An Efficient Synthesis of 5-Amido-3-Hydroxy 4-Pyrones as Inhibitors of Matrix Metalloproteinases

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Supporting Information

General Method: All reagents were used as supplied commercially unless otherwise noted. All reactions were performed in oven-dried glassware. Flash column chromatography was performed on Merck silica gel 60 (230–400 mesh ASTM). Nuclear magnetic resonance spectra (^{1}H NMR and ^{13}C NMR) were recorded on a Varian XL-400 or XL-500 Spectrometer. Visible spectra were recorded in CH_2Cl_2 using a Perkin-Elmer Lambda 25 spectrophotometer. Infrared spectra were collected on a Nicolet AVATAR 360 FT-IR instrument at the Department of Chemistry and Biochemistry, University of California, San Diego (laboratory of Prof. D. Hendrickson). Mass spectra were acquired at the Small Molecule Mass Spectrometry Facility at the Department of Chemistry and Biochemistry, University of California, San Diego. A ThermoFinnigan MAT 900XL mass spectrometer was used to acquire the data for the high resolution mass spectra (HRMS). Elemental analysis was performed by NuMega Laboratories, San Diego, California.

Preparation of Compound (2): To a stirred solution of (**1**) (3-bromopyruvic acid, 10 g, 60 mmol) in trimethyl orthoformate (30 mL, 180 mmol) was added H_2SO_4 (conc. 0.6 mL). The reaction mixture was stirred at room temperature overnight (23 h). The

mixture was diluted with CH_2Cl_2 (150 mL) and then washed with water and brine. After dried over anhydrous $MgSO_4$, the organic solution was concentrated and the residue was recrystallized from CH_2Cl_2 and hexane. The product was a white solid (13.0 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.27 (t, J = 7.2 Hz, 6H), 3.45–3.65 (m, 4H), 3.62 (s, 2H), 9.00 (s, 1H); ^{13}C NMR ($CDCl_3$): δ 169.03, 100.33, 59.73, 30.35, 15.24; ESI-MS: m/z = 262.82 $[M+Na]^+$for $C_7H_{13}BrO_4Na$; HRMS for $C_7H_{14}O_4Br$ $[M+H]^+$: calcd. 241.0070, found 241.0066.

Preparation of Compound (3): Compound (**2**) (2.41 g, 10 mmol) and 4-nitrophenyl trifluoroacetate (2.82 g, 12 mmol) were stirred in pyridine (10 mL) under N_2 overnight. After diluted with water (50 mL), the mixture was extracted with Et_2O (3×50 mL). The combined extracts were washed with 5% NaOH (5×50 mL) then dried over anhydrous $MgSO_4$. After concentration, the residue was recrystallized from hexane. The product was a white solid (2.38 g, 65%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.29 (d, J = 5.0 Hz, 2H), 7.33 (d, J = 5.0 Hz, 2H), 3.77 (s, 2H), 3.70–3.76 (m, 2H), 3.60–3.65 (m, 2H), 1.31 (t, J = 7.2 Hz, 6H); ^{13}C NMR ($CDCl_3$): δ 165.09, 154.91, 125.26, 122.39, 100.22, 59.12, 31.07, 15.26. ESI-MS: m/z = 383.93 $[M+Na]^+$for $C_{13}H_{16}BrNO_6Na$; HRMS for $C_{13}H_{17}NO_6Br$ $[M+H]^+$: calcd. 362.0234, found 362.0235.

Preparation of Compound (4a): To a suspension of NaH (336 mg, 8.4 mmol) in dry THF (25 mL) was added ethyl acetoacetate (1.0 mL, 8 mmol) over 10 min via a syringe. Compound (**3**) (1.44 g, 3.99 mmol) in THF (10 mL) was added slowly to the above solution at room temperature over 15 min. The mixture was refluxed for 4 h. The reaction mixture turned orange and some precipitate formed. After removal of solvent, water (50 mL) was added to the residue. The mixture was extracted with $CHCl_3$. The organic extract was dried over $MgSO_4$ and then concentrated. The residue was purified by column chromatography with eluent EtOAc/Hexane = 10–20%. The product **4a** was a colorless oil (700 mg, 64%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.32 (s, 2H), 4.24 (q, J = 7.0 Hz, 2H), 3.58-3.65 (m, 4H), 2.19 (s, 3H), 1.29 (t, J = 7.0 HZ, 3H), 1.19 (t, J = 7.2 Hz, 6H); ^{13}C NMR ($CDCl_3$): δ 184.22, 176.33, 165.39, 110.86, 91.73, 71.82, 61.49, 58.83, 20.65, 15.83, 14.58 ; ESI-MS: m/z = 272.87 for $C_{13}H_{21}O_6$ $[M+H]^+$; HRMS for $C_{13}H_{21}O_6$ $[M+H]^+$: calcd. 273.1333, found 273.1328.

Preparation of Compound (4b): 4b was prepared similarly to **4a**. A colorless oil, Yield 76%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.30 (s, 2H), 3.59 (q, J = 7.0 Hz, 4H), 2.15 (s, 3H), 1.49 (s, 9H), 1.19 (t, J = 7.0 Hz, 6H); ^{13}C NMR ($CDCl_3$): δ 184.38, 174.69, 164.86, 112.71, 91.73, 82.11, 71.91, 58.67, 28.37, 20.08, 15.68; ESI-MS: *m/z* = 323.04 for $C_{15}H_{24}NaO_6$ $[M+Na]^+$; HRMS for $C_{15}H_{24}O_6$ $[M]^+$: calcd. 300.1567, found 300.1572.

Preparation of Compound (5): To a stirred solution of compound **4a** (1.0 g, 3.66 mmol) in THF (10 mL) was added formic acid (5 mL) and H_2O (1 mL). The mixture was heated to reflux for 2 h. After evaporation of the solvents to dryness, the residue was purified by column chromatography with eluent EtOAc/Hexane = 30–40% (silica gel column was pretreated with a 5% solution of maltol in CH_3OH/CH_2Cl_2 = 10/90 to remove metal ions from the silica gel). The product was a white solid (480 mg, 67%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.77 (s, 1H), 6.51 (s, 1H), 4.39 (q, J = 7.0 Hz, 2H), 2.45 (s, 3H), 1.37 (t, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 170.90, 167.95, 164.14, 145.56, 137.10, 62.26, 19.88, 14.65; ESI-MS: *m/z* = 198.97 $[M+H]^+$ for $C_9H_{11}O_5$; HRMS for $C_9H_{10}O_5$ $[M]^+$: calcd. 198.0523, found 198.0525.

Preparation of Compound (8) from 4a: To a stirred solution of compound (**4a**) (2.30 g, 8.4 mmol) in ethanol/THF (10 mL/10 mL) was added a NaOH (4.0 g, 100 mmol) water (10 mL) solution. The mixture was stirred at room temperature for 1 h. After evaporation of most THF and ethanol, the residue was diluted with water/Et_2O. The mixture was first acidified with acetic acid and extracted with CH_2Cl_2. The water layer was further acidified with HCl (conc.) and then extracted CH_2Cl_2. The organic extracts were combined and dried over $MgSO_4$. After concentration, the crude product was purified by column chromatography with eluent EtOAc/Hexane = 20–40%. The product was light yellow oil (1.22 g, 60%). ^{1}H NMR (400 MHz, $CDCl_3$, two conformations were observed, likely due to an intramolecular hydrogen bonding): δ 17.45 (s, ~0.63×1H, carboxylic acid proton), 15.80 (s, ~0.37×1H, carboxylic acid proton), 4.30 (s, 0.37×2H), 4.24 (s, 0.63×2H), 3.60–3.75 (m, 4H), 2.60 (s, 3H), 1.19–1.23 (m, 6H); ^{13}C NMR ($CDCl_3$): δ 200.77, 194.75, 192.83, 186.60, 173.51, 163.27, 102.02, 100.01, 93.08, 92.51, 67.72, 67.17, 59.00, 58.72, 25.46, 24.23, 15.42,

15.37 ; ESI-MS: *m/z* = 243.10 $[M-H]^+$for $C_{11}H_{15}O_6$. HRMS $[M+H]^+$ for $C_{11}H_{17}O_6$: calcd. 245.1020, found 245.1023.

Preparation of Compound (8) from 4b: To a solution of compound **4b** (200 mg, 0.67 mmol) in CH_2Cl_2 (30 mL) was added trifluoroacetic acid (0.5 mL). The mixture was stirred at room temperature for 30 min. The mixture was washed with water (30 mL) then brine (2_30 mL). After dried over $MgSO_4$, the organic solution was concentrated to dryness. ^{1}H NMR showed the pure product (158 mg, 97%).

Preparation of Compound (7b): To a stirred solution of compound (**8**) (2.06 g, 8.40 mmol), 4-phenylbenzylamine (1.6 g, 8.70 mmol), and *N*,*N*-dimethylaminopyridine (DMAP, 320 mg, 2.62 mmol) in CH_2Cl_2 (180 mL) was added DCC (1.95 g, 9.46 mmol) at room temperature. The mixture was stirred for 5 h under N_2 atmosphere. The reaction mixture was concentrated and recrystallized from CH_2Cl_2/Hexane to give compound (**7b**) as a white solid (2.15 g, 63%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 13.23 (s, 1H), 7.57 (t, *J* = 7.2 Hz, 4H), 7.43 (t, J = 7.6 Hz, 2H), 7.34 (m, 3H), 4.65 (d, *J* = 6.0 Hz, 2H), 4.21 (s, 2H), 3.67 (q, *J* = 7.0 Hz, 4H), 2.59 (s, 3H), 1.22 (t, *J* = 7.0 Hz, 6H) ; ^{13}C NMR ($CDCl_3$): δ 189.02, 175.31, 165.96, 141.43, 140.35, 133.90, 128.96, 128.00, 127.70, 127.17, 92.90, 67.26, 58.63, 48.07, 18.27, 15.80; ESI-MS: *m/z* = 409.98 $[M+H]^+$for $C_{24}H_{27}NO_5$; HRMS $[M+H]^+$for $C_{24}H_{27}NO_5$: calcd. 409.1884, found 409.1890.

Preparation of Compound (7a): Compound **7a** was prepared similarly to **7b**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.40 (s, 1H), 4.32 (s, 2H), 3.60 (q, *J* = 7.0 Hz, 4H), 2.95 (d, *J* = 5.2 Hz, 3H), 2.51 (s, 3H), 1.18 (t, *J* = 7.0 Hz, 6H) ; ^{13}C NMR ($CDCl_3$): δ 198.70, 183.64, 169.66, 96.71, 92.25, 70.41, 58.58, 32.36, 27.39, 15.68; ESI-MS: *m/z* = 257.86 $[M+H]^+$for $C_{12}H_{20}NO_5$; HRMS $[M+H]^+$for $C_{12}H_{19}NO_5$: calcd. 257.1258, found 257.1262.

Preparation of Compound (9b): Compound (**7b**) (820 mg, 2.0 mmol) was stirred in formic acid/H_2O (5 mL/0.5 mL) at 80 – 90 ^{o}C for 2 h. The mixture was diluted with CH_2Cl_2, washed with water and then brine. After dried with anhydrous $MgSO_4$, the CH_2Cl_2 solution was concentrated. The product was a yellow solid obtained by

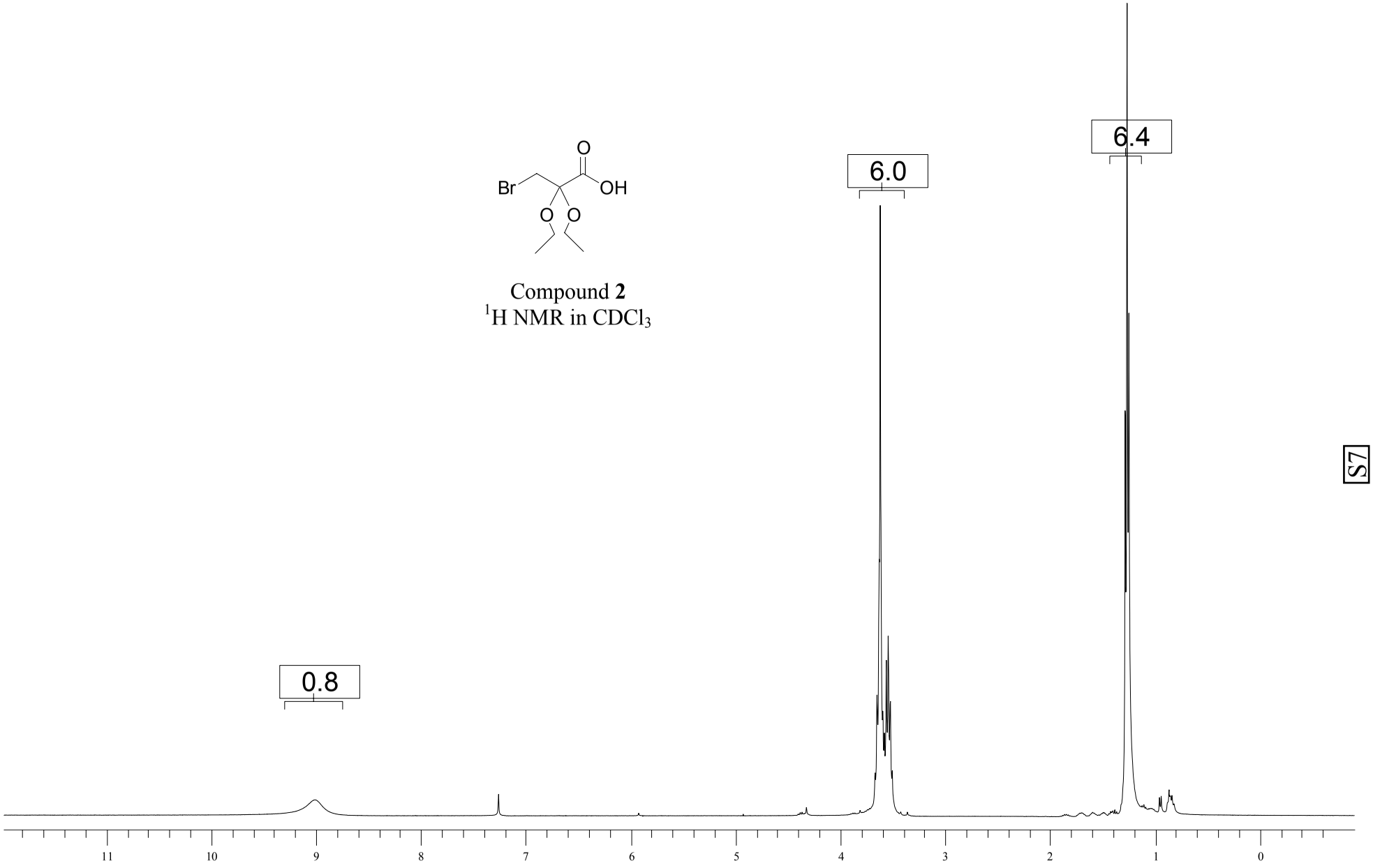
recrystalization from CH_2Cl_2/hexane. Yield 550 mg (82%). ^{1}H NMR (400 MHz, d6-DMSO): δ 13.70 (s, 1H), 8.21 (s, 1H), 7.65–7.71 (m, 4H), 7.43-7.48 (m, 4H), 7.35–7.38 (m, 2H), 4.88 (d, J = 7.0 Hz, 2H), 2.68 (s, 3H); ^{13}C NMR (d^6-DMSO): δ 178.57, 177.04, 163.28, 140.57, 140.25, 138.70, 135.71, 134.85, 129.68, 129.12, 128.31, 127.93, 127.39, 97.29, 47.83, 18.91; ESI-MS: m/z = 335.98 $[M+H]^+$for $C_{20}H_{18}NO_4$; HRMS $[M]^+$for $C_{20}H_{17}NO_4$: calcd. 335.1152, found 335.1149; UV-Vis (CH_2Cl_2, λ_{max}): 246, 318 nm; IR (KBr): ν 1144, 1556, 1614, 2843, 2917, 3346, 3412 cm^{-1}; Anal. Calcd for $C_{20}H_{17}NO_4$: C, 71.63; H, 5.11; N, 4.18; Found C, 71.25; H, 5.48; N, 4.48.

Preparation of Compound (9a): Compound **9a** was prepared similarly to **9b** starting with 300 mg of **7b**, and was purified by column chromatography on silica gel. The product was a yellow solid. Yield 145 mg (68%). ^{1}H NMR (400 MHz, d^6-DMSO): δ 13.01 (s, 1H), 8.11 (s, 1H), 7.32 (s, 1H), 3.16 (d, J = 6.0 Hz, 3H), 2.59 (s, 3H); ^{13}C NMR (d^6-DMSO): δ 178.08, 177.76, 163.53, 138.80, 134.42, 96.97, 31.46, 18.38; ESI-MS: m/z = 184.14 $[M+H]^+$for $C_8H_{10}NO_4$; HRMS $[M]^+$for $C_8H_9NO_4$: calcd. 183.0526, found 183.0529; Anal. Calcd for $C_8H_9NO_4$: C, 52.46; H, 4.95; N, 7.65; Found C, 52.59; H, 5.34; N, 7.68.

Preparation of Compound (10): To a stirred solution of compound (**9b**) (200 mg, 0.6 mmol) in THF (50 mL), was added hexamethyldisiloxane (HMDO, 0.425 mL, 2.0 mmol) and P_4S_{10} (100 mg, 0.2 mmol). The mixture was deoxygenated and then refluxed for 5 h. After addition of water (~20 mL), the reaction mixture was concentrated to remove THF. The mixture was extracted with CH_2Cl_2. The extract was washed with water, dried over $MgSO_4$ and then concentrated. The crude product was purified by column chromatography (silica gel column was pretreated with a 5% solution of maltol in CH_3OH/CH_2Cl_2 = 10/90 to remove metal ions from the silica gel) with eluent EtOAc/hexane = 20–40%. The product was a yellow solid (34 mg, 16%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 14.75 (s, 1H), 7.65 (d, J = 8.0 Hz, 2H), 7.59 (d, J = 8.0 Hz, 2H), 7.38-7.48 (m, 5H), 7.34 (s, 1H), 7.22 (s, 1H), 4.87 (d, J = 5.5 Hz, 2H), 2.87 (s, 3H);); ^{13}C NMR ($CDCl_3$): δ (The carbonyl group didn't show up due to short scan time) 141.84, 140.38, 133.58, 129.15, 128.28, 127.96, 127.93, 127.33, 48.19, 18.85; ESI-MS: m/z = 351.96 for $C_{20}H_{18}NO_3S$ $[M+H]^+$; HRMS for

$C_{20}H_{17}NO_3S$ $[M]^+$: calcd. 351.0924, found 351.0927; UV-Vis (CH_2Cl_2, λ_{max}): 258, 376 nm; IR (KBr): ν 1172, 1683, 2741, 2925, 3105, 3264, 3432 cm^{-1}.

Inhibitor Activity Assays against MMPs. All MMP enzymes (MMP-1, MMP-2, MMP-3, and MMP-9) and the assay kit were purchased from BIOMOL International. The assays were carried out following the procedure provided with the kit. Experiments were performed using a Bio-Tek Flx 800 fluorescence plate reader and Nunc white 96-well plates. Each inhibitor was dissolved in DMSO and further diluted 500-fold in assay buffer. MMP-1, MMP-2, MMP-3, and MMP-9 were incubated individually with different concentrations of different inhibitors for 1 h at 37 °C, followed by addition of a fluorescent substrate (Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH_2 (0.4 mM in assay; Mca = 7-methoxycoumarin-4-yl)-acetyl; Dpa = N-3-(2,4-dinitrophenyl)-L-α-β-diaminopropionyl) to initiate the assay. Reactions were agitated by shaking for 1 sec after each fluorescence measurement. Mca fluorescence (λ_{ex} = 340 nm, λ_{em} = 400 nm) was measured at 60-second intervals for 30 min. Experiments were repeated at least three times. Enzyme activity percentages at an inhibitor concentration were calculated against the control experiment (no inhibitor present).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Br
OH
O
O
Compound **2**
^{1}H NMR in $CDCl_3$
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6.0
6.4
11
10
9
8
7
6
5
4
3
2
1
0

169.015
Br
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O
OH
Compound 2
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150
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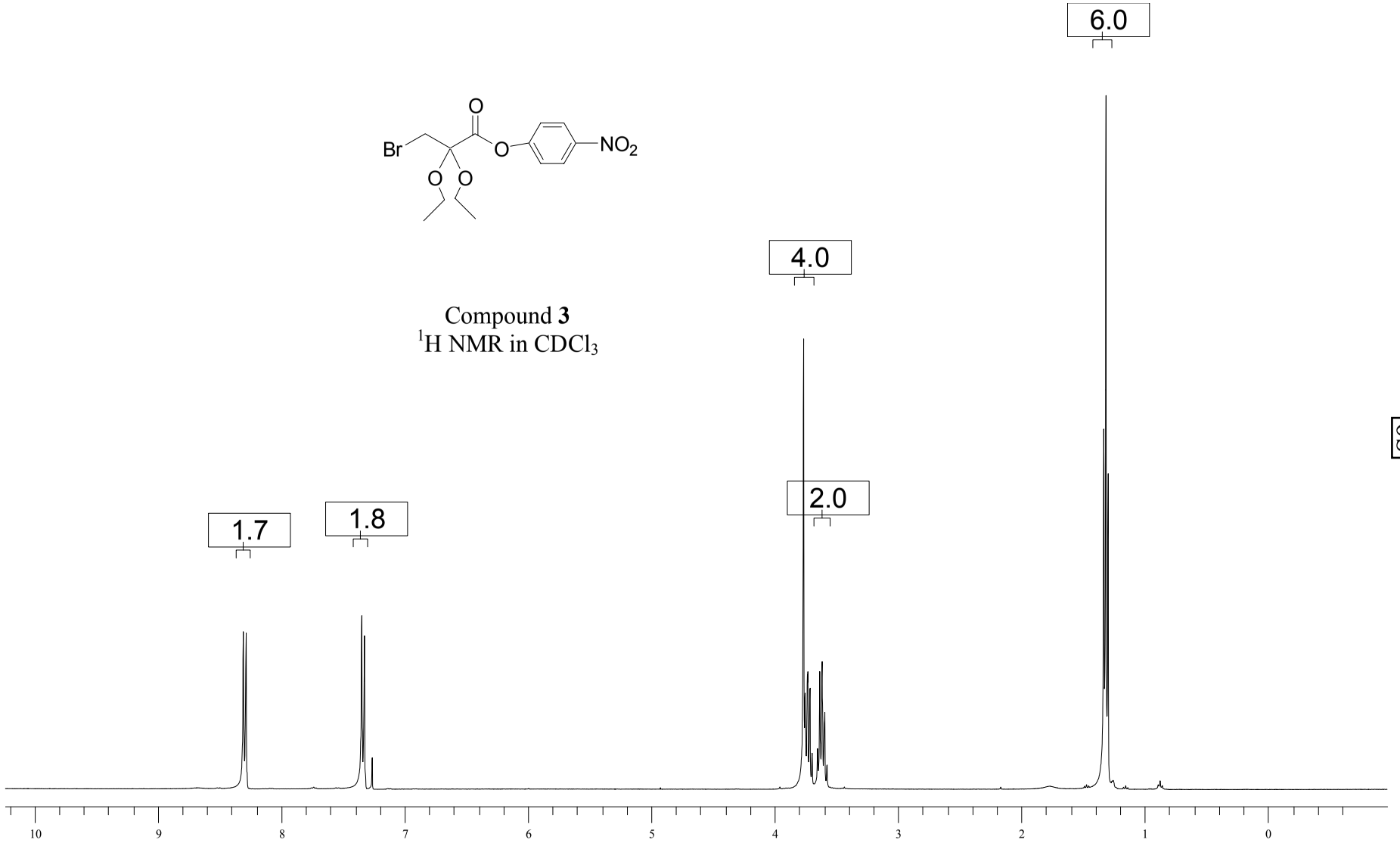
0



Compound **3**
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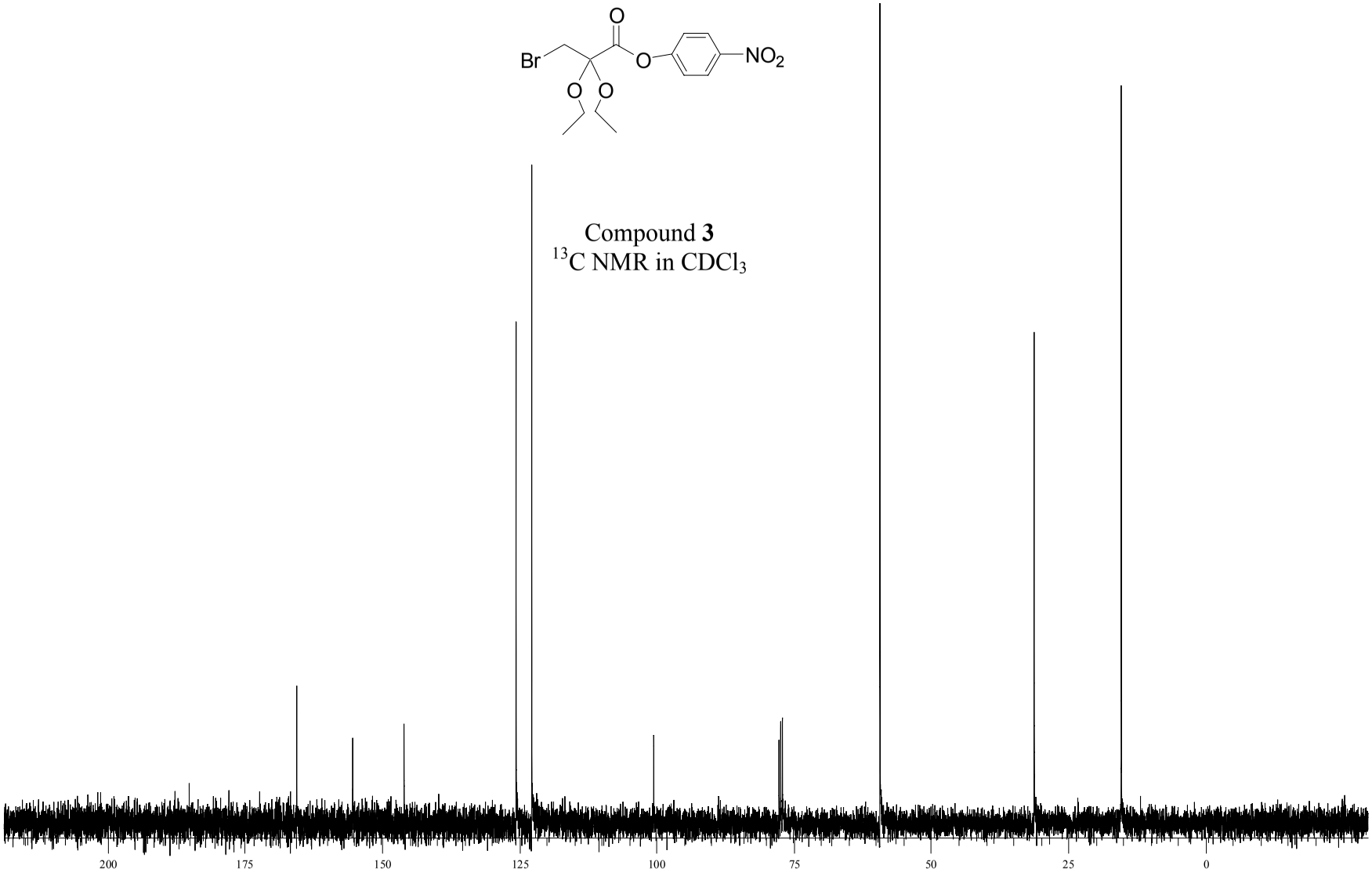
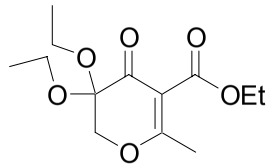


Compound **3**
^{13}C NMR in $CDCl_3$



Compound **4a**
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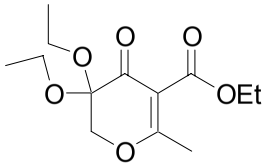
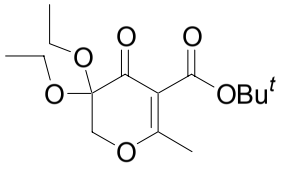


Compound **4a**
^{13}C NMR in $CDCl_3$



Compound **4b**
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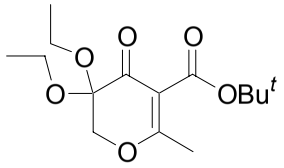
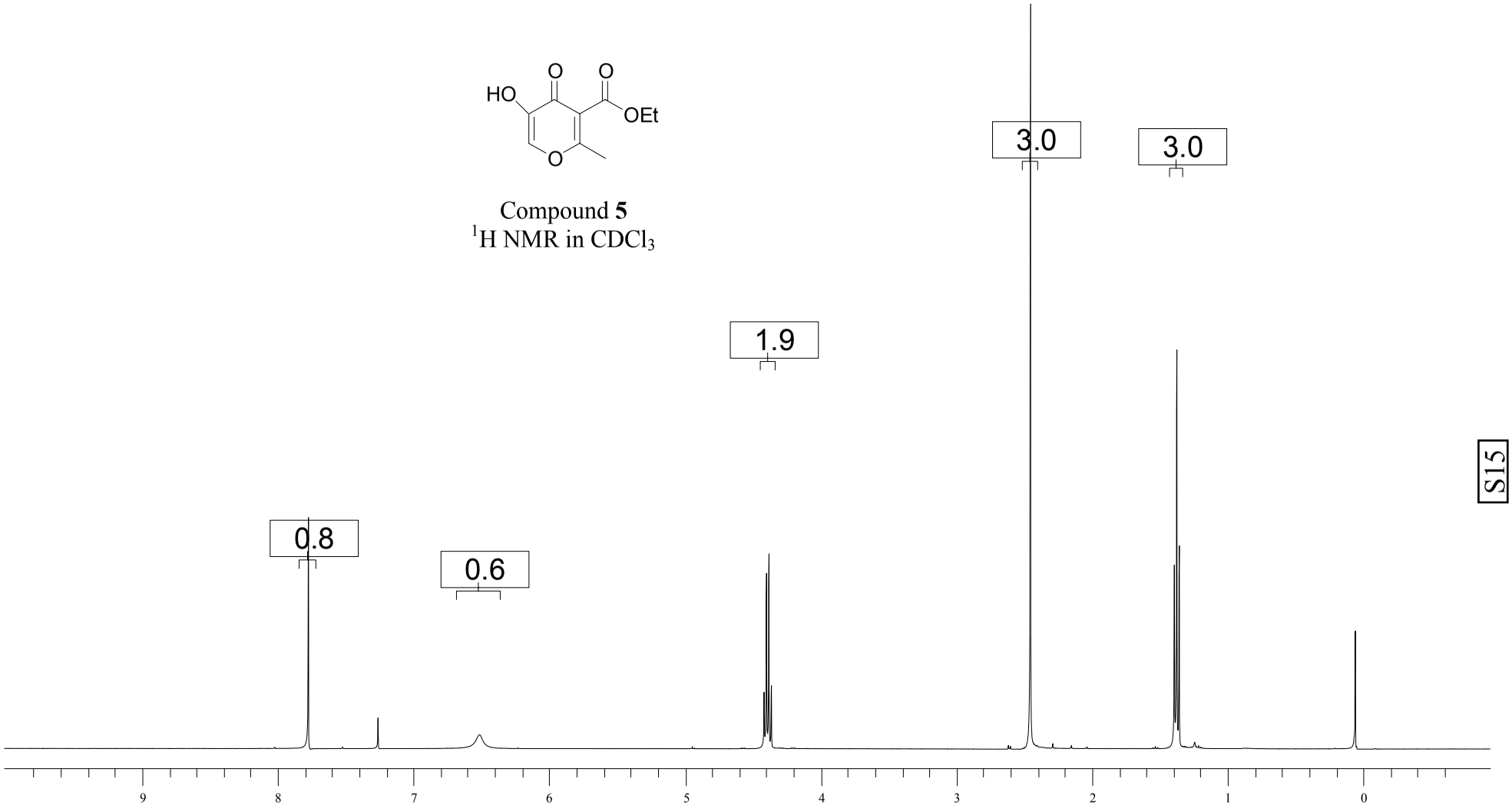


Compound **4b**
^{13}C NMR in $CDCl_3$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OEt
O
Compound 5
1H NMR in CDCl3
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3.0
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8
7
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5
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3
2
1
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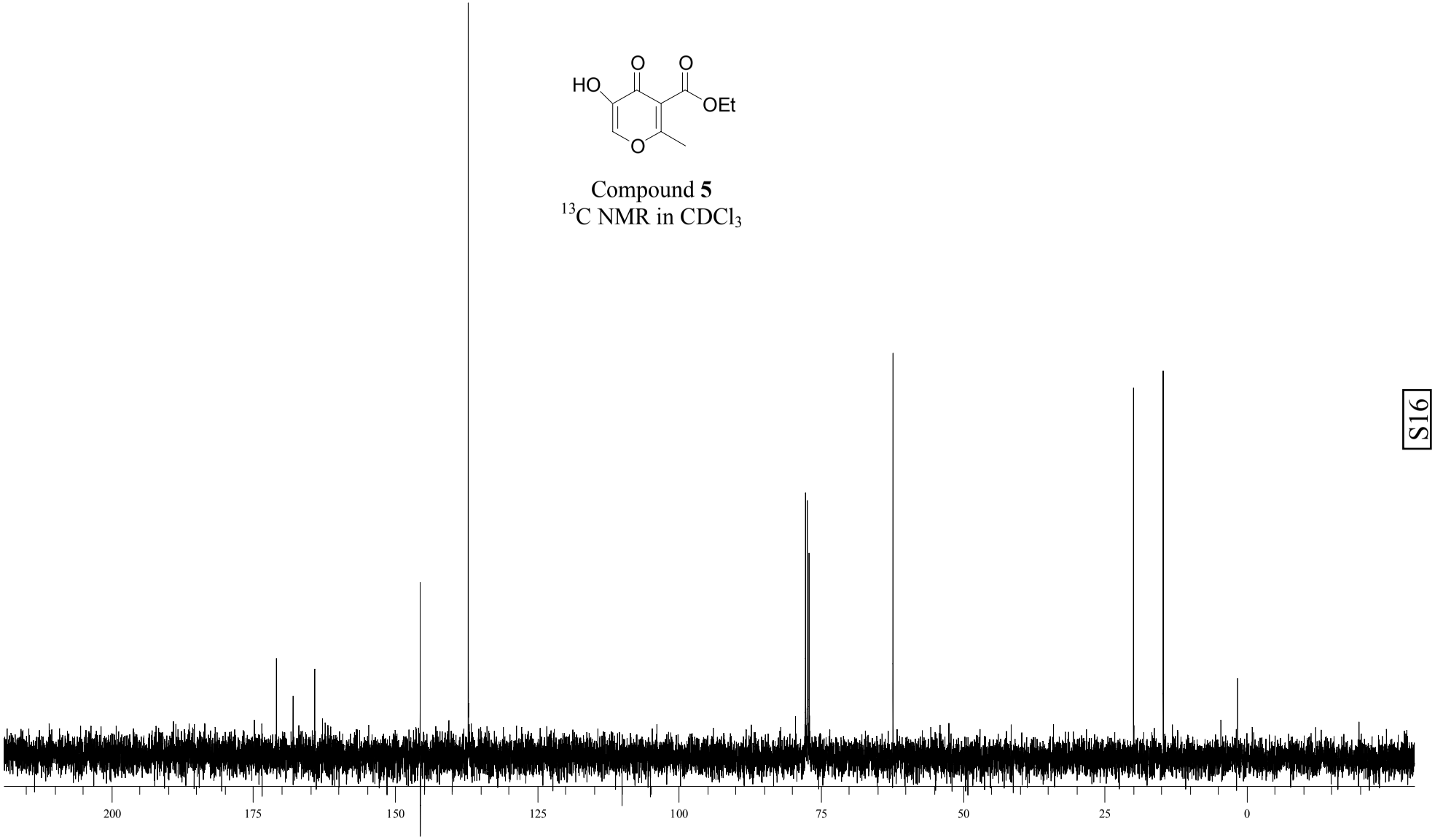

Compound **5**
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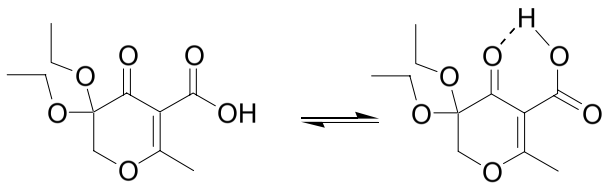
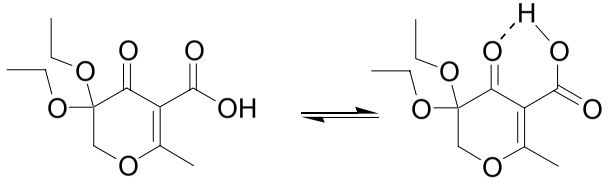
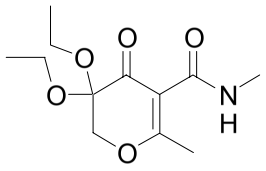


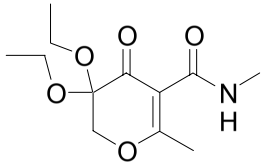
Compound **8**
^{1}H NMR in $CDCl_3$



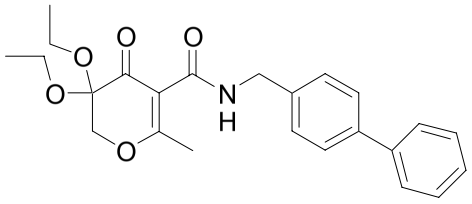
Compound **8**
^{13}C NMR in $CDCl_3$



Compound **7a**
^{1}H NMR in $CDCl_3$



Compound **7a**
^{13}C NMR in $CDCl_3$



Compound **7b**
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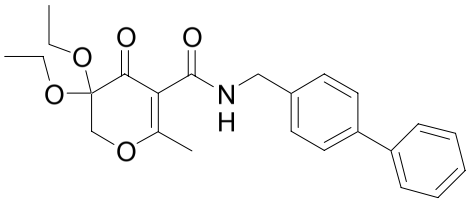
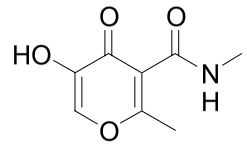
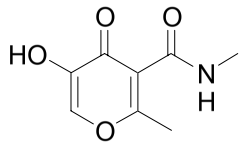


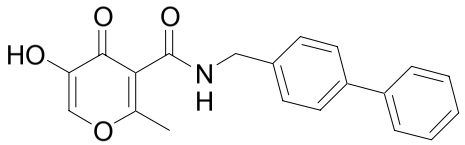
Compound **7b**
^{13}C NMR in $CDCl_3$



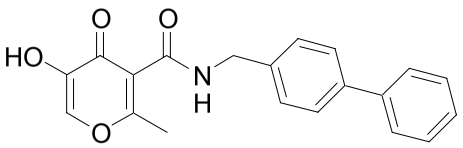
Compound **9a**
^{1}H NMR in d6-DMSO



Compound **9a**
^{13}C NMR in d6-DMSO



Compound **9b**
^{1}H NMR in d6-DMSO



Compound **9b**
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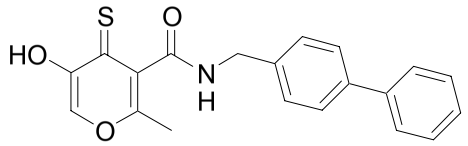
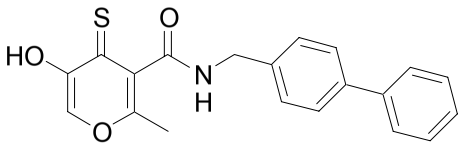


Compound **10**
^{1}H NMR in $CDCl_3$



Compound **10**
^{13}C NMR in $CDCl_3$