Endogenous Enzymes Enable Antimicrobial Activity

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Materials and Methods

Materials. Chemicals were purchased from Sigma–Aldrich (Milwaukee, WI), Acros Organics (New Jersey, NJ) or Combi-Blocks (San Diego, CA) and used without further purification. All glassware was flame- or oven-dried, and all reactions were performed under a N_2(g) atmosphere unless indicated otherwise. Dichloromethane (DCM), tetrahydrofuran (THF), diethyl ether (Et_2O), and triethylamine (TEA) were dried over a column of activated alumina and accessed under a blanket of Ar(g). Dimethylformamide (DMF) was dried over a column of activated alumina, purified further through an isocyanate scrubbing column, and accessed under a blanket of Ar(g). Dry acetone was obtained by shaking with activated Drierite (Ca_2SO_4(s)) and subsequent distillation from fresh Drierite under an atmosphere of N_2(g). Column chromatography was performed using 40–63 Å silica gel, 230–400 mesh from Silicycle (Québec City, Canada). Thin-layer chromatography (TLC) was performed with EMD 250-µm silica gel 60-F_{254} plates and visualized by using UV light or staining with $KMnO_4$, bromocresol green, or ceric ammonium nitrate.

Conditions. All reactions were performed in air at ambient temperature (~22 °C) and pressure (1.0 atm) unless indicated otherwise.

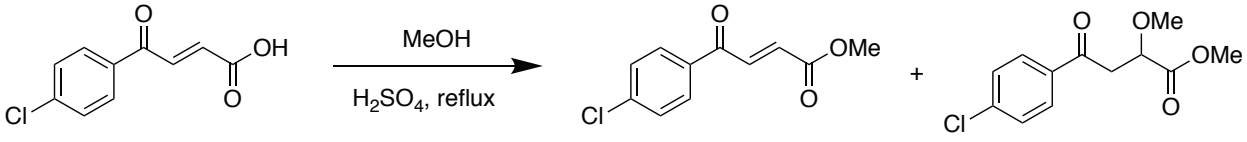
Solvent Removal. The phrase "under reduced pressure" refers to removal of solvent and other volatile materials with a rotary evaporator at water-aspirator pressure (<20 torr) and a water bath at <40 °C. Residual solvent was removed from samples at high vacuum (<0.1 torr). The phrase "high vacuum" refers to the vacuum achieved by using a mechanical belt-drive oil pump.

NMR Spectroscopy. ^{1}H and ^{13}C NMR spectra were obtained at ambient temperature with an Avance Neo (500 MHz) or Avance-III HD Nanobay spectrometer (400 MHz) from Bruker (Billerica, MA) at the Department of Chemistry Instrumentation Facility (DCIF) and were referenced to TMS or residual protic solvent.

Mass Spectrometry. High-resolution mass spectrometry data were obtained with an AccuTOF 4G LC-plus from JEOL (Tokyo, Japan) equipped with an ionSense (Saugus, MA) DART (Direct Analysis in Real Time) at the Department of Chemistry Instrumentation Facility (DCIF). LC–MS data were acquired with a 6125B mass spectrometer from Agilent Technologies (Santa Clara, CA) attached to an Agilent 1260 Infinity LC with an electrospray ionization source in the positive mode.

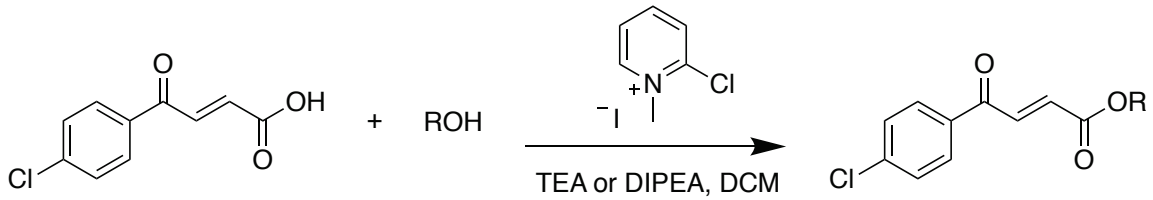
UV–Vis Spectroscopy. Absorbance measurements were made with an infinite M1000 plate reader from Tecan (Männedorf, Switzerland).

Synthetic Methods



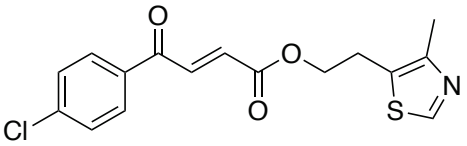
Methyl (*E*)-4-(4-chlorophenyl)-4-oxobut-2-enoate (2). (*E*)-4-(4-Chlorophenyl)-4-oxobut-2-enoic acid (210 mg, 1.0 mmol) was dissolved in methanol (10 mL). Concentrated sulfuric acid (0.1 mL) was added, and the solution was heated at reflux under air for 6 h. The reaction mixture was concentrated under reduced pressure and taken up into ethyl acetate. The organic phase was extracted with saturated aqueous $NaHCO_3$ (2×), H_2O (2×), and brine (1×), dried with $MgSO_4$(s), and filtered before the solvent was removed under reduced pressure. The products were separated

by column chromatography to afford methyl (*E*)-4-(4-chlorophenyl)-4-oxobut-2-enoate (25% yield, R_f = 0.25 in 10% v/v EtOAc in hexanes) as a light yellow solid and methyl 4-(4-chlorophenyl)-2-methoxy-4-oxobutanoate (73% yield, R_f = 0.17 in 10% v/v EtOAc in hexanes) as a white solid. *Methyl (E)-4-(4-chlorophenyl)-4-oxobut-2-enoate (**2**):* **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 7.97–7.93 (m, 2H), 7.88 (d, J = 15.6 Hz, 1H), 7.52–7.47 (m, 2H), 6.90 (d, J = 15.6 Hz, 1H), 3.86 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$, δ): 195.95, 179.92, 139.31, 136.15, 135.04, 132.67, 130.38, 129.43, 52.58. **HRMS–DART** (*m*/*z*): $[M + H]^+$ calcd for $C_{11}H_{10}ClO_3^+$, 225.0313; found, 225.0316. *Methyl 4-(4-chlorophenyl)-2-methoxy-4-oxobutanoate (**S1**):* **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 7.90–7.85 (m, 2H), 7.46–7.40 (m, 2H), 4.41 (dd, J = 7.9, 4.3 Hz, 1H), 3.78 (s, 3H), 3.46 (s, 3H), 3.45–3.39 (m, 1H), 3.28 (dd, J = 17.0, 4.3 Hz, 1H). **^{13}C NMR** (101 MHz, $CDCl_3$, δ): 195.20, 172.56, 140.04, 135.02, 129.73, 129.10, 76.38, 59.08, 52.36, 41.54. **HRMS–DART** (*m*/*z*): $[M + H]^+$ calcd for $C_{12}H_{13}ClO_4^+$, 257.0575; found, 257.0574.



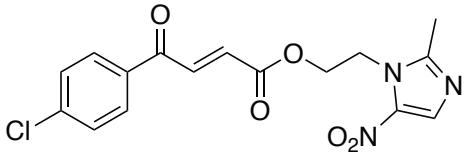
General Procedure A for the Synthesis of Esters Derived from (*E*)-4-(4-Chlorophenyl)-4-oxobut-2-enoic acid. (*E*)-4-(4-Chlorophenyl)-4-oxobut-2-enoic acid (210 mg, 1.0 mmol), an alcohol (1.0 mmol), and Mukaiyama's reagent (307 mg, 1.2 mmol) were dissolved in DCM (10 mL). TEA (0.28 mL, 2.0 mmol) was added in one portion, and the resulting solution was stirred until the reaction was complete according to TLC (4–8 h). Solvent was removed under reduced pressure, and the residue was dissolved by sonication in ethyl acetate. The organic phase was extracted with 3 M HCl (1×), saturated aqueous $NaHCO_3$ (2×), H_2O (1×), and brine (1×), dried with $MgSO_4$(s), and filtered before solvent was removed under reduced pressure. Column chromatography was performed with a gradient of hexanes/EtOAc to afford pure compounds.

General Procedure B for the Synthesis of Esters Derived from (*E*)-4-(4-Chlorophenyl)-4-oxobut-2-enoic acid. (*E*)-4-(4-Chlorophenyl)-4-oxobut-2-enoic acid (210 mg, 1.0 mmol), an alcohol (1.0 mmol), and Mukaiyama's reagent (307 mg, 1.2 mmol) were dissolved in DCM (10 mL). DIPEA (0.52 mL, 3.0 mmol) was added in one portion, and the resulting solution was stirred overnight. Solvent was removed under reduced pressure, and the residue was dissolved by sonication in DCM. The organic phase was extracted with saturated aqueous $NaHCO_3$ (2×), H_2O (1×), and brine (1×), dried with $MgSO_4$(s), and filtered before solvent was removed under reduced pressure. Column chromatography was performed with a gradient of DCM/MeOH to afford pure compounds.

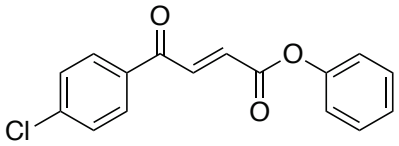


2-(4-Methylthiazol-5-yl)ethyl (*E*)-4-(4-Chlorophenyl)-4-oxobut-2-enoate (3). Following general procedure A, the title product was obtained as a brown solid (90% yield, R_f = 0.36 in 50%

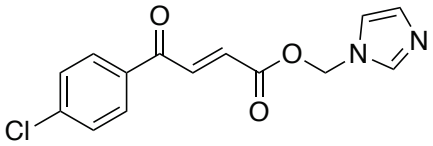
v/v EtOAc in hexanes). **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 8.61 (s, 1H), 7.97–7.91 (m, 2H), 7.87 (d, J = 15.5 Hz, 1H), 7.54–7.47 (m, 2H), 6.89 (d, J = 15.5 Hz, 1H), 4.41 (t, J = 6.6 Hz, 2H), 3.20 (t, J = 6.6 Hz, 2H), 2.44 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$, δ): 188.14, 165.27, 150.30, 150.14, 140.76, 136.50, 134.98, 132.44, 130.37, 129.47, 126.40, 65.07, 25.88, 15.12. **HRMS–DART** (m/z): $[M + H]^+$ calcd for $C_{16}H_{15}ClNO_3S^+$, 336.0456; found, 336.0458.



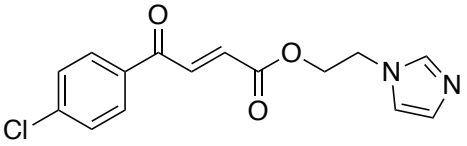
2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl (*E*)-4-(4-Chlorophenyl)-4-oxobut-2-enoate (4). Following general procedure B, the title product was obtained as a brown solid (86% yield, R_f = 0.29 in 5% v/v MeOH in DCM). **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 7.98 (s, 1H), 7.96–7.91 (m, 2H), 7.84 (d, J = 15.5 Hz, 1H), 7.53–7.47 (m, 2H), 6.82 (d, J = 15.5 Hz, 1H), 4.68 (t, J = 5.2 Hz, 2H), 4.59 (t, J = 5.3 Hz, 2H), 2.53 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$, δ): 187.68, 164.89, 157.22, 150.88, 140.95, 137.24, 134.78, 133.46, 131.26, 130.39, 129.52, 63.56, 45.06, 14.58. **HRMS–DART** (m/z): $[M + H]^+$ calcd for $C_{16}H_{15}ClN_3O_5^+$, 364.0695; found, 364.0693.



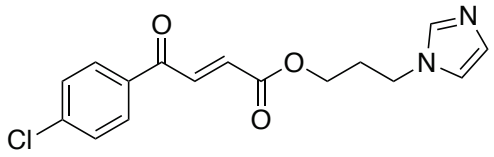
Phenyl (*E*)-4-(4-Chlorophenyl)-4-oxobut-2-enoate (5). Following general procedure A, the title product was obtained as a light pink solid (88% yield, R_f =0.34 in 10% v/v EtOAc in hexanes). **^{1}H NMR** (500 MHz, $CDCl_3$, δ): 8.04 (d, J = 15.5 Hz, 1H), 8.01–7.97 (m, 2H), 7.54–7.49 (m, 2H), 7.46–7.40 (m, 2H), 7.33–7.26 (m, 1H), 7.20–7.15 (m, 2H), 7.10 (d, J = 15.5 Hz, 1H). **^{13}C NMR** (126 MHz, $CDCl_3$, δ): 188.06, 164.01, 150.49, 140.84, 137.46, 134.93, 132.31, 130.44, 129.74, 129.50, 126.45, 121.45. **HRMS–DART** (m/z): $[M + H]^+$ calcd for $C_{16}H_{12}ClO_3^+$, 287.0472; found, 287.0469.



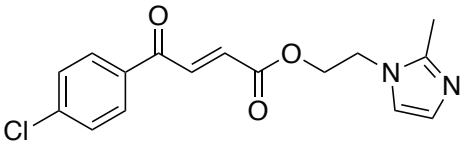
(1*H*-Imidazol-1-yl)methyl (*E*)-4-(4-Chlorophenyl)-4-oxobut-2-enoate (6). Following general procedure B, the title product was obtained as a light orange solid (36% yield, R_f = 0.54 in 10% v/v MeOH in DCM). **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 8.61 (s, 2H), 7.30 (d, J = 1.2 Hz, 2H), 7.08 (s, 1H), 7.11–7.00 (m, 1H), 6.93 (d, J = 1.4 Hz, 2H), 5.40 (s, 4H). **^{13}C NMR** (101 MHz, $CDCl_3$, δ): 188.19, 165.37, 141.20, 137.30, 132.05, 130.69, 129.82, 128.29, 119.62, 64.39, 44.56, 20.55, 12.57. **HRMS–DART** (m/z): $[M + H]^+$ calcd for $C_{14}H_{12}ClN_2O_3^+$, 291.0533; found, 291.0531.



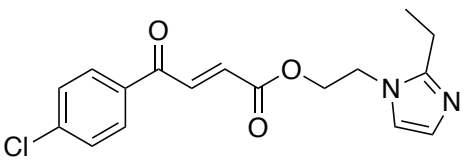
2-(1*H*-Imidazol-1-yl)ethyl (*E*)-4-(4-Chlorophenyl)-4-oxobut-2-enoate (7). Following general procedure B, the title product was obtained as a light brown solid (75% yield, R_f = 0.45 in 10% v/v MeOH in DCM). **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 7.95–7.90 (m, 2H), 7.85 (d, J = 15.5 Hz, 1H), 7.53 (s, 1H), 7.52–7.47 (m, 2H), 7.10 (s, 1H), 6.98 (s, 1H), 6.88 (d, J = 15.5 Hz, 1H), 4.51 (t, J = 5.3 Hz, 2H), 4.29 (t, J = 5.3 Hz, 2H). **^{13}C NMR** (101 MHz, $CDCl_3$, δ): 187.89, 164.98, 140.86, 137.51, 137.05, 134.85, 131.71, 130.38, 130.23, 129.50, 119.19, 64.24, 45.74. **HRMS–DART** (m/z): $[M + H]^+$ calcd for $C_{15}H_{14}ClN_2O_3^+$, 305.0687; found, 305.0689.



3-(1*H*-Imidazol-1-yl)propyl (*E*)-4-(4-Chlorophenyl)-4-oxobut-2-enoate (8). Following general procedure B, the title product was obtained as an orange semisolid (50% yield, R_f = 0.42 in 10% v/v MeOH in DCM). **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 7.98–7.93 (m, 2H), 7.87 (d, J = 15.5 Hz, 1H), 7.53–7.48 (m, 3H), 7.09 (s, 1H), 6.94 (s, 1H), 6.90 (d, J = 15.5 Hz, 1H), 4.25 (t, J = 6.0 Hz, 2H), 4.10 (t, J = 6.9 Hz, 2H), 2.21 (p, J = 6.5 Hz, 2H). **^{13}C NMR** (101 MHz, $CDCl_3$, δ): 188.13, 165.33, 140.82, 137.32, 136.69, 134.93, 132.25, 130.41, 130.09, 129.48, 118.89, 62.07, 43.77, 30.31. **HRMS–DART** (m/z): $[M + H]^+$ calcd for $C_{16}H_{16}ClN_2O_3^+$, 319.0844; found, 319.0844.

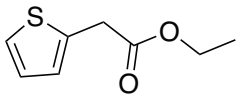


2-(2-Methyl-1*H*-imidazol-1-yl)ethyl (*E*)-4-(4-Chlorophenyl)-4-oxobut-2-enoate (9). Following general procedure B, the title product was obtained as an orange solid (51% yield, R_f = 0.44 in 10% v/v MeOH in DCM). **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 7.95–7.91 (m, 2H), 7.85 (d, J = 15.5 Hz, 1H), 7.53–7.47 (m, 2H), 6.95–6.84 (m, 3H), 4.48 (t, J = 5.6 Hz, 2H), 4.19 (t, J = 5.6 Hz, 2H), 2.43 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$, δ): 186.00, 165.04, 162.72, 137.01, 134.86, 131.72, 130.37, 129.51, 127.94, 127.70, 119.45, 64.00, 44.68, 13.24. **HRMS–DART** (m/z): $[M + H]^+$ calcd for $C_{16}H_{15}ClN_2O_3^+$, 319.0844; found, 319.0845.

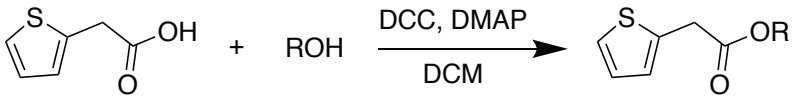


2-(2-Ethyl-1*H*-imidazol-1-yl)ethyl (*E*)-4-(4-Chlorophenyl)-4-oxobut-2-enoate (10). Following general procedure B, the title product was obtained as an orange solid (84% yield, R_f =

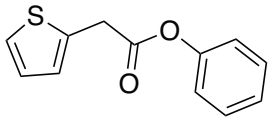
0.47 in 10% v/v MeOH in DCM). **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 7.95–7.90 (m, 2H), 7.85 (d, J = 15.5 Hz, 1H), 7.53–7.47 (m, 2H), 6.98 (d, J = 1.4 Hz, 1H), 6.89–6.84 (m, 2H), 4.48 (t, J = 5.6 Hz, 2H), 4.20 (t, J = 5.6 Hz, 2H), 2.72 (q, J = 7.5 Hz, 2H), 1.37 (t, J = 7.5 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$, δ): 188.19, 165.37, 149.84, 141.20, 137.30, 135.17, 132.06, 130.69, 129.82, 128.29, 119.63, 77.48, 64.39, 44.56, 20.56, 12.57. **HRMS–DART** (m/z): $[M + H]^+$ calcd for $C_{17}H_{18}ClN_2O_3$, 333.1001; found, 333.1002.



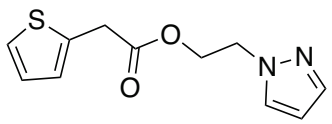
Ethyl 2-(Thiophen-2-yl)acetate. Ethyl 2-(thiophen-2-yl)acetate was prepared as described previously.[1]



General procedure for the Synthesis of Esters Derived from Thiophen-2-yl-acetic acid. Thiophen-2-yl-acetic acid (142 mg, 1.0 mmol), 4-dimethylaminopyridine (3.0 mg, 0.025 mmol) and alcohol 2.0 mmol) were cooled to 0 °C. A solution of dicyclohexylcarbodiimide (227 mg, 1.1 mmol) in DCM (1.1 mL) was added, and the resulting solution was stirred on ice for 15 min and then at room temperature for 3 h. The reaction mixture was filtered, and the filtrate was concentrated under reduced pressure. The crude mixture was taken up in EtOAc and washed twice with 1 M HCl (2×) (except for reactions involving imidazolyl alcohols), twice with saturated aqueous $NaHCO_3$ (1×), and brine (1×). The organic layer was dried over $MgSO_4$(s), concentrated under reduced pressure, and purified by chromatography on silica gel, eluting with a gradient of hexanes/EtOAc or DCM/MeOH to afford pure compounds.

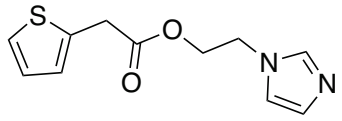


Phenyl 2-(Thiophen-2-yl)acetate (S2). Following the general procedure, the title compound was obtained as a white solid (95% yield, R_f = 0.17 in 10% v/v EtOAc in hexanes). **^{1}H NMR** (500 MHz, $CDCl_3$, δ): 7.44–7.39 (m, 2H), 7.32–7.27 (m, 2H), 7.17–7.13 (m, 2H), 7.11–7.08 (m, 1H), 7.06–7.03 (m, 1H), 4.12 (s, 2H). **^{13}C NMR** (126 MHz, $CDCl_3$, δ): 168.92, 150.68, 134.40, 129.46, 127.17, 126.98, 126.02, 125.35, 121.42, 35.61. **HRMS–DART** (m/z): $[M + H]^+$ calcd for $C_{12}H_{11}O_2S^+$, 219.0474; found, 219.0470.

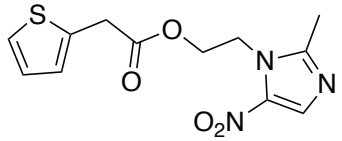


2-(1*H*-Pyrazol-1-yl)ethyl 2-(Thiophen-2-yl)acetate (S3). Following the general procedure, the title compound was obtained as a pale yellow oil (76% yield, R_f = 0.20 in 30% v/v EtOAc in

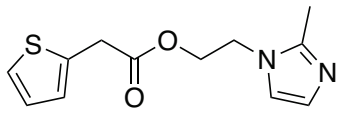
hexanes). **^{1}H NMR** ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.51 (d, J = 1.8 Hz, 1H), 7.20 (d, J = 5.2 Hz, 1H), 6.94 (dd, J = 5.2, 3.6 Hz, 1H), 6.89 (d, J = 3.5 Hz, 1H), 6.21 (t, J = 2.1 Hz, 1H), 4.47 (t, J = 5.3 Hz, 2H), 4.35 (t, J = 5.3 Hz, 2H), 3.81 (s, 2H). **^{13}C NMR** (101 MHz, $CDCl_3$, δ): 170.01, 139.90, 134.56, 129.84, 127.08, 126.94, 125.18, 105.80, 63.61, 50.67, 35.26. **HRMS–DART** (*m*/*z*): $[M + H]^+$ calcd for $C_{11}H_{13}N_2O_2S^+$, 237.0692; found, 237.0691.



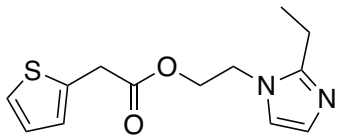
2-(1*H*-Imidazol-1-yl)ethyl 2-(Thiophen-2-yl)acetate (S4). Following the general procedure, the title compound was obtained as a brown oil (61% yield, R_f = 0.45 in 10% v/v MeOH in DCM). **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 7.39 (s, 1H), 7.22 (dd, J = 5.2, 1.2 Hz, 1H), 7.03 (s, 1H), 6.96 (dd, J = 5.2, 3.5 Hz, 1H), 6.92–6.89 (m, 1H), 6.84 (s, 1H), 4.35 (dd, J = 5.8, 4.7 Hz, 2H), 4.17 (dd, J = 5.8, 4.7 Hz, 2H), 3.83 (s, 2H). **^{13}C NMR** (101 MHz, $CDCl_3$, δ): 170.00, 137.44, 134.28, 129.87, 127.25, 127.08, 125.40, 119.10, 64.00, 45.69, 35.30. **HRMS–DART** (*m*/*z*): $[M + H]^+$ calcd for $C_{11}H_{13}N_2O_2S^+$, 237.0692; found, 237.0690.



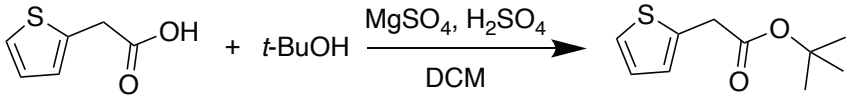
2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl 2-(Thiophen-2-yl)acetate (S5). Following the general procedure, the title compound was obtained as a light brown solid (68% yield, R_f = 0.17 in 50% v/v EtOAc in hexanes). **^{1}H NMR** (500 MHz, $CDCl_3$, δ): 7.95 (s, 1H), 7.22 (d, J = 5.2 Hz, 1H), 6.95 (dd, J = 5.1, 3.6 Hz, 1H), 6.87 (d, J = 3.6 Hz, 1H), 4.57 (t, J = 5.1 Hz, 2H), 4.47 (t, J = 5.1 Hz, 2H), 3.80 (s, 2H), 2.35 (s, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$, δ): 169.98, 151.08, 138.59, 134.05, 133.38, 127.35, 127.18, 125.56, 63.43, 45.08, 35.30, 14.26. **HRMS–DART** (*m*/*z*): $[M + H]^+$ calcd for $C_{12}H_{14}N_3O_4S^+$; found, 296.0700 296.0669.



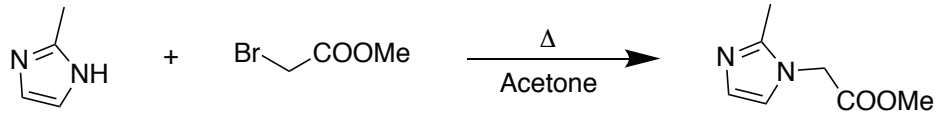
2-(2-Methyl-1*H*-imidazol-1-yl)ethyl 2-(Thiophen-2-yl)acetate (S6). Following the general procedure, the title compound was obtained as a brown oil (70% yield, R_f = 0.31 in 10% v/v MeOH in DCM). **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 7.22 (dd, J = 5.2, 1.2 Hz, 1H), 6.95 (dd, J = 5.1, 3.5 Hz, 1H), 6.91–6.87 (m, 2H), 6.75 (d, J = 1.4 Hz, 1H), 4.33 (t, J = 5.5 Hz, 2H), 4.09 (t, J = 5.5 Hz, 2H), 3.82 (s, 2H), 2.34 (s, 3H).**^{13}C NMR** (101 MHz, $CDCl_3$, δ): 170.11, 144.81, 134.28, 127.72, 127.25, 127.08, 125.40, 119.31, 63.82, 44.60, 35.29, 13.09. **HRMS–DART** (*m*/*z*): $[M + H]^+$ calcd for $C_{12}H_{15}N_2O_2{}^+S$, 251.0849; found, 251.0847.



2-(2-Ethyl-1*H*-imidazol-1-yl)ethyl 2-(Thiophen-2-yl)acetate (S7). Following the general procedure, the title compound was obtained as a brown oil (86% yield, R_f = 0.39 in 10% v/v MeOH in DCM). **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 7.22 (dd, J = 5.2, 1.3 Hz, 1H), 6.96 (dd, J = 5.2, 3.5 Hz, 1H), 6.93 (d, J = 1.4 Hz, 1H), 6.90 (dd, J = 3.5, 1.3 Hz, 1H), 6.76 (d, J = 1.4 Hz, 1H), 4.34 (t, J = 5.6 Hz, 2H), 4.10 (t, J = 5.6 Hz, 2H), 3.83 (s, 2H), 2.65 (q, J = 7.5 Hz, 2H), 1.33 (t, J = 7.5 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$, δ): 170.16, 149.45, 134.31, 127.76, 127.26, 127.09, 125.42, 119.23, 63.91, 44.19, 35.32, 20.12, 12.19. **HRMS–DART** (m/z): $[M + H]^+$ calcd for $C_{13}H_{17}N_2O_2S^+$, 265.1005; found, 265.1005.

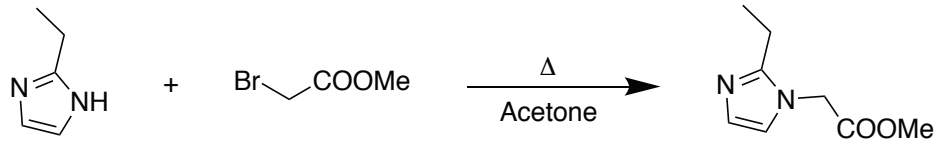


Synthesis of *tert*-Butyl 2-(Thiophen-2-yl)acetate (S8). Sulfuric acid (0.11 mL, 2.0 mmol) was added to dry magnesium sulfate (0.96g, 2.0 mmol) in dichloromethane (5 mL), and the resulting solution was stirred for 15 min. After this time, thiophen-2-yl-acetic acid (284 mg, 2.0 mmol) and *tert*-butanol (0.96mL, 10.0 mmol) were added sequentially, and the resulting solution was stirred for 18 h at room temperature. The reaction was quenched with a sodium bicarbonate solution, and extracted with ethyl acetate. The organic phase was dried with $MgSO_4$(s) and the solvent was removed under reduced pressure. The residue was separated via column chromatography to obtain the desired product as a brown solid. (22% yield, R_f = 0.39 in 20% v/v EtOAc in hexanes). **^{1}H NMR** (500 MHz, $CDCl_3$, δ): 7.20 (dd, J = 5.1, 1.3 Hz, 1H), 6.95 (dd, J = 5.2, 3.4 Hz, 1H), 6.92 (dq, J = 3.4, 1.0 Hz, 1H), 3.74 (d, J = 1.0 Hz, 2H), 1.47 (s, 10H). **^{13}C NMR** (126 MHz, $CDCl_3$, δ): 169.56, 135.76, 126.51, 126.33, 124.68, 81.25, 36.73, 27.88. **HRMS–DART** (m/z): $[M + H]^+$ calcd for $C_{10}H_{15}O_2S^+$, 199.0787; found, 199.0785.

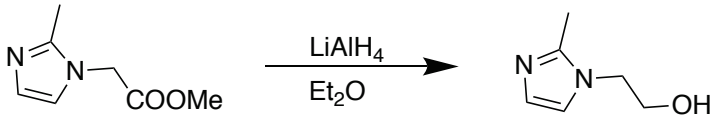


Synthesis of Methyl 2-(2-Methyl-1*H*-imidazol-1-yl)acetate (S9). 2-Methylimidazole (4.11 g, 0.05 mol) were dissolved in warm dry acetone (250 mL), and methylbromoacetate (5.7 mL, 0.06 mol) was added to the resulting solution in a single portion. The mixture was heated at reflux for 3 h, when the mixture turned into a milky white suspension. Solvent was removed under reduced pressure, and the residue was redissolved in methanol (50 mL) and heated at reflux for 1 h to quench unreacted methylbromoacetate. Solvent was removed under reduced pressure and co-evaporated twice with acetone to remove the last traces of methanol. The gooey solid was triturated with Et_2O (3 × 20 mL) with mechanical stirring, and dissolved in Et_2O (100 mL) with ammonia in MeOH (7 M, 20 mL) with stirring for 1 h. Solvent was removed under reduced pressure and co-evaporated with DCM under high vacuum. The product was isolated by column chromatography under an isocratic system (2% v/v MeOH in DCM) to give the title compound as a light orange oil (29% yield, R_f = 0.28 in 10% v/v MeOH in DCM). **^{1}H NMR** (500 MHz, $CDCl_3$, δ): 6.92 (s, 1H),

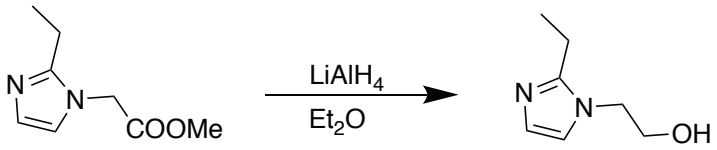
6.82 (s, 1H), 4.59 (s, 2H), 3.77 (s, 3H), 2.33 (s, 3H). **^{13}C NMR** (126 MHz, $CDCl_3$, δ): 168.04, 145.37, 127.75, 120.25, 52.89, 47.42, 12.94. **HRMS–DART** (*m*/*z*): $[M + H]^+$ for $C_7H_{11}N_2O_2^+$, 155.0815; found, 155.0819.



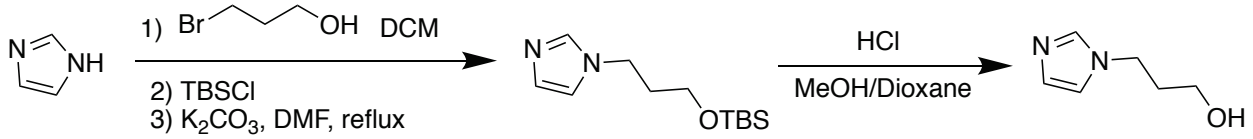
Synthesis of Methyl 2-(2-Ethyl-1*H*-imidazol-1-yl)acetate (S10). 2-Ethylimidazole (4.81 g, 0.05 mol) were dissolved in warm dry acetone (250 mL). Methylbromoacetate (5.7 mL, 0.06 mol) was added to the resulting solution in a single portion. The mixture was heated at reflux for 3 h until the mixture turned into a milky white suspension. Solvent was removed under reduced pressure, and the residue was redissolved in methanol (50 mL) and heated at reflux for 1 h to quench unreacted methylbromoacetate. Solvent was removed under reduced pressure and co-evaporated twice with acetone to remove the last traces of methanol. The gooey solid was triturated with Et_2O (3 × 20 mL) with mechanical stirring, and dissolved in Et_2O (100 mL) with ammonia in MeOH (7 M, 20 mL) under stirring for 1 h. Solvent was removed under reduced pressure and co-evaporated with DCM under high vacuum. The product was isolated by column chromatography under an isocratic system (2% v/v MeOH in DCM) to give the title compound as a yellow oil (33% yield, R_f = 0.35 in 10% v/v MeOH in DCM). **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 6.90 (d, J = 1.4 Hz, 1H), 6.77 (d, J = 1.5 Hz, 1H), 4.56 (s, 2H), 3.71 (s, 3H), 2.55 (q, J = 7.5 Hz, 2H), 1.26 (t, J = 7.5 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$, δ): 168.07, 149.68, 127.50, 120.10, 52.71, 46.88, 19.85, 11.71. **HRMS–DART** (*m*/*z*): $[M + H]^+$ calcd for $C_8H_{13}N_2O_2^+$, 169.0972; found, 169.0972.



Synthesis of 2-(2-Methyl-1*H*-imidazol-1-yl)ethan-1-ol (S11). Lithium aluminum hydride (0.76 g, 20 mmol) was dissolved in Et_2O (70 mL), and the resulting solution was cooled to 0 ºC. The solution was added dropwise to a solution of methyl 2-(2-methyl-1*H*-imidazol-1-yl)acetate (1.70 g, 11.0 mmol) in Et_2O (40 mL) on ice over 15 min, and allowed to stir at room temperature >1 h. The solution was cooled on an ice bath, and an aqueous solution (3.0 mL) of 1.5 M NaOH was added dropwise with concomitant evolution of heat. Once all the solution was added, the resulting solution was allowed to stir for 30 min, and solvent was removed under reduced pressure. The residue was dissolved in DCM, dried with Na_2SO_4(s), and filtered. Solvent was removed to afford pure product as an off-pink solid (quantitative). **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 6.79 (d, J = 1.4 Hz, 1H), 6.67 (d, J = 1.4 Hz, 1H), 5.93 (s, 1H), 3.91 (t, J = 5.1 Hz, 2H), 3.82 (t, J = 5.0 Hz, 2H), 2.26 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$, δ): 144.89, 126.58, 119.65, 61.34, 48.97, 13.03. **HRMS–DART** (*m*/*z*): $[M + H]^+$ calcd for $C_6H_{10}N_2O^+$, 127.0866; found, 127.0870.



Synthesis of 2-(2-Ethyl-1*H*-imidazol-1-yl)ethan-1-ol (S12). Lithium aluminum hydride (0.76 g, 20.0 mmol) was dissolved in Et_2O (70 mL), and the resulting solution was cooled to 0 °C. The solution was added dropwise to a solution of methyl 2-(2-methyl-1*H*-imidazol-1-yl)acetate (1.70 g, 11.0 mmol) in Et_2O (40 mL) on ice over 15 min, and allowed to stir at room temperature >1 h. The solution was cooled down on an ice bath, and an aqueous solution (3.0 mL) of 1.5 M NaOH was added dropwise with concomitant evolution of heat. Once all the solution was added, it was allowed to stir for 30 min, and solvent was removed under reduced pressure. The residue was dissolved in DCM, dried with Na_2SO_4(s), and filtered. Solvent was removed under reduced pressure to afford pure product as an off-yellow solid (quantitative). **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 6.82 (d, J = 1.4 Hz, 1H), 6.73 (d, J = 1.4 Hz, 1H), 5.36 (s, 1H), 3.94 (t, J = 5.3 Hz, 2H), 3.83 (t, J = 5.3 Hz, 2H), 2.61 (q, J = 7.6 Hz, 2H), 1.23 (t, J = 7.6 Hz, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$, δ): 149.54, 126.70, 119.63, 61.50, 48.49, 20.12, 12.18. **HRMS–DART** (*m*/*z*): $[M + H]^+$ calcd for $C_7H_{13}N_2O^+$, 141.1022; found, 141.1021.



Synthesis of 3-(1*H*-Imidazol-1-yl)propan-1-ol (S13). Imidazole (1.50 g, 22.0 mmol) was dissolved in DCM (20 mL) and 3-bromopropanol (1 mL, 11.0 mmol) was added in one portion to this mixture and it was allowed to stir for 3 h. TBSCl (1.66 g, 11.0 mmol) was added in one portion, and allowed to stir overnight. The solvent was removed under reduced pressure with a water bath at room temperature. K_2CO_3 (5.00 g, 36.1 mmol) was added. The residue was dissolved in DMF (10 mL), and the resulting solution was heated at reflux for 6 h. The reaction mixture was partitioned into DCM and water, the organic layer was washed with a saturated LiCl solution in water (5×), dried with Na_2SO_4(s), and filtered. Solvent was removed under reduced pressure, and purified using column chromatography under an isocratic system (6% v/v MeOH in DCM) to give 1-(3-((*tert*-butyldimethylsilyl)oxy)propyl)-1*H*-imidazole as a volatile yellow semisolid (44% yield, R_f = 0.43 in 6% v/v MeOH in DCM) that was used immediately in the next step. 1-(3-((*tert*-butyldimethylsilyl)oxy)propyl)-1*H*-imidazole (1.14 g, 4.74 mmol) was dissolved in MeOH (10 mL). 4 M HCl in dioxane (1.5 mL) was added dropwise to the resulting solution, and the mixture was allowed to stir overnight. $NaHCO_3$(s) was added until a pH ~8 was attained, and solvent was removed under reduced pressure. The residue was taken up in DCM and removed under reduced pressure. The resulting oil was triturated with hexanes (2×) to afford the title product as a light-orange oil (quantitative). **^{1}H NMR** (500 MHz, $CDCl_3$, δ): 7.47 (s, 1H), 7.17–6.85 (m, 2H), 4.12 (t, J = 6.7 Hz, 2H), 3.59 (t, J = 5.8 Hz, 2H), 3.12 (s, 1H), 1.99 (p, J = 6.3 Hz, 2H). **^{13}C NMR** (126 MHz, $CDCl_3$, δ): 137.21, 128.59, 119.12, 57.55, 43.55, 33.45. **HRMS–DART** (*m*/*z*): $[M + H]^+$ for $C_6H_{11}N_2O^+$, 127.0866; found, 127.0865.

Biological Methods

Esterase Activity Screen with 2-Thiopheneacetic Acid (2TA). Bacterial cells (*M. smegmatis* mc^2155, or *E. coli* DH10B) from an overnight culture were inoculated at 1% v/v into 15 mL of Luria–Bertani medium. The resulting culture were grown in a shaking incubator at 37 °C to log phase ($OD_{600\ nm}$ = 0.4–0.8) and then removed from the shaking incubator. Cells were pelleted by centrifugation at 3260*g* for 3 min, and the supernatant was discarded. The cell pellet was resuspended in 5 mL of water and pelleted again. The resulting pellet was resuspended in 1.5 mL of water (thereby concentrating the cells 10-fold), and 45 μL of this suspension was combined with 5 μL of ester substrate and 50 μL of 2× assay buffer (which was 10 mM HEPES–NaOH buffer, pH 7.4, containing 8 mM $TbCl_3$, 700 μM TOPO, and 0.2% w/v Triton X-100), and the luminescence was measured immediately. A blank solution lacking the analyte but including 45 μL of cell suspension, 5 μL of water, and 50 μL of 2× assay buffer was also prepared in triplicate. A calibration curve was prepared by combining 45 μL of cell suspension, a known amount of 2TA, 50 μL of 2× assay buffer, and water to a final volume of 100 μL. The luminescence of samples, blanks, and calibration-curve solutions was then monitored. The luminescence of the sample solutions was normalized against that of the blank solutions, and the concentration of liberated 2TA in the sample solutions was determined by using the slope of the calibration curve.

General Procedure for Viability Assay with Resazurin Dye. A 5-mL culture of bacterial cells was inoculated from a single colony taken from solid medium. The culture was allowed to grow overnight. Then, the $OD_{600\ nm}$ was measured, and the culture was diluted to $OD_{600\ nm}$ = 0.02 (for *E. coli* and *B. subtilis*) or 0.04 (for *M. smegmatis*). Stocks of compounds to be tested were prepared at 50 mM in DMSO and then diluted to 1 mM working stocks in medium. To each well of a sterile 96-well plate, 10 μL of resazurin dye (AlamarBlue from Bio-Rad Laboratories) was added, and then the appropriate amount of compound working stock was added to designated wells. Medium was added as needed to bring the volume of each well to 20 μL, then 80 μL of diluted overnight culture was added to each well. The plate was then sealed with clear polyolefin sealing tape (Thermo Fisher Scientific) and placed in the plate reader, pre-heated to 37 °C. The plate reader was programmed to shake the plate for 3 s before every measurement and take measurements every 2–3 min with an excitation wavelength of 545 nm and emission wavelength of 590 nm.

Determination of Sulfurol Ester Cleavage in Bacterial Lysates. To prepare bacterial lysate, a 5-mL culture of bacterial cells was inoculated from a single colony taken from solid medium. The culture was allowed to grow overnight and then lysed with a Qsonica sonicator set to 80% amplitude using 10 cycles of 1 s on, 1 s off.

To determine the accuracy and recovery of the methodology, authentic solutions of sulfurol ester, sulfurol, and *trans*-3-(4-chlorobenzoyl)acrylic acid were spiked into cell lysate in the concentrations described in the table below. Stock solutions of sulfurol esters was prepared at a concentration of 50 mM in DMSO, and stock solutions of sulfurol and *trans*-3-(4-chlorobenzoyl)acrylic acid were prepared at a concentration of 100 μM in DMSO. Aliqouts (10 μL) of these stock solutions were then added to 1.5-mL sterile Eppendorf tube, and 490 μL of lysate was added to yield a total volume of 500 μL and the targeted concentrations. A blank sample was prepared by combining 10 μL of DMSO and 490 μL of lysate. Each tube was then mixed by inversion, and 300 μL of dichloromethane was added immediately to each tube to extract the spiked compounds. The tubes were mixed by inversion, and the dichloromethane was allowed to settle to the bottom. A pipettor was used to extract 150 μL of the settled dichloromethane and

transfer it into a fresh 1.5-µL Eppendorf tube. The solvent was removed under a stream of N_2(g), and 75 µL of 1:1 acetonitrile/water were then added to each tube. The tube was mixed vigorously by vortexing and then subjected to centrifugation at 23100*g* for 3 min. Following centrifugation, 50 µL was transferred to an HPLC vial equipped with a vial insert.

% hydrolyzed	[sulfurol ester] (µM)	[sulfurol] (µM)	[*trans*-3-(4-chlorobenzoyl)acrylic acid] (µM)
0	200	0	0
10	180	20	20
25	150	50	50
50	100	100	100
75	50	150	150
90	20	180	180
100	0	200	200

Samples to determine the extent of hydrolysis in the lysate were prepared by adding 490 µL of lysate to a 1.5-mL Eppendorf vial and then adding sulfurol ester in DMSO to a final concentration of 200 µM. The volume was brought to 500 µL with DMSO to match the total volume of DMSO that was used to obtain the standard curve. The vial was then incubated at 37 °C for 2 h. After incubation, 300 µL of dichloromethane was added to each vial. The vials were mixed with inversion, and the dichloromethane was allowed to settle to the bottom. A pipettor was used to extract 150 µL of the settled dichloromethane and transfer it into a fresh 1.5-µL Eppendorf tube. The solvent was removed under a stream of N_2(g), and 75 µL of 1:1 acetonitrile/water were then added to each tube. The tube was mixed vigorously by vortexing and then pelleted via centrifugation at 23100*g* for 3 min. Following centrifugation, 50 µL was transferred to an HPLC vial equipped with a vial insert.

Samples and calibration samples were transferred to the LC–MS instrument. Separation was achieved on an Agilent Poroshell 120 SB C18 (50 mm × 2.1 mm i.d., 2.7 µm pore size) running a linear gradient of 0.1% v/v formic acid in 90:10 to 10:90 water/acetonitrile over 6 min, followed by a 1-min equilibration at the initial conditions.

Calibration curves and hydrolysis samples were prepared in the above manner in lysate from *E. coli* DH10B, *B. subtilis* OI1085, and *M. smegmatis* mc^2155. Peaks correlating to the sulfurol ester, sulfurol, or *trans*-3-(4-chlorobenzoyl)acrylic acid were integrated using SpectraGryph software. The acid peak was found to be too variable, particularly from *M. smegmatis* lysate, for reliable quantification (see: Figure S8); however, the ratio of the area of the sulfurol ester to the sulfurol peak was found to be linear in all lysates from 25–100% hydrolyzed (Figure S7), where % hydrolyzed was calculated as moles of sulfurol divided by moles of sulfurol ester. These calibration curves were then used to determine the % hydrolyzed in the samples.

Determination of Minimum Inhibitory Concentration. A 5-mL culture of bacterial cells was inoculated from a single colony taken from solid medium and allowed to grow overnight. After overnight growth, the bacteria was diluted to $OD_{600\ nm}$ 0.02–0.04. Wells were prepared in triplicate. Solutions of antibiotic were prepared at a 50 mM concentration in DMSO then diluted to 0.5 mM in medium. Final antibiotic compound concentrations were 400, 300, 200, 100, and 50 µM. Control wells containing no antibiotic and medium alone were also prepared and monitored to ensure bacterial cell growth and non-contamination, respectively. The $OD_{600\ nm}$ was determined at the initial time point and after 24 h. For *M. smegmatis*, an additional 30-h time point was taken due to slower growth of *M. smegmatis* cells. Values for the MIC of acid **1** and sulfurol ester **3** were

>400 μM for *E. coli* DH10B, 400 and 200 μM (respectively) for *B. subtilis* OI1085, and >400 and 300 μM (respectively) for *M. smegmatis* mc2155. Notably, the solubility limit of sulfurol ester **3** was 400 μM.

Creation of Sequence Similarity Network (SSN). Annotated carboxylesterase enzymes were identified by searching the NCBI protein database using the search term "carboxylesterase" and the desired species (*Bacillus subtilis* subsp. subtilis str. 168, *Escherichia coli* str. K-12 substr. DH10B, or *Mycolicibacterium smegmatis* MC2 155). The resulting sequences were analyzed with the Enzyme Function Initiative's Enzyme Similarity Tool (EFI-EST; http://efi.igb.illinois.edu/efi-est/index.php)[2] and subsequently analyzed using Cytoscape 3.8.1.[3] To determine the appropriate edge threshold, E-values were tested in increments of 5 ranging from 15 to 85 (Figure S9). In the range from 35–80, one and only one esterase from *B. subtilis* was observed to persist in a cluster containing esterases from *M. smegmatis* as well. Therefore, a final threshold was set at 37, which is just above the E-value at which multiple clusters exhibited esterases from both *M. smegmatis* and *B. subtilis* but unlikely to be too discriminatory.

Creation of pET22b-pnbA and pET22b-MSmegEsterase. A culture of *B. subtilis* subsp. *subtilis* 168 cells was inoculated from a single colony on an LB agar plate. The culture was grown overnight, then 1.5 mL was pelleted by centrifugation at 23100*g*. The spent medium was removed by aspiration, and the pellet was resuspended in 50 μL of DMSO. The resuspended pellet was held at 98 °C for 5 min to lyse the bacteria and liberate genomic DNA. The mixture was then cooled to room temperature and pelleted again at 23100*g* for 5 min to remove cellular debris. The supernatant was removed and saved for use as template DNA. The *pnbA* gene was cloned by the polymerase chain reaction (PCR) using primers pnbA_fp and pnbA_rp. The PCR reaction product was purified by using a GeneJET PCR purification kit from Thermo Fisher Scientific. Empty plasmid pET-22b(+) (Novagen) was digested with *Sal*I-HF (NEB) and *Nde*I (NEB) as per the manufacturer's directions, and the restriction enzymes were heat-inactivated. The *pnbA* PCR product was then combined with the digested vector in an isothermal assembly with Gibson Assembly 2× Master Mix (NEB) to yield pET22b-pnbA.

Using the same technique, pET22b-MSmegEsterase was created by inoculating a culture from a single colony taken from an 7H10 agar plate and growing overnight. From this culture, 1.5 mL was pelleted by centrifugation at 23100*g*. The spent medium was removed by aspiration, and the pellet was resuspended in 50 μL of DMSO. The resuspended pellet was held at 98 °C for 5 min to lyse the bacteria and liberate the genomic DNA. The mixture was then cooled to room temperature and pelleted again at 23100*g* for 5 min to remove cellular debris. The supernatant was removed and saved for use as template DNA. The gene encoding A0QYI2 was cloned by the PCR using primers A0QYI2_f and A0QYI2_r. The PCR reaction product was purified by using a GeneJET PCR purification kit from Thermo Fisher Scientific. Empty plasmid pET-22b(+) (Novagen) was digested with *Sal*I-HF (NEB) and *Nde*I (NEB) as per the manufacturer's directions, and the restriction enzymes were heat-inactivated. The PCR product encoding A0QYI2 was then combined with the digested vector in an isothermal assembly with Gibson Assembly 2× Master Mix (NEB) to yield pET22b-MSmegEsterase.

Primers (5′→3′)

pnbA_fp: GTTTAACTTTAAGAAGGAGATATACATATGACTCATCAAATAGTAACG
pnbA_rp: GCGGCCGCAAGCTTGTCGACTTATTCTCCTTTTGAAGGG
A0QYI2_f: tttgtttaactttaagaaggagatatacatATGGCGAACGCCGCCCGGATC
A0QYI2_r: ggtgctcgagtgcggccgcaagcttgtcgaTTACCGGAAGTTGAGTACCTGGTCACCCC

Heterologous Expression of Esterases in *E. coli* DH10B. Chemically competent *E. coli* DH10B was transformed with plasmids pET22b-pnbA and pCS6 or pET22b-MSmegEsterase. Plasmid pCS6 was a gift from Matthew Bennett (Addgene plasmid #55752; http://n2t.net/addgene:55752; RRID:Addgene_55752) and directs the expression of a gene encoding T7 RNA polymerase (T7RNAP) under the control of an arabinose promoter.[4] Transformants were plated on LB agar medium containing ampicillin (100 μg/mL) and spectinomycin (25 μg/mL). An overnight culture was inoculated in LB medium containing ampicillin (100 μg/mL), spectinomycin (25 μg/mL), and glucose (2% w/v). From this overnight, cultures for viability assays were inoculated to $OD_{600\ nm}$ = 0.02. Cultures intended for "leaky" expression contained CAMHB, ampicillin (100 μg/mL), and spectinomycin (25 μg/mL). Cultures intended for expression contained ampicillin (100 μg/mL), spectinomycin (25 μg/mL), and arabinose (0.05% w/v).

To generate a control strain that expressed T7RNAP but not pnbA, the procedure described above was followed except that empty pET-22b(+) was used instead of pET22b-pnbA.

Lysates of *pnbA*-Expressing *E. coli* DH10B. *E. coli* DH10B cells expressing the esterase from either *M. smegmatis* mc^2155 or *B. subtilis* subsp. *subtilis* 168 were lysed as described above. As a control, *E. coli* DH10B cells expressing pCS6 and empty pET-22b(+) were treated in the same manner.

Nonlinear Regression Fit of Viability Curves. Fits were performed with R software using the minpack.lm nonlinear least-squares regression package. Data were fitted to eq 1, where a = the upper asymptote, v = parameter affecting near which asymptote the maximum growth occurs, k = the growth rate, and l = the point of inflection on the ordinate. The variable t represents the time in minutes and is plotted on the ordinate. The fit was used solely to determine the ordinate inflection point (variable l), and no conclusions about growth rates were drawn from the fit.

$$\mathrm{RFU} = a\left(l + ve^{k(l-t)}\right)^{\left(-1/v\right)} \quad (1)$$

Table S1. Annotated Carboxylesterases in the Sequence Similarity Network in Figure 3A. Each Carboxylesterase is Indicated in Figure 3A as a Circle or "Node." A UniProt ID can be Associated with Multiple NCBI IDs with Identical Protein Sequences.

UniProt ID	Organism	PFAM	Description	Associated NCBI IDs
P96688	*Bacillus subtilis* (strain 168)	PF12697	Uncharacterized carboxylesterase nap	NP_388425.1|BAA19378.1|CAB12351.1|P96688.1|WP_003242638.1
P94407	*Bacillus subtilis* (strain 168)	PF00561	AB hydrolase superfamily protein YclE	CAB12160.2|P94407.2|WP_003246662.1|NP_388248.2
P94396	*Bacillus subtilis* (strain 168)	PF00561	Uncharacterized hydrolase YcgS	P94396.2|WP_003246384.1|CAB12120.1|NP_388208.1
P70981	*Bacillus subtilis* (strain 168)	PF12146	Probable aminopeptidase YbaC	WP_003235060.1|BAA11005.1|P70981.2|NP_387995.2|CAB11890.2
P70948	*Bacillus subtilis* (strain 168)	PF00326	Putative esterase YitV	NP_388996.1|CAB12955.1
P54549	*Bacillus subtilis* (strain 168)	PF12146	Uncharacterized protein YqjL	P54549.1|CAB14315.1|NP_390264.1|WP_003230373.1
P37967	*Bacillus subtilis* (strain 168)	PF00135	Para-nitrobenzyl esterase	WP_003243926.1|P37967.2|AAB39889.1|NP_391319.1|CAB15444.1
O52202	*Mycolicibacterium smegmatis*	None	Uncharacterized protein	AAB96637.1
O34592	*Bacillus subtilis* (strain 168)	PF12697	AB hydrolase superfamily protein YdjP	CAB12447.1|O34592.1|NP_388509.1|WP_003244040.1
O32234	*Bacillus subtilis* (strain 168)	PF00561	AB hydrolase superfamily protein YvaM	O32234.1|CAB15369.1|NP_391244.1|WP_003228381.1
O32232	*Bacillus subtilis* (strain 168)	PF12146	Carboxylesterase	WP_003242610.1|CAB15367.2|O32232.2
O31581	*Bacillus subtilis* (strain 168)	PF00561	AB hydrolase superfamily protein YfhM	NP_388739.1|WP_003243419.1|O31581.1|CAB12687.1
O31452	*Bacillus subtilis* (strain 168)	PF12697	Carboxylesterase YbfK	CAB12020.1|NP_388108.1|O31452.1|BAA33123.1|WP_003246261.1
O31431	*Bacillus subtilis* (strain 168)	PF00561	Uncharacterized protein YbdG	BAA33096.1|WP_003234896.1|CAB11993.2|O31431.2|NP_388081.2
O07937	*Bacillus subtilis* (strain 168)	PF00561	Uncharacterized hydrolase YraK	NP_390568.2|CAB14632.2|O07937.2|WP_003229849.1
O07015	*Bacillus subtilis* (strain 168)	PF12697	Sigma factor SigB regulation protein RsbQ	CAB15415.1|O07015.1|WP_003228292.1|NP_391290.1
O06734	*Bacillus subtilis* (strain 168)	PF00561| PF08386	AB hydrolase superfamily protein YisY	NP_388971.1|WP_003245141.1|CAB12930.1|O06734.1
O05235	*Bacillus subtilis* (strain 168)	PF00561| PF08386	Uncharacterized hydrolase YugF	CAB07918.1|O05235.1|NP_391020.1|CAB15131.1|WP_003228858.1
I7GGN0	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00561	Alpha/beta hydrolase fold protein	AFP42964.1
I7GGJ9	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF12697	Alpha/beta fold hydrolase	AFP42834.1

I7GGB1	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00135	Carboxylic ester hydrolase	AFP42569.1
I7GG82	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF06441	Epoxide hydrolase	AFP43101.1
I7GFL3	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF06441	Epoxide hydrolase	AFP41949.1
I7GE94	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF12697	Alpha/beta hydrolase fold protein	AFP41184.1
I7GCR4	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00561	Alpha/beta hydrolase fold protein	AFP41041.1|WP_011730025.1
I7GCD6	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00561	Alpha/beta hydrolase fold protein	AFP40259.1
I7GBY1	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF12697	Alpha/beta hydrolase fold protein	AFP43147.1
I7GBL2	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00561	Alpha/beta hydrolase fold protein	WP_011731517.1|AFP43007.1
I7GBG7	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00561	Haloacetate dehalogenase H-1	WP_011731476.1|AFP42952.1
I7GAN5	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00135	Carboxylic ester hydrolase	AFP40216.1|WP_011729367.1
I7G9U1	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00561	Alpha/beta hydrolase fold protein	AFP39891.1
I7G952	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF12697	Alpha/beta hydrolase fold protein	AFP39716.1
I7G7E3	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00561	Alpha/beta hydrolase fold protein	AFP39176.1|WP_011728576.1
I7G7D2	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF12697	AB hydrolase-1 domain-containing protein	AFP39166.1
I7G720	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF12697	Putative peroxidase (Non-heme peroxidase)| BpoB| alpha/beta hydrolase family	AFP41517.1|WP_011730384.1
I7G6F9	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF12697	Salicylate esterase	AFP38224.1|WP_011727914.1
I7G5M0	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00135	Carboxylic ester hydrolase	WP_011727668.1|AFP37869.1
I7G5G0	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00561	Alpha/beta hydrolase fold protein	AFP38411.1
I7G5A2	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00211| PF00561	Lignin peroxidase LipJ	AFP40822.1
I7G4B5	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF12146	Alpha/beta hydrolase fold protein	AFP38011.1
I7G3B1	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00135	Carboxylic ester hydrolase	WP_011726970.1
I7G317	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF12697	Bromoperoxidase BpoC	WP_014876968.1|AFP37556.1

I7G2M2	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF06441	Epoxide hydrolase	WP_011726720.1|AFP36659.1
I7G234	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00135	Carboxylic ester hydrolase	WP_011727065.1|AFP37076.1
I7G086	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF12697	Putative hydrolase	AFP38942.1
I7FXZ7	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00561	Lipase/esterase lipG	AFP37787.1
I7FVK9	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00561	Alpha/beta hydrolase fold protein	AFP42963.1|WP_011731487.1
I7FP61	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF12697	Alpha/beta hydrolase fold protein	AFP43075.1|WP_011731568.1
I7FP13	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF12697	Alpha/beta hydrolase fold protein	AFP40438.1|WP_011729531.1
I7FNE2	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00486| PF00561	Alpha/beta hydrolase fold protein	AFP40223.1
I7FMU7	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00135	Carboxylesterase	AFP40043.1
I7FMD4	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00561	Epoxide hydrolase EphE	AFP42455.1
I7FM79	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00561	Epoxide hydrolase EphA	WP_011731041.1|AFP42380.1
I7FM61	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF12697	Alpha/beta hydrolase fold protein	AFP42360.1
I7FJS6	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	None	Conserved hypothetical membrane protein	AFP38998.1
I7FID2	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	None	Uncharacterized protein	AFP41135.1
I7FGW1	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF12697	Alpha/beta hydrolase fold protein	WP_011727803.1|AFP38088.1
I7FGP2	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00561	Alpha/beta hydrolase fold protein	AFP38023.1
I7FDF1	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF12697	Alpha/beta hydrolase fold protein	AFP36928.1
I7FD31	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00135	Carboxylic ester hydrolase	AFP39390.1
I7FCR0	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00561	Alpha/beta hydrolase fold protein	AFP39305.1
I7FC89	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF12697	Alpha/beta hydrolase fold protein	AFP39145.1
I7FBN9	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00561	Alpha/beta hydrolase fold protein	AFP38925.1
I7FA07	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF12697	Putative hydrolase| alpha/beta fold LipV	AFP38370.1

I7F8W6	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF12697	Carboxylesterase	AFP38005.1
C4ZUR7	*Escherichia coli* (strain K12 / MC4100 / BW2952)	PF00561	Pimeloyl-[acyl-carrier protein] methyl ester esterase	WP_001060070.1
B1X758	*Escherichia coli* (strain K12 / DH10B)	PF00561	Pimeloyl-[acyl-carrier protein] methyl ester esterase	B1X758.1|ACB04470.1
A0R7G7	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF12697	Putative hydrolase	ABK71430.1|WP_011731621.1
A0R7C4	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF06441	Epoxide hydrolase 1	ABK74036.1
A0R797	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF12697	Hydrolase| alpha/beta hydrolase fold family protein	ABK71718.1
A0R733	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00561	Oxidoreductase	ABK74727.1
A0R6Z0	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00561	Epoxide hydrolase	WP_011731488.1|ABK74436.1
A0R6Y9	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00561	Epoxide hydrolase	ABK73666.1
A0R6X8	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00561	Epoxide hydrolase	ABK72816.1
A0R6L0	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF12697	Alpha/beta hydrolase| putative	WP_011731381.1|ABK70030.1
A0R619	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF01083	Cutinase domain-containing protein	A0R619.1
A0R5T4	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00135	Carboxylic ester hydrolase	WP_011731192.1
A0R5G5	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00561	Hydrolase| alpha/beta fold family protein	ABK73054.1|WP_011731100.1
A0R589	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00561	Epoxide hydrolase	ABK70428.1
A0R568	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF12697	Hydrolase| alpha/beta hydrolase fold family protein	WP_011731027.1|ABK70319.1
A0R403	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF06441	Epoxide hydrolase	ABK72315.1
A0R2R7	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF12697	Hydrolase| alpha/beta fold family protein	ABK74195.1
A0R1M8	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	None	Uncharacterized protein	WP_011730102.1|ABK74316.1
A0R1C9	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00561	Non-heme bromoperoxidase BPO-A2	ABK71131.1
A0R1A3	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00144	Carboxylesterase| putative	ABK71637.1

A0R0R2	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00211|PF00561	Hydrolase| alpha/beta hydrolase fold family protein	ABK75261.1|WP_011729865.1
A0QZM4	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF12697	Putative hydrolase	ABK72475.1
A0QZ43	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00561	Hydrolase	ABK74851.1|WP_011729398.1
A0QZ01	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00135	Carboxylic ester hydrolase	ABK75312.1
A0QYI2	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00135	Carboxylic ester hydrolase	WP_011729246.1|ABK69668.1
A0QY24	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00561	Hydrolase| alpha/beta hydrolase fold family protein	ABK71451.1
A0QXK5	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF12697	Hydrolase	ABK73823.1|WP_011728989.1
A0QWN1	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00135	Carboxylic ester hydrolase	WP_011728750.1|ABK73819.1
A0QWE5	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00561	Hydrolase	ABK69734.1|WP_011728682.1
A0QW20	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00561	Alpha/beta hydrolase fold	ABK72302.1
A0QW10	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF12697	AB hydrolase-1 domain-containing protein	WP_011728568.1|ABK70069.1
A0QVJ1	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	None	Uncharacterized protein	WP_011728457.1|ABK75569.1
A0QVD3	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF12697	Putative carboxylesterase protein	ABK74379.1|WP_011728413.1
A0QVB5	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00561	Hydrolase| alpha/beta fold family protein| putative	WP_011728396.1|ABK76041.1
A0QTW0	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00561	Haloacetate dehalogenase H-1	ABK70097.1|WP_011728061.1
A0QTR8	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF12697	Hydrolase| alpha/beta fold family protein	ABK72057.1|WP_011728027.1
A0QTD1	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF12697	Salicylate esterase	ABK72895.1
A0QSZ4	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF12697	Hydrolase| alpha/beta fold family protein| putative	ABK73343.1
A0QSS9	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00561	Alpha/beta hydrolase fold	WP_011727758.1|ABK70732.1
A0QSR7	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF12146	Alpha/beta hydrolase fold	WP_011727748.1|ABK75088.1
A0QSR0	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF12697	Carboxylesterase	ABK72861.1|WP_011727741.1
A0QSC7	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00135	Carboxylic ester hydrolase	ABK75119.1

A0QS51	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00561	Hydrolase| alpha/beta fold family protein	ABK71811.1
A0QRG4	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF12697	Hydrolase| alpha/beta fold family protein	ABK75828.1
A0QQ34	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF00135	Carboxylic ester hydrolase	ABK75576.1
A0QPN5	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF12697	Alpha/beta hydrolase fold	ABK74583.1|WP_011726959.1
A0QNW0	*Mycolicibacterium smegmatis* (strain ATCC 700084 / mc^2)	PF06441	Epoxide hydrolase 1	ABK72822.1
A0A2U9Q0T8	*Mycolicibacterium smegmatis* (strain MKD8)	PF06441	Epoxide hydrolase 1	WP_003898281.1
A0A2U9PXN4	*Mycolicibacterium smegmatis* (strain MKD8)	PF06441	Epoxide hydrolase	WP_003897072.1
A0A2U9PRT3	*Mycolicibacterium smegmatis* (strain MKD8)	PF00561	Hydrolase| alpha/beta hydrolase fold family protein	WP_003894961.1
A0A2U9PL24	*Mycolicibacterium smegmatis* (strain MKD8)	PF00561	Hydrolase| alpha/beta fold family protein	WP_003892740.1

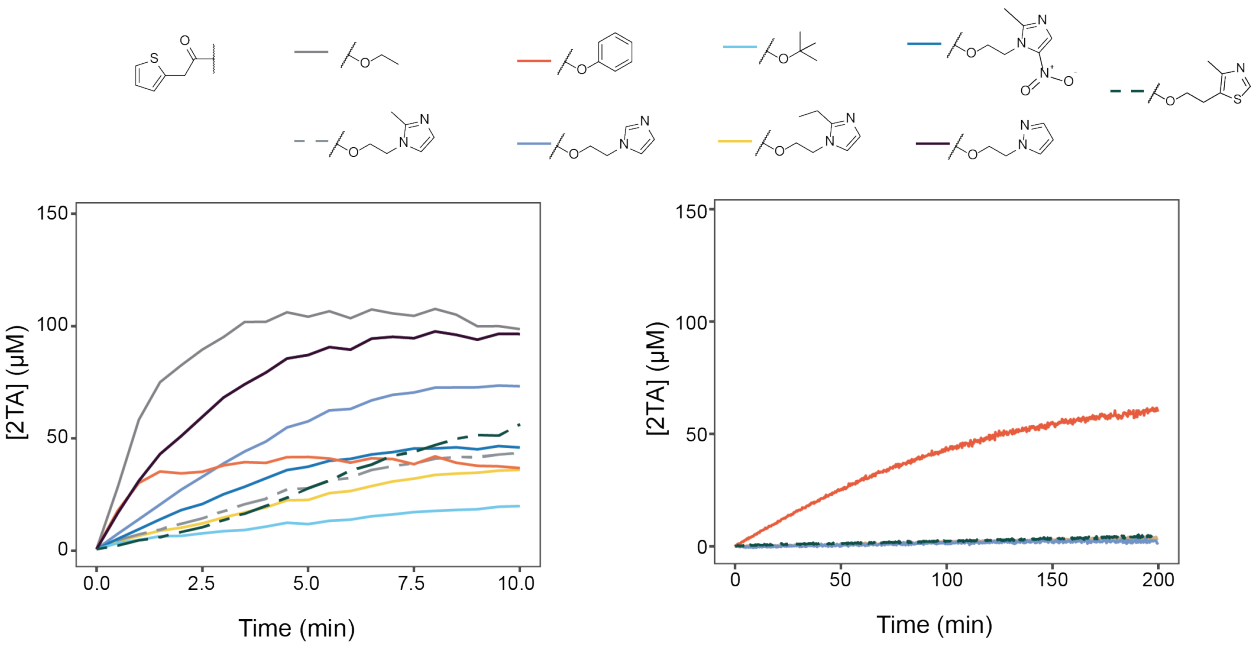


Figure S1. Graphs showing esterase activity of lysates from *M. smegmatis* mc^{2}155 and *E. coli* DH10B cells. Although all esters were cleaved to some degree in the *M. smegmatis* lysate, only the phenol ester was hydrolyzed in the *E. coli* cell lysate. Note: Only the ethyl, imidazole, sulfurol, and phenol esters were tested in *E. coli*.

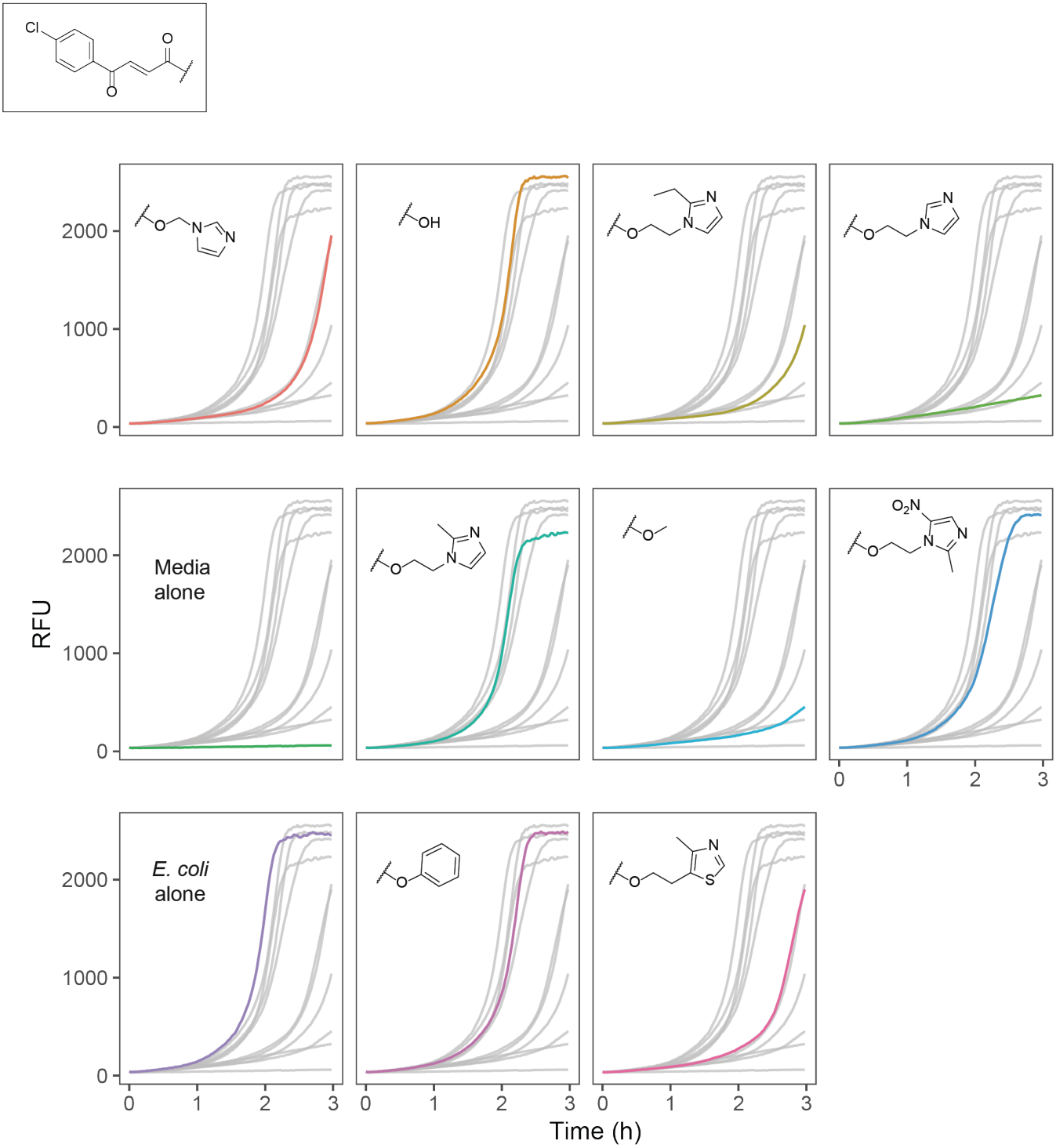


Figure S2. Graphs showing a screen of the antibacterial activity of esters of *trans*-3-(4-chlorobenzoyl)acrylic acid against *E. coli* DH10B cells. Compounds were at a concentration of 50 µM. Lines represent the average of triplicate or duplicate preparations. Colored lines represent the compound drawn in the inset; gray lines represent the other compounds.

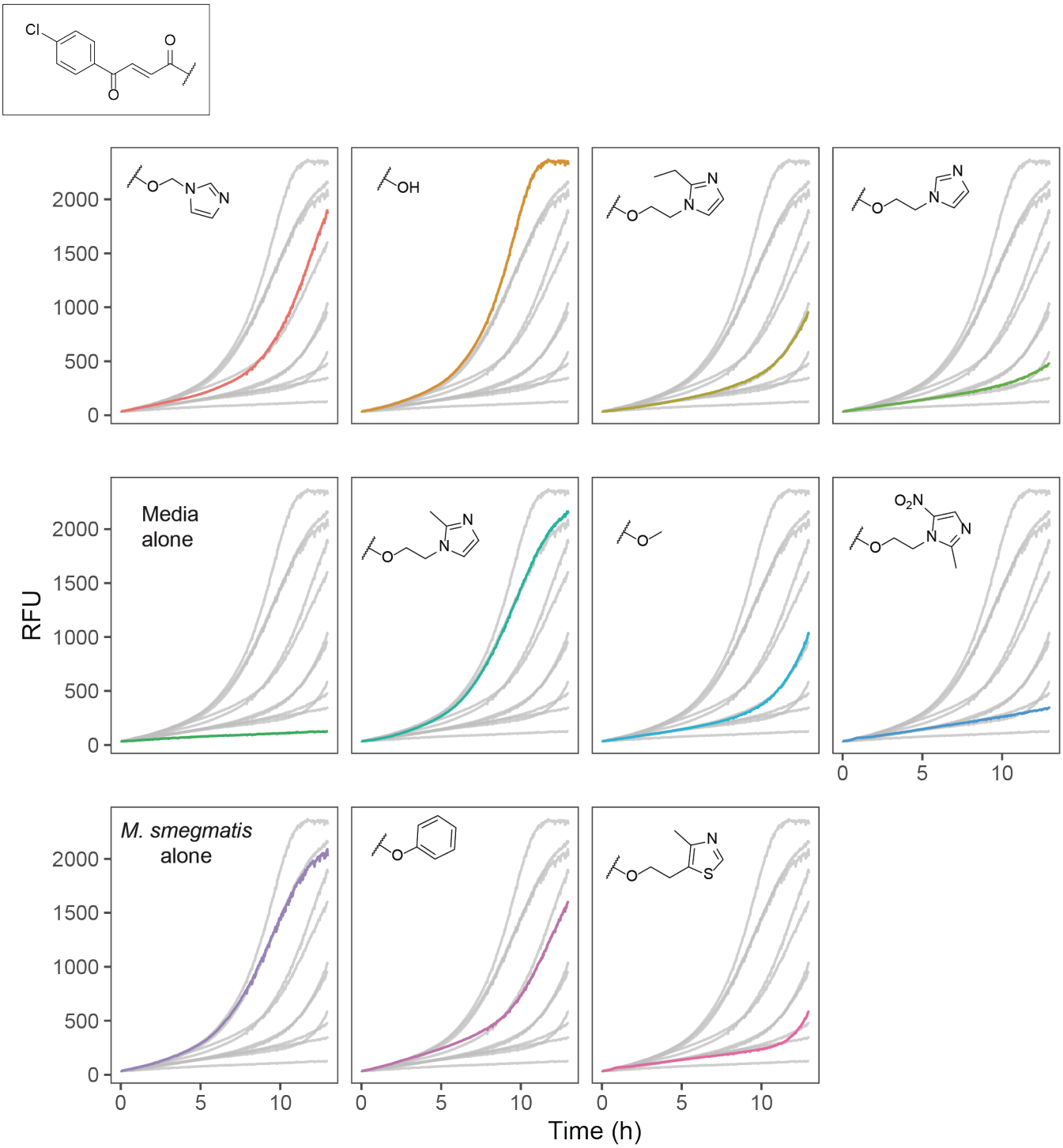


Figure S3. Graphs showing a screen of the antibacterial activity of esters of *trans*-3-(4-chlorobenzoyl)acrylic acid against *M. smegmatis* mc^2155 cells. Compounds were at a concentration of 50 µM. Lines represent the average of triplicate or duplicate preparations. Colored lines represent the compound drawn in the inset; gray lines represent the other compounds.

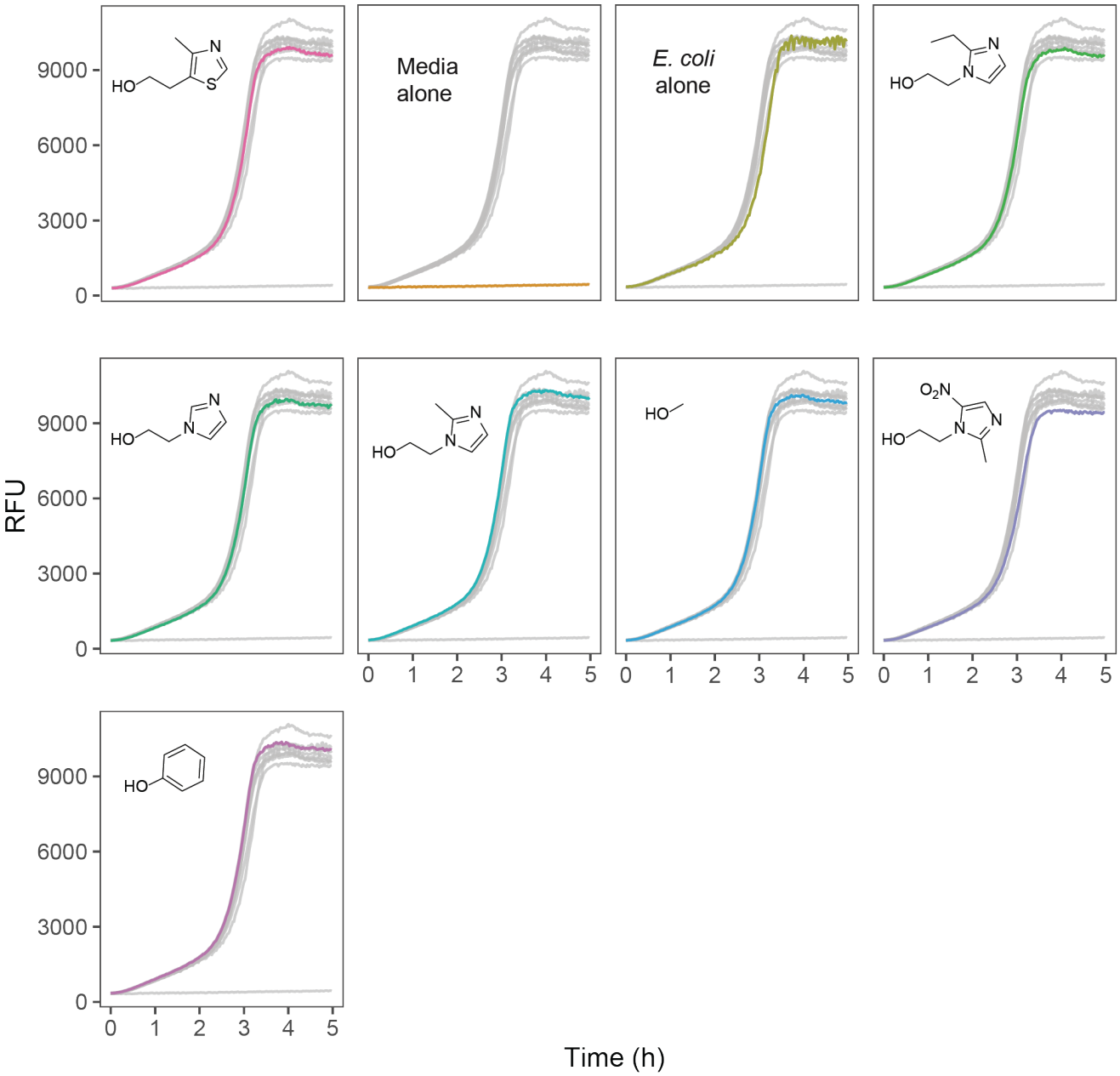


Figure S4. Graphs showing a screen of the antibacterial activity of alcohols against *E. coli* DH10B cells. Compounds were at a concentration of 100 µM. Lines represent the average from triplicate preparations. Colored lines represent the compound drawn in the inset; gray lines represent the other compounds.

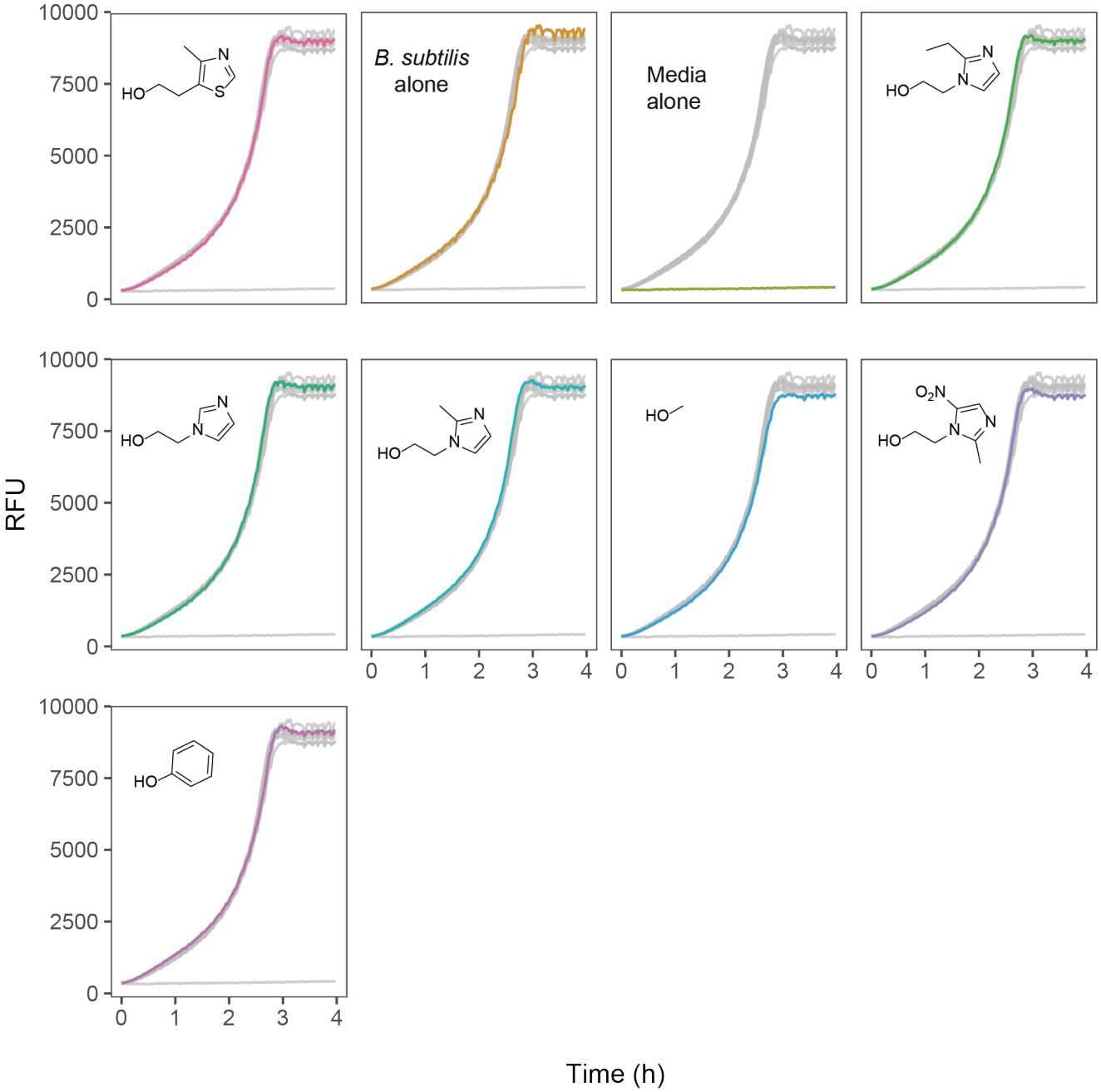


Figure S5. Graphs showing a screen of the antibacterial activity of alcohols against *B. subtilis* OI1085 cells. Compounds were at a concentration of 100 µM. Lines represent average of triplicate preparations, with the exception of those for phenol and 2-(2-methyl-1*H*-imidazol-1-yl)ethan-1-ol, which were tested in duplicate. Colored lines represent the compound drawn in the inset; gray lines represent the other compounds.

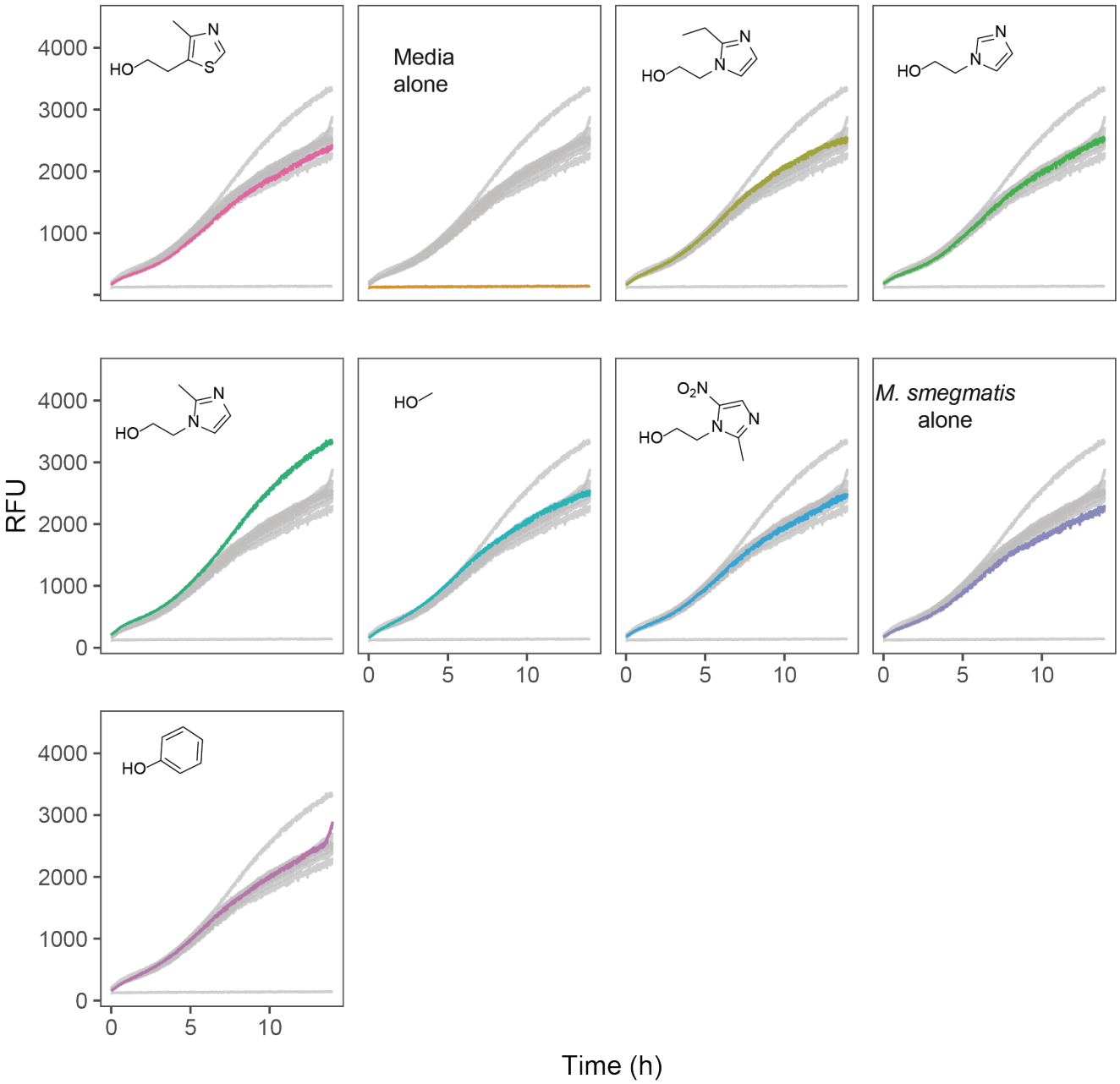


Figure S6. Graphs showing a screen of the antibacterial activity of alcohols against *M. smegmatis* mc^2155 cells. Compounds were at a concentration of 100 µM. Lines represent average of triplicate preparations. Colored lines represent the compound drawn in the inset; gray lines represent the other compounds.

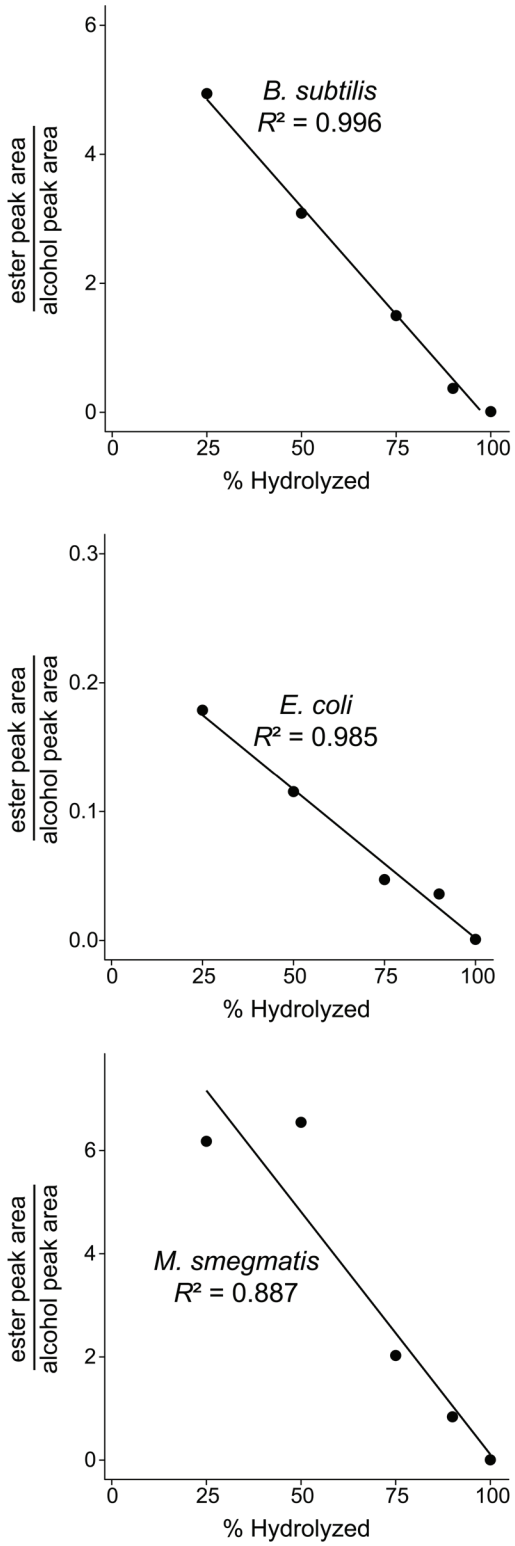


Figure S7. Calibration of sulfurol ester:sulfurol peak-area ratio versus % hydrolyzed. Curves were used to determine the extent of hydrolysis in incubated samples of the sulfurol ester in cell lysate of the corresponding bacteria. Note: At <25% hydrolysis, the relationship was no longer linear.

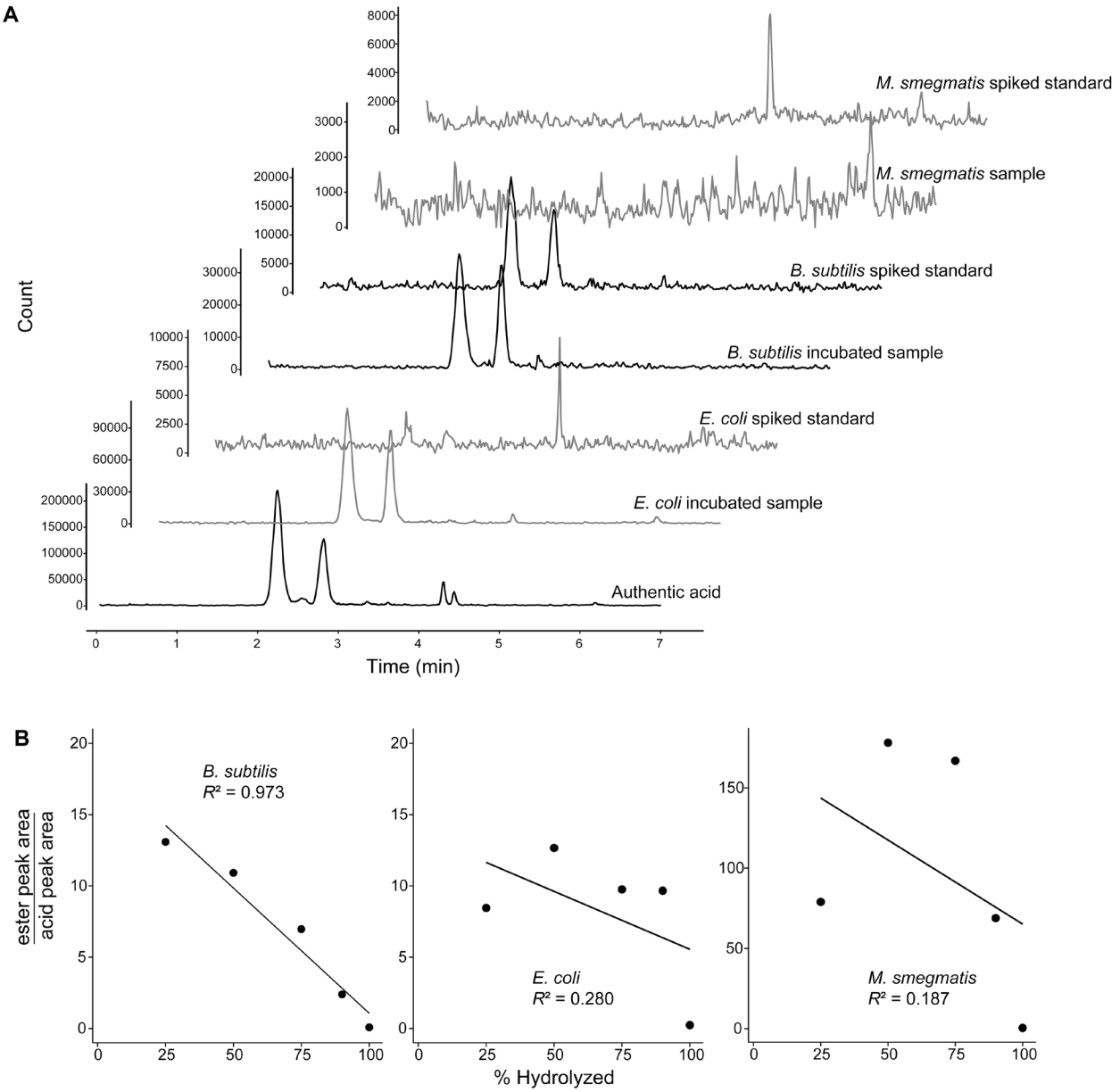


Figure S8. LC–MS spectra and calibration curves of *trans*-3-(4-chlorobenzoyl)acrylic acid demonstrating its unsuitability for semiquantitative determination of ester hydrolysis. (A) The traces labeled "spiked standard" were prepared at 200 µM in the indicated bacterial lysate and immediately extracted with dichloromethane and prepared for injection as described above. The traces labeled "incubated sample" were samples that were incubated with 200 µM of the sulfurol ester, held at 37° C for 2 h, and finally extracted with dichloromethane and injected as described above. (B) Calibration curves showing the correlation or lack of correlation of the sulfurol ester:acid peak area ratio as a function of the % hydrolyzed. Whereas the *B. subtilis* standard and sample injections show good correspondence between the major peaks in both the sample and standard chromatograms as well as a strong correlation, *E. coli* and *M. smegmatis* show little correspondence between the major peaks in the sample and standard and weak correlation between the area ratio and % hydrolyzed.

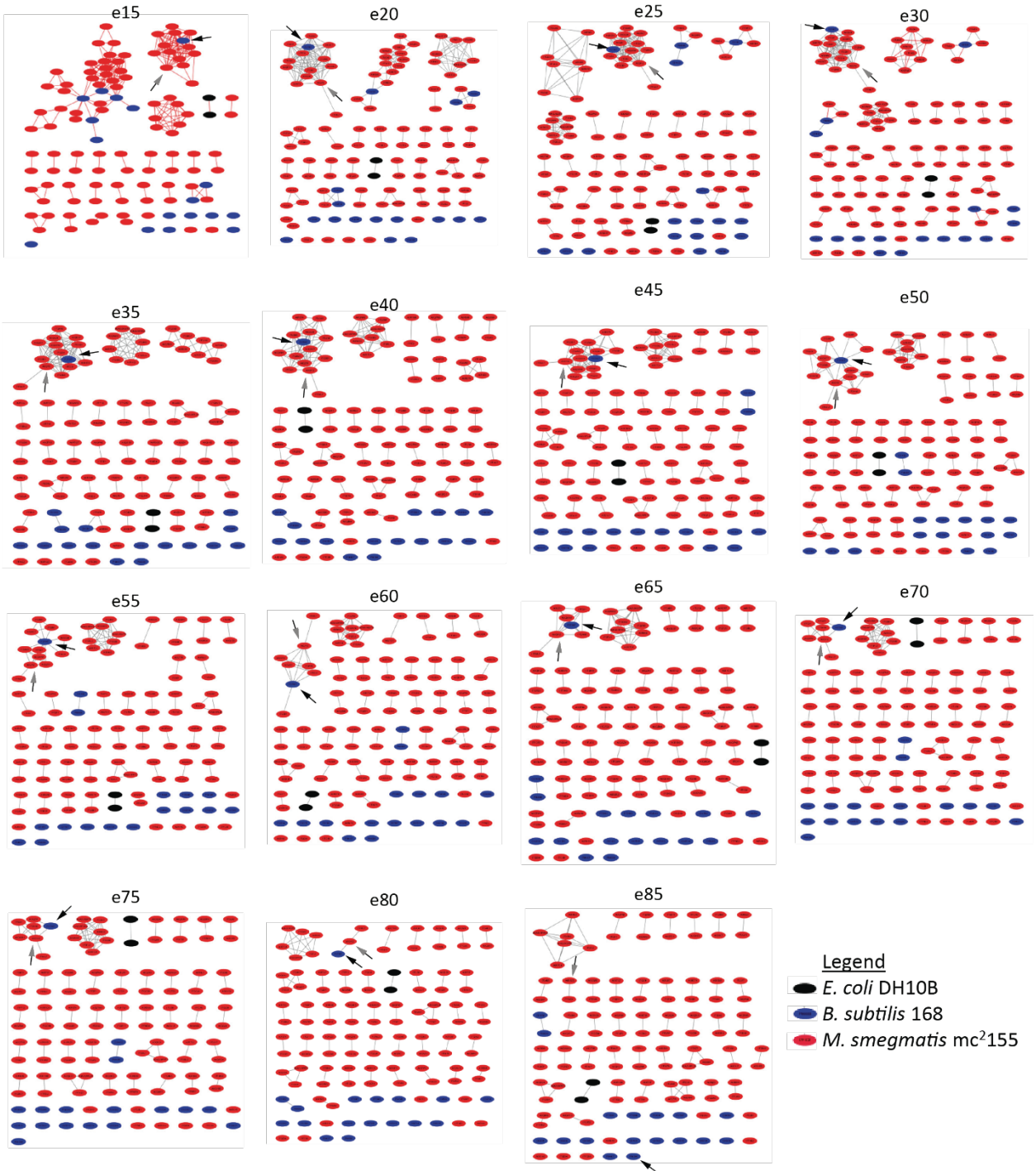


Figure S9. Determination of the E-value threshold for SSN. Black arrows indicate esterase pnbA from *B. subtilis*; gray arrows indicate esterase A0QIY2 from *M. smegmatis*. The cluster containing both of these esterases persists from E-values ranging from 15–80. A final threshold value of 37 was chosen because this value was just above the value at which multiple clusters containing esterases from both *M. smegmatis* and *B. subtilis* were found.

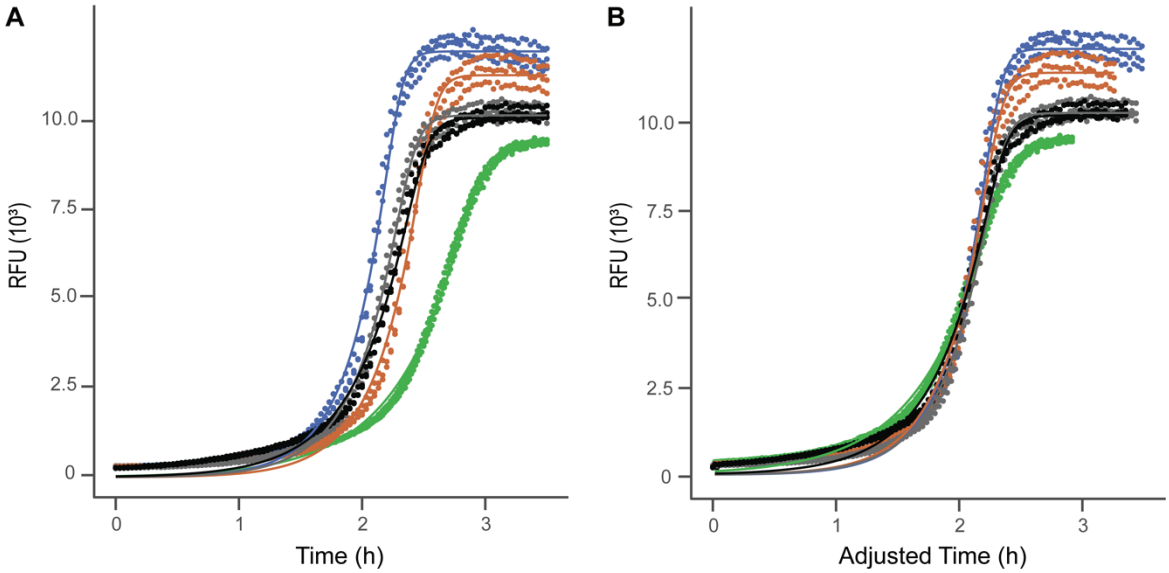


Figure S10. Viability curves of *E. coli* DH10B cells with empty pet-22b(+) and pSC6 (blue and gray) *E. coli* DH10B with pET22b-pnbA and pSC6 (sienna), *E coli* DH10B cells with pET22b-MSmegEsterase and pSC6 (black), and *E. coli* DH10B (green). (A) Viability curves versus unadjusted time. Fits to Richards curve are also illustrated. (B) Viability curves and fits plotted after adjusting the time to have an identical inflection point.

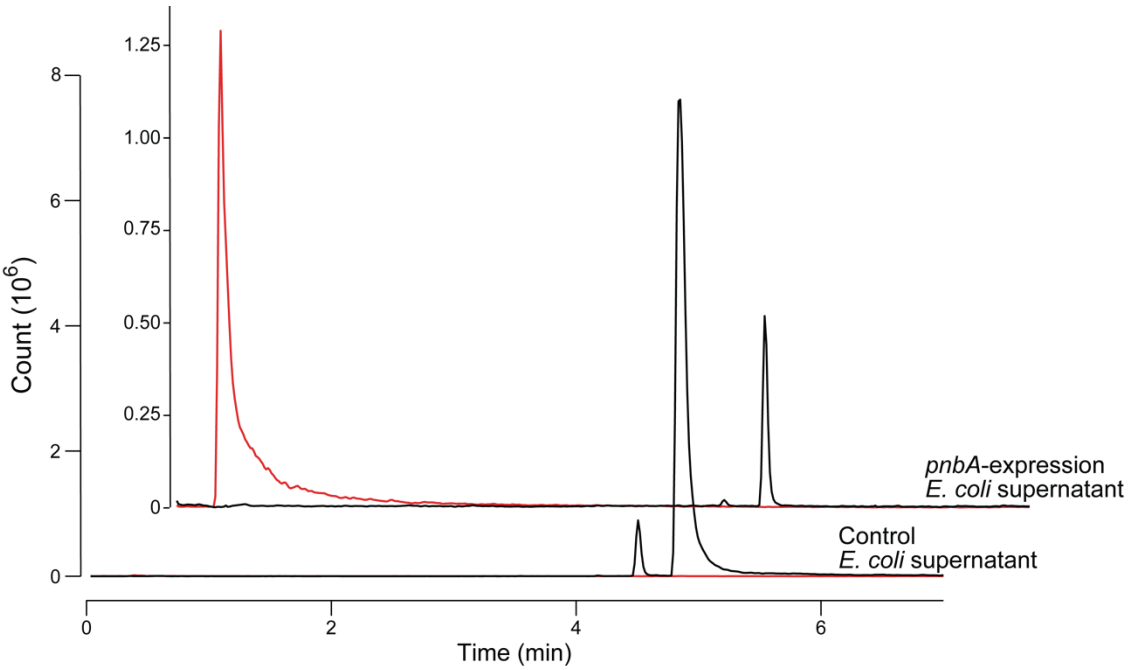
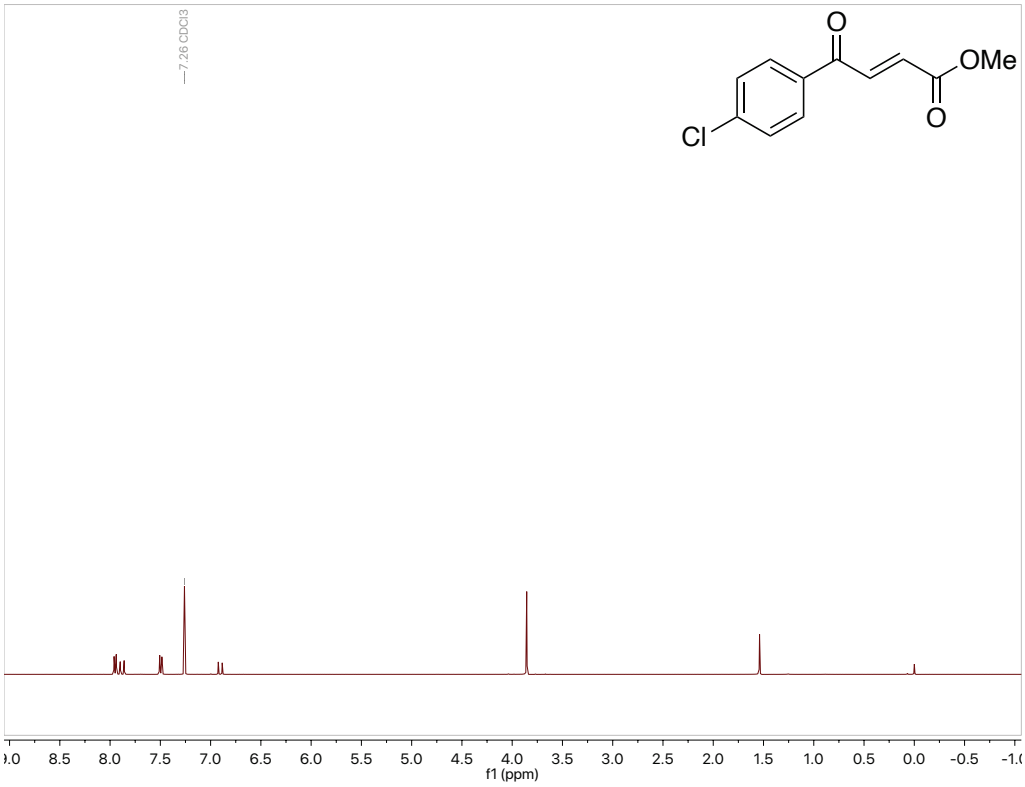
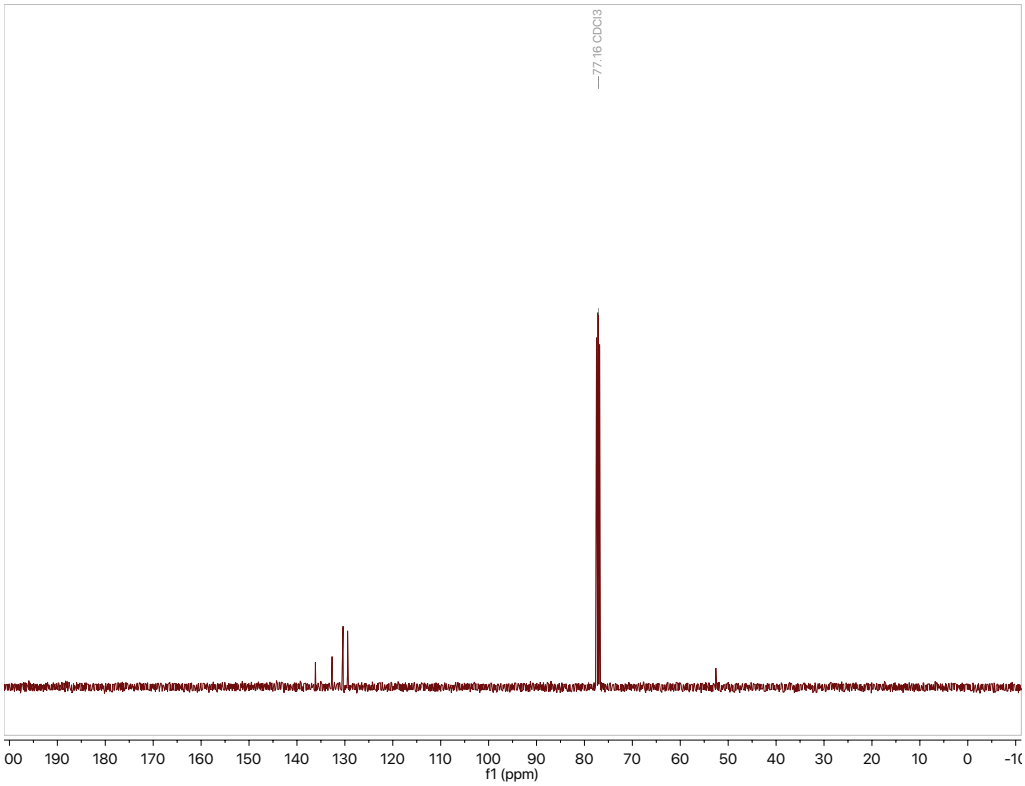


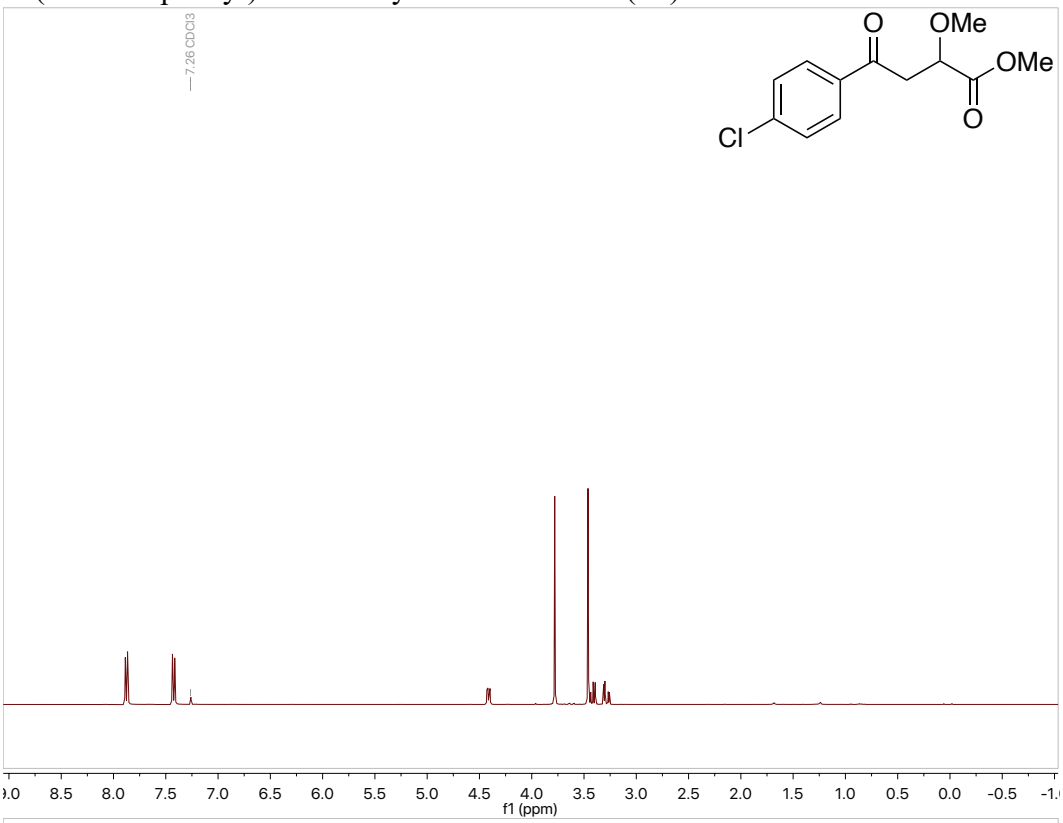
Figure S11. Extracted ion chromatograms of sulfurol ester **3** mixed with various spent culture supernatants. EICs of m/z = 144 (corresponding to sulfurol) are in red; EICs of m/z = 336 (corresponding to sulfurol ester **3**) are in black. Sulfurol is observed only in the supernatant of *pnbA*-expressing *E. coli*, suggesting that pnbA carboxylesterase is present in the cell culture supernatant.

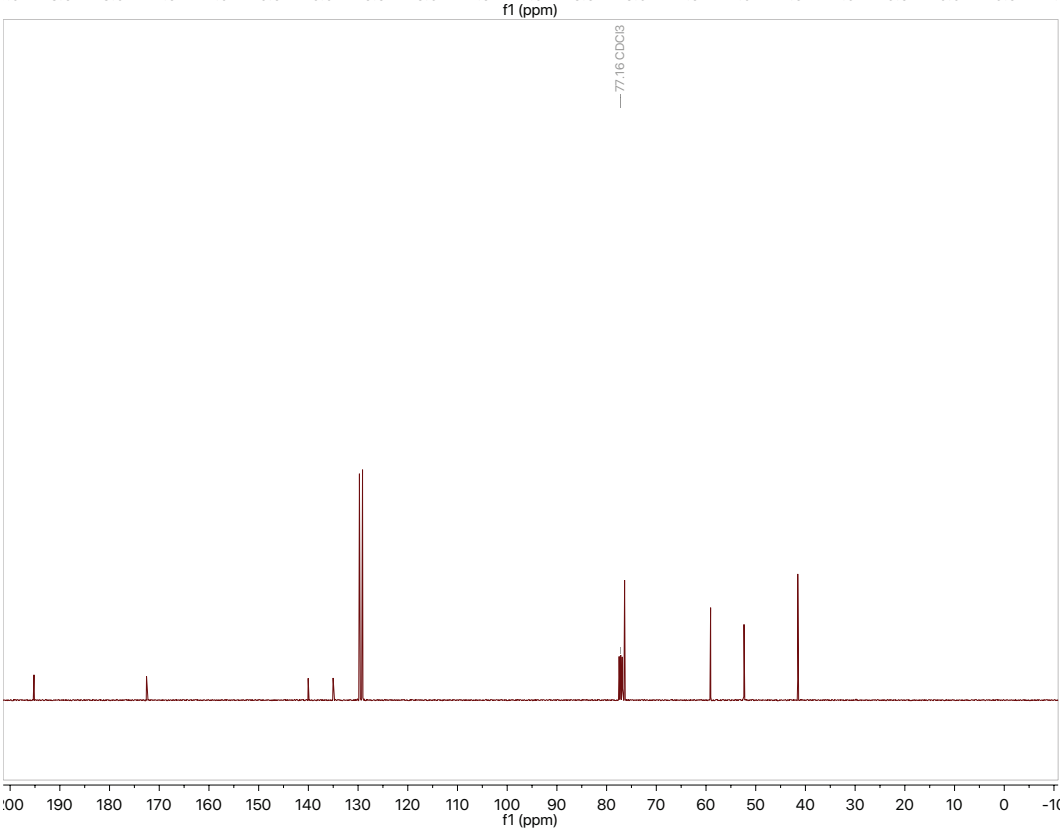
Methyl (*E*)-4-(4-Chlorophenyl)-4-oxobut-2-enoate (**2**)



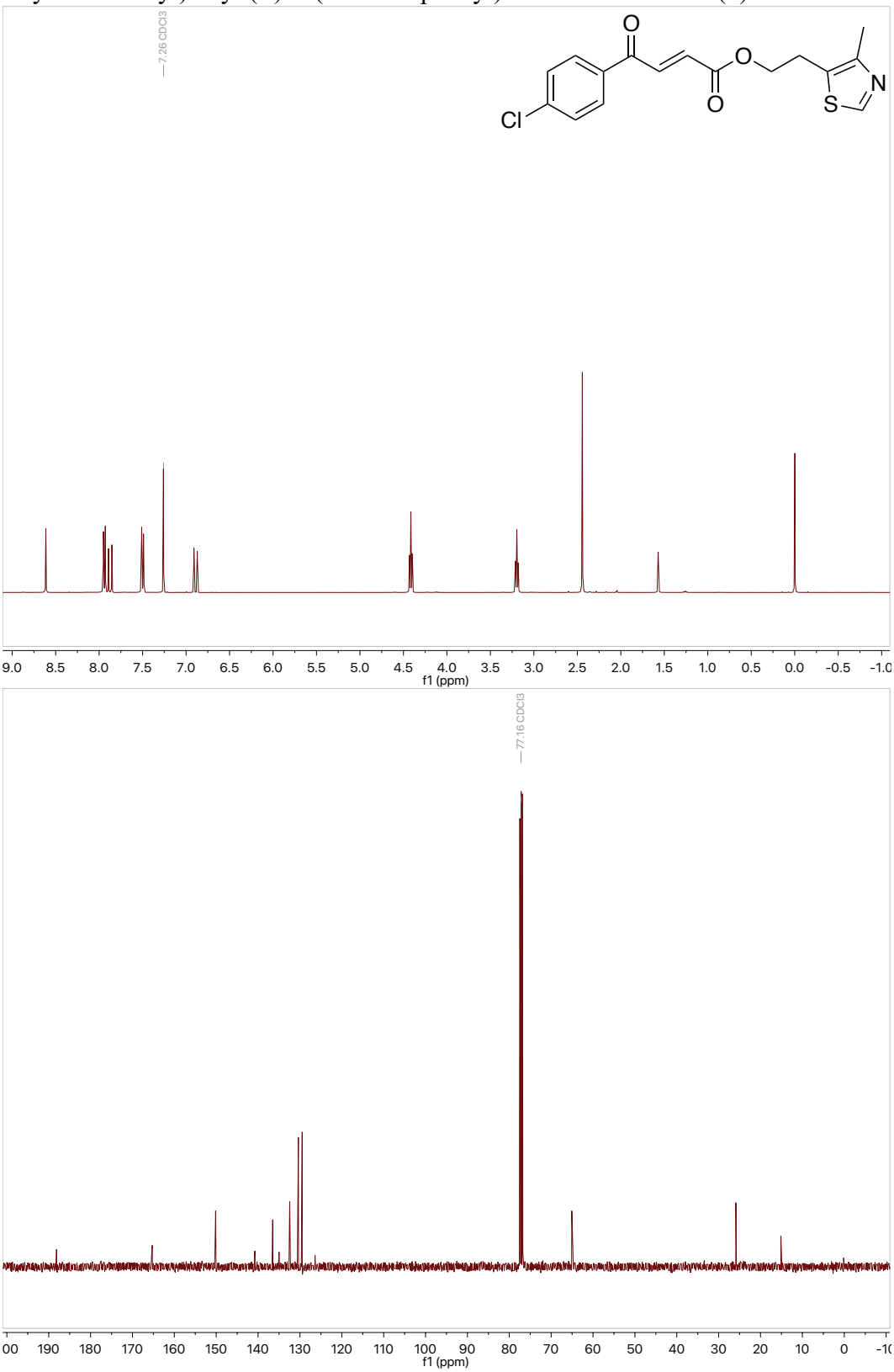


Methyl 4-(4-Chlorophenyl)-2-methoxy-4-oxobutanoate (**S1**)

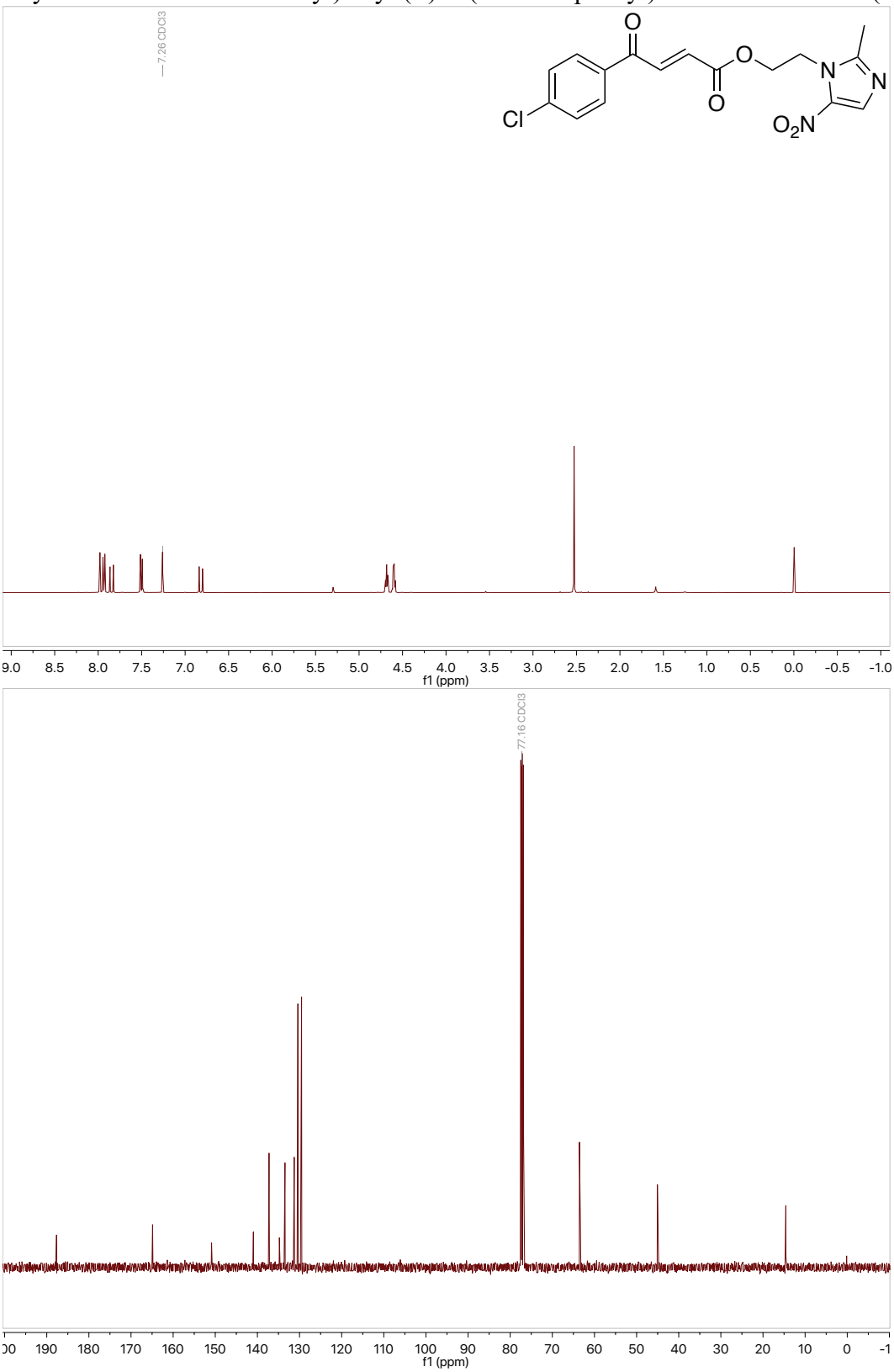




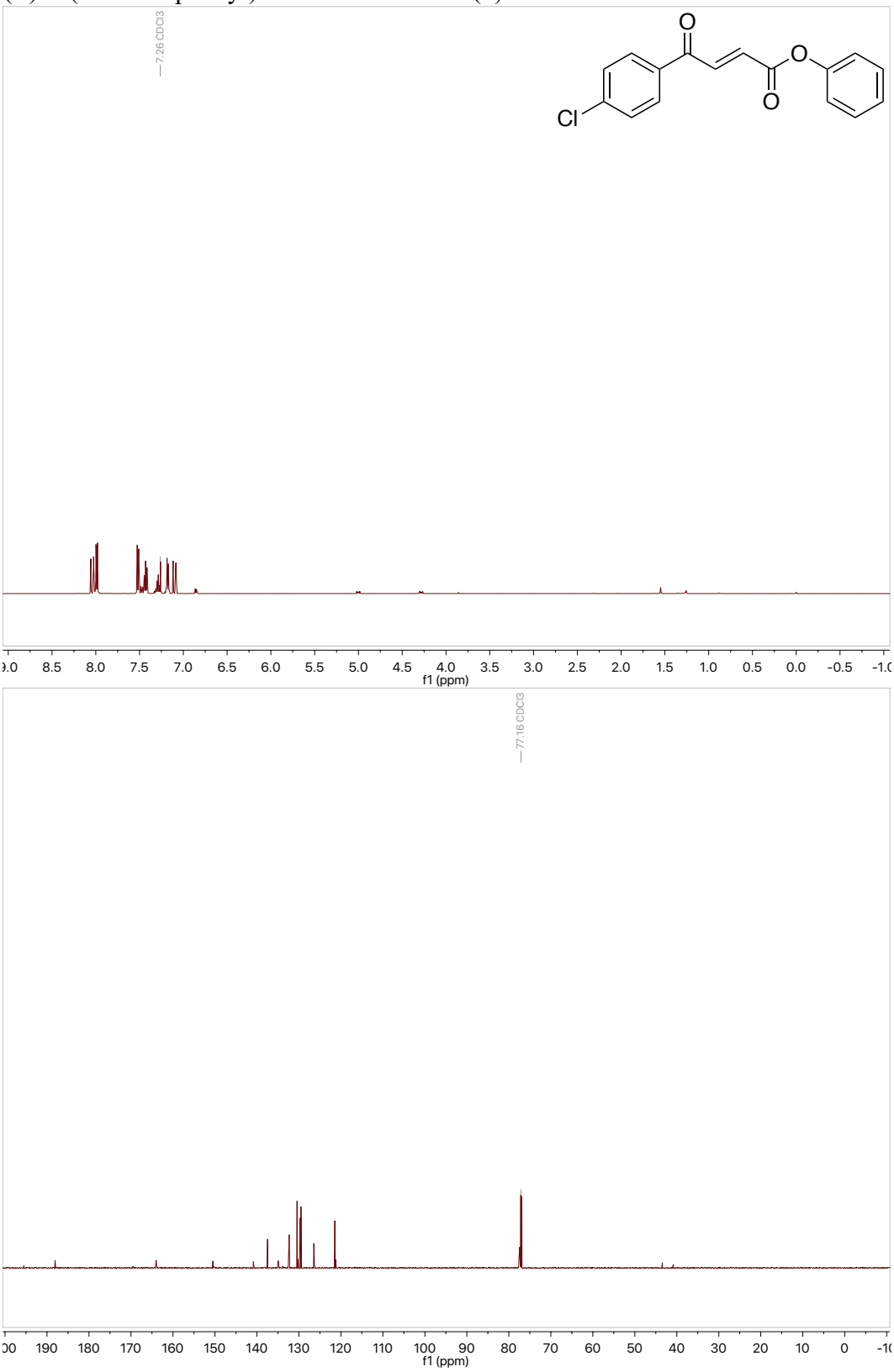
2-(4-Methylthiazol-5-yl)ethyl (*E*)-4-(4-Chlorophenyl)-4-oxobut-2-enoate (**3**)



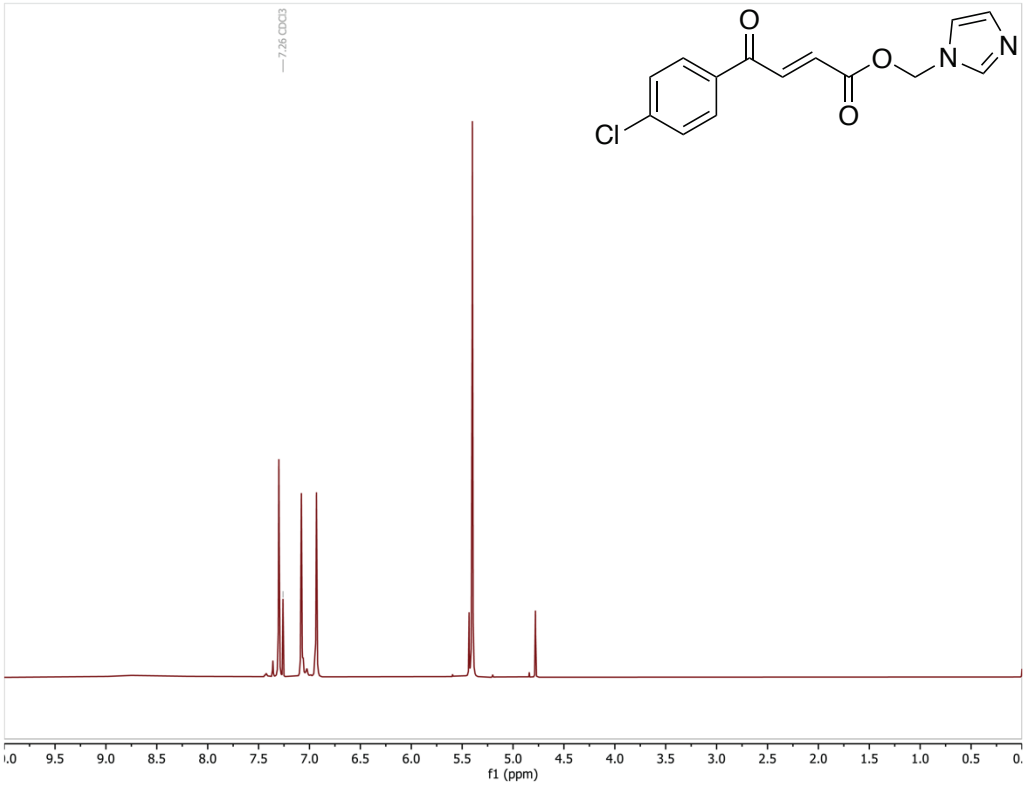
2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl (*E*)-4-(4-Chlorophenyl)-4-oxobut-2-enoate (**4**)

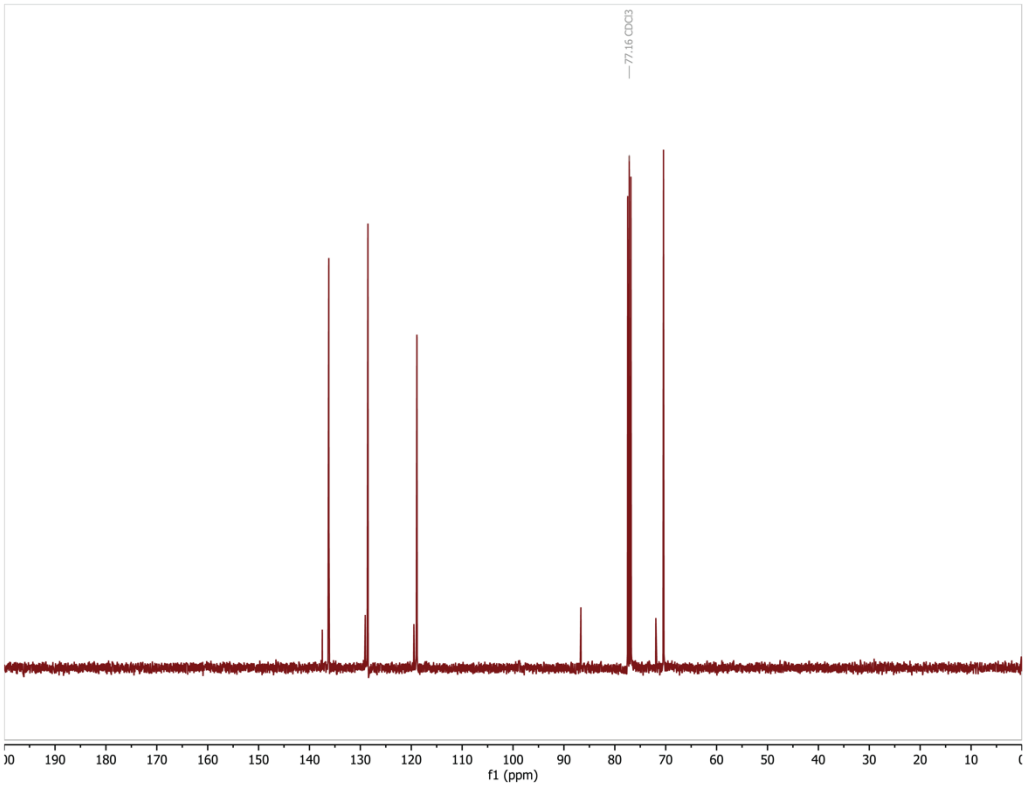


Phenyl (*E*)-4-(4-Chlorophenyl)-4-oxobut-2-enoate (**5**)

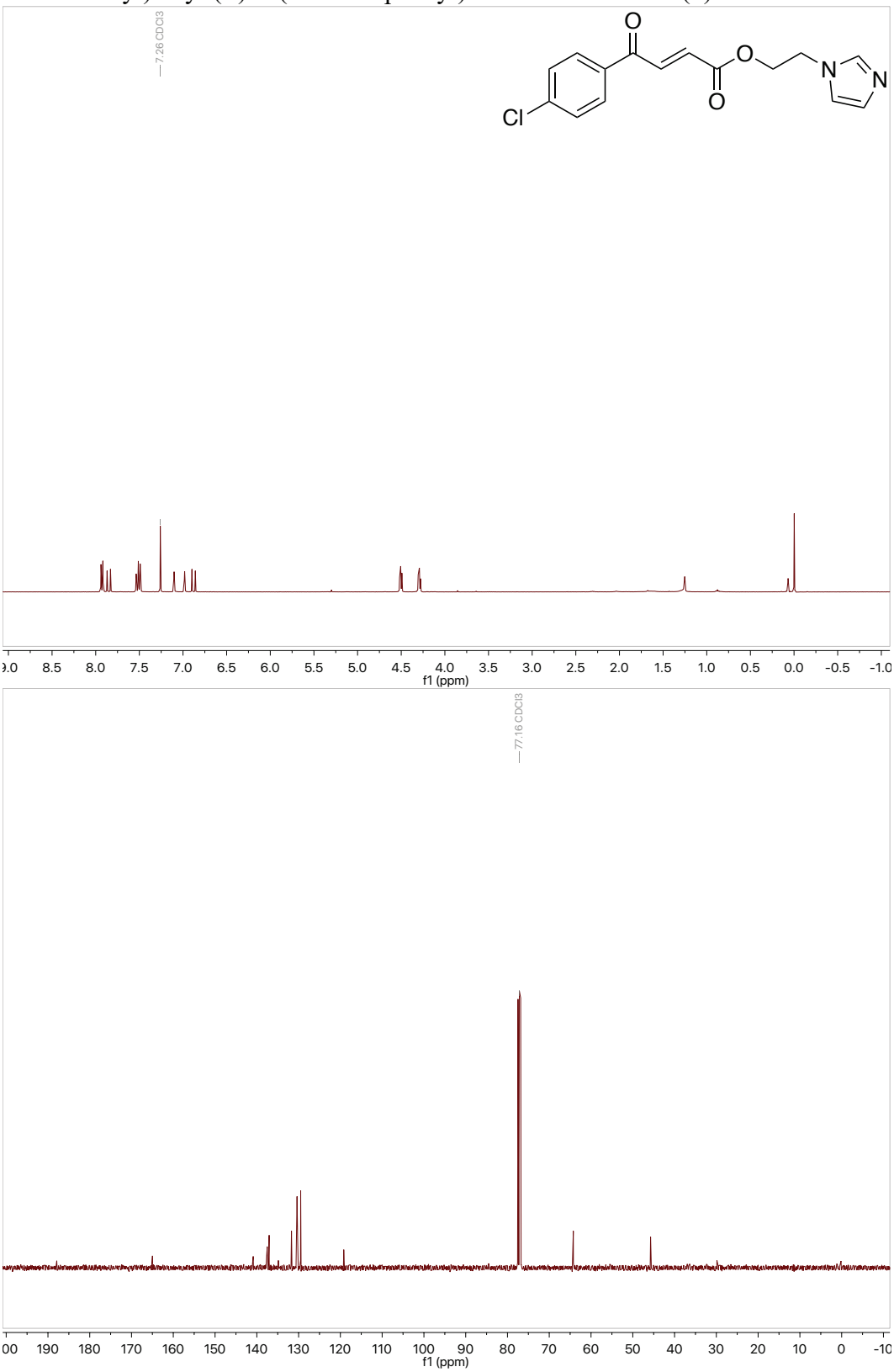


(1*H*-Imidazol-1-yl)methyl (*E*)-4-(4-Chlorophenyl)-4-oxobut-2-enoate (**6**)

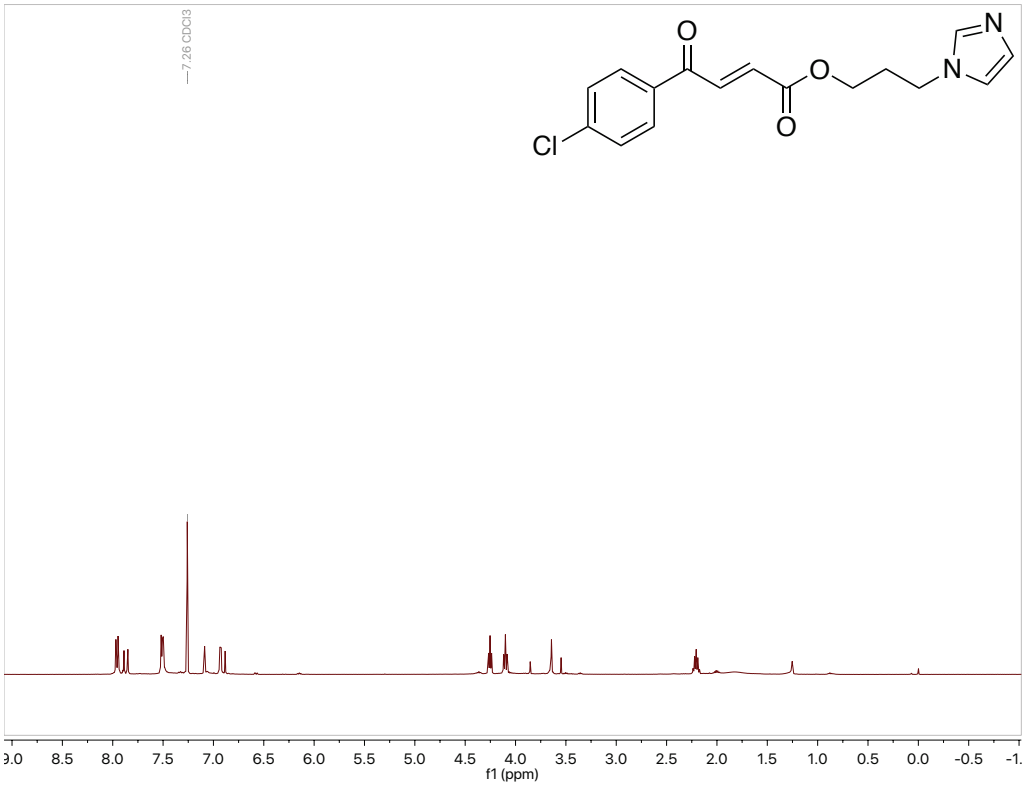


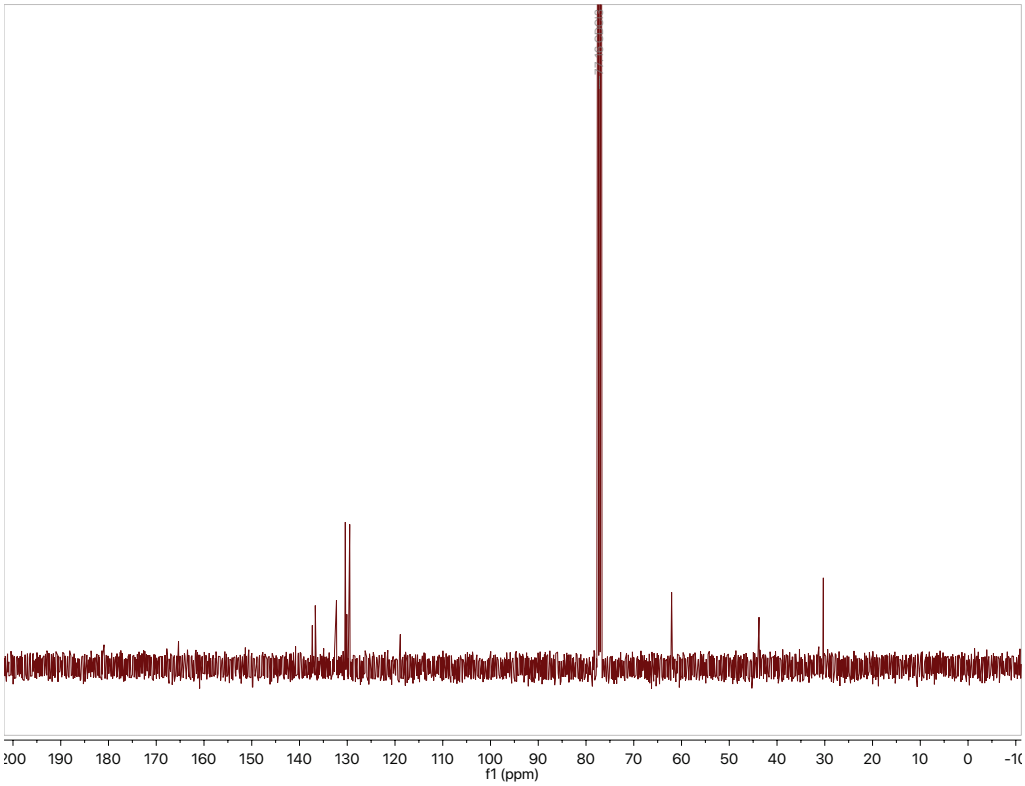


2-(1*H*-Imidazol-1-yl)ethyl (*E*)-4-(4-Chlorophenyl)-4-oxobut-2-enoate (**7**)

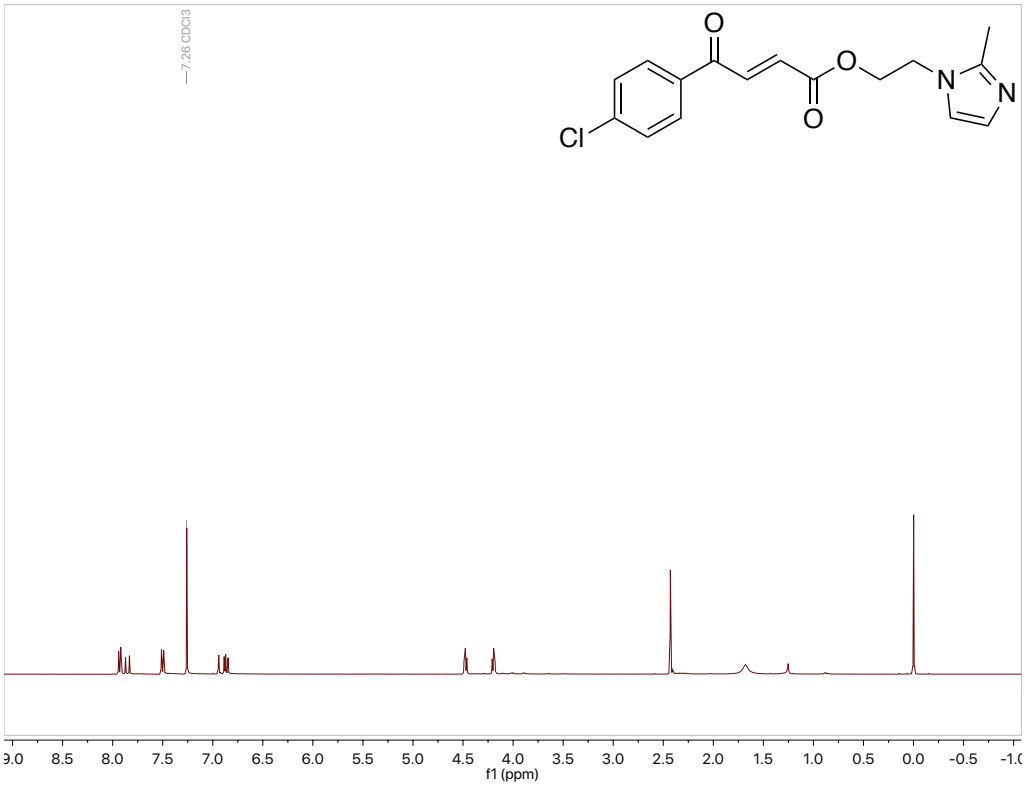


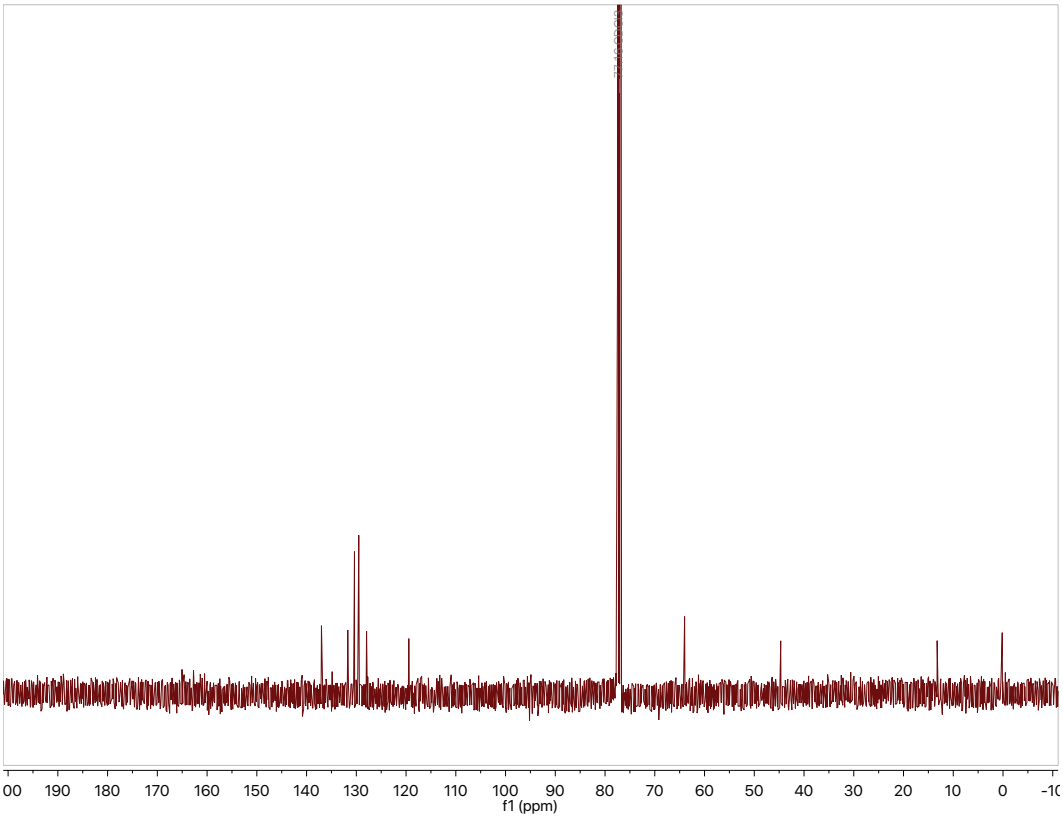
3-(1*H*-Imidazol-1-yl)propyl (*E*)-4-(4-Chlorophenyl)-4-oxobut-2-enoate (**8**)



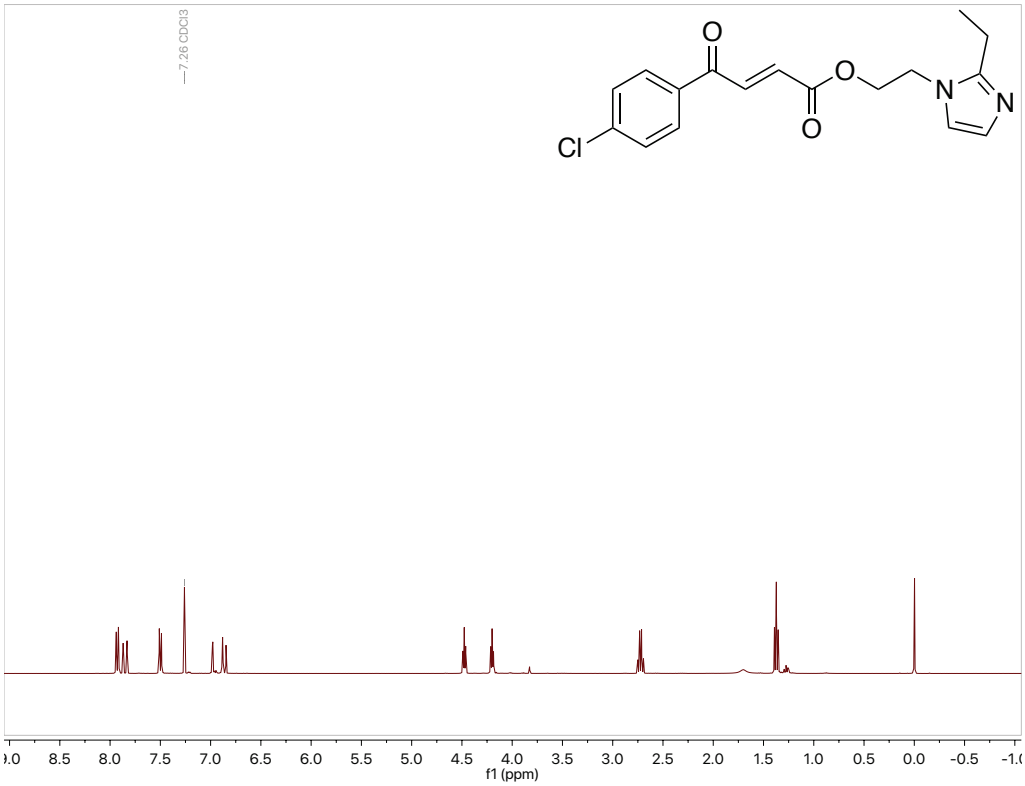


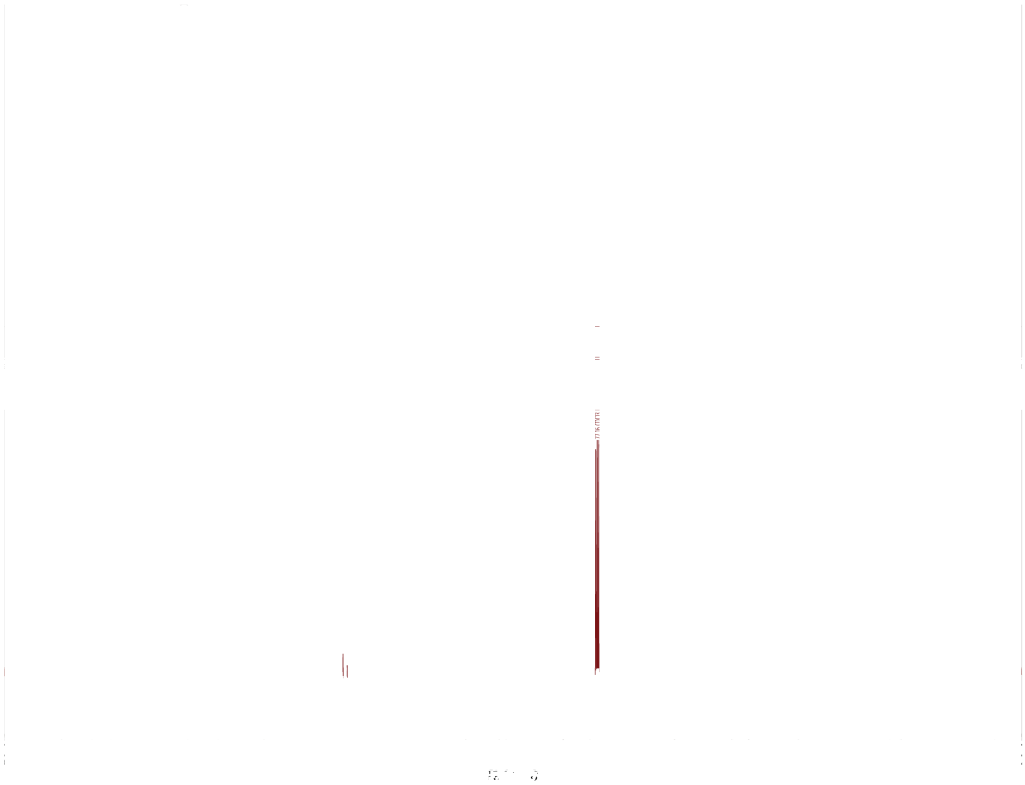
2-(2-Methyl-1*H*-imidazol-1-yl)ethyl (*E*)-4-(4-Chlorophenyl)-4-oxobut-2-enoate (**9**)



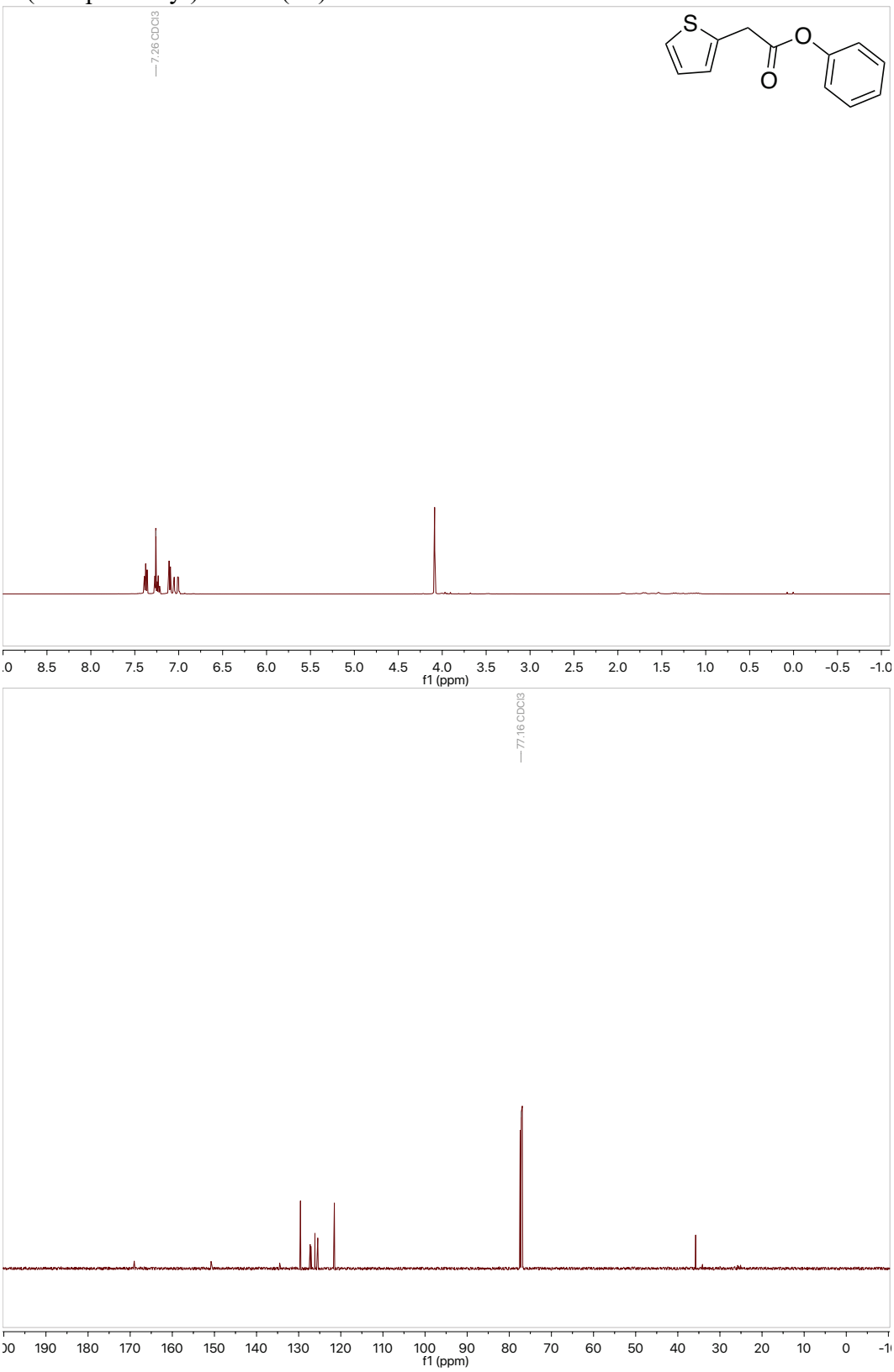


2-(2-Ethyl-1*H*-imidazol-1-yl)ethyl (*E*)-4-(4-Chlorophenyl)-4-oxobut-2-enoate (**10**)

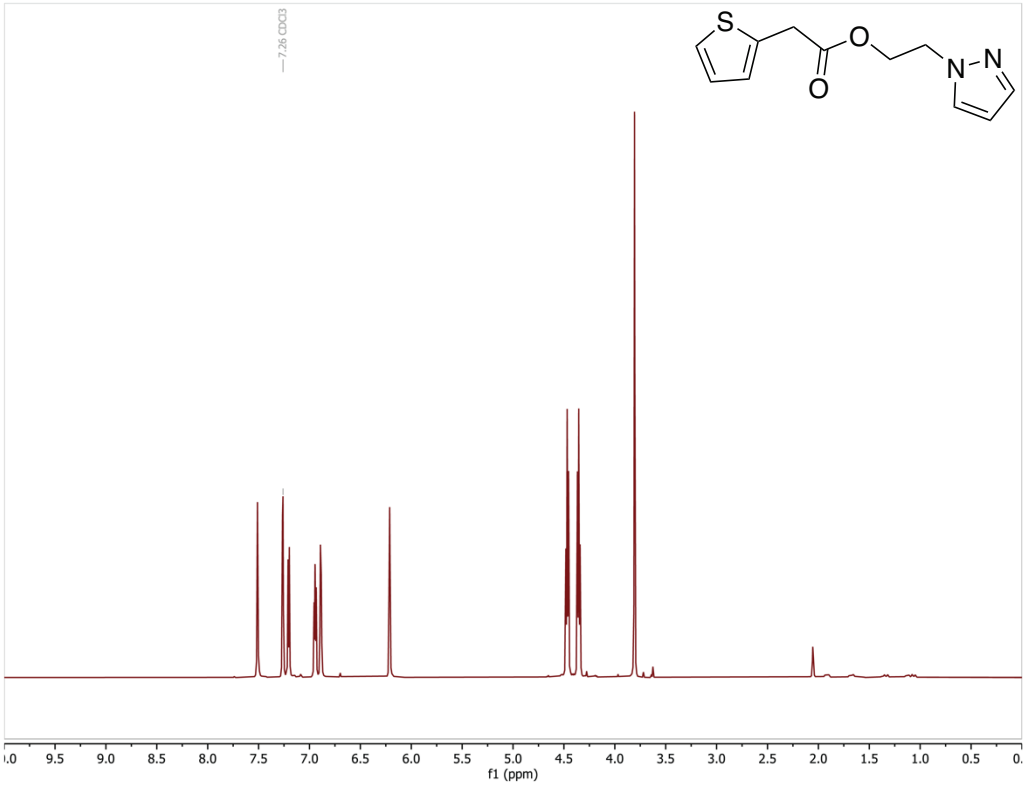


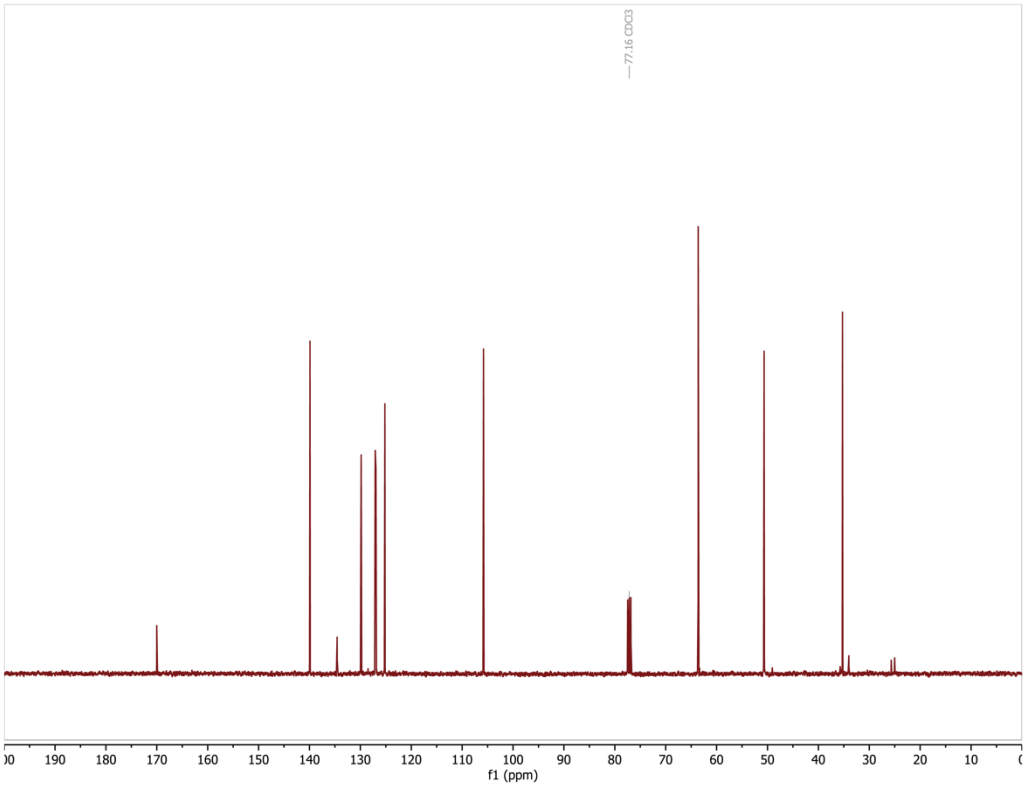


Phenyl 2-(Thiophen-2-yl)acetate (**S2**)

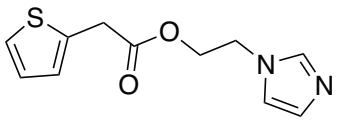


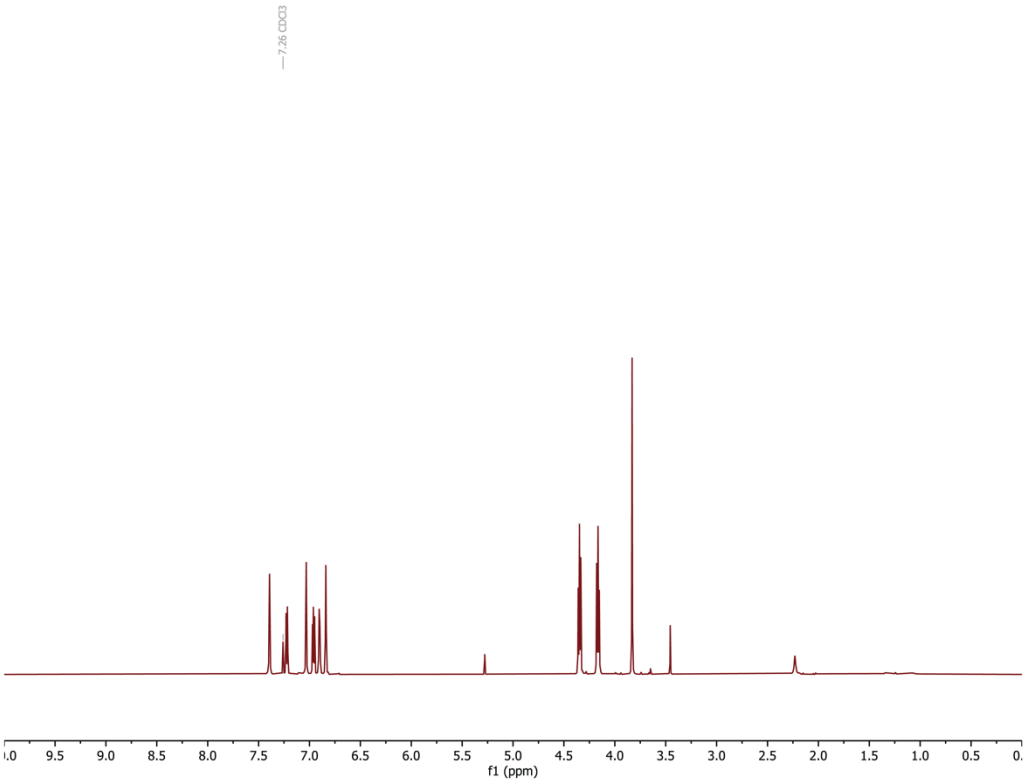
2-(1*H*-Pyrazol-1-yl)ethyl 2-(Thiophen-2-yl)acetate (**S3**)



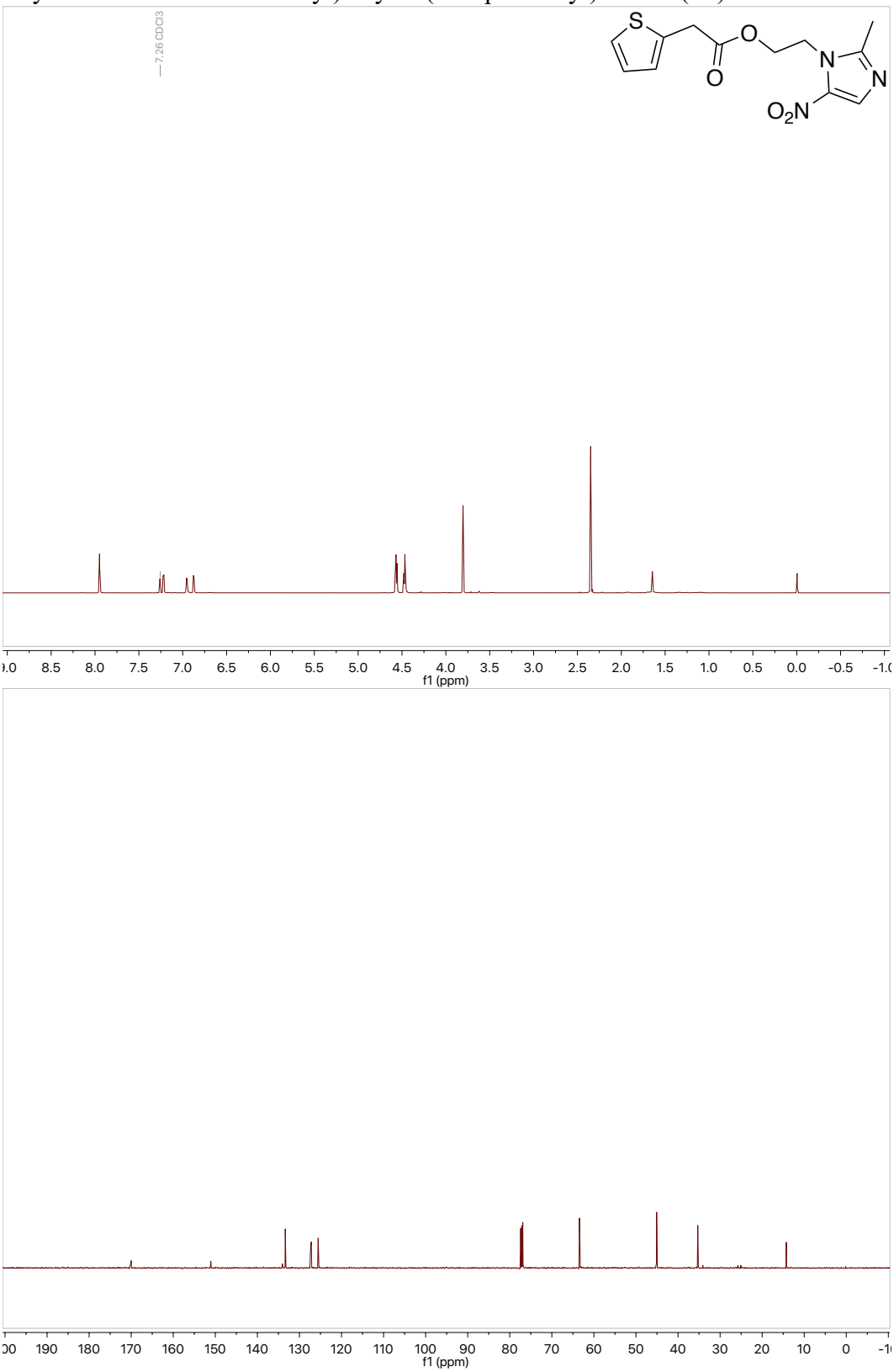


2-(1*H*-Imidazol-1-yl)ethyl 2-(Thiophen-2-yl)acetate (**S4**)

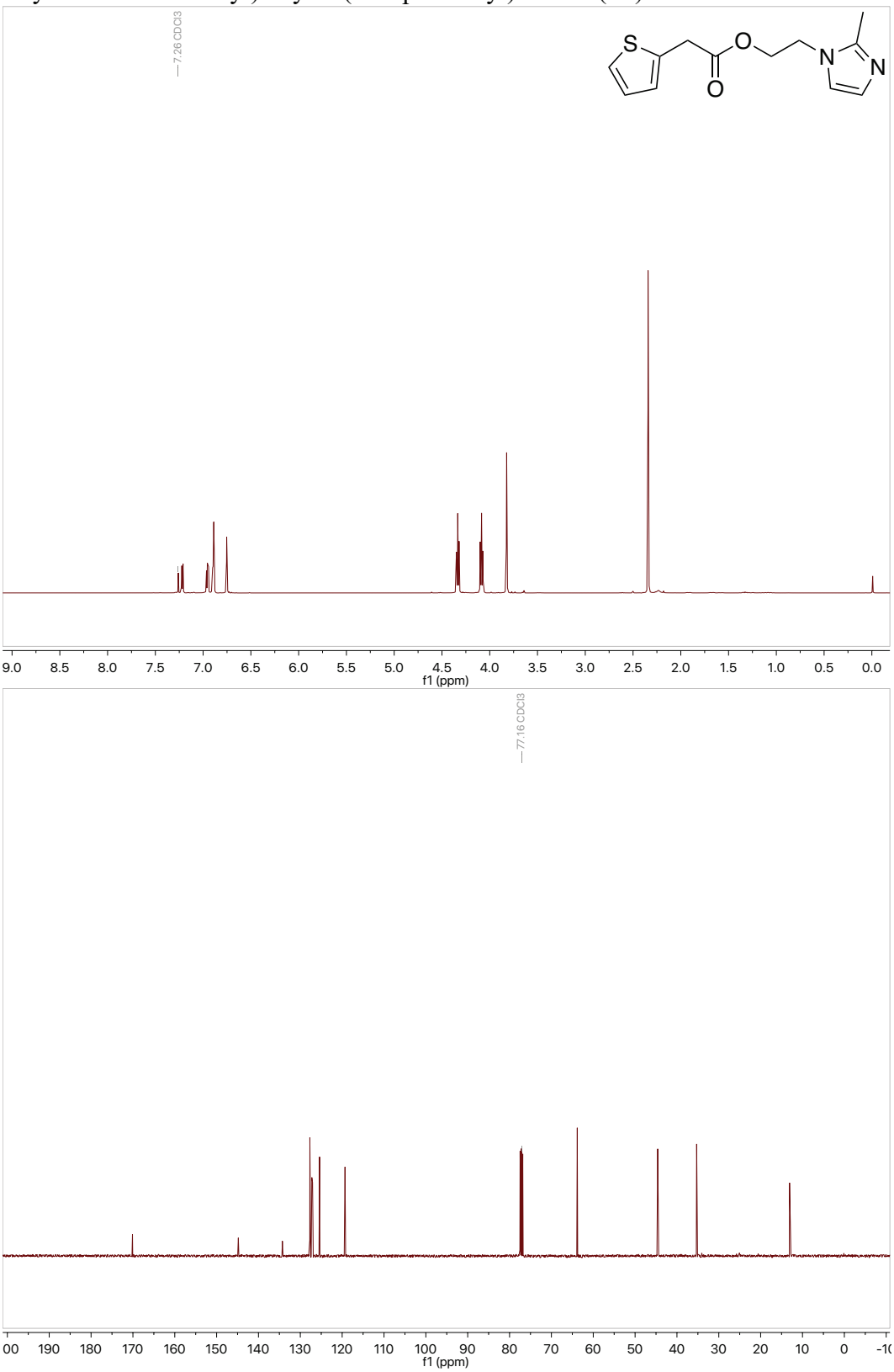




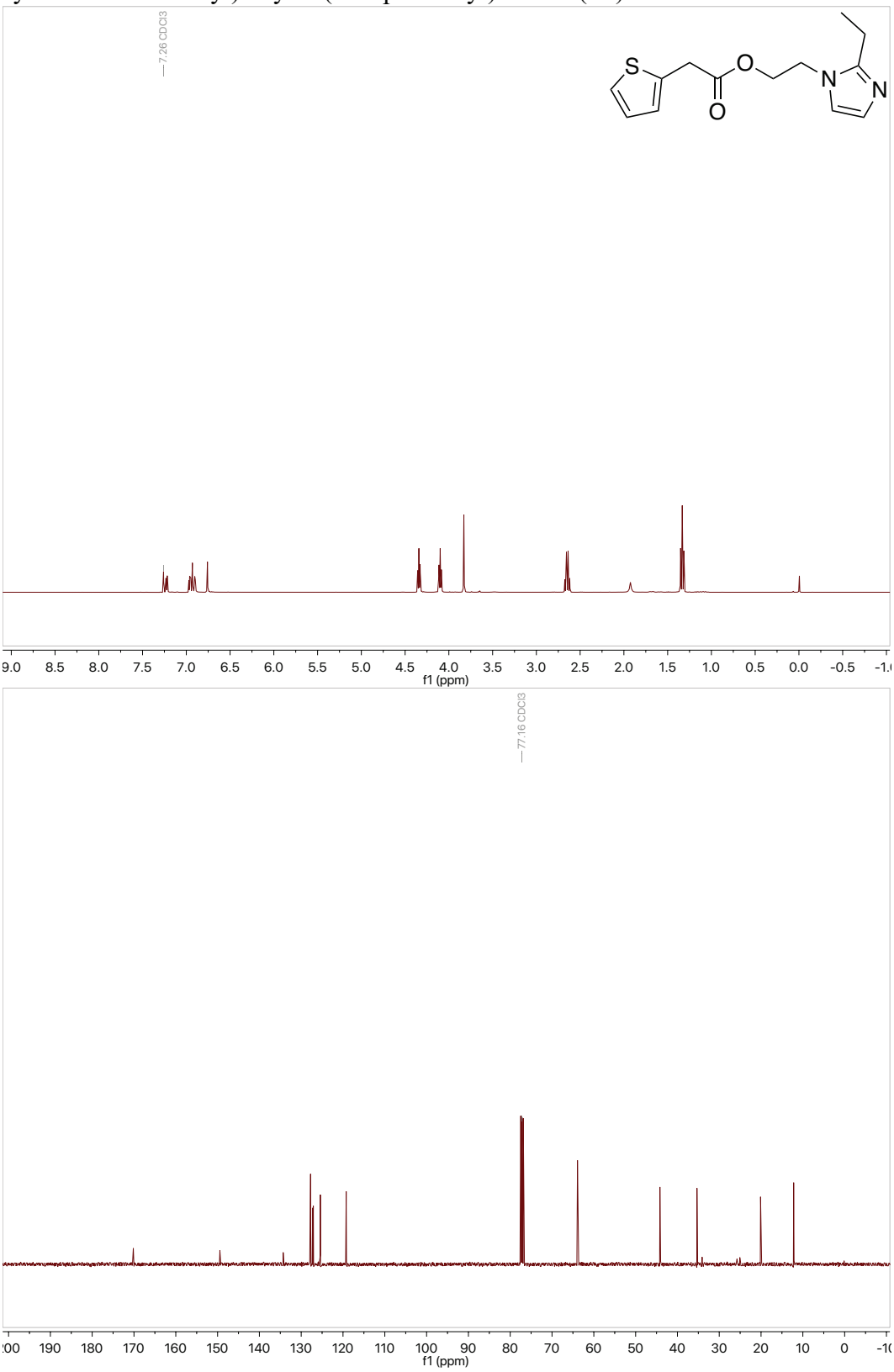
2-(2-Methyl-5-nitro-1*H*-imidazol-1-yl)ethyl 2-(Thiophen-2-yl)acetate (**S5**)



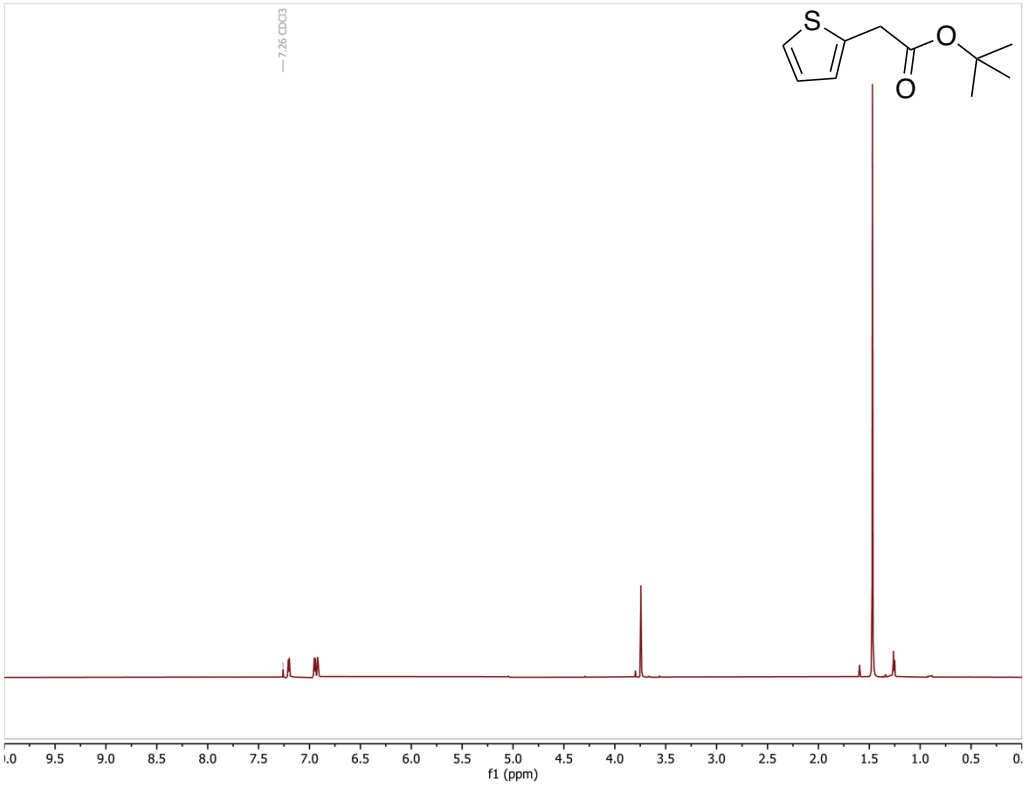
2-(2-Methyl-1*H*-imidazol-1-yl)ethyl 2-(Thiophen-2-yl)acetate (**S6**)

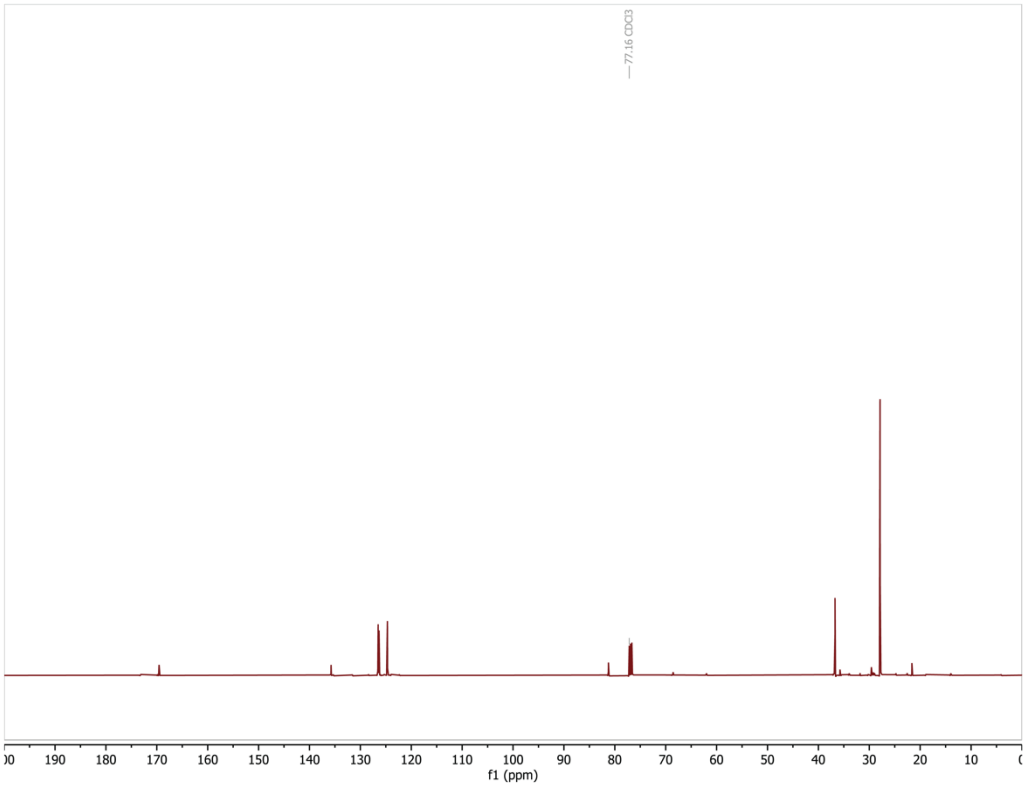


2-(2-Ethyl-1*H*-imidazol-1-yl)ethyl 2-(Thiophen-2-yl)acetate (**S7**)

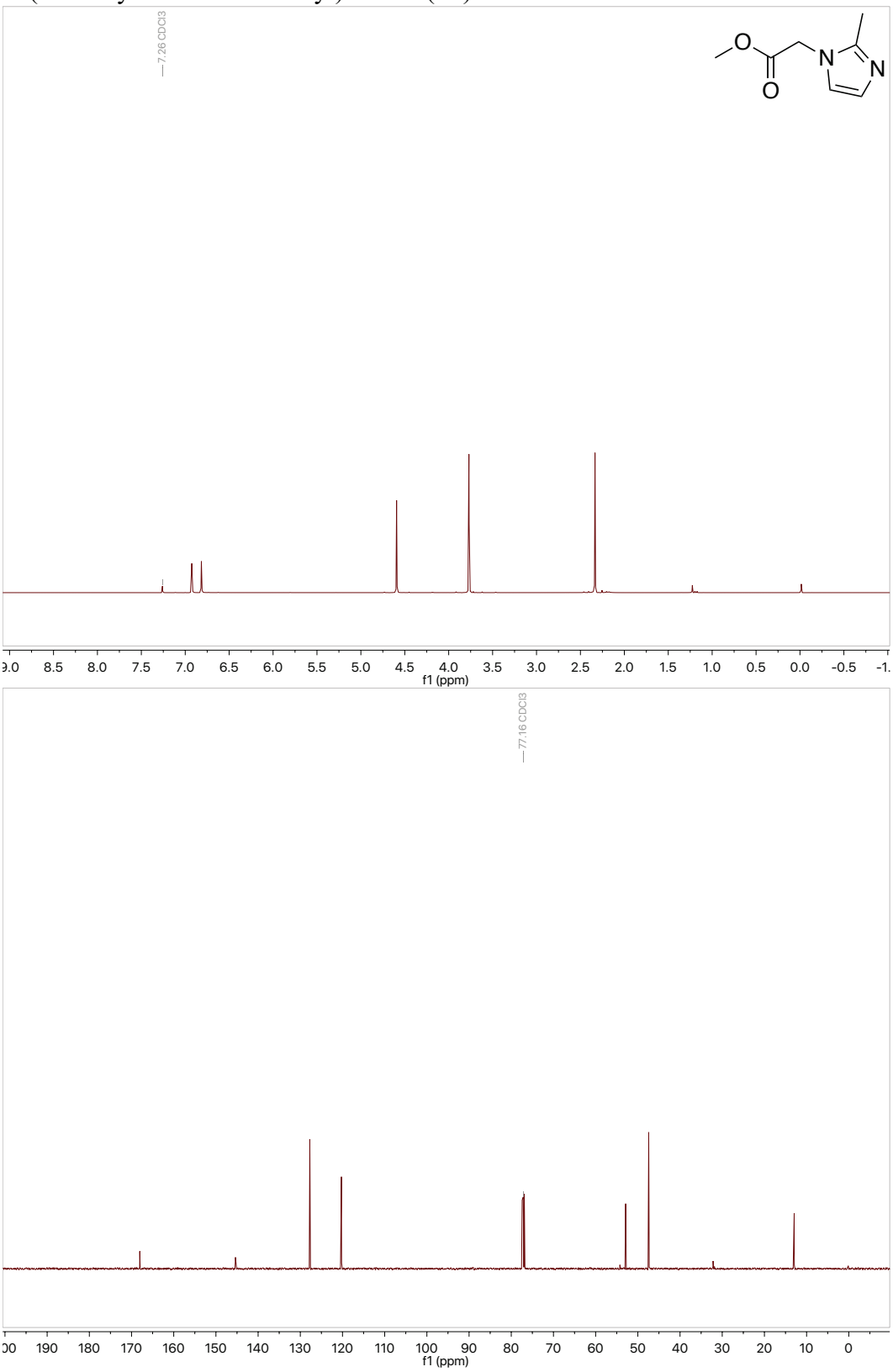


tert-Butyl 2-(Thiophen-2-yl)acetate (**S8**)

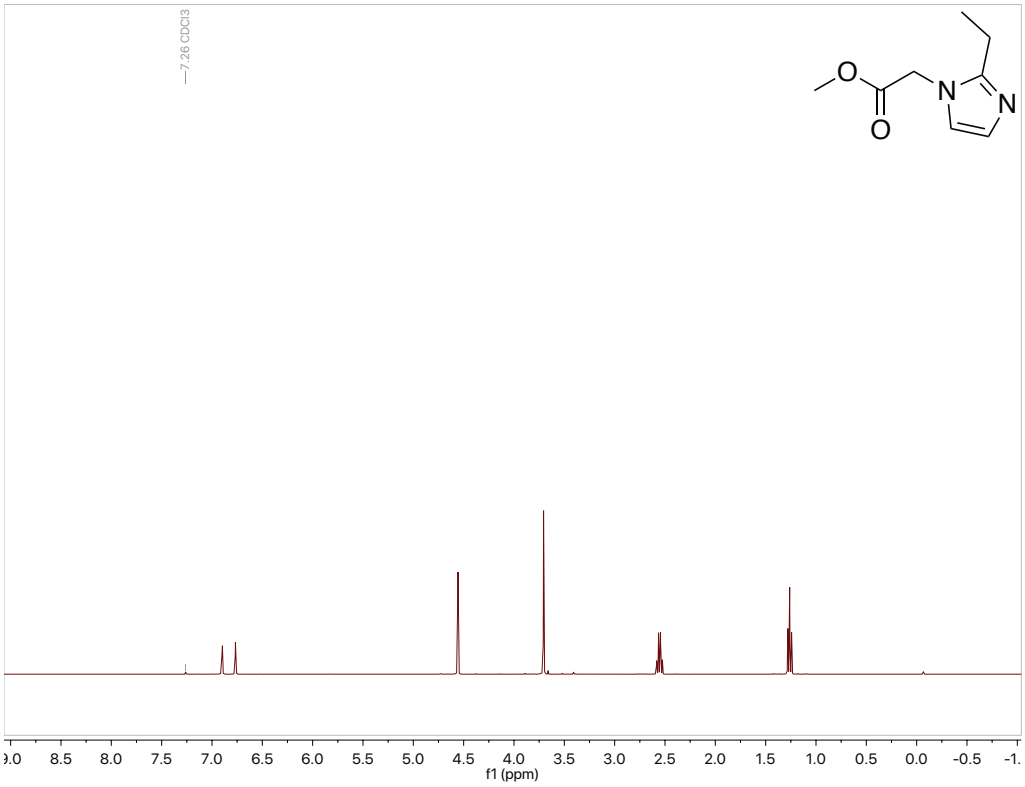


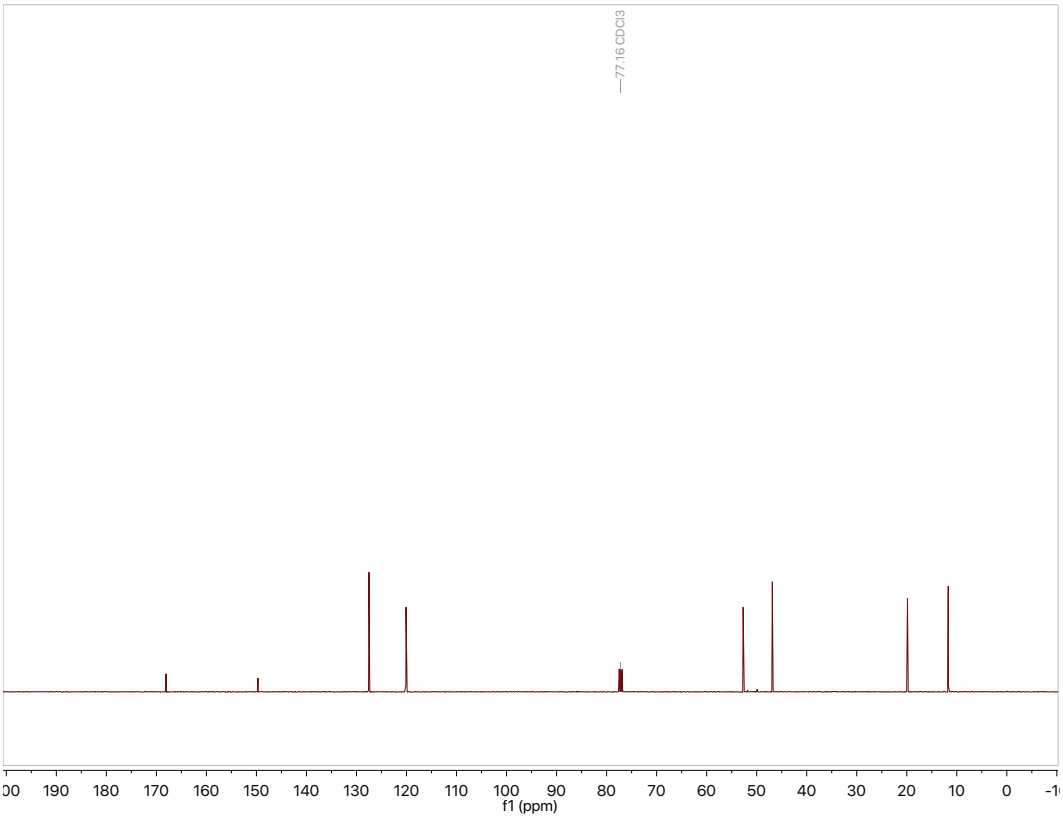


Methyl 2-(2-Methyl-1*H*-imidazol-1-yl)acetate (**S9**)

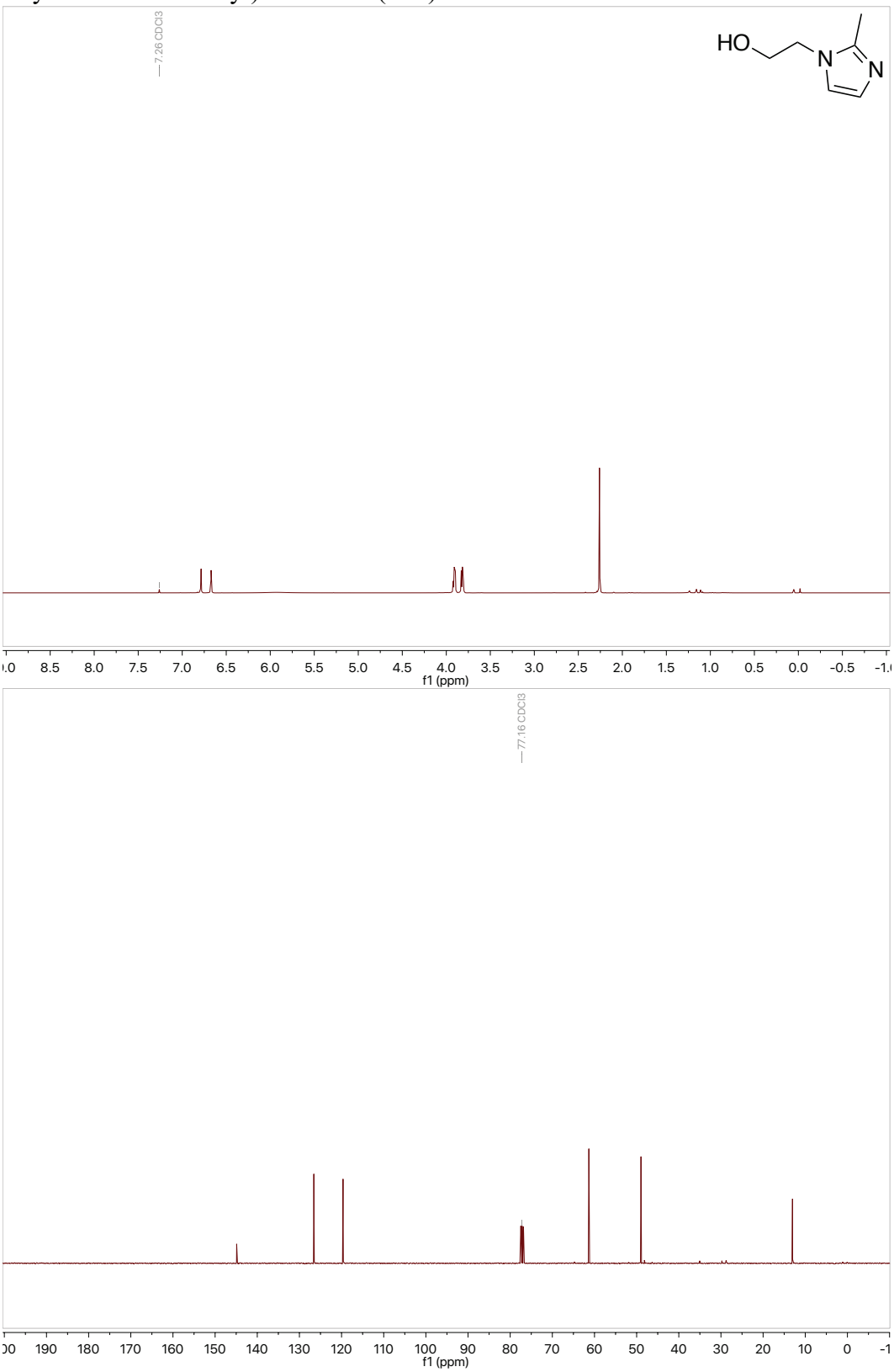


Methyl 2-(2-Ethyl-1*H*-imidazol-1-yl)acetate (**S10**)

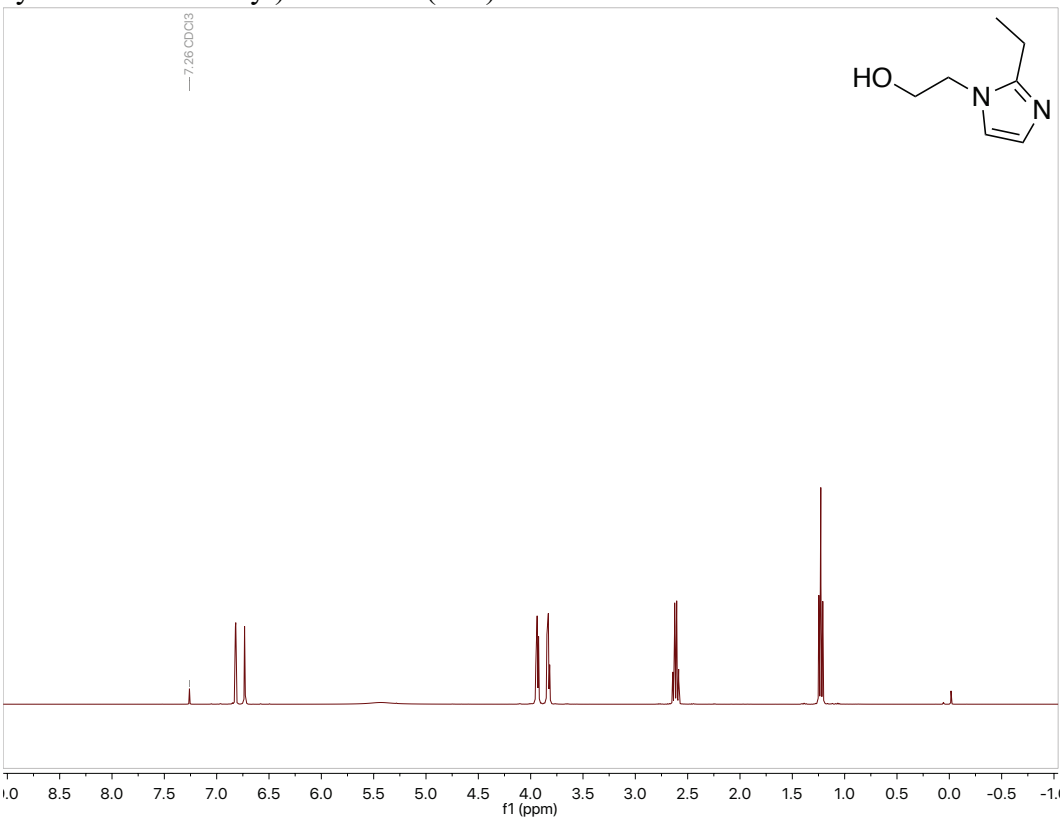


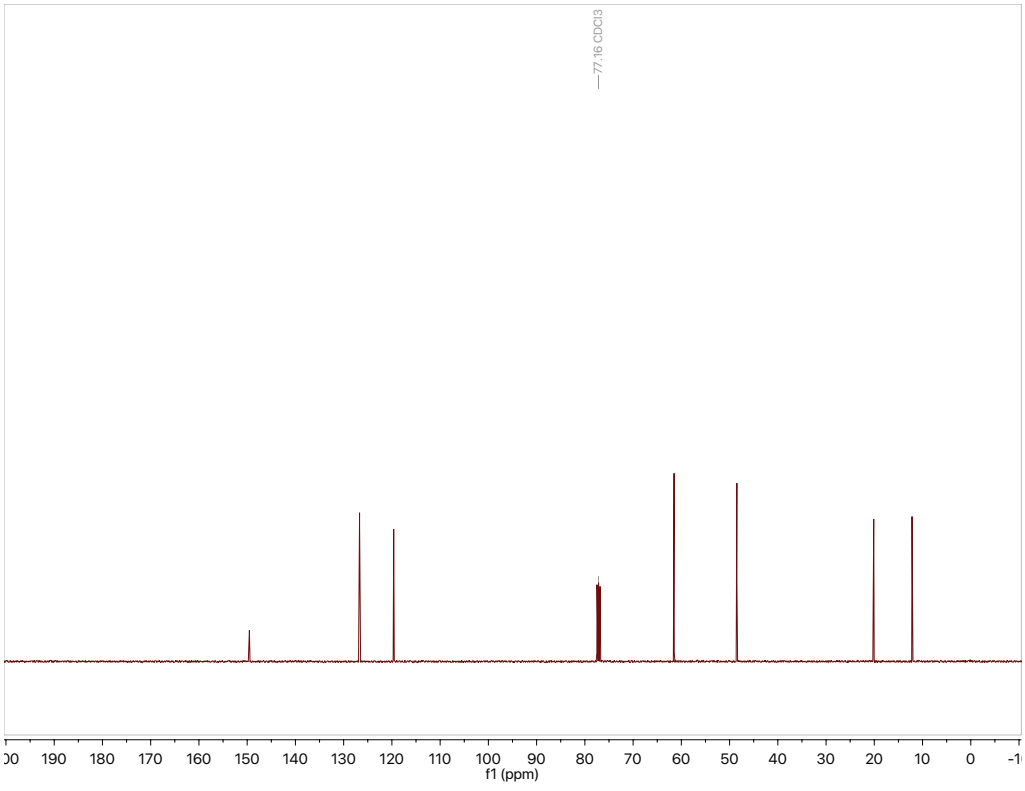


2-(2-Methyl-1*H*-imidazol-1-yl)ethan-1-ol (**S11**)

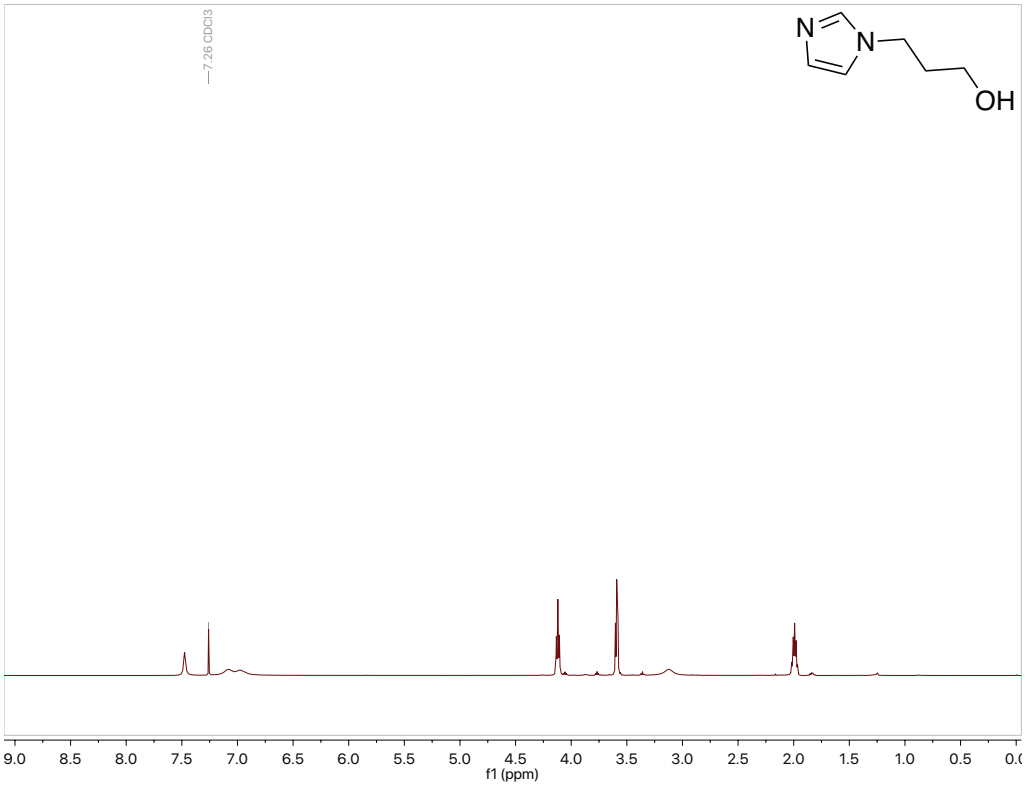


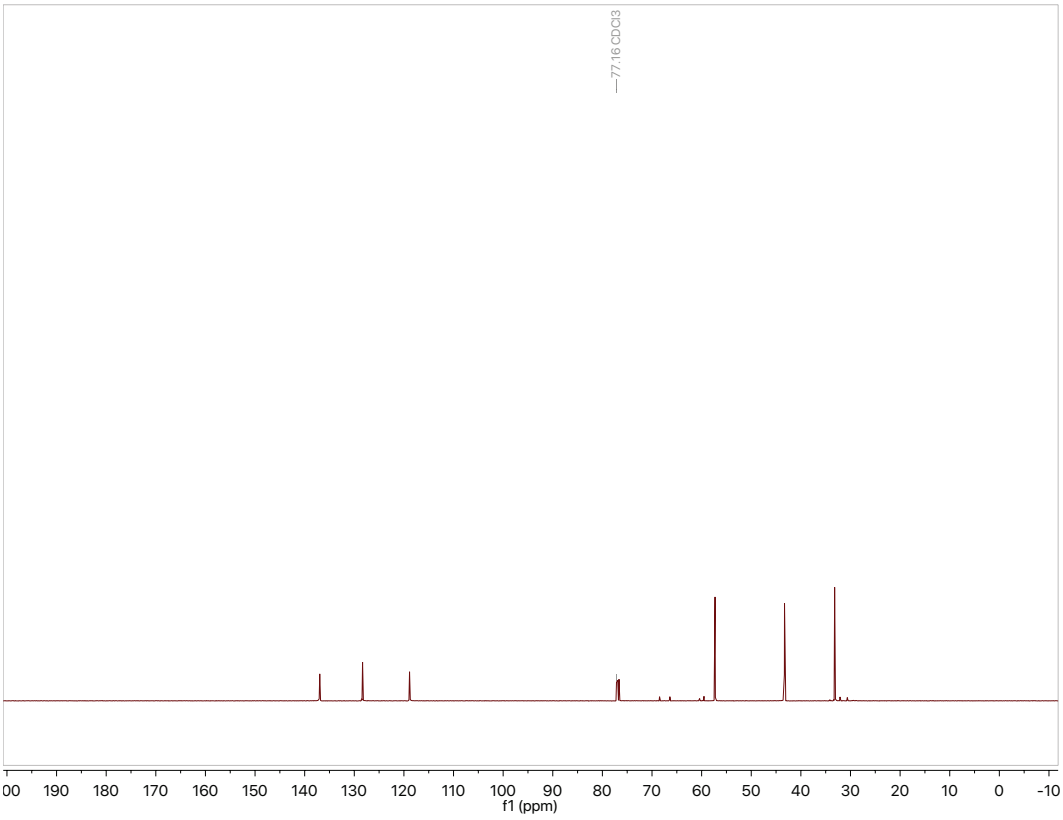
2-(2-Ethyl-1*H*-imidazol-1-yl)ethan-1-ol (**S12**)





3-(1*H*-Imidazol-1-yl)propan-1-ol (**S13**)





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