Supporting information

Detection of GNAQ Mutations and Reduction of Cell Viability in Uveal Melanoma Cells with Functionalized Gold Nanoparticles.

Christian Posch[a,b,§], Alfonso Latorre[c, §], Michelle B. Crosby[d], Anna Celli[a], Ana Latorre[c], Igor Vujic[a,b], Martina Sanlorenzo[a,e], Gary A. Green[a], Jingly Weier[a], Mitchel Zekhtser[a], Jeffrey Ma[a], Gabriela Monico[a], Devron H. Char[f], Denis Jusufbegovic[f], Klemens Rappersberger[b], Álvaro Somoza[b, §], Susana Ortiz-Urda[a, §,*].

[a] University of California San Francisco, Department of Dermatology, Mount Zion Cancer Research Center, 2340 Sutter Street N461, 94115, San Francisco, United States.

[b] The Rudolfstiftung Hospital, Department of Dermatology, Juchgasse 25, 1030 Vienna, Austria

[c] Instituto Madrileño de Estudios Avanzados en Nanociencia (IMDEA Nanociencia), & CNB-CSIC-IMDEA Nanociencia Associated Unit "Unidad de Nanobiotecnología", 28049 Madrid, Spain

[d] North County Eye Surgery Associates, 320 Santa Fe Drive Suite 104, Encinitas, 92024, United States.

[e] Department of Medical Sciences, Section of Dermatology, University of Turin, Via Cherasco 23, 10100, Torino, Italy

[f] Departments of Ophthalmology, Stanford University, University of California San Francisco and California Pacific Medical Center, 45 Castro Street, 94114 San Francisco, United States.

§ Authors contributed equally to this work.

* To whom correspondence should be addressed:

Susana Ortiz-Urda MD PhD, University of California San Francisco, Department of Dermatology, Mount Zion Cancer Research Center, 2340 Sutter Street N419, CA-94115, San Francisco, United States

E-mail: ortizsm@derm.ucsf.edu, phone: +1 (415) 476 8502, fax: +1 (415) 476 88373

CONTENTS:

1. Supporting figures

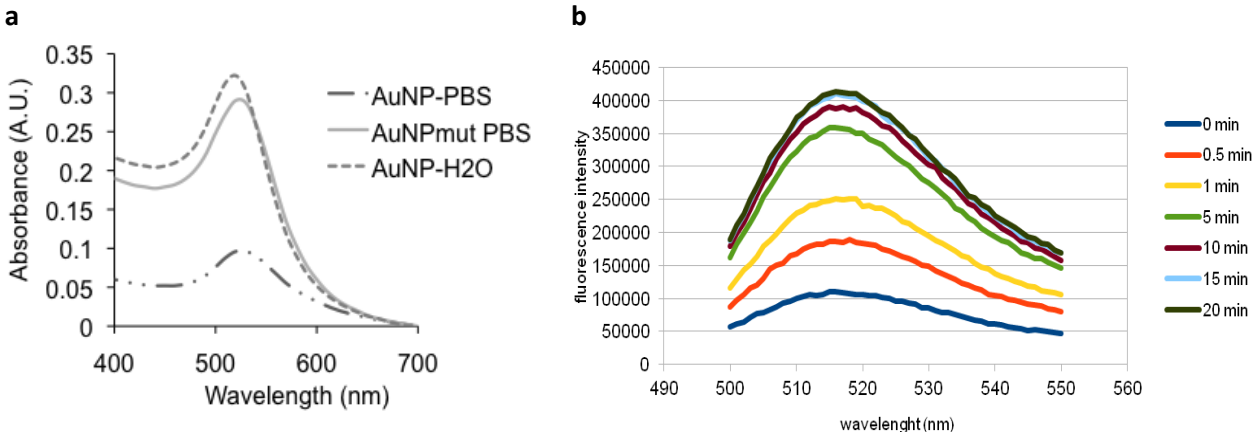
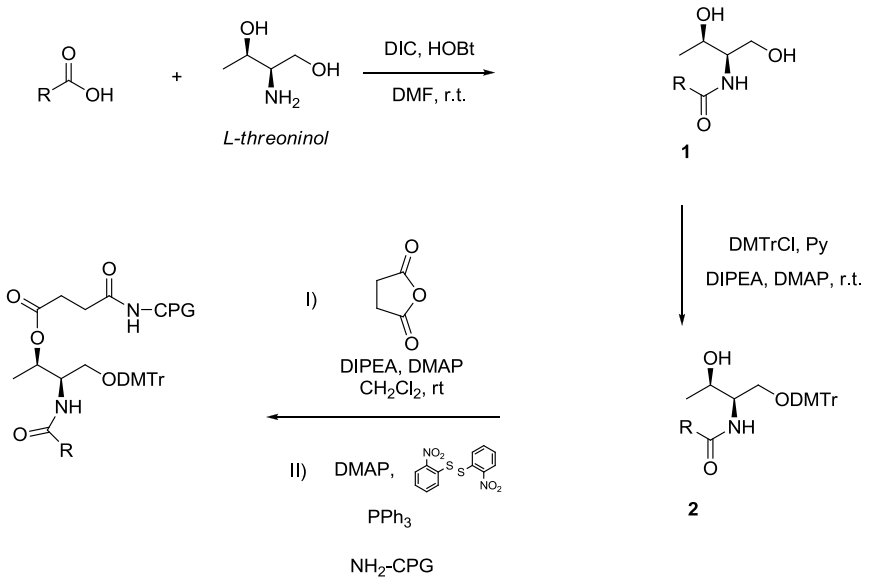


Fig. S1a UV-VIS spectra of functionalized AuNPs in PBS. The plasmon band at 520 nm revealed that they remain stable after modification with oligonucleotides. **b** Peak fluorescence of functionalized AuNPs at 518nm after 20min incubation with the matching mRNA sequence.

2. General.[1]H and [13]C NMR spectra were recorded in $CDCl_3$ at 300 and 75 MHz, respectively. All reactions were monitored by thin layer chromatography that was performed on precoated sheets of silica gel 60, and flash column chromatography was done with silica gel 60 (230-400 mesh) of Merck. Eluting solvents are indicated in the text. Solvents were dried over 4Å molecular sieves. All other reagent were purchased from Aldrich and used without further purification. The UV/VIS and fluorescence spectra were recorded at room temperature with the Synergy H4 microplate reader. Ultrapure reagent grade water (18.2MΩ, Wasserlab), was used in all experiments.

3. Synthesis of the modified solid supports $CPG^{Dithiolane}$ and CPG^{Methyl}.

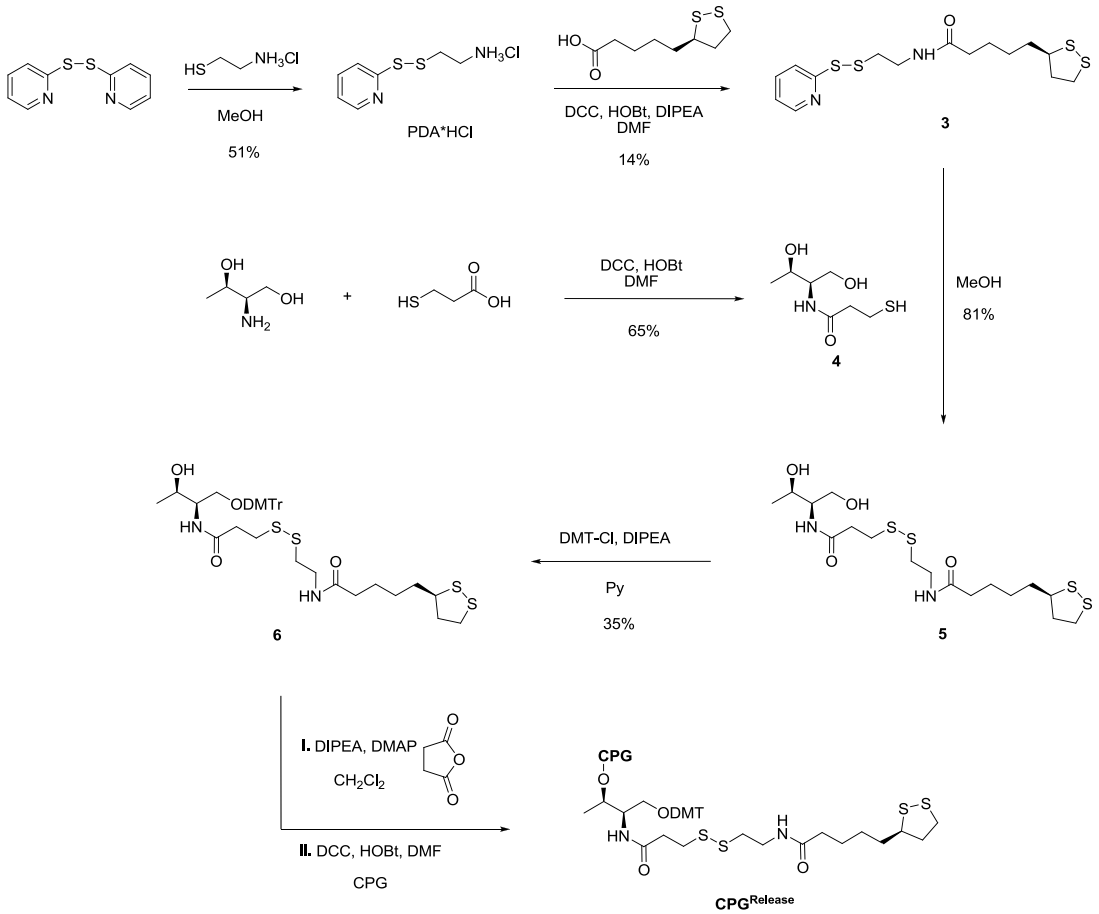


The synthesis of **$CPG^{Dithiolane}$** and **CPG^{Methyl}** were achieved following the protocol reported in our previous work starting from lipoic acid[1] and acetic acid[2] respectively. Briefly, corresponding acid and L-threoninol were coupled in the presence of DIC and HOBt, to give the amide **1**. The primary alcohol of **1**, was protected with DMTCl in the presence of DIPEA and DMAP to give compound **2**. Finally, the secondary alcohol was attached to the CPG in two consecutives steps: Firstly, a carboxyl acid moiety was introduced by the reaction of the secondary alcohol and succinic anhydride using DIPEA and DMAP. Finally, resulted compound

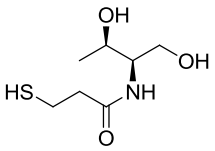
[1]. Latorre A, Posch C, Garcimartín Y, Ortiz-Urda S and Somoza Á. Single-point mutation detection in RNA extracts using gold nanoparticles modified with hydrophobic molecular beacon-like structures. Chem. Commun., 2014 Oct 13; 50: 3018-20.
[2]. Somoza Á, Terrazas M, Eritja R, Chem. Commun. Modified siRNAs for the study of the PAZ domain. 2010 Feb 16; 46: 4270-72.

was attached to the CPG trough an amide bond formed in the presence of DMAP, DTNP and PPh_3.

4. Synthesis of the modified solid support bearing the "release" modification ($CPG^{Release}$).



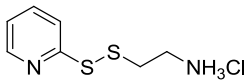
***N*-((2*R*,3*R*)-1,3-dihydroxybutan-2-yl)-3-mercaptopropanamide (3).**



To a stirred mixture of 3-mercaptopropionic acid (100 mg, 0.94 mmol), *N*-hydroxybenzotriazole (135 mg, 1 mmol) and *N*,*N*′-Dicyclohexylcarbodiimide (206 mg, 1 mmol) in DMF (3 mL) under N_2, L-threoninol (100 mg, 0.94 mmol) was added at room temperature. After 16 h the reaction mixture was quenched with MeOH and the solvent evaporated in vacuum. To the residue 30 mL of $CH_2Cl_2$was added, and the solid filtered off. After solvent evaporation and

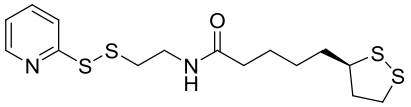
flash chromatography (eluent CH_2Cl_2/MeOH 15:1) compound **3** was obtained as a colorless oil, in 65% yield; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.55 (s, 1H), 4.17 (qd, *J* = 6.1, 2.0 Hz, 1H), 4.03 – 3.64 (m, 3H), 3.02 – 2.67 (m, 2H), 2.57 (t, *J* = 6.6 Hz, 2H), 1.63 (t, *J* = 8.3 Hz, 1H), 1.20 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (75MHz, $CDCl_3$) δ172.2, 67.7, 63.9, 55.1, 40.3, 29.6, 20.5;MS (ESI):*m/z* (%) 176 (M^+-OH, 11), 194 (M^++1, 10), 216 (M^++Na, 100); HRMS (ESI) calcd for $C_7H_{15}NO_3S$ (M^++1) 216.0660, found 216.0664.

2-(Pyridyldithio)-ethylaminehydrochloride(PDA*HCl).



PDA*HCl was synthesized as reported[3] with some modifications. To a stirred solution of aldrithiol (213 mg, 0.96 mmol) in MeOH (1.1 mL), 2-mercaptoethylamine hydrochloride (109 mg, 0.96 mmol was added. After stirring 1h, the solvent was evaporated and the residue washed with cold AcOEt three times. PDA*HCl was obtained as a white solid in 51% yield: ^{1}H NMR (300 MHz, d^6-CD_3OD)δ8.57 (d, *J* = 5.0 Hz, 1H), 7.83 (t, *J* = 7.7 Hz, 1H), 7.69 (d, *J* = 8.0 Hz, 1H), 7.35 (dd, *J* = 7.5, 5.0 Hz, 1H), 3.18 (t, *J* = 6.1 Hz, 2H), 3.07 (t, *J*= 6.8 Hz, 2H); MS (ESI):*m/z* (%) 107 (100), 153 (79), 187 (M^+-Cl, 12); HRMS (ESI) calcdfor $C_7H_{11}NS_2$(M^++1) 187.0366, found187.0391.

(*R*)-5-(1,2-dithiolan-3-yl)-N-(2-(pyridin-2-yldisulfanyl)ethyl)pentanamide (4).



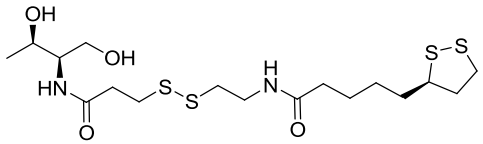
To a stirred mixture of (*R*)-(+)-α-Lipoic acid (134mg, 0.65 mmol), *N*-hydroxybenzotriazole (96 mg, 0.71 mmol) and *N*,*N*′-Dicyclohexylcarbodiimide (147, 0.71 mmol) in DMF (1.7 mL) under N_2, PDA*HCl (145 mg, 0.65 mmol) and DIPEA (147 μL, 0.71 mmol) were added at room temperature. After 16 h the reaction mixture was quenched with MeOH and the solvent evaporated in vacuum. To the residue 30 mL of CH_2Cl_2 was added, and the solid filtered off.

---

3 Zugates GT, Anderson DG, Little S R,. Lawhorn IEB, Langer R. Synthesis of Poly(β-amino ester)s with Thiol-Reactive Side Chains for DNA Delivery. J. Am. Chem. Soc. 2006 Sep 12; 128 (39): 12726-12734.

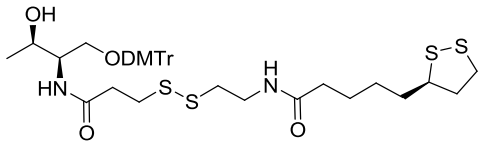
After solvent evaporation and flash chromatography (eluent CH_2Cl_2/AcOEt 1:1) compound **4** was obtained as a colorless oil, in 44% yield;^{1}H NMR (300 MHz, $CDCl_3$) δ 8.48 (dd, *J* = 3.7, 1.1 Hz, 1H), 7.70 – 7.56 (m, 1H),7.51 (d, *J* = 8.0 Hz, 1H),7.23 – 7.08 (m, 2H),3.64 – 3.46 (m, 3H),3.22 – 3.02 (m, 2H),2.96 – 2.85 (m, 2H),2.43 (dq, *J* = 12.5, 6.4 Hz, 1H),2.21 (t, *J* = 7.4 Hz, 2H),1.97 – 1.80 (m, 1H),1.78 – 1.57 (m, 5H),1.54 – 1.38 (m, 2H);^{13}C NMR (75MHz, $CDCl_3$)δ172.9, 159.2, 149.7, 137.1, 121.4, 121.2, 95.7, 77.5, 77.1, 76.7, 56.4, 40.3, 39.0, 38.5, 37.3, 36.6, 34.7, 29.0, 25.4; MS (ESI):*m/z* (%) 225 (52), 264 (10), 375 (M^++1, 100); HRMS (ESI) calcdfor $C_{15}H_{23}N_2S_4$(M^++1) 375.0675, found375.0687.

***N*-(2-((3-((2*R*,3*R*)-1,3-dihydroxybutan-2-ylamino)-3-oxopropyl)disulfanyl)ethyl)-5-((*R*)-1,2-dithiolan-3-yl)pentanamide (5).**



To a solution of disulfide **4** (45 mg, 0.12 mmol) in MeOH (1mL) under N_2, a solution of thiol **3** (23mg, 0.12 mmol) in MeOH (1 mL) was added and stirred for 2 h. The solvent was evaporated and the residue purified by flash chromatography (CH_2Cl_2/MeOH 20:1) to obtain compound **5** as colorless oil in 88% yield; ^{1}H NMR (300 MHz, $CDCl_3$)δ6.88 (d, *J* = 8.3 Hz, 1H), 6.61 (t, *J* = 5.6 Hz, 1H), 4.23 – 4.03 (m, 1H), 3.91-3.74 (m, 5H), 3.62 – 3.47 (m, 3H), 3.02 (t, *J* = 6.8 Hz, 2H), 2.83 (t, *J* = 6.5 Hz, 2H), 2.68 (t, *J* = 6.7 Hz, 2H), 2.45 (dq, *J* = 12.4, 6.4 Hz, 1H), 2.22 (t, *J* = 7.4 Hz, 2H), 1.90 (dq, *J* = 13.7, 6.9 Hz, 1H), 1.78 – 1.55 (m, 5H), 1.45 (m, 3H), 1.19 (d, *J* = 6.4 Hz, 3H).^{13}C NMR (75 MHz, CDCl3) δ 173.9, 171.9, 68.6, 64.6, 56.4, 55.1, 40.2, 38.6, 38.5, 38.3, 36.5, 36.3, 34.9, 34.5, 28.8, 25.3, 20.4; MS (ESI):*m/z* (%) 301 (13), 457 (M^++1, 14),479 (M^++Na, 100); HRMS (ESI) calcd for $C_{17}H_{33}N_2O_4S_4$(M^++1) 457.1334, found 457.1317; HRMS (ESI) calcd for $C_{17}H_{32}N_2O_4NaS_4$ (M^+) 479.1166, found 479.1137.

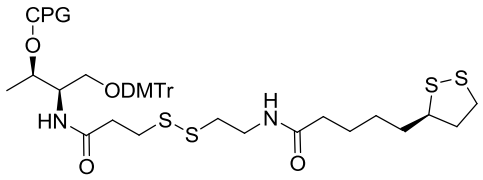
***N*-(2-((3-((2*R*,3*R*)-1-(bis(4-methoxyphenyl)(phenyl)methoxy)-3-hydroxybutan-2-ylamino)-3-oxopropyl)disulfanyl)ethyl)-5-((*R*)-1,2-dithiolan-3-yl)pentanamide (6).**



To a solution of compound **7** (74 mg, 0.16 mmol) in pyridine (0.8 mL) at 0 ºC, DIPEA (43 µL, 0.24 mmol) and 4,4'-dimethoxytrithylchloride (64 mg, 0.19 mmol) were added. The mixture was stirred and allowed to reach room temperature slowly. After 16 h, the solvent was

evaporated and the residue purified by flash chromatography (eluent Hex/AcOEt 1:9 using silica gel deactivated with Et_3N) to obtain compound **6** in 35% yield as a yellow solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ7.39 – 7.27 (m, 2H), 7.27 – 7.11 (m, 7H), 6.76 (d, *J* = 8.8 Hz, 4H), 6.41 – 6.21 (m, 2H), 4.13 – 3.96 (m, 2H), 3.95 – 3.83 (m, 1H), 3.71 (s, 6H), 3.42-3.51 (m, 3H), 3.28 (ddd, *J* = 36.7, 9.6, 4.1 Hz, 2H), 3.13 – 2.97 (m, 2H), 2.92 (t, *J* = 6.8 Hz, 2H), 2.73 (t, *J* = 6.2 Hz, 2H), 2.57 (td, *J* = 6.9, 2.7 Hz, 2H), 2.45 – 2.26 (m, 1H), 2.10 (t, *J* = 7.4 Hz, 2H), 1.80 (dq, *J* = 13.5, 6.9 Hz, 1H), 1.57 (qd, *J* = 13.2, 11.1, 6.8 Hz, 4H), 1.46 – 1.26 (m, 2H), 1.07 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (75 MHz, CDCl3) δ ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.2, 171.3, 158.7, 144.4, 135.4, 130.0, 128.1, 128.0, 127.0, 113.3, 86.8, 68.4, 64.9, 56.4, 55.3, 53.8, 40.2, 38.5, 38.4, 38.1, 36.5, 36.3, 34.6, 34.0, 28.9, 25.4, 20.1; MS (ESI): *m/z* (%) 303 (100), 781 (M^++Na, 15); HRMS (ESI) calcd for $C_{38}H_{50}N_2O_6S_4$ (M^++Na) 781.2455, found 781.2443.

Solid Support (CPG^{Release}) Preparation.



To a solution of compound **6** (50 mg, 0.067 mmol) in CH_2Cl_2(0.5 mL), succinic anhydride (9.0 mg, 0.087 mmol), DIPEA (17 µL, 0.093 mmol) and DMAP (catalytic amount) were added under N_2 at room temperature. The mixture was stirred during 16 h, washed with water and dried with $MgSO_4$. After solvent evaporation, the residue obtained was dissolved in DMF (1.5 mL), and HBOt (9 mg, 0.067 mmol) and DCC (14 mg, 0.067 mmol) were added. This mixture was added to 500 mg of CPG (500 Å) and stirred during 2h. The solvent was removed and the CPG washed with CH_2Cl_2 and MeOH gently. Once the CPG was dry, 3 mL of a 1:1 mixture of capping reagents used on oligonucleotide synthesis [CAP MIX A: Acetic anhydride (400 µL)/ Py (600 µL)/ THF (500 µL); CAP MIX B: 1-Methylimidazol (400 µL)/ THF (1 mL)] was added and stirred. After 25 min, the modified CPG was washed with MeOH, CH_3CN, and dried.

The CPG loading was calculated using the trityl quantification method. To 10 mg of the modified CPG was added 5 mL of a detritylation solution (3 mL of perchloric acid and 2 mL of EtOH) and stirred during 30 min. Then, 10 µL of the mixture was diluted to 1mL, and the absorbance was measured at 498 nm to quantify the trityl cation. Functionalization (F) was determined by Lambert_Beer law. The extinction coefficient

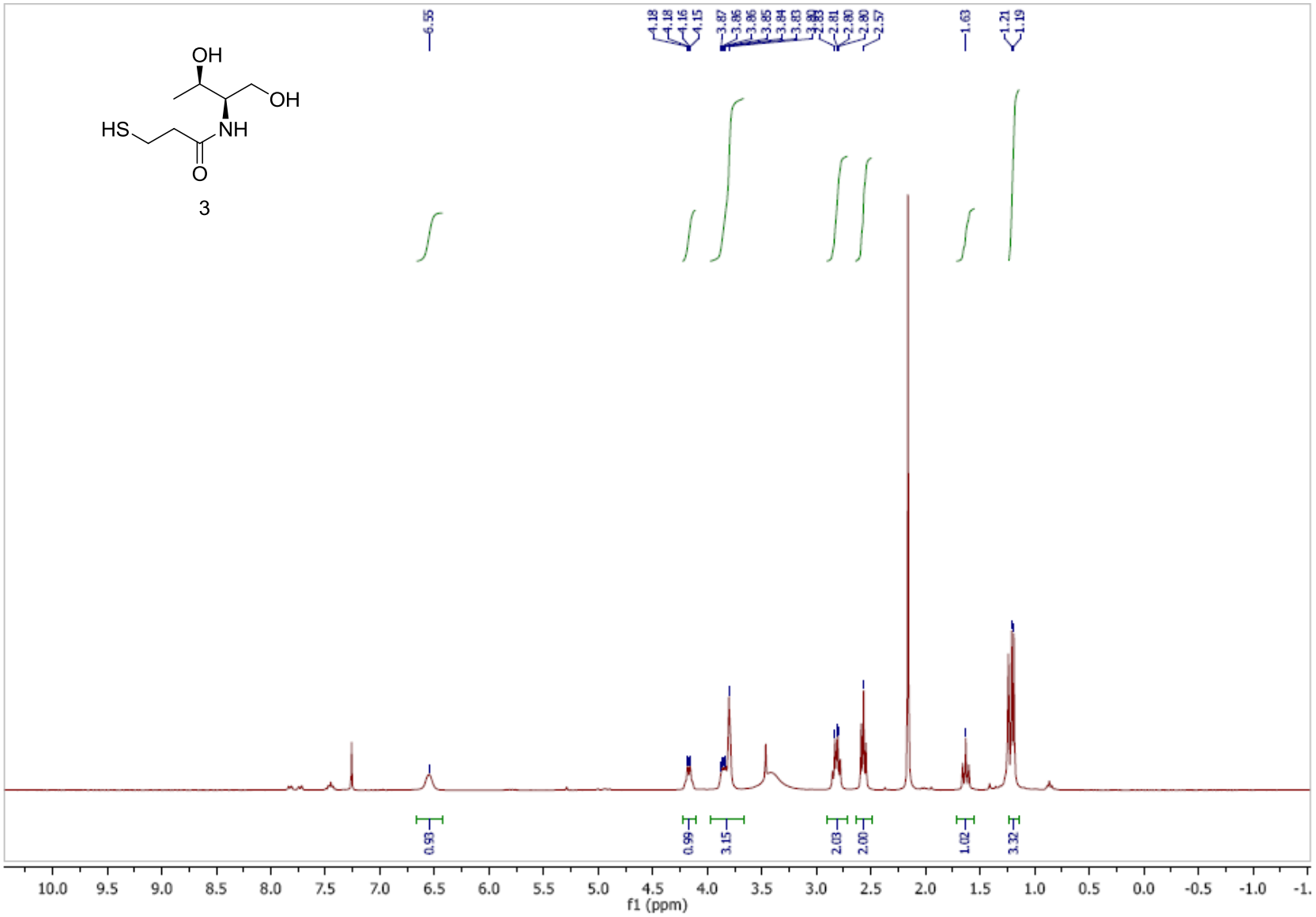
(ε) at this wavelength is 70000 mol^{-1} dm^3 cm^{-1}.The quantity of linker calculated by this method was 11.4 µM/gr.

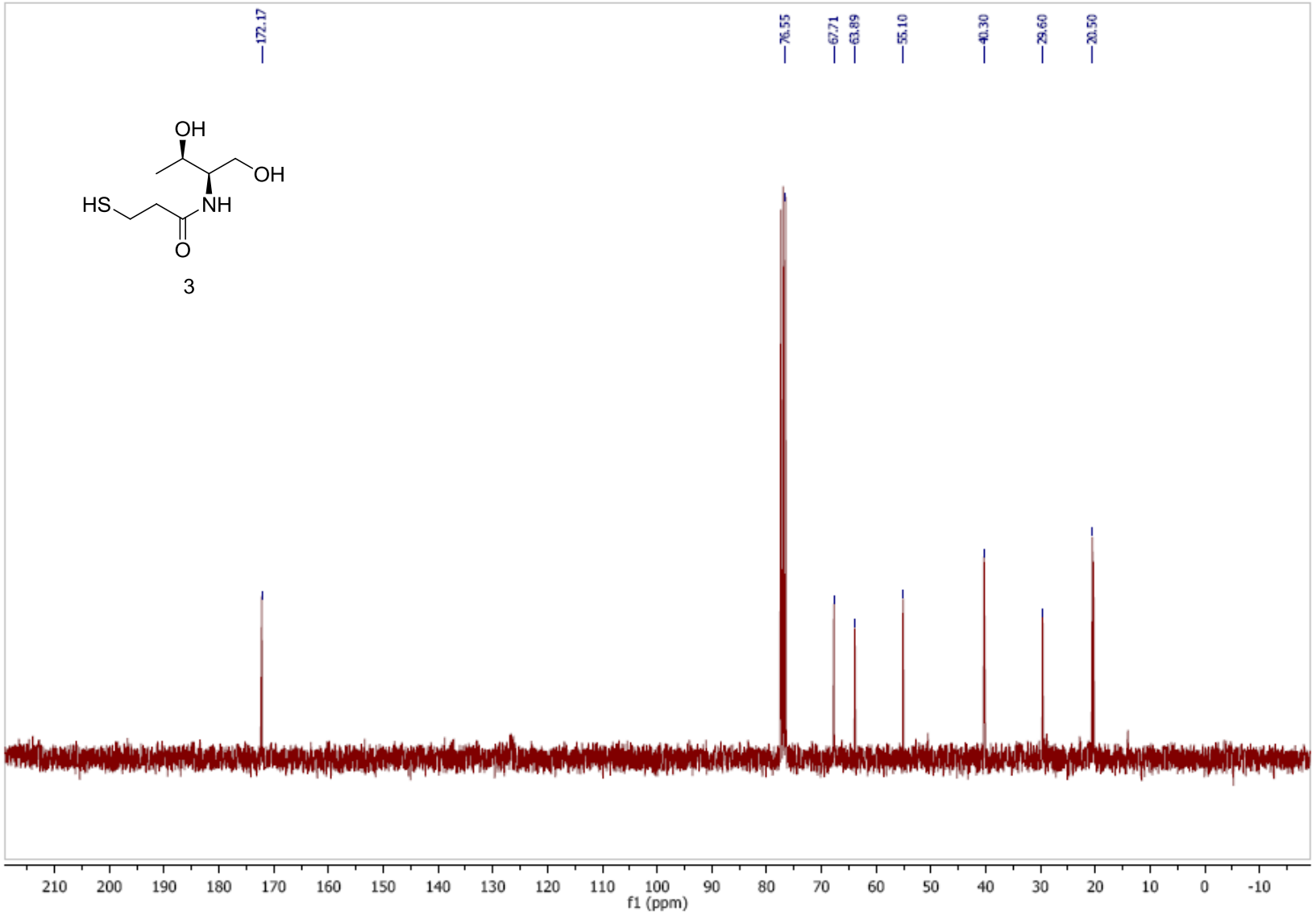
5. Sequences and MALDI data of oligonucleotides

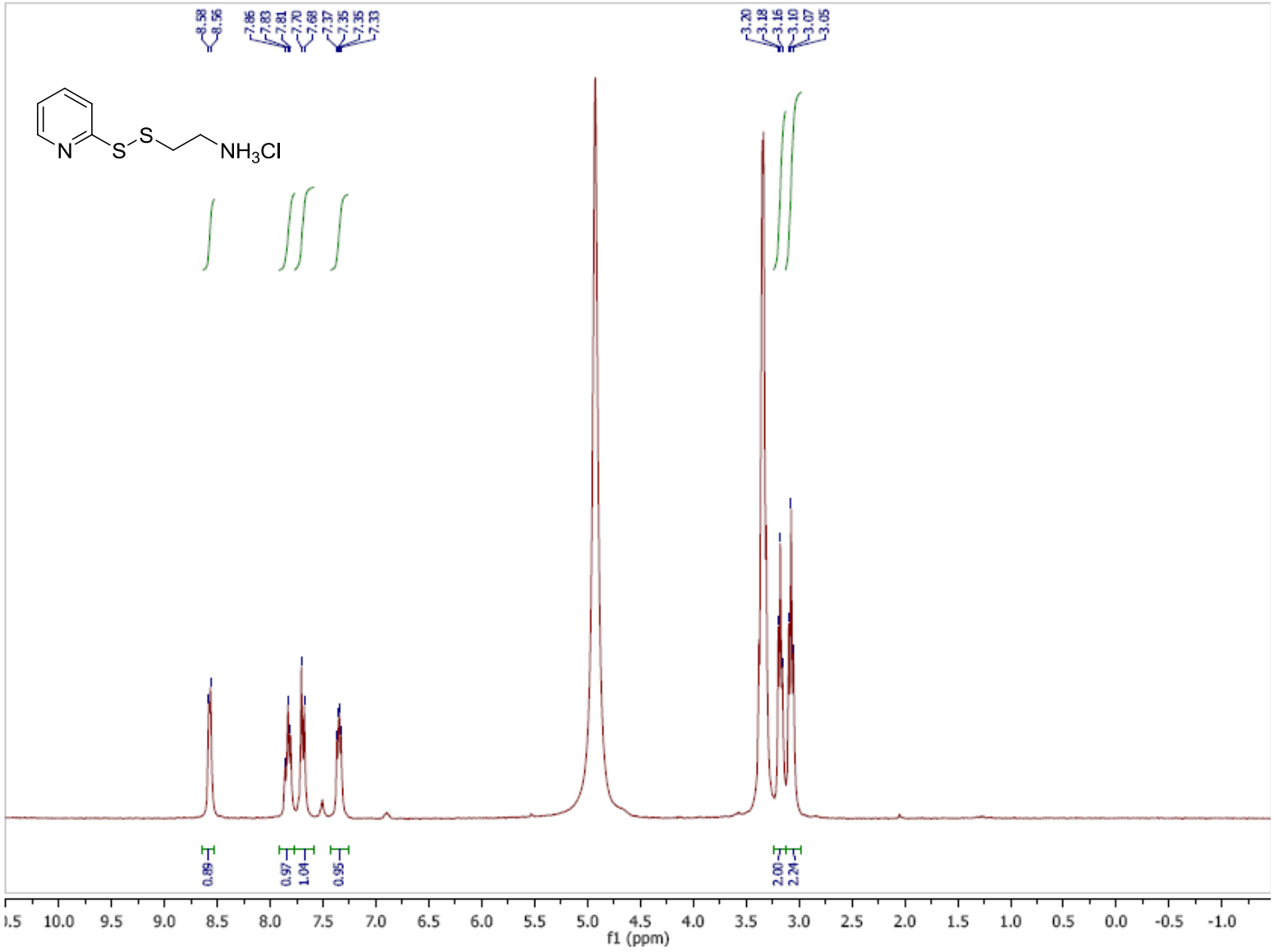
Sequences

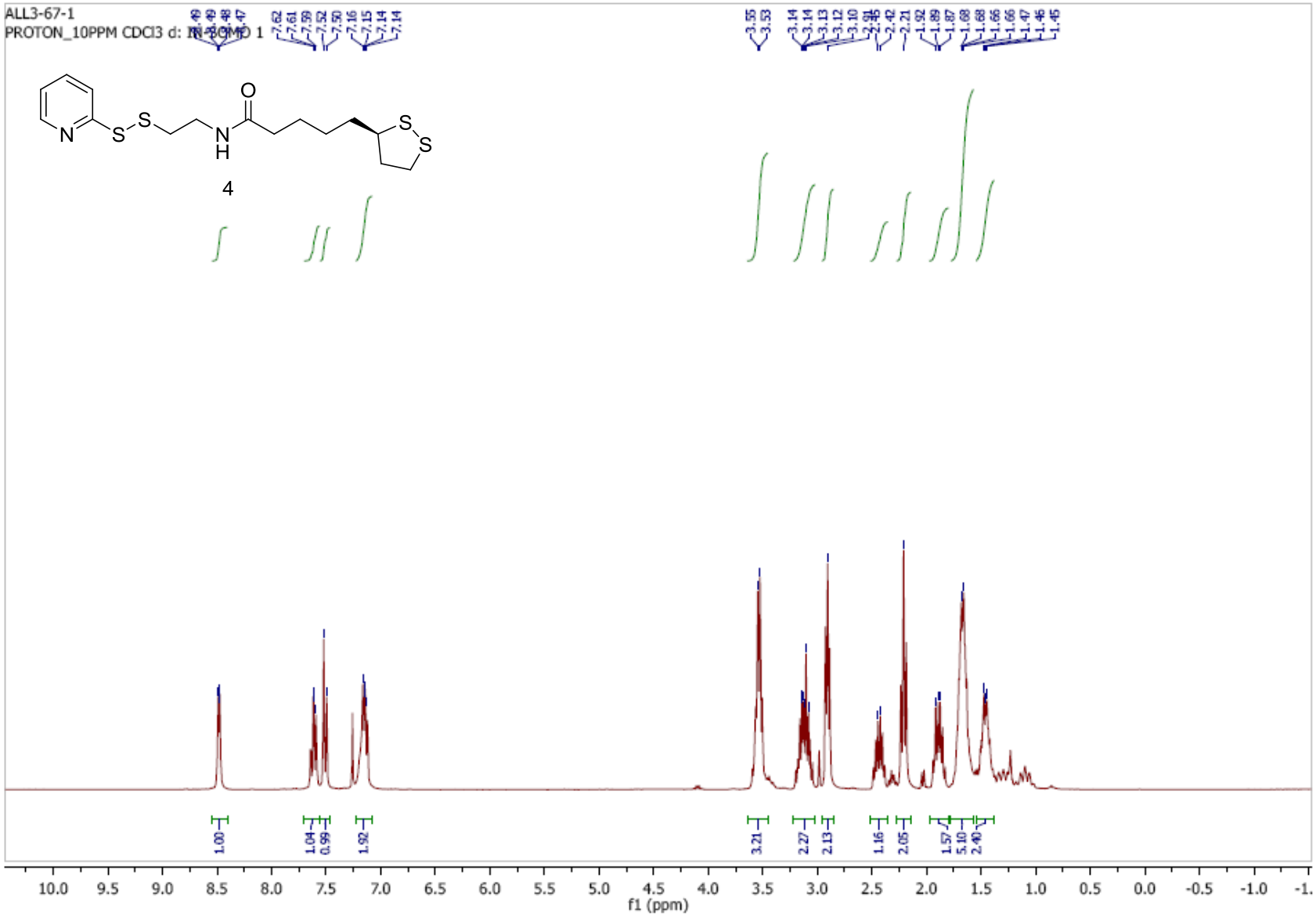
Entry	**Name**	**Sequence 5'-3'**
1	Molecular Beacon	Fluorescein-CCGTCTGACCTTGGGCCCCCGACGGTTTTTT-Dithiolane
2	Taget MUT	GGGGGCCCAAGGTCA
3	Target WT	GGGGGCCAAAGGTCA
4	PolyT-Dithio	Fluorescein-TTTTTTTTTT-Dithiolane
5	PolyT-Rel	Fluorescein-TTTTTTTTTT-Release
6	SiRNA Guide	UAGGGGGCCUAAGGUCAGAT-Methyl
7	SiRNA Passenger	UCUGACCUUAGGCCCCCUATTTTT-Release

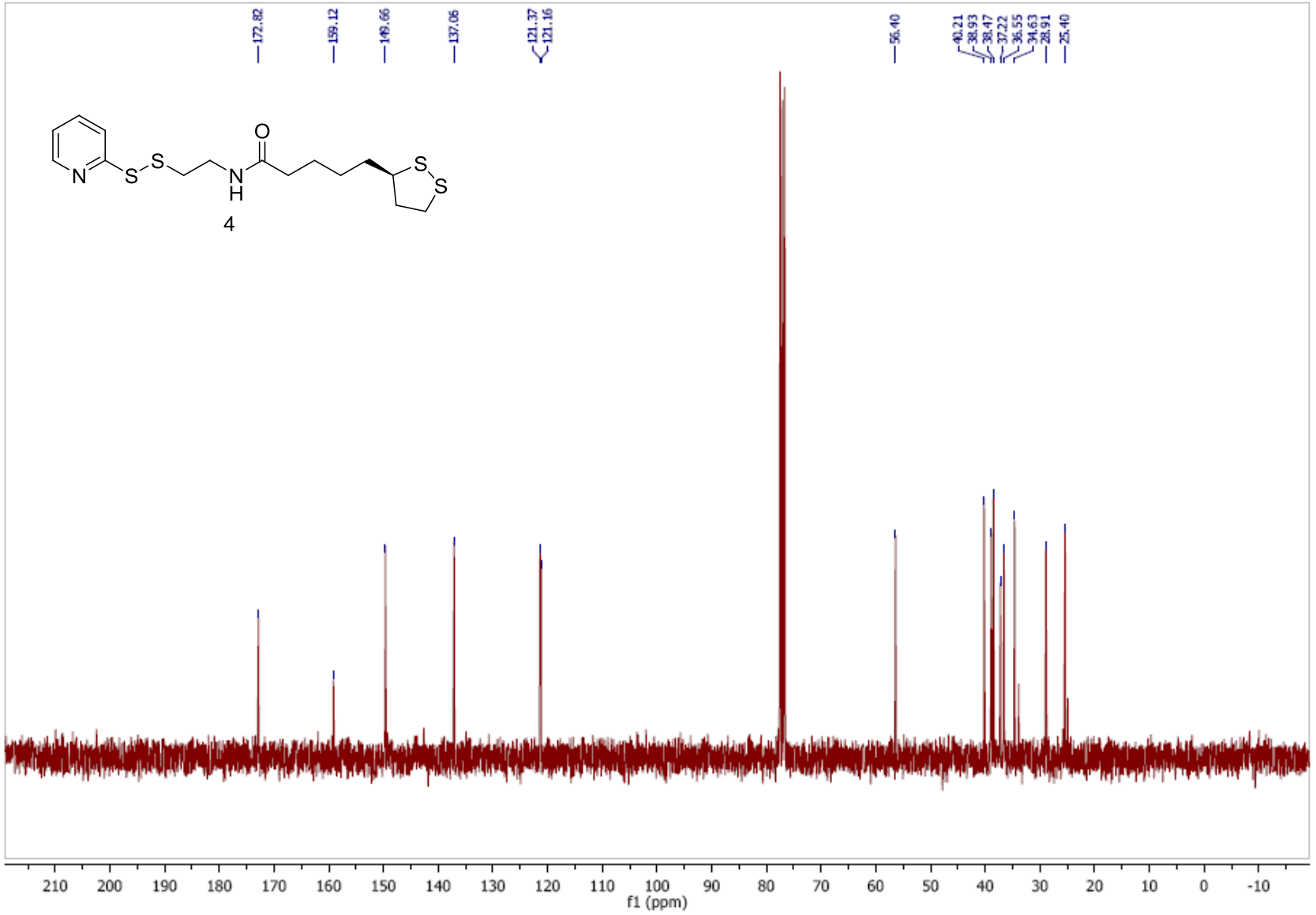
6. NMR SPECTRA

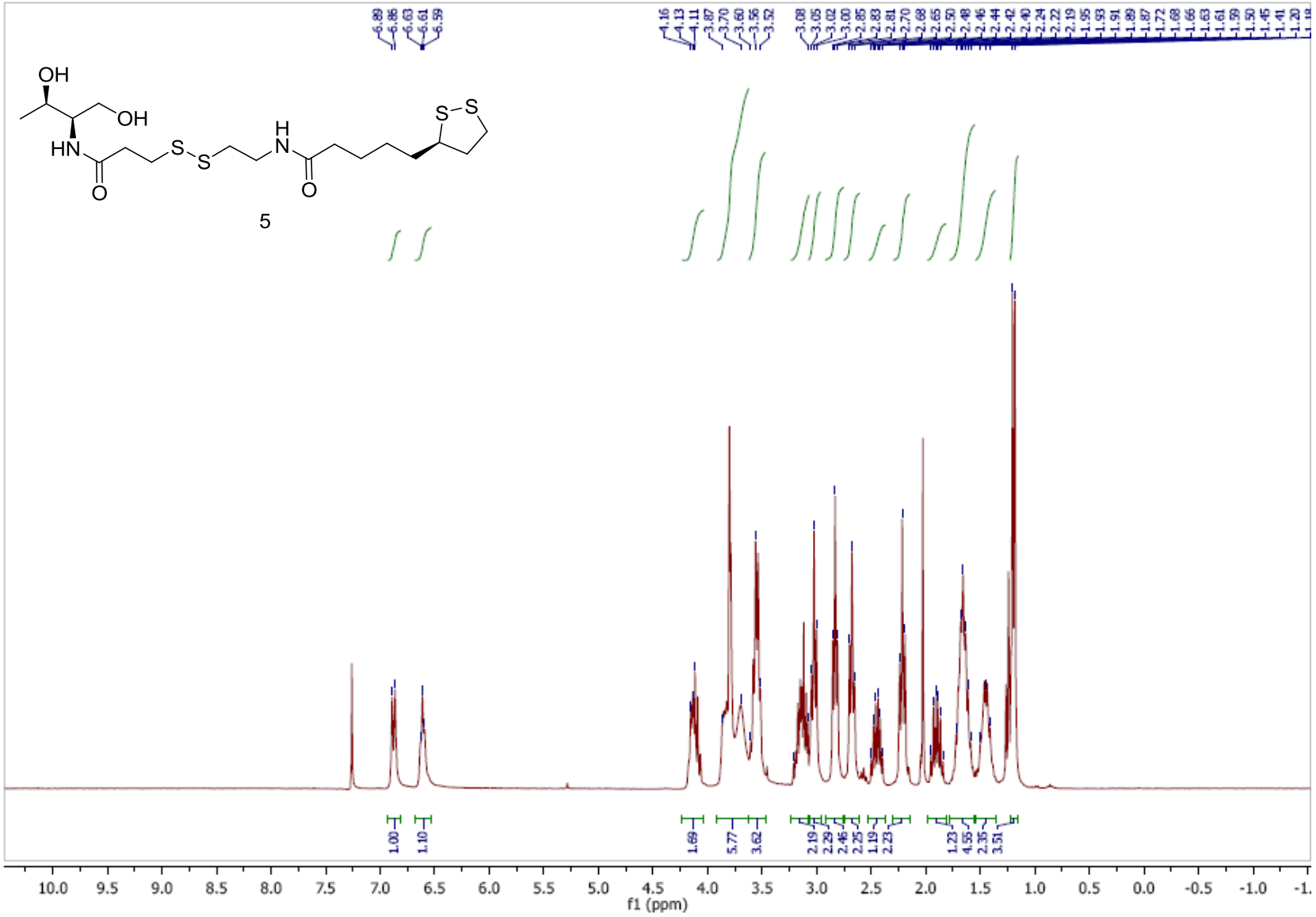

OH
OH
HS
NH
O
3
6.55
4.18
4.18
4.16
4.15
3.87
3.86
3.86
3.85
3.84
3.83
2.81
2.80
2.80
2.57
1.63
1.21
1.19
0.93
0.99
3.15
2.03
2.00
1.02
3.32
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
f1 (ppm)

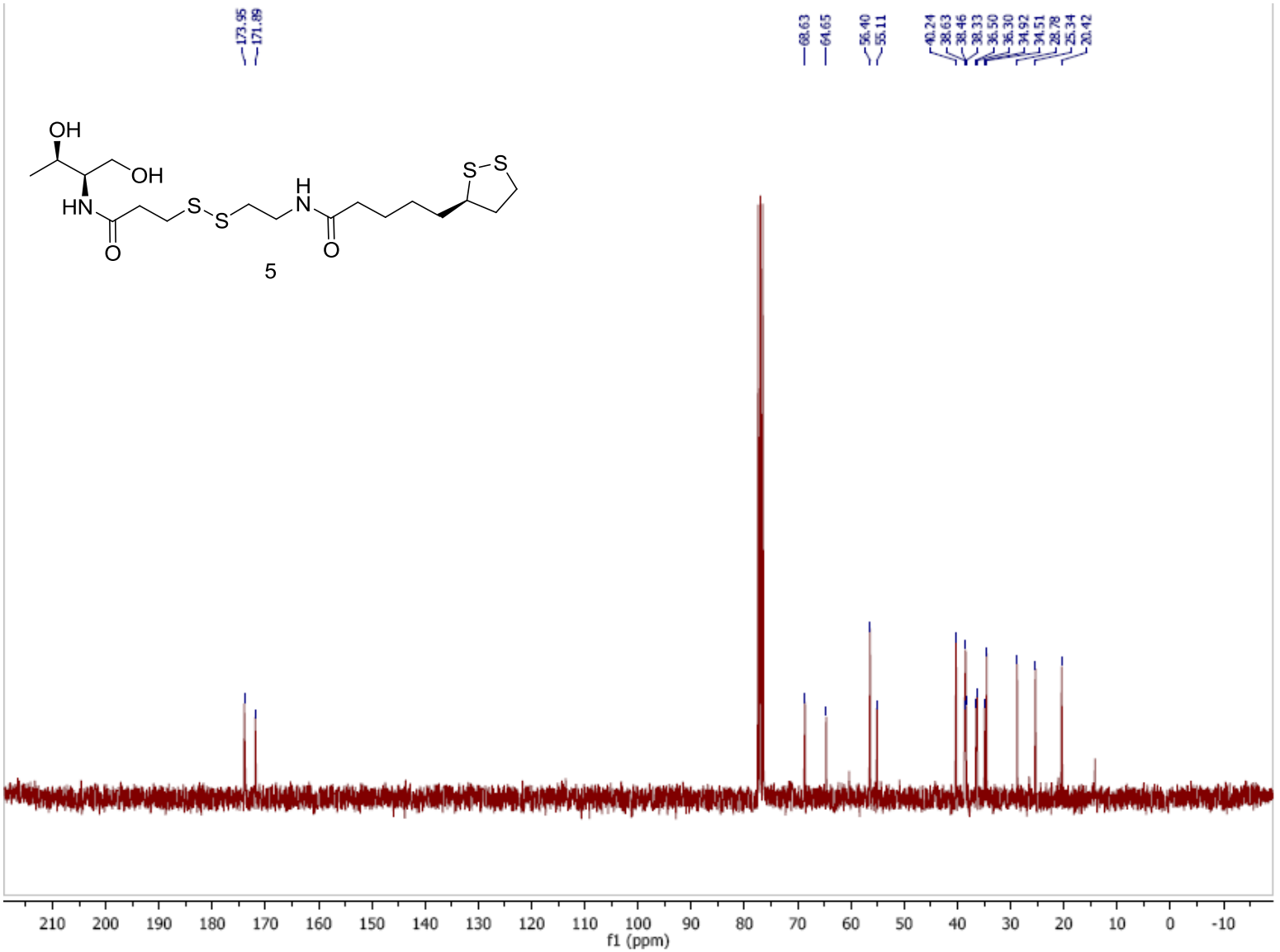

172.17
76.55
67.71
63.89
55.10
40.30
29.60
20.50
OH
OH
HS
NH
O
3
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

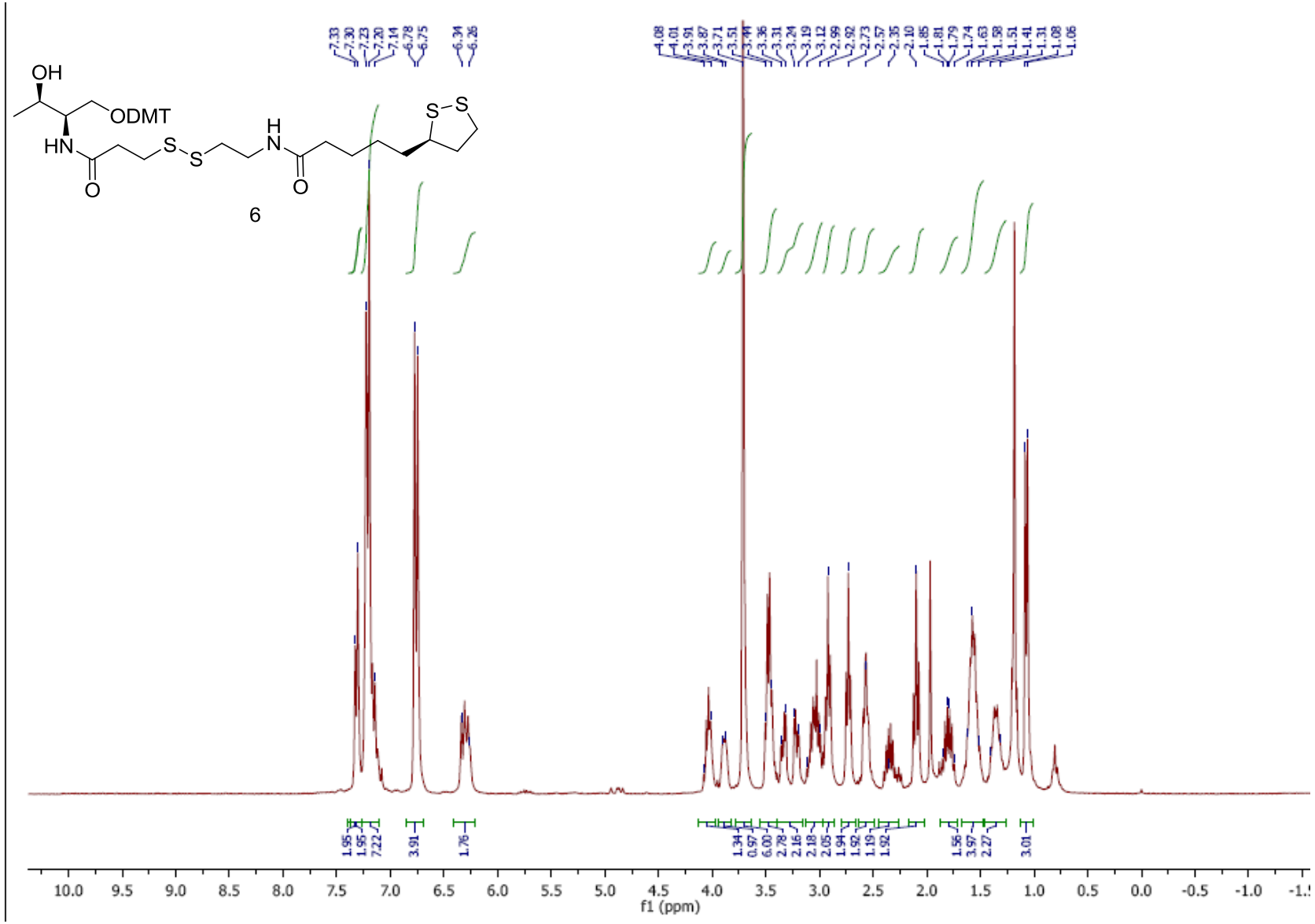

8.58
8.56
7.86
7.83
7.81
7.70
7.68
7.37
7.35
7.35
7.33
3.20
3.18
3.16
3.10
3.07
3.05
N
S
S
NH3Cl
0.89
0.97
1.04
0.95
2.00
2.24
f1 (ppm)

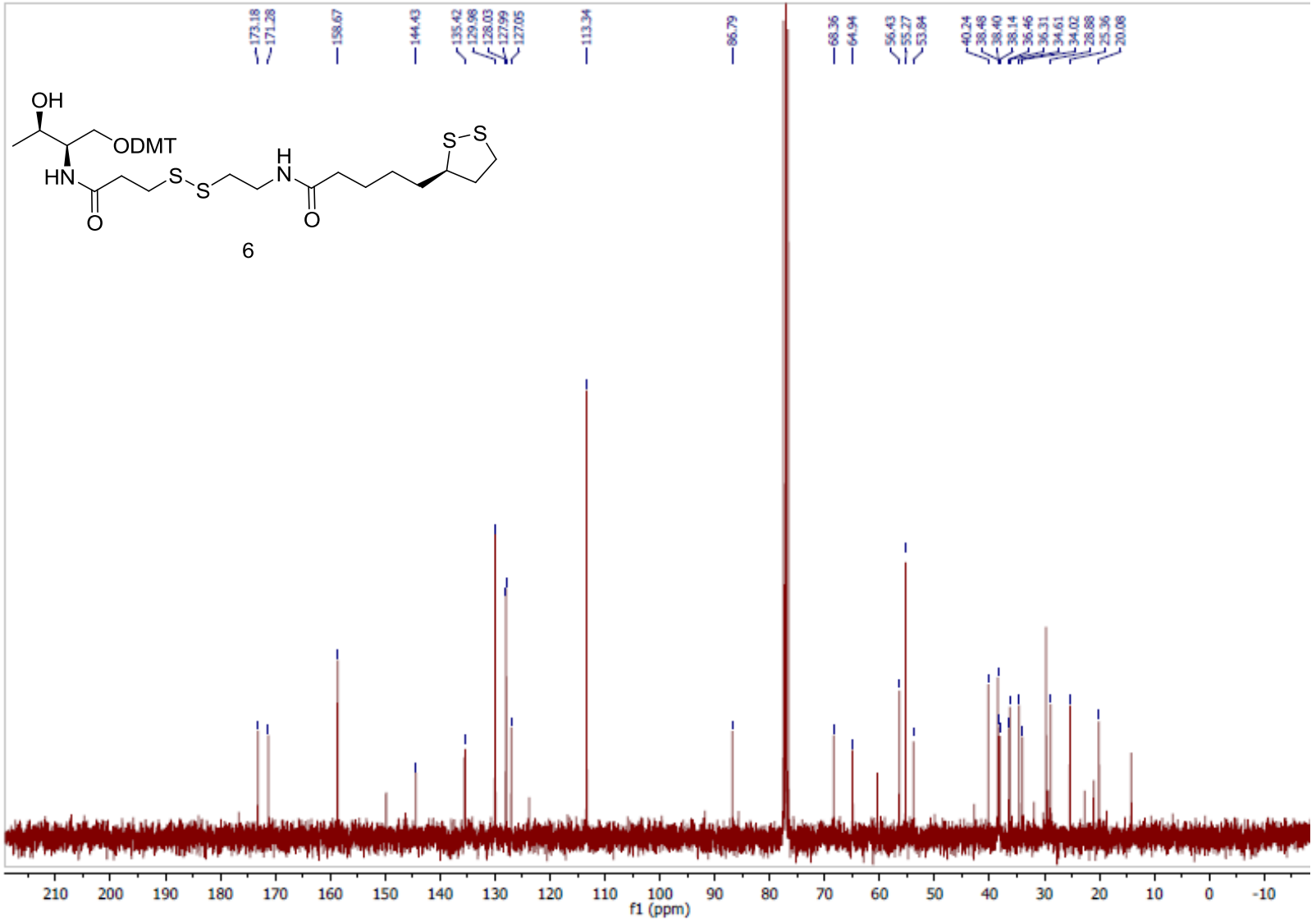

ALL3-67-1
PROTON_10PPM CDCl3 d: 20 SCANS 1
4
f1 (ppm)


172.82
159.12
149.66
137.06
121.37
121.16
56.40
40.21
38.93
38.47
37.22
36.55
34.63
28.91
25.40
O
N
H
S
S
S
S
4
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)


OH
OH
HN
S
S
H
N
S
S
O
O
5
6.89
6.86
6.63
6.61
6.59
4.16
4.13
4.11
3.87
3.70
3.60
3.56
3.52
3.08
3.05
3.02
3.00
2.85
2.83
2.81
2.70
2.68
2.65
2.50
2.48
2.46
2.44
2.42
2.40
2.24
2.22
2.19
1.95
1.93
1.91
1.89
1.87
1.72
1.68
1.66
1.63
1.61
1.59
1.50
1.45
1.41
1.20
1.00
1.10
1.69
5.77
3.62
2.19
2.29
2.46
2.25
1.19
2.23
1.23
4.55
2.35
3.51
10.0
9.5
9.0
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
0.0
-0.5
-1.0
f1 (ppm)


173.95
171.89
68.63
64.65
56.40
55.11
40.24
38.63
38.46
38.33
36.50
36.30
34.92
34.51
28.78
25.34
20.42
OH
OH
HN
O
S
S
H
N
O
S
S
5
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)


OH
ODMT
HN
O
S
S
H
N
O
S
S
6
7.33
7.30
7.23
7.20
7.14
6.78
6.75
6.34
6.26
4.08
4.01
3.91
3.87
3.71
3.51
3.44
3.36
3.31
3.24
3.19
3.12
2.99
2.92
2.73
2.57
2.35
2.10
1.85
1.81
1.79
1.74
1.63
1.58
1.51
1.41
1.31
1.08
1.06
1.95
1.95
7.22
3.91
1.76
1.34
0.97
6.00
2.78
2.16
2.18
2.05
1.94
1.92
1.19
1.92
1.56
3.97
2.27
3.01
10.0
9.5
9.0
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
0.0
-0.5
-1.0
f1 (ppm)


173.18
171.28
158.67
144.43
135.42
129.98
128.03
127.99
127.05
113.34
86.79
68.36
64.94
56.43
55.27
53.84
40.24
38.48
38.40
38.14
36.46
36.31
34.61
34.02
28.88
25.36
20.08
OH
ODMT
HN
O
S
S
H
N
O
S
S
6
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)