Electronic Supplementary Information

Azide-alkyne cycloaddition for universal post-synthetic modifications of nucleic acids and effective synthesis of bioactive nucleic acid conjugates

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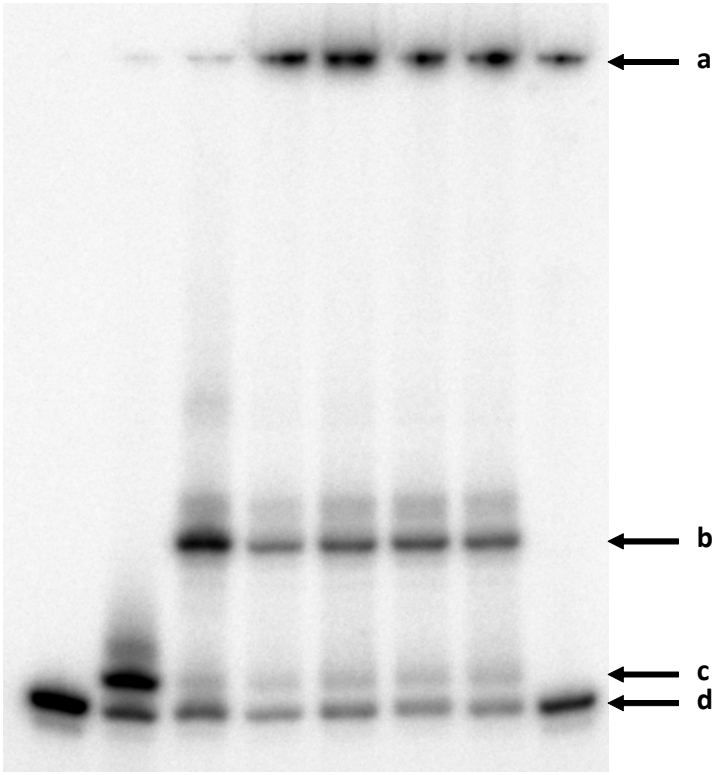
Contents

(A)

Maleimide Linker:	N/A	N/A	**3e**	**3a**	**3b**	**3c**	**3d**	N/A
Cystamine Linker:	-	+	+	+	+	+	+	-
POC (b) Yield (%):	N/A	N/A	72	27	27	37	30	0



(B)

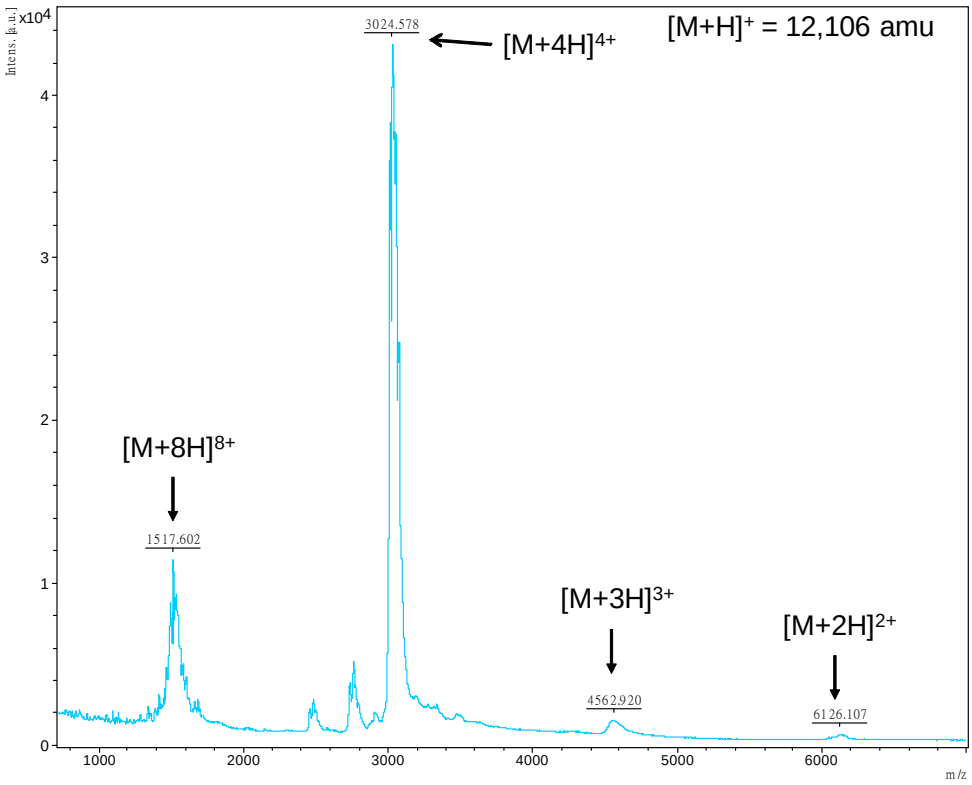


Fig. S1 Exploring potential of the *N*-maleoyl amino acid succinimidyl esters (3) for synthesis of POCs by Michael addition of a thiolate peptide. (A) The ^{32}P-labeled 3' primer DNA with or without previous modifications with cystamine and **3** were reacted with a cysteine-containing Tat peptide under the conditions of Michael addition and analyzed the reaction products by 20% urea-PAGE. **a**, the multiple peptide-linked DNA conjugate; **b**, the POC product; **c**, the cystamine-modified 3' primer DNA; **d**, the 3' primer DNA. (B) MALDI-TOF analysis of the denaturing PAGE-purified Product **a** from the far right reaction in Fig. S1A. The molecular mass for the 3' primer DNA is 6,038 amu and for the cysteine-containing Tat peptide is 1,517 amu. With four of the peptides covalently linked to the 3' primer DNA, the molecular mass of the DNA conjugate is equal to the 12,106 amu. It is noted that, even though the unusual MALDI-TOF peak distribution of Product **a** as shown here, the observed peaks could not be attributed to $[M+H]^+$, $[2M+H]^{2+}$, $[3M+H]^{3+}$, etc, because Product **a** had been purified by denaturing urea-PAGE prior to the MALDI-TOF analysis. We thus ruled out the possible presence of DNA conjugates with lower molecular mass as the identity of Product **a**.

CuAAC:	-	-	-	+	+	+	+	+	+
Alkyne : azido-DNA ratio:	-	-	-	0.33	1	10	20	40	20
DNA substrate:	-	1	2	3	3	3	3	3	3
Cu ligand:	-	-	-	4	4	4	4	4	5
Yield (%):	N/A	76	83	16	37	56	80	82	N/A

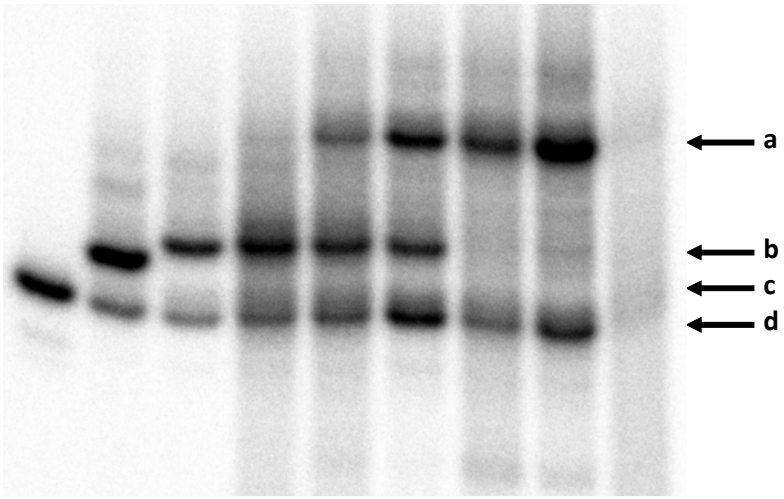


Fig. S2 Determination of the optimal alkyne : azido-DNA ratio and the appropriate copper ligand in the CuAAC reaction. The studied DNA was the 3' primer DNA and was labelled with 32-P at the 5' end before the reactions. The reaction products were analyzed by 20% urea-PAGE and visualized by an Amersham Typhoon PhosphorImager. 1, ethylenediamine; 2, ethylenediamine + **6d**; 3, ethylenediamine + **6d** + **10**; 4, THPTA; 5, NTB; **a**, the CuAAC reaction product between the azido 3' primer DNA and **10**; **b**, the **6d**-ethylenediamine-3' primer DNA conjugate; **c**, the ethylenediamine-modified 3' primer DNA; **d**, the 3' primer DNA.

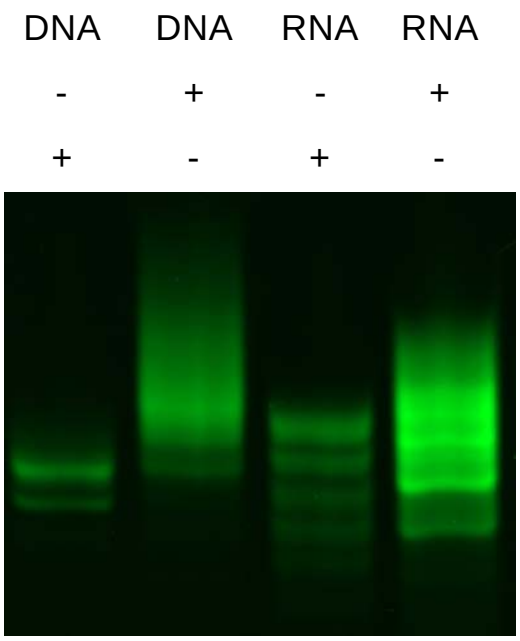


Fig. S3 The products of the CuAAC reactions between azido nucleic acids and the fluorophore 15, or between alkynyl nucleic acids and the other fluorophore 16 as analyzed by fluorescence imaging. The nucleic acid conjugates acquired from the CuAAC reactions were separated by 20% urea-PAGE and visualized by an Amersham Typhoon PhosphorImager through detection of fluorescein signals (Reference 14 in the text). DNA, the 3' primer DNA; RNA, the 17-mer RNA; alkynyl substrate, ethylenediamine + **19**; azido substrate, ethylenediamine + **6d**.

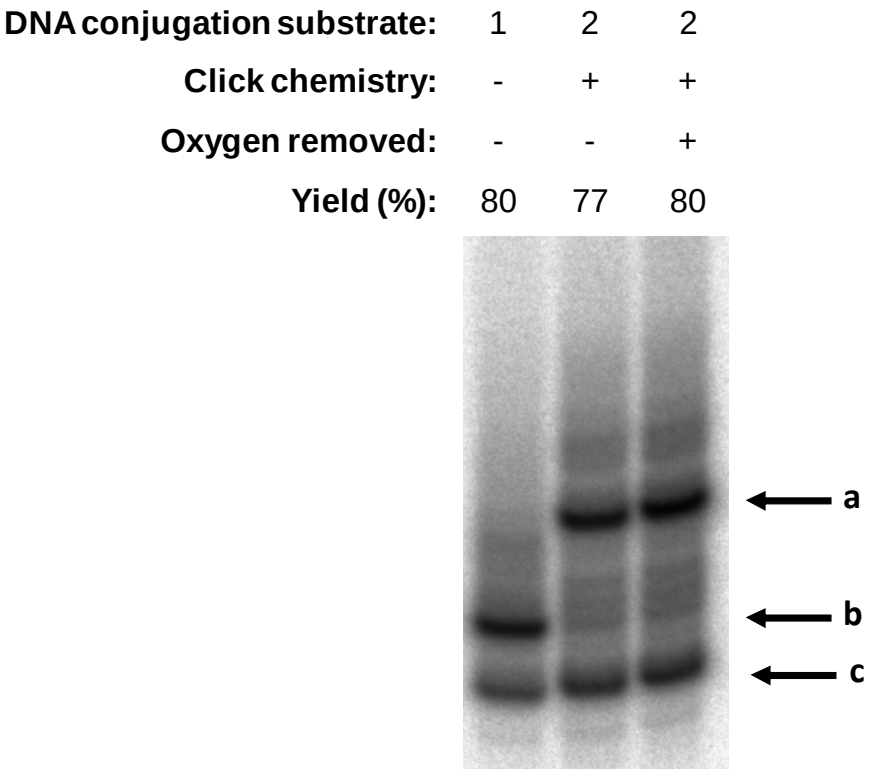


Fig. S4 Oxygen not the major cause of nucleic acid degradation in the CuAAC reactions. The DNA, the 3' primer DNA, was labeled with ^{32}P at the 5' end, and modified with ethylenediamine by the two-step phosphoramidation reaction and with **6d** by the amidation reaction, sequentially. The afforded DNA conjugate was further subjected to the CuAAC reaction with **10** in the presence/absence of oxygen to attain the final reaction products. The CuAAC reaction products were analyzed by 20% urea-PAGE and visualized by an Amersham Typhoon PhosphorImager. DNA conjugation substrate: 1, ethylenediamine + **6d**; 2, ethylenediamine + **6d** + **10**. a, the triazole product of the CuAAC reaction between the azido 3' primer DNA and **10**; b, the azido 3' primer DNA; c, the 3' primer DNA.

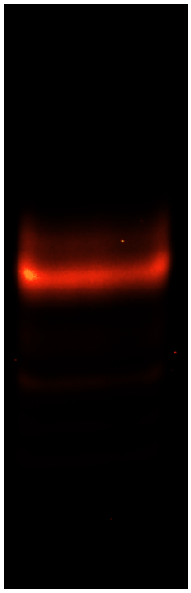


Fig. S5 Product analysis of the SPAAC reaction between the azido 3’ primer DNA and the cyclooctyne Alkyne MegaStokes dye 608 by fluorescence imaging.

The azido DNA was prepared by the two-step phosphoramidation reaction to modify the 3’ primer DNA with ethylenediamine and subsequently by amidation of the ethylenediamine-modified 3’ primer DNA with **6d**. The DNA conjugates obtained from the SPAAC reaction were purified by the first 20% urea-PAGE, analyzed by the second 20% urea-PAGE and finally visualized by an Amersham Typhoon PhosphorImager with the settings of the excitation wavelength at 488 nm and the emission wavelength at 580 nm.

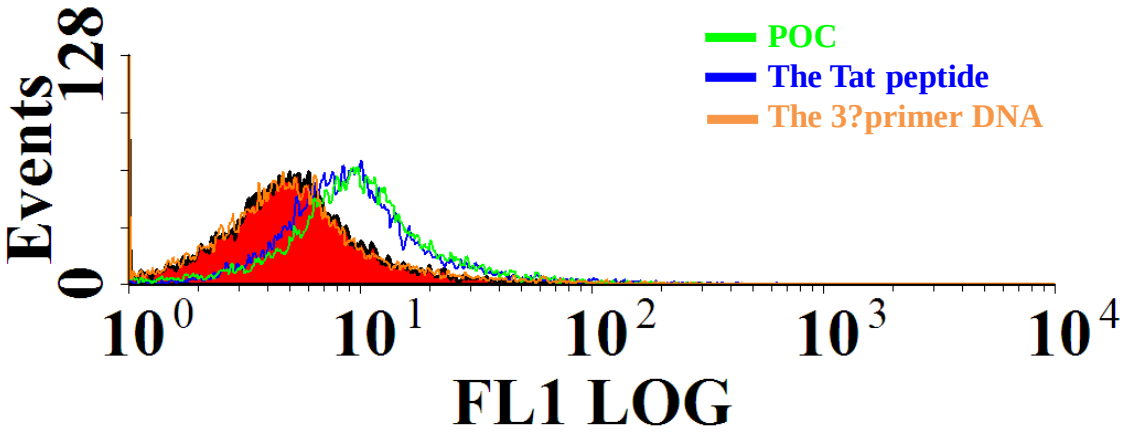
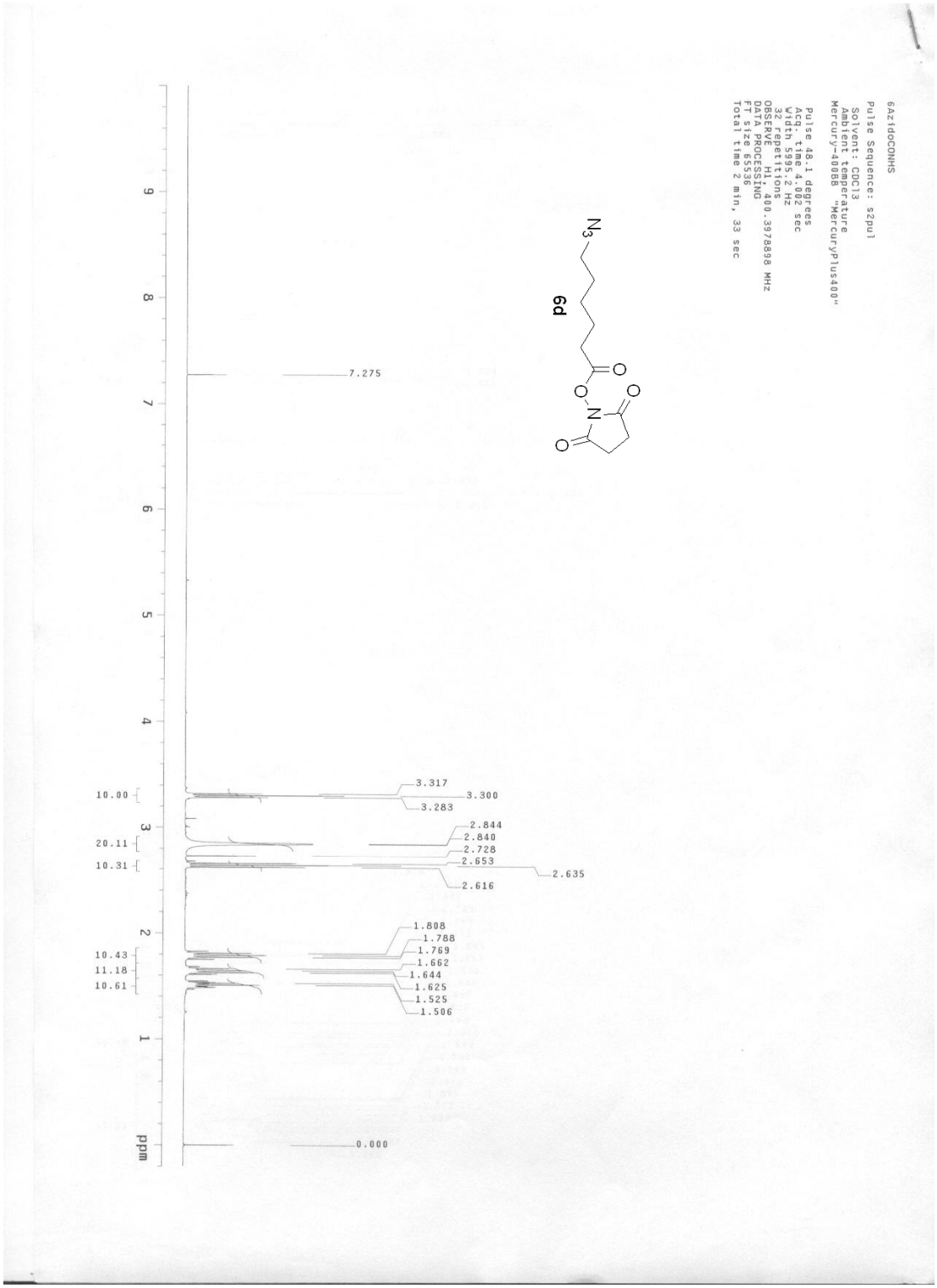


Fig. S6 Associations of the CuAAC-prepared POC with human A549 cells analyzed by flow cytometry. A549 cells were incubated with 5 μM of the FITC-labeled inoculates (the Tat peptide, the 3’ primer DNA and the CuAAC-prepared POC, respectively) for 24 h and followed by analysis of flow cytometry. Cells were washed and harvested with trypsin/EDTA before the analysis.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32 repetitions
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3.300
3.283
2.844
2.840
2.728
2.653
2.635
2.616
1.808
1.788
1.769
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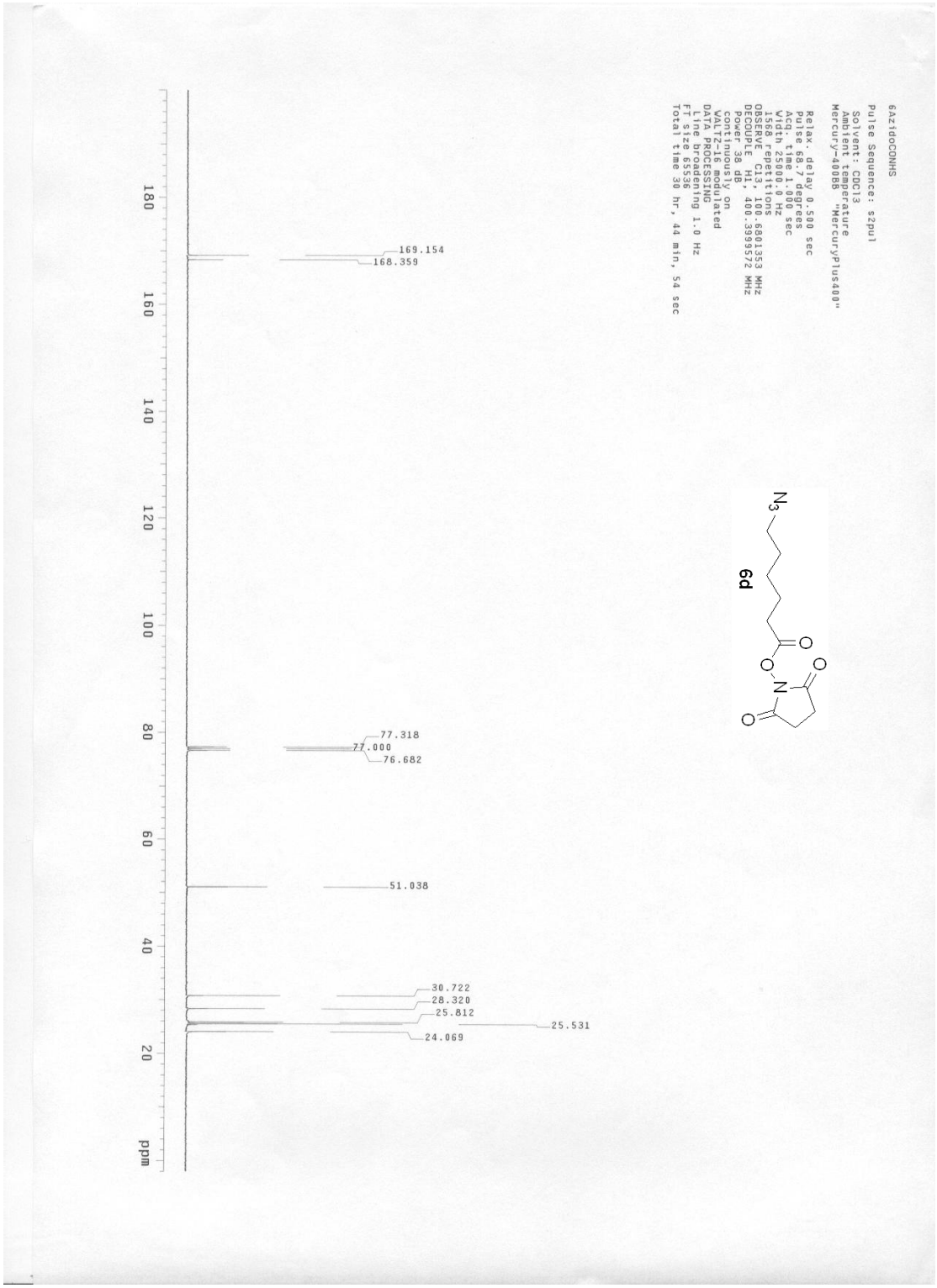
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Width 15060.0 Hz
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continuously on
WALTZ-16 modulated
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77.000
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25.531
24.069
180
160
140
120
100
80
60
40
20
ppm
6d
N3
O
O
O
N
O
O

Mass Spectrum SmartFormula Report

Analysis Info

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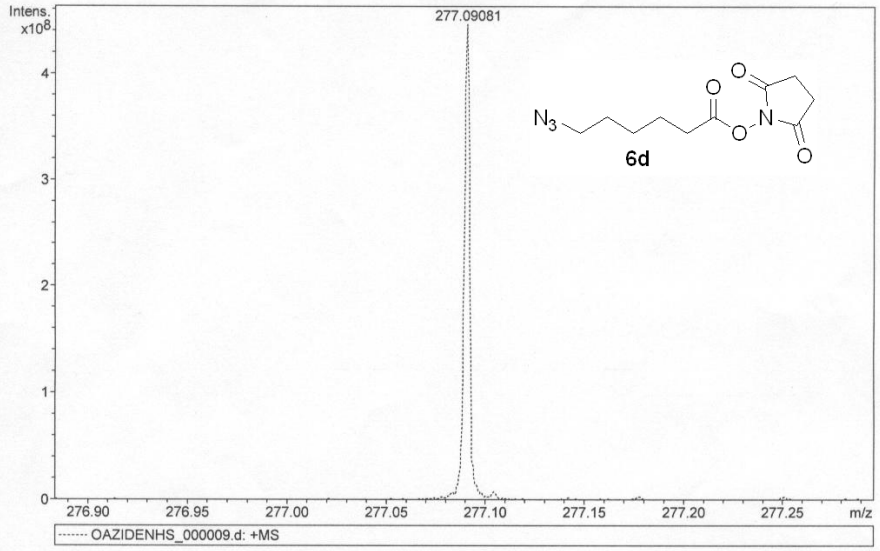
Comment	ESI Positive	



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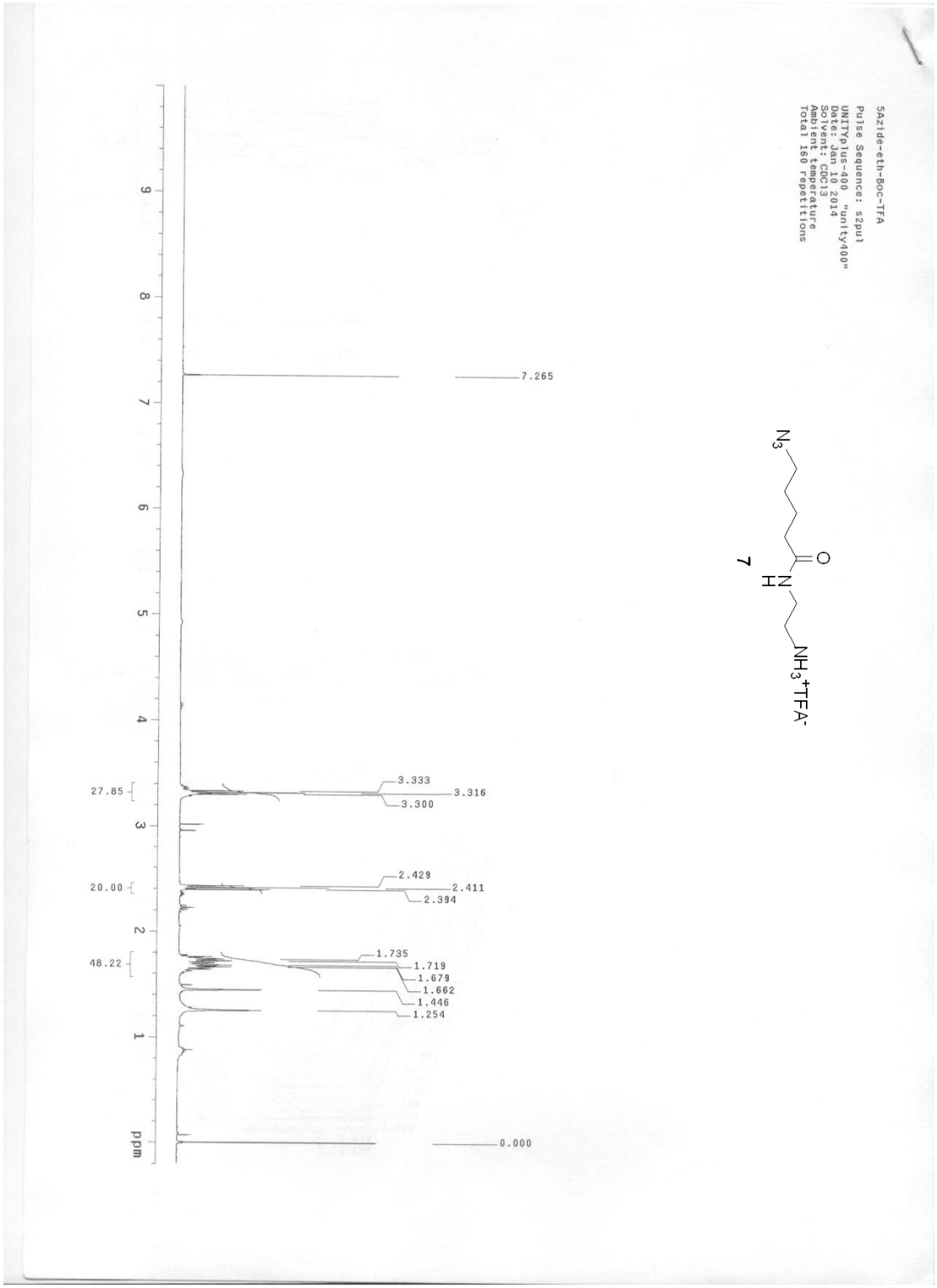
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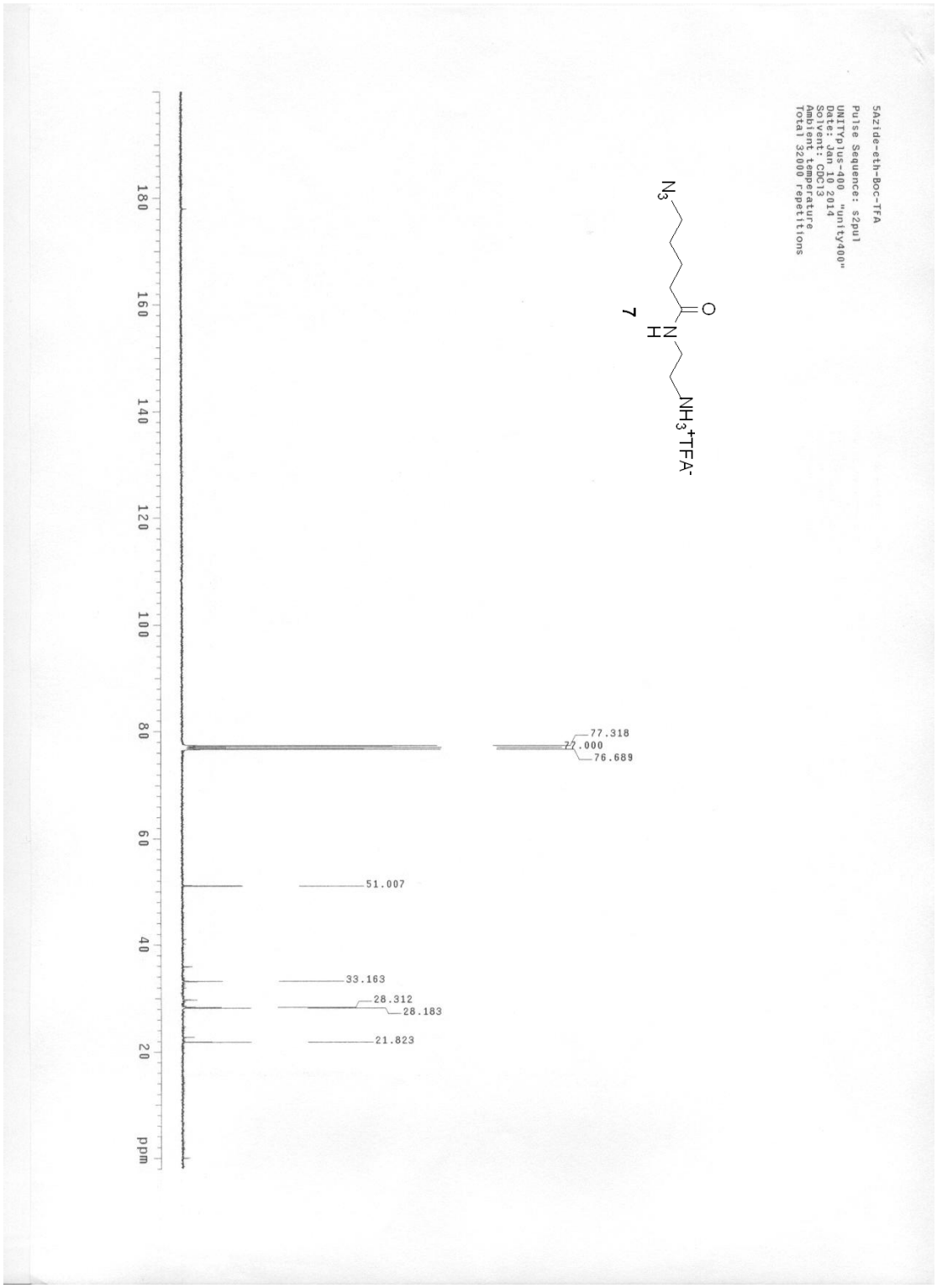
N
H
NH3+TFA-
7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Ambient temperature
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Mass Spectrum SmartFormula Report

Analysis Info

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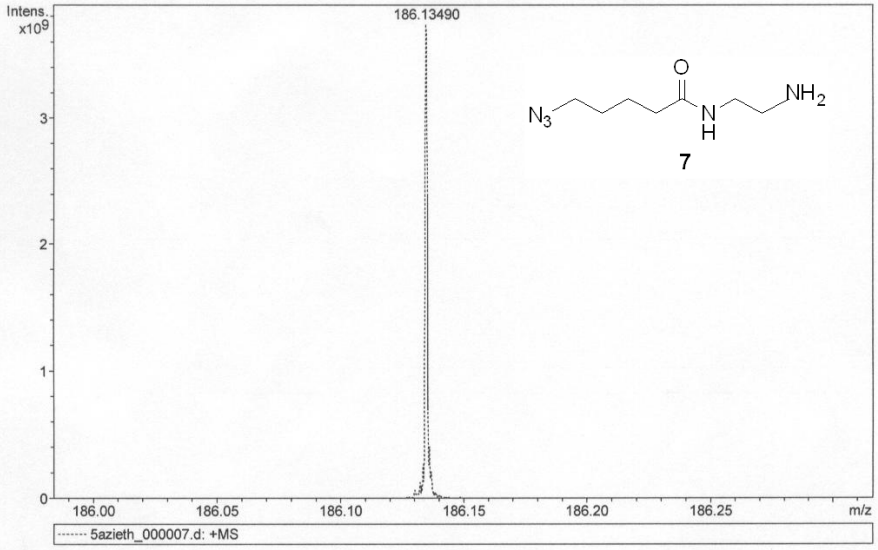

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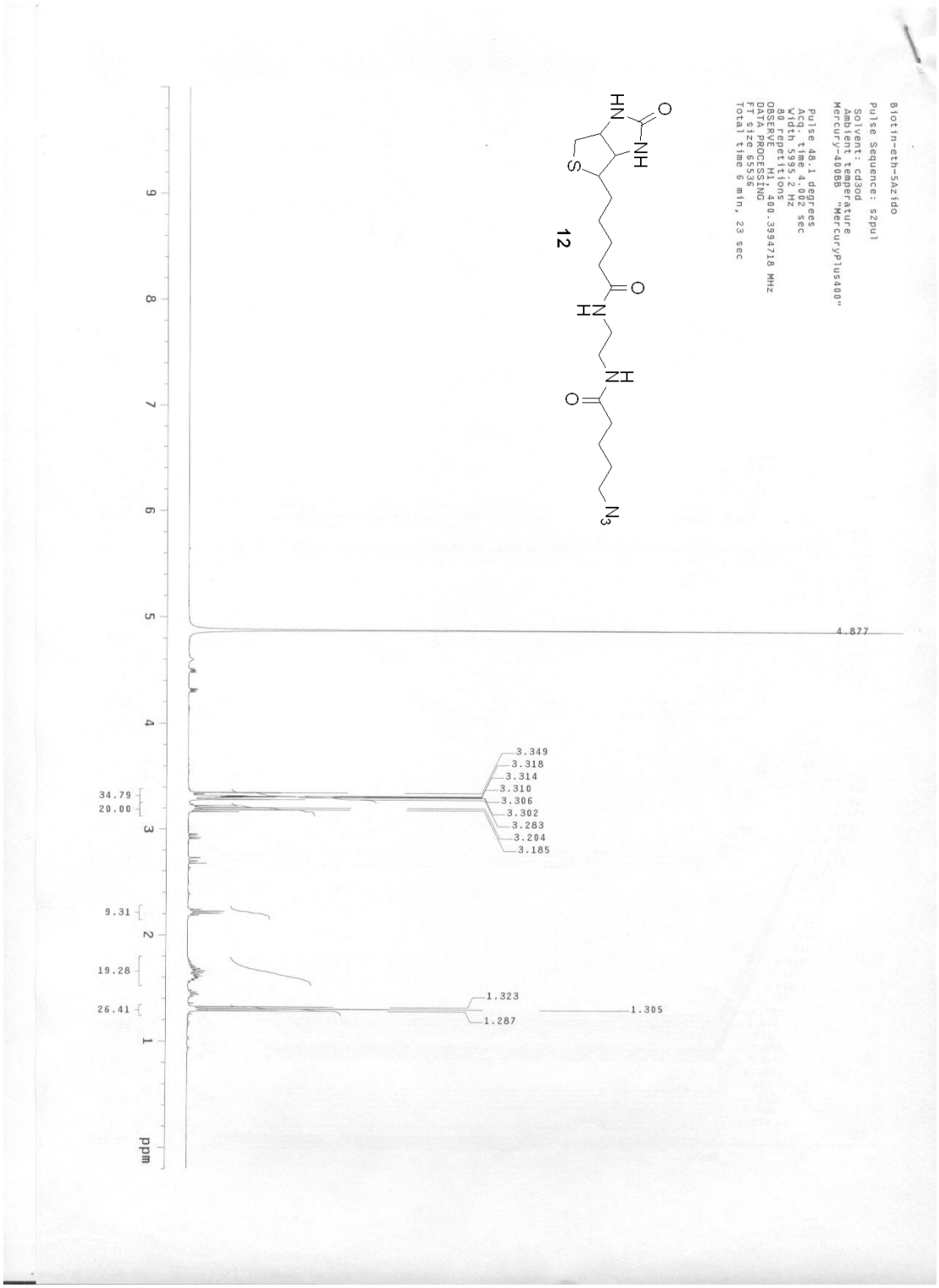
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3.310
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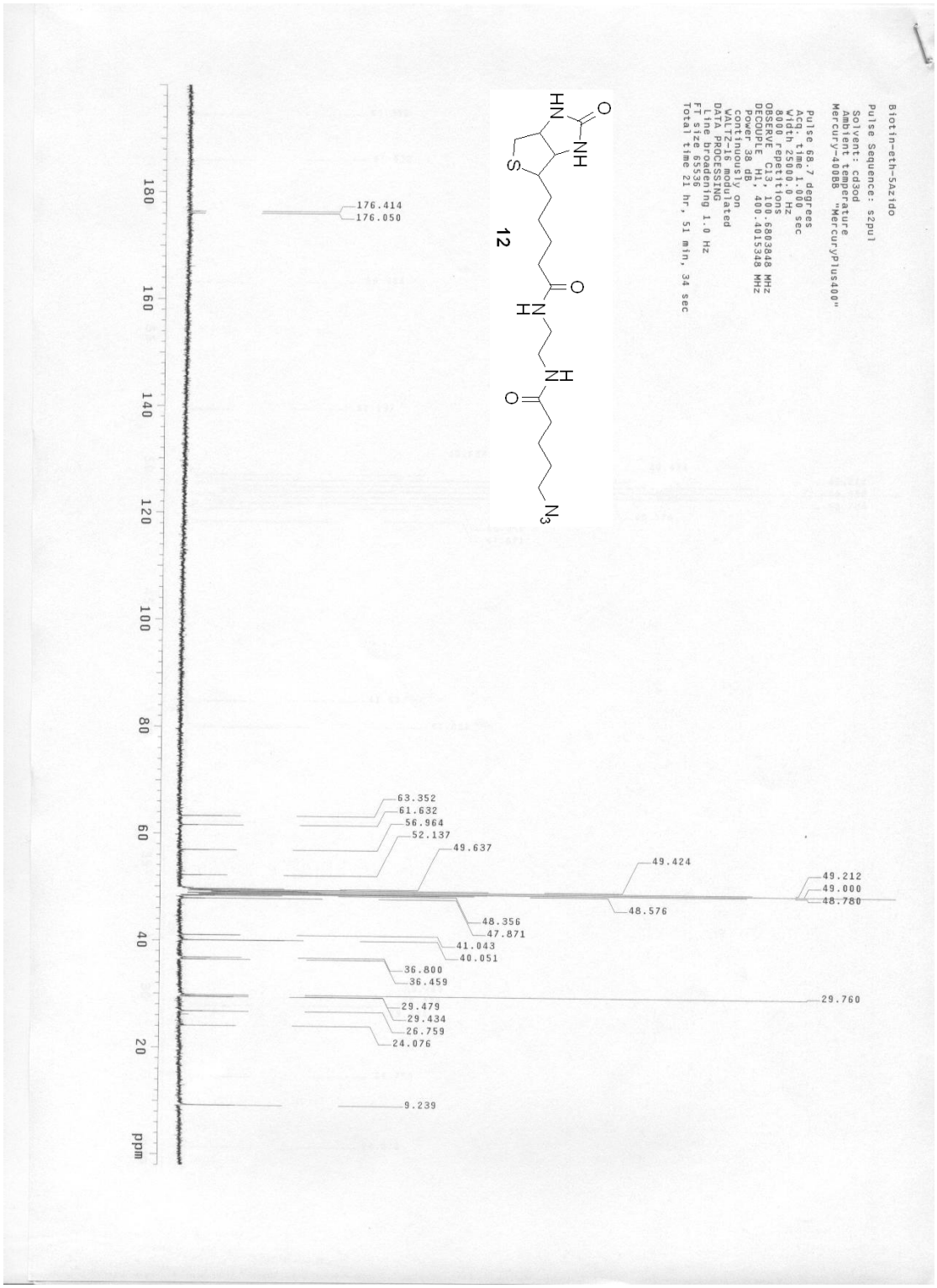
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8000 repetitions
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DECOUPLE H1, 400.4015348 MHz
Power 38 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
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63.352
61.632
56.964
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48.576
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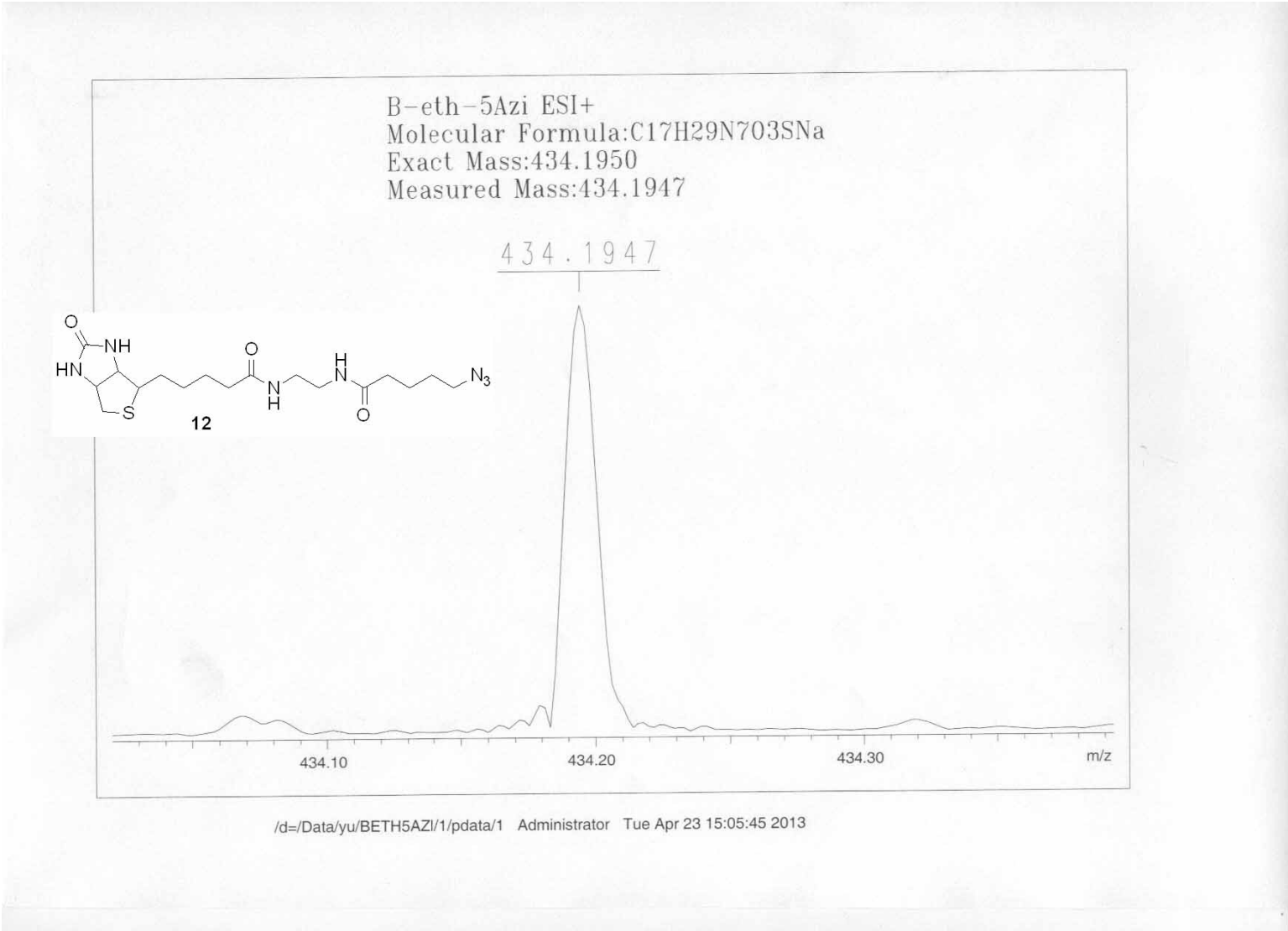
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Measured Mass:434.1947
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HN
S
O
N
H
H
N
O
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434.20
434.30
m/z
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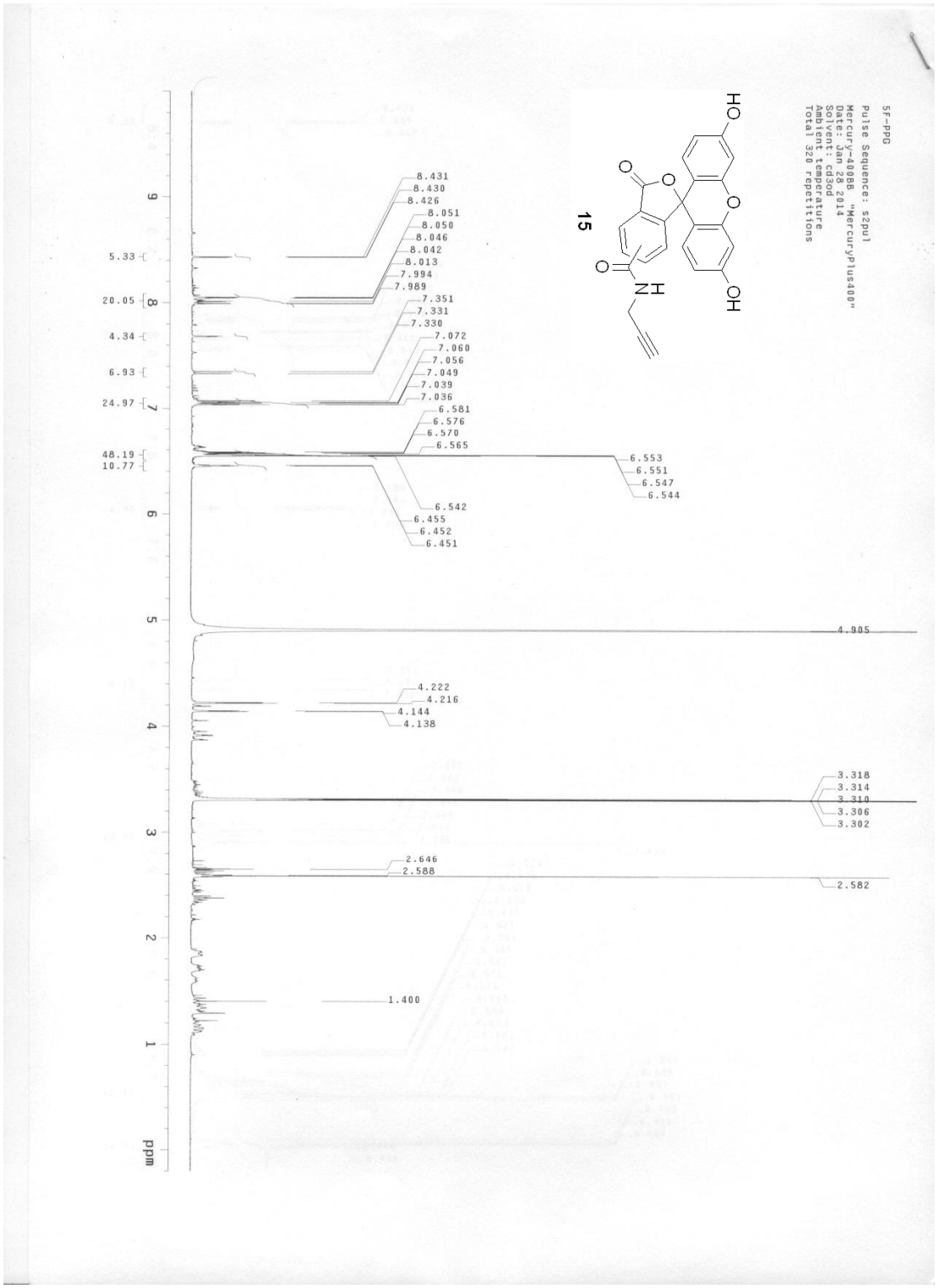
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8.050
8.046
8.042
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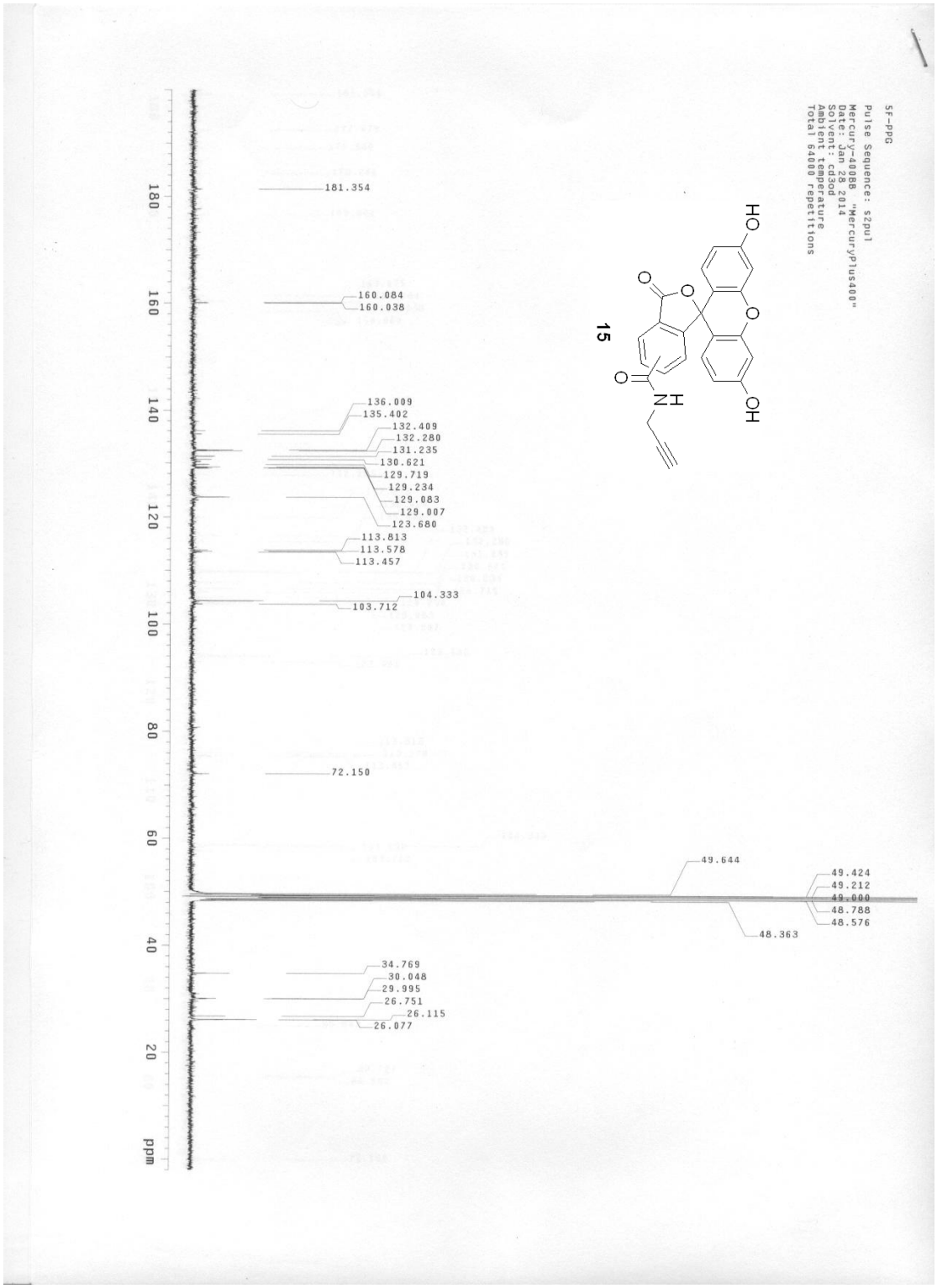
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131.235
130.621
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129.234
129.083
129.007
123.680
113.813
113.578
113.457
104.333
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48.363
34.769
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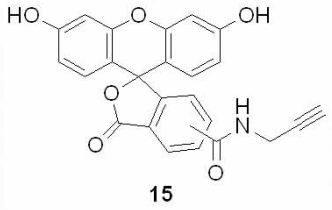

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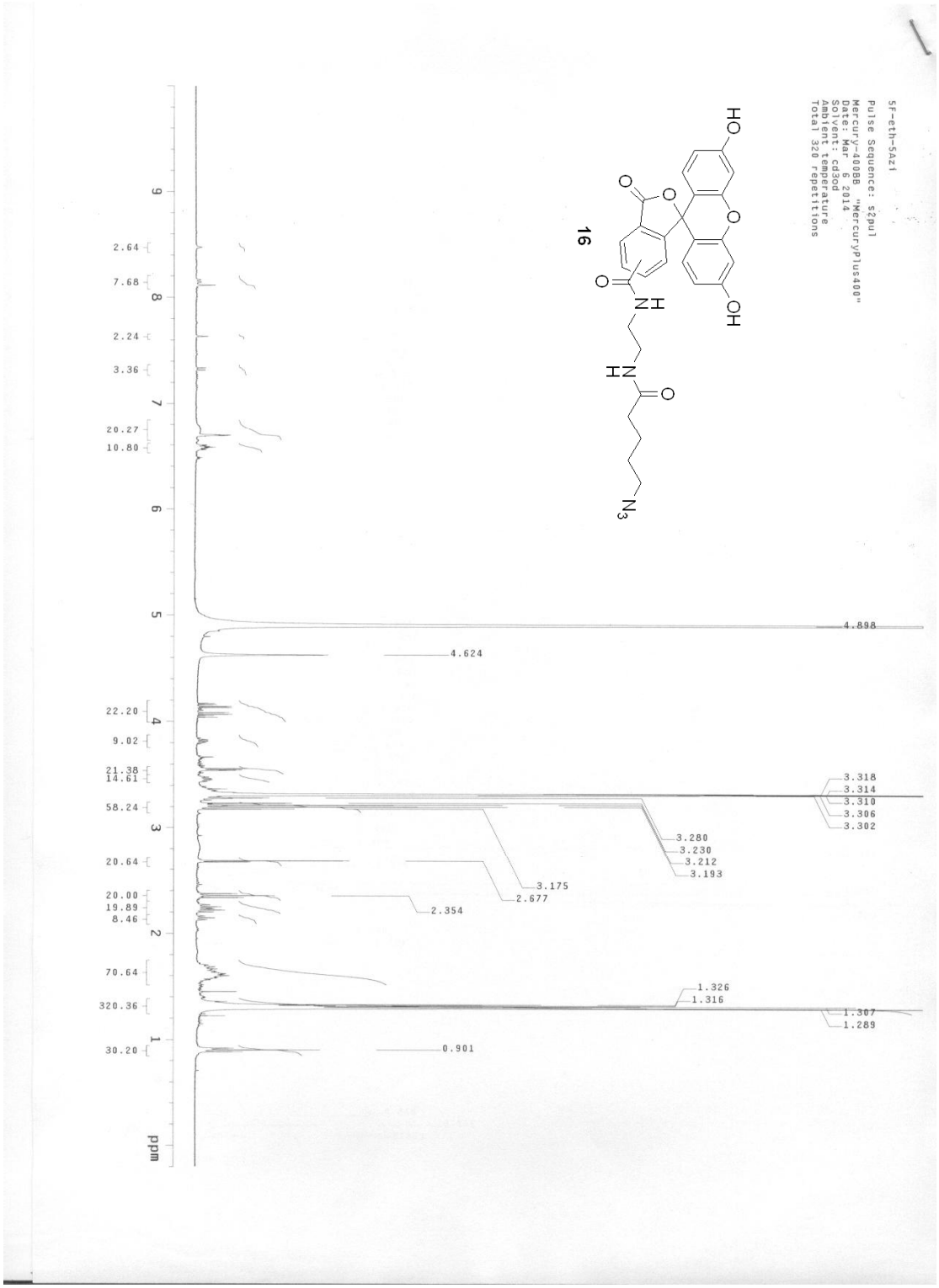
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NH
HN
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3.310
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3.230
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1.316
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3.36
20.27
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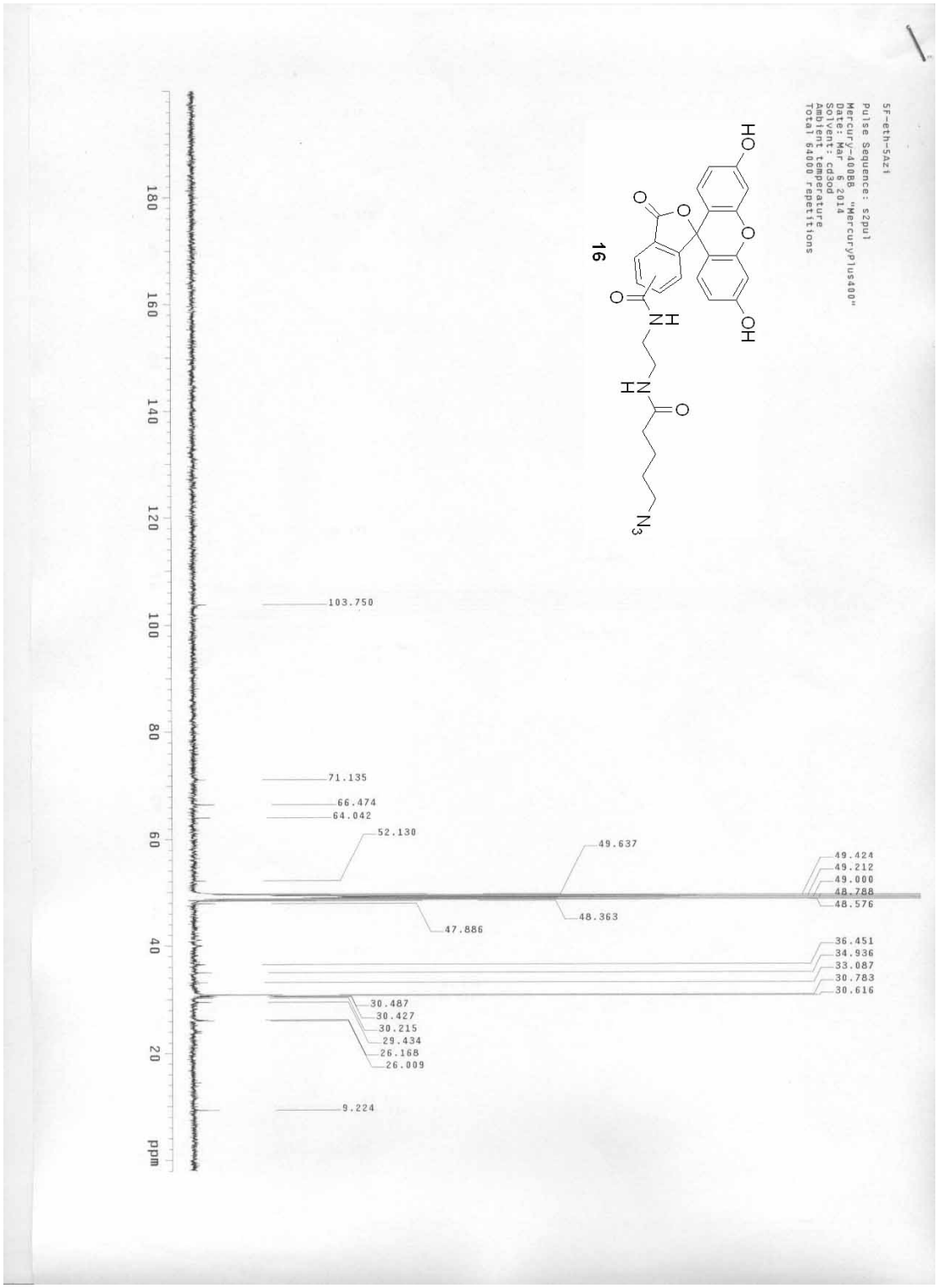
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36.451
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Mass Spectrum SmartFormula Report

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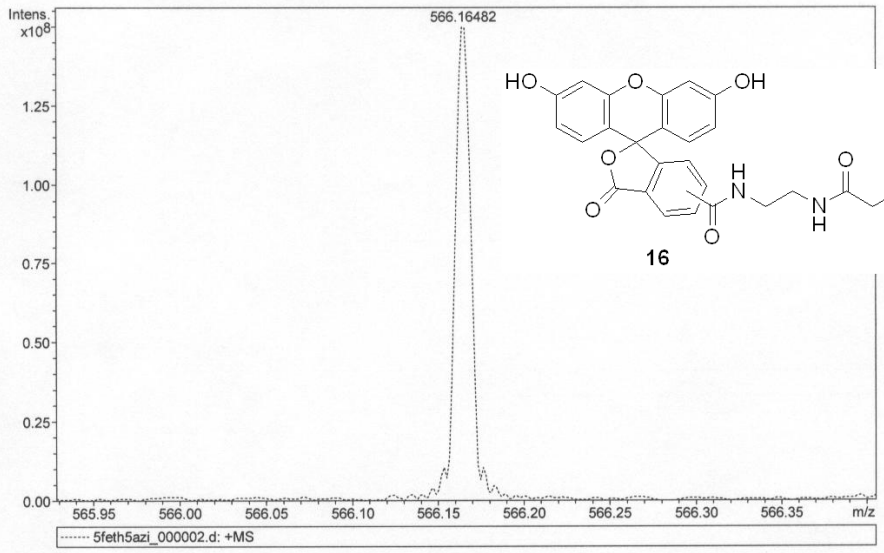

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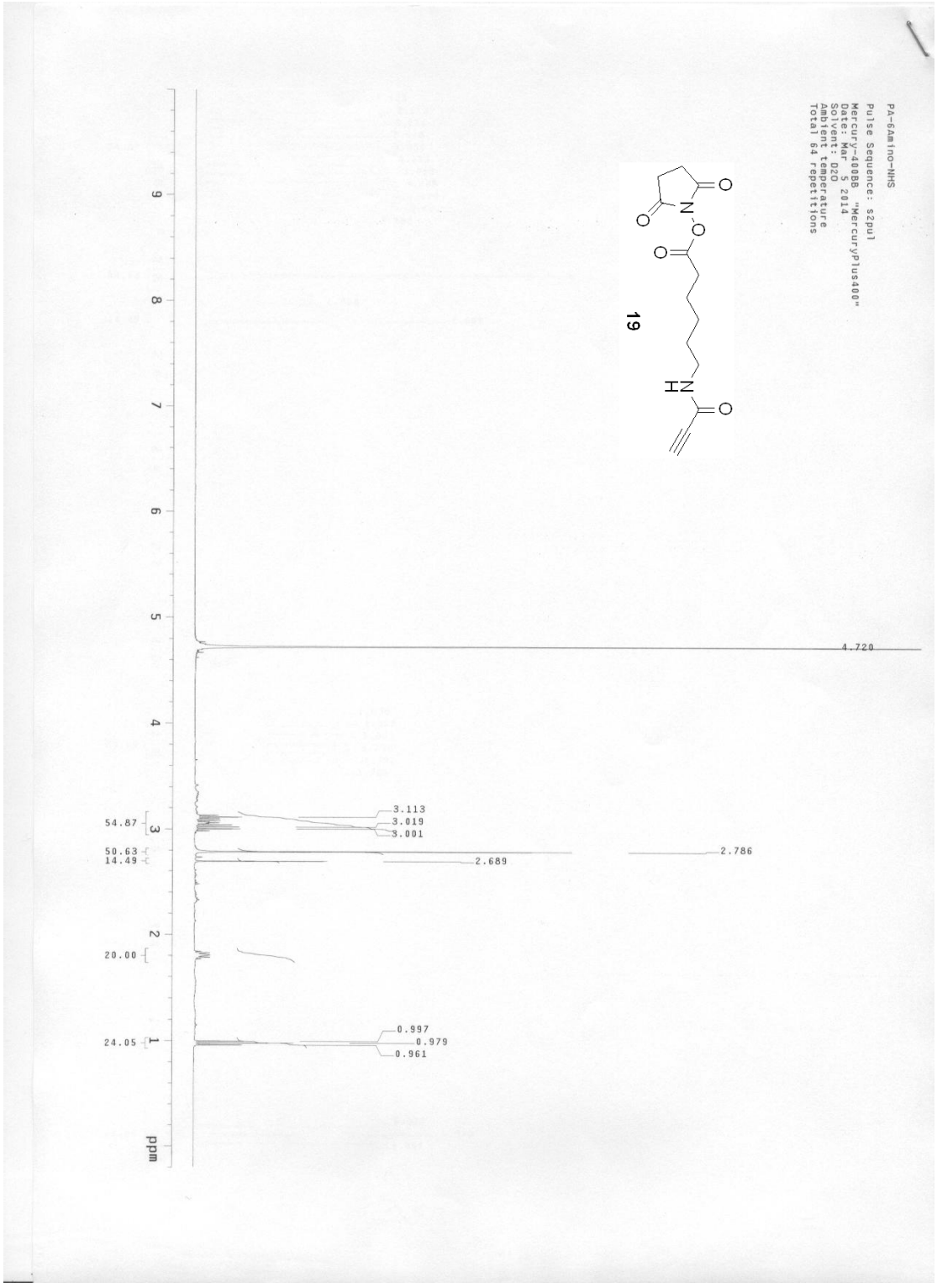
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Solvent: D2O
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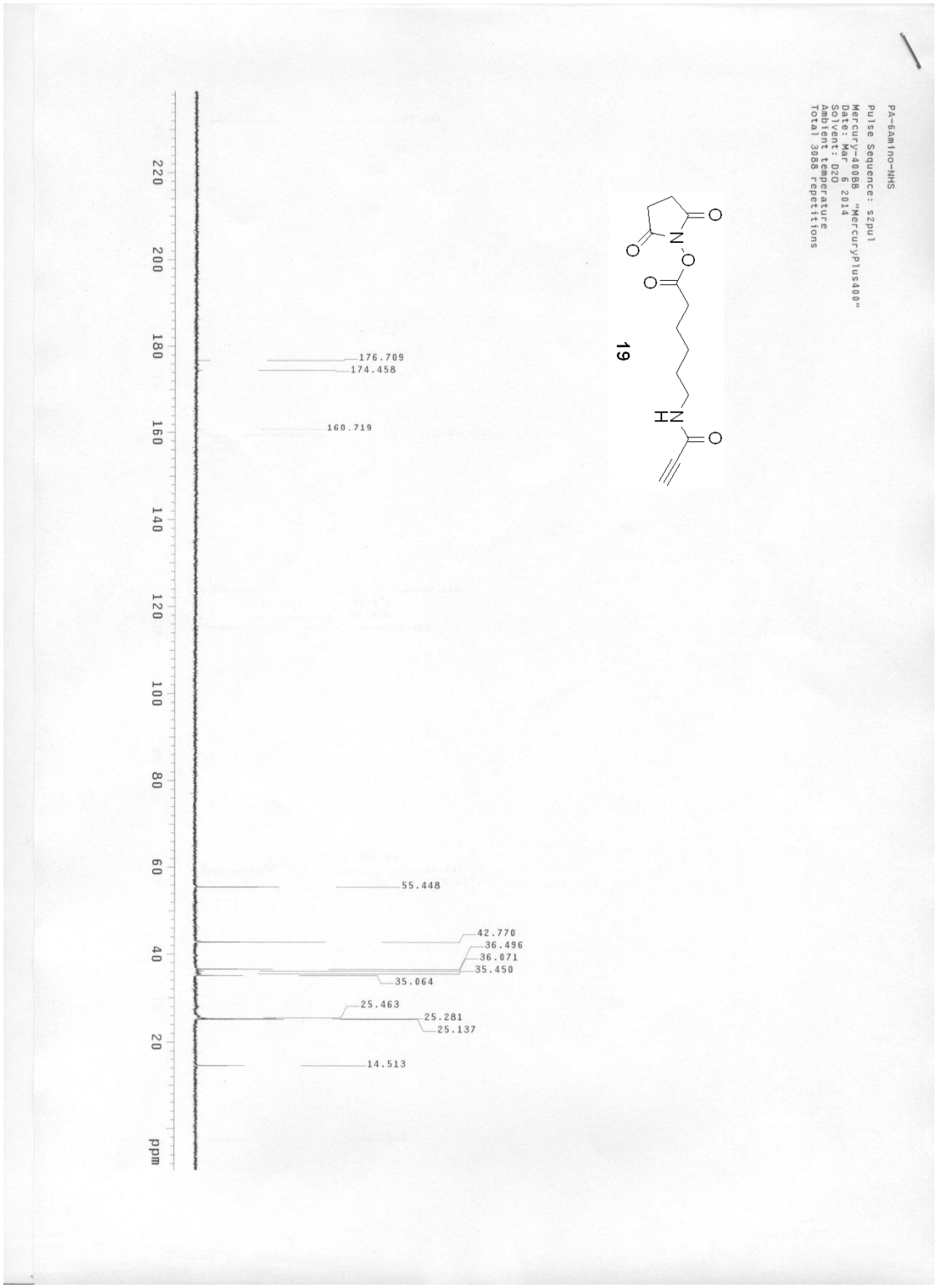
ppm


PA-6Amino-NHS
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Mercury-400BB "MercuryPlus400"
Date: Mar 6 2014
Solvent: D2O
Ambient temperature
Total 3088 repetitions
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42.770
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36.071
35.450
35.064
25.463
25.281
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14.513
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180
160
140
120
100
80
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20
ppm

Mass Spectrum SmartFormula Report

Analysis Info

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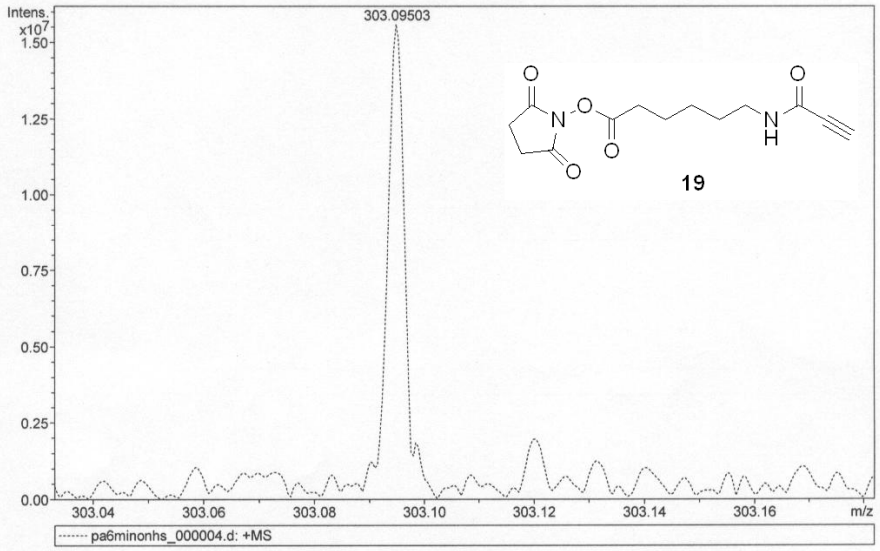
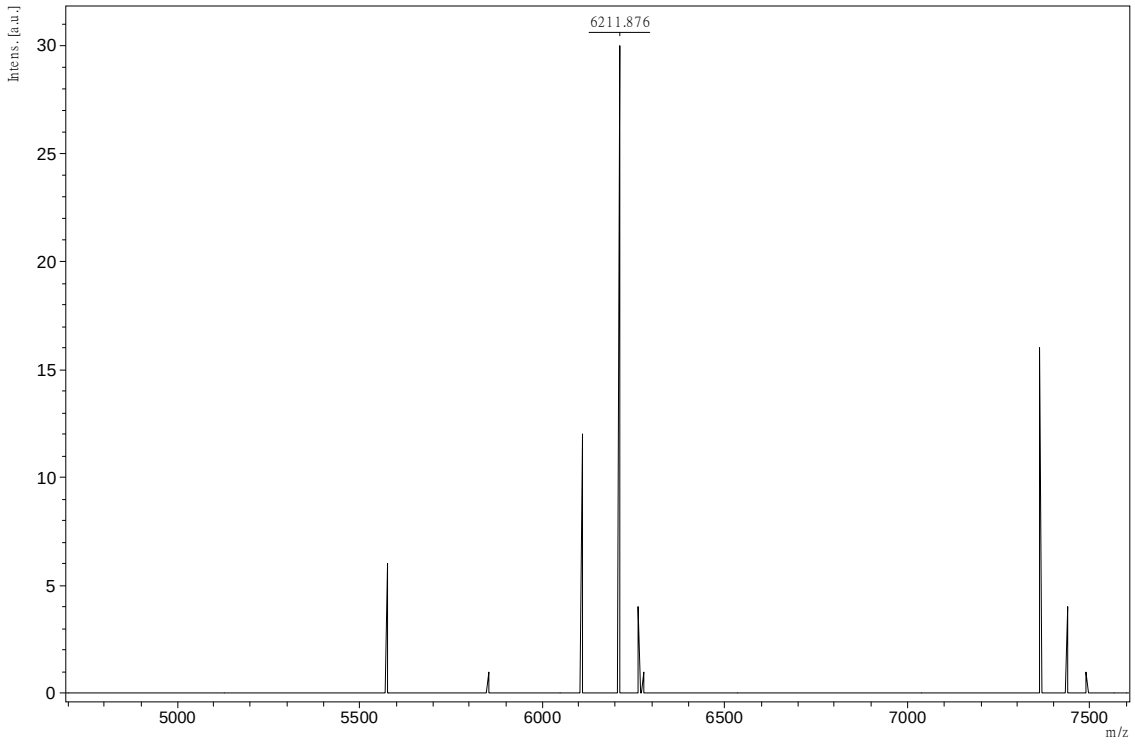
Sample Name	PA-6Amino NHS	Instrument:	FT-MS solariX
Comment	ESI Positive		



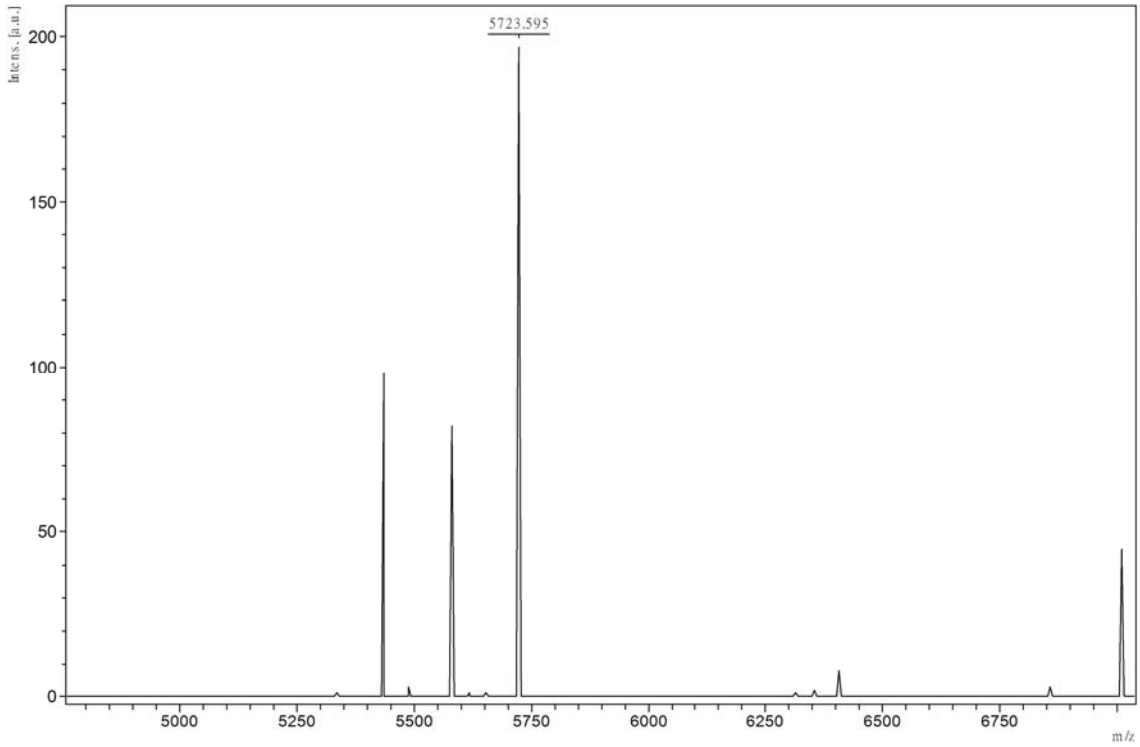
19

Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
303.09503	1	C 13 H 16 N 2 Na O 5	100.00	303.09514	0.11	0.36	187.8	6.5	even	ok

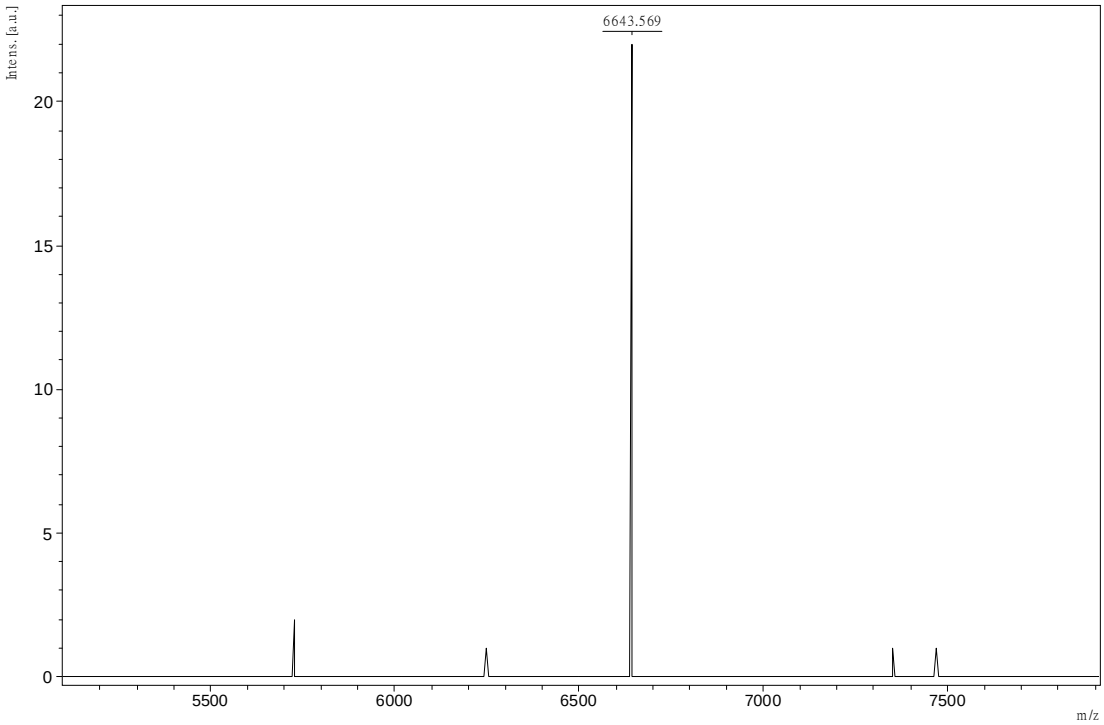
The 3’ primer DNA-ethylenediamine-**6d** conjugate.



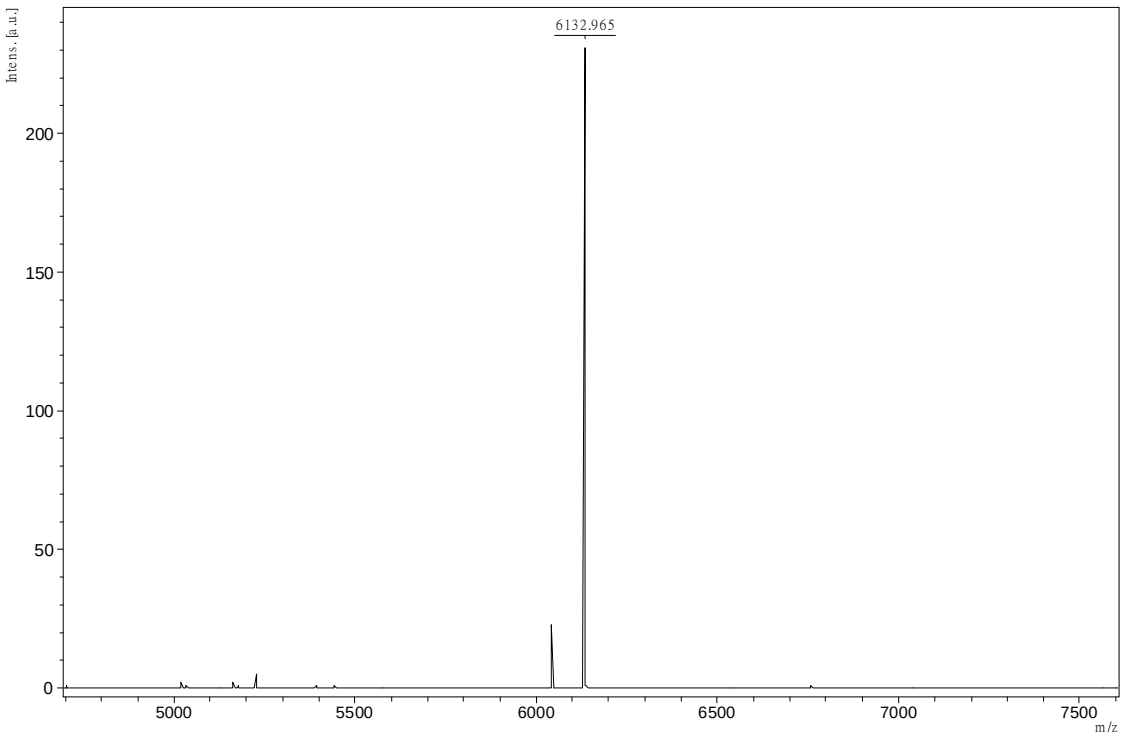
The 17-mer RNA-cystamine-**6d** conjugate.



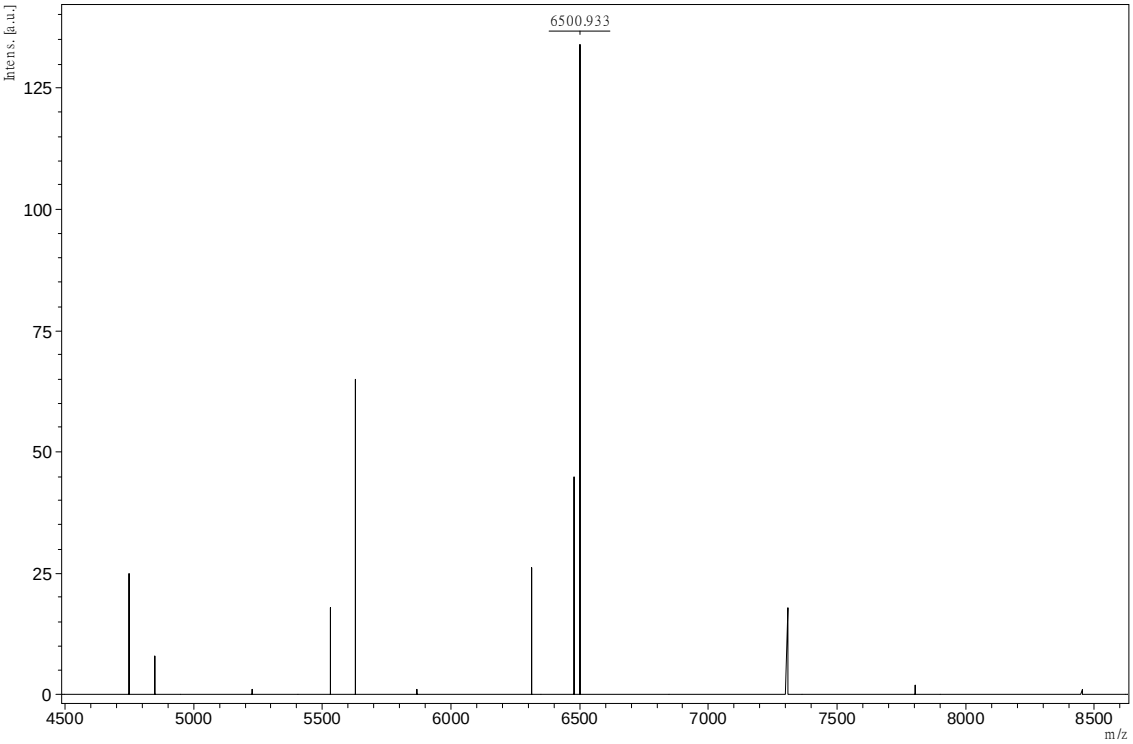
The CuAAC reaction product of the 3’ primer DNA-ethylenediamine-**6d** conjugate and **15**.



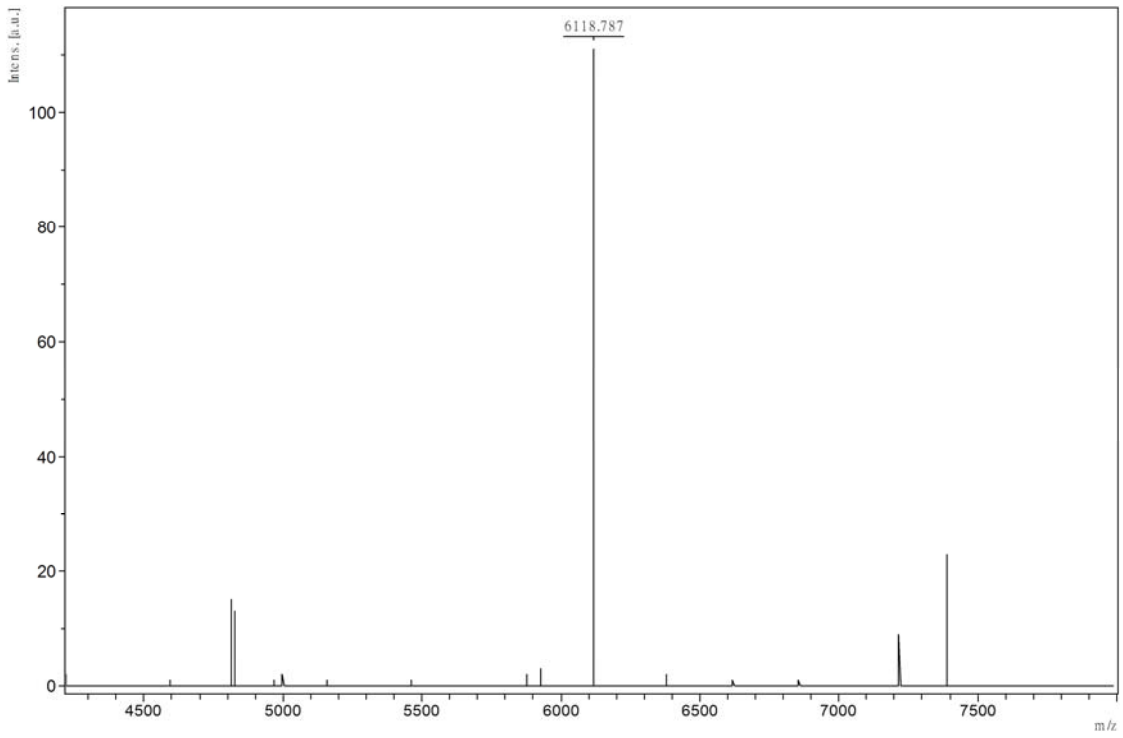
The CuAAC reaction product of the 17-mer RNA-cystamine-**6d** conjugate and **15**.



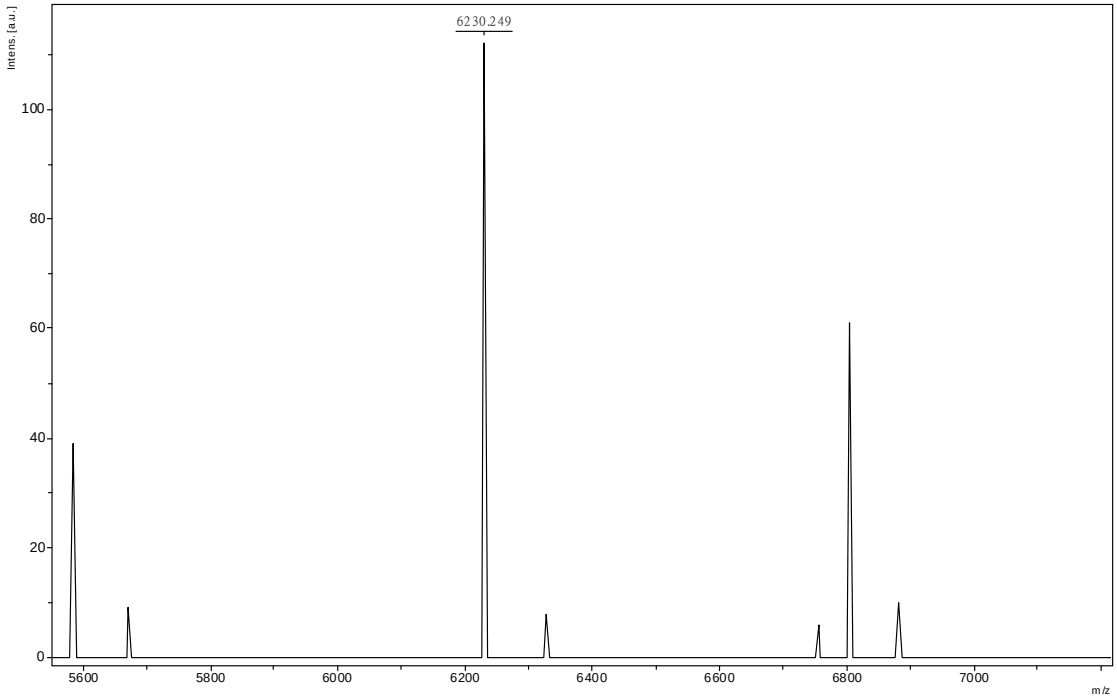
The CuAAC reaction product of the 3’ primer DNA-ethylenediamine-**6d** conjugate and **10**.



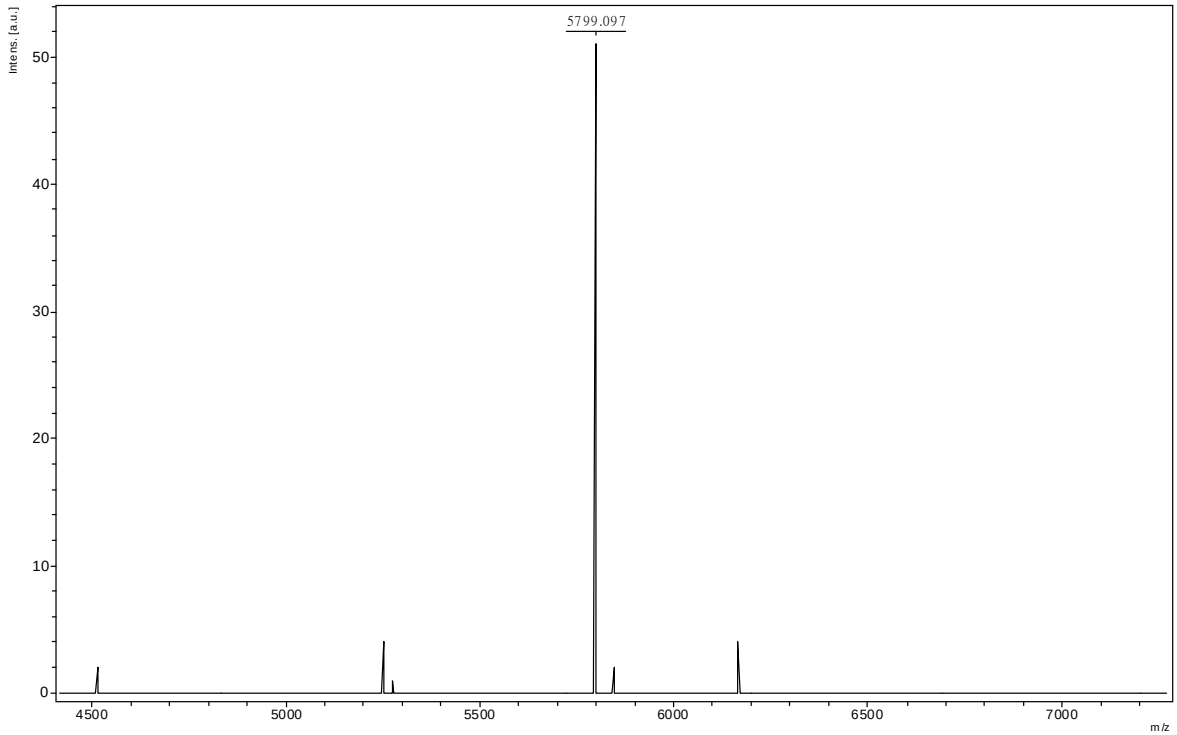
The CuAAC reaction product of the 17-mer RNA-cystamine-**6d** conjugate and **10**.



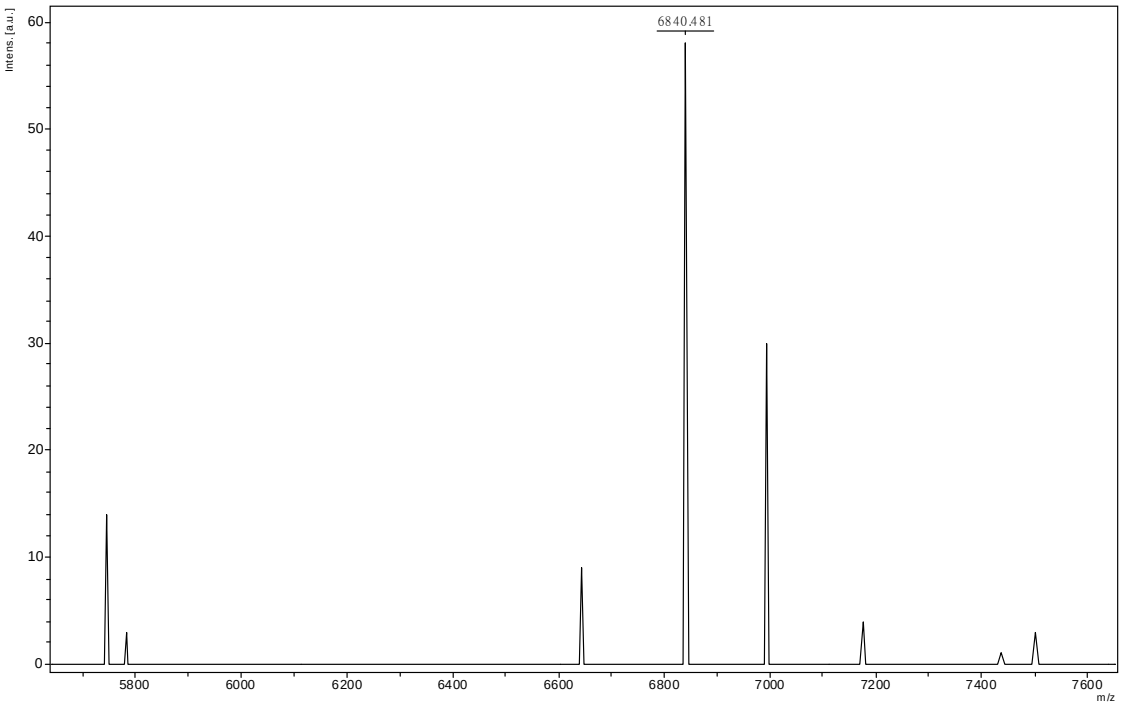
The 3’ primer DNA-ethylenediamine-**19** conjugate.



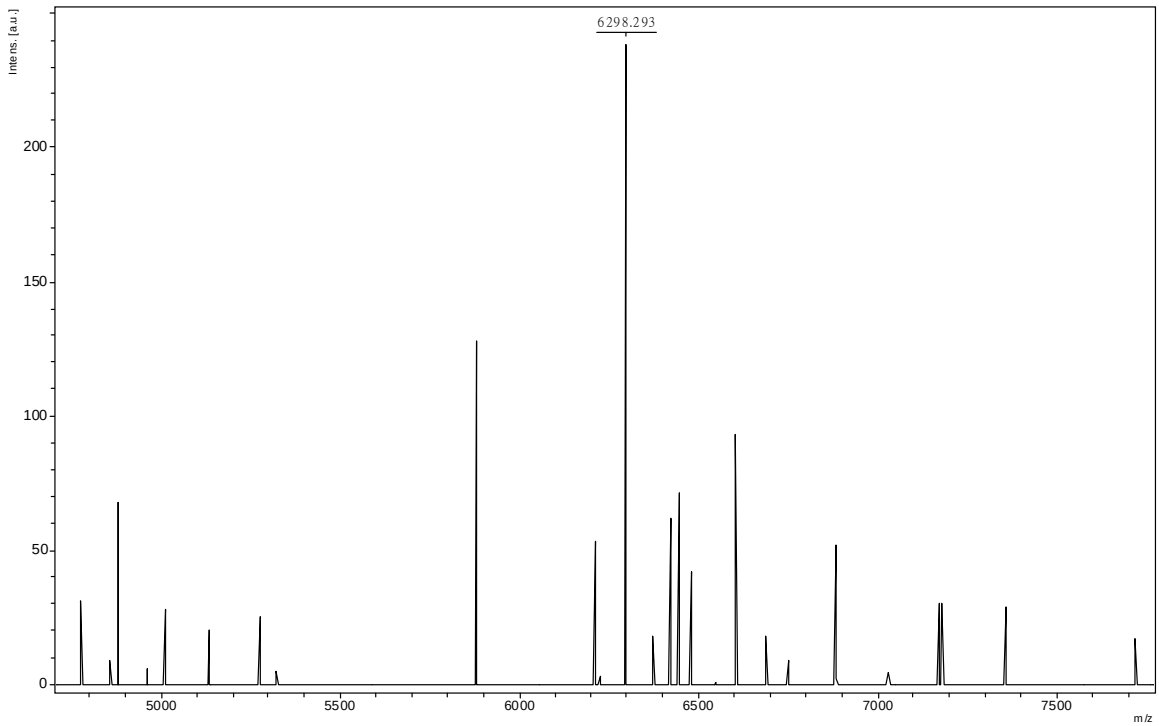
The 17-mer RNA-cystamine-**19** conjugate.



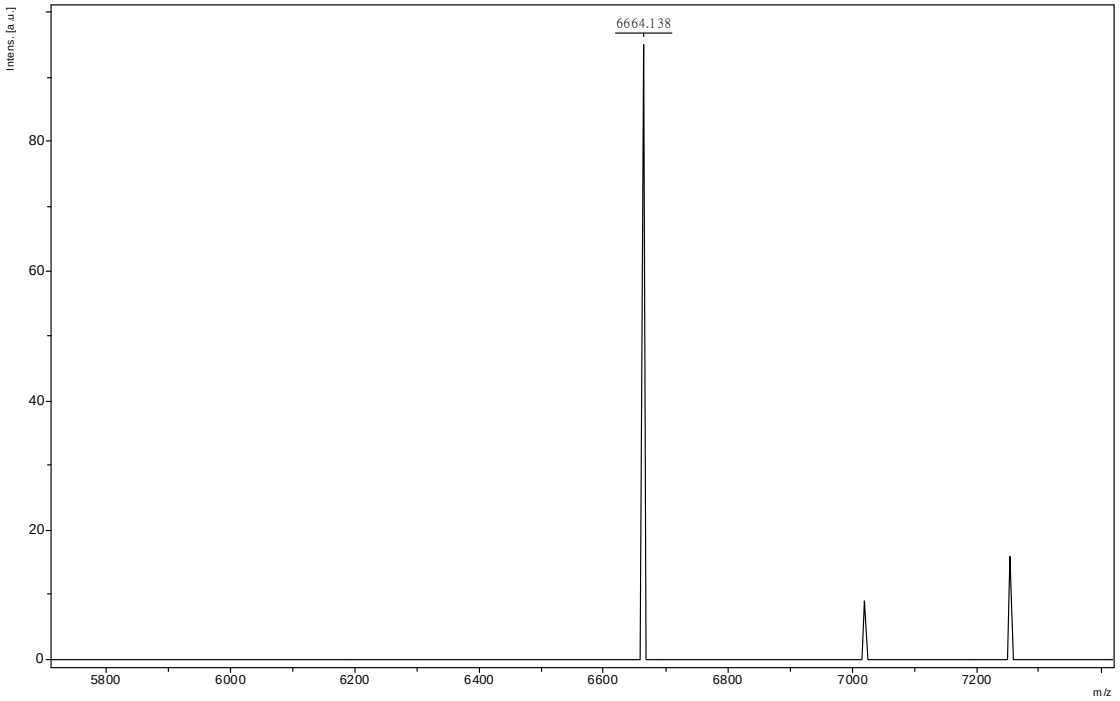
The CuAAC reaction product of the 3’ primer DNA-ethylenediamine-**19** conjugate and **16**.



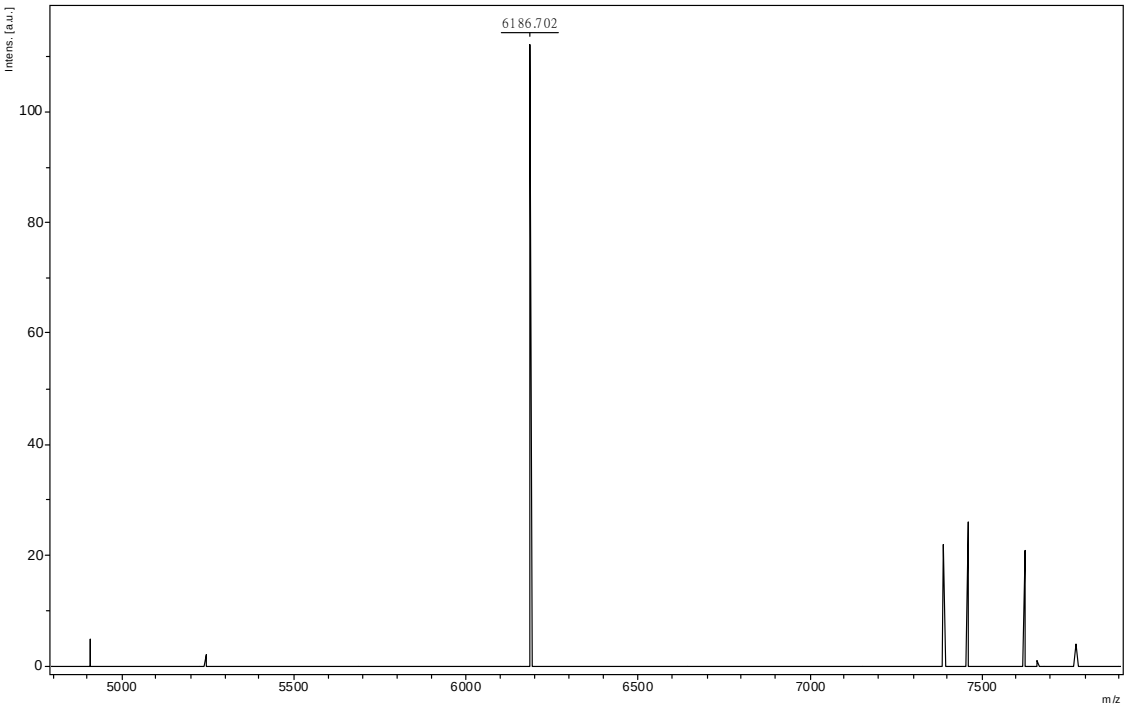
The CuAAC reaction product of the 17-mer RNA-cystamine-**19** conjugate and **16**.



The CuAAC reaction product of the 3’ primer DNA-ethylenediamine-**19** conjugate and **12**.



The CuAAC reaction product of the 17-mer RNA-cystamine-**19** conjugate and **12**.



The CuAAC reaction product of the 3’ primer DNA-ethylenediamine-**6d** conjugate and the alkynyl Tat peptide.