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Bicyclic isothioureas for conjugation with tubulin targeted anticancer agents

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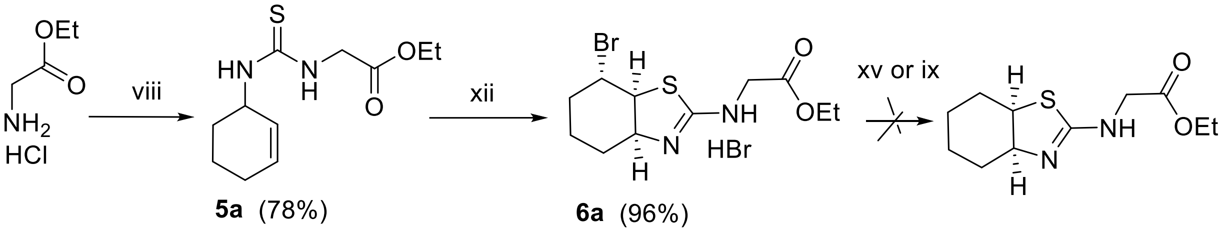
General information

All starting materials and reagents were purchased as high-grade commercial products and used without further purification; the solvents were technical grade and distilled from standard drying agents. Liquid column chromatography was performed using silica gel Acros (40–60 μm). Thin-layer chromatography (TLC) was performed on Silufol-UV254 silica gel sheets. ^{1}H and ^{13}C NMR spectra were recorded on Agilent 400-MR spectrometer (400.0 MHz for ^{1}H; 100.6 MHz for ^{13}C) at 28°C. Chemical shifts (δ) are reported in ppm referenced to residual solvent peak ($CDCl_3$, δ_H=7.24 ppm, δ_C=77.0 ppm). Liquid chromatography (LC) and ElectroSpray ionization mass spectrometry (ESI-MS) data were obtained on an Agilent 1100 LC/MSD with an Agilent 1100 SL quadrupole mass spectrometer, eluting with 0.05 % TFA in H_2O and 0.05 % TFA in CH_3CN (positive-ion monitoring mode). Melting points were determined using a capillary melting point apparatus and were uncorrected.

Synthetic procedures and compound characterization data

Ethyl *N*-[(cyclohex-2-en-1-ylamino)carbonothioyl]glycinate (5a).

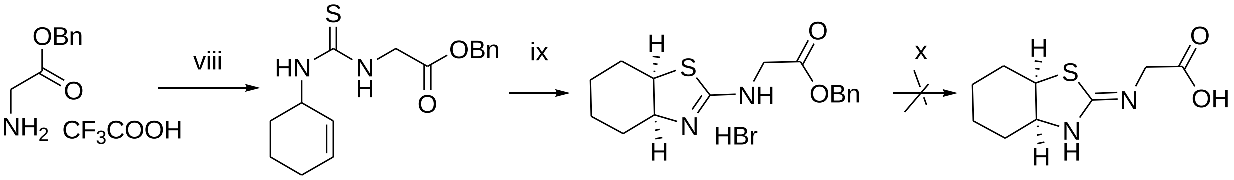


Reagents and conditions: viii, 3-isothiocyanatocyclohexene, DIPEA, CH_2Cl_2, 22°C, 12 h; xii, Br_2, CH_2Cl_2, 22 °C, 8 h; xv, LiOH, THF/MeOH/H_2O, 22°C; ix, AcBr, MeOH, CH_2Cl_2, 22 °C, 12 h.

A solution of ethyl glycinate hydrochloride (0.202 g, 1.97 mmol) in CH_2Cl_2 (10 ml) was treated with DIPEA (0.685 ml, 3.93 mmol) and 3-isothiocyanatocyclohexene (274 mg, 1.97 mmol). The mixture was stirred overnight at room temperature, concentrated at reduced pressure and purified by column chromatography on SiO_2 (eluent: 1:5 ethyl acetate–hexane) to afford thiourea **5a** as yellowish oil (273 mg, yield 78%). ^{1}H NMR (400 MHz, $CDCl_3$, δ): 1.30 (t, 3H, *J* = 7.1 Hz, CH_3), 1.62–1.74 (m, 3H), 1.92–2.05 (m, 3H), 4.23 (q, 2H, *J* = 7.1 Hz, CH_2O_2C), 4.40 (dd, 2H, *J* = 4.8, 2.2 Hz, $NHCH_2$), 4.59 (m, 1H, H1), 5.66 (ddt, 1H, *J* = 10.0, 3.6, 2.2 Hz, H2), 5.90 (dtd, 1H, *J* = 10.0, 3.6, 1.6 Hz, H3), 6.35 (br.s., 1H, NH), 6.45 (br.s., 1H, NH). ^{13}C NMR (100 MHz, CDCl$_3$, δ): 13.90 (CH$_3$), 19.52, 24.56, 28.57, 46.19 (NHCH$_2$), 49.40 (C^1), 61.57 (CH$_2$O$_2$), 126.50 (C=C), 131.28 (C=C), 170.65 (CO$_2$), 181.27 (C=S).

Ethyl (3a*RS*,7*RS*,7a*RS*)-*N*-(7-bromo-3a,4,5,6,7,7a-hexahydro-1,3-benzothiazol-2-yl)-glycinate hydrobromide (**6a**). A solution of urea **5a** (53 mg, 0.22 mmol) in CH$_2$Cl$_2$ (10 ml) cooled on an ice bath was treated with Br$_2$ (12 µl, 0.22 mmol), then the reaction was stirred at room temperature for 2 h. The sediment was filtered off and washed with CH$_2$Cl$_2$ (2×5 ml) to afford product **6a** as white solid (84 mg, yield 96%). M.p. 249–251°C (decomp.). ^{1}H NMR (400 MHz, CDCl$_3$, δ): 1.23 (t, 3H, *J* = 7.1 Hz, CH$_3$), 1.43–1.53 (m, 1H), 1.60–1.68 (m, 1H), 1.72–1.85 (m, 2H), 2.12–2.24 (m, 2H), 4.17 (q, 2H, *J* = 7.1 Hz, CH$_2$O$_2$C), 4.21–4.26 (m, 2H, H^7+H^{7a}), 4.29–4.32 (m, 1H, *J* = 3.7 Hz, H^{3a}), 4.36 (s, 2H, CH$_2$CO$_2$), 10.31 (br.s., 1H, NH), 10.49 (br.s., 1H, NH). ^{13}C NMR (100 MHz, CDCl$_3$, δ): 4.05 (CH$_3$), 20.44, 24.92, 34.55, 46.04 (NHCH$_2$), 55.21 (CH$_2$O$_2$), 56.36 (C^{7a}), 61.49 (C^7), 62.85 (C^{3a}), 167.27 (CO$_2$), 170.14 (C^2).

Benzyl *N*-[(cyclohex-2-en-1-ylamino)carbonothioyl]glycinate (**5b**).



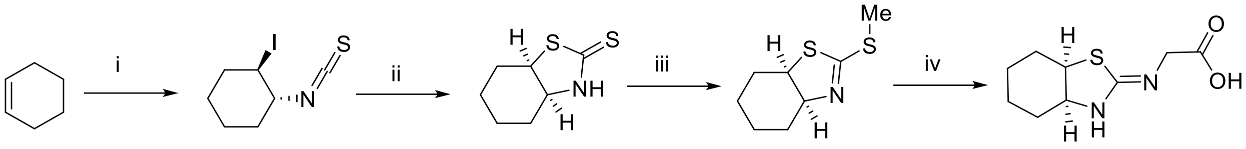
5b (71%) **6b** (95%)

Reagents and conditions: viii, 3-isothiocyanatocyclohexene, DIPEA, CH$_2$Cl$_2$, 22 °C, 12h; ix, AcBr, MeOH, CH$_2$Cl$_2$, 22°C, 12 h; x, H$_2$, Pd/C, MeOH, 22°C, 12 h.

A solution of benzyl 2-aminoacetate trifluoroacetic acid salt (1.28 g, 4.60 mmol) in CH$_2$Cl$_2$ (10 ml) was treated with DIPEA (1.95 ml, 9.19 mmol) and 3-isothiocyanatocyclohexene (767 mg, 5.51 mmol). The mixture was stirred overnight at room temperature, concentrated at reduced pressure and purified by column chromatography on SiO$_2$ (eluent: CH$_2$Cl$_2$) to afford thiourea **5b** as yellowish solid (987 mg, yield 71%). M.p. 84–86°C. ^{1}H NMR (400 MHz, CDCl$_3$, δ): 1.59–1.69 (m, 3H), 1.87–2.04 (m, 3H), 4.44 (m, 2H, *J* = 4.6, 2.8 Hz, CH$_2$COOBn), 5.18 (s, 2H, CH$_2$Ph), 5.63 (m, 1H, *J* = 10.0, 2.3 Hz, H2), 5.86 (d, 1H, *J* = 10.0 Hz, H3), 6.59 (br.s, 2H, NH+H1), 7.31–7.39 (m, 5H, Ph). ^{13}C NMR (100 MHz, CDCl$_3$, δ): 19.40, 24.40, 28.43, 45.96 (NHCH$_2$), 49.34 (C^1), 66.88 ($\underline{\mathrm{C}}$H$_2$Ph), 126.65 (C=C), 127.86 (C^{3,5Ph}), 128.14 (C^{4Ph}), 128.25 (C ^{2,6Ph}), 130.93 (C=C), 134.72 (C^{1Ph}), 170.30 (COOBn), 181.40 (C=S).

Benzyl (3a*RS*,7a*SR*)-*N*-(3a,4,5,6,7,7a-hexahydro-1,3-benzothiazol-2-yl)glycinate hydrobromide (**6b**). A solution of acetyl bromide (123 µl, 1.68 mmol) in CH$_2$Cl$_2$ (5 ml) was treated with MeOH (68 µl, 1.68 mmol) and stirred at room temperature in the dark. In 10 min, a solution of thiourea **5b** (340 mg, 1.12 mmol) in CH$_2$Cl$_2$ (5 ml) was added, and the mixture was stirred for more 12 h, then concentrated at reduced pressure and purified by column chromatography on SiO$_2$ (eluent: gradient 0→3% MeOH in CH$_2$Cl$_2$) to afford thiourea **6b** as yellowish solid (322 mg, yield 95%). M.p. 194–196°C (decomp.). ^{1}H NMR (400 MHz, CDCl$_3$, δ): 1.34–1.42 (m, 1H), 1.44–1.51 (m, 1H), 1.57–1.68 (m, 2H), 1.71–1.86 (m, 2H), 1.93–2.01 (m, 1H), 2.05–2.13 (m, 1H), 3.90 (dt, 1H, *J* = 8.4, 5.4 Hz, H^{7a}), 4.13 (m, 2H, CH$_2$CO$_2$Bn), 4.20 (m, 1H, *J* = 5.4 Hz, H^{3a}), 5.2 (m, 2H, *J* = 12.0 Hz, CH$_2$Ph), 7.36–7.41 (m, 5H, Ph). ^{13}C NMR (100 MHz, CDCl$_3$, δ): 20.13, 22.12, 27.08, 28.63, 47.55 (C^7), 49.49 ($NHCH_2$), 61.43 (C^3), 67.96 (CH_2Ph), 128.61 ($C^{3,5Ph}$), 128.63 (C^{4Ph}), 128.67 ($C^{2,6Ph}$), 134.53 (C^{1Ph}), 166.65 (COOBn), 167.99 (C^2).

(3a*RS*,7a*SR*)-2-Methylthio-3a,4,5,6,7,7a-hexahydrobenzo[*d*]thiazole (**8**) was prepared by modified literature procedures [S1-S5].



7 (70%) (73%) **8** (72%) **1** (68%)

Reagents and conditions: i, $Hg(SCN)_2$, I_2, C_6H_6, 22°C, 18 h; ii, $Na_2S{\times}9H_2O$, Me_2CO, H_2O, 22 °C, 45 min; iii, MeI, Me_2CO, Δ, 3 h, then K_2CO_3; iv, glycine, MeOH, H_2O, Δ, 5 h.

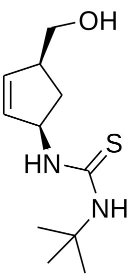
An iodine (12.53 g, 49.4 mmol) solution in benzene (30 ml) was slowly added to a stirred suspension of $Hg(SCN)_2$ (7.82 g, 24.7 mmol) in benzene (15 ml) at room temperature. In 30 min, cyclohexene (5 ml, 49.4 mmol) was added dropwise maintaining the temperature at 18-22°C, and this wasstirred 18 h in the dark. The precipitate of HgI_2 was filtered off and the filtrate was washed with saturated aqueous solution of KI, then with saturated aqueous solution of $Na_2S_2O_3$ and finally with brine. The organic layer was separated, dried over Na_2SO_4, concentrated in vacuum to give 11.5 g of crude intermediate ***trans*-1-iodo-2-isothiocyanatocyclohexane (7)** as the 7:3 mixture with ***trans*-1-iodo-2-thiocyanatocyclohexane** as yellow oil, which was used in the next step without purification. Characteristic signals ^1H NMR (400 MHz, $CDCl_3$, δ): 3.83 (td, 1H, J = 9.3, 4.0 Hz, CHN), 4.14 (td, 1H, J = 9.7, 4.0 Hz, CHI). For ***trans*-1-iodo-2-thiocyanatocyclohexane**: 3.50 (td, J = 9.2, 3.8 Hz, CHS), 4.30 (td, J = 9.3, 3.8 Hz, CHI). The spectral shifts correspond with literature data [S1].

To a solution of crude ***trans*-1-iodo-2-isothiocyanatocyclohexane** (2.74 g, 10.3 mmol) in acetone was added a solution of $Na_2S{\times}9H_2O$ (2.71 g, 11.3 mmol) in water (5 ml) and stirred at room temperature for 45 min, then the solvent was removed in vacuum, and the residue oil was triturated with petroleum ether (40-70°C) at reflux. The precipitate was filtered, washed with petroleum ether (40-70°C) (3×10 ml) and dried in air to give **(3a*RS*,7a*SR*)-hexahydrobenzo[*d*] thiazole-2(3*H*)-thione** as white solid (1.3 g, yield 73%). M.p. 115–117°C (lit. 111–113°C [S2]). ^1H NMR (400 MHz, $CDCl_3$, δ): 1.28–1.38 (m, 1H), 1.43–1.62 (m, 2H), 1.67–1.86 (m, 3H), 1.91–2.07 (m, 2H), 3.81 (dt, 1H, J = 8.9, 5.5 Hz, CHS), 4.20 (ddd, 1H, J = 5.2 Hz, CHN), 8.13 (br.s., 1H, NH).

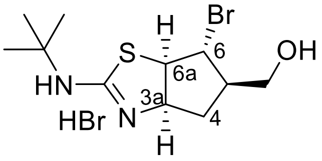
A solution of the above thione (1.22 g, 7 mmol) and iodomethane (437 μl, 7 mmol) in dry acetone (200 ml) was refluxed for 3 h. After cooling, the precipitate was filtered, washed with acetone, then suspended in aqueous saturated solution of K_2CO_3 and extracted with CH_2Cl_2 (4×20 ml). The organic layers were dried over Na_2SO_4, concentrated in vacuum to give **(3a*RS*,7a*SR*)-2-methylthio-3a,4,5,6,7,7a-hexahydrobenzo[*d*]thiazole (8)** as colorless oil (947 mg, yield 72%) [S3]. ^1H NMR (400 MHz, $CDCl_3$, δ): 1.22–1.32 (m, 1H), 1.38-1.52 (m, 2H), 1.53–1.64 (m, 2H), 1.65–1.82 (m, 2H), 2.06–2.13 (m, 1H), 2.50 (s, 3H, SMe), 3.79 (dt, 1H, J = 8.8, 5.5 Hz, CHS), 4.03 (q, 1H, J = 5.5 Hz, CHN). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 15.14 (SMe), 21.36, 22.83, 28.38, 29.18, 52.98 (C^{7a}), 73.93 (C^{3a}), 166.11 (C^2).

***N*-[(3a*RS*,7a*SR*,*Z*)-Hexahydro-1,3-benzothiazol-2(3*H*)-ylidene]glycine** (**1**). To a solution of compound **8** (400 mg, 2.14 mmol) in MeOH (4 ml) was added glycine (160 mg, 2.14 mmol) dissolved in water (5 ml). The mixture was refluxed for 6 h, then cooled, concentrated at reduced pressure and purified by column chromatography on SiO_2 (eluent: 20% methanol in CH_2Cl_2) to afford acid **1** as yellowish solid (310 mg, yield 68%). M.p. 209–211°C. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 1.25-1.35 (m, 1H), 1.41–1.58 (m, 2H), 1.61–1.78 (m, 3H), 1.90–1.98 (m, 1H), 2.05–2.15 (m, 1H), 3.68 (dt, 1H, *J* = 9.2, 5.5 Hz, H^{7a}), 3.88 (m, 2H, *J* = 16.2 Hz, CH_2CO_2), 4.07 (m, 1H, *J* = 4.9 Hz, H^{3a}), 11.59 (br.s, 1H), 13.87 (br. s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 19.63, 22.33, 26.72, 29.08, 47.78 (CH_2COOH), 50.66 (C^{7a}), 60.41 (C^{3a}), 170.69 (C^2), 171.51 (COOH).

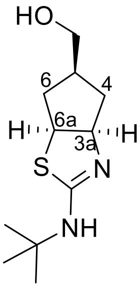
1-*tert*-Butyl-3-((1*S*,4*R*)-4-(hydroxymethyl)cyclopent-2-en-1-yl)thiourea (9).



((1*S*,4*R*)-4-aminocyclopent-2-en-1-yl)methanol hydrochloride (600 mg, 4 mmol) in CH_2Cl_2 (10 ml) was treated with DIPEA (768 μl, 4.4 mmol) and *t*-BuNCS (1 ml, 8 mmol). The mixture was stirred at room temperature for 24 h, concentrated at reduced pressure and purified by column chromatography on SiO_2 (eluent: 3% methanol in CH_2Cl_2) to afford thiourea **9** as white solid (782 mg, yield 86%). M.p. 175–177°C (decomp.). ^{1}H NMR (400 MHz, $CDCl_3$, δ): 1.31(s, 9H, *t*-Bu), 1.54 (dt, 1H, *J* = 14.1, 2.1 Hz, H^{5a}), 2.46 (dt, 1H, *J* = 14.1, 9.0 Hz, H^{5b}), 2.57 (dd,1H, *J* = 5.3, 3.9 Hz, H^4), 2.83 (m, 1H, *J* = 8.0, 2.5 Hz, H^1), 3.58 (ddd, 1H, *J* = 10.2, 5.2, 3.6 Hz, CH_2O), 3.69 (ddd, 1H, *J* = 10.2, 3.6, 2.7 Hz, CH_2O), 5.4 (t, 1H, *J* = 8.9 Hz, OH), 5.76 (m, 1H, *J* = 5.4, 2.5, 1.3 Hz, H^2), 5.87 (dt, 1H, *J* = 5.4, 2.2 Hz, H^3), 6.02 (br.s, 1H, NH), 6.51 (d, 1H, *J* = 8.4, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 29.2($C(\underline{C}H_3)_3$), 33.9 (C^5), 46.2 (C^4), 53.4 ($\underline{C}(CH_3)_3$), 60.9 (C^1), 63.7 (C^6), 132.8 (C^2), 134.6 (C^3), 178.2 (C=S). MS (ESI), m/z: 229 $[M+H]^+$.

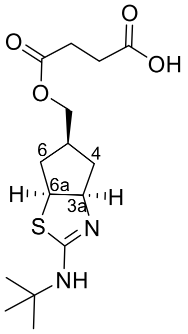


((3a*R*,5*R*,6*R*,6a*R*)-6-Bromo-2-*tert*-butylamino-3a,5,6,6a-tetrahydro-4*H*-cyclopenta[*d*]-thiazol-5-yl)methanol hydrobromide (**10**). Compound **9** (785 mg, 3.44 mmol) in CH_2Cl_2 (4 ml) was treated with Br_2 (178 μl, 3.44 mmol) under ice cooling. The reaction mass was stirred at room temperature for 24 h in the dark, concentrated at reduced pressure and purified by column chromatography on SiO_2 (eluent: 3% methanol in CH_2Cl_2) to afford product **10** as white solid (932 mg, yield 70%). M.p. 109–111°C. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 1.34 (s, 9H, *t*-Bu), 1.68 (ddd, 1H, *J* = 13.3, 10.7, 6.4 Hz, H4a), 2.25 (m, 1H, *J* = 10.7, 7.6, 5.2, 4.2 Hz, H5), 2.51 (dt, 1H, *J* = 13.3, 7.6 Hz, H^{4b}), 3.70 (dd, 1H, *J* = 11.1, 5.3 Hz, CH_2O), 3.79 (dd, 1H, *J* = 11.1, 4.3 Hz, CH_2O), 3.91 (dd, 1H, *J* = 9.8, 8.5 Hz, H6), 4.16 (m, 1H, *J* = 8.5 Hz, H^{6a}), 4.84 (td, 1H, *J* = 8.6, 6.3 Hz, H^{3a}). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 28.9 ($C(CH_3)_3$), 35.2, 49.5, 53.0, 58.1, 62.0, 62.7, 77.9, 154.1 (C^2).

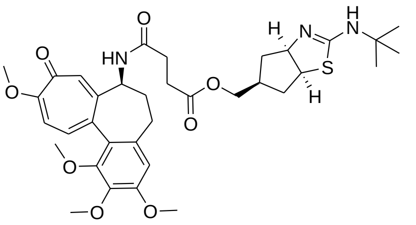


((3a*R*,5*S*,6a*S*)-2-*tert*-Butylamino-3a,5,6,6a-tetrahydro-4*H*-cyclopenta[*d*]-thiazol-5-yl)-methanol (2). Bromide **10** (443 mg, 1.14 mmol) in toluene (10 ml) was treated with Bu_3SnH (614 μl, 2.28 mmol) and AIBN (21 mg, 0.012 mmol). The reaction mixture was heated at 100 °C under Ar atmosphere for 5 h, then concentrated at reduced pressure and purified by column chromatography on SiO_2 (eluent: 2% Et_3N in hexane, then 0–2% methanol in CH_2Cl_2) to afford procuct **2** as colorless oil (225 mg, yield 86%). ^{1}H NMR (400 MHz, $CDCl_3$, δ): 1.39 (s, 9H, *t*-Bu), 1.60–1.77 (m, 2H), 2.22 (m, 1H, *J* = 9.6, 6.5 Hz, H^5), 2.32 (dt, 1H, *J*= 13.8, 7.3

Hz), 2.42 (dt, 1H, *J* = 13.7, 7.2 Hz), 3.58 (d, 2H, *J*= 6.2 Hz, H^7), 4.09 (q, 1H, *J* = 8.3 Hz, H^{3a}), 4.61 (ddd, 1H, *J* = 8.8, 7.1, 5.7 Hz, H^{6a}), 6.08 (br.s, 2H, NH+OH). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 28.8 ($C(CH_3)_3$), 35.7, 38.3, 41.5, 50.3, 54.7, 64.5, 67.9, 166.6 (C^2).



4-(((3a*R*,5*S*,6a*S*)-2-*tert*-Butylamino-3a,5,6,6a-tetrahydro-4*H*-cyclopenta[*d*]thiazol-5-yl)methoxy)-4-oxobutanoic acid (**11**). Bicyclic alcohol **2** (100 mg, 0.44 mmol) in CH_2Cl_2 (10 ml) was treated with DMAP (5 mg, 0.044 mmol) and succinic anhydride (132 mg, 1.31 mmol). The mixture was stirred at room temperature for 8 h, concentrated at reduced pressure and purified by column chromatography on SiO_2 (eluent: 7% methanol in CH_2Cl_2) to afford ester **11** as colorless oil (123 mg, yield 86%). ^{1}H NMR (400 MHz, $CDCl_3$, δ): 1.45 (s, 9H, *t*-Bu), 1.75–1.89 (m, 2H), 2.22–2.41 (m, 3H), 2.52–2.76 (m, 4H), 4.04 (ddd, 1H, *J* = 10.0, 8.5, 7.0 Hz, H3a), 4.17 (dd, 1H, *J* = 11.3, 3.9 Hz, $CH_2OC(O)$), 4.25 (dd, 1H, *J* = 11.3, 4.5 Hz, $CH_2OC(O)$), 4.57 (td, 1H, *J* = 7.6, 5.3 Hz, H^{6a}), 9.79 (br.s, 1H, COOH). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 28.9 ($C(CH_3)_3$), 29.1, 29.4, 34.7, 37.8, 38.5, 49.7, 55.3, 64.3, 64.4, 168.9 (C^2), 172.0 (COOR), 175.0 (COOH).



***N*-Deacetyl-*N*-[4-(((3a*R*,5*S*,6a*S*)-2-*tert*-butylamino-3a,5,6,6a-tetrahydro-4*H*-cyclopenta[*d*]thiazol-5-yl)methoxy)-4-oxobutanoyl]colchicine (12).** A solution of compound **11** (53 mg, 0.16 mmol) in CH_2Cl_2 (10 ml) was treated with EEDQ (48 mg, 0.19 mmol) and *N*-deacetylcolchicine (52 mg, 0,14 mmol). The mixture was stirred at room temperature for 24 h, concentrated at reduced pressure and purified by column chromatography on SiO_2 (eluent: 7% methanol in CH_2Cl_2) to afford product **12** as yellowish amorphous solid (43 mg, yield 40%). ^{1}H NMR (400 MHz, $CDCl_3$, δ): 1.44 (s, 9H, *t*-Bu), 1.66–1.75 (m, 1H), 1.99–2.08 (m, 2H), 1.20–1.29 (m, 1H), 2.35–2.62 (m, 7H), 2.70–2.80 (m, 2H, CH_2CO), 3.65 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 4.00–4.08 (m, 1H), 4.09 (dd, 1H, *J* = 11.3, 5.0 Hz, H^{7a}), 4.25 (dd, 1H, *J* = 11.3, 4.5 Hz, H^{7b}), 4.58 (ddd, 1H, *J* = 12.3, 6.4, 6.2 Hz, H^{6a}), 4.67 (ddd, 1H, *J* = 12.4, 6.8, 5.8 Hz, H^7colch), 6.53 (s, 1H, H^4colch), 6.79 (d, 1H, *J* = 10.9 Hz, H^{11}colch), 7.25 (d, 1H, *J* = 10.9 Hz, H^{12}colch), 7.45 (s, 1H, H^8colch), 7.57 (br s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 28.94 ($C(\underline{C}H_3)_3$), 31.56, 36.59, 38.28, 49.63, 52.52 (C^7colch), 53.41, 56.07 (OCH_3), 56.24 (OCH_3), 60.37, 61.34 (OCH_3), 61.53 (OCH_3), 64.63, 64,72, 107.19 (C^4colch), 112.04 (C^{11}colch), 125.69, 131.12 (C^8colch), 134.83, 135.36, 136.31, 141.44, 151.07, 153.34, 153.61, 163.83, 168.39, 172.28, 172.93, 179.34 (C^9colch). MS (ESI), m/z: 669 $[M+H]^+$. MS (MALDI-TOF), m/z: 668 $[M]^+$.

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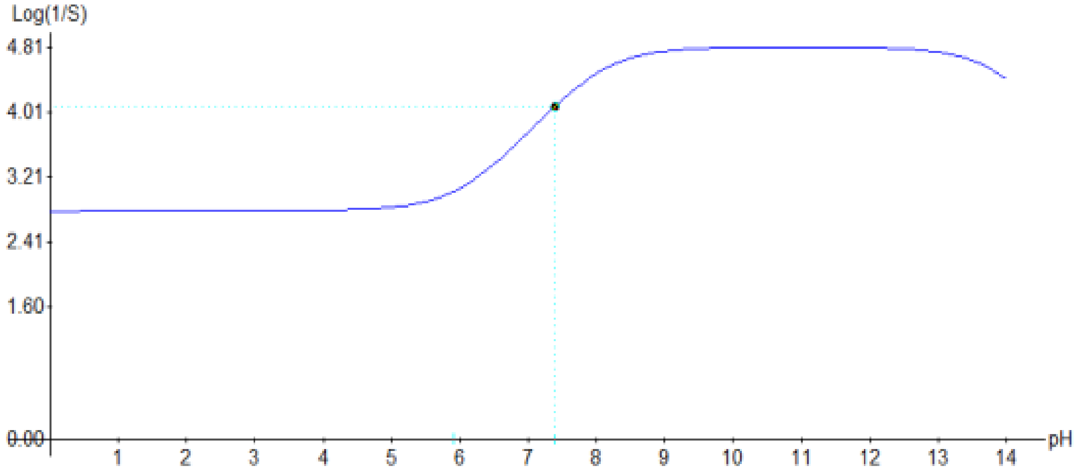
S4 F. Winternitz, M. Mousseron and R. Dennilauler, *Bull. Soc. Chim. Fr.*, 1956, **8**, 1228.

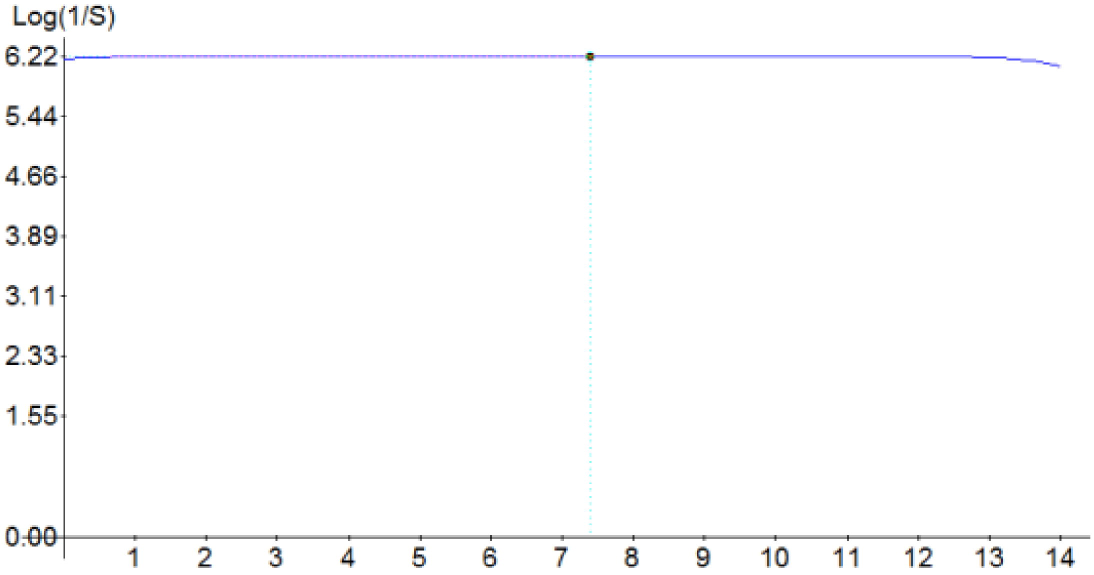
S5 D. J. Whittingham and A. F. McKay, *Patent US 2881169 A*, 1958.

Solubility estimation for compound **12** and tubuloclustin **4b** was made using ACD/Labs Software Solubility Database.

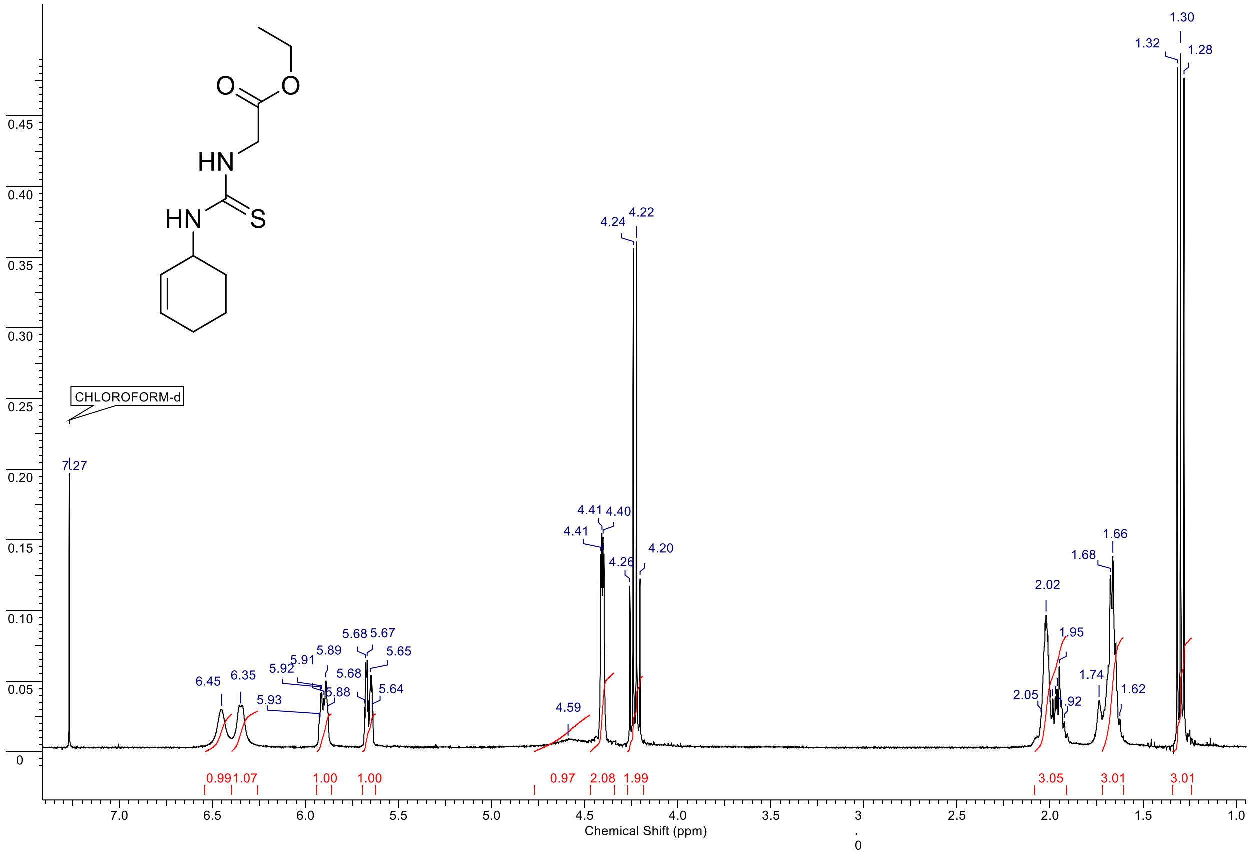
For compound **12**:



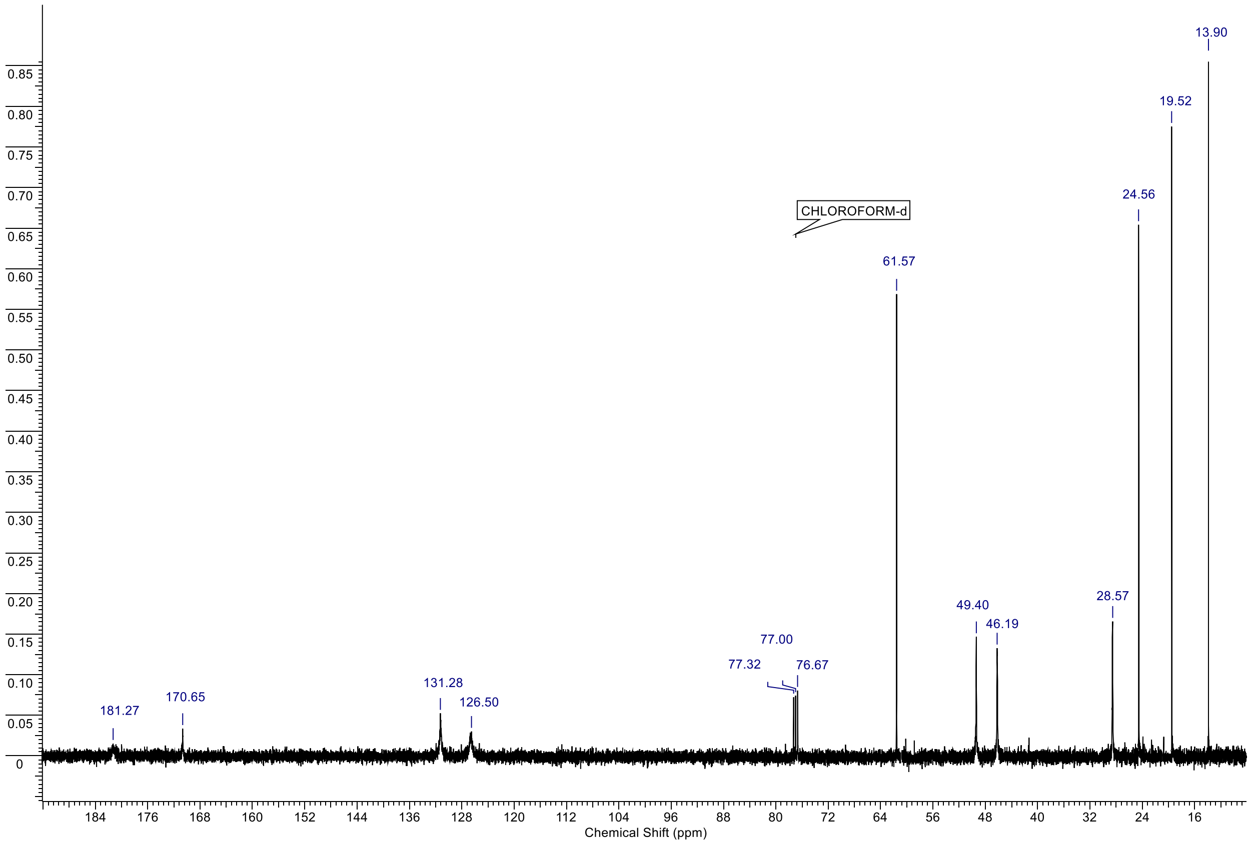
For tubuloclustin **4b**:



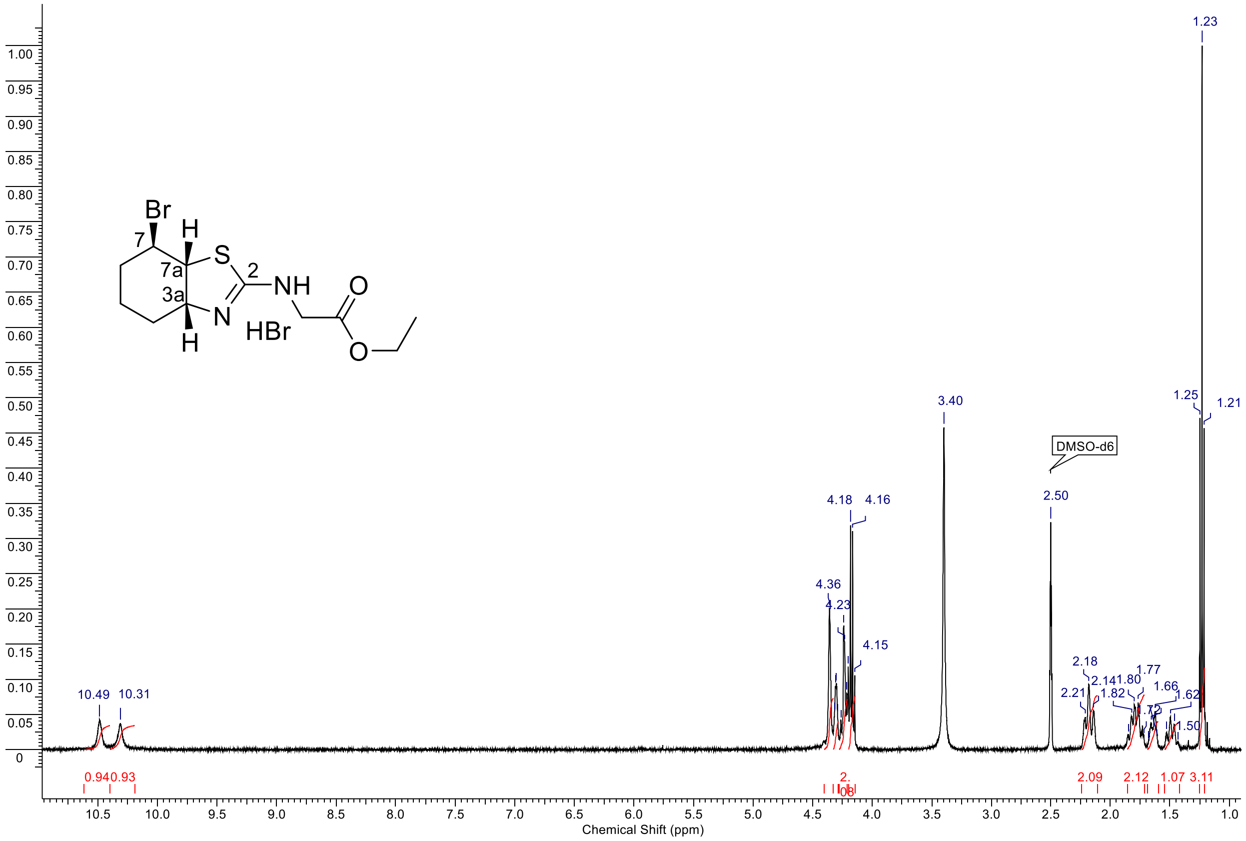
^{1}H NMR spectrum of compound **5a**



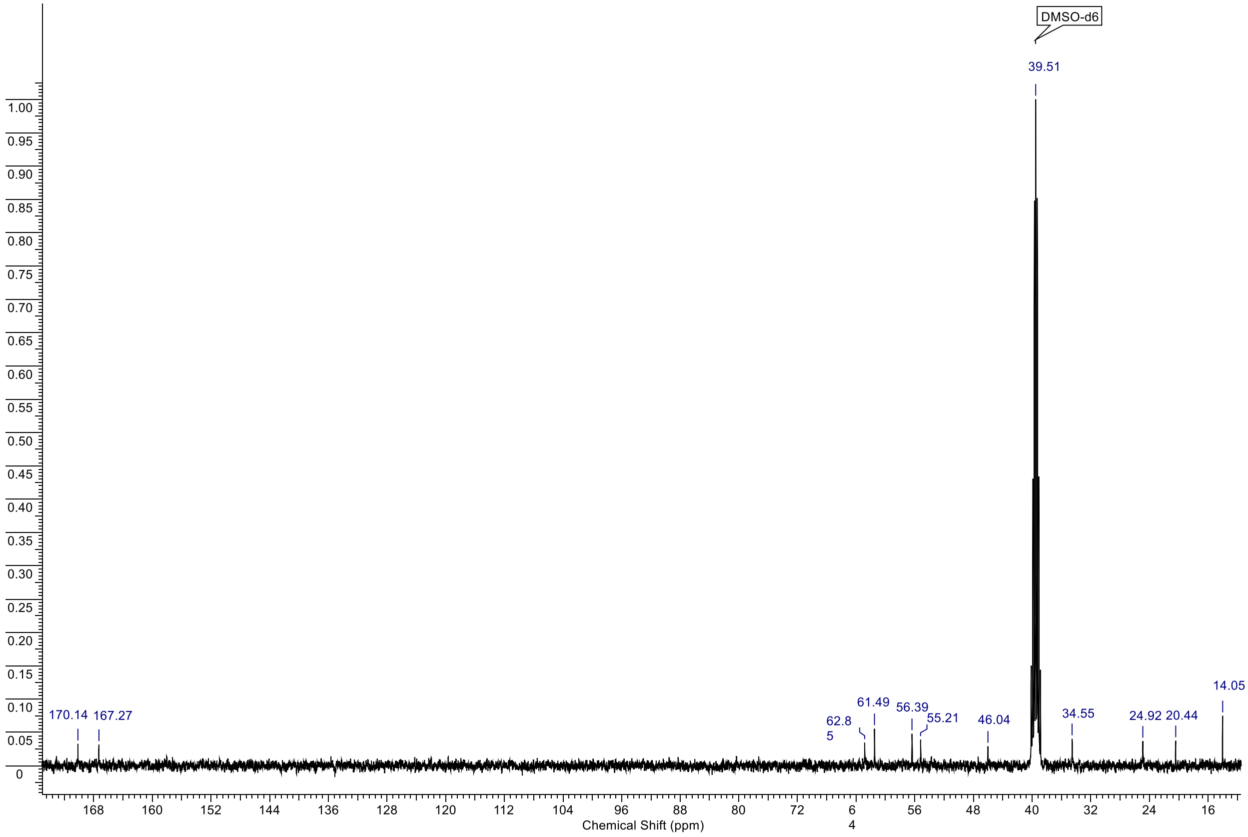
^{13}C NMR spectrum of compound **5a**



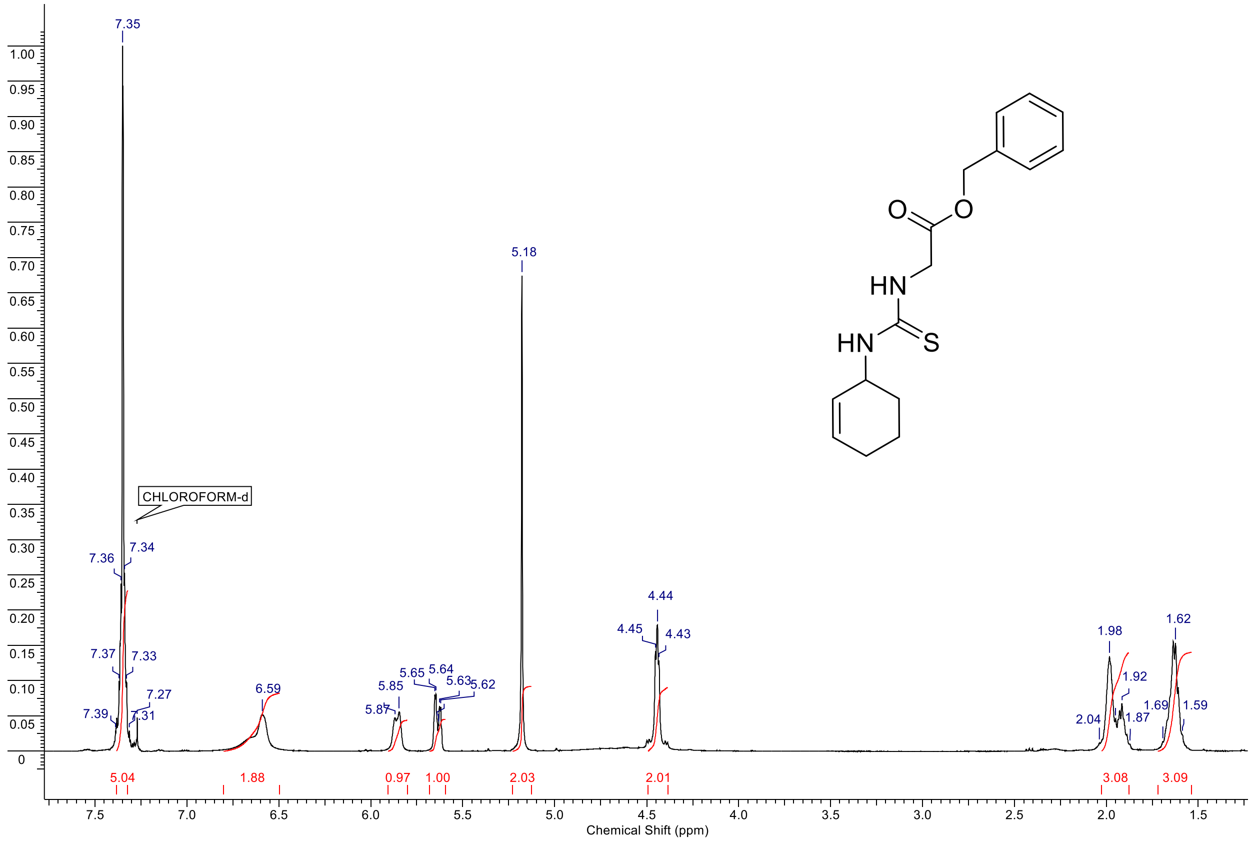
^{1}H NMR spectrum of compound **6a**



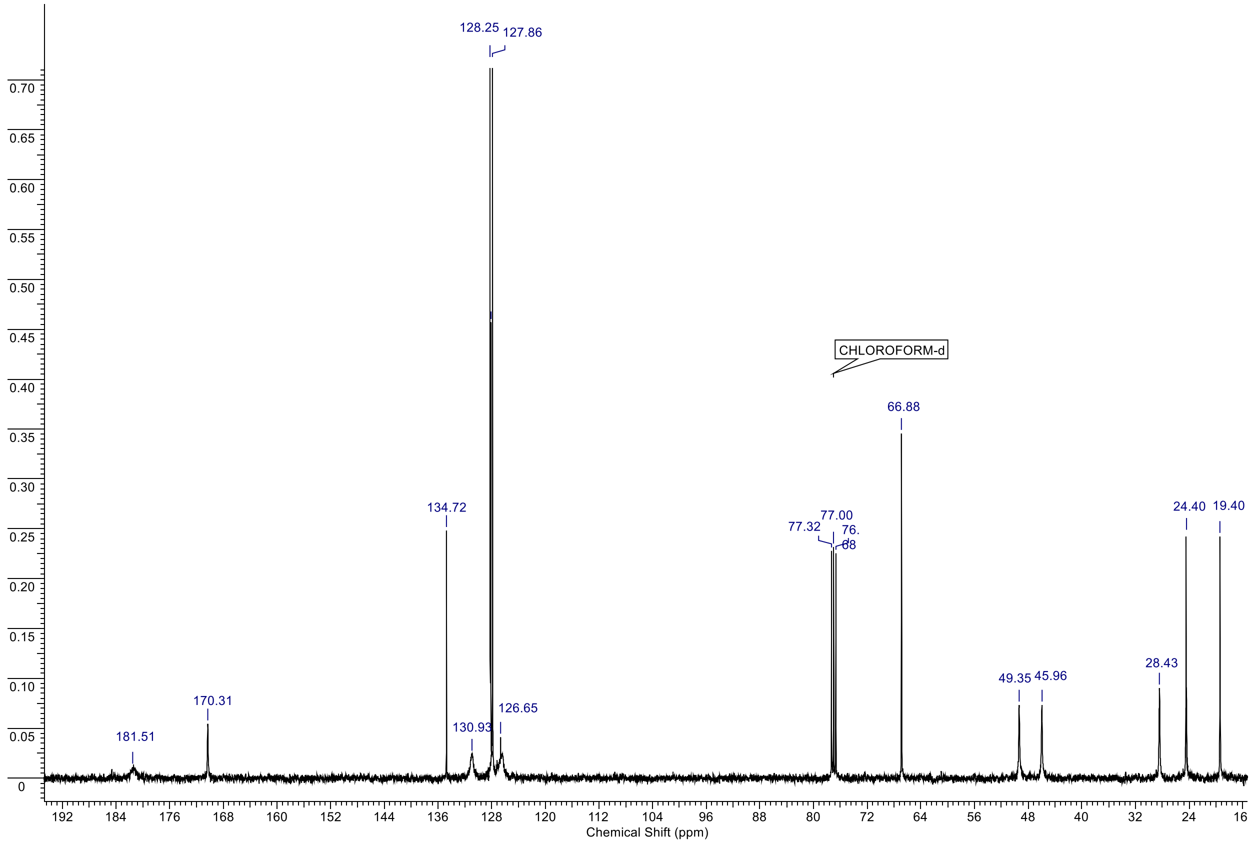
^{13}C NMR spectrum of compound **6a**



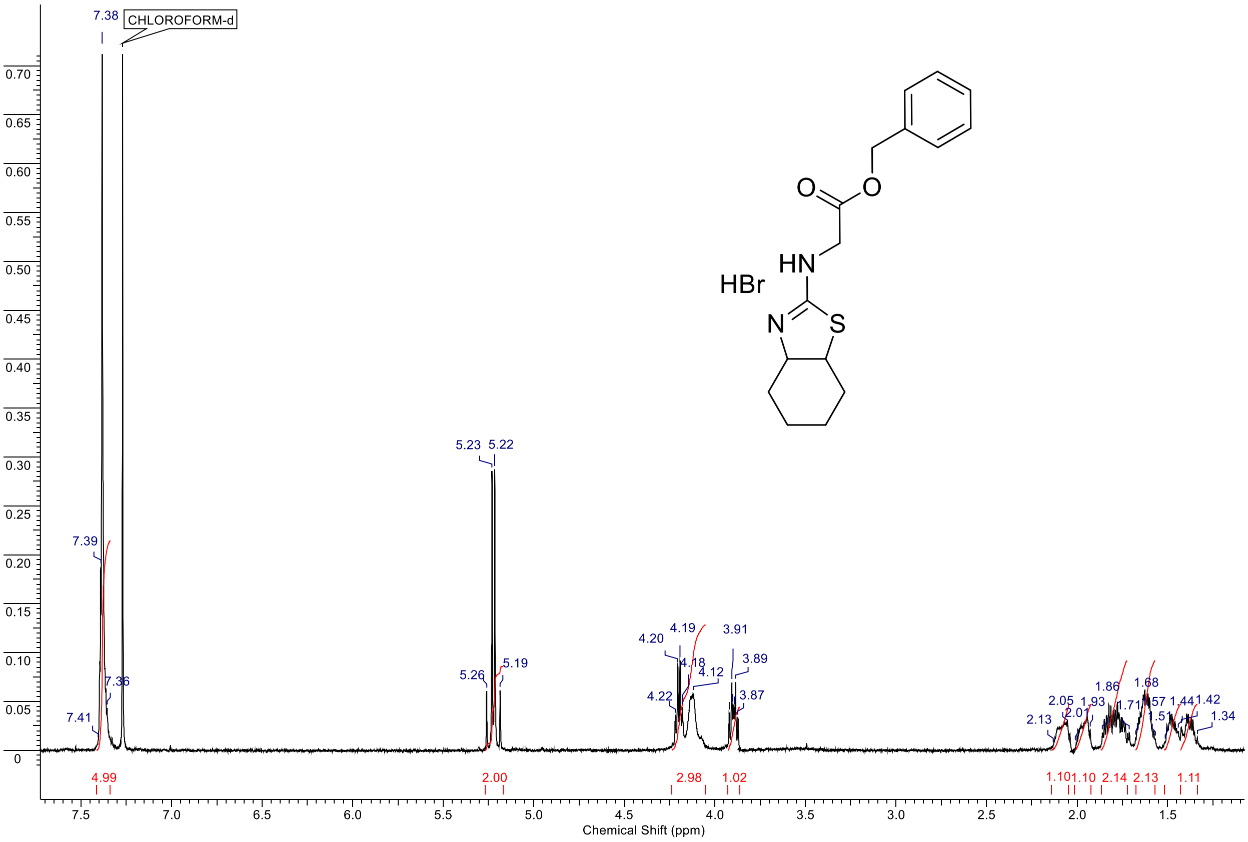
^{1}H NMR spectrum of compound **5b**



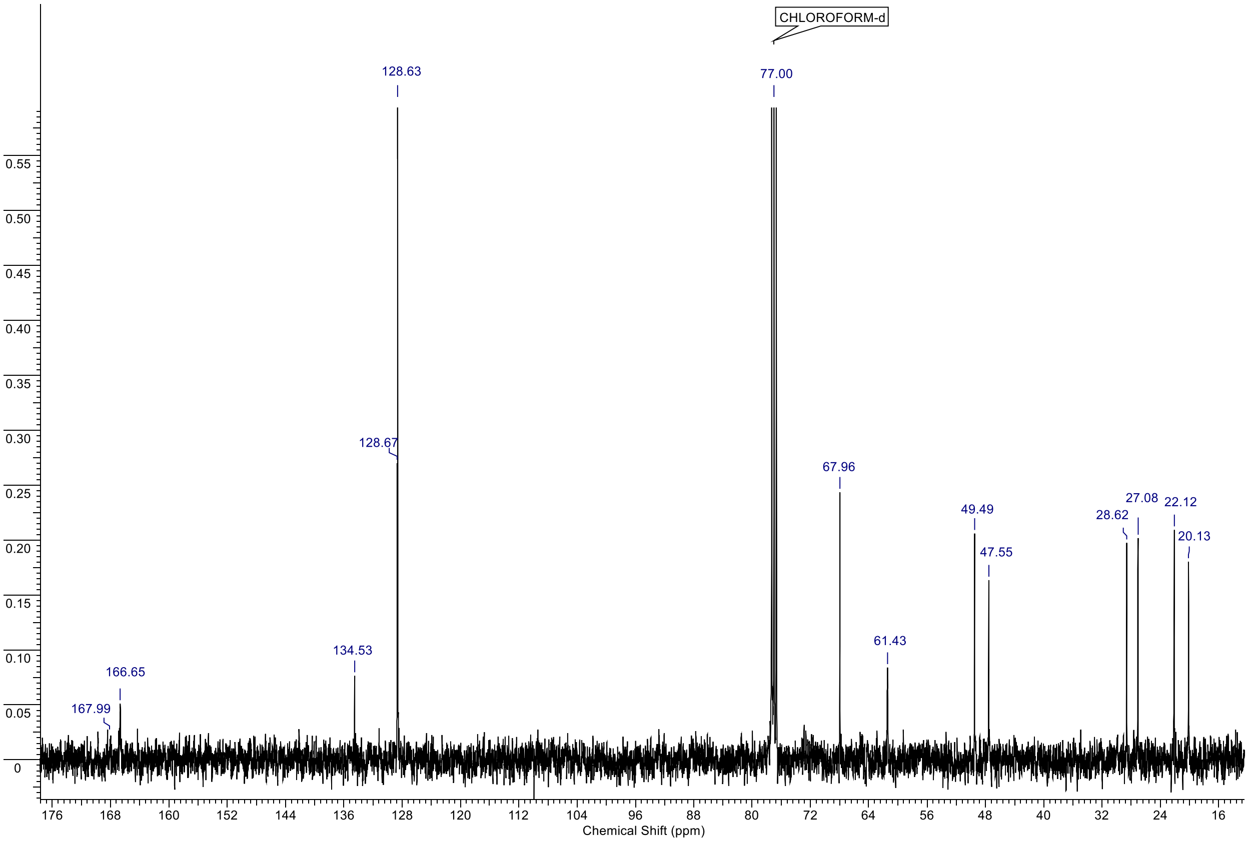
^{13}C NMR spectrum of compound **5b**



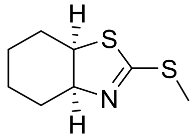
^{1}H NMR spectrum of compound **6b**

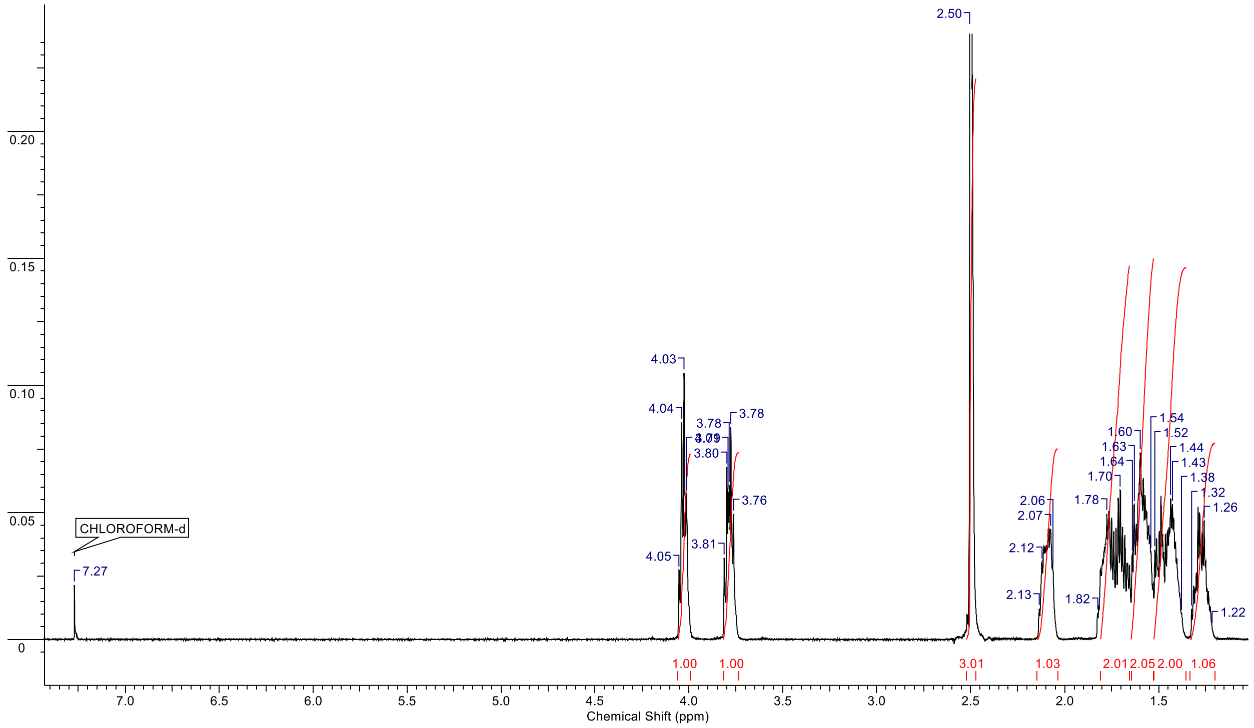


^{13}C NMR spectrum of compound **6b**

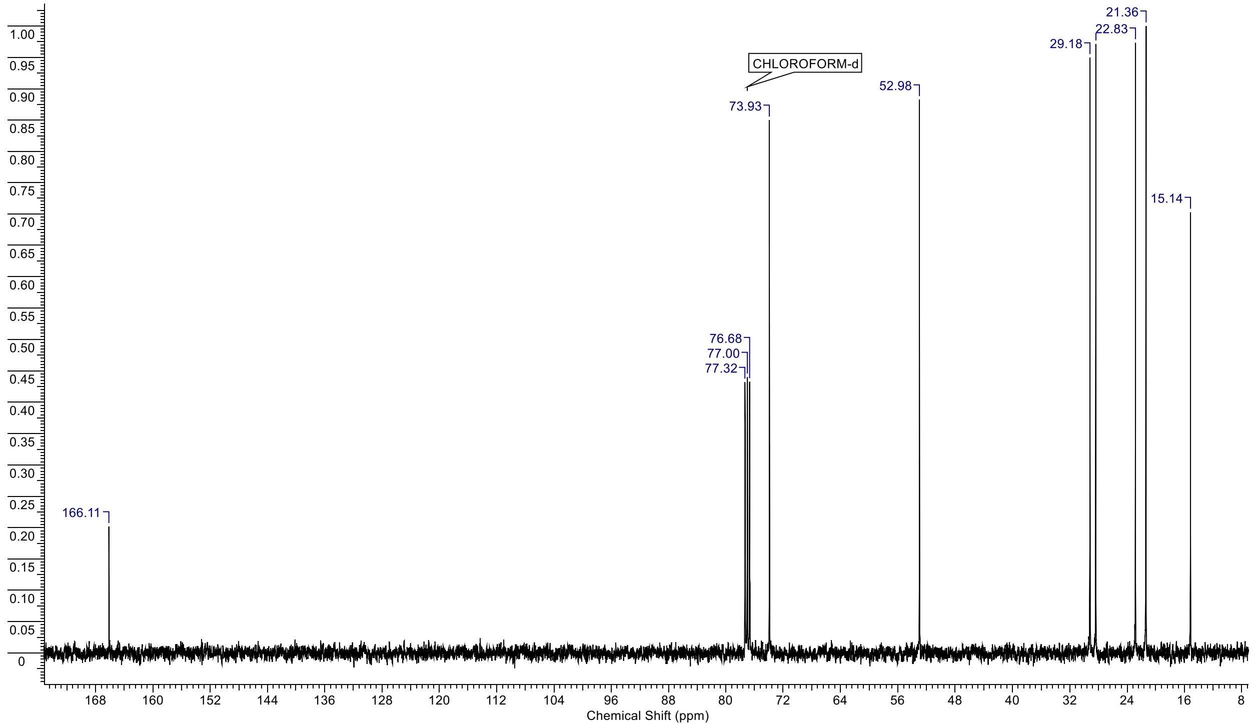


^{1}H NMR spectrum of compound **8**

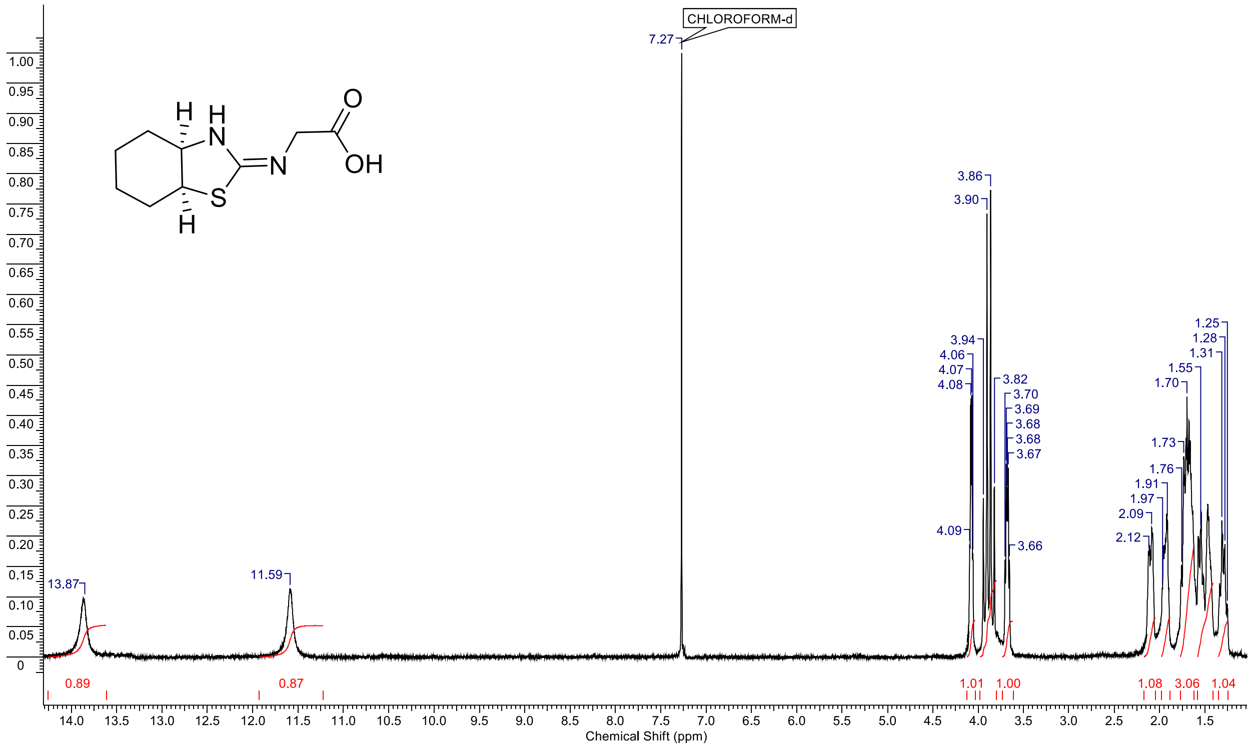




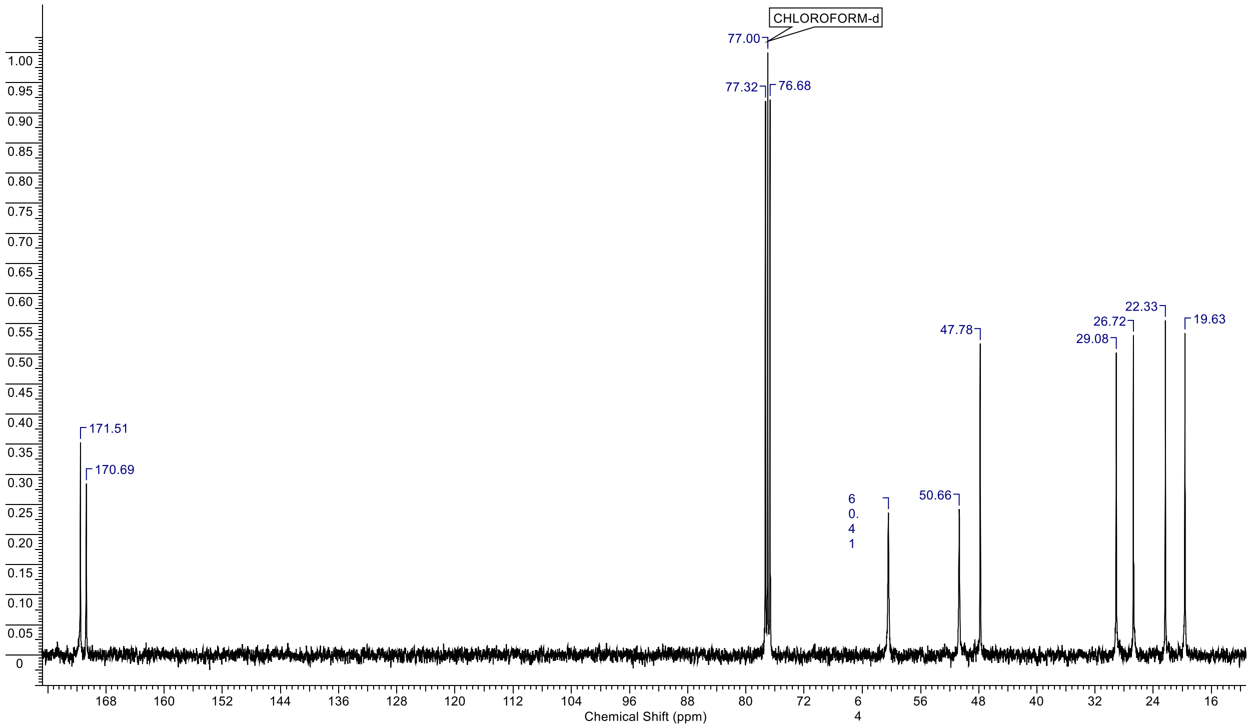
^{13}C NMR spectrum of compound **8**



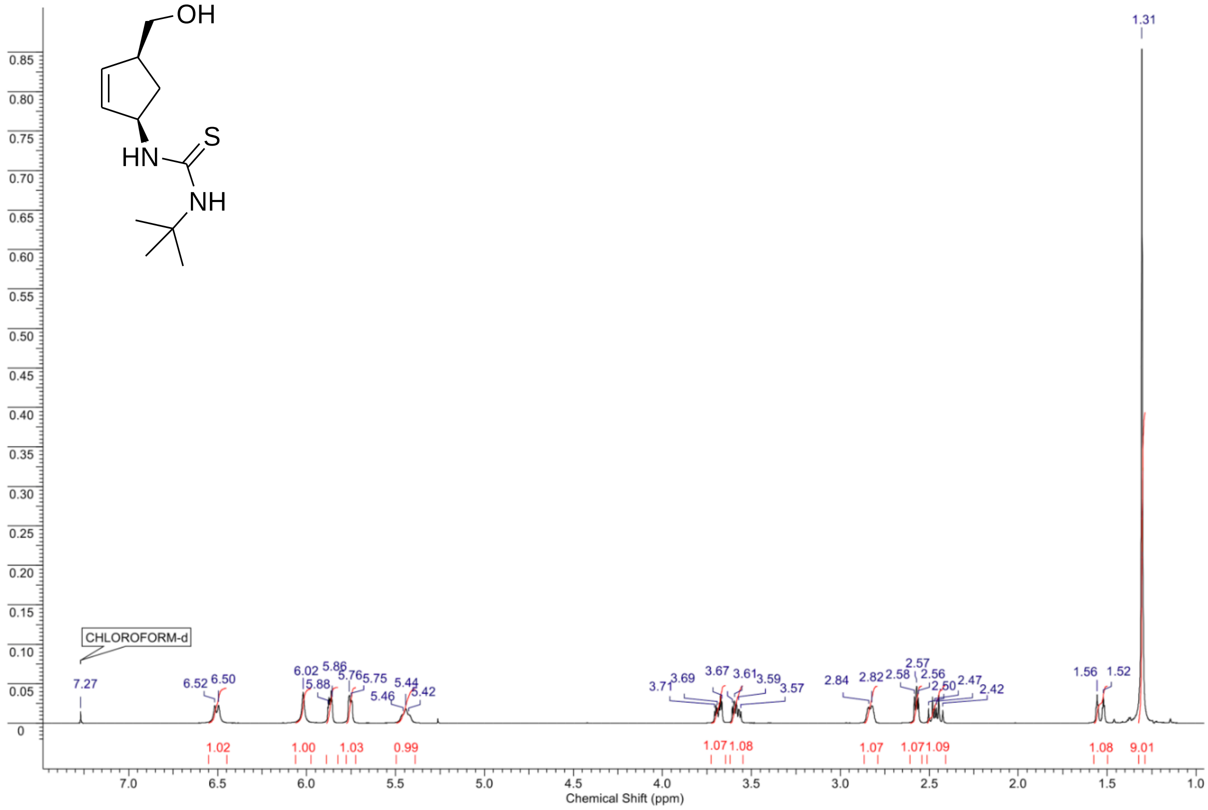
^{1}H NMR spectrum of compound **1**



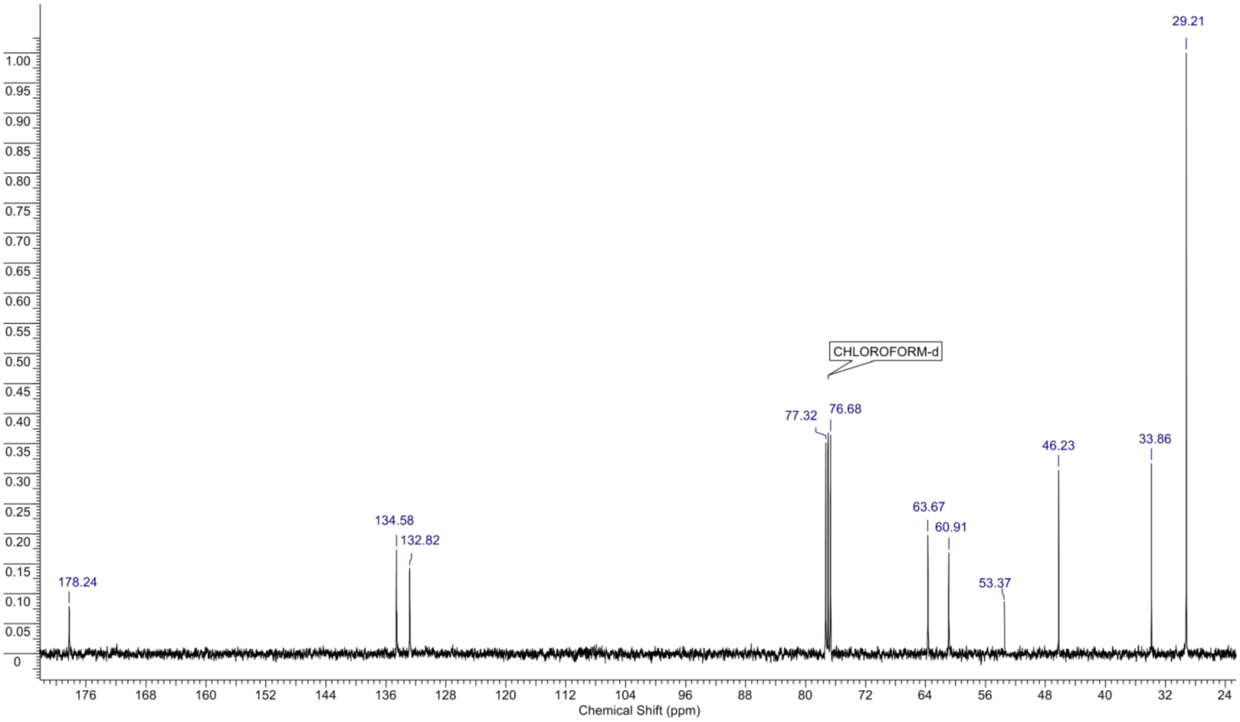
^{13}C NMR spectrum of compound **1**



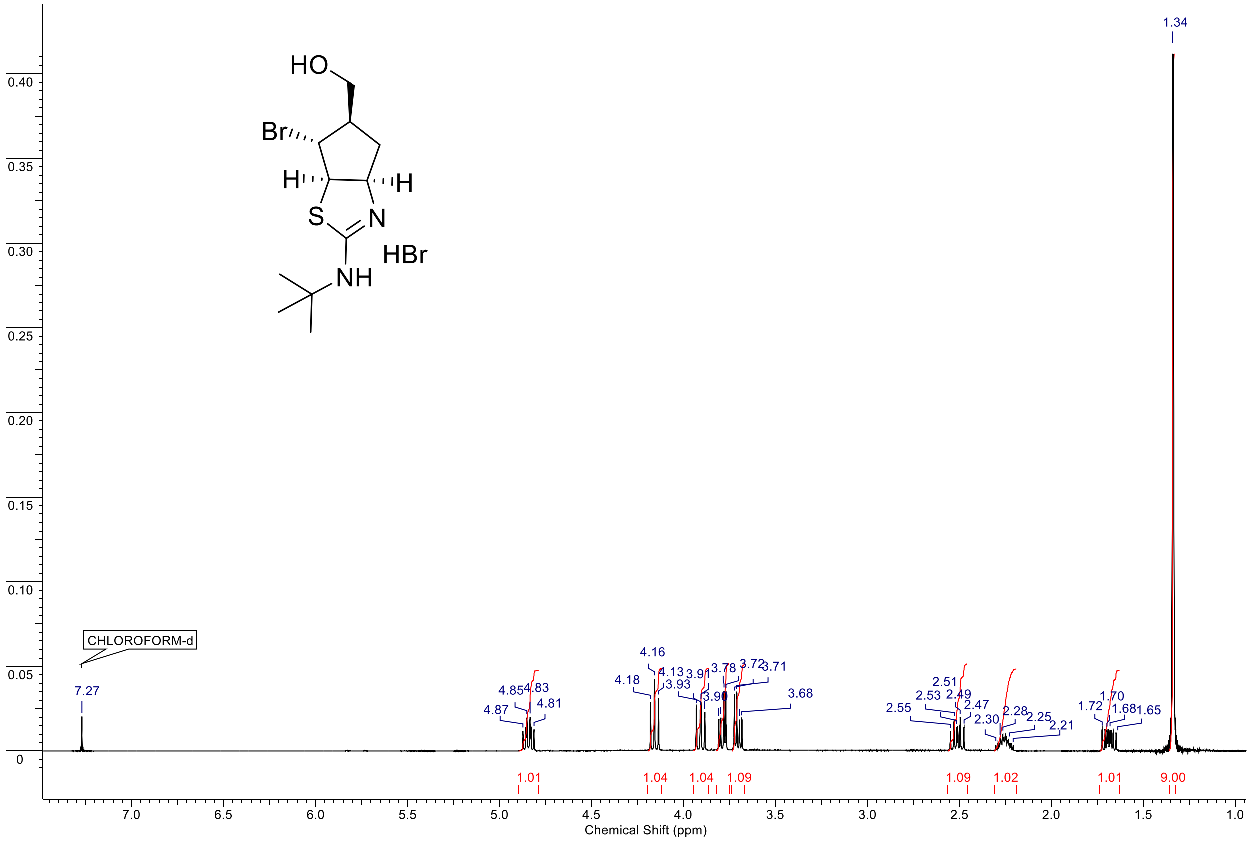
^{1}H NMR spectrum of compound **9**



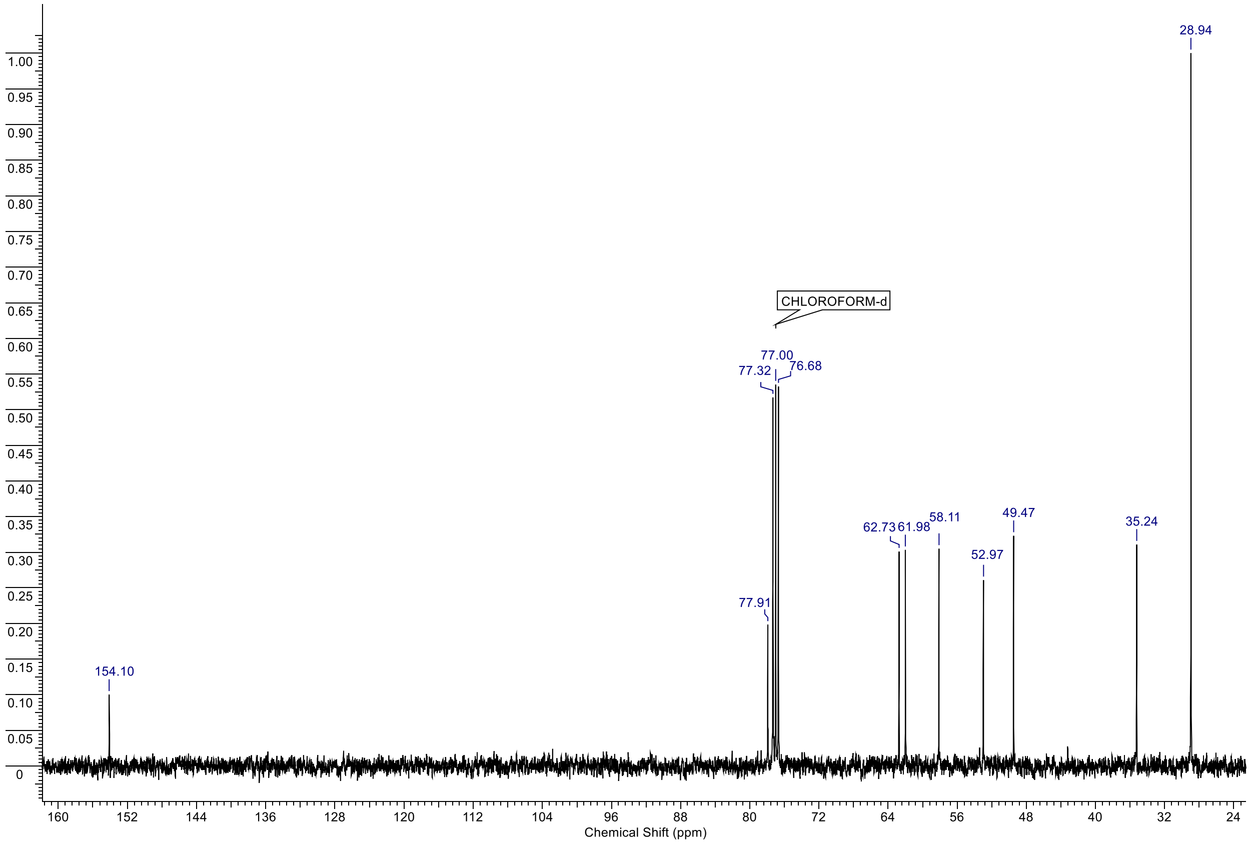
^{13}C NMR spectrum of compound **9**



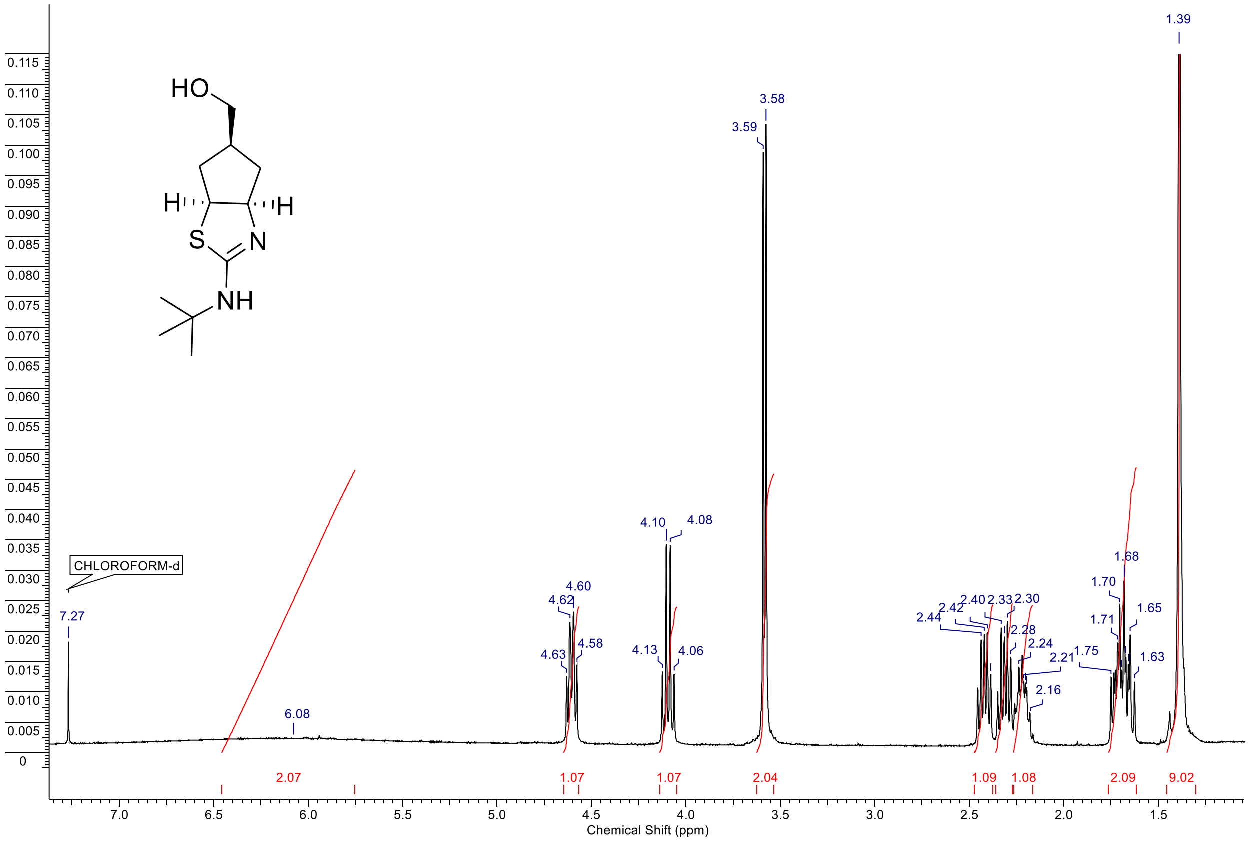
^{1}H NMR spectrum of compound **10**



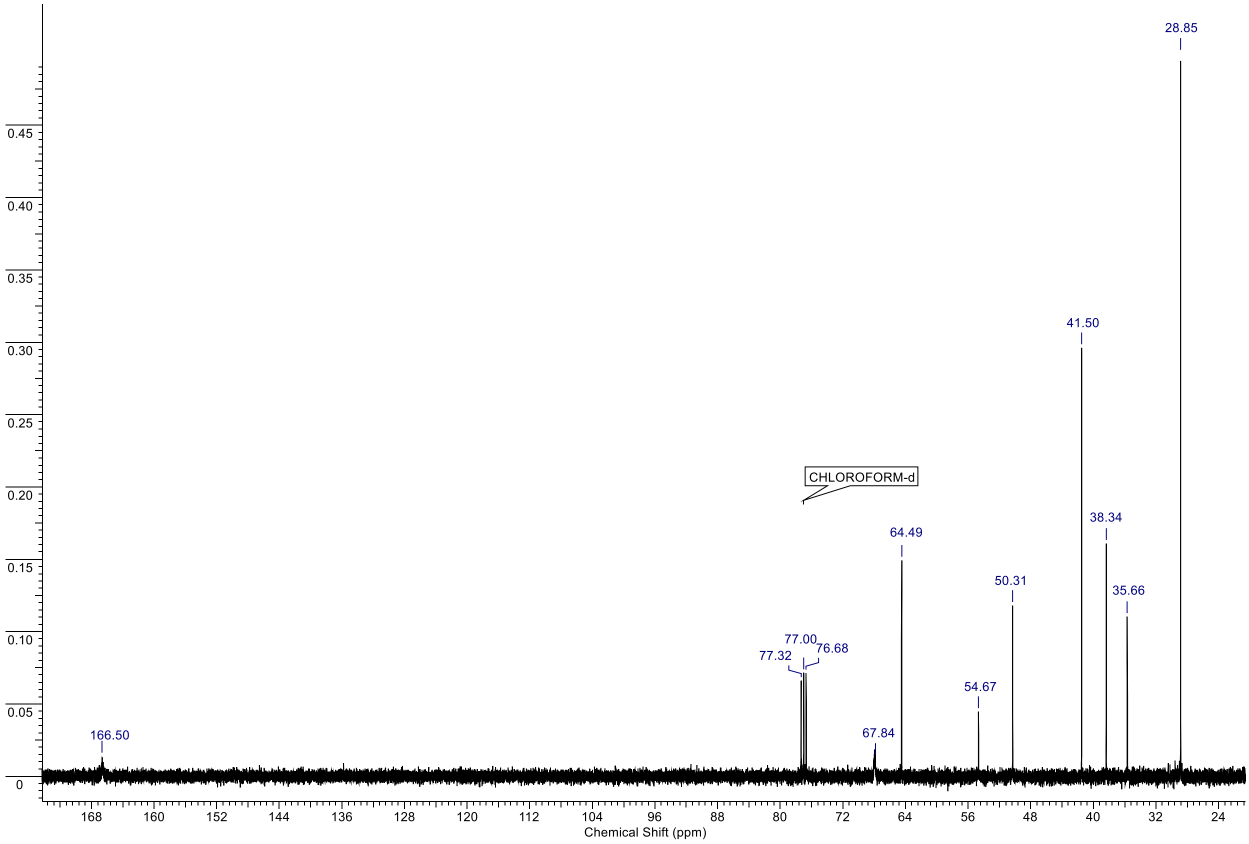
^{13}C NMR spectrum of compound **10**



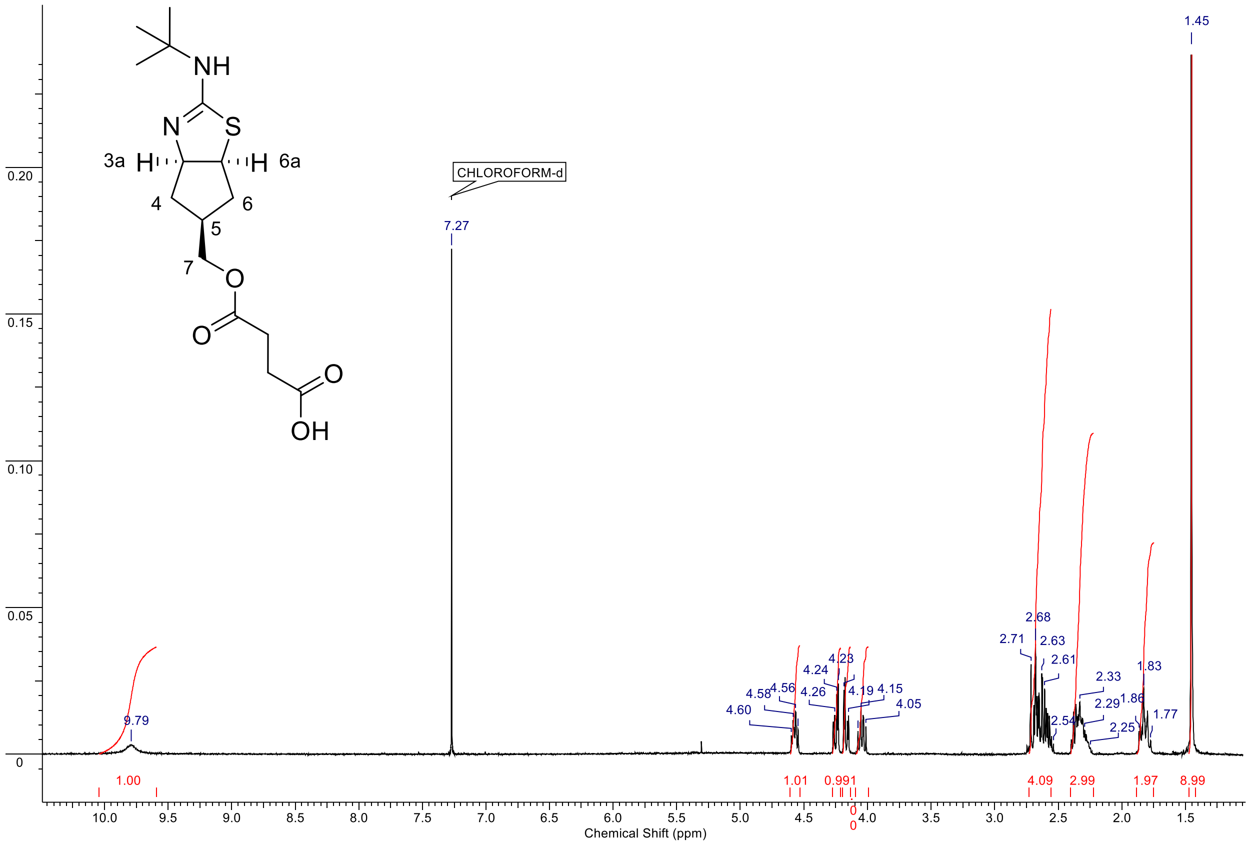
^{1}H NMR spectrum of compound **2**



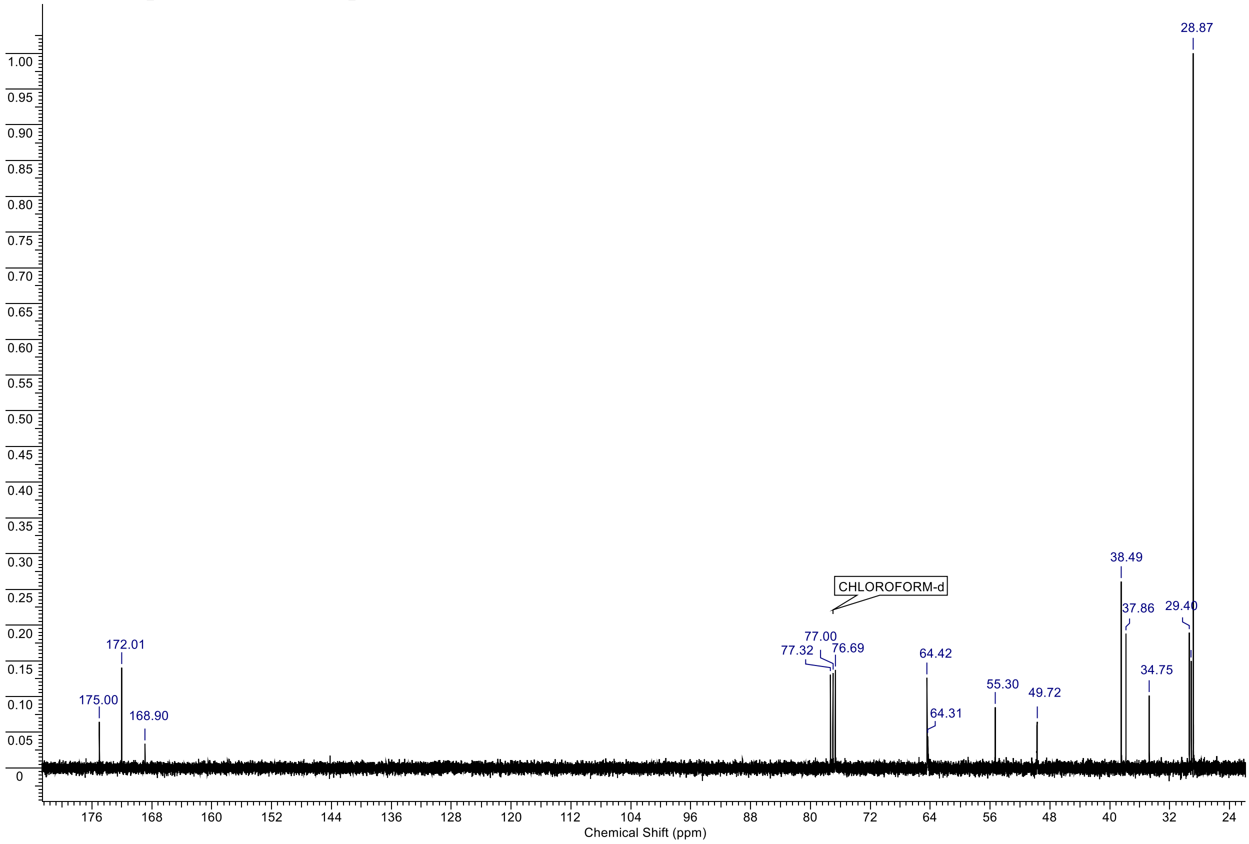
^{13}C NMR spectrum of compound **2**



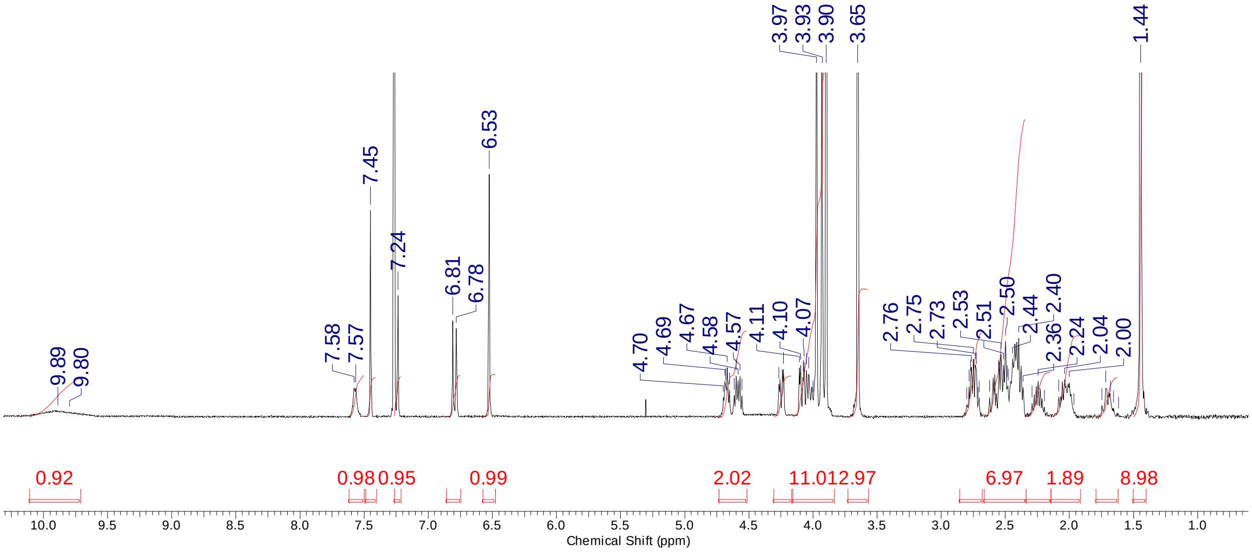
^{1}H NMR spectrum of compound **11**



^{13}C NMR spectrum of compound **11**



^{1}H NMR spectrum of compound **12**



LC-MS spectrum of compound **12**