**Electronic supplementary materials** *Mendeleev Commun.*, 2023, **33**, 325–327

# A new way of synthesizing heterocyclic primary sulfonamide probes for carbonic anhydrase

**Vasilisa Krivovicheva, Andrey Bubyrev, Stanislav Kalinin, Dmitry Dar'in, Maxim Gureev, Daniela Vullo, Mikhail Krasavin, Mikhail Korsakov and Claudiu T. Supuran**

## Table of Contents

## Experimental Procedures

### General information

NMR spectroscopic data were recorded with a Bruker Avance III 400 MHz spectrometer (400.13 MHz for 1 H, 100.61 MHz for $^{13}C\{^1H\}$ and 376.50 MHz for $^{19}F\{^1H\}$), and with a Bruker Avance III 500 MHz spectrometer (125.73 MHz for $^{13}C\{^1H\}$) in $CDCl_3$, Acetone-$d_6$ and DMSO-$d_6$ and were referenced to residual solvent proton signals ($\delta_H$ = 7.26, 2.05 and 2.50, respectively) and solvent carbon signals ($\delta_C$ = 77.16, 29.84 and 39.52, respectively). Melting points were determined with a melting point apparatus REACH Devices RD-MP in the open capillary tubes. HRMS were recorded using a Shimadzu LCMS9030 Q-TOF spectrometer (ionization by electrospray, positive and negative detection). The solvents were dried according to standard protocols. Single crystal X-ray data were obtained using an Agilent Technologies SuperNova Atlas and an Agilent Technologies Xcalibur Eos diffractometers at a temperature of 100 K. Column chromatography was carried out with silica gel grade 60 (0.040−0.063 mm) 230−400 mesh. TLC was performed on aluminum-backed pre-coated plates with silica gel 60 $F_{254}$ with suitable solvent system and was visualized using UV fluorescence. An oil bath was used for all the reactions required heating.

### Synthetic procedure for the preparation of 2

*N,N-Bis(4-methoxybenzyl)methanesulfonamide (**4**)*

To a stirred ice-cooled solution of bis(4-methoxybenzyl)amine **3** (14.5 g, 1 equiv., 56.5 mmol) and triethylamine (9.4 mL, 1.25 equiv., 70.6 mmol) in THF (55 mL), a solution of methanesulfonyl chloride (4.6 mL, 1.05 equiv., 59.3 mmol) in THF (20 mL) was added dropwise during 30 minutes at 0 °C. A formation of white precipitate was observed. The reaction mixture was removed from the ice bath and stirring was continued for 24 hours until full conversion was observed (TLC *n*-hexane/ethyl acetate = 8/2). The precipitate was filtered and washed with THF. The filtrate was evaporated and a light-yellow solid residue was recrystallized from *n*-hexane/chloroform in order to afford compound **4**. Yield: 14.3 g (76 %). White solid; mp 102–104 °C. $^1H$ NMR (400 MHz, $CDCl_3$) δ 7.24 (d, *J* = 8.6 Hz, 4H), 6.89 (d, *J* = 8.6 Hz, 4H), 4.26 (s, 4H), 3.81 (s, 6H), 2.73 (s, 3H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 159.5, 130.2, 127.6, 114.2, 55.4, 49.2, 40.3. HRMS (ESI) *m/z*: $[M+Na]^+$ Calc. for $C_{17}H_{21}NNaO_4S$ 358.1083; Found 358.1087.

*N,N-Bis(4-methoxybenzyl)-2-oxopropane-1-sulfonamide (**5**)*

A 250 mL Schlenk tube fitted with a rubber septum was charged with dry THF (100 mL) and HMDS (23.2 mL, 2.6 equiv., 110.6 mmol), and then cooled to –30 °C under an inert atmosphere while 0.89 M solution of *n*-butyllithium in *n*-hexane (105.2 mL, 2.2 equiv., 93.6 mmol) was added dropwise over 3 min. After 20 min, the resulting solution was cooled to –84 °C and a solution of compound **4** (14.3 g, 1 equiv., 42.5 mmol) in dry THF (50 mL) was added dropwise over 10 min. The bright red reaction mixture was stirred at –84 °C for 1 hour, and a solution of ethyl acetate (6.7 mL, 1.6 equiv., 68.1 mmol) in THF (20 mL) was added dropwise over 5 minutes. After 40 min full consumption of the starting material was observed (TLC *n*-hexane/DCM/acetone = 5/5/1). The white reaction mixture was quenched by the addition of 1 M HCl and semi-saturated sodium chloride solution (70 mL). The aqueous layer was separated and extracted with ethyl acetate (30 mL). The combined organic layers were washed with brine (90 mL), dried over anhydrous $Na_2SO_4$, filtered, and concentrated under reduced pressure to afford 16.1 g of yellow oil. The crude product **5** was used in the next step without any further purification. Characterization sample was obtained by crystallization from *n*-hexane/DCM. White solid; mp 73-74 °C. $^1H$ NMR (400 MHz, $CDCl_3$) δ 7.24 – 7.17 (m, 4H), 6.90 – 6.82 (m, 4H), 4.29 (s, 4H), 3.82 (s, 2H), 3.81 (s, 2H), 2.38 (s, 3H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 197.3, 159.6, 130.3, 127.3, 114.2, 64.7, 55.4, 50.3, 31.3. HRMS (ESI) *m/z*: $[M+Na]^+$ Calc. for $C_{19}H_{23}NNaO_5S$ 400.1189; Found 400.1193.

*1-Diazo-N,N-bis(4-methoxybenzyl)-2-oxopropane-1-sulfonamide (**2**)*

To a solution of potassium carbonate (20.2 g, 3.4 equiv., 146.0 mmol) and sodium azide (7.3 g, 2.6 equiv., 112.3 mmol) in water (55 mL), 3-(chlorosulfonyl)benzoic acid (16.1 g, 1.72 equiv., 73.0 mmol) was added portionwise. The 'SAFE' cocktail was stirred for 10 min and added to a solution of compound **5** (16.1 g, 1 equiv., 42.5 mmol) in acetonitrile (40 mL). The stirred reaction mixture was left for 18 hours (TLC *n*-hexane/DCM/acetone = 5:5:1). The aqueous layer was extracted with chloroform (3×30 mL). The combined organic layers were washed with semi-saturated sodium chloride solution (80 mL) and brine (80 mL), dried over anhydrous $Na_2SO_4$, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, eluent: *n*-hexane/ethyl acetate, gradient from 0.5 to 1% of ethyl acetate). Yield over two steps: 15.2 g (89%). Yellow solid; mp 74-76 °C. $^1H$ NMR (400 MHz, $CDCl_3$) δ 7.22 – 7.13 (m, 4H), 6.90 – 6.81 (m, 4H), 4.42 (s, 4H), 3.81 (s, 6H), 2.13 (s, 3H). $^{13}C\{^1H\}$ (101 MHz, $CDCl_3$) δ 186.3, 159.7, 130.0, 127.3, 114.3, 55.5, 51.3, 26.7. HRMS (ESI) *m/z*: $[M+Na]^+$ Calc. for $C_{19}H_{21}N_3NaO_5S$ 426.1094; Found 426.1101.

**Synthetic procedure for the preparation of 7**

*1-Diazo-N,N-bis(4-methoxybenzyl)methanesulfonamide (**6**)*

To a stirred ice-cooled solution of diazo keto sulfonamide **2** (4.0 g, 1 equiv., 9.9 mmol) in dry methanol (35 mL), potassium carbonate (0.11 g, 8 mol %., 0.8 mmol) was added at 0 °C. The reaction mixture was stirred for 30 min. After full consumption of the starting material (TLC *n*-hexane/DCM/acetone = 5/5/1), the mixture was diluted with water and extracted with chloroform (1×30, 2×15 mL). The combined organic layers were washed with water and brine, dried over anhydrous $Na_2SO_4$, filtered, and concentrated under reduced pressure to afford CH-diazomethane sulfonamide, which was used without further purification. Yield: 3.4 g, 95%. Bright yellow solid; mp

74–76 °C. $^{1}$H NMR (400 MHz, $CDCl_3$) δ 7.24 (d, *J* = 8.6 Hz, 4H), 6.89 (d, *J* = 8.6 Hz, 4H), 4.78 (s, 1H), 4.24 (s, 4H), 3.82 (s, 6H). $^{13}$C{$^{1}$H} NMR (101 MHz, $CDCl_3$) δ 159.5, 130.2, 127.1, 114.2, 55.3, 54.8, 49.5. HRMS (ESI) *m/z*: $[M+Na]^+$ Calc. for $C_{17}H_{19}N_3NaO_4S$ 384.0988; Found 384.0992.

To a stirred ice-cooled CH-diazomethane sulfonamide (3.19 g, 8.8 mmol) without solvent, methyl propiolate (4 mL) was added at 0 °C. The reaction mixture was removed from the ice bath and stirred for 30 min until full conversion of **2** was observed (TLC *n*-hexane/ethyl acetate = 1:1, 2 times). The mixture triturated with diethyl ether (2×5 mL) and dried under reduced pressure to afford compound **6**. Yield: 3.10 g (78%). White solid; mp 152–153 °C. $^{1}$H NMR (400 MHz, $CDCl_3$) δ 11.32 (s, 1H), 7.11 – 7.02 (m, 5H), 6.85 – 6.72 (m, 4H), 4.33 (s, 4H), 3.96 (s, 3H), 3.78 (s, 6H). $^{13}$C{$^{1}$H} NMR (101 MHz, $CDCl_3$) δ 159.4, 130.2, 127.4, 114.0, 109.6, 55.4, 52.9, 50.2. HRMS (ESI) *m/z*: $[M+H]^+$ Calc. for $C_{21}H_{24}N_3O_6S$ 446.1380; Found 446.1385.

*Methyl 3-sulfamoyl-1H-pyrazole-5-carboxylate (**7**).*

A solution of compound **6** (0.22 mmol) in trifluoroacetic acid (2 mL) was stirred at 80 °C for 1 h. All volatiles were removed under vacuum, the residue was diluted with dry toluene and evaporated again. The residue was triturated with DCM (4×0.8 mL) and dried under reduced pressure to afford primary sulfonamide **7**. Yield: 36 mg (78%). White solid; mp 188–190 °C.$^{1}$H NMR (400 MHz, DMSO-$d_6$) δ 14.67 (s, 1H), 7.55 (s, 2H), 7.06 (s, 1H), 3.87 (s, 3H). $^{13}$C{$^{1}$H} NMR (126 MHz, DMSO-$d_6$) δ 158.8, 155.0, 134.4, 107.6, 52.4. HRMS (ESI) *m/z*: $[M-H]^-$ Calc. for $C_5H_6N_3O_4S$ 204.0084; Found 204.0084.

## Synthetic procedure for the preparation of 9

*N,N-Bis(4-methoxybenzyl)-5-methyl-1,2,3-thiadiazole-4-sulfonamide (**8**)*

A solution of compound **2** (158 mg, 1 equiv., 0.40 mmol) and the Lawesson's reagent (111 mg, 0.7 equiv., 0.27 mmol) in dry toluene (7 ml) was refluxed overnight. The reaction mixture was concentrated *in vacuo* and purified by column chromatography (silica gel, eluent n-hexane/acetone, gradient from 0 to 15% of acetone) to afford thiadiazole **8**. Yield: 137 mg (83 %). White oil. $^{1}$H NMR (400 MHz, CDCl3) δ 7.13 – 6.97 (m, 4H), 6.86 – 6.63 (m, 4H), 4.51 (s, 4H), 3.76 (s, 6H), 2.78 (s, 3H). $^{13}$C{$^{1}$H} NMR (101 MHz, $CDCl_3$) δ 159.3, 158.5, 155.3, 130.0, 127.2, 113.9, 55.3, 51.2, 10.4. HRMS (ESI) m/z: $[M+H]^+$ Calc. for $C_{19}H_{22}N_3O_4S_2$ 420.1046; Found 420.1048.

*5-Methyl-1,2,3-thiadiazole-4-sulfonamide (**9**)*

A solution of compound **8** (137 mg, 0.38 mmol) in trifluoroacetic acid (2 ml) was stirred at 80 °C for 1 h. All volatiles were removed under vacuum, the residue was diluted with dry toluene and evaporated again. The crude product was recrystallized from n-hexane/DCM to afford compound **9**. Yield: 43 mg (73%). White solid; mp 149–150 °C. $^{1}$H NMR (400 MHz, Acetone-$d_6$) δ 7.23 (s, 2H), 2.87 (s, 3H). $^{13}$C{$^{1}$H} NMR (101 MHz, Acetone-$d_6$) δ 160.9, 155.8, 10.3. HRMS (ESI) m/z: $[M - H]^-$ Calc. for $C_3H_4N_3O_2S_2$ 177.9750; Found 117.9745.

## Synthetic procedure for the preparation of 11

*Methyl 3-(N,N-bis(4-methoxybenzyl)sulfamoyl)-1-nonyl-1H-pyrazole-5-carboxylate(**10**)*

Compound **6** (80 mg, 1 equiv., 0.18 mmol), potassium carbonate (37 mg, 1.5 equiv., 0.27 mmol) and tetrabuthylammonium bromide (22 mg, 5 mol%, 0.01 mmol) were sonicated for 15 min, and 1-bromononane (377 µl, 11 equiv., 1.98 mmol) was added. The reaction mixture was stirred over 2 days, adsorbed onto silica and then purified by column chromatography (silica gel, eluent: *n*-hexane/ethyl acetate, gradient from 0.5 to 25% of ethyl acetate) to afford compound **10**. Yield: 52 mg (51%). White solid; mp 90–91 °C. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.14 – 7.00 (m, 5H), 6.83 – 6.66 (m, 4H), 4.57 (t, *J* = 7.5 Hz, 2H), 4.32 (s, 4H), 3.91 (s, 3H), 3.77 (s, 6H), 1.83 (p, *J* = 7.5 Hz, 2H), 1.41 – 1.19 (m, 12H), 0.88 (t, *J* = 6.5 Hz, 3H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 159.3, 150.2, 132.9, 130.2, 127.6, 114.3, 113.8, 112.1, 55.3, 53.0, 52.5, 49.9, 31.9, 30.6, 29.6, 29.3, 29.3, 26.6, 22.8, 14.2. HRMS (ESI) m/z: $[M+Na]^+$ Calc. for $C_{30}H_{41}N_3NaO_6S$ 594.2608; Found 594.2614.

*Methyl 1-nonyl-3-sulfamoyl-1H-pyrazole-5-carboxylate (**11**)*

A solution of compound **10** (39 mg, 0.07 mmol) in trifluoroacetic acid (2 ml) was stirred at 80 °C for 1 h. All volatiles were removed under vacuum, the residue was diluted with dry toluene and evaporated again. The residue was adsorbed onto silica and then purified by column chromatography (silica gel, eluent: chloroform/methanol, gradient from 0 to 0.5% of methanol) to afford compound **11**. Yield: 17 mg (75%). White-yellow solid; mp 89–90 °C. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.23 (s, 1H), 5.02 (s, 2H), 4.59 (t, *J* = 7.4 Hz, 2H), 3.91 (s, 3H), 1.85 (p, *J* = 7.4 Hz, 2H), 1.38 – 1.17 (m, 12H), 0.88 (t, *J* = 6.7 Hz, 3H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 159.2, 151.0, 133.4, 111.1, 53.2, 52.6, 31.9, 30.6, 29.5, 29.3, 29.2, 26.6, 22.8, 14.2. HRMS (ESI) m/z: $[M+Na]^+$ Calc. for $C_{14}H_{25}N_3NaO_4S$ 354.1458; Found 354.1458.

## Synthetic procedure for the preparation of 13

*Methyl 3-(N,N-bis(4-methoxybenzyl)sulfamoyl)-1-phenyl-1H-pyrazole-5-carboxylate (**12**)*

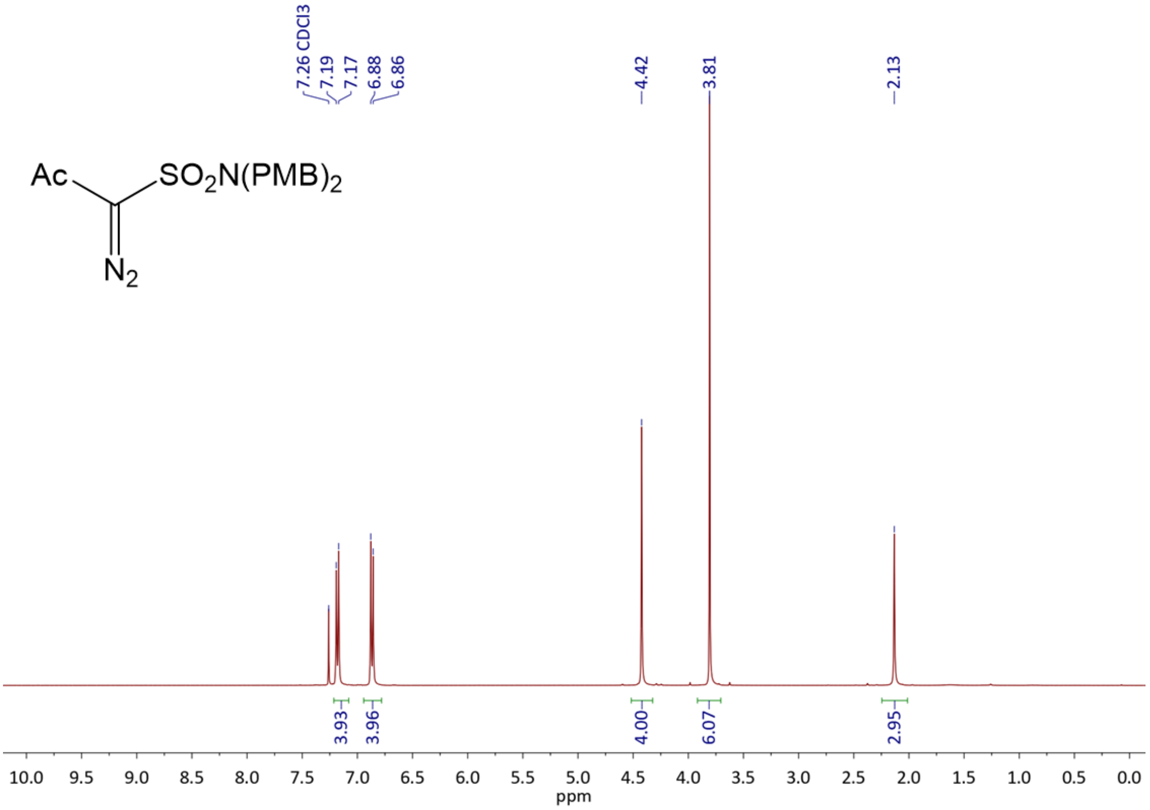
Compound **6** (90 mg, 1 equiv., 0.20 mmol), phenylboronic acid (74 mg, 3 equiv., 0.61 mmol), copper(II) acetate (83 mg, 2.25 equiv., 0.46 mmol), pyridine (49 µl, 3 equiv., 0.61 mmol) and molecular sieves 3 Å (175 mg) in dry DCM (7 ml) were stirred at room temperature with calcium chloride tube open to atmosphere for 9 days until full conversion was observed (TLC *n*-hexane/DCM/acetone = 5/5/1). The mixture was adsorbed onto celite® and purified by column chromatography (silica gel, eluent: *n*-hexane/ethyl acetate, gradient from 30 to 40% of ethyl acetate) to afford compound **12**. Yield: 96 mg (91%). White solid; mp 122–123 °C. $^{1}$H NMR (400 MHz, $CDCl_3$) δ 7.54 – 7.44 (m, 3H), 7.43 – 7.31 (m, 2H), 7.26 (s, 1H), 7.17 – 7.07 (m, 4H), 6.81 – 6.75 (m, 4H), 4.39 (s, 4H), 3.82 (s, 3H), 3.78 (s, 6H). $^{13}$C{$^{1}$H} NMR (101 MHz, $CDCl_3$) δ 159.4, 158.6, 152.4, 139.5, 134.2, 130.4, 129.7, 128.8, 127.5, 126.1, 114.2, 113.9, 112.8, 55.4, 52.6, 50.1. HRMS (ESI) m/z: $[M+Na]^+$ Calc. for $C_{27}H_{27}N_3NaO_6S$ 544.1513; Found 544.1520.

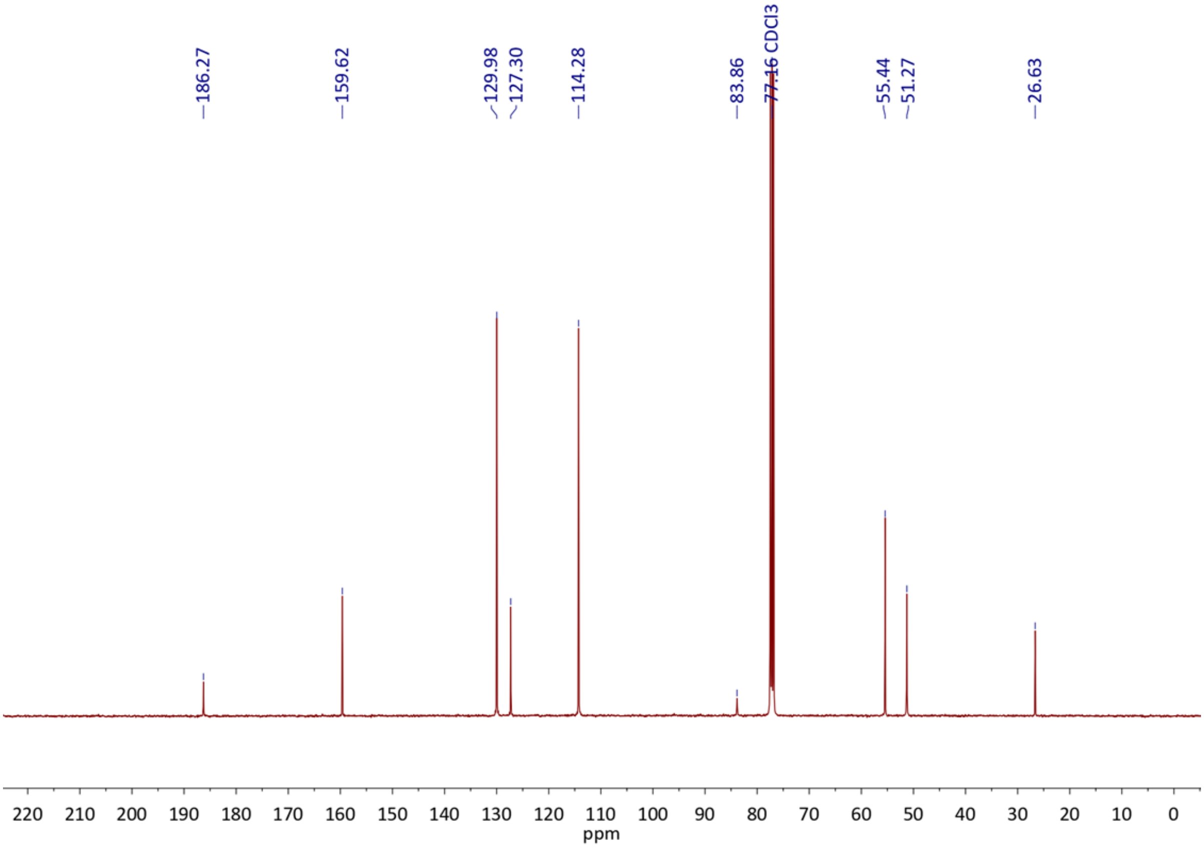
*Methyl 1-phenyl-3-sulfamoyl-1H-pyrazole-5-carboxylate (**13**)*

A solution of compound **12** (62 mg, 1 equiv., 0.12 mmol) in trifluoroacetic acid (2 ml) was stirred at 80 °C for 1 h. All volatiles were removed under vacuum, the residue was diluted with dry toluene and evaporated again. The residue was adsorbed onto silica and then purified by column chromatography (silica gel, eluent: chloroform/methanol, gradient from 0 to 1 % of methanol) to afford compound **13**. Yield: 27 mg (81 %). Orange semi-solid. $^{1}$H NMR (400 MHz, Acetone-$d_6$) δ 7.63 – 7.49 (m, 5H), 7.30 (s, 1H), 6.89 (s, 2H), 3.80 (s, 3H). $^{13}$C{$^{1}$H} NMR (101 MHz, acetone-$d_6$) δ 159.2, 155.4, 140.7, 135.5, 130.1, 129.6, 126.9, 111.7, 52.8. HRMS (ESI) m/z: $[M+Na]^+$ Calc. for $C_{11}H_{11}N_3NaO_4S$ 304.0362; Found 304.0360.

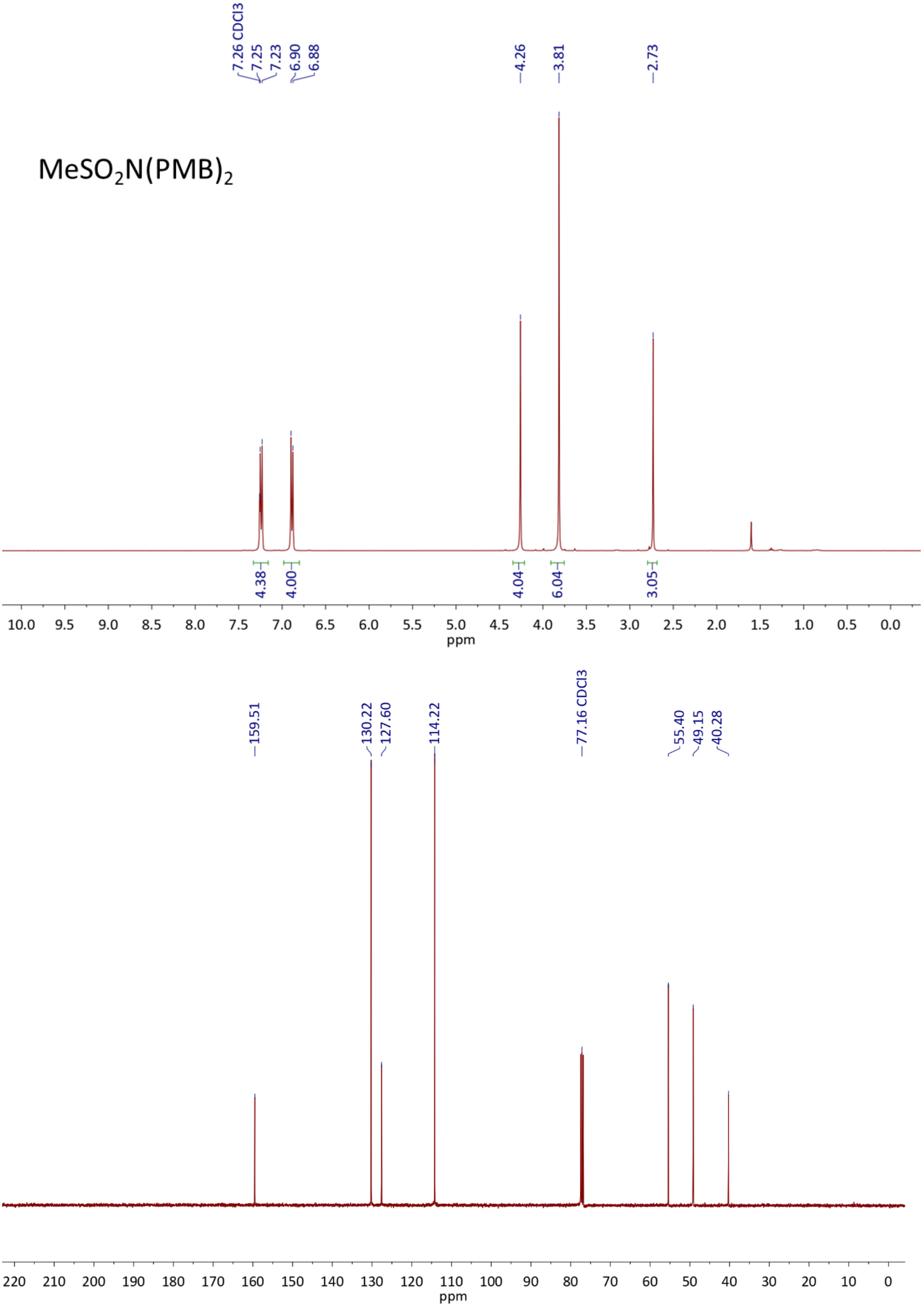
## NMR spectra

Copies of $^{1}H$ (400.13 MHz, $CDCl_3$) and $^{13}C\{^{1}H\}$ (100.61 MHz, $CDCl_3$) spectra of ***2***

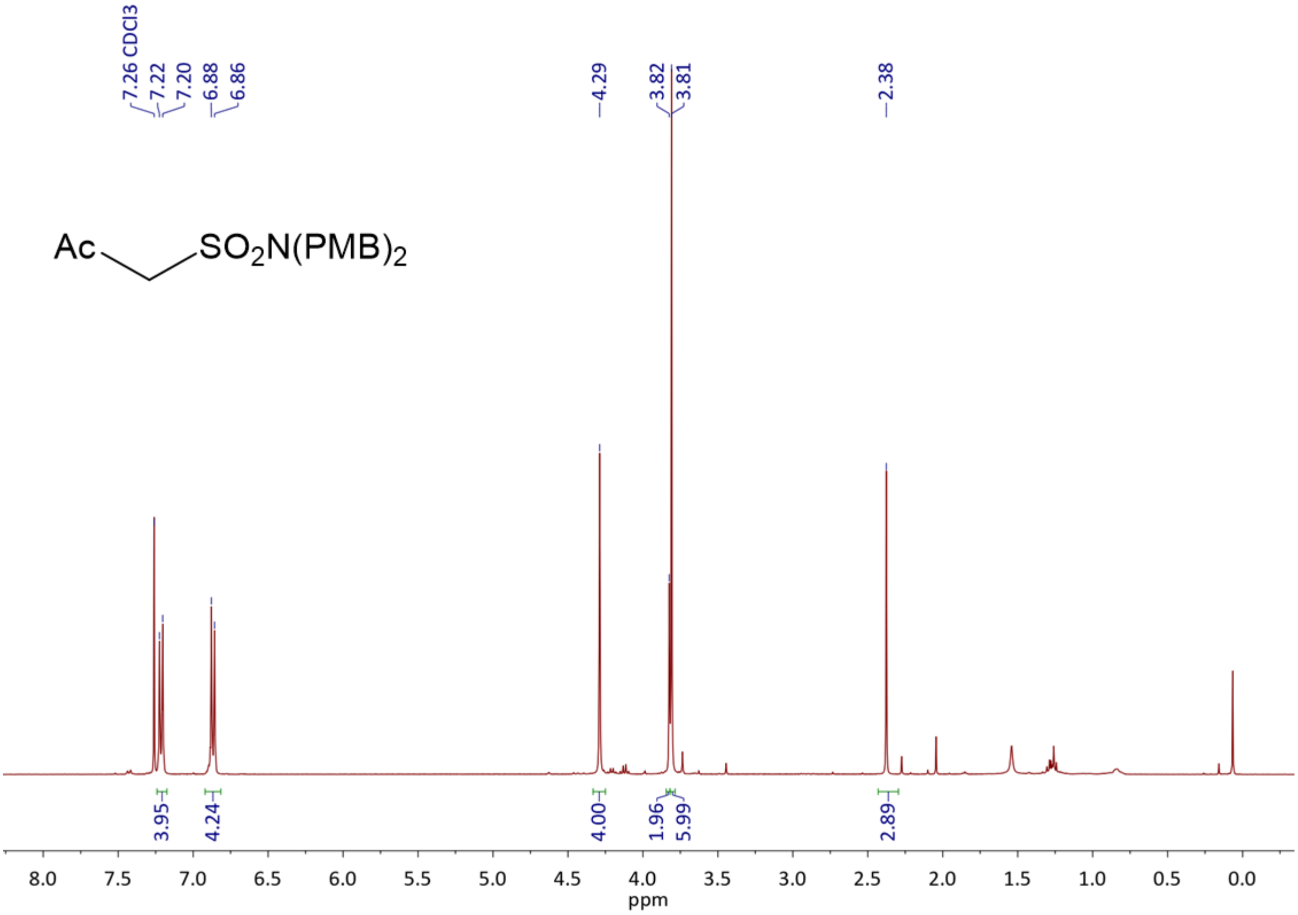


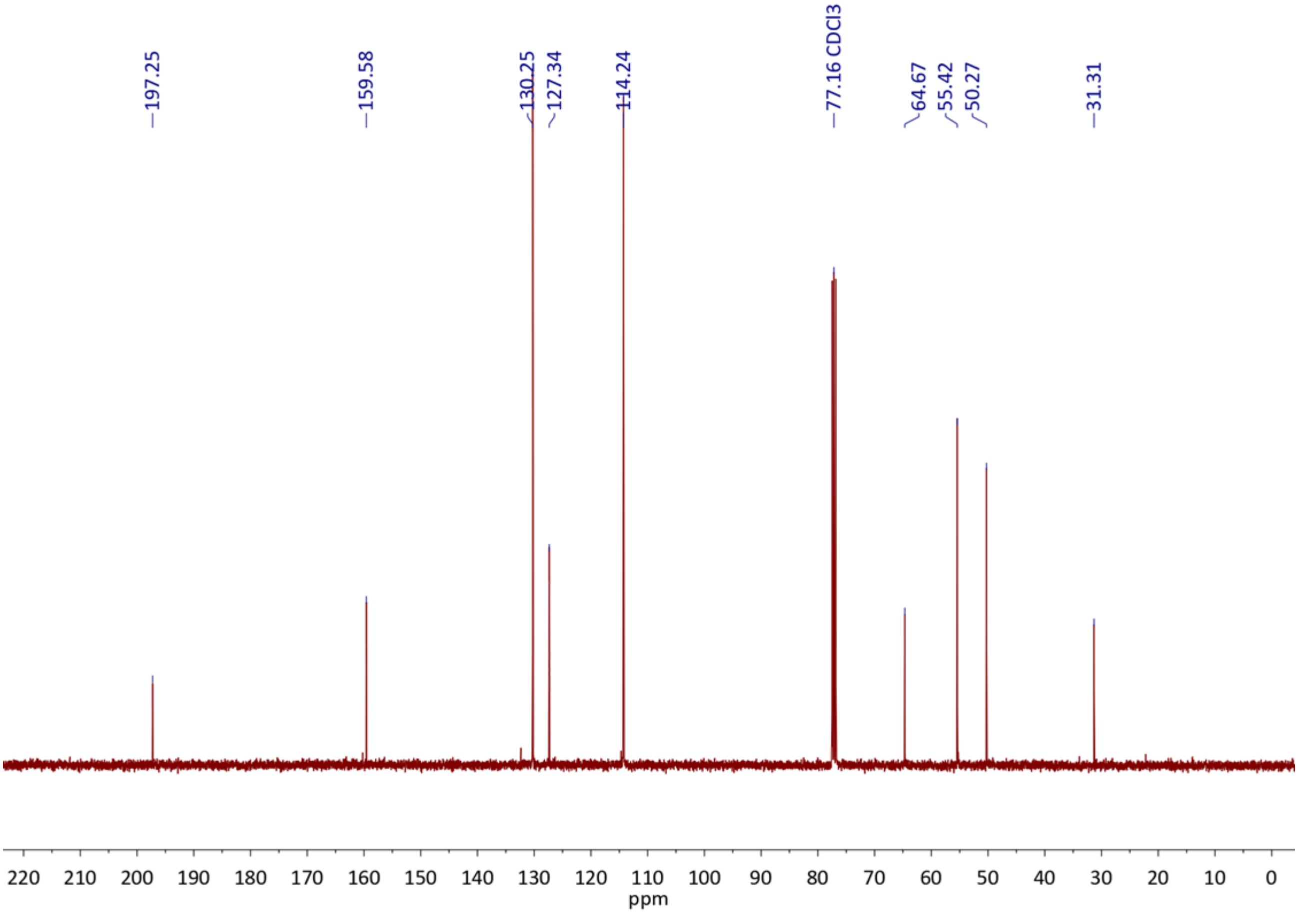


Copies of $^1H$ (400.13 MHz, $CDCl_3$) and $^{13}C\{^1H\}$ (100.61 MHz, $CDCl_3$) spectra of ***4***

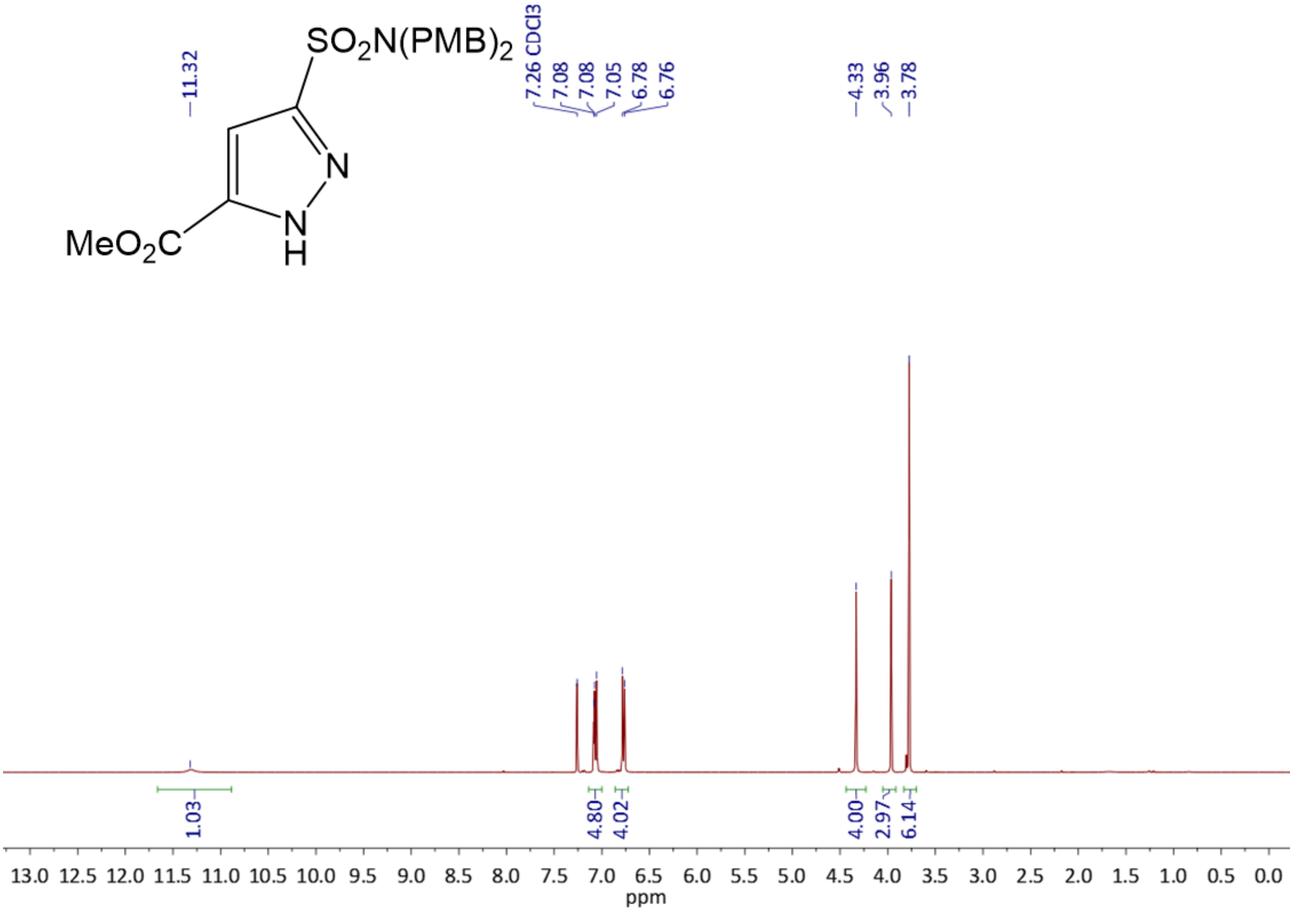


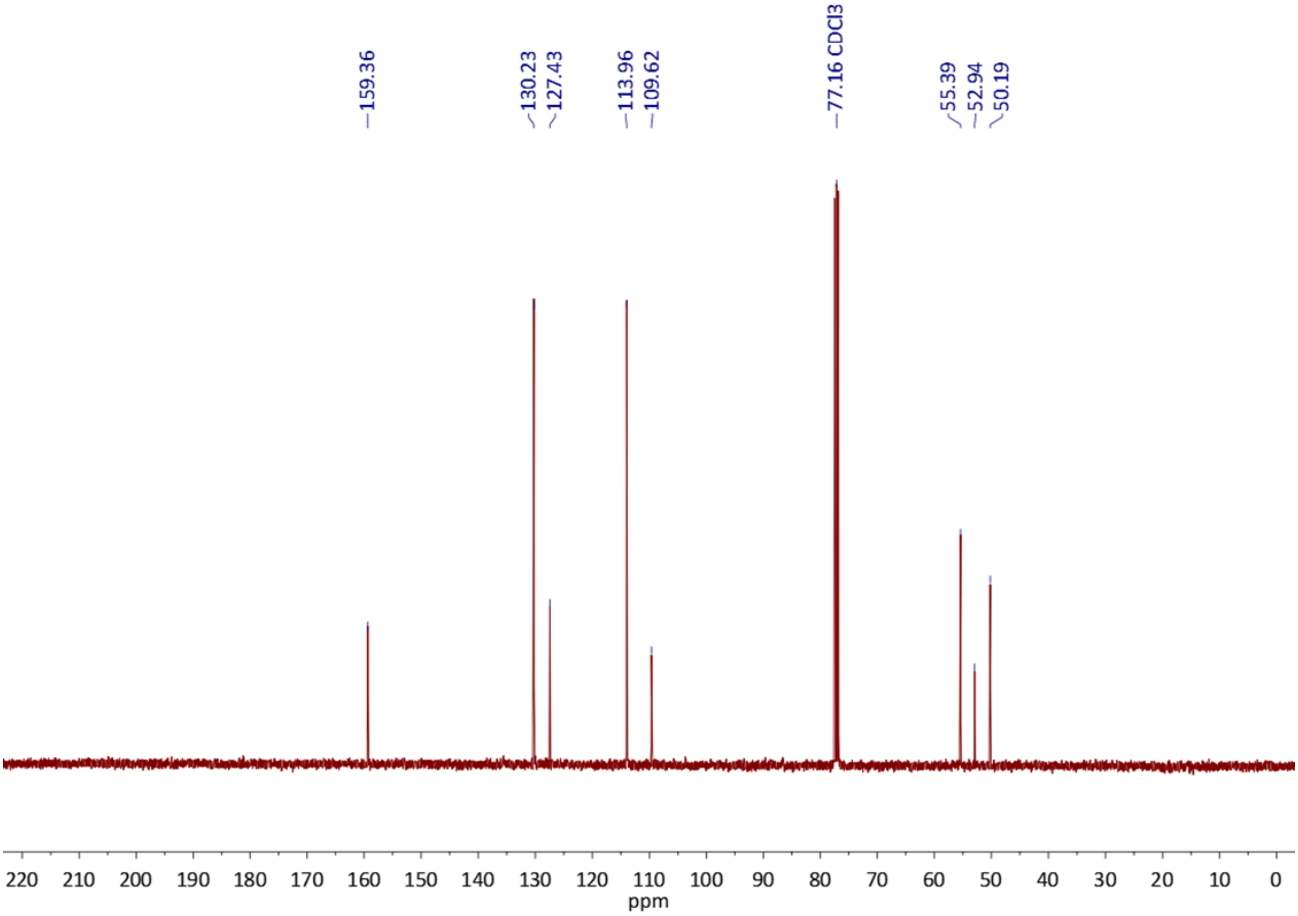
Copies of $^1$H (400.13 MHz, CDCl$_3$) and $^{13}$C{$^1$H} (100.61 MHz, CDCl$_3$) spectra of ***5***



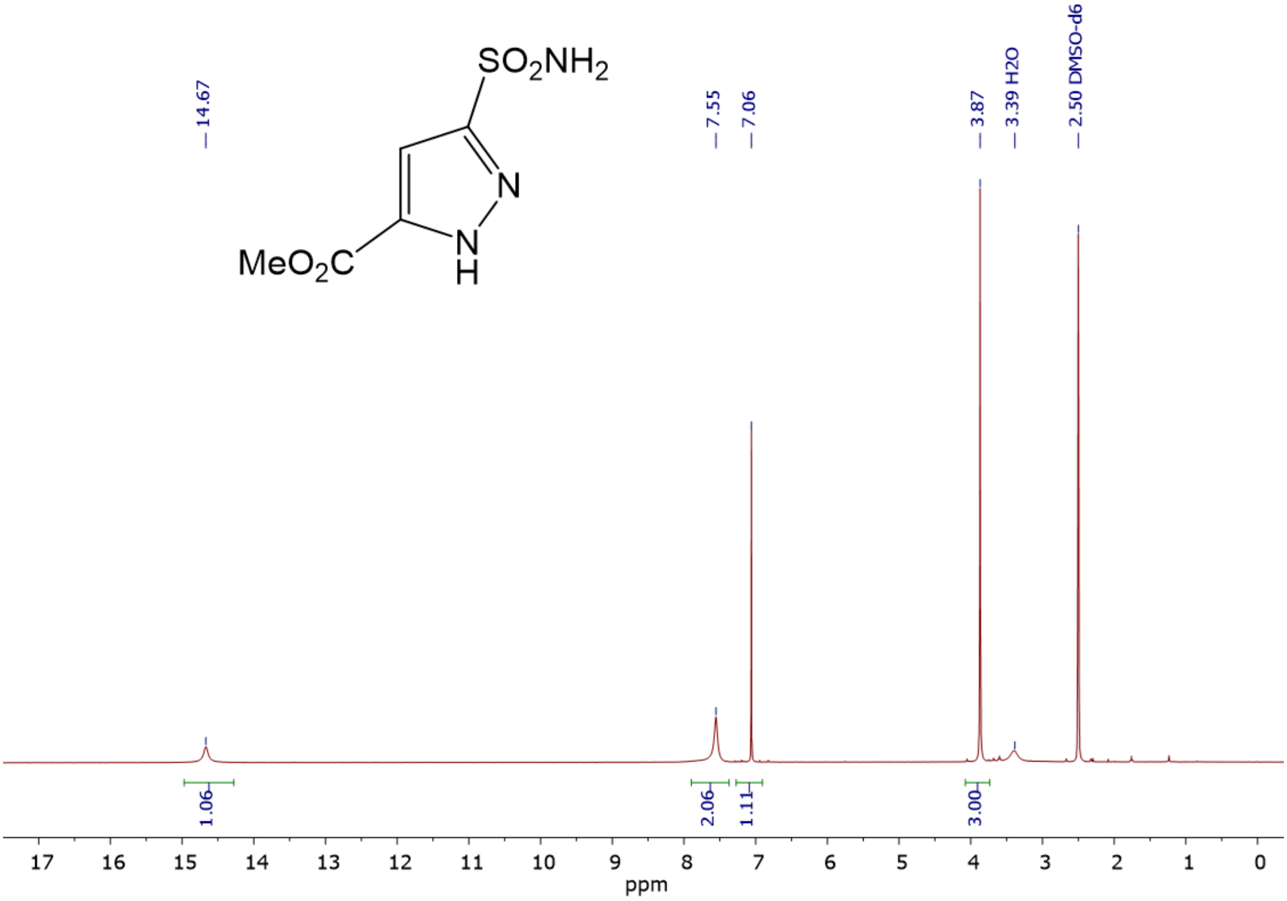


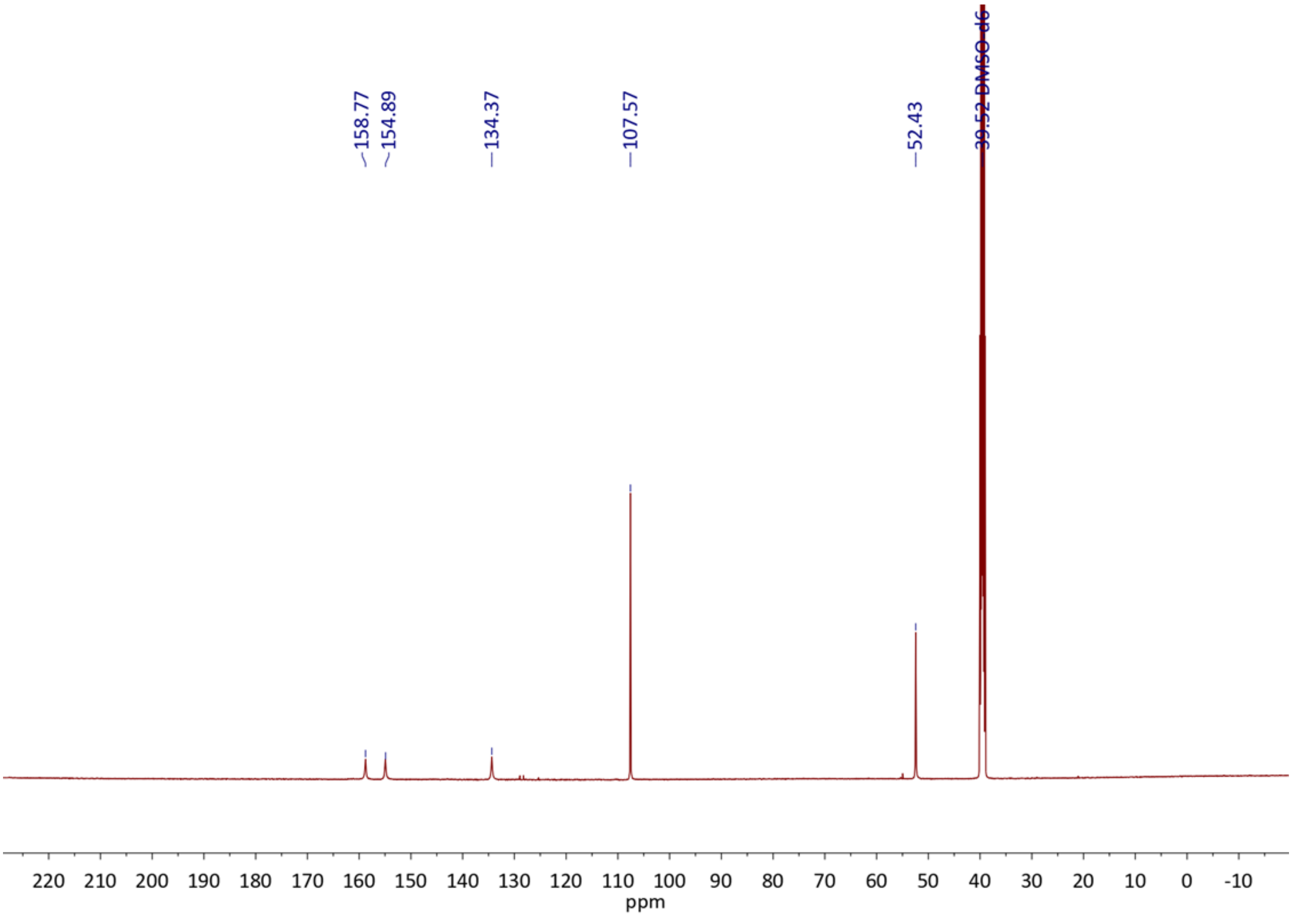
Copies of $^{1}$H (400.13 MHz, CDCl$_3$) and $^{13}$C{$^{1}$H} (100.61 MHz, CDCl$_3$) spectra of ***6***



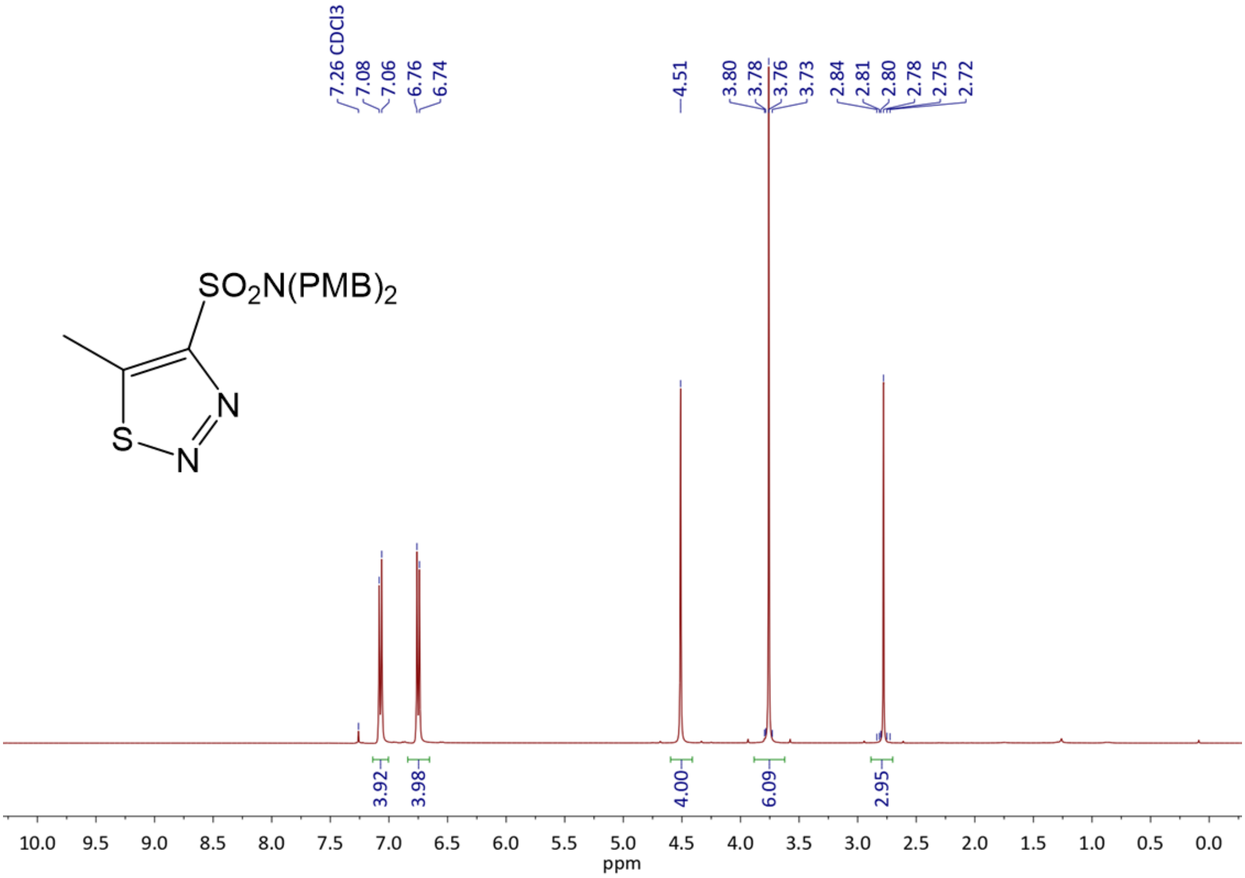


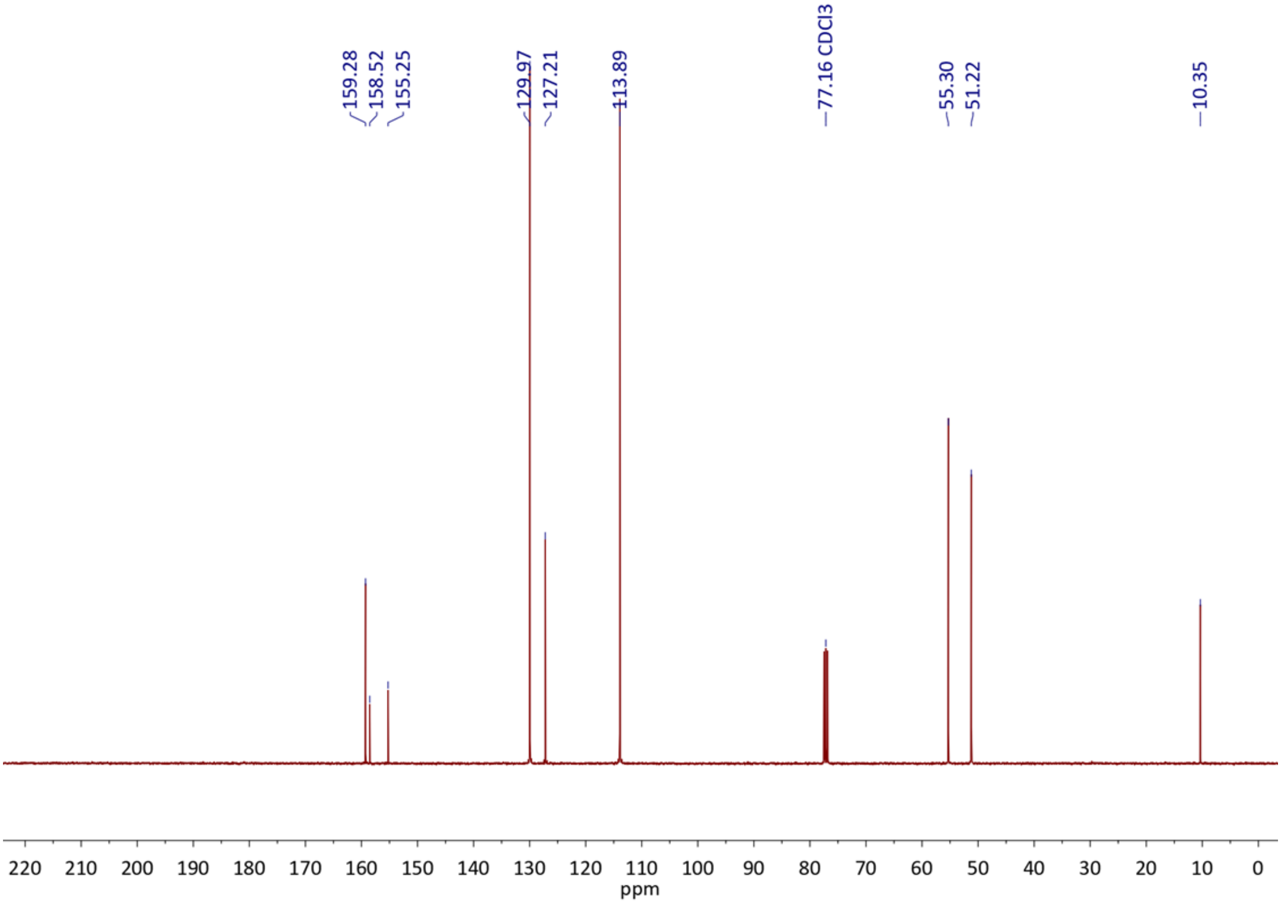
Copies of $^{1}$H (400.13 MHz, DMSO-*d*$_6$) and $^{13}$C{$^{1}$H} NMR (125.73 MHz, DMSO-*d*$_6$) spectra of ***7***



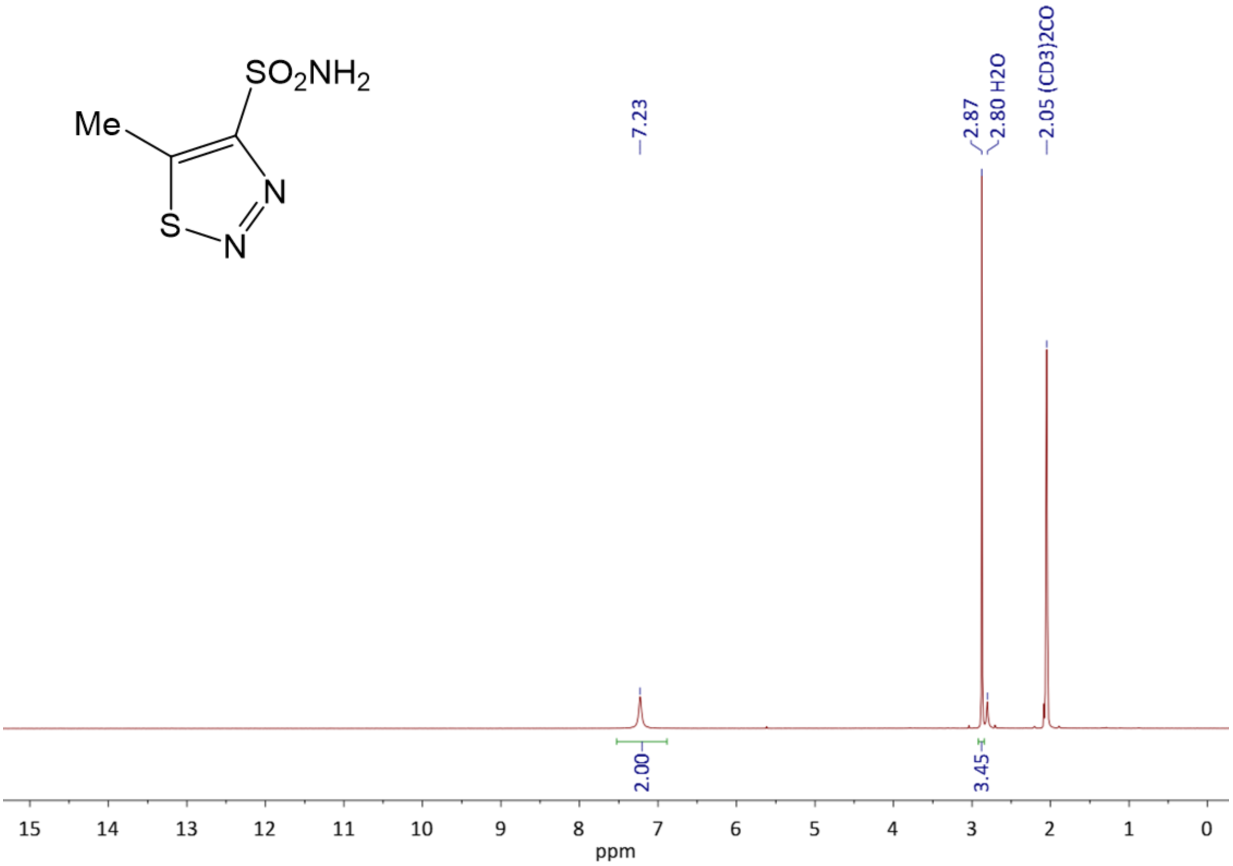


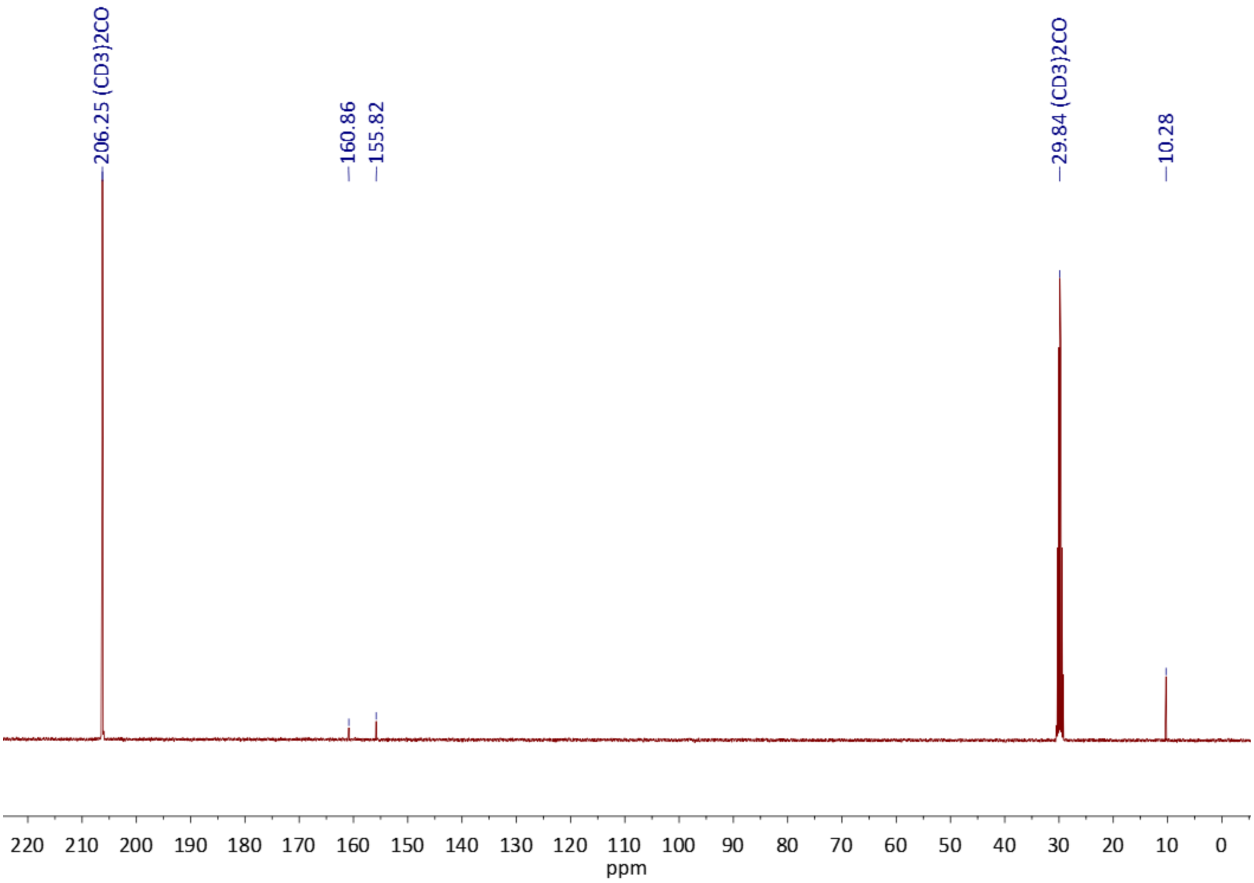
Copies of $^1$H (400.13 MHz, CDCl$_3$) and $^{13}$C{$^1$H} (100.61 MHz, CDCl$_3$) spectra of ***8***



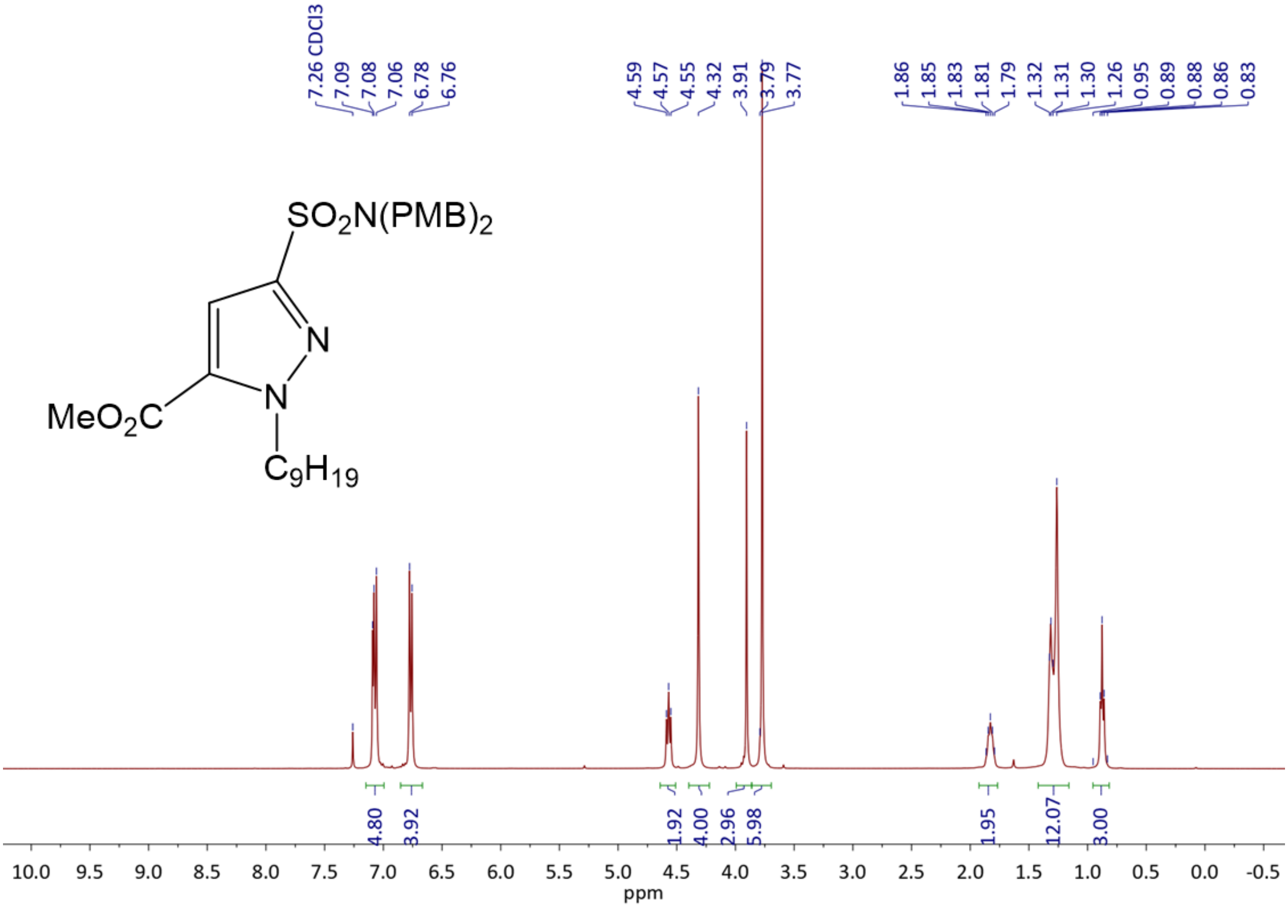


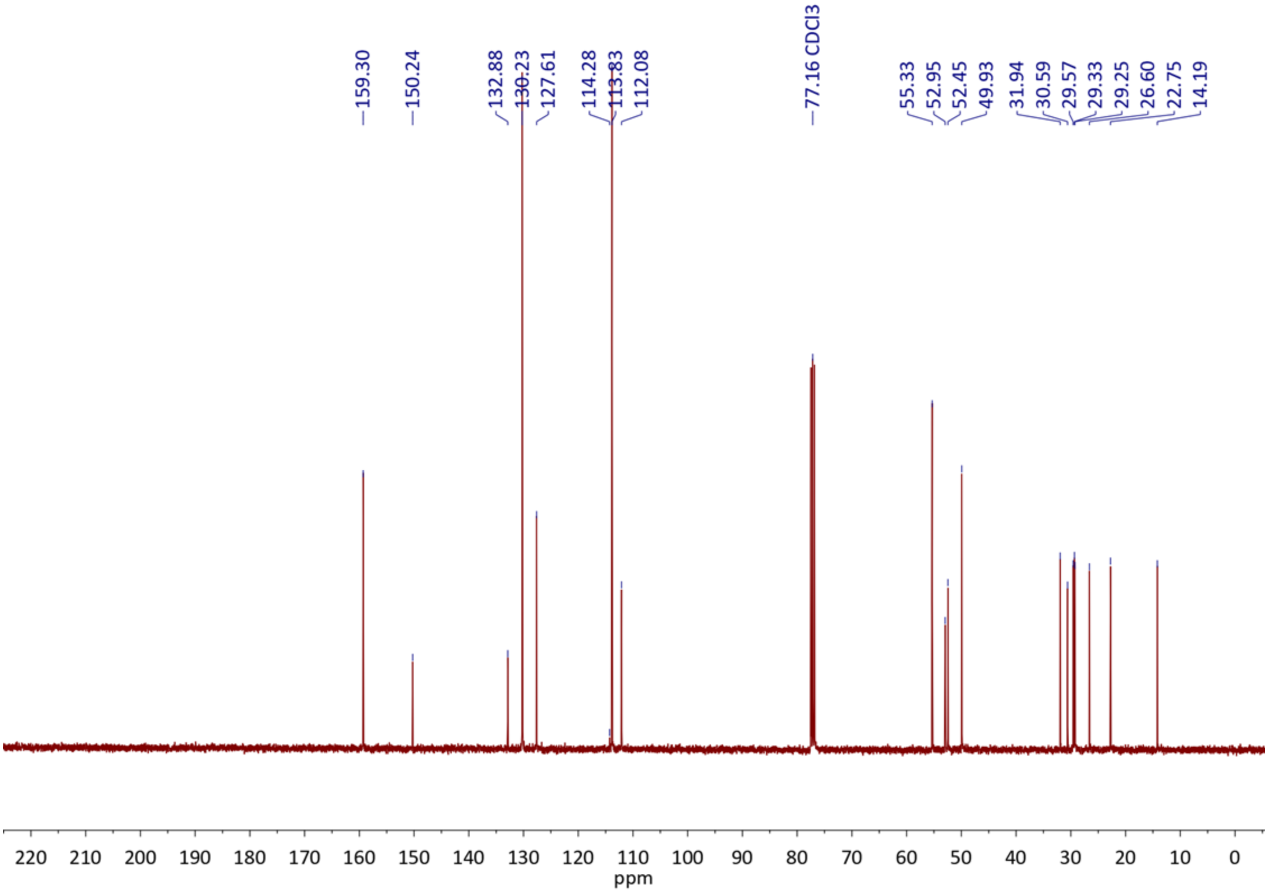
Copies of $^1$H (400.13 MHz, acetone-*d$_6$*) and $^{13}$C{$^1$H} (100.61 MHz, acetone-*d$_6$*) spectra of ***9***



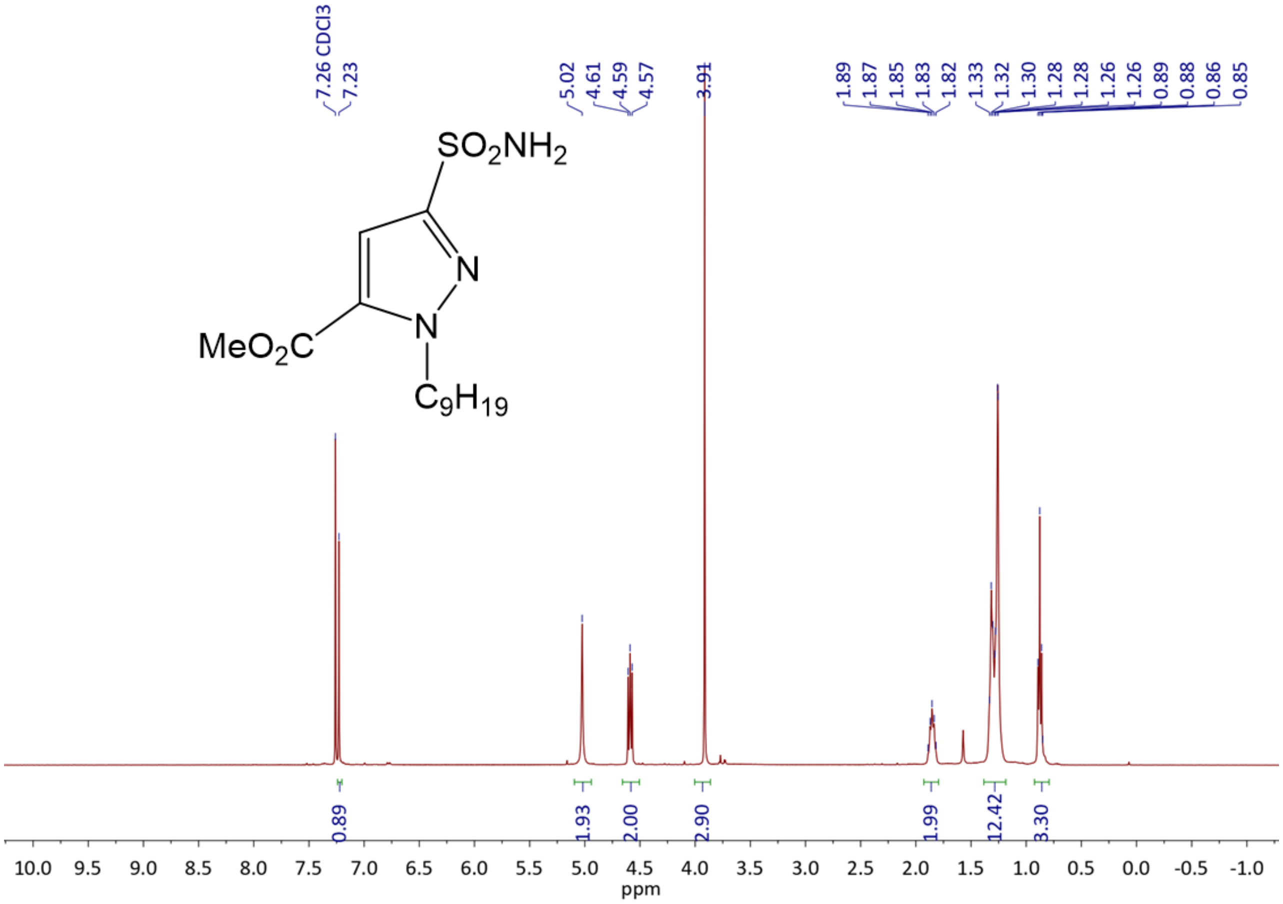


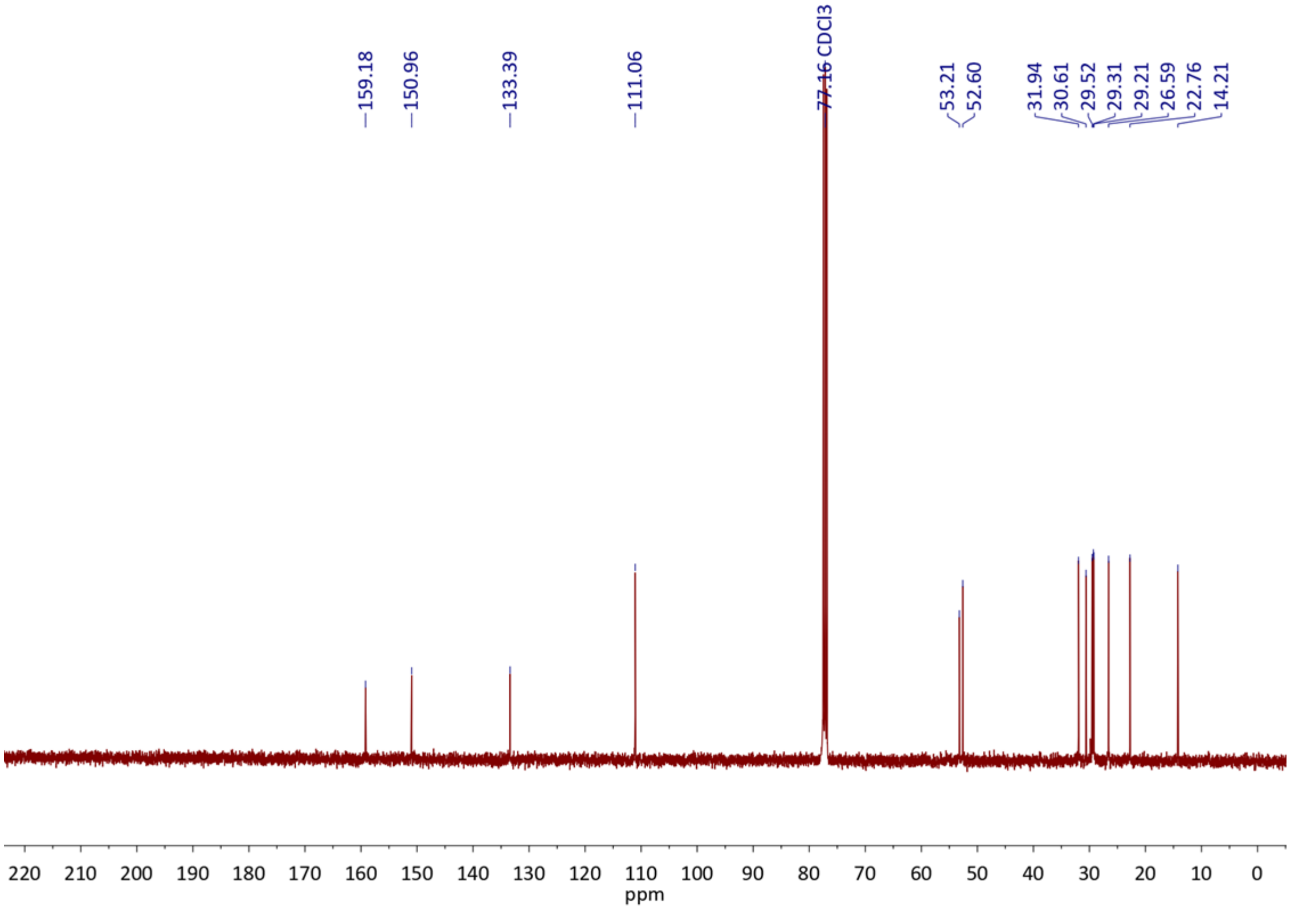
Copies of $^{1}H$ (400.13 MHz, $CDCl_3$) and $^{13}C\{^{1}H\}$ (100.61 MHz, $CDCl_3$) spectra of ***10***



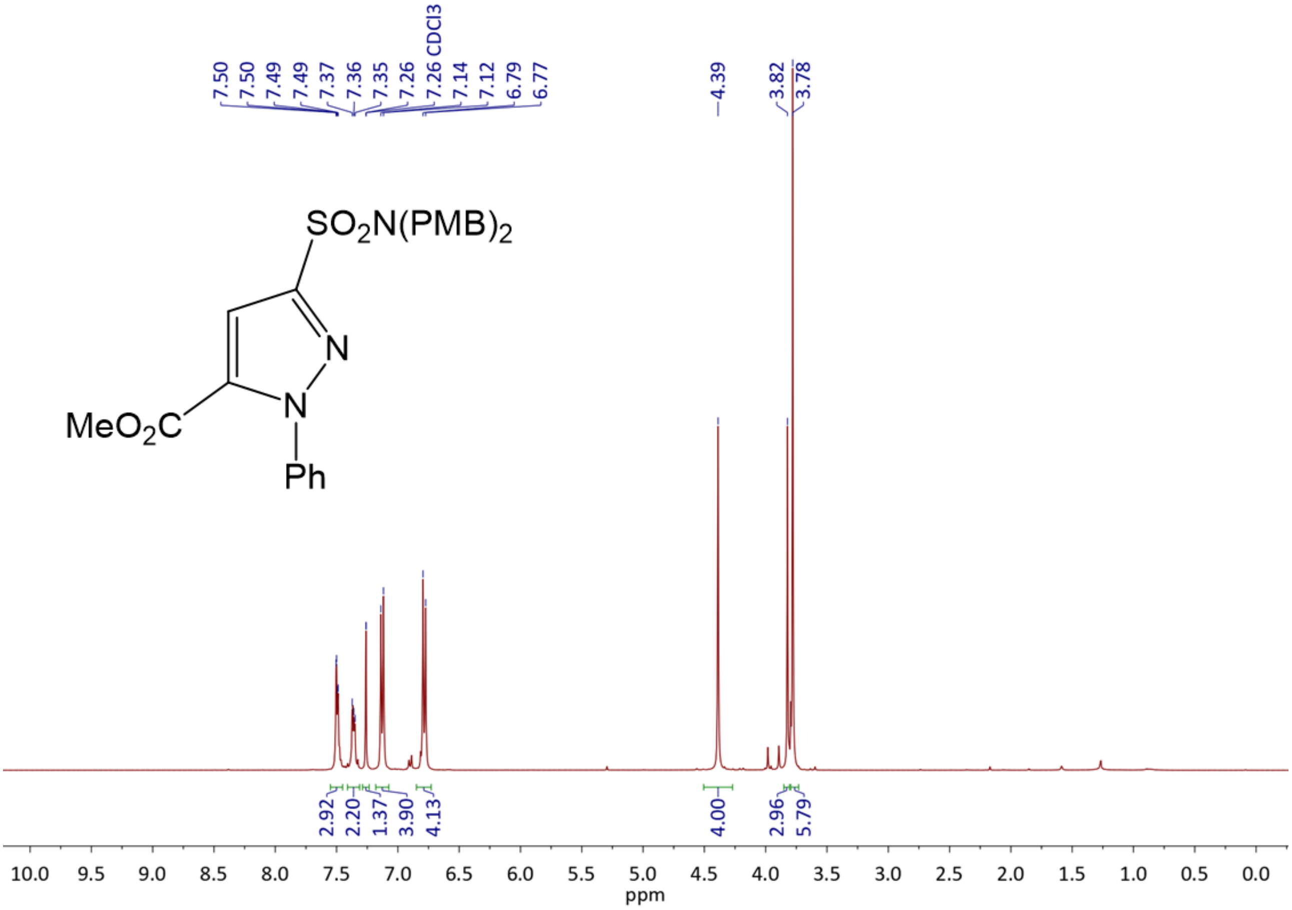


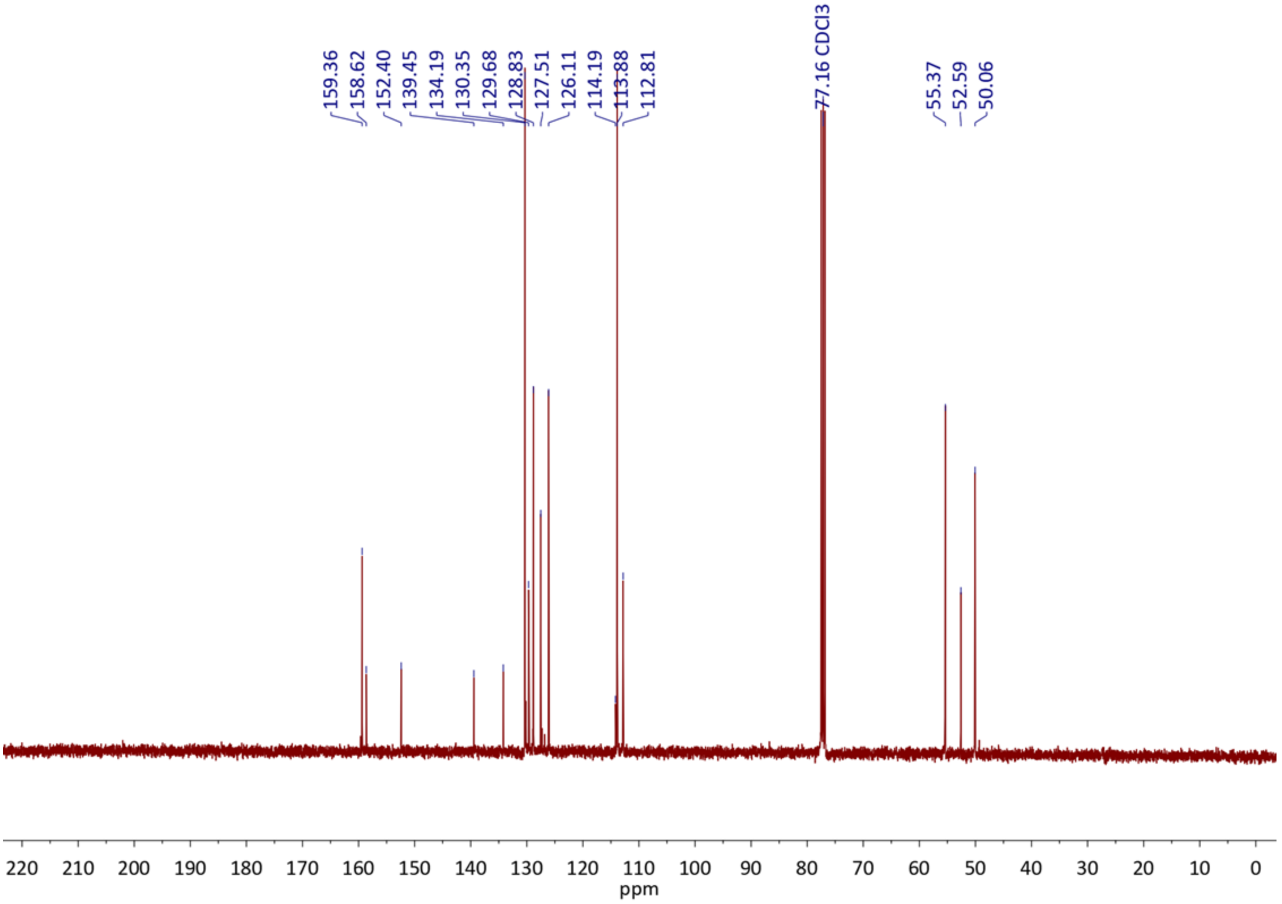
Copies of $^1$H (400.13 MHz, CDCl$_3$) and $^{13}$C{$^1$H} (100.61 MHz, CDCl$_3$) spectra of ***11***



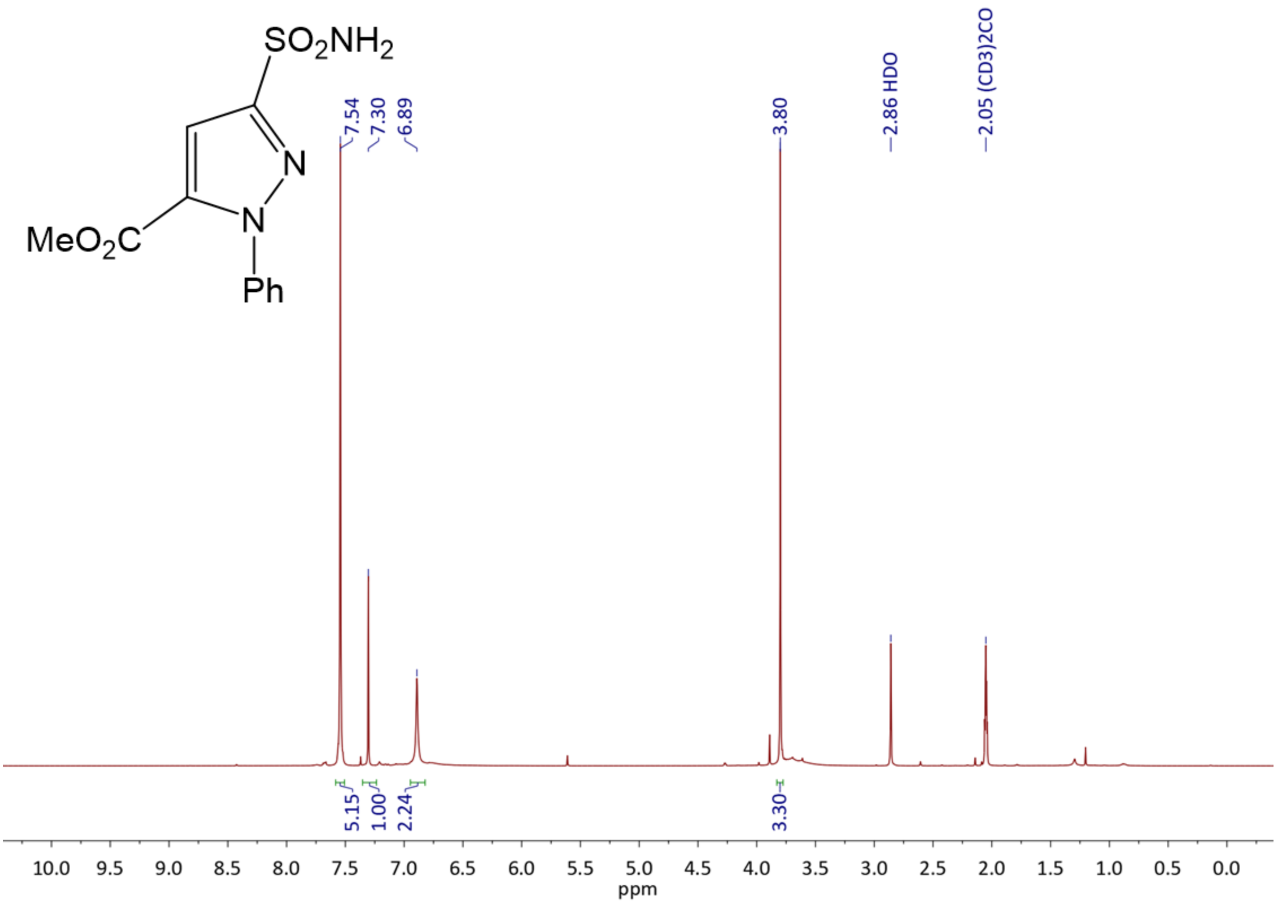


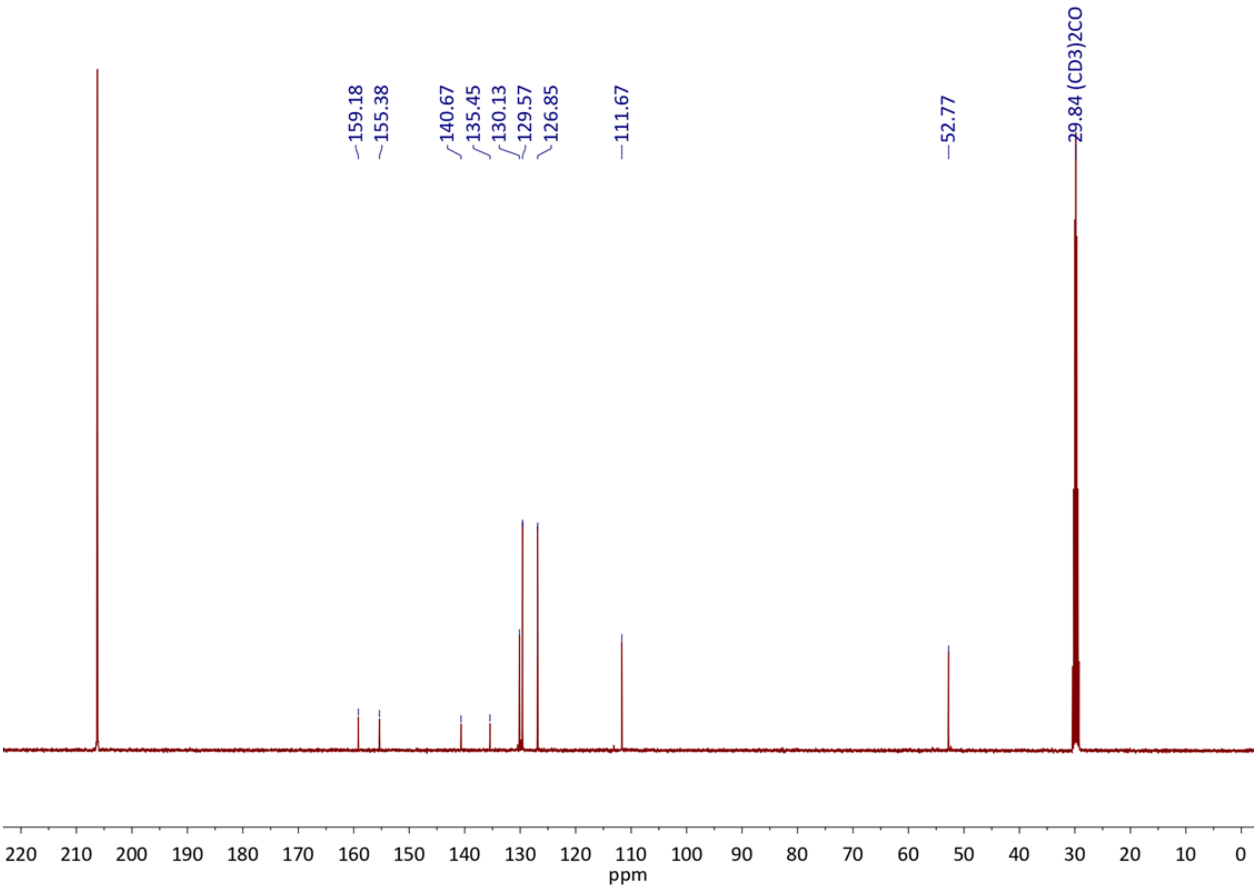
Copies of $^1$H (400.13 MHz, CDCl$_3$) and $^{13}$C{$^1$H} (100.61 MHz, CDCl$_3$) spectra of ***12***





Copies of $^{1}$H (400.13 MHz, acetone-$d_6$) and $^{13}$C{$^{1}$H} (100.61 MHz, acetone-$d_6$) spectra of ***13***





## Carbonic anhydrase inhibition assay

An instrument of Applied Photophysics stopped-flow was utilized for assaying the carbonic anhydrase catalyzed $CO_2$ hydration activity[S1]. Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20mM Hepes (pH 7.5) as buffer, and 20mM $Na_2SO_4$ (for maintaining constant the ionic strength), following the initial rates of the CA-catalyzed $CO_2$ hydration reaction for a period of 10–100 s. The $CO_2$ concentrations ranged from 1.7 to 17mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor at least six traces of the initial 5–10% of the reaction have been used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.1 mM) were prepared in distilled deionized water and dilutions up to 0.01nM were done thereafter with the assay buffer. Inhibitor and enzyme solutions were preincubated together for 15 min at room temperature prior to assay, in order to allow for the formation of the EI complex. The inhibition constants were subsequently obtained by nonlinear least-squares methods using PRISM 3 and the ChengPrusoff equation and represent the mean from at least three different determinations. All CA isoforms were recombinant ones obtained in-house as reported earlier[S2–S5].

## Molecular modelling

### Protein structure preparation

Protein structure model (hCA IX) is available in the RCSB Protein Data Bank (PDB: 4Q06). The protein model was prepared with use of Protein Prepwizard protocol (included in Schrödinger suite). This procedure includes: adding of missing aminoacids sidechains, assignment of bond orders, adding hydrogens, deletion of water molecules and optimization of H-bond network[S6]. All operations with proteins and ligands were performed in OPLS4[7] forcefield.

### Ligand structure preparation

Three-dimensional structures of ligands were generated in the same forcefield – OPLS4[S7] with use of LigPrep module. For all ligands, $pK_a$ values were calculated and likely protonation states determined with the use of Epik module of the Schrödinger Suite[S8].

### Molecular Docking Procedure and best-fitting structure selection

Docking method were used for prioritization of compounds into the binding cleft of hCA IX. Protein-ligand complexes with hCA IX were obtained with use of Glide methodology of molecular docking.

Docking gridbox generated with use of reference compound structure, located in hCA IX model (PDB: 4Q06). To enhance docking quality was applied following docking constraints: binding of sulfamide to zinc ion and (optional) priority of sulfamide substituent positioning in direction of zinc-interacting histidines. Docking gridbox was positioned in accordance to reference ligand structure. Gridbox side size is 12 Å.

For each ligand was generated 15 resulting docking poses. Best-fitting binding pose was selected on basis of the GlideScore/Emodel values, reference ligand interactions representation and docking solutions clustering.

### MM-GBSA

For all best-fitting docking solutions was calculated binding free energy and its components, such as: Gibbs free energy terms like lipophilic contacts, strain energy, and other parameters, with use of MM-GBSA method. Solvation model is VSGB, protein flexibility radius is 6 Å around ligand.

## Crystal structure data

X-Ray Single Crystal Analysis was performed on a SuperNova diffractometer. Crystals were kept at 100(2) K during data collection. Using Olex2[S9], the structures were solved with the SHELXT[S10] structure solution program using Intrinsic Phasing and refined with the SHELXL[S11] refinement package using Least Squares minimization.

## References

S1 R. G. Khalifah, *J. Biol. Chem.*, 1971, **246**, doi: 10.1016/s0021-9258(18)62326-9.

S2 B. L. Wilkinson, L. F. Bornaghi, T. A. Houston, A. Innocente, C. T. Supuran and S. A. Poulsen, *J. Med. Chem.*, 2006, **49**, 6539.

S3 M. Lopez, A. J. Salmon, C. T. Supuran and S.-A. Poulsen, *Curr. Pharm. Des.*, 2010, **16**, 3277.

S4 B. L. Wilkinson, L. F. Bornaghi, T. A. Houston, A. Innocenti, D. Vullo, C. T. Supuran and S.-A. Poulsen, *J. Med. Chem.*, 2007, **50**, 1651.

S5 N. Pala, L. Micheletto, M. Sechi, M. Aggarwal, F. Carta, R. McKenna and C. T. Supuran, *ACS Med. Chem. Lett.*, 2014, **5**, 927

S6 G. Madhavi Sastry, M. Adzhigirey, T. Day, R. Annabhimoju and W. Sherman, *J. Comput. Aided. Mol. Des.*, 2013, **27**, 221.

S7 C. Lu, C. Wu, D. Ghoreishi, W. Chen, L. Wang, W. Damm, G. A. Ross, M. K. Dahlgren, E. Russell, C. D. Von Bargen, R. Abel, R. A. Friesner and E. D. Harder, *J. Chem. Theory Comput.*, 2021, **17**, 4291.

S8 J. R. Greenwood, D. Calkins, A. P. Sullivan and J. C. Shelley, *J. Comput. Aided. Mol. Des.*, 2010, **24**, 591.

S9 O. V. Dolomanov, L. J. Bourhis, R. J. Gildea, J. A. K. Howard and H. Puschmann, *J. Appl. Crystallogr.*, 2009, **42**, 339.

S10 G. M. Sheldrick, *Acta Crystallogr.*, 2015, **A71**, 3.

S11 G. M. Sheldrick, *Acta Crystallogr.*, 2015, **C71**, 3.