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Supplementary Materials

A Bioorthogonally Synthesized and Disulfide-Containing Fluorescence Turn-On Chemical Probe for Measurements of Butyrylcholinesterase Activity and Inhibition in the Presence of Physiological Glutathione

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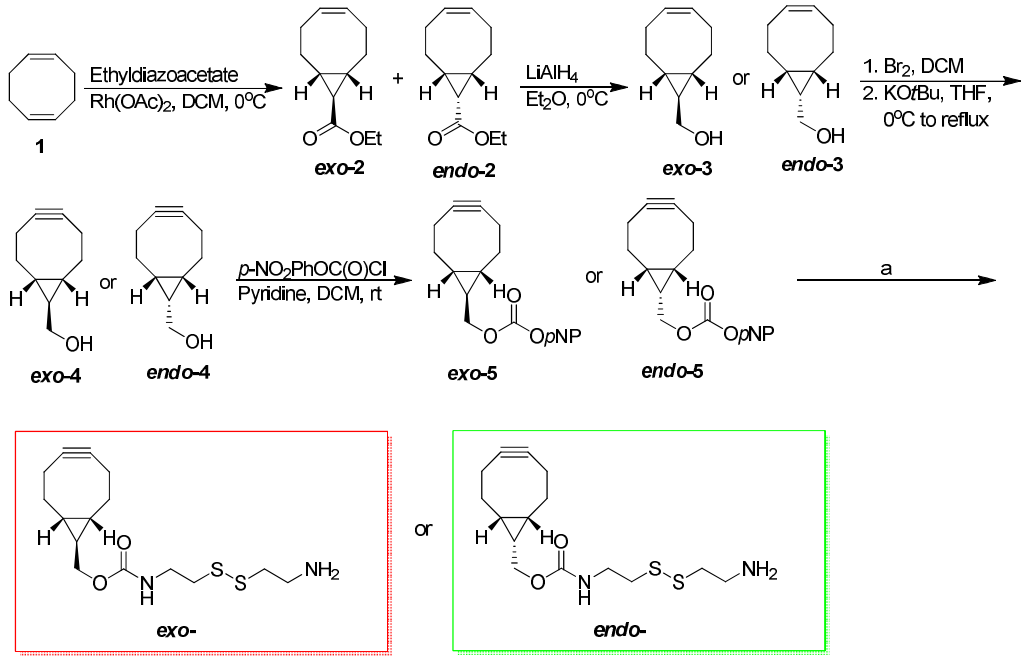
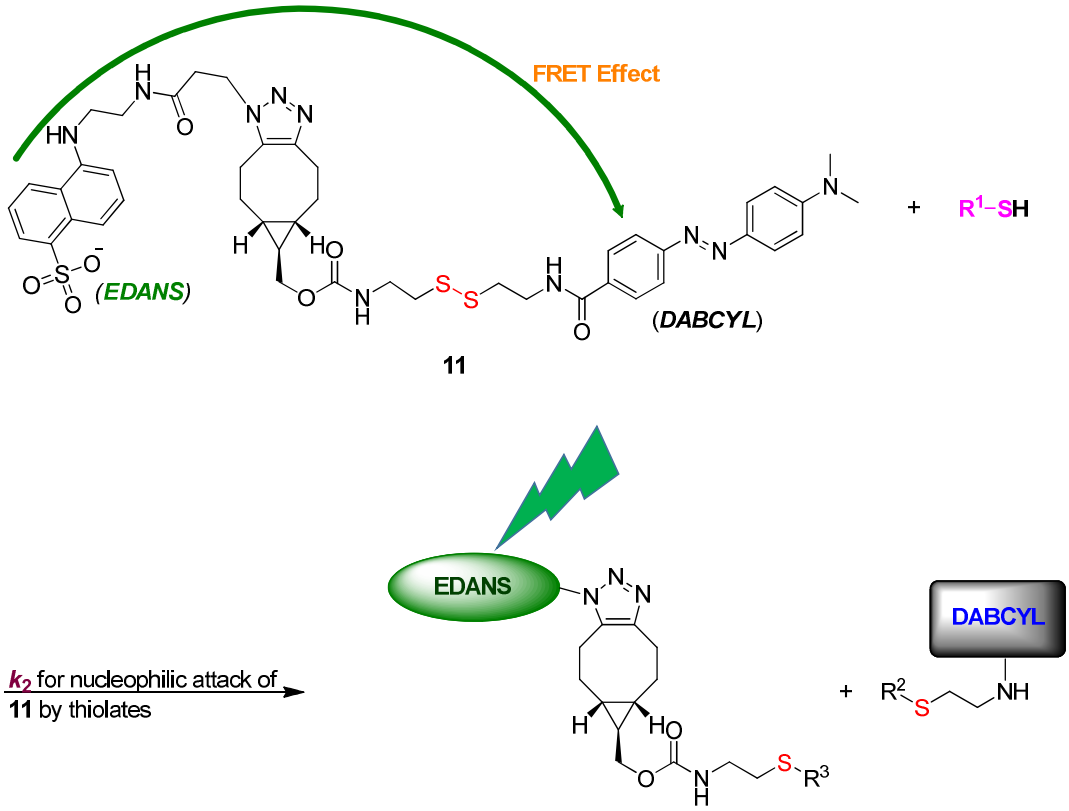
† Authors contributed equally to this study.

Contents

Schemes



Scheme S1. Synthesis of the key bicyclononyne derivatives (**6**). Syntheses of **2** - **5** were reported previously [1]. a: cystamine, Et_3N, MeOH/DCM, 50˚C.



Scheme S2. Obliteration of the FRET effect in **11** and release of the EDANS fluorescence initiated by nucleophilic attack of **11** by thiolates (highlighted in pink in Scheme 1 and here). R^1-SH: thiol-containing reactants; R^2 and R^3: H or thiolate groups. The disulfide group in **11** is highlighted in red. k_2, the second-order rate constant for the S_N2 nucleophilic substitution reaction of **11** with a thiolate.

Figures

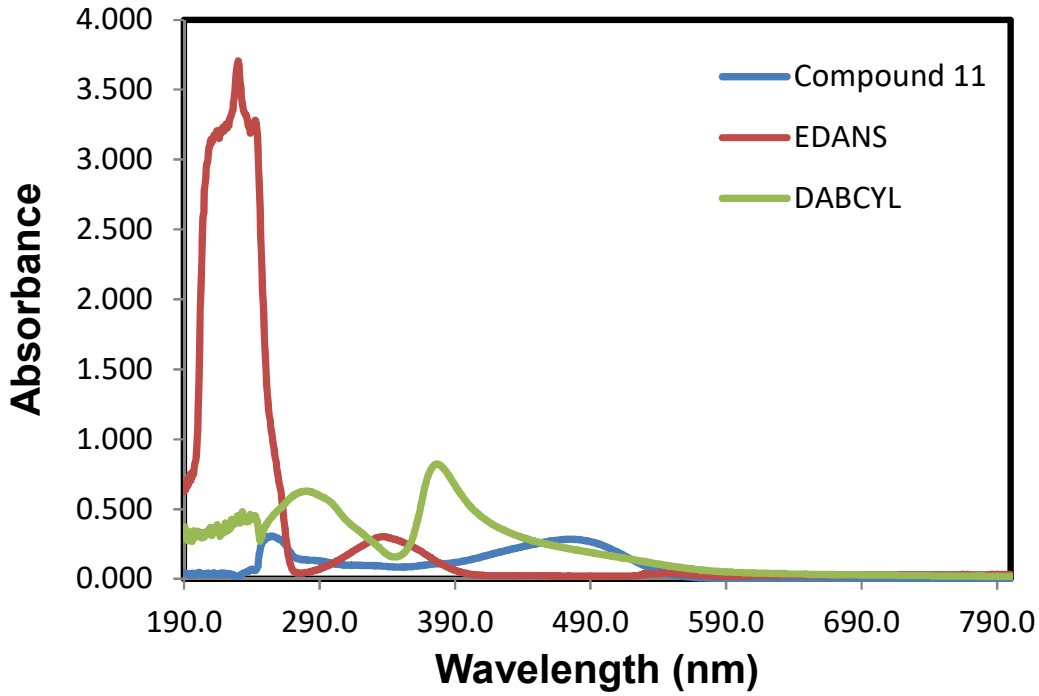


Figure S1. The UV-Vis spectra for 50 μM of the FRET probe **11** (—, the blue curve), EDANS (—, the red curve) and DABCYL (—, the green curve) in phosphate buffered saline (PBS).

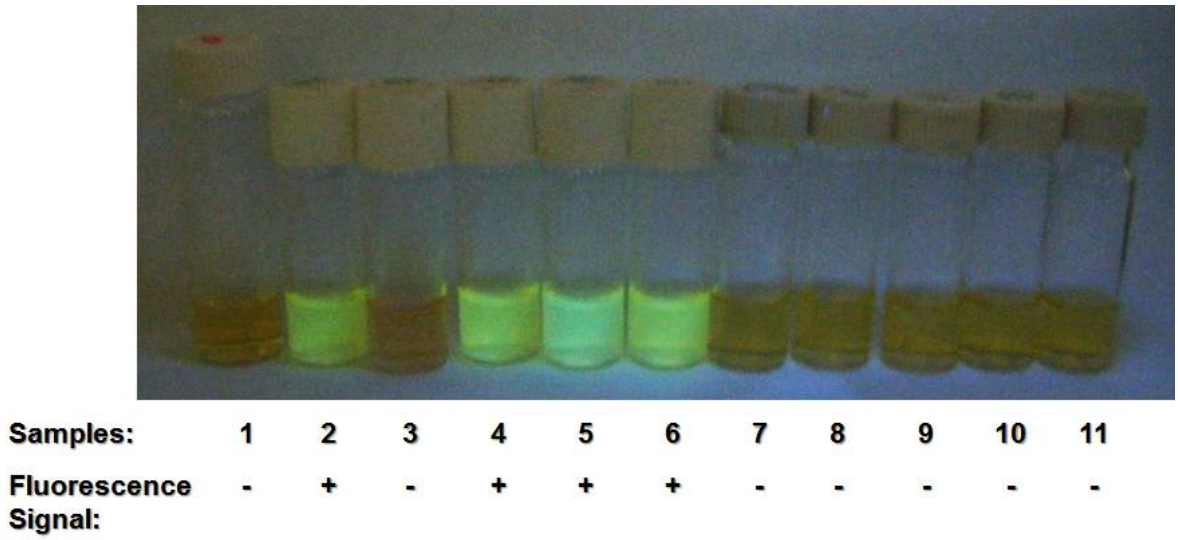


Figure S2. Visualization of the EDANS fluorescence to explore the properties of the fluorescent emission from **11** in the presence of various reactants. Each vial contained 25 μM of **11** in PBS in the presence or the absence of an indicated reactant (50 mM).

The reactions were carried out in the dark at rt for 1 h before the photograph was taken. Samples: 1, **11** in the absence of reactants; 2, **11** + 2-mercaptoethanol; 3, **11** + GSH; 4, **11** + L-cysteine; 5, **11** + 2-aminoethanethiol; 6, **11** + DTT; 7, **11** + L-methionine; 8, **11** + L-lysine; 9. **11** + glycine; 10, **11** + L-serine; 11, **11** + L-glutamate.

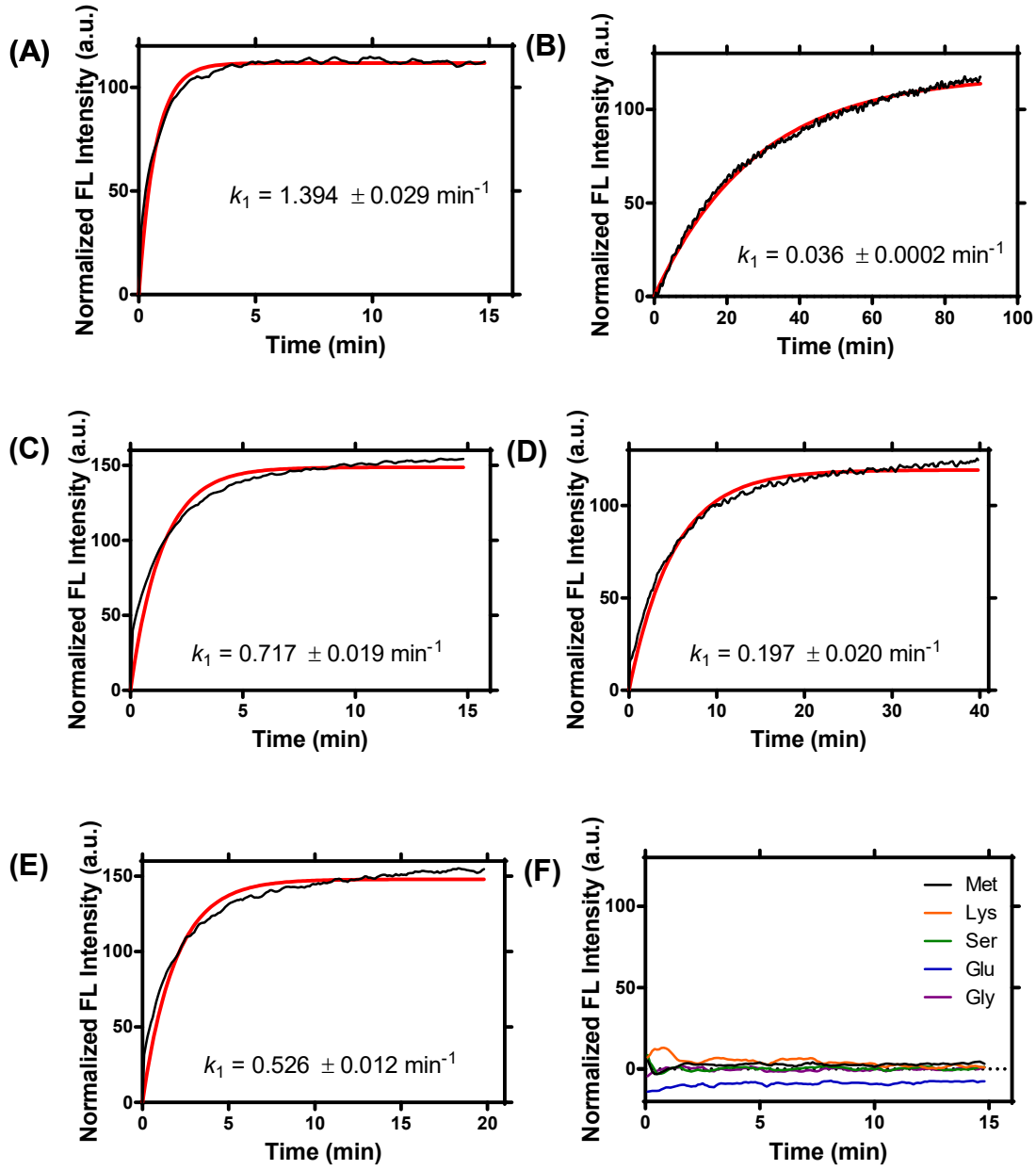


Figure S3. Pseudo-first-order reactions of **11** (25 μM) with 50 mM of (**A**) DTT, (**B**) GSH, (**C**) L-cysteine, (**D**) 2-mercaptoethanol, (**E**) 2-aminoethanethiol, or (**F**) five non-thiol amino acids (L-methionine, L-lysine, L-serine, L-glutamate, and glycine) in PB. Progress of each reaction was monitored by measuring fluorescence emission at.

505 nm at specific time intervals. Data of normalized EDANS fluorescence (FL) intensity *vs* time were fitted to a single-exponential equation for first-order kinetics $F(t) = F_0 + F_{max}(1 - e^{-k_1 t})$ [$F(t)$, EDANS fluorescence at a specific time point t] to afford the values of the first-order rate constant k_1 (GraphPad, La Jolla, CA, USA) illustrated in the graphs. In Panels S3A-S3E, the black lines represent original FL changes in the reaction time courses, and the red curves show the results calculated by the single-exponential equation. The normalized FL intensity data at 505 nm were acquired by subtracting a background FL_{505} intensity of **11** from the original FL_{505} intensity measurements.

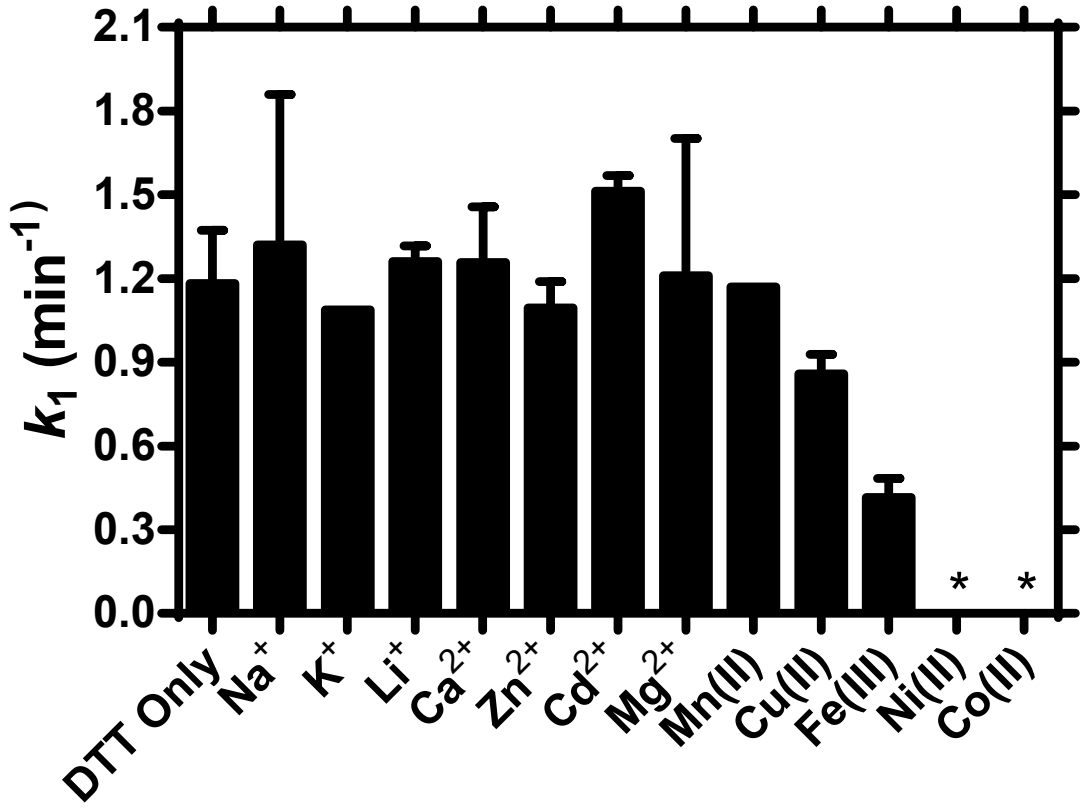


Figure S4. Effects of metal ion (1 mM) on the pseudo-first-order reactions of **11** (25 μM) with 50 mM DTT in PB. The k_1 values were determined by a method analogous to that as described above. The symbol * indicates that **11** had no detectable EDANS fluorescence change in presence of Ni(II) or Co(II); the EDANS fluorescence change was so insignificant so that the values of averaged k_1 and standard deviation could not be determined.

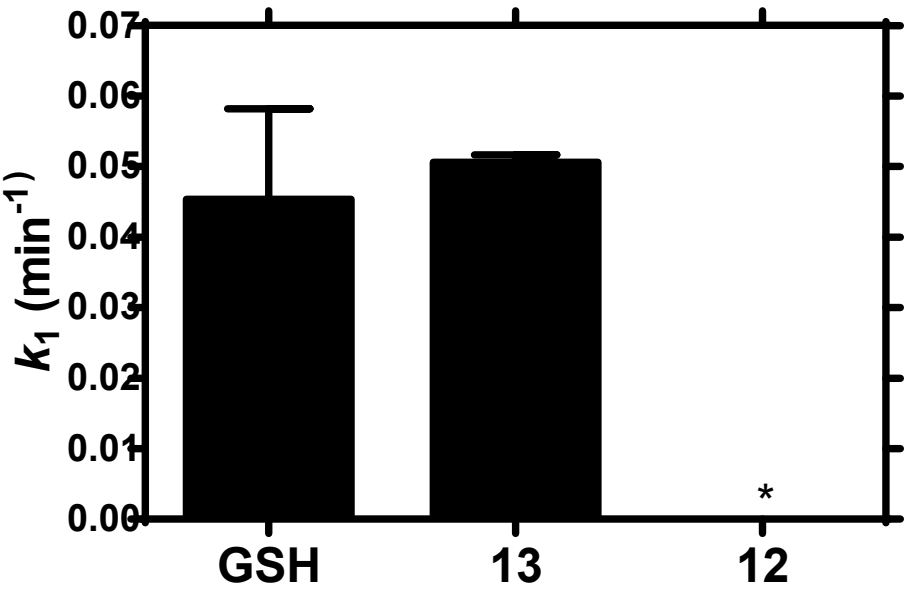


Figure S5. Kinetic analysis of the pseudo-first-order reactions of **11** (25 μM) with 50 mM of one of the structure-modified GSH derivatives **12** and **13** in PB. The k_1 values were again determined by a method similar to that as described above. The symbol * indicates that the reaction of **11** with **12** had no detectable EDANS fluorescence and very low reactivity. Therefore, the values of averaged k_1 and standard deviation could not be determined for the reaction.

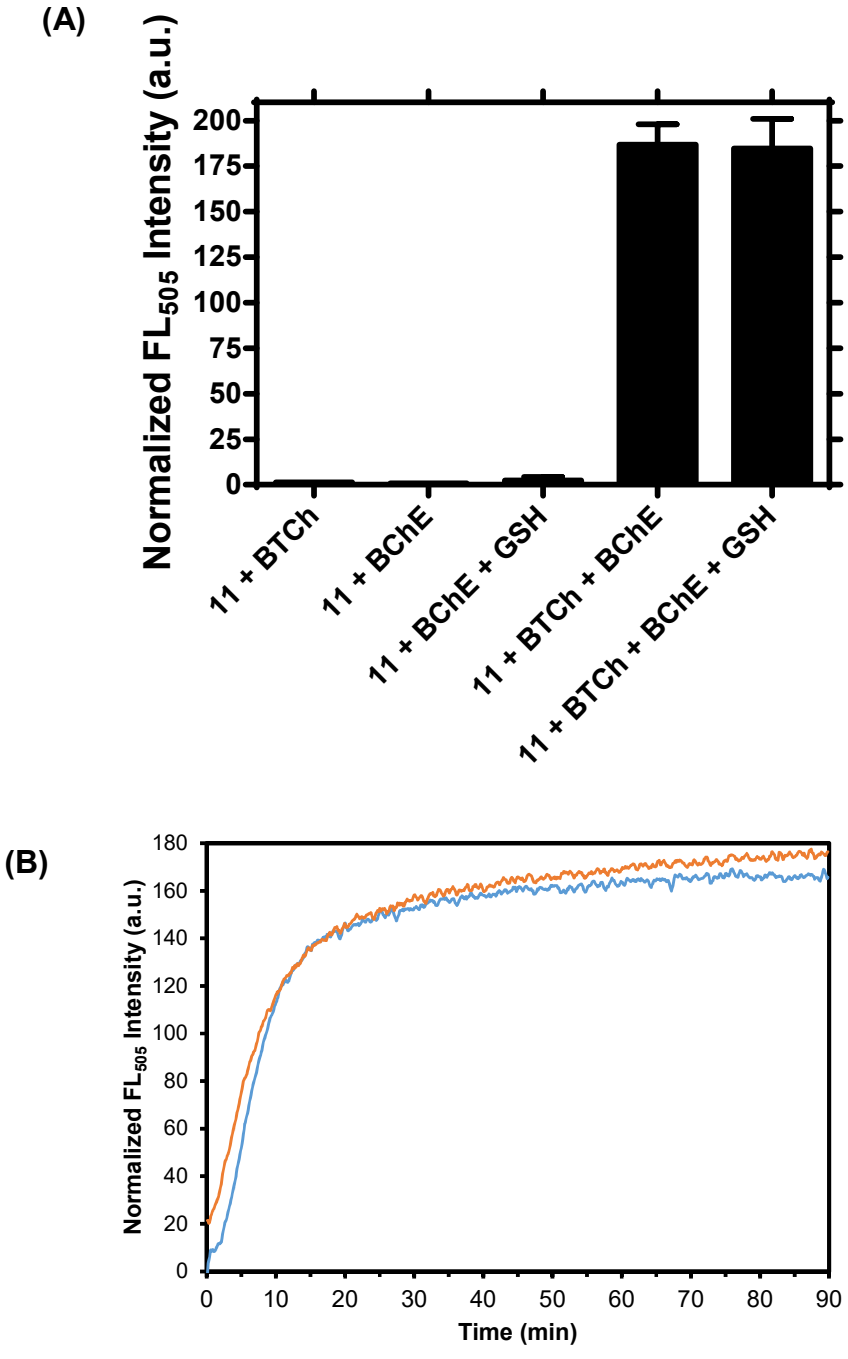


Figure S6. Presence of 50 μM or 50 mM of GSH in the BChE-BTCh-**11** reaction did not affect release of the EDANS fluorescence. BChE activity was determined by measuring the EDANS fluorescence released from **11** (**A**) at the end of the reactions or (**B**) at the time-dependent increments measured by the spectrofluorometer. An enzymatic reaction consisted of BChE (182 U L^{-1}), BTCh (5 mM), **11** (25 μM), and GSH [50 μM in (**A**) or 50 mM in (**B**)], if present, in PB. The reactions were carried out at rt in (**A**) or 37°C in (**B**) for 90 min. The normalized FL intensity data at 505 nm were acquired by subtracting a background FL_{505} intensity of **11** from the original FL_{505} intensity readings. Each reaction was analyzed three times in order to acquire the averaged FL_{505} values and standard deviation (the error

bar) in (**A**). In (**B**), the time-fluorescence curves and enzyme activities of the BChE-BTCh-**11** reaction were almost identical in the presence or absence of 50 mM GSH (the initial velocities v_i: 12.9 ± 2.0 min^{-1} vs 12.2 ± 1.6 min^{-1}, respectively).

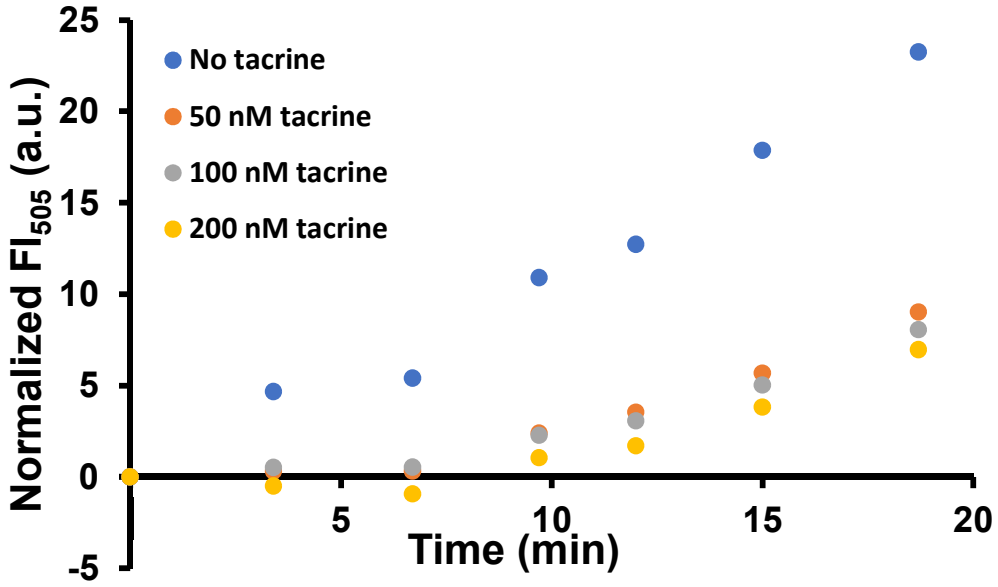


Figure S7. Tacrine inhibition on BChE catalysis analyzed by the fluorescence assay based on **11**. (A) Time-course kinetic analysis of tacrine inhibition on BChE (182.2 U L^{-1}) catalysis was performed in the presence of BTCh (250 μM) in PB. Each BChE reaction contained 0, 50, 100 or 200 nM of tacrine.

Table

Table S1. Comparison on analytical performance of optical assays for BChE activity quantification.

Probe	Linear Range	LOD	Detection Mode	Reference
2,6-Dichloroindophenol Acetate	72–36600 U L^{-1}	72 U L^{-1}	Colorimetry	[2]
Gold Nanorod	0.042–8.4 mU L^{-1}	0.018 mU L^{-1}	Colorimetry	[3]
Resorufin Butyrate	0.3–60 U L^{-1}	0.3 U L^{-1}	Fluorometry	[4]
2-(2-(5,6-Dimethoxy-1,3-dioxoisoindolin-2-yl) Acetoxy)-*N*,*N*,*N*-trimethylethan-1-ammonium Iodide	1,000–10,000 U L^{-1}	1,000 U L^{-1}	Fluorometry	[5]
BChE-FP	N/A	N/A	Fluorometric	[6]
CdTe Quantum Dots	10-1000 U L^{-1}	10 U L^{-1}	Fluorometric	[7]
Carbon Quantum Dots	60.0–220.0 U L^{-1}	2.7 U L^{-1}	Fluorometric	[8]
CdTe Quantum Dots	4-400 U L^{-1}	0.96 U L^{-1}	Fluorometric	[9]
Prussian Blue	2,000-15,000 U L^{-1}	800 U L^{-1}	Colorimetry	[10]
Carbon Quantum Dots	100–5,000 U L^{-1}	40 U L^{-1}	Fluorometric	[11]
Gold Nanoclusters	5-100 ng mL^{-1}	4 ng mL^{-1}	Fluorometric	[12]
G-Quadruplex DNA	1-1,000 ng mL^{-1}	0.15 ng mL^{-1}	Fluorometric	[13]
2,3-Dicyano-1,4-phenylene Diacrylate	0.2-9 U L^{-1}	0.06 U L^{-1}	Fluorometric	[14]
MnO_2 Nanosheets	10-500 U L^{-1}	0.035 U L^{-1}	Fluorometric	[15]
Compound **11**	4.3-182.2 U L^{-1}	4.3 U L^{-1}	Fluorometric	This work

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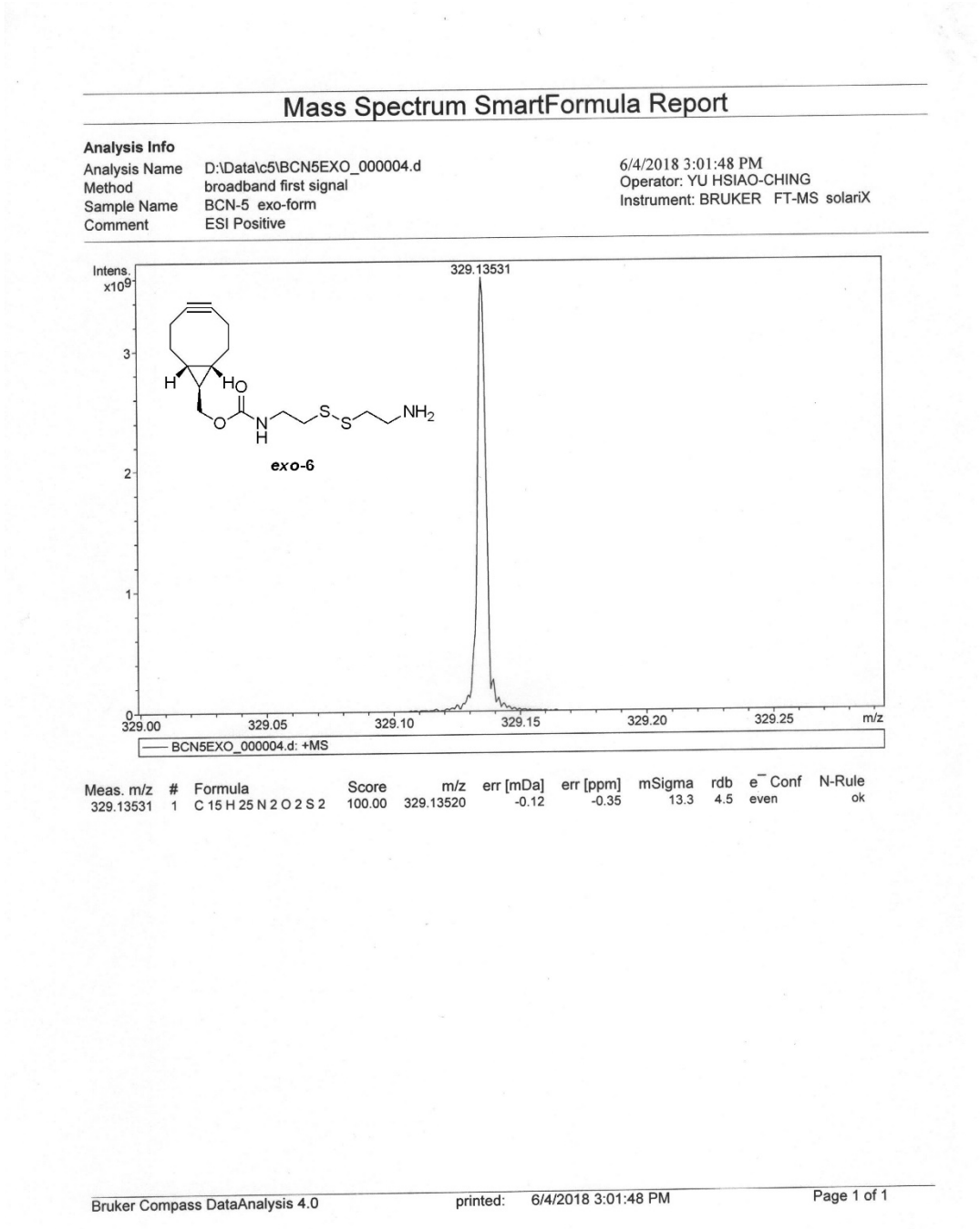
Spectra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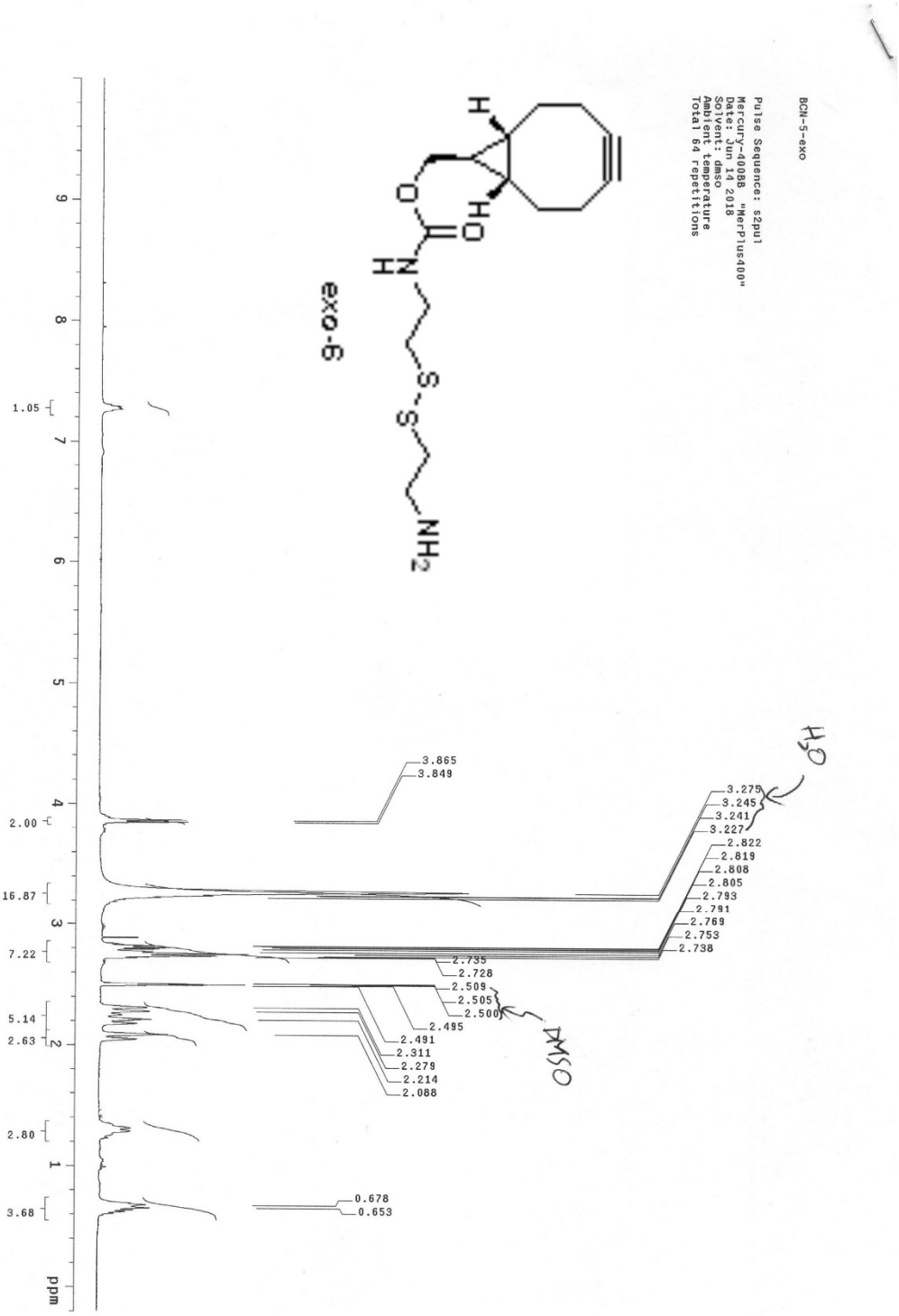
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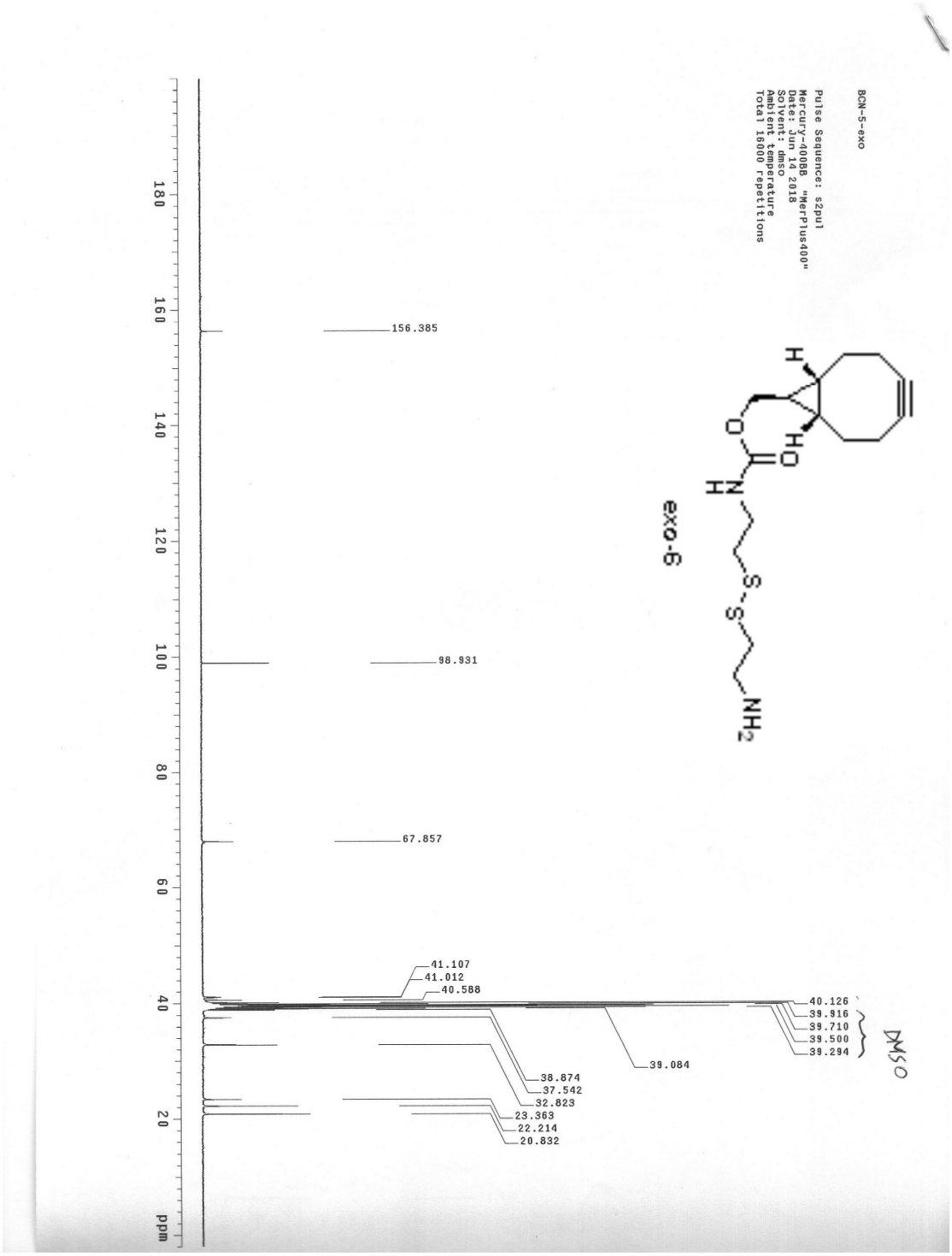
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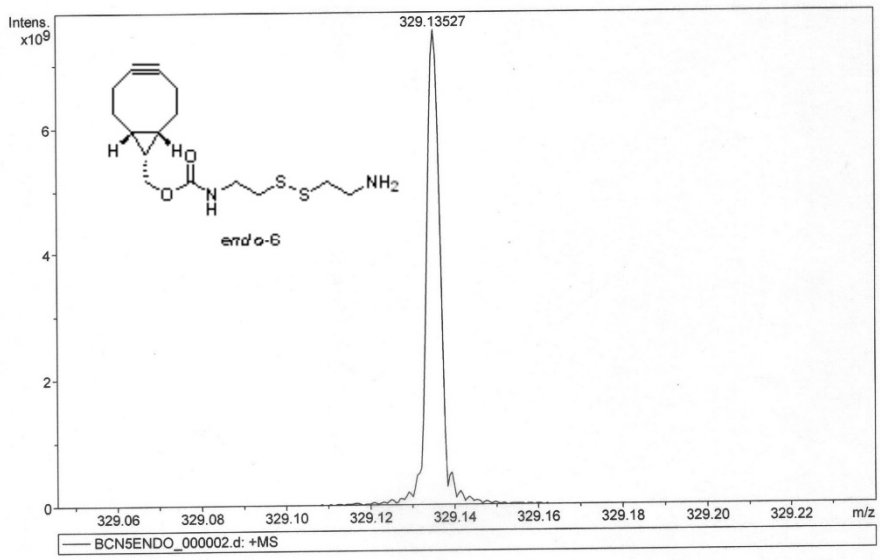

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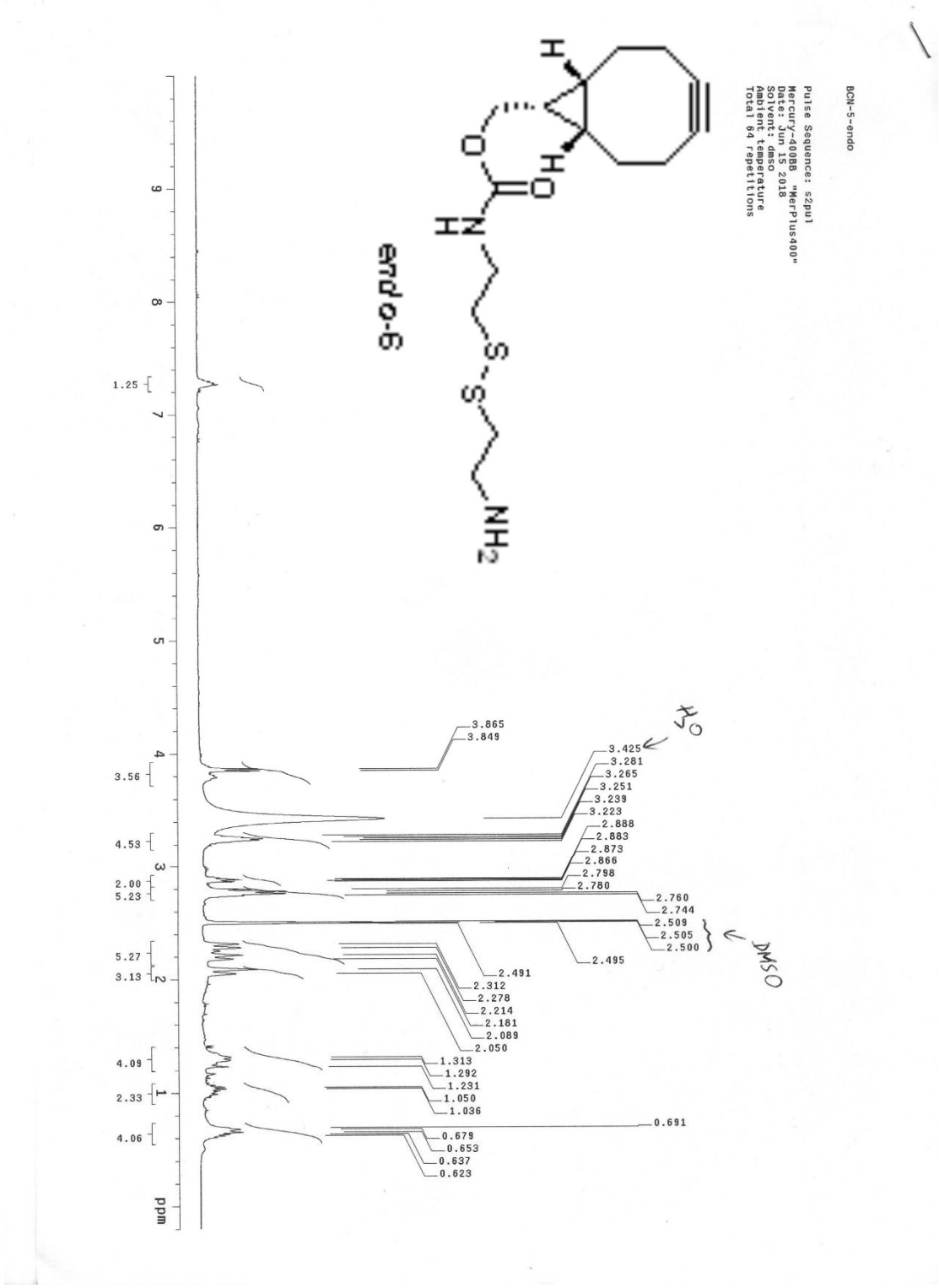
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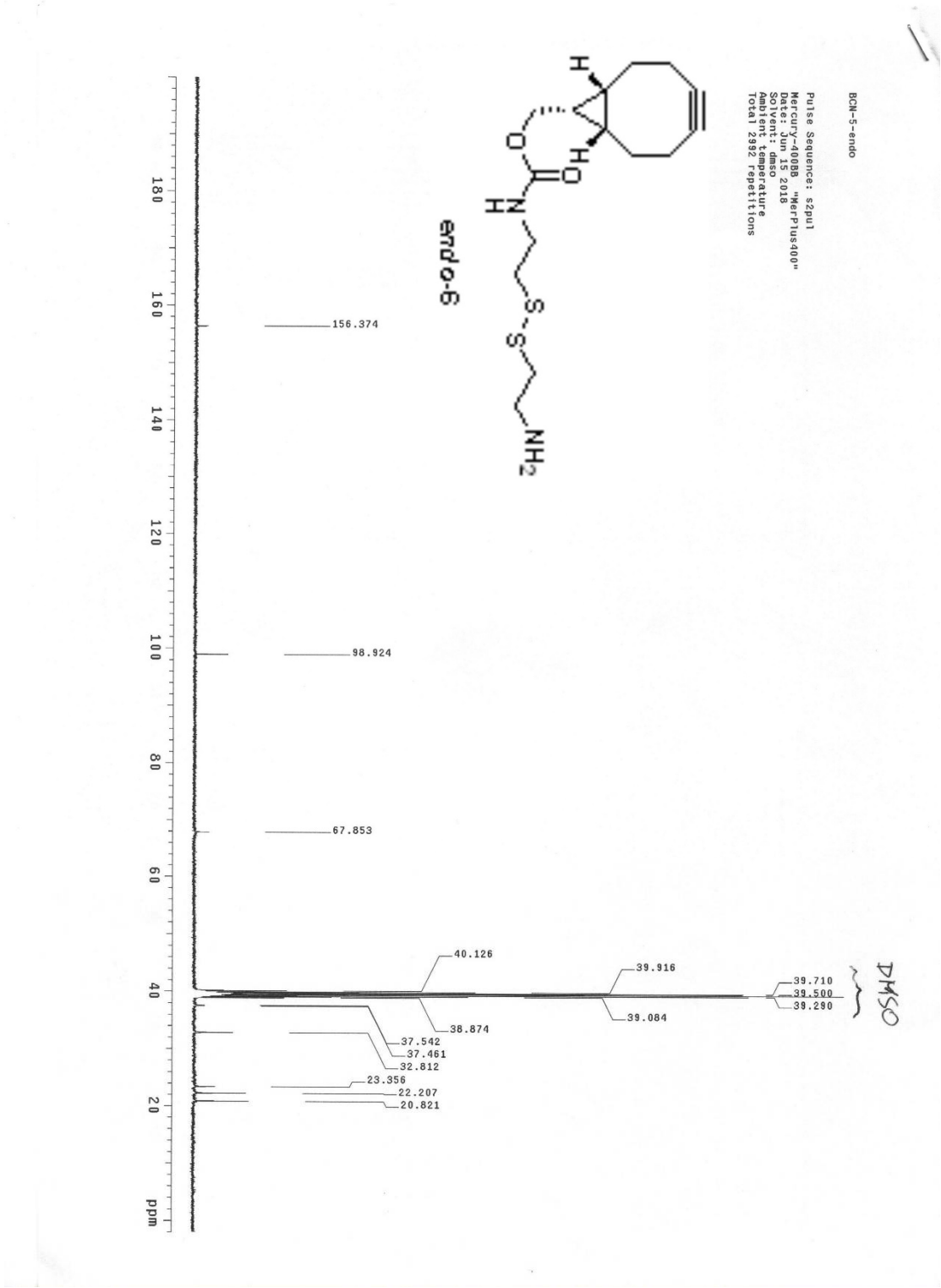
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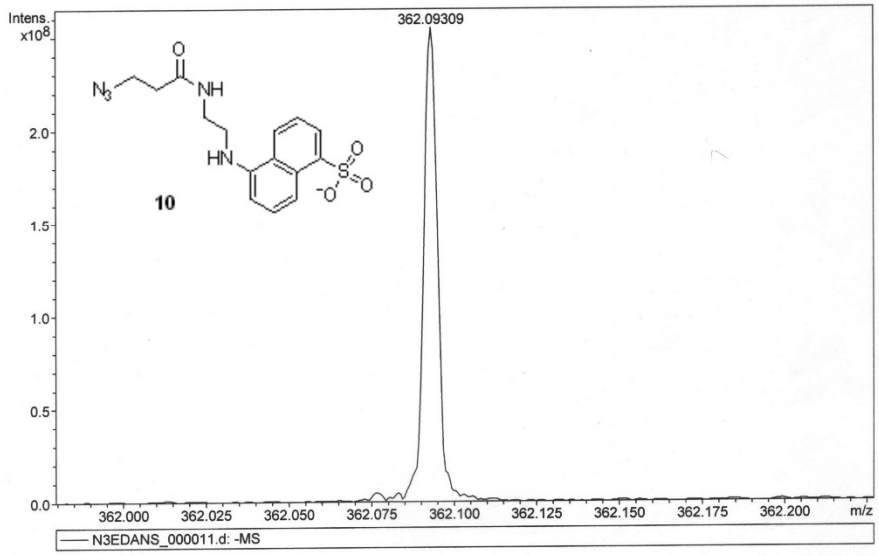

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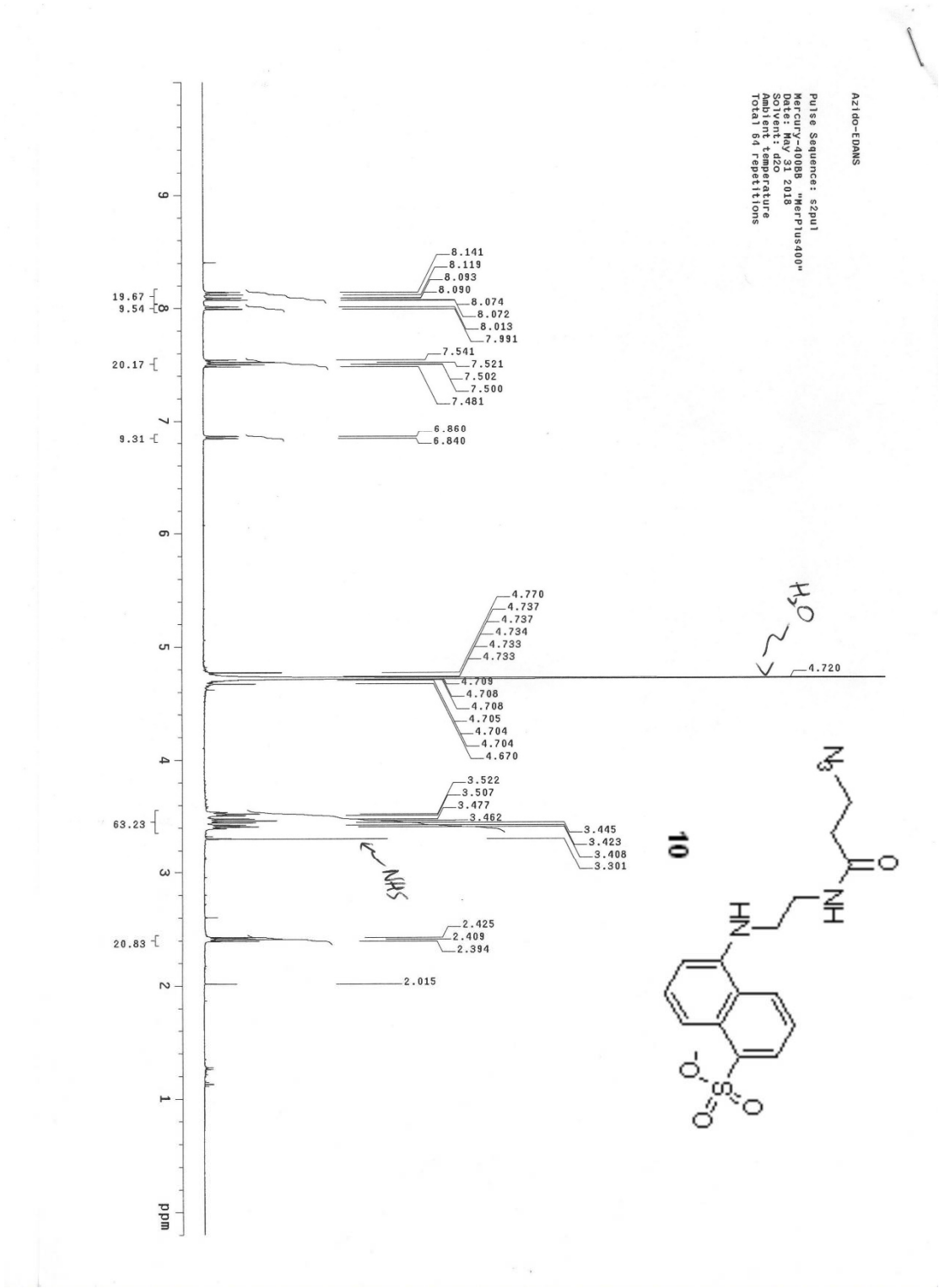
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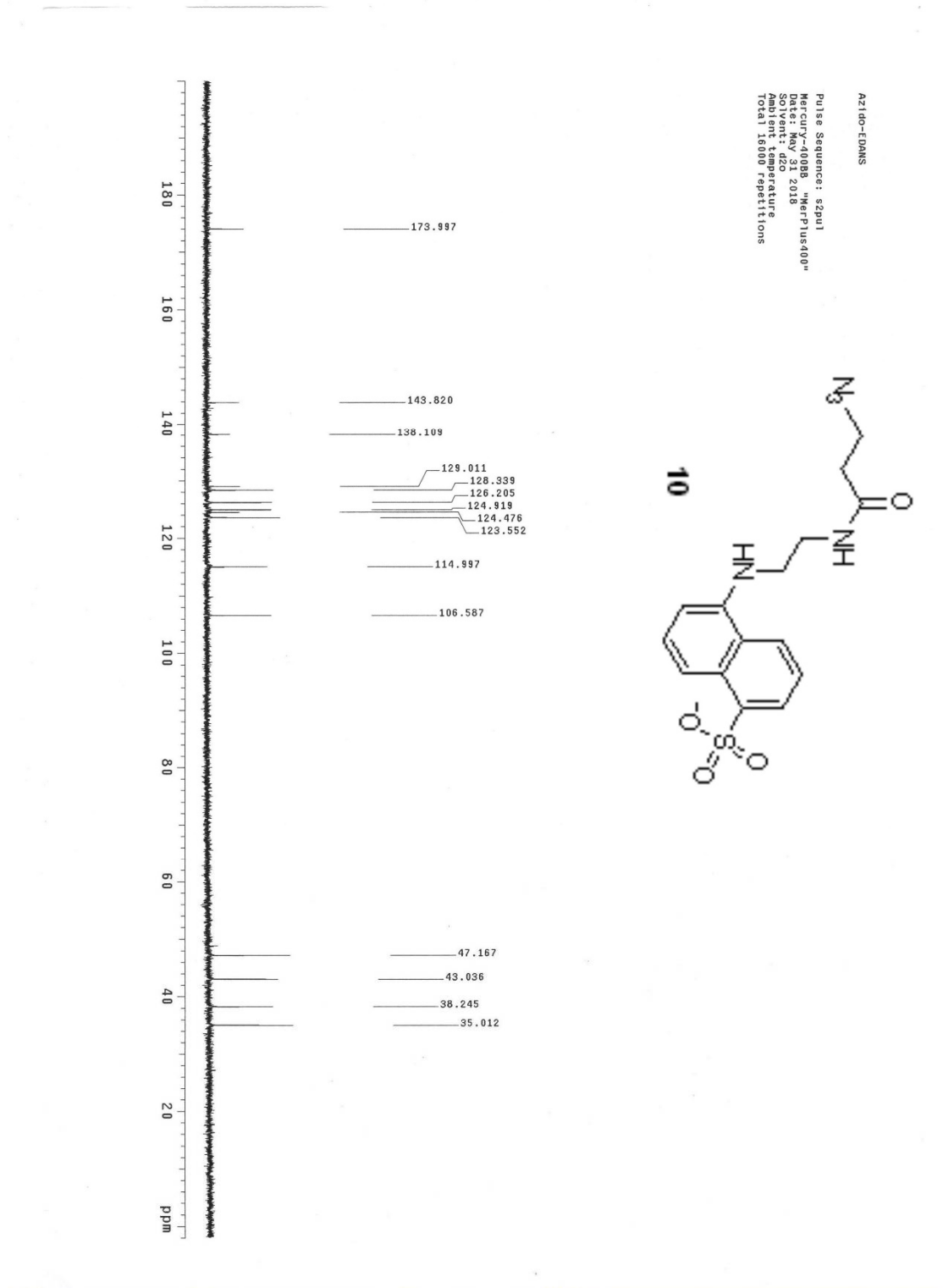
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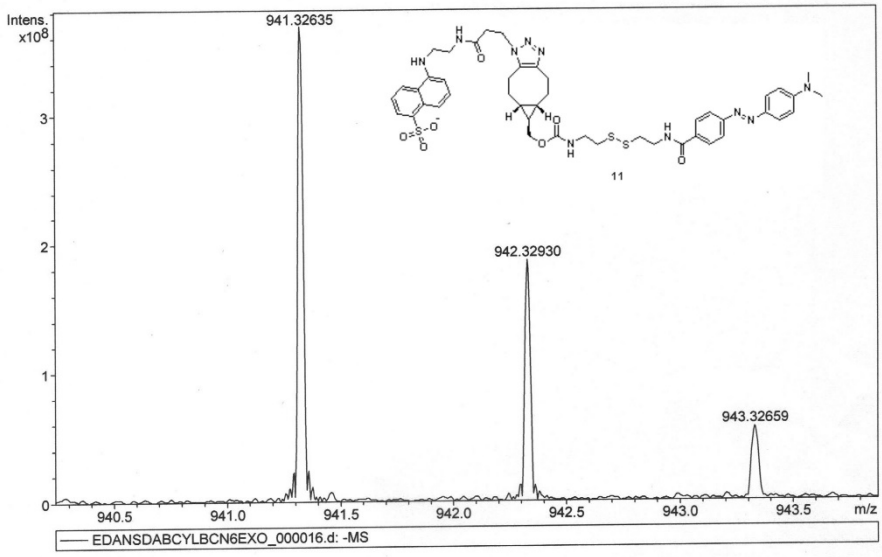

Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
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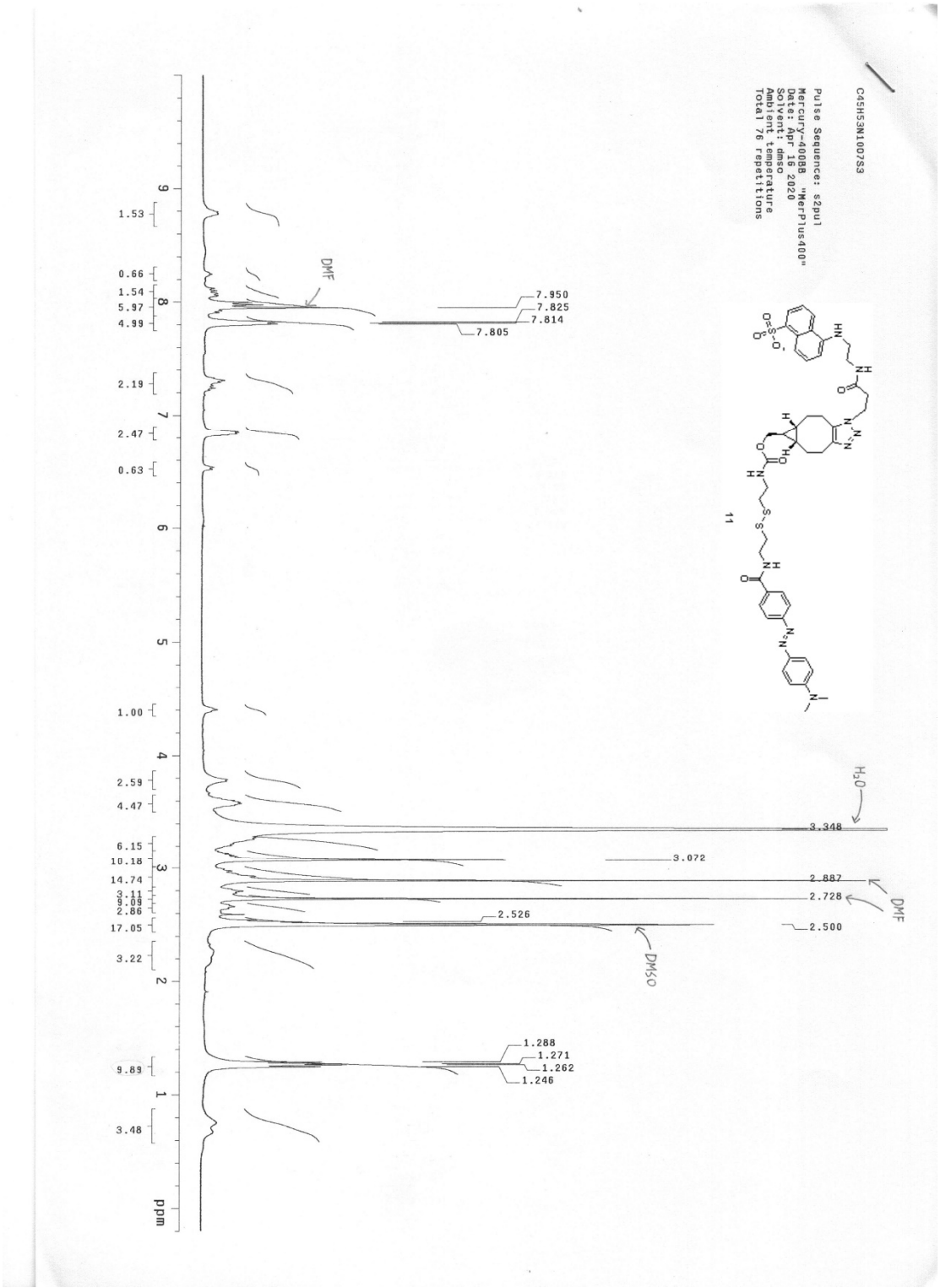
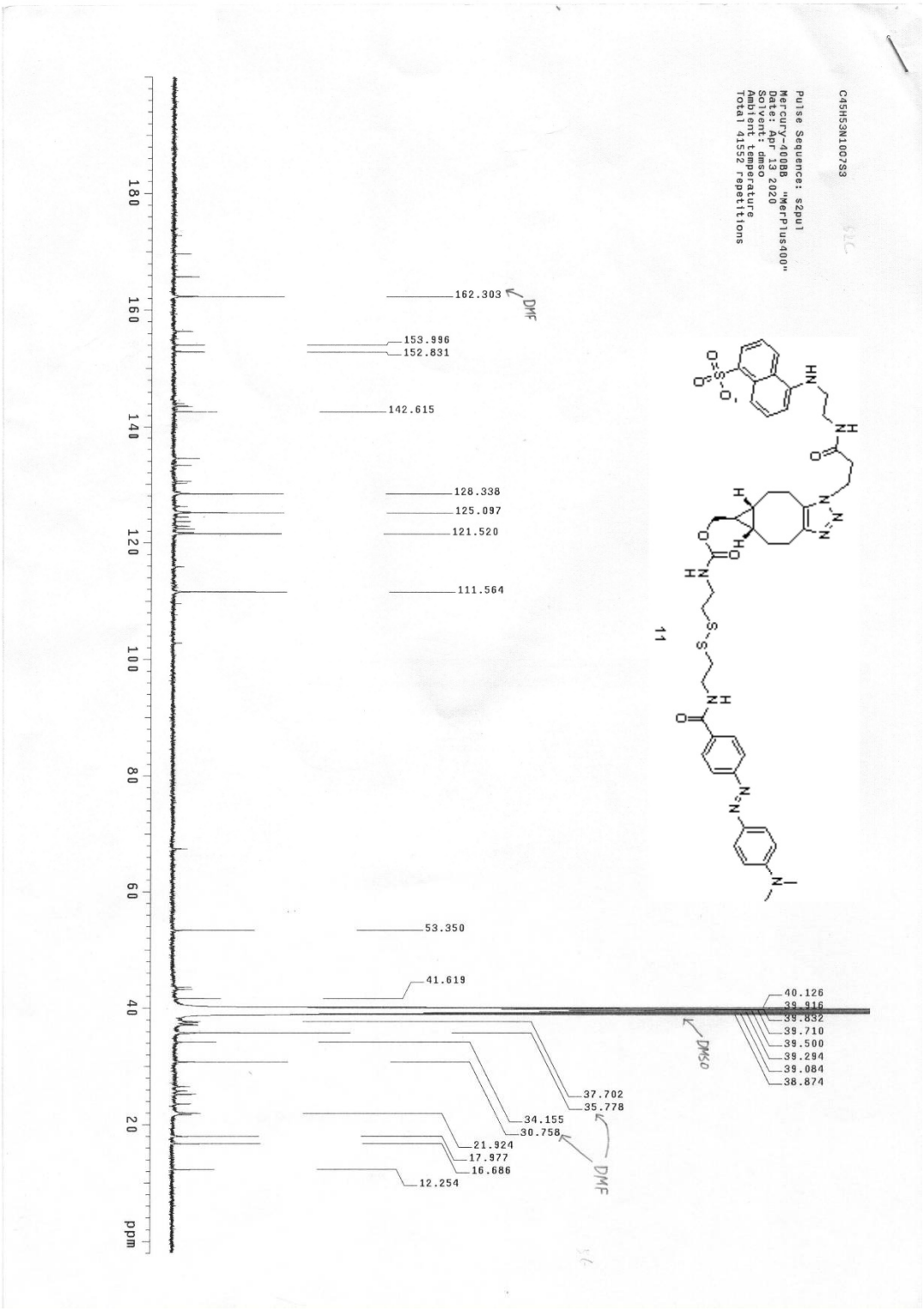
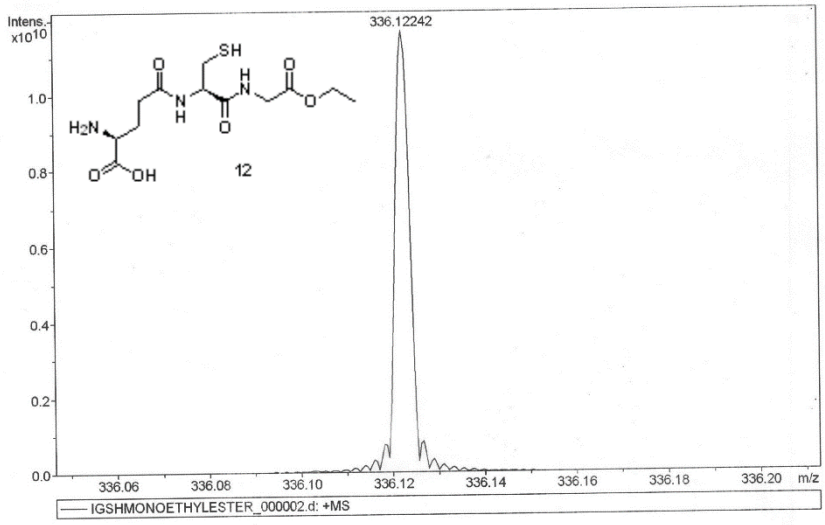

C45H53N10O7S3
Pulse Sequence: s2pul
Mercury-400BB "MerPlus400"
Date: Apr 16 2020
Solvent: dmso
Ambient temperature
Total 76 repetitions
11
DMF
7.950
7.825
7.814
7.805
H2O
3.348
3.072
2.887
2.728
DMF
2.526
2.500
DMSO
1.288
1.271
1.262
1.246
1.53
0.66
1.54
5.97
4.99
2.19
2.47
0.63
1.00
2.59
4.47
6.15
10.18
14.74
3.11
9.09
2.86
17.05
3.22
9.89
3.48
ppm


C45H53N10O7S3
Pulse Sequence: s2pul
Mercury-400BB "MerPlus400"
Date: Apr 13 2020
Solvent: dmso
Ambient temperature
Total 41552 repetitions
162.303
DMF
153.996
152.831
142.615
128.338
125.097
121.520
111.564
53.350
41.619
40.126
39.916
39.832
39.710
39.500
39.294
39.084
38.874
DMSO
37.702
35.778
34.155
30.758
DMF
21.924
17.977
16.686
12.254
11
180
160
140
120
100
80
60
40
20
ppm

Mass Spectrum SmartFormula Report

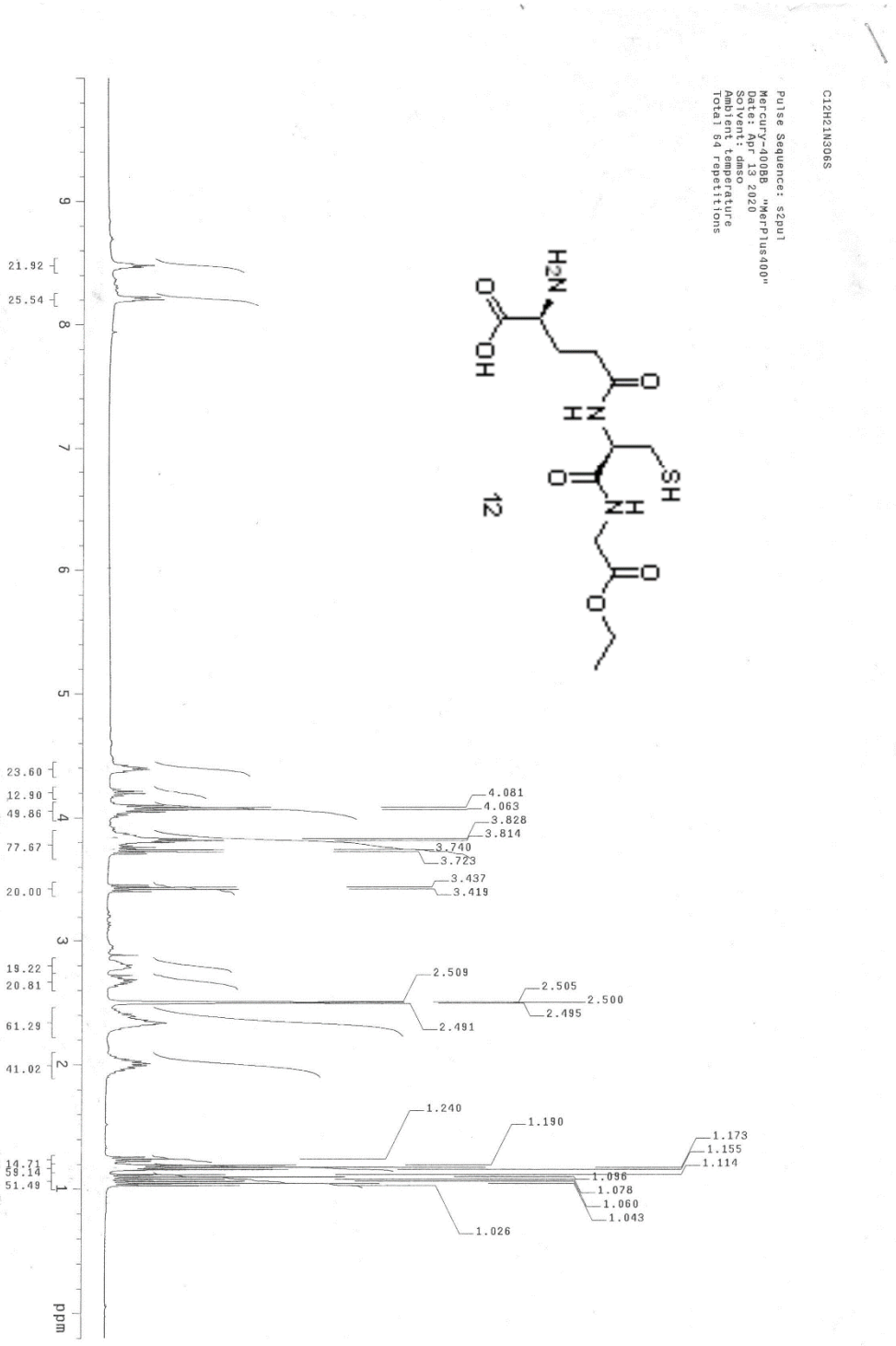
Analysis Info

Analysis Name	D:\Data\a4\IGSHMONOETHYLESTER_000002.d	6/12/2020 3:43:39 PM
Method	broadband first signal	Operator: YU HSIAO-CHING
Sample Name	I. GSH monoethyl ester	Instrument: BRUKER FT-MS solariX
Comment	ESI Positive	



12

Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
336.12242	1	C 12 H 22 N 3 O 6 S	100.00	336.12238	-0.03	-0.10	20.4	3.5	even	ok


C12H21N3O6S
Pulse Sequence: s2pul
Mercury-400BB "MerPlus400"
Date: Apr 13 2020
Solvent: dmso
Ambient temperature
Total 64 repetitions
12
21.92
25.54
23.60
12.90
49.86
77.67
20.00
19.22
20.81
61.29
41.02
14.71
59.14
51.49
4.081
4.063
3.828
3.814
3.740
3.723
3.437
3.419
2.509
2.505
2.500
2.495
2.491
1.240
1.190
1.173
1.155
1.114
1.096
1.078
1.060
1.043
1.026
ppm