Supporting information for

pH-sensitive Metal-free Carbon Monoxide Prodrugs with Tunable and Predictable Release Rates

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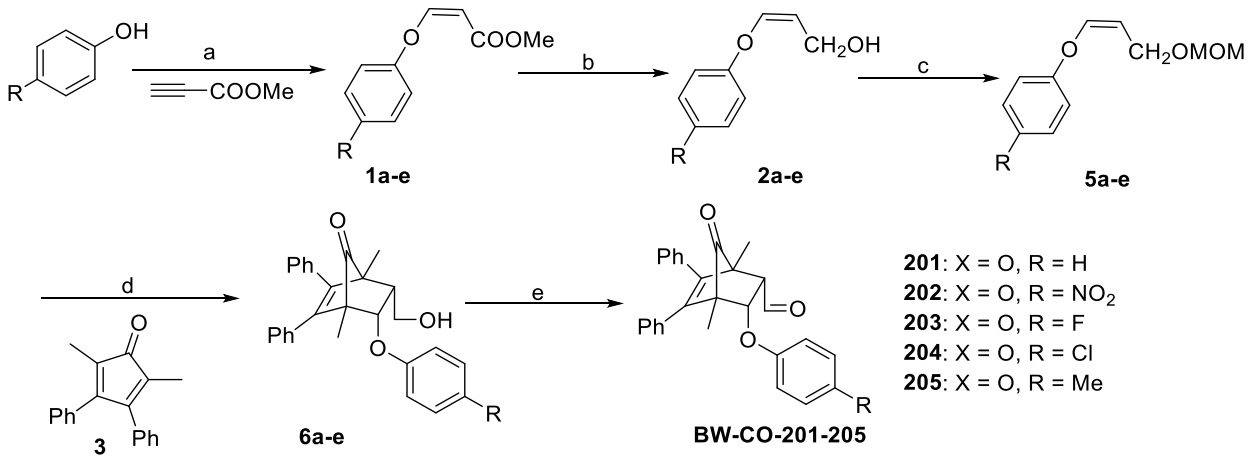
I. Chemistry

1.1 Chemical synthesis

General information: All reagents and solvents were of reagent grade. Column chromatography was carried out using flash silica gel (Sorbent 230–400 mesh) and P-2 Gel (Bio-Gel, particle size range 45- 90 μm). TLC analyses were conducted on silica gel plates (Sorbent Silica G UV254). NMR spectra were recorded at 400 MHz for ^{1}H and 100 MHz for ^{13}C on an Avance Bruker instrument. Chemical shifts (δ values) and coupling constants (J values) are given in ppm and hertz, respectively, using the respective solvent (^{1}H NMR, ^{13}C NMR) or TMS as the internal reference.



Scheme 1. The synthesis of metal free CO prodrugs. Reagents and conditions: a) K_2CO_3, H_2O, r.t., overnight, 40-92%; b) DIBAL, CH_2Cl_2, -78 °C, 1-2 h, 75%-80%; c) DIPEA, MOMCl, CH_2Cl_2, r.t., 3 h, 85-90%; d) xylene, 170 °C, 12 h; then HCl, MeOH, reflux, 1h, 50-60%; e) CH_2Cl_2, PCC, reflux, 30 min-1h, 60-73%.

General procedure for the synthesis of compounds 1a-e

To a solution of substituted phenol (1 equiv.) and K_2CO_3 (1.1 equiv.) in water (30 mL), was added methyl propiolate (1 equiv.) dropwise at room temperature. The resulting mixture was stirred at room temperature overnight, and then extracted with ethyl acetate (3 × 40 mL). The combined organic layers were washed with 5% of NaOH and brine successively, and dried over anhydrous Na_2SO_4. After filtration and concentration, the obtained residue was purified on a silica gel column to afford the title compounds.

1a [1] (colorless oil, yield: 90%): ^{1}H NMR ($CDCl_3$): δ 7.31 (t, *J* = 8.0 Hz, 2H), 7.10 (t, *J* = 8.0 Hz, 1H), 7.06 (d, *J* = 8.0 Hz, 1H), 6.98 (d, *J* = 6.9 Hz, 1H), 5.20 (d, *J* = 6.9 Hz, 1H), 3.80 (s, 3H).

1b (white solid, without purification, yield: 40%): ^{1}H NMR ($CDCl_3$) δ 8.31 (d, *J* = 8.0 Hz, 2H), 7.25 (d, *J* = 8.0 Hz, 2H), 6.93 (d, *J* = 6.9 Hz, 1H), 5.39 (d, *J* = 6.9 Hz, 1H), 3.79 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 164.4, 160.9, 150.9, 144.3, 126.0, 117.5, 102.9, 51.5. HRMS $(ESI)^+$ calculated for $C_{10}H_9NO_5Na$ $[M+Na]^+$: m/z 246.0378, found 246.0367.

1c (colorless oil, yield: 92%): ^{1}H NMR ($CDCl_3$): δ 7.12-7.00 (m, 4H), 6.82 (d, *J* = 6.9 Hz, 1H), 5.19 (d, *J* = 7.0 Hz, 1H), 3.77 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 165.4, 154.6, 119.2, 119.1, 116.6, 116.3, 116.2, 116.2, 115.8, 115.6, 99.6, 51.3. HRMS $(ESI)^+$ calculated for $C_{10}H_9FO_3Na$ $[M+Na]^+$: m/z 219.0433, found 219.0430.

1d (colorless oil, yield: 80%): ^{1}H NMR ($CDCl_3$): δ 7.33 (d, *J* = 8.8 Hz, 2H), 7.06 (d, *J* = 8.8 Hz, 2H), 6.82 (d, *J* = 6.8 Hz, 1H), 5.21 (d, *J* = 6.8 Hz, 1H), 3.76 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 164.9, 155.6, 153.6, 130.0, 129.9, 119.0, 100.4, 51.3. HRMS $(ESI)^+$ calculated for $C_{10}H_9ClO_3Na$ $[M+Na]^+$: m/z 235.0138, found 235.0128.

1e (colorless oil, yield: 85%): ^{1}H NMR ($CDCl_3$) δ 7.04-7.02 (m, 2H), 6.89-6.86 (m, 2H), 6.75 (d, *J* = 7.2 Hz, 1H), 5.03 (d, *J* = 6.8 Hz, 1H), 3.62 (s, 3H), 2.21 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 164.8, 154.9, 154.5, 134.0, 130.0, 117.1, 98.7, 50.7, 20.3. HRMS $(ESI)^+$ calculated for $C_{11}H_{12}O_3Na$ $[M+Na]^+$: m/z 215.0684, found 215.0679.

General procedure for the synthesis of compounds 2a-e

To a solution of **1a-e** (1 equiv.) in dry CH_2Cl_2 (50 mL) was added DIBAL (2.8 equiv.) dropwise under N_2 at -78 °C. The resulting reaction mixture was stirred for another 1 h at -78 °C. before being was poured into saturated Rochelle salt solution (50 mL) carefully. The resulting mixture was stirred for another 30 min at room temperature. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 40 mL). The combined organic layer was washed with brine, and dried over anhydrous Na_2SO_4. After filtration and concentration, the obtained residue was purified on a silica gel column to afford the title compound.

2a (colorless oil, yield: 75%). ^{1}H NMR ($CDCl_3$): δ 7.35 (t, *J* = 7.9 Hz, 2H), 7.10 (t, *J* = 7.3 Hz, 1H), 7.03 (d, *J* = 8.0 Hz, 2H), 6.51 (d, *J* = 6.1 Hz, 1H), 5.11 (dd, *J* = 13.2, 6.6 Hz, 1H), 4.41 (dd, *J* = 6.8, 1.0 Hz, 2H). ^{13}C NMR ($CDCl_3$): δ 157.1, 142.5, 129.7, 123.2, 116.5, 111.1, 56.3. HRMS $(ESI)^+$ calculated for $C_{10}H_9O_2Na$ $[M+Na]^+$: m/z 173.0578, found 173.0571.

2b (colorless oil, yield: 78%): ^{1}H NMR ($CDCl_3$): δ 8.22 (d, *J* = 8.0 Hz, 2H), 7.09 (d, *J* = 8.0 Hz, 2H), 6.53 (d, *J* = 6.1 Hz, 1H), 5.29 (q, *J* = 8.0 Hz, 1H), 4.40 (t, *J* = 5.5 Hz, 2H), 1.94 (t, *J* = 5.4 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ 161.5, 143.04, 140.1, 126.0, 116.1, 114.5, 56.0. HRMS $(ESI)^+$ calculated for $C_9H_9NO_4Na$ $[M+Na]^+$: m/z 218.0419, found 218.0429.

2c (colorless oil, yield: 80%): ^{1}H NMR ($CDCl_3$): δ 7.07-6.84 (m, 4H), 6.39 (d, *J* = 6.1 Hz, 1H), 5.07 (q, *J* = 6.8 Hz, 1H), 4.37 (t, *J* = 5.6 Hz, 2H), 2.31 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 159.9, 157.5, 153.2, 142.7, 117.9, 117.8, 116.3, 116.0, 111.1, 56.0. HRMS $(ESI)^+$ calculated for $C_9H_9FO_2Na$ $[M+Na]^+$: m/z 191.0484, found 191.0491.

2d (colorless oil, yield: 76%): ^{1}H NMR ($CDCl_3$): δ 7.28 (t, *J* = 9.2 Hz, 2H), 6.95 (d, *J* = 9.2 Hz, 2H), 6.43 (d, *J* = 6.0 Hz, 1H), 5.12 (q, *J* = 6.8 Hz, 1H), 4.38 (t, *J* = 5.6 Hz, 2H), 1.81 (t, *J* = 5.6 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ 155.6, 142.1, 129.7, 128.2, 117.8, 111.7, 56.2. HRMS $(ESI)^+$ calculated for $C_9H_9ClO_2Na$ $[M+Na]^+$: m/z 207.0189, found 207.0195.

2e (colorless oil, yield: 80%): ^{1}H NMR ($CDCl_3$) δ 7.12-7.10 (m, 2H), 6.91-6.89 (m, 2H), 6.42 (d, 1H), 5.09-5.04 (m, 1H), 4.42-4.40 (m, 2H), 3.33-3.00 (brs, 1H), 2.32 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 155.0, 142.4, 132.4, 130.0, 116.3, 110.6, 55.8, 20.5. HRMS (ESI)+ calculated for $C_{10}H_{12}O_2Na$ $[M+Na]^+$: m/z 187.0735, found 187.0733.

The general procedure for the synthesis of compounds 5a-e

To a solution of **2a-e** (1 equiv.) and DIPEA (1.6 equiv.) in dry CH_2Cl_2 (30 mL) was added MOMCl (1.5 equiv.) dropwise at room temperature, and the resulting solution was stirred for another 3 h at

room temperature. Then the reaction mixture was washed with water and brine, and was dried over anhydrous Na_2SO_4. After filtration and concentration, the obtained residue was purified on a silica gel column to afford the title compound as colorless oil.

5a (colorless oil, yield: 90%): ^{1}H NMR ($CDCl_3$): δ 7.38 – 7.30 (m, 2H), 7.10 (t, *J* = 8.0 Hz, 1H), 7.02 (d, *J* = 8.0 Hz, 2H), 6.55 (dt, *J* = 6.1, 1.3 Hz, 1H), 5.05 (dd, *J* = 13.2, 6.9 Hz, 1H), 4.71 (s, 2H), 4.34 (dd, *J* = 7.0, 1.1 Hz, 2H), 3.42 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 157.2, 143.1, 129.7, 123.1, 116.6, 108.2, 95.9, 60.5, 55.3.

5b (colorless oil, yield: 85%):^{1}H NMR ($CDCl_3$): δ 8.24 (d, J = 8.0Hz, 2H), 7.10 (d, J = 8.0Hz, 2H), 6.58 (dd, *J* = 6.1, 0.9 Hz, 1H), 5.26 (q, *J* = 6.3 Hz, 1H), 4.67 (s, 2H), 4.32 (d, *J* = 6.8 Hz, 2H), 3.39 (d, *J* = 2.0 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 161.5, 143.1, 140.7, 126.0, 116.2, 112.0, 96.0, 60.2, 55.3. HRMS $(ESI)^+$ calculated for $C_{11}H_{13}NO_5Na$ $[M+Na]^+$: m/z 262.0691, found 262.0701.

5c (colorless oil, yield: 87%): ^{1}H NMR ($CDCl_3$): δ 7.08- 6.87 (m, 4H), 6.45 (dt, *J* = 6.4, 1.2 Hz, 1H), 5.08-4.97 (m, 1H), 4.69 (s, 2H), 4.31 (dd, *J* = 7.2, 1.2 Hz, 2H), 3.40 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 159.8, 157.5, 153.3, 143.4, 117.9, 117.8, 116.2, 116.0, 108.3, 95.9, 60.3, 55.2. HRMS $(ESI)^+$ calculated for $C_{11}H_{13}FO_3Na$ $[M+Na]^+$: m/z 235.0746, found 235.0747.

5d (colorless oil, yield: 90%): ^{1}H NMR ($CDCl_3$): δ 7.28 (d, *J* = 6.8 Hz, 2H), 6.95 (d, *J* = 6.8 Hz, 2H), 6.47 (d, *J* = 6.4 Hz, 1H), 5.08 (q, *J* = 7.2 Hz, 1H), 4.69 (s, 2H), 4.31 (d, *J* = 6.8 Hz, 2H), 3.40 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 155.7, 142.7, 129.6, 128.1, 117.8, 109.0, 95.9, 60.3, 55.3. HRMS $(ESI)^+$ calculated for $C_{11}H_{13}ClO_3Na$ $[M+Na]^+$: m/z 251.0451, found 251.0461.

5e (colorless oil, yield:88%): ^{1}H NMR ($CDCl_3$): δ 7.10 (d, *J* = 8.4 Hz, 2H), 6.92-6.89 (m, 2H), 6.49 (dt, *J* = 6.4, 1.2 Hz, 1H), 5.02-4.97 (m, 1H), 4.68 (s, 2H), 4.33(dd, *J* = 6.8, 1.2 Hz, 2H), 3.40 (s, 3H), 2.31 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 155.1, 143.5, 132.4, 130.0, 116.4, 107.5, 95.8, 60.4, 55.1, 20.5. HRMS $(ESI)^+$ calculated for $C_{12}H_{16}O_3Na$ $[M+Na]^+$: m/z 231.0997, found 231.1001.

General procedure for the synthesis of compounds 6a-e

A solution of **5a-e** (6 equiv.) and dienone compound **3** (1 equiv.) in xylene (2 mL) was heated to 170-180 ºC in a sealed tube for 12 h. Then the reaction mixture was concentrated under vacuum, and the obtained residue was purified on a silica gel column (hexane/ether = 10:1) to afford a yellowish oil, which was dissolved in MeOH (5 mL) containing 1 mL of HCl aqueous solution (37%). The resulting solution was heated under reflux for 1 h. Then the reaction mixture was concentrated, and the obtained residue was taken up with ethyl acetate (40 mL). The organic layer was then washed with brine, and was dried over anhydrous Na_2SO_4. After filtration and concentration, the obtained residue was purified on a silica gel column to afford the title compound.

6a (white solid, yield: 50%): ^{1}H NMR ($CDCl_3$): δ 7.38-7.31 (m, 2H), 7.25-7.21 (m, 8H), 7.15-7.12 (m, 2H), 7.04-7.01 (m, 3H), 5.08 (d, *J* = 8.5 Hz, 1H), 4.01-3.96 (m, 1H), 3.88-3.82 (m, 1H), 2.78-2.66 (m, 1H), 1.47 (s, 3H), 1.32 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 203.5, 159.1, 141.4, 134.8, 130.0, 129.9, 129.7, 128.2, 127.9, 127.2, 127.2, 121.9, 115.6, 81.9, 60.0, 57.1, 52.7, 12.1, 11.0. HRMS (ESI)$^+$ calculated for $C_{28}H_{26}O_3Na$ $[M+Na]^+$: m/z 433.1780, found 433.1767.

6b (yellowish solid, yield: 51%): ^{1}H NMR ($CDCl_3$): δ 8.23 (d, *J* = 8.0 Hz, 2H), 7.28-7.22 (m, 6H), 7.19-7.05 (m, 6H), 5.17 (d, *J* = 8.0 Hz, 1H), 3.95-3.82 (m, 2H), 2.80 (q, *J* = 8.0 Hz, 1H), 1.48 (s, 3H), 1.34 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 202.5, 164.1, 142.1, 141.8, 141.0, 134.6, 134.5, 129.9, 129.6, 128.3, 128.0, 127.5, 127.4, 126.1, 115.5, 82.7, 59.9, 59.6, 57.2, 52.3, 12.1, 11.0. HRMS (ESI)$^+$ calculated for $C_{28}H_{25}NO_5Na$ $[M+Na]^+$: m/z 478.1630, found 478.1630.

6c (white solid, yield: 60%): ^{1}H NMR ($CDCl_3$): δ 7.27-7.16 (m, 8H), 7.16-7.09 (m, 2H), 7.02-6.91 (m, 4H), 4.98 (d, *J* = 8.4 Hz, 1H), 4.02-3.97 (m, 1H), 3.92-3.79 (m, 1H), 2.77-2.71 (m, 1H), 1.45 (s, 3H), 1.31 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 203.3, 159.0, 156.6, 155.3, 155.3, 141.5, 141.1, 134.9, 134.7, 130.0, 129.7, 128.2, 127.9, 127.3, 127.2, 117.0, 116.9, 116.4, 116.1, 83.2, 60.0, 60.0, 57.0, 52.6, 12.0, 11.1. HRMS (ESI)$^+$ calculated for $C_{28}H_{25}FO_3Na$ $[M+Na]^+$: m/z 451.1685, found 451.1695.

6d (white solid, yield: 55%): ^{1}H NMR ($CDCl_3$): δ 7.28-7.20 (m, 8H), 7.20- 7.15 (m, 2H), 7.15-7.10 (m, 2H), 6.98- 6.87 (m, 2H), 5.01 (d, *J* = 8.4 Hz, 1H), 3.99-3.93 (m, 1H), 3.89-3.83 (m, 1H), 2.77-2.71 (m, 1H), 1.46 (s, 3H), 1.31 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 203.2, 157.7, 141.5, 141.2,

134.8, 134.7, 130.8, 130.0, 129.7, 129.7, 128.2, 127.9, 127.3, 127.3, 126.8, 119.4, 117.0, 82.6, 60.0, 59.9, 57.1, 52.5, 12.1, 11.0. HRMS $(ESI)^+$ calculated for $C_{28}H_{25}ClO_3Na$ $[M+Na]^+$: m/z 467.1390, found 467.1374.

6e (white solid, yield: 54%): ^1H NMR ($CDCl_3$) δ 7.26-7.19 (m, 8H), 7.13-7.09 (m, 4H), 6.91 (m, 2H), 5.02 (d, *J* = 8.0 Hz, 1H), 4.01-3.79 (m, 2H), 2.73-2.71 (m, 1H), 2.30 (s, 3H), 2.28 (s, 1H), 1.44 (s, 3H), 1.30 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 203.8, 157.1, 141.5, 141.4, 135.0, 131.4, 130.4, 130.1, 129.8, 128.2, 128.0, 127.3, 127.2, 115.5, 82.2, 60.1, 57.1, 52.8, 20.6, 12.2, 11.1.

General procedure for the synthesis of BW-CO-201-205

A mixture of **6a-f** (1 equiv.) and PCC (4 equiv.) in CH_2Cl_2 (20 mL) was heated under reflux for 30 min. Then the reaction mixture was filtered through a short (Ø = 2 cm, L = 2 cm) silica gel column under reduced pressure, and the obtained filtrate was dried directly to afford the title compound as white solid.

BW-CO-201 (white solid, yield: 60%): ^1H NMR ($CDCl_3$): δ 9.63 (d, *J* = 4.8 Hz, 1H), 7.34-7.27 (m, 5H), 7.24-7.21 (m, 5H), 7.08-6.99 (m, 3H), 6.98-6.89 (m, 2H), 5.13 (d, *J* = 8.1 Hz, 1H), 3.14 (dd, *J* = 8.1, 4.8 Hz, 1H), 1.43 (s, 3H), 1.41 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 200.3, 200.1, 157.2, 142.4, 140.1, 134.4, 134.1, 130.0, 129.7, 128.3, 128.0, 127.6, 127.6, 122.4, 115.4, 82.1, 60.9, 59.8, 58.1, 11.5, 10.5. HRMS $(ESI)^+$ calculated for $C_{28}H_{24}O_3Na$ $[M+Na]^+$: m/z 431.1623, found 431.1620.

BW-CO-202 (white solid, yield: 65%): ^1H NMR ($CDCl_3$): δ 9.62 (d, *J* = 4.8 Hz, 1H), 8.24 (d, *J* = 8.0 Hz, 2H), 7.32-7.28 (m, 4H), 7.27-7.18 (m, 4H), 7.10-6.89 (m, 4H), 5.23 (d, *J* = 8.0 Hz, 1H), 3.23 (dd, *J* = 8.0, 4.8 Hz, 1H), 1.45 (s, 3H), 1.44 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 199.1, 199.1, 162.0, 142.7, 141.8, 140.7, 136.1, 134.0, 133.7, 131.3, 129.8, 129.7, 128.5, 128.2, 127.9, 126.2, 115.4, 83.1, 60.8, 59.8, 58.0, 11.5, 10.6. HRMS $(ESI)^+$ calculated for $C_{28}H_{23}NO_5Na$ $[M+Na]^+$: m/z 476.1474, found 476.1479.

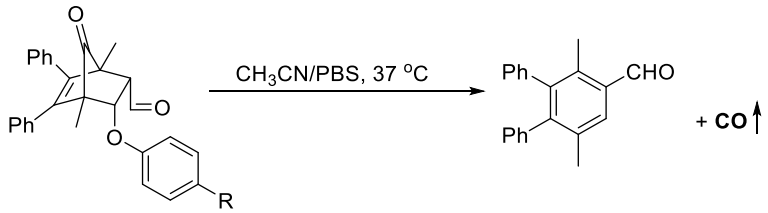
BW-CO-203 (white solid, yield: 73%): ^1H NMR ($CDCl_3$): δ 9.66 (d, *J* = 5.2 Hz, 1H), 7.28-7.19 (m, 7H), 7.04-6.98 (m, 5H), 6.88 (dd, *J* = 9.2, 4.2 Hz, 2H), 5.06 (d, *J* = 8.0 Hz, 1H), 3.14 (dd, *J* =

8.0, 4.8 Hz, 1H), 1.43 (s, 3H), 1.42 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 200.1, 200.0, 159.3, 156.9, 153.5, 142.2, 140.3, 134.3, 134.0, 129.9, 129.7, 128.4, 128.1, 127.7, 127.6, 116.7, 116.7, 116.6, 116.3, 83.3, 61.0, 59.9, 58.0, 11.5, 10.6. HRMS (ESI)$^+$ calculated for $C_{28}H_{23}FO_3Na$ $[M+Na]^+$: m/z 449.1529, found 449.1529.

BW-CO-204 (white solid, yield: 70%): ^{1}H NMR ($CDCl_3$): δ 9.64 (d, *J* = 4.8 Hz, 1H), 7.30-7.20 (m, 10H), 7.04-7.02 (m, 2H), 6.88 (d, *J* = 8.8 Hz, 2H), 5.09 (d, *J* = 8.0 Hz, 1H), 3.15 (dd, *J* = 8.4, 4.8 Hz, 1H), 1.43 (s, 3H), 1.42 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 199.9, 199.9, 155.9, 142.2, 140.3, 134.3, 134.0, 129.9, 129.9, 129.7, 128.4, 128.1, 127.69, 127.7, 127.5, 116.7, 82.8, 60.9, 59.8, 58.0, 11.5, 10.6. HRMS (ESI)$^+$ calculated for $C_{28}H_{23}ClO_3Na$ $[M+Na]^+$: m/z 465.1233, found 465.1248.

BW-CO-205 (white solid, yield: 63%): ^{1}H NMR ($CDCl_3$): δ 9.63 (d, *J* = 4.9 Hz, 1H), 7.28-7.21 (m, 8H), 7.11 (d, *J* = 8.2 Hz, 2H), 7.04-7.01 (m, 2H), 6.84 (d, *J* = 8.2 Hz, 2H), 5.09 (d, *J* = 8.4 Hz, 1H), 3.13 (dd, *J* = 8.1, 4.9 Hz, 1H), 2.31 (s, 3H), 1.43 (s, 3H), 1.41 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 200.4, 200.2, 155.1, 142.4, 140.0, 134.4, 134.1, 131.8, 130.4, 130.0, 129.7, 128.3, 128.0, 127.6, 127.5, 115.2, 82.3, 60.9, 59.8, 58.1, 20.5, 11.5, 10.5. HRMS (ESI)$^+$ calculated for $C_{29}H_{26}O_3Na$ $[M+Na]^+$: m/z 445.1780, found 445.1788.

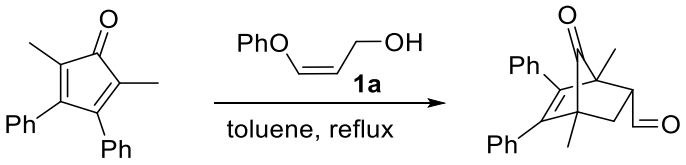
Preparation of the inactive control product



BW-CO-201-205 **7**

A solution of **BW-CO-201-205** (10 mg) in CH_3CN/PBS (10 mL, 2:1) was incubated at 37 °C overnight. Then the reaction mixture was extracted with ethyl acetate (3 × 30 mL). The combine organic layer was washed with 5% NaOH and brine, and was dried over anhydrous Na_2SO_4. After filtration and concentration, the obtained residue was purified on a silica gel column to afford

compound **7** as a white solid (yield: 90%). ^{1}H NMR ($CDCl_3$): δ 10.42 (s, 1H), 7.79 (s, 1H), 7.19-7.11 (m, 6H), 6.95-6.92 (m, 4H), 2.40 (s, 3H), 2.16 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 192.9, 147.3, 143.5, 139.9, 139.6, 136.3, 134.3, 133.3, 131.8, 130.1, 129.2, 127.7, 127.6, 126.4, 126.3, 20.7, 16.3. HRMS (ESI)$^+$ calculated for $C_{21}H_{18}ONa$ $[M+Na]^+$: m/z 309.1255, found 309.1243.



3 **4**

A solution of **3** and **1a** in toluene was heated under reflux for 2 h. Then the reaction mixture was dried directly under vacuum, and the obtained residue was purified on a silica gel column to afford compound **4** as a white solid. ^{1}H NMR ($CDCl_3$) δ 9.78 (d, J = 1.7 Hz, 1H), 7.27-7.14 (m, 6H), 7.11-6.97 (m, 2H), 6.94-6.79 (m, 2H), 3.05-3.01 (m, 1H), 2.47 (dd, J = 12.5, 5.1 Hz, 1H), 1.99 (dd, J = 12.5, 9.3 Hz, 1H), 1.57 (s, 3H), 1.33 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 204.0, 200.8, 144.7, 139.2, 133.9, 133.8, 129.8, 129.0, 128.2, 128.1, 127.5, 127.4, 100.0, 55.8, 54.8, 52.8, 31.7, 12.1, 11.8. HRMS (ESI)$^+$ calculated for $C_{22}H_{20}O_2Na$ $[M+Na]^+$: m/z 339.1361, found 339.1375.

1.2 The determination of the stereochemistry of the DA reaction product

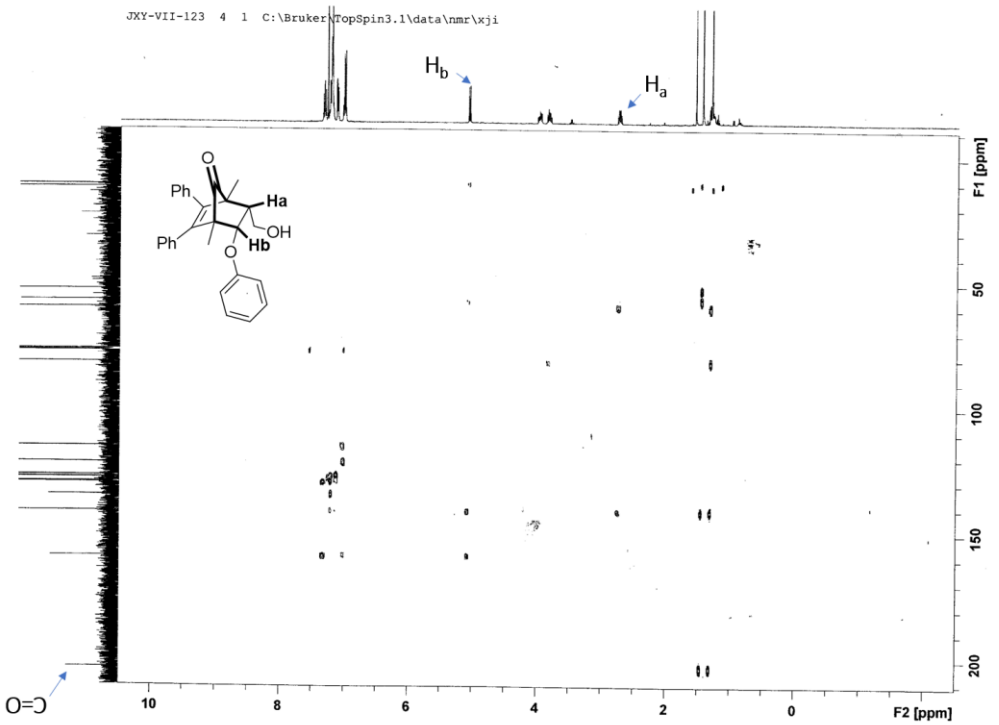


Figure S1. The HMBC spectrum of **6a** in $CDCl_3$

The DA reaction between **5a-e** and **3** yielded product **6a-e** predominantly as *endo* form, which was confirmed by HMBC experiment (O=C-C-C-Ha).[2] The specific example used for illustration is **6a.** No correlation between the carbonyl carbon and proton Ha or Hb was observed, indicating that compound **6a** existed in *endo*-form (Figure S1). In addition, the *endo* form can be further confirmed by comparing the chemical shift of proton H_c in **BW-CO-201-205** with the ones (H_d and H_e) in a reported compound with similar structure (Figure S2).[3]

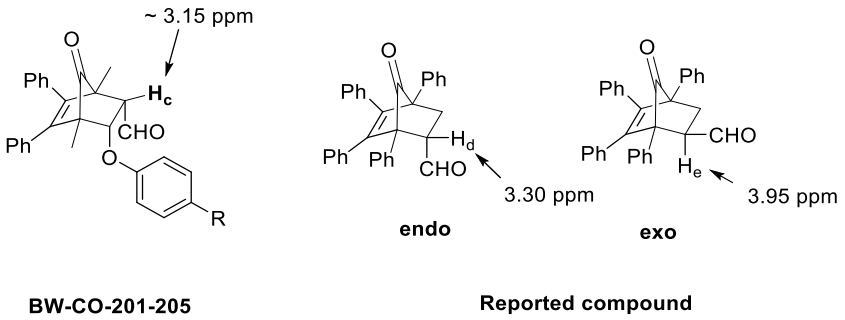


Figure S2. The comparison of the chemical shift among protons Hc, Hd and He.

1.3 Stability of BW-CO-203 in $CDCl_3$

Compound **BW-CO-203** was dissolved in $CDCl_3$, and was incubated at 37 ºC. The ^1H NMR of the solution was taken at intervals. As shown in Figure S3, no elimination product was observed even after one week of incubation.

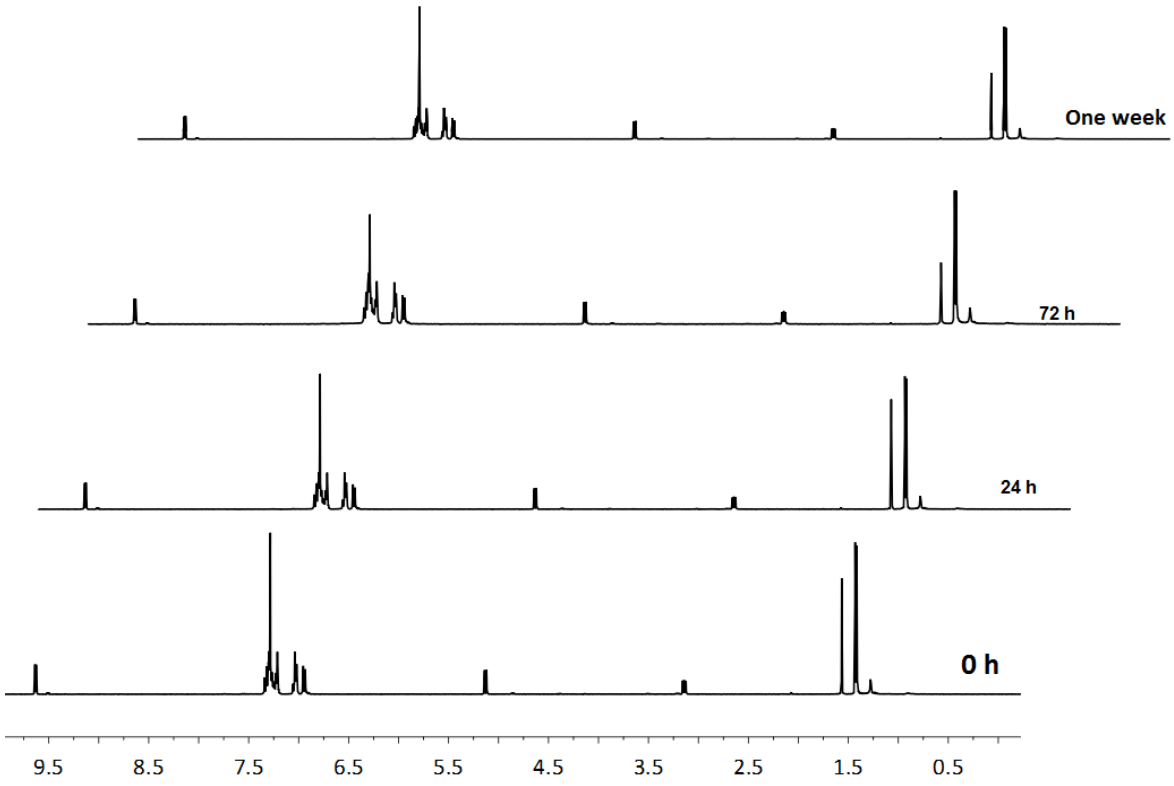


Figure S3. The ^1H NMR spectra of **BW-CO-201** at different incubation time point

1.4 CO-Myoglobin assay

In order to confirm CO release, **BW-CO-203** was chosen for CO-myoglobin assay. For such a purpose, a solution of myoglobin (0.5 mg/ml) in PBS (10 mL, pH = 7.4) was degassed by bubbling with nitrogen for at least 20 min. To this degassed solution was added a solution of **BW-CO-203** (0.45 mg) in DMSO (1 mL), and the resulting solution was incubated at 37 ºC for 2 h. Then a solution of sodium dithionite (1 mL, 22 mg/mL) was added to yield a reddish solution, which was cooled down to 0 ºC with an ice bath, and was stirred for another 1 h. Afterwards, the

UV-vis spectra of the resulting pinkish red solution were taken to confirm CO release. The results are shown in Figure S4.

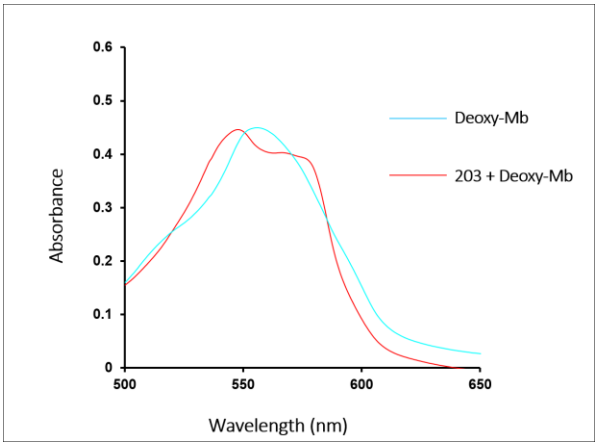
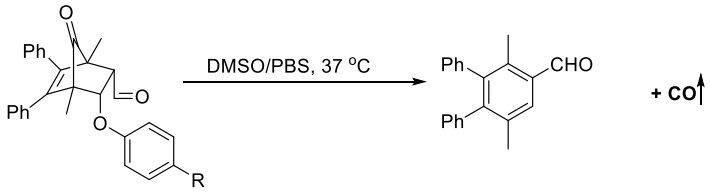


Figure S4. The CO-myoglobin assay.

1.5 CO release kinetics

The CO release kinetics of **BW-CO-201-205** was determined in 30% of DMSO in PBS (pH = 7.4) at 37 °C by monitoring the consumption of CO prodrugs and the formation of compound **7** using HPLC. For prodrug **BW-CO-202**, the kinetics was determined by monitoring the formation of 4-nitrophenol using UV spectrum, which has a specific absorbance at 400 nm. The HPLC eluent used is acetonitrile/H_2O containing 0.05% trifluoroacetic acid (v/v). Two columns were used: Column 1: Waters C18, 3.5 μm, 4.6 × 100 mm (for **BW-CO-201**, **203** and **205**); Column 2: Shimadzu C18, 3 μm, 4.6 × 50 mm (for **BW-CO-204**). Each experiment was triplicated.



BW-CO-201-205 **7**

i) **The retention time (t_R) of product 7 under different eluent conditions**

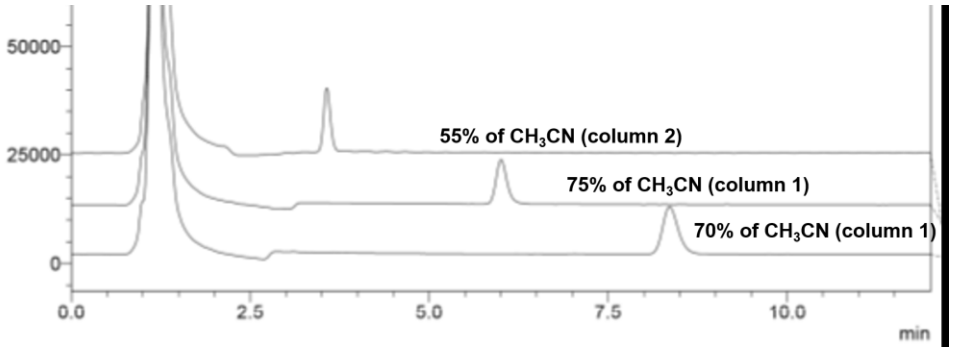


Figure S5. The t_R of compound **7** under different conditions

During the kinetics study, two HPLC with different columns (1 and 2) were used. The much shorter retention time of **7** (~3.5 min *vs* 6 min) with a lower ratio of CH_3CN (55% *vs* 75%) is attributed to the length and particle size differences between column 1 (particle size: 3.5 μm, column length: 100 mm) and 2 (particle size:3 μm; column length: 50 mm).

i) The CO release kinetics of BW-CO-201

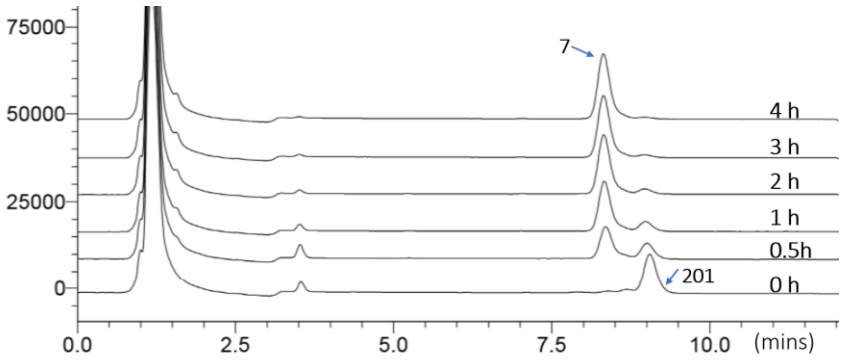


Figure S6. The HPLC spectra of **BW-CO-201** at different incubation time point (Eluent condition: 70% of acetonitrile; column 1)

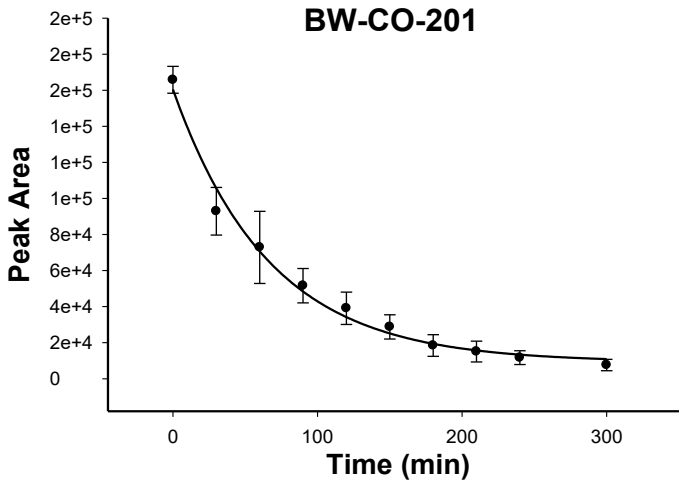


Figure S7. CO release kinetics of **BW-CO-201**

ii) The CO release kinetic of BW-CO-202

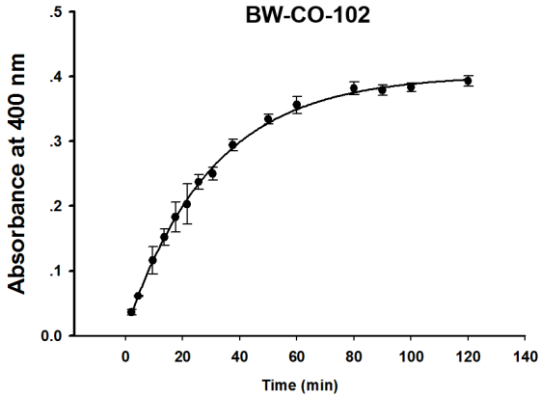


Figure S8. CO release kinetics of **BW-CO-202**

iii) The CO release kinetic of BW-CO-203

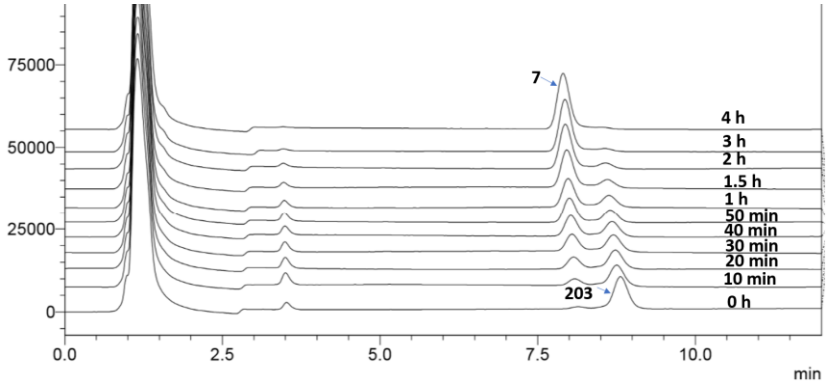


Figure S9. The HPLC spectra of **BW-CO-203** at different incubation time point (Eluent condition: 70% of acetonitrile; column 1)

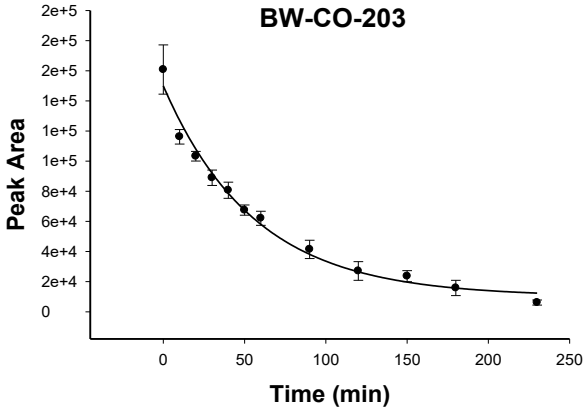


Figure S10. CO release kinetics of **BW-CO-203**

iv) The CO release kinetic of BW-CO-204

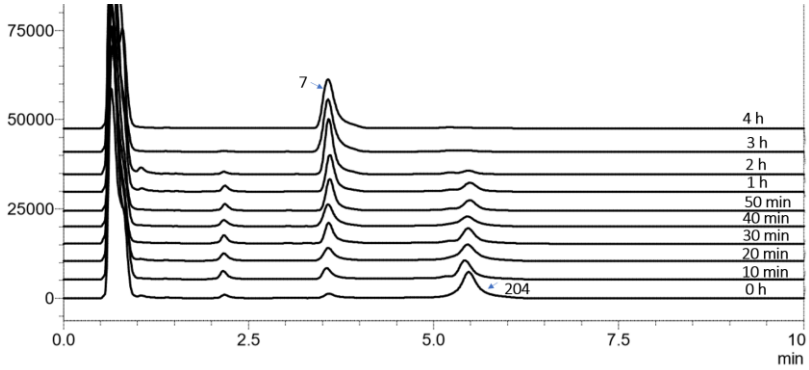


Figure S11. The HPLC spectra of **BW-CO-204** at different incubation time point (Eluent condition: 55% of acetonitrile, column 2)

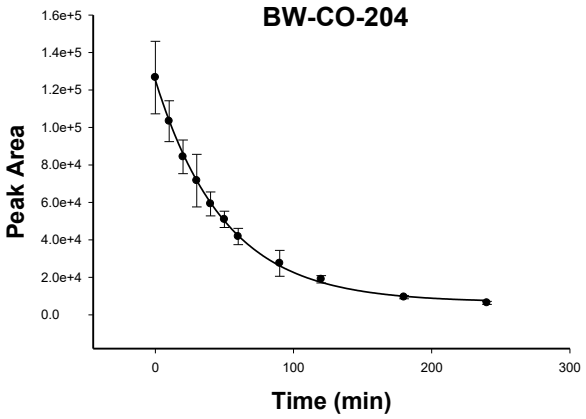


Figure S12. CO release kinetics of **BW-CO-204**

v) The CO release kinetic of BW-CO-205

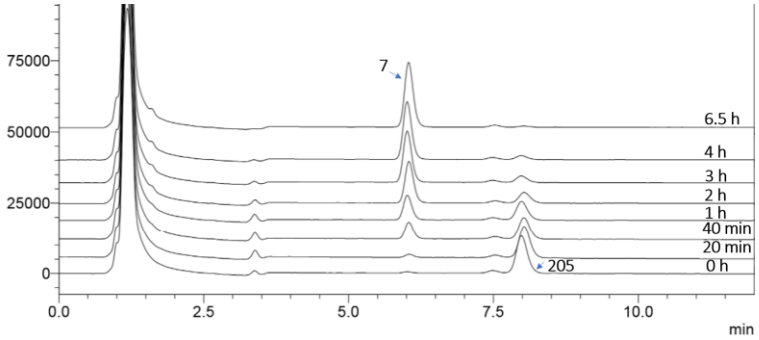


Figure S13. The HPLC spectra of **BW-CO-205** at different incubation time point (Eluent condition: 75% of acetonitrile; column 1)

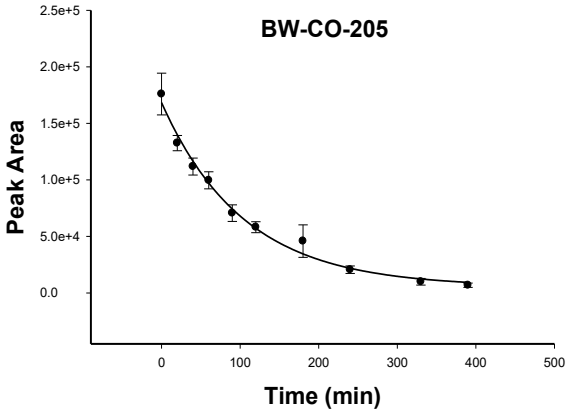


Figure S14. CO release kinetics of **BW-CO-205**

1.6 CO release kinetics of BW-CO-203 in different pH buffer solutions

BW-CO-203 was chosen to study the pH effects on CO release kinetics by analyzing the disappearance of the CO prodrugs using HPLC (The same HPLC conditions aforementioned were used). Specifically, a solution of **BW-CO-203** in 30% of DMSO in different pH buffer was incubated at 37 ^{o}C, and the solution was taken for HPLC analysis at intervals. The buffer with different pH values (traceable to SRM of NIST and PTB) were purchase form Aldrich. The simulated gastric fluid without Pepsin was prepared in house. Specifically, 200 mg of NaCl and 0.7 mL of HCl (37%, aq.) was dissolved in 80 ml of DI water, and was transferred into a 100 mL of volumetric flask. Additional DI water was added to make a final volume of 100 mL.

II. Biology

A mouse macrophage cell line, RAW 264.7 (ATCC® TIB-71™), was used for the in vitro studies. RAW 264.7 cells were maintained in DMEM (Dulbecco's Modified Eagle's Medium) supplemented with 10% fetal bovine serum (MidSci; S01520HI) and 1% penicillin-streptomycin (Sigma-Aldrich; P4333) at 37 °C with 5% CO_2. The media was changed every other day. All the experiments were done within 10 passages of RAW 264.7 cells. All the tested compounds were dissolved in 100% DMSO to yield the respective stock solution.

2.1 Cytotoxicity studies

Raw 264.7 cells were seeded in 96-well plates at an initial density of 10,000/well and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 °C under 5% CO_2 for 24 h. Then RAW 264.7 cells were incubated in DMEM containing 1% DMSO and compounds (0 – 50 µM) for 24 hours. Then 10 µL of Cell Counting Kit-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt, WST-8, purchased from Sigma Aldrich)[4] solution was added to each well and incubated for another three hours at 37 °C. The absorbance at 570 nm was measured by using Perkin Elmer 1420 Victor 3 multilabel counter. The cell viability was measured and the results were shown in Figure S14-15.

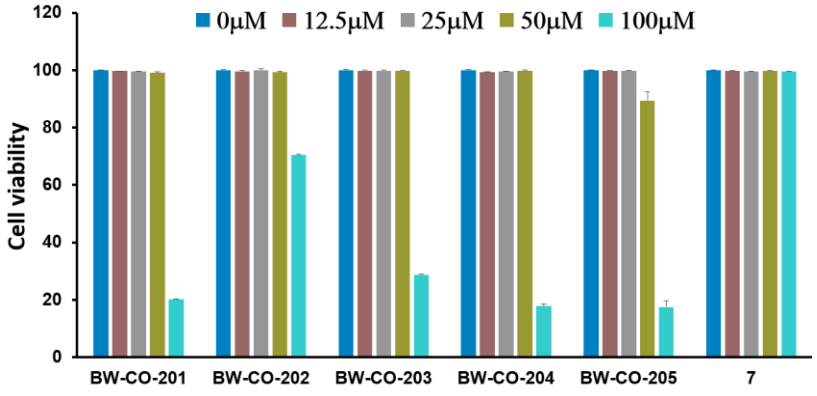


Figure S15. The cytotoxicity of **BW-CO-201-205** and **7** to Raw 264.7 cells

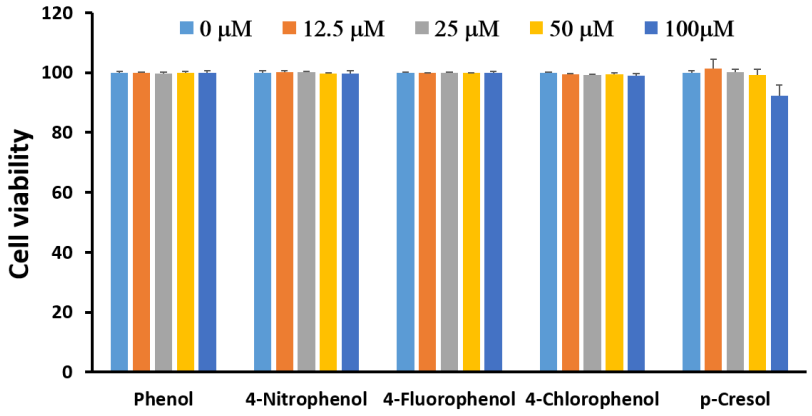


Figure S16. The cytotoxicity of various substituted phenol to Raw 264.7 cells

2.2 Anti-inflammatory effects

RAW 264.7 cells were seeded in 96-well plates at an initial density of 10,000/well one day before the experiment. LPS was used to initiate the inflammatory response in RAW 264.7 cells. RAW 264.7 cells were pre-treated with different concentrations of **BW-CO-203/205** or their respective inactive control compounds for 4 hours. All the final samples contained 1% DMSO. Thereafter, LPS was added into the cell culture media to make a final concentration of 1 μg/mL. The cells were then incubated at 37 ºC for another 1 h, and the cell culture supernatant was collected afterwards. Cell culture without LPS treatment was used as the control. The concentrations of TNF-α in the cell culture supernatant were determined by a commercial ELISA kit (ELISA Ready-SET-Go!®-eBioscience).

2.3. Fluorescent cell imaging of CO release

RAW 264.7 cells were seeded on coverslips in 6-well plate at an initial density of 10,000/well one day before the imaging experiment. Compounds were dissolved in DMSO as stock solution. Final concentration of 1 μM of **COP-1** (Stock solution in DMSO: 200 μM) and 50 μM of **BW-CO-203/205** (Stock solution in DMSO: 10 mM) were added into the cell culture. All the final samples contained 1% DMSO. After adding the compounds, the cells were incubated under 37 °C for 5 h. The cell samples were then fixed with 4% paraformaldehyde. The cells were then washed with PBS again twice and the coverslips with cells were immersed in DI water. The coverslips

were mounted onto glass slides using the mounting media without DAPI (ProLong® Live Antifade Reagent; P36974). The fluorescent imaging was performed under FITC channel (excitation: 490 nm, emission: 525 nm) using a Zeiss fluorescent microscope (Axio Vert. A1).

2.4. Fluorescence quantification in Raw 264.7 cells

Raw 264.7 cells were seeded in 96-well plates at an initial density of 10,000/well one day before the experiment. CO prodrugs and COP-1 were dissolved in DMSO. Final concentration of 1 μM of **COP-1** (Stock solution in DMSO: 200 μM) and 50 μM of **BW-CO-203/205** (Stock solution in DMSO: 10 mM) were added into the cell culture. All the final samples contained 1% DMSO. The cells were then incubated for 5 h at 37 °C. Then, the fluorescence intensity (λ_{ex} = 485 nm, λ_{em} = 525 nm) of each well was measured by using a Perkin Elmer 1420 Victor 3 multilabel counter. The results were normalized to the control group (COP-1 only). Each experiment was repeated six times and the results are expressed as mean ± SEM (n=6).

III. NMR spectra

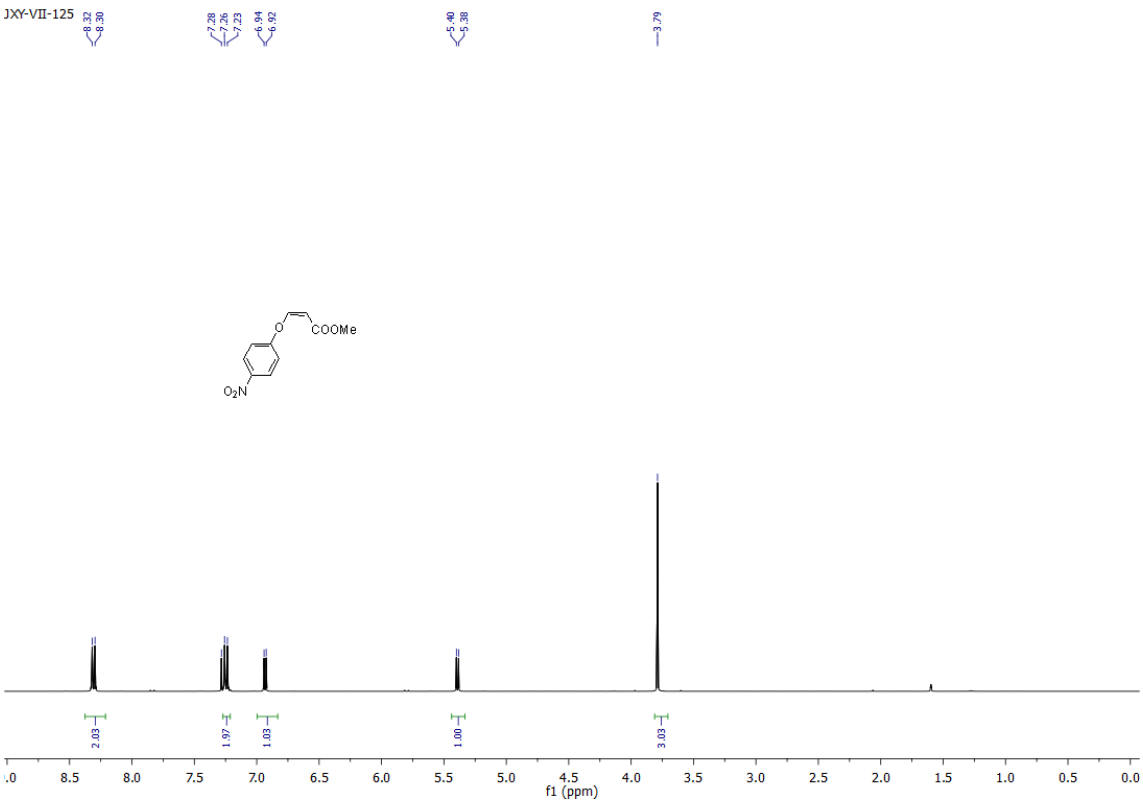


Figure S17. The ^{1}H NMR spectrum of **1b** in $CDCl_3$

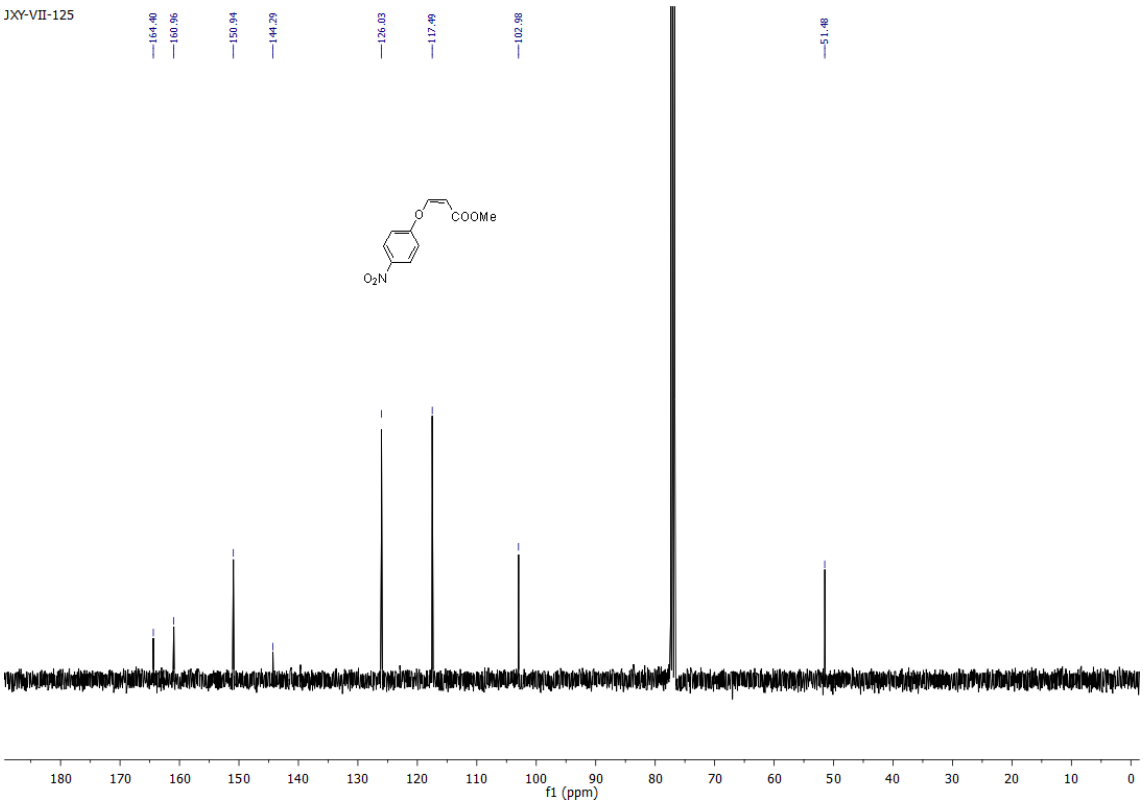


Figure S18. The ^{13}C NMR spectrum of **1b** in $CDCl_3$

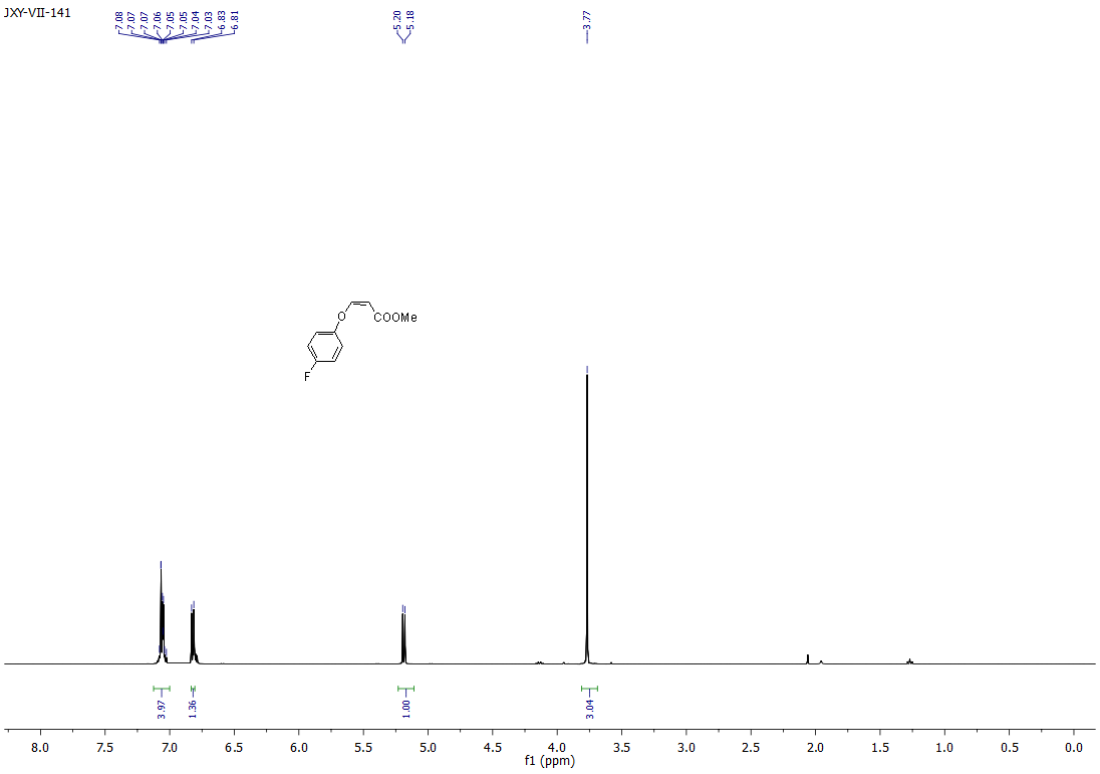


Figure S19. The ^{1}H NMR spectrum of **1c** in $CDCl_3$

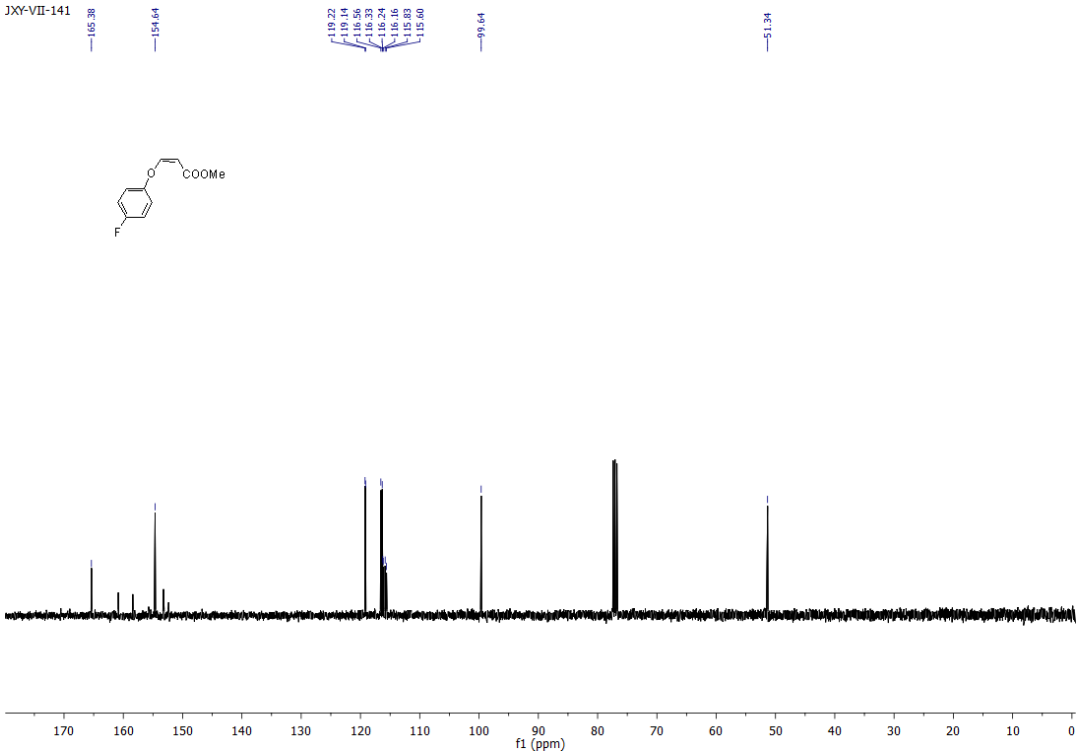


Figure S20. The ^{13}C NMR spectrum of **1c** in $CDCl_3$

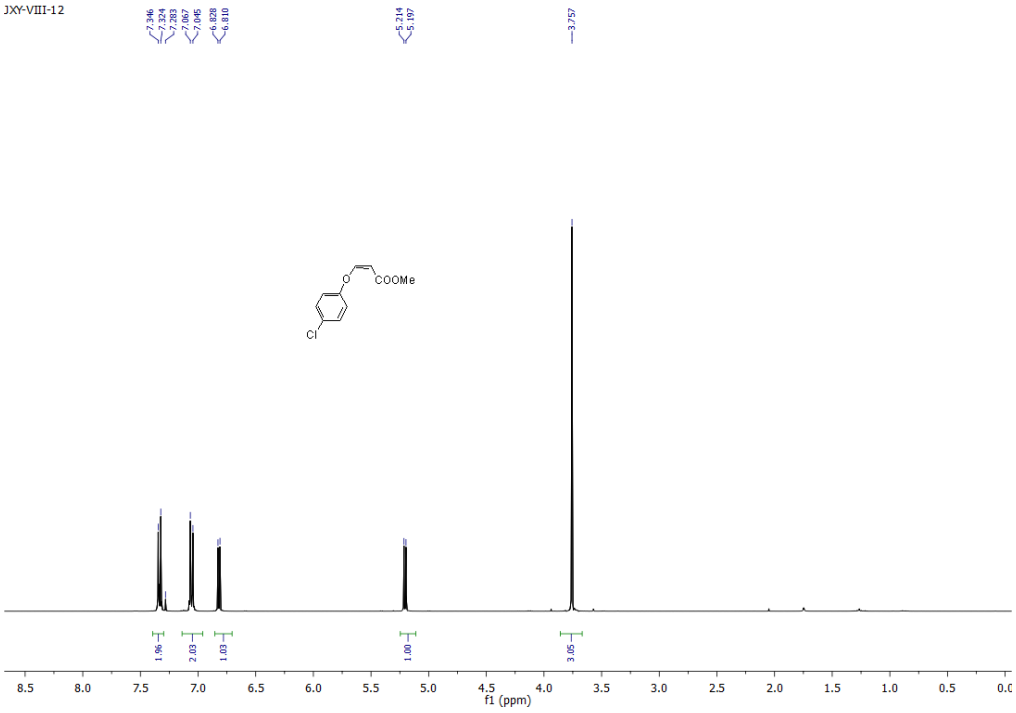


Figure S21. The ^{1}H NMR spectrum of **1d** in $CDCl_3$

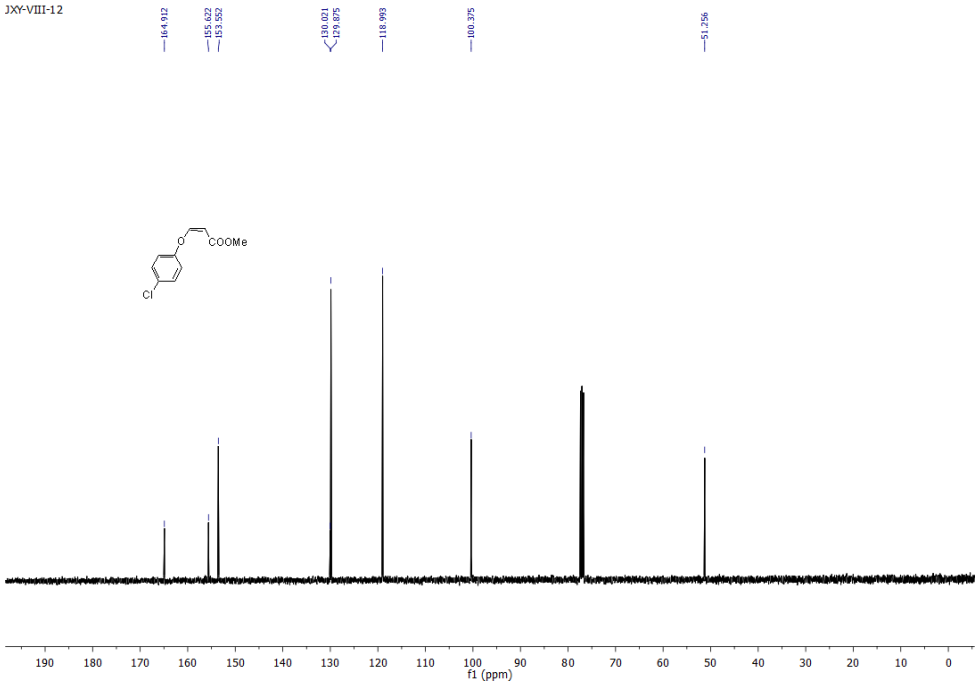


Figure S22. The ^{13}C NMR spectrum of **1d** in $CDCl_3$

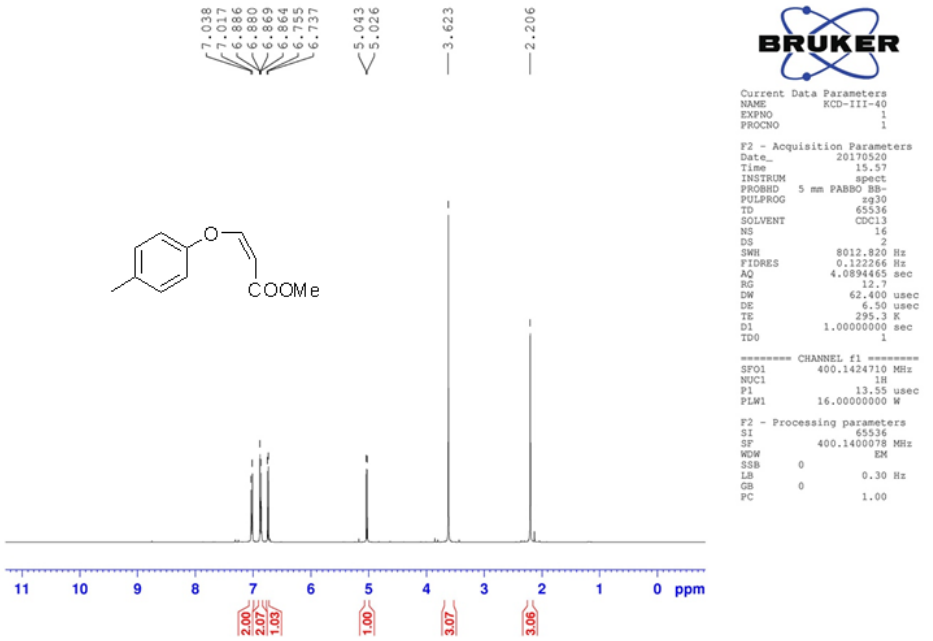


Figure S23. The ^{1}H NMR spectrum of **1e** in $CDCl_3$

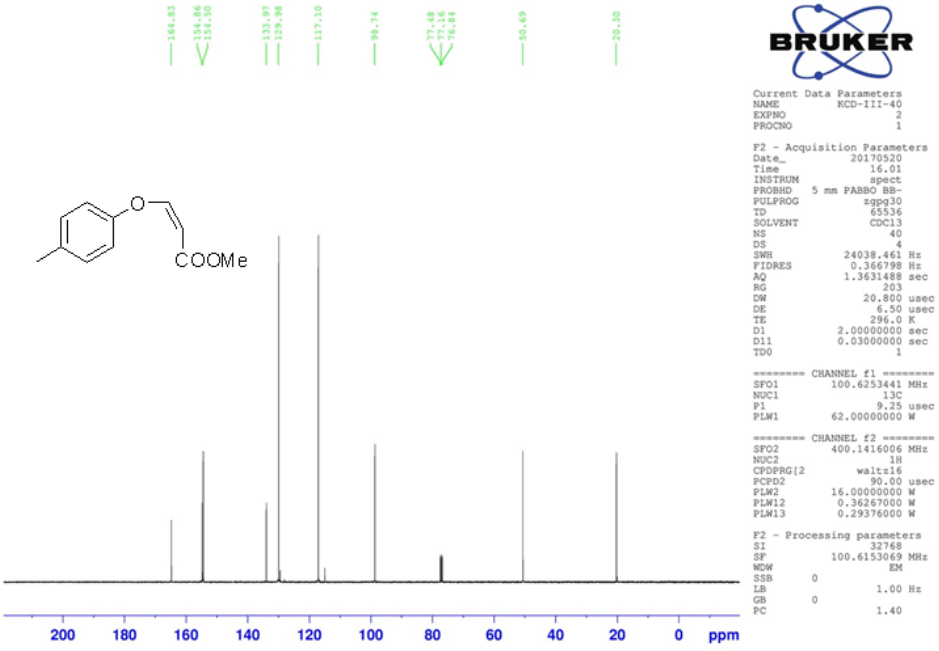


Figure S24. The ^{13}C NMR spectrum of **1e** in $CDCl_3$

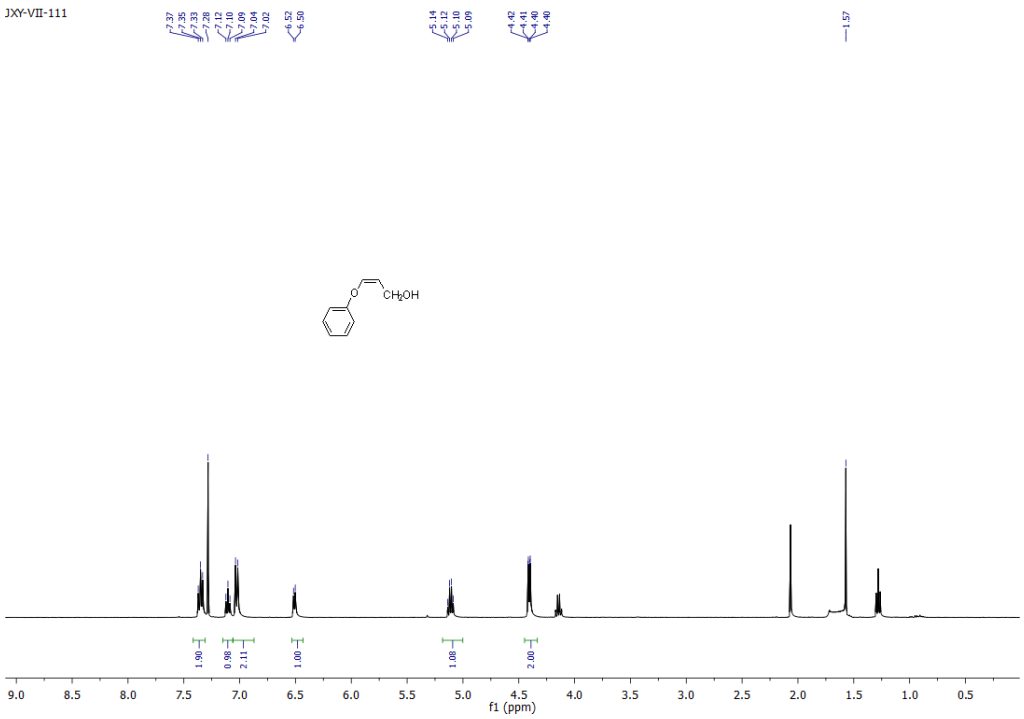


Figure S25. The ^{1}H NMR spectrum of **2a** in $CDCl_3$

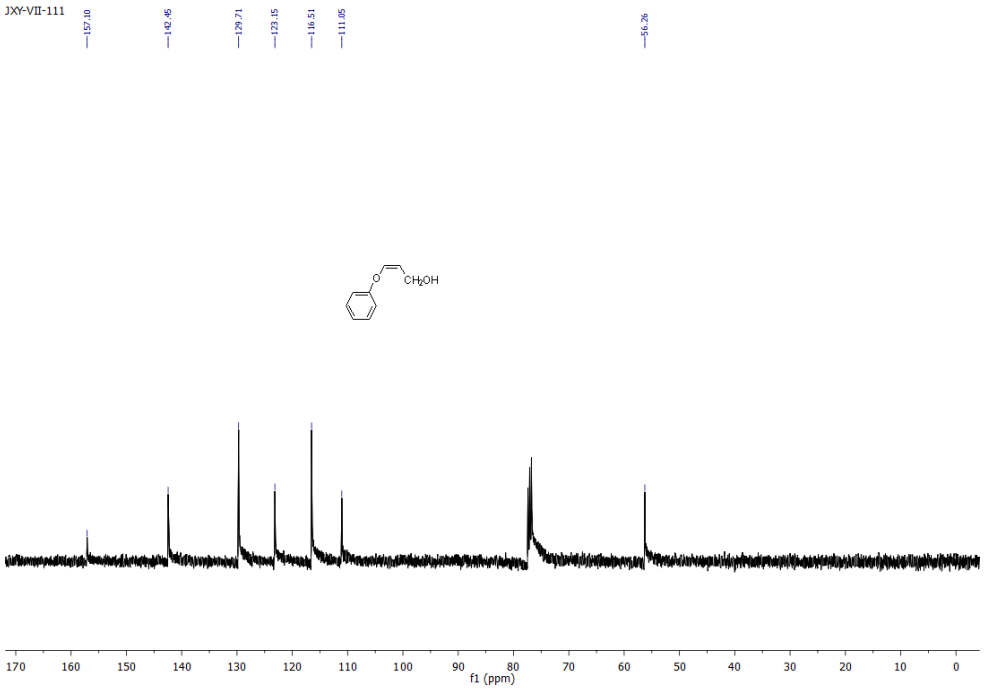


Figure S26. The ^{13}C NMR spectrum of **2a** in $CDCl_3$

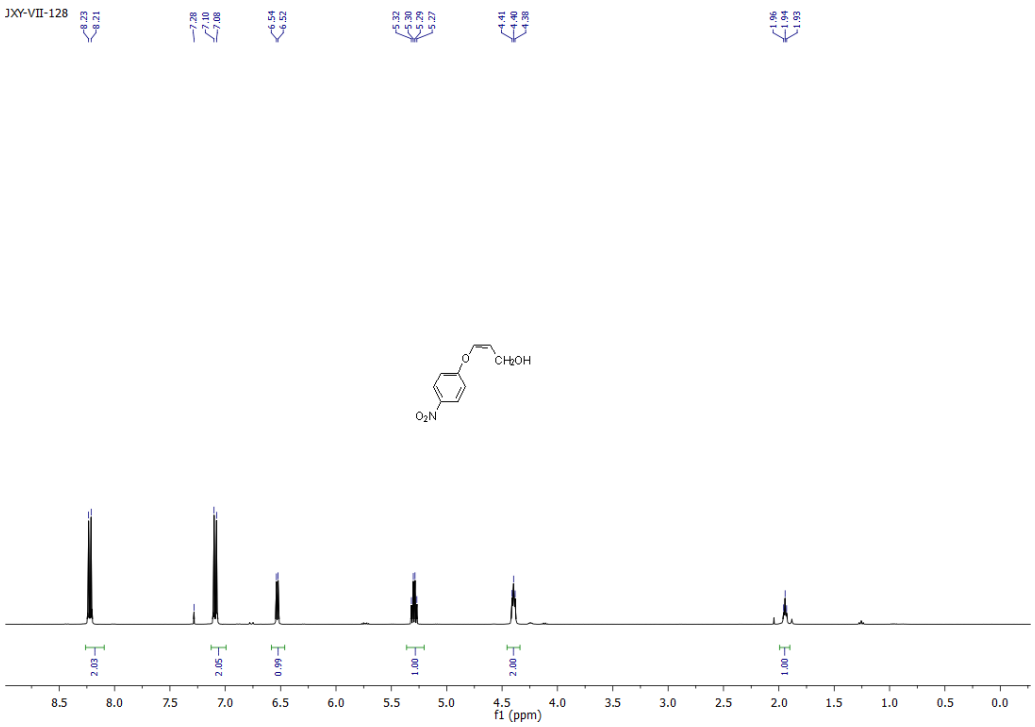


Figure S27. The ^{1}H NMR spectrum of **2b** in $CDCl_3$

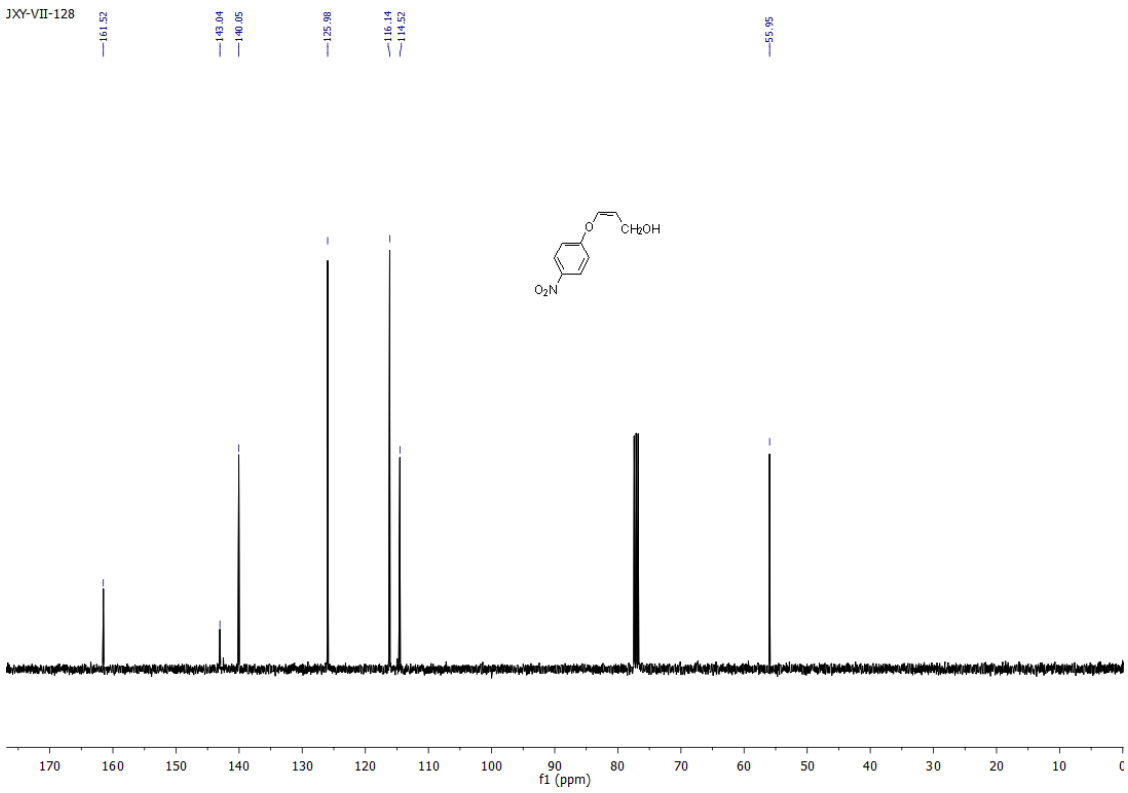


Figure S28. The ^{13}C NMR spectrum of **2b** in $CDCl_3$

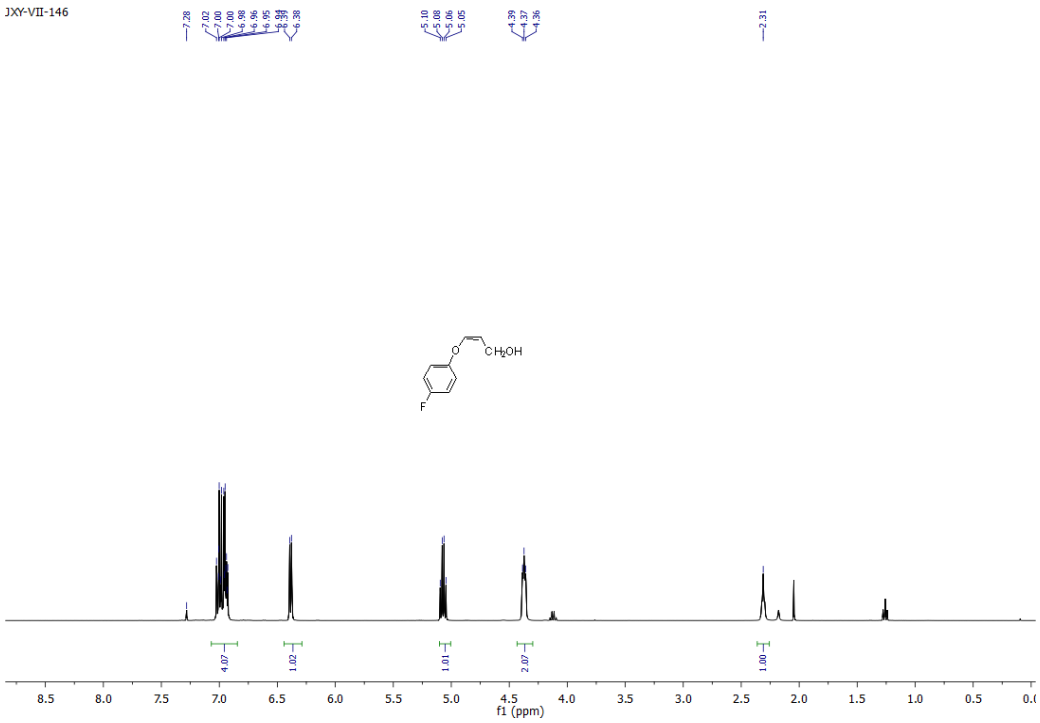


Figure S29. The ^{1}H NMR spectrum of **2c** in $CDCl_3$

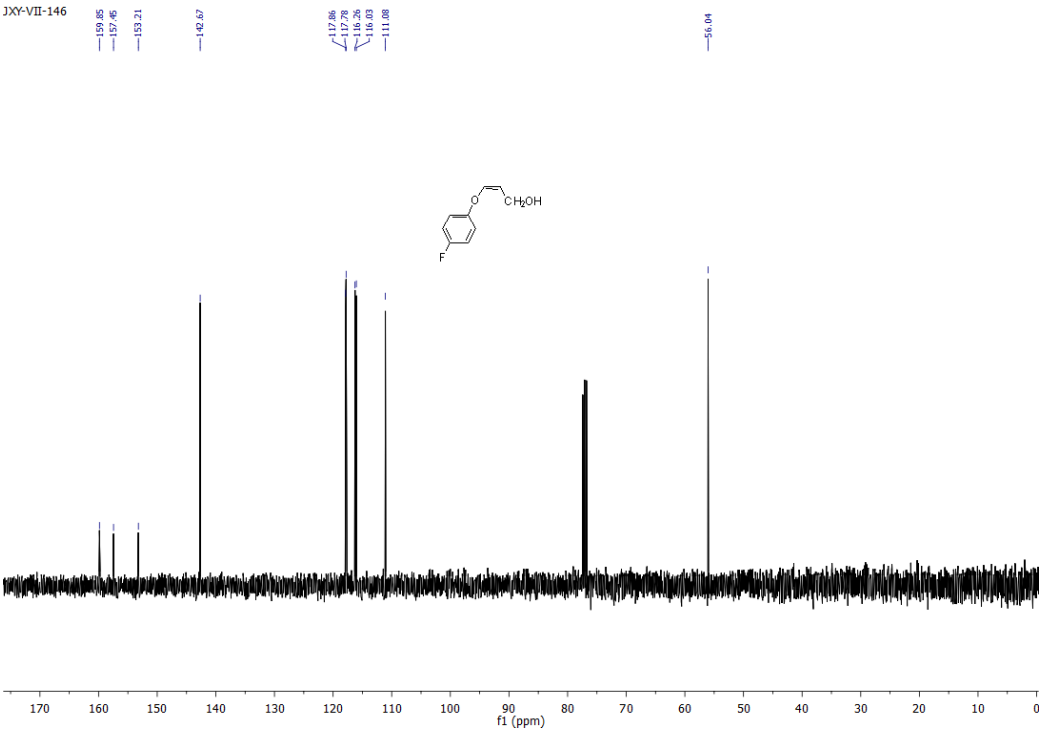


Figure S30. The ^{13}C NMR spectrum of **2c** in $CDCl_3$

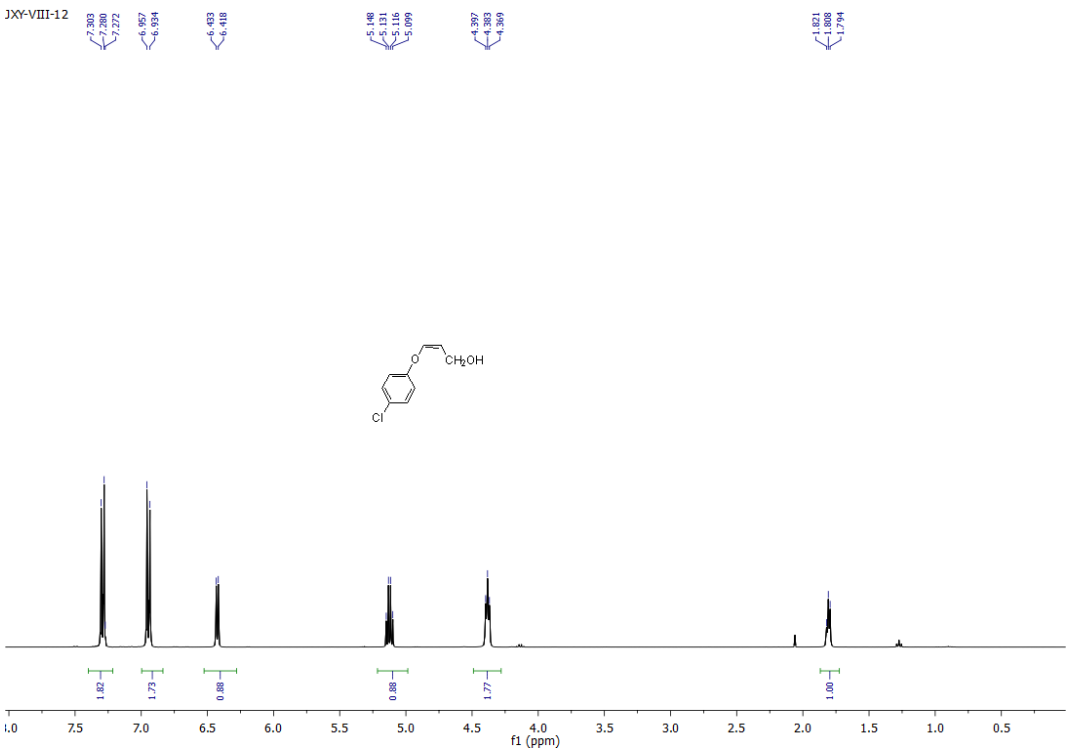


Figure S31. The ^{1}H NMR spectrum of **2d** in $CDCl_3$

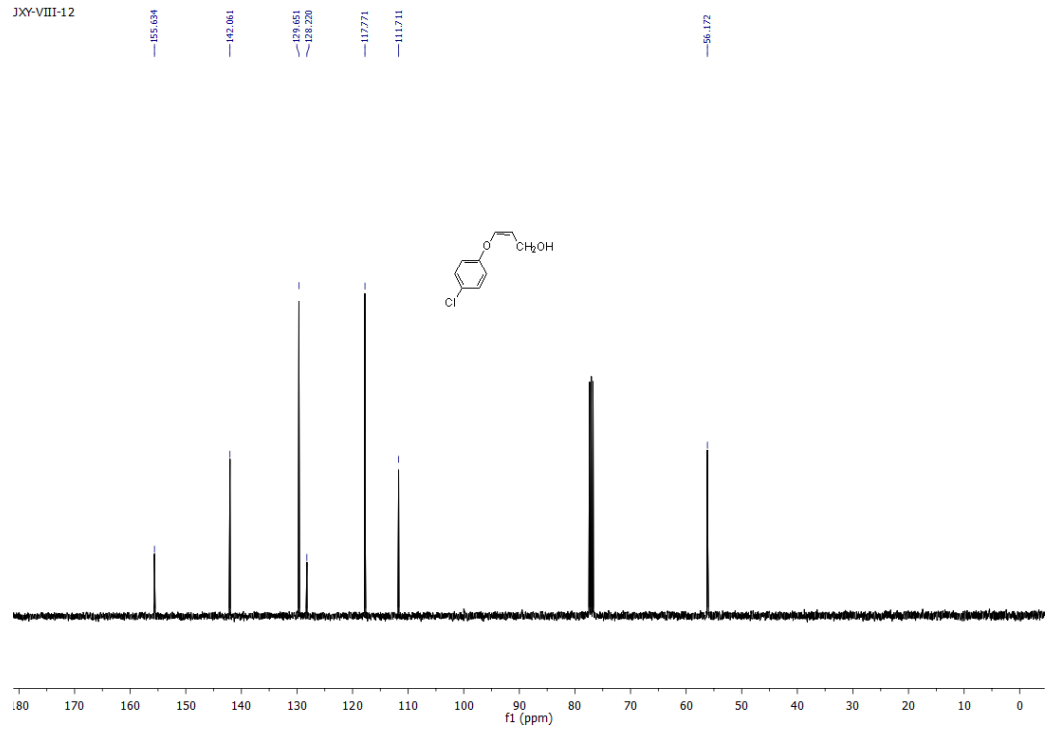


Figure S32. The ^{13}C NMR spectrum of **2d** in $CDCl_3$

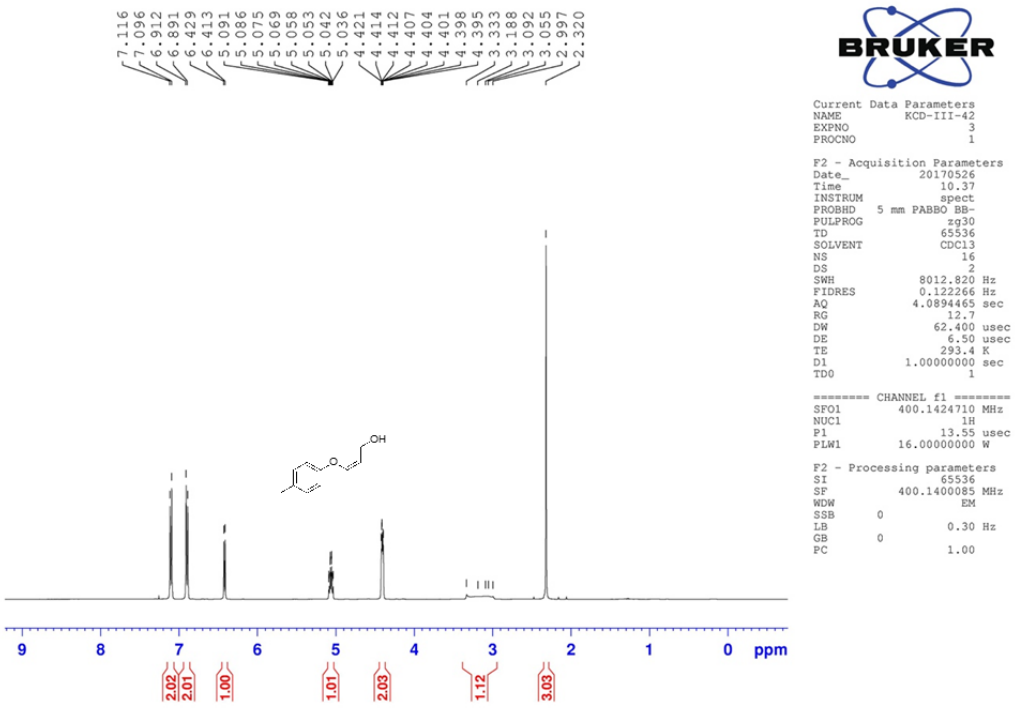


Figure S33. The ^{1}H NMR spectrum of **2e** in $CDCl_3$

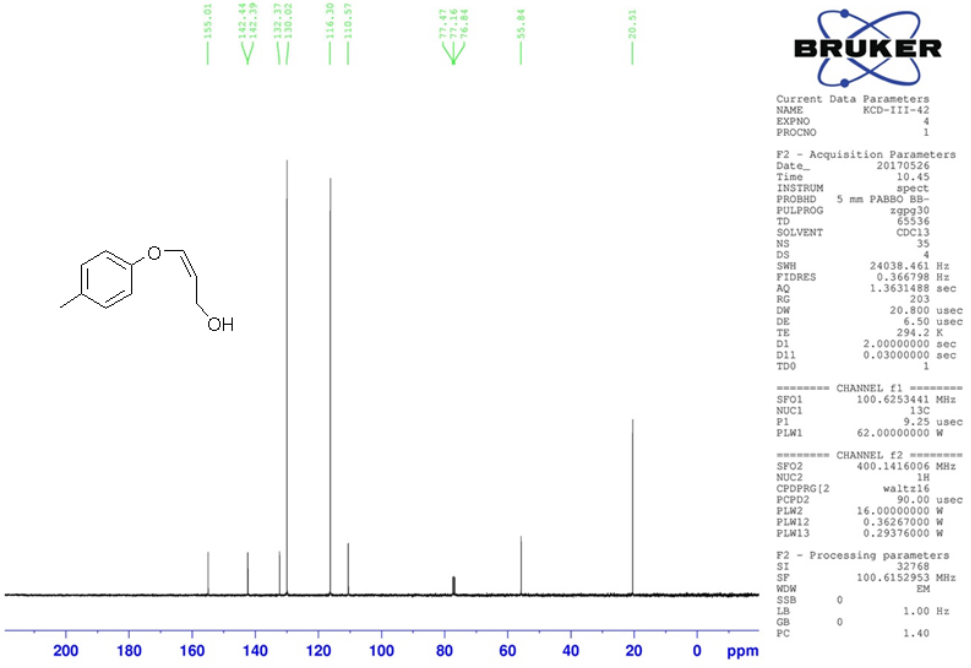


Figure S34. The ^{13}C NMR spectrum of **2e** in $CDCl_3$

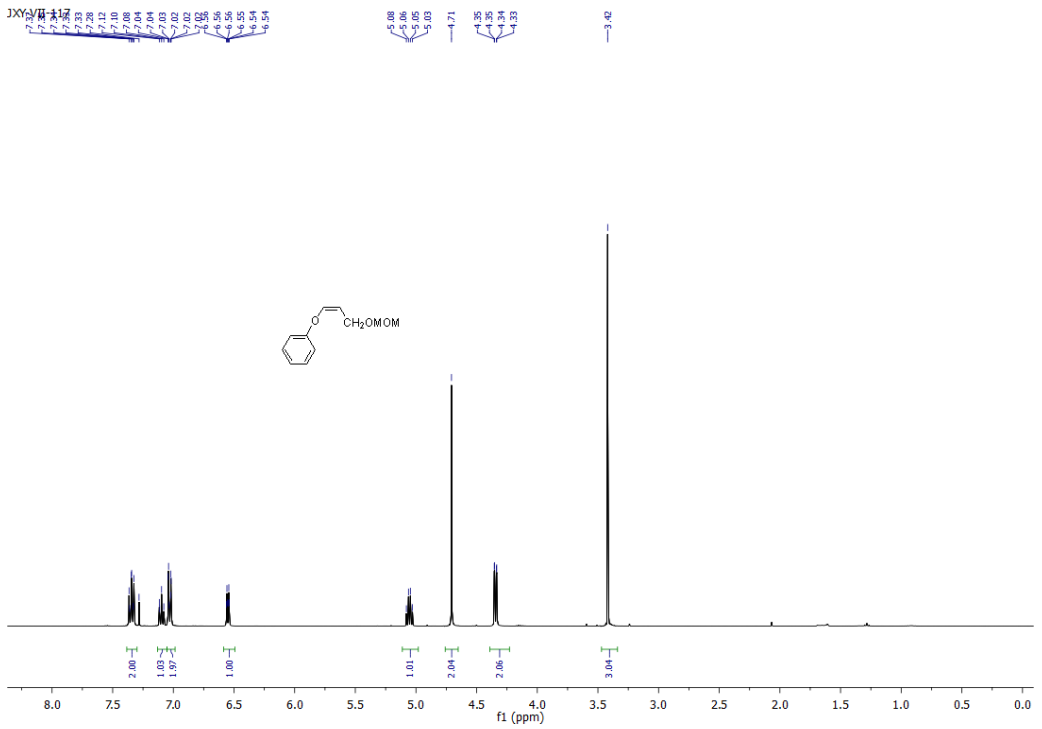


Figure S35. The ^{1}H NMR spectrum of **5a** in $CDCl_3$

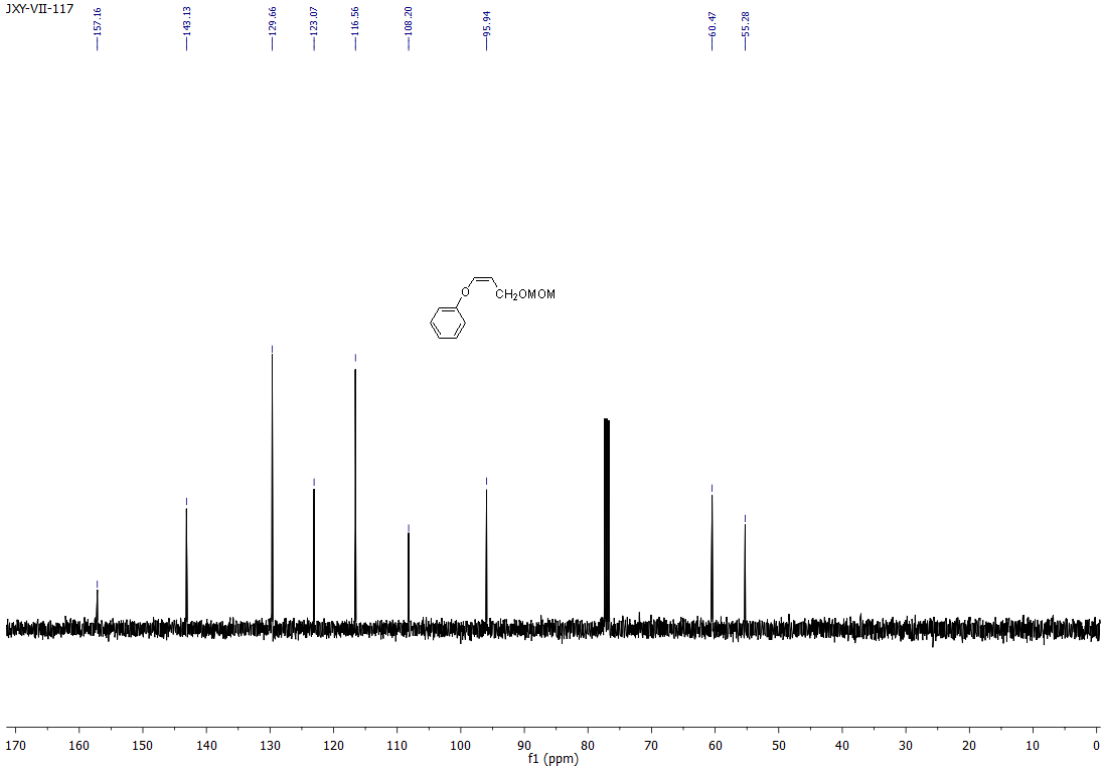


Figure S36. The ^{13}C NMR spectrum of **5a** in $CDCl_3$

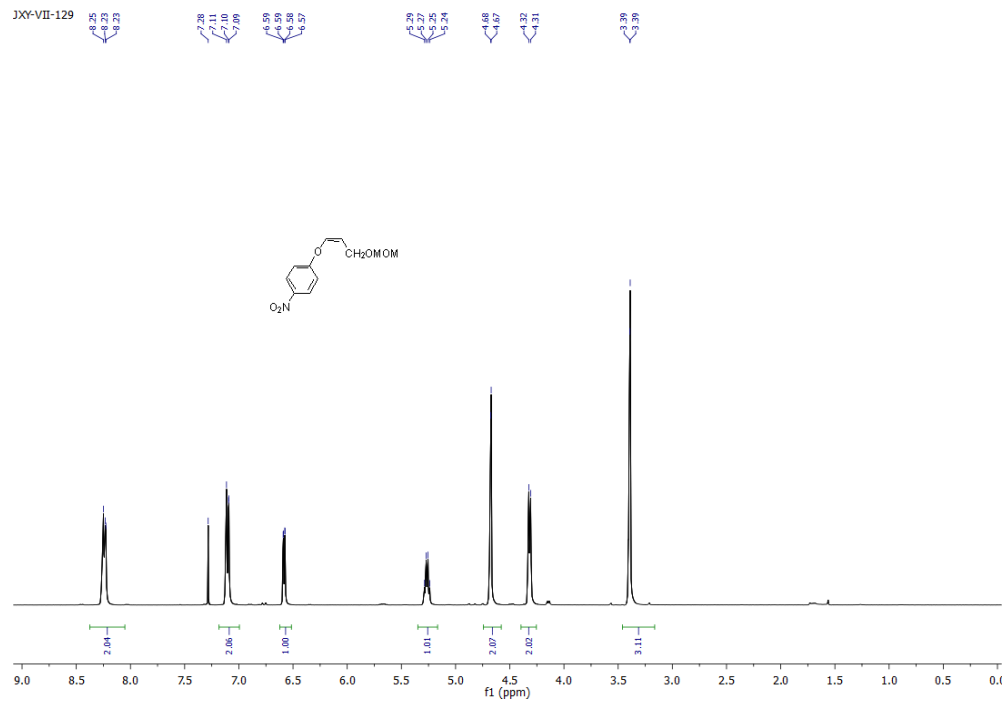


Figure S37. The ^{1}H NMR spectrum of **5b** in $CDCl_3$

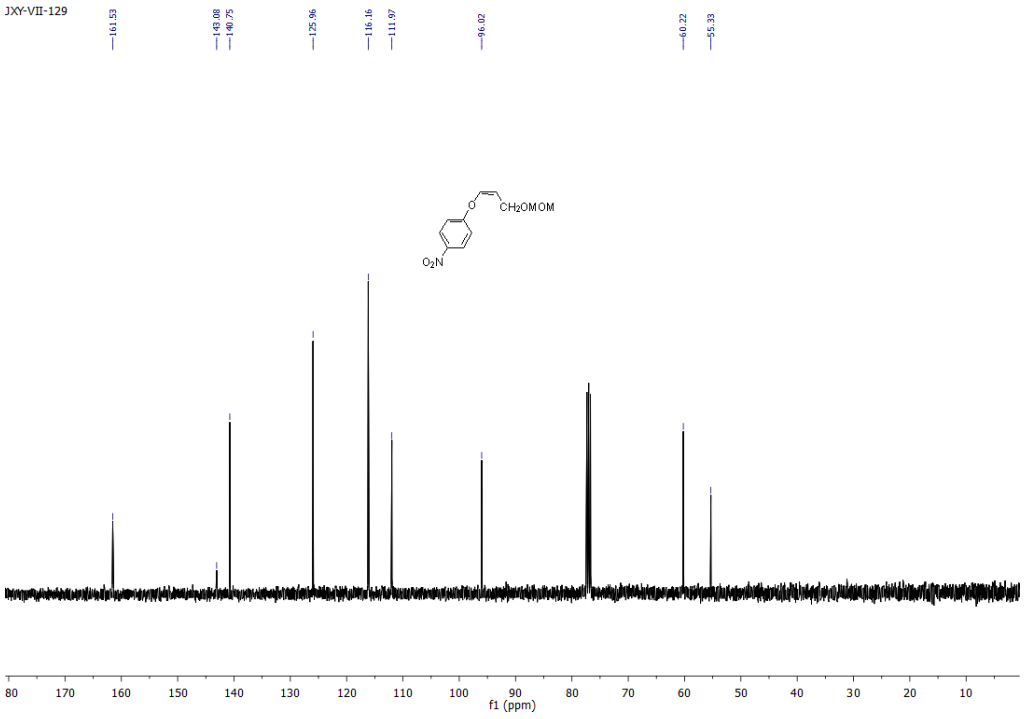


Figure S38. The ^{13}C NMR spectrum of **5b** in $CDCl_3$

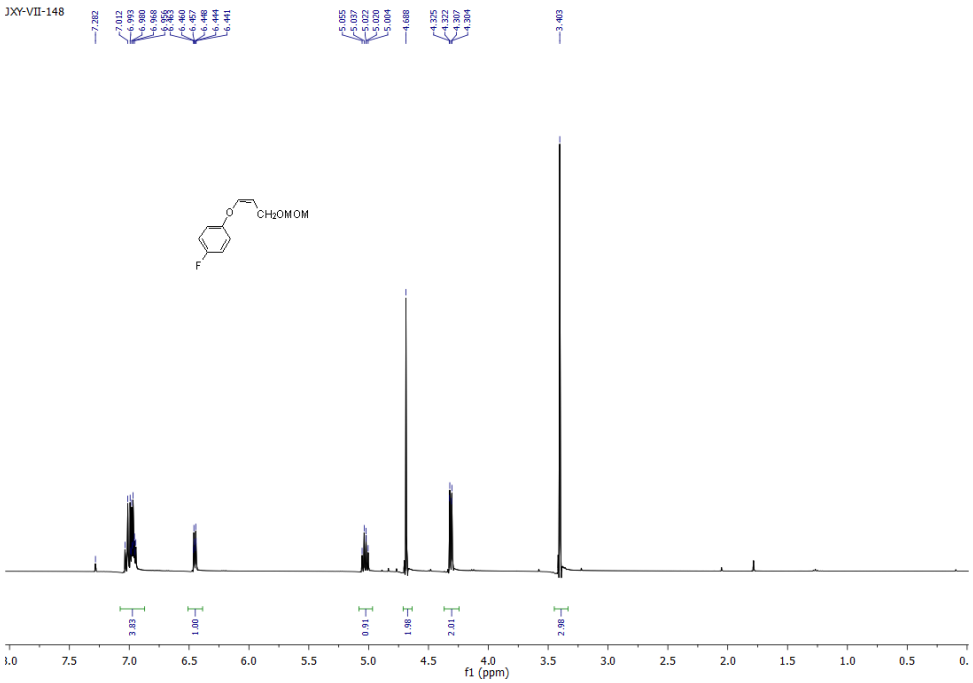


Figure S39. The ^{1}H NMR spectrum of **5c** in $CDCl_3$

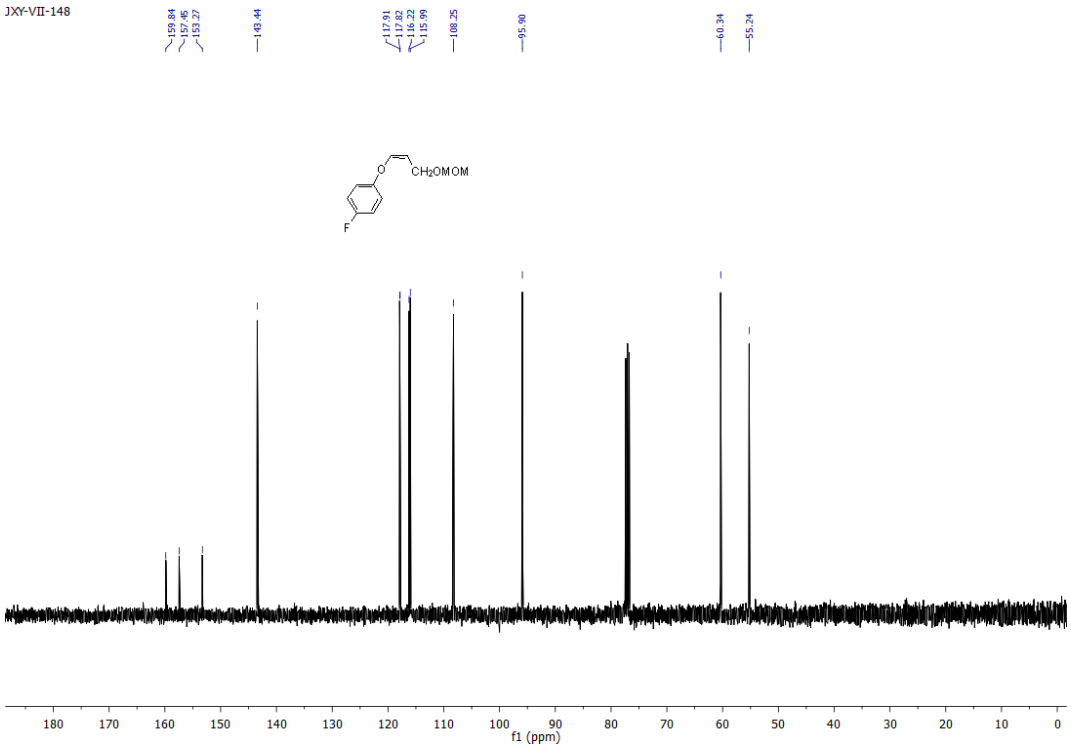


Figure S40. The ^{13}C NMR spectrum of **5c** in $CDCl_3$

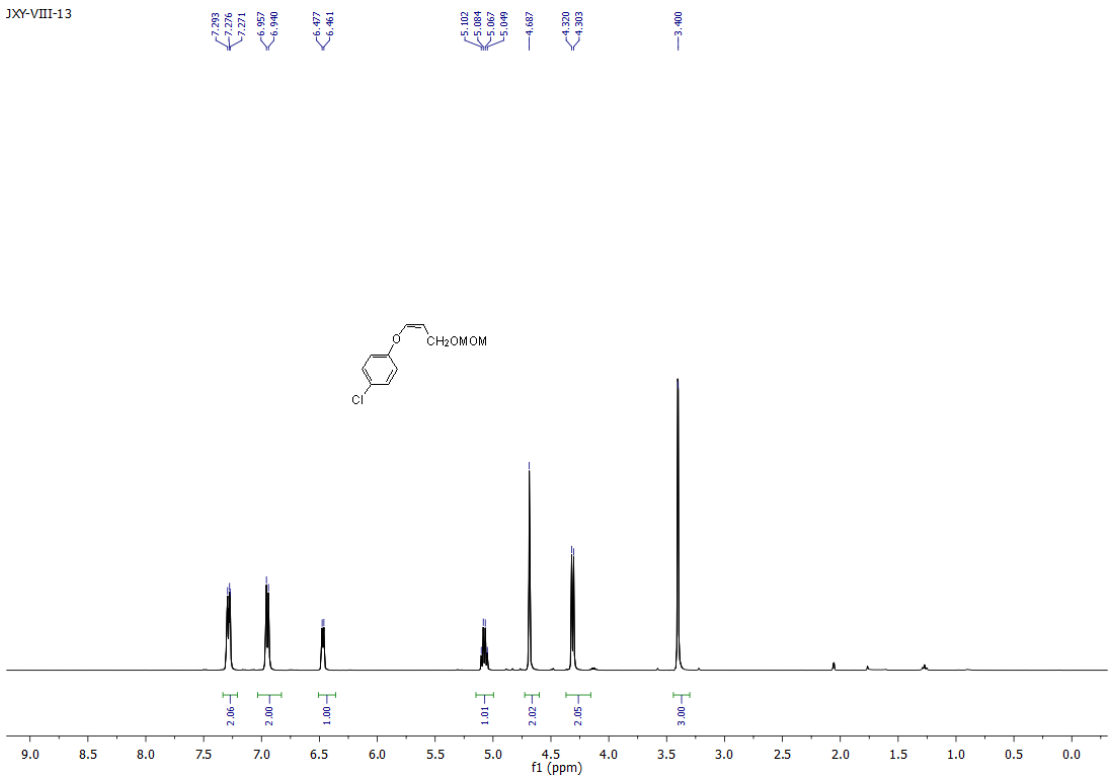


Figure S41. The ^{1}H NMR spectrum of **5d** in $CDCl_3$

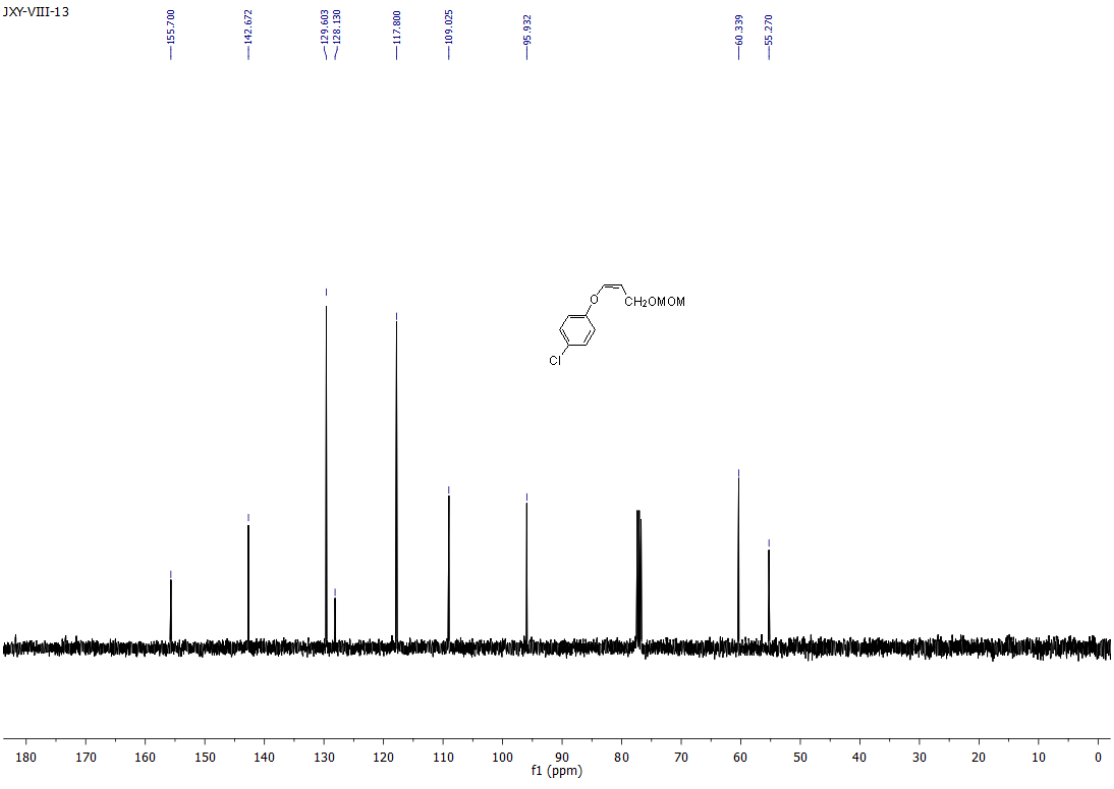


Figure S42. The ^{13}C NMR spectrum of **5d** in $CDCl_3$

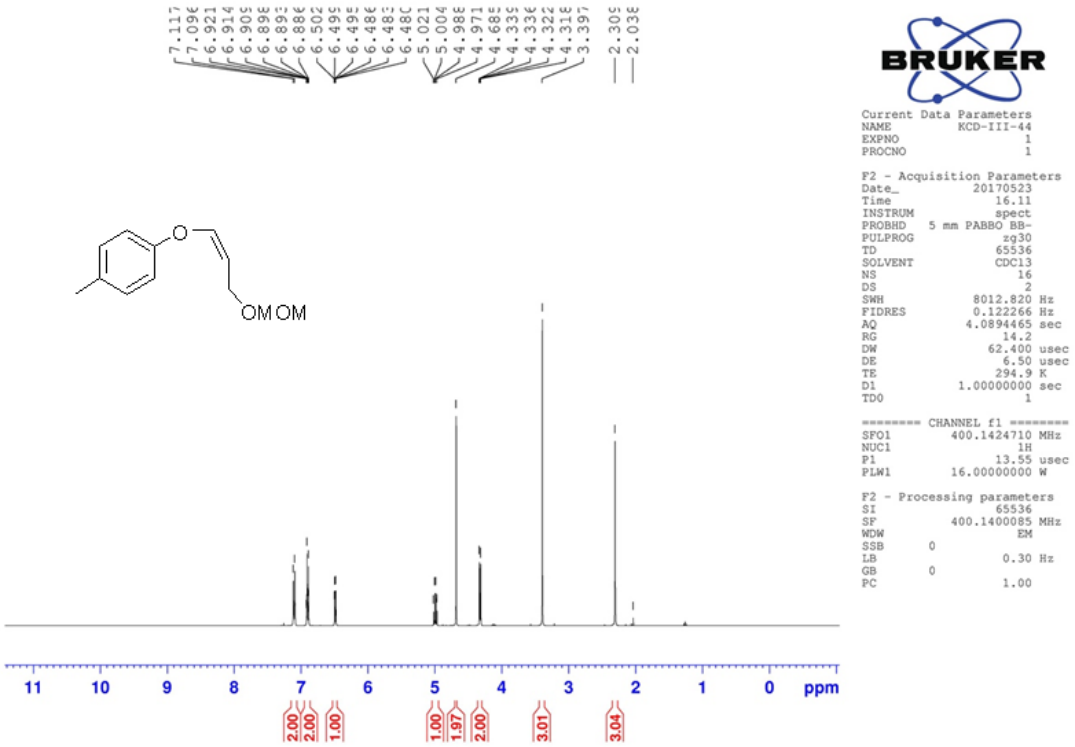


Figure S43. The ^{1}H NMR spectrum of **5e** in $CDCl_3$

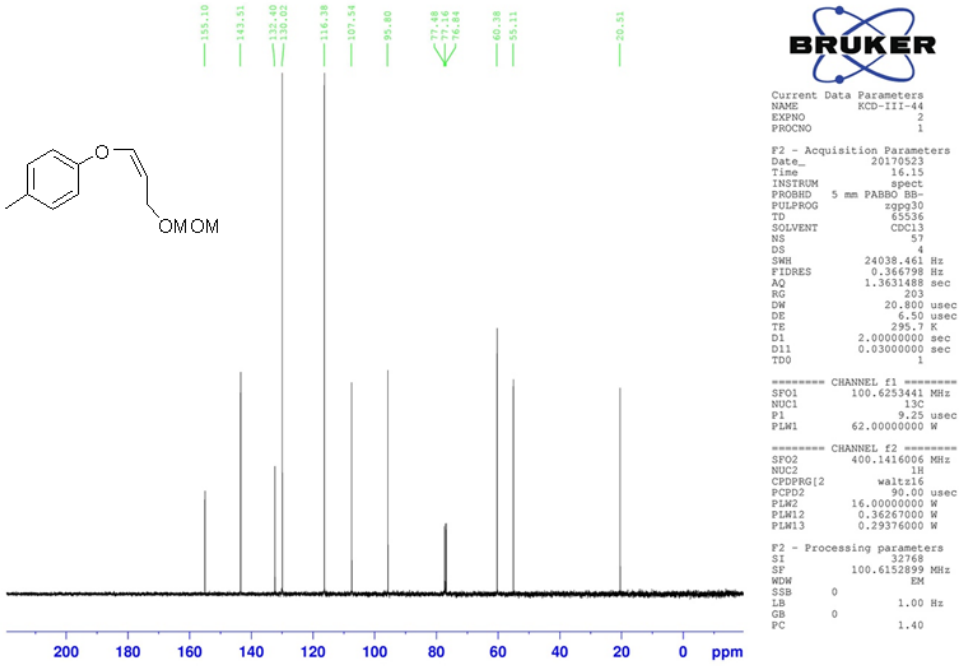


Figure S44. The ^{13}C NMR spectrum of **5e** in $CDCl_3$

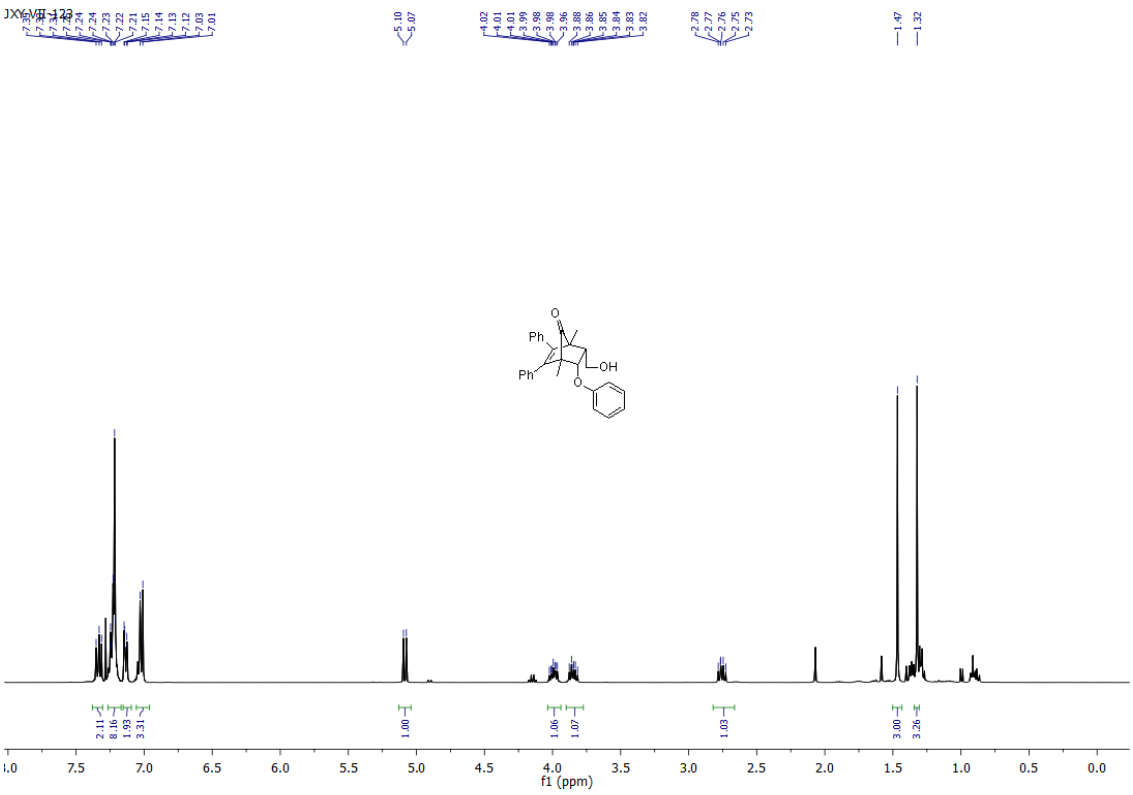


Figure S45. The ^{1}H NMR spectrum of **6a** in $CDCl_3$

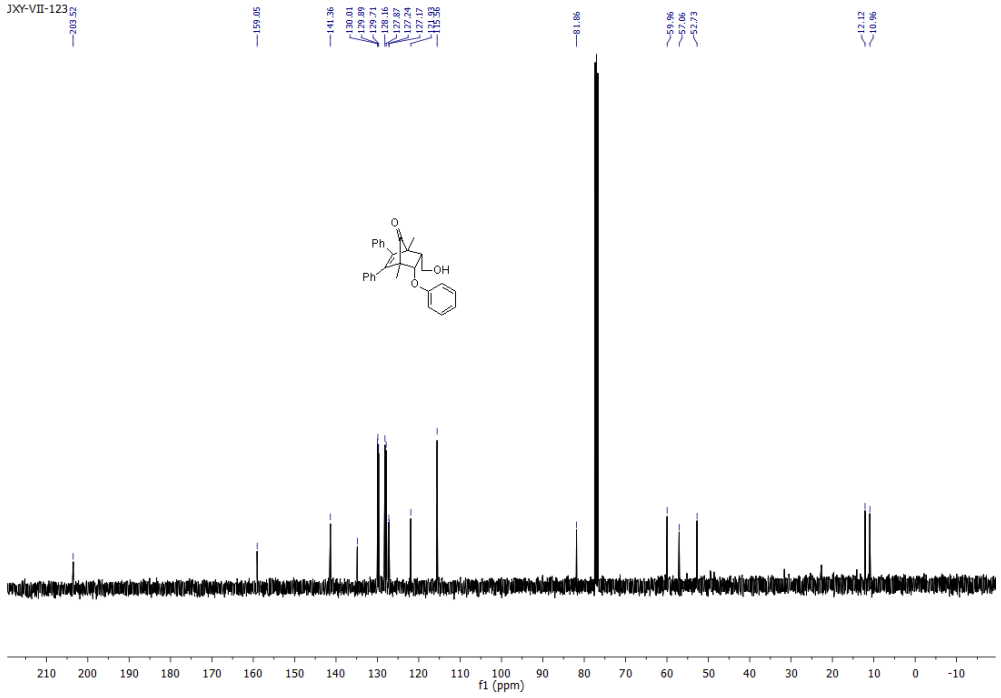


Figure S46. The ^{13}C NMR spectrum of **6a** in $CDCl_3$

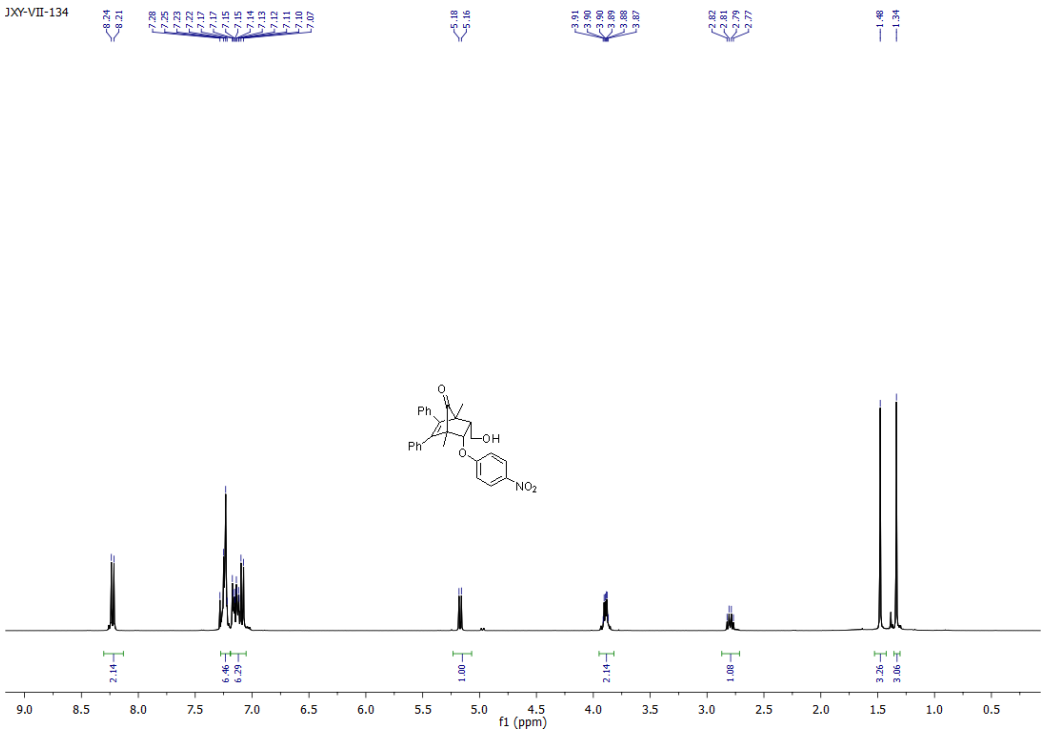


Figure S47. The ^{1}H NMR spectrum of **6b** in $CDCl_3$

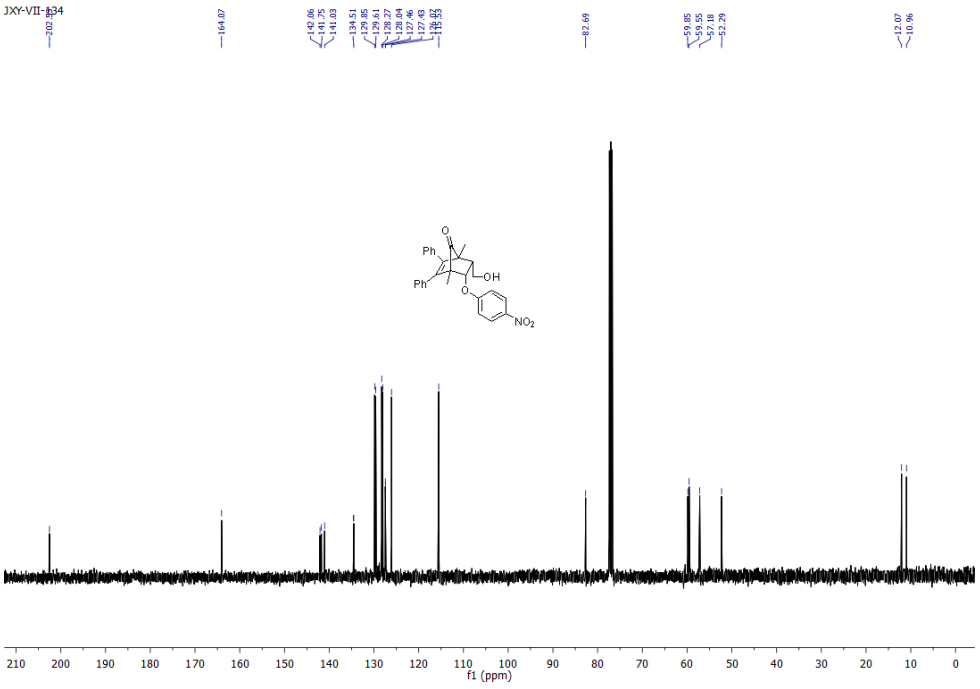


Figure S48. The ^{13}C NMR spectrum of **6b** in $CDCl_3$

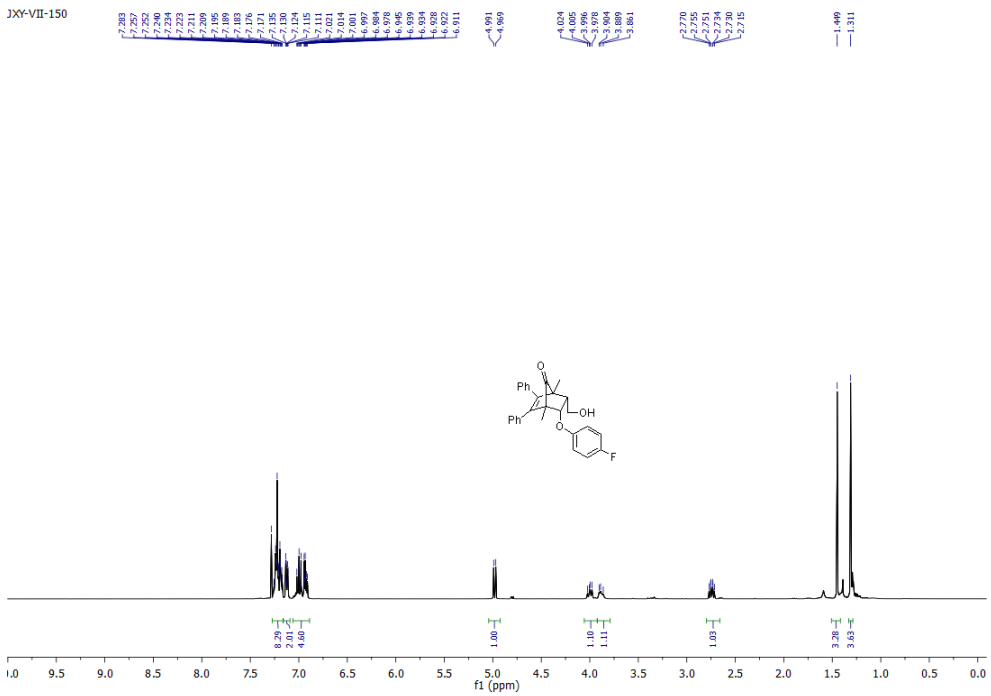


Figure S49. The ^{1}H NMR spectrum of **6c** in $CDCl_3$

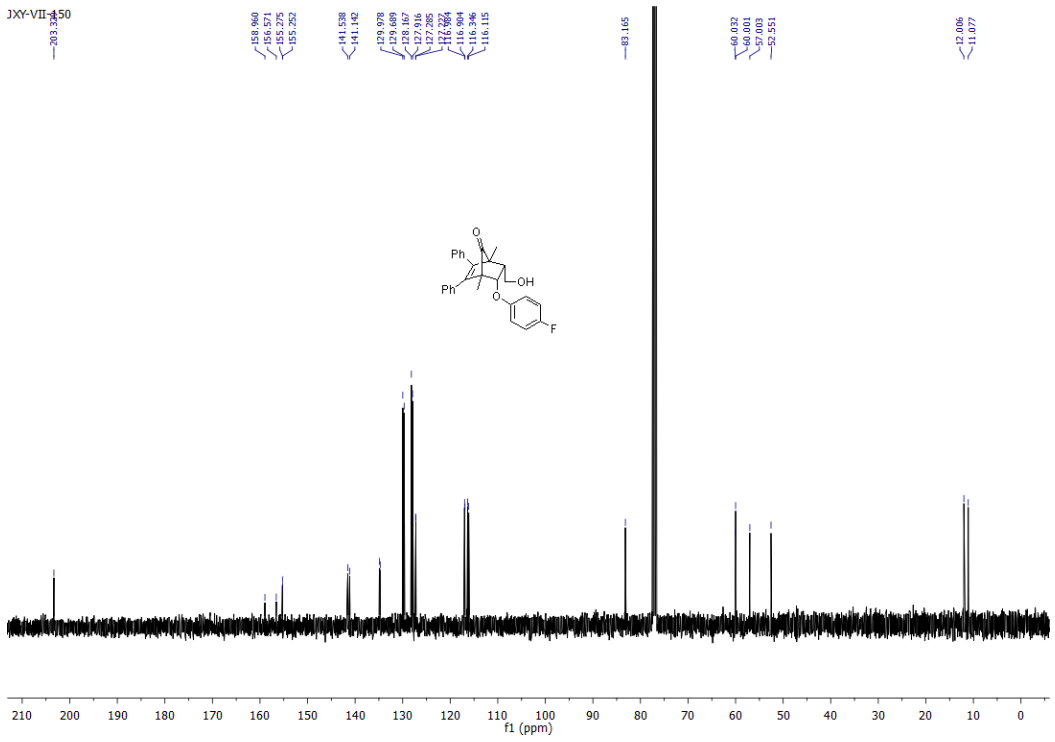


Figure S50. The ^{13}C NMR spectrum of **6c** in $CDCl_3$

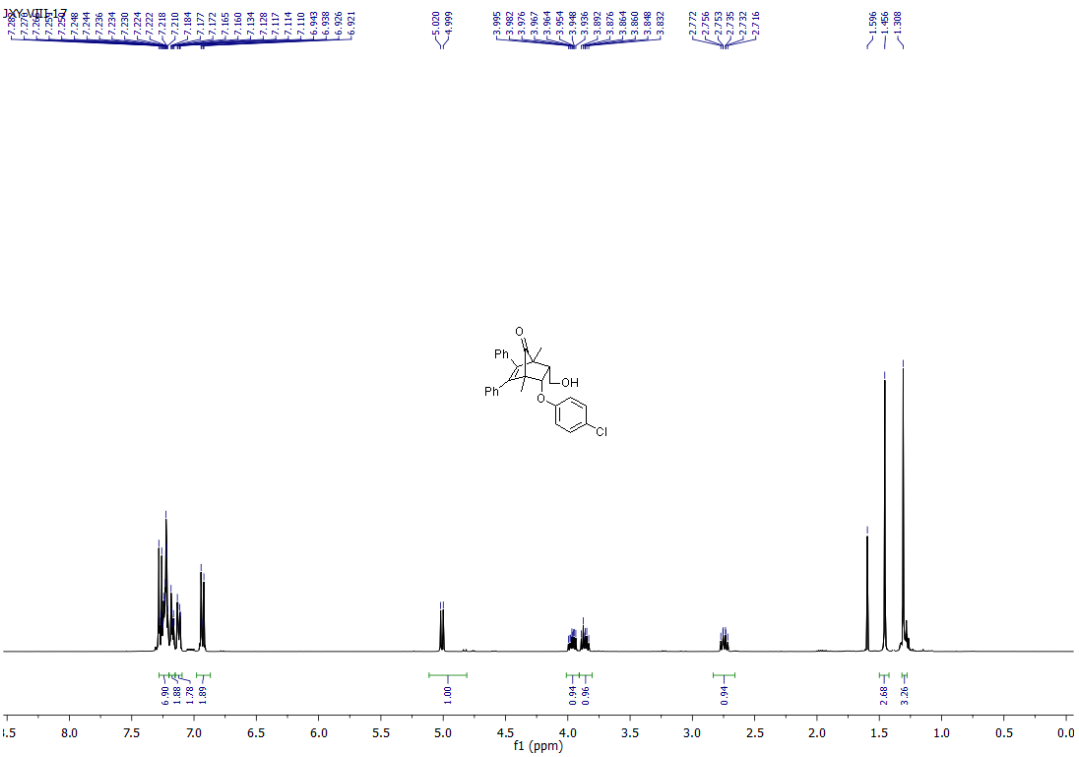


Figure S51. The ^{1}H NMR spectrum of **6d** in $CDCl_3$

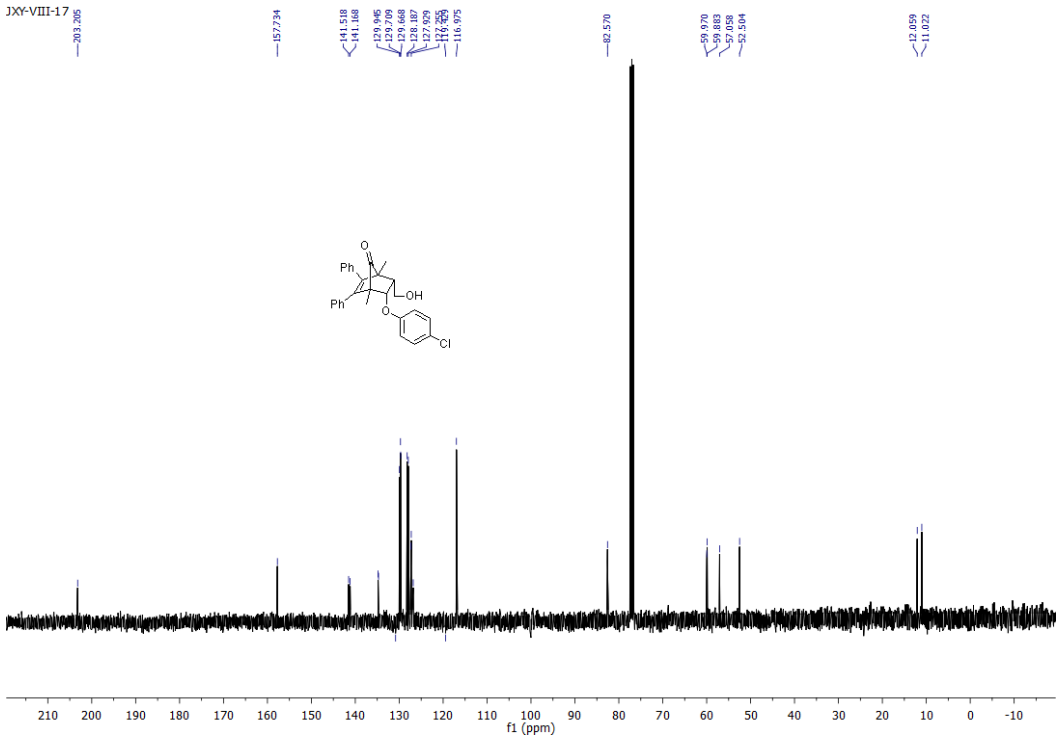


Figure S52. The ^{13}C NMR spectrum of **6d** in $CDCl_3$

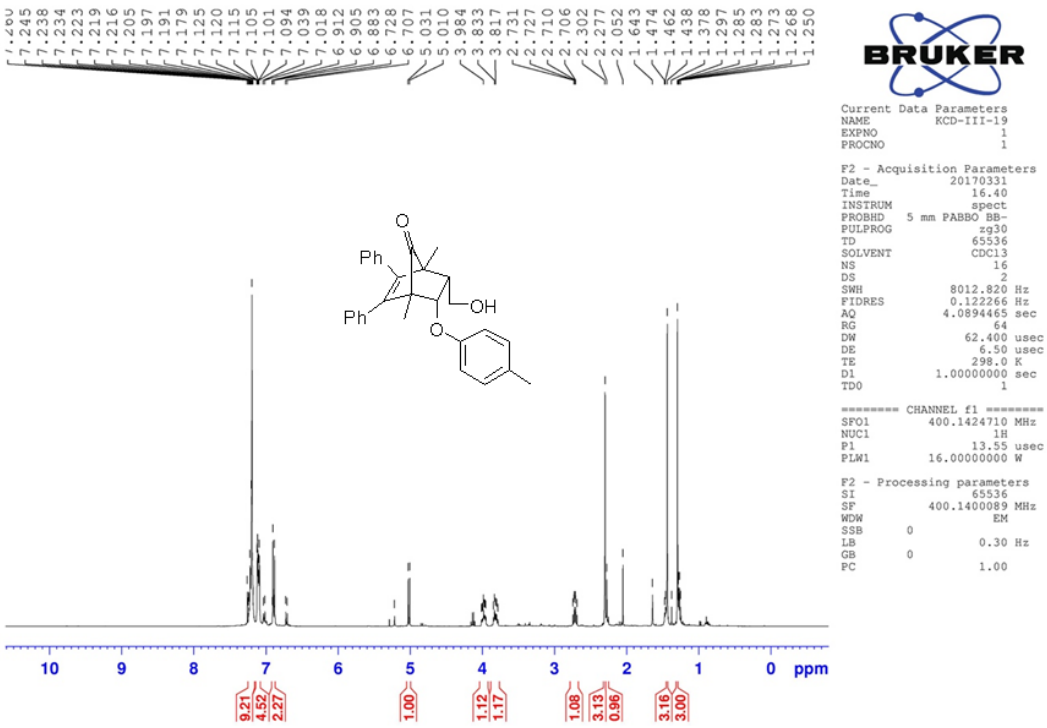


Figure S53. The ^{1}H NMR spectrum of **6e** in $CDCl_3$

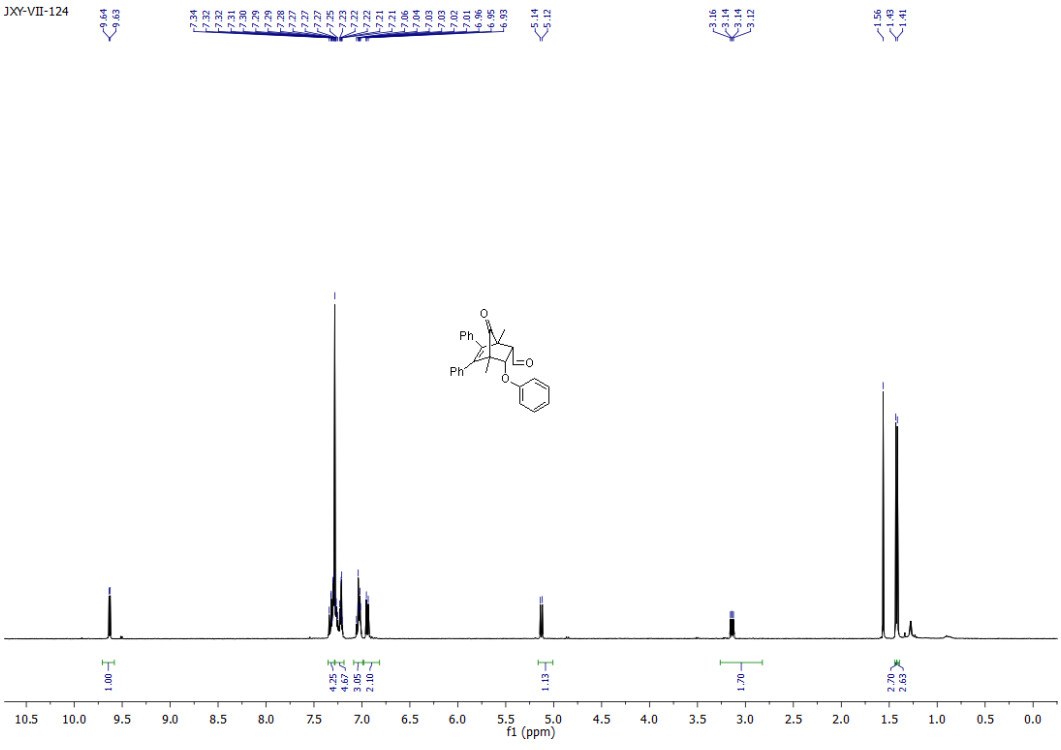


Figure S54. The ^{1}H NMR spectrum of **BW-CO-201** in $CDCl_3$

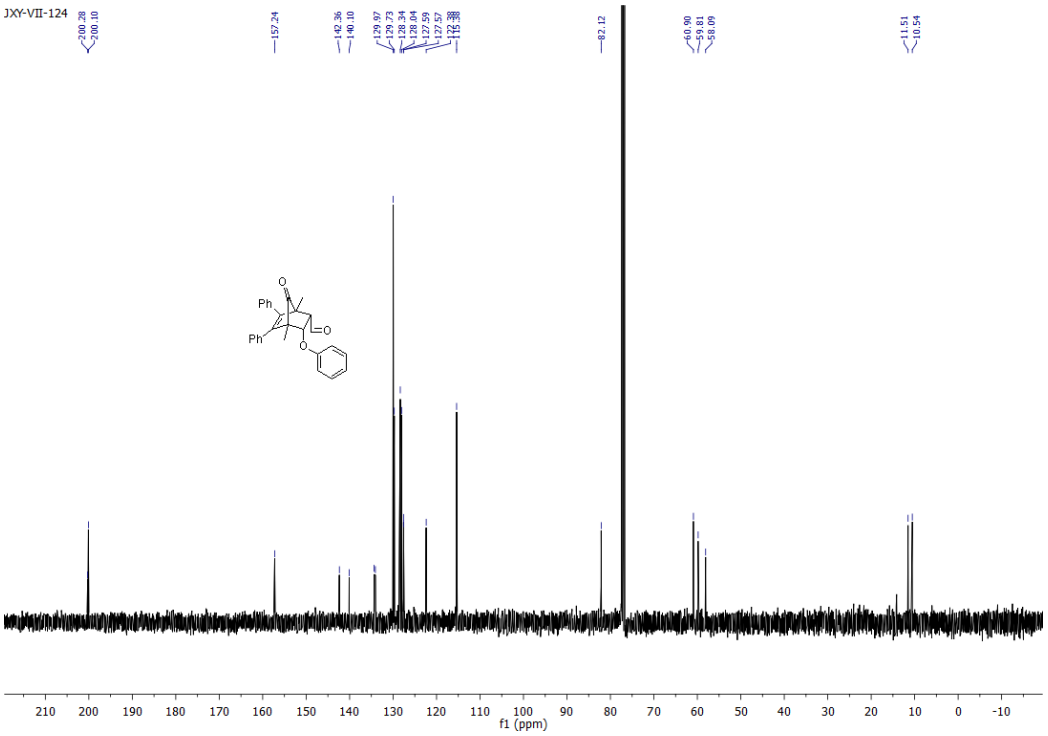


Figure S55. The ^{13}C NMR spectrum of **BW-CO-201** in $CDCl_3$

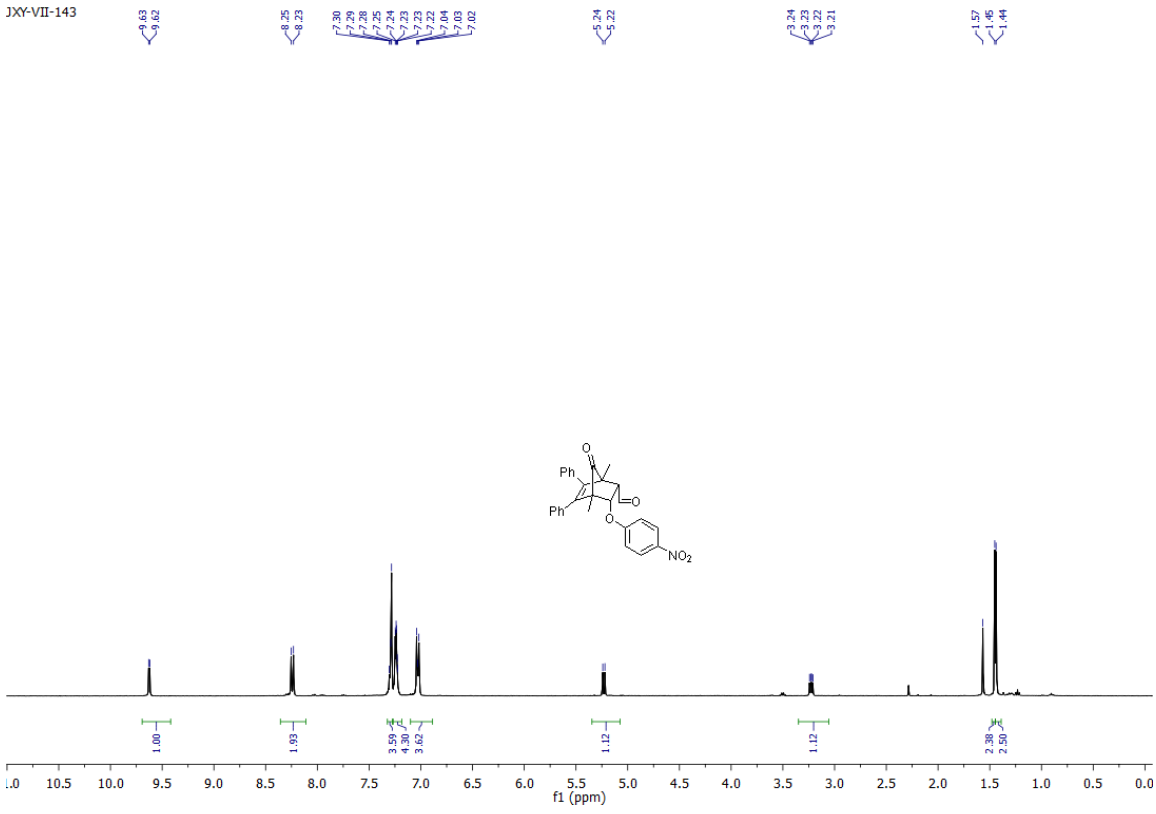


Figure S56. The ^{1}H NMR spectrum of **BW-CO-202** in $CDCl_3$

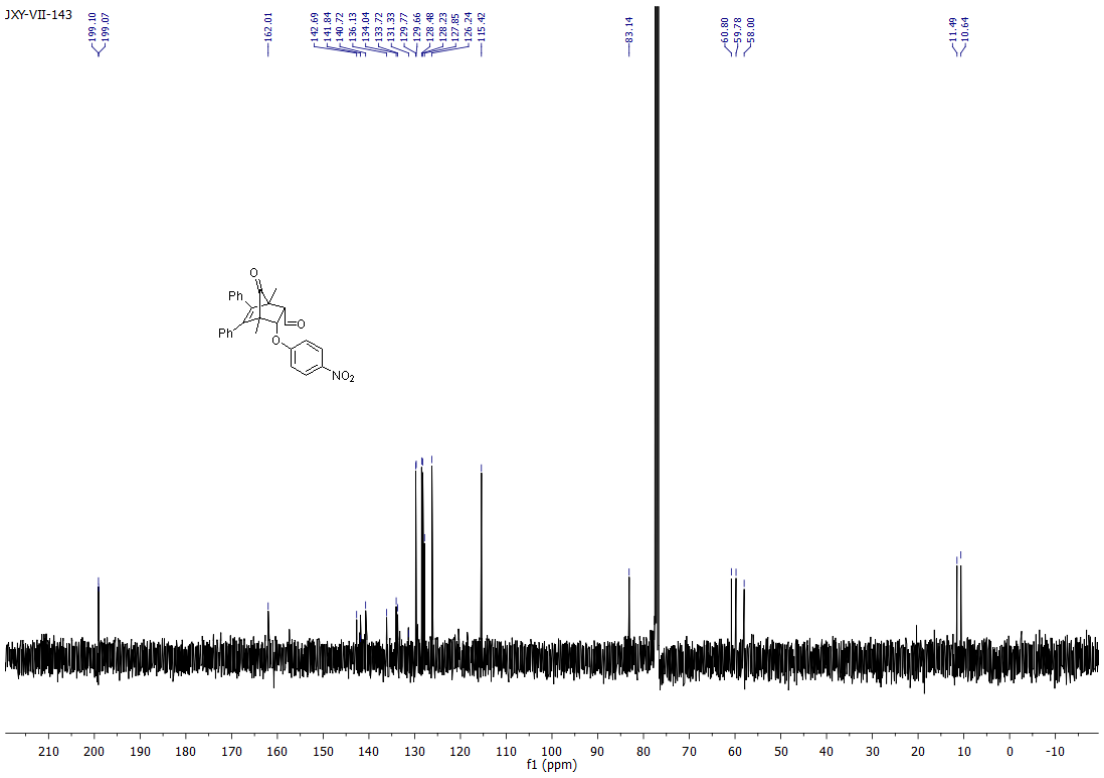


Figure S57. The ^{13}C NMR spectrum of **BW-CO-202** in $CDCl_3$

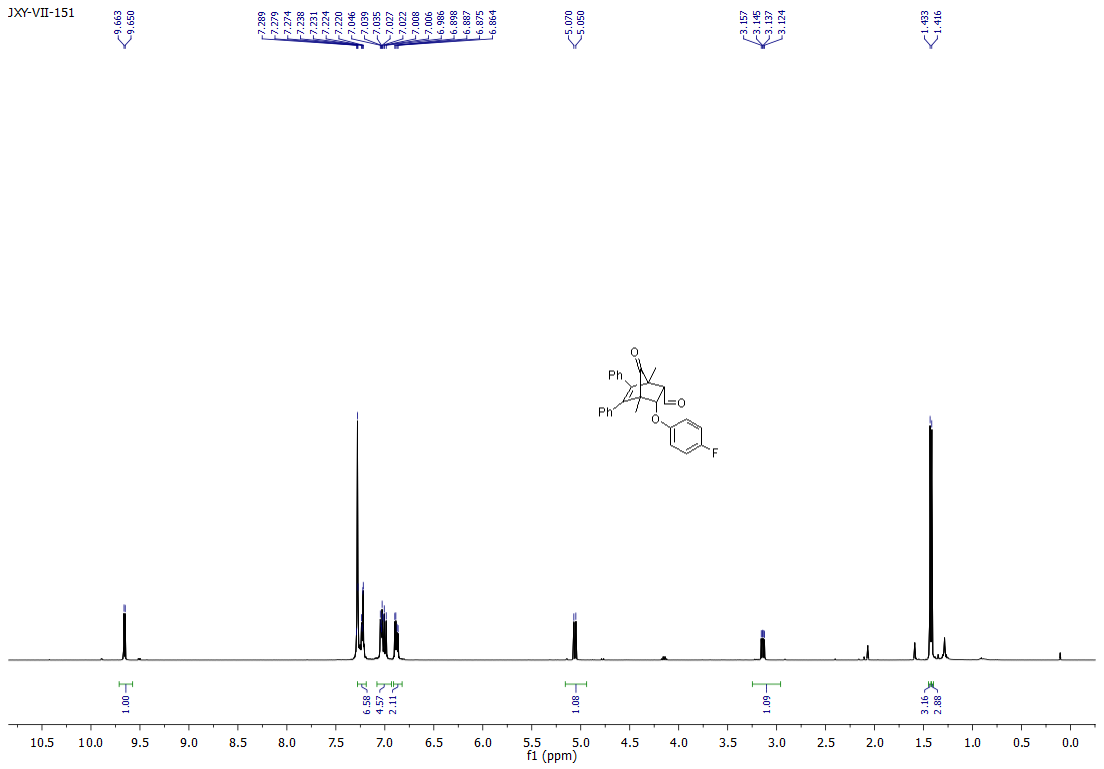


Figure S58. The ^{1}H NMR spectrum of **BW-CO-203** in $CDCl_3$

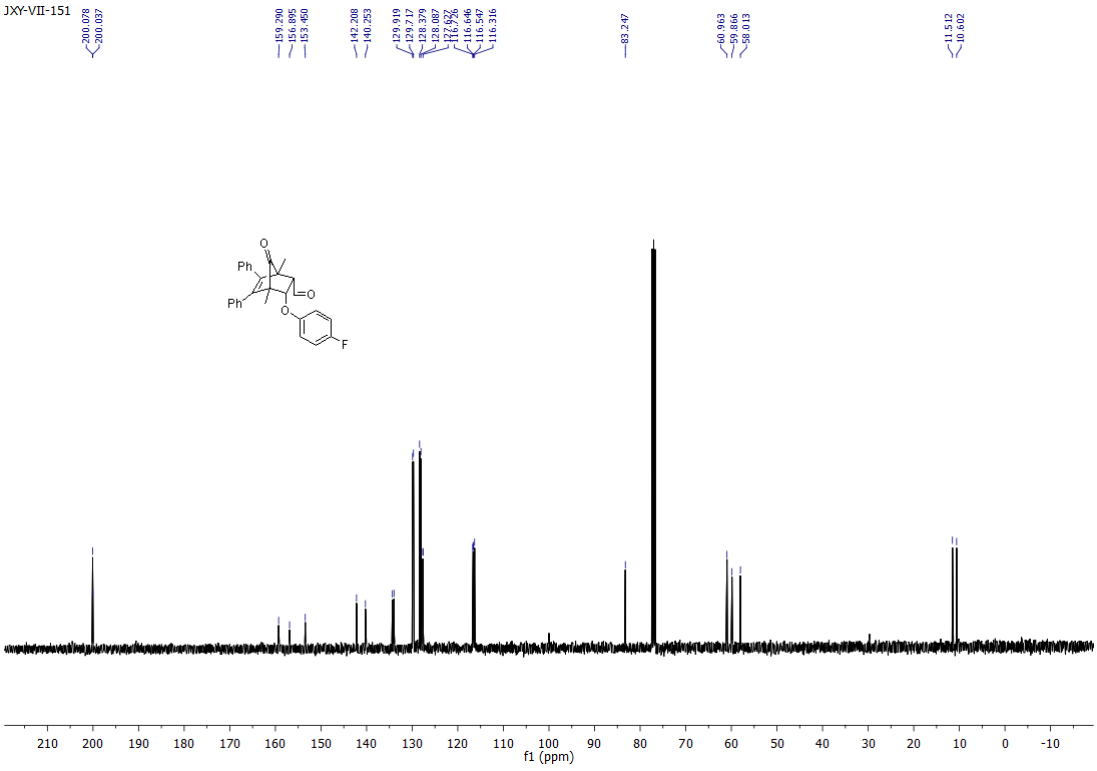


Figure S59. The ^{13}C NMR spectrum of **BW-CO-203** in $CDCl_3$

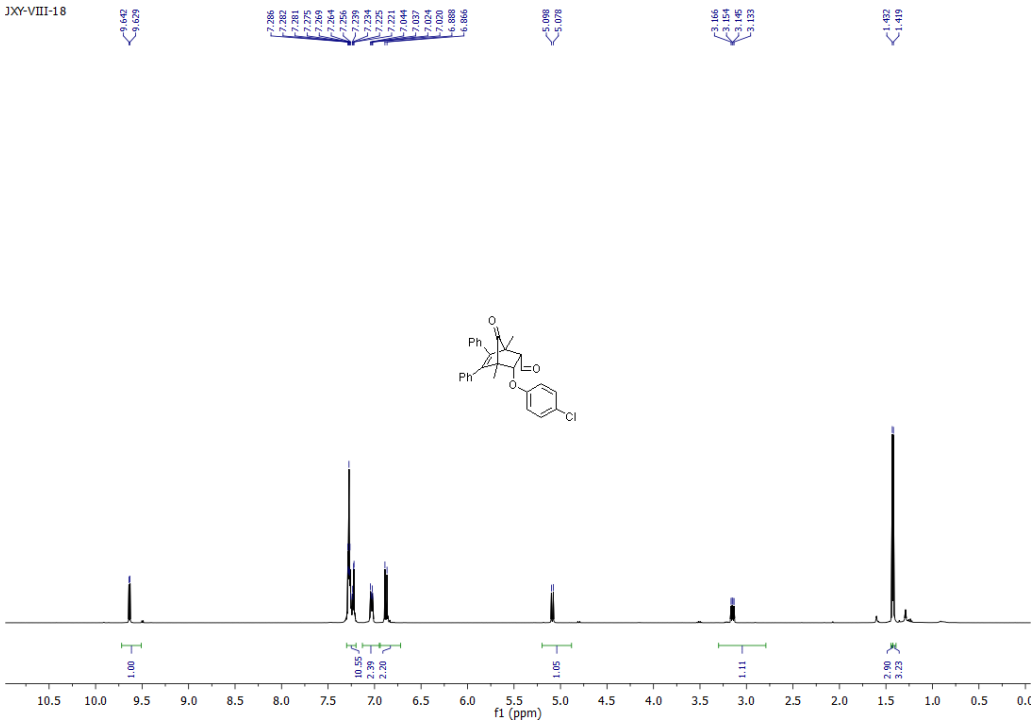


Figure S60. The ^{1}H NMR spectrum of **BW-CO-204** in $CDCl_3$

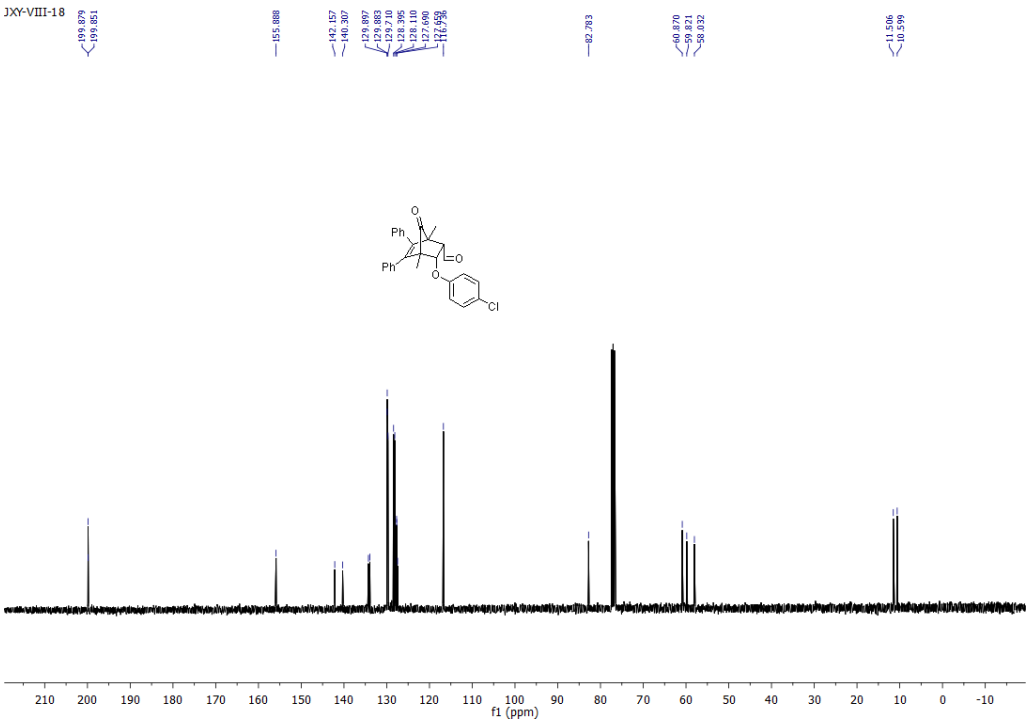


Figure S61. The ^{13}C NMR spectrum of **BW-CO-204** in $CDCl_3$

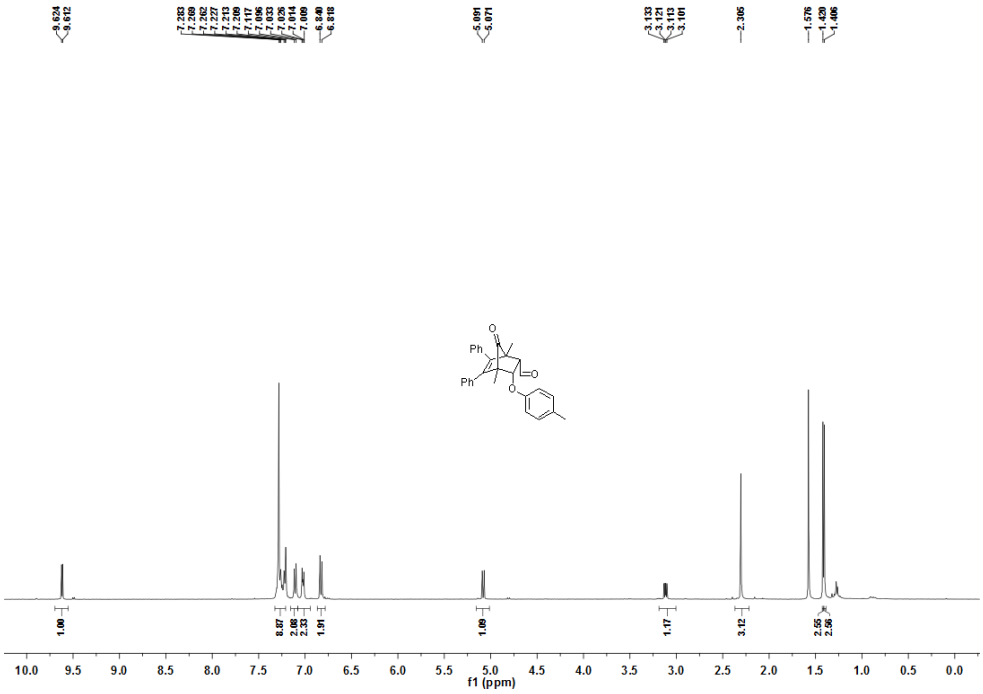


Figure S62. The ^{1}H NMR spectrum of **BW-CO-205** in $CDCl_3$

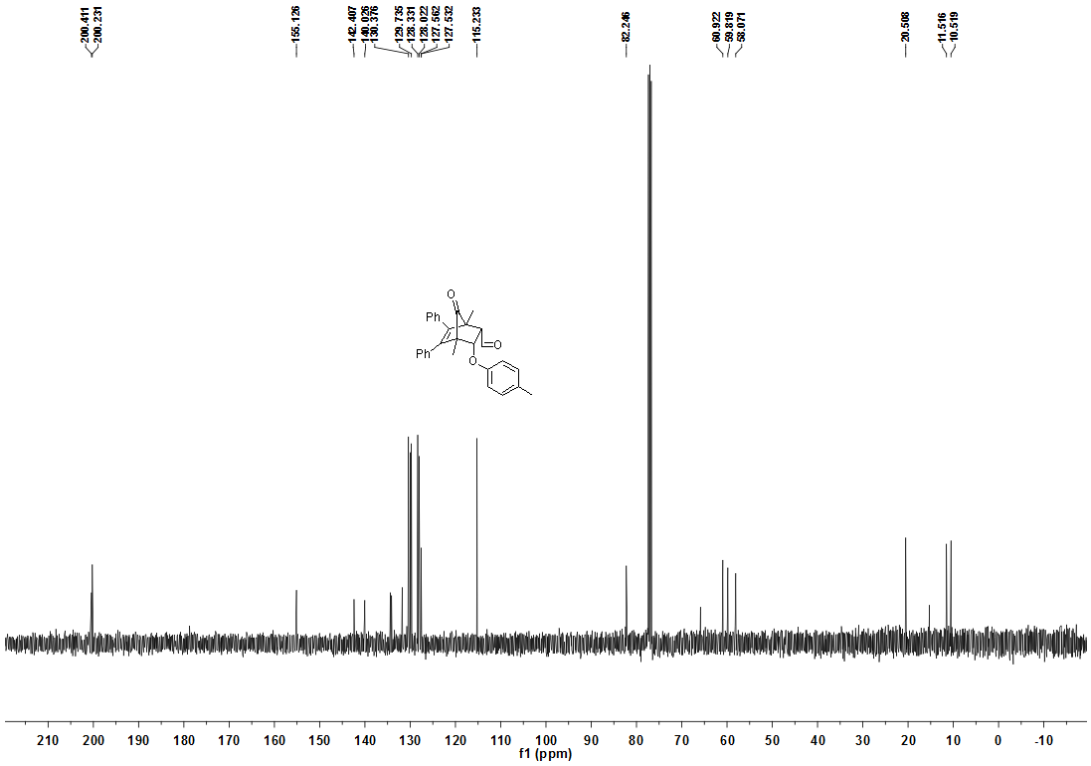


Figure S63. The ^{13}C NMR spectrum of **BW-CO-205** in $CDCl_3$

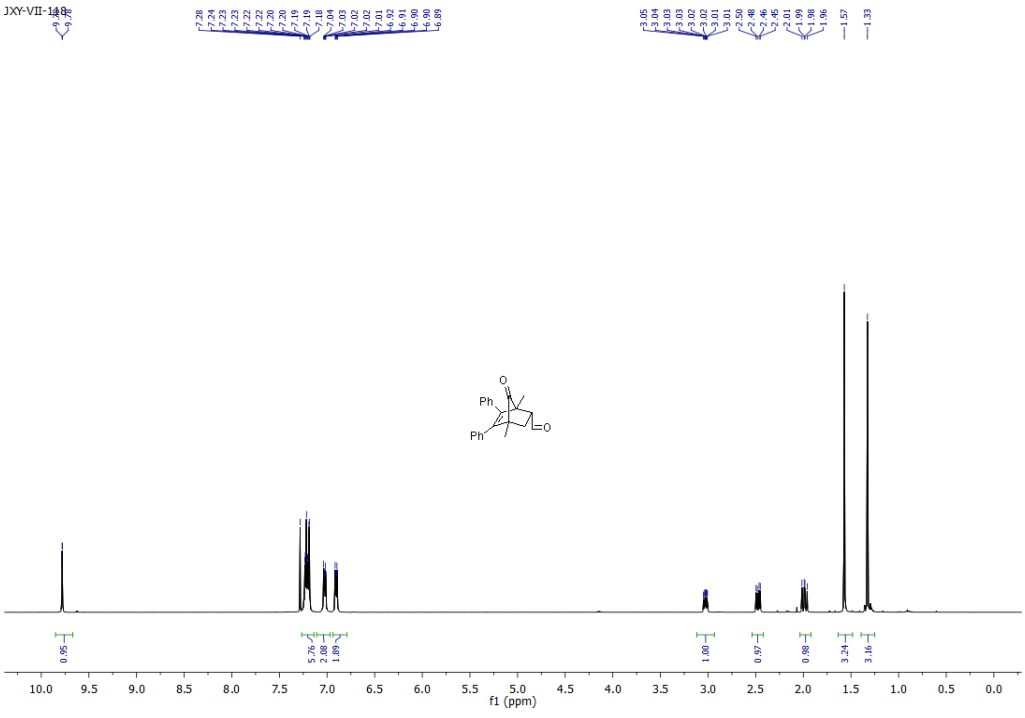


Figure S64. The ^{1}H NMR spectrum of **4** in $CDCl_3$

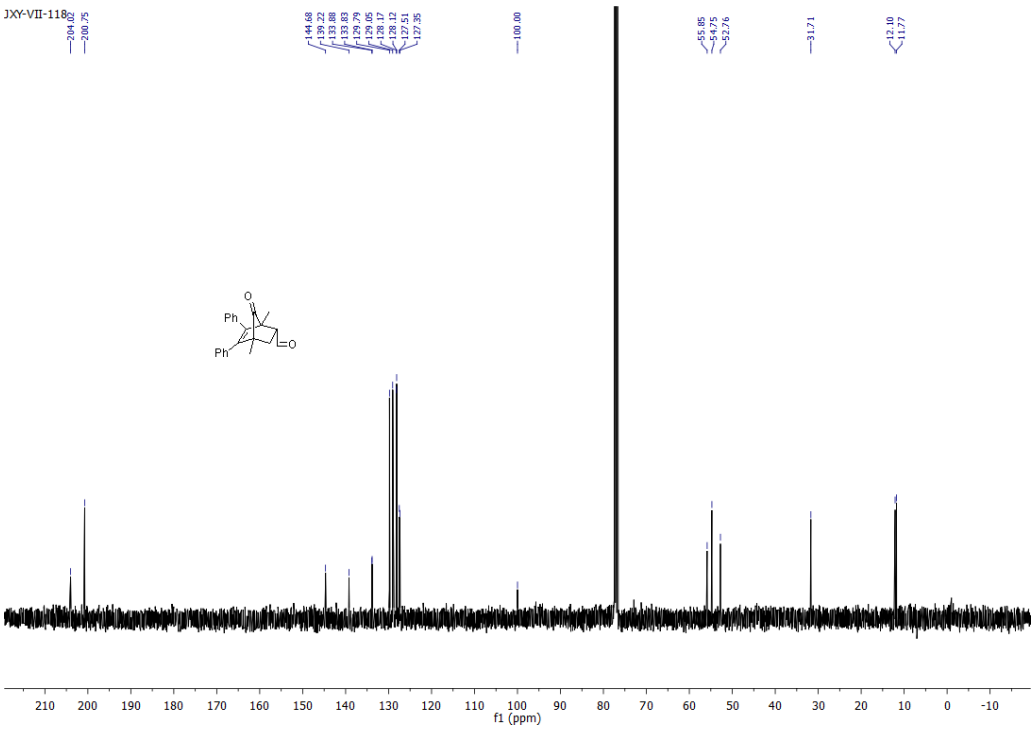


Figure S65. The ^{13}C NMR spectrum of **4** in $CDCl_3$

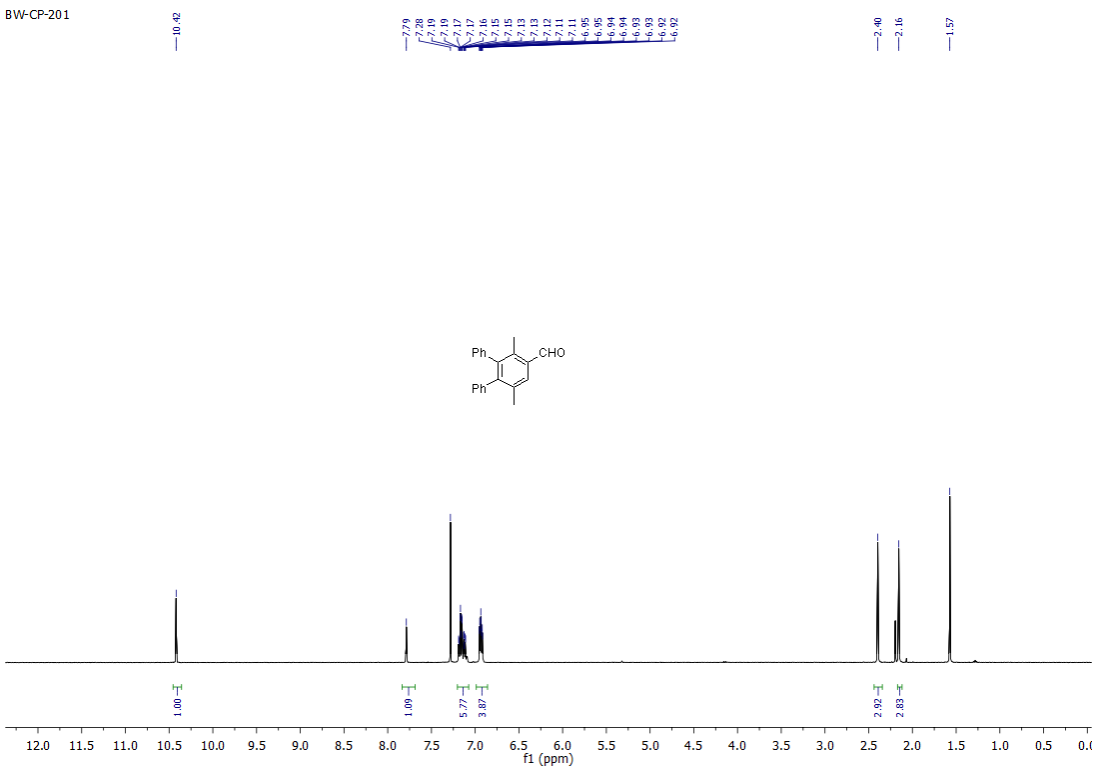


Figure S66. The ^{1}H NMR spectrum of **7** in $CDCl_3$

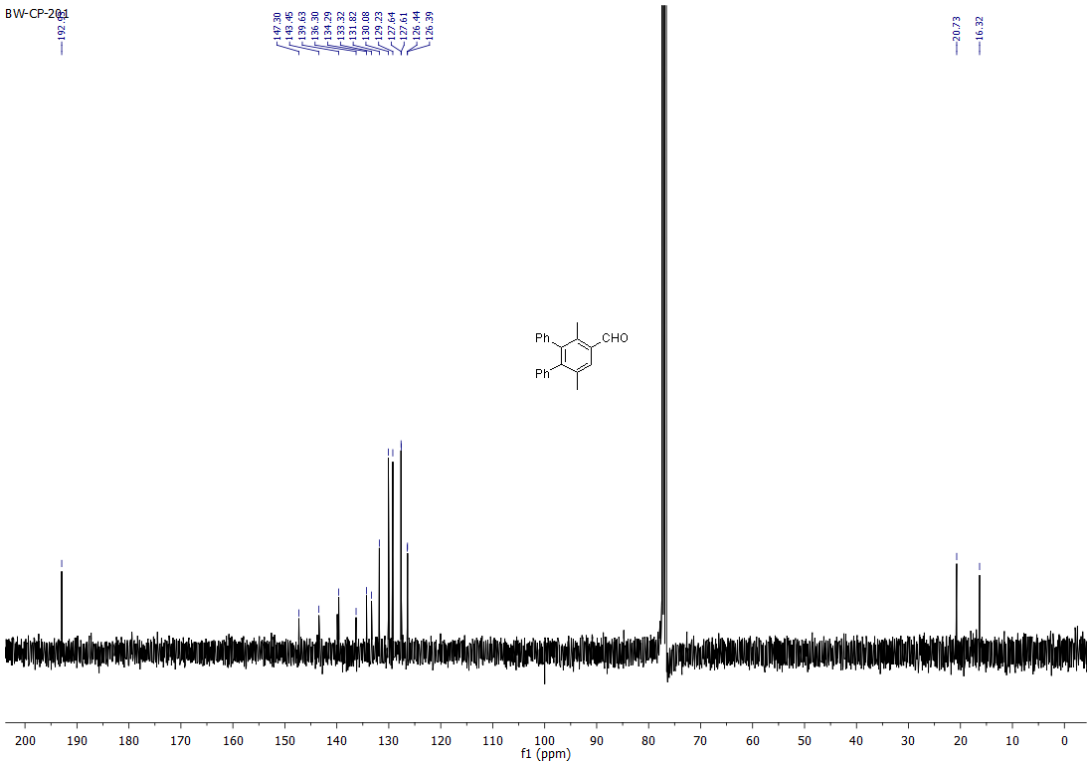


Figure S67. The ^{13}C NMR spectrum of **7** in $CDCl_3$

IV. References

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