Supporting Information

Resin- and magnetic nanoparticle-based free radical probes for glycan capture, isolation, and structural characterization

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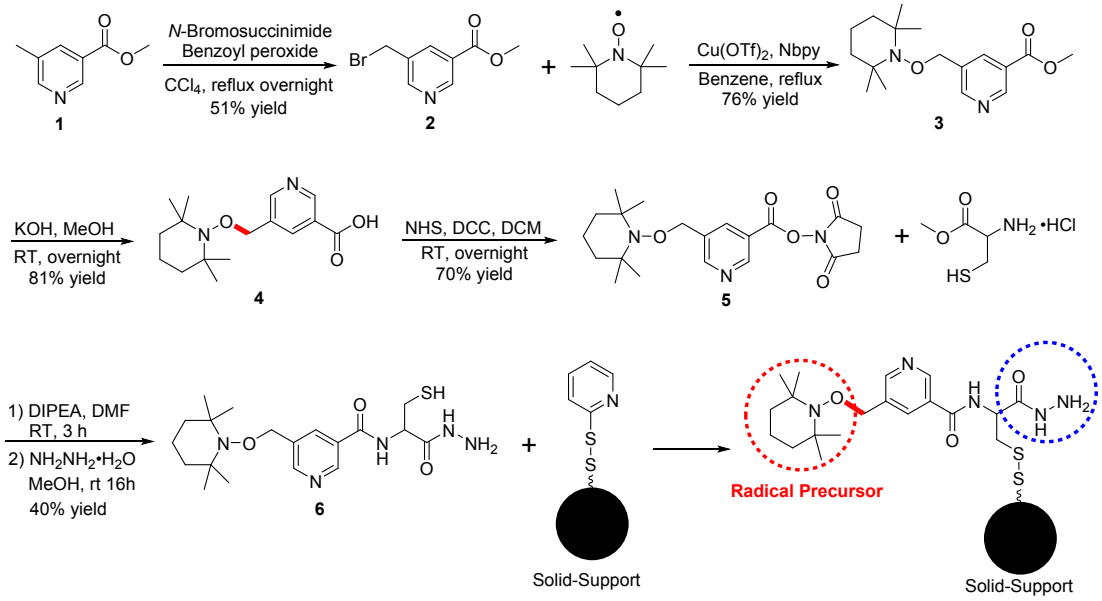
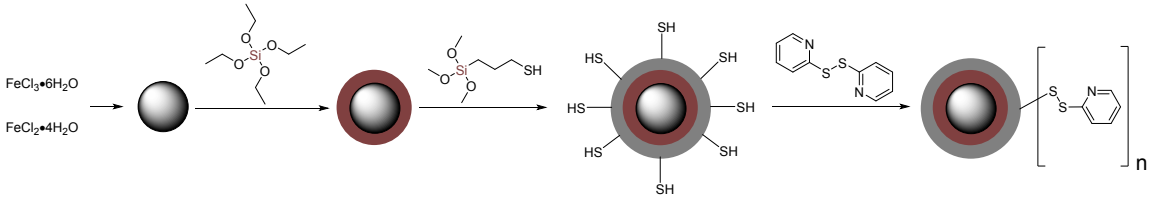
Preparation of the solid-supported free radical probe (SS-FRAGS)

The SS-FRAGS was synthesized according to the procedure reported previously.[1-3] Compound **5** was synthesized according to the procedure reported previously.[4-5] The SS-FRAGS is generally accomplished by benzylic bromination with NBS, coupling with TEMPO, hydrolysis of the ester group, activation of the carboxylic acid by *N*-hydroxysuccinimide, amidation reaction with cysteine, hydrazinolysis of the ester, and finally coupling with the activated solid-support. The preparation of the magnetic nanoparticle (Scheme S1) was achieved by following the previously reported procedures.[6]



To a solution of cysteine (0.353 g, 2 mmol) and *N,N*-diisopropylethylamine (0.366 mL, 2 mmol). in anhydrous dimethylformamide (5 mL) was added slowly a solution of **5** (0.080 g, 0.2 mmol) in 5 ml anhydrous dimethylformamide under argon protection. After the reaction mixture was stirred at room temperature for three hours, the reaction was quenched by adding H_2O. The reaction mixture was extracted with 20 mL CH_2Cl_2 (×3). The extract was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. Without further purification, 1 ml hydrazine and 5 mL methanol were added to the crude product. The reaction mixture was stirred at room temperature overnight. The reaction mixture was concentrated under vacuum and purified by flash chromatography on silica gel (0% - 10% methanol in dichloromethane) to give **6** as a white solid (0.0327 mg, 40%). ^{1}HNMR (300 MHz, $CDCl_3$, δ ppm): 8.96 (s, 1H), 8.78 (s, 1H), 8.10 (s, 1H), 7.73 (br, 1H), 4.91 (s, 2H), 4.90 (m, 1H), 3.97 (br, 2H), 3.22 (m, 1H), 2.87 (m, 1H), 1.78 (t, 1H), 1.58 (m, 1H), 1.50 (m, 4H), 1.36 (m, 1H), 1.24 (s, 6H), 1.16 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm), 16.97, 20.20, 25.63, 33.03, 39.61, 60.15, 75.39, 121.29, 134.15, 136.42, 150.18, 154.12, 160.89, 168.88. ESI-MS: $[M+H]^+$, 410.2.

To a solution of **7** in 1 mL 50% methanol was added one equivalent of dithiothreitiol to break dimers of **7** into monomers. Thiopropyl Sepharose™ 6B resins (0.5 mL, 15 μmol) was washed with deionized water and suspended in 50% methanol. 500 μL of solution of compound 7 was added to the suspension. The suspensions were placed on a rocking incubator overnight at room temperature. The resin-supported free radical probe was washed by 50% methanol, water, and 20% ethanol successively. The resin-supported free radical probe was stored in 20% ethanol at 4 °C for further use. The magnetic nanoparticle-supported free radical probe was prepared by replacing resin with magnetic nanoparticles.



Scheme S1. Preparation of the thio-activated magnetic nanoparticle.

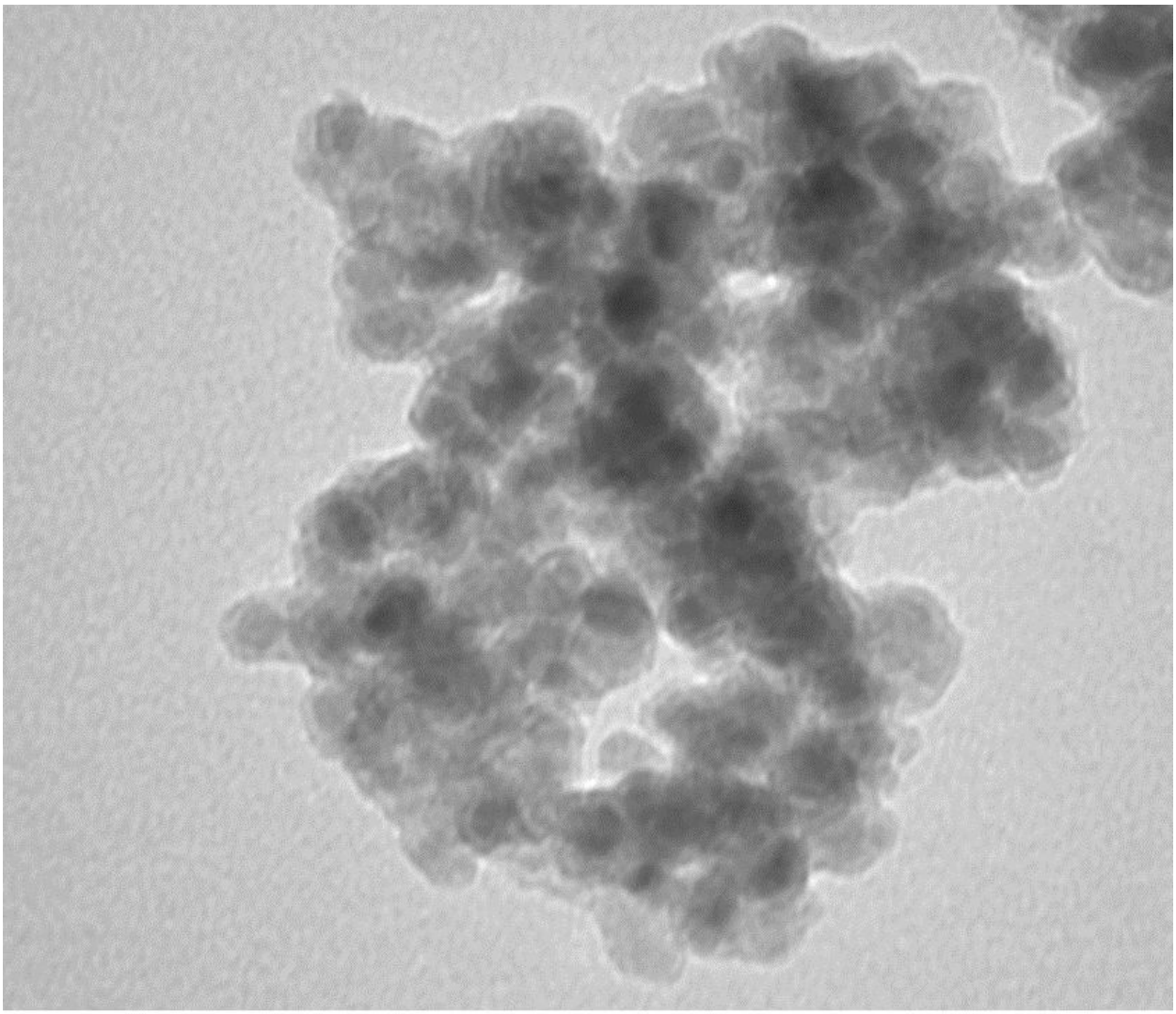


Figure S1. TEM image of synthesized Fe_3O_4 nanoparticles.

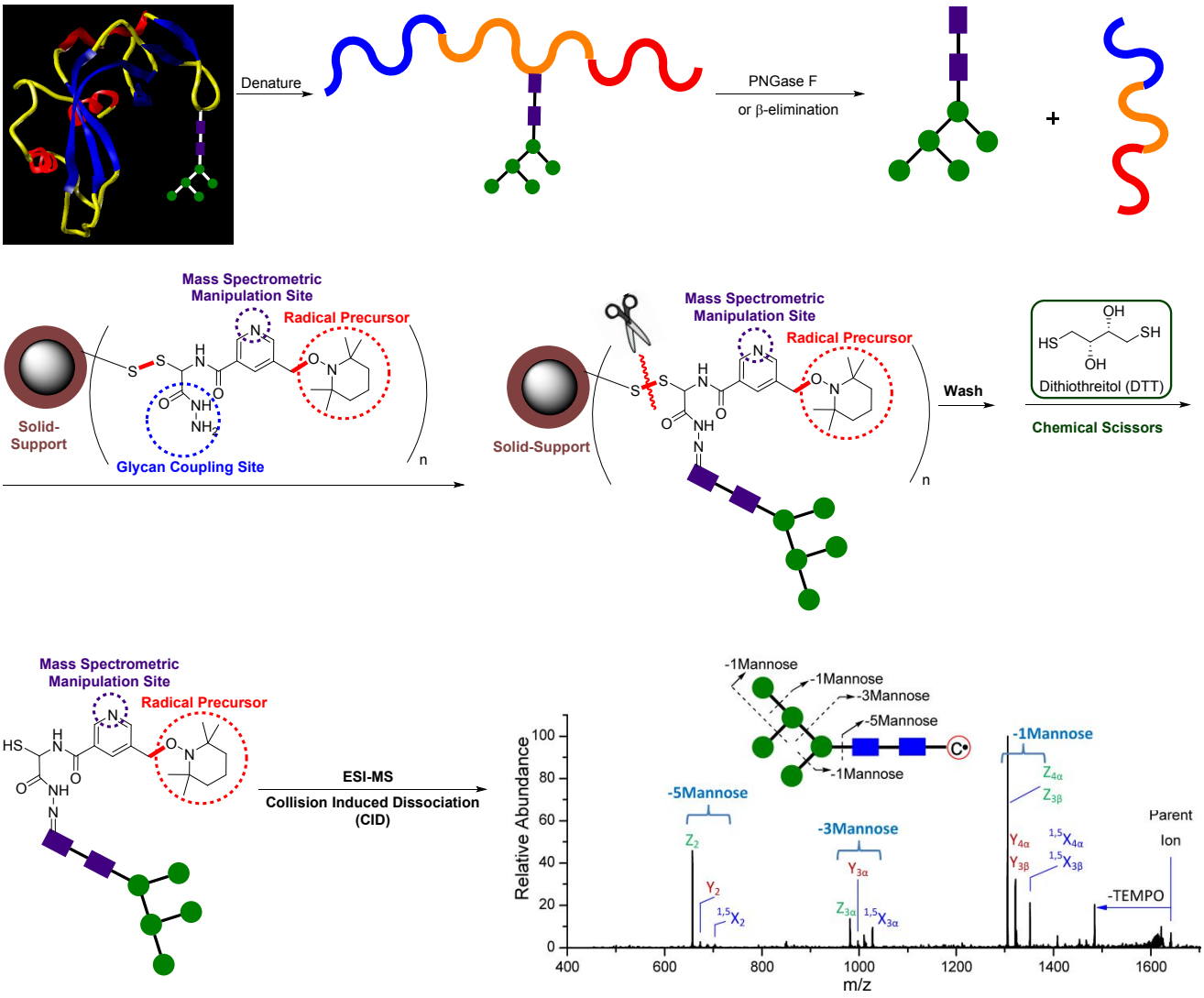


Figure S2. Schematic illustration of glycan enrichment and MS analysis using solid-supported free radical probes. First, glycoproteins are denatured to make the glycosylation site more accessible. Second, glycans are released either by PNGase F (*N*-linked glycans) or β-elimination (*O*-linked glycans). Third, glycans are conjugated to the solid-support via the reduction reaction between the glycans and probe. Fourth, impurities and excess reactants are washed away. Finally, conjugated glycans are released from the solid-support through the cleavage of the disulfide bond followed by the ionization and collision induced dissociation.

References:

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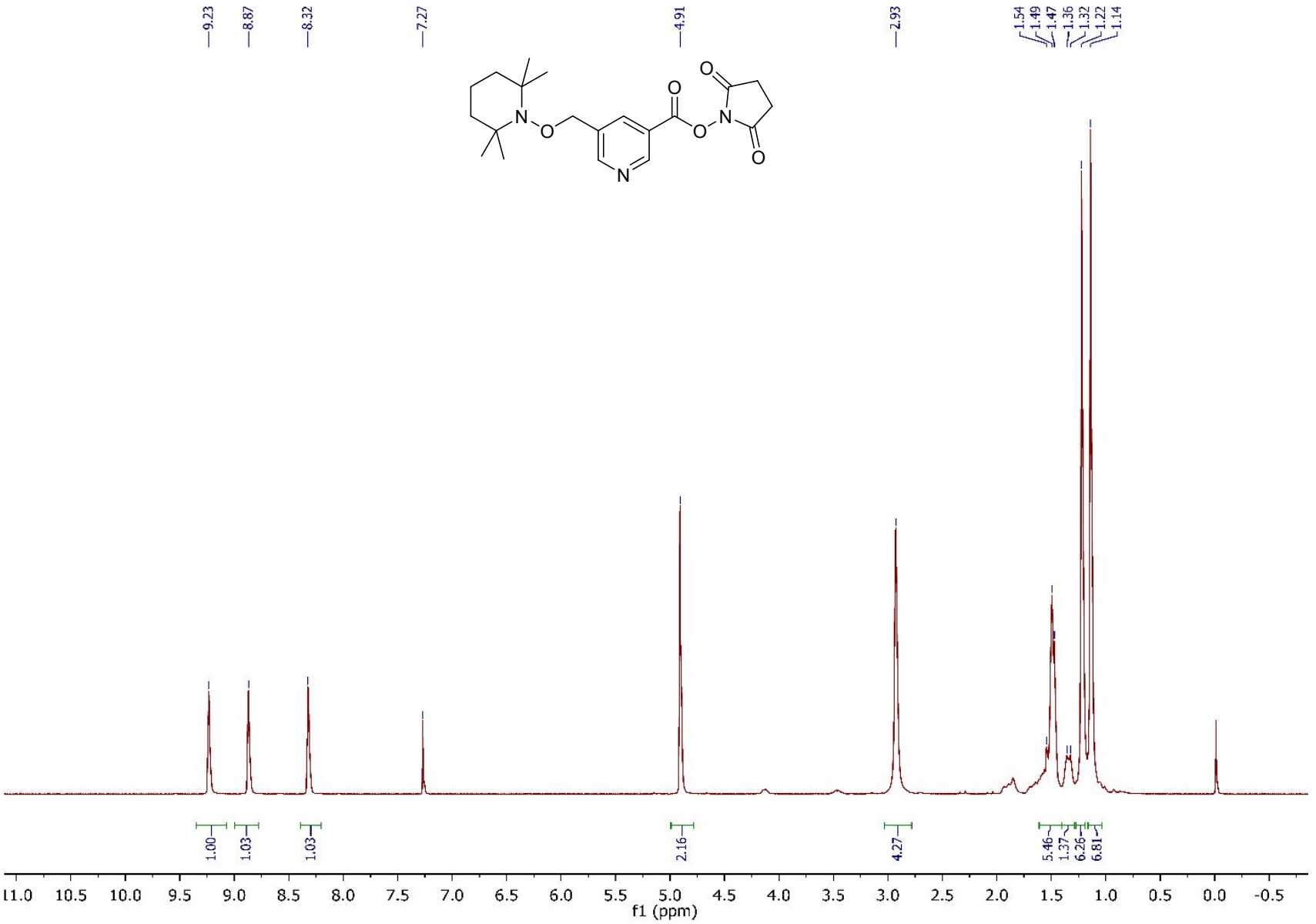
6. Patil, U. S.; Qu, H. O.; Caruntu, D.; O'Connor, C. J.; Sharma, A.; Cai, Y.; Tarr, M. A. Labeling Primary Amine Groups in Peptides and Proteins with N-Hydroxysuccinimidyl Ester Modified Fe3O4@SiO2 Nanoparticles Containing Cleavable Disulfide-Bond Linkers. *Bioconjugate Chemistry* **2013,** *24*, 1562-1569.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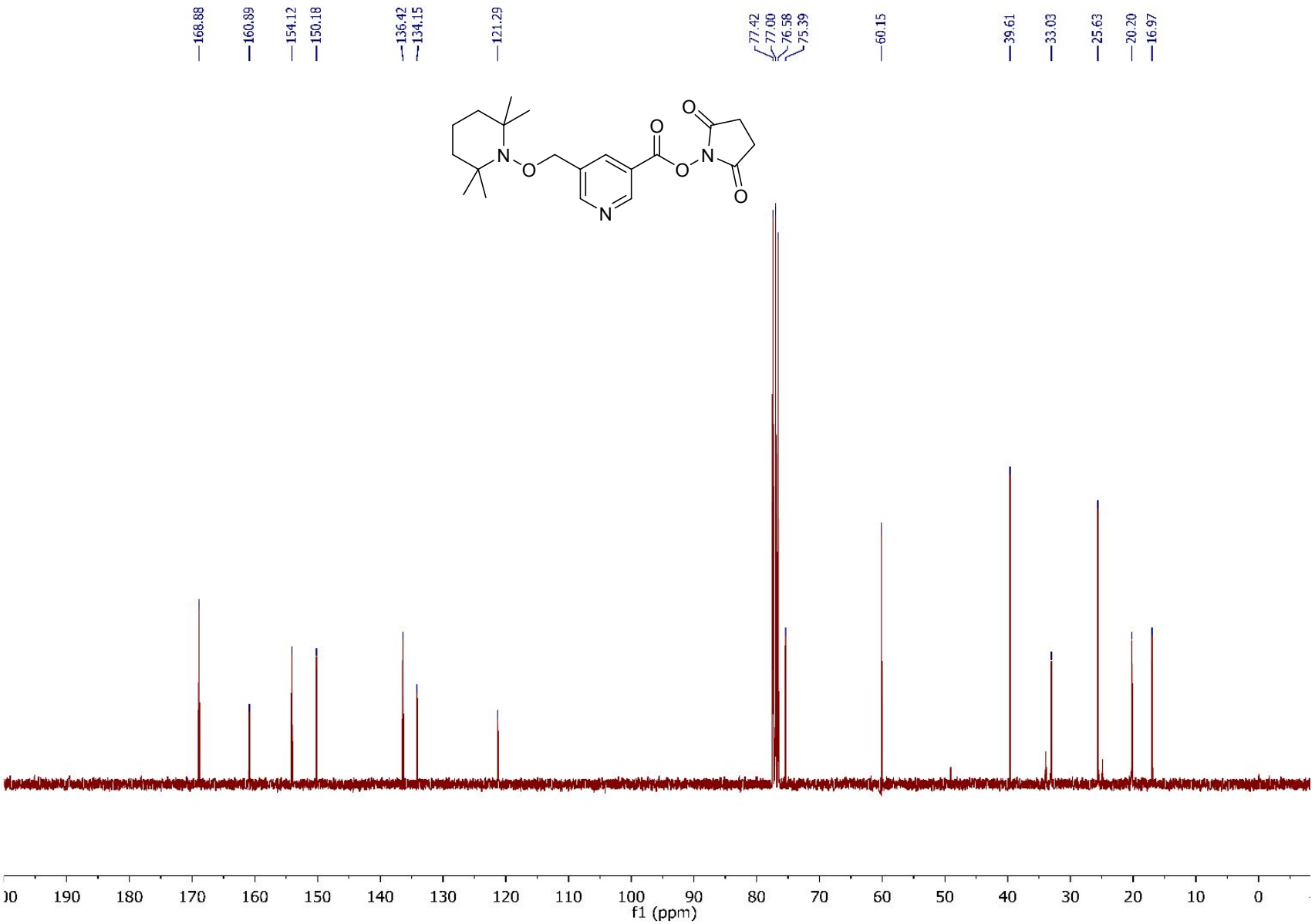
8.32
7.27
4.91
2.93
1.54
1.49
1.47
1.36
1.32
1.22
1.14
1.00
1.03
1.03
2.16
4.27
5.46
1.37
6.26
6.81
f1 (ppm)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10
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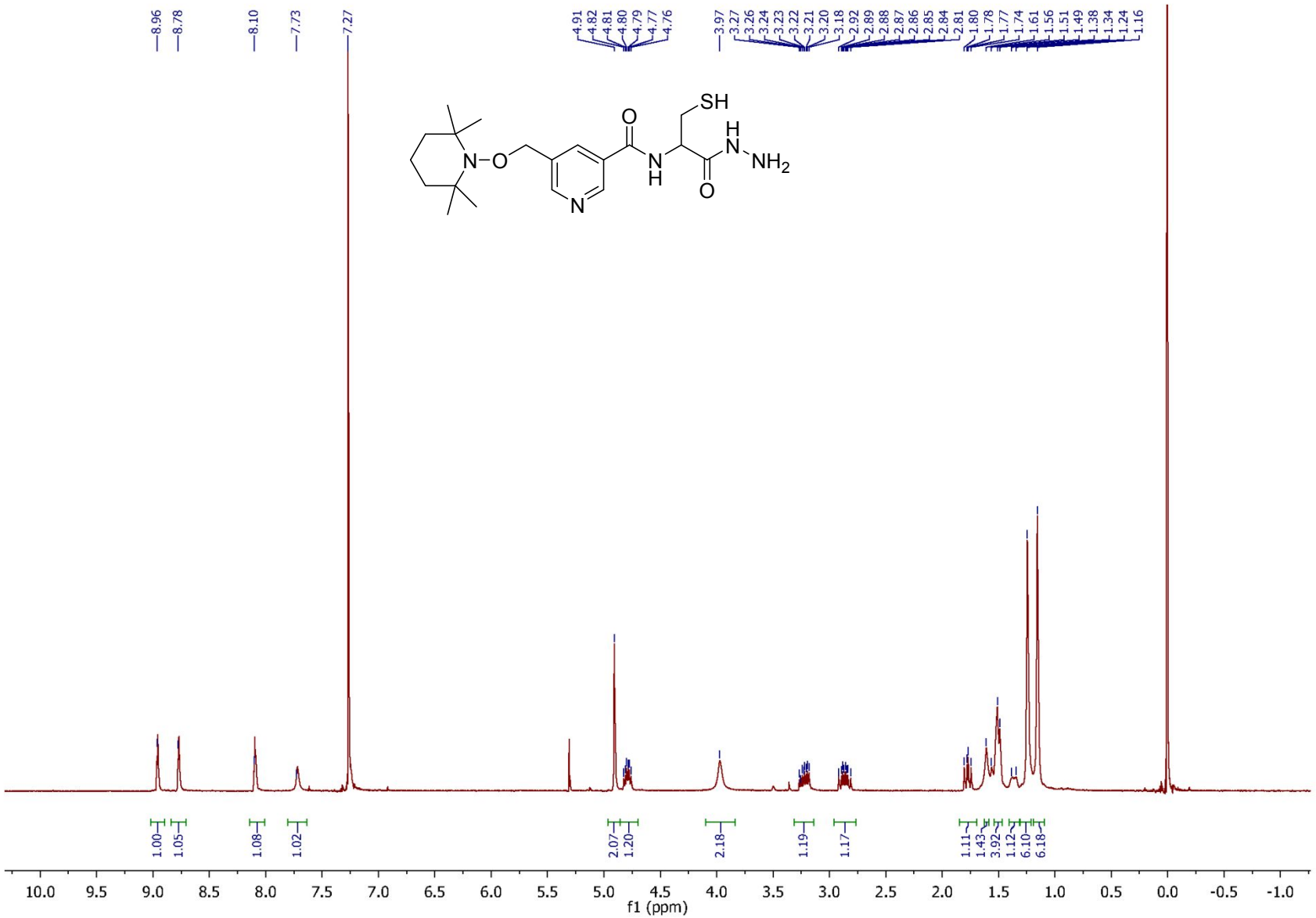
f1 (ppm)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2.86
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1.61
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1.49
1.38
1.34
1.24
1.16
SH
O
H
N
NH2
N–O
N
H
O
N
1.00
1.05
1.08
1.02
2.07
1.20
2.18
1.19
1.17
1.11
1.43
3.92
1.12
6.10
6.18
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
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2.5
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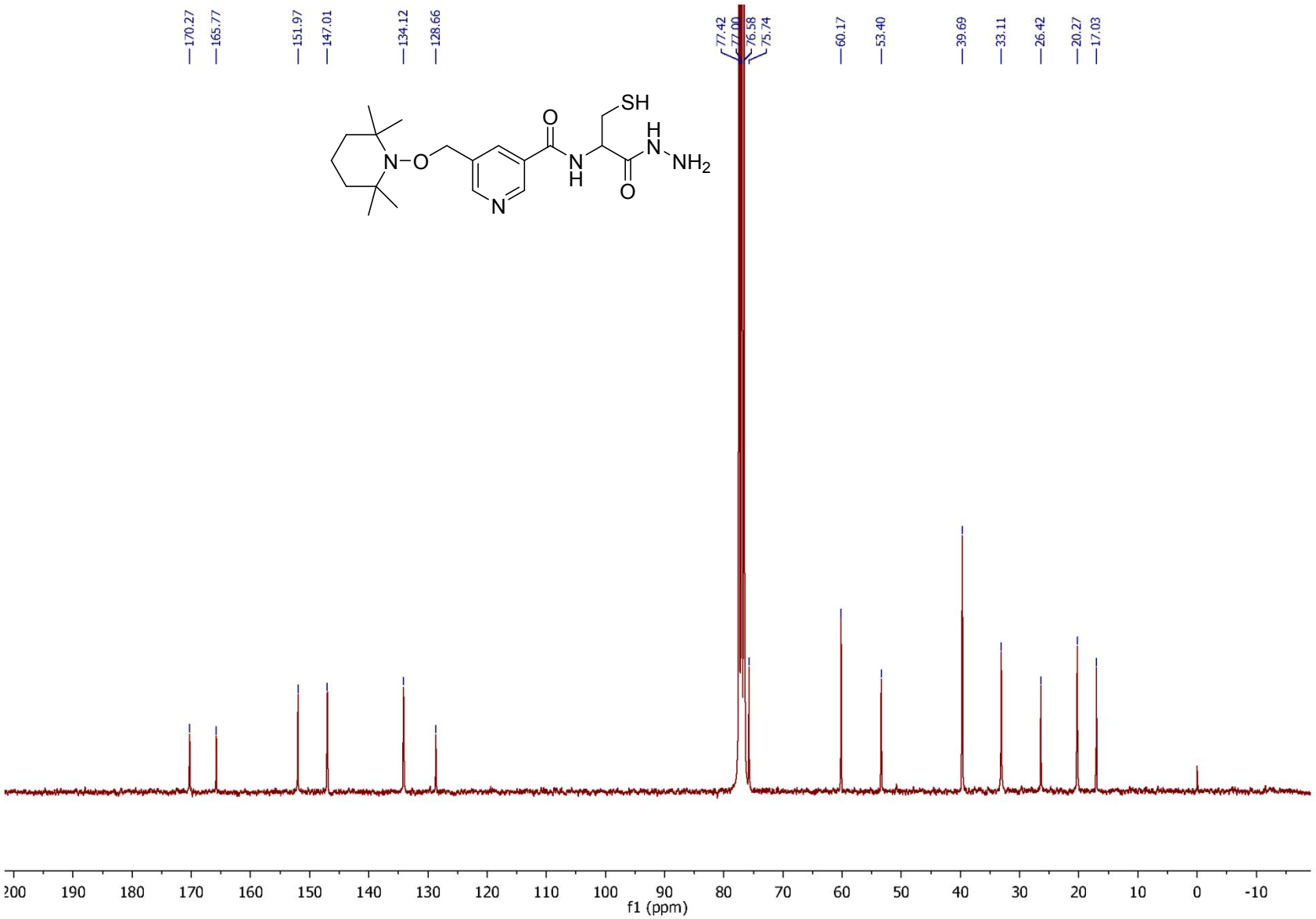
-1.0
f1 (ppm)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128.66
77.42
77.00
76.58
75.74
60.17
53.40
39.69
33.11
26.42
20.27
17.03
SH
O
H
N
NH2
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H
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200
190
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170
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-10
f1 (ppm)