Supplementary Information

1,4,5-Trisubstituted-Carboxylated 1,2,3-Triazoles: An Unconventional Class of Ribonuclease A Inhibitor

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1. General Information

All reagents were commercially purchased and used without purification. Column chromatographic separations were done using silica gel (230–400 mesh). Solvents were dried and distilled following standard procedures. Thin-layer chromatography (TLC) was carried out on precoated plates (Merck silica gel 60, f254) and the spots were visualized with UV light or by charring the plates dipped in 5 % vanillin in MeOH solutuion. ^{1}H NMR (400 MHz and 500 MHz) and ^{13}C NMR (100 MHz and 125 MHz) spectra were recorded on Bruker NMR spectrometer using $CDCl_3$, DMSO-d_6, D_2O as the solvent and the multiplicity were marked as s-singlet, d-doublet, dd-doublet of doublet, t-triplet, q-quartet, m-multiplet, bs-broad singlet and b-broad. Trimethylsilane (TMS) was used as the internal standard for $CDCl_3$. On the other hand, 1,4-dioxane was used as the internal standard for D_2O for the water suppressed case and multiplicity was marked as the same as for $CDCl_3$. DEPT-135 experiments have been carried out to identify the methylene carbons and quaternary carbons. Chemical shifts were measured in parts per million (ppm, δ scale) referenced to 7.26 ppm and 77.16 in $CDCl_3$; 2.50 ppm and 39.52 ppm in DMSO-d_6; 3.75 ppm and 67.19 ppm in D_2O for ^{1}H NMR and ^{13}C NMR respectively. High resolution mass spectra were obtained from Xevo G2QTof mass spectrometer and Agilent quadruple-equipped TOF mass spectrometer in ESI+ mode. Bovine pancreatic RNase A, yeast tRNA, 2′ , 3′ -cCMP were purchased commercially. UV–vis measurements were made using a UV–vis spectrophotometer (Model Lambda 25). Concentrations of the solutions were made using a UV–vis spectrophotometrically using the following data: $\varepsilon_{278.5} = 9800\ M^{-1}\ cm^{-1}$ (RNase A)[1] and $\varepsilon_{268} = 8500\ M^{-1}\ cm^{-1}$ (2′, 3′ -cCMP).[2] All the azides **4a-g** were synthesized following the reported literature procedure.[3-7]

2. General Experimental Procedure

2-1. General procedure for the synthesis of 1,4,5-trisubstituted triazolylated ester derivatives: Aryl/alkyl azides **4a-h** (1 eq.) and thioglycolate/oxyacetate modified alkyne derivatives **2** or **3** (1 eq.) were mixed and heated under 100^{o}C under solvent-free conditions. After completion of the reaction (TLC), the reaction mixture was allowed to stand at room temperature and the crude products were purified by column chromatography on silica gel eluting with hexane/ethyl acetate in different ratios to obtain the pure products **5a-h** or **7a-h** respectively

2-2. General procedure for base-promoted hydrolysis of esters: To a well-stirred solution of ester **5a-h** or **7a-h** (1 mmol) in MeOH (5 mL) was added aq. solution of NaOH (1 M; 1 mL

per acid moiety) dropwise at 0°C and the mixture was stirred at room temperature for 45 min. After completion of reaction (TLC), methanol was removed under reduced pressure. The residue was dissolved in 10 mL of distilled water and neutralized with amberlite® IR-120(H^+) ion exchange resin and filtered, and the filtrate was evaporated under reduced pressure to generate the pure acid-derivatives **6a-e, 6h, 6f', 6g'** or **8a-e, 8h, 8f', 8g'** respectively.

3. Synthesis of 1,4-Functionalized But-2-ynes (2,3)

Dimethyl 2,2'-[but-2-yne-1,4-diylbis(sulfanediyl)] diacetate (2): To a suspension of NaH (60% dispersion in mineral oil, 2.5 mmol) in anhyd DMF (5 mL) was added methyl thioglycolate (1.2 mmol) dropwise at 0°C under N_2 atmosphere. The resulting suspension was stirred at room temperature for 30 min while the solution turned yellow. The reaction mixture was cooled to 0 °C and a solution of ditosylated compound **1A** (0.394g, 1 mmol) in anhyd DMF was added to the mixture. After 6 h the reaction mixture was partitioned between EtOAc (3x10 mL) and satd aq $NaHCO_3$ solution (50 mL). The organic phases were pooled together, washed with brine, dried over anhyd Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to get a residue, which was purified over silica gel to afford the thioglycolate derivative **2** (0.307g, 78%); [Eluent: EtOAc: pet ether (1: 4)]; colourless gum; ^{1}H NMR (400MHz, $CDCl_3$): δ 3.41 (s, 2H, CH_2S), 3.44 (s, 2H, SCH_2CO_2Me), 3.75 (s, 3H, CO_2Me); ^{13}C NMR (125MHz, $CDCl_3$): δ 20.7 (2×CH_2S), 32.7 (2×SCH_2CO_2Me), 52.6 (2×CH_3), 78.9 (2×CCH_2S), 170.6 (2×CO_2Me). HRMS [ES^+, $(M + H)^+$]: for $C_{10}H_{14}O_4S_2$ found 263.0386, calcd 263.0412.

Diethyl 2,2'-[but-2-yne-1,4-diylbis(oxy)]diacetate (3): To a suspension of NaH (60% dispersion in mineral oil, 2 mmol) in anhyd DMF (5 mL) was added 2-butyne-1,4-diol (0.086g, 1 mmol) dropwise at 0°C under N_2 atmosphere. The resulting suspension was stirred at 0°C for 2 h and a solution of ethyl bromo acetate (1.2 mmol) in anhyd DMF was added to the mixture. After 4 h the reaction mixture was worked up followining the procedure described for compound **2**. The residue was purified over silica gel to afford the oxydiacetate derivative **3** (0.052g, 60%). [Eluent: EtOAc: pet ether (1: 4)]; orange yellow liquid; ^{1}H NMR (500MHz, $CDCl_3$): δ 1.21-1.24 (3H, m, CH_2CH_3), 4.10 (2H, s, CH_2O), 4.14-4.18 (2H, m, CH_2CH_3), 4.29 (2H, s, OCH_2); ^{13}C NMR (125MHz, $CDCl_3$): δ 14.1 (2×CH_3), 58.4 (2×CH_2O), 60.9 (2×CH_2CH_3), 66.3 (2×OCH_2CO_2Et), 82.3 (2×CCH_2O), 169.8 (2×CO_2Et). HRMS [ES^+, $(M + H)^+$]: for $C_{12}H_{18}O_6$ found 259.1181, calcd 259.1182.

4. Synthesis of 1,4,5-TTs modified with Thioglycolate (5a-h)

Dimethyl 1-benzyl-1*H*-1,2,3-triazole-4,5-di(methylenethioglycolate) (5a): Following the general procedure compound **4a** (0.2g, 1.5mmol) was converted to compound **5a** (0.16g, 80%). [Eluent: EtOAc: pet ether (2: 3)]; yellow gum; ^{1}H NMR (500MHz, $CDCl_3$): δ 3.13 (2H, s, CH_2S), 3.31 (2H, s, CH_2S), 3.70 (6H, s, 2×CH_3), 3.77 (2H, s, SCH_2CO_2Me), 3.94 (2H, s, SCH_2CO_2Me), 5.67 (2H, s, $C_6H_5CH_2$), 7.19-7.21 (2H, m, H_{arom}), 7.32-7.34 (3H, m, H_{arom}); ^{13}C NMR (125MHz, $CDCl_3$): δ 23.2, 26.1 (2×CH_2S), 32.5, 33.2 (2×SCH_2CO_2Me), 52.5 ($C_6H_5CH_2$), 52.5, 52.7 (2×CH_3), 127.5, 128.6, 129.2 (CH_{arom}), 129.6 ($C_{triazole}$), 134.6 (C_{arom}), 143.9 ($C_{triazole}$), 170.4, 170.8 (2×CO_2Me). HRMS [ES^+, $(M + H)^+$]: for $C_{17}H_{22}N_3O_4S_2$ found 396.1032, calcd 396.1052.

Dimethyl 1-(2-pyridyl)-1*H*-1,2,3-triazole-4,5-di(methylenethioglycolate) (5b): Following the general procedure compound **4b** (0.200g, 1.5mmol) was converted to compound **5b** (0.15g, 76%). [Eluent: EtOAc : pet ether (4:1)]; pale yellow gum; ^{1}H NMR (400MHz, $CDCl_3$): δ 3.13 (2H, s, CH_2S), 3.28 (2H, s, CH_2S), 3.66 (6H, s, 2xCH_3), 3.93 (2H, s, SCH_2CO_2Me), 4.01 (2H, s, SCH_2CO_2Me), 5.73 (2H, s, $C_5H_4NCH_2$), 7.11-7.12 (1H, m, H_{arom}), 7.18-7.20 (1H, m, H_{arom}), 7.62-7.65 (1H, m, H_{arom}), 8.49-8.50 (1H, m, H_{arom}); ^{13}C NMR (125MHz, $CDCl_3$): δ 23.1, 26.0 (2×CH_2S), 32.5, 33.1 (2×SCH_2CO_2Me), 52.4, 52.6 (2xCH_3), 53.8 ($C_5H_4NCH_2$), 122.1, 123.2 (CH_{arom}), 130.4 ($C_{triazole}$), 137.2 (CH_{arom}), 143.5 ($C_{triazole}$), 149.7 (CH_{arom}), 154.6 (C_{arom}), 170.4, 170.7 (2×CO_2Me). HRMS [ES^+, $(M + H)^+$]: for $C_{16}H_{20}N_4O_4S_2$ found 397.1003, calcd 397.1004.

Dimethyl 1-phenyl-1*H*-1,2,3-triazole-4,5-di(methylenethioglycolate) (5c): Following the general procedure compound **4c** (0.200 g, 1.7 mmol) was converted to compound **5c** (0.17 g, 84%). [Eluent: EtOAc: pet ether (1: 1)]; orange yellow gum; ^{1}H NMR (400MHz, $CDCl_3$): δ 3.16 (2H, s, CH_2S), 3.38 (2H, s, CH_2S), 3.65 (3H, s, CH_3), 3.73 (3H, s, CH_3), 4.02 (2H, s, SCH_2CO_2Me), 4.08 (2H, s, SCH_2CO_2Me), 7.55 (5H, s, H_{arom}); ^{13}C NMR (125MHz, $CDCl_3$): δ 24.0, 26.5 (2×CH_2S), 33.4, 33.5 (2×SCH_2CO_2Me), 52.6, 52.7 (2×CH_3), 125.6, 129.7, 130.1 (CH_{arom}), 131.1 ($C_{triazole}$), 136.2 (C_{arom}), 143.4 ($C_{triazole}$), 170.2, 170.9 (2×CO_2Me). HRMS [ES^+, $(M + H)^+$]: for $C_{16}H_{19}N_3O_4S_2$ found 382.0898, calcd 382.0895.

Dimethyl 1-(4-methoxyphenyl)-1*H*-1,2,3-triazole-4,5-di(methylenethioglycolate) (5d): Following the general procedure compound **4d** (0.200 g, 1.3 mmol) was converted to compound **5d** (0.16 g, 80%). [Eluent: EtOAc : pet ether (2:3)]; brown gum; (400MHz, $CDCl_3$): δ 3.17 (2H, s,

CH_2S), 3.38 (2H, s, CH_2S), 3.67 (3H, s, CH_3), 3.73 (3H, s, CH_3), 3.88 (3H, s, CH_3), 3.98 (2H, s, SCH_2CO_2Me), 4.07 (2H, s, SCH_2CO_2Me), 7.04 (2H, d, *j*=8.8 Hz, H_{arom}), 7.46 (2H, d, *j*=8.8 Hz, H_{arom}); ^{13}C NMR (125MHz, $CDCl_3$): δ 23.9, 26.5 (2×CH_2S), 33.4, 33.4 (2×SCH_2CO_2Me) 52.6, 52.7 (2×CH_3), 55.8 (OCH_3), 114.8, 127.1 (CH_{arom}), 129.0 (C_{arom}), 131.2 ($C_{triazole}$), 143.1 ($C_{triazole}$), 160.8 (C_{arom}), 170.3, 170.9 (2×CO_2Me). HRMS [ES^+, $(M + H)^+$]: for $C_{17}H_{22}N_3O_5S_2$ found 412.0994, calcd 412.1001.

Dimethyl 1-(4-nitrophenyl)-1*H*-1,2,3-triazole-4,5-di(methylenethioglycolate) (5e): Following the general procedure compound **4e** (0.200 g, 1.2 mmol) was converted to compound **5e** (0.16 g, 81%). [Eluent: EtOAc : pet ether (2:3)]; brown gum; (400MHz, $CDCl_3$): δ 3.20 (2H, s, CH_2S), 3.22 (2H, s, CH_2S), 3.64 (3H, s, CH_3), 3.68 (3H, s, CH_3), 4.02 (2H, s, SCH_2CO2Me), 4.06 (2H, s, SCH_2CO2Me), 7.84 (2H, d, *j*=8.8 Hz, H_{arom}), 8.39 (2H, d, *j*=8.8 Hz, H_{arom}); ^{13}C NMR (125MHz, $CDCl_3$): δ 23.8, 26.0 (2×CH_2S), 33.2 (2×SCH_2CO_2Me), 52.5, 52.6 (2×CH_3), 125.1, 125.7 (CH_{arom}),131.0 ($C_{triazole}$), 140.9 (C_{arom}), 144.2 ($C_{triazole}$) 148.1 (C_{arom}), 170.0, 170.6 (2×CO_2Me). HRMS [ES^+, $(M + H)^+$]: for $C_{16}H_{18}N_4O_6S_2$ found 427.0750, calcd 427.0746.

Dimethyl 1-(4-methylbenzoate)-1*H*-1,2,3-triazole-4,5-di(methylenethioglycolate) (5f): Following the general procedure compound **4f** (0.200 g, 1.13 mmol) was converted to compound **5f** (0.16 g, 82%). [Eluent: EtOAc : pet ether (7:3)]; orange yellow gum; (400MHz, $CDCl_3$): δ 3.16 (2H, s, CH_2S), 3.34 (2H, s, CH_2S), 3.64 (3H, s, CH_3), 3.69 (3H, s, CH_3), 3.94 (3H, s, CO_2CH_3), 4.03-4.04 (4H, m, 2xSCH_2CO_2Me), 7.66 (2H, d, *j*=8.4 Hz, H_{arom}), 8.20 (2H, d, *j*=8.4 Hz, H_{arom}); ^{13}C NMR (125MHz, $CDCl_3$): δ 23.9, 26.2 (2×CH_2S), 33.3 (2×SCH_2CO_2Me), 52.5, 52.6, 52.7 (3×CH_3), 125.1 (CH_{arom}), 130.9 (C_{arom}),131.1 (CH_{arom}), 131.5 ($C_{triazole}$), 139.6 (C_{arom}) 143.9 ($C_{triazole}$), 165.9, 170.1, 170.8 (3×CO_2Me). HRMS [ES^+, $(M + H)^+$]: for $C_{18}H_{21}N_3O_6S_2$ found 440.0946, calcd 440.0950.

Dimethyl 1-(2-ethylacetate)-1*H*-1,2,3-triazole-4,5-di(methylenethioglycolate) (5g): Following the general procedure compound **4g** (0.200 g, 1.6 mmol) was converted to compound **5g** (0.16 g, 80%). [Eluent: EtOAc : pet ether (3:2)]; pale yellow gum; (400MHz, $CDCl_3$): δ 1.19 (3H, t, *j* = 7.2 Hz, $CO_2CH_2CH_3$), 3.05 (2H, s, CH_2S), 3.19 (2H, s, CH_2S), 3.61, 3.63 (6H, s, 2×CH_3), 3.87 (2H, s, SCH_2CO_2Me), 3.92 (2H, s, SCH_2CO_2Me), 4.15 (2H, q, *j* = 7.2 Hz, $CO_2CH_2CH_3$), 5.21 (2H, s, CH_2CO_2Et); ^{13}C NMR (125MHz, $CDCl_3$): δ 14.2 (CH_3), 23.1, 26.0 (2×CH_2S), 32.3, 33.0 (2×SCH_2CO_2Me), 49.8 (CH_2CO_2Et), 52.6, 52.8 (2×CH_3), 62.5 ($CO_2CH_2CH_3$), 130.7 ($C_{triazole}$), 143.6 ($C_{triazole}$),

166.5 (CO_2Et), 170.5, 170.9 (2×CO_2Me) HRMS [ES^+, $(M + H)^+$]: for $C_{14}H_{21}N_3O_4S_2$ found 392.0947, calcd 392.0950.

Dimethyl 1-(2-uracil-2-ethyl)-1*H*-1,2,3-triazole-4,5-di(methylenethioglycolate) (5h): Following the general procedure compound **4h** (0.200 g, 1.1 mmol) was converted to compound **5h** (0.14 g, 72%). [Eluent: MeOH : EtOAc (1 : 19)]; cream gum; (400MHz, $CDCl_3$): δ 3.17 (2H, s, CH_2S), 3.27 (2H, s, CH_2S), 3.72 (3H, s, CH_3), 3.73 (3H, s, CH_3), 3.94 (2H, s, SCH_2), 3.96 (2H, s, SCH_2), 4.38-4.40 (2H, m, CH_2CH_2U), 4.64-4.67 (2H, m, CH_2CH_2U) 5.55 (1H, d, j = 8 Hz, H_{uracil}), 6.95 (1H, d, j = 8 Hz, H_{uracil}), 9.48 (1H, brs, NH); ^{13}C NMR (125MHz, $CDCl_3$): 22.9 (CH_2S), 26.0 (CH_2S), 32.7 (SCH_2CO_2Me), 33.1 (SCH_2CO_2Me), 46.4 (CH_2CH_2U), 48.3 (CH_2CH_2U), 52.6, 52.3 (2×CH_3), 102.6 (CH_{uracil}), 131.0 ($C_{triazole}$), 143.6 ($C_{triazole}$), 144.7 (CH_{uracil}), 151.0 (CO_{uracil}), 163.5 (CO_{uracil}), 170.4, 170.8 (2×CO_2Me). HRMS [ES^+, $(M + H)^+$]: for $C_{16}H_{21}N_5O_6S_2$ found 444.1011, calcd 444.1011.

5. Synthesis of 1,4,5-TTs modified with thioglycolicacid (6a-e, 6f′, 6g′, 6h)

(1-benzyl-1*H*-1,2,3-triazole-4,5-diyl)di(methylenethioglycolicacid) (6a): Following the general procedure compound **5a** (0.16 g, 0.40 mmol) was converted to compound **6a** (0.10 g, 65%). orange yellow gum; ^1H NMR (500MHz, DMSO-d_6): δ 3.20 (2H, s, CH_2S), 3.26 (2H, s, CH_2S), 3.89 (2H, s, SCH_2CO_2H), 3.96 (2H, s, SCH_2CO_2H), 5.62 (2H, s, $C_6H_5CH_2$), 7.23-7.24 (2H, m, CH_{arom}), 7.34-7.36 (3H, m, CH_{arom}); ^{13}C NMR (125MHz, DMSO-*d6*): δ 21.9, 25.0 (2×CH_2S), 33.9, 34.1 (2×SCH_2CO_2H), 50.9 ($C_6H_5CH_2$), 127.5, 127.9, 128.7 (CH_{arom}), 130.8 ($C_{triazole}$), 135.6 (C_{arom}), 142.6 ($C_{triazole}$), 171.4, 171.5 (2×CO_2H). HRMS [ES^+, $(M + H)^+$]: for $C_{15}H_{17}N_3O_4S_2$ found 368.0724, calcd 368.0739.

(1-(2-pyridyl)-1*H*-1,2,3-triazole-4,5-diyl)di(methylenethioglycolicacid) (6b): Following the general procedure compound **5b** (0.15 g, 0.38 mmol) was converted to compound **6b** (0.09 g, 62%). colourless gum;^1H NMR (400MHz, D_2O): δ 3.11 (2H, s, CH_2S), 3.23 (2H, s, CH_2S), 3.88 (2H, s, SCH_2CO_2H), 3.91 (2H, s, SCH_2CO_2H), 5.81 (2H, s, $C_5H_4NCH_2$), 7.25 (1H, brs, H_{arom}), 7.42 (1H, brs, H_{arom}), 7.87 (1H, brs, H_{arom}), 8.48 (1H, brs, H_{arom}); ^{13}C NMR (125MHz, D_2O): δ 23.0, 25.8 (2×CH_2S), 37.1, 37.2 (2×SCH_2CO_2H), 53.6 ($C_5H_4NCH_2$), 123.3, 124.6 (CH_{arom}), 133.2 ($C_{triazole}$), 139.4 (CH_{arom}), 144.1 ($C_{triazole}$), 149.6 (CH_{arom}), 153.9 (C_{arom}), 177.1, 177.7 (2×CO_2H). HRMS [ES^+, $(M + H)^+$]: for $C_{14}H_{16}N_4O_4S_2$ found 369.0675, calcd 369.0691.

(1-phenyl-1*H*-1,2,3-triazole-4,5-diyl)di(methylenethioglycolicacid) (6c): Following the general procedure compound **5c** (0.17 g, 0.45 mmol) was converted to compound **6c** (0.11 g, 63%). colourless gum; ^{1}H NMR (500MHz, D_2O): δ 3.16 (2H, s, CH_2S), 3.36 (2H, s, CH_2S), 4.00 (2H, s, SCH_2CO_2H), 4.03 (2H, s,SCH_2CO_2H), 7.54-7.56 (2H, m, H_{arom}), 7.63-7.65 (3H, m, H_{arom}); ^{13}C NMR (125MHz, D_2O): δ 23.6, 26.0 (2×CH_2S), 34.9, 35.1 (2×SCH_2CO_2H), 126.2, 130.5, 131.3 (CH_{arom}), 133.8 ($C_{triazole}$), 135.6 (C_{arom}), 143.5 ($C_{triazole}$), 175.1, 175.7 (2×CO_2H). HRMS [ES^+, $(M + H)^+$]: for $C_{14}H_{16}N_3O_4S_2$ found 354.0575, calcd 354.0582.

(1-(4-methoxyphenyl)-1*H*-1,2,3-triazole-4,5-diyl)di(methylenethioglycolicacid) (6d): Following the general procedure compound **5d** (0.16 g, 0.38 mmol) was converted to compound **6d** (0.08 g, 55%). colourless gum; ^{1}H NMR (500MHz, DMSO-d_6): δ 3.20 (2H, s, CH_2S), 3.37 (2H, s, CH_2S), 3.84 (3H, s, OCH_3), 3.97 (2H, s, SCH_2CO_2H), 3.98 (2H, s, SCH_2CO_2H), 7.13 (2H, d, j = 9 Hz, H_{arom}), 7.52 (2H, d, j = 9 Hz, H_{arom}); ^{13}C NMR (125MHz, DMSO-d_6): δ 23.2, 25.6 (2×CH_2S), 33.8, 33.9 (2×SCH_2CO_2H), 56.0 (OCH_3), 115.1, 127.2 (CH_{arom}), 129.5 (C_{arom}), 132.1 ($C_{triazole}$), 142.9 ($C_{triazole}$), 160.5 (C_{arom}), 171.4, 171.8 (2×CO_2H). HRMS [ES^+, $(M + H)^+$]: for $C_{15}H_{18}N_3O_5S_2$ found 384.0675, calcd 384.0688.

(1-(4-nitrophenyl)-1*H*-1,2,3-triazole-4,5-diyl)di(methylenethioglycolicacid) (6e): Following the general procedure compound **5e** (0.16 g, 0.37 mmol) was converted to compound **6e** (0.09 g, 58%). yellow gum;^{1}H NMR (400MHz, DMSO-d_6): δ 3.21 (2H, s, CH_2S), 3.38 (2H, s, CH_2S), 3.99 (2H, s, SCH_2CO_2H), 4.15 (2H, s, SCH_2CO_2H), 8.00 (2H, d, j = 8.8 Hz, H_{arom}), 8.45 (2H, d, j = 8.8 Hz, H_{arom}); ^{13}C NMR (125MHz, DMSO-d_6): δ 22.7, 24.9 (2×CH_2S), 33.3 (2×SCH_2CO_2H), 125.0, 126.0 (CH_{arom}), 131.9 ($C_{triazole}$), 140.9 (C_{arom}), 143.6 ($C_{triazole}$), 147.6 (C_{arom}), 170.8, 171.2 (2×CO_2H).

(1-(4-benzoicacid)-1*H*-1,2,3-triazole-4,5-diyl)di(methylenethioglycolicacid) (6f'): Following the general procedure compound **5f** (0.16 g, 0.36 mmol) was converted to compound **6f'** (0.09 g, 55%). cream gum;^{1}H NMR (500MHz, DMSO-d_6): δ 3.17 (2H, s, CH_2S), 3.33 (2H, s, CH_2S), 4.00 (2H, s, SCH_2CO_2H), 4.08 (2H, s, SCH_2CO_2H), 7.74 (2H, d, j = 8.5 Hz, H_{arom}), 8.12 (2H, d, j = 8 Hz, H_{arom}); ^{13}C NMR (125MHz, DMSO-d_6): δ 22.6, 25.1 (2×CH_2S), 34.1, 34.3 (2×SCH_2CO_2H), 124.6, 130.4 (CH_{arom}), 131.8 (C_{arom}), 133.8 ($C_{triazole}$), 138.6 (C_{arom}), 143.1 ($C_{triazole}$), 166.9, 171.0, 171.4 (3×CO_2H). HRMS [ES^+, $(M + H)^+$]: for $C_{14}H_{16}N_3O_4S_2$ found 398.0476, calcd 398.0481.

(1-(2-aceticacid)-1*H*-1,2,3-triazole-4,5-diyl)di(methylenethioglycolicacid) (6g′): Following the general procedure compound **5g** (0.16 g, 0.41 mmol) was converted to compound **6g′** (0.11 g, 70%). colourless gum;^{1}H NMR (400MHz, D_2O): δ 3.28 (2H, s, CH_2S), 3.34 (2H, s, CH_2S), 3.96 (2H, s, SCH_2CO_2H), 4.03 (2H, s, SCH_2CO_2H), 5.33 (2H, s, CH_2CO_2H); ^{13}C NMR (125MHz, D_2O): δ 23.0, 25.7 (2×CH_2S), 33.6, 33.8 (2×SCH_2CO_2H), 50.7 (CH_2CO_2H), 132.9 ($C_{triazole}$), 143.5 ($C_{triazole}$), 171.4, 174.6, 174.8 (3×CO_2H). HRMS [ES$^+$, $(M + H)^+$]: for $C_{10}H_{13}N_3O_6S_2$ found 336.0322, calcd 336.0324.

(1-(2-uracil-2-ethyl)-1*H*-1,2,3-triazole-4,5-diyl)di(methylenethioglycolicacid) (6h): Following the general procedure compound **5h** (0.14 g, 0.31 mmol) was converted to compound **6h** (0.08 g, 60%); mp 190-192^{0}C white solid; ^{1}H NMR (500MHz, DMSO-d_6): δ 3.15, 3.18 (4H, s, 2×CH_2S), 3.86 (2H, s, SCH_2CO_2H), 4.04 (2H, s, SCH_2CO_2H), 4.15-4.17 (2H, m, CH_2CH_2U), 4.61-4.64 (2H, m, CH_2CH_2U), 5.44 (1H, d, j = 8 Hz, H_{uracil}), 7.29 (1H, d, j = 8 Hz, H_{uracil}); ^{13}C NMR (125MHz, DMSO-d_6): 22.1, 25.4 (2×CH_2S), 34.4, 34.6 (2×SCH_2CO_2H), 46.2 (CH_2CH_2U), 47.6 (CH_2CH_2U), 101.5 (CH_{uracil}), 131.7 ($C_{triazole}$), 142.9 ($C_{triazole}$), 145.8 (CH_{uracil}), 151.4 (CO_{uracil}), 164.1 (CO_{uracil}), 171.7, 172.1 (2×CO_2H). HRMS [ES$^+$, $(M + H)^+$]: for $C_{14}H_{17}N_5O_6S_2$ found 416.0697, calcd 416.0698.

6. Synthesis of 1,4,5-TTs modified with Oxyacetate (7a-h)

Diethyl 1-benzyl-1*H*-1,2,3-triazole-4,5-di(methyleneoxyacetate) (7a): Following the general procedure compound **4a** (0.200 g, 1.5 mmol) was converted to compound **7a** (0.15 g, 77%). [Eluent: EtOAc : pet ether (1:1)]; pale yellow gum; ^{1}H NMR (400MHz, $CDCl_3$): δ 1.24-1.28 (6H, m, 2×CH_3), 4.02, 4.11 (4H, s, 2×CH_2O), 4.16-4.21 (4H, m, 2×CH_2CH_3), 4.68, 4.74 (4H, s, 2×OCH_2CO_2Et), 5.69 (2H, s, $C_6H_5CH_2$), 7.29-7.30 (5H, m, H_{arom}); ^{13}C NMR (125MHz, $CDCl_3$): δ 14.3, 14.3 (2×CH_3), 52.6 ($C_6H_5CH_2$), 60.2 (CH_2O), 61.0, 61.1 (2×CH_2CH_3), 64.3 (CH_2O), 67.3, 67.4 (2×OCH_2CO_2Et), 127.9, 128.4, 129.0 (CH_{arom}), 131.6 ($C_{triazole}$), 135.0 (C_{arom}), 143.5 ($C_{triazole}$), 169.9, 170.3 (2×CO_2Et). HRMS [ES$^+$, $(M + H)^+$]: for $C_{19}H_{25}N_3O_6$ found 392.1848, calcd 392.1822.

Diethyl 1-(2-pyridyl)-1*H*-1,2,3-triazole-4,5-di(methyleneoxyacetate) (7b): Following the general procedure compound **4b** (0.200 g, 1.5 mmol) was converted to compound **7b** (0.16 g, 78%). [Eluent: EtOAc : pet ether (9:1)]; colourless gum;^{1}H NMR (400MHz, $CDCl_3$): δ 1.22-1.28 (6H, m, 2×CH_3), 4.05, 4.13 (4H, s, 2×CH_2O), 4.15-4.21 (4H, m, 2×CH_2CH_3), 4.77, 4.84 (4H, s, 2×OCH_2CO_2Et), 5.80 (2H, s, $C_5H_4NCH_2$), 7.12-7.14 (1H, m, H_{arom}), 7.19-7.22 (1H, m,

H_{arom}), 7.62-7.66 (1H, m, H_{arom}), 8.53 (1H, m, H_{arom}); ^{13}C NMR (125MHz, $CDCl_3$): δ 14.2, 14.3 (2×CH_3), 54.0 ($C_5H_4NCH_2$), 60.4 (CH_2O), 61.0, 61.1 (2×CH_2CH_3), 64.2 (CH_2O), 67.4, 67.5 (OCH_2CO_2Et), 122.1 (CH_{arom}), 123.1 (CH_{arom}), 132.3 ($C_{triazole}$), 137.2 (CH_{arom}), 143.4 ($C_{triazole}$), 149.7 (CH_{arom}), 155.0 (C_{arom}), 170.0, 170.1 (2×CO_2Et). HRMS [ES^+, $(M + H)^+$]: for $C_{18}H_{24}N_4O_6$ found 393.1785, calcd 393.1774.

Diethyl 1-phenyl-1*H*-1,2,3-triazole-4,5-di(methyleneoxyacetate) (7c): Following the general procedure compound **4c** (0.200 g, 1.7 mmol) was converted to compound **7c** (0.15 g, 77%). [Eluent: EtOAc : pet ether (3:2)]; yellow gum; ^1H NMR (400MHz, $CDCl_3$): δ 1.21-1.30 (6H, m, 2×CH_3), 4.13-4.24 (8H, m, 2×CH_2CH_3; 2×CH_2O), 4.75 (2H, s, OCH_2CO_2Et), 4.86 (2H, s, OCH_2CO_2Et), 7.53 (3H, d, j =8 Hz, H_{arom}), 7.71 (2H, d, j =8 Hz, H_{arom}); ^{13}C NMR (125MHz, $CDCl_3$): δ 14.2, 14.3 (2×CH_3), 60.1 (CH_2O), 61.0, 61.1 (2×CH_2CH_3), 64.2 (CH_2O), 67.5, 67.7 (2×OCH_2CO_2Et), 125.0, 129.6, 129.8 (CH_{arom}), 132.2 ($C_{triazole}$), 136.3 (C_{arom}), 144.1 ($C_{triazole}$), 170.0, 170.3 (2×CO_2Et). HRMS [ES^+, $(M + H)^+$]: for $C_{18}H_{23}N_3O_6$ found 378.1688, calcd 378.1665.

Diethyl 1-(4-methoxyphenyl)-1*H*-1,2,3-triazole-4,5-di(methyleneoxyacetate) (7d): Following the general procedure compound **4d** (0.200 g, 1.3 mmol) was converted to compound **7d** (0.15 g, 75%). [Eluent: EtOAc : pet ether (3:2)]; white gum; ^1H NMR (400MHz, $CDCl_3$): δ 1.21-1.29 (6H, m, 2×CH_3), 3.85 (3H, s, OCH_3), 4.15-4.23 (8H, m, 2×CH_2CH_3; 2×CH_2O), 4.71, 4.85 (4H, s, 2×OCH_2CO_2Et), 7.01 (2H, d, j = 8.8 Hz, H_{arom}), 7.60 (2H, d, j = 8.8 Hz, H_{arom}); ^{13}C NMR (125MHz, $CDCl_3$): δ 14.2, 14.3 (2×CH_3), 55.7 (OCH_3), 60.1 (CH_2O), 61.0, 61.1 (2×CH_2CH_3), 64.2 (CH_2O), 67.5, 67.6 (2×OCH_2CO_2Et), 114.7, 126.5 (CH_{arom}), 129.2 (C_{arom}), 132.2 ($C_{triazole}$), 143.8 ($C_{triazole}$), 160.6 (C_{arom}), 170.0, 170.3 (2×CO_2Et). HRMS [ES^+, $(M + H)^+$]: for $C_{19}H_{25}N_3O_7$ found 408.1768, calcd 408.1771.

Diethyl 1-(4-nitrophenyl)-1*H*-1,2,3-triazole-4,5-di(methyleneoxyacetate) (7e): Following the general procedure compound **4e** (0.200 g, 1.2 mmol) was converted to compound **7e** (0.16 g, 78%). [Eluent: EtOAc : pet ether (9:11)]; dark yellow gum; ^1H NMR (400MHz, $CDCl_3$): δ 1.231.31 (6H, m, 2×CH_3), 4.15-4.27 (8H, m, 2×CH_2CH_3; 2×CH_2O), 4.84, 4.87 (4H, s, 2×OCH_2CO_2Et), 8.12 (2H, d, j = 8 Hz, H_{arom}), 8.42 (2H, d, j = 8 Hz, H_{arom}); ^{13}C NMR (125MHz, $CDCl_3$): δ 14.3 (2×CH_3), 60.0 (OCH_2), 61.1, 61.2 (2×CH_2CH_3), 64.2 (OCH_2), 67.6, 68.0 (2×OCH_2CO_2Et), 125.2, 125.3 (CH_{arom}), 132.3 ($C_{triazole}$), 141.0 (C_{arom}), 145.0 ($C_{triazole}$), 148.1

(C_{arom}), 170.0, 170.2 (2×CO_2Et). HRMS [ES^+, $(M + H)^+$]: for $C_{18}H_{23}N_4O_8$ found 423.1548, calcd 423.1516.

Diethyl 1-(4-methylbenzoate)-1*H*-1,2,3-triazole-4,5-di(methyleneoxyacetate) (7f): Following the general procedure compound **4f** (0.200 g, 1.13 mmol) was converted to compound **7f** (0.16 g, 80%). [Eluent: EtOAc : pet ether (11:9)]; yellow gum;^{1}H NMR (500MHz, $CDCl_3$): δ 1.24-1.60 (6H, m, 2×CH_3), 3.96 (3H, s, CO_2CH_3), 4.16-4.25 (8H, m, 2×CH_2CH_3; 2×CH_2O), 4.80, 4.88 (4H, s, 2×OCH_2CO_2Et), 7.89 (2H, d, j = 8.5 Hz, H_{arom}), 8.23 (2H, d, j = 8.5 Hz, H_{arom}); ^{13}C NMR (125MHz, $CDCl_3$): δ 14.3, 14.3 (2×CH_3), 52.6 (CO_2CH_3), 60.0 (CH_2O), 61.1, 61.2 (2xCH_2CH_3), 64.2 (CH_2O), 67.6, 67.8 (2×OCH_2CO_2Et), 124.6, 131.1 (CH_{arom}), 131.3 (C_{arom}), 132.2 ($C_{triazole}$), 139.8 (C_{arom}), 144.6 ($C_{triazole}$), 166.1 (CO_2Me), 170.0, 170.2 (2xCO_2Et). HRMS [ES^+, $(M + H)^+$]: for $C_{20}H_{25}N_3O_8$ found 436.1721, calcd 436.1720.

Diethyl 1-(2-ethylacetate)-1*H*-1,2,3-triazole-4,5-di(methyleneoxyacetate) (7g): Following the general procedure compound **4g** (0.200 g, 1.6 mmol) was converted to compound **7g** (0.15 g, 77%). [Eluent: EtOAc : pet ether (7:3)]; colourless gum; ^{1}H NMR (400MHz, $CDCl_3$): δ 1.24-1.30 (9H, m, 3×CH_3), 4.06, 4.11 (4H, s, 2×CH_2O), 4.15-4.26 (6H, m, 3×CH_2CH_3), 4.76, 4.83 (4H, s, 2×OCH_2CO_2Et), 5.35 (2H, s, CH_2CO_2Et); ^{13}C NMR (100MHz, $CDCl_3$): δ 14.2, 14.2, 14.3 (3×CH_3), 50.1 (CH_2CO_2Et), 60.5 (CH_2O), 61.0, 61.2, 62.3 (3×CH_2CH_3), 64.1 (CH_2O), 67.2, 67.2 (2×OCH_2CO_2Et), 132.6 ($C_{triazole}$), 142.9 ($C_{triazole}$), 166.8, 170.0, 170.3 (3×CO_2Et) . HRMS [ES^+, $(M + H)^+$]: for $C_{16}H_{25}N_3O_8$ found 388.1738, calcd 388.1720.

Diethyl 1-(2-uracil-2-ethyl)-1*H*-1,2,3-triazole-4,5-di(methyleneoxyacetate) (7h): Following the general procedure compound **4h** (0.200 g, 1.1 mmol) was converted to compound **7h** (0.13 g, 67%). [Eluent: MeOH : EtOAc (1:19)]; colourless gum;^{1}H NMR (400MHz, $CDCl_3$): δ 1.25-1.29 (6H, m, 2×CH_3), 4.10 (2H, s, CH_2O), 4.16-4.22 (6H, m, 2×CH_2CH_3; CH_2O), 4.32-4.35 (2H, m, CH_2CH_2U), 4.73 (2H, s, OCH_2CO_2Et), 4.79-4.81 (4H, m, OCH_2CO_2Et, CH_2CH_2U), 5.47 (1H, d, j = 8 Hz, H_{uracil}), 6.80 (1H, d, j = 8 Hz, H_{uracil}), 9.33 (1H, brs, NH); ^{13}C NMR (125MHz, $CDCl_3$): δ 14.3 (2×CH_3), 46.8, 48.6 (2×CH_2CH_2U), 60.0 (CH_2O), 61.1, 61.3 (2×CH_2CH_3), 64.1 (CH_2O), 67.3, 67.7 (2×OCH_2CO_2Et), 102.3 (CH_{uracil}), 132.7 ($C_{triazole}$), 143.1 ($C_{triazole}$), 144.6 (CH_{uracil}), 151.0 (CO_{uracil}), 163.7 (CO_{uracil}), 170.1, 170.2 (2×CO_2Et).

7. Synthesis of 1,4,5-TTs modified with Oxyaceticacid (8a-e, 8f′, 8g′, 8h)

(1-benzyl-1*H*-1,2,3-triazole-4,5-diyl)di(methyleneoxyaceticacid) (8a): Following the general procedure compound **7a** (0.15 g, 0.29 mmol) was converted to compound **8a** (0.11 g, 70%). pale yellow gum; ^{1}H NMR (500MHz, D_2O): δ 3.95 (2H, s, CH_2O), 4.13 (2H, s, CH_2O), 4.67 (2H, s, OCH_2CO_2H), 4.71 (2H, s, OCH_2CO_2H), 5.59 (2H, s, $C_6H_5CH_2$), 7.19-7.20 (2H, m, H_{arom}), 7.27-7.28 (3H, m, H_{arom}); ^{13}C NMR (125MHz, D_2O): δ 52.9 ($C_6H_5CH_2$), 59.9, 63.1 (2×CH_2O), 67.2, 68.0 (2×OCH_2CO_2H), 128.2, 129.1, 129.6 (CH_{arom}), 133.3 ($C_{triazole}$), 135.2 (C_{arom}), 143.7 ($C_{triazole}$), 175.0, 175.5 (2×CO_2H). HRMS [ES$^+$, $(M + H)^+$]: for $C_{15}H_{17}N_3O_6$ found 336.1193, calcd 336.1196.

(1-(2-pyridyl)-1*H*-1,2,3-triazole-4,5-diyl)di(methyleneoxyaceticacid) (8b): Following the general procedure compound **7b** (0.16 g, 0.41 mmol) was converted to compound **8b** (0.11 g, 68%). brown gum; ^{1}H NMR (400MHz, D_2O): δ 3.89 (2H, s, CH_2O), 4.07 (2H, s, CH_2O), 4.75, 4.77 (4H, s, 2×OCH_2), 5.93 (2H, s, $C_5H_4NCH_2$), 7.45 (1H, brs, H_{arom}), 7.57 (1H, s, H_{arom}), 8.04-8.05 (1H, m, H_{arom}), 8.54 (1H, brs, H_{arom}); ^{13}C NMR (125MHz, D_2O): δ 52.7 ($C_5H_4NCH_2$), 59.7, 62.9 (2×CH_2O), 68.7, 69.0 (2×OCH_2CO_2H), 124.5 (C_{arom}), 125.4 (C_{arom}), 134.0 ($C_{triazole}$), 141.6 (C_{arom}), 143.8 ($C_{triazole}$), 147.8 (C_{arom}), 152.6 (C_{arom}), 176.4, 176.8 (2×CO_2H). HRMS [ES$^+$, $(M + H)^+$]: for $C_{14}H_{16}N_4O_6$ found 337.1144, calcd 337.1148.

(1-phenyl-1*H*-1,2,3-triazole-4,5-diyl)di(methyleneoxyaceticacid) (8c): Following the general procedure compound **7c** (0.16 g, 0.42 mmol) was converted to compound **8c** (0.11 g, 67%). pale yellow gum; ^{1}H NMR (400MHz, D_2O): δ 4.08 (2H, s, CH_2O), 4.25 (2H, s, CH_2O), 4.73 (2H, s, OCH_2CO_2H), 4.85 (2H, s, OCH_2CO_2H), 7.58 (2H, brs, H_{arom}), 7.63 (3H, brs, H_{arom}); ^{13}C NMR (125MHz, D_2O): δ 60.1, 63.3 (2×CH_2O), 67.6, 67.8 (2×OCH_2CO_2H), 125.8, 130.4, 131.3 (CH_{arom}), 134.0 ($C_{triazole}$), 135.6 (C_{arom}), 143.8 ($C_{triazole}$), 170.5, 175.0 (2×CO_2H). HRMS [ES$^+$, $(M + H)^+$]: for $C_{14}H_{15}N_3O_6$ found 322.1036, calcd 322.1039.

(1-(4-methoxyphenyl)-1*H*-1,2,3-triazole-4,5-diyl)di(methyleneoxyaceticacid) (8d): Following the general procedure compound **7d** (0.15 g, 0.36 mmol) was converted to compound **8d** (0.09 g, 60%). White gum; ^{1}H NMR (400MHz, DMSO-d_6): δ 3.84 (3H, s, OCH_3), 4.05 (2H, s, CH_2O), 4.11 (2H, s, CH_2O), 4.66 (2H, s, OCH_2CO_2H), 4.71 (2H, s, OCH_2CO_2H), 7.14 (2H, d, j = 8.8 Hz, H_{arom}), 7.62 (2H, d, j = 8.8 Hz, H_{arom}), ^{13}C NMR (125MHz, DMSO-d_6): δ 56.0 (OCH_3), 59.7, 63.1 (2×CH_2O), 67.2, 67.4 (2×OCH_2CO_2H), 115.1, 126.7 (CH_{arom}), 129.3 (C_{arom}), 133.0

($C_{triazole}$), 143.7 ($C_{triazole}$), 160.4 (C_{arom}), 171.7, 171.9 (2×CO_2H). HRMS [ES^+, $(M + H)^+$]: for $C_{14}H_{15}N_3O_6$ found 352.1138, calcd 352.1145.

(1-(4-nitrophenyl)-1*H*-1,2,3-triazole-4,5-diyl)di(methyleneoxyaceticacid) (8e): Following the general procedure compound **7e** (0.16 g, 0.38 mmol) was converted to compound **8e** (0.09 g, 56%). lemon yellow gum;^{1}H NMR (400MHz, DMSO-d_6): δ 4.09 (2H, s, CH_2O), 4.10 (2H, s, CH_2O), 4.75 (2H, s, OCH_2CO_2H), 4.80 (2H, s, OCH_2CO_2H), 8.12 (2H, d, j = 8 Hz, H_{arom}), 8.45 (2H, d, j = 8 Hz, H_{arom}); ^{13}C NMR (125MHz, DMSO-d_6): δ 59.5, 62.9 (2×CH_2O), 67.5, 67.9 (2×OCH_2CO_2H), 125.5, 125.9 (CH_{arom}), 133.4 ($C_{triazole}$), 141.3 (C_{arom}), 144.6 ($C_{triazole}$), 148.1 (C_{arom}), 171.8, 172.0 (2×CO_2H). HRMS [ES^+, $(M + H)^+$]: for $C_{14}H_{14}N_4O_8$ found 367.0882, calcd 367.0890.

(1-(4-benzoicacid)-1*H*-1,2,3-triazole-4,5-diyl)di(methyleneoxyaceticacid) (8f′): Following the general procedure compound **7f** (0.16 g, 0.37 mmol) was converted to compound **8f′** (0.10 g, 63%); mp 120-122^{0}C; cream solid; ^{1}H NMR (500MHz, D_2O): δ 3.99 (2H, s, CH_2O), 4.14 (2H, s, CH_2O), 4.86 (4H, s, 2×OCH_2CO_2H), 7.72 (2H, d, j = 7.5 Hz, H_{arom}), 8.13 (2H, d, j = 7 Hz, H_{arom}); ^{13}C NMR (125MHz, D_2O): δ 58.2, 61.4 (2×CH_2O), 65.6, 67.3 (2×OCH_2CO_2H), 124.0, 129.6 (CH_{arom}), 132.5 (C_{arom}), 133.9 ($C_{triazole}$), 137.0 (C_{arom}), 142.7 ($C_{triazole}$), 170.5 (CO_2H), 174.6, 175.1 (2×CO_2H). HRMS [ES^+, $(M + H)^+$]: for $C_{15}H_{16}N_3O_8$ found 366.0943, calcd 366.0937.

(1-(2-aceticacid)-1*H*-1,2,3-triazole-4,5-diyl)di(methyleneoxyaceticacid) (8g′): Following the general procedure compound **7g** (0.15 g, 0.39 mmol) was converted to compound **8g′** (0.10 g, 66%). cream gum;^{1}H NMR (400MHz, D_2O): δ 4.15 (2H, s, CH_2O), 4.23 (2H, s, CH_2O), 4.79 (2H, s, OCH_2CO_2H), 4.84 (2H, s, OCH_2CO_2H), 5.30 (2H, s, CH_2CO_2H); ^{13}C NMR (125MHz, D_2O): δ 51.3 (CH_2CO_2H), 60.1, 63.2 (2×CH_2O), 67.4, 67.5 (2×OCH_2CO_2H), 133.8 ($C_{triazole}$), 143.2 ($C_{triazole}$), 171.9 (CO_2H), 174.5, 174.8 (2×CO_2H). HRMS [ES^+, $(M + H)^+$]: for $C_{10}H_{13}N_3O_8$ found 304.0770, calcd 304.0781.

(1-(2-uracil-2-ethyl)-1*H*-1,2,3-triazole-4,5-diyl)di(methyleneoxyaceticacid) (8h):Following the general procedure compound **7h** (0.13 g, 0.34 mmol) was converted to compound **8h** (0.07 g, 56%). colourless gum; ^{1}H NMR (500MHz, D_2O): δ 4.13, 4.17 (4H, s, 2×CH_2O), 4.33 (2H, brs, CH_2CH_2U), 4.75, 4.79 (4H, s, 2×OCH_2CO_2H), 4.83 (2H, brs, CH_2CH_2U), 5.67 (1H, d, j = 7.5 Hz, H_{uracil}), 7.21 (1H, d, j = 7.5 Hz, H_{uracil}); ^{13}C NMR (125MHz, D_2O): δ 47.5 (CH_2CH_2U), 49.1 (CH_2CH_2U), 59.6,

62.9 (2×CH_2O), 67.4, 68.1 (2×OCH_2CO_2H), 102.3 (CH_{uracil}), 134.2 ($C_{triazole}$), 143.4 ($C_{triazole}$), 147.2 (CH_{uracil}), 152.5 (CO_{uracil}), 167.2 (CO_{uracil}), 175.0, 175.2 (2×CO_2H). HRMS [ES^+, $(M + H)^+$]: for $C_{14}H_{17}N_5O_8$ found 384.1180, calcd 384.1155.

8. Biophysical studies

8-1. Agarose gel electrophoresis assay

Inhibition of RNase A was assayed qualitatively by the degradation of tRNA in an agarose gel. In this method, 20 µL of RNase A (2 µM) was mixed with 20 µL of compounds **6a-e, 6f′-g′, 6h** and **8a-e, 8f′-g′, 8h** (from lane 3 to 8: 0.048 mM, 0.096 mM, 0.114 mM, 0.192 mM, 0.216 mM and 0.24 mM respectively) and the resulting solutions was incubated for 3 h. 40 µL of tRNA solution (10 mg/mL tRNA, freshly dissolved in RNase free water) was added to the incubated mixtures and further incubated for another 30 min. Then 10 µL of sample buffer (containing 10 % glycerol and 0.025 % bromophenol blue) was added to this mixture and 20 µL from each solution was taken and loaded into a 1.1 % agarose gel. The gel was run using 0.04 M Tris-Aceticacid-EDTA (TAE) buffer (pH 8.0). The residual tRNA was visualized by ethidium bromide staining under UV light. The band intensities in gel were analysed by using ImageJ software.[8]

8-2. Enzyme kinetics

The inhibition of RNase A by compounds **6a-e, 6f′-g′, 6h** and **8a-e, 8f′-g′, 8h** were determined individually by a spectrophotometric method as described by Anderson and co-workers.[2] The assay was performed in 0.1 M MES–NaOH buffer (pH 6.0) containing 0.1 M NaCl, by using 2′,3′ - cCMP as the substrate. The inhibition constants were determined from the initial velocity data using Lineweaver–Burk plots.[9] For the Lineweaver–Burk plot, the reciprocal of the initial velocity was plotted against the reciprocal of the substrate concentration at a constant inhibitor concentration according to the following Michaelis–Menten equation:

$$1/v = K_m/V_{max} (1 + [I]/K_i) \, 1/[S] + 1/V_{max} (1 + [I]/K_i)$$

where v is the initial velocity, [S] the substrate concentration, [I] the inhibitor concentration, Km the Michaelis constant, K_i the inhibition constant, and Vmax the maximum velocity. Steady-state kinetics experiments were performed with two fixed inhibitor concentrations and another in the absence of an inhibitor with various substrate (2′,3′ - cCMP) concentrations. The slopes from the double reciprocal plot were again plotted against the corresponding inhibitor concentrations [I], to generate the inhibition constants (K_i) for the competitive inhibitors.

8-3. Docking studies

The crystal structure of RNase A (PDB ID: 1FS3) was downloaded from the Protein Data Bank[10] and used for docking studies after removal of water molecules and any other ions. The 3D structures of the inhibitors were generated in Chem3D Pro and minimum energy conformations were obtained with the help of the MM2 force field using a gradient of 0.005 kcal/mole by 1000 iterations with all other default parameters. The ligands were docked with the protein using AutoDockTools-1.5.6. and AutoDock Vina.[11] Docked conformations were visualized using PyMol.[12]

9. References

1 M. Sela and C. B. Anfinsen, *Biochim. Biophys. Acta*, 1957, **24**, 229-235.

2 D. G. Anderson, G. G. Hammes and F. G. Walz Jr, *Biochemistry*, 1968, **7**, 1637-1645.

3 V. V. Rostovetsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chem. Int. Ed.,* 2002, **41,** 2596-2599.

4 V. Bevilacqua, M. King, M. Chaumontet, M. Nothisen, S. Gabillet, D. Buisson, C. Puente, A. Wagner and F. Taran, *Angew. Chem. Int. Ed.,* 2014*,* **53***,* 5872 –5876.

5 (a) T. B. Silva, K. N. K. Ji, F. P. Pauli, R. M. S. Galvao, A. F. M. Faria, M. L. Bello, J. A. L. C. Resende, V. R. Campos, L. S. M. Forezi, F. C. Silva, R. X. Faria and V. F. Ferreira, *Bioorg. Chem.*, 2021, **116**, 105250; (b) L. Hradilová, M. Poláková, B. Dvoráková, M. Hajdúch and L. Petruš, *Carbohydr. Res.*, 2012, **361**, 1-6.

6 L. S. Campbell-Verduyn, L. Mirfeizi, R. A. Dierckx, P. H. Elsinga and B. L. Feringa, *Chem. Commun.*, 2009, 2139–2141.

7 A. Singh, C. Biot, A. Viljoen, C. Dupont, L. Kremer, K. Kumar and V. Kumar, *Chem Biol Drug Des.,* 2017, **89**, 856–861.

8 C. Schneider, W. Rasband and K. Eliceiri, *Nat Methods*, 2012, **9**, 671–675.

9 H. Lineweaver and D. Burk, *J. Am. Chem. Soc.*, 1934, **56**, 658-666.

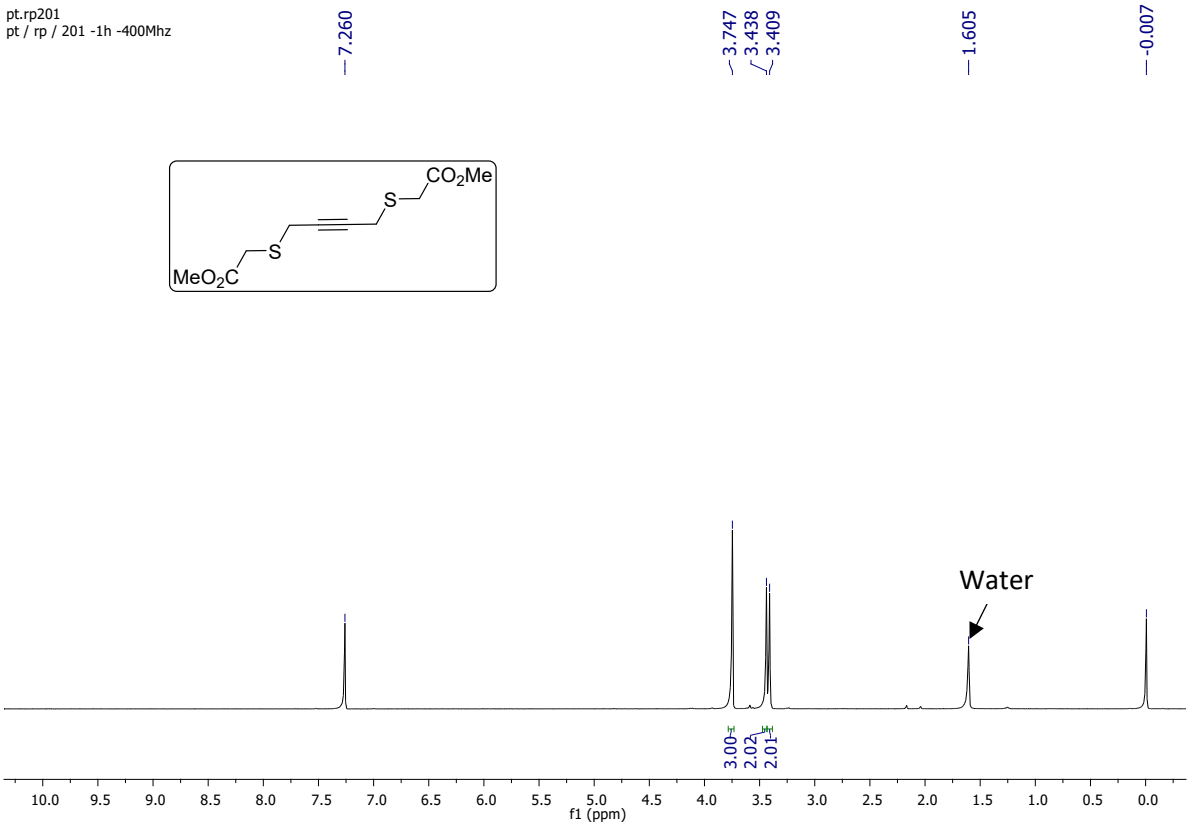
10 H. M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T. N. Bhat, H. Weissig, L. N. Shindyalov and P. E. Bourne, *Nucleic acids res*, 2000, **28**, 235-242.

11 O. Trott and A. J. Olson, *J. Comput. Chem.*, 2010, **31**, 455-461.

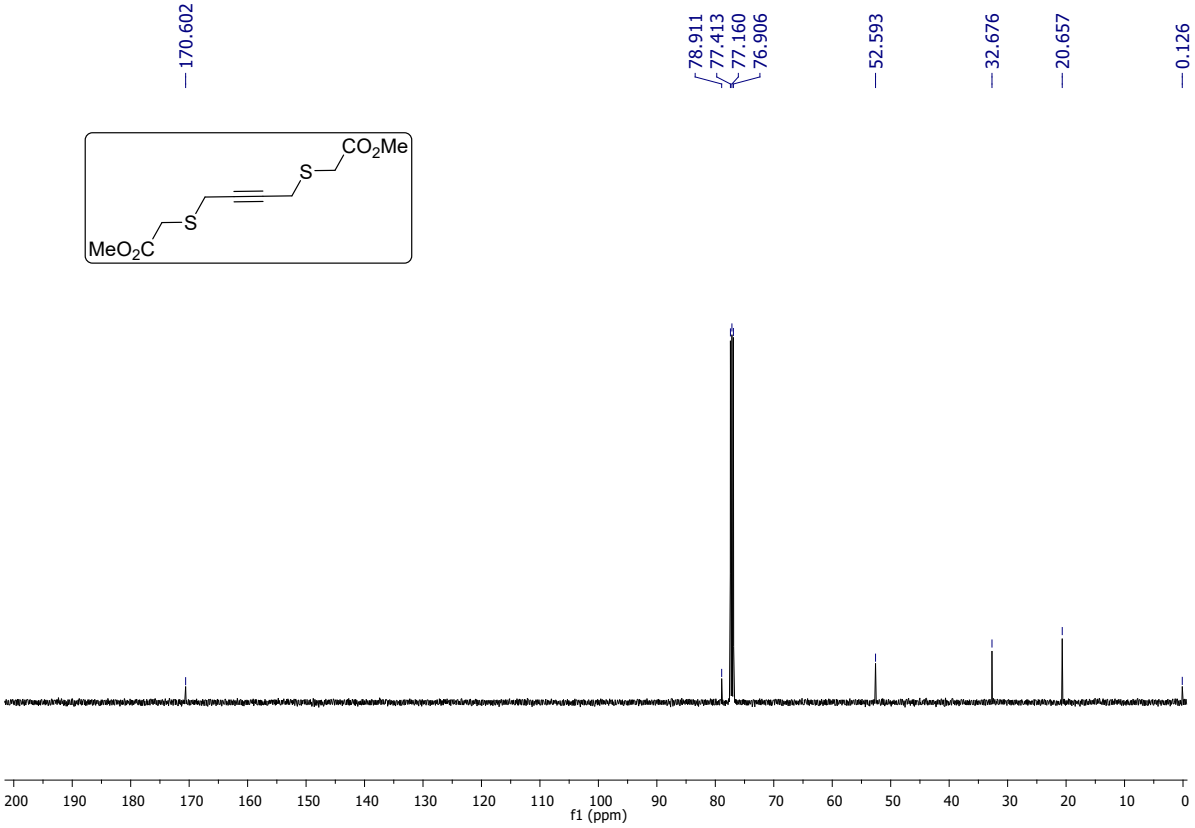
12 W. L. De Lano, The PyMOL Molecular Graphics System (Version 2.0), San Carlos, CA, 2006.

10. ^{1}H NMR, ^{13}C NMR and DEPT 135 NMR Spectra of All Compounds

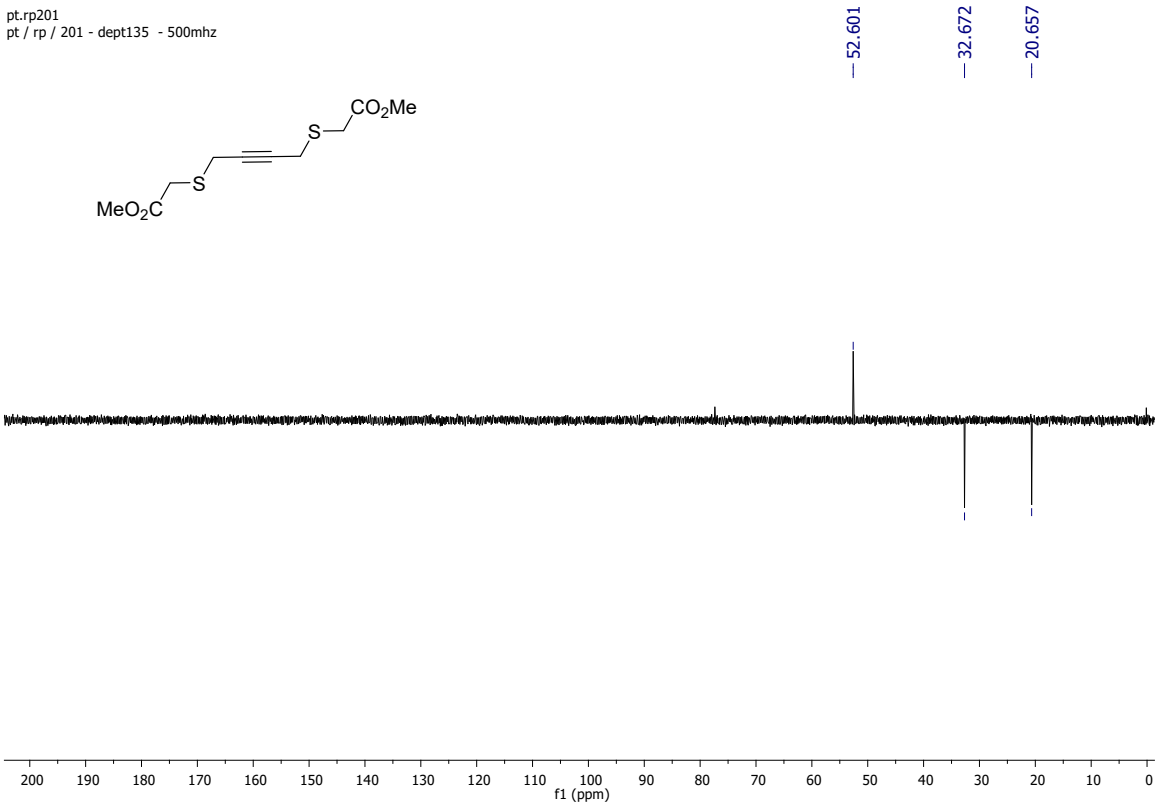
^{1}H-NMR spectra of compound **2** (400MHz, $CDCl_3$, 25°C)



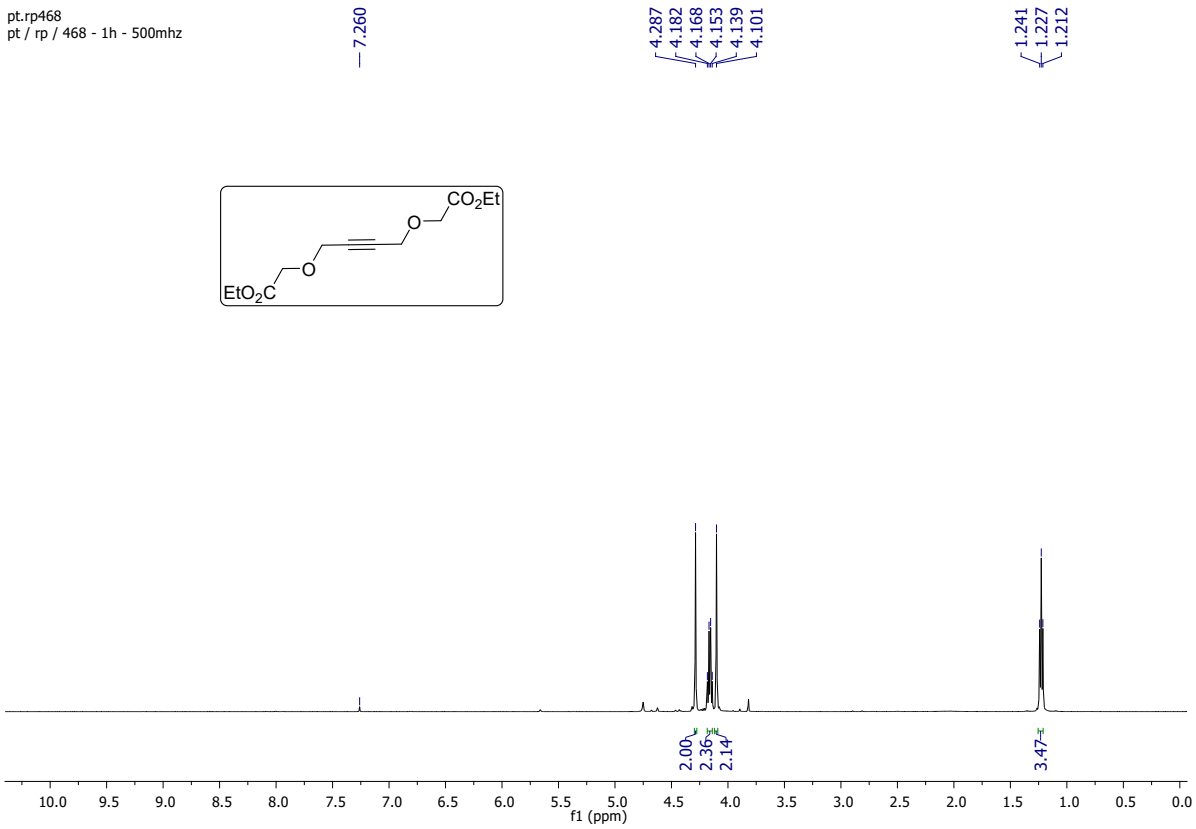
^{13}C-NMR spectra of compound **2** (125MHz, $CDCl_3$, 25°C)



DEPT-135 spectra of compound **2** (125MHz, $CDCl_3$, 25°C)



^{1}H-NMR spectra of compound **3** (500MHz, $CDCl_3$, 25^{o}C)



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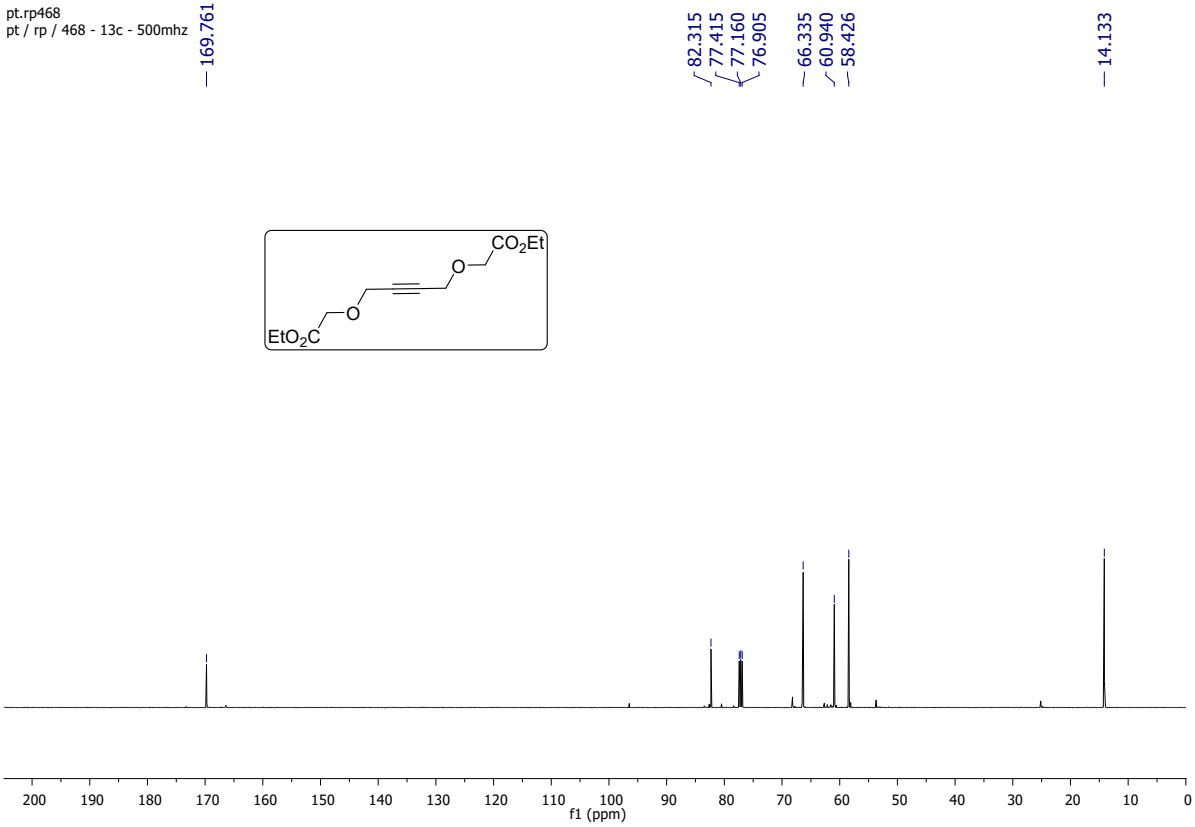
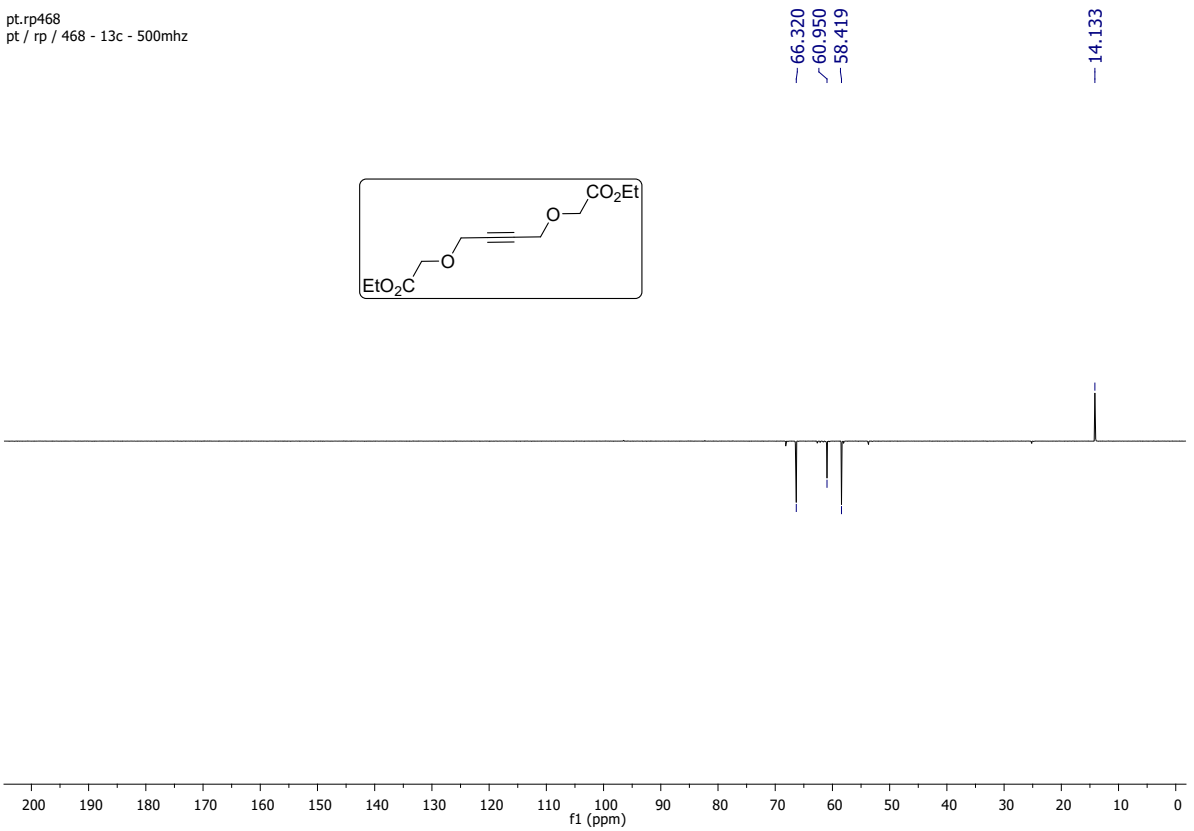
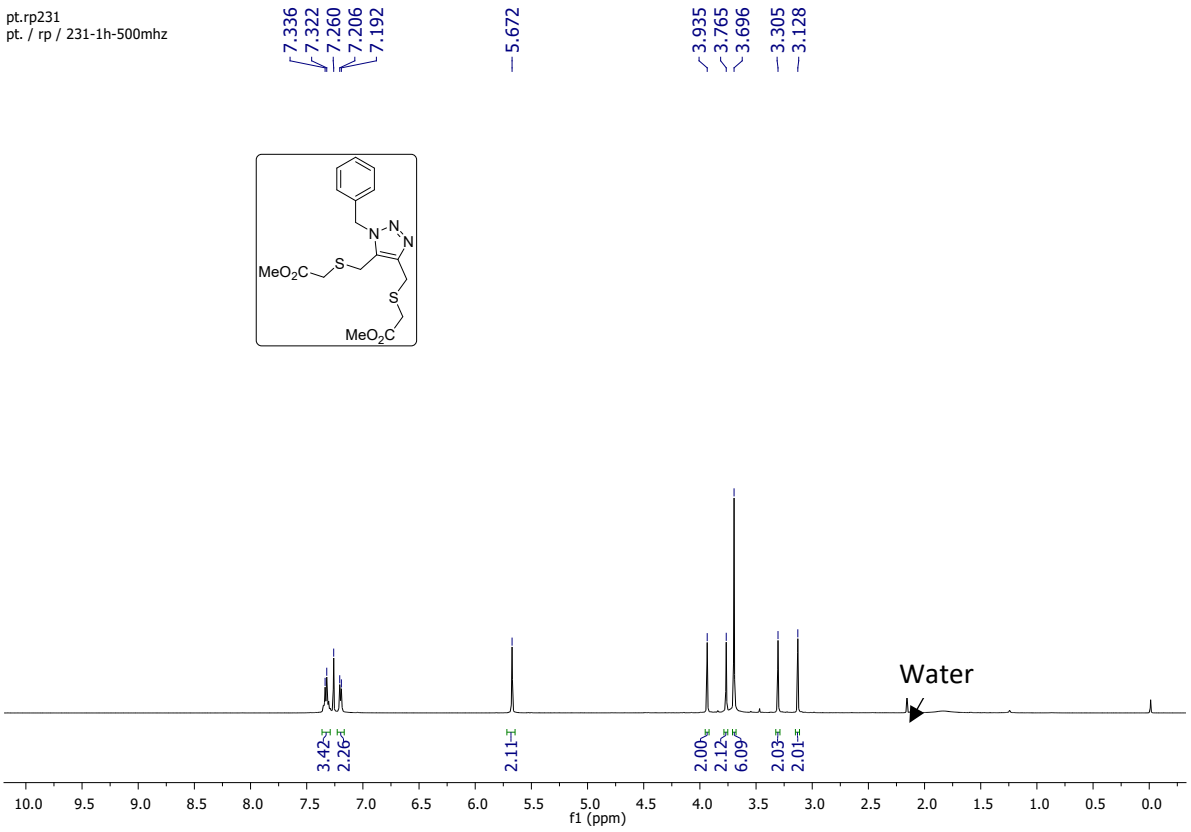


DEPT-135 spectra of compound **3** (125MHz, $CDCl_3$, 25°C)



^{1}H-NMR spectra of compound **5a** (500MHz, $CDCl_3$, 25°C)



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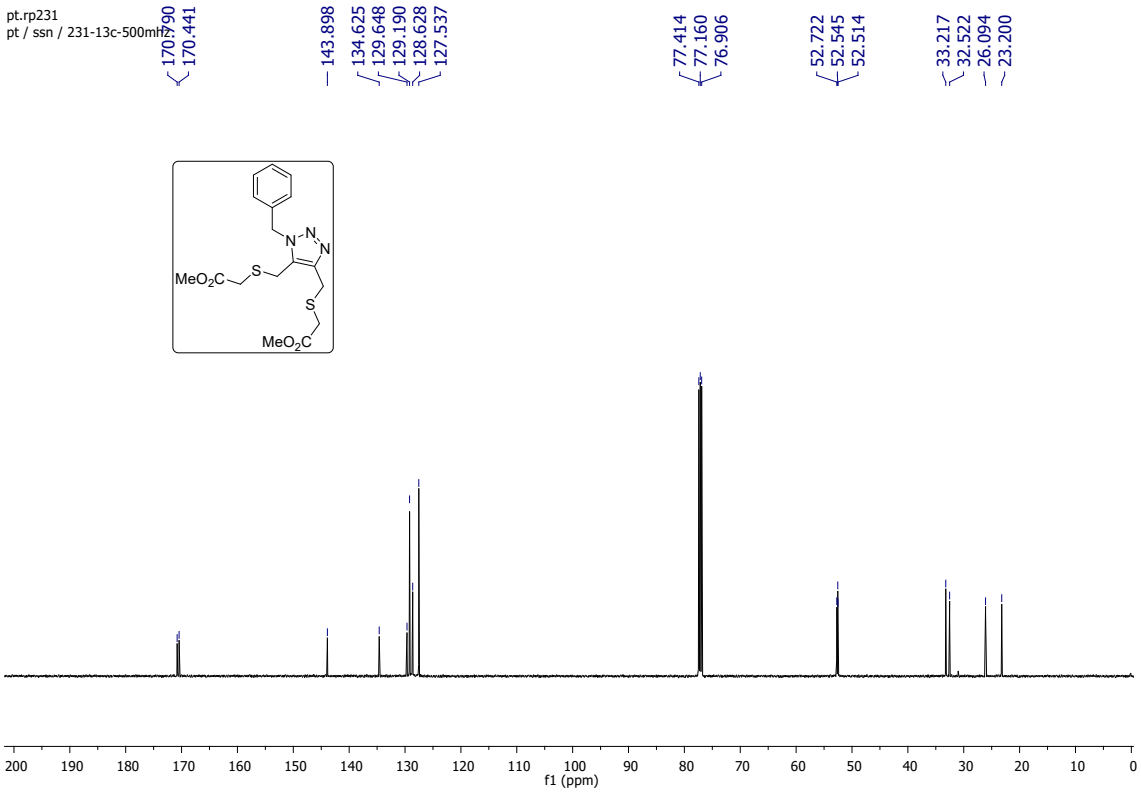
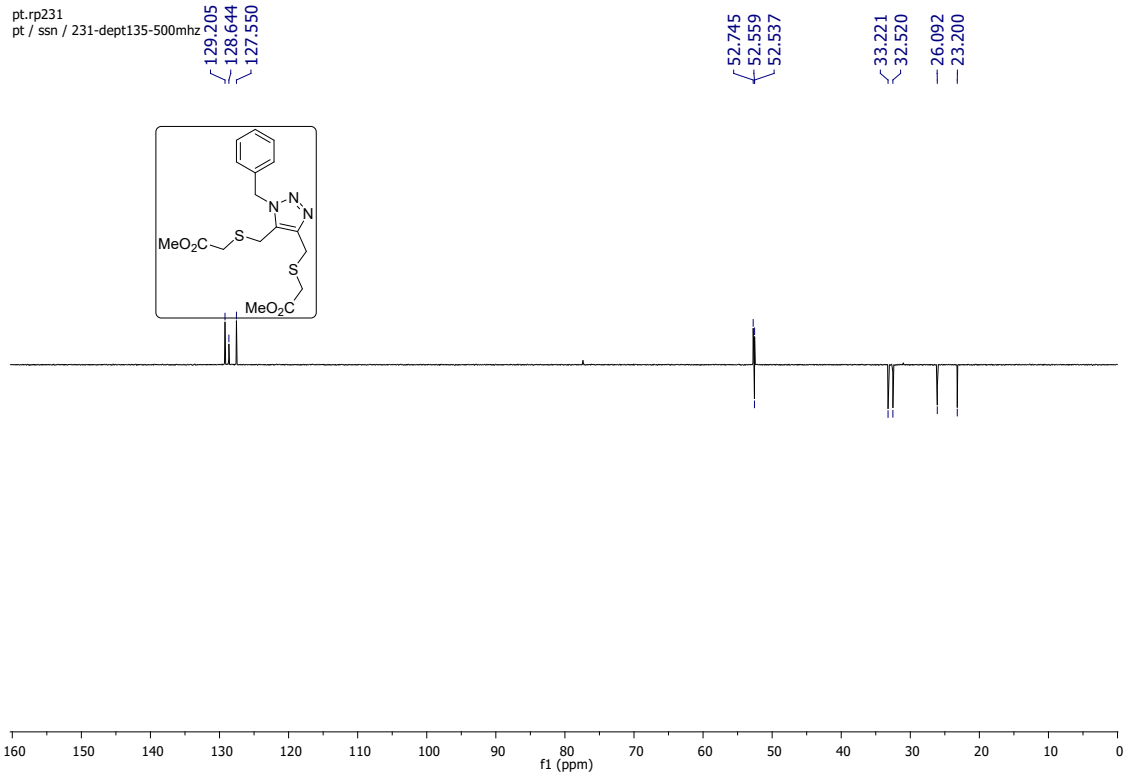
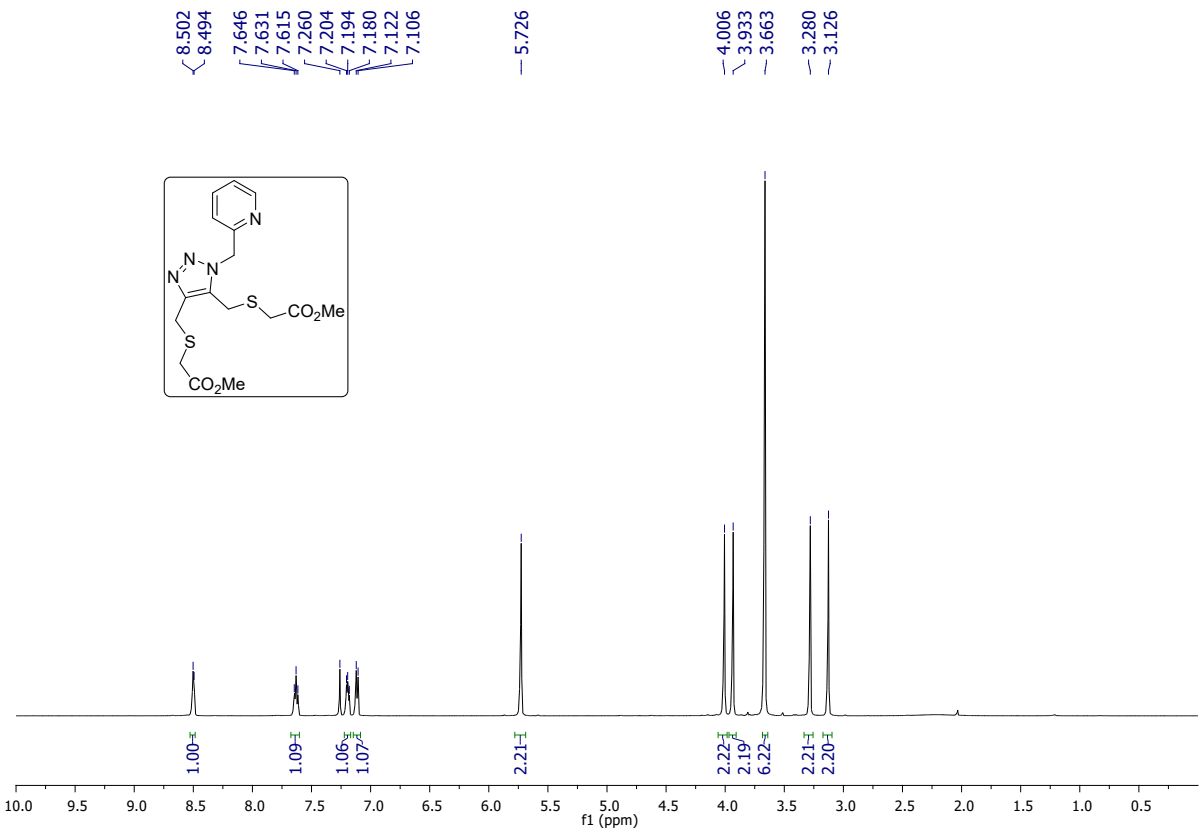


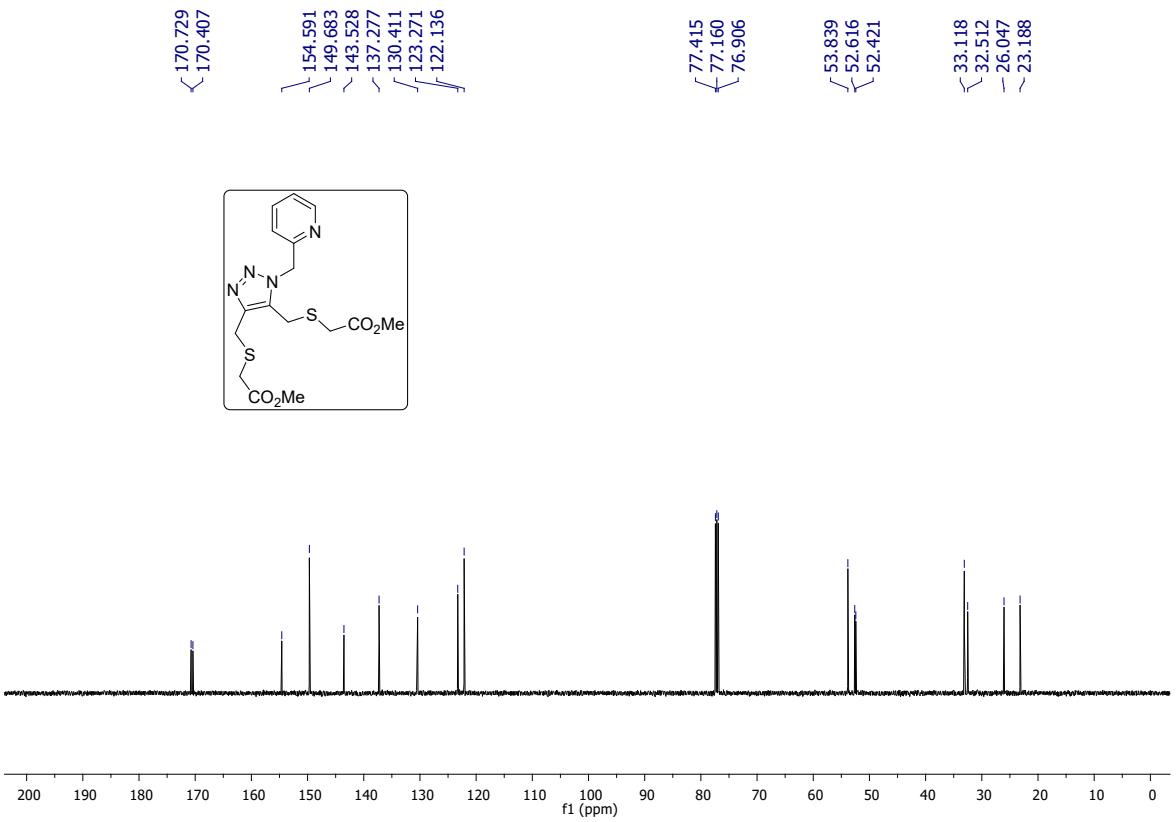
DEPT-135 spectra of compound **5a** (125MHz, $CDCl_3$, 25°C)



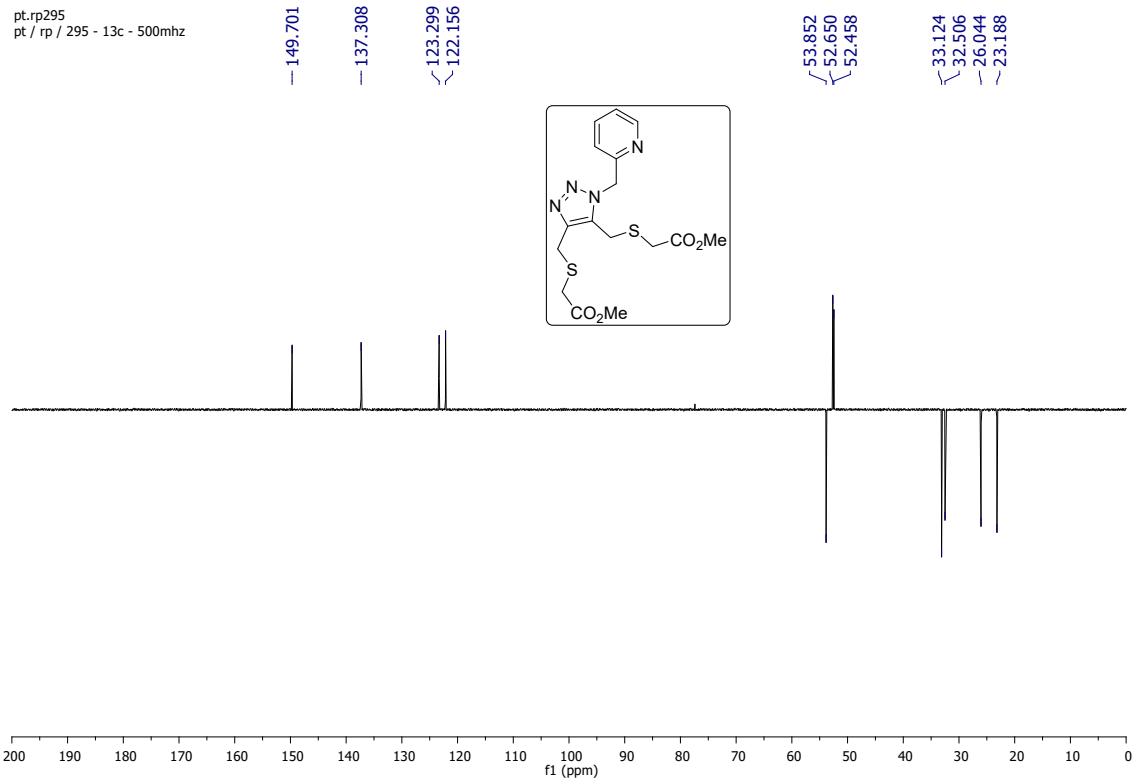
^{1}H-NMR spectra of compound **5b** (400MHz, $CDCl_3$, 25^{o}C)



^{13}C-NMR spectra of compound **5b** (125MHz, $CDCl_3$, 25°C)



DEPT-135 spectra of compound **5b** (125MHz, $CDCl_3$, 25°C)



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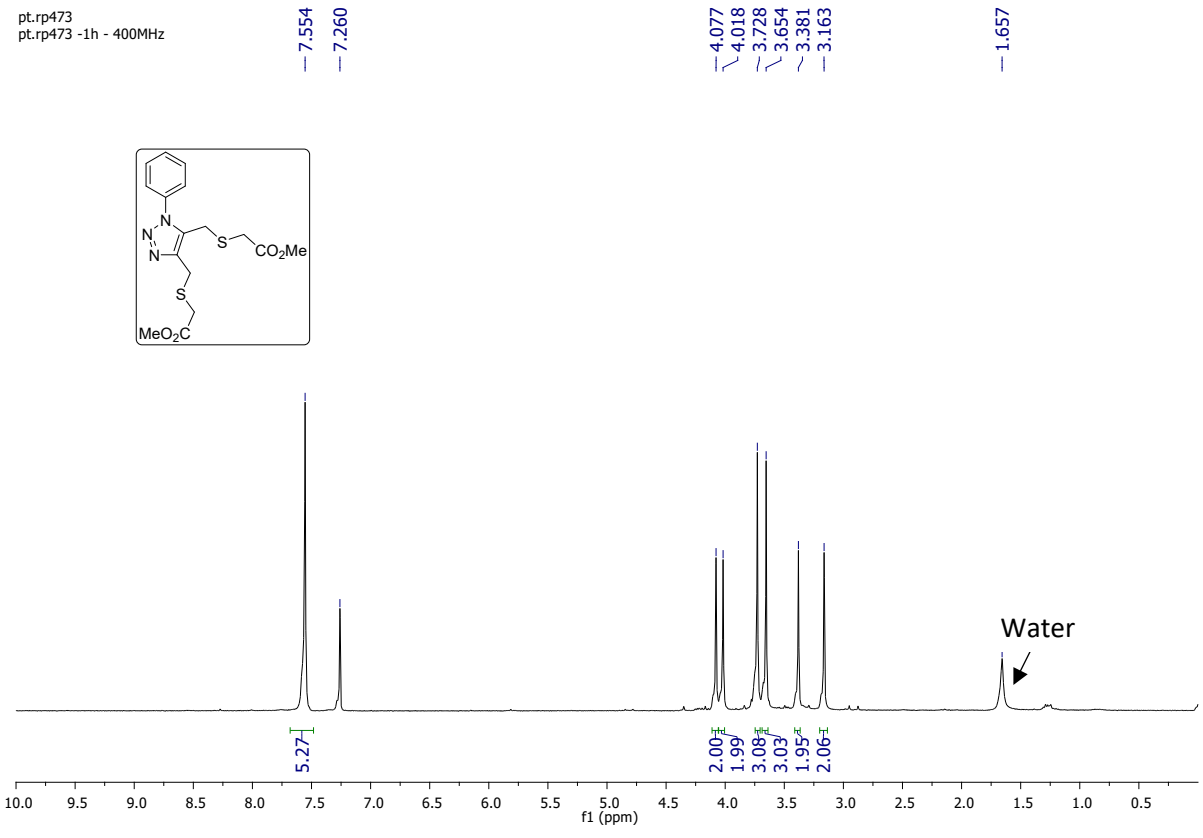

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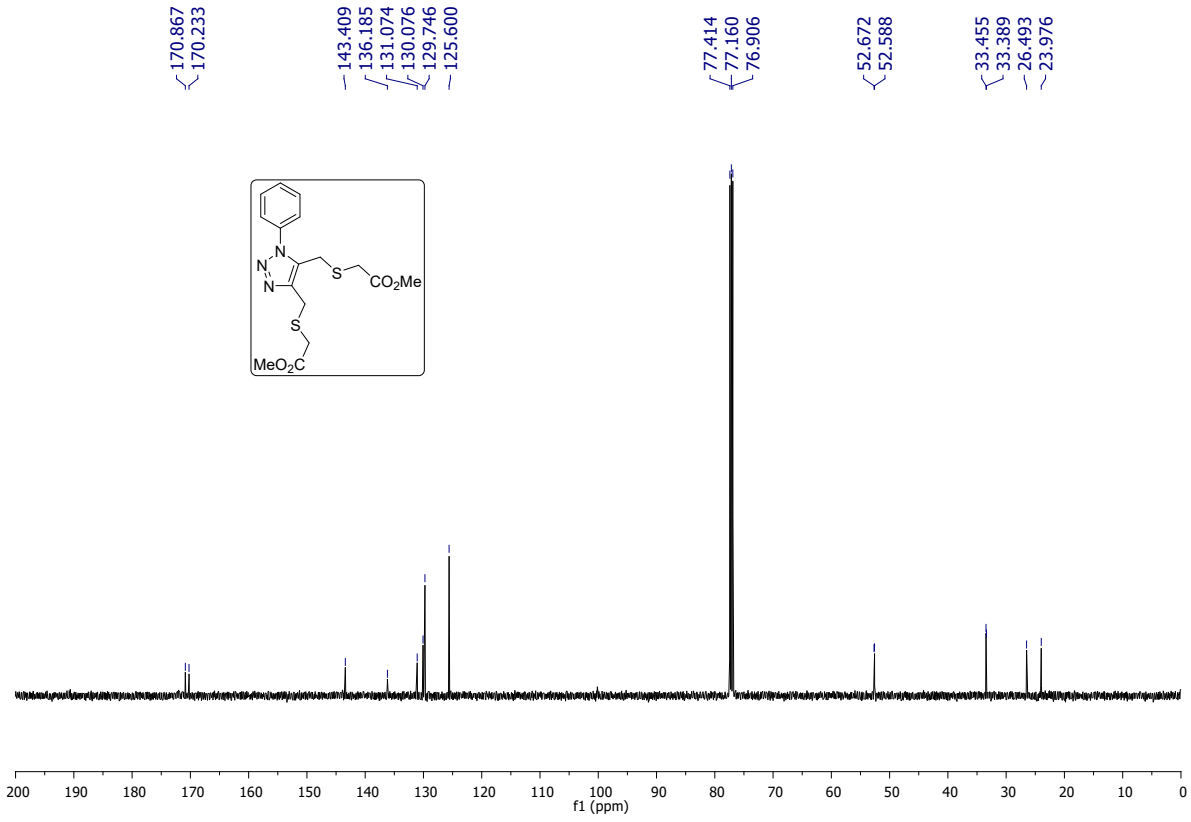

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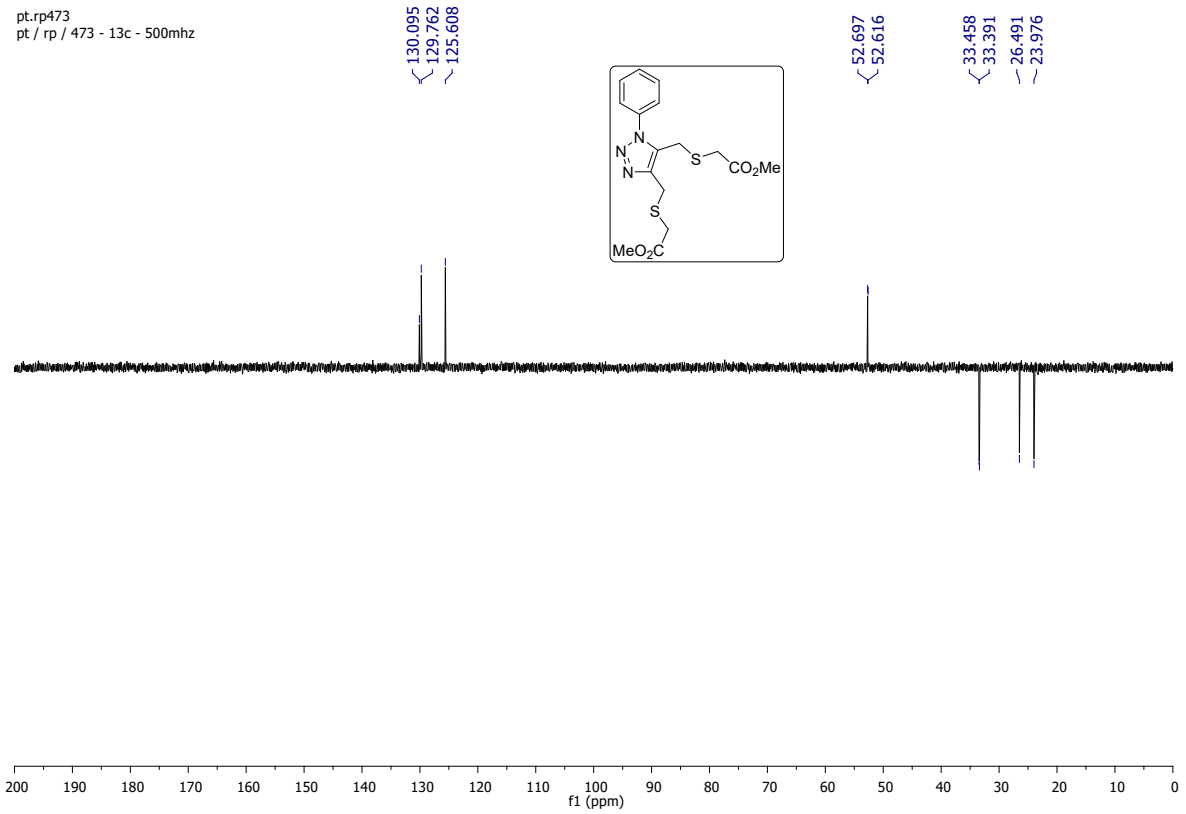

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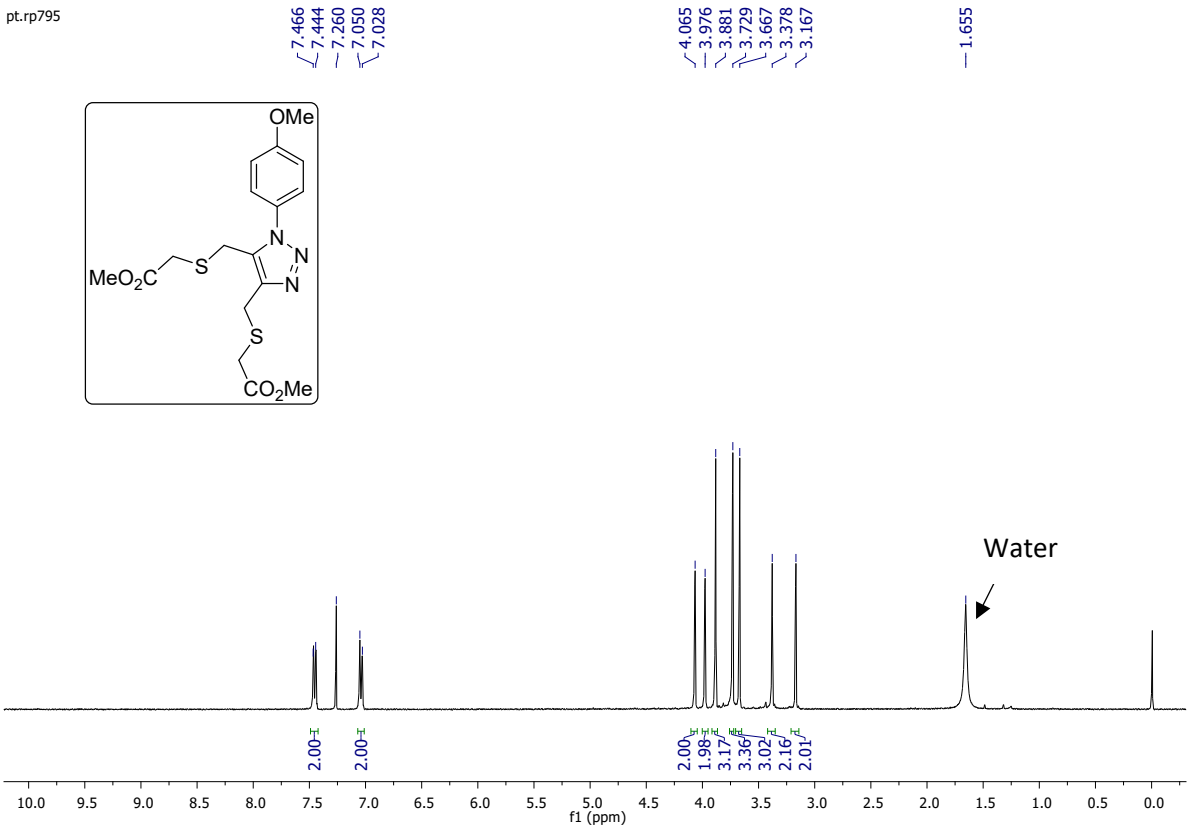

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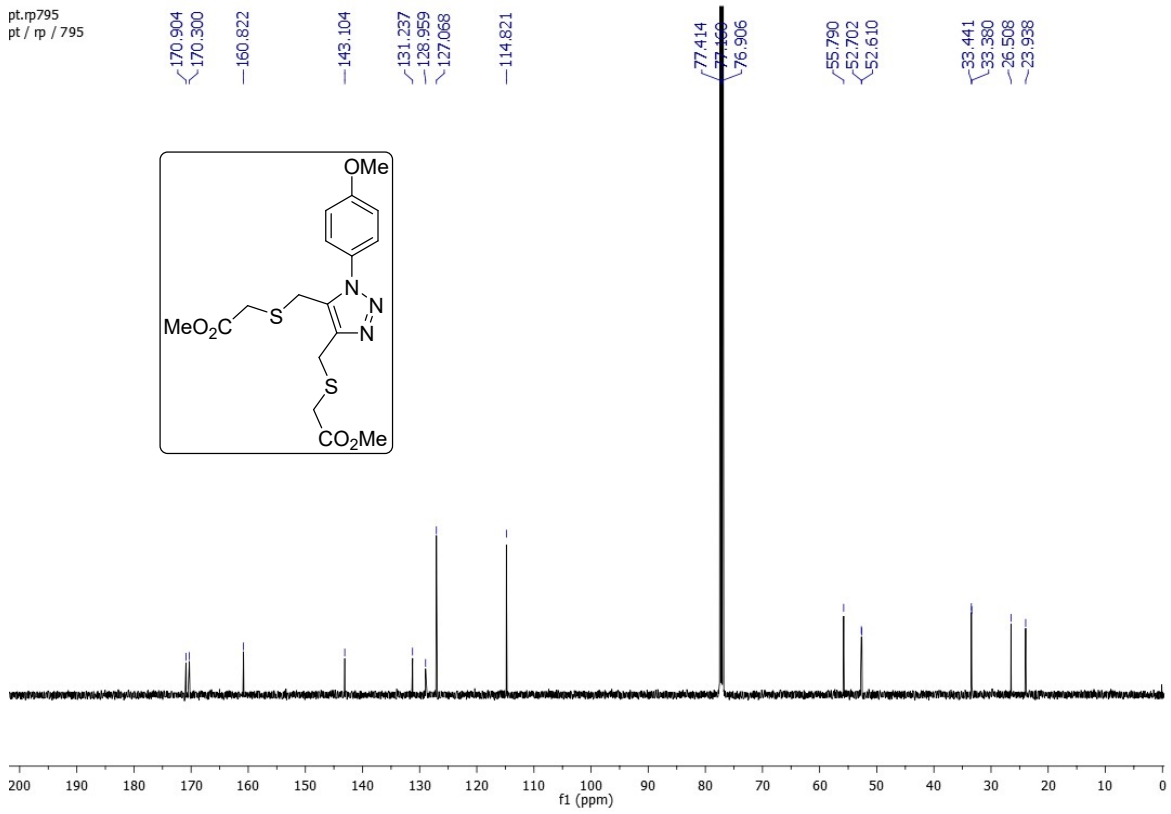

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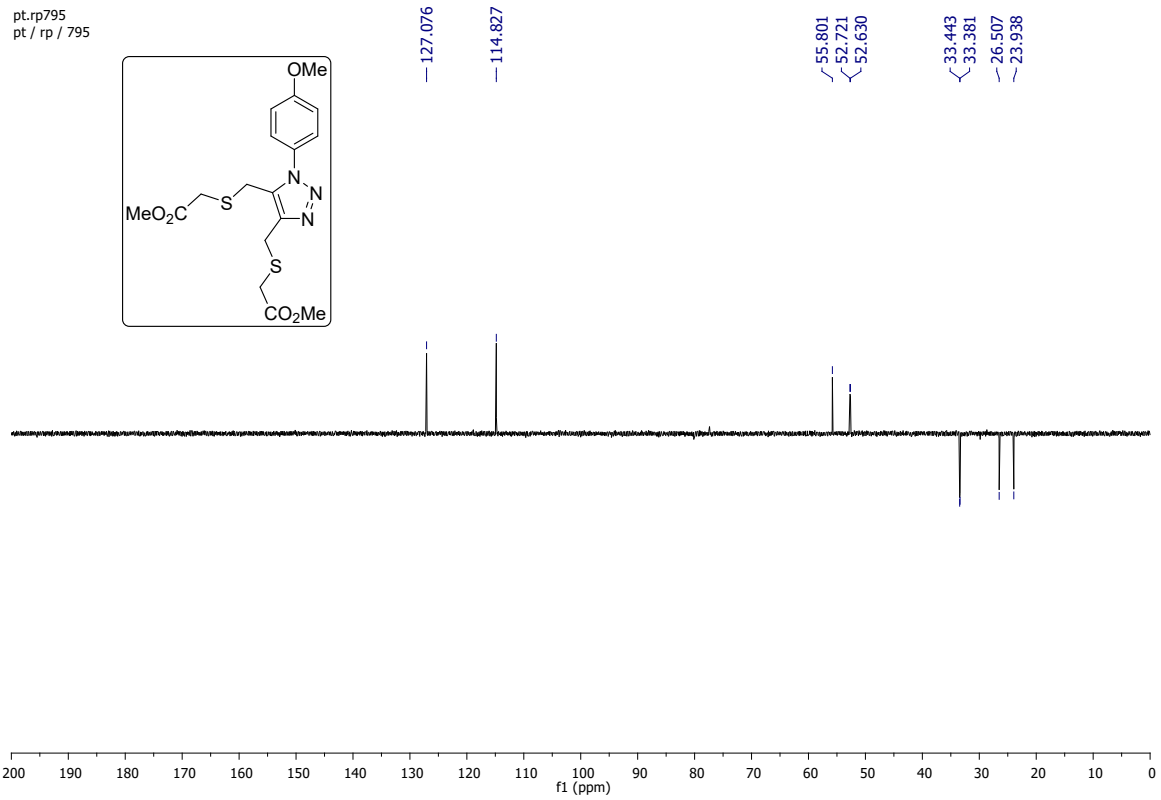

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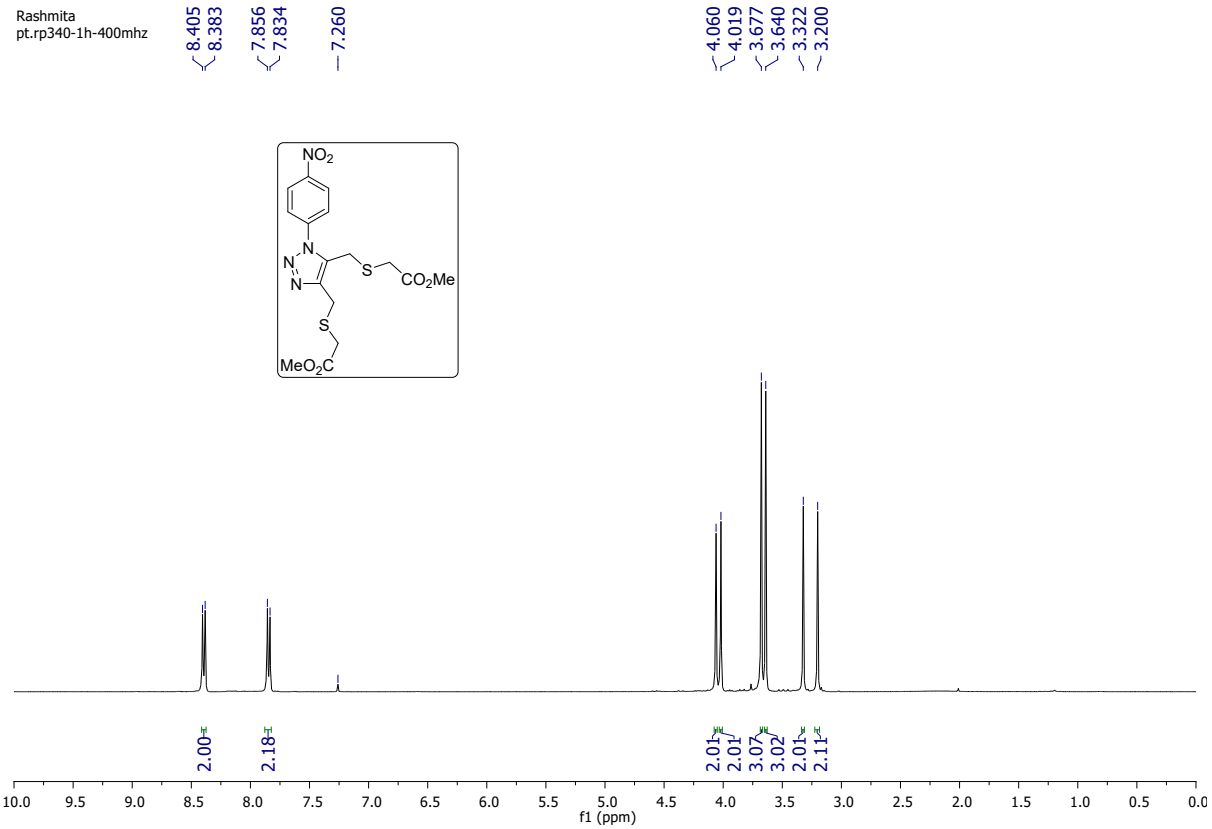

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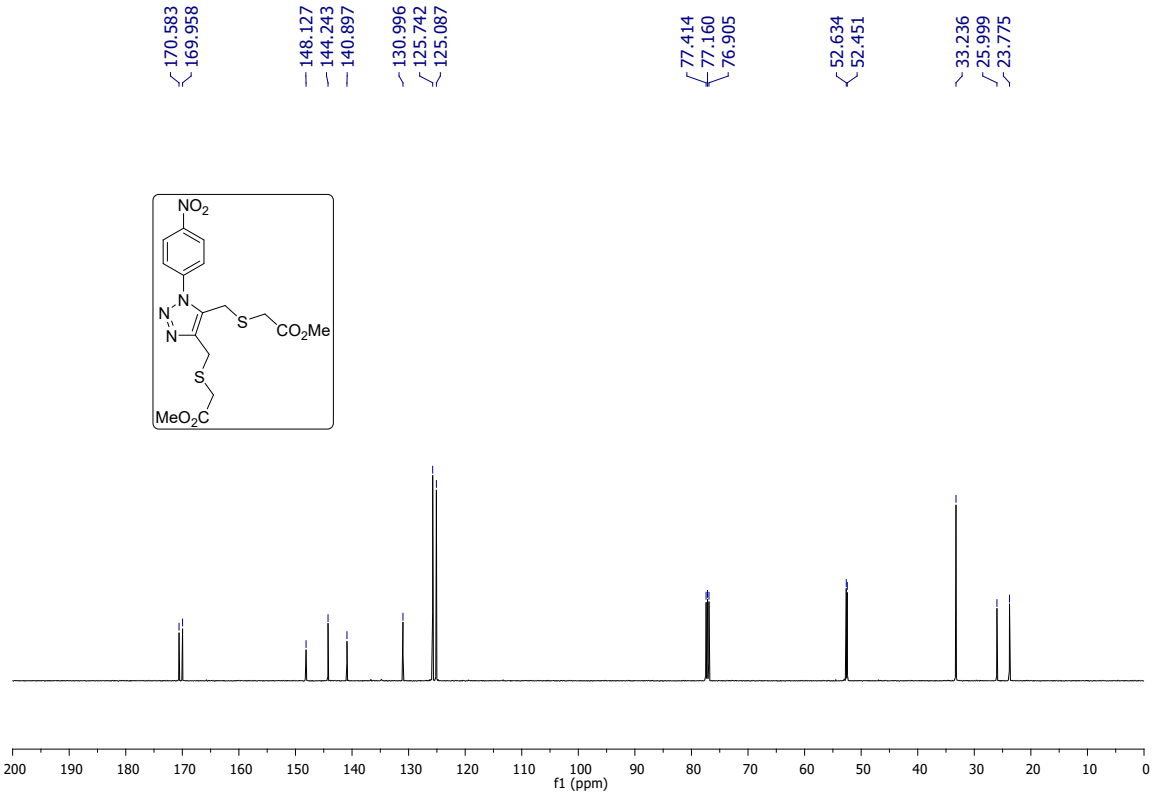

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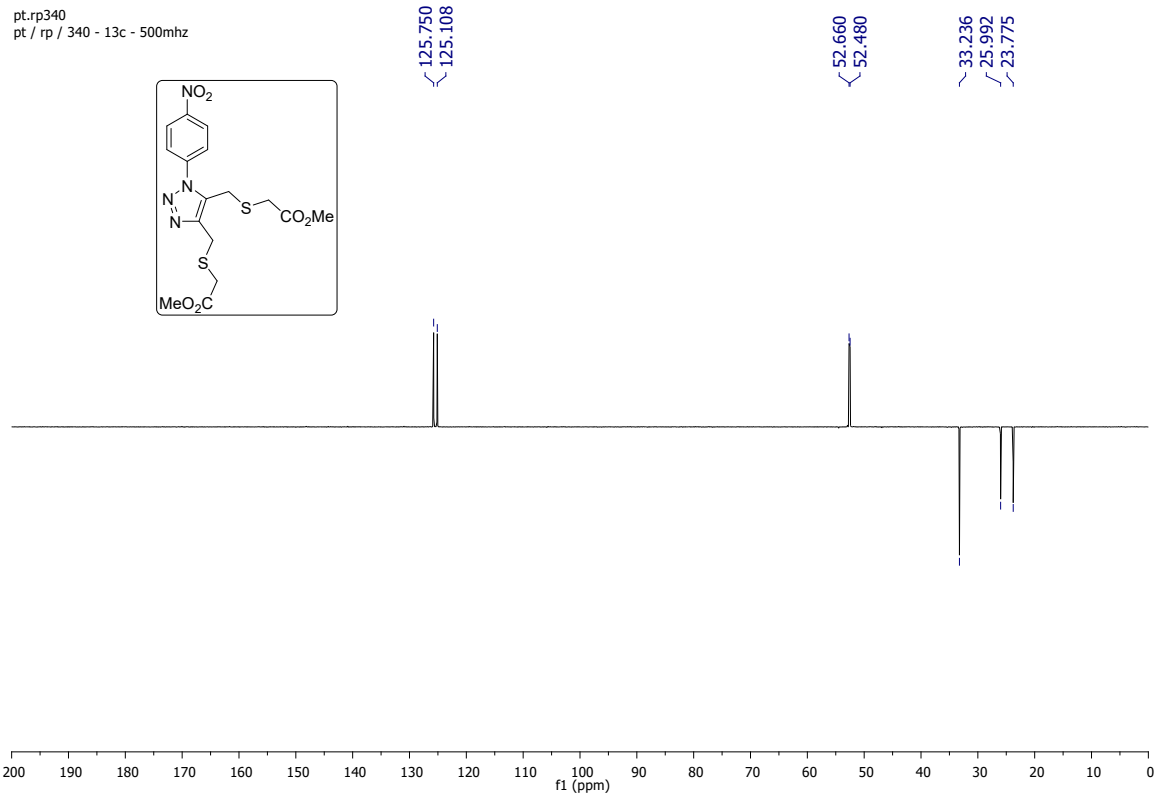
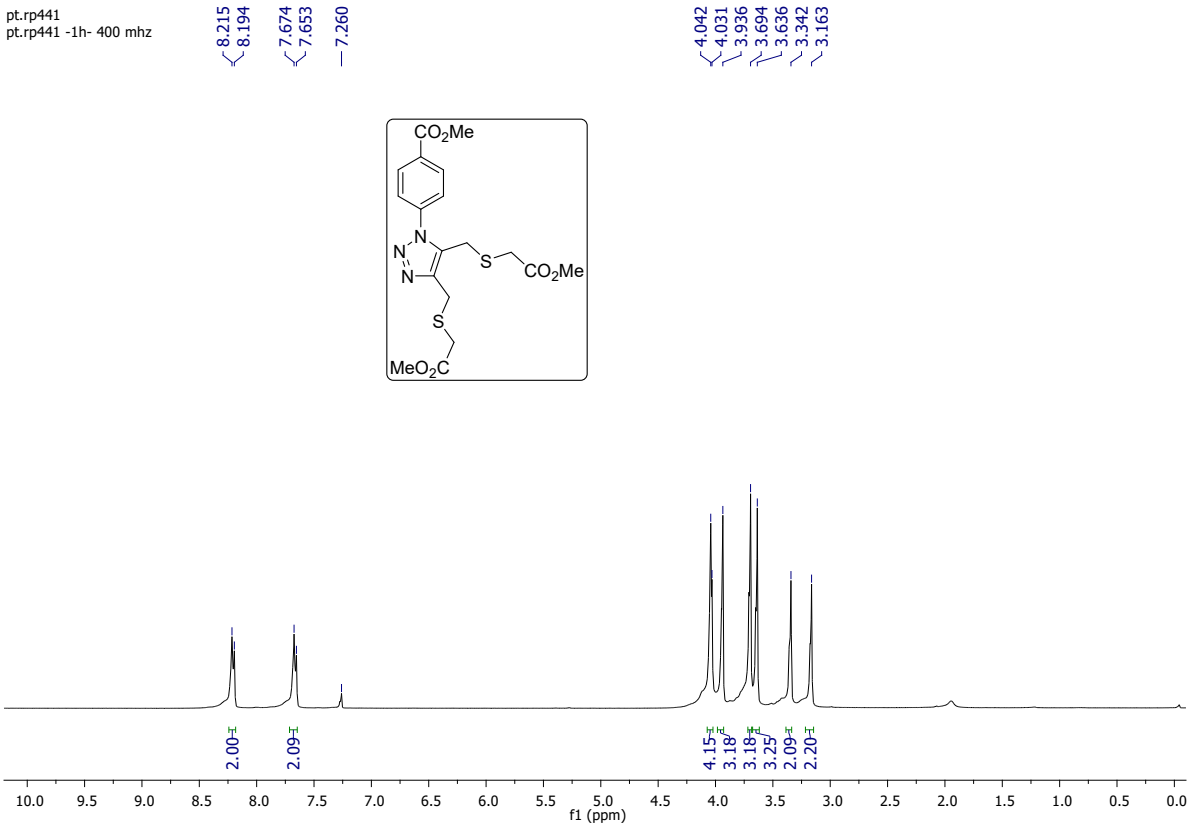


^{1}H-NMR spectra of compound **5f** (400MHz, $CDCl_3$, 25°C)



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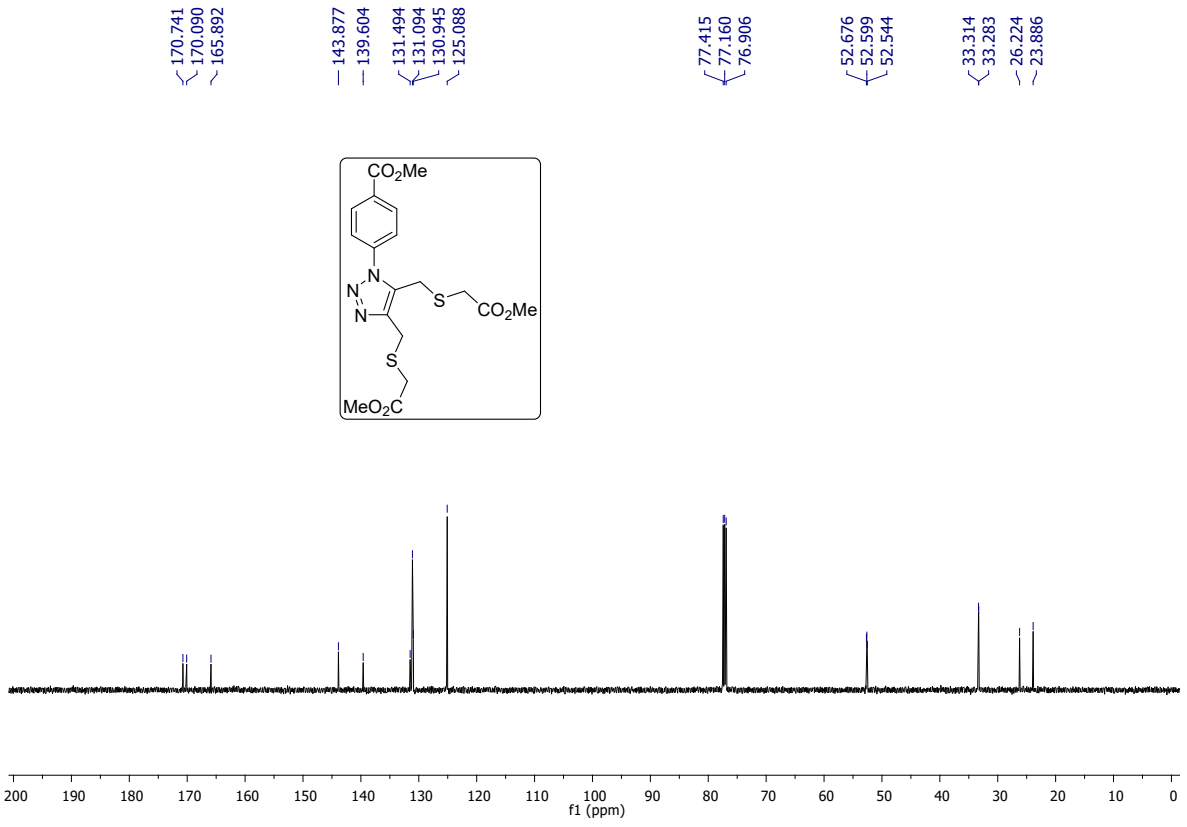

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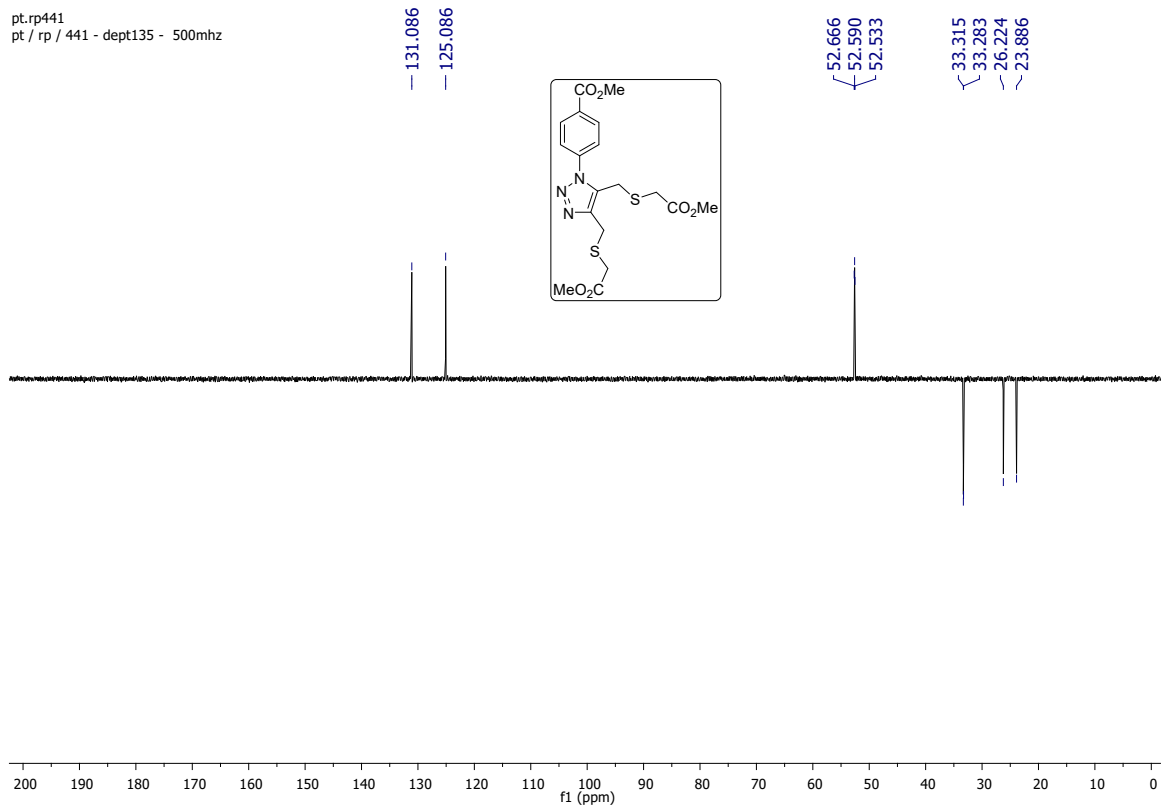
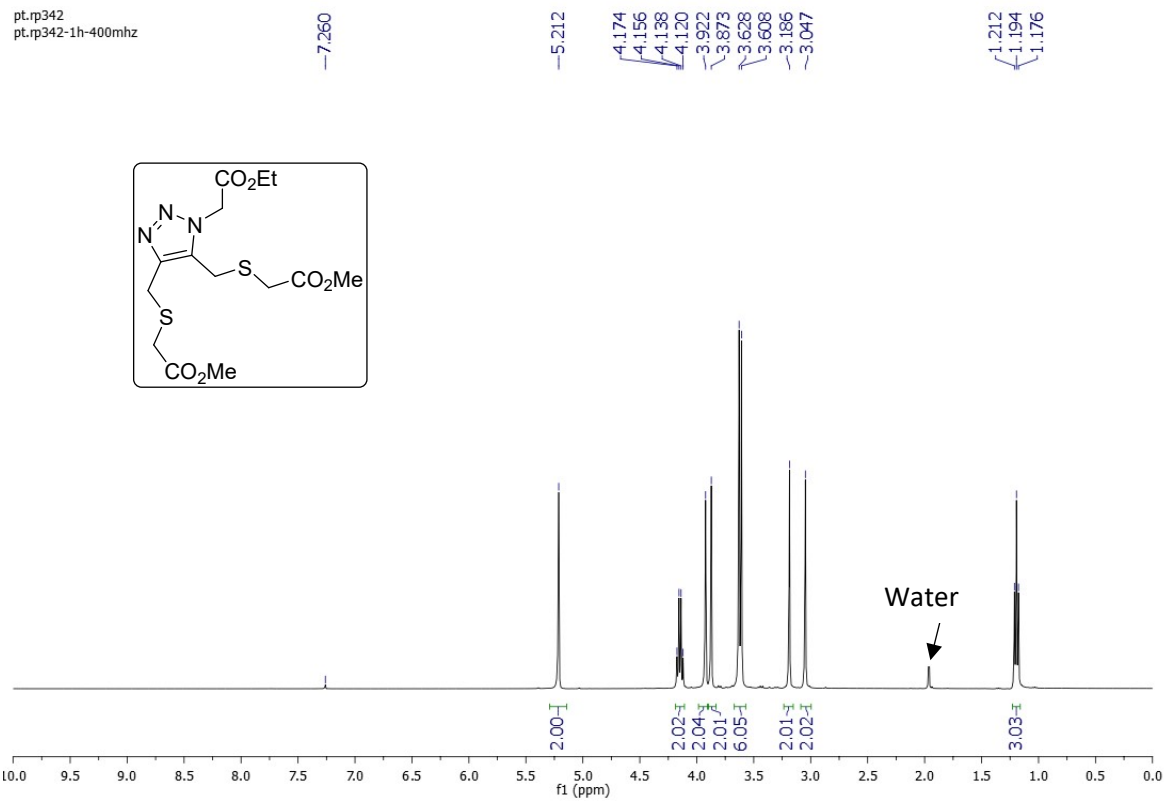


^{1}H-NMR spectra of compound **5g** (400MHz, $CDCl_3$, 25^{o}C)



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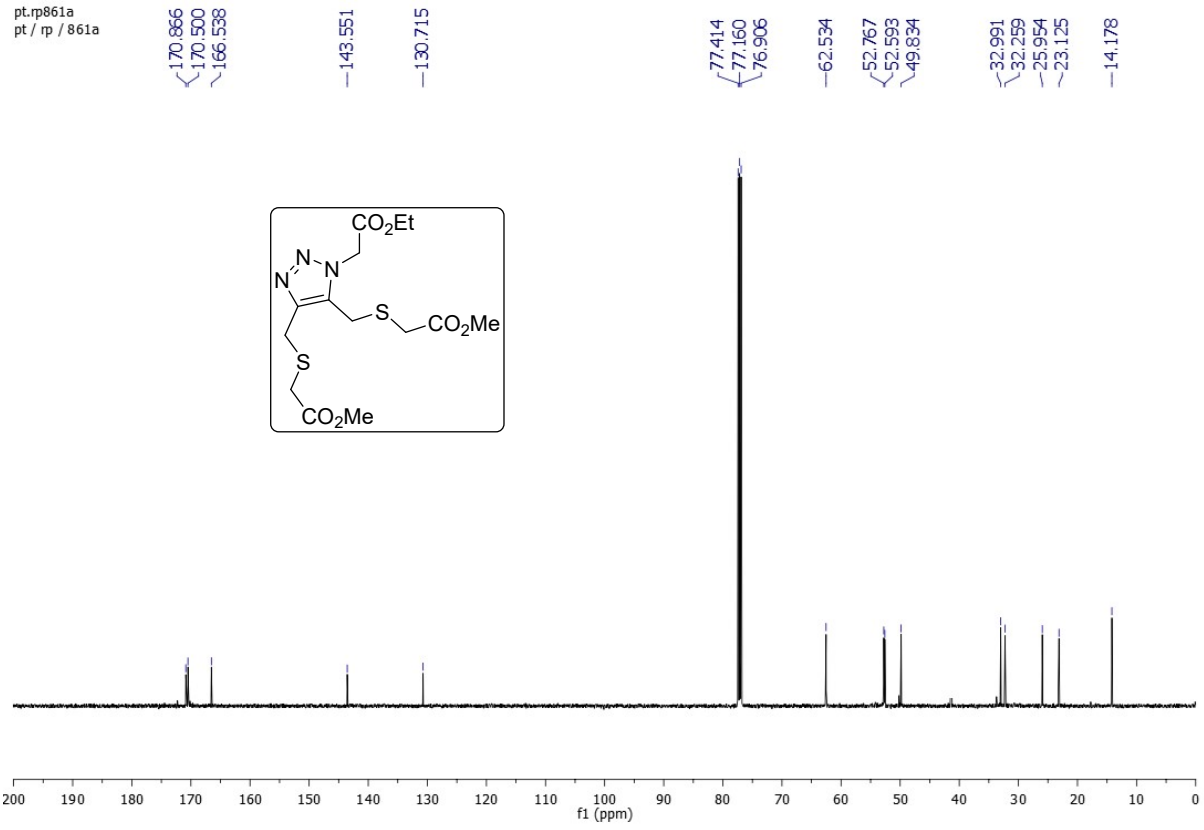

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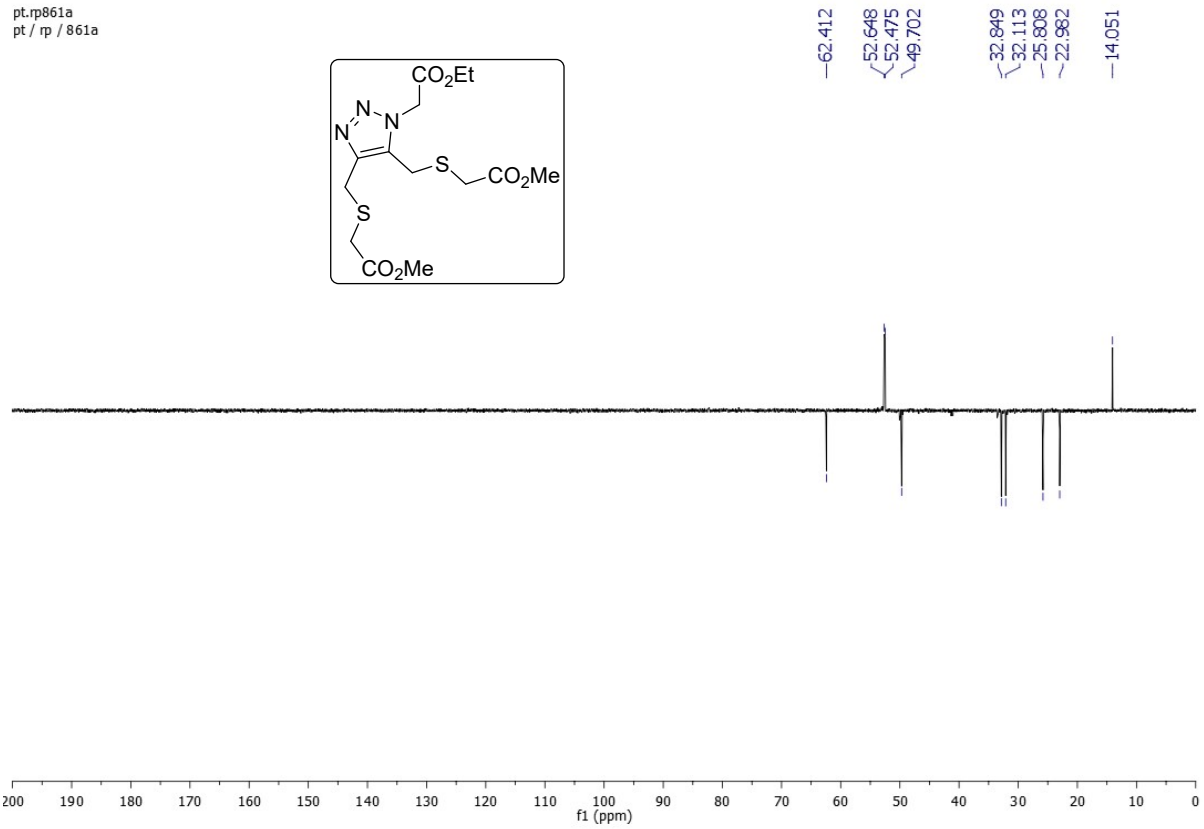
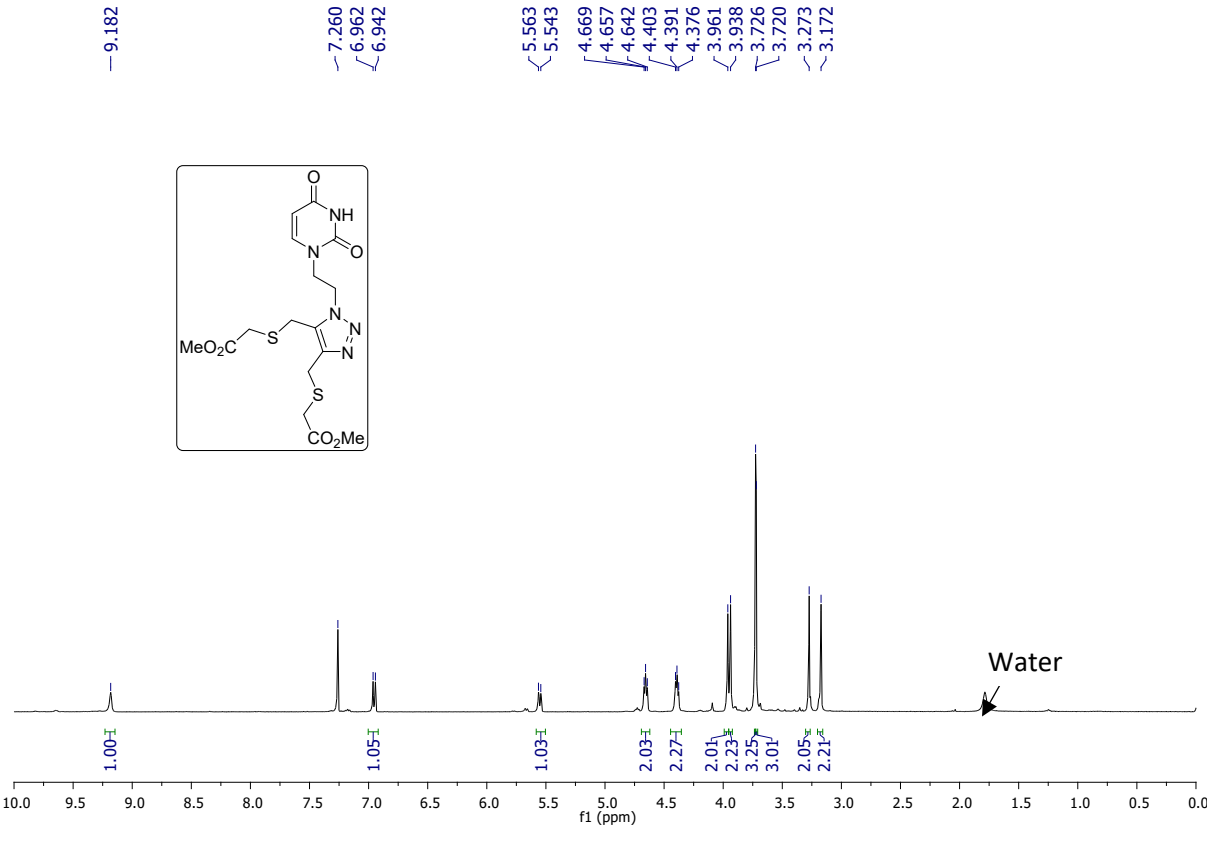
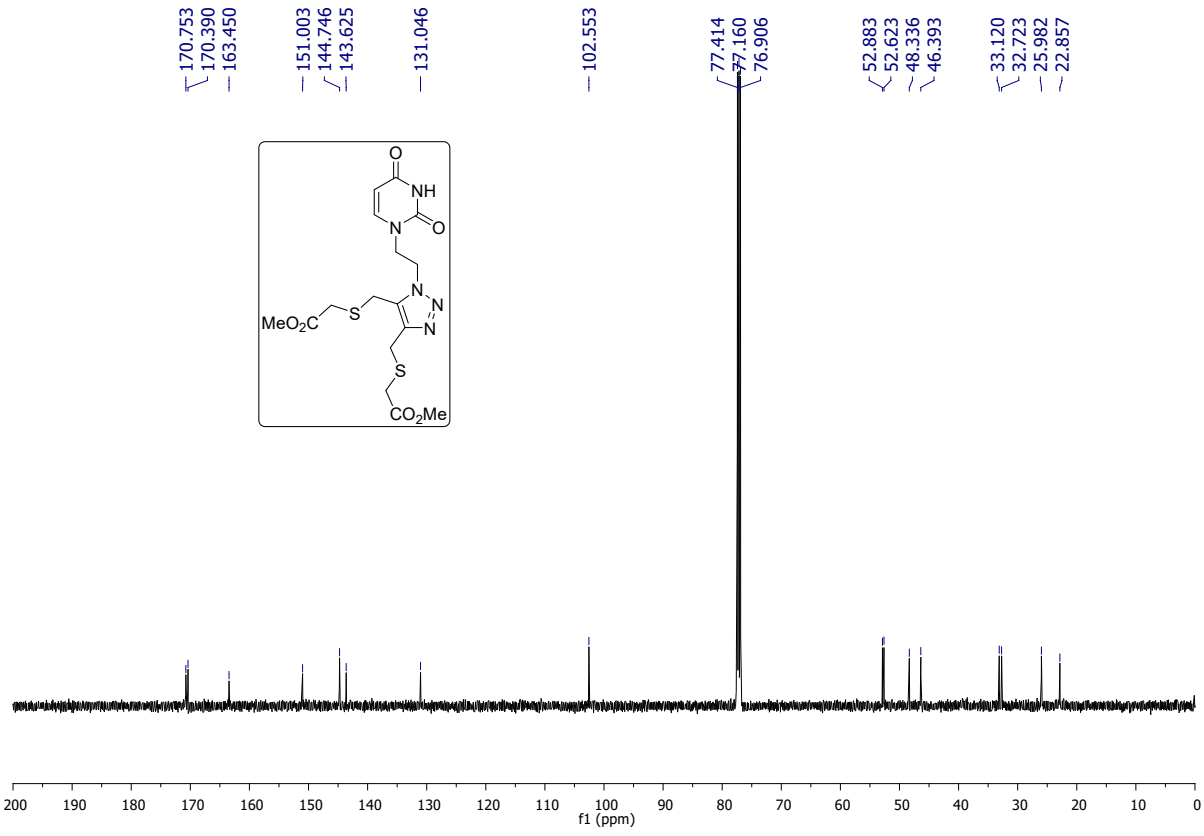


^{1}H-NMR spectra of compound **5h** (400MHz, $CDCl_3$, 25^{o}C)



^{13}C-NMR spectra of compound **5h** (125MHz, $CDCl_3$, 25^{o}C)



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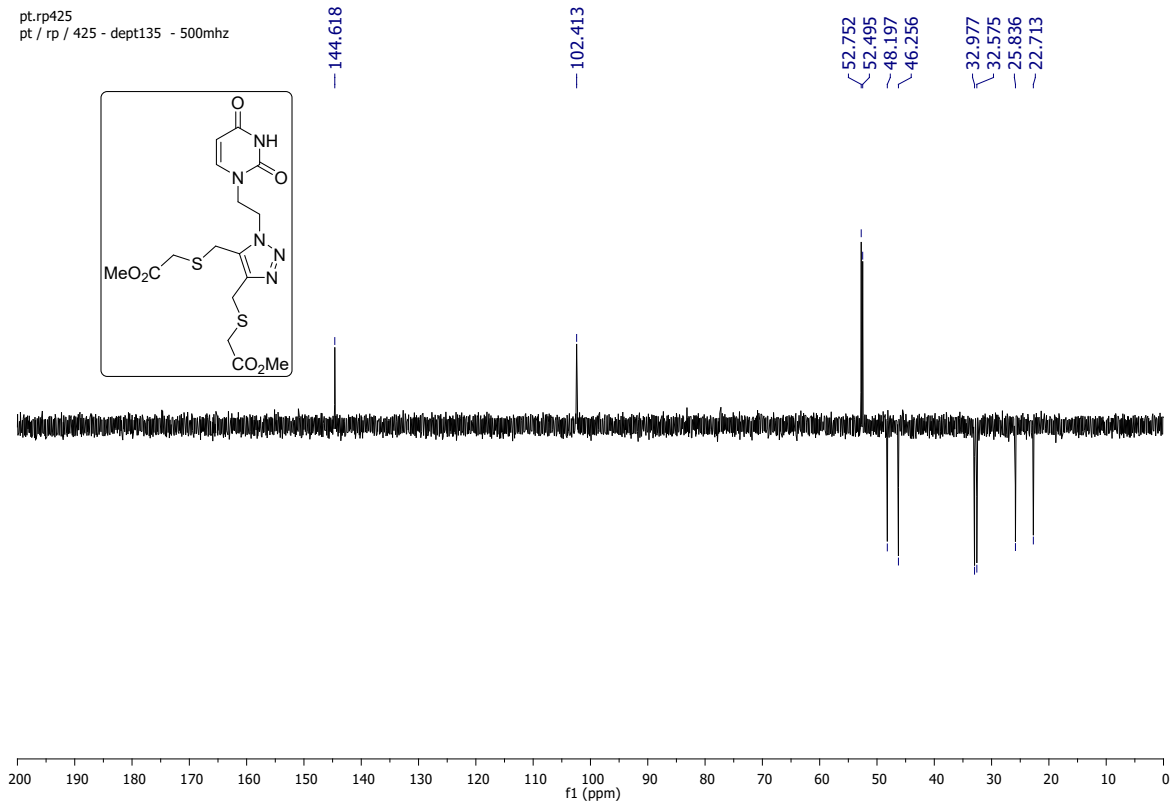
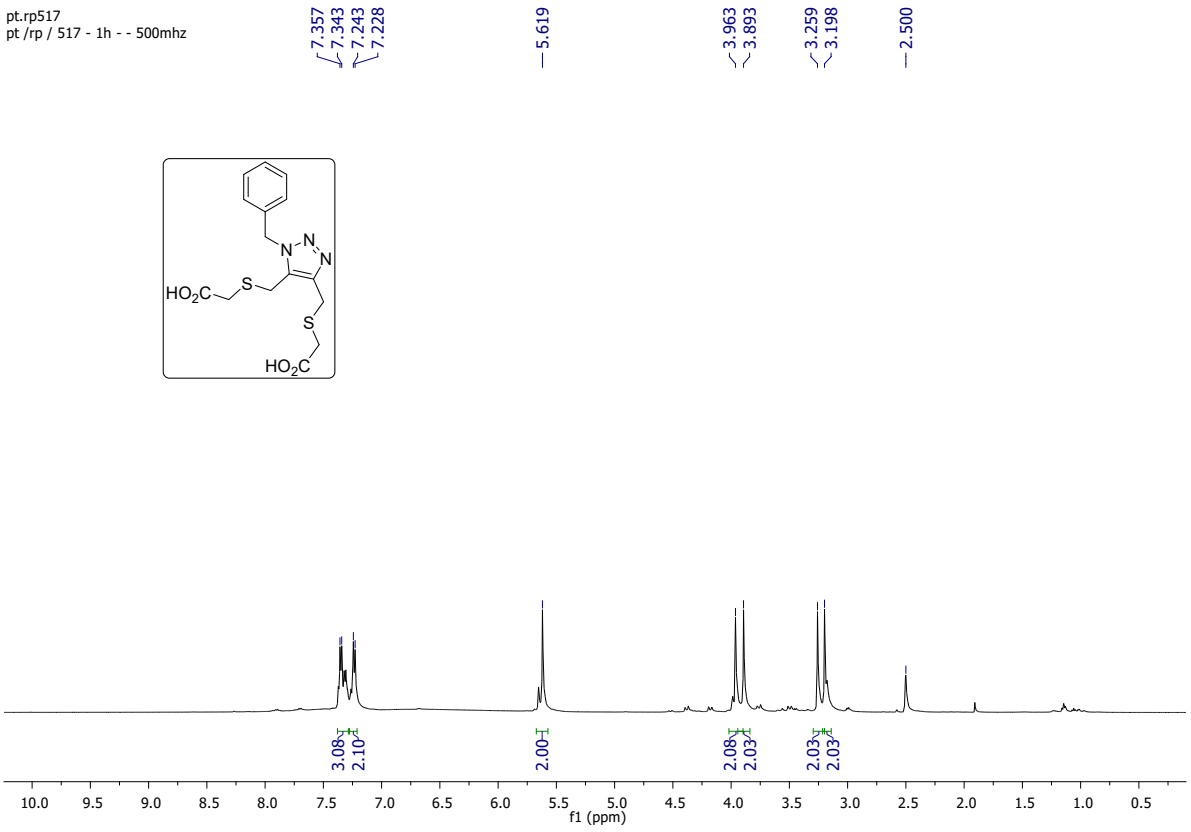


^{1}H-NMR spectra of compound **6a** (500MHz, DMSO-d_6, 25°C)



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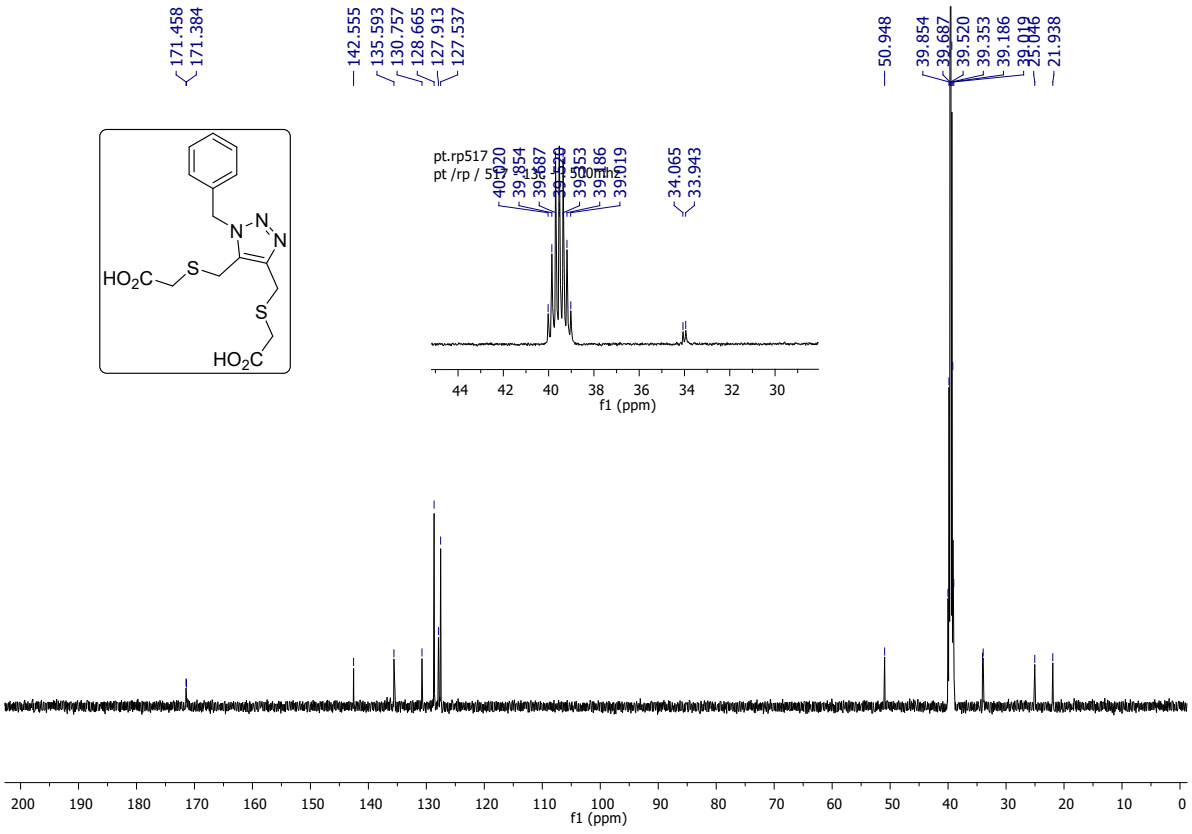

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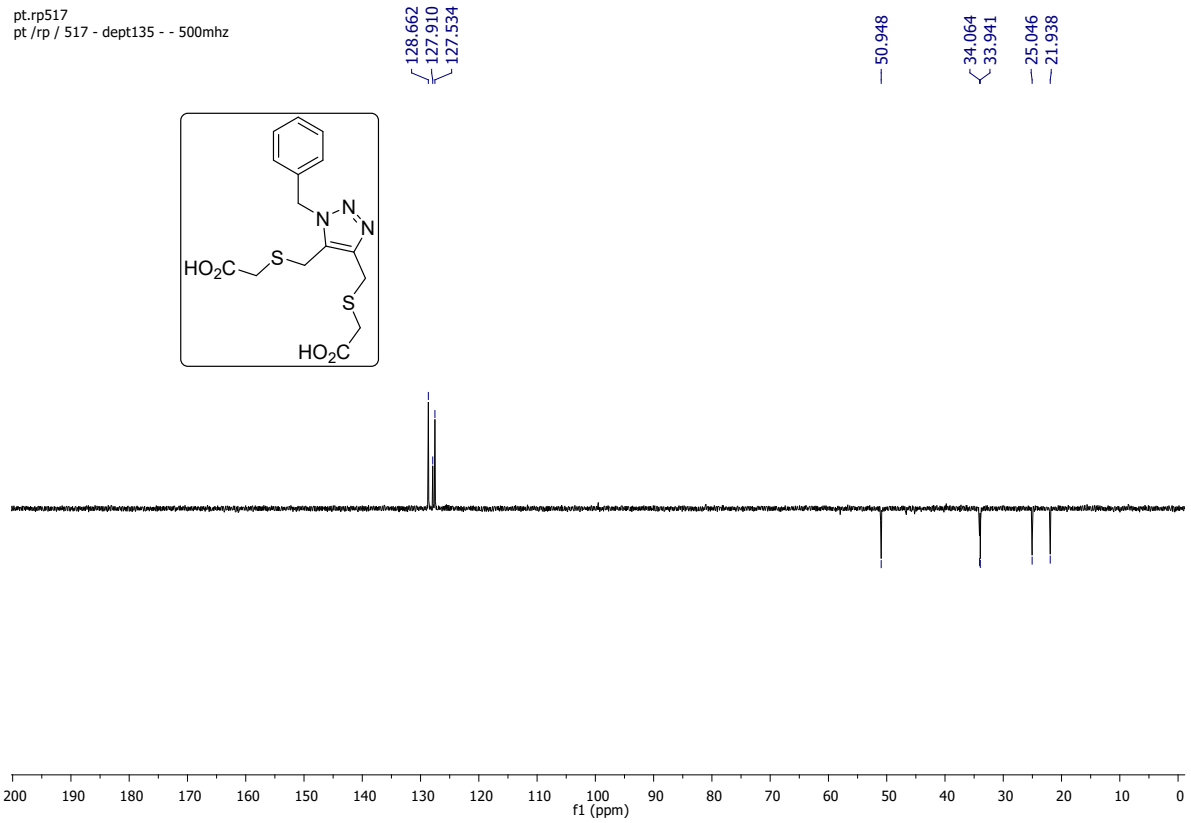
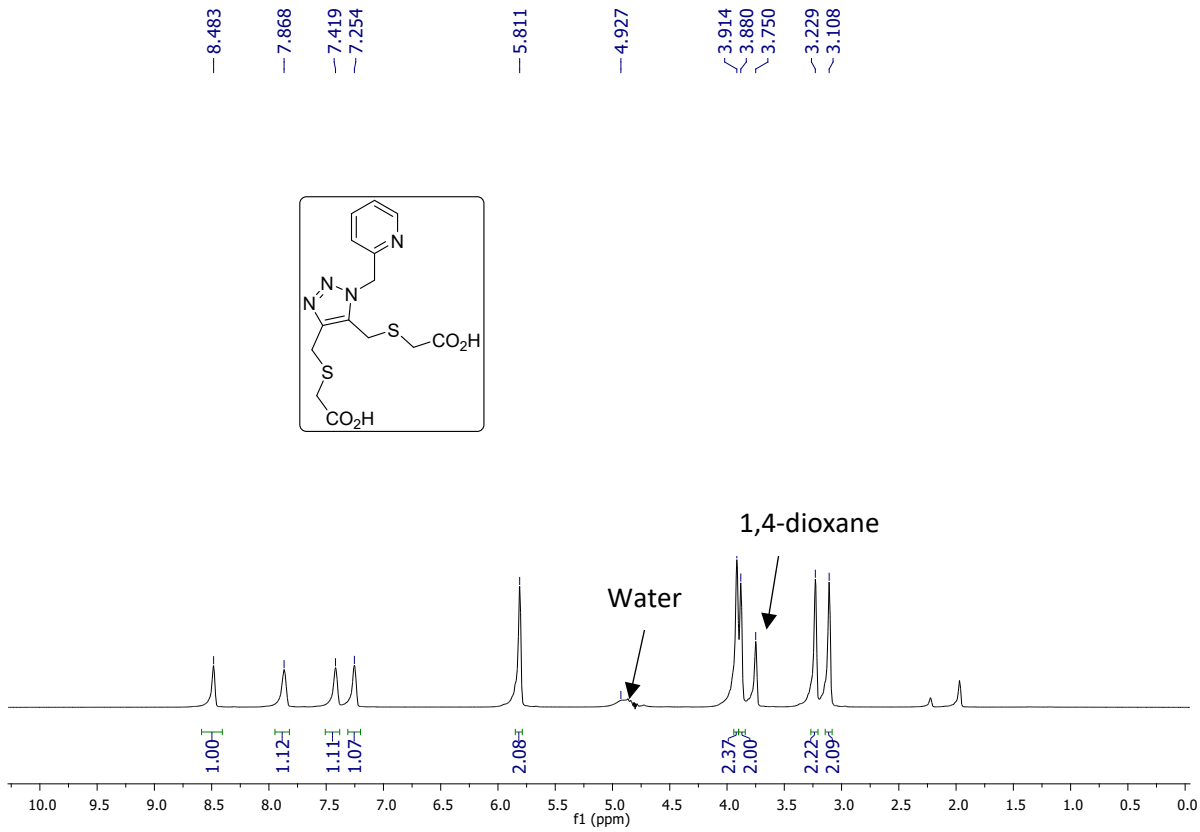


^{1}H-NMR spectra of compound **6b** (400MHz, D_2O, 25°C)



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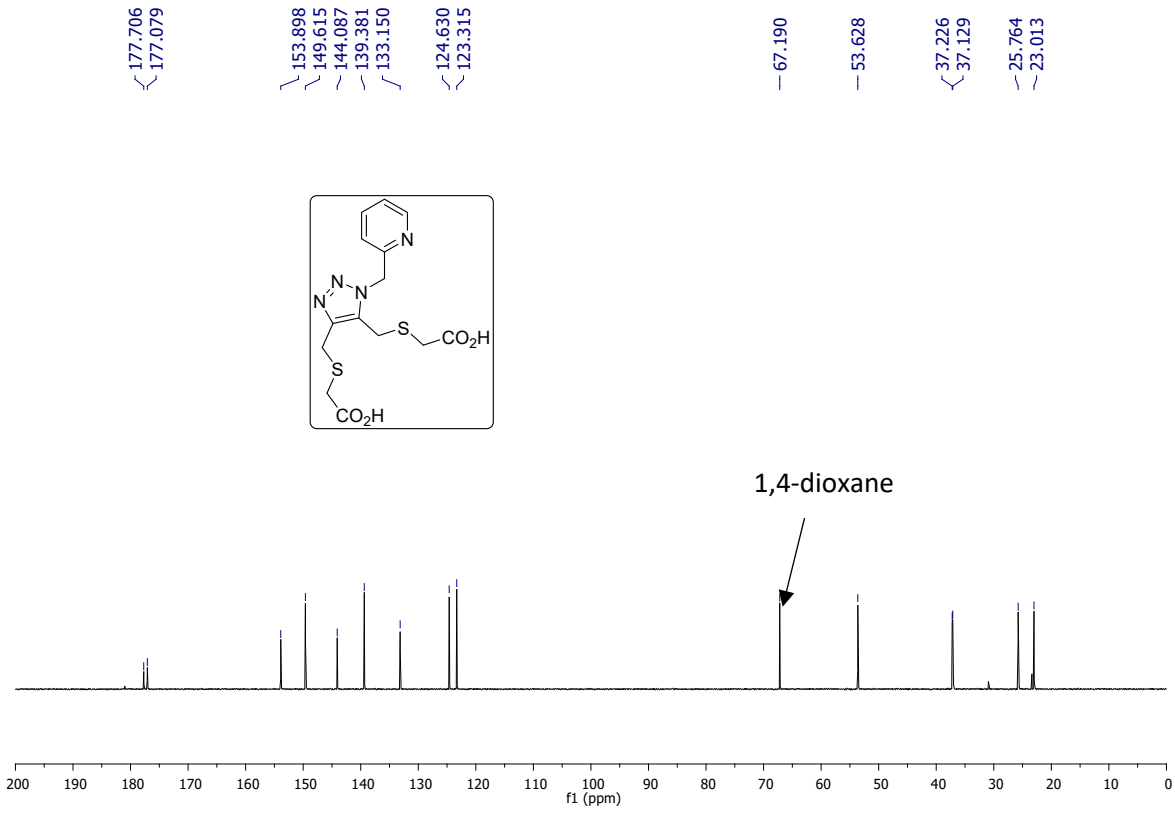
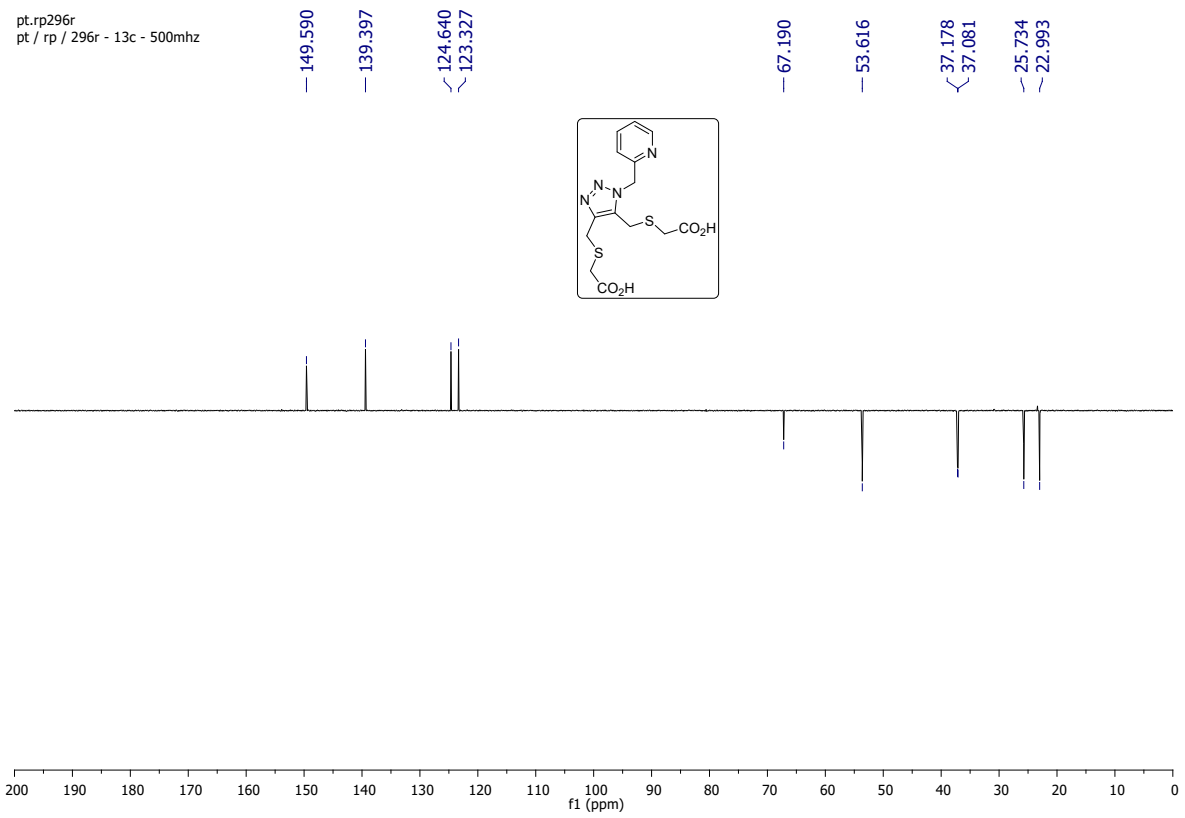


DEPT-135 spectra of compound **6b** (125MHz, D_2O, 25°C)



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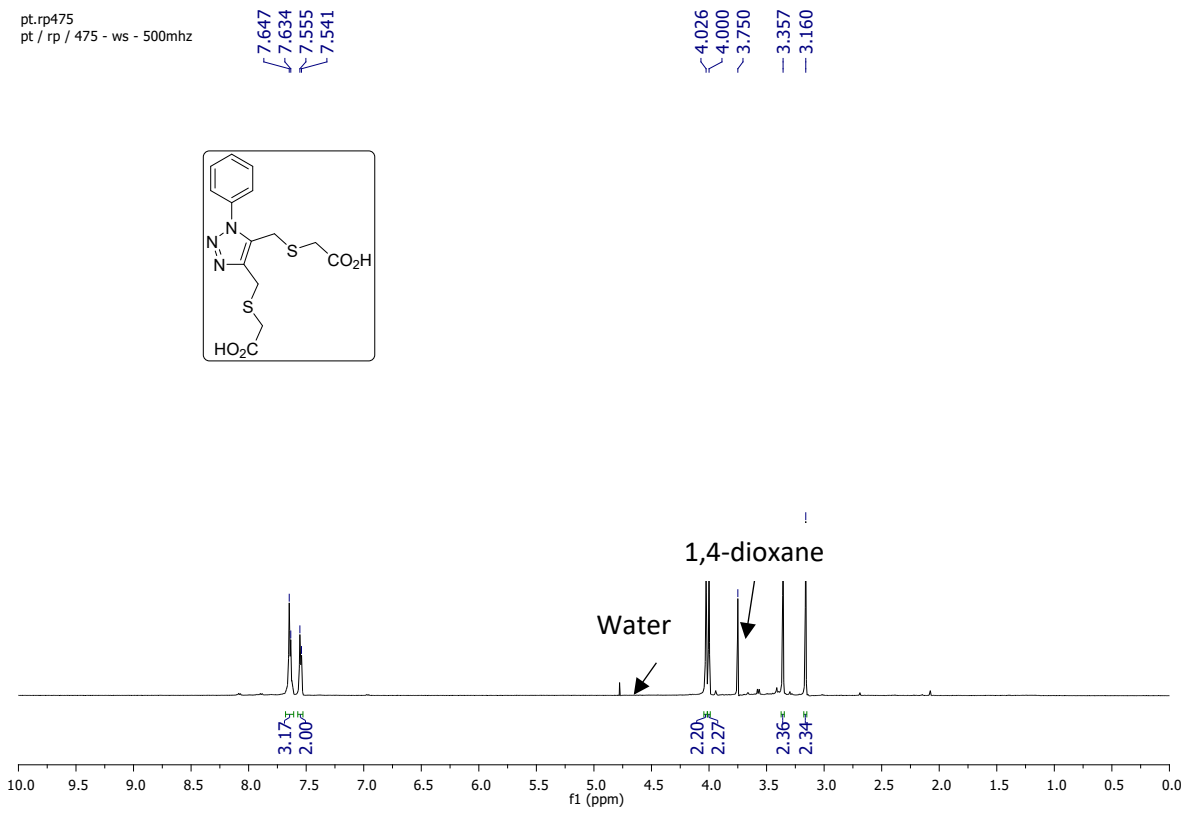

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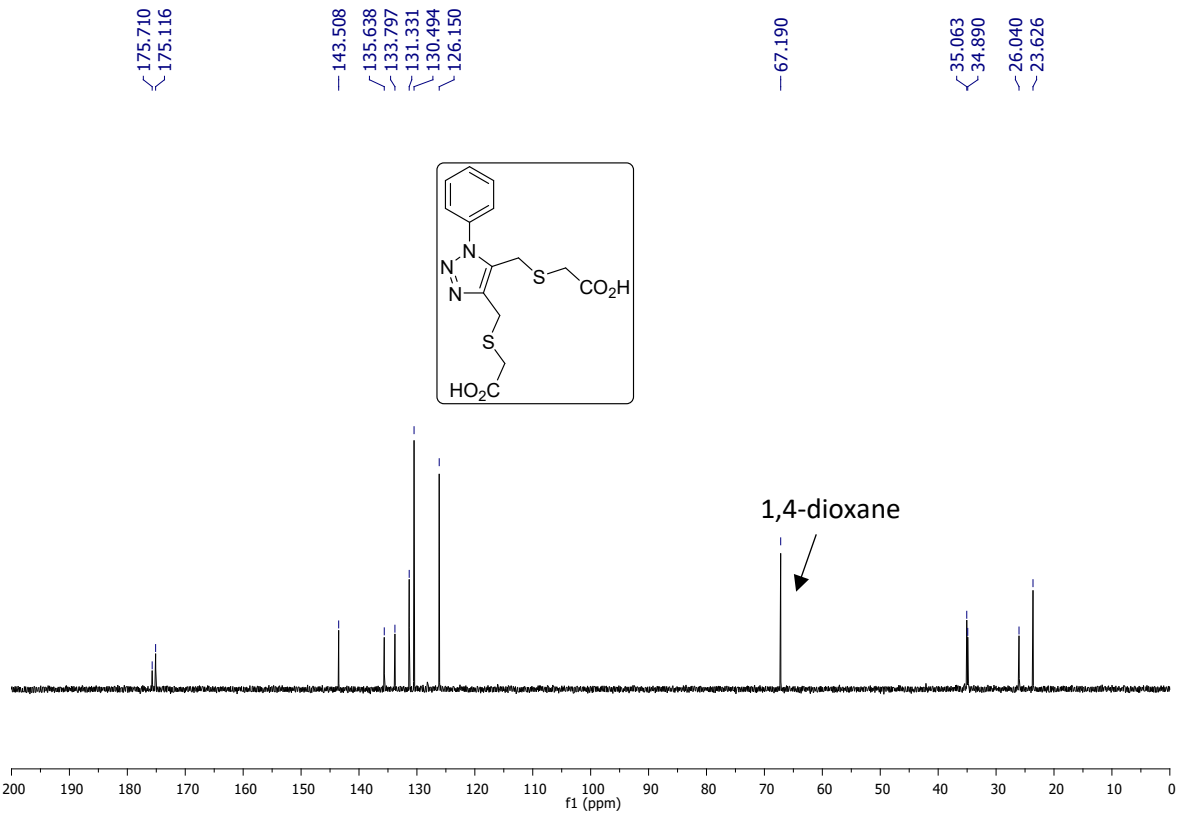
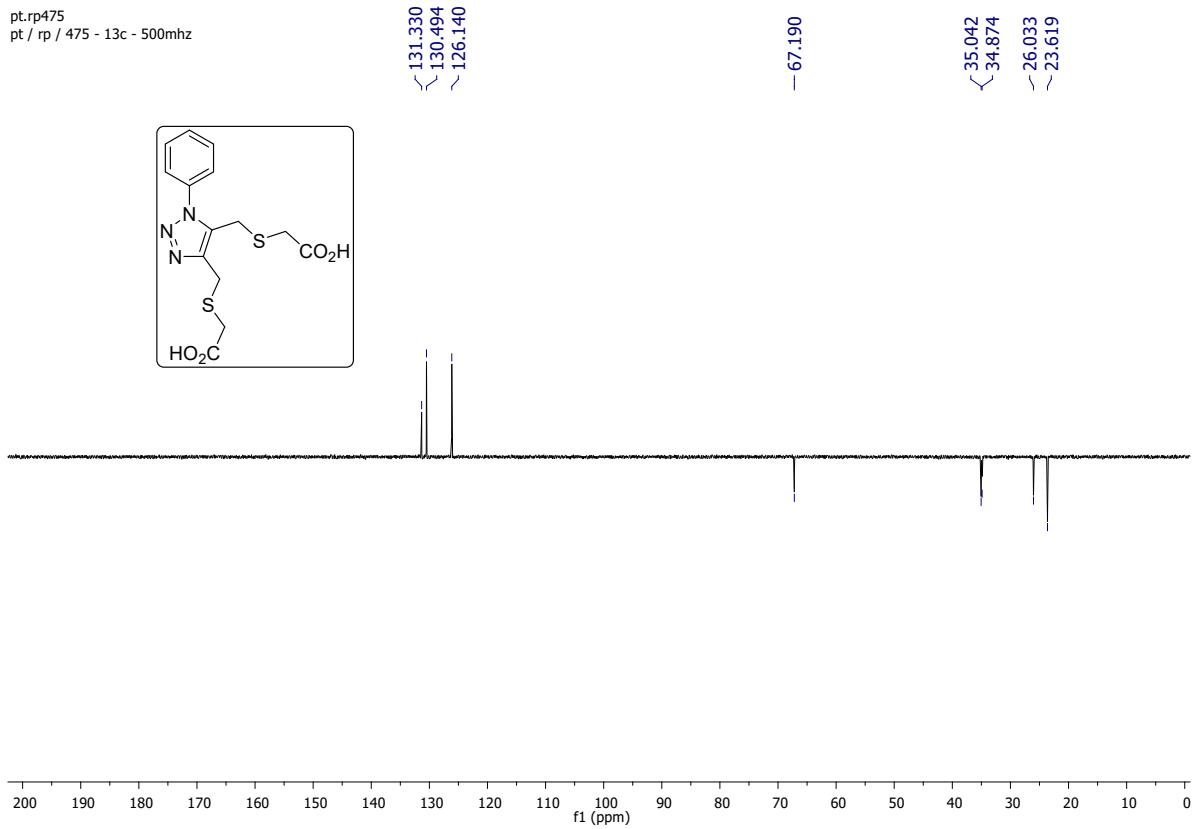


DEPT-135 spectra of compound **6c** (125MHz, D_2O, 25°C)



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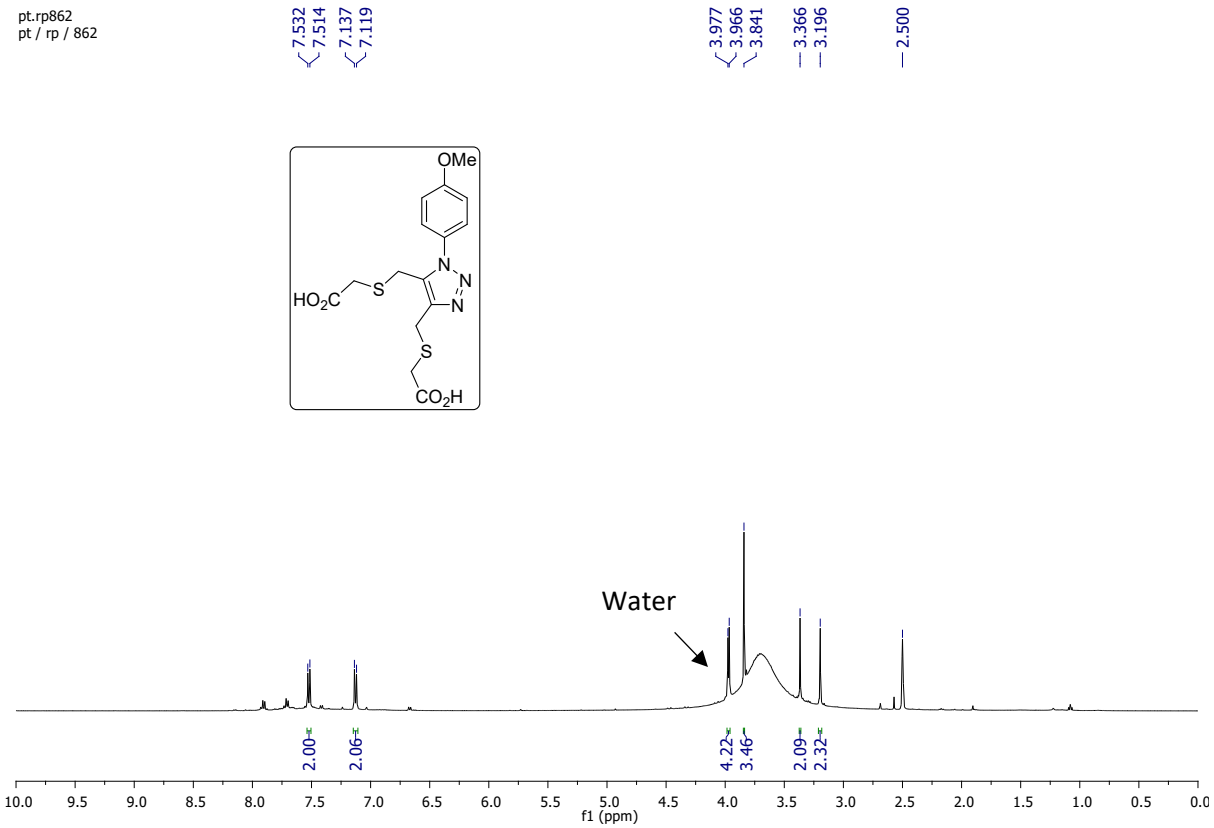

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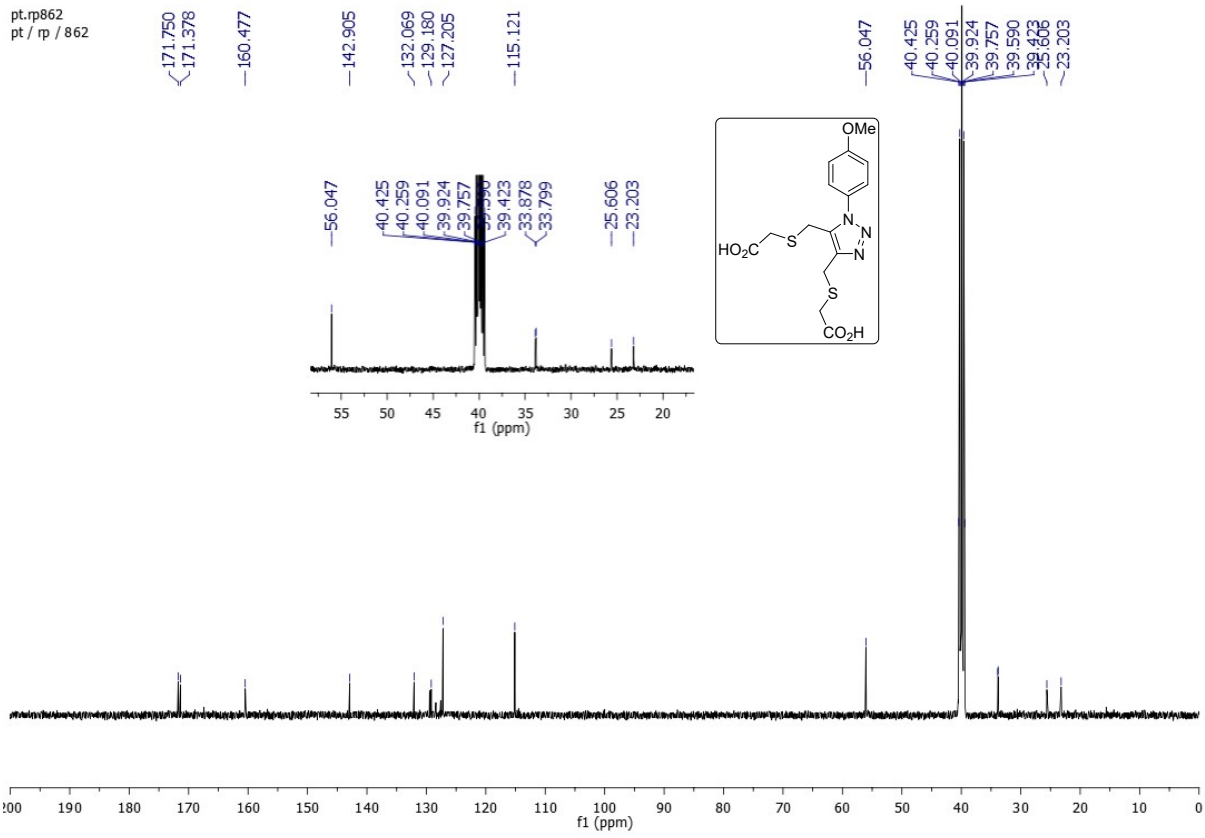
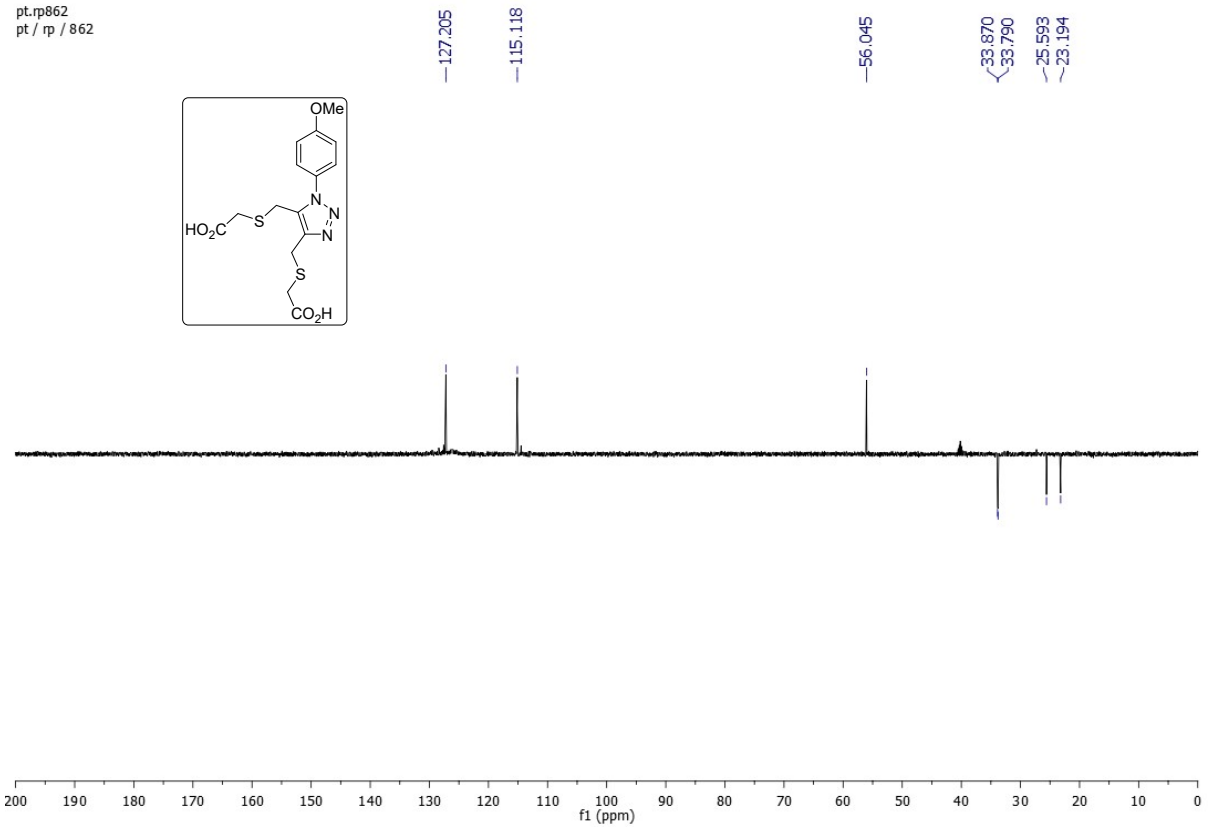


DEPT-135 spectra of compound **6d** (125MHz, DMSO-d_6, 25°C)



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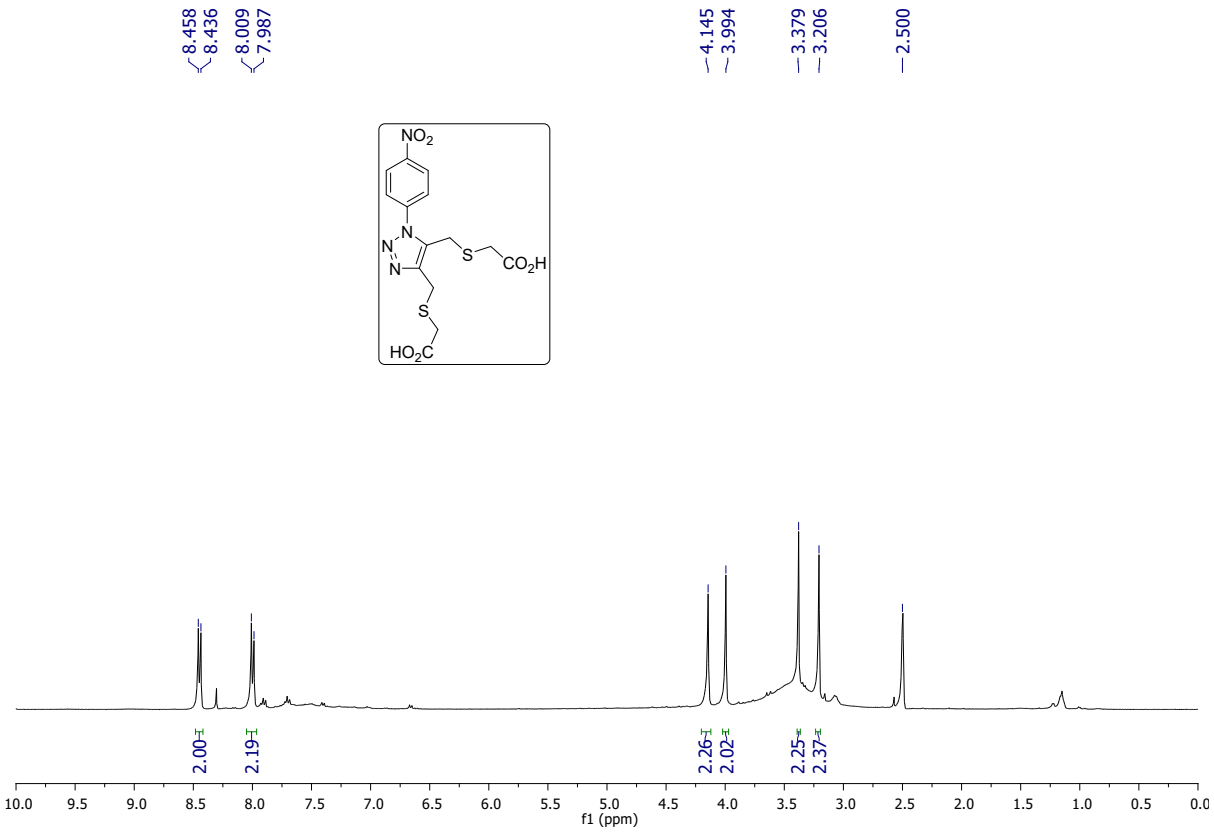
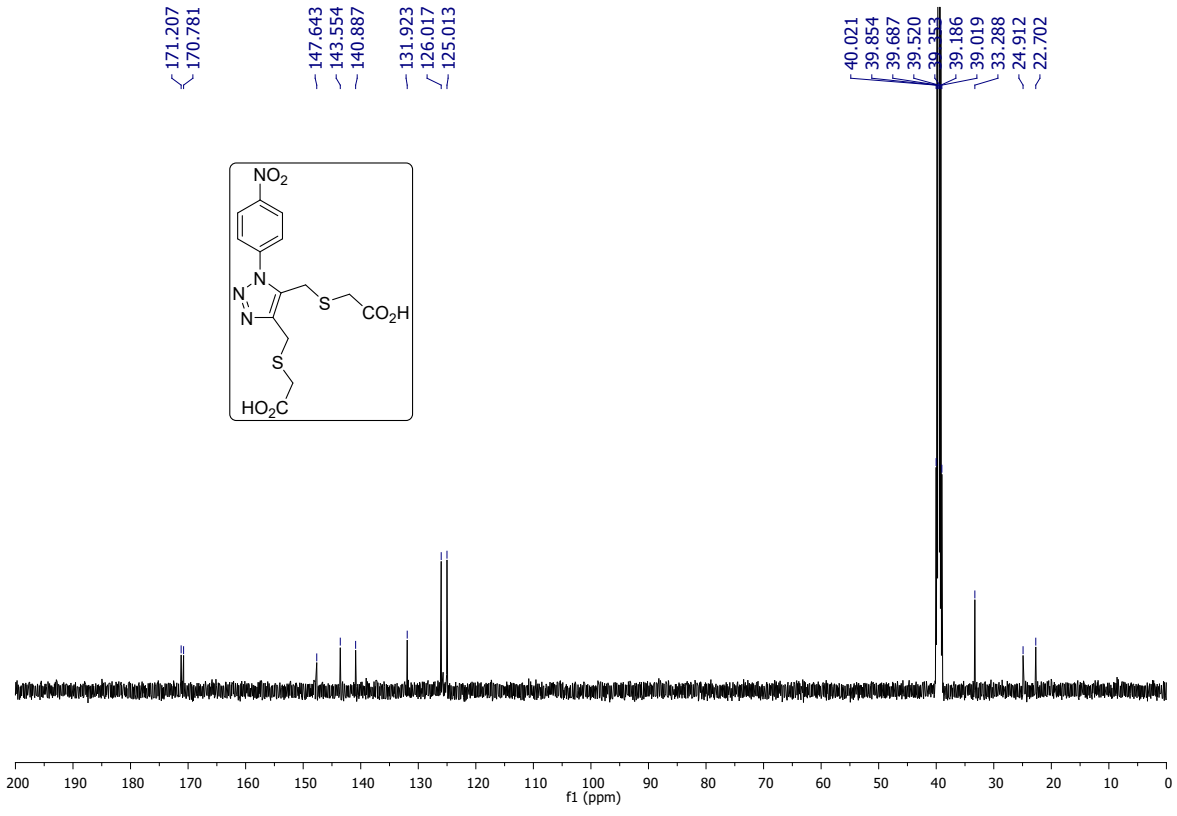
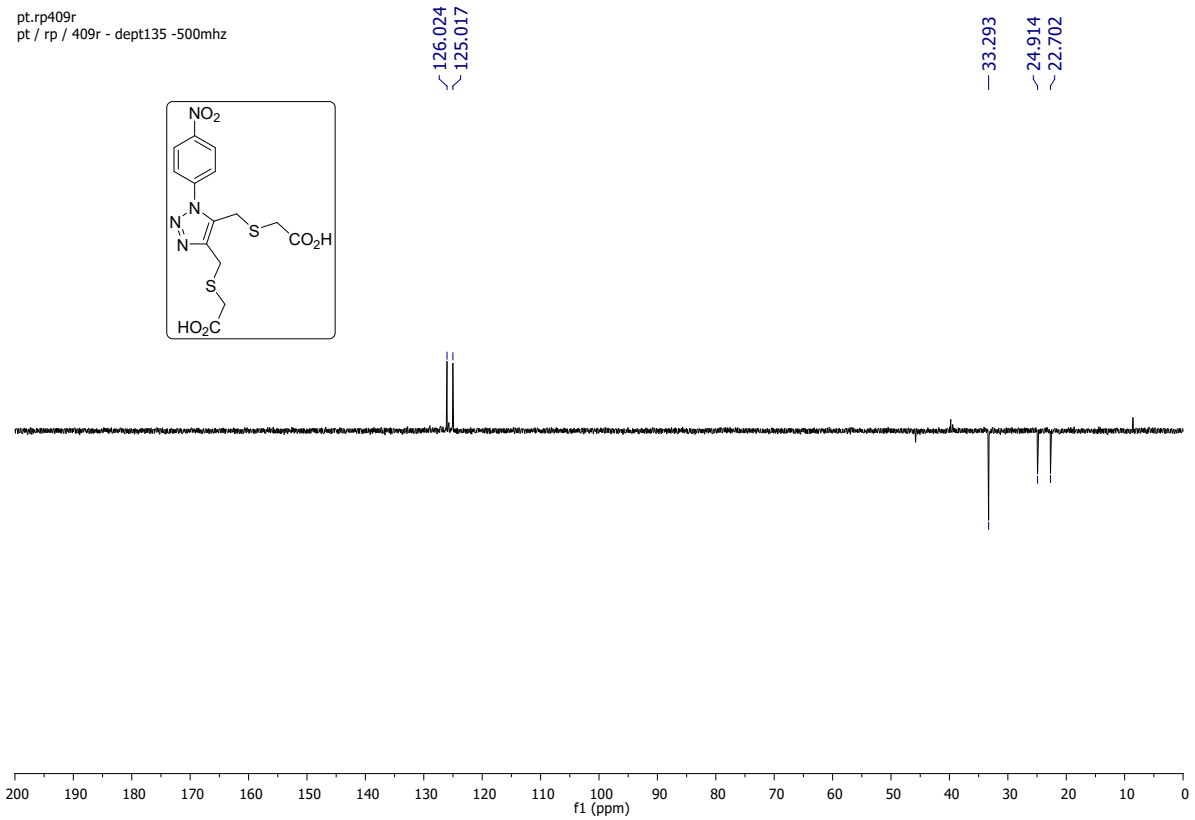


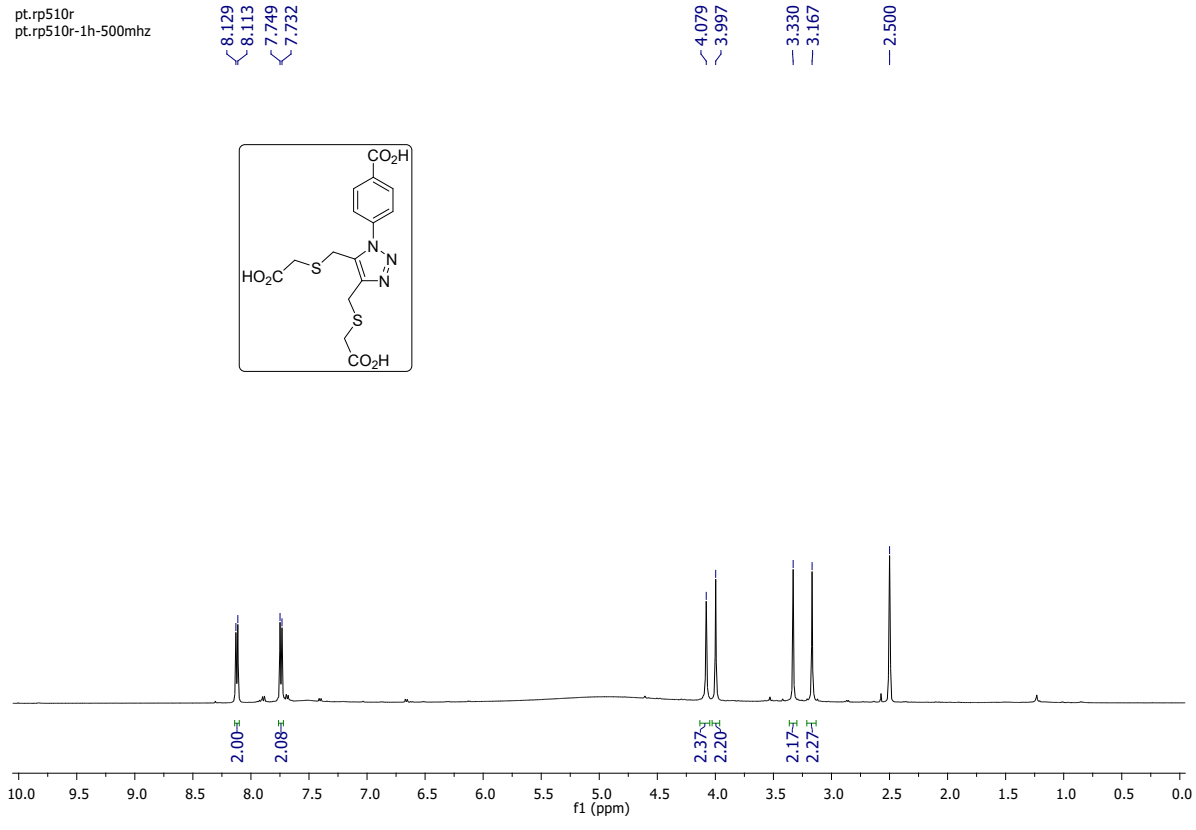
^{13}C-NMR spectra of compound **6e** (125MHz, DMSO-d_6, 25°C)



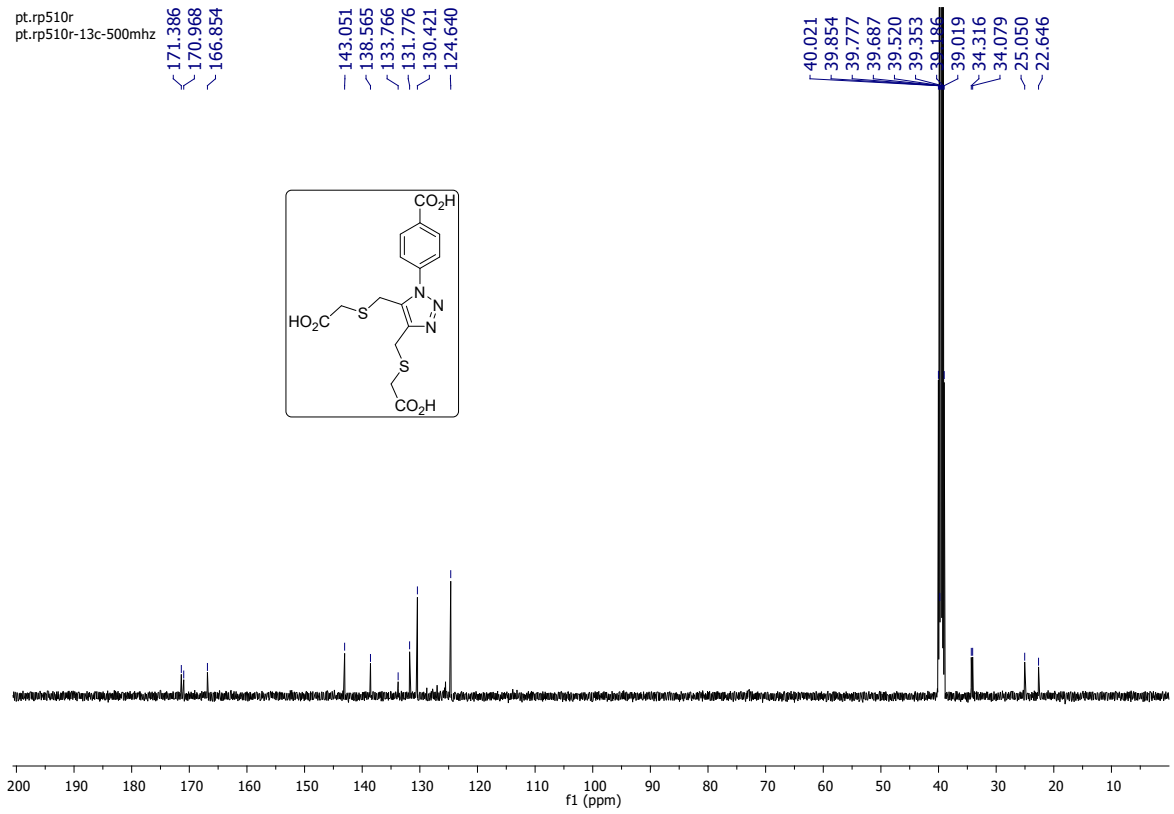
DEPT-135 spectra of compound **6e** (125MHz, DMSO-d_6, 25°C)



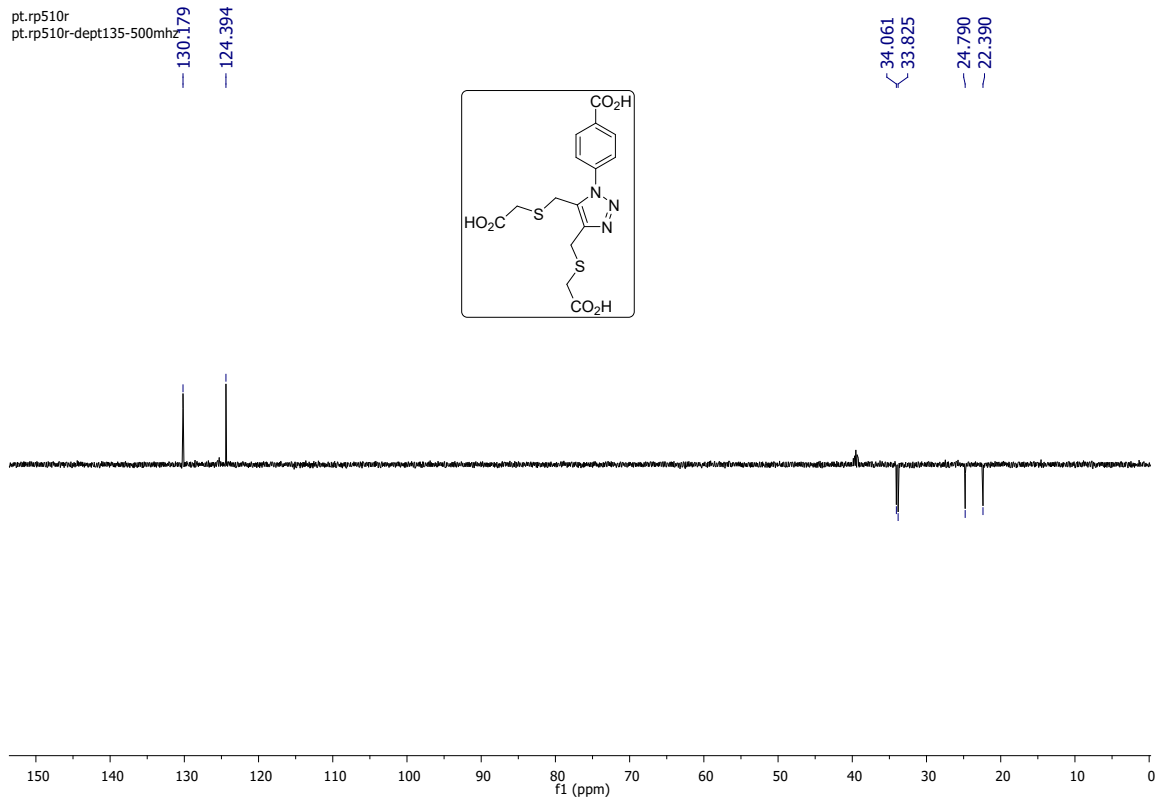
^{1}H-NMR spectra of compound **6f'** (500MHz, DMSO-d_6, 25^{o}C)



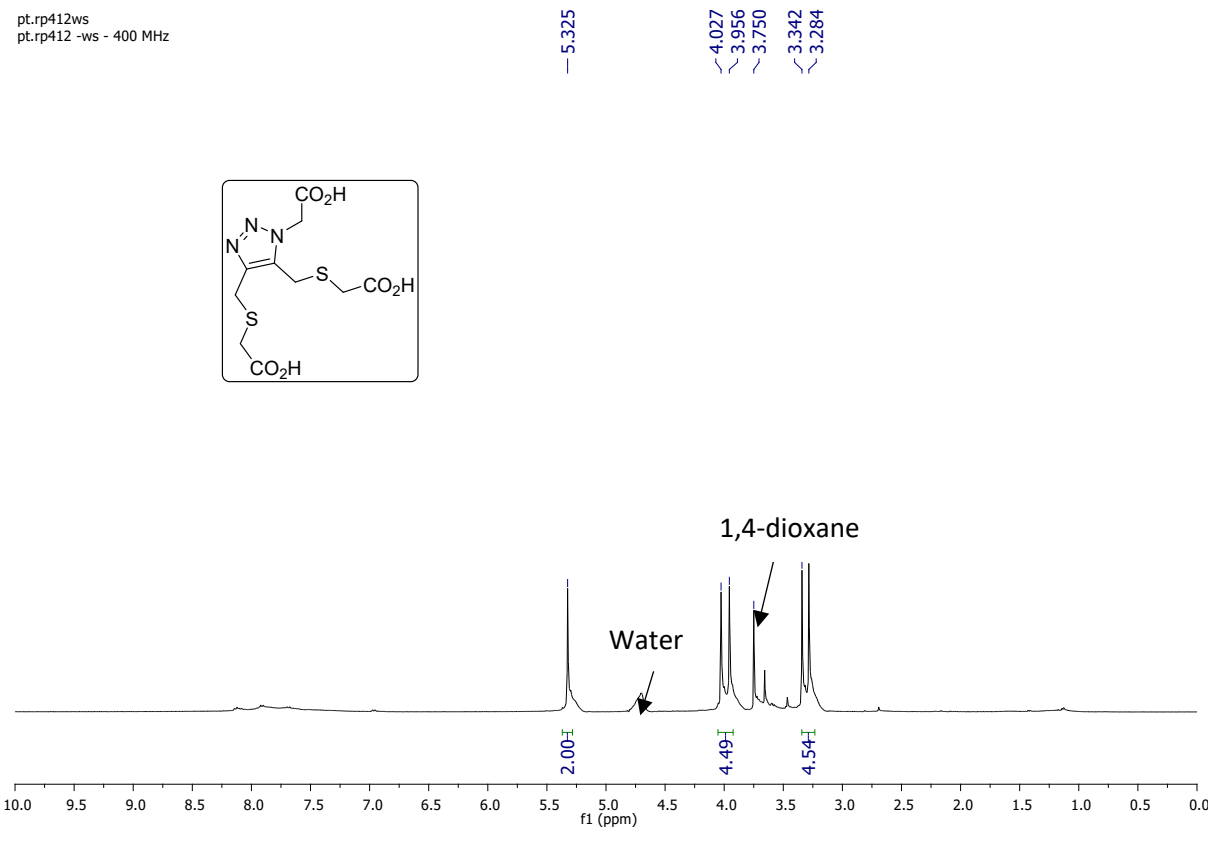
^{13}C-NMR spectra of compound **6f'** (125MHz, DMSO-d_6, 25°C)



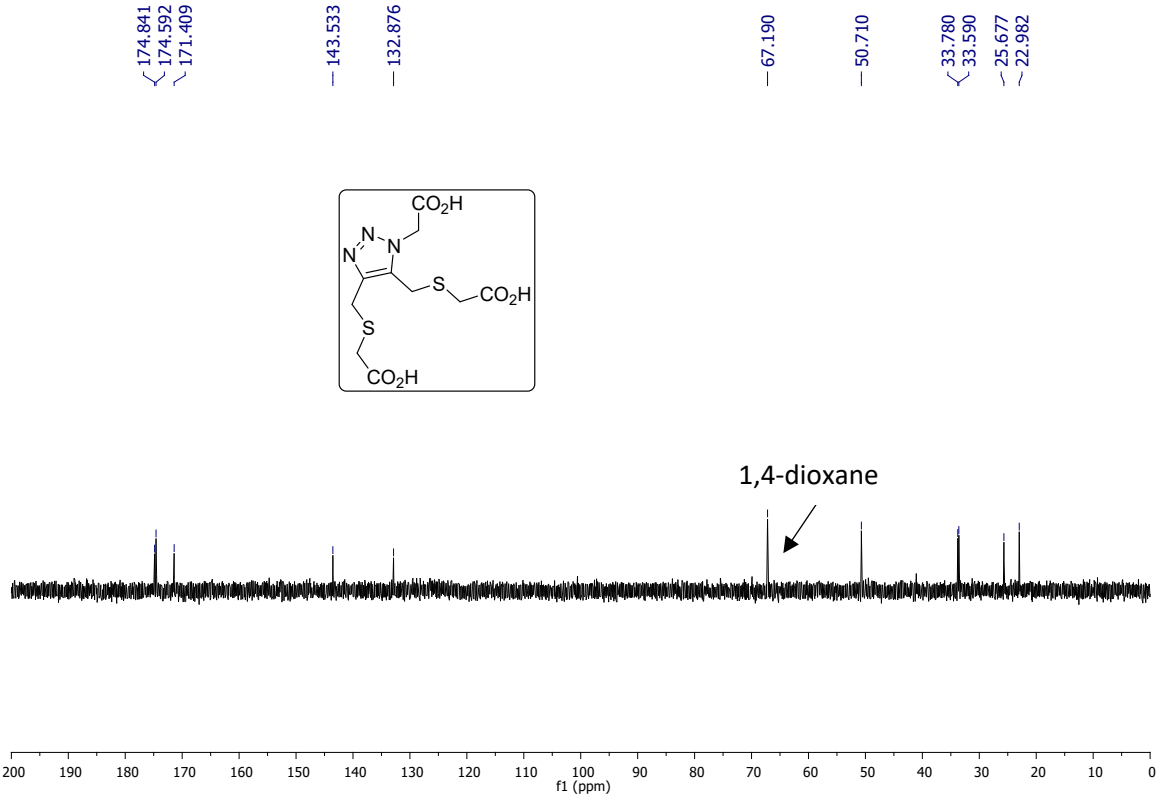
DEPT-135 spectra of compound **6f'** (125MHz, DMSO-d_6, 25°C)



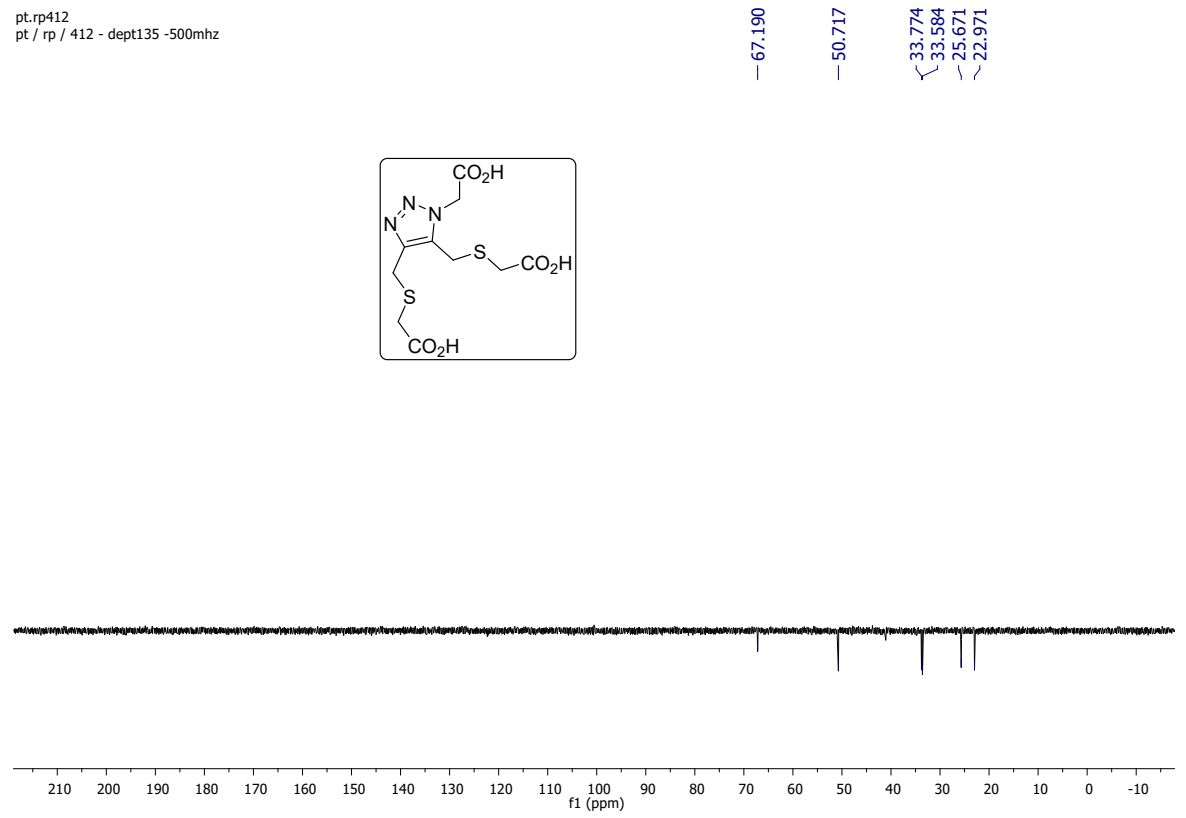
^{1}H-NMR spectra of compound **6g'** (400MHz, D_2O, 25°C)



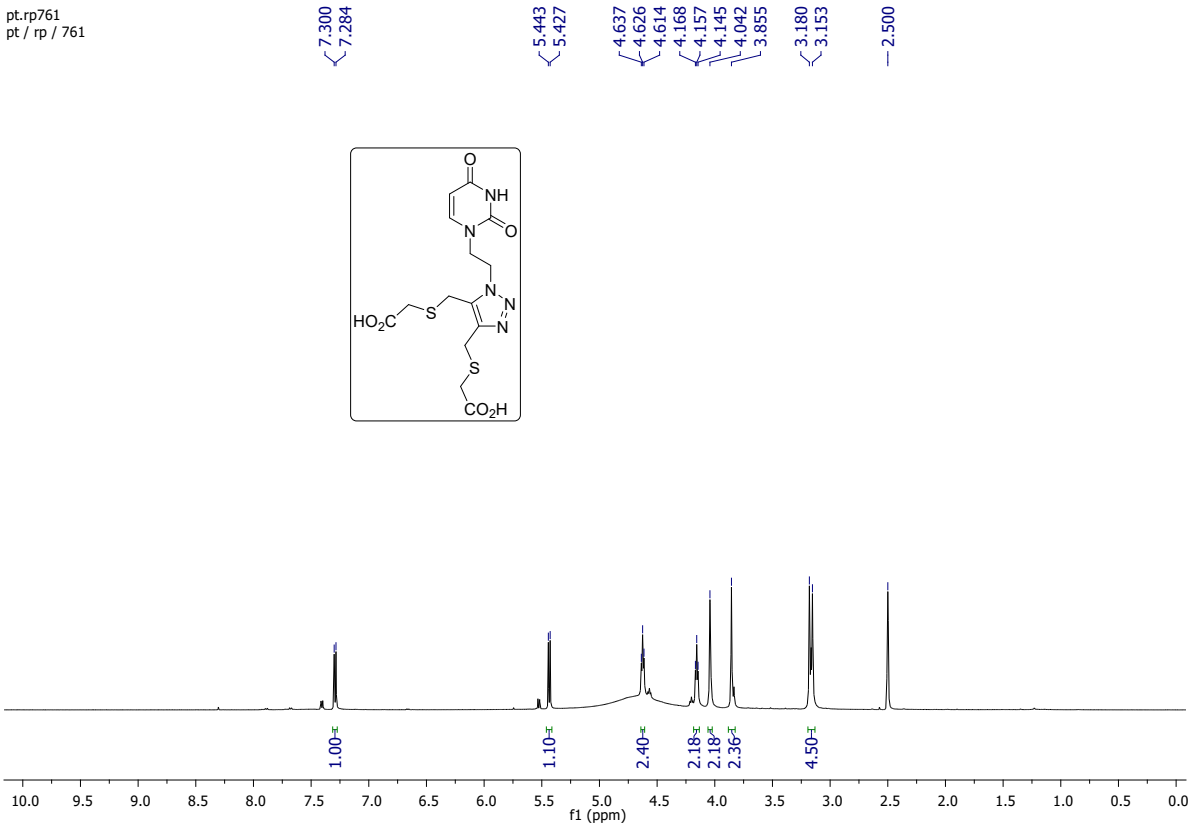
^{13}C-NMR spectra of compound **6g'** (125MHz, D_2O, 25°C)



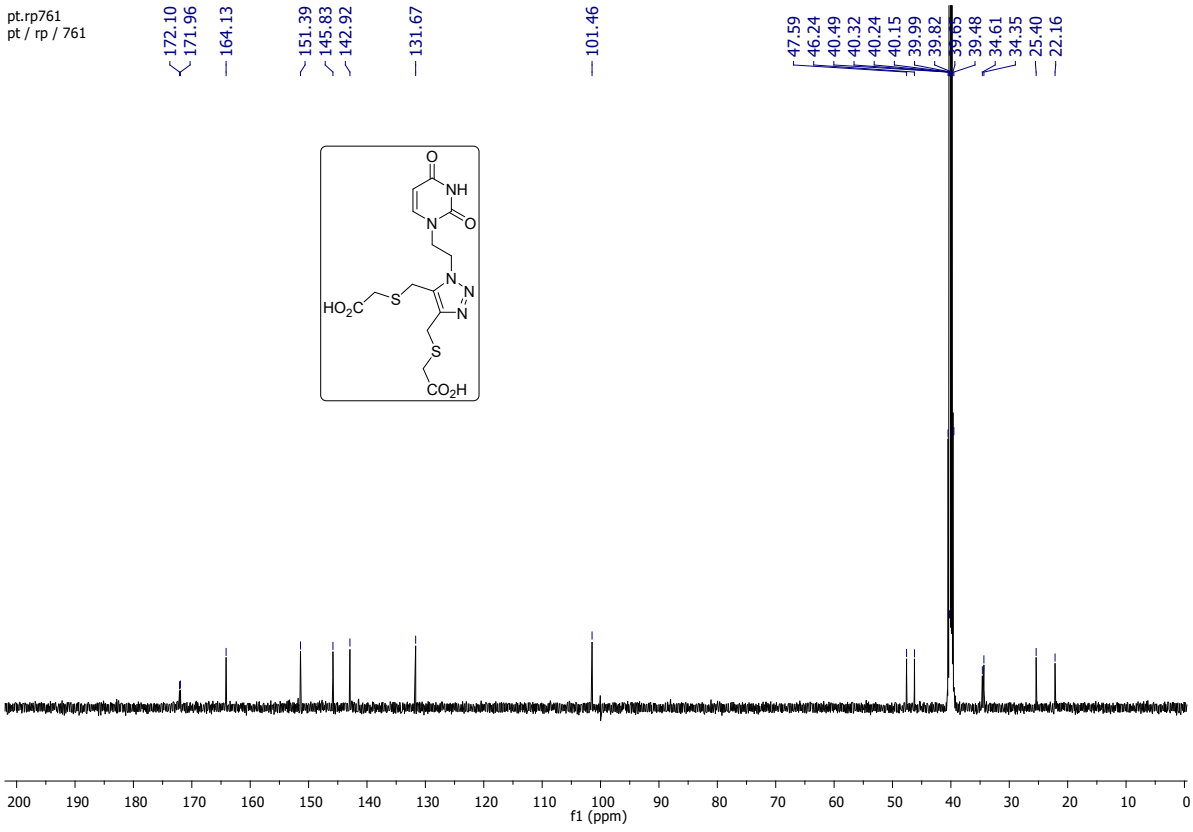
DEPT-135 spectra of compound **6g'** (125MHz, D_2O, 25°C)



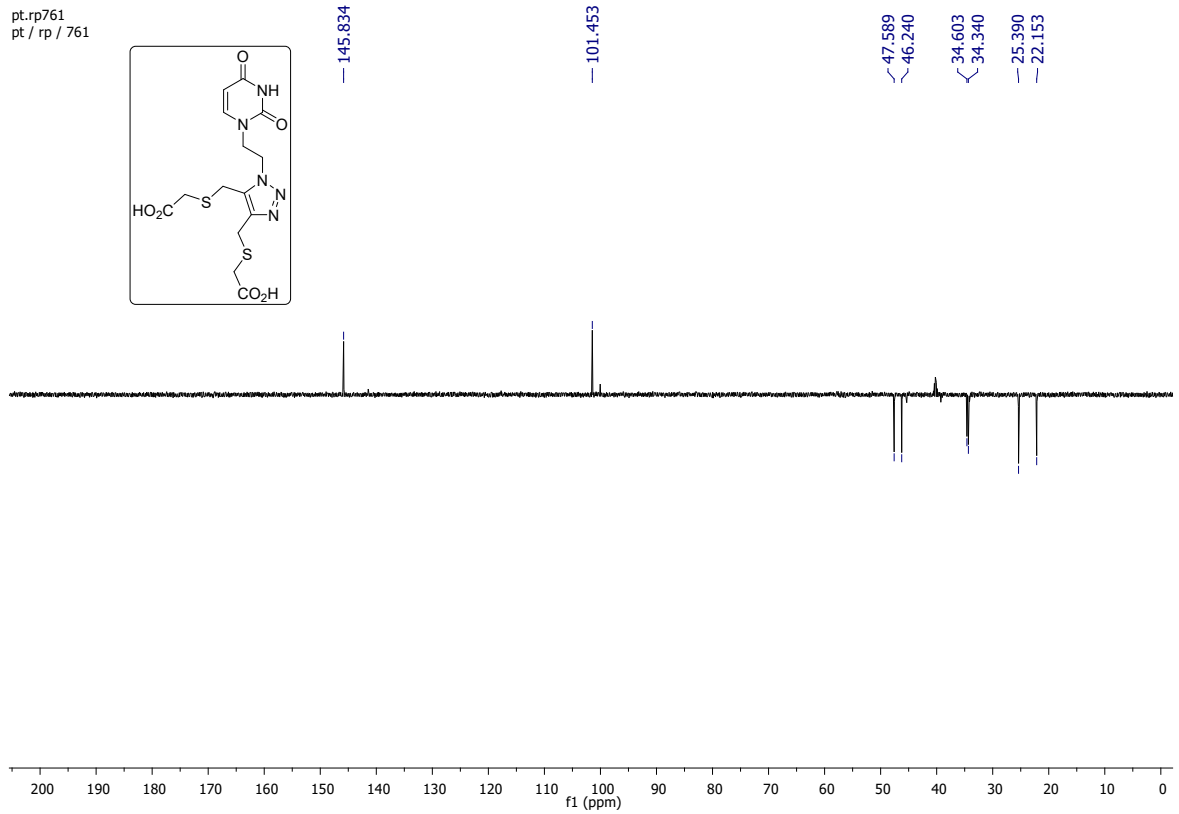
^{1}H-NMR spectra of compound **6h** (500MHz, DMSO-d_6, 25^{o}C)



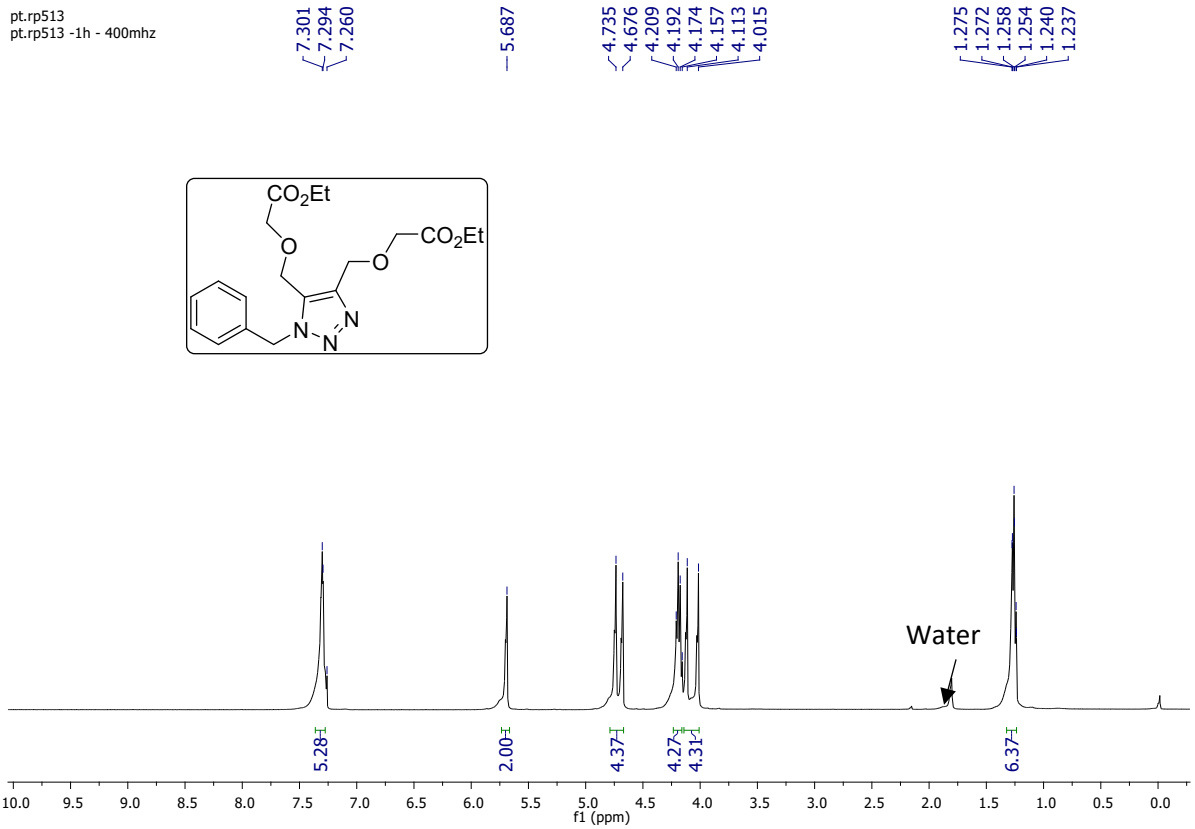
^{13}C-NMR spectra of compound **6h** (125MHz, DMSO-d_6, 25°C)



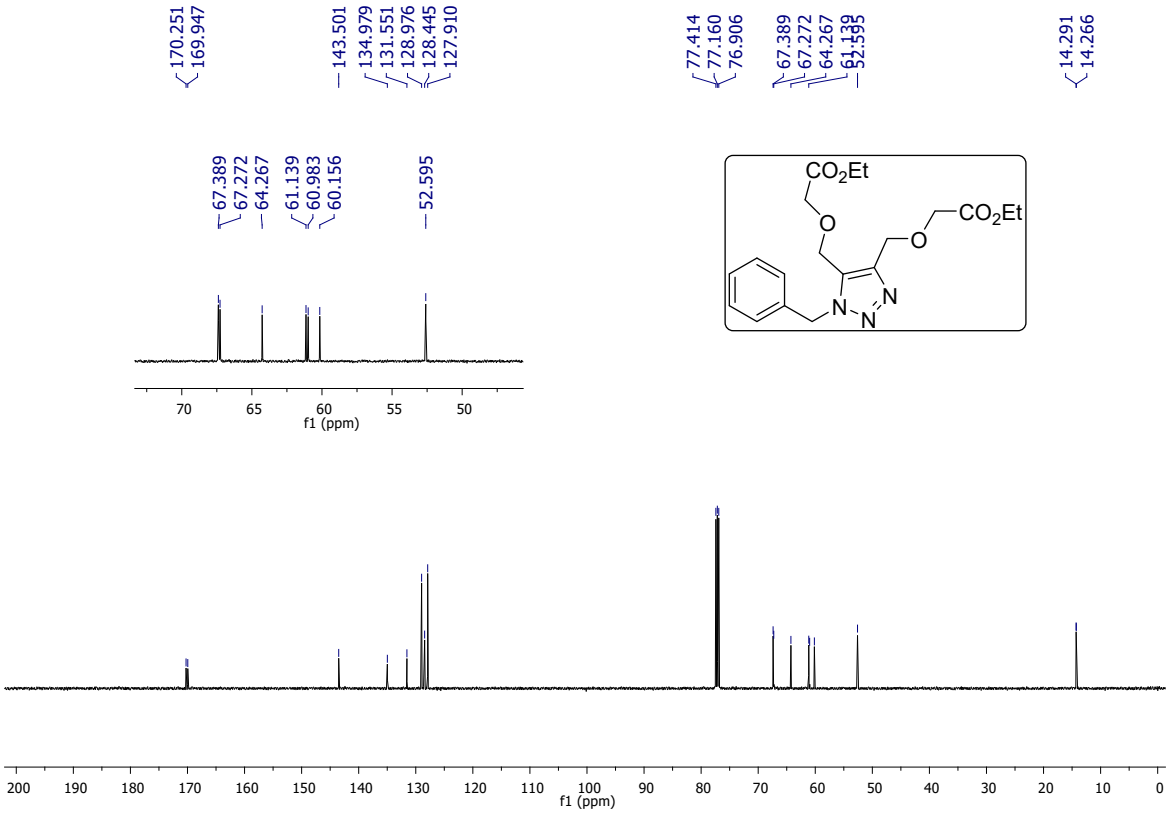
DEPT-135 spectra of compound **6h** (125MHz, DMSO-d_6, 25°C)



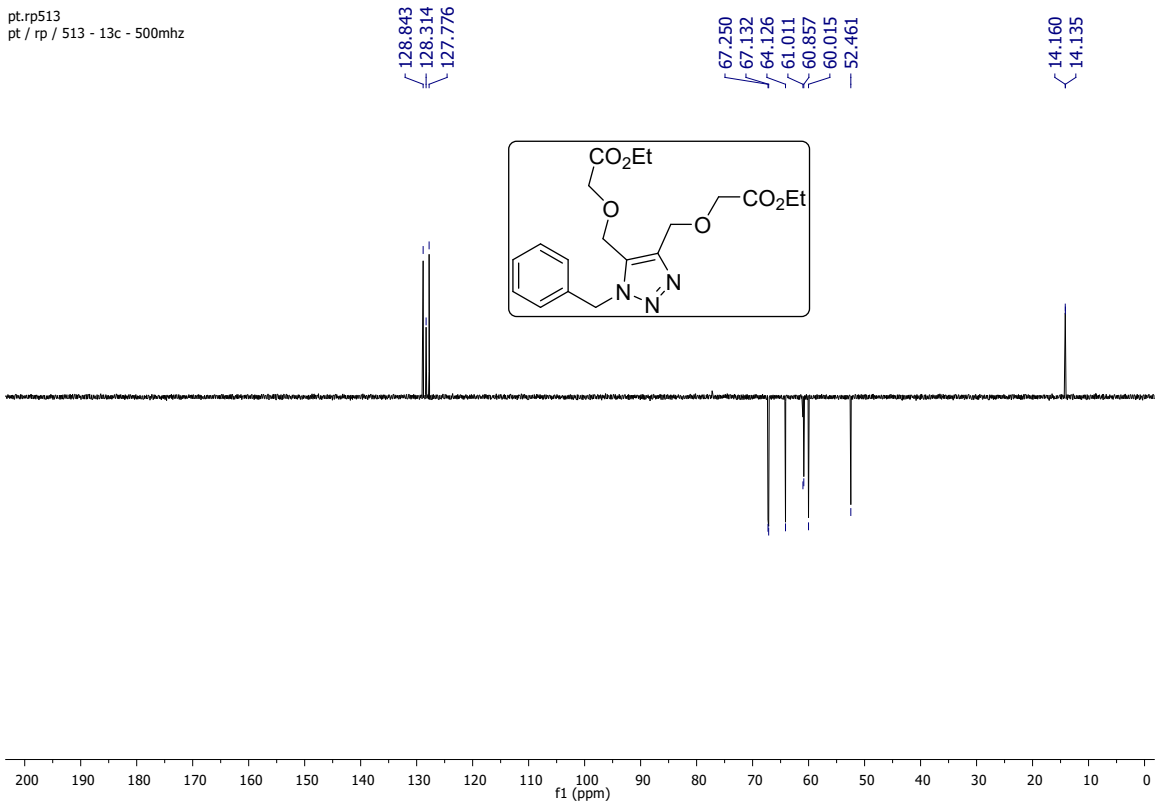
^{1}H-NMR spectra of compound **7a** (400MHz, $CDCl_3$, 25°C)



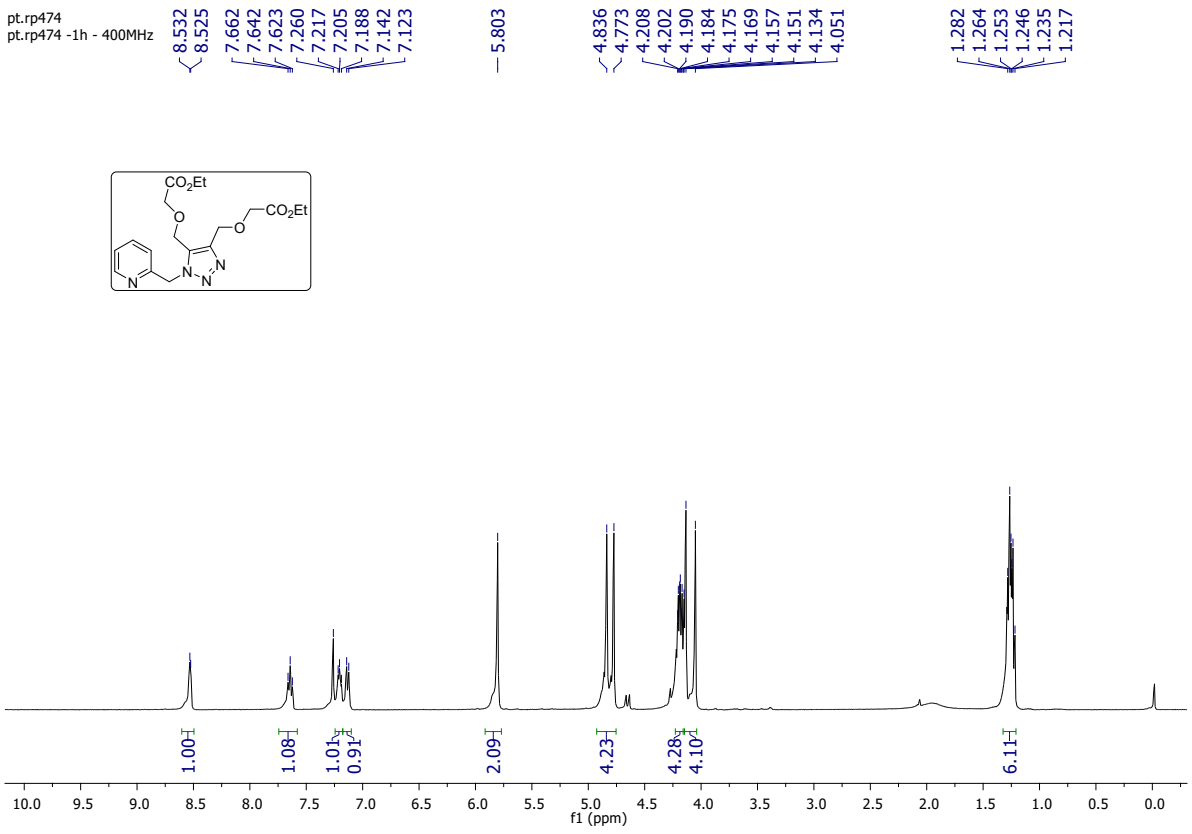
^{13}C-NMR spectra of compound **7a** (125MHz, $CDCl_3$, 25°C)



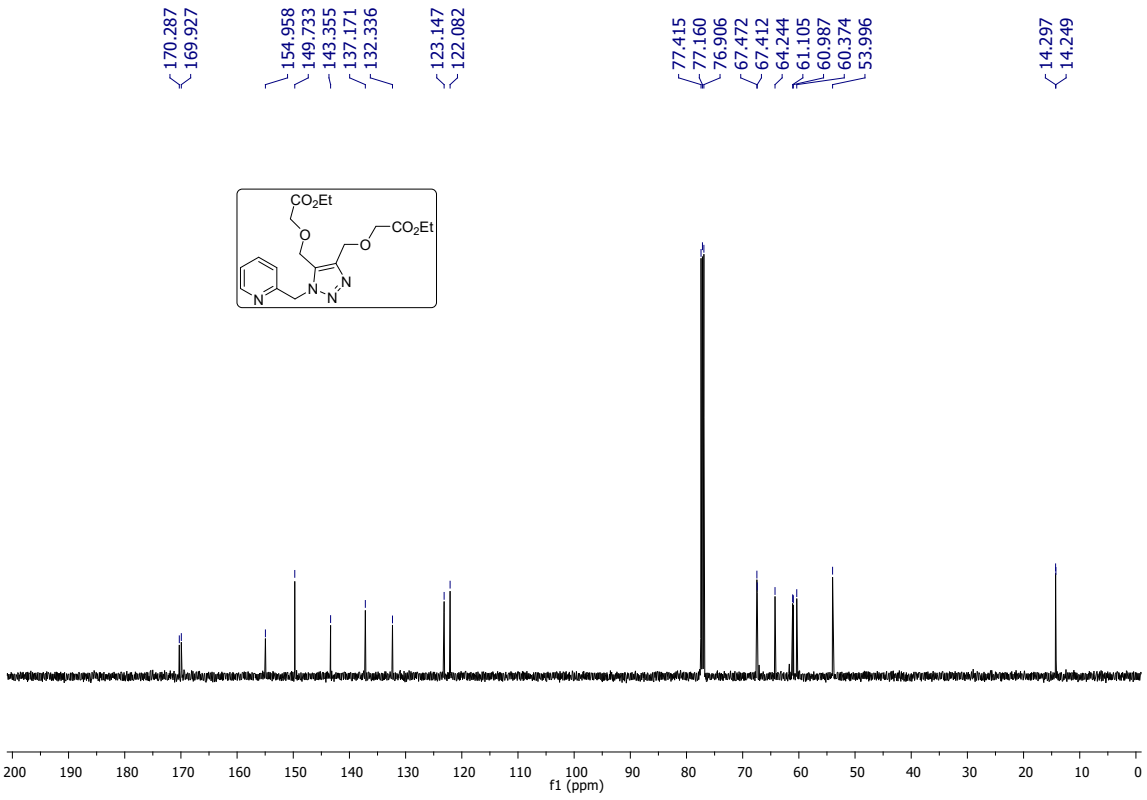
DEPT-135 spectra of compound **7a** (125MHz, $CDCl_3$, 25°C)



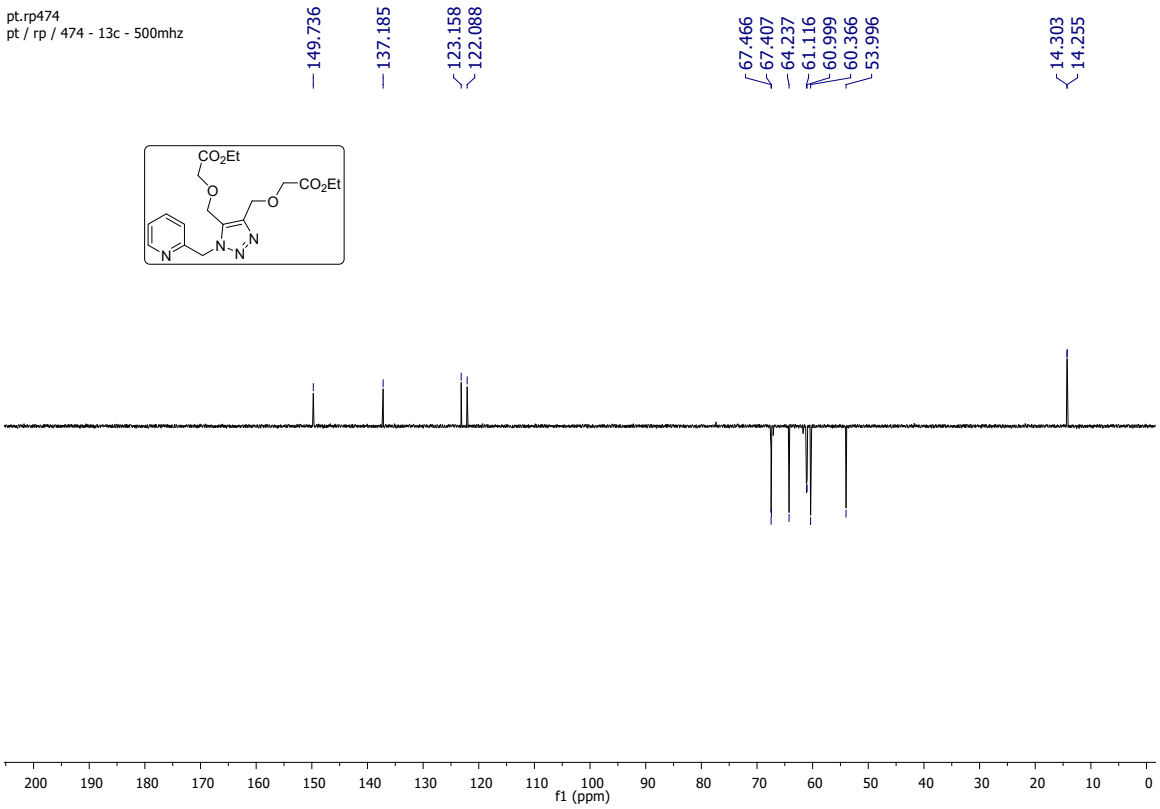
^{1}H-NMR spectra of compound **7b** (400MHz, $CDCl_3$, 25°C)



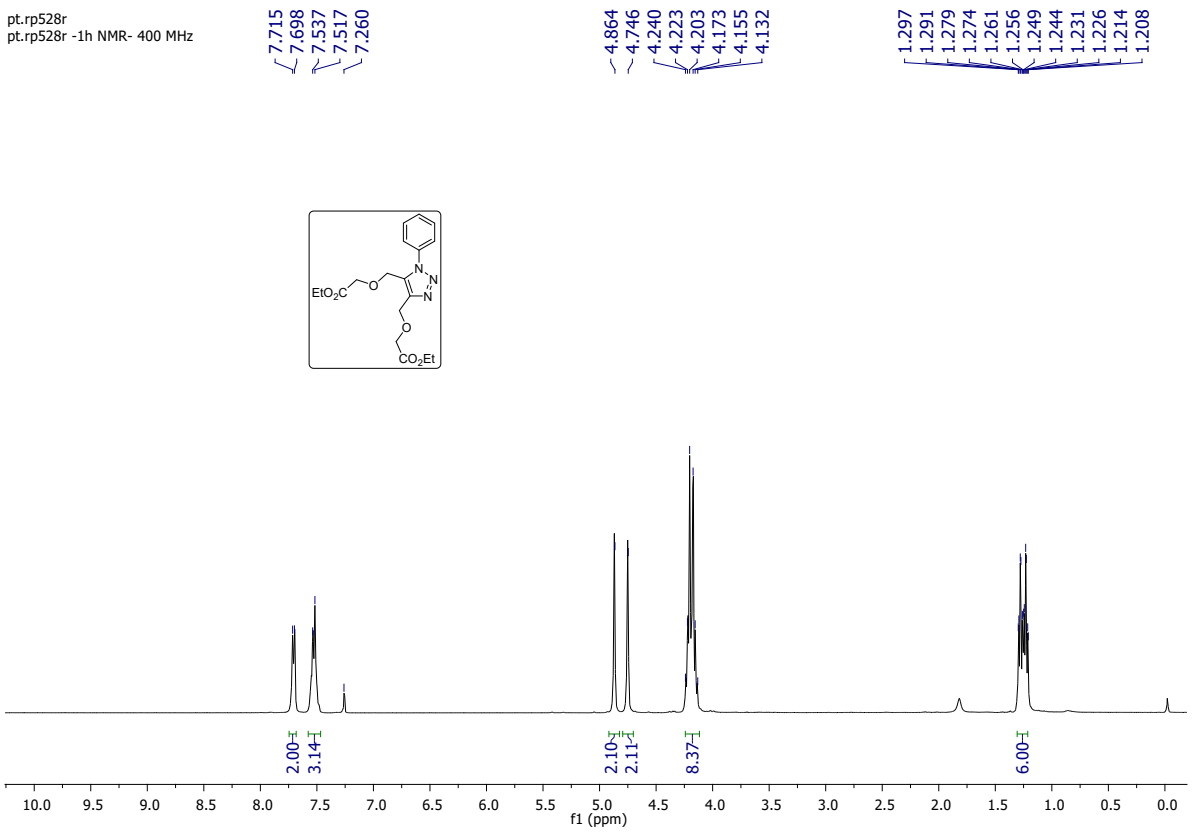
^{13}C-NMR spectra of compound **7b** (125MHz, $CDCl_3$, 25^{o}C)



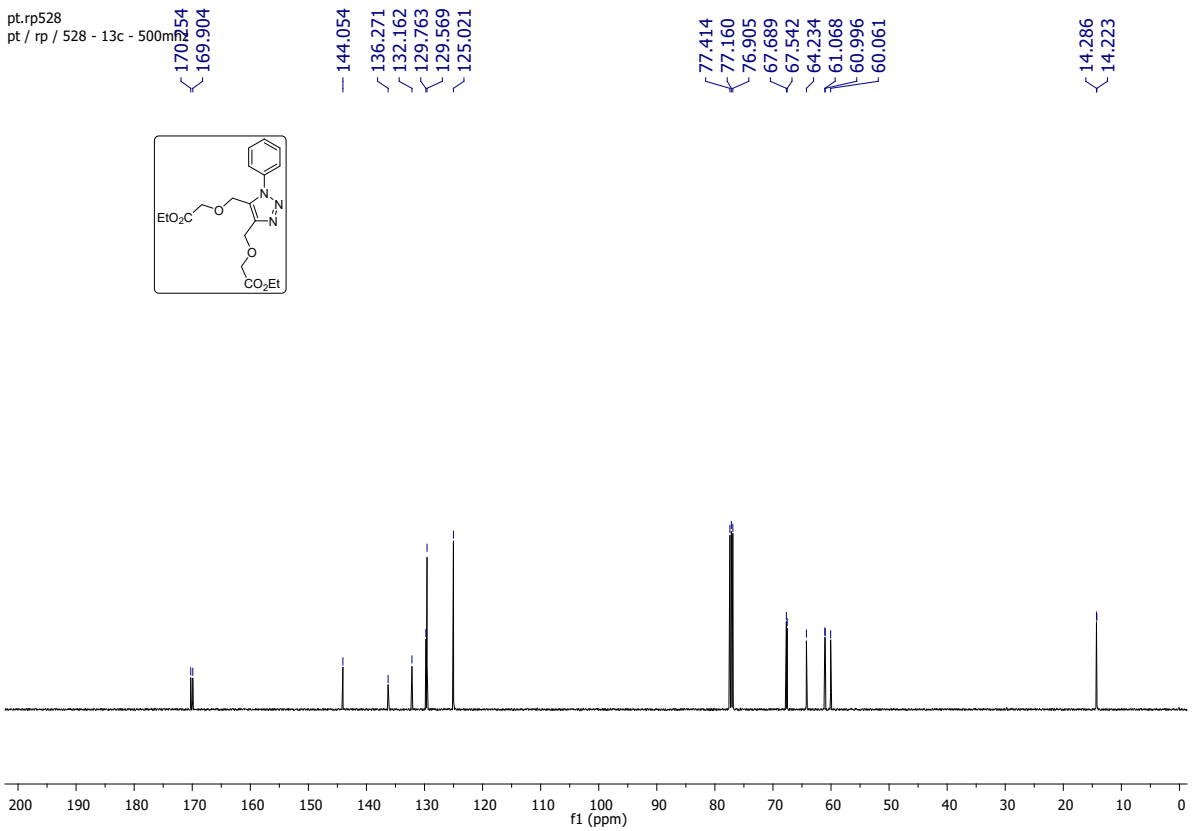
DEPT-135 spectra of compound **7b** (125MHz, $CDCl_3$, 25°C)



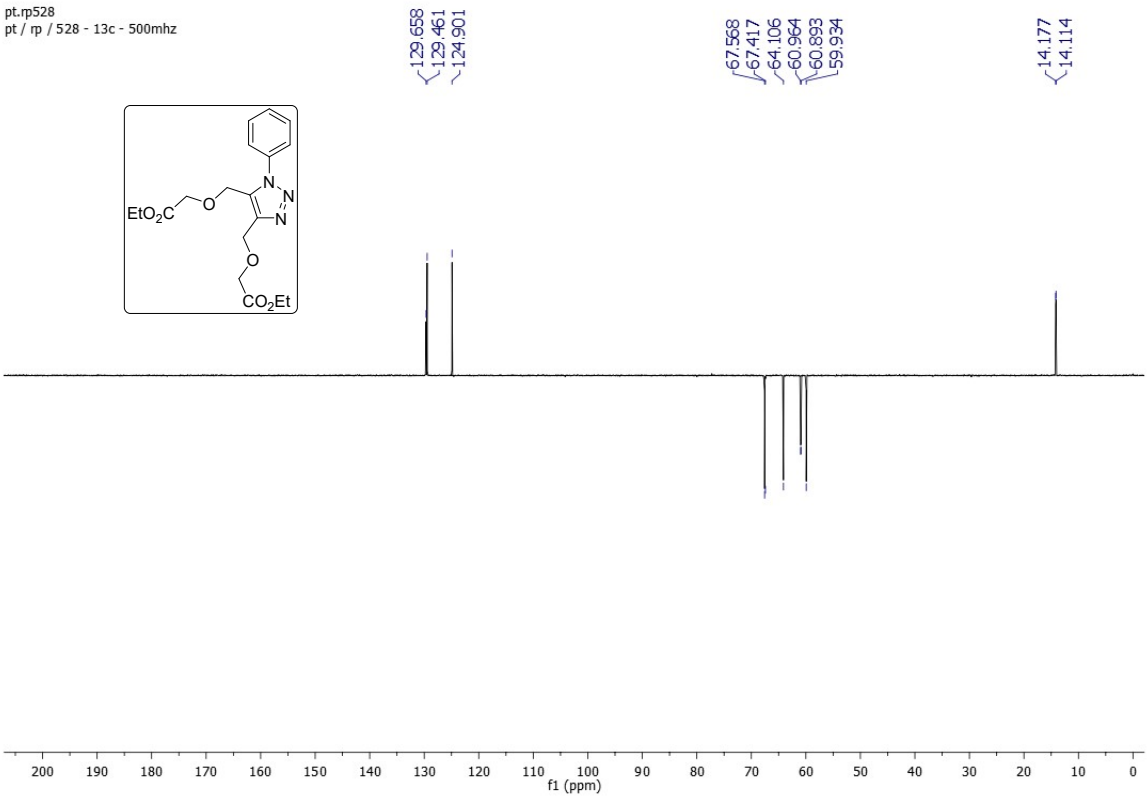
^{1}H-NMR spectra of compound **7c** (400MHz, $CDCl_3$, 25°C)



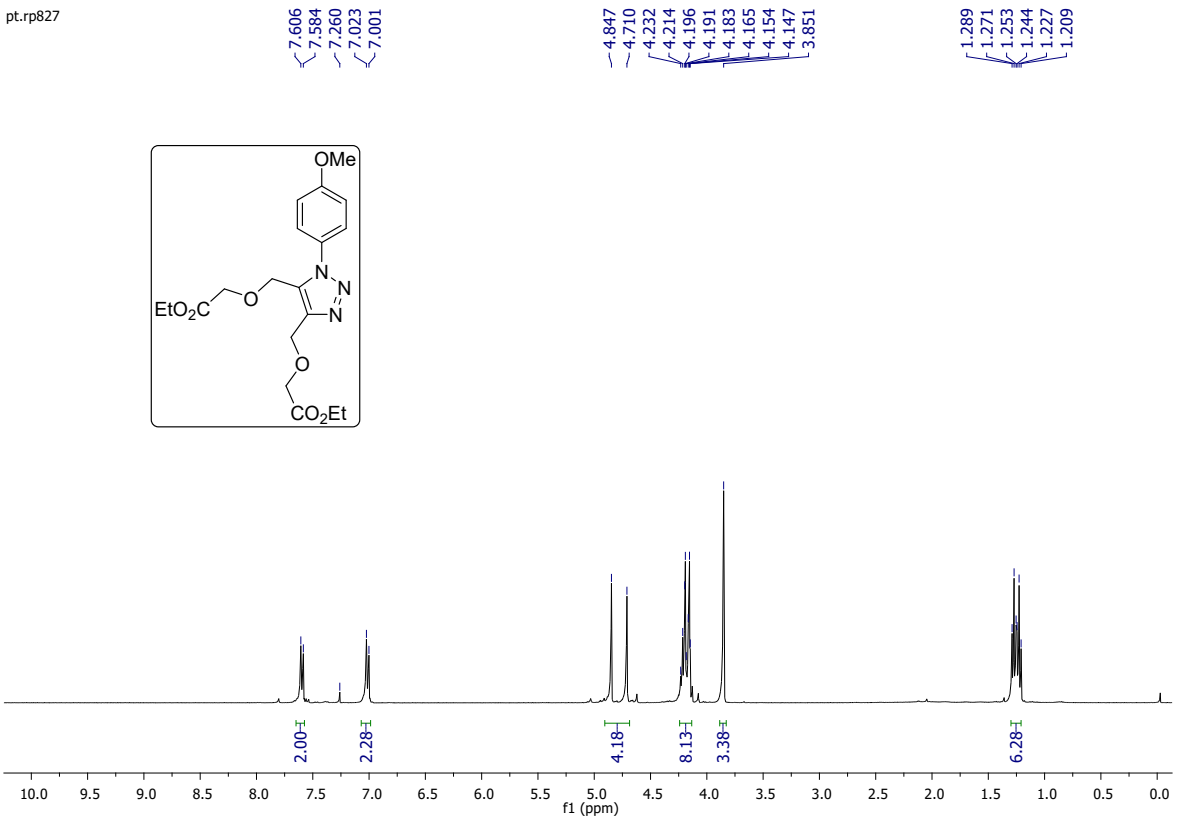
^{13}C-NMR spectra of compound **7c** (125MHz, $CDCl_3$, 25^{o}C)



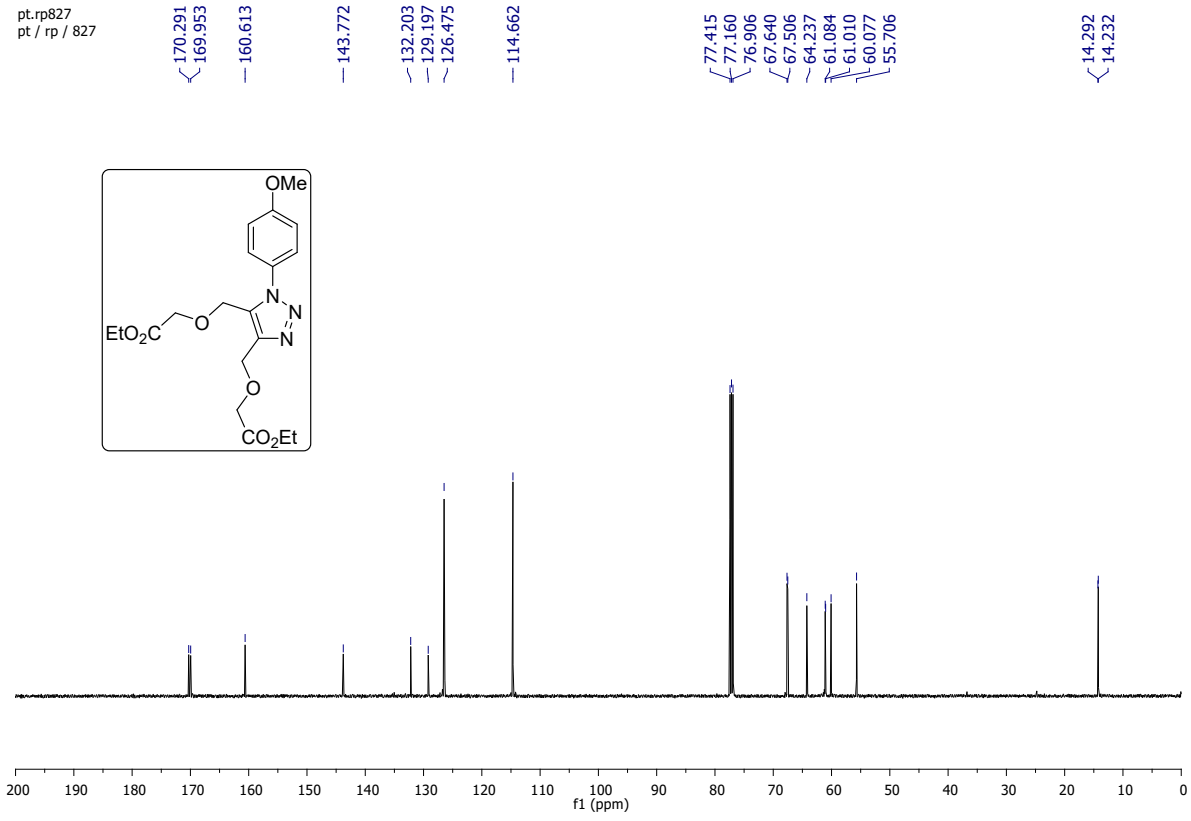
DEPT-135 spectra of compound **7c** (125MHz, $CDCl_3$, 25°C)



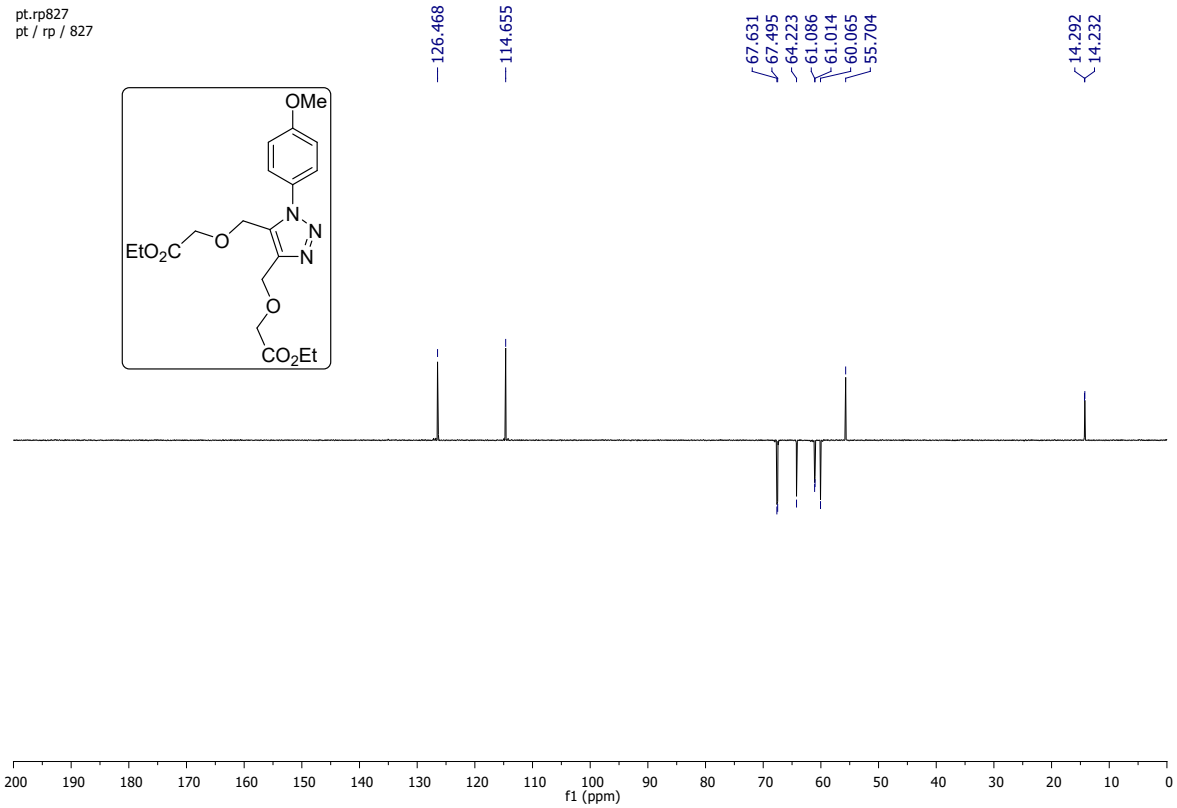
^{1}H-NMR spectra of compound **7d** (400MHz, $CDCl_3$, 25°C)



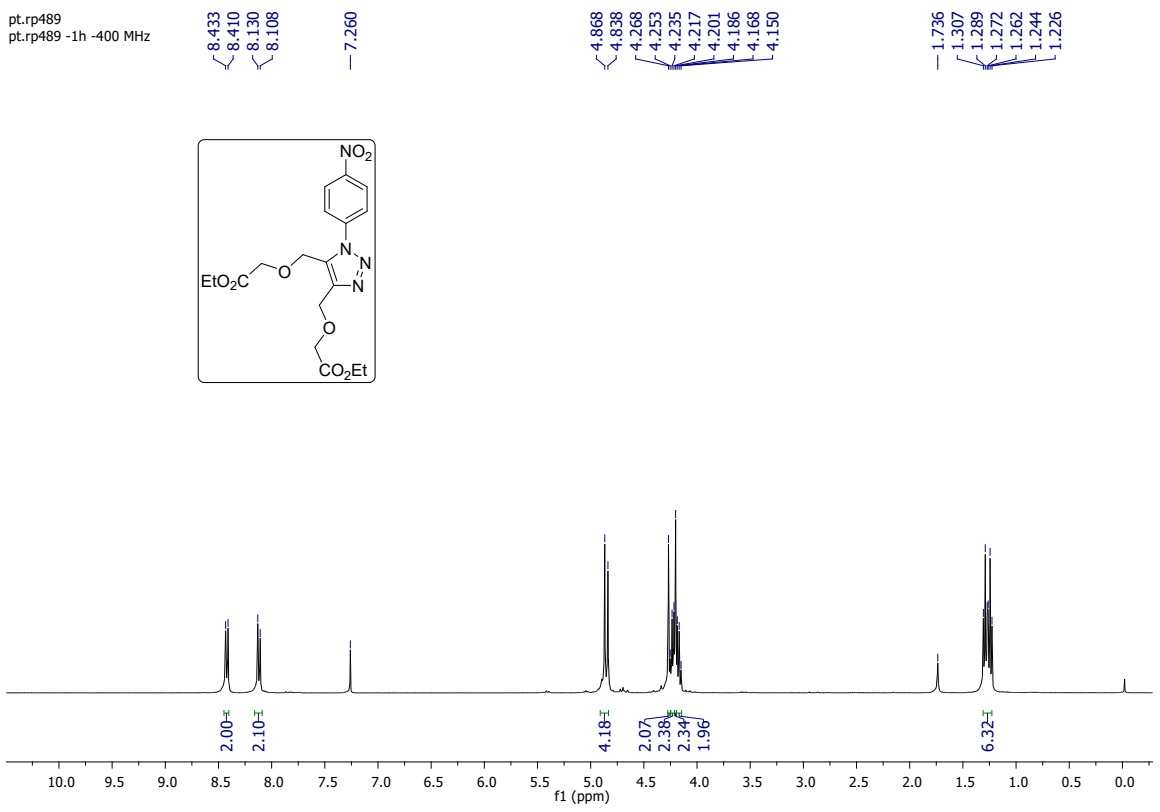
^{13}C-NMR spectra of compound **7d** (125MHz, $CDCl_3$, 25^{o}C)



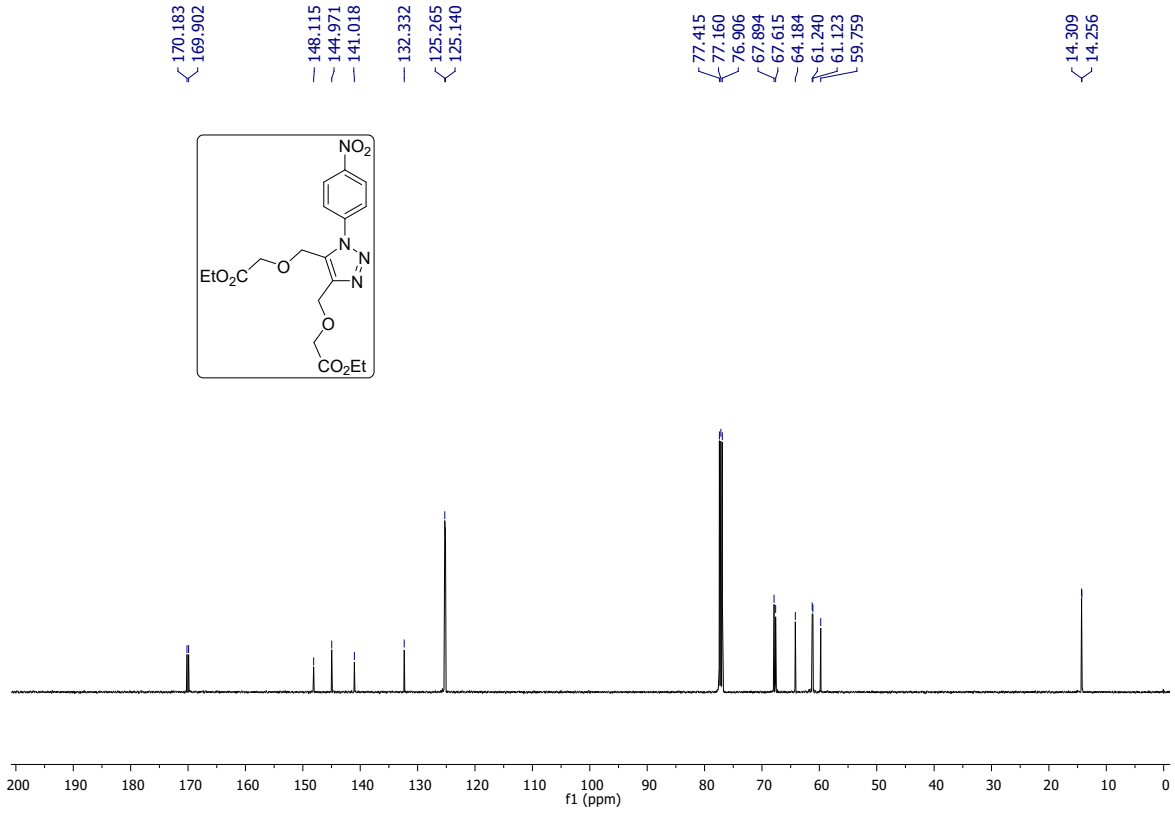
DEPT-135 spectra of compound **7d** (125MHz, $CDCl_3$, 25°C)



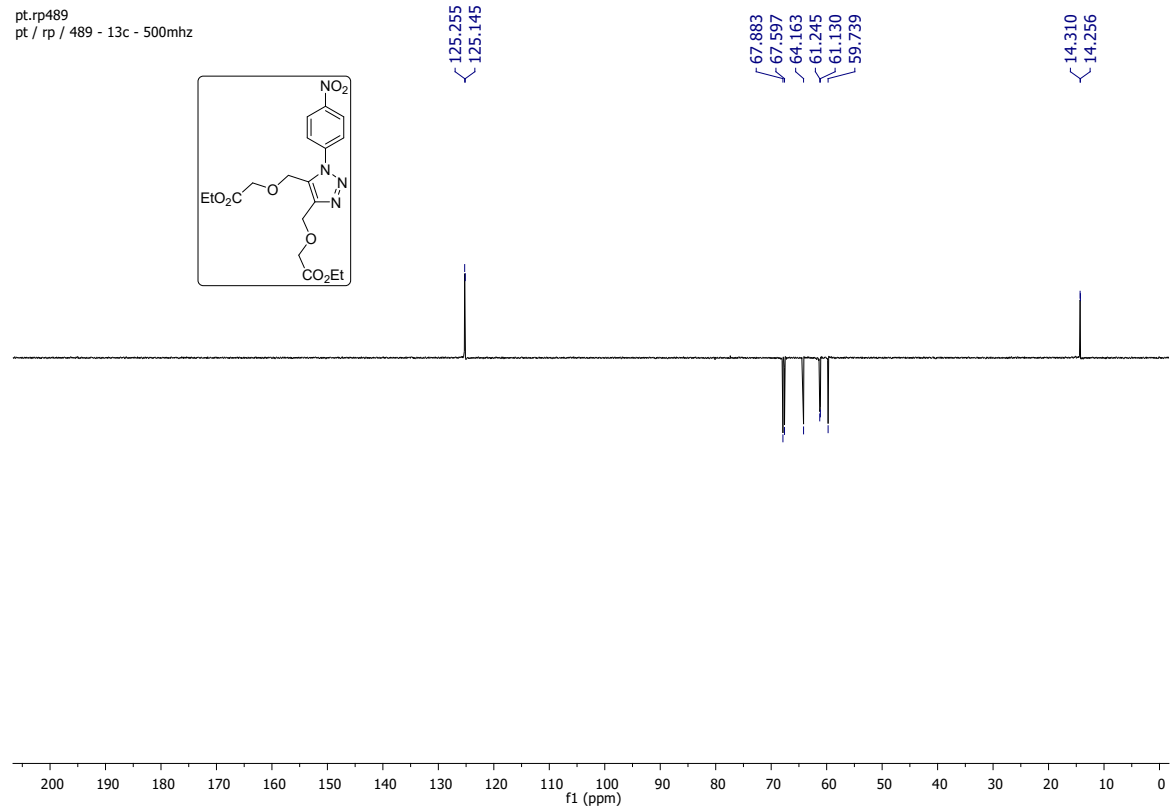
^{1}H-NMR spectra of compound **7e** (400MHz, $CDCl_3$, 25°C)



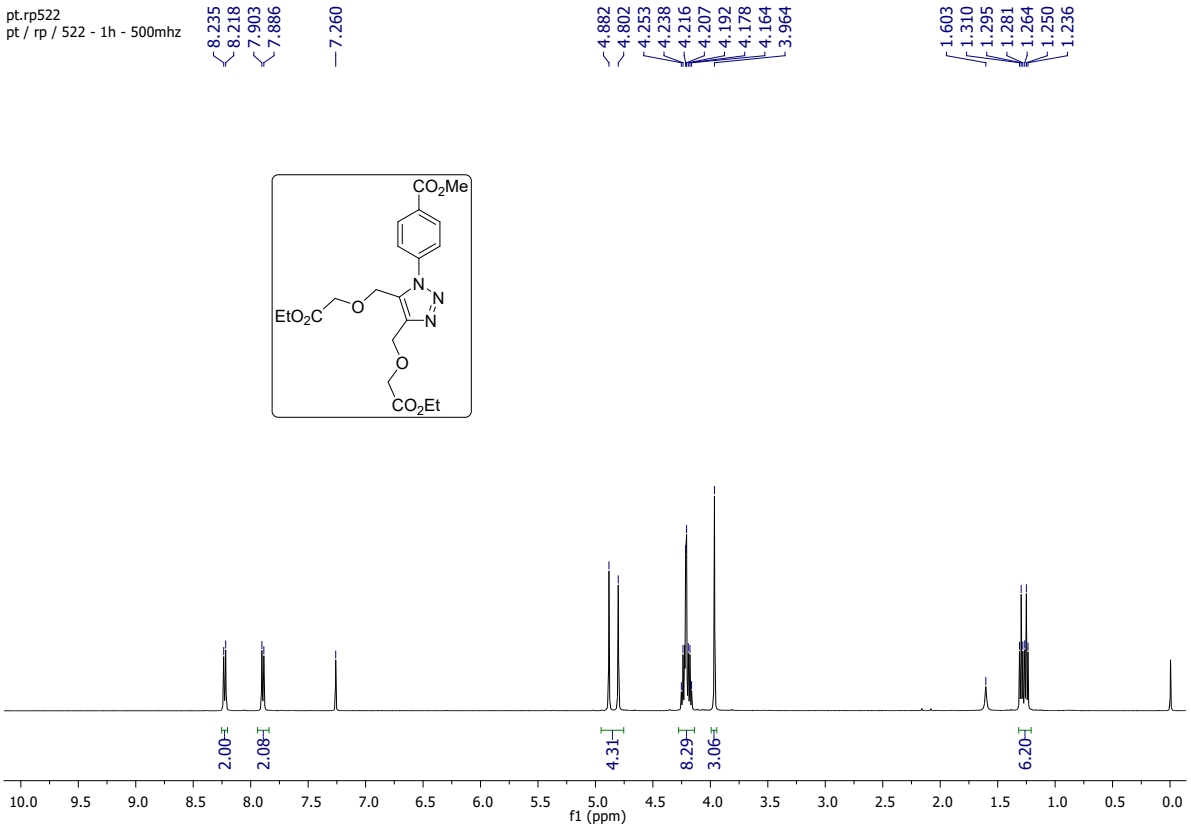
^{13}C-NMR spectra of compound **7e** (125MHz, $CDCl_3$, 25°C)



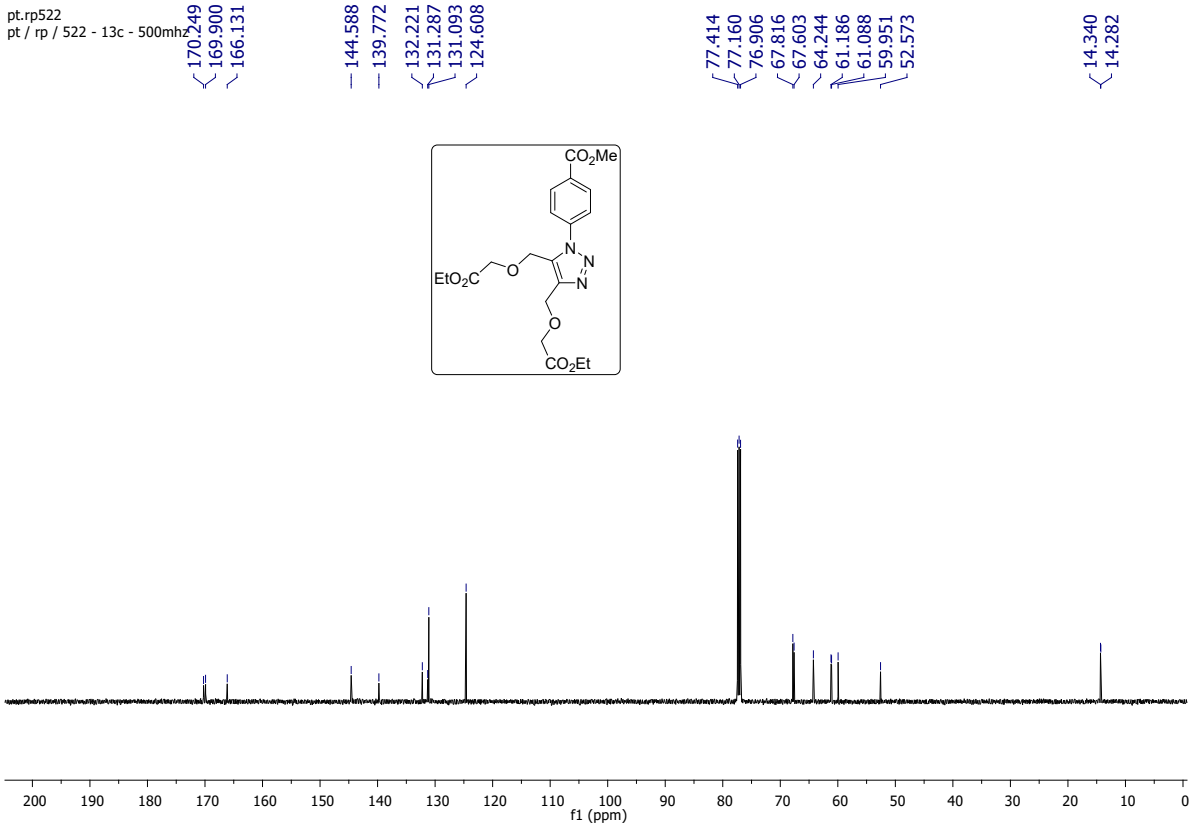
DEPT-135 spectra of compound **7e** (125MHz, $CDCl_3$, 25°C)



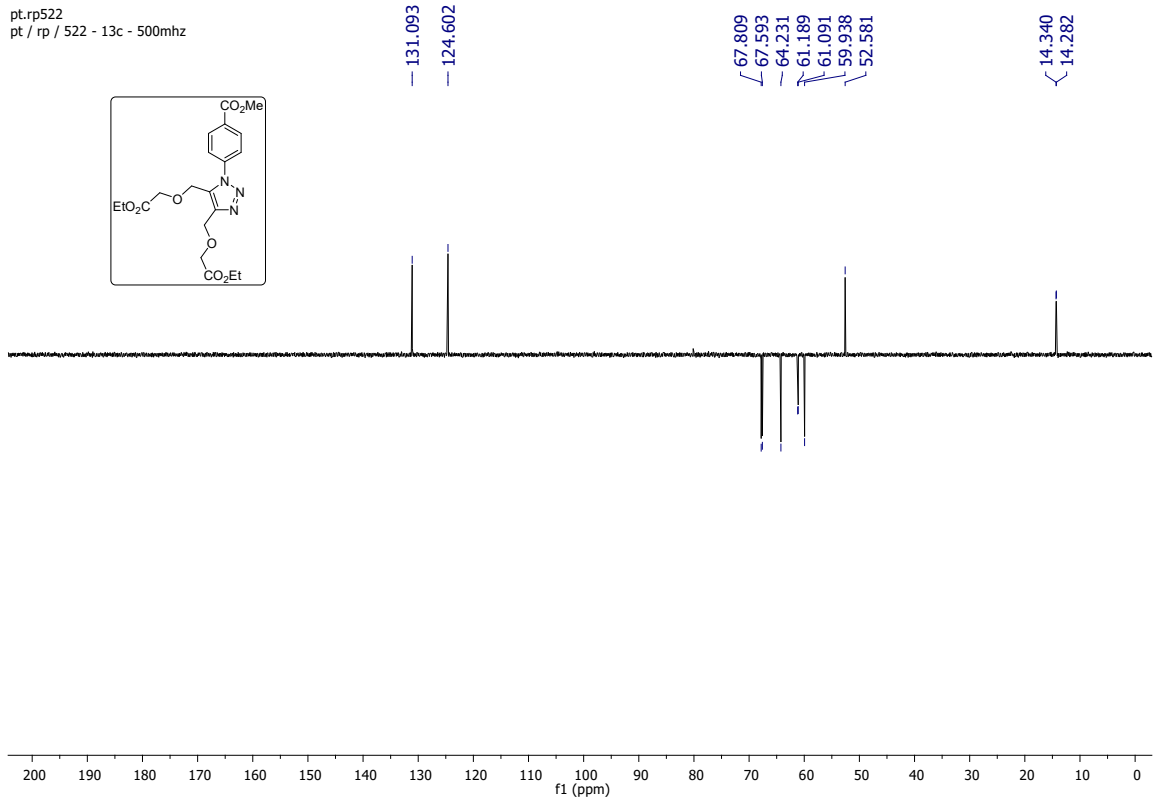
^{1}H-NMR spectra of compound **7f** (400MHz, $CDCl_3$, 25°C)



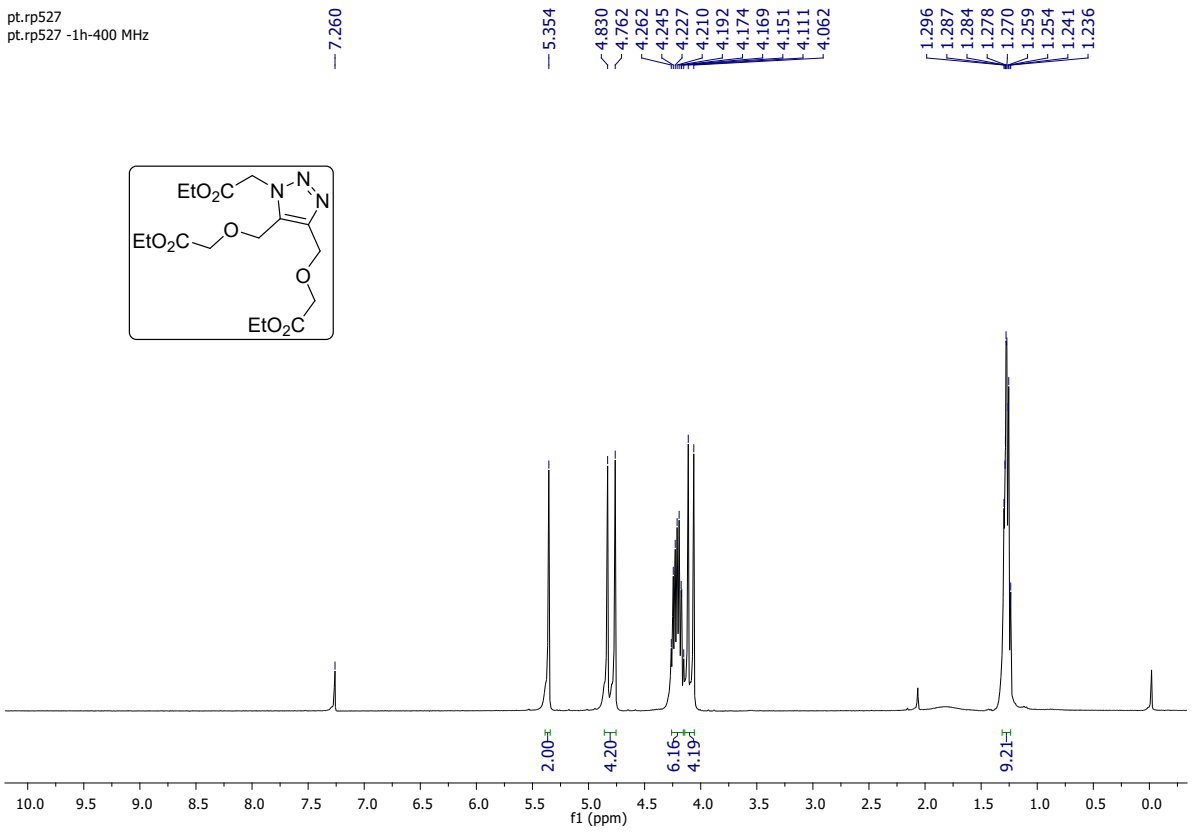
^{13}C-NMR spectra of compound **7f** (125MHz, $CDCl_3$, 25^{o}C)



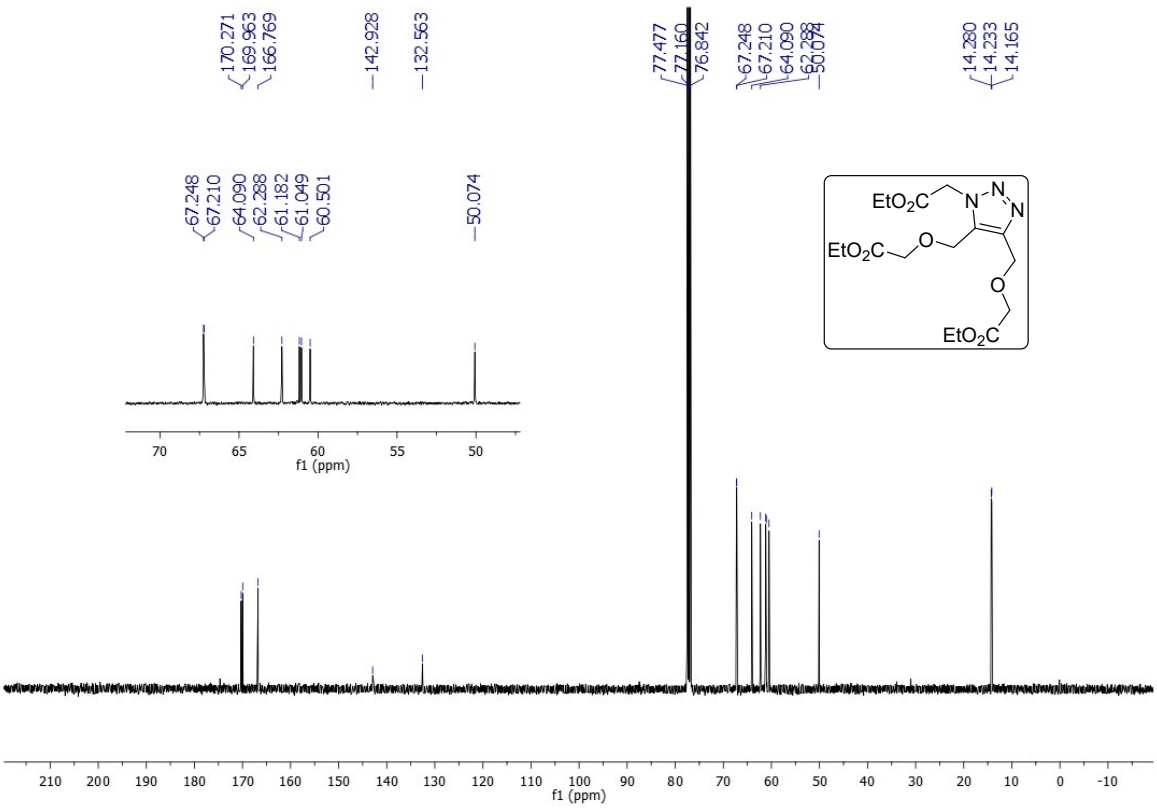
DEPT-135 spectra of compound **7f** (125MHz, $CDCl_3$, 25°C)



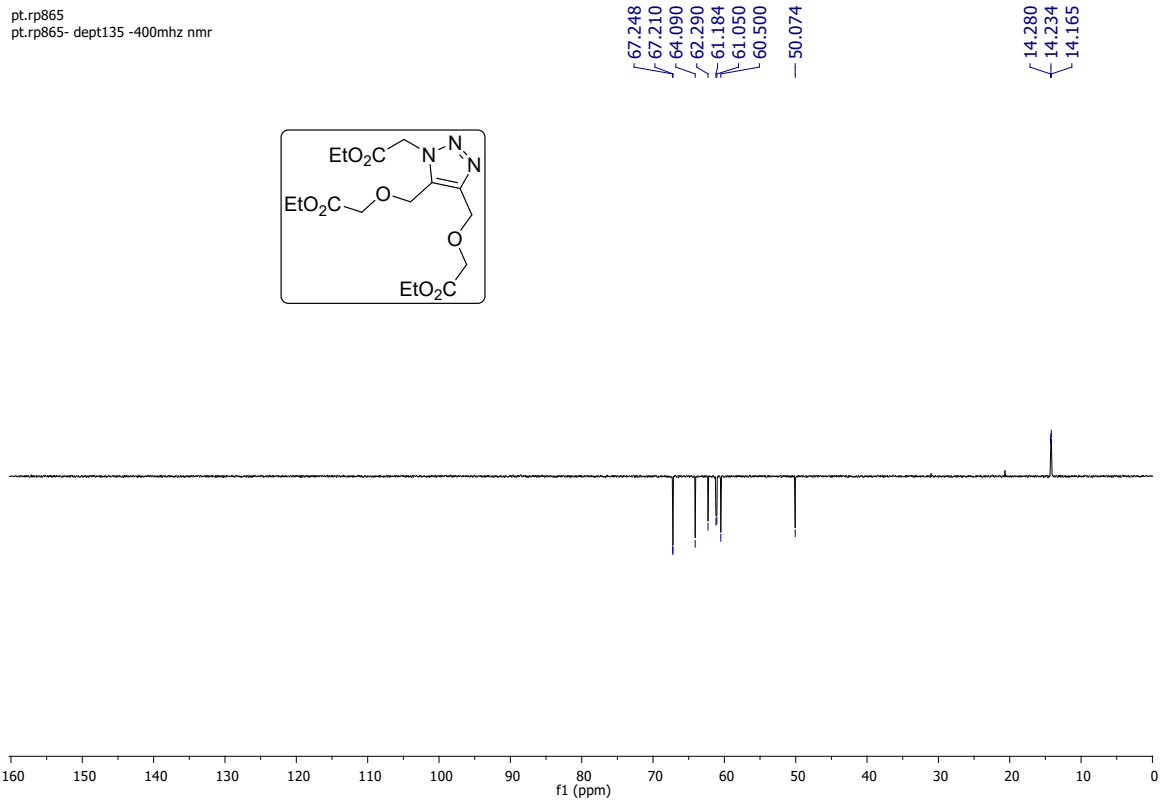
^{1}H-NMR spectra of compound **7g** (400MHz, $CDCl_3$, 25°C)



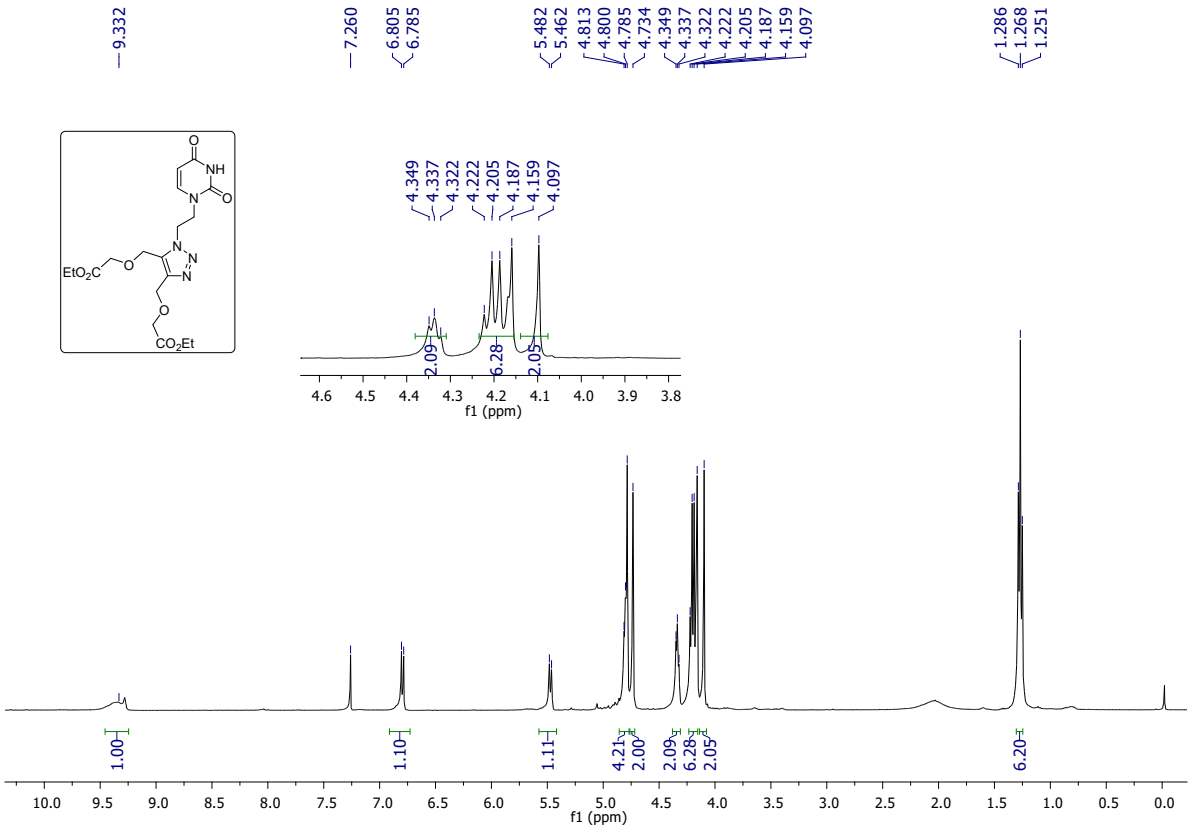
^{13}C-NMR spectra of compound **7g** (100MHz, $CDCl_3$, 25°C)



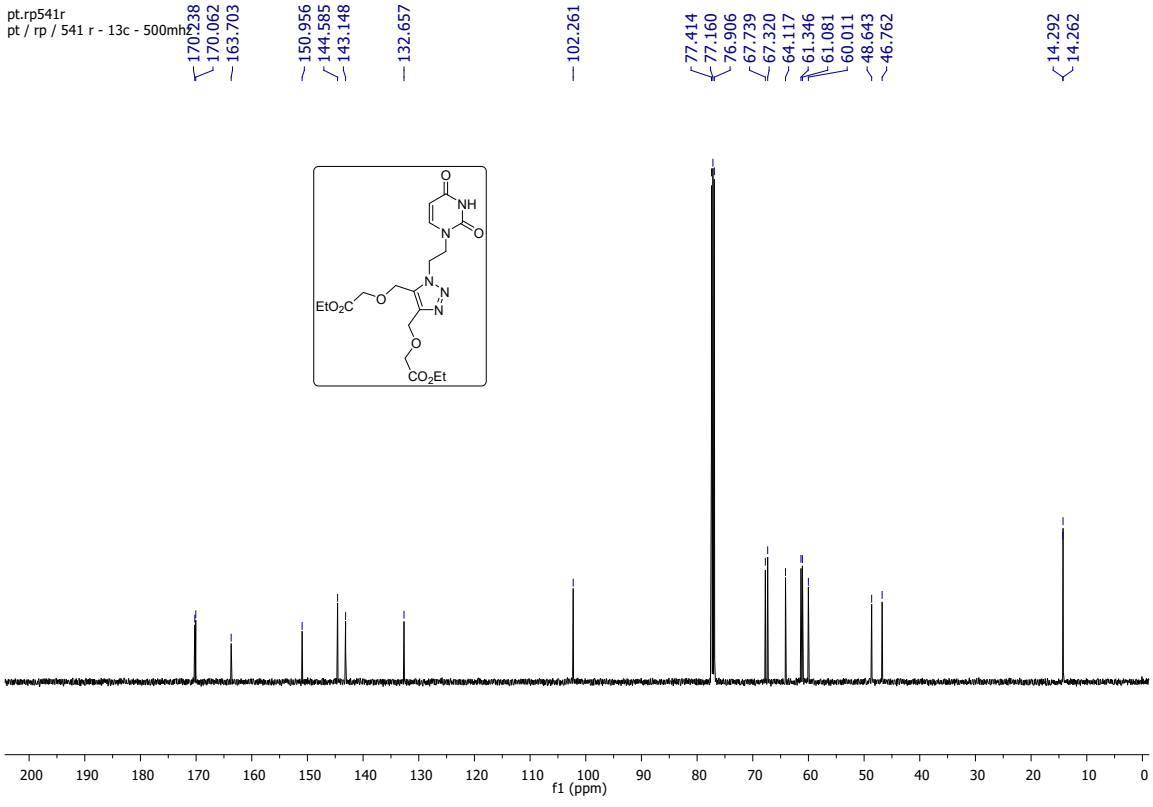
DEPT-135 spectra of compound **7g** (125MHz, $CDCl_3$, 25°C)



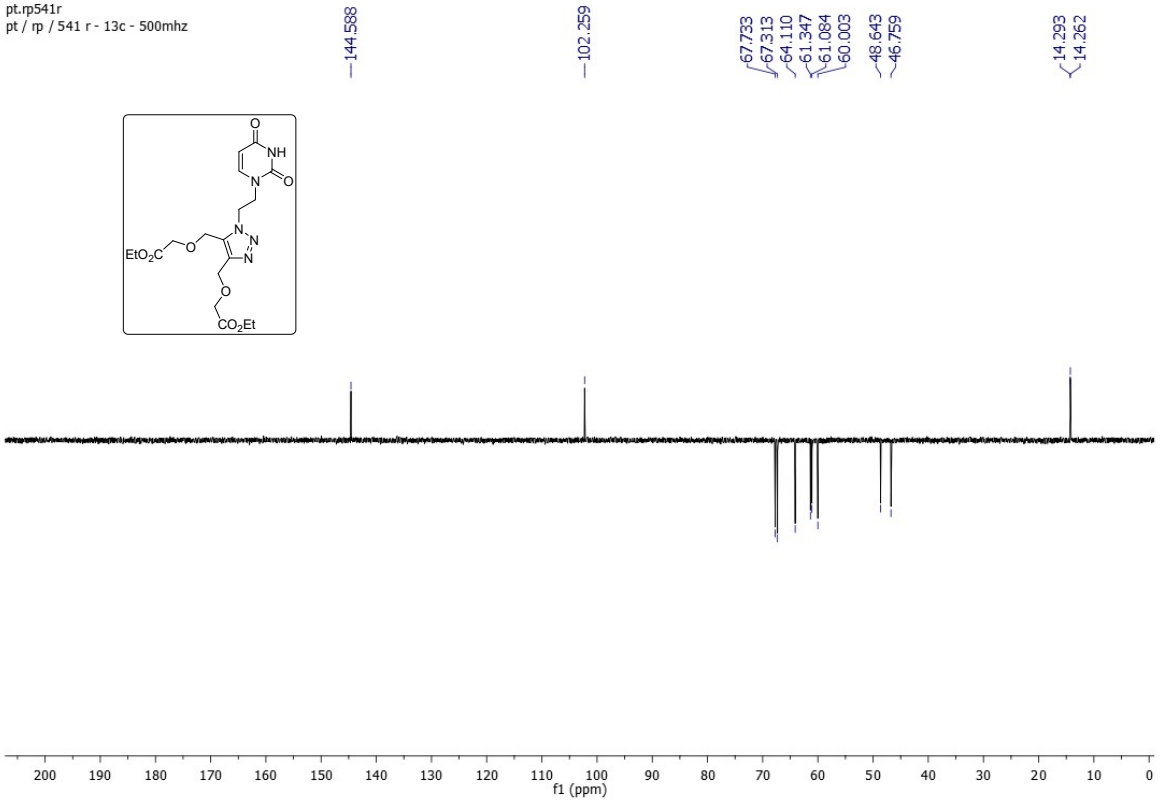
^{1}H-NMR spectra of compound **7h** (400MHz, $CDCl_3$, 25^{o}C)



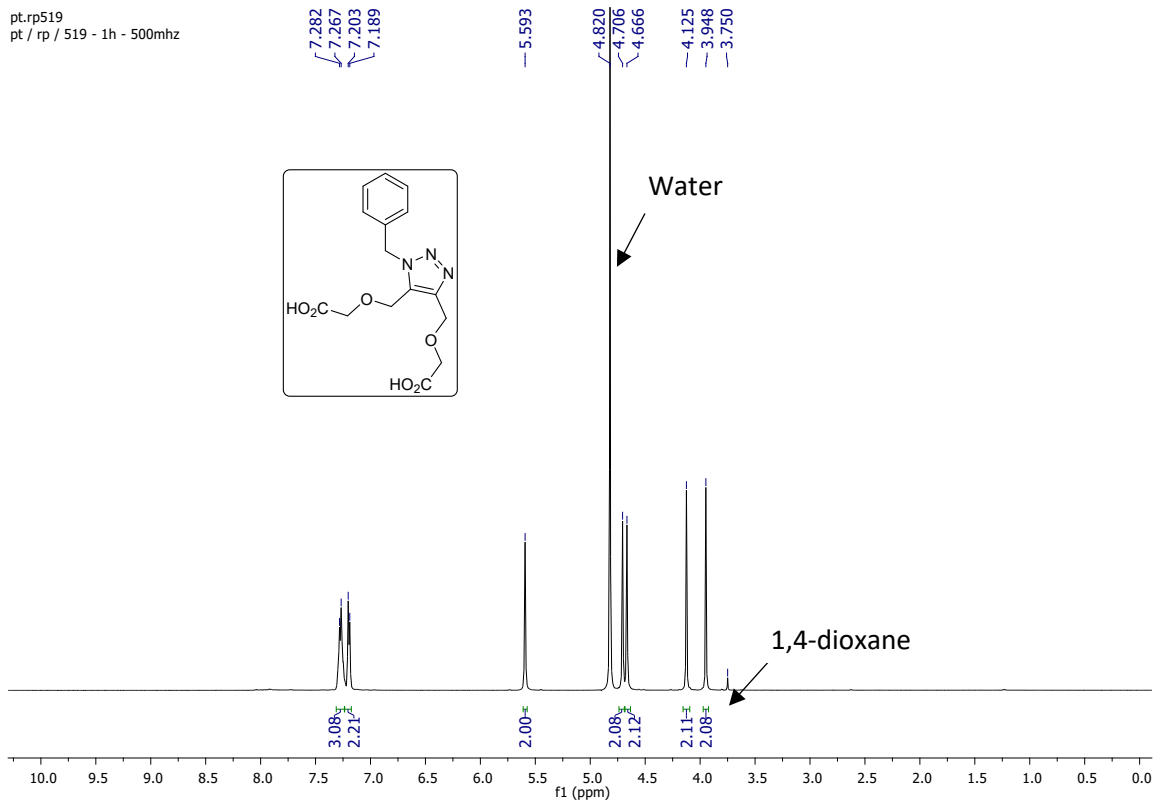
^{13}C-NMR spectra of compound **7h** (125MHz, $CDCl_3$, 25^{o}C)



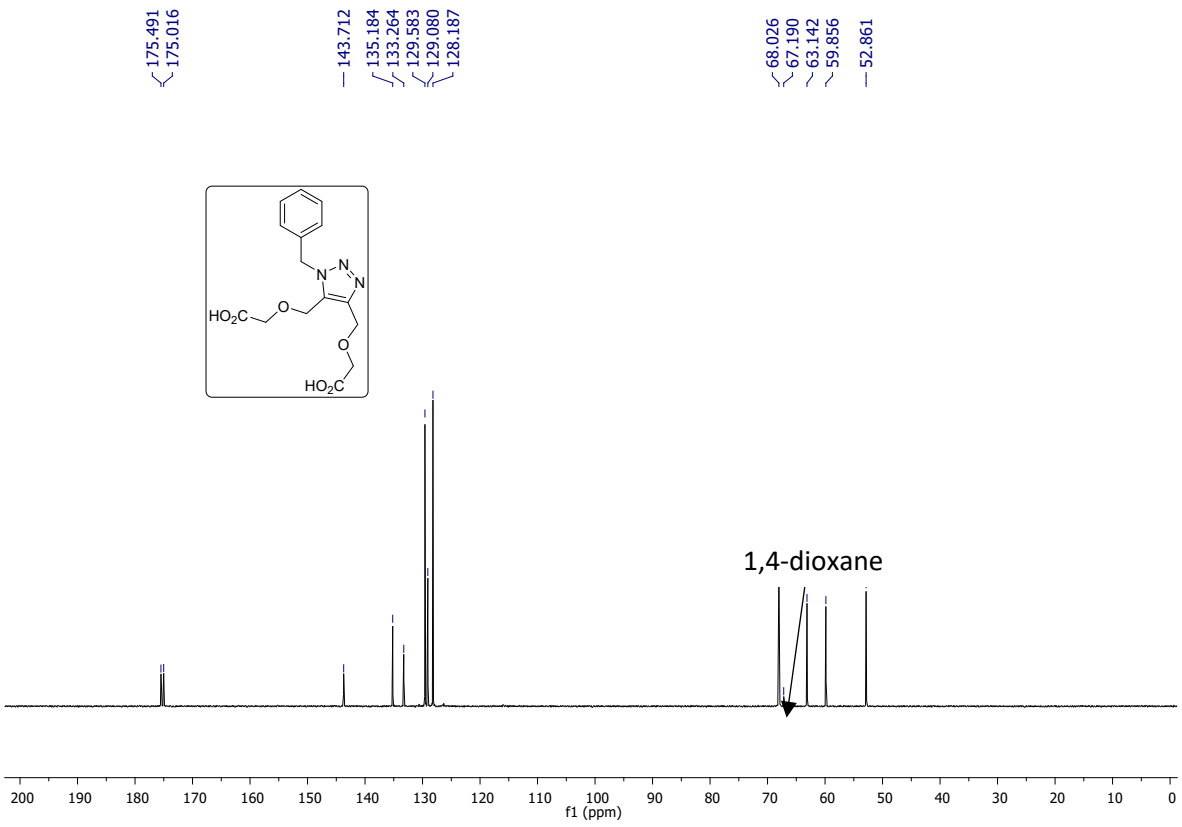
DEPT-135 spectra of compound **7h** (125MHz, $CDCl_3$, 25°C)



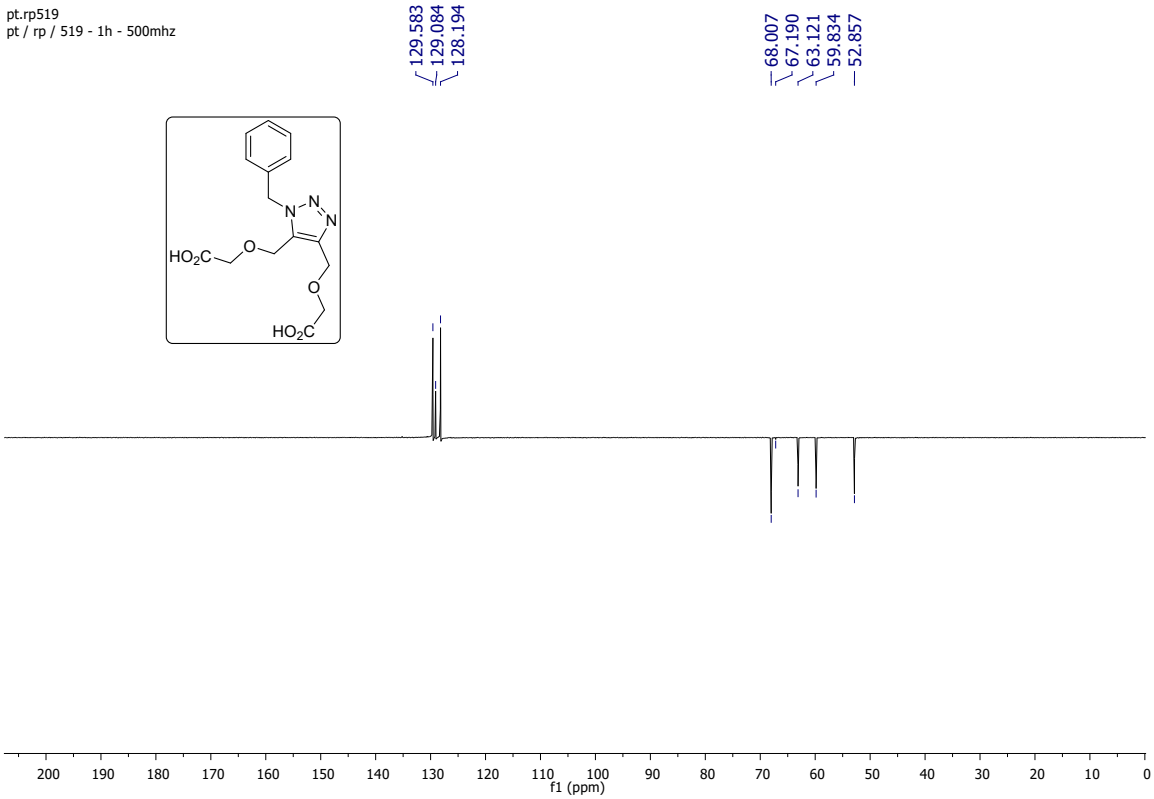
^{1}H-NMR spectra of compound **8a** (500MHz, D_2O, 25°C)



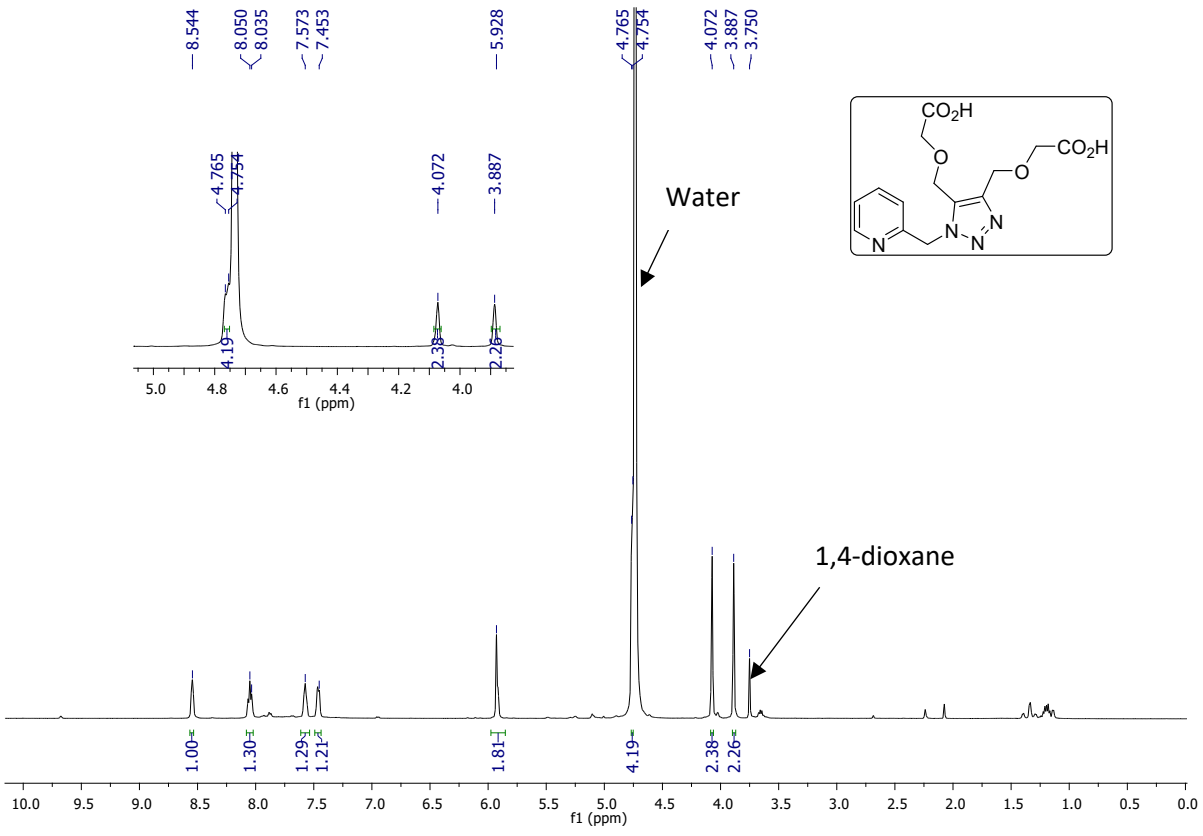
^{13}C-NMR spectra of compound **8a** (125MHz, D_2O, 25°C)



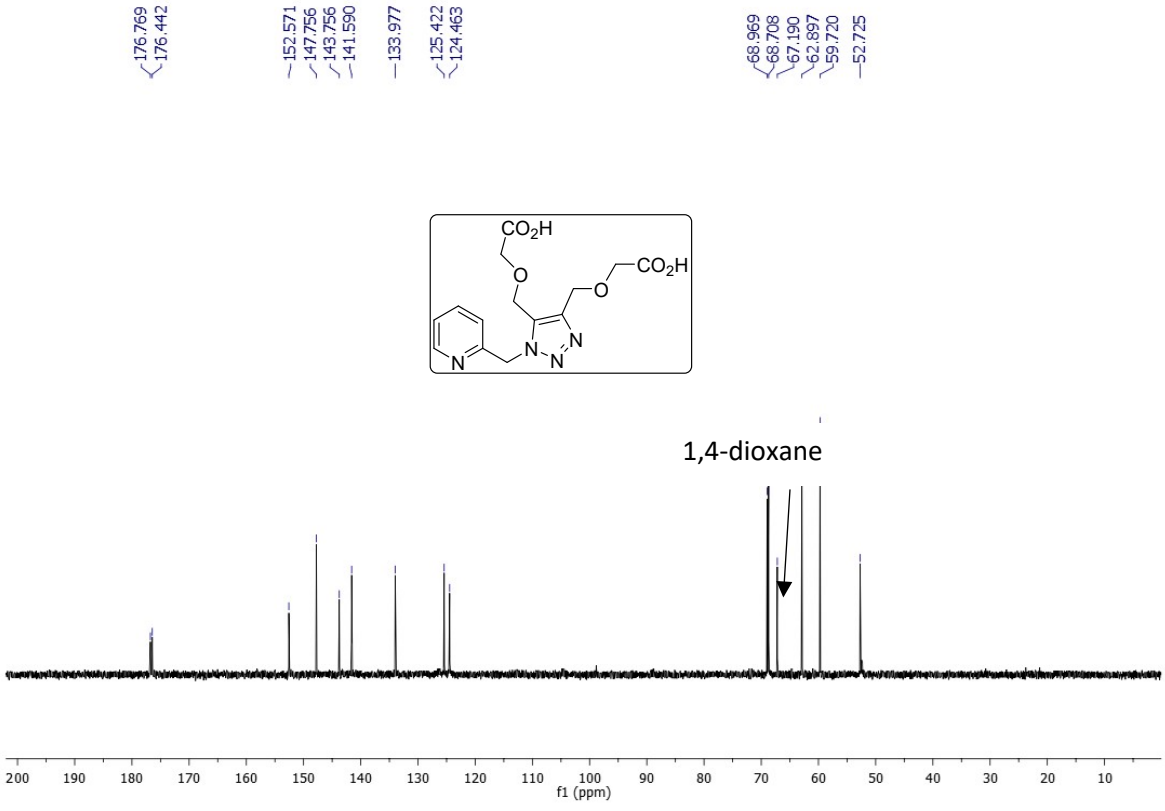
DEPT-135 spectra of compound **8a** (125MHz, D_2O, 25°C)



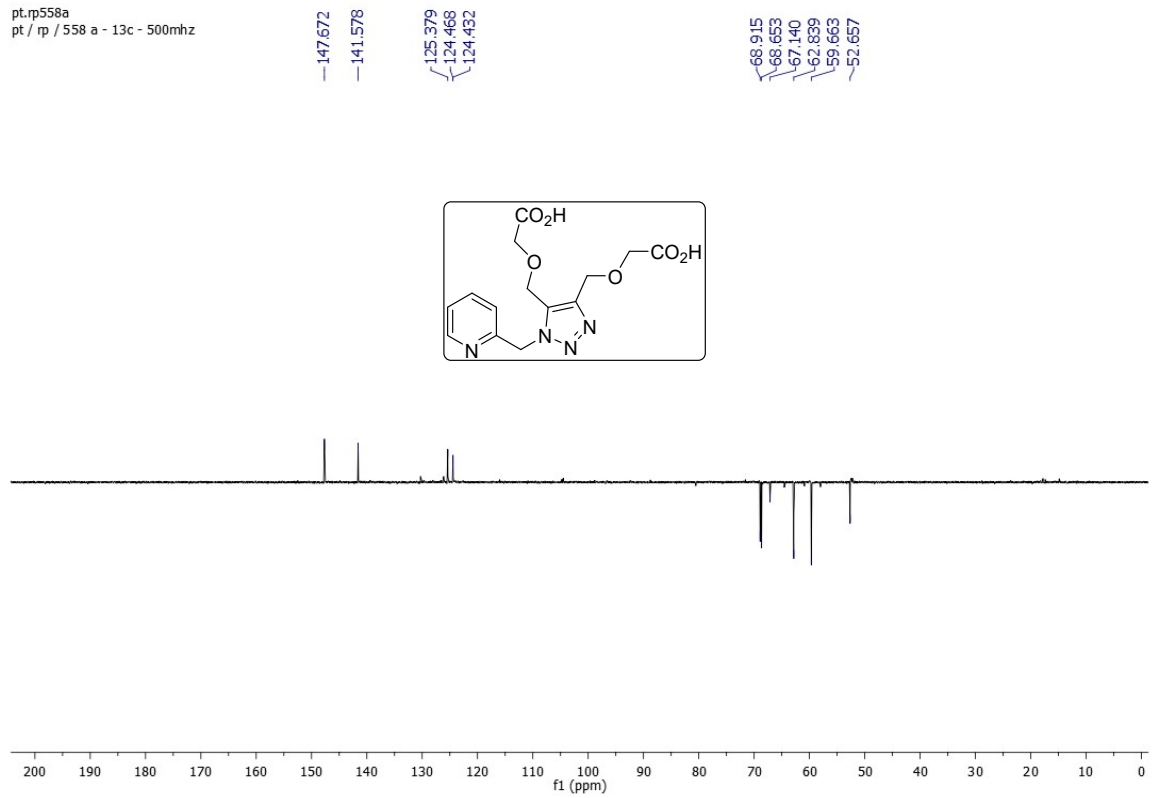
^{1}H-NMR spectra of compound **8b** (400MHz, D_2O, 25°C)



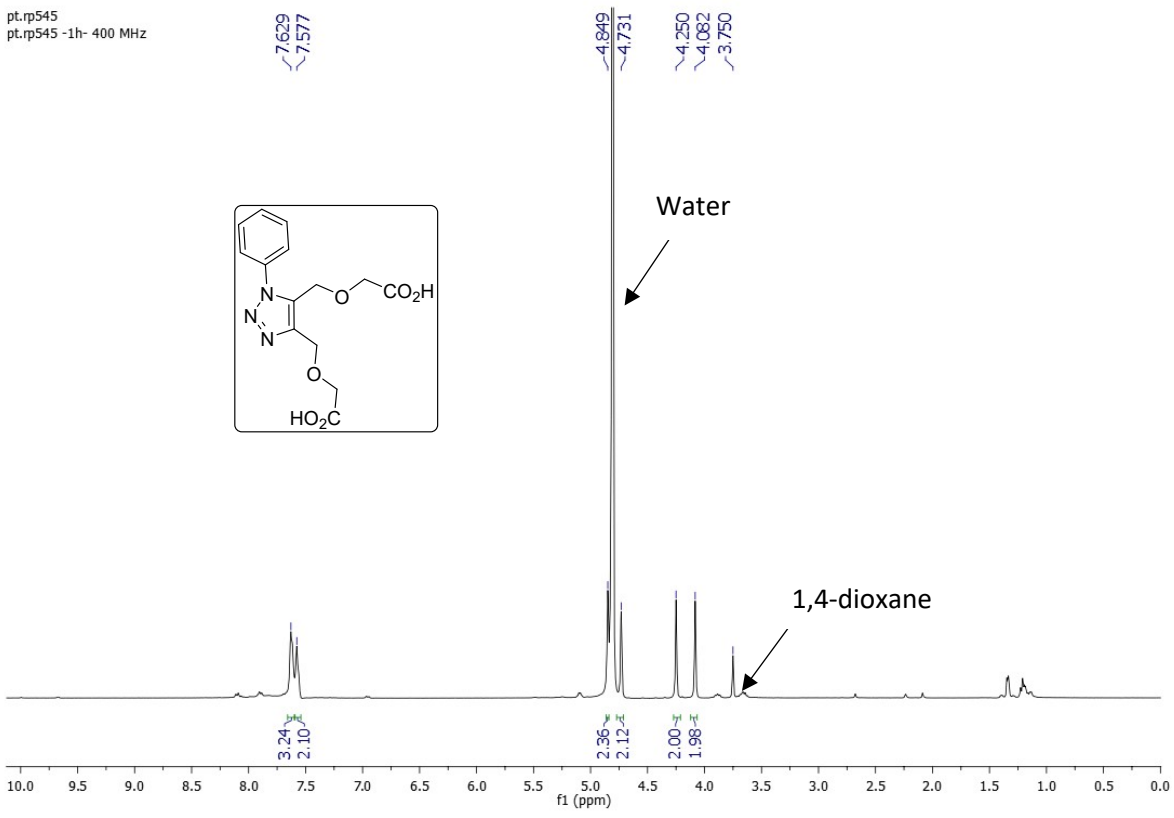
^{13}C-NMR spectra of compound **8b** (125MHz, D_2O, 25°C)



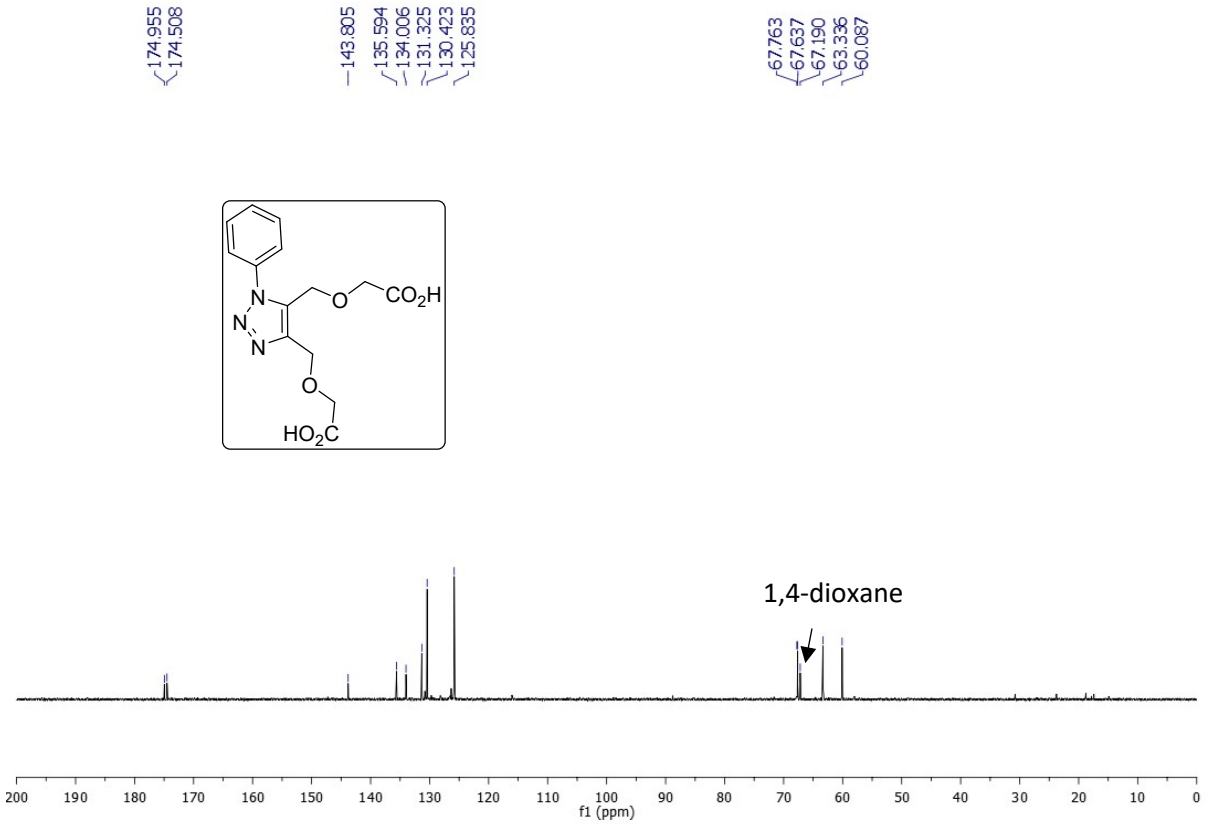
DEPT-135 spectra of compound **8b** (125MHz, D_2O, 25°C)



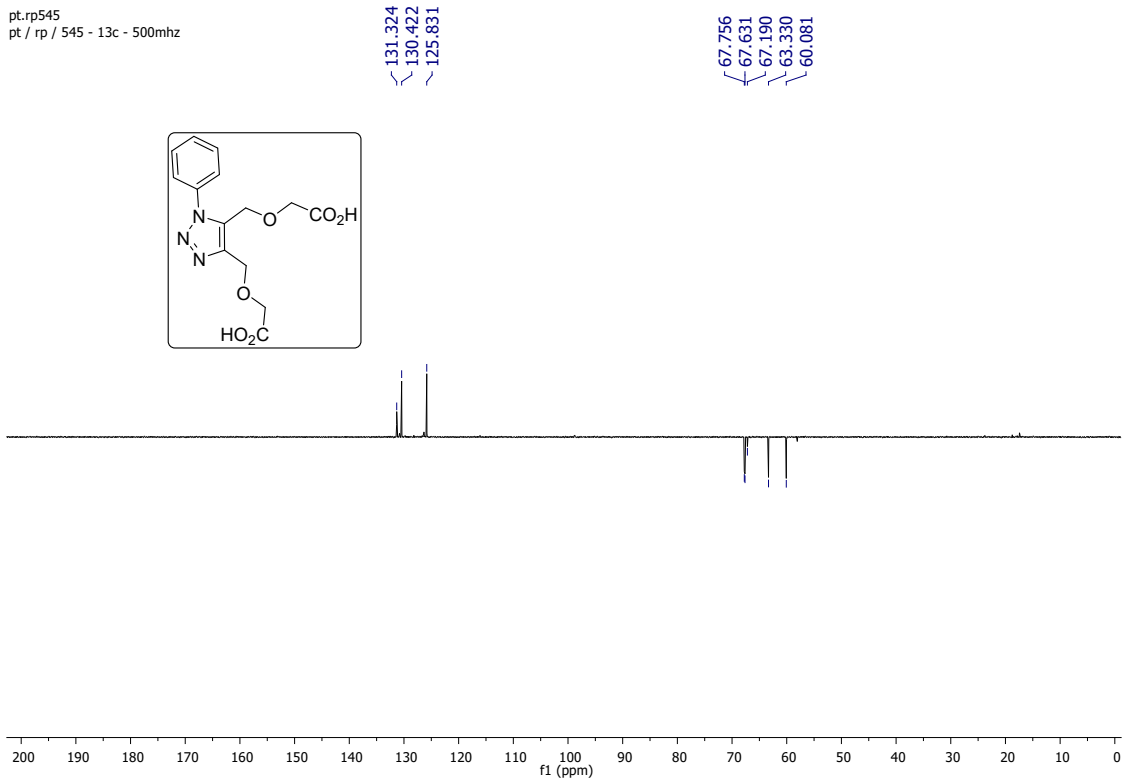
^{1}H-NMR spectra of compound **8c** (400MHz, D_2O, 25^{o}C)



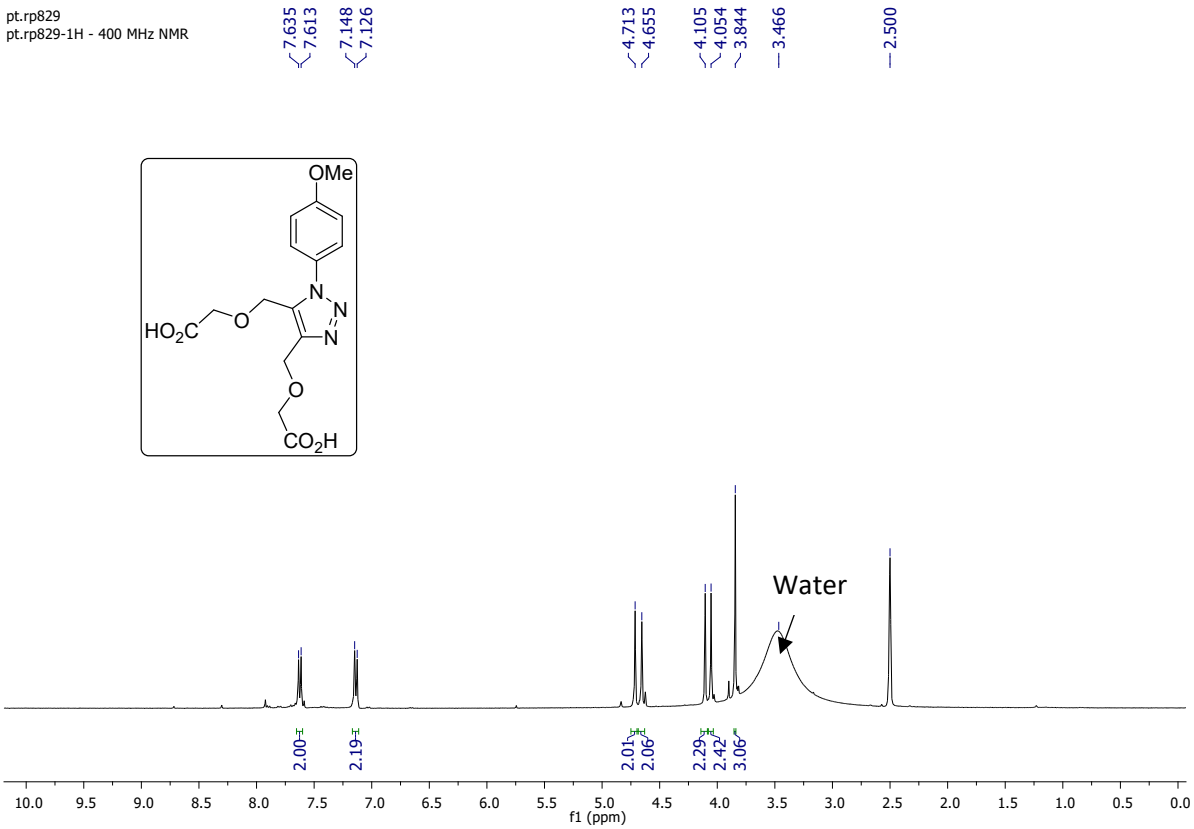
^{13}C-NMR spectra of compound **8c** (125MHz, D_2O, 25°C)



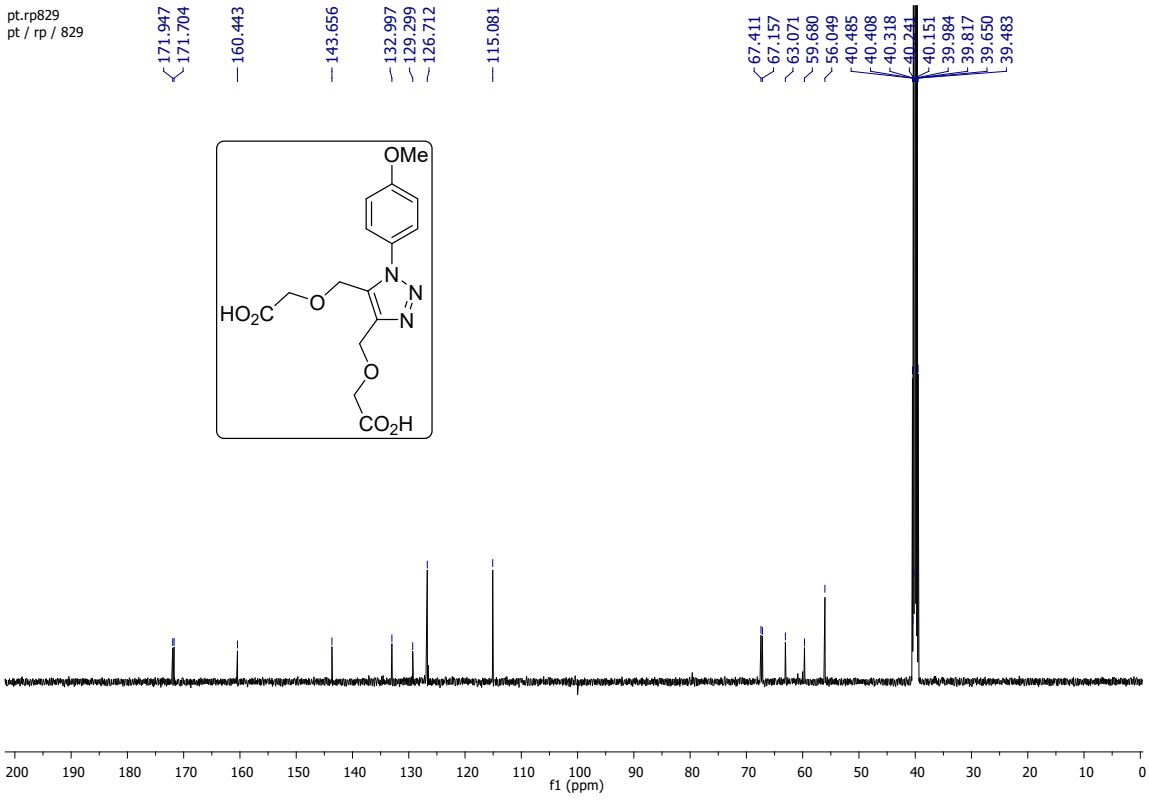
DEPT-135 spectra of compound **8c** (125MHz, D_2O, 25°C)



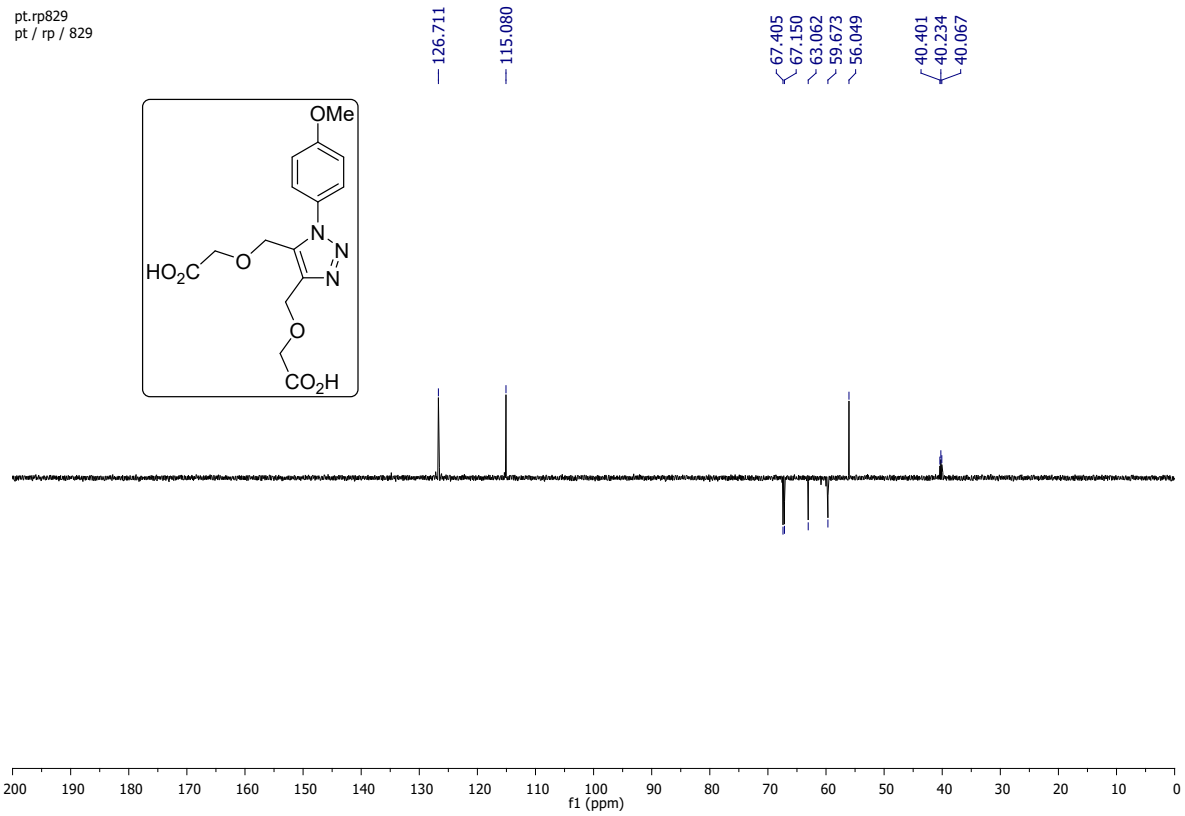
^{1}H-NMR spectra of compound **8d** (400MHz, DMSO-d_6, 25°C)



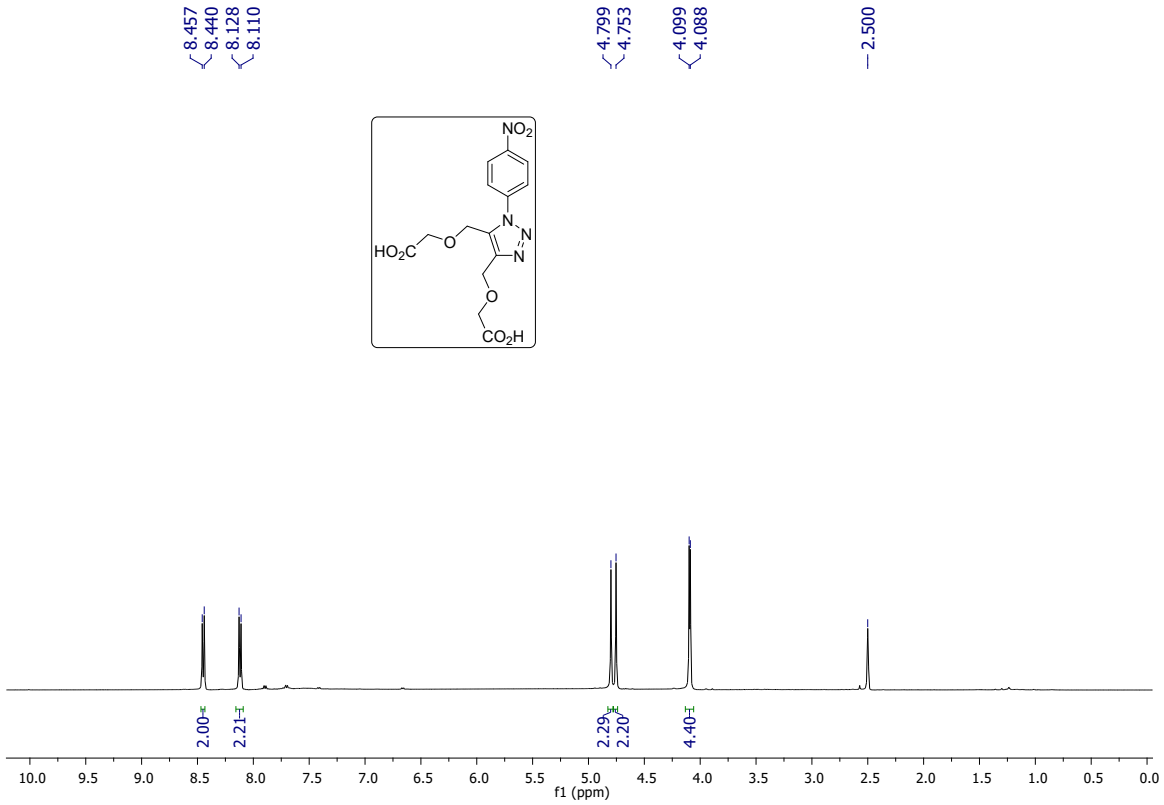
^{13}C-NMR spectra of compound **8d** (125MHz, DMSO-d_6, 25°C)



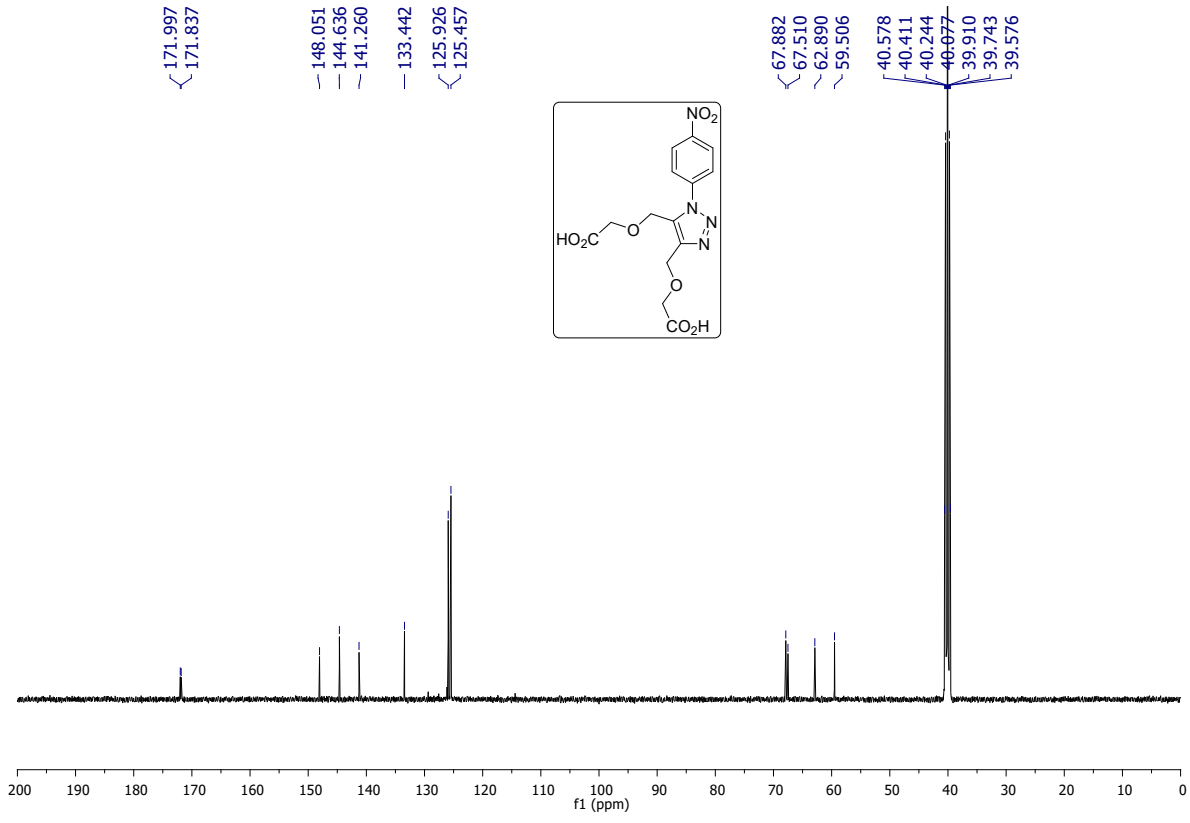
DEPT-135 spectra of compound **8d** (125MHz, DMSO-d_6, 25°C)



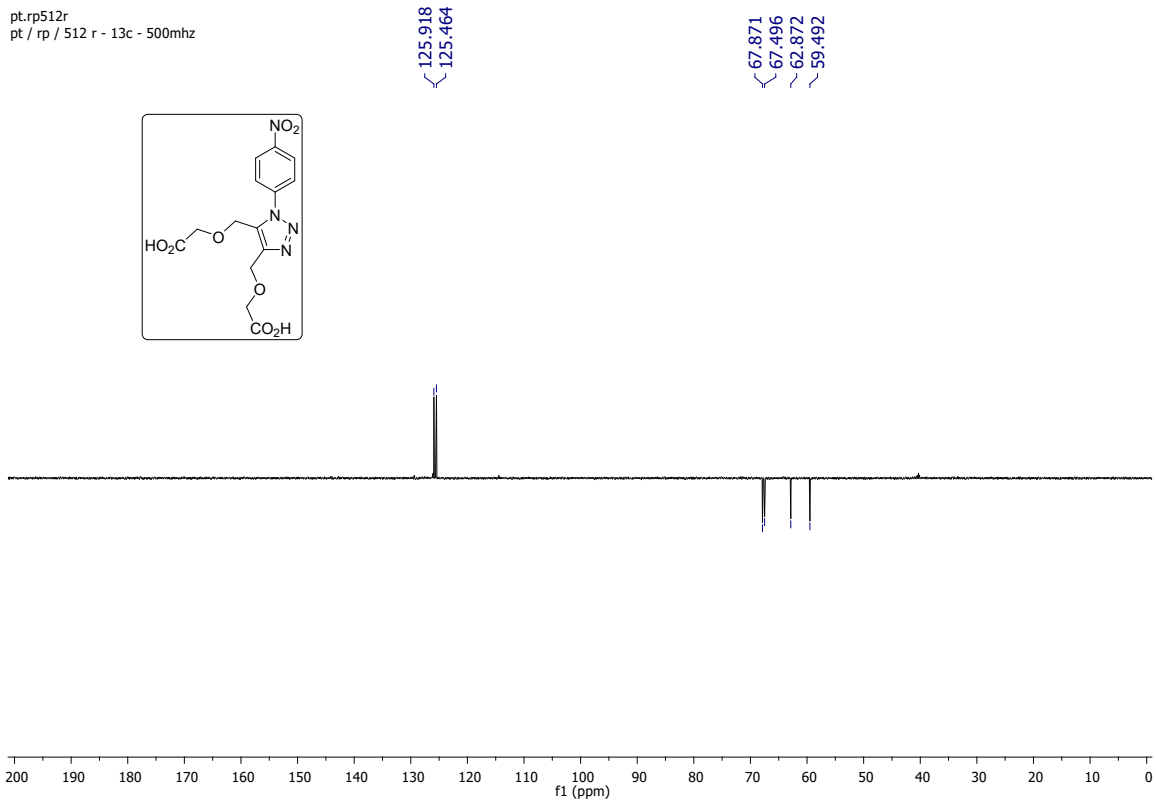
^{1}H-NMR spectra of compound **8e** (400MHz, DMSO-d_6, 25^{o}C)



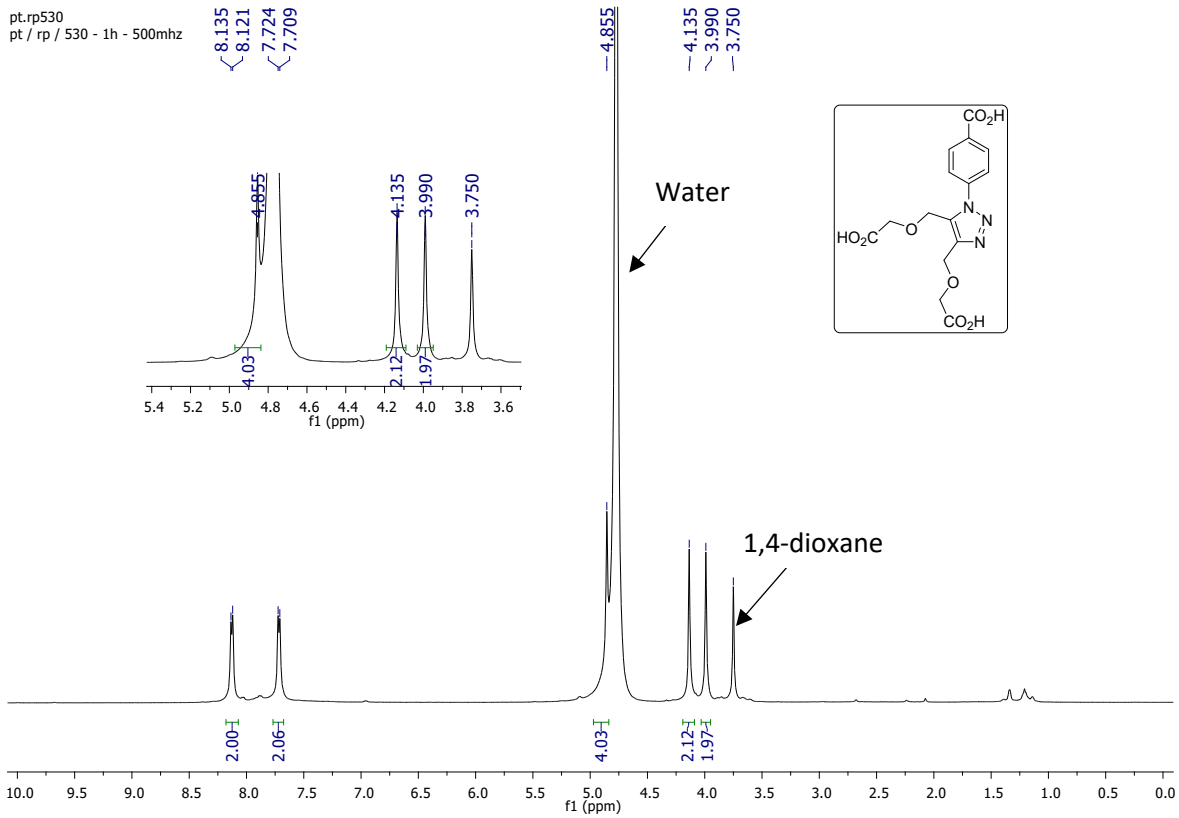
^{13}C-NMR spectra of compound **8e** (125MHz, DMSO-d_6, 25°C)



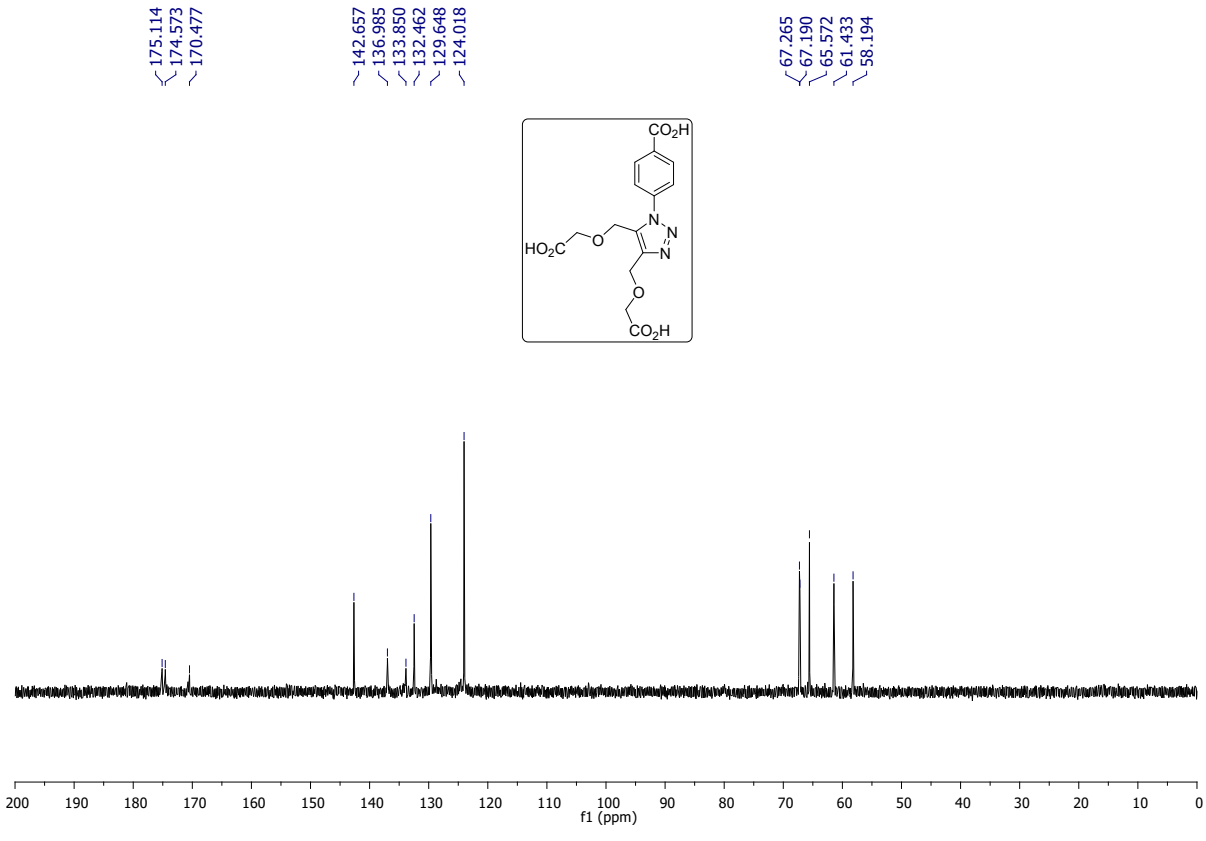
DEPT-135 spectra of compound **8e** (125MHz, DMSO-d_6, 25°C)



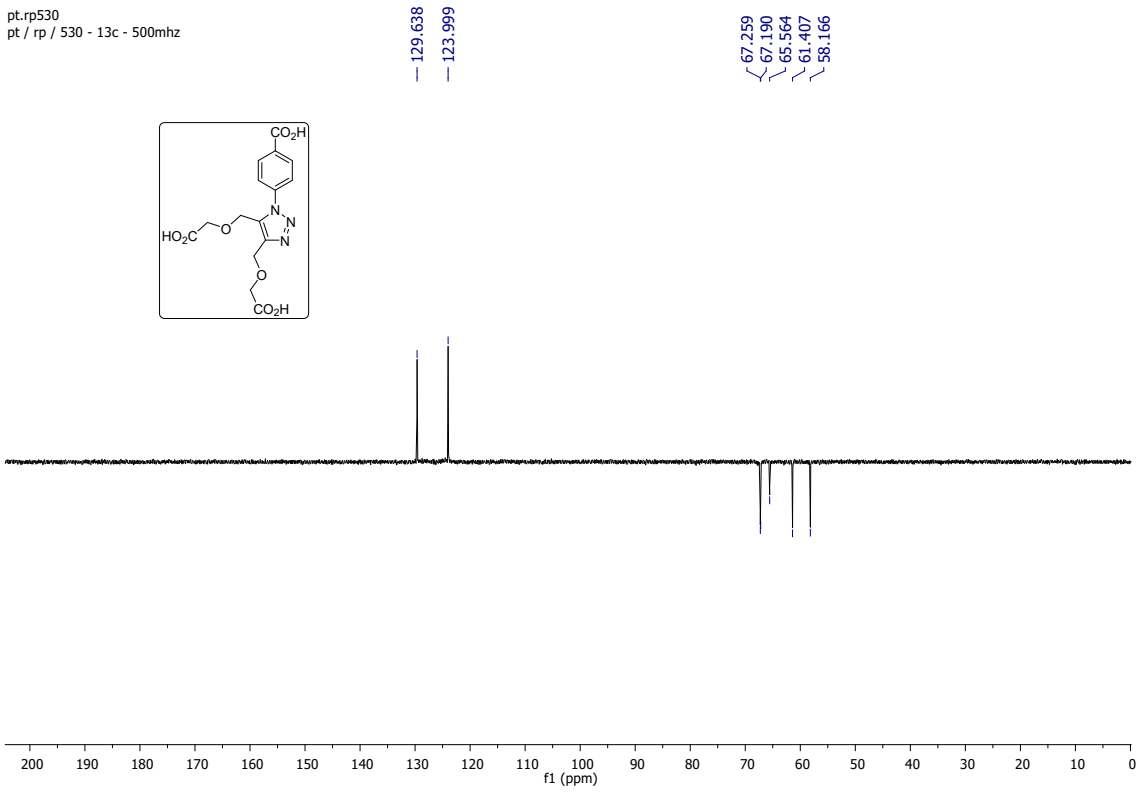
^{1}H-NMR spectra of compound **8f'** (500MHz, D_2O, 25°C)



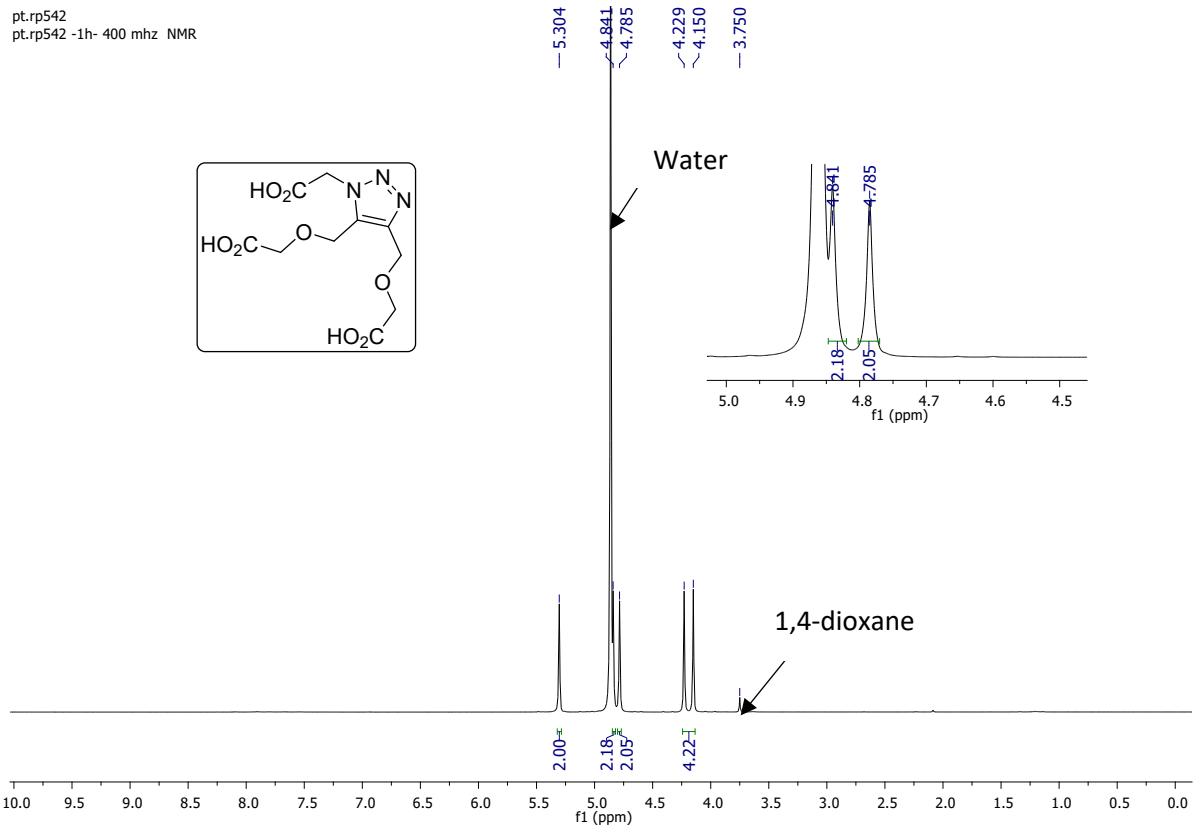
^{13}C-NMR spectra of compound **8f'** (125MHz, D_2O, 25°C)



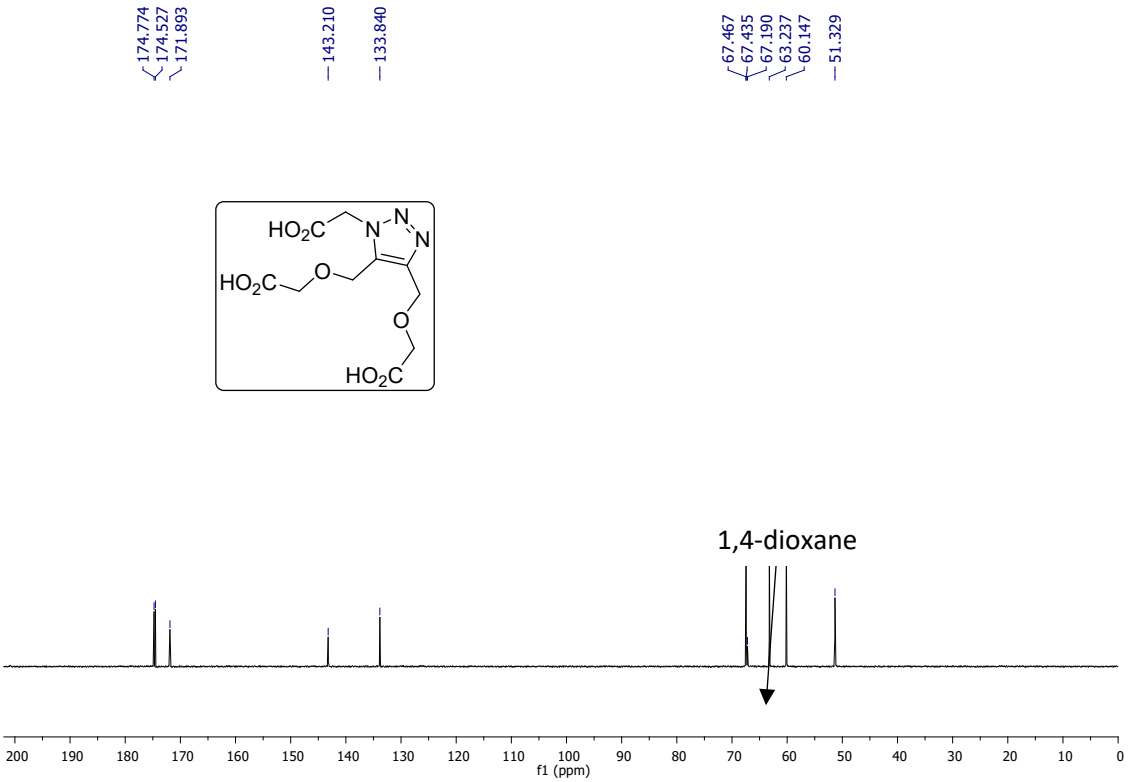
DEPT-135 spectra of compound **8f'** (125MHz, D_2O, 25°C)



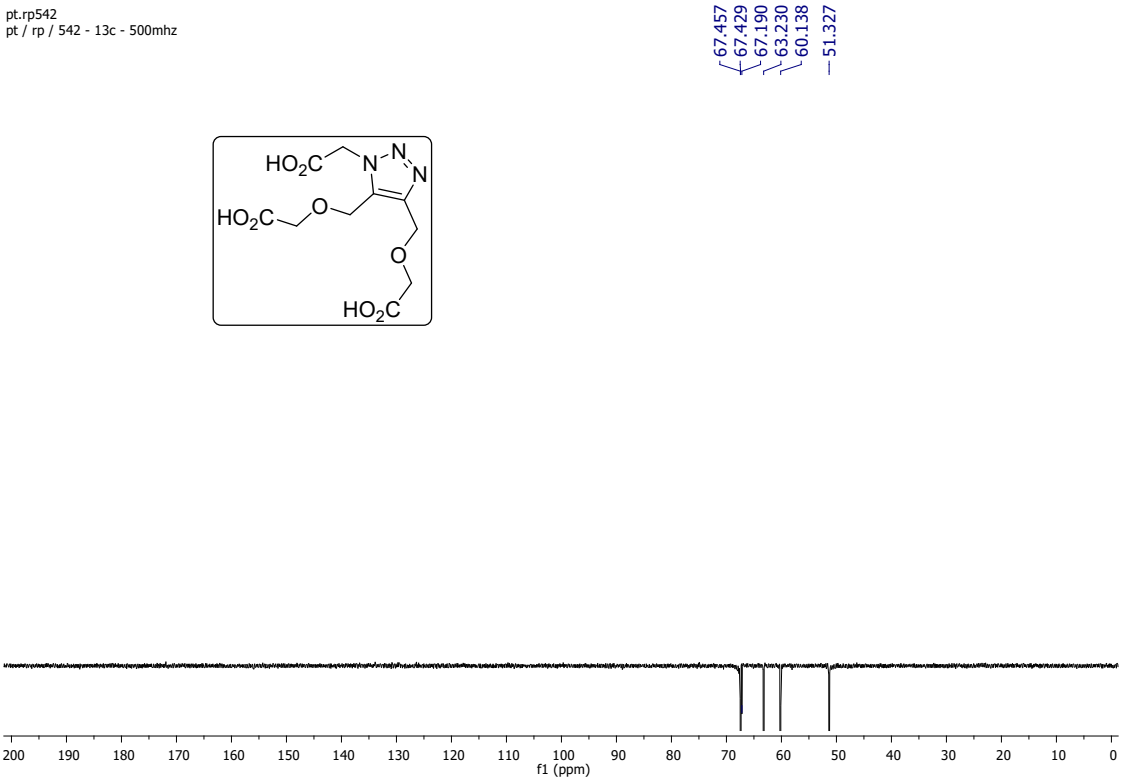
^{1}H-NMR spectra of compound **8g'** (400MHz, D_2O, 25°C)



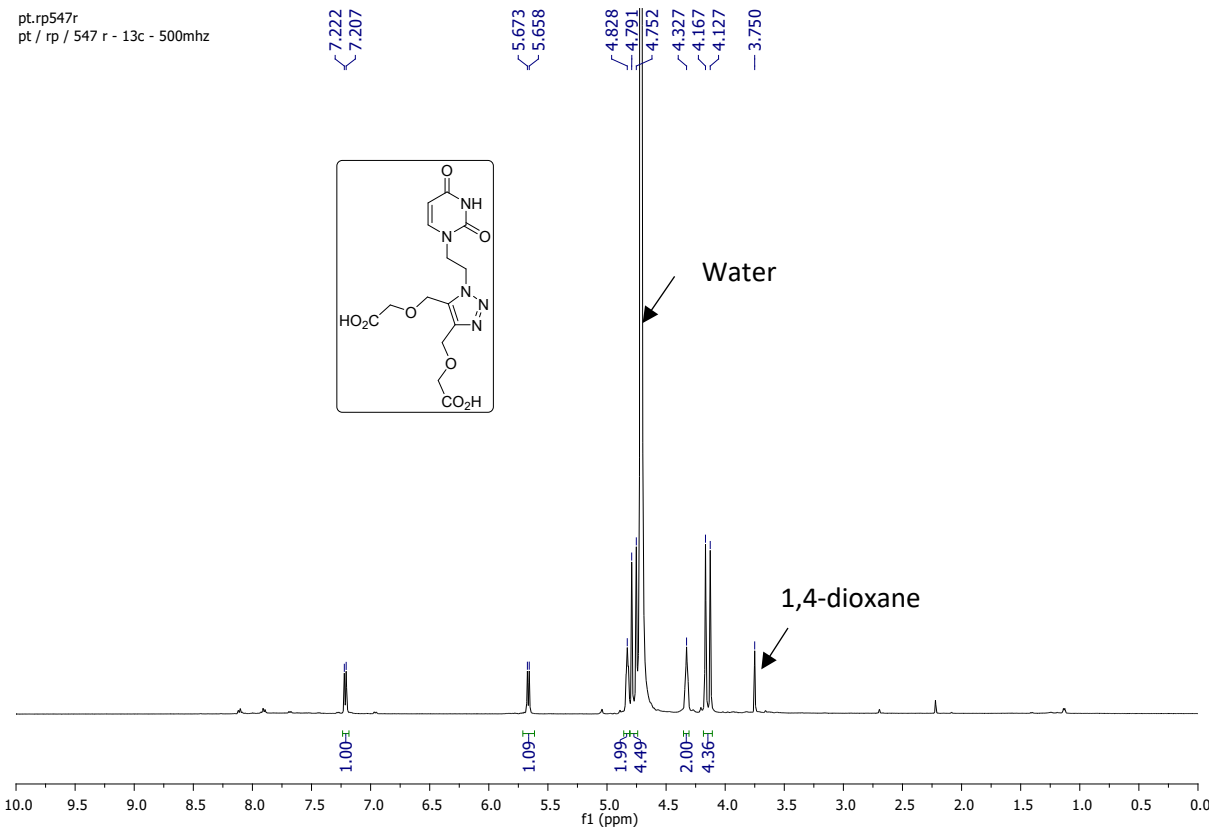
^{13}C-NMR spectra of compound **8g'** (125MHz, D_2O, 25°C)



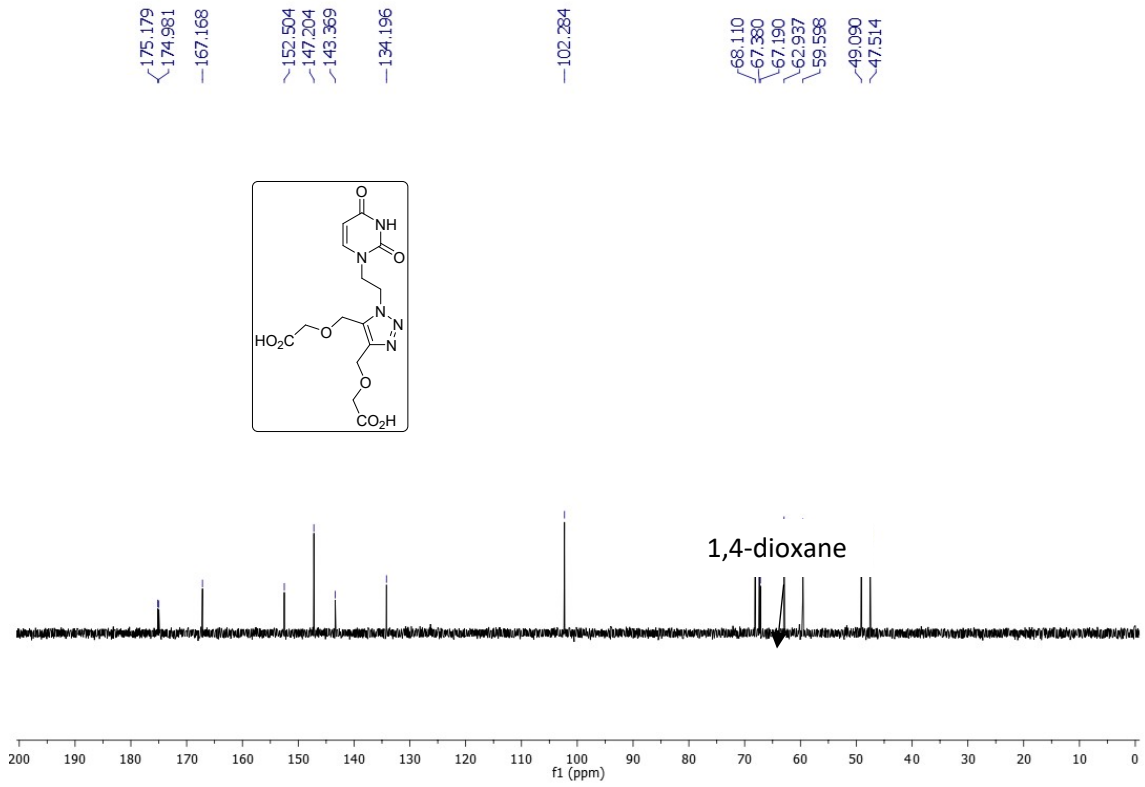
DEPT-135 spectra of compound **8g'** (125MHz, D_2O, 25°C)



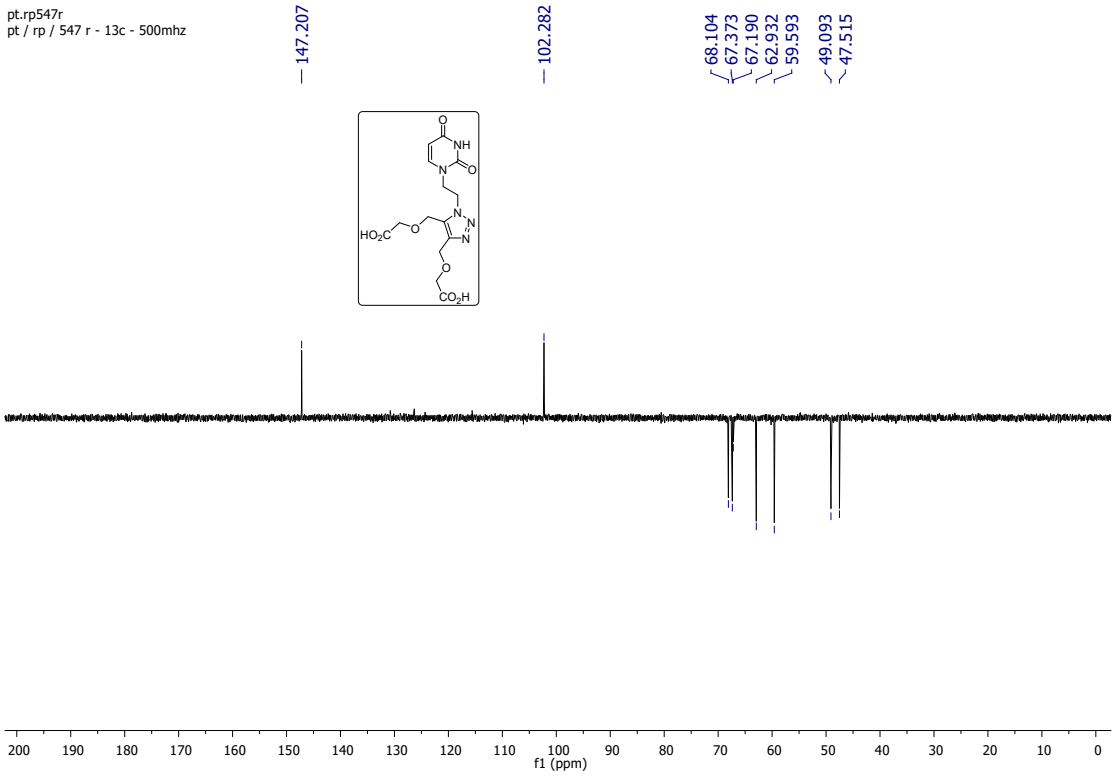
^{1}H-NMR spectra of compound **8h** (500MHz, D_2O, 25°C)



^{13}C-NMR spectra of compound **8h** (125MHz, D_2O, 25^{o}C)



DEPT-135 spectra of compound **8h** (125MHz, D_2O, 25°C)



11. Lineweaver-Burk Plots of all the Synthesized Inhibitors (Fig. S1-S2)

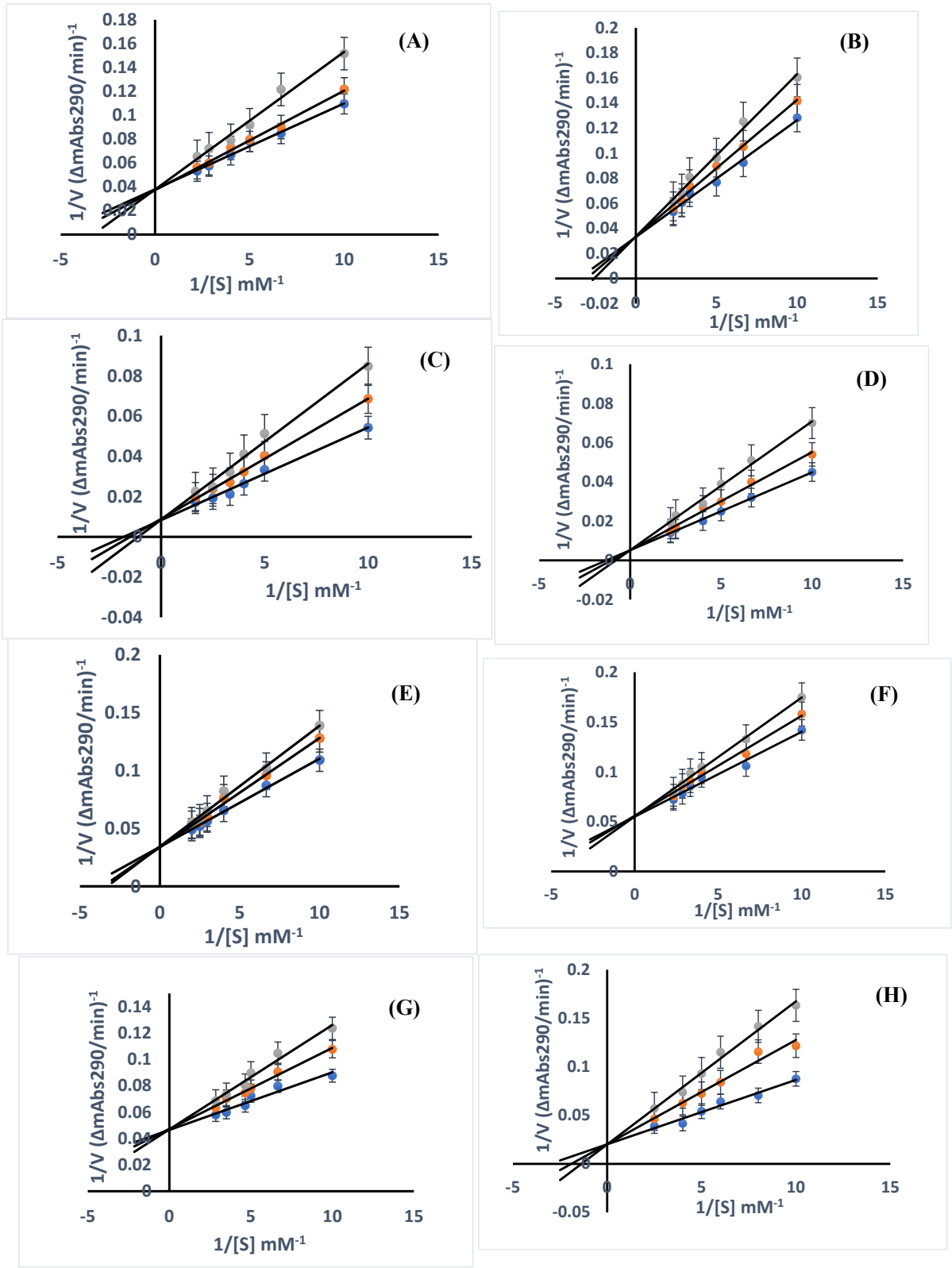


Fig. S1 Lineweaver-Burk plots for inhibition of RNase A by **(A)** inhibitor **6a** at different concentrations [0.00 µM (●), 6.00 µM (●) and 12.00 µM (●)] with 2′,3′ -cCMP (0.10–0.43 mM) and RNase A (10 µM); **(B)** inhibitor **6b** at different concentrations [0.00 µM (●), 6.00 µM (●) and 12.00 µM (●)] with 2′,3′ -cCMP (0.10–0.45 mM) and RNase A (10 µM); **(C)** inhibitor **6c** at different concentrations [0.00 µM (●),6 µM (●) and 12 µM (●)] with 2′,3′ -cCMP (0.10–0.60 mM) and RNase A (10 µM); **(D)**

inhibitor **6d** at different concentrations [0.00 µM (●), 6 µM (●) and 12 µM (●)] with 2′,3′-cCMP (0.10–0.50 mM) and RNase A (10 µM); **(E)** inhibitor **6e** at different concentrations [0.00 µM (●), 6 µM (●) and 12 µM (●)] with 2′,3′-cCMP (0.10–0.43 mM) and RNase A (10 µM); **(F)** inhibitor **6f'** at different concentrations [0.00 µM (●), 6 µM (●) and 12 µM (●)] with 2′,3′-cCMP (0.10–0.35 mM) and RNase A (10 µM); **(G)** inhibitor **6g'** at different concentrations [0.00 µM (●), 6 µM (●) and 12 µM (●)] with 2′,3′-cCMP (0.10–0.40 mM) and RNase A (10 µM); **(H)** inhibitor **6h** at different concentrations [0.00 µM (●), 6 µM (●) and 12 µM (●)] with 2′,3′-cCMP (0.10–0.40 mM) and RNase A (10 µM); Error bars represent SD (n = 3).

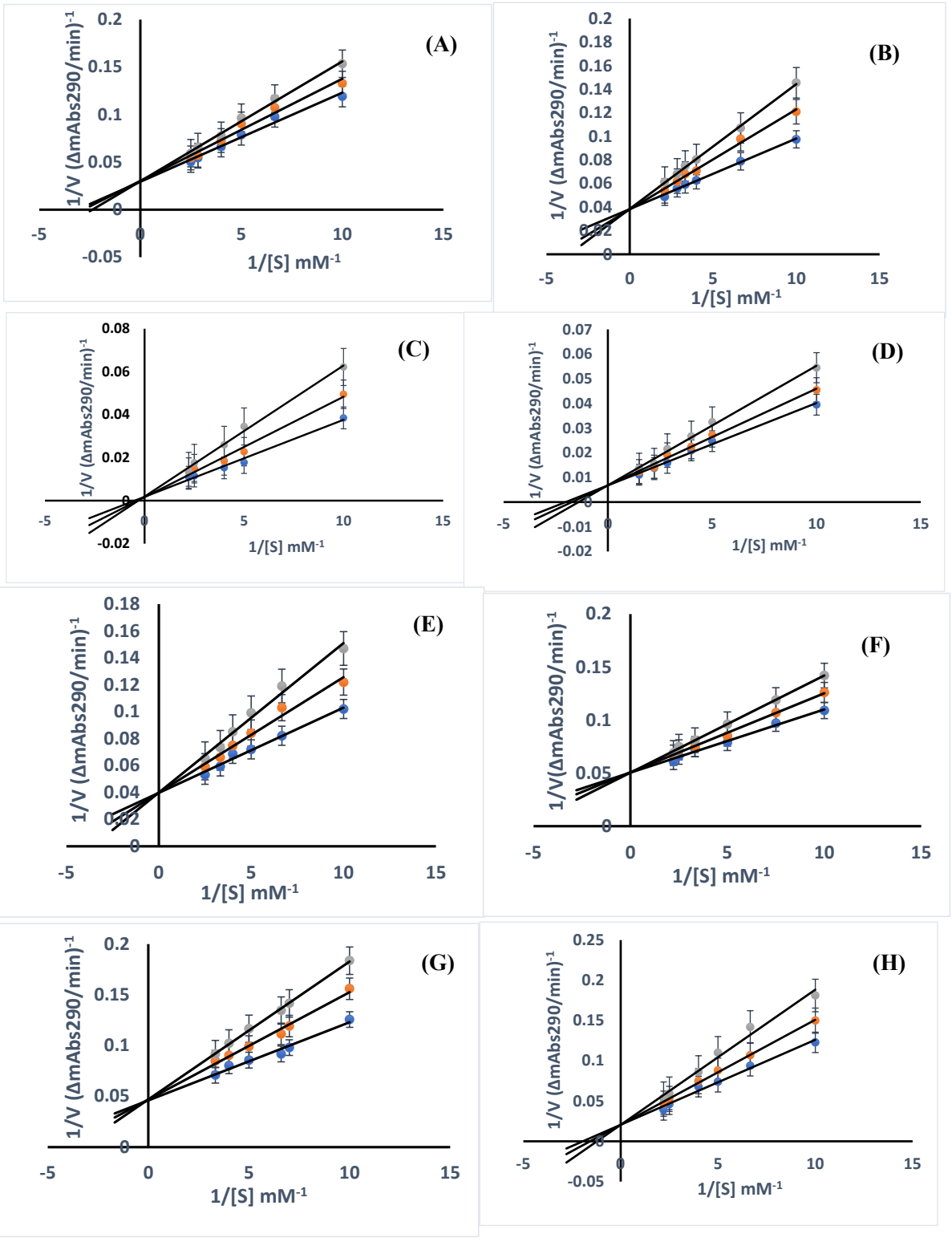


Fig. S2 Lineweaver-Burk plots for inhibition of RNase A by **(A)** inhibitor **8a** at different concentrations [0.00 µM (●), 6 µM (●) and 12 µM (●)] with 2′ ,3′ -cCMP (0.10–0.40 mM) and RNase A (10 µM); **(B)** inhibitor **8b** at different concentrations [0.00 µM (●), 6 µM (●) and 18 µM (●)] with 2′ ,3′ -cCMP (0.10–0.47 mM) and RNase A (10 µM); **(C)** inhibitor **8c** at different concentrations [0.00 µM (●), 6 µM (●) and 12 µM (●)] with 2′ ,3′ -cCMP (0.10–0.45 mM) and RNase A (10 µM); **(D)** inhibitor **8d** at different concentrations [0.00 µM (●), 6 µM (●) and 12 µM (●)] with 2′ ,3′ -cCMP (0.10–0.40 mM) and RNase A (10 µM); **(E)** inhibitor **8e** at different concentrations [0.00 µM (●), 6 µM (●) and 12 µM (●)] with

2′,3′ -cCMP (0.10–0.45 mM) and RNase A (10 µM); **(F)** inhibitor **8f′** at different concentrations [0.00 µM (●), 6 µM (●) and 12 µM (●)] with 2′ ,3′ -cCMP (0.10–0.30 mM) and RNase A (10 µM); **(G)** inhibitor **8g′** at different concentrations [0.00 µM (●),6 µM (●) and 12 µM (●)] with 2′ ,3′ -cCMP (0.10–0.45 mM) and RNase A (10 µM); **(H)** inhibitor **8h** at different concentrations [0.00 µM (●),6 µM (●) and 12 µM (●)] with 2′ ,3′ -cCMP (0.10–0.45 mM) and RNase A (10 µM); Error bars represent SD (n = 3).

12. Docked Poses of Synthesized Inhibitors (Fig. S3-S5)

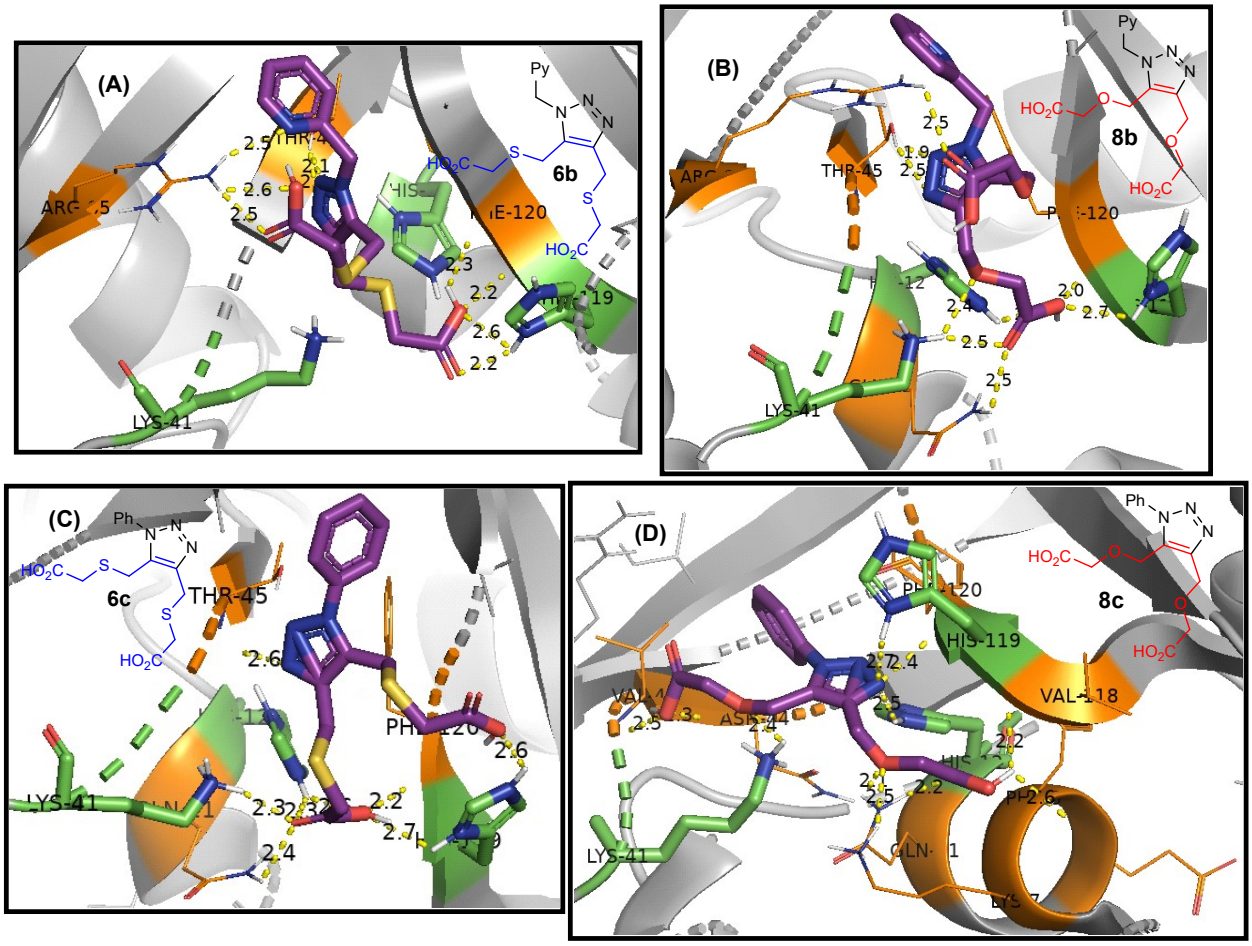


Fig. S3 Docked poses of (**A**) inhibitor **6b**, (**B**) inhibitor **8b**, (C) inhibitor **6c**, (**D**) inhibitor **8c** with RNaseA (PDB ID: 1FS3), where amino acid residues of active site and other subsites are highlighted in green and orange, respectively.

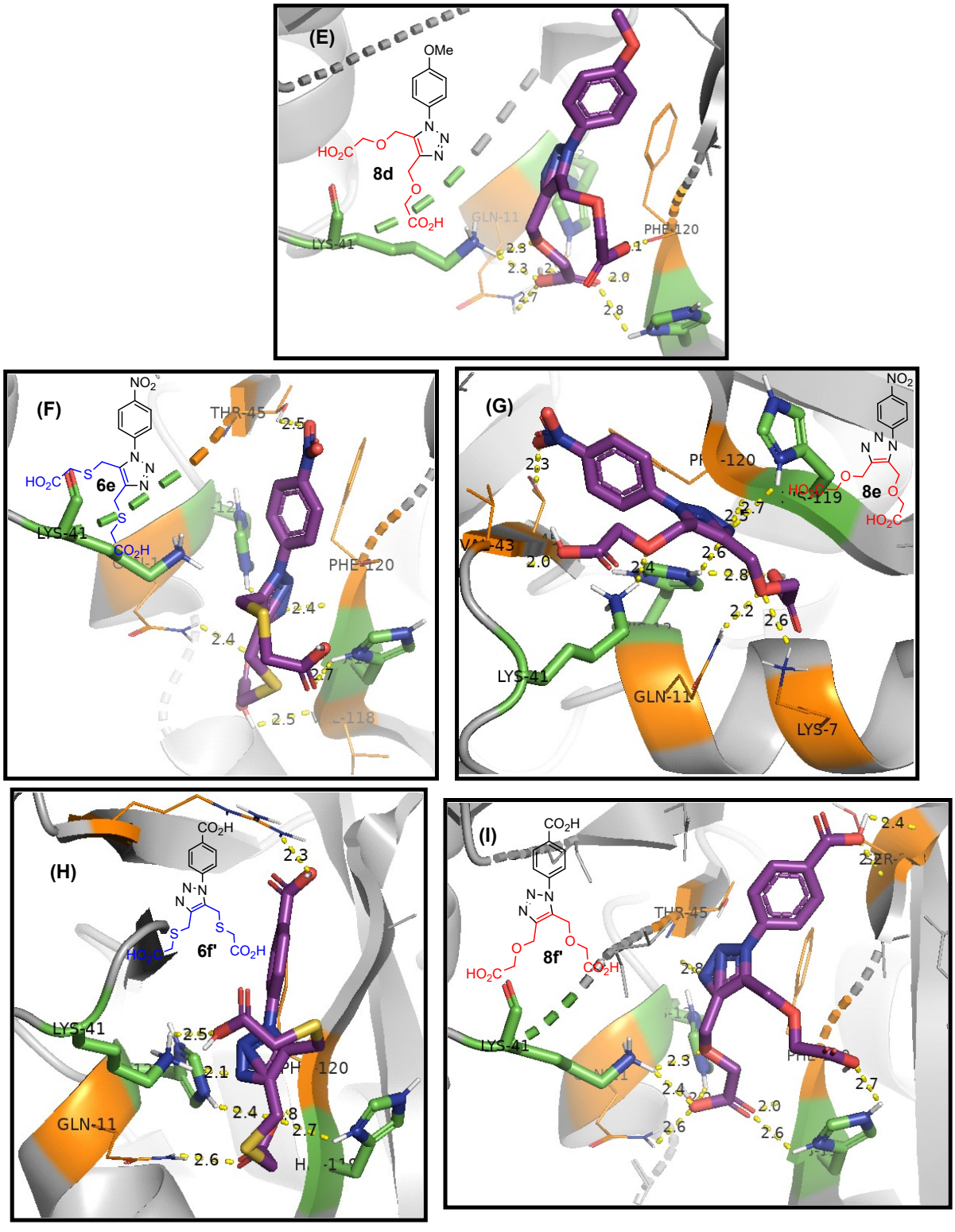


Fig. S4 Docked poses of (**E**) inhibitor **8d**, (**F**) inhibitor **6e**, (**G**) inhibitor **8e**, (**H**) inhibitor **6f' (I)** inhibitor **8f'** with RNaseA (PDB ID: 1FS3), where amino acid residues of active site and other subsites are highlighted in green and orange, respectively.

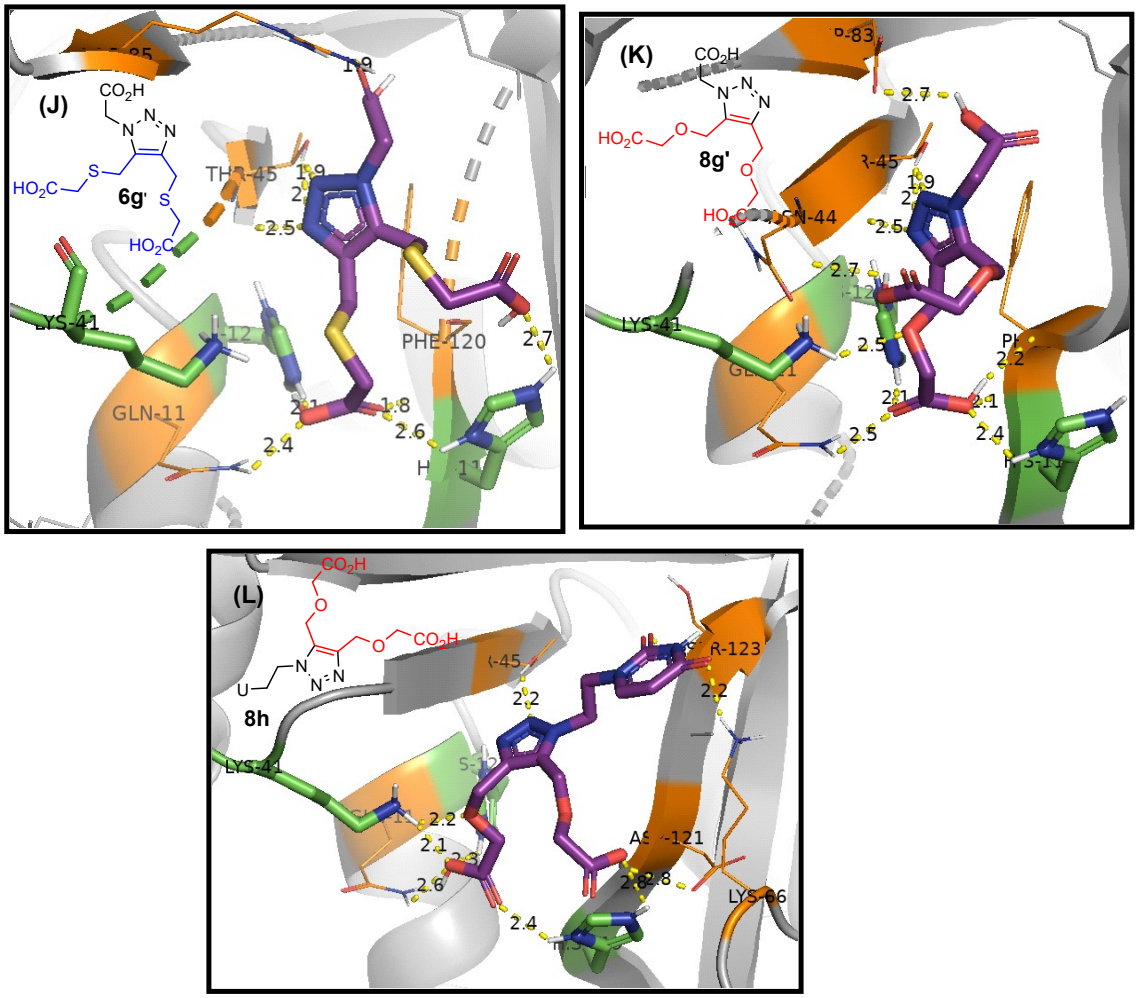


Fig. S5 Docked poses of (**J**) inhibitor **6g'**, (**K**) inhibitor **8g'**, (**L**) inhibitor **8h** with RNaseA (PDB ID: 1FS3), where amino acid residues of active site and other subsites are highlighted in green and orange, respectively.

13. Hydrogen Bonding Distances of all the Synthesized Inhibitors with Amino acid Residues of RNase A (Table S1-S4)

Table S1. Hydrogen bonding distance (Å) of compound **6a-d** with amino acid residues of RNase A (1FS3)

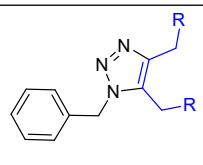
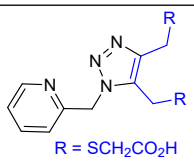
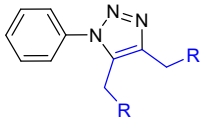
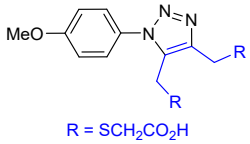
RNase A (1FS3)	 R = SCH_2CO_2H **6a**	 R = SCH_2CO_2H **6b**	 R = SCH_2CO_2H **6c**	 R = SCH_2CO_2H **6d**
Gln11 $NH\varepsilon^2$	2.3 [OH of -CO_2H]		2.4 [CO of -CO_2H]	2.5 [OH of -CO_2H]
His12 $NH\varepsilon^2$	2.2 [OH of -CO_2H]		2.3 [CO of -CO_2H] 2.6 [OH of -CO_2H]	2.3 [OH of -CO_2H]
Arg39 $NH\eta^1$	2.0 [OH of -CO_2H]			
Arg39 $NH\eta^2$	2.5 [OH of -CO_2H]			
Lys41 $NH\zeta$	2.4 [N2 of triazole] 2.1 [N3 of triazole]		2.3 [CO of -CO_2H]	2.3 [OH of -CO_2H]
Thr45 $NH\alpha$			2.6 [N3 of triazole]	2.6 [N3 of triazole]
Thr45 $OH\gamma^1$		2.1 [N2 of triazole] 2.4 [N3 of triazole]		
Arg85 $NH\eta^1$		2.5 [N of pyridine] 2.6 [OH of CO_2H] 2.5 [CO of CO_2H]		
His119 $NH\delta^1$	2.3 [CO of CO_2H]	2.2 [CO of CO_2H] 2.6 [OH of CO_2H]	2.7 [OH of -CO_2H]	2.7 [CO of -CO_2H]
His119 $NH\varepsilon^2$			2.6 [OH of -CO_2H]	2.6 [OH of -CO_2H]
His119 $NH\alpha$		2.2 [OH of CO_2H]		
Phe120 $NH\alpha$	2.2 [CO of CO_2H]		2.2 [OH of -CO_2H]	2.1 [CO of -CO_2H]
Phe120 $CO\alpha$		2.3 [OH of CO_2H]		
Asp121 $CO\gamma$				2.4 [OH of -CO_2H]
Asp121 $CO\alpha$				2.3 [OH of -CO_2H]

Table S2. Hydrogen bonding distance (Å) of compound **6e, 6f'-g', 6h** with amino acid residues of RNase A (1FS3)

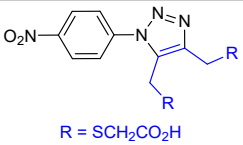
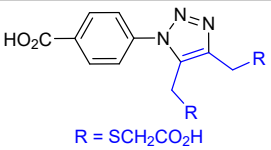
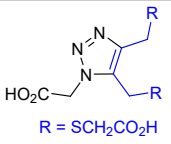
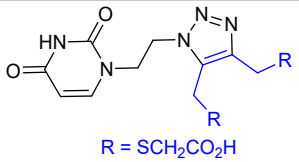
RNase A (1FS3)	 R = SCH_2CO_2H **6e**	 R = SCH_2CO_2H **6f'**	 R = SCH_2CO_2H **6g'**	 R = SCH_2CO_2H **6h**
Lys7 NHζ				2.1 [OH of -CO_2H]
Gln11 NHε^2	2.4 [CO of -CO_2H]	2.6 [CO of CO_2H]	2.4 [OH of CO_2H]	2.6 [CO of CO_2H]
His12 NHε^2	2.2 [N3 of triazole]	2.4 [OH of -CO_2H]	2.1 [OH of CO_2H]	2.1 [CO of CO_2H] 2.5 [OH of -CO_2H]
Lys41 NHζ		2.1 [N3 of triazole] 2.5 [OH of -CO_2H]		2.4 [CO of CO_2H] 2.8 [N2 of triazole]
Thr45 OHγ^1			1.9 [N2 of triazole] 2.4 [N3 of triazole]	2.1 [O2 of uracil]
Thr45 Oγ^1	2.5 [O of NO_2]			2.3 [NH of uracil]
Thr45 NHα			2.5 [N3 of triazole]	2.3 [O2 of uracil]
Arg85 NHη^1		2.3 [OH of -CO_2H of phenyl]	1.9 [CO of -CH_2CO_2H]	2.6 [O4 of uracil]
Val118 COα	2.5 [OH of CO_2H]			
His119 NHδ^1	2.7 [CO of CO_2H]	2.7 [OH of -CO_2H]	2.6 [CO of CO_2H]	2.8 [OH of -CO_2H]
His119 NHε^2			2.7 [OH of CO_2H]	
Phe120 NHα	2.4 [N3 of triazole]	1.8 [OH of -CO_2H]	1.8 [CO of CO_2H]	2.0 [OH of -CO_2H]

Table S3. Hydrogen bonding distance (Å) of compound **8a-d** with amino acid residues of RNase A (1FS3)

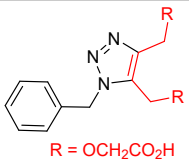
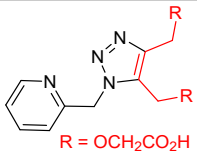
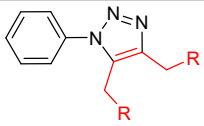
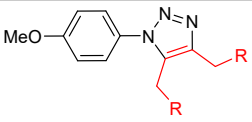
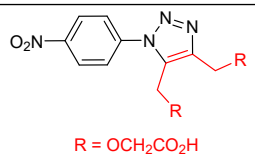
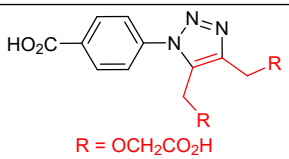
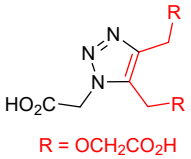
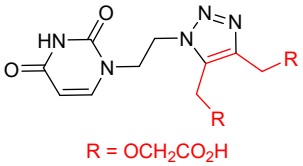
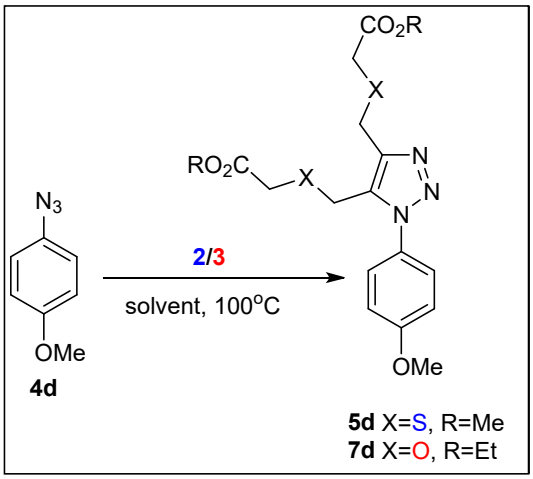
RNase A (1FS3)	 R = OCH_2CO_2H **8a**	 R = OCH_2CO_2H **8b**	 R = OCH_2CO_2H **8c**	 R = OCH_2CO_2H **8d**
Lys7 NHζ			2.5 [O of 'CH_2O']	
Phe8 COα			2.6 [OH of -CO_2H]	
Gln11 NHε^2	2.5 [OH of -CO_2H]	2.5 [CO of -CO_2H]	2.1 [O of 'CH_2O'] 2.2 [CO of -CO_2H]	2.7 [OH of -CO_2H]
His12 NHε^2	2.0 [OH of -CO_2H]	2.5 [OH of -CO_2H]	2.5 [N1 of triazole]	2.3 [OH of -CO_2H]
Arg39 NHη^1	2.4 [CO of -CO_2H]			
Arg39 NHη^2	2.1 [CO of -CO_2H]			
Lys41 NHζ	2.6 [O of 'CH_2O']	2.4 [O of 'CH_2O'] 2.5 [CO of -CO_2H]	2.4 [O of 'CH_2O']	2.3 [O of 'CH_2O'] 2.3 [OH of -CO_2H]
Val43 NHα			2.5 [OH of -CO_2H]	
Asn44 COα			2.3 [OH of -CO_2H]	
Thr45 NHα				
Thr45 OHγ^1		1.9 [N2 of triazole] 2.5 [N3 of triazole]		
Arg85 NHη^1		2.5 [CO of -CO_2H]		
Val118 COα			2.2 [OH of -CO_2H]	
His119 NHδ^1	2.2 [CO of CO_2H]	2.7 [OH of CO_2H]	2.7 [N3 of triazole]	2.8 [CO of -CO_2H]
Phe120 NHα		2.0 [OH of CO_2H]	2.4 [N3 of triazole]	2.1 [CO of -CO_2H] 2.0 [CO of -CO_2H]

Table S4. Hydrogen bonding distance (Å) of compound **8e, 8f'-g', 8h** with amino acid residues of RNase A (1FS3)

RNase A (1FS3)	 **8e**	 **8f'**	 **8g'**	 **8h**
Lys7 NHζ	2.6 [O of 'CH$_2$O']			
Gln11 NHε^2	2.2 [O of 'CH$_2$O']	2.6 [OH of CO$_2$H]	2.5 [CO of -CO$_2$H]	2.6 [OH of CO$_2$H]
His12 NHε^2	2.6 [N3 of triazole] 2.8 [OH of -CO$_2$H]	2.2 [OH of -CO$_2$H]	2.1 [CO of -CO$_2$H]	2.3 [OH of -CO$_2$H]
Lys41 NHζ	2.4 [O of 'CH$_2$O']	2.3 [O of 'CH$_2$O'] 2.4 [OH of -CO$_2$H]	2.5 [O of 'CH$_2$O']	2.1 [OH of -CO$_2$H] 2.2 [O of 'CH$_2$O']
Val43 NHα	2.0 [OH of -CO$_2$H]			
Asn44 NHα			2.5 [N3 of triazole]	
Asn44 COα			2.7 [OH of -CO$_2$H]	
Thr45 NHα	2.3 [O of NO$_2$]			
Thr45 OHγ^1			1.9 [N2 of triazole] 2.3 [N3 of triazole]	2.2 [N2 of triazole]
Lys66 NHζ				2.2 [O4 of uracil]
Asp83 COγ			2.7 [OH of -CH$_2$CO$_2$H]	
His119 NHδ^1	2.7 [N3 of triazole]	2.6 [CO of -CO$_2$H]	2.4 [OH of -CO$_2$H]	2.4 [CO of -CO$_2$H]
His119 NHε^2		2.7 [OH of -CO$_2$H]		2.8 [OH of -CO$_2$H]
Phe120 NHα	2.5 [N3 of triazole]	2.0 [CO of -CO$_2$H]	2.1 [OH of CO$_2$H]	
Phe120 COα			2.2 [OH of CO$_2$H]	
Asp121 COγ				2.8 [OH of -CO$_2$H]
Ser123 NHα		2.2 [OH of -CO$_2$H of phenyl]		2.3 [O2 of uracil]
Ser123 COα		2.4 [OH of -CO$_2$H of phenyl]		

14. A Comparative Study of the Synthesis of Triazoles 5d and 7d in the Absence and Presence of Solvents



Scheme 3S Synthesis of **5d** and **7d** in different solvent medium

Synthesis of compound 5d in absence and presence of solvent

Entry	Solvent	Time (h)	Yield (%)
1	Without solvent	12	80%
2	DMF	24	70%
3	Water	33	62%
4	Toluene	36	57%

Synthesis of compound 7d in absence and presence of solvent

Entry	Solvent	Time (h)	Yield (%)
1	Without solvent	10	75%
2	DMF	20	72%
3	Water	32	67%
4	Toluene	36	55%