Supporting Information

Thiol-Retaining N-Terminal Cysteine Chemistry for Dual Modification and Bicyclic Peptide Construction

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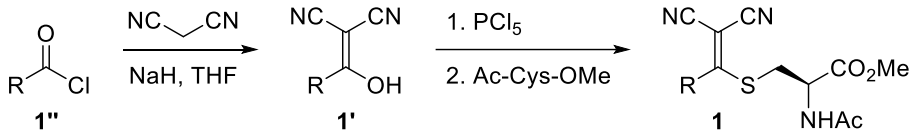
Table of Contents

Chemical Synthesis

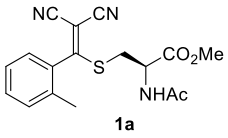
General procedure for TAMM synthesis



1'' **1'** **1**

To a suspension of NaH (288 mg, 12 mmol, 6 equiv.) in anhydrous THF (10 mL) under nitrogen atmosphere at 0 °C was added a solution of malononitrile (397 mg, 6 mmol, 3 equiv.) in anhydrous THF (10 mL) in a dropwise manner. After 1 hour at 0 °C, a solution of **1″** (2 mmol, 1 equiv.) in anhydrous THF (10 mL) was added into the mixture at 0 °C in a dropwise manner. After the addition, the reaction was allowed to warm up to room temperature. After 2 hours at room temperature, the solvent was removed under reduced pressure. The mixture was acidified to pH 1-2 using $HCl_{(aq)}$. The mixture was extracted with EtOAc (3 ×100 mL). The combined organic layers were washed with brine (3 ×50 mL), dried over Na_2SO_4, filtered and concentrated. Silica gel column chromatography was performed to isolate **1′**.

To a solution of **1′** (1 mmol, 1.0 equiv.) in anhydrous acetonitrile (20 ml) under nitrogen atmosphere was added PCl_5 (624 mg, 3 mmol, 3 equiv.). After 6 hours at 65 °C, the solvent was removed under reduced pressure. The residue was dissolved in DCM (60 mL), washed with water (3 × 20 mL) and brine (20 mL), dried over Na_2SO_4, filter and concentrate. The residue was then dissolved in acetonitrile (15 mL), followed by addition of Ac-Cys-OMe (213 mg, 1.2 mmol, 1.2 equiv.) and NaHCO3 (252 mg, 3 mmol, 3 equiv.). After overnight at room temperature, the solvent was removed under reduced pressure. The mixture was purified by silica gel column chromatography to afford **1**.



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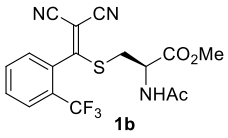

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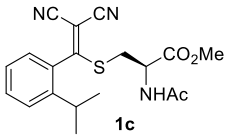

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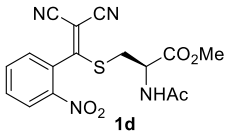

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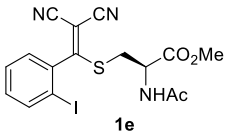

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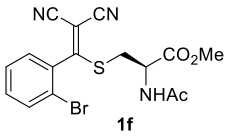

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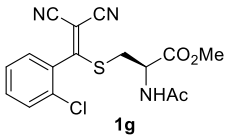

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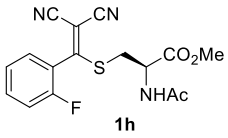

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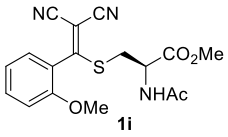

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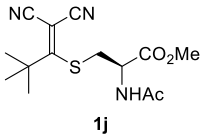

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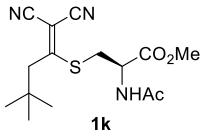
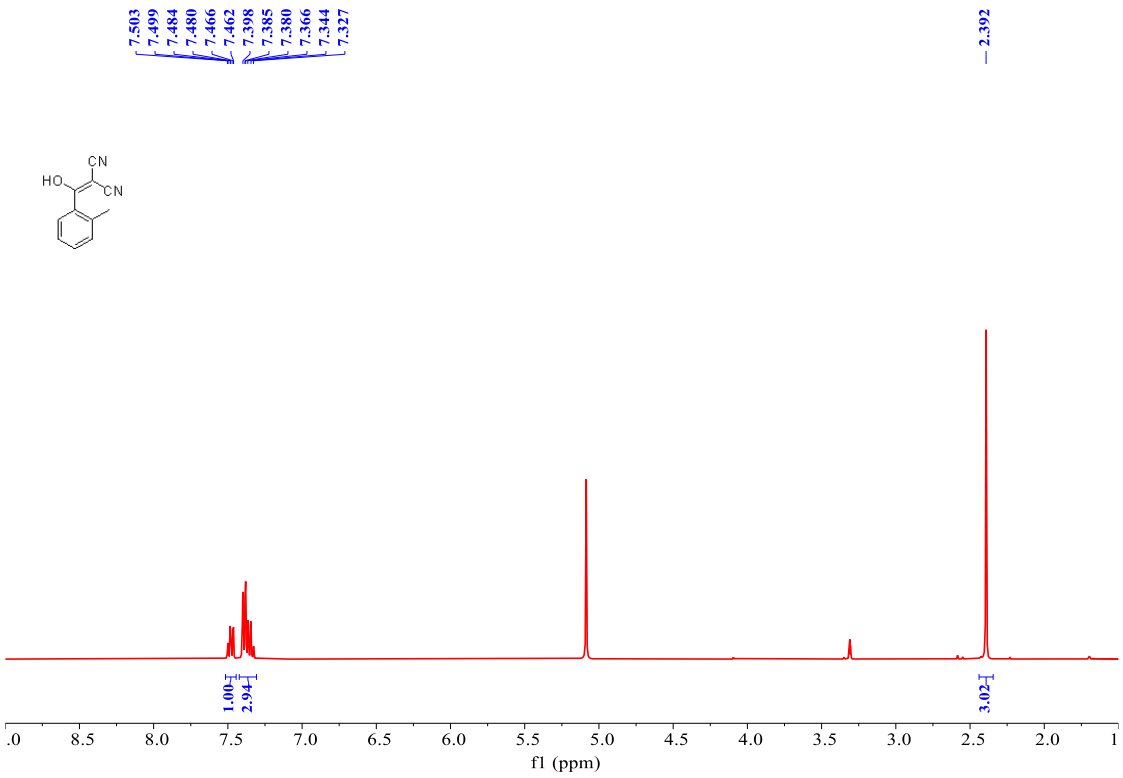


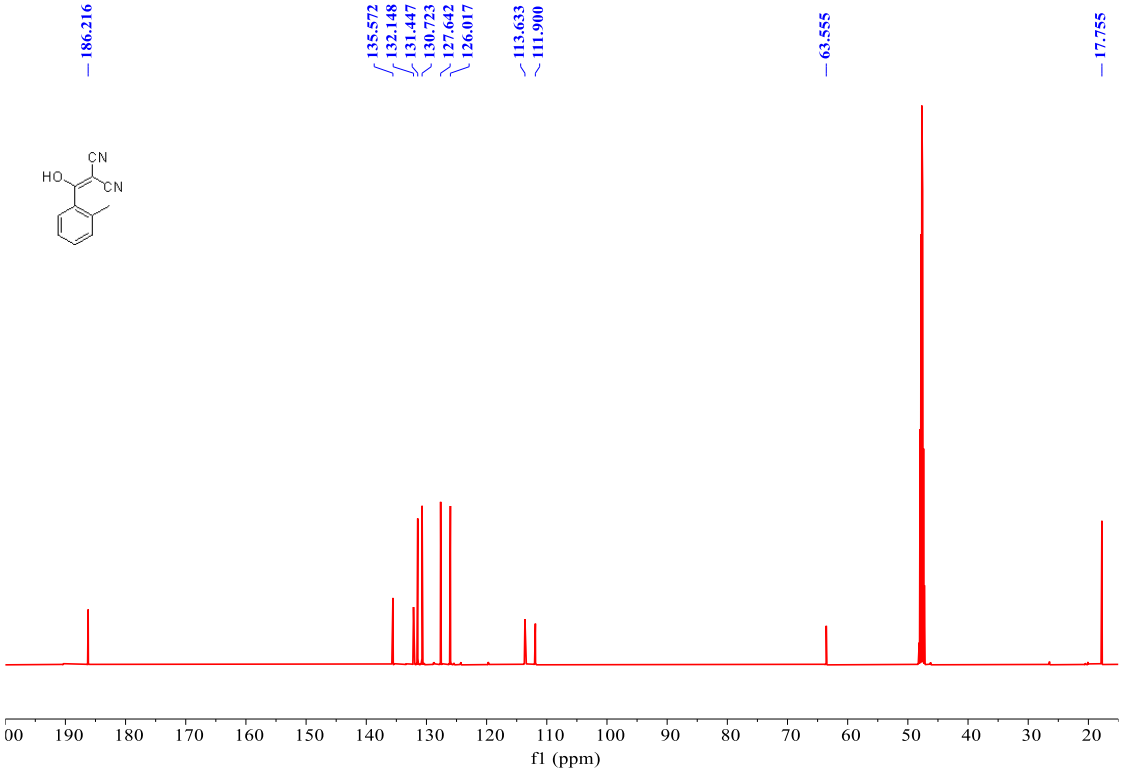
1k

TAMM 1a

Compound **1a'** (360 mg, 1.96 mmol, 98% yield) was obtained as a light yellow solid from silica gel column chromatography (DCM: MeOH=10:1). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.482 (td, J = 7.40, 1.60 Hz, 1H), 7.398 – 7.327 (m, 3H), 2.392 (s, 3H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 186.216, 135.572, 132.148, 131.447, 130.723, 127.642, 126.017, 113.633, 111.900, 63.555, 17.755. ESI-(-)-HRMS $(M-H)^-$ calculated for $C_{11}H_8N_2O$: 183.05639; found: 183.05632 (-3.1 ppm). R_f (DCM: MeOH=10:1) = 0.2.

Compound **1a** (133 mg, 0.38 mmol, 38% yield) was obtained as a light yellow solid from silica gel column chromatography (EA: PE=2:1). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.426 – 7.388 (m, 1H), 7.328 (d, J = 7.60 Hz, 2H), 7.082 (dd, J = 22.40, 7.60 Hz, 1H), 6.621 – 6.518 (m, 1H), 4.584 (dtd, J = 11.00, 6.70, 5.60, 2.80 Hz, 1H), 3.682 (dd, J = 6.80, 3.20 Hz, 3H), 3.108 – 2.814 (m, 2H), 2.284 (s, 3H), 1.960 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-d) δ 181.951, 181.771, 170.186, 169.524, 169.480, 134.975, 134.684, 131.714, 131.653, 131.585, 131.560, 131.455, 127.514, 127.379, 127.074, 126.963, 111.850, 111.587, 80.585, 80.496, 53.147, 53.102, 51.149, 51.078, 35.686, 35.660, 22.850, 22.833, 18.944, 18.909. ESI-(+)-HRMS $(M+H)^+$ calculated for $C_{17}H_{17}N_3O_3S$: 344.10634; found: 344.10592 (+0.4 ppm). R_f (EA: PE=3:1) = 0.55.

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135.572
132.148
131.447
130.723
127.642
126.017
113.633
111.900
63.555
17.755
HO
CN
CN
f1 (ppm)


7.426
7.407
7.388
7.337
7.318
7.119
7.100
7.063
7.043
6.621
6.604
6.588
6.571
6.561
6.554
6.538
6.529
6.518
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4.611
4.599
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4.557
4.551
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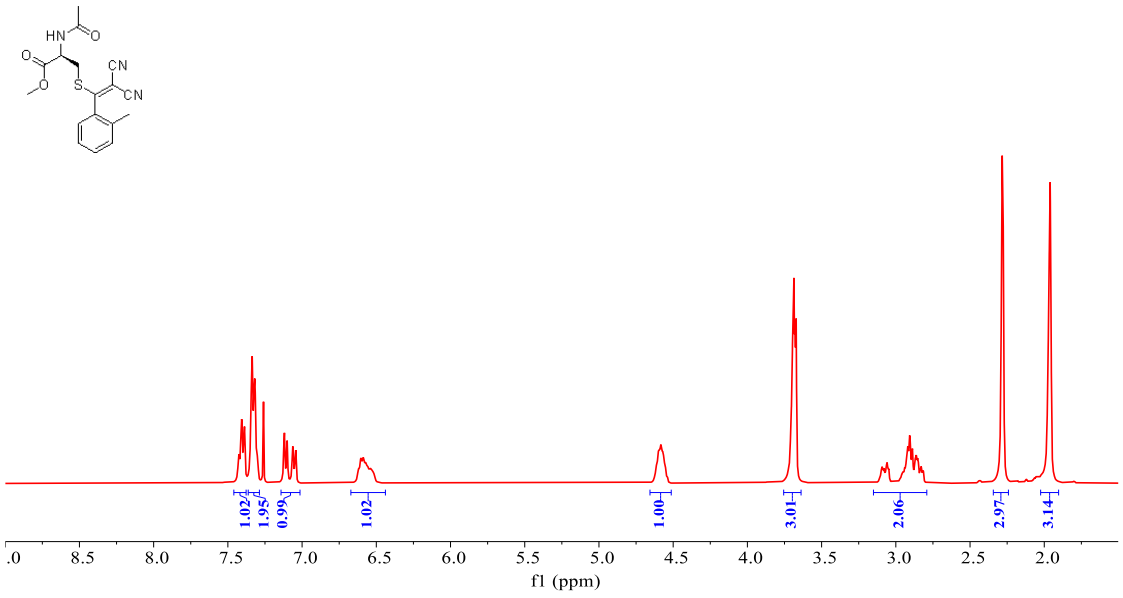
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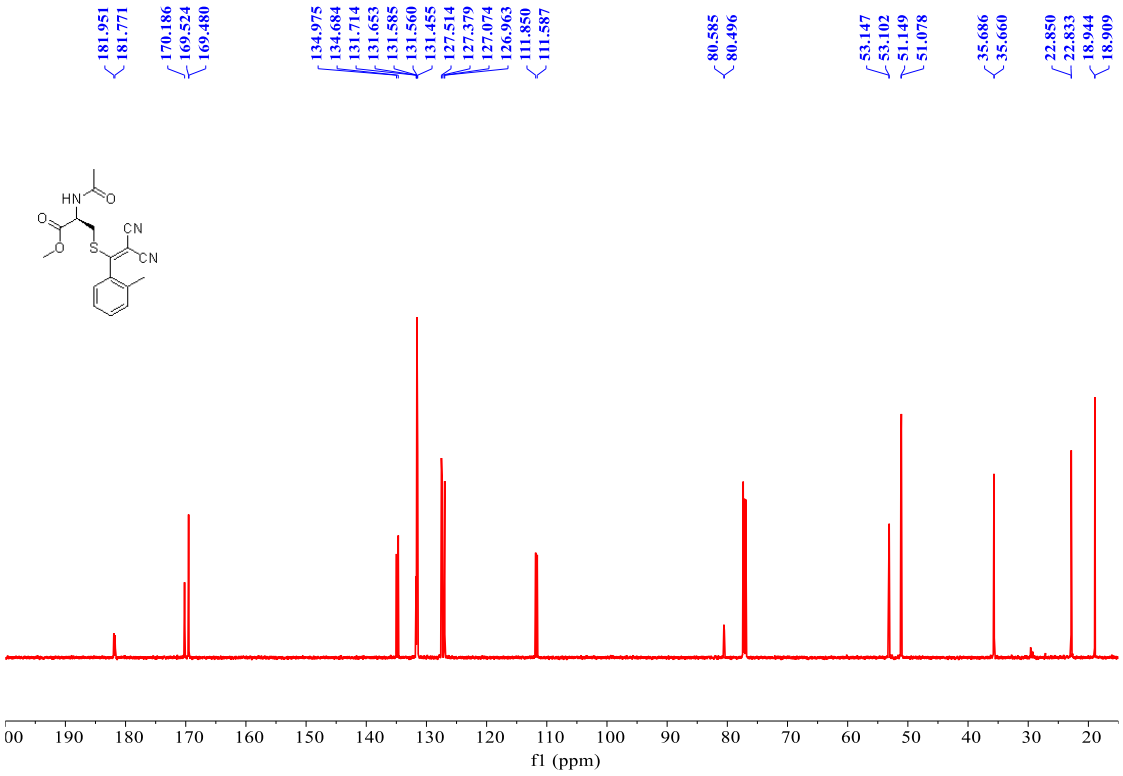
f1 (ppm)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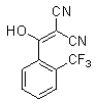
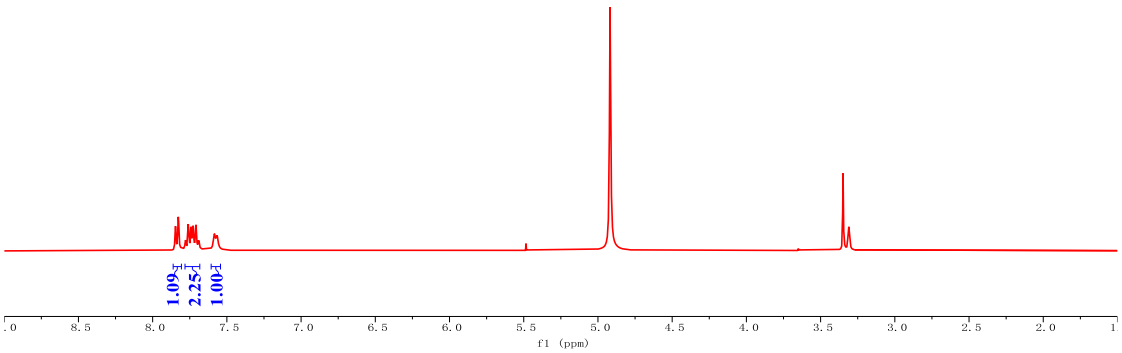
53.102
51.149
51.078
35.686
35.660
22.850
22.833
18.944
18.909
HN
O
O
O
S
CN
CN
00 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
f1 (ppm)

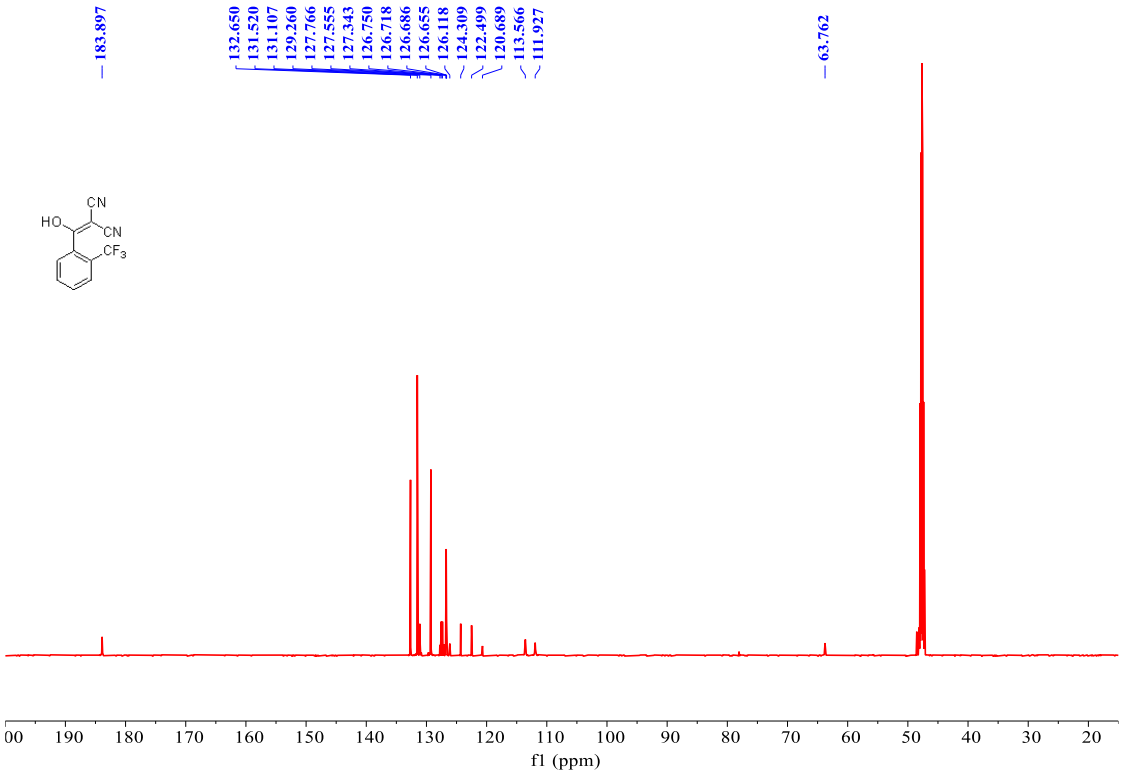
TAMM 1b

Compound **1b'** (471 mg, 1.98 mmol, 99% yield) was obtained as a light yellow solid from silica gel column chromatography (DCM: MeOH=10:1). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.846 – 7.817 (m, 1H), 7.779 – 7.687 (m, 2H), 7.575 (d, *J* = 7.20 Hz, 1H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 183.897, 132.650, 131.520, 131.107, 129.260, 127.766, 127.555, 127.343, 126.750, 126.718, 126.686, 126.655, 126.118, 124.309, 122.499, 120.689, 113.566, 111.927, 63.762. ^{19}F NMR (376 MHz, Methanol-d_4) δ -60.637. ESI-(-)-HRMS $(M-H)^-$ calculated for $C_{11}H_5F_3N_2O$: 237.02812; found: 237.02815 (-0.1 ppm). R_f (DCM: MeOH=5:1) = 0.3.

Compound **1b** (92 mg, 0.23 mmol, 23% yield) was obtained as a light yellow solid from silica gel column chromatography (EA: PE=2:1). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.870 (dd, *J* = 8.00, 2.80 Hz, 1H), 7.796 – 7.690 (m, 2H), 7.326 – 7.260 (m, 1H), 6.292 (dd, *J* = 10.40, 6.40 Hz, 1H), 4.717 – 4.639 (m, 1H), 3.775 (d, *J* = 20.80 Hz, 3H), 3.073 (td, *J* = 12.80, 4.80 Hz, 1H), 2.906 (ddd, *J* = 16.00, 11.20, 5.60 Hz, 1H), 2.028 (d, *J* = 20.00 Hz, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 178.142, 178.004, 170.289, 170.066, 169.449, 169.418, 133.391, 133.265, 131.925, 131.891, 130.436, 130.352, 129.274, 129.202, 127.953, 127.921, 127.870, 127.838, 125.786, 123.970, 123.923, 122.154, 122.106, 120.339, 111.397, 111.195, 111.167, 82.460, 82.421, 53.520, 53.507, 51.047, 50.914, 36.325, 36.190, 23.157, 23.021. ^{19}F NMR (376 MHz, Chloroform-*d*) δ -59.366, -59.467. ESI-(+)-HRMS $(M+H)^+$ calculated for $C_{17}H_{14}F_3N_3O_3S$: 398.07807; found: 398.07710 (+2.4 ppm). R_f (EA: PE=3:1) = 0.5.



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124.309
122.499
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HO
CN
CF3
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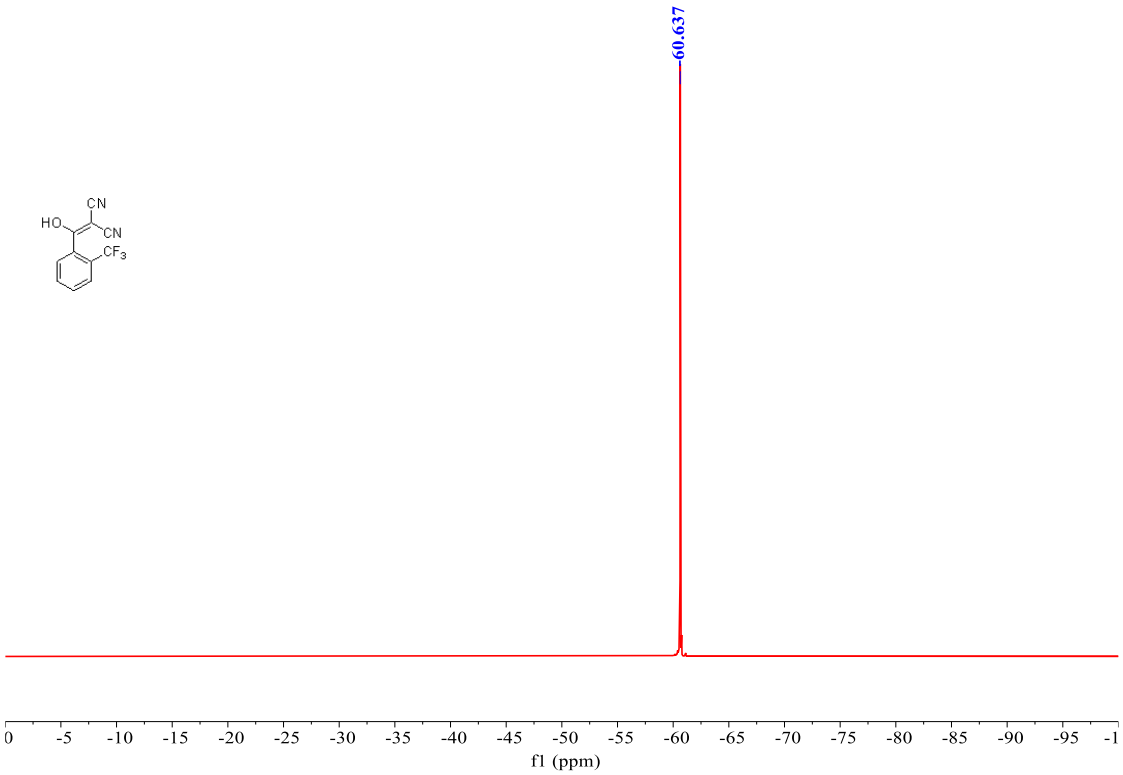
f1 (ppm)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HO
CN
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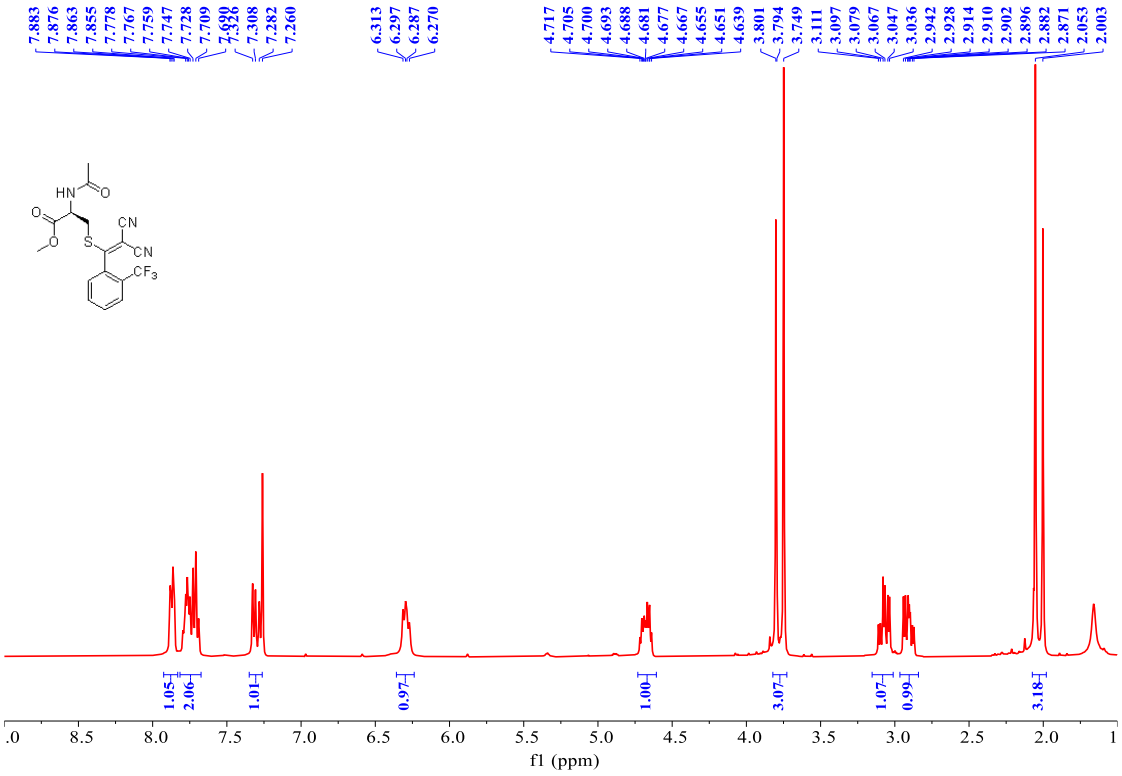
f1 (ppm)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O
O
S
CN
CN
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1.00
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1.07
0.99
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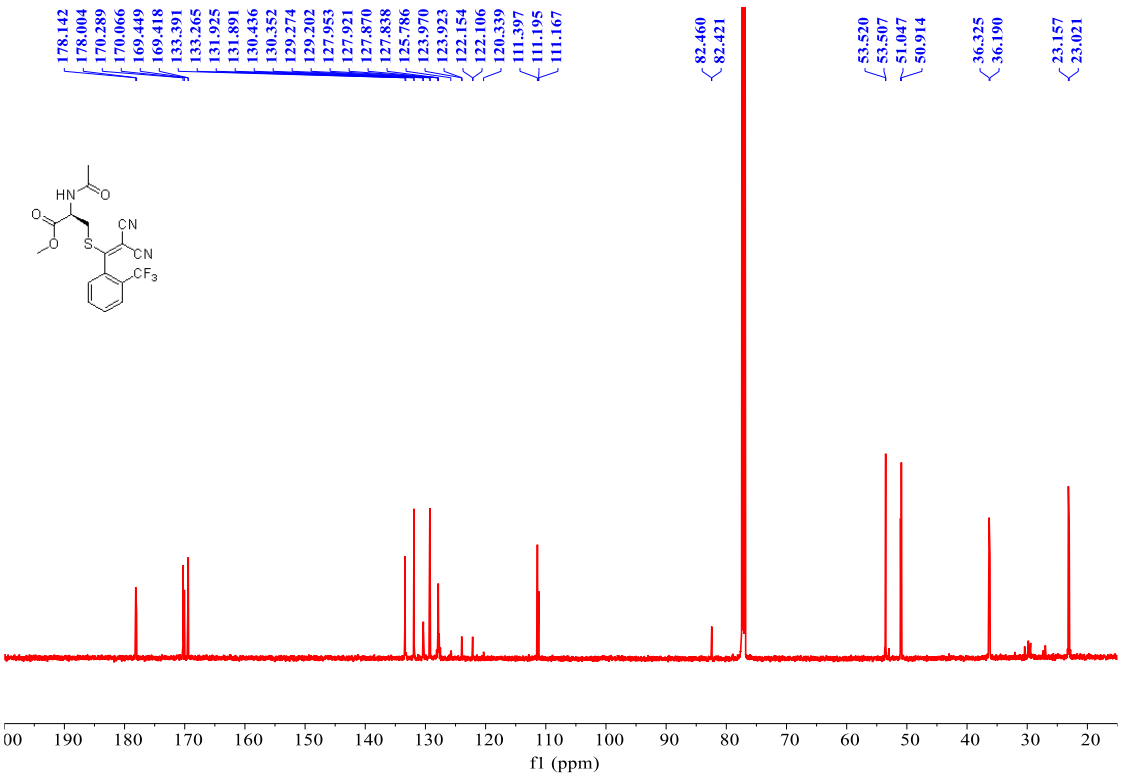
f1 (ppm)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127.921
127.870
127.838
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123.970
123.923
122.154
122.106
120.339
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111.195
111.167
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82.421
53.520
53.507
51.047
50.914
36.325
36.190
23.157
23.021
HN
O
O
O
S
CN
CN
CF3
00
190
180
170
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150
140
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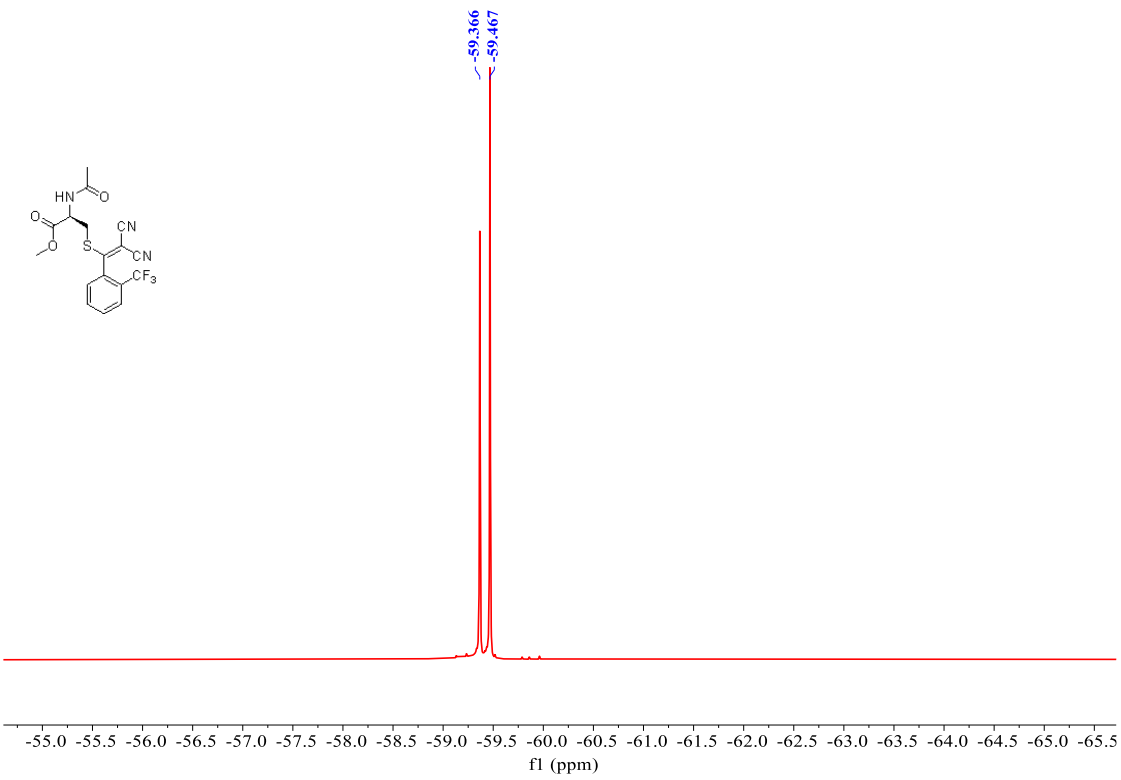
110
100
90
80
70
60
50
40
30
20
f1 (ppm)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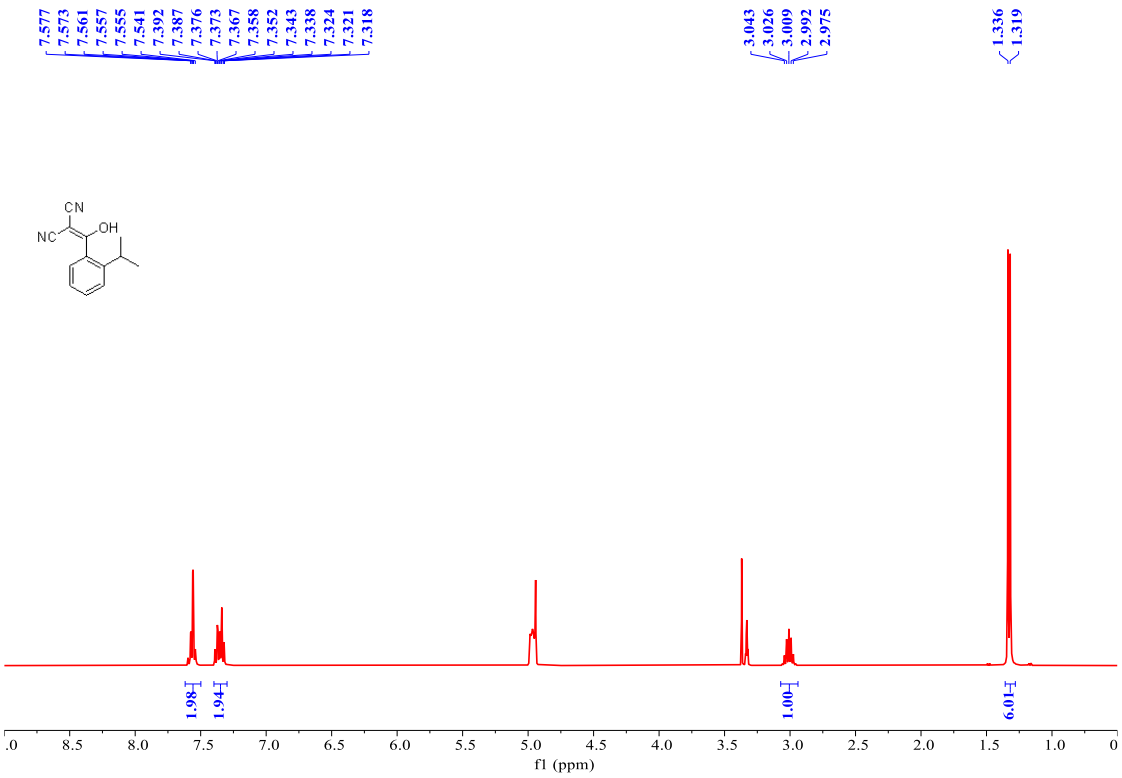
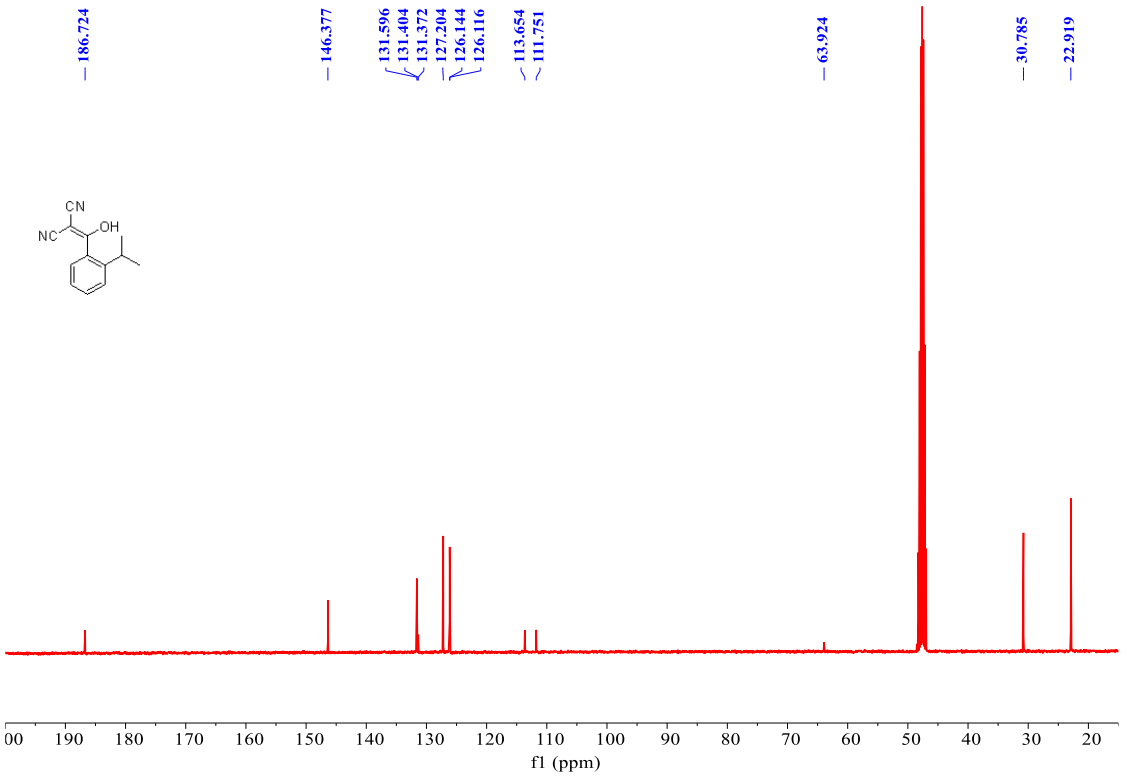
-55.0 -55.5 -56.0 -56.5 -57.0 -57.5 -58.0 -58.5 -59.0 -59.5 -60.0 -60.5 -61.0 -61.5 -62.0 -62.5 -63.0 -63.5 -64.0 -64.5 -65.0 -65.5
f1 (ppm)

TAMM 1c

Compound **1c'** (201 mg, 1.9 mmol, 95% yield) was obtained as a light yellow solid from silica gel column chromatography (DCM: MeOH=10:1). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.577 – 7.541 (m, 2H), 7.392– 7.3 (m, 2H), 3.009 (p, J = 6.83 Hz, 1H), 1.328 (d, J = 6.80 Hz, 6H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 186.724, 146.377, 131.596, 131.404, 131.372, 127.204, 126.144, 126.116, 113.654, 111.751, 63.924, 30.785, 22.919. ESI-(-)-HRMS $(M-H)^-$ calculated for $C_{13}H_{12}N_2O$: 211.08769; found: 211.08787 (+0.8 ppm). R_f (DCM: MeOH=5:1) = 0.4.

Compound **1c** (85 mg, 0.23 mmol, 23% yield) was obtained as a light yellow solid from silica gel column chromatography (EA: PE=2:1). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.518 – 7.450 (m, 2H), 7.353 – 7.301 (m, 1H), 7.016 (ddd, J = 22.40, 8.00, 1.60 Hz, 1H), 6.461 (dd, J = 10.00, 7.60 Hz, 1H), 4.709 – 4.632 (m, 1H), 3.724 (d, J = 16.40 Hz, 3H), 2.944 – 2.929 (m, 1H), 3.128 – 2.803 (m, 2H), 1.990 (d, J = 9.20 Hz, 3H), 1.270 – 1.226 (m, 6H). ^{13}C NMR (101 MHz, Chloroform-d) δ 182.303, 182.088, 170.134, 170.072, 169.528, 169.466, 145.811, 145.634, 132.008, 131.983, 130.688, 130.440, 127.285, 127.155, 127.127, 127.025, 126.988, 111.959, 111.618, 80.969, 80.921, 53.292, 53.221, 50.953, 50.867, 36.165, 36.077, 30.732, 30.679, 24.778, 24.717, 23.421, 23.345, 22.970, 22.919. ESI-(+)-HRMS $(M+H)^+$ calculated for $C_{19}H_{21}N_3O_3S$: 372.13764; found: 372.03724 (+1.1 ppm). R_f (EA: PE=3:1) = 0.45.

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146.377
131.596
131.404
131.372
127.204
126.144
126.116
113.654
111.751
63.924
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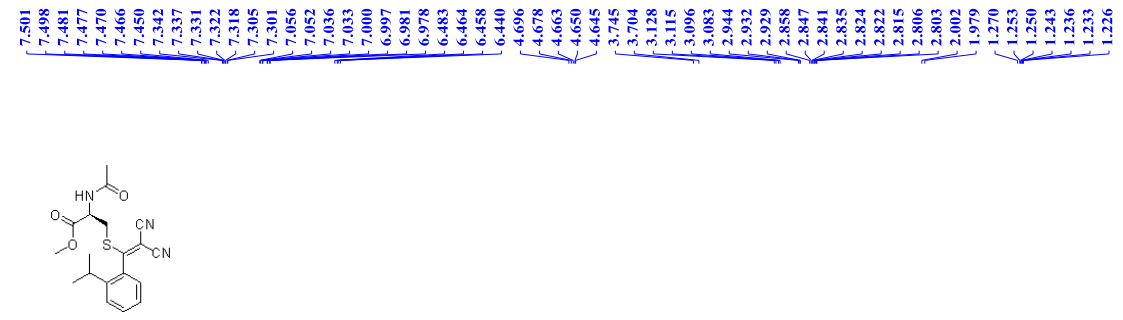
22.919
CN
NC
OH
f1 (ppm)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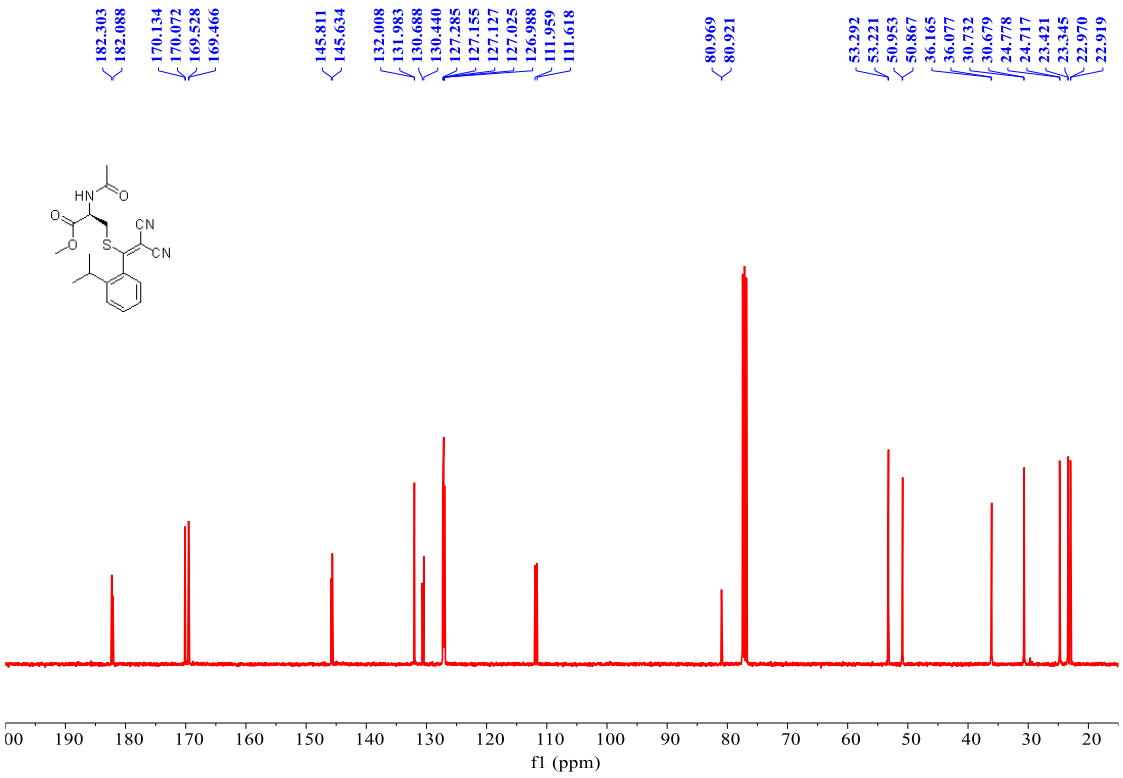
7.481
7.477
7.470
7.466
7.450
7.342
7.337
7.331
7.322
7.318
7.305
7.301
7.056
7.052
7.036
7.033
7.000
6.997
6.981
6.978
6.483
6.464
6.458
6.440
4.696
4.678
4.663
4.650
4.645
3.745
3.704
3.128
3.115
3.096
3.083
2.944
2.932
2.929
2.858
2.847
2.841
2.835
2.824
2.822
2.815
2.806
2.803
2.002
1.979
1.270
1.253
1.250
1.243
1.236
1.233
1.226

HN
O
O
O
S
CN
CN
2.00
1.03
0.99
0.97
1.00
2.93
0.45
1.02
1.45
2.98
6.17
f1 (ppm)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182.088
170.134
170.072
169.528
169.466
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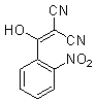
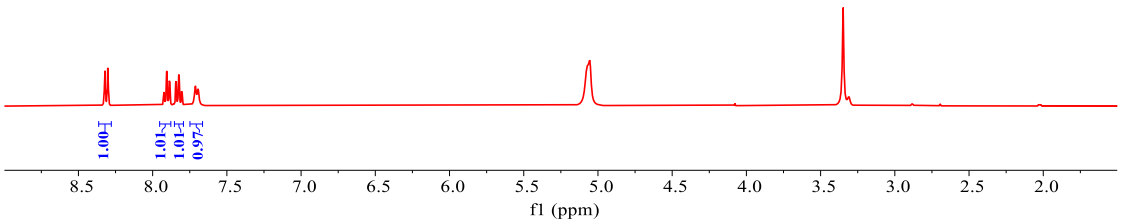
145.634
132.008
131.983
130.688
130.440
127.285
127.155
127.127
127.025
126.988
111.959
111.618
80.969
80.921
53.292
53.221
50.953
50.867
36.165
36.077
30.732
30.679
24.778
24.717
23.421
23.345
22.970
22.919
HN
O
O
O
S
CN
CN
00 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
f1 (ppm)

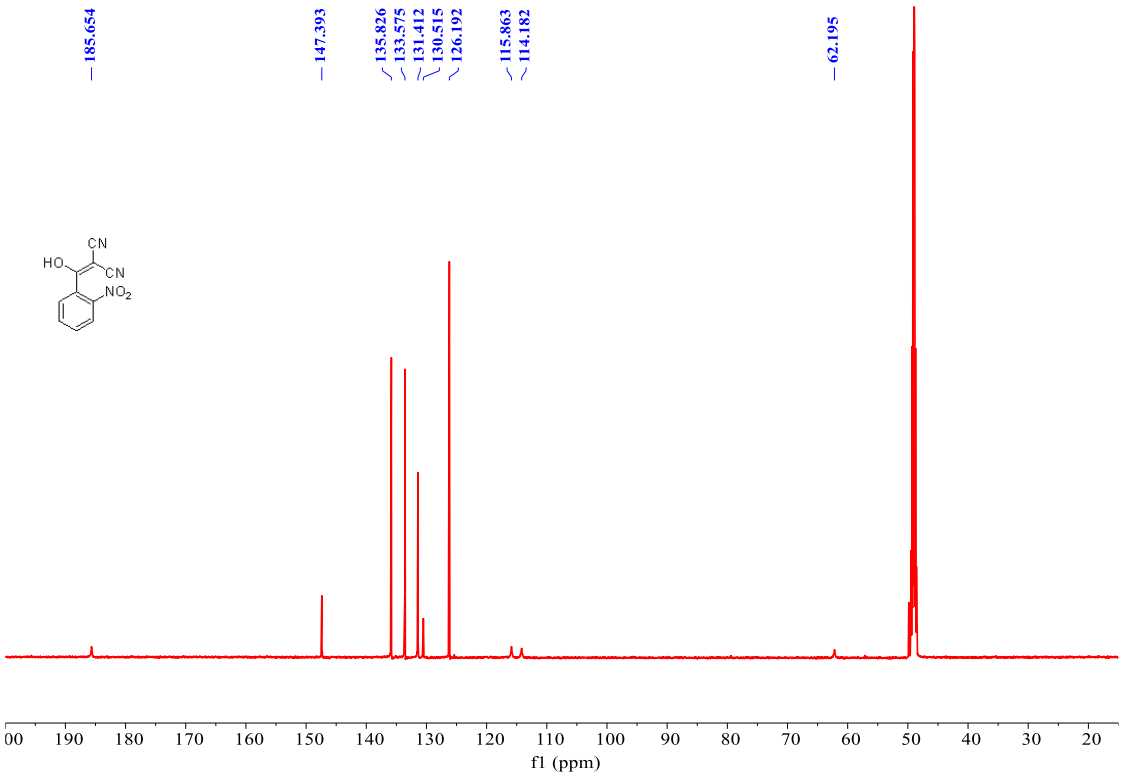
TAMM 1d

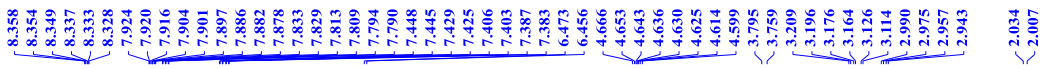
Compound **1d'** (408 mg, 1.9 mmol, 95% yield) was obtained as a light yellow solid from silica gel column chromatography (DCM: MeOH=10:1). ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.310 (d, J = 8.40 Hz, 1H), 7.903 (t, J = 6.80 Hz, 1H), 7.821 (t, J = 7.80 Hz, 1H), 7.70 (d, J = 7.20 Hz, 1H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 185.654, 147.393, 135.826, 133.575, 131.412, 130.515, 126.192, 115.863, 114.182, 62.195. ESI-(-)-HRMS $(M-H)^-$ calculated for $C_{10}H_5N_3O_3$: 214.02581; found: 214.02610 (-1.4 ppm). R_f (DCM: MeOH=10:1) = 0.2.

Compound **1d** (269 mg, 0.72 mmol, 72% yield) was obtained as a light yellow solid from silica gel column chromatography (EA: PE=2:1). ^{1}H NMR (400 MHz, Chloroform-d) δ 8.349 (dt, J = 8.40, 1.80 Hz, 1H), 7.901 (tt, J = 7.60, 1.40 Hz, 1H), 7.811 (td, J = 7.80, 1.60 Hz, 1H), 7.415 (ddd, J = 16.80, 7.60, 1.40 Hz, 1H), 6.464 (d, J = 6.80 Hz, 1H), 4.666 – 4.599 (m, 1H), 3.777 (d, J = 14.40 Hz, 3H), 3.209 – 2.994 (m, 2H), 2.020 (d, J = 10.80 Hz, 3H). ^{13}C NMR (151 MHz, Chloroform-d) δ 178.281, 178.192, 170.319, 169.339, 146.256, 135.188, 135.160, 132.953, 132.908, 129.964, 129.892, 127.942, 127.837, 126.154, 126.065, 111.361, 111.118, 111.084, 81.099, 81.080, 53.437, 53.414, 51.232, 51.101, 36.145, 36.088, 22.973, 22.901. ESI-(+)-HRMS $(M+H)^+$ calculated for $C_{16}H_{14}N_4O_5S$: 375.07577; found: 375.07505 (+1.9 ppm). R_f (EA: PE=3:1) = 0.4.






185.654
147.393
135.826
133.575
131.412
130.515
126.192
115.863
114.182
62.195
HO
CN
CN
NO2
f1 (ppm)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8.354
8.349
8.337
8.333
8.328
7.924
7.920
7.916
7.904
7.901
7.897
7.886
7.882
7.878
7.833
7.829
7.813
7.809
7.794
7.790
7.448
7.445
7.429
7.425
7.406
7.403
7.387
7.383
6.473
6.456
4.666
4.653
4.643
4.636
4.630
4.625
4.614
4.599
3.795
3.759
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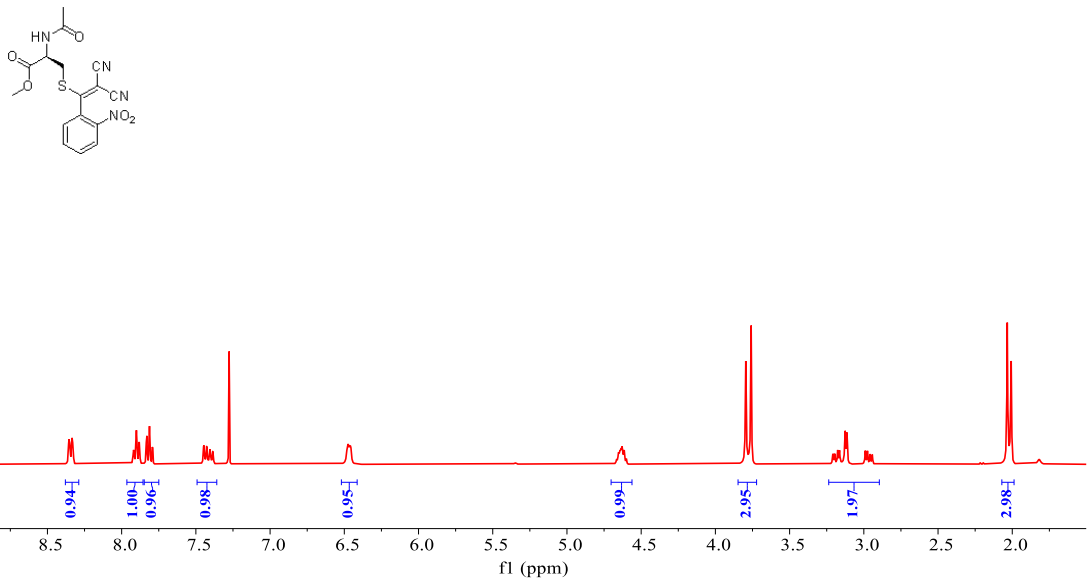
2.975
2.957
2.943
2.034
2.007


HN
O
O
O
S
CN
CN
NO2
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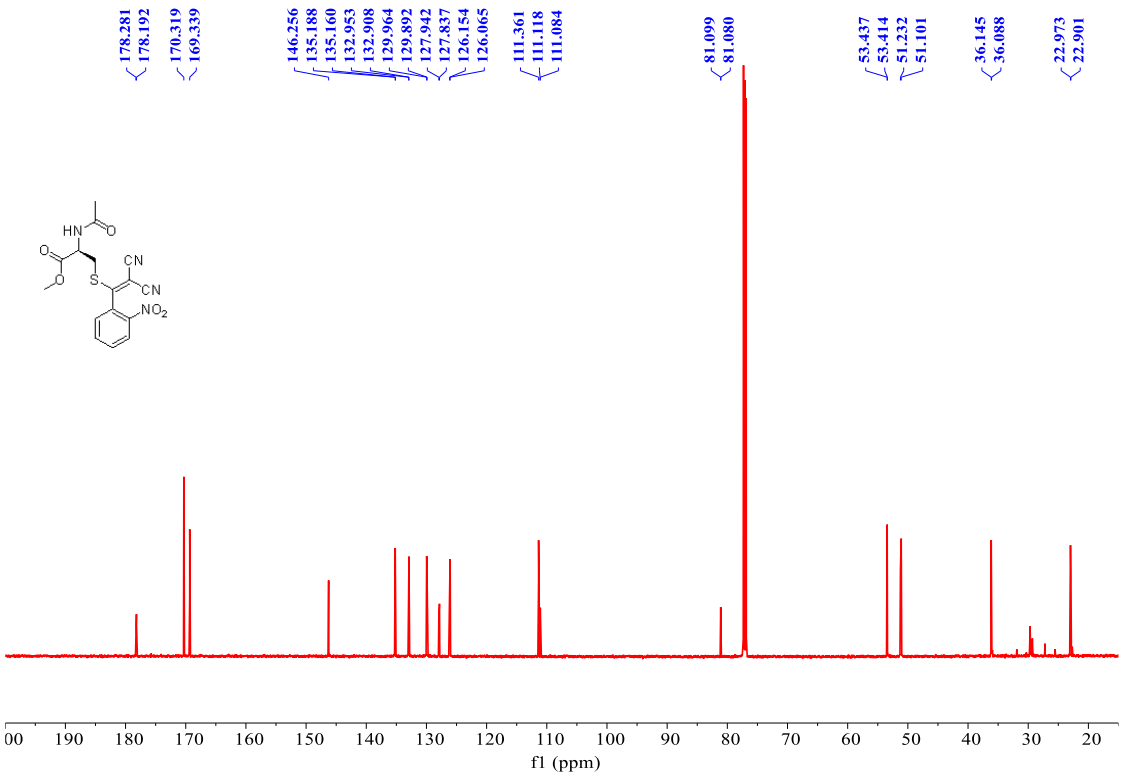
1.00
0.96
0.98
0.95
0.99
2.95
1.97
2.98
f1 (ppm)


178.281
178.192
170.319
169.339
146.256
135.188
135.160
132.953
132.908
129.964
129.892
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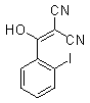
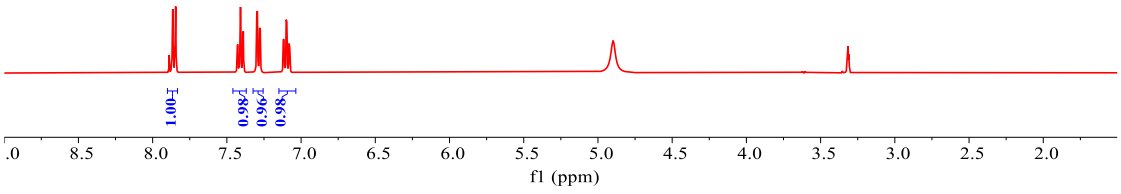
127.837
126.154
126.065
111.361
111.118
111.084
81.099
81.080
53.437
53.414
51.232
51.101
36.145
36.088
22.973
22.901
HN
O
O
O
S
CN
CN
NO2
00 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
f1 (ppm)

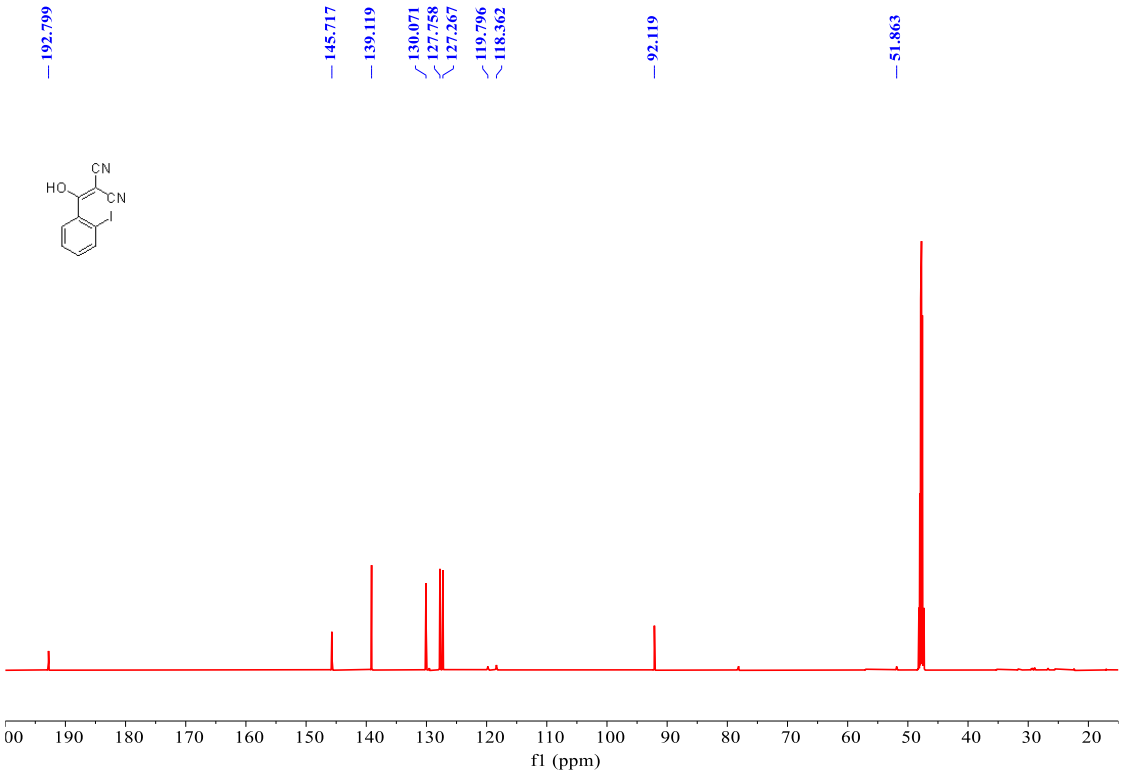
TAMM 1e

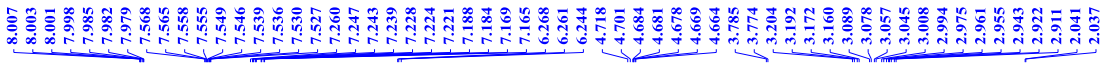
Compound **1e'** (550 mg, 1.86 mmol, 93% yield) was obtained as a light yellow solid from silica gel column chromatography (DCM: MeOH=10:1). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.856 (dd, *J* = 8.00, 1.20 Hz, 1H), 7.409 (td, *J* = 7.40, 1.20 Hz, 1H), 7.287 (dd, *J* = 7.20, 1.60 Hz, 1H), 7.098 (td, *J* = 7.80, 1.60 Hz, 1H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 192.799, 145.717, 139.119, 130.071, 127.758, 127.267, 119.796, 118.362, 92.119, 51.863. ESI-(-)-HRMS $(M-H)^-$ calculated for $C_{10}H_5IN_2O$: 294.93738; found: 294.93748 (-0.3 ppm). R_f (DCM: MeOH=10:1) = 0.2.

Compound **1e** (104 mg, 0.28 mmol, 28% yield) was obtained as a light yellow solid from silica gel column chromatography (EA: PE=2:1). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.992 (dt, *J* = 7.60, 1.20 Hz, 1H), 7.556 (tdd, *J* = 7.60, 3.60, 1.20 Hz, 1H), 7.247 – 7.165 (m, 2H), 6.265 (dd, *J* = 10.00, 7.20 Hz, 1H), 4.731 – 4.652 (m, 1H), 3.780 (d, *J* = 4.40 Hz, 3H), 3.204 – 2.911 (m, 2H), 2.039 (d, *J* = 1.60 Hz, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 182.038, 181.923, 170.105, 169.982, 169.483, 169.437, 162.611, 140.593, 140.495, 137.405, 137.315, 132.583, 132.561, 129.459, 129.350, 128.588, 128.394, 111.416, 111.325, 95.153, 94.995, 81.959, 81.840, 53.408, 53.351, 51.085, 50.873, 36.108, 35.951, 23.155, 23.071. ESI-(+)-HRMS $(M+H)^+$ calculated for $C_{16}H_{14}IN_3O_3S$: 455.98733; found: 455.98668 (+1.4 ppm). Rf (EA: PE=2:1) = 0.3.






192.799
145.717
139.119
130.071
127.758
127.267
119.796
118.362
92.119
51.863
CN
HO
CN
I
fl (ppm)


8.007
8.003
8.001
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7.982
7.979
7.568
7.565
7.558
7.555
7.549
7.546
7.539
7.536
7.530
7.527
7.260
7.247
7.243
7.239
7.228
7.224
7.221
7.188
7.184
7.169
7.165
6.268
6.261
6.244
4.718
4.701
4.684
4.681
4.678
4.669
4.664
3.785
3.774
3.204
3.192
3.172
3.160
3.089
3.078
3.057
3.045
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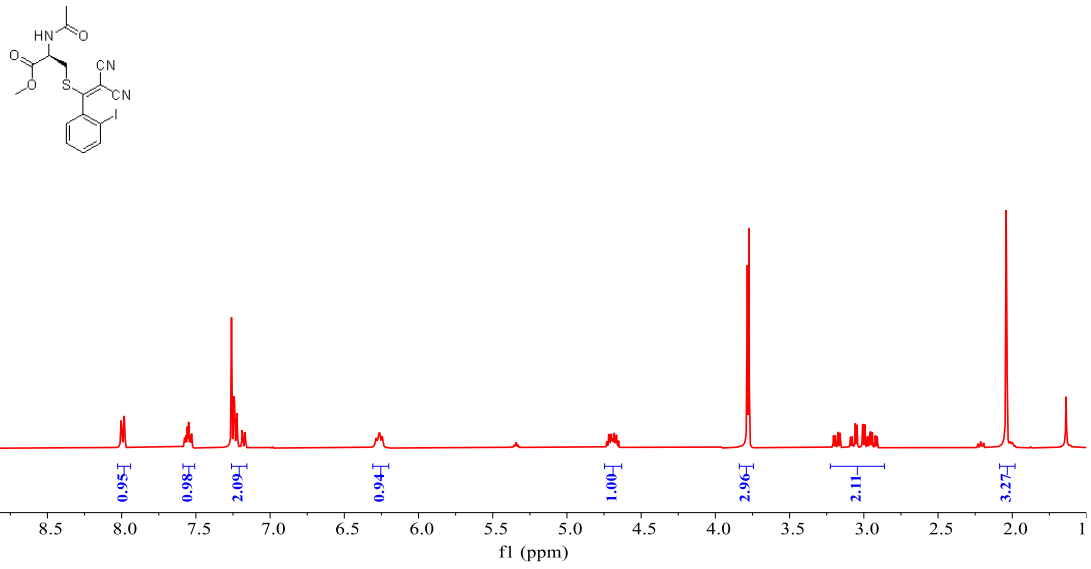
2.975
2.961
2.955
2.943
2.922
2.911
2.041
2.037


HN
O
O
O
S
CN
CN
I
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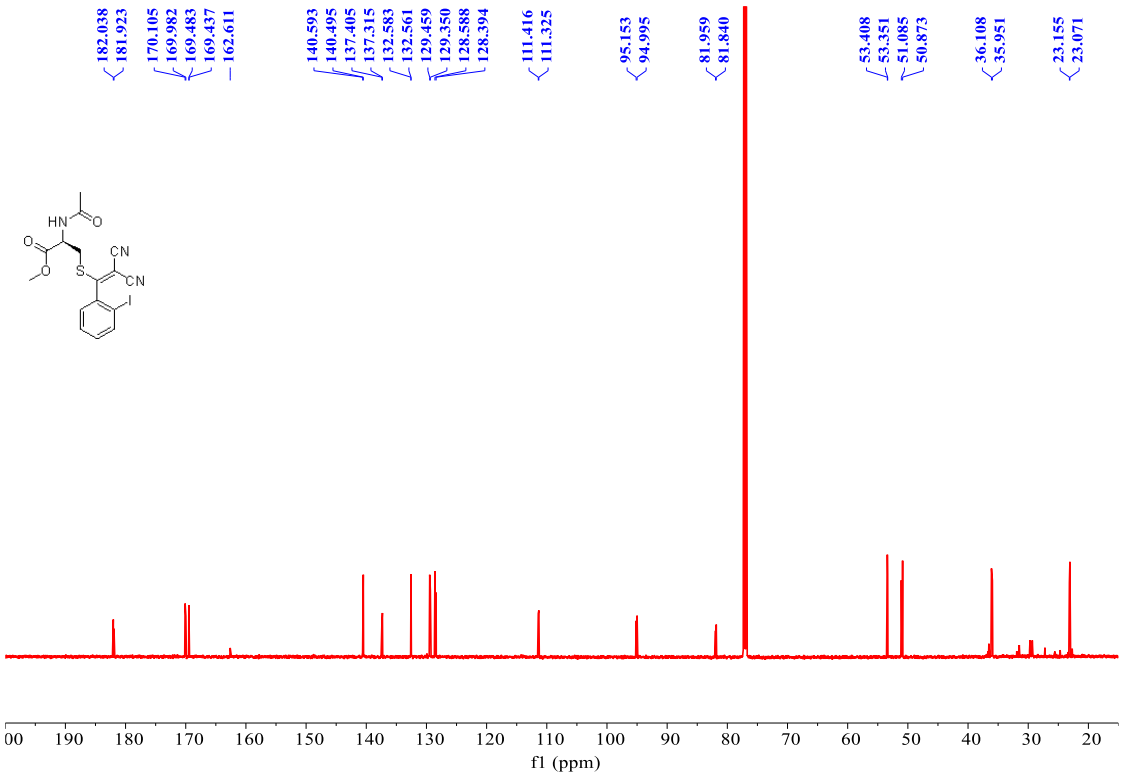
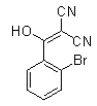
0.98
2.09
0.94
1.00
2.96
2.11
3.27
fl (ppm)

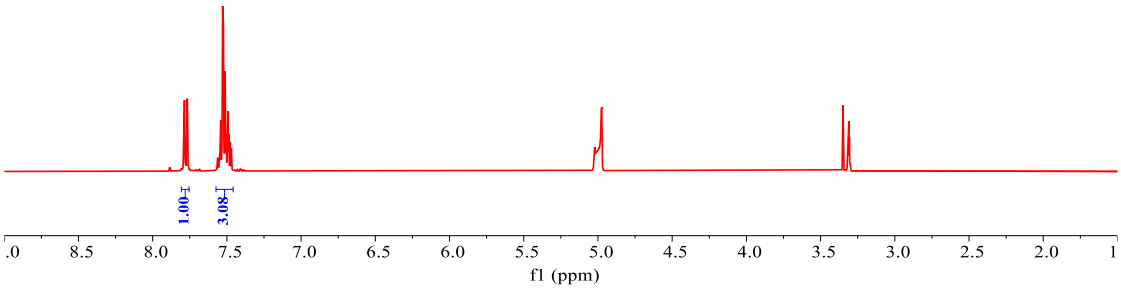

182.038
181.923
170.105
169.982
169.483
169.437
162.611
140.593
140.495
137.405
137.315
132.583
132.561
129.459
129.350
128.588
128.394
111.416
111.325
95.153
94.995
81.959
81.840
53.408
53.351
51.085
50.873
36.108
35.951
23.155
23.071
HN
O
O
O
S
CN
CN
I
00
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)

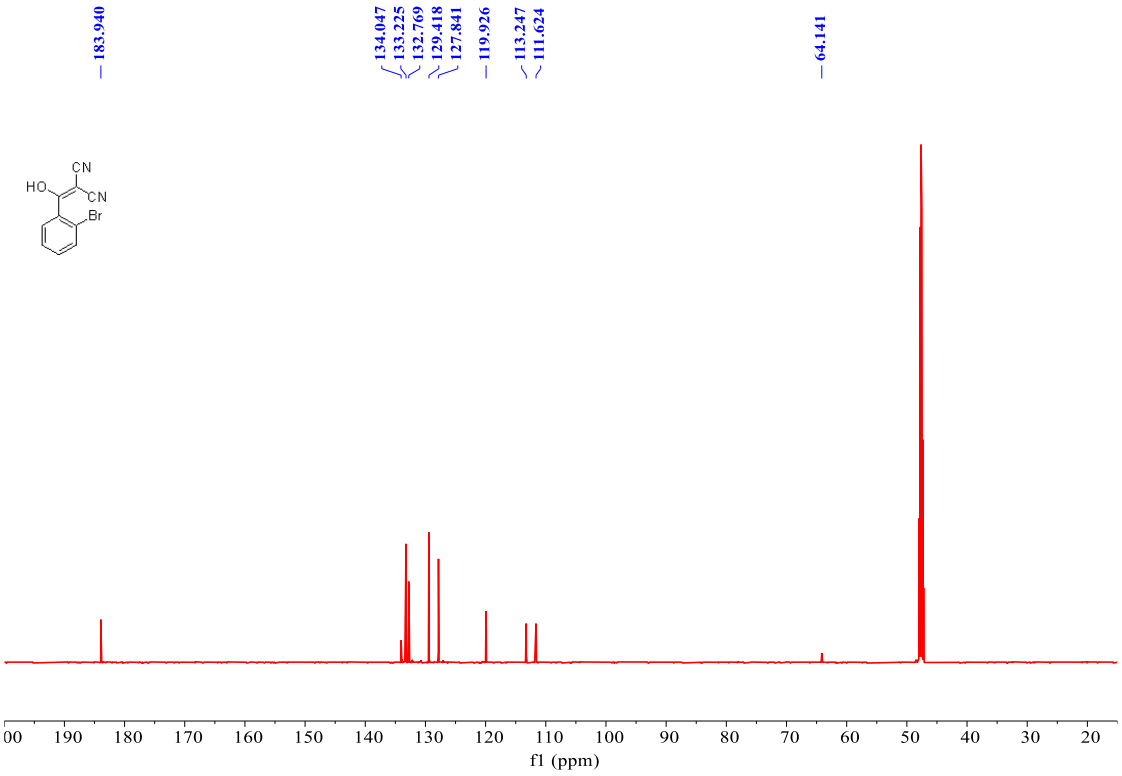
TAMM 1f

Compound **1f'** (476 mg, 1.92 mmol, 96% yield) was obtained as a light yellow solid from silica gel column chromatography (DCM: MeOH=10:1). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.776 (dt, J = 7.60, 1.00 Hz, 1H), 7.563 – 7.470 (m, 3H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 183.940, 134.047, 133.225, 132.769, 129.418, 127.841, 119.926, 113.247, 111.624, 64.141. ESI-(-)-HRMS $(M-H)^-$ calculated for $C_{10}H_5BrN_2O$: 246.95125; found: 246.95214 (-3.6 ppm). R_f (DCM: MeOH=10:1) = 0.4.

Compound **1f** (106 mg, 0.26 mmol, 26% yield) was obtained as a light yellow solid from silica gel column chromatography (EA: PE=2:1). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.722 (d, J = 9.60 Hz, 1H), 7.501 (td, J = 7.40, 1.60 Hz, 1H), 7.706 (td, J = 7.80, 1.00 Hz, 1H), 7.279 – 7.184 (m, 1H), 6.518 (t, J = 6.60 Hz, 1H), 4.678 – 4.590 (m, 1H), 3.718 (d, J = 10.40 Hz, 3H), 3.176 – 2.915 (m, 2H), 1.986 (d, J = 2.80 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-d) δ 179.865, 179.627, 170.271, 170.183, 169.502, 169.462, 134.128, 134.034, 133.272, 133.170, 132.944, 129.399, 129.195, 128.739, 128.677, 120.811, 120.646, 111.546, 111.372, 81.793, 81.638, 53.297, 51.213, 50.973, 35.942, 35.812, 23.016, 22.987. ESI-(+)-HRMS $(M+H)^+$ calculated for $C_{16}H_{14}BrN_3O_3S$: 408.00120; found: 408.00100 (+0.5 ppm). R_f (EA: PE=3:1) = 0.45.



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134.047
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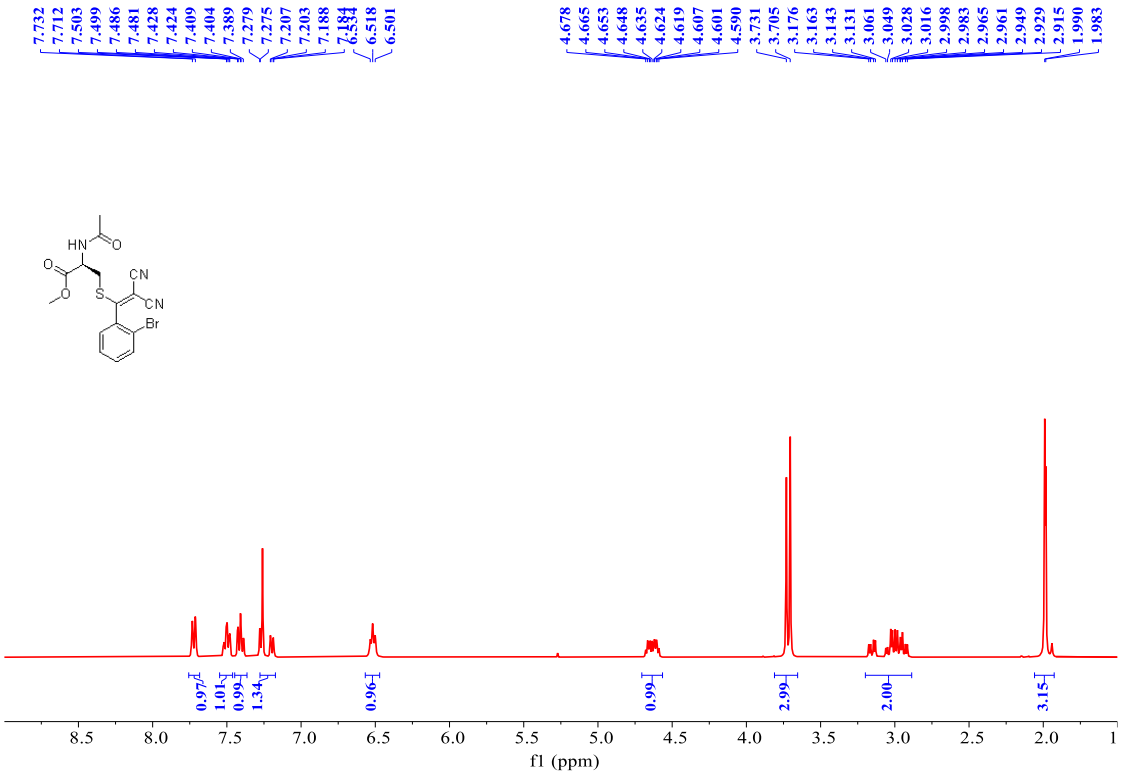
129.418
127.841
119.926
113.247
111.624
64.141
HO
CN
CN
Br
f1 (ppm)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7.275
7.207
7.203
7.188
7.184
6.534
6.518
6.501
4.678
4.665
4.653
4.648
4.635
4.624
4.619
4.607
4.601
4.590
3.731
3.705
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3.131
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3.028
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1.990
1.983
HN
O
O
O
S
CN
CN
Br
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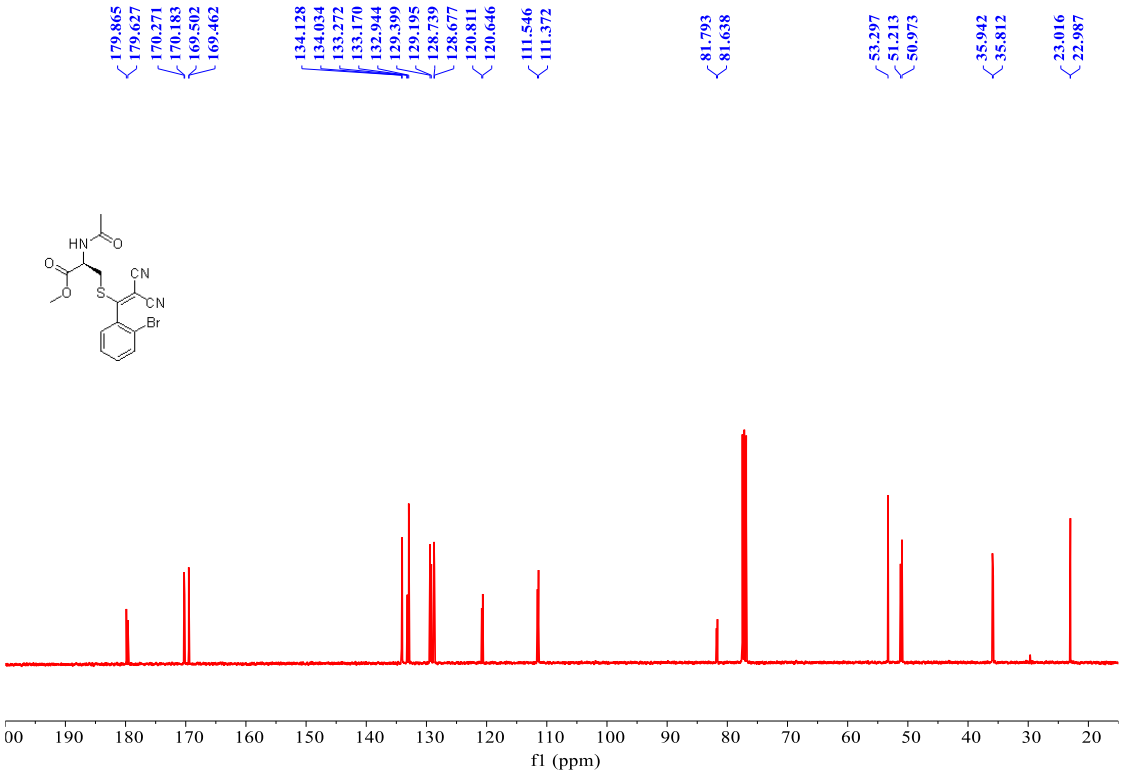
0.99
1.34
0.96
0.99
2.99
2.00
3.15
f1 (ppm)


179.865
179.627
170.271
170.183
169.502
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129.195
128.739
128.677
120.811
120.646
111.546
111.372
81.793
81.638
53.297
51.213
50.973
35.942
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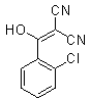
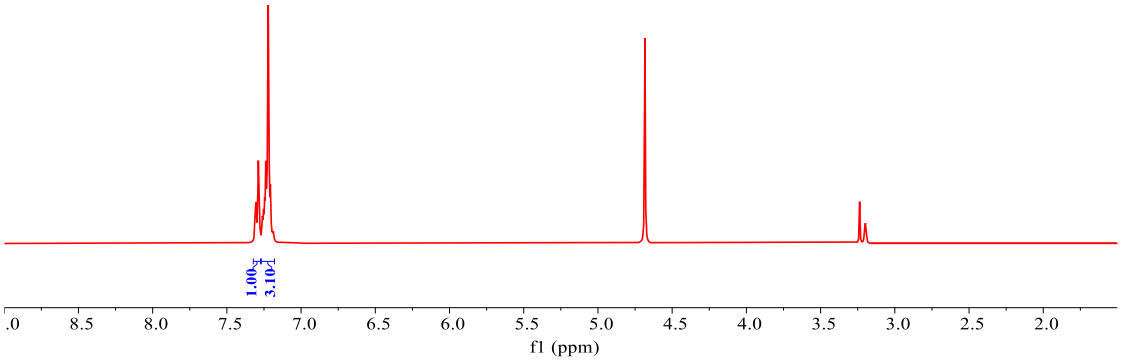
23.016
22.987
HN
O
O
O
S
CN
CN
Br
00 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
f1 (ppm)

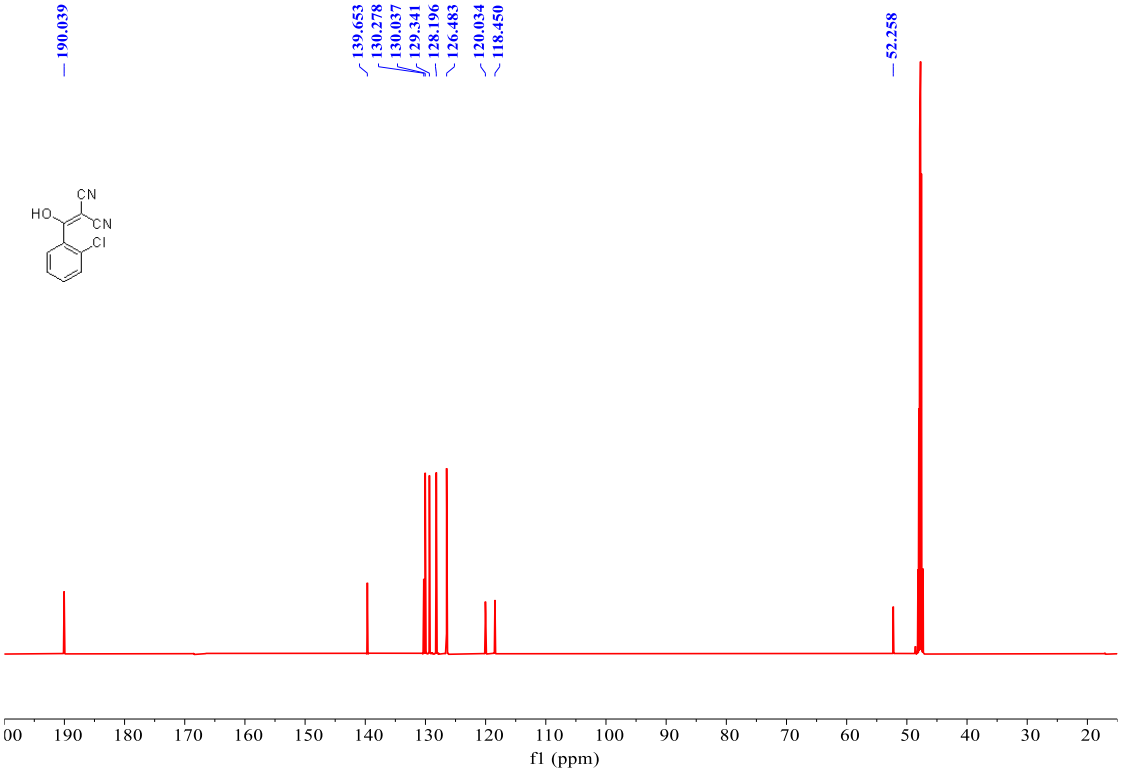
TAMM 1g

Compound **1g'** (396 mg, 1.94 mmol, 97% yield) was obtained as a light yellow solid from silica gel column chromatography (DCM: MeOH=10:1). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.309 – 7.279 (m, 1H), 7.261 – 7.183 (m, 3H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 190.039, 139.653, 130.278, 130.037, 129.341, 128.196, 126.483, 120.034, 118.450, 52.258. ESI-(-)-HRMS $(M-H)^-$ calculated for $C_{10}H_5ClN_2O$: 203.00176; found: 203.00239 (-3.1 ppm). R_f (DCM: MeOH=5:1) = 0.4.

Compound **1g** (113 mg, 0.31 mmol, 31% yield) was obtained as a light yellow solid from silica gel column chromatography (EA: PE=2:1). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.565 – 7.429 (m, 3H), 7.294 – 7.199 (m, 1H), 6.510 (t, *J* = 6.80 Hz, 1H), 4.669 – 4.581 (m, 1H), 3.717 (d, *J* = 11.20 Hz, 3H), 3.165 – 2.47 (m, 2H), 1.984 (d, *J* = 3.0 Hz, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 178.417, 178.142, 170.263, 170.189, 169.503, 169.453, 133.998, 131.619, 131.427, 131.153, 131.078, 130.968, 130.864, 129.492, 129.300, 128.180, 128.118, 111.585, 111.380, 81.937, 81.764, 53.295, 53.268, 51.300, 51.058, 35.869, 35.772, 22.975. ESI-(+)-HRMS $(M+H)^+$ calculated for $C_{16}H_{14}ClN_3O_3S$: 364.05172; found: 364.05138 (+0.9 ppm). R_f (EA: PE=3:1) = 0.60.



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139.653
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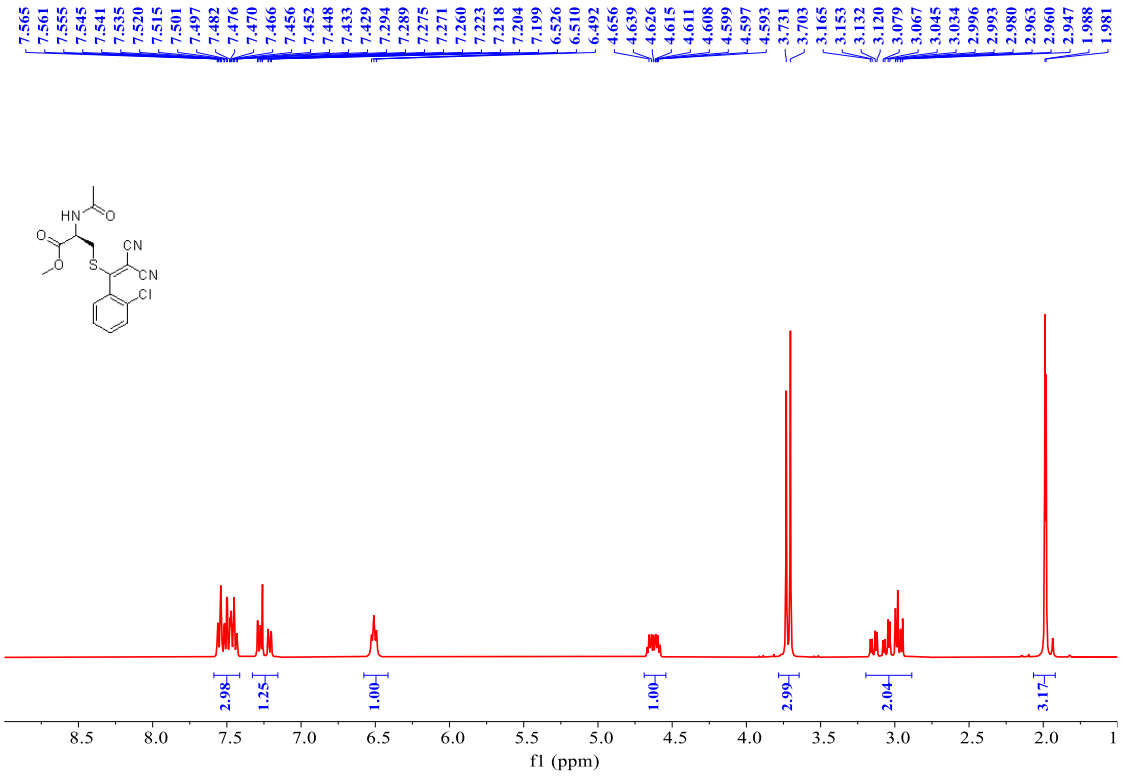
130.037
129.341
128.196
126.483
120.034
118.450
52.258
HO
CN
CN
Cl
f1 (ppm)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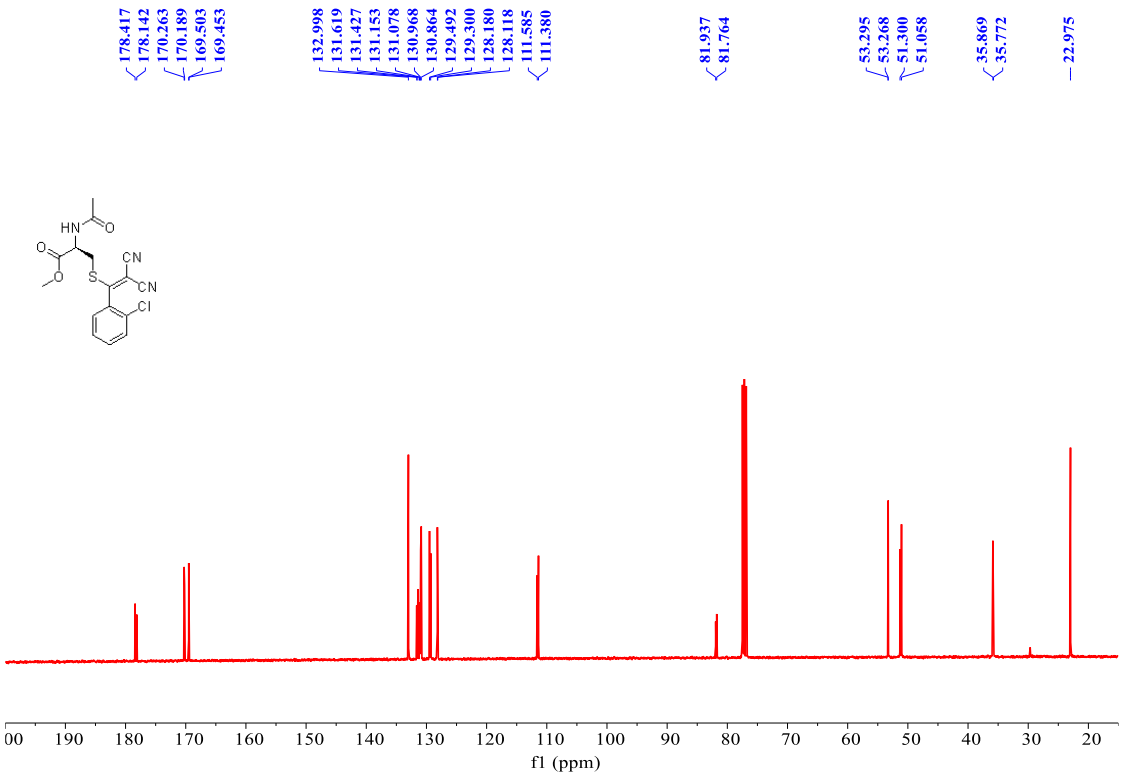
7.561
7.555
7.545
7.541
7.535
7.520
7.515
7.501
7.497
7.482
7.476
7.470
7.466
7.456
7.452
7.448
7.433
7.429
7.294
7.289
7.275
7.271
7.260
7.223
7.218
7.204
7.199
6.526
6.510
6.492
4.656
4.639
4.626
4.615
4.611
4.608
4.599
4.597
4.593
3.731
3.703
3.165
3.153
3.132
3.120
3.079
3.067
3.045
3.034
2.996
2.993
2.980
2.963
2.960
2.947
1.988
1.981
HN
O
O
O
S
CN
CN
Cl
2.98
1.25
1.00
1.00
2.99
2.04
3.17
f1 (ppm)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178.142
170.263
170.189
169.503
169.453
132.998
131.619
131.427
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131.078
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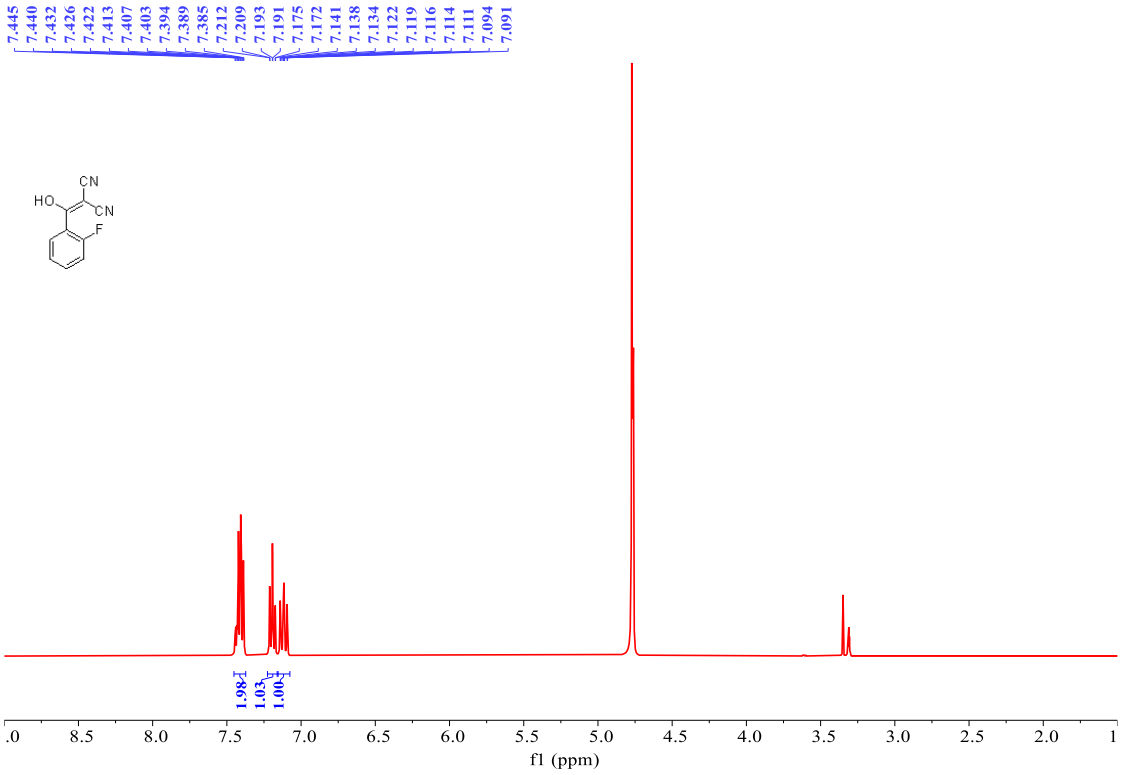
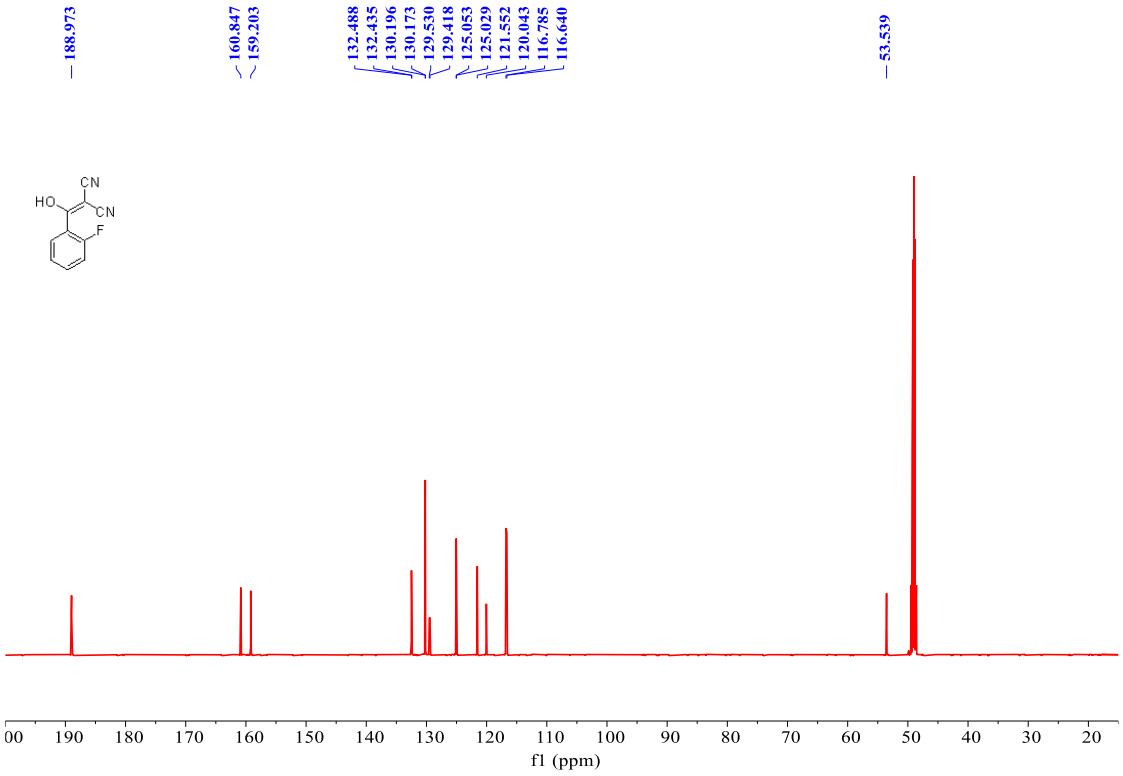
130.864
129.492
129.300
128.180
128.118
111.585
111.380
81.937
81.764
53.295
53.268
51.300
51.058
35.869
35.772
22.975
HN
O
O
O
S
CN
CN
Cl
00
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)

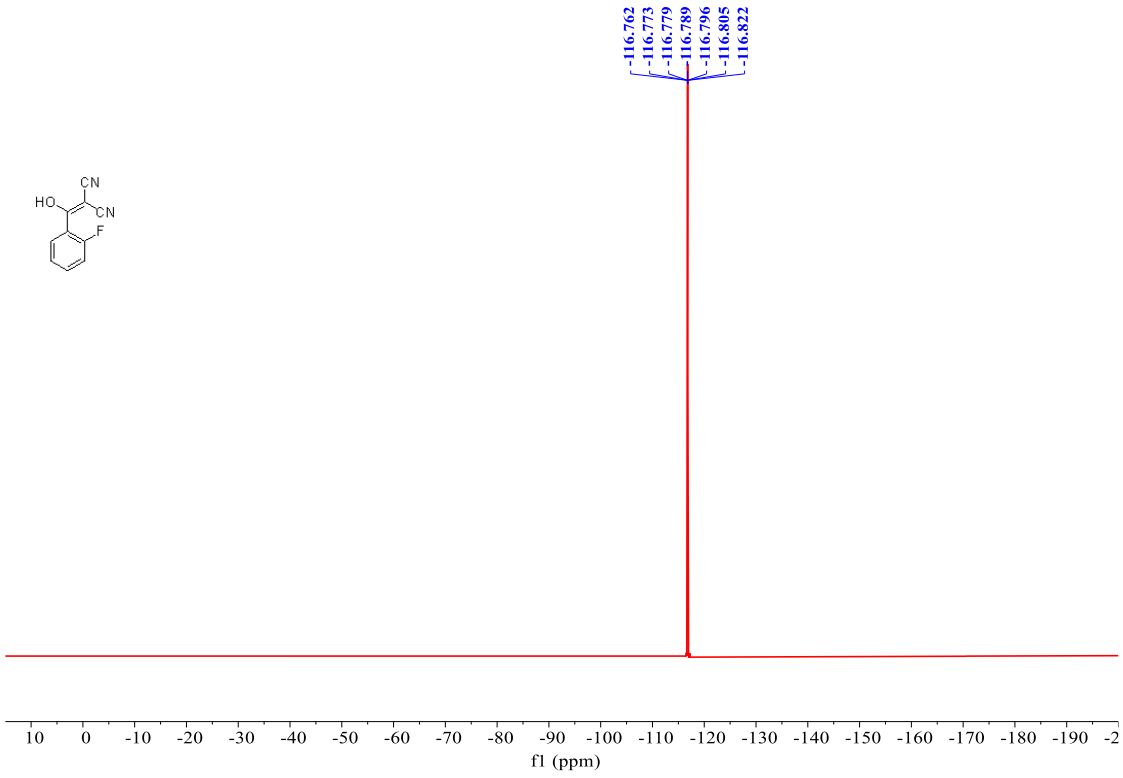
TAMM 1h

Compound **1h'** (368 mg, 1.96 mmol, 98% yield) was obtained as a light yellow solid from silica gel column chromatography (DCM: MeOH=10:1). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.445 – 7.385 (m, 2H), 7.192 (td, *J* = 7.60, 1.20 Hz, 1H), 7.141 – 7.091 (m, 1H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 188.973, 160.847, 159.203, 132.488, 132.435, 130.196, 130.173, 129.530, 129.418, 125.053, 125.029, 121.552, 120.043, 116.785, 116.640, 53.539. ^{19}F NMR (376 MHz, Methanol-d_4) δ -116.794 (dt, *J* = 9.75, 6.33 Hz). ESI-(-)-HRMS (M-H)$^-$ calculated for $C_{10}H_5FN_2O$: 187.03131; found: 187.03128 (+0.2 ppm). R_f (DCM: MeOH=5:1) = 0.4.

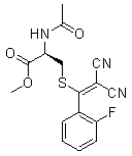
Compound **1h** (87 mg, 0.25 mmol, 25% yield) was obtained as a light yellow solid from silica gel column chromatography (EA: PE=2:1). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.604 – 7.547 (m, 1H), 7.356 – 7.232 (m, 3H), 6.545 (d, *J* = 7.20 Hz, 1H), 4.62 (dt, *J* = 7.20, 3.60 Hz, 1H), 3.727 (s, 3H), 3.178 (dd, *J* = 14.00, 4.80 Hz, 1H), 3.07 (dd, *J* = 14.00, 5.60 Hz, 1H), 1.979 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 174.453, 170.276, 169.472, 159.085, 157.405, 134.659, 134.604, 130.040, 125.576, 125.552, 119.922, 119.829, 111.889, 111.571, 82.534, 53.265, 51.675, 36.132, 22.900. ^{19}F NMR (376 MHz, Chloroform-*d*) δ -111.298 (q, *J* = 7.52, 6.77 Hz). ESI-(-)-HRMS (M-H)$^-$ calculated for $C_{16}H_{14}FN_3O_3S$: 346.06671; found: 346.06695 (-0.7 ppm). R_f (EA: PE=3:1) = 0.40.

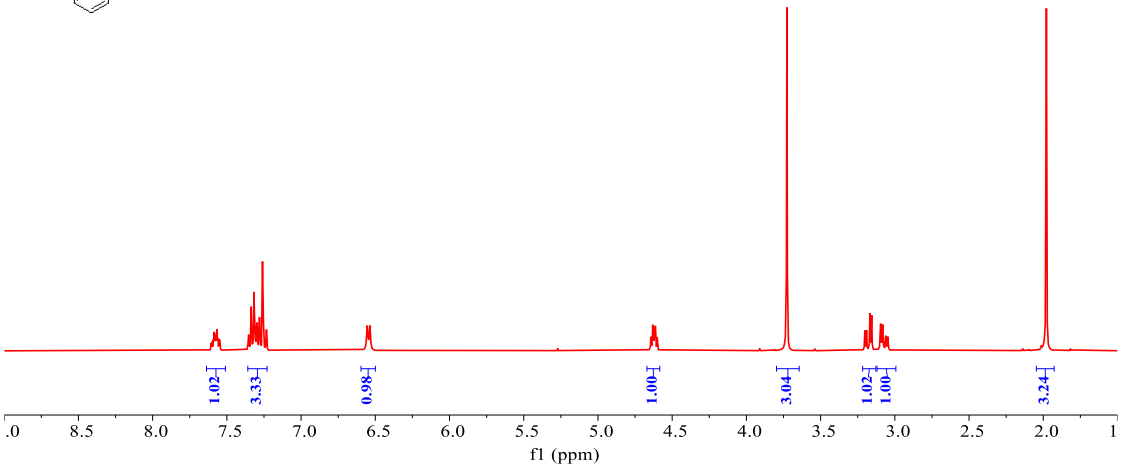



188.973
160.847
159.203
132.488
132.435
130.196
130.173
129.530
129.418
125.053
125.029
121.552
120.043
116.785
116.640
53.539
CN
HO
CN
F
00 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
f1 (ppm)

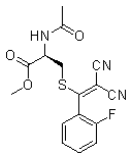

-116.762
-116.773
-116.779
-116.789
-116.796
-116.805
-116.822
CN
HO
CN
F
10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -2
f1 (ppm)

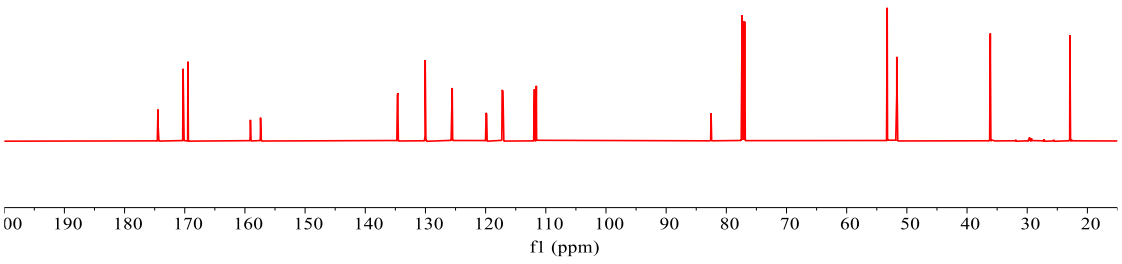

7.604
7.599
7.591
7.587
7.583
7.581
7.577
7.573
7.570
7.568
7.566
7.560
7.552
7.547
7.356
7.353
7.336
7.334
7.317
7.311
7.300
7.296
7.280
7.277
7.256
7.253
7.235
7.232
6.554
6.536
4.643
4.631
4.625
4.618
4.613
4.600
3.727
3.202
3.190
3.167
3.156
3.096
3.082
3.061
3.047
1.979

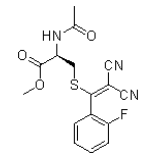

HN
O
O
O
S
CN
CN
F


1.02
3.33
0.98
1.00
3.04
1.02
1.00
3.24
.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
f1 (ppm)


174.453
170.276
169.472
159.085
157.405
134.659
134.604
130.040
125.576
125.552
119.922
119.829
111.889
111.571
82.534
53.265
51.675
36.132
22.900


HN
O
O
O
S
CN
CN
F


00
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)

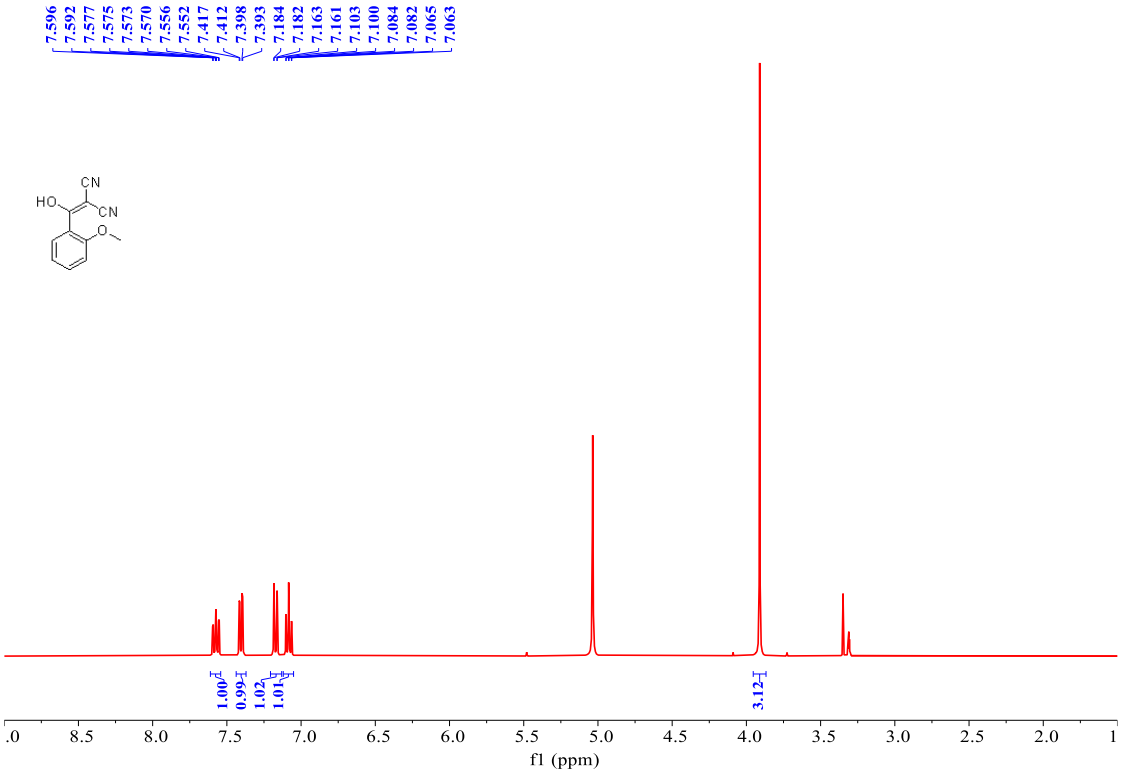

HN
O
O
O
S
CN
CN
F

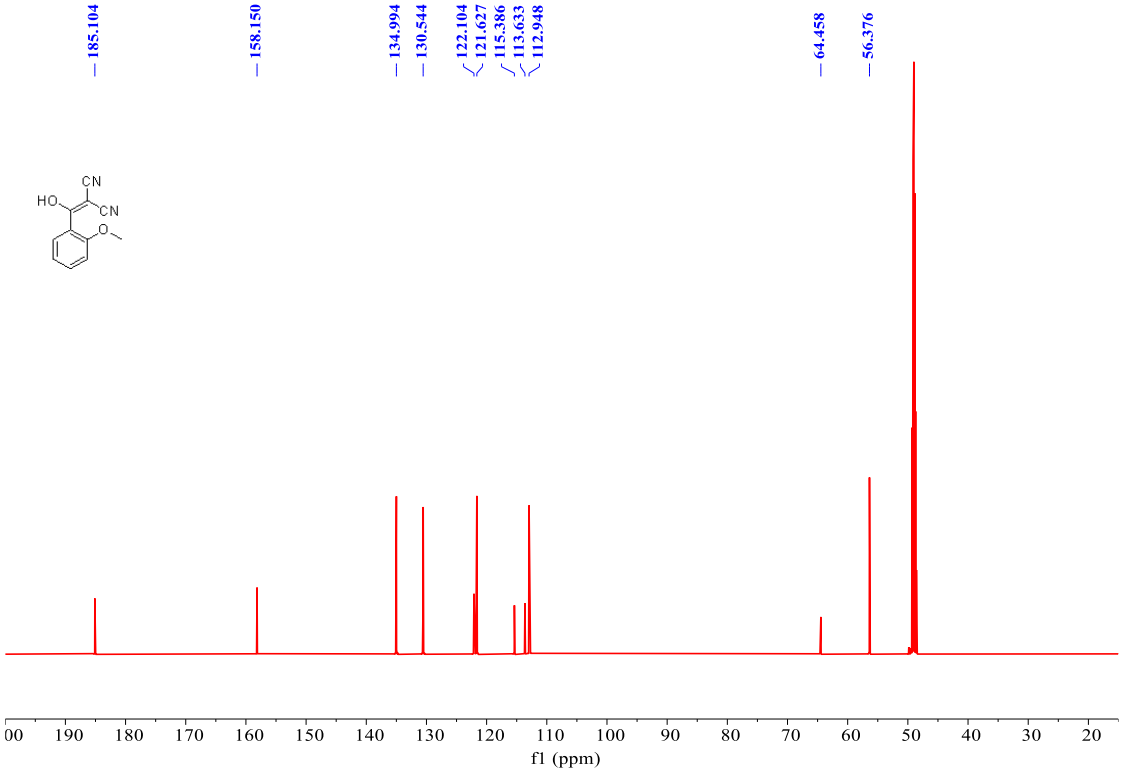
-111.267
-111.288
-111.308
-111.326
10
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
-2
f1 (ppm)

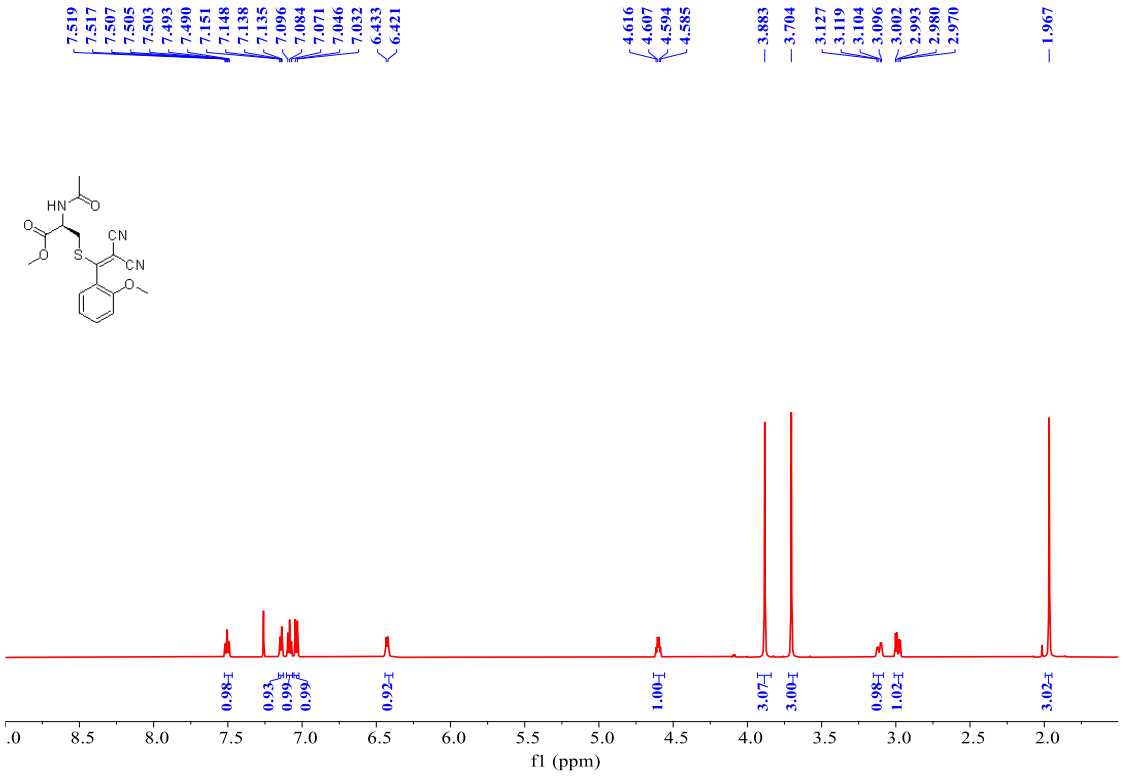
TAMM 1i

Compound **1i'** (380 mg, 1.9 mmol, 95% yield) was obtained as a light yellow solid from silica gel column chromatography (DCM: MeOH=10:1). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.574 (ddd, *J* = 9.20, 7.60, 1.60 Hz, 1H), 7.405 (dd, *J* = 7.60, 2.00 Hz, 1H), 7.172 (dd, *J* = 8.40, 0.80 Hz, 1H), 7.083 (td, *J* = 7.60, 0.80 Hz, 1H), 3.911 (s, 3H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 185.104, 158.150, 134.994, 130.544, 122.104, 121.627, 115.386, 113.633, 112.948, 64.458, 56.376. ESI-(-)-HRMS $(M-H)^-$ calculated for $C_{11}H_8N_2O_2$: 199.05130; found: 199.05143 (-0.6 ppm). R_f (DCM: MeOH=10:1) = 0.25.

Compound **1i** (108 mg, 0.3 mmol, 30% yield) was obtained as a light yellow solid from silica gel column chromatography (EA: PE=2:1). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.505 (ddd, *J* = 9.60, 7.20, 1.20 Hz, 1H), 7.143 (dd, *J* = 7.80, 1.80 Hz, 1H), 7.084 (t, *J* = 7.50 Hz, 1H), 7.039 (d, *J* = 8.40 Hz, 1H), 6.427 (d, *J* = 7.20 Hz, 1H), 4.616 – 4.585 (m, 1H),, 3.883 (s, 3H), 3.704 (s, 3H), 3.112 (dd, *J* = 13.80, 4.80 Hz, 1H), 2.986 (dd, *J* = 13.20, 5.40 Hz, 1H), 1.967 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 178.461, 170.036, 169.739, 155.793, 133.845, 129.478, 121.529, 120.627, 112.455, 112.228, 112.059, 81.209, 55.997, 53.119, 51.531, 35.766, 22.933. ESI-(+)-HRMS $(M+H)^+$ calculated for $C_{17}H_{17}N_3O_4S$: 360.10125; found: 360.10087 (+1.0 ppm). R_f (EA: PE=3:1) = 0.30.




185.104
158.150
134.994
130.544
122.104
121.627
115.386
113.633
112.948
64.458
56.376
HO
CN
CN
O
f1 (ppm)


7.519
7.517
7.507
7.505
7.503
7.493
7.490
7.151
7.148
7.138
7.135
7.096
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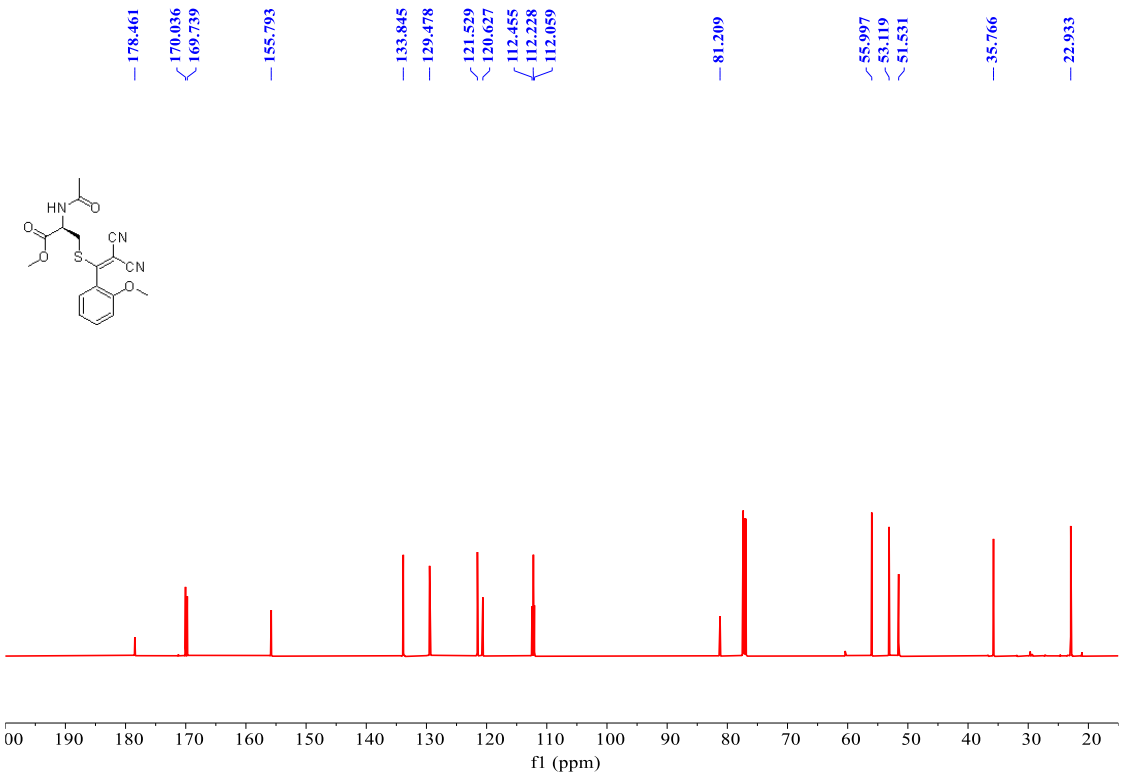
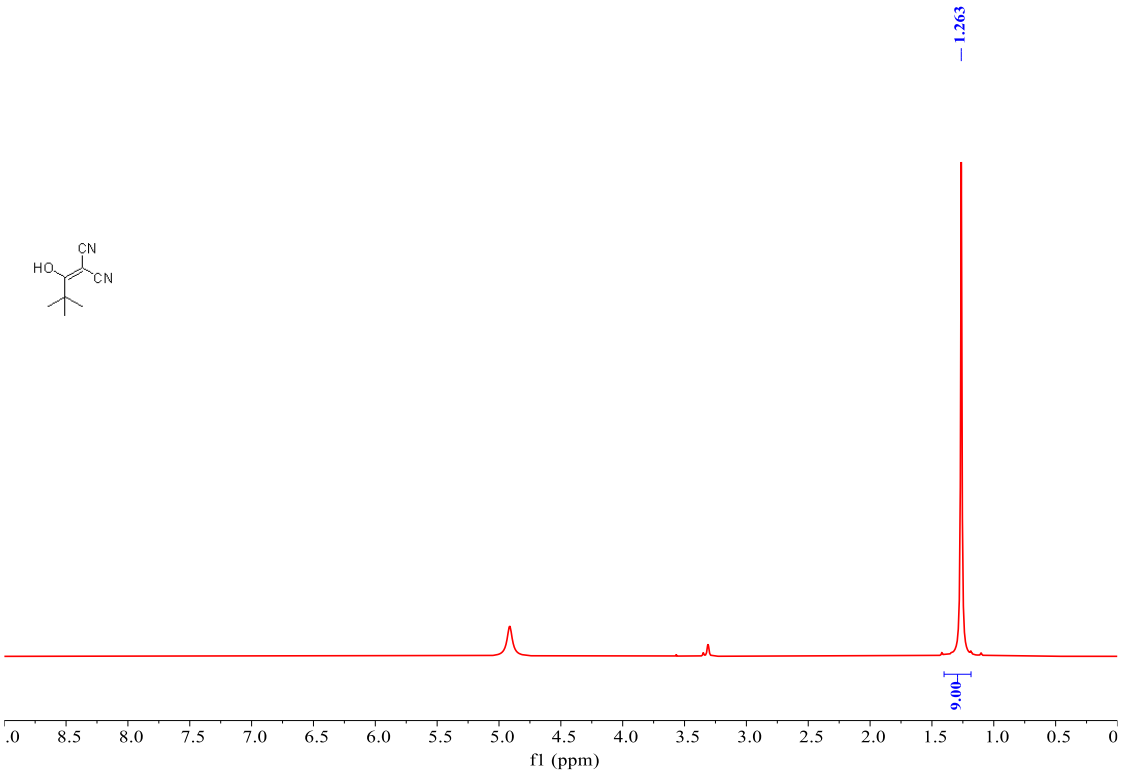
7.071
7.046
7.032
6.433
6.421
4.616
4.607
4.594
4.585
3.883
3.704
3.127
3.119
3.104
3.096
3.002
2.993
2.980
2.970
1.967
HN
O
O
O
S
CN
CN
O
0.98
0.93
0.99
0.99
0.92
1.00
3.07
3.00
0.98
1.02
3.02
f1 (ppm)

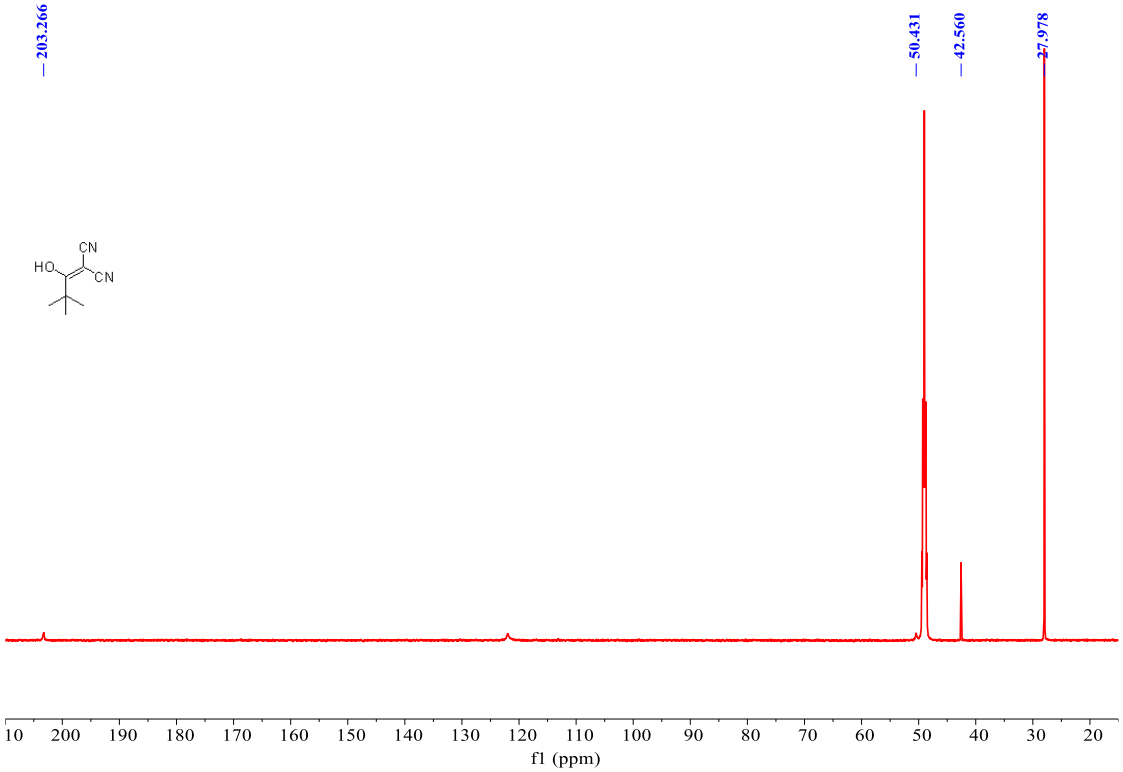

178.461
170.036
169.739
155.793
133.845
129.478
121.529
120.627
112.455
112.228
112.059
81.209
55.997
53.119
51.531
35.766
22.933
HN
O
O
O
S
CN
CN
O
00 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
f1 (ppm)

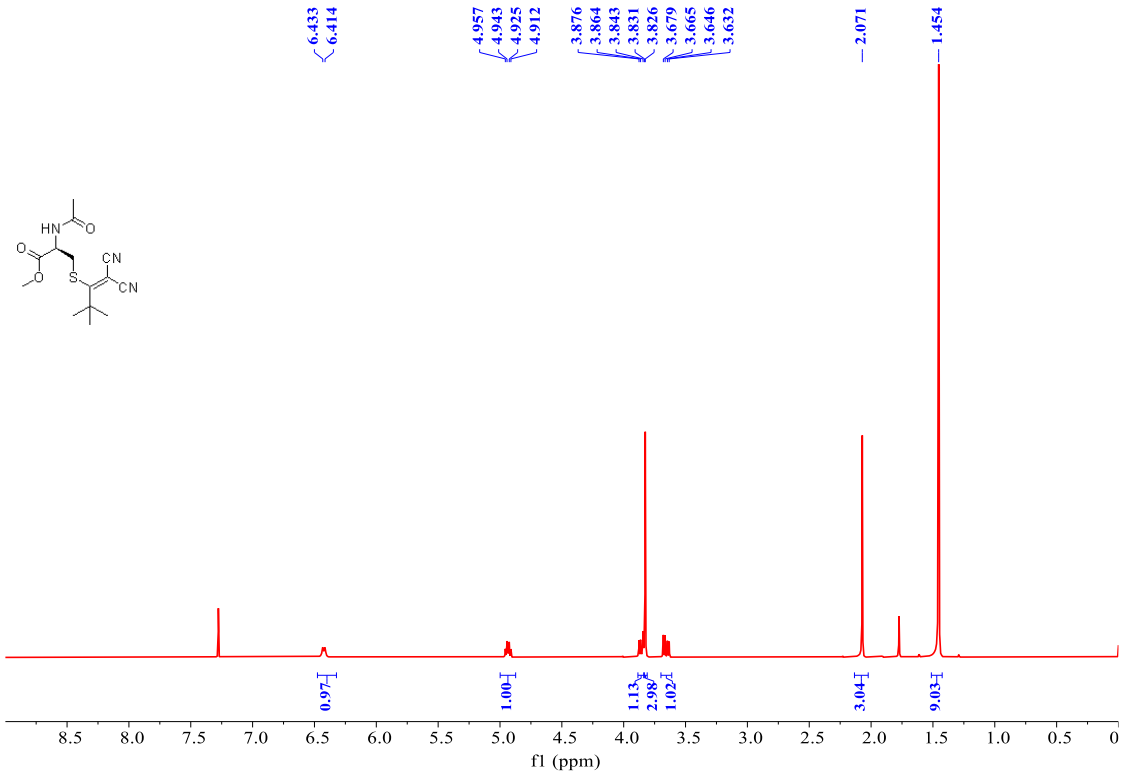
TAMM 1j

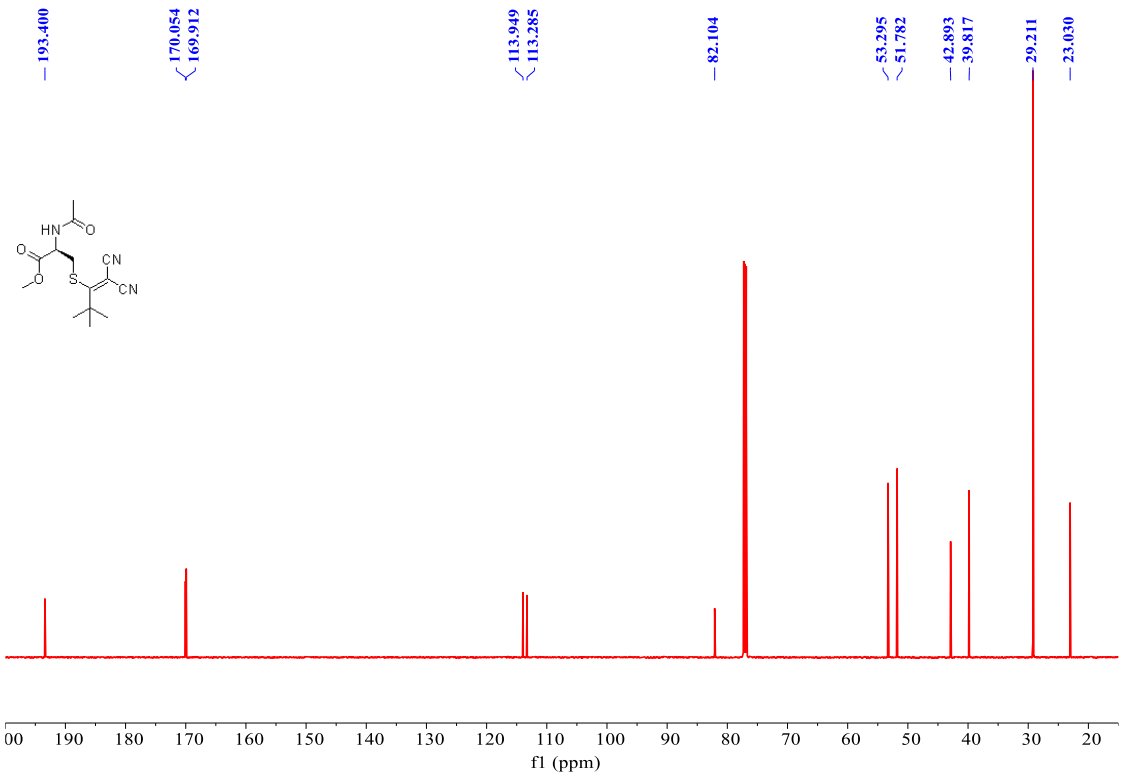
Compound **1j'** (297 mg, 1.98 mmol, 99% yield) was obtained as a light yellow solid from silica gel column chromatography (DCM: MeOH=10:1). ^{1}H NMR (400 MHz, Methanol-d_4) δ 1.263 (s, 9H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 203.266, 50.431, 42.560, 27.978. ESI-(-)-HRMS $(M-H)^-$ calculated for $C_8H_{10}N_2O$: 149.07204; found: 149.07198 (+0.4 ppm). R_f (DCM: MeOH=5:1) = 0.3.

Compound **1j** (102 mg, 0.33 mmol, 33% yield) was obtained as a light yellow solid from silica gel column chromatography (EA: PE=2:1). ^{1}H NMR (400 MHz, Chloroform-d) δ 6.423 (d, J = 7.60 Hz, 1H), 4.957 – 4.912 (m, 1H), 3.876 – 3.831 (m, 1H), 3.826 (s, 3H), 3.656 (dd, J = 13.20, 5.60 Hz, 1H), 2.071 (s, 3H), 1.454 (s, 9H). ^{13}C NMR (151 MHz, Chloroform-d) δ 193.400, 170.054, 169.912, 113.949, 113.285, 82.104, 53.295, 51.782, 42.893, 39.817, 29.211, 23.030. ESI-(+)-HRMS $(M+H)^+$ calculated for $C_{14}H_{19}N_3O_3S$: 310.12199; found: 310.12164 (+1.1 ppm). R_f (EA: PE=2:1) = 0.40.




203.266
50.431
42.560
27.978
HO
CN
CN
10 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
f1 (ppm)

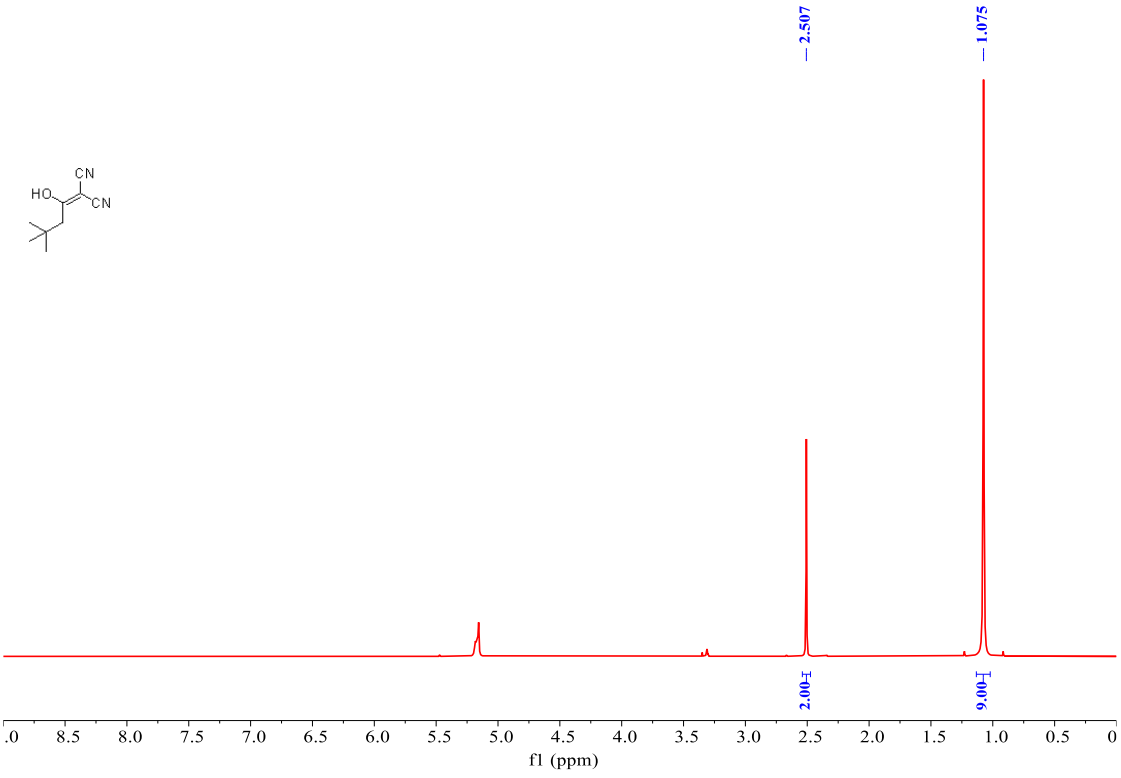

6.433
6.414
4.957
4.943
4.925
4.912
3.876
3.864
3.843
3.831
3.826
3.679
3.665
3.646
3.632
2.071
1.454
HN
O
O
O
S
CN
CN
0.97
1.00
1.13
2.98
1.02
3.04
9.03
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0
f1 (ppm)

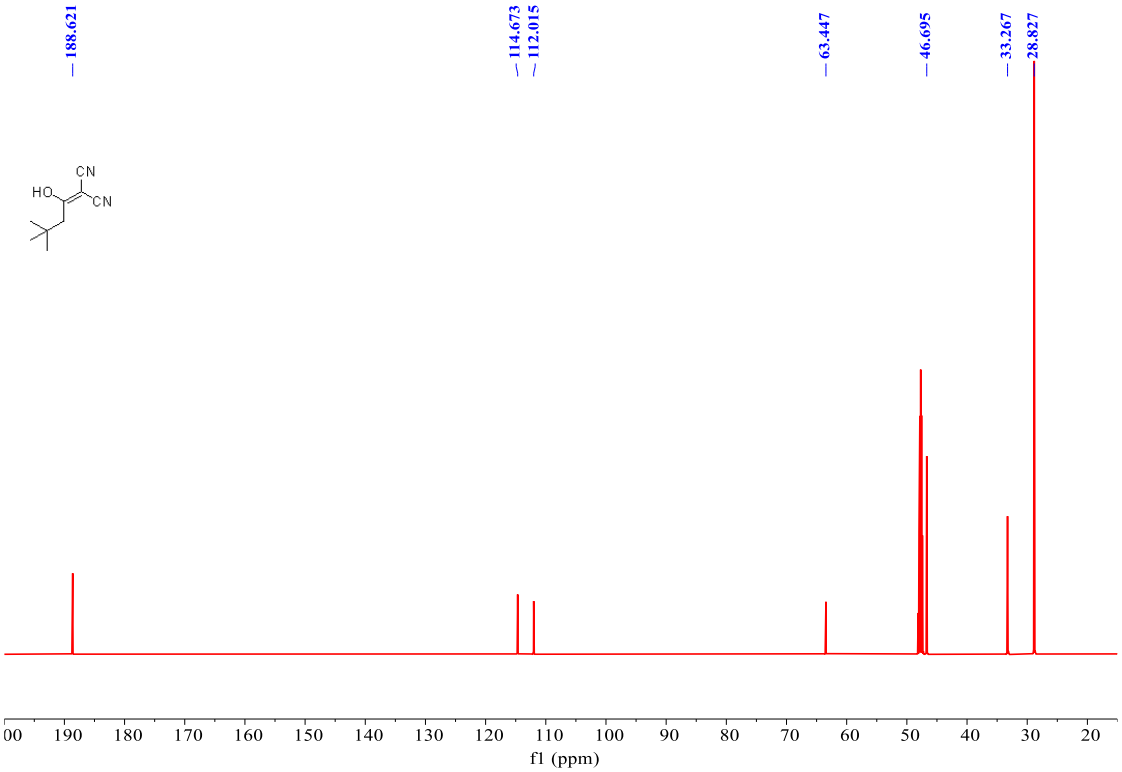

193.400
170.054
169.912
113.949
113.285
82.104
53.295
51.782
42.893
39.817
29.211
23.030
HN
O
O
O
S
CN
CN
00 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
f1 (ppm)

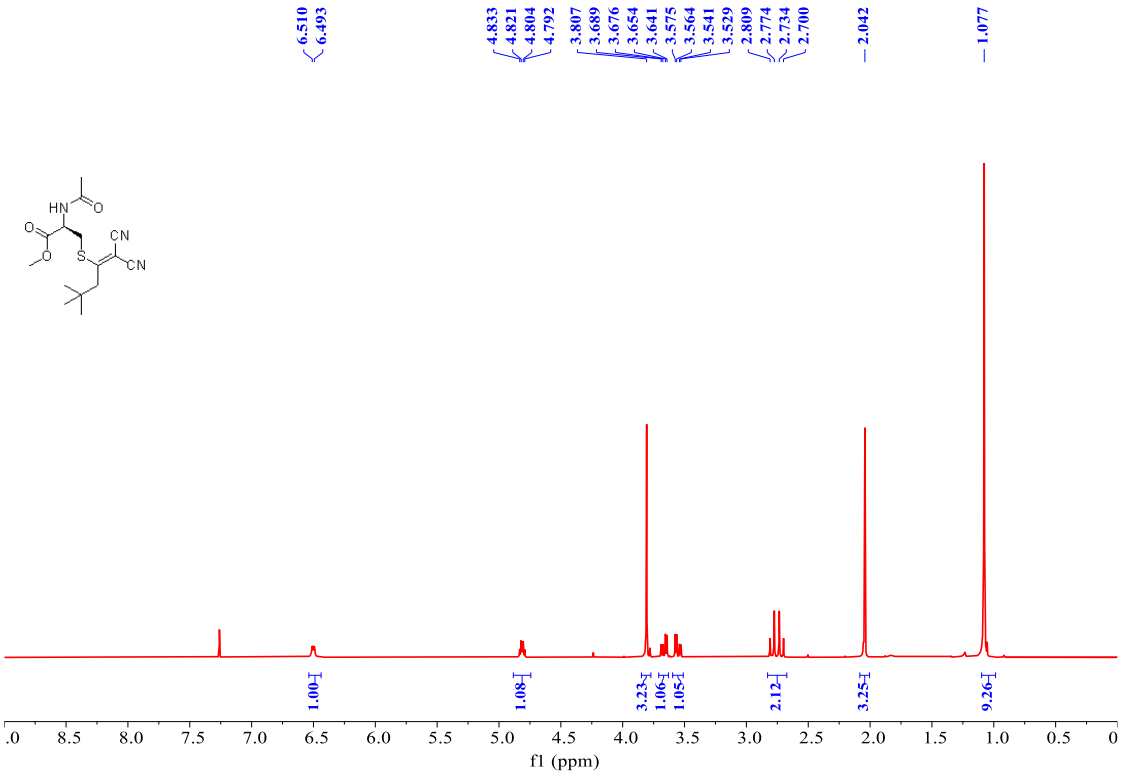
TAMM 1k

Compound **1k'** (318 mg, 1.94 mmol, 97% yield) was obtained as a light yellow solid from silica gel column chromatography (DCM: MeOH=10:1). ^{1}H NMR (400 MHz, Methanol-d_4) δ 2.507 (s, 2H), 1.075 (s, 9H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 188.621, 114.673, 112.015, 63.447, 46.695, 33.267, 28.827. ESI-(-)-HRMS $(M-H)^-$ calculated for $C_9H_{12}N_2O$: 163.08769; found: 163.08773 (-2.4 ppm). R_f (DCM: MeOH=10:1) = 0.2.

Compound **1k** (45 mg, 0.14 mmol, 14% yield) was obtained as a light yellow solid from silica gel column chromatography (EA: PE=2:1). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.502 (d, *J* = 6.80 Hz, 1H), 4.833 – 4.792 (m, 1H), 3.807 (s, 3H), 3.665 (dd, *J* = 14.00, 5.20 Hz, 1H), 3.552 (dd, *J* = 13.60, 4.40 Hz, 1H), 2.809 – 2.700 (m, 2H), 2.042 (s, 3H), 1.077 (s, 9H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 180.690, 170.331, 169.536, 112.927, 112.031, 83.391, 53.339, 52.268, 46.906, 35.389, 35.343, 29.757, 22.953. ESI-(+)-HRMS $(M+H)^+$ calculated for $C_{15}H_{21}N_3O_3S$: 324.13764; found: 324.13724 (+1.2 ppm). Rf (EA: PE=3:1) = 0.55.




188.621
114.673
112.015
63.447
46.695
33.267
28.827
HO
CN
CN
00 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
f1 (ppm)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3.541
3.529
2.809
2.774
2.734
2.700
2.042
1.077
HN
O
O
O
S
CN
CN
1.00
1.08
3.23
1.06
1.05
2.12
3.25
9.26
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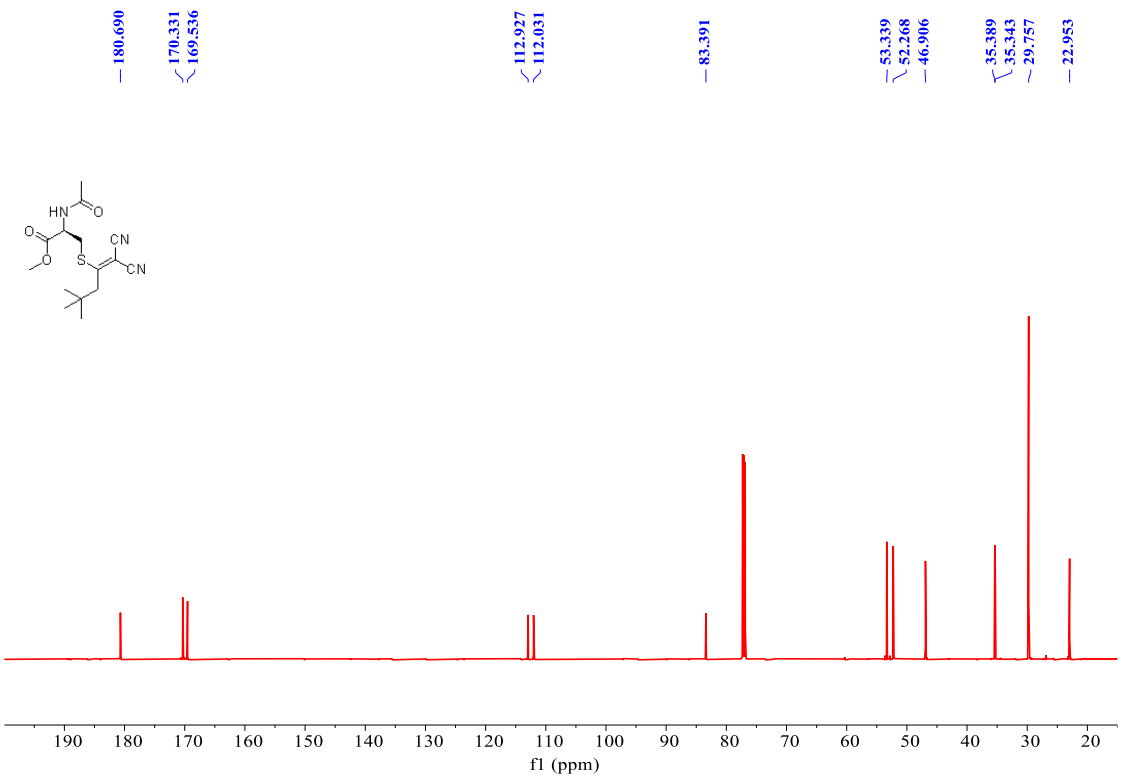
f1 (ppm)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O
O
O
S
CN
CN
190
180
170
160
150
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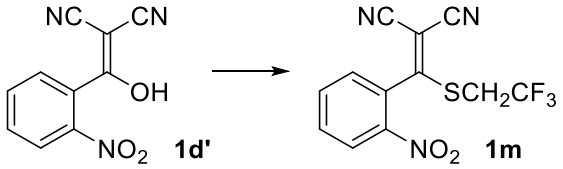
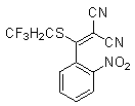
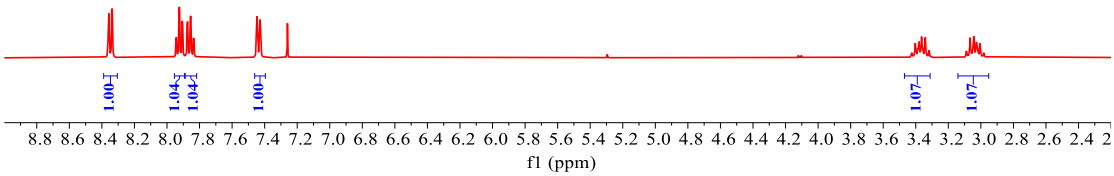
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)

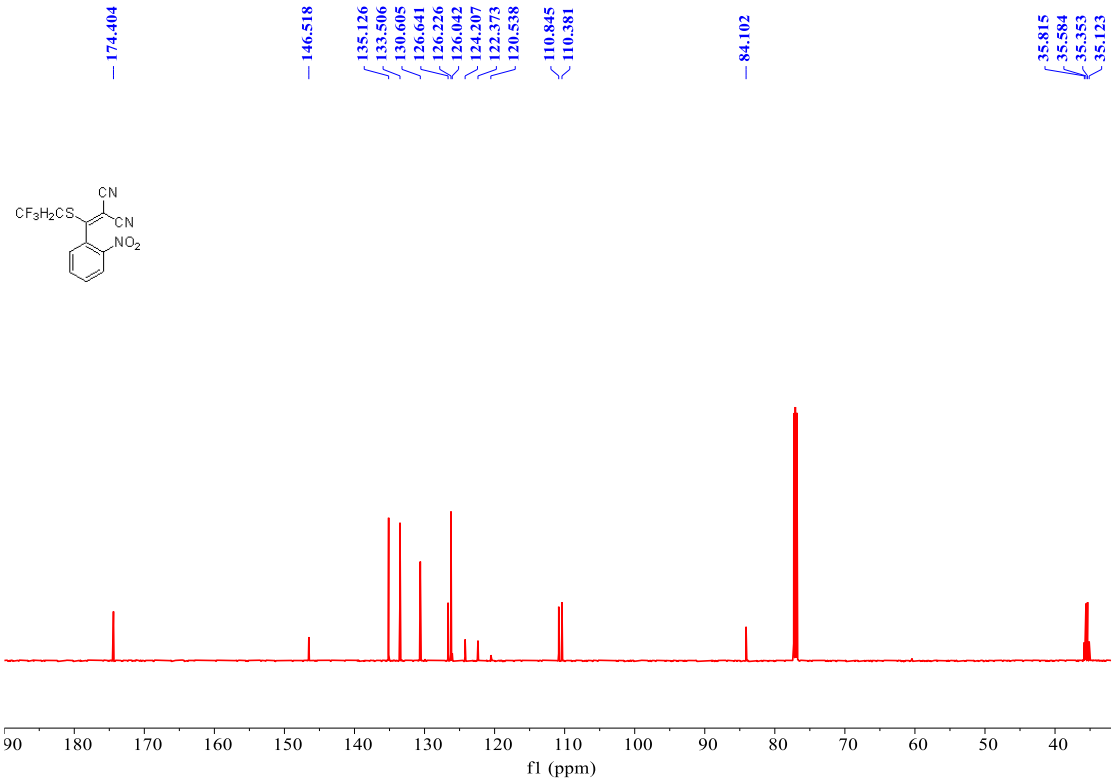
Synthesis of TAMM 1l



To a solution of **1d'** (557 mg, 2.6 mmol, 1.0 equiv.) in anhydrous acetonitrile (30 mL) was added PCl_5 (1.62 g, 7.8 mmol, 3.0 equiv.). Under nitrogen atmosphere, the reaction was stirred at 65 °C for 6 hours and then concentrated. The residue was dissolved in DCM (60 mL), washed with water (3 × 20 mL) and brine (20 mL), dried over Na_2SO_4, filter and concentrate. The residue was then dissolved in acetonitrile (10 mL), followed by addition of 2,2,2-trifluoroethanthiol (225 μL, 2.6 mmol, 1.0 equiv.) and DIPEA (466 μL, 2.6 mmol, 1.0 equiv.). The reaction was stirred at room temperature for overnight before concentrated under reduced pressure. The crude mixture was purified by silica gel column chromatography (EA: PE=2:1) to give compound **1m** (150 mg, 0.48 mmol, 18 % yield). ^{1}H NMR (400 MHz, Chloroform-d) δ 8.348 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.924 (ddd, *J* = 7.8, 7.6, 1.4 Hz, 1H), 7.856 (ddd, *J* = 8, 7.8, 1.6 Hz, 1H), 7.438 (dd, *J* = 7.60, 1.60 Hz, 1H), 3.371 (dq, *J* = 15.4, 9.2 Hz, 1H), 3.035 (dq, *J* = 15.4, 9.2 Hz, 1H). ^{13}C NMR (151 MHz, Chloroform-d) δ 174.404, 146.518, 135.126, 133.506, 130.605, 126.641, 126.226, 126.042, 124.207, 122.373, 120.538, 110.845, 110.381, 84.102, 35.815, 35.584, 35.353, 35.123. ^{19}F NMR (376 MHz, Chloroform-d) δ-65.544 (t, *J* = 9.2 Hz). ESI-(-)-HRMS $(M-H)^-$ calculated for $C_{12}H_6F_3N_3O_2S$: 312.00601; found: 312.00587 (+0.45 ppm). R_f (EA: PE=1:3) = 0.3.



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110.381
84.102
35.815
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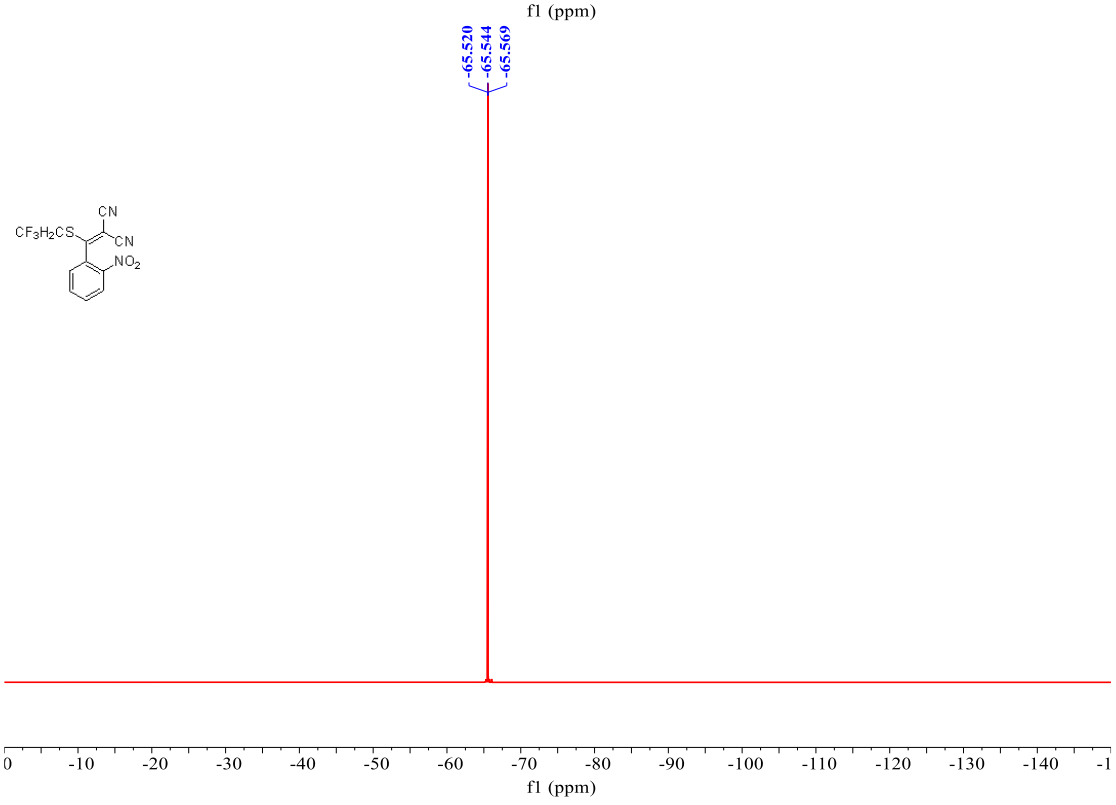
CF3H2CS
CN
NO2
90 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40
f1 (ppm)


-65.520
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CN
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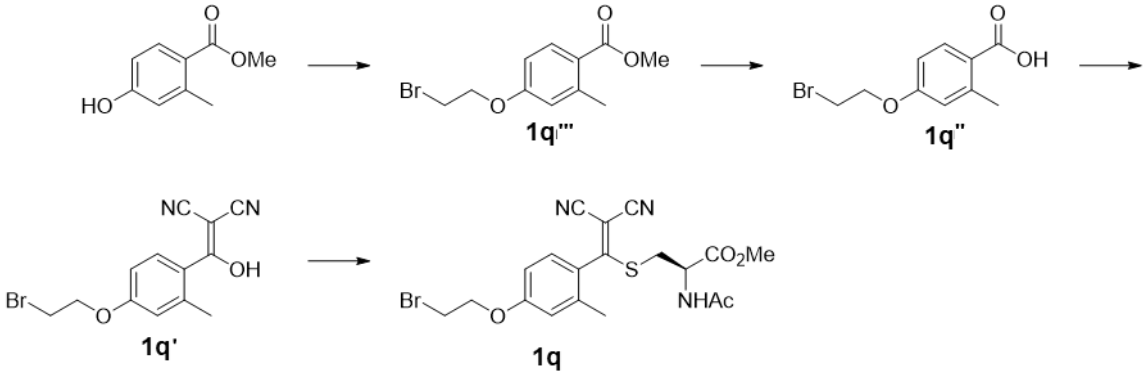
0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140
f1 (ppm)

Synthesis of TAMM 1q

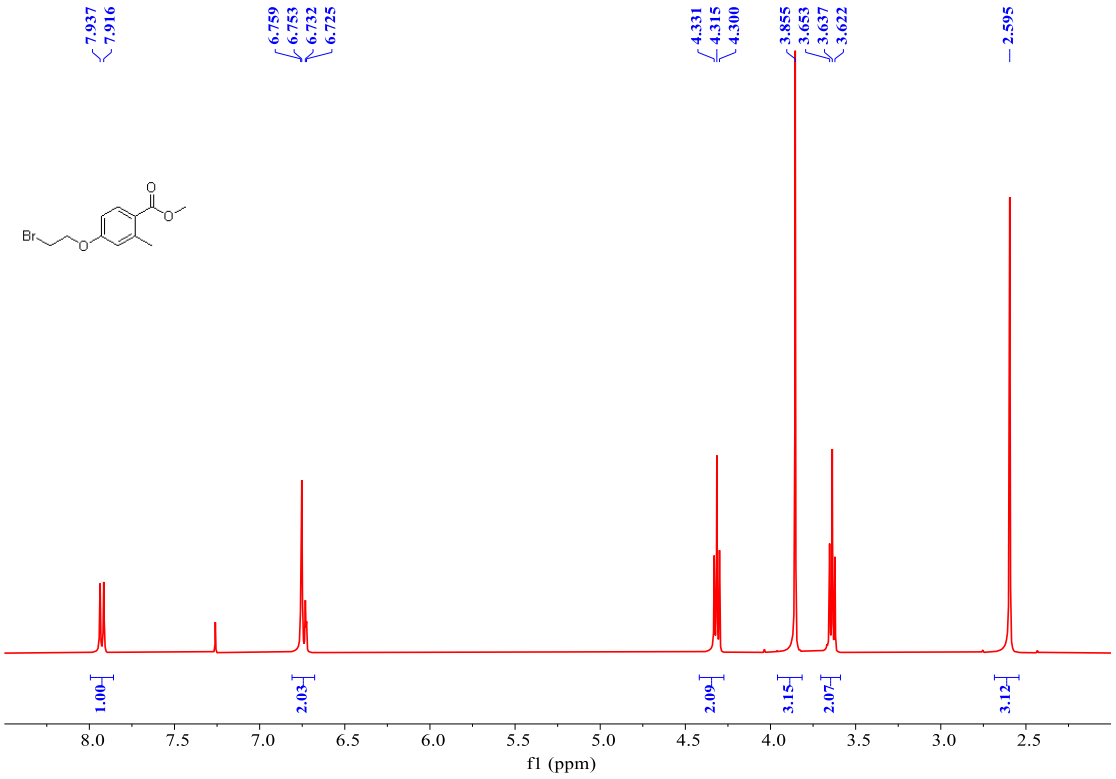


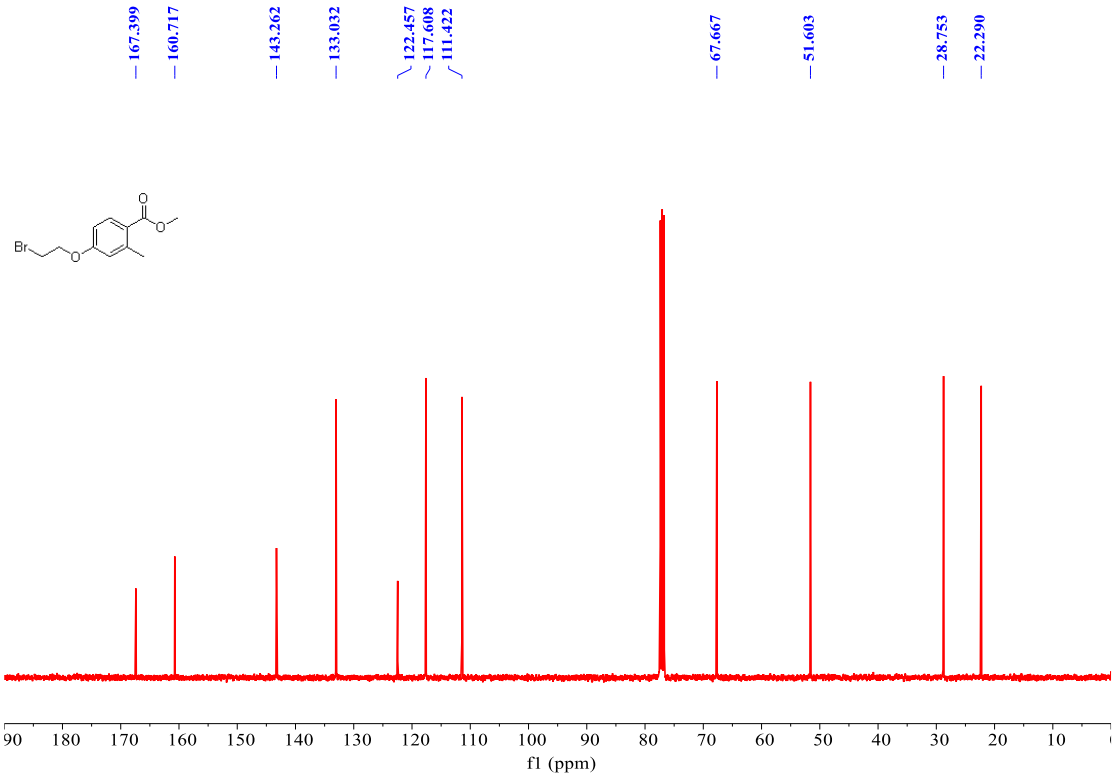
To a 2-mL acetone solution of methyl 4-hydroxy-2-methylbenzoate (1.66 g, 10 mmol, 1.0 eq) were added 1,2-dibromoethane (1.04 mL, 12 mmol, 1.2 eq) and K_2CO_3 (4.16 g, 30 mmol, 3.0 eq). The reaction mixture was refluxed for 6 hours before filtration to remove K_2CO_3. The solution was concentrated under reduced pressure before purification by silica gel column chromatography to afford **1q‴** (403 mg, 1.5 mmol, 15% yield). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.926 (d, *J* = 8.40 Hz, 1H), 6.759-6.725 (m, 2H), 4.315 (t, *J* = 6.20 Hz, 2H), 3.855 (s, 3H), 3.637 (t, *J* = 6.20 Hz, 2H), 2.595 (s, 3H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 167.399, 160.717, 143.262, 133.032, 122.457, 117.608, 111.422, 67.667, 51.603, 28.753, 22.290. R_f (EA: PE=1:10) = 0.20.

To a 5-mL methanol solution of **1q‴** (404 mg, 1.5 mmol, 1.0 eq) was slowly added sodium hydroxide (178 mg, 4.4 mmol, 3.0 eq) aqueous solution (5 mL). The reaction mixture was refluxed and stirred at 80 °C for 3 hours. The mixture was acidified to pH 1-2 with concentrated aqueous HCl before extraction with EtOAc (3 ×100 mL). The combined organic phases were washed with brine (3 ×50 mL), dried over Na_2SO_4, filtered and concentrated. The crude mixture was purified by silica gel column chromatography (DCM: MeOH=20:1) to afford **1q″** (302 mg, 1.17 mmol, 79% yield). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.856 (m, 1H), 6.653-6.634 (m, 2H), 4.217 (t, *J* = 6.10 Hz, 2H), 3.540 (t, *J* = 6.10 Hz, 2H), 2.482 (s, 3H).^{13}C NMR (151 MHz, Chloroform-*d*) δ 173.447, 164.775, 147.328, 137.406, 126.389, 121.509, 115.277, 71.619, 32.737, 26.095. ESI-(-)-HRMS $(M-H)^-$ calculated for $C_{10}H_{11}BrO_3$: 256.98188; found: 256.98278 (-3.5 ppm). R_f (DCM: MeOH=10:1) = 0.6.

To a mixture of **1q″** (302 mg, 1.17 mmol, 1.0 equiv.) and thionyl chloride (15 ml) was added DMF (50 μL) drop by drop at the room temperature. After two hours, the reaction mixture was concentrated under reduced pressure to give a white solid. The white solid was then dissolved in anhydrous THF (20 mL) and added dropwise into a mixture of NaH and malononitrile. The mixture of NaH and malononitrile was prepared by dropwise addition of an anhydrous THF solution (10 mL) of malononitrile (232 mg, 3.5 mmol, 3.0 equiv.) into a 10-mL anhydrous THF suspension of NaH (168.5 mg, 7.0 mmol, 6.0 equiv.) at 0 °C, followed by one hour incubation at 0 °C. After two hours, THF was removed under reduced pressure. The mixture was acidified to pH 1-2 using concentrated $HCl_{(aq)}$. The reaction mixture was extracted with EtOAc (3 ×100 mL). The combined organic phases were washed with brine (3 ×50 mL), dried over Na_2SO_4, filtered and concentrated. The crude mixture was purified by silica gel column chromatography (DCM: MeOH=10:1) to give **1q′** (241 mg, 0.79 mmol, 67% yield). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.228 (d, *J* = 8.30 Hz, 1H), 6.811 (d, *J* = 2.40 Hz, 1H), 6.770 (dd, *J* = 8.30, 2.40 Hz, 1H), 4.289 (t, *J* = 5.60 Hz, 2H), 3.676 (t, *J* = 5.60 Hz, 2H), 2.349 (s, 3H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 192.541, 159.229, 136.988, 131.837, 128.582, 116.272, 111.219, 67.921, 29.269, 18.340. ESI-(-)-HRMS $(M-H)^-$ calculated for $C_{13}H_{11}BrN_2O_2$: 304.99311; found:304.99431 (-3.9 ppm). R_f (DCM: MeOH=1:1) = 0.2.

To a 20-mL acetonitrile solution of **1q′** (241 mg, 0.79 mmol, 1.0 equiv.) was added PCl_5 (492 mg, 2.4 mmol, 3.0 equiv.). The reaction mixture was stirred at 65 °C under nitrogen atmosphere for six hours. The solvent was then removed under reduced pressure. The residue was dissolved in DCM (60 mL), washed with water (3 × 20 mL) and brine (20 mL), dried over Na_2SO_4, filtered, and concentrated to give a yellow solid. To an acetonitrile solution (5 mL) of the yellow solid (32 mg, 0.1 mmol, 1.0 equiv.) were added Ac-Cys-OMe (18 mg, 0.1 mmol, 1.0 equiv.) and Et_3N (28 μL, 0.2 mmol, 2.0 equiv.) at 0 °C. After ten min, acetonitrile was removed under reduced pressure. The reaction mixture was purified by silica gel column chromatography (EA: PE=2:1) to give **1q** (24 mg, 0.05 mmol,52% yield). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.085-7.023 (m, 1H), 6.880-6.855 (m, 2H), 6.256 (br, 1H), 4.646 (m, 1H), 4.319 (t, *J* = 6.10 Hz, 2H), 3.762 (br, 3H), 3.652 (t, *J* = 6.10 Hz, 2H), 3.172-3.023 (m, 1H), 2.917 (dd, *J* = 13.20, 5.20 Hz, 1H), 2.295 (s, 3H), 2.026 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 181.297, 181.186, 169.986, 169.968, 169.588, 169.538, 160.428, 137.630, 137.315, 129.667, 129.553, 124.596, 117.664, 117.478, 113.246, 113.081, 112.110, 111.766, 81.052, 67.880, 53.305, 51.272, 51.210, 35.954, 28.677, 23.056, 19.409, 19.370. ESI-(+)-HRMS $(M+H)^+$ calculated for $C_{19}H_{20}BrN_3O_4S$: 466.04307; found: 466.04207 (+2.1 ppm). Rf (EA: PE=2:1) = 0.5.

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22.290
Br
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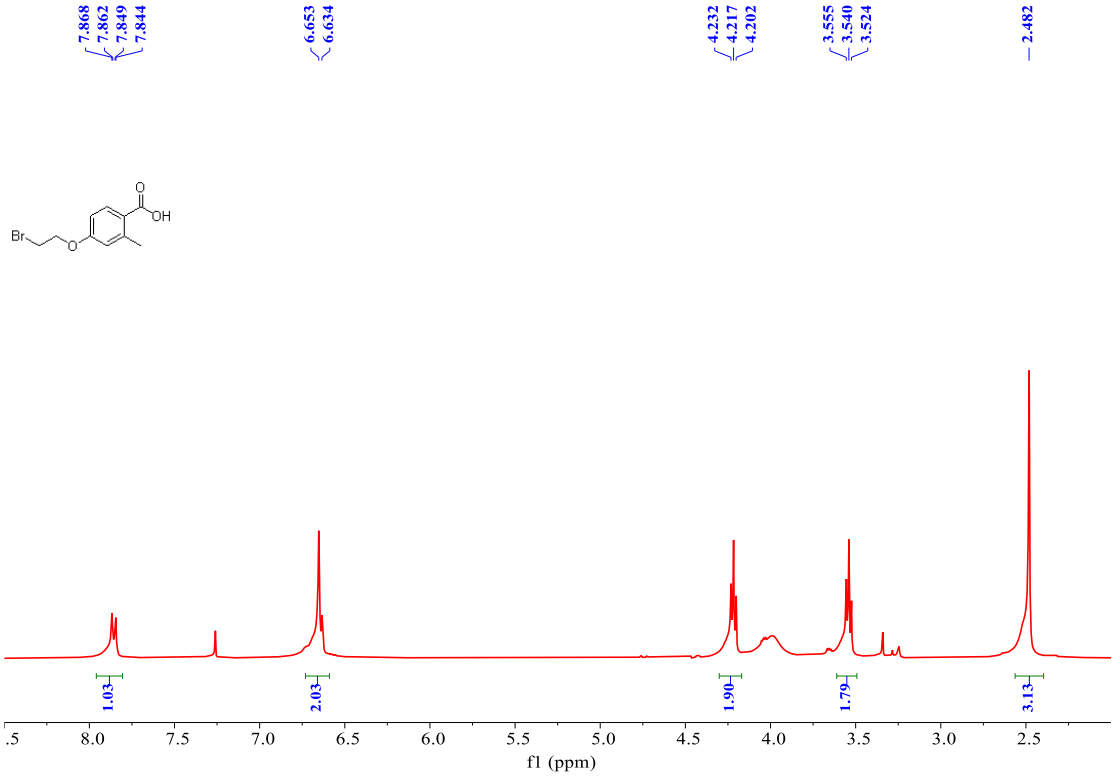
f1 (ppm)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2.482
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OH
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2.03
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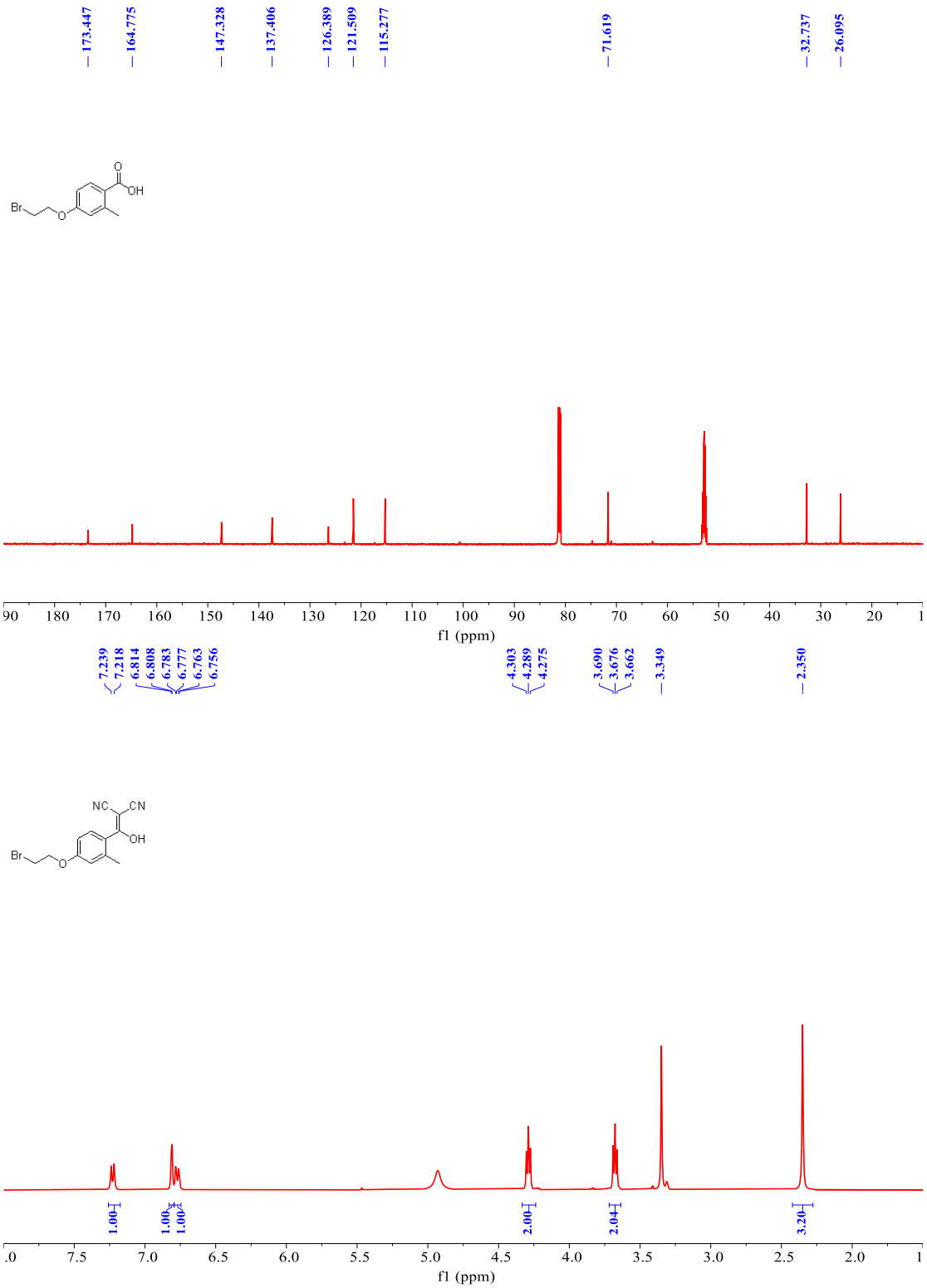
f1 (ppm)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126.389
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26.095
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OH
Br
O
90 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 1
f1 (ppm)
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7.218
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6.808
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6.777
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3.662
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2.350
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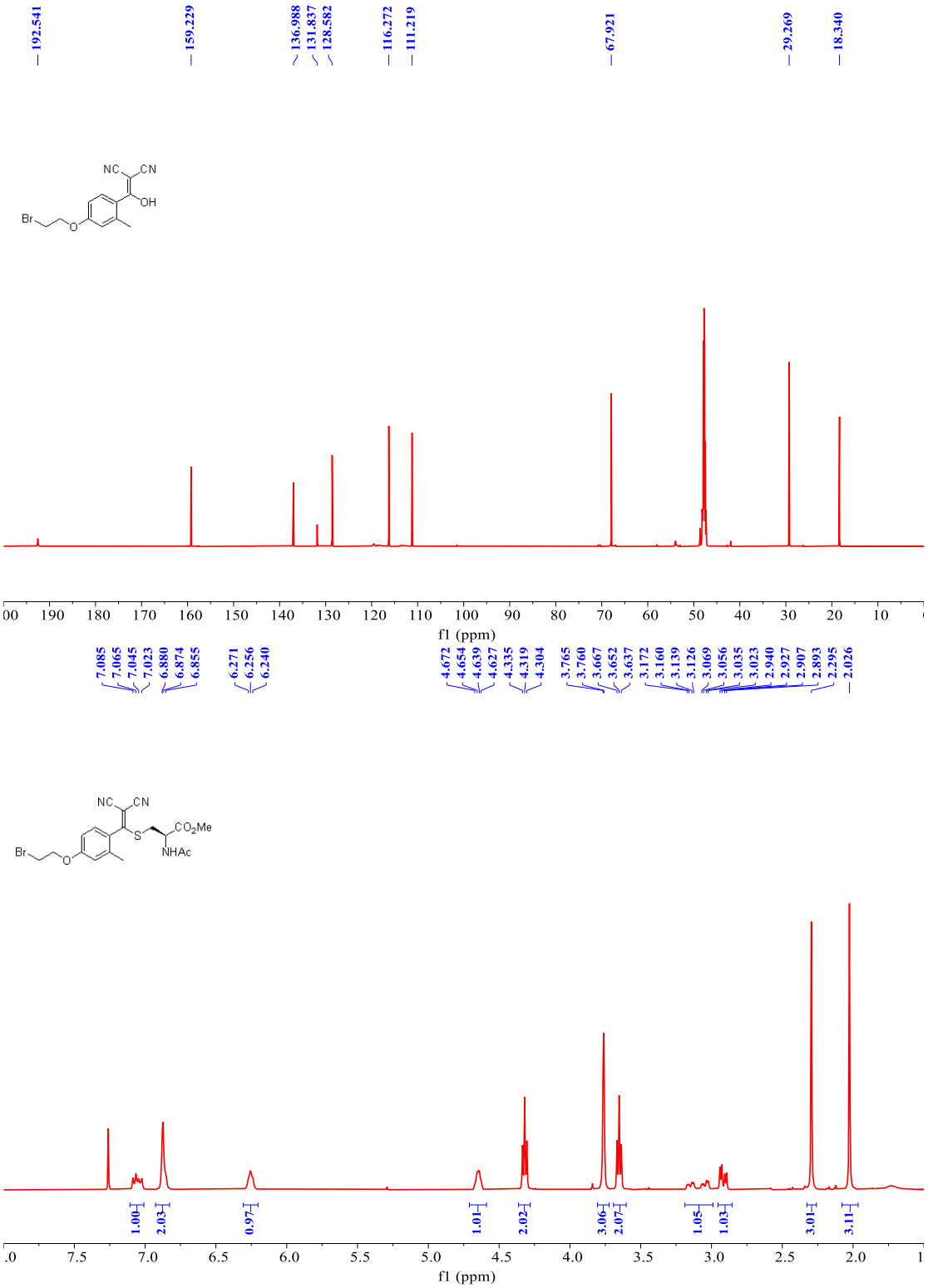
f1 (ppm)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Br
O
f1 (ppm)
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4.627
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4.304
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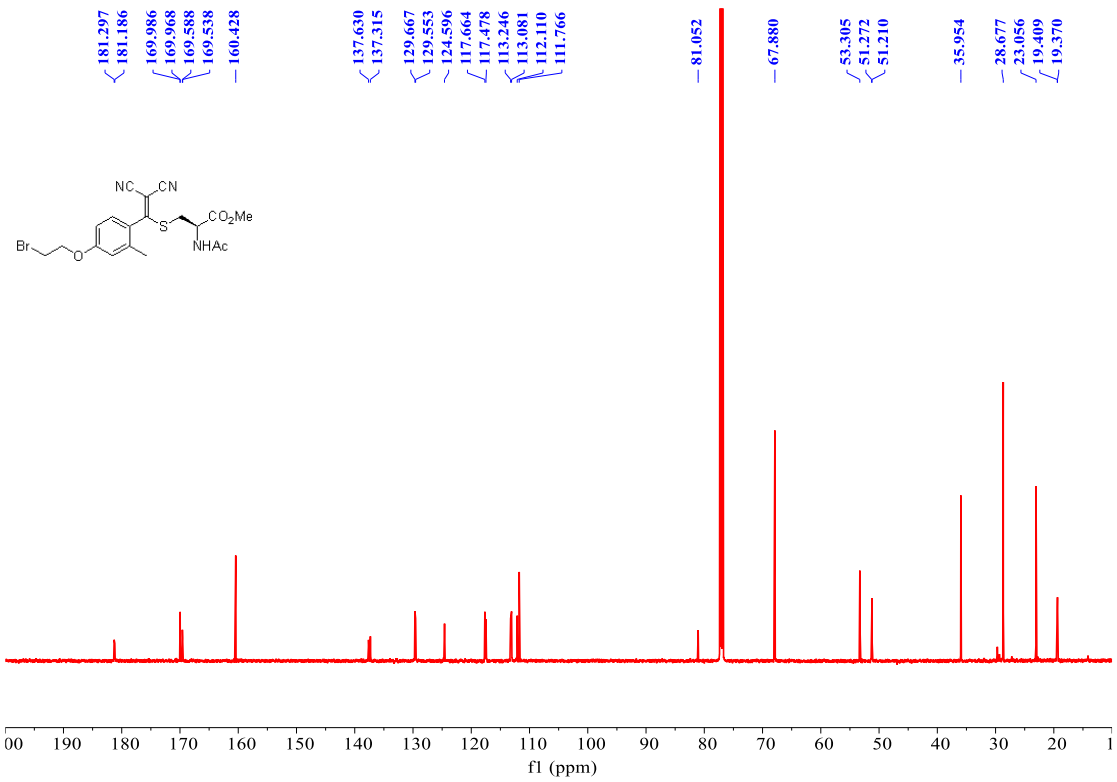
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35.954
28.677
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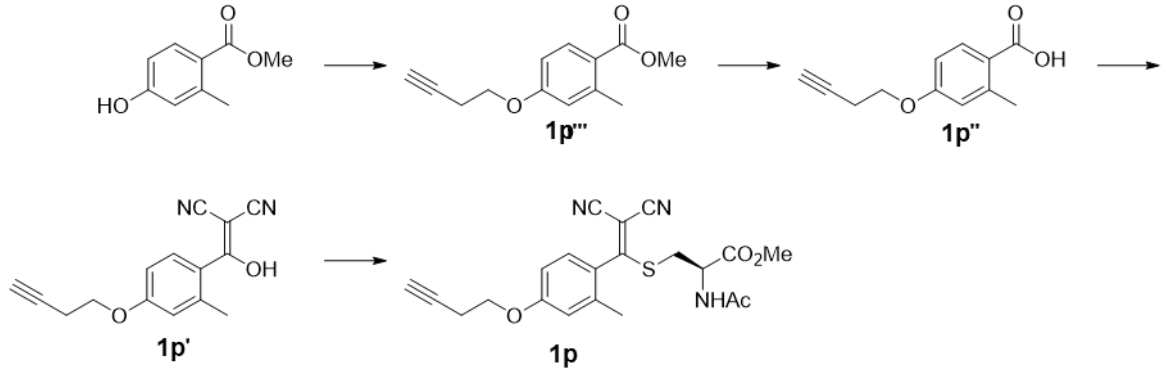
f1 (ppm)

Synthesis of TAMM 1p

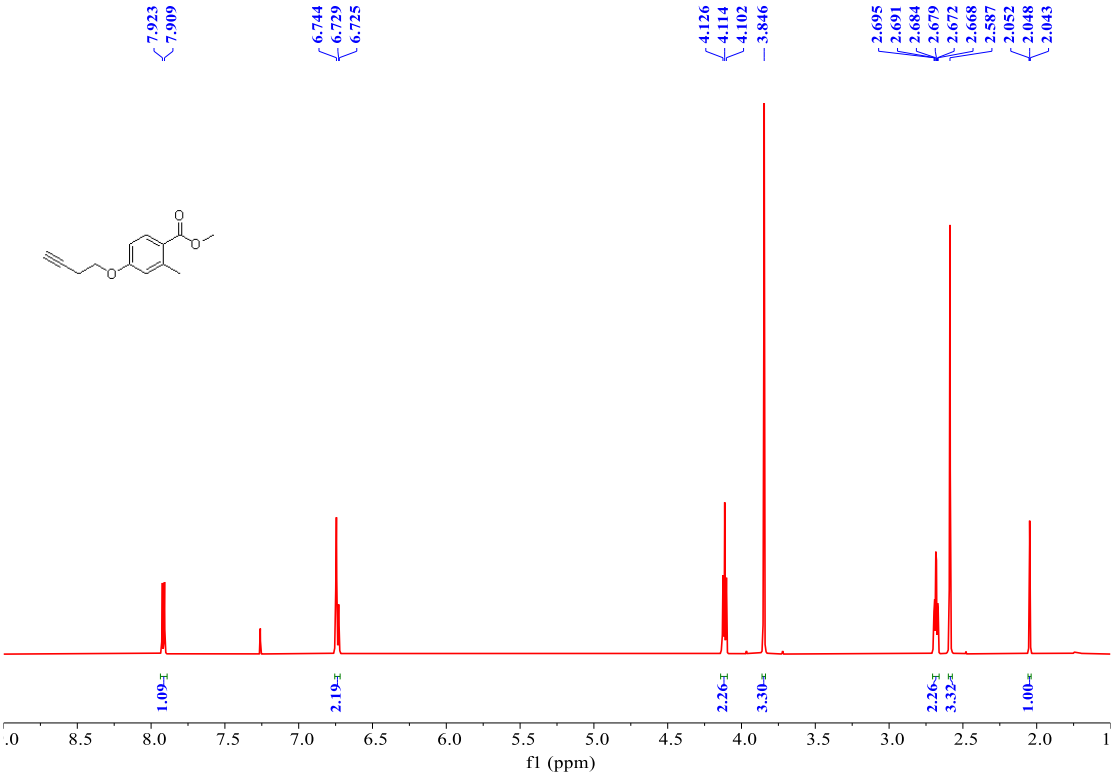


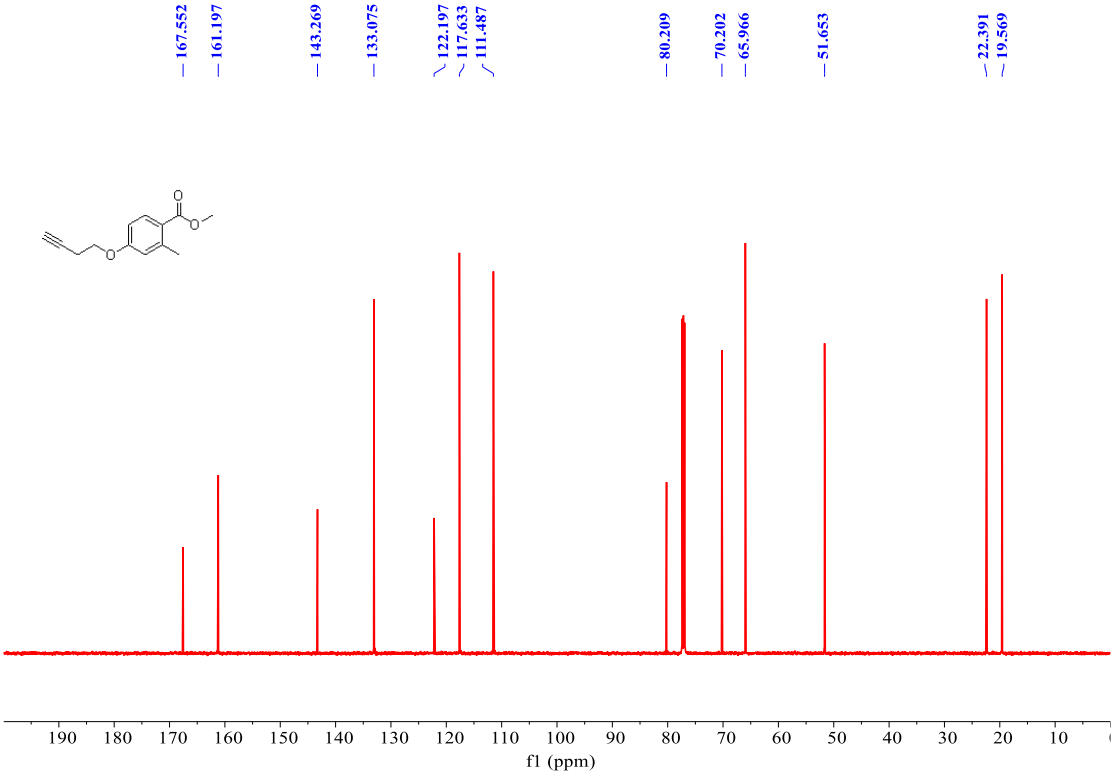
To a solution of methyl 4-hydroxy-2-methylbenzoate (166 mg,1.0 mmol, 1.0 equiv.), K_2CO_3 (414 mg, 3.0 mmol, 3.0 equiv.) in acetone (20 mL) was added but-3-yn-1-yl 4-methylbenzenesulfonate (530 µL, 3.0 mmol, 3.0 equiv.). After 24 hours at 80 °C, acetone was then removed under reduced pressure. The residue was purified by flash chromatography (SiO_2, 20% EtOAc in PE) to yield **1p‴** as a yellow powder (72 mg, 0.33 mmol, 33%). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.92 (d, *J* = 8.4 Hz, 1H), 6.74 – 6.73 (m, 2H), 4.1 (t, *J* = 7.1 Hz, 2H), 3.85 (s, 3H), 2.68 (td, *J* = 7.1, 2.6 Hz, 2H), 2.59 (s, 3H), 2.0 (t, *J* = 2.6 Hz, 1H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 167.6, 161.2, 143.3, 133.1, 122.2, 117.6, 111.5, 80.2, 70.2, 66.0, 51.7, 22.4, 19.6. ESI-(+)-HRMS $(M+H)^+$ calculated for $C_{13}H_{15}O_3$: 219.1016; found:219.1016. R_f (EA: PE=1:10) = 0.20.

To a solution of **1p‴** (44 mg, 0.2 mmol, 1.0 equiv.) in MeOH (3 mL) was added a solution of NaOH (24 mg, 0.6 mmol, 3.0 equiv.) in water (3 mL). After two hours at 80 ℃, complete conversion was confirmed by TLC. MeOH was then removed under reduced pressure. Then the mixture was acidified to pH 1–2 using 1 N $HCl_{(aq)}$. The mixture was extracted with EtOAc (3 ×20 mL). The combined organic phases were washed with brine (20 mL), dried over Na_2SO_4, filtered, and concentrated to **1p″** as a white solid (39 mg, 0.19 mmol, 95%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.92 (d, *J* = 9.60 Hz, 1H), 6.80 – 6.78 (m, 2H), 4.12 (t, *J* = 6.65 Hz, 2H), 2.66 (td, *J* = 6.7, 2.6 Hz, 2H), 2.57 (s, 3H), 2.34 (t, *J* = 2.6 Hz, 1H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 169.2, 161.5, 142.9, 133.0, 122.1, 117.7, 111.1, 79.9, 69.6, 65.9, 21.1, 18.7. ESI-(-)-HRMS $(M-H)^-$ calculated for $C_{12}H_{11}O_3$: 203.0714; found: 203.0714. Rf (DCM: MeOH=10:1) = 0.6.

To a solution of **1p″** (30 mg, 0.15 mmol, 1.0 equiv.) in $SOCl_2$ (2 mL) was added DMF (5 µL). After 1 hours at room temperature, $SOCl_2$ was removed under reduced pressure to yield 4-(but-3-yn-1-yloxy)-2-methylbenzoyl chloride as a colorless oil. The colorless oil was then dissolved in anhydrous THF (2 mL) and added dropwise into a mixture of NaH and malononitrile. The mixture of NaH and malononitrile was prepared by dropwise addition of an anhydrous THF solution (3 mL) of malononitrile (20 mg, 0.3 mmol, 2.0 equiv.) into a 10-mL anhydrous THF suspension of NaH (144 mg, 6.0 mmol, 2.0 equiv.) at 0 °C, followed by one hour incubation at 0 °C. After two hours, THF was removed under reduced pressure. The mixture was acidified to pH 1-2 using concentrated $HCl_{(aq)}$. The reaction mixture was extracted with EtOAc (3 ×100 mL). The combined organic phases were washed with brine (3 ×50 mL), dried over Na_2SO_4, filtered and concentrated. The crude mixture was purified by silica gel column chromatography (DCM: MeOH=10:1) to give **1p′** as a light-yellow solid (25 mg, 0.1 mmol, 68%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.30 (d, *J* = 8.4 Hz, 1H), 6.89 (s, 1H), 6.85 (d, *J* = 8.4 Hz, 1H), 4.12 (t, *J* = 6.7 Hz, 2H), 2.66 (td, *J* = 6.7, 2.7 Hz, 2H), 2.36 (s, 3H), 2.33 (t, *J* = 2.7 Hz, 1H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 162.0, 139.1, 130.7, 128.5, 117.8, 112.9, 81.3, 71.0, 67.4, 20.1, 19.5. ESI-(-)-HRMS $(M-H)^-$ calculated for $C_{15}H_{11}N_2O_2$: 251.0826; found: 251.0829. R_f (DCM: MeOH=10:1) = 0.2.

To a solution of **1p′** (25 mg, 0.1 mmol, 1.0 equiv.) in acetonitrile (6 mL) was added PCl_5 (62 mg, 0.3 mmol, 3.0 equiv.). After five hours at 60 ℃ under argon atmosphere, the mixture was concentrated. The residue was dissolved in DCM (20 mL), washed with water (3×10 mL) and brine (3×10 mL), dried over Na_2SO_4, filtered, and concentrated. The resulting yellow solid was then dissolved in acetonitrile (3 mL), N-acetyl-L-cysteine methyl ester (35 mg, 0.2 mmol, 2.0 equiv.) and $NaHCO_3$ (25 mg, 0.3 mmol, 3.0 equiv.) were added. The reaction was stirred at room temperature for two hours. The acetonitrile was removed under reduced pressure. Brine (15 mL) was added. The mixture was extracted with EtOAc (3×20 mL). The combined organic phases were washed with brine (20 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by flash chromatography (SiO_2, 10% MeOH in EtOAc) to yield **1p** as an oil (9 mg, 0.023 mmol, 23%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.04 (m, 1H), 6.86 (s, 1H), 6.84 (s, 1H), 6.45 (d, *J* = 8.0 Hz, 1H), 4.92 – 4.46 (m, 1H), 4.10 (t, *J* = 6.8 Hz, 2H), 3.73 (s, 3H), 3.23 – 2.66 (m, 3H), 2.70 – 2.66 (m, 2H), 2.28 (s, 3H), 2.5 (m, 1H), 2.01 (s, 3H). ^{13}C NMR (101 MHz, Methanol-d_4) δ 181.7, 170.4, 169.7, 160.9, 137.6, 129.7, 124.3, 117.6, 113.2, 112.2, 111.9, 80.1, 70.3, 66.1, 53.3, 51.3, 36.0, 26.9, 23.1, 19.5, 19.4. ESI-(-)-HRMS $(M-H)^-$ calculated for $C_{21}H_{21}N_3O_4S$: 412.1326; found: 412.1314. Rf (PE: EA=1:1) = 0.3.

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19.569
O
O
O
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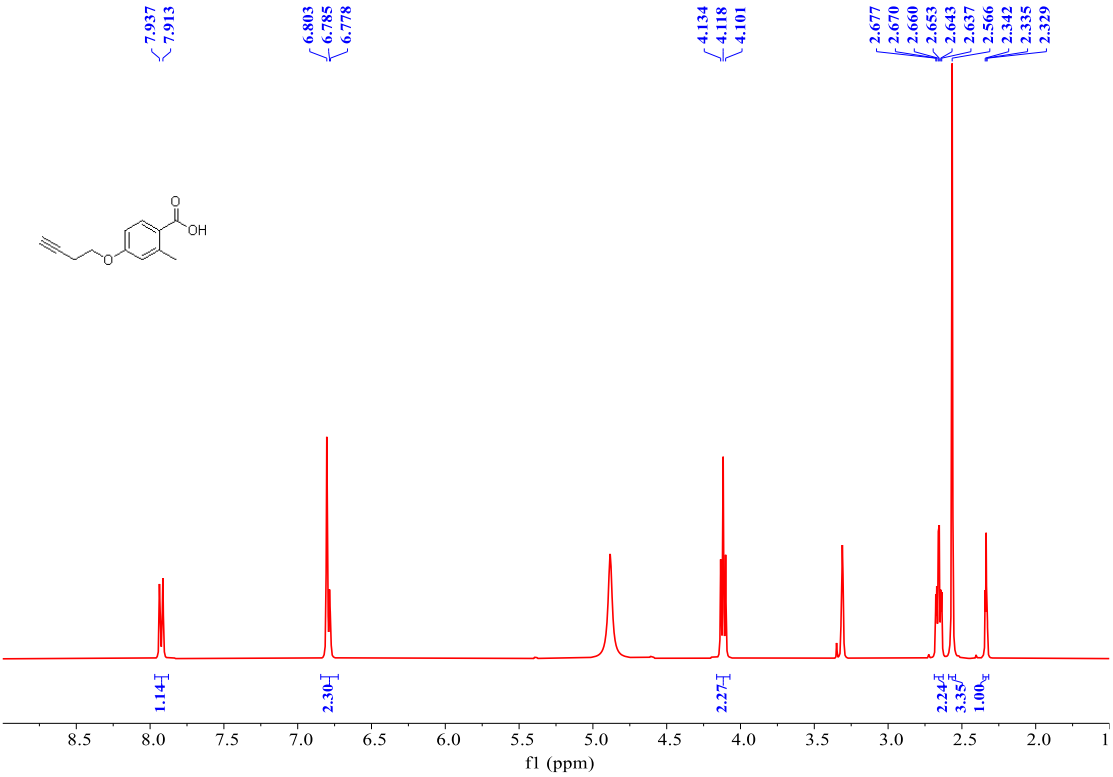
f1 (ppm)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4.101
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2.643
2.637
2.566
2.342
2.335
2.329
O
OH
1.14
2.30
2.27
2.24
3.35
1.00
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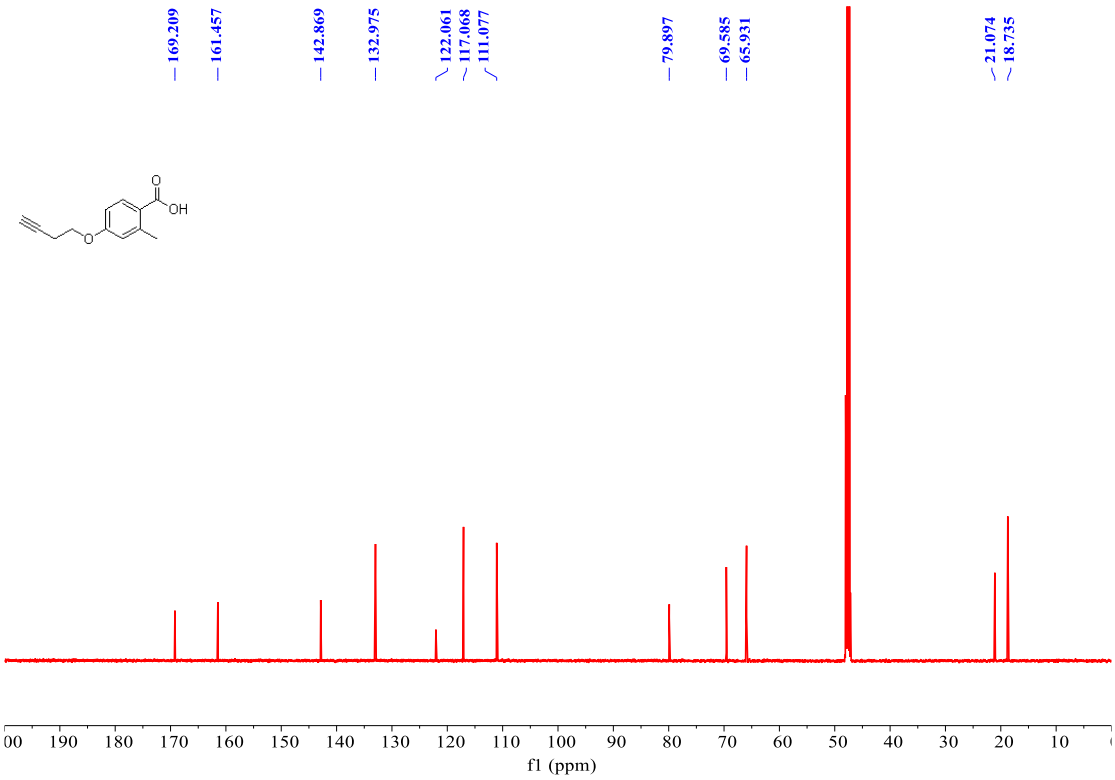
f1 (ppm)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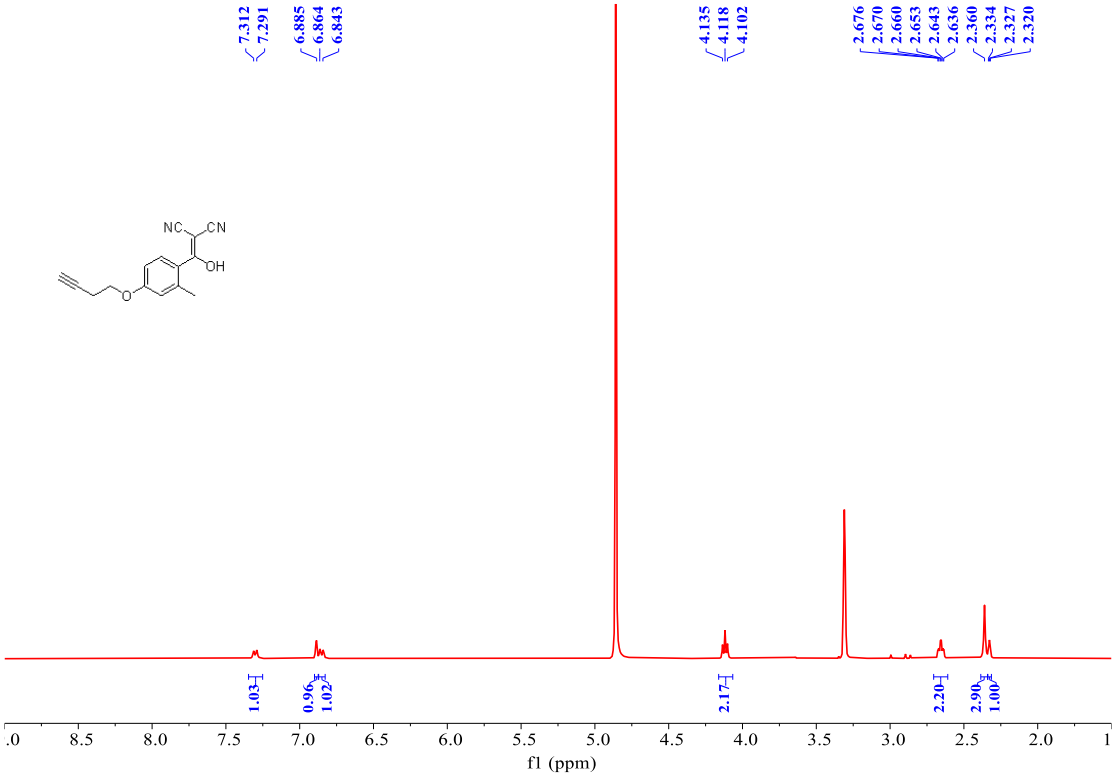
OH
O
f1 (ppm)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2.334
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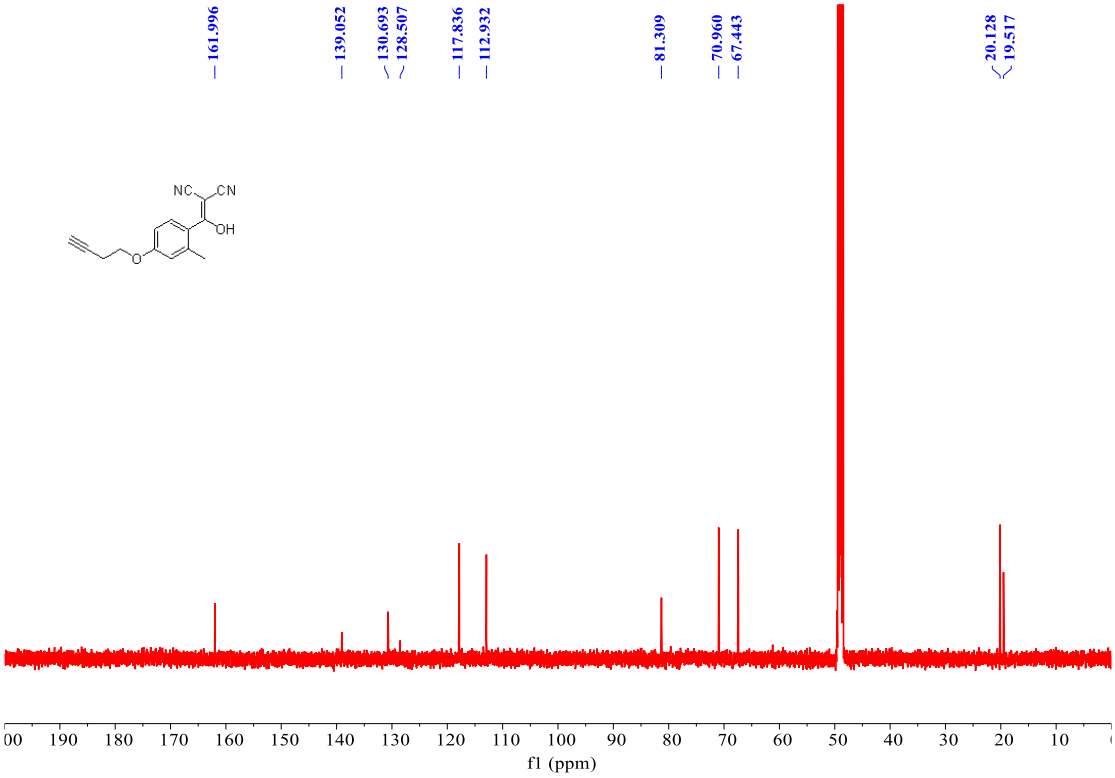
f1 (ppm)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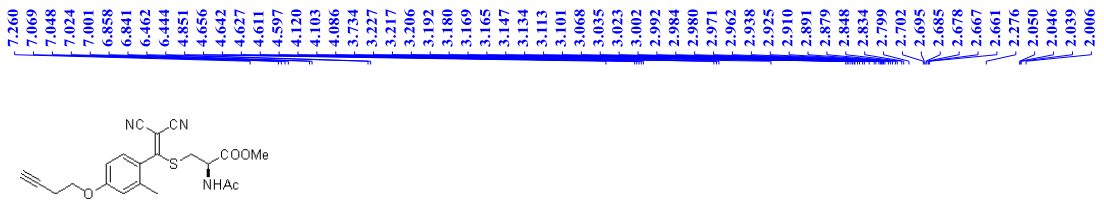
O
f1 (ppm)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3.217
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3.192
3.180
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3.165
3.147
3.134
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3.101
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3.023
3.002
2.992
2.984
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2.695
2.685
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COOMe
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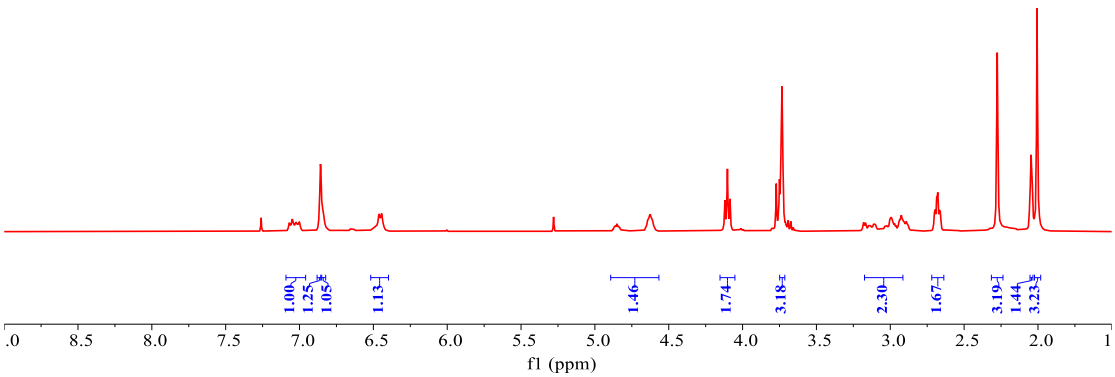
O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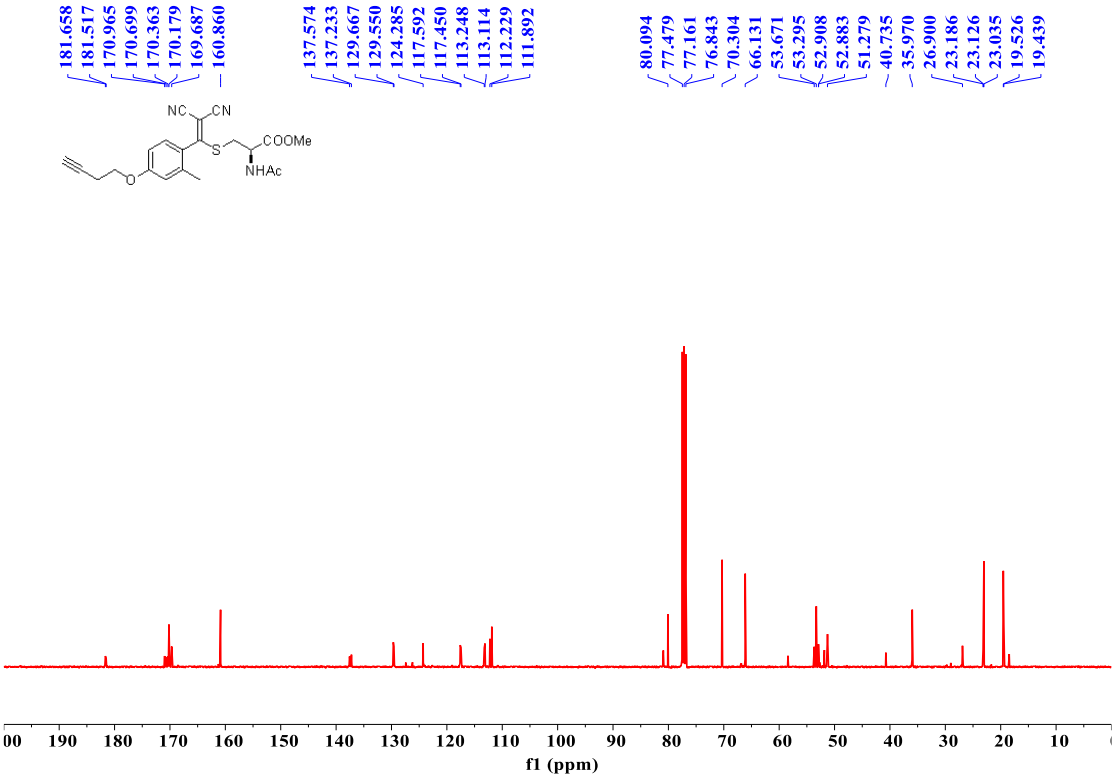
3.23
f1 (ppm)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66.131
53.671
53.295
52.908
52.883
51.279
40.735
35.970
26.900
23.186
23.126
23.035
19.526
19.439
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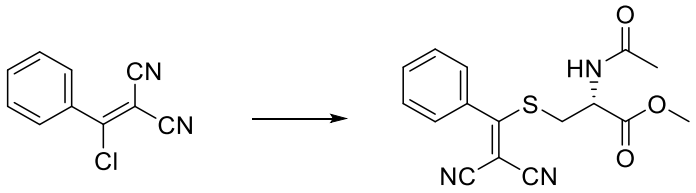
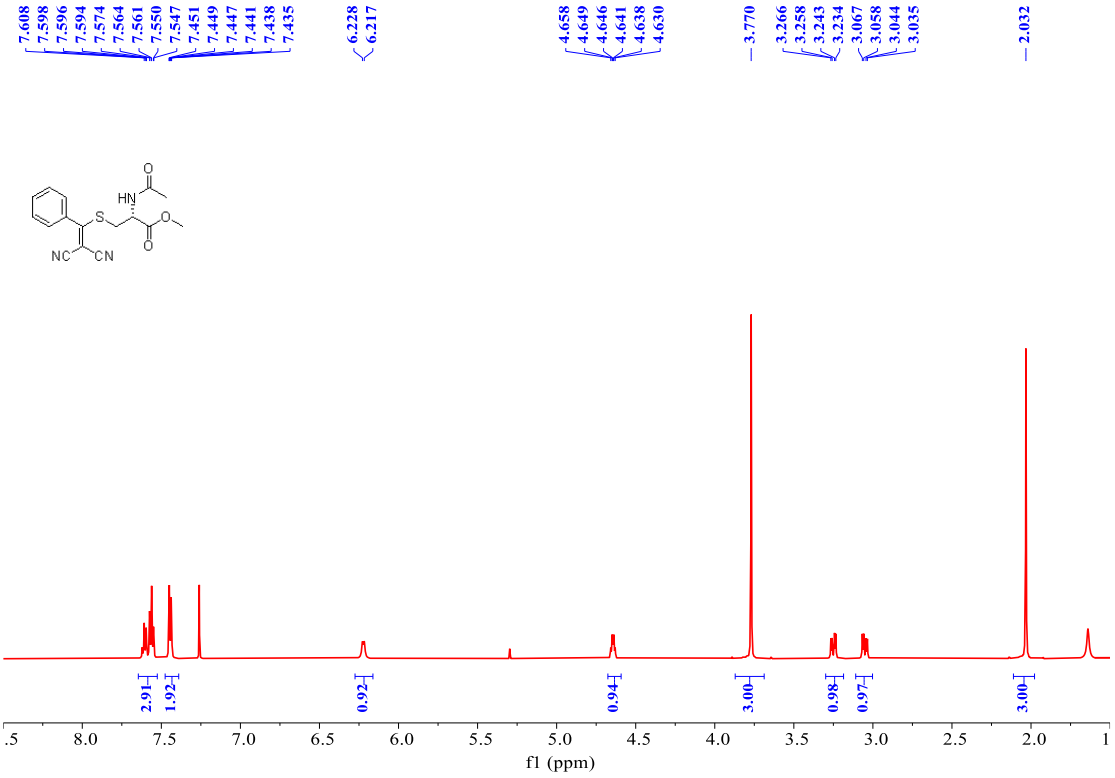
f1 (ppm)

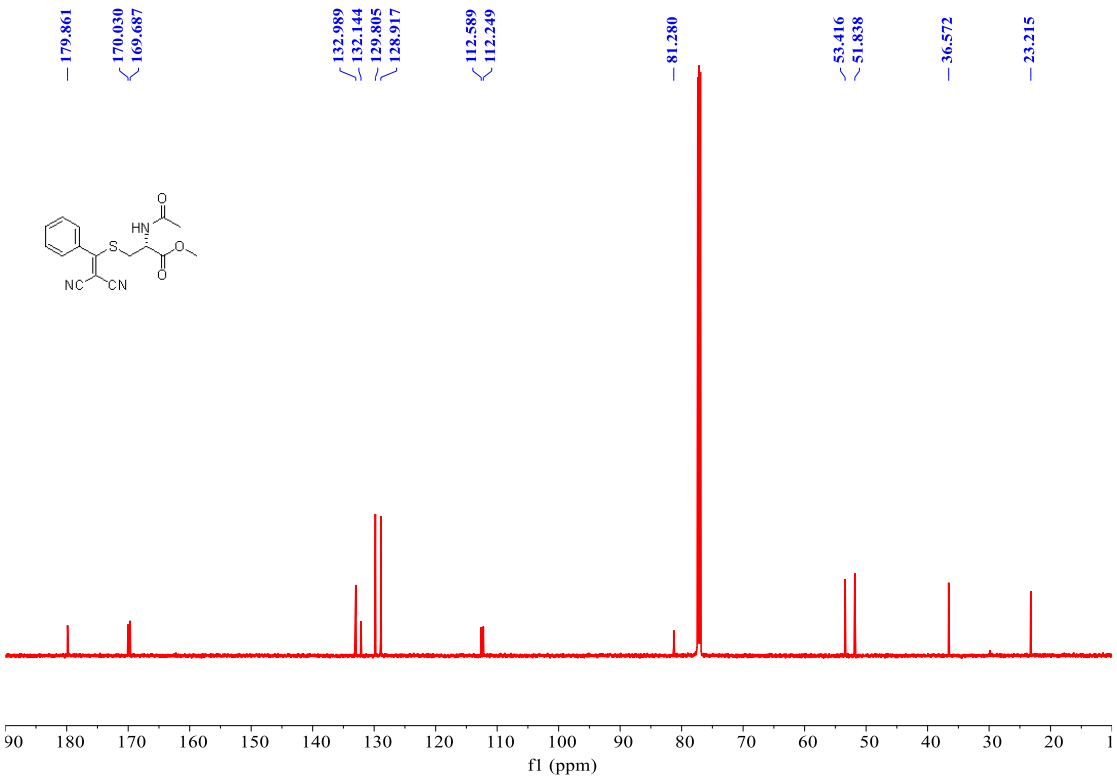
Synthesis of TAMM 1m



1m' **1m**

To a solution of **1m'** (35 mg, 0.19 mmol, 1.0 equiv.) in acetonitrile was added N-acetyl-L-cysteine methyl ester (39 mg, 0.22 mmol, 1.2 equiv.) and $NaHCO_3$ (50 mg, 0.60 mmol, 3.0 equiv.). The reaction was stirred at room temperature for overnight before concentrated under reduced pressure. The residue was purified by flash chromatography (SiO_2, 50% PE in EtOAc) to yield **1m** as an oil (53 mg, 0.16 mmol, 84%). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.61 – 7.55 (m, 3H), 7.45 – 7.44 (m, 2H), 6.22 (d, *J* = 6.6 Hz, 1H), 4.64 (dt, *J* = 6.6, 5.1 Hz, 1H), 3.77 (s, 3H), 3.25 (dd, *J* = 13.8, 5.1 Hz, 1H), 3.05 (dd, *J* = 13.8, 5.1 Hz, 1H), 2.03 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 179.9, 170.0, 169.7, 133.0, 132.1, 129.80, 129.0, 112.6, 112.2, 81.3, 53.4, 51.8, 36.6, 23.2. Rf (PE: EA=1:2) = 0.3.

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O
O
NC
CN
90
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170
160
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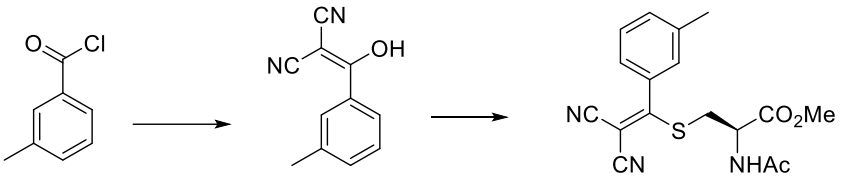
100
90
80
70
60
50
40
30
20
1
f1 (ppm)

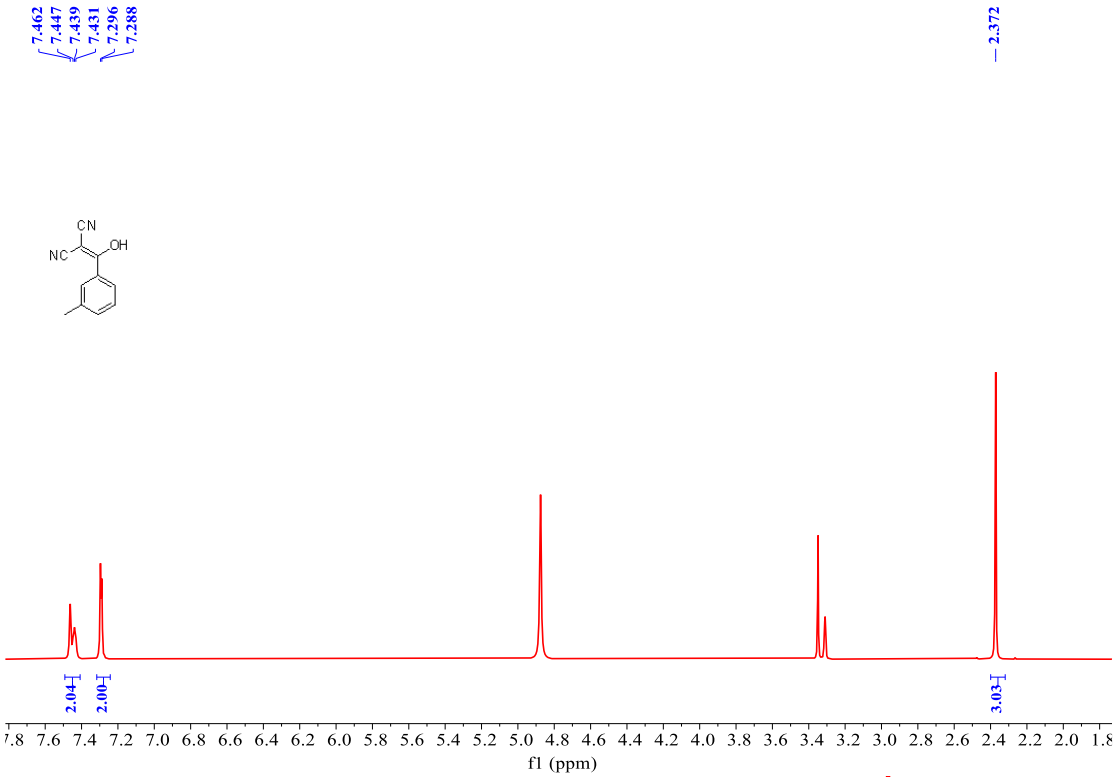
Synthesis of TAMM 1n



1n'' **1n'** **1n**

To a suspension of NaH (1440 mg, 60 mmol, 6 equiv.) in anhydrous THF (10 mL) under nitrogen atmosphere at 0 °C was added a solution of malononitrile (1982 mg, 30 mmol, 3 equiv.) in anhydrous THF (10 mL) in a dropwise manner. After 1 hour at 0 °C, a solution of **1n''** (1550 mg, 10 mmol, 1 equiv.) in anhydrous THF (10 mL) was added into the mixture at 0 °C in a dropwise manner. After the addition, the reaction was allowed to warm up to room temperature. After 2 hours at room temperature, the solvent was removed under reduced pressure. The mixture was acidified to pH 1-2 using $HCl_{(aq)}$. The mixture was extracted with EtOAc (3 ×100 mL). The combined organic layers were washed with brine (3 ×50 mL), dried over Na_2SO_4, filtered and concentrated. Silica gel column chromatography (DCM: MeOH=10:1) was performed to isolate **1n'** (1830 mg, 9.9 mmol, 99%). ^{1}H NMR (600 MHz, Methanol-d_4) δ 7.46 – 7.43 (m, 2H), 7.29 (d, J = 4.8 Hz, 2H), 2.37 (s, 3H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 191.9, 139.2, 139.1, 132.6, 129.1, 129.1, 125.8, 121.5, 119.9, 53.2, 21.4. R_f (DCM: MeOH=6:1) = 0.3.

To a solution of **1n'** (1800 mg, 9.8 mmol, 1.0 equiv.) in anhydrous acetonitrile (50 ml) under nitrogen atmosphere was added PCl_5 (6115 mg, 3 mmol, 3 equiv.) was added. After 6 hours at 65 °C, the solvent was removed under reduced pressure. The residue was dissolved in DCM (60 mL), washed with water (3 × 20 mL) and brine (20 mL), dried over Na_2SO_4, filter and concentrate. The residue was (199 mg, 0.98 mmol, 1.3 equiv.) then dissolved in acetonitrile (15 mL), followed by addition of Ac-Cys-OMe (137 mg, 0.77 mmol, 1.0 equiv.) and $NaHCO_3$ (192 mg, 2.3 mmol, 3 equiv.). After overnight at room temperature, the solvent was removed under reduced pressure. The mixture was purified by silica gel column chromatography (EA: PE=2:1) to afford **1n** (264 mg, 0.77 mmol, 99%). ^{1}H NMR (600 MHz, Chloroform-d) δ 7.45 – 7.39 (m, 2H), 7.22 (d, J = 6.6 Hz, 2H), 6.27 (d, J = 6.6 Hz, 1H), 4.66 (dt, J = 7.2, 5.1 Hz, 1H), 3.77 (s, 3H), 3.26 – 3.23 (m, 1H), 3.07 – 3.04 (m, 1H), 2.43 (s, 3H), 2.03 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-d) δ 180.2, 170.0, 169.7, 139.9, 133.8, 132.1, 129.6, 129.2, 126.0, 112.7, 112.3, 80.9, 53.4, 51.8, 36.6, 23.2, 21.5. R_f (EA: PE=2:1) = 0.3.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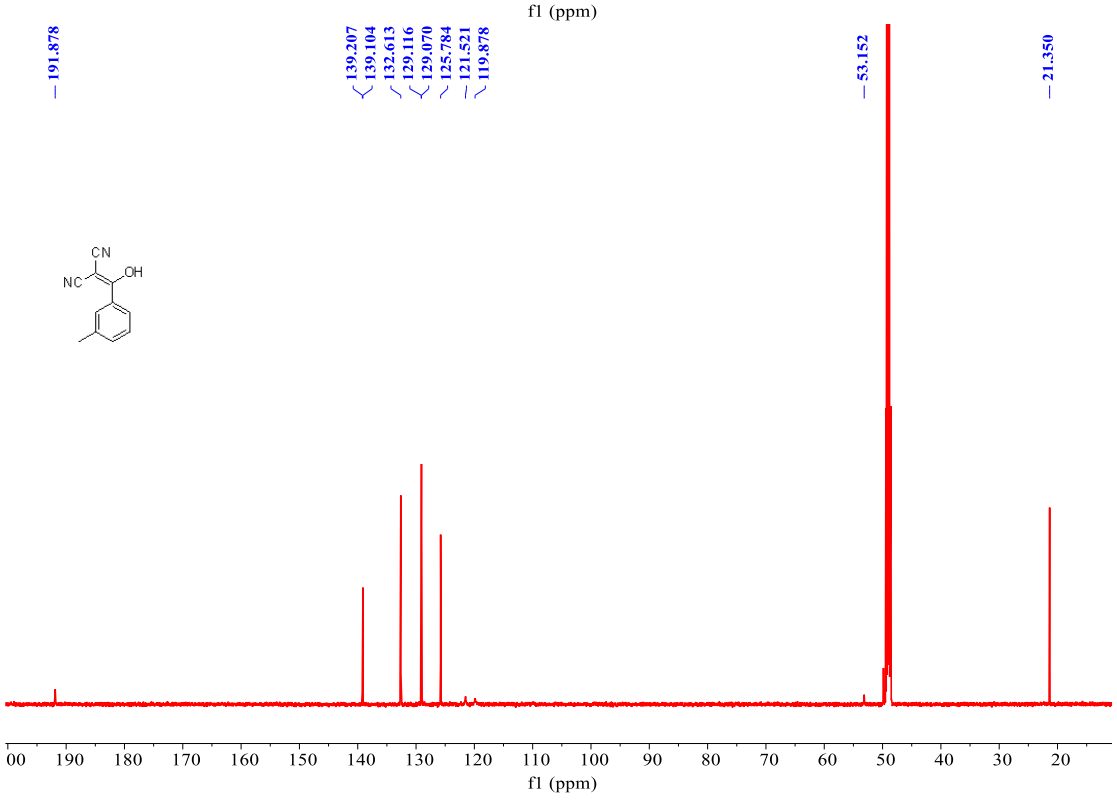
f1 (ppm)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f1 (ppm)


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3.768
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3.252
3.250
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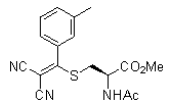
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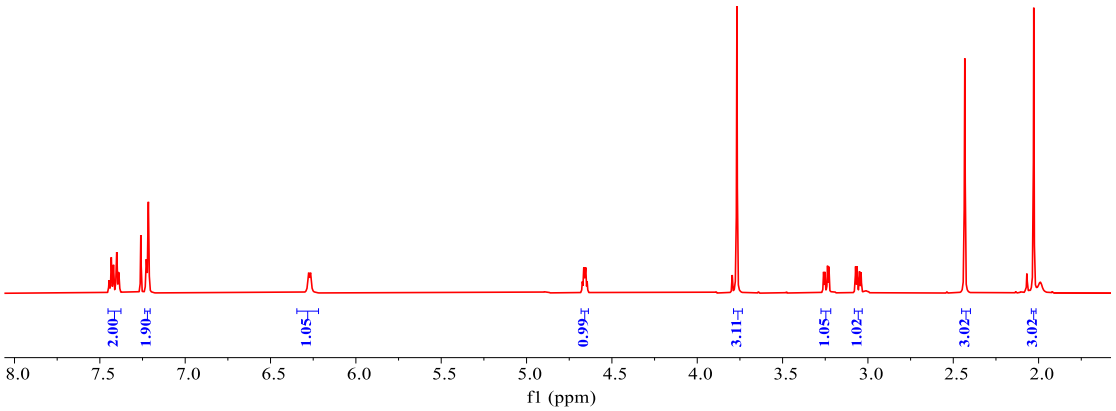
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4.0
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3.0
2.5
2.0
f1 (ppm)


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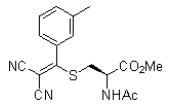
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S
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NHAc

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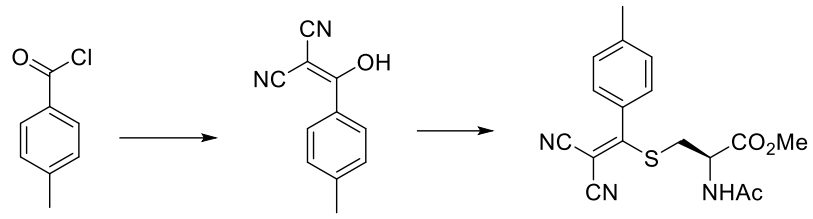
30
20
f1 (ppm)

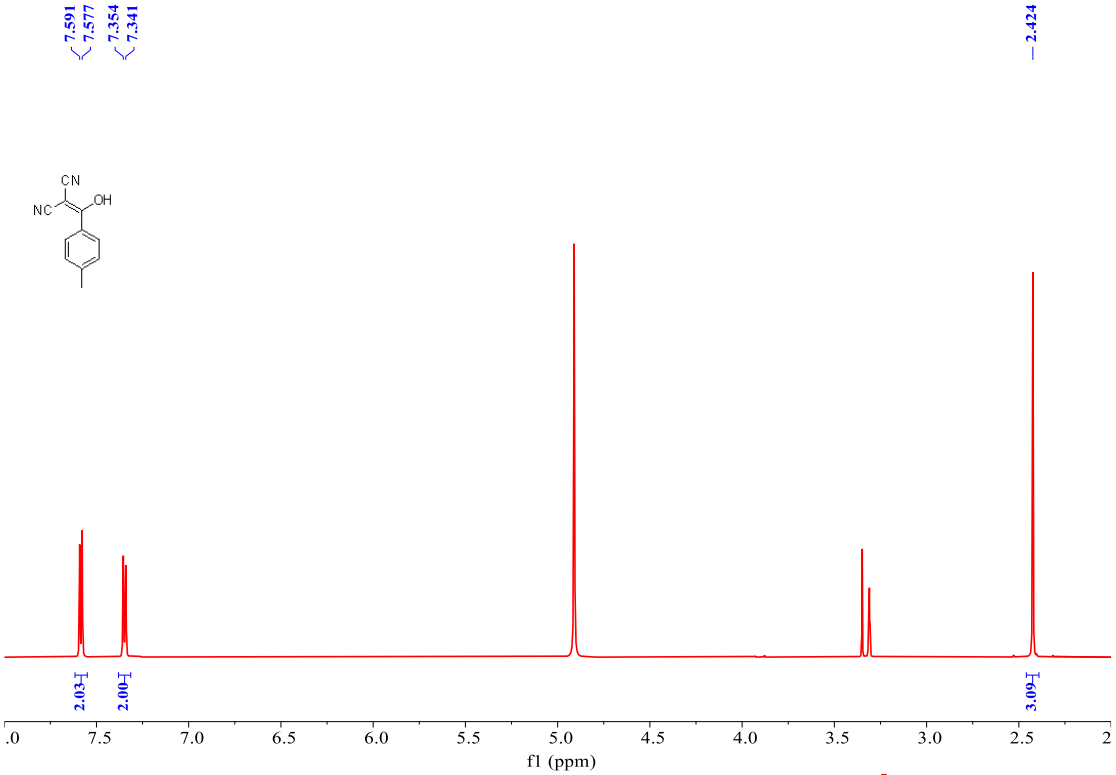
Synthesis of TAMM 1o



1o'' **1o'** **1o**

To a suspension of NaH (1440 mg, 60 mmol, 6 equiv.) in anhydrous THF (10 mL) under nitrogen atmosphere at 0 °C was added a solution of malononitrile (1982 mg, 30 mmol, 3 equiv.) in anhydrous THF (10 mL) in a dropwise manner. After 1 hour at 0 °C, a solution of **1o''** (1550 mg, 10 mmol, 1 equiv.) in anhydrous THF (10 mL) was added into the mixture at 0 °C in a dropwise manner. After the addition, the reaction was allowed to warm up to room temperature. After 2 hours at room temperature, the solvent was removed under reduced pressure. The mixture was acidified to pH 1-2 using $HCl_{(aq)}$. The mixture was extracted with EtOAc (3 ×100 mL). The combined organic layers were washed with brine (3 ×50 mL), dried over Na_2SO_4, filtered and concentrated. Silica gel column chromatography (DCM: MeOH=10:1) was performed to isolate **1o'** (1835 mg, 9.9 mmol, 99%). ^{1}H NMR (600 MHz, Methanol-d_4) δ 7.58 (d, *J* = 8.4 Hz, 2H), 7.35 (d, *J* = 7.8 Hz, 2H), 2.42 (s, 3H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 187.2, 144.8, 131.3, 130.4, 129.2, 117.1, 115.3, 60.4, 21.6. R_f (DCM: MeOH=10:1) = 0.3.

To a solution of **1o'** (810 mg, 4.4 mmol, 1.0 equiv.) in anhydrous acetonitrile (50 ml) under nitrogen atmosphere was added PCl_5 (2746 mg, 13.2 mmol, 3 equiv.) was added. After 6 hours at 65 °C, the solvent was removed under reduced pressure. The residue was dissolved in DCM (60 mL), washed with water (3 × 20 mL) and brine (20 mL), dried over Na_2SO_4, filter and concentrate. The residue was (129 mg, 0.64 mmol, 1.5 equiv.) then dissolved in acetonitrile (15 mL), followed by addition of Ac-Cys-OMe (75 mg, 0.42 mmol, 1.0 equiv.) and $NaHCO_3$ (107 mg, 1.3 mmol, 3 equiv.). After overnight at room temperature, the solvent was removed under reduced pressure. The mixture was purified by silica gel column chromatography (EA: PE=2:1) to afford **1o** (102 mg, 0.30 mmol, 71%). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.35 (s, 4H), 6.25 (d, *J* = 6.6 Hz, 1H), 4.66 (dt, *J* = 7.2, 4.8 Hz, 1H), 3.77 (s, 3H), 3.27 (dd, *J* = 14.1, 4.8 Hz, 1H), 3.08 (dd, *J* = 14.1, 4.8 Hz, 1H), 2.44 (s, 3H), 2.03 (s, 3H). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 179.8, 169.9, 169.6, 144.1, 130.4, 129.1, 128.9, 112.8, 112.4, 80.5, 53.3, 51.8, 36.6, 23.1, 21.70. R_f (EA: PE=2:1) = 0.4.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2.00
3.09
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6.0
5.5
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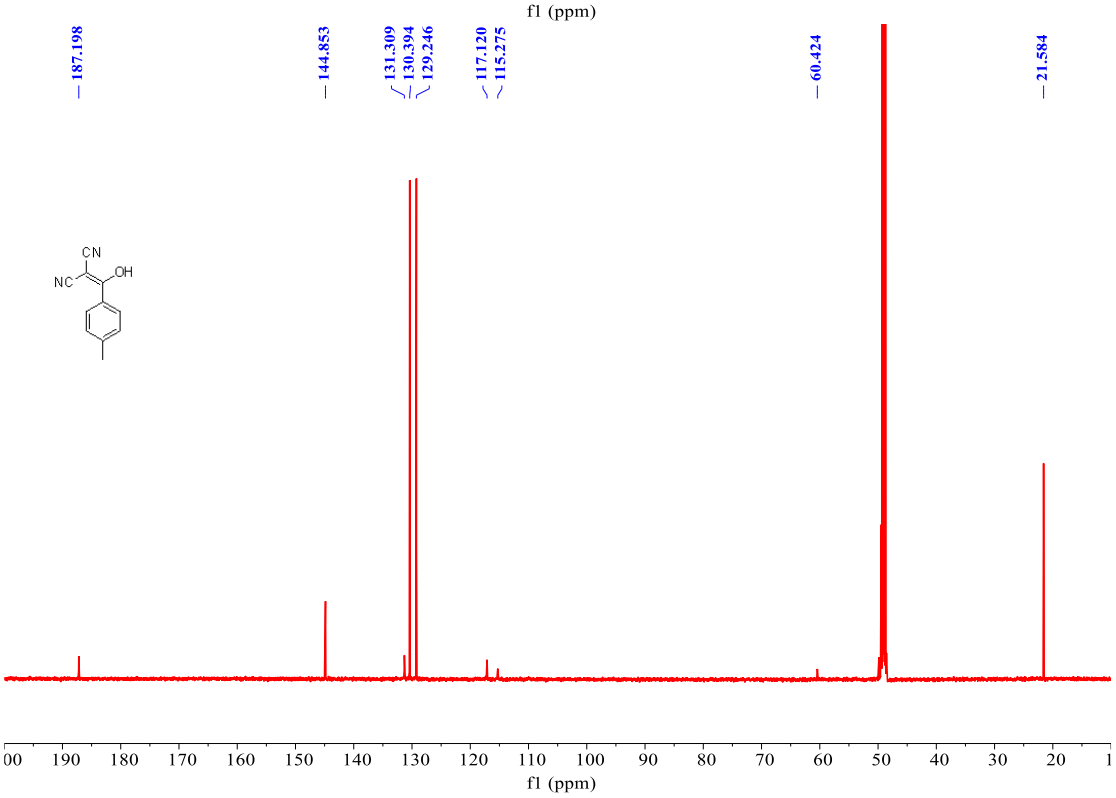
4.5
4.0
3.5
3.0
2.5
2
f1 (ppm)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60.424
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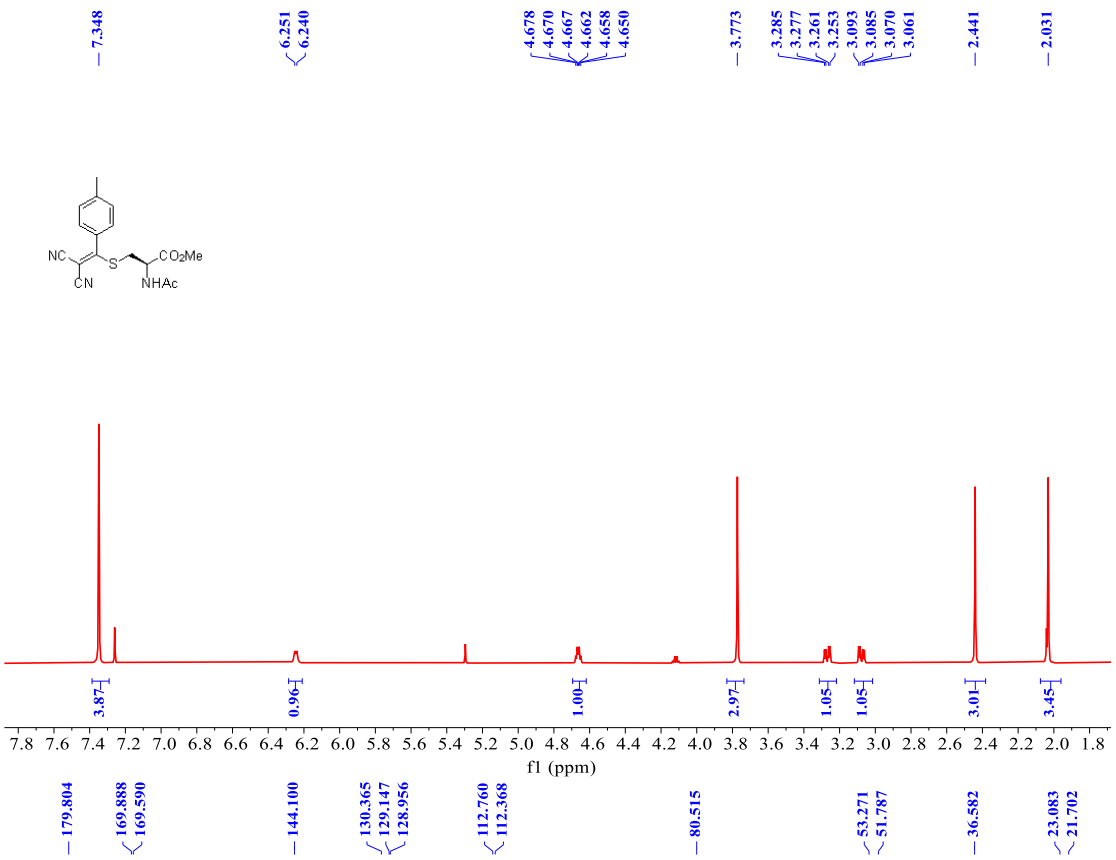
70
60
50
40
30
20
1
f1 (ppm)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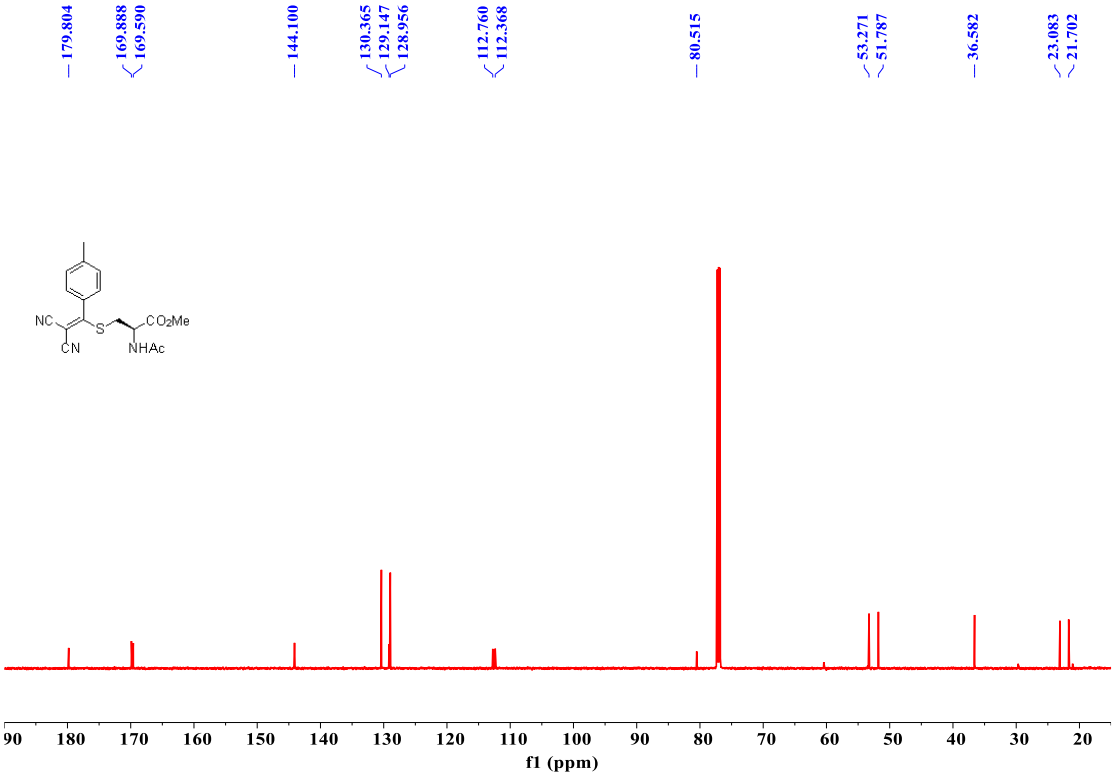
3.253
3.093
3.085
3.070
3.061
2.441
2.031
NC
CN
S
CO2Me
NHAc
3.87
0.96
1.00
2.97
1.05
1.05
3.01
3.45
7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8
f1 (ppm)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144.100
130.365
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112.760
112.368
80.515
53.271
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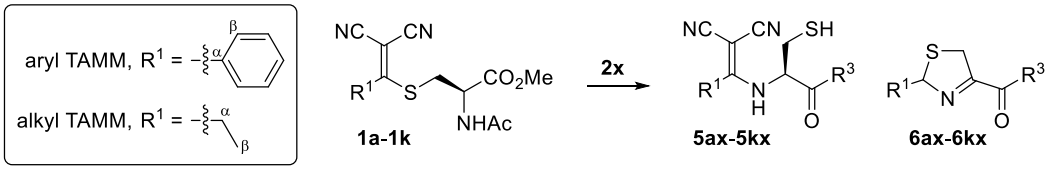
36.582
23.083
21.702
NC
CN
S
CO2Me
NHAc
90 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
f1 (ppm)

Reaction of TAMM 1 with 2x



A 200-µL reaction mixture was prepared by mixing 186 µL of 0.1 M $NaHCO_{3(aq)}$ or PBS with Ac-Cys-OMe (2 µL, 50 mM, 10 equiv.; final concentration = 500 µM), TCEP (2 µL, 50 mM, 10 equiv.; final concentration = 500 µM), **TAMM 1** (8 µL, 5 mM, 4 equiv.; final concentration = 200 µM) and peptide **2x** (2 µL, 5 mM, 1 equiv.; final concentration = 50 µM). The reaction was incubated at 37 °C for the indicated time and analyzed by LC-MS using chromatography condition A.

HPLC characterization of peptides

Analytic HPLC analyses were performed on a Shimadzu LCMS-2020 with a C18 column (Agilent, #770450-902, 5 µm, 4.6×250 mm) at 40 °C and detection of absorbance at 280 nm.

- Chromatography condition A: water (containing 0.1% v/v formic acid) and methanol as the mobile phases at the flow rate of 1 mL/min, 10% to 40% methanol from 0 to 8 min, 40% to 50% methanol from 8 to 12 min, 50% to 90% methanol from 12 to 16 min, 90% methanol for 2 min, 90% to 10% methanol from 18 to 19 min, and 10% methanol from 19 to 24 min.
- Chromatography condition B: water (containing 0.1% v/v formic acid) and methanol as the mobile phases at the flow rate of 1 mL/min, 10% to 70% methanol from 0 to 6 min, 70% to 90% methanol from 6 to 9 min, 90% methanol for 3 min, 90% to 10% methanol from 12 to 13 min, and 10% methanol from 13 to 17 min.
- Chromatography condition C: water (containing 0.1% v/v formic acid) and acetonitrile (containing 0.1% v/v formic acid) as the mobile phases at the flow rate of 1 mL/min, isocratic 10% acetonitrile for 5 min, then 10% to 90% acetonitrile from 5 to 30 min, 90% to 10% acetonitrile from 30 to 31 min, and 10% acetonitrile from 31 to 40 min.

Mechanistic investigation of o-TAMM properties

Effective Reaction Barrier

The reaction system under consideration

$$A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B \overset{k_2}{\rightarrow} C$$

This corresponds to the system of differential equations:

$$\frac{d[A]}{dt} = -k_1[A] + k_{-1}[B]$$
$$\frac{d[B]}{dt} = k_1[A] - (k_{-1} + k_2)[B]$$
$$\frac{d[C]}{dt} = k_2[B]$$

with initial conditions $[A](0) = a_0$, $[B](0) = 0$, and $[C](0) = 0$.

Derivation of the solution under general conditions

The system of differential equations can be written in matrix form as

$$\frac{d}{dt}\begin{bmatrix}[A]\\[B]\end{bmatrix} = \mathbf{M}\begin{bmatrix}[A]\\[B]\end{bmatrix}$$

where the coefficient matrix $\mathbf{M}$ is

$$\mathbf{M} = \begin{bmatrix}-k_1 & k_{-1}\\ k_1 & -(k_{-1}+k_2)\end{bmatrix}$$

(Note: we only need to consider the first two differential equations for $[A]$ and $[B]$ since the third one for $[C]$ is related through mass conservation, $[A] + [B] + [C] = a_0$.)

The eigenvalues λ_1, λ_2 are found from the characteristic equation $\det(\mathbf{M} - \lambda\mathbf{I}) = 0$:

$$\lambda_{1,2} = \frac{-(k_1 + k_{-1} + k_2) \pm \sqrt{(k_1 + k_{-1} + k_2)^2 - 4k_1k_2}}{2}$$

The general solution for $[A]$ and $[B]$ takes the form:

$$\begin{bmatrix}[A](t)\\[B](t)\end{bmatrix} = c_1 e^{\lambda_1 t}\mathbf{v}_1 + c_2 e^{\lambda_2 t}\mathbf{v}_2$$

Here, $\mathbf{v}_1, \mathbf{v}_2$ are eigenvectors. For eigenvalue λ_1, we have:

$$\mathbf{M} - \lambda_1\mathbf{I} = \begin{bmatrix}-k_1 - \lambda_1 & k_{-1}\\ k_1 & -(k_{-1}+k_2) - \lambda_1\end{bmatrix}$$

For eigenvector $\mathbf{v}_1 = \begin{bmatrix}v_{11}\\ v_{12}\end{bmatrix}$: $(-k_1 - \lambda_1)v_{11} + k_{-1}v_{12} = 0$, giving $v_{11} = \frac{k_{-1}}{k_1+\lambda_1}v_{12}$.

Choosing $v_{12} = k_1 + \lambda_1$ yields $v_{11} = k_{-1}$, resulting in:

$$\mathbf{v}_1 = \begin{bmatrix}k_{-1}\\ k_1 + \lambda_1\end{bmatrix}$$

Similarly, for λ_2:

$$\mathbf{v}_2 = \begin{bmatrix}k_{-1}\\ k_1 + \lambda_2\end{bmatrix}$$

This gives the solution:

$$[A](t) = c_1 k_{-1} e^{\lambda_1 t} + c_2 k_{-1} e^{\lambda_2 t}$$
$$[B](t) = c_1(k_1 + \lambda_1)e^{\lambda_1 t} + c_2(k_1 + \lambda_2)e^{\lambda_2 t}$$

The coefficients c_i can be determined from the initial conditions $[A](0) = a_0$ and $[B](0) = 0$:

$$c_1 = \frac{a_0(k_1 + \lambda_2)}{k_{-1}(\lambda_2 - \lambda_1)}, \qquad c_2 = -\frac{a_0(k_1 + \lambda_1)}{k_{-1}(\lambda_2 - \lambda_1)}$$

Therefore,

$$[A](t) = -\frac{a_0(k_1 + \lambda_2)}{\lambda_1 - \lambda_2}e^{\lambda_1 t} + \frac{a_0(k_1 + \lambda_1)}{\lambda_1 - \lambda_2}e^{\lambda_2 t} = -\frac{a_0}{\lambda_1 - \lambda_2}\left[(k_1 + \lambda_2)e^{\lambda_1 t} - (k_1 + \lambda_1)e^{\lambda_2 t}\right]$$

$$[B](t) = -\frac{a_0(k_1+\lambda_2)(k_1+\lambda_1)}{k_{-1}(\lambda_1-\lambda_2)}e^{\lambda_1 t} + \frac{a_0(k_1+\lambda_1)(k_1+\lambda_2)}{k_{-1}(\lambda_1-\lambda_2)}e^{\lambda_2 t} = \frac{a_0 k_1}{\lambda_1-\lambda_2}[e^{\lambda_1 t} - e^{\lambda_2 t}]$$
$$[C](t) = a_0 - [A](t) - [B](t)$$

(The expression in terms of a_0 and k_i without eigenvalues λ_i is too complicated to show in full.)

(Re-)evaluating the solution under the rapid pre-equilibrium conditions

Let's now examine the effect of $k_1, k_{-1} \gg k_2$ on the eigenvalues, which are determined from $\det(\mathbf{M} - \lambda\mathbf{I}) = 0$:

$$\lambda^2 + (k_1 + k_{-1} + k_2)\lambda + k_1 k_2 = 0$$
$$s = k_1 + k_{-1} + k_2$$
$$\Delta = s^2 - 4k_1 k_2$$
$$\lambda_{1,2} = \frac{-s \pm \sqrt{\Delta}}{2}$$

Under $k_1, k_{-1} \gg k_2$,

$$s = k_1 + k_{-1} + k_2 \approx k_1 + k_{-1}$$
$$\Delta = (k_1 + k_{-1} + k_2)^2 - 4k_1 k_2 \approx (k_1 + k_{-1})^2 - 4k_1 k_2$$
$$\sqrt{\Delta} \approx (k_1 + k_{-1})\left(1 - \frac{4k_1 k_2}{(k_1 + k_{-1})^2}\right)^{1/2} \approx (k_1 + k_{-1}) - \frac{2k_1 k_2}{k_1 + k_{-1}}$$
$$\lambda_1 \approx -\frac{k_1 k_2}{k_1 + k_{-1}}$$
$$\lambda_2 \approx -(k_1 + k_{-1})$$

(Binomial approximation $(1+x)^n \approx 1 + nx$ for $|x| \ll 1$ is used.)

As $(k_1 + k_{-1})$ is large, the $e^{\lambda_2 t}$ term decays rapidly (***fast mode***), whereas the $e^{\lambda_1 t} \approx e^{-k_{\text{eff}} t}$ term dominates the decay behavior of $[A](t)$, $[B](t)$, and $[C](t)$ (***slow mode***). Consequently, while $[A](t)$, $[B](t)$, and $[C](t)$ are originally biexponential, under $k_1, k_{-1} \gg k_2$, they simplify to single exponential functions (i.e., omitting the fast mode):

$$[A](t) = \frac{a_0 k_{-1}}{k_1 + k_{-1}} e^{-\frac{k_1 k_2}{k_1 + k_{-1}} t}$$
$$[B](t) = \frac{a_0 k_1}{k_1 + k_{-1}} e^{-\frac{k_1 k_2}{k_1 + k_{-1}} t}$$
$$[C](t) = a_0\left(1 - e^{-\frac{k_1 k_2}{k_1 + k_{-1}} t}\right)$$

It should be noted that this result could be similarly obtained under quasi-steady-state approximation (QSSA, not discussed here).

Effective reaction barrier under the rapid pre-equilibrium conditions

Following the above discussion, under $k_1, k_{-1} \gg k_2$, the formation of C follows:

$$\frac{d[C]}{dt} = k_2[B] = k_2 \cdot \frac{a_0 k_1 e^{-k_{\text{eff}} t}}{k_1 + k_{-1}} = a_0 k_{\text{eff}} \cdot e^{-k_{\text{eff}} t}$$

with

$$k_{\text{eff}} = \frac{k_1 k_2}{k_1 + k_{-1}}$$

Thus, **k_{eff} is the effective rate constant for C's formation**. To find its activation energy $\Delta G^{\ddagger}_{\text{eff}}$, transition state theory is considered here: $k_i = \frac{k_B T}{h} e^{-\Delta G_i^{\ddagger}/RT}$. Substituting these k_i into k_{eff} gives:

$$
\begin{aligned}
k_{\text{eff}} &= \frac{(\frac{k_B T}{h} e^{-\Delta G_1^{\ddagger}/RT})(\frac{k_B T}{h} e^{-\Delta G_2^{\ddagger}/RT})}{\frac{k_B T}{h} e^{-\Delta G_1^{\ddagger}/RT} + \frac{k_B T}{h} e^{-\Delta G_{-1}^{\ddagger}/RT}} \\
&= \frac{k_B T}{h} \cdot \frac{e^{-(\Delta G_1^{\ddagger} + \Delta G_2^{\ddagger})/RT}}{e^{-\Delta G_1^{\ddagger}/RT} + e^{-\Delta G_{-1}^{\ddagger}/RT}} \\
&= \frac{k_B T}{h} \cdot \frac{e^{-\Delta G_2^{\ddagger}/RT}}{e^{\Delta G_1^{\ddagger}/RT}(e^{-\Delta G_1^{\ddagger}/RT} + e^{-\Delta G_{-1}^{\ddagger}/RT})} \\
&= \frac{k_B T}{h} \cdot \frac{e^{-(\Delta G_1^{\ddagger} + \Delta G_2^{\ddagger})/RT}}{e^{-\Delta G_1^{\ddagger}/RT} + e^{-\Delta G_{-1}^{\ddagger}/RT}}
\end{aligned}
$$

Since $k_{\text{eff}} = \frac{k_B T}{h} e^{-\Delta G_{\text{eff}}^{\ddagger}/RT}$, we focus on:

$$
e^{-\Delta G_{\text{eff}}^{\ddagger}/RT} = \frac{e^{-\Delta G_2^{\ddagger}/RT}}{1 + e^{-(\Delta G_{-1}^{\ddagger} - \Delta G_1^{\ddagger})/RT}}
$$

or

$$
\Delta G_{\text{eff}}^{\ddagger} = \Delta G_2^{\ddagger} + RT \ln\left(1 + e^{-(\Delta G_{-1}^{\ddagger} - \Delta G_1^{\ddagger})/RT}\right)
$$

or equivalently,

$$
\Delta G_{\text{eff}}^{\ddagger} = \Delta G_2^{\ddagger} + RT \ln\left(1 + \frac{1}{K}\right)
$$

where K is the equilibrium constant for $A \rightleftharpoons B$.

Case 1: Moderate K:

- Either $k_1 > k_{-1}$ or $k_1 < k_{-1}$ (i.e., $K > 1$ or $K < 1$)
- $\Delta G_{\text{eff}}^{\ddagger}$ depends primarily on $\Delta G_2^{\ddagger}$ (slow step) and is further modulated by the difference in $(\Delta G_{-1}^{\ddagger} - \Delta G_1^{\ddagger})$
- $\Delta G_{\text{eff}}^{\ddagger}$ is generally larger than $\Delta G_2^{\ddagger}$

Case 2: Large K:

- $k_1 \gg k_{-1}$ and $K \gg 1$ (i.e., the equilibrium strongly lies on B)
- $\ln\left(1 + e^{-(\Delta G_{-1}^{\ddagger} - \Delta G_1^{\ddagger})/RT}\right) \approx \ln(1 + 0) = 0$

- $\Delta G_{\text{eff}}^{\ddagger} \approx \Delta G_2^{\ddagger}$. In other words, only the barrier from the reactive intermediate B is important; the precursor A is not contributing to the reaction kinetics as it is much higher in energy.

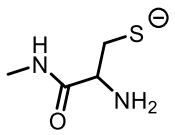
Case 3: Small K:

- $k_1 \ll k_{-1}$ and $K \ll 1$ (i.e., $\frac{1}{K} \gg 1$ and $1 + \frac{1}{K} \approx \frac{1}{K}$)
- $\Delta G_{\text{eff}}^{\ddagger} \approx \Delta G_2^{\ddagger} + RT \cdot \frac{\Delta G_1^{\ddagger} - \Delta G_{-1}^{\ddagger}}{RT} = \Delta G_2^{\ddagger} + \Delta G_1^{\ddagger} - \Delta G_{-1}^{\ddagger} = \Delta G_2^{\ddagger} + \Delta G_{\text{B-A}}$, as $\Delta G_{\text{B-A}} = \Delta G_1^{\ddagger} - \Delta G_{-1}^{\ddagger}$. In other words, the effective barrier is the sum of the barrier of the slow step ($\Delta G_2^{\ddagger}$) and the energy difference between the reactive intermediate and its lower-energy precursor ($\Delta G_{\text{B-A}}$), i.e., $\Delta G_{\text{eff}}^{\ddagger} \approx \Delta G_2^{\ddagger} + \Delta G_{\text{B-A}}$.
- This scenario applies to the TAMM reactions studied here.

Conformation scan and Cartesian coordinates of optimized geometries

Two-dimensional conformational scans of the dihedral angles were conducted at the GFN2-Xtb [1] level using Gaussian 09 [2] through the external command.

The Global Optimizer Algorithm (GOAT) implemented in ORCA 6.0 [1, 3] was used to find low a set of low-energy conformers for each species. These structures were then further optimized at the level of ωB97X-D/6-31+G(d,p) in vacuum, and the electronic energies in water were calculated at the same level using the SMD model in Gaussian 09.[2] Thermal correction, used for free energy calculation, was evaluated using Shermo 2.3 [4] with a scaling factor for zero-point energy = 0.9523, low-frequency vibration modes (< 100 cm^{-1}) raised to 100 cm^{-1}, and temperature = 298.15 K. Electronic energies are given in Hartrees and coordinates in Ångstroms. Transition states were labelled with a superscript double dagger.



E (vac) = -740.733367673

E (H_2O) = -740.825573221

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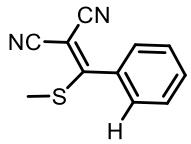
C	1.493598	0.518197	0.439333
H	2.281458	1.273408	0.340515
H	1.216552	0.480797	1.504901
C	0.284500	1.069167	-0.342489
N	0.106600	2.497045	-0.058229
H	-0.878974	2.713167	-0.199363
H	0.274188	2.645970	0.934391
H	0.510966	0.954675	-1.411273
C	-1.030908	0.300065	-0.098652
O	-2.112666	0.893886	0.028856
N	-0.930412	-1.037378	-0.078666
H	0.030956	-1.431374	-0.127673
C	-2.081432	-1.877951	0.129341
H	-2.842408	-1.703790	-0.640282
H	-1.759604	-2.921277	0.085276
H	-2.550369	-1.690204	1.103376



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E (H_2O) = -931.457429057

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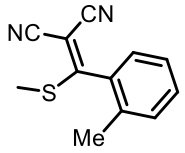
C	1.102973	-2.170674	-0.173384
N	0.751786	-3.272947	-0.256103
C	2.974266	-0.615789	-0.017041
N	4.120928	-0.445771	0.021839
C	-2.949129	0.193149	-1.086098
C	-1.579123	0.427742	-1.098194
C	-0.786228	-0.004947	-0.029844
C	-1.377101	-0.662214	1.052315

C	-2.751467	-0.882567	1.065530
C	-3.537606	-0.457299	-0.001980
H	-3.557630	0.514930	-1.924831
H	-1.115458	0.928896	-1.943105
H	-3.204657	-1.394791	1.907613
H	-4.607704	-0.637001	0.006693
S	1.301426	1.863930	-0.066242
C	-0.099379	2.852972	0.531581
H	-0.486348	2.453165	1.469394
H	-0.898081	2.918899	-0.206518
H	0.314974	3.847202	0.708761
H	-0.760235	-1.002522	1.878123



E (vac) = -970.757532500

E (H_2O) = -970.768597255

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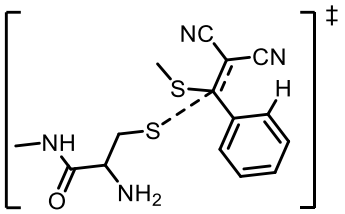
C	-1.130462	-2.147133	-0.265910
N	-0.739263	-3.238449	-0.303814
C	-3.032869	-0.613354	-0.145981
N	-4.178206	-0.435242	-0.101295
C	2.748487	-0.124258	-1.524614
C	1.375746	0.081108	-1.448076
C	0.723031	0.016164	-0.215097
C	1.432617	-0.255682	0.965272
C	2.811717	-0.451955	0.864695
C	3.466901	-0.387898	-0.361622
H	3.250452	-0.082425	-2.485335
H	0.799550	0.284275	-2.346224
H	3.378940	-0.666539	1.765892
H	4.538987	-0.549609	-0.408669
S	-1.365149	1.880024	-0.080420
C	0.137275	2.883029	0.087681
H	0.699662	2.609084	0.981053
H	-0.221441	3.909166	0.187519
H	0.773230	2.804283	-0.793972
C	0.729197	-0.363172	2.293942
H	0.033996	0.466800	2.458304
H	0.146670	-1.289048	2.348600

H	1.448502	-0.371641	3.115592

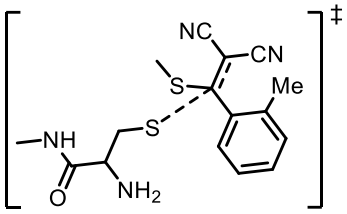


E (vac) = -1672.20217180

E (H_2O) = -1672.27971003

C	-0.456857	0.637024	-0.122876
C	0.031987	1.927354	-0.415400
C	-0.064897	2.466048	-1.728356
N	-0.149027	2.939807	-2.789397
C	0.768409	2.714941	0.503330
N	1.347758	3.393730	1.253897
S	1.316390	-0.803359	-1.294353
C	1.472131	-2.368959	-0.371767
C	-3.122444	-1.661721	-1.584221
C	-1.932595	-1.223877	-1.017801
C	-1.690851	0.141549	-0.826236
C	-2.684965	1.053444	-1.195449
C	-3.875225	0.615190	-1.772838
C	-4.097935	-0.742724	-1.972326
H	-3.282958	-2.724933	-1.735578
H	-1.167644	-1.937985	-0.739807
H	-4.626712	1.343096	-2.063240
H	-5.023505	-1.086313	-2.425512
S	-0.439693	-0.026285	1.526281
C	1.251830	0.205444	2.133944
H	1.432499	-0.597190	2.852867
H	1.394991	1.177577	2.602929
H	1.932865	0.089268	1.288929
H	0.891235	-3.152172	-0.870803
H	1.037404	-2.236578	0.631973
C	2.897328	-2.943526	-0.222981
N	2.796258	-4.321007	0.250016
H	3.713239	-4.614907	0.576529
H	2.215210	-4.331729	1.085162
H	3.353513	-2.961079	-1.220699
C	3.823375	-2.097773	0.679135
O	4.368573	-2.579778	1.675388
N	4.034671	-0.826649	0.277920

H	3.378972	-0.461321	-0.421030
C	4.803976	0.091817	1.088160
H	4.855882	1.052184	0.572734
H	4.346600	0.243700	2.073699
H	5.815227	-0.294316	1.243682
H	-2.531265	2.112657	-1.023565

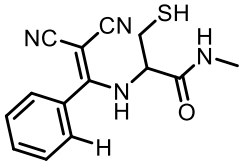


E (vac) = -1711.50955342

E (H_2O) = -1711.58351215

C	-0.400605	0.544206	-0.268926
C	0.178524	1.765292	-0.666750
C	-0.161119	2.333652	-1.923735
N	-0.458265	2.837679	-2.931776
C	1.236193	2.408841	0.021655
N	2.100706	2.961188	0.575692
S	1.337728	-1.011656	-1.285084
C	1.532801	-2.496197	-0.245642
C	-3.116522	-1.411355	-2.119754
C	-1.886469	-1.039386	-1.589101
C	-1.725713	0.167287	-0.904097
C	-2.842200	1.016440	-0.724074
C	-4.068214	0.619927	-1.267583
C	-4.215359	-0.575099	-1.962766
H	-3.207381	-2.351040	-2.656097
H	-1.010763	-1.662884	-1.724077
H	-4.928016	1.272461	-1.137103
H	-5.182370	-0.847423	-2.376536
S	-0.455288	-0.070675	1.400518
C	1.167801	0.229876	2.144725
H	1.245431	-0.478934	2.972802
H	1.280596	1.249226	2.509578
H	1.942870	0.004840	1.412070
H	0.980375	-3.332781	-0.685849
H	1.082251	-2.299834	0.739918
C	2.978422	-2.999491	-0.047510
N	2.931308	-4.349439	0.509289
H	3.829819	-4.538540	0.947687

H	2.264395	-4.358325	1.277620
H	3.448557	-3.057776	-1.037812
C	3.870635	-2.069614	0.808516
O	4.479813	-2.496978	1.793412
N	3.974268	-0.794696	0.384628
H	3.323381	-0.501509	-0.349986
C	4.711502	0.203566	1.129751
H	4.386521	1.192809	0.801165
H	4.517789	0.100170	2.202253
H	5.790864	0.100001	0.972556
C	-2.783363	2.329675	0.019552
H	-2.174751	2.262448	0.924395
H	-2.354750	3.114634	-0.611240
H	-3.792590	2.639912	0.305874

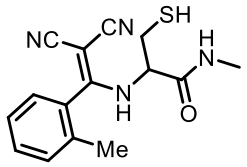


E (vac) = -1234.07614451

E (H_2O) = -1234.09961488

C	4.164869	-2.679351	-0.868737
N	3.407031	-1.453812	-0.687129
C	2.152063	-1.475371	-0.208975
O	1.536794	-2.508851	0.041865
C	1.498462	-0.098249	0.030689
C	2.141428	0.629072	1.236096
S	3.732246	1.440630	0.866284
N	0.097776	-0.373767	0.272581
C	-0.979855	0.365130	-0.028131
C	-0.977005	1.688897	-0.455170
C	0.183791	2.512273	-0.516393
N	1.119174	3.201625	-0.563372
C	-2.183535	2.317707	-0.891712
N	-3.143691	2.859480	-1.256555
C	-2.267215	-0.368079	0.123487
C	-3.305247	0.161995	0.893921
C	-4.488859	-0.551176	1.047406
C	-4.645201	-1.790174	0.429662
C	-3.612161	-2.321353	-0.339195
C	-2.422229	-1.616619	-0.488463
H	4.422415	-3.135843	0.091954

H	3.575000	-3.396158	-1.442881
H	5.078842	-2.445455	-1.414945
H	3.882882	-0.561055	-0.733541
H	1.606537	0.511834	-0.869904
H	1.448419	1.382470	1.612739
H	2.328068	-0.082668	2.044937
H	3.194981	2.465480	0.177426
H	-0.062253	-1.340559	0.546563
H	-3.186507	1.127581	1.374062
H	-5.290745	-0.135693	1.648270
H	-5.572980	-2.340900	0.546313
H	-3.732071	-3.283149	-0.826559
H	-1.620739	-2.028029	-1.095487



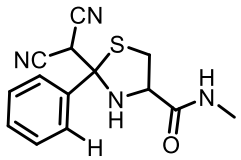
E (vac) = -1273.38671562

E (H_2O)

= -1273.4095775

C	4.162058	-2.783059	-0.607481
N	3.450944	-1.521082	-0.502767
C	2.193452	-1.466972	-0.035476
O	1.540344	-2.460952	0.274503
C	1.585796	-0.055530	0.104847
C	2.270420	0.757470	1.228973
S	3.873273	1.492353	0.762690
N	0.183069	-0.266310	0.394022
C	-0.873609	0.479705	0.045194
C	-0.848915	1.762517	-0.484441
C	0.330746	2.540367	-0.668892
N	1.286753	3.185708	-0.816073
C	-2.068951	2.389589	-0.886823
N	-3.043805	2.921650	-1.225380
C	-2.186414	-0.192181	0.272400
C	-2.549274	-1.305379	-0.504513
C	-3.779012	-1.910049	-0.234654
C	-4.616979	-1.435685	0.770141
C	-4.245353	-0.328205	1.527082
C	-3.030535	0.295428	1.271556
H	5.099214	-2.610778	-1.136958
H	4.374761	-3.203510	0.380070

H	3.559854	-3.503012	-1.164991
H	3.956340	-0.650208	-0.612807
H	1.692587	0.471225	-0.847455
H	1.602752	1.559024	1.548013
H	2.454955	0.116339	2.095223
H	3.349932	2.456488	-0.018516
H	-0.006609	-1.212748	0.715536
H	-4.086706	-2.763869	-0.831575
H	-5.567231	-1.926686	0.954333
H	-4.899180	0.054124	2.303380
C	-1.664277	-1.828952	-1.609955
H	-2.237650	-2.463692	-2.288999
H	-1.234695	-1.011652	-2.197765
H	-0.834249	-2.425014	-1.215162
H	-2.732863	1.169795	1.842244

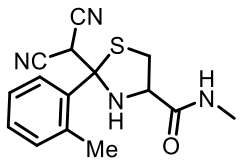


E (vac) = -1234.06363389

E (H_2O) = -1234.09425124

C	2.556464	-2.463676	-1.028872
C	1.573106	-1.486066	-0.890443
C	1.635679	-0.569321	0.159048
C	2.703166	-0.634737	1.058472
C	3.690736	-1.602695	0.912630
C	3.617659	-2.524160	-0.131094
C	0.587676	0.538994	0.264728
C	1.215708	1.866652	-0.277852
C	1.686778	1.715983	-1.666249
N	2.072666	1.594174	-2.749189
C	0.283881	3.003630	-0.188905
N	-0.459498	3.887545	-0.131700
H	2.495735	-3.170678	-1.850104
H	0.756415	-1.415689	-1.600350
H	2.758368	0.058464	1.893726
H	4.513344	-1.642280	1.619425
H	4.386071	-3.282371	-0.243137
C	-1.613121	0.266591	1.739971
C	-1.526677	-0.501526	0.401293
N	-0.611079	0.220691	-0.460158

S	0.088407	0.811828	2.069490
H	-1.099907	1.041937	-0.816063
H	-2.276299	1.129260	1.637719
H	-1.954495	-0.369220	2.559442
H	-1.127614	-1.506777	0.583282
H	2.090092	2.099212	0.340365
C	-2.892778	-0.558617	-0.279008
O	-3.328323	0.412019	-0.882258
N	-3.587657	-1.707540	-0.119325
C	-4.909397	-1.875111	-0.696973
H	-5.368683	-2.769435	-0.274443
H	-5.525544	-1.005769	-0.459910
H	-4.857626	-1.971938	-1.785641
H	-3.132086	-2.499870	0.304028

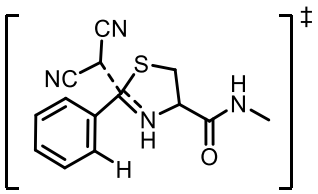


E (vac) = -1273.36823406

E (H_2O) = -1273.39862664

C	-3.689931	-1.613101	-0.425838
C	-2.805539	-0.546423	-0.615181
C	-1.537871	-0.613600	-0.001074
C	-1.225820	-1.718948	0.796026
C	-2.120700	-2.767782	0.970847
C	-3.362525	-2.718375	0.350391
C	-0.473856	0.488658	-0.133170
H	-4.665950	-1.567699	-0.901650
C	-3.311865	0.622770	-1.432820
H	-1.848020	-3.610435	1.597879
H	-4.076423	-3.526521	0.473683
C	1.674344	0.386989	-1.723900
C	1.681268	-0.486512	-0.453319
N	0.770771	0.122558	0.494434
S	-0.073358	0.804303	-1.960455
H	1.255452	0.905725	0.930556
H	2.272078	1.288873	-1.567906
H	2.033823	-0.151443	-2.603512
H	1.326799	-1.494378	-0.703084
C	3.074295	-0.528339	0.172235
O	3.471167	0.390507	0.875015

N	3.835453	-1.598439	-0.150356
H	3.416636	-2.362459	-0.655758
C	5.190413	-1.738339	0.352908
H	5.194779	-1.959396	1.424492
H	5.685657	-2.546612	-0.186272
H	5.738976	-0.808065	0.193533
C	-1.003320	1.811287	0.517298
H	-1.908025	2.121726	-0.012071
C	-1.374177	1.601898	1.927888
C	-0.033940	2.913575	0.403332
N	-1.690216	1.434581	3.027344
N	0.736328	3.771321	0.312941
H	-0.267589	-1.731921	1.301587
H	-4.057022	0.280361	-2.154653
H	-2.536493	1.137220	-2.002953
H	-3.811310	1.359433	-0.791010

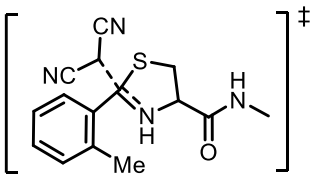


E (vac) = -1234.03091663

E (H_2O) = -1234.06601201

C	4.055247	-0.903384	-0.168448
C	2.761750	-1.185115	0.252421
C	1.664401	-0.607867	-0.398597
C	1.888844	0.283558	-1.460869
C	3.183424	0.571863	-1.865500
C	4.269200	-0.027056	-1.226475
C	0.305998	-0.922202	0.044138
C	0.294605	1.005475	1.677328
C	1.628920	1.076206	2.131672
N	2.737860	1.038825	2.489378
C	-0.175674	1.867020	0.681410
N	-0.645229	2.416940	-0.240402
H	4.895206	-1.351078	0.350410
H	2.613126	-1.841293	1.103450
H	3.345735	1.272914	-2.677074
H	5.280560	0.202187	-1.545501
C	-1.814103	-1.896728	1.027180
C	-2.009266	-1.342767	-0.388391
N	-0.771318	-0.633134	-0.687424

S	-0.029361	-2.233776	1.167806
H	-0.703826	-0.020677	-1.487423
H	-2.098294	-1.158192	1.781661
H	-2.382338	-2.812008	1.182235
H	-2.127038	-2.167021	-1.102122
H	-0.442012	0.676571	2.404924
C	-3.306224	-0.528461	-0.462187
O	-4.363650	-1.144804	-0.403038
N	-3.208303	0.806276	-0.573752
C	-4.401838	1.633721	-0.596817
H	-4.934348	1.580845	0.357361
H	-4.099836	2.663371	-0.787940
H	-5.079426	1.296936	-1.384480
H	-2.308071	1.274858	-0.511612
H	1.066990	0.789938	-1.956491



E (vac) = -1273.33738894

E (H_2O) = -1273.37027838

C	3.991534	-1.405419	-0.314461
C	2.680218	-1.449333	0.128911
C	1.717769	-0.541460	-0.341878
C	2.105413	0.470329	-1.249065
C	3.431362	0.484821	-1.686762
C	4.367402	-0.437458	-1.240289
C	0.337122	-0.704175	0.145620
C	0.621948	1.121282	1.838416
C	1.848809	1.779764	1.585652
N	2.872320	2.263883	1.310243
C	-0.564448	1.779262	1.498735
N	-1.571325	2.224150	1.103175
H	4.712987	-2.122534	0.061472
H	2.400457	-2.214943	0.844802
H	3.739574	1.265476	-2.375312
H	5.390862	-0.384552	-1.596358
C	-1.853351	-1.673968	1.047106
C	-1.937871	-1.193180	-0.396314
N	-0.747951	-0.358718	-0.561799
S	-0.075306	-1.999984	1.280643

H	-0.651164	0.204871	-1.393283
H	-2.183419	-0.903874	1.749312
H	-2.418070	-2.592709	1.199840
H	-1.856968	-2.054411	-1.071255
H	0.592301	0.463170	2.699963
C	-3.293067	-0.554951	-0.727830
O	-4.196895	-1.305803	-1.078757
N	-3.405604	0.775578	-0.605780
C	-4.688719	1.422134	-0.814222
H	-5.433479	1.065243	-0.096083
H	-4.551653	2.496437	-0.690142
H	-5.059145	1.213553	-1.820470
H	-2.683632	1.304769	-0.110755
C	1.186177	1.562561	-1.738758
H	1.775561	2.413566	-2.083748
H	0.579087	1.236413	-2.594944
H	0.522310	1.936945	-0.954867

Phage Display Screening

Modification of phage surface proteins

A mixture of TCEP (2 μL, 50 mM) and CX_9CX_4C phage library (98 μL in PBS, pH 7.4, ~10^{13} pfu/mL) was gently shaken at 37 °C for 30 min. Then an ice-cold PEG/NaCl solution (20% v/v) was added into the reaction mixture, and the precipitated phages were collected by centrifugation and resuspended into $NaHCO_3$(aq) (93 μL, 0.1 M). The CX_9CX_4C library solution was then treated with TCEP (2 μL, 50 mM), NAC-OMe (4 μL, 50 mM) and Me-TAMM-Br **1q** (1 μL, 50 mM). The reaction mixture was incubated at 37 °C for 1 h. An ice-cold PEG/NaCl solution (20% v/v) was then added into the reaction mixture, and the precipitated phages were collected by centrifugation and resuspended into PBS (99.5 μL, pH 7.4). After that, GSSG (0.5 μL, 100 mM) was added into the phage solution, and the oxidation reaction was carried out at 37 °C for 2 h. Finally, an ice-cold PEG/NaCl solution (20% v/v) was added into the reaction mixture, and the precipitated phages were collected by centrifugation and resuspended into PBS (1 mL, pH 7.4). The obtained modified phage solution was stored at 4 °C for the phage selection.

Binder selection by phage display

CX_9CX_4C phage library was modified by the Me-TAMM-Br **1q**. After the reaction, the modified phage was incubated with 0.5 mM GSSG at 37 °C for two hours. The oxidized phages were recovered through precipitation with ice-cold PEG/NaCl solution (20% v/v). The precipitated phages were resuspended in 1 mL PBS. The methods of phage screening and biotinylation of protein were the same as described previously.[5, 6]

(1) Protein biotinylation

After thawing the stored protein at 4°C, aliquot of the protein stock solution into a 1.5 mL centrifuge tube. Then add a 5-fold molar excess of Sulfo-NHS-LC-Biotin (ThermoFisher, #21335) dissolved in 1× PBS, and incubate on a shaking table at room temperature for 30 min. After that, use an ultrafiltration tube to centrifuge and remove excess biotin. The resulting biotinylated target protein is quantified using a Nanodrop and stored at -80°C for future use.

(2) Phage screening

100 μL streptavidin-coated magnetic beads (ThermoFisher, #11205D; neutravidin-coated magnetic beads for the second round, Biomag Biotechnology,# BMJ2800-2) were washed three times with binding buffer (10 mM Tris-Cl, 150 mM NaCl, 10 mM $MgCl_2$, 1 mM $CaCl_2$, pH 7.4) in a 1.5 mL microcentrifuge tube and the washed beads were re-suspended completely (the tube was removed from the magnet) with 100 μL binding buffer, followed by distributing into two 1.5 mL microcentrifuge tubes equally. Then the biotinylated target protein (1st round 5 μg, 2nd round 5 μg and 3rd round 2 μg) was added to one of the two microcentrifuge tubes and the same volume of 1×PBS (without protein) was added to the other one. The tubes were incubated on a slowly rotating wheel for 15 min at room temperature. These beads were washed three times with the binding buffer to remove unbound proteins and re-suspended with 1 mL blocking buffer (binding buffer with 0.3% v/v Tween-20 and 3% w/v BSA), then incubated on a slowly rotating wheel at room temperature for 2 hours. In parallel, the modified phage library (10^{11}~10^{12} pfu.) dissolved in 900 μL 1×PBS was blocked by addition of 2 mL blocking buffer and incubated on a slowly rotating wheel at room temperature for 2 hours. Then the blocked phages were split equally into two 10 mL tubes, into which the blocked beads with and without the immobilized proteins added, respectively. The two tubes were incubated on a slowly rotating wheel at room temperature for 30 min. After that, the unbound phages in the

supernatant were removed (the co-incubated solution were transferred to 1.5 mL tubes placed in a magnet). The beads were washed for nine times with the washing buffer (binding buffer with 0.1% v/v Tween-20) and twice with binding buffer. During the period, the tubes were replaced at least three times. The buffer was removed completely in the last washing step, and then 200 μL×2 elution buffer (50 mM glycine, pH 2.2) was added to elute the phages that bounded on the beads by re-suspending the beads on a mini-vortex finder and incubated for 5 min×2. Then the supernatant was transferred into a 1.5 mL microcentrifuge tube containing 25 μL×2 neutralization buffer (1 M Tris-Cl, pH 8.0). The eluted phage was diluted to infect exponential TG1 cells for monitoring the phage titer, followed by amplification and purification. The purified phage was further modified with Me-TAMM and oxidized by GSSG for the next round of selection.

Preparation of next-generation sequencing (NGS) samples

Phage vectors were extracted from *E. coli* TG1 cells using a commercial plasmid purification kit (CWBIO, #29124). At first, the phage vector DNA was then amplified with the junction primers by PCR (Primers sequence 5'→3':
NGS-F1:
TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTTCTATGCGGCCCAGCCGGCCATG
NGS-R1:
GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTTTCAACAGTCTATGCGGC).
and then the purified PCR products also coupled with the primers designed to include distinct barcodes by PCR. (Primers sequence 5'→3':
i5-index-1:
AATGATACGGCGACCACCGAGATCTACACCCAACTTCTCGTCGGCAGCGTCAGATGT i7-index-1: CAAGCAGAAGACGGCATACGAGATGTTGTTCGGTCTCGTGGGCTCGGAGATG;
i5-index-2:
AATGATACGGCGACCACCGAGATCTACACGTGGTATGTCGTCGGCAGCGTCAGATGT
i7-index-2:
CAAGCAGAAGACGGCATACGAGATCGGTTGTTGTCTCGTGGGCTCGGAGATG
i5-index-3:
AATGATACGGCGACCACCGAGATCTACACGTCAACAGTCGTCGGCAGCGTCAGATGT
i7-index-3:
CAAGCAGAAGACGGCATACGAGATACTGAGGTGTCTCGTGGGCTCGGAGATG).
The PCR reaction mixture consisted of PrimeSTAR DNA polymerase (GenStar, #A064-10), 500 nM of each primer, 10 ng of phage vector DNA, and sterile water to a final volume of 20 μL. The PCR amplification was carried out under the following conditions: initial denaturation at 95 °C for 5 min, followed by 35 cycles of 98 °C for 15 seconds, 55 °C for 1 minute, 72 °C for 30 seconds, and a final extension at 72 °C for 5 min. The PCR products were separated by 3% agarose gel electrophoresis and purified using a commercial gel extraction kit (Omega Bio-tek, #D2500-03). Sequencing was performed by Novogene.

Surface plasmon resonance (SPR) assay

Biotinylated proteins were immobilized onto the CAPture chip (Cytiva, #28920234). A range of peptide concentrations in the running buffer (50 μM EDTA, 0.05% v/v Tween 20, PBS, pH 7.4) were then introduced at a flow rate of 30 μL/min using the automated sample handling system. Following the experiment, sensorgram data were analyzed using the Biacore 8K Evaluation software, applying a 1:1 binding model for K_D estimation.

Supplementary Figures

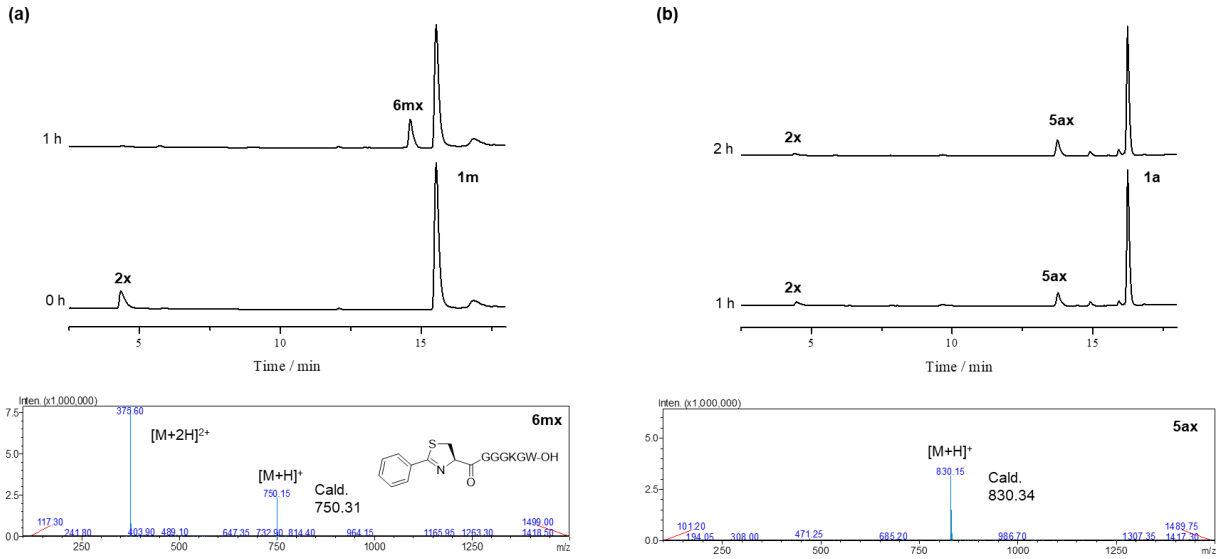


Figure S1. Different reactivity of unsubstituted and ortho-substituted TAMMs

HPLC chromatograms of reactions between 50 μM of **2x** and 400 μM of unsubstituted TAMM **1m** (a) or **1a** (b) at 37 °C in PBS containing 500 μM TCEP and 500 μM Ac-Cys-OMe under chromatography condition A.

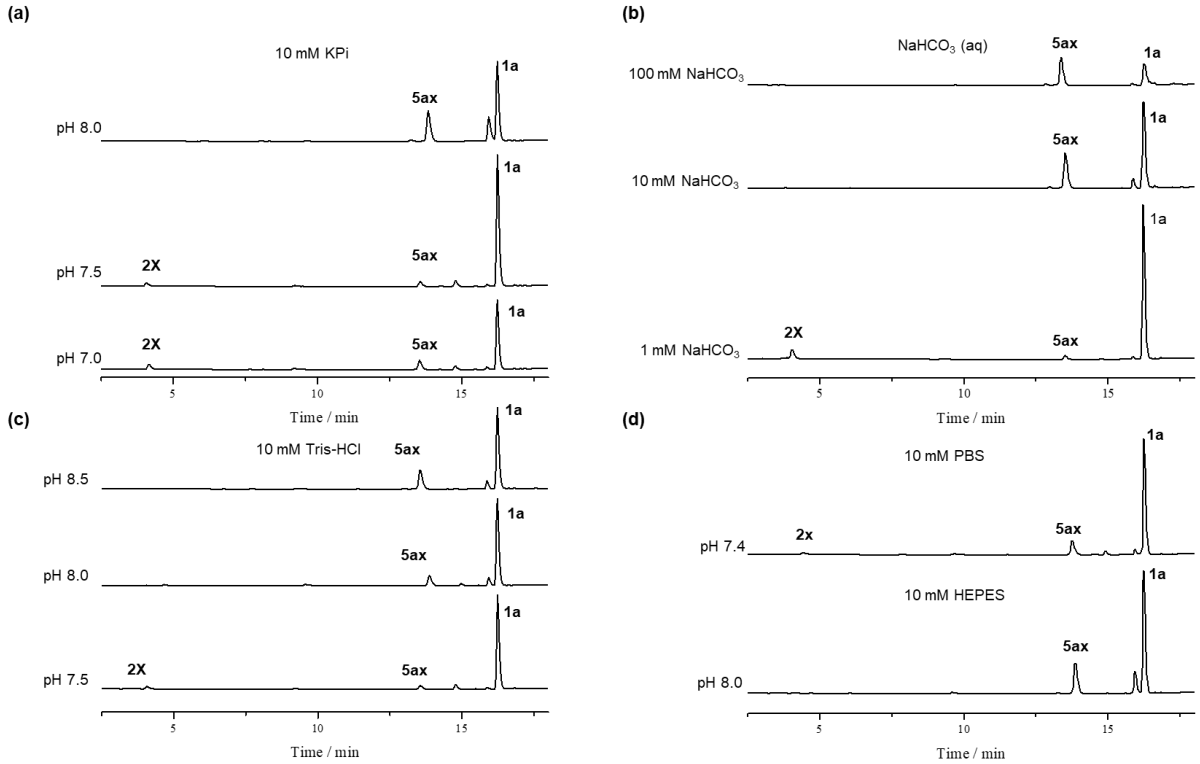


Figure S2. Effect of media compositions and pH values for reactions with 1a

HPLC chromatograms of reactions between 50 μM **2x** and 400 μM **1a** at 37 °C in 10 mM KPi (a), 10 mM Tris-HCl (b), designated concentration of $NaHCO_{3(aq)}$ (c) or 10 mM PBS / HEPES (d) containing 500 μM TCEP and 500 μM Ac-Cys-OMe under chromatography condition A.

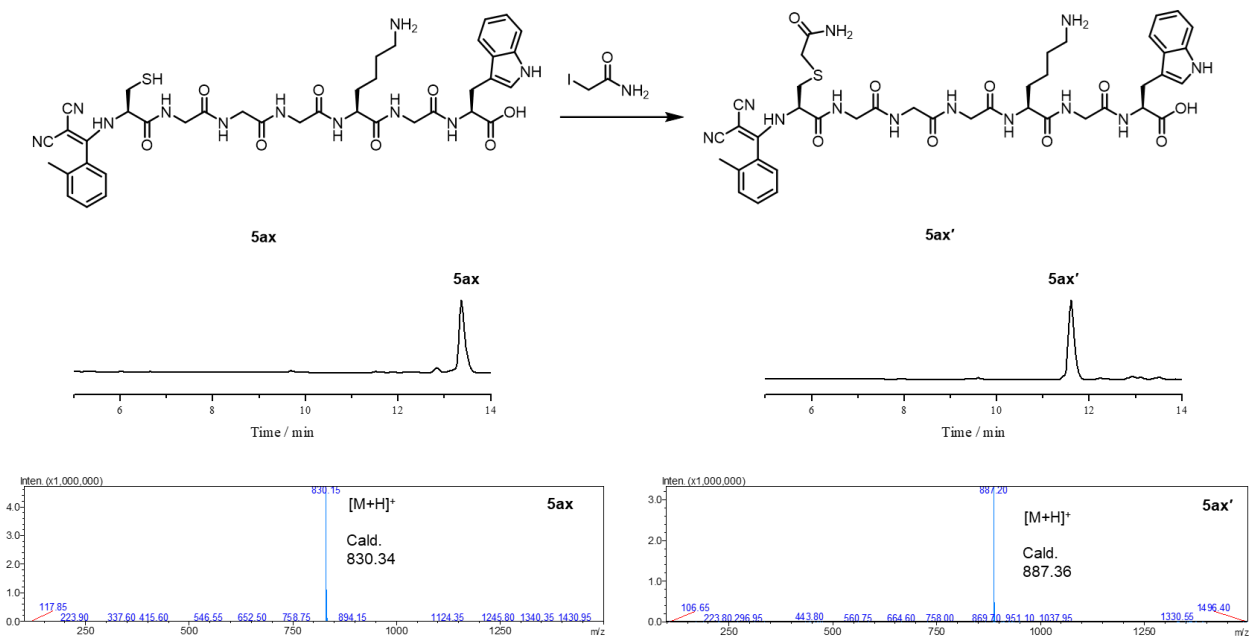


Figure S3. Reaction of 5ax with iodoacetamide

HPLC chromatograms and mass spectra of the reaction between 50 μM **5ax** and 1 mM iodoacetamide in 0.1 M $NaHCO_3$(aq) containing 500 μM TCEP under chromatography condition A.

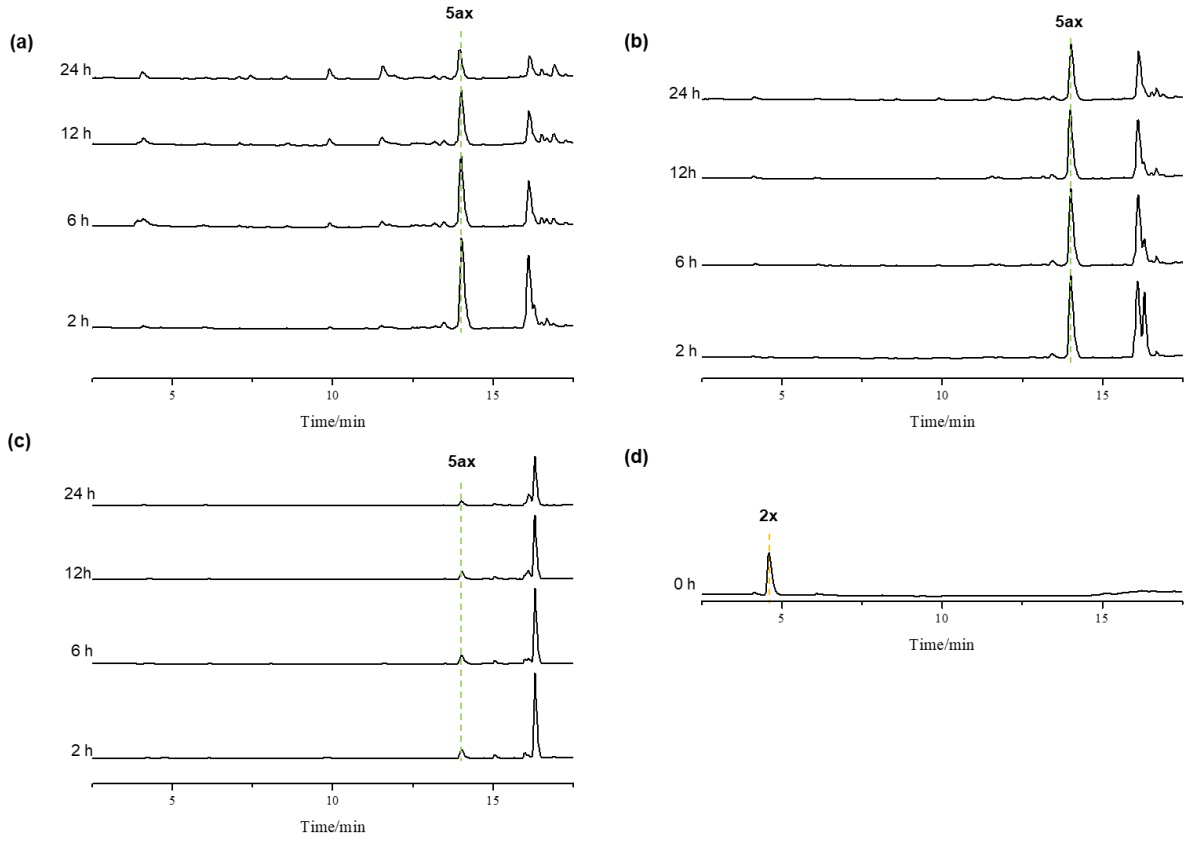


Figure S4. Reaction of 1a and 2x and stability of 5ax in the reaction system

Conversions were quantified by HPLC for reactions of peptide 50 μM **2x** and 200 μM **1a** in the presence of 500 μM TCEP and 500 μM Ac-Cys-OMe under 37 °C in 0.1 M $NaHCO_3$(aq) (a), 25 °C in 0.1 M $NaHCO_3$(aq) (b), or 37 °C in PBS (c). (d) The reaction starting point of raw material peptide **2x**. HPLC chromatograms showing the reaction under chromatography condition A.

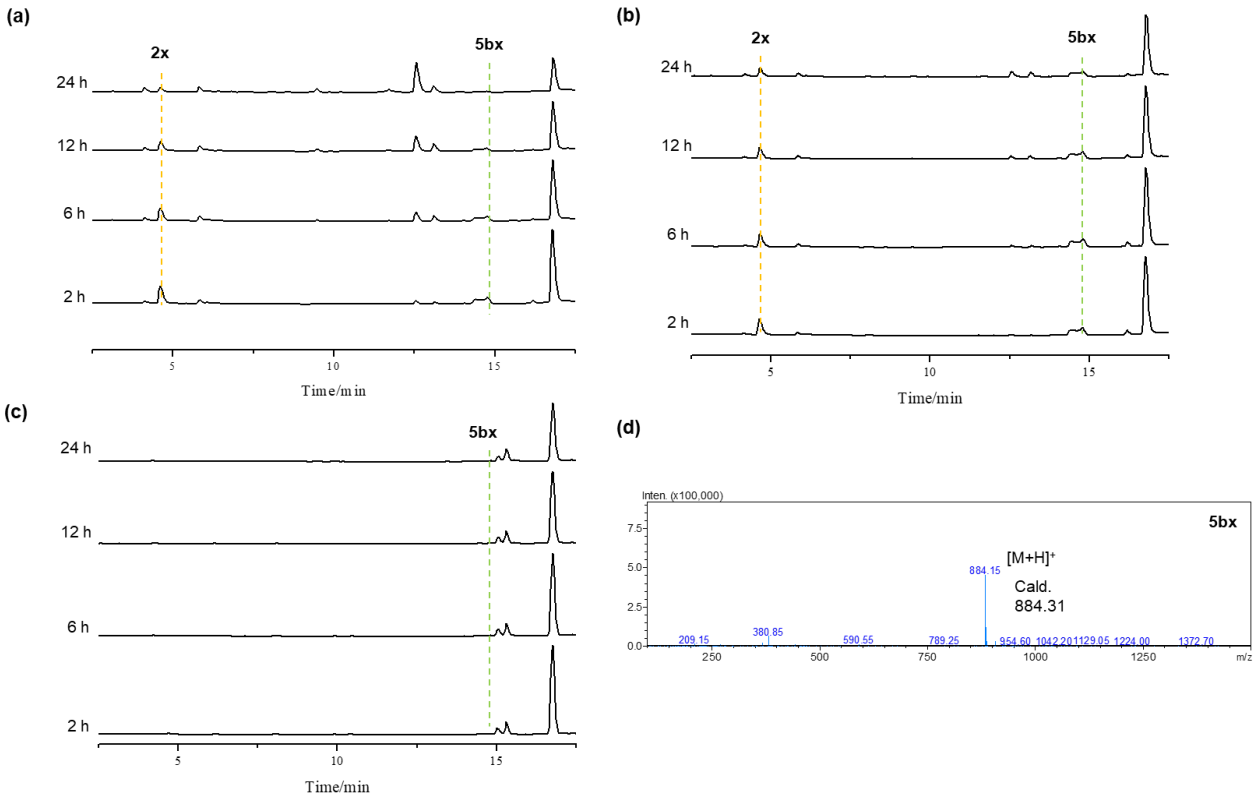


Figure S5. Reaction of 1b and 2x

Conversions were quantified by HPLC for reactions of peptide 50 μM **2x** and 200 μM **1b** in the presence of 500 μM TCEP and 500 μM Ac-Cys-OMe under 37 °C in 0.1 M $NaHCO_3$(aq) (a), 25 °C in 0.1 M $NaHCO_3$(aq) (b), or 37 °C in PBS (c). (d) The mass spectrum of **5bx**. HPLC chromatograms showing the reaction under chromatography condition A.

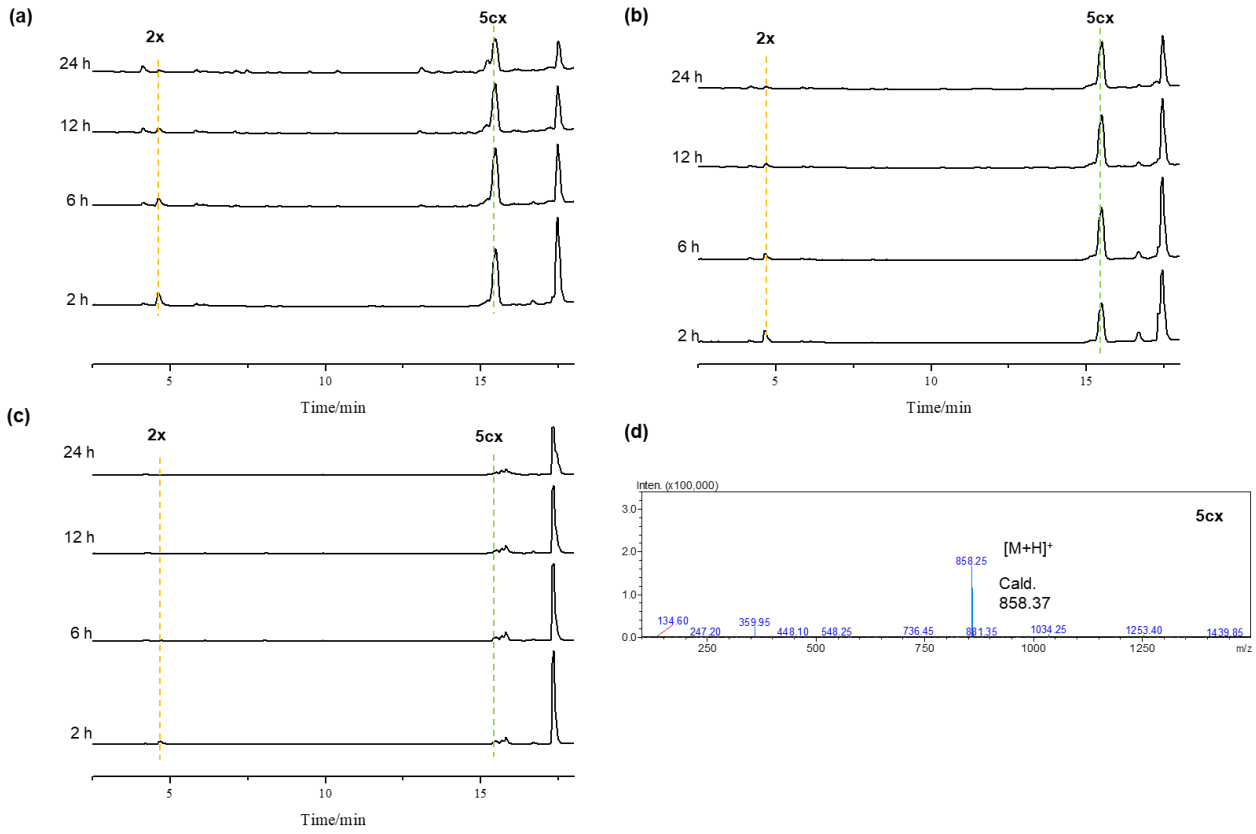


Figure S6. Reaction of 1c and 2x

Conversions were quantified by HPLC for reactions of peptide 50 μM **2x** and 200 μM **1c** in the presence of 500 μM TCEP and 500 μM Ac-Cys-OMe under 37 °C in 0.1 M $NaHCO_3$(aq) (a), 25 °C in 0.1 M $NaHCO_3$(aq) (b), or 37 °C in PBS (c). (d) The mass spectrum of **5cx**. HPLC chromatograms showing the reaction under chromatography condition A.

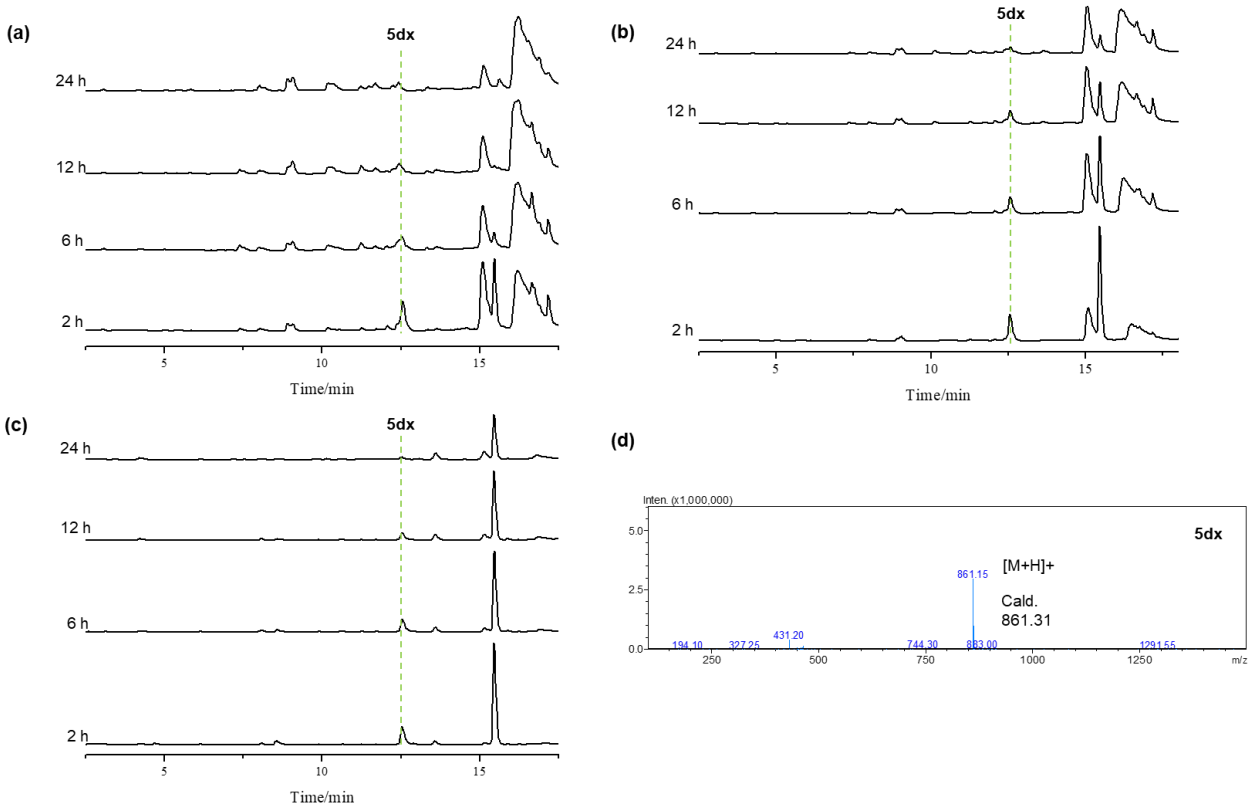


Figure S7. Reaction of 1d and 2x

Conversions were quantified by HPLC for reactions of peptide 50 μM **2x** and 200 μM **1d** in the presence of 500 μM TCEP and 500 μM Ac-Cys-OMe under 37 °C in 0.1 M $NaHCO_3$(aq) (a), 25 °C in 0.1 M $NaHCO_3$(aq) (b), or 37 °C in PBS (c). (d) The mass spectrum of **5dx**. HPLC chromatograms showing the reaction under chromatography condition A.

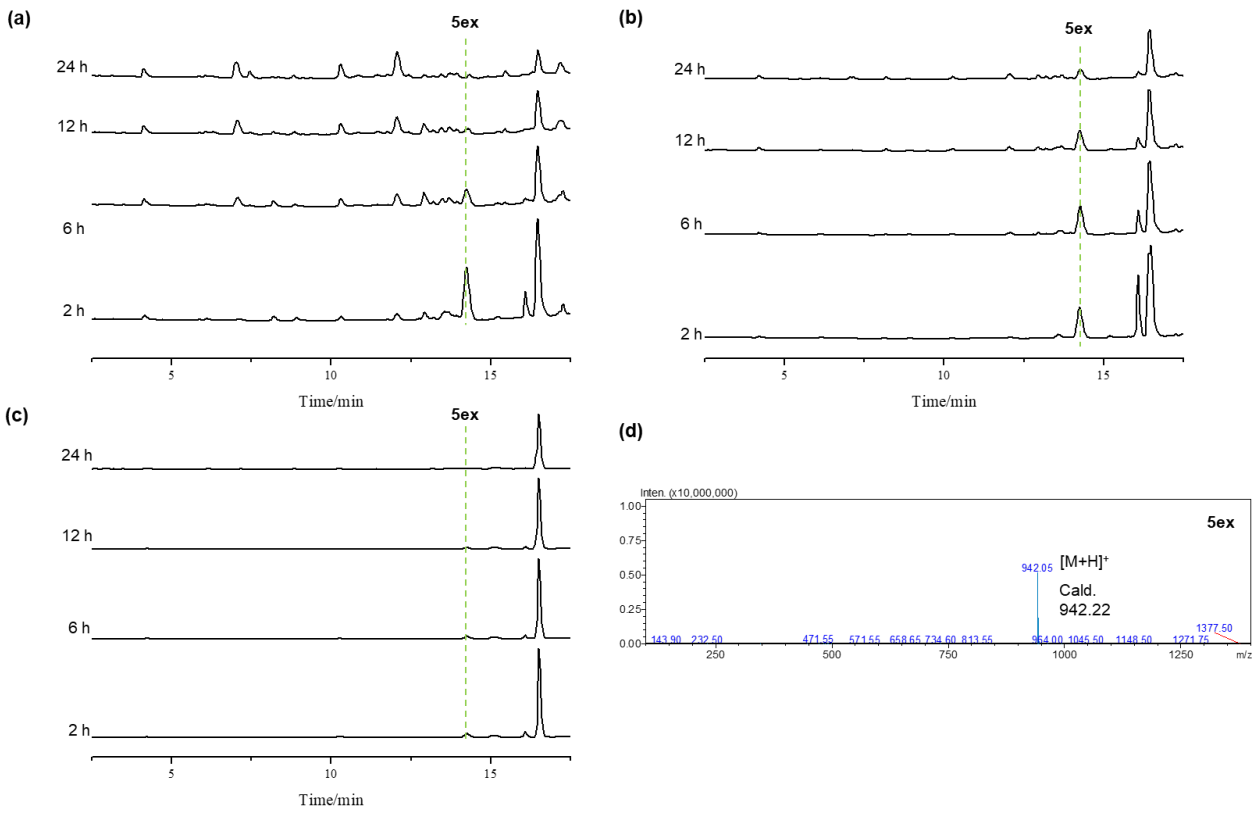


Figure S8. Reaction of 1e and 2x

Conversions were quantified by HPLC for reactions of peptide 50 μM **2x** and 200 μM **1e** in the presence of 500 μM TCEP and 500 μM Ac-Cys-OMe under 37 °C in 0.1 M $NaHCO_3$(aq) (a), 25 °C in 0.1 M $NaHCO_3$(aq) (b), or 37 °C in PBS (c). (d) The mass spectrum of **5ex**. HPLC chromatograms showing the reaction under chromatography condition A.

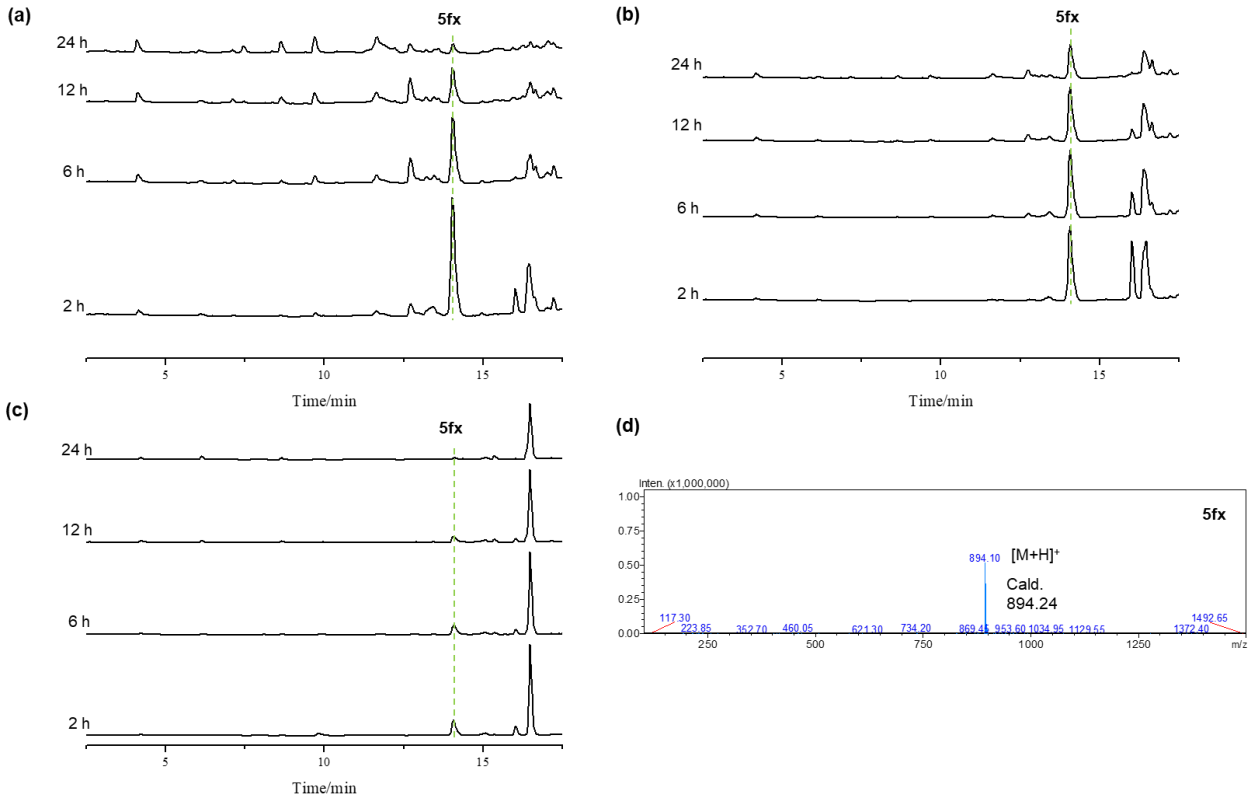


Figure S9. Reaction of 1f and 2x

Conversions were quantified by HPLC for reactions of peptide 50 μM **2x** and 200 μM **1f** in the presence of 500 μM TCEP and 500 μM Ac-Cys-OMe under 37 °C in 0.1 M $NaHCO_3$(aq) (a), 25 °C in 0.1 M $NaHCO_3$(aq) (b), or 37 °C in PBS (c). (d) The mass spectrum of **5fx**. HPLC chromatograms showing the reaction under chromatography condition A.

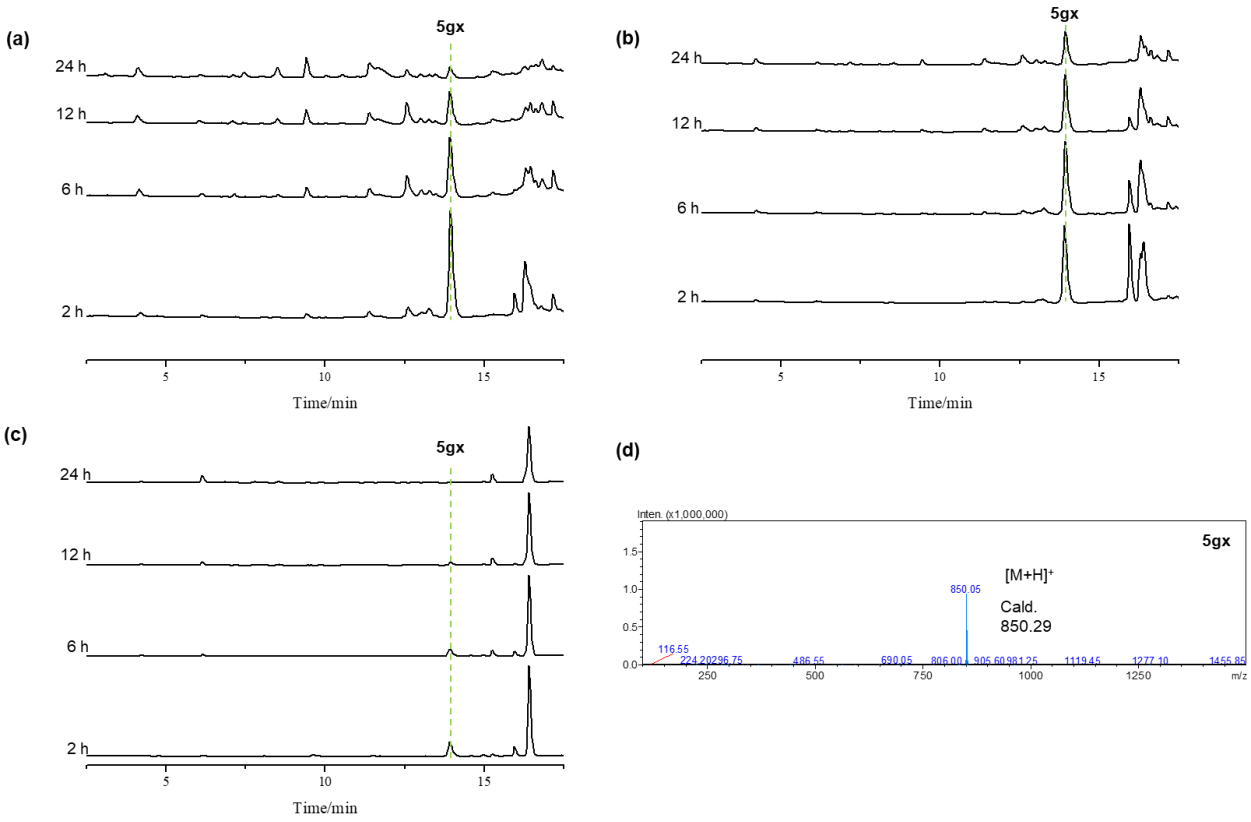


Figure S10. Reaction of 1g and 2x

Conversions were quantified by HPLC for reactions of peptide 50 μM **2x** and 200 μM **1g** in the presence of 500 μM TCEP and 500 μM Ac-Cys-OMe under 37 °C in 0.1 M $NaHCO_3$(aq) (a), 25 °C in 0.1 M $NaHCO_3$(aq) (b), or 37 °C in PBS (c). (d) The mass spectrum of **5gx**. HPLC chromatograms showing the reaction under chromatography condition A.

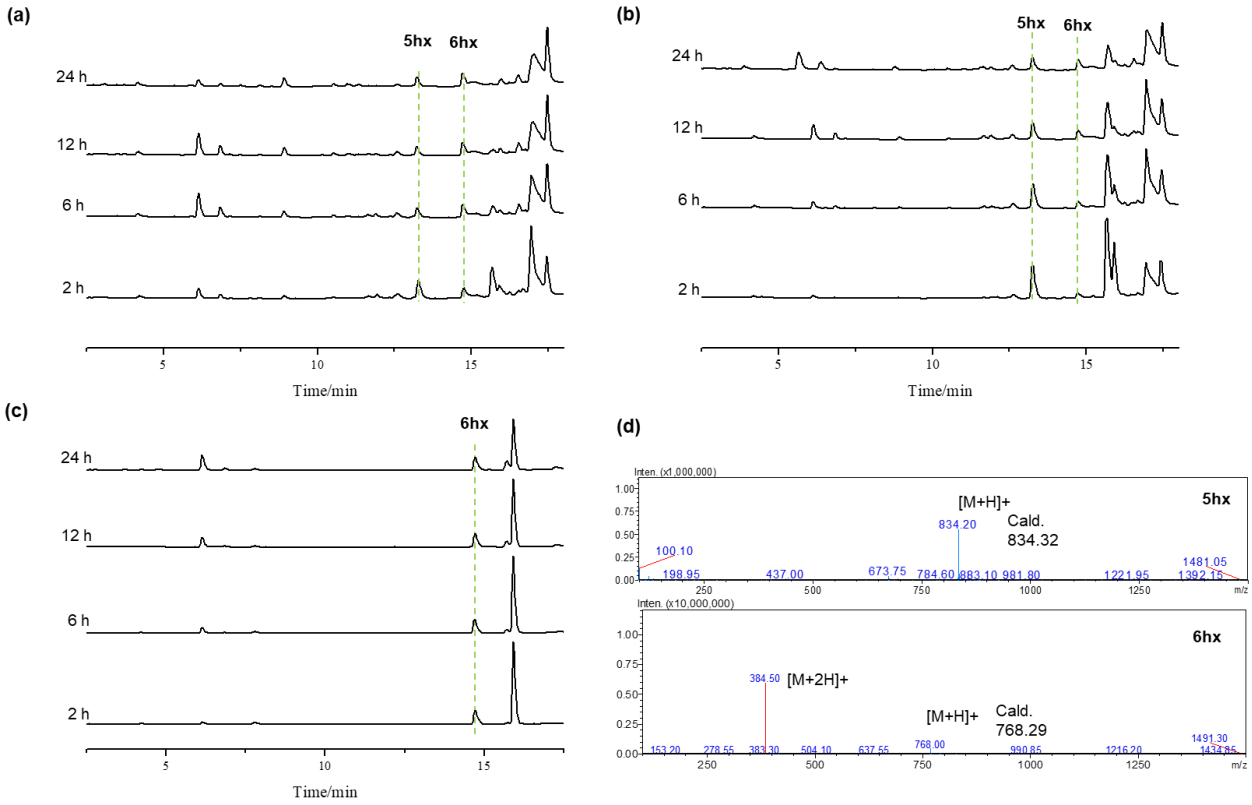


Figure S11. Reaction of 1h and 2x

Conversions were quantified by HPLC for reactions of peptide 50 μM **2x** and 200 μM **1h** in the presence of 500 μM TCEP and 500 μM Ac-Cys-OMe under 37 °C in 0.1 M $NaHCO_3$(aq) (a), 25 °C in 0.1 M $NaHCO_3$(aq) (b), or 37 °C in PBS (c). (d) The mass spectrum of **5hx** and **6hx**. HPLC chromatograms showing the reaction under chromatography condition A.

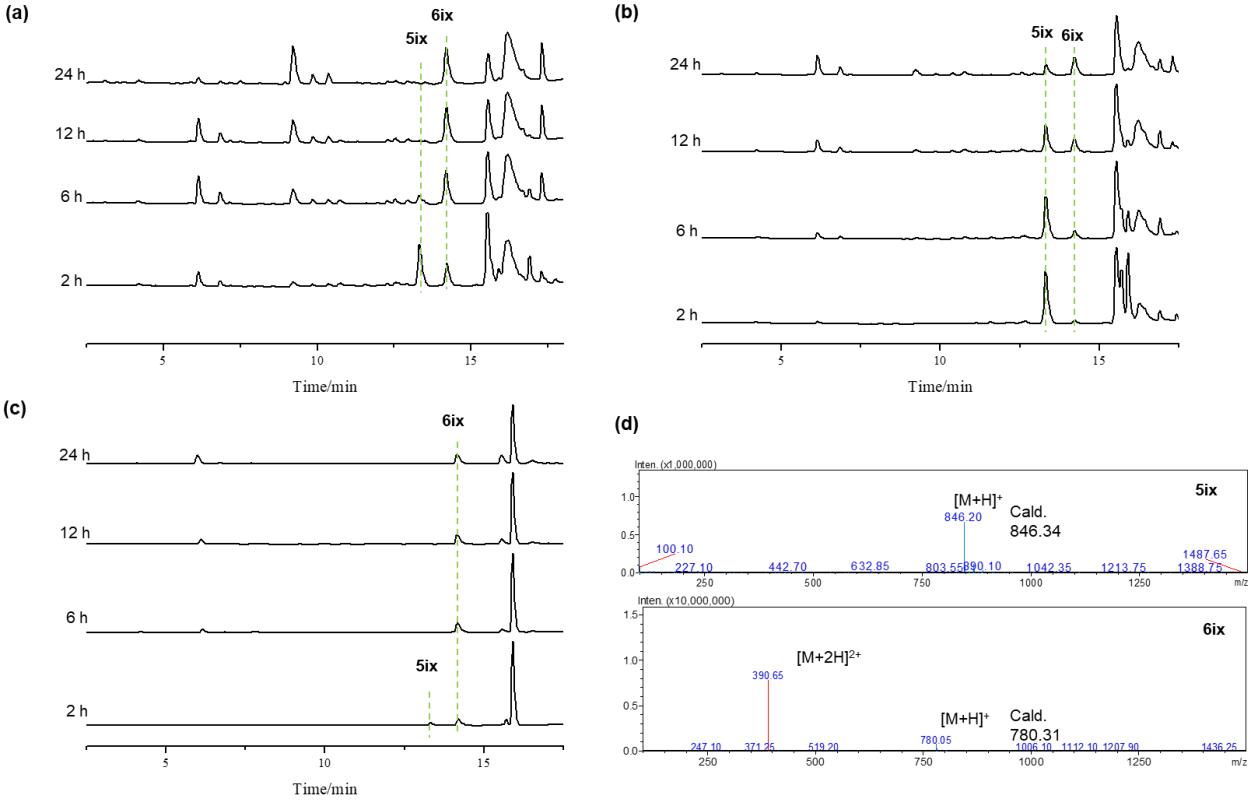


Figure S12. Reaction of 1i and 2x

Conversions were quantified by HPLC for reactions of peptide 50 μM **2x** and 200 μM **1i** in the presence of 500 μM TCEP and 500 μM Ac-Cys-OMe under 37 °C in 0.1 M $NaHCO_3$(aq) (a), 25 °C in 0.1 M $NaHCO_3$(aq) (b), or 37 °C in PBS (c). (d) The mass spectrum of **5ix** and **6ix**. HPLC chromatograms showing the reaction under chromatography condition A.

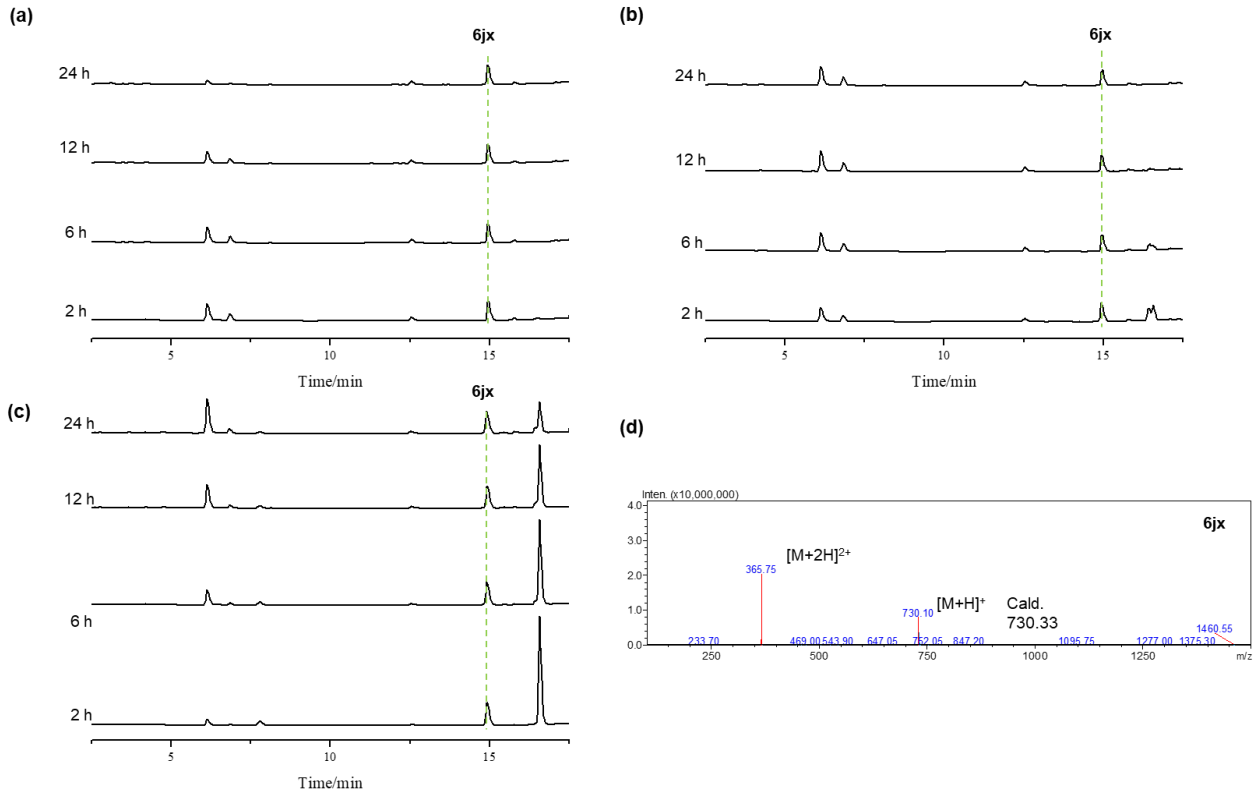


Figure S13. Reaction of 1j and 2x

Conversions were quantified by HPLC for reactions of peptide 50 μM **2x** and 200 μM **1j** in the presence of 500 μM TCEP and 500 μM Ac-Cys-OMe under 37 °C in 0.1 M $NaHCO_3$(aq) (a), 25 °C in 0.1 M $NaHCO_3$(aq) (b), or 37 °C in PBS (c). (d) The mass spectrum of **6jx**. HPLC chromatograms showing the reaction under chromatography condition A.

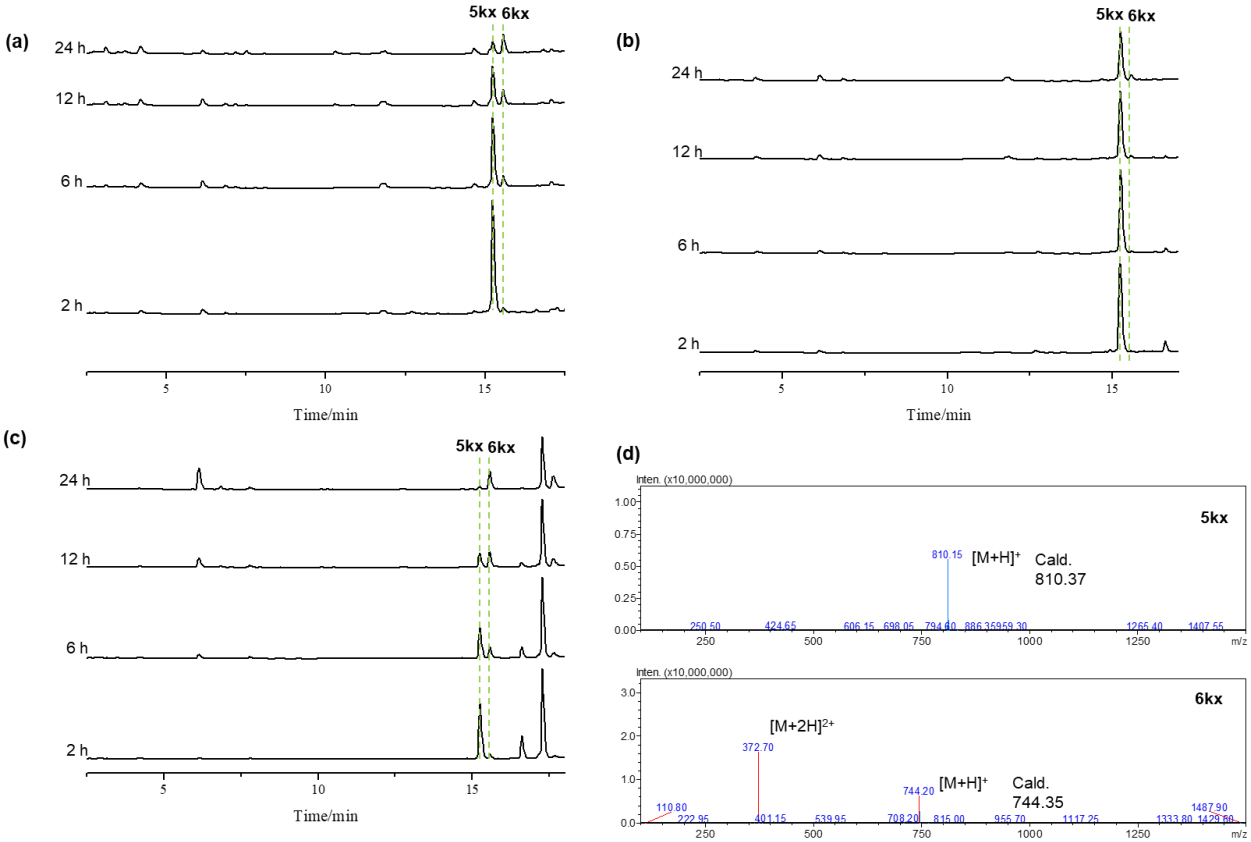


Figure S14. Reaction of 1k and 2x

Conversions were quantified by HPLC for reactions of peptide 50 μM **2x** and 200 μM **1k** in the presence of 500 μM TCEP and 500 μM Ac-Cys-OMe under 37 °C in 0.1 M $NaHCO_3$(aq) (a), 25 °C in 0.1 M $NaHCO_3$(aq) (b), or 37 °C in PBS (c). (d) The mass spectrum of **5kx** and **6kx**. HPLC chromatograms showing the reaction under chromatography condition A.

Table S1. Conversions of 1a~1k reaction with 2x

Entry	R =	Condition	Conversion* (2 h)	Conversion* (6 h)	Conversion* (12 h)	Conversion* (24 h)
1a	2-methylphenyl	0.1 M $NaHCO_{3(aq)}$ 37 °C	93% **5ax** 0% **6ax**	83% **5ax** 0% **6ax**	60% **5ax** 0% **6ax**	43% **5ax** 0% **6ax**
		0.1 M $NaHCO_{3(aq)}$ 25 °C	87% **5ax** 0% **6ax**	81% **5ax** 0% **6ax**	73% **5ax** 0% **6ax**	68% **5ax** 0% **6ax**
		PBS 37 °C	45% **5ax** 0% **6ax**	47% **5ax** 0% **6ax**	42% **5ax** 0% **6ax**	29% **5ax** 0% **6ax**
1b	2-trifluoromethylphenyl	0.1 M $NaHCO_{3(aq)}$ 37 °C	22% **5bx** 0% **6bx**	19% **5bx** 0% **6bx**	14% **5bx** 0% **6bx**	1% **5bx** 0% **6bx**
		0.1 M $NaHCO_{3(aq)}$ 25 °C	15% **5bx** 0% **6bx**	18% **5bx** 0% **6bx**	14% **5bx** 0% **6bx**	12% **5bx** 0% **6bx**
		PBS 25 °C	5% **5bx** 0% **6bx**	6% **5bx** 0% **6bx**	7% **5bx** 0% **6bx**	6% **5bx** 0% **6bx**
1c	2-isopropyl	0.1 M $NaHCO_{3(aq)}$ 37 °C	60% **5cx** 0% **6cx**	61% **5cx** 0% **6cx**	53% **5cx** 0% **6cx**	37% **5cx** 0% **6cx**
		0.1 M $NaHCO_{3(aq)}$ 25 °C	45% **5cx** 0% **6cx**	60% **5cx** 0% **6cx**	61% **5cx** 0% **6cx**	56% **5cx** 0% **6cx**
		PBS 25 °C	26% **5cx** 0% **6cx**	30% **5cx** 0% **6cx**	23% **5cx** 0% **6cx**	9% **5cx** 0% **6cx**
1d	2-nitrophenyl	0.1 M $NaHCO_{3(aq)}$ 37 °C	28% **5dx** 0% **6dx**	15% **5dx** 0% **6dx**	6% **5dx** 0% **6dx**	0% **5dx** 0% **6dx**
		0.1 M $NaHCO_{3(aq)}$ 25 °C	25% **5dx** 0% **6dx**	24% **5dx** 0% **6dx**	16% **5dx** 0% **6dx**	1% **5dx** 0% **6dx**
		PBS 25 °C	64% **5dx** 0% **6dx**	50% **5dx** 0% **6dx**	33% **5dx** 0% **6dx**	14% **5dx** 0% **6dx**
1e	2-iodophenyl	0.1 M $NaHCO_{3(aq)}$ 37 °C	55% **5ex** 0% **6ex**	18% **5ex** 0% **6ex**	0% **5ex** 0% **6ex**	0% **5ex** 0% **6ex**
		0.1 M $NaHCO_{3(aq)}$ 25 °C	44% **5ex** 0% **6ex**	39% **5ex** 0% **6ex**	28% **5ex** 0% **6ex**	17% **5ex** 0% **6ex**
		PBS 25 °C	34% **5ex** 0% **6ex**	28% **5ex** 0% **6ex**	20% **5ex** 0% **6ex**	0% **5ex** 0% **6ex**
1f	2-bromophenyl	0.1 M $NaHCO_{3(aq)}$ 37 °C	72% **5fx** 0% **6fx**	43% **5fx** 0% **6fx**	24% **5fx** 0% **6fx**	6% **5fx** 0% **6fx**
		0.1 M $NaHCO_{3(aq)}$ 25 °C	58% **5fx** 0% **6fx**	51% **5fx** 0% **6fx**	43% **5fx** 0% **6fx**	26% **5fx** 0% **6fx**
		PBS 25 °C	51% **5fx** 0% **6fx**	28% **5fx** 0% **6fx**	26% **5fx** 0% **6fx**	8% **5fx** 0% **6fx**
1g	2-chlorophenyl	0.1 M $NaHCO_{3(aq)}$ 37 °C	73% **5gx** 0% **6gx**	44% **5gx** 0% **6gx**	23% **5gx** 0% **6gx**	8% **5gx** 0% **6gx**
		0.1 M $NaHCO_{3(aq)}$ 25 °C	55% **5gx** 0% **6gx**	53% **5gx** 0% **6gx**	40% **5gx** 0% **6gx**	24% **5gx** 0% **6gx**
		PBS 25 °C	51% **5gx** 9% **6gx**	30% **5gx** 17% **6gx**	17% **5gx** 24% **6gx**	4% **5gx** 26% **6gx**
1h	2-fluorophenyl	0.1 M $NaHCO_{3(aq)}$ 37 °C	51% **5hx** 36% **6hx**	22% **5hx** 31% **6hx**	22% **5hx** 29% **6hx**	21% **5hx** 29% **6hx**
		0.1 M $NaHCO_{3(aq)}$ 25°C	77% **5hx** 14% **6hx**	57% **5hx** 23% **6hx**	44% **5hx** 29% **6hx**	30% **5hx** 28% **6hx**
		PBS 25 °C	0% **5hx** 90% **6hx**	0% **5hx** 90% **6hx**	0% **5hx** 92% **6hx**	0% **5hx** 90% **6hx**
1i	2-methoxyphenyl	0.1 M $NaHCO_{3(aq)}$ 37 °C	58% **5ix** 34% **6ix**	16% **5ix** 51% **6ix**	0% **5ix** 63% **6ix**	0% **5ix** 58% **6ix**
		0.1 M $NaHCO_{3(aq)}$ 25°C	90% **5ix** 10% **6ix**	72% **5ix** 14% **6ix**	46% **5ix** 23% **6ix**	23% **5ix** 40% **6ix**
		PBS 25 °C	20% **5ix** 49% **6ix**	0% **5ix** 70% **6ix**	0% **5ix** 73% **6ix**	0% **5ix** 72% **6ix**
1j	*tert*-butyl	0.1 M $NaHCO_{3(aq)}$ 37 °C	0% **5jx** 86% **6jx**	0% **5jx** 87% **6jx**	0% **5jx** 82% **6jx**	0% **5jx** 82% **6jx**
		0.1 M $NaHCO_{3(aq)}$ 25°C	0% **5jx** 82% **6jx**	0% **5jx** 77% **6jx**	0% **5jx** 74% **6jx**	0% **5jx** 74% **6jx**
		PBS 37 °C	0% **5jx** 95% **6jx**	0% **5jx** 93% **6jx**	0% **5jx** 90% **6jx**	0% **5jx** 90% **6jx**
1k	neopentyl	0.1 M $NaHCO_{3(aq)}$ 37 °C	95% **5kx** 5% **6kx**	57% **5kx** 9% **6kx**	33% **5kx** 14% **6kx**	11% **5kx** 17% **6kx**
		0.1 M $NaHCO_{3(aq)}$ 25°C	96% **5kx** 4% **6kx**	86% **5kx** 5% **6kx**	70% **5kx** 5% **6kx**	53% **5kx** 15% **6kx**
		PBS 37 °C	63% **5kx** 7% **6kx**	34% **5kx** 14% **6kx**	16% **5kx** 19% **6kx**	6% **5kx** 27% **6kx**

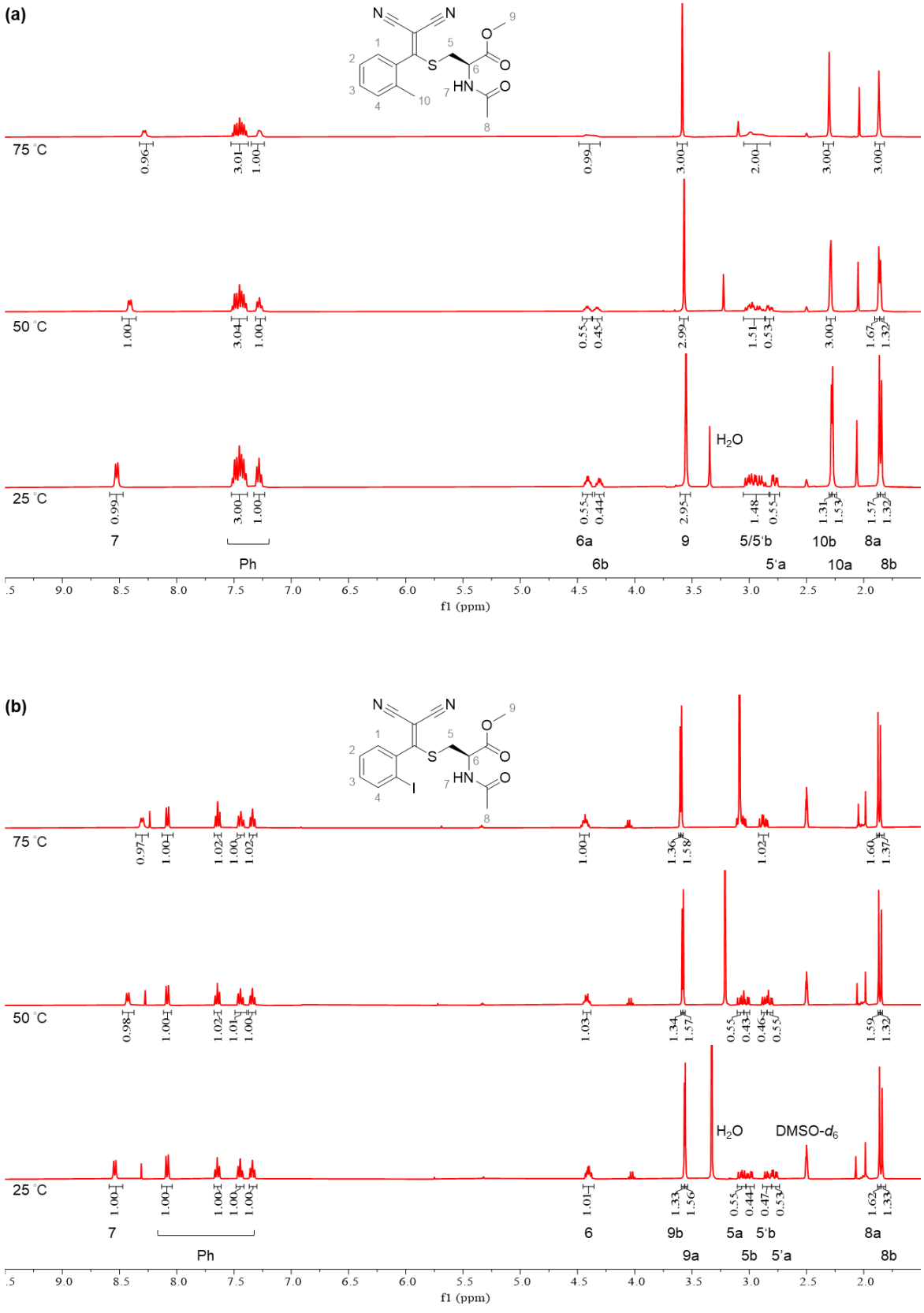


Figure S15. ^{1}H NMR of 1a and 1e at different temperatures

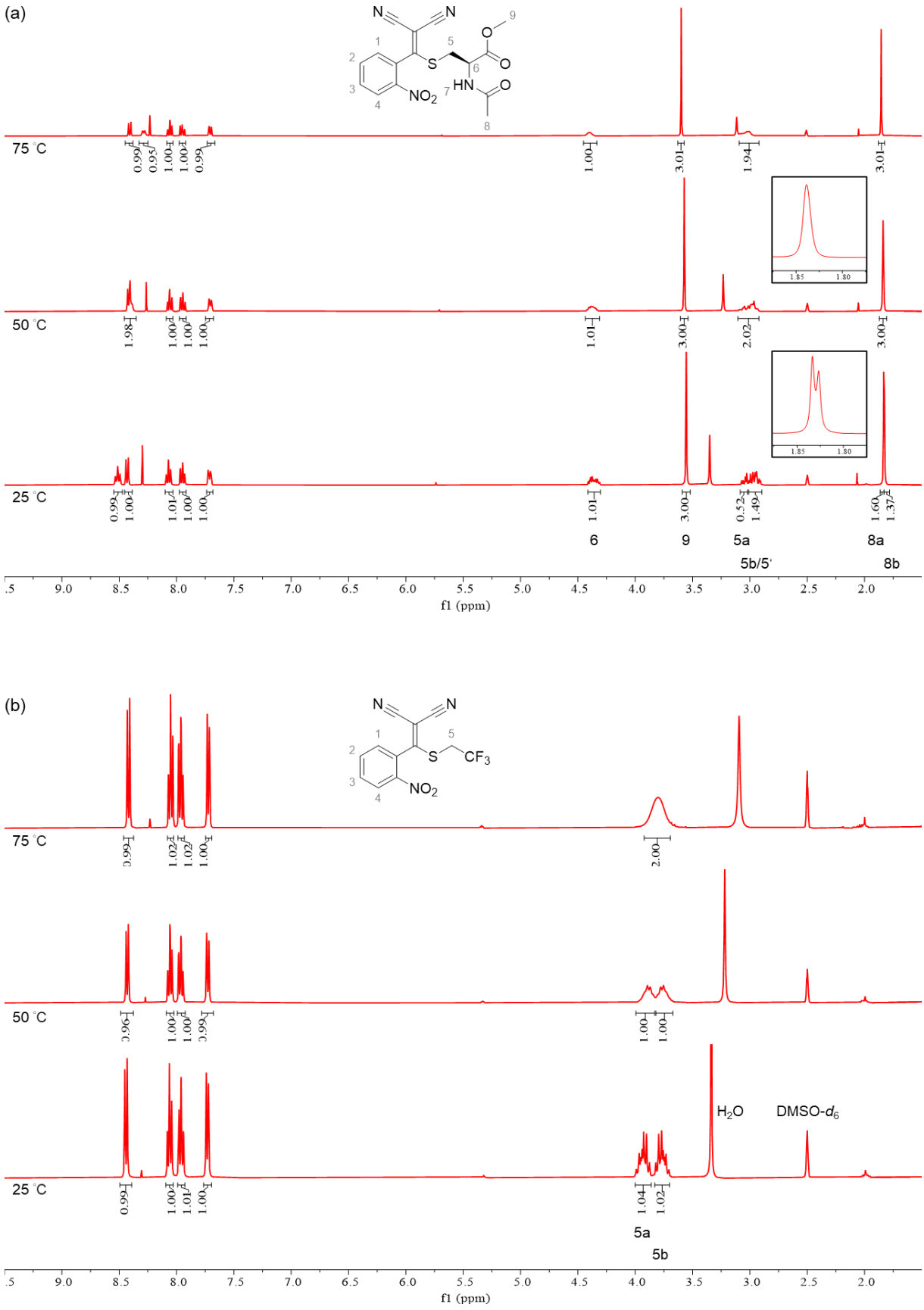


Figure S16. ^{1}H NMR of 1d and 1l at different temperatures

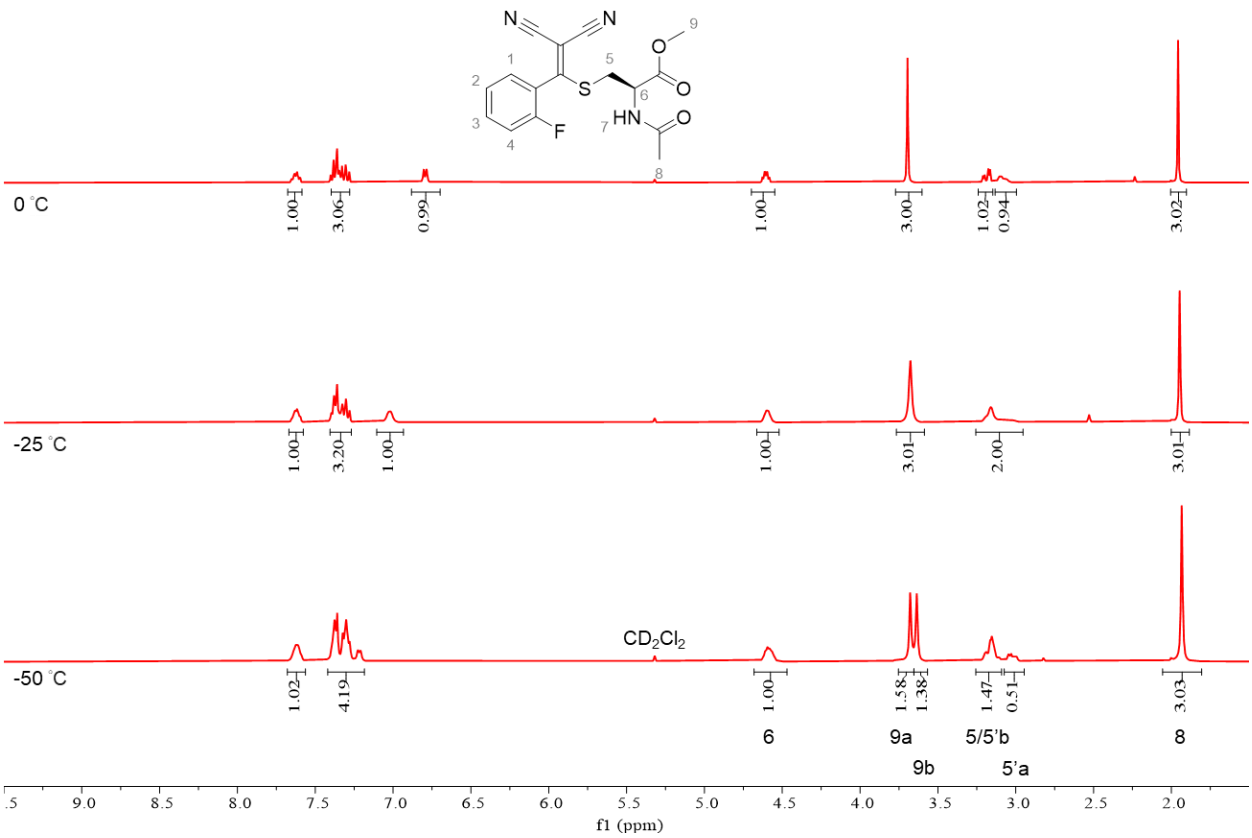


Figure S17. ^{1}H NMR of 1h at different temperatures

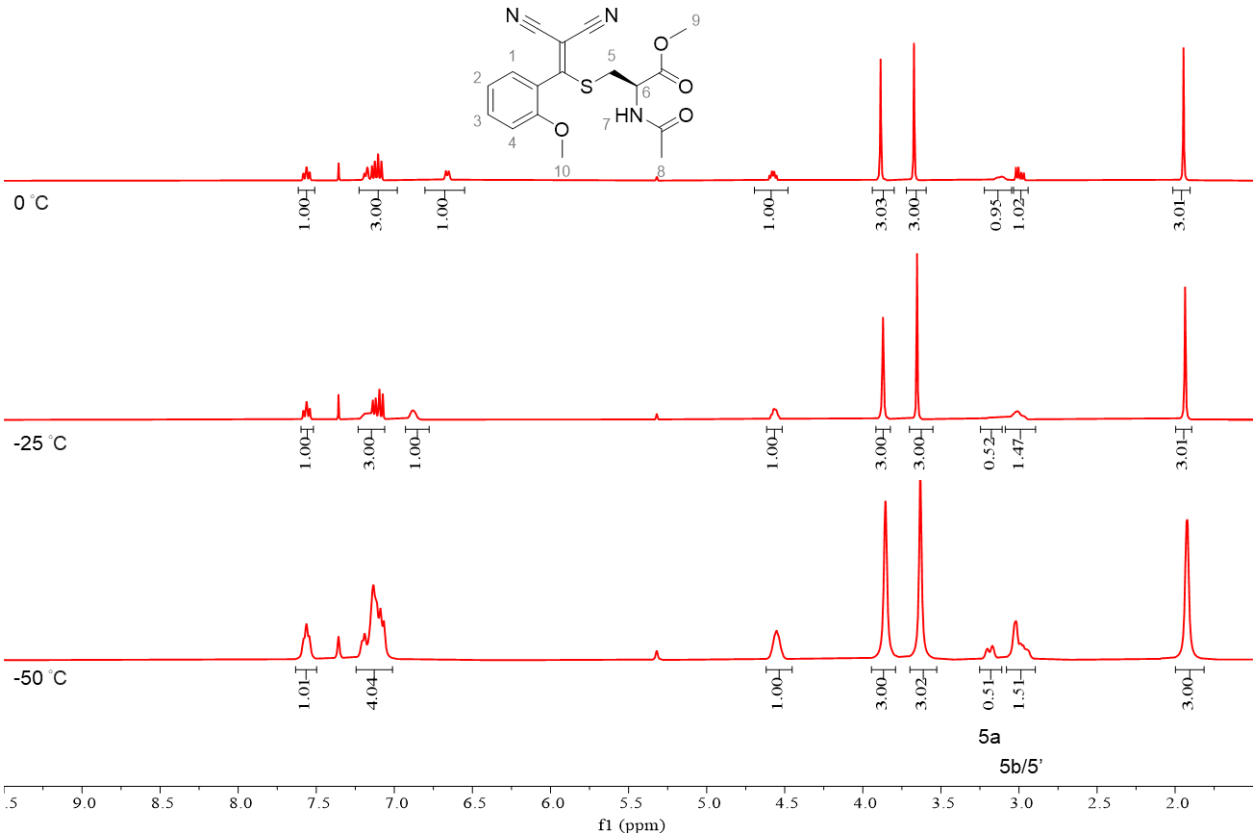
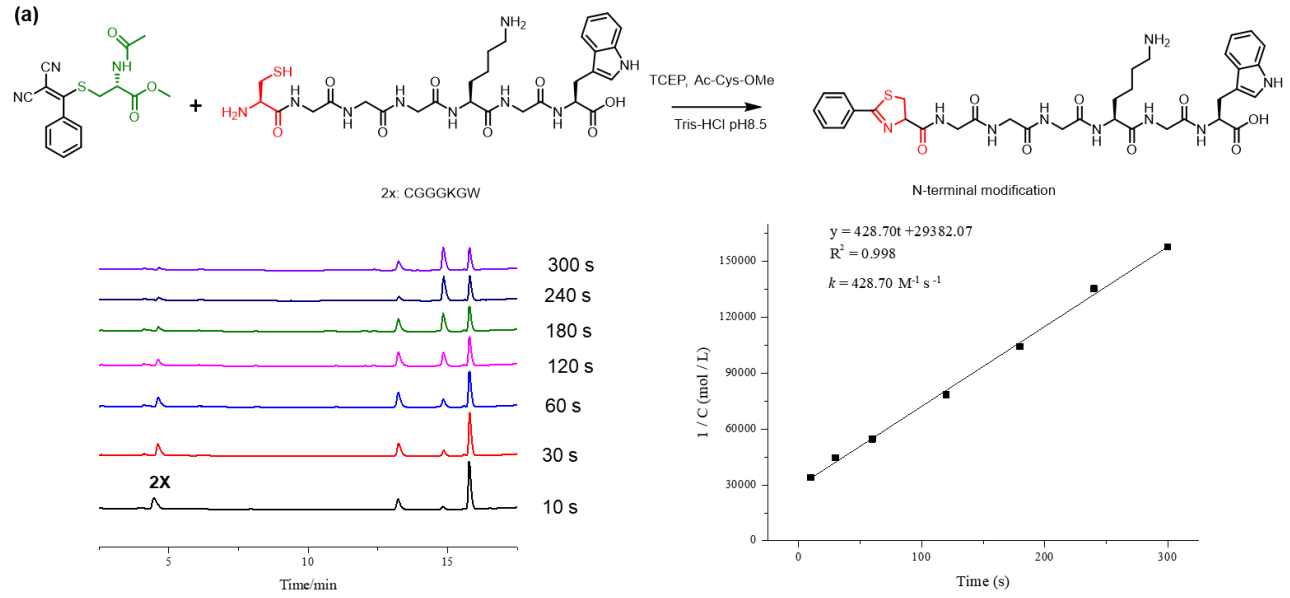
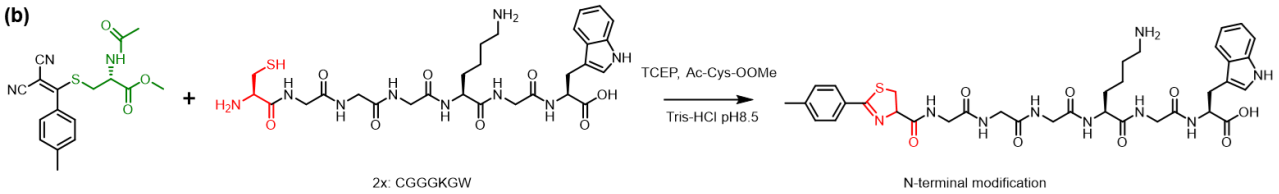


Figure S18. ^{1}H NMR of 1i at different temperatures

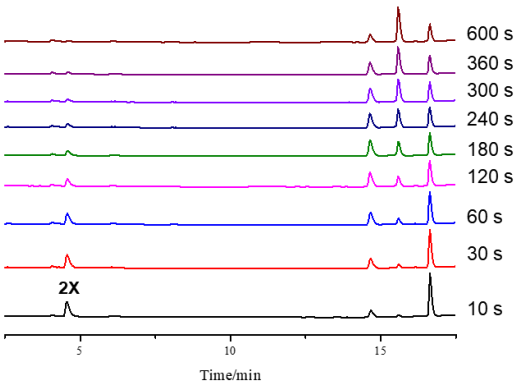


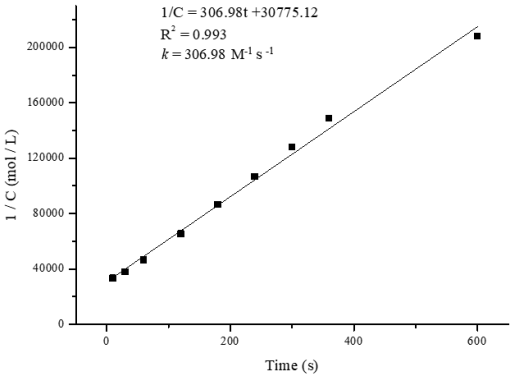
(b)



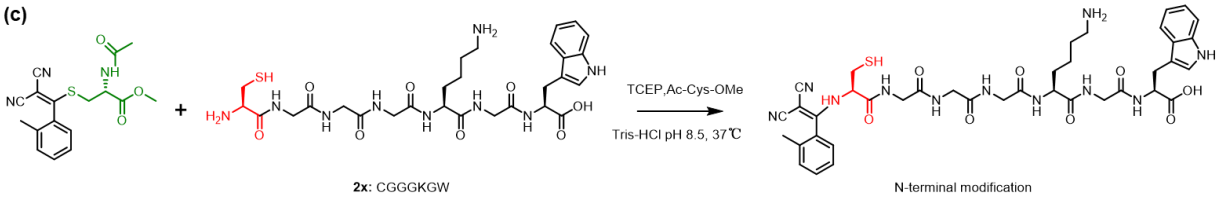
2x: CGGGKGW

N-terminal modification



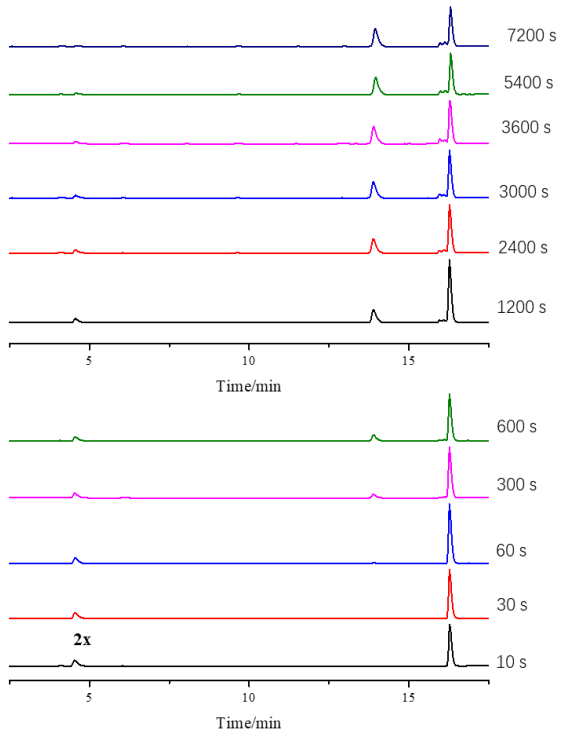


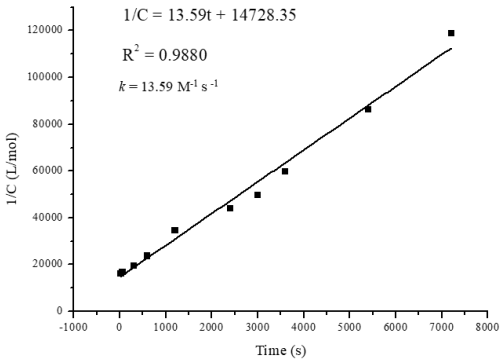
(c)



2x: CGGGKGW

N-terminal modification





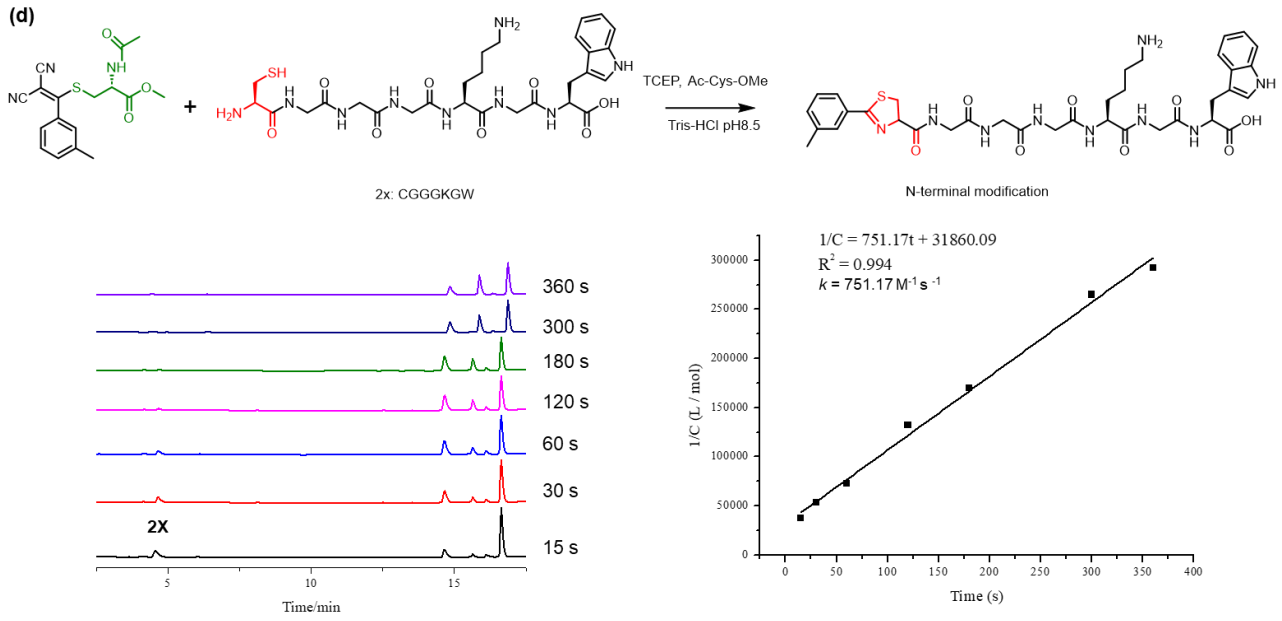


Figure S19. The reaction rates *k* obtained from the tests of different TAMM molecules reacting with 2x

HPLC chromatograms and the reaction rate equation showing the reaction of 50 μM peptide **2x** with 100 μM different **TAMM** in 10 mM Tris-HCl (pH 8.5) containing 500 μM TCEP and 500 μM Ac-Cys-OMe under chromatography condition A.

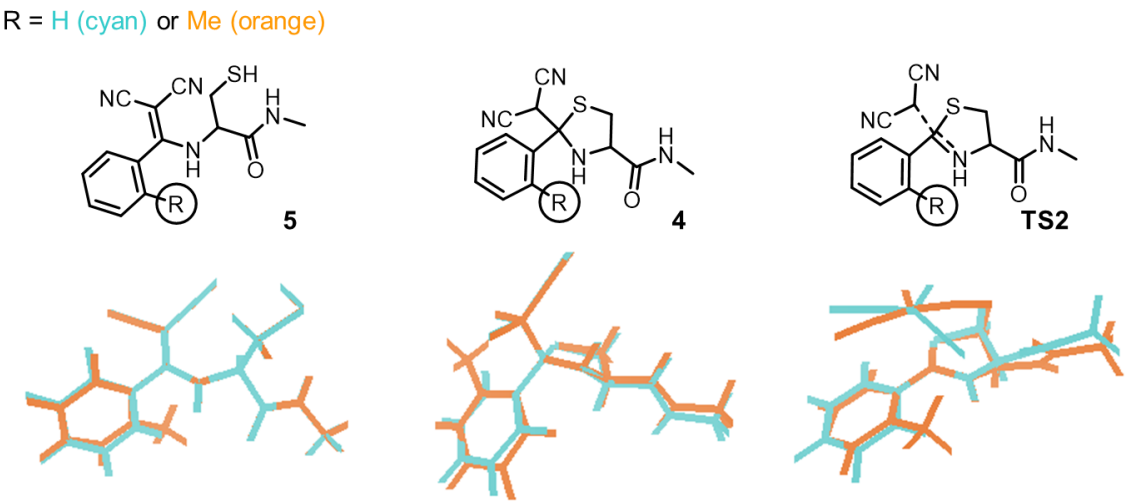


Figure S20. Lowest energy conformers of 4, 5, and TS2 by DFT calculation

The lowest energy states/conformers of unsubstituted **4xa**/**5xa**/TS2-**xa** and *ortho*-methyl **4ya**/**5ya**/**TS2-ya**.

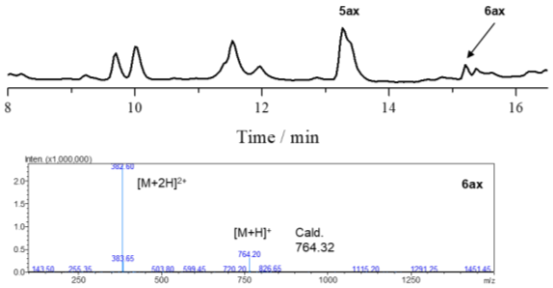


Figure S21. Transformation of 5ax to 6ax

HPLC chromatograms showing the reaction of 50 μM peptide **2x** and 400 μM **1j** in 0.1 M $NaHCO_{3(aq)}$ containing 500 μM TCEP and 1 mM Ac-Cys-OMe under chromatography condition A.

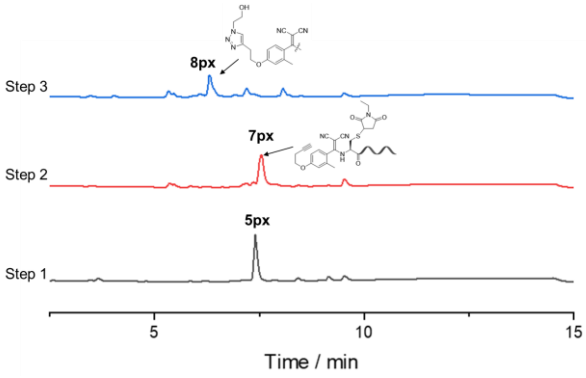


Figure S22. The results of HPLC chromatograms for 2x dual functionalization

HPLC chromatograms showing the results of step1 **2x** (50 μM) and **1p** (100 μM, 2 equiv.) in 0.1 M $NaHCO_3$(aq) in the presence of TCEP (1 mM, 20 equiv.) and Ac-Cys-OMe (0.5 mM, 10 equiv.) to form **5px**, step2 the obtained **5py** and **Maleimide** (1 mM) in 0.1 M $NaHCO_3$(aq) to form **7px**, and step3 the obtained **7px** and **2-azidoethanol** in presence of 1mM $CuSO_4$ and 1mM BTTTA to form **8px**.Chromatography condition B.

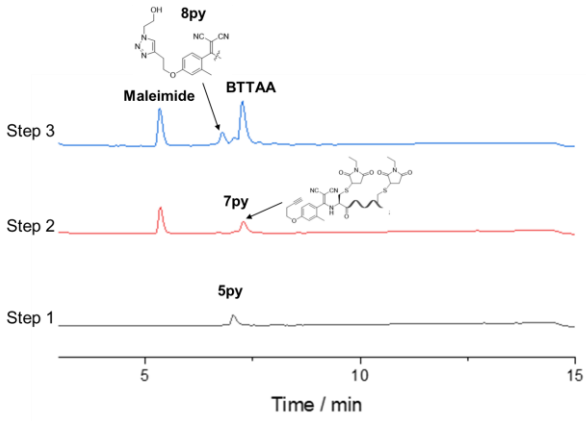


Figure S23. The results of HPLC chromatograms for 2y dual functionalization

HPLC chromatograms showing the results of step1 **2y** (50 μM) and **1n** (100 μM, 2 equiv.) in 0.1 M $NaHCO_3$(aq) in the presence of TCEP (1 mM, 20 equiv.) and Ac-Cys-OMe (0.5 mM, 10 equiv.) to

form **5py**, step2 the obtained **5py** and **Maleimide** (1 mM) in 0.1 M $NaHCO_3$(aq) to form **7py**, and step3 the obtained **7py** and **2-azidoethanol** in presence of 1mM $CuSO_4$ and 1mM BTTTA to form **8py**.Chromatography condition B.

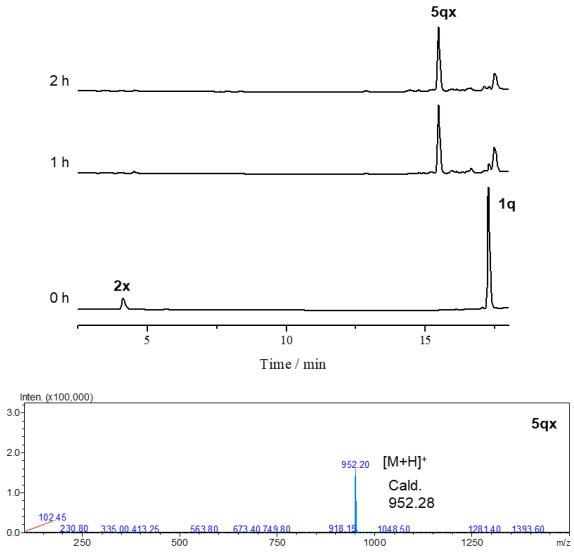


Figure S24. The results of the chemical reaction between 2x and 1q

HPLC chromatograms showing the reaction of **2x** (50 μM) and **1q** (200 μM, 4 equiv.) in 0.1 M $NaHCO_3$(aq) in the presence of TCEP (0.5 mM, 10 equiv.) and Ac-Cys-OMe (1 mM, 20 equiv.) to form **5qx**. Chromatography condition A.

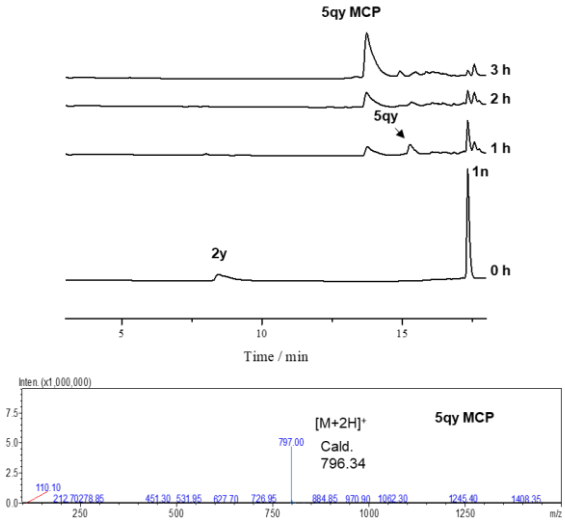


Figure S25. The results of the chemical reaction between 2y and 1q

HPLC chromatograms showing the reaction of **2y** (50 μM) and **1q** (200 μM, 4 equiv.) in 0.1 M $NaHCO_3$(aq) in the presence of TCEP (0.5 mM, 10 equiv.) and Ac-Cys-OMe (1 mM, 10 equiv.) to form **5qy** and **5qy MCP**. Chromatography condition A.

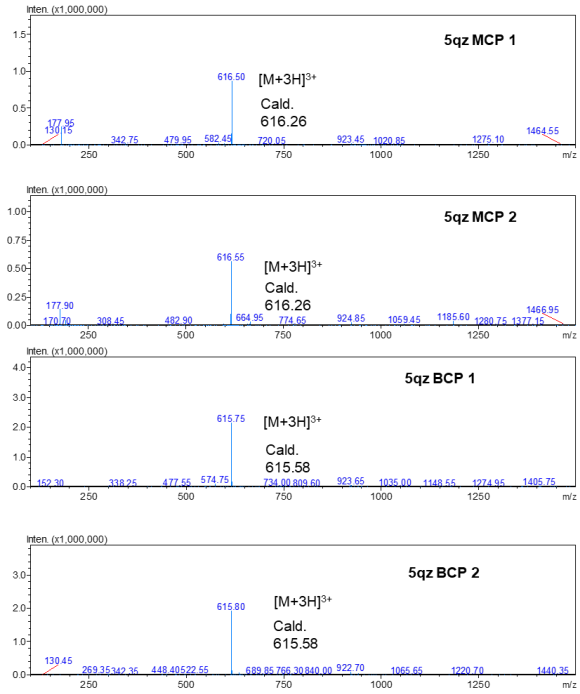


Figure S26. The mass spectrum results of the construction of bicyclic peptide

The mass spectrum results of **5qz MCPs** and **5qz BCPs**.

Round	Recovery rate*		Enrichment
	Control	Experimental	
1st	1.20×10^{-7}	2.40×10^{-8}	0.2
2nd	1.79×10^{-7}	1.06×10^{-4}	593.2
3rd	2.09×10^{-6}	3.43×10^{-3}	1641.1

*Recovery rate refers to the ratio of the phage titer recovered from either the experimental group (magnetic beads with immobilized target proteins) or the control group (magnetic beads without target proteins) relative to the initial phage titer that was introduced into the system. Enrichment = Experimental / Control.

Figure S27. Enrichment from KEAP1 selection.

next-generation sequencing of the 1st round

NO.	Sequence	Abundance	Percentage(%)
A1-1	C T G W E P E T G E C R E T Q C	730195	6.03%
A1-2	C H N P A T G E L V C E S A C C	103814	0.86%
A1-3	C E T S P E T G E T C G D S E C	55648	0.46%
A1-4	C V N G R Y M L R I C G R T G L	39389	0.33%
A1-5	C R A P H P V T G V C L T Y E C	38619	0.32%
A1-6	C P A L L P D T G E C E E E L C	23728	0.20%
A1-7	C T G H I R M R H E C S P C C C	18248	0.15%
A1-8	C Q V L V V D T G E C E C R S C	17764	0.15%
A1-9	C R D V E T G E L T C P Y P E C	15169	0.13%
A1-10	C Q G F R G R K Q S C T S W E C	14354	0.12%
A1-11	C A G P K E S M P T C G D G E C	12891	0.11%
A1-12	C H I Q L E T G E S C A G E R G	12683	0.10%
A1-13	C G D K A E W R A G C L F A E C	12641	0.10%
A1-14	C S G D C C E L G A C S E Q P C	12416	0.10%
A1-15	C N M Y D R D T G E C G T Y E C	10723	0.09%
A1-16	C H G L M R R E W A C G H G M C	10140	0.08%
A1-17	C N N S D P E T G E C R R E Q C	9940	0.08%
A1-18	C V R I V G W L E E C F G R T C	9790	0.08%
A1-19	C L A R N P E D G V C H V E L C	9647	0.08%
A1-20	C N L E T G I D C E C T R P E C	9328	0.08%

next-generation sequencing of the 2nd round

NO.	Sequence	Abundance	Percentage(%)
A2-1	C T G W E P E T G E C R E T Q C	8092190	58.83%
A2-2	C E T S P E T G E T C G D S E C	1135923	8.26%
A2-3	C H N P A T G E L V C E S A C C	546924	3.98%
A2-4	C N N S D P E T G E C R R E Q C	494210	3.59%
A2-5	C R D V E T G E L T C P Y P E C	435808	3.17%
A2-6	C L R D P E T G E N C P E S S C	206927	1.50%
A2-7	C R W E H P S T G E C E V Q A C	172876	1.26%
A2-8	C P A L L P D T G E C E E E L C	158791	1.15%
A2-9	C M D L E T G E R S C N R V E C	105732	0.77%
A2-10	C Y S E S P E T G E C M A W D C	95988	0.70%
A2-11	C E R E M E T G E A C G C F E C	88349	0.64%
A2-12	C N M Y D R D T G E C G T Y E C	82765	0.60%
A2-13	C Y T V H P E N G E C L C R E C	65038	0.47%
A2-14	C Q V L V V D T G E C E C R S C	62313	0.45%
A2-15	C S W L D H E N G E C V E E R C	62194	0.45%
A2-16	C R D E A T G E L V C G E L G C	54911	0.40%
A2-17	C L A R N P E D G V C H V E L C	46969	0.34%
A2-18	C M P D T G E G C E C I P S T C	46451	0.34%
A2-19	C I D Q E S G E D V C K S F E C	46283	0.34%
A2-20	C E S Y D P E D G T C S A L R C	41605	0.30%

next-generation sequencing of the 3rd round

NO.	Sequence	Abundance	Percentage(%)
A3-1	C R D V E T G E L T C P Y P E C	2159914	18.49%
A3-2	C E T S P E T G E T C G D S E C	1807262	15.47%
A3-3	C N N S D P E T G E C R R E Q C	1679681	14.38%
A3-4	C T G W E P E T G E C R E T Q C	1348847	11.55%
A3-5	C L R D P E T G E N C P E S S C	605379	5.18%
A3-6	C M P D T G E G C E C I P S T C	362892	3.11%
A3-7	C H N P A T G E L V C E S A C C	251011	2.15%
A3-8	C M D L E T G E R S C N R V E C	207638	1.78%
A3-9	C Y S E S P E T G E C M A W D C	141414	1.21%
A3-10	C R W E H P S T G E C E V Q A C	110853	0.95%
A3-11	C I N L E T G E S Q C E M A E C	100605	0.86%
A3-12	C S R D P E T G E G C E Y K N C	92353	0.79%
A3-13	C Y T V H P E N G E C L C R E C	88666	0.76%
A3-14	C Y D V G P E T G E C E G E C C	76175	0.65%
A3-15	C I D Q E S G E D V C K S F E C	63227	0.54%
A3-16	C E R E M E T G E A C G C F E C	62832	0.54%
A3-17	C N M Y D R D T G E C G T Y E C	54936	0.47%
A3-18	C N L E T G I D C E C T R P E C	52089	0.45%
A3-19	C S W L D H E N G E C V E E R C	47400	0.41%
A3-20	C D I E T G V E C D C L Y D R C	35205	0.30%

Figure S28. HTS sequencing results of phages enriched over three rounds from KEAP1 selection.

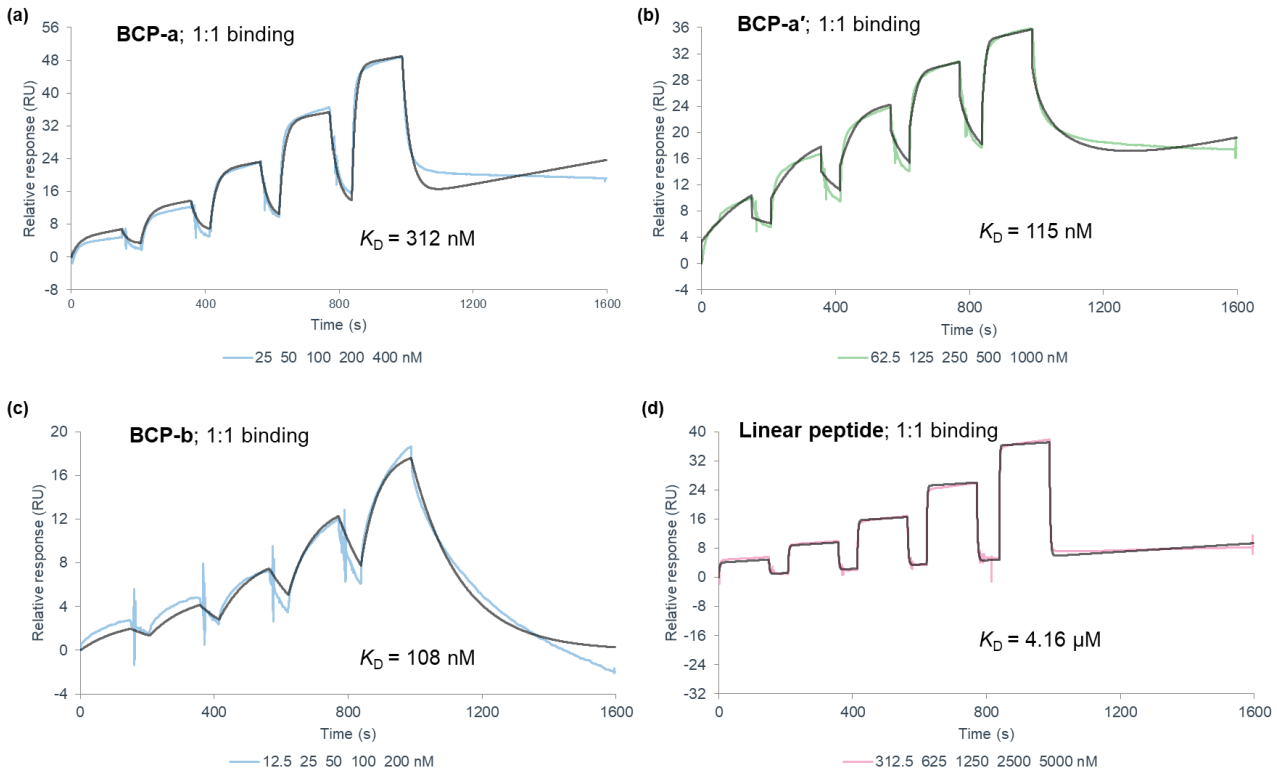


Figure S29. SPR results of bicyclic peptide and linear peptide binding to KEAP1.

Reference

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