Electrochemical Control of Rapid Bioorthogonal Tetrazine Ligations for Selective Functionalization of Microelectrodes

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Supporting Information

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1 Reagents

The following chemicals were purchased from commercial sources and used as received. *Sigma Addrich*: 11-Bromo-1-undecanol, (*E*)-cyclooct-4-enyl 2,5-dioxo-1-pyrrolidinyl carbonate, ferrocene carboxylic acid, 4-chloro-1-naphthol solution (For HRP detection on Western Blots), horseradish peroxidase and benzonitrile. Alfa Aesar: 1-Nonanethiol. *Koptec*: Ethanol. *Conju-Probe*: TCO-PEG_3-amine

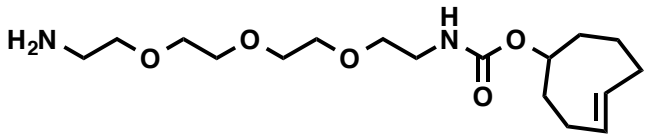


Figure S1: Molecular Structure of TCO-PEG_3-amine

2 Substrates

Gold substrates were prepared by sputtering of a titanium adhesion layer (10-20 nm) followed by gold (150 – 200 nm). Silicon was pre-cleaned with an RF-bias (30 sec, 400 W). Depositions were carried out on a Denton Discovery Sputter System.
Interdigitated array (IDA) electrodes were purchased from Abtech Scientific (www.abtechsci.com).

3 Formation of Mixed SAM

3.1 On macroscopic planar electrodes

Gold substrates were pre-cleaned for 10 minutes in hot piranha (1 vol 30% by mass aqueous H_2O_2 : 3 vol H_2SO_4), and rinsed with copious amounts of deionized water (**Warning**: Piranha solution reacts violently, even explosively, with organic materials. It should not be stored or combined with significant quantities of organic material.). Freshly cleaned gold substrates were then immersed in deposition solutions made by dissolving the desired ratio of tetrazine-terminated thiol (**1** or **2**) and 1-nonanethiol in ethanol for 24-36 hours. After deposition, the monolayer-coated samples were rinsed in ethanol and water to remove excess absorbate and dried with N_2 to remove residual solvent.

3.2 On interdigitated array electrodes

The IDA were electrochemically cleaned by i) holding its electrochemical potential at +1.65 V versus Ag/AgCl/(gel) in 0.5 M aqueous H_2SO_4 for 15 seconds ii) 2 potential sweeps (scan rate 50 mV/s) between -0.2 V and -1.4 V versus Ag/AgCl/(gel) in 50 mM aqueous KOH. After rinsing with deionized water, the freshly cleaned electrodes were immersed into a 0.1 mM solution of **1** for 24 h.

4 Electrochemical measurements

4.1 On macroscopic planar electrodes

The electrochemical cell area was defined by pressing down on the sample with a cylindrically bored Teflon™ cone pressed against the sample. The bore was filled with ca. 5mL of electrolyte, degased with argon for 1 minute, and a platinum counter electrode and a glass frit-isolated Ag/AgCl/(3 M)KCl reference electrode were suspended above the cell. For electrochemical measurements a wavenano potentiostat (Pine) and for data analysis the aftermath software package (Pine) was used. All electrochemical measurements were performed at room temperature.

4.2 On interdigitated array electrodes

The IDA was mounted on the holder and dipped into an electrochemical cell with ca. 5 mL of electrolyte, platinum counter electrode and Ag/AgCl/(gel) reference electrode. The electrolyte was degased with argon for 1 minute. CVs were taken between 0.2 Vto 0.9 V starting at 0.2 V. For electrochemical measurements a wavenano potentiostat (Pine) and for data analysis the aftermath software package (Pine) was used. All electrochemical measurements were performed at room temperature.

5 Synthesis

5.1 Synthesis of 1,2,4,5-tetrazines alkane thiols

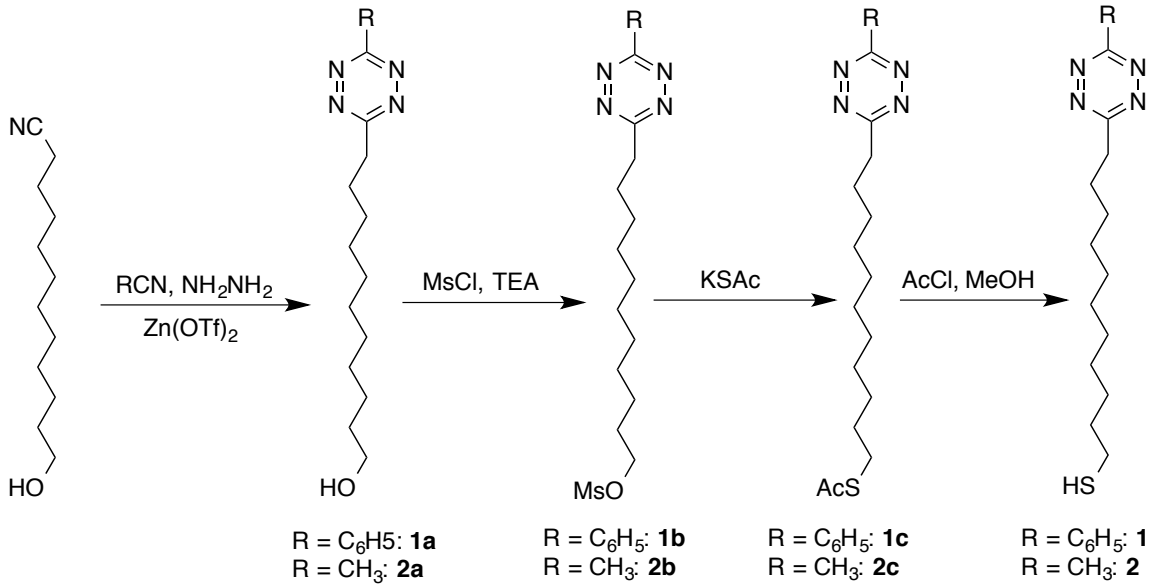
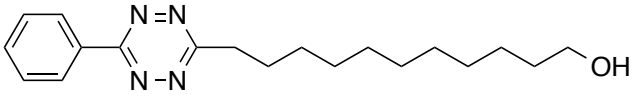


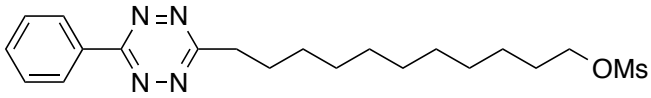
Figure S2: Synthesis 1,2,4,5-tetrazines alkane thiols **1** and **2** used for SAM formation.

5.1.1 Synthesis of **1a**



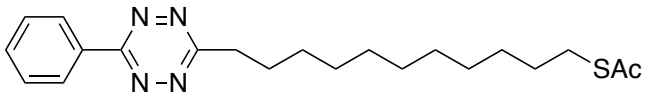
Following a previously developed procedure (*Angew. Chem. Int. Ed*. **2012**, *51*, 5222 – 5225), in a 50 mL flask equipped with a stir bar, $Zn(OTf)_2$ (71 mg, 0.2 mmol), 12-hydroxydodecanenitrile (980 mg, 5 mmol), acetonitrile (103 mg, 1 mmol), and anhydrous hydrazine (3.3 mL, 30 mmol) were added. The reaction was protected with a shield. Under N_2 gas, the mixture was stirred in an oil bath at 70 °C for 40 hours. The reaction solution was cooled with ice water, and sodium nitrite (40 mmol, 2.8 g) dissolved in 20 mL of ice water was slowly added, followed by slow addition of 1M HCl during which time the solution turned bright red and gas evolved. Addition of 1M HCl continued until gas evolution ceased and the pH value was 3. The solution was extracted with ethyl acetate (50 mL × 3) and the combined organic layers were dried over Na_2SO_4 and evaporated. The residue was purified by silica column to afford the product **1a** (203 mg) as pink solid, with a yield of 62 %. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.64 – 8.55 (m, 2H), 7.65 – 7.55 (m, 3H), 3.63 (t, *J* = 6.3 Hz, 2H), 3.38 – 3.32 (m, 2H), 1.98 (dt, *J* = 15.3, 7.6 Hz, 2H), 1.59 – 1.51 (m, 2H), 1.46 (dt, *J* = 14.9, 7.0 Hz, 2H), 1.39 – 1.25 (m, 13H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.37, 164.29, 132.68, 131.95, 129.37, 129.37, 128.03, 128.03, 63.23, 34.94, 32.92, 29.69, 29.62, 29.53, 29.53, 29.39, 29.28, 28.46, 25.85. HRMS $[M+H]^+$ m/z calcd. for $[C_{19}H_{29}N_4O]^+$ 329.2336, found 329.2335.

5.1.2 Synthesis of **1b**



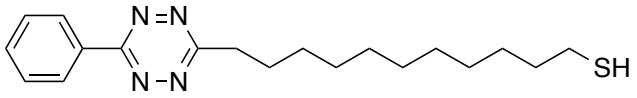
In a 50 mL flask **1a** (63.5 mg, 0.2 mmol) was dissolved in anhydrous methylene chloride, with added triethylamine (26 μL, 0.24 mmol), followed by the addition of (15 μL, 0.24 mmol) at 0 °C. The reaction solution was stirred at room temperature for 20 min, and checked for completion by TLC. The reaction solution was washed with water. The organic layer was dried over Na_2SO_4 and evaporated. The residues were purified by silica column chromatography to afford the product **1b** (71 mg) as pink solid, with a yield of 88 %. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.63 – 8.56 (m, 2H), 7.65 – 7.55 (m, 3H), 4.22 (t, *J* = 6.6 Hz, 2H), 3.40 – 3.30 (m, 2H), 3.00 (s, 3H), 1.98 (dt, *J* = 15.3, 7.6 Hz, 2H), 1.74 (dt, *J* = 14.7, 6.6 Hz, 2H), 1.50 – 1.42 (m, 2H), 1.41 – 1.34 (m, 4H), 1.33 – 1.25 (m, 8H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.35, 164.29, 132.69, 131.94, 129.37, 129.37, 128.02, 128.02, 70.34, 37.48, 34.92, 29.55, 29.50, 29.49, 29.37, 29.26, 29.23, 29.13, 28.44, 25.54. HRMS $[M+H]^+$ m/z calcd. for $[C_{20}H_{31}N_4O_3S]^+$ 407.2111, found 407.2112.

5.1.3 Synthesis of **1c**



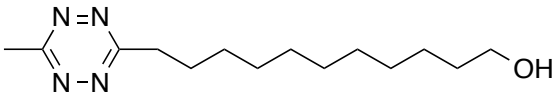
In a 50 mL flask **1b** (71 mg, 0.178 mmol) was dissolved in 10 mL anhydrous DMF, with added potassium thioacetate (22.2 mg, 0.2 mmol). The reaction solution was stirred under N_2 at 100 °C for 2 hours, and checked for completion by TLC. The reaction solution was washed with water (50 mL), extracted with ethyl acetate (50 mL x 3). The organic layer was dried over Na_2SO_4 and evaporated. The residues were purified by silica column chromatography to afford the product **1c** (64 mg) as pink solid with, a yield of 95 %. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.64 – 8.55 (m, 2H), 7.65 – 7.55 (m, 3H), 3.38 – 3.32 (m, 2H), 2.89 – 2.82 (m, 2H), 2.31 (s, 3H), 1.98 (dt, *J* = 15.3, 7.6 Hz, 2H), 1.55 (m, 2H), 1.48 – 1.41 (m, 2H), 1.32 (dt, *J* = 34.0, 7.1 Hz, 12H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 196.32, 170.39, 164.31, 132.69, 131.98, 129.38, 129.38, 128.04, 128.04, 34.96, 30.82, 29.63, 29.63, 29.58, 29.54, 29.41, 29.30, 29.24, 28.95, 28.47. $[M+H]^+$ m/z calcd. for $[C_{21}H_{31}N_4OS]^+$ 387.2213, found 387.2214.

5.1.4 Synthesis of **1**



In a 50 mL flask **1c** (64 mg, 0.165 mmol) was dissolved in 5 mL anhydrous methanol, under Argon, with added acetyl chloride 50 μL (30 eq). The reaction solution was stirred under argon at room temperature for 3 hours, and checked for completion by TLC. The reaction solvent was removed. The residues were purified by silica column chromatography under N_2 to afford the product **1** (28 mg) as pink solid, with a yield of 49 %. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.63 – 8.58 (m, 2H), 7.65 – 7.57 (m, 3H), 3.38 – 3.32 (m, 2H), 2.52 (dd, *J* = 14.7, 7.5 Hz, 2H), 1.99 (t, *J* = 7.6 Hz, 2H), 1.59 (dd, *J* = 14.9, 7.4 Hz, 2H), 1.49 – 1.42 (m, 2H), 1.31 (m, *J* = 28.2, 13.7 Hz, 13H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.24, 164.17, 132.56, 131.84, 129.25, 129.25, 127.90, 127.90, 34.81, 34.06, 29.50, 29.48, 29.41, 29.27, 29.16, 29.06, 28.38, 28.33, 24.69. $[M+H]^+$ m/z calcd. for $[C_{19}H_{29}N_4S]^+$ 345.2107, found 345.2109.

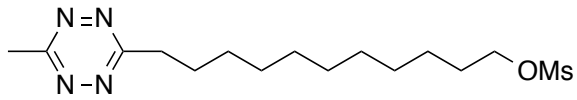
5.1.5 Synthesis of **2a**



In a 50 mL flask $Zn(OTf)_2$ (71 mg, 0.2 mmol), 12-hydroxydodecanenitrile (980 mg, 5 mmol), acetonitrile (41 mg, 1 mmol), and anhydrous hydrazine (3.3 mL, 30 mmol) were added. The reaction was protected with a shield. Under N_2 gas, the mixture was stirred in an oil bath at 70 °C for 40 hours. The reaction solution was cooled

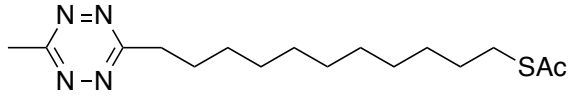
with ice water, and sodium nitrite (40 mmol, 2.8 g) dissolved in 20 mL of ice water was slowly added, followed by slow addition of 1M HCl during which time the solution turned bright red, and gas evolved. Addition of 1M HCl continued until gas evolution ceased and the pH value was 3. The solution was extracted with ethyl acetate (50 mL × 3) and the combined organic layers were dried over Na_2SO_4 and evaporated. The residue was purified by silica column to afford the product **2a** (138 mg) product as pink solid, with a yield of 52 %. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.61 (t, *J* = 6.7 Hz, 2H), 3.29 – 3.22 (m, 2H), 3.02 (s, 3H), 1.90 (dd, *J* = 15.2, 7.7 Hz, 2H), 1.82 (s, 1H), 1.53 (dd, *J* = 14.5, 6.8 Hz, 2H), 1.43 – 1.36 (m, 2H), 1.36 – 1.18 (m, 14H). ^{13}C NMR (126 MHz, cdcl$_3$) δ 170.22, 167.37, 63.04, 34.78, 32.84, 29.62, 29.54, 29.47, 29.46, 29.29, 29.18, 28.43, 25.80, 21.17. $[M+H]^+$ m/z calcd. for $[C_{14}H_{26}N_4O]^+$ 267.2185, found 267.2181.

5.1.6 Synthesis of **2b**



In a 50 mL flask **2a** (53 mg, 0.2 mmol) was dissolved in anhydrous methylene chloride, with added triethylamine (26 μL, 0.24 mmol), followed by the addition of methanesulfonyl chloride (15 μL, 0.24 mmol) at 0 °C. The reaction solution was stirred at room temperature for 20 min, and checked for completion by TLC. The reaction solution was washed with water. The organic layer was dried over Na_2SO_4 and evaporated. The residues were purified by silica column chromatography to afford the product **2b** (60 mg) as pink solid, with a yield of 88 %. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.21 (td, *J* = 6.6, 4.0 Hz, 2H), 3.27 (td, *J* = 7.9, 3.2 Hz, 2H), 3.02 (s, 3H), 3.01 – 2.97 (s, 3H), 1.96 – 1.86 (m, 2H), 1.78 – 1.69 (m, 2H), 1.45 – 1.22 (m, 14H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.26, 167.43, 70.33, 37.46, 34.83, 29.52, 29.48, 29.47, 29.32, 29.22, 29.22, 29.11, 28.47, 25.52, 21.24. HRMS $[M+H]^+$ m/z calcd. for $[C_{15}H_{28}N_4O_3S]^+$ 345.1955, found 345.1954.

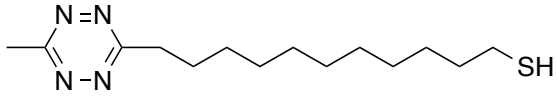
5.1.7 Synthesis of **2c**



In a 50 mL flask **2b** (60 mg, 0.174 mmol) was dissolved in 10 mL anhydrous DMF, with added potassium thioacetate (22.2 mg, 0.2 mmol). The reaction solution was stirred under N_2 at 100 °C for 2 hours, and checked for completion by TLC. The reaction solution was washed with water (50 mL), extracted with ethyl acetate (50 mL x 3). The organic layer was dried over Na_2SO_4 and evaporated. The residues were purified by silica column chromatography to afford the product **2c** (55 mg) as pink solid, with a yield of 98 %. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.30 – 3.22 (m, 2H), 3.01 (s, 3H), 2.84 (t, *J* = 7.3 Hz, 2H), 2.30 (s, 3H), 1.91 (dt, *J* = 15.4, 7.6 Hz, 2H), 1.59 – 1.48 (m, 2H), 1.39 (d, *J* = 8.7 Hz, 2H), 1.36 – 1.30 (m, 4H), 1.25 (s, 8H). ^{13}C NMR (126 MHz,

$CDCl_3$) δ 196.21, 170.25, 167.39, 34.82, 30.77, 29.59, 29.56, 29.52, 29.48, 29.33, 29.23, 29.23, 29.18, 28.89, 28.47, 21.22. HRMS $[M+H]^+$ m/z calcd. for $[C_{16}H_{29}N_4OS]^+$ 325.2060, found 325.2057.

5.1.8 Synthesis of **2**



In a 50mL flask **2c** (55 mg, 0.17 mmol) was dissolved in 5 mL anhydrous methanol, under Argon, with added acetyl chloride 50 μL (30 eq). The reaction solution was stirred under Argon at room temperature for 3 hours, and checked for completion by TLC. The reaction solvent was removed. The residues were purified by silica column chromatography under N_2 to afford the product **2** (25 mg) as pink solid, with a yield of 53 %. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.35 – 3.22 (m, 2H), 3.04 (s, 3H), 2.52 (dd, *J* = 14.7, 7.4 Hz, 2H), 1.93 (dd, *J* = 14.8, 7.3 Hz, 2H), 1.61 (dd, *J* = 14.4, 7.1 Hz, 2H), 1.48 – 1.20 (m, 15H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.31, 167.45, 34.87, 34.19, 29.63, 29.61, 29.54, 29.38, 29.27, 29.19, 28.52, 28.51, 24.82, 21.27. HRMS $[M+H]^+$ m/z calcd. for $[C_{14}H_{26}N_4S]^+$ 283.1951, found 283.1950.

5.2 Synthesis of TCO-modified ferrocene

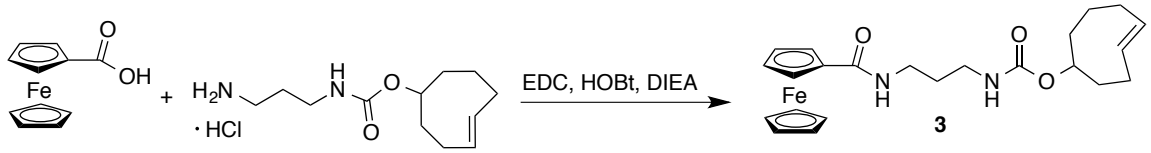


Figure S3: Synthesis of the TCO-modified ferrocene redox probe for IEDDAR at tetrazine-terminated SAMs.

In a 2-dram vial equipped with stir bar, ferrocene carboxylic acid (2.89 mg, 12.56 μmol) was dissolved into 100 μL of dry DMF. To this solution was added (*E*)-cyclooct-4-en-1-yl (3-aminopropyl)carbamate hydrochloride (TCO-Amine) (3.0 mg, 11.92 μmol), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) (2.41 mg 12.56 μmol),1-hydroxybenzotriazole (HOBt) (1.7 mg, 12.56 μmol) and N-N-diisopropylethylamine (DIEA) (5.97 μL, 34.25 μmol). The reaction solution was stirred at room temperature for 3 hours, and checked for completion by TLC. The reaction solution was diluted in methylene chloride and washed with water. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by silica column chromatography to afford the product **3** (2.5 mg), with a yield of 50 %. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.58 (s, 1H), 5.70 – 5.48 (m, 2H), 5.07 (d, J = 7.3 Hz, 1H), 4.72 (t, J = 2.0 Hz, 2H), 4.35 (t, J = 2.0 Hz, 2H), 4.21 (d, J = 1.0 Hz, 5H), 3.42 (qd, J = 6.0, 1.3 Hz, 2H), 3.30 (dq, J = 6.0, 1.8 Hz, 2H), 2.41 – 2.32 (m, 3H), 2.07 – 2.00 (m, 1H), 2.00 – 1.89 (m, 3H), 1.84 – 1.63 (m, 5H), 1.31 – 1.20 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 157.1, 135.0, 133.0, 129.7, 129.7, 80.8, 76.4, 76.3, 70.6, 69.9, 68.3, 41.1,

38.7, 37.3, 34.3, 32.6, 31.0. HRMS $[M+H]^+$ m/z calcd. for $[C_{23}H_{31}FeN_2O_3^+]$439.1679, found 439.1673.

5.3 Synthesis of TCO-modified horseradish peroxidase

Horseradish peroxidase (HRP) was modified with a TCO-NHS linker following general NHS conjugation methods. Briefly, 150 µl of HRP solution (0.1 mM, phosphate buffer, pH = 6.5) and 6µl of (*E*)-cyclooct-4-enyl 2,5-dioxo-1-pyrrolidinyl carbonate solution (100 mM, DMF) were added to 1.5 ml of carbonate buffer (pH = 8.6, 15 % v/v DMF) and the mixture was gently stirred for 4 h at room temperature. Thereafter, a buffer exchange with phosphate buffer (0.1 M, pH = 6.5) in a 10 kD spin filter was performed.

6 Supplementary voltammetric data

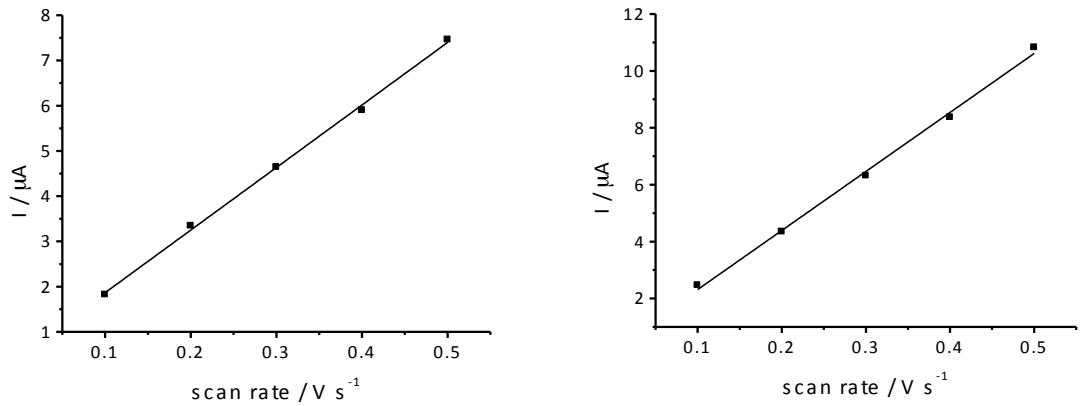


Figure S4: Plot of the anodic peak current versus scan rate and a linear fit (solid line) obtained from CVs of SAMs on gold formed from 0.1 mM ethanolic solution of $CH_3(CH_2)_8SH$ and **1** (left) and **2** right (right) ($\chi_{1,2}$ = 0.05).

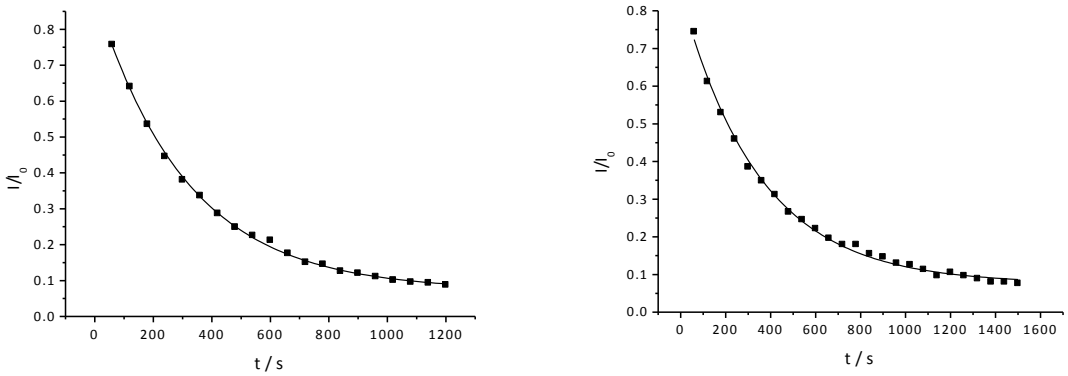


Figure S5: Plot of peak current vs time for a mixed-SAM of **1** (left) and **2** (right) during functionalization with 1 µM TCO-PEG_3-Amine.

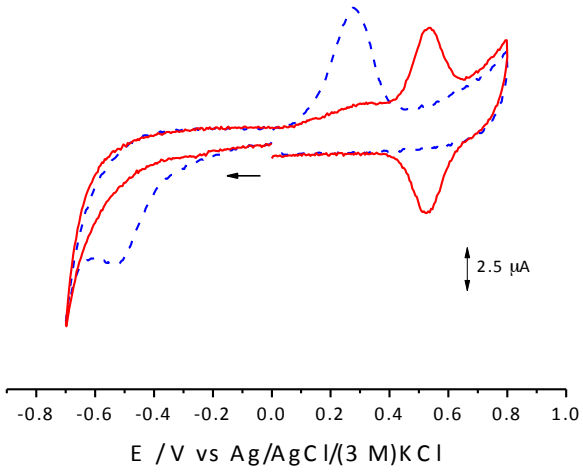


Figure S6: CVs (0.5 V/s) of a SAM on gold formed from 0.1 mM ethanolic solution of $CH_3(CH_2)_8SH$ and **1** (χ_1 = 0.05), before (blue dashed line) and after (solid red line) the SAM was contacted to a 1 µM solution of **3** for 30 minutes. Electrolyte buffered 0.1 M NaCl (pH = 4). From the charge passed resulting from the redox process of tetrazine Q_{Tz} = 1.83 x 10^{-6} A s^{-1} and ferrocene Q_{Fc} = 0.90 x 10^{-6} A s^{-1} the number of surface bound tetrazine (N_{Tz} = 1.9 x 10^{-11} mol) and ferrocene (N_{Fc} = 1.8 x 10^{-11} mol) was calculated (N = Q/*F* z; *F* = Faraday Constant, z = no. of electrons). N_{Fc}/N_{Tz} gives an approximate yield of 95 % for the surface coupling reaction.

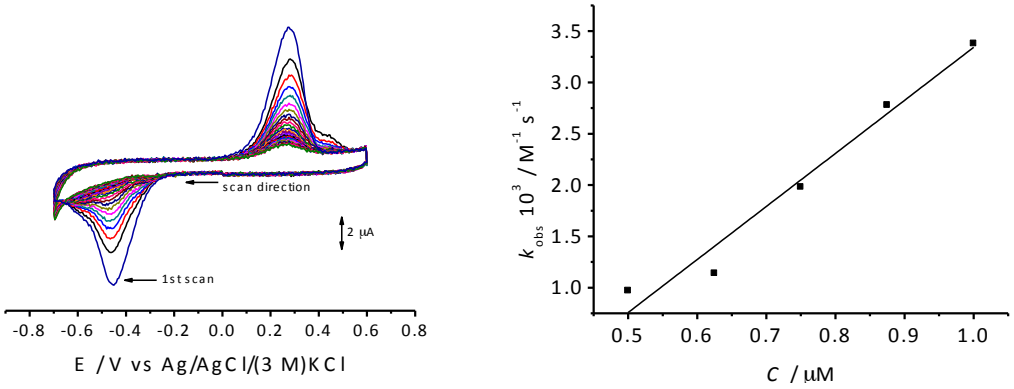


Figure S7: Left: CVs (0.5 V/s scan rate) of a mixed SAM on gold formed from a 0.1 mM ethanolic solution of **2** and $CH_3(CH_2)_8SH$ (χ_1 = 0.05). Scans were taken once every minute during the 20 min functionalization of the monolayer with TCO-PEG_3-Amine. Electrolyte 0.1 M phosphate buffer (pH = 7) and 1 µM TCO-PEG_3-Amine. Right: Plot of k_{obs} versus the concentration of TCO-PEG_3-Amine for mixed monolayers of **2** and $CH_3(CH_2)_8SH$ (χ_1 = 0.05). The slope of the linear fit (solid line) allows estimation of a second-order rate constant for the reaction of k = 5200 M^{-1} s^{-1}.

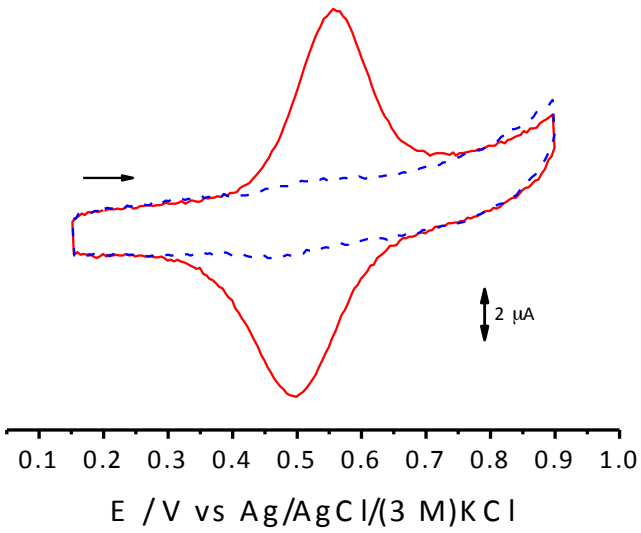
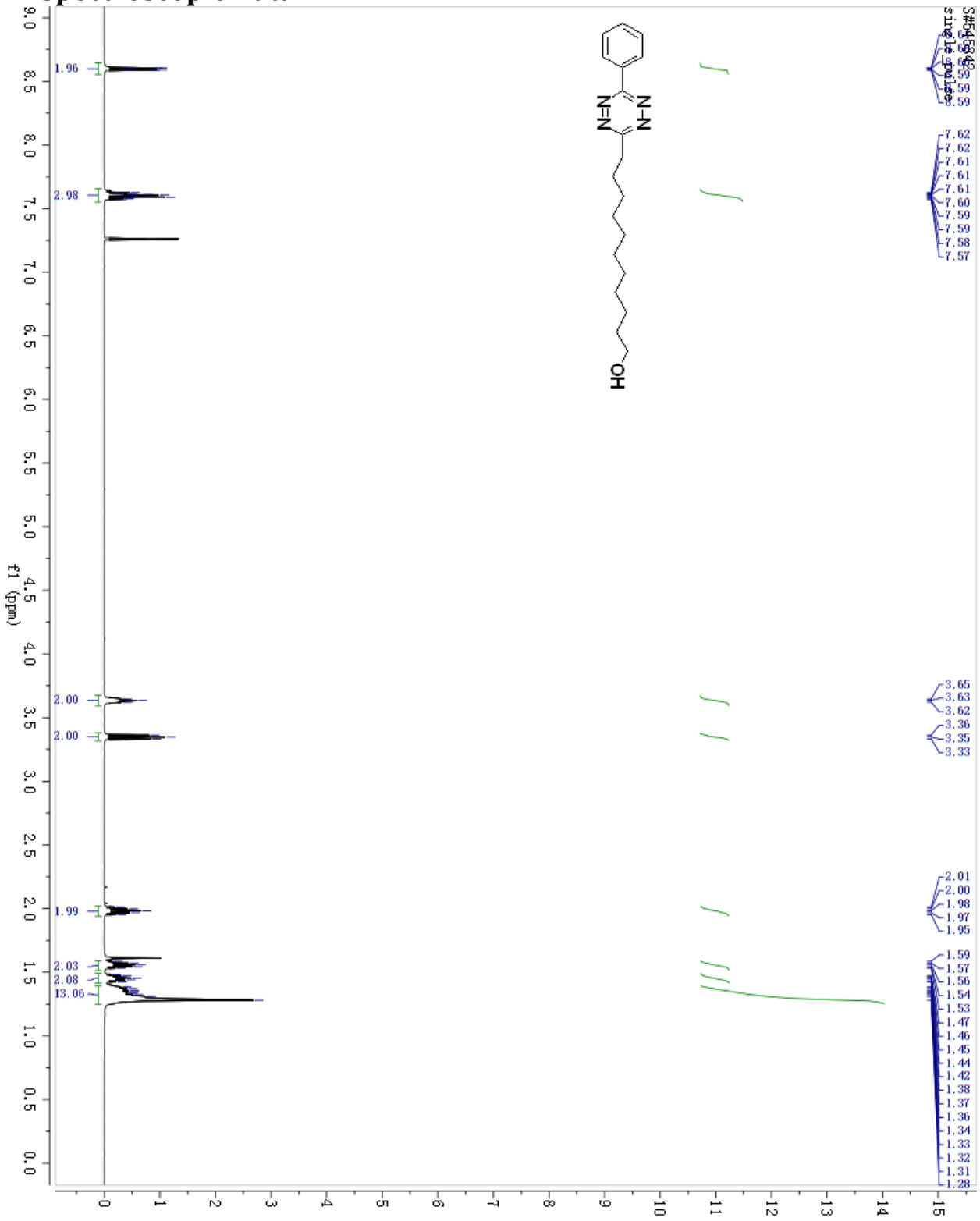
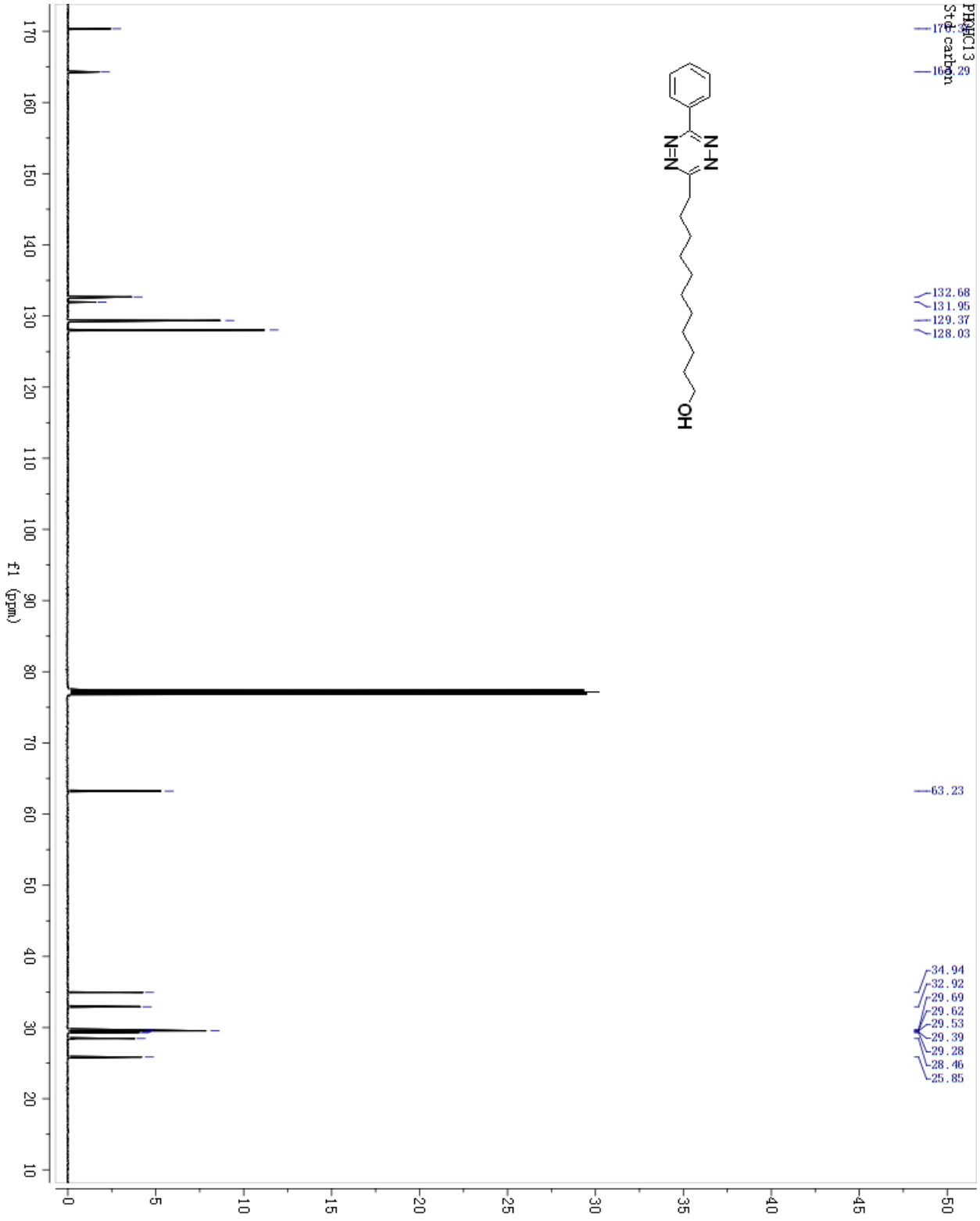
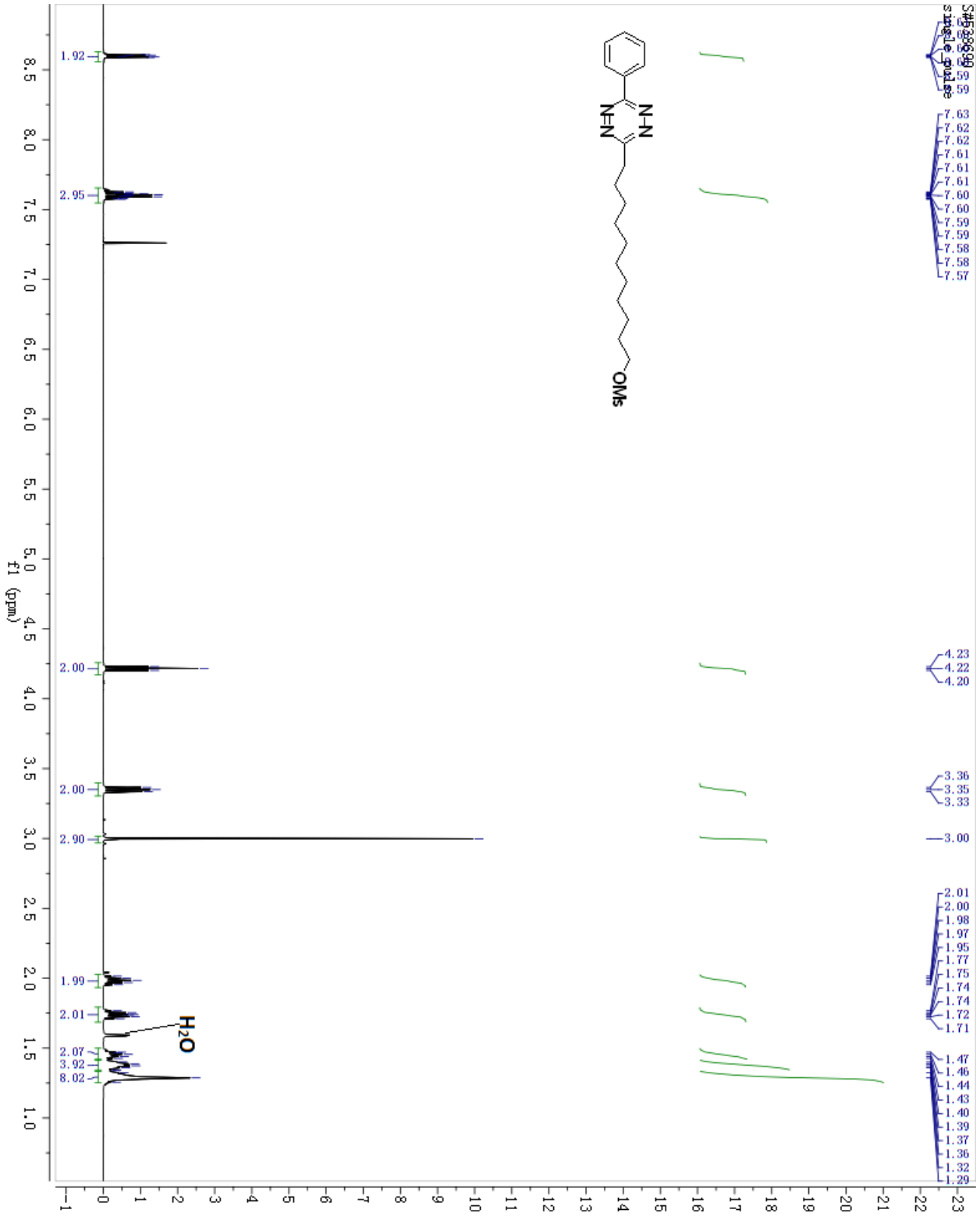


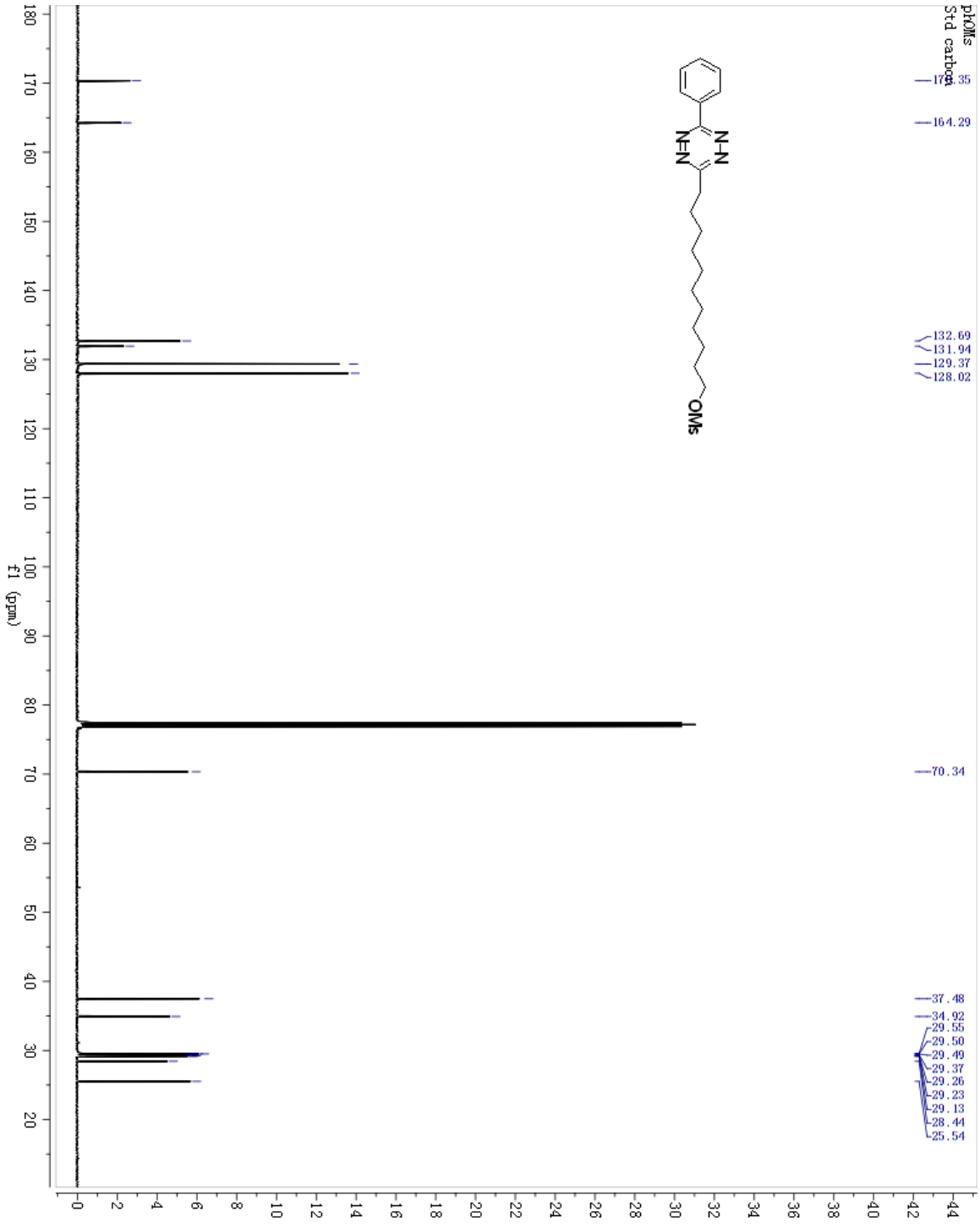
Figure S8: CVs (1 V/s scan rate) of a mixed SAM on gold formed from a 0.1 mM ethanolic solution of **1**. Electrolyte buffered 0.1 M NaCl (pH = 4). CV recorded after the SAM was contacted to a 1 μM solution of **3** for 15 minutes, while the potential was held at -600 mV (dashed blue line). CV recorded after the identical SAM was contacted to a 1 μM solution of **3** for 15 minutes, while the potential was held at open circuit (solid red line).

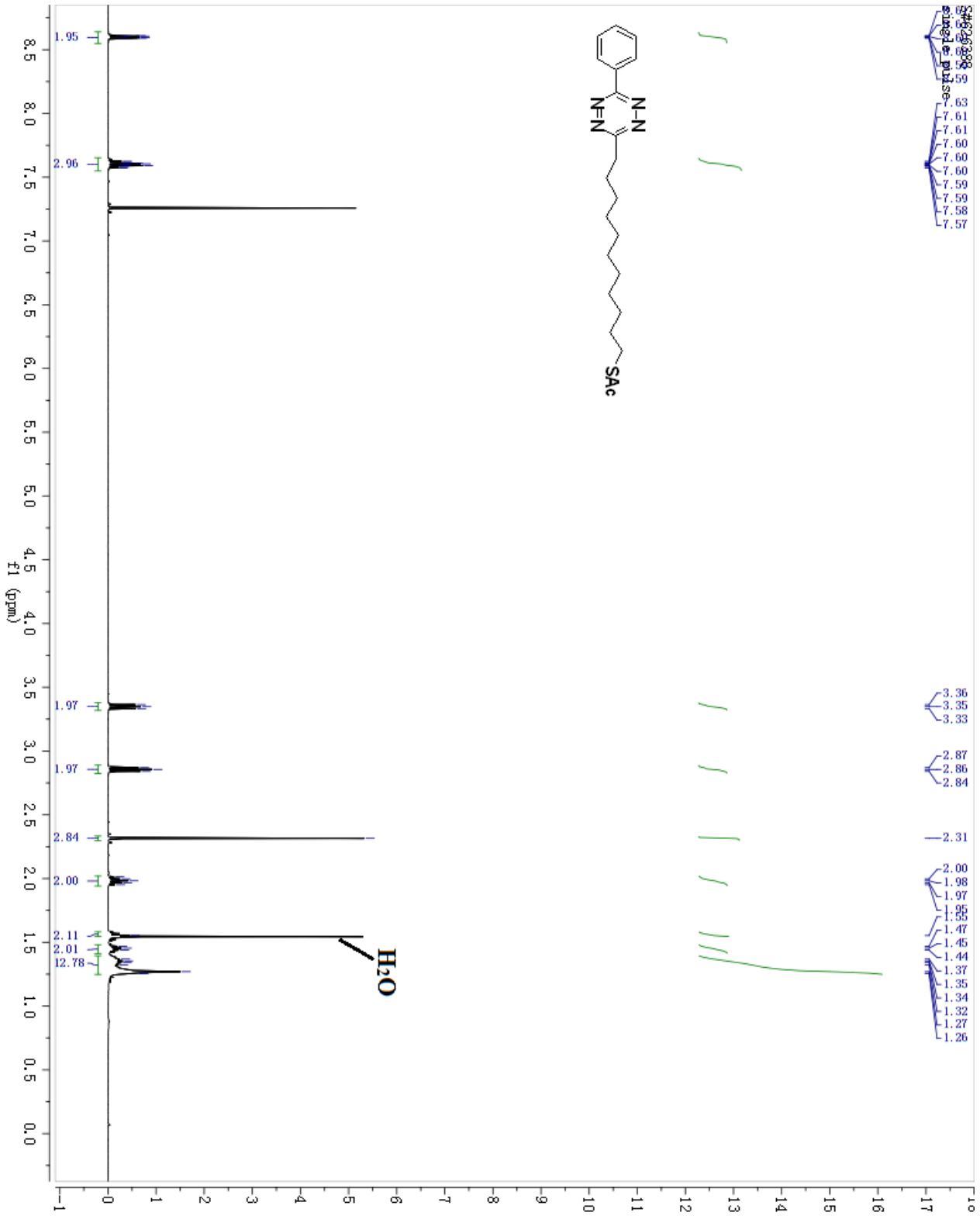
7 Spectroscopic Data

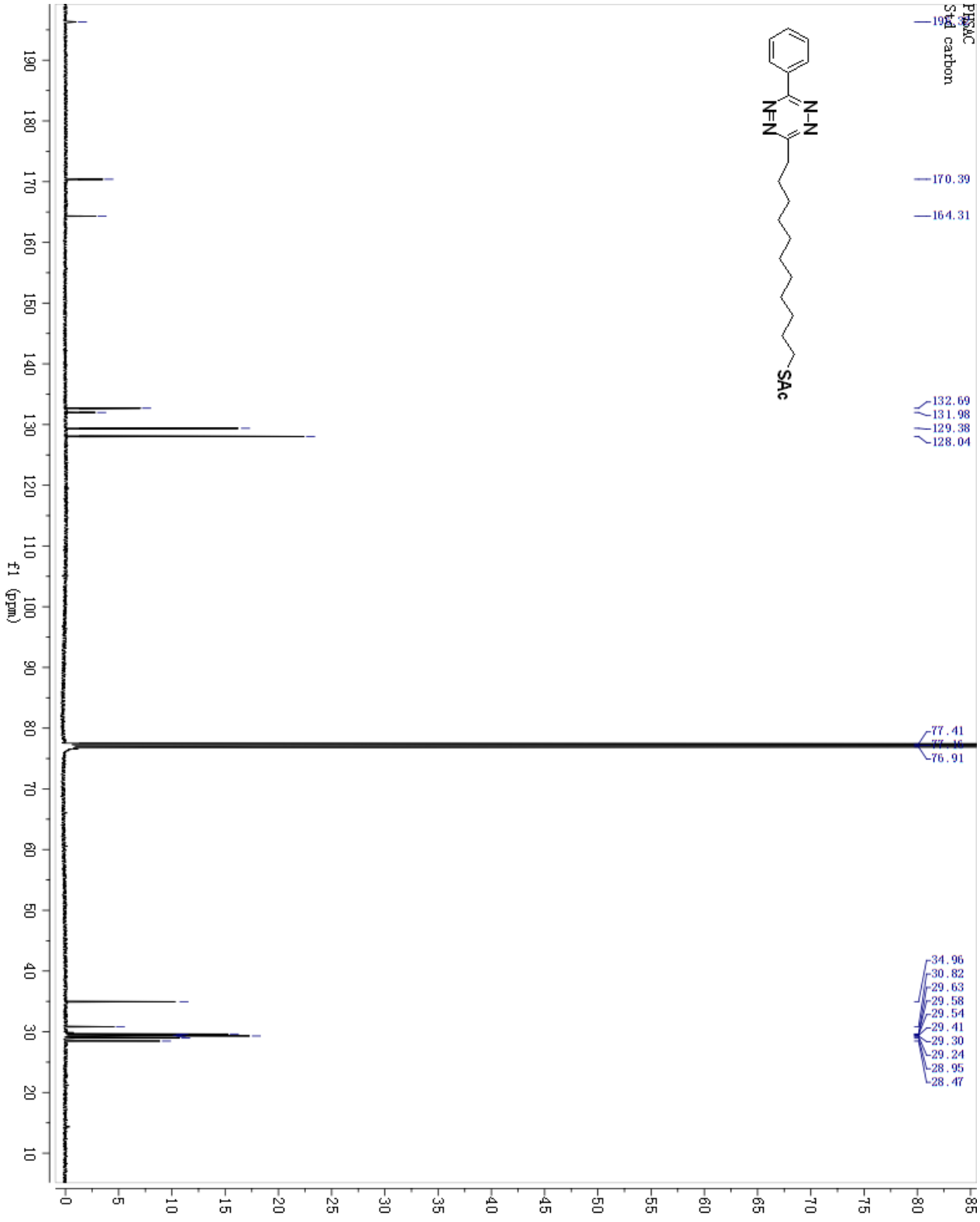


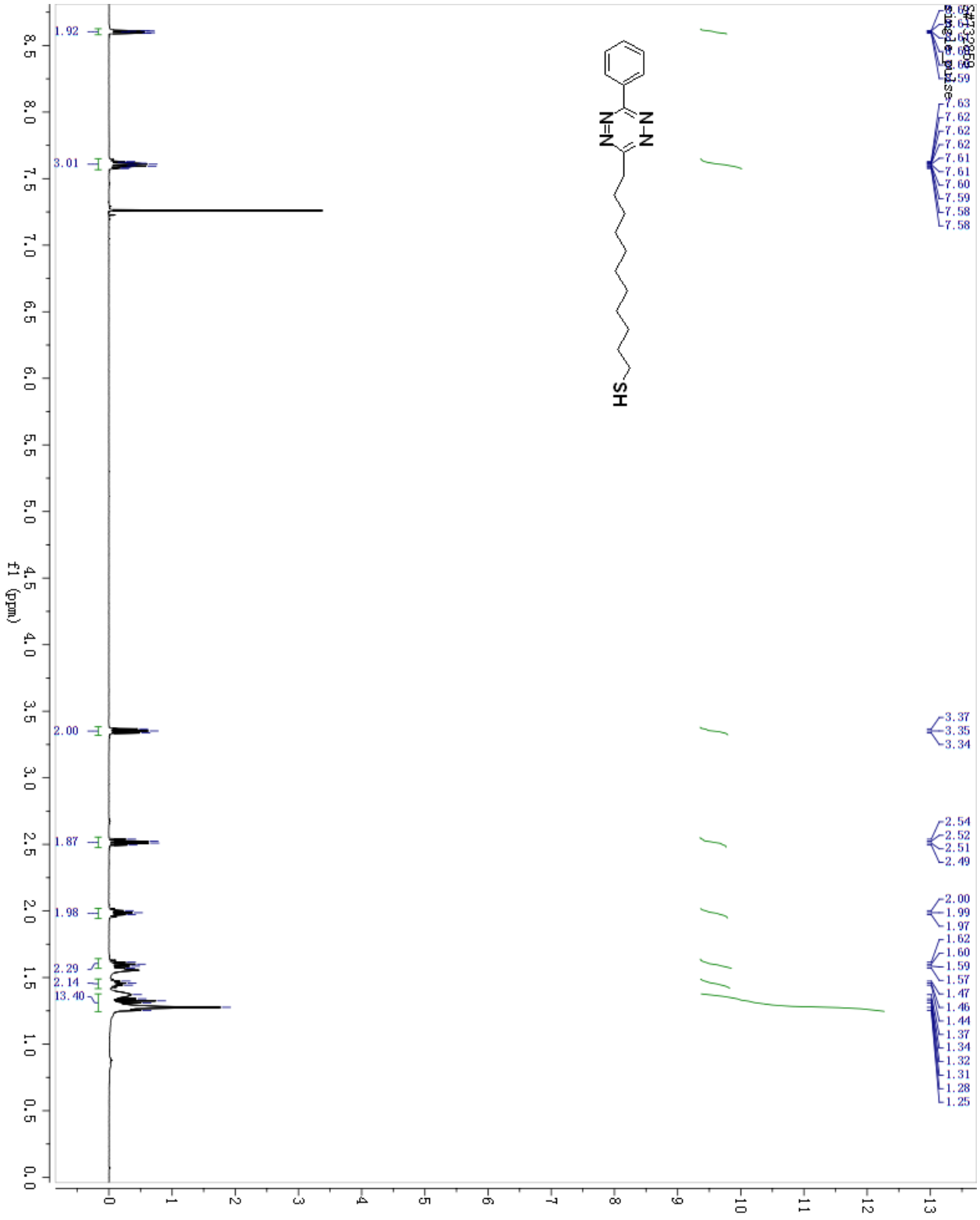

PH-HC13
Std carbon
170.29
132.68
131.95
129.37
128.03
63.23
34.94
32.92
29.69
29.62
29.53
29.39
29.28
28.46
25.85
f1 (ppm)
OH

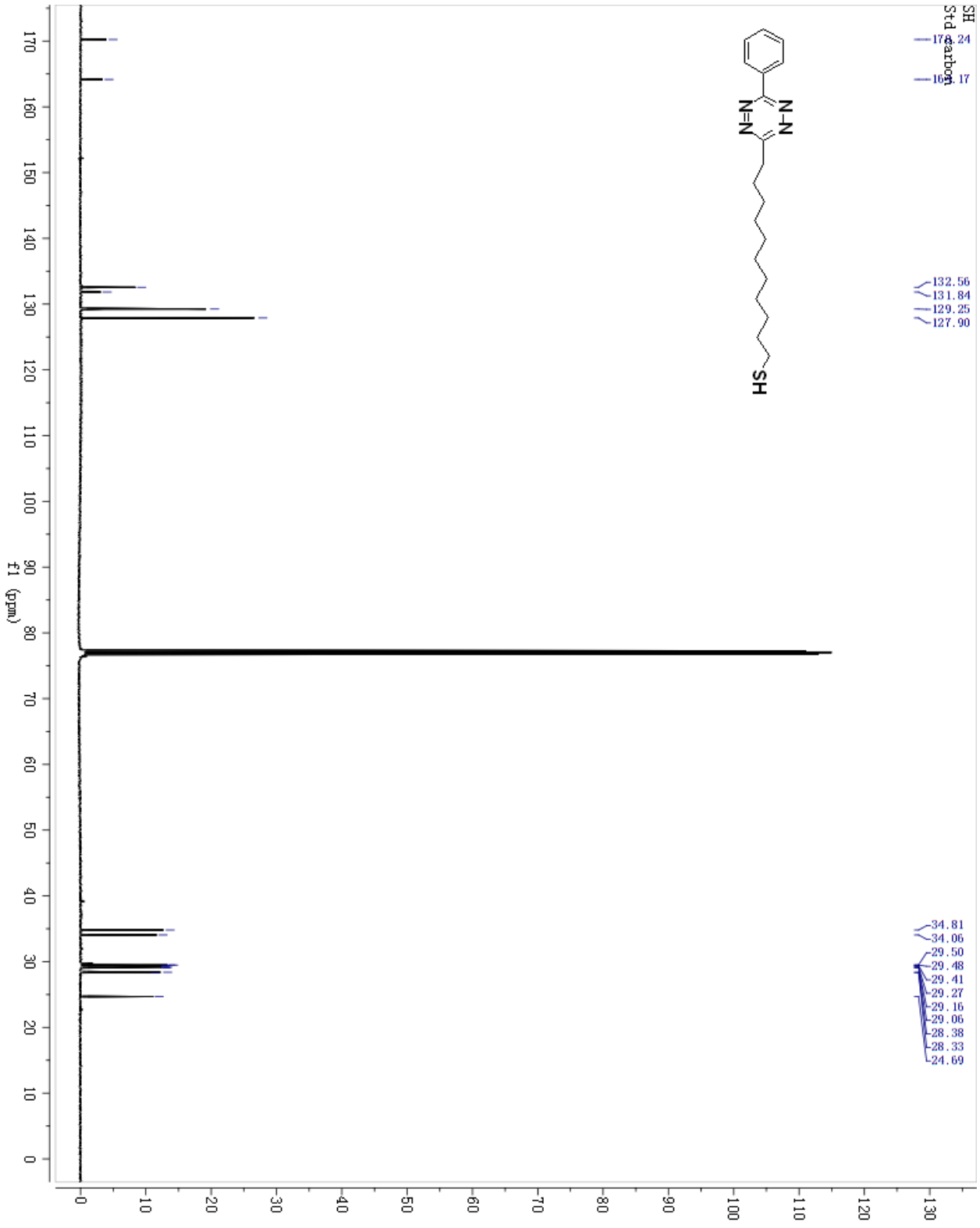

OMs
H2O
f1 (ppm)

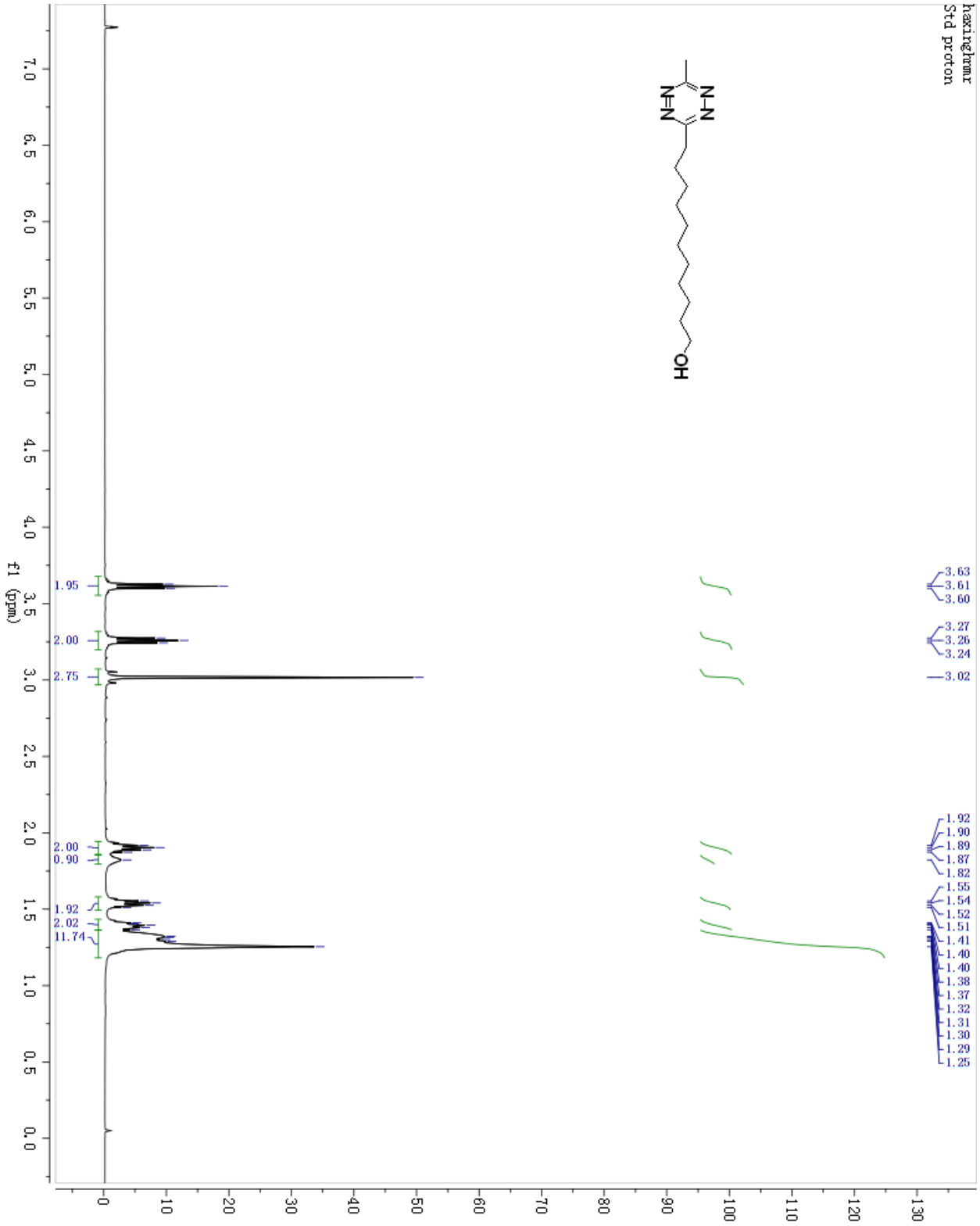

phOMs
Std carbon
172.35
164.29
132.69
131.94
129.37
128.02
70.34
37.48
34.92
29.55
29.50
29.49
29.37
29.26
29.23
29.13
28.44
25.54
OMs
f1 (ppm)

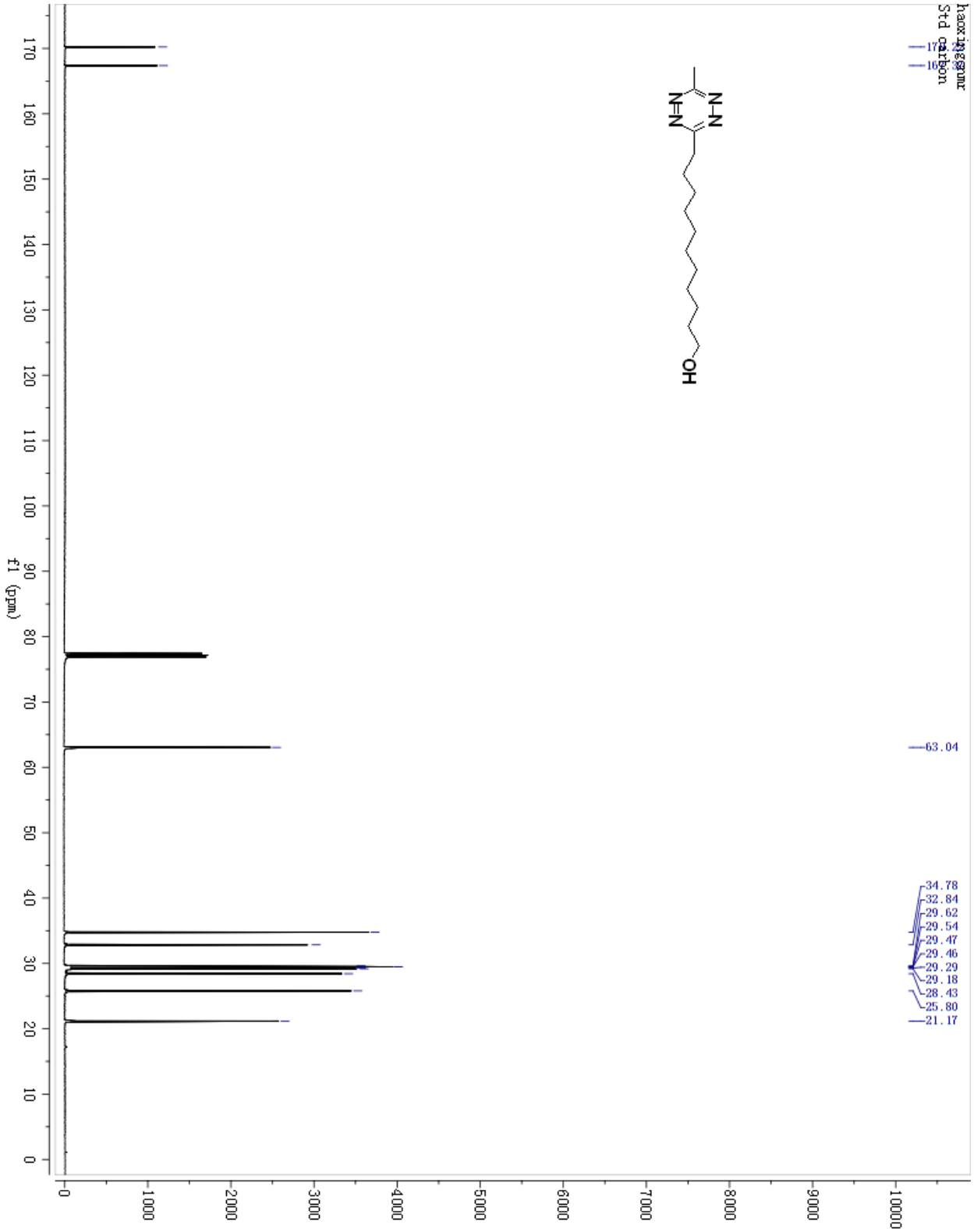

1.95
2.96
1.97
1.97
2.84
2.00
2.11
2.01
12.78
H2O
N-N
N=N
SAc
7.63
7.61
7.61
7.60
7.60
7.60
7.59
7.59
7.58
7.57
3.36
3.35
3.33
2.87
2.86
2.84
2.31
2.00
1.98
1.97
1.95
1.55
1.47
1.45
1.44
1.37
1.35
1.34
1.32
1.27
1.26
f1 (ppm)

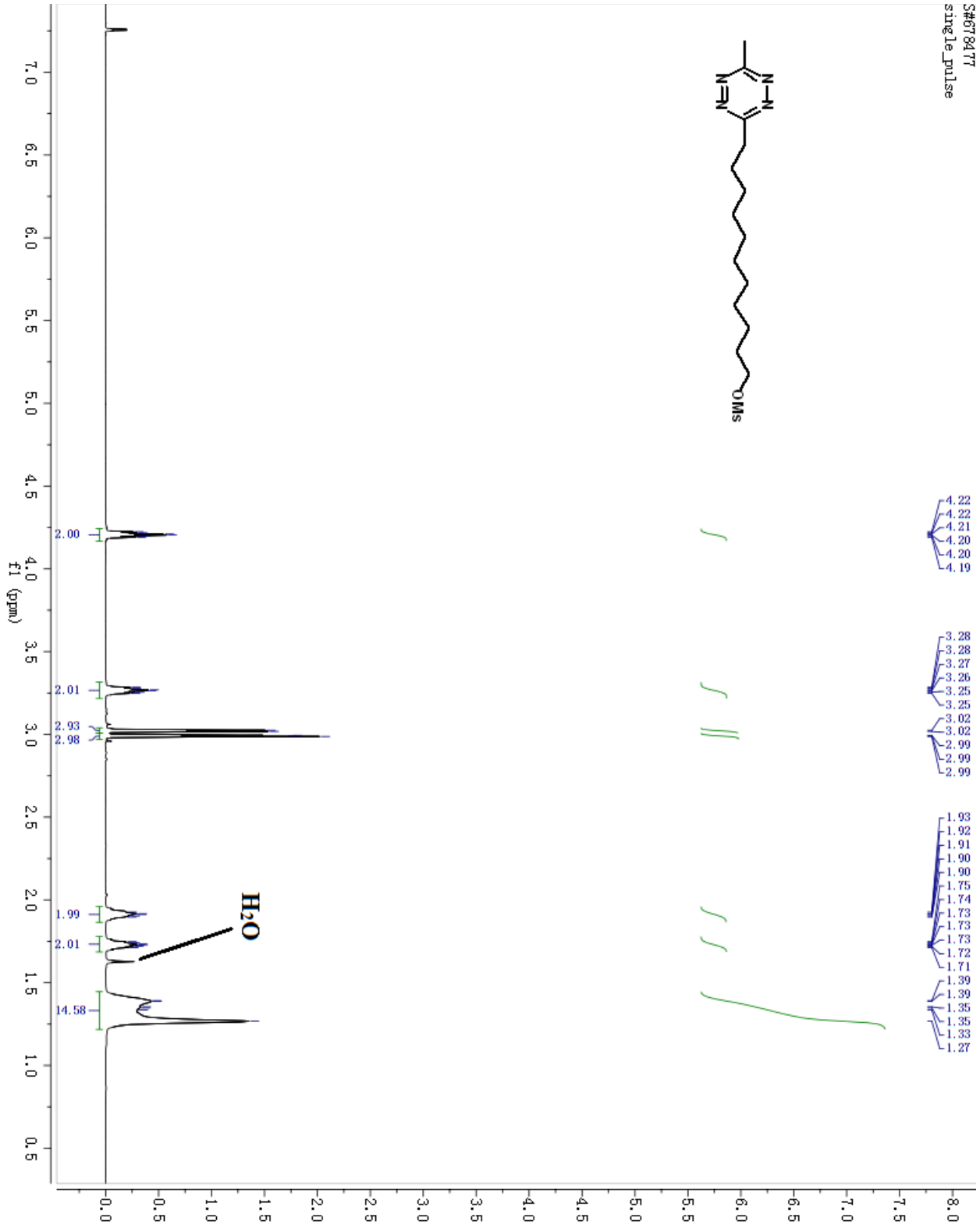

PHSAC
Std carbon
N-N
N=N
SAc
197.
170.39
164.31
132.69
131.98
129.38
128.04
77.41
76.91
34.96
30.82
29.63
29.58
29.54
29.41
29.30
29.24
28.95
28.47
f1 (ppm)

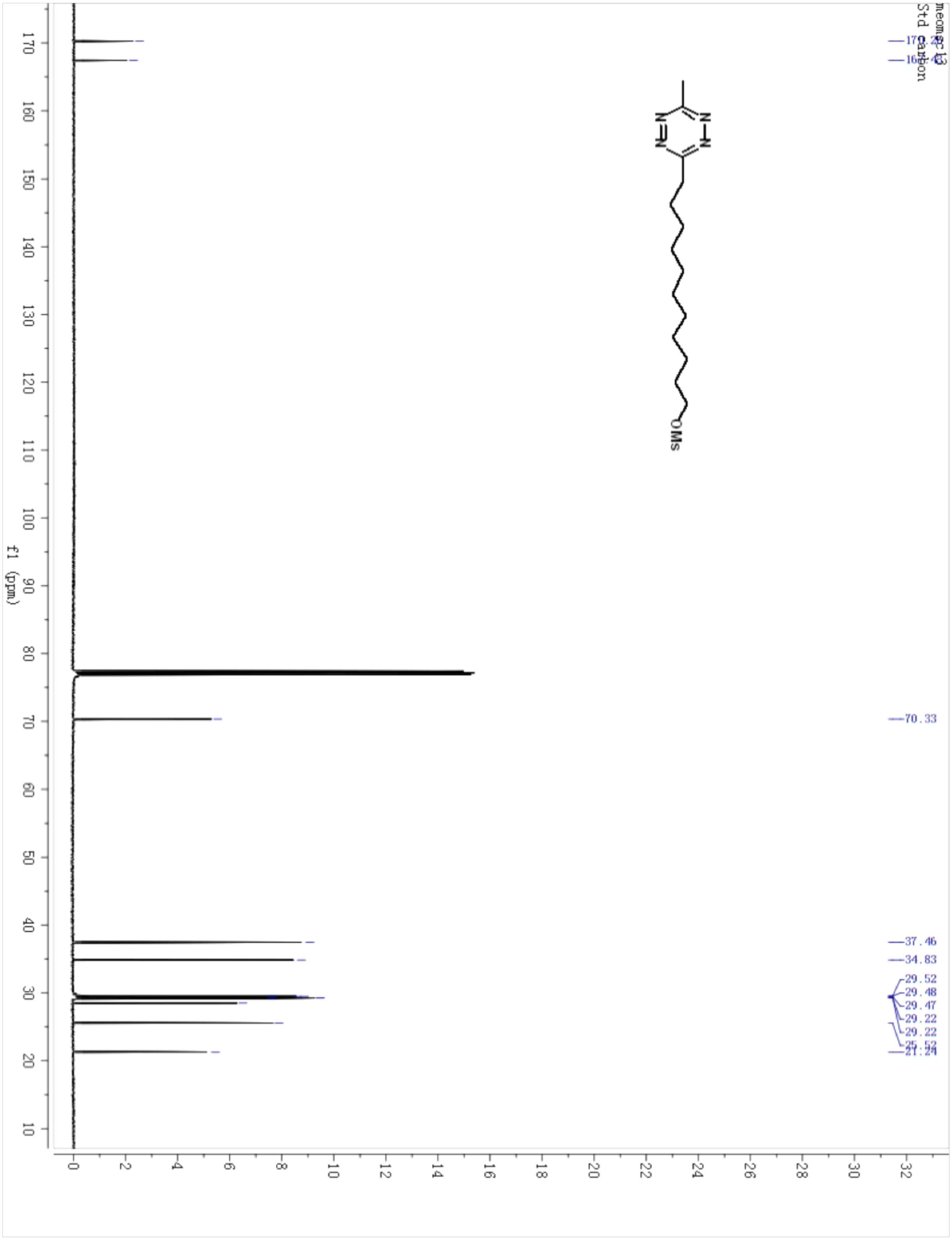

1.92
3.01
2.00
1.87
1.98
2.29
2.14
13.40
f1 (ppm)
SH

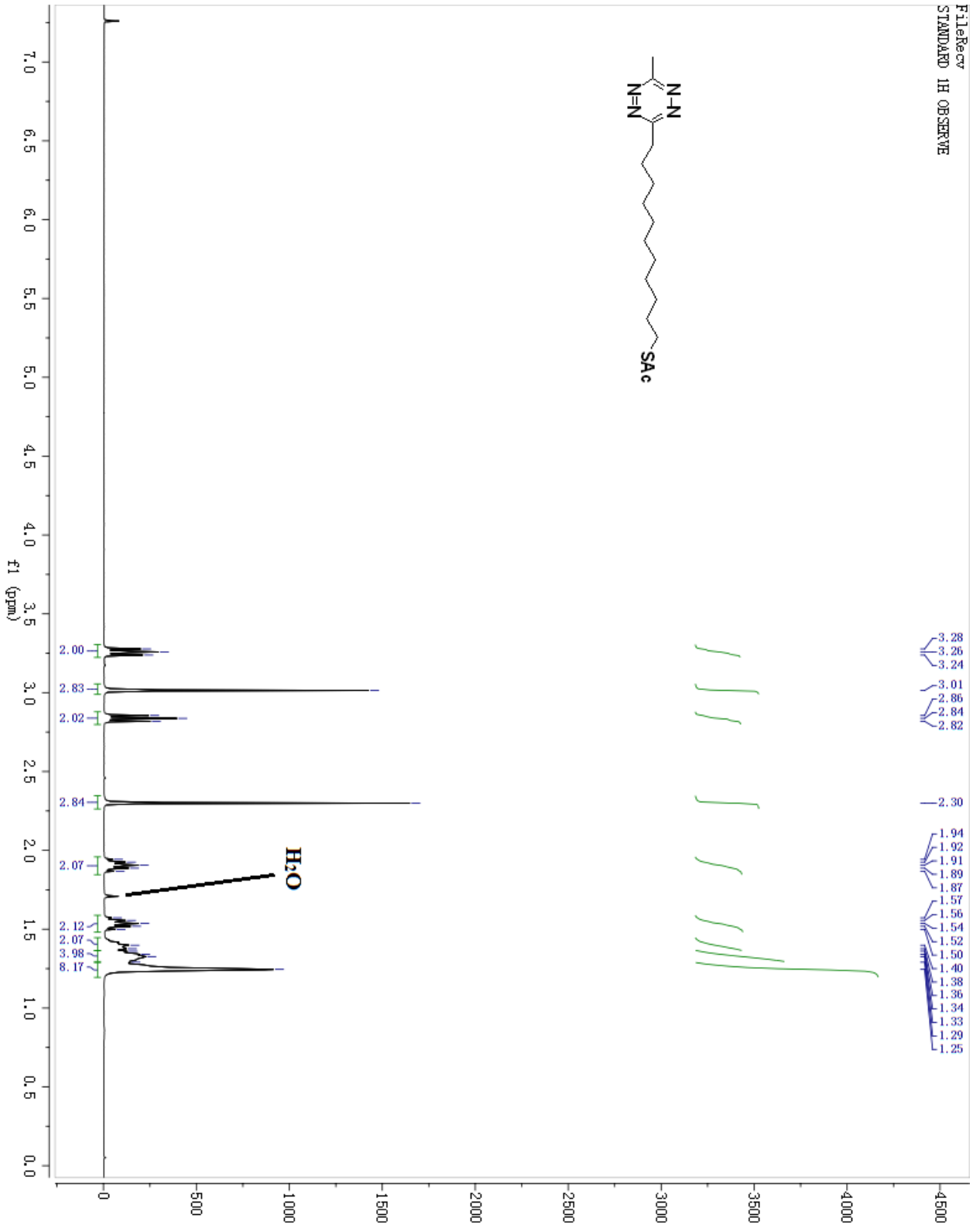

SH
Std Carbon
178.24
163.17
132.56
131.84
129.25
127.90
34.81
34.06
29.50
29.48
29.41
29.27
29.16
29.06
28.38
28.33
24.69
HS
f1 (ppm)


haxinghmar
Std proton
f1 (ppm)
1.95
2.00
2.75
2.00
0.90
1.92
2.02
11.74
3.63
3.61
3.60
3.27
3.26
3.24
3.02
1.92
1.90
1.89
1.87
1.82
1.55
1.54
1.52
1.51
1.41
1.40
1.40
1.38
1.37
1.32
1.31
1.30
1.29
1.25
OH


haoxingguanr
Std carbon
17
16
63.04
34.78
32.84
29.62
29.54
29.47
29.46
29.29
29.18
28.43
25.80
21.17
N=N
N-N
OH
f1 (ppm)


S#678477
single_pulse
OMs
H2O
2.00
2.01
2.93
2.98
1.99
2.01
14.58
4.22
4.22
4.21
4.20
4.20
4.19
3.28
3.28
3.27
3.26
3.25
3.25
3.02
3.02
2.99
2.99
2.99
1.93
1.92
1.91
1.90
1.90
1.75
1.74
1.73
1.73
1.73
1.72
1.71
1.39
1.39
1.35
1.35
1.33
1.27
f1 (ppm)


Std Carbon
70.33
37.46
34.83
29.52
29.48
29.47
29.22
29.22
21.24
OMs
f1 (ppm)


FileRecv
STANDARD 1H OBSERVE
N-N
N=N
SAc
H2O
f1 (ppm)
2.00
2.83
2.02
2.84
2.07
2.12
2.07
3.98
8.17
3.28
3.26
3.24
3.01
2.86
2.84
2.82
2.30
1.94
1.92
1.91
1.89
1.87
1.57
1.56
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1.52
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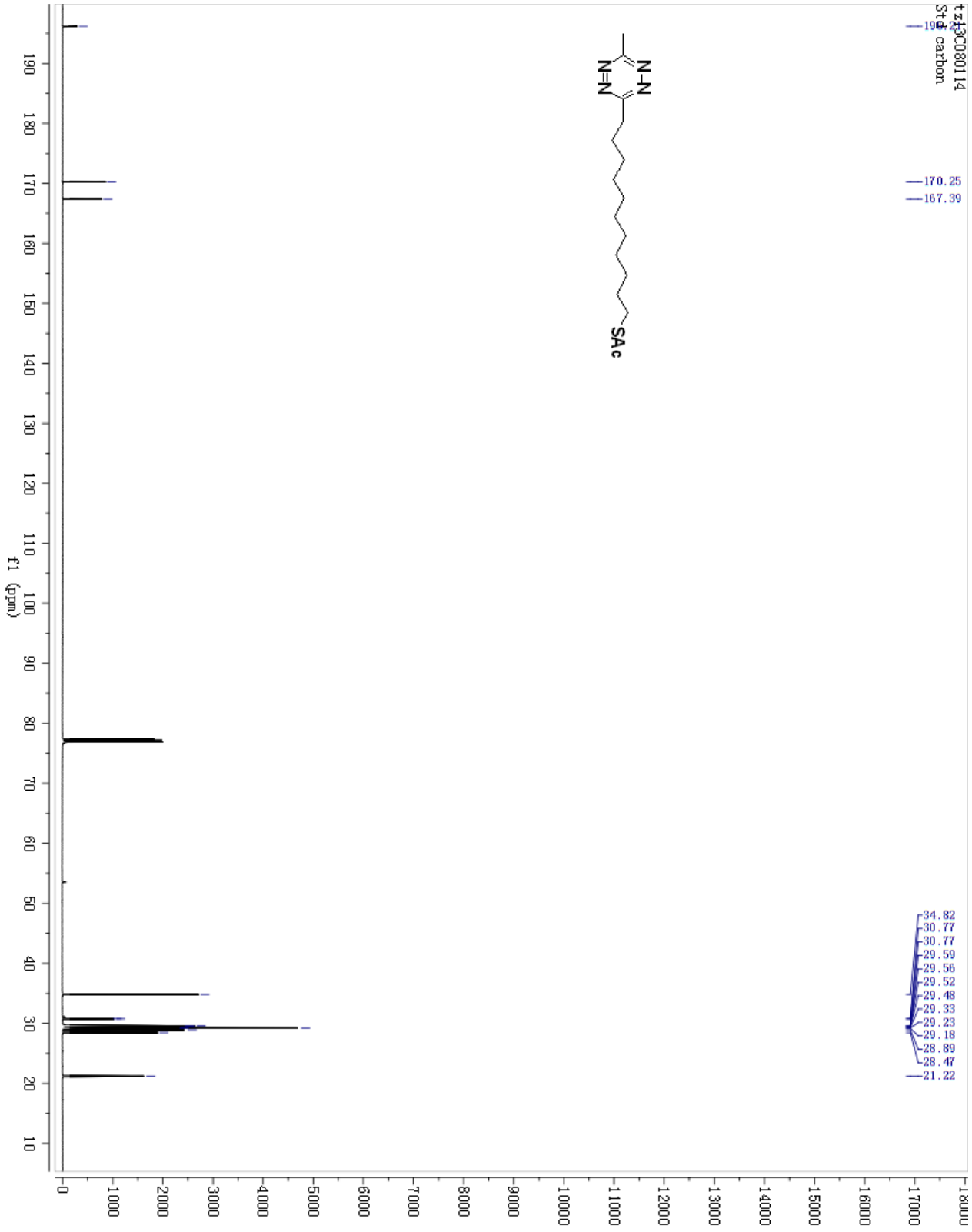
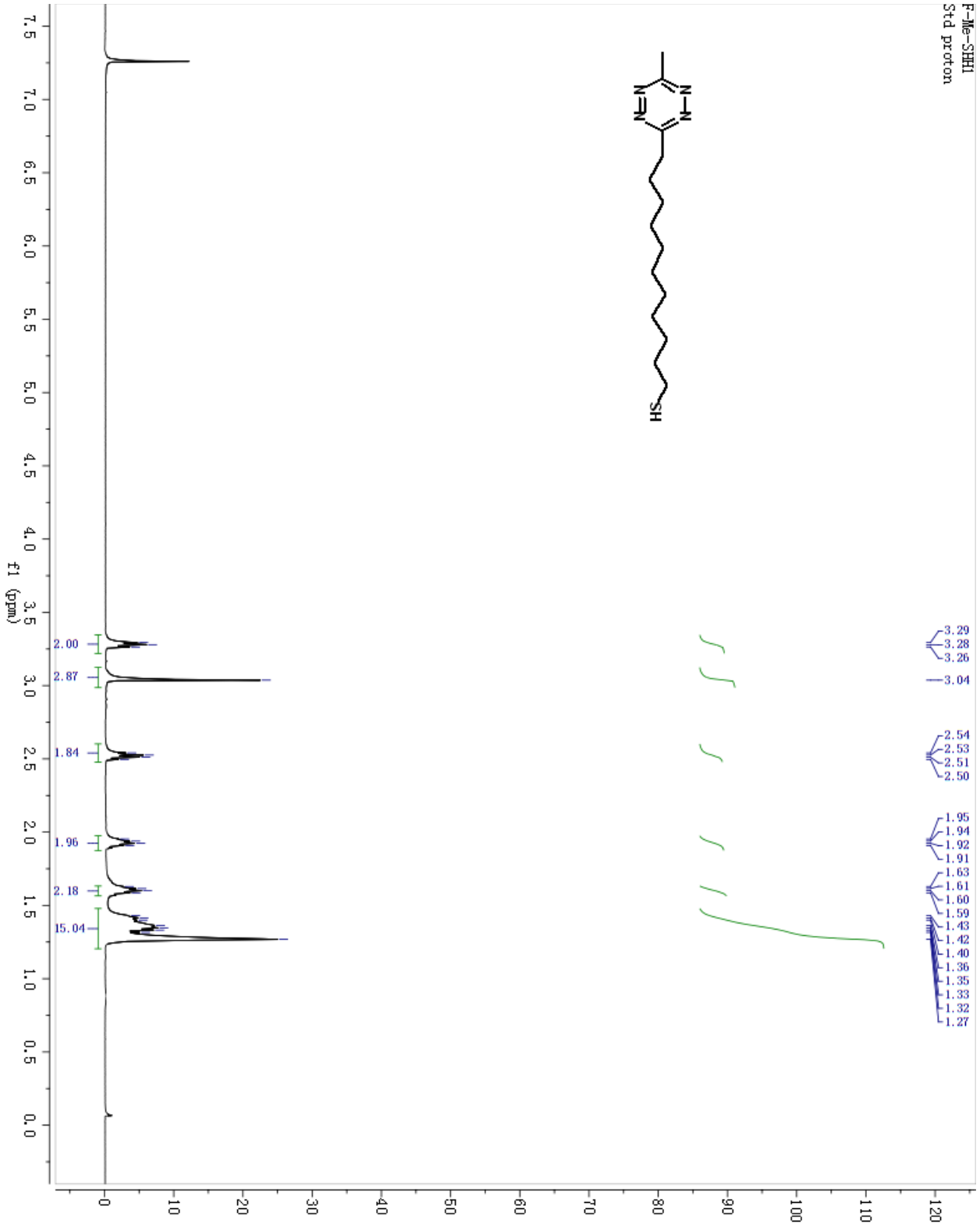
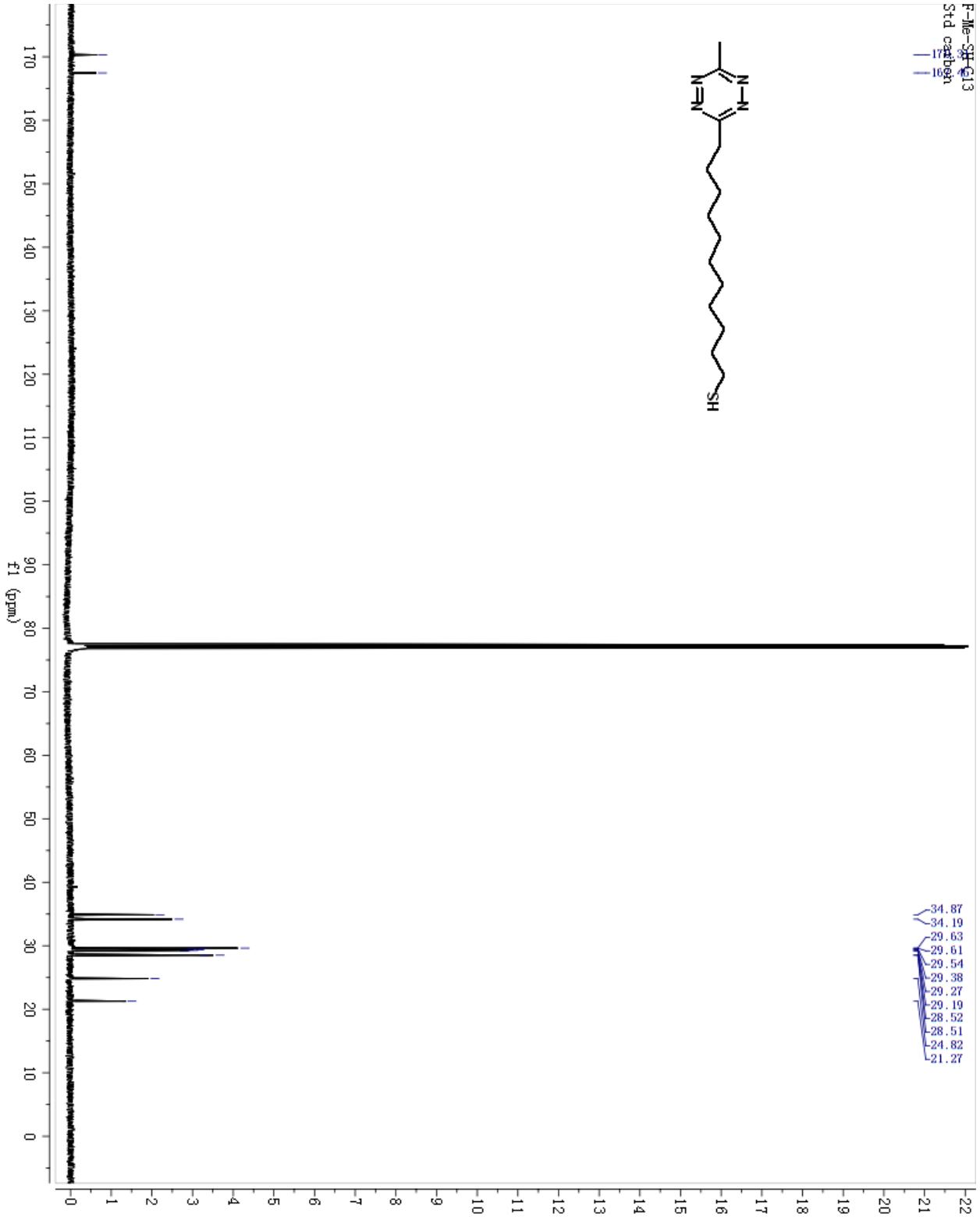
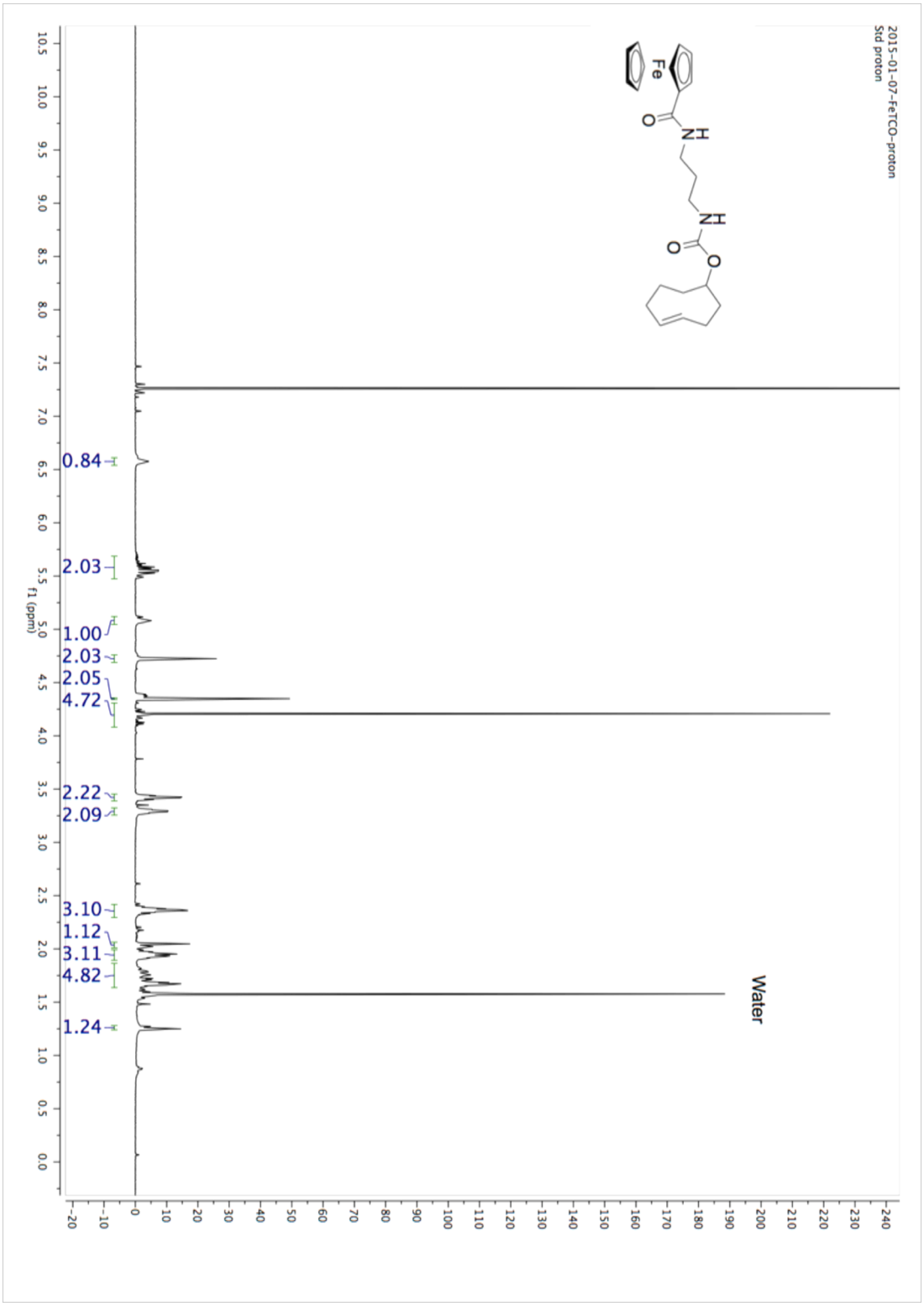
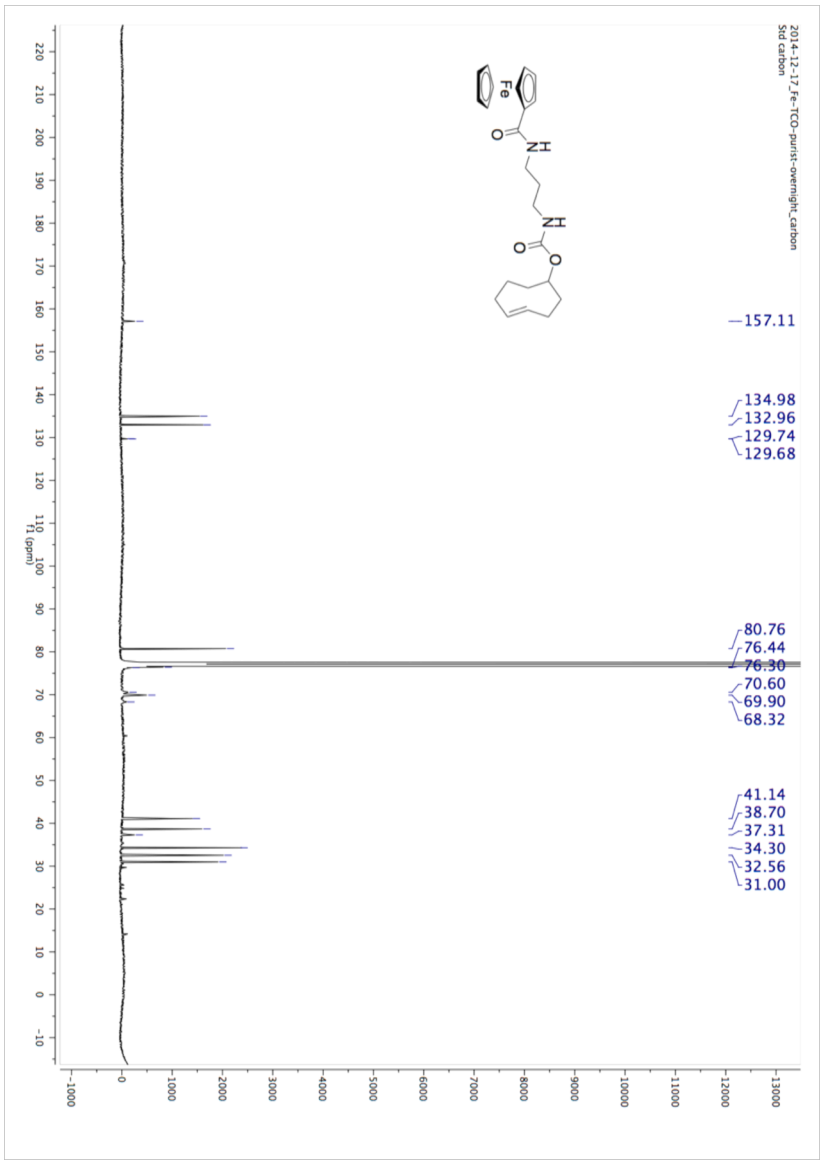
1.38
1.36
1.34
1.33
1.29
1.25


tzl3C080114
Std carbon
190.22
170.25
167.39
34.82
30.77
30.77
29.59
29.56
29.52
29.48
29.33
29.23
29.18
28.89
28.47
21.22
N-N
N=N
SAc
f1 (ppm)


F-Me-SHH1
Std proton
f1 (ppm)
2.00
2.87
1.84
1.96
2.18
15.04
3.29
3.28
3.26
3.04
2.54
2.53
2.51
2.50
1.95
1.94
1.92
1.91
1.63
1.61
1.60
1.59
1.43
1.42
1.40
1.36
1.35
1.33
1.32
1.27
SH


F-Me-SH-C13
Std carbon
170.38
169.47
34.87
34.19
29.63
29.61
29.54
29.38
29.27
29.19
28.52
28.51
24.82
21.27
SH
f1 (ppm)


2015-01-07-FeTCO-proton
Std proton
Fe
O
N
H
N
H
O
O
Water
0.84
2.03
1.00
2.03
2.05
4.72
2.22
2.09
3.10
1.12
3.11
4.82
1.24
f1 (ppm)


2014-12-17_Fe-TCO-purist-overnight_carbon
Std carbon
Fe
O
N
H
N
H
O
O
157.11
134.98
132.96
129.74
129.68
80.76
76.44
76.30
70.60
69.90
68.32
41.14
38.70
37.31
34.30
32.56
31.00
f1 (ppm)