Organocatalysts of Oxidative Protein Folding Inspired by Protein Disulfide Isomerase

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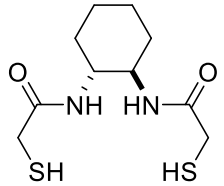
I. General

Commercial reagents were used without further purification. β-Mercaptoethanol (βME), oxidized β-mercaptoethanol (βME^{ox}), and diethylenetriamine were from Sigma–Aldrich (St. Louis, MO). RNase A was from Sigma–Aldrich and purified further by cation-exchange chromatography. The RNase A substrate 6-FAM–dArUdAdA–6-TAMRA was from Integrated DNA Technologies (Coralville, IA). All glassware was oven- or flame-dried, and reactions were performed under N_2(g) unless stated otherwise. Dichloromethane (DCM) and tetrahydrofuran (THF) were dried over a column of alumina. Triethylamine was dried over a column of alumina and purified further by passage through an isocyanate scrubbing column. Flash chromatography was performed with columns of 40–63 Å silica, 230–400 mesh from Silicycle (Québec City, Canada). Thin-layer chromatography (TLC) was performed on plates of EMD 250-μm silica 60-F_{254}. The term "concentrated under reduced pressure" refers to the removal of solvents and other volatile materials using a rotary evaporator at water aspirator pressure (<20 torr) while maintaining the water-bath temperature below 40 °C. Residual solvent was removed from samples at high vacuum (<0.1 torr). The term "high vacuum" refers to vacuum achieved by a mechanical belt-drive oil pump. Analytical samples of all protein folding catalysts were obtained with a preparative HPLC instrument from Shimadzu (Kyoto, Japan), which was equipped with a C18 reverse-phase preparative column, a Prominence diode array detector, and fraction collector. Equilibrium and reduction potential assays were performed using an analytical HPLC instrument from Waters (Milford, MA), which was equipped with a Waters 996 photodiode array detector, Empower 2 software, and a Varian C18 reverse-phase column. Thiol pK_a values were determined with a Varian Cary 60 UV–Vis spectrophotometer. Fluorescence was measured with an Infinite M1000 plate reader from Tecan (Männedorf, Switzerland). Calculations and rate constants were performed with Prism 6 software from GraphPad (La Jolla, CA). All NMR spectra were acquired at ambient temperature with a Bruker DMX-400 Avance spectrometer and Bruker III 500ii with

cryoprobe spectrometer at the National Magnetic Resonance Facility at Madison (NMRFAM), and were referenced to TMS or residual solvent.

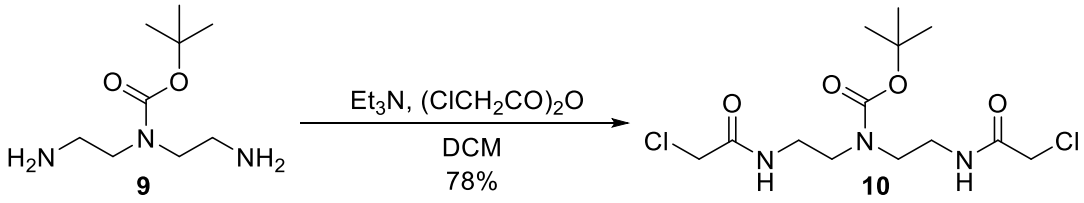
II. Chemical synthesis



BMC, **1**

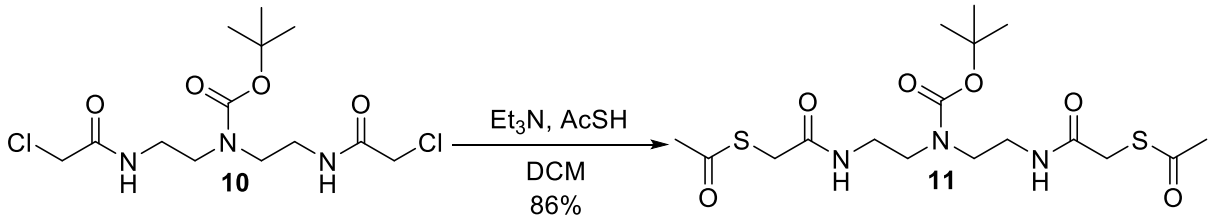
BMC (**1**) was synthesized as a racemate from (±)-*trans*-1,2-diaminocyclohexane as described previously.[1] An analytically pure sample of BMC was obtained by reverse-phase HPLC using a preparatory C18 column and a linear gradient of 10−80% v/v acetonitrile (0.1% v/v TFA) in water (0.1% v/v TFA) over 45 min. BMC eluted at 23 min and, after lyophilization, was isolated as a white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.83 (d, J = 5.3 Hz, 2H), 3.52−3.48 (m, 2H), 3.09−2.99 (m, 4H), 2.60 (t, J = 7.9 Hz, 2H), 1.79−1.77 (m, 2H), 1.66−1.65 (m, 2H), 1.24−1.20 (m, 4H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ = 169.2, 52.2, 31.7, 27.3, 24.3; **HRMS** (ESI) calculated for $[C_{10}H_{19}N_2O_2S_2]^+$ (M + H^+) requires m/z = 263.0883, found 263.0895



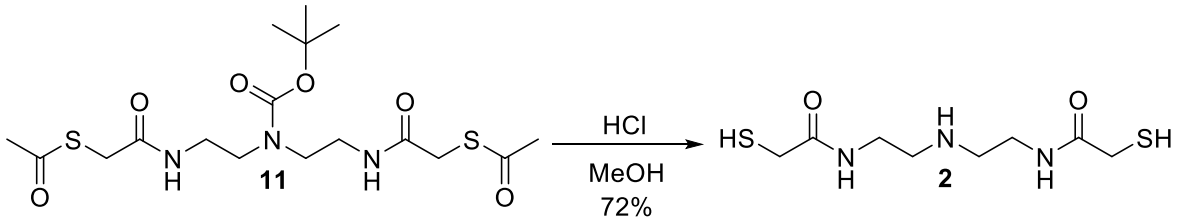
To a flame-dried round-bottom flask was added **9** (0.847 g, 4.166 mmol), which was synthesized as described previously.[2] Fifty mL of dichloromethane was then added, and the resulting solution was cooled to 0 °C under an atmosphere of N_2(g). Next, triethylamine (2.3 mL, 16.667 mmol) and chloroacetic anhydride (1.567 g, 9.167 mmol) were added, and the reaction mixture was stirred for 30 min before being quenched by the addition of 50 mL of saturated $NaHCO_3$(aq). The organic layer was extracted and washed with water (2 × 25 mL). The organic extract was then dried over $MgSO_4$(s), filtered, and concentrated under reduced pressure, and the product was purified by column chromatography (silica, EtOAc) yielding **10** as a colorless oil (1.154 g, 78%).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.36 (br, s, 1H), 6.99 (br, s, 1H), 4.09−3.98 (m, 4H), 3.48−3.38 (m, 8H), 1.49 (s, 9H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ = 166.9, 166.4, 156.6, 81.0, 47.1, 46.1, 42.5, 39.8, 38.8, 28.3; **HRMS** (ESI) calculated for $[C_{13}H_{23}Cl_2N_3O_4Na]^+$ (M + Na^+) requires m/z = 378.0958, found 378.0938



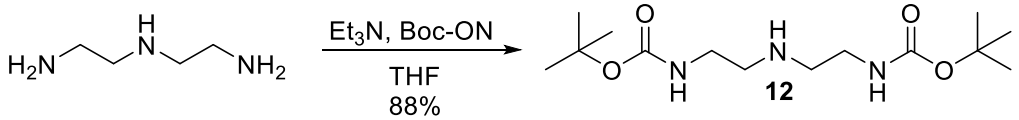
Compound **10** (1.154 g, 3.239 mmol) was placed in a round-bottom flask and dissolved in 30 mL of dichloromethane, and the resulting solutions was placed under an atmosphere of N_2(g). Triethylamine (2.3 mL, 16.208 mmol) and thioacetic acid (0.5 mL, 7.131 mmol) were then added, and the resulting solution was stirred under N_2(g). After 16 h, the reaction mixture was concentrated, and the product was purified by column chromatography (silica, EtOAc), giving **11** as a colorless oil (2.792 g, 86%).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.81 (br, s, 2H), 3.56 (s, 4H), 3.42−3.29 (m, 8H), 2.41 (s, 6H), 1.49 (s, 9H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ = 195.1, 194.7, 168.2, 156.3, 80.3, 47.9, 47.1, 39.7, 38.9, 32.9, 30.1, 28.3; **HRMS** (ESI) calculated for $[C_{17}H_{29}N_3O_6S_2Na]^+$ (M + Na^+) requires *m*/*z* = 458.1390, found 458.1405



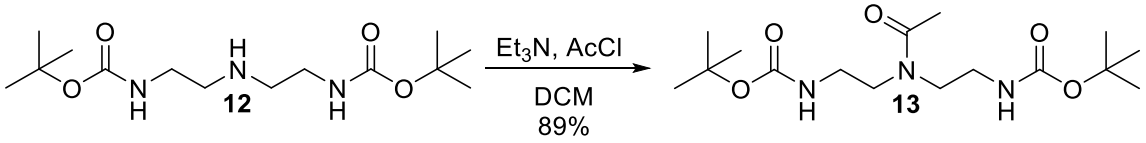
A flame-dried round-bottom flask was charged with **11** (0.178 g, 0.409 mmol) and placed under an atmosphere of N_2(g). Four mL of anhydrous methanol followed by 2 mL of 3 N HCl in methanol were then added, and the reaction mixture was stirred under N_2(g). Upon confirmation by TLC that the Boc group had been removed, the reaction mixture was concentrated under reduced pressure, and the product was purified by reverse-phase HPLC using a preparatory C18 column and a linear gradient of 10−50% v/v acetonitrile (0.1% v/v TFA) in water (0.1% v/v TFA) over 55 min. Dithiol **2** eluted as its TFA salt at 12.5 min and, after lyophilization, was isolated as a white solid (0.108 g, 72%).

^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.76 (br, s, 2H), 8.33 (br, s, 2H), 3.5203.35 (m, 8H), 3.15 (d, *J* = 7.7 Hz, 4H), 2.88 (t, *J* = 7.7 Hz, 2H); **^{13}C NMR (100 MHz, DMSO-d_6)** δ = 170.4, 46.1, 35.5, 27.2; **HRMS** (ESI) calculated for $[C_8H_{18}N_3O_2S_2]^+$ (M + H^+) requires *m*/*z* = 252.0835, found 252.0839



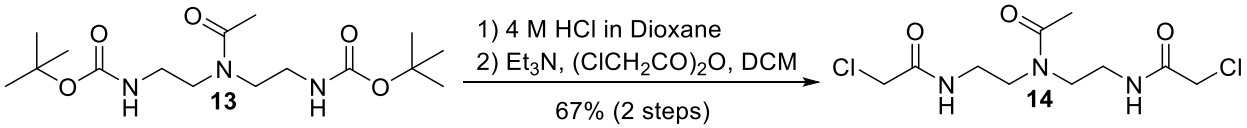
Synthesis of compound **12** was accomplished by closely following a procedure reported previously.[3] Specifically, diethylenetriamine (2.003 g, 19.415 mmol) and triethylamine (8.1 mL, 58.245 mmol) were dissolved in 100 mL of THF, and the resulting solutions was cooled to 0 °C in an ice bath and placed under an atmosphere of N_2(g). Next, a solution of 2-(boc-oxyimino)-2-phenylacetonitrile (Boc-ON) (9.563 g, 38.832 mmol) in 40 mL of THF was added dropwise. The reaction mixture was stirred for 1 h on ice and then for another 1 h at room temperature. The solvent was removed under reduced pressure, and the residue was dissolved in 200 mL of dichloromethane and washed with 5% w/v NaOH. The organic extract was then dried with $MgSO_4$(s), filtered, concentrated under reduced pressure, and the product was purified by column chromatography (silica, 10% v/v methanol in dichloromethane, 1% ammonium hydroxide), yielding **12** as a colorless oil (5.184 g, 88%).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 4.95 (br, s, 2H), 3.22 (q, J = 5.9 Hz, 4H), 2.73 (t, J = 5.9 Hz, 4 H), 1.45 (s, 18H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ = 156.3, 79.4, 49.0, 40.5, 28.6; **HRMS** (ESI) calculated for $[C_{14}H_{30}N_3O_4]^+$ (M + H^+) requires m/z = 304.2231, found 304.2230



Compound **12** (0.419 g, 1.381 mmol) was placed in a flame-dried round-bottom flask, dissolved in 15 mL of anhydrous dichloromethane, and cooled to 0 °C in an ice bath under an inert atmosphere. Triethylamine (0.72 mL, 5.17 mmol) and acetyl chloride (0.16 mL, 2.29 mmol) were then added, and the resulting solution was stirred at 0 °C for 1 h and at room temperature for another 2 h. The reaction mixture was then concentrated under reduced pressure, and the product was purified by column chromatography (silica, EtOAc), yielding **13** as a colorless oil (0.425 g, 89%).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.20 (br, s, 1H), 5.11 (br, s, 1H), 3.47–3.43 (m, 4H), 3.32–3.25 (m, 4H), 2.12 (s, 3H), 1.43 (s, 18H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ = 172.2, 156.6, 156.2, 79.8, 79.5, 49.4, 45.8, 39.7, 39.3, 28.6, 28.5, 21.6; **HRMS** (ESI) calculated for $[C_{16}H_{32}N_3O_5]^+$ (M + H^+) requires m/z = 346.2337, found 346.2338



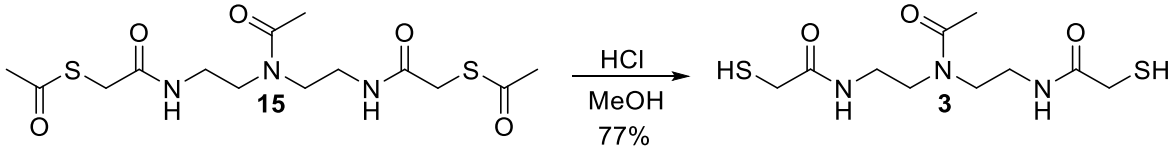
Forty mL of 4 M HCl in dioxane was added to a round-bottom flask containing **13** (0.425 g, 1.230 mmol). The resulting solution was stirred overnight and then concentrated under reduced pressure. The product was then partially dissolved in 20 mL of dichloromethane, and the resulting slurry was cooled to 0 °C in an ice bath and placed under an atmosphere of N_2(g).

Triethylamine (1.1 mL, 7.9 mmol) and chloroacetic anhydride (0.463 g, 2.706 mmol) were then added, and the reaction mixture was stirred for 30 min before being quenched by the addition of 50 mL of saturated $NaHCO_3$(aq). The organic layer was extracted and washed with water (2 × 25 mL). The organic extract was then dried with $MgSO_4$(s), filtered, and concentrated under reduced pressure, and the product was purified by column chromatography (silica, 10% v/v methanol in dichloromethane), yielding **14** as a colorless oil (0.245 g, 67%).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.33 (br, s, 1H), 7.06 (br, s, 1H), 4.06 (s, 2H), 4.02 (s, 2H), 3.60−3.56 (m, 2H), 3.52−3.47 (m, 6H), 2.15 (s, 3H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ = 172.5, 167.2, 166.9, 48.7, 45.4, 42.6, 39.6, 38.8, 21.5; **HRMS** (ESI) calculated for $[C_{10}H_{18}Cl_2N_3O_3]^+$ ($M + H^+$) requires m/z = 298.0713, found 298.0720

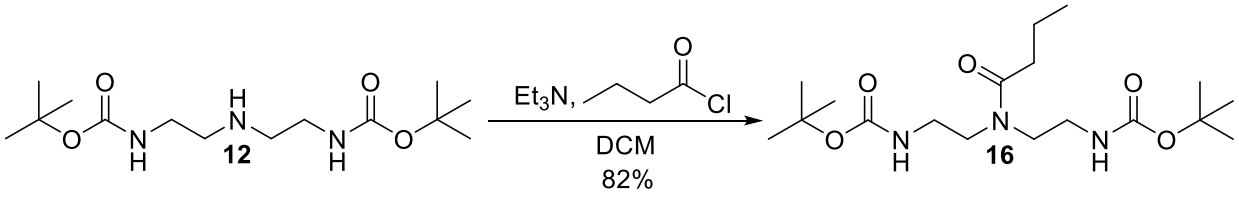
A round-bottom flask was charged with **14** (0.246 g, 0.824 mmol) dissolved in 15 mL of dichloromethane, and placed under an atmosphere of N_2(g). Triethylamine (0.57 mL, 4.12 mmol) and thioacetic acid (0.13 mL, 1.81 mmol) were then added, and the reaction mixture was stirred under N_2(g). After 16 h, the mixture was concentrated under reduced pressure, and the product was purified by column chromatography (silica, 10% v/v methanol in dichloromethane), providing **15** as a yellow oil (0.286 g, 92%).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.91 (br, s, 2H), 3.58 (s, 2H), 3.54 (s, 2H), 3.52−3.35 (m, 8H), 2.44 (s, 3H), 2.41 (s, 3H), 2.09 (s, 3H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ = 195.8, 195.2, 172.6, 168.9, 168.7, 48.8, 45.1, 39.4, 38.9, 33.1, 33.0, 30.5, 30.4, 21.6; **HRMS** (ESI) calculated for $[C_{14}H_{24}N_3O_5S_2]^+$ ($M + H^+$) requires m/z = 378.1152, found 378.1150



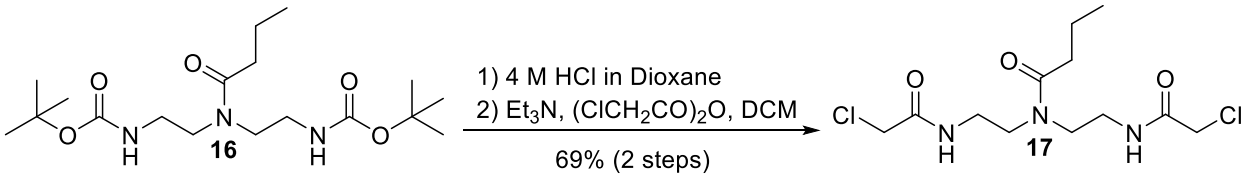
A flame-dried round-bottom flask was charged with **15** (0.159 g, 0.421 mmol) and placed under an atmosphere of N_2(g). Four mL of anhydrous methanol followed by 2 mL of 3 N HCl in methanol were then added, and the resulting solution was stirred under N_2(g). After 16 h, the reaction mixture was concentrated under reduced pressure, and the product was purified by reverse-phase HPLC using a preparatory C18 column and a linear gradient of 10−80% v/v acetonitrile (0.1% v/v TFA) in water (0.1% v/v TFA) over 45 min. Dithiol **3** eluted at 17 min and, after lyophilization, was isolated as a colorless oil (95.11 mg, 77%).

^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.19 (t, J = 6.0 Hz, 1H), 8.07 (t, J = 5.9 Hz, 1H), 3.33–3.26 (m, 4H), 3.24–3.16 (m, 4H), 3.11 (d, J = 8.6 Hz, 2H), 3.06 (d, J = 8.6 Hz, 2H), 2.74 (t, J = 8.6 Hz, 1H), 2.71 (t, J = 8.6 Hz, 1H), 1.99 (s, 3H); **^{13}C NMR (100 MHz, DMSO-d_6)** δ = 170.1, 170.0, 169.9, 47.8, 44.65, 37.7, 36.9, 27.1, 27.0, 21.3; **HRMS** (ESI) calculated for $[C_{10}H_{20}N_3O_3S_2]^+$ (M + H$^+$) requires m/z = 294.0942, found 294.0941



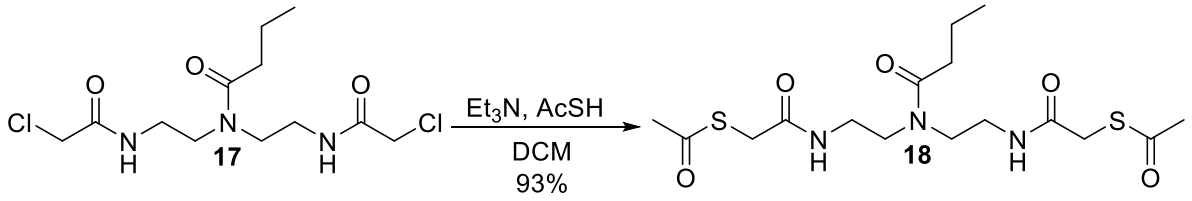
Compound **12** (0.619 g, 2.040 mmol) was placed in a flame-dried round-bottom flask and dissolved in 20 mL of anhydrous dichlormethane, and the resulting solution was cooled to 0 °C in an ice bath under an atmosphere of N_2(g). Triethylamine (1.4 mL, 10.2 mmol) and butyryl chloride (0.25 mL, 2.45 mmol) were then added, and the reaction mixture was stirred at 0 °C for 1 h and at room temperature for another 2 h. The reaction mixture was then concentrated under reduced pressure, and the product was purified by column chromatography (silica, EtOAc) yielding **16** as a colorless oil (0.625 g, 82%).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.39 (br, s, 1H), 5.30 (br, s, 1H), 3.48–3.42 (m, 4H), 3.32–3.25 (m, 4H), 2.32 (t, J = 7.5Hz, 2H), 1.65 (sex, J = 7.5 Hz, 2H), 1.43 (s, 9H), 1.42 (s, 9H), 0.95 (t, J = 7.5 Hz, 3H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ = 174.7, 156.6, 156.1, 79.6, 79.3, 48.4, 45.7, 39.6, 39.3, 34.9, 28.4, 18.8, 13.9; **HRMS** (ESI) calculated for $[C_{18}H_{36}N_3O_5]^+$ (M + H$^+$) requires m/z = 374.2650, found 374.2655



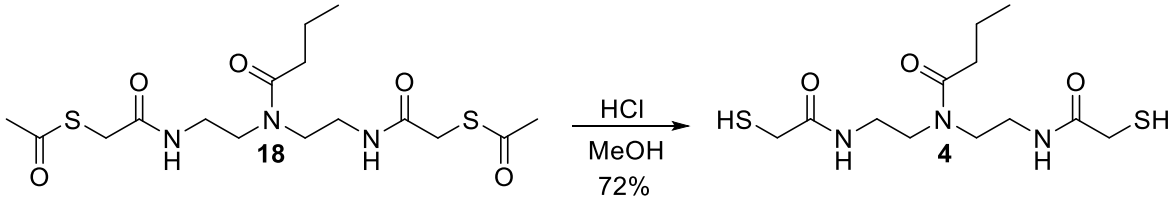
Fifty mL of 4 M HCl in dioxane was added to a round-bottom flask containing **16** (0.625 g, 1.673 mmol). The reaction mixture was left to stir overnight and then concentrated under reduced pressure. The compound was then partially dissolved in 20 mL of dichloromethane, cooled to 0 °C in an ice bath, and placed under an atmosphere of N_2(g). Triethylamine (1.2 mL, 8.4 mmol) and chloroacetic anhydride (0.629 g, 3.681 mmol) were then added, and the reaction mixture was stirred for 30 min before being quenched by the addition of 50 mL of saturated $NaHCO_3$(aq). The organic layer was extracted and washed twice with 25 mL of water. The organic extract was then dried with anhydrous $MgSO_4$(s), filtered, and concentrated under reduced pressure, and the product was purified by column chromatography (silica, 10% v/v methanol in dichloromethane), yielding **17** as a colorless oil (0.377 g, 69%).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.48 (br, s, 1H), 7.26 (br, s, 1H), 4.06 (s, 2H), 4.00 (s, 2H), 3.58 (t, J = 6.1 Hz, 2H), 3.52−3.46 (m, 6H), 2.34 (t, J = 7.4 Hz, 2H), 1.66 (sex, J = 7.4 Hz, 2H), 0.96 (t, J = 7.4 Hz, 3H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ = 175.0, 167.1, 166.9, 47.6, 45.2, 42.5, 39.5, 38.7, 34.9, 18.9, 14.0; **HRMS** (ESI) calculated for $[C_{12}H_{22}Cl_2N_3O_3]^+$ (M + H^+) requires m/z = 326.1033, found 326.1036



A round-bottom flask was charged with **17** (0.377 g, 1.154 mmol) dissolved in 20 mL of dichloromethane, and placed under an atmosphere of N_2(g). Triethylamine (0.80 mL, 5.77 mmol) and thioacetic acid (0.18 mL, 2.54 mmol) were then added, and the resulting solution was stirred under N_2(g). After 16 h, the reaction mixture was concentrated under reduced pressure, and the product was purified by column chromatography (silica, 10% v/v methanol in dichloromethane), providing **18** as a yellow oil (0.435 g, 93%).

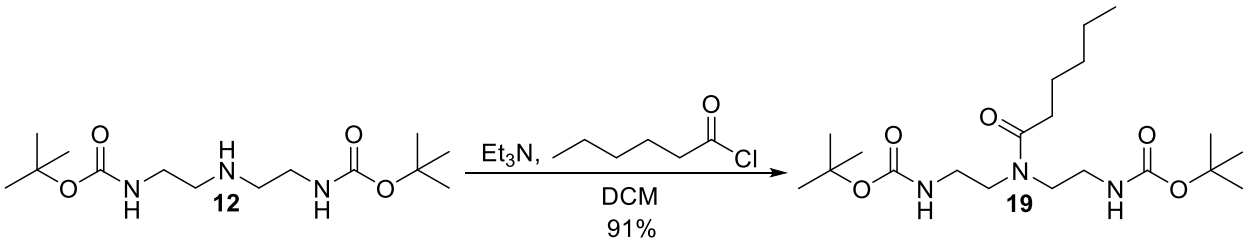
^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.13 (br, s, 2H), 3.59 (s, 2H), 3.56 (s, 2H), 3.51−3.37 (m, 8H), 2.42 (s, 3H), 2.41 (s, 3H), 2.32 (t, J = 7.5 Hz, 2H), 1.64 (sex, J = 7.5 Hz, 2H), 0.96 (t, J = 7.5 Hz, 3H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ = 195.6, 195.0, 174.9, 168.8, 168.6, 47.6, 45.1, 39.4, 38.8, 34.9, 33.0, 30.3, 30.2, 18.8, 13.9; **HRMS** (ESI) calculated for $[C_{16}H_{28}N_3O_5S_2]^+$ (M + H^+) requires m/z = 406.1465, found 406.1459



A flame-dried round-bottom flask was charged with **18** (0.111 g, 0.274 mmol) and placed under an atmosphere of N_2(g). Six mL of anhydrous methanol followed by 3 mL of 3 N HCl in methanol was then added, and the resulting solution was stirred under N_2(g). After 16 h, the reaction mixture was concentrated under reduced pressure, and the product was purified by reverse-phase HPLC using a preparatory C18 column and a linear gradient of 10−80% v/v acetonitrile (0.1% v/v TFA) in water (0.1% v/v TFA) over 45 min. Dithiol **4** eluted at 22 min and, after lyophilization, was isolated as a colorless oil (63.42 mg, 72%).

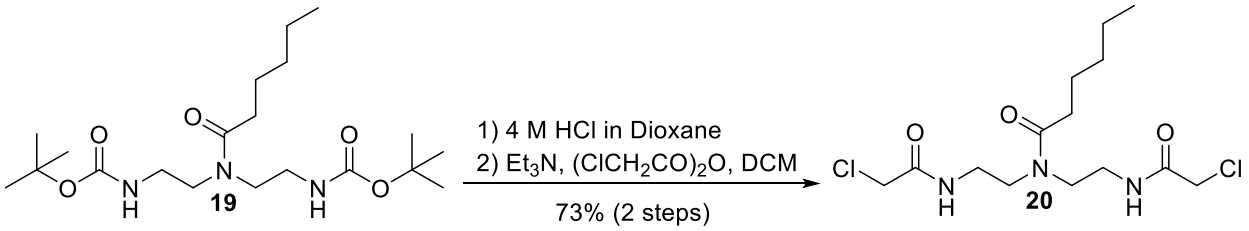
^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.19 (t, J = Hz, 1H), 8.05 (t, J = Hz, 1H), 3.34−3.28 (m, 4H), 3.23−3.15 (m, 4H), 3.09 (d, J = 8.0 Hz, 2H), 3.06 (d, J = 8.0 Hz, 2H), 2.74 (t, J = 8.0 Hz, 1H), 2.71 (t, J = 8.0 Hz, 1H), 2.27 (t, J = 7.4 Hz, 2H), 1.51 (sex, J = 7.4 Hz, 2H), 0.87 (t, J = 7.4

Hz, 3H); **^{13}C NMR (100 MHz, DMSO-d_6)** δ = 172.3, 170.0, 169.7, 46.8, 45.0, 37.8, 37.0, 34.0, 27.1, 27.0, 18.3, 13.8; **HRMS** (ESI) calculated for $[C_{12}H_{24}N_3O_3S_2]^+$ (M + H^+) requires *m*/*z* = 322.1254, found 322.1258



Compound **12** (1.534 g, 5.056 mmol) was placed in a flame-dried round-bottom flask and dissolved in 50 mL of anhydrous dichloromethane, and the resulting solution was cooled to 0 °C in an ice bath under an atmosphere of N_2(g). Triethylamine (2.1 mL, 15.2 mmol) and hexanoyl chloride (0.78 mL, 5.56 mmol) were then added, and the reaction mixture was stirred at 0 °C for 1 h and at room temperature for another 2 h. The reaction mixture was then concentrated under reduced pressure, and the product was purified by column chromatography (silica, 50% v/v EtOAc in Hexanes) yielding **19** as a colorless oil (1.848 g, 91%).

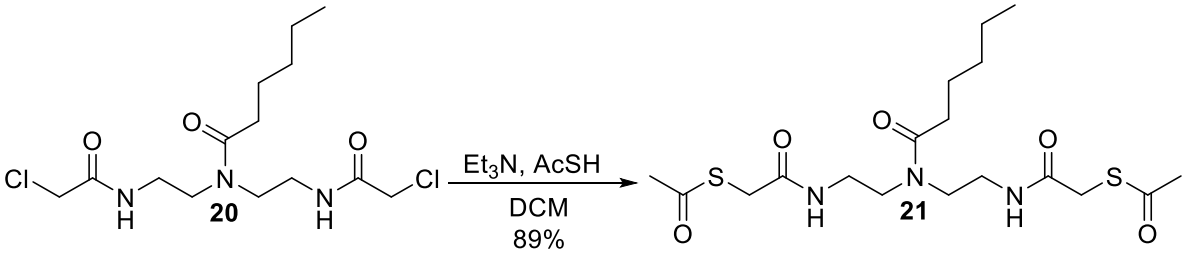
^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.12 (br, s, 1H), 5.06 (br, s, 1H), 3.48–3.43 (m, 4H), 3.32–3.24 (m, 4H), 2.32 (t, *J* = 7.6 Hz, 2H), 1.62 (quin, *J* = 7.6 Hz, 2H), 1.43 (s, 18H), 1.34–1.26 (m, 4H), 0.90 (t, *J* = 6.7 Hz, 3H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ = 174.7, 156.4, 156.0, 79.5, 79.2, 48.3, 45.6, 39.5, 39.2, 32.9, 31.5, 28.3, 25.0, 22.5, 13.9; **HRMS** (ESI) calculated for $[C_{20}H_{40}N_3O_5]^+$ (M + H^+) requires *m*/*z* = 402.2963, found 402.2966



Fifty mL of 4 M HCl in dioxane was added to a round-bottom flask containing **19** (0.867 g, 2.159 mmol). The resulting solution was stirred overnight and then concentrated under reduced pressure. The product was then partially dissolved in 40 mL of dichloromethane, and the resulting slurry was cooled to 0 °C in an ice bath and placed under an atmosphere of N_2(g). Triethylamine (1.8 mL, 12.9 mmol) and chloroacetic anhydride (0.923 g, 5.398 mmol) were then added, and the reaction mixture was stirred for 30 min before being quenched by the addition of 50 mL of saturated $NaHCO_3$(aq). The organic layer was extracted and washed with water (2 × 30 mL). The organic extract was then dried over $MgSO_4$(s), filtered, and concentrated under

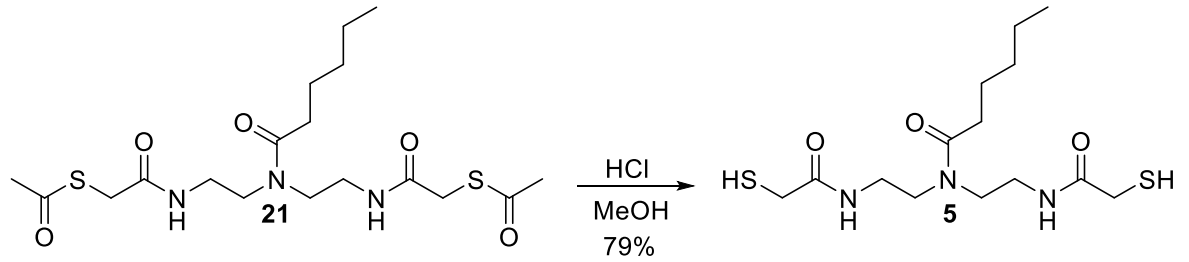
reduced pressure, and the product was purified by column chromatography (silica, 10% v/v methanol in dichloromethane), yielding **20** as a colorless oil (0.558 g, 73%).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.36 (br, s, 1H), 7.08 (br, s, 1H), 4.06 (s, 2H), 4.00 (s, 2H), 3.58 (t, J = 6.1 Hz, 2H), 3.52–3.46 (m, 6H), 2.35 (t, J = 7.4 Hz, 2H), 1.63 (quin, J = 7.4 Hz, 2H), 1.34–1.26 (m, 4H), 0.90 (t, J = 6.7 Hz, 3H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ = 175.2, 167.1, 166.9, 47.7, 45.3, 42.6, 39.7, 38.8, 33.1, 31.7, 25.3, 22.7, 14.1; **HRMS** (ESI) calculated for $[C_{14}H_{26}Cl_2N_3O_3]^+$ ($M + H^+$) requires m/z = 354.1346, found 354.1342



A round-bottom flask charged with **20** (0.558 g, 1.575 mmol) was dissolved with 20 mL of dichloromethane and placed under N_2(g). Triethylamine (1.1 mL, 7.9 mmol) and thioacetic acid (0.25 mL, 3.47 mmol) were then added, and the resulting solution was stirred under N_2(g). After 16 h, the reaction mixture was concentrated under reduced pressure, and the product was purified by column chromatography (silica, 10% v/v methanol in dichloromethane), providing **21** as a colorless oil (0.608 g, 89%).

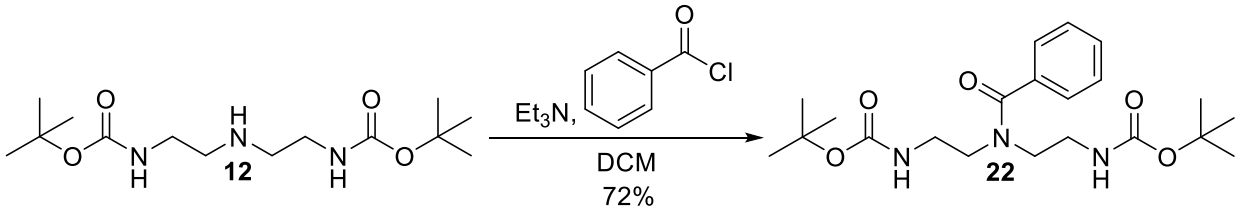
^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.03–6.98 (m, 2H), 3.57 (s, 2H), 3.55 (s, 2H), 3.51–3.36 (m, 8H), 2.42 (s, 2H), 2.41 (s, 2H), 2.32 (t, J = 7.4 Hz, 2H), 1.61 (quin, J = 7.4 Hz, 2H), 1.36–1.28 (m, 4H), 0.90 (t, J = 6.7 Hz, 3H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ = 195.7, 195.1, 175.2, 168.8, 168.6, 47.8, 45.3, 39.6, 38.9, 33.1, 33.0, 32.9, 31.6, 30.4, 30.3, 25.2, 22.6, 14.1; **HRMS** (ESI) calculated for $[C_{18}H_{32}N_3O_5S_2]^+$ ($M + H^+$) requires m/z = 434.1778, found 434.1780



A flame-dried round-bottom flask was charged with **21** (0.149 g, 0.344 mmol) and placed under an atmosphere of N_2(g). Six mL of anhydrous methanol followed by 3 mL of 3 N HCl in methanol were then added, and the resulting solution was stirred under N_2(g). After 24 h, the reaction mixture was concentrated under reduced pressure, and the product was purified by reverse-phase HPLC using a preparatory C18 column and a linear gradient of 10–80% v/v

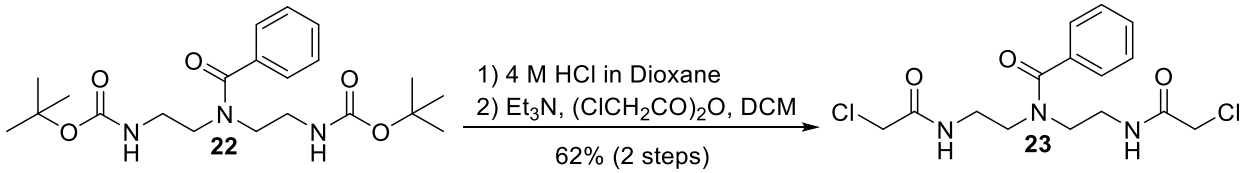
acetonitrile (0.1% v/v TFA) in water (0.1% v/v TFA) over 45 min. Dithiol **5** eluted at 28 min and, after lyophilization, was isolated as a colorless oil (94.98 mg, 79%).

^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.21 (t, J = 5.8 Hz, 1H), 8.07 (t, J = 5.8 Hz, 1H), 3.31 (t, J = 6.6 Hz, 2H), 3.29 (t, J = 6.6 Hz, 2H) 3.22=3.15 (m, 4H), 3.08 (d, J = 8.0 Hz, 2H), 3.06 (d, J = 8.0 Hz, 2H), 2.75 (t, J = 8.0 Hz, 1H), 2.72 (t, J = 8.0 Hz, 1H), 2.27 (t, J = 7.5 Hz, 2H), 1.48 (quin, J = 7.5 Hz, 2H), 1.32–1.20 (m, 4H), 0.86 (t, J = 6.9 Hz, 3H); **^{13}C NMR (100 MHz, DMSO-d_6)** δ = 172.5, 170.0, 169.7, 46.8, 44.7, 37.8, 37.0, 32.0, 31.1, 27.2, 27.1, 24.6, 22.1, 14.0; **HRMS** (ESI) calculated for $[C_{14}H_{28}N_3O_3S_2]^+$ (M + H^+) requires m/z = 350.1567, found 350.1565



Compound **12** (1.391 g, 4.585 mmol) was placed in a flame-dried round-bottom flask and dissolved in 50 mL of anhydrous dichloromethane, and the resulting solution was cooled to 0 °C in an ice bath under an atmosphere of N_2(g). Triethylamine (1.9 mL, 13.8 mmol) and benzoyl chloride (0.64 mL, 5.50 mmol) were then added, and the resulting solution was stirred at 0 °C for 1 h and at room temperature for another 2 h. The reaction mixture was then concentrated under reduced pressure, and the product was purified by column chromatography (silica, EtOAc), yielding **22** as a colorless oil (1.848 g, 91%).

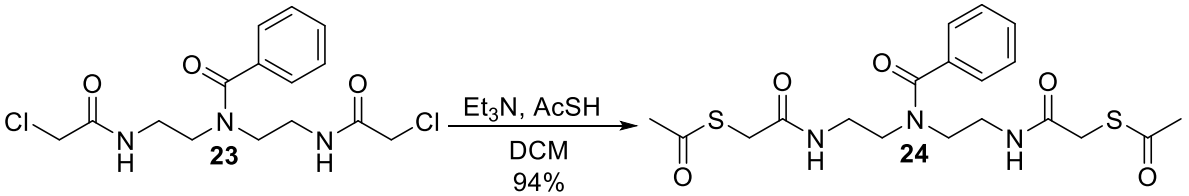
^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.40–7.35 (m, 5H), 5.12 (br, s, 1H), 5.06 (br, s, 1H), 3.67–3.24 (m, 8H), 1.44 (s, 18H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ = 173.4, 156.7, 155.8, 136.4, 129.6, 128.7, 126.8, 50.0, 45.0, 39.5, 38.9, 28.6, 28.4; **HRMS** (ESI) calculated for $[C_{21}H_{34}N_3O_5]^+$ (M + H^+) requires m/z = 408.2493, found 408.2491



Fifty mL of 4 M HCl in dioxane was added to a round-bottom flask containing **22** (0.713 g, 1.750 mmol). The resulting solution was stirred under N_2(g) overnight and then concentrated under reduced pressure. The product was then partially dissolved in 55 mL of dichloromethane, and the resulting slurry was cooled to 0 °C in an ice bath and placed under an atmosphere of N_2(g). Triethylamine (1.5 mL, 10.5 mmol) and chloroacetic anhydride (0.748 g, 4.375 mmol) were then added, and the reaction mixture was stirred for 30 min before being quenched by the addition of 55 mL of saturated $NaHCO_3$(aq). The organic layer was extracted and washed with

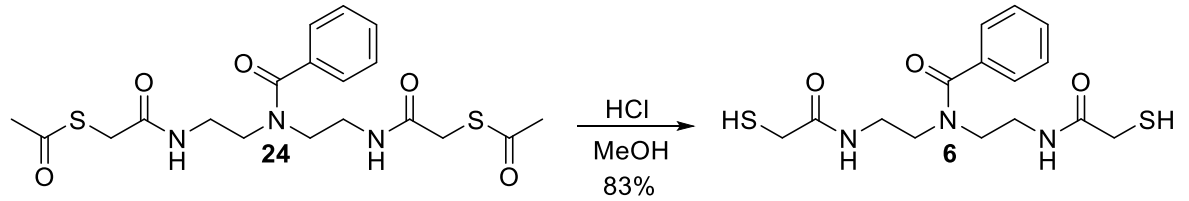
water (2 × 40 mL). The organic extract was then dried with anhydrous $MgSO_4$(aq), filtered, and concentrated under reduced pressure, and the product was purified by column chromatography (silica, 10% v/v methanol in dichloromethane), yielding **23** as a colorless oil (0.391 g, 62%).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.44–7.41 (m, 3H), 7.37–7.35 (m, 2H), 6.90 (br, s, 2H), 4.05 (s, 2H), 3.98 (s, 2H), 3.80–3.41 (m, 8H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ = 173.4, 167.1, 166.3, 135.6, 129.9, 128.8, 126.5, 48.9, 44.6, 42.5, 39.0, 38.3; **HRMS** (ESI) calculated for $[C_{15}H_{20}Cl_2N_3O_3]^+$ (M + H^+) requires *m*/*z* = 360.0877, found 360.0888



A round-bottom flask charged with **23** (0.391 g, 1.085 mmol) was dissolved with 15 mL of dichloromethane and placed under N_2(g). Triethylamine (0.76 mL, 5.43 mmol) and thioacetic acid (0.17 mL, 2.39 mmol) were then added, and the reaction was stirred under N_2(g). After 24 h, the reaction mixture was concentrated under reduced pressure, and the product was purified by column chromatography (silica, 10% v/v methanol in dichloromethane) providing **24** as a colorless oil (0.448 g, 94%).

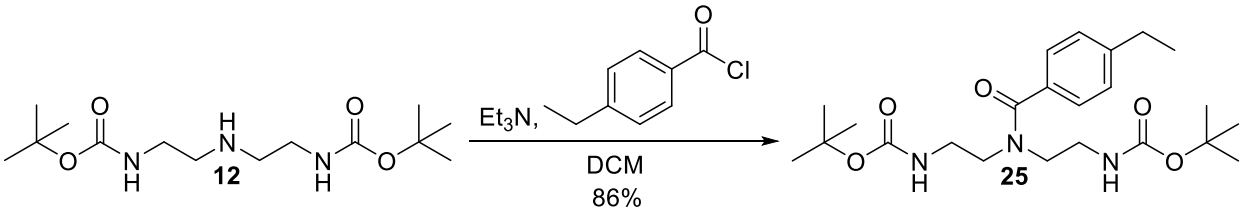
^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.43–7.42 (m, 3H), 7.37–7.35 (m, 2H), 6.98 (br, s, 1H), 6.80 (br, s, 1H), 3.72–3.32 (m, 12H), 2.37 (s, 6H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ = 195.6, 195.4, 173.4, 168.9, 168.4, 135.8, 129.8, 128.6, 126.6, 49.3, 44.8, 39.2, 38.5, 32.9, 30.3; **HRMS** (ESI) calculated for $[C_{19}H_{26}N_3O_5S_2]^+$ (M + H^+) requires *m*/*z* = 440.1309, found 440.1310



A flame-dried round-bottom flask was charged with **24** (0.131 g, 0.298 mmol) and placed under an atmosphere of N_2(g). Six mL of anhydrous methanol followed by 3 mL of 3 N HCl in methanol was then added, and the resulting solution was stirred under N_2(g). After 24 h, the reaction mixture was concentrated under reduced pressure, and the product was purified by reverse-phase HPLC using a preparatory C18 column and a linear gradient of 10–80% v/v acetonitrile (0.1% v/v TFA) in water (0.1% v/v TFA) over 45 min. Dithiol **6** eluted at 25 min and, after lyophilization, was isolated as a colorless oil (87.92 mg, 83%).

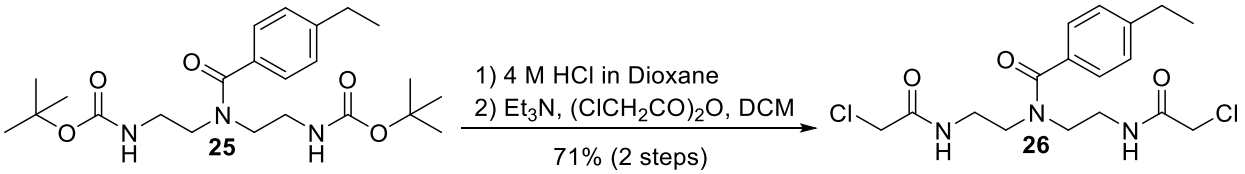
^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ = 8.23 (br, s, 1H), 8.08 (br, s, 1H), 7.42–7.40 (m, 3H), 7.36–7.34 (m, 2H), 3.52–3.50 (m, 2H), 3.36–3.34 (m, 2H), 3.28–3.25 (m, 2H), 3.14–3.10 (m, 4H),

3.01 (d, J = 8.0Hz, 2H), 2.76 (t, J = 8.0 Hz, 1H), 2.68 (t, J = 8.0 Hz, 1H); **^{13}C NMR (100 MHz, DMSO-d_6)** δ = 171.1, 169.9, 169.6, 136.9, 129.0, 128.3, 126.6, 48.3, 44.1, 37.3, 36.7, 27.2, 27.0; **HRMS** (ESI) calculated for $[C_{15}H_{22}N_3O_3S_2]^+$ (M + H$^+$) requires m/z = 356.1098, found 356.1096



Compound **12** (1.592 g, 5.247 mmol) was placed in a flame-dried round-bottom flask and dissolved in 60 mL of anhydrous dichlormethane, and the resulting solution was cooled to 0 °C in an ice bath under an atmosphere of N_2(g). Triethylamine (2.2 mL, 15.7 mmol) and 4-ethylbenzoyl chloride (0.93 mL, 6.30 mmol) were then added, and the resulting solution was stirred at 0 °C for 1 h and at room temperature for another 2 h. The reaction mixture was then concentrated under reduced pressure, and the product was purified by column chromatography (silica, 50 % EtOAc v/v in hexanes), yielding **25** as a white solid (1.965 g, 86%).

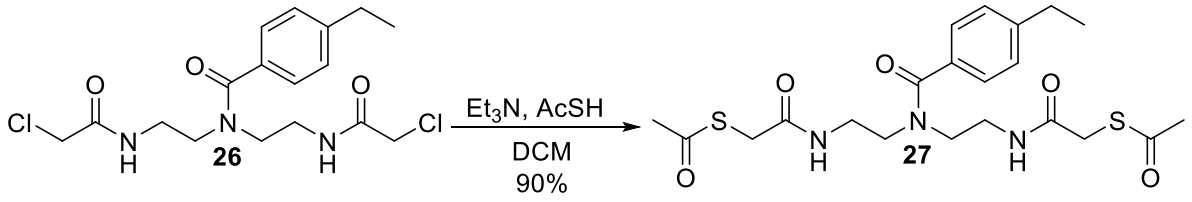
^{1}H NMR (400 MHz, CDCl$_3$) δ = 7.29 (d, J = 7.7 Hz, 2H), 7.20 (d, J = 7.7 Hz, 2H), 5.15 (br, s, 1H), 5.07 (br, s, 1H), 3.65−3.25 (m, 8H), 2.65 (q, J = 7.6 Hz, 2H), 1.44 (2, 18H), 1.23 (t, J = 7.6 Hz, 3H); **^{13}C NMR (100 MHz, CDCl$_3$)** δ = 173.6, 156.6, 155.9, 133.7, 128.1, 126.9, 79.6, 50.0, 45.0, 39.4, 39.1, 28.8, 28.5, 15.4; **HRMS** (ESI) calculated for $[C_{23}H_{38}N_3O_5]^+$ (M + H$^+$) requires m/z = 436.2806, found 436.2806



Fifty mL of 4 M HCl in dioxane was added to a round-bottom flask containing **25** (0.689 g, 1.584 mmol). The resulting solution was stirred overnight and then concentrated under reduced pressure. The product was then partially dissolved in 60 mL of dichloromethane, and the resulting slurry was cooled to 0 °C in an ice bath and placed under an atmosphere of N_2(g). Triethylamine (1.1 mL, 7.9 mmol) and chloroacetic anhydride (0.677 g, 3.960 mmol) were then added, and the reaction mixture was stirred for 30 min before being quenched by the addition of 60 mL of saturated $NaHCO_3$(aq). The organic layer was extracted and washed twice with 40 mL of water. The organic extract was then dried with anhydrous $MgSO_4$(s), filtered, and concentrated under reduced pressure, and the product was purified by column chromatography (silica, 10% v/v methanol in dichloromethane), yielding **26** as a colorless oil (0.437 g, 71%).

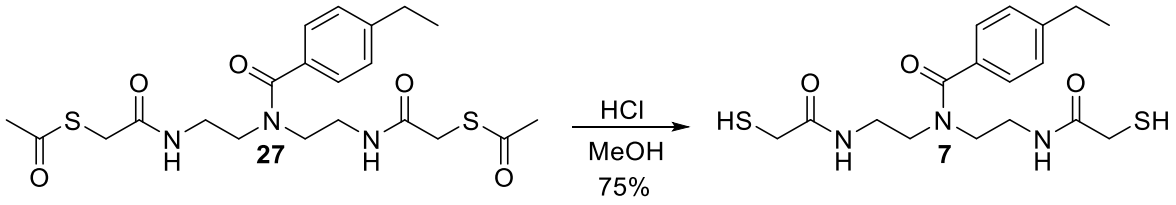
^{1}H NMR (400 MHz, CDCl$_3$) δ = 7.61 (br, s, 1H), 7.28 (d, J = 7.8 Hz, 2H), 7.23 (d, J = 7.8 Hz, 2H), 7.10 (br, s, 1H), 3.97 (s, 4H), 3.73−3.41 (m, 8H), 2.67 (q, J = 7.7 Hz, 2H), 1.24 (t, J = 7.7

Hz, 3H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ = 173.6, 167.1, 166.5, 146.3, 132.9, 128.2, 126.7, 48.9, 44.6, 42.5, 38.5, 28.7, 15.4; **HRMS** (ESI) calculated for $[C_{17}H_{24}Cl_2N_3O_3]^+$ (M + H^+) requires *m*/*z* = 388.1190, found 388.1192



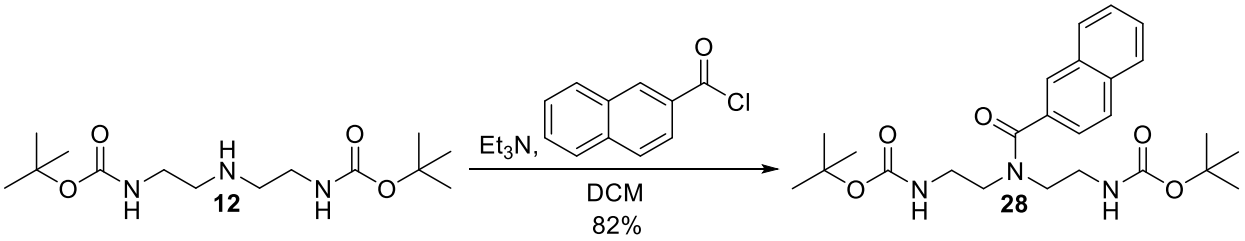
A round-bottom flask was charged with **26** (0.437 g, 1.125 mmol) dissolved in 15 mL of dichloromethane and placed under an atmosphere of N_2(g). Triethylamine (0.78 mL, 5.62 mmol) and thioacetic acid (0.18 mL, 2.48 mmol) were then added, and the resulting solution was stirred under N_2(g). After 24 h, the reaction mixture was concentrated under reduced pressure, and the product was purified by column chromatography (silica, 10% v/v methanol in dichloromethane), providing **27** as a yellow solid (0.473 g, 90%).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.29 (d, *J* = 8.0 Hz, 2H), 7.23 (d, *J* = 8.0 Hz, 2H), 7.02 (br, s, 1H), 6.84 (br, s, 1H), 3.68−3.33 (m, 12H), 2.68 (q, *J* = 7.6 Hz, 2H), 2.37 (s, 6H), 1.25 (t, *J* = 7.6 Hz, 3H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ = 195.6, 173.7, 169.0, 168.5, 146.3, 133.2, 128.2, 126.9, 49.5, 44.9, 39.3, 38.7, 33.0, 30.4, 28.8, 15.5; **HRMS** (ESI) calculated for $[C_{21}H_{30}N_3O_5S_2]^+$ (M + H^+) requires *m*/*z* = 468.1622, found 468.1626



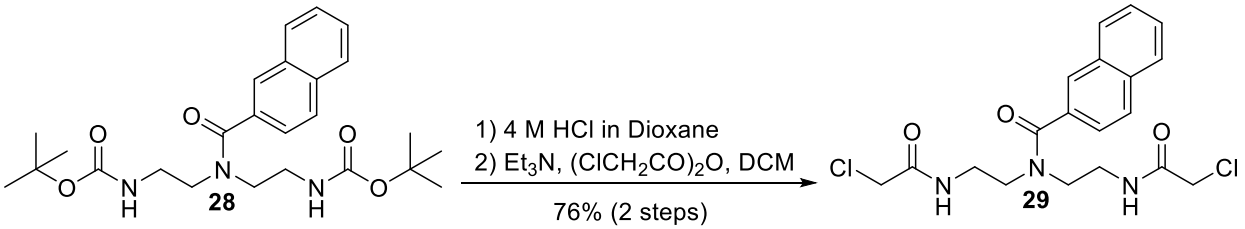
A flame-dried round-bottom flask was charged with **27** (0.147 g, 0.314 mmol) and placed under an inert atmosphere. Six mL of anhydrous methanol followed by 3 mL of 3 N HCl in methanol were then added, and the resulting solution was stirred under N_2(g). After 24 h, the reaction mixture was concentrated under reduced pressure, and the product was purified by reverse-phase HPLC using a preparatory C18 column and a linear gradient of 10−80% v/v acetonitrile (0.1% v/v TFA) in water (0.1% v/v TFA) over 45 min. Dithiol **7** eluted at 30 min and, after lyophilization, was isolated as a white solid (90.32 mg, 75%).

^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.20 (br, s, 1H), 8.06 (br, s, 1H), 7.28 (d, *J* = 7.8 Hz, 2H), 7.24 (d, *J* = 7.8 Hz, 2H), 3.54−3.46 (m, 2H), 3.35−3.29 (m, 4H), 3.14−3.02 (m, 6H), 2.74−2.60 (m, 4H), 1.19 (t, *J* = 7.5 Hz, 3H); **^{13}C NMR (100 MHz, DMSO-d_6)** δ = 171.2, 169.8, 169.6, 144.6, 134.2, 127.6, 126.7, 48.4, 44.1, 37.3, 36.8, 28.0, 27.1, 15.4; **HRMS** (ESI) calculated for $[C_{17}H_{26}N_3O_3S_2]^+$ (M + H^+) requires *m*/*z* = 384.1411, found 384.1411



Compound **12** (0.203 g, 0.669 mmol) was placed in a flame-dried round-bottom flask and dissolved in 10 mL of anhydrous dichlormethane, and the resulting solution was cooled to 0 °C in an ice bath under an atmosphere of N_2(g). Triethylamine (0.28 mL, 2.01 mmol) and 2-naphthoyl chloride (0.153 g, 0.803 mmol) were then added, and the resulting solution was stirred at 0 °C for 1 h and at room temperature for another 2 h. The reaction mixture was then concentrated under reduced pressure, and the product was purified by column chromatography (silica, 50 % EtOAc v/v in Hexanes), yielding **28** as a white solid (0.251 g, 82%).

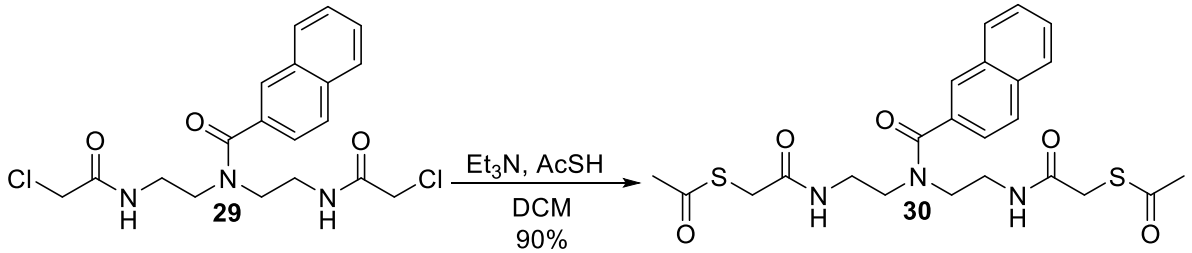
^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.90−7.87 (m, 1H), 7.85−7.82 (m, 3H), 7.53−7.49 (m, 2H), 7.47−7.47 (d, J = 8.5 Hz, 1H), 5.35 (br, s, 1H), 5.11 (br, s, 1H), 3.73−3.65 (m, 2H), 3.51−3.40 (m, 4H), 3.27−3.19 (m, 2H), 1.45 (s, 9H), 1.39 (s, 9H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ = 173.1, 156.6, 155.8, 133.6, 133.5, 132.7, 128.4, 127.8, 127.0, 126.7, 126.5, 124.1, 79.4, 49.9, 45.1, 39.2, 38.8, 28.5, 28.4; **HRMS** (ESI) calculated for $[C_{25}H_{36}N_3O_5]^+$ (M + H^+) requires m/z = 458.2650, found 458.2642



Fifteen mL of 4 M HCl in dioxane was added to a round-bottom flask containing **28** (0.251 g, 0.549 mmol). The resulting solution was stirred overnight and then concentrated under reduced pressure. The product was then partially dissolved in 20 mL of dichloromethane, and the resulting slurry was cooled to 0 °C in an ice bath and placed under an atmosphere of N_2(g). Triethylamine (0.46 mL, 3.28 mmol) and chloroacetic anhydride (0.235 g, 1.373 mmol) were then added, and the reaction mixture was stirred for 30 min before being quenched by the addition of 20 mL of saturated $NaHCO_3$(aq). The organic layer was extracted and washed with water (2 × 10 mL). The organic extract was then dried over anhydrous $MgSO_4$(s), filtered, and concentrated under reduced pressure, and the product was purified by column chromatography (silica, 10% v/v methanol in dichloromethane), yielding **29** as a colorless oil (0.171 g, 76%).

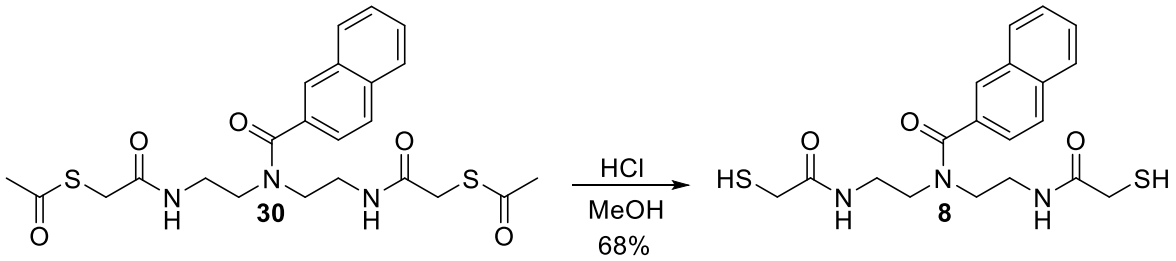
^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.91−7.86 (m, 4H), 7.58−7.53 (m, 2H), 7.45−7.43 (m, 2H), 6.90 (br, s, 1H), 4.06−3.43 (m, 12H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ = 173.4, 167.1, 166.5, 133.6, 133.0, 132.7, 128.8, 128.4, 128.0, 127.4, 127.1, 126.5, 123.7, 49.0, 44.7, 42.6, 38.8, 38.4;

HRMS (ESI) calculated for $[C_{19}H_{22}Cl_2N_3O_3]^+$ (M + H^+) requires *m*/*z* = 410.1033, found 410.1033



A round-bottom flask was charged with **29** (0.171 g, 0.417 mmol) dissolved in 5 mL of dichloromethane, and the resulting solution was placed under an atmosphere of N_2(g). Triethylamine (0.29 mL, 2.08 mmol) and thioacetic acid (0.1 mL, 1.40 mmol) were then added, and the resulting solution was stirred under N_2(g). After 24 h, the reaction mixture was concentrated under reduced pressure, and the product was purified by column chromatography (silica, 10% v/v methanol in dichloromethane), providing **30** as a yellow solid (0.184 g, 90%).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.87−7.82 (m, 4H), 7.53−7.49 (m, 2H), 7.44 (d, *J* = 8.4 Hz, 1H), 7.35 (br, s, 1H), 7.10 (br, s, 1H), 3.69−3.28 (m, 12H), 2.30 (s, 6H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ = 195.5, 173.3, 168.9, 168.4, 133.5, 133.1, 132.6, 128.5, 127.9, 127.2, 126.9, 126.5, 123.9, 49.3, 44.8, 39.2, 38.5, 32.9, 30.3; **HRMS** (ESI) calculated for $[C_{23}H_{28}N_3O_5S_2]^+$ (M + H^+) requires *m*/*z* = 490.1465, found 490.1464



A flame-dried round-bottom flask was charged with **30** (0.184 g, 0.376 mmol) and placed under an atmosphere of N_2(g). Six mL of anhydrous methanol followed by 3 mL of 3 N HCl in methanol were then added, and the resulting solution was stirred under N_2. After 24 h, the reaction mixture was concentrated under reduced pressure, and the product was purified by reverse-phase HPLC using a preparatory C18 column and a linear gradient of 10−80% v/v acetonitrile (0.1% v/v TFA) in water (0.1% v/v TFA) over 45 min. Dithiol **8** eluted at 32 min and, after lyophilization, was isolated as a white solid (103.69 mg, 68%).

^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.29 (t, *J* = 5.7 Hz, 1H), 8.06 (t, *J* = 5.7 Hz, 1H), 7.97−7.93 (m, 4H), 7.59−7.55 (m, 2H), 7.50 (dd, *J* = 8.5, 1.6 Hz, 1H), 3.59–3.57 (m, 2H), 3.43−3.34 (m, 4H), 3.16 (m, 4H), 3.00 (d, *J* = 8.0 Hz, 2H), 2.80 (t, *J* = 8.0 Hz, 1H), 2.66 (t, *J* = 8.0 Hz, 1H); **^{13}C NMR (100 MHz, DMSO-d_6)** δ = 171.1, 170.0, 169.7, 134.3, 132.9, 132.3, 128.3, 128.0, 127.8,

126.9, 126.7, 125.9, 124.5, 48.5, 44.2, 37.3, 36.8, 27.3, 27.1; **HRMS** (ESI) calculated for $[C_{19}H_{24}N_3O_3S_2]^+$ (M + H^+) requires *m*/*z* = 406.1254, found 406.1256

III. Determination of thiol pK_a values

The thiol pK_a values for **2** and **7** were determined by following closely a procedure reported previously that exploits the elevated absorbance of the deprotonated thiolate at 238 nm.[4] A plot of A_{238} *vs* pH was recorded (Fig. S1), and pK_a values were determined by fitting these data to eq 1, which was derived from Beer's law and the definition of the acid dissociation constant.[1,5]

$$A_{238} = C_T\left(\frac{\varepsilon_{S^-}^{S^-}10^{(pH-pKa2)}+\varepsilon_{SH}^{S^-}+\varepsilon_{SH}^{SH}10^{(pKa1-pH)}}{10^{(pH-pKa2)}+1+10^{(pKa1-pH)}}\right) \quad (1)$$

In eq 1, C_T is the total thiol concentration, ε_{SH}^{SH} is the extinction coefficient of the doubly protonated form of **2**, **5**, or **7**, $\varepsilon_{SH}^{S^-}$ is the extinction coefficient of the singly protonated form of **2**, **5**, or **7**, and $\varepsilon_{S^-}^{S^-}$ is the extinction coefficient of the doubly deprotonated form of **2**, **5**, or **7**.

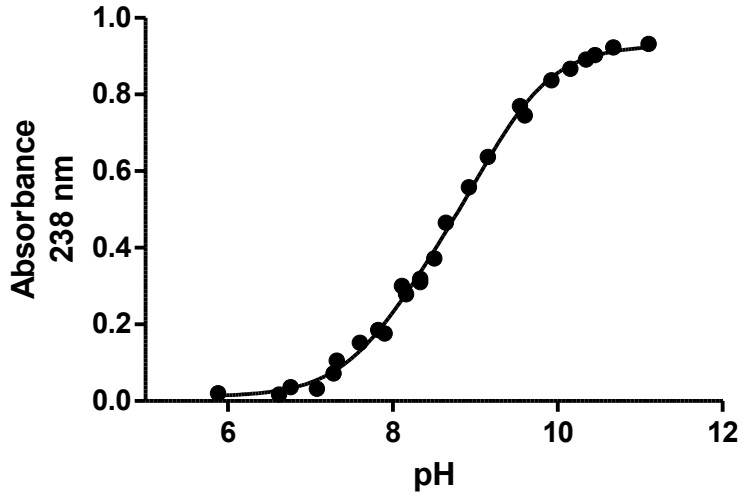


Fig. S1 Effect of pH on absorbance by **2** at 238 nm in 0.10 M potassium phosphate buffers. pK_a values of 8.0 ± 0.2 and 9.2 ± 0.1, and extinction coefficients of ε_{SH}^{SH} = 9.20, $\varepsilon_{SH}^{S^-}$ = 3996, $\varepsilon_{S^-}^{S^-}$ = 9296 $M^{-1}cm^{-1}$ with $r^2 > 0.99$ were determined by fitting the data to eq 1.

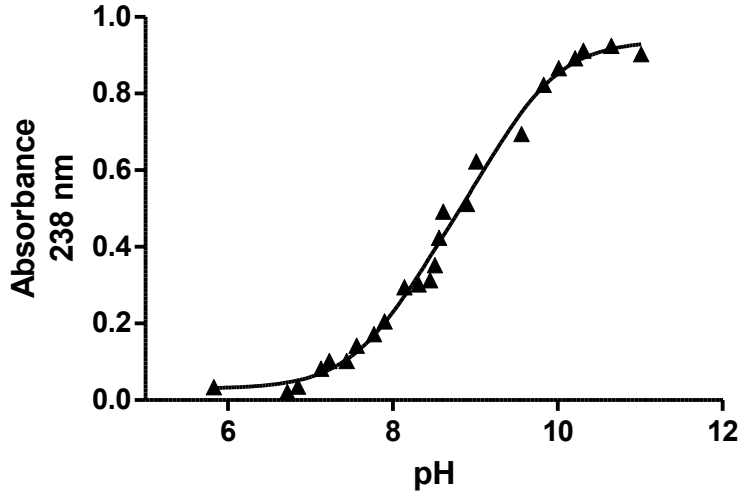


Fig. S2 Effect of pH on absorbance by **5** at 238 nm in 0.10 M potassium phosphate buffers. pK_a values of 8.1 ± 0.3 and 9.3 ± 0.3, and extinction coefficients of ε_{SH}^{SH} = 10.23, ε_{SH}^{S-} = 4610, ε_{S-}^{S-} = 9386 $M^{-1}cm^{-1}$ with $r^2 > 0.99$ were determined by fitting the data to eq 1.

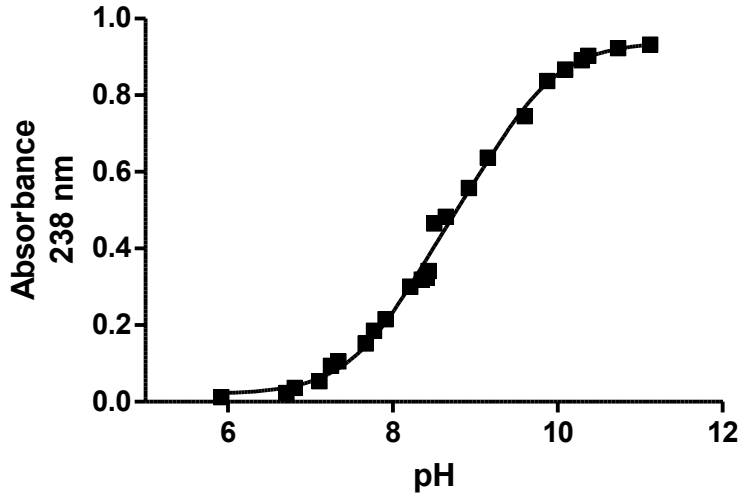


Fig. S3 Effect of pH on absorbance by **7** at 238 nm in 0.10 M potassium phosphate buffers. pK_a values of 8.1 ± 0.2 and 9.4 ± 0.2, and extinction coefficients of ε_{SH}^{SH} = 8.13, ε_{SH}^{S-} = 4940, ε_{S-}^{S-} = 9390 $M^{-1}cm^{-1}$ with $r^2 > 0.99$ were determined by fitting the data to eq 1.

IV. Determination of disulfide $E°'$ values

The reduction potentials of BMC, **2**, **5**, and **7** were determined as described previously.[1,5,6] Briefly, an equilibrium was established between reduced BMC (or **2**, **5**, or **7**) and βME^{ox}, and analyzed with analytical HPLC. The equilibrium concentrations were determined by integration of the peaks corresponding to βME and βME^{ox}. From these concentrations, the K_{eq} for the reaction was determined and the reduction potential of BMC (or **2**, **5**, or **7**) was then calculated using the Nernst equation and $E_{\beta ME}{}^{ox\circ\prime} = -0.260$ V. The values reported are the mean (±SE) of three separate measurements for each compound

V. Assay for disulfide-bond isomerization activity

sRNase was prepared as described previously.[7] Bovine liver PDI was from Sigma–Aldrich (product #P3818). The activation of sRNase A in the presence of refolding catalysts was determined as described previously.[1] Refolding reactions were performed at 30 °C in 50 mM Tris–HCl buffer, pH 7.6, containing GSH (1.0 mM), GSSG (0.2 mM), and catalyst (1.0 mM). Organocatalysts were delivered from 100-fold concentrated stock solutions in DMSO. Reactions were initiated by the addition of sRNase A to a final concentration of 5 μg/mL. Reaction progress was monitored by quantifying the cleavage of the RNase A substrate 6-FAM–dArUdAdA–6-TAMRA at 493/515 nm, as described previously.[8] Assays were performed in triplicate at ambient temperature in a black polystyrene 96-well plate, in 200 μL of 0.10 M MES-NaOH buffer, pH 6.0, containing NaCl (0.10 M). The resulting fluorescence data were fitted to the equation $k_{cat}/K_M = (\Delta I/\Delta t)/(I_f - I_0)[E]$, in which $\Delta I/\Delta t$ is the initial reaction velocity, I_0 is the fluorescence intensity before addition of any ribonuclease, I_f is the fluorescence intensity after complete substrate hydrolysis, and [E] is the total ribonuclease concentration. Reaction progress was monitored every hour until the increase in activity leveled off (~5 h). In Fig. 4A, data were fitted to eq 2.[1]

$$[\text{active RNase A}] = [\text{sRNase A}]_{t=0}(1 - e^{[\text{catalyst}]tk_{obs}}) \quad (2)$$

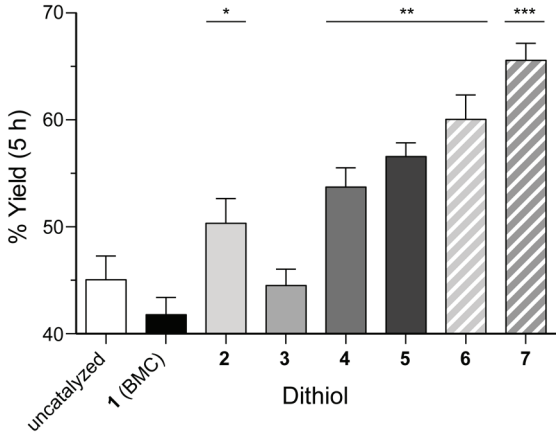
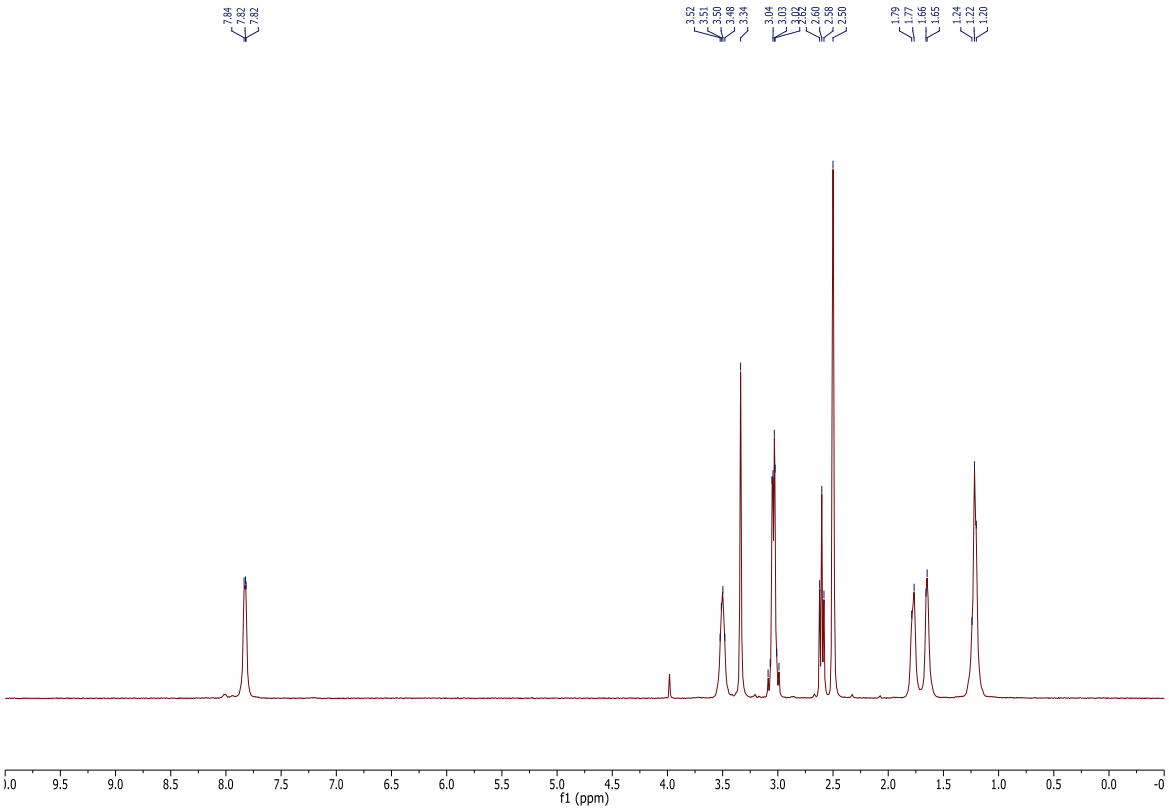
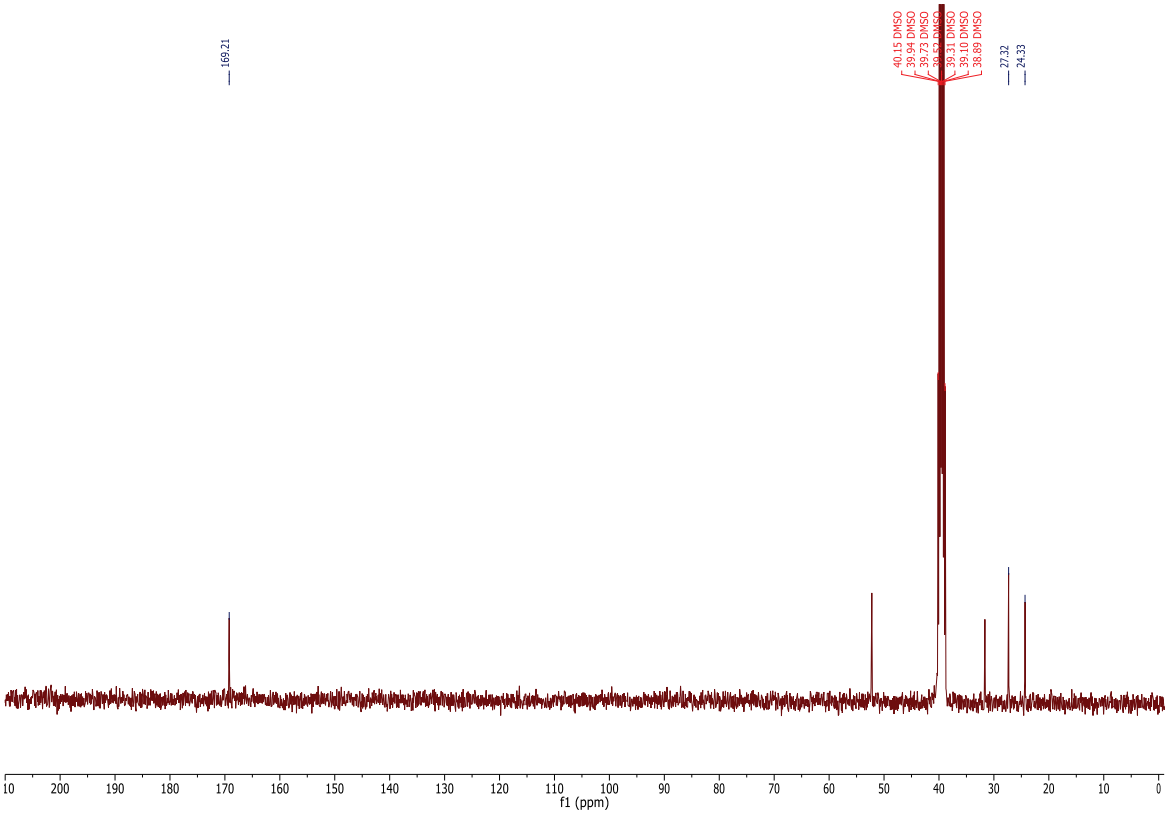


Fig. S4. Yield of native RNase A achieved by PDI mimics **1**–**7** after 5 h (*p < 0.05, **p <0.01, ***p < 0.005). Values are listed in Table 1.

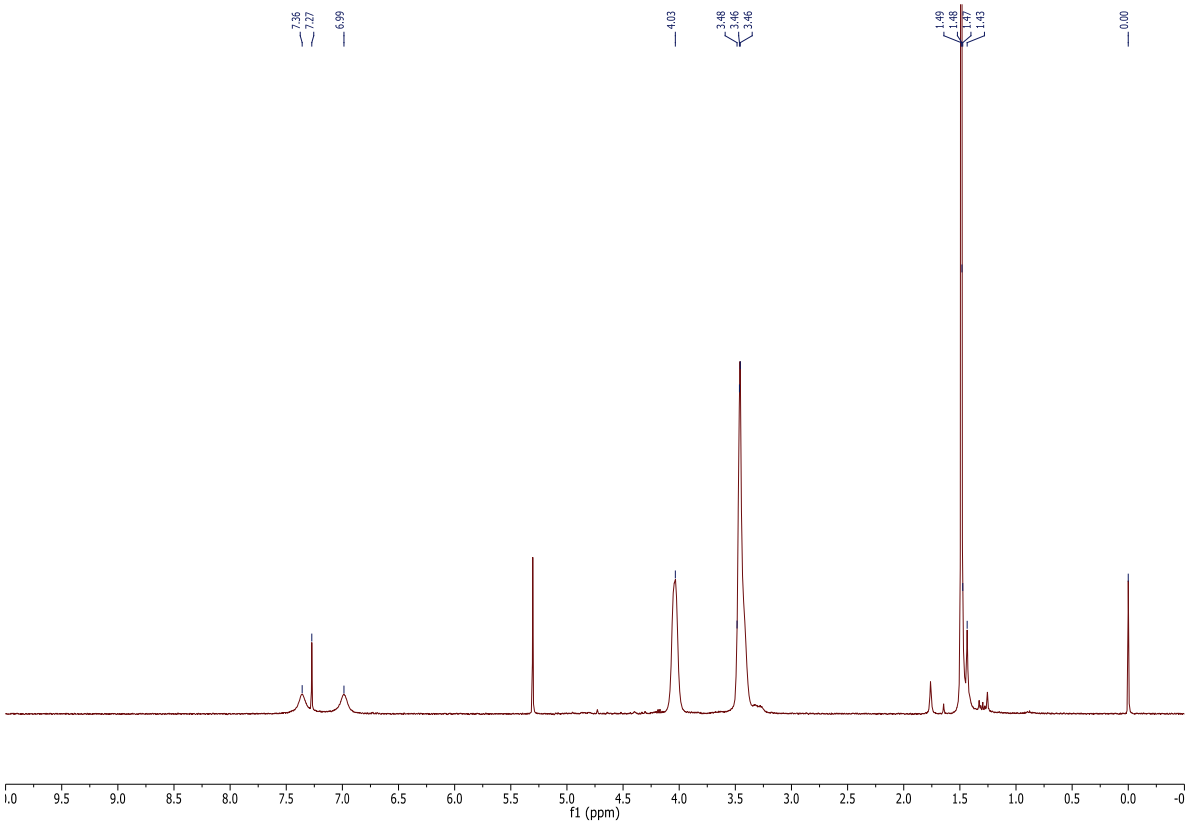
VI. NMR spectra

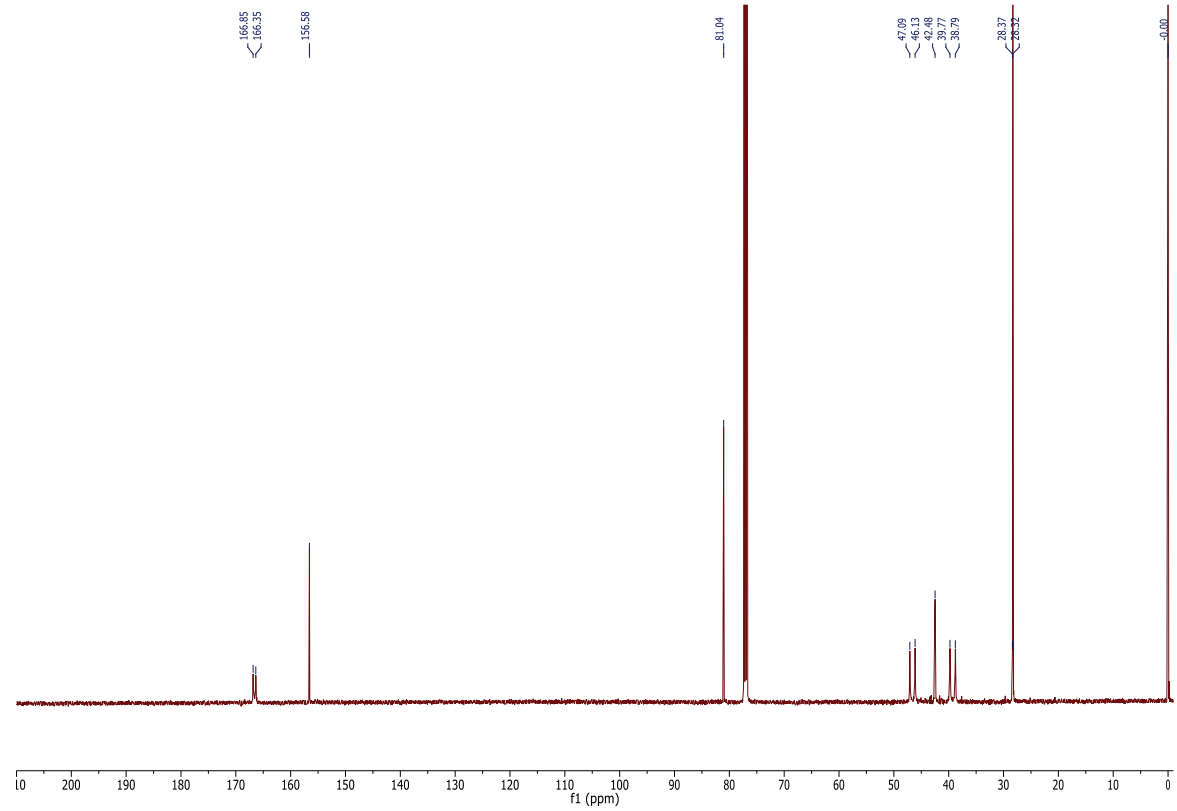
^{1}H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6) of **1 (BMC)**



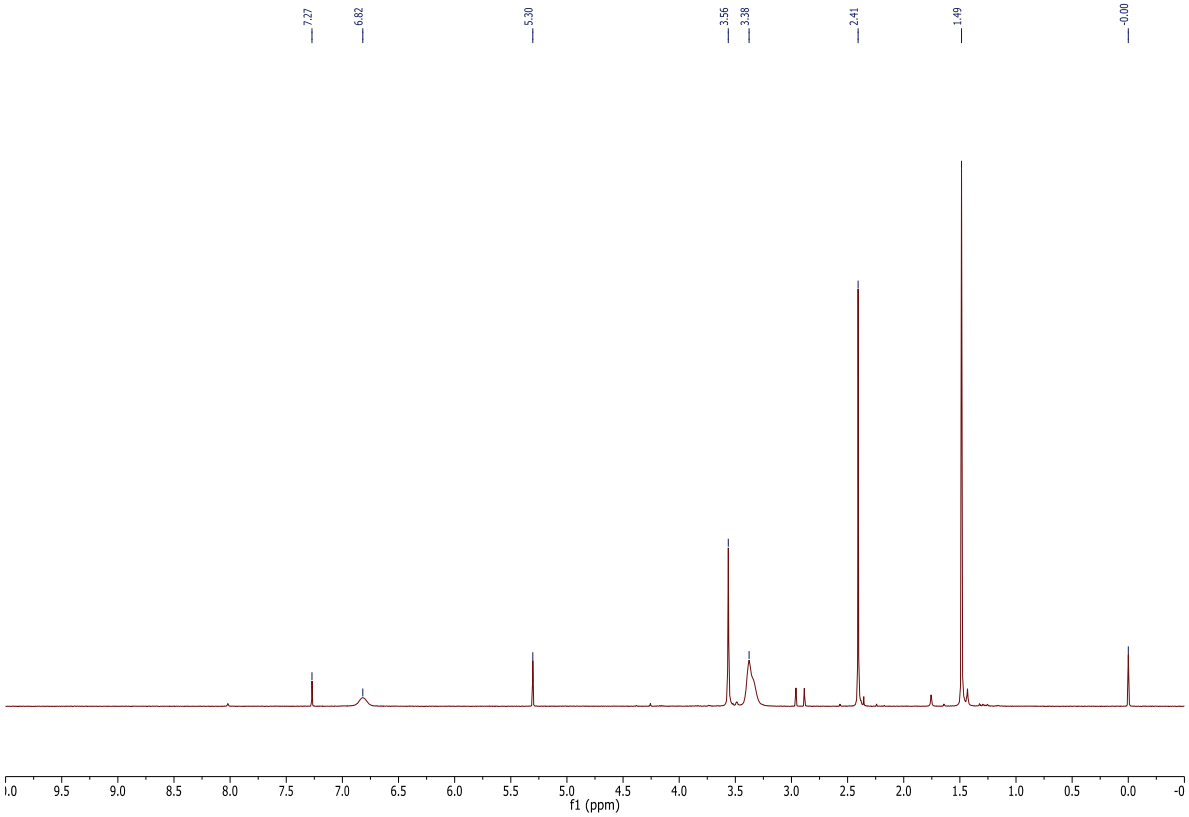


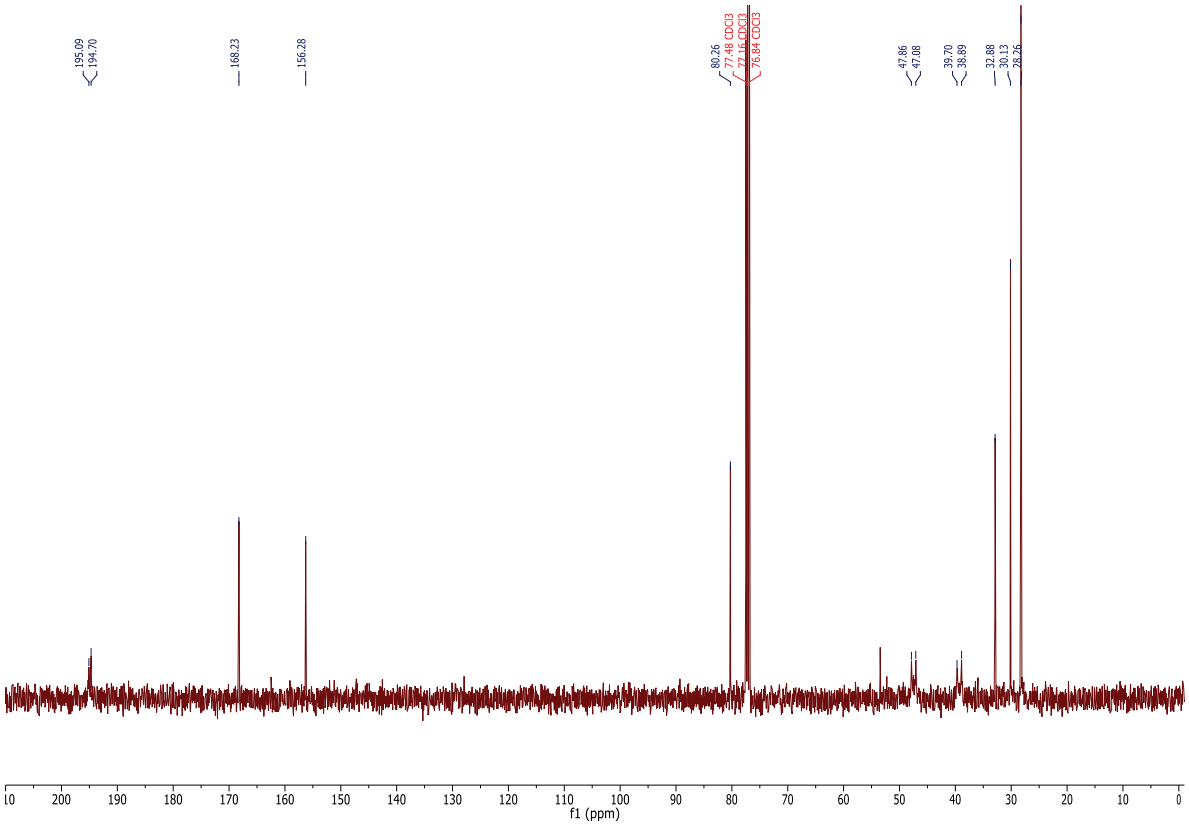
^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of **10** in dichloromethane



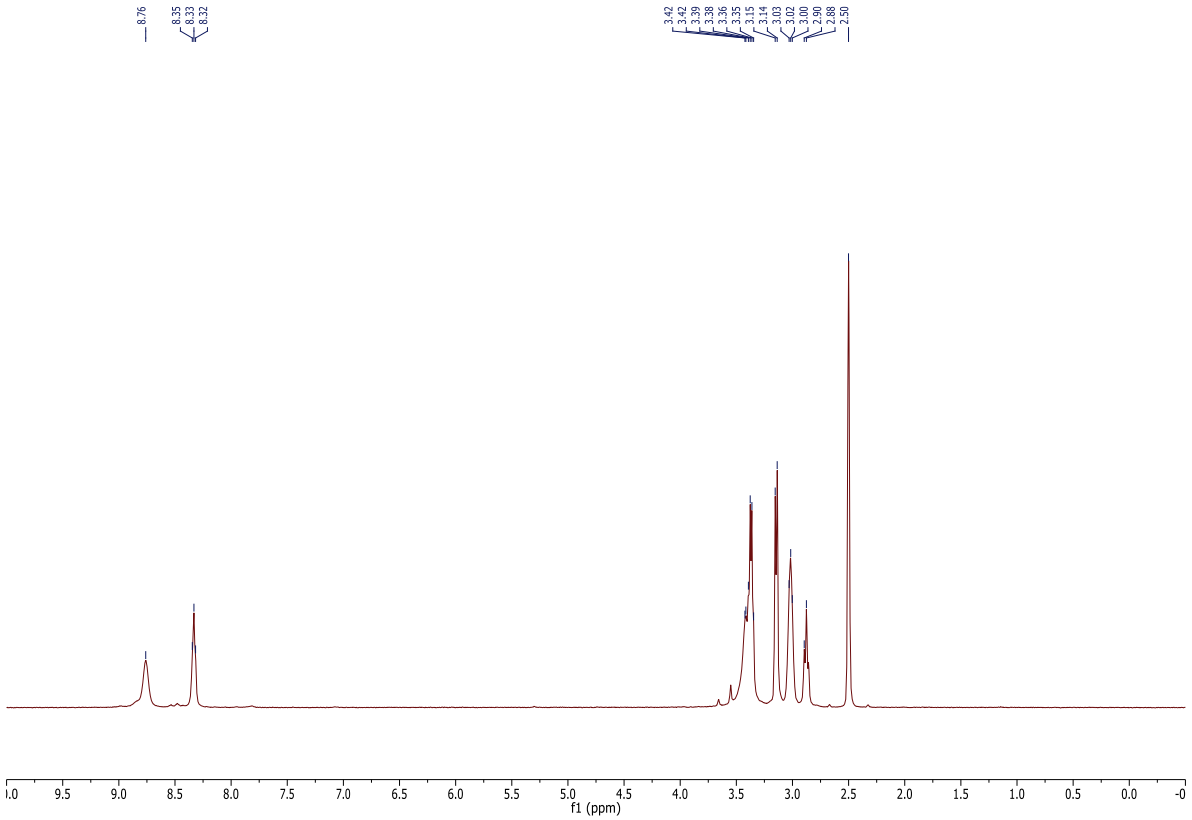


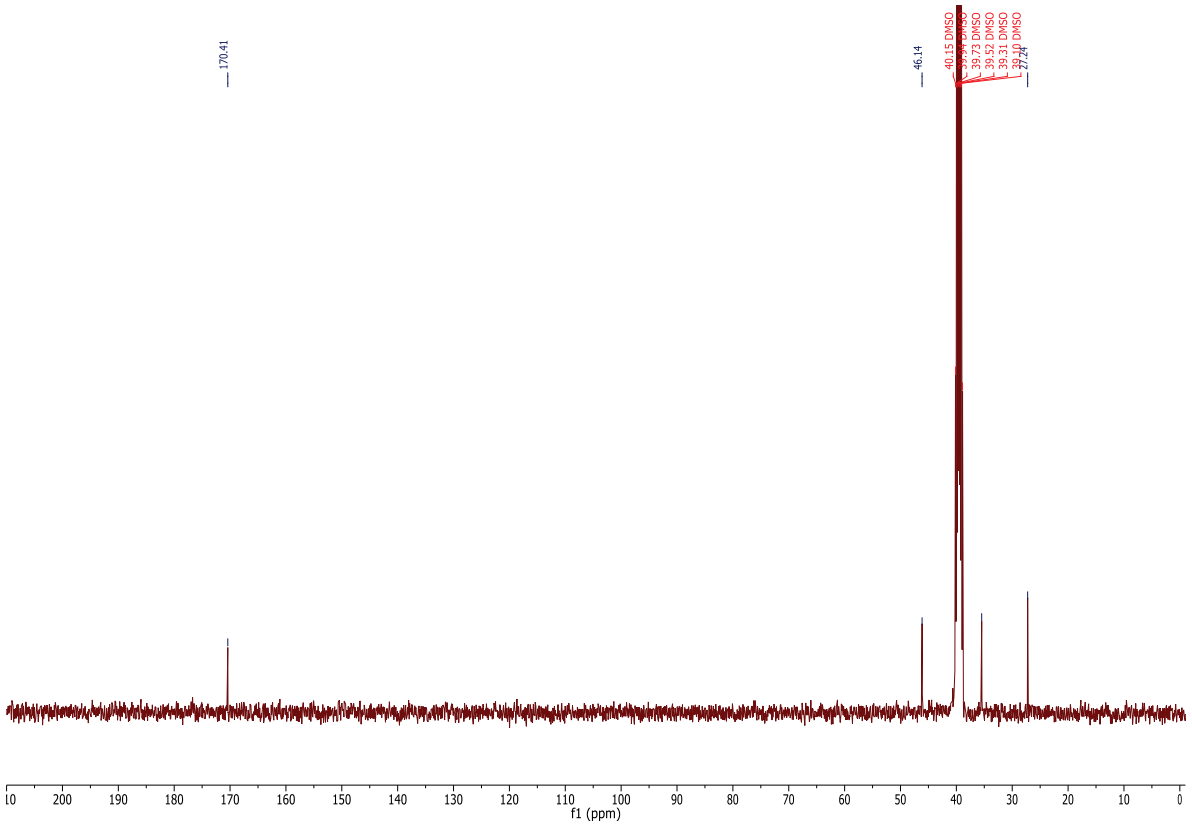
^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of **11** in dichloromethane



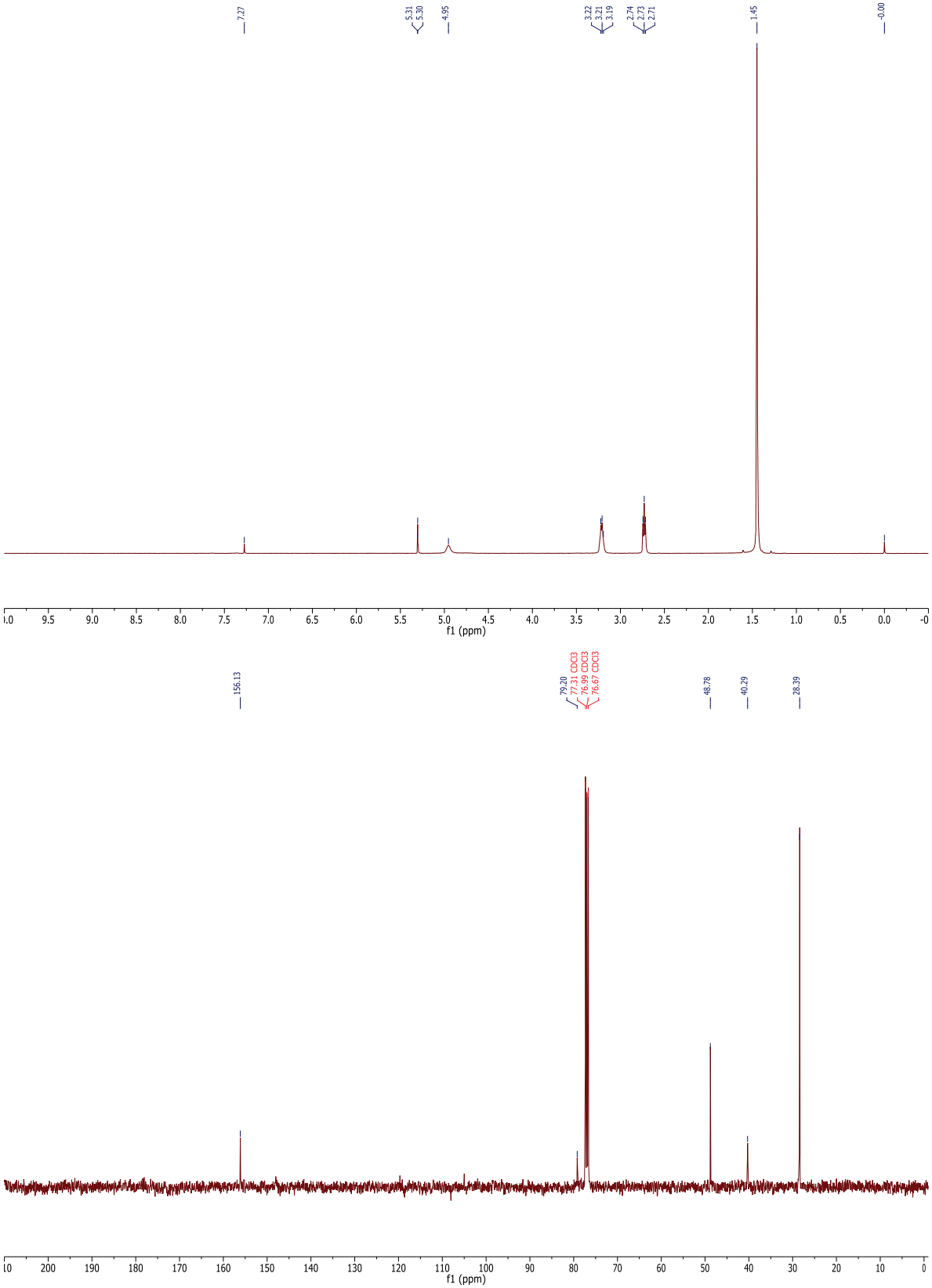


^{1}H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6) of **2**

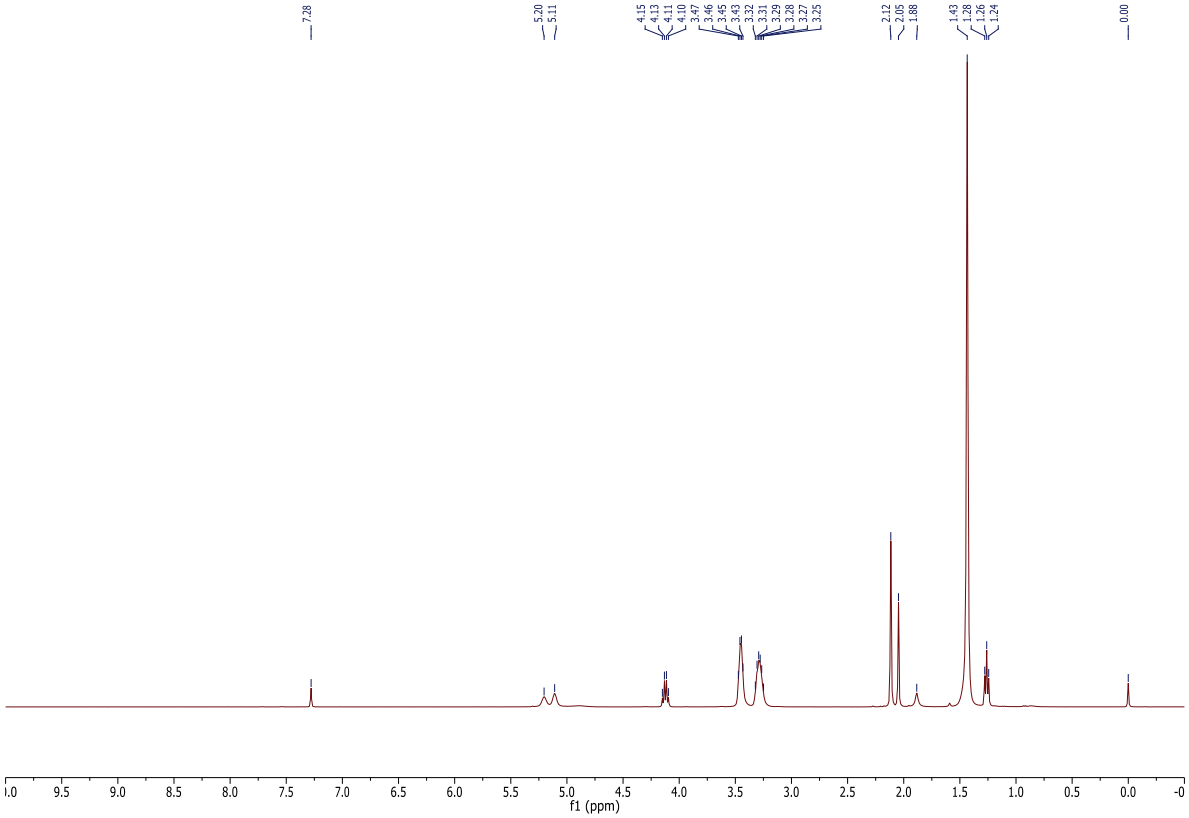


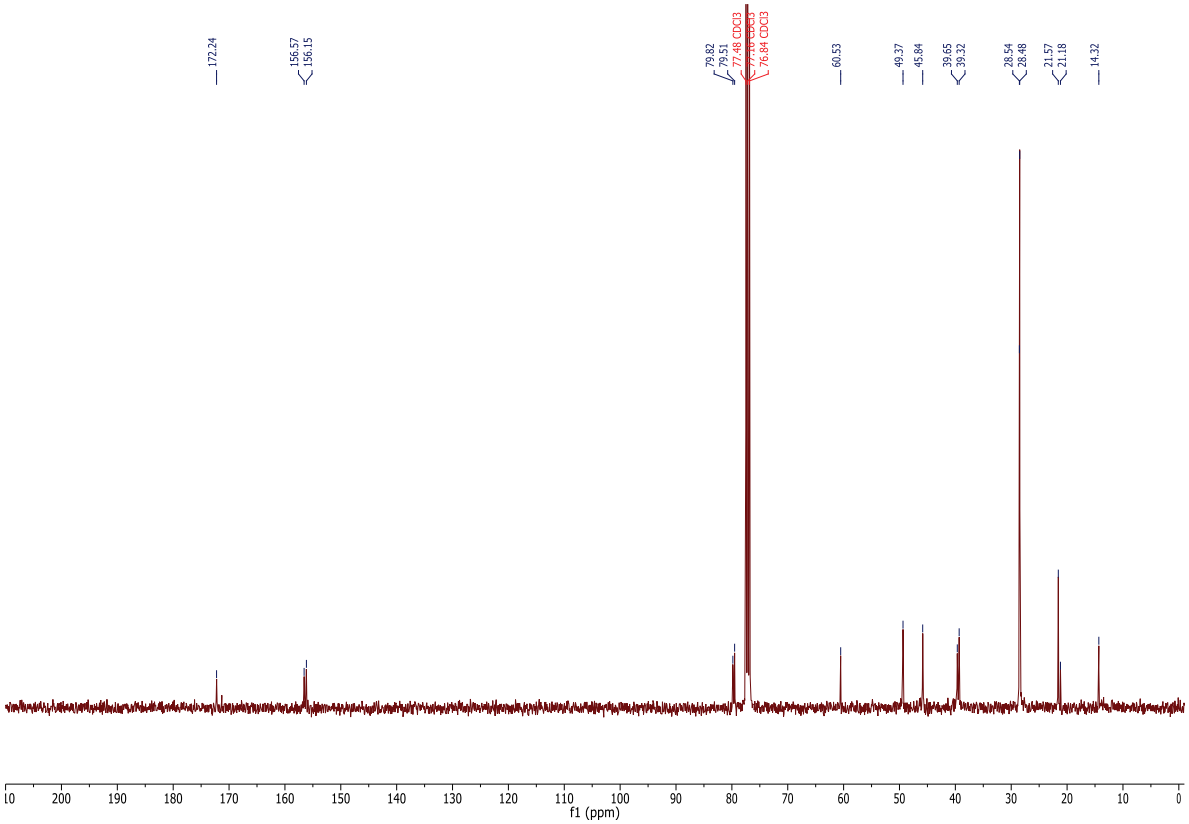


^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of **12** in dichloromethane

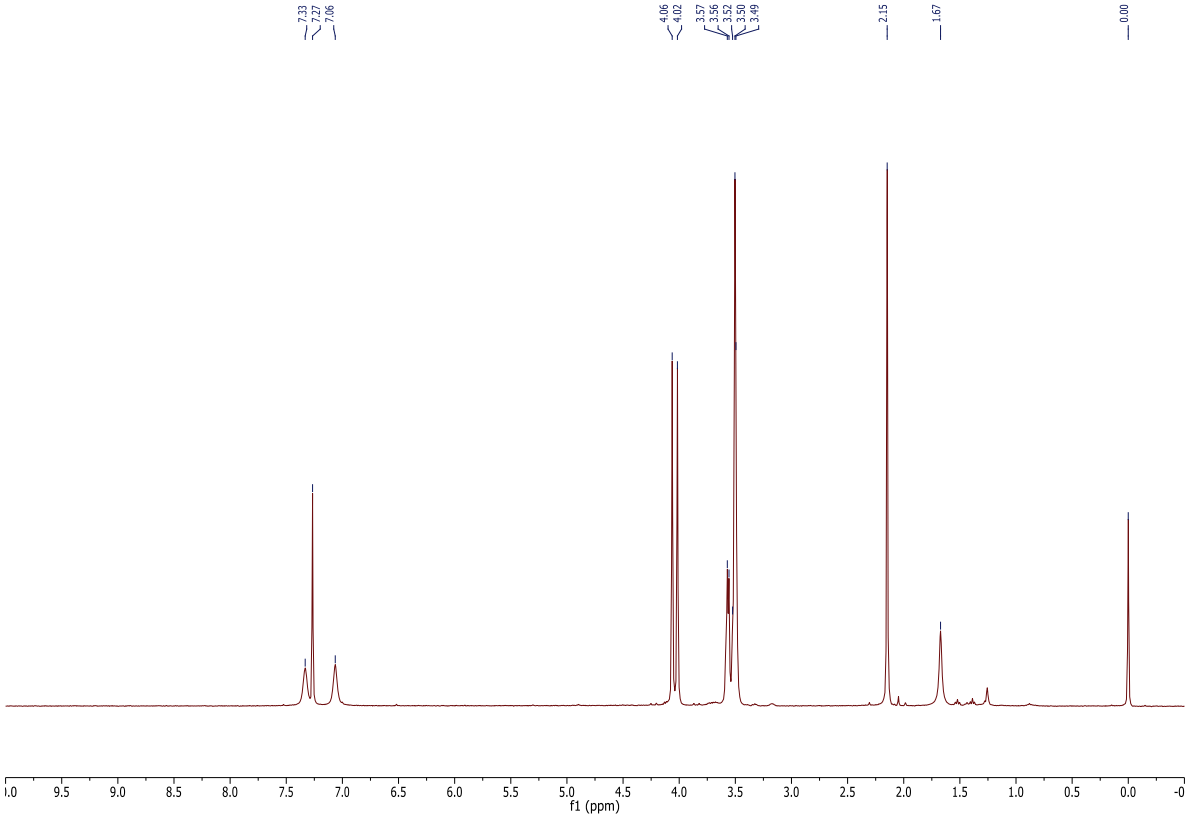


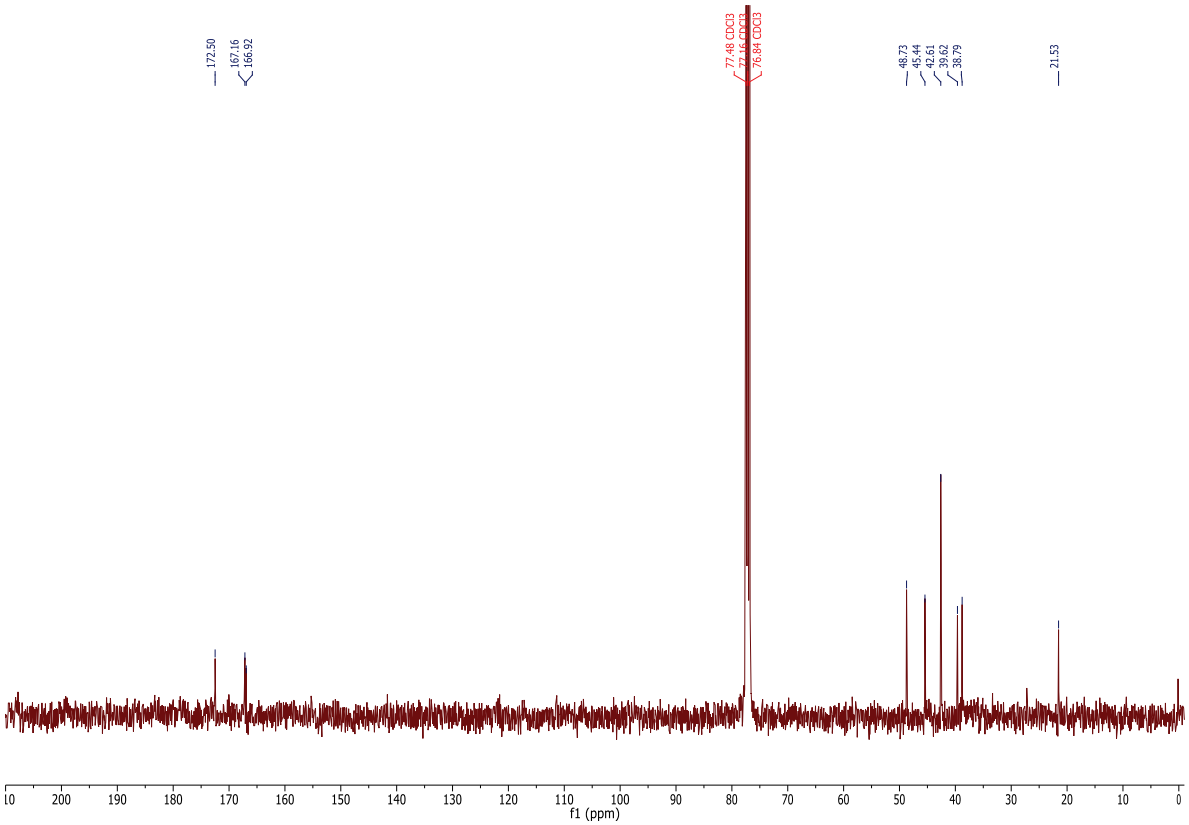
^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of **13** in ethyl acetate



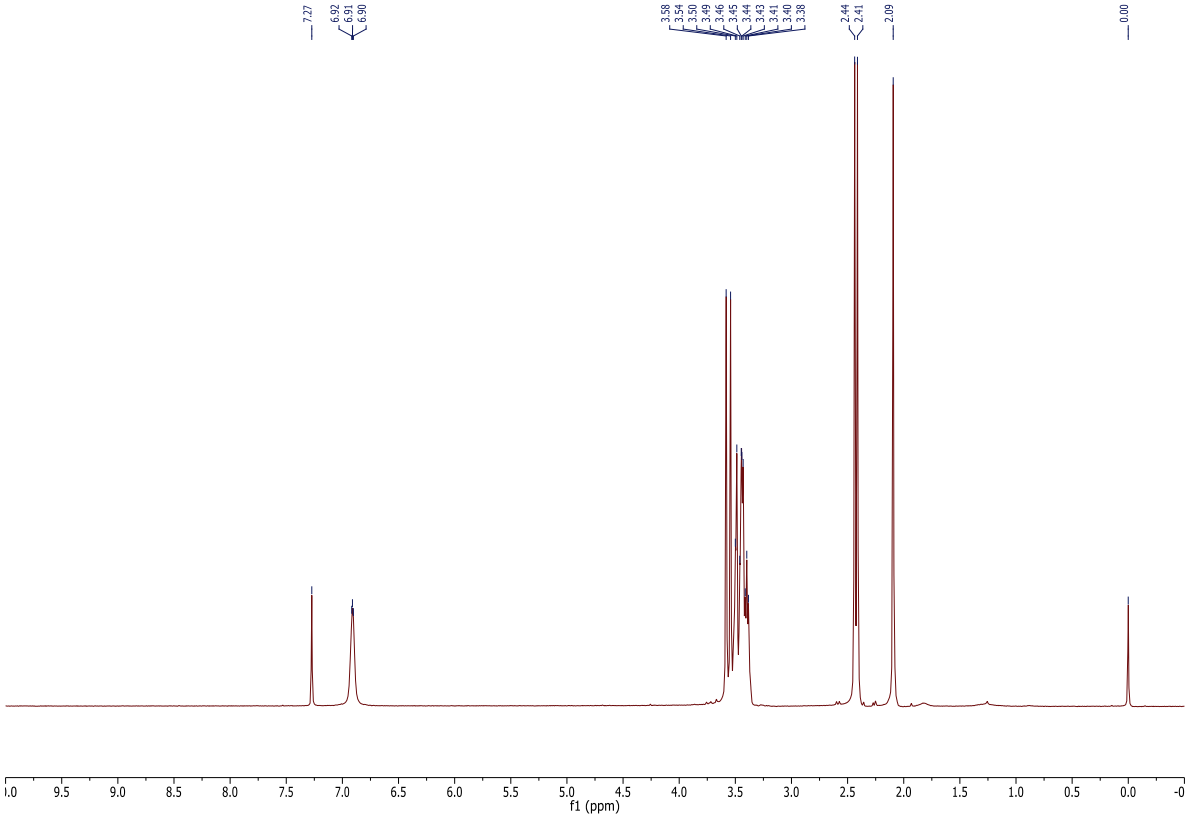


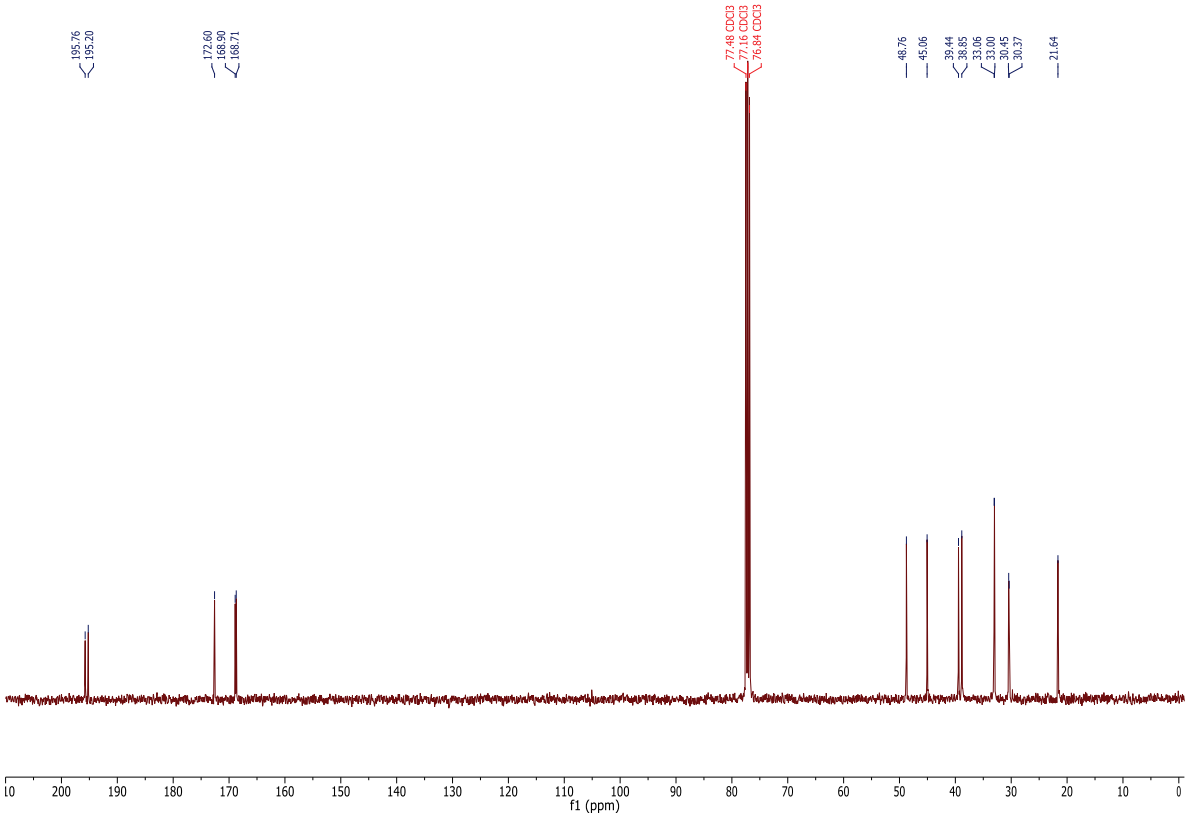
^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of **14**



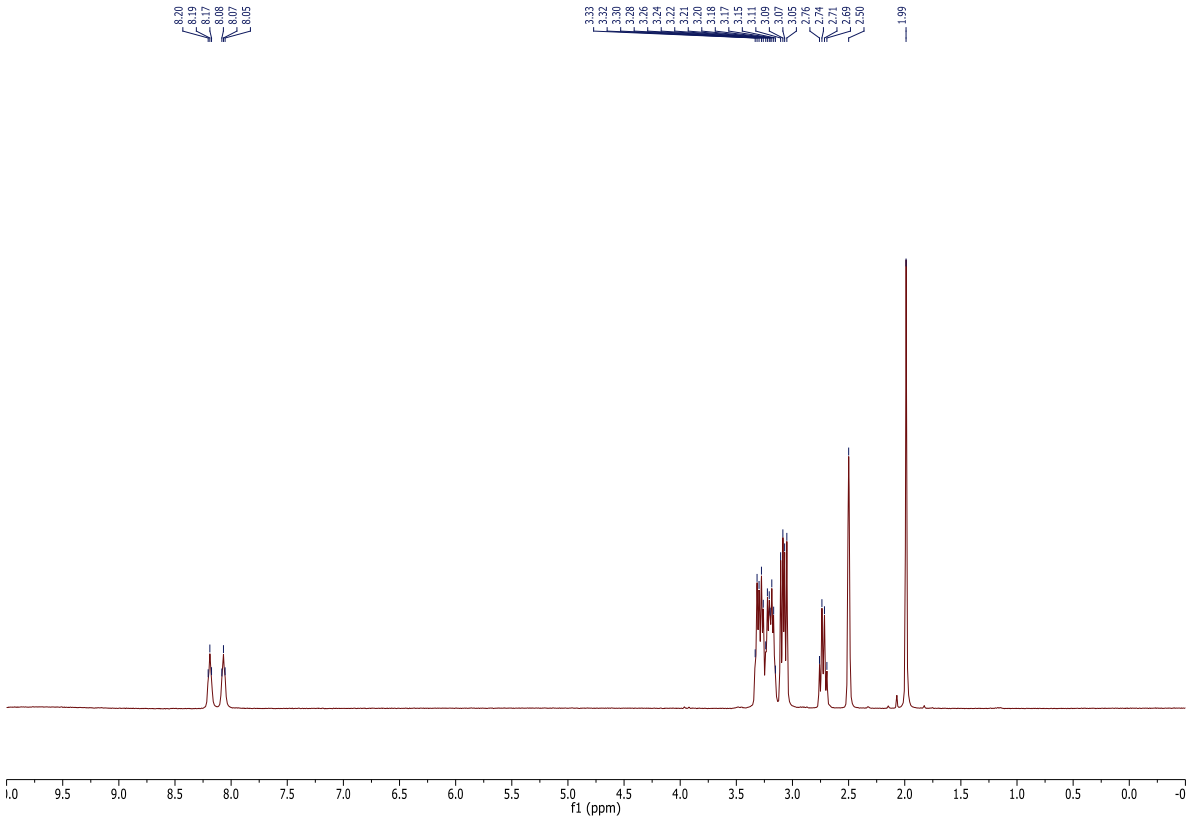


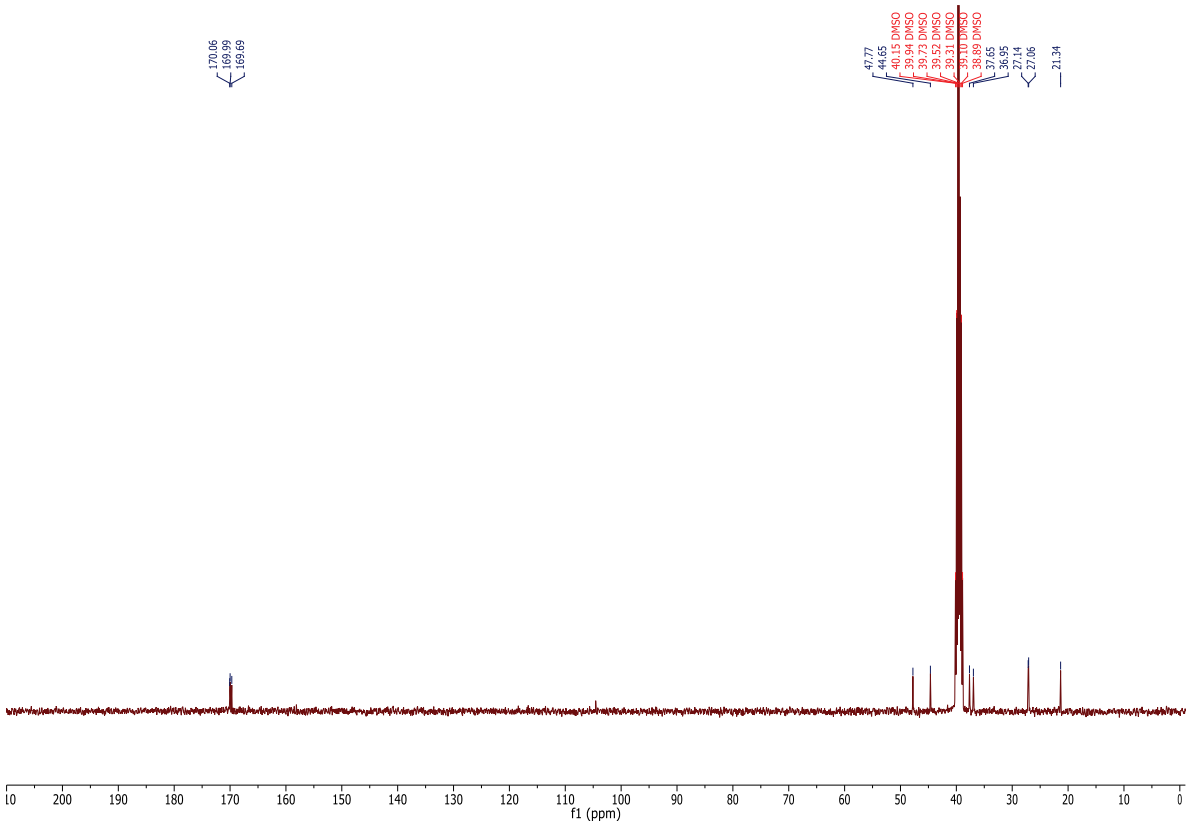
^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of **15**



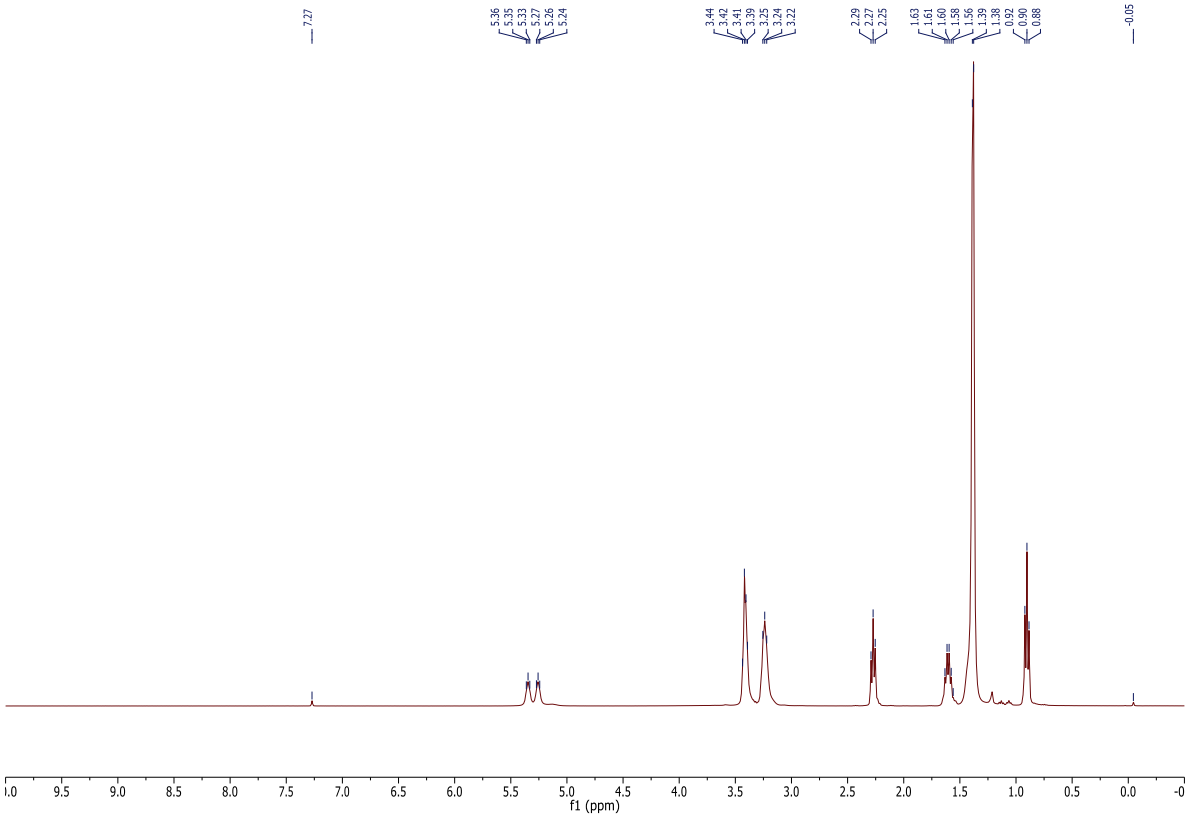


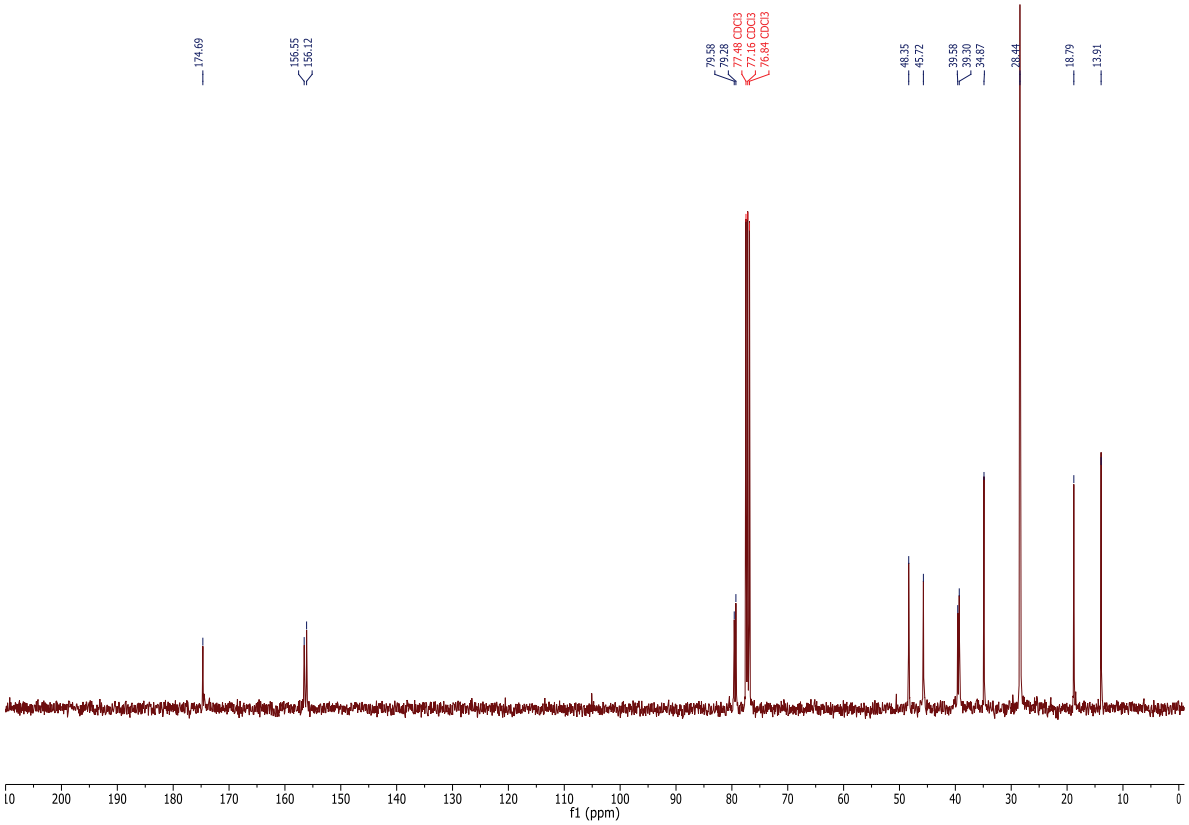
^{1}H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6) of **3**



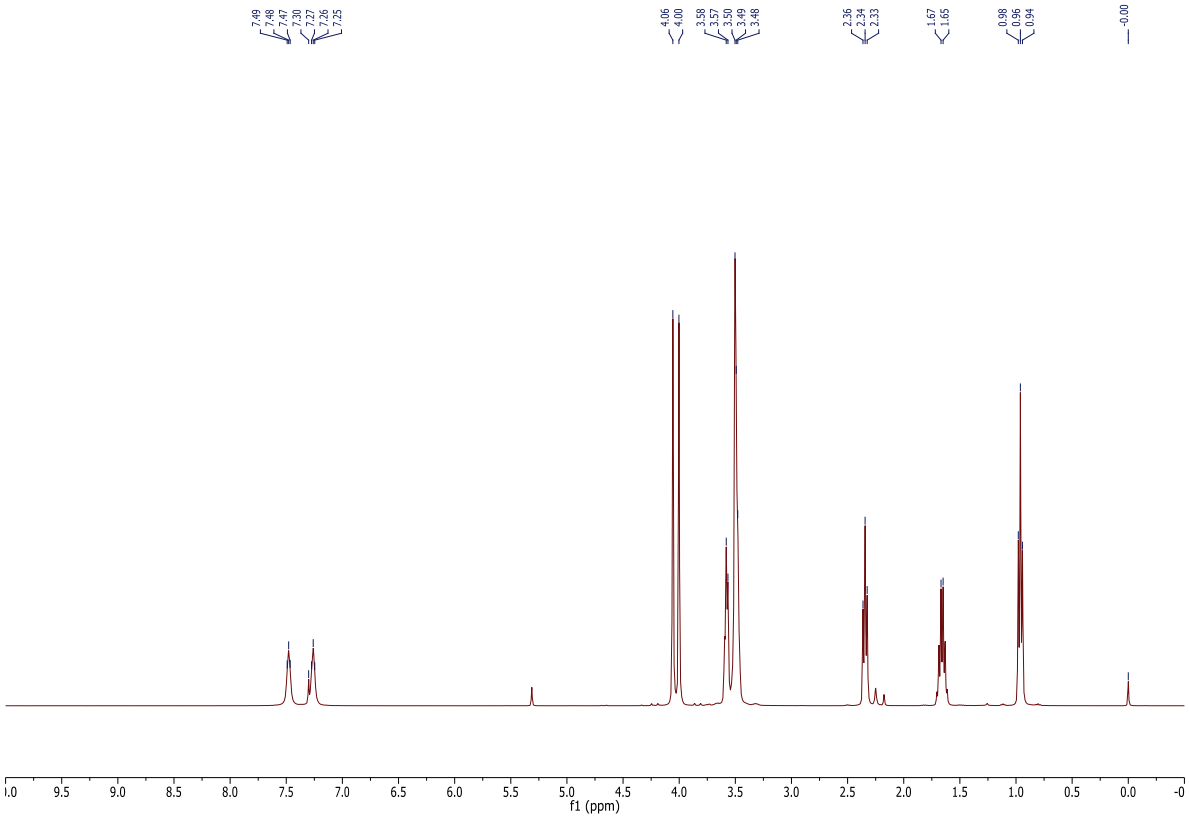


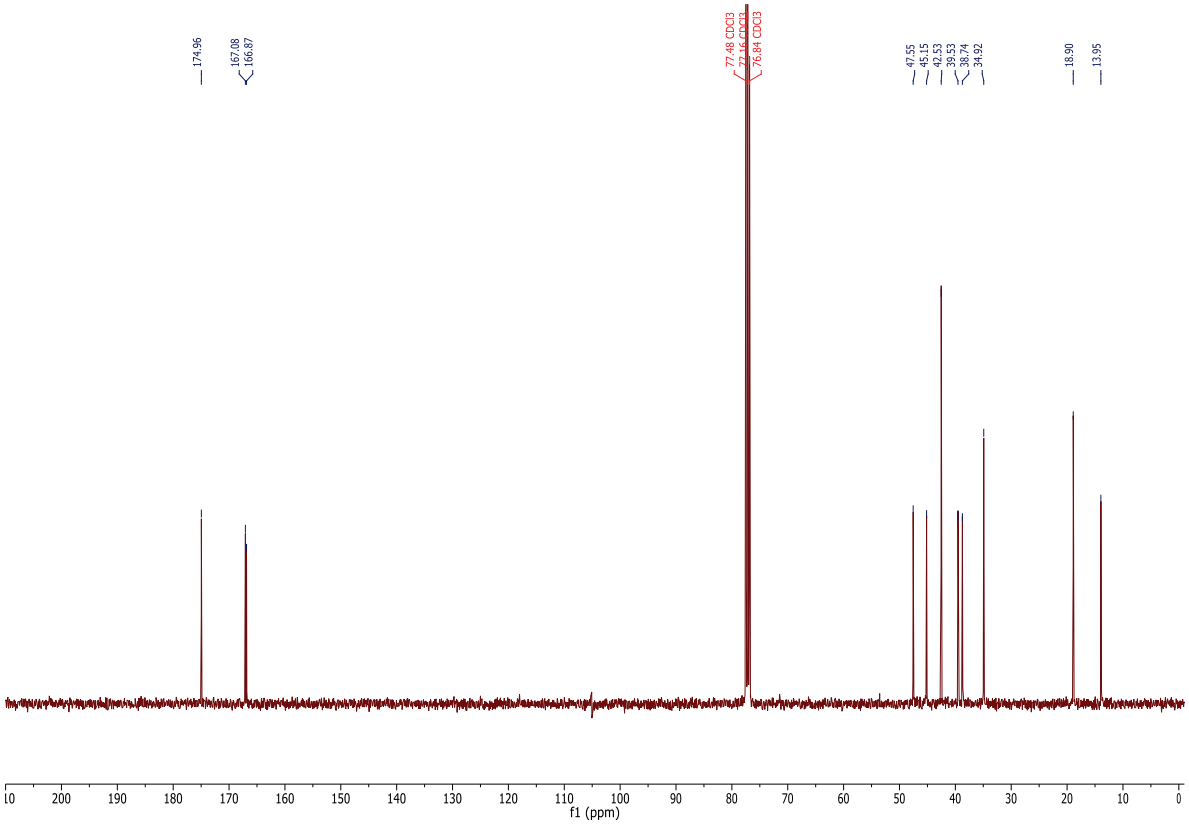
^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of **16**



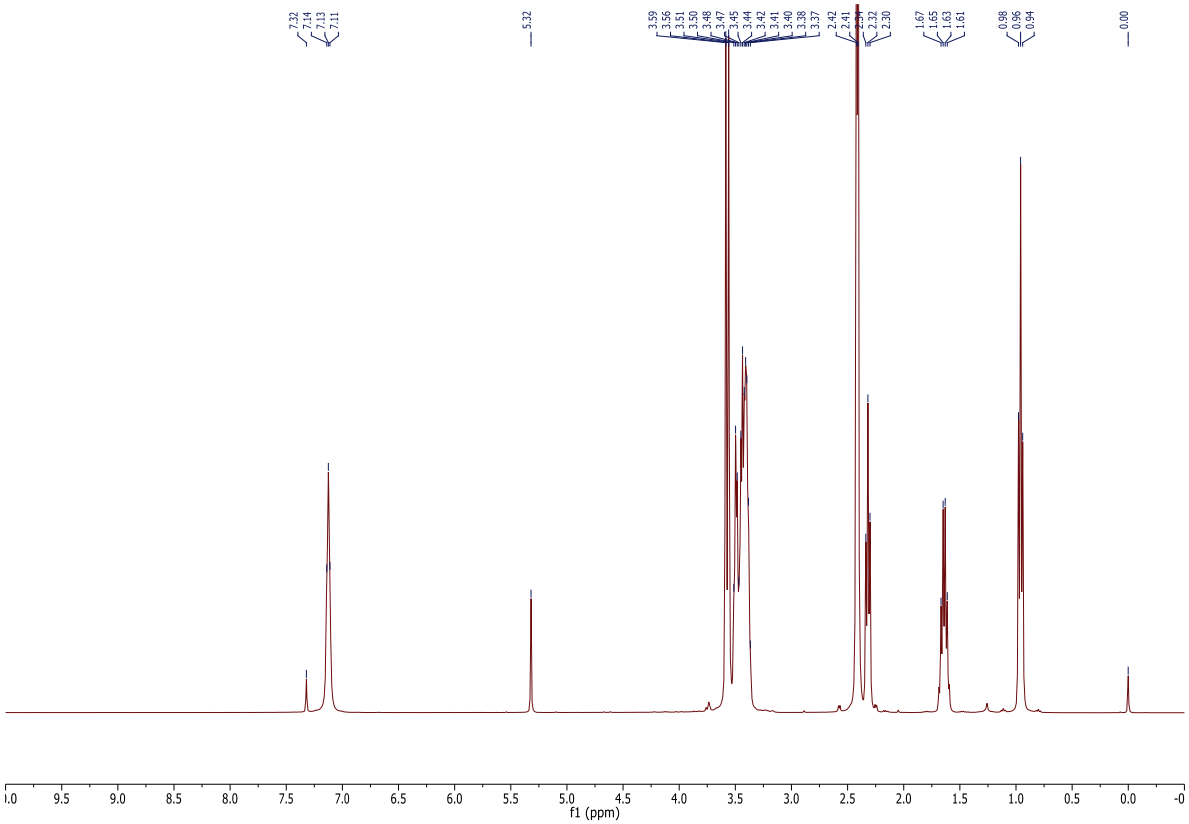


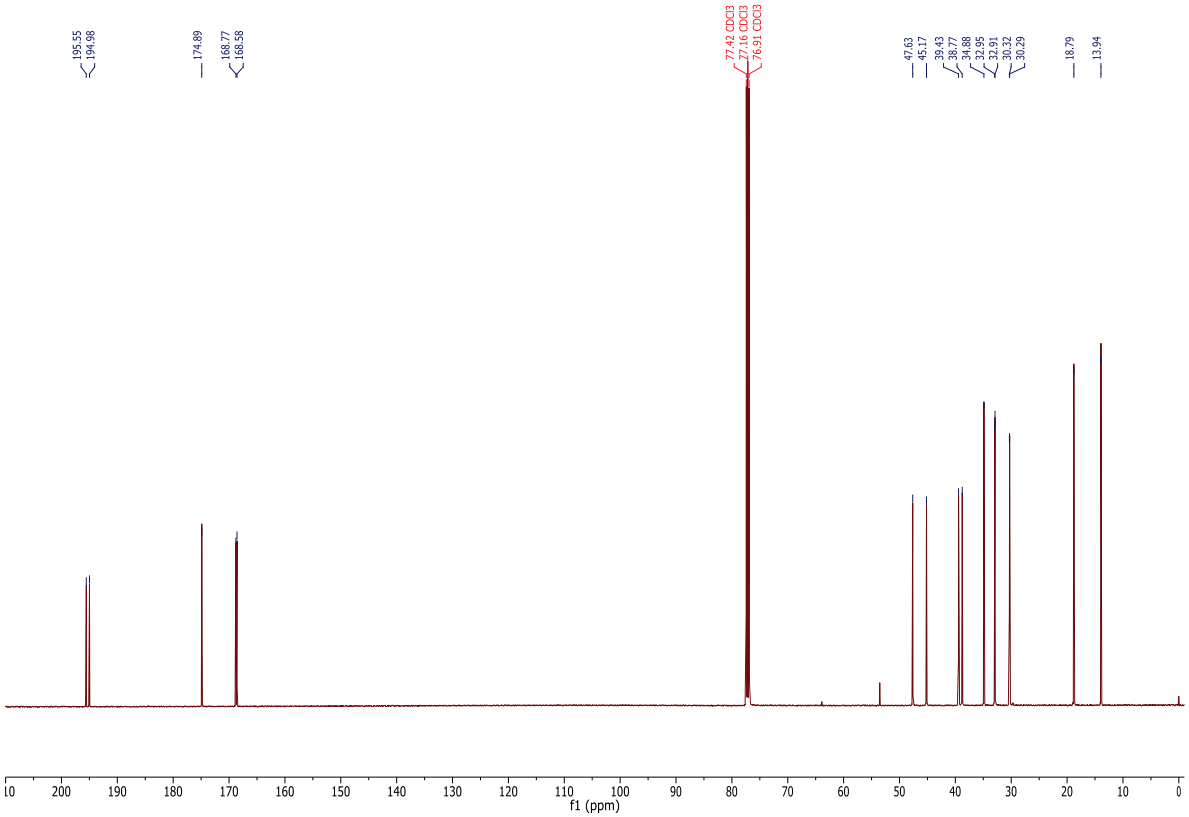
^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of **17**



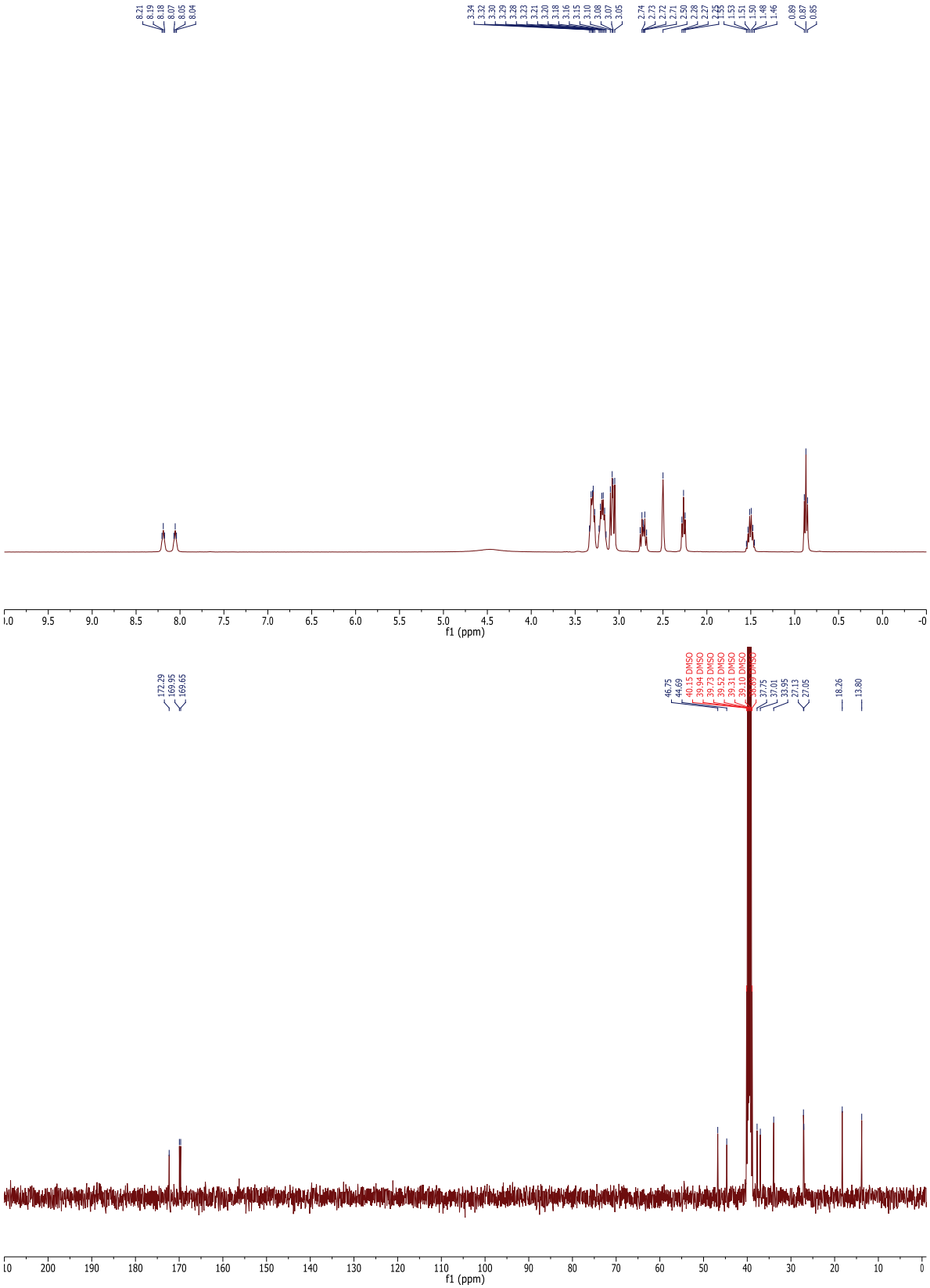


^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of **18** in dichloromethane

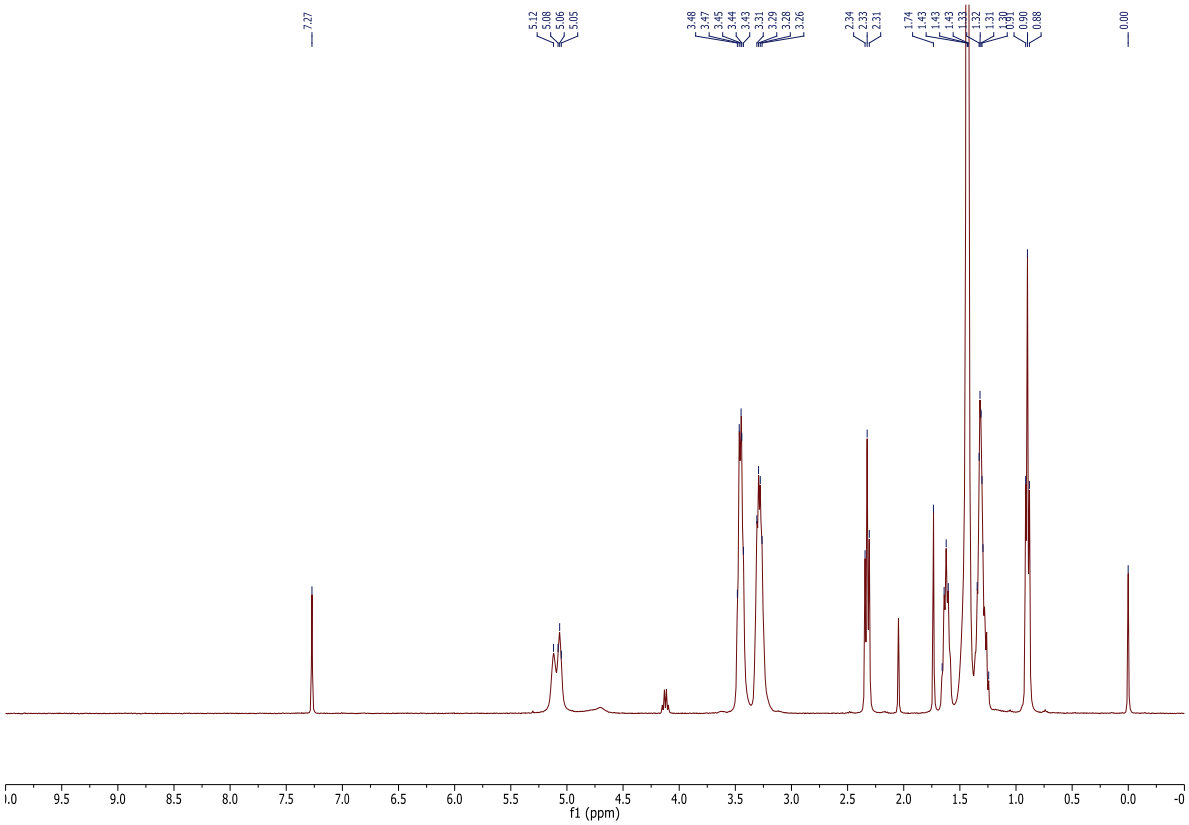


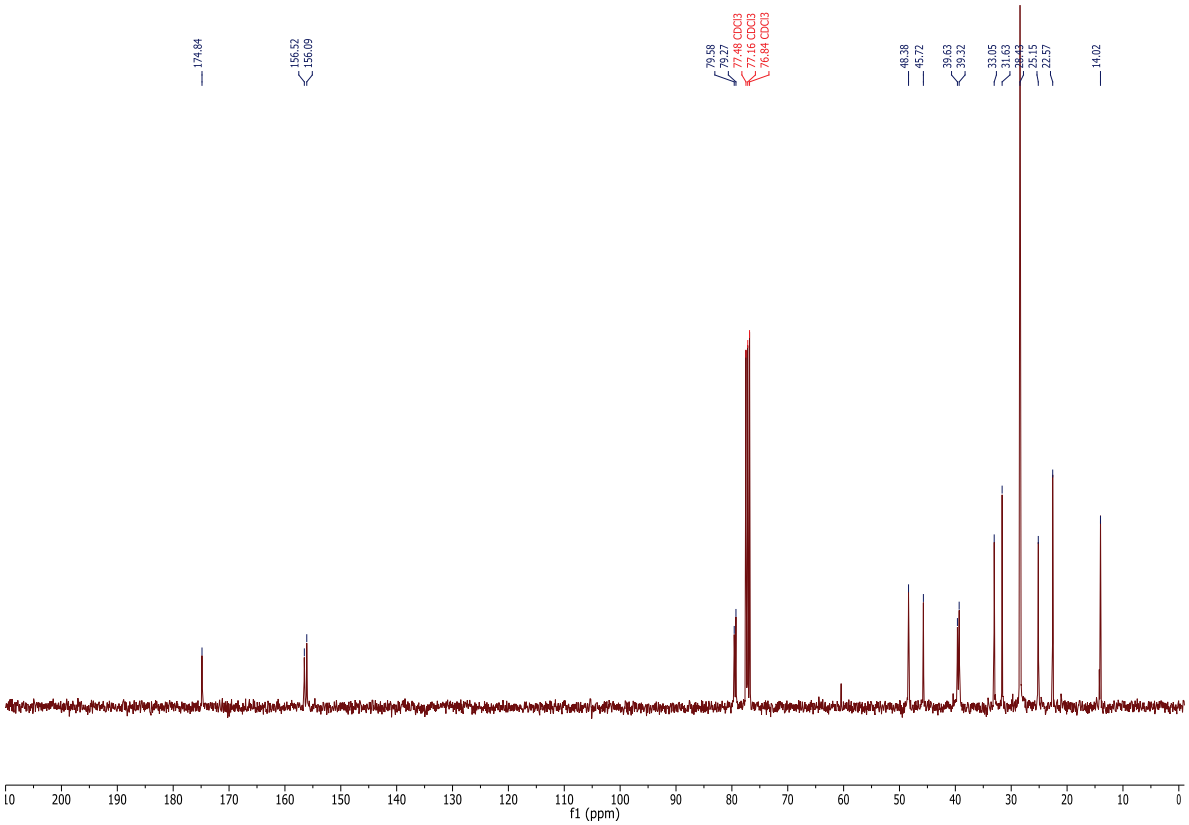


^{1}H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6) of **4**

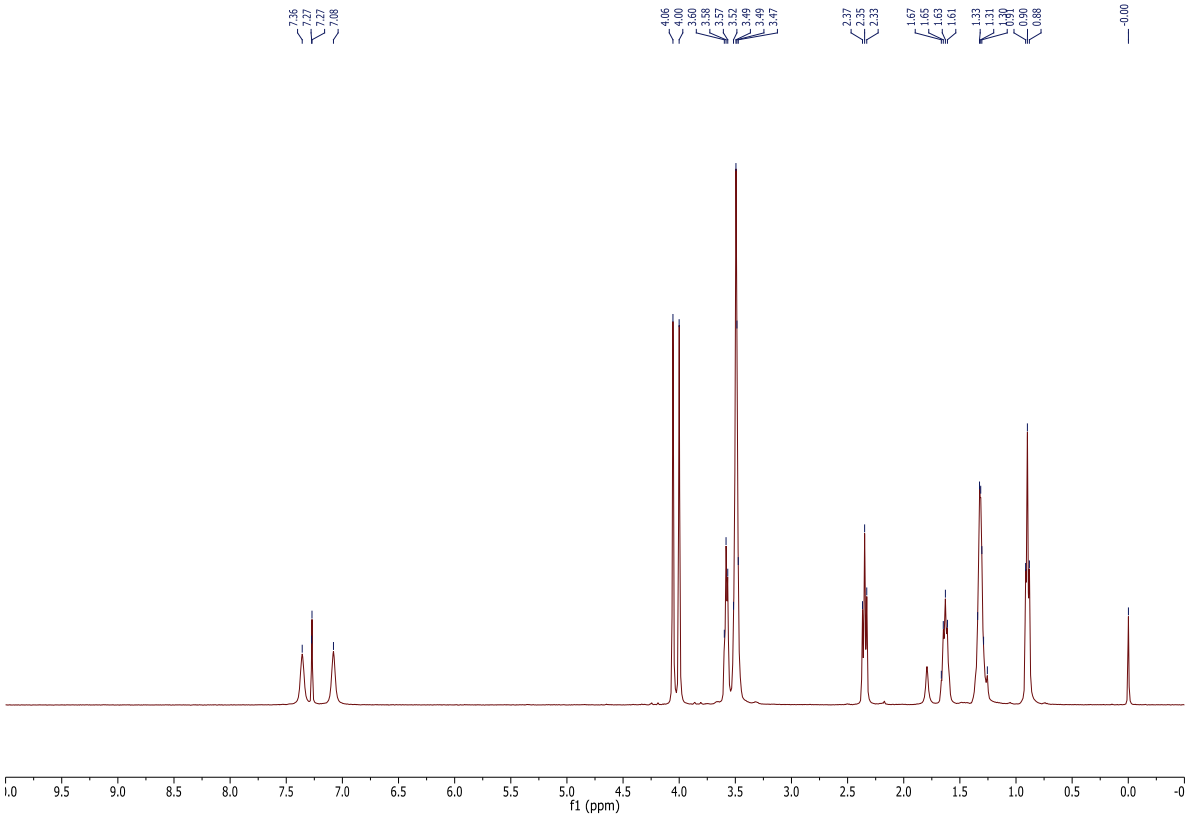


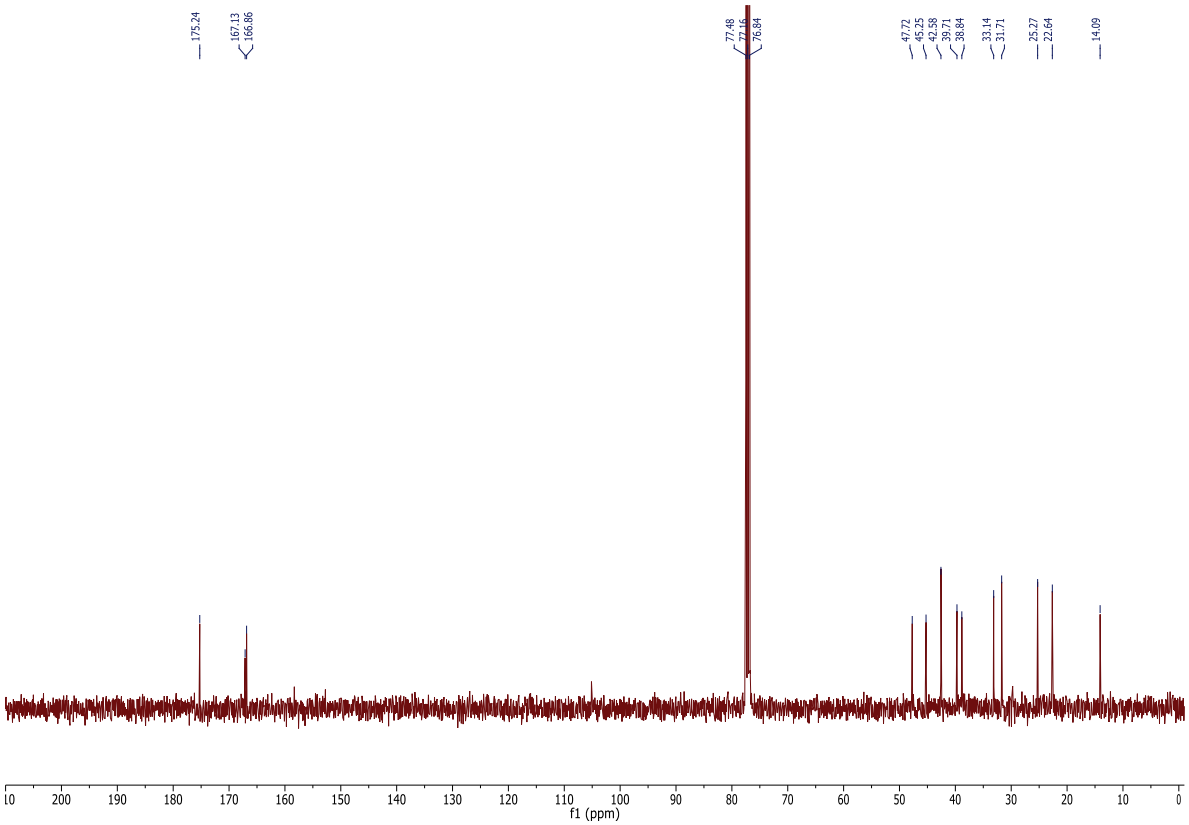
^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of **19** in ethyl acetate



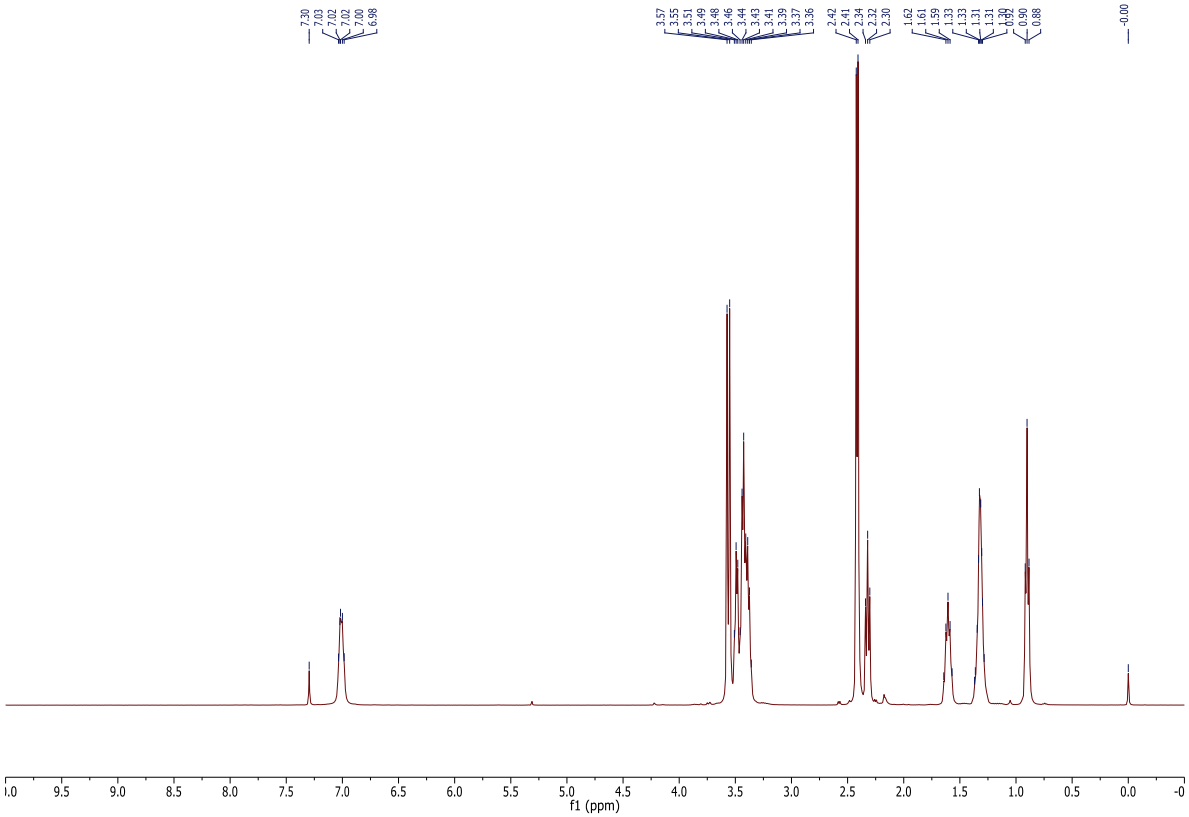


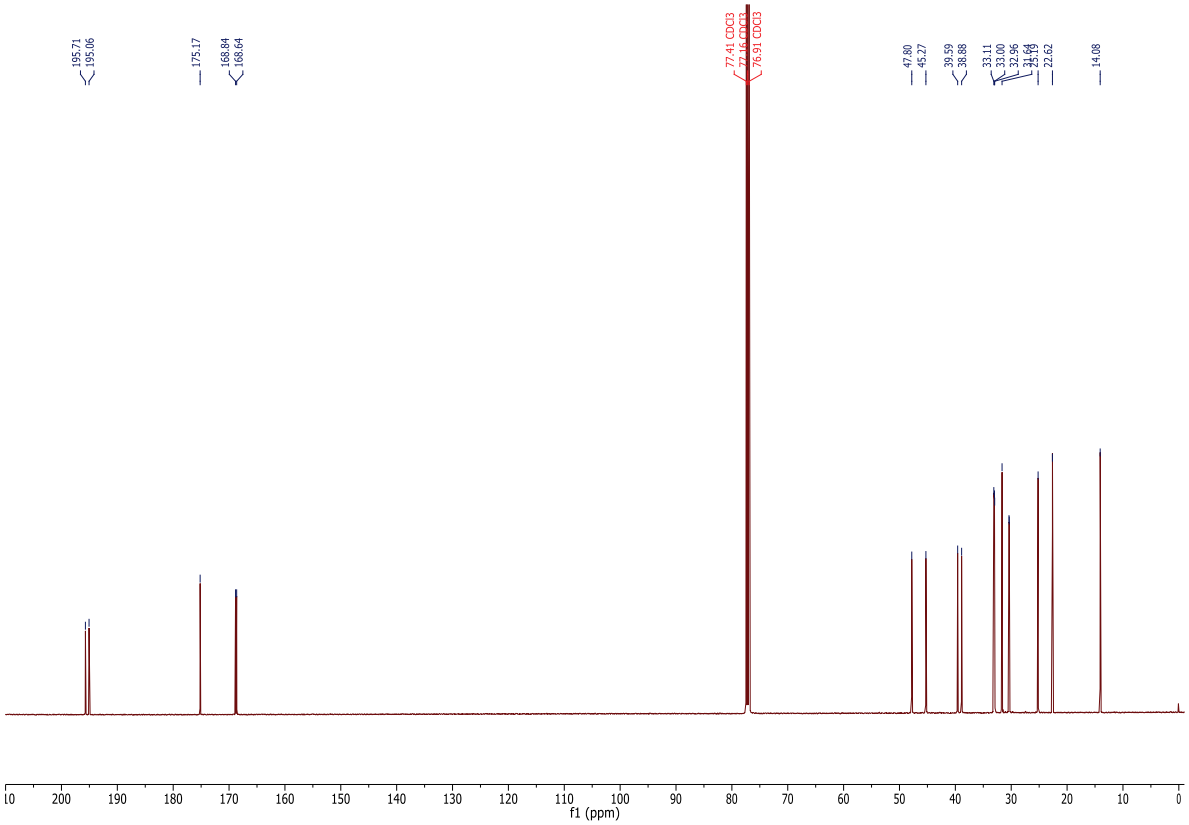
^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of **20**



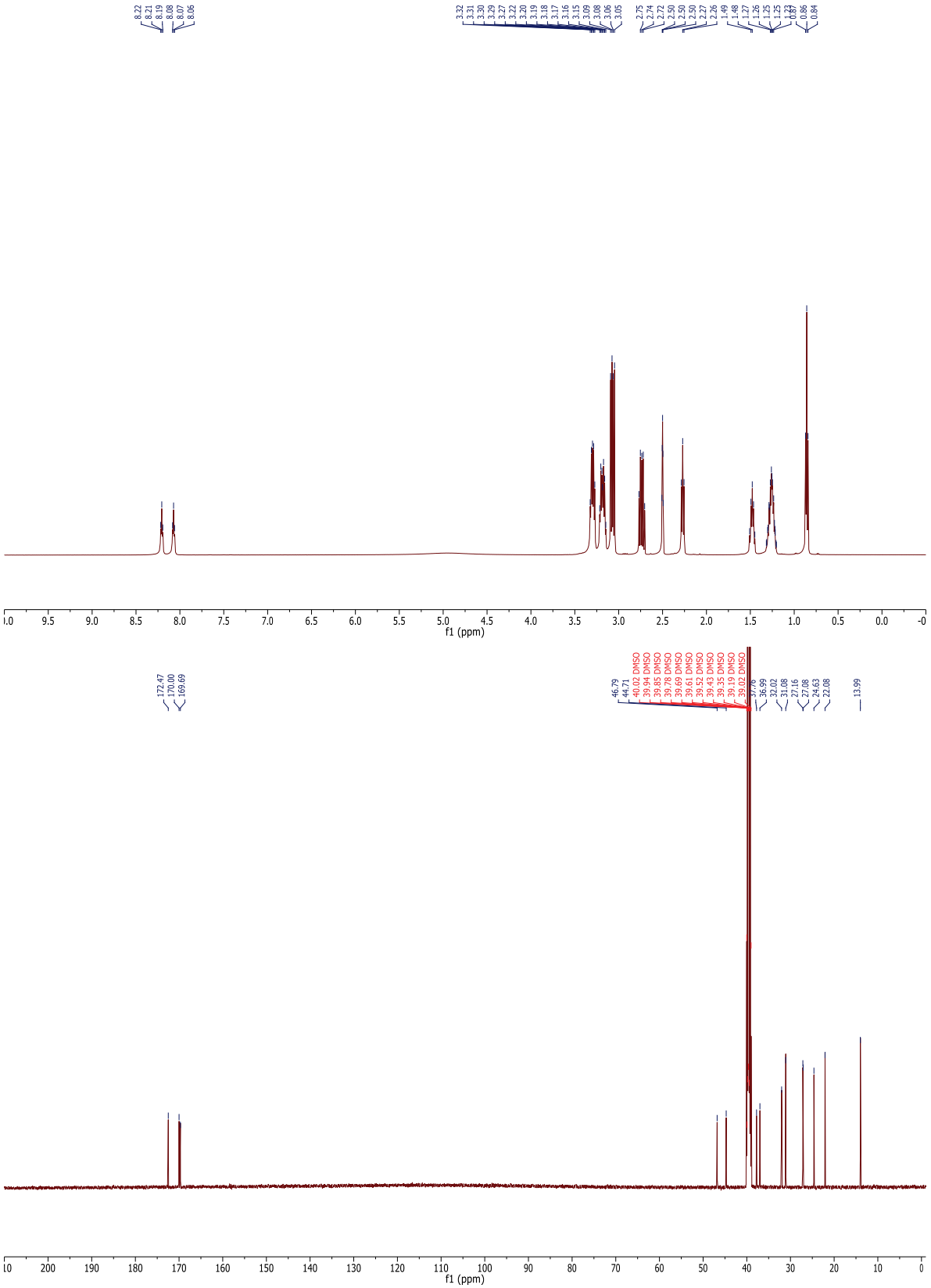


^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of **21**

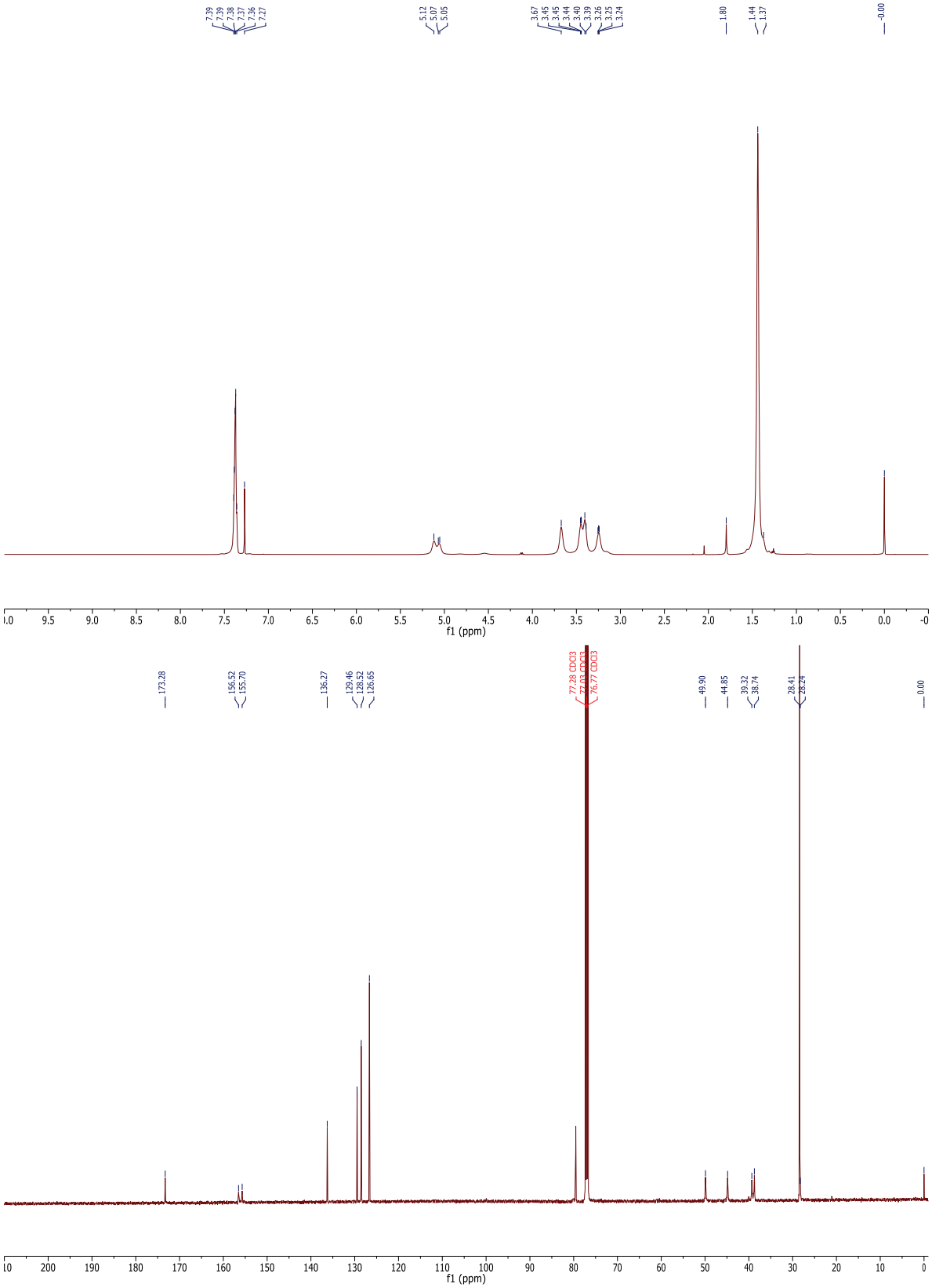




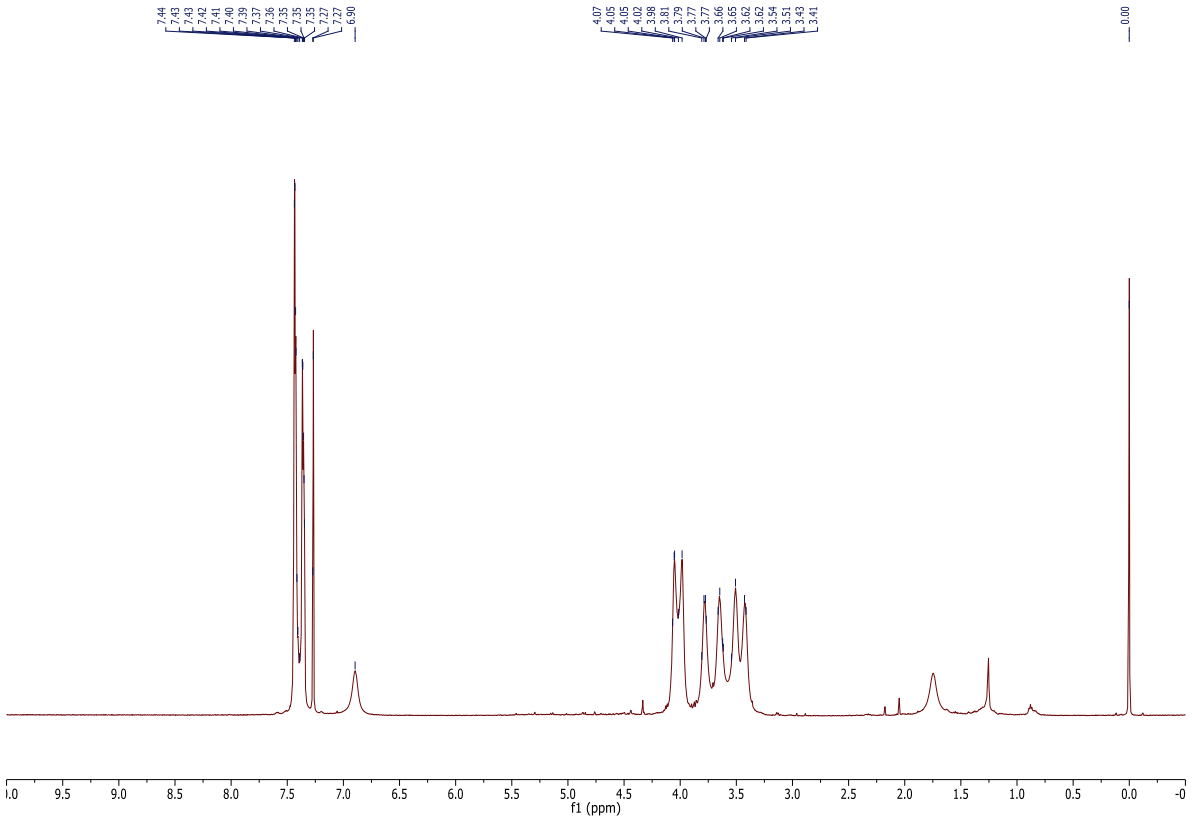
^{1}H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6) of **5**

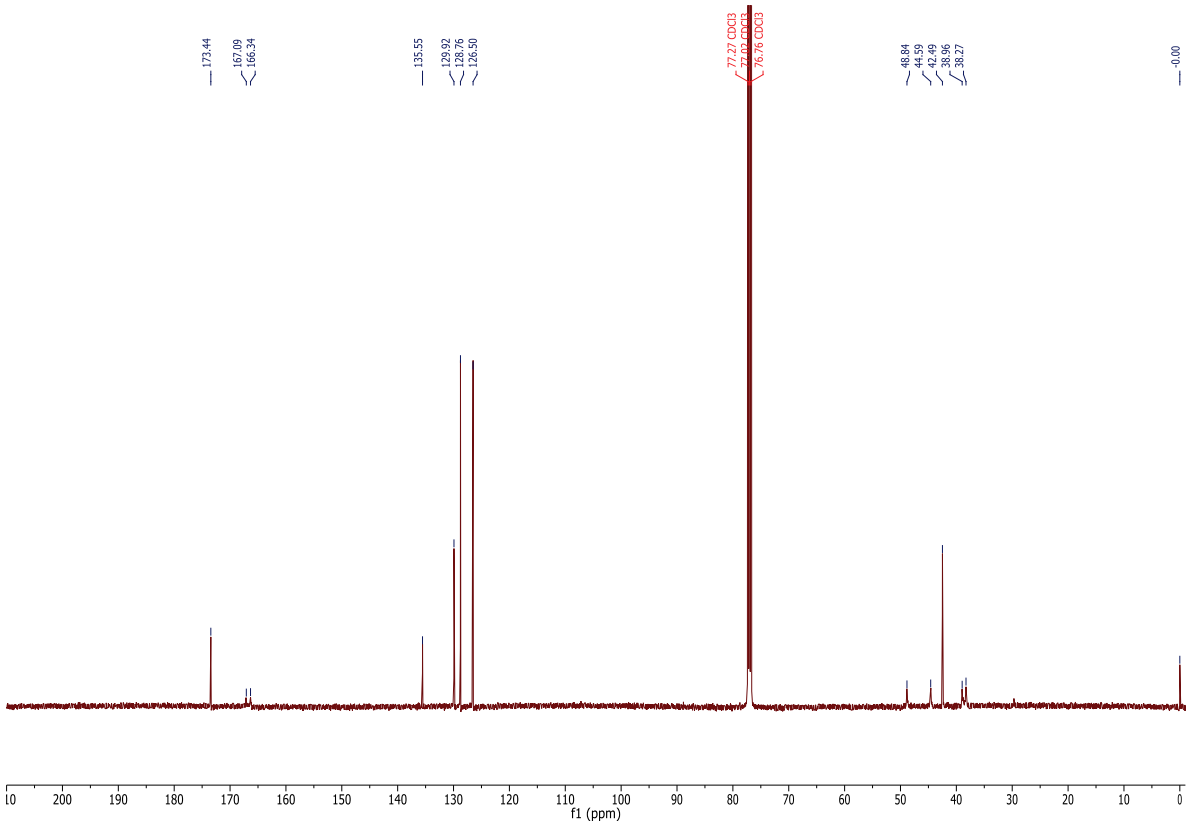


^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of **22**

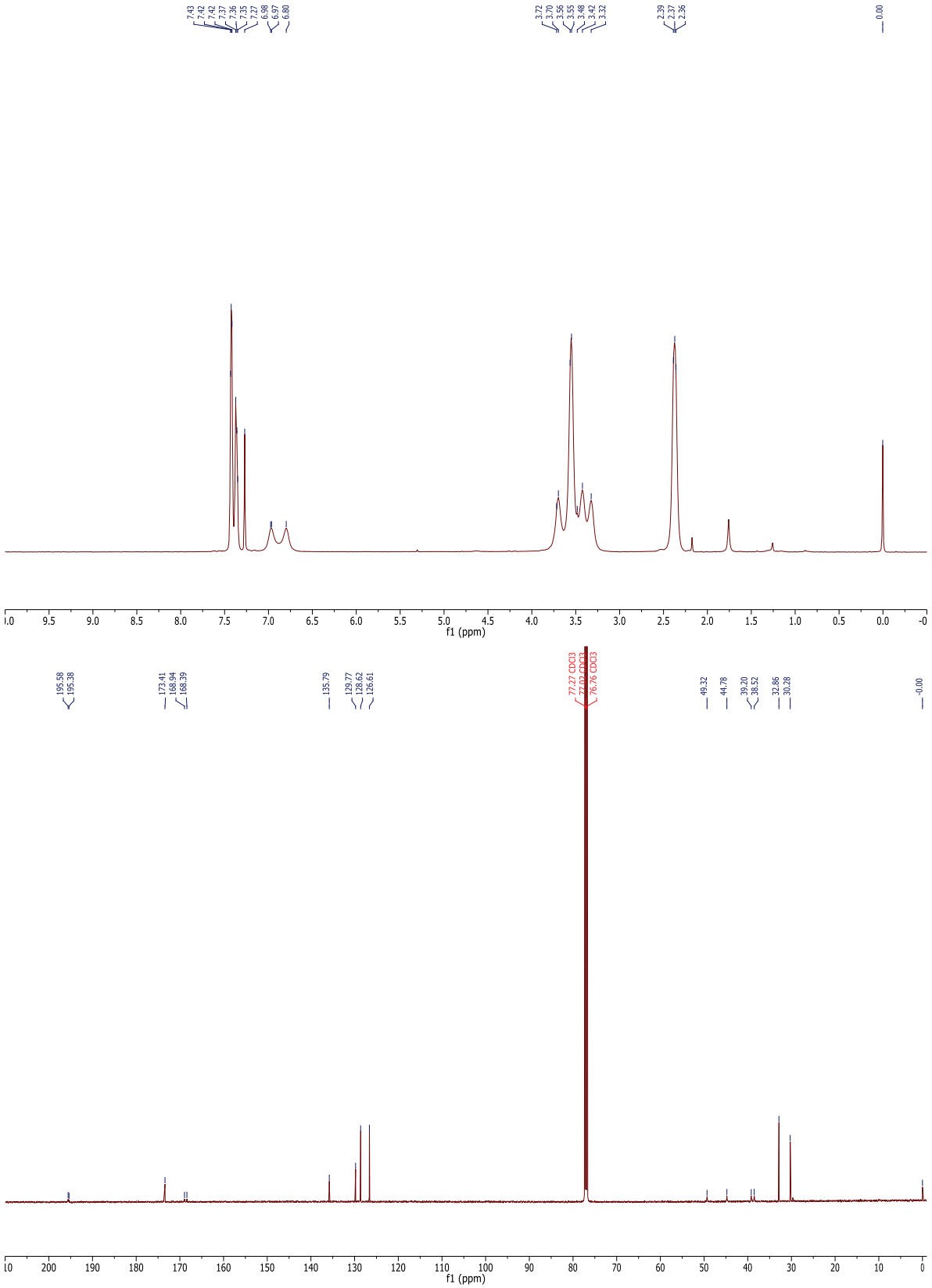


^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of **23**

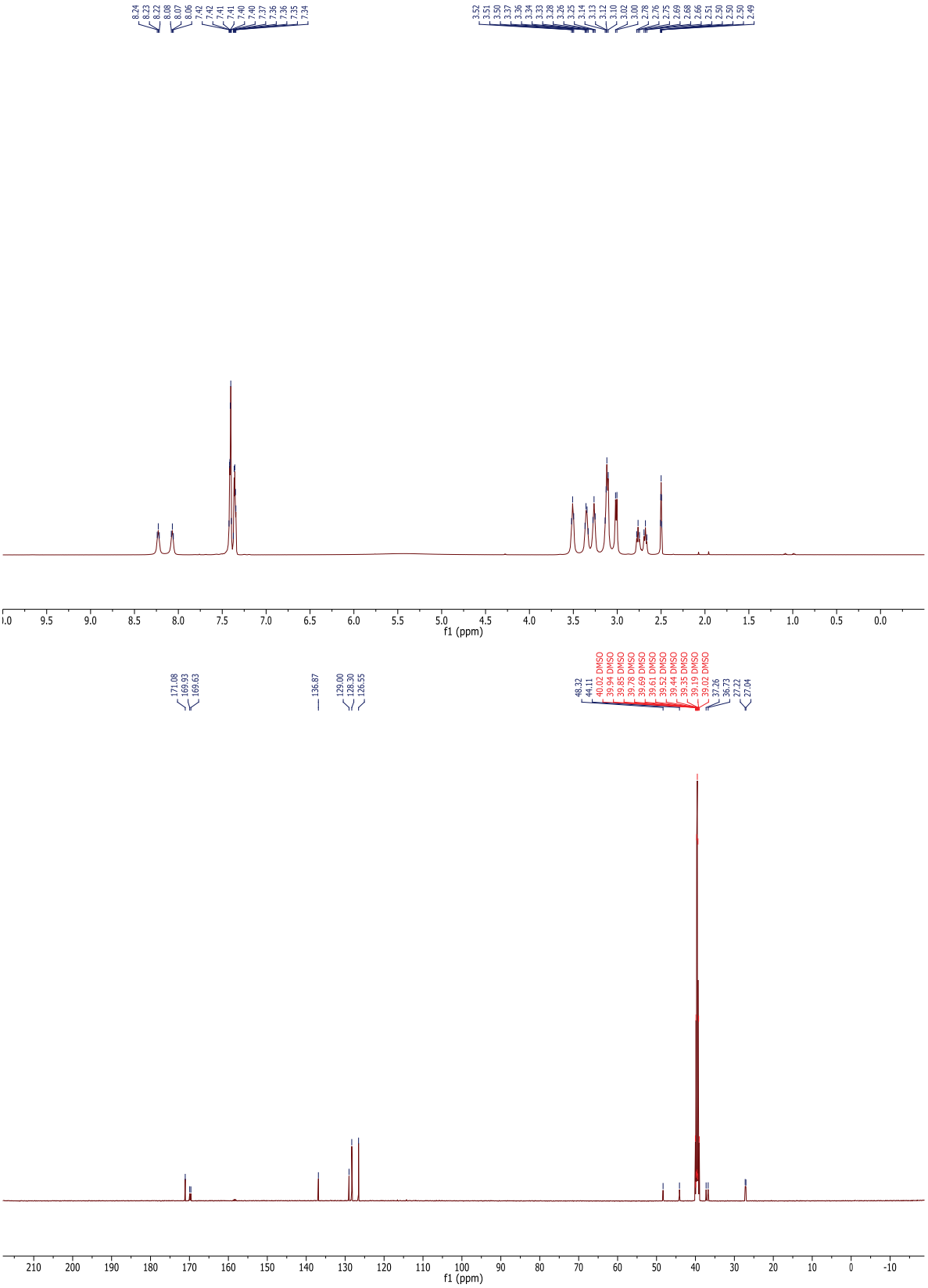




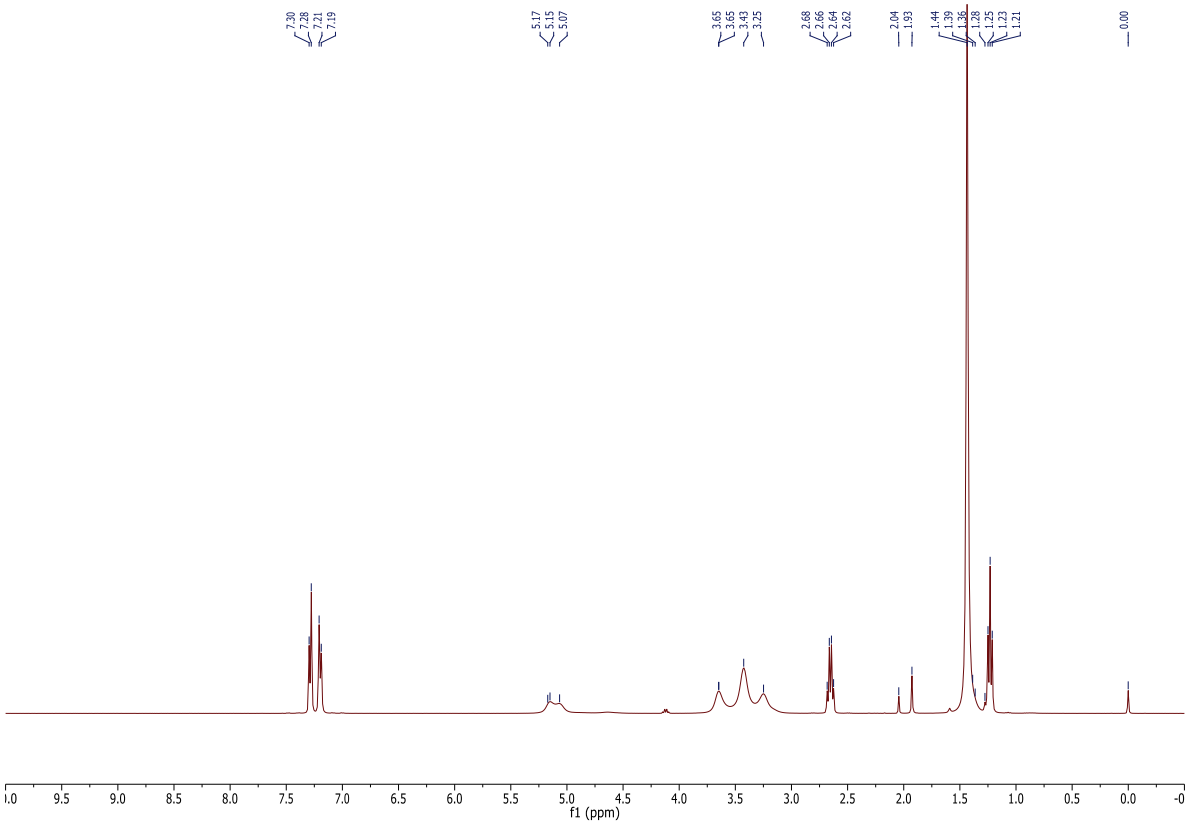
^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of **24**

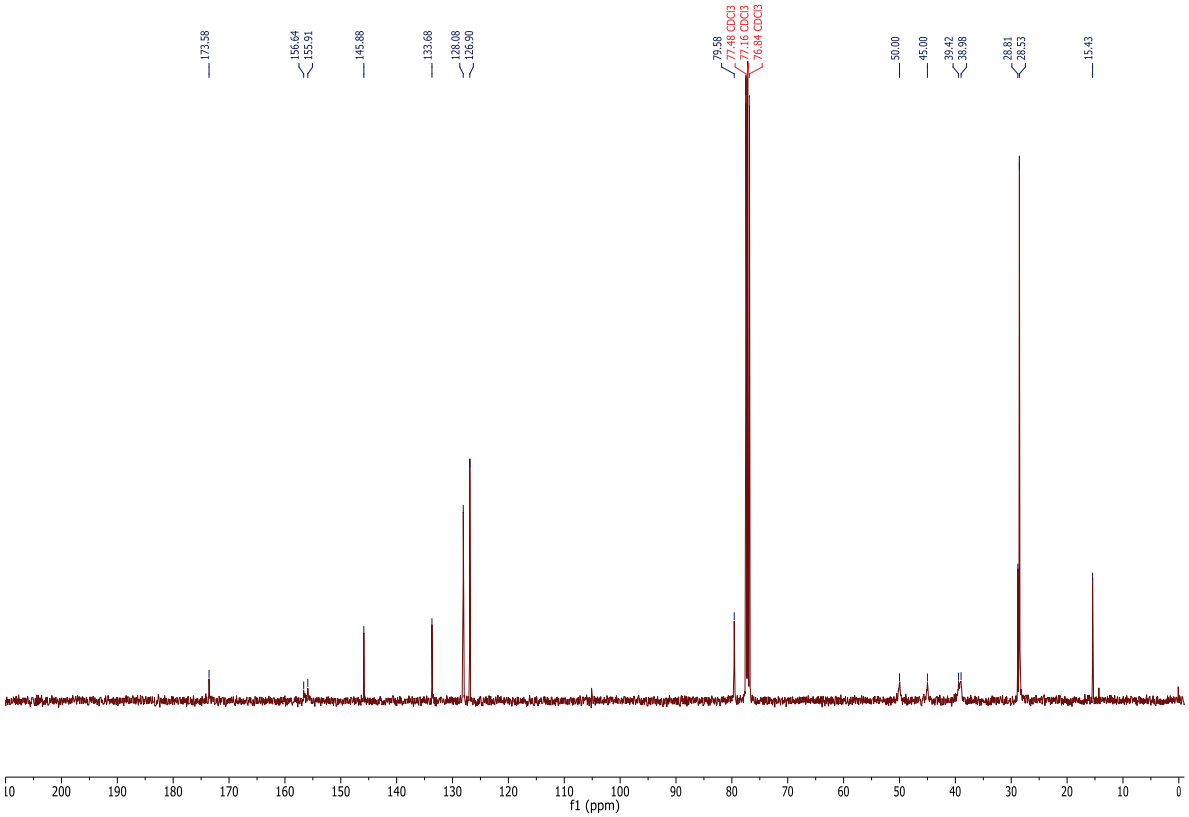


^{1}H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6) of **6**

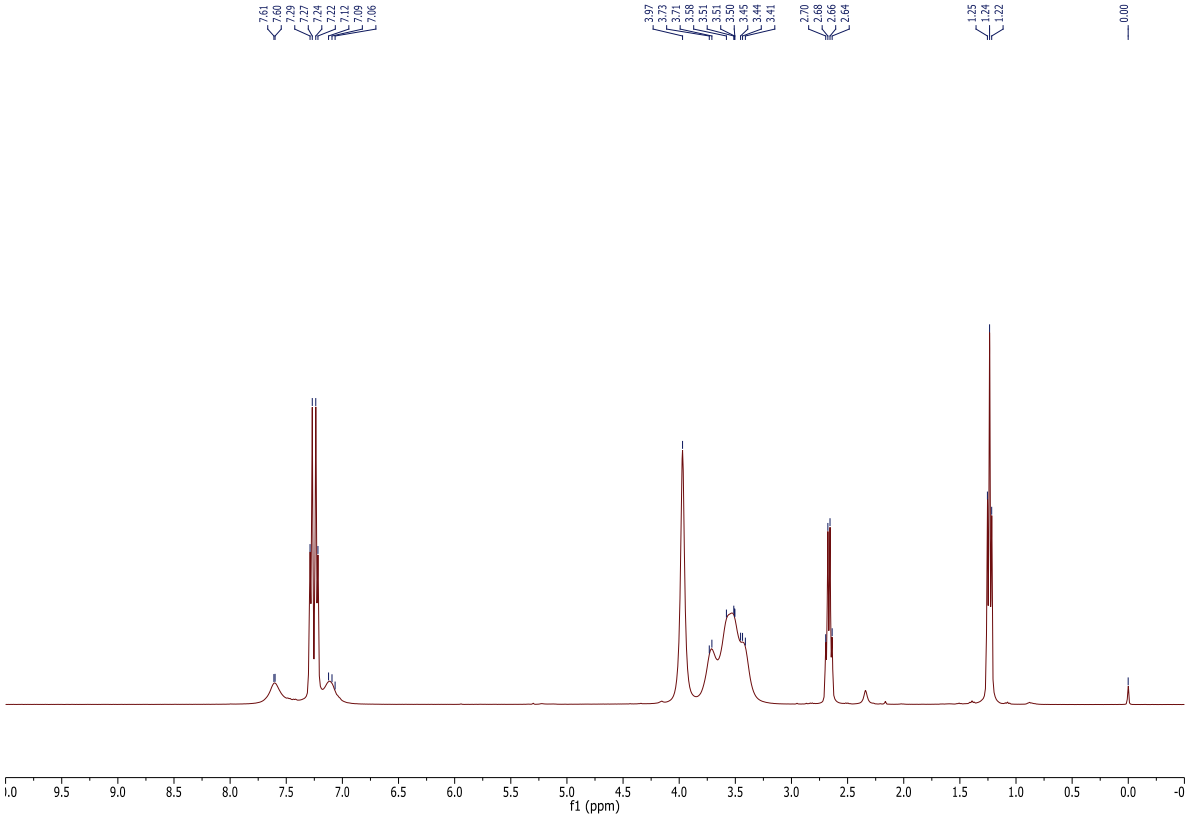


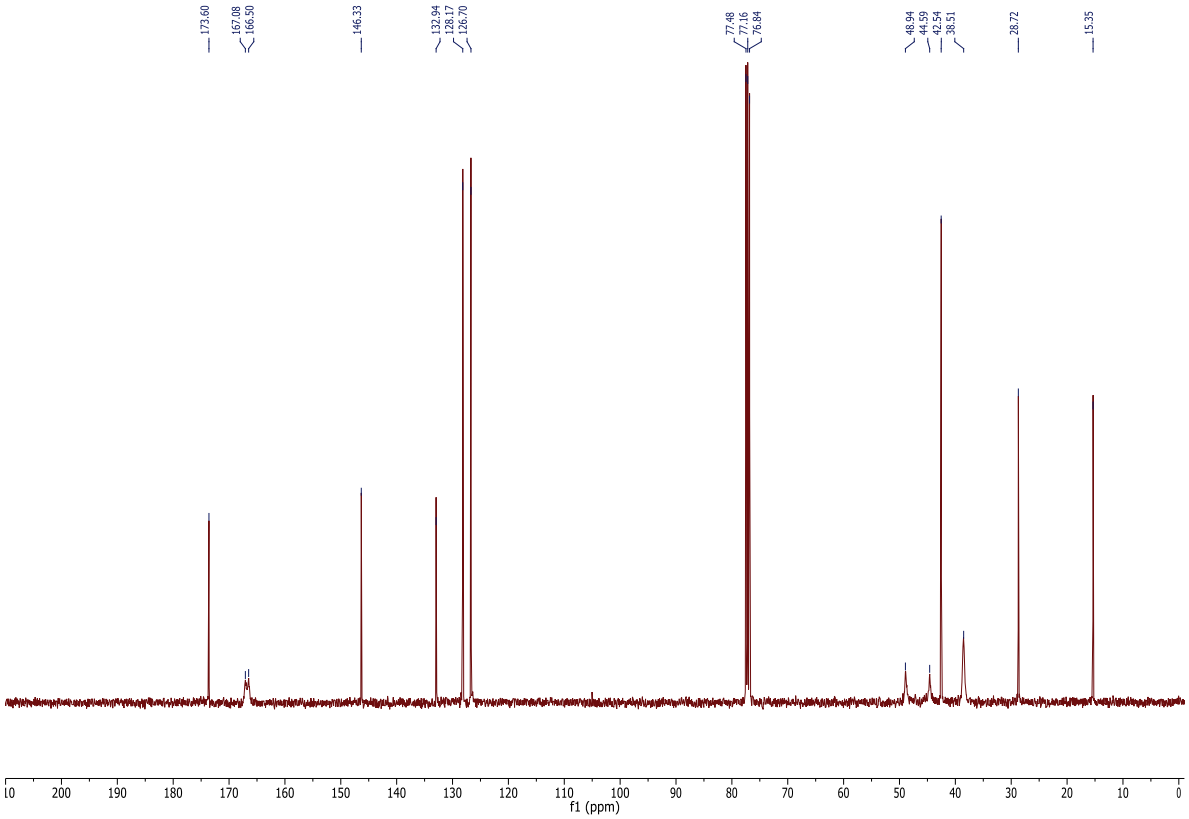
^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of **25**



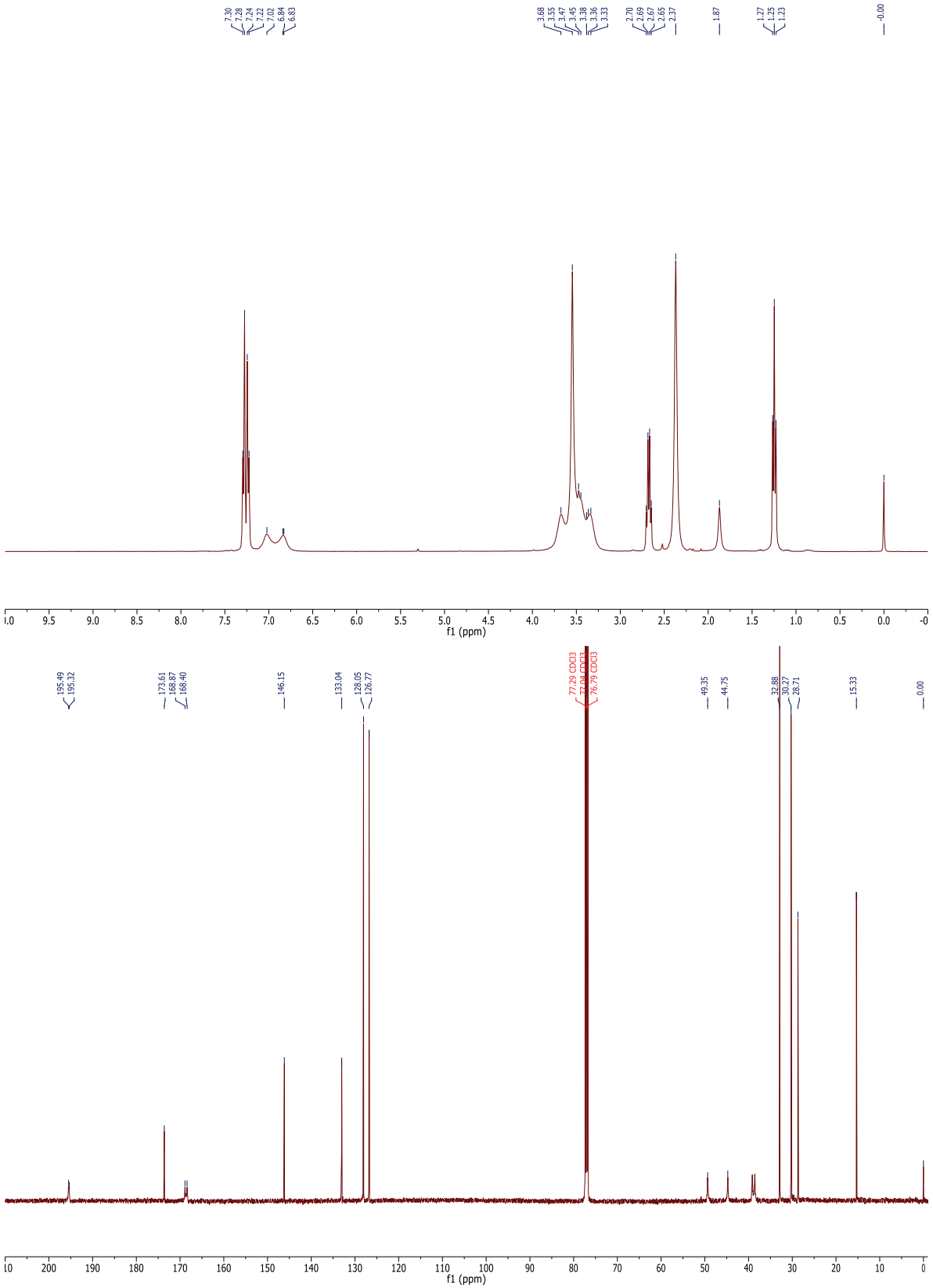


^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of **26**

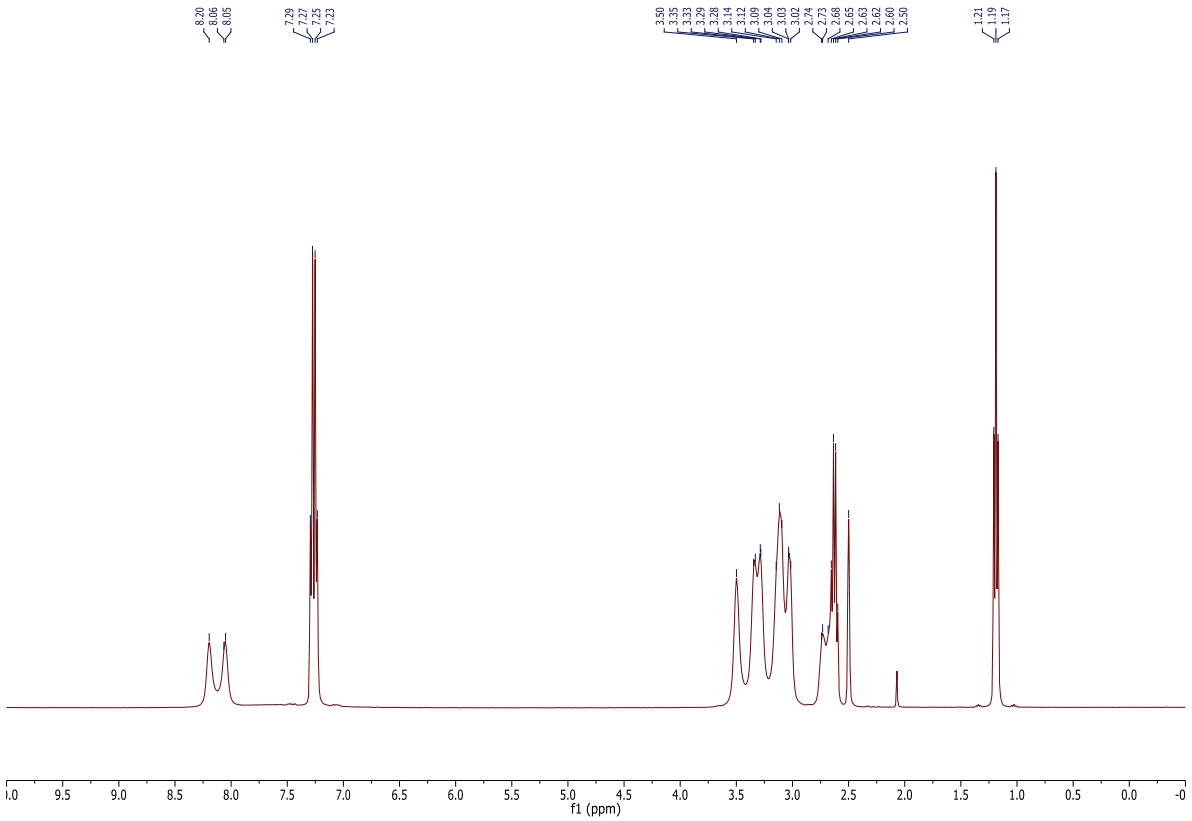


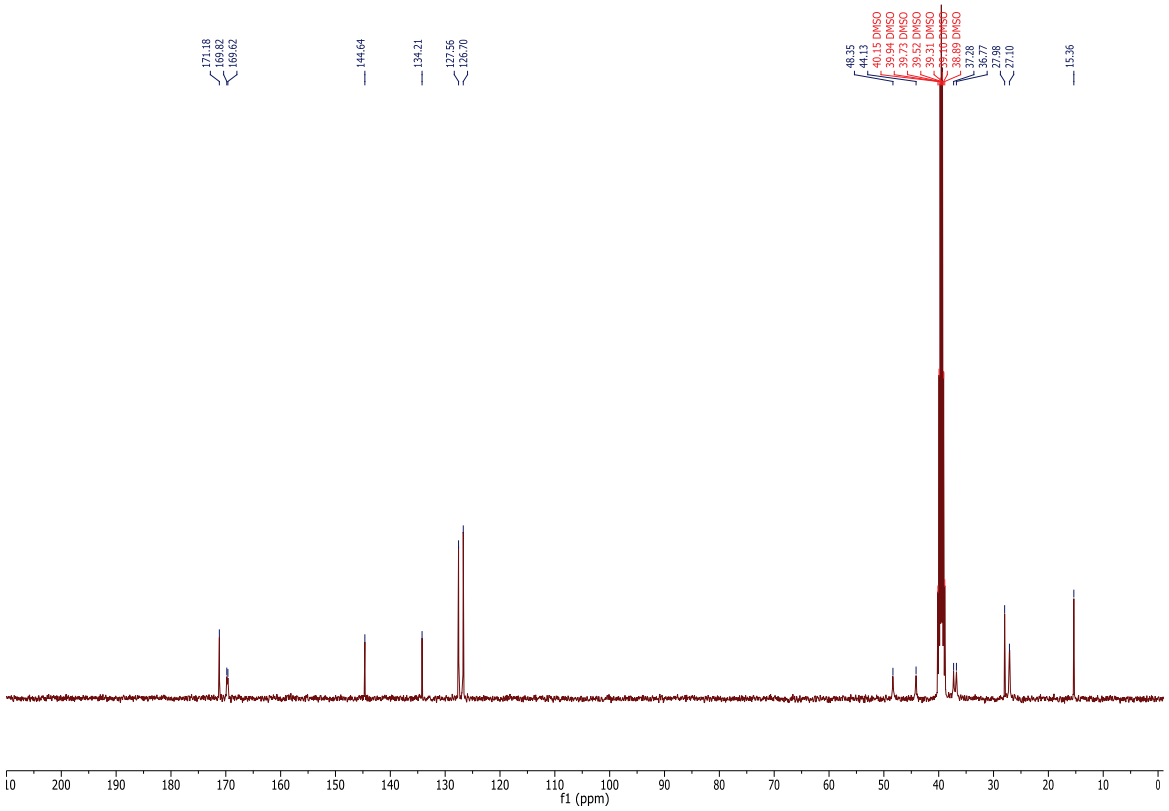


^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of **27**

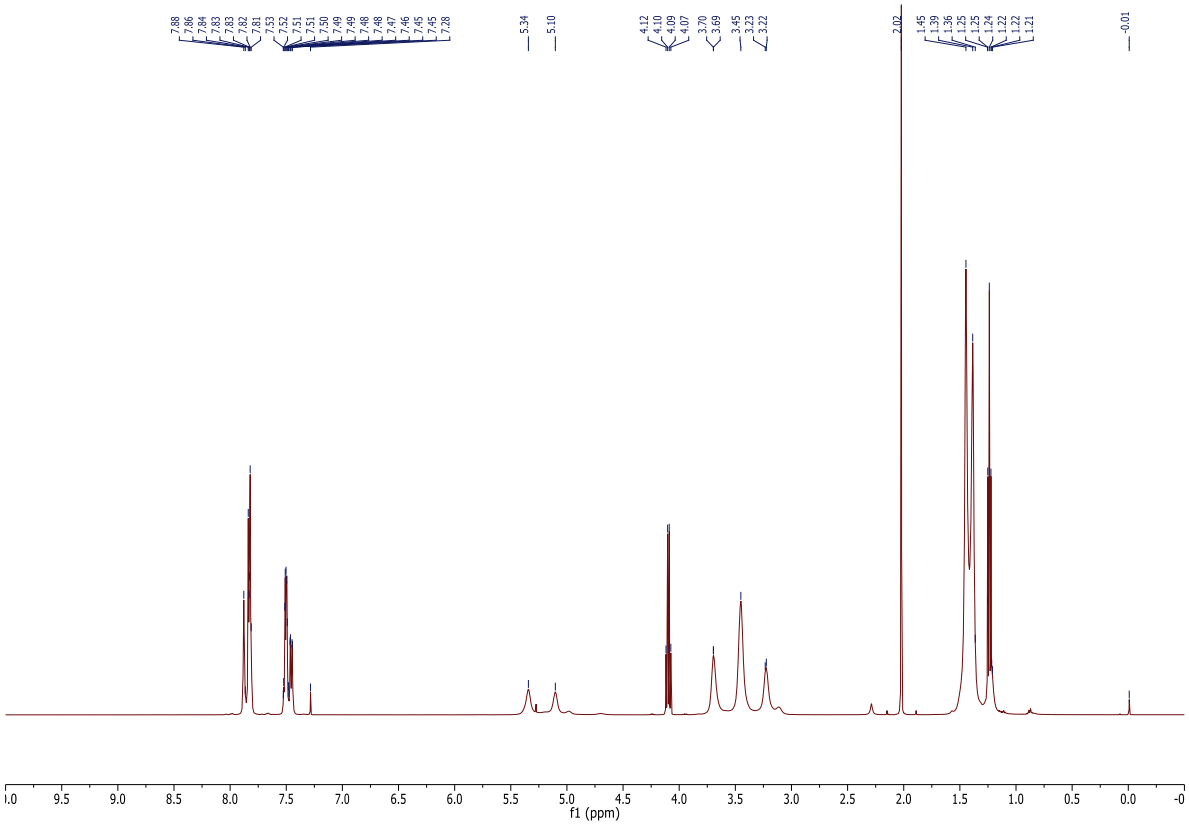


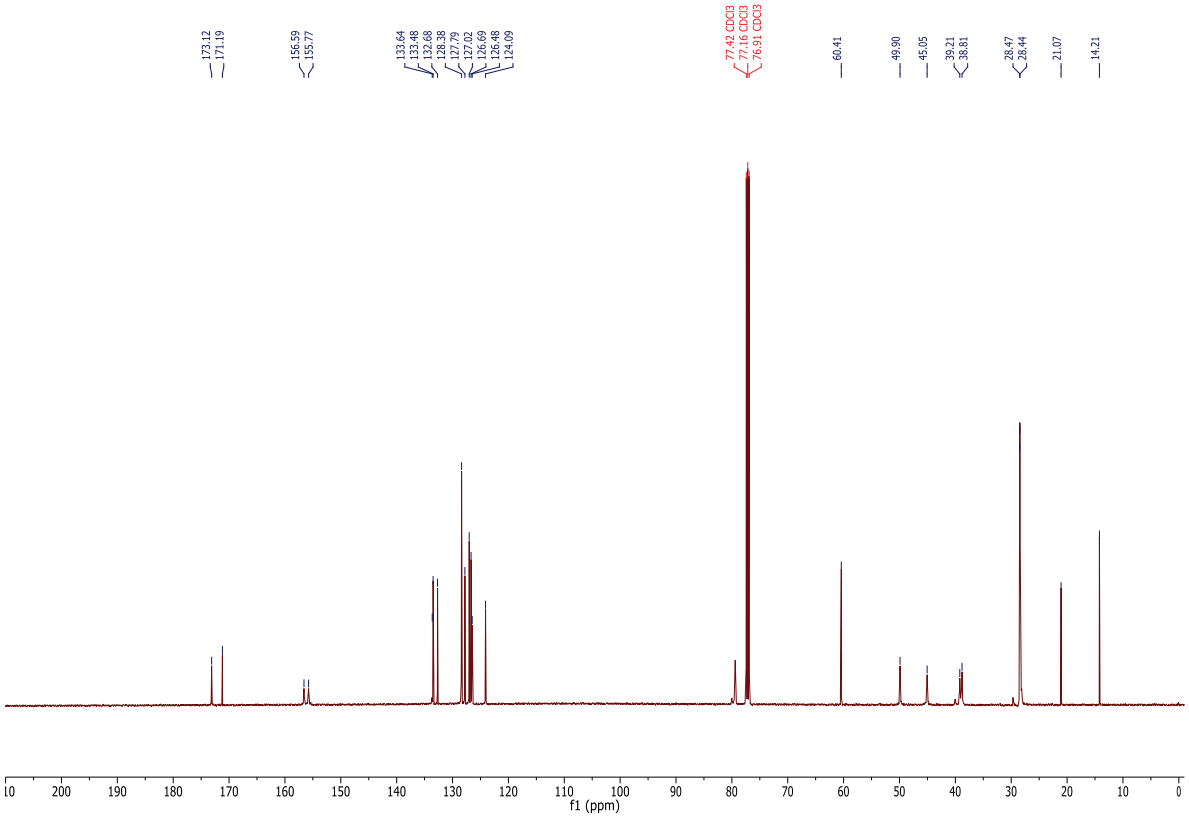
^{1}H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6) of **7**



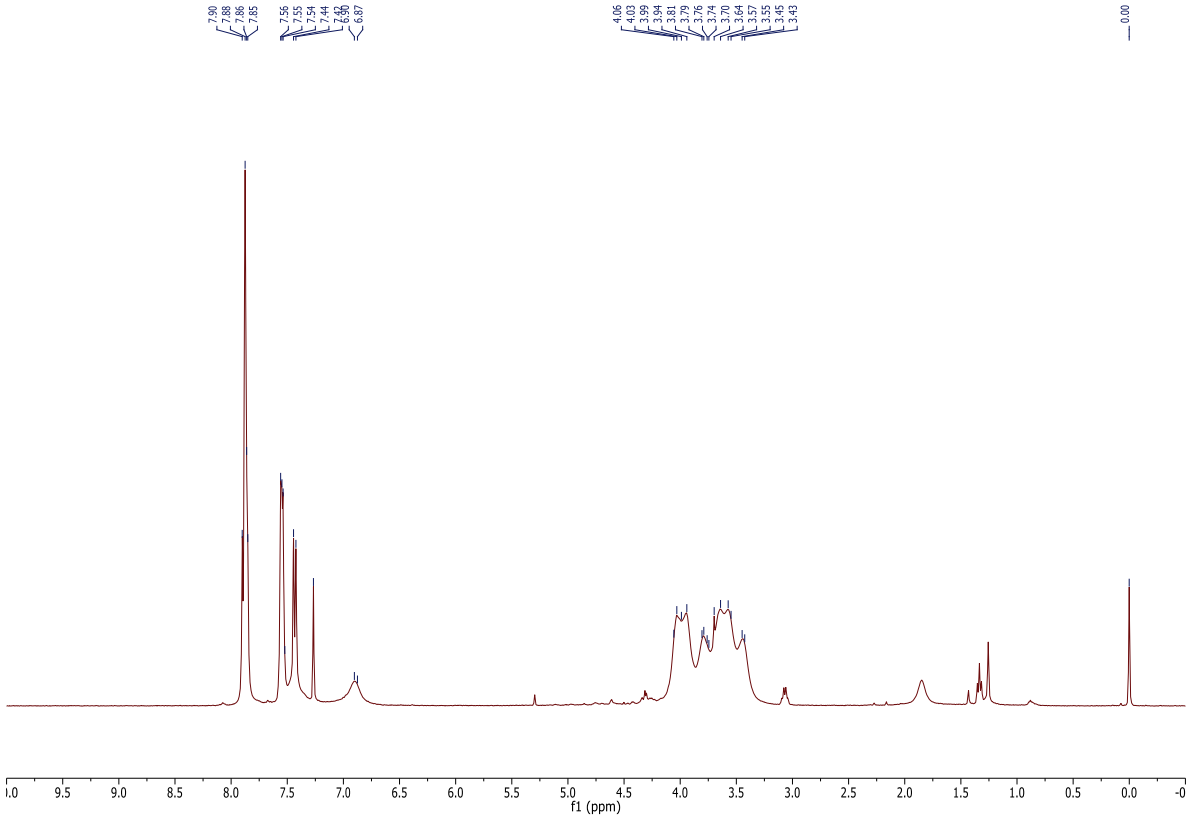


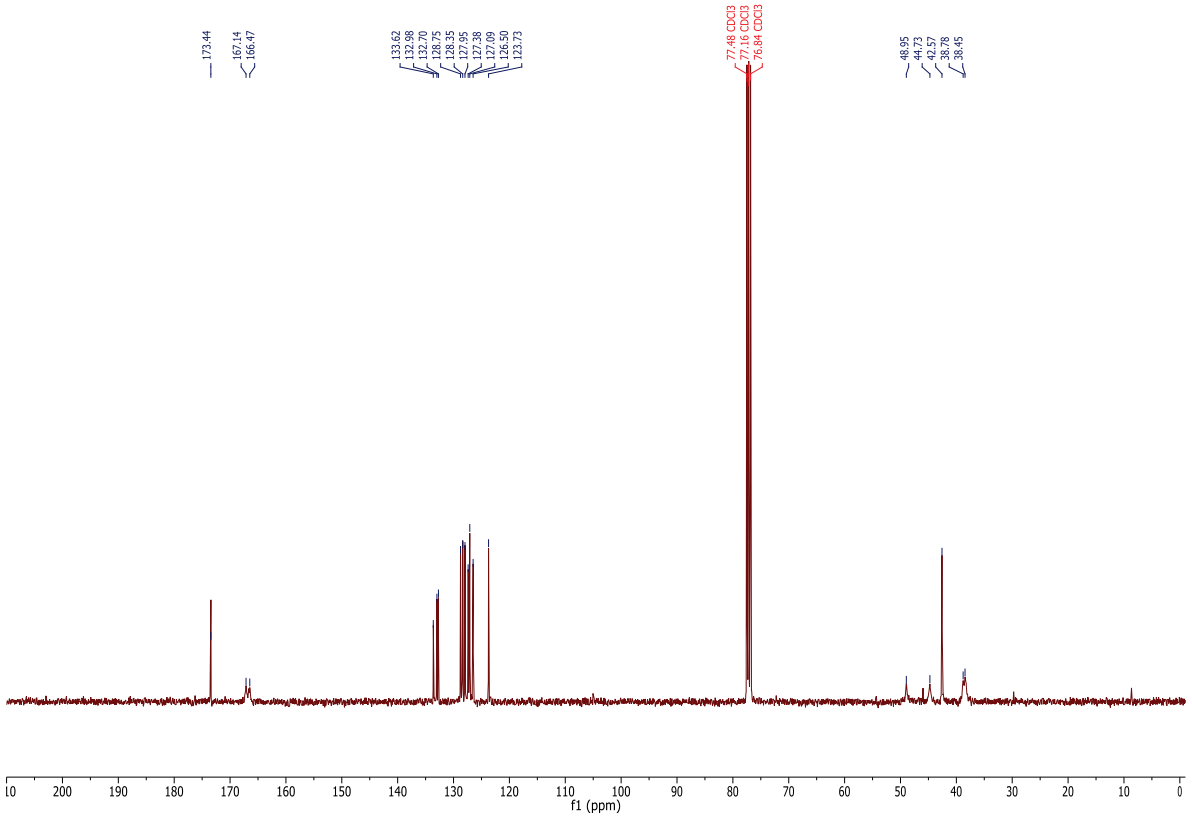
^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of **28** in ethyl acetate



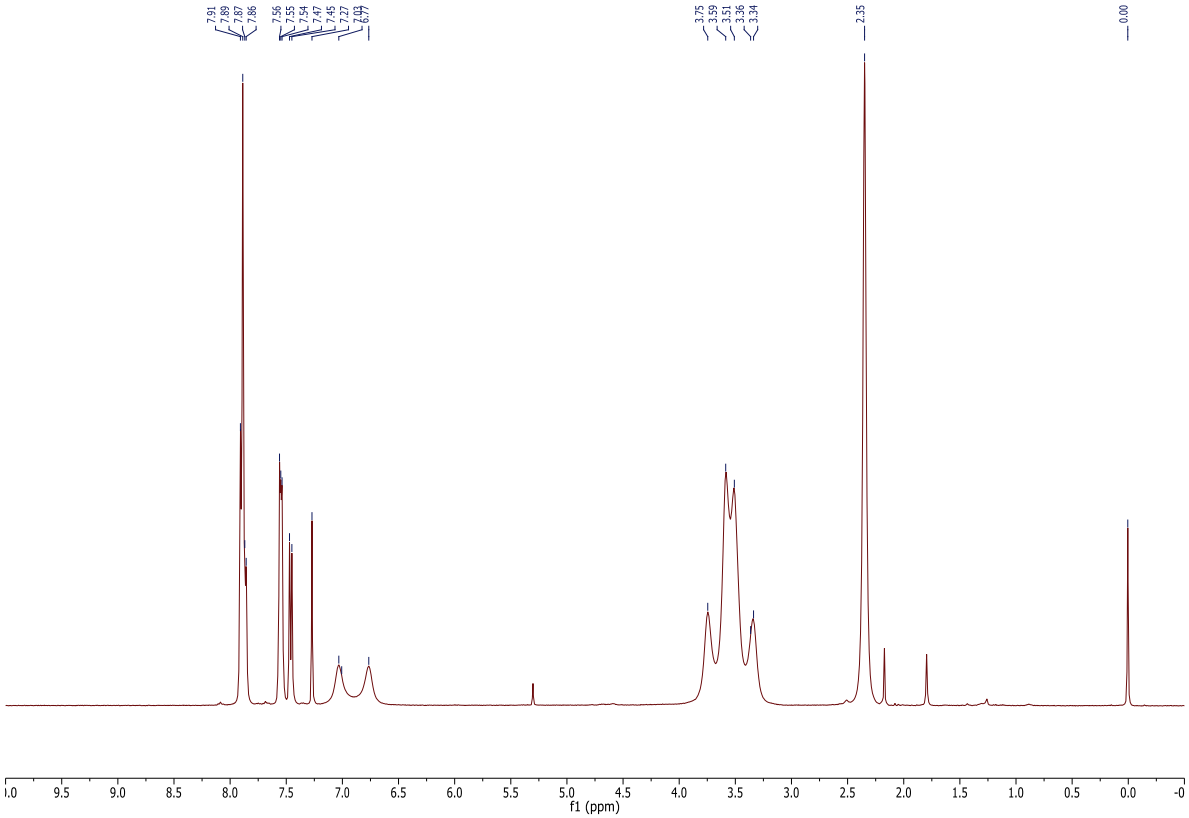


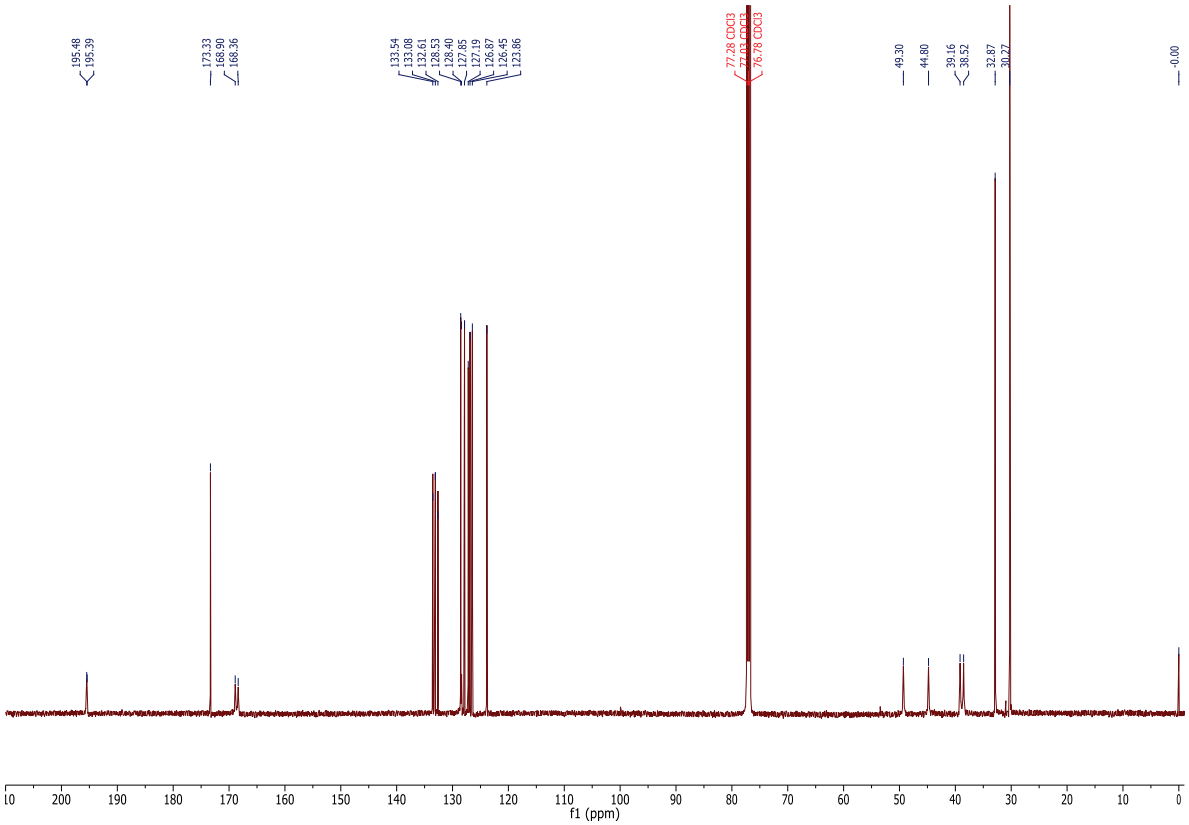
^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of **29**



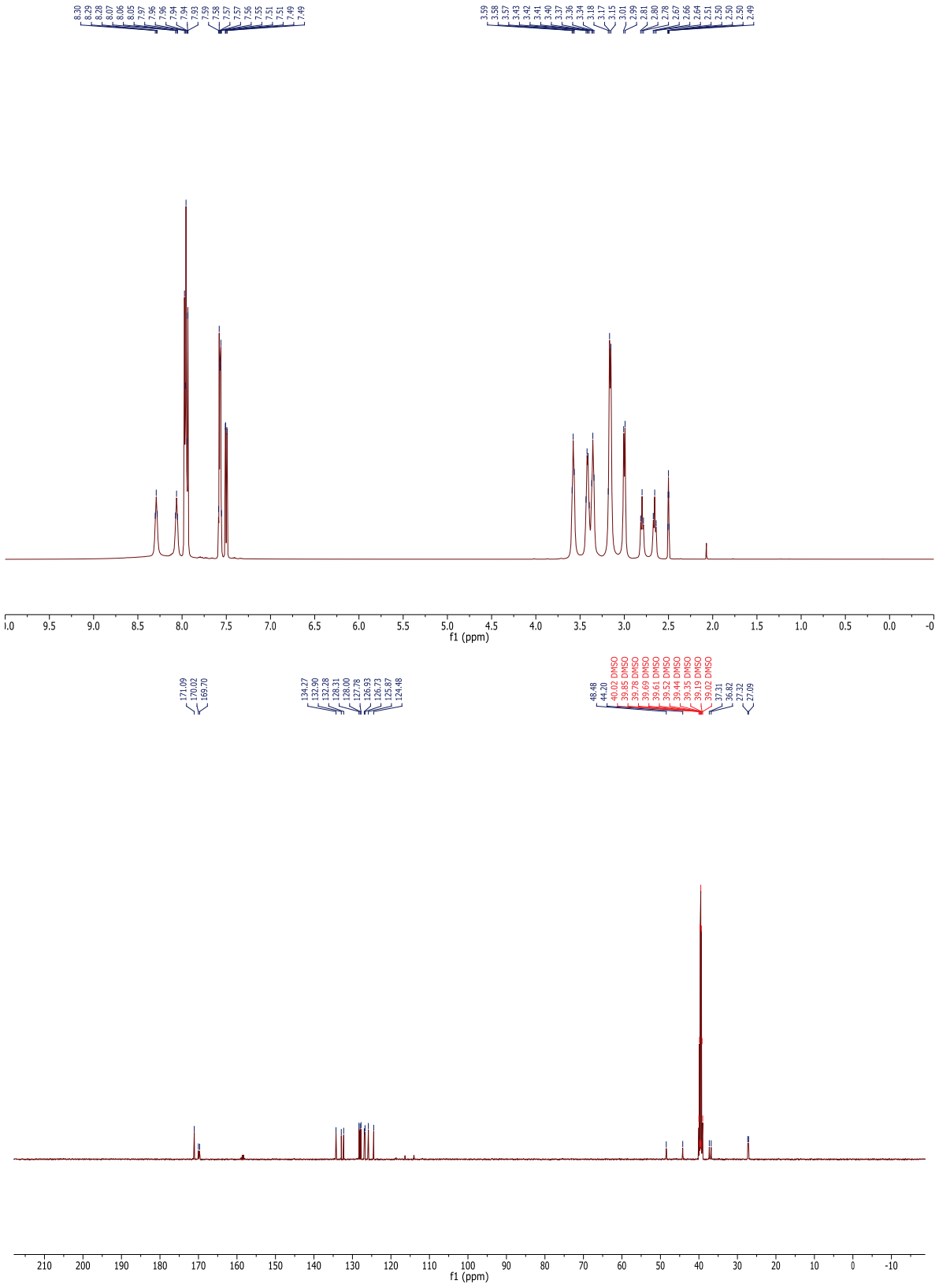


^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) of **30**





^{1}H NMR (DMSO-d_6) and ^{13}C NMR (DMSO-d_6) of **8**



VII. References

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