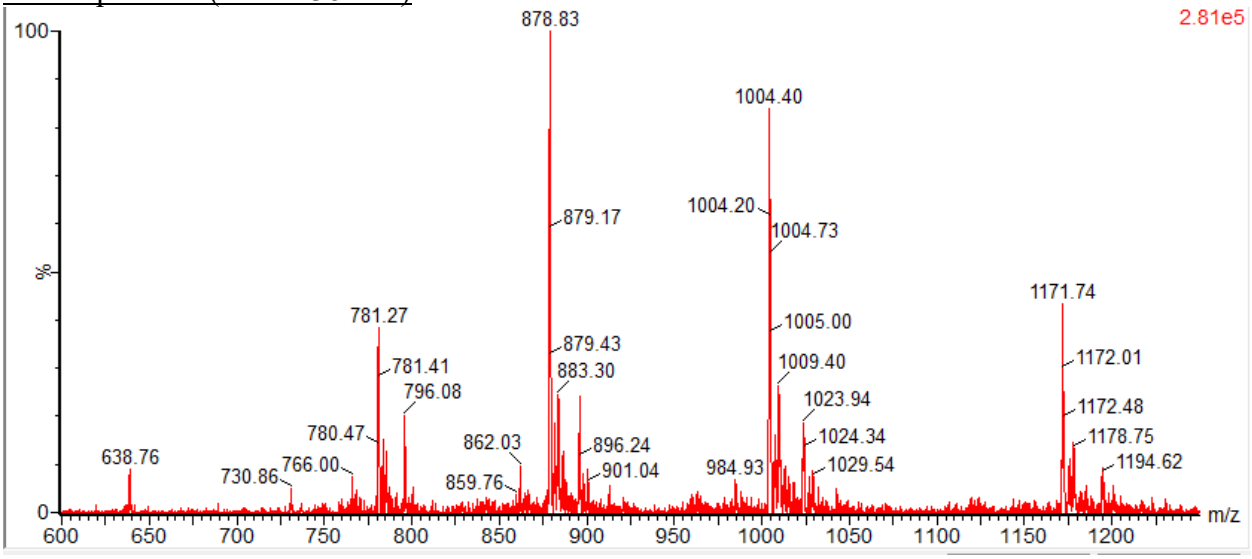


Exact Mass: 7035.16
Molecular Weight: 7038.63

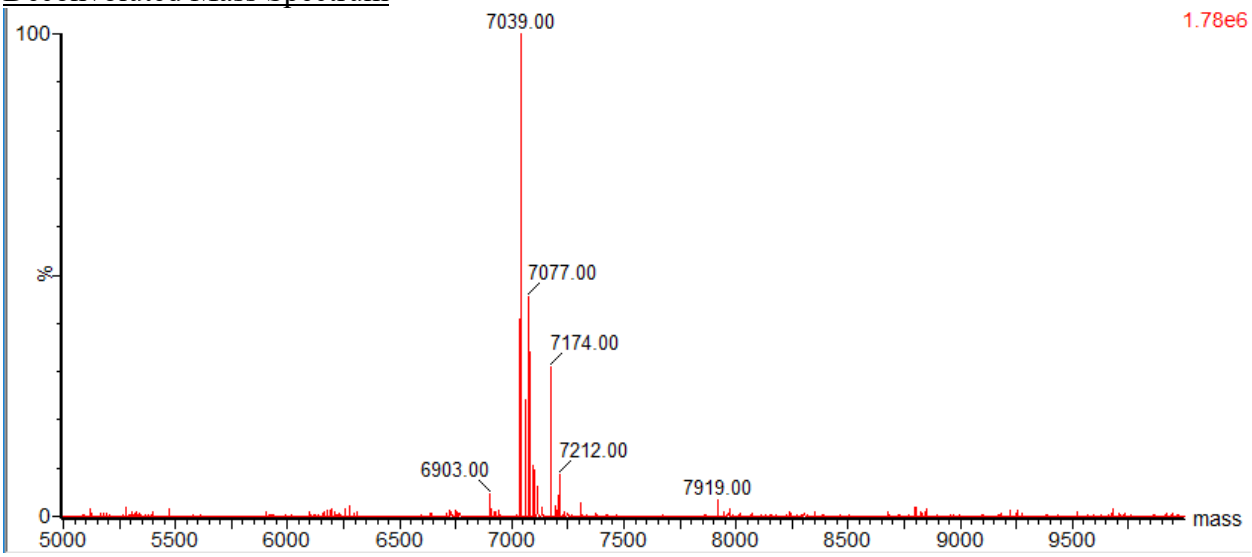
A260 Chromatogram



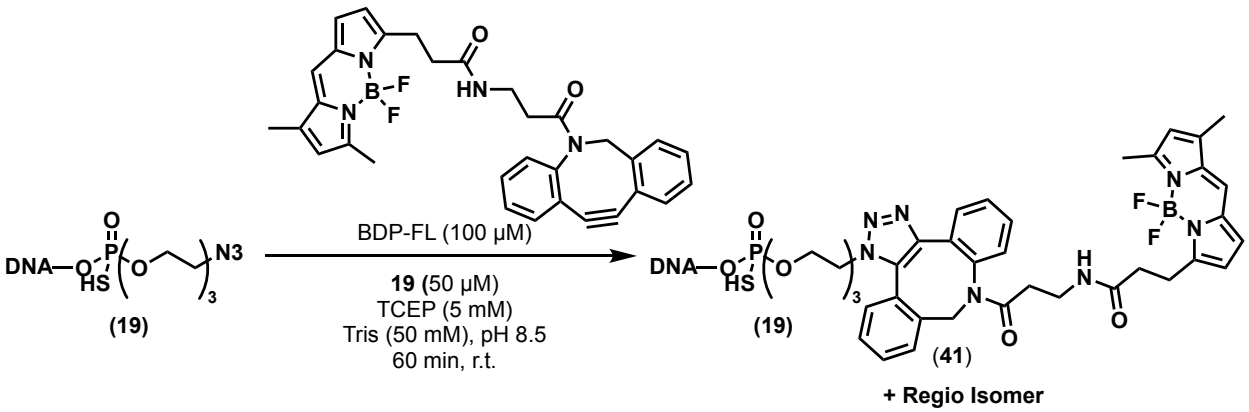
Mass Spectrum (RT= 1.38 min)



Deconvoluted Mass Spectrum



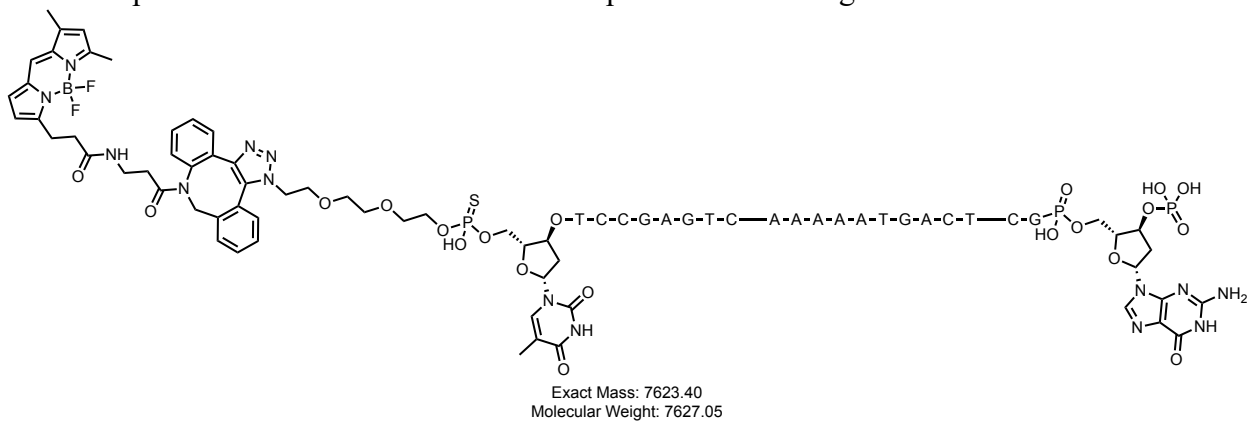
41 SPAAC Reaction:



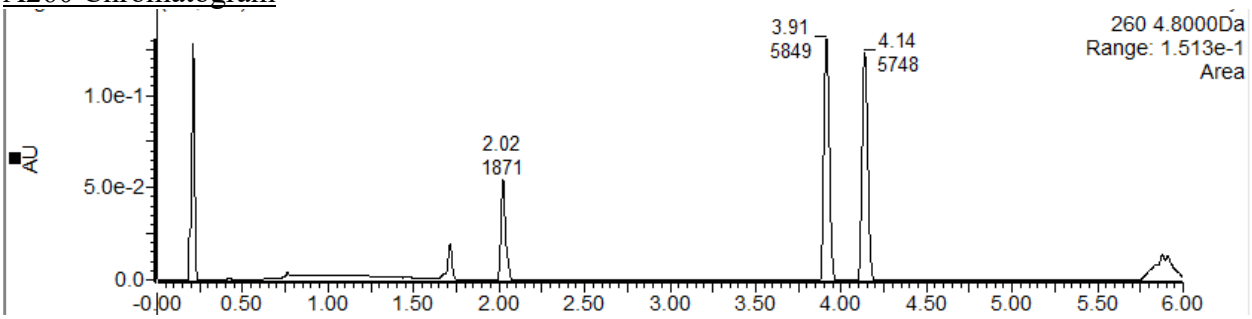
Compound **19** (5 nmol) was synthesized by *General Procedure 2* and isolated crude after ethanol precipitation. Solid **19** was dissolved (98 µL) in tris buffer (50 mM) at pH 8.5. To this solution BDP-FL (2 µL) was added from stock solution (5 mM) in DMA, to a final concentration of 100 µM. This solution was vortexed and transferred to an HPLC vial. This reaction was injected onto the H-Class HPLC after 60 minutes. SPAAC product **41** was characterized below.

LCMS Characterization of 41

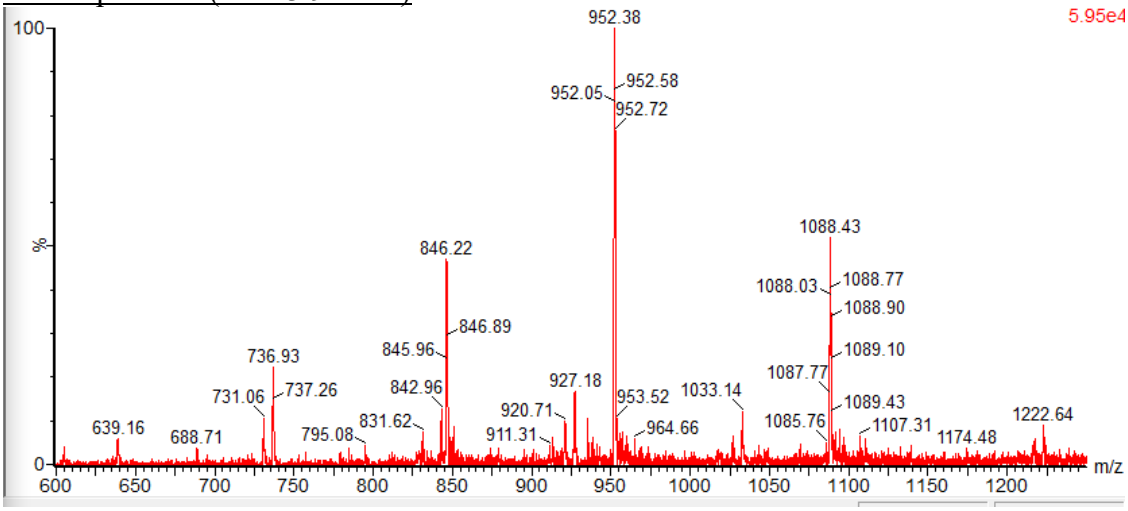
Produced from **19**
Yield (A260): 86%
Expected Mass: 7627.05
Expected (M-8)/8 Ion: 951.925
Found Deconvoluted Mass: 7627.0
Found (M-6)/6 Ion: 952.05
*The two peaks at 3.91and 4.14 minutes correspond to the two regioisomers of **41.**



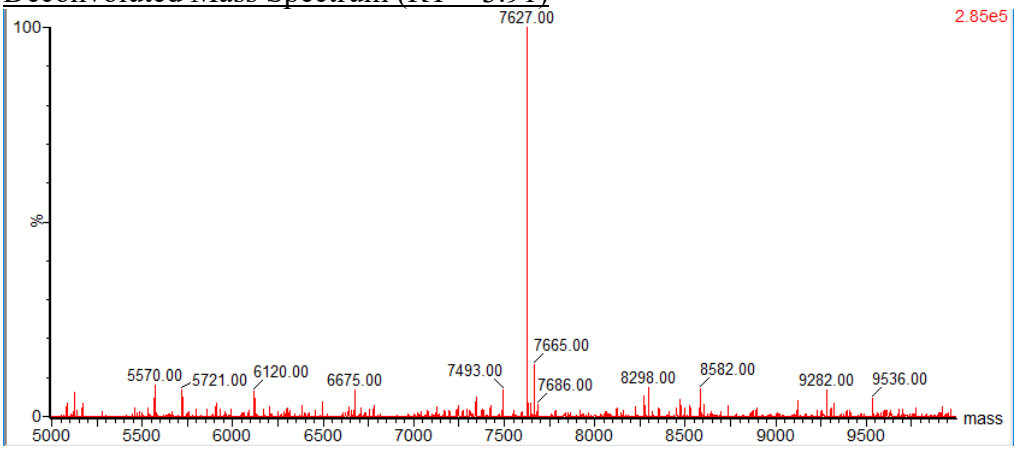
A260 Chromatogram



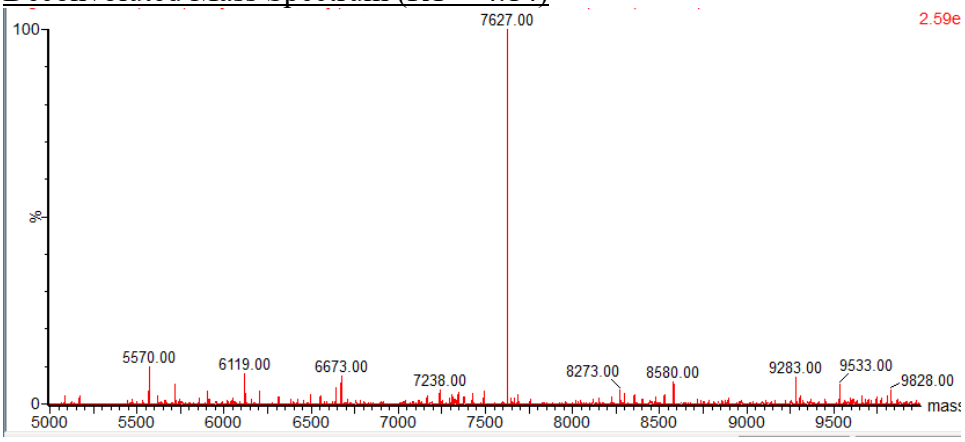
Mass Spectrum (RT= 3.91 min)



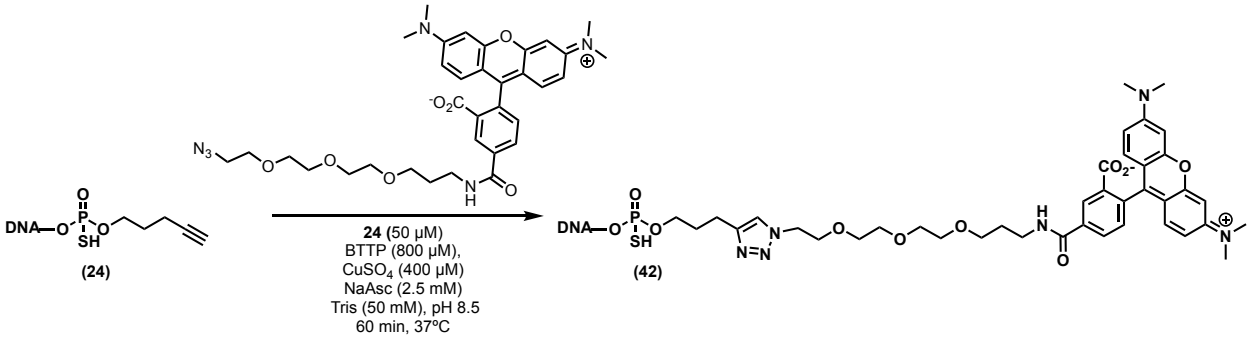
Deconvoluted Mass Spectrum (RT = 3.91)



Deconvoluted Mass Spectrum (RT = 4.14)



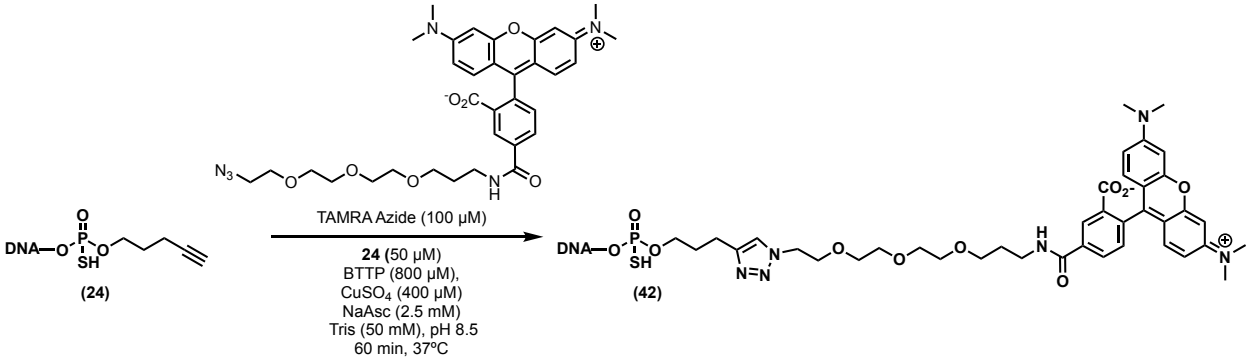
42 CuAAC Reaction:



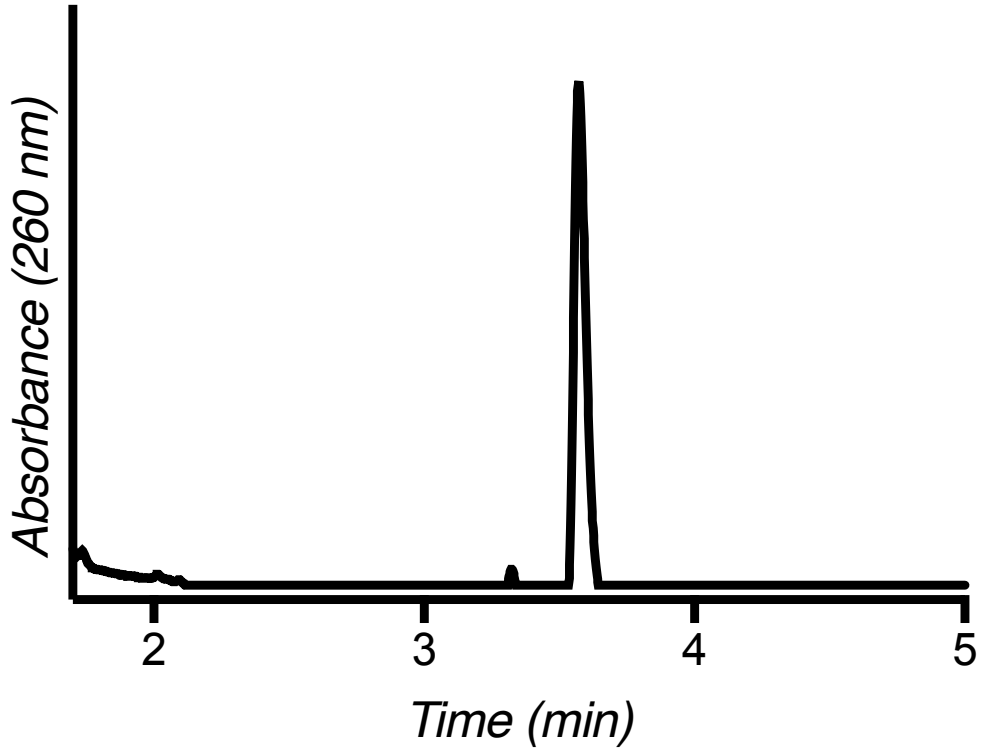
Compound **24** (5 nmol) was synthesized by *General Procedure 2* and isolated crude after ethanol precipitation. Solid **24** was dissolved (92.5 µL) in tris buffer (50 mM) at pH 8.5. To this solution TAMRA Azide (2 µL) was added from stock solution (5 mM) in DMA, (final concentration of 100 µM). Next, BTTP (2 µL) was added from a stock solution (40 mM in water). Next, $CuSO_4$ (1 µL) was added from a stock solution (40 µM in water) (a final concentration of 400 µM). The solution was capped and vortexed. Finally, sodium ascorbate (2.5 uL) was added from a stock solution (100 mM in water). This reaction solution was vortexed and incubated for 1 hour at 37 ºC. This reaction was injected onto the H-Class HPLC after 60 minutes. CuAAC product **42** was characterized below.

LCMS Characterization of 42

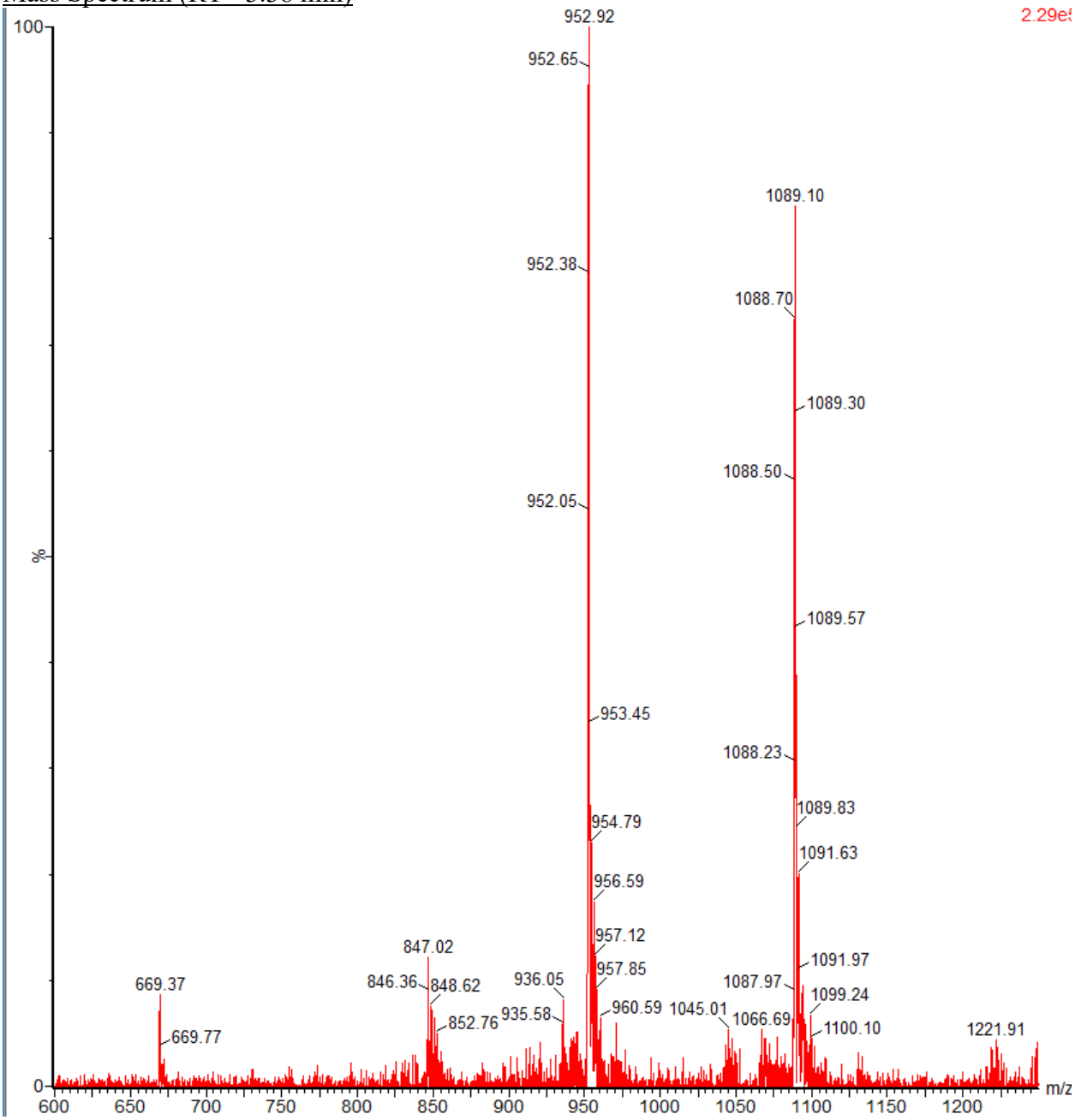
Produced from **24**
Yield (A260): >90%
Expected Mass: 7630.3
Expected (M-8)/8 Ion: 952.75
Found Deconvoluted Mass: 7631.00
Found (M-8)/8 Ion: 952.92



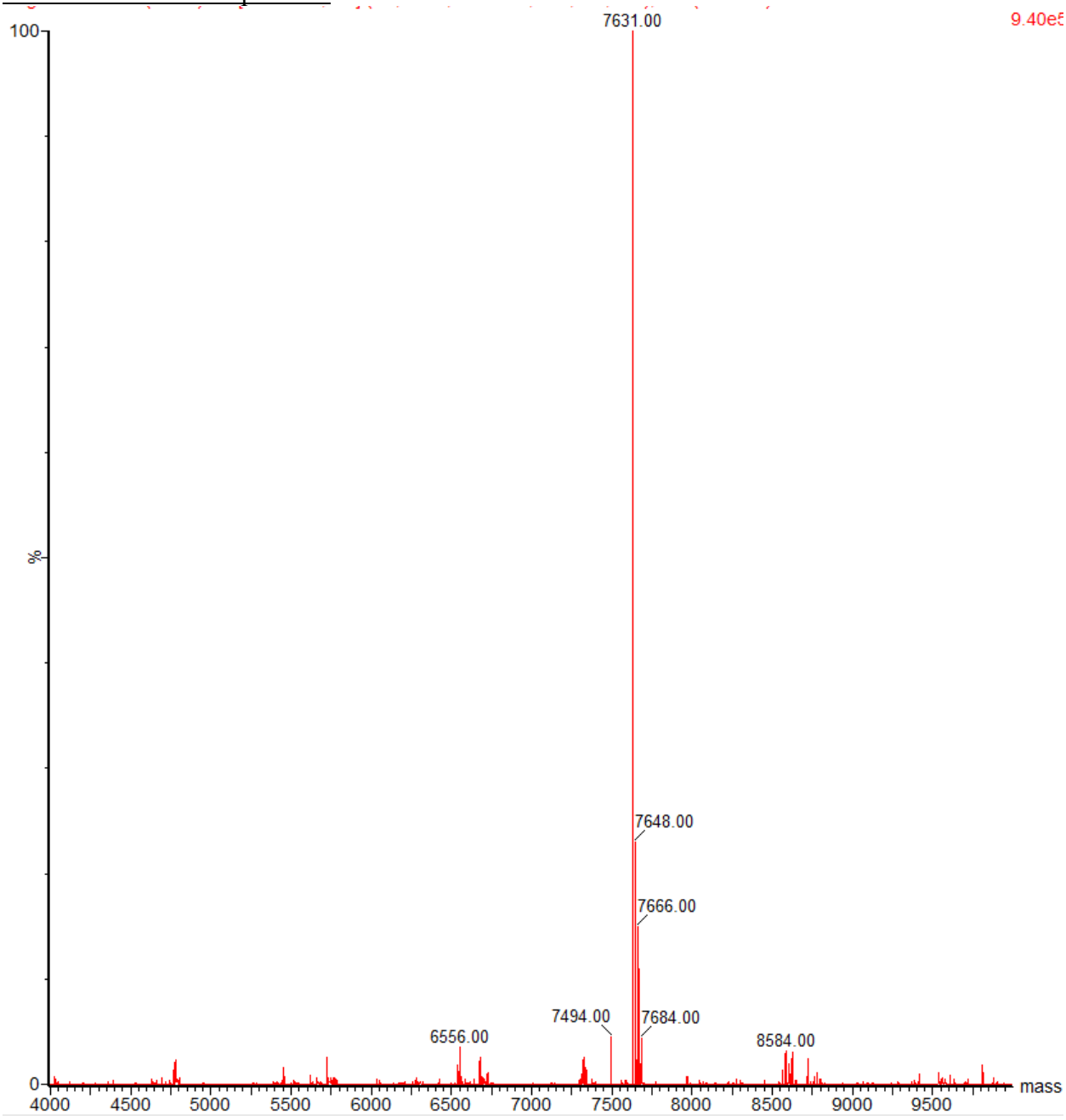
A260 Chromatogram



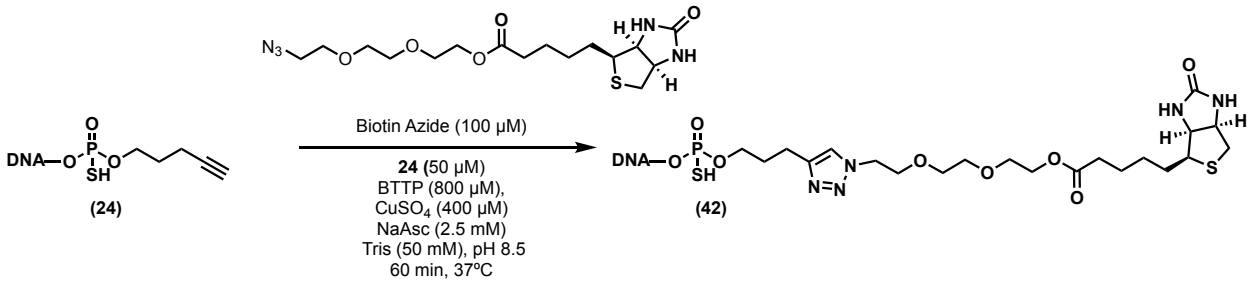
Mass Spectrum (RT= 3.38 min)



Deconvoluted Mass Spectrum



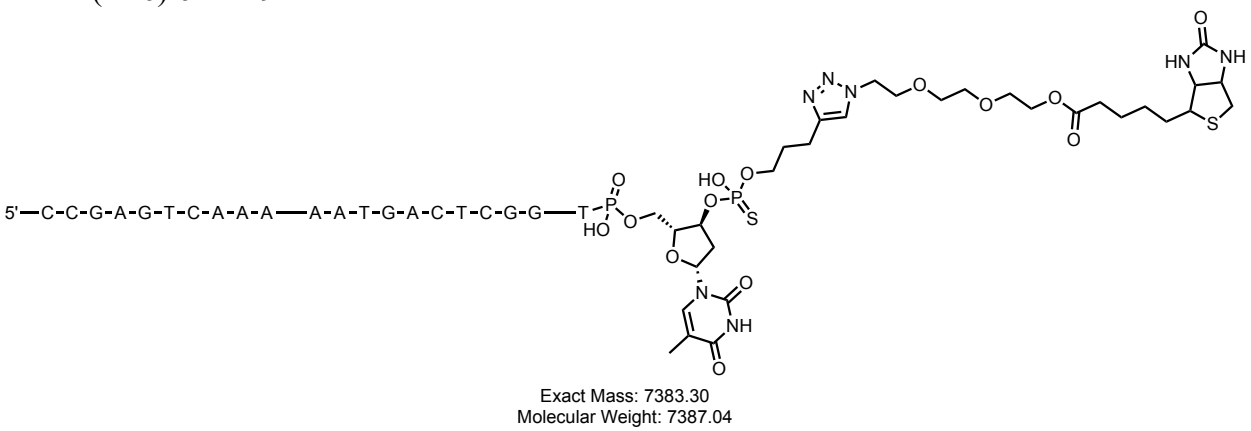
43 CuAAC Reaction:



Compound **24** (5 nmol) was synthesized by *General Procedure 2* and isolated crude after ethanol precipitation. Solid **24** was dissolved (92.5 µL) in tris buffer (50 mM) at pH 8.5. To this solution Biotin Azide (2 µL) was added from stock solution (5 mM) in DMA, (final concentration of 100 µM). Next, BTTP (2 µL) was added from a stock solution (40 mM in water). Next, $CuSO_4$ (1 µL) was added from a stock solution (40 µM in water) (a final concentration of 400 µM). The solution was capped and vortexed. Finally, sodium ascorbate (2.5 uL) was added from a stock solution (100 mM in water). This reaction solution was vortexed and incubated for 1 hour at 37 ºC. This reaction was injected onto the H-Class HPLC after 60 minutes. CuAAC product **43** was characterized below.

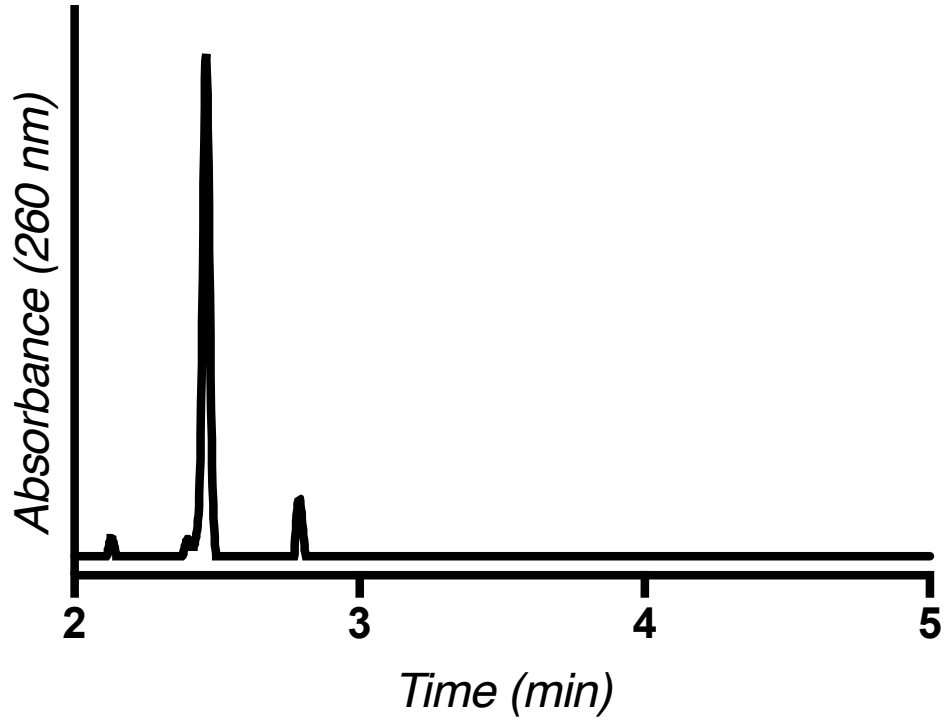
LCMS Characterization of 43

Produced from **24**
Yield (A260): 90%
Expected Mass: 7387.04
Expected (M-8)/8 Ion: 921.9
Found Deconvoluted Mass: 7387
Found (M-6)/6 Ion: 922.4

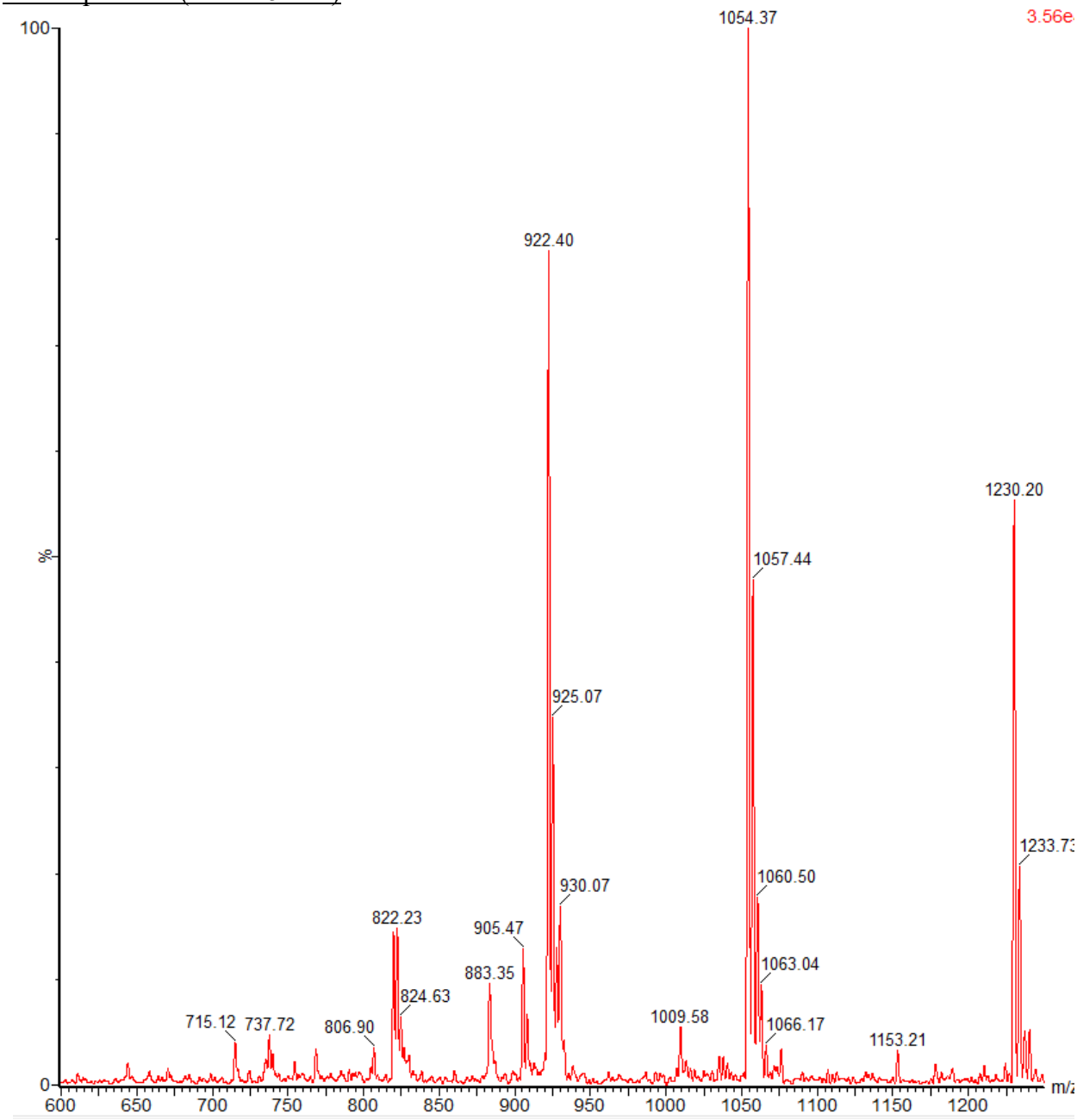


Exact Mass: 7383.30
Molecular Weight: 7387.04

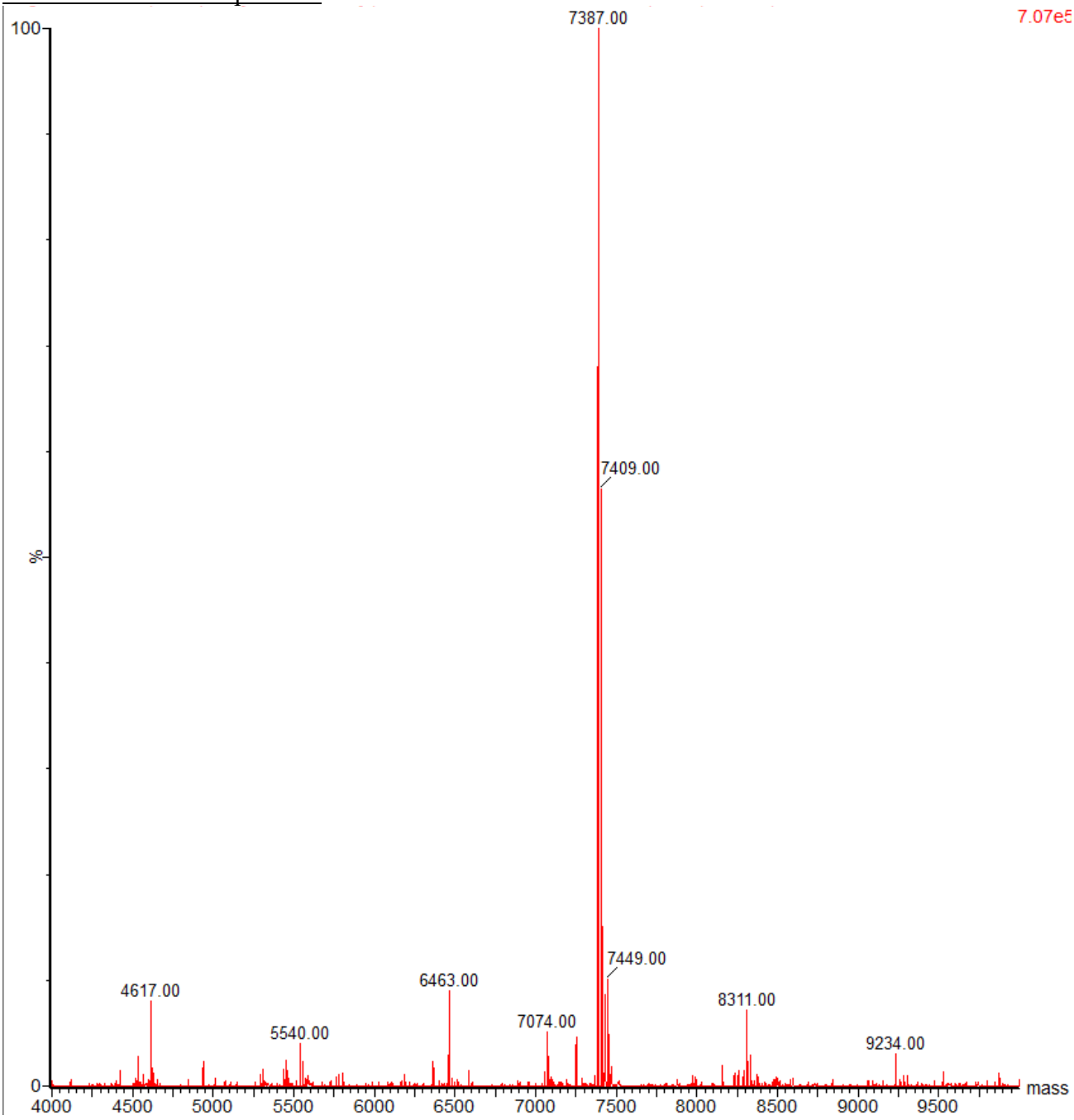
A260 Chromatogram



Mass Spectrum (RT= 2.5 min)



Deconvoluted Mass Spectrum



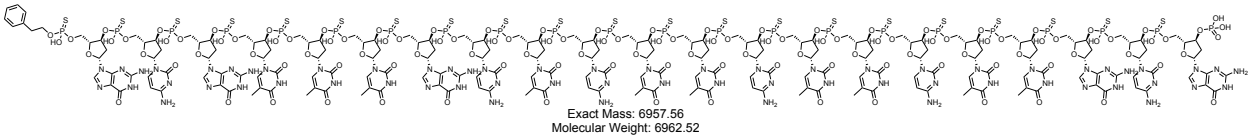
Phosphorothioate (PS) compatibility:

Protocol for SENDR on PS DNA:

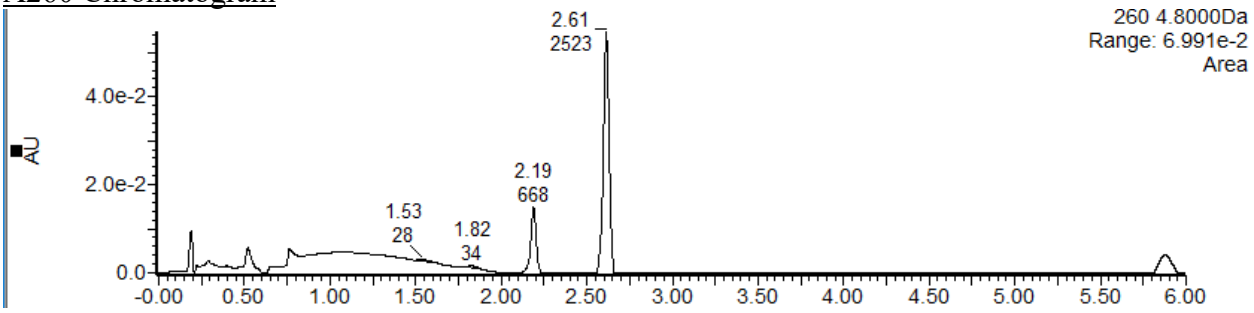
SENDR phsophorothioate (PS) compatibility was analyzed by performing reactions in microcentrifuge tube as described in *General Procedure 2.* Briefly, 5 nmol of PS DNA **44** was loaded onto 100 µL of equilibrated resin. This loaded resin was washed with DMA (500 µL twice) and dry THF (500 µL three times) and dried under vacuum.
To the loaded and dried resin, PSI-module in MeCN (150 mM, 250 µL) was added. Then DBU (450 mM, 18 µL) was added to the reaction mixture. The reaction tube was vortexed for 30 seconds and incubated at 37ºC for 60 minutes. The reactions were worked up by aspirating and discarding the reaction solution, and washing the resin bed with MeCN (500 µL) and 1:1 MeCN:PBS (500 µL). Elute buffer (300 µL) was added to the resin bed and the tube was agitated by orbital shaker for 5-10 minutes. The DNA containing elution buffer was collected by carefully pipetting the supernatant. DNA was isolate *via* ethanol precipitation. Ethanol precipitation was performed by adding 10% v/v of a NaCl solution (30 µL, 5M) to the elute buffer and three volumes of cold ethanol (-20 ºC) were added to the tube (~1000µL) and incubated for 18 hours at -20 ºC. The tube was then centrifuged at 13,000 rpm for 15 minutes to pellet the DNA, and the extra ethanol was decanted off. The tubes were dried *via* speed vacuum, the DNA was dissolved, and HPLC-MS analysis was performed.

LCMS Characterization of 45

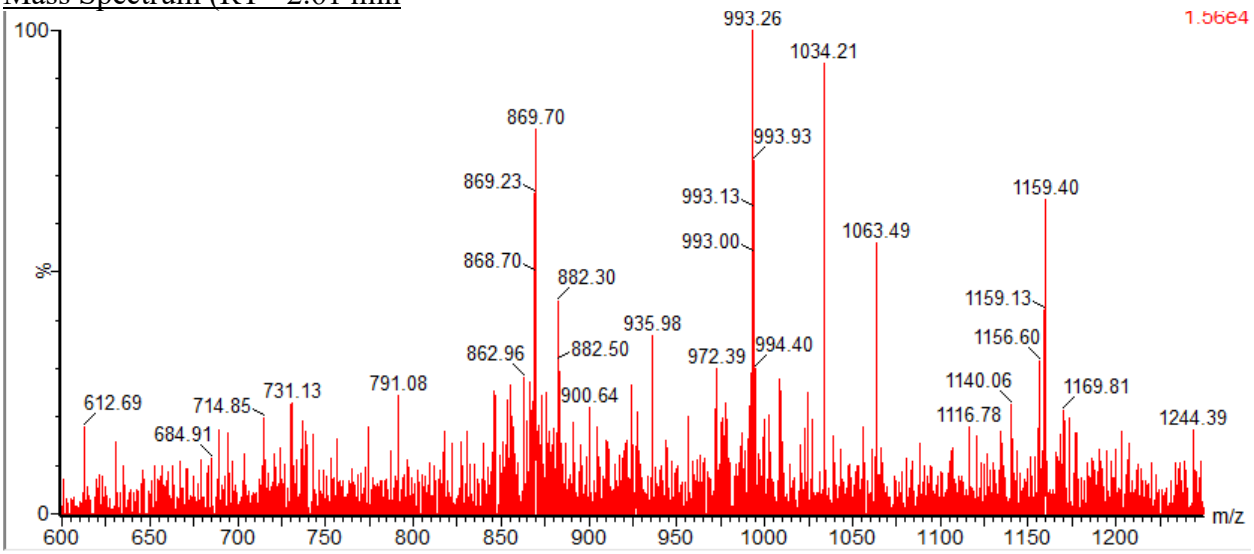
Produced from **44** by General Procedure **2:**
Using **Ψ-1**
Yield (A260): 70%
Expected Mass: 6962.52
Expected (M-6)/6 Ion: 1159.6
Found Deconvoluted Mass: 6963.0
Found (M-6)/6 Ion: 1159.4



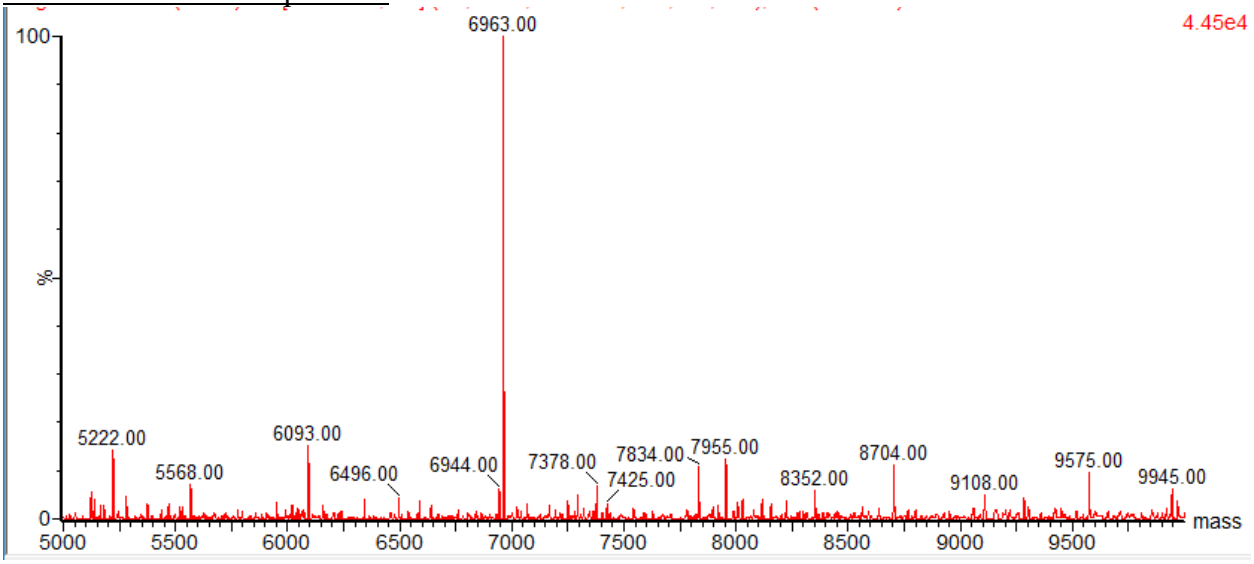
A260 Chromatogram



Mass Spectrum (RT= 2.61 min

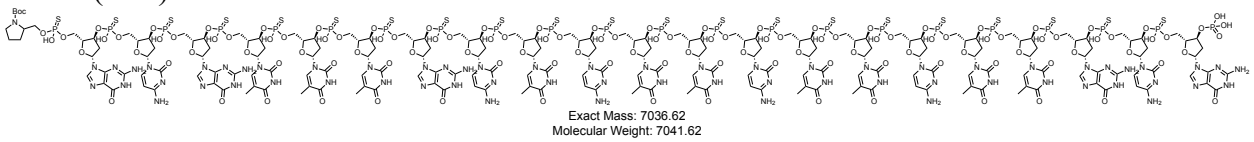


Deconvoluted Mass Spectrum

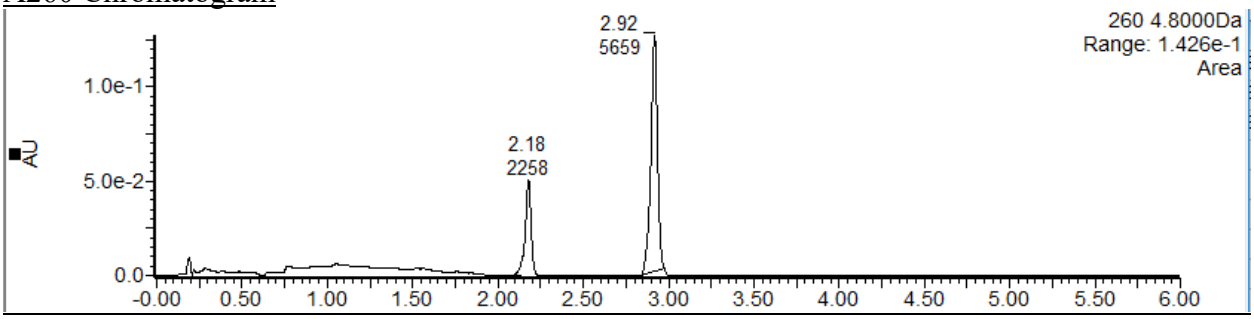


LCMS Characterization of 46

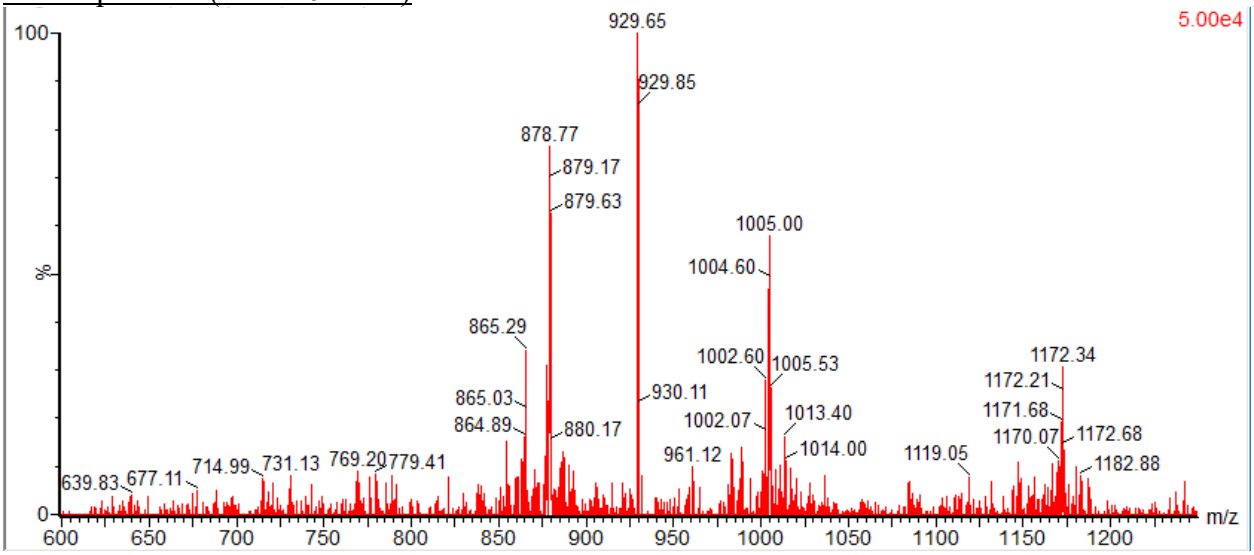
Produced from **44** by General Procedure **2:**
Using **Ψ-2**
Yield (A260): 71%
Expected Mass: 7041.62
Expected (M-6)/6 Ion: 1172.6
Found Deconvoluted Mass: 7040
Found (M-6)/6 Ion: 1172.7



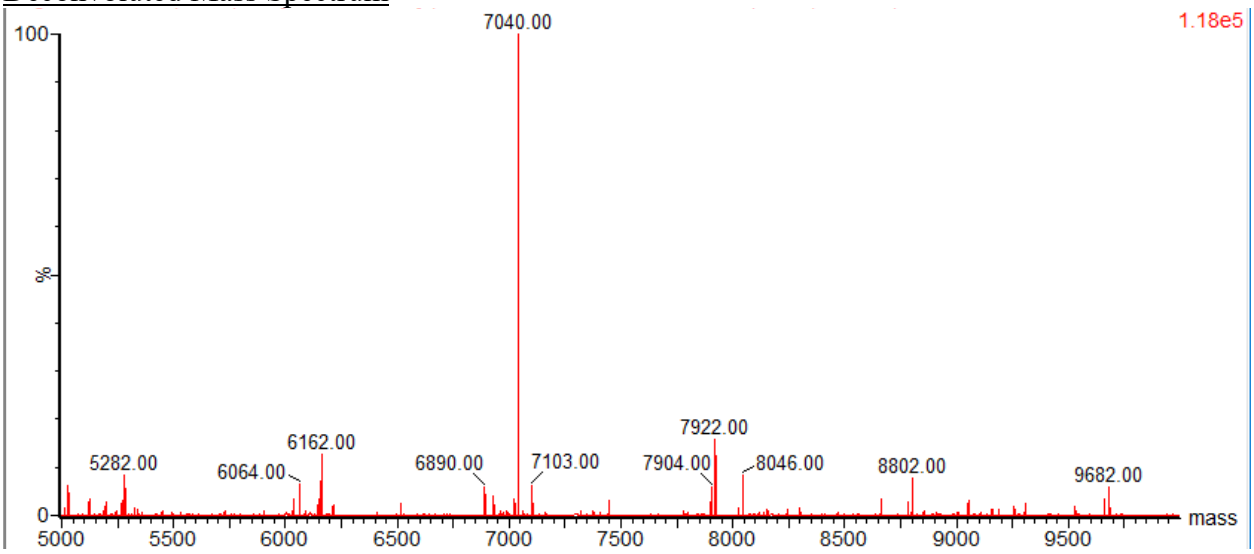
A260 Chromatogram



Mass Spectrum (RT= 2.92 min)

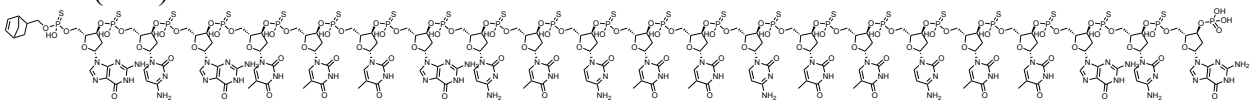


Deconvoluted Mass Spectrum

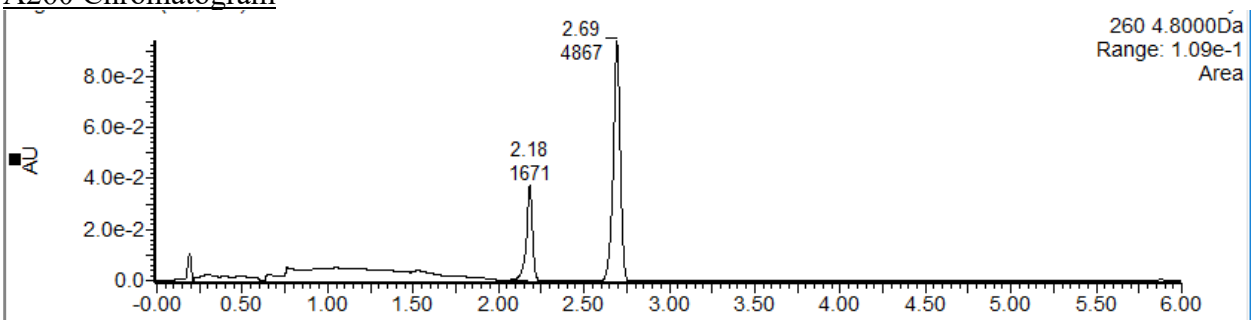


LCMS Characterization of 47

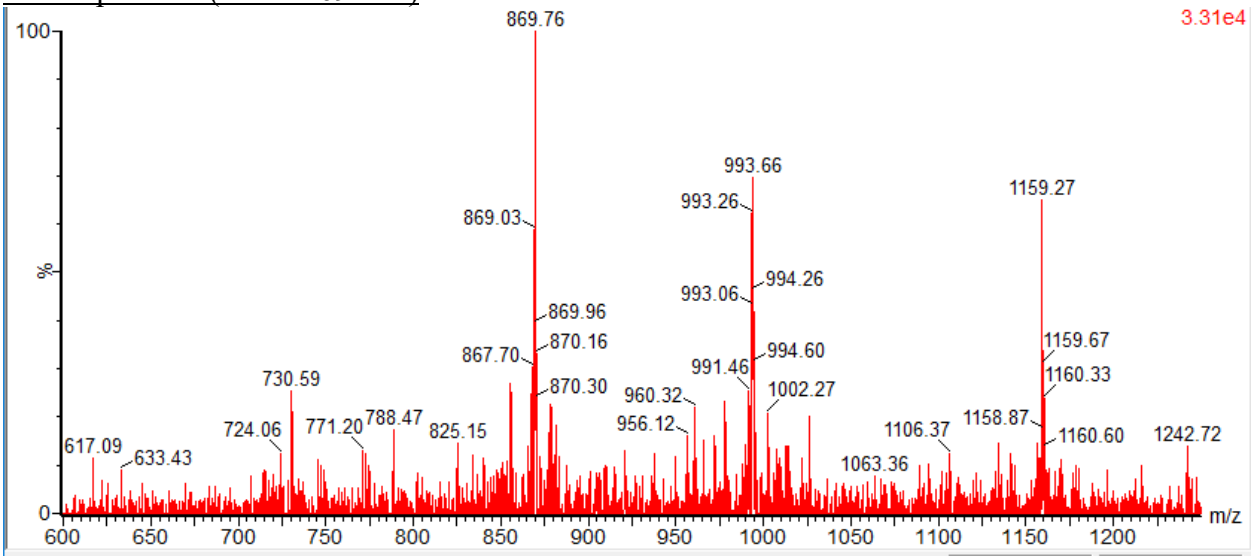
Produced from **44** by General Procedure **2:**
Using **Ψ-12**
Yield (A260): 74%
Expected Mass: 6964.54
Expected (M-6)/6 Ion: 1158.9
Found Deconvoluted Mass: 6964.0
Found (M-6)/6 Ion: 1159.2



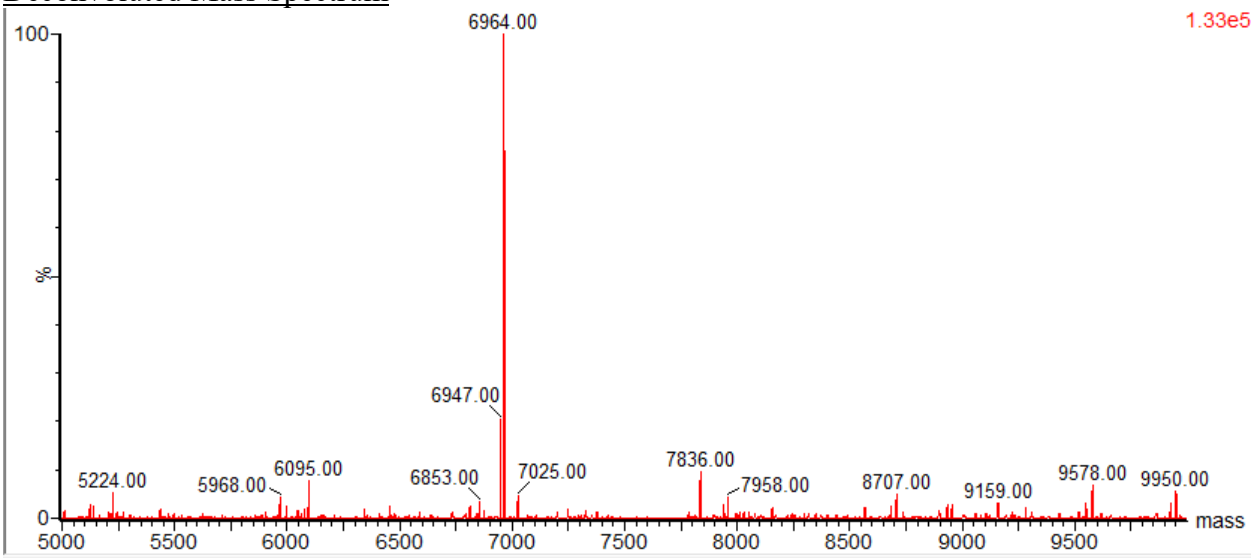
A260 Chromatogram



Mass Spectrum (RT = 2.69 min)

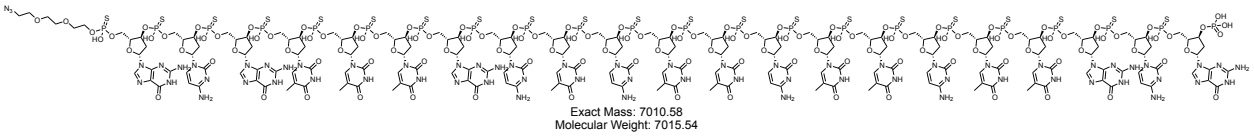


Deconvoluted Mass Spectrum

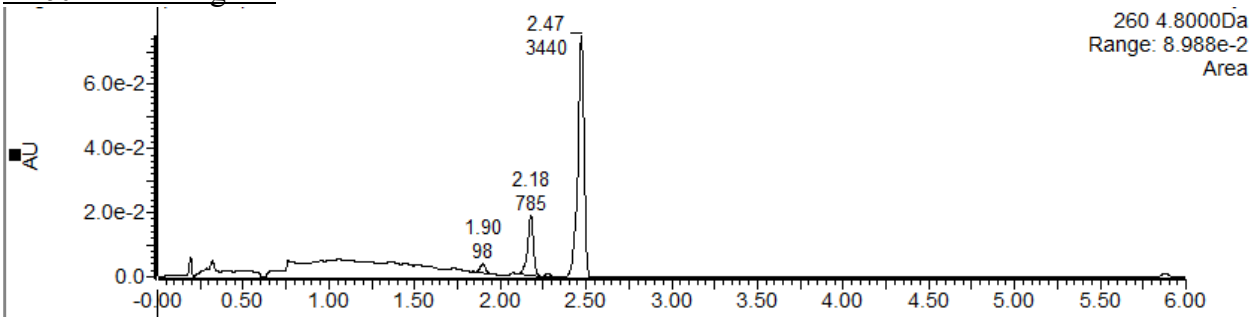


LCMS Characterization of 48

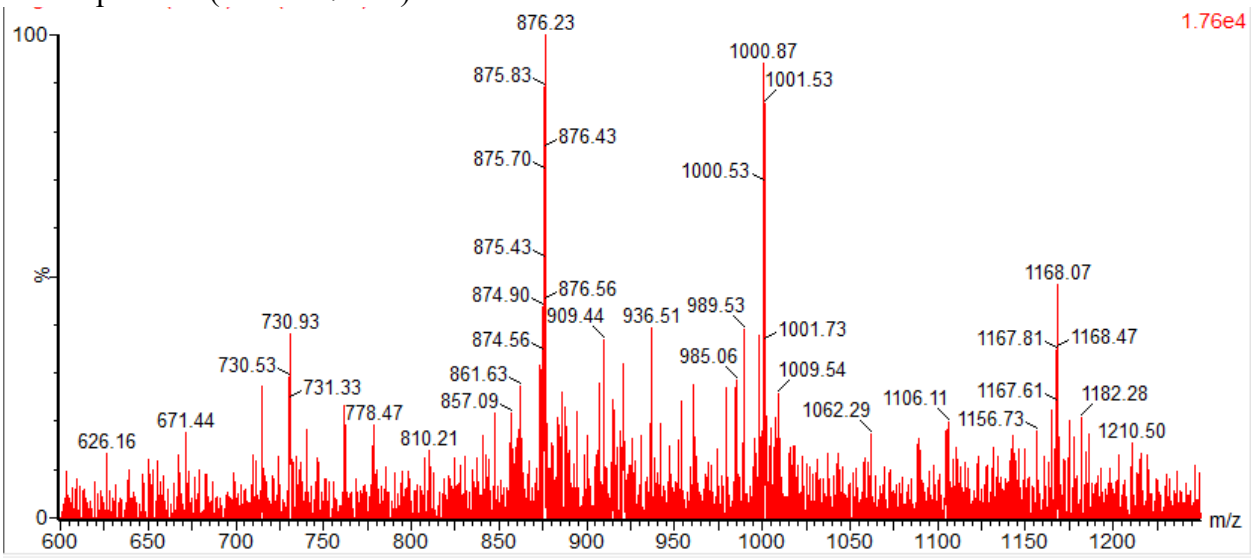
Produced from **44** by General Procedure **2:**
Using **Ψ-3**
Yield (A260): 81%
Expected Mass: 7015.54
Expected (M-6)/6 Ion: 1168.3
Found Deconvoluted Mass: 7015
Found (M-6)/6 Ion: 1168.07



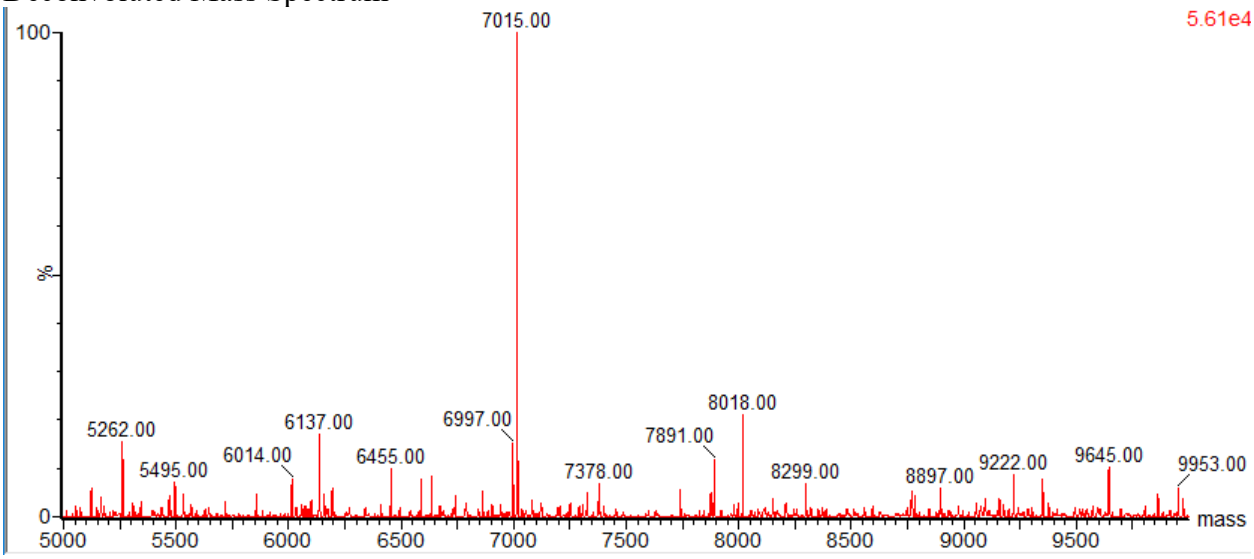
A260 Chromatogram



Mass Spectrum (RT= 2.47 min)



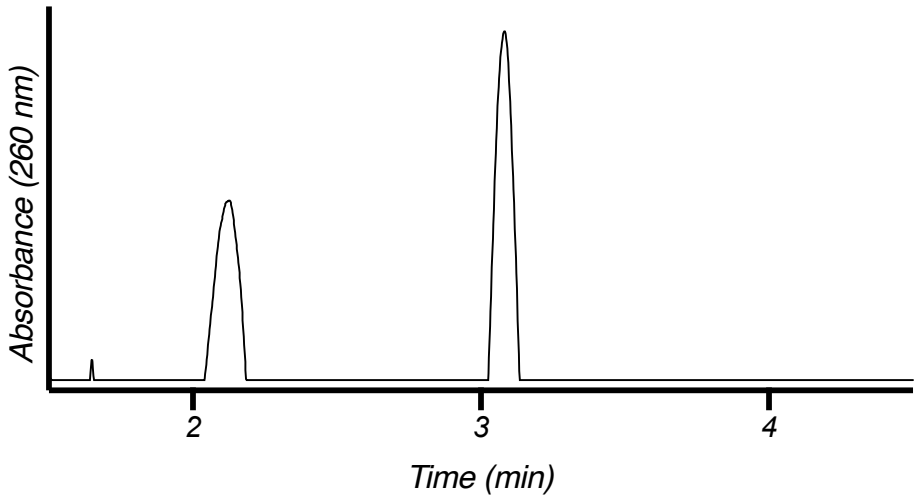
Deconvoluted Mass Spectrum



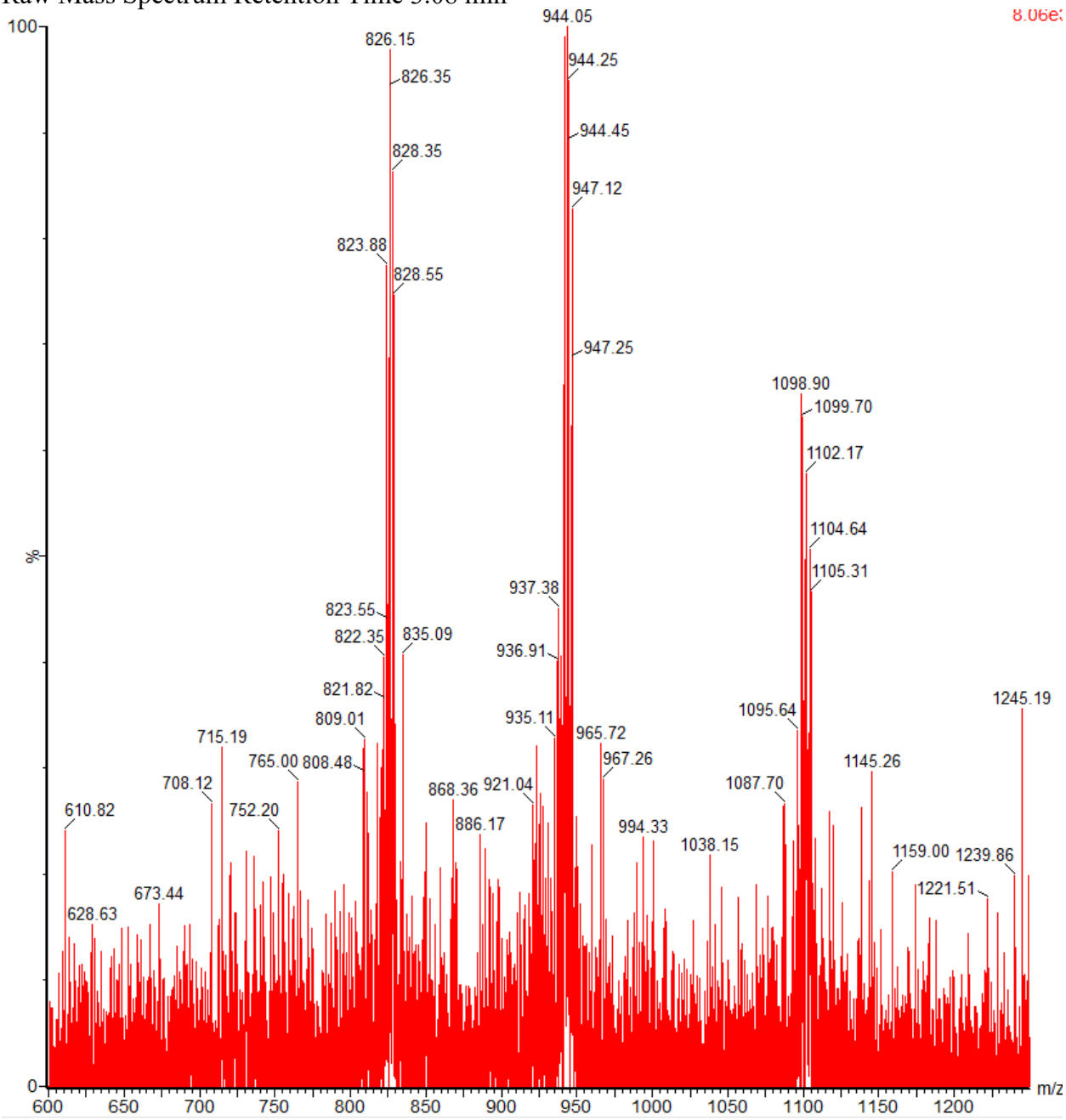
LCMS Characterization of 50

Produced from **49** by General Procedure **3:**
Using **Ψ-16**
Yield (A260): 66%
Expected Mass: 6604
Expected (M-6)/6 Ion: 1099.6
Found Deconvoluted Mass: 6601.0
Found (M-6)/6 Ion: 1099.7

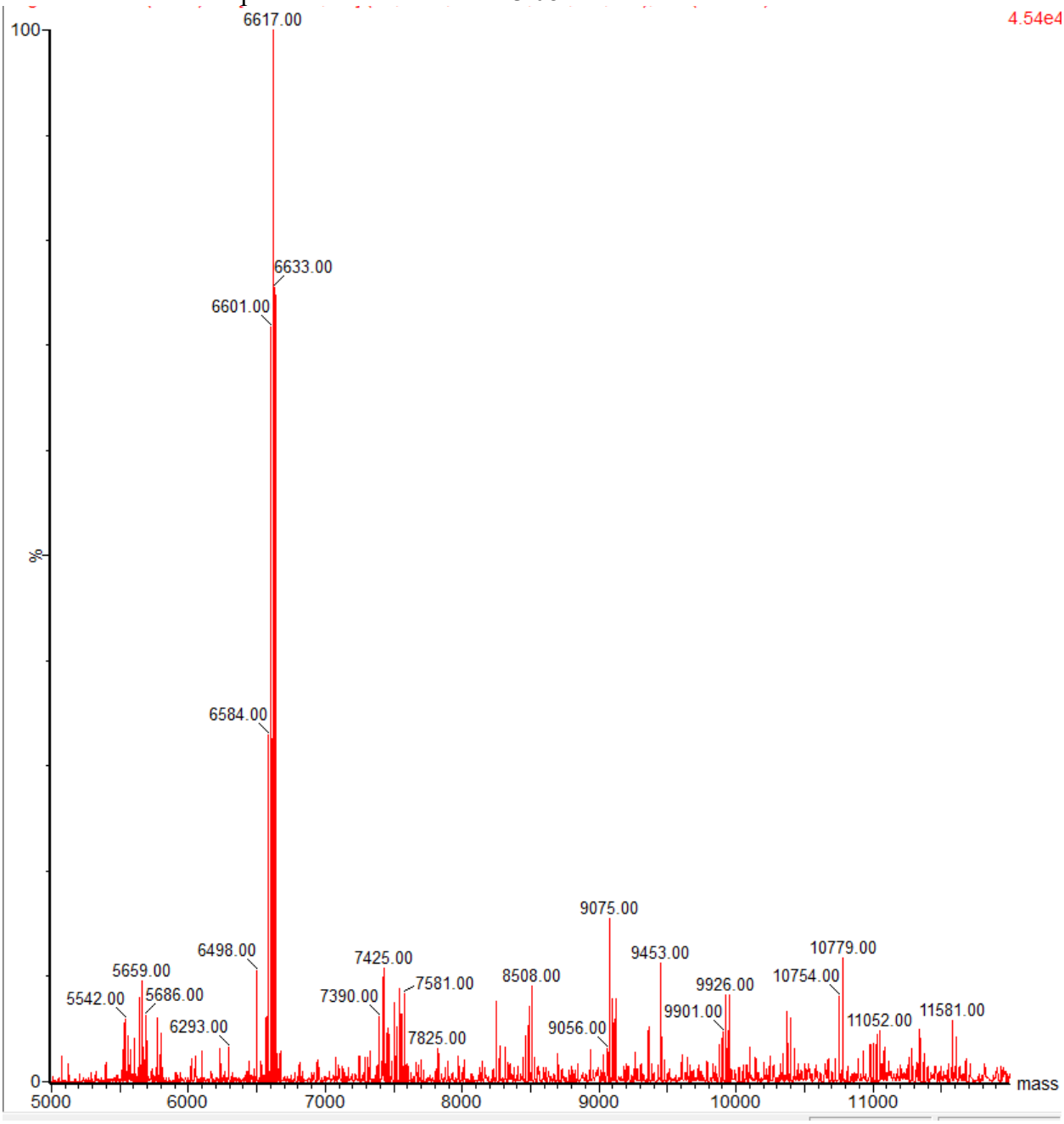
MW: 6616



Raw Mass Spectrum Retention Time 3.08 min

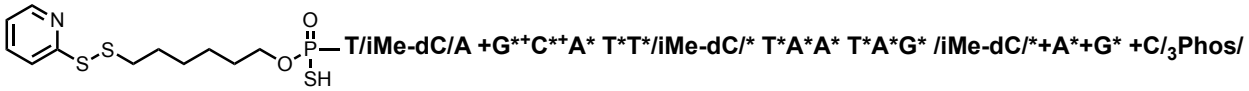


Deconvultued Mass Spectrum Retention Time 3.08 Minutes



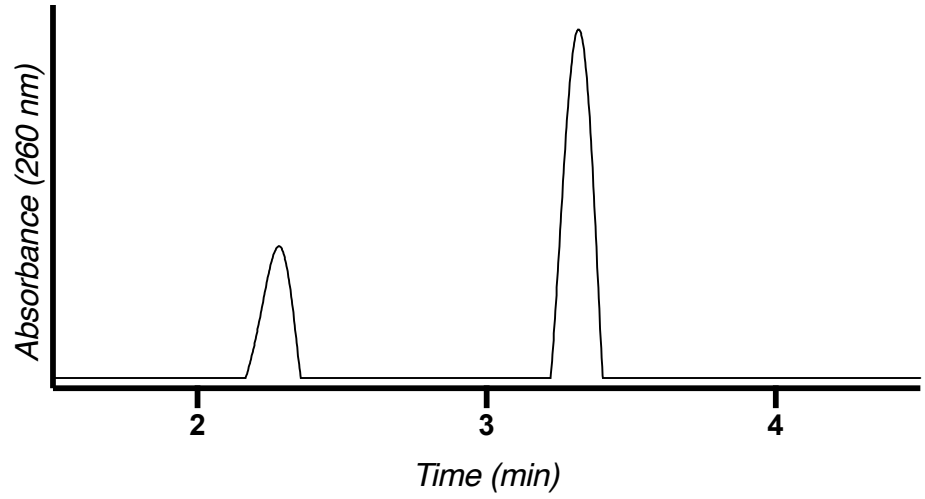
LCMS Characterization of 51

Produced from **49** by General Procedure **3:**
Using **Ψ-8**
Yield (A260): 68%
Expected Mass: 6632
Expected (M-8)/8 Ion: 828
Found Deconvoluted Mass: 6636.0
Found (M-8)/6 Ion: 828.42

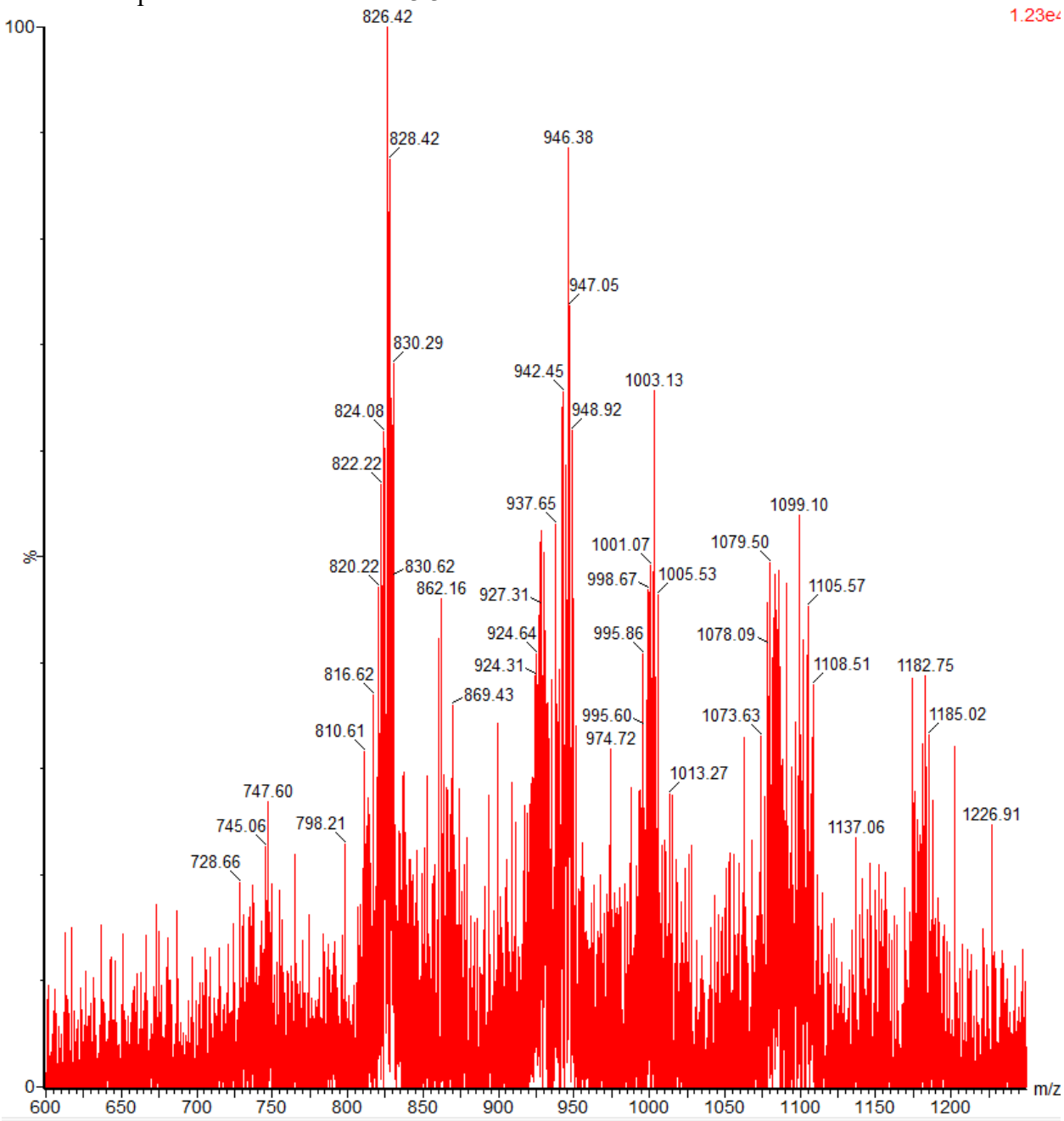


MW: 6632

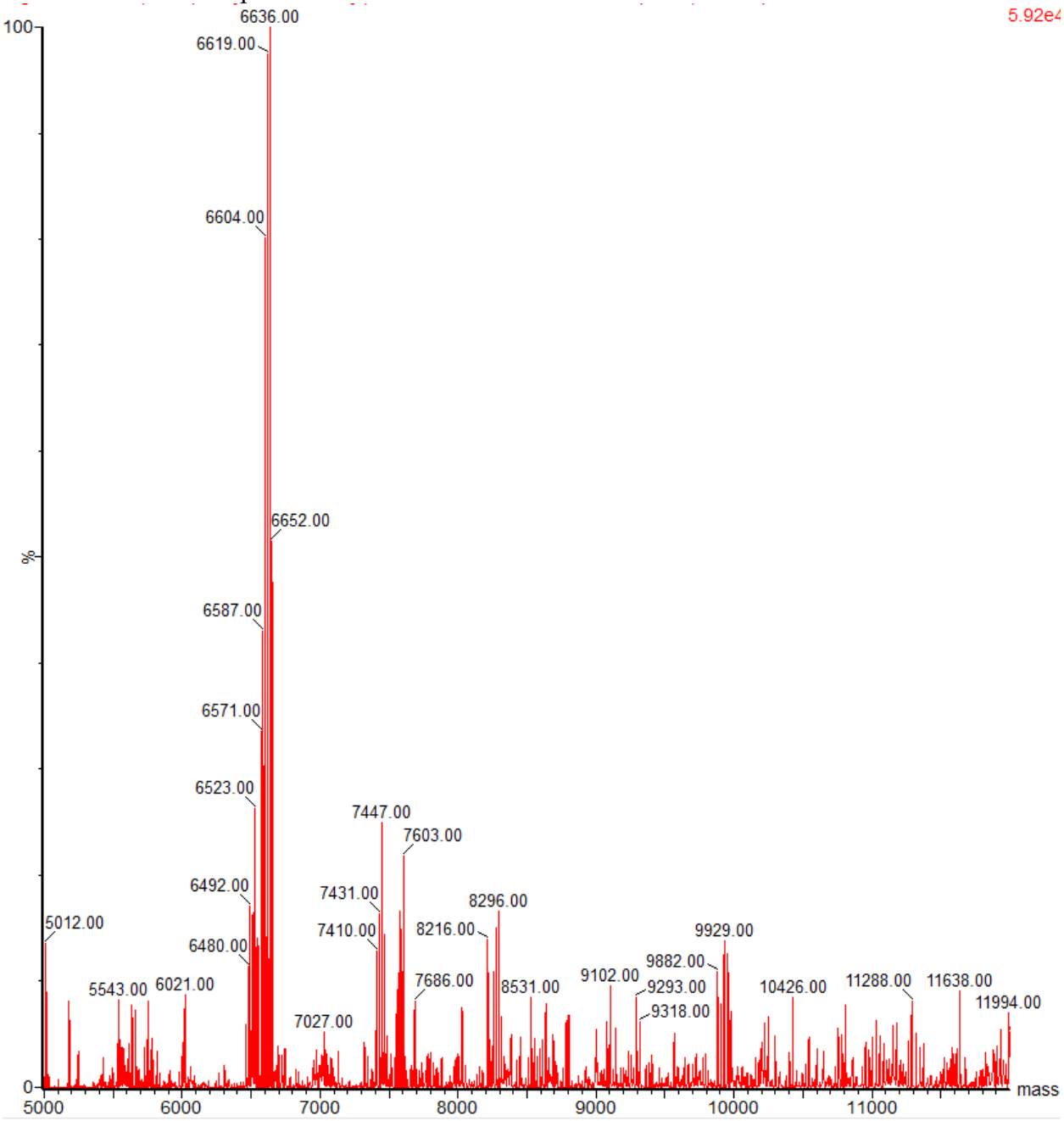
A260



Raw Mass Spectrum Retention Time 3.32 Minutes



Deconvoluted Mass Spectrum Retention Time 3.32 Minutes



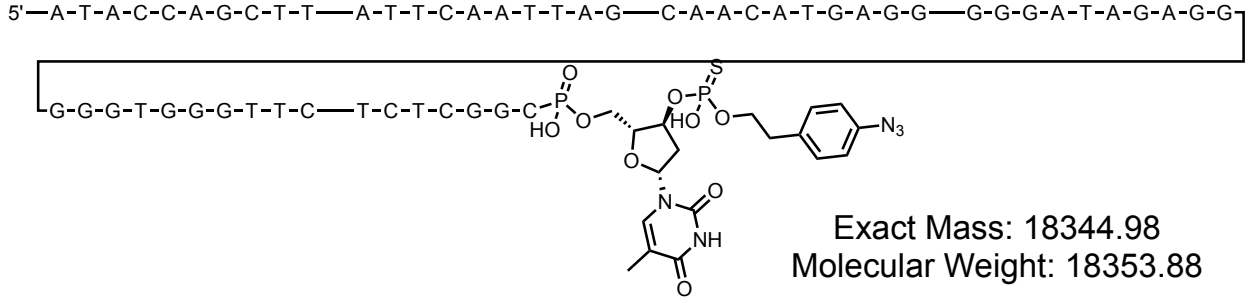
SENDR on Aptamers:

Aptamer compatibility protocol:

SENDR Aptamer compatibility was analyzed by performing reactions in microcentrifuge tube as described in *General Procedure 2.* Briefly, 5 nmol of aptamer **52** was loaded onto 100 µL of equilibrated resin. This loaded resin was washed with DMA (500 µL twice) and dry THF (500 µL three times) and dried under vacuum.
To the loaded and dried resin, Using **Ψ-4** in MeCN (150 mM, 250 µL) was added. Then DBU (450 mM, 18 µL) was added to the reaction mixture. The reaction tube was vortexed for 30 seconds and incubated at 37ºC for 60 minutes. The reactions were worked up by aspirating and discarding the reaction solution, and washing the resin bed with MeCN (500 µL) and 1:1 MeCN:PBS (500 µL). Elute buffer (300 µL) was added to the resin bed and the tube was agitated by orbital shaker for 5-10 minutes. The DNA containing elution buffer was collected. DNA was isolate *via* ethanol precipitation. Ethanol precipitation was performed by adding 10% v/v of a NaCl solution (30 µL, 5M) to the elute buffer and three volumes of cold ethanol (-20 ºC) were added to the tube (~1000µL) and incubated for 18 hours at -20 ºC. The tube was then centrifuged at 13,000 rpm for 15 minutes to pellet the DNA, and the extra ethanol was decanted off. The tubes were dried *via* speed vacuum and the DNA was dissolved and HPLC-MS analysis was performed.

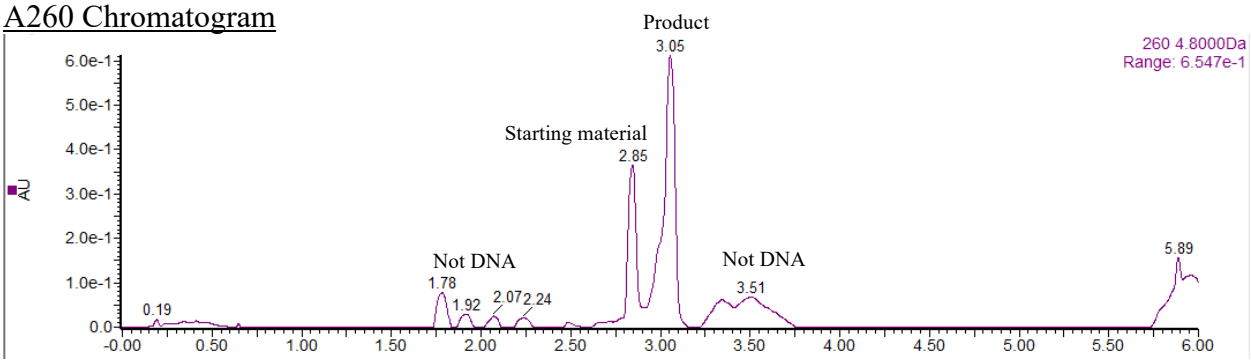
LCMS Characterization of 53

Produced from **52** by General Procedure **2:**
Using **Ψ-4**
Yield (A260): 70%
Expected Mass: 18353.88
Expected (M-15)/15 Ion: 1221.99
Found Deconvoluted Mass: 18350
Found (M-6)/6 Ion: 1221.84

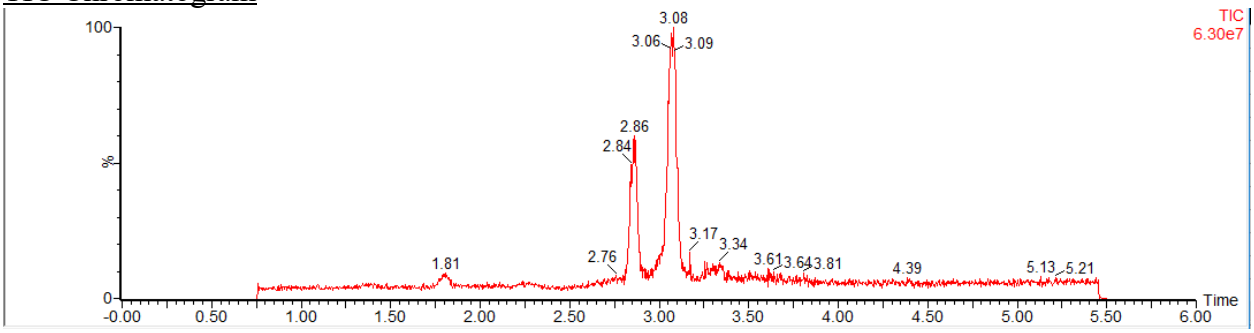


Exact Mass: 18344.98
Molecular Weight: 18353.88

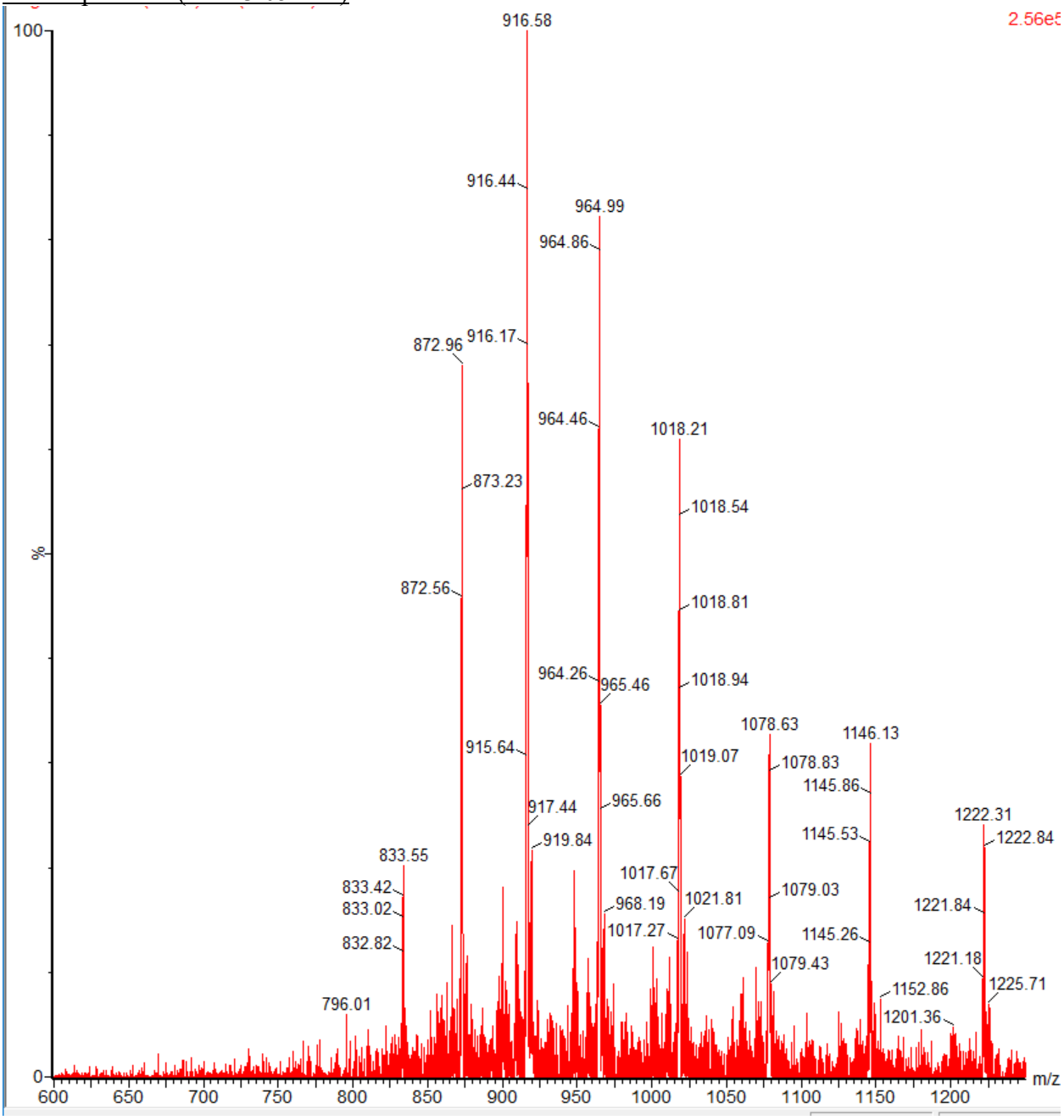
A260 Chromatogram



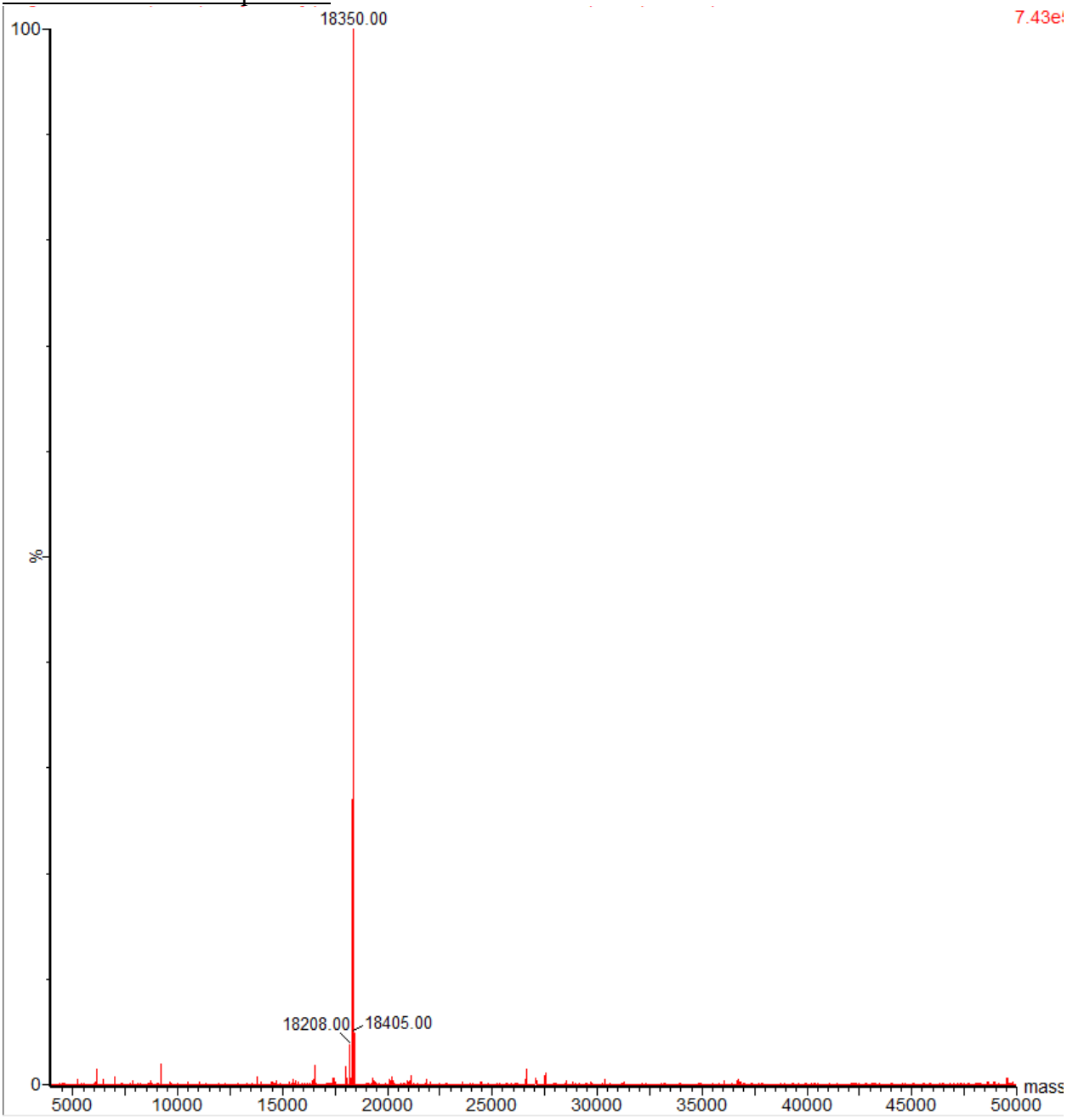
TIC Chromatogram



Mass Spectrum (RT= 3.05 min)



Deconvoluted Mass Spectrum



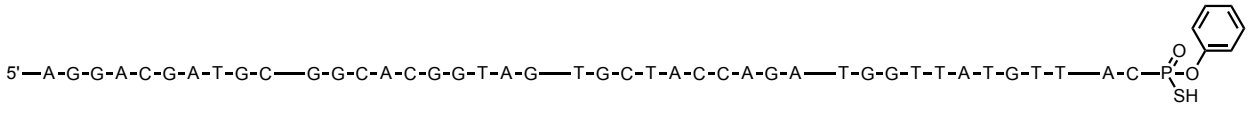
Synthesis of Electrophile Modified hNE Aptamers **R/S-(55)**

Aptamer **54** was used in two SENDR protocols according to General Procedure 3 with both enantiomers of **Ψ-14.** Reaction conditions were slightly altered to increase conversion, PSI was used at 150 mM and the reaction was run at 37ºC for 60 minutes. Modified aptamers **R-(55)** and **S-(55)** were isolated by ethanol precipitation. After ethanol precipitation unlabeled aptamer **54** was selectively degraded by ExoIII. In short, 10 ug of **S-(55)** or **R-(55)** were loaded into a PCR tube in 40 μL water. To each tube CutSmart® (5 μL, 10x), water (3 μL), and ExoIII (2μL) was added. The reactions were vortexed and incubated at 37ºC for 60 minutes at which point the ExoIII was thermally inactivated at 75ºC for 10 minutes. The resulting DNA constructs were isolated by ZymoSpin oligo clean and concentrator column.

Analysis of **S-55**

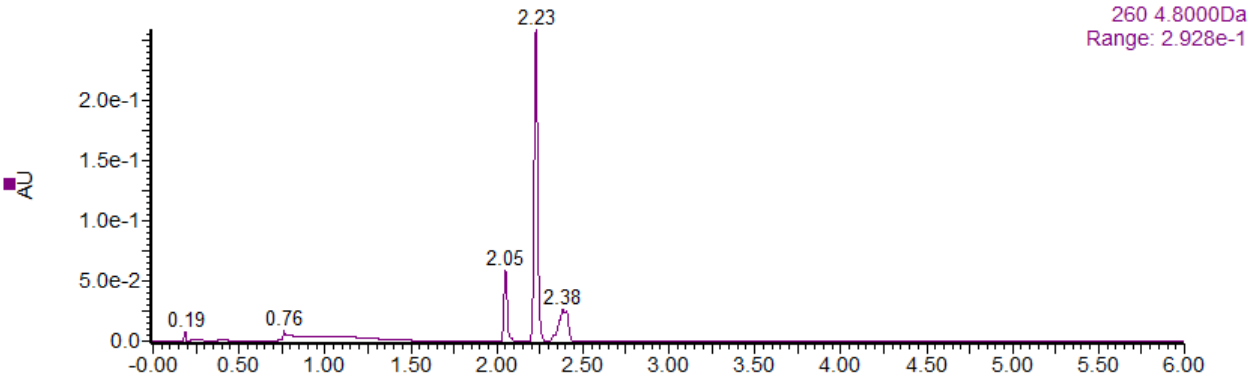
LCMS Characterization of S-55/ After SENDR

Produced from **54** by General Procedure **2:**
Using **(-)Ψ-14**
Yield (A260): 76%
Expected Mass: 13286.6
Found Deconvoluted Mass: 13288.0

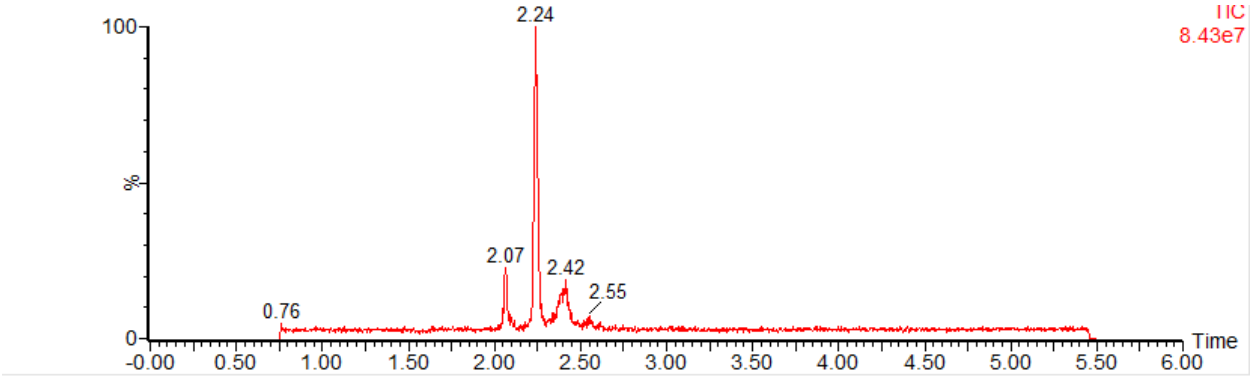


Exact Mass: 13280.13
Molecular Weight: 13286.59

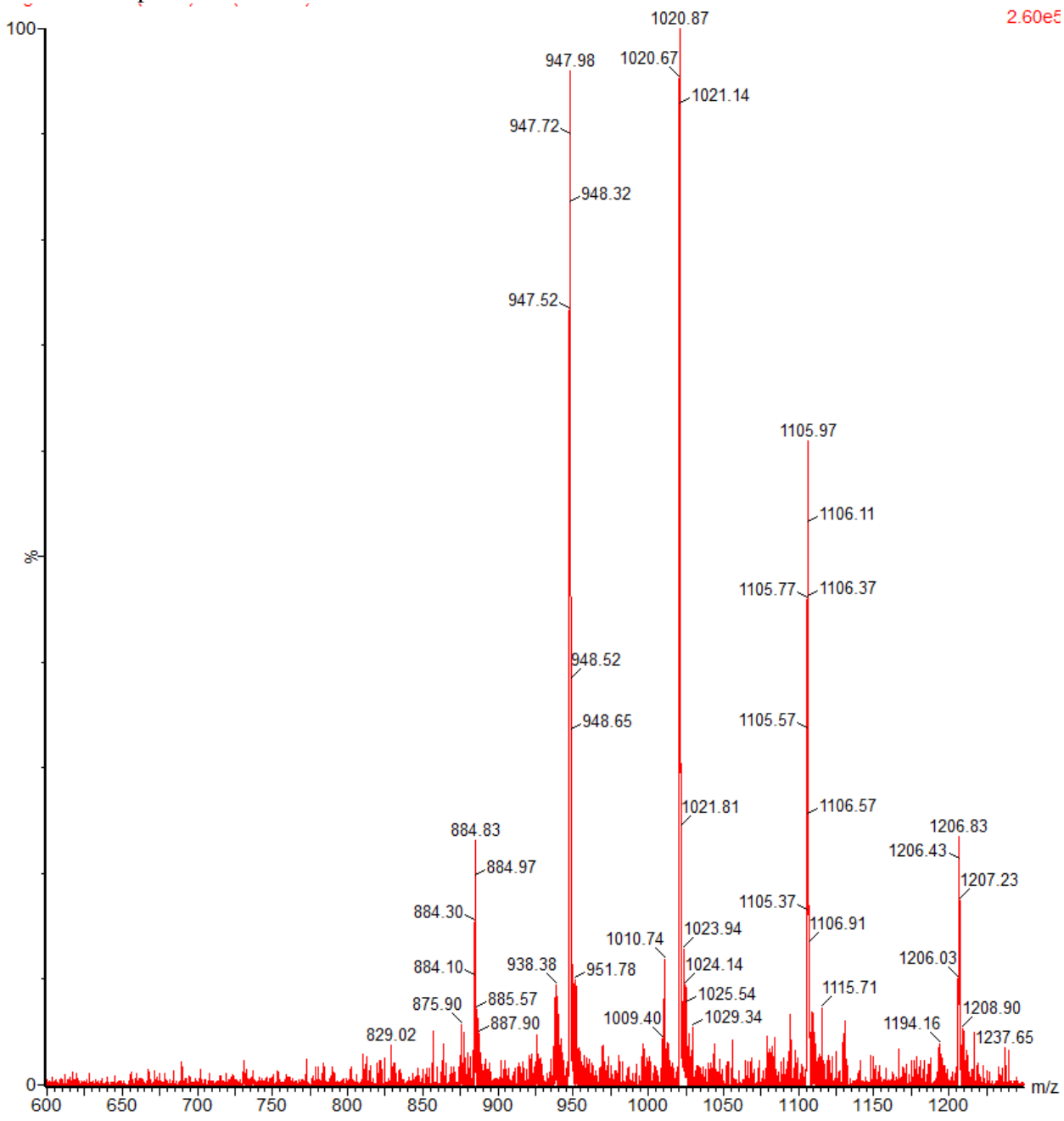
A260



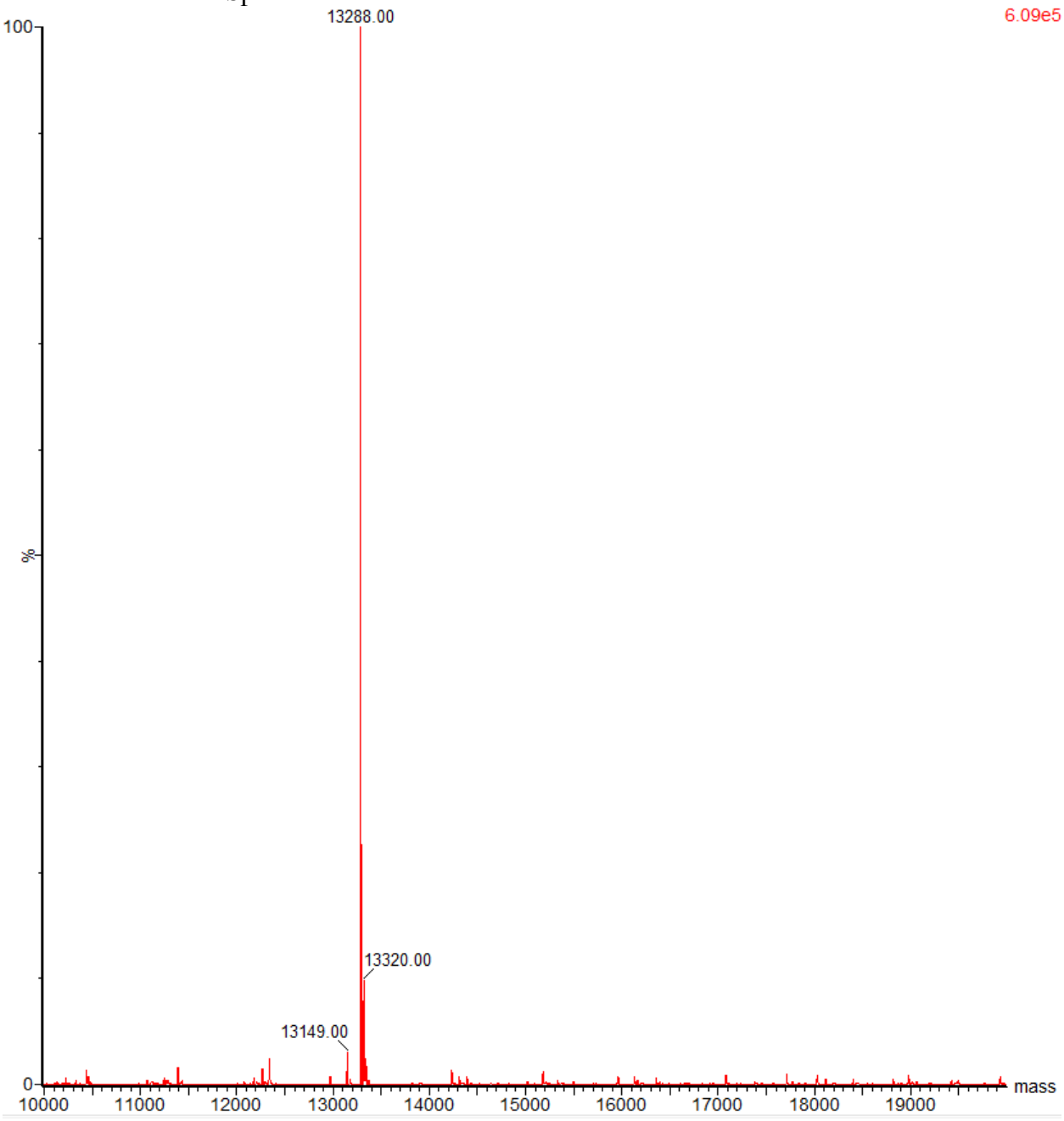
TIC



Raw Mass Spectrum RT = 2.24



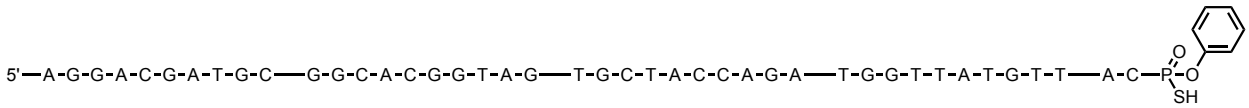
Deconvoluted Mass Spectrum



Analysis of **S-55**

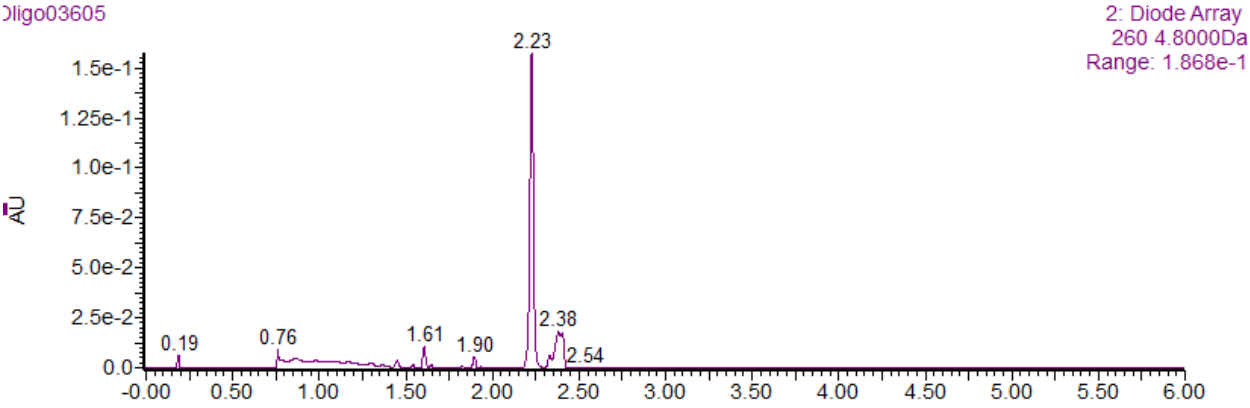
LCMS Characterization of S55/ After ExoIII Digestion

Using **(-)Ψ-14**
Yield (A260): 91%
Expected Mass: 13286.6
Found Deconvoluted Mass: 13287.0

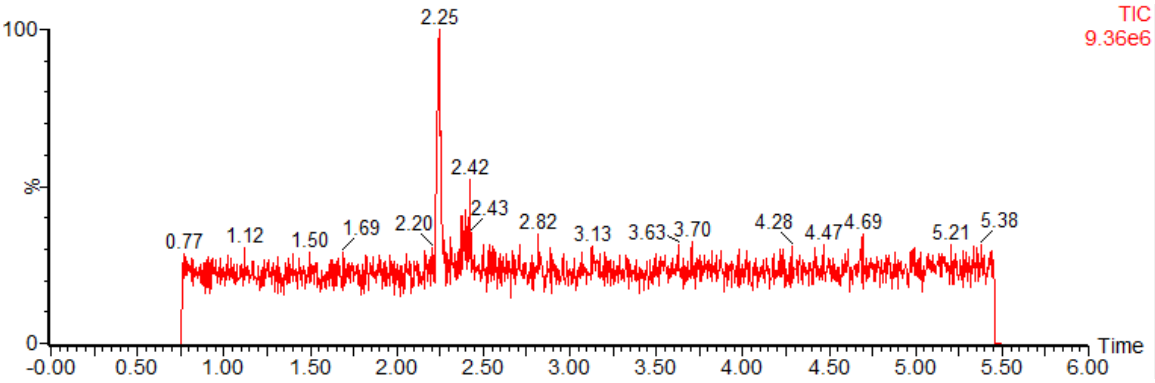


Exact Mass: 13280.13
Molecular Weight: 13286.59

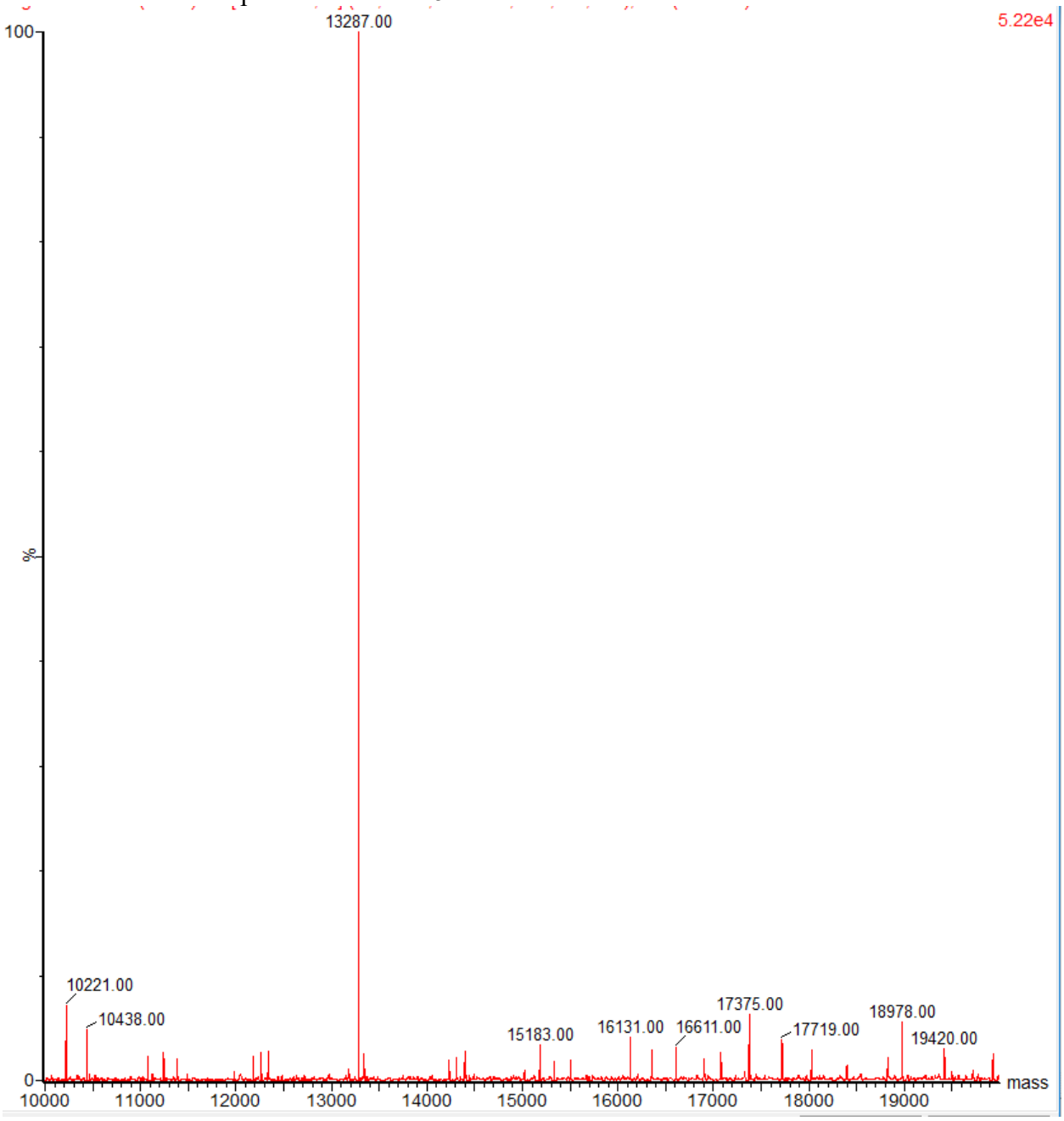
A260



TIC



Deconvoluted Mass Spectrum RT = 2.25



Analysis of **R-55**

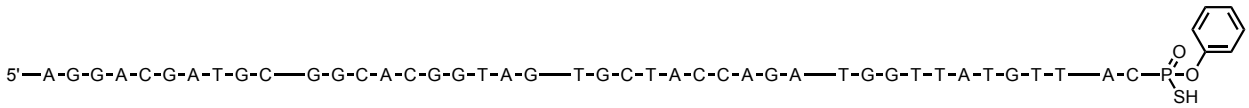
LCMS Characterization of R-55/ SEDNR

Using **(+)Ψ-14**

Yield (A260): 63%

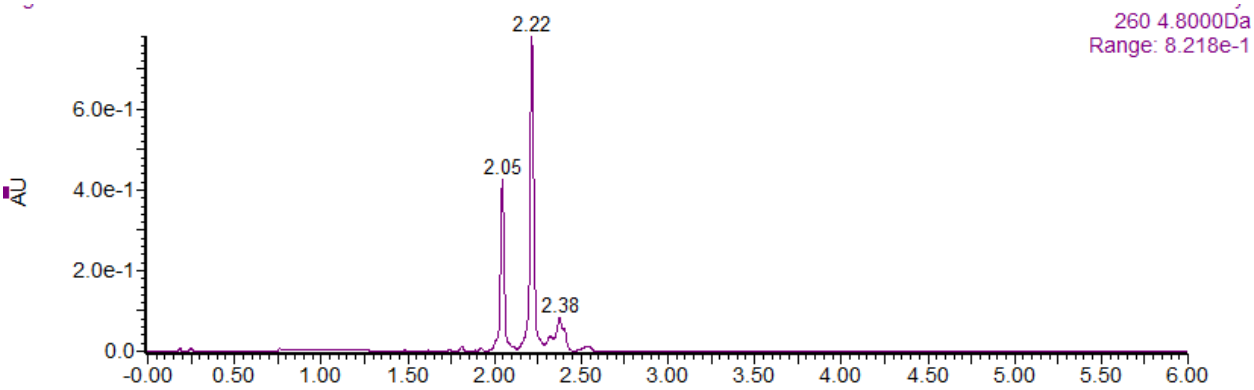
Expected Mass: 13286.6

Found Deconvoluted Mass: 13286.0

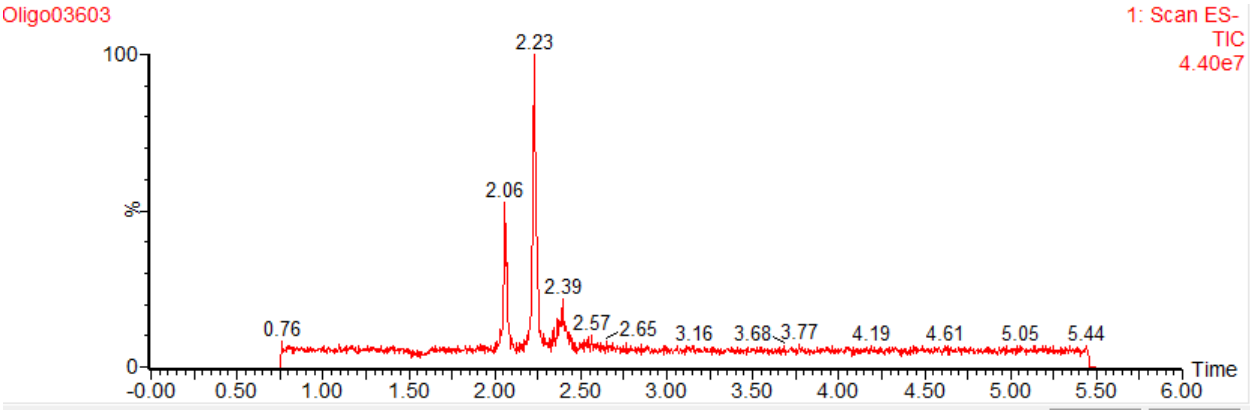


Exact Mass: 13280.13
Molecular Weight: 13286.59

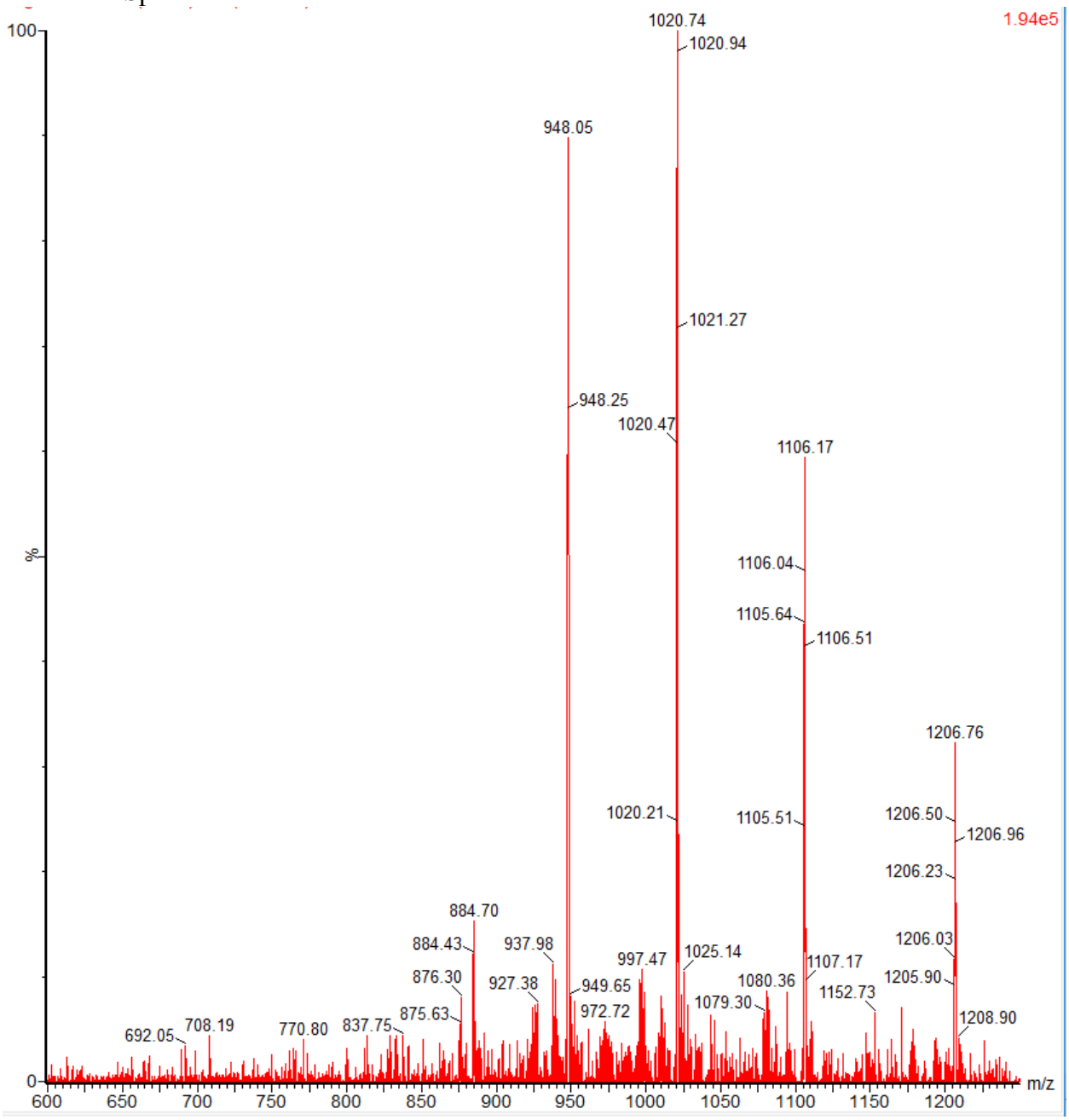
A260



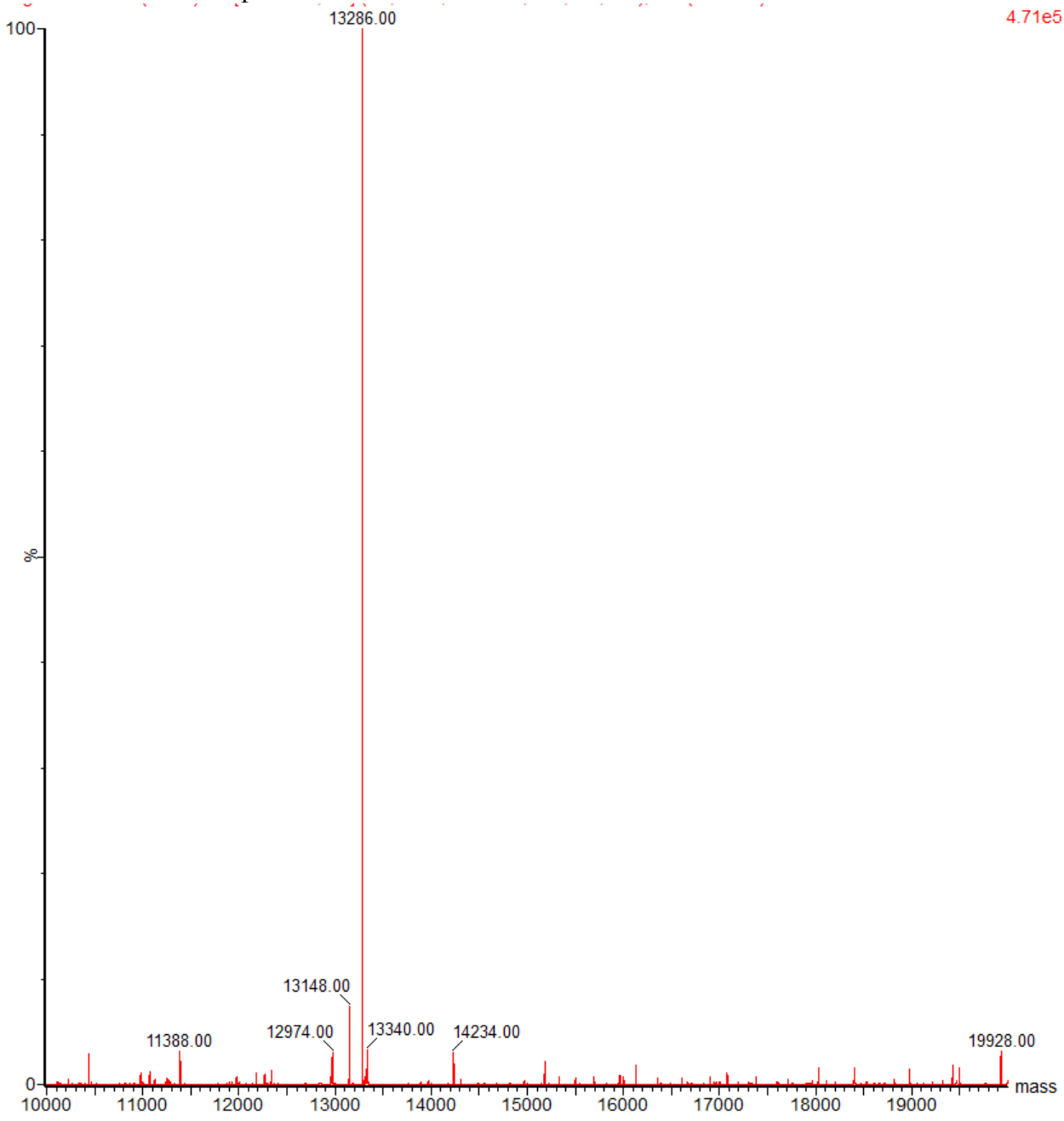
TIC



Raw Mass Spectrum RT = 2.22 minutes



Deconvoluted Mass Spectrum RT = 2.22 Minutes

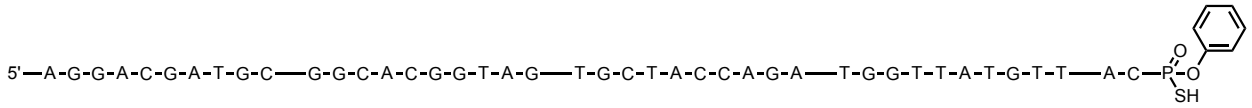


Analysis of **R-55**

LCMS Characterization of R-55/ After EXOIII

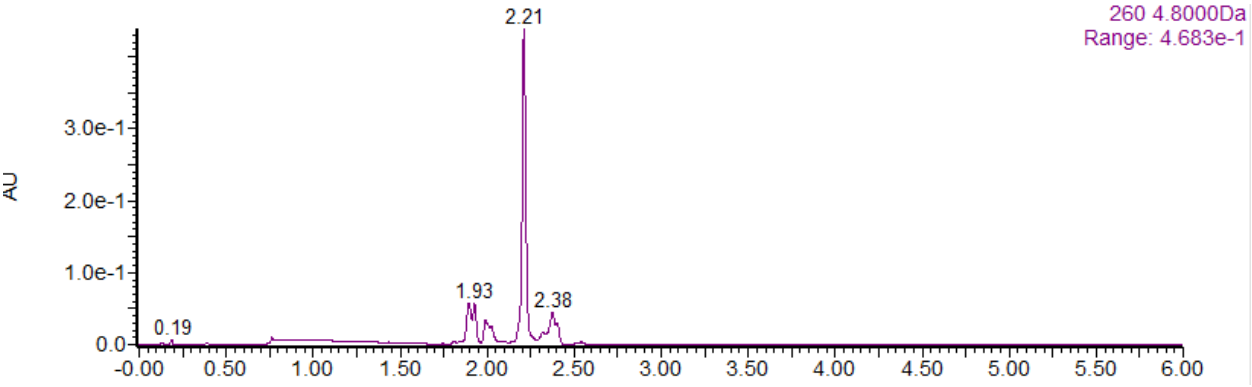
Using (+)Ψ-14

Yield (A260): 83%
Expected Mass: 13286.6
Found Deconvoluted Mass: 13287.0

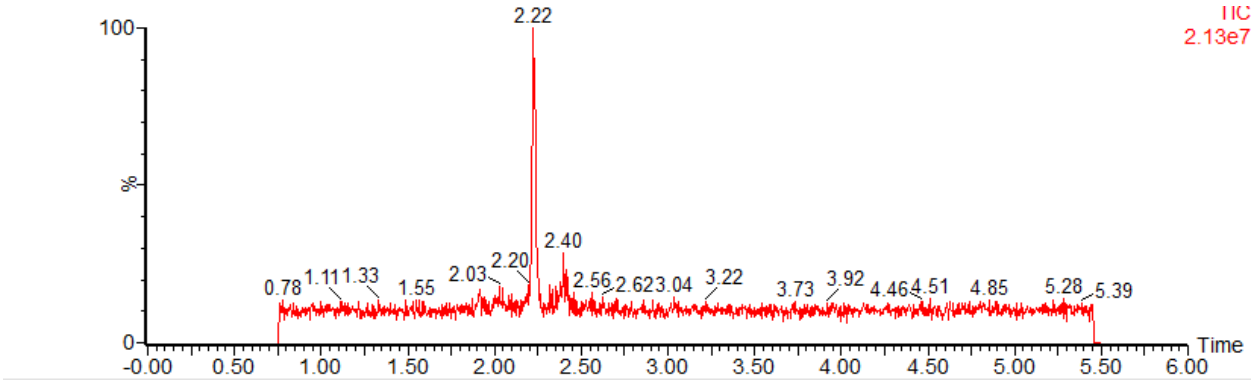


Exact Mass: 13280.13
Molecular Weight: 13286.59

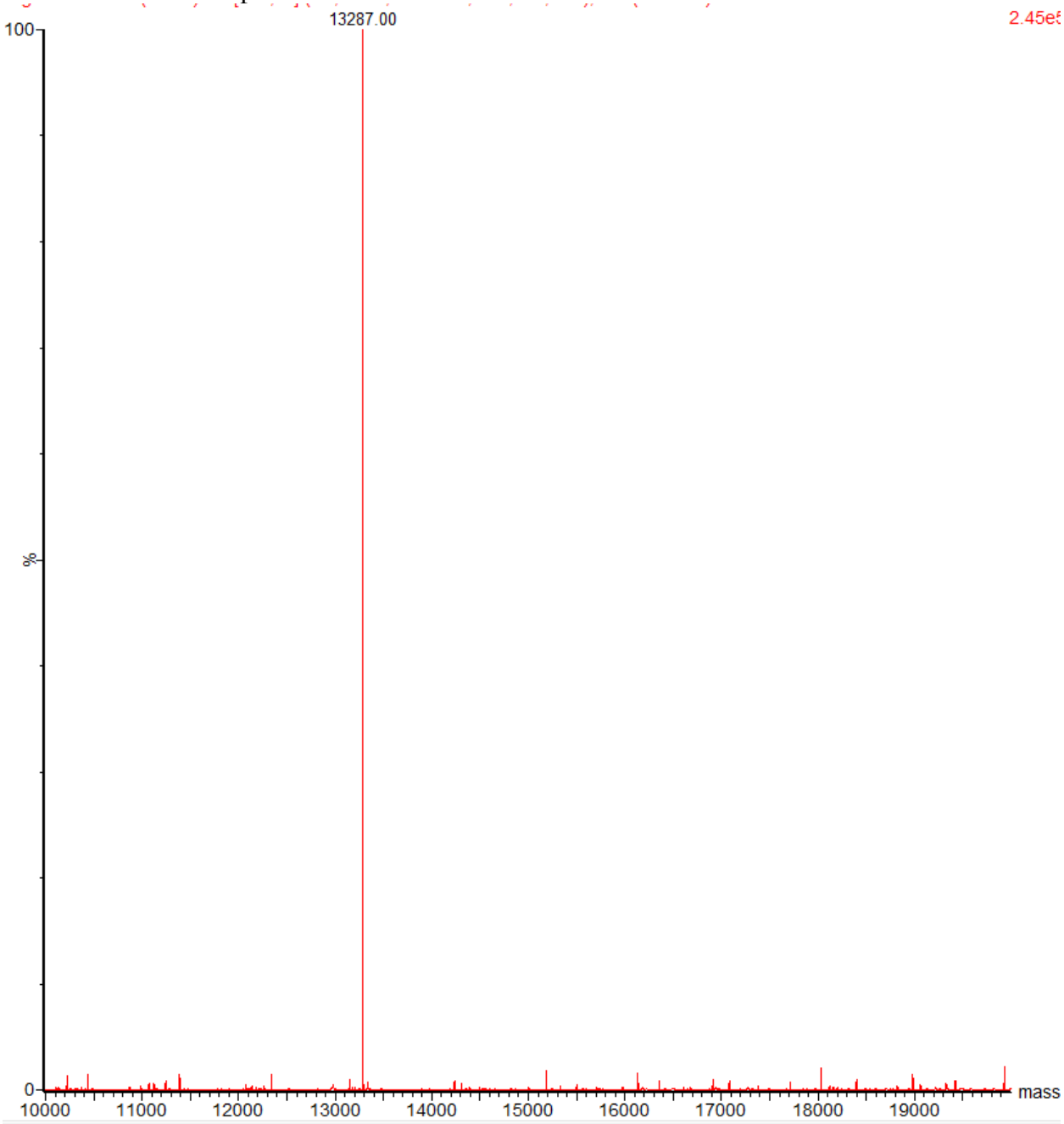
A260



TIC



Deconvoluted Mass Spectrum RT = 2.21



hNE Inhibition Assay

Protocol

R/S-(55) were used directly in the assay after isolation, without further purification. Activity of human neutrophil elastase (hNE) was measured using the method described previously[2] with slight modifications in a total volume of 10 μL in a reaction buffer of PBS (pH 7.4) and 0.05% (v/v) Nonidet™ P 40 Substitute (Sigma). Final composition of each reaction was 5 nM hNE (Elastin Products Corp.), 50 μM AAPV-aminomethylcoumarin (AMC) substrate (Millipore), and various concentrations of compounds. hNE was incubated with inhibitors for 10 min at room temperature before addition of AAPV-AMC. Residual proteolytic activity was measured kinetically at 25 °C using a Synergy H1 microplate reader (BioTek) for a total of 30 min at 30 sec intervals (Excitation: 380 nm, Emission: 460 nm). Only data points reflecting linear substrate conversion were used to determine relative protease activity. IC_{50} values were obtained by fitting the data to a concentration-response inhibition, log (inhibitor) vs. response – variable slope (four parameters) using GraphPad Prism.

Raw Data

Raw data for **R-(55)**

Log [M]	R-(55)		
-6	0.08443	0.09821	0.1046
-6.30103	0.08173	0.09096	0.09701
-6.60206	0.1038	0.1246	0.1173
-6.90309	0.09301	0.1166	0.1337
-7.20412	0.1193	0.144	0.1471
-7.50515	0.1201	0.1623	0.1527
-7.80618	0.1355	0.1876	0.1953
-8.10721	0.1741	0.2032	0.2049
-8.40824	0.2159	0.2269	0.2179
-8.70927	0.2347	0.2387	0.2242
-9.0103	0.2237	0.248	0.2462

Raw Data for **S-(55)**

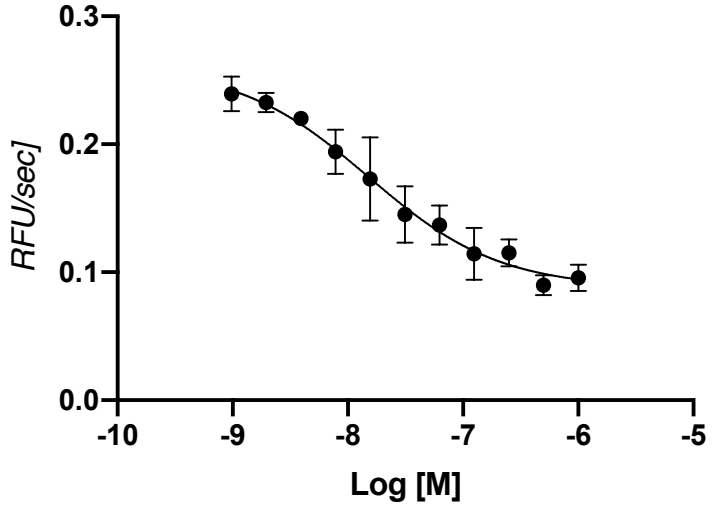
Log [M]	S-(55)		
-6	0.1285	0.1268	0.1318
-6.301	0.1127	0.1152	0.1286
-6.6021	0.13	0.1229	0.1433
-6.9031	0.1256	0.1381	0.1447
-7.2041	0.1514	0.1531	0.1821
-7.5052	0.177	0.1934	0.2086
-7.8062	0.2347	0.2358	0.2644
-8.1072	0.253	0.2727	0.298
-8.4082	0.3032	0.3237	0.3083
-8.7093	0.3229	0.3286	0.3441
-9.0103	0.3415	0.3645	0.3692

Raw Data for **54**

Log [M]	54		
-6	0.1285	0.1268	0.1318
-6.301	0.1127	0.1152	0.1286
-6.6021	0.13	0.1229	0.1433
-6.9031	0.1256	0.1381	0.1447
-7.2041	0.1514	0.1531	0.1821
-7.5052	0.177	0.1934	0.2086
-7.8062	0.2347	0.2358	0.2644
-8.1072	0.253	0.2727	0.298
-8.4082	0.3032	0.3237	0.3083
-8.7093	0.3229	0.3286	0.3441
-9.0103	0.3415	0.3645	0.3692

Plotted Data

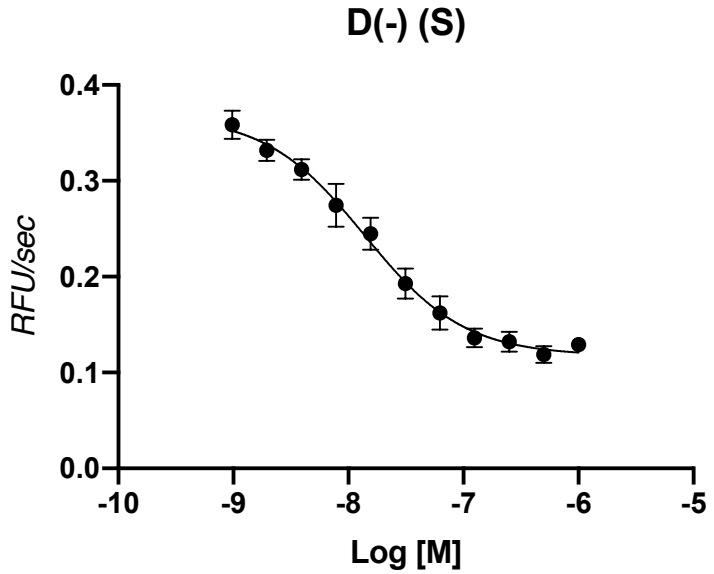
Plotted Data for R-(55)



Non Linear Fit Parameters

Best-fit values	
Bottom	0.08754
Top	0.2613
LogIC50	-7.820
HillSlope	-0.7621
IC50	1.513e-008
Span	0.1737
95% CI (profile likelihood)	
Bottom	0.002397 to 0.1062
Top	0.2324 to 0.5491
LogIC50	-9.462 to -7.536
HillSlope	-1.303 to -0.2260
IC50	3.451e-010 to 2.912e-008
Goodness of Fit	
Degrees of Freedom	29
R squared	0.9290
Sum of Squares	0.006909
Sy.x	0.01543

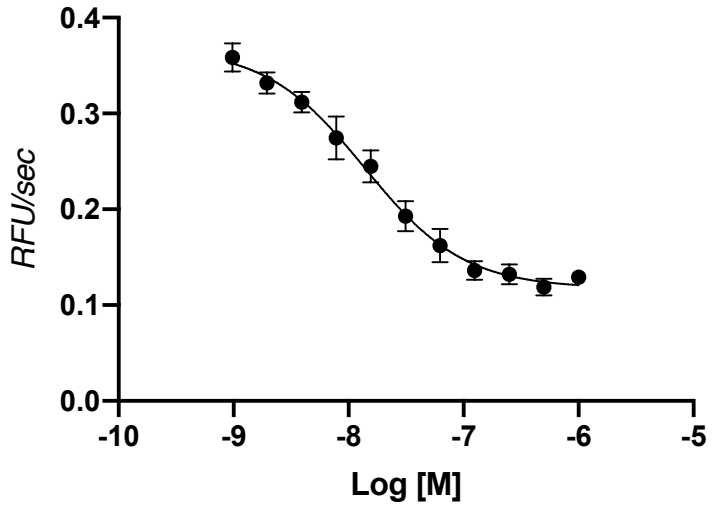
Plotted Data for S-(55)



Non-Linear Fit Parameters

Best-fit values	
Bottom	0.1175
Top	0.3686
LogIC50	-7.858
HillSlope	-1.005
IC50	1.386e-008
Span	0.2510
95% CI (profile likelihood)	
Bottom	0.1021 to 0.1292
Top	0.3464 to 0.4059
LogIC50	-8.016 to -7.742
HillSlope	-1.305 to -0.7440
IC50	9.635e-009 to 1.811e-008
Goodness of Fit	
Degrees of Freedom	29
R squared	0.9793
Sum of Squares	0.005105
Sy.x	0.01327

Plotted Data for 54



Nonlinear Fit Parameters

Best-fit values	
Bottom	0.1175
Top	0.3686
LogIC50	-7.858
HillSlope	-1.005
IC50	1.386e-008
Span	0.2510
95% CI (profile likelihood)	
Bottom	0.1021 to 0.1292
Top	0.3464 to 0.4059
LogIC50	-8.016 to -7.742
HillSlope	-1.305 to -0.7440
IC50	9.635e-009 to 1.811e-008
Goodness of Fit	
Degrees of Freedom	29
R squared	0.9793
Sum of Squares	0.005105
Sy.x	0.01327

SENDR on Biosynthetically Derived DNA

Construction of 56

PCR Amplification:

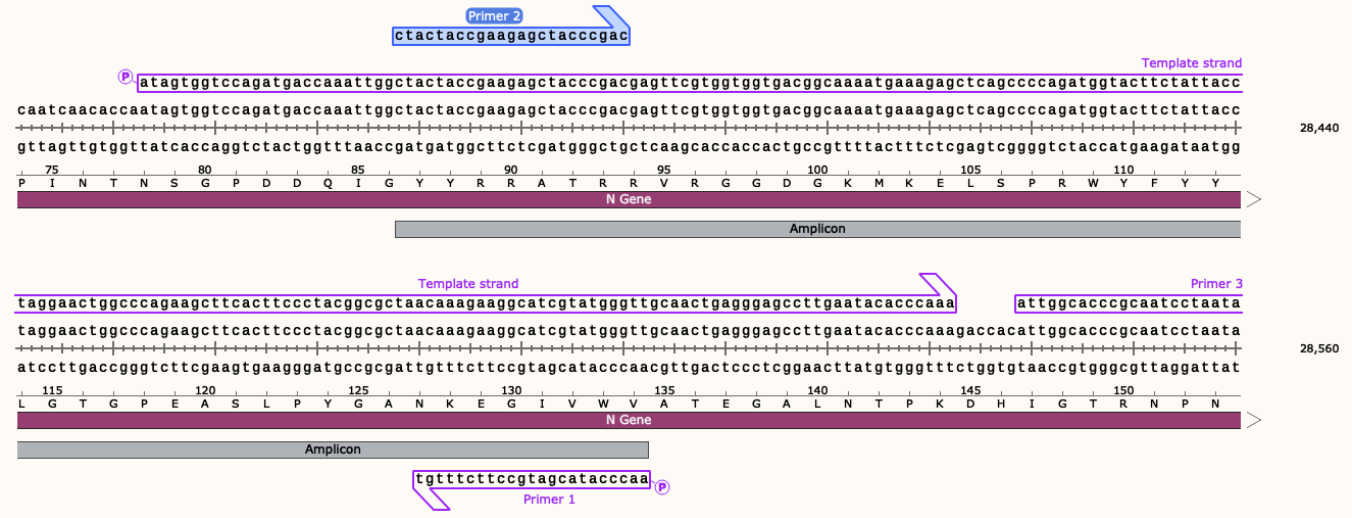
To a PCR tube template strand **(SI-18)** (2 μL, 5 ng from 2 ng/μL stock), primer 1 **(SI-19)** (2.5 μL from a 10 μM stock), primer 2 **(SI-20)** (2.5 μL from a 10 μM stock), water (18 μL) were added on ice. To this mixture Q5® High Fidelity 2x Master Mix (NEB) was added (25 μL). Tubes were place into a preheated (95ºC) thermocycler. An initial denaturing step of 95 °C for 30 was performed. This was followed by 30 rounds standard PCR protocol, with a denaturing step at 95 °C for 10 seconds, an annealing step at 59 °C for 15 seconds and an elongation step at 72 °C for 20 minute. A final extension step was performed at 752 °C for 2 minutes. The PCR products were purified by ZymoSpin Oligo Clean and Concentrator and eluted in 50 μL and carried forward.

Template Strand: **SI-18**
/5Phos/atagtggtccagatgaccaaattggctactaccgaagagctacccgacgagttcgtggtggtgacggcaaaatgaaagagctca
gccccagatggtacttctattacctaggaactggcccagaagcttcacttccctacggcgctaacaaagaaggcatcgtatgggttgcaact
gagggagccttgaatacacccaaa

Primer 1: **SI-19**
/5Phos/ aacccatacgatgccttctttgt

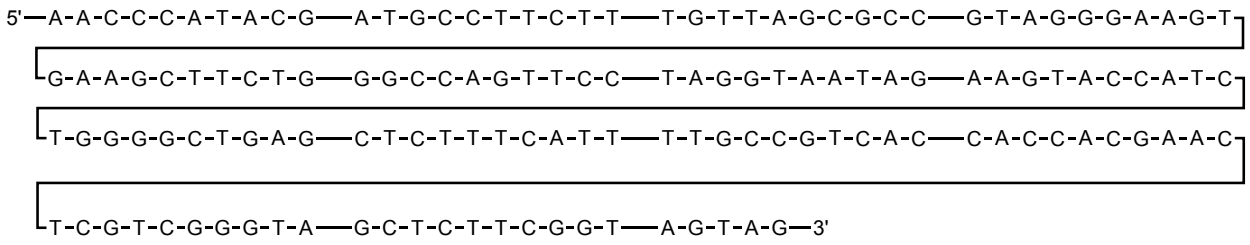
Primer 2: **SI-20**
ctactaccgaagagctacccgac

PCR Map

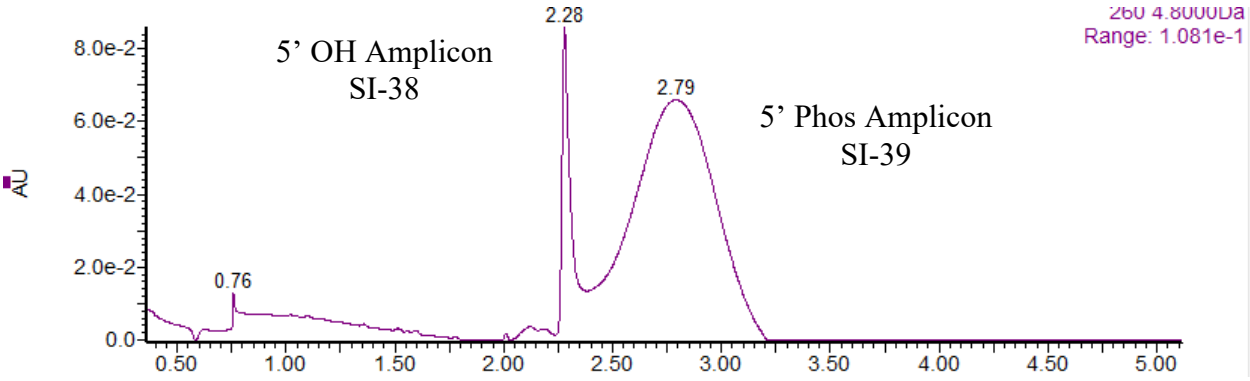


Analysis of Amplicons

5’ Phosphorylated Amplicon

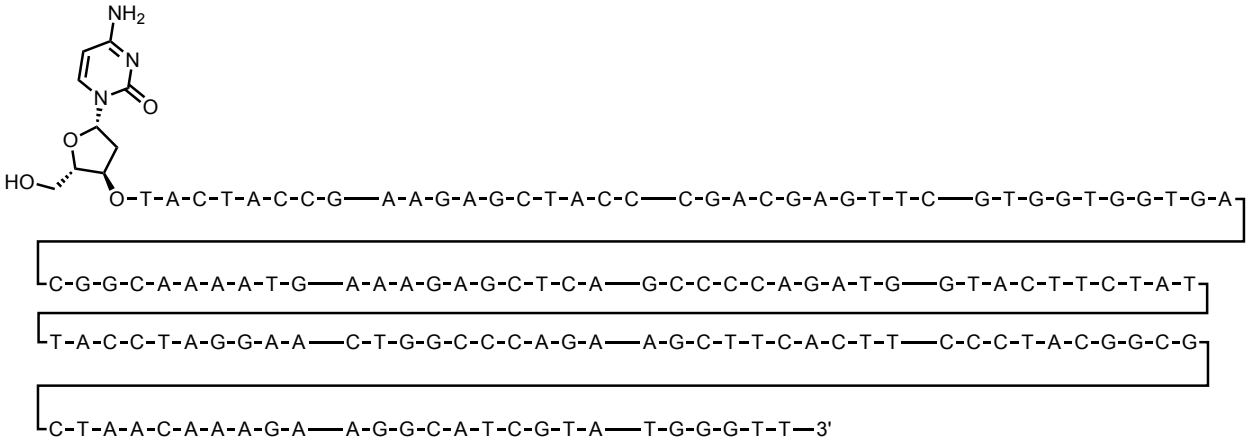


HPLC

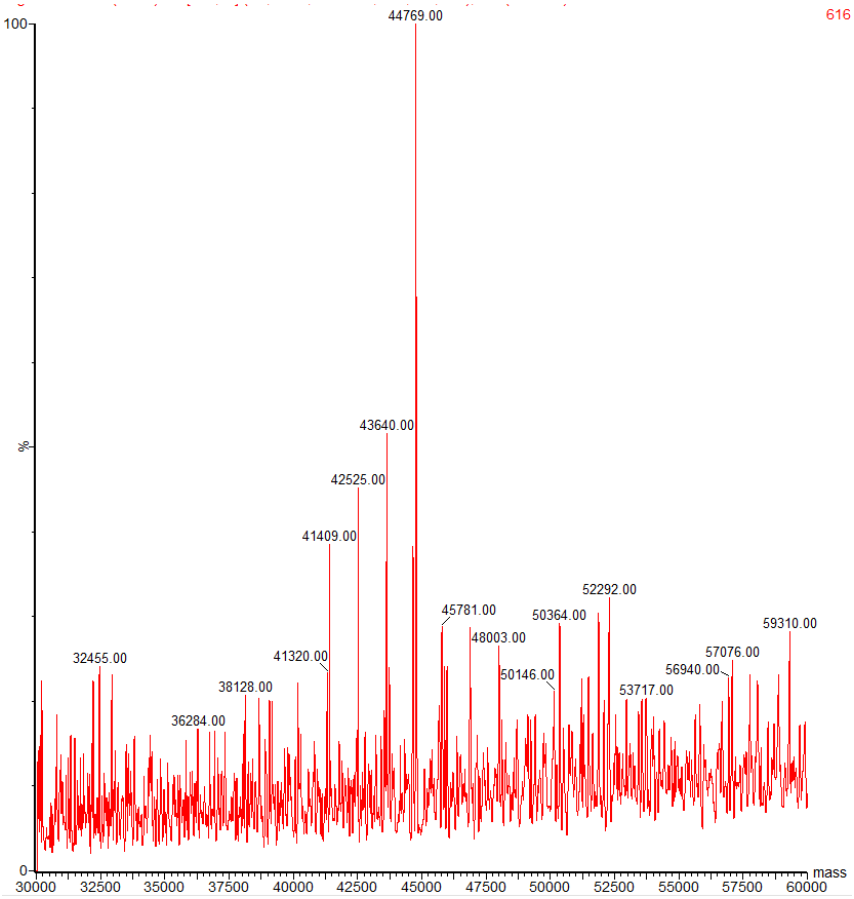


Deconvoluted Mass Spectrum RT=2.28 min

5’ OH Amplicon **SI-38**

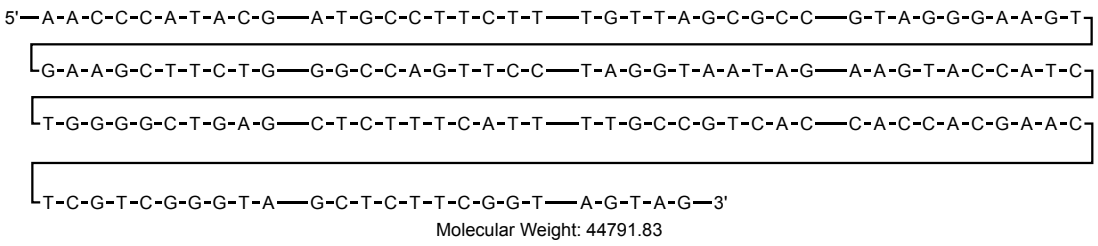


Molecular Weight: 44761.98

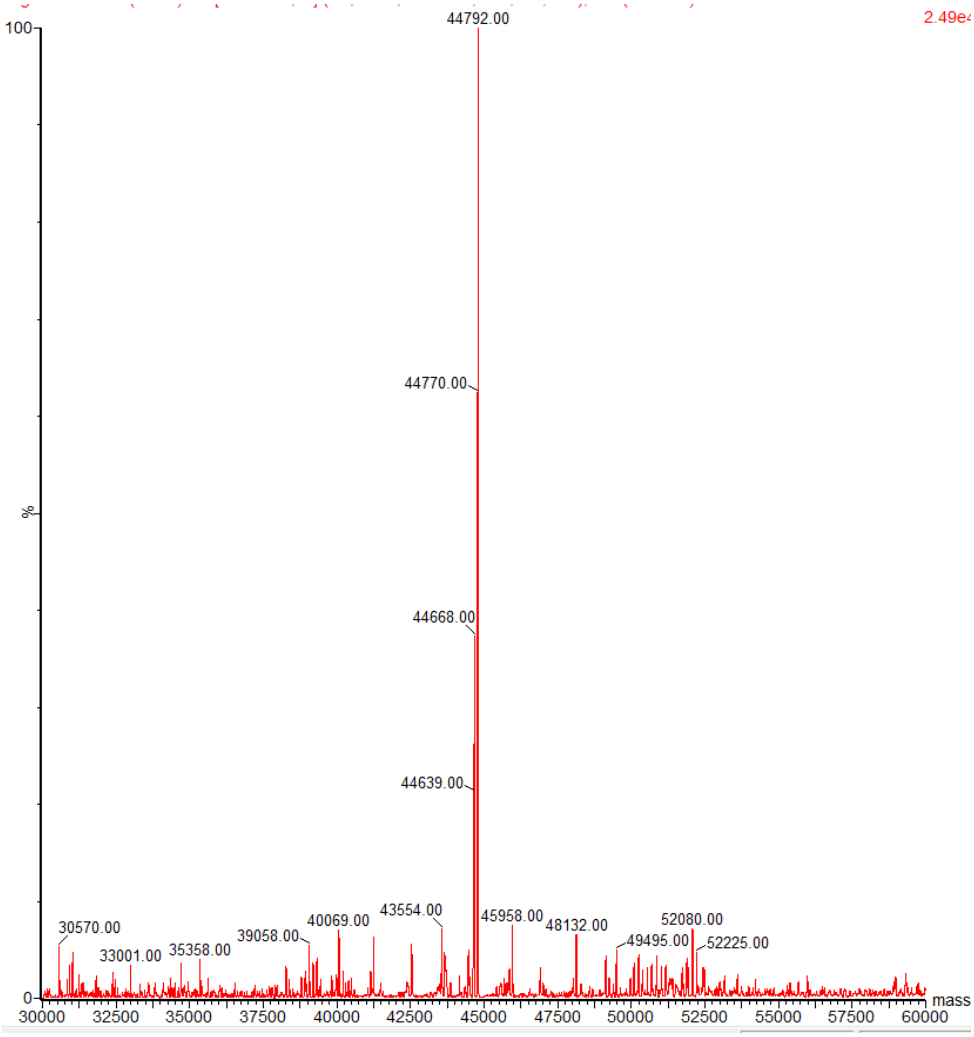


Deconvoluted Mass Spectrum RT=2.79 min

5’ Phosphorylated Amplicon **SI-39**



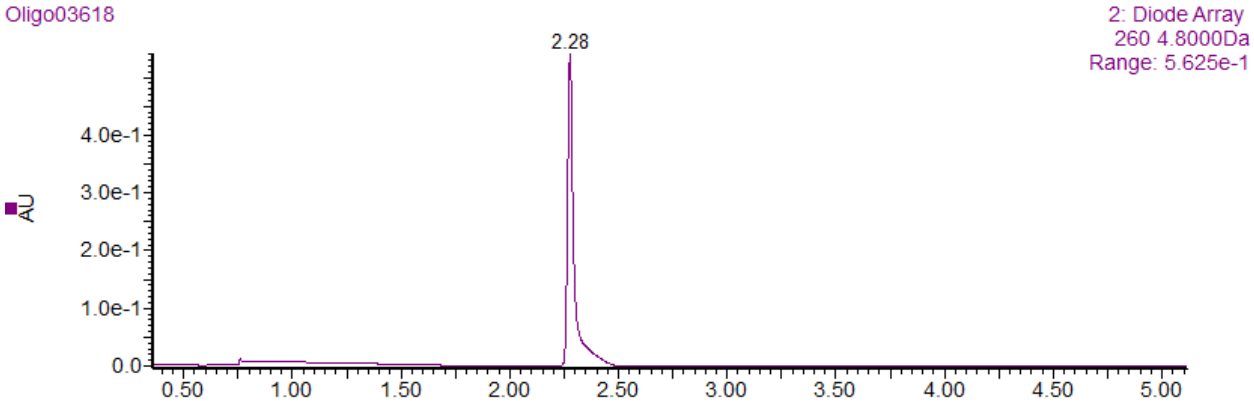
Molecular Weight: 44791.83



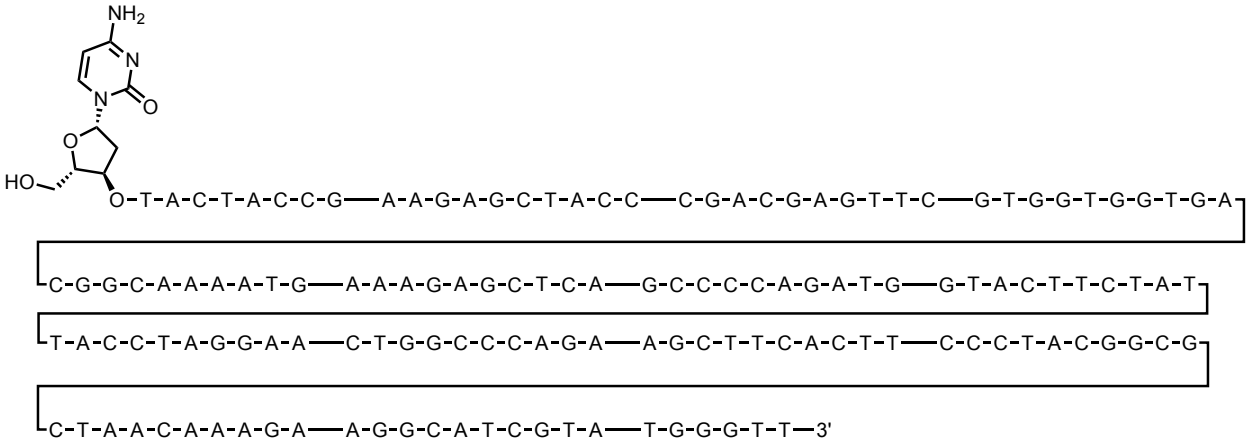
Lambda Exonuclease Degradation:

To the DNA mixture from the previous step (40 μL), CutSmart® (5 μL, 10x), water (3 μL), and Lambda Exonuclease (2 μL) was added in a PCR tube. This mixture was vortexed and incubated at 37ºC for 30 minutes at which point the lambda exonuclease was thermally denatured at 75ºC for 10 minutes. The resulting ssDNA (not phosphorylated) was isolated using a ZymoSpin Oligo clean and concentrator column.

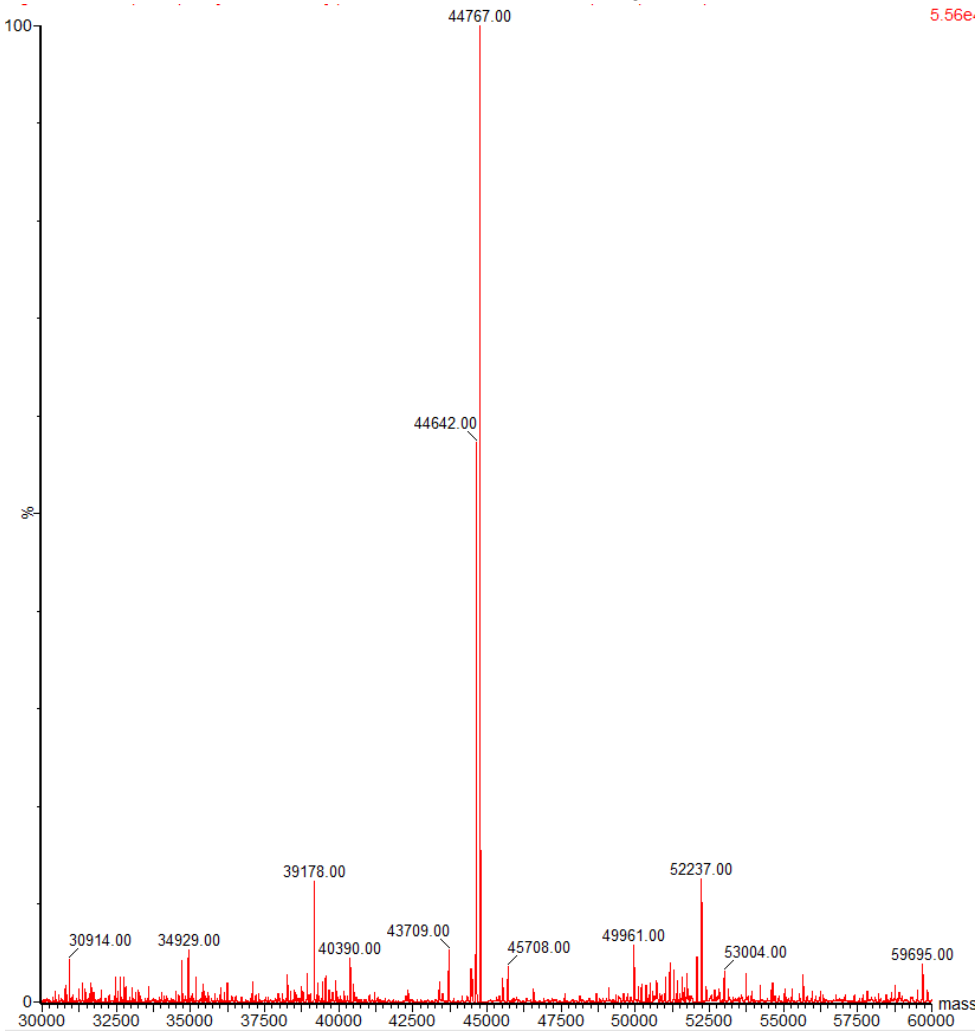
Analysis of Lambda Exonuclease Digestion:



Remaining ssDNA **SI-38**



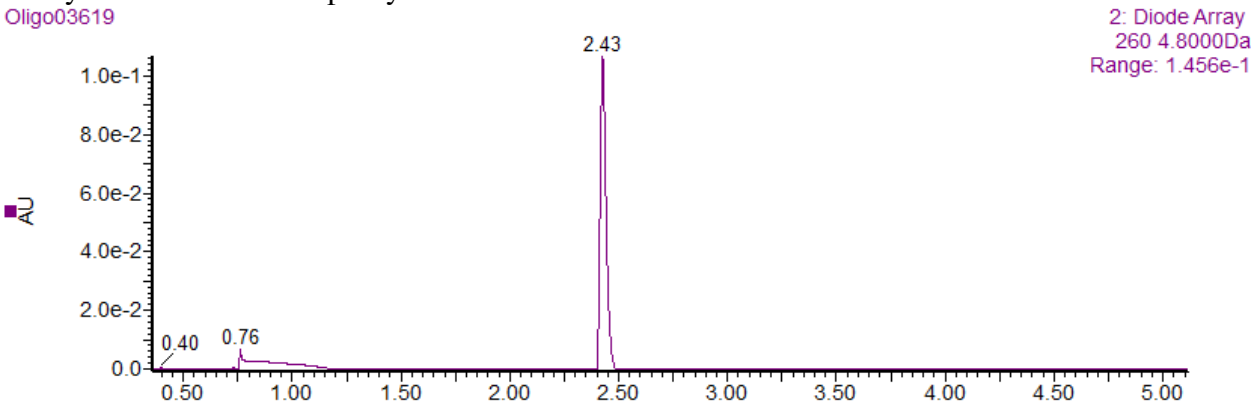
Molecular Weight: 44761.98



T4PNK Phosphorylation:

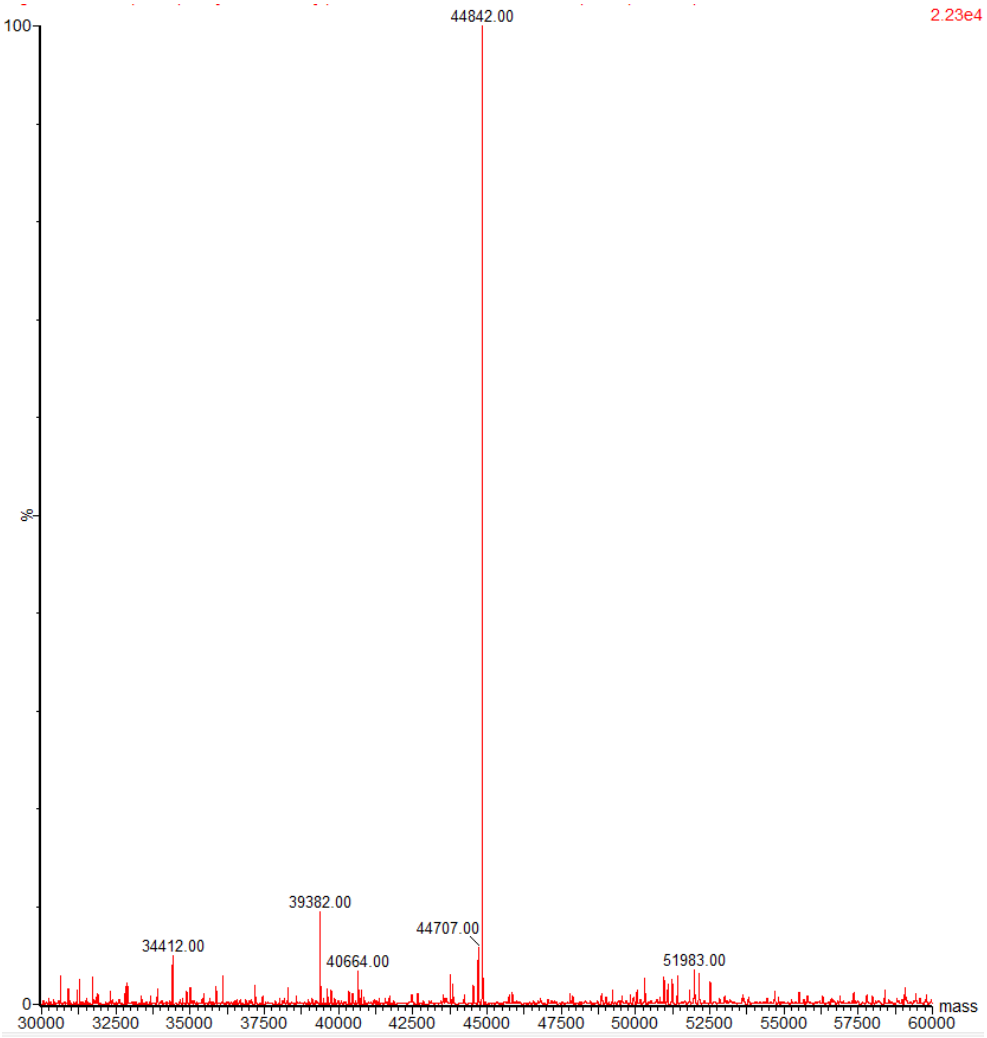
To the DNA mixture from the previous step (30 μL), CutSmart® (5 μL, 10x), DTT (5 μL, 50 mM Stock), ATP (5 μL from 10 mM stock), water (3 uL) and T4PNK (2 μL) was added in a PCR tube. This mixture was vortexed and incubated at 37ºC for 60 minutes at which point the T4PNK was thermally denatured at 75ºC for 10 minutes. The resulting ssDNA **56** was isolated using a ZymoSpin Oligo clean and concentrator column.

Analysis of T4PNK Phosphorylation **56**:



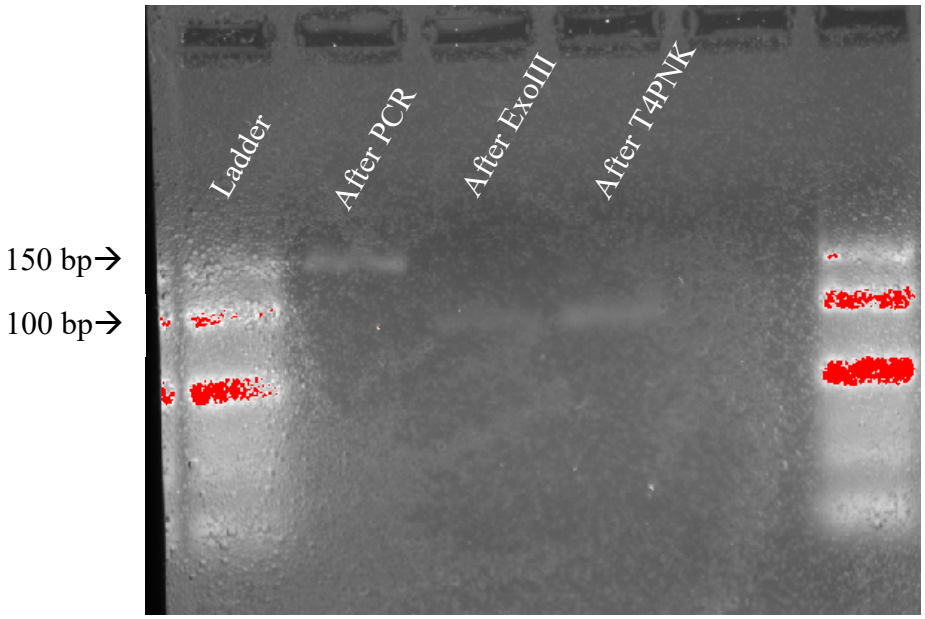
DNA **56**

Molecular Weight: 44841.96



Analysis of PCR and Enzymatic Modifications by DNA Gel:

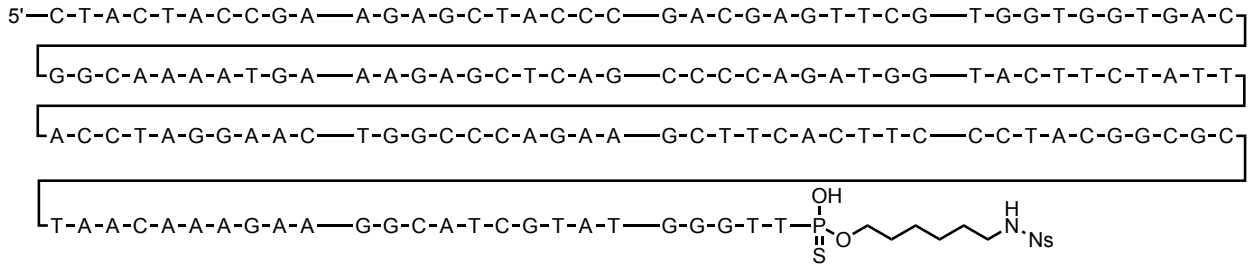
Without purification, 1 µL of each DNA sample was added to 4 µL water and 1 µL Gel Loading Dye, Purple (6X), no SDS. Laddther was Thermofisher O'RangeRuler 10 bp DNA Ladder. Then, 5 µL of each sample was loaded onto a 5% agarose gel, which had been freshly cast with 7.5 g UltraPure Agarose, 150 mL 1X TAE buffer and 15 µL 10,000X SYBR™ Safe DNA Gel Stain. The gel was run in 1X TAE buffer at 150 V for 30 minutes.



SENDR: 56→57

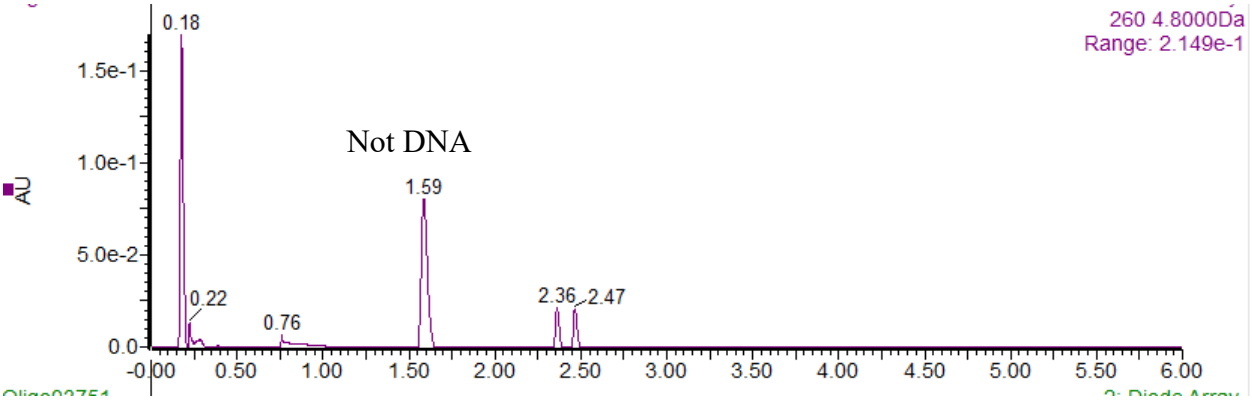
DNA **56** was used in SENDR Protocol without variation.

Analysis of **57:**
Produced from **56** by General Procedure **2:**
Using **Ψ-7**
Yield (A260): 54%
Expected Mass: 45222.23
Found Deconvoluted Mass: 45224.00

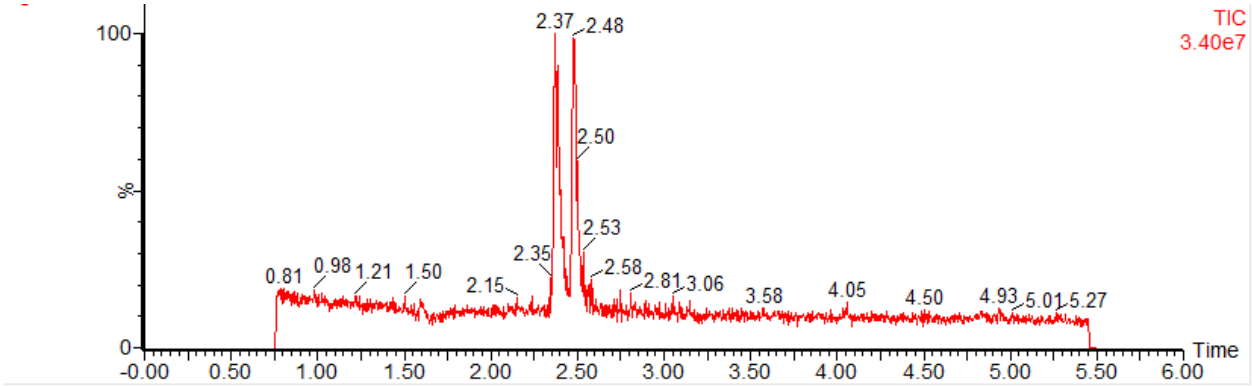


Molecular Weight: 45222.33

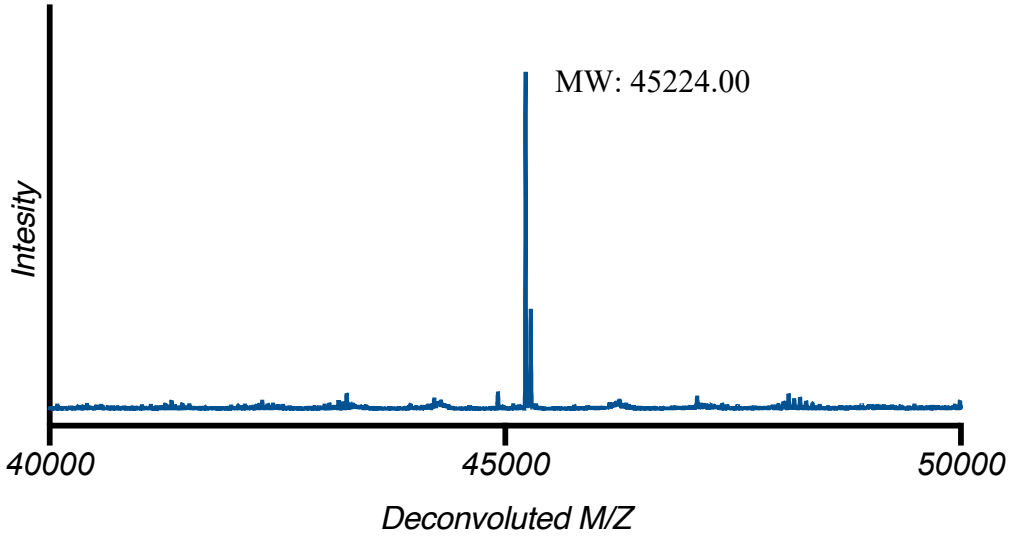
A260



TIC



Deconvoluted Mass Spectrum RT=2.48



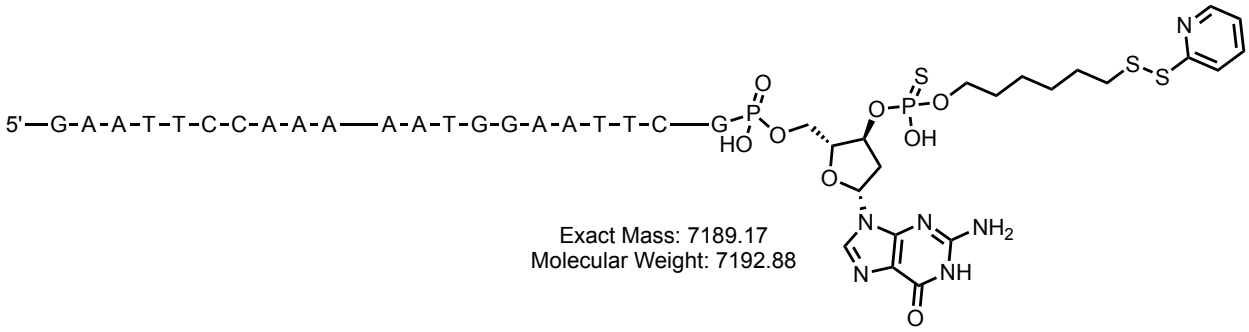
DNA BSA Conjugation

Procedure for the creation of 59

DNA **59** was synthesized using *General Procedure 2.* Briefly, 100 nmol of DNA **58** was loaded onto two tubes each containing 100 µL of equilibrated resin. This loaded resin was washed with DMA (500 µL twice) and dry THF (500 µL three times) and dried under vacuum. These tubes were manipulated in parallel.
Ψ-8 in MeCN (150 mM, 250 µL) was added to the loaded and dried resin. Then DBU (450 mM, 18 µL) was added to the reaction mixture. The reaction tube was vortexed for 30 seconds incubated at 37 ºC for 60 minutes. The reactions were worked up by aspirating and discarding the reaction solution, and washing the resin bed with MeCN (500 µL) and 1:1 MeCN:PBS (500 µL). Elute buffer (300 µL) was added to the resin bed and the tube was agitated by orbital shaker for 5-10 minutes. The DNA containing elution buffer was collected. DNA was isolate *via* ethanol precipitation. Ethanol precipitation was performed by adding 10% v/v of a NaCl solution (30 µL, 5M) to the elute buffer and three volumes of cold ethanol (-20ºC) were added to the tube (~1000µL) and incubated for 18 hours at -20 ºC. The tube was then centrifuged at 13,000 rpm for 15 minutes to pellet the DNA, and the extra ethanol was decanted off. The tubes were dried *via* speed vacuum and the DNA was dissolved and HPLC-MS analysis was performed.

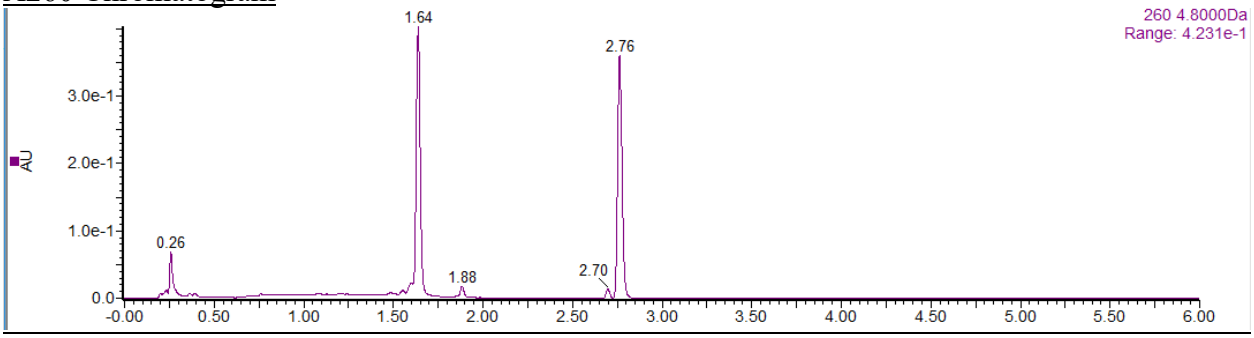
LCMS Characterization of 59

Produced from **58** by General Procedure **2:**
Using **Ψ-8**
Conversion (A260): 48%
Expected Mass: 7192.88
Expected (M-6)/6 Ion: 1197.81
Found Deconvoluted Mass: 7193.0
Found (M-6)/6 Ion: 1197.74
Total DNA Recovery: 60 nmol (60%)

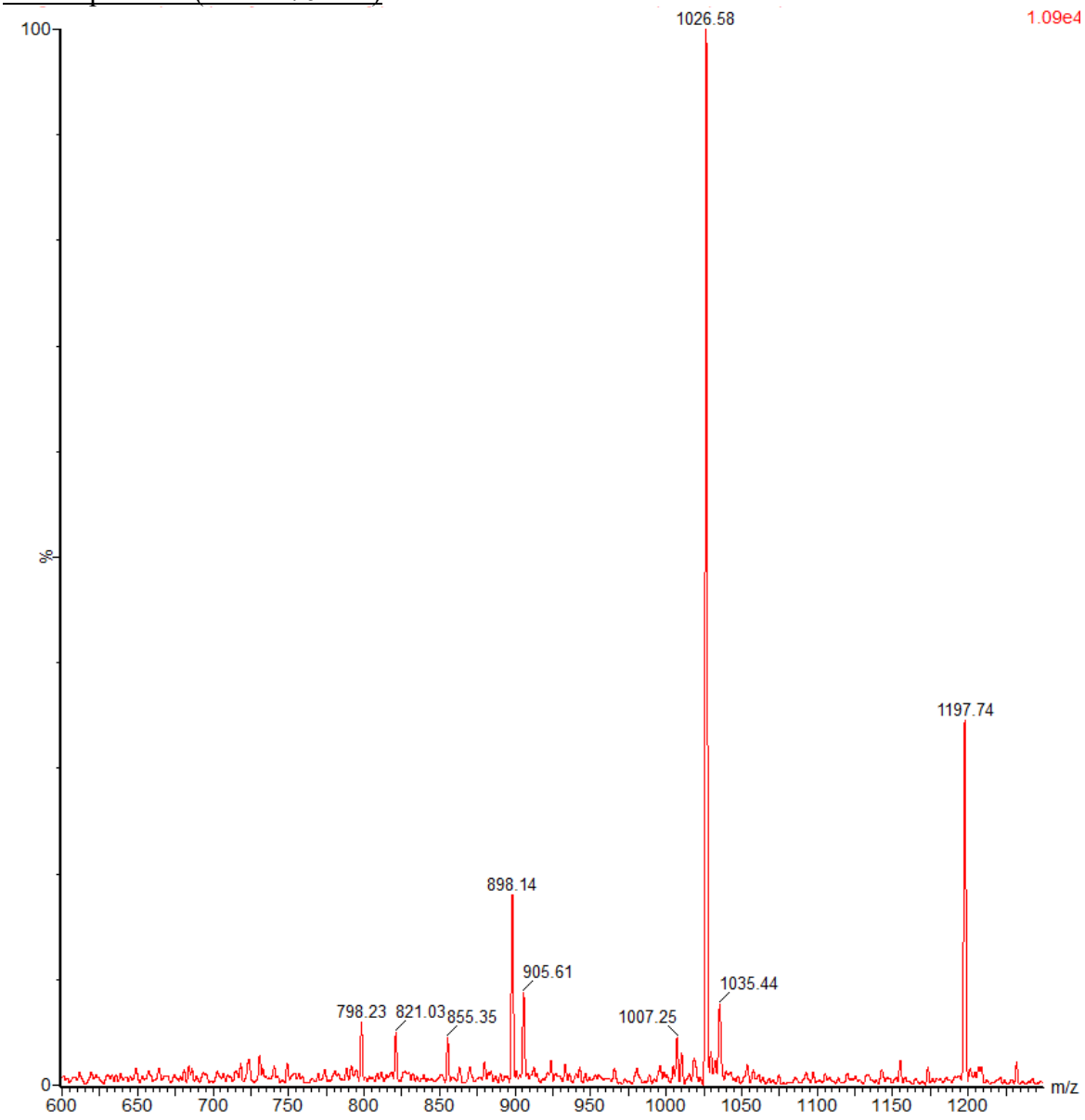


Exact Mass: 7189.17
Molecular Weight: 7192.88

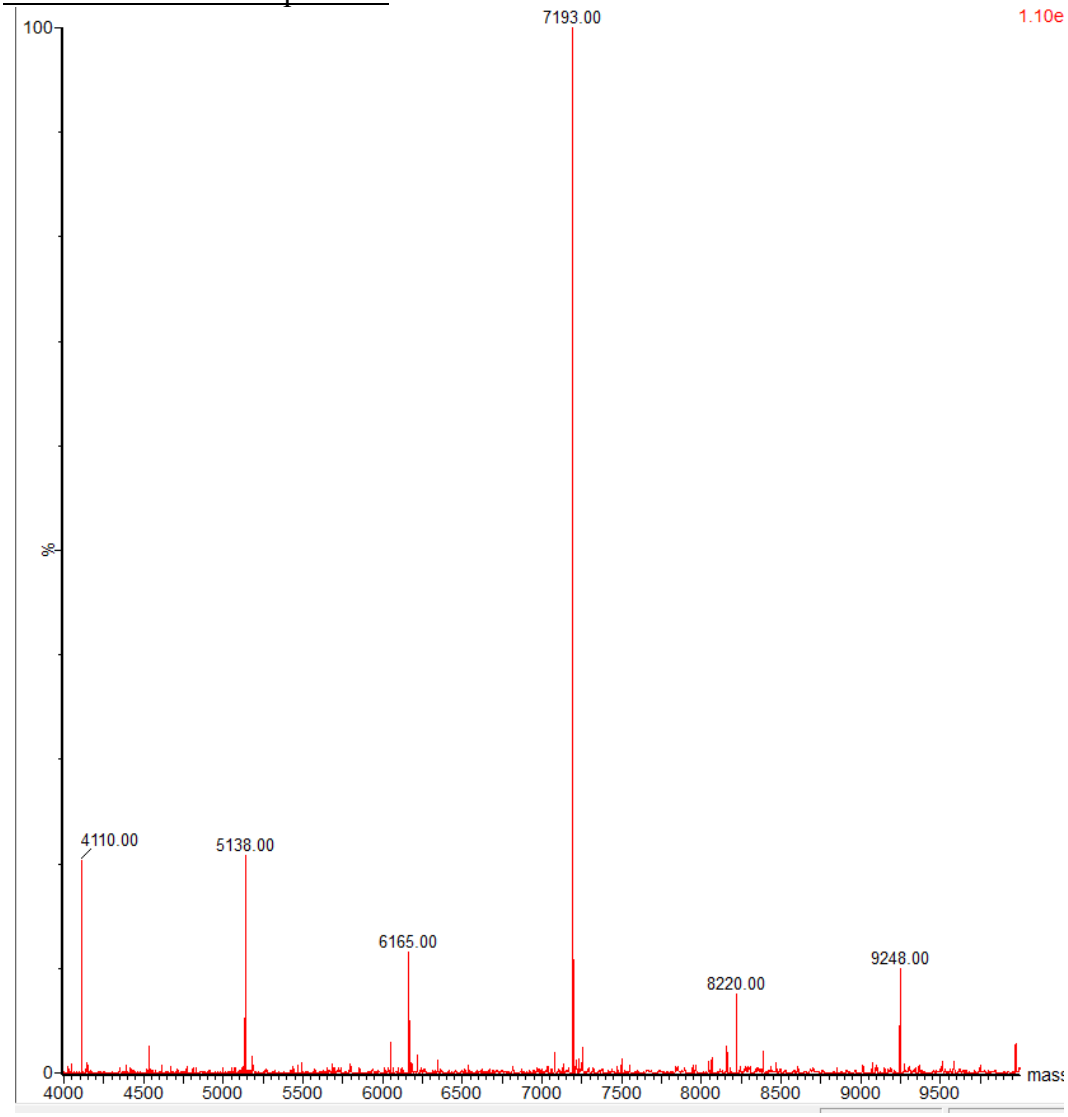
A260 Chromatogram



Mass Spectrum (RT= 2.76 min)



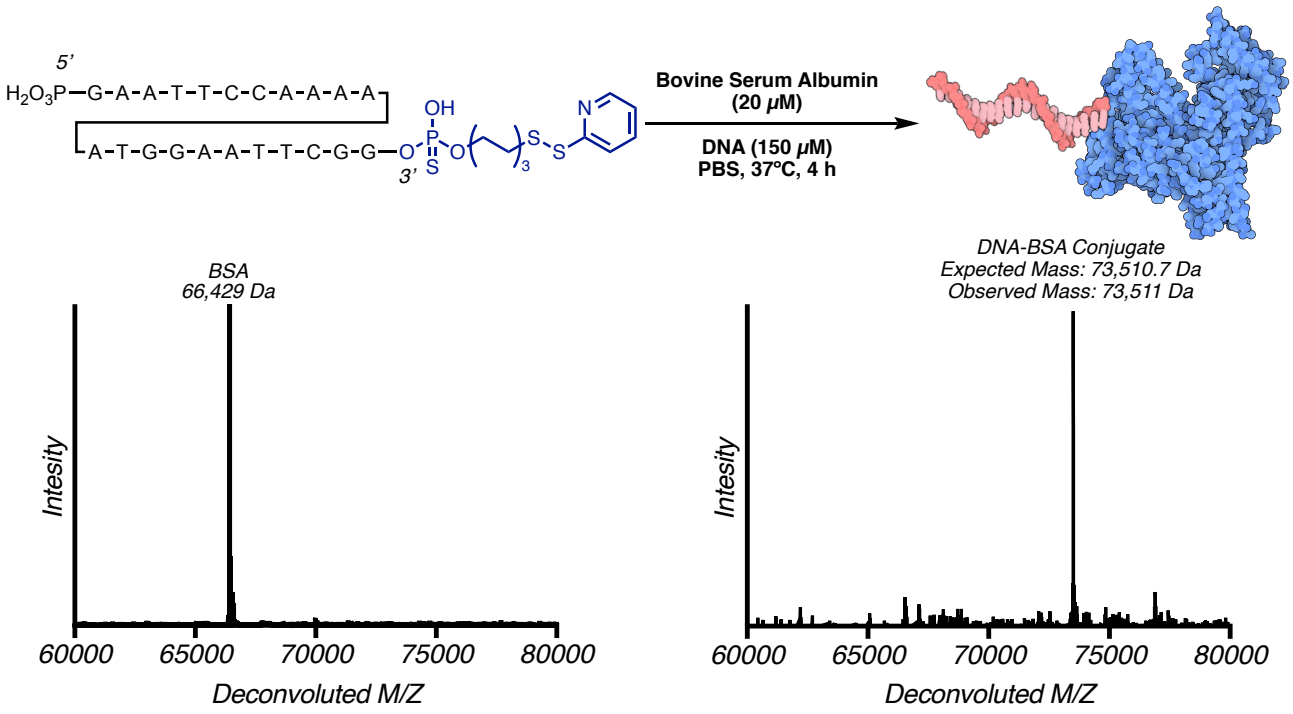
Deconvoluted Mass Spectrum



Procedure for the creation of 60

Compound **59** was synthesized by *General Procedure 2* and isolated crude after ethanol precipitation. DNA **59** was dissolved to 300 μM in PBS. Solid BSA was dissolved in PBS (2.5 mg/mL, ~40 μM). these two solutions were combined in a PCR tube (25 μL of each for a reaction volume of 50 μL) and the resulting reaction mixture was incubated in a thermocycler for 4 hours. The crude ligation solution was diluted to 0.5 mg/mL with respect to BSA and injected for intact protein analysis. Deconvolution across the entire mass peak showed no detectible unmodified BSA remaining.

Analysis of 60



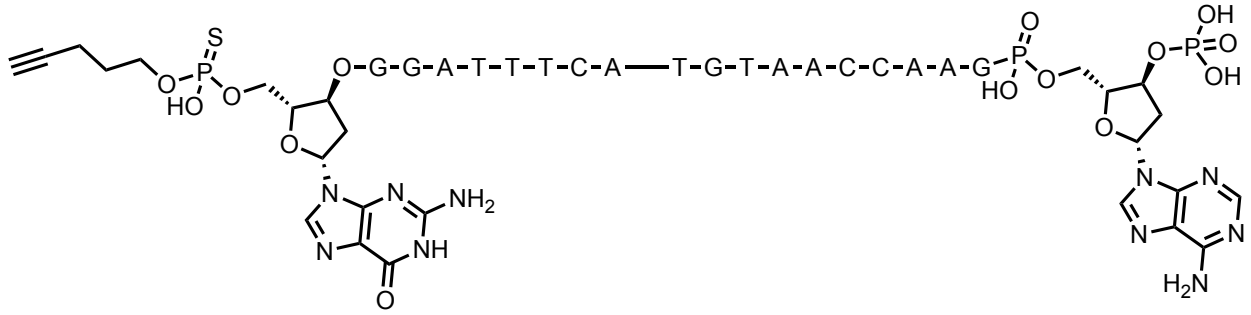
DNA DVD Conjugation

Procedure for the creation of SI-40

DNA **SI-40** was synthesized using *General Procedure 2.* Briefly, 100 nmol of DNA **61** was loaded onto two tubes containing 100 µL of equilibrated resin. This loaded resin was washed with DMA (500 µL twice) and dry THF (500 µL three times) and dried under vacuum. These tubes were manipulated in parallel.
Ψ-5 in MeCN (150 mM, 250 µL) was added to the loaded and dried resin. Then DBU (450 mM, 18 µL) was added to the reaction mixture. The reaction tube was vortexed for 30 seconds incubated at 37 ºC for 60 minutes. The reaction was worked up by aspirating and discarding the reaction solution, and washing the resin bed with MeCN (500 µL) and 1:1 MeCN:PBS (500 µL). Elute buffer (300 µL) was added to the resin bed and the tube was agitated by orbital shaker for 5-10 minutes. The DNA containing elution buffer was collected. DNA was isolate *via* ethanol precipitation. Ethanol precipitation was performed by adding 10% v/v of a NaCl solution (30 µL, 5M) to the elute buffer and three volumes of cold ethanol (-20 ºC) were added to the tube (~1000µL) and incubated for 18 hours at -20 ºC. The tube was then centrifuged at 13,000 rpm for 15 minutes to pellet the DNA, and the extra ethanol was decanted off. The tubes were dried *via* speed vacuum and the DNA was dissolved and HPLC-MS analysis was performed.

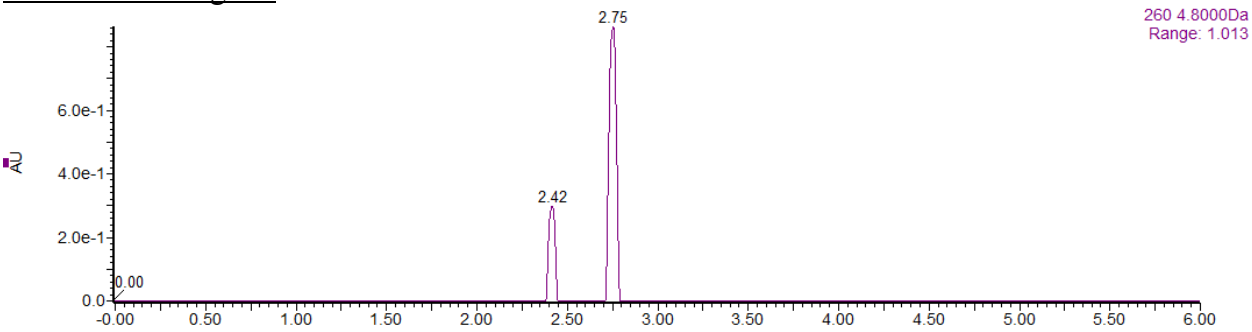
LCMS Characterization of SI-40

Produced from **61** by General Procedure **2:**
Crude Conversion (A260): 73%
Expected Mass: 6407.2
Expected (M-6)/6 Ion: 1066.34
Found Deconvoluted Mass: 6407.0
Found (M-6)/6 Ion: 1066.5
Crude Isolated Recovery: 44%

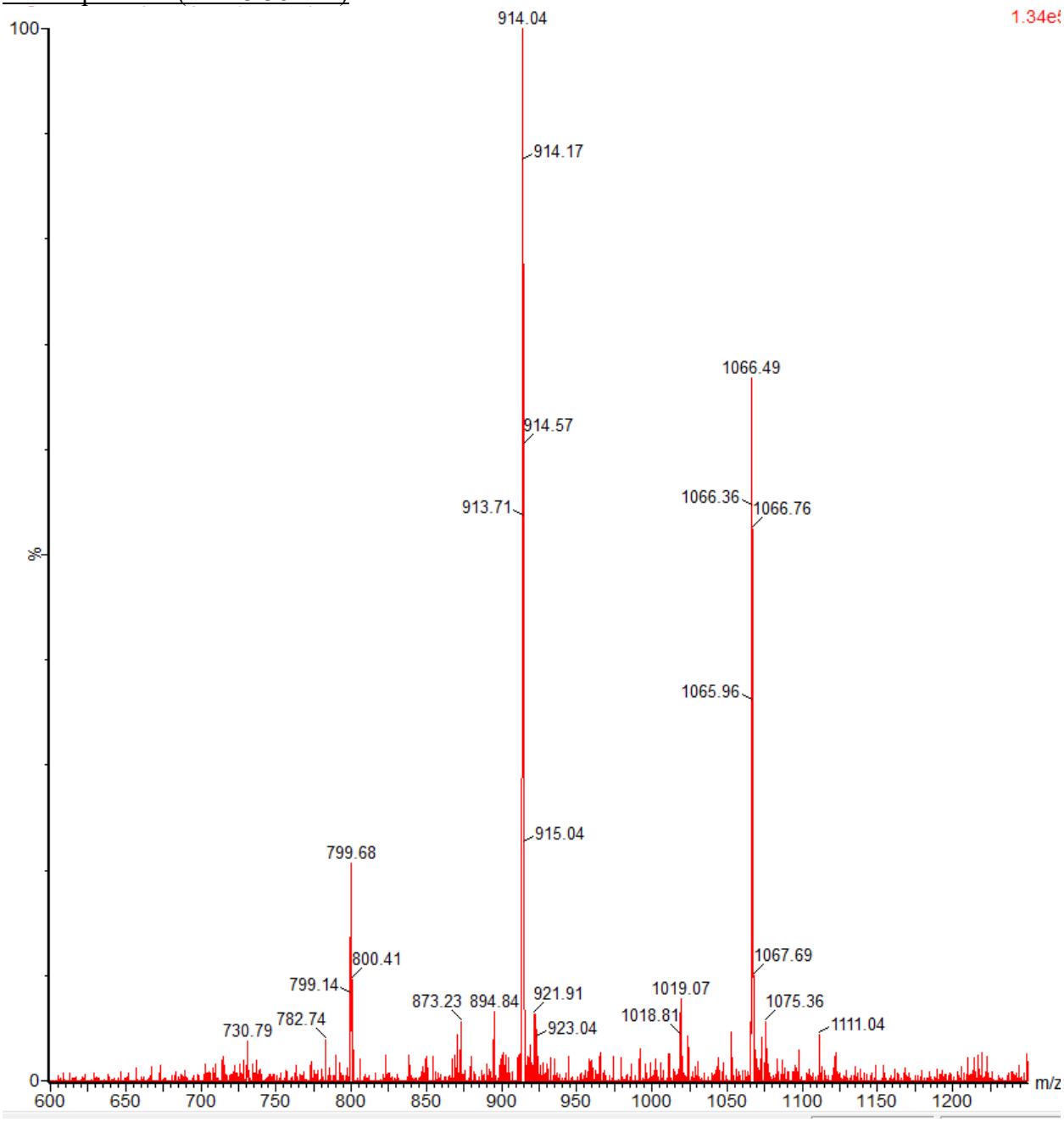


Exact Mass: 6404.04
Molecular Weight: 6407.20

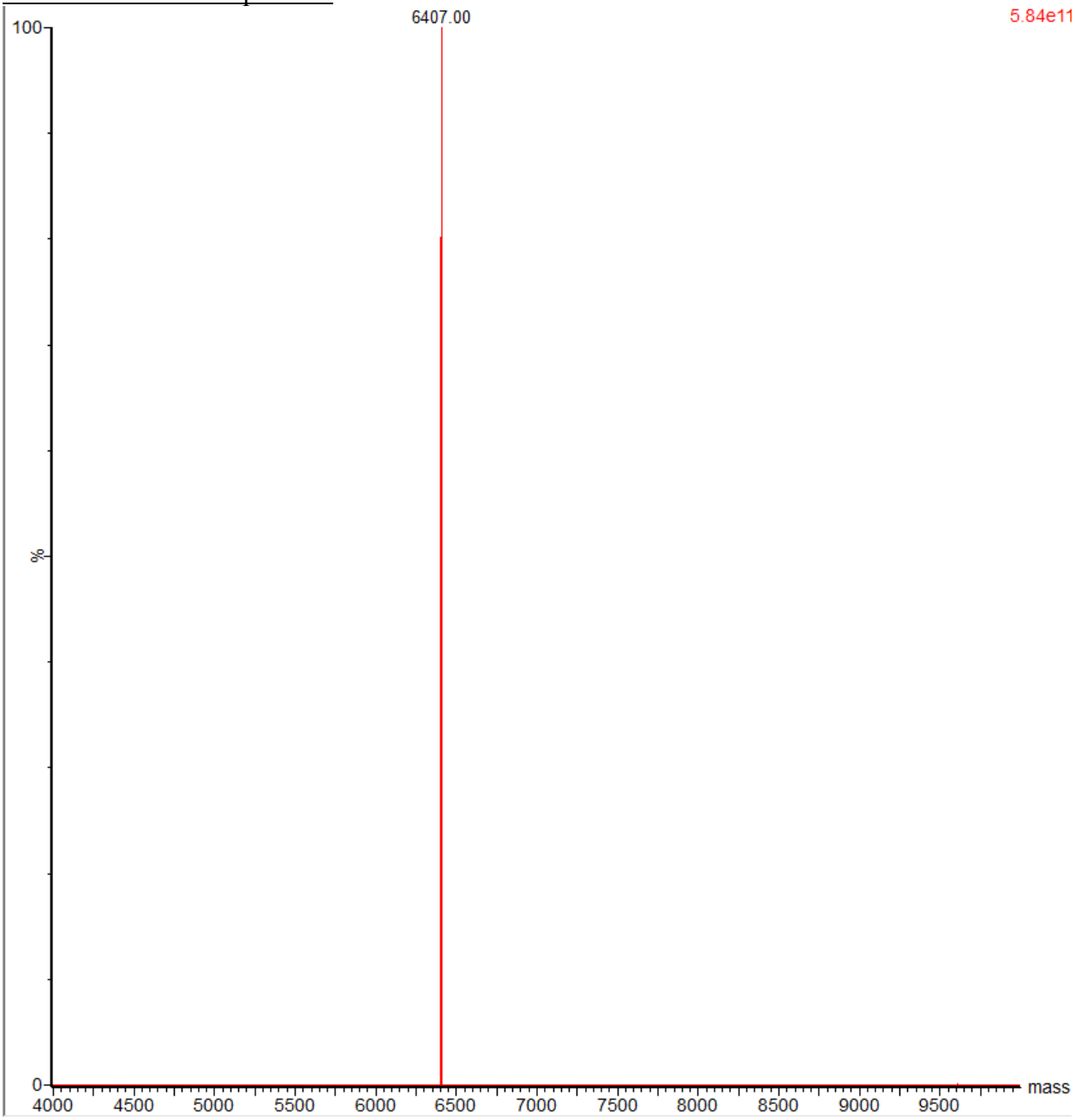
A260 Chromatogram



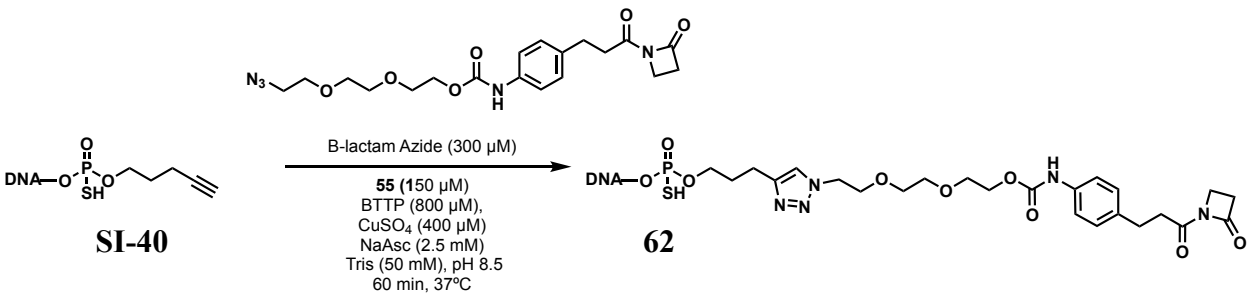
Mass Spectrum (RT= 3.38 min)



Deconvoluted Mass Spectrum



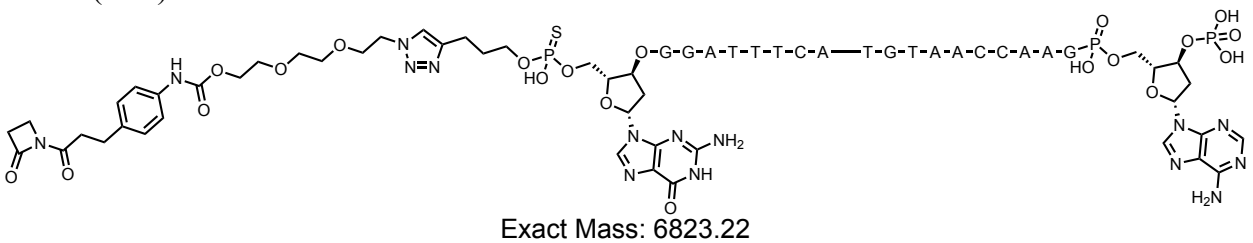
Procedure for the creation 62



Crude isolated DNA **SI-40** was purified using RP HPLC. After purification 15 nMol (15% total starting material) of pure **SI-40** was obtained. To **SI-40** a CuAAC reaction was performed. Compound **SI-40** (15 nmol) was dissolved (92.5 µL) in tris buffer (50 mM) at pH 8.5. To this solution the β-lactam azide **SI-53** (2 µL) was added from stock solution (30 mM) in DMA, (final concentration of 300 µM). Next, BTTP (2 µL) was added from a stock solution (40 mM in water). Next, $CuSO_4$ (1 µL) was added from a stock solution (40 µM in water) (a final concentration of 400 µM). The solution was capped and vortexed. Finally, sodium ascorbate (2.5 uL) was added from a stock solution (100 mM in water). This reaction solution was vortexed and incubated for 1 hour at 37 ºC. DNA **62** was ethanol precipitated, to remove excess small molecules, as previously described. Mass recovery and conversion for this transformation was assumed to be quantitative for subsequent protein conjugation.

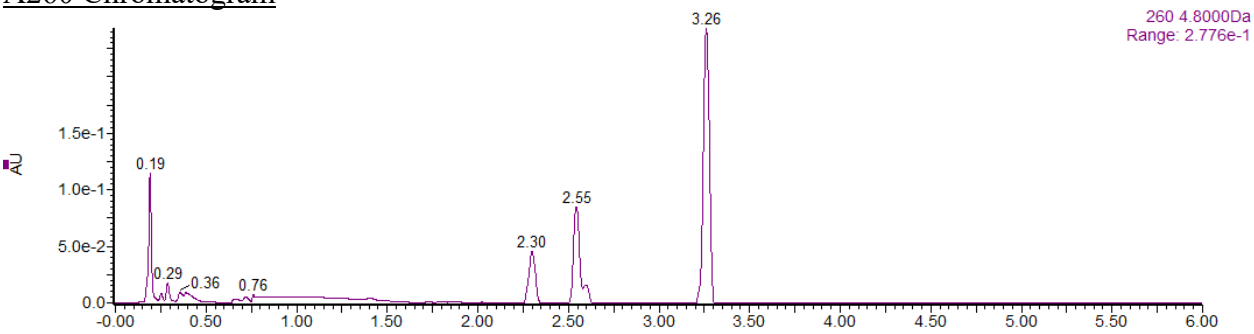
LCMS Characterization of 62

Produced from **SI-40:**
Crude Conversion (A260): >85%
Expected Mass: 6826.63
Expected (M-6)/6 Ion: 1136.20
Found Deconvoluted Mass: 6827 + Cu Adducts
Found (M-6)/6 Ion: 1136.4

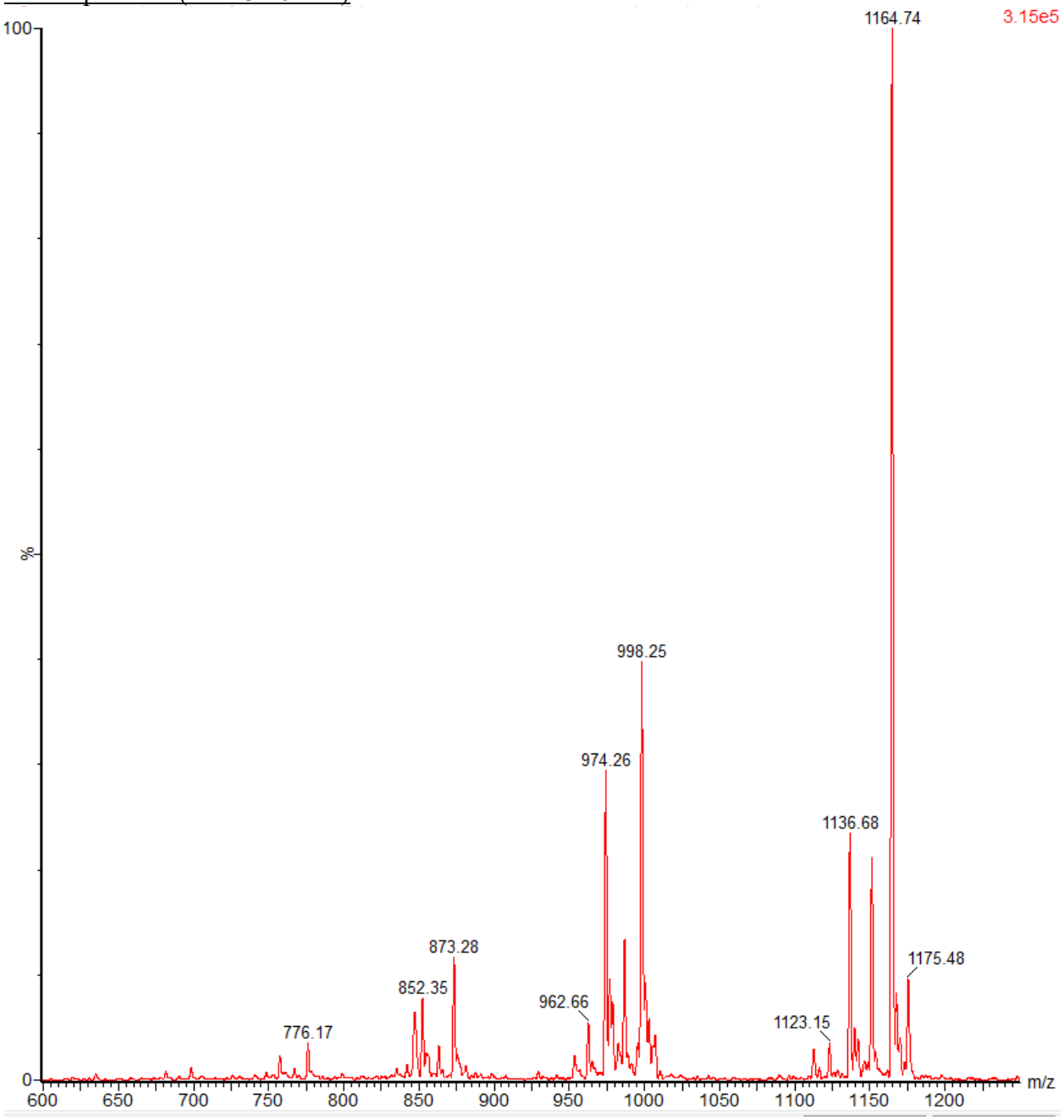


Exact Mass: 6823.22
Molecular Weight: 6826.63

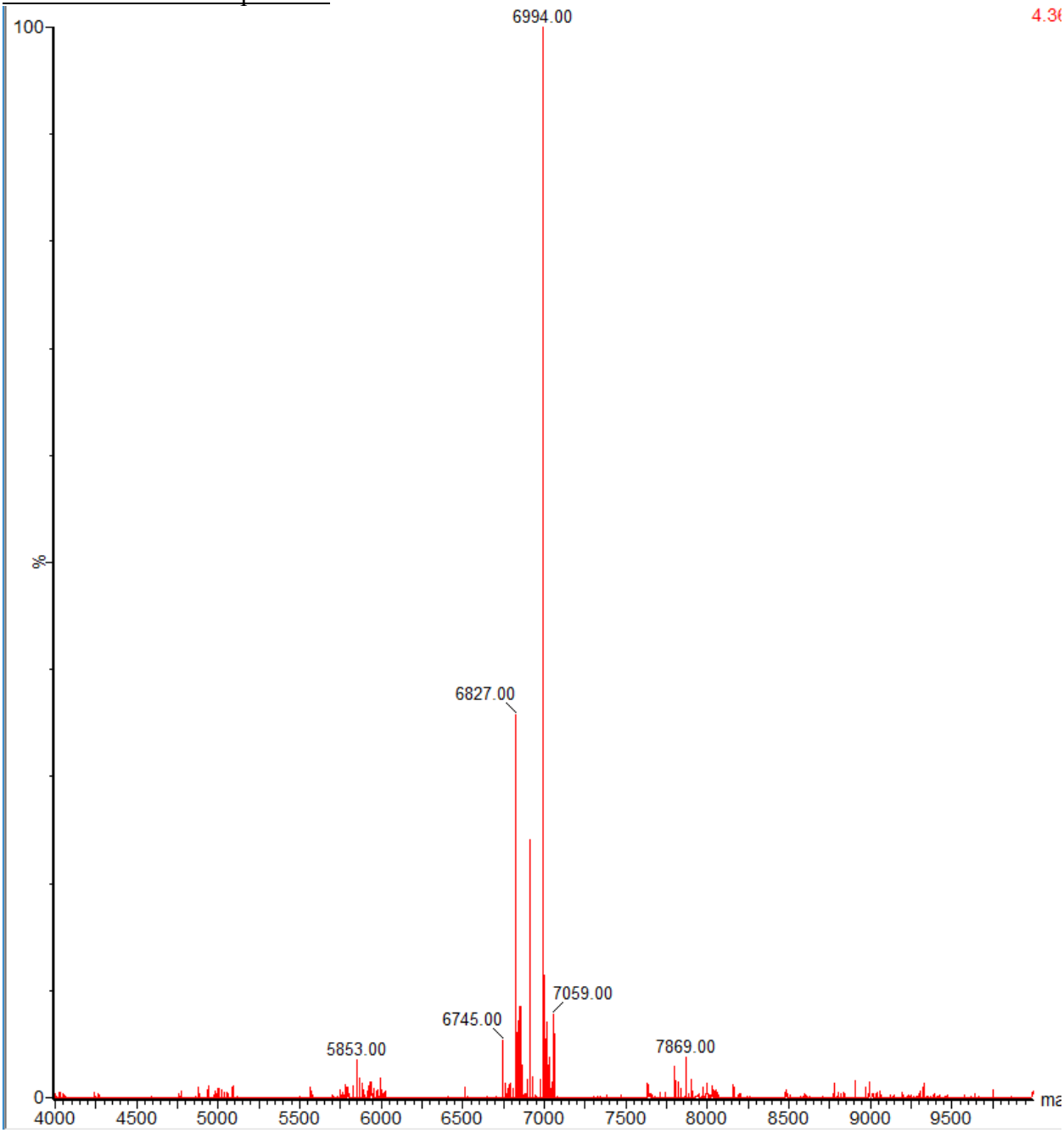
A260 Chromatogram



Mass Spectrum (RT= 3.26 min)



Deconvoluted Mass Spectrum

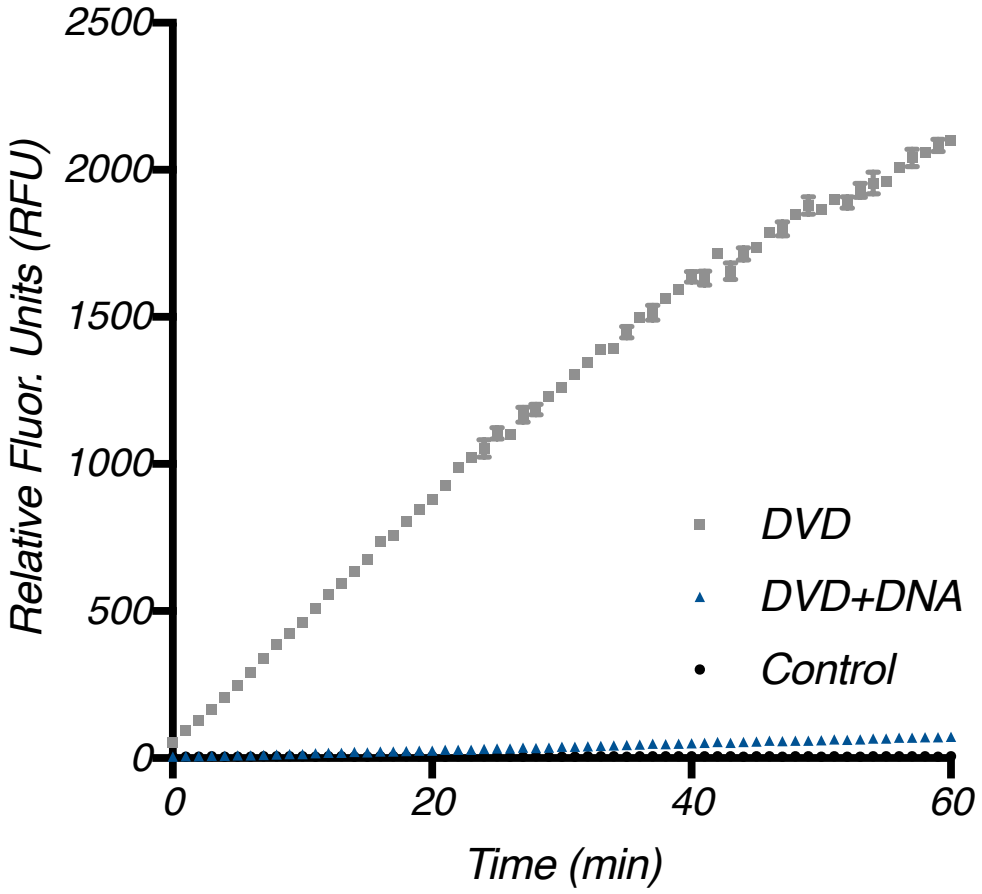


DNA-DVD conjugation procedure

DVD in storage solution was buffer exchanged into PBS by amicon spin filter (30 kDA) and concentrated to 25 μM (~5 mg/mL). This solution was used to dissolve (50 μL) solid DNA **62** to a presumptive concertation of 300 μM. In addition, a second tube containing 50 μL of DVD was aliquoted and taken through an identical process to serve as a positive control. Dissolving **62** with DVD solution was performed by vortexing the solution. This reaction mixture was incubated at 37 ºC for 8 hours to produce DNA-DVD conjugate **63**. The crude reactions mixture was analyzed for conjugation efficiency as previously described by Rader *et. al*.[3]

Methodol Assay

The methodol assay for conjugation confirmation was performed as described by Rader and co-workers. Briefly, aliquots (12.5 µL) of the reaction and control solutions were diluted (0.2 mg/mL relative to original antibody concentration) in PBS to a final volume of 310 µL. Each sample were dispensed (98 µL) in triplicate into a black 96-well plate. Three blank wells, containing PBS were also dispensed (98 µL) into the black plate. A plate reader was prepared, the wavelength of excitation (λext) was set to 330 nm and wavelength of emission (λem) was set to 452 nm. The instrument was programed to record every minute for 60 minutes and shake the plate in between. Finally, methodol (10 mM in ethanol) was added (2µL) to each well using a multichannel pipette and the plate was immediately loaded into the plate reader and data collection initiated. Signal was determined by normalizing against the blank wells. Measurements in triplicate were averaged and plotted along with standard deviation. Standard deviation was usually smaller than the marker size. Protocol adapted from: A. R. Nanna and C. Rader, *Methods Mol Biol*, **2019**, *2033*, 39-52.

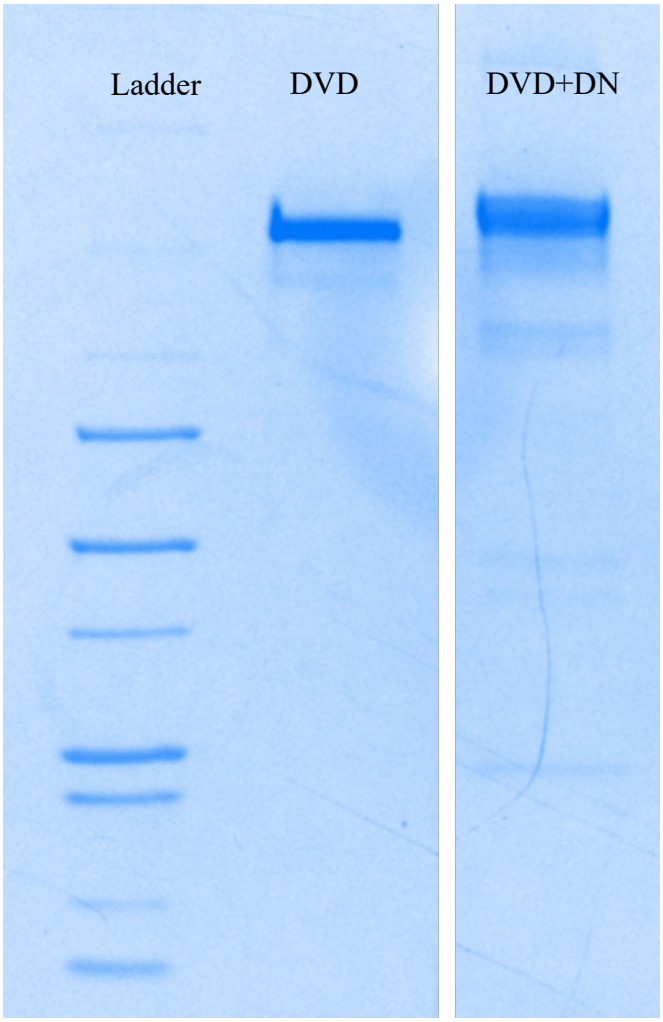


Methodol Assay Raw Data:

Time (min)	Control			DVD			DVD+DNA		
0	4.813	6.357	5.112	56.766	53.479	51.822	5.719	3.785	4.994
1	5.779	3.962	6.109	95.476	96.689	89.292	6.114	5.435	6.685
2	5.863	5.366	5.509	125.36	133.37	127.32	8.667	6.217	7.122
3	5.548	6.566	6.439	166.31	167.88	166.5	8.773	7.422	7.704
4	5.777	4.774	6.747	206.87	205.98	204.8	7.166	7.792	7.928
5	6.511	4.014	6.368	249.11	248.03	243.76	8.767	9.713	10.174
6	4.227	5.487	5.596	291.87	290.45	286.37	10.22	9.39	7.816
7	5.7	5.688	7.117	336.98	335.9	336.92	9.428	12.136	11.267
8	7.151	4.483	7.073	391.48	386.59	383.17	9.949	14.497	13.91
9	6.632	6.401	5.278	437.88	414.02	416.41	14.196	13.759	14.397
10	6.318	5.399	7.315	471.17	458.82	456.27	13.645	14.922	12.905
11	6.58	4.204	6.119	511.83	511.51	501.86	16.444	15.726	17.444
12	5.014	6.556	6.253	561.34	548.15	561.01	15.801	19.052	19.132
13	5.269	6.264	6.618	597.37	590.15	590.84	18.221	17.48	18.54
14	4.601	7.207	5.439	637.05	636.35	631.93	20.223	21.872	20.629
15	6.21	4.36	6.972	683.76	669.1	672.47	19.044	19.496	19.355
16	6.395	6.288	6.027	747.01	732.83	727.67	21.755	24.39	22.252
17	6.729	5.695	5.602	760.87	747.73	763.86	22.258	23.619	22.843
18	4.902	7.298	5.627	810.16	811.91	793.71	22.088	25.07	25.425
19	6.954	6.659	5.49	854.11	837.49	844.61	28.222	23.736	26.629
20	6.867	5.246	6.748	886.09	874.09	874.37	26.365	25.273	26.677
21	5.383	5.222	6.976	936.53	920.21	923.47	26.192	29.141	29.523
22	6.852	6.222	6.126	1005.7	980.58	983.83	27.259	31.357	29.347
23	5.513	6.902	6.138	1037.5	1015.6	1007.4	28.634	28.21	30.814
24	5.68	5.703	6.87	1085.9	1027.3	1046.5	30.017	32.884	31.785
25	6.004	7.747	6.449	1115.8	1081.8	1115.6	34.948	30.753	35.451
26	6.952	4.984	4.724	1114.8	1093.5	1097.8	33.571	31.443	34.195
27	5.418	7.521	4.372	1195.4	1159.5	1147.9	34.929	36.269	36.388
28	5.892	6.805	7.725	1206.3	1176.2	1175.5	33.615	36.81	33.069
29	5.745	5.655	4.88	1230	1219.6	1237.1	35.952	35.135	38.308
30	4.098	6.869	5.767	1251.1	1258.7	1277	38.678	36.053	41.436
31	6.541	5.135	5.41	1307.8	1299.2	1307.4	36.99	41.447	38.118
32	7.755	6.277	4.241	1350.1	1336.7	1347.9	41.741	38.865	42.119
33	4.777	6.663	6.414	1402.9	1378.5	1385	39.111	44.909	43.161
34	5.165	7.52	6.512	1403.6	1373.3	1402.5	41.781	45.707	44.158
35	6.156	4.771	6.389	1457.2	1426.4	1463.8	42.858	45.501	45.584
36	7.009	5.765	5.657	1492.6	1495.6	1506.8	45.72	46.675	47.945
37	7.032	5.683	5.893	1533.3	1484.8	1524	47.531	50.302	49.256
38	4.559	8.857	5.67	1564.5	1556.7	1570.4	48.533	44.907	50.719
39	5.461	7.194	5.188	1602	1578.7	1595.3	45.227	49.651	52.87
40	6.613	6.042	7.168	1636	1617	1654.6	47.746	50.65	53.776
41	5.345	6.697	5.75	1653.5	1605.3	1635.6	51.599	53.259	51.561
42	7.773	6.158	5.62	1715.4	1702.6	1735.8	53.903	55.252	55.449
43	5.055	6.443	7.933	1669.5	1621.4	1674.1	52.7	53.942	51.127
44	4.025	6.35	5.12	1728.1	1690.6	1723.1	51.876	57.908	56.334
45	5.898	5.723	6.062	1730.9	1738.4	1741	56.076	57.615	55.425
46	6.305	6.259	6.21	1781.5	1786.7	1793	60.277	58.895	57.506
47	5.971	6.406	4.709	1825.4	1794.8	1779.1	56.55	56.585	60.932
48	5.629	7.202	5.665	1862.6	1831.8	1848.1	56.339	61.973	59.941
49	6.416	8.093	6.528	1885.7	1846.9	1905	58.923	59.38	59.887
50	6.283	5.27	6.002	1855.1	1871	1868.2	59.419	60.198	62.85
51	6.523	6.58	5.649	1905.1	1891.5	1898.9	60.782	64.608	66.772
52	7.091	6.33	4.083	1909	1869.8	1888.8	62.243	62.215	63.381
53	4.3	5.85	4.851	1948.5	1940.2	1903.4	64.338	64.485	63.638
54	5.291	7.323	7.095	1995.6	1924.7	1945.8	65.252	69.041	65.932
55	6.488	5.384	6.398	1977.5	1948.2	1958.5	64.439	68.919	65.464
56	6.149	4.663	7.725	2007.1	2016.1	1998.5	64.769	73.471	71.441
57	6.4	6.638	6.978	2071.5	2010.7	2038.2	67.684	72.716	70.504
58	6.732	5.518	6.723	2068.9	2048.8	2062.4	71.295	70.981	73.276
59	7.388	4.446	5.686	2099.6	2090.8	2060.4	68.77	73.455	73.328
60	6.68	4.384	7.986	2100.4	2110.4	2092.1	67.195	76.714	72.241

SDS PAGE Analysis

SDS Page was performed to confirm mass increase of the DVD-DNA construct. For this analysis 1 µg of the reaction and the control were aliquoted into PCR tubes. To these tubes 6X Lamelli buffer (6µL) was added and finally the tubes were diluted with water (to 24 µL). These reactions were heated at 95 ºC for 10 minutes before being loaded into separate lanes on a precast Bio-Rad 4-20% SDS PAGE Gel. To another lane of Bio-Rad Precsion Plus Protein standard was added (7 µL). The rest of the lanes were loaded with 6x Lamelli buffer (6µL). The gel was run at 200V for 30 minutes at which point it was stained with Coomassie protein stain and distained with water over night. Finally, the gel was imaged on a Bio-Rad gel imager. Protocol adapted from: A. R. Nanna and C. Rader, *Methods Mol Biol*, **2019**, *2033*, 39-52.



Creation of Dual Labeled Probes:

Procedure For the Creation of 65-67

64→SI-41

SENDR was used to access the dual label probes. The first tag was appended to the 3' end of DNA **64** using *General Procedure 2.* Briefly, 25 nmol of precursor **64** was loaded onto 100 µL of equilibrated resin. This loaded resin was washed with DMA (500 µL twice) and dry THF (500 µL three times) and dried under vacuum. **Ψ-5** in MeCN (150 mM, 250 µL) was added to the loaded and dried resin. Then DBU (450 mM, 18 µL) was added to the reaction mixture. The reaction tube was vortexed for 30 seconds and incubated at 37 ºC for various amounts of time. The reactions were worked up by aspirating and discarding the reaction solution, and washing the resin bed with MeCN (500 µL) and 1:1 MeCN:PBS (500 µL). Elute buffer (300 µL) was added to the resin bed and the tube was agitated by orbital shaker for 5-10 minutes. The DNA containing elution buffer was collected by carefully pipetting the supernatant. DNA was isolate *via* ethanol precipitation. Ethanol precipitation was performed by adding 10% v/v of a NaCl solution (30 µL, 5M) to the elute buffer and three volumes of cold ethanol (-20ºC) were added to the tube (~1000µL) and incubated for 18 hours at -20 ºC. The tube was then centrifuged at 13,000 rpm for 15 minutes to pellet the DNA, and the extra ethanol was decanted off. The tubes were dried *via* speed vacuum and the DNA was dissolved and HPLC-MS analysis was performed.

SI-41→65

Dephosphorylation of the 5' end of **SI-41** to generate **65** along with degradation of unlabeled 3' DNA was carried out as follows. The crude reaction mixture from the previous step was dissolved in water (120 µL). This solution was aliquoted (40µL) into three separate PCR tubes. To these tubes, Cut Smart 10X Buffer was added (5µL) and the tubes were vortexed. Finally, rSAP (2.5 µL) and ExoIII (2.5 uL) was added to each tube. These tubes were vortexed, spun down and placed in a thermocycler. They were incubated at 37 ºC for 60 min, then the enzymes were deactivated at 70 ºC for 10 minutes. The reaction mixtures were isolated by zymo spin column.

65→66

65 was loaded onto resin and modified with SENDR by *General Procedure 2* using **Ψ-17.** After this reaction, the crude reaction mixture is ethanol precipitated, pelleted, dried, dissolved and analyzed by HPLC-MS.

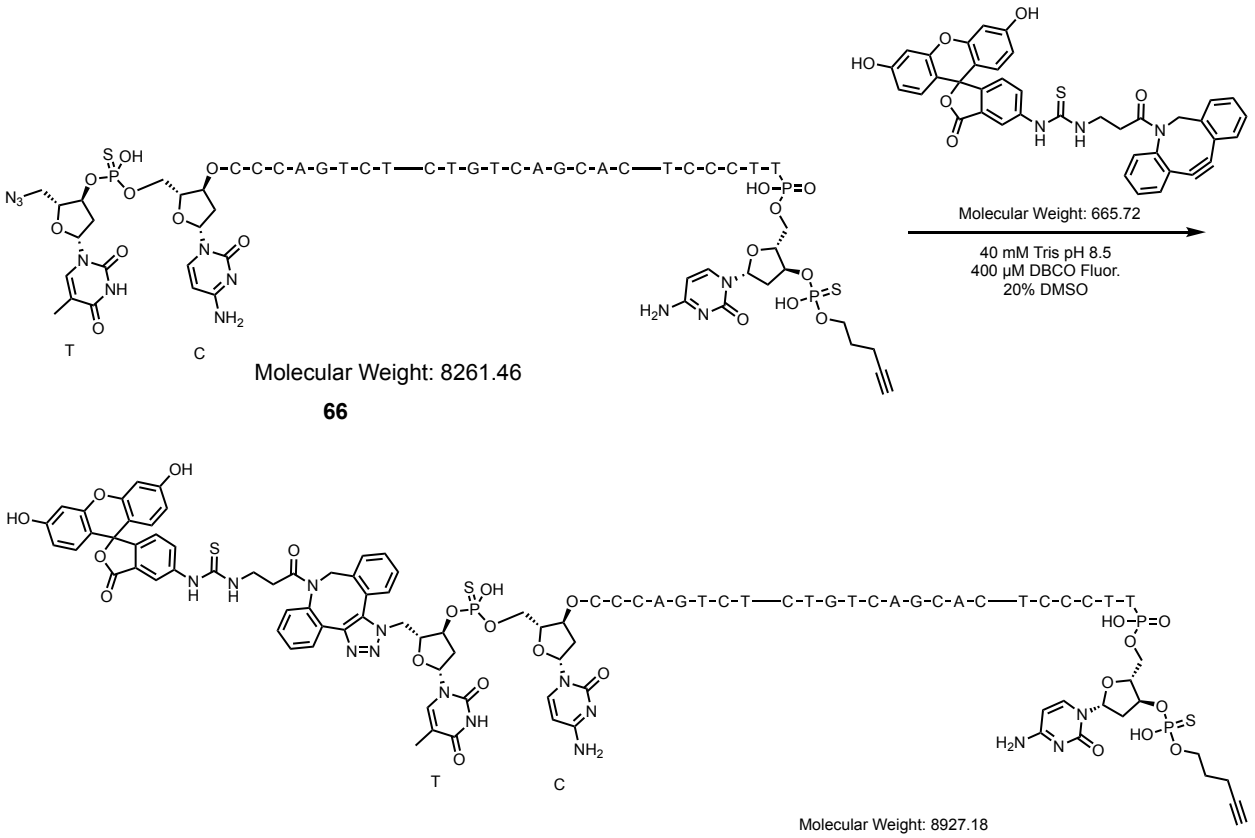
Lambda Exo Cleanup of 66

66 was loaded into a PCR tube (5 µg) in 30 uL water. To each tube CutSmart (5 µL at 10x), ATP (5µL at 5 mM) and DTT (5µL at 50 mM) was added. Finally Lambda exonuclease (2.5 µL) and T4PNK (2.5 µL) were added. These tubes were incubated for 90 minutes at 37ºC at which point the enzymes were thermally deactivated at 75ºC for 10 minutes.

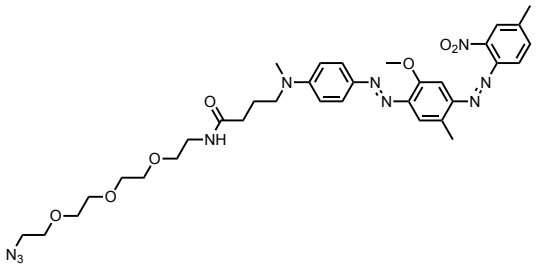
66→SI-42→67

66 was added to a PCR tube in 38 uL water. To this tube DMSO (8 uL), Tris buffer (2 µL, 1M at pH 8.5) and DBCO Fluorescein (2 µL, 10 mM, in DMSO) were added. This reaction was allowed to incubate at 37ºC for 120 minutes and excess regents were removed by zymo spin column. Resulting in FAM labeled probe **SI-42.** To **SI-42** in water (30 µL), Tris Buffer (1 uL, 1 M pH 8.5), BTTP (2 µL, 20 mM in water), $CuSO_4$ (1 µL, 20 mM, in water), DMSO (14 µL), $MgCL_2$ (1 µL, 1 M in water), and BHQ-Azide **SI-61** (1 µL, 5 mM in DMSO) were added. Finally, NaAsc (1 µL 100mM) was added and the reaction was incubated at 37ºC for 60 minutes. The final dual labeled probe **67** was isolated by ethanol precipitation and analyzed by HPLC MS.

66→SI-42



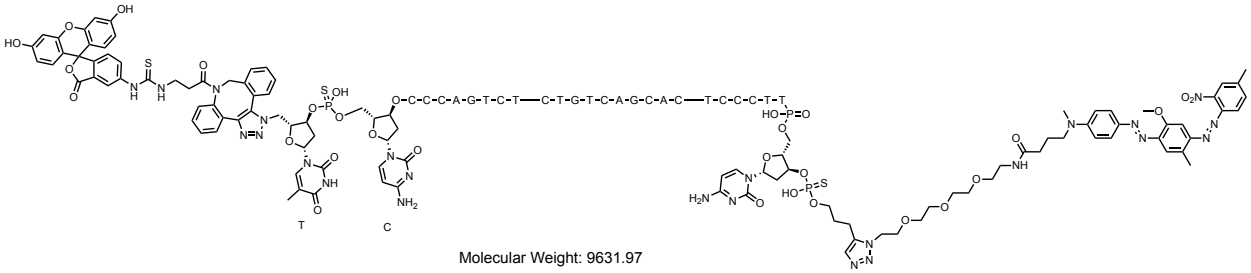
SI-42→67



Molecular Weight: 704.79

SI-42

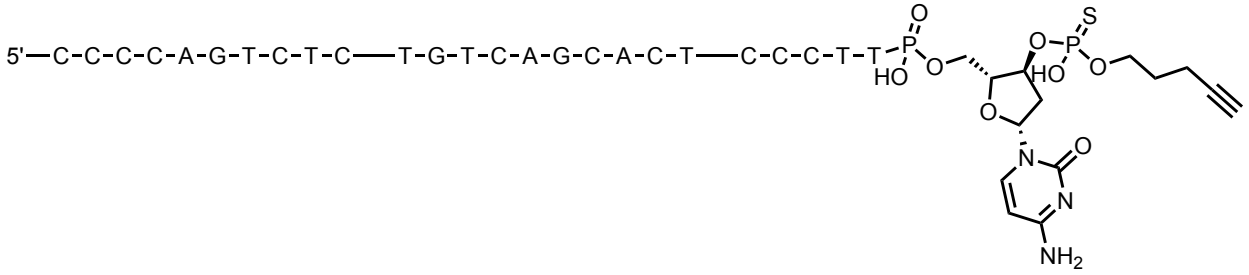
100 μM BHQ_1 Azide
800 μM BTTP
400 μM $CuSO_4$
20 mM Tris pH 8.5
20 mM $MgCl_2$
2 mM NaAsc
15% DMSO



Molecular Weight: 9631.97

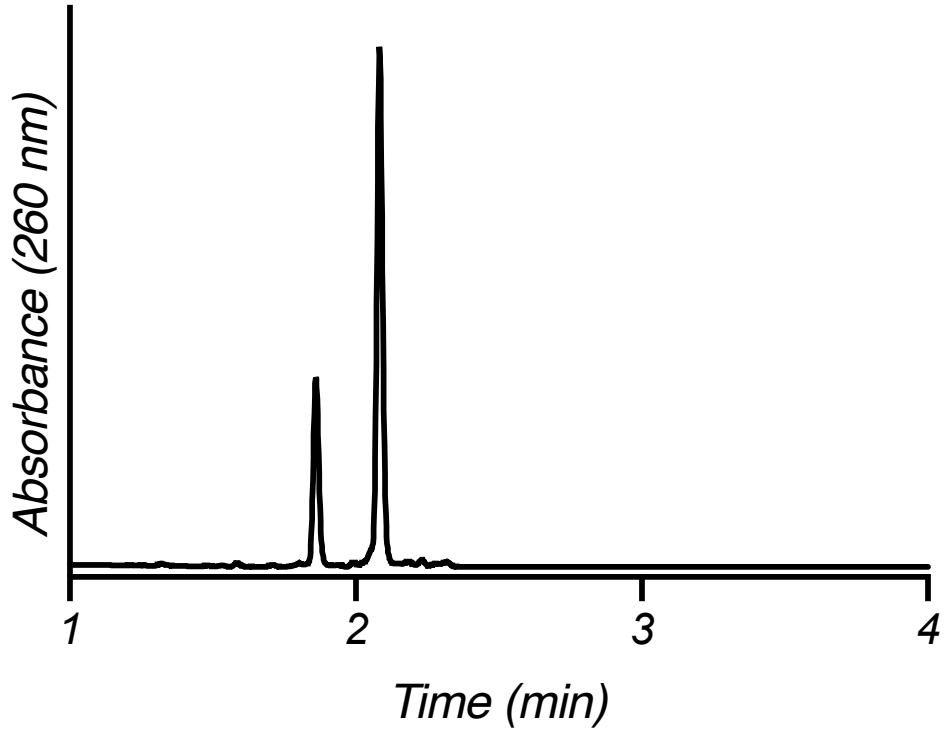
LCMS Characterization of SI-41

Produced from **64** by General Procedure **3:**
Using **Ψ-5**
Expected Mass: 7996.2
Expected (M-7)/7 Ion: 1141.3
Found Deconvoluted Mass: 7996.0
Found (M-7)/7 Ion: 1140.93

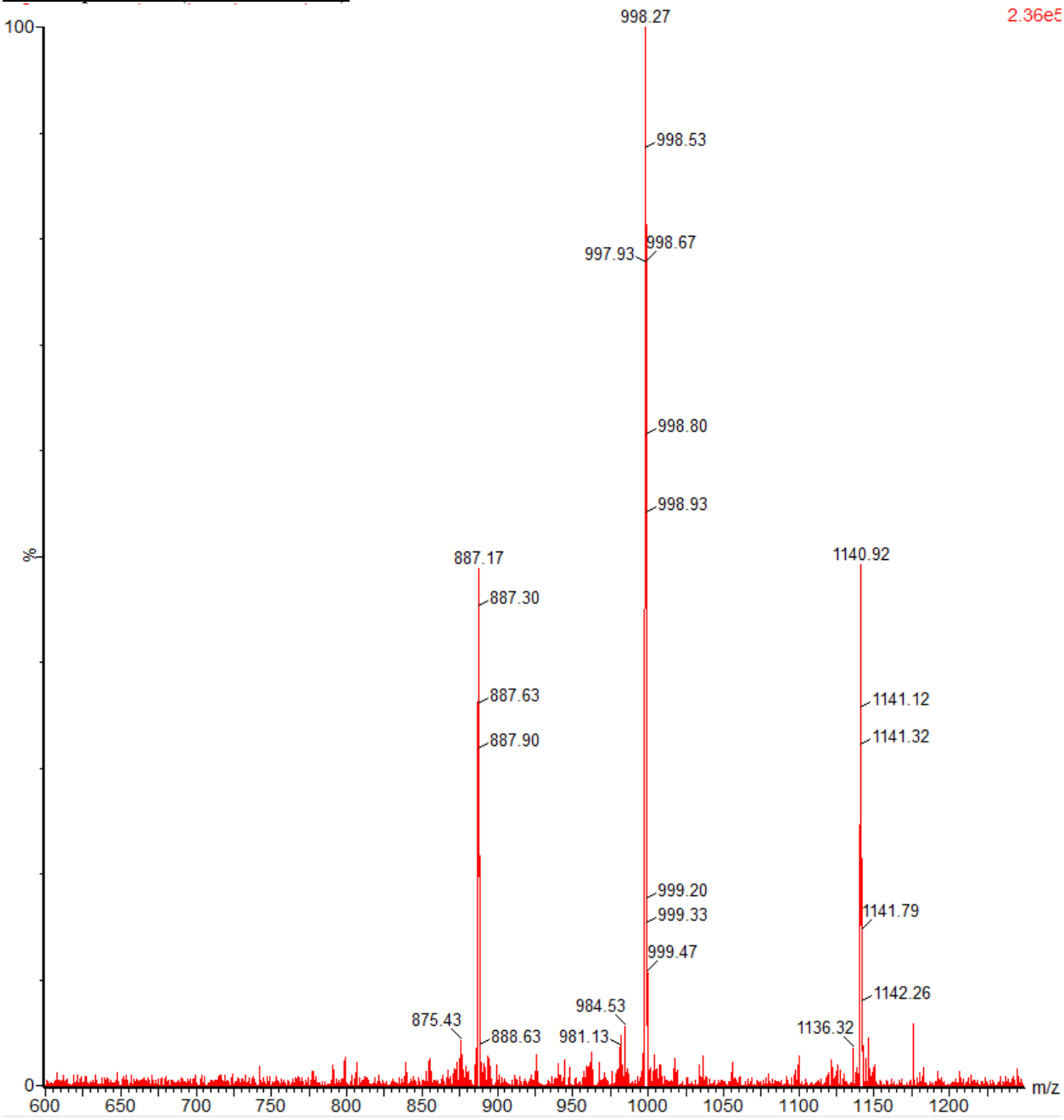


Molecular Weight: 7996.17 C

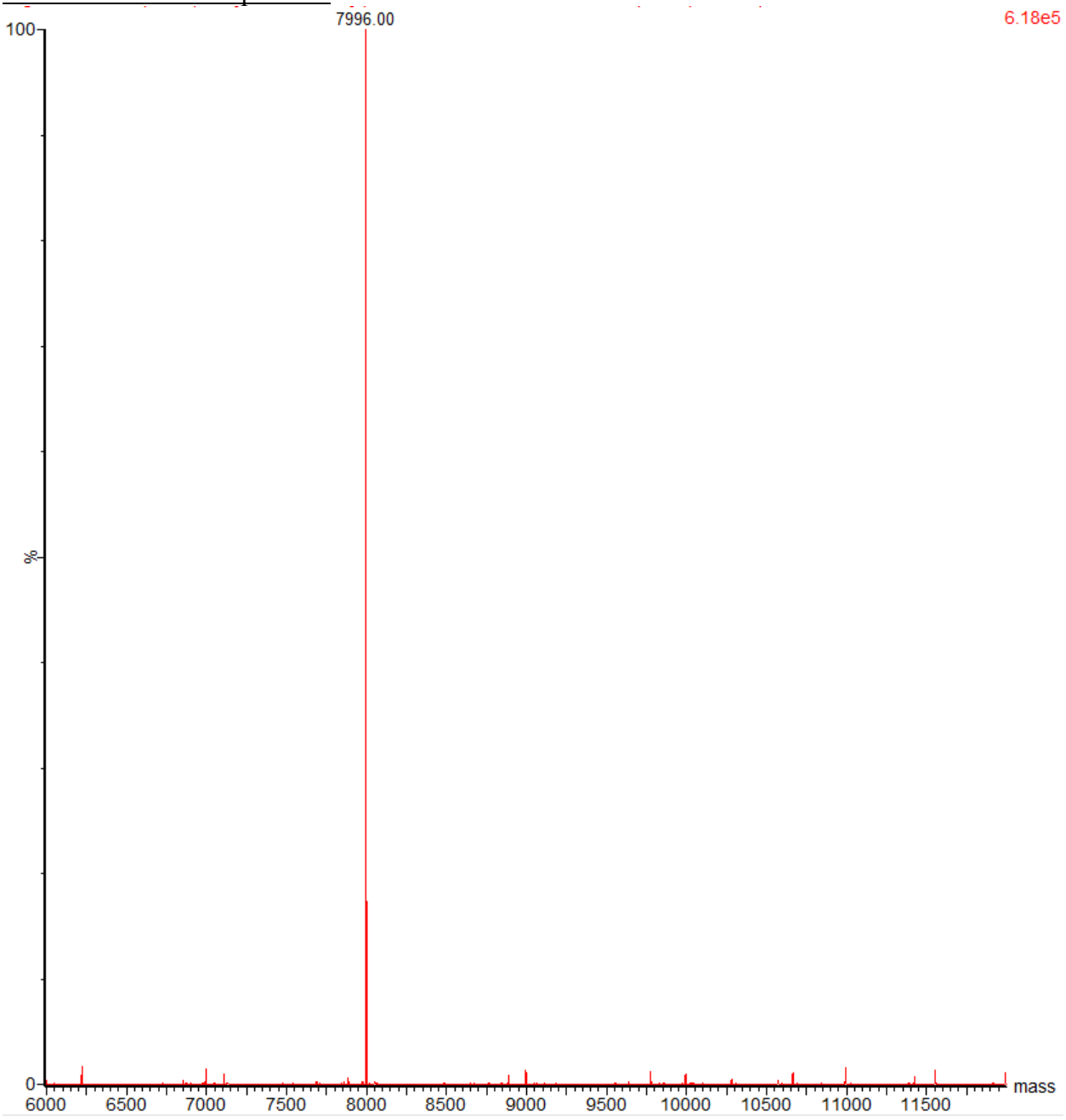
A260 Chromatogram



Mass Spectrum (RT= 2.10 min)

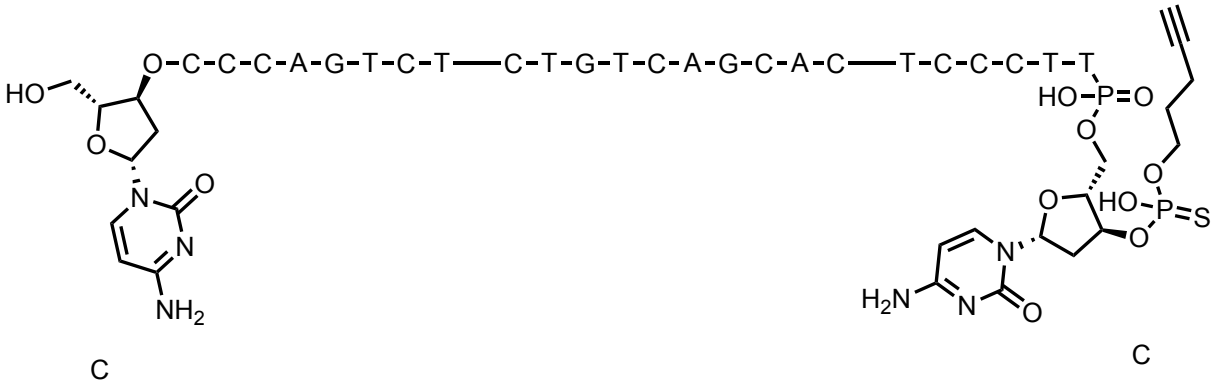


Deconvoluted Mass Spectrum



LCMS Characterization of 65

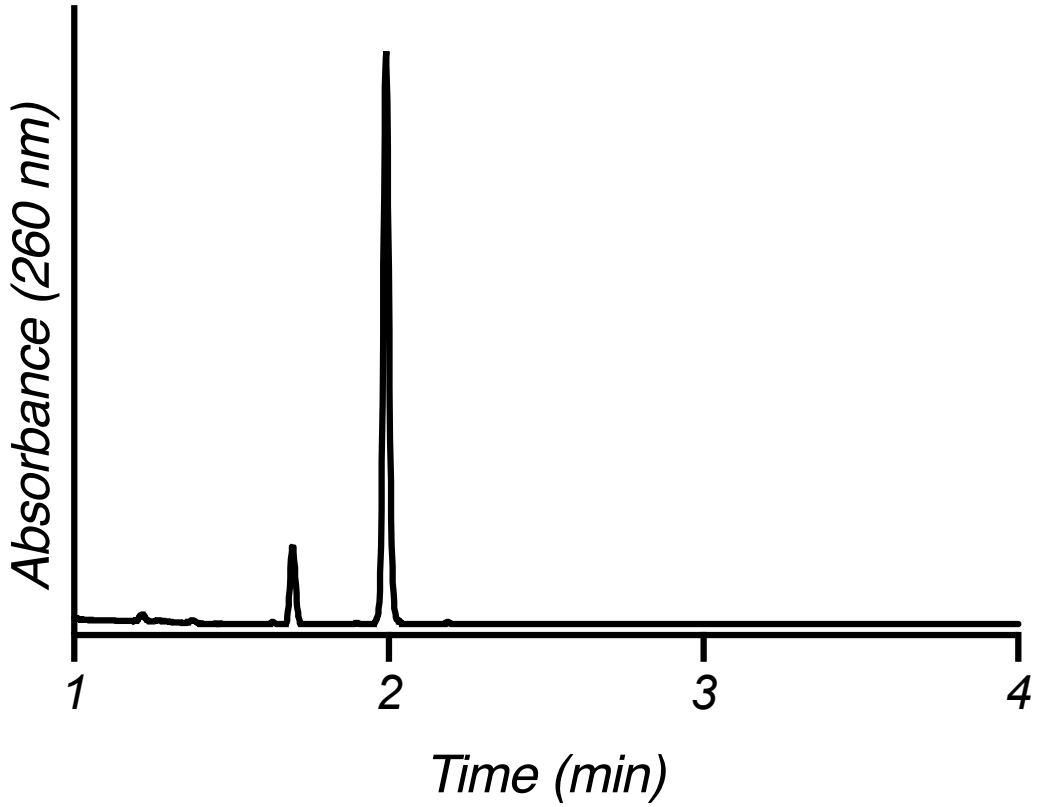
Produced from **SI-41** by rSAP and ExoIII**:**
Expected Mass: 7916.2
Expected (M-7)/7 Ion: 1129.88
Found Deconvoluted Mass: 7917.0
Found (M-7)/7 Ion: 1129.65



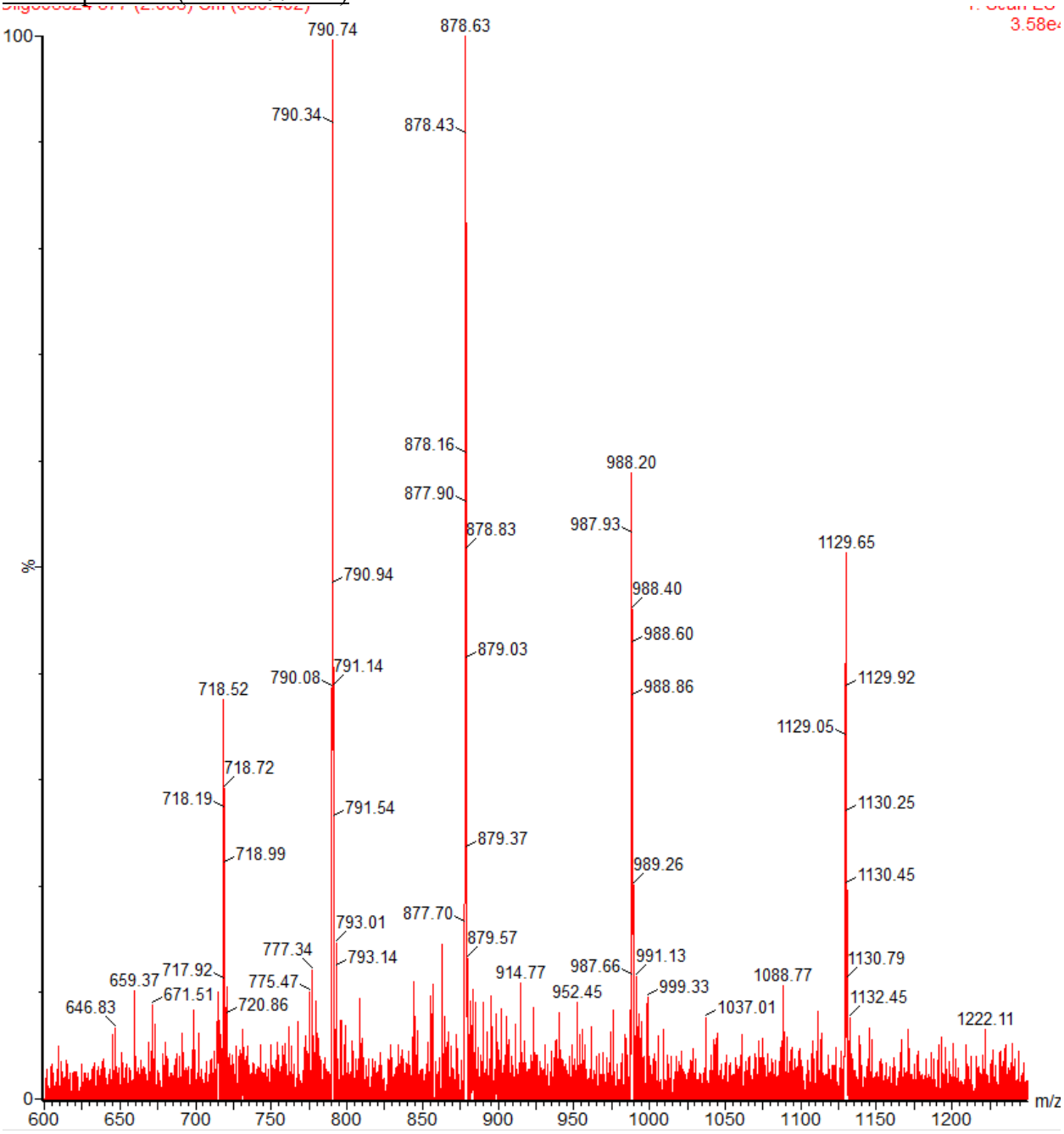
C C

Molecular Weight: 7916.19

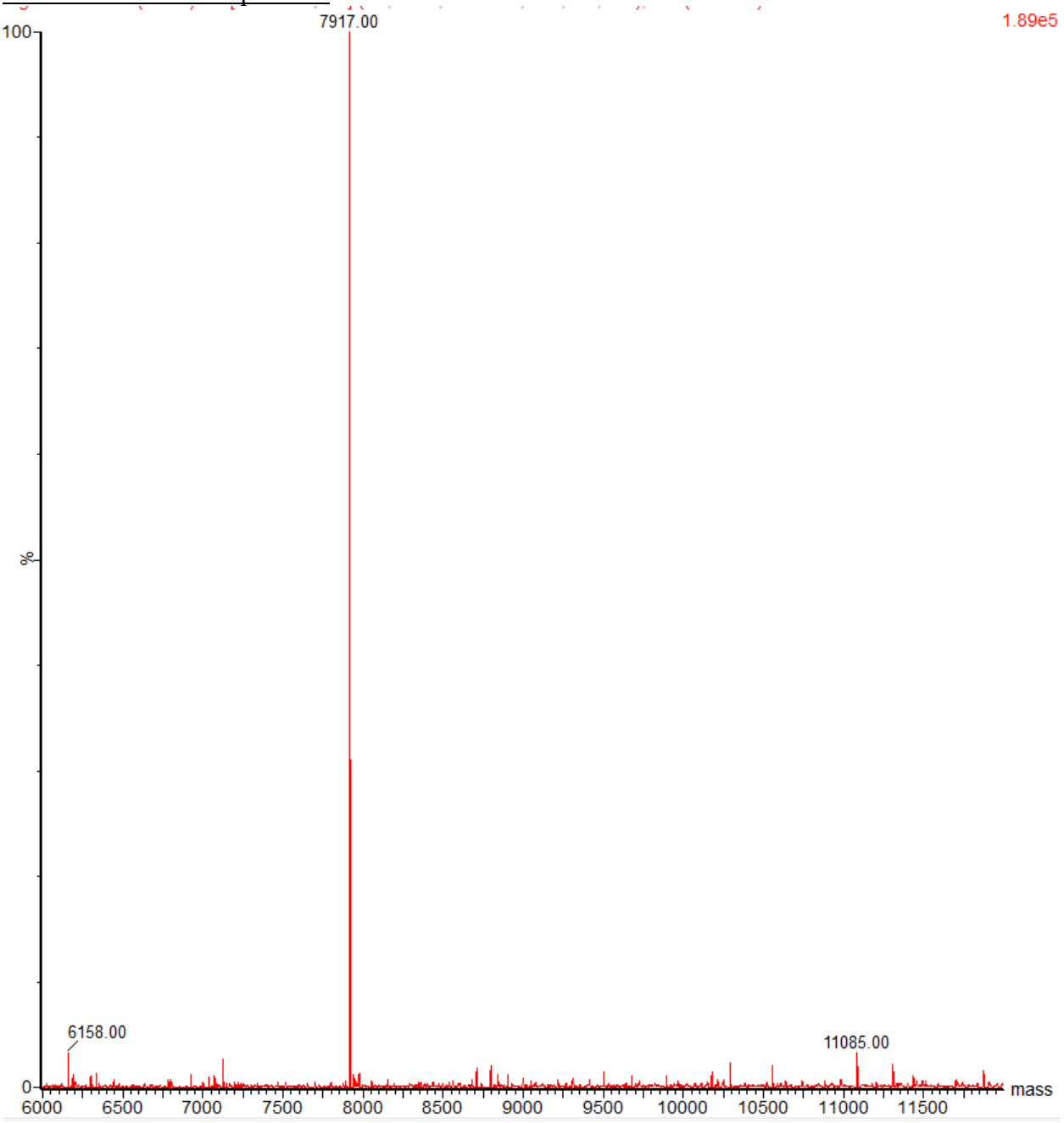
A260 Chromatogram



Mass Spectrum (RT= 2.07 min)

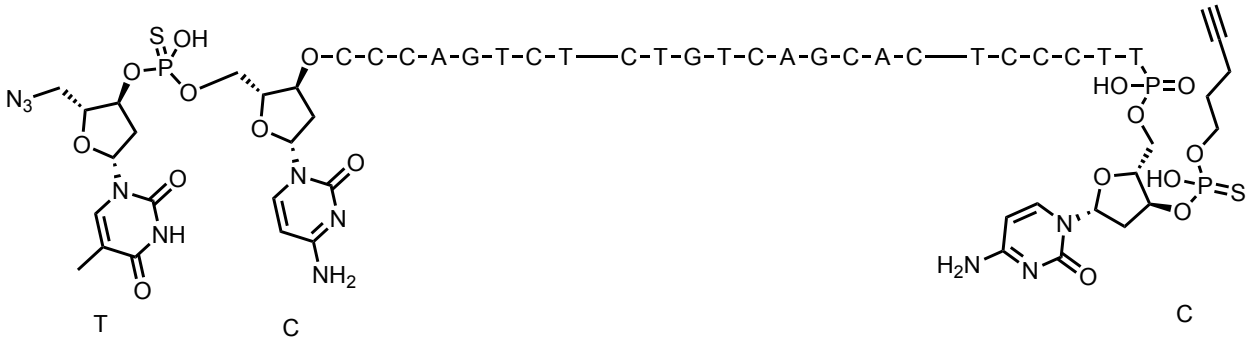


Deconvoluted Mass Spectrum



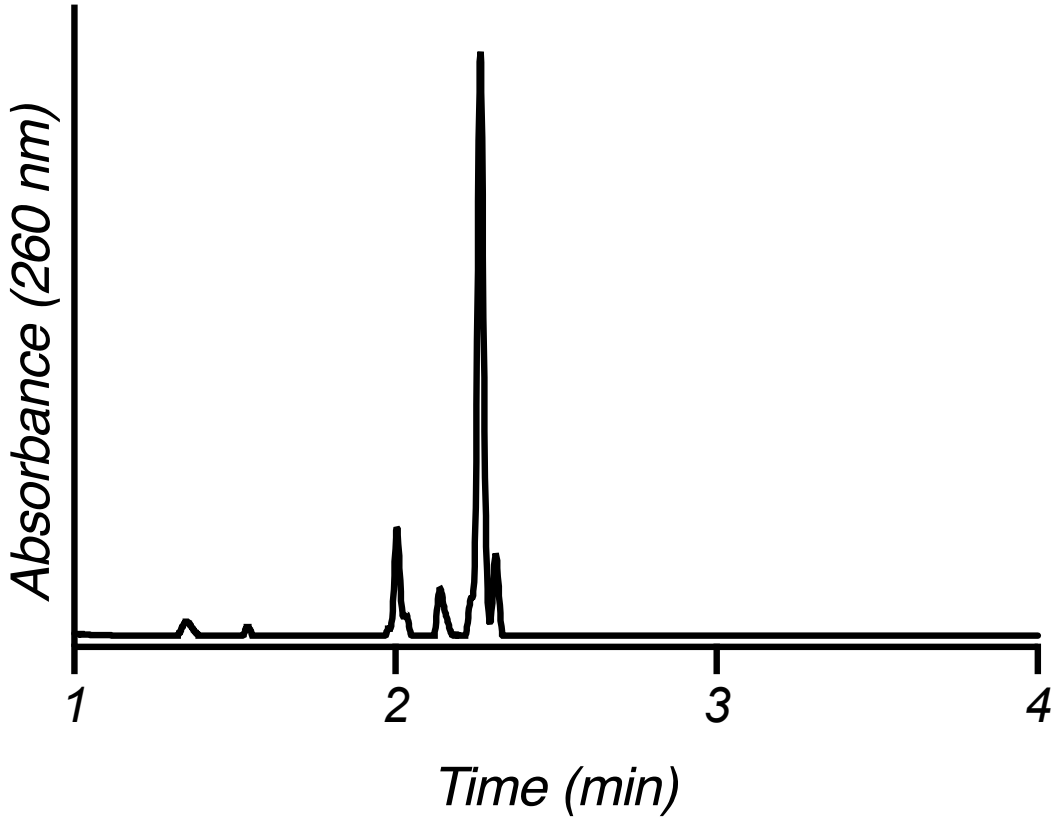
LCMS Characterization of 66

Produced from **59** by General Procedure **2:**
Using **Ψ-17**
Expected Mass: 8261.5
Expected (M-7)/7 Ion: 1179.2
Found Deconvoluted Mass: 8262.0
Found (M-7)/7 Ion: 11790.1

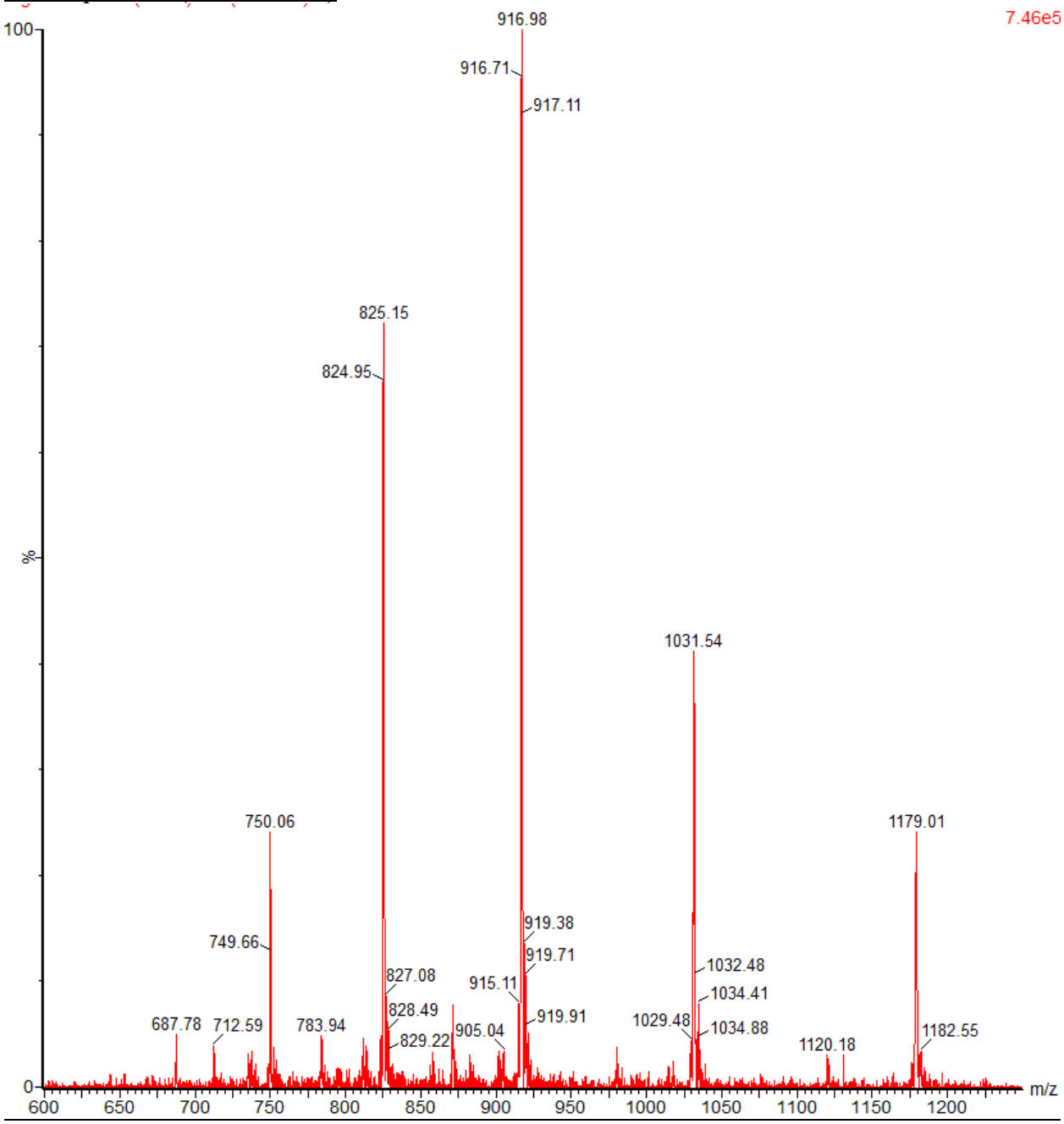


Molecular Weight: 8261.46

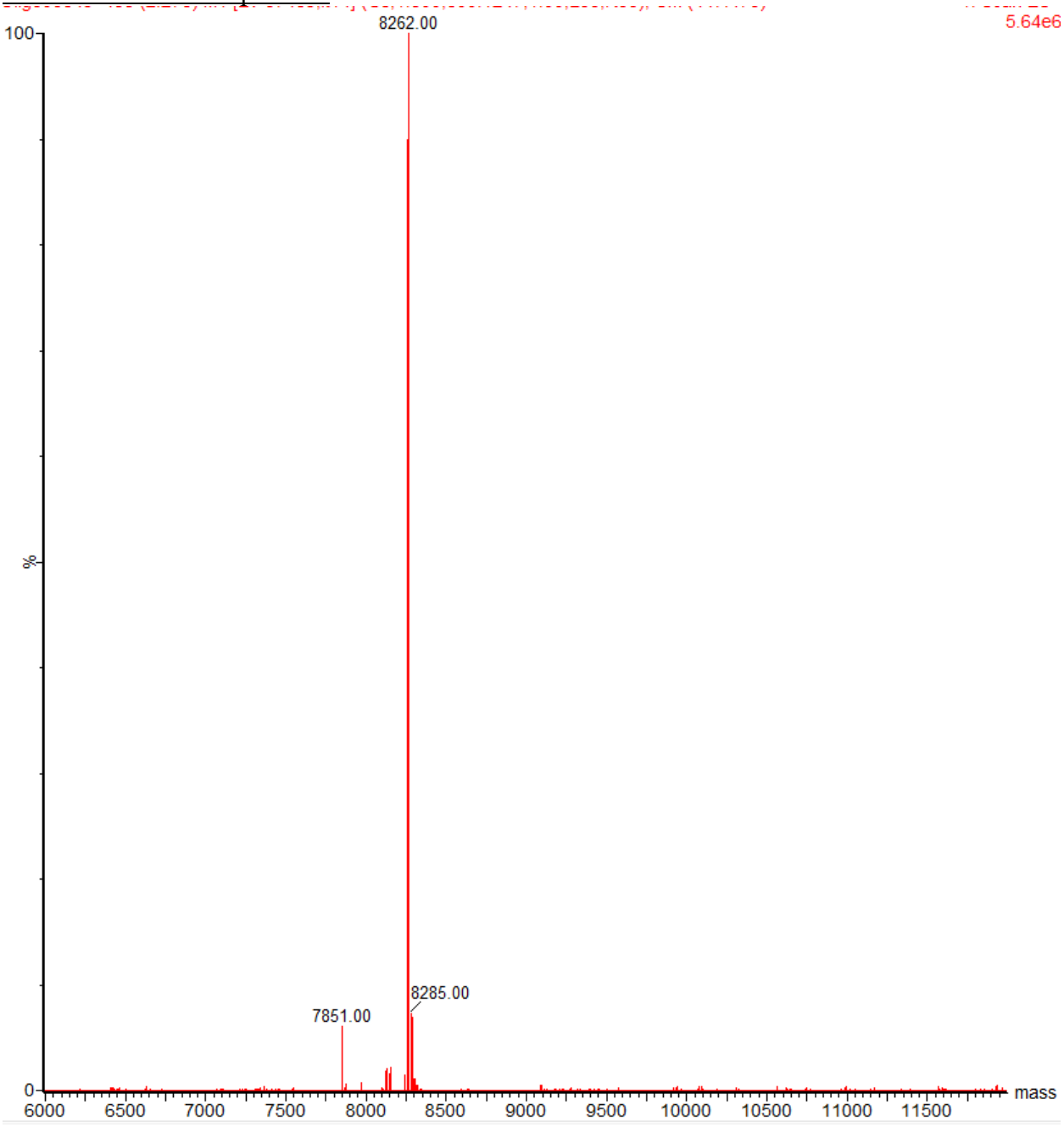
A260 Chromatogram



Mass Spectrum (RT= 2.2 min)

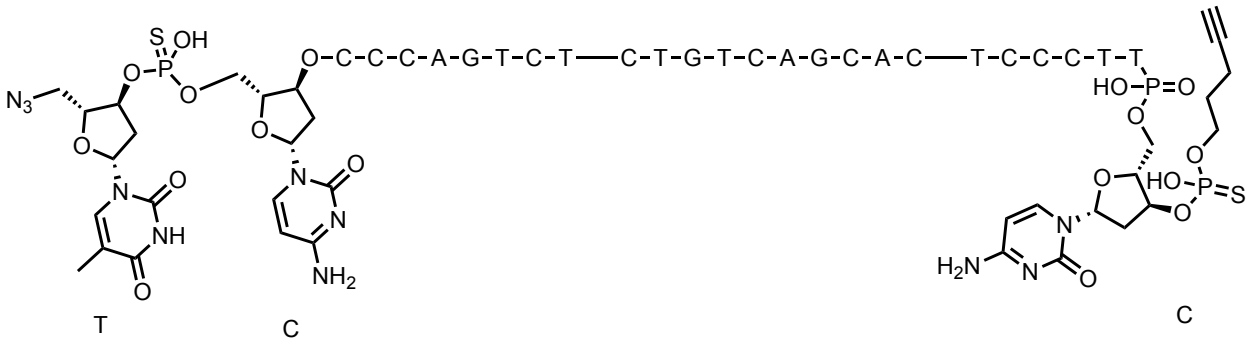


Deconvoluted Mass Spectrum



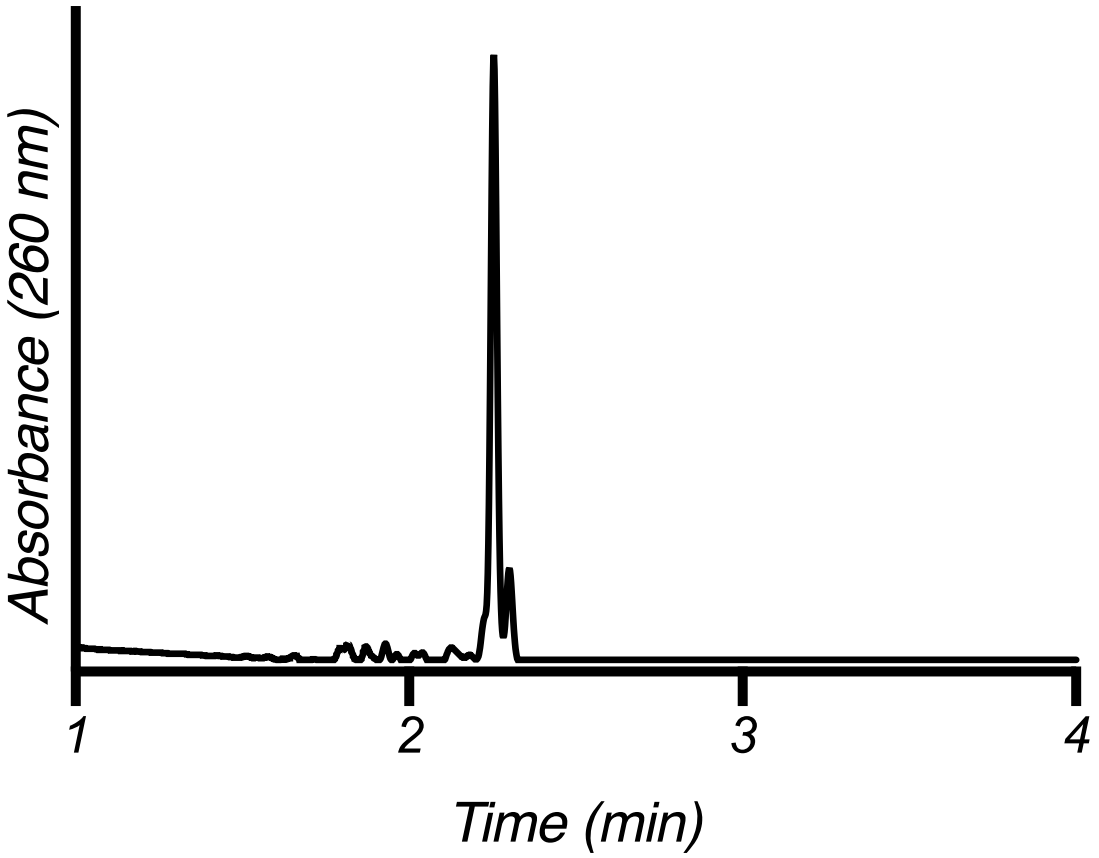
LCMS Characterization of 66 After Enzymatic Cleanup

Produced from **66:**
Expected Mass: 8261.5
Expected (M-7)/7 Ion: 1179.2
Found Deconvoluted Mass: 8262.0
Found (M-7)/7 Ion: 11790.1

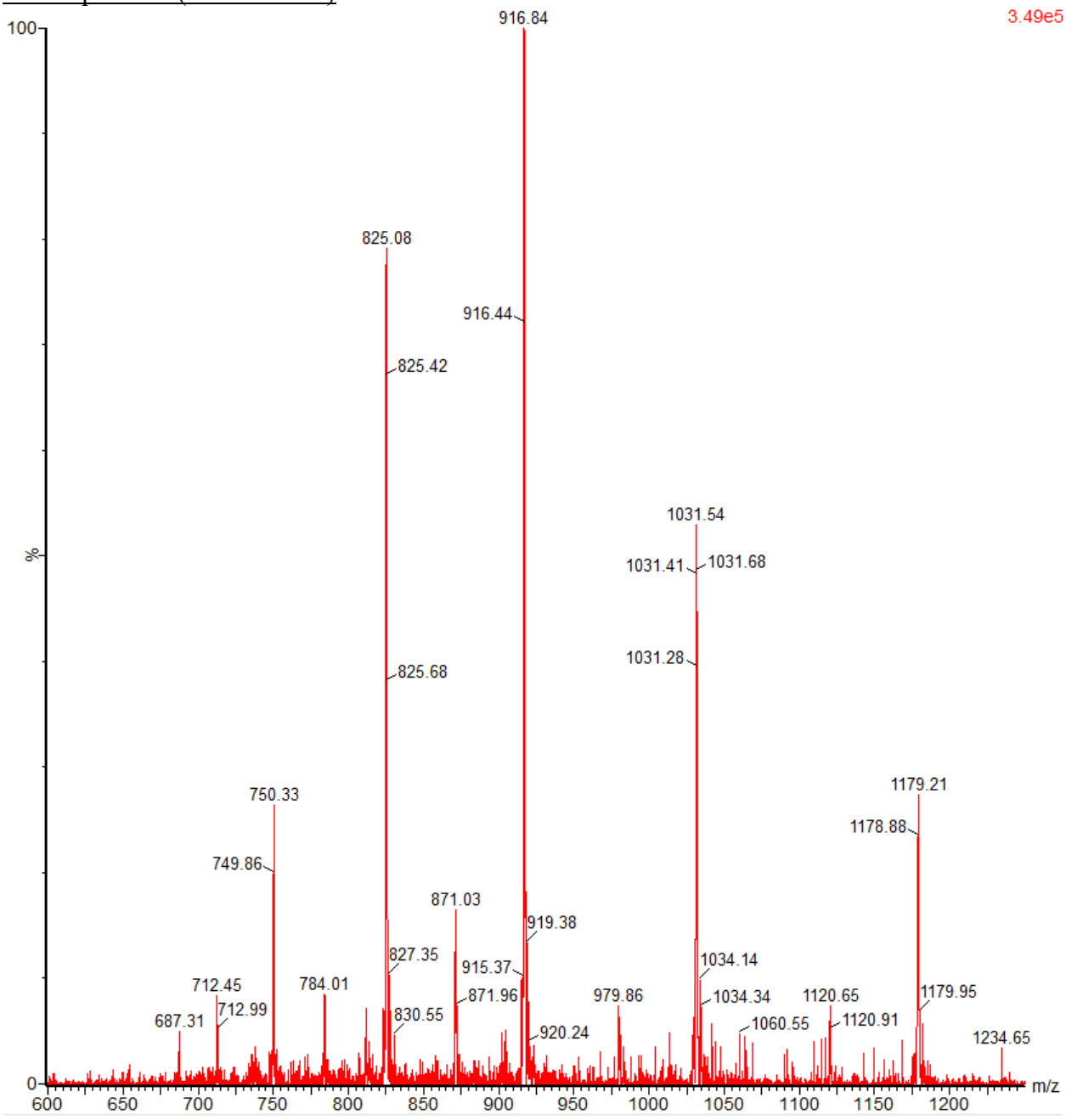


Molecular Weight: 8261.46

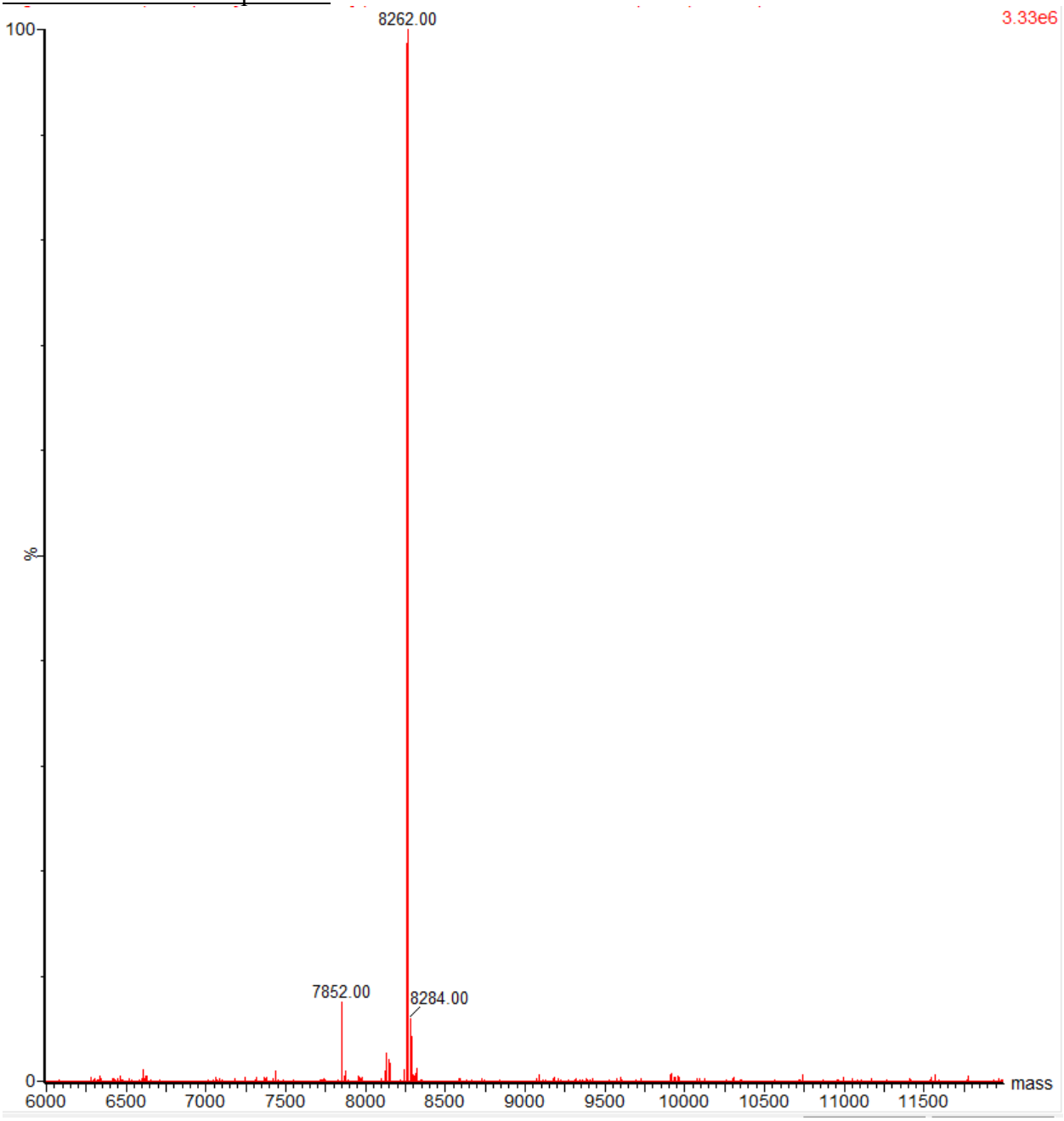
A260 Chromatogram



Mass Spectrum (RT= 2.2 min)

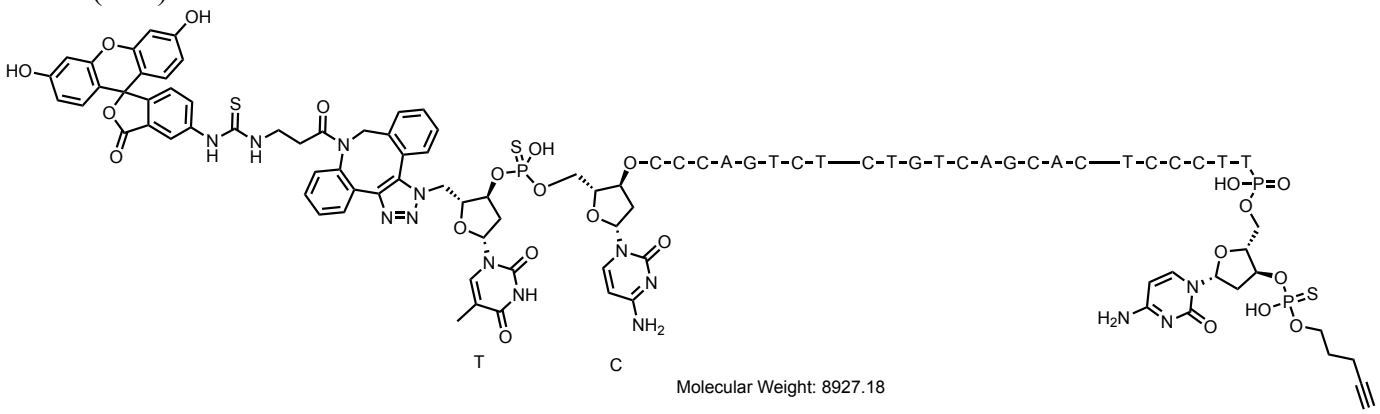


Deconvoluted Mass Spectrum



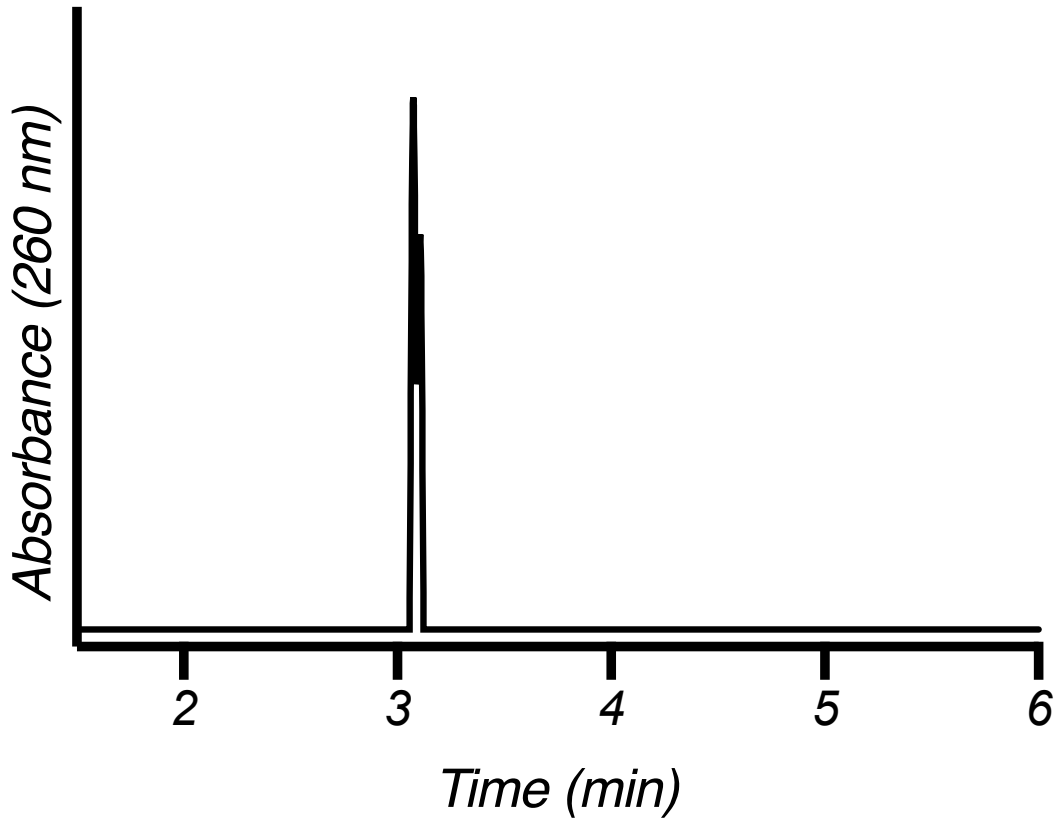
LCMS Characterization of SI-42

Produced from **66** by General Procedure **SPAAC:**
Expected Mass: 8927.2
Expected (M-8)/8 Ion: 1114.9
Found Deconvoluted Mass: 8927.00
Found (M-7)/7 Ion: 1114.51

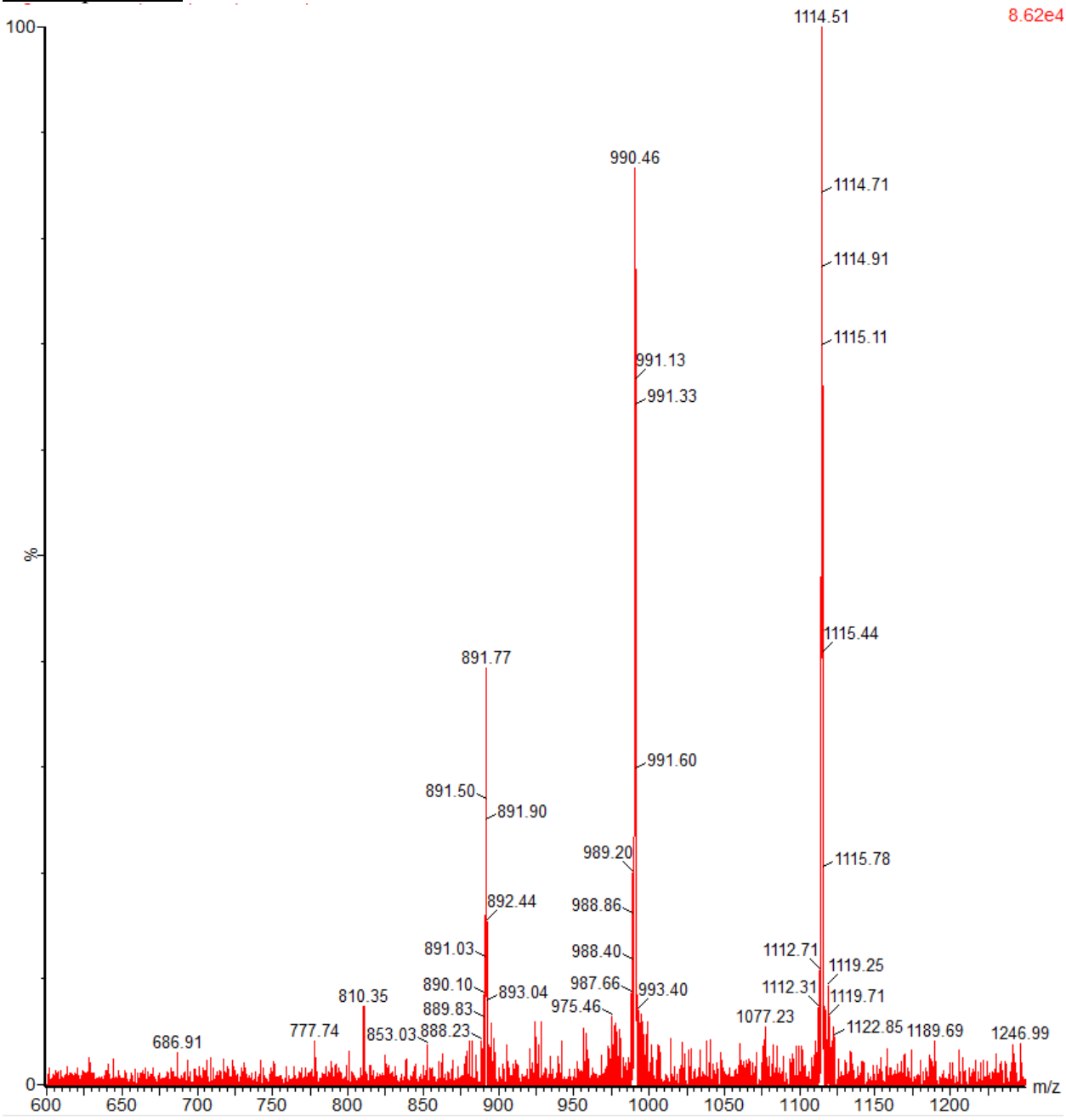


+Isomer

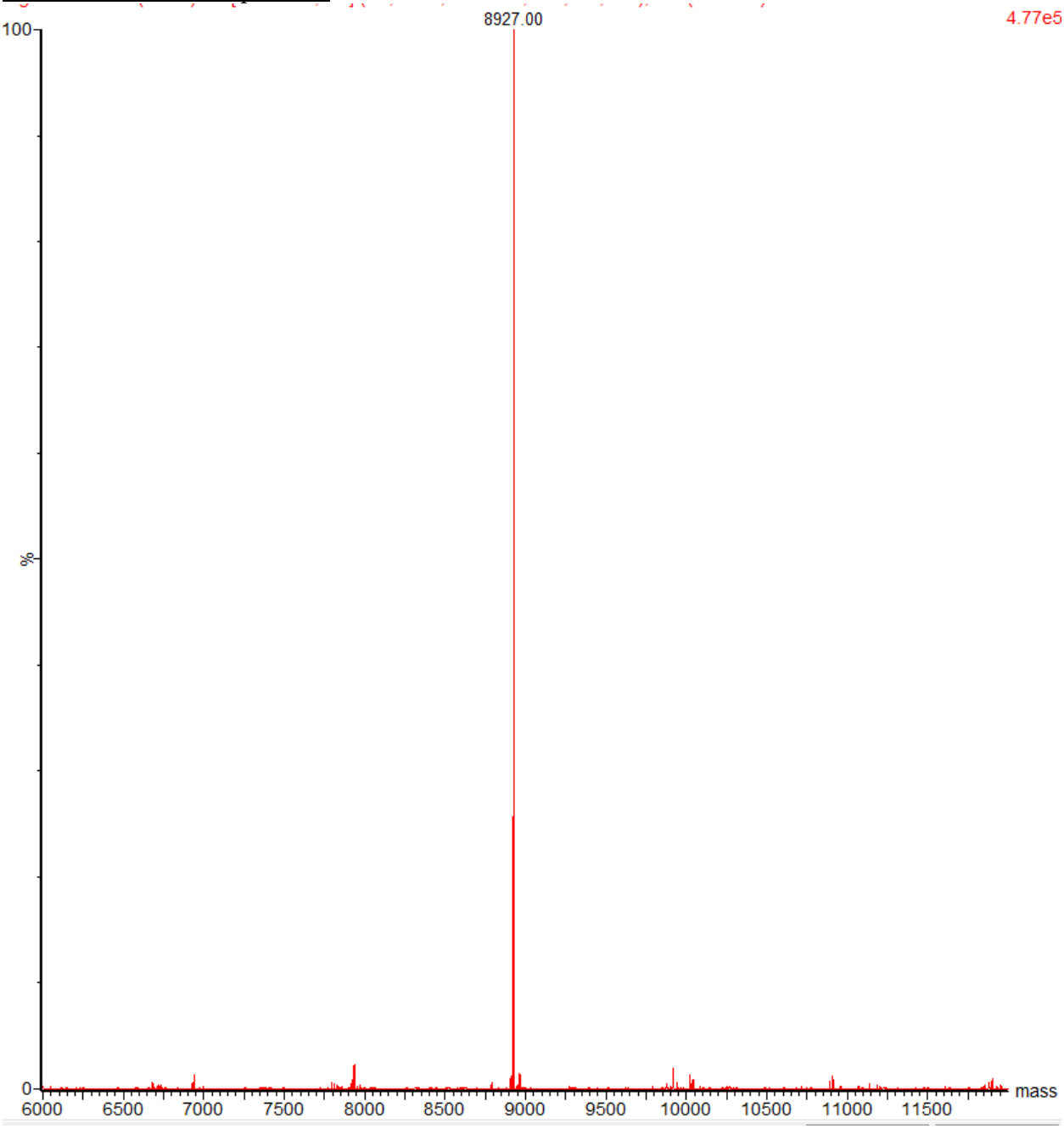
A260 Chromatogram



Mass Spectrum

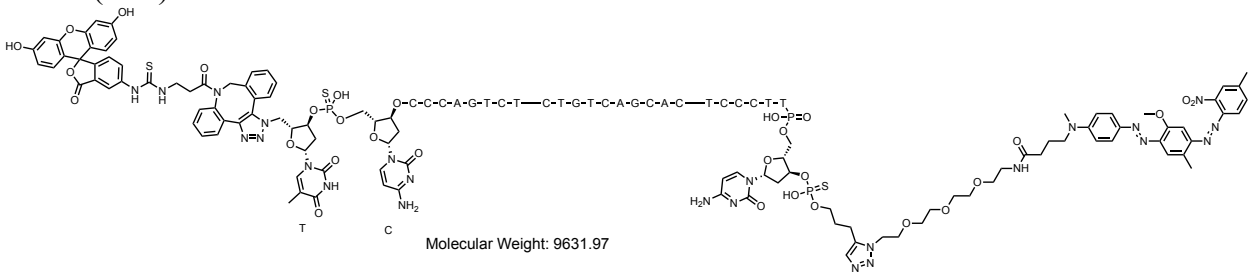


Deconvoluted Mass Spectrum



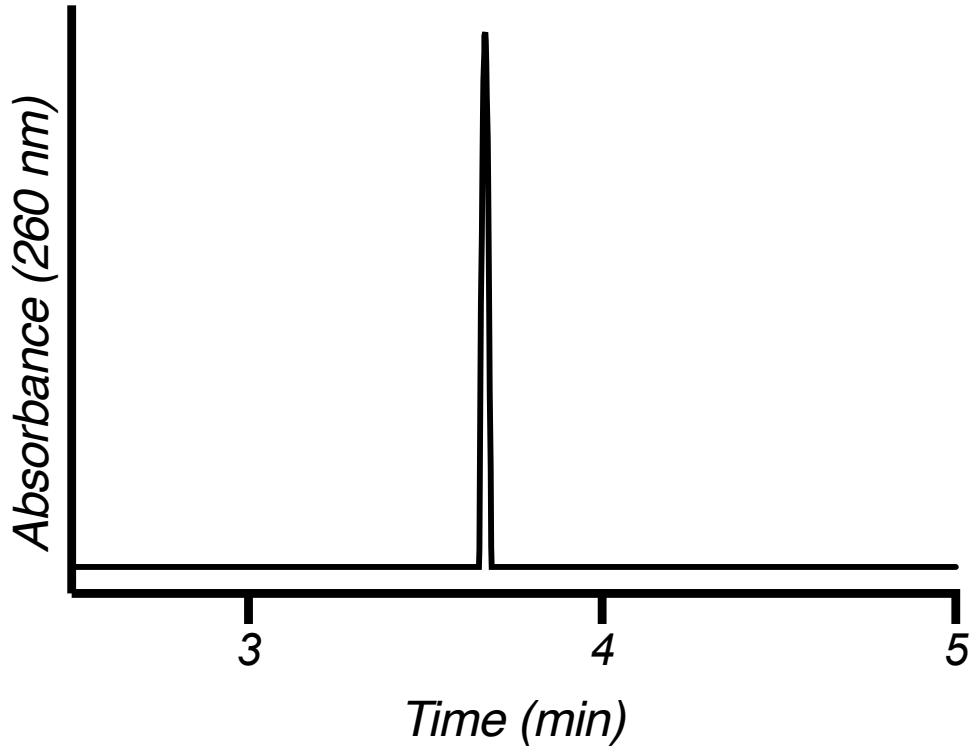
LCMS Characterization of 67

Produced from **SI-42** by General Procedure **CuAAC**
Using **Ψ-67**
Expected Mass: 9631.97
Expected (M-8)/8 Ion: 1202.98
Found Deconvoluted Mass: 9631.0
Found (M-7)/7 Ion: 1202.7

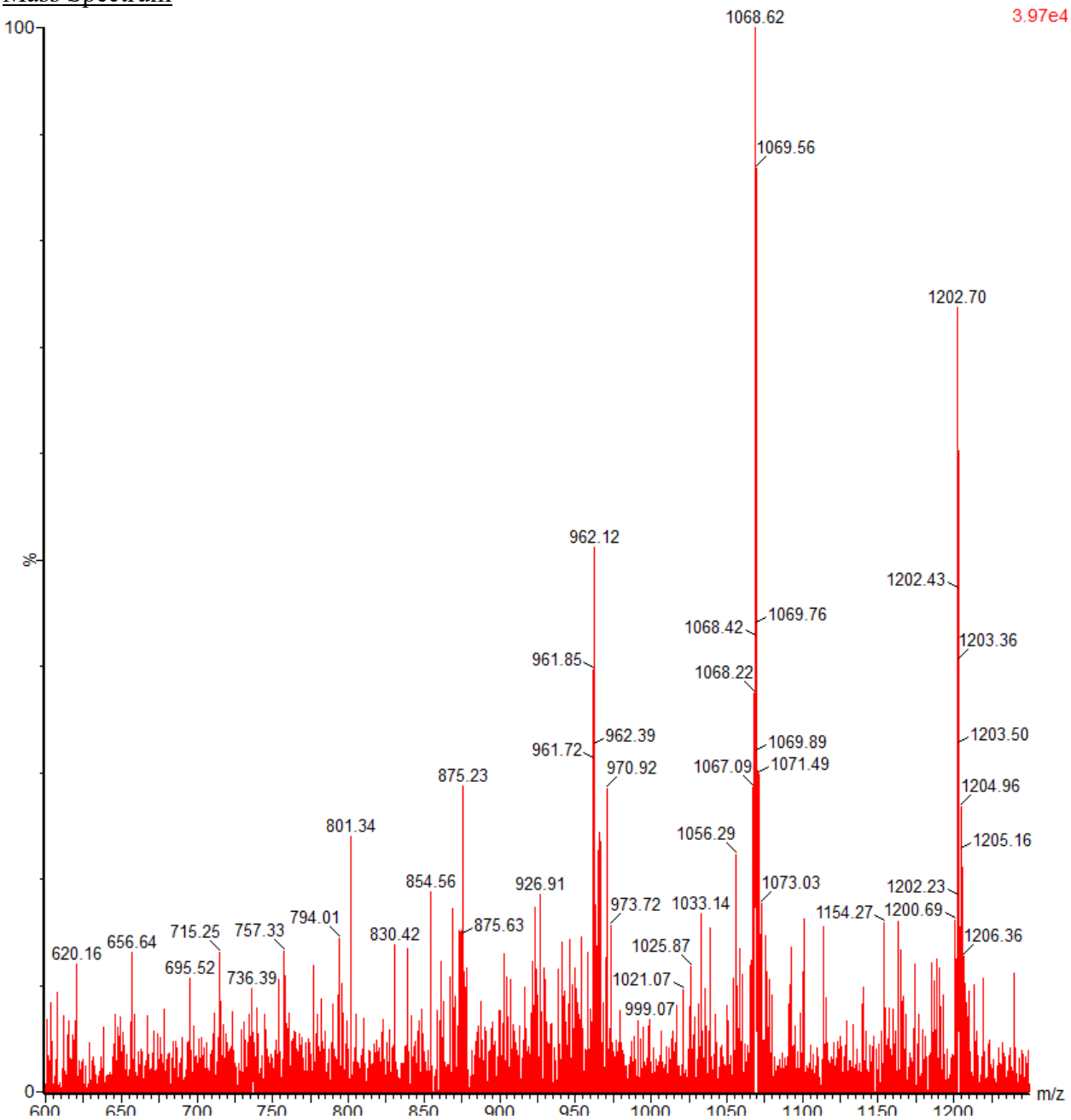


+Isomer

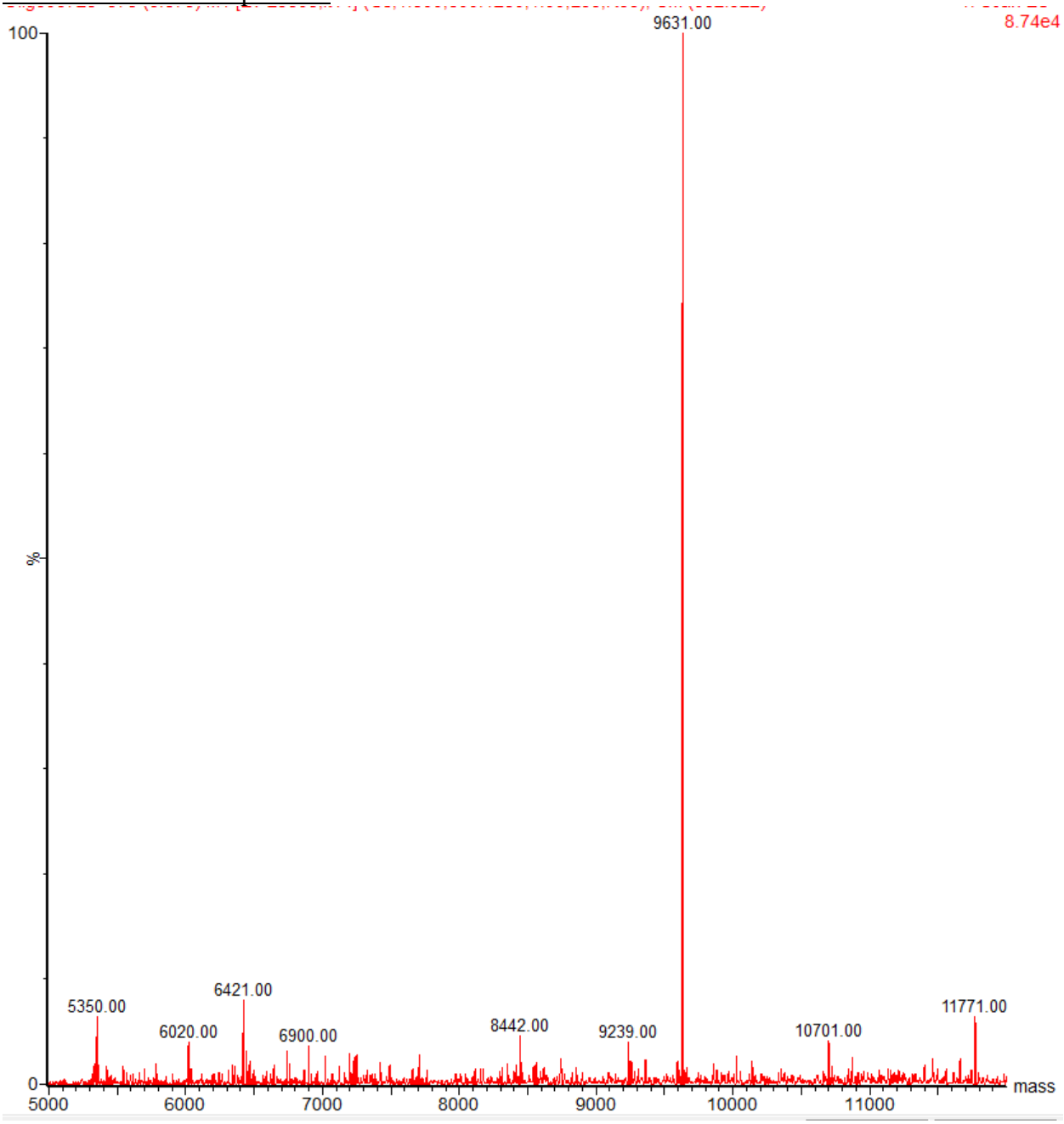
A260 Chromatogram



Mass Spectrum

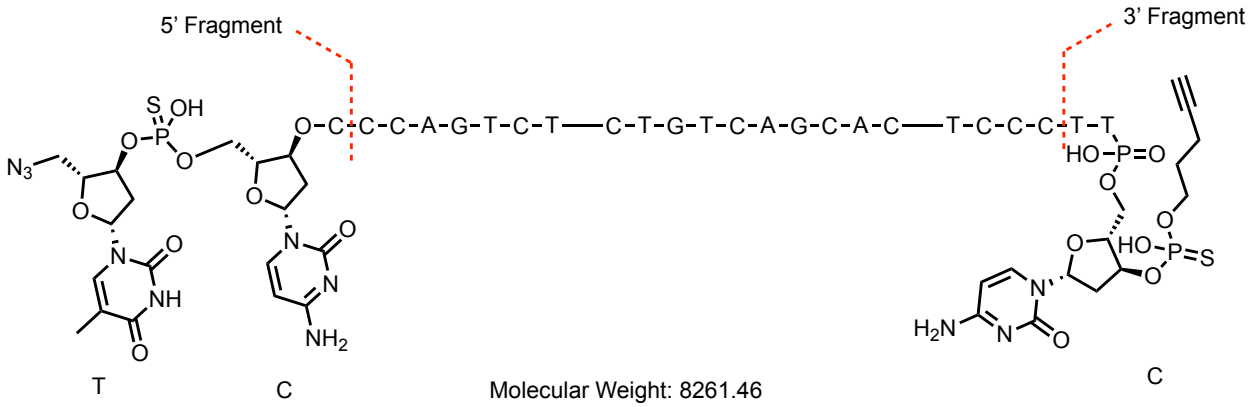


Deconvoluted Mass Spectrum

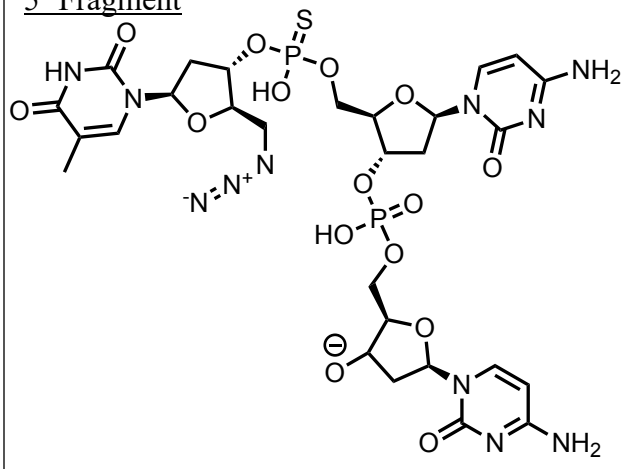


Process for Mass Fragmentation of 66

A crude solution of **66** in water (100 μM) was injected on the Waters I-Class ToF for peak identification. The MS cone voltage was then increased from 5 mV to 30 mV and the same sample was reinjected to fragment the modified oligonucleotide. Diagnostic fragments were then extracted, and their presence confirmed ion the total ion current. They are plotted below along with total ion current.

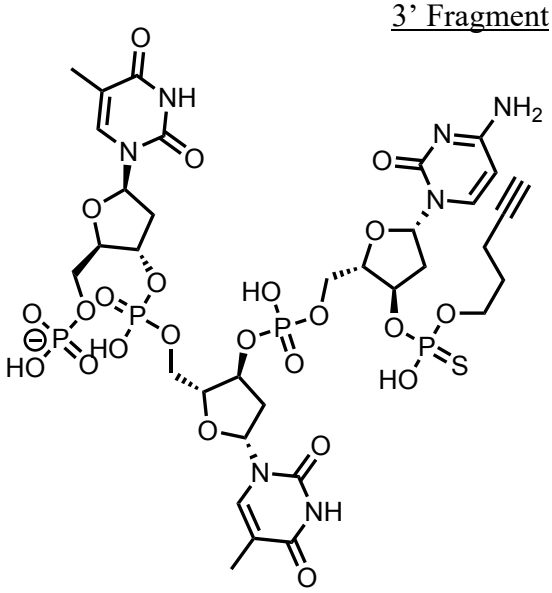


5’ Fragment

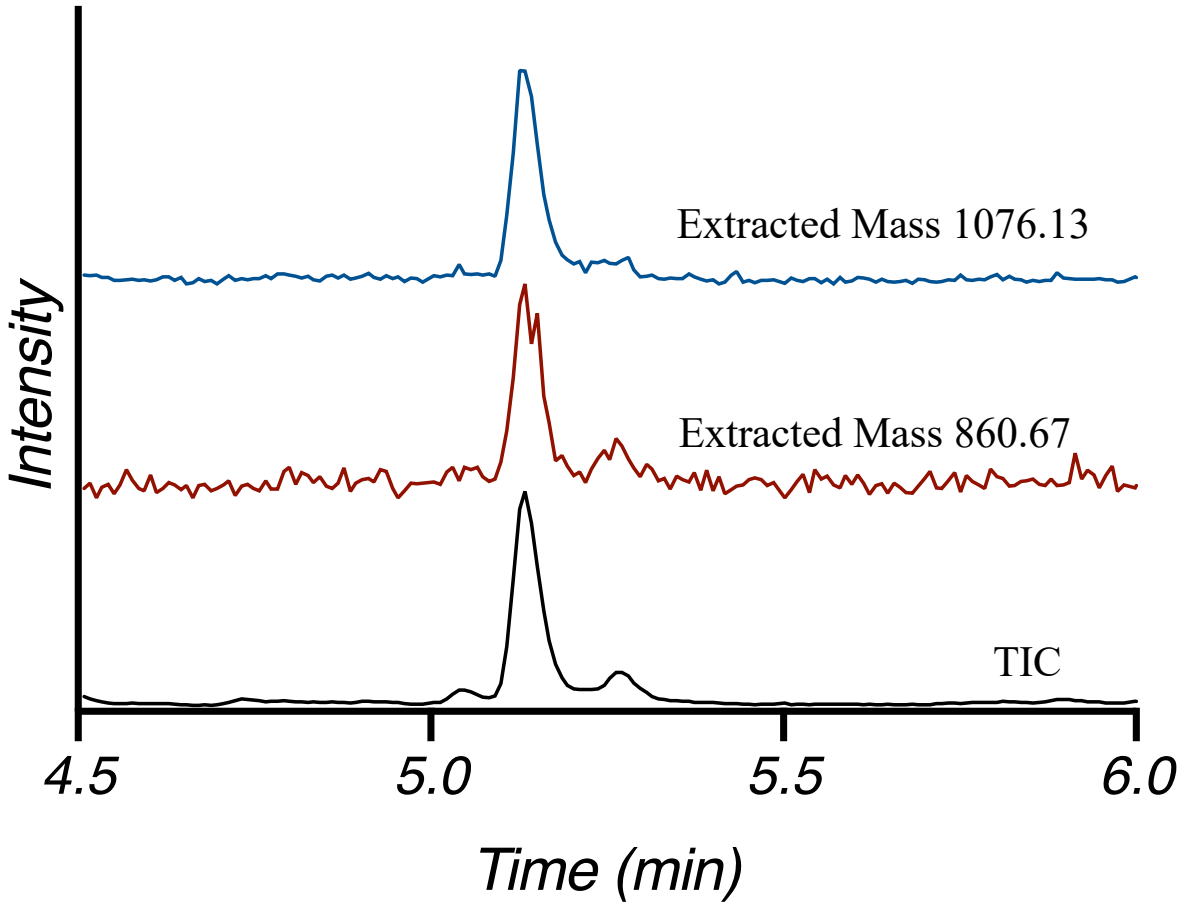


Exact Mass: 860.16
Molecular Weight: 860.67

3’ Fragment



Exact Mass: 1076.13
Molecular Weight: 1076.72

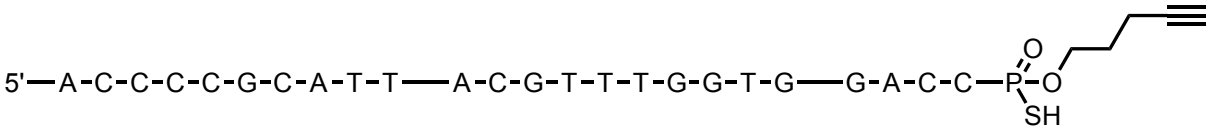

Extracted Mass 1076.13
Extracted Mass 860.67
TIC
Intensity
4.5
5.0
5.5
6.0
Time (min)

nCov Probe Production

Probes were produced in an analogous manner to procedure outlined above for the production of **66.**

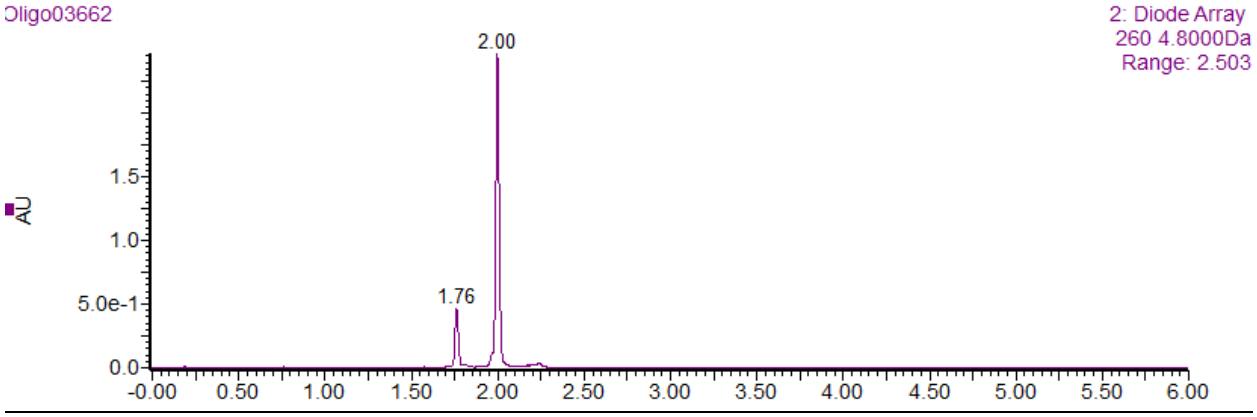
LCMS Characterization of SI-43

Produced from **SI-13** by General Procedure **3:**
Using **Ψ-5**
Expected Mass: 7546.89
Expected (M-8)/8 Ion: 942.4
Found Deconvoluted Mass: 7546
Found (M-8)/8 Ion: 942.05

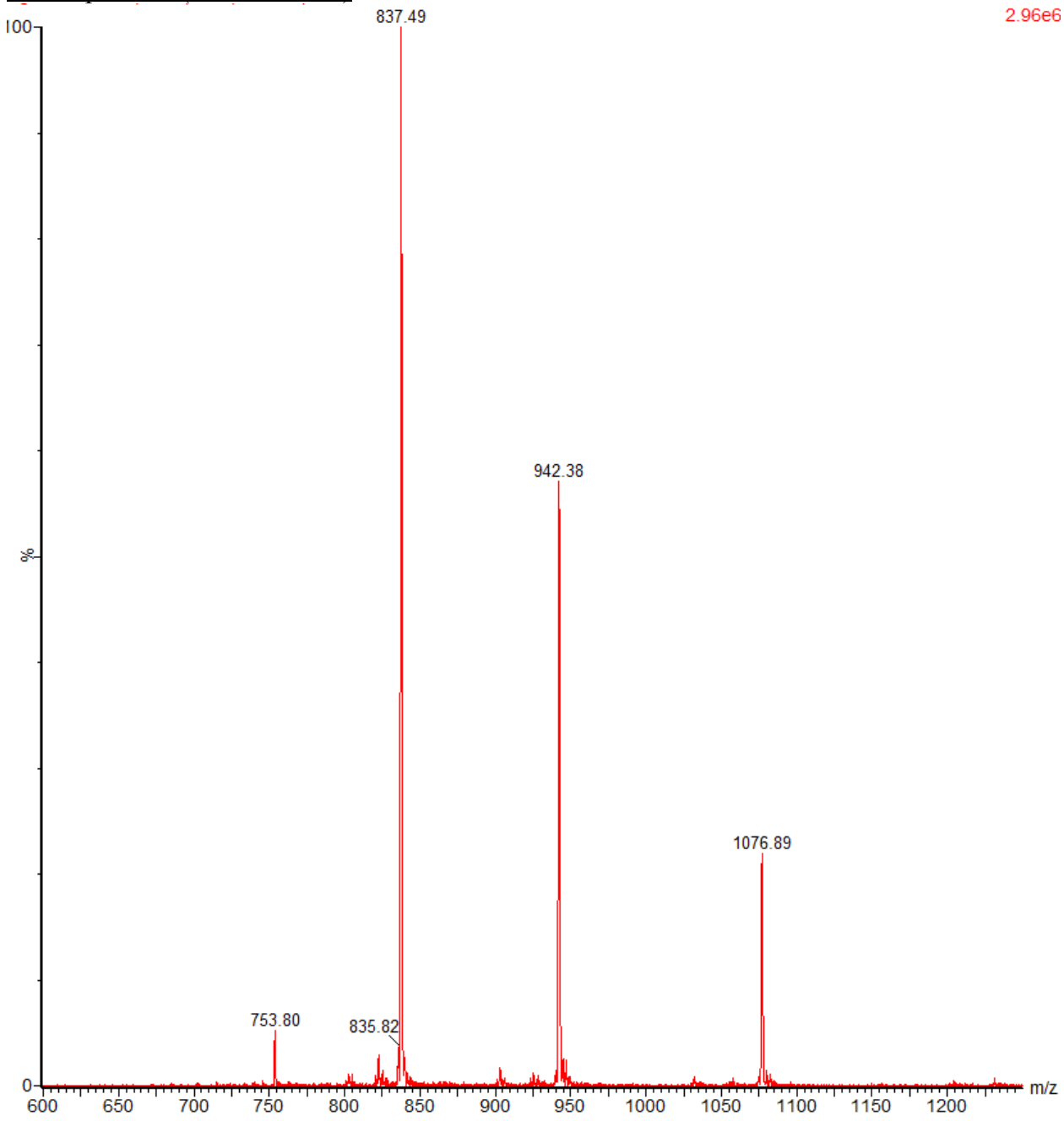


Molecular Weight: 7546.89

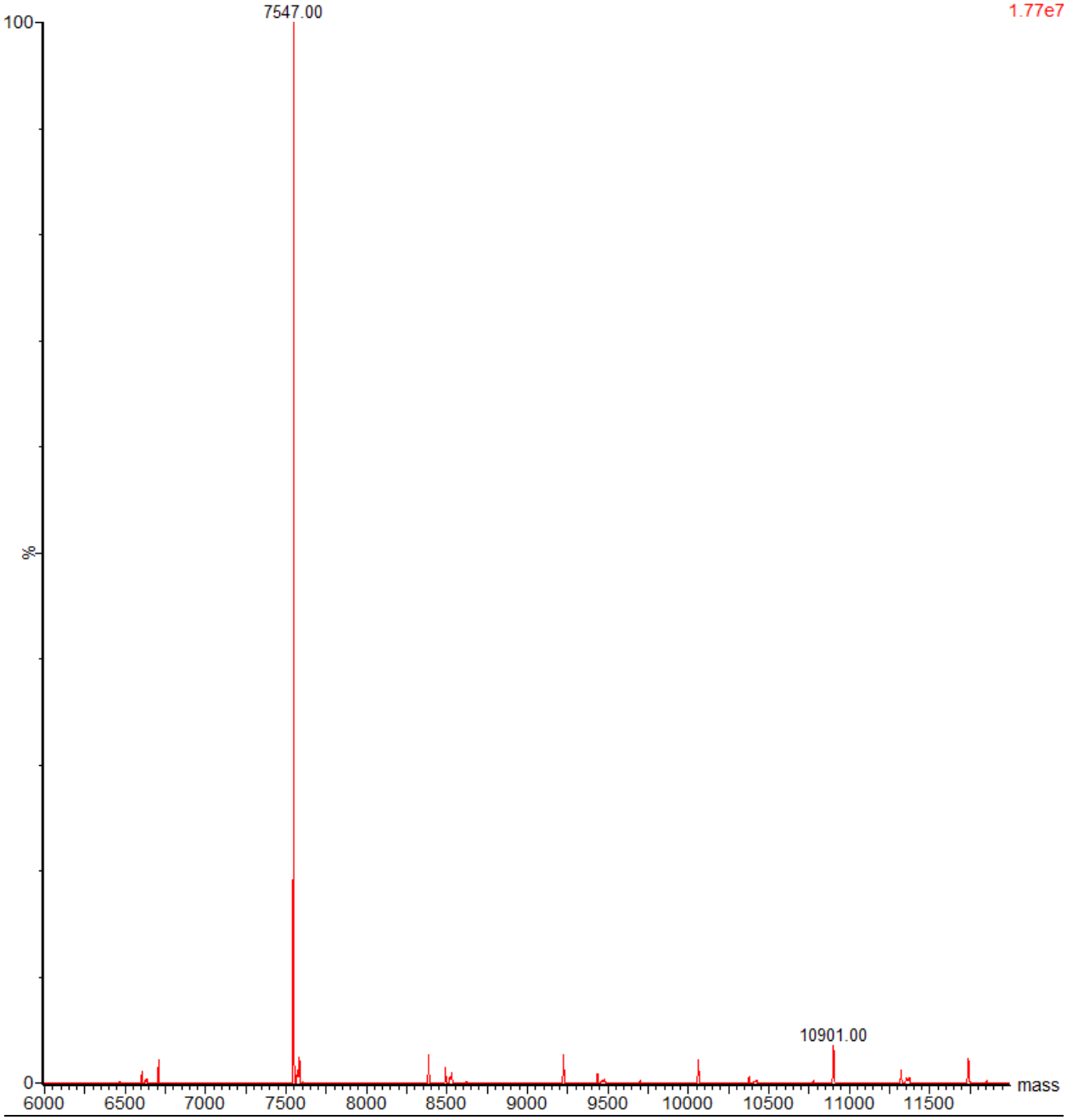
A260 Chromatogram



Mass Spectrum (RT= 2.00 min)

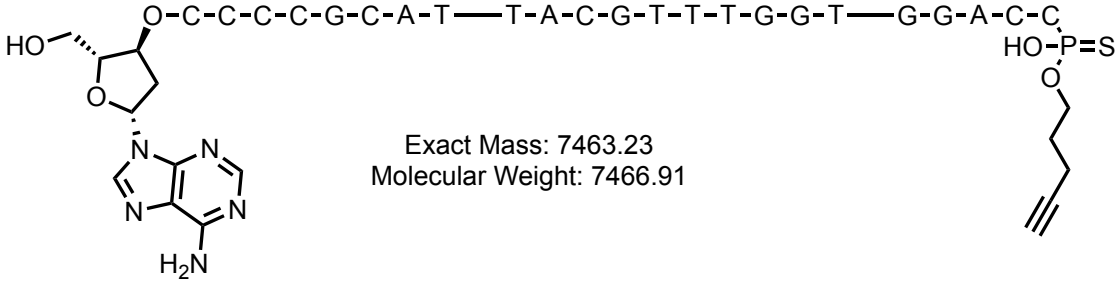


Deconvoluted Mass Spectrum



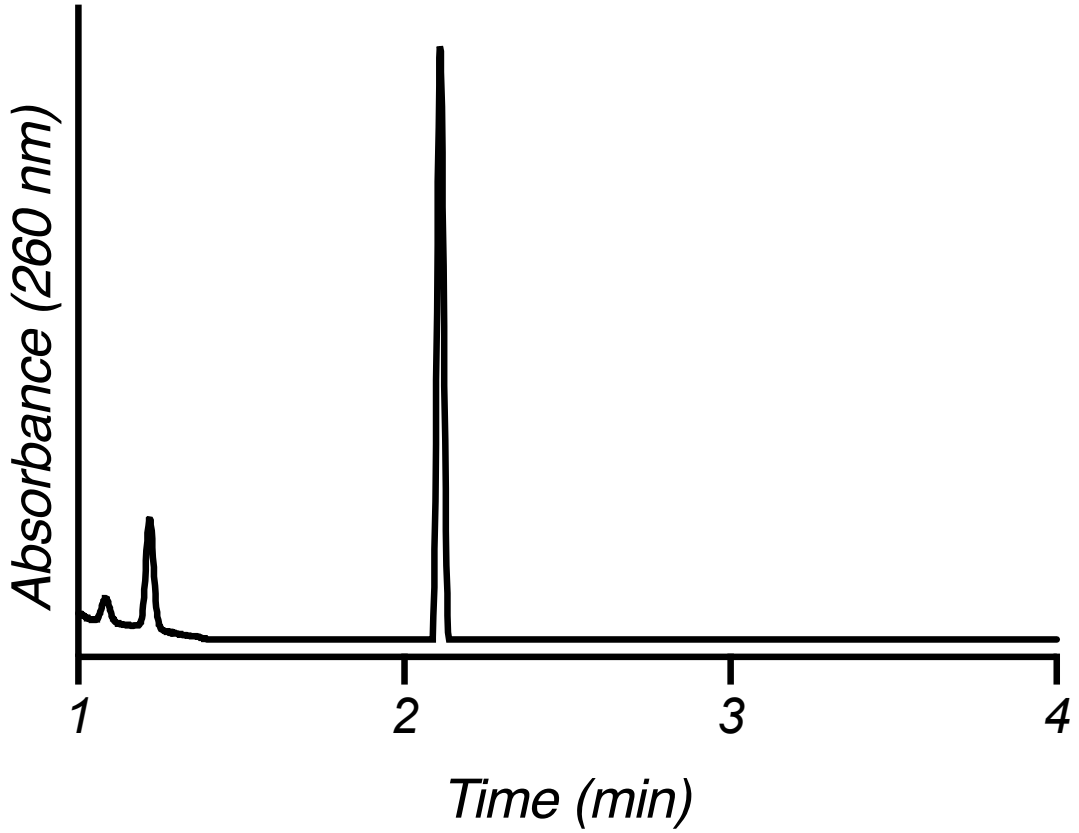
LCMS Characterization of SI-44

Produced from **SI-43** by rSAP and EXOIII**:**
Expected Mass: 7466.91
Expected (M-8)/8 Ion: 932.4
Found Deconvoluted Mass: 7467.00
Found (M-8)/8 Ion: 932.5

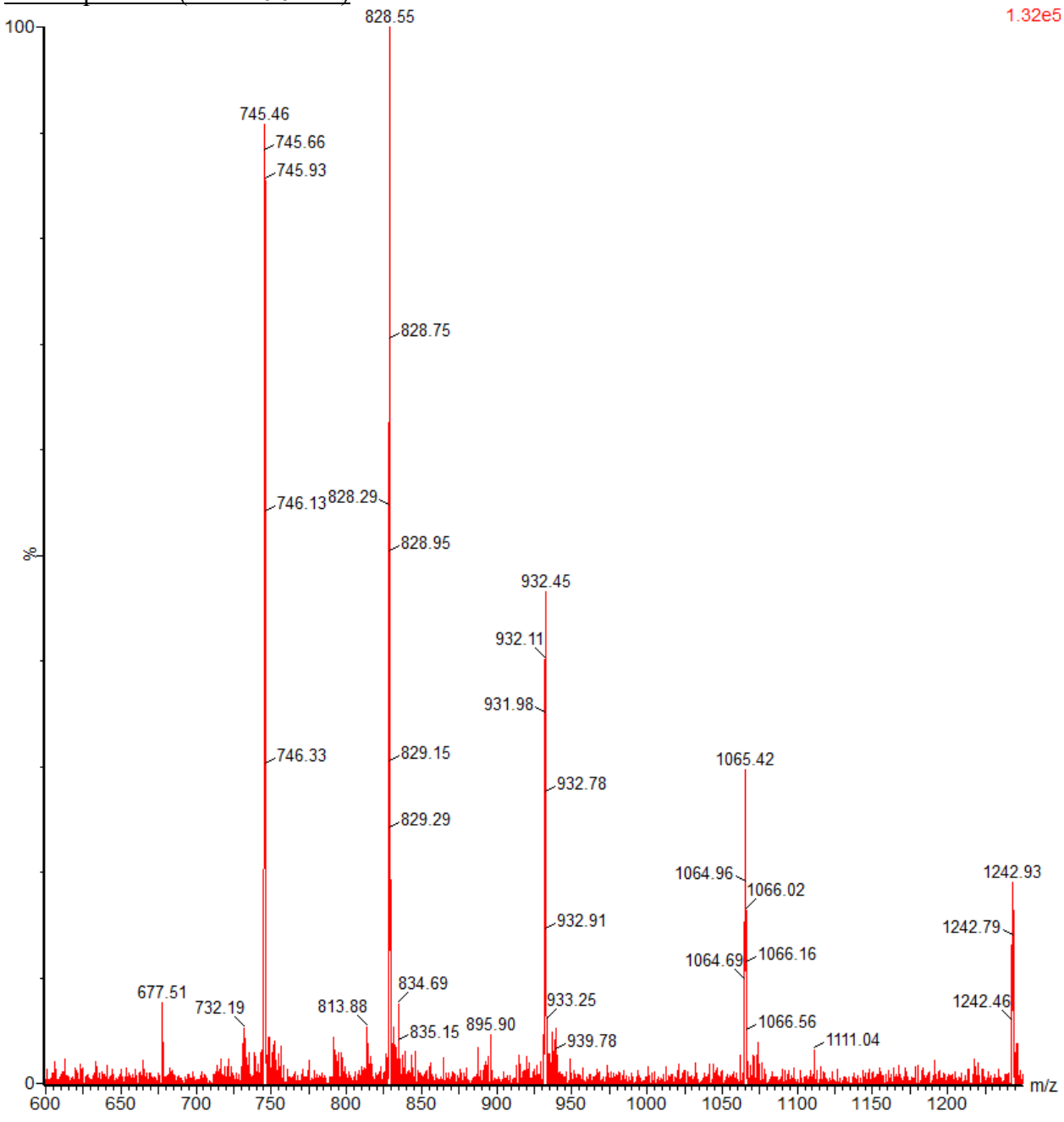


Exact Mass: 7463.23
Molecular Weight: 7466.91

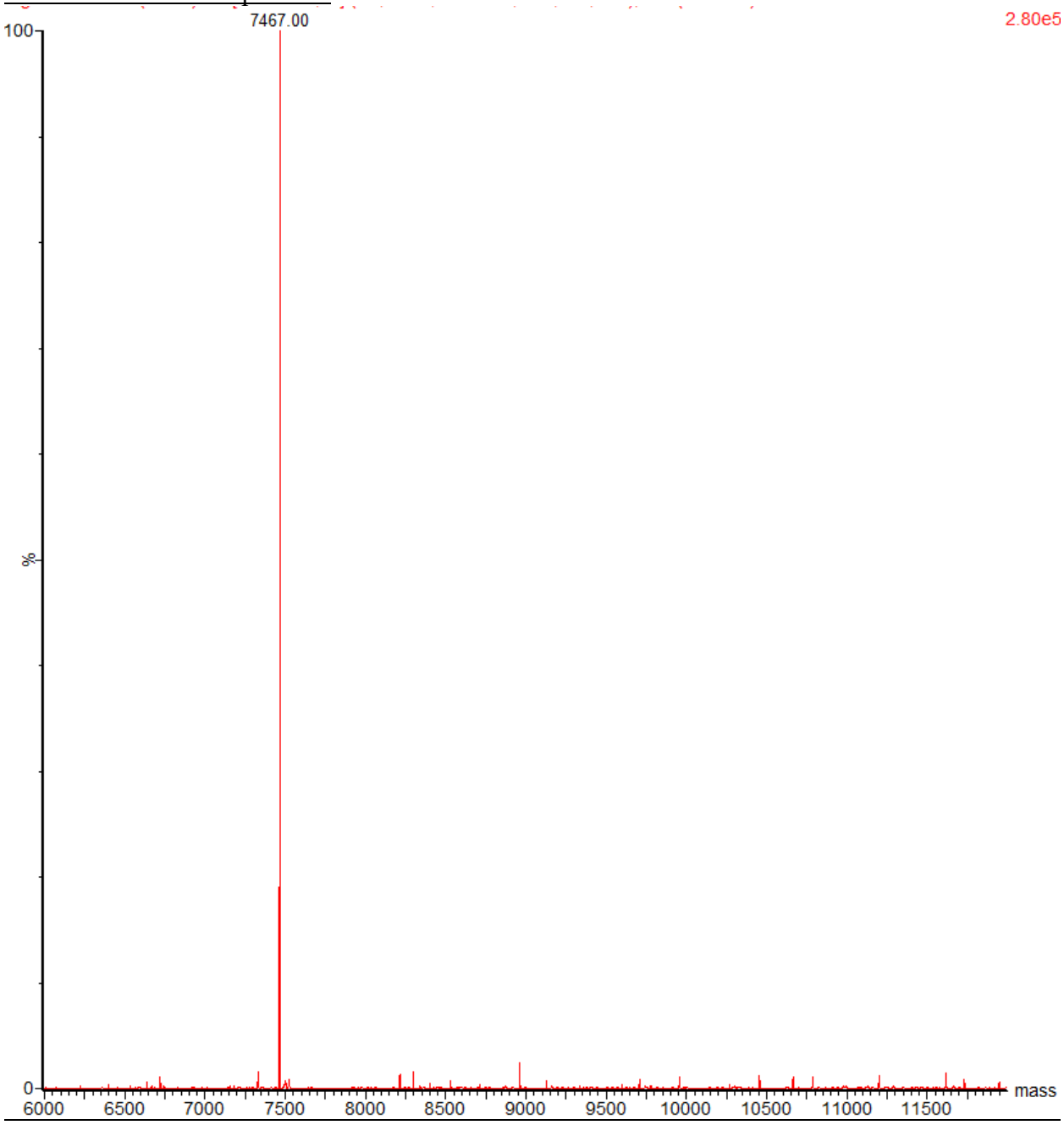
A260 Chromatogram



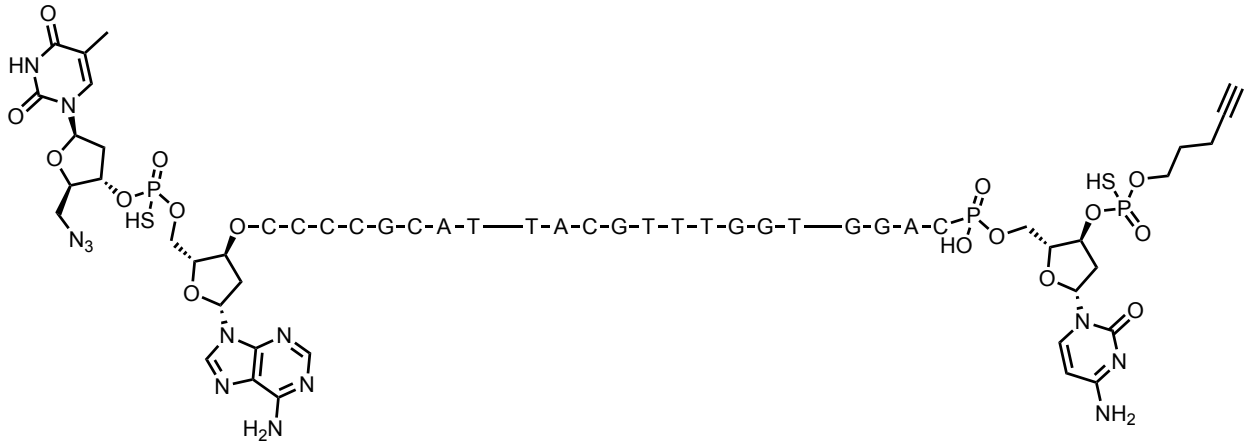
Mass Spectrum (RT= 1.96 min)



Deconvoluted Mass Spectrum

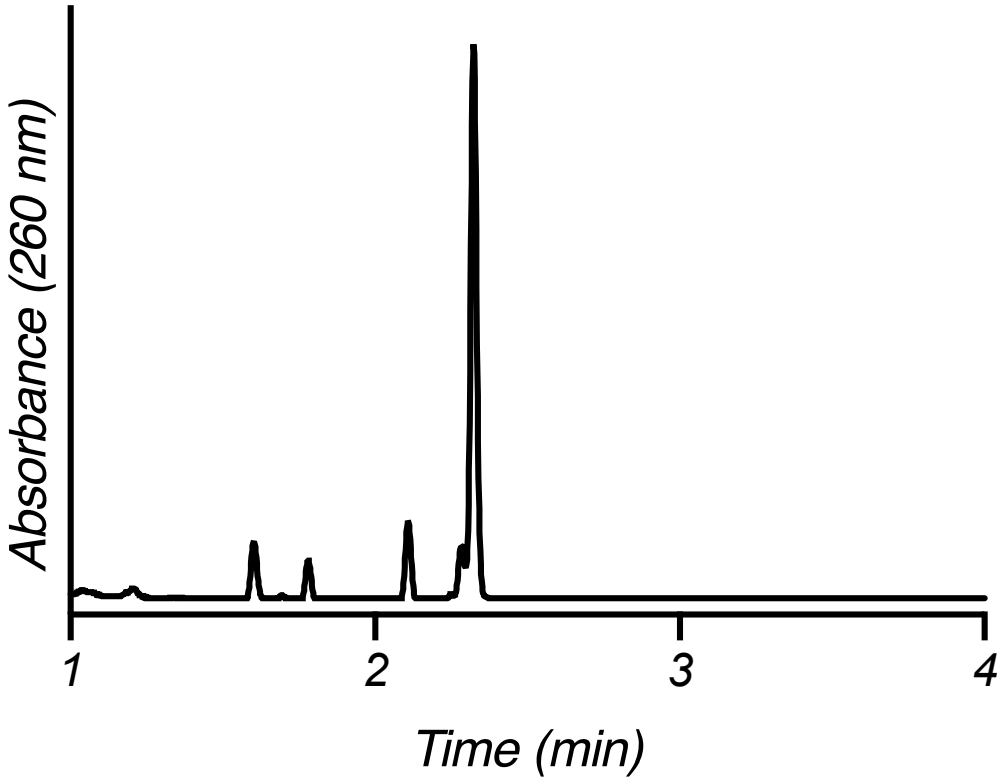


LCMS Characterization of **68** after SENDR with **Ψ-17** and Lambda Exo Cleanup
Produced from **SI-44:**
Using **Ψ-17**
Expected Mass: 7812.2
Expected (M-6)/6 Ion: 975.525
Found Deconvoluted Mass: 7813.0
Found (M-6)/6 Ion: 975.4

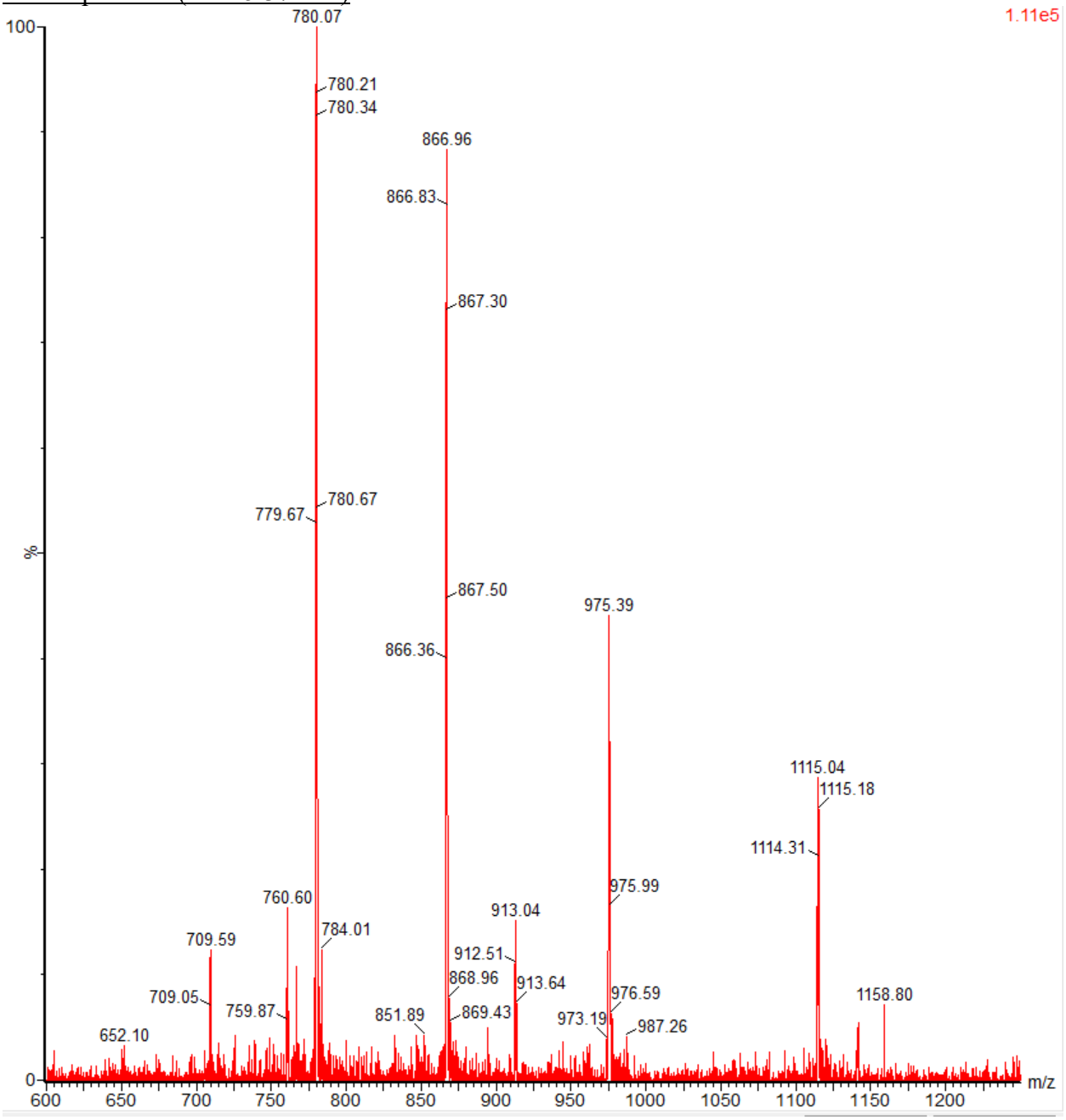


Molecular Weight: 7812.18

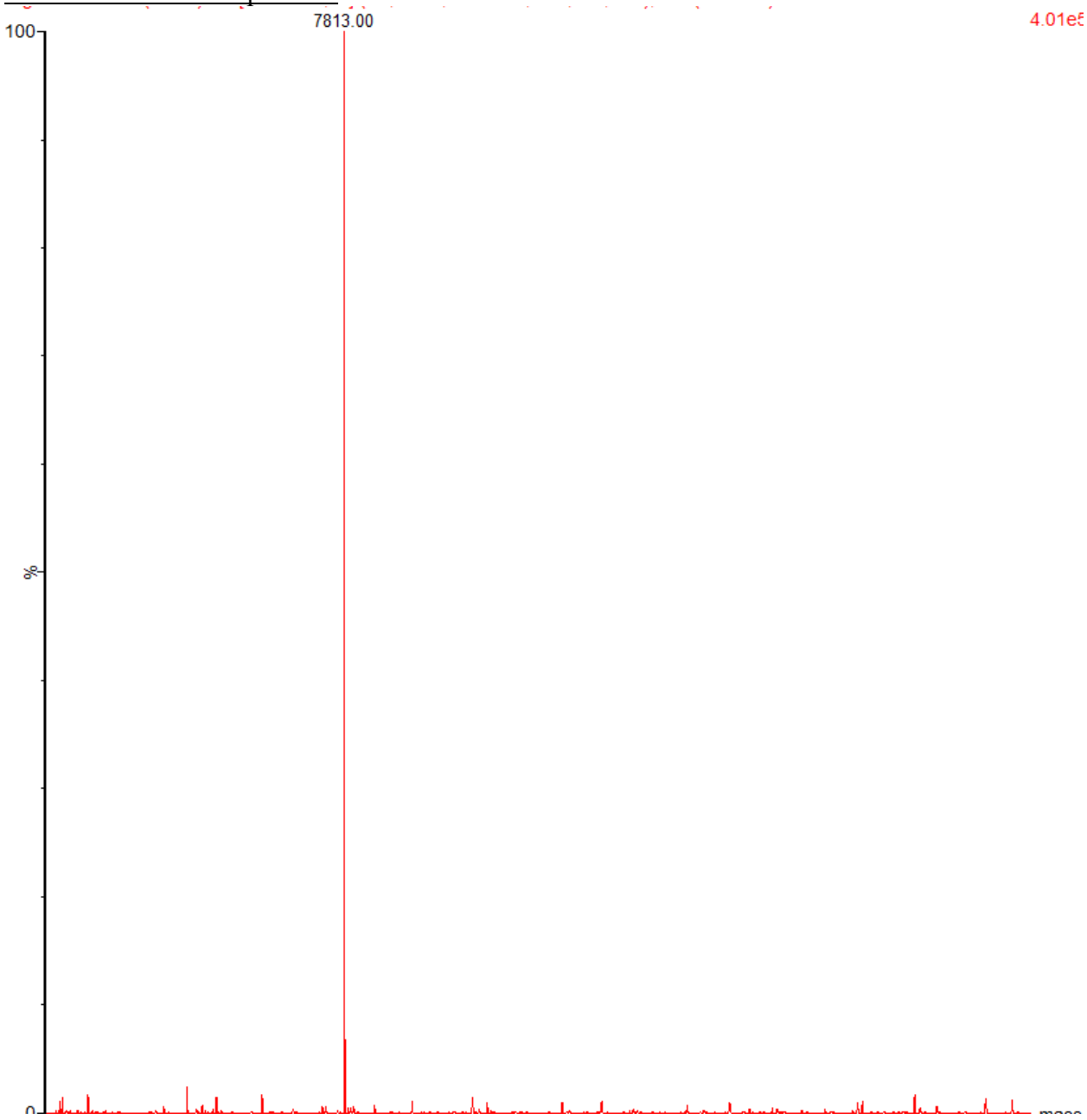
A260 Chromatogram



Mass Spectrum (RT= 5.37 min)

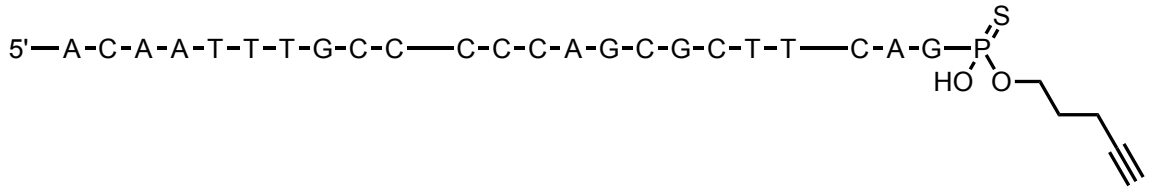


Deconvoluted Mass Spectrum



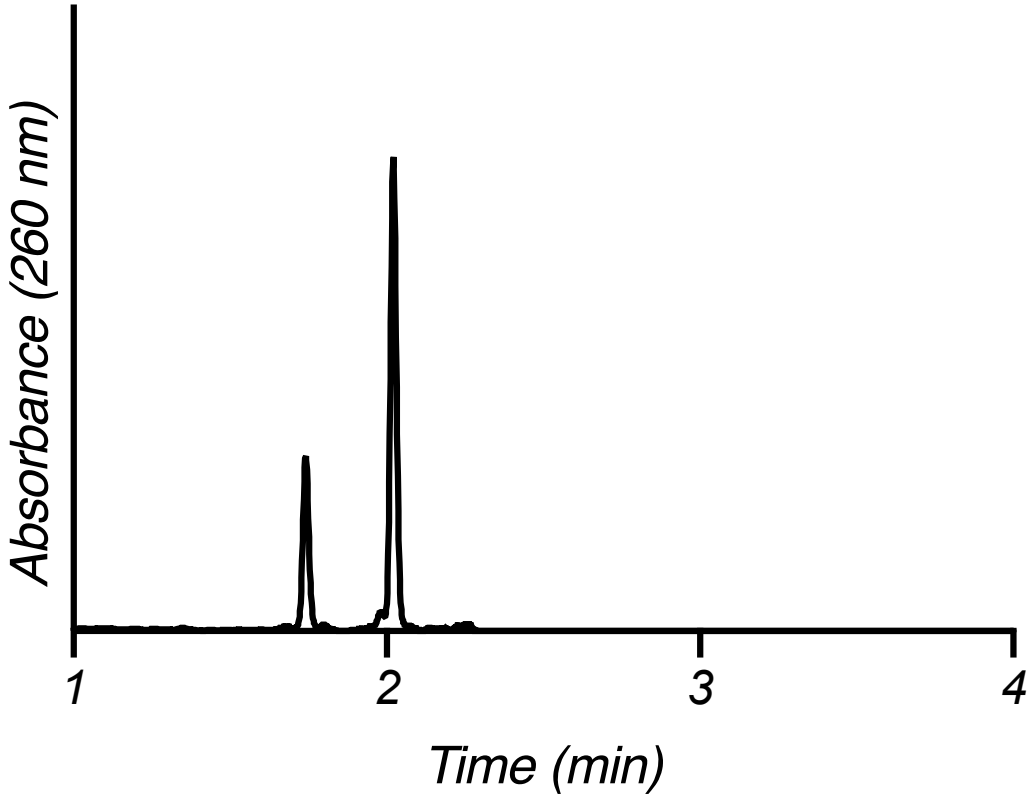
LCMS Characterization of SI-45

Produced from **SI-14:**
Using **Ψ-5**
Expected Mass: 7186.67
Expected (M-8)/8 Ion: 897.33
Found Deconvoluted Mass: 7186.00
Found (M-8)/8 Ion: 897.17

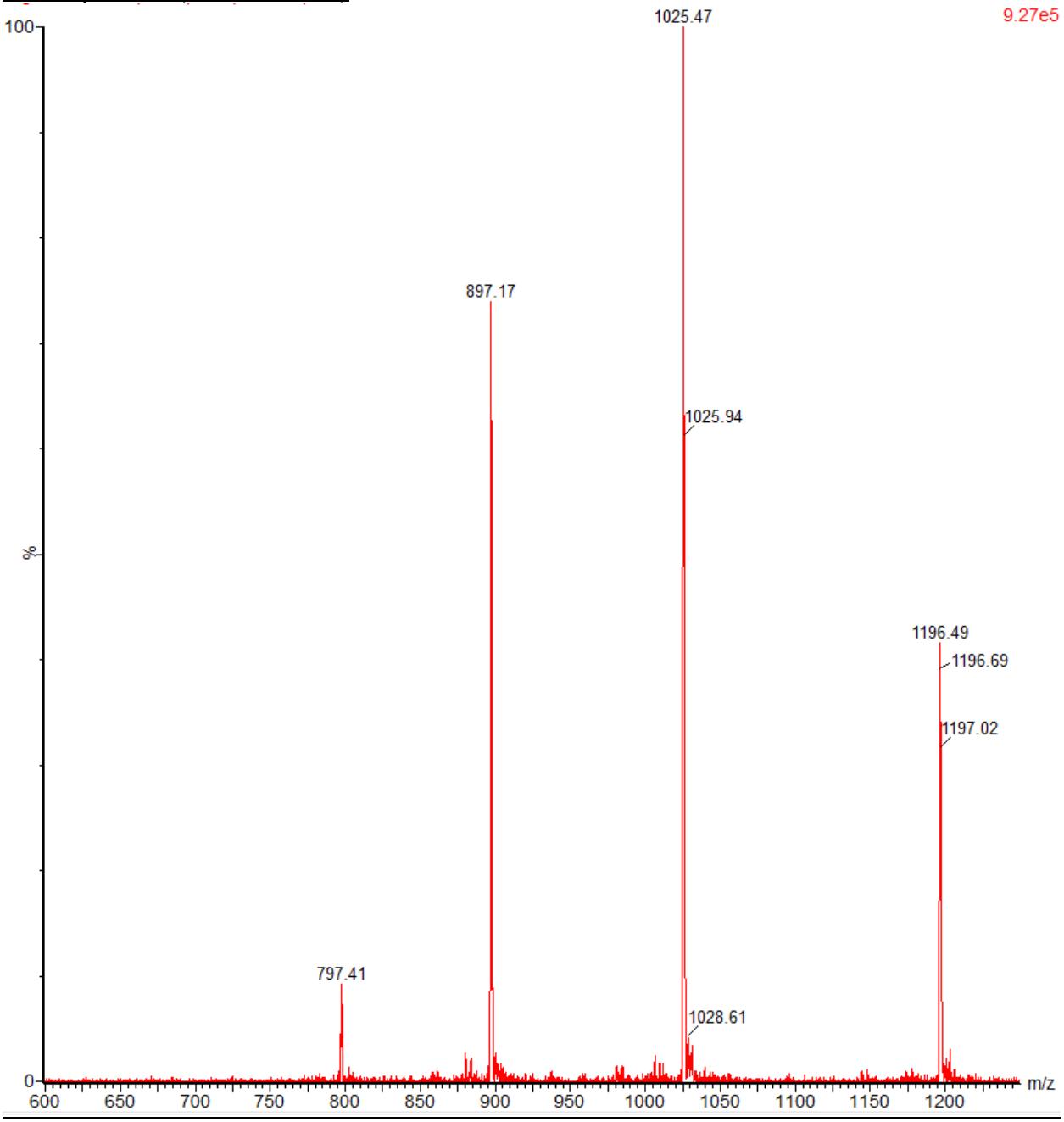


Exact Mass: 7183.15
Molecular Weight: 7186.67

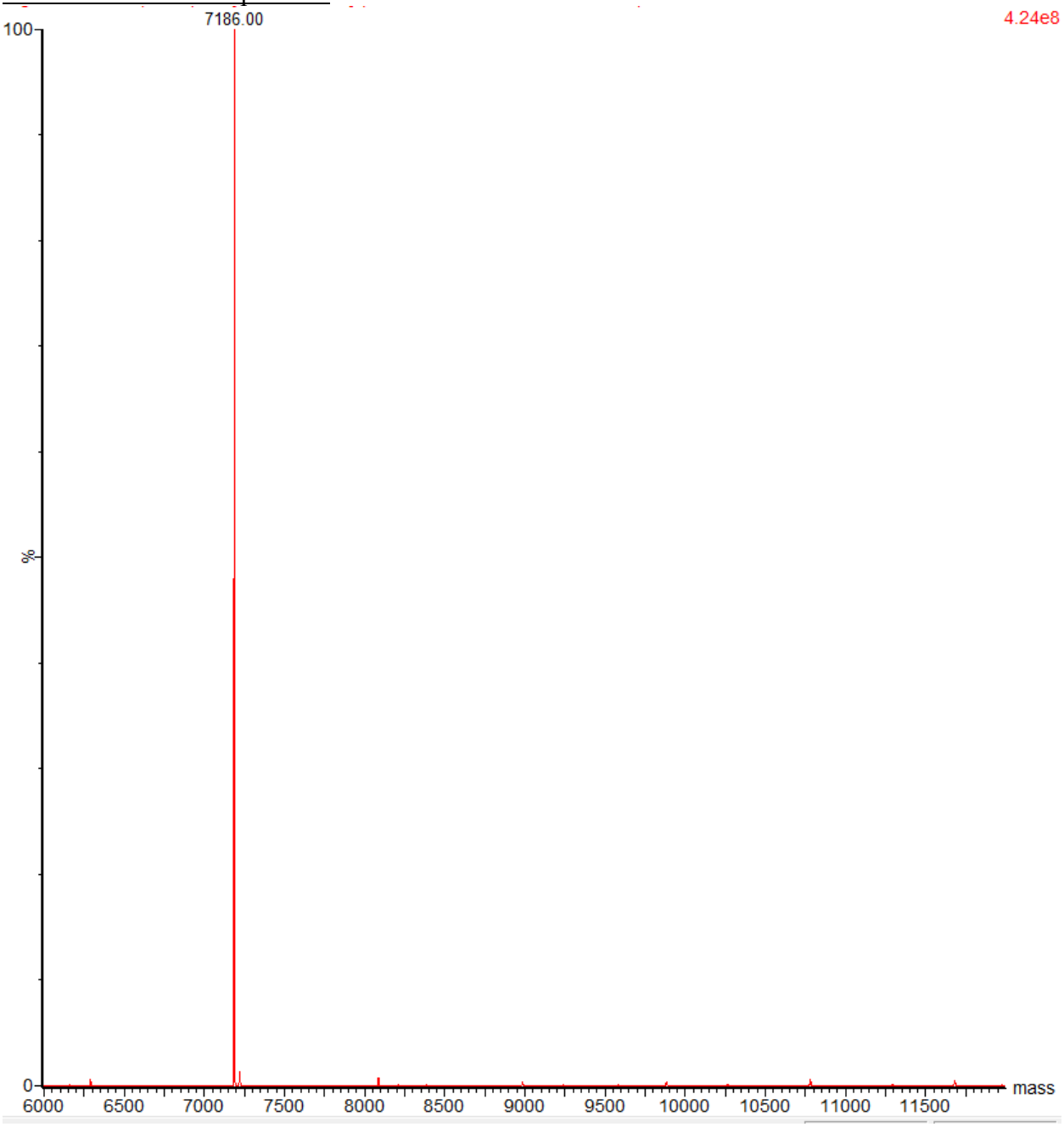
A260 Chromatogram



Mass Spectrum (RT= 2.00 min)

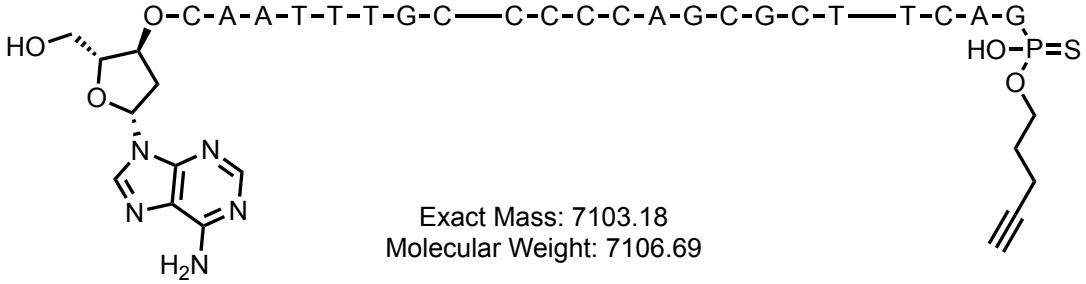


Deconvoluted Mass Spectrum



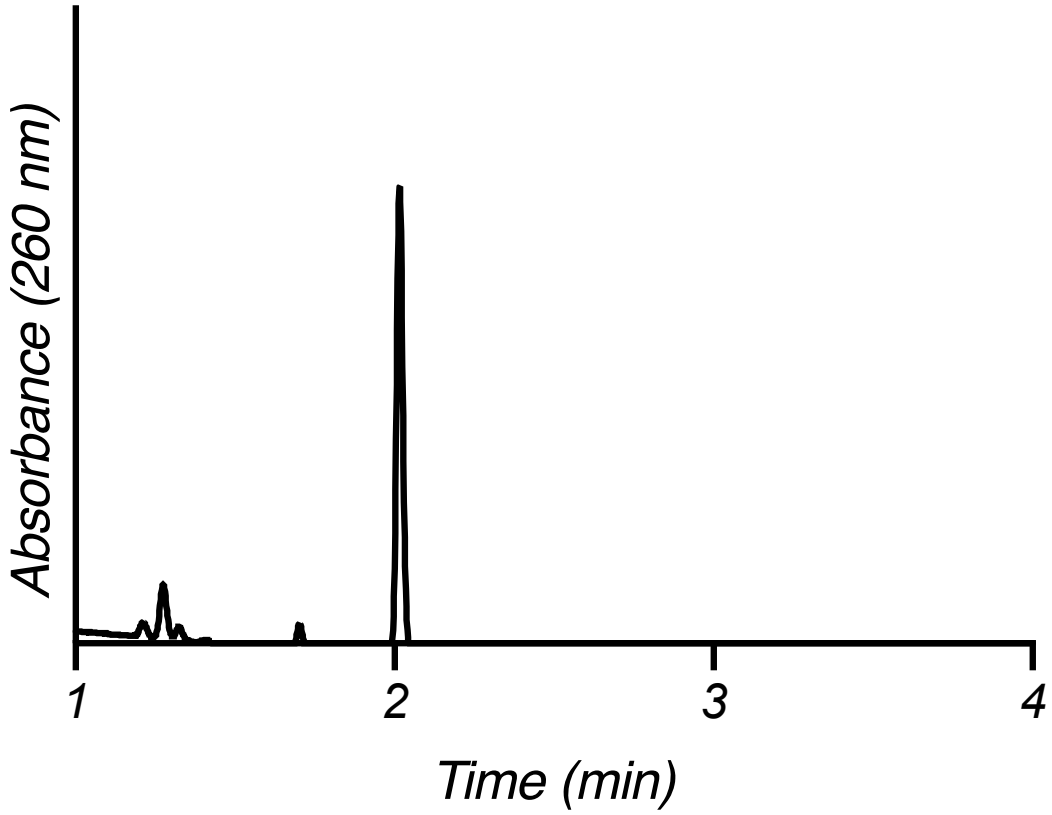
LCMS Characterization of SI-46

Produced from **SI-45** by rSAP**:**
Expected Mass: 7106.69
Expected (M-8)/8 Ion: 887.33
Found Deconvoluted Mass: 886.97
Found (M-8)/8 Ion: 7107.00

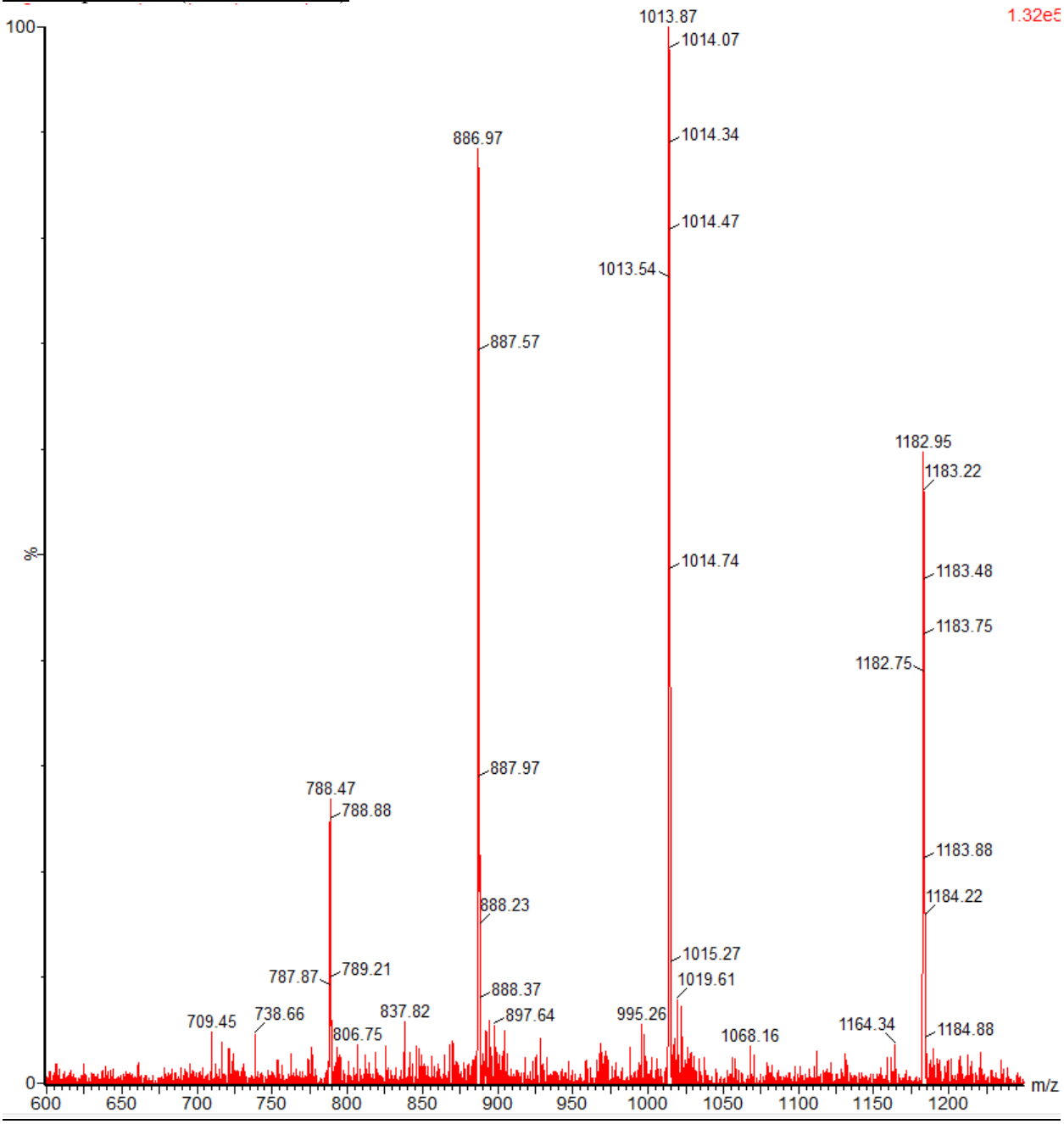


Exact Mass: 7103.18
Molecular Weight: 7106.69

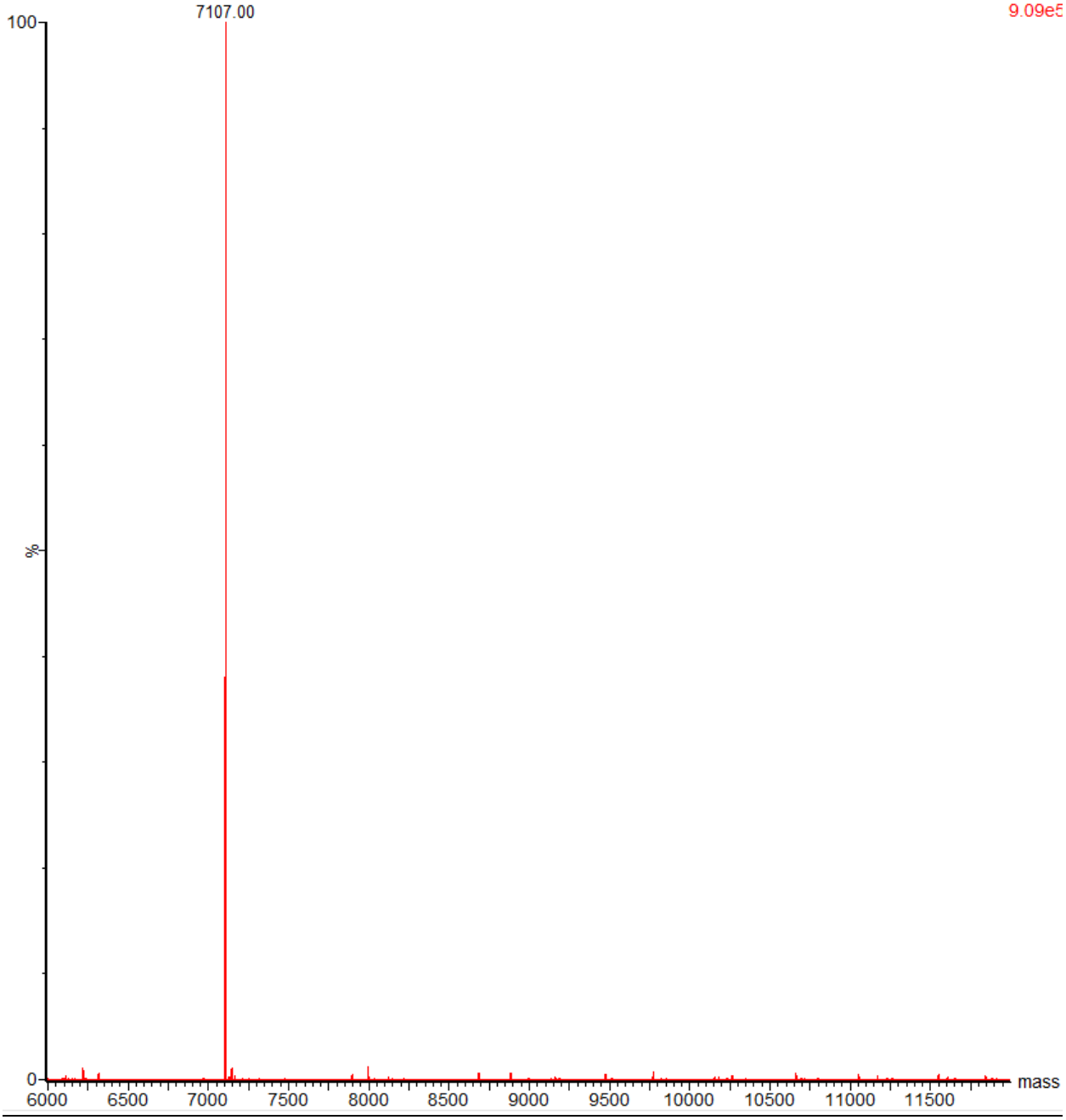
A260 Chromatogram



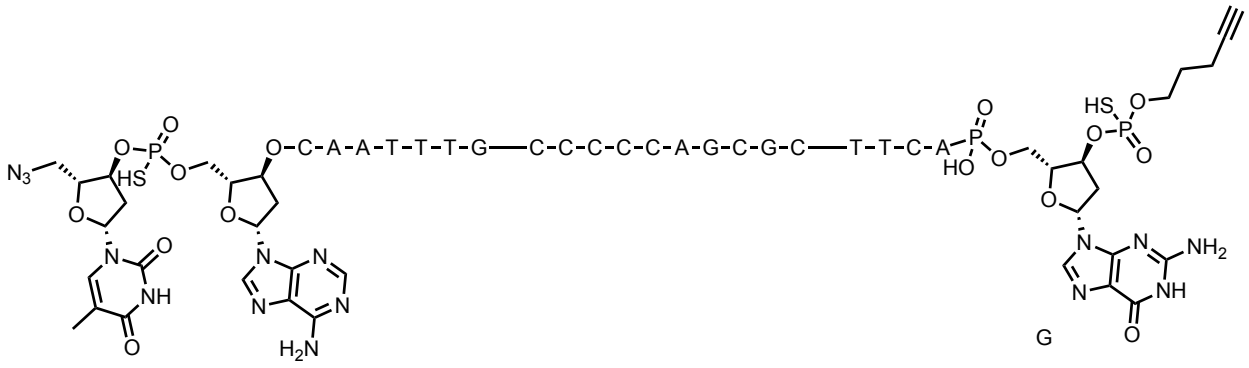
Mass Spectrum (RT= 1.97 min)



Deconvoluted Mass Spectrum

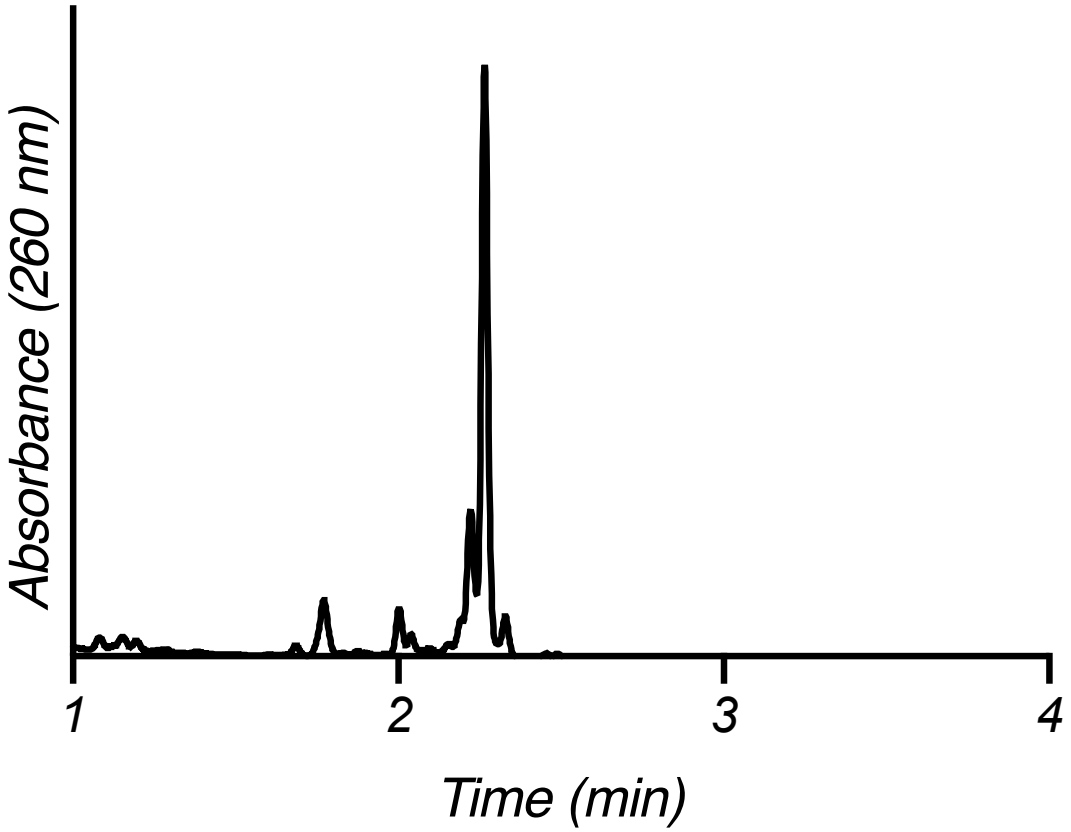


LCMS Characterization of 69 After SENDR with **Ψ-17** and Lambda Exo Cleanup:
Produced from **SI-46:**
Using **Ψ-17**
Expected Mass: 7451.96
Expected (M-6)/6 Ion: 1240.98
Found Deconvoluted Mass: 7452.00
Found (M-6)/6 Ion: 1240.59

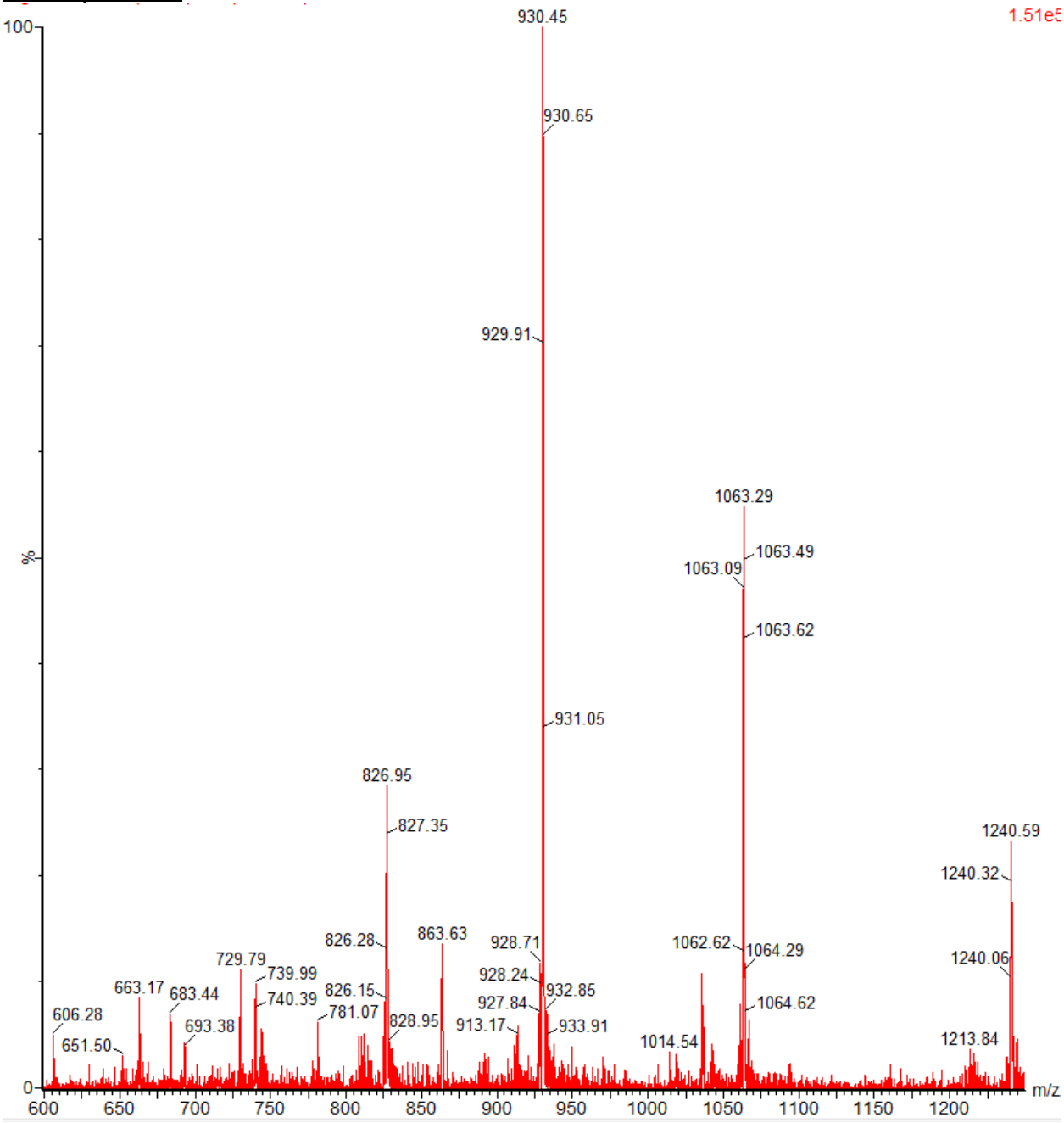


Molecular Weight: 7451.96

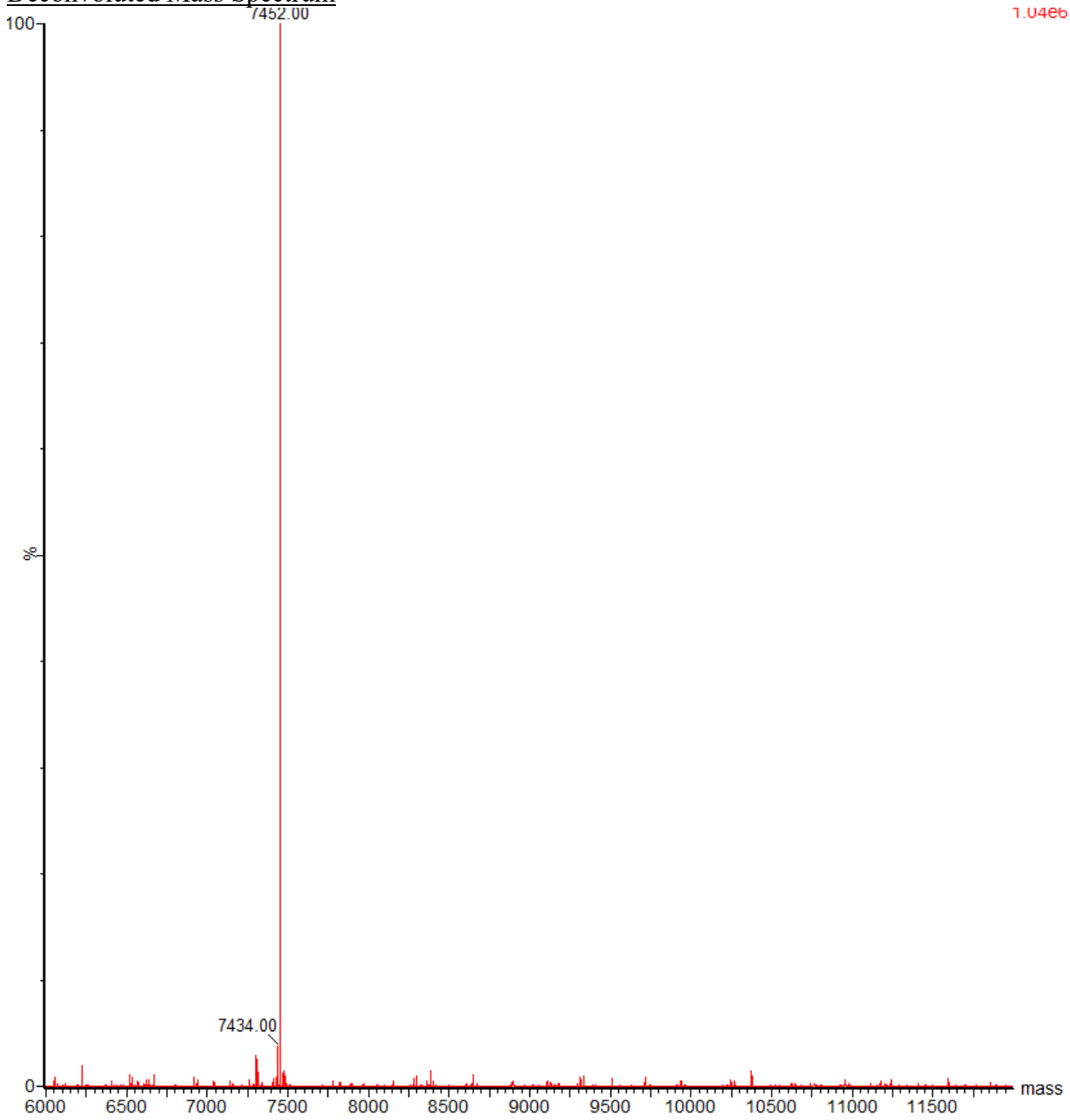
A260 Chromatogram



Mass Spectrum

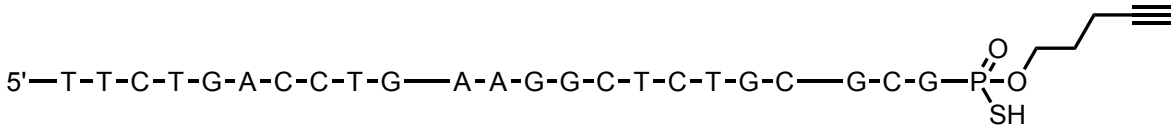


Deconvoluted Mass Spectrum



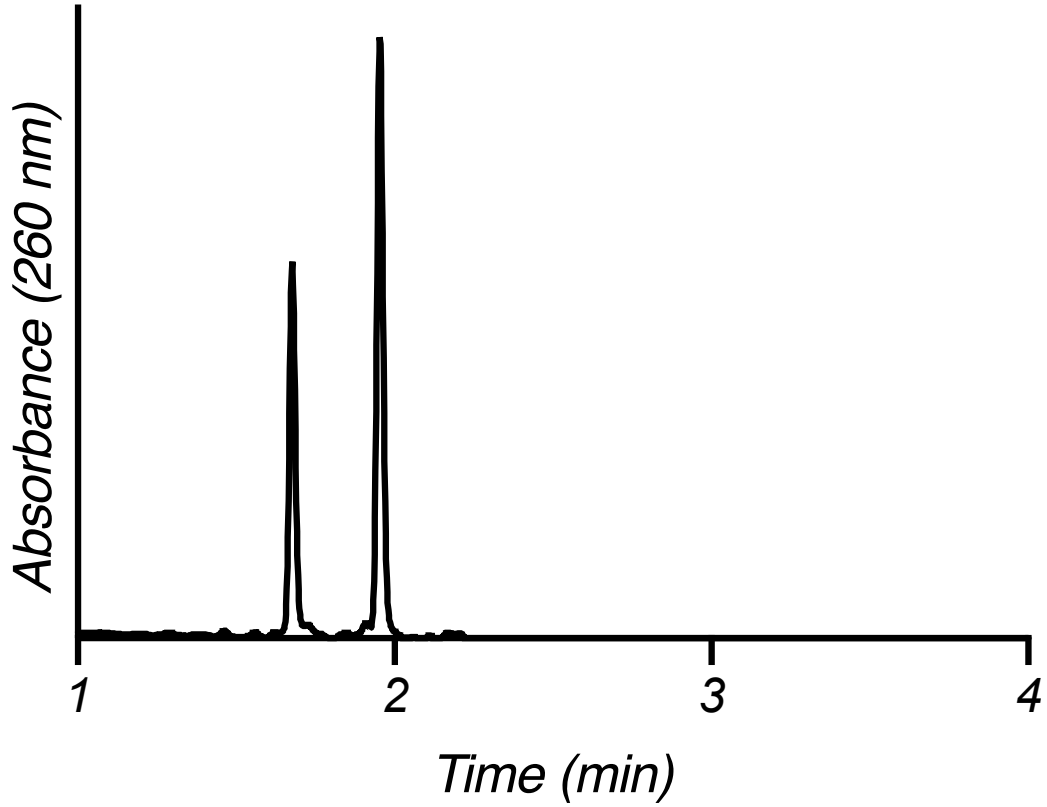
LCMS Characterization of SI-47

Produced from **SI-15 by** General Procedure **3:**
Using **Ψ-5**
Expected Mass: 7273.7
Expected (M-6)/6 Ion: 1211.166
Found Deconvoluted Mass: 7274.00
Found (M-6)/6 Ion: 1211.03

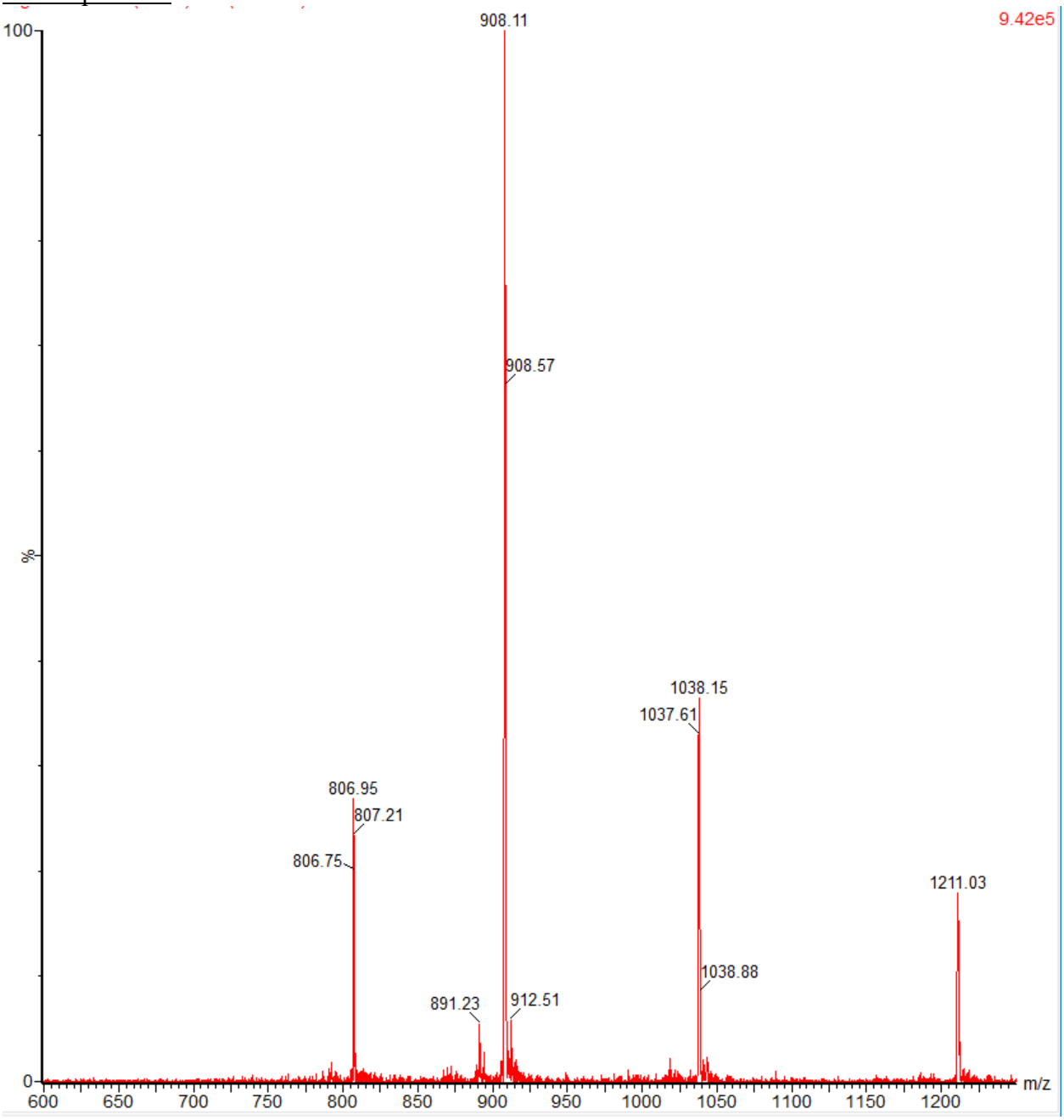


Molecular Weight: 7273.70

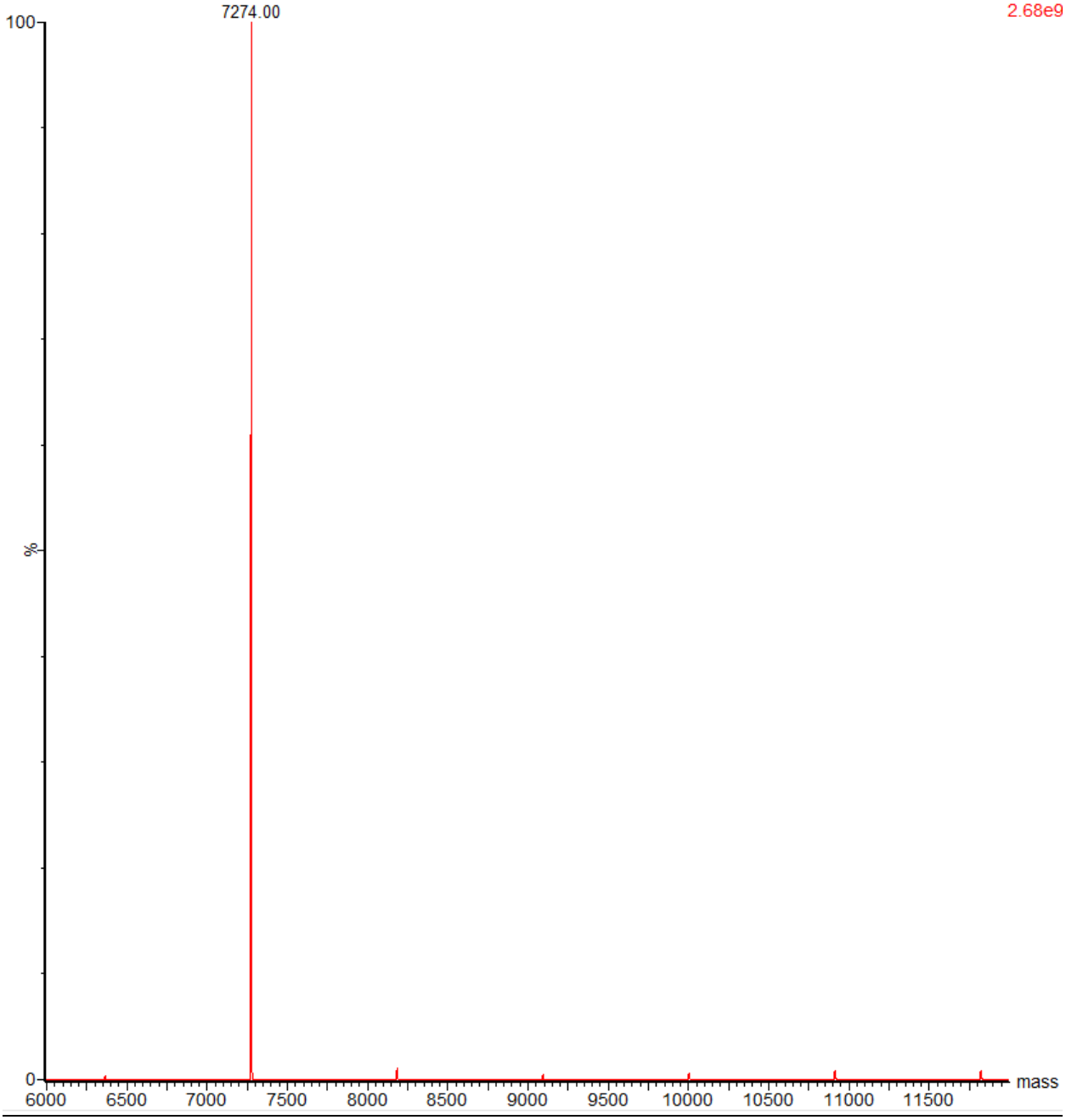
A260 Chromatogram



Mass Spectrum

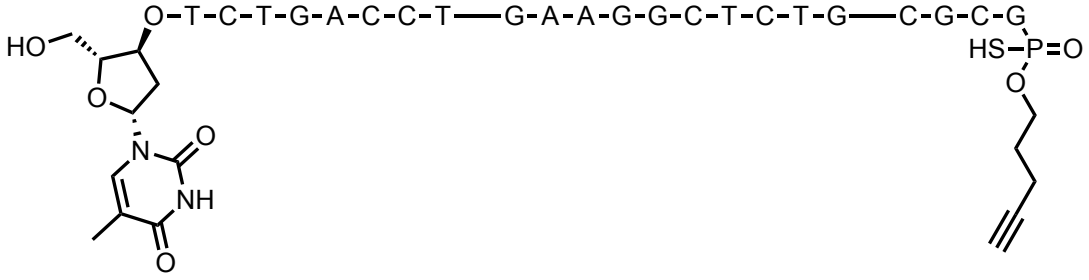


Deconvoluted Mass Spectrum



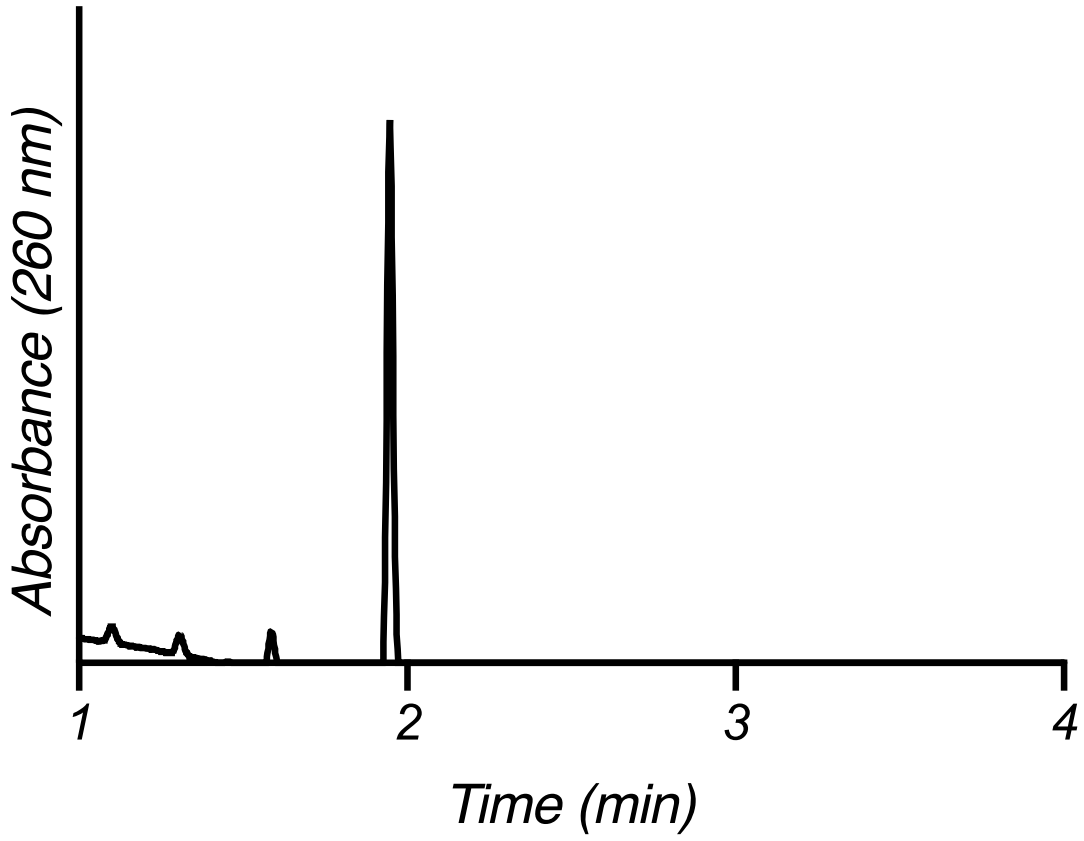
LCMS Characterization of SI-48

Produced from **SI-47** by rSAP and EXOIII**:**
Expected Mass: 7193.72
Expected (M-6)/6 Ion: 1197.95
Found Deconvoluted Mass: 7193.00
Found (M-6)/6Ion: 1197.76

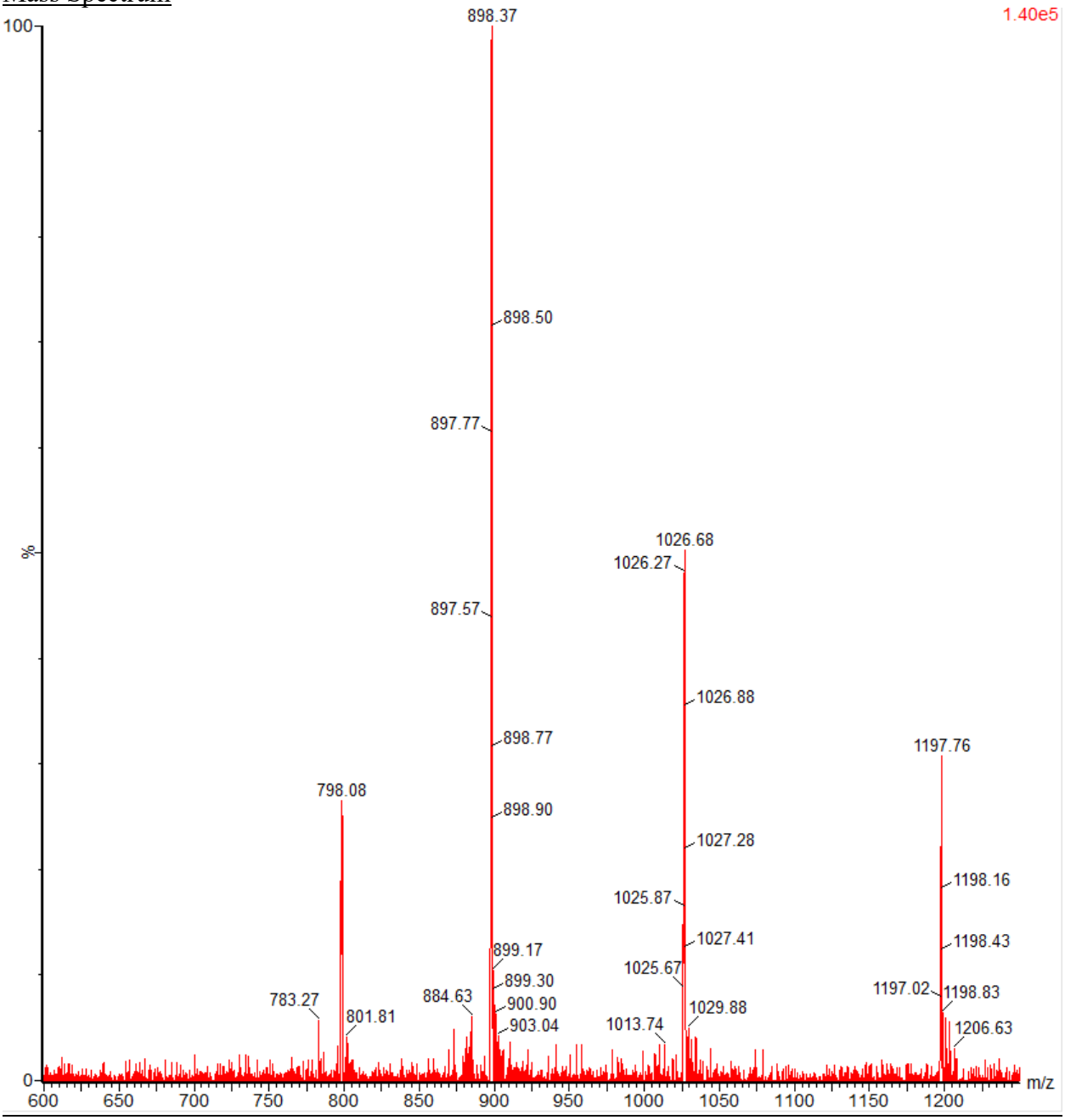


Molecular Weight: 7193.72

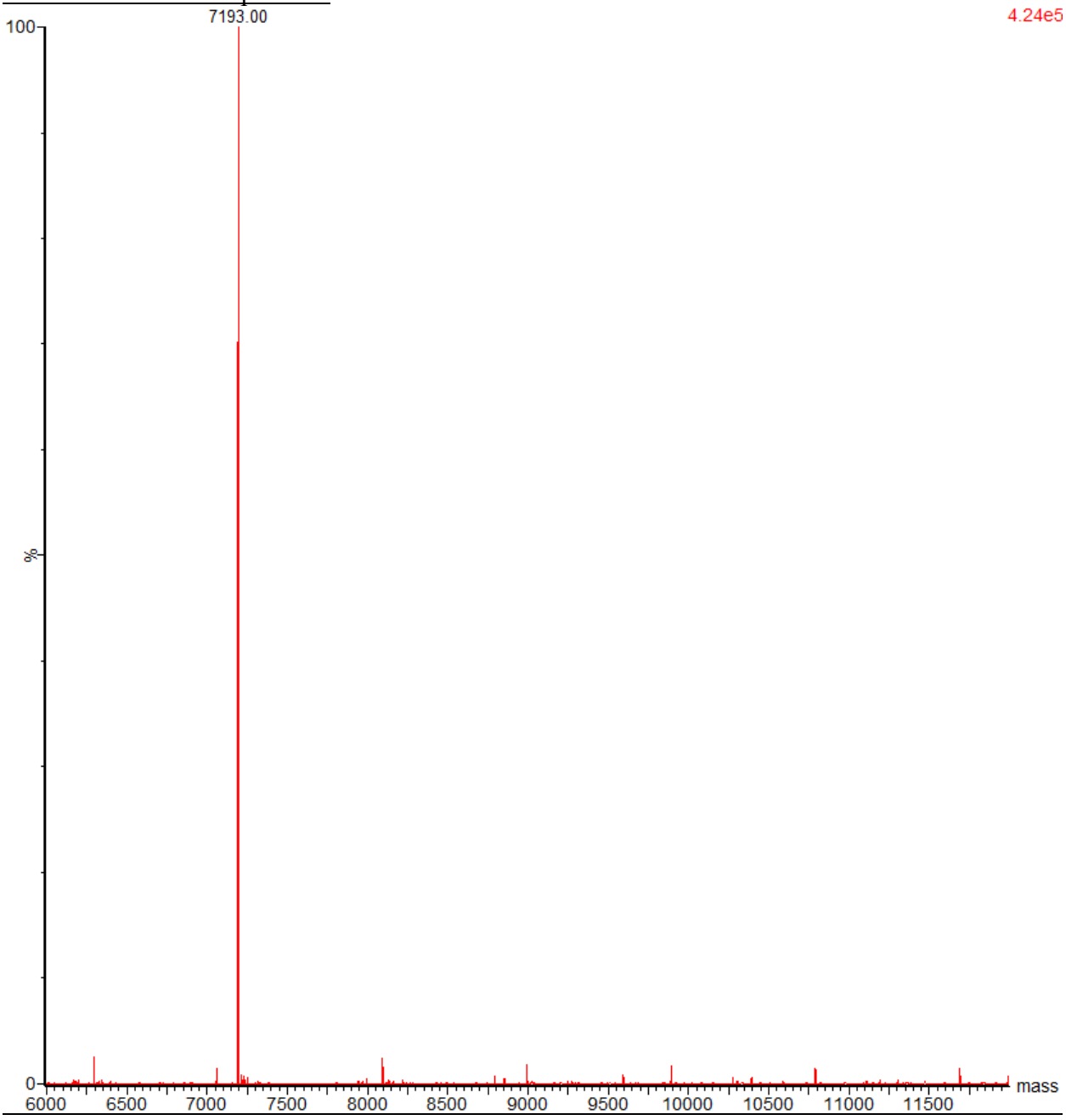
A260 Chromatogram



Mass Spectrum

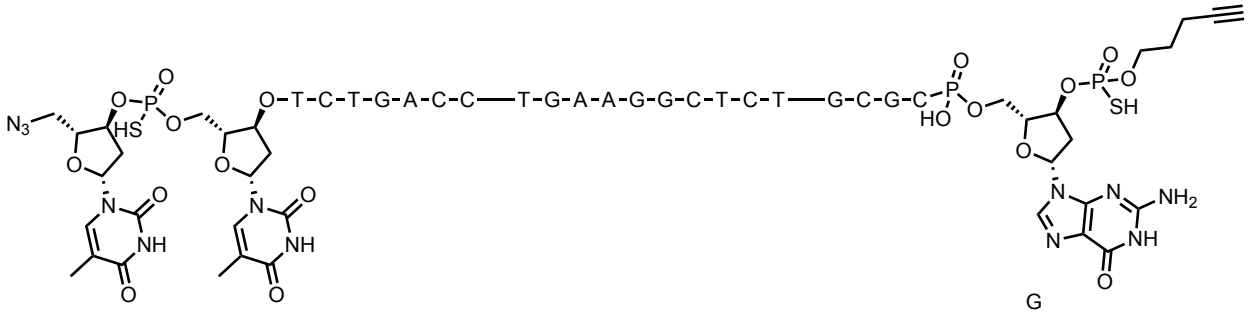


Deconvoluted Mass Spectrum



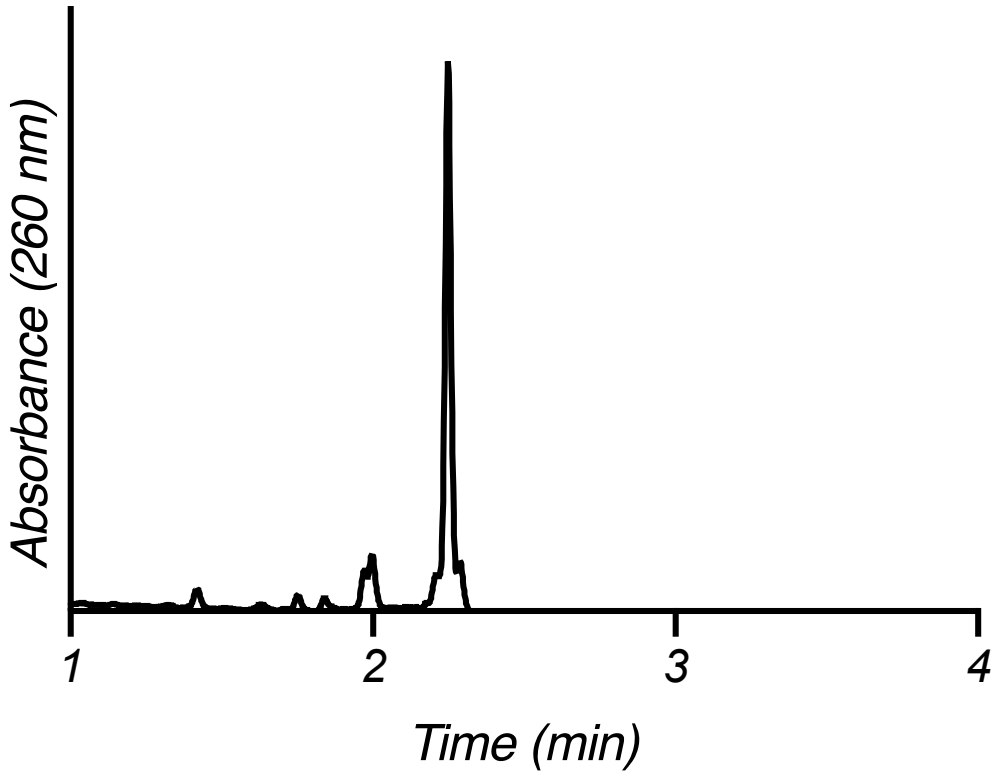
LCMS Characterization of 70 after SENDR with **Ψ-17** and lambda exo cleanup:

Produced from **SI-48:**
Using **Ψ-17**
Expected Mass: 7538.99
Expected (M-7)/7 Ion: 1075.5
Found Deconvoluted Mass: 7539.00
Found (M-6)/6 Ion: 1075.6

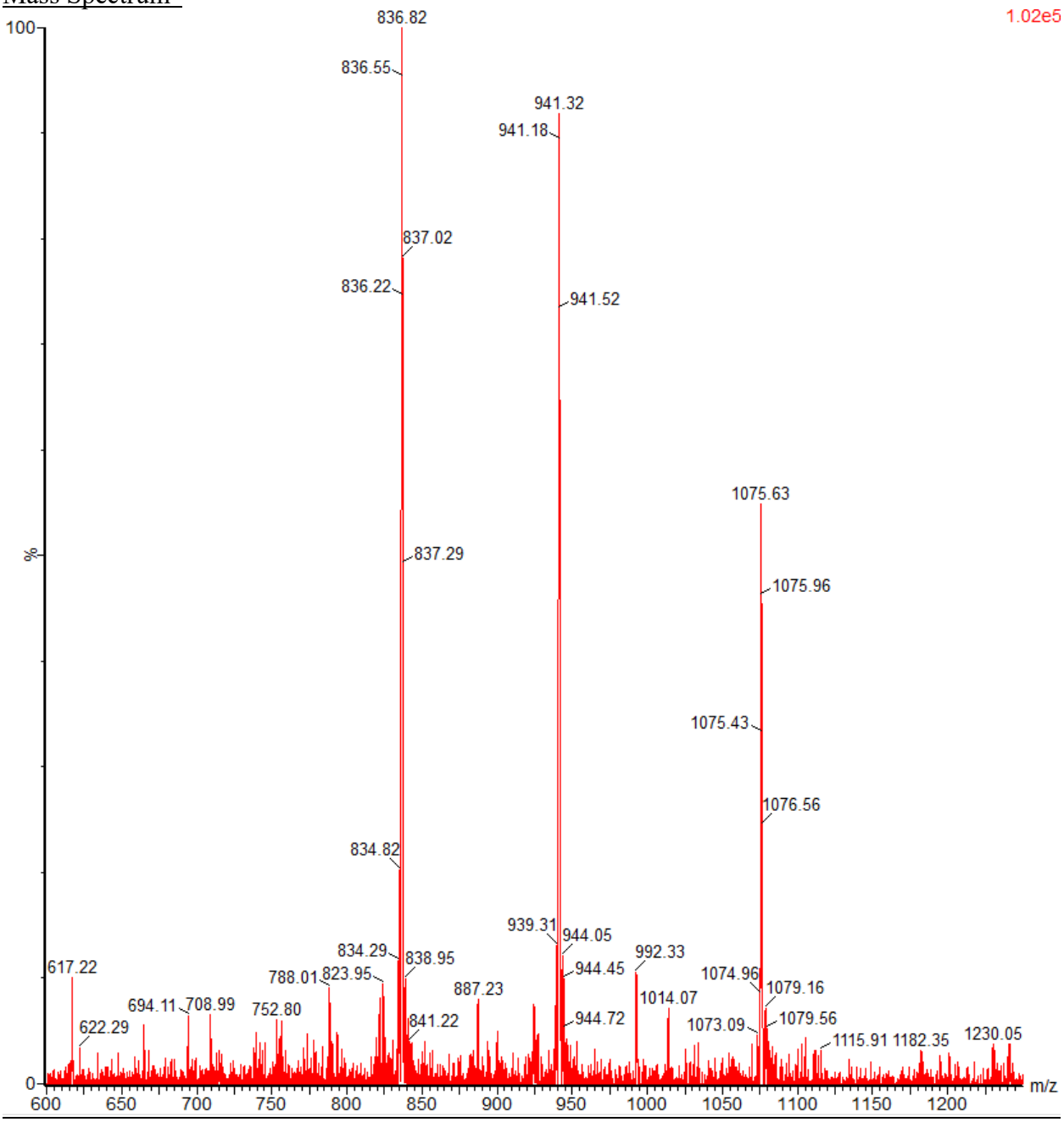


Exact Mass: 7535.21
Molecular Weight: 7538.99

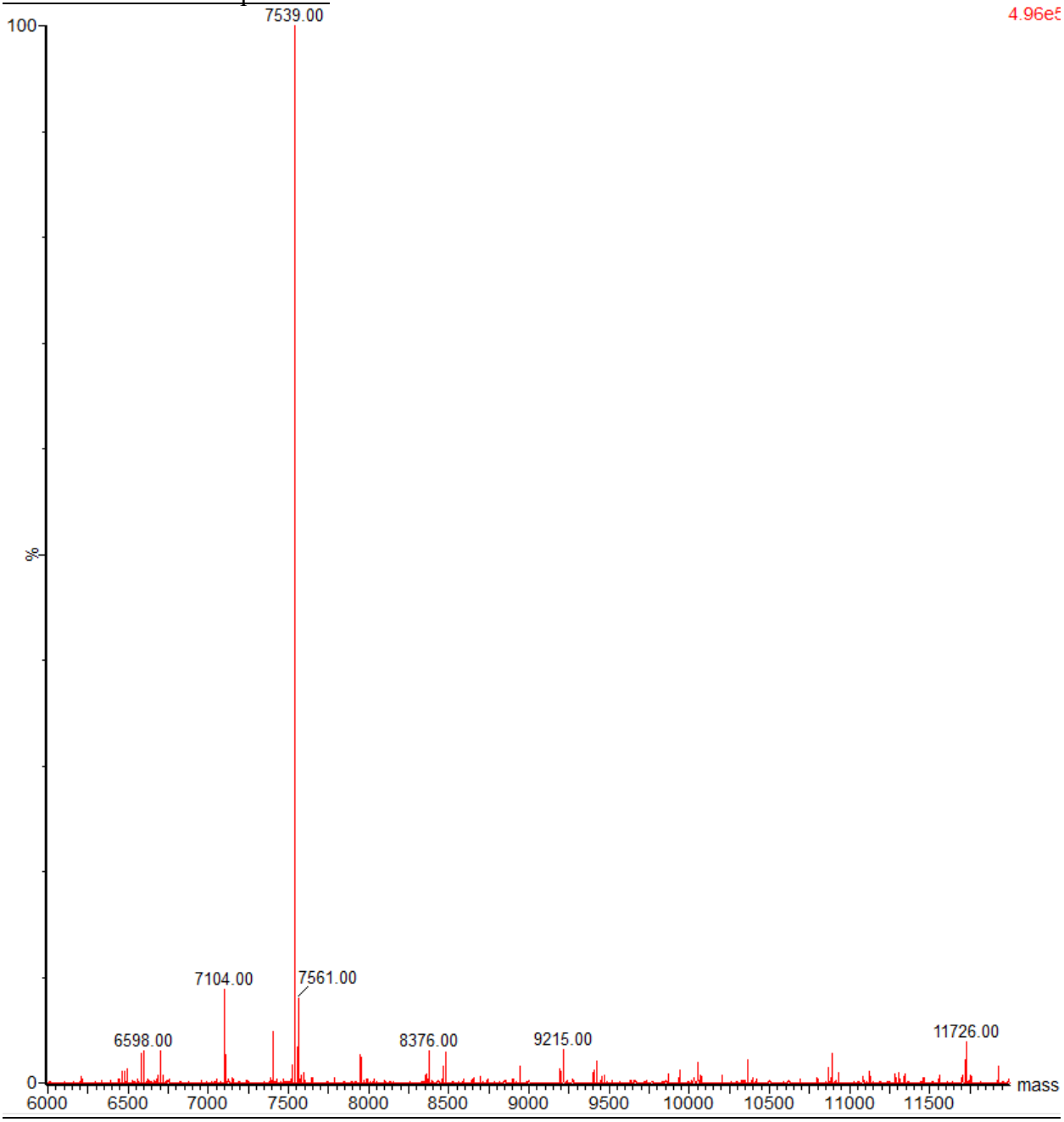
A260 Chromatogram



Mass Spectrum



Deconvoluted Mass Spectrum



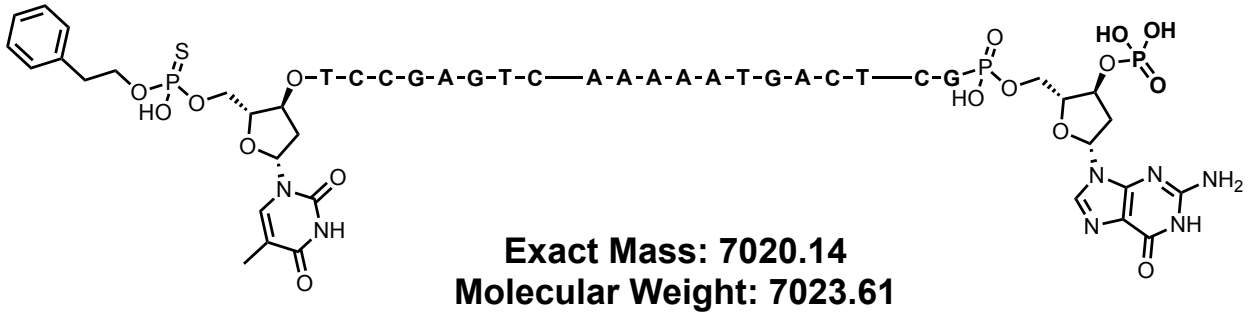
Comparison Between Kit and Microtube Format

Procedure

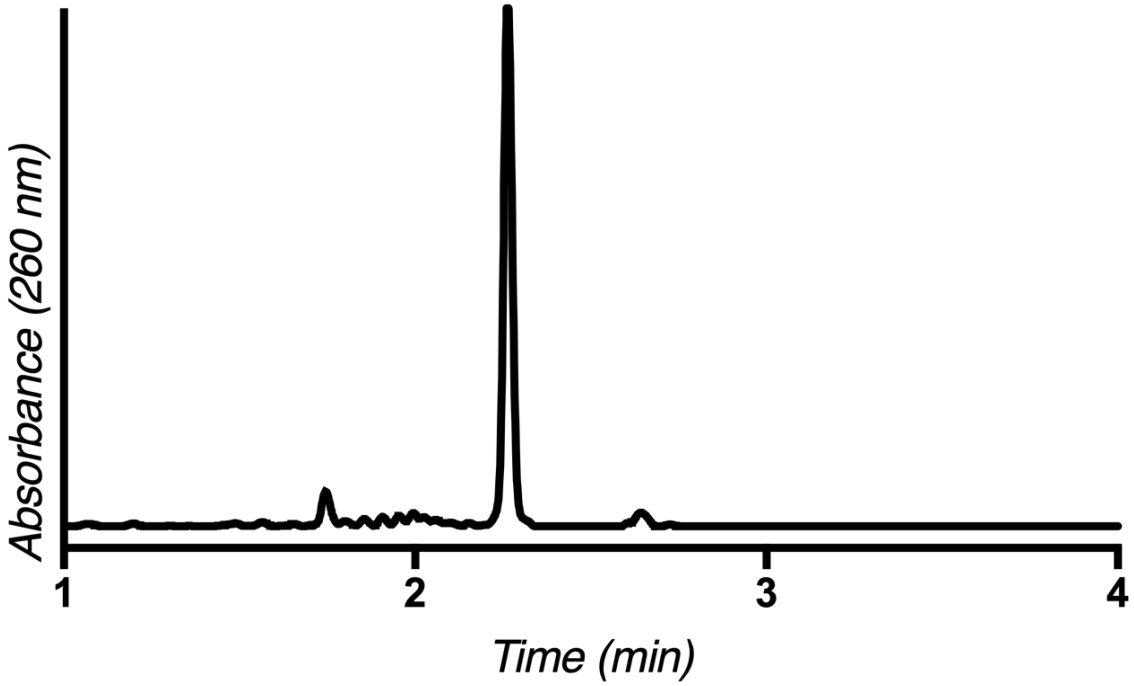
DNA **3** was synthesized in cartridges according to *General Procedure 3*. Briefly, the resin (100 μL) was loaded into a fritted cartridge (Bio-Rad, Bio-Spin column, 1.2 mL, Cat. Num. 7326008). PBS (500 μL) was added to the resin bed and allowed to flow through with gravity. The column was capped on the bottom and DNA was loaded (5 nmol in 200 μL PBS) onto the resin bed. The column was then capped (on top), vortexed and agitated for 5 minutes. The load buffer was allowed to flow out with gravity. The Resin bed was washed with DMA (500 μL twice) and THF (500 μL three times), and the solvent was allowed to flow through with gravity each time. The top cap was replaced, and the resin was dried for 2 hours under vacuum (placed on a lyophilizer). A new cap was replaced on the bottom and Ψ-1 was added (300 mM in MeCN, 125 μL) and then DBU was added (900 mM in MeCN, 125 μL). The top cap was replaced, the cartridge was vortexed and the column was incubated at 37 ºC for 60 minutes. After the reaction the caps were removed, and the reaction mixture was allowed to flow to waste. The reaction was worked up by washing the resin with PBS and 1:1 PBS:MeCN (500 μL each). The cap washed replaced and elute buffer added (300 μL). The cartridge as vortexed for 30 seconds and agitated for 10 minutes. The cap was removed and the elute buffer was collected. Finally, the DNA was isolated by ethanol precipitation and analyzed by HPLC MS.

LCMS Characterization of 3

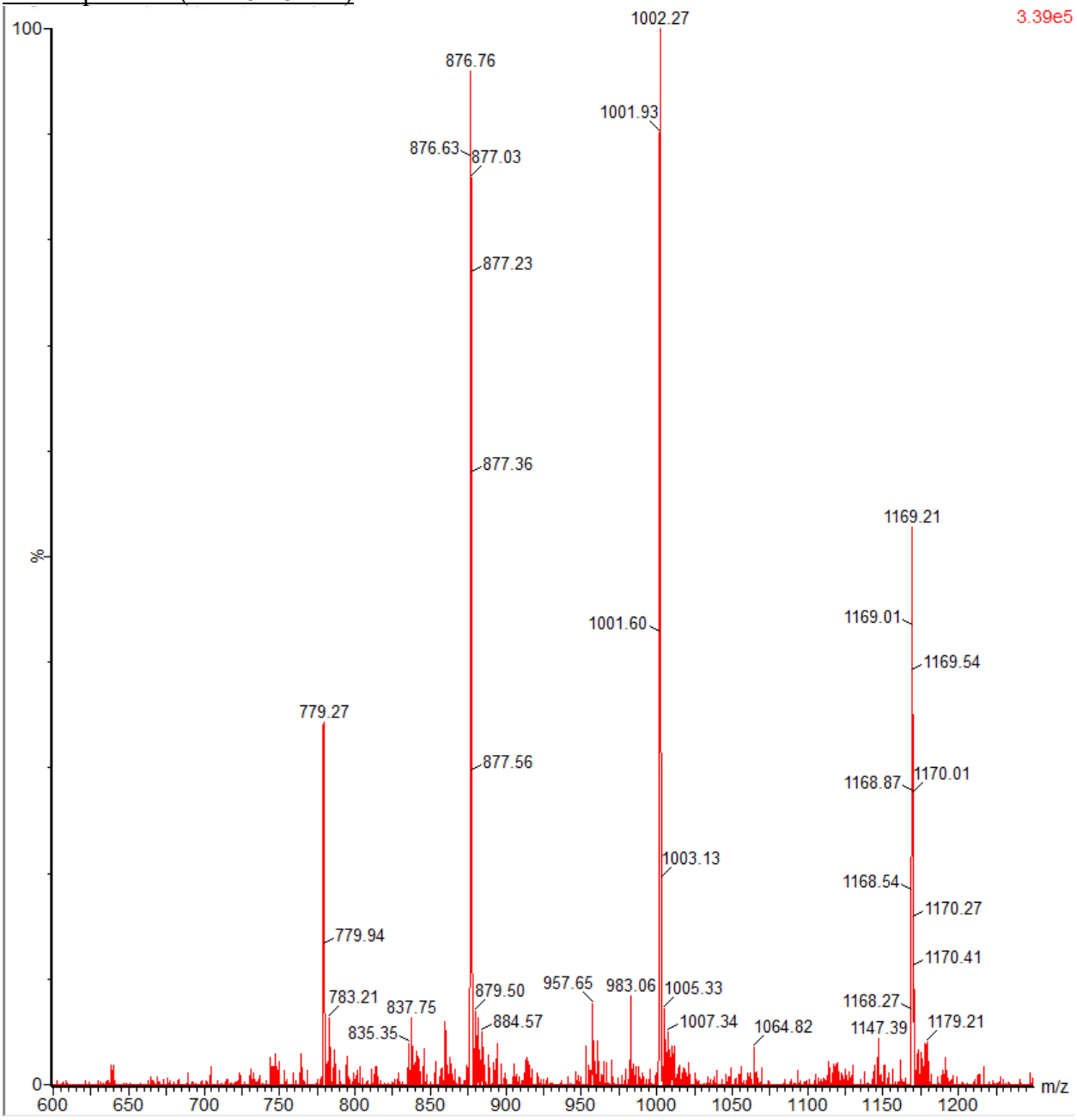
Produced from **1** by General Procedure **3:**
Using **Ψ-1**
Yield (A260): >95%
Expected Mass: 7023.6
Expected (M-4)/4 Ion: 1169.02
Found Deconvoluted Mass: 7024.00
Found (M-6)/6 Ion: 1169.2



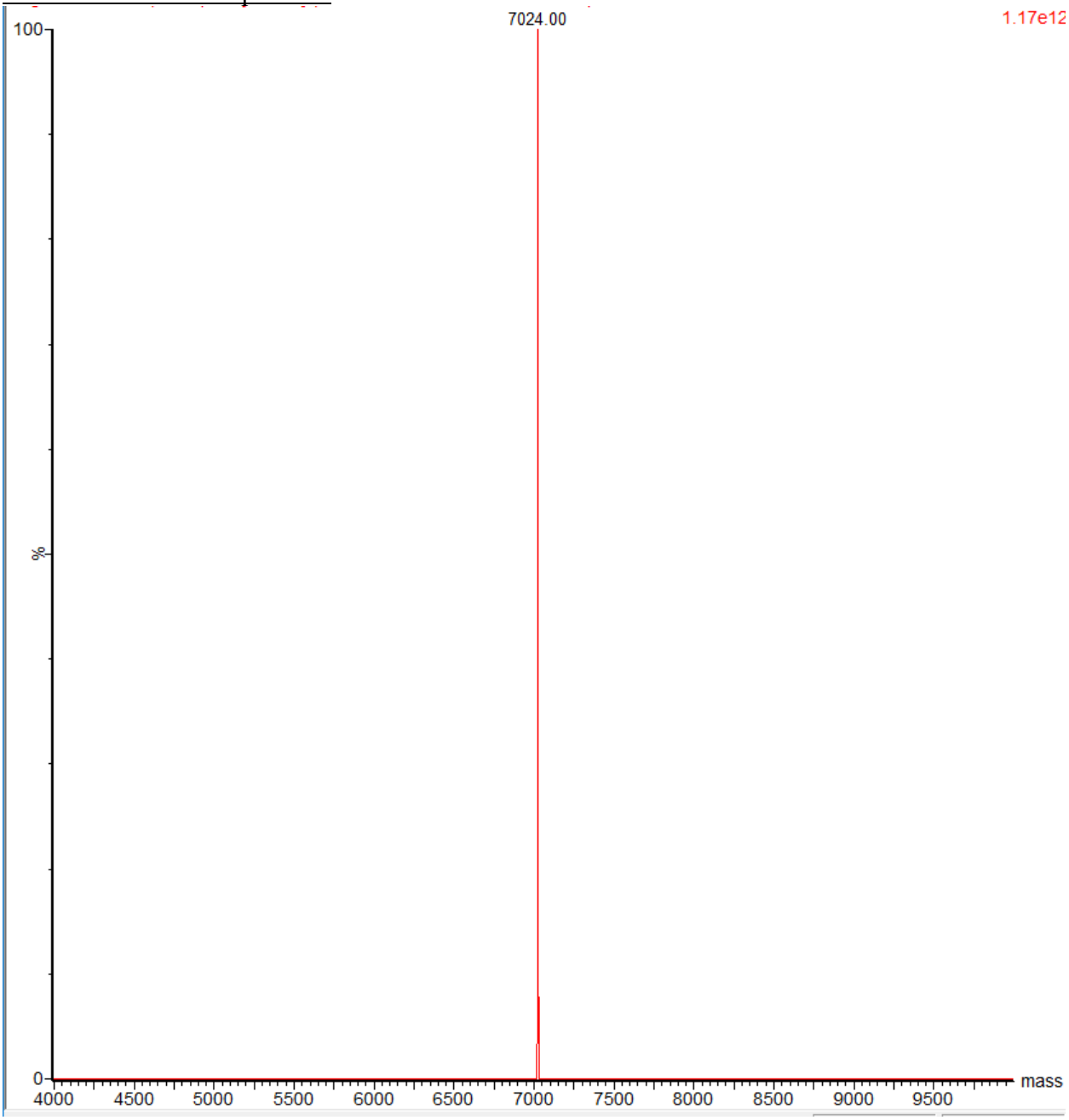
A260 Chromatogram



Mass Spectrum (RT= 5.28 min)

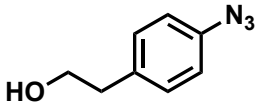


Deconvoluted Mass Spectrum



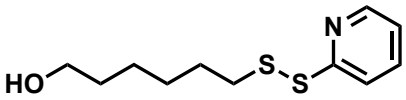
Synthesis of Small Molecule Alcohols for use in Ψ-Module Synthesis

Synthesis of SI-49



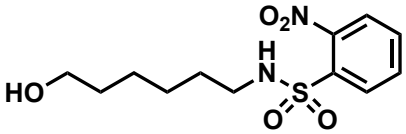
The synthesis of **SI-49** was carried out according to known literature procedures[4]

Synthesis of SI-50



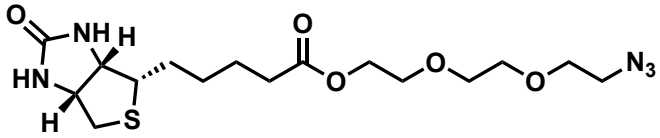
The synthesis of **SI-50** was carried out according to known literature procedures[5]

Synthesis of SI-51



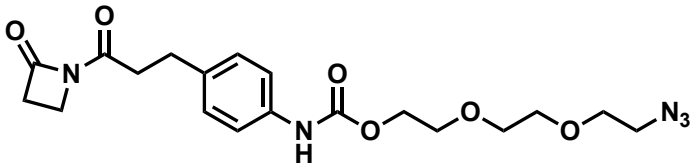
The synthesis of **SI-51** was carried out according to known literature procedures[6]

Synthesis of SI-52



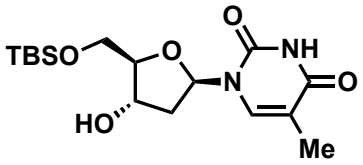
The synthesis of **SI-52** was carried out according to known literature procedures[7]

Synthesis of SI-53



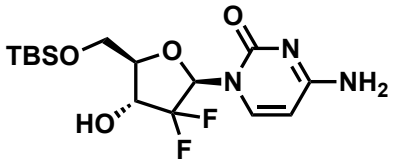
The synthesis of **SI-53** was carried out according to known literature procedures[8-9]

Synthesis of SI-54



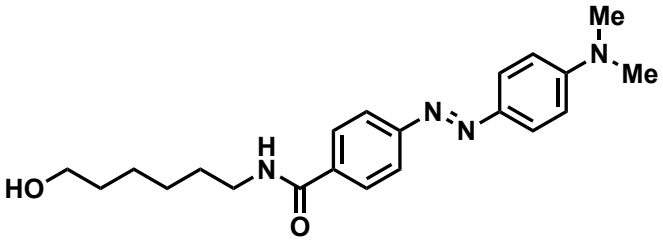
The synthesis of **SI-54** was carried out according to known literature procedures[10]

Synthesis of SI-55



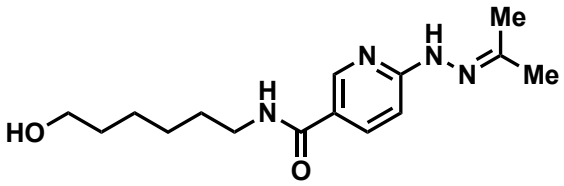
The synthesis of **SI-55** was carried out according to known literature procedures[11]

Synthesis of SI-56



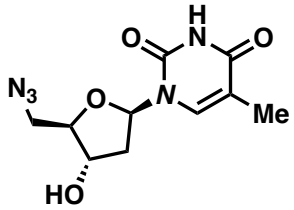
The synthesis of **SI-56** was carried out according to known literature procedures[12]

Synthesis of SI-57



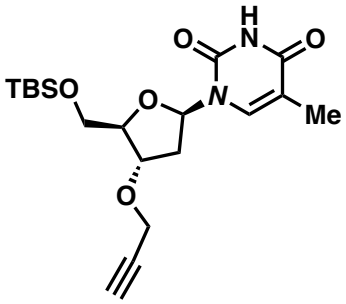
The synthesis of **SI-57** was carried out according to known literature procedures[13]

Synthesis of SI-58



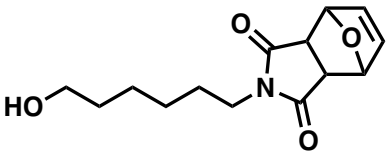
The synthesis of **SI-58** was carried out according to known literature procedures[14]

Synthesis of SI-59



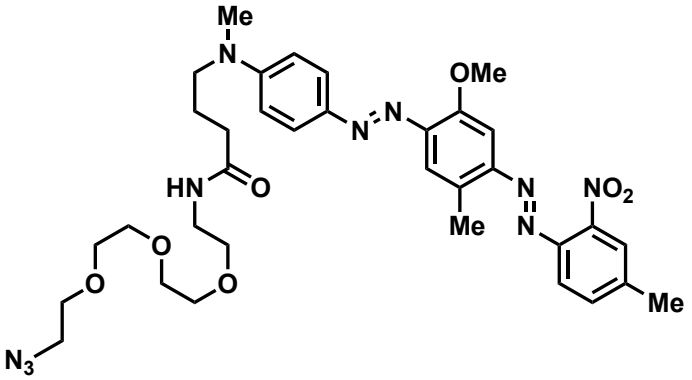
The synthesis of **SI-59** was carried out according to known literature procedures[15]

Synthesis of SI-60



The synthesis of **SI-60** was carried out according to known literature procedures[16]

Synthesis of SI-61



The synthesis of **SI-61** was carried out according to known literature procedures[9]

References

1. Knouse, K. W.; deGruyter, J. N.; Schmidt, M. A.; Zheng, B.; Vantourout, J. C.; Kingston, C.; Mercer, S. E.; McDonald, I. M.; Olson, R. E.; Zhu, Y.; Hang, C.; Zhu, J.; Yuan, C.; Wang, Q.; Park, P.; Eastgate, M. D.; Baran, P. S., Unlocking P(V): Reagents for chiral phosphorothioate synthesis. *Science* **2018,** *361* (6408), 1234-1238.
2. Zheng, Q.; Woehl, J. L.; Kitamura, S.; Santos-Martins, D.; Smedley, C. J.; Li, G.; Forli, S.; Moses, J. E.; Wolan, D. W.; Sharpless, K. B., SuFEx-enabled, agnostic discovery of covalent inhibitors of human neutrophil elastase. *Proc Natl Acad Sci U S A* **2019,** *116* (38), 18808-18814.
3. Nanna, A. R.; Rader, C., Engineering Dual Variable Domains for the Generation of Site-Specific Antibody-Drug Conjugates. *Methods Mol Biol* **2019,** *2033*, 39-52.
4. Bressy, C.; Belkheira, M.; El Abed, D.; Pons, J.-M., Chemoselective Organoclick–Click Sequence. *Synthesis* **2018,** *50* (21), 4254-4262.
5. Filippov, D.; van der Marel, G.; van Delft, P.; van Schie, E.; Meeuwenoord, N.; Overkleeft, H., Oligonucleotide Conjugates by Means of Copper-Free Click Chemistry - Expanding the Repertoire of Strained Cyclooctyne Phosphoramidites. *Synthesis* **2011,** *2011* (17), 2724-2732.
6. Miki, K.; Kimura, A.; Oride, K.; Kuramochi, Y.; Matsuoka, H.; Harada, H.; Hiraoka, M.; Ohe, K., High-contrast fluorescence imaging of tumors in vivo using nanoparticles of amphiphilic brush-like copolymers produced by ROMP. *Angew Chem Int Ed Engl* **2011,** *50* (29), 6567-70.
7. Ramos-Soriano, J.; Reina, J. J.; Perez-Sanchez, A.; Illescas, B. M.; Rojo, J.; Martin, N., Cyclooctyne [60]fullerene hexakis adducts: a globular scaffold for copper-free click chemistry. *Chem Commun (Camb)* **2016,** *52* (69), 10544-6.
8. Palanki, M. S.; Bhat, A.; Bolanos, B.; Brunel, F.; Del Rosario, J.; Dettling, D.; Horn, M.; Lappe, R.; Preston, R.; Sievers, A.; Stankovic, N.; Woodnut, G.; Chen, G., Development of a long acting human growth hormone analog suitable for once a week dosing. *Bioorg Med Chem Lett* **2013,** *23* (2), 402-6.
9. Ghosh, A. K.; Doung, T. T.; McKee, S. P.; Thompson, W. J., N,N`-dissuccinimidyl carbonate: a useful reagent for alkoxycarbonylation of amines. *Tetrahedron Letters* **1992,** *33* (20), 2781-2784.
10. Beigelman, L.; Serebryany, V.; Karpeisky, A.; Matulic-Adamic, J., Synthesis of 1,4-Anhydro-2-deoxy-d-ribitol Derivatives from Thymidine Using Improved Preparation of Furanoid Glycals. *Synthesis* **2002,** *2002* (12), 1652-1654.
11. Labroli, M. A.; Dwyer, M. P.; Shen, R.; Popovici-Muller, J.; Pu, Q.; Wyss, D.; McCoy, M.; Barrett, D.; Davis, N.; Seghezzi, W.; Shanahan, F.; Taricani, L.; Beaumont, M.; Malinao, M. C.; Parry, D.; Guzi, T. J., The identification of novel 5'-amino gemcitabine analogs as potent RRM1 inhibitors. *Bioorg Med Chem* **2014,** *22* (7), 2303-10.
12. Katritzky, A. R.; Chen, Q. Y.; Tala, S. R., Convenient preparations of azo-dye labeled amino acids and amines. *Org Biomol Chem* **2008,** *6* (13), 2400-4.
13. Grotzky, A.; Nauser, T.; Erdogan, H.; Schluter, A. D.; Walde, P., A fluorescently labeled dendronized polymer-enzyme conjugate carrying multiple copies of two different types of active enzymes. *J Am Chem Soc* **2012,** *134* (28), 11392-5.

14. Skakuj, K.; Bujold, K. E.; Mirkin, C. A., Mercury-Free Automated Synthesis of Guanidinium Backbone Oligonucleotides. *J Am Chem Soc* **2019,** *141* (51), 20171-20176.
15. Lolk, L.; Pohlsgaard, J.; Jepsen, A. S.; Hansen, L. H.; Nielsen, H.; Steffansen, S. I.; Sparving, L.; Nielsen, A. B.; Vester, B.; Nielsen, P., A click chemistry approach to pleuromutilin conjugates with nucleosides or acyclic nucleoside derivatives and their binding to the bacterial ribosome. *J Med Chem* **2008,** *51* (16), 4957-67.
16. Fuhrmann, A.; Broi, K.; Hecht, S., Lowering the Healing Temperature of Photoswitchable Dynamic Covalent Polymer Networks. *Macromol Rapid Commun* **2018,** *39* (1).