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N-(4-Methoxyphenyl)-substituted bicyclic isothioureas: effect on morphology of cancer cells

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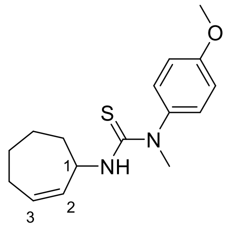
Chemistry

All starting materials, reagents and solvents were high-grade and were used as purchased. Reactions sensitive to moisture and/or oxygen were carried out under anhydrous argon. Liquid column chromatography was performed using silica gel Acros (40–60 μm). Thin-layer chromatography (TLC) was performed on Silufol-UV254 silica gel sheets, and spots were visualized with UV light (λ = 254 nm) or stained with iodine vapor or aqueous potassium permanganate solution. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400/ Agilent 400-MR spectrometer at 27°C in $CDCl_3$ at 400 and 100 MHz correspondingly. Chemical shifts (δ) are reported in parts per million (ppm) referenced to residual protonated solvent peak ($CDCl_3$, δ = 7.27 for ^{1}H NMR) or to carbon resonances in the NMR solvent ($CDCl_3$, δ = 77.0 for ^{13}C NMR). MS spectra were recorded on a Agilent 1100 SL quadrupole mass spectrometer (electrospray ionization) with detector Sedex 75 ELSD (positive ion mode).

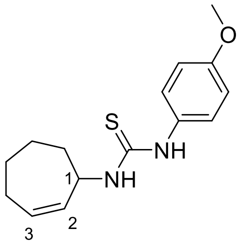


3-(Cyclohept-2-en-1-yl)-1-(4-methoxyphenyl)-1-methylthiourea (3a). 1-Isothiacyanatocyclohept-2-ene (**2a**) solution was obtained by analogy to the procedure described for **3b** from cycloheptene (**1a**) (0.89 ml, 7.66 mmol, 2 equiv.), NBS (0.68 g, 3.83 mmol, 1 equiv.) and potassium thiocyanate (0.372 g, 3.83 mmol, 1 equiv.). The reaction control was carried out by ^{1}H NMR of the reaction mixture: the resonance signal at 4.40 ppm (m, 1H, *J* = 9.5, 1.9 Hz) instead of 4.93 ppm confirmed the conversion of intermediate 3-bromocycloheptene into isothiocyanate **2a**. The solution of **2a** was treated with DIPEA (1.33 ml, 7.66 mmol, 2 equiv.) and 4-methoxy-*N*-methylaniline (0.52 g, 3.83 mmol, 1 equiv.). The reaction mixture was stirred overnight at room temperature, concentrated and purified by column chromatography (eluent: $CHCl_3$) to give thiourea **3a** as yellow amorphous solid (0.44 g, yield 40% from **1a**).

^{1}H NMR (δ, $CDCl_3$): 1.19 – 1.28 (m, 1H), 1.41 (m, 1H, *J* = 12.4, 10.2, 3.5 Hz), 1.58 – 1.63 (m, 1H), 1.64 – 1.68 (m, 1H), 1.70 – 1.76 (m, 1H), 1.82 (m, 1H, *J* = 9.4, 6.2, 2.9 Hz), 2.01 – 2.08 (m, 2H), 3.60 (s, 3H, NMe), 3.82 (s, 3H, OMe), 5.05 (td, 1H, *J* = 7.7, 1.6 Hz, H^1), 5,36 – 5.42 (m, 1H, H^2), 5.38 (br.s., 1H, NH), 5.70 (m, 1H, *J* = 11.9, 6.5, 5.4, 2.3 Hz, H^3), 6.95 (m, 2H, *J* = 8.9 Hz H^{Ar}), 7.10 (m, 2H, *J*= 8.9 Hz, H^{Ar}). ^{13}C NMR (δ, $CDCl_3$): 26.68, 27.13, 28.35, 33.36, 43.23 (NMe), 55.44 (C^1), 56.22 (OMe), 115.57 ($C^{3,5Ar}$), 128.08 ($C^{2,6Ar}$), 131.96 (C^3), 134.42 (C^2), 135.04 (C^{1Ar}), 159.14 (C^{4Ar}), 180.75 (C=S).

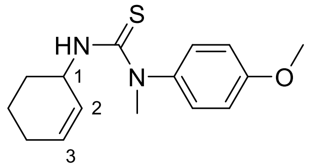
MS (ESI) m/z: 197 $[C_9H_{13}N_2OS]^+$, 291 $[M+H]^+$.

1-(Cyclohept-2-en-1-yl)-3-(4-methoxyphenyl)thiourea (3'a). The solution of **2a** prepared by the same procedure as for compound **3a** was treated with DIPEA (1.33 ml, 7.66 mmol, 2 equiv.) and 4-methoxyaniline (0.471 g, 3.83 mmol, 1 equiv.). The reaction mass was stirred overnight at room temperature, concentrated and purified by column chromatography (eluent: $CHCl_3$) to give thiourea **3'a** as yellow amorphous solid (0.168 g, yield 16%).



^{1}H NMR (δ, $CDCl_3$): 1.21 – 1.32 (m, 1H), 1.45 – 1.54 (m, 1H), 1.60 – 1.70 (m, 2H), 1.76 – 1.83 (m, 1H), 1.87 – 1.92 (m, 1H), 2.05-2.10 (m, 2H), 3.77 (s, 3H, OMe), 5.04 (m, 1H, H^1), 5.48 (dd, 1H, J = 11.5, 2.7 Hz, H^2), 5.75 (m, 1H, J = 11.5, 6.0, 5.9, 2.2 Hz, H^3), 5.92 (br.s., 1H, NH), 6.89 (m, 2H, J = 8.8 Hz, H^{Ar}), 7.11 (m, 2H, d, J = 8.8 Hz, H^{Ar}), 8.27 (br.s., 1H, NH). ^{13}C NMR (δ, $CDCl_3$): 26.60, 27.18, 28.38, 33.38, 55.41 (C^1), 56.01 (OMe), 115.12 ($C^{3,5Ar}$), 127.43 ($C^{2,6Ar}$), 128.45 (C^3), 132.44 (C^2), 133.70 (C^{1Ar}), 158.65 (C^{4Ar}), 179.58 (C=S). MS (ESI) m/z: 183 $[C_8H_{11}N_2OS]^+$, 277 $[M+H]^+$.

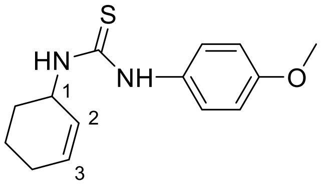
3-(Cyclohex-2-en-1-yl)-1-(4-methoxyphenyl)-1-methylthiourea (3b). Cyclohexene (**1b**) (0.247 ml, 2.44 mmol, 2 equiv.) dissolved in CCl_4 (10 ml) was treated at stirring with *N*-bromosuccinimide (NBS) (0.215 g, 1.22 mmol, 1 equiv.) and azobis(isobutyronitrile) (AIBN, 2 mg, 10.4 mmol, cat. equiv.). The reaction mixture was refluxed for 1.5 h, then cooled to 0°C and filtered, the filtrate was washed with CCl_4 (5 ml). The reaction control was carried out by ^{1}H NMR. Characteristic signals of 3-bromocyclohexene in the ^{1}H NMR (δ, CCl_4+ $CDCl_3$): 1.66 – 1.73 (m, 1H), 1.90 – 2.12 (m, 2H), 2.15 – 2.26 (m, 3H), 4.83 – 4.87 (m, 1H, J = 3.9 Hz, H^1), 5.80 – 5.85 (m, 1H, J = 9.9, 3.9 Hz, H^2), 5.90 – 5.95 (m, 1H, J = 9.9 Hz, H^3). A solution of 3-bromocyclohexene in CCl_4 from the previous step was treated dropwise with the solution of potassium thiocyanate (0.1 g, 1.8 mmol, 1 equiv.) in absolute MeOH (4 ml) at ice cooling and was stirred at room temperature. After 2 h the mixture was filtered through SiO_2, washed with hexane (20 ml). The solution of 3-isothiocyanatocyclohexene **2b** was used directly for the next step without evaporation and further purification. The conversion was controlled by ^{1}H NMR analysis of the reaction solution. The presence of the resonance signal at 4.23 ppm (m, 1H, H^1) instead of 4.87 ppm confirms the conversion of bromide into isothiocyanate.



A solution of **2b** was treated with DIPEA (0.42 ml, 2.42 mmol, 2 equiv.) and *N*-methyl-4-methoxyaniline (0.167 g, 1.22 mmol, 1 equiv.). The reaction mass was stirred overnight at room temperature, concentrated and purified by column chromatography (eluent: 1:6 ethyl acetate : hexane by v/v) to give thiourea **3b** as white amorphous solid (0.205 g, yield 61%).

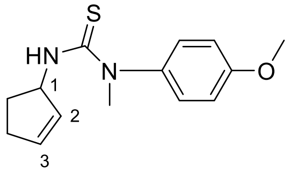
^{1}H NMR (δ, $CDCl_3$): 1.38 – 1.50 (m, 2H), 1.55 – 1.65 (m, 1H), 1.87 – 1.95 (m, 3H), 3.62 (s, 3H, NMe), 3.84 (s, 3H, OMe), 4.98 (br.s. 1H, NH), 5.25 (m, 1H, *J* = 8 Hz, H^1), 5.52 (dd, 1H, *J* = 10.5, 1.7 Hz, H^2), 5.74 (dtd, 1H *J* = 10.5, 3.4, 1.5 Hz, H^3), 6.96 (m, 2H, *J* = 8.9 Hz, H^{Ar}), 7.11 (m, 2H, *J* = 8.9 Hz, H^{Ar}). ^{13}C NMR (δ, $CDCl_3$): 19.74, 24.75, 28.99, 43.38 (NMe), 50.69 (C^1), 55.47 (OMe), 115.62 (C^{3,5Ar}), 127.44 (C^3), 128.08 (C^{2,6Ar}), 130.93 (C^2), 135.18 (C^{1Ar}), 159.15 (C^{4Ar}), 181.06 (C=S). MS (ESI) m/z: 197 $[C_9H_{13}N_2OS]^+$, 277 $[M+H]^+$.

1-(Cyclohex-2-en-1-yl)-3-(4-methoxyphenyl)thiourea (3'b). The solution of **2b** prepared by the same procedure as for compound **3b** was treated with DIPEA (0.42 ml, 2.42 mmol, 2 equiv.) and 4-methoxyaniline (0.150 g, 1.22 mmol, 1 equiv.). The reaction mixture was stirred overnight at room temperature, concentrated and purified by column chromatography (eluent: 1:6 ethyl acetate : hexane by v/v) to give thiourea **3'b** as white amorphous solid (0.234 g, yield 73%).



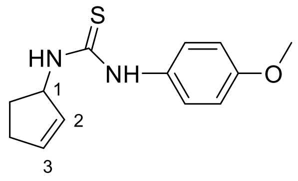
^{1}H NMR (δ, $CDCl_3$): 1.48 – 1.72 (m, 4H), 1.97 – 2.05 (m, 2H), 3.83 (s, 3H, OMe), 4.97 (m, 1H, H^1), 5.61 (m, 1H, *J* = 10.0, 2.1 Hz, H^2), 5.7 – 5.9 (br.s., 1H, NH), 5.84 (dtd, 1H *J* = 10.0, 3.7, 1.9 Hz, H^3), 6.94 (m, 2H, *J* = 9.0 Hz, H^{Ar}), 7.14 (m, 2H, d, *J* = 9.0 Hz, H^{Ar}), 7.61 (br.s., 1H, NH). ^{13}C NMR (δ, $CDCl_3$): 19.69, 24.65, 28.84, 50.40 (C^1), 55.40 (OMe), 115.16 (C^{3,5Ar}), 126.81 (C^3), 127.33(C^{2,6Ar}), 128.48 (C^2), 131.39(C^{1Ar}), 158.64 (C^{4Ar}), 179.85 (C=S). MS (ESI) m/z: 263 $[M+H]^+$.

3-(Cyclopent-2-en-1-yl)-1-(4-methoxyphenyl)-1-methylthiourea (3c). 1-Isothiacyanatocyclopentene **2c** solution was obtained by analogy to the procedure described for **3b** from cyclopentene **1c** (0.33 ml, 3.7 mmol, 2 equiv.), NBS (0.318 g, 1.8 mmol, 1 equiv.) and potassium thiocyanate (0.175 g, 1.8 mmol, 1 equiv.). The reaction control was carried out by ^{1}H NMR of the reaction mixture: the resonance signal at 4.66 – 4.72 ppm (m, 1H, H^3) instead of 5.17 ppm confirmed the conversion of intermediate 3-bromocyclopentene into isothiocyanate **2c**. The solution of **2c** was treated with DIPEA (0.64 ml, 3.7 mmol, 2 equiv.) and 4-methoxy-*N*-methylaniline (0.25 g, 1.8 mmol, 1 equiv.). The reaction mixture was stirred overnight at room temperature, concentrated and purified by column chromatography (eluent: $CHCl_3$) to give thiourea **3c** as yellow amorphous solid (0.24 g, yield 52%).



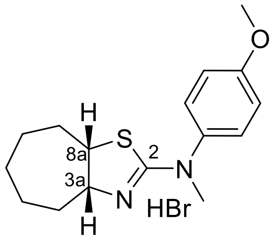
^{1}H NMR (δ, $CDCl_3$): 1.32 (m, 1H, *J* = 13.5, 8.6, 5.8 Hz), 2.09 – 2.25 (m, 2H), 2.32 (ddd, 1H, *J* = 13.2, 8.7, 4.5 Hz), 3.53 (s, 3H, NMe), 3.74 (s, 3H, OMe), 5.19 (d, 1H, *J* = 8.0 Hz, NH), 5.39 (m, 1H, *J* = 7.9, 5.6, 2.2 Hz, H^1), 5.53 (m, 1H, *J* = 6,0, 2.2 Hz, H^2), 5.75 (m, 1H, *J* = 6.0, 2.2 Hz, H^3), 6.88 (m, 2H, *J* = 9.0 Hz, H^{Ar}), 7.03 (m, 2H, *J* = 9.0 Hz, H^{Ar}). ^{13}C NMR (δ, $CDCl_3$): 30.7, 31.1, 43.2 (NMe), 55.3 (OMe), 61.7 (C^1), 115.4 ($C^{3,5Ar}$), 127.8 ($C^{2,6Ar}$), 130.7 (C^{1Ar}), 134.5, 134.8, 158.9 (C^{4Ar}), 181.0 (C=S). MS (ESI) m/z: 263 $[M+H]^+$.

1-(Cyclopent-2-en-1-yl)-3-(4-methoxyphenyl)thiourea (3'c). The solution of **2c** prepared by the same procedure as for compound **3c** was treated with DIPEA (0.64 ml, 3.7 mmol, 2 equiv.) and 4-methoxyaniline (0.221 g, 1.8 mmol, 1 equiv.). The reaction mass was stirred overnight at room temperature, concentrated and purified by column chromatography (eluent: 1:10 ethyl acetate : hexane by v/v) to give thiourea **3’c** as beige amorphous solid (0.34 g, yield 75%).



^{1}H NMR (δ, $CDCl_3$): 1.53 (ddt, 1H, J = 13.8, 8.5, 5.6 Hz), 2.24 – 2.36 (m, 2H), 2.44 (m, 1H, J = 13.2, 8.8, 8.1, 4.3 Hz), 3.80 (s, 3H, OMe), 5.48 (m, 1H, H^1), 5.69 (m, 1H, J = 5.7, 2.2 Hz, H^2), 5.75 (br.s., 1H, NH), 5.91 (m, 1H, J = 5.7, 2.2 Hz, H^3), 6.91 (m, 2H, J = 8.9 Hz, H^{Ar}), 7.12 (m, 2H, J = 8.9 Hz, H^{Ar}), 7.89 (br.s., 1H, NH). ^{13}C NMR (δ, $CDCl_3$): 31.0, 31.01, 55.4 (OMe), 61.7 (C^1), 115.2 ($C^{3,5Ar}$), 127.4 ($C^{2,6Ar}$), 128.4, 130.4 (C^{1Ar}), 135.3, 158.7 (C^{4Ar}), 180.1 (C=S).
MS (ESI) m/z: 249 $[M+H]^+$.

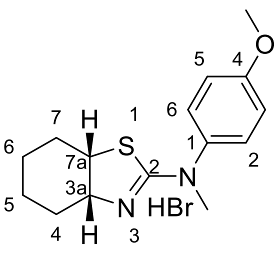
(±)-(3a*R*,8a*S*)-N-(4-Methoxyphenyl)-*N*-methyl-3a,5,6,7,8,8a-hexahydro-4*H*-cyclohepta[*d*]-thiazol-2-amine hydrobromide (4a) was prepared from thiourea **3a** (0.32 g, 1.1 mmol, 1 equiv.) by analogy to the procedure described for **4b**. The reaction mass was concentrated and the residue purified by column chromatography (eluent: CH_2Cl_2, then gradient 2-5% MeOH in CH_2Cl_2) to give the product as yellow amorphous solid (0.23 g, yield 57%).



^{1}H NMR (δ, $CDCl_3$): 1.25 – 1.41 (m, 3H), 1.72 – 1.89 (m, 6H), 2.09 (m, 1H, J = 14.1, 8.6, 3.9 Hz), 2.70 (br. s., 1H, NH), 3.33 (s, 3H, NMe), 3.80 (s, 3H, OMe), 3.96 (ddd, 1H, J = 9.8, 8.5, 3.7 Hz, H^{8a}), 4.45 (ddd, 1H, J = 10.2, 8.5, 3.9 Hz, H^{3a}), 6.85 (m, 2H, J = 9.0 Hz, H^{Ar}), 7.18 (m, 2H, J = 9.0 Hz, H^{Ar}). The coupling constant $J^{3a\text{-}8a}$ ~9.5 Hz **4a** is consistent with *cis* configuration of the rings on the basis of Karplus relationship [S1].[1]

^{13}C NMR (δ, $CDCl_3$): 24.86, 27.39, 30.19, 30.46, 31.56, 44.83 (NMe), 51.75 (C^{8a}), 55.54 (OMe), 67.37 (C^{3a}), 115.04 ($C^{3,5Ar}$), 127.02 ($C^{2,6Ar}$), 136.14 (C^{1Ar}), 160.24 (C^{4Ar}), 170.87 (C^2).
MS (ESI) m/z: 291 $[M+H-Br]^+$.

(±)-(3a*R*,7a*S*)-*N*-(4-Methoxyphenyl)-*N*-methyl-3a,4,5,6,7,7a-hexahydrobenzo[*d*]thiazol-2-amine hydrobromide (4b). A solution of acetyl bromide (25 µl, 0.33 mmol, 3 equiv.) in CH_2Cl_2 (5 ml) was treated with methanol (13 µl, 0.33 mmol, 3 equiv.) and stirred at room temperature in darkness. In 10 min, a solution of thiourea **3b** (30 mg, 0.11 mmol, 1 equiv.) in CH_2Cl_2 (5 ml) was added,

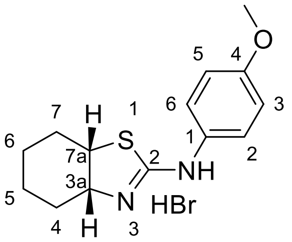


and stirring was continued for 12 h. The reaction mixture was concentrated to give the product as colorless oil (35 mg, yield 90%).

^{1}H NMR (δ, $CDCl_3$): 1.29 – 1.46 (m, 2H), 1.54 – 1.76 (m, 3H), 1.80 – 1.89 (m, 2H), 2.25 – 2.33 (m, 1H), 3.65 (s, 3H, NMe) 3.81 (s, 3H, OMe), 3.82 (m, 1H, *J* = 7.6, 5.3 Hz, H^{7a}), 4.27 (m, 1H, *J* = 5.5, 5.3 Hz, H^{3a}), 4.93 (br.s., 1H, NH), 6.90 (m, 2H, *J* = 8.9 Hz, H^{Ar}), 7.16 (m, 2H, *J* = 8.9 Hz, H^{Ar}). The cycle junction in products **4b,4'b** was assigned as *cis* in accordance with our earlier study and confirmed by the values of observed coupling constants ($J^{3a\text{-}7a}$ ~5 Hz) for bridgehead proton signals [S2].[2] ^{13}C NMR (δ, $CDCl_3$): 20.80, 22.21, 27.89, 28.70, 43.30 (NMe), 50.66 (C^{7a}), 55.49 (OMe), 65.36 (C^{3a}), 114.77 (C^{3,5Ar}), 127.27 (C^{2,6Ar}), 137.20 (C^{1Ar}), 159.53 (C^{4Ar}), 169.76 (C^{2}).

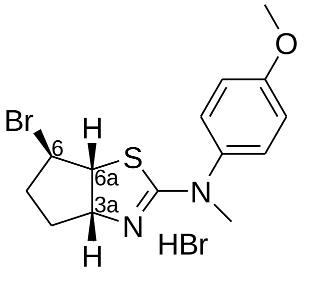
MS (ESI) m/z: 277 [M+H-Br]$^{+}$.

(±)-(3a*R*,7a*S*)-*N*-(4-Methoxyphenyl)-3a,4,5,6,7,7a-hexahydrobenzo[*d*]thiazol-2-amine hydrobromide (4'b) was prepared from thiourea **3'b** (0.088 g, 0.33 mmol, 1 equiv.) by analogy to the procedure described for **4b**. The reaction mixture was concentrated, and the residue purified by column chromatography (eluent: $CHCl_3$, then 2% MeOH in $CHCl_3$) to give the product as beige oil (0.110 g, yield 96%).



^{1}H NMR (δ, $CDCl_3$): 1.29 – 1.45 (m, 2H), 1.51 – 1.63 (m, 2H),1.67 – 1.84 (m, 2H), 1.91 – 2.05 (m. 2H), 3.74 (s, 3H, OMe), 3.85 (ddd, 1H, *J* = 8.2, 5.7, 5.4 Hz, H^{7a}), 4.19 (m, 1H, *J* = 5.1, 5.4 Hz, H^{3a}), 6.83 (m, 2H, *J* = 8.9 Hz, H^{Ar}), 7.14 (m, 2H, *J* = 8.9 Hz, H^{Ar}), 10.11 (br.s., 2H, 2NH). ^{13}C NMR (δ, $CDCl_3$): 20.11, 22.13, 27.18, 28.78, 48.33 (C^{7a}), 55.49 (OMe), 60.65 (C^{3a}), 114.69 (C^{3,5Ar}), 125.63 (C^{2,6Ar}), 129.07 (C^{1Ar}), 159.38 (C^{4Ar}), 174.20 (C^{2}). MS (ESI) m/z: 263 [M+H-Br]$^{+}$.

(±)-(3a*R*,6a*R*,6*R*)-6-Bromo-*N*-(4-methoxyphenyl)-*N*-methyl-3a,5,6,6a-tetrahydro-4*H*-cyclopenta[*d*]thiazol-2-amine hydrobromide (5). A solution of **3c** (0.1 g, 0.38 mmol, 1 equiv.) in CH_2Cl_2 (10 ml) was treated with Br_2 (20 µl, 0.38 mmol, 1 equiv.) and stirred the in darkness at room temperature for 12 h. The reaction mass was washed with aqueous saturated solution of Na_2SO_3 (3x20ml). The organic layer was dried over Na_2SO_4, concentrated and purified by column chromatography (eluent: $CHCl_3$) to yield the product as yellow oil (0.080 g, 50%).



^{1}H NMR (δ, $CDCl_3$): 1.97-2.05 (m, 2H), 2.25-2.34 (m, 1H), 2.34-2.48(m,1H), 3.32 (s, 3H, NMe), 3.82 (s, 3H, OMe), 4.28 (m, 1H, H^{6}), 4.41 (dd, 1H, *J* = 8.0, 1.5 Hz, H^{6a}), 5.14 (td, 1H, *J* = 8.0, 1.9 Hz, H^{3a}), 6.87 (m, 2H, *J* = 8.9 Hz, H^{Ar}), 7.17 (m, 2H, *J* = 8.9 Hz, H^{Ar}). The values of coupling constants ($J^{3a\text{-}6a}$ ~7.8-8.0 Hz) in ^{1}H NMR spectra of compounds **5,5'** correspond with literature data for similar *cis*-bicyclic compounds [S3].[3] The configuration of bromine atom was assigned as *trans*-

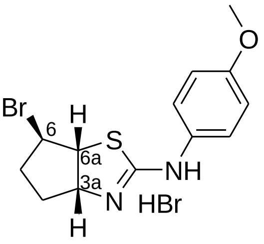
towards thiazol ring on the basis of ^{1}H NOESY1D spectra: H^{6a} – H^{3a} (1.4%); H^{3a} – H^{6a} (2.3%); H^{3a} – H^6 and H^6 – H^{3a} correlations were not observed.

^{13}C NMR (δ, $CDCl_3$): 32.7, 33.8, 41.0 (NMe), 55.4 (OMe), 58.0 (C^{6a}), 63.5 (C^6), 78.5 (C^{3a}), 114.2, 128.1, 138.7, 158.6, 160.5 (C^2). MS (ESI) m/z: 341 $[M+H-Br]^+$, 343$[M+2+H-Br]^+$.

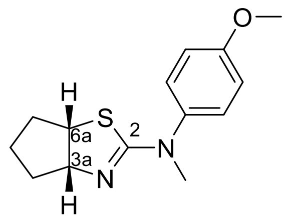
(±)-(3a*R*,6a*R*,6*R*)-6-Bromo-*N*-(4-methoxyphenyl)-3a,5,6,6a-tetrahydro-4*H*-cyclopenta[*d*]-thiazol-2-amine hydrobromide (5') was prepared by analogy to **5** from **3'c** (0.1 g, 0.4 mmol, 1 equiv.) and Br_2 (21 µl, 0.4 mmol, 1 equiv.) in CH_2Cl_2 (10 ml) Column chromatography of the reaction mixture (eluent: 3% MeOH in CH_2Cl_2) yielded the major C^6-diastereomer **5'** as yellow oil (0.115 g, 71%).

^{1}H NMR (δ, $CDCl_3$): 1.87-1.93 (m, 1H), 2.0 – 2.06 (m, 1H), 2.29-2.45 (m, 2H), 3.78 (s, 3H, OMe), 4.33 – 4.37 (m, 1H, H^6), 4.42 (dd, 1H, *J* = 7.8, 1.8 Hz, H^{6a}), 4.85 (m, 1H, *J* = 7.8, 5.8, 1.9 Hz, H^{3a}), 6.81 (m, 2H, *J* = 8.9 Hz, H^{Ar}), 7.05 (m, 2H, *J* = 8.9 Hz, H^{Ar}), 7.60 (br.s., 1H, NH). ^{13}C NMR (δ, $CDCl_3$): 32.5, 33.7, 55.4 (OMe), 57.4 (C^{6a}), 60.1 (C^6), 70.0 (C^{3a}), 114.1, 123.0, 138.1, 156.1, 159.5 (C^2).

MS (ESI) m/z: 327 $[M+H-Br]^+$, 329$[M+2+H-Br]^+$.



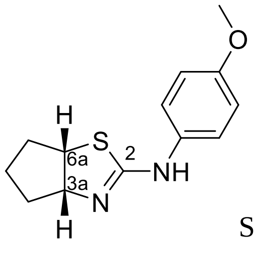
(±)-(3a*R*,6a*S*)-*N*-(4-Methoxyphenyl)-*N*-methyl-3a,5,6,6a-tetrahydro-4*H*-cyclopenta[*d*]-thiazol-2-amine (6). A solution of thiazol **5** (18 mg, 43 µmol, 1 equiv.) in toluene (10 ml) was treated with tri-*n*-butyltin hydride (29 µl, 0.11 mmol, 2.5 equiv) and azobis(isobutyronitrile) (AIBN, 2 mg, 0.012 mmol, catalytic amount). The mixture was heated at 100 ^{0}C under argon atmosphere during 5 h, then concentrated and purified by column chromatography (eluent: ethyl acetate: hexane 1:1 and 1% Et_3N) to yield the product as colorless oil (8 mg, 71%).



^{1}H NMR (δ, $CDCl_3$):1.53 – 1.60 (m, 1H), 1.70 – 1.84 (m, 2H), 1.89 – 1.97 (m, 3H), 3.35 (s, 3H, NMe), 3.81 (s, 3H, OMe), 4.08 (td, 1H, *J* = 8.2, 3.9 Hz, H^{6a}), 4.96 (dt, 1H, *J* = 8.2, 5.3 Hz, H^{3a}), 6.86 (m, 2H, *J* = 9.0 Hz, H^{Ar}), 7.19 (m, 2H, *J* = 9.0 Hz, H^{Ar}). ^{13}C NMR (δ, $CDCl_3$): 23.6, 35.1, 36.5, 41.0, 53.5 (C^{6a}), 55.4 (OMe), 79.5 (C^{3a}), 114.1, 128.1, 139.2, 158.3, 162.6 (C^2).

MS (ESI) m/z: 263 $[M+H]^+$.

(±)-(3a*R*,6a*S*)-*N*-(4-Methoxyphenyl)-3a,5,6,6a-tetrahydro-4*H*-cyclopenta[*d*]thiazol-2-amine (6') was prepared from thiazol **5'** (57 mg, 0.14 mmol, 1 equiv.) by analogy to the procedure for **6.** Purification of the reaction mass by column chromatography (eluent: ethyl acetate: hexane 1:2 and 1% Et_3N) yielded the product **6’** as yellow oil (24 mg, 69%).



^{1}H NMR (δ, $CDCl_3$):1.74 – 1.81 (m, 1H), 1.83 – 1.97 (m, 3H), 2.01 – 2.10 (m, 2H), 3.80 (s, 3H, OMe), 4.21 (td, 1H, J = 8.2, 2.8 Hz, H^{6a}), 4.78 (ddd, 1H, J = 8.2, 6.1, 2.6 Hz, H^{3a}), 6.86 (m, 2H, J = 8.9 Hz, H^{Ar}), 7.15 (m, 2H, J = 8.9 Hz, H^{Ar}), 7.25 (br.s., 1H, NH). ^{13}C NMR (δ, $CDCl_3$): 23.5, 34.6, 36.0, 49.8 (C^{6a}), 55.4 (OMe), 68.3 (C^{3a}), 114.1, 123.0, 139.5, 156.0, 162.0 (C^2). MS (ESI) m/z: 249 $[M+H]^+$.

Biological testing

Immunofluorescence staining of cellular microtubules. A549 human lung epithelial carcinoma cells (CCL-185™) were cultured under a 5% CO_2 humidified atmosphere with Dulbecco´s Modified Eagle medium (DMEM) at 37°C in 12-well plates on 11 mm glass coverslips (~20000 cells per coverslip). Cells were incubated with tested compounds at 100 μM (and 10 μM for compound 4c) for 24 h (0.5 % DMSO served as a negative control). The cells were fixed and stained as described in [4]. Fixed cells were labeled with mouse monoclonal antibody against α-tubulin (Sigma, St. Louis, USA) at a dilution of 1:300, followed by incubation of Alexa Fluor488 labeled goat anti-mouse IgG (Invitrogen, Germany) at a dilution of 1:300. Images of all samples were acquired with a Nikon Diaphot 300 inverted microscope (Nikon GmbH, Germany) equipped with a cooled charge-couple device camera system (SenSys; Photometrics, Germany).

References

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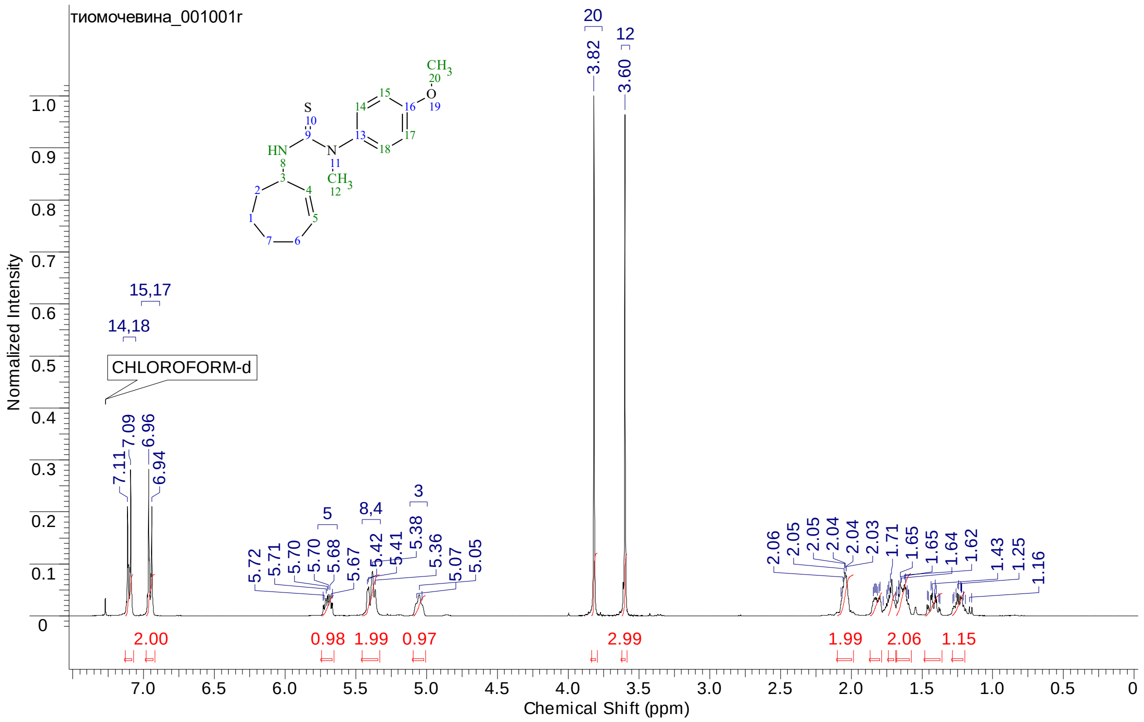
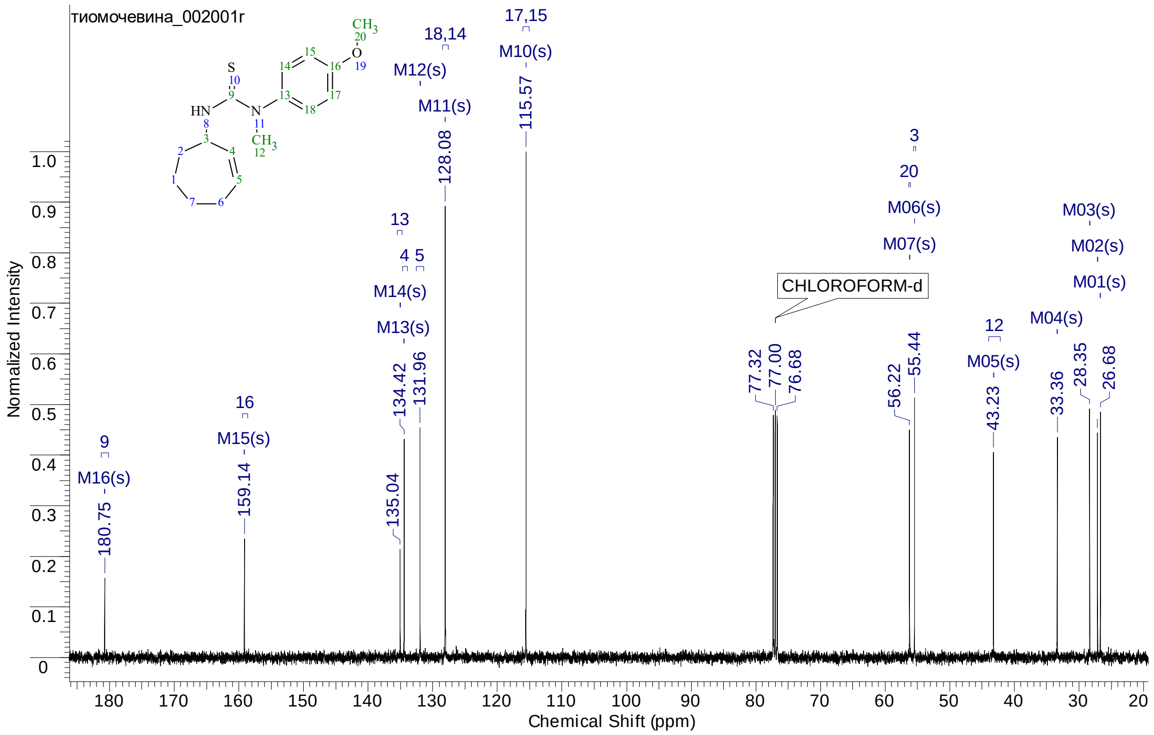
[S3] (*a*) N.-E. Alom, Y. A. Rina and W. Li, *Org. Lett.*, 2017, **19**, 6204; (*b*) J.-Y. Wu, Z.-B. Luo, L.-X. Dai and X.-L. Hou, *J. Org. Chem.*, 2008, **73**, 9137.

[S4] A. Al-Haddad, M. A. Shonn, B. Redlich, A. Blocker, J. K. Burkhardt, H. Yu., J.A. Hammer, III, D.G. Weiss, W. Steffen, G. Griffiths and S.A. Kuznetsov, *Mol. Biol. Cell*, 2001, **12**, 2742.

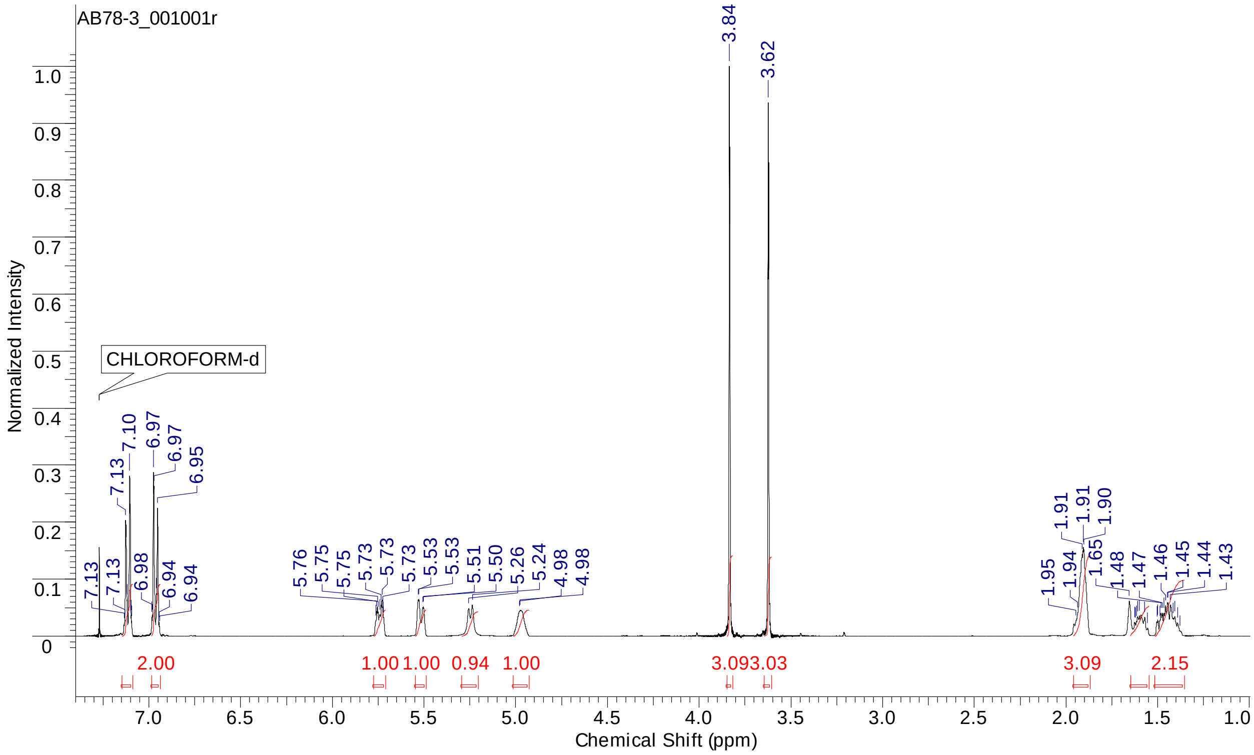
^{1}H NMR and ^{13}C NMR spectra of novel compounds

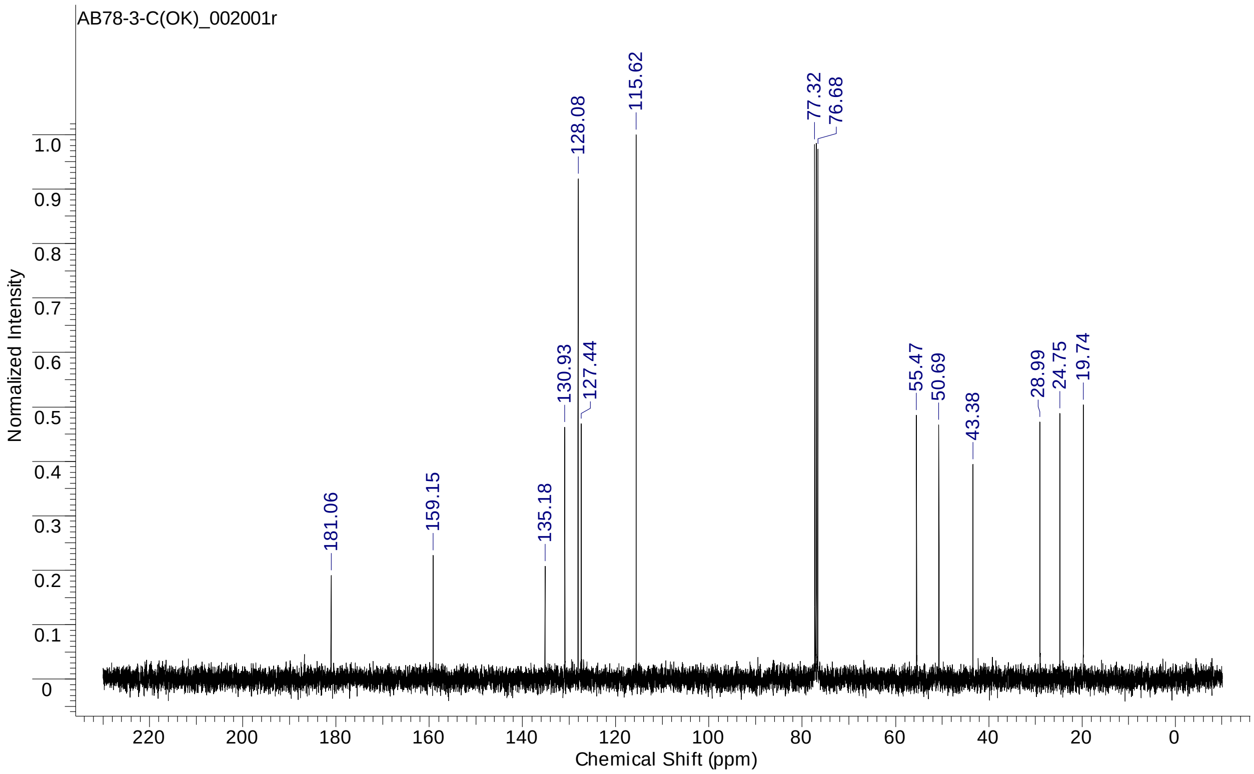
^{1}H NMR and ^{13}C NMR spectra of compound **3a** in $CDCl_3$



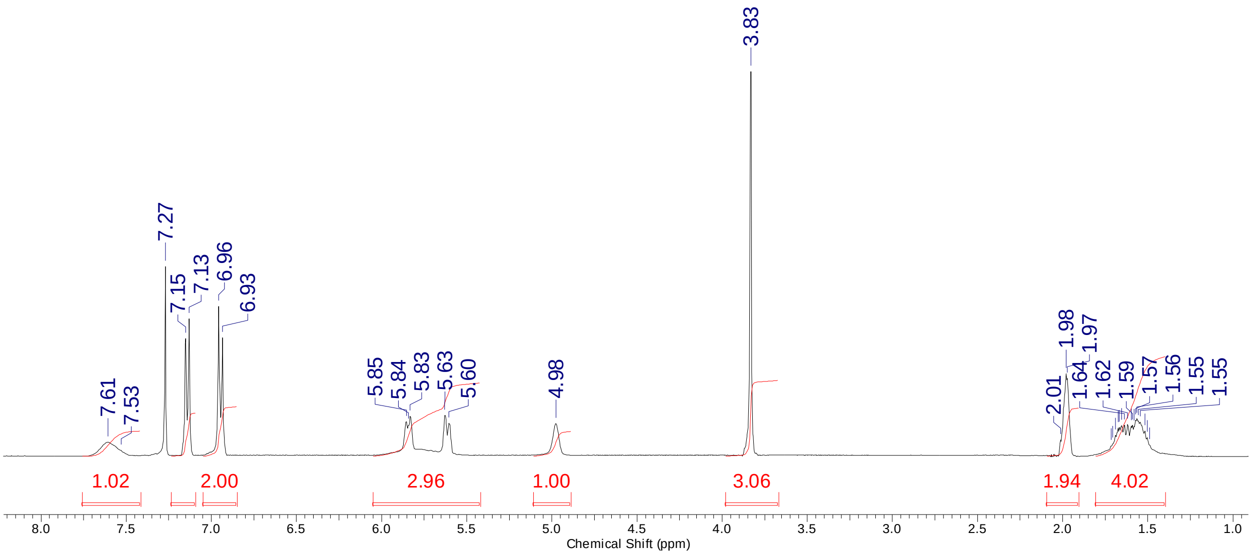


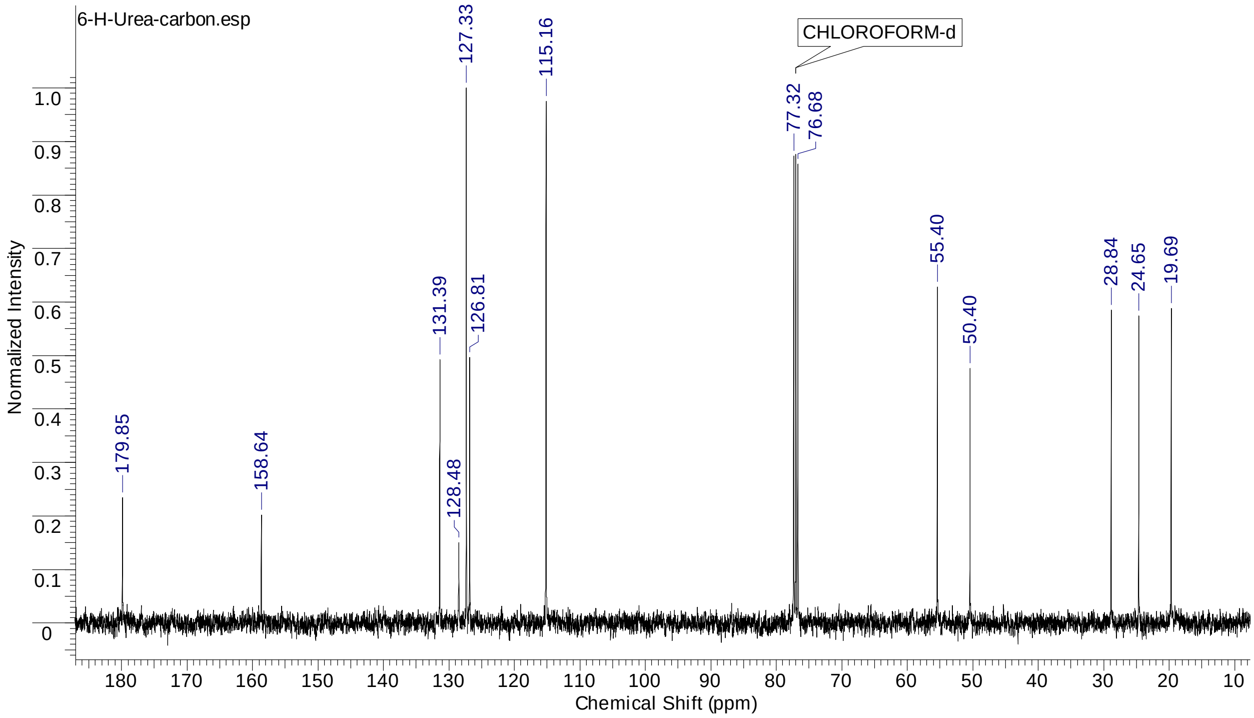
^{1}H NMR and ^{13}C NMR spectra of compound **3b** in $CDCl_3$



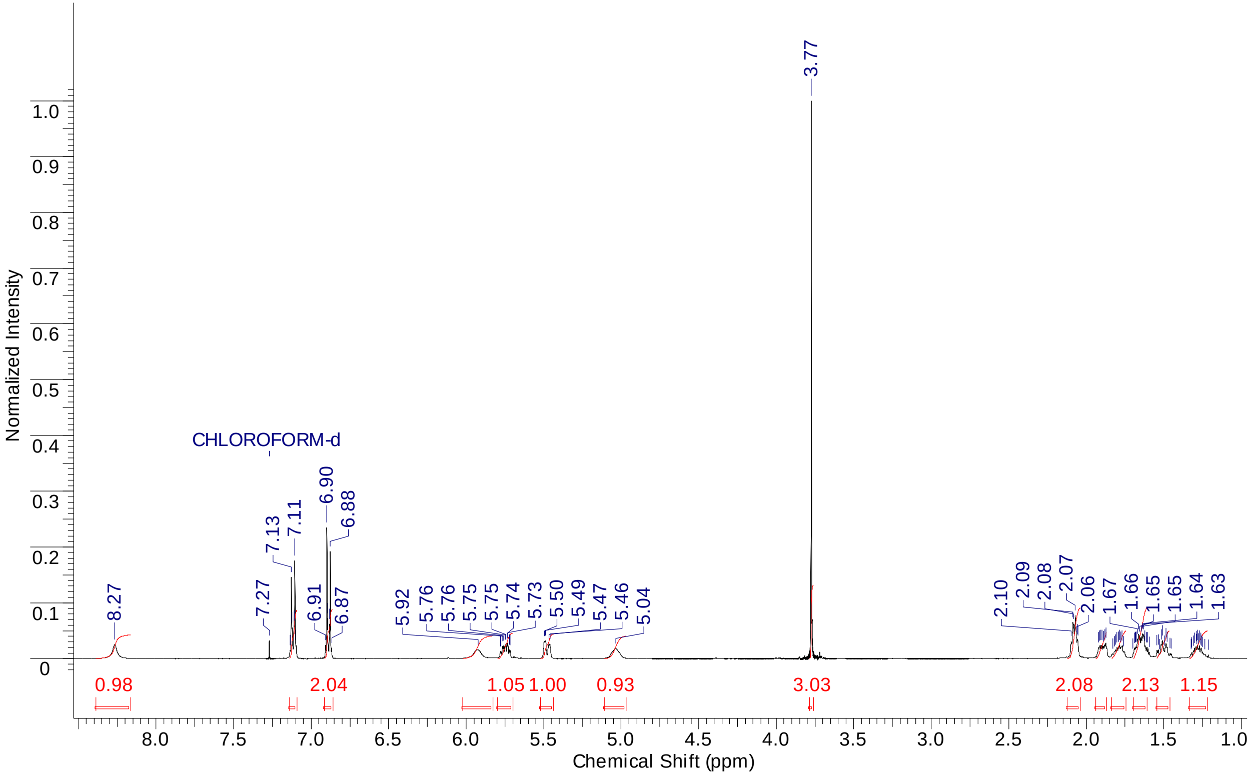


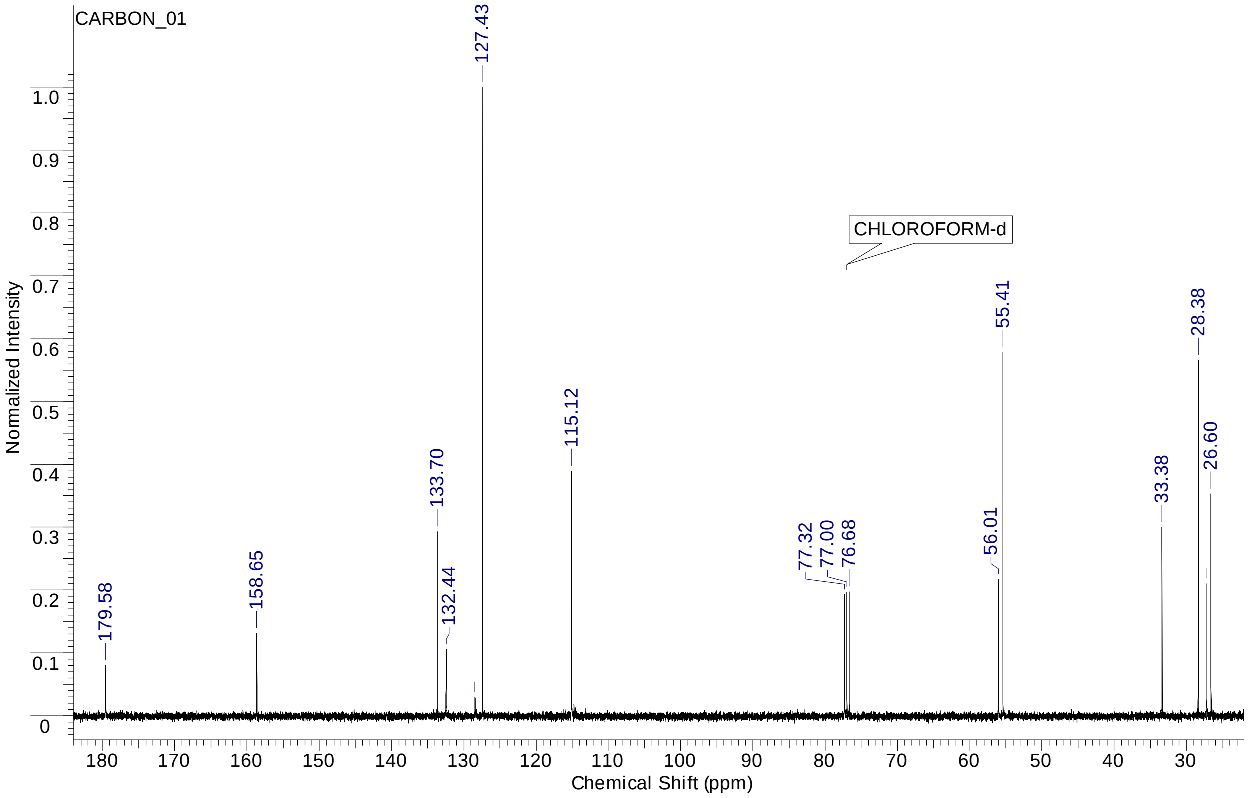
^{1}H NMR and ^{13}C NMR spectra of compound **3'b** in $CDCl_3$



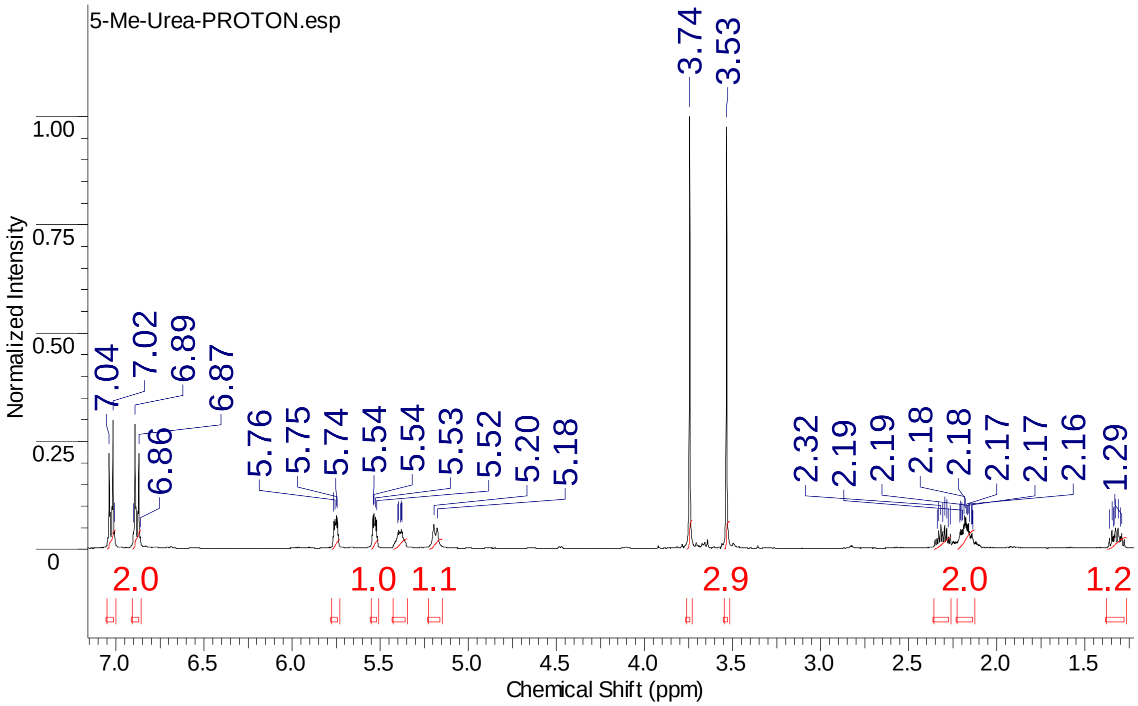


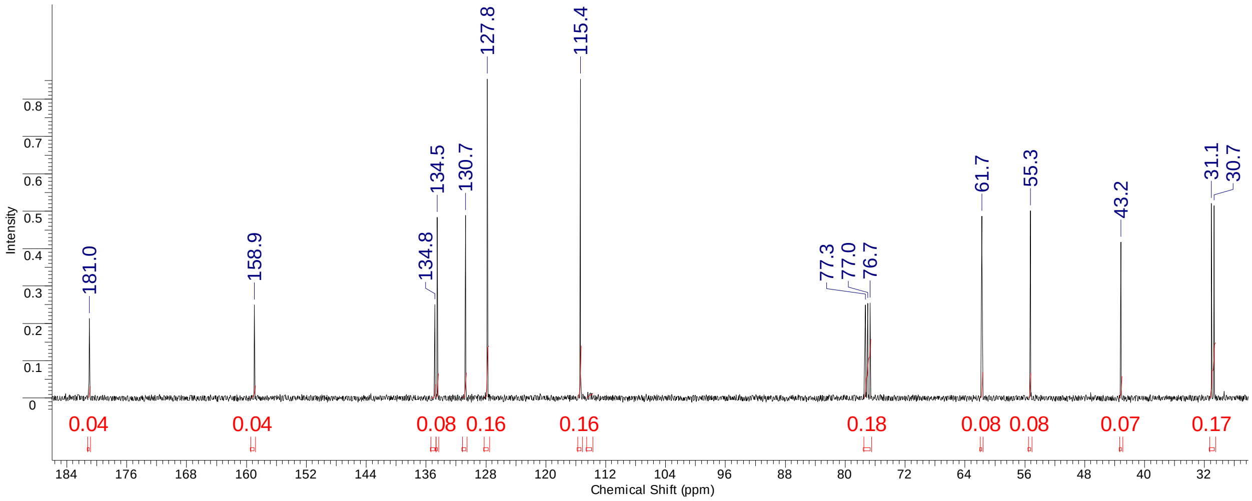
^{1}H NMR and ^{13}C NMR spectra of compound **3'a** in $CDCl_3$



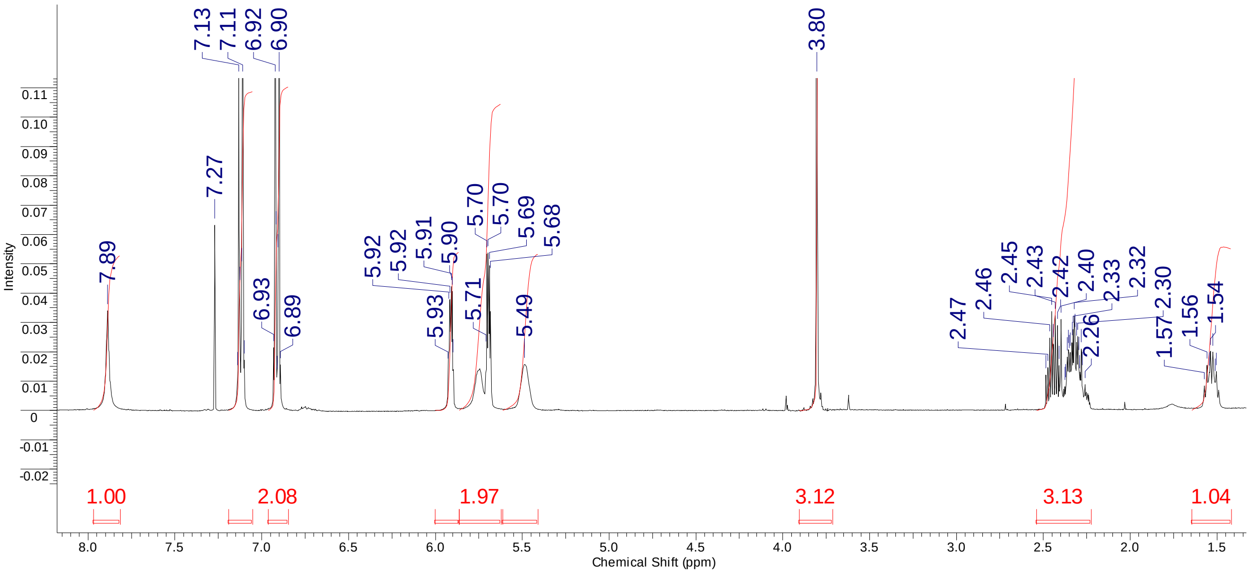


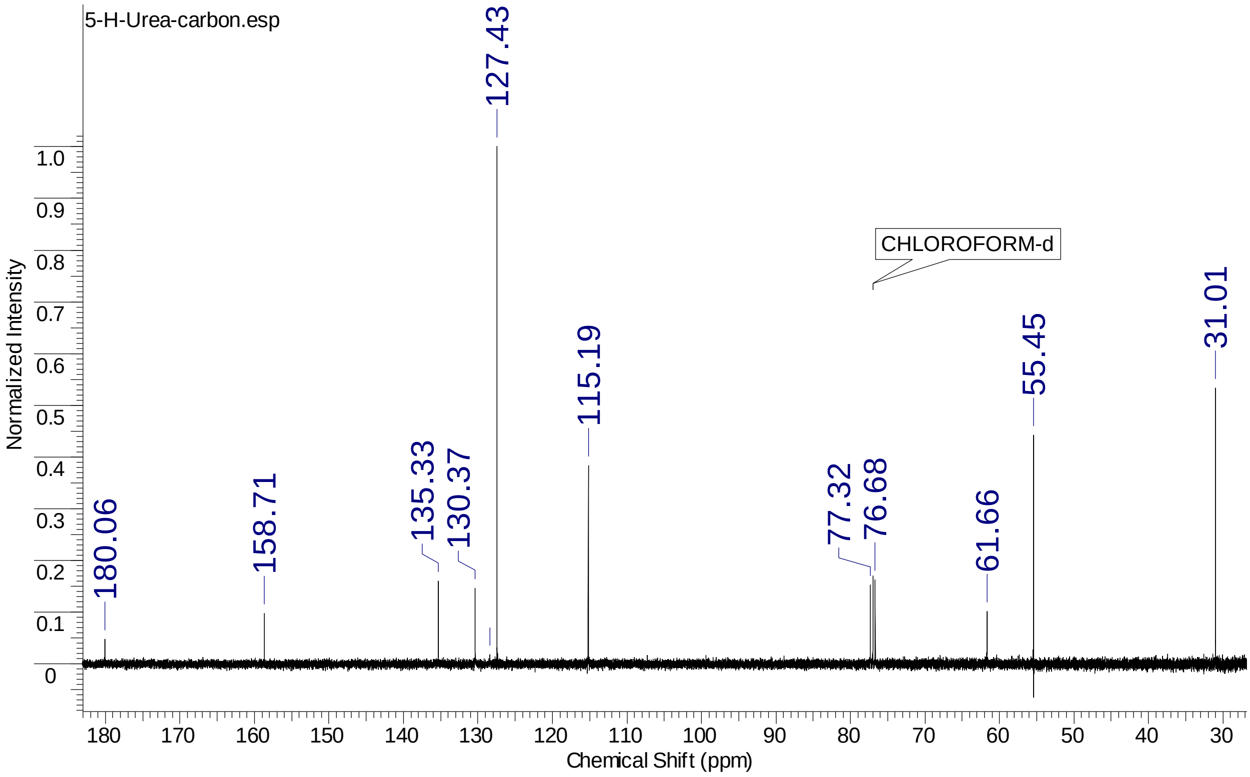
^{1}H NMR and ^{13}C NMR spectra of compound **3c** in $CDCl_3$



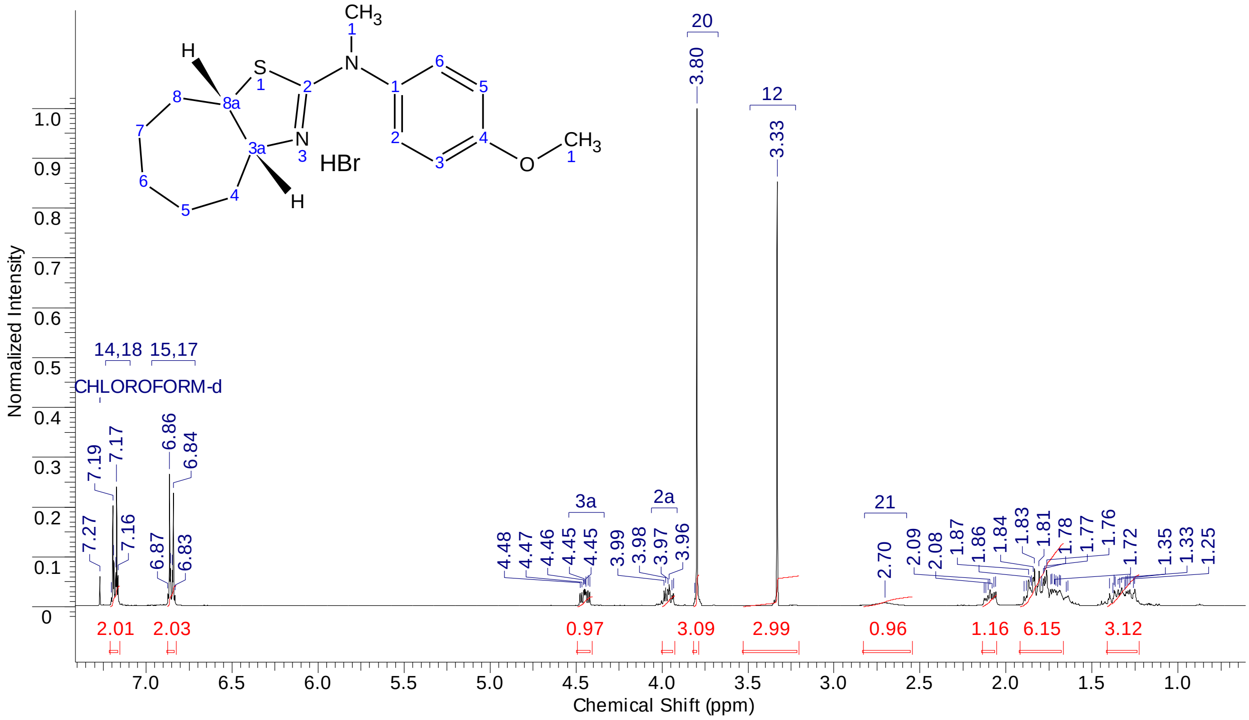


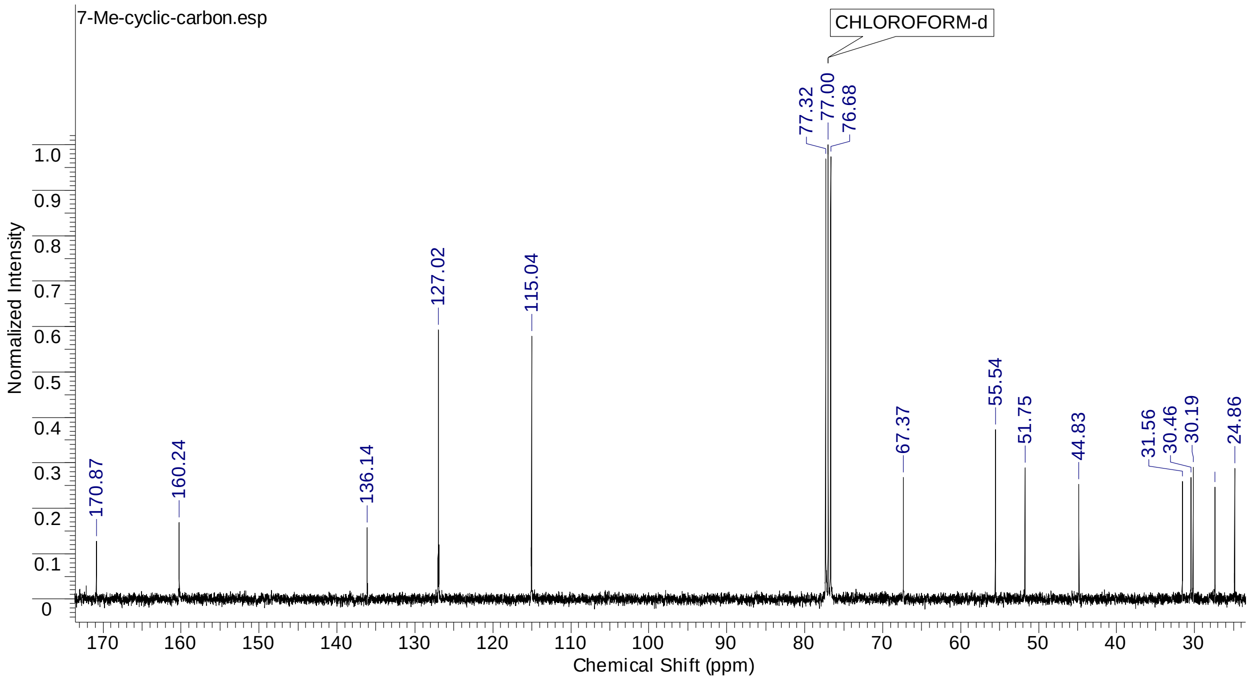
^{1}H NMR and ^{13}C NMR spectra of compound **3'c** in $CDCl_3$



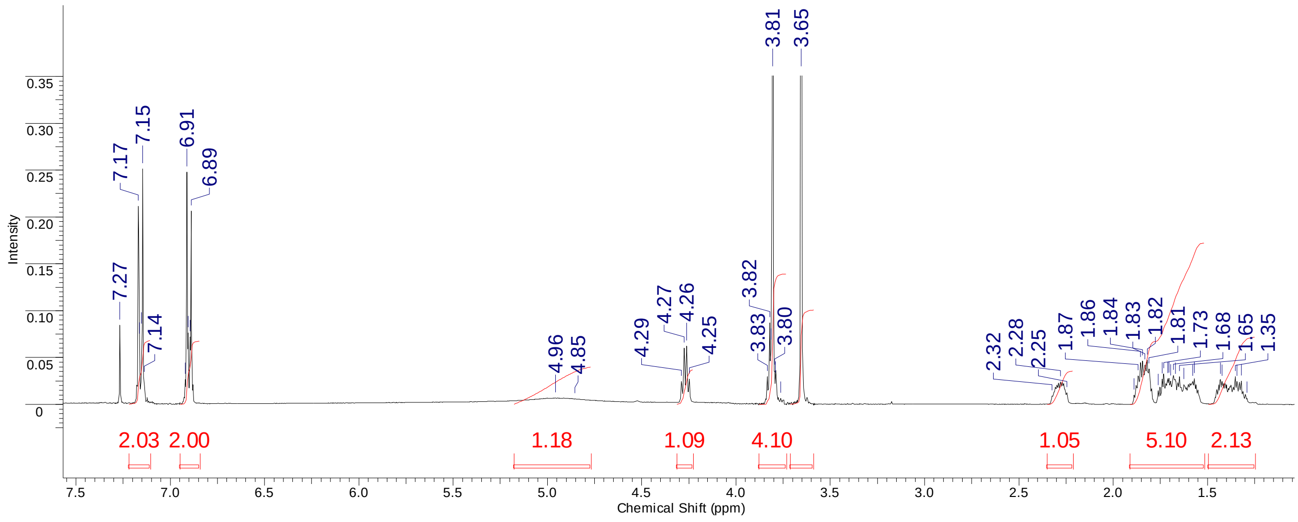


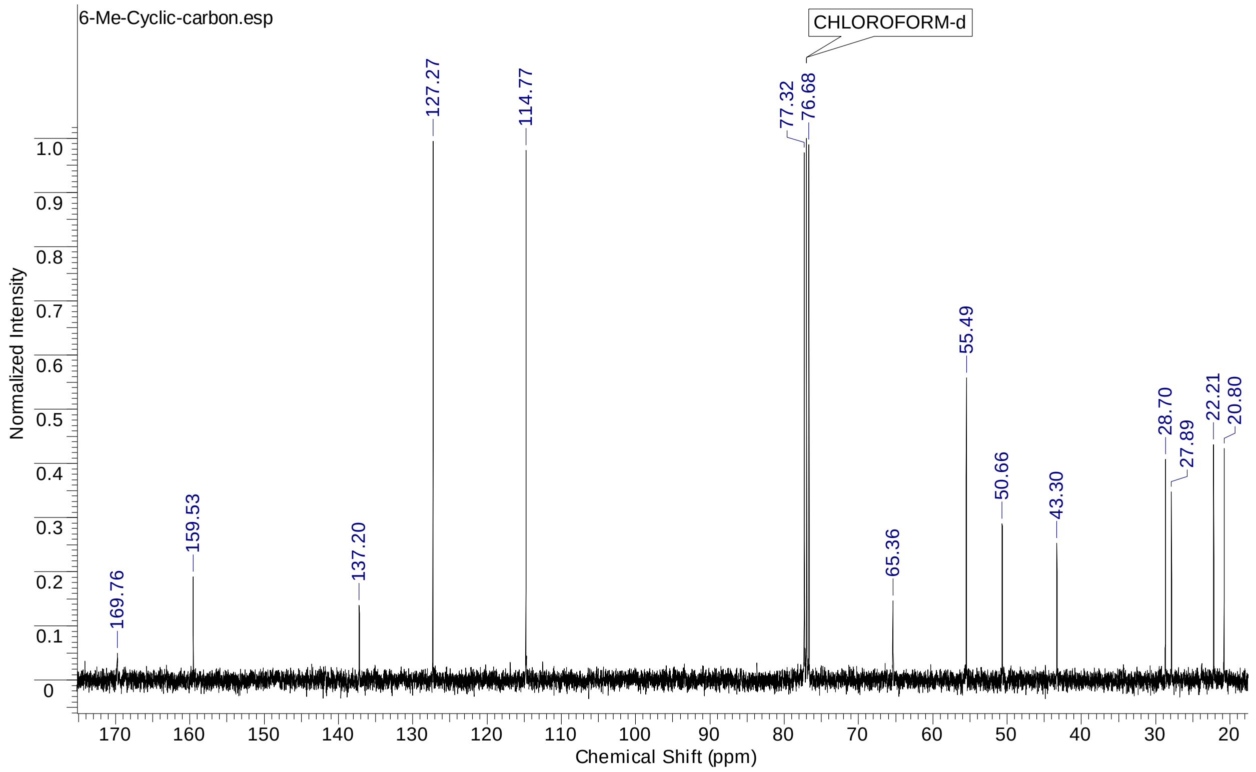
^{1}H NMR and ^{13}C NMR spectra of compound **4a** in $CDCl_3$



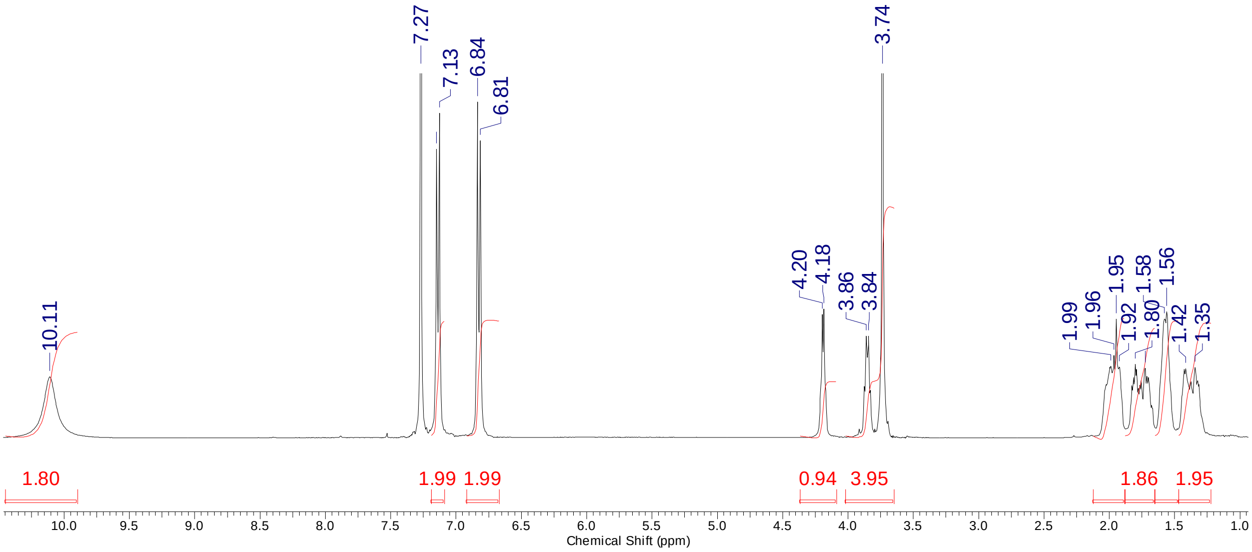


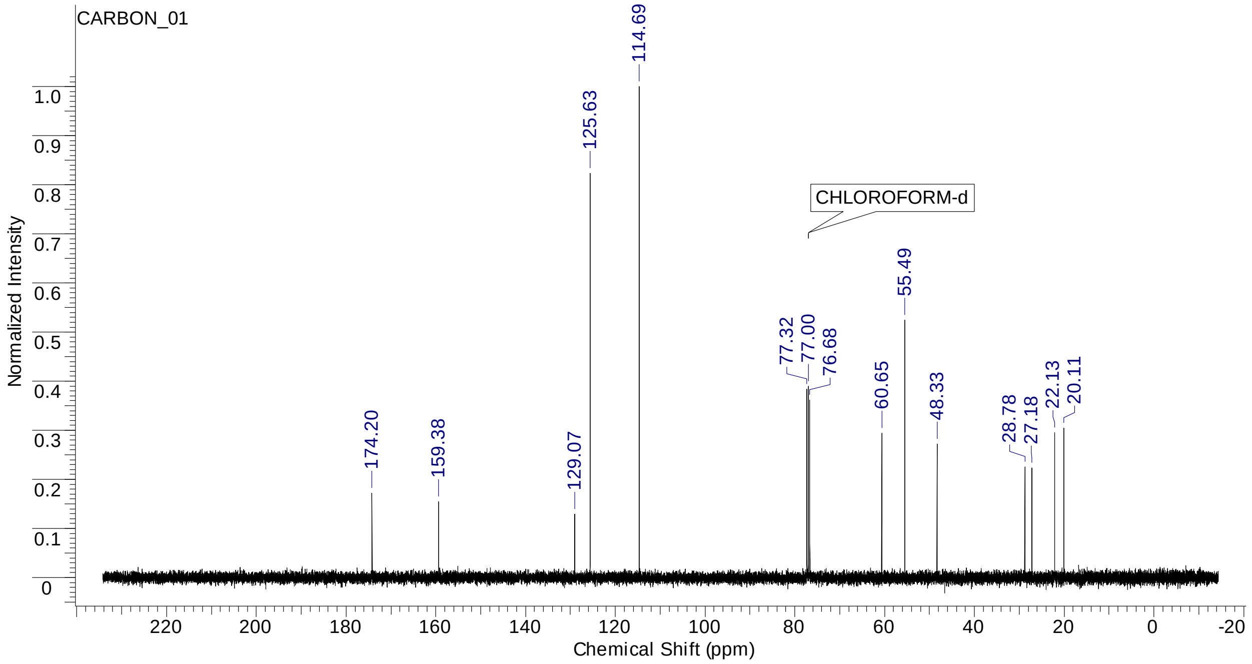
^{1}H NMR and ^{13}C NMR spectra of compound **4b** in CDCl$_3$



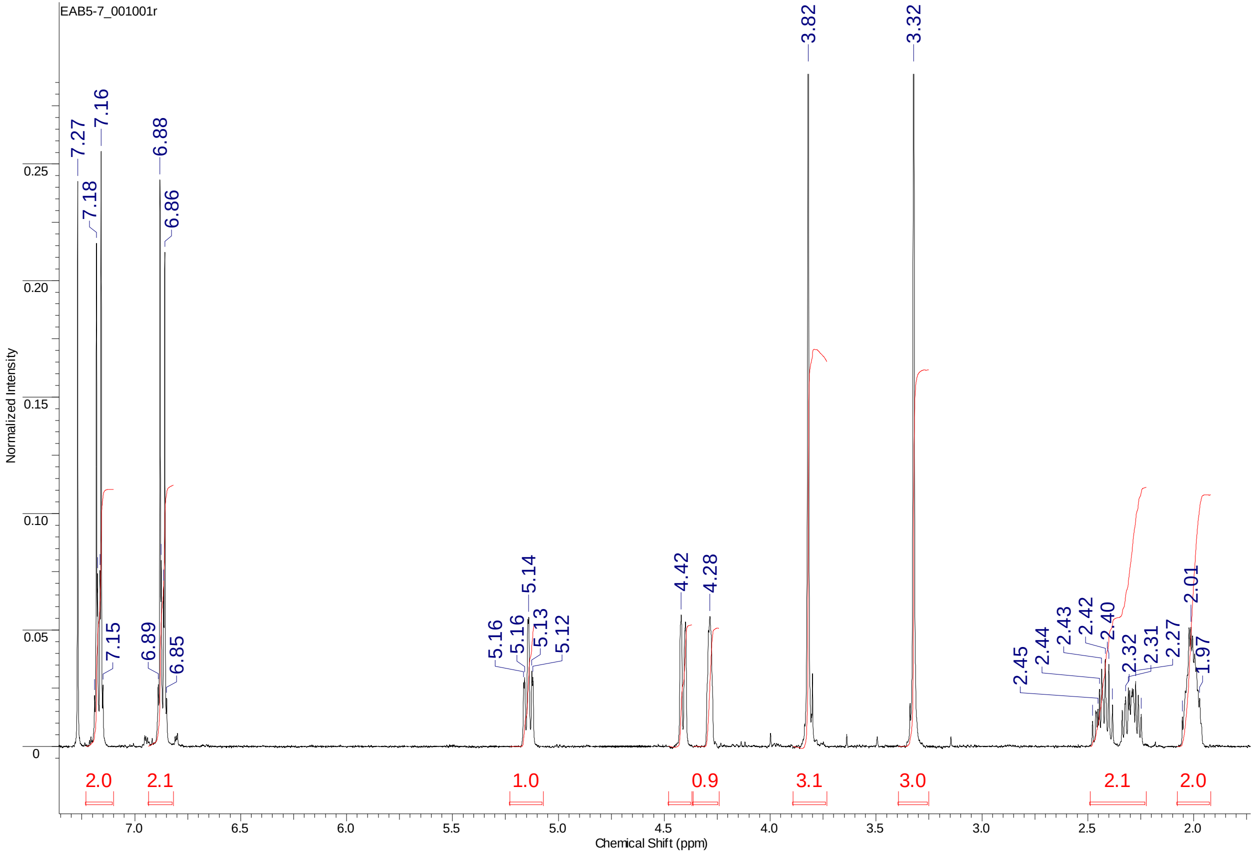


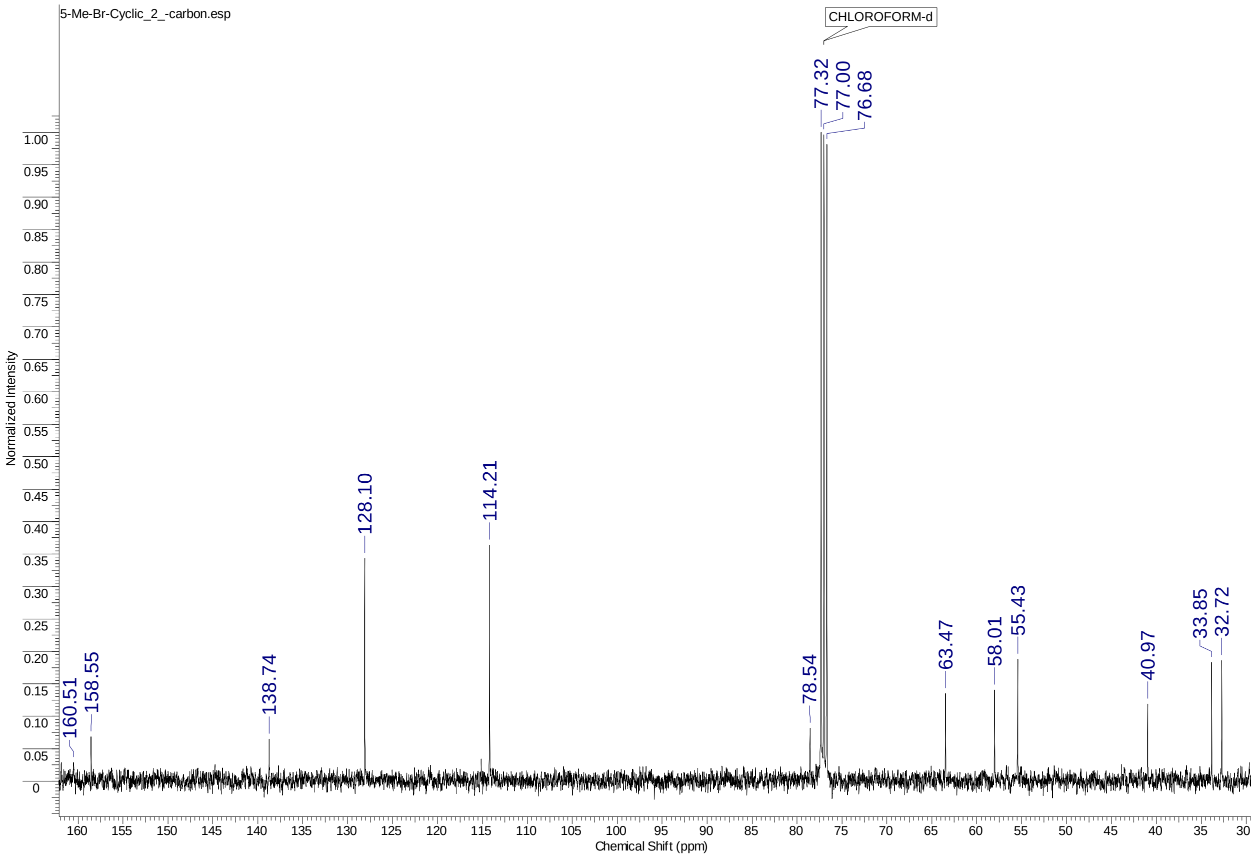
^{1}H NMR and ^{13}C NMR spectra of compound **4'b** in $CDCl_3$



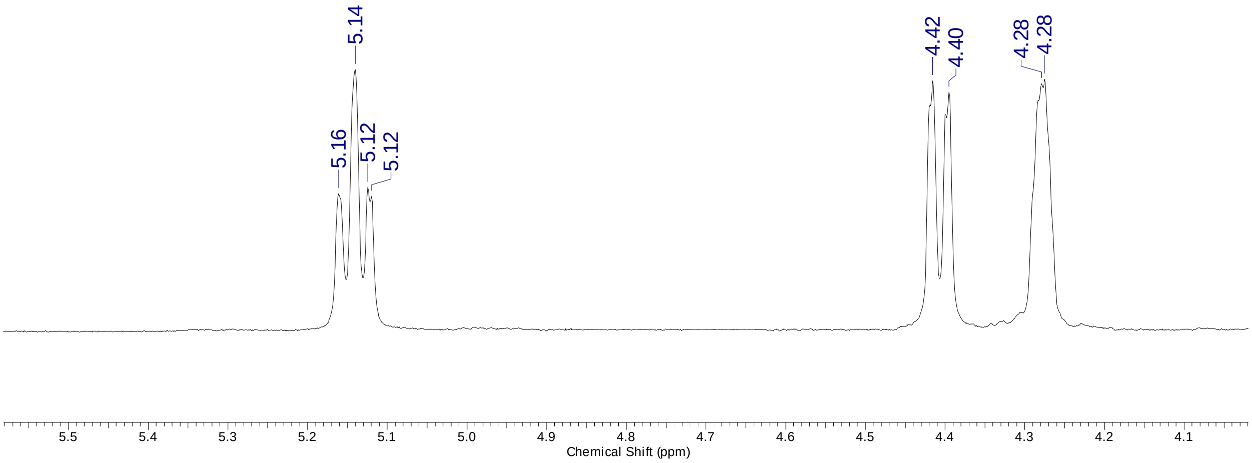


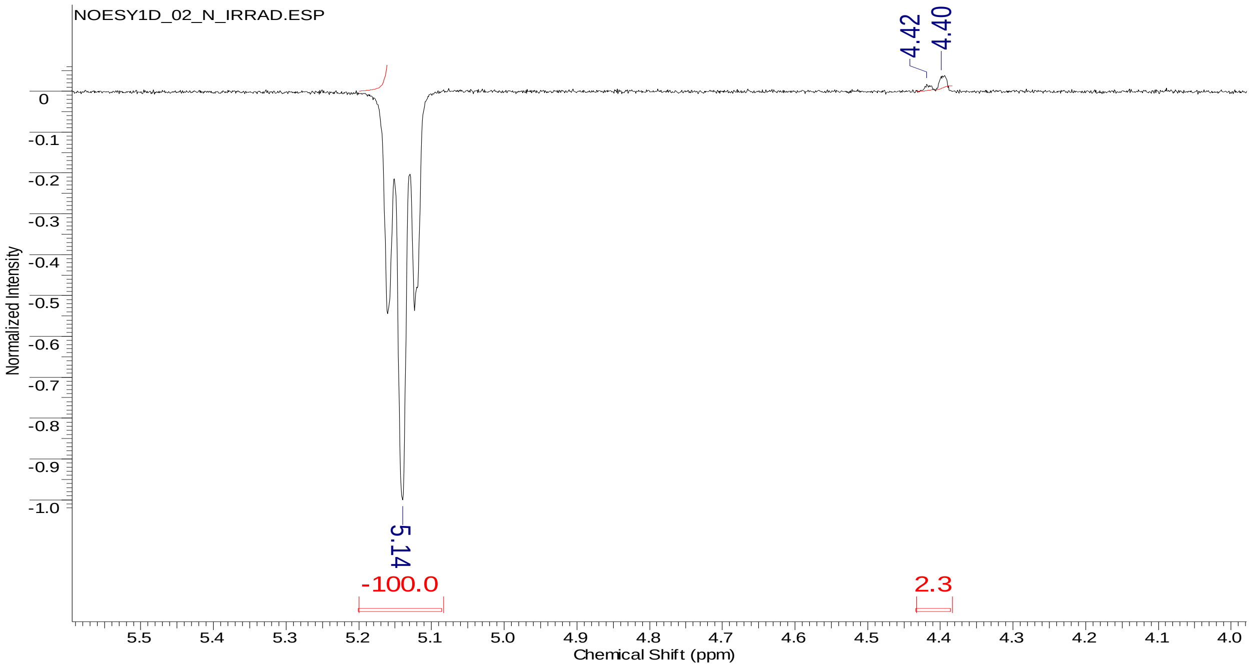
^{1}H NMR and ^{13}C NMR spectrum of compound **5** in $CDCl_3$



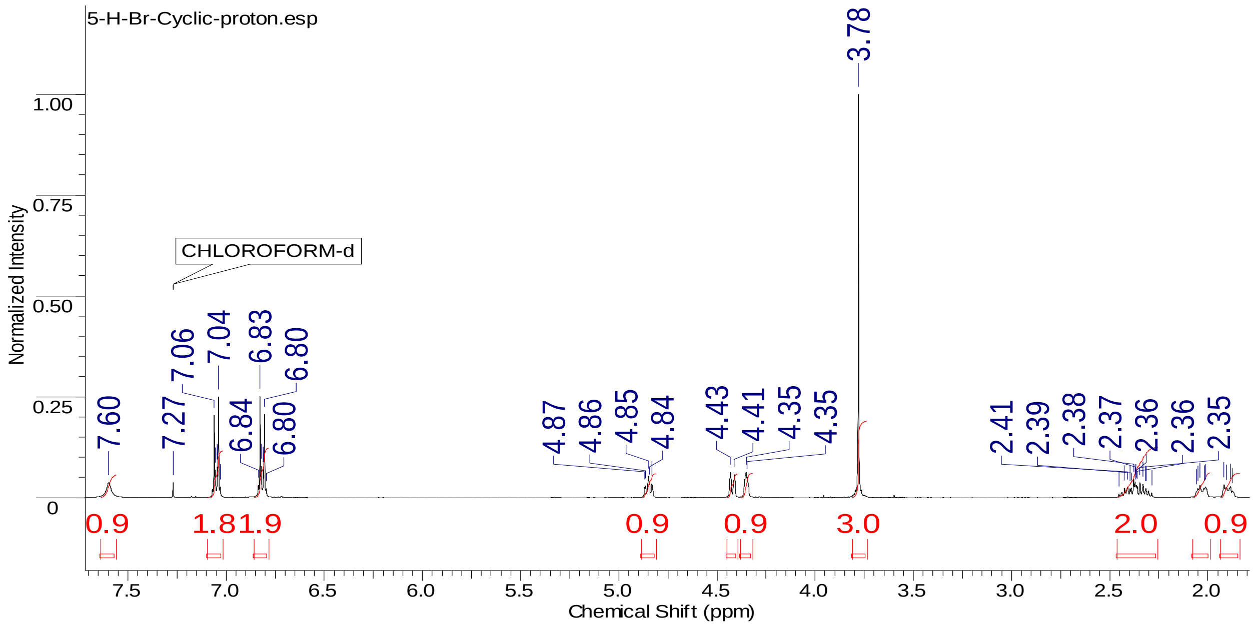


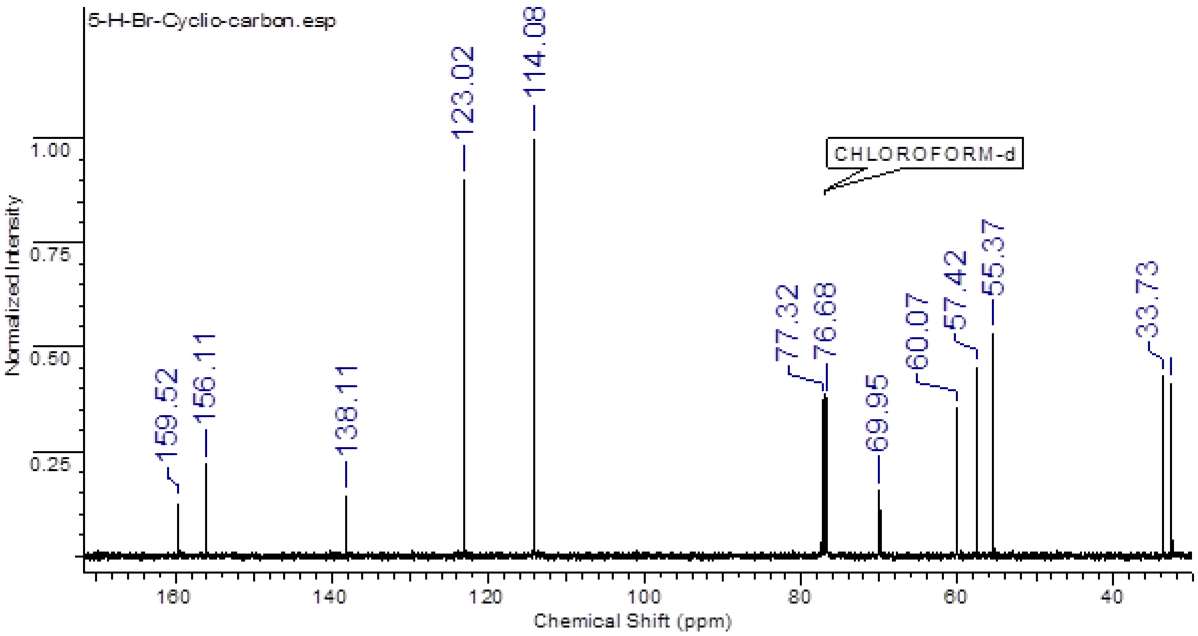
The fragments of ^1H NMR and NOESY 1D spectra of compound **5** in CDCl3
$H^{3a} – H^{6a}$ (2.3%); $H^{3a} – H^{6}$ is not observed.



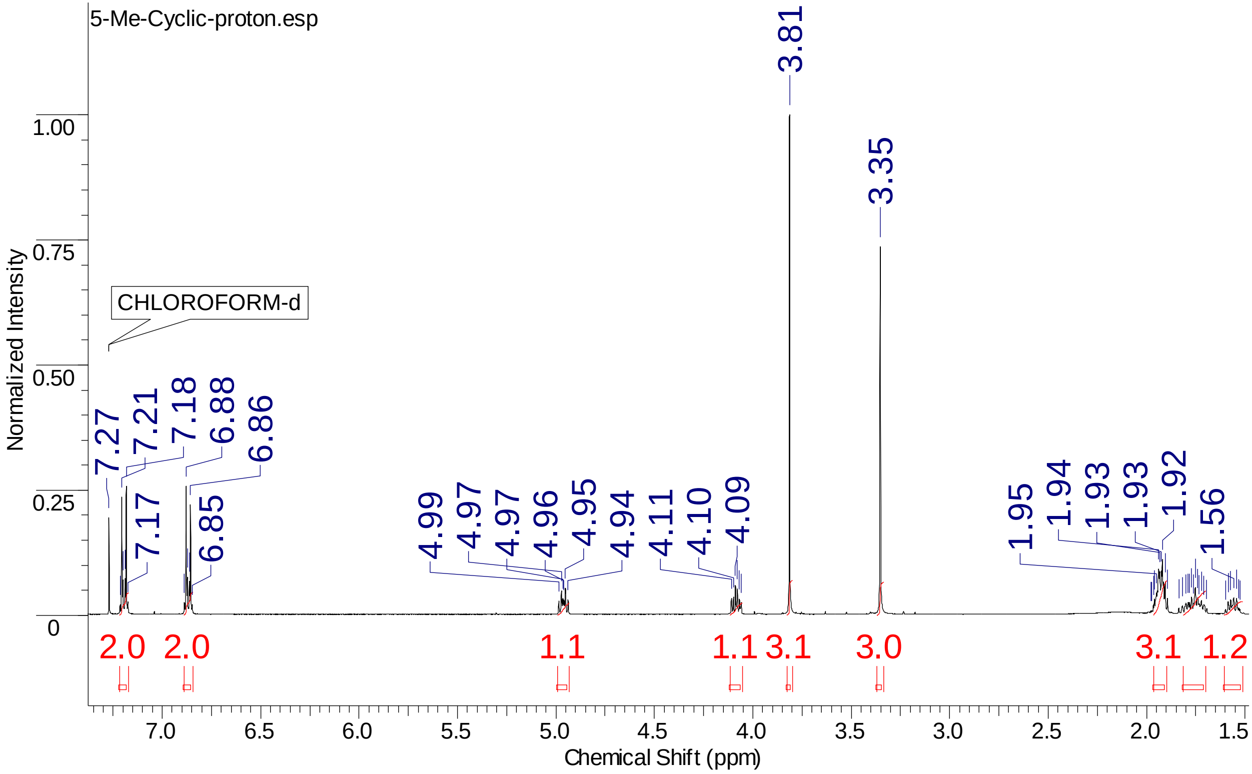


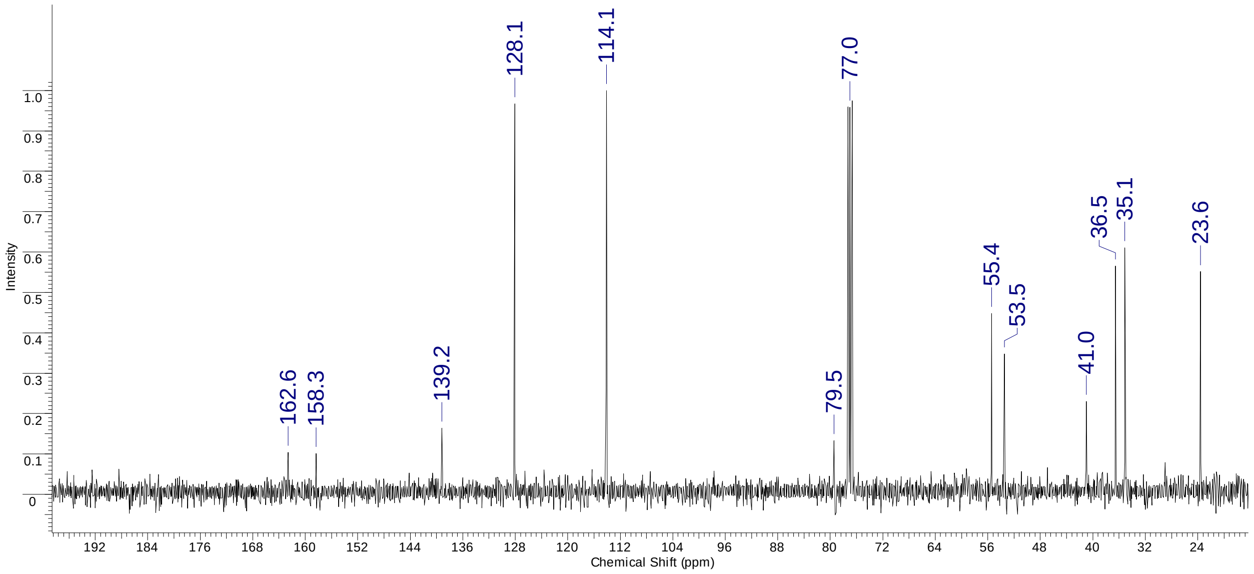
^{1}H NMR and ^{13}C NMR spectra of compound **5'** in $CDCl_3$





^{1}H NMR and ^{13}C NMR spectra of compound **6** in CDCl$_3$





^{1}H NMR and ^{13}C NMR spectra of compound **6'** in $CDCl_3$