Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Conversion of *S*-phenylsulfonylcysteine residues to mixed disulfides at pH 4.0: Utility in Protein thiol blocking and in Protein-*S*-nitrosothiol detection.

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Supplementary

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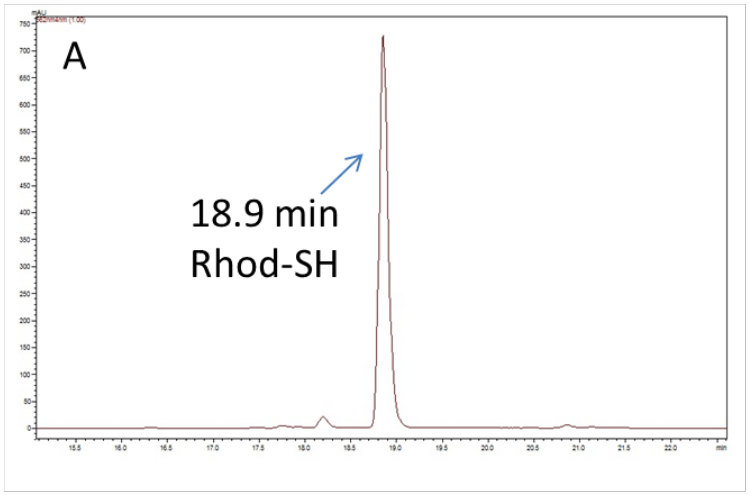
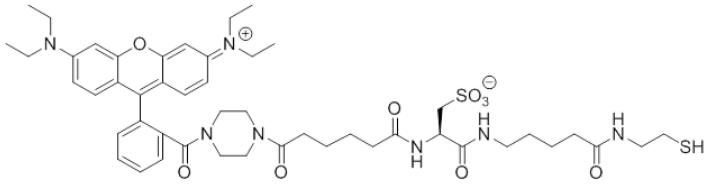
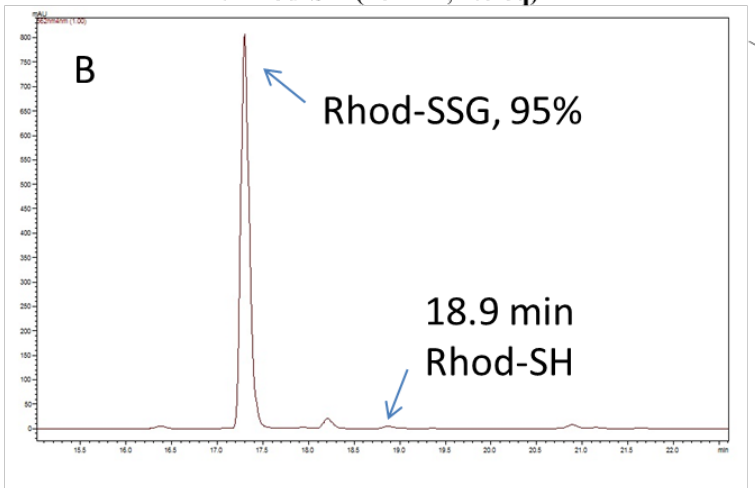
Rhod-SH

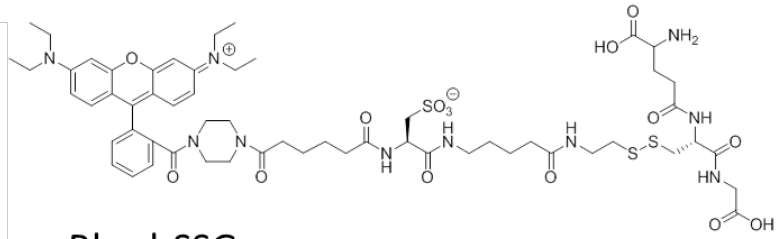




Rhod-SH

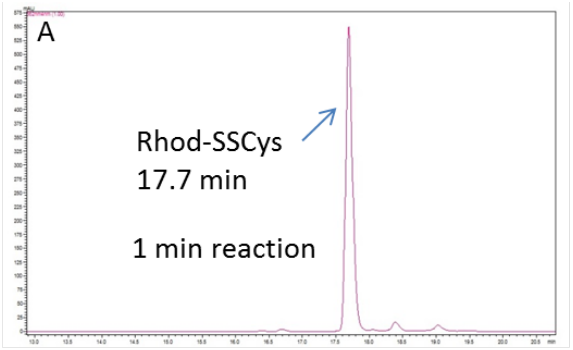
GSNO —(1. $PhSO_2Na$ (30 min, 250 eq); 2. Rhod-SH (10 min, 1.0 eq))→ GSSDye

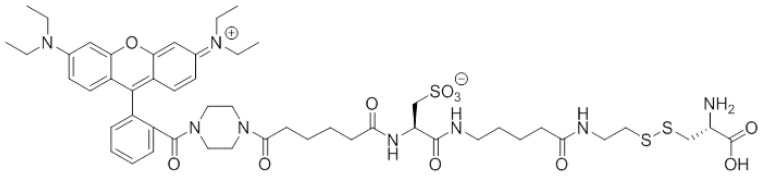




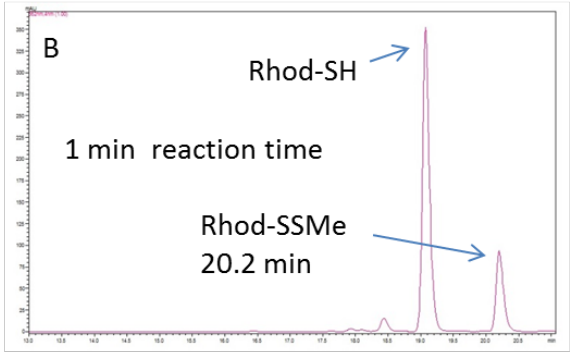
Rhod-SSG
Rt= 17.3 min
HRMS-EI (m/z): calcd $C_{58}H_{80}N_{10}O_{15}S_3$
$[M+H]^+$ 1254.5040, found 1254.5031.

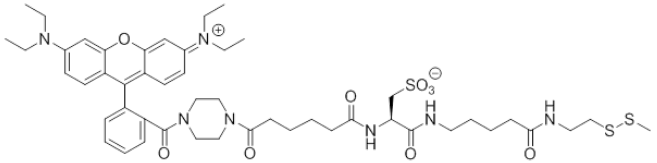
Fig. S1. HPLC traces (λ= 562 nm) of (A) Rhod-SH (**1**), and (B) a reaction solution of GSNO after addition of $PhSO_2Na$ (30 min) and Rhod-SH(**1**) (10 min).



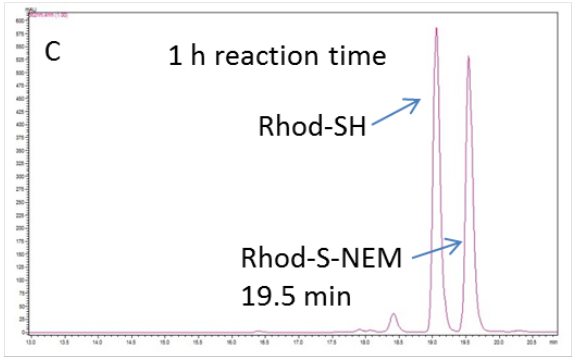


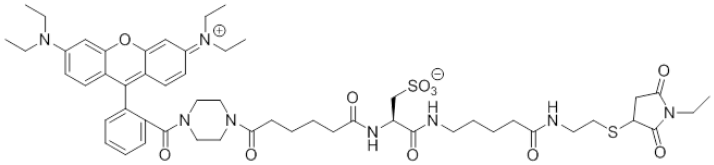
Rhod-SSCys: Rt= 17.7 min
HRMS (m/z): calc for $C_{51}H_{70}N_8O_{11}S_3$
$[M+H]^+$ 1067.4399, found 1067.4375





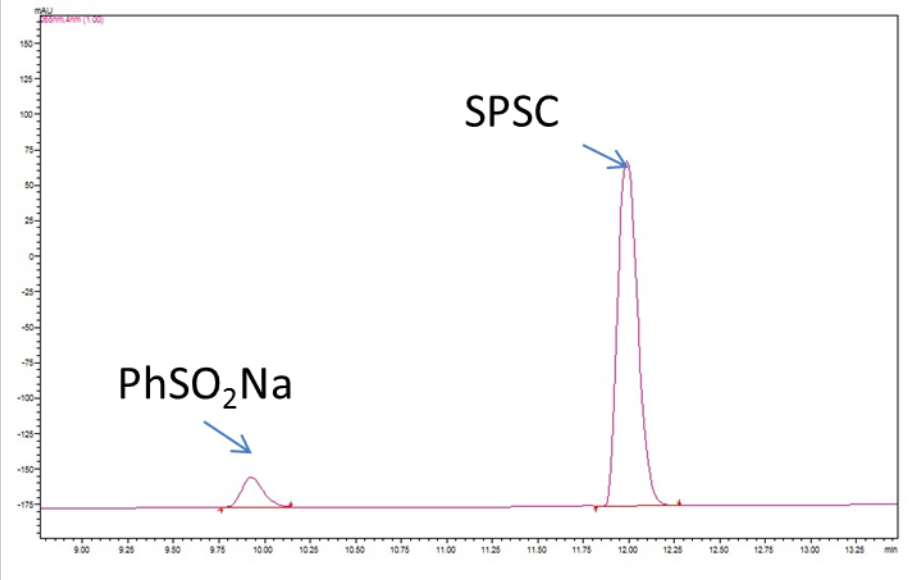
Rhod-SSMe: Rt= 20.2 min
HRMS (m/z): calc for $C_{49}H_{67}N_7O_9S_3$
$[M+H]^+$ 994.4235, found 994.4200





Rhod-S-NEM: Rt= 19.5 min
HRMS (m/z): calc for $C_{54}H_{72}N_8O_{11}S_2$
$[M+H]^+$ 1073.4835, found 1073.4808

Fig. S2. HPLC traces (λ= 562 nm) of reactions of Rhod-SH (**1**) with (A) SPSC, (B) MMTS, and (C) NEM.



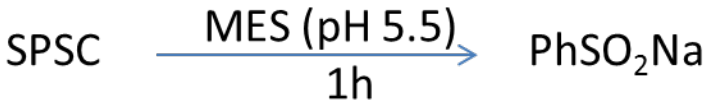


Fig. S3. HPLC trace (λ= 265 nm) of a solution of SPSC (2 mM) in MES (100 mM, 1 mM EDTA, pH 5.5) after 1h.

Table S1. Theoretical vs actual masses of compounds from digests.

	Compound (elution time)		**Mass (amu)**
Figure 4: BSA-SS-Rhod digest	FKDLGEEHFKGLVLIAFSQYLQQ C(SSRhod)PFDEHVK^{+6} (3.7 min)	Th	769.3826, 769.5497, 769.7168, 769.8838, 770.0509, 770.2178, 770.3848, 770.5518, 770.7188.
		Actual	769.3911, 769.5609, 769.7225, 769.8898, 770.0550, 770.2207, 770.3838, 770.5471, 770.7094
	GLVLIAFSQYLQQ C(SSRhod)PFDEHVK^{+3} (3.85 min)	Th	1127.5567, 1127.8910, 1128.2255, 1128.5599, 1128.8944, 1129.2289, 1129.5598
		Actual	1127.5512, 1127.8867, 1128.2192, 1128.5535, 1128.8840, 1129.2197, 1129.5514,
	GLVLIAFSQYLQQ C(SSRhod)PFDEHVK^{+4} (3.85 min)	Th	845.9194, 846.1701, 846.4207, 846.6711, 846.9215, 847.1719, 847.4223
		Actual	845.9240, 846.1748, 846.4239, 846.6744, 846.9241, 847.1736, 847.4232
	Rhod-SSMe^{+1} (4.35 min)	Th	994.4236, 995.4268, 996.4295, 997.4228
		Actual	994.4111, 995.4203, 996.4112, 997.4112
	Rhod-SSRhod^{+2} (4.68 min)	Th	947.428, 947.9295, 948.4299, 948.9303,
		Actual	947.4277, 947.9293, 948.4300, 948.9286
	RhodSSRhod (-Et)$^{+2}$ (4.5 min)	Actual	622.6106, 622.9450, 623.2793, 623.6106, 623.9449
		Th	622.6205, 622.9442, 623.2832, 623.6112, 623.9528
Figure S8-A: BSA-SS-Rhod Digest	LQQC(SSRhod)PF^{+2} (15.3 min)	Th	840.8848, 841.3863, 841.8866, 842.387, 842.8874,
		Actual	840.8743, 841.3811, 841.8846, 842.3793, 842.8869
	QQC(SSRhod)PF (14.6 min)	Th	784.3428, 784.8442, 785.3445, 785.8448, 786.3451,
		Actual	784.3383, 784.8316, 785.3378, 785.8434, 786.3346
Figure S8-B: AdhR-SS-Rhod digest	C(SSRhod)MR^{+2} (9.2 min)	Th	677.7944, 678.2958, 678.7954, 679.2956
		Actual	677.7856, 678.2901, 678.7923, 679.2922
	C(SSRhod)MR^{+3} (9.2 min)	Th	452.1987 452.533, 452.866, 453.1995
		Actual	452.1965, 452.5309, 452.8636, 453.1978
	C(SSRhod)M^{+2} (13.4 min)	Th	599.7438, 600.2453, 600.7446, 601.2449, 601.7447
		Actual	599.7400, 600.2376, 600.7399, 601.2314, 601.7334
	C(SSRhod)$^{+2}$ (12.6 min)	Th	534.2236, 534.7251, 535.2245, 535.7248,
		Actual	534.2193, 534.7196, 535.2185, 535.7168
AdhR-SS-Rhod Glu-C digest	FIKC(SSRhod)MRNAGLSIE^{+3} (6.2 min)	Th	809.7292, 810.0635, 810.3973, 810.731, 811.0646, 811.3982, 811.7318
		Actual	809.7371, 810.0702, 810.4053, 810.7385, 811.0735, 811.4062, 811.7391

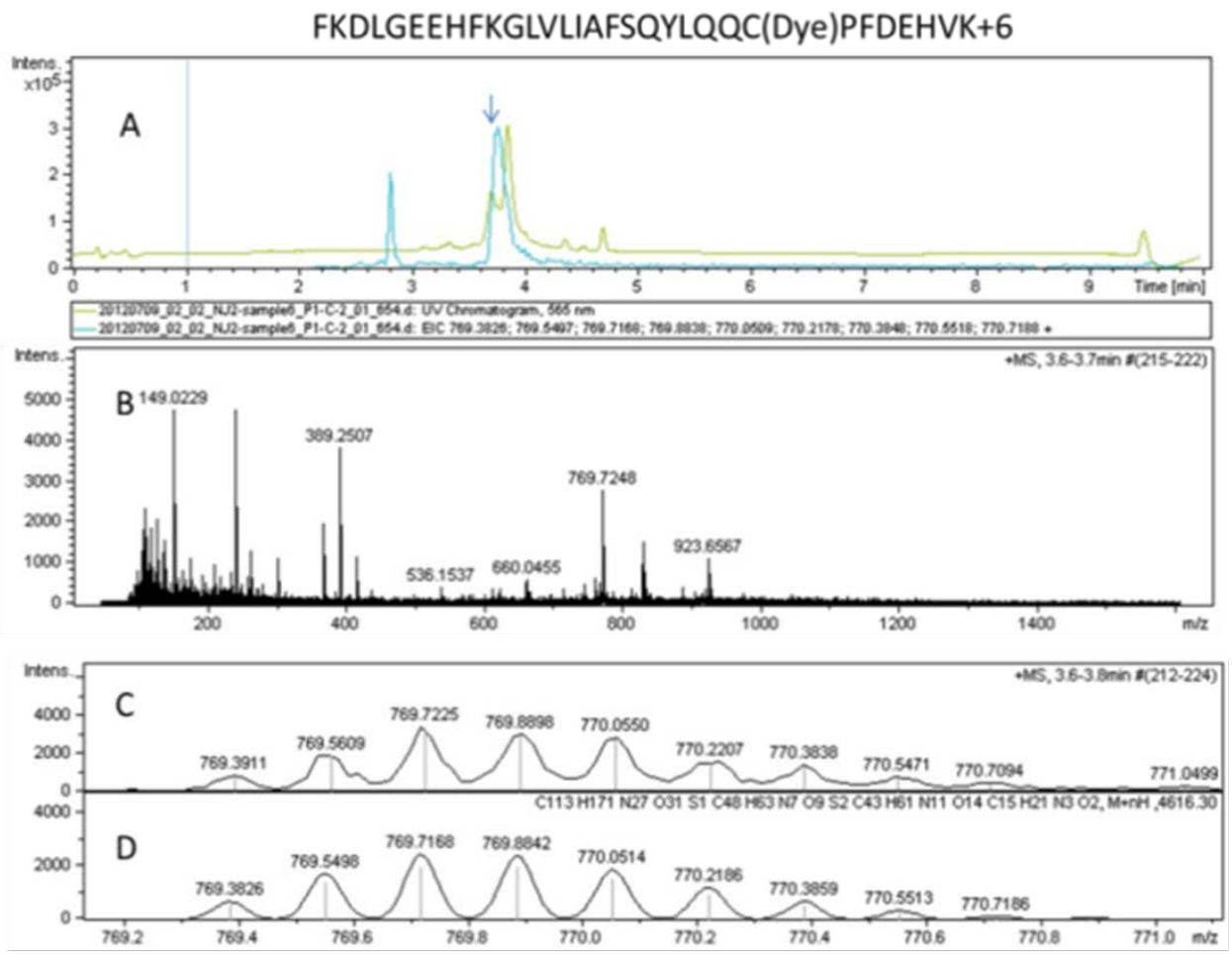


Fig. S4. Representative mass spectrometry identification of Rhod-SH labeled peptide FKDLGEEHFKGLVLIAFSQYLQQC(SSRhod)PFDEHVK^{+6}. A) Green= HPLC absorbance trace @ 565 nm, blue= extracted ion content for 769.3826, 769.5497, 769.7168, 769.8838, 770.0509, 770.2178, 770.3848, 770.5518, 770.7188. B) Mass spectrum of selected region under arrow in A. C) Expanded mass spectrum of selected area in B. D) Simulated pattern for FKDLGEEHFKGLVLIAFSQYLQQC(Dye)PFDEHVK^{+6}.

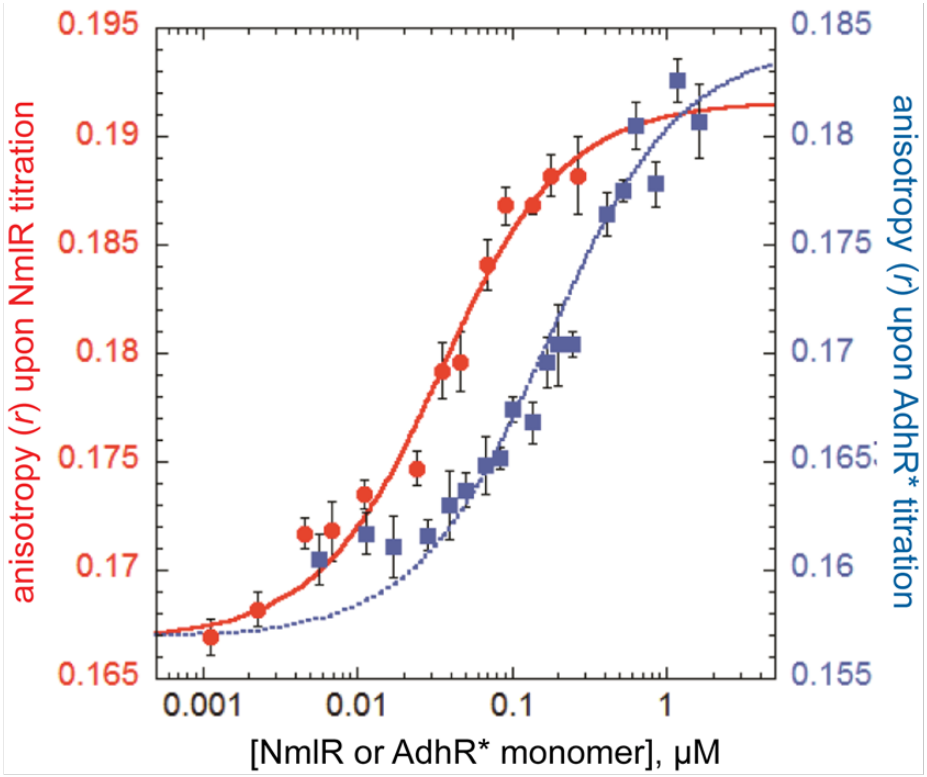


Fig. S5. Recombinant *S. pneumoniae* NmlR and *B. subtilis* AdhR* bind to NmlO-containing DNA. The anisotropy (r_i) of the fluorescein fluorescence is shown as a function of added NmlR (*red* circles) or AdhR* (*blue* squares) monomer, with the continuous line a fit to a nondissociable dimer binding model (K_{dim} fixed at a value of 10^{12} M^{-1}) using DynaFit.[1] The parameters obtained for NmlR are K_{DNA}=6.7 (±0.1) x 10^7 M^{-1}, r_{DNA}=0.167; $r_{P2•D}$=0.192 ±0.001 and AdhR*, K_{DNA}=1.2 (±0.2) x 10^7 M^{-1}, r_{DNA}=0.157; $r_{P2•D}$=0.184 ±0.001. The sequence of the NmlO is 5'-CTTGA**CTTGGAG**TCAA**CTCAAAG**TTATATG[F]-3' which was annealed to a complementary ssDNA, synthesized and purified as previously described.[2] The pseudo 2-fold symmetric operator is highlighted in bold, with the [F] is fluorescein. The 18-bp *nml* and *adh* operator core sequences are very similar to one another: 5-**CTTXGAG**XXXX**CTCXAAG**-3'; adh core, 5'-**CTTAAAG**XXXX**CTTTAAG**-3'), respectively. Conditions: 10 mM Hepes, 0.2 M NaCl, 1 mM DTT, 5 nM 30-bp NmlO DNA, pH 7.0, 25 °C.

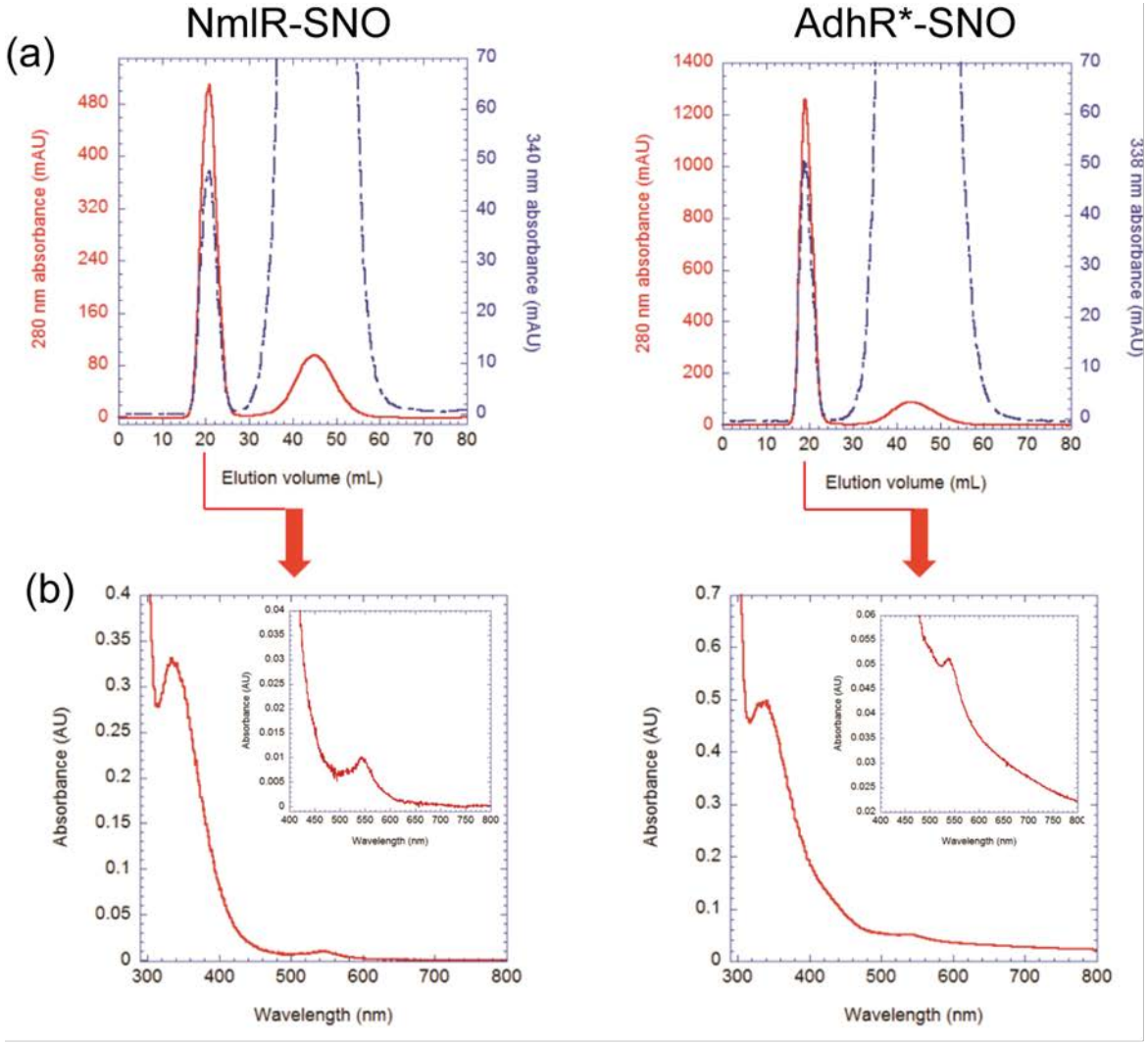


Fig. S6. *S. pneumoniae* NmlR and *B. subtilis* AdhR* form C52 SNO adducts. Both proteins were reacted with a 10-fold excess of GSNO (see Methods) for 1 h anaerobically and in darkness followed by (**a**) injection onto a HiPrep 26/10 desalting column. This results in elution of AdhR*-SNO or NmlR-SNO (first peak), followed by excess GSNO (second peak). (**b**) Following desalting, fractions in the first peak on each chromatogram were concentrated to ≈400-500 µL and the UV-Vis spectrum was measured. S-NO bonds possess a characteristic 330-350 nm absorbance band with an extinction coefficient, ε ≈1,000 M^{-1} cm^{-1}.[3] *Inset*, expanded view of the 400-800 nm region of the spectra. S-NO bonds also exhibit a weak 550-600 nm absorbance band with an extinction coefficient, ε ≈20 M^{-1} cm^{-1}.[3] NmlR and AdhR* possess 280 nm molar absorptivities of 7,450 and 12,950 M^{-1} cm^{-1}, respectively.

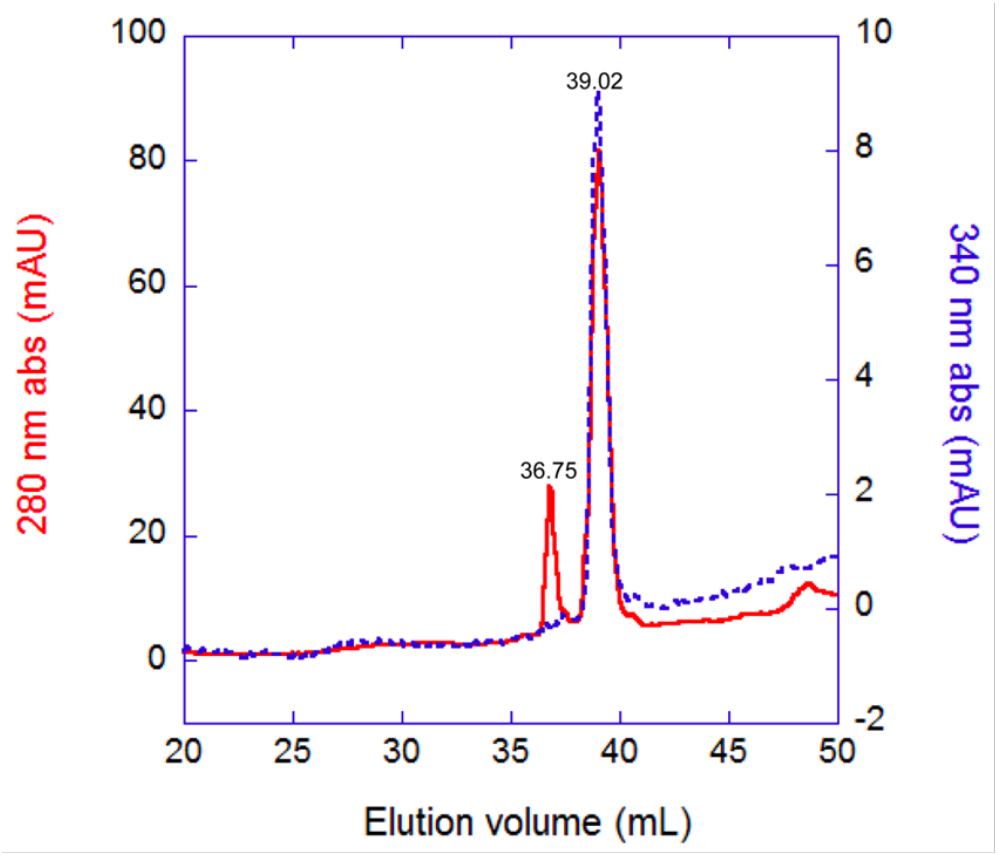


Fig. S7. Reverse-phase (µRPC C2/C18) chromatogram of NmlR reacted with GSNO. The first peak (Ev=36.75 mL) exhibits only 280 nm absorbance, and ESI/MS showed that this peak is NmlR-S-S-G, where G is glutathione (13,767.5 D observed). The second peak (Ev=39.02 mL) exhibits both 280 nm and 340 nm absorbance, and ESI/MS showed that this peak is NmlR-SNO (13,492.3 D obs) and NmlR-SH (13,461.8 D obs). Buffers were 0.1% formic acid (Buffer A) and 0.1% formic acid, 60% acetonitrile (Buffer B) with a very shallow gradient was run to Buffer B to produce the chromatogram shown.

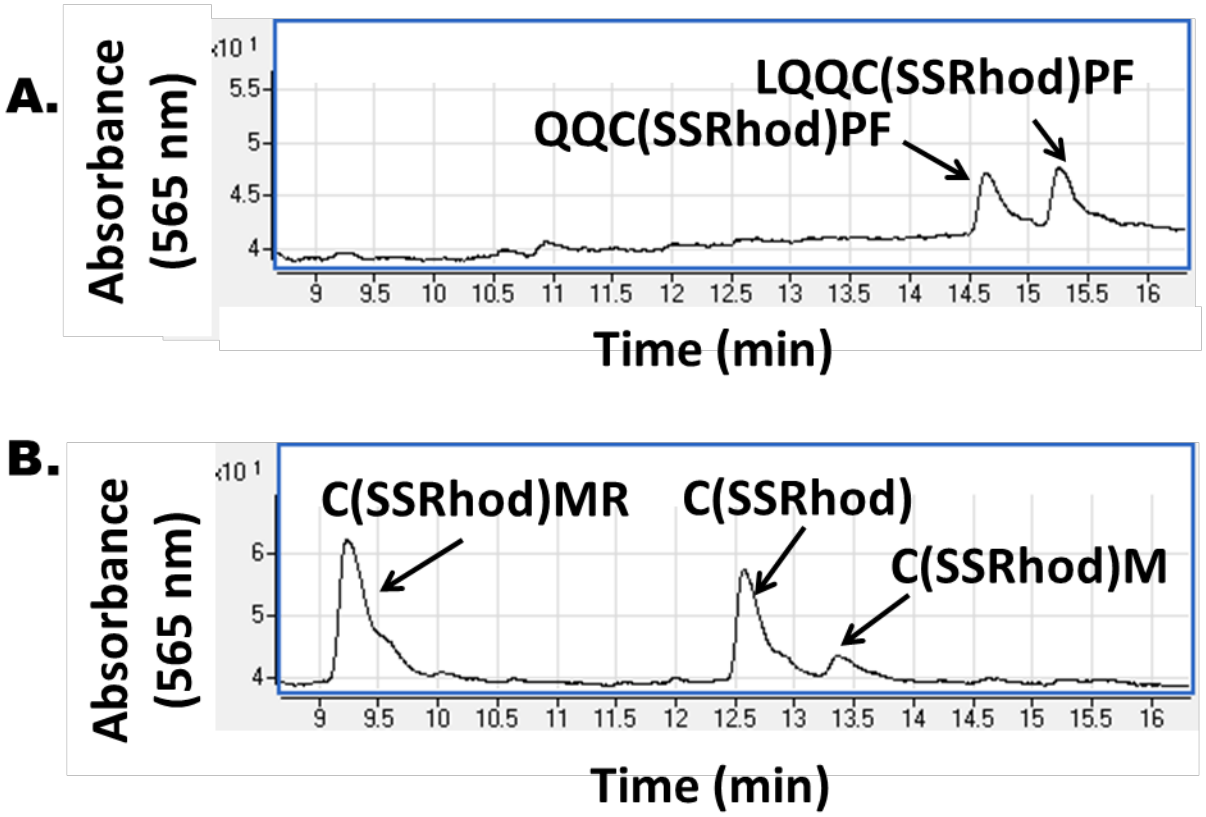


Fig. S8. (A) Absorbance (565nm) vs time (min) for peptides obtained after labelling a mixture of BSA-SNO and AdhR*-SH (50 µM each) by the thiosulfonate switch followed by trypsinolysis **(B)** Absorbance (565nm) vs time (min) for peptides obtained after labeling a mixture of BSA and AdhR*-SNO (50 µM each) by TST followed by trypsinolysis. Note that the C(SSRhod) observed from the tryptic digestion is an artifact from Rhod-SH (**1**) reacting with residual **SPSC**.

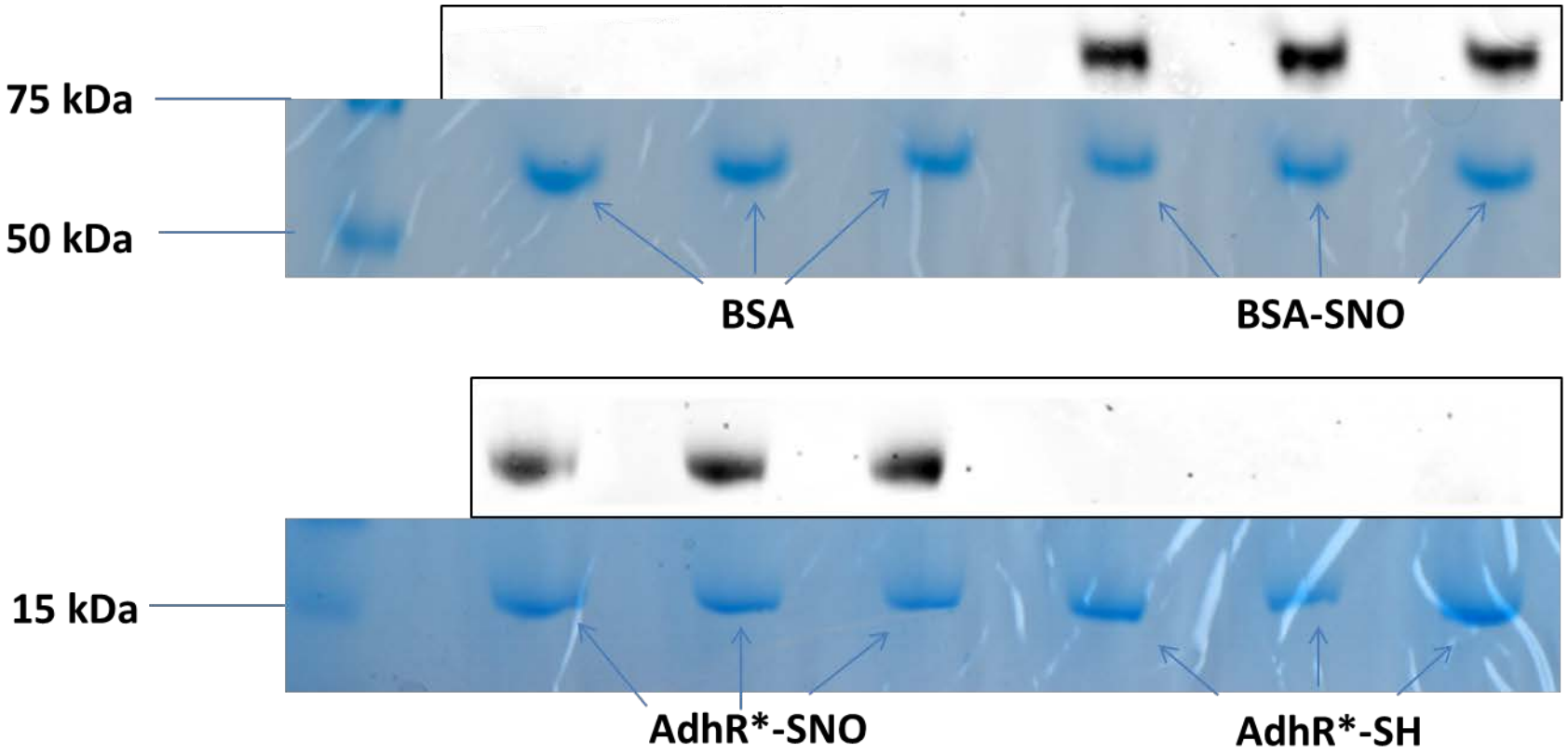


Fig. S9. 1DGE gel fluorescent images and loading controls from Coomassie staining for Fig 8 and 9.

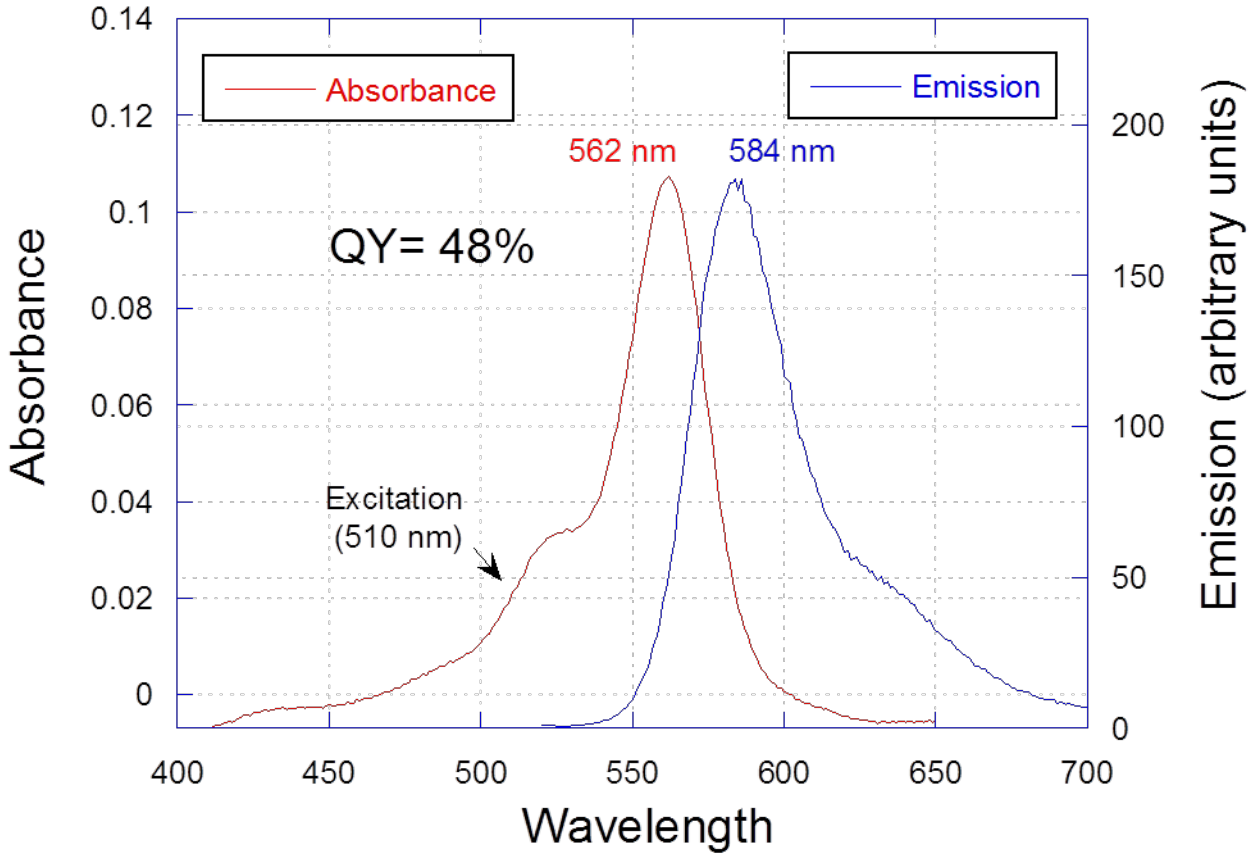
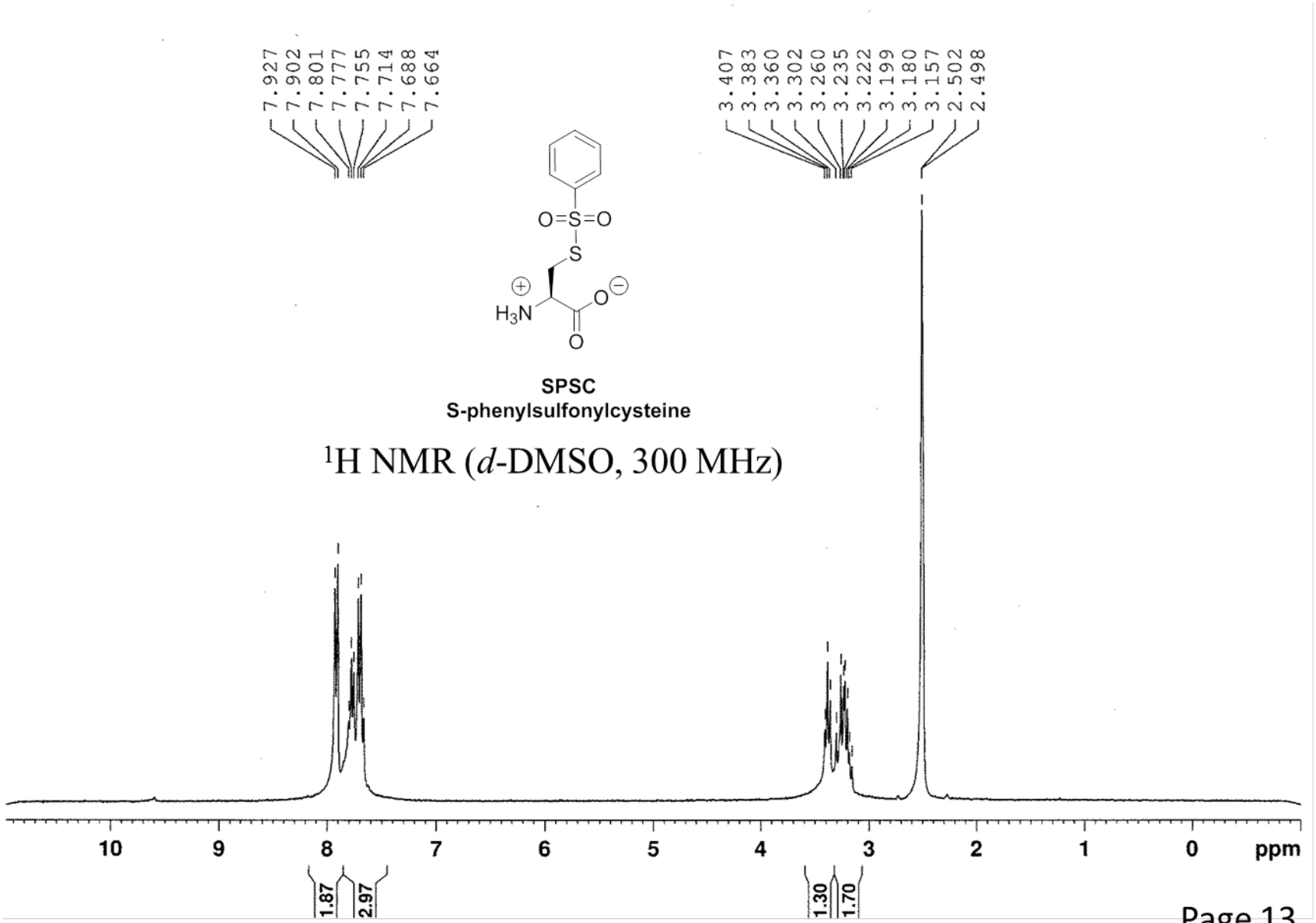


Fig. S10. Absorbance (red) and emission (blue, excitation= 510 nm) of a solution of Rhod-SH, **1,** in ethanol.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3.260
3.235
3.222
3.199
3.180
3.157
2.502
2.498
O=S=O
S
H3N
O
O
SPSC
S-phenylsulfonylcysteine
1H NMR (d-DMSO, 300 MHz)
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8
7
6
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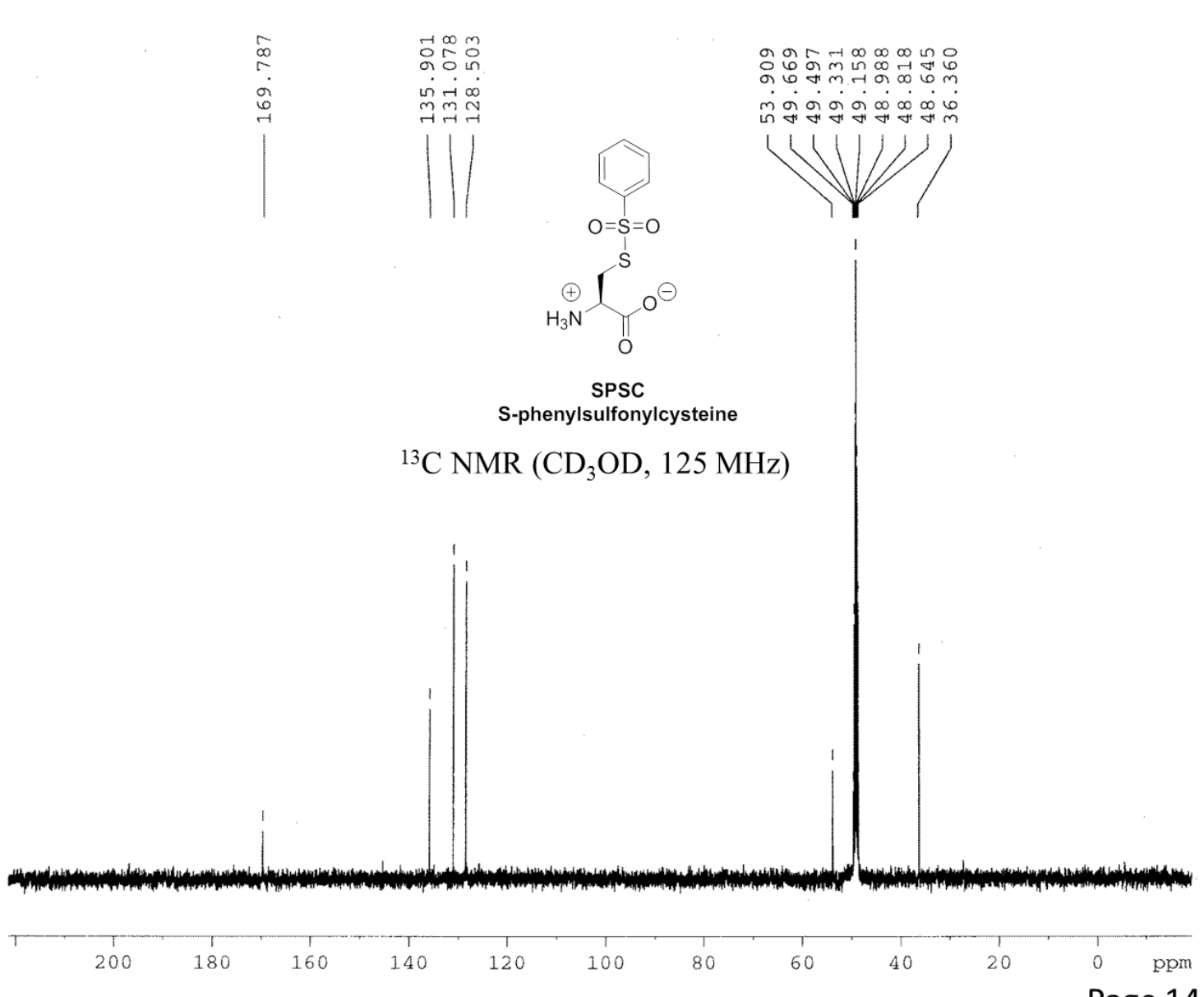
1.70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O=S=O
S
H3N
O
O
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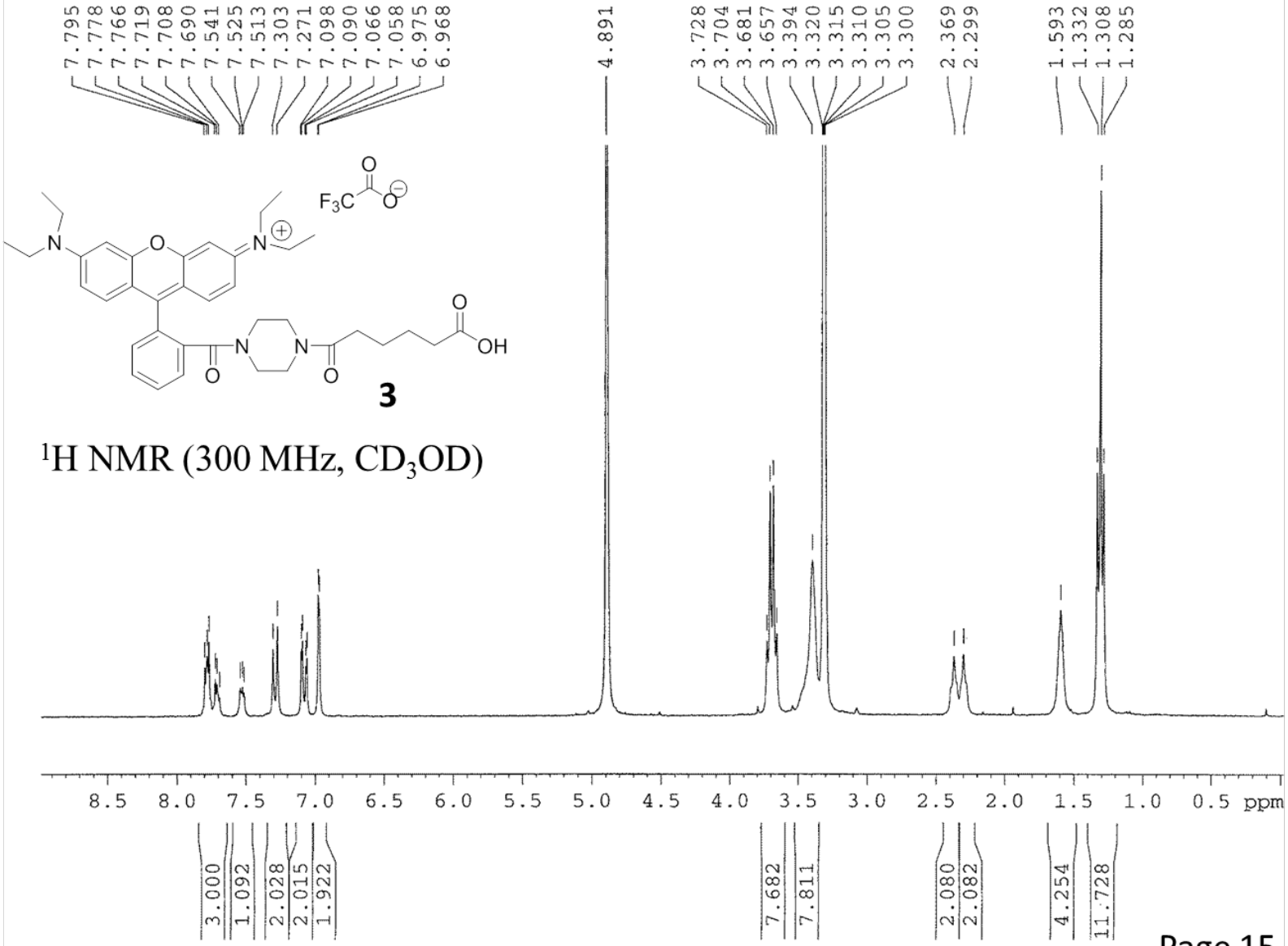
0
ppm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6.975
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3.704
3.681
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3.320
3.315
3.310
3.305
3.300
2.369
2.299
1.593
1.332
1.308
1.285
F3C
O
OH
N
3
1H NMR (300 MHz, CD3OD)
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
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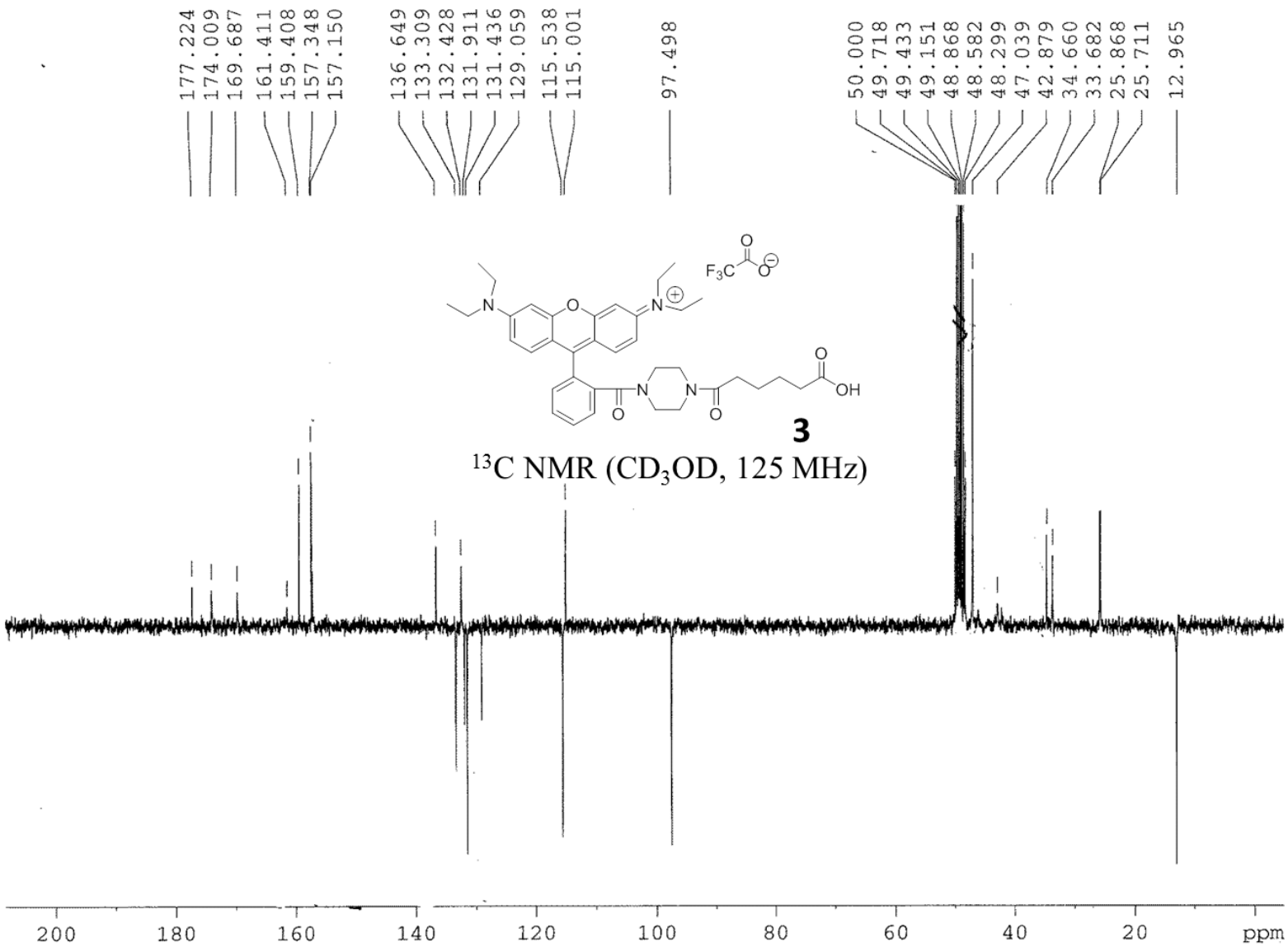
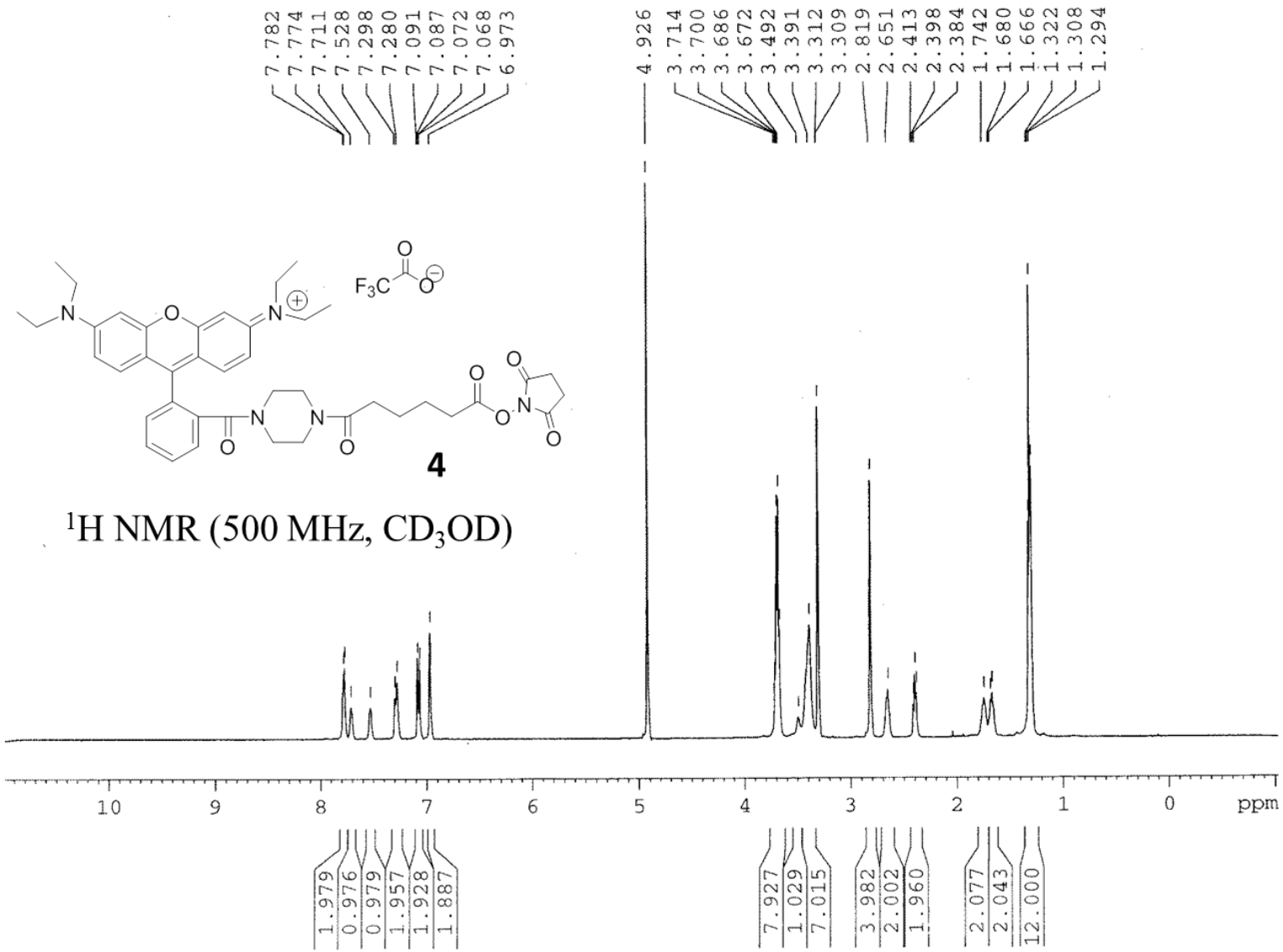


3

^{13}C NMR (CD_3OD, 125 MHz)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3.672
3.492
3.391
3.312
3.309
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2.651
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2.398
2.384
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F3C
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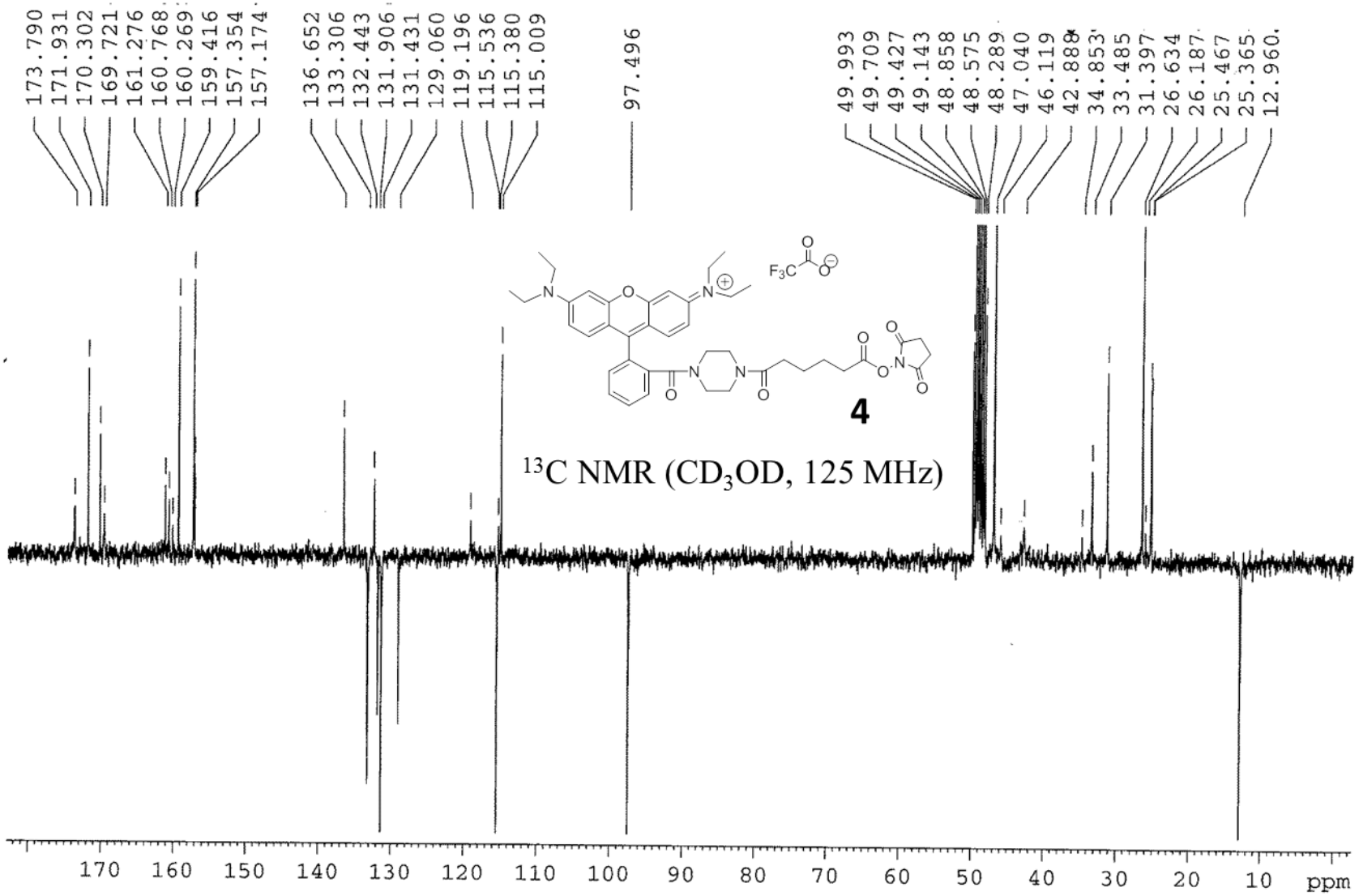
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97.496
49.993
49.709
49.427
49.143
48.858
48.575
48.289
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26.187
25.467
25.365
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13C NMR (CD3OD, 125 MHz)
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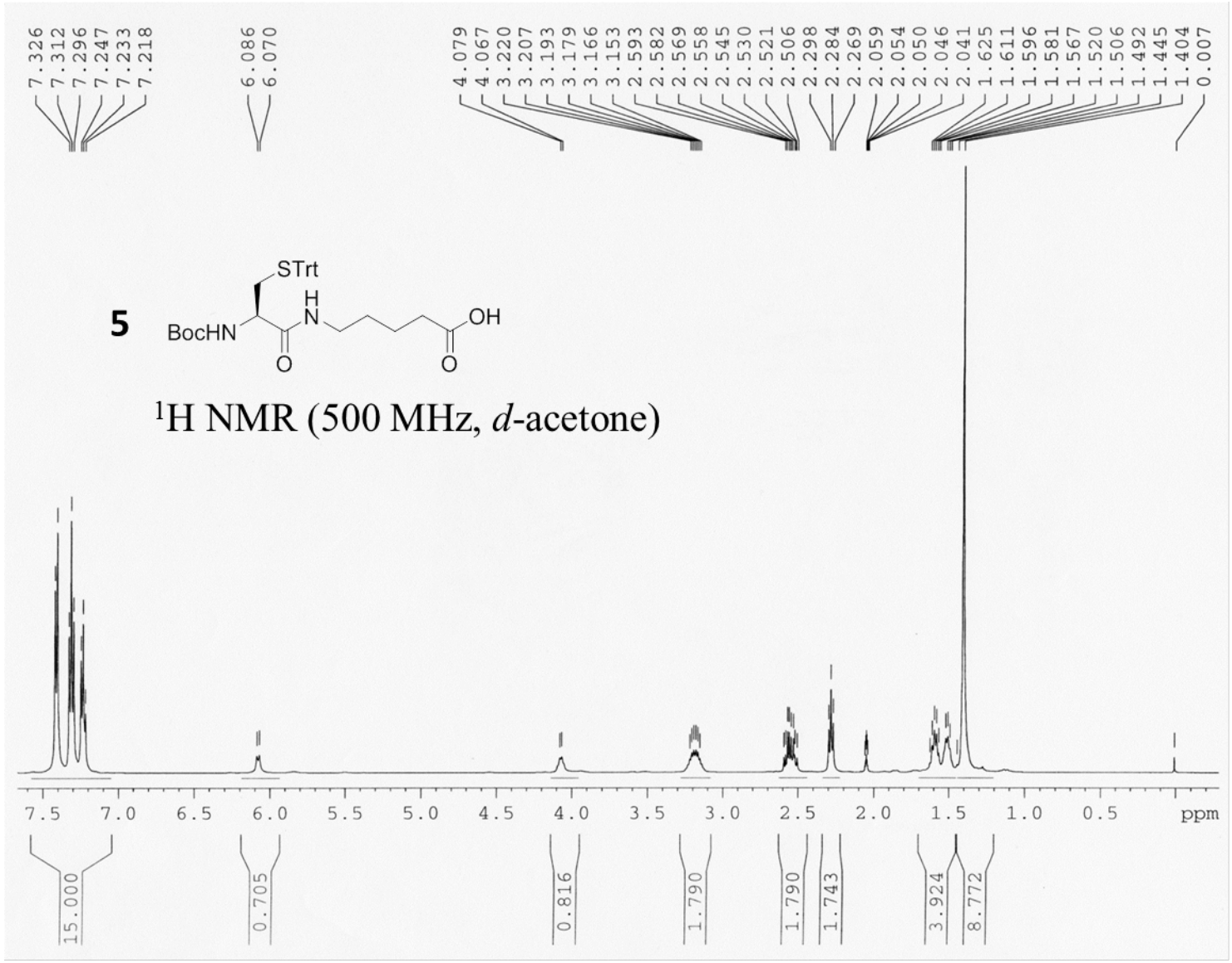
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2.046
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1.611
1.596
1.581
1.567
1.520
1.506
1.492
1.445
1.404
0.007
5
STrt
H
N
BocHN
OH
O
O
1H NMR (500 MHz, d-acetone)
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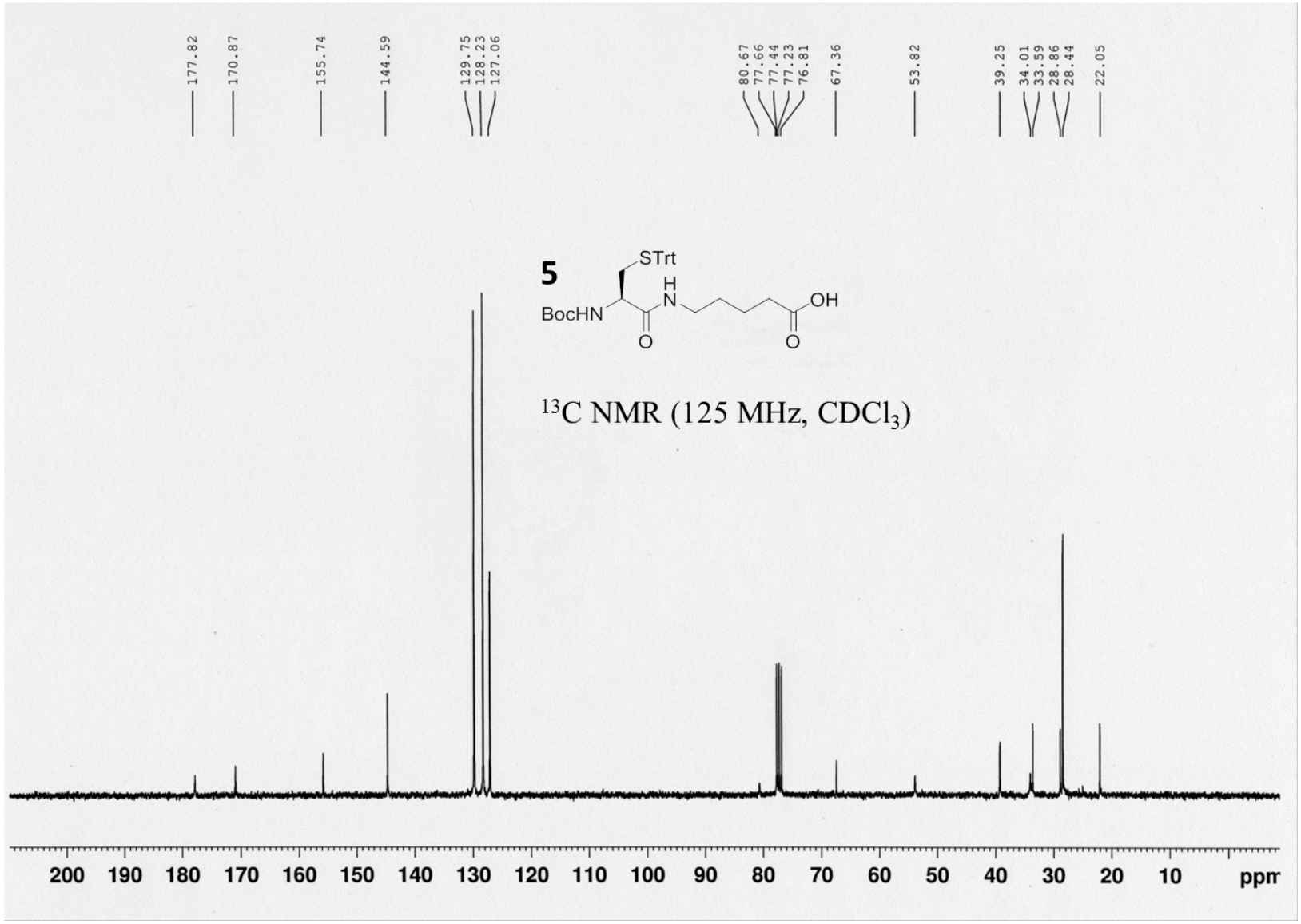
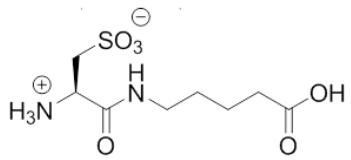
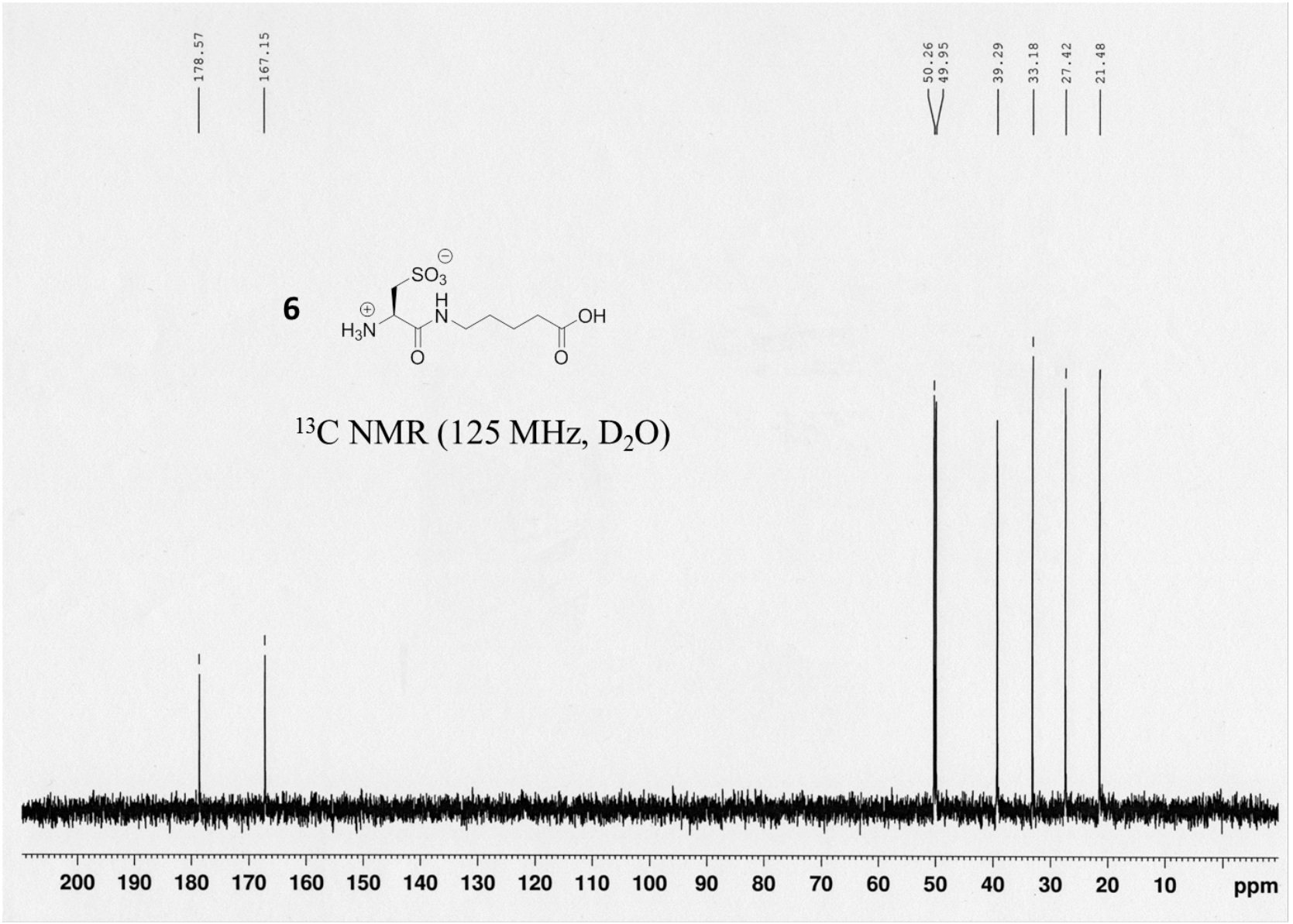
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
15.000
0.705
0.816
1.790
1.790
1.743
3.924
8.772

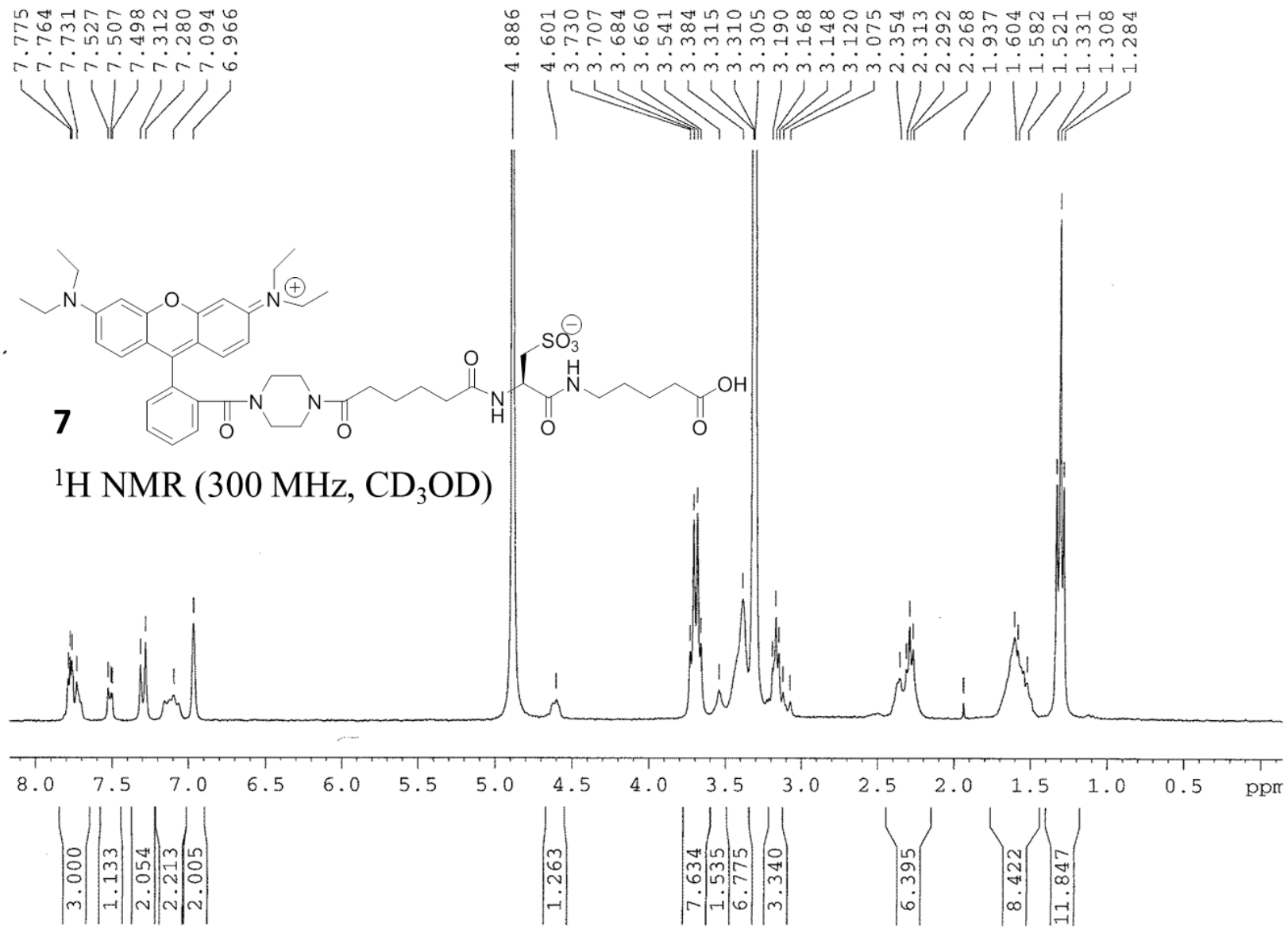

177.82
170.87
155.74
144.59
129.75
128.23
127.06
80.67
77.66
77.44
77.23
76.81
67.36
53.82
39.25
34.01
33.59
28.86
28.44
22.05
5
STrt
H
N
BocHN
OH
O
O
13C NMR (125 MHz, CDCl3)
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

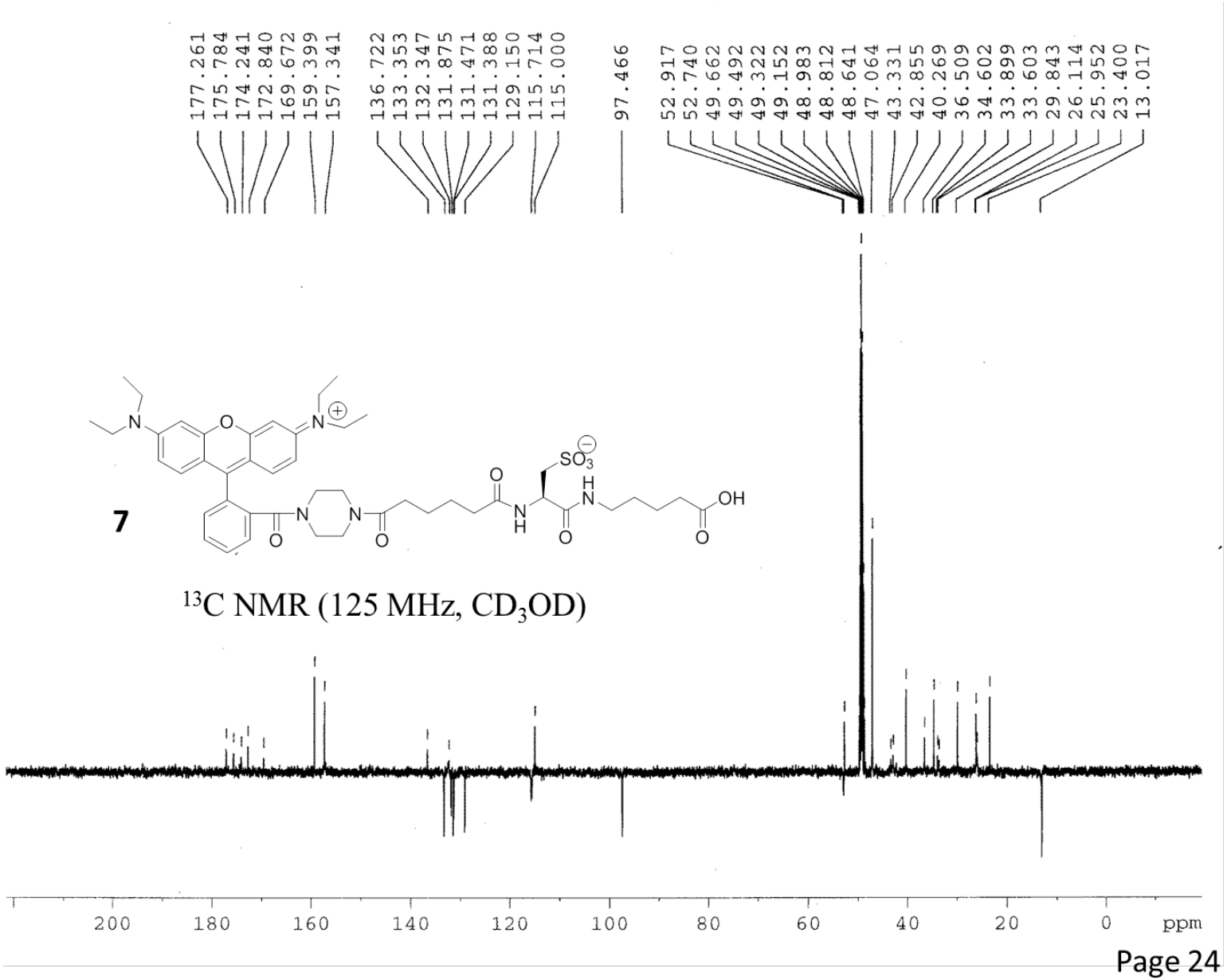
6

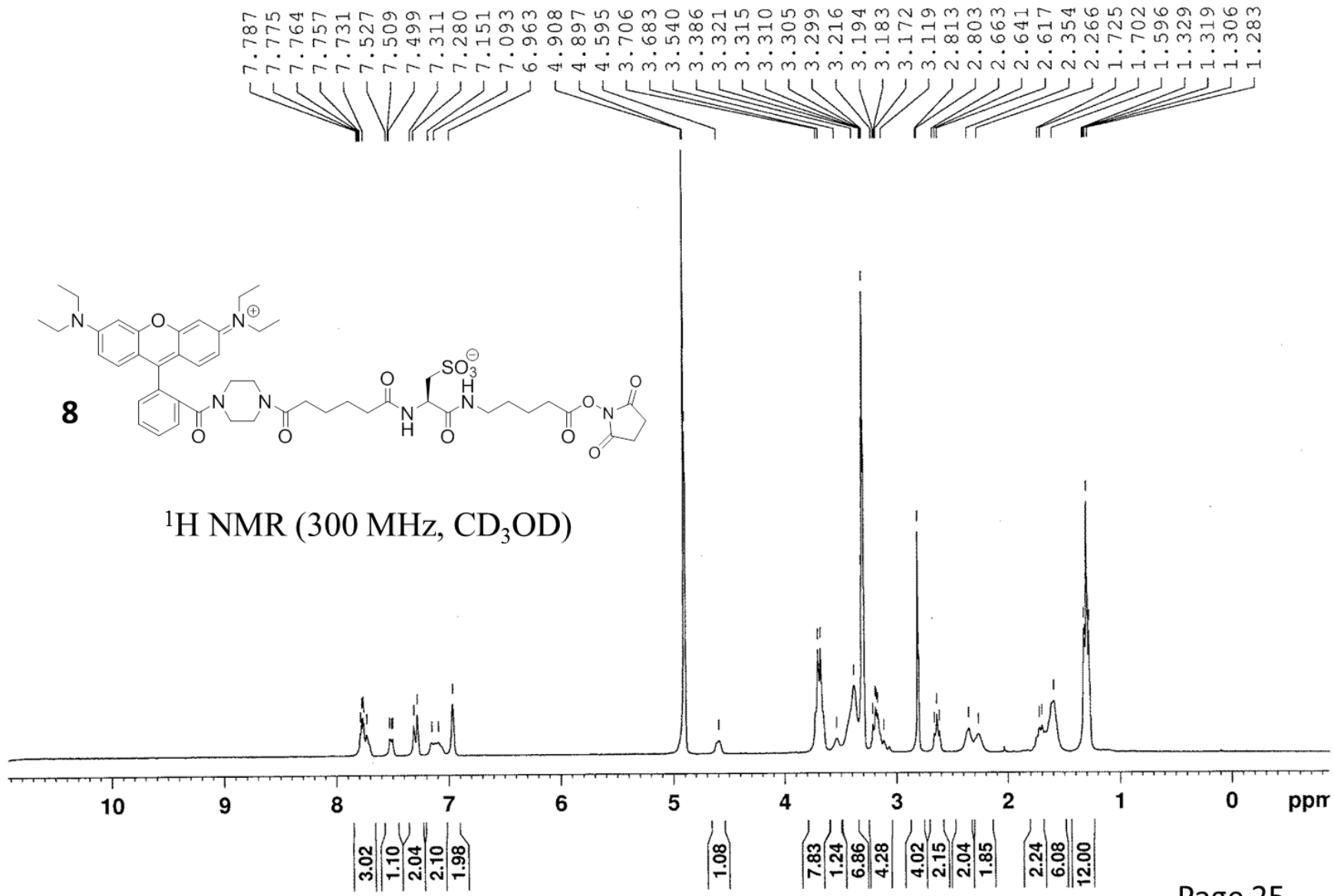


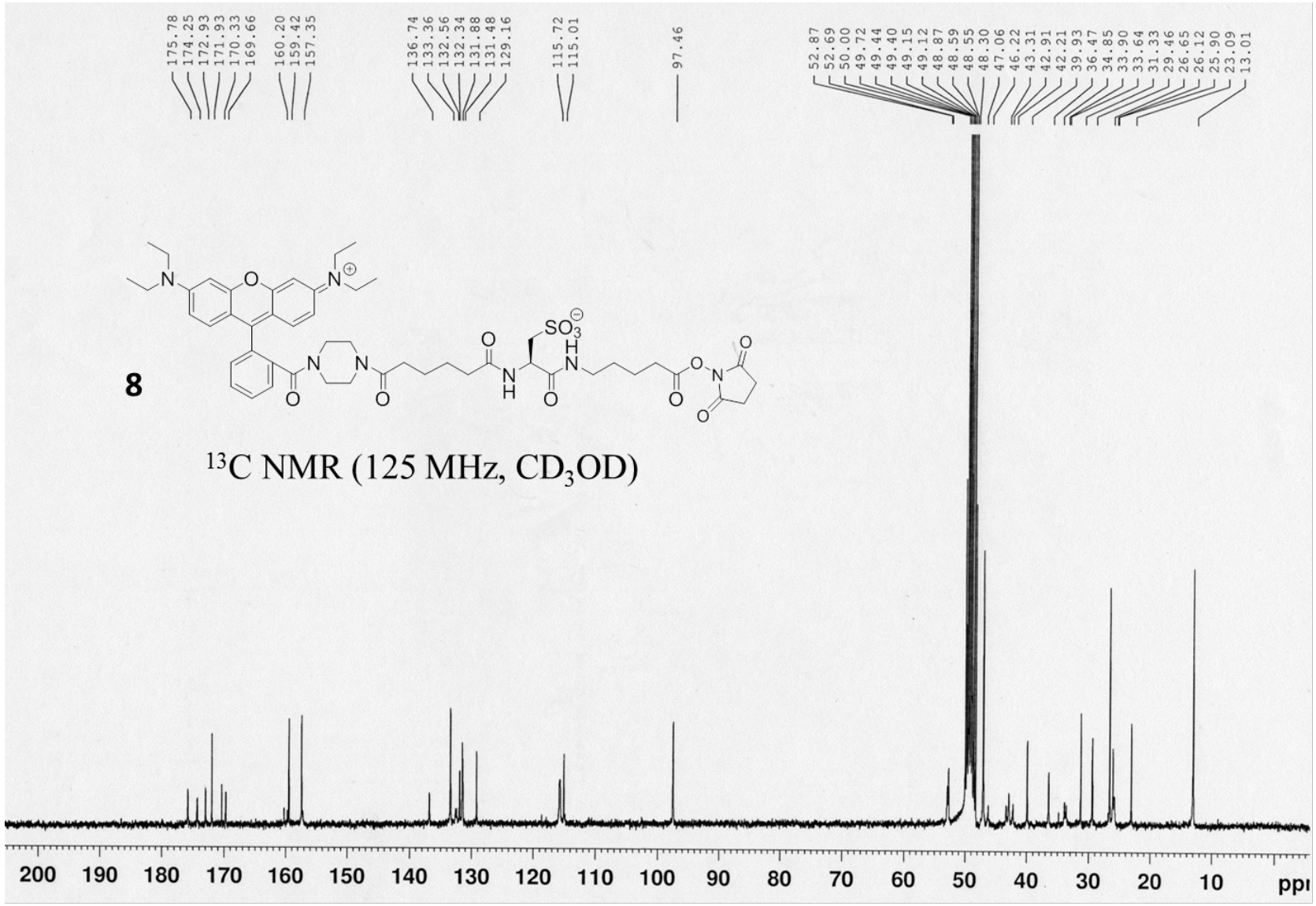
^{1}H NMR (500 MHz, D_2O)

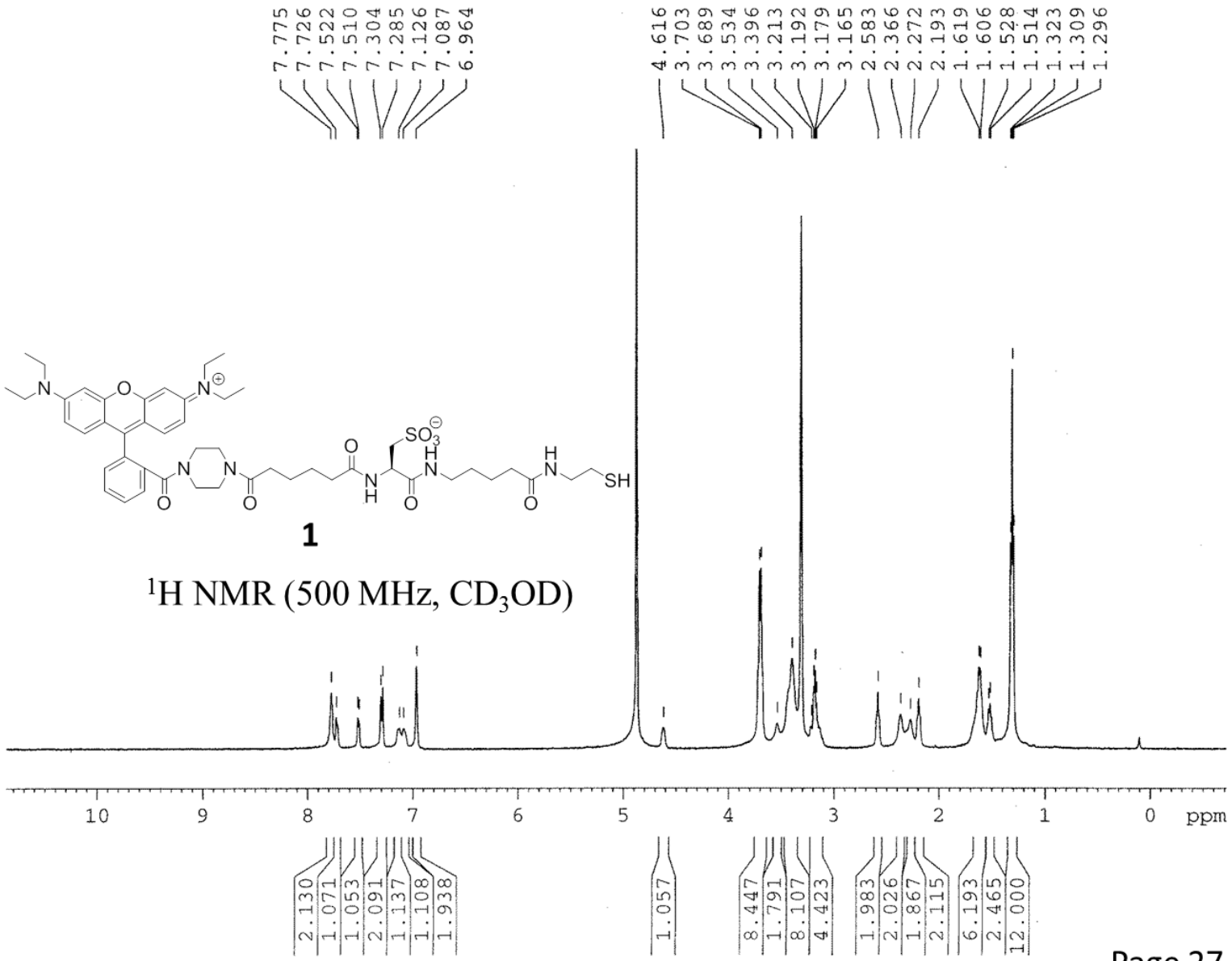

178.57
167.15
50.26
49.95
39.29
33.18
27.42
21.48
6
SO3
H3N
H
N
OH
O
O
13C NMR (125 MHz, D2O)
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

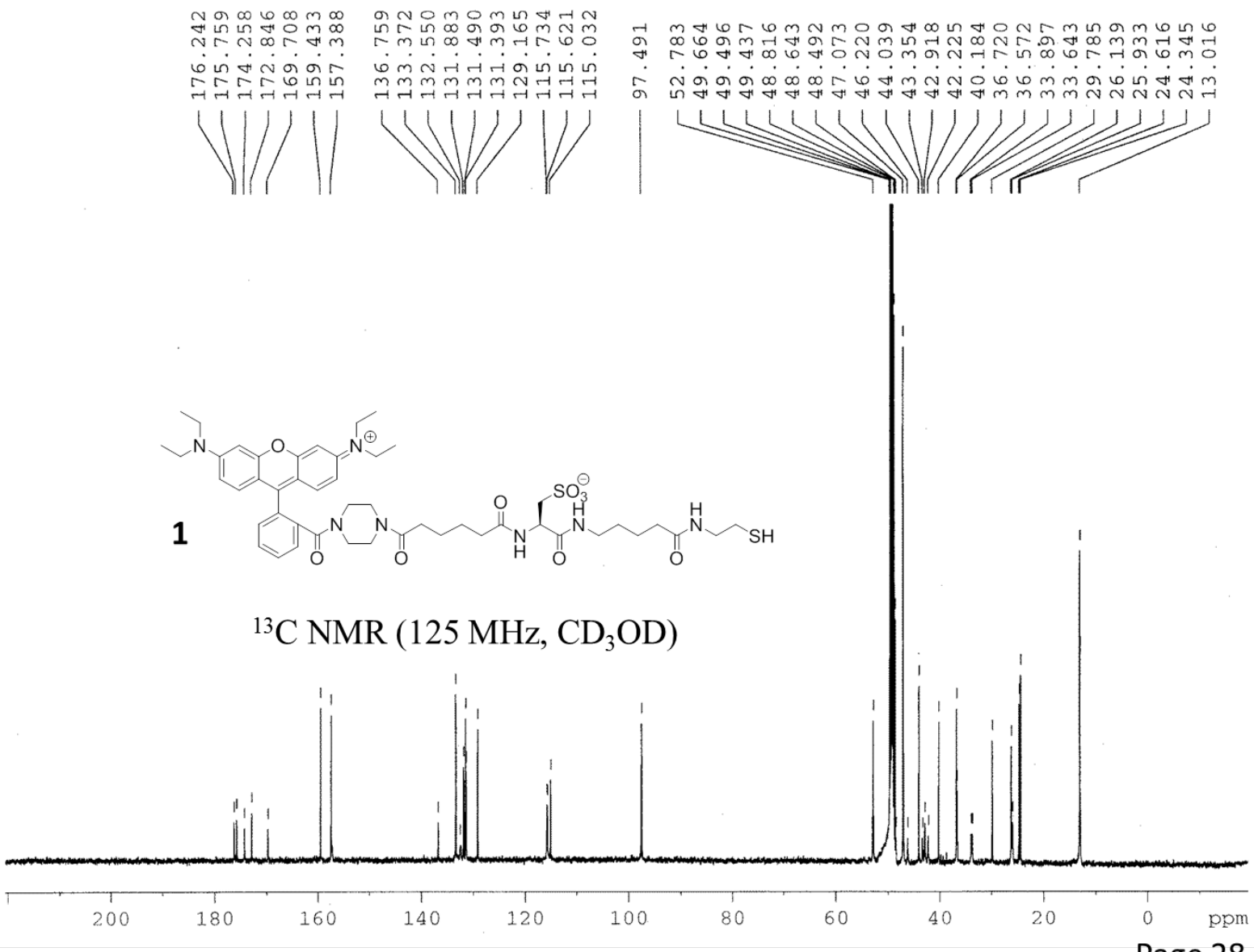

7.775
7.764
7.731
7.527
7.507
7.498
7.312
7.280
7.094
6.966
4.886
4.601
3.730
3.707
3.684
3.660
3.541
3.384
3.315
3.310
3.305
3.190
3.168
3.148
3.120
3.075
2.354
2.313
2.292
2.268
1.937
1.604
1.582
1.521
1.331
1.308
1.284
7
^{1}H NMR (300 MHz, CD_3OD)
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
3.000
1.133
2.054
2.213
2.005
1.263
7.634
1.535
6.775
3.340
6.395
8.422
11.847


177.261
175.784
174.241
172.840
169.672
159.399
157.341
136.722
133.353
132.347
131.875
131.471
131.388
129.150
115.714
115.000
97.466
52.917
52.740
49.662
49.492
49.322
49.152
48.983
48.812
48.641
47.064
43.331
42.855
40.269
36.509
34.602
33.899
33.603
29.843
26.114
25.952
23.400
13.017
7
^{13}C NMR (125 MHz, CD_3OD)
200
180
160
140
120
100
80
60
40
20
0
ppm


7.787
7.775
7.764
7.757
7.731
7.527
7.509
7.499
7.311
7.280
7.151
7.093
6.963
4.908
4.897
4.595
3.706
3.683
3.540
3.386
3.321
3.315
3.310
3.305
3.299
3.216
3.194
3.183
3.172
3.119
2.813
2.803
2.663
2.641
2.617
2.354
2.266
1.725
1.702
1.596
1.329
1.319
1.306
1.283
8
^{1}H NMR (300 MHz, CD_3OD)
10
9
8
7
6
5
4
3
2
1
0
ppm
3.02
1.10
2.04
2.10
1.98
1.08
7.83
1.24
6.86
4.28
4.02
2.15
2.04
1.85
2.24
6.08
12.00


175.78
174.25
172.93
171.93
170.33
169.66
160.20
159.42
157.35
136.74
133.36
132.56
132.34
131.88
131.48
129.16
115.72
115.01
97.46
52.87
52.69
50.00
49.72
49.44
49.40
49.15
49.12
48.87
48.59
48.55
48.30
47.06
46.22
43.31
42.91
42.21
39.93
36.47
34.85
33.90
33.64
31.33
29.46
26.65
26.12
25.90
23.09
13.01
8
^{13}C NMR (125 MHz, CD_3OD)
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppı


7.775
7.726
7.522
7.510
7.304
7.285
7.126
7.087
6.964
4.616
3.703
3.689
3.534
3.396
3.213
3.192
3.179
3.165
2.583
2.366
2.272
2.193
1.619
1.606
1.528
1.514
1.323
1.309
1.296
1
1H NMR (500 MHz, CD3OD)
10
9
8
7
6
5
4
3
2
1
0
ppm
2.130
1.071
1.053
2.091
1.137
1.108
1.938
1.057
8.447
1.791
8.107
4.423
1.983
2.026
1.867
2.115
6.193
2.465
12.000


176.242
175.759
174.258
172.846
169.708
159.433
157.388
136.759
133.372
132.550
131.883
131.490
131.393
129.165
115.734
115.621
115.032
97.491
52.783
49.664
49.496
49.437
48.816
48.643
48.492
47.073
46.220
44.039
43.354
42.918
42.225
40.184
36.720
36.572
33.897
33.643
29.785
26.139
25.933
24.616
24.345
13.016
1
SH
13C NMR (125 MHz, CD3OD)
200
180
160
140
120
100
80
60
40
20
0
ppm

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2. G. C. Campanello, Z. Ma, N. E. Grossoehme, A. J. Guerra, B. P. Ward, R. D. Dimarchi, Y. Ye, C. E. Dann and D. P. Giedroc, *J. Mol. Biol.,* 2013 **425**, 1143-57.

3. D. L. Williams, *Acc. Chem. Res*., 1999, **32**, 869-876.