**Supporting information for**

# Esterase-sensitive and pH-controlled Carbon Monoxide Prodrugs for Treating Systemic Inflammation

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## 1. The synthesis of compound 2 and 3

*Synthesis of (Z)-3-(phenylsulfonyl)prop-2-en-1-ol (**2**)*

To a solution of methyl (Z)-3-(phenylsulfonyl)acrylate (**1**, 2.0 mmol, 452 mg) in dichloromethylene (15 mL) at -78 °C was slowly added DIBAL solution in dichloromethylene (1M, 6 mL). The reaction was then slowly warmed to room temperature and stirred for another 2 h. The reaction was then carefully quenched with a saturated solution of potassium sodium tartrate (20 mL), and the resulting mixture was stirred at room temperature for 30 min. The organic layer was separated, and the aqueous layer was extracted with $CH_2Cl_2$ (2 × 30 mL). The combined organic phase was dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure. The resulting residue was purified on a silica gel column (hexane: EtOAc = 2:1) to afford the desired compound **2** as colorless oil in 43% yield (170 mg). $^1$H NMR ($CDCl_3$): δ 7.95 – 7.87 (m, 2H), 7.70 – 7.65 (m, 1H), 7.64 – 7.49 (m, 2H), 6.54 – 6.41 (m, 1H), 6.27 -6.31 (m, 1H), 4.77 (dd, J = 5.5, 2.0 Hz, 2H), 2.43 (s, 1H) ppm. $^{13}$C NMR ($CDCl_3$): δ 146.2, 140.6, 133.8, 129.7, 129.5, 127.4, 58.5 ppm. HRMS calcd for $C_9H_{10}O_3SNa$ $[M+Na]^+$: 221.0244; found: 221.0248.

*Synthesis of 3-(phenylsulfonyl)acrylaldehyde (**3**)*

To a solution of compound **2** (1.0 mmol, 198 mg) in dichloromethylene (5 mL) at room temperature was added DMP (1.5 mmol, 640 mg) and the mixture was stirred at room temperature for 1 h. The solution was then filtered through a short column (4 cm) filled with silica gel, and the filtrate was concentrated to afford the crude product 3-(phenylsulfonyl)acrylaldehyde (**3**) as a colorless oil, which was used for the next step without further purification. $^1$H NMR ($CDCl_3$): δ 10.86 (d, *J* = 6.9 Hz, 1H), 8.01-7.92 (m, 2H), 7.82 – 7.69 (m, 1H), 7.69 – 7.53 (m, 2H), 7.04 (d, *J* = 11.6 Hz, 1H), 6.33 (dd, *J* = 11.6, 6.9 Hz, 1H). $^3$C NMR ($CDCl_3$): δ 189.8, 142.7, 136.2, 134.7, 129.9, 128.1 ppm.

## 2. The CO release of 6a/c in the presence of porcine liver esterase

3',6'-dimethyl-[1,1':2',1''-terphenyl]-4'-carbaldehyde (**7**). A solution of **6a-c** (6 mg) and porcine liver esterase (15 mg) in 30% of DMSO/PBS (50 mL) was incubated at 37 °C for 48 h. Then EtOH (60 mL) was added, and the solution was centrifuged to get rid of the protein. The obtained solution was concentrated under vacuum and was acidified with 5% of HCl. Then the resulting mixture was extracted with ethyl acetate (3 × 20 mL). The obtained organic layer was dried over anhydrous

$Na_2SO_4$. After filtration and concentration, the residue was purified on a silica gel column (hexane: EtOAc = 10 :1) to afford the **7** as a white solid. $^1$H NMR ($CDCl_3$): δ 10.42 (s, 1H), 7.79 (s, 1H), 7.11-7.19 (m, 6H), 6.98 – 6.85 (m, 4H), 2.40 (s, 3H), 2.15 (s, 3H) ppm. $^{13}$C NMR ($CDCl_3$): δ 192.9, 147.3, 143.4, 139.9, 139.6, 136.3, 134.3, 133.3, 131.8, 130.1, 129.2, 127.6, 127.6, 126.4, 126.4, 20.7, 16.3 ppm. HRMS calcd for $C_{21}H_{18}ONa$ $[M+Na]^+$: 309.1255; found: 309.1248.

## 3. Myoglobin-CO assay

A previously reported myoglobin-CO assay (two-compartment assay) was employed to confirm CO release from **5**.[1] Specifically, a myoglobin solution in PBS (0.01 M, pH = 7.4) (1.7 mg/mL, 2.9 mL) was degassed by bubbling with nitrogen for at least 20 min and a freshly prepared solution of sodium dithionite (17 mg/mL, 300 μL) was added to the myoglobin solution. Then the CO prodrug (10 mM, 0.1 mL) was added to the inner vial. After incubation for 2 h at 37 °C, the solution was cooled in an ice bath for 10 min to increase the solubility of CO in water. Then the UV-vis absorption spectra was recorded (Figure S1). The maximal absorption peak of deoxy-Mb at 560 nm is converted to two maximal absorption peaks of Mb-CO at 540 and 578 nm, indicating the formation of CO bounded myoglobin when it is treated with **5.**

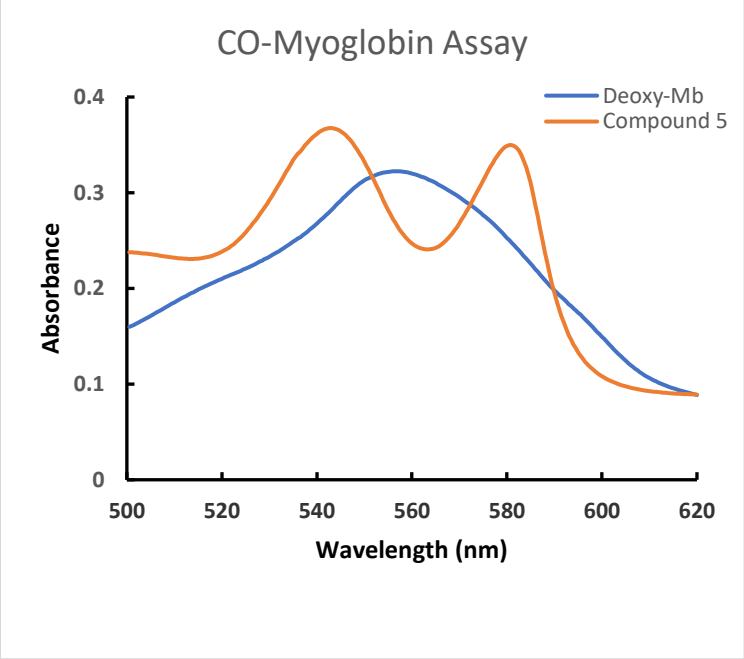



**Figure S1.** CO myoglobin assay. Blue curve: absorbance for deoxy-Mb. Orange curve: deoxy-Mb treated with compound **5**

## 4. CO release kinetics of 6a-d

A solution of compound **5** only (20 μM) or **6a-d** (20 μM) and porcine liver esterase (3 U/mL) in 5% of DMSO in PBS (pH = 7.4) was sealed and incubated at 37 °C. At each defined time point, 250 μL of the reaction mixture was taken out and added into a vial containing 500 μL ethanol. The mixture was incubated in an acetone dry ice bath (-78 °C) for 5 min, and centrifuged for 9 min (14.5 × 1000 rp) to precipitate out the esterase. The resulting supernatant was then analyzed by HPLC (column: Waters C18 3.5 μM, 4.6×100 mm, injection loop volume: 20μL). CO release was determined by monitoring the formation of the product **7**. The mobile phase was acetonitrile ACN/$H_2O$ (containing 0.05% trifluoroacetic acid). Detailed conditions are summarized in Table S1, and the results are summarized in Figures S2-6.

**Table S1.** The HPLC condition used for analysis

| | Compound **5** | **6a** | **6b** | **6c** | **6d** |
|---|---|---|---|---|---|
| Eluent conditions | 0~8 min, 85% ~ 95% ACN; 8-10 min, 95% ~ 85% | | | | |
| $t_R$ (min) | Prodrug: 3.2 ±0.2; Product: 4.6 ± 0.2; | Prodrug: 3.4 ± 0.2; Intermediate: 3.2 ±0.2; Product: 4.6 ± 0.2; | Prodrug: 5.6 ± 0.2; Intermediate: 2.9 ± 0.2, 3.3 ± 0.2; Product: 4.6 ± 0.2; | Prodrug: 3.8 ±0.2; Product: 4.6 ± 0.2; | Prodrug: 7.5 ±0.2; |

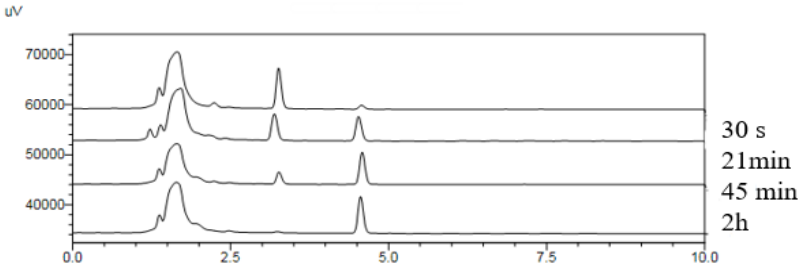



**Figure S2**. HPLC chromatogram of Compound **5**

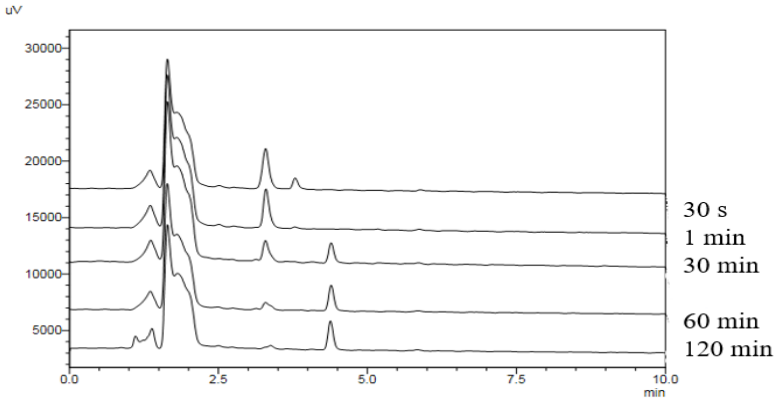



**Figure S3**. HPLC chromatogram of **6a**

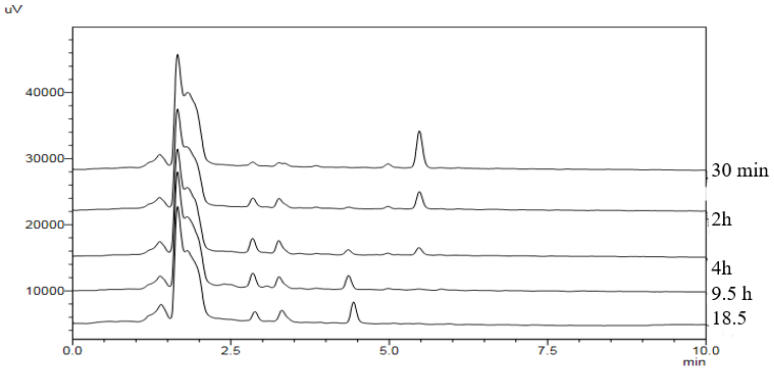



**Figure S4**. HPLC chromatogram of **6b**

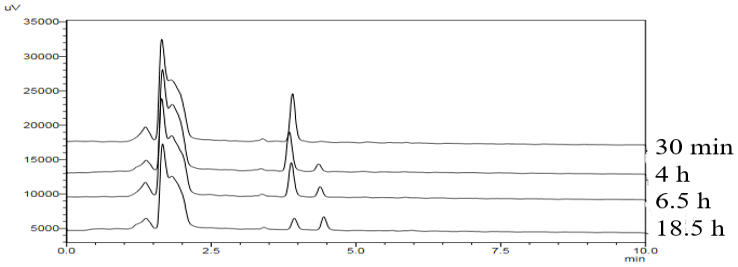



**Figure S5**. HPLC chromatogram of **6c**

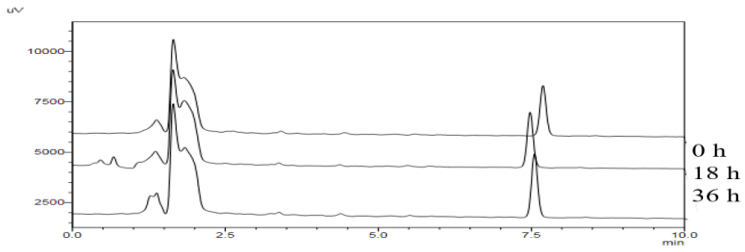



**Figure S6**. HPLC chromatogram of **6d**

## 5. Stability test of 6a-d

A solution of **6a-d** (20 μM) in 5% of DMSO in PBS (pH = 7.4) or Simulated Gastric Fluid (0.2% (w/v), sodium chloride in 0.7% (v/v), hydrochloric acid) was sealed and incubated at 37 °C. The stability was then analyzed by HPLC, and the results are summarized in Figure S7-10.

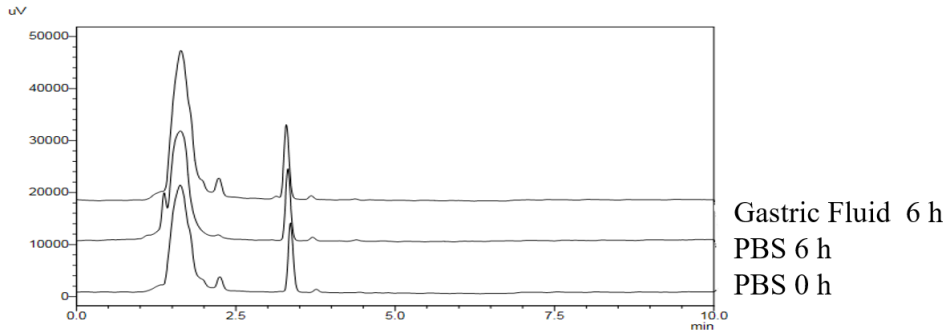



**Figure S7**. Stability study of **6a**

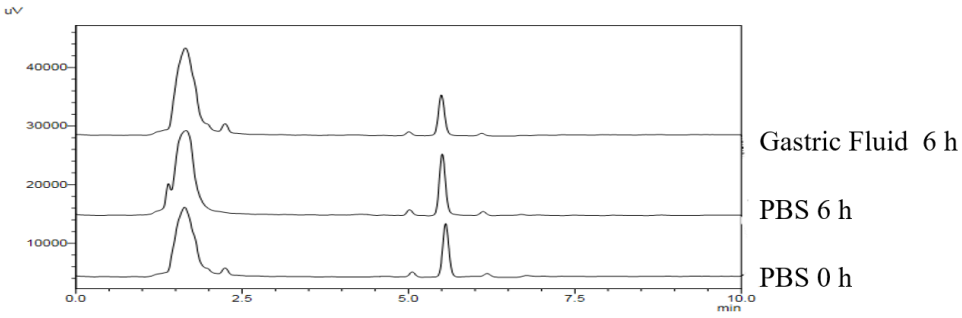



**Figure S8**. Stability study of **6b**

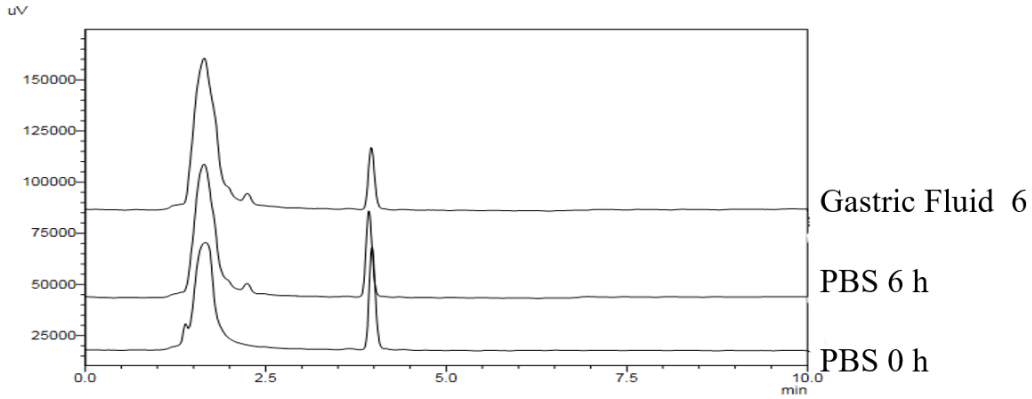



**Figure S9**. Stability study of **6c**

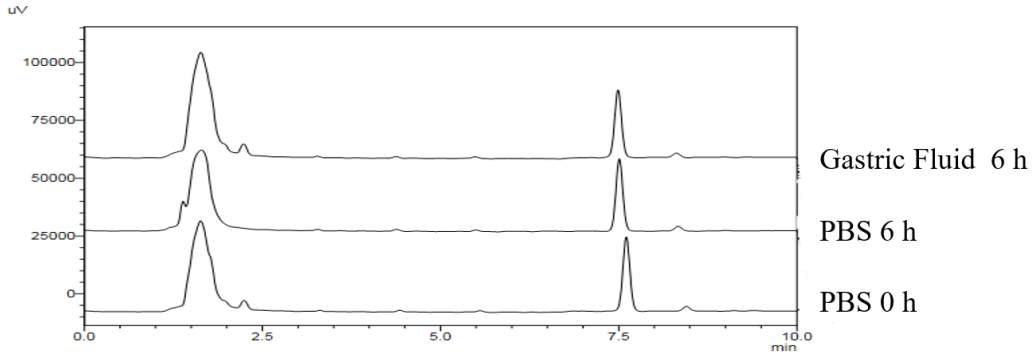



**Figure S10**. Stability study of **6d**

## 6. The CO release kinetics of 6a in serums

The CO release profiles of **6a** in mice, rat, rabbit and human plasma were tested using HPLC. **6a** was dissolved in DMSO to make a 10mM stock solution. 2.85 mL of plasma was added to a 10 mL EP tube and pre-incubated at 37 °C for 5 min. 150 μL of stock solution of **6a** was added to the plasma to yield a final concentration of 500 μM with 5% of DMSO. The spiked plasma samples were incubated at 37 °C. 100 μL aliquot of the incubation mixture was removed and was diluted with 300 μL of acetonitrile at different time points. The samples were vortexed and centrifuged at 20000 rpm for 10 min at 4°C. The supernatant was transferred to an auto-sampler vails for HPLC analysis (Agilent 1100 Series). HPLC conditions: column: Chromplus C18 column (150 mm × 4.6 mm, 5μm); Eluent conditions: 75% of ACN for 1 min, 75% to 90% of ACN in 5 min, 90% of ACN for 4 min, then 90% to 75% of ACN in 3.5min; Flow rate: 1 ml/min. Column temperature:

30 ℃. Detection wavelength: 220 nm. The results were summarized in Figures S11-18. Experiments were conducted in triplicate.

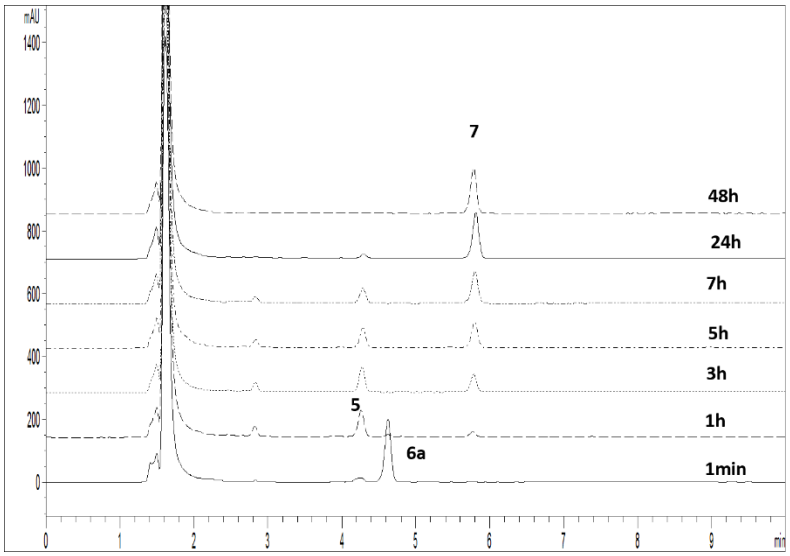



**Figure S11**. The CO release profile of **6a** in rabbit serum (HPLC chromatogram)

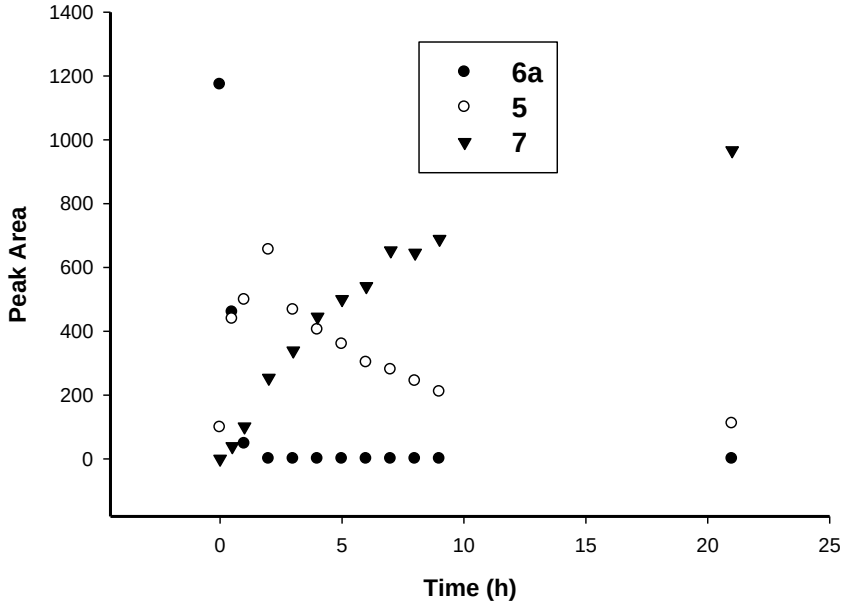



**Figure S12**. The plot of peak area of **6a**, **5** and **7** in Figure S11 against different time points.

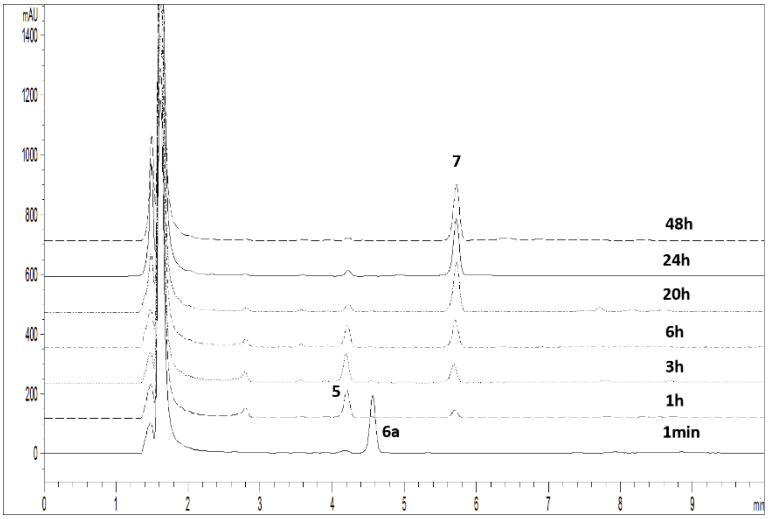



**Figure S13**. The CO release profile of **6a** in mouse serum (HPLC chromatogram)

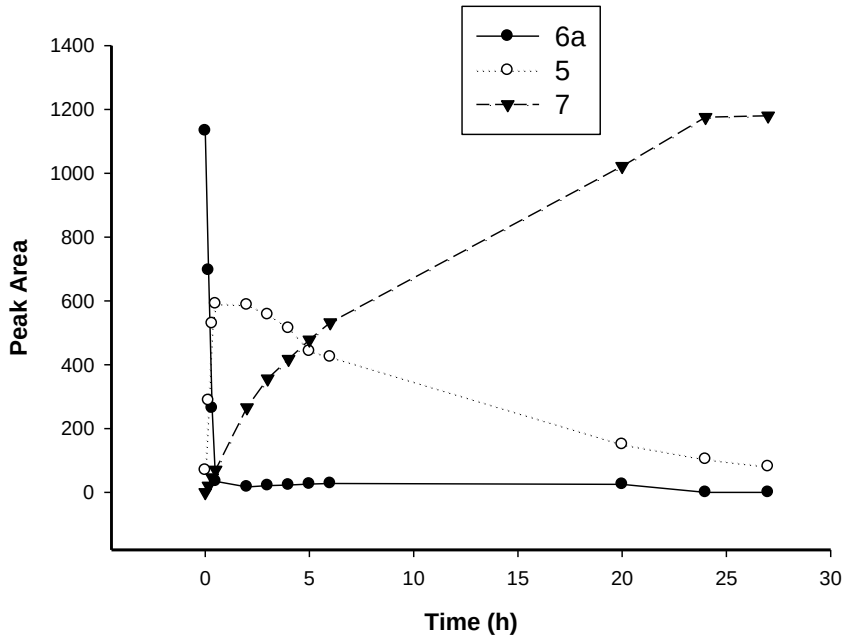



**Figure S12**. The plot of peak area of **6a**, **5** and **7** in Figure S13 against different time points.

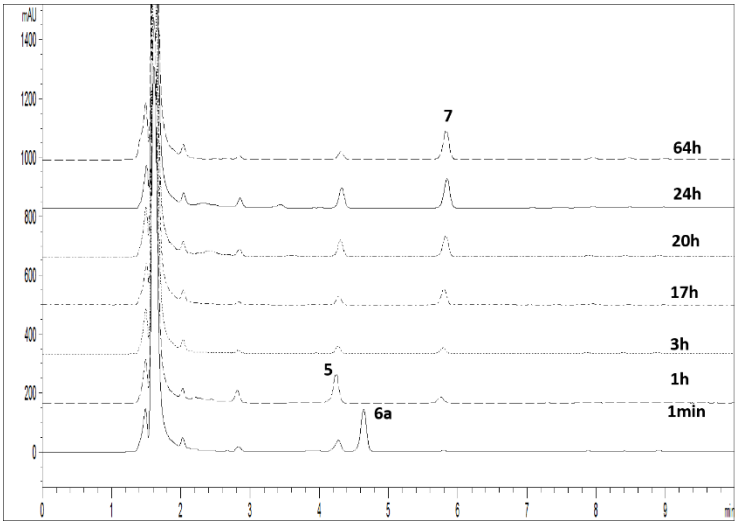



**Figure S15**. The CO release profile of **6a** in rat serum (HPLC chromatogram)

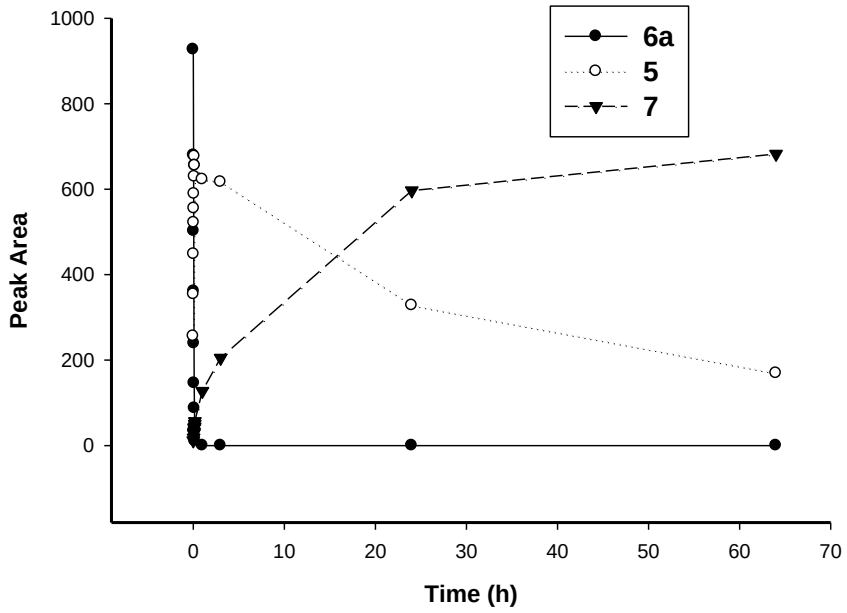



**Figure S16**. The plot of peak area of **6a**, **5** and **7** in Figure S15 against different time points.

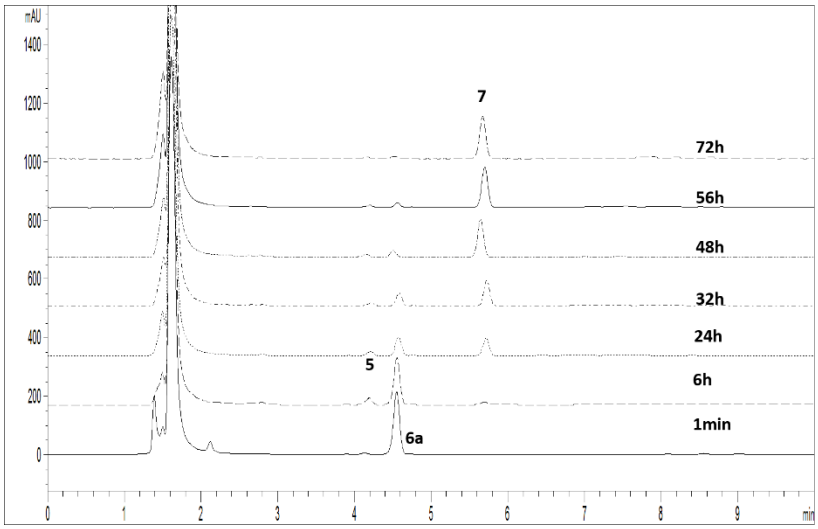



**Figure S17**. The CO release profile of **6a** in human serum (HPLC chromatogram)

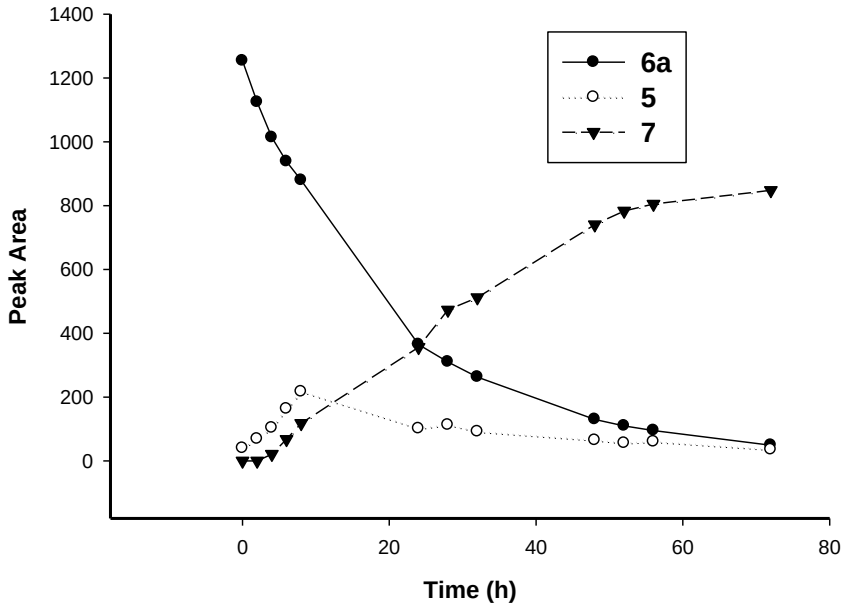
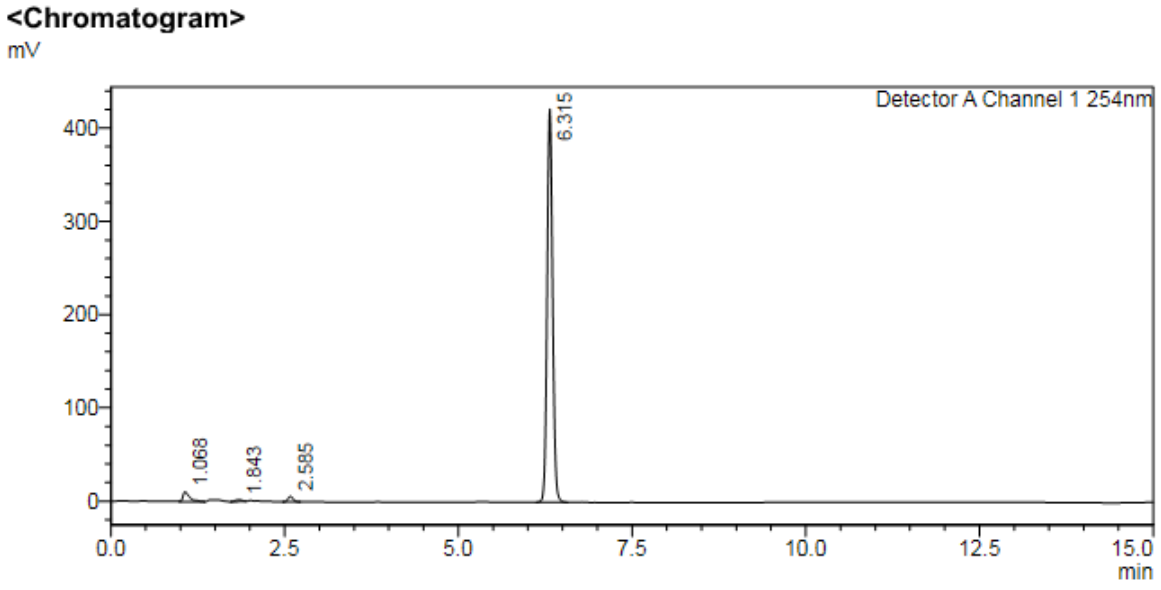


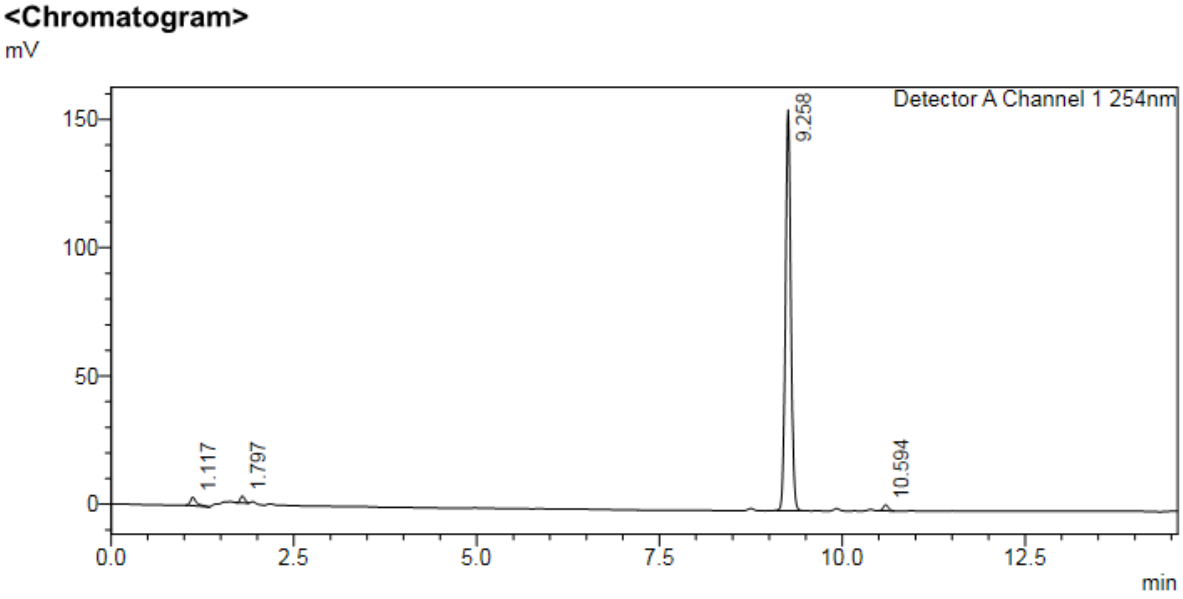

**Figure S18**. The plot of peak area of **6a**, **5** and **7** in Figure S17 against different time points.

## 7. Purity Study of final CO prodrugs




| Peak# | Ret. Time | Area | Height | Conc. | Unit | Mark | Name |
|---|---|---|---|---|---|---|---|
| 1 | 1.068 | 69486 | 10545 | 2.857 | | | |
| 2 | 1.843 | 11914 | 1734 | 0.490 | | | |
| 3 | 2.585 | 29837 | 5750 | 1.227 | | | |
| 4 | 6.315 | 2321276 | 421555 | 95.427 | | | |
| Total | | 2432514 | 439583 | | | | |

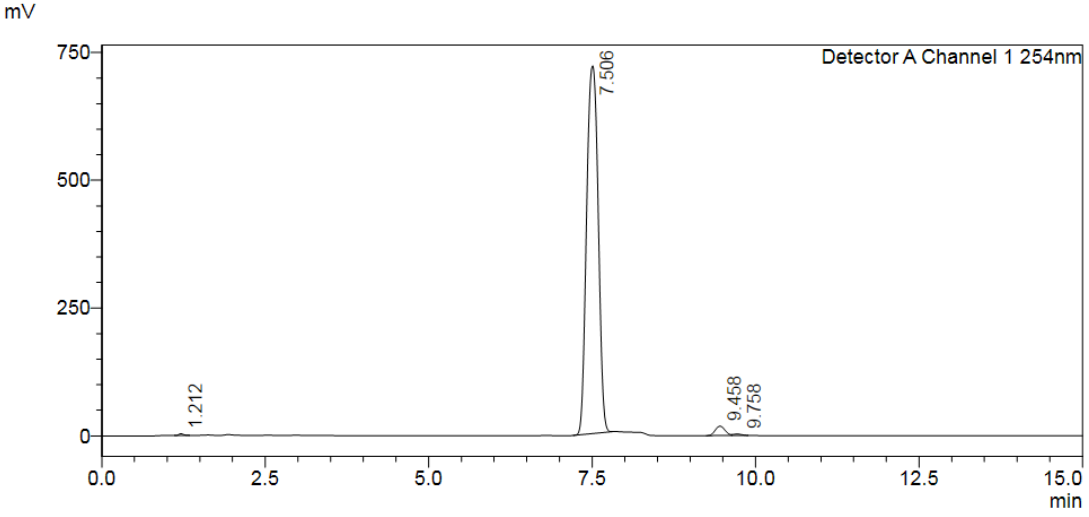
**Figure S19**. HPLC chromatogram of compound **6a**



| Peak# | Ret. Time | Area | Height | Conc. | Unit | Mark | Name |
|---|---|---|---|---|---|---|---|
| 1 | 1.117 | 19075 | 3330 | 2.246 | | | |
| 2 | 1.797 | 10621 | 2529 | 1.251 | | | |
| 3 | 9.258 | 807851 | 156157 | 95.124 | | | |
| 4 | 10.594 | 11714 | 2308 | 1.379 | | | |
| Total | | 849260 | 164324 | | | | |

**Figure S20**. HPLC chromatogram of compound **6b**

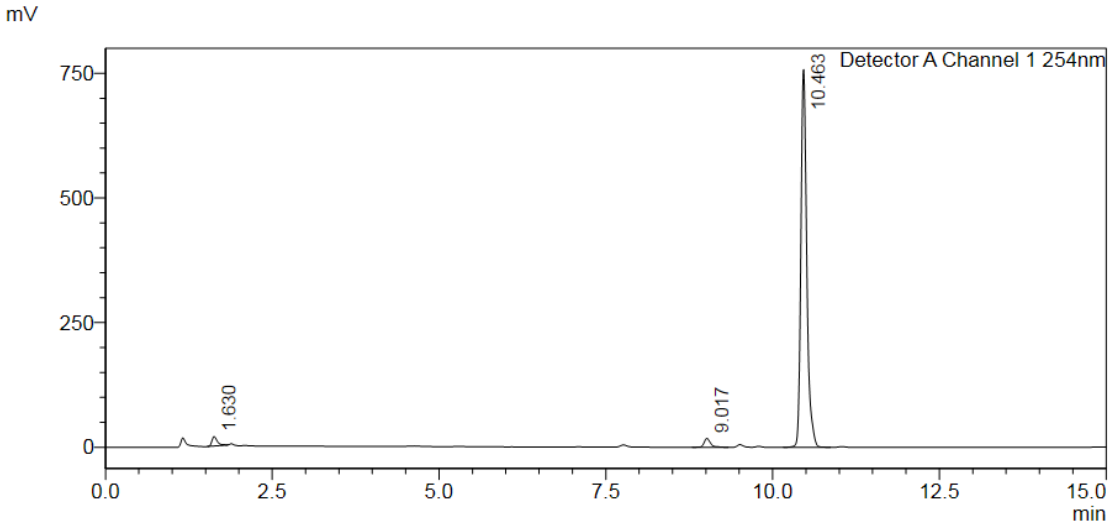
**<Chromatogram>**




| Peak# | Ret. Time | Area | Height | Conc. | Unit | Mark | Name |
|---|---|---|---|---|---|---|---|
| 1 | 1.212 | 14949 | 3021 | 0.167 | | | |
| 2 | 7.506 | 8743648 | 719633 | 97.468 | | | |
| 3 | 9.458 | 189049 | 18203 | 2.107 | | | |
| 4 | 9.758 | 23098 | 2173 | 0.257 | | V | |
| Total | | 8970743 | 743031 | | | | |

**Figure S21**. HPLC chromatogram of compound **6c**

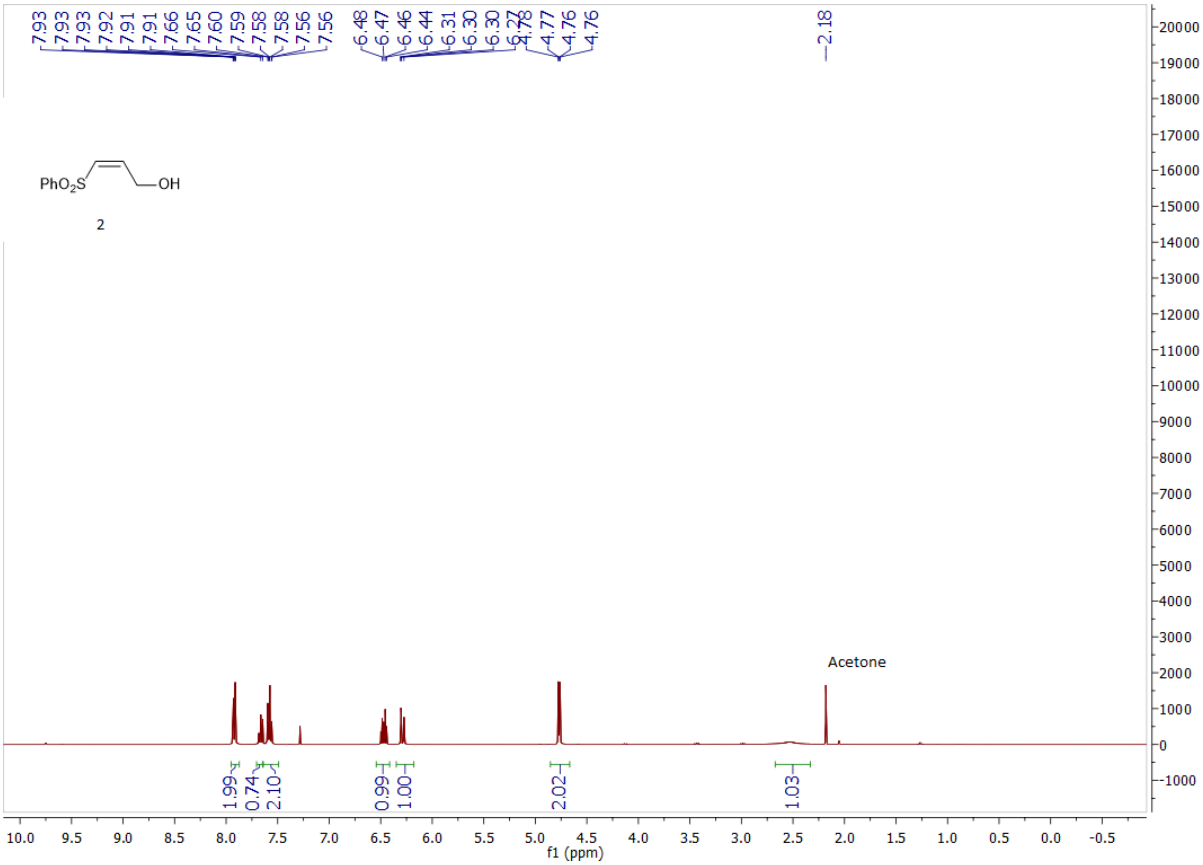
<Chromatogram>

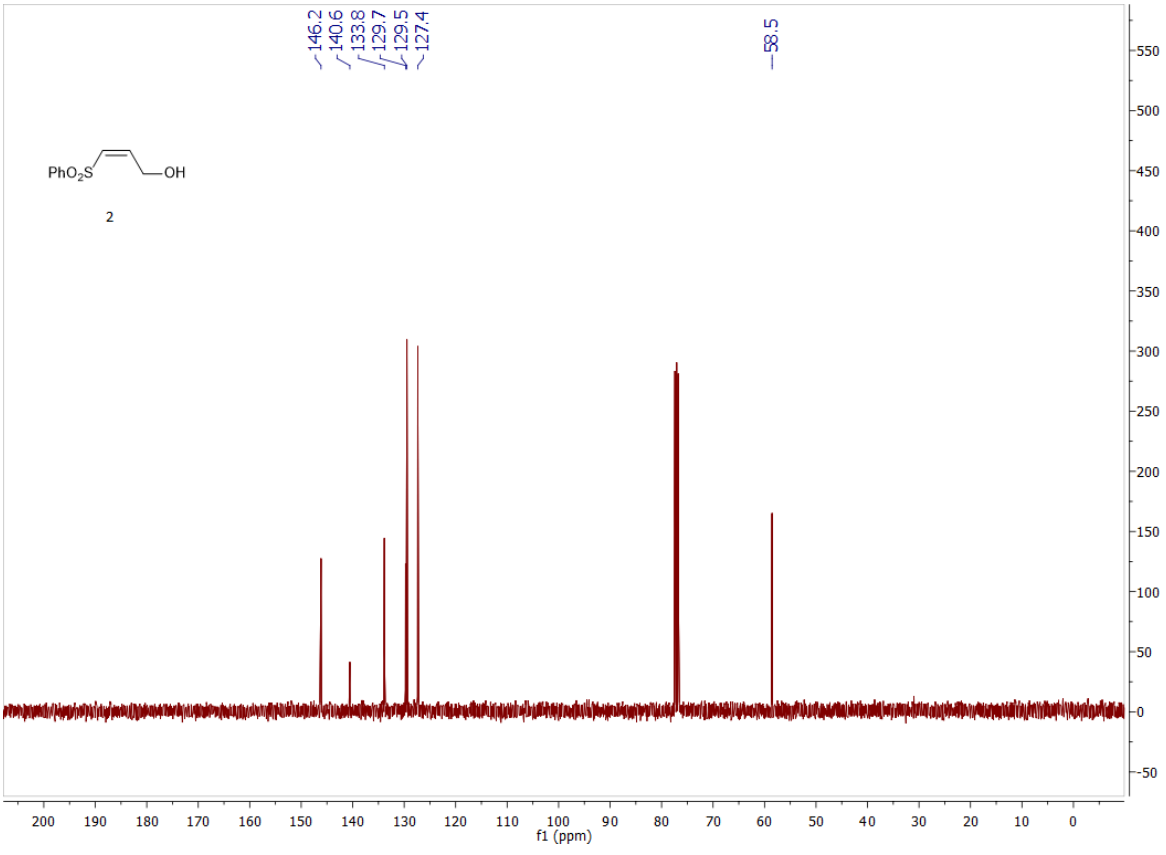


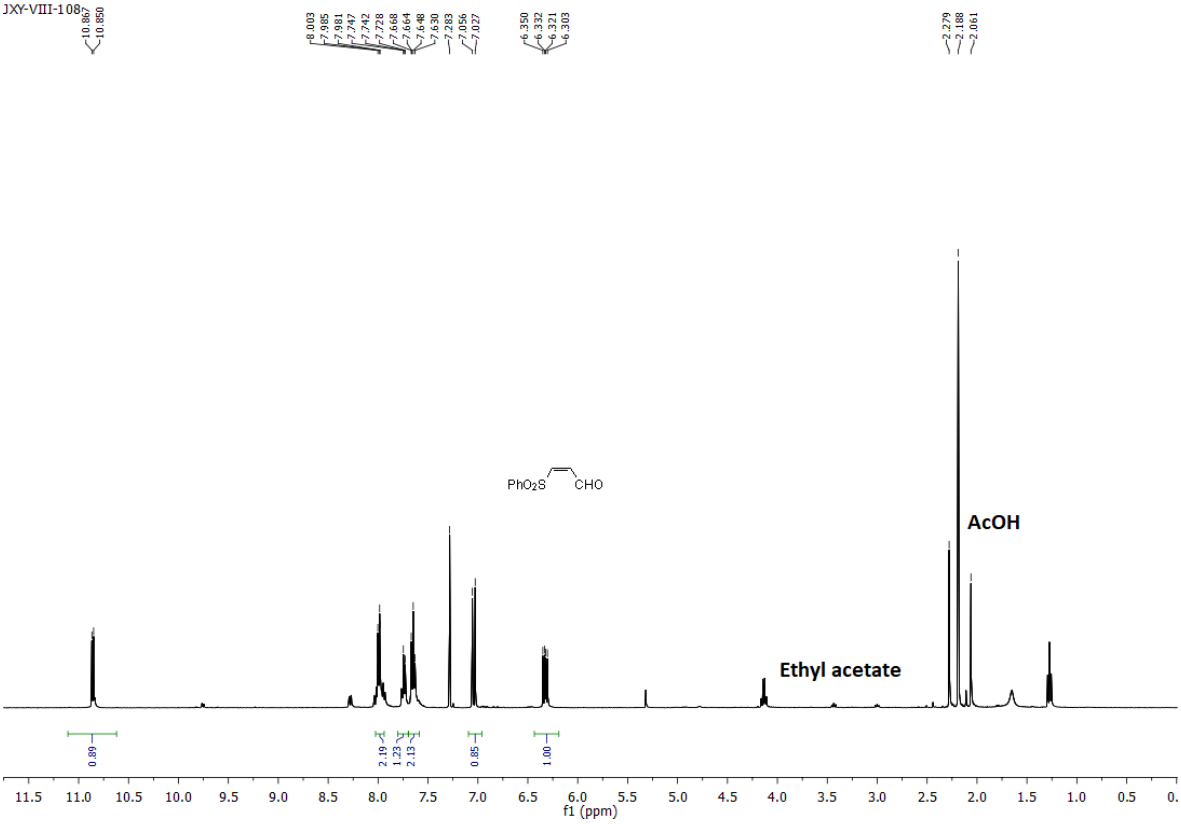

| Peak# | Ret. Time | Area | Height | Conc. | Unit | Mark | Name |
|---|---|---|---|---|---|---|---|
| 1 | 1.630 | 108542 | 17885 | 2.209 | | | |
| 2 | 9.017 | 116853 | 16937 | 2.378 | | | |
| 3 | 10.463 | 4687856 | 718109 | 95.413 | | | |
| Total | | 4913251 | 752932 | | | | |

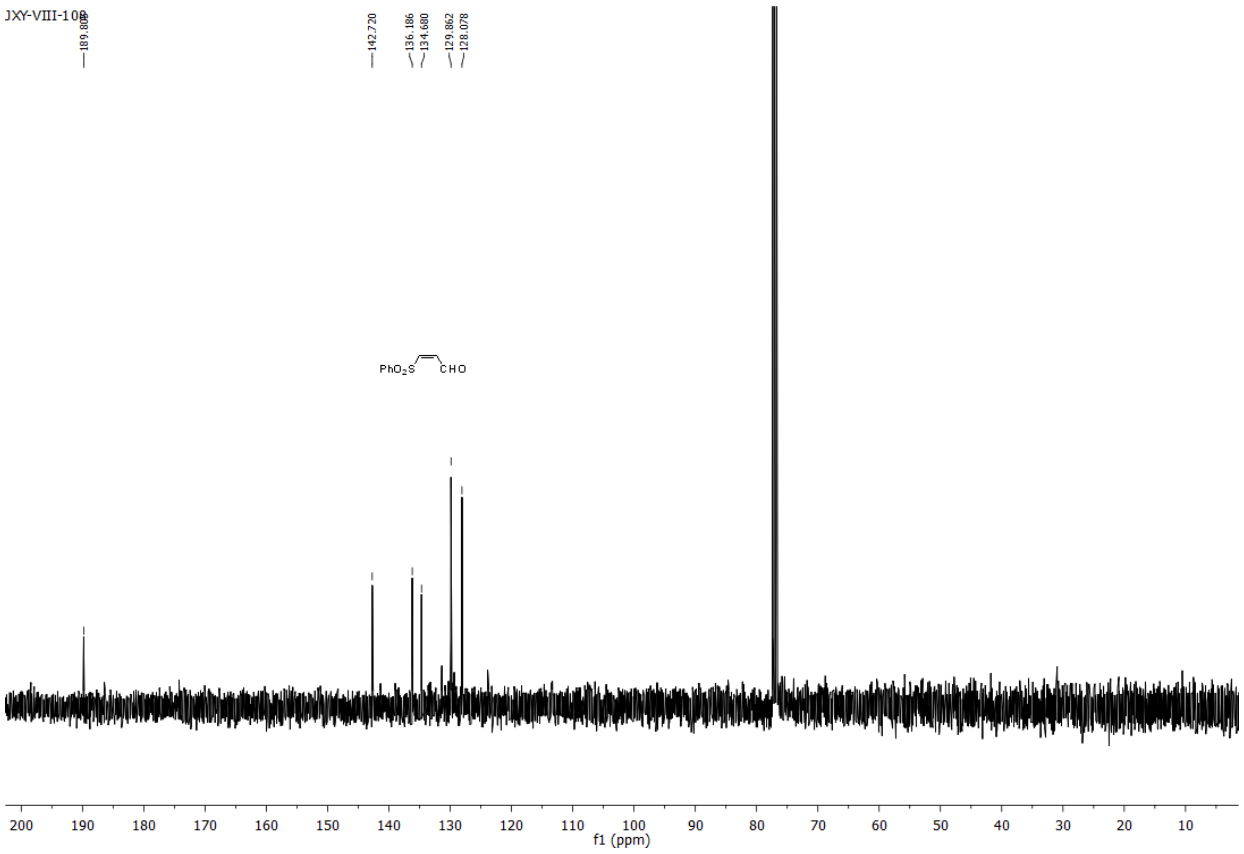
**Figure S22**. HPLC chromatogram of compound **6d**

## 8. NMR spectra


7.93
7.93
7.93
7.92
7.91
7.91
7.66
7.65
7.60
7.59
7.58
7.58
7.56
7.56
6.48
6.47
6.46
6.44
6.31
6.30
6.30
6.27
6.26
4.78
4.77
4.76
4.76
2.18
PhO2S
OH
2
Acetone
1.99
0.74
2.10
0.99
1.00
2.02
1.03
f1 (ppm)


146.2
140.6
133.8
129.7
129.5
127.4
58.5
PhO2S
OH
2
f1 (ppm)


JXY-VIII-108
10.867
10.850
8.003
7.985
7.981
7.747
7.742
7.728
7.668
7.664
7.648
7.630
7.283
7.056
7.027
6.350
6.332
6.321
6.303
2.279
2.188
2.061
PhO2S
CHO
AcOH
Ethyl acetate
0.89
2.19
1.23
2.13
0.85
1.00
11.5
11.0
10.5
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.
f1 (ppm)


JXY-VIII-10
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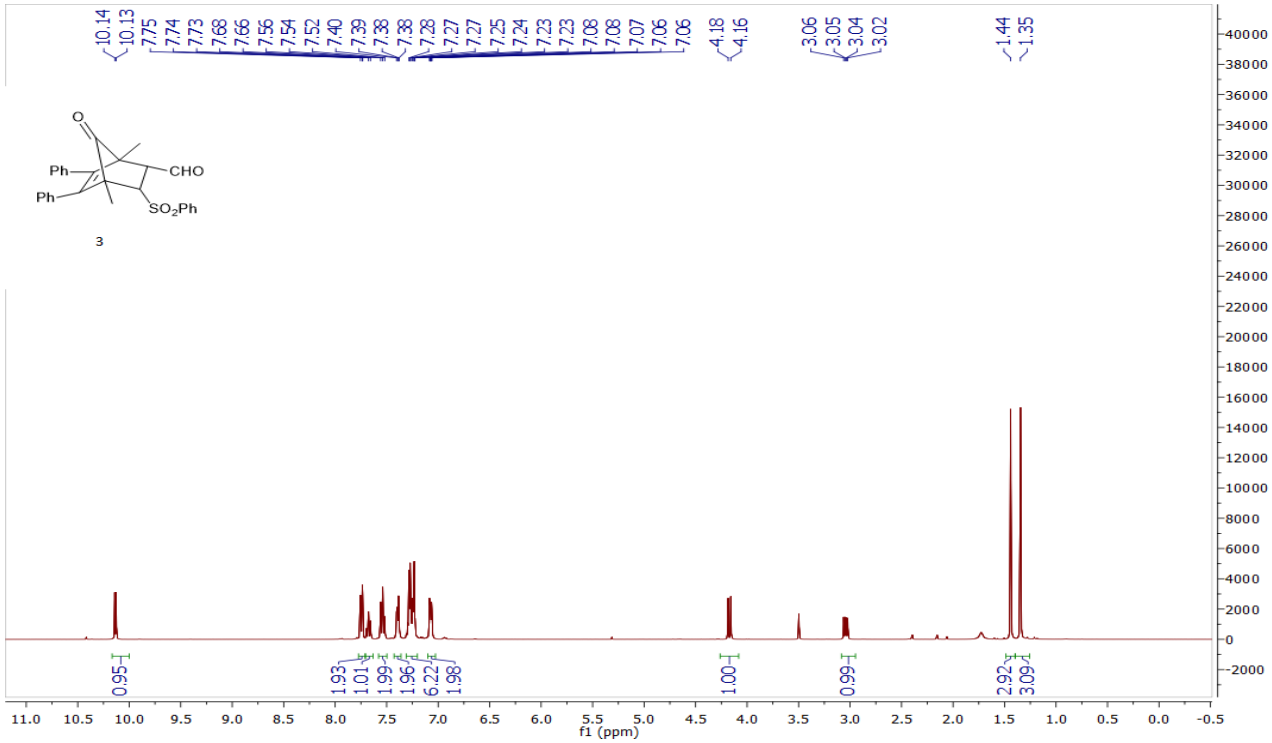
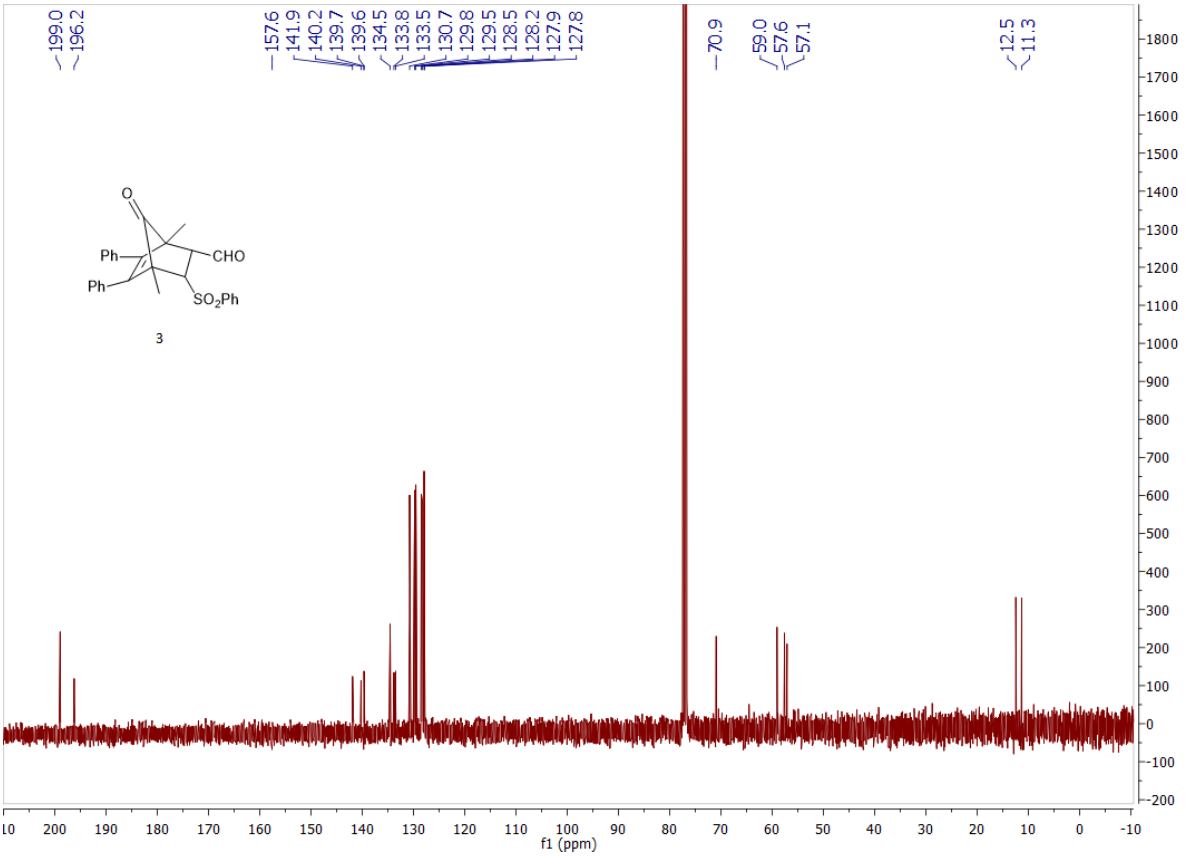
136.186
134.680
129.862
128.078
PhO2S
CHO
f1 (ppm)


10.14
10.13
7.75
7.74
7.73
7.68
7.66
7.56
7.54
7.52
7.40
7.39
7.38
7.38
7.28
7.27
7.27
7.25
7.24
7.23
7.23
7.08
7.08
7.07
7.06
7.06
4.18
4.16
3.06
3.05
3.04
3.02
1.44
1.35
O
Ph
Ph
CHO
SO2Ph
3
0.95
1.93
1.01
1.99
1.96
6.22
1.98
1.00
0.99
2.92
3.09
f1 (ppm)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196.2
157.6
141.9
140.2
139.7
139.6
134.5
133.8
133.5
130.7
129.8
129.5
128.5
128.2
127.9
127.8
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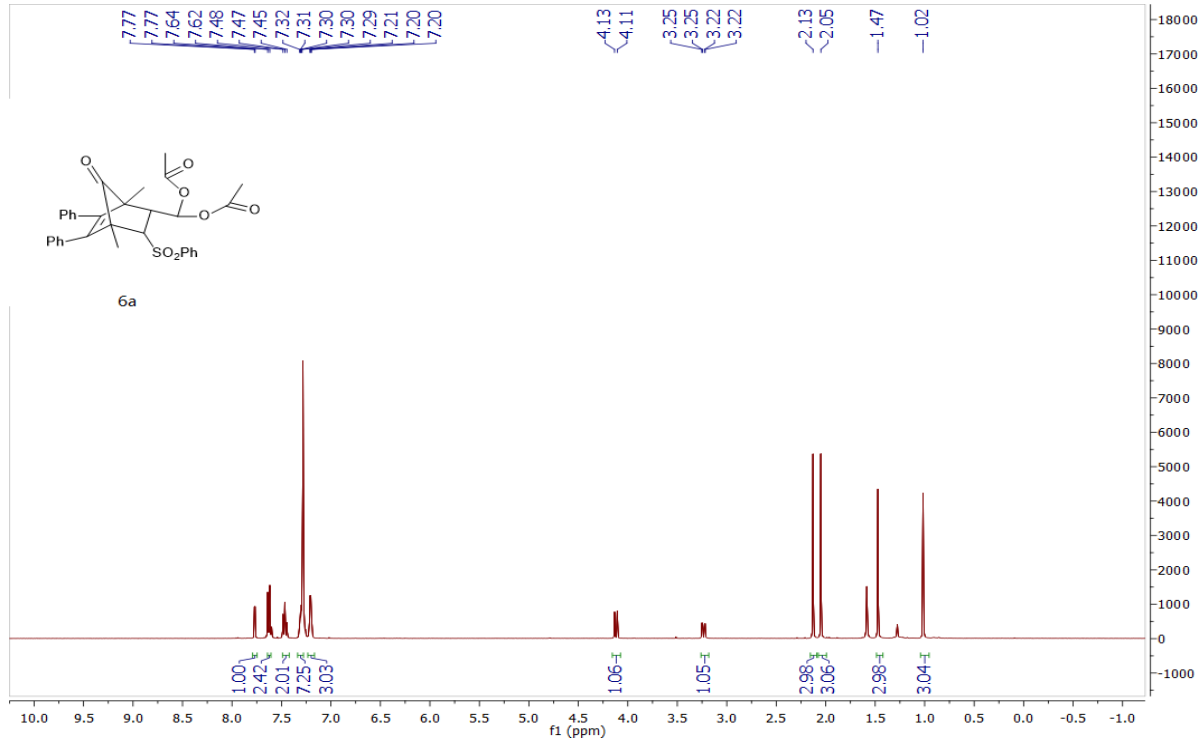
57.6
57.1
12.5
11.3
O
Ph
Ph
CHO
SO2Ph
f1 (ppm)

3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7.77
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7.47
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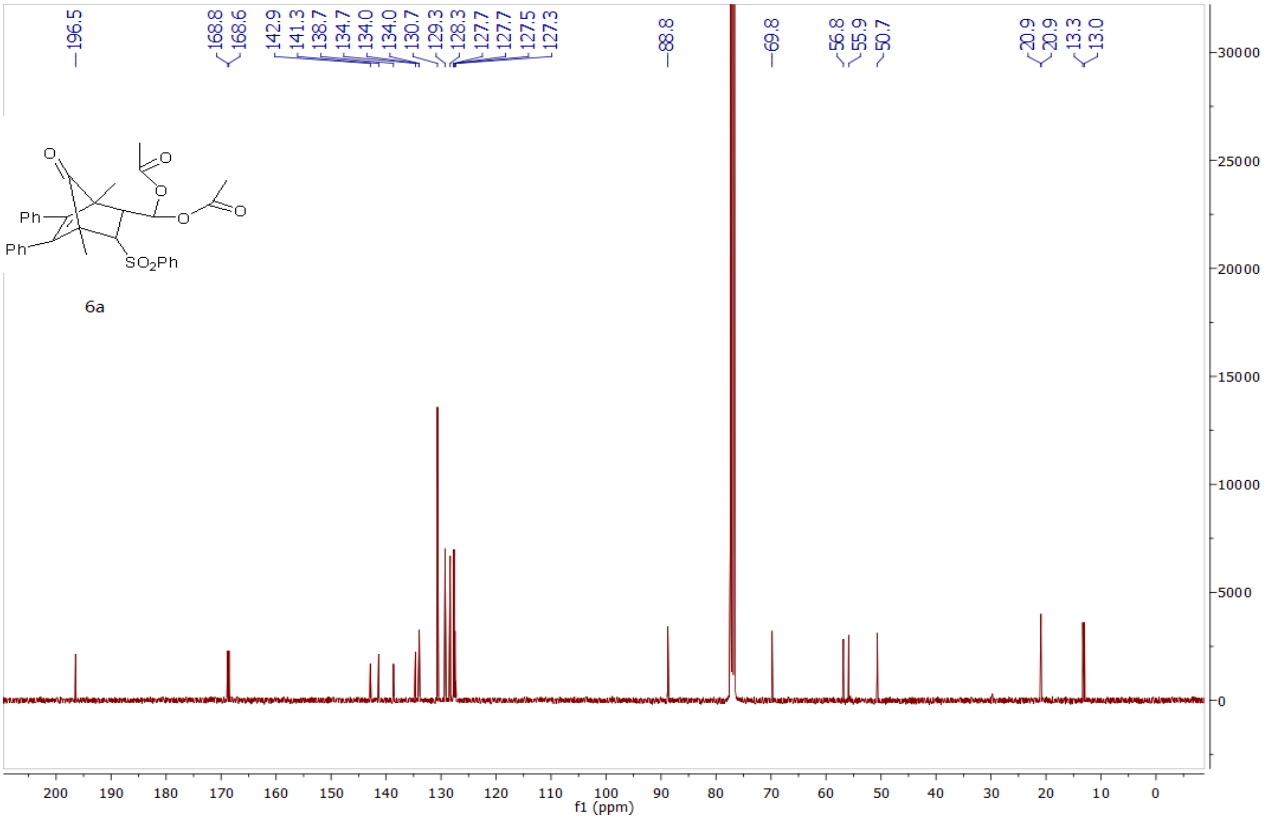
7.32
7.31
7.30
7.30
7.29
7.21
7.20
7.20
4.13
4.11
3.25
3.25
3.22
3.22
2.13
2.05
1.47
1.02
O
O
O
O
Ph
Ph
SO2Ph
1.00
2.42
2.01
7.25
3.03
1.06
1.05
2.98
3.06
2.98
3.04
f1 (ppm)

6a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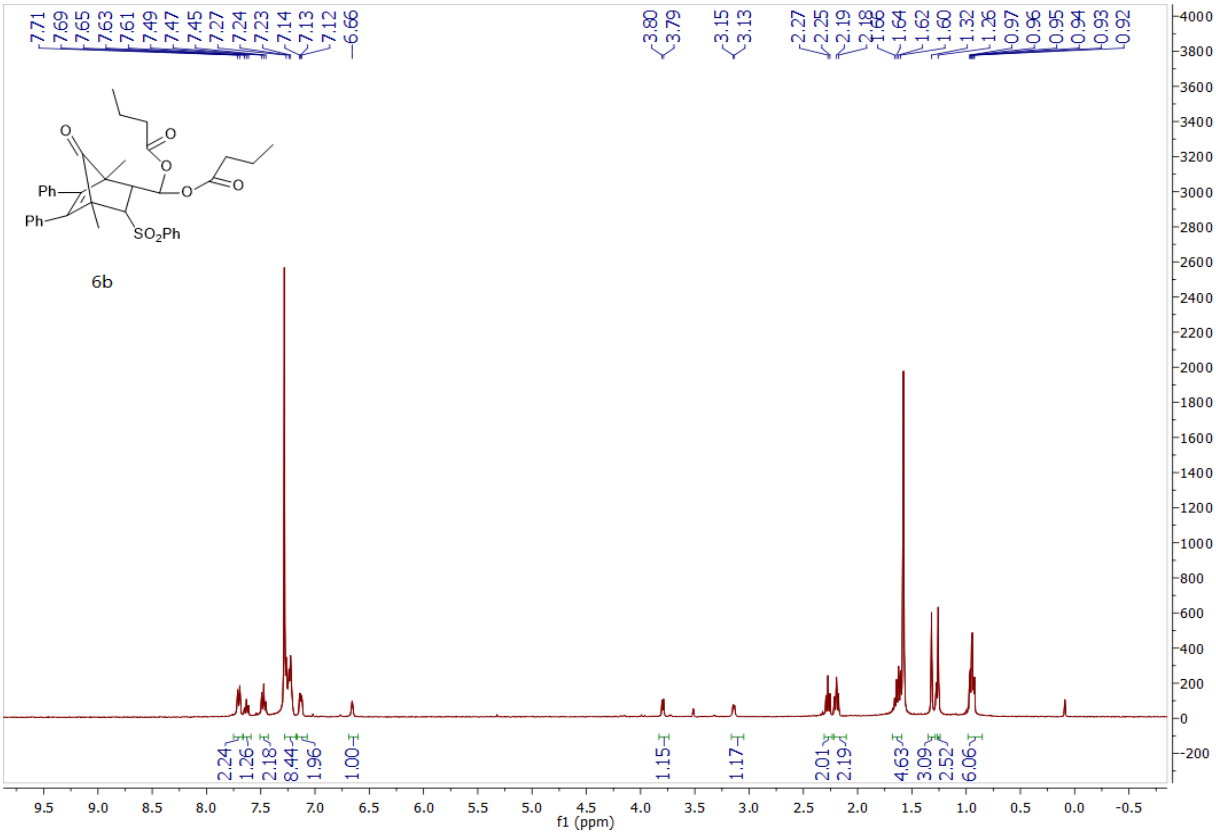
168.8
168.6
142.9
141.3
138.7
134.7
134.0
134.0
130.7
129.3
128.3
127.7
127.7
127.5
127.3
88.8
69.8
56.8
55.9
50.7
20.9
20.9
13.3
13.0
Ph
Ph
SO2Ph
6a
f1 (ppm)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7.61
7.49
7.47
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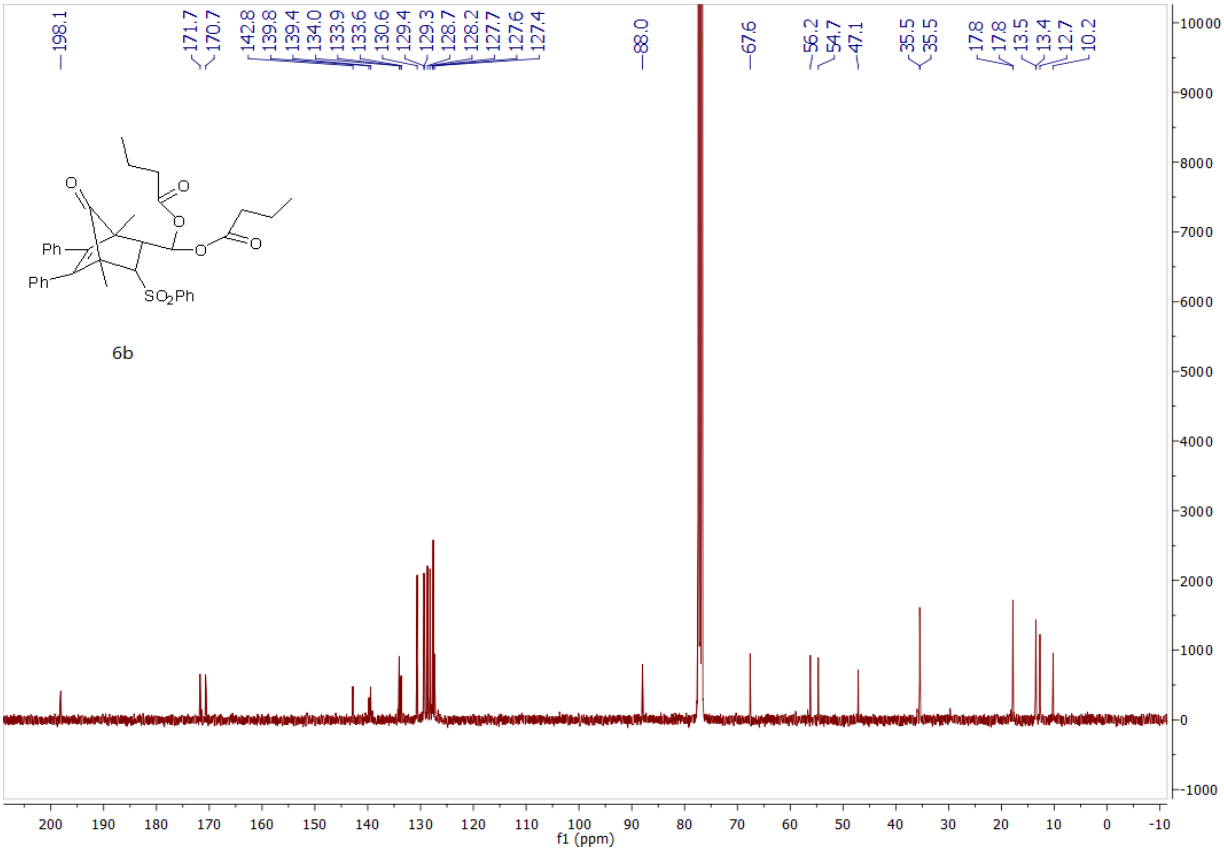
7.24
7.23
7.14
7.13
7.12
6.66
3.80
3.79
3.15
3.13
2.27
2.25
2.19
1.64
1.62
1.60
1.32
1.26
0.97
0.96
0.95
0.94
0.93
0.92
Ph
Ph
SO2Ph
6b
2.24
1.26
2.18
8.44
1.96
1.00
1.15
1.17
2.01
2.19
4.63
3.09
2.52
6.06
f1 (ppm)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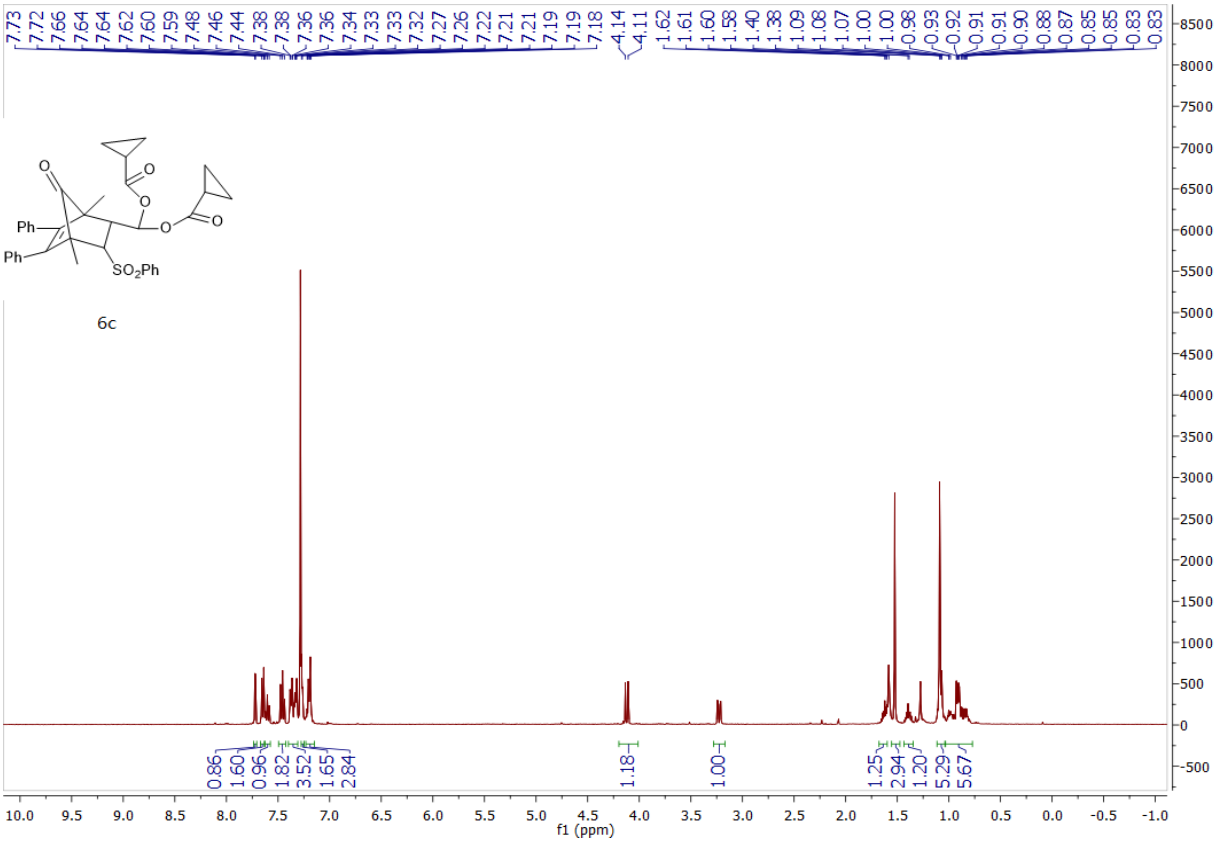
139.4
134.0
133.9
133.6
130.6
129.4
129.3
128.7
128.2
127.7
127.6
127.4
88.0
67.6
56.2
54.7
47.1
35.5
35.5
17.8
17.8
13.5
13.4
12.7
10.2
Ph
Ph
SO2Ph
6b
f1 (ppm)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7.62
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7.59
7.48
7.46
7.44
7.38
7.38
7.36
7.36
7.34
7.33
7.33
7.32
7.27
7.26
7.22
7.21
7.21
7.19
7.18
4.14
4.11
1.62
1.61
1.60
1.58
1.40
1.38
1.09
1.08
1.07
1.00
1.00
0.98
0.93
0.92
0.91
0.91
0.90
0.88
0.87
0.85
0.85
0.83
0.83
Ph
Ph
SO2Ph
6c
0.86
1.60
0.96
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3.52
1.65
2.84
1.18
1.00
1.25
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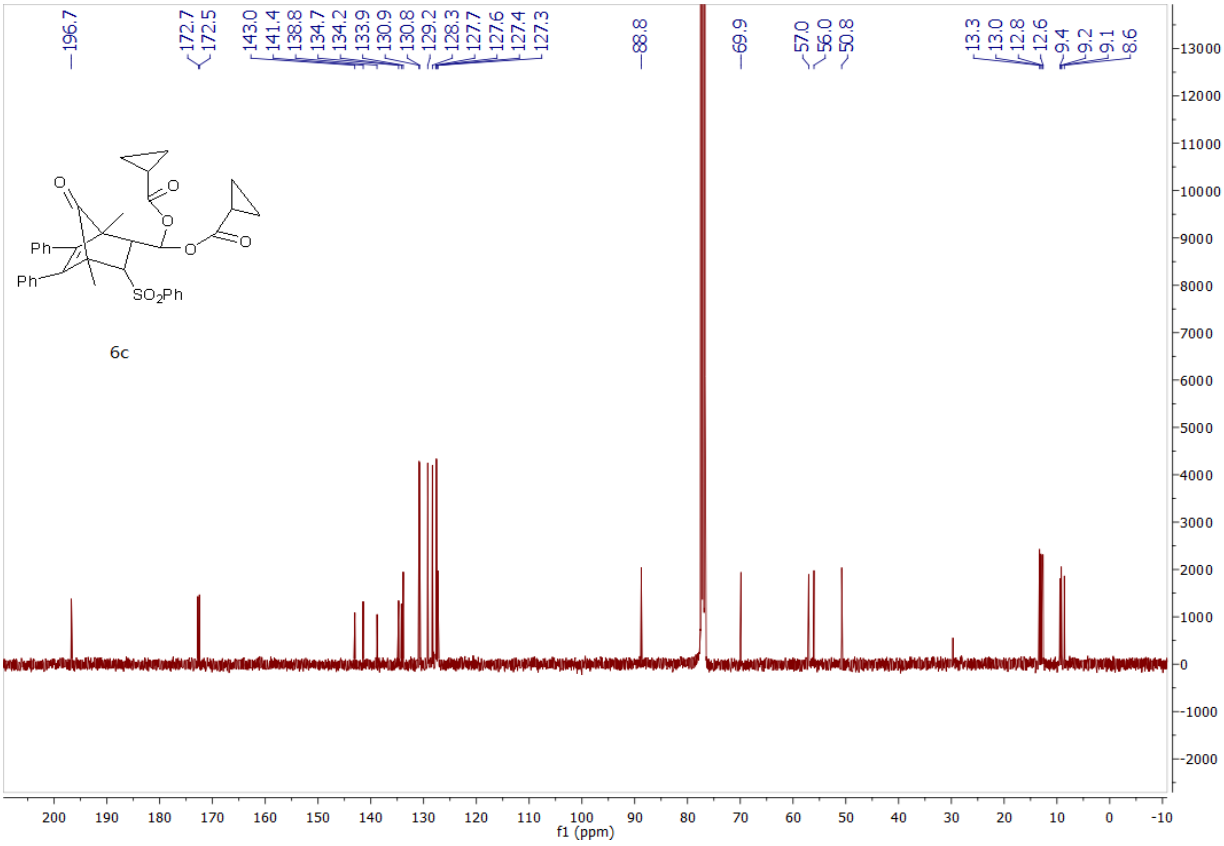
1.20
5.29
5.67
f1 (ppm)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141.4
138.8
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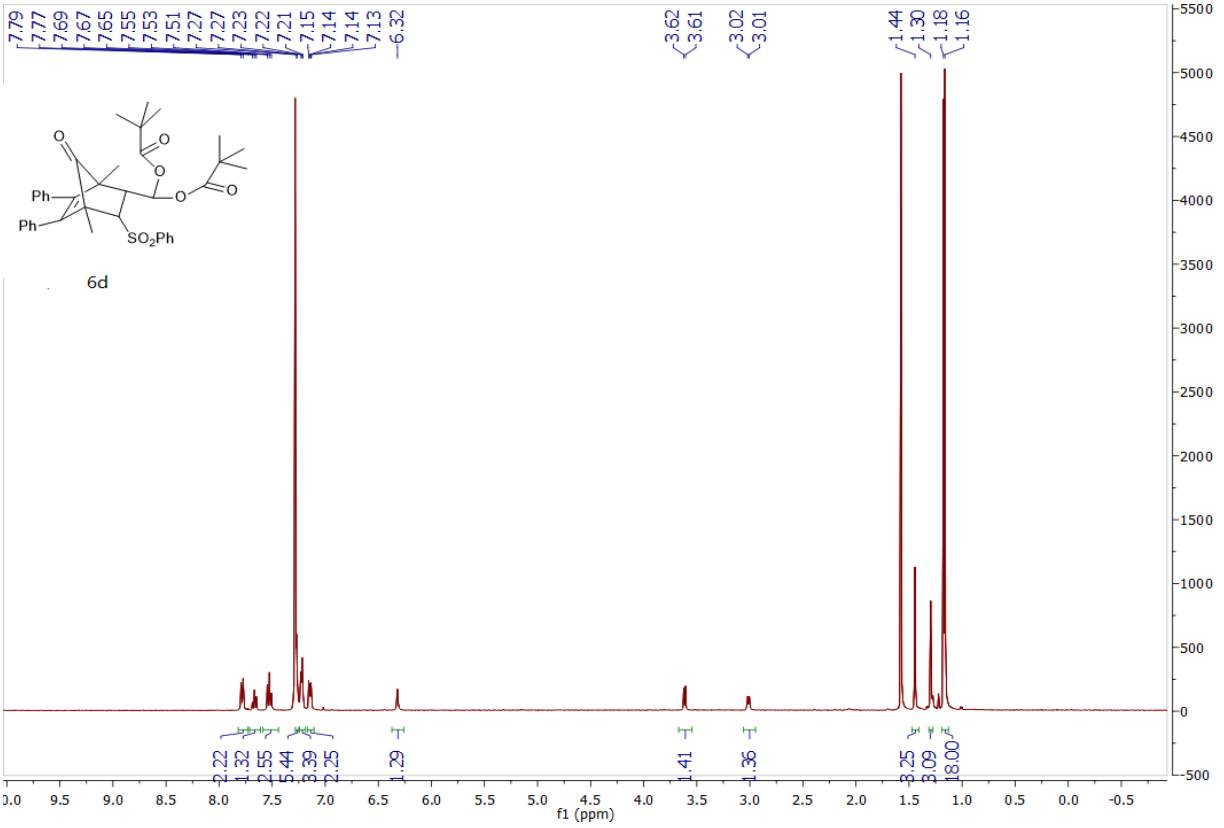
134.2
133.9
130.9
130.8
129.2
128.3
127.7
127.6
127.4
127.3
88.8
69.9
57.0
56.0
50.8
13.3
13.0
12.8
12.6
9.4
9.2
9.1
8.6
Ph
Ph
SO2Ph
f1 (ppm)

6c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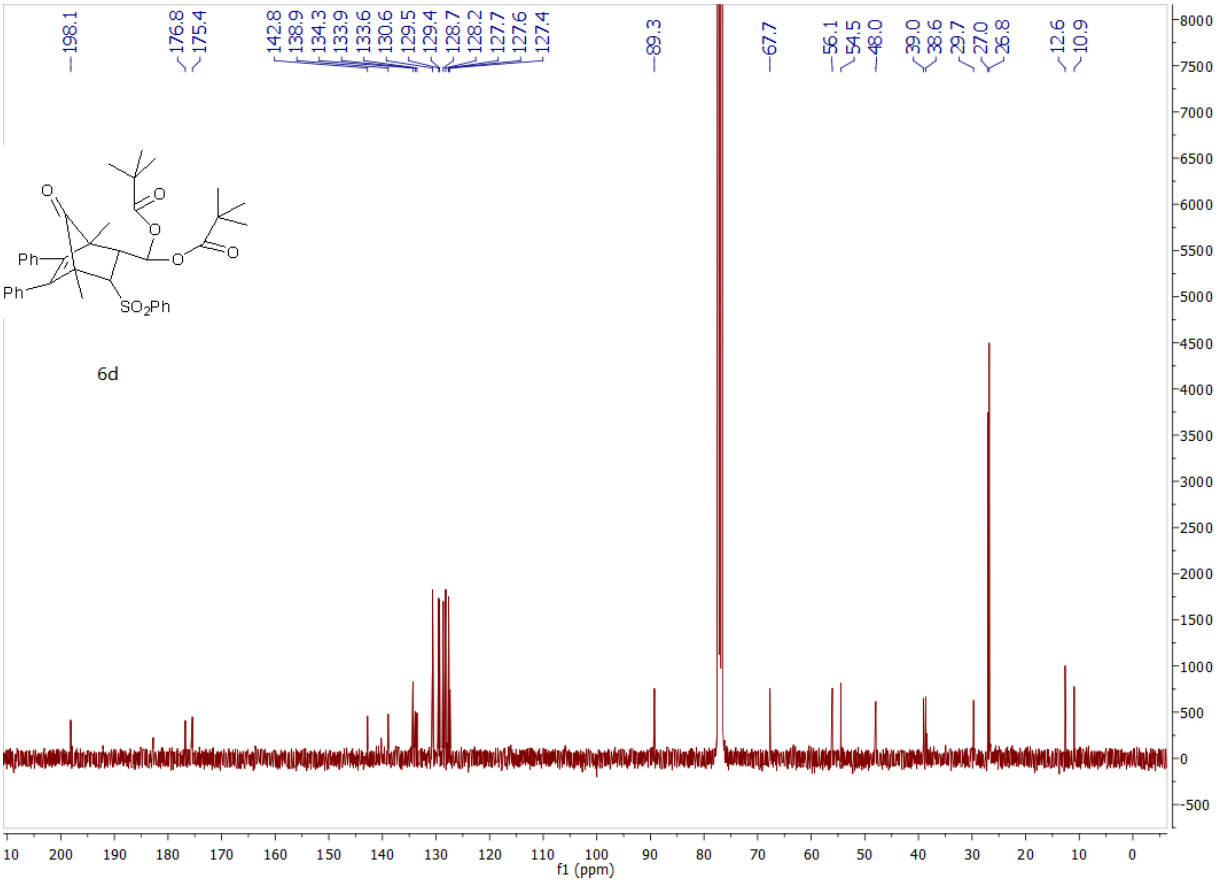
7.55
7.53
7.51
7.27
7.27
7.23
7.22
7.21
7.15
7.14
7.13
6.32
3.62
3.61
3.02
3.01
1.44
1.30
1.18
1.16
Ph
Ph
SO2Ph
2.22
1.32
2.55
5.44
3.39
2.25
1.29
1.41
1.36
8.25
3.09
18.00
f1 (ppm)

6d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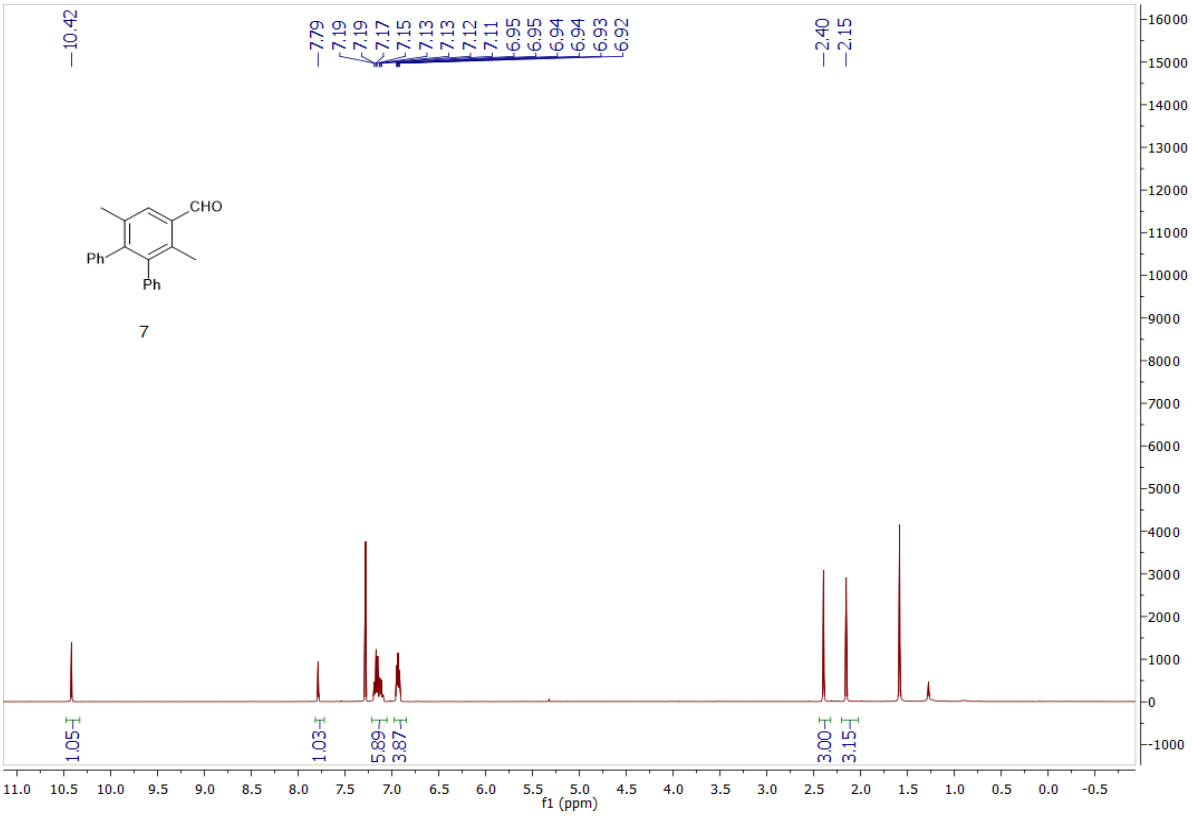
176.8
175.4
142.8
138.9
134.3
133.9
133.6
130.6
129.5
129.4
128.7
128.2
127.7
127.6
127.4
89.3
67.7
56.1
54.5
48.0
39.0
38.6
29.7
27.0
26.8
12.6
10.9
Ph
Ph
SO2Ph
6d
f1 (ppm)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7.19
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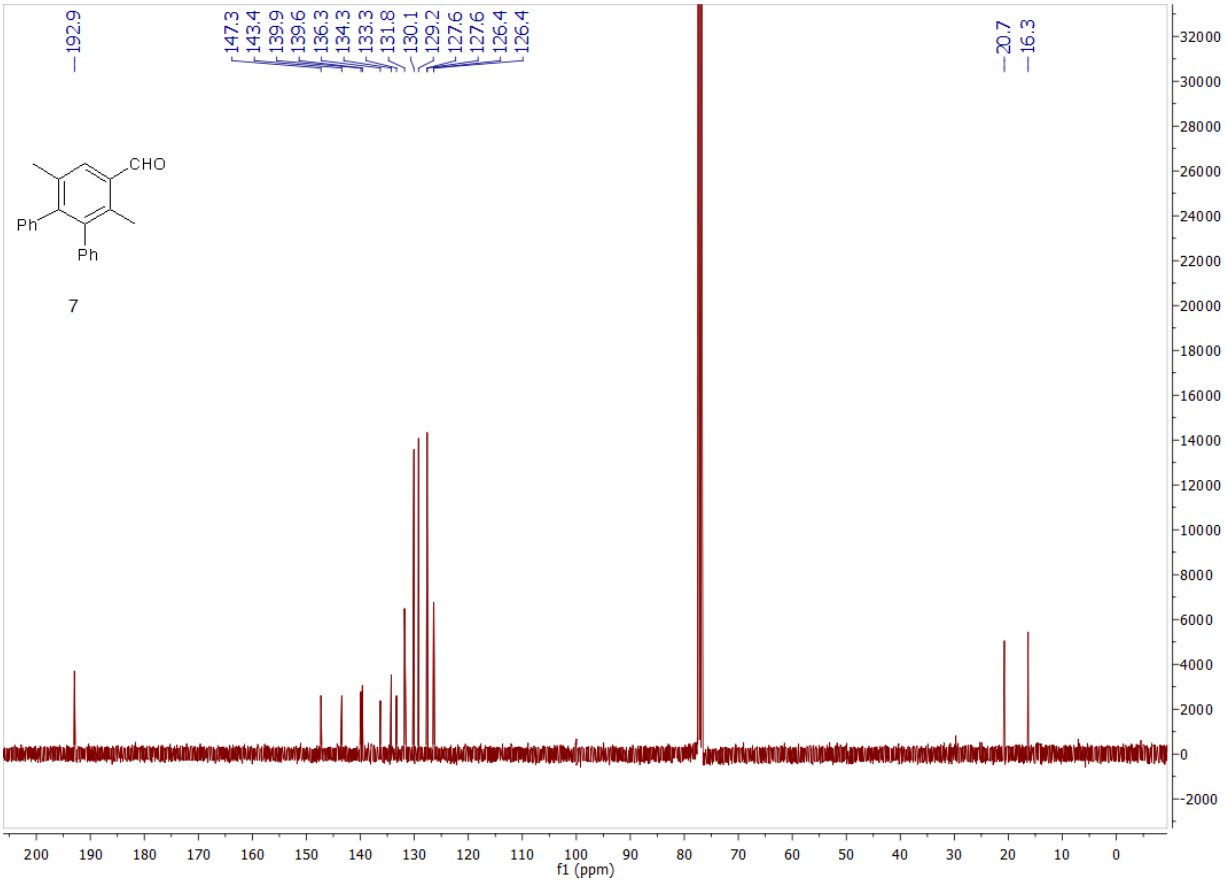
7.17
7.15
7.13
7.13
7.12
7.11
6.95
6.95
6.94
6.94
6.93
6.92
2.40
2.15
CHO
Ph
Ph
7
1.05
1.03
5.89
3.87
3.00
3.15
f1 (ppm)




## 9. Reference

[1] X. Ji, C. Zhou, K. Ji, R. E. Aghoghovbia, Z. Pan, V. Chittavong, B. Ke, B. Wang. *Angew. Chem. Int. Ed.* **2016**, *55*, 15846-15851.