

Supporting Information

Hydroxylamine as an Oxygen Nucleophile: Substitution of Sulfonamide by Hydroxyl Group in Benzothiazole-2-sulfonamides

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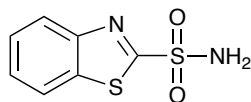
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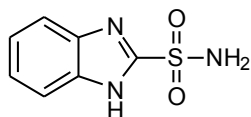
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I. Synthesis of reagents



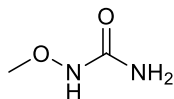
Benzothiazole-2-sulfonamide¹

3.368 g (0.020 mol) of 2-mercaptobenzothiazole was added to a stirred 2 M HCl (50 mL) cooled on an ice bath (0 °C). To this suspension was added 2.1 M NaOCl (10 mL) cooled on ice (<5 °C). After 2 hours of stirring the reaction mixture was filtered and the solid precipitate was redissolved in acetone (50 mL) cooled on an ice bath (<5 °C). To this solution 25% NH₃ (5 mL, 66.8 mmol) was added while stirring. After an hour 0.5 M HCl (20 mL) was added and acetone was removed by rotary evaporation. The water layer was then extracted with EtOAc (2 × 50 mL) and the combined organic phases were dried with Na₂SO₄. After removal of solvent in vacuo a brown/yellowish oil was obtained, which was purified by column chromatography on silica gel using ethyl acetate/n-heptane eluent (from 1:9 to 3:7). The product was recrystallized from n-heptane, to give a white solid. Mp 159-161 °C (decomposition); ¹H NMR (400 MHz, DMSO-d₆) δ 8.32 (bs, 2H), 8.24 (ddd, J = 8.0, 1.5, 0.5 Hz, 1H), 8.16 (ddd, J = 8.0, 1.5, 0.5 Hz, 1H), 7.50-7.71 (m, 2H); ¹³C NMR (101 MHz, DMSO-d₆) δ 169.9, 152.2, 136.1, 128.0, 127.9, 124.7, 123.7 ppm. LC-MS analysis t_r: 13.57, MS+ m/z (rel. intensity: 217(10), 216(10), 215 (100, M+1).



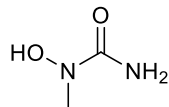
Benzimidazole-2-sulfonamide

The compound was synthesized using a previously reported method². Mp: 209-211 °C (decomposition) (Lit: 214³), ¹H NMR (400 MHz, DMSO-d₆) δ 13.42 (s, 1H), 8.01 (s, 2H), 7.57 (s, 2H), 7.42 – 7.21 (m, 2H).



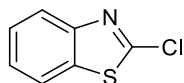
Methoxyurea

To a stirred solution of 164 mg (1.96 mmol) methoxylamine hydrochloride in H₂O (25 mL) was added a solution of 242 mg (2.98 mmol) KOCN in H₂O (10 mL) at 0 °C. After two hours, the solvent was removed under reduced pressure. The white solid was extracted with methanol and filtered. 132 mg (1.47 mmol 74.8%) of white solid was obtained by concentrating the solution in vacuo. Mp 73-75 °C; ¹H NMR (400 MHz, DMSO-d₆) δ 8.98 (s, 1H), 6.33 (s, 2H), 3.49 (s, 3H); ¹³C NMR (101 MHz, DMSO-d₆) δ 161.2, 63.8 ppm.



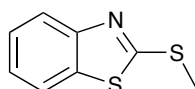
N-methylhydroxyurea

To a stirred solution of 833 mg (9.98 mmol) N-methylhydroxylamine hydrochloride in H₂O (40 mL) was added a solution of 1.217 g KOCN in H₂O (20 mL) at 0 °C. After 75 minutes the solution was concentrated in vacuo. The white solid was extracted with methanol and filtered, and the filtrate concentrated in vacuo. The product was purified by column chromatography on silica gel using methanol/CH₂Cl₂ eluent (from 1:99 to 1:9). A clear yellowish viscous oil (321 mg 3.13 mmol, 31.5%) was obtained. ¹H NMR (400 MHz, DMSO-d₆) δ 9.30 (s, 1H), 6.22 (s, 2H), 2.91 (s, 3H); ¹³C NMR (101 MHz, DMSO-d₆) δ 162.5, 38.5 ppm.



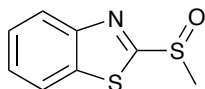
2-Chlorobenzothiazole

To H₂O (45 mL) cooled on ice were slowly added conc. HCl (1.05 mL), 5.03 g (33.4 mmol, 1 eq) 2-amino-benzothiazole and 6.87 g (101 mmol, 3eq) NaNO₂ at -5 °C. The reaction mixture was stirred for 3 hours at room temperature, followed by stirring for 30 minutes at 45 °C on a water bath until no further gas bells appeared. The aqueous layer was washed three times with DCM (3 × 100 mL), sat. NaHCO₃ (3 × 100 mL), brine (100 mL) and dried with Na₂SO₄. After filtration the organic layer was evaporated to give crude product which was redissolved in *n*-heptane and filtered. By concentrating the organic solution 4.02 g (23.4 mmol, 70%) of red viscous oil was obtained. ¹H NMR (400 MHz, CDCl₃) δ 7.93 (ddd, *J* = 8.0, 1.0, 0.5 Hz, 1H), 7.74 (ddd, *J* = 8.0, 1.0, 0.5 Hz, 1H), 7.39-7.44 (m, 2H); ¹³C NMR (101 MHz, CDCl₃) δ 153.2, 151.0, 136.1, 126.7, 125.7, 122.8, 121.1 ppm.



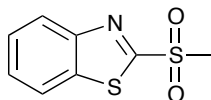
2-(Methylmercapto)-benzothiazole

The compound was synthesized according to previously reported procedure⁴. Mp: 43-44 °C; ¹H NMR (400 MHz, CDCl₃) δ 7.87 (ddd, *J* = 8.0, 1.0, 0.5 Hz, 1H), 7.76-7.73 (m, 1H), 7.44-7.38 (m, 1H), 7.30-7.26 (m, 1H), 2.79 (s, 3H); ¹³C NMR (101 MHz, CDCl₃) δ 168.0, 153.3, 135.1, 126.0, 124.1, 121.4, 120.9, 15.9 ppm.



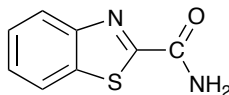
2-(Methylsulfinyl)-benzothiazole

The compound was synthesized according to previously reported procedure⁴. Mp: 70-71 °C; ¹H NMR (400 MHz, CDCl₃) δ 8.07 (ddd, *J* = 8.0, 1.0, 0.5 Hz, 1H), 8.01 (ddd, *J* = 8.0, 1.5, 0.5 Hz, 1H), 7.60-7.47 (m, 2H), 3.09 (s, 3H); ¹³C NMR (101 MHz, CDCl₃) δ 178.4, 153.7, 136.0, 127.0, 126.3, 124.0, 122.3, 43.2 ppm.



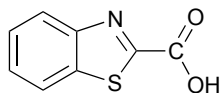
2-(Methylsulfonyl)-benzothiazole

This compound was synthesized according to previously reported procedure⁴. Mp: 88-90 °C; ¹H NMR (400 MHz, CDCl₃) δ 8.23-8.20 (m, 1H), 8.04-8.00 (m, 1H), 7.68-7.58 (m, 2H), 3.42 (s, 3H); ¹³C NMR (101 MHz, CDCl₃) δ 166.4, 152.4, 136.6, 128.1, 127.7, 125.4, 122.4, 42.4 ppm.



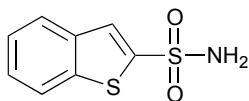
Benzothiazole-2-amide

To a mechanically stirred solution of ethyl benzothiazole-2-carboxylate (210 mg, 1.01 mmol) in ethanol (3 mL), was added NH₃ in ethanol (2 M, 8.5 mL, 17 mmol). The mixture was refluxed for 8 h, then cooled down and the precipitation collected by filtration to afford 110 mg (61%) of an orange solid. Mp: 228-229 °C; ¹H NMR (400 MHz, DMSO-d₆) δ 8.46 (bs, 1H), 8.21-8.16 (m, 1H), 8.12-8.08 (m, 1H), 8.05 (bs, 1H), 7.63-7.51 (m, 2H); ¹³C NMR (101 MHz, DMSO-d₆) δ 165.4, 161.8, 153.3, 136.8, 127.4, 127.3, 124.4, 123.4 ppm.



Benzothiazole-2-carboxylic acid

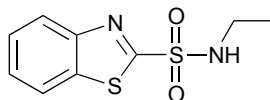
To a solution of ethyl benzothiazole-2-carboxylate (203 mg, 0.98 mmol) in THF (3 mL) was dropwise added 1 M NaOH (3 mL, 3 mmol). The mixture was stirred for 1 h at room temperature. THF was removed in vacuo and the precipitation was acidified with 1 M HCl to pH 3. The precipitation was collected by filtration, washed with cold water (5 mL) to afford 140 mg (80%) of yellowish crystalline product. Mp: 108 °C decomposition. ¹H NMR (400 MHz, DMSO-d₆) δ 8.09-8.02 (m, 2H), 7.55-7.42 (m, 2H); ¹³C NMR (101 MHz, DMSO-d₆) δ 168.9, 162.2, 153.6, 136.8, 126.6, 126.5, 124.4, 122.9 ppm.



Benzothiophene-2-sulfonamide

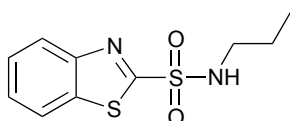
The compound was synthesized according to previously reported procedure². Mp: 202-203 °C; ¹H NMR (400 MHz, DMSO-d₆) δ 8.07-8.03 (m, 1H), 8.01-7.97 (m, 1H), 7.90 (d, *J* = 0.5 Hz, 1H), 7.86 (s, 2H), 7.52-7.43 (m, 2H); ¹³C NMR (101 MHz, DMSO-d₆) δ 146.2, 140.7, 138.0, 127.3, 127.1, 126.0, 125.8, 123.4 ppm. HRMS (ESI) calcd for (M + Na)⁺: 235.98159, found: 235.98221.

Preparation of *N*-substituted benzothiazole-2-sulfonamides: following the previously reported procedure



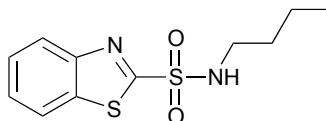
N-ethylbenzothiazole-2-sulfonamide

Mp: 144-146 °C; ¹H NMR (400 MHz, CDCl₃) δ 8.20-8.16 (m, 1H), 8.00-7.96 (m, 1H), 7.65-7.54 (m, 2H), 5.01 (s, 1H), 3.32 (m, *J* = 7.0, 6.0 Hz, 2H), 1.25-1.19 (m, 3H); ¹³C NMR (101 MHz, CDCl₃) δ 166.2, 152.3, 136.4, 127.5, 125.0, 122.2, 110.0, 39.2, 15.3 ppm. HRMS (ESI) calcd for (M + Na)⁺: 265.00814, found: 265.00805.



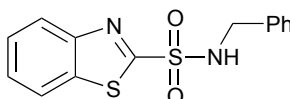
N-propylbenzothiazole-2-sulfonamide

Mp: 97-98 °C; ¹H NMR (400 MHz, CDCl₃) δ 8.20-8.15 (m, 1H), 8.01-7.96 (m, 1H), 7.65-7.54 (m, 2H), 5.06 (s, 1H), 3.22 (q, *J* = 7.0, 6.0 Hz, 2H), 1.66-1.53 (m, 2H), 0.93 (t, *J* = 7.5 Hz, 3H); ¹³C NMR (CDCl₃, 126 MHz) δ 166.1, 152.3, 136.4, 127.6, 127.4, 125.0, 122.2, 45.8, 23.1, 11.0 ppm. HRMS (ESI) calcd for (M + Na)⁺: 279.02379, found: 279.02529.



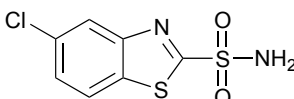
N-butylbenzothiazole-2-sulfonamide

Mp: 114-116 °C; ¹H NMR (400 MHz, CDCl₃) δ 8.19-8.14 (m, 1H), 7.99-7.94 (m, 1H), 7.64-7.52 (m, 2H), 3.25 (t, *J* = 7.0 Hz, 2H), 1.59-1.50 (m, 2H), 1.41-1.29 (m, 2H), 0.87 (t, *J* = 7.5 Hz, 3H); ¹³C NMR (101 MHz, CDCl₃) δ 166.1, 152.3, 136.4, 127.6, 127.4, 125.0, 122.2, 43.8, 31.7, 19.6, 13.5 ppm. HRMS (ESI) calcd for (M + Na)⁺: 293.03944, found: 293.03935.



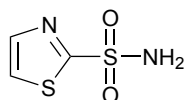
N-benzylbenzothiazole-2-sulfonamide

Mp: 130-130 °C; ¹H NMR (400 MHz, CDCl₃) δ 8.18-8.14 (m, 1H), 8.00-7.96 (m, 1H), 7.65-7.54 (m, 2H), 7.32-7.21 (m, 5H), 5.40 (s, 1H), 4.45 (s, 2H); ¹³C NMR (CDCl₃, 126 MHz) δ 166.0, 152.2, 136.4, 135.7, 128.7, 128.1, 128.1, 127.6, 127.4, 125.0, 122.1, 48.0 ppm. HRMS (ESI) calcd for (M + Na)⁺: 327.02379, found: 327.02399.



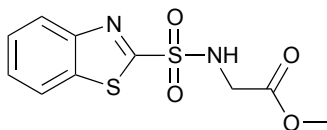
5-Chlorobenzothiazole-2-sulfonamide

Mp: 204-206 °C decomposition. ¹H NMR (400 MHz, DMSO-d₆) δ 8.39 (s, 2H), 8.30-8.25 (m, 2H), 7.66 (dd, *J* = 9.0, 2.0 Hz, 1H); ¹³C NMR (101 MHz, DMSO-d₆) δ 172.1, 153.1, 134.9, 132.8, 128.2, 125.3, 124.0 ppm. HRMS (ESI) calcd for (M + Na)⁺: 270.93787, found: 270.93712.



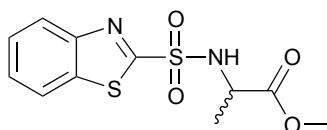
Thiazole-sulfonamide²

Mp: 118-119 °C; ¹H NMR (400 MHz, DMSO-d₆) δ 8.06 (s, 2H), 8.03 (d, *J* = 3.0, 1H), 8.01 (d, *J* = 3.0 Hz, 1H); ¹³C NMR (126 MHz, DMSO-d₆) δ 168.9, 144.2, 125.9 ppm. MS *m/z*: 164 (M + H)⁺.



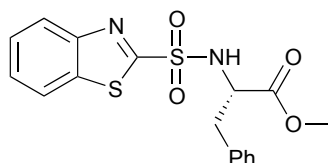
Methyl benzothiazole-2-sulfoglycinate

Mp: 111-113 °C; ¹H NMR (400 MHz, CDCl₃) δ 8.19-8.15 (m, 1H), 7.99-7.96 (m, 1H), 7.64-7.53 (m, 2H), 5.96 (s, 1H), 4.16 (s, 2H), 3.67 (s, 3H); ¹³C NMR (101 MHz, CDCl₃) δ 169.0, 165.3, 152.3, 136.3, 127.7, 127.5, 125.1, 122.2, 52.8, 44.7 ppm. HRMS (ESI) calcd for (M + Na)⁺: 308.99797, found: 308.99907.



Methyl benzothiazole-2-DL-sulfonalaninate

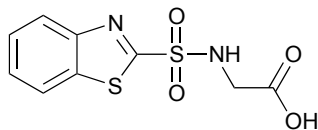
Mp: 117-118 °C; ¹H NMR (400 MHz, CDCl₃) δ 8.16-8.13 (m, 1H), 7.98 (ddd, *J* = 8.0, 1.5, 0.5 Hz, 1H), 7.64-7.54 (m, 2H), 5.86 (d, *J* = 8.0 Hz, 1H), 4.49 (dq, *J* = 8.0, 7.0 Hz, 1H), 3.62 (s, 3H), 1.52 (d, *J* = 7.0 Hz, 3H); ¹³C NMR (101 MHz CDCl₃) δ 172.2, 165.7, 152.2, 136.3, 127.7, 127.4, 125.0, 122.2, 52.9, 52.4, 20.0 ppm. HRMS (ESI) calcd for (M + Na)⁺: 323.01362, found: 323.01387.



Methyl benzothiazole-2-L-sulfonphenylalaninate

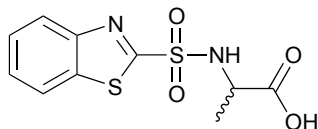
Mp: 110-111 °C; ¹H NMR (400 MHz, DMSO-d₆) δ 9.46 (s, 1H), 8.19 – 8.14 (m, 1H), 8.08 – 8.03 (m, 1H), 7.65 – 7.55 (m, 2H), 7.12 – 7.06 (m, 2H), 7.02 (dd, *J* = 8.4, 6.8 Hz, 2H), 6.96 – 6.89 (m, 1H), 4.24 (dd, *J* = 9.6, 5.4 Hz, 1H), 3.39 (s, 3H), 2.97 (dd, *J* = 13.8, 5.4 Hz, 1H), 2.77 (dd, *J* = 13.8, 9.7 Hz, 1H); ¹³C NMR (101 MHz, DMSO-d₆) δ 171.4, 166.9, 152.2, 136.5, 136.2, 129.4, 128.4, 128.0, 127.9, 126.9, 124.8, 123.5, 58.3, 52.5, 37.8 ppm. HRMS (ESI) calcd for (M + Na)⁺: 399.04492, found: 399.04640.

Hydrolysis reactions



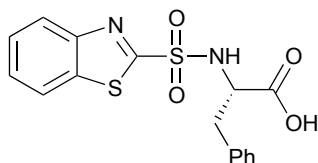
Benzothiazole-2-sulfon-*N*-glycine

To a solution of methyl benzothiazole-2-sulfon-glycinate (201 mg, 0.702 mmol) in THF (4 mL) was slowly added 1 M NaOH (4 mL, 4 mmol). The reaction mixture was stirred for 2 h, acidified to pH 4 and the volume was reduced by 40% in vacuo. The aqueous phase was extracted with EtOAc (3 × 5 mL) followed by drying of the combined organic layers with Na₂SO₄. The solvent was removed in vacuo and a yellowish solid was obtained (176 mg, 0.646 mmol) yielding 92% of product. Mp: 177-178 °C. ¹H NMR (400 MHz, Methanol-d₄) δ 8.15-8.07 (m, 2H), 7.66-7.55 (m, 2H), 4.01 (s, 2H); ¹³C NMR (101 MHz, Methanol-d₄) δ 170.5, 167.0, 152.2, 136.2, 127.3, 127.1, 124.2, 122.2, 43.9 ppm. HRMS (ESI) calcd for (M + Na)⁺: 294.98232, found: 294.98340.



Benzothiazole-2-sulfon-*N-DL*-alanine

The hydrolysis reaction of benzothiazole-2-sulfon-*N-DL*-alanine was performed using the same method as for benzothiazole-2-sulfon-*N*-glycine. Starting from methyl benzothiazole-2-*DL*-sulfonalaninate (40.7 mg, 0.136 mmol), benzothiazole-2-sulfon-*N-DL*-alanine (34.1 mg, 0.119 mmol, 88%) was obtained as a yellowish solid. Mp: 174-175 °C. ¹H NMR (400 MHz, Acetone-d₆) δ 8.23-8.18 (m, 1H), 8.13 (ddd, *J* = 8.0, 1.5, 0.5 Hz, 1H), 7.69-7.60 (m, 2H), 4.41 (q, *J* = 7.5 Hz, 1H), 1.49 (d, *J* = 7.5 Hz, 3H); ¹³C NMR (101 MHz, Acetone-d₆) δ 172.2, 167.1, 152.5, 136.2, 127.5, 127.4, 124.6, 122.7, 52.1, 18.6 ppm. HRMS (ESI) calcd for (M + Na)⁺: 308.99797, found: 308.99858.



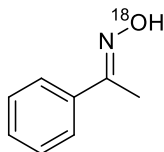
Benzothiazole-2-sulfon-*N-L*-phenylalanine

The hydrolysis reaction of benzothiazole-2-sulfon-*N-L*-phenylalanine was performed using the same method as for benzothiazole-2-sulfon-*N*-glycine. Starting with methyl benzothiazole-2-*L*-sulfonphenylalaninate (204 mg, 0.542 mmol), benzothiazole-2-sulfon-*N-L*-phenylalanine (171 mg, 0.473 mmol, 81%) was obtained as a yellowish solid. Mp: 150-151 °C. ¹H NMR (400 MHz, DMSO-d₆) δ 9.46 (s, 1H), 8.19-8.13 (m, 1H), 8.09-8.00 (m, 1H), 7.66-7.53 (m, 2H), 7.13-7.06 (m, 2H), 7.02 (dd, *J* = 8.5, 7.0 Hz, 2H), 6.96-6.89 (m, 1H), 4.24 (dd, *J* = 9.5, 5.5 Hz, 1H), 3.39 (s, 3H), 2.97 (dd, *J* = 14.0, 5.5 Hz, 1H), 2.77 (dd, *J* = 14.0, 9.5 Hz, 1H); ¹³C NMR (101 MHz, Methanol-d₄) δ 166.9, 152.0, 136.3, 136.3, 128.9, 127.7, 127.2, 127.0, 126.1, 124.2, 122.1, 38.2, 29.3 ppm. HRMS (ESI) calcd for (M + Na)⁺: 385.02927, found: 385.02981.

Preparation of H¹⁸ONH₂⁵

NaN¹⁸O₂

To a solution of 501.2 mg (7.2 mmol, 1 eq) NaNO₂ in H₂¹⁸O (600 μL, 30 mmol, 4.1 eq) cooled at 0 °C, was dropwise added conc. HCl. After 24 hours 25.0 mg of NaOH was added at once and the mixture was stirred for an additional hour. The solvent was removed under vacuo, yielding a solid which was allowed to dry.



¹⁸O-enriched acetophenone

To a solution of dry NaN¹⁸O₂ dissolved in anhydrous THF (7 mL) was dropwise added 1.58 mL (14.4 mmol 2 eq) of borane dimethyl sulfide (BMS) over 10 minutes. The reaction mixture was stirred overnight at room temperature and then cooled to -5 °C. Water (3.2 mL) was slowly added to this solution over 15 minutes, followed by 6 M HCl (3.2 mL) over 5 minutes. After being stirred for 45 minutes at room temperature, NaOH solution was added together with 850 μL of acetophenone. Then, the reaction mixture was heated to 83 °C and left stirring overnight. The aqueous layer was saturated with NaCl and extracted with diethylether (3 × 30 mL). The combined organic layers were dried using Na₂SO₄, filtered and concentrated in vacuo. The crude product was purified by column chromatography (1:19-3:7 EtOAc / *n*-heptane), yielding 103.5 mg (0.77 mmol, 11%) of clear colorless oil which solidified over time. Mp: 57.0-58.1 °C. LC-MS analysis: t_r: , MS⁺ (rel. intensity: 139(8 M(¹⁸O)+2), 138(100 M(¹⁸O)+1), 137(6 M(¹⁶O)+2), 136(59 M(¹⁶O)+1) (also see Figure S62).

¹⁸O-enriched hydroxylamine

A mixture of 60.0 mg (0.29 mmol) acetophenone oxime and 2 M HCl (10 mL) H¹⁸O-NH₂ was refluxed for 2 hours. The solvent was removed in vacuo using the rotary evaporator. The remaining residue was dissolved in ethylacetate (10 mL). The salt was filtered, and the filtrate evaporated to afford 20.1 mg of slightly yellow solid.

II. Products of the cleavage reaction

Detection of 2-hydroxybenzothiazole:

NMR time course for the detection of 2-hydroxybenzothiazole.

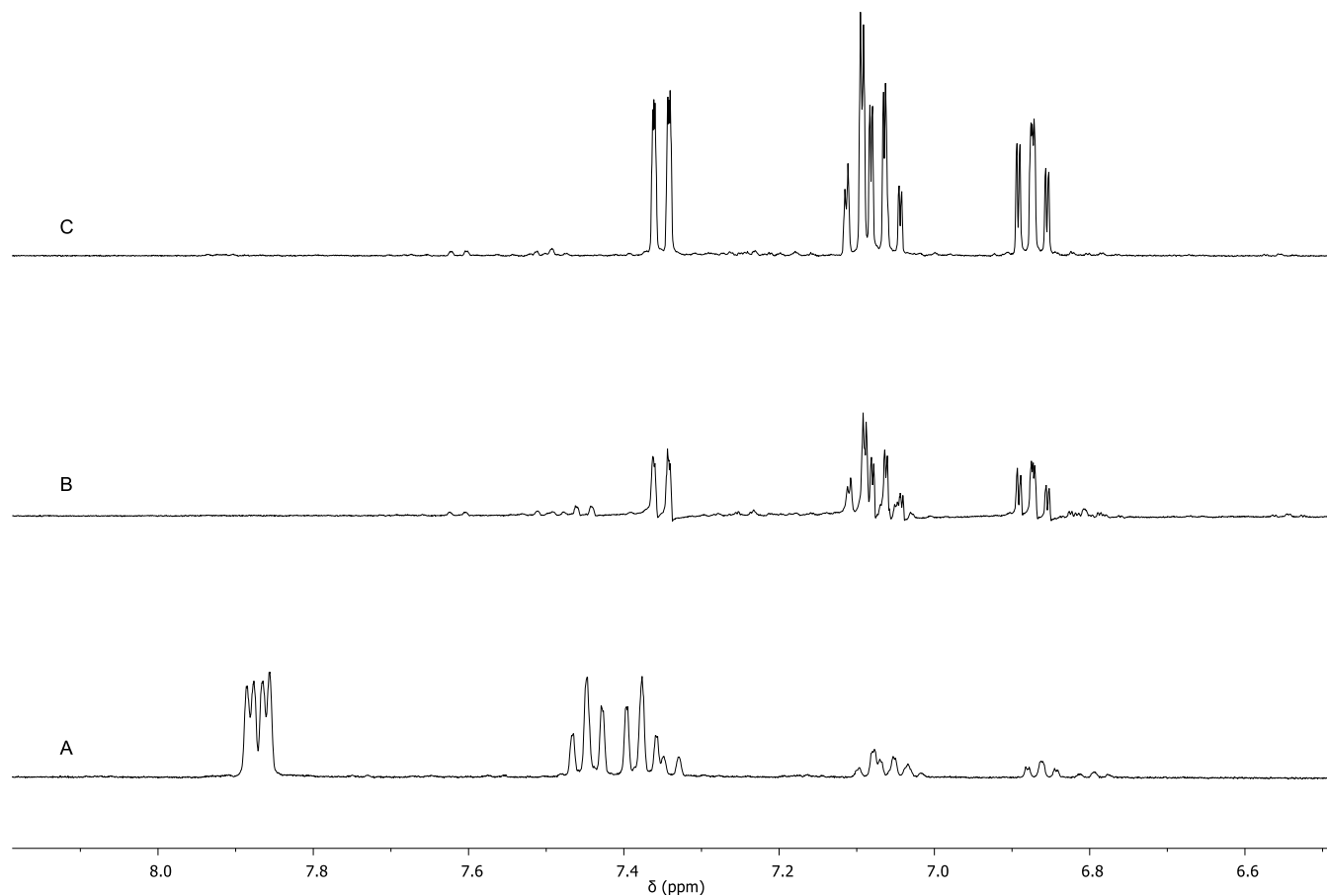


Figure S1. Detection of 2-hydroxybenzothiazole, by doping the product. **A**; reaction mixture after 10 minutes, **B**; reaction mixture after 3 hours, **C**; reaction mixture after 3 hours doped with 2-hydroxybenzothiazole.

Detection of ethylamine

Figure S2 represents the detection of ethylamine.

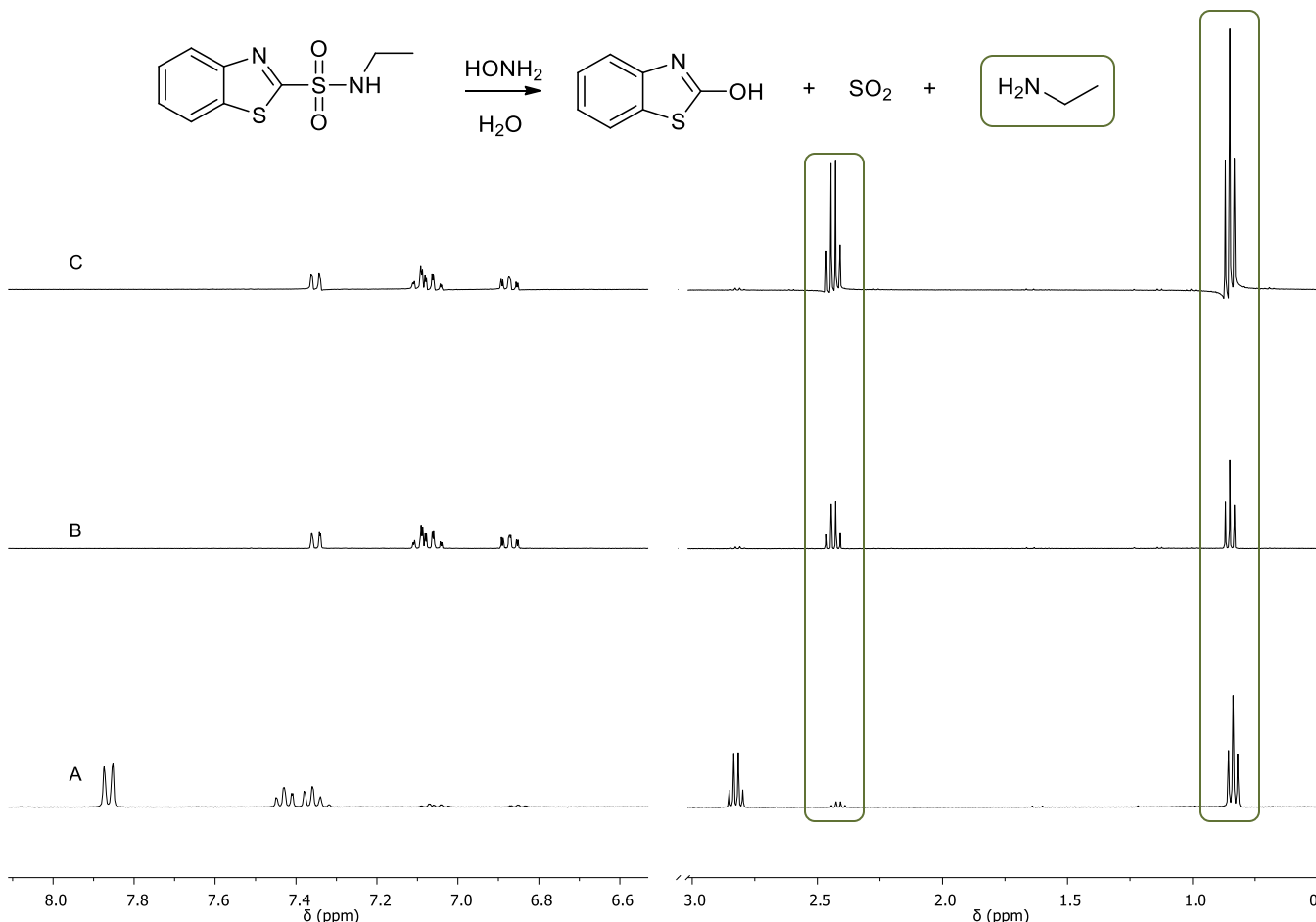
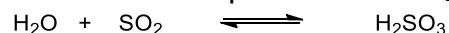


Figure S2. Detection of ethylamine, by doping the product into the reaction mixture. **A**; reaction mixture after 10 minutes, **B**; reaction mixture after 6 hours, **C**; reaction mixture after 6 hours doped with ethylamine.

Detection of SO₂ using new fuchsin

To detect the inorganic SO₂, a colorimetric detection method was used based on the color change of new fuchsin solution when exposed to SO₂. This method is described in literature as a specific indicator of SO₂ that is able to detect this inorganic compound in low quantities⁶. After the standard cleavage reaction with BTA and hydroxylamine was completely done (confirmed by ¹H NMR and LC-MS), an aliquot was taken from this solution and added into the solution of new fuchsin. Using UV/VIS spectroscopy, the absorbance of this solution was measured, resulting in the red line (figure S3). A control experiment that uses SO₂ as a reference, represented by the green line, gave a similar absorbance. By dissolving SO₂ in water an equilibrium is formed with sulfurous acid, H₂SO₃ (Scheme S1). A mixture of BTA and color reagent also gave a similar absorption signature (purple line) as was observed with the SO₂ control experiment. For this reason, it was necessary to check the reaction mixture for full conversion of BTA in order to verify the formation of SO₂. As the dark blue line shows, an aqueous solution of hydroxylamine does not give any significant absorbance. Since the product of the reaction is also a sulphur containing compound, this should obviously not result into any significant absorbance when mixed with the color reagent (light blue line).

Scheme S1. Equilibrium of SO₂ in water



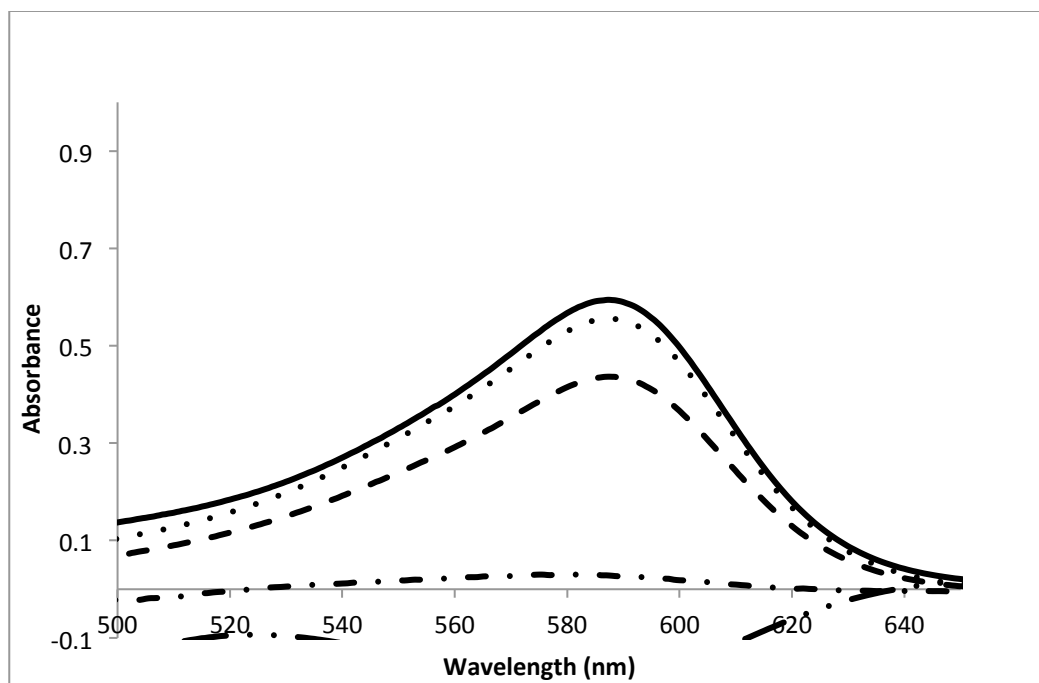


Figure S3. Detection of SO₂ using a new fuchsine based color reagent. **Solid line**; absorbance of the reaction mixture with color reagent, **Dotted line**; absorbance of the H₂SO₃ stock solution and color reagent, **Dashed line**; absorbance of BTA and color reagent, **Small dashed dotted line**; absorbance of 2-hydroxybenzothiazole and color reagent, **Large dashed dotted line**; absorbance of HONH₂ and color reagent.

Oxygen labelling experiments

Detection of the product

As shown in the previous section, 2-hydroxybenzothiazole is formed when BTA is exposed to an excess of hydroxylamine. There are three possible oxygen donors; dioxygen from air, water or hydroxylamine. A possible reaction involving oxygen from the air was excluded by successfully performing a standard cleavage reaction under N_2 atmosphere.

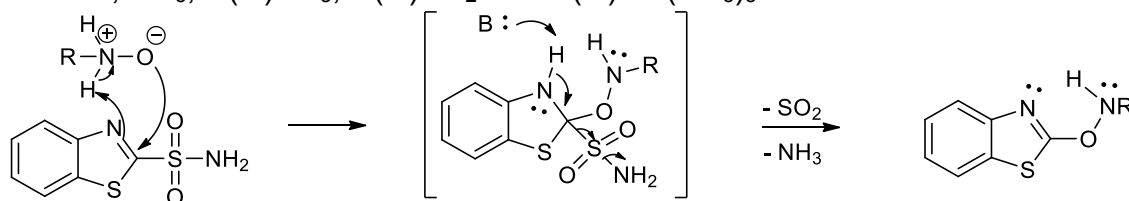
To investigate if oxygen is derived from water, $H_2^{18}O$ was used as a solvent. If the oxygen donor in this reaction derives from water, then the incorporation of the heavy oxygen atom should be observed by mass spectrometry when performing the standard cleavage in $H_2^{18}O$. Figure S4 shows the mass of 2-hydroxybenzothiazole in positive ion mode when the cleavage reaction was done using non-labeled water. As illustrated, the m/z of the product corresponds to 152 in positive ion mode. When $H_2^{18}O$ is employed as a solvent for the standard cleavage reaction, no significant change in m/z is observed (Figure S5). This excludes water as the oxygen donor and leaves the assumption that oxygen is derived from hydroxylamine. To fully prove this hypothesis, ^{18}O -enriched (~65% ^{18}O labeled) hydroxylamine was synthesised. Figure S6 shows the cleavage reaction of BTA using the ^{18}O labeled hydroxylamine in non-labeled water. A significant amount (~65% ^{18}O labeled) of heavy oxygen is incorporated into the 2-hydroxybenzothiazole, confirming the origin of oxygen in the final product.

Detection of the intermediate

Results from several LC-MS analyses on the cleavage of BTA using different reagents strongly suggested the formation of an aryloxyamine intermediate (Scheme S2). In addition to oxygen-labeling experiments that provided the evidence that the oxygen atom in product derives from hydroxylamine, we precisely looked on potential intermediates of the substitution reaction. When ^{18}O -labeled hydroxylamine was used to cleave BTA, we found that the intermediate also had an incorporated heavy oxygen atom (Figure S7).

Figure S7-S9 illustrate the incorporation of ^{18}O into the key intermediate of the reaction between BTA and hydroxylamine in water.

Scheme S2. Reaction mechanism illustrating the forming an aryloxyamine intermediate product. $R = H, CH_3, C(O)CH_3, C(O)NH_2$ and $C(O)OC(CH_3)_3$.



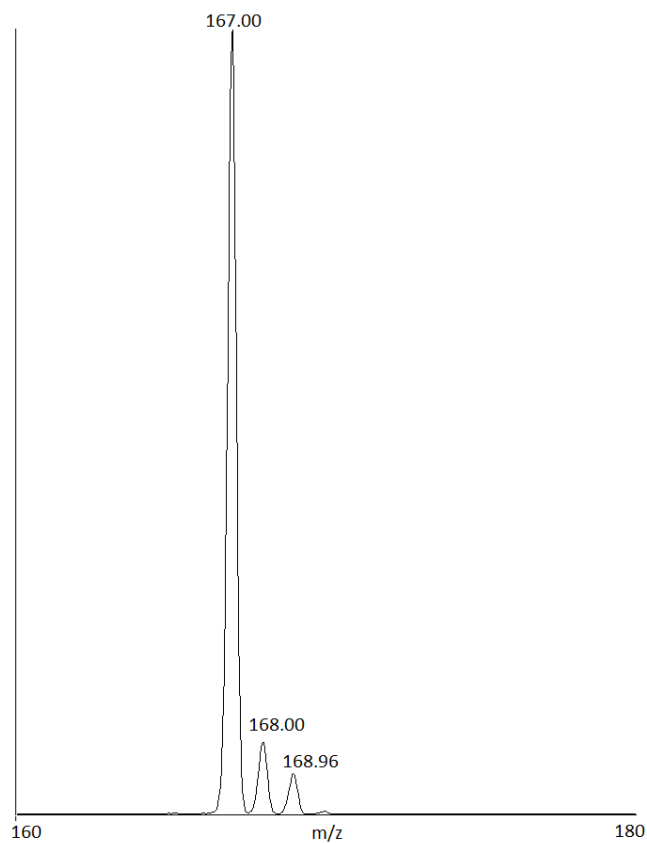


Figure S7. MS⁺ spectrum showing the mass of the intermediate product when BTA is cleaved using non-labeled hydroxylamine in H₂O.

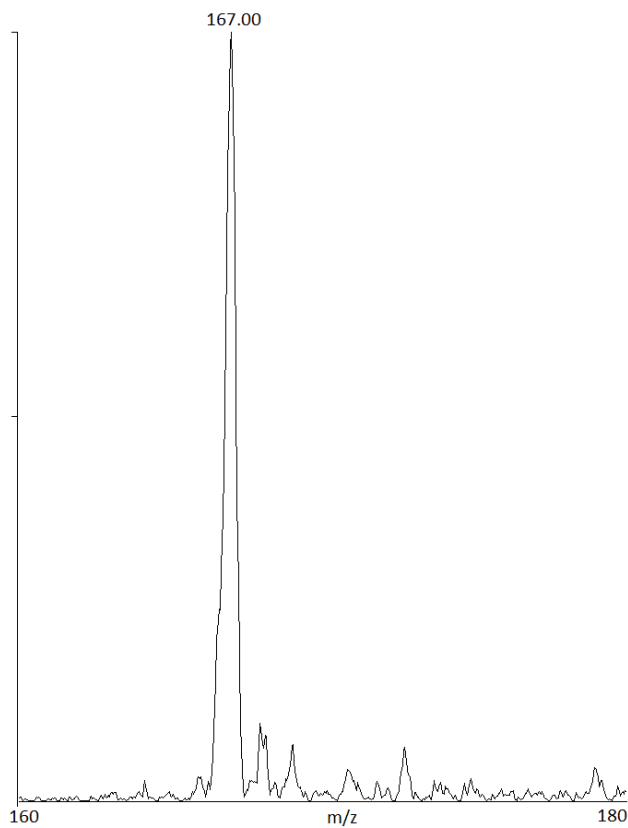


Figure S8. MS⁺ spectrum showing the mass of the intermediate product when BTA is cleaved using non-labeled hydroxylamine in H₂¹⁸O.

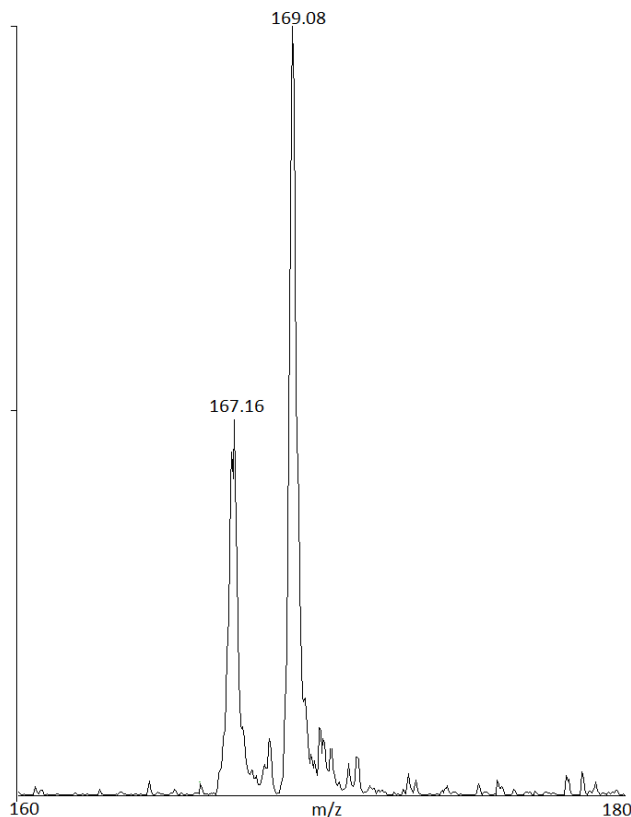
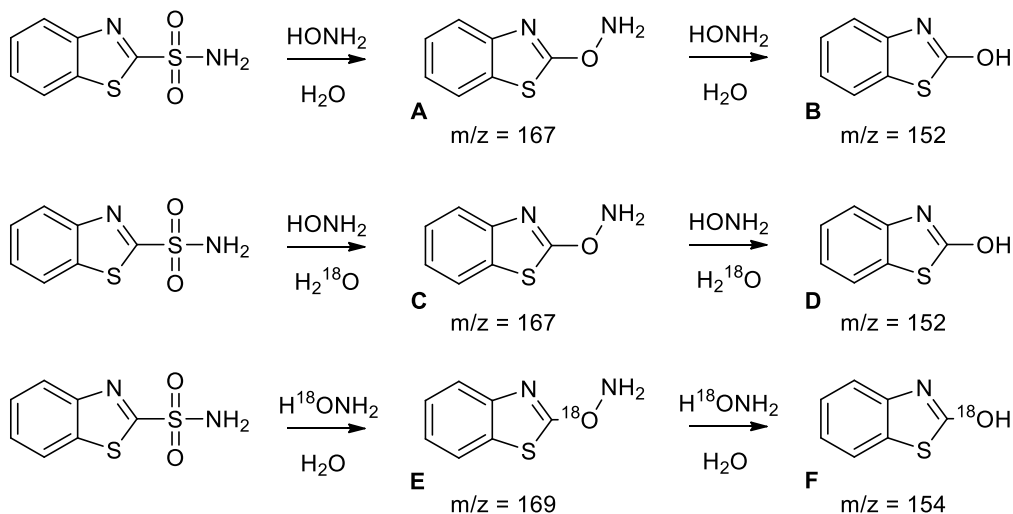


Figure S9. MS⁺ spectrum showing the mass of the intermediate product when BTA is cleaved using ¹⁸O labeled hydroxylamine (~70% ¹⁸O, ~30% ¹⁶O) in H₂O.

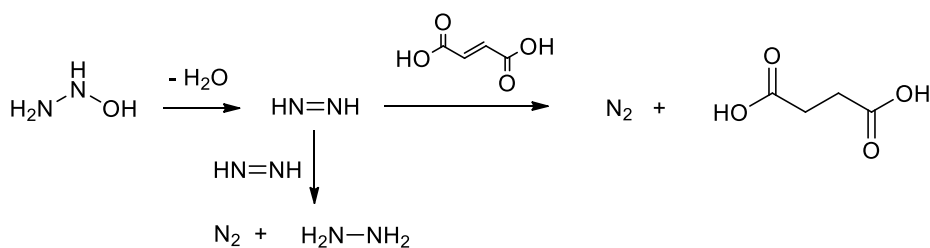
Scheme S3. Overview on ¹⁸O-labeling reactions, showing the experimentally observed values of m/z for intermediates and products. **A** is illustrated in figure S4, **B** is illustrated in figure S7, **C** is illustrated in figure S5, **D** is illustrated in figure S8, **E** is illustrated in figure S6, **F** is illustrated in figure S9.



An indirect detection of diazene

Next, we identified diazene (also known as diimide) as an intermediate of the substitution reaction (Scheme S4). Diazene is a known reducing reagent, capable of reducing double bonds to saturated ones. This property of diazene was used to reduce fumaric acid to succinic acid. Using ^1H NMR spectroscopy, the newly formed succinic acid can easily be distinguished from fumaric acid (Figure S10). The solution was analysed by ^1H NMR, showing a clear peak at aliphatic side (D, Figure S10), which was confirmed to be succinic acid by doping sodium succinate into the sample (E, Figure S10). When a different reagent (e.g. hydroxyurea) was used for the reaction with BTA, no succinate was formed (A, Figure S10). Not surprisingly, BTA does not react with fumaric acid to form succinate (B Figure S10); same should apply to hydroxylamine (C, Figure S10).

Scheme S4. Formation of diazene and the subsequent reaction with fumarate to produce succinate.



III. Cleavage reaction using different substrates

NMR time-course of hydroxylamine-mediated cleavage of benzothiazole-2-sulfonamide

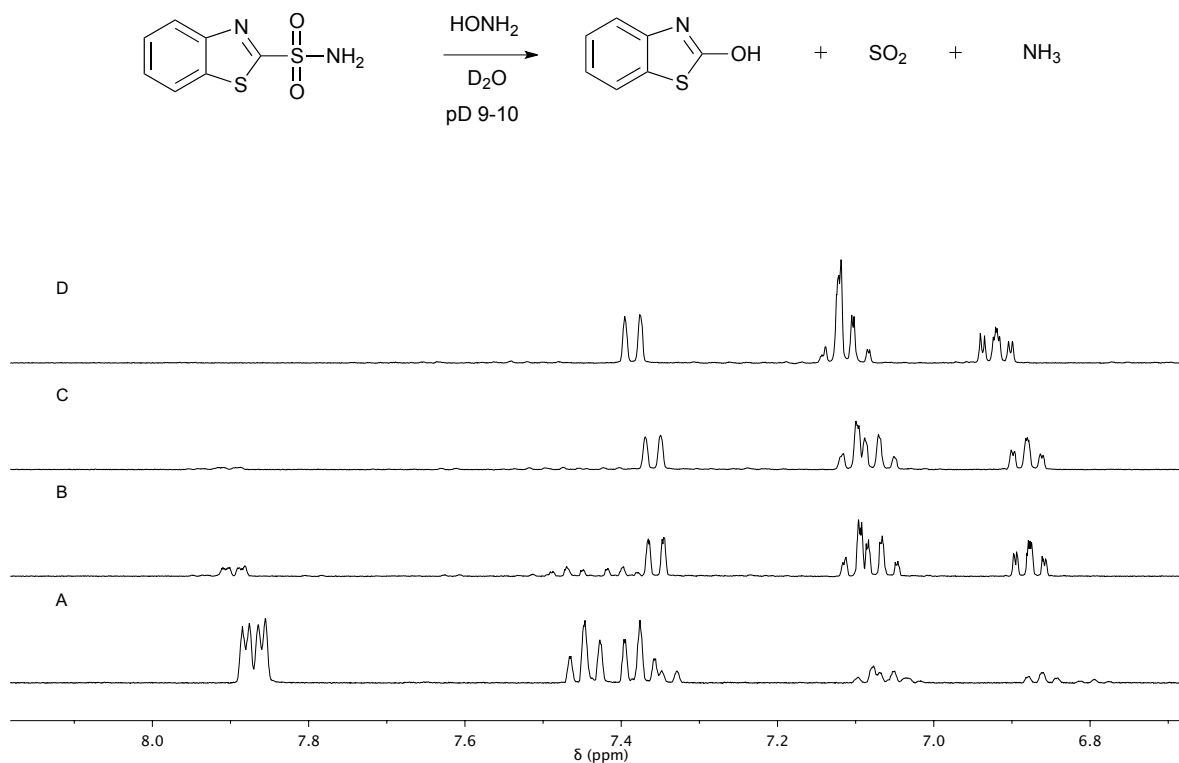


Figure S11. ¹H-NMR time course of hydroxylamine mediated cleavage of BTA after A: 10 min, B: 2 h, C: 3 h, D: doping of 2-hydroxybenzothiazole.

NMR time-course of hydroxylamine-mediated cleavage of *N*-propyl benzothiazole-2-sulfonamide

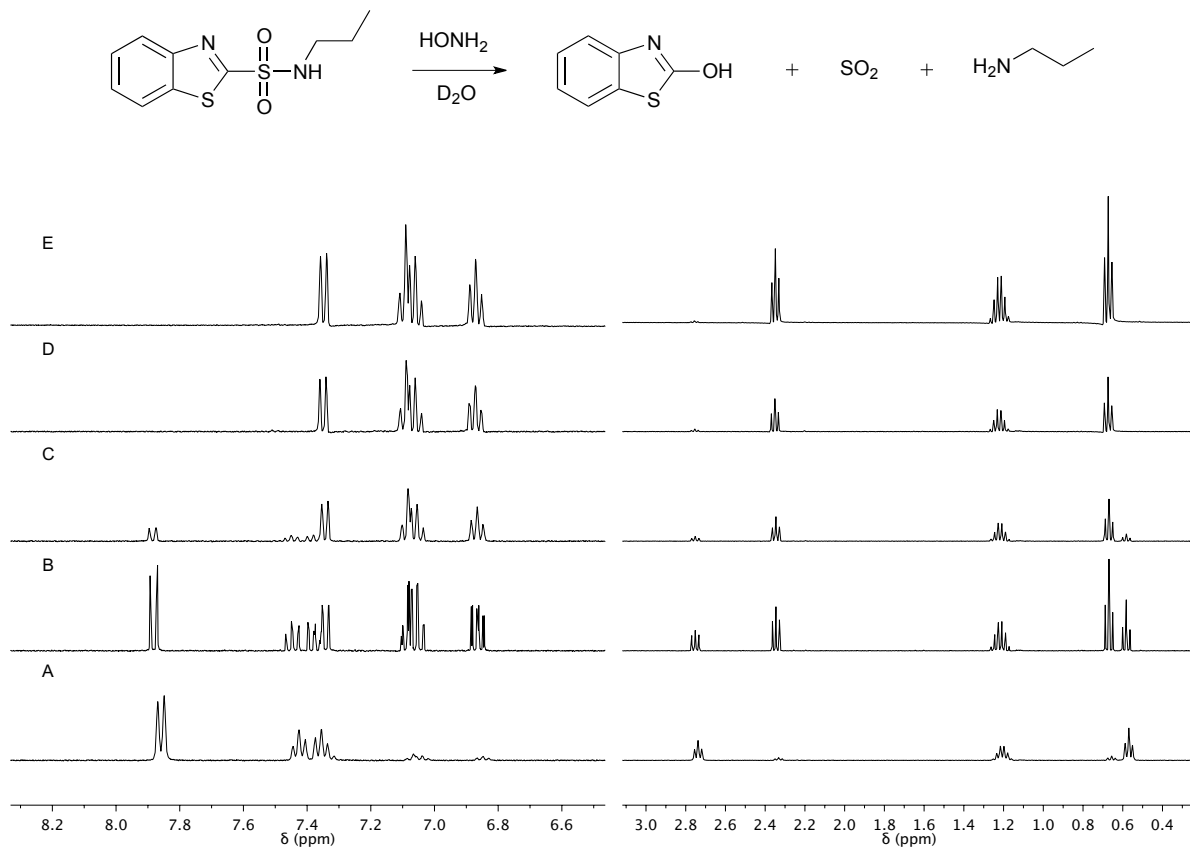


Figure S12. ¹H-NMR time course of hydroxylamine mediated cleavage of BTA after A: 10 min, B: 2 h, C: 4 h, D: 6 h, E: doping of 2-hydroxybenzothiazole (L), doping of propylamine (R).

NMR time-course of hydroxylamine-mediated cleavage of benzothiazole-2-sulfon-N-glycine

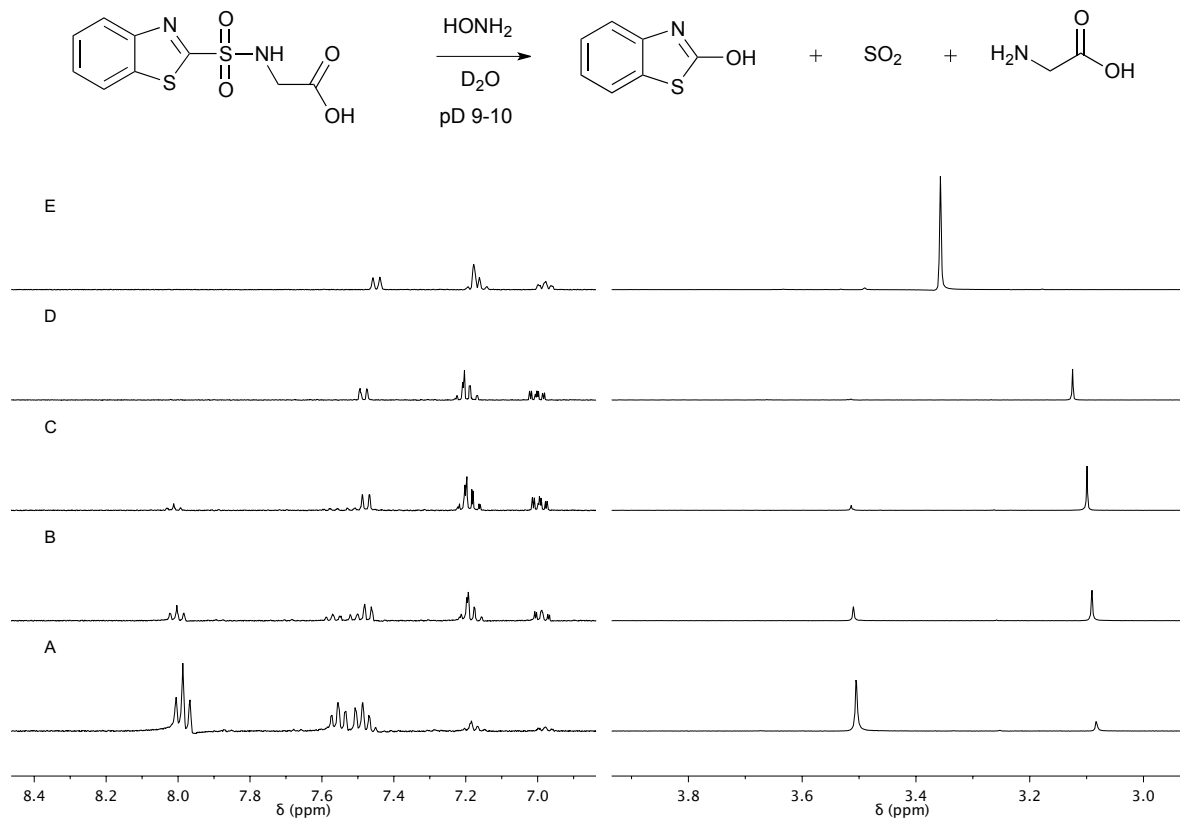


Figure S13. ¹H-NMR time course of hydroxylamine mediated cleavage of benzothiazole-2-sulfon-N-glycine after A: 10 min, B: 2 h, C: 4 h, D: 6 h, E: doping of 2-hydroxybenzothiazole (L), doping of glycine (R).

NMR time-course of hydroxylamine-mediated cleavage of benzothiazole-2-sulfon-*N*-*DL*-alanine

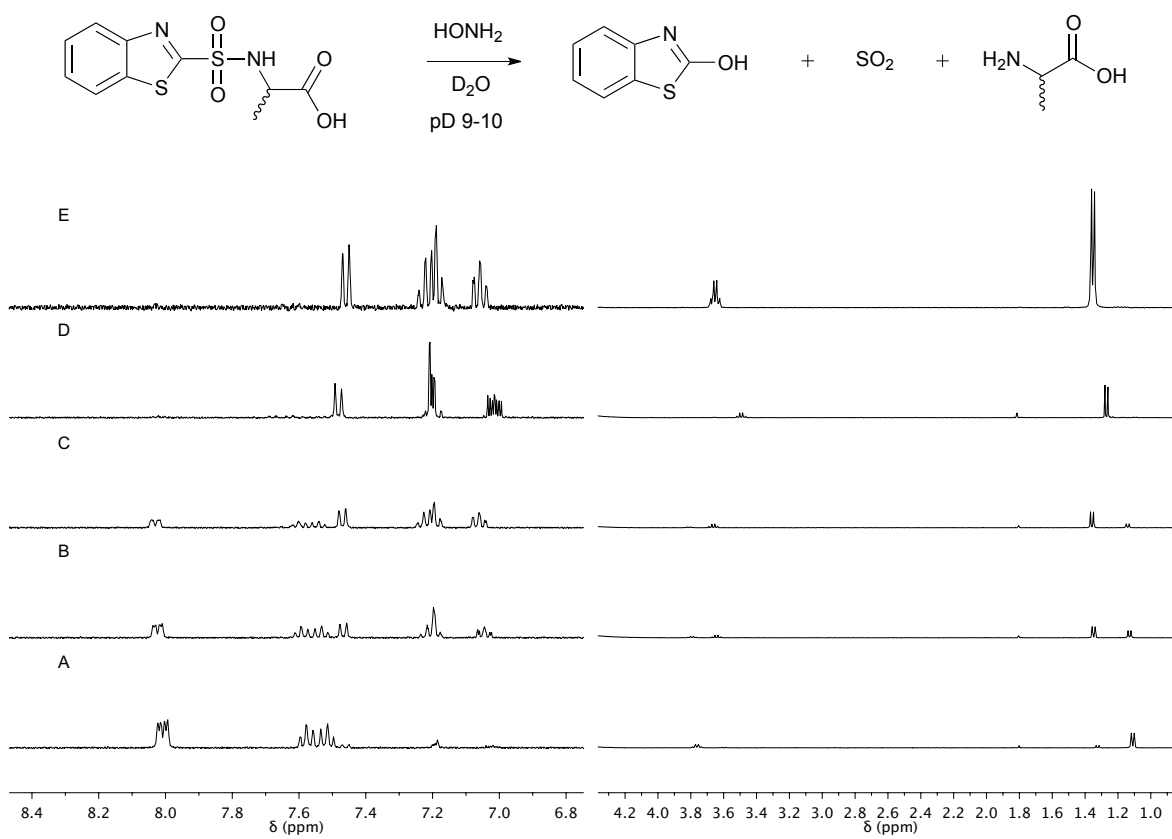


Figure S14. ¹H-NMR time course of hydroxylamine mediated cleavage of benzothiazole-2-sulfon-*N*-*DL*-alanine after A: 10 min, B: 2 h, C: 4 h, D: 6 h, E: doping of 2-hydroxybenzothiazole (L), doping of *DL*-alanine (R).

NMR time-course of hydroxylamine-mediated cleavage of benzothiazole-2-sulfon-*N-L*-phenylalanine

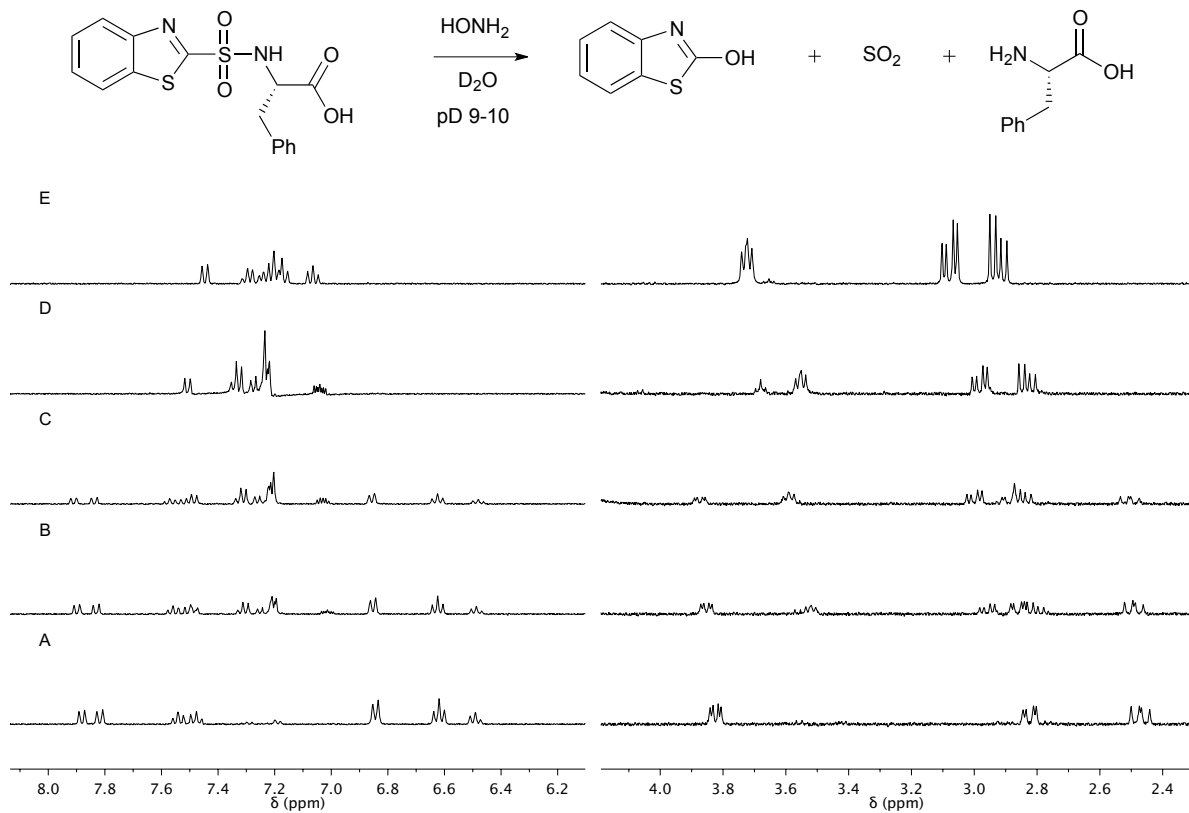


Figure S15. ¹H-NMR time course of hydroxylamine mediated cleavage of benzothiazole-2-sulfon-*N-L*-phenylalanine after A: 10 min, B: 2 h, C: 4 h, D: 7 h, E: doping of 2-hydroxybenzothiazole (L), doping of *L*-phenylalanine (R).

NMR time-course of hydroxylamine-mediated cleavage of methyl benzothiazole-2-sulfonglycinate

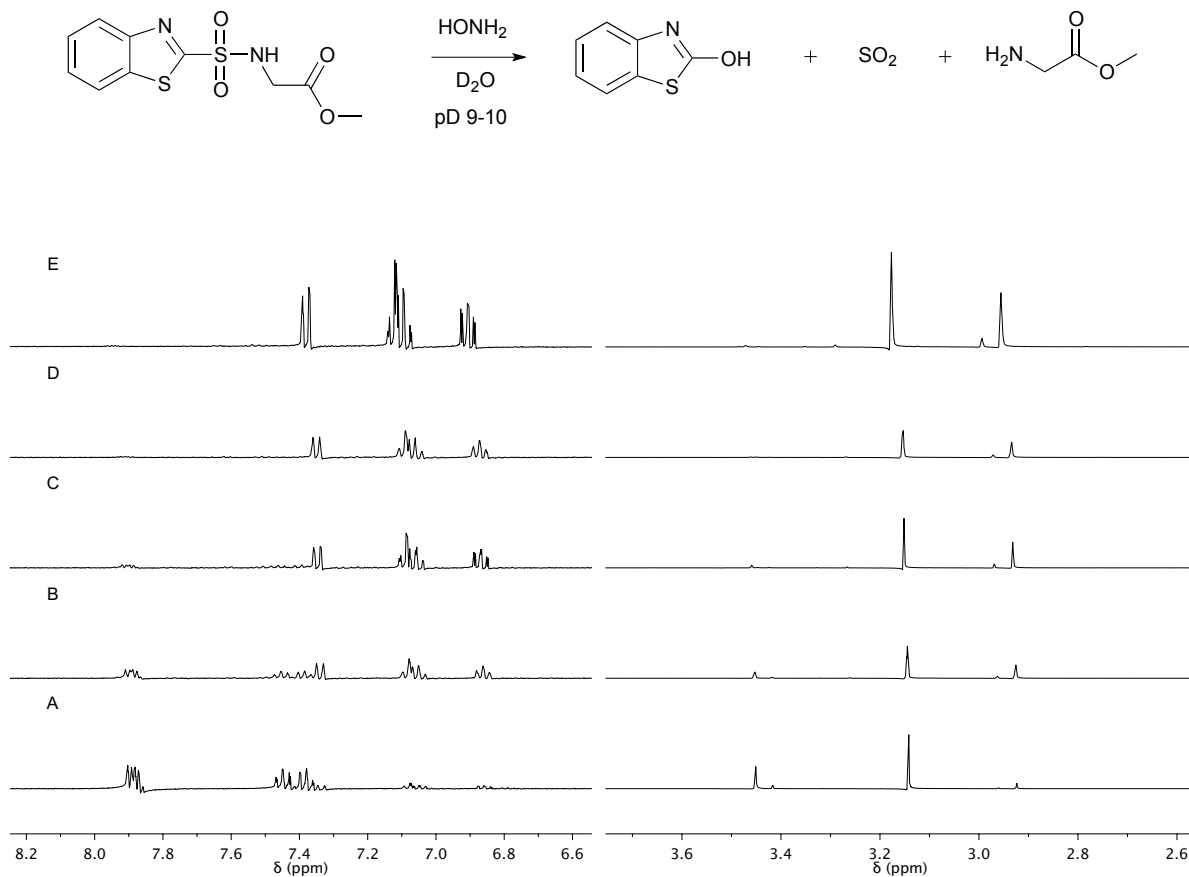


Figure S16: ¹H-NMR time course of hydroxylamine mediated cleavage of methyl benzothiazole-2-sulfoglycinate after A: 10 min, B: 2 h, C: 4 h, D: 6 h, E: doping of 2-hydroxybenzothiazole (L), doping of glycine methyl ester hydrochloride (R).

NMR time-course of hydroxylamine-mediated cleavage of 5-chloro-benzothiazole-2-sulfonamide

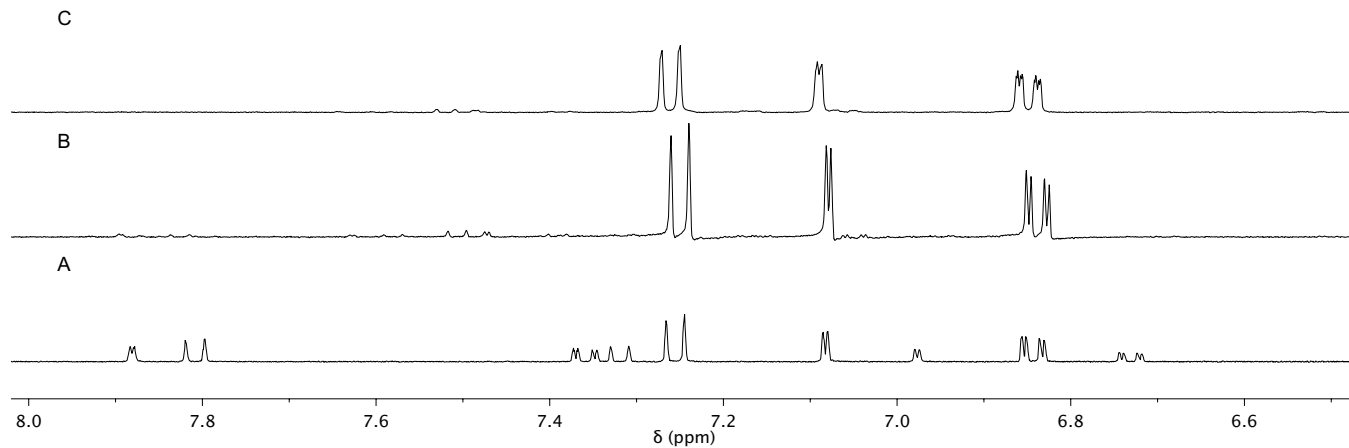
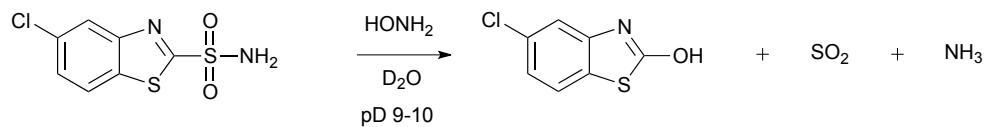


Figure S17: ¹H-NMR time course of hydroxylamine mediated cleavage of 5-chloro-benzothiazole-2-sulfonamide after A: 10 min, B: 30 min, C: doping of 2-hydroxy-5-chloro-benzothiazole.

IV. Additional NMR data and LC-MS data

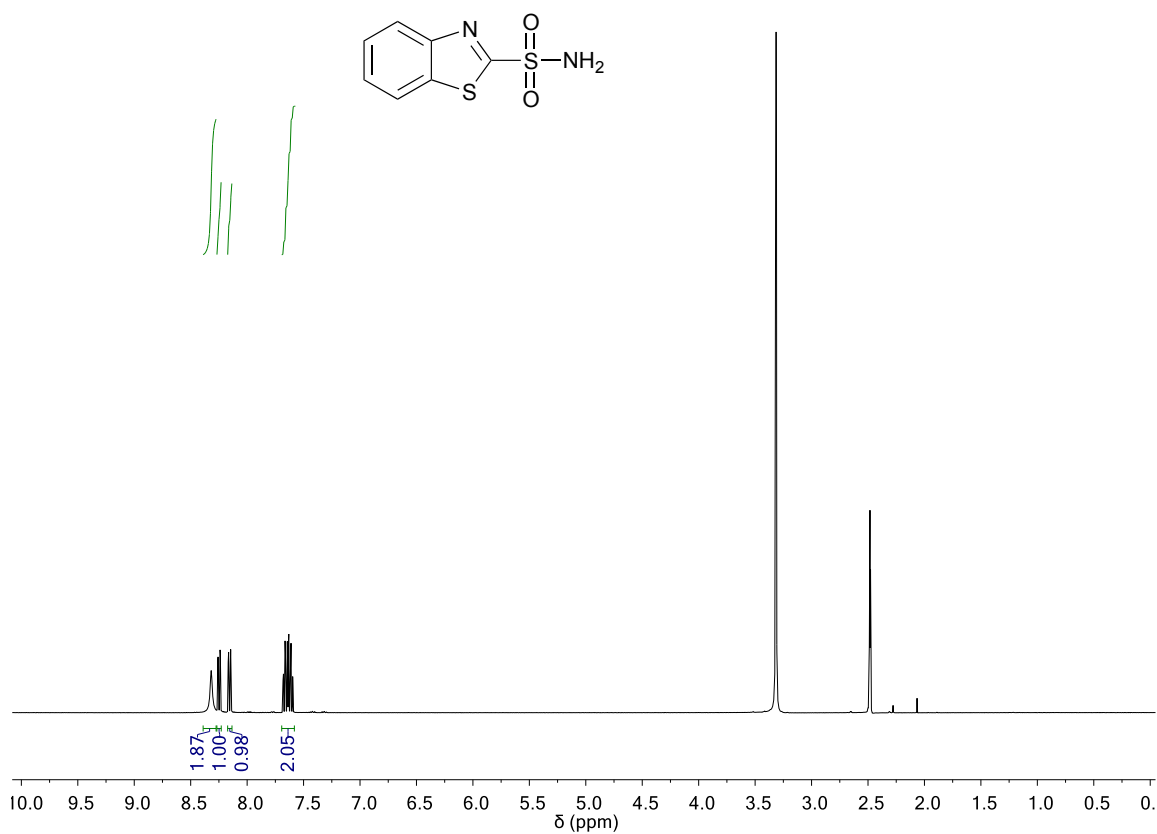


Figure S18. $^1\text{H NMR}$ of benzothiazole-2-sulfonamide.

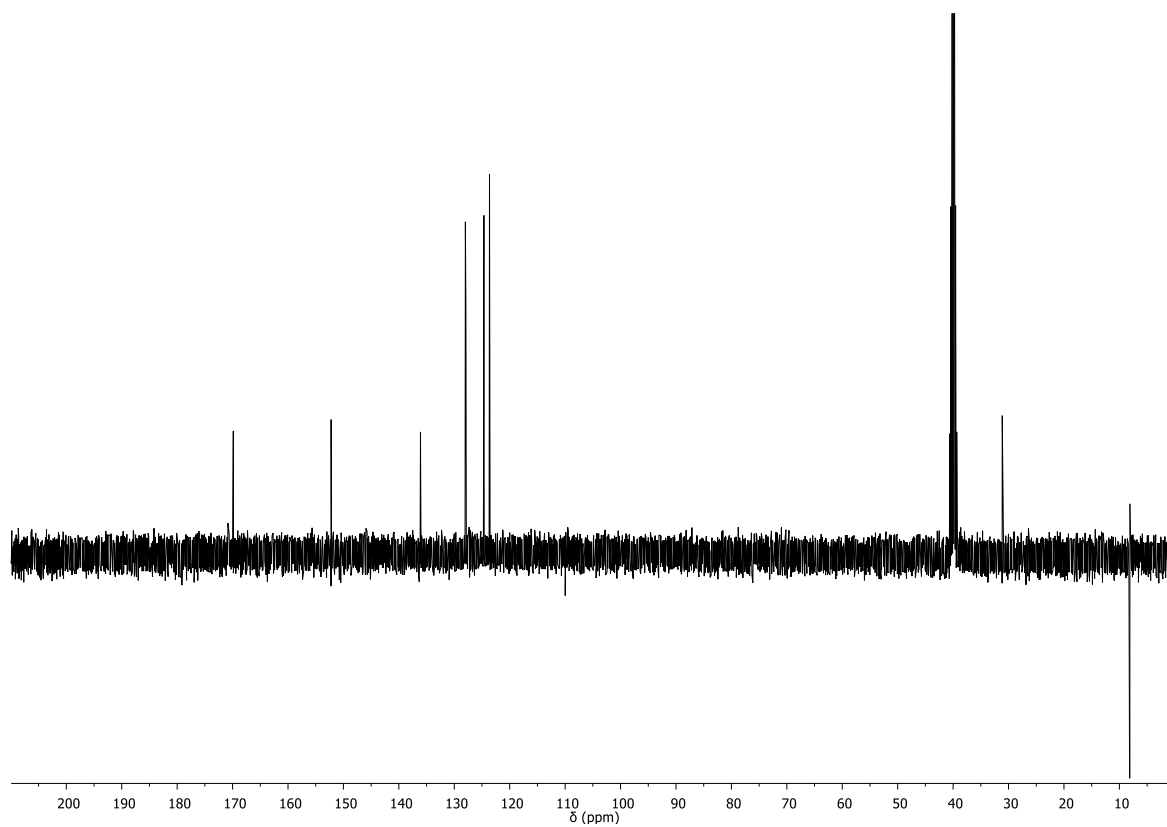


Figure S19. $^{13}\text{C NMR}$ of benzothiazole-2-sulfonamide.

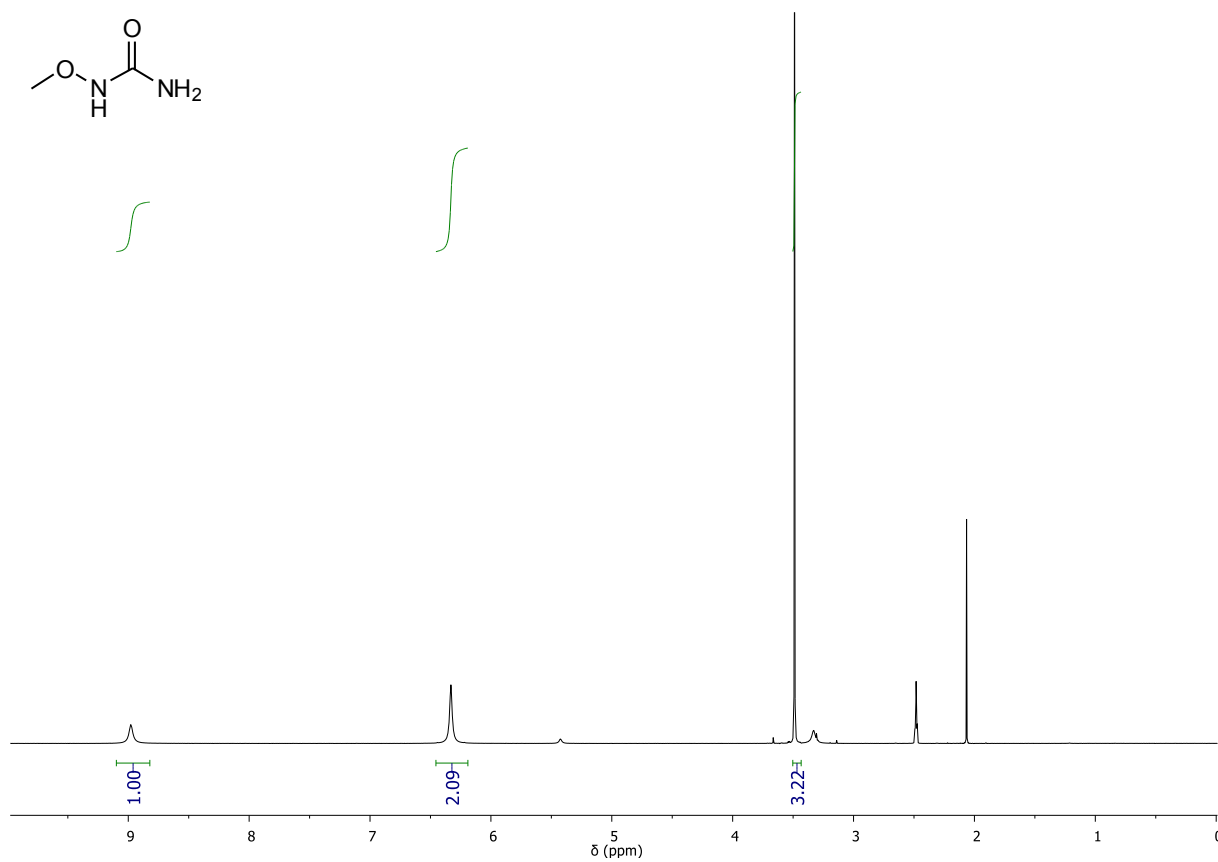


Figure S20. ¹H NMR of methoxyurea.

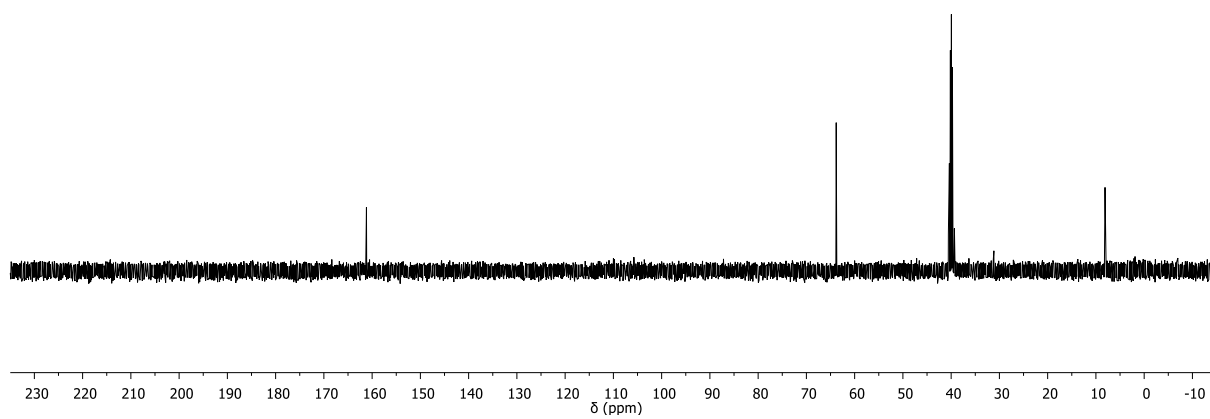


Figure S21. ¹³C NMR of methoxyurea.

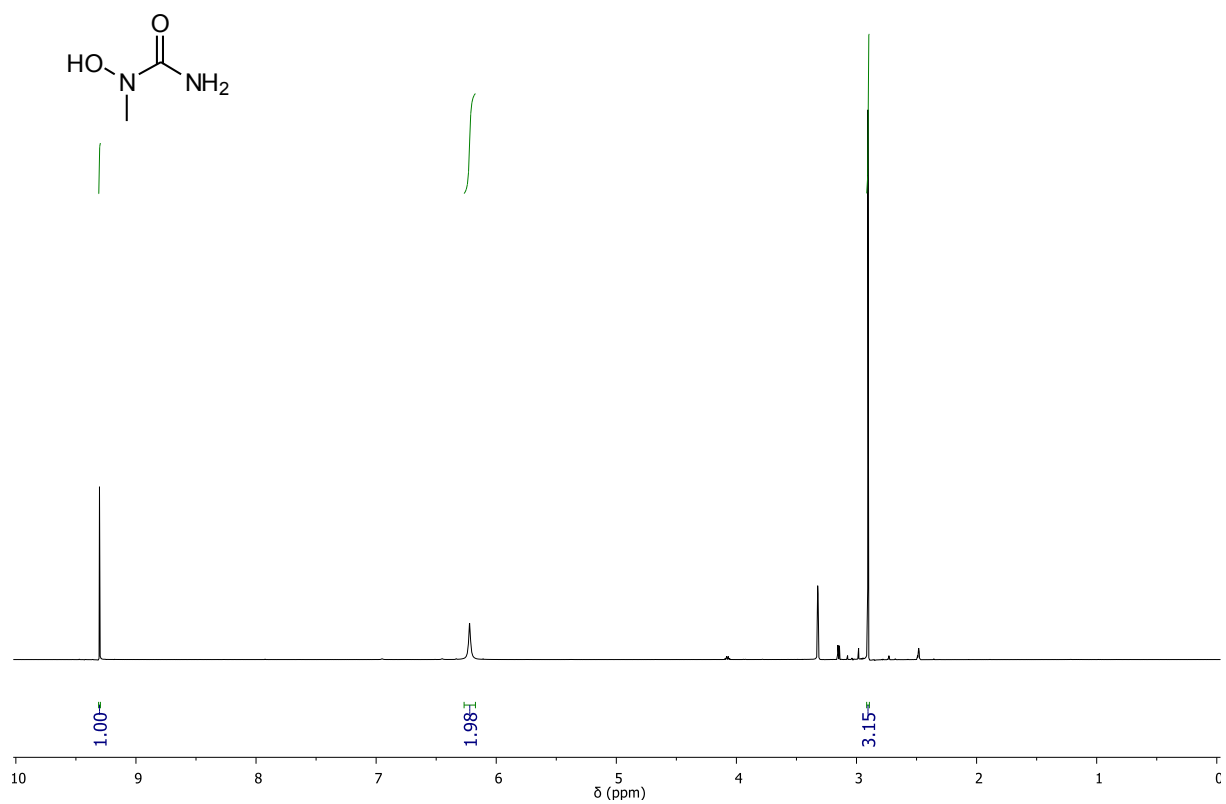


Figure S22. ¹H NMR of *N*-methyl hydroxyurea.

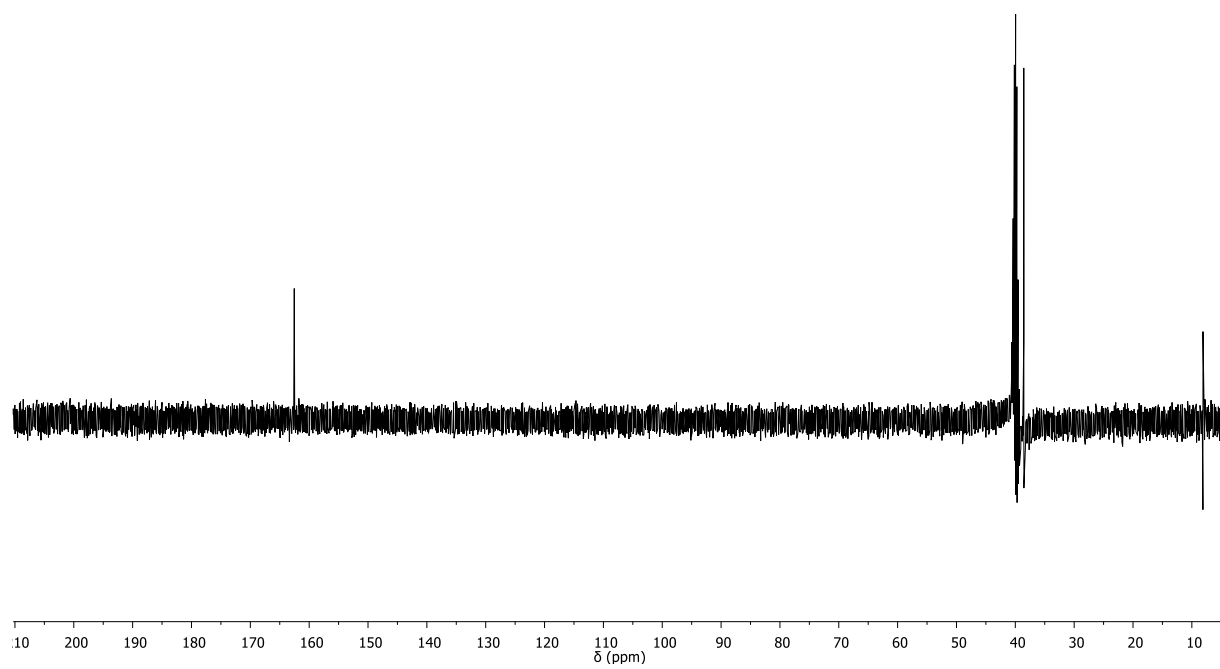


Figure S23. ¹³C NMR of *N*-methyl hydroxyurea.

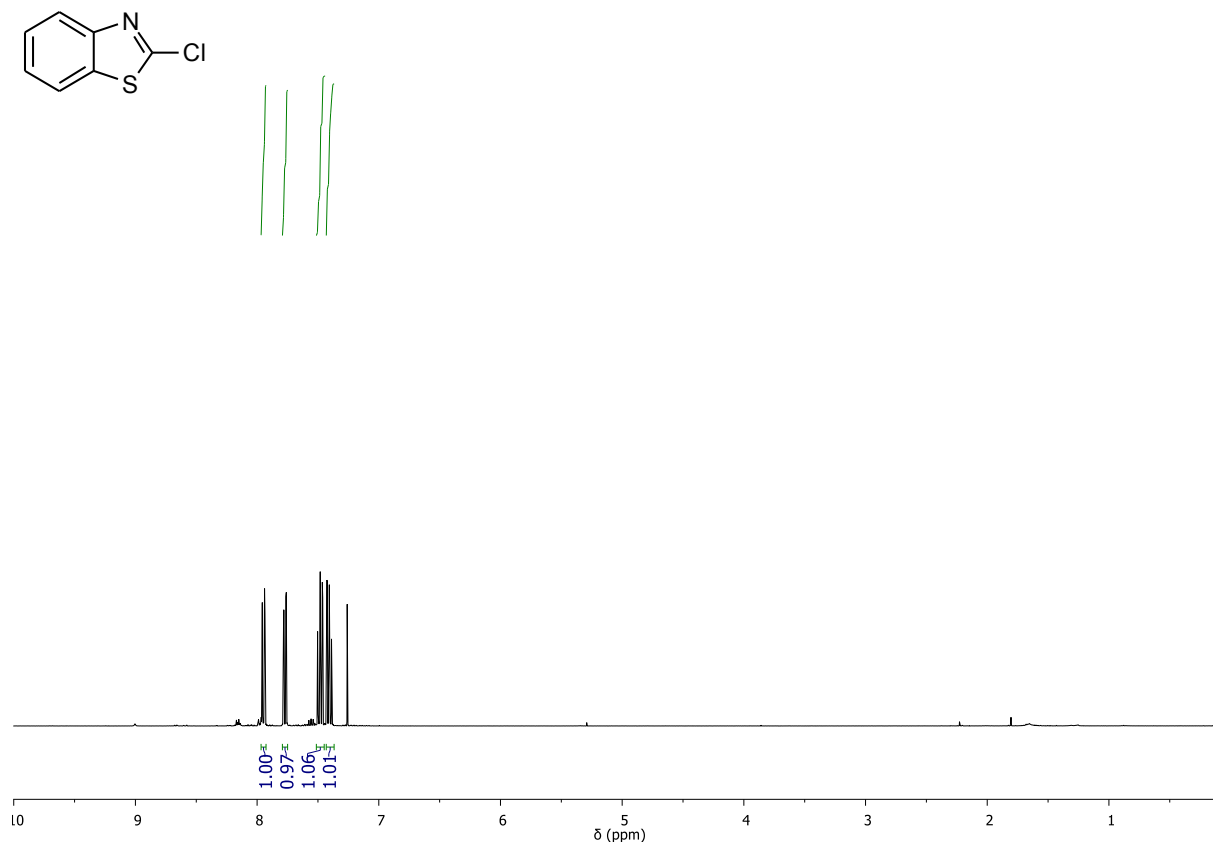


Figure S24. ¹H NMR of 2-chlorobenzothiazole.

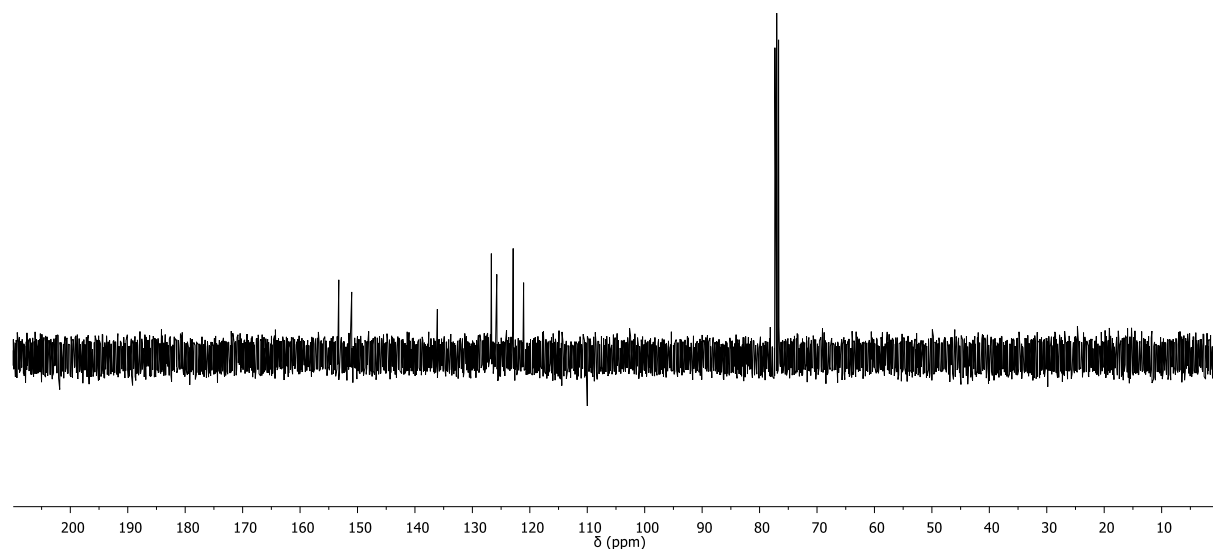


Figure S25. ¹³C NMR of 2-chlorobenzothiazole.

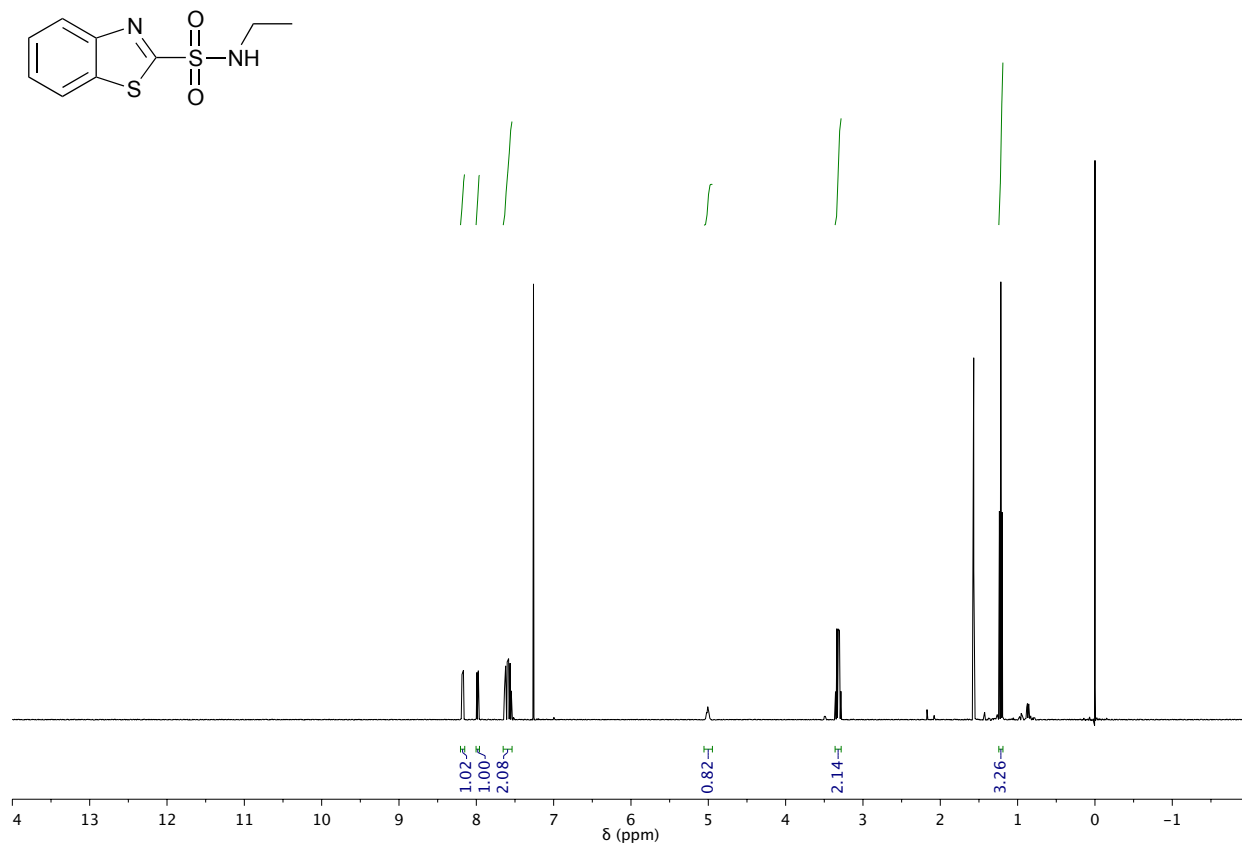


Figure S26. ¹H-NMR spectrum of *N*-ethyl-benzothiazole-2-sulfonamide.

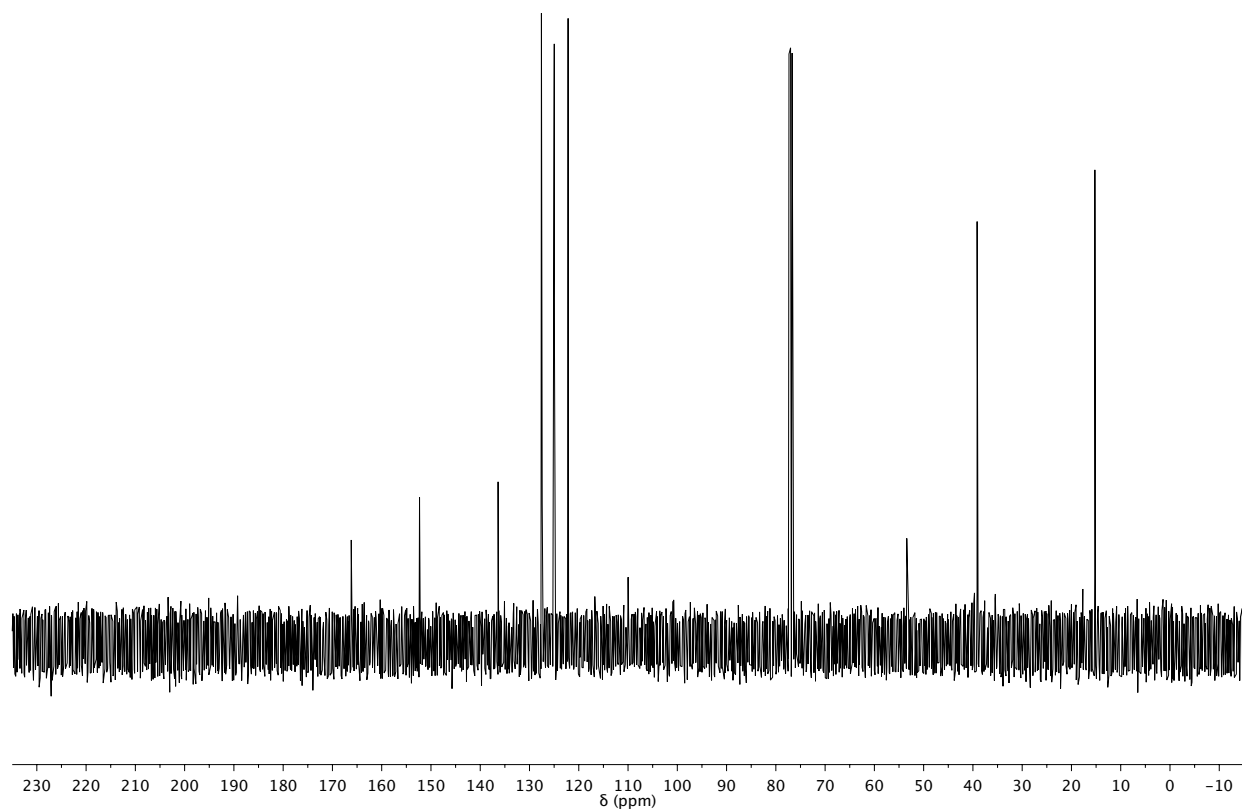


Figure S27. ¹³C-NMR spectrum of *N*-ethyl-benzothiazole-2-sulfonamide.

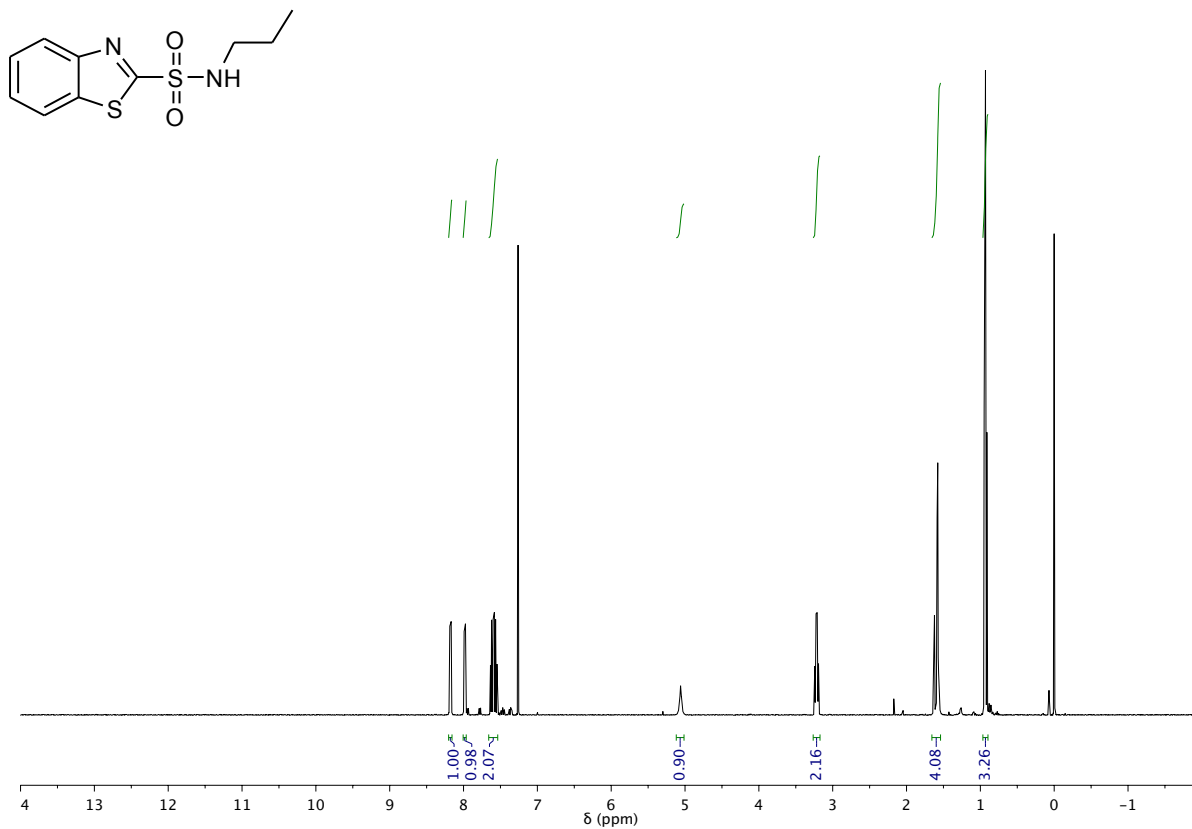


Figure S28. ¹H-NMR spectrum of *N*-propyl-benzothiazole-2-sulfonamide.

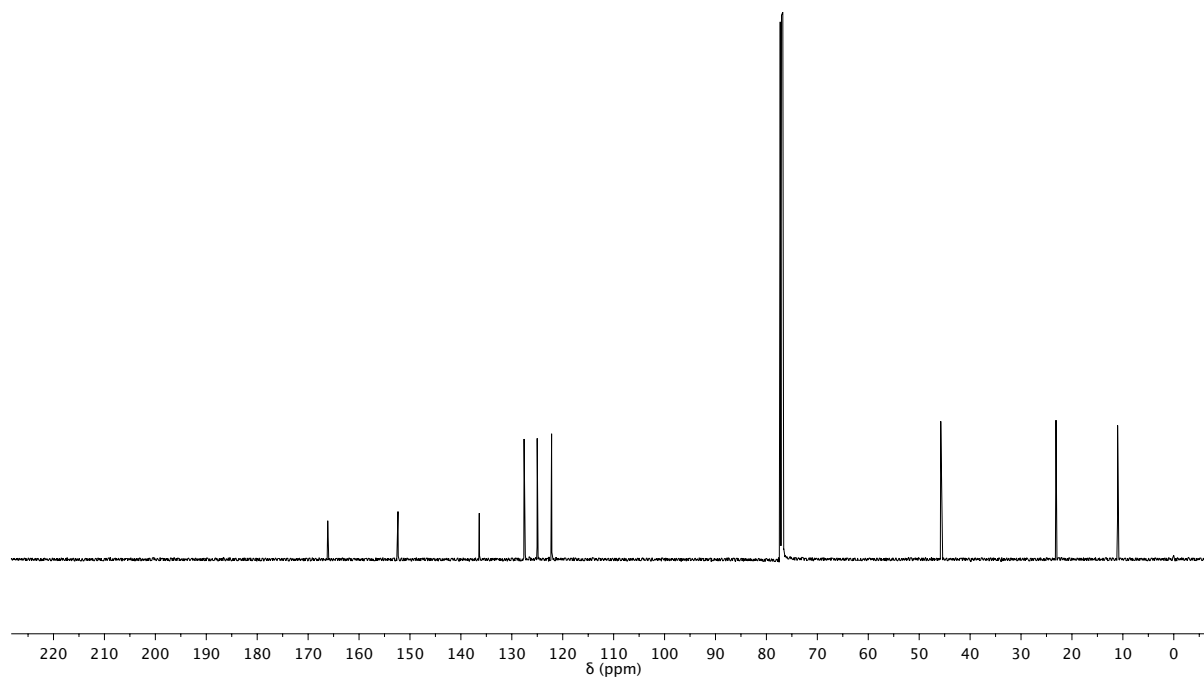


Figure 29. ¹³C-NMR spectrum of *N*-propyl-benzothiazole-2-sulfonamide.

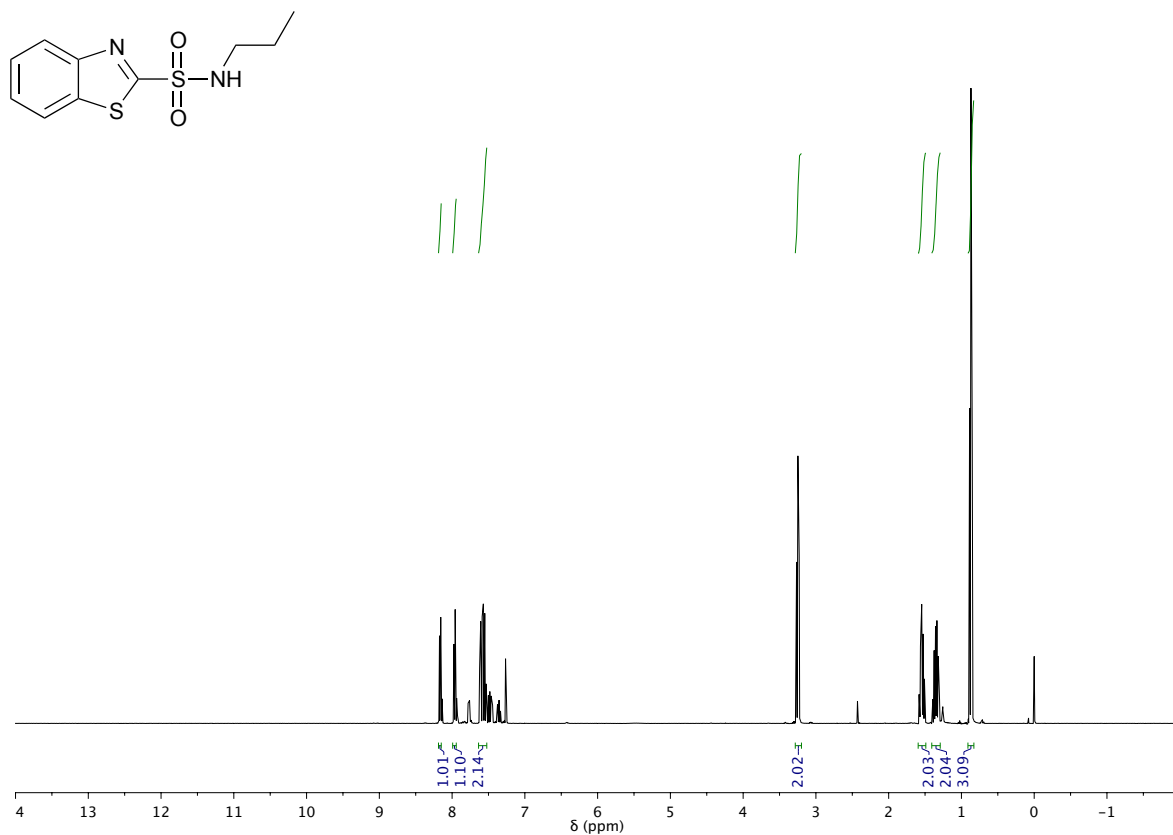


Figure 30. ¹H-NMR spectrum of *N*-Butyl-benzothiazole-2-sulfonamide.

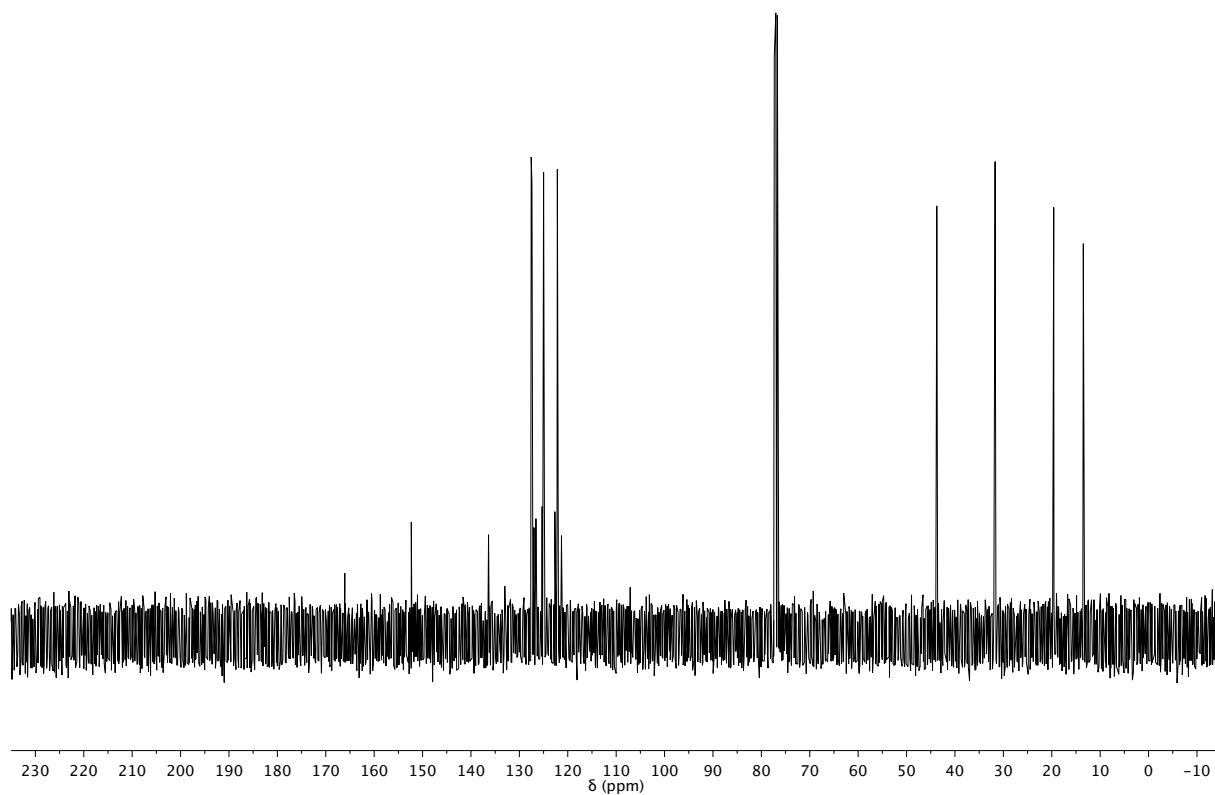


Figure 31. ¹³C-NMR spectrum of *N*-butyl-benzothiazole-2-sulfonamide.

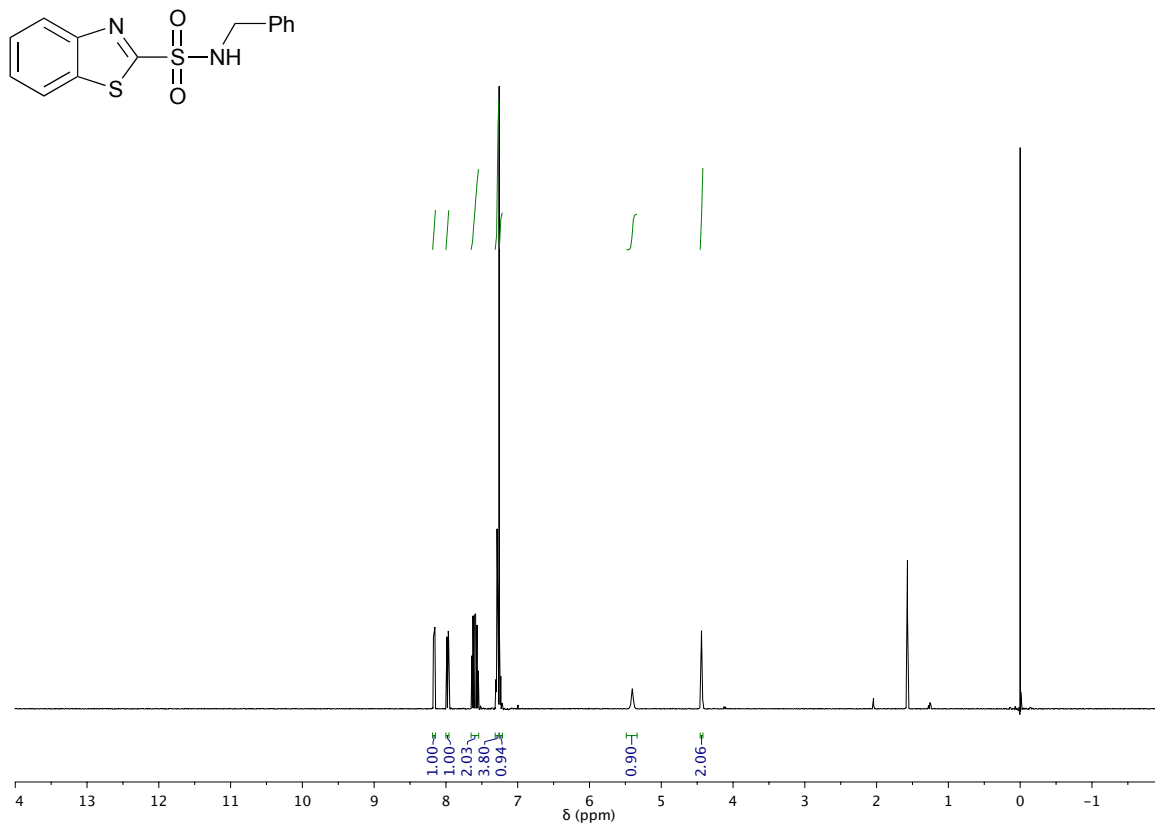


Figure S32. ¹H-NMR spectrum of *N*-benzyl-benzothiazole-2-sulfonamide.

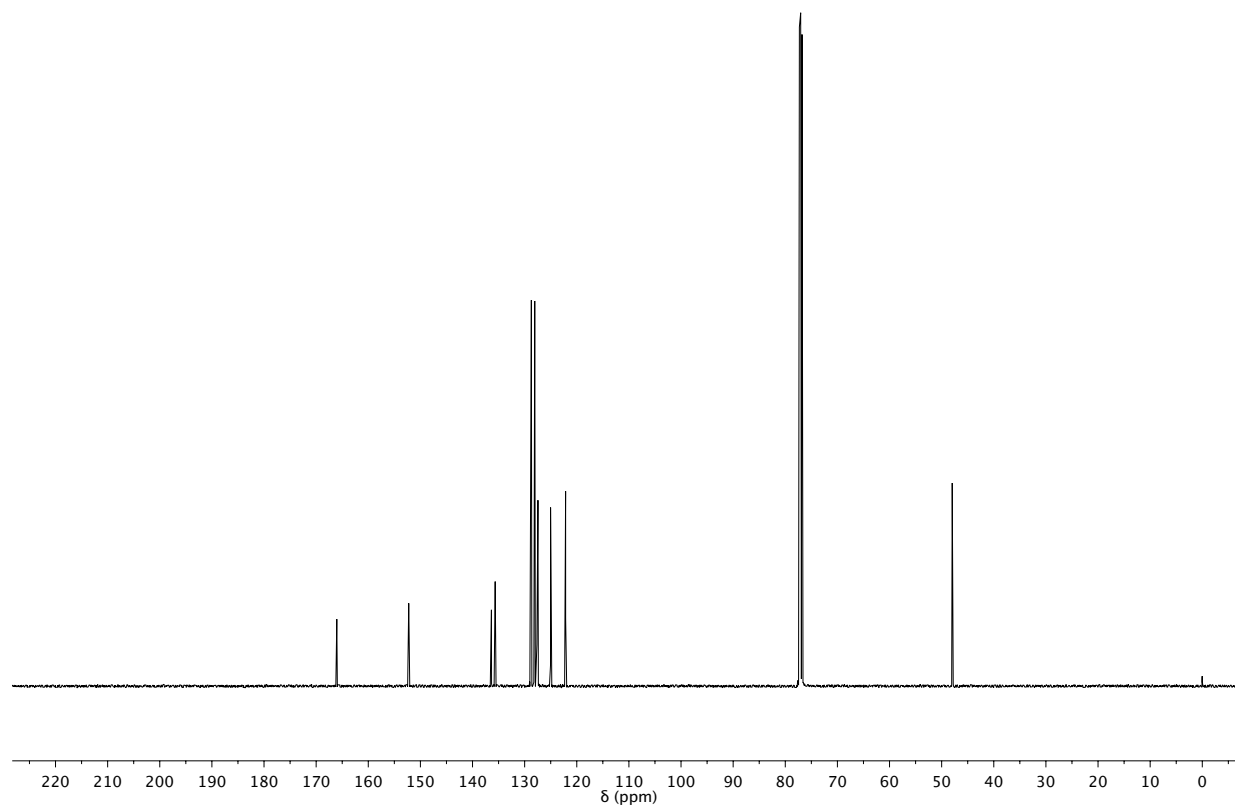


Figure S33. ¹³C-NMR spectrum of *N*-benzyl-benzothiazole-2-sulfonamide.

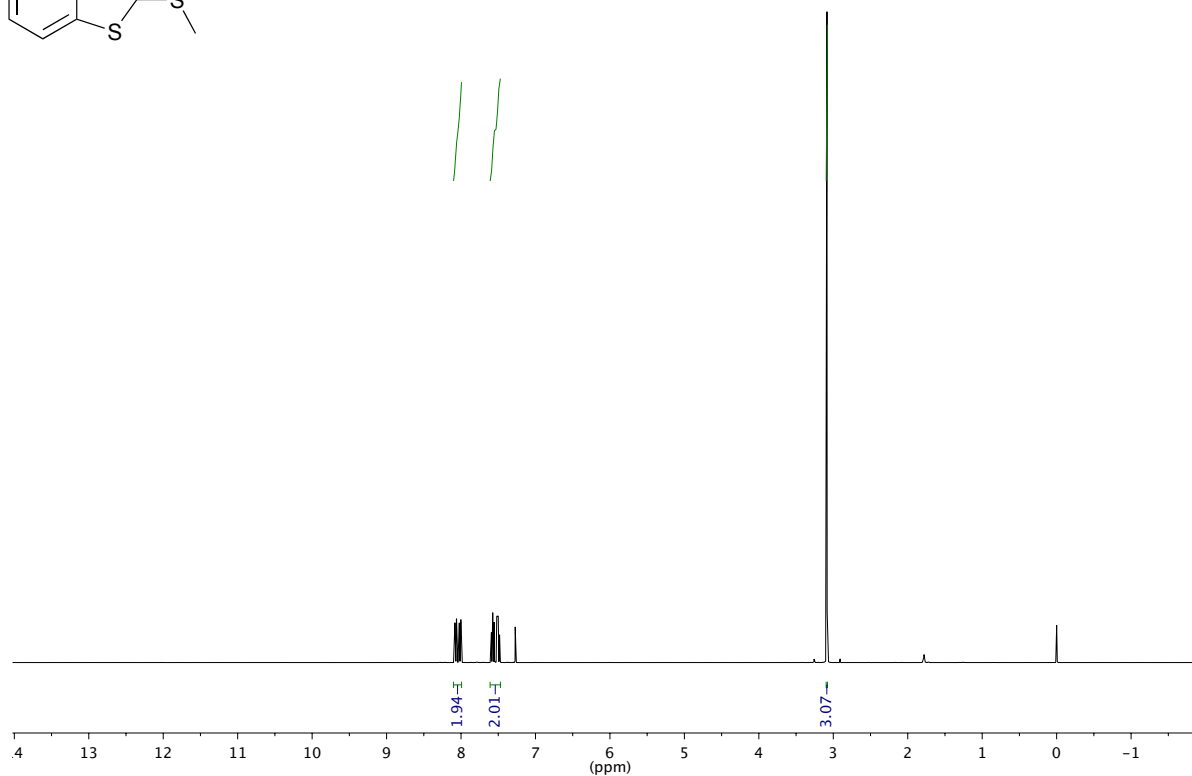
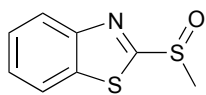


Figure S34. ¹H-NMR spectrum of 2-(sulfinylmethyl)-benzothiazole.

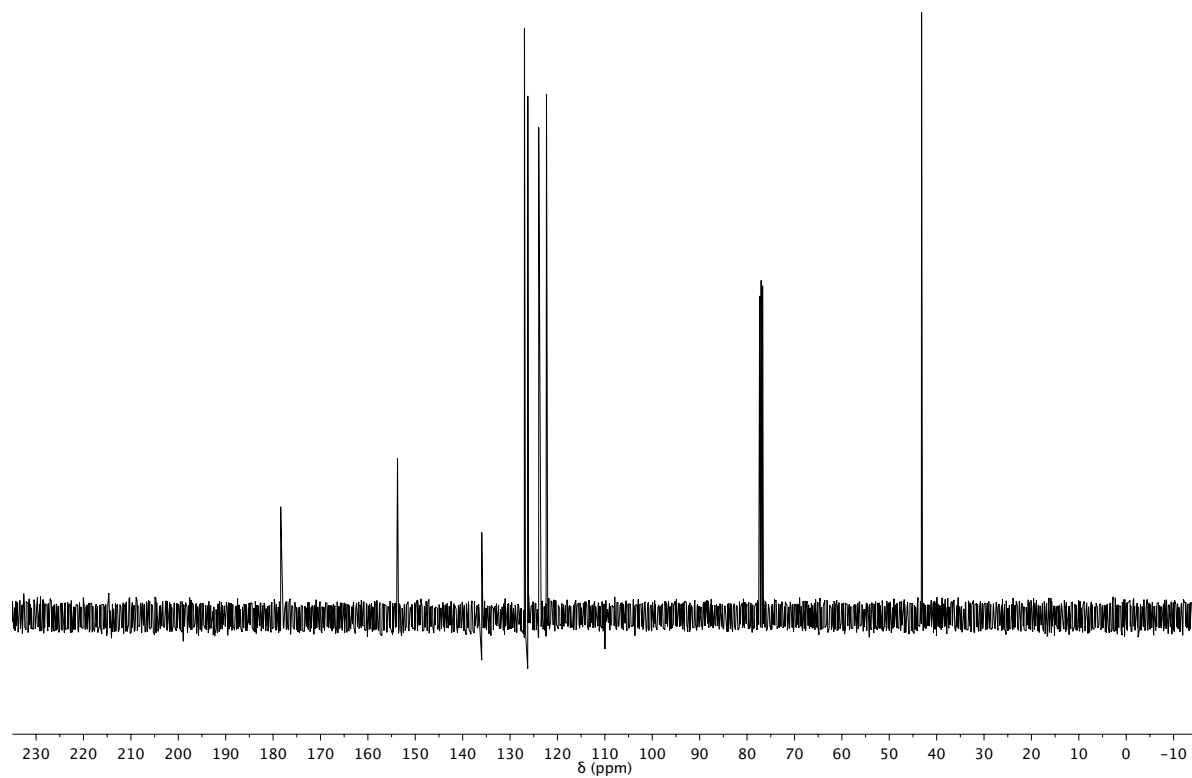


Figure S35. ¹³C-NMR spectrum of 2-(sulfinylmethyl)-benzothiazole.

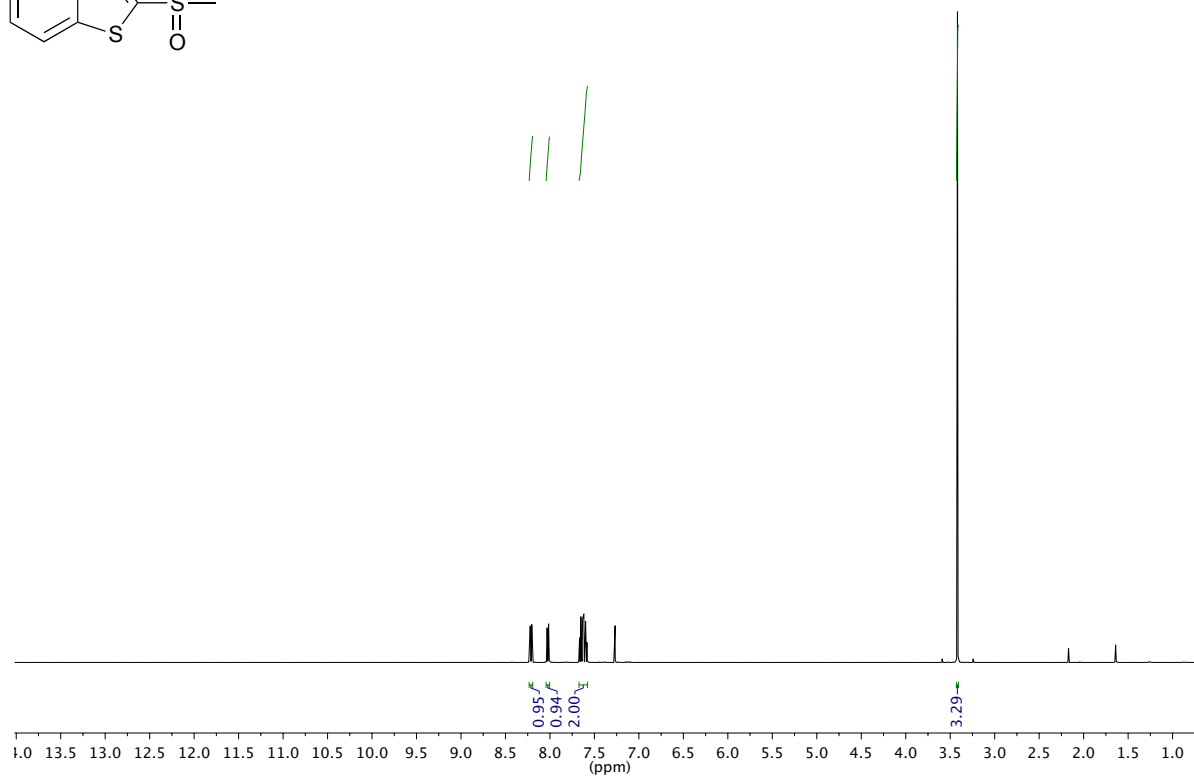
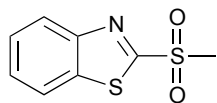


Figure S36. ¹H-NMR spectrum of 2-(sulfonylmethyl)-benzothiazole.

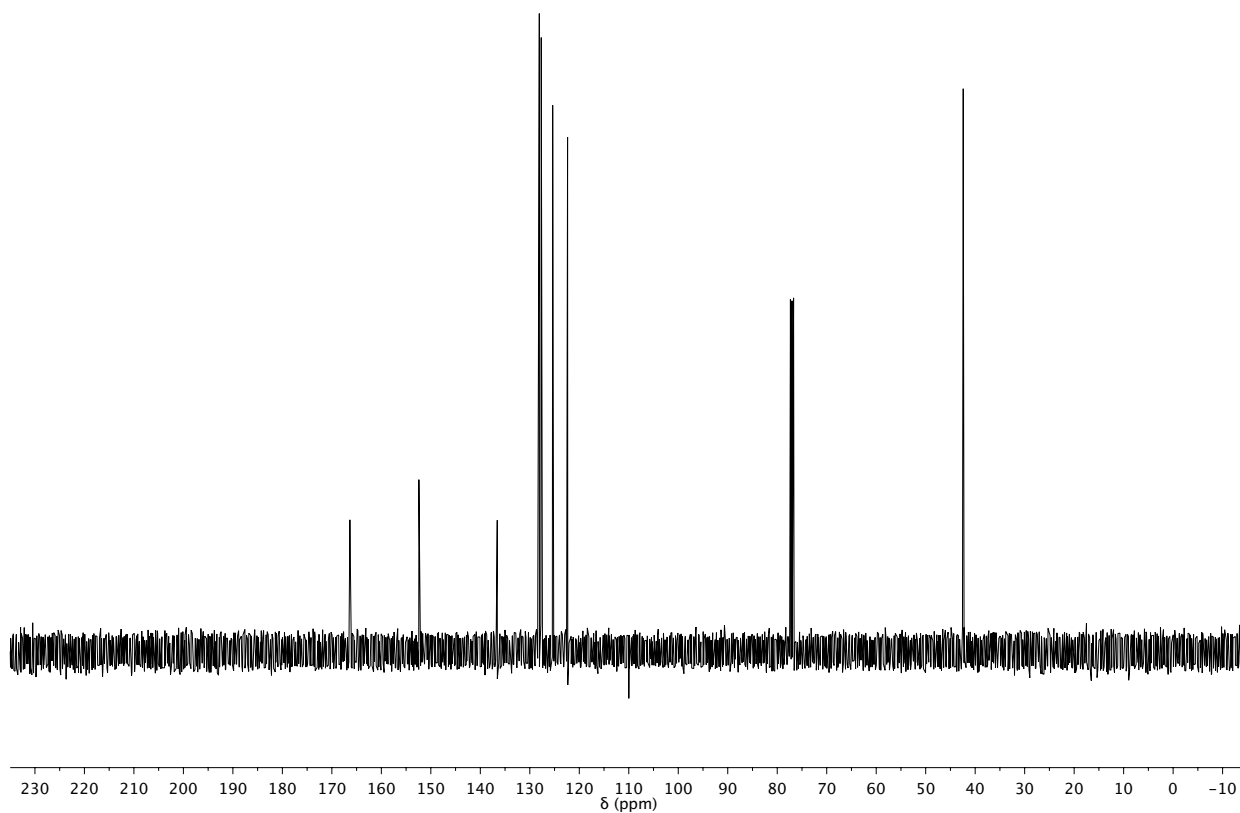


Figure S37. ¹³C-NMR spectrum of 2-(sulfonylmethyl)-benzothiazole.

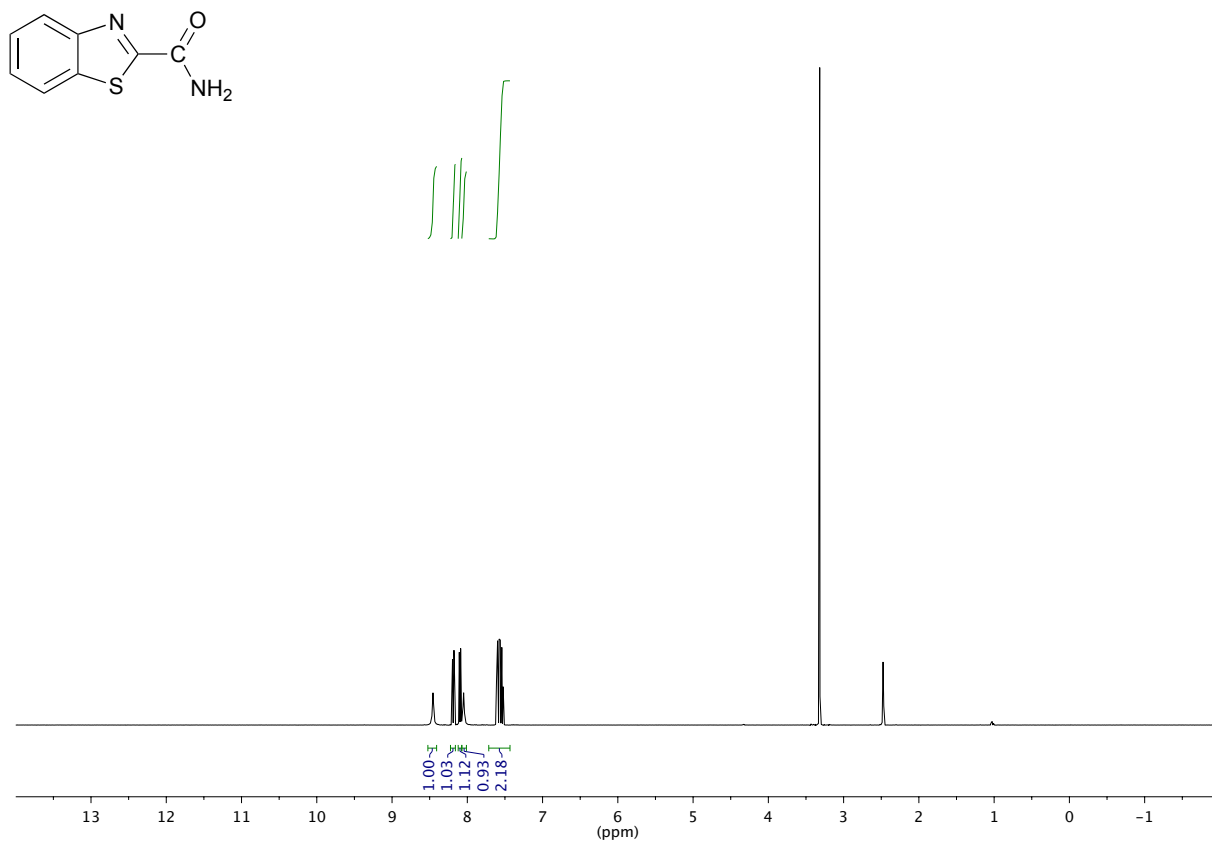


Figure S38. ^1H -NMR spectrum of benzothiazole-2-amide.

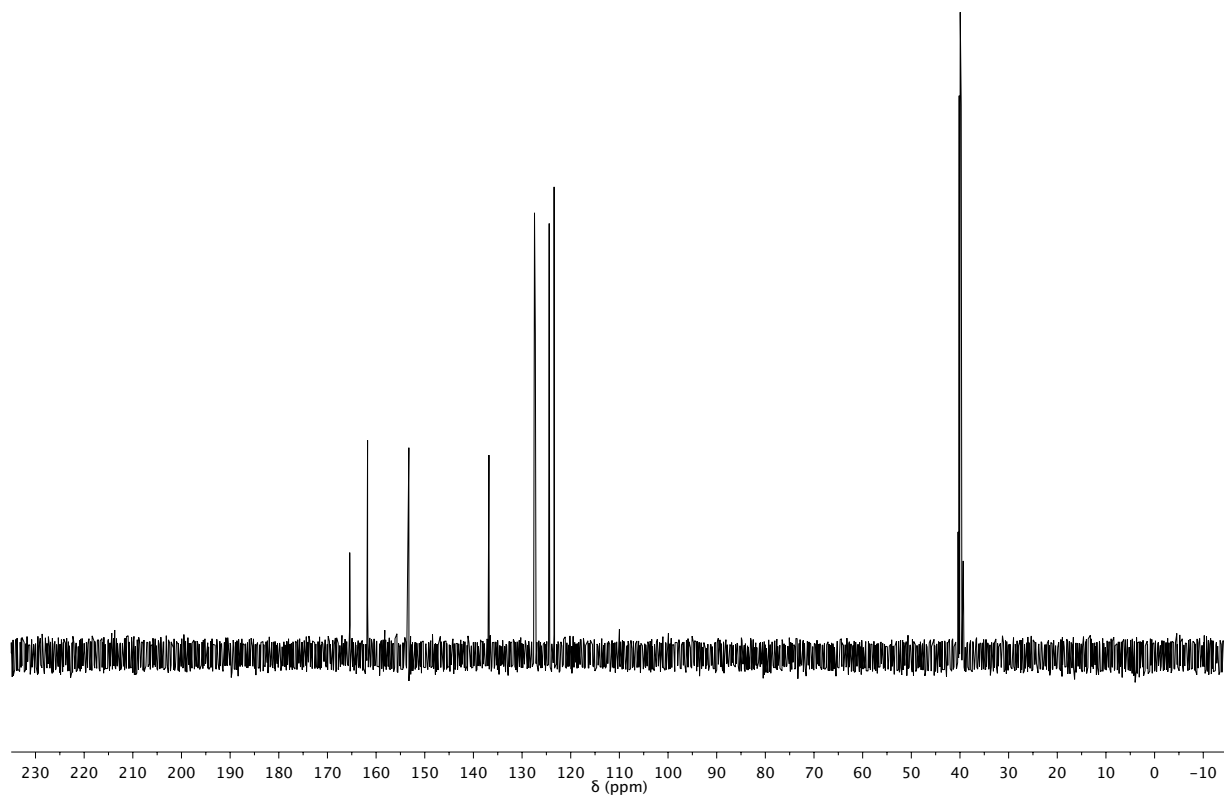


Figure S39. ^{13}C -NMR spectrum of benzothiazole-2-amide.

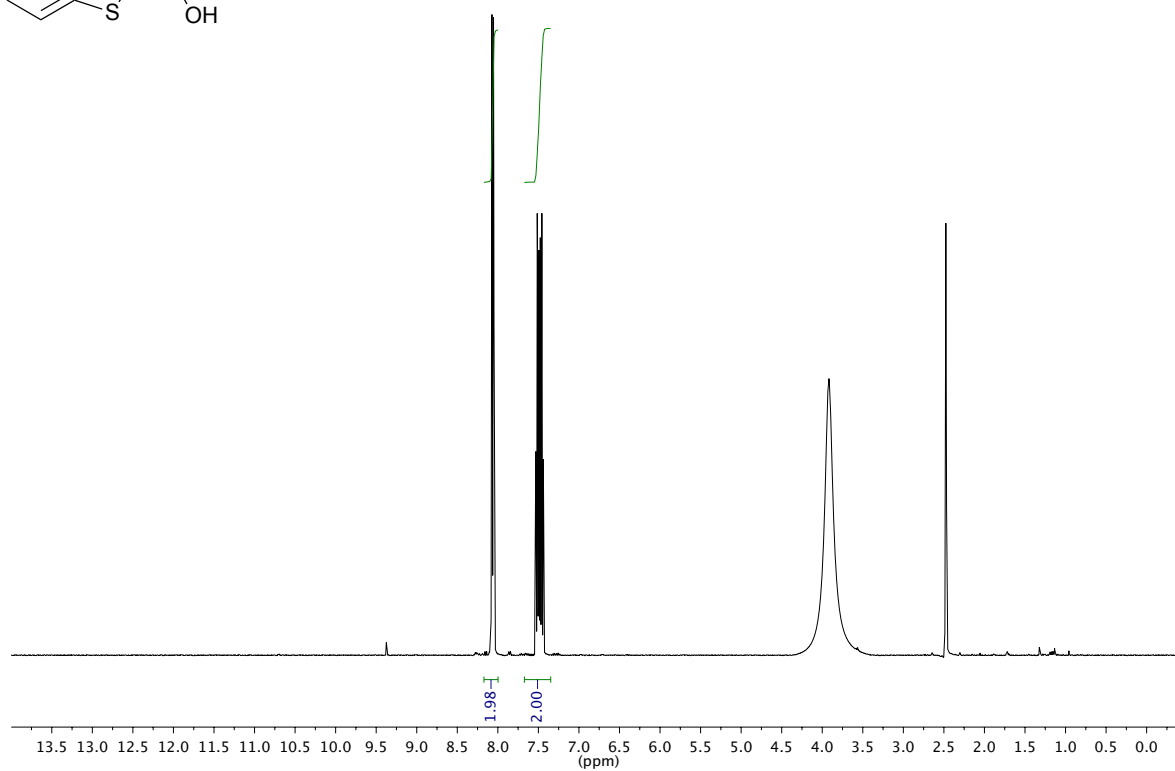
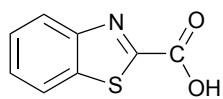


Figure S40. $^1\text{H-NMR}$ spectrum of benzo[1,2-b]thiazole-2-carboxylic acid.

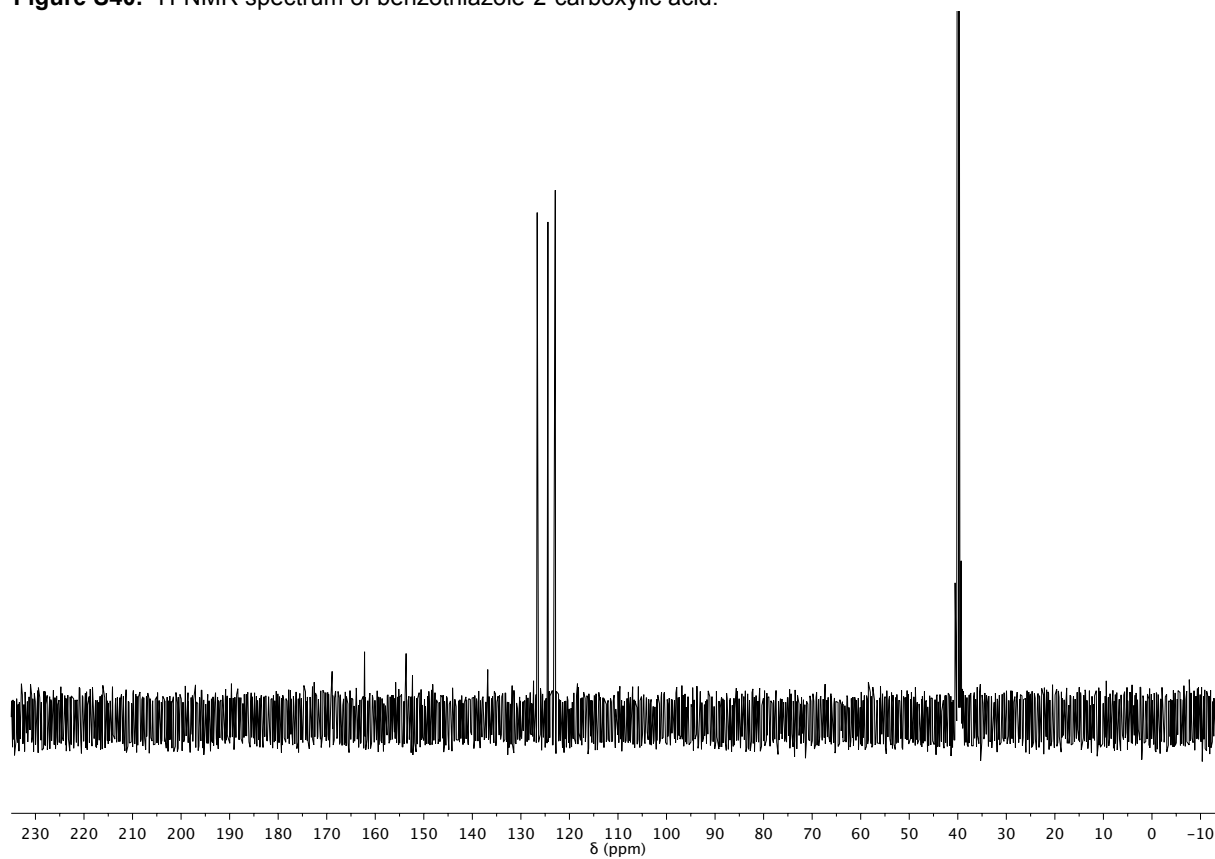


Figure S41. $^{13}\text{C-NMR}$ spectrum of benzo[1,2-b]thiazole-2-carboxylic acid.

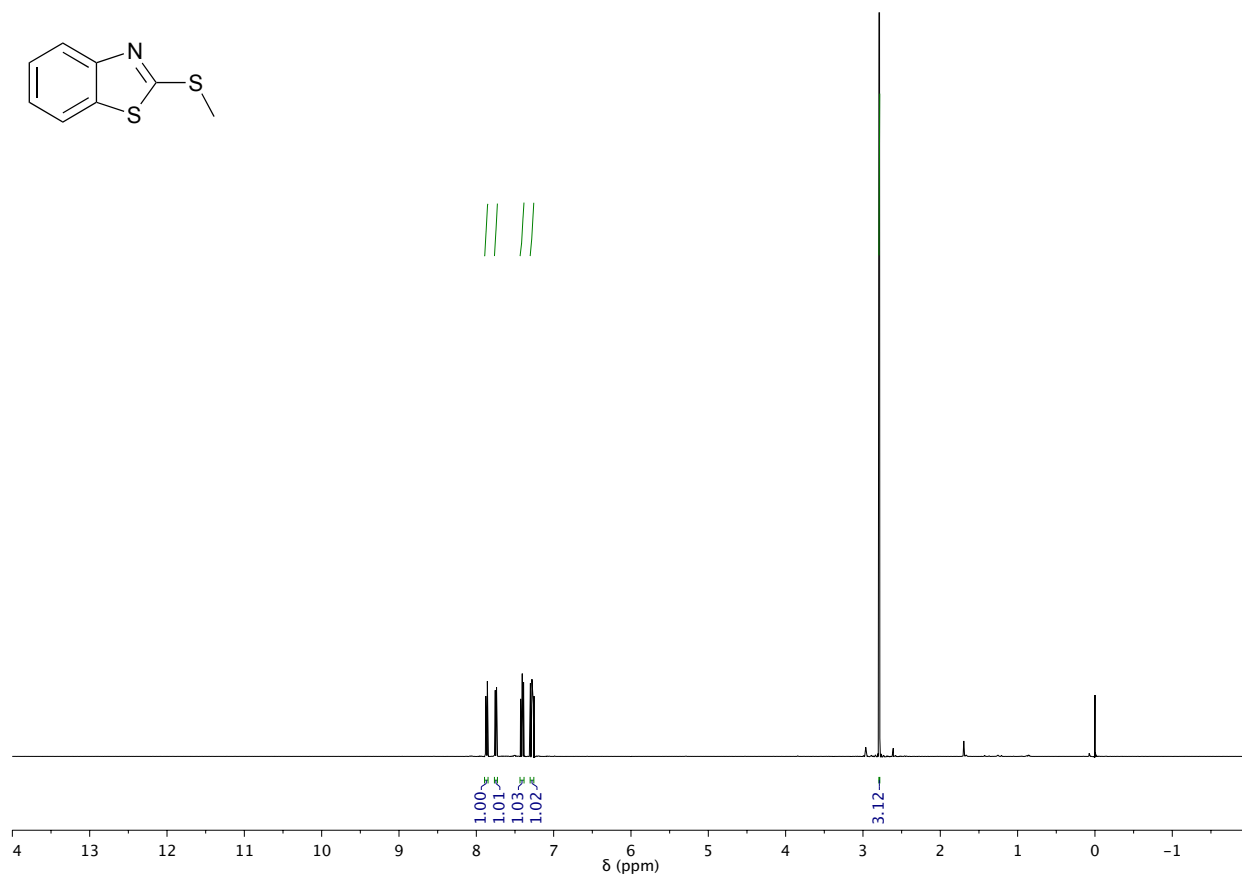


Figure S42. ¹H-NMR spectrum of methyl benzothiazole-2-sulfide.

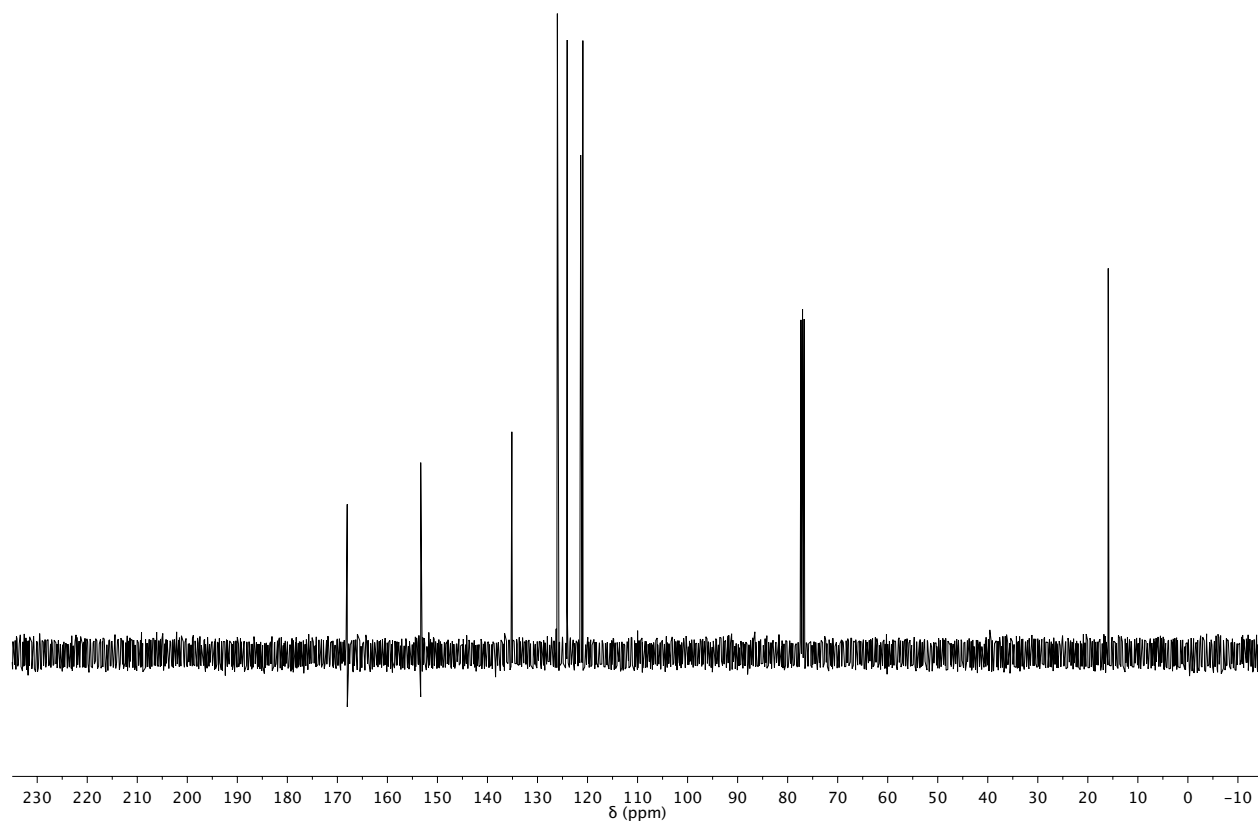


Figure S43. ¹³C-NMR spectrum of methyl benzothiazole-2-sulfide.

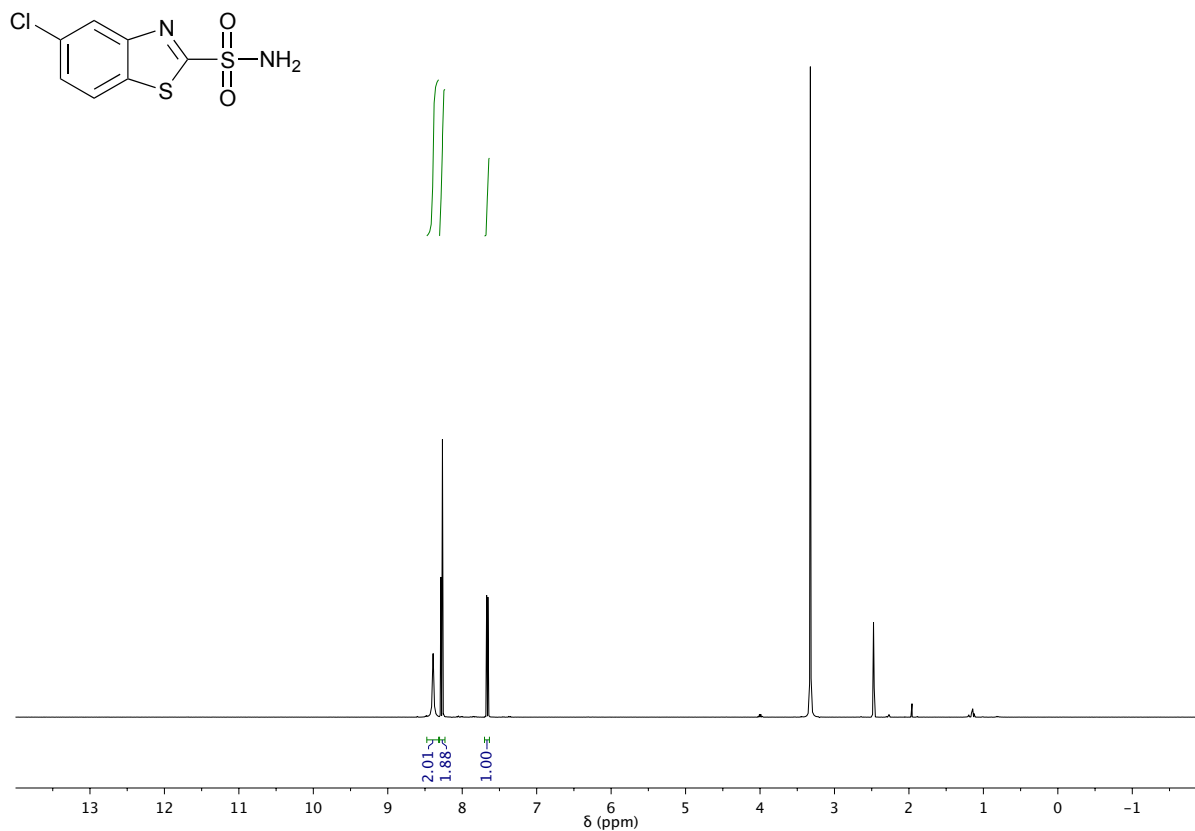


Figure S44. ¹H-NMR spectrum of 5-Chloro-benzothiazole-2-sulfonamide.

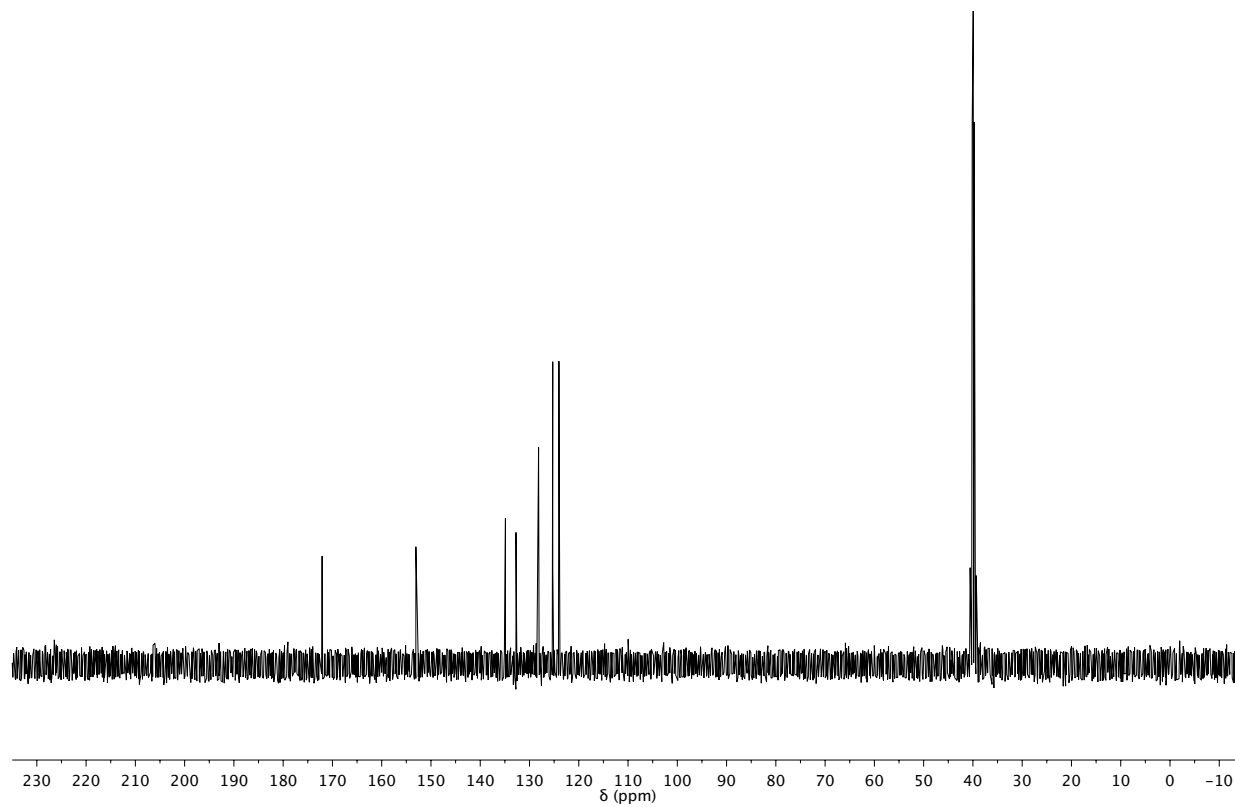


Figure S45. ¹³C-NMR spectrum of 5-Chloro-benzothiazole-2-sulfonamide.

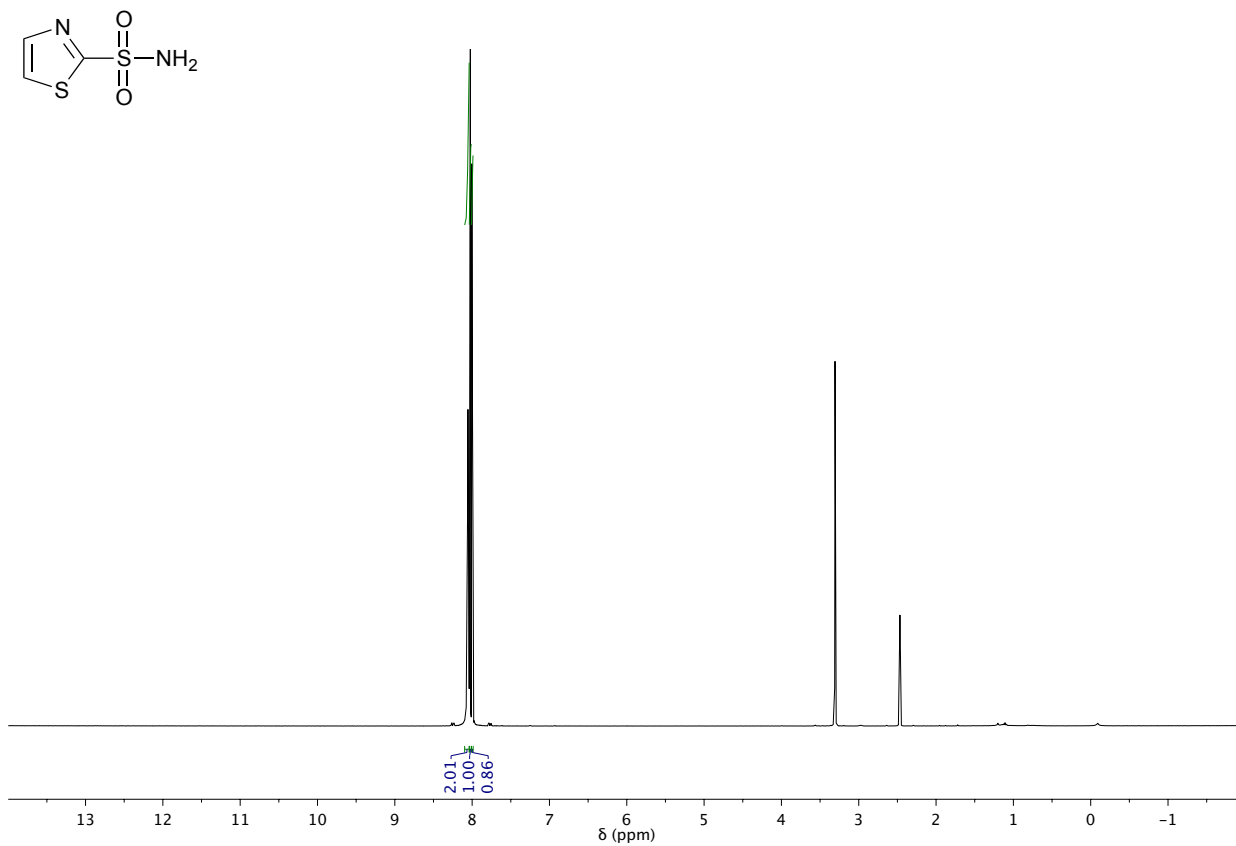


Figure S46. ¹H-NMR spectrum of thiazole-2-sulfonamide.

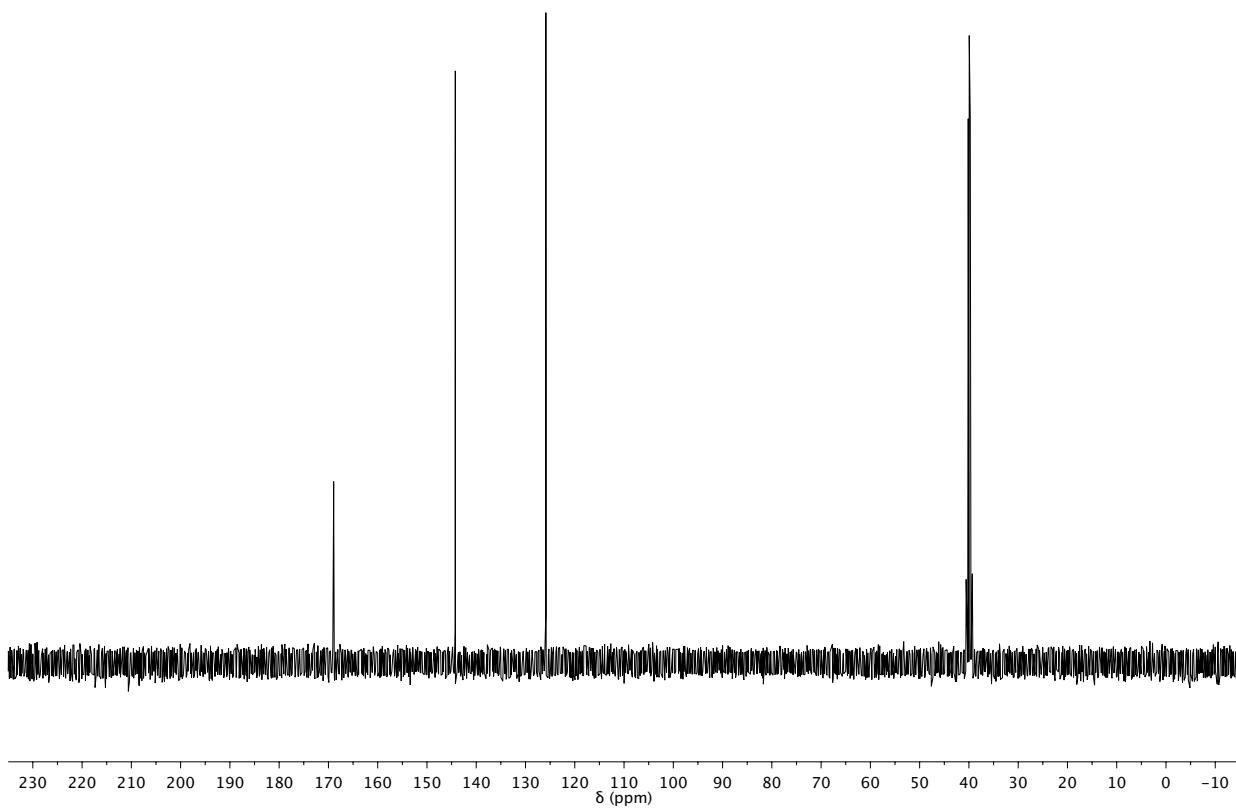


Figure S47. ¹³C-NMR spectrum of thiazole-2-sulfonamide.

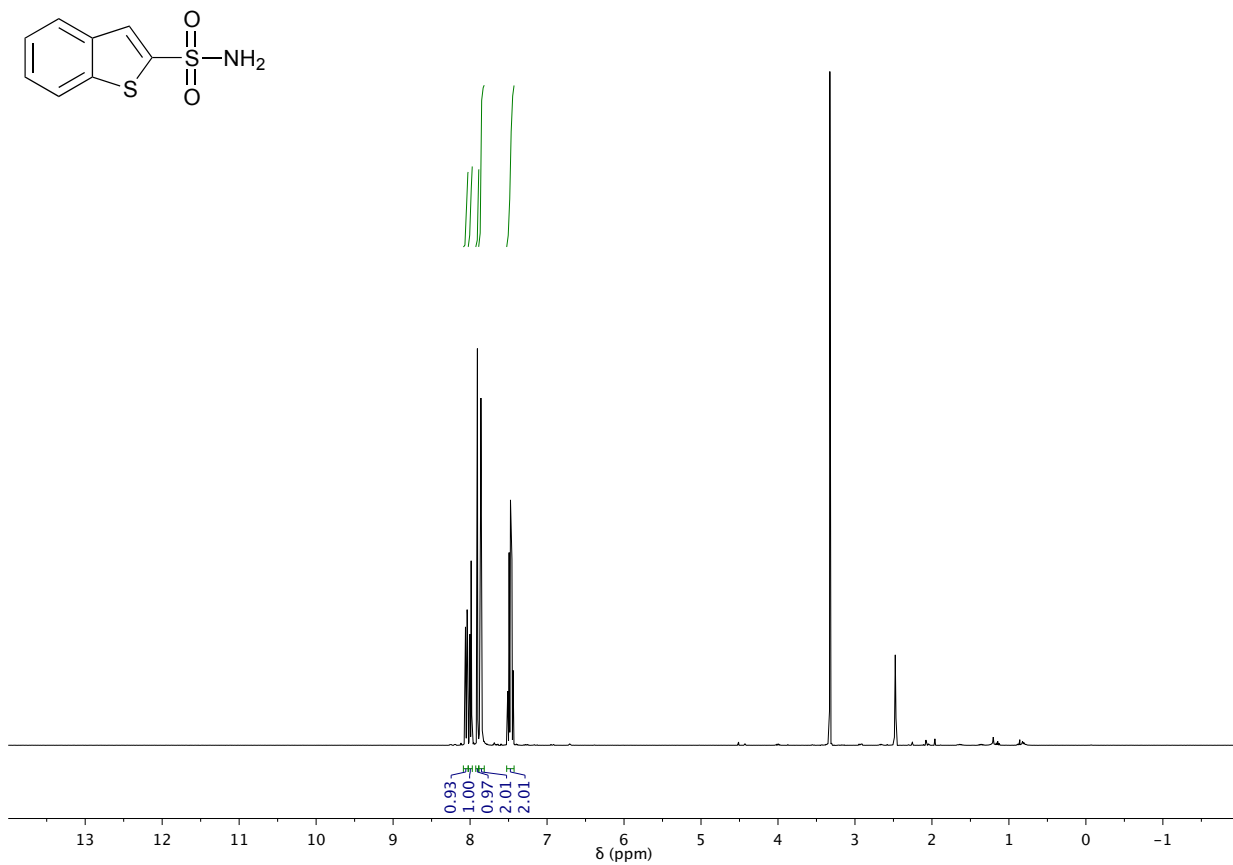


Figure S48. ¹H-NMR spectrum of Benzothiophene-2-sulfonamide.

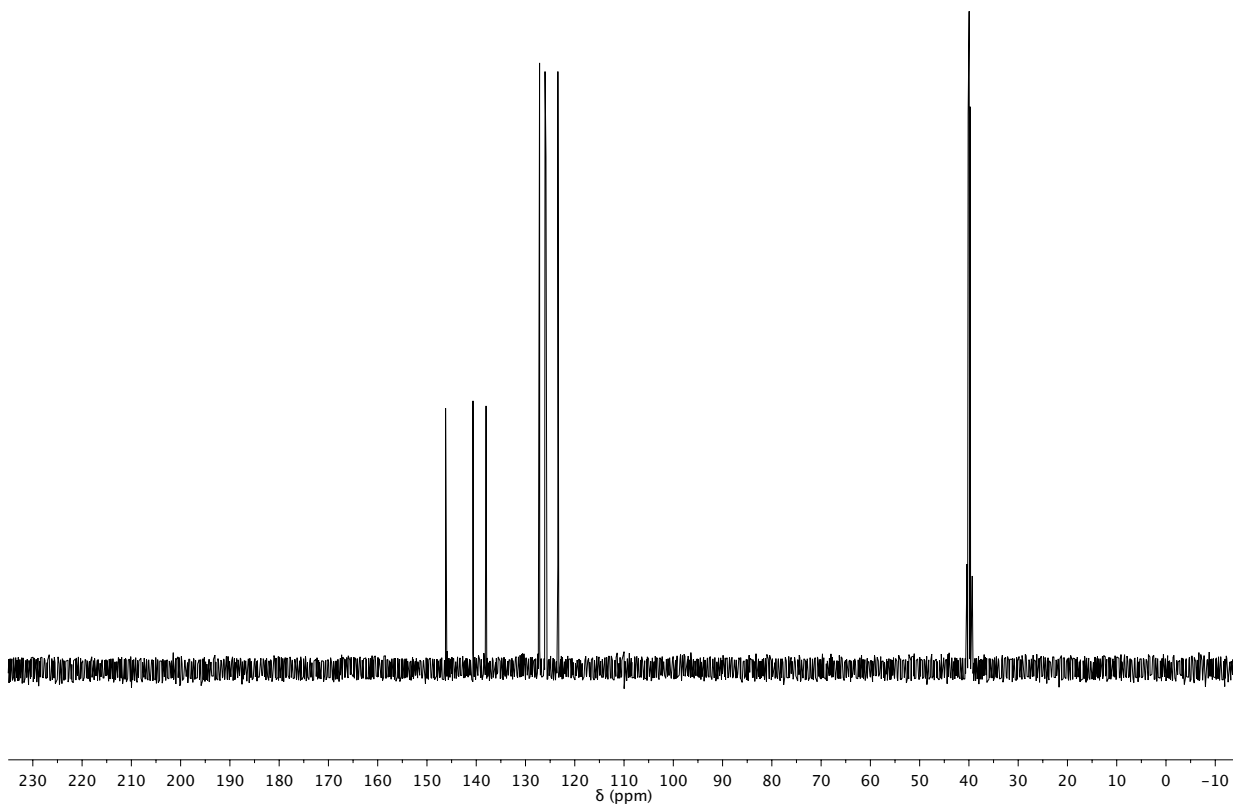


Figure S49. ¹³C-NMR spectrum of Benzothiophene-2-sulfonamide.

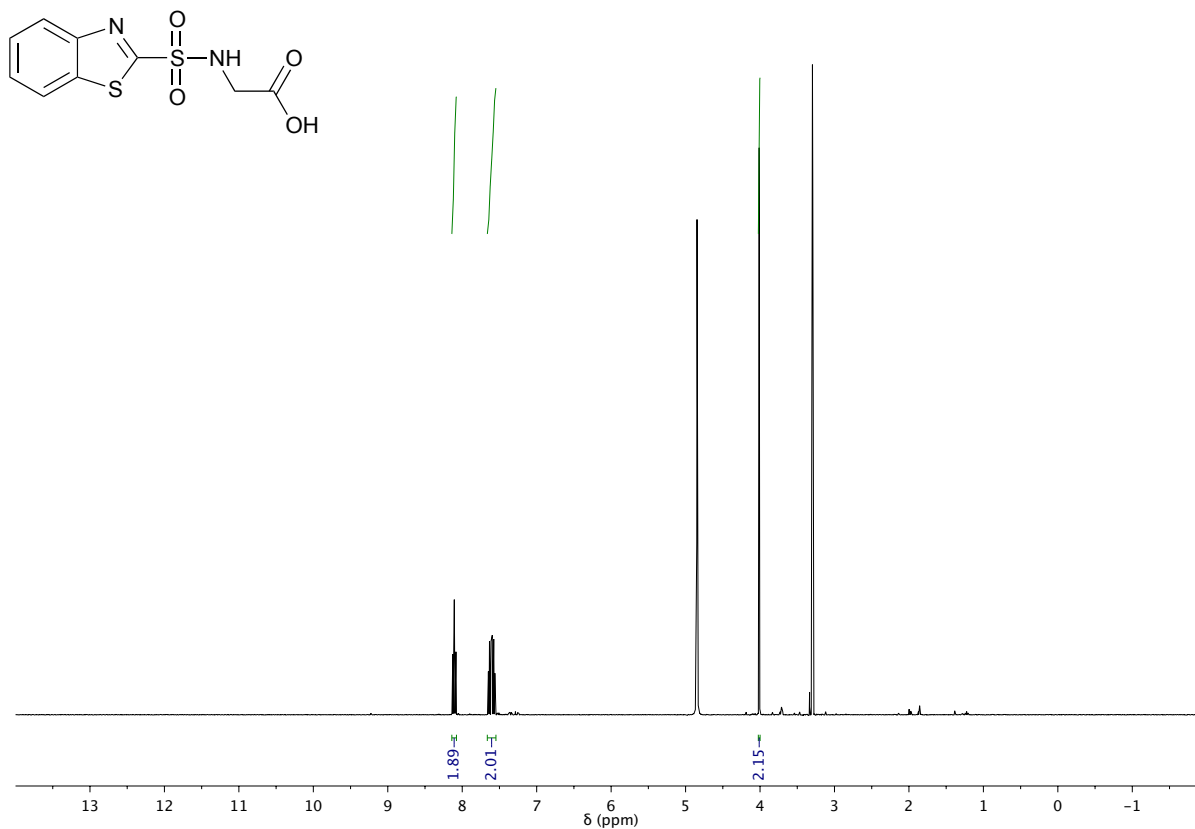


Figure S50. ¹H-NMR spectrum of benzothiazole-2-sulfon-N-glycine.

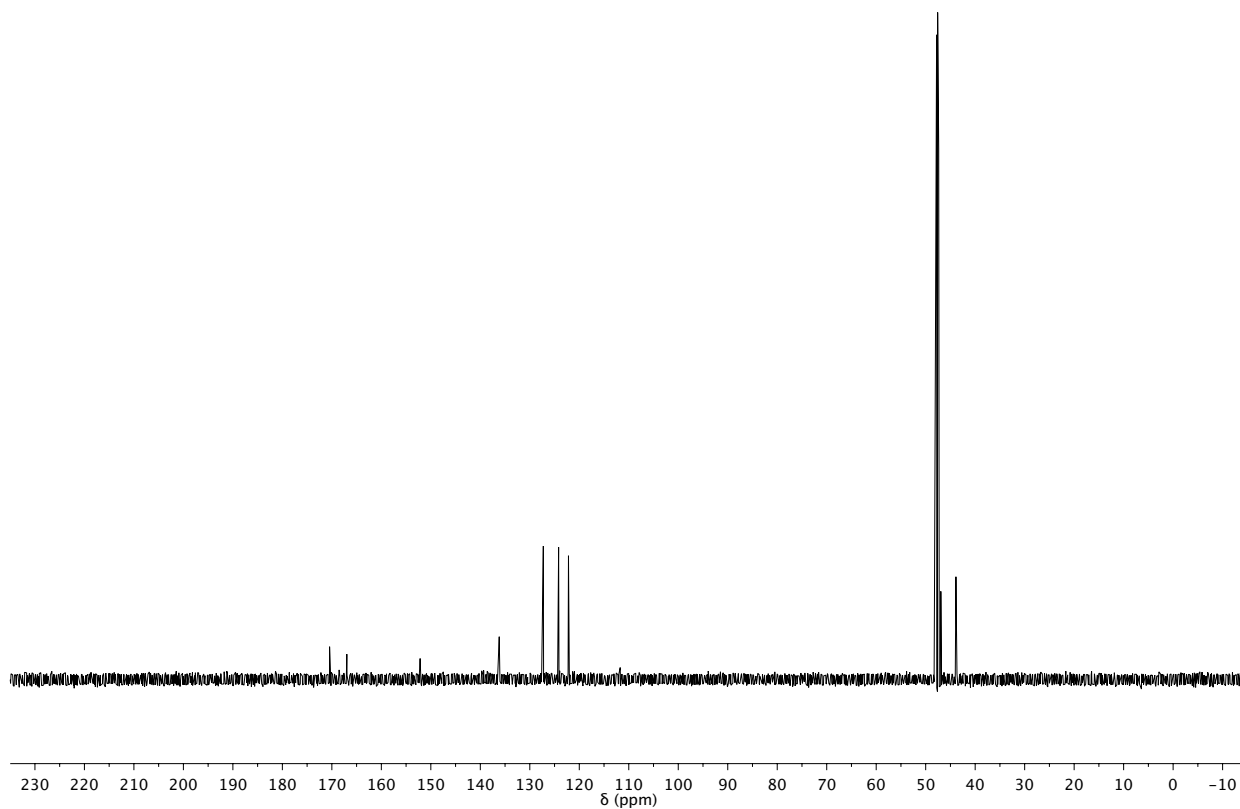


Figure S51. ¹³C-NMR spectrum of benzothiazole-2-sulfon-N-glycine.

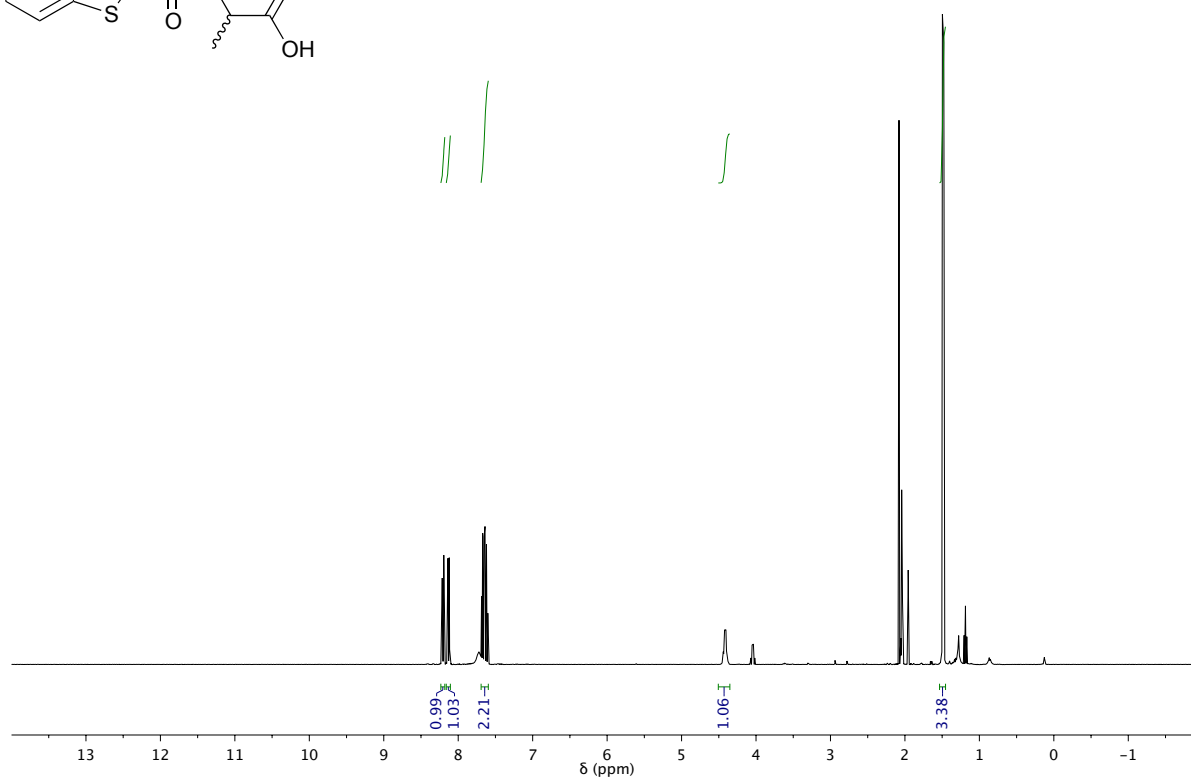
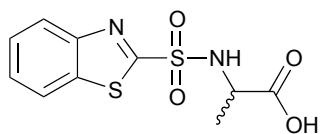


Figure S52. ¹H-NMR spectrum of benzothiazole-2-sulfon-N-DL-Alanine.

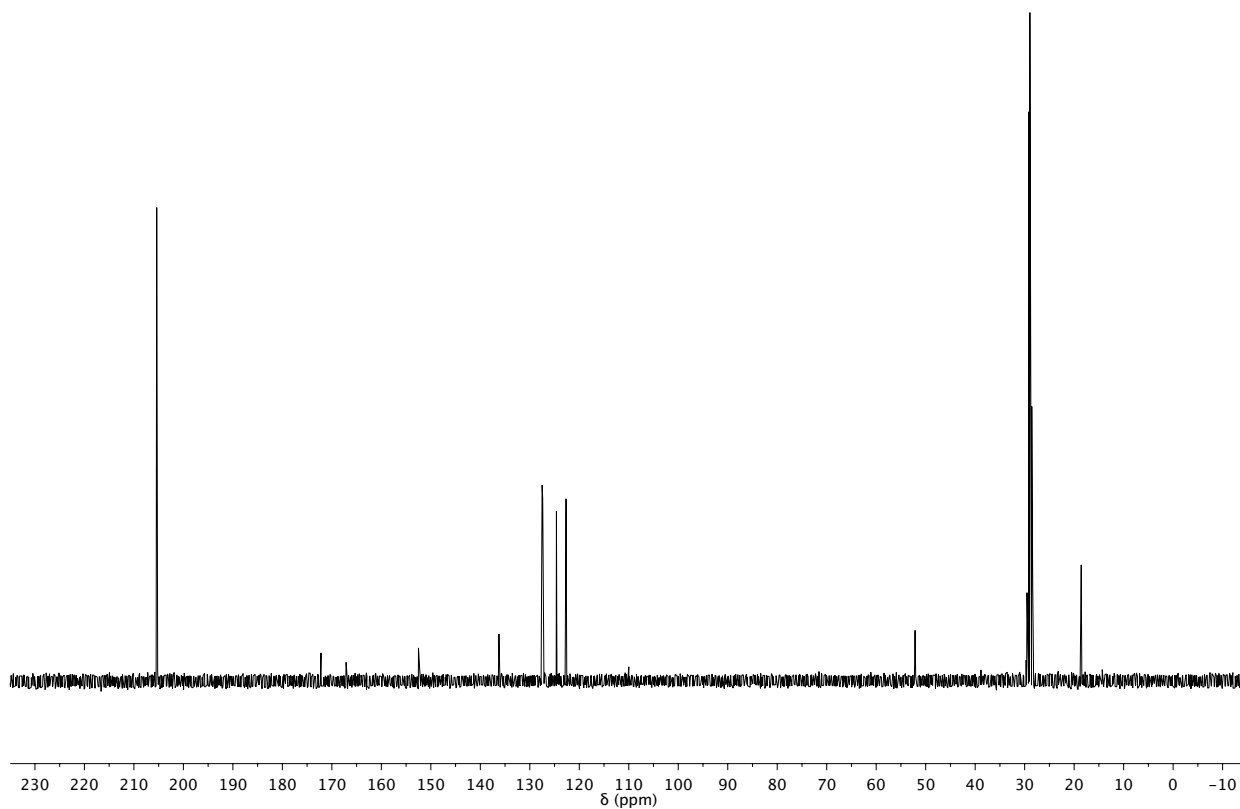


Figure S53. ¹³C-NMR spectrum of benzothiazole-2-sulfon-N-DL-Alanine.

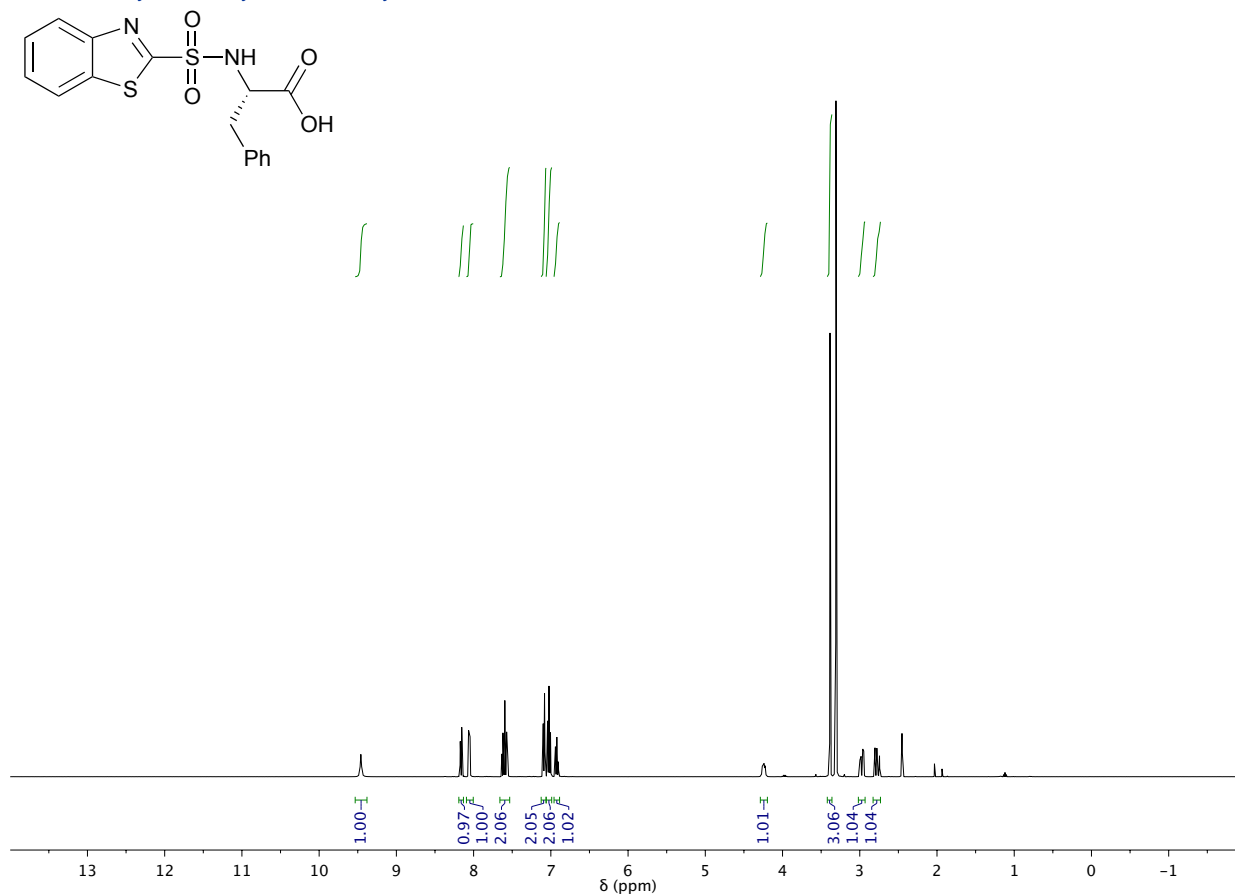


Figure S54. ¹H-NMR spectrum of benzothiazole-2-sulfon-*N*-phenylalanine.

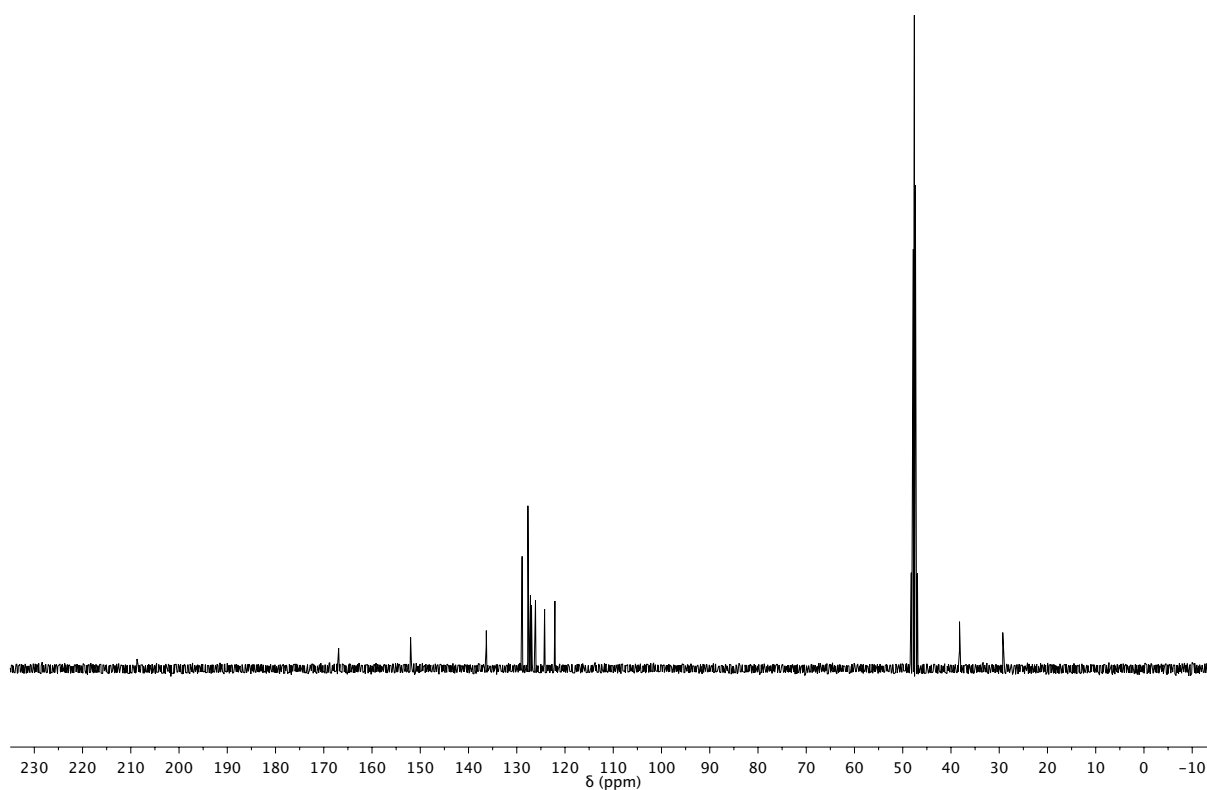


Figure S55. ¹³C-NMR spectrum of benzothiazole-2-sulfon-*N*-phenylalanine.

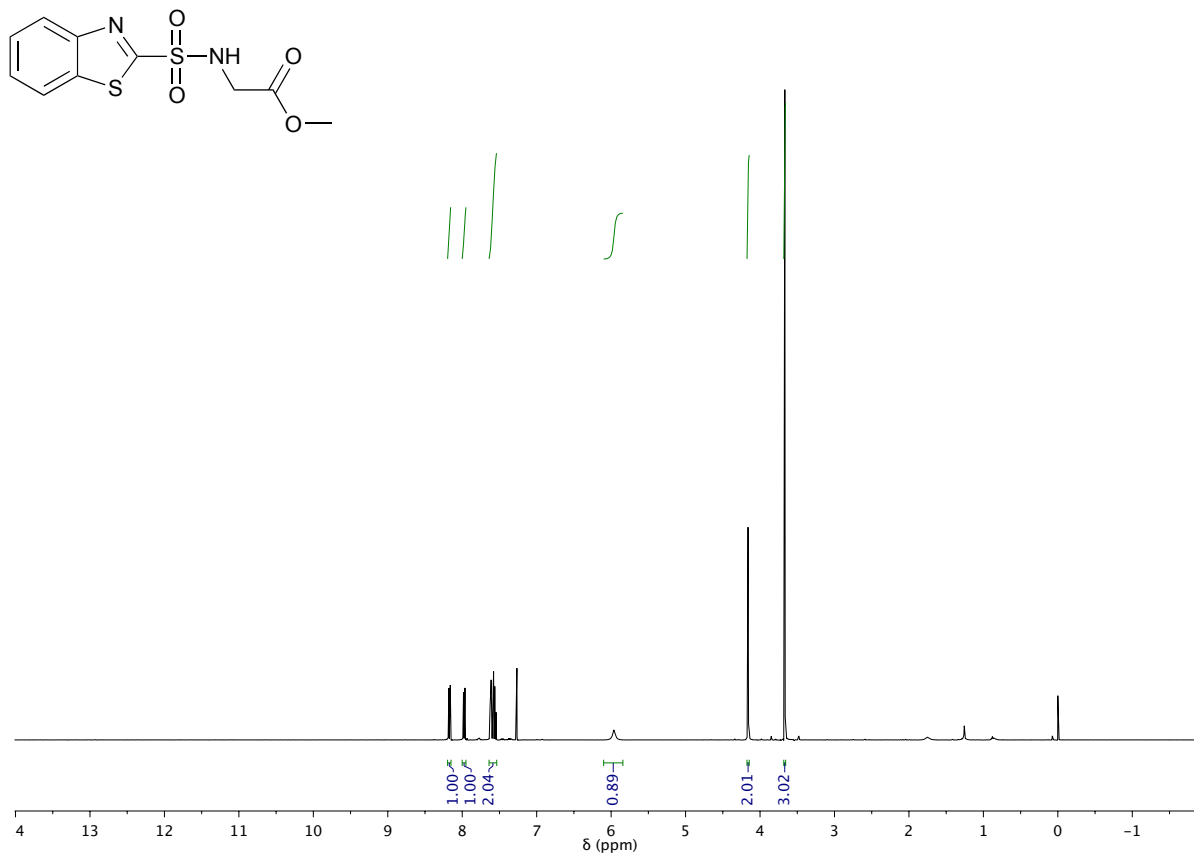


Figure 56. ¹H-NMR spectrum of methyl benzothiazole-2-sulfonglycinate.

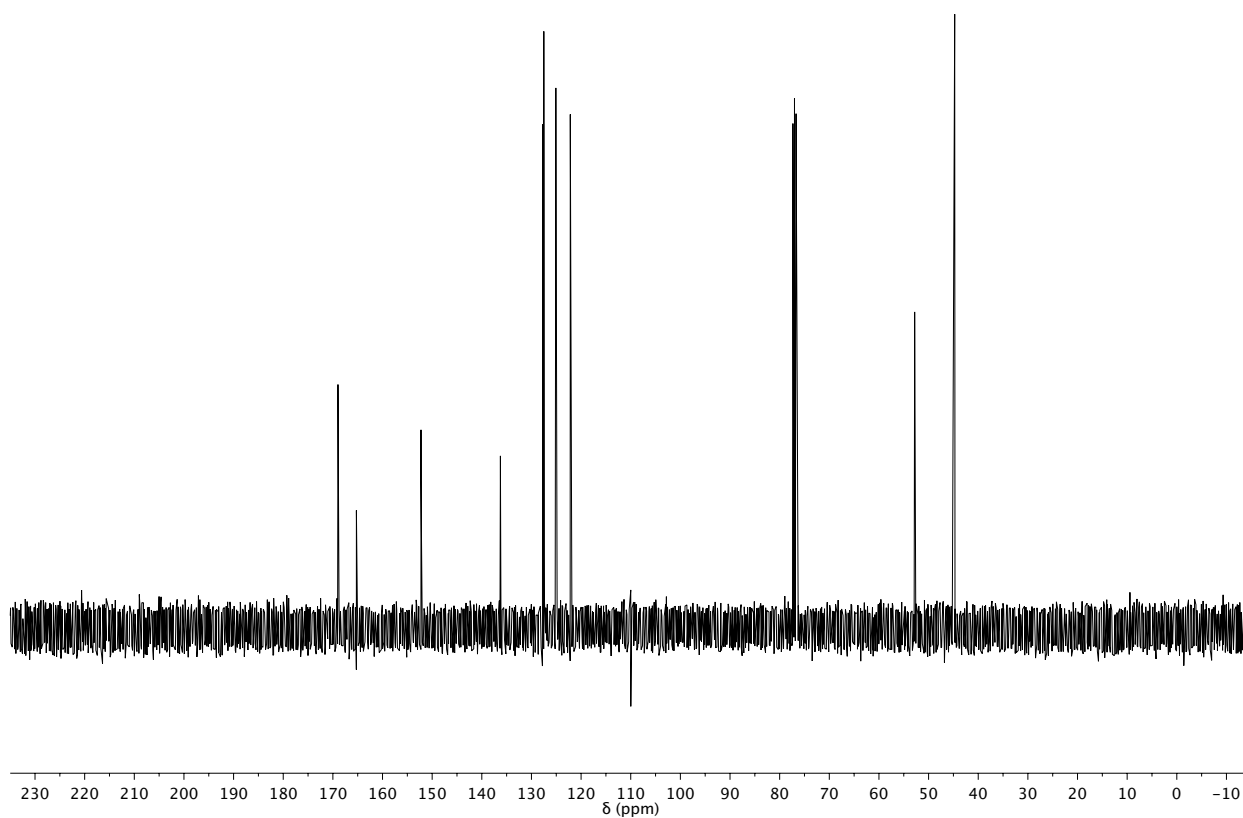


Figure 57. ¹³C-NMR spectrum of methyl benzothiazole-2-sulfonglycinate.

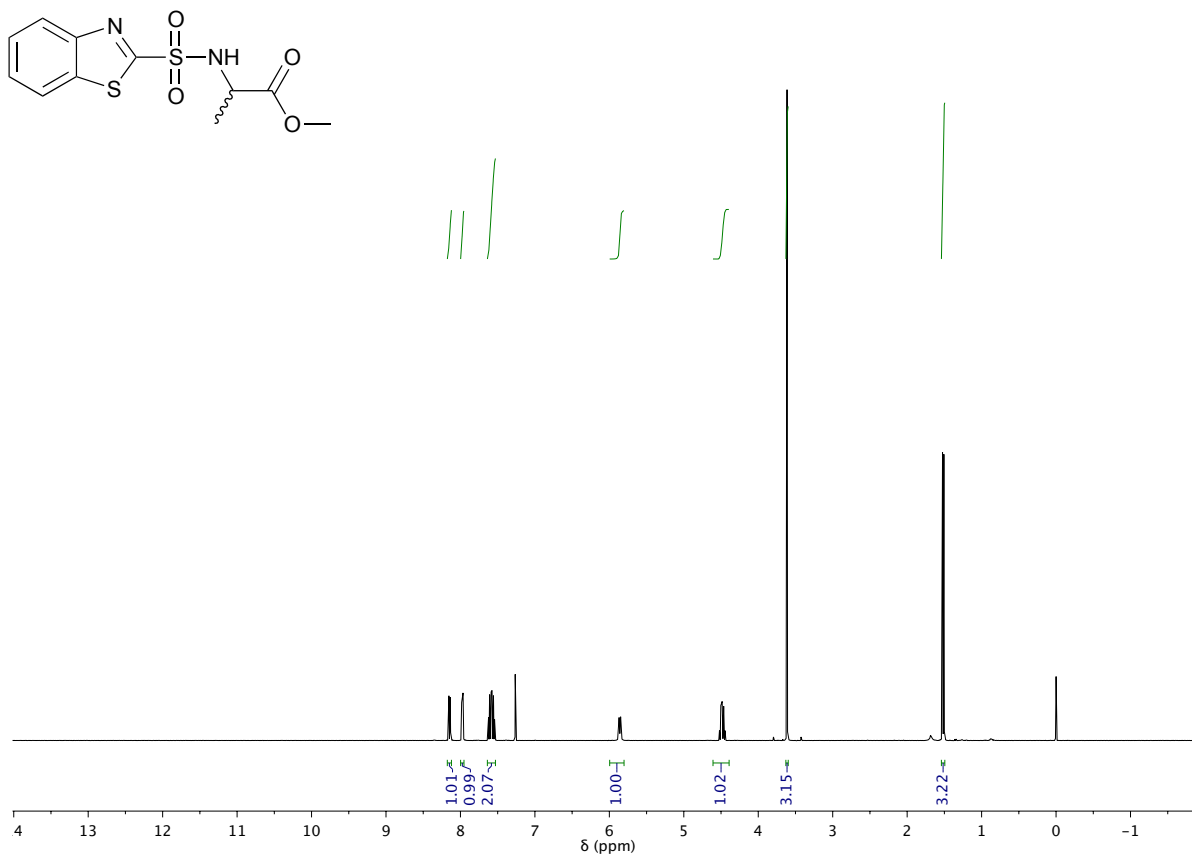


Figure 58. ¹H-NMR spectrum of methyl benzothiazole-2-sulfon-*DL*-alaninate.

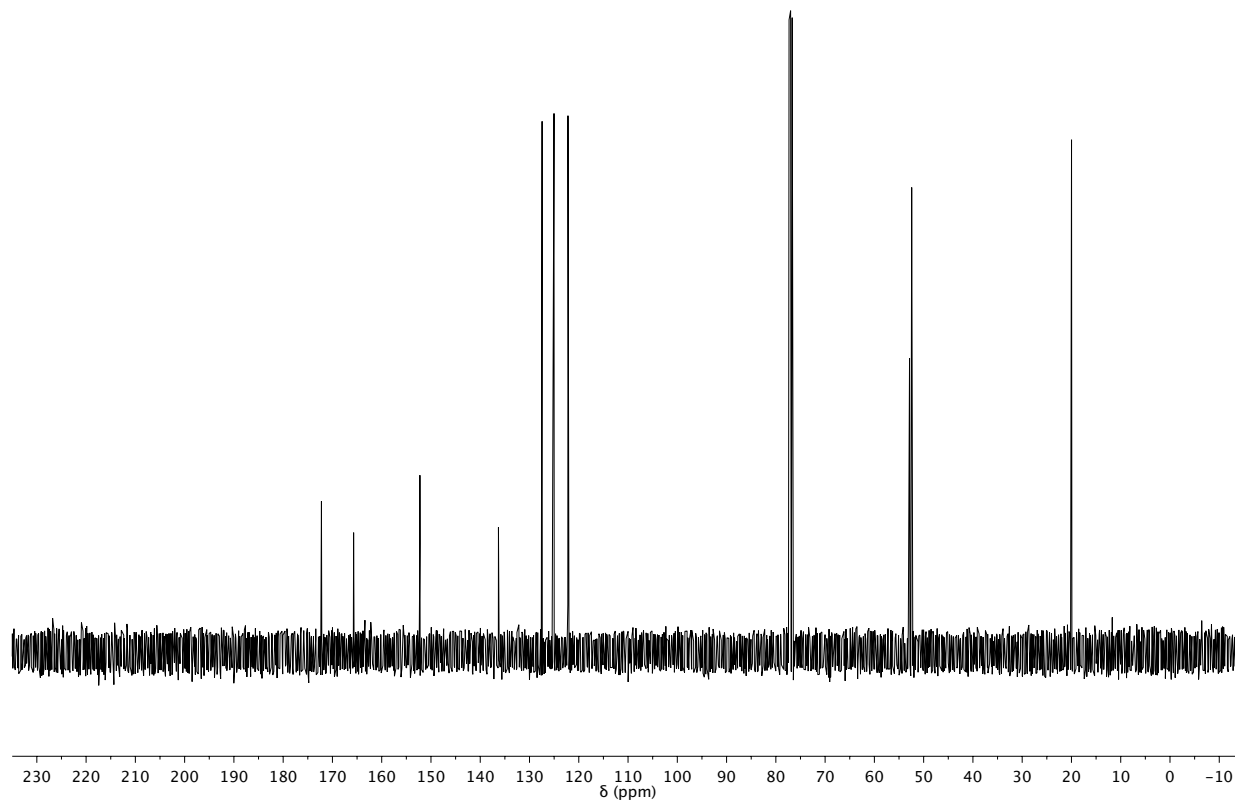


Figure 59. ¹³C-NMR spectrum of methyl benzothiazole-2-sulfon-*DL*-alaninate.

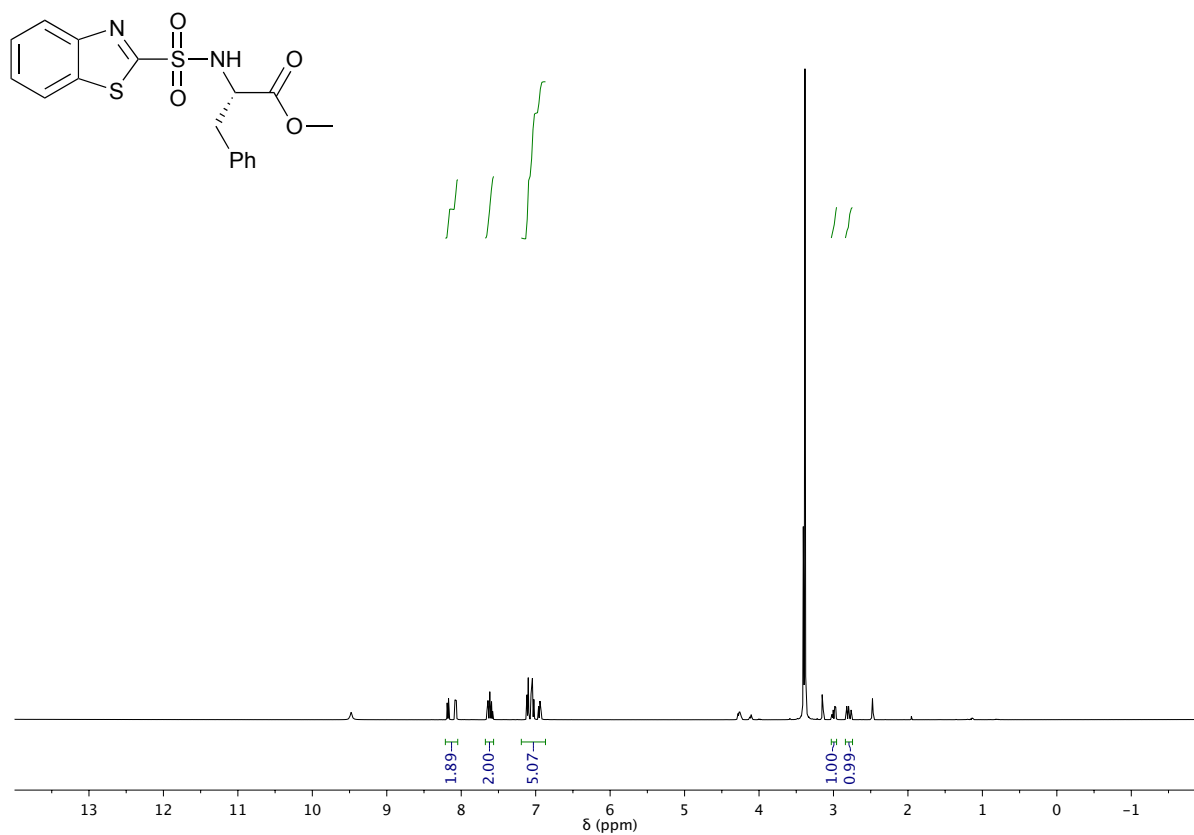


Figure 60. $^1\text{H-NMR}$ spectrum of methyl benzothiazole-2-sulfon-*L*-phenylalaninate.

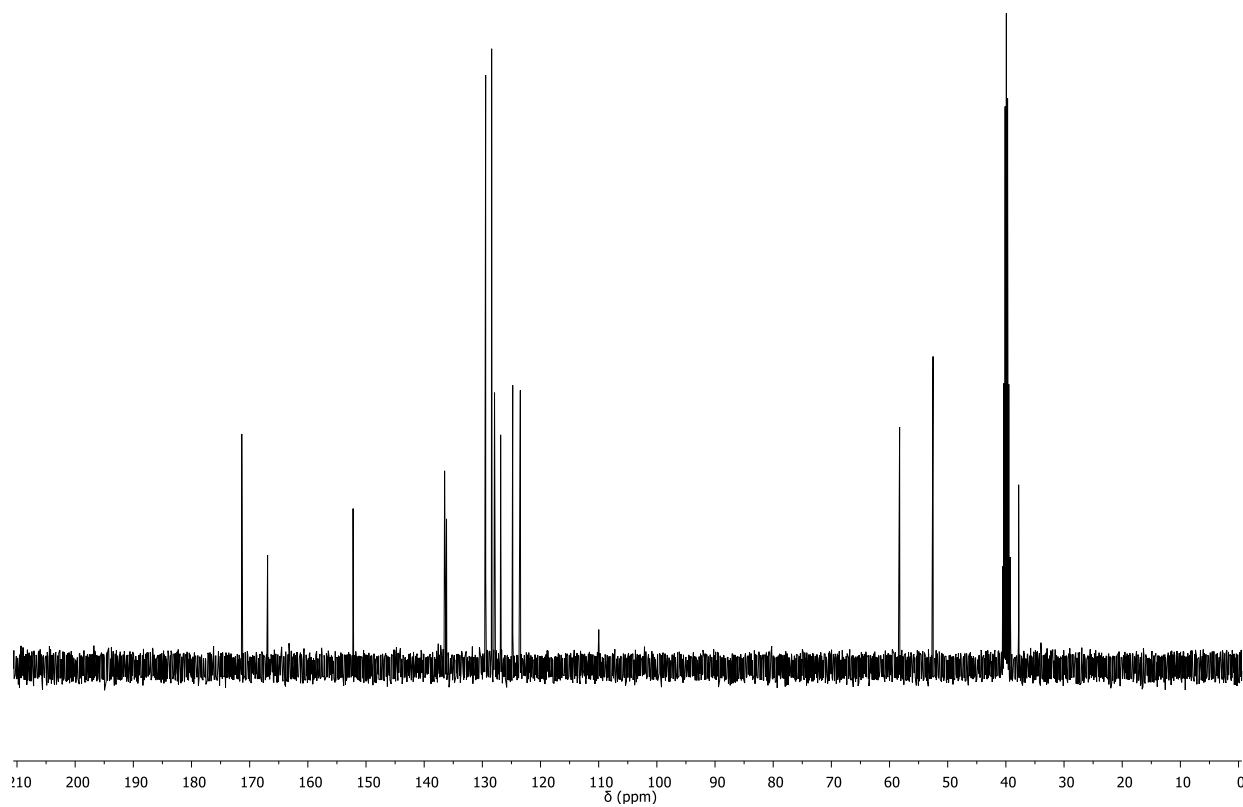


Figure 61. $^{13}\text{C-NMR}$ spectrum of methyl benzothiazole-2-sulfon-*L*-phenylalaninate.

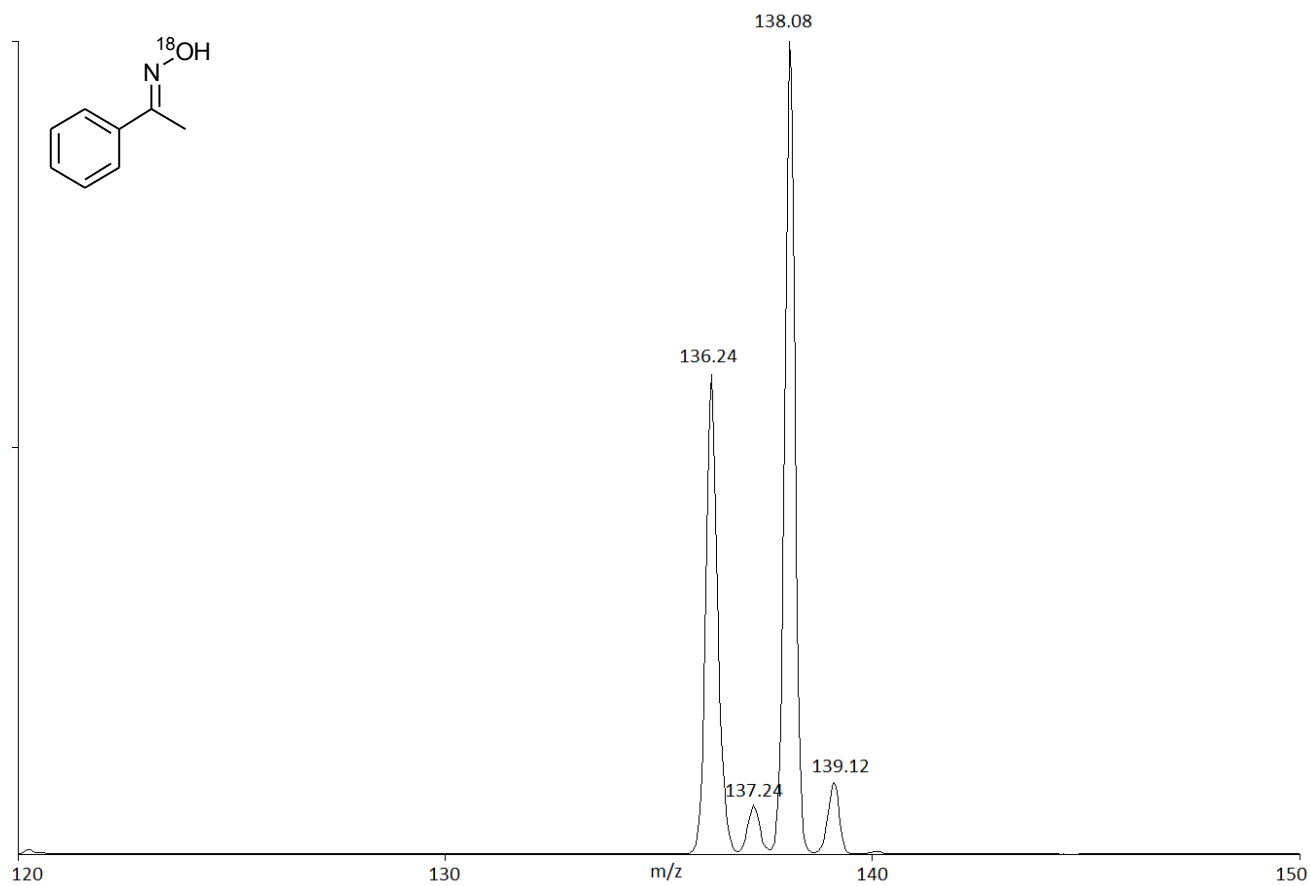


Figure S62. MS spectrum of acetophenone oxime with ~70% ^{18}O incorporation.

Collected LC-MS data

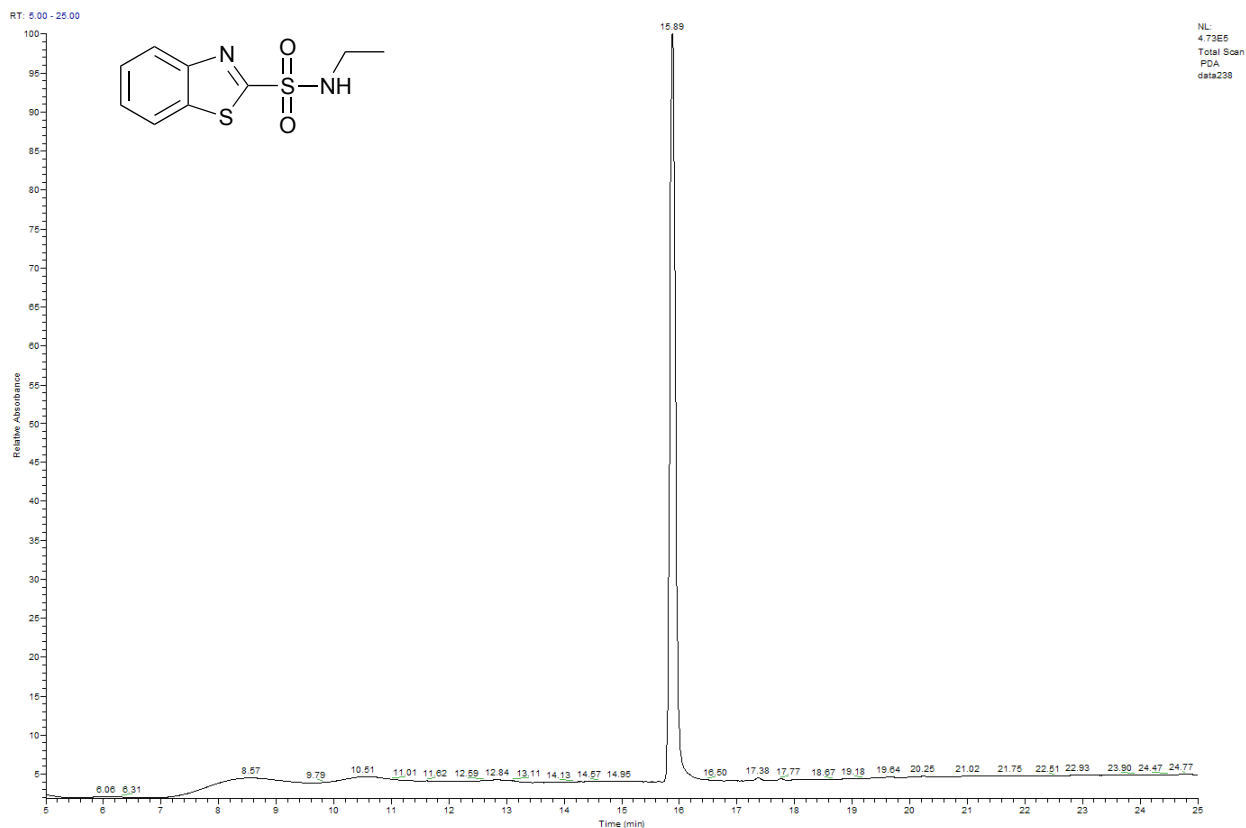


Figure 63. LC-MS-UV spectrum of *N*-ethyl-benzothiazole-2-sulfonamide.

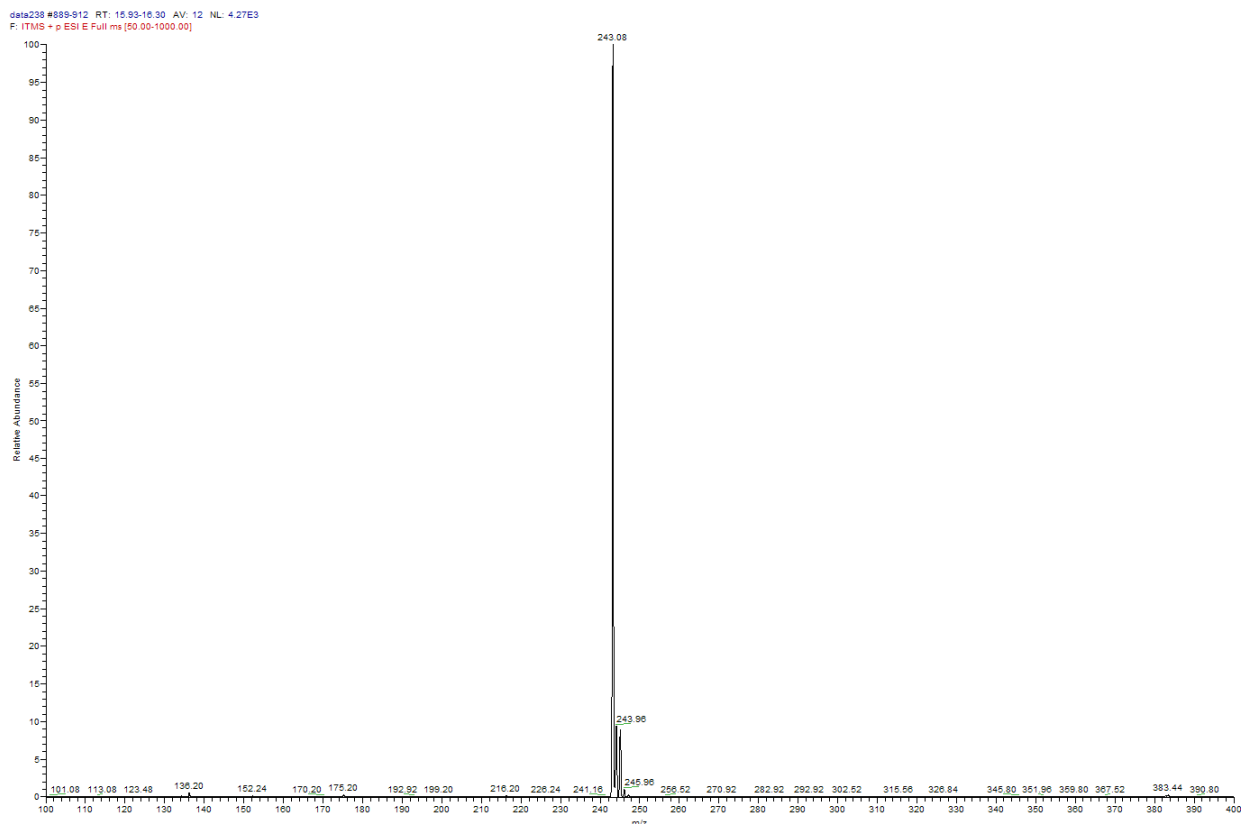


Figure S64. LC-MS spectrum of *N*-ethyl-benzothiazole-2-sulfonamide.

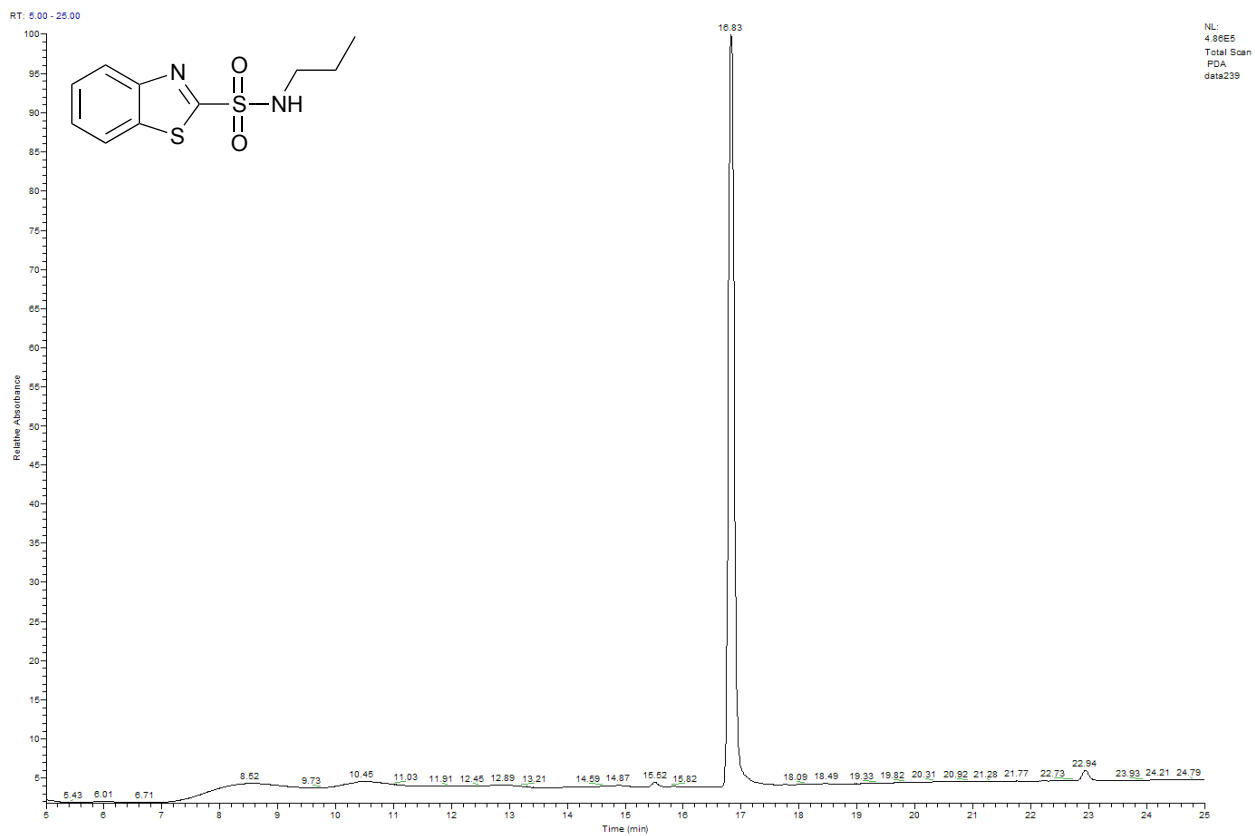


Figure S65. LC-MS-UV spectrum of *N*-propyl-benzothiazole-2-sulfonamide.

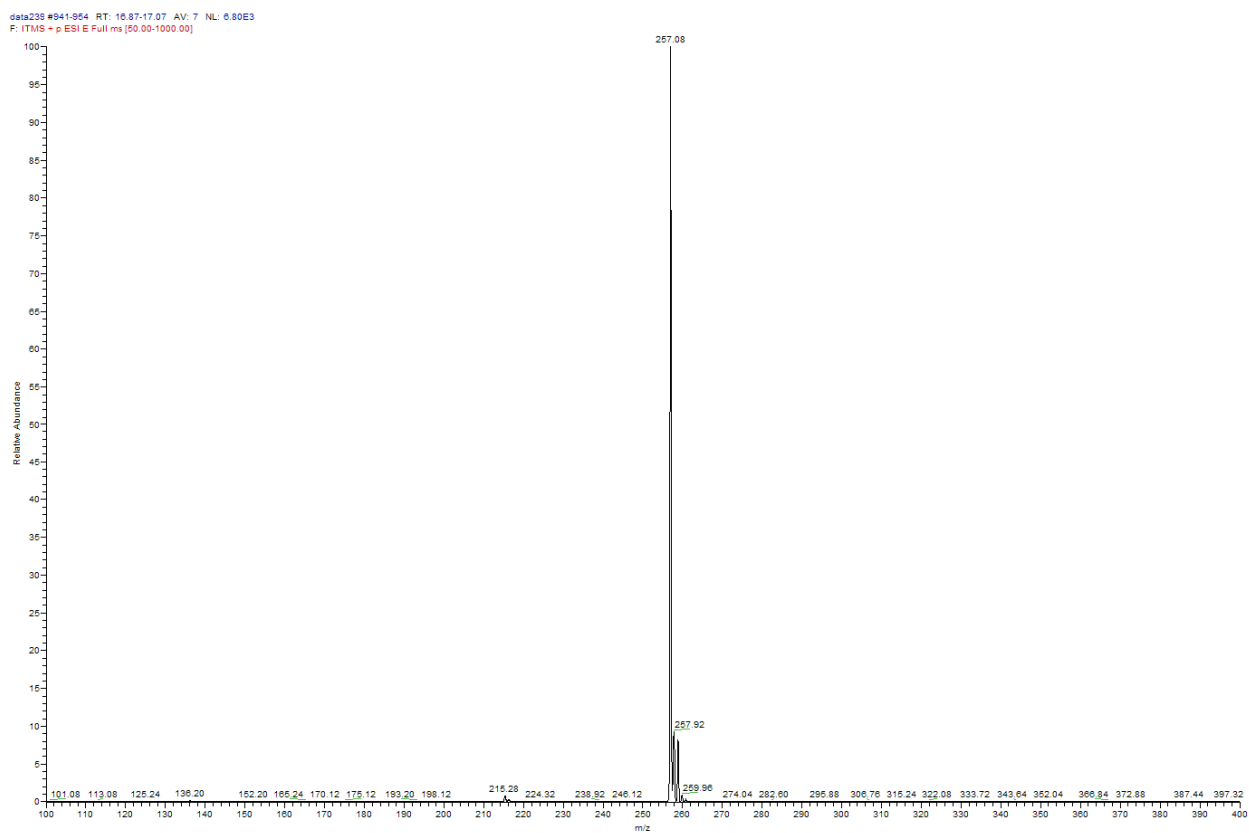


Figure S66. LC-MS spectrum of *N*-propyl-benzothiazole-2-sulfonamide

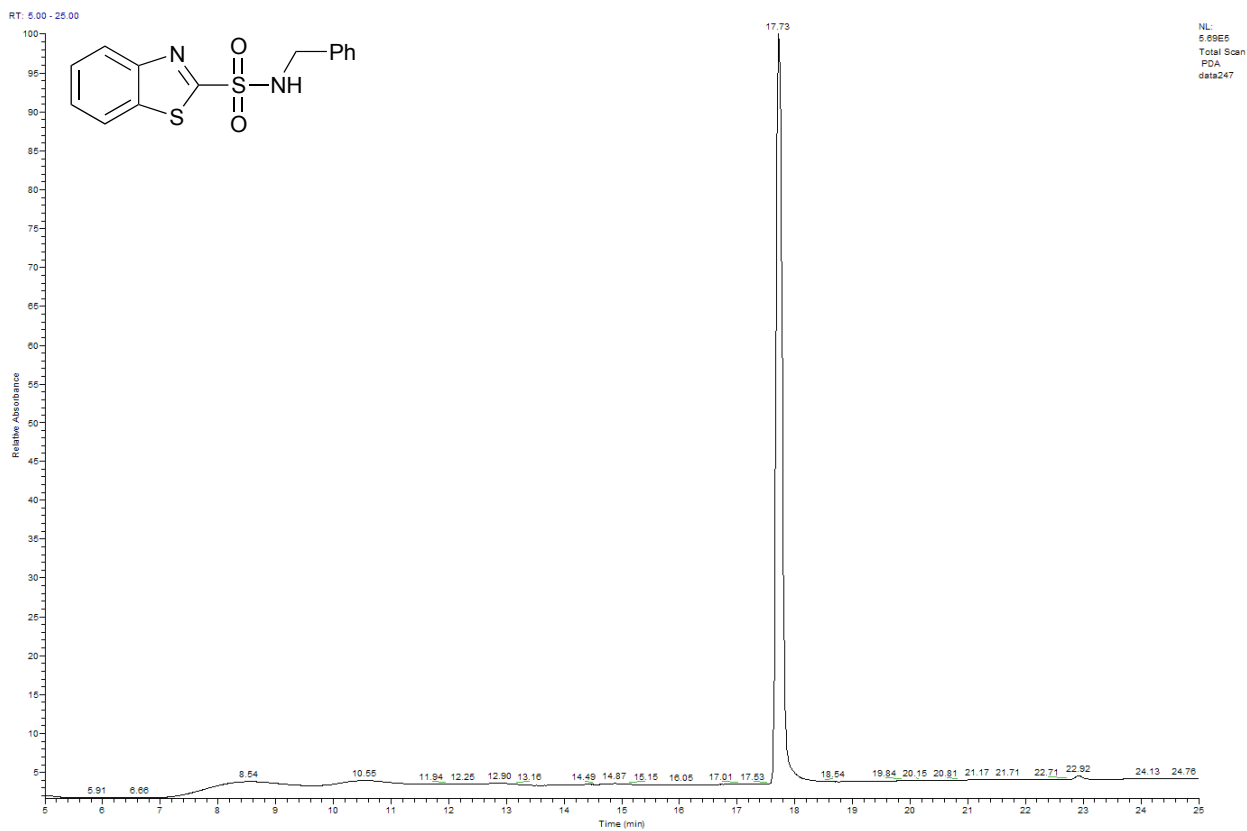


Figure S67. LC-MS-UV spectrum of *N*-benzyl-benzothiazole-2-sulfonamide.

data247 #992:1010 RT: 17.80-18.06 AV: 9 NL: 2.32E3
F: ITMS + p ESI E Full ms [50.00-1000.00]

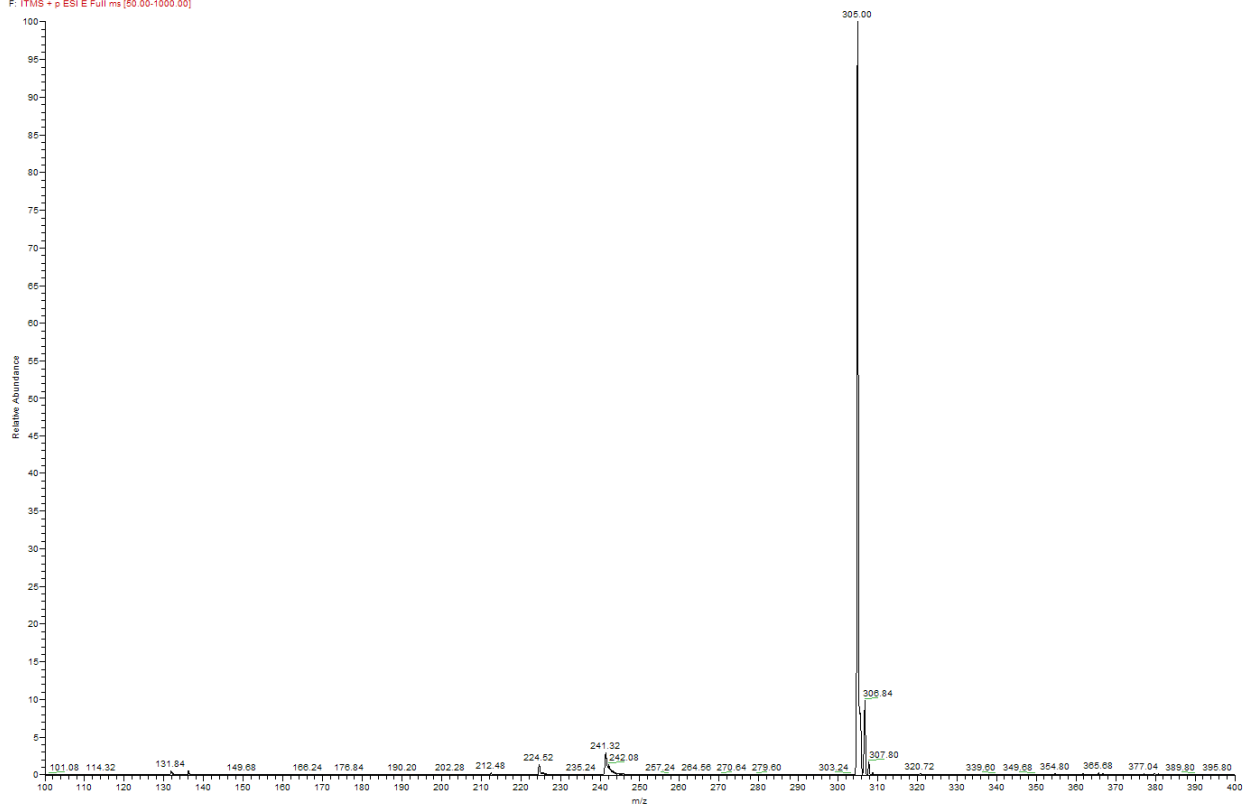


Figure S68. LC-MS spectrum of *N*-benzyl-benzothiazole-2-sulfonamide.

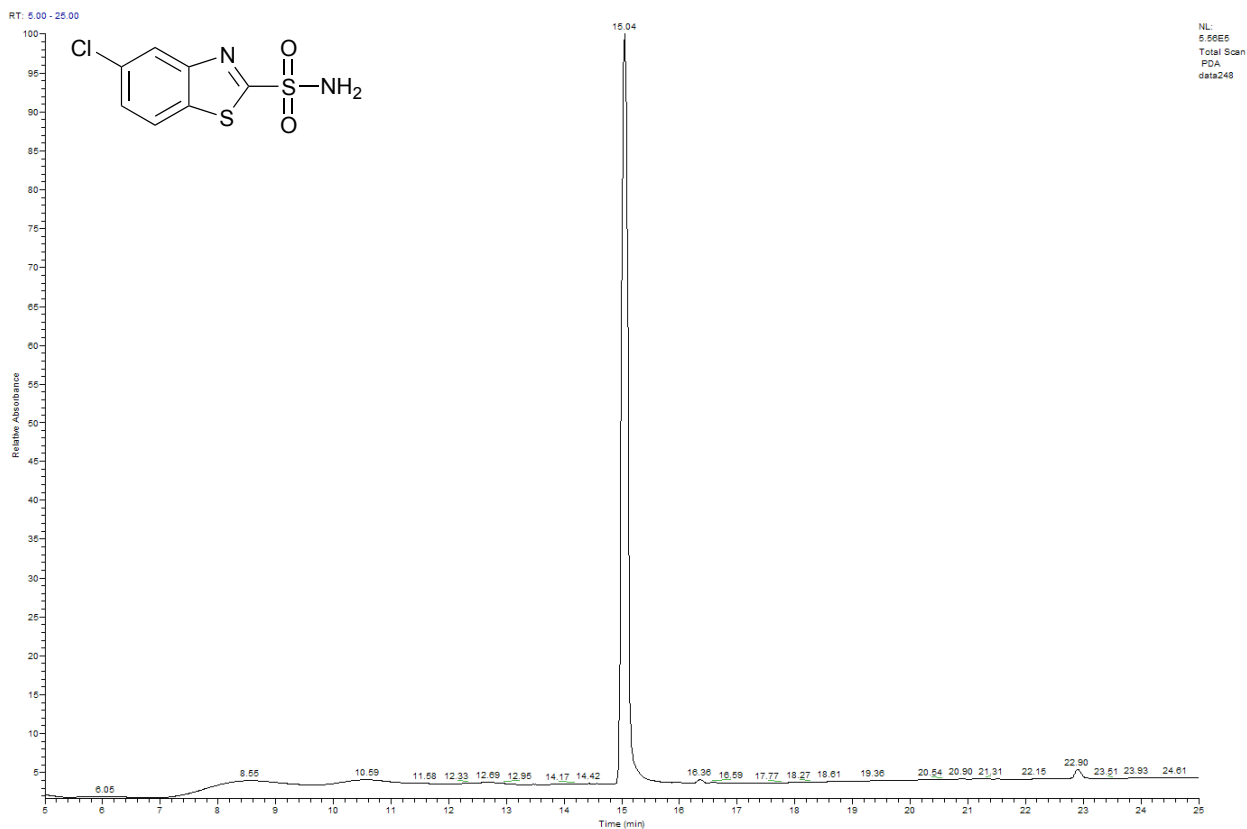


Figure S69. LC-MS-UV spectrum of 5-Chloro-benzothiazole-2-sulfonamide.

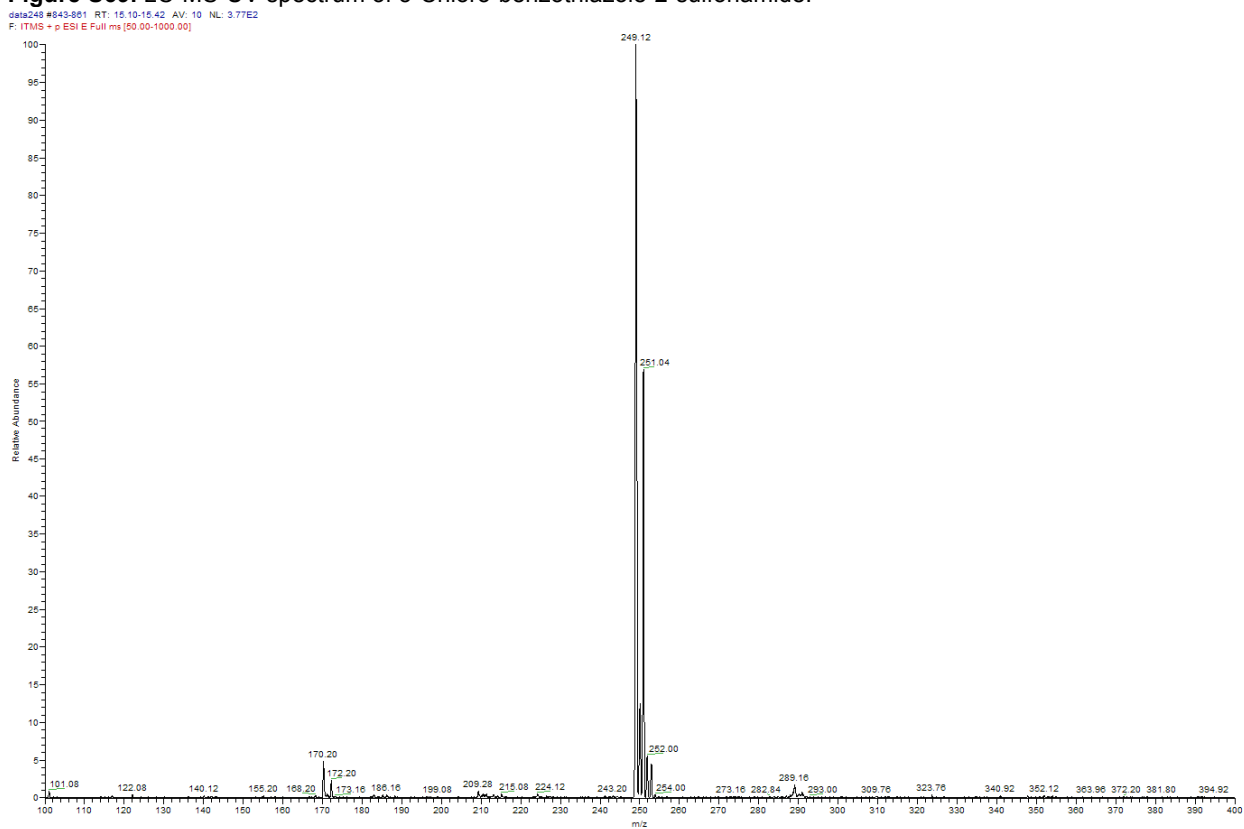


Figure S70. LC-MS spectrum of 5-Chloro-benzothiazole-2-sulfonamide.

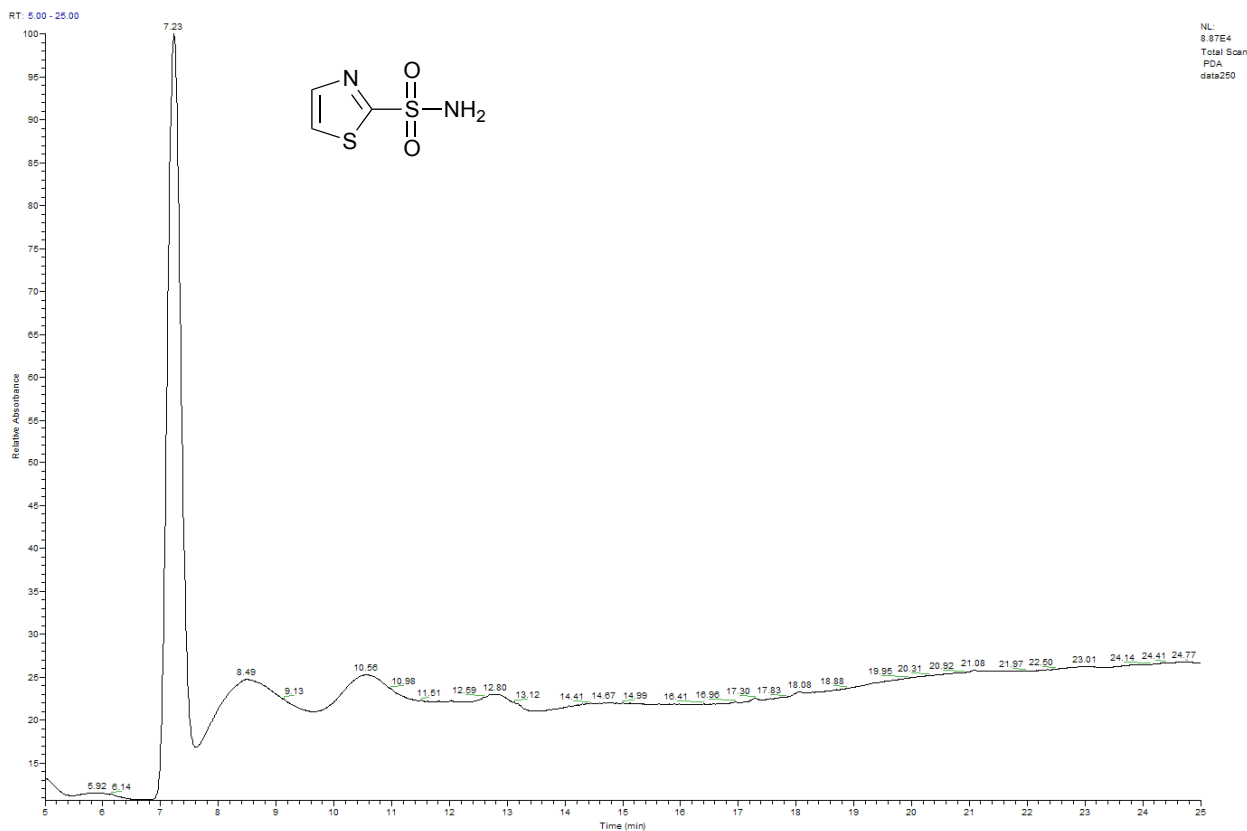


Figure S71. LC-MS-UV spectrum of thiazole-2-sulfonamide.

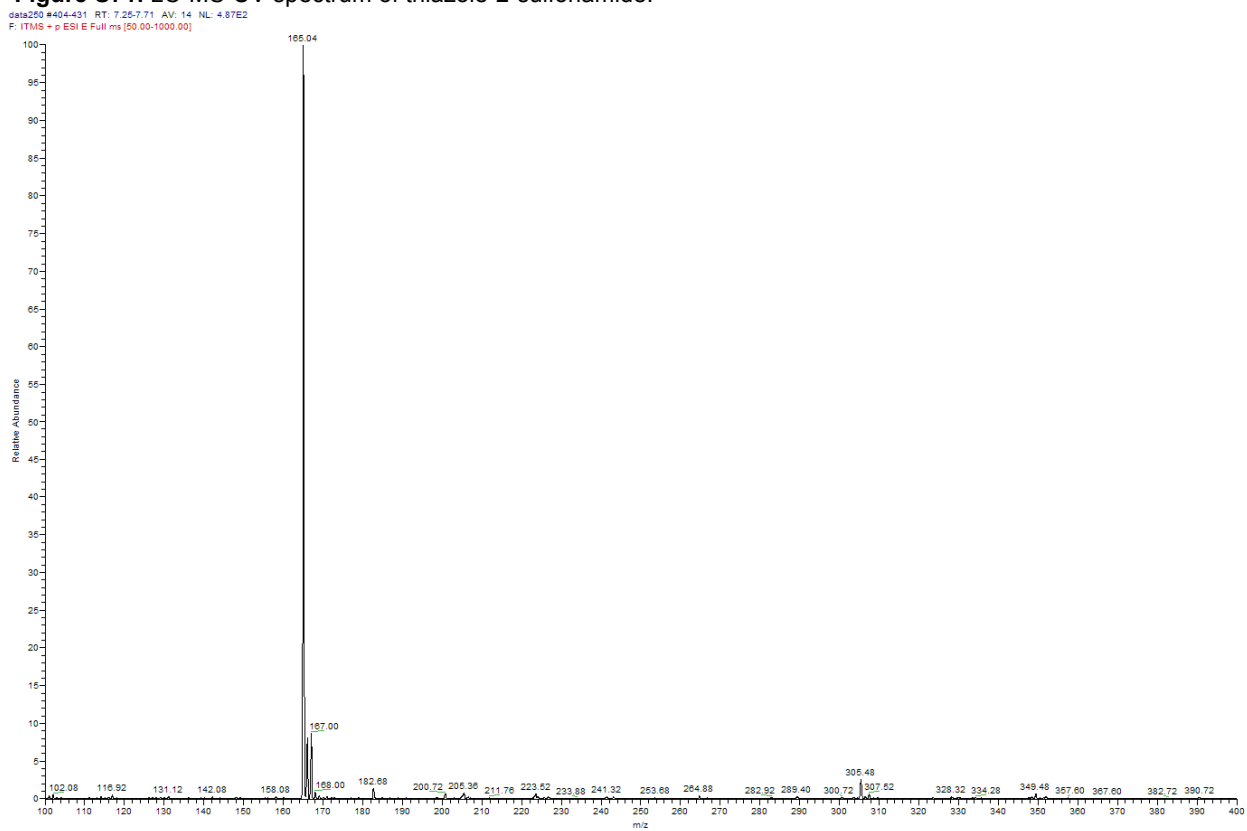


Figure S72. LC-MS spectrum of thiazole-2-sulfonamide.

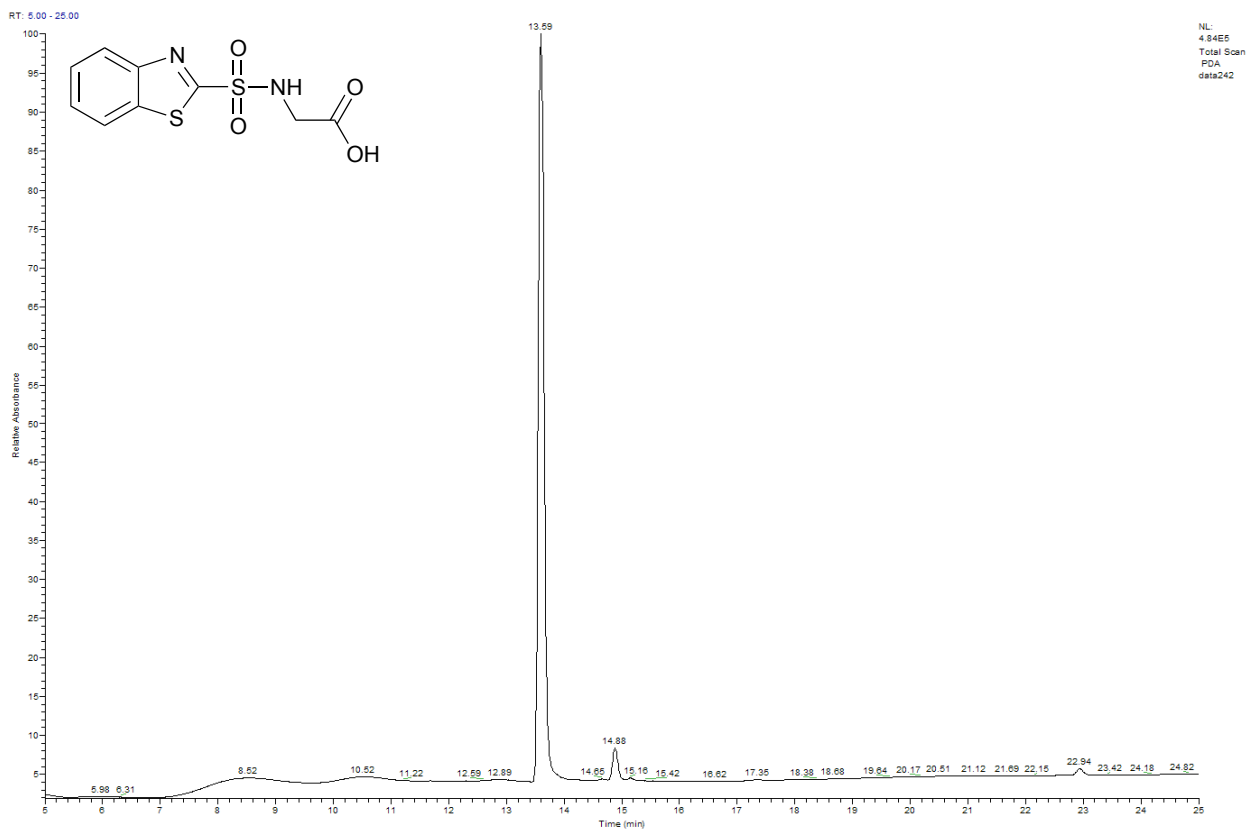


Figure S73. LC-MS-UV spectrum of benzothiazole-2-sulfon-*N*-glycine.

data242 #760-782 RT: 13.64-13.97 Av: 11 NL: 2.00E3
F: ITMS + p ESI E Full ms [50.00-1000.00]

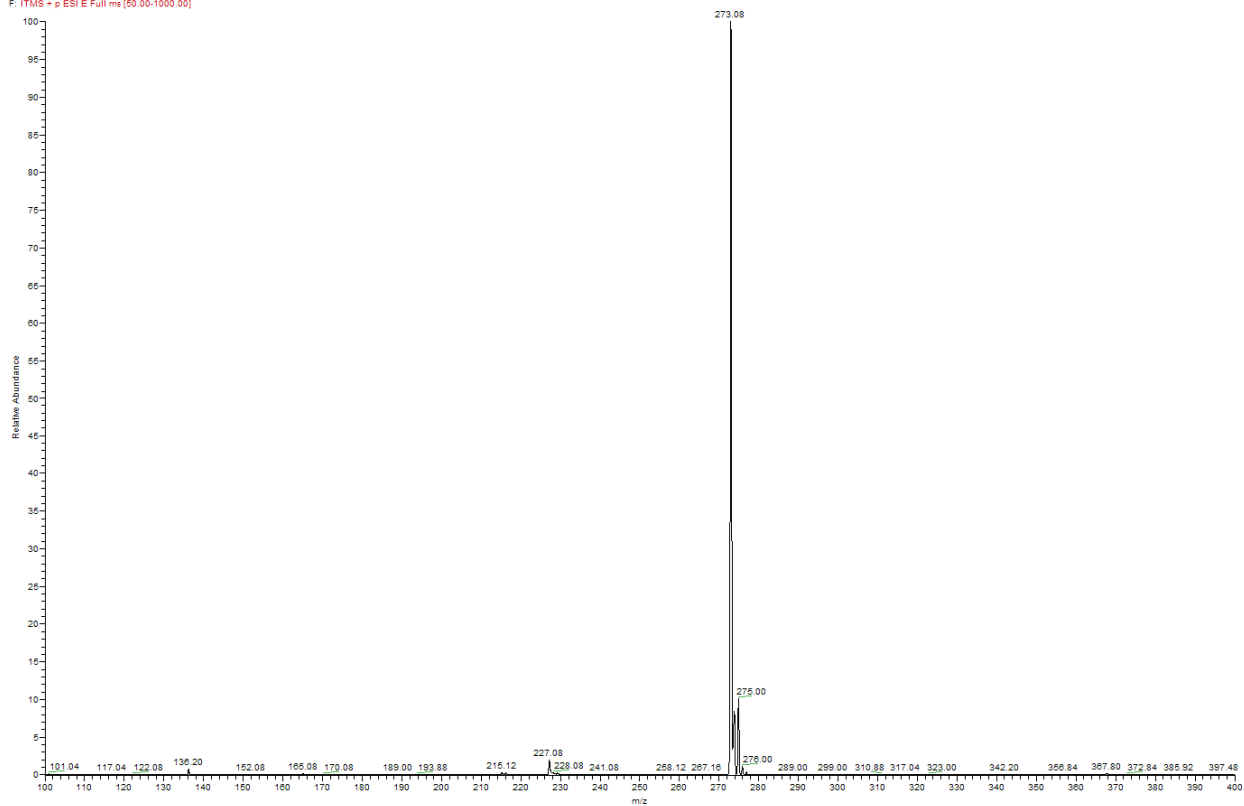


Figure S74. LC-MS spectrum of benzothiazole-2-sulfon-*N*-glycine.

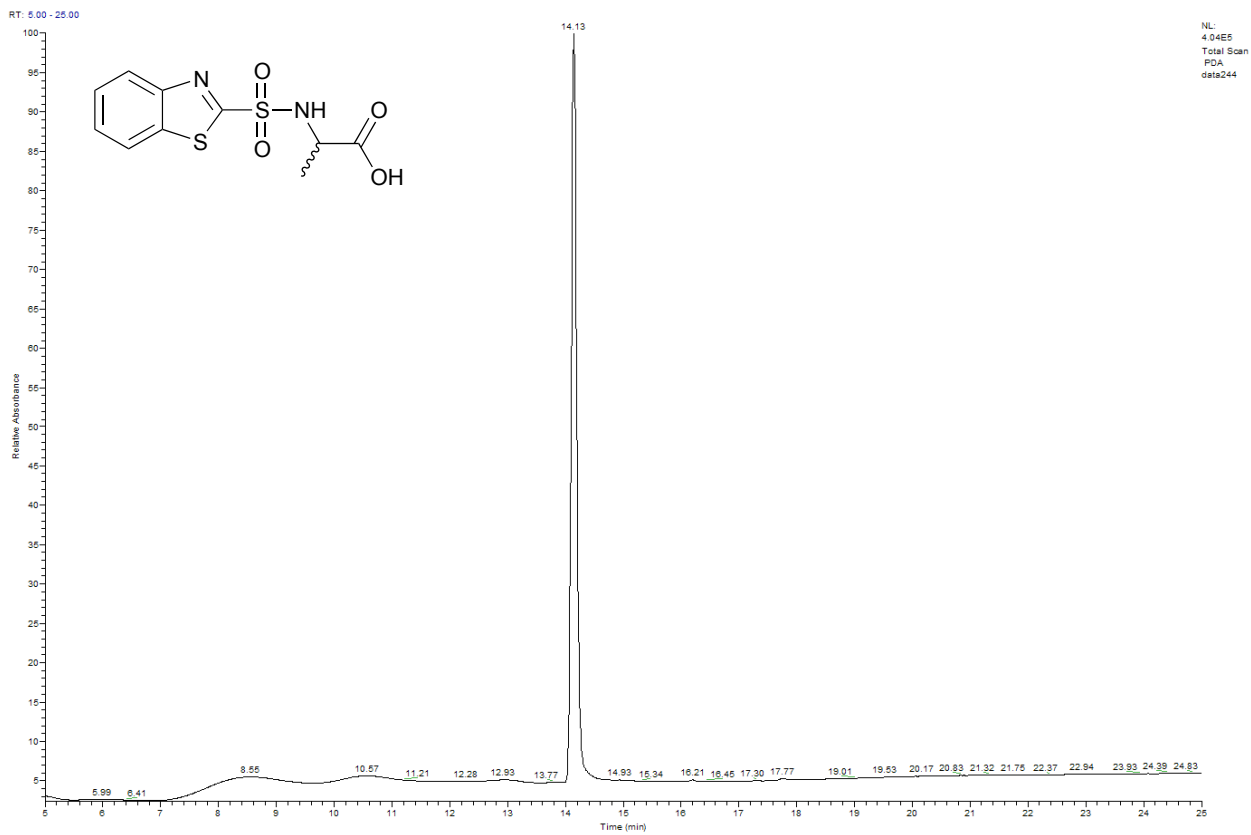


Figure S75. LC-MS-UV spectrum of benzothiazole-2-sulfon-*N-DL*-Alanine.

data244 4783-811 RT: 14.21-14.50 AV: 10 NL: 3.25E3
F: ITMS + p ESI E Full ms [50.00-1000.00]

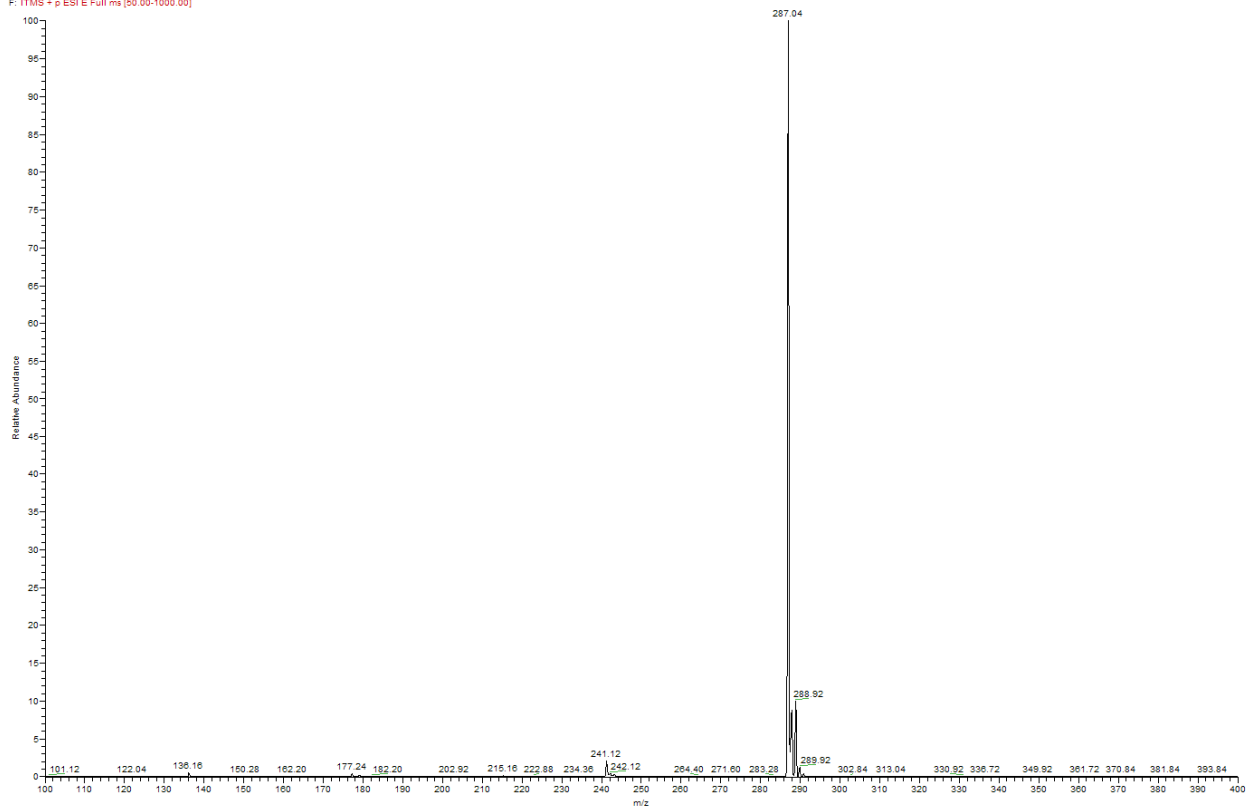


Figure S76. LC-MS spectrum of benzothiazole-2-sulfon-*N-DL*-Alanine.

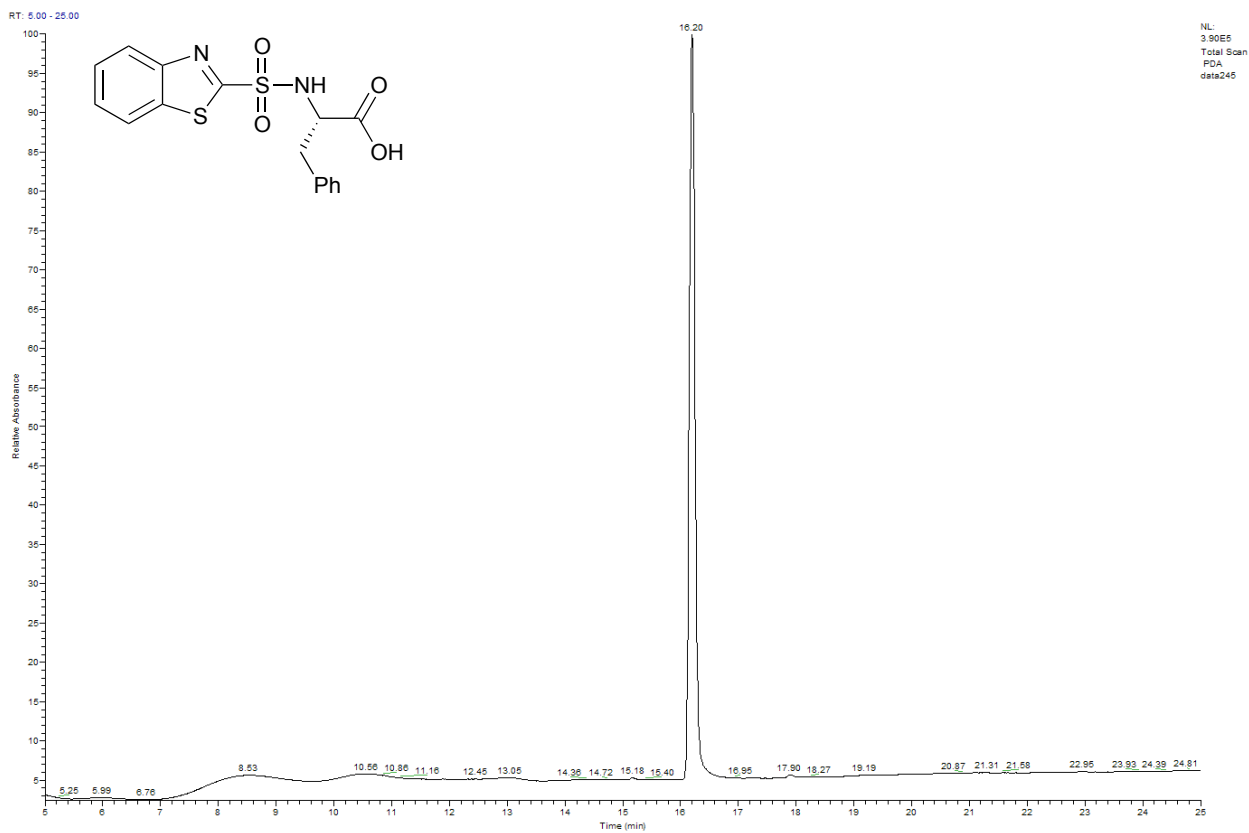


Figure S77. LC-MS-UV spectrum of benzothiazole-2-sulfon-N-phenylalanine.



Figure S78. LC-MS spectrum of benzothiazole-2-sulfon-N-phenylalanine.

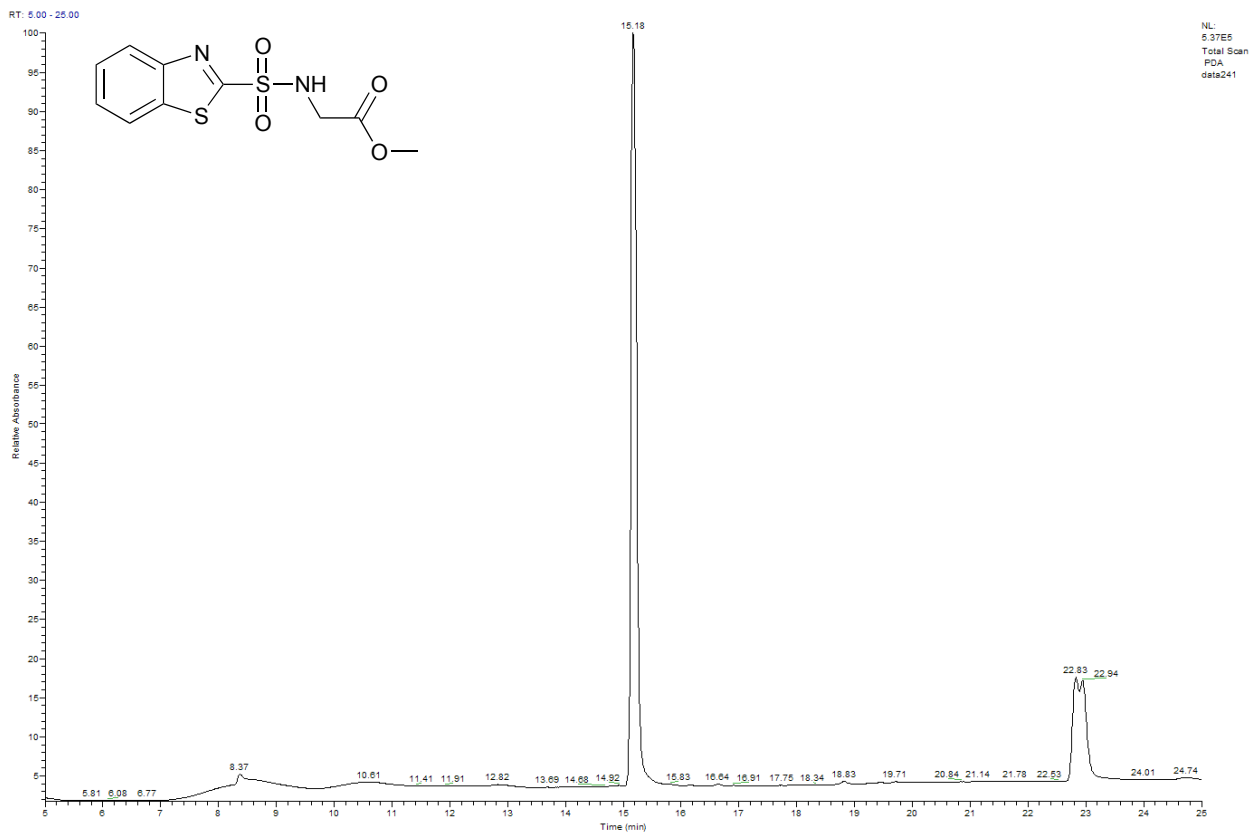


Figure S79. LC-MS-UV spectrum of methyl benzothiazole-2-sulfonglycinate.

data241 4952.856 RT: 16.23-16.52 Av: 8 NL: 6.32E3
F: ITMS + p ESI E Full ms [50.00-1000.00]

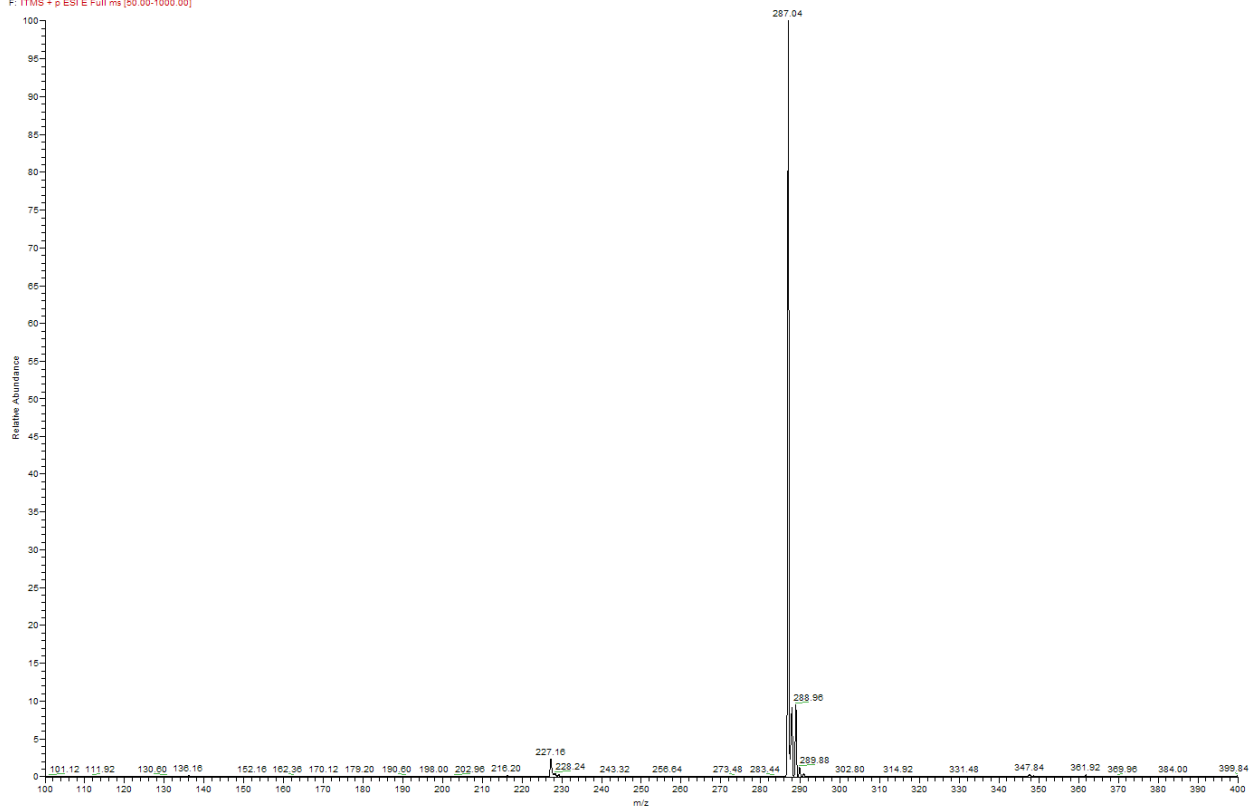


Figure S80. LC-MS spectrum of methyl benzothiazole-2-sulfonglycinate.

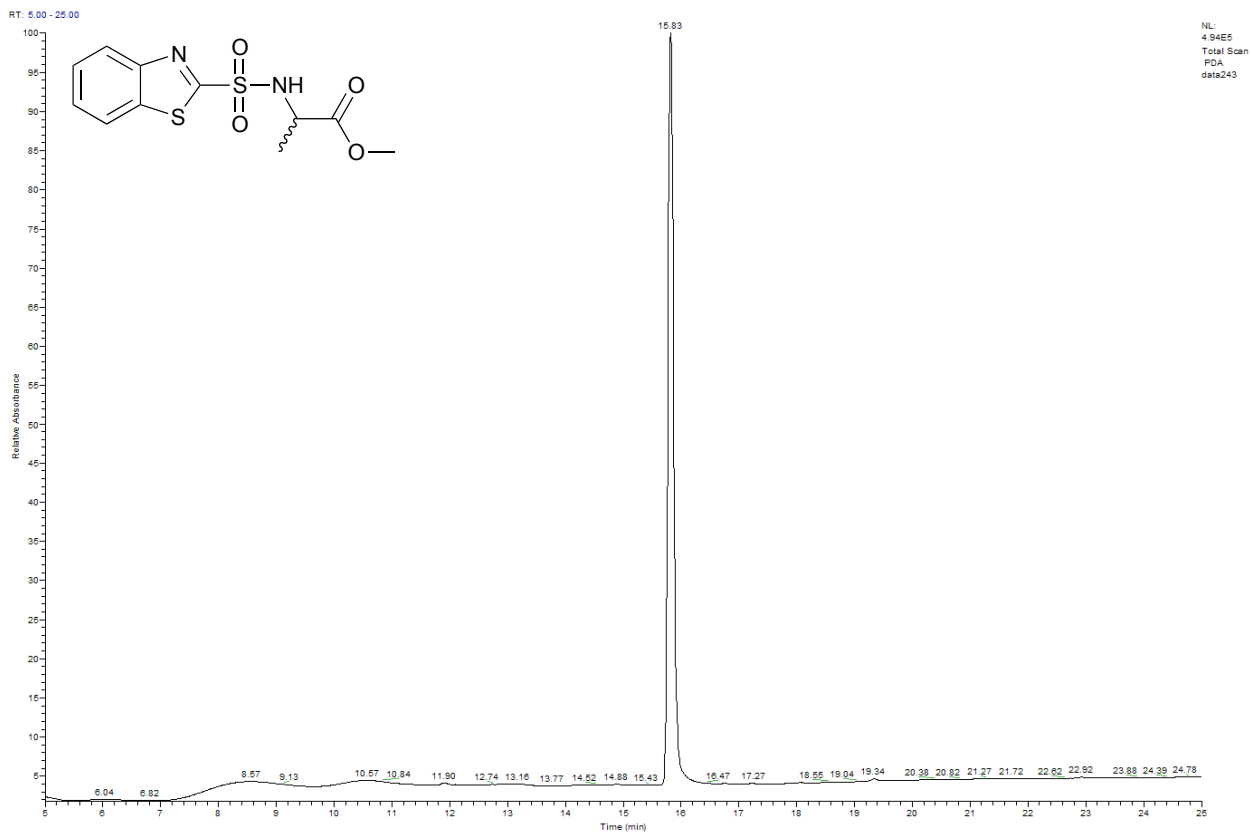


Figure S81. LC-MS-UV spectrum of methyl benzothiazole-2-sulfon-*DL*-alaninate.

data243 4889-902 RT: 16.93-16.12 Av: 7 NL: 1.35E4
F: ITMS + p ESI E Full ms [50.00-1000.00]

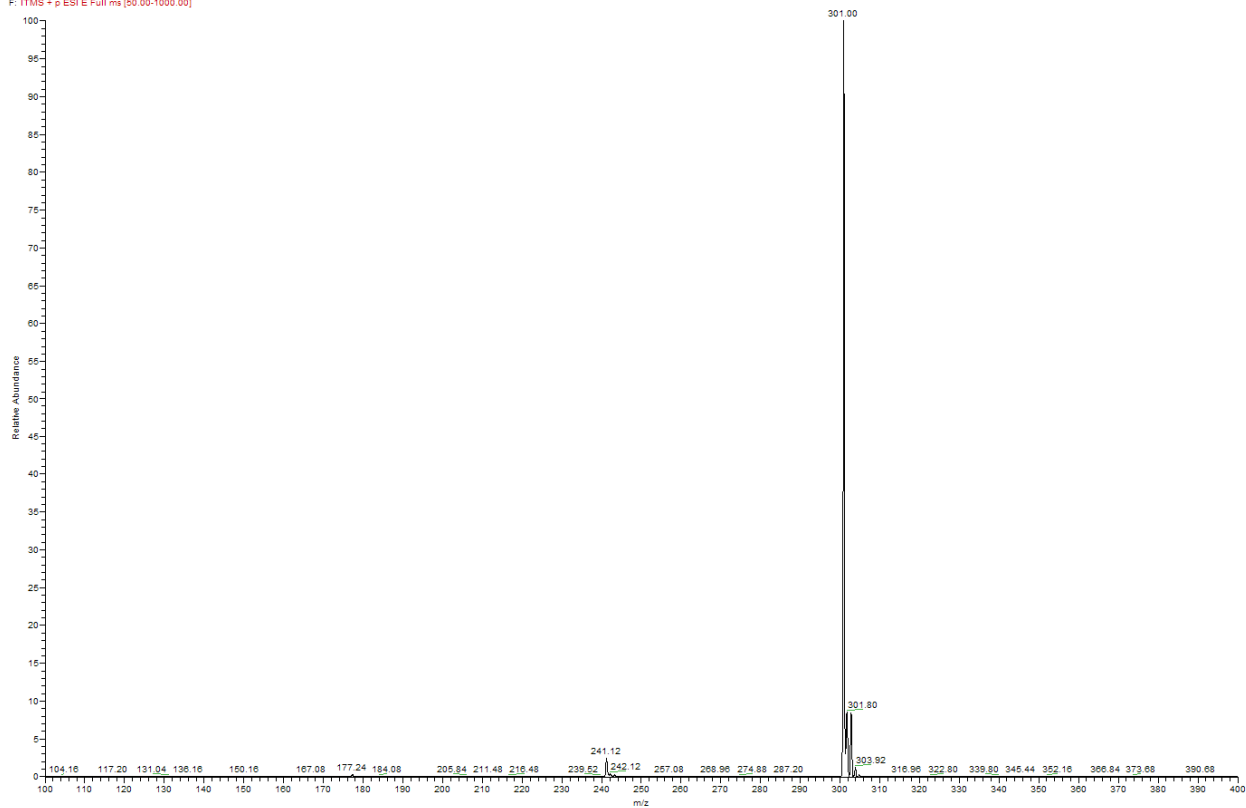


Figure S82. LC-MS spectrum of methyl benzothiazole-2-sulfon-*DL*-alaninate.

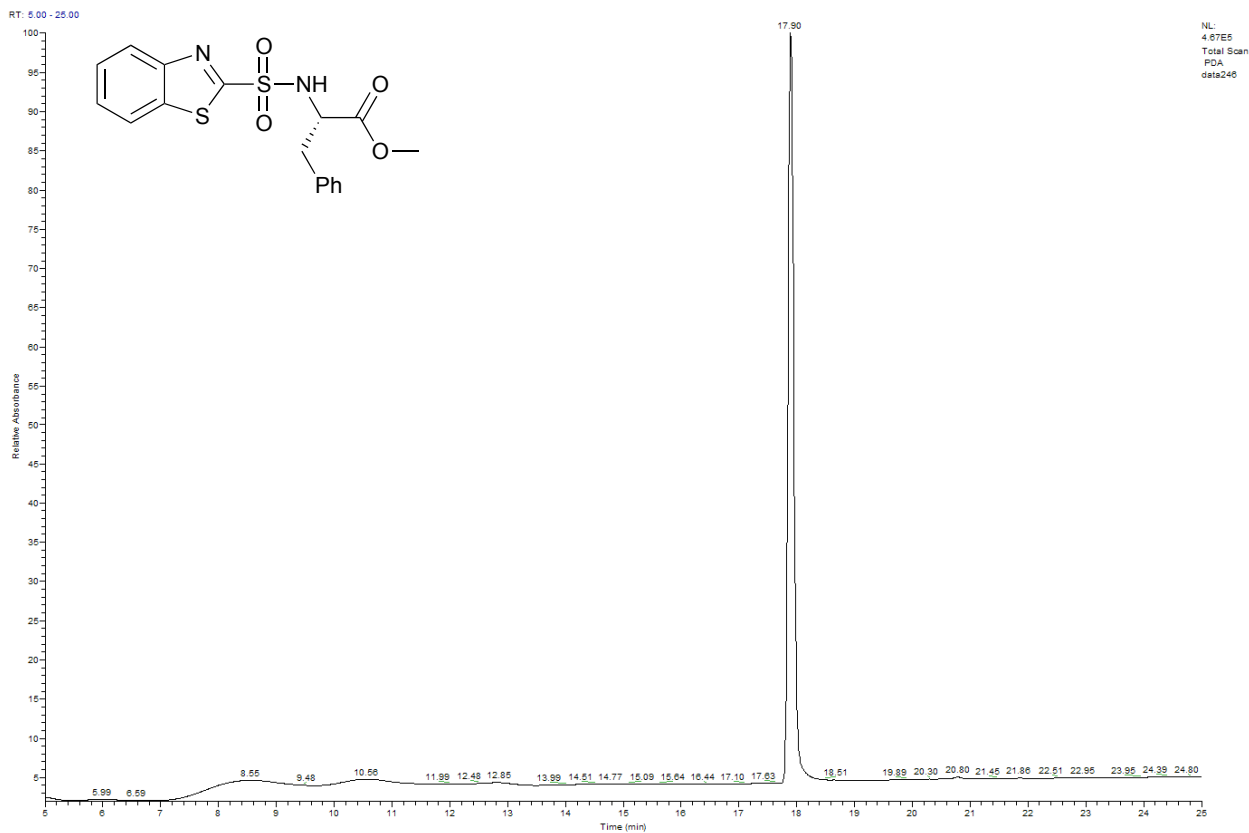


Figure S83. LC-MS-UV spectrum of methyl benzothiazole-2-sulfon-*L*-phenylalaninate.

data248 #1001-1025 RT: 17.84-18.33 AV: 13 NL: 1.31E4
F: ITMS - p ESI E Full ms [50.00-1000.00]

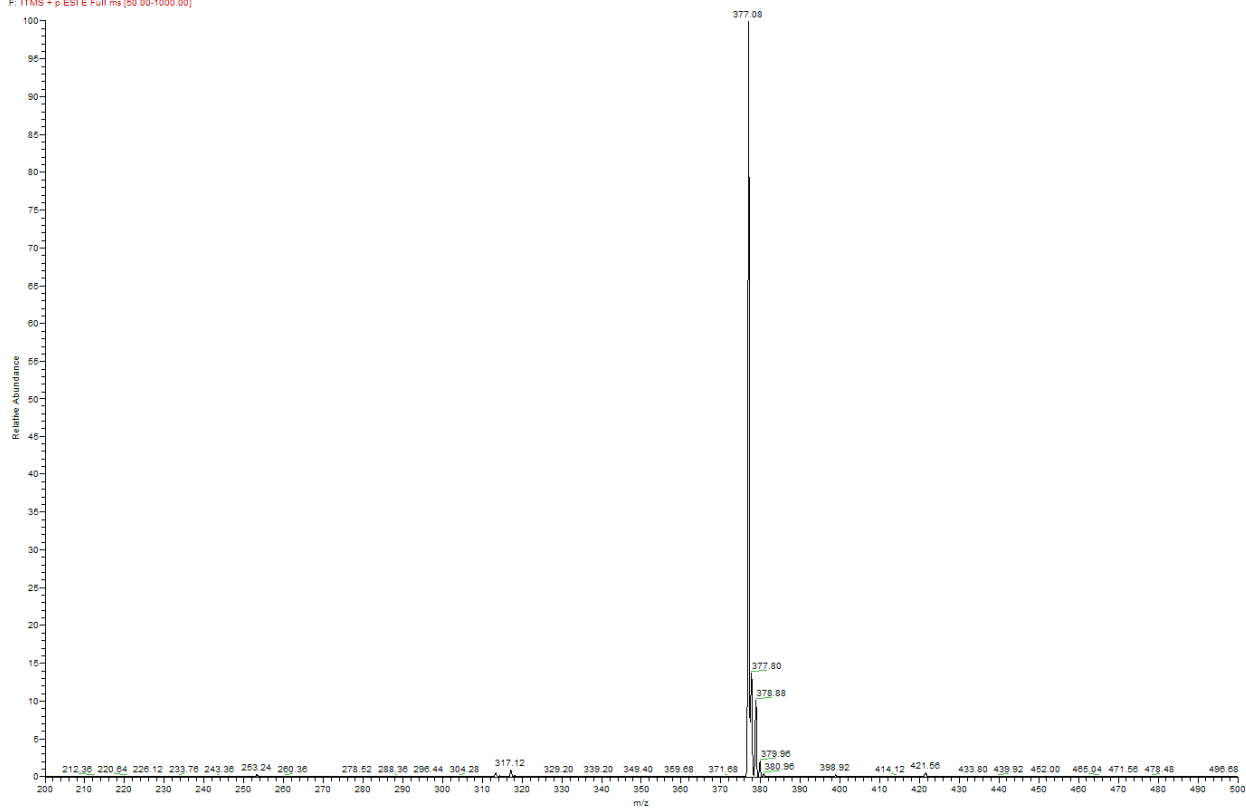


Figure S84. LC-MS- spectrum of methyl benzothiazole-2-sulfon-*L*-phenylalaninate.

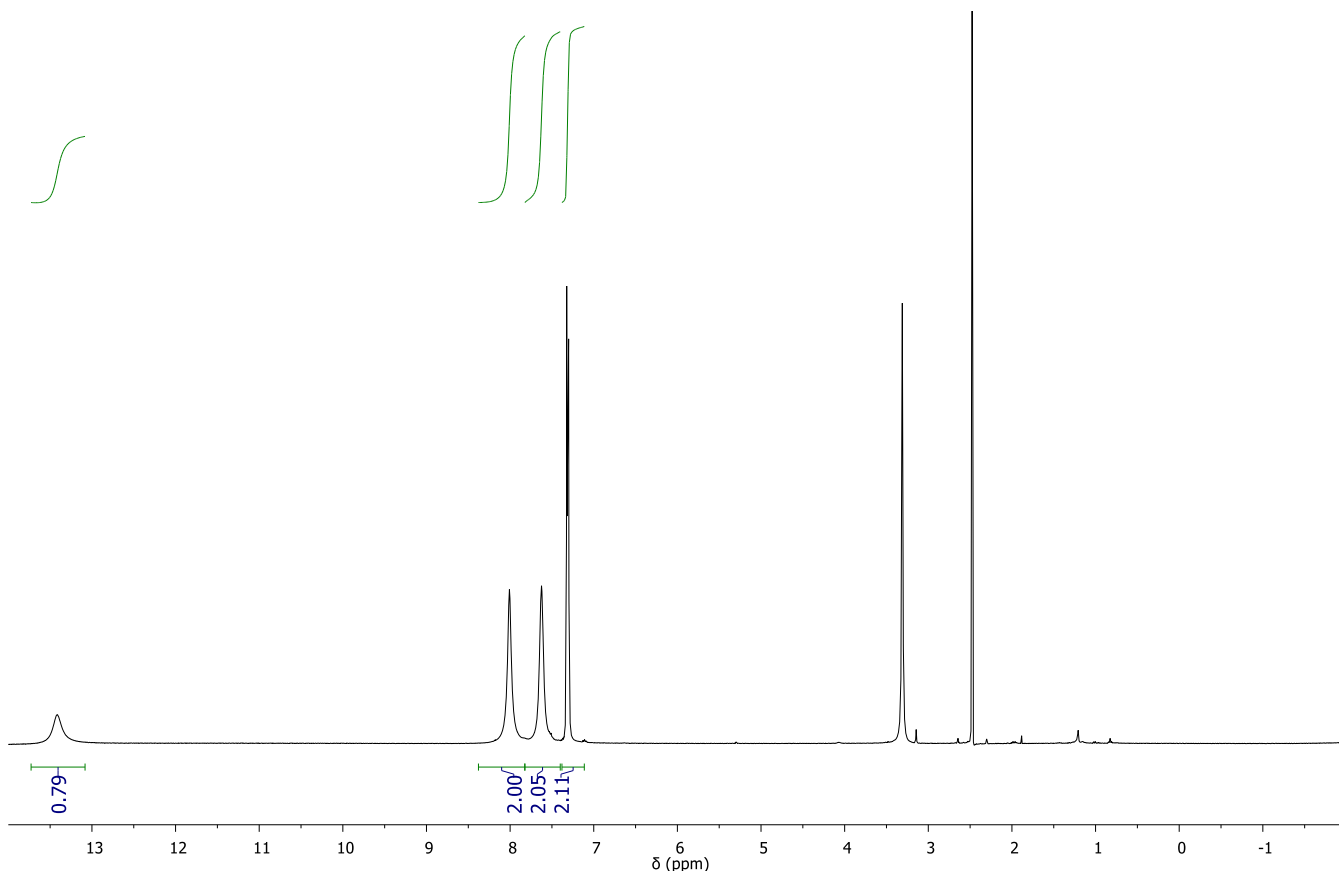
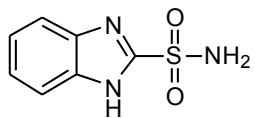


Figure 85. ¹H-NMR spectrum of benzimidazole-2-sulfonamide.

V. References

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