Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry
This journal is © The Royal Society of Chemistry 2012

Supporting Information

Efficient Synthesis of RITA and its Analogues: Derivation of Analogues with Improved Antiproliferative Activity *via* Modulation of p53/miR-34a Pathway

Jinshun Lin,[a,b] Xiuli Jin,[b] Yiwen Bu,[e] Deliang Cao,[e] Nannan Zhang,[a,b] Shangfu Li,[b] Qinsheng Sun,[b] Chunyan Tan,[b,c] Chunmei Gao*[b,c] and Yuyang Jiang*[b,d]

Table of contents

This journal is © The Royal Society of Chemistry 2012

Biological assays

Cell culture and reagents

Human cell lines, including K562 cells, A549 cells were obtained from Cell Resources Center of Shanghai Institutes for Biological Science, Chinese Academy of Science. NCM460 cells were obtained from Hanbio Biotechnology Co.LTD (China). The HCT116 human colon cancer cell lines ($p53^{+/+}$ and $p53^{-/-}$) and MCF-7 cells were kindly provided by Dr. Cao Deliang (Southern Illinois University School of Medicine, USA). They were maintained in Dulbecco's modified Eagle's medium (DMEM) with 10 % fetal bovine serum, 100 μg/mL penicillin, and 100 μg/mL streptomycin at 37 ℃ in a humidified atmosphere of 5 % CO_2.

Cell proliferation assay and colony formation assay

Cells were seeded at a density of 5 × 10^3/100 μL medium in 96-well microtiter plate and treated with the synthesized compounds at different concentrations for 72 h, ranging from 0.0125 to 50 μM. Viable cells were incubated with MTT (5 mg/mL) for 5 h, formazan precipitate was dissolved in 100 μL of DMSO and the absorbance at 490 nm was measured by Multimode Detector DTX880 (Beckman Coulter).

For colony formation assays, HCT116 cells at the exponential phase were plated into 24-well culture plate (200–300 cells/well) and allowed to adhere for 10 h before treatment. Culture medium containing RITA and compound **1f** ranging from 0.0625 to 5 μM was added to cells and incubated for 14 days. Cells were then rinsed with PBS, stained with 1.0 % crystal violet and photographed with a digital camera (Bio-Rad).

Cell morphological assay

HCT116 cells were seeded to each well of a 24-well plate and treated with 2 μM RITA and compound **1f** for 48 h in a 5 % CO_2 incubator at 37 ℃. Cells were then rinsed with PBS and fixed with MeOH-HAc (3:1 v/v) for 10 min at 4 ℃. Hoechst 333258 staining solution (5 μg/mL) was added to each well and incubated for 5 min at 37 ℃, and then detected under a fluorescence microscope (Olympus IX51, Olympus Corporation, Japan).

Flow cytometry assay

For the apoptosis assay, untreated and drug-treated cells were collected by centrifugation and washed twice with ice-cold PBS. Apoptotic cells were measured by the Annexin VFITC/PI apoptosis detection kit (Beyotime Company) according to the protocol described using flow cytometry (Moflo XDP,

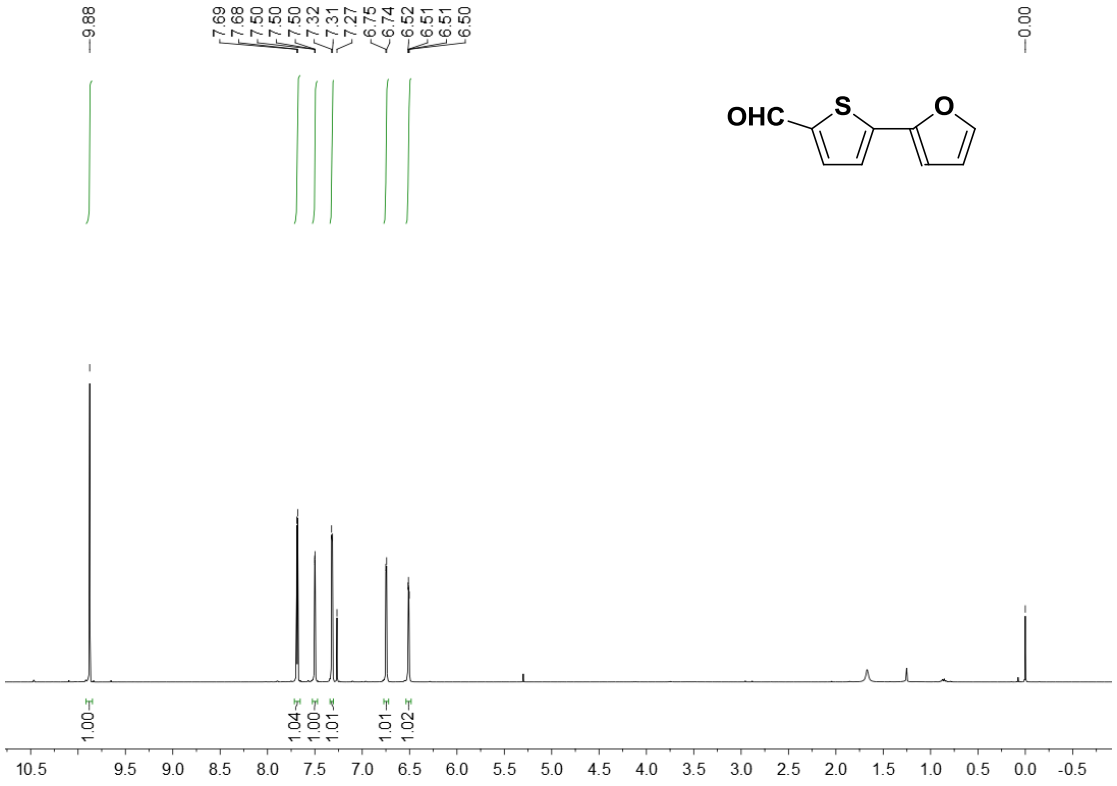
This journal is © The Royal Society of Chemistry 2012

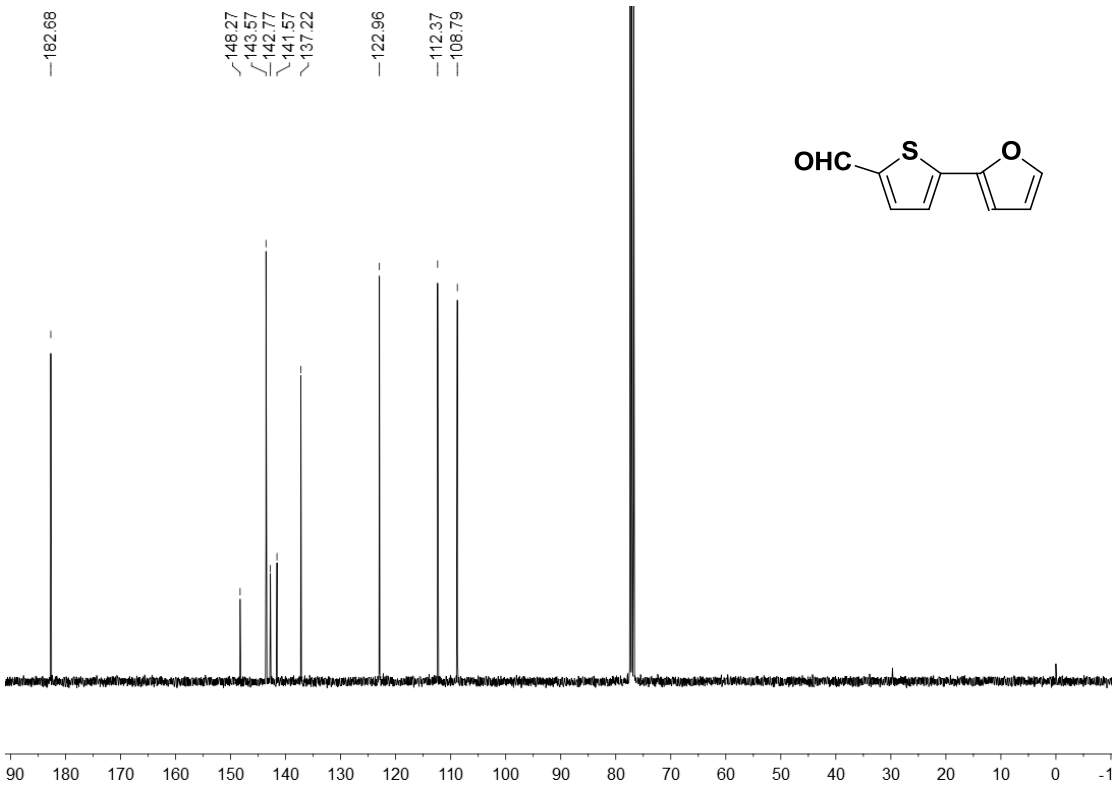
Beckman Coulter).[1]

For the cell cycle profile assay, RITA and compound **1f**-treated and control cells were collected and washed twice with ice-cold PBS. The cells were fixed with 70 % ethanol at 4 ℃ overnight, and resuspended in propidium iodide (50 μg/mL PI in 0.1 % sodium citrate plus 0.03 % v/v NP-40) and incubated for 30 min in the dark. The tubes were packed with tin foil on ice. The cell cycle profile was analyzed with flow cytometry (Moflo XDP, Beckman Coulter).

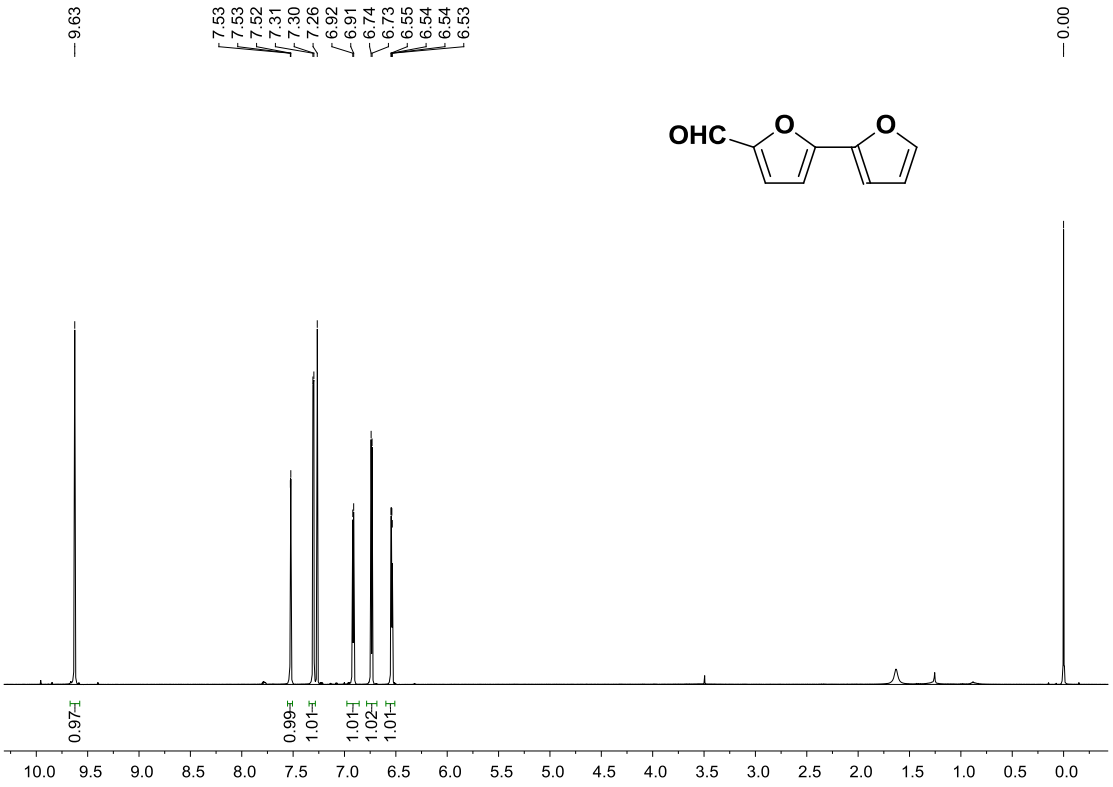
1. C. Zhang, C. Tan, X. Zu, X. Zhai, F. Liu, B. Chu, X. Ma, Y. Chen, P. Gong and Y. Jiang, *Eur. J. Med. Chem.*, 2011, **46**, 1404.

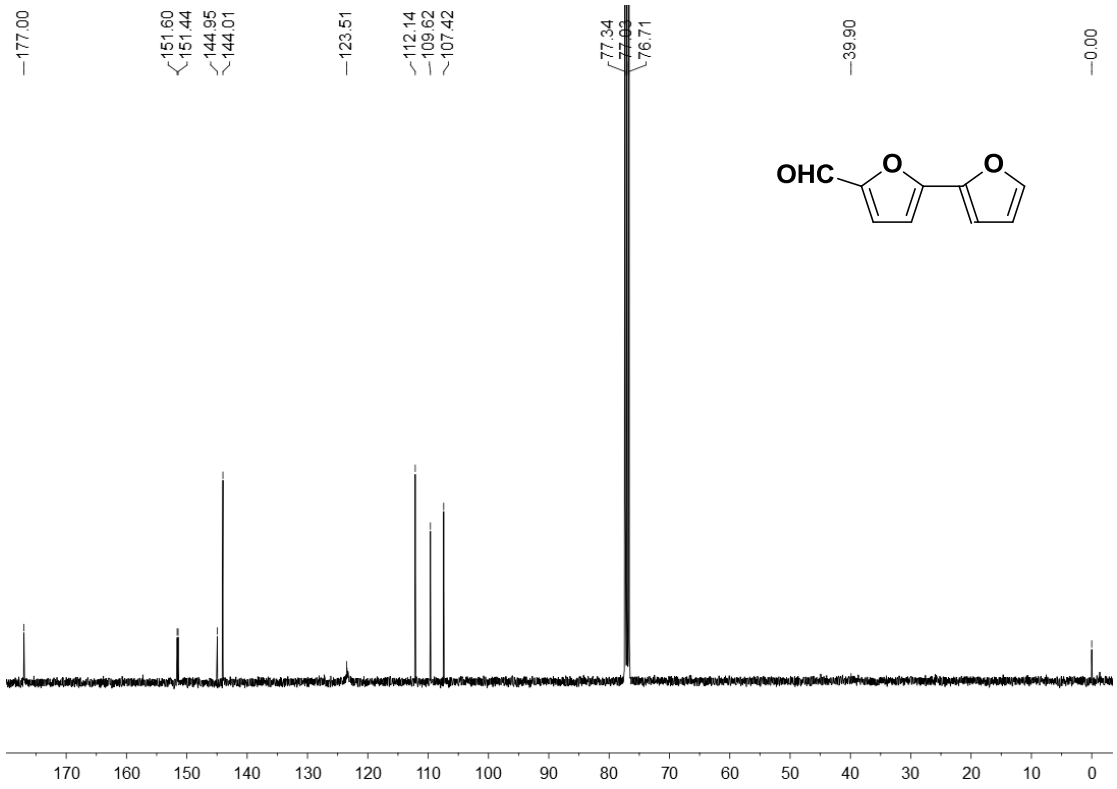
VI. Spectra

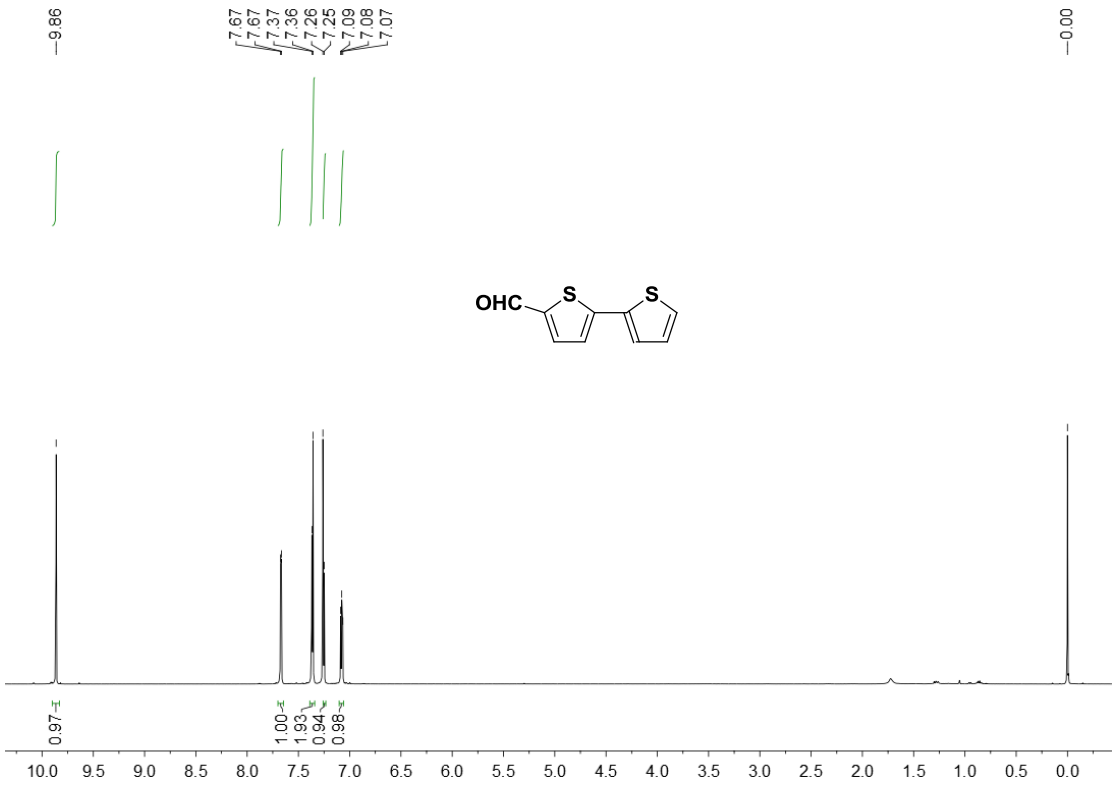

9.88
7.69
7.68
7.50
7.50
7.50
7.32
7.31
7.27
6.75
6.74
6.52
6.51
6.51
6.50
0.00
OHC
S
O
1.00
1.04
1.00
1.01
1.01
1.02
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5

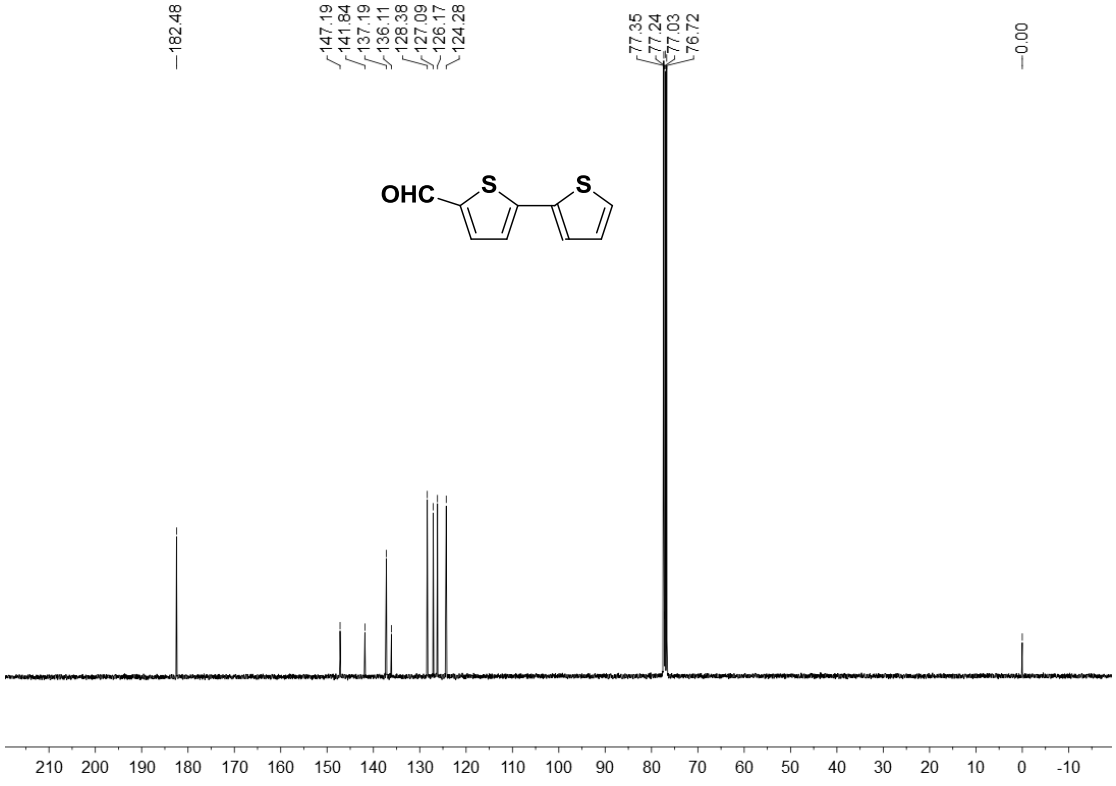

182.68
148.27
143.57
142.77
141.57
137.22
122.96
112.37
108.79
OHC
S
O
90
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-1

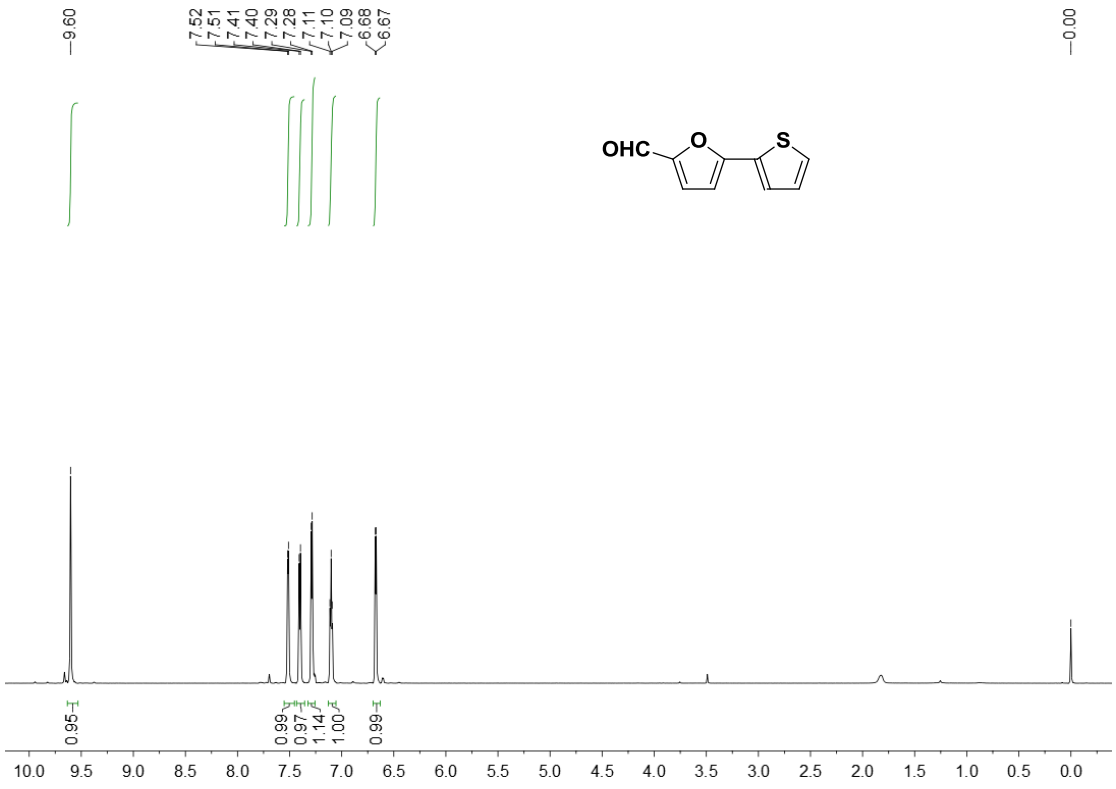
This journal is © The Royal Society of Chemistry 2012

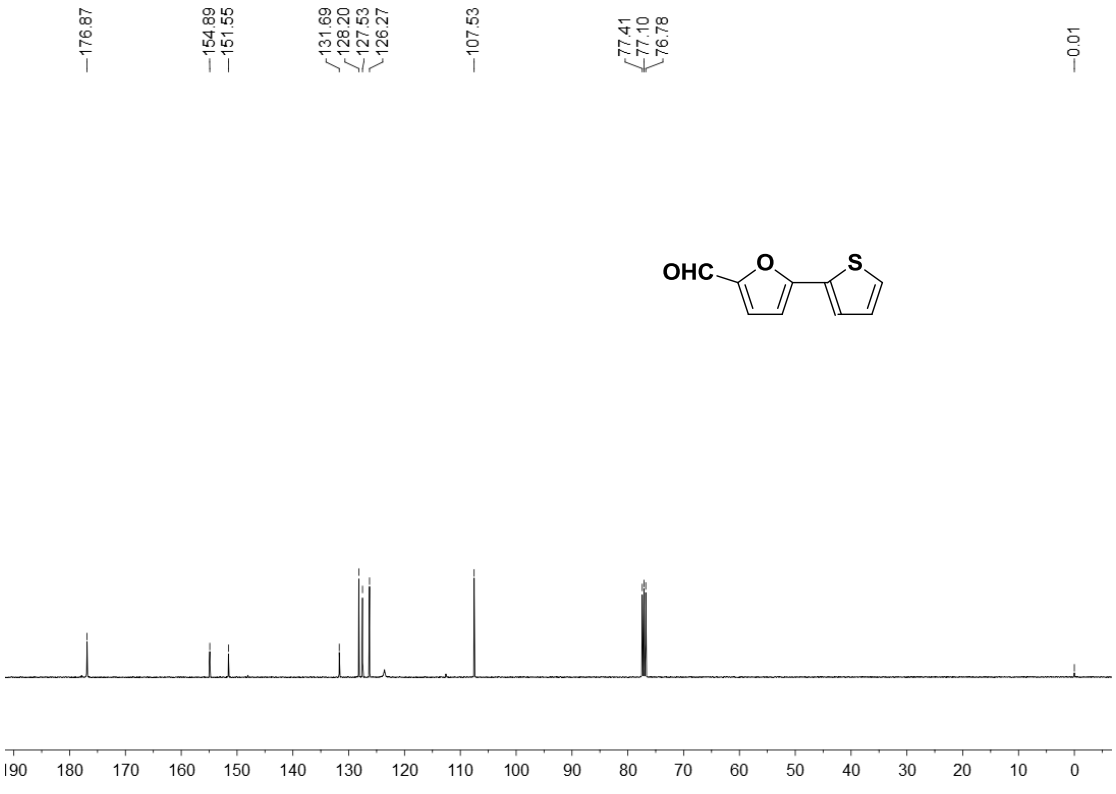


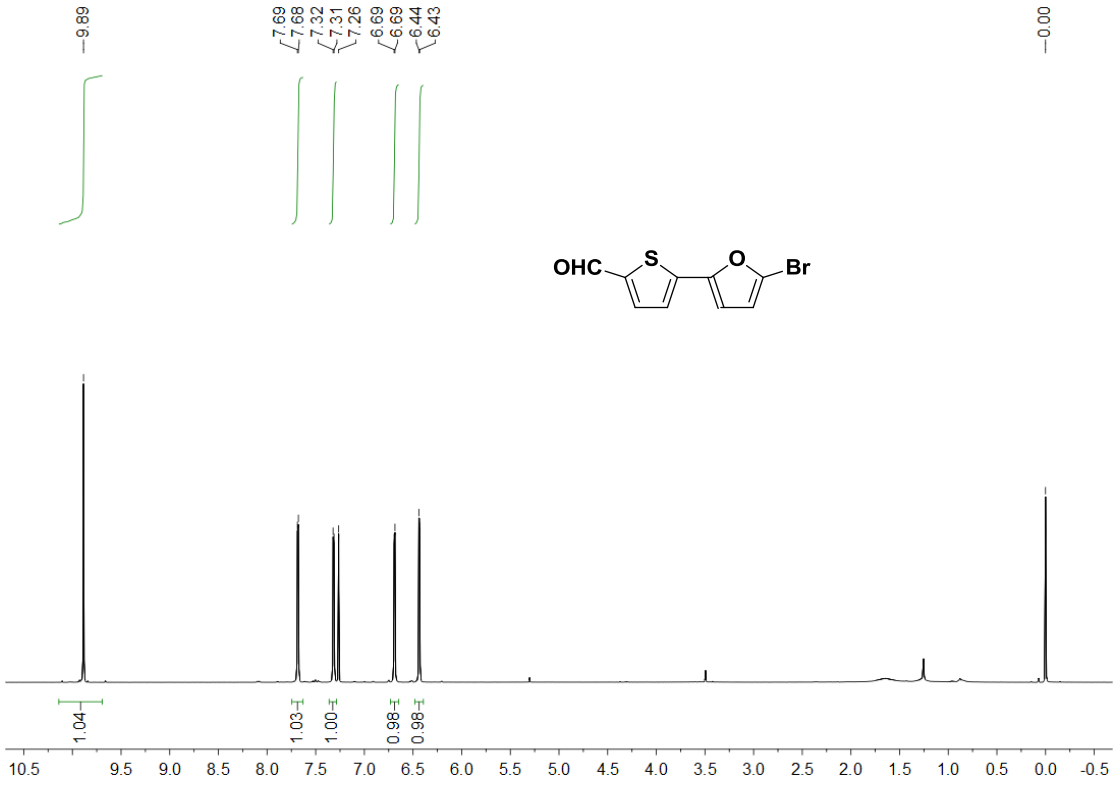


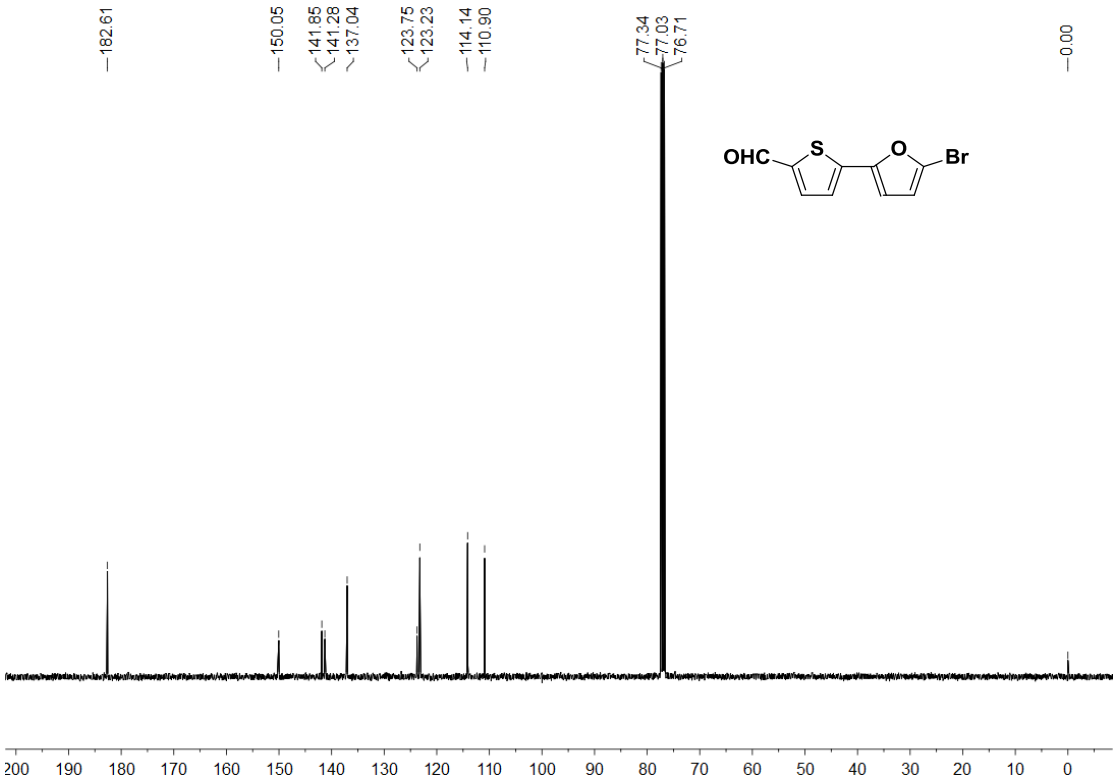

9.86
7.67
7.67
7.37
7.36
7.26
7.25
7.09
7.08
7.07
0.00
OHC
S
S
0.97
1.00
1.93
0.94
0.98
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

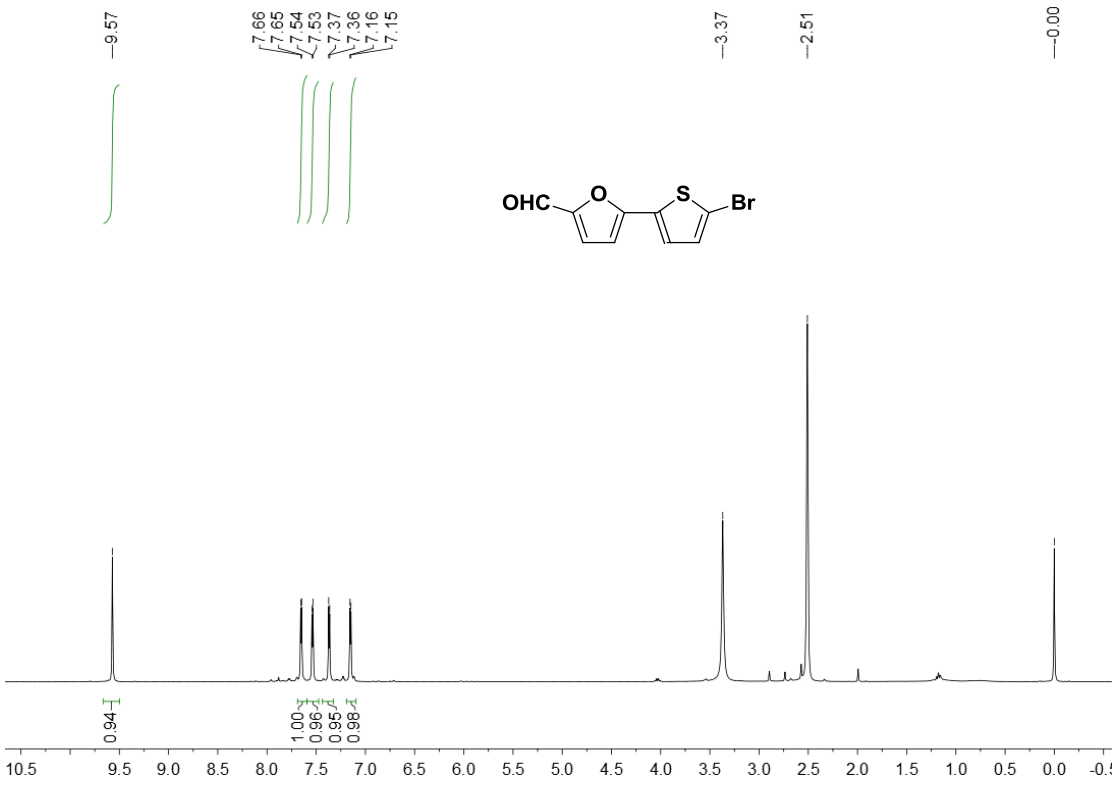

182.48
147.19
141.84
137.19
136.11
128.38
127.09
126.17
124.28
77.35
77.24
77.03
76.72
0.00
OHC
S
S
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10

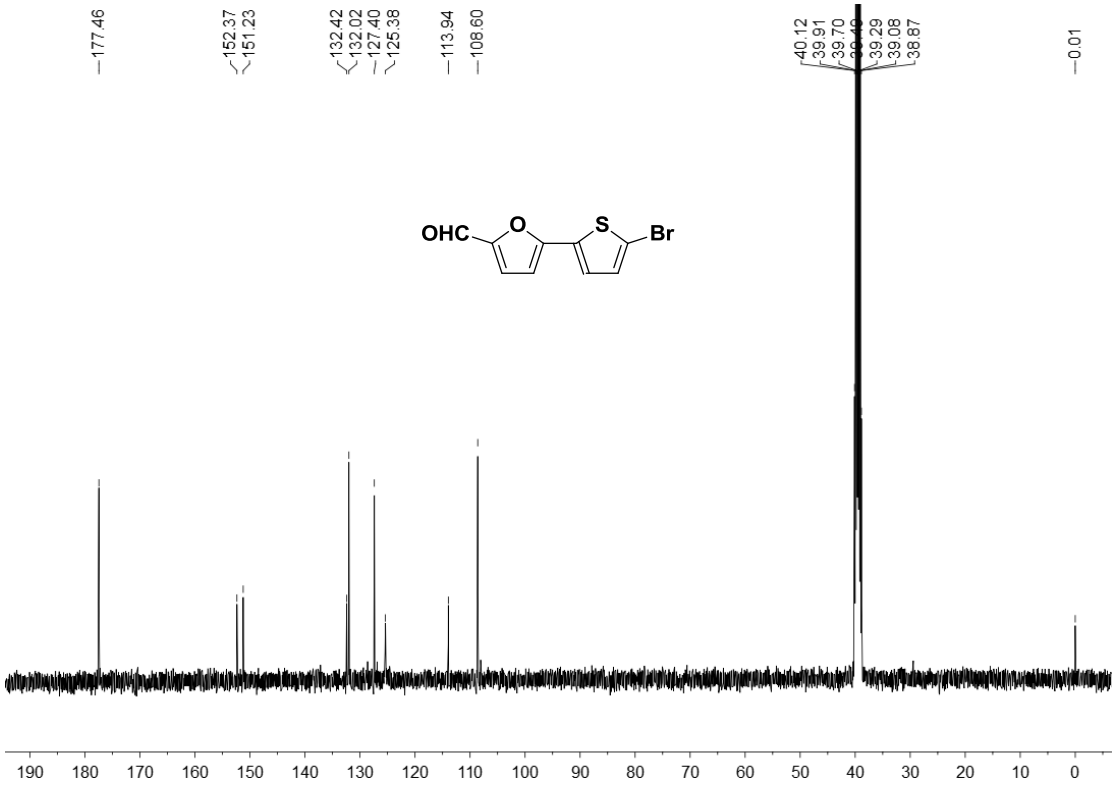

9.60
7.52
7.51
7.41
7.40
7.29
7.28
7.11
7.10
7.09
6.68
6.67
0.00
OHC
O
S
0.95
0.99
0.97
1.14
1.00
0.99
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

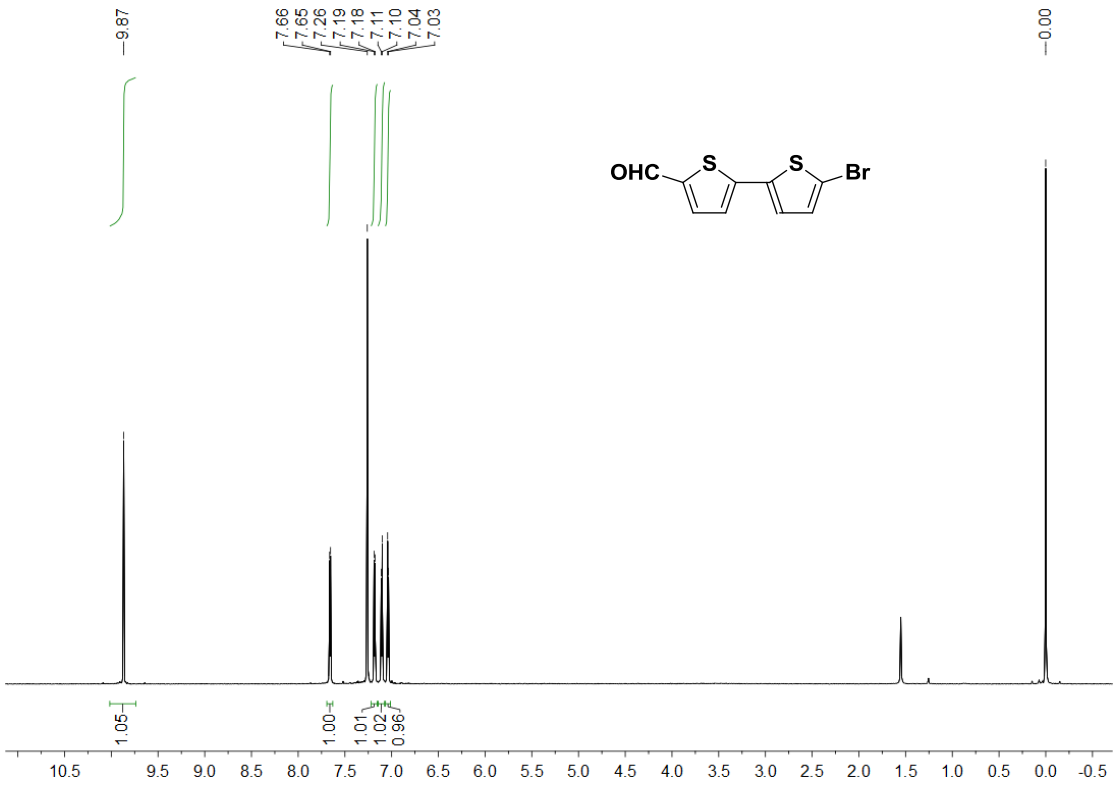

176.87
154.89
151.55
131.69
128.20
127.53
126.27
107.53
77.41
77.10
76.78
0.01
OHC
O
S
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

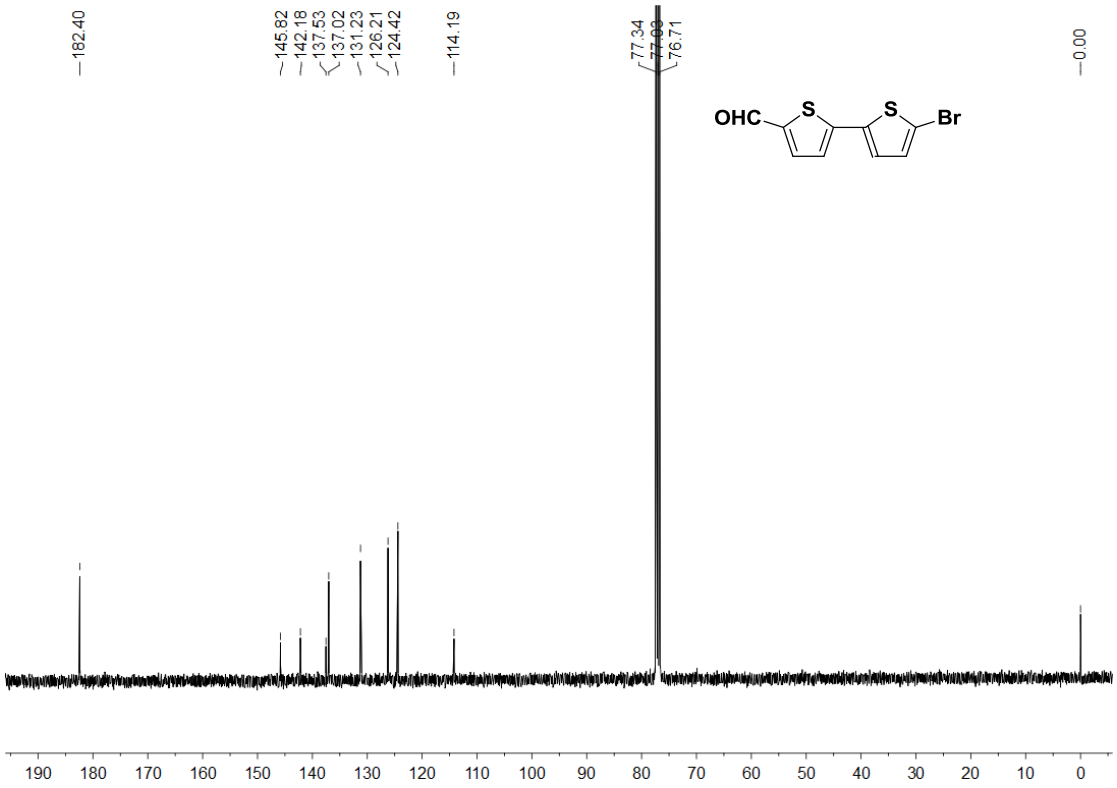

9.89
7.69
7.68
7.32
7.31
7.26
6.69
6.69
6.44
6.43
0.00
OHC
S
O
Br
1.04
1.03
1.00
0.98
0.98
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5


182.61
150.05
141.85
141.28
137.04
123.75
123.23
114.14
110.90
77.34
77.03
76.71
0.00
OHC
S
O
Br
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

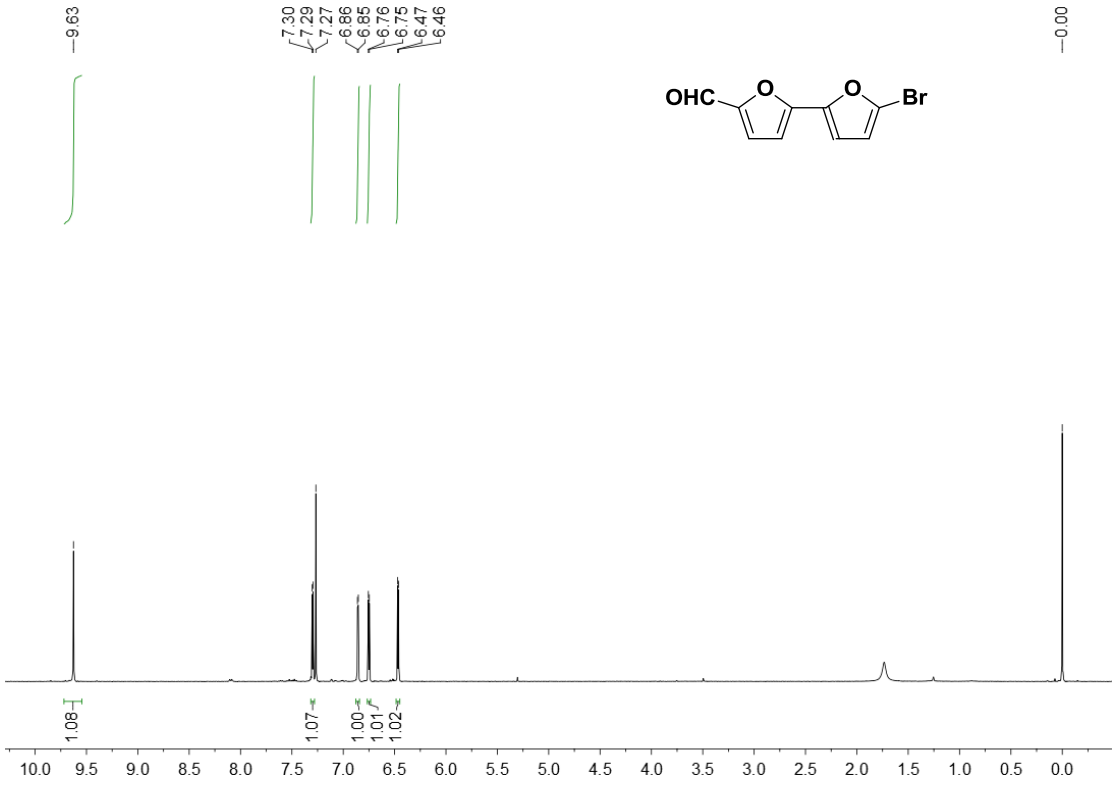

9.57
7.66
7.65
7.54
7.53
7.37
7.36
7.16
7.15
3.37
2.51
0.00
OHC
O
S
Br
0.94
1.00
0.96
0.95
0.98
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

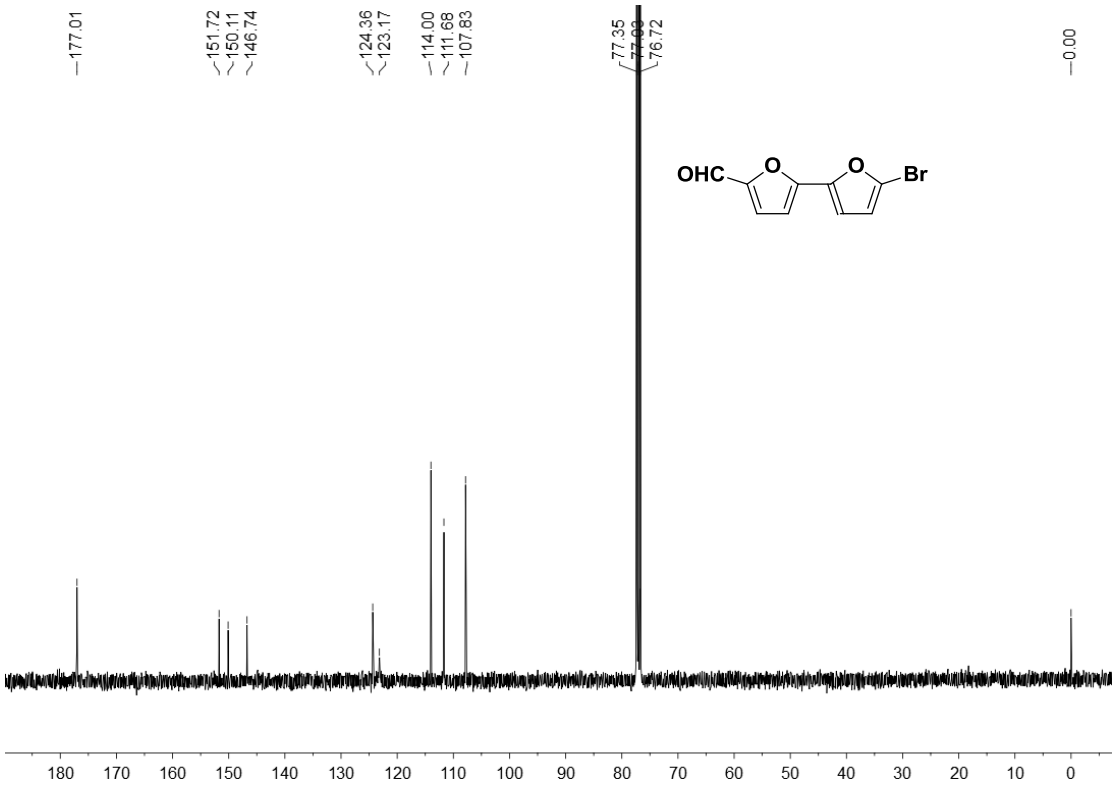

177.46
152.37
151.23
132.42
132.02
127.40
125.38
113.94
108.60
40.12
39.91
39.70
39.29
39.08
38.87
0.01
OHC
O
S
Br
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

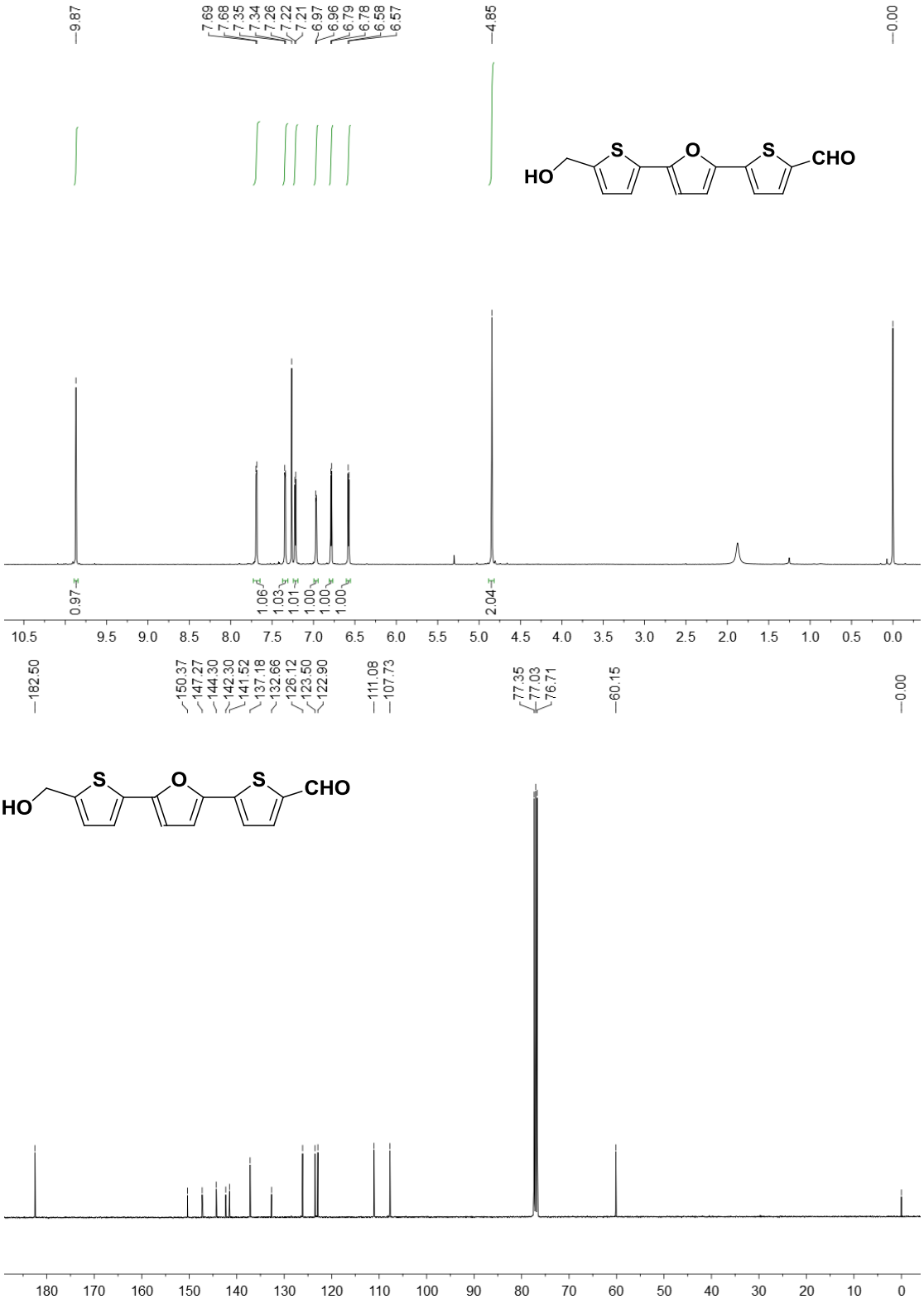

9.87
7.66
7.65
7.26
7.19
7.18
7.11
7.10
7.04
7.03
0.00
OHC
S
S
Br
1.05
1.00
1.01
1.02
0.96
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5


182.40
145.82
142.18
137.53
137.02
131.23
126.21
124.42
114.19
77.34
77.00
76.71
0.00
OHC
S
S
Br
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

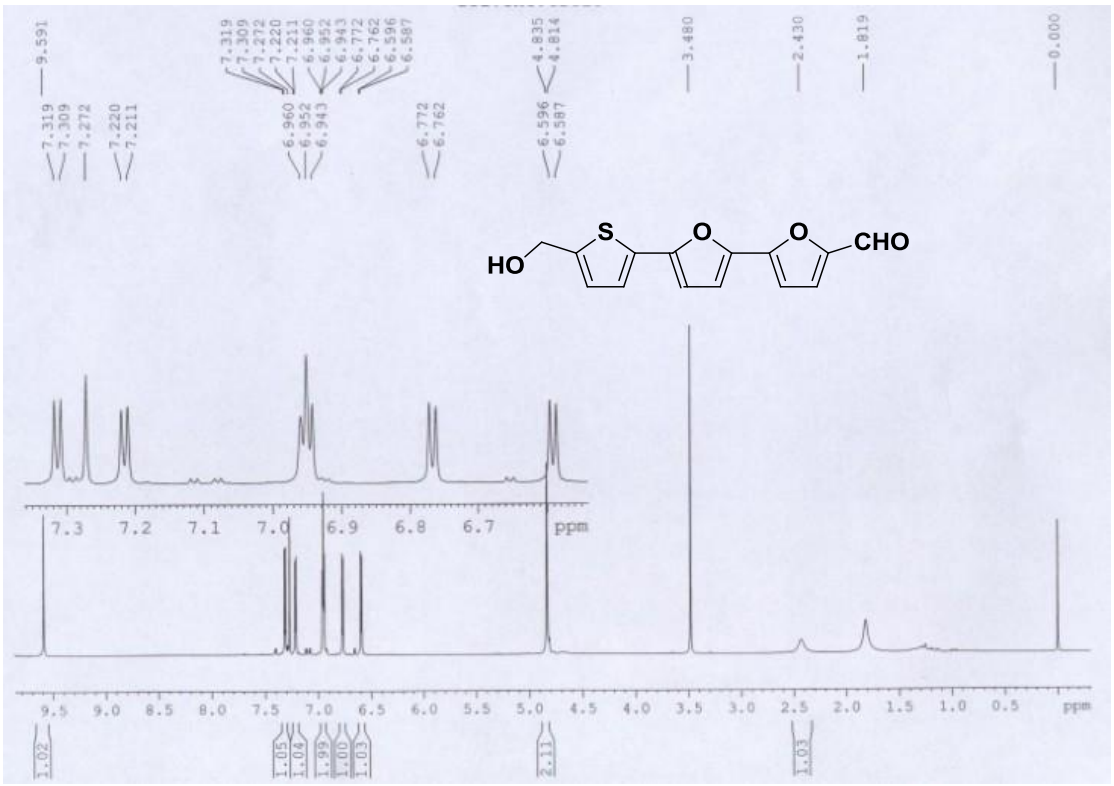
This journal is © The Royal Society of Chemistry 2012

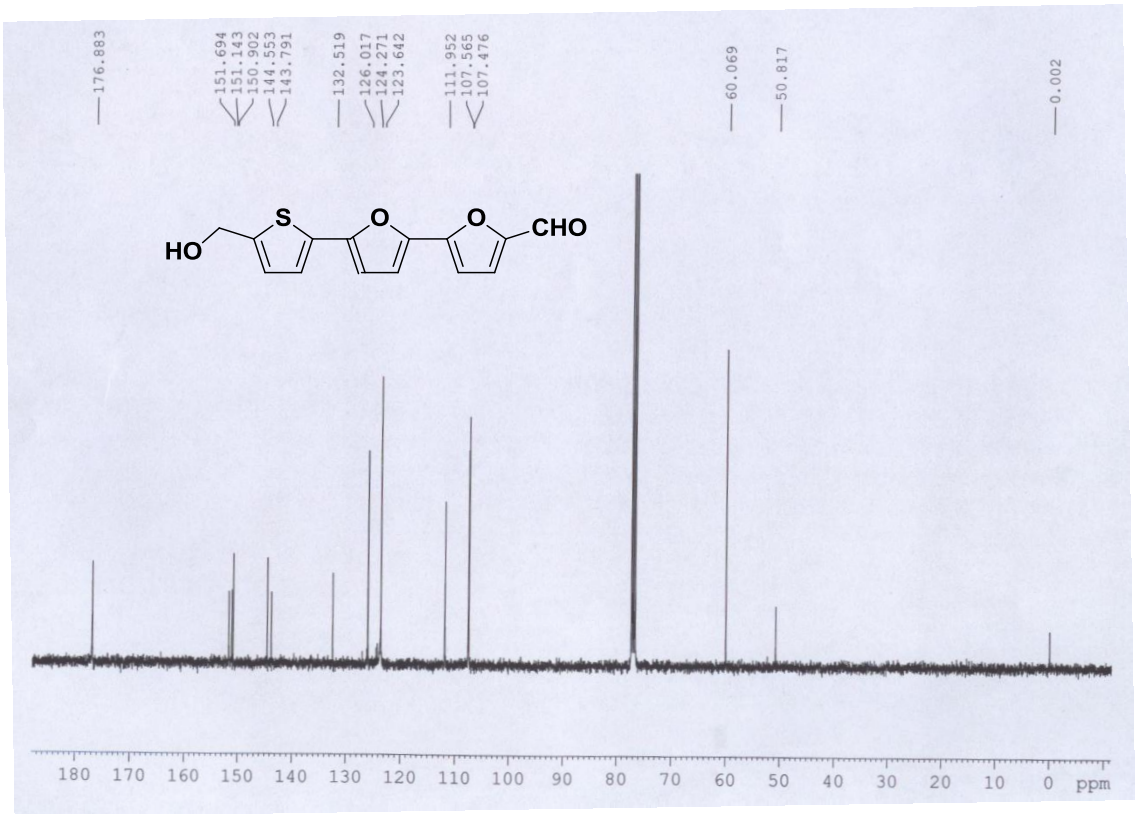




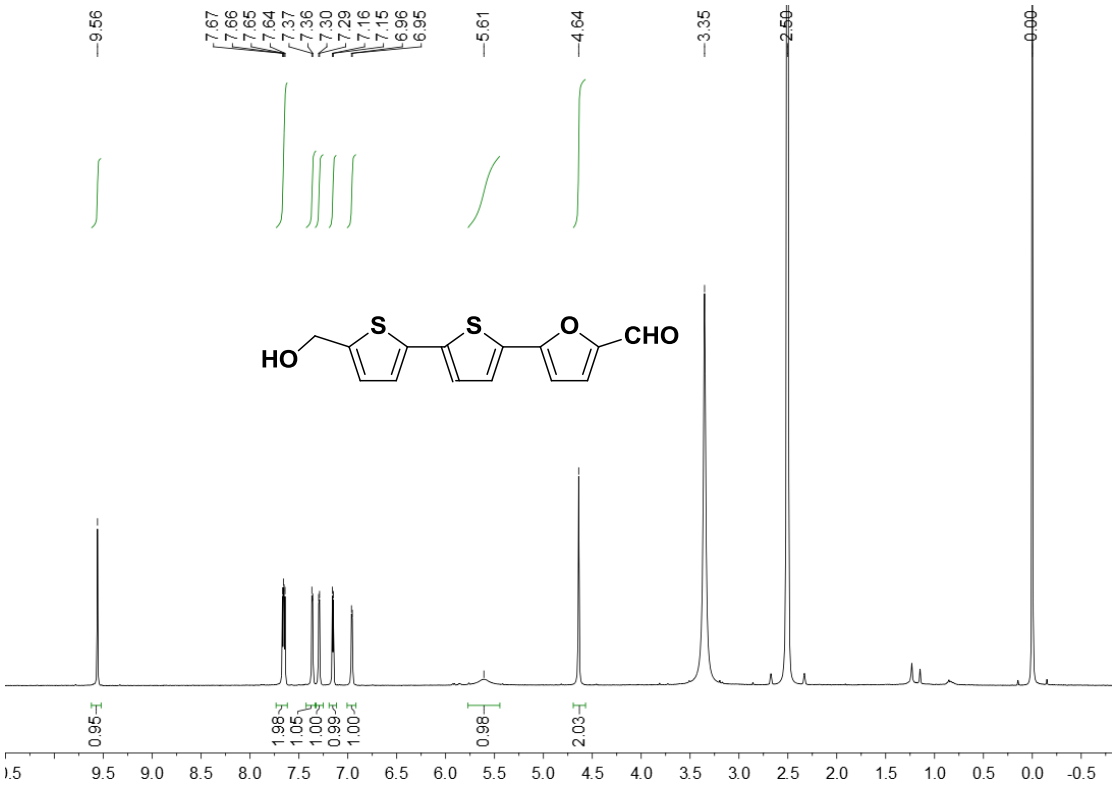

9.87
7.69
7.68
7.35
7.34
7.26
7.22
7.21
6.97
6.96
6.79
6.78
6.58
6.57
4.85
0.00
HO
S
O
S
CHO
0.97
1.06
1.03
1.01
1.00
1.00
1.00
2.04
182.50
150.37
147.27
144.30
142.30
141.52
137.18
132.66
126.12
123.50
122.90
111.08
107.73
77.35
77.03
76.71
60.15
0.00

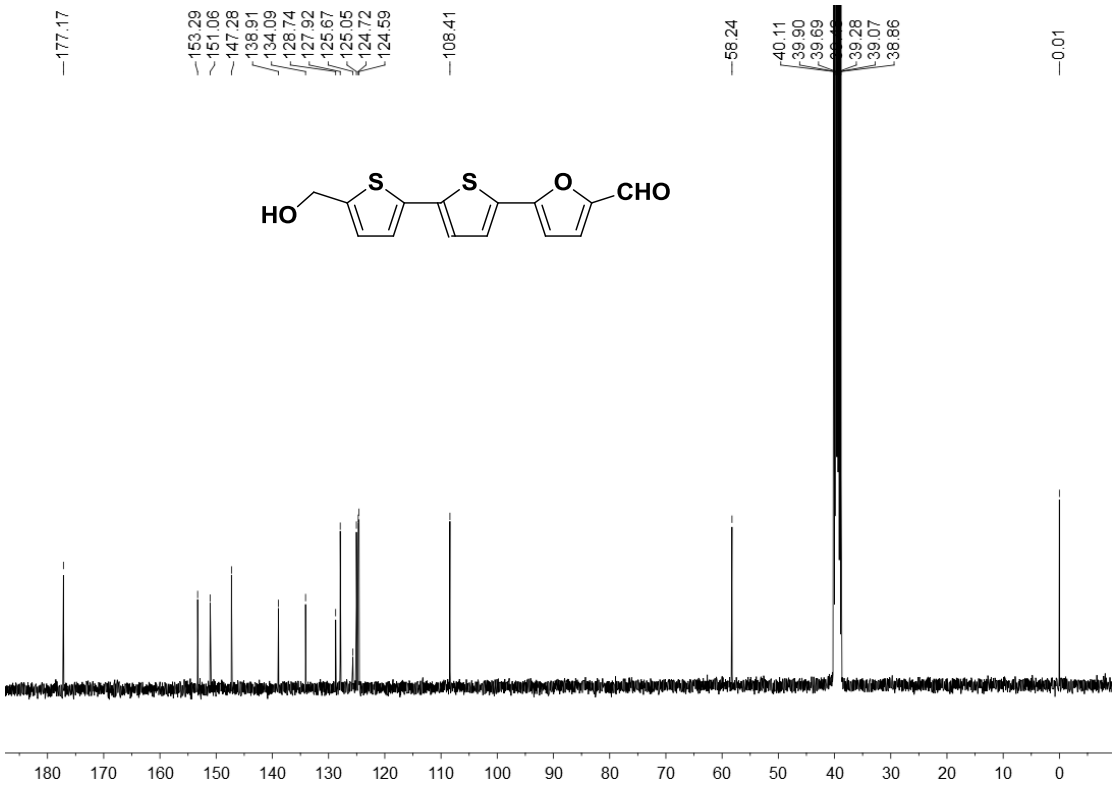
This journal is © The Royal Society of Chemistry 2012



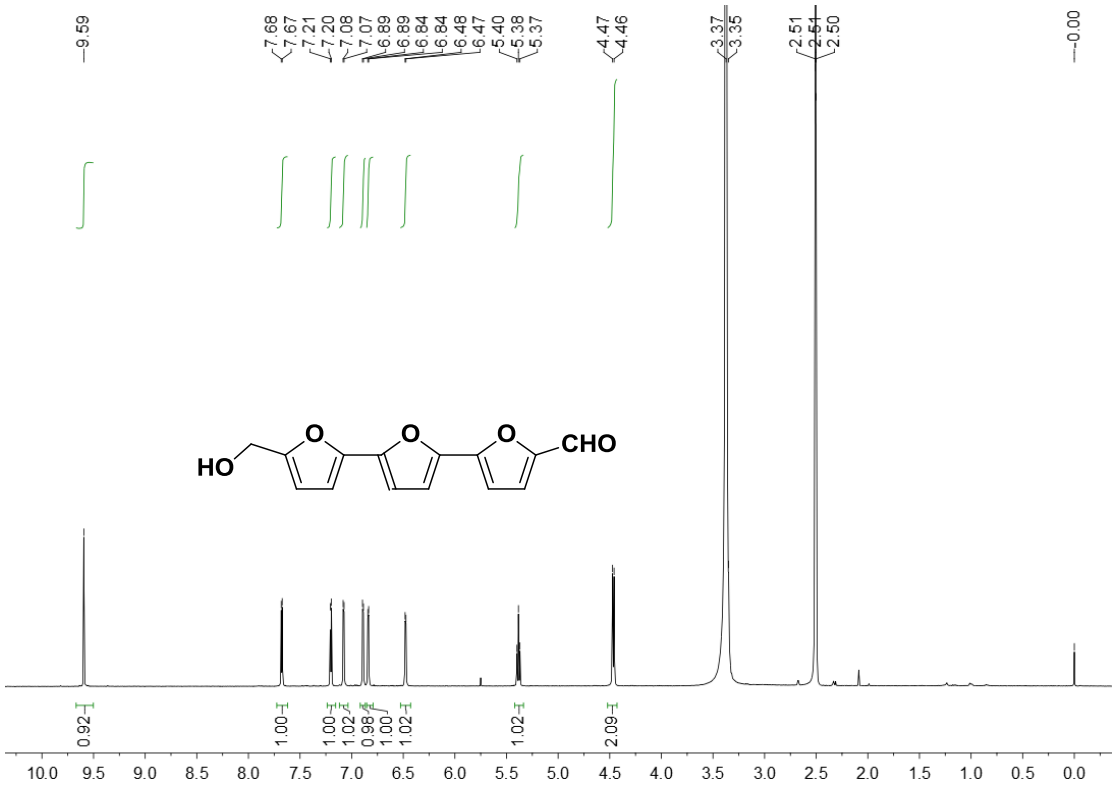


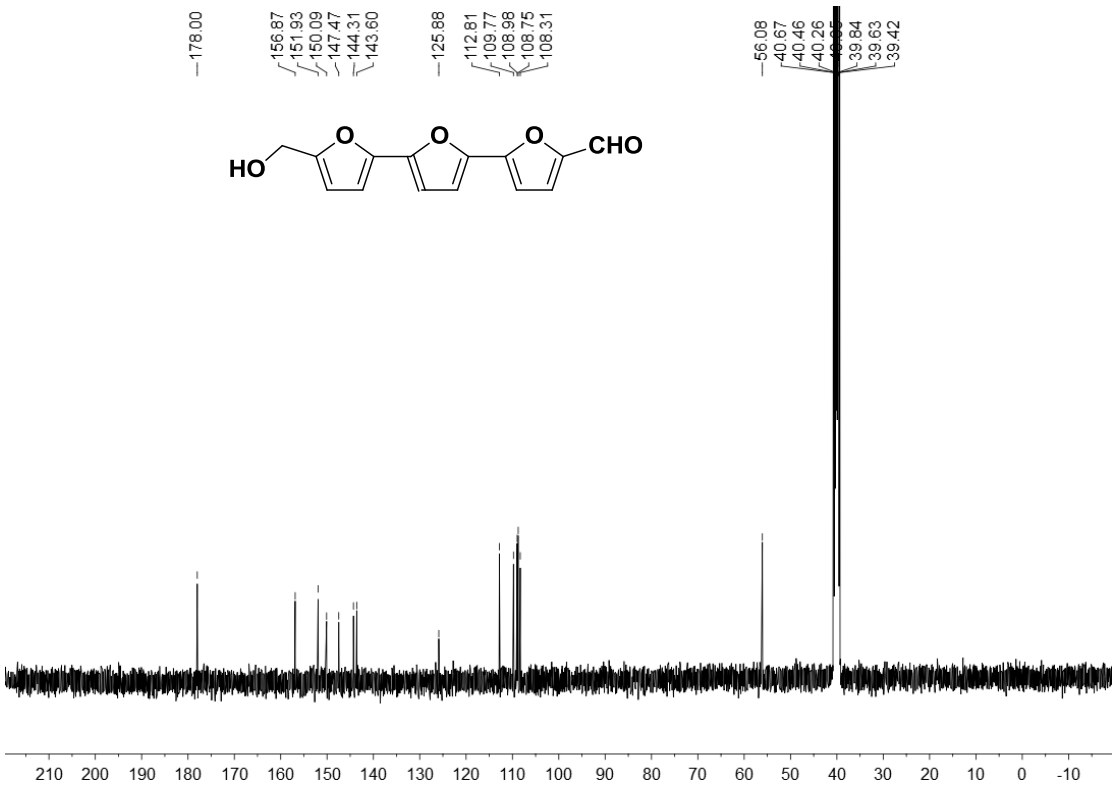
This journal is © The Royal Society of Chemistry 2012

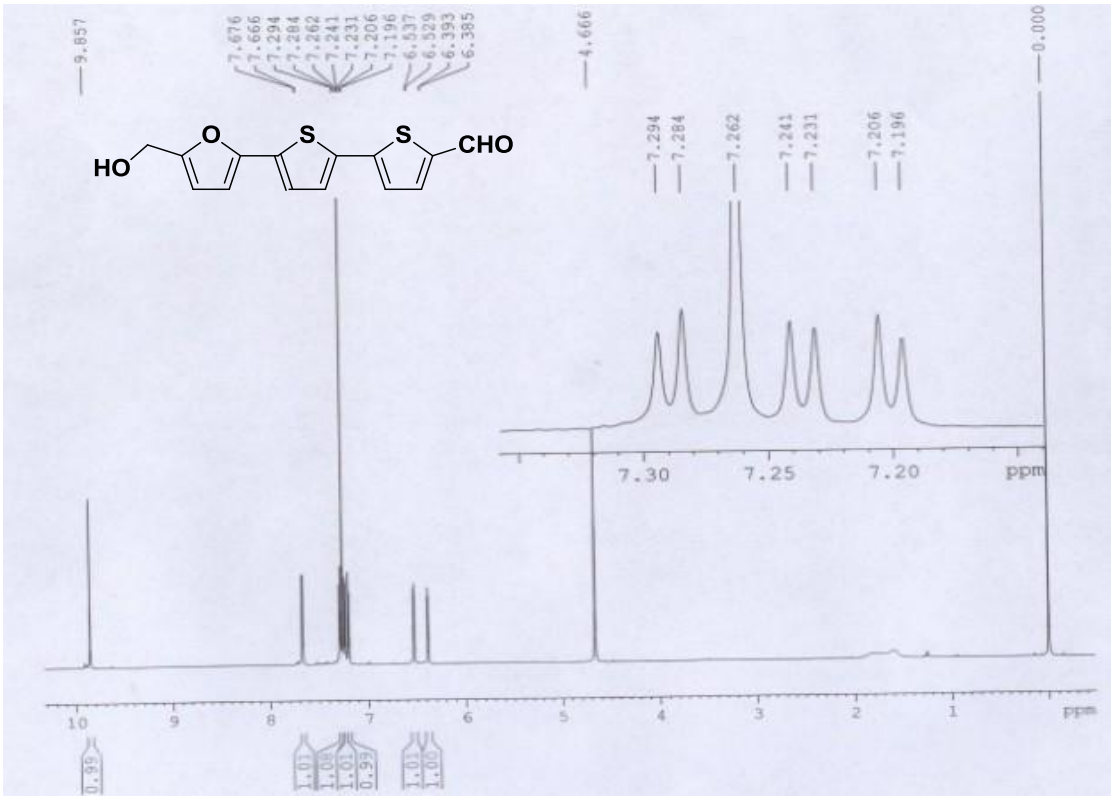


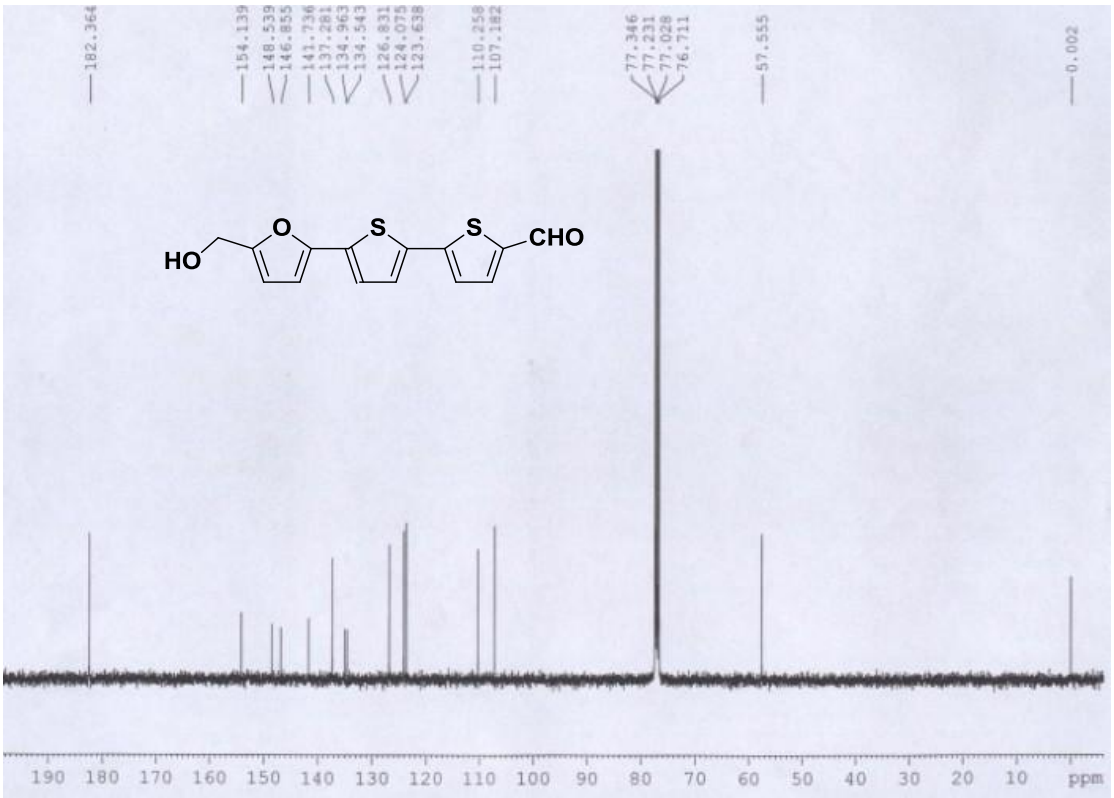


This journal is © The Royal Society of Chemistry 2012

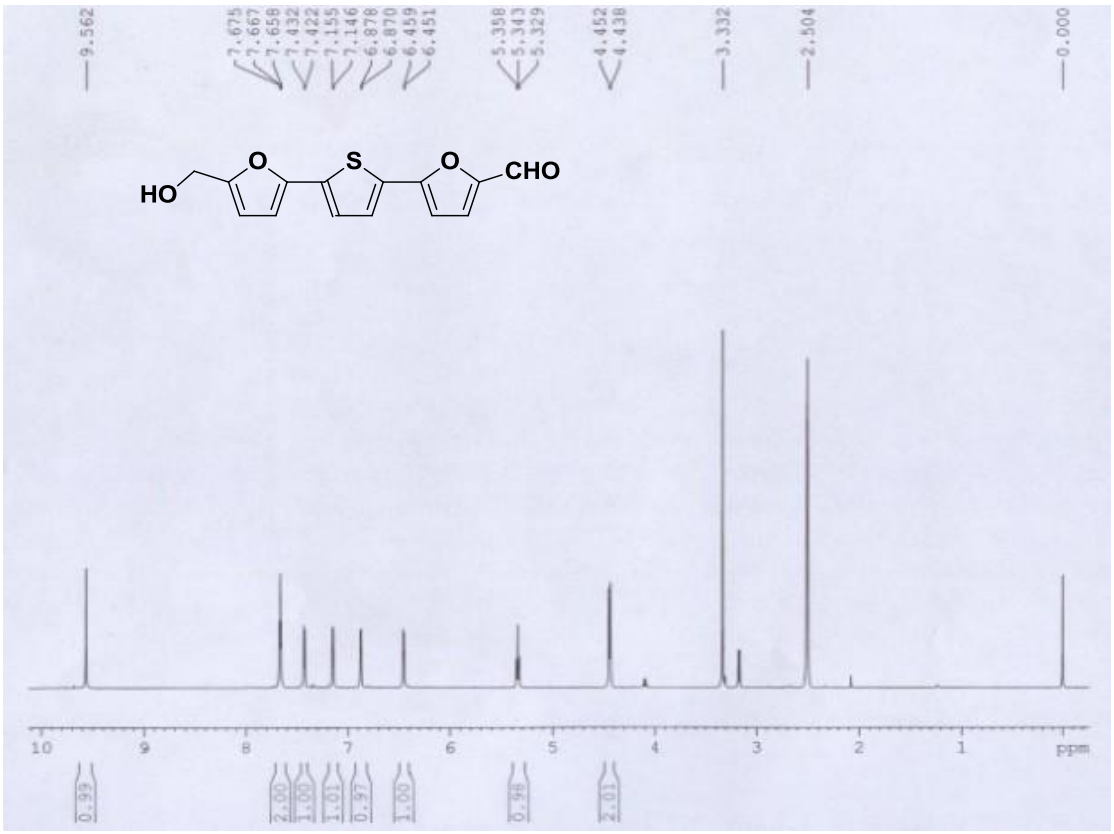


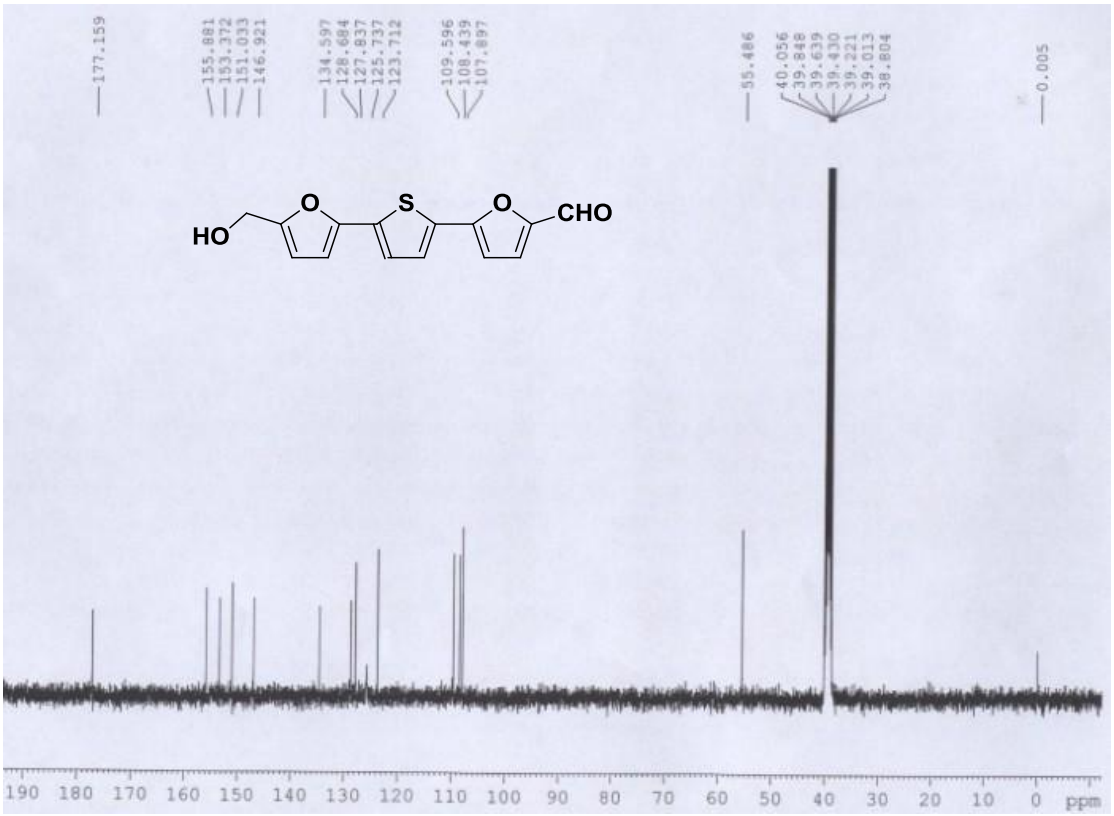


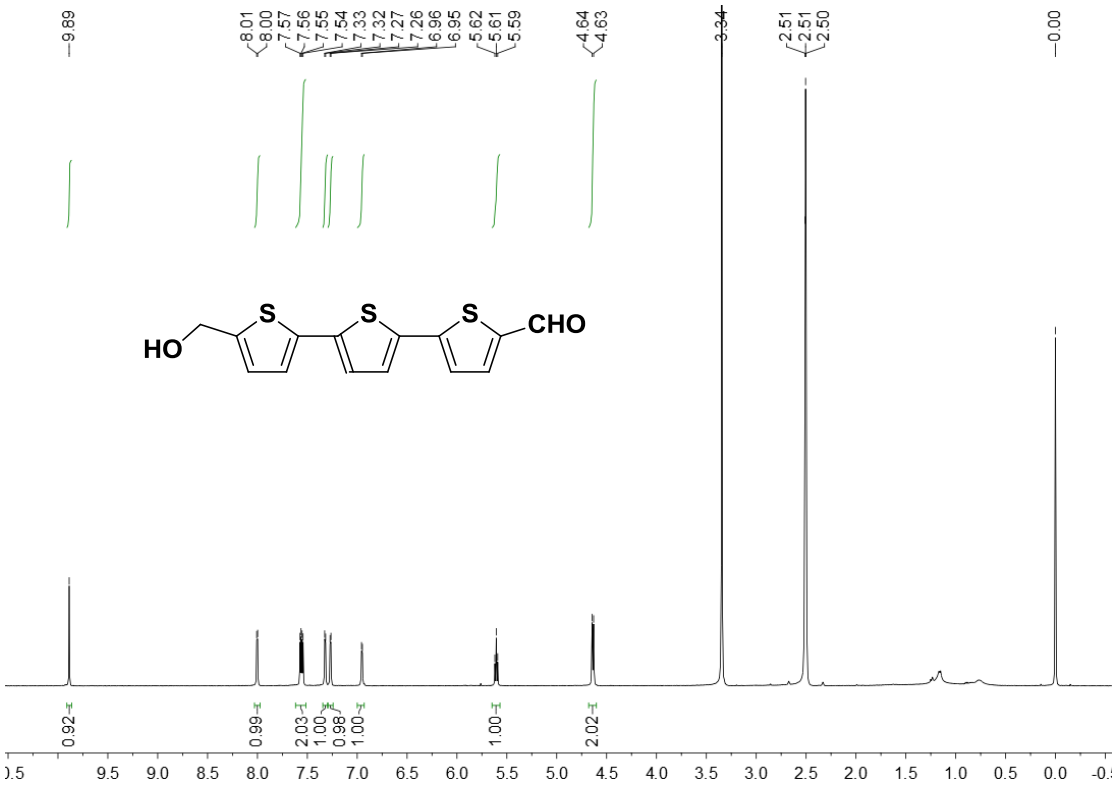

9.857
7.676
7.666
7.294
7.284
7.262
7.241
7.231
7.206
7.196
6.537
6.529
6.393
6.385
4.666
0.000
HO
O
S
S
CHO
7.294
7.284
7.262
7.241
7.231
7.206
7.196
7.30
7.25
7.20
ppm
10
9
8
7
6
5
4
3
2
1
ppm

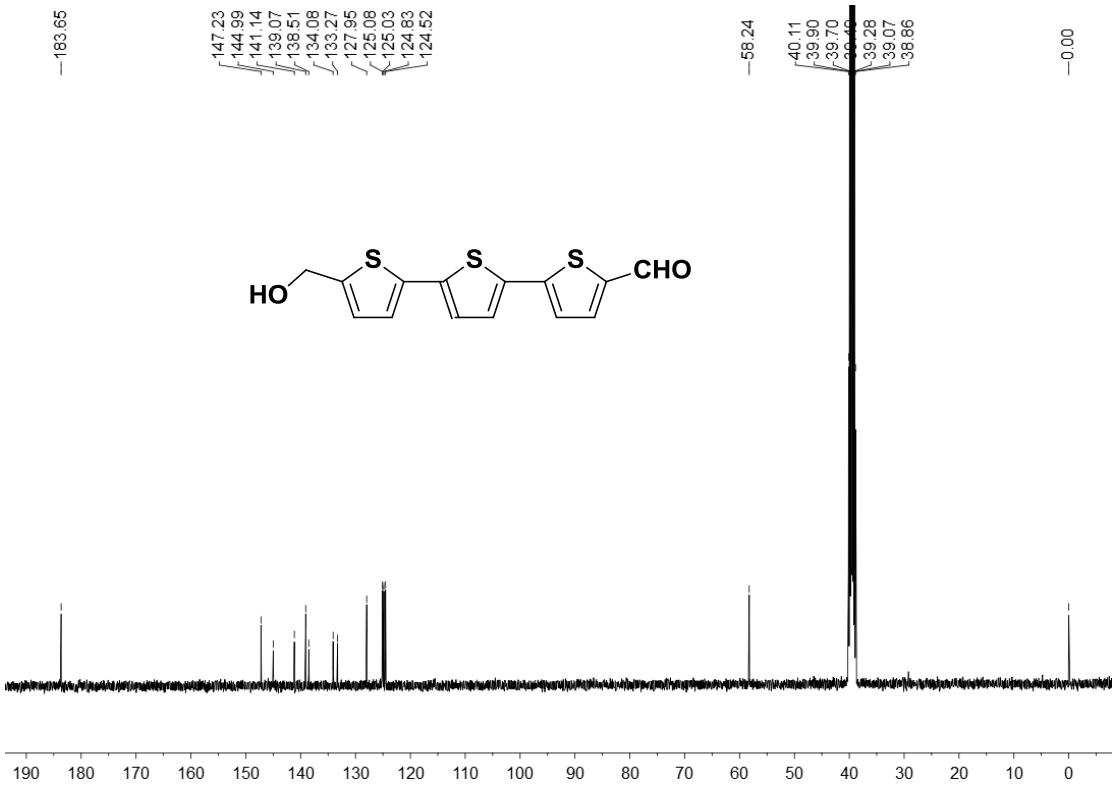

182.364
154.139
148.539
146.855
141.736
137.281
134.963
134.543
126.831
124.075
123.638
110.258
107.182
77.346
77.231
77.028
76.711
57.555
0.002
HO
O
S
S
CHO
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

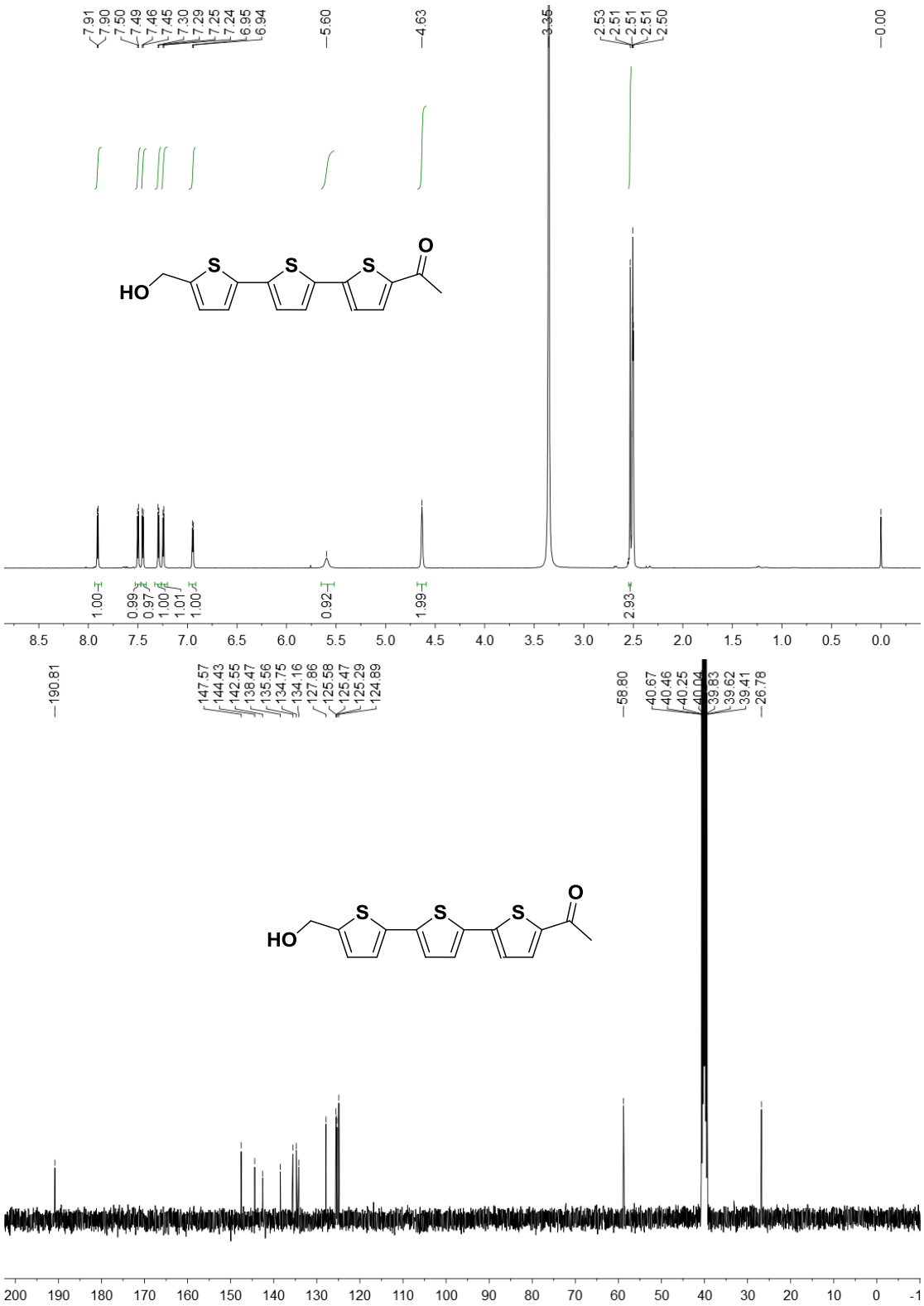
This journal is © The Royal Society of Chemistry 2012

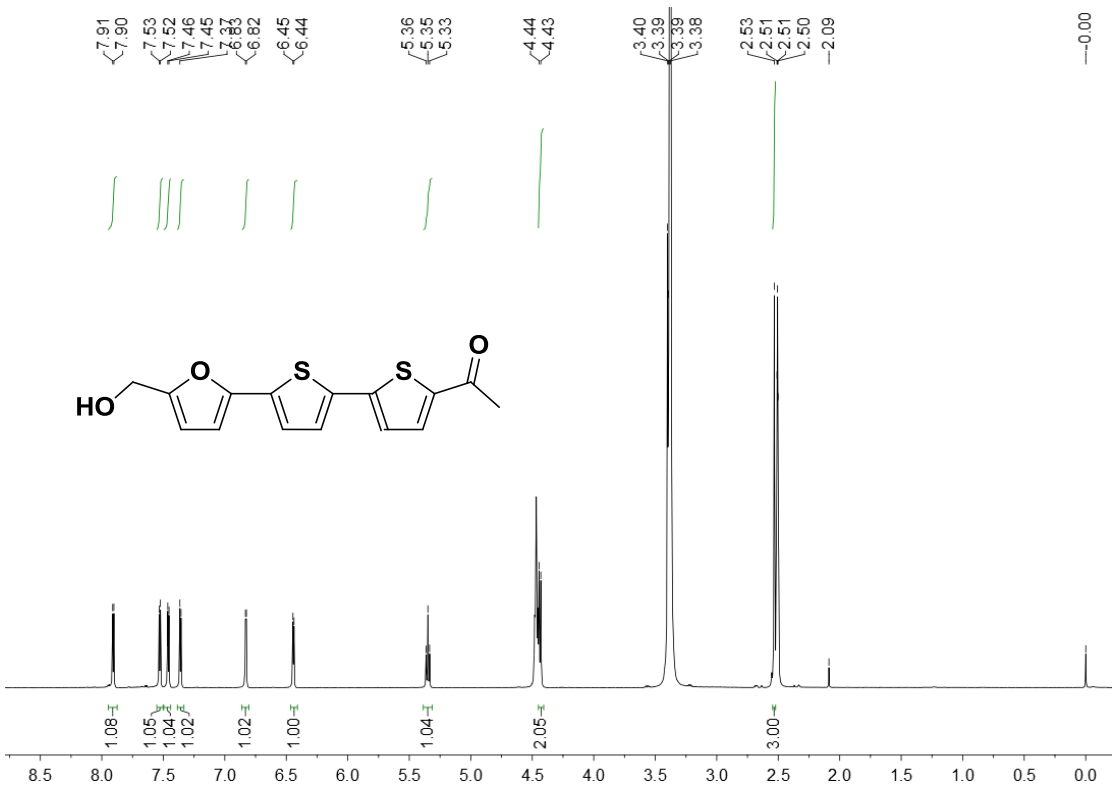


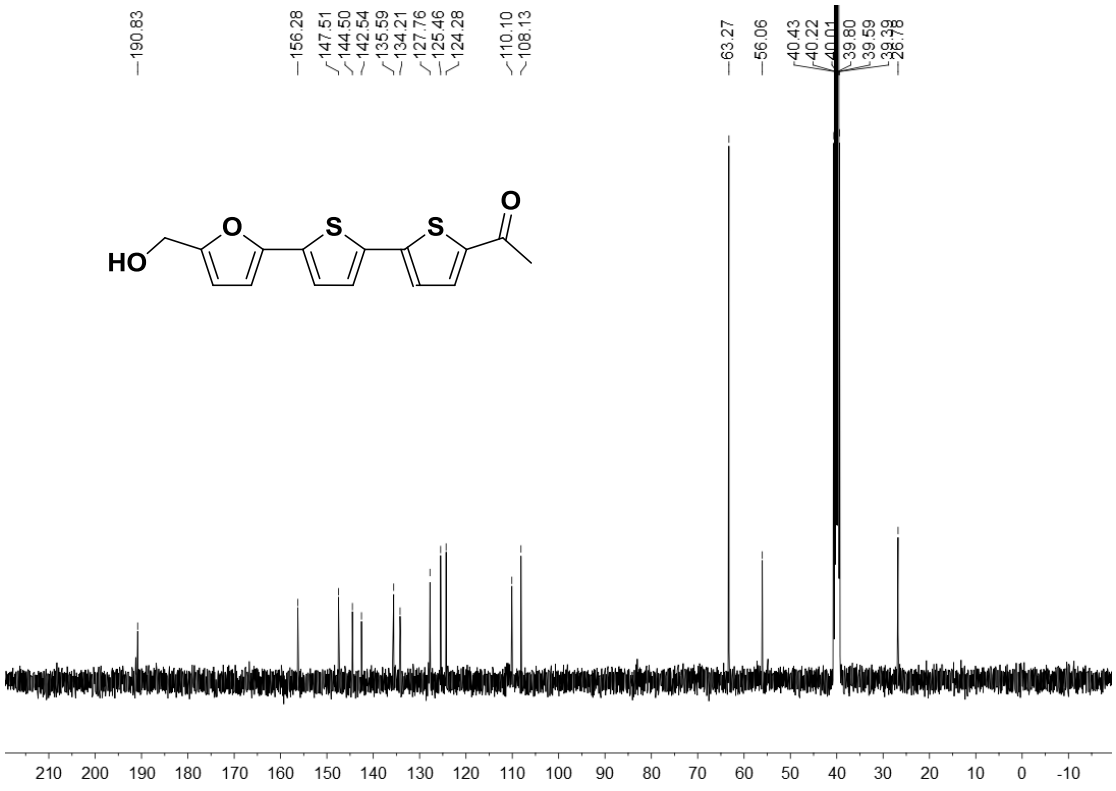


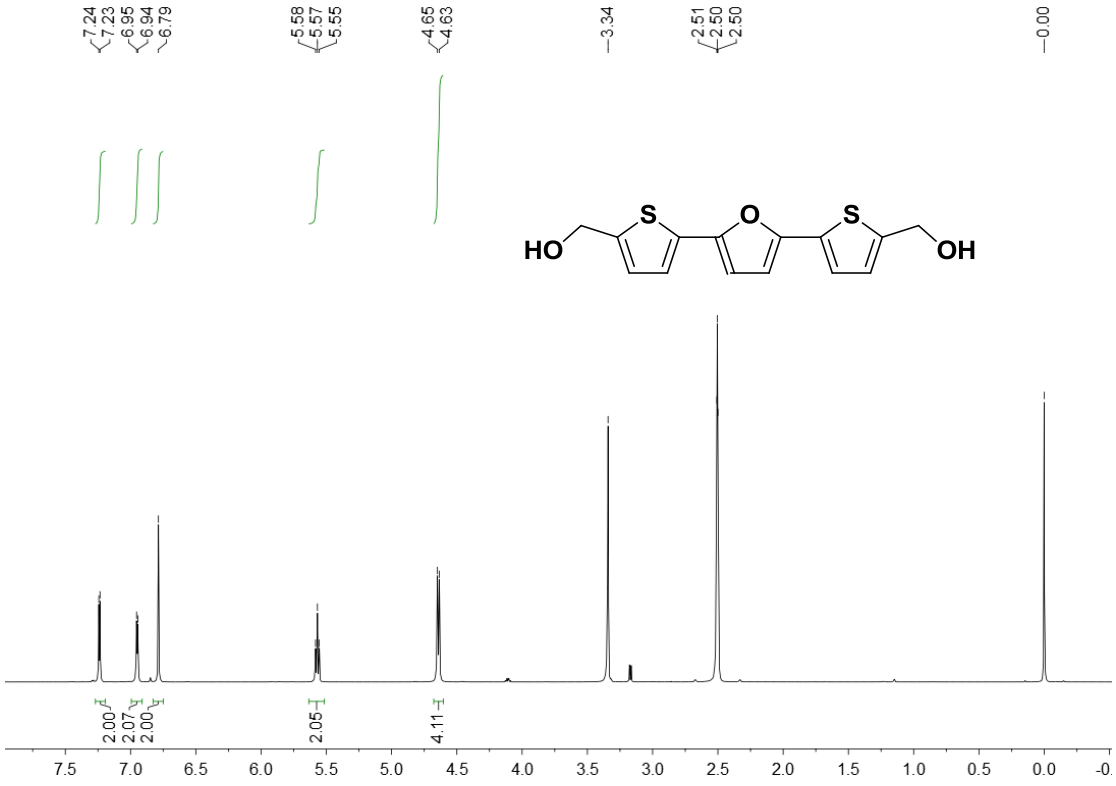

9.89
8.01
8.00
7.57
7.56
7.55
7.54
7.33
7.32
7.27
7.26
6.96
6.95
5.62
5.61
5.59
4.64
4.63
3.34
2.51
2.51
2.50
0.00
HO
S
S
S
CHO
0.92
0.99
2.03
1.00
0.98
1.00
1.00
2.02

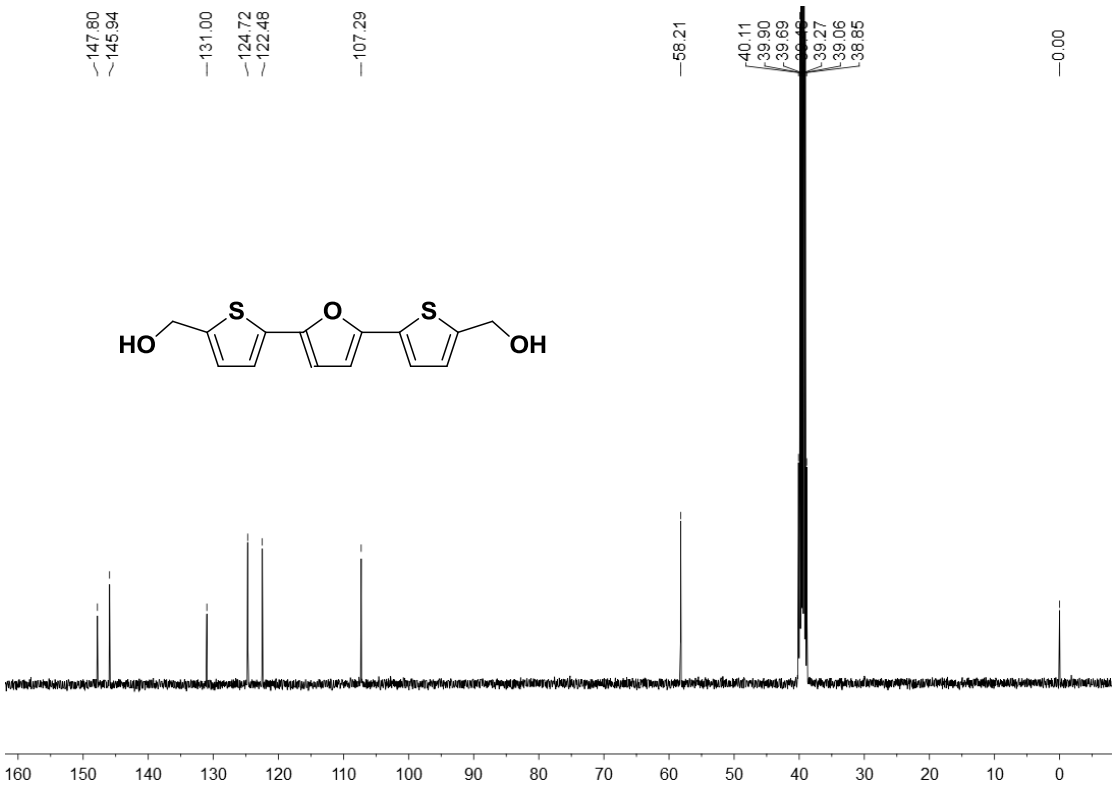

183.65
147.23
144.99
141.14
139.07
138.51
134.08
133.27
127.95
125.08
125.03
124.83
124.52
58.24
40.11
39.90
39.70
39.28
39.07
38.86
0.00
HO
S
S
S
CHO


7.91
7.90
7.50
7.49
7.46
7.45
7.30
7.29
7.25
7.24
6.95
6.94
5.60
4.63
3.35
2.53
2.51
2.51
2.51
2.50
0.00
HO
S
S
S
O
1.00
0.99
0.97
1.00
1.01
1.00
0.92
1.99
2.93
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
190.81
147.57
144.43
142.55
138.47
135.56
134.75
134.16
127.86
125.58
125.47
125.29
124.89
58.80
40.67
40.46
40.25
40.04
39.83
39.62
39.41
26.78
HO
S
S
S
O
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-1

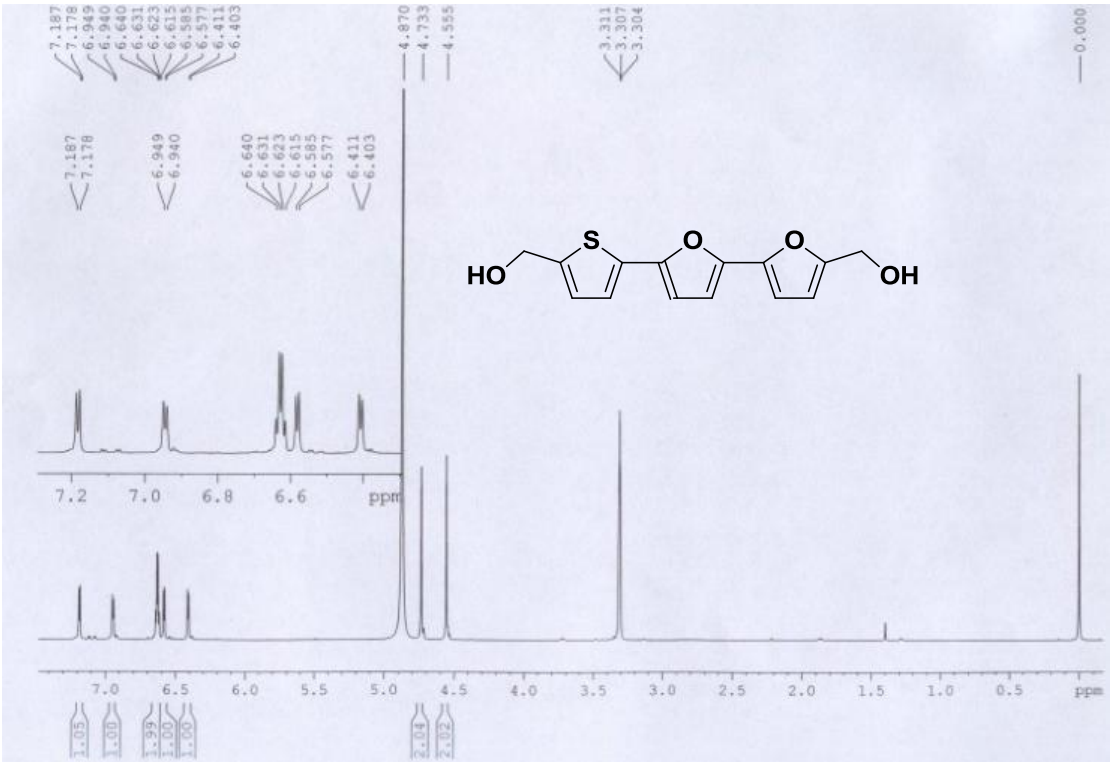

7.91
7.90
7.53
7.52
7.46
7.45
6.83
6.82
6.45
6.44
5.36
5.35
5.33
4.44
4.43
3.40
3.39
3.39
3.38
2.53
2.51
2.51
2.50
2.09
0.00
HO
O
S
S
O
1.08
1.05
1.04
1.02
1.02
1.00
1.04
2.05
3.00
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

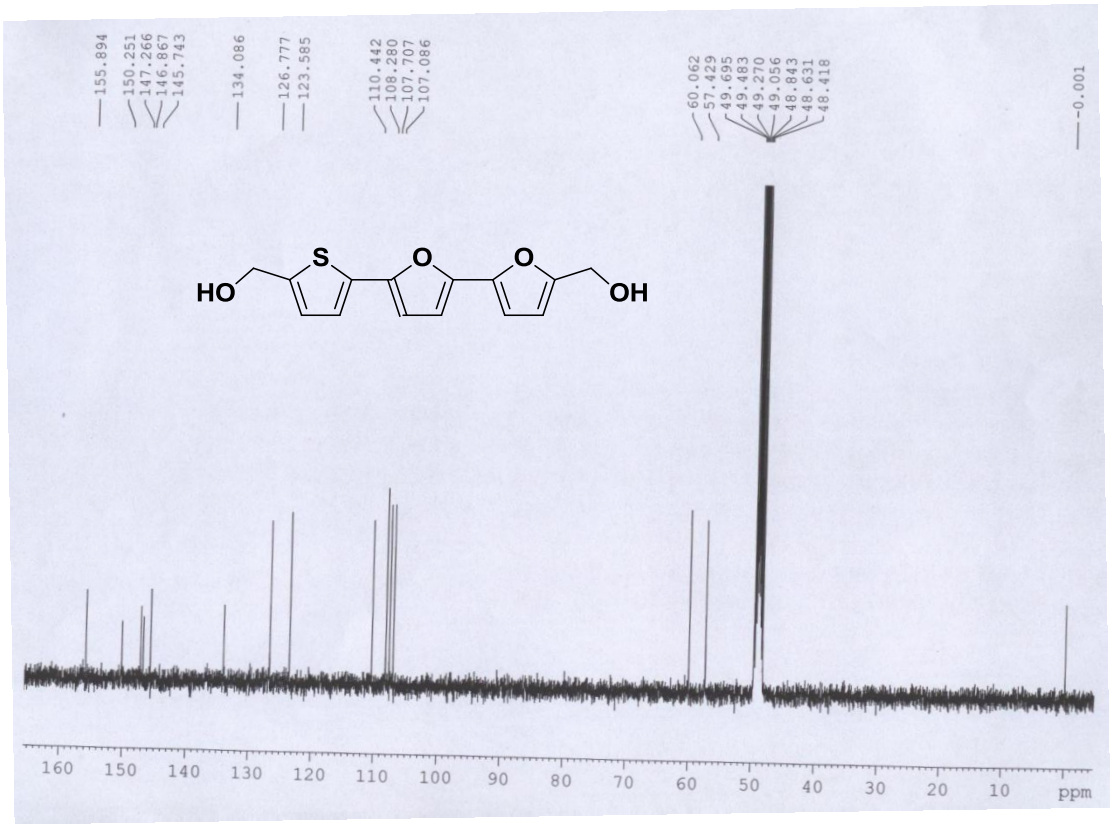

190.83
156.28
147.51
144.50
142.54
135.59
134.21
127.76
125.46
124.28
110.10
108.13
63.27
56.06
40.43
40.22
40.01
39.80
39.59
39.39
26.78
HO
O
S
S
O
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10

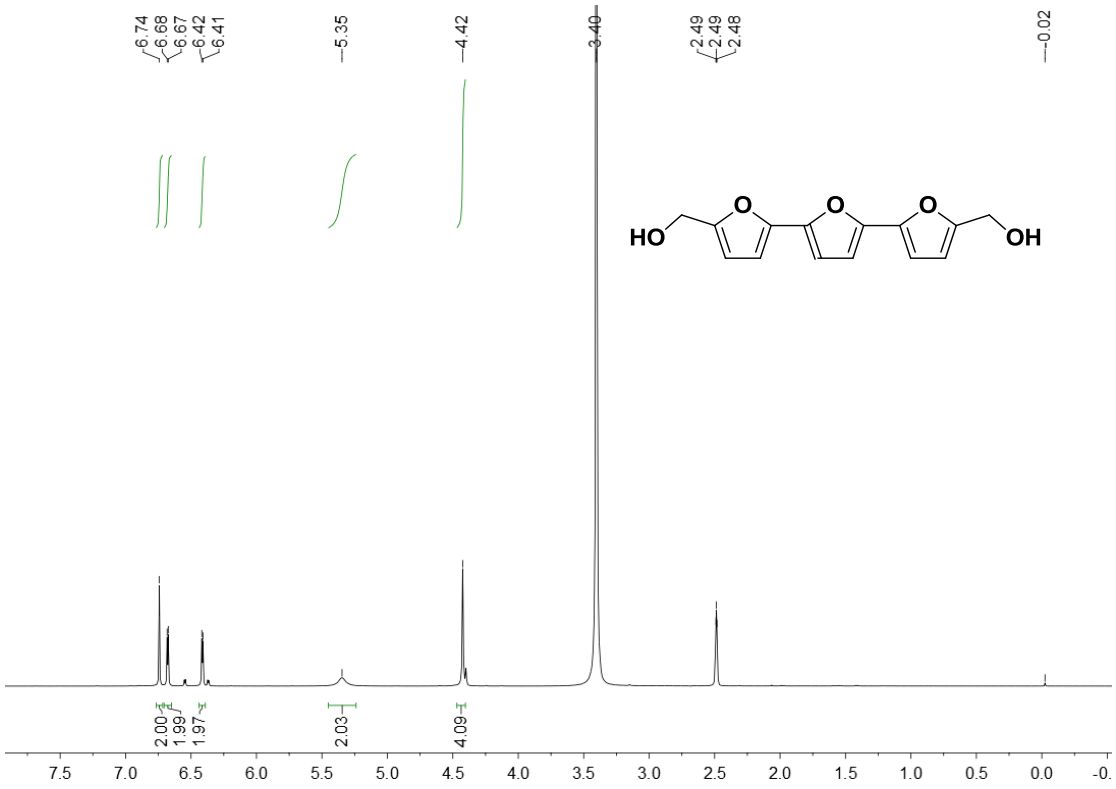

7.24
7.23
6.95
6.94
6.79
5.58
5.57
5.55
4.65
4.63
3.34
2.51
2.50
2.50
0.00
HO
S
O
S
OH
2.00
2.07
2.00
2.05
4.11
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

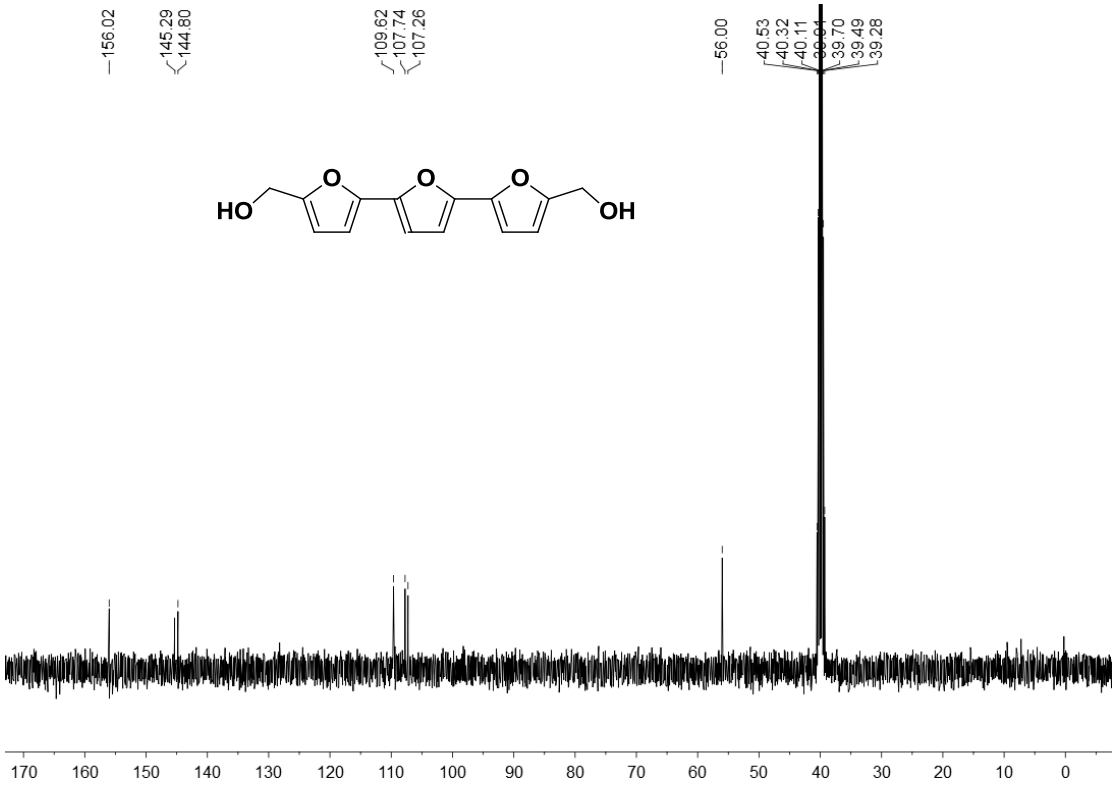

147.80
145.94
131.00
124.72
122.48
107.29
58.21
40.11
39.90
39.69
39.27
39.06
38.85
0.00
HO
S
O
S
OH
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

This journal is © The Royal Society of Chemistry 2012

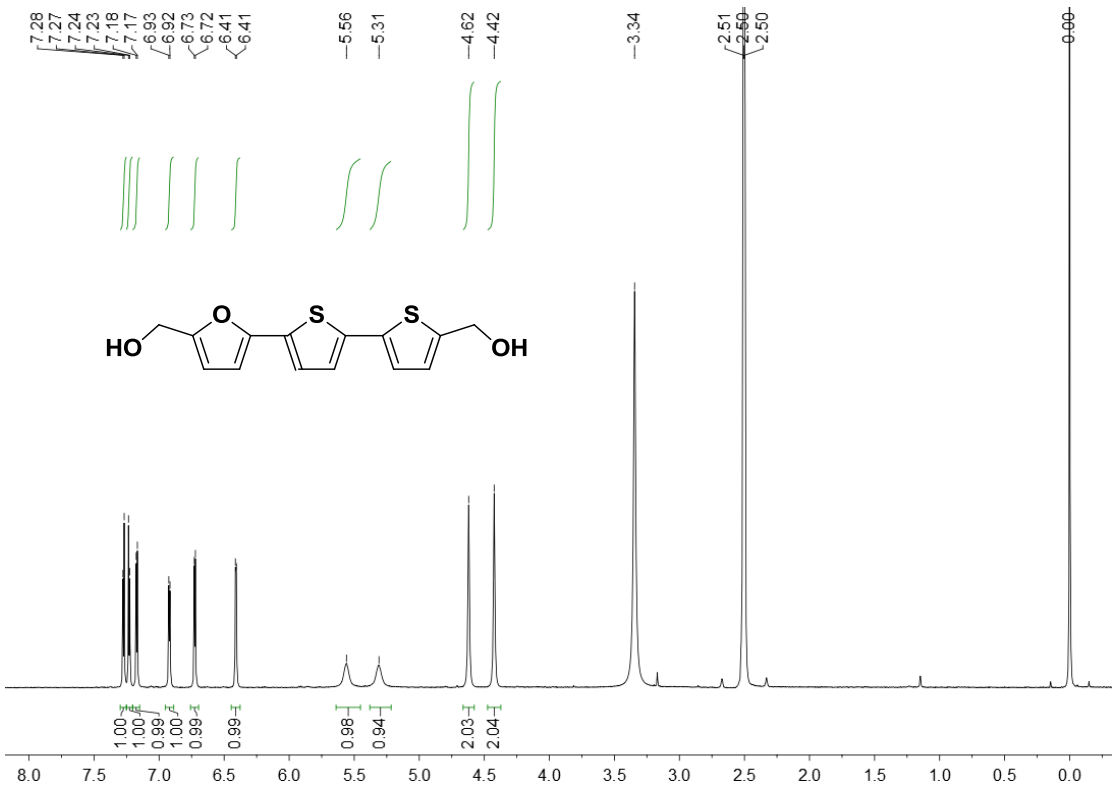


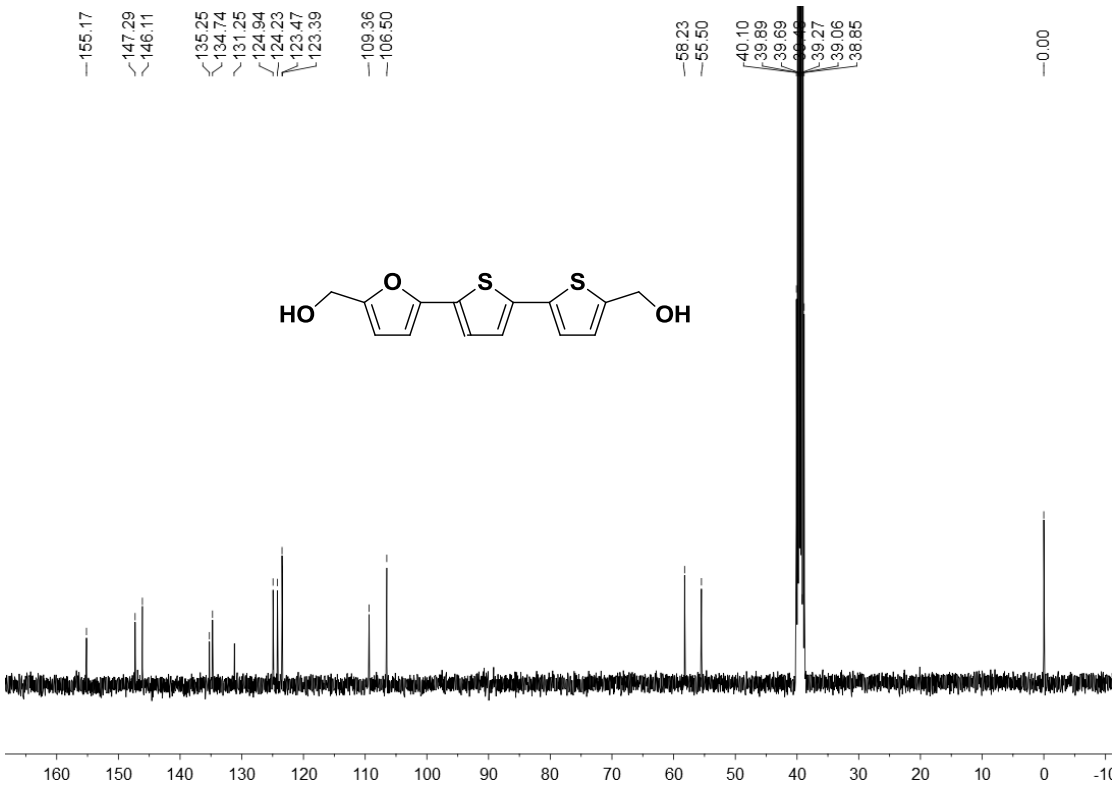



6.74
6.68
6.67
6.42
6.41
5.35
4.42
3.40
2.49
2.49
2.48
0.02
HO
O
O
O
OH
2.00
1.99
1.97
2.03
4.09
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

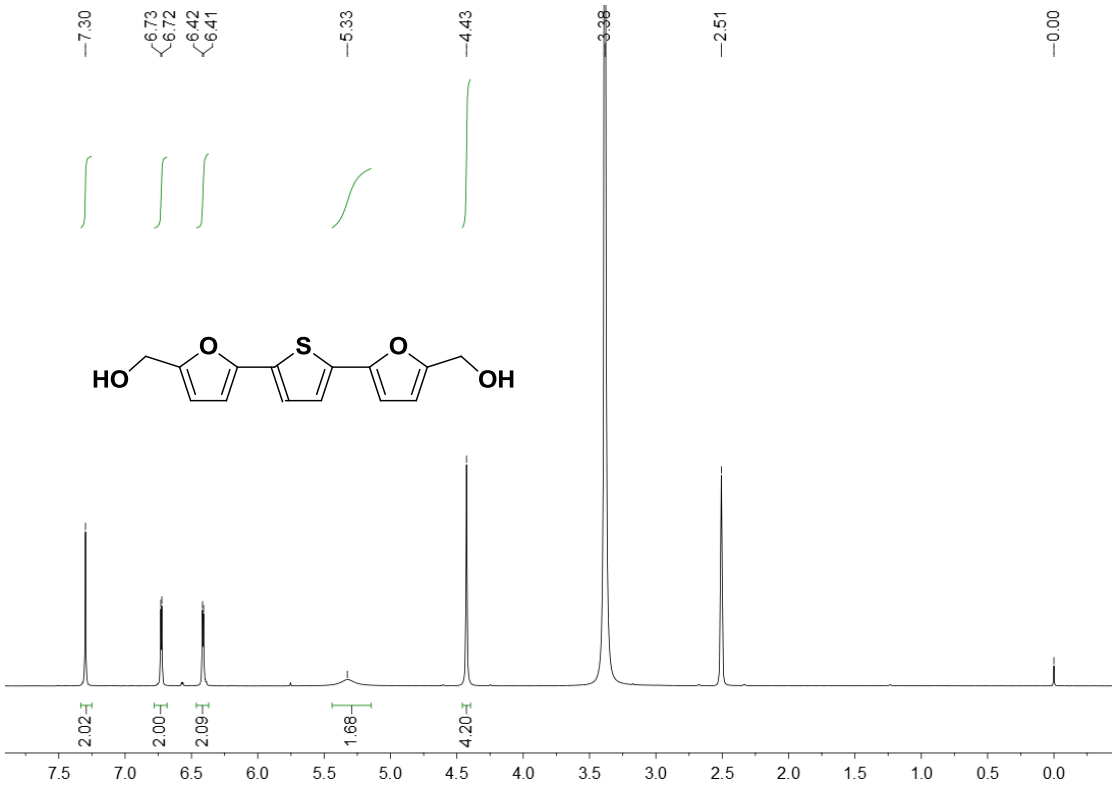

156.02
145.29
144.80
109.62
107.74
107.26
56.00
40.53
40.32
40.11
39.91
39.70
39.49
39.28
HO
O
O
O
OH
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

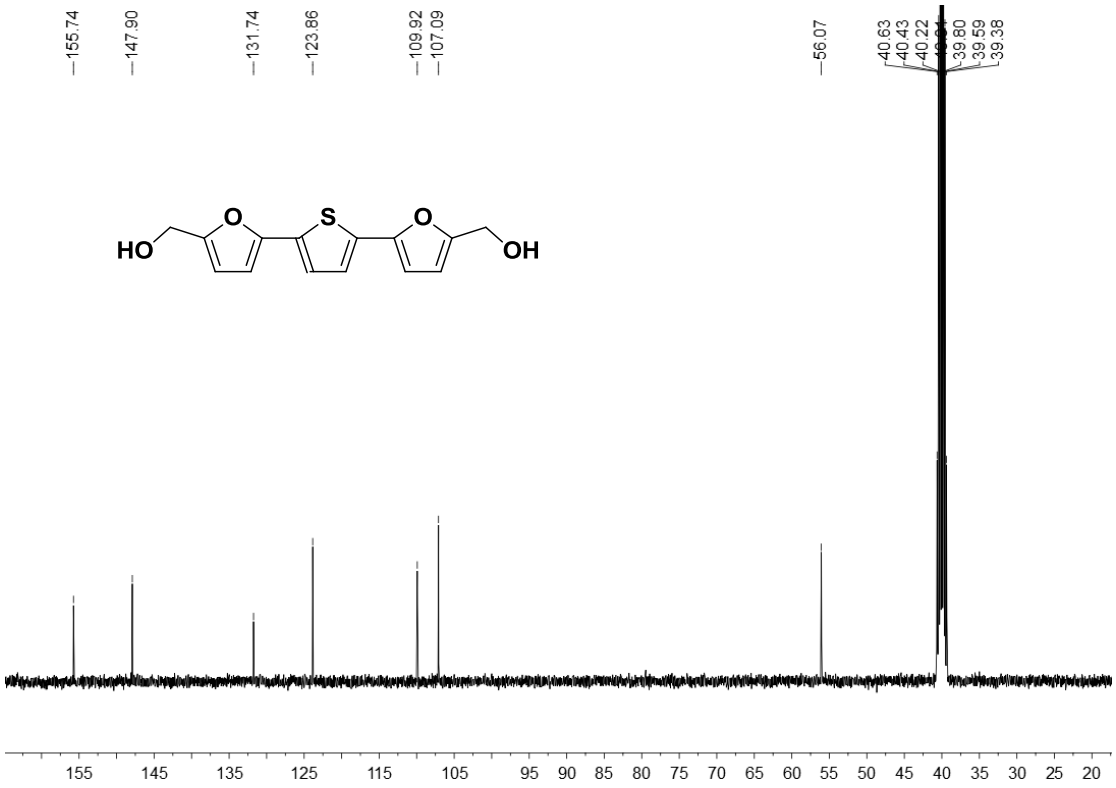
This journal is © The Royal Society of Chemistry 2012

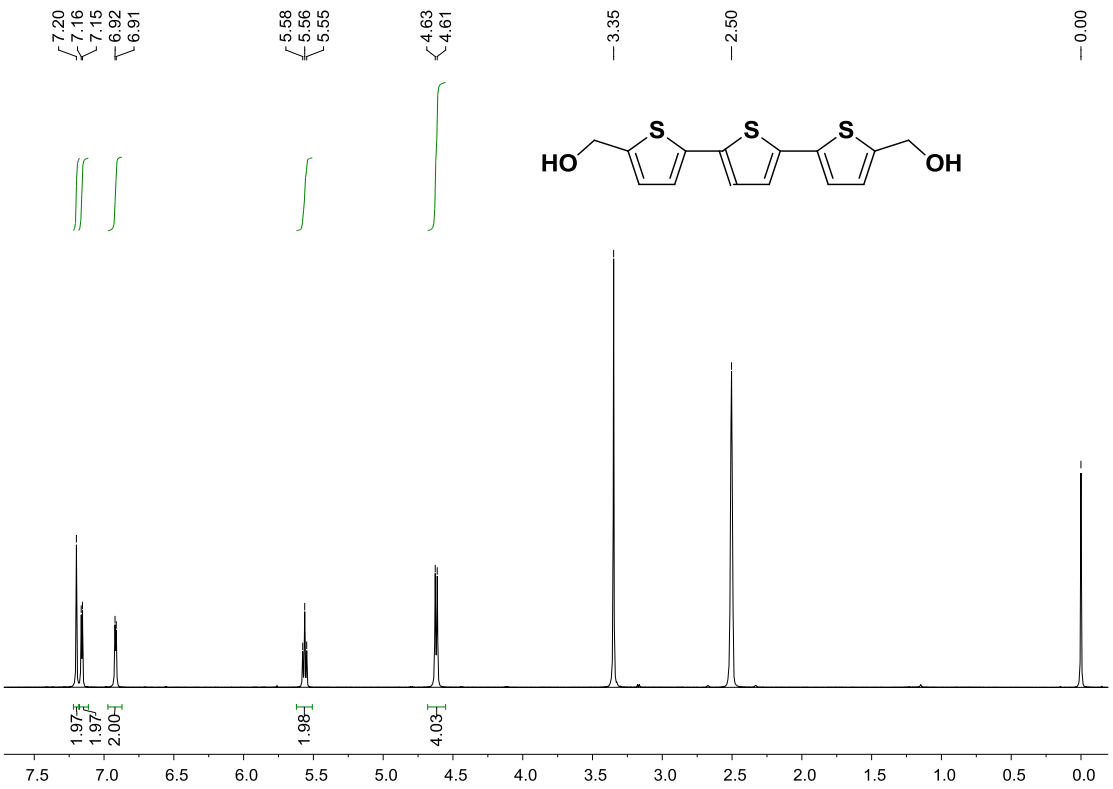


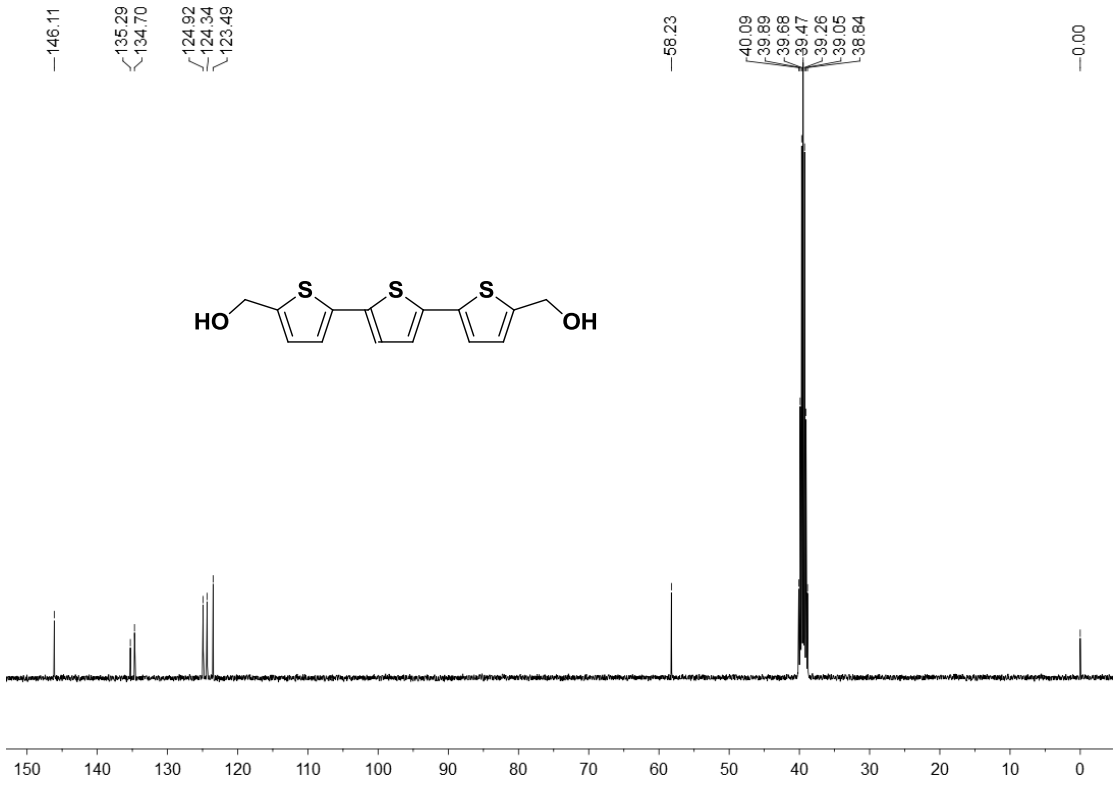


This journal is © The Royal Society of Chemistry 2012

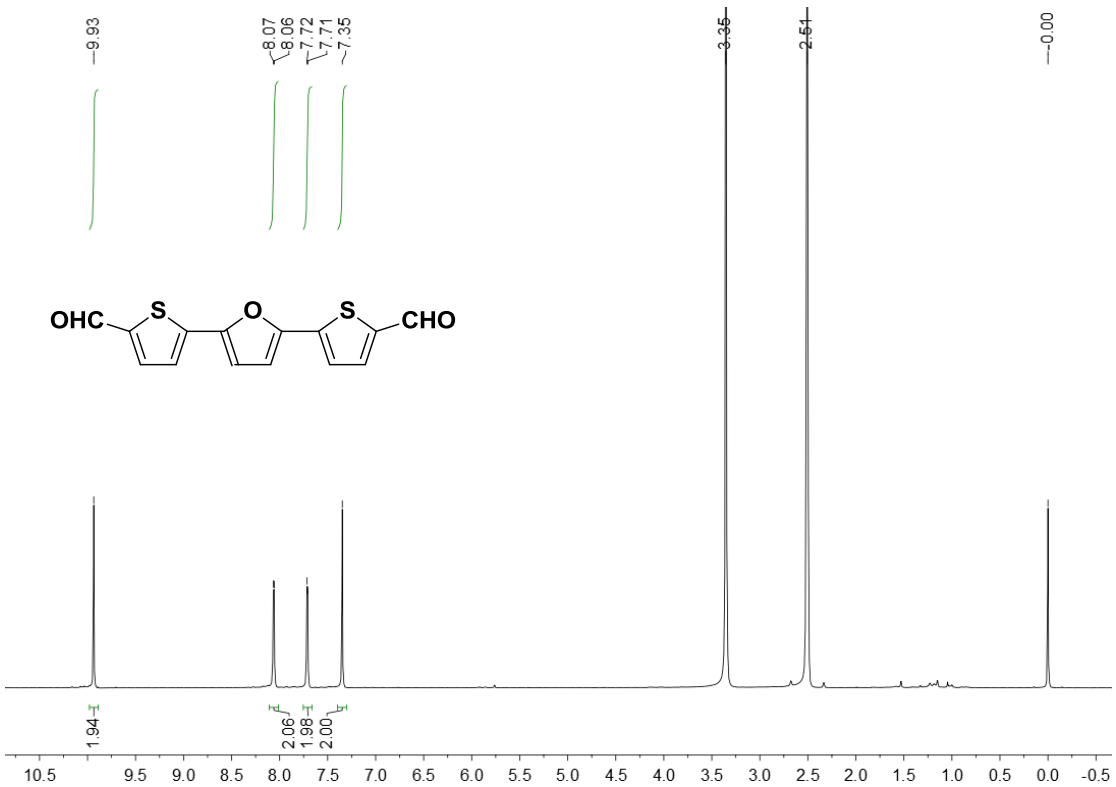


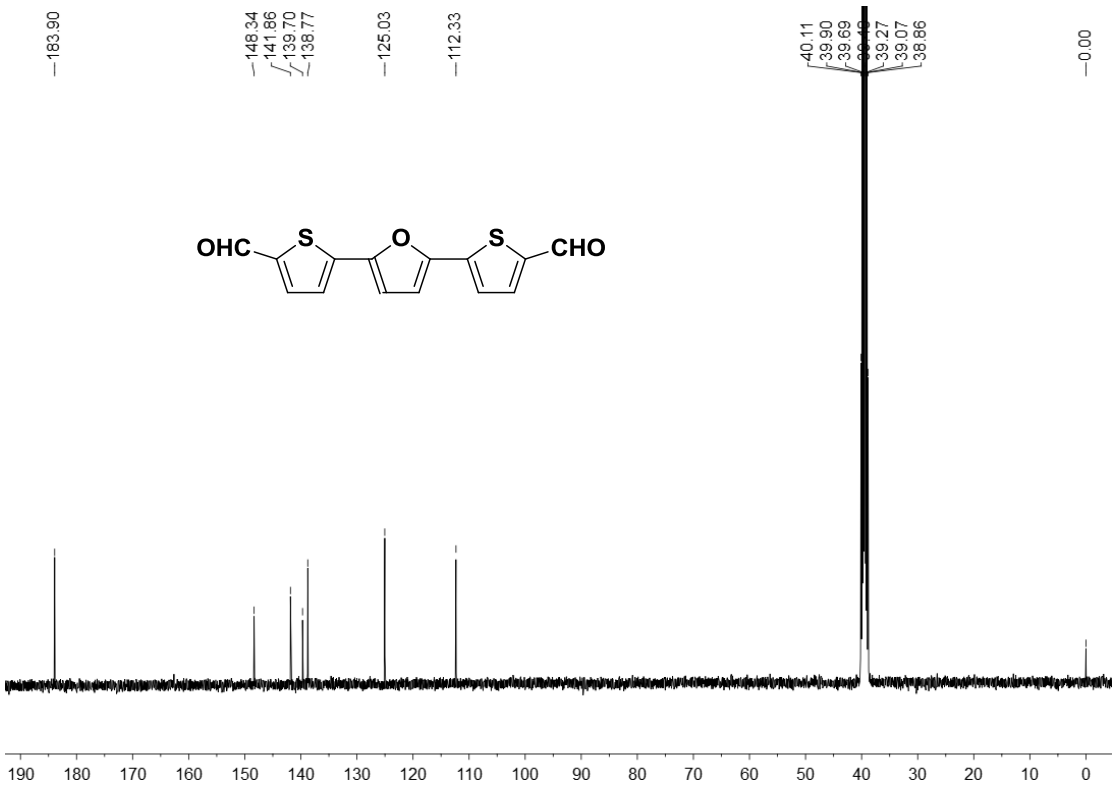


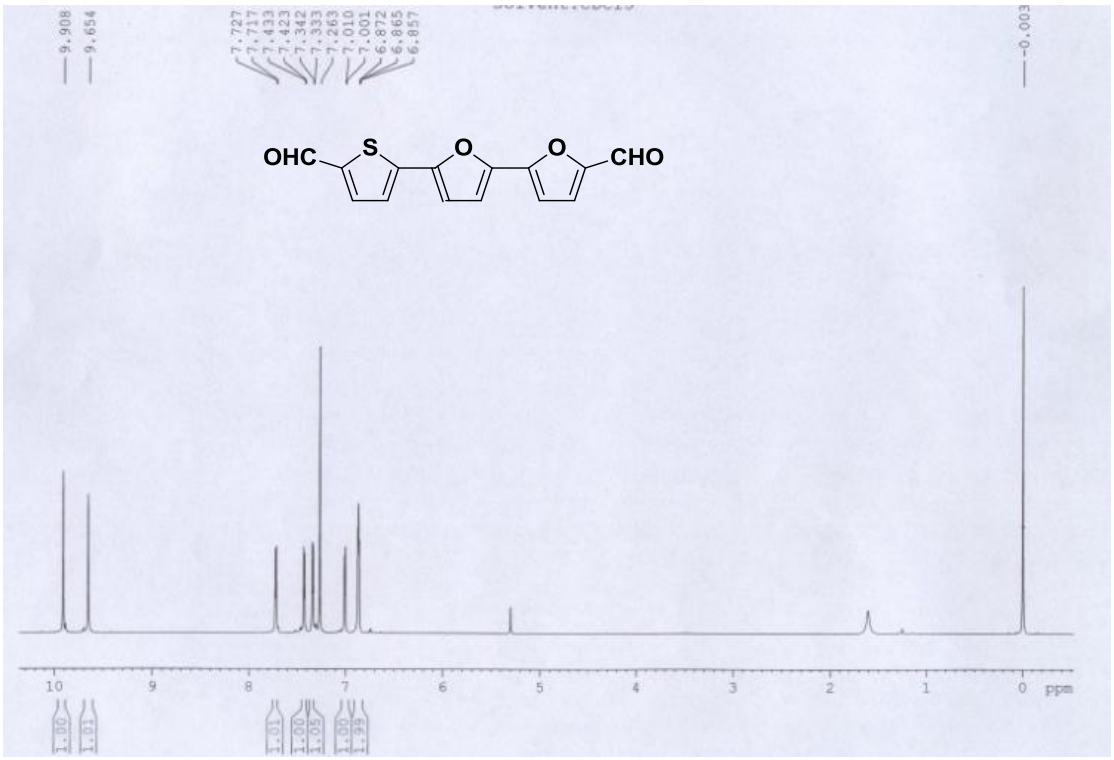

7.20
7.16
7.15
6.92
6.91
5.58
5.56
5.55
4.63
4.61
3.35
2.50
0.00
HO
S
S
S
OH
1.97
1.97
2.00
1.98
4.03
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

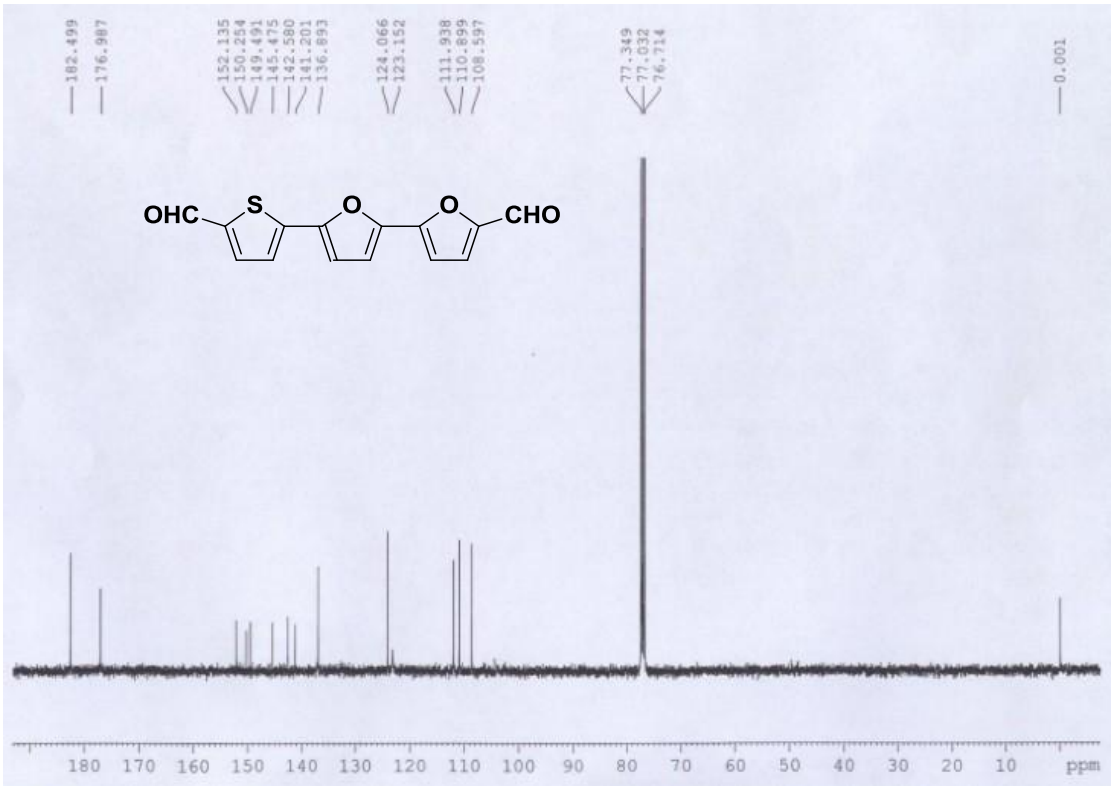

146.11
135.29
134.70
124.92
124.34
123.49
58.23
40.09
39.89
39.68
39.47
39.26
39.05
38.84
0.00
HO
S
S
S
OH
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

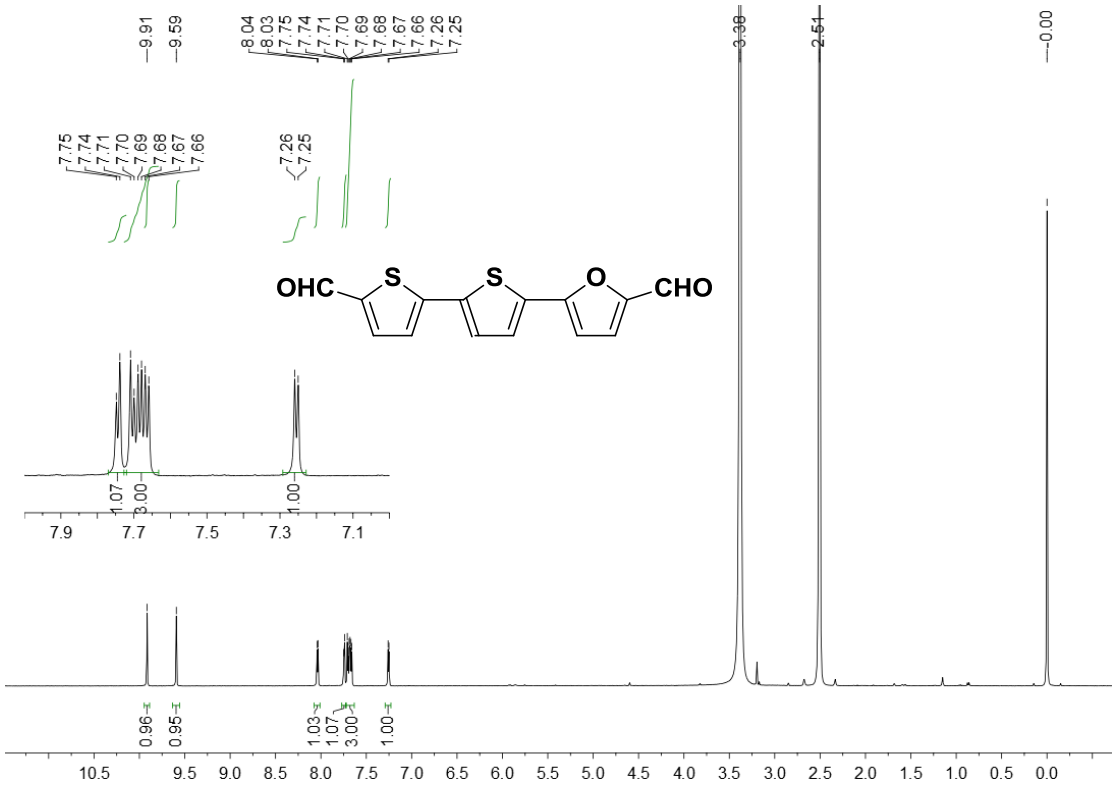
This journal is © The Royal Society of Chemistry 2012

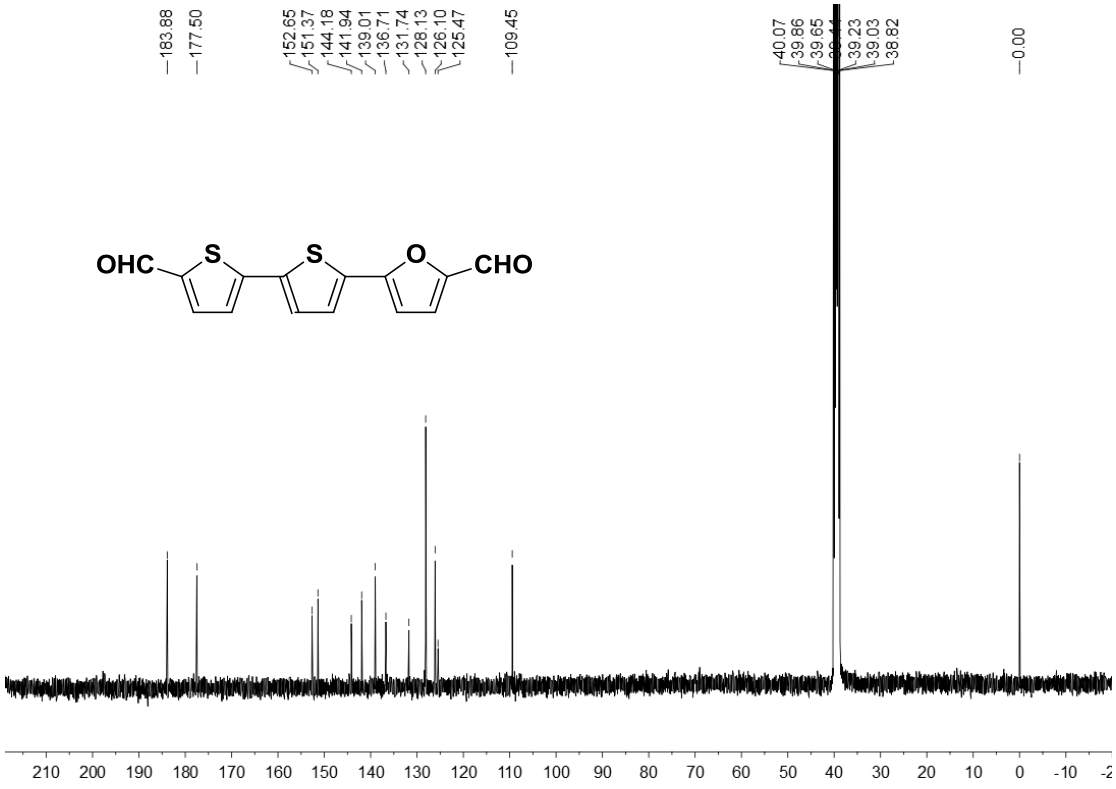


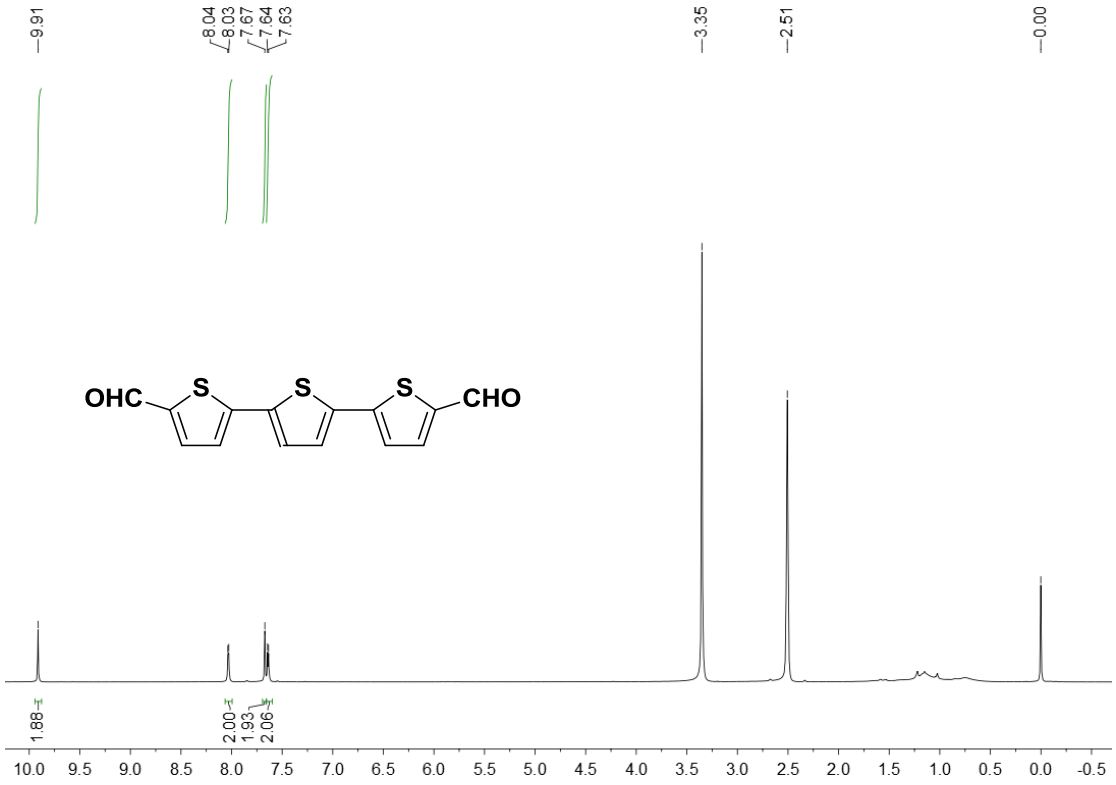


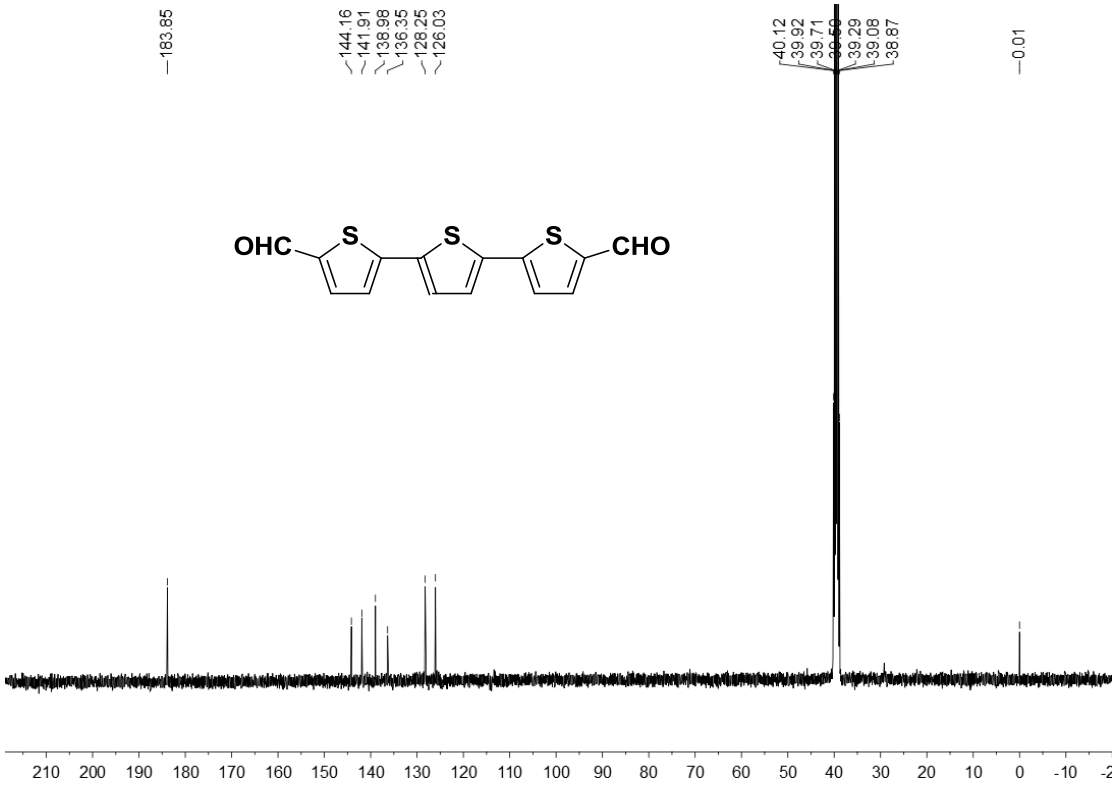

9.908
9.654
7.727
7.717
7.433
7.423
7.342
7.333
7.263
7.010
7.001
6.872
6.865
6.857
-0.003
OHC
S
O
O
CHO
10
9
8
7
6
5
4
3
2
1
0
ppm
1.00
1.01
1.01
1.00
1.05
1.00
1.99

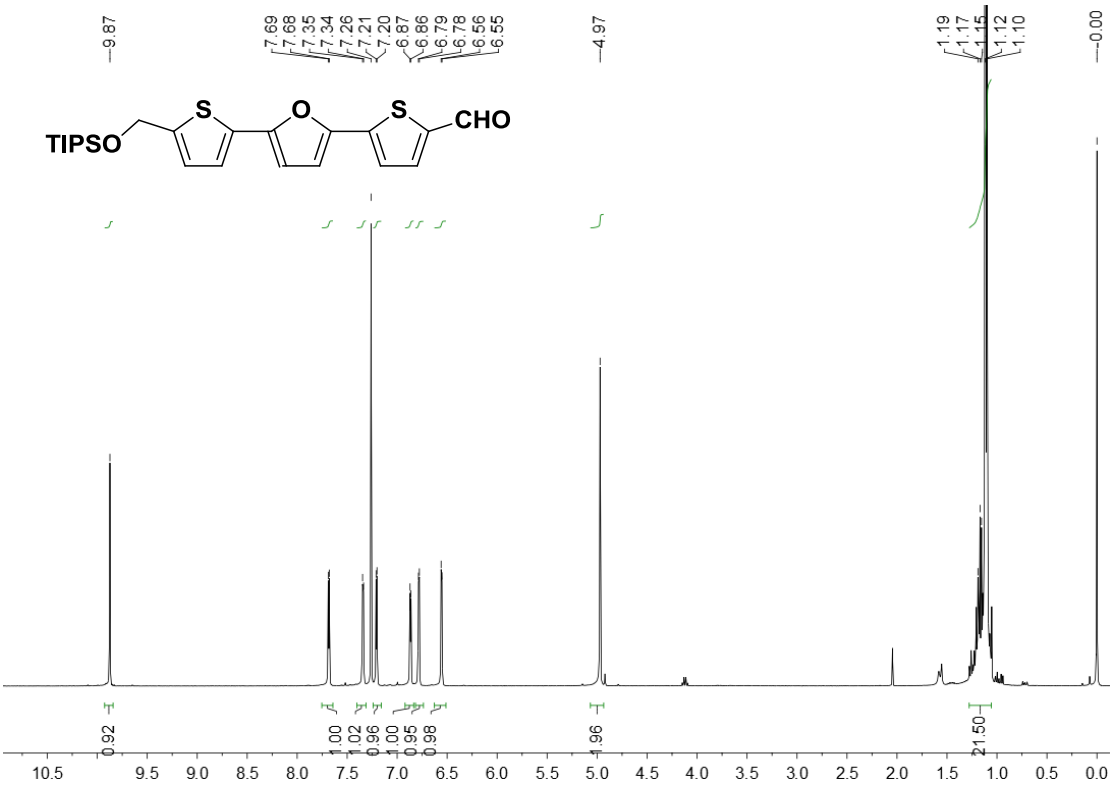

182.499
176.987
152.135
150.254
149.491
145.475
142.580
141.201
136.893
124.066
123.152
111.938
110.899
108.597
77.349
77.032
76.714
0.001
OHC
S
O
O
CHO
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

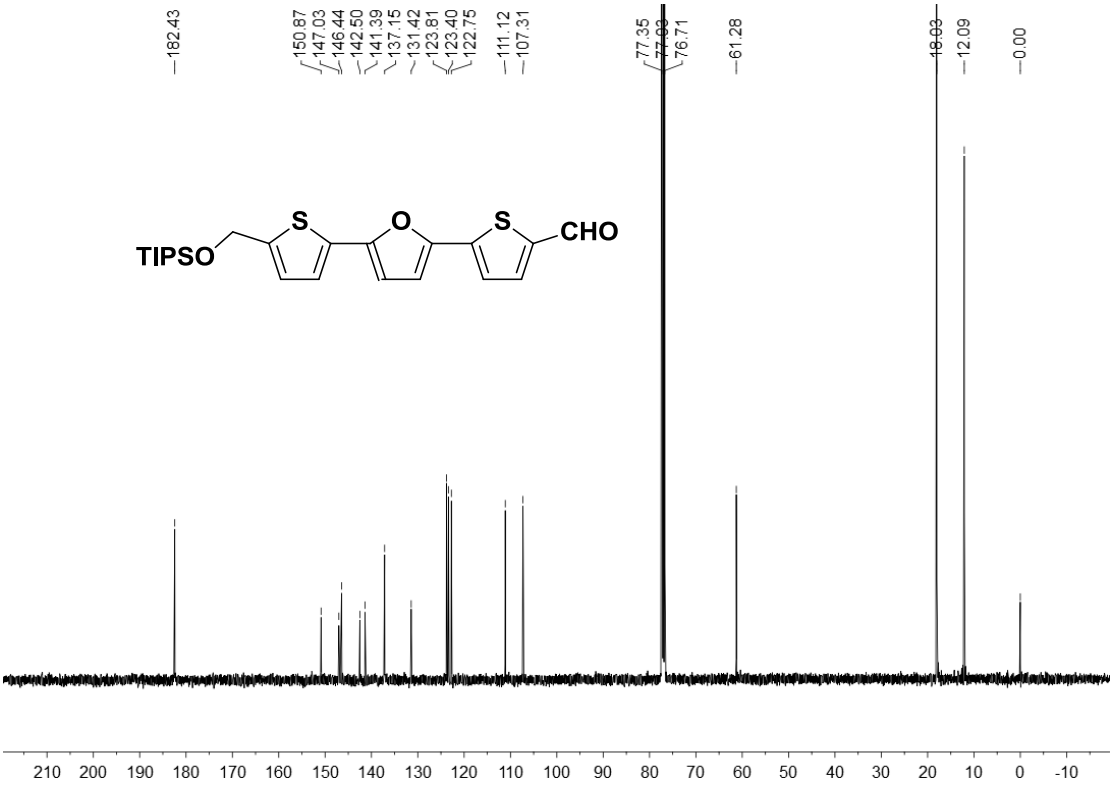

9.91
9.59
8.04
8.03
7.75
7.74
7.71
7.70
7.69
7.68
7.67
7.66
7.26
7.25
3.30
2.61
0.00
OHC
S
S
O
CHO
1.07
3.00
1.00
0.96
0.95
1.03
1.07
3.00
1.00


183.88
177.50
152.65
151.37
144.18
141.94
139.01
136.71
131.74
128.13
126.10
125.47
109.45
40.07
39.86
39.65
39.23
39.03
38.82
0.00
OHC
S
S
O
CHO

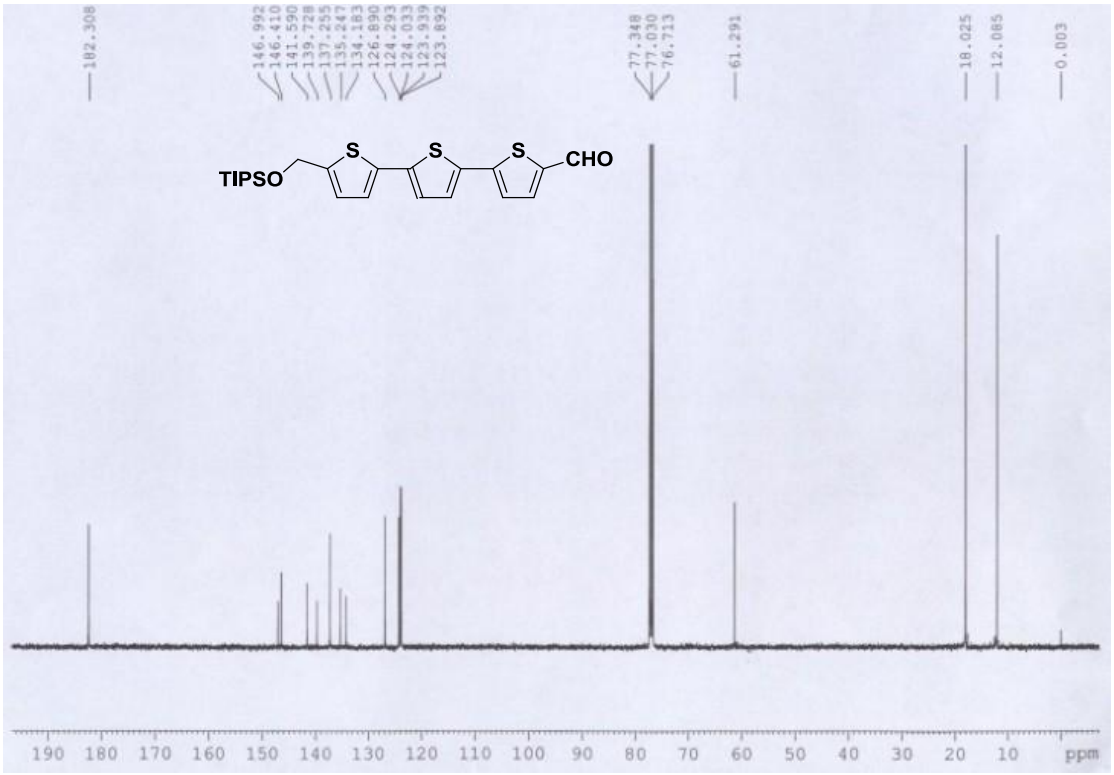

9.91
8.04
8.03
7.67
7.64
7.63
3.35
2.51
0.00
OHC
S
S
S
CHO
1.88
2.00
1.93
2.06
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5

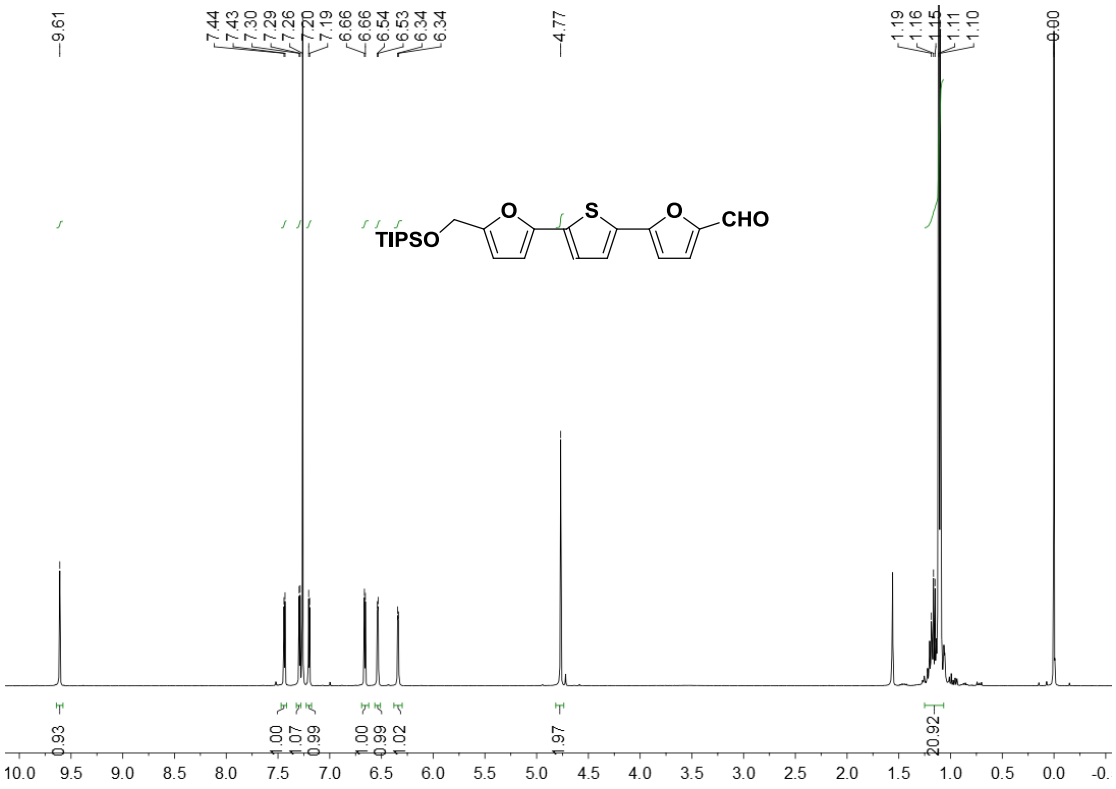

183.85
144.16
141.91
138.98
136.35
128.25
126.03
40.12
39.92
39.71
39.50
39.29
39.08
38.87
0.01
OHC
S
S
S
CHO
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10 -2

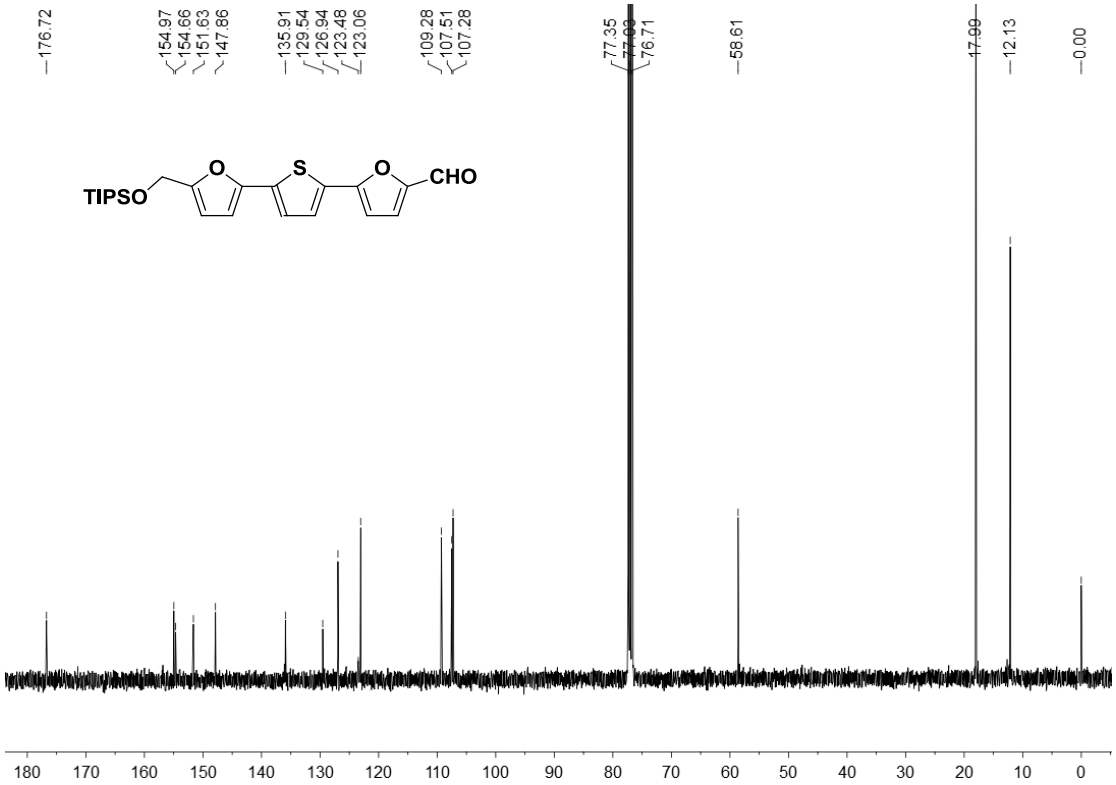

9.87
7.69
7.68
7.35
7.34
7.26
7.21
7.20
6.87
6.86
6.79
6.78
6.56
6.55
4.97
1.19
1.17
1.15
1.12
1.10
0.00
TIPSO
S
O
S
CHO
0.92
1.00
1.02
0.96
1.00
0.95
0.98
1.96
21.50

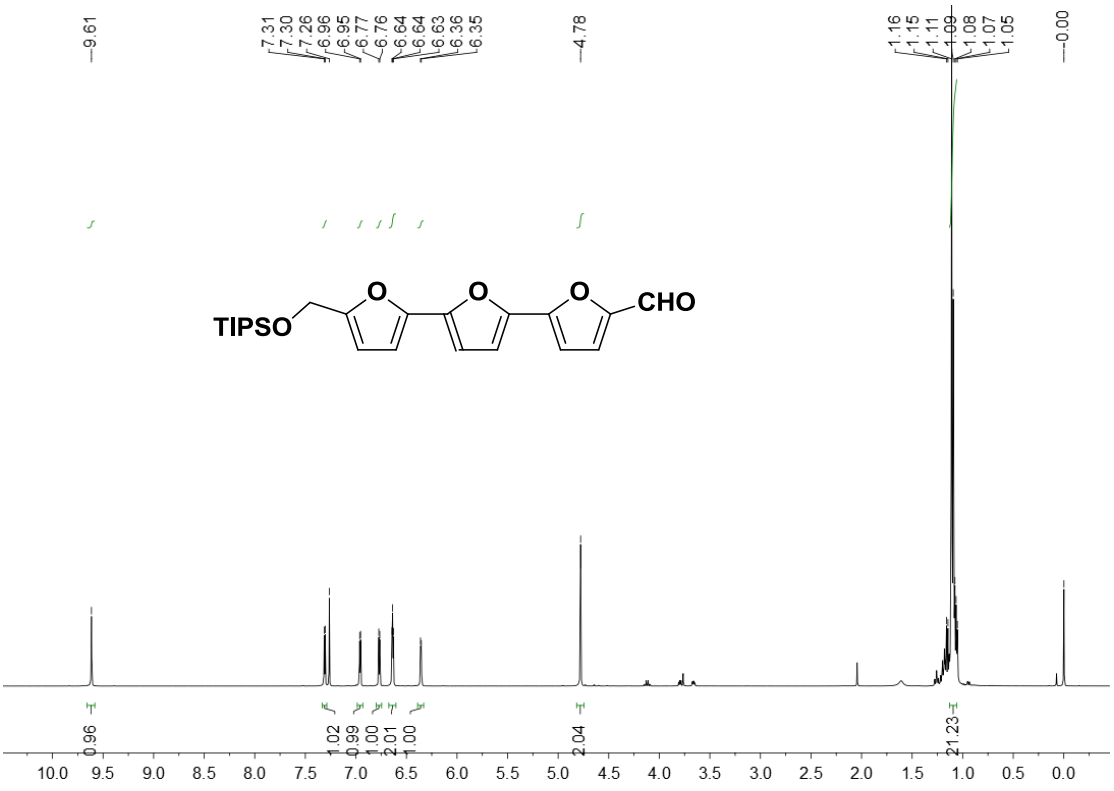

182.43
150.87
147.03
146.44
142.50
141.39
137.15
131.42
123.81
123.40
122.75
111.12
107.31
77.35
77.03
76.71
61.28
18.03
12.09
0.00
TIPSO
S
O
S
CHO

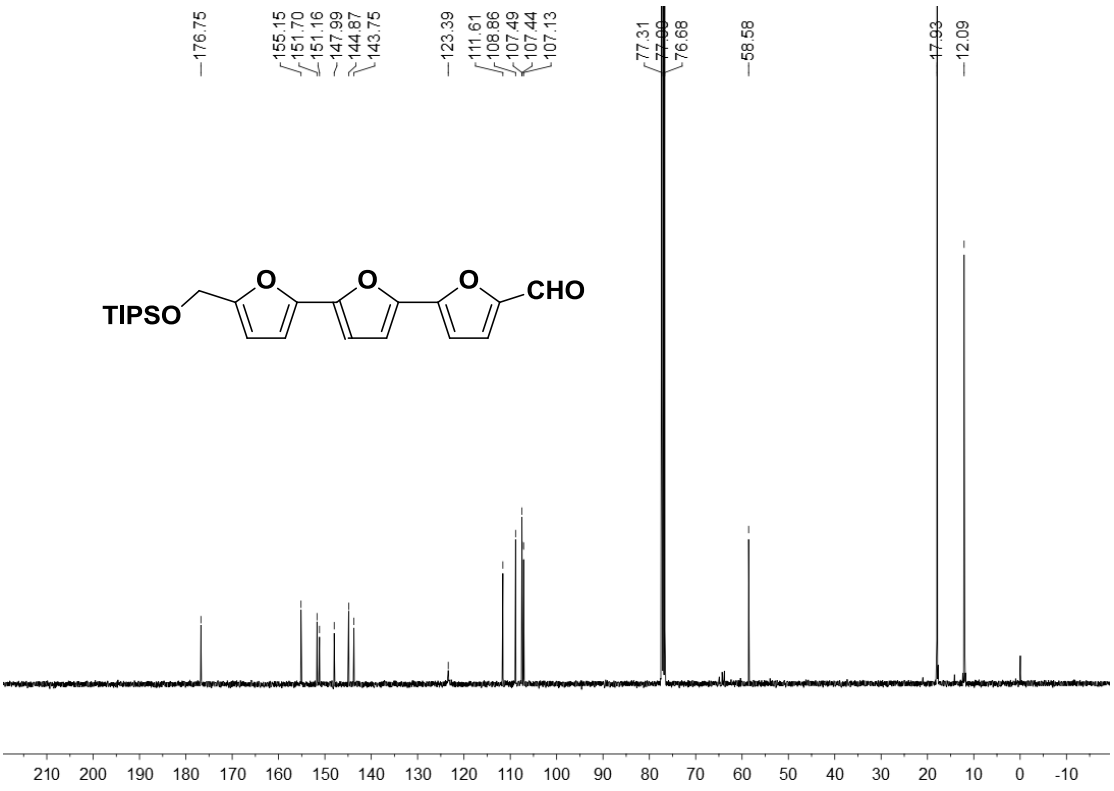

9.849
7.662
7.652
7.259
7.255
7.246
7.221
7.211
7.090
7.080
7.068
7.059
6.837
6.827
4.949
1.113
1.098
1.091
0.000
TIPSO
S
S
S
CHO
7.5
7.0
ppm
10
9
8
7
6
5
4
3
2
1
0
ppm
0.93
1.00
0.96
1.00
1.00
0.96
1.00
1.96
21.23


182.308
146.992
146.410
141.590
139.728
137.255
135.247
134.183
126.890
124.293
124.033
123.939
123.892
77.348
77.030
76.713
61.291
18.025
12.085
0.003
TIPSO
S
S
S
CHO
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

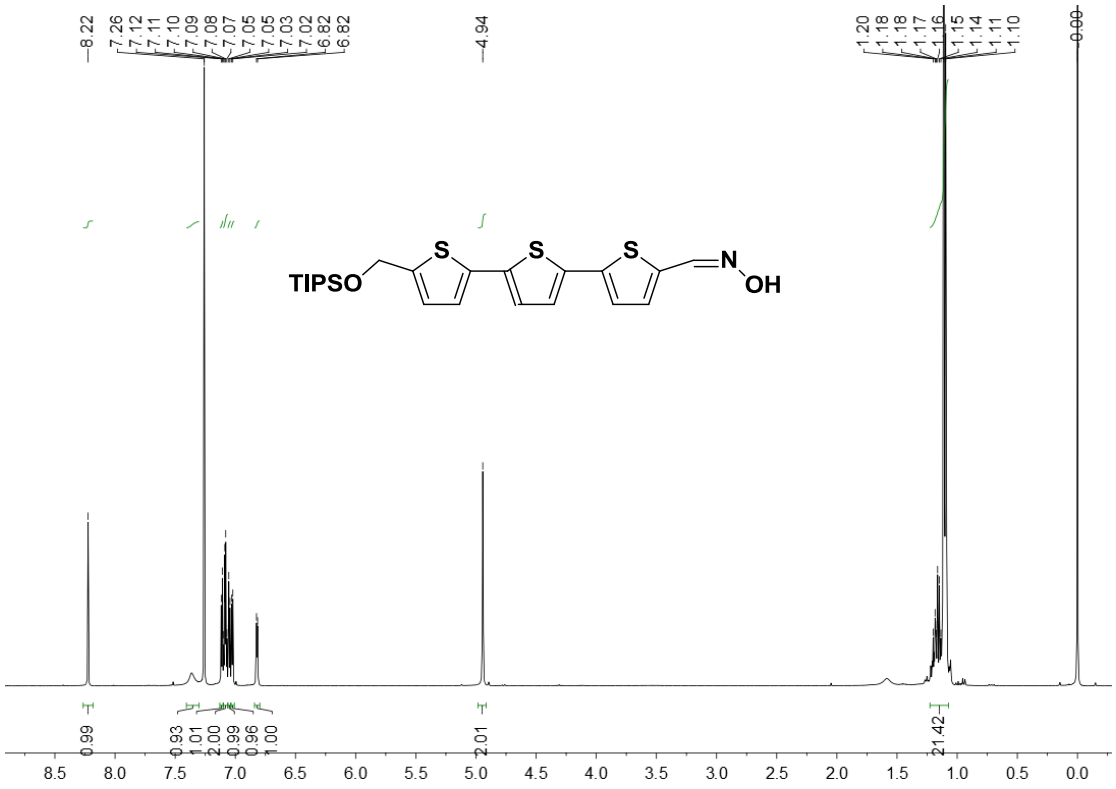

9.61
7.44
7.43
7.30
7.29
7.26
7.20
7.19
6.66
6.66
6.54
6.53
6.34
6.34
4.77
1.19
1.16
1.15
1.11
1.10
0.00
TIPSO
O
S
O
CHO
0.93
1.00
1.07
0.99
1.00
0.99
1.02
1.97
20.92
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

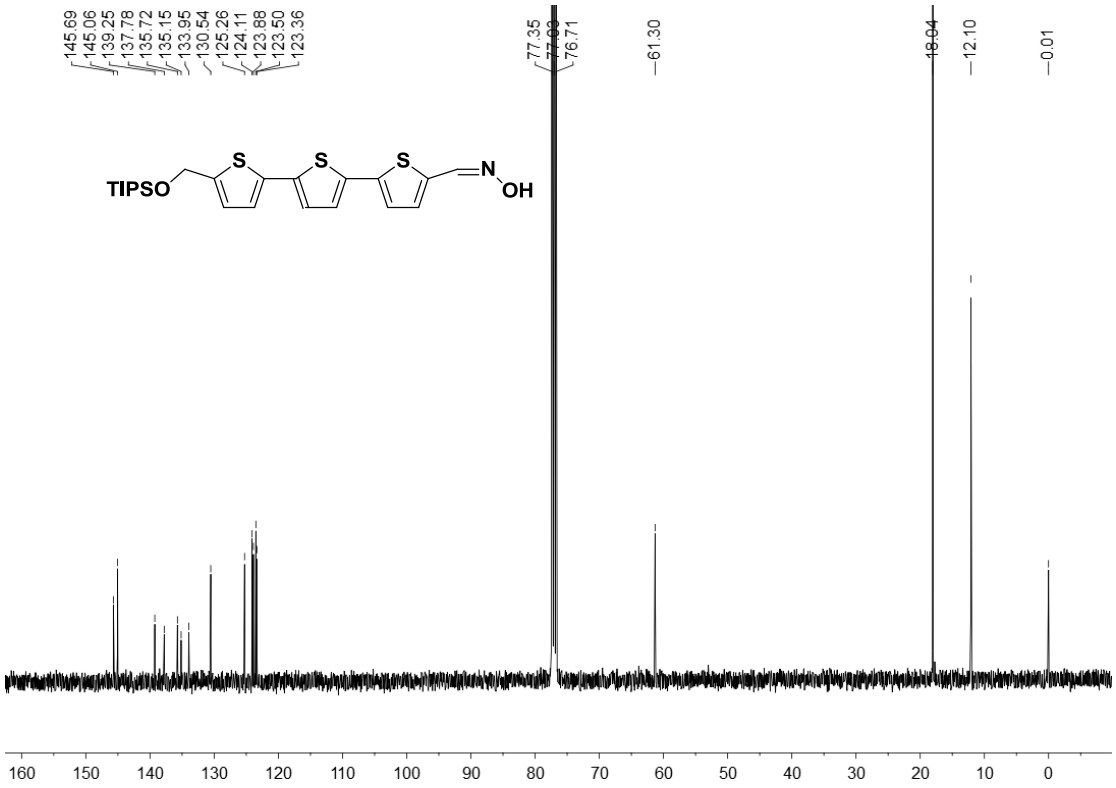

176.72
154.97
154.66
151.63
147.86
135.91
129.54
126.94
123.48
123.06
109.28
107.51
107.28
77.35
77.00
76.71
58.61
17.99
12.13
0.00
TIPSO
O
S
O
CHO
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0


9.61
7.31
7.30
7.26
6.96
6.95
6.77
6.76
6.64
6.64
6.63
6.36
6.35
4.78
1.16
1.15
1.11
1.09
1.08
1.07
1.05
0.00
TIPSO
O
O
O
CHO
0.96
1.02
0.99
1.00
2.01
1.00
2.04
21.23
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0

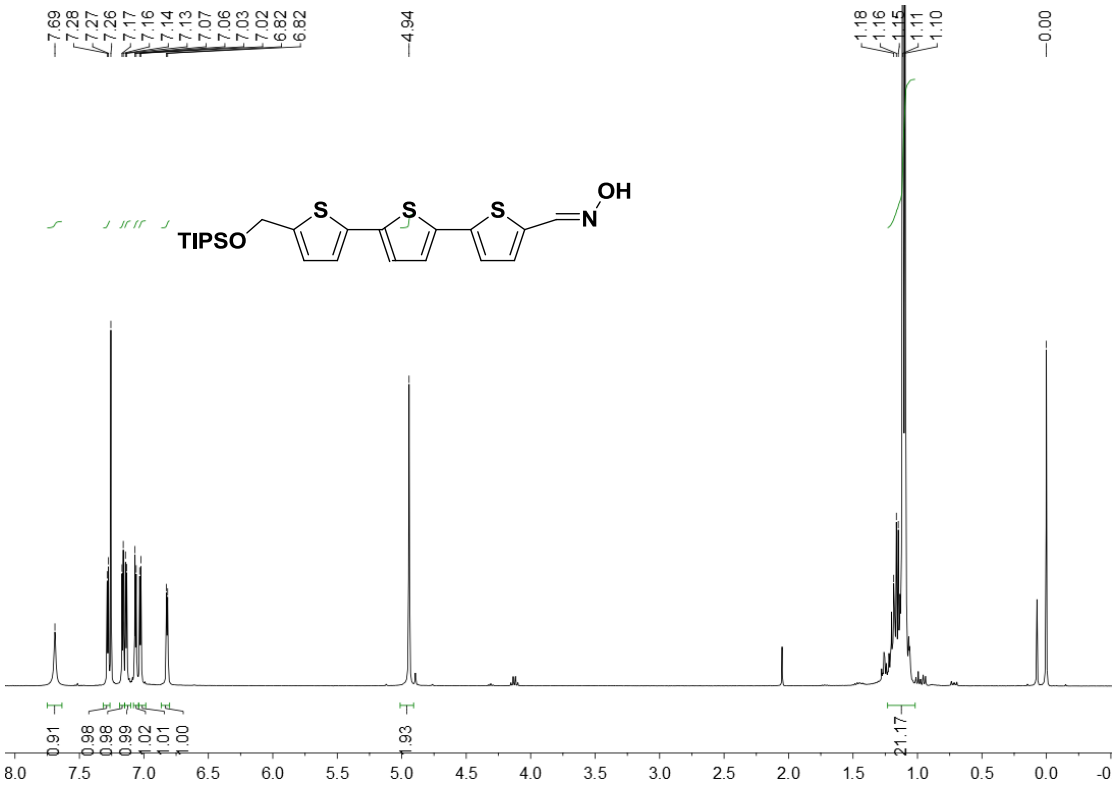

176.75
155.15
151.70
151.16
147.99
144.87
143.75
123.39
111.61
108.86
107.49
107.44
107.13
77.31
77.00
76.68
58.58
17.93
12.09
TIPSO
O
O
O
CHO
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10

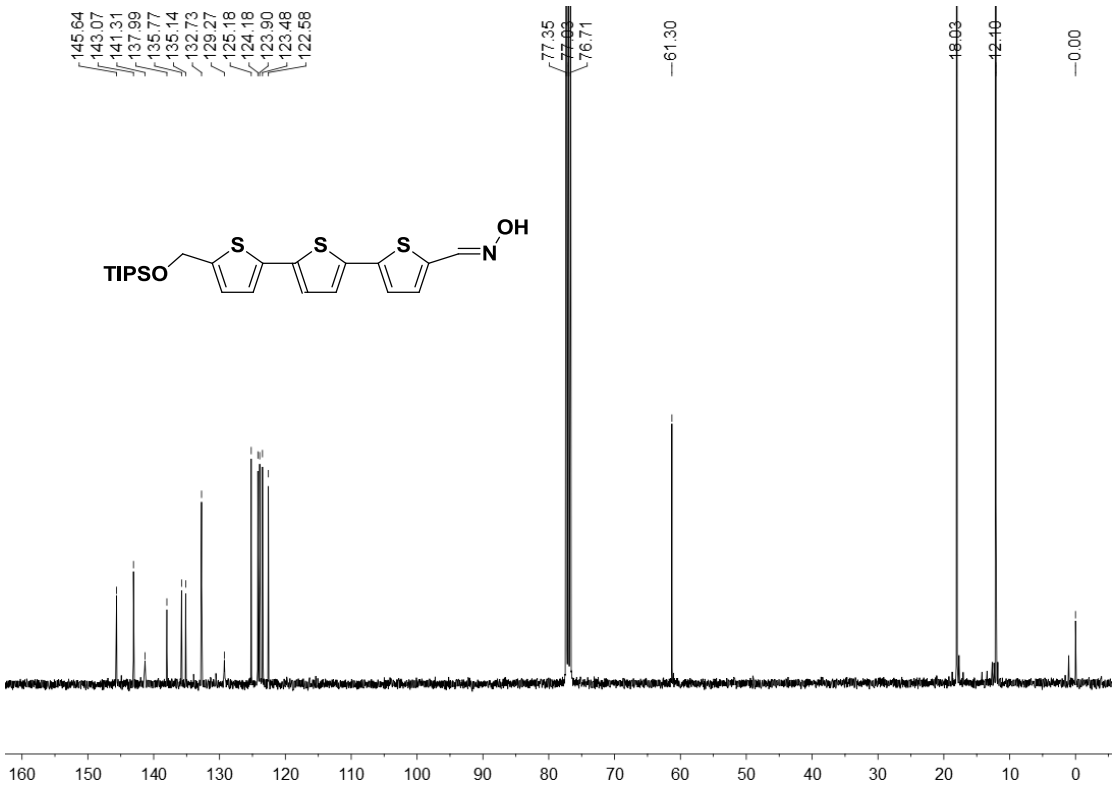
This journal is © The Royal Society of Chemistry 2012



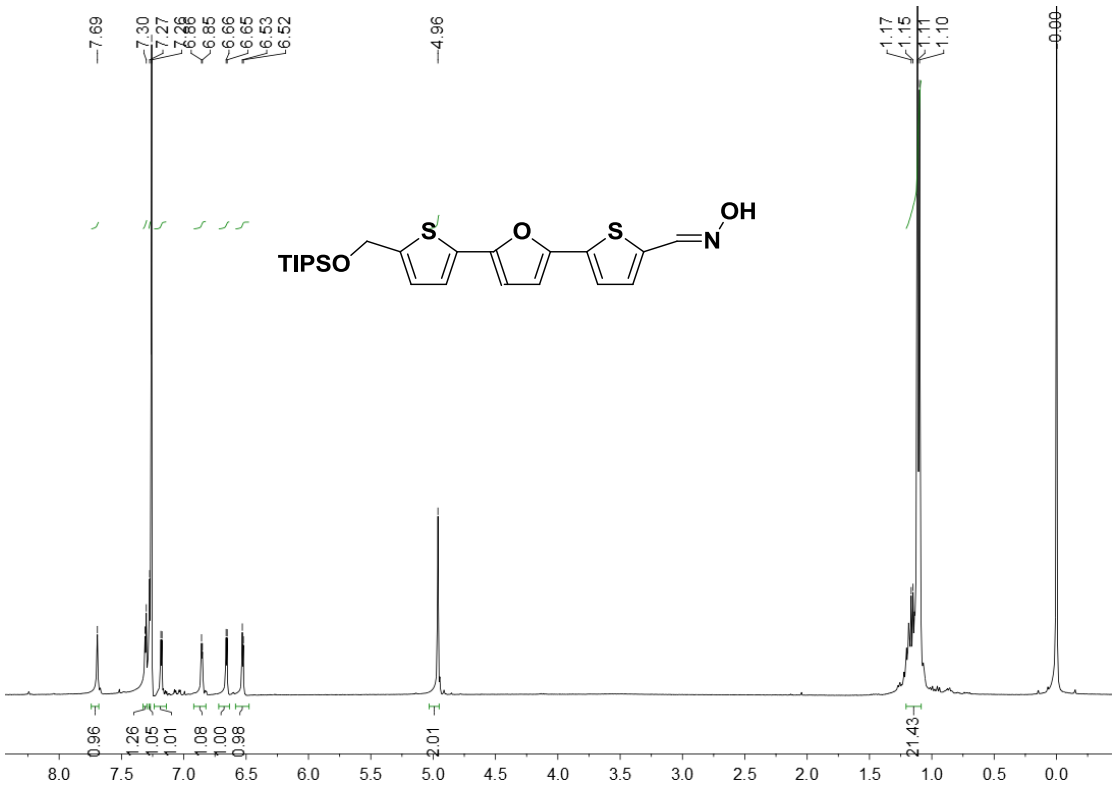


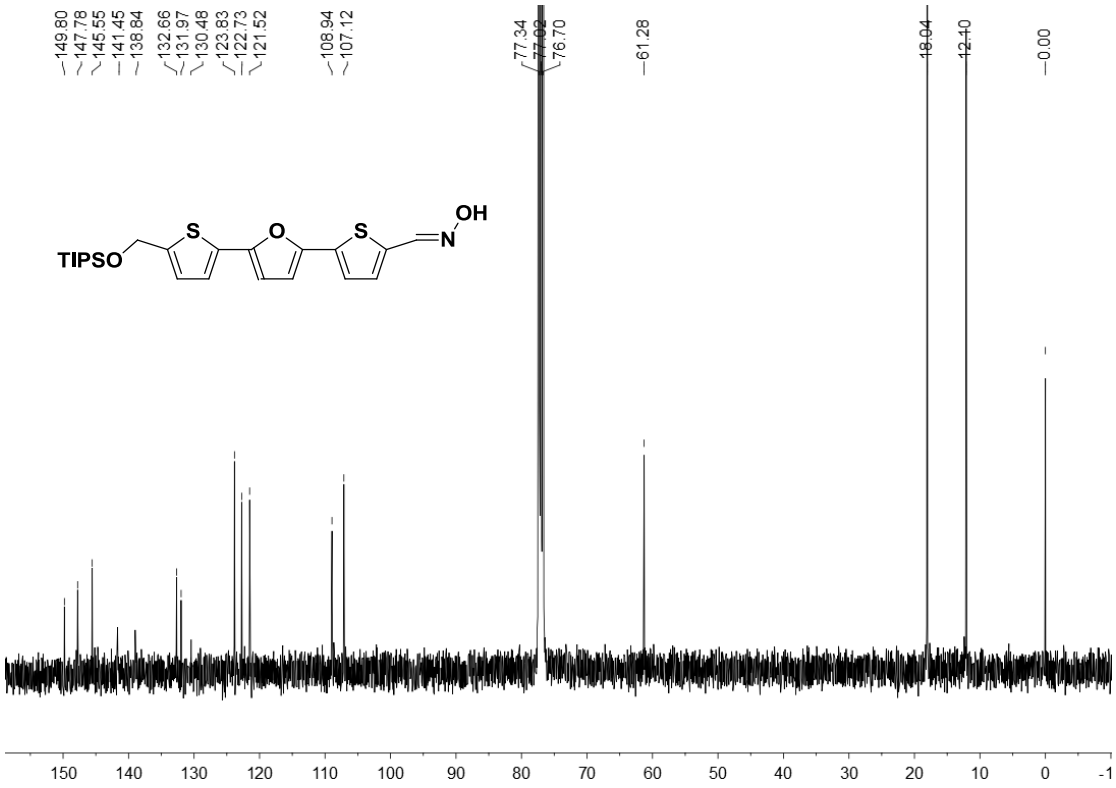
This journal is © The Royal Society of Chemistry 2012

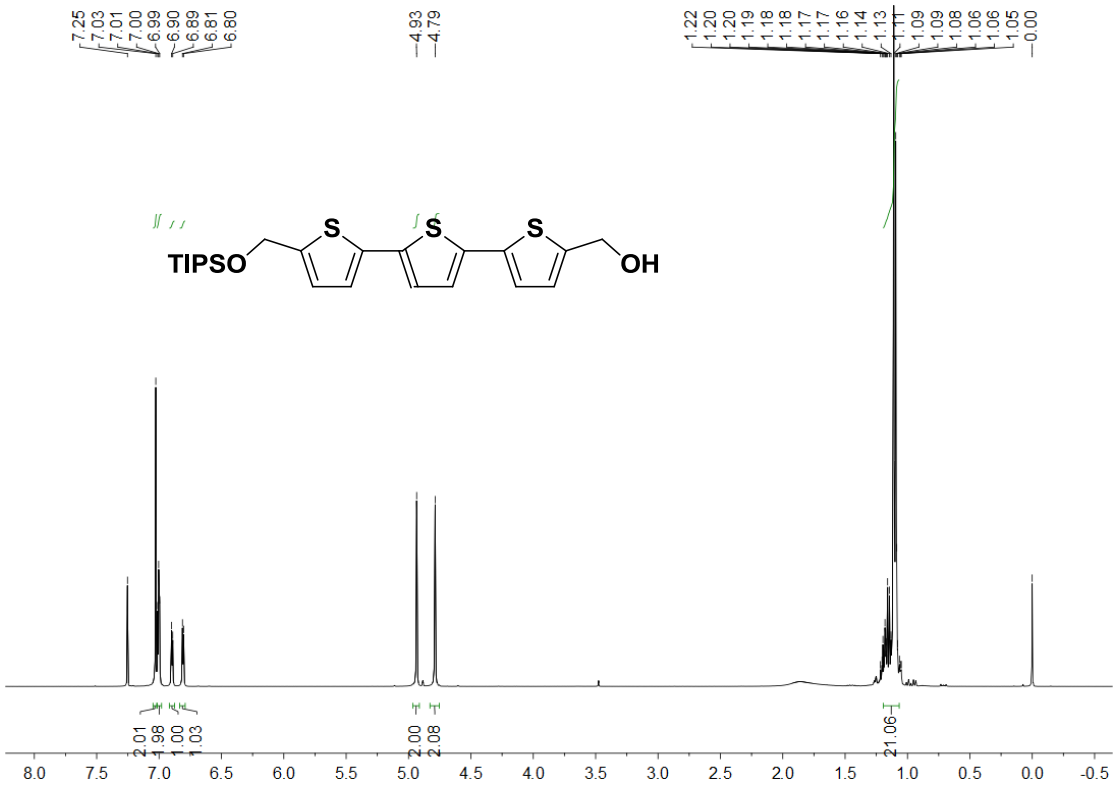


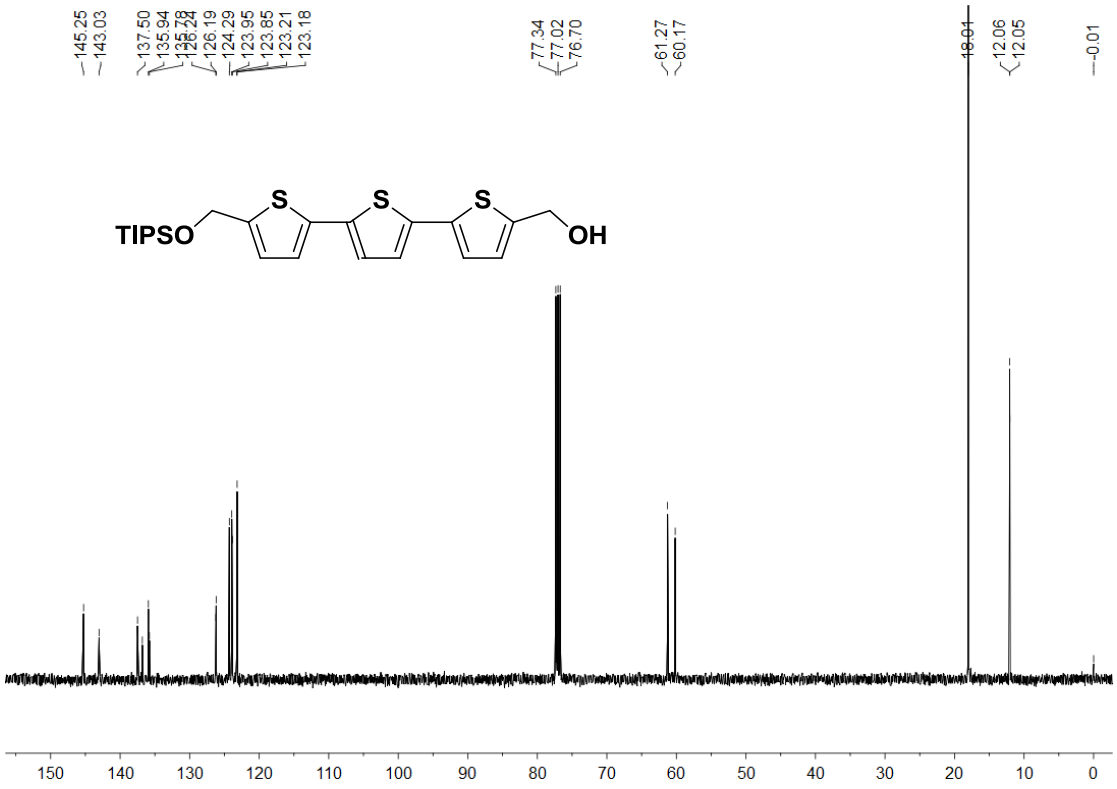


This journal is © The Royal Society of Chemistry 2012






7.25
7.03
7.01
7.00
6.99
6.90
6.89
6.81
6.80
4.93
4.79
1.22
1.20
1.20
1.19
1.18
1.18
1.17
1.17
1.16
1.14
1.13
1.09
1.09
1.08
1.06
1.06
1.05
0.00
TIPSO
S
S
S
OH
2.01
1.98
1.00
1.03
2.00
2.08
21.06
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5


145.25
143.03
137.50
135.94
126.19
124.29
123.95
123.85
123.21
123.18
77.34
77.02
76.70
61.27
60.17
18.01
12.06
12.05
0.01
TIPSO
S
S
S
OH
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0