Cascade reactions of indigo with oxiranes and aziridines: efficient access to dihydropyrazinodiindoles and spiro-oxazocinodiindoles

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Supplementary Information

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Experimental Section

General Experimental Information

Reagents and solvents were purchased reagent grade and used without further purification unless otherwise stated. All reactions were performed in standard oven-dried glassware under a nitrogen atmosphere unless otherwise stated. Melting point temperatures are expressed in degrees Celsius (˚C) and are uncorrected. ^{1}H and ^{13}C NMR spectra ($CDCl_3$/DMSO) were recorded either at 500 MHz and 125 MHz, or 400 MHz and 101 MHz, respectively, with chemical shifts (δ) reported as parts per million relative to TMS (δ = 0.00 ppm), $CDCl_3$ (δ = 7.26; 77.0 ppm) or DMSO-d_6 (δ = 2.50 ppm; 39.51 ppm). Coupling constants (*J*) are reported in Hertz (Hz). Multiplicities are reported as singlets (s), doublets (d), triplets (t), doublet of doublets (dd) or multiplet (m). Electrospray (ESI single quadrupole) mass spectra have their ion mass to charge values (*m/z*) stated with their relative abundances as a percentage in parentheses. Peaks assigned to the molecular ion are denoted as $[M+H]^+$ or $[M+Na]^+$. Infrared (IR) spectra were recorded neat. UV-visible spectra were recorded as solutions in CH_2Cl_2 at 25 ˚C. Optical rotations were recorded in $CHCl_3$ or CH_2Cl_2 at room temperature. Thin layer chromatography (TLC) was performed using silica gel F254 aluminium sheets. Column chromatography was performed using silica gel 60 (0.063-0.200 mm). Eluents are reported in volume to volume (v:v) ratios. Solvent extracts and chromatographic fractions were concentrated by rotary evaporation *in vacuo*. Indigo dye was purchased from Sigma Aldrich and used without further purification. *N,N'*-Ditosyl-2,2'-biaziridine,[1] 2,2'-bioxirane,[2] and (±)-*N*-tosyl-2-bromomethylaziridine[3] were prepared by reported procedures. Pet. spirit had a bp range of 40-60 ˚C.

General Procedure A

A suspension of indigo (262 mg, 1.00 mmol) in anhydrous DMF (40 mL) was sonicated at 60 ˚C for one hour. The resulting hot suspension was transferred by cannula under positive pressure of nitrogen into a 100 mL round-bottomed flask containing a magnetic stir bar, pre-dried and ground Cs_2CO_3 (1.206 g, 3.700 mmol), and fitted with a rubber septum. The resulting dark-green to amber solution was stirred for one hour in an oil bath, pre-heated to strictly 85-87 ˚C under dry nitrogen. The nitrogen flow was cut, and the electrophile (5.0 mmol) injected through the septum either neat, or as a solution in DMF (2 mL), and the mixture stirred at 85-87 ˚C for the required time. The resulting intensely-coloured solution was quenched over crushed ice (ca. 100 g), and the flask rinsed with EtOAc (ca. 10 mL) to remove adhered products. Upon warming to RT, the emulsion was saturated with solid NaCl, and extracted with EtOAc (4×60 mL) or $CHCl_3$ (4×60 mL) until the aqueous phase became clear. The combined organic fractions were condensed to ca. 150 mL, then extracted with brine (4×40 mL), dried ($MgSO_4$), and filtered through a 2 cm plug of celite, and the solvent removed *in vacuo*.

Products from the reaction of indigo with *(S)*-epichlorohydrin (7)

(*S*)-6-(hydroxymethyl)-6,7-dihydropyrazino[1,2-*a*:4,3-*a*']diindole-13,14-dione (**8**)

Prepared following ***General Procedure A***, using (*S*)-epichlorohydrin (462 mg, 5.0 mmol), and a 30 min reaction time. The resulting crude residue was dissolved in a minimum of hot $CHCl_3$, and gradual dilution with a tenfold volume of hot pet. spirit precipitated an intense blue powder, which was collected on a Hirsch funnel and combined with **Fraction 5** (see below). The combined solids were dried *in vacuo* to furnish the dihydropyrazinodiindole **8** (141 mg, 44%) as a dark blue powder, mp >350 ˚C. R_F (9:1 CH_2Cl_2:EtOAc) 0.14. UV-Vis (CH_2Cl_2) λ_{max}/nm (ε, $M^{-1}cm^{-1}$) 241 (12773), 303 (6339), 579 (2819). ^{1}H-NMR (DMSO-d_6, 500 MHz) 7.56-7.64 (m, 4H, ArH), 7.35 (d, *J* = 8.0 Hz, 2H, H4, H9), 6.99-7.06 (m, 2H, ArH), 5.28 (s, 1H, CH_2O**H**), 4.66 (s, 1H, H6), 4.44 (d, *J* = 12.0 Hz, 1H, H7a), 3.71 (dd, *J* = 3.5, 12.0 Hz, 1H, H7b), 3.47-3.57 (m, 2H, C**H$_2$**OH). ^{13}C-NMR (125 MHz) δ 179.9, 179.7, 150.2, 149.4, 135.3, 135.0, 123.9, 123.5, 123.1, 122.0, 121.5, 120.8, 120.6, 120.4, 110.9, 110.5, 59.9, 51.1, 39.5. IR (neat) 3432 (br, m), 2937 (w), 2876 (w), 1689 (s), 1607 (s), 1561 (s), 1470 (m), 1465 (m), 1369 (m), 1340 (w), 1298 (s), 1180 (s), 1158 (m), 1144 (s), 743 (s). HR-ESI-MS calcd. for $C_{19}H_{14}N_2O_3Na^+$ = 341.0902 found 341.0888. $[\alpha]_D^{29}$ = -6.4 ˚ (*c* 0.10, $CHCl_3$).

(7*S*,9a*R*)-7,8-dihydro-6*H*-7,9a-epoxy[1,5]oxazocino[5,4-*a*:3,2-*b*']diindol-15(14*H*)-one (**9**)

The mother liquor from compound **8** was concentrated, and subjected to flash chromatography (gradient elution, 5-50% EtOAc:CH_2Cl_2) to afford five major fractions. **Fraction 1** was subjected to PTLC (10% EtOAc:CH_2Cl_2), and the selected band scraped off and soaked in EtOAc. The silica was removed by filtration and the luminescent, yellow solution was combined with **Fraction 2** and the solvent removed to furnish the title compound **9** as small, bright orange crystals (101 mg, 32%), mp 189-191 ˚C (dec). X-ray quality crystals were grown by slow evaporation of a saturated solution in EtOAc at -20 ˚C. R_F (9:1 CH_2Cl_2:EtOAc) 0.57. UV-Vis (CH_2Cl_2) λ_{max}/nm (ε, $M^{-1}cm^{-1}$) 236 (8964), 287 (5169), 364 (2651), 483 (3573). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.81 (d, *J* = 7.7 Hz, 1H, H1), 7.49 (m, 2H, ArH), 7.35 (t, *J* = 7.7 Hz, 1H, ArH), 7.08 (d, *J* = 8.3 Hz, 1H, ArH), 7.02 (t, *J* = 7.5 Hz, 2H, ArH), 6.92 (d, *J* = 7.9 Hz, 1H, ArH), 5.08 - 5.16 (m, 1H, H7), 4.37 (t, *J* = 7.2 Hz, 1H, H8b), 4.26 (dd, *J* = 7.0 Hz, 3 Hz, H8a) 4.20 (dd, *J* = 14.0, 3.0 Hz, 1H, H6b), 3.74 (d, *J* = 14 Hz, 1H, H6a).^{13}C NMR (125 MHz, $CDCl_3$) δ 188.1, 152.3, 146.8, 145.3, 144.7, 133.7, 132.2, 125.2, 124.3, 123.9, 122.8, 122.0, 119.9, 110.9, 110.6, 73.9, 65.4, 51.6. IR (neat) 3310 (br, w) 2966 (w), 2953 (w), 2893 (w), 1689 (s), 1614 (s), 1576 (s), 1098 (m), 995 (s), 747 (s). HR-ESI-MS calcd. for $C_{19}H_{14}N_2O_3Na^+$ = 341.0902 found 341.0900. $[\alpha]_D^{27}$ = -6.5 ˚ (*c* 0.17, $CHCl_3$).

(7*R*,9a*R*)-14-((*E*)-3-hydroxyprop-1-en-1-yl)-7,8-dihydro-6*H*-7,9a-epoxy[1,5]oxazocino[5,4-*a*:3,2-*b*']diindol-15(14*H*)-one (**10**)

Fraction 3 was subjected to PTLC (developed twice; 20% EtOAc:CH_2Cl_2, then EtOAc) and the selected band scraped off and soaked in EtOAc. The silica was removed, and the solution combined with **Fraction 4** to give the title compound **10** as red-orange crystals (75.2 mg, 21%), mp 152-154 ˚C (dec.). X-ray quality crystals were grown by slow evaporation of a saturated solution in 9:1 CH_2Cl_2 : hexane at RT. R_F 0.21 (20% EtOAc:CH_2Cl_2). UV-Vis (CH_2Cl_2) λ_{max}/nm (ε, $M^{-1}cm^{-1}$) 243 (18482), 274 (20838), 355 (1472), 500 (1174). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (d, *J* = 7.6 Hz, 1H, ArH), 7.48 – 7.55 (m, 2H, 2×ArH), 7.45 (t, *J* = 7.7 Hz, 1H, ArH), 7.30 – 7.36 (m, 2H, 2×ArH), 7.17 (t, *J* = 7.4 Hz, 1H, ArH), 7.06 (d, *J* = 8.3 Hz, 1H, ArH), 7.02 (t, *J* = 7.4 Hz, 1H ArH), 6.87 (d, *J* = 14.2 Hz, 1H, H1'), 6.07 (dt, *J* = 6.5, 14.0 Hz, 1H, H2'), 5.01 – 5.05 (m, 1H, H7), 4.21 – 4.44 (m, 6H, H6b, H8a, H8b, H3'a, H3'b, OH), 3.74 (d, *J* = 14.3 Hz, 1H, H6a). ^{13}C NMR (125 MHz, $CDCl_3$) δ 185.1, 152.7, 151.4, 149.7, 134.2, 131.9, 130.8, 125.5, 124.9, 124.5, 124.3, 123.6, 121.6, 120.0, 119.1, 111.7, 109.9, 73.4, 67.6, 61.9, 52.2. IR (neat) 3349 (br, m), 1726 (m), 1466 (s), 1265 (s), 702 (s). HR-ESI-MS calcd. for $C_{22}H_{19}N_2O_4^+$ = 375.1345 found 375.1357. $[\alpha]_D^{29}$ = -6.7 ˚ (*c* 0.11, $CHCl_3$).

14-((2-oxo-1,3-dioxolan-4-yl)methyl)-7,8-dihydro-6*H*-7,9a-epoxy[1,5]oxazocino[5,4-*a*:3,2-*b'*]diindol-15(14*H*)-one (±**11**)

Prepared following ***General Procedure A***, using (±)-epichlorohydrin (5.0 mmol, 462 mg), and a 2 h reaction time. Following the above separation protocol, the racemic products (±)-**3** (134.4 mg, 43%), (±)-**4** (19.8 mg, 6%), and (±)-**5** (27.9 mg, 7%) were isolated. **Fraction 4** was condensed, and recrystallisation from a minimum volume of EtOAc afforded cyclic carbonate **11** (27 mg, 6%) as luminescent orange crystals. X-ray quality crystals were grown from slow evaporation of a saturated solution in 9:1 EtOAc:pet spirit at 4 ˚C. mp 146-149 ˚C (dec.) UV-Vis (CH_2Cl_2) λ_{max}/nm (ε, $M^{-1}cm^{-1}$) 238 (17364), 344 (5999), 499 (2531). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (d, *J* = 7.1 Hz, 1H), 7.52 – 7.41 (m, 3H), 7.33 (d, *J* = 8.1 Hz, 1H), 7.13 (td, *J* = 7.4, 0.8 Hz, 1H), 7.04 – 6.94 (m, *J* = 16.6, 7.8 Hz, 2H), 5.48 (ddd, *J* = 14.9, 8.2, 1.1 Hz, 1H), 5.20 (d, *J* = 9.2 Hz, 1H), 5.00 – 4.94 (m, 1H), 4.33 – 4.22 (m, 4H), 4.22 – 4.08 (m, 2H), 3.68 (d, *J* = 14.3 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 182.2, 178.3, 155.0, 152.9, 148.0, 146.6, 134.3, 132.7, 128.3, 124.8, 124.6, 124.2, 123.4, 119.9, 112.0, 111.1, 110.2, 81.3, 73.4, 69.0, 67.9, 55.5, 52.4. IR (neat) 3431 (w), 1799 (m), 1734 (s), 1611 (s), 1466 (s), 1065 (s), 774 (m). HRESI-MS calcd. for $C_{23}H_{18}N_2O_6Na^+$ = 441.1063 found 441.1082 (+4.3 ppm); calcd. for $C_{23}H_{19}N_2O_6^+$ = 419.1243 found 419.1257.

(7*R*,9a*R*)-(((*S*)-2-oxo-1,3-dioxolan-4-yl)methyl)-7,8-dihydro-6*H*-7,9a-epoxy[1,5]oxazocino[5,4-*a*:3,2-*b'*]diindol-15(14*H*)-one (**11**)

Prepared following ***General Procedure A***, using *(S*)-epichlorohydrin **7** (462 mg, 5.0 mmol) and a 30 min reaction time. Following the above separation protocol, products **8** (133 mg, 41%), and **9** (97 mg, 30%) were isolated. **Fraction 4** was subjected to PTLC (20% EtOAc:CH_2Cl_2) and the selected band scraped off and soaked in EtOAc. Recrystallisation from a minimum volume of EtOAc afforded cyclic carbonate **11** (87 mg, 20%) as luminescent orange crystals. $[\alpha]_D^{29}$ = -6.1 ˚ (*c* 0.09, $CHCl_3$).

(*R*)-6-(hydroxymethyl)-6,7-dihydropyrazino[1,2-*a*:4,3-*a*']diindole-13,14-dione (*R*-**8**)

Prepared following ***General Procedure A***, using of (*R*)-glycidyl tosylate (1.141 g, 5.0 mmol), and a 10 min reaction time. The crude residue was dissolved in a minimum of hot $CHCl_3$, and gradual dilution with a tenfold volume of hot pet. spirit precipitated an intense blue powder, which was collected on a Hirsch funnel. The mother liquor was condensed, and a second recrystallization of the residue afforded a further crop of product. The combined solids were dried *in vacuo* to furnish the title compound *(R)*-**8** as a dark blue powder (242.3 mg, 76%). Spectral characteristics were identical to those obtained from its enantiomer. $[\alpha]_D^{29}$ = +3.7˚ (*c* 0.33, CH_2Cl_2).

(±)-6-(hydroxymethyl)-6,7-dihydropyrazino[1,2-*a*:4,3-*a*']diindole-13,14-dione (±**8**)

Prepared following ***General Procedure A***, using (±)-epibromohydrin (685 mg, 5.0 mmol), and a 10 min reaction time. The crude residue was dissolved in a minimum of hot $CHCl_3$, and gradual dilution with a tenfold volume of hot pet. spirit precipitated an intense blue powder, which was collected on a Hirsch funnel. The mother liquor was condensed, and a second recrystallization of the residue afforded a second crop of product. The combined solids were dried *in vacuo* to furnish the title compound as a dark blue powder (266.9 mg, 84%). Spectral characteristics were identical to those noted for (*S*)-**8**.

Products from the reaction of indigo with 2,2'-bioxirane

(7*S*,8*R*,9a*S*)-8-(hydroxymethyl)-7,8-dihydro-6*H*-7,9a-epoxy[1,5]oxazocino[5,4-*a*:3,2-*b*']diindol-15(14*H*)-one (**12**)

Prepared following ***General Procedure A***, using (2*S*,2'*S*)-2,2'-bioxirane (281 mg, 3.3 mmol), and a 30 min reaction time. The crude residue was separated into five fractions on a plug of silica (70 g), eluting sequentially with 1) CH_2Cl_2 (250 mL each) 2) 20% EtOAc:CH_2Cl_2 3) 40% EtOAc:CH_2Cl_2 4) 60% EtOAc:CH_2Cl_2 5) 80% EtOAc:CH_2Cl_2. **Fractions 2** and **3** were combined, and subjected to flash chromatography on 25 g silica, and elution with 20% EtOAc:CH_2Cl_2 afforded the *mono*-substituted spirocycle **12** as a luminescent orange powder (216 mg, 62%), mp 155-156 ˚C (dec.). X-ray quality crystals

were grown from slow evaporation of a hexane/ethyl acetate solution. R_F (20% EtOAc:CH_2Cl_2) 0.33. UV-Vis (CH_2Cl_2) λ_{max}/nm (ε, $M^{-1}cm^{-1}$) 244 (12573), 365 (2296), 485 (2882). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 (d, *J* = 7.6 Hz, 1H, H1), 7.50 (dd, *J* = 10.4, 4.4 Hz, 2H, H3, H10), 7.34 (dd, *J* = 11.1, 4.4 Hz, 1H, H12), 7.10 (d, *J* = 8.4 Hz, 1H, H4), 7.01 (dd, *J* = 16.6, 7.7 Hz, 2H, H2, H11), 6.90 (d, *J* = 7.9 Hz, 1H, H13), 5.02 – 4.97 (m, 1H, H7), 4.74 (dd, *J* = 13.0, 6.1 Hz, 1H, H8), 4.37 (dd, *J* = 14.1, 2.7 Hz, 1H, H6b), 4.19 – 4.12 (m, 1H, H1'a), 3.95 (dd, *J* = 11.3, 5.7 Hz, 1H, H1'b), 3.69 (dd, *J* = 14.0, 2.3 Hz, 1H, H6a). ^{13}C NMR (101 MHz, $CDCl_3$) δ 184.1, 151.8, 145.2, 144.5, 138.7, 134.0, 132.4, 125.3, 124.2, 124.0, 123.5, 122.3, 121.0, 120.1, 117.8, 111.2, 110.7, 109.6, 77.8, 75.6, 60.4, 48.3. IR (neat) 3420 (w), 2922 (w), 1715 (m), 1612 (s), 1317 (m), 735 (s). HRESI-MS calcd. for $C_{20}H_{17}N_2O_4^+$ 349.1188, found 349.1192. $[\alpha]_D^{25}$ = -7.2˚ (*c* 0.21, CH_2Cl_2).

(7*S*,8*R*,9a*S*)-14-((*S*)-2-hydroxy-2-((*S*)-oxiran-2-yl)ethyl)-8-(hydroxymethyl)-7,8-dihydro-6*H*-7,9a-epoxy[1,5]oxazocino[5,4-*a*:3,2-*b*']diindol-15(14*H*)-one (**13**)

Further elution of **Fractions 2** and **3** with 40% EtOAc:CH_2Cl_2 gave a residue which was combined with **Fractions 4** and **5** and concentrated, and flash chromatography of this residue (20% EtOAc:CH_2Cl_2; 20 g silica) gave the *di*-alkylated spirocycle **13** (82 mg, 19%) as a pale lime-green, amorphous solid. R_F (40% EtOAc:CH_2Cl_2) 0.63. UV-Vis (CH_2Cl_2) λ_{max}/nm (ε, $M^{-1}cm^{-1}$) 323 (3820), 350 (4772), 425 (1723). ^{1}H NMR (400 MHz, DMSO) δ 7.65 – 7.46 (m, 1H), 7.26 – 7.19 (m, 1H), 7.12 – 7.00 (m, 1H), 6.82 (t, *J* = 7.3 Hz, 1H), 5.83 (d, *J* = 4.8 Hz, 1H), 5.22 (d, *J* = 5.7 Hz, 1H), 4.61 (t, *J* = 6.0 Hz, 1H), 4.54 (d, *J* = 12.6 Hz, 1H), 4.21 (dt, *J* = 11.6, 5.8 Hz, 1H), 4.11 – 3.96 (m, 1H), 3.83 (dd, *J* = 9.2, 6.4 Hz, 1H), 3.73 (dd, *J* = 9.2, 5.0 Hz, 1H), 3.24 (dt, *J* = 11.6, 5.9 Hz, 1H), 2.78 (dd, *J* = 12.3, 10.3 Hz, 1H), 2.73 – 2.68 (m, 1H), 2.61 – 2.54 (m, 1H), 2.33 (dd, *J* = 5.2, 2.7 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ 193.5, 158.4, 139.2, 133.9, 131.9, 130.6, 125.5, 122.6, 120.2, 120.1, 118.8, 118.6, 118.2, 110.5, 109.4, 81.9, 75.9, 70.8, 70.6, 62.8, 53.7, 44.1, 43.2, 37.4. IR (neat) 3566 (w), 2922 (w), 1737 (m), 1608 (m), 1155 (s), 748 (s). HRESI-MS (+) calcd. for $C_{24}H_{23}N_2O_6^+$ 435.1556, found 435.1575. $[\alpha]_D^{25}$ = -8.5˚ (*c* 0.32, CH_2Cl_2).

Synthesis of (*R*)-*N*-tosyl-2-chloromethylaziridine (14)

(*S*)-*N*-(3-chloro-2-hydroxypropyl)-4-methylbenzenesulfonamide (**23**)

To a well-stirred suspension of chloramine-T trihydrate (3.59 g, 12.8 mmol) in dry acetonitrile (20 mL) in a 50 mL round-bottomed flask fitted with a rubber septum was injected (*S*)-epichlorohydrin (2.36 g, 25.5 mmol). The mixture was warmed to 50 ˚C in an oil bath, and stirred vigorously for 24 h. Sat. $Na_2S_2O_3$ solution (5 mL) was added and the acetonitrile removed, then the wet residue was diluted with EtOAc (40 mL) and extracted with water (40 mL). The aqueous phase was extracted with further EtOAc (3×20 mL) and the combined organic fractions washed sequentially with sat. $Na_2S_2O_3$ solution (2×10 mL), water (2×10 mL) and brine (2×40 mL). The organic fraction was dried ($MgSO_4$) and the solvent removed to give a clear

syrup, which solidified upon standing to give a pale ivory solid. The resulting chlorohydrin **23** (3.25 g, 97%) was found to be >90% pure by NMR, and was used directly for subsequent steps. R_F (9:1 CH_2Cl_2:EtOAc) 0.25. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, *J* = 8.3 Hz, 2H, 2×ArH), 7.33 (d, *J* = 8.0 Hz, 2H, 2×ArH), 4.79 (t, *J* = 6.2 Hz, 1H, NH), 3.94 (dq, *J* = 10.7, 5.2 Hz, 1H, H2), 3.63 – 3.47 (m, 2H, CH_2Cl), 3.22 – 3.14 (m, 1H, H1a), 3.07 – 2.99 (m, 1H, H1b), 2.58 (d, *J* = 5.2 Hz, 1H, OH), 2.44 (s, 3H, $ArCH_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 143.9 (**Cq**-SO_2), 136.4 (**Cq**-CH_3), 130.1 (ArCH), 127.2 (ArCH), 70.0 (C2), 46.8 (C3), 45.9 (C1), 21.6 (Ts**C**H_3). ESI-MS (+) 286 (100%, [^{35}Cl]M+Na^+), 288 (33%, [^{37}Cl]M+Na^+]. $[\alpha]_D^{21}$ = -19.7 ˚ (*c* 0.64, CH_2Cl_2).

(*S*)-1-chloro-3-((4-methylphenyl)sulfonamido)propan-2-yl methanesulfonate (**24**)

To a solution of **23** (1.331 g, 5.05 mmol) and NEt_3 (1.1 mL, 7.9 mmol) in dry CH_2Cl_2 (30 mL) was added slowly dropwise methanesulfonyl chloride (0.58 mL, 7.5 mmol) at 0 ˚C, and the resulting pale golden solution allowed to warm to RT overnight with stirring. The mixture was partitioned with 1N HCl solution (10 mL) and the resulting organic phase washed with 1N HCl (2×20 mL), then sat. $NaHCO_3$ solution (1×20 mL), and the resulting solution dried ($MgSO_4$) and the solvent removed to give chloromesylate **24** (1.566 g, 91%) as a clear yellow oil. NMR showed the mesylate to be >90% pure, and was used directly for the next step. R_F (9:1 CH_2Cl_2:EtOAc) 0.62. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, *J* = 8.3 Hz, 2H, 2×ArH), 7.34 (d, *J* = 8.1 Hz, 2H 2×ArH), 4.93 (d, *J* = 6.5 Hz, 1H, NH), 4.89 – 4.80 (m, 1H, H2), 3.77 (d, *J* = 5.4 Hz, 2H, H1a, H1b), 3.68 (s, 3H, SO_2CH_3), 3.37 – 3.29 (m, 2H, H3a, H3b), 2.44 (s, 3H, $ArCH_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 144.2 (**Cq**-SO_2), 135.7 (**Cq**-CH_3), 130.2 (ArCH), 127.1 (ArCH), 78.9 (C2), 52.6 (Ms**C**H_3), 44.5 (C3), 43.1 (C1), 21.6 (Ts**C**H_3). ESI-MS (+) 364 (100%, [^{35}Cl]M+Na^+), 366 (33%, [^{37}Cl]M+Na^+]. $[\alpha]_D^{21}$ = +14.1˚ (*c* 0.22, CH_2Cl_2).

(*R*)-*N*-tosyl-2-chloromethylaziridine (**14**)

To a 0 ˚C solution of **24** (621 mg, 1.82 mmol) in dry CH_2Cl_2 (160 mL) was added pre-dried and ground cesium carbonate (976 mg, 3.00 mmol), and the mixture stirred vigorously for 2 h at 0 ˚C, then allowed to warm to room temperature overnight. The mixture was partitioned with water (100 mL), and the resulting organic layer separated and washed with brine (2×50 mL), dried ($MgSO_4$), and the solvent removed to give a clear, bluish oil. Flash chromatography (20 g silica, 20% EtOAc:pet spirit) gave the desired aziridine **14** (325 mg, 73%) as a colourless, viscous oil which slowly crystallised on standing, mp 42-44 ˚C. R_F (20% EtOAc:Pet Spirit) 0.40. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 7.82 (d, *J* = 8.0 Hz, 2H, H2', H6'), 7.33 (d, *J* = 8.0 Hz, 2H, H3', H5'), 3.39-3.50 (m, 2H, C**H**$_2$Cl), 3.04 (quint, *J* = 5.0 Hz, 1H, H2), 2.74 (d, *J* = 7.0 Hz, 1H, H1b) 2.43 (s, 3H, TsC**H**$_3$), 2.24 (d, *J* = 4.5 Hz, 1H, H1a). ^{13}C-NMR (125 MHz) δ 145.2 (Ar C1'), 134.6 (Ar C4'), 130.0 (Ar C2', C6'), 128.4 (Ar C3', C5'), 43.7 (**C**H_2Cl), 39.9 (C3), 33.1 (C2), 21.9 (Ts**C**H_3). ESI-MS (+) 268 (100%, [^{35}Cl]M+Na^+), 270 (33%, [^{37}Cl]M+Na^+]. $[\alpha]_D^{21}$ = +61.5˚ (*c* 0.71, CH_2Cl_2).

Products from the reaction of indigo with (*R*)-*N*-tosyl-2-chloromethylaziridine (14)

(*R*)-*N*-((13,14-dioxo-6,7,13,14-tetrahydropyrazino[1,2-*a*:4,3-*a*']diindol-6-yl)methyl)-4-methylbenzenesulfonamide (**15**)

A suspension of indigo (131 mg, 0.500 mmol) in anhydrous DMF (20 mL) was sonicated at 60 ˚C for one hour. The resulting hot suspension was transferred by cannula under positive pressure of nitrogen into a 50 mL round-bottomed flask containing a magnetic stir bar, pre-dried and ground Cs_2CO_3 (603 mg, 1.85 mmol), and fitted with a rubber septum. The resulting dark-green to amber solution was stirred for one hour in an oil bath, pre-heated to strictly 85-87 ˚C under dry nitrogen. The nitrogen flow was cut, and a solution of (*R*)-*N*-tosyl-2-chloromethylaziridine **14** (627 mg, 2.56 mmol) in DMF (2 mL) was injected through the septum, and the mixture stirred at 85-87 ˚C for 30 min. The resulting intensely-coloured solution was quenched over crushed ice (ca. 50 g), and the flask rinsed with EtOAc (ca. 10 mL) to remove adhered products. Upon warming to RT, the emulsion extracted with EtOAc (5×40 mL) until the aqueous phase became clear. The combined organic fractions were washed with brine (4×40mL), dried ($MgSO_4$), and filtered through a 2 cm plug of celite, and the solvent removed *in vacuo*. The crude residue was fractionated on silica (60 g) using $CHCl_3$ as eluent to afford two major fractions. Removal of the solvent from **Fraction 2** afforded the *N*,*N*-cyclised *mono*-adduct **15** (108.2 mg, 46%) as a glassy, intense-blue solid, mp 165-167 ˚C (dec.). R_F (9:1 $CHCl_3$:MeCN) 0.23. UV-VIS (CH_2Cl_2) λ_{max}/nm (ε, $M^{-1}cm^{-1}$) 236 (18847), 302 (14297), 380 (2329), 576 (8879). ^{1}H NMR (500 MHz, DMSO) δ 8.07 (d, *J* = 6.0 Hz, 1H, NH), 7.66 – 7.59 (m, 6H, 2×TsH, 4×ArH), 7.32 (d, *J* = 7.8 Hz, 3H, 2×TsH, ArH), 7.25 (d, *J* = 8.3 Hz, 1H, ArH), 7.05 (dd, *J* = 14.5, 7.2 Hz, 2H, 2×ArH), 4.67 (s, 1H, H6), 4.43 (d, *J* = 12.3 Hz, 1H, H7a), 3.71 (d, *J* = 9.4 Hz, 1H, H7b), 3.08 – 2.96 (m, 1H, CH_2a), 2.93 – 2.83 (m, 1H, CH_2b), 2.32 (s, 3H, $TsCH_3$). ^{13}C NMR (126 MHz, DMSO) δ 180.43, 180.40, 151.1, 149.6, 143.5, 138.0, 136.0, 135.9, 130.5, 130.3, 127.1, 126.9, 124.6, 124.5, 124.3, 124.2, 123.6, 122.9, 122.4, 121.6, 121.4, 121.3, 111.6, 110.9, 106.9, 88.1, 73.8, 50.5, 50.4, 21.5. IR (neat) 3566 (w), 2987 (w), 1737 (w), 1697 (m), 1609 (s), 1159 (s), 1132 (s) 754 (s). HR-ESI-MS calcd. for $C_{26}H_{21}N_3O_4SNa^+$ 472.1331, found 472.1319. $[\alpha]_D^{25}$ = -9.3˚ (*c* 0.41, $CHCl_3$).

Fraction 1 was concentrated to dryness then dissolved in acetonitrile (20.0 mL), of which 10.0 mL was subjected to preparative RP-HPLC over 5×2.0 mL injections, using a Prominence Preparatory Liquid Chromatograph (Shimadzu) with PDA detector, and a Shim-Pack GIS C18 5 μm column (Shimadzu) with 150×20 mm I.D. Gradient elution from 80% to 0% Solvent A (0.1% TFA in H_2O) in 60 minutes with Solvent B (0.1% TFA in MeCN) at a flow rate of 10.0 mL/min afforded three major fractions at T_R = 35 min, 37 min, and 47.7 min, and their purity was confirmed by analytical RP-HPLC, using a Prominence LC-2030C 3D system (Shimadzu) with PDA detector, and a Shim-Pack GIS C18 5 μm column (Shimadzu) with 4.6×150 mm I.D. Gradient elution from 80% to 0% Solvent A (0.1% TFA in H_2O) in 30 minutes with

Solvent B (0.1% TFA in MeCN) at a flow rate of 1.0 mL/min showed the three fractions (T_R = 16.5, 17.2, and 21.4 min, respectively) to be free of neighbouring impurities.

N-(((*R*)-13,14-dioxo-6,7,13,14-tetrahydropyrazino[1,2-*a*:4,3-*a*']diindol-6-yl)methyl)-4-methyl-*N*-(((*S*)-1-tosylaziridin-2-yl)methyl)benzenesulfonamide (**16**)

Removal of the solvent from the fraction collected at T_R = 35 min afforded the *di*-substituted *N,N'*-cyclised indigo derivative **16** (27.4 mg, 8%) as an intense blue-purple solid, mp 121-123 ˚C (dec.). R_F (9:1 $CHCl_3$:MeCN) 0.492. UV-Vis (CH_2Cl_2) λ_{max}/nm (ε, $M^{-1}cm^{-1}$) 281 (37018), 360 (2022), 578 (7659). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 – 7.71 (m, 2H, 2×ArH), 7.60 (d, *J* = 8.1 Hz, 2H, 2×TsH), 7.57 – 7.48 (m, *J* = 8.0 Hz, 4H, 2×TsH, 2×ArH), 7.29 (d, *J* = 8.1 Hz, 2H, 2×TsH), 7.21 (d, *J* = 8.0 Hz, 2H, 2×TsH), 7.16 (s, 2H, 2×ArH), 7.02 (s, 2H, 2×ArH), 4.63 (s, 1H, H6), 4.47 (d, *J* = 10.8 Hz, 1H, H7a), 3.64 (d, *J* = 15.8 Hz, 1H, H4'a), 3.51 – 3.37 (m, 1H, H7b), 3.31 – 3.19 (m, 1H, H6a*a*), 3.05 – 2.95 (m, 1H, H4'b), 2.95 – 2.86 (m, 2H, H6a*b*, H3'), 2.57 (d, *J* = 6.9 Hz, 1H, H2'a), 2.38 (d, *J* = 3.1 Hz, 6H, 2×$TsCH_3$), 2.21 (d, *J* = 4.1 Hz, 1H, H2'b). ^{13}C NMR (126 MHz, DMSO) δ 180.4, 180.1, 150.9, 149.0, 145.4, 144.5, 143.1, 135.7, 133.9, 130.6, 130.3, 130.1, 128.0, 127.7, 127.0, 124.1, 121.4, 111.6, 110.9, 60.6, 54.4, 49.5, 48.9, 47.3, 40.8, 21.5, 21.4. IR (neat) 2987 (w), 1738 (w), 1697 (m), 1609 (s), 1132 (s), 754 (s). HRESI-MS calcd. for $C_{36}H_{32}N_4O_6S_2Na^+$ 703.1655 found 703.1661. $[\alpha]_D^{25}$ = -12.6˚ (*c* 0.11, $CHCl_3$).

N-(3-chloro-2-((4-methylphenyl)sulfonamido)propyl)-*N*-(((*R*)-13,14-dioxo-6,7,13,14-tetrahydropyrazino[1,2-*a*:4,3-*a*']diindol-6-yl)methyl)-4-methylbenzenesulfonamide (**17**)

Removal of the solvent from the fraction collected at T_R = 37 min afforded the *di*-substituted *N,N'*-cyclised indigo derivative **17** (33.3 mg, 9%) as an intense blue-purple solid, mp 132-134 ˚C (dec.). R_F (9:1 $CHCl_3$:MeCN) 0.493. UV-Vis (CH_2Cl_2) λ_{max}/nm (ε, $M^{-1}cm^{-1}$) 574 (8850). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.82 (d, *J* = 7.9 Hz, 2H), 7.75 (d, *J* = 7.3 Hz, 2H), 7.54 (d, *J* = 8.2 Hz, 3H), 7.49 (t, *J* = 7.7 Hz, 1H), 7.36 (d, *J* = 7.9 Hz, 2H), 7.27 (s, 1H), 7.12 (d, *J* = 8.1 Hz, 1H), 7.01 (dt, *J* = 22.7, 7.6 Hz, 3H), 5.69 (s, 1H), 4.60 (d, *J* = 10.3 Hz, 1H), 4.39 (d, *J* = 12.1 Hz, 1H), 4.00 (s, 1H), 3.86 (d, *J* = 11.6 Hz, 1H), 3.57 – 3.37 (m, 4H), 3.19 (dd, *J* = 15.1, 6.8 Hz, 1H), 2.65 (d, *J* = 14.3 Hz, 1H), 2.42 (s, 3H), 2.38 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 181.2, 180.5, 150.7, 148.4, 145.2, 144.3, 137.3, 135.7, 133.2, 130.4, 130.2, 127.7, 127.4, 125.7, 125.2, 125.1, 123.0, 121.8, 121.6, 110.7, 108.9, 77.2, 53.9, 53.1, 51.0, 49.5, 45.4, 40.2, 21.8, 21.7. IR (neat) 3013 (w), 1743 (w), 1608 (m), 1304 (m), 756 (s). HRESI-MS calcd. for $C_{36}H_{33}N_4O_6S_2{}^{35}ClNa^+$ 739.1428 found 739.1448. $[\alpha]_D^{25}$ = -11.4˚ (*c* 0.12, $CHCl_3$)

(±)-*N*-((13,14-dioxo-6,7,13,14-tetrahydropyrazino[1,2-*a*:4,3-*a*']diindol-6-yl)methyl)-4-methylbenzenesulfonamide (±**15**)

Prepared following ***General Procedure A***, using (±)-*N*-tosyl-2-bromomethylaziridine (1.00 g, 3.45 mmol), and a 5 min reaction time.* The crude residue was fractionated on silica (80 g) using 5% MeCN:$CHCl_3$ as eluent. Removal of the solvent from **Fraction B** afforded the *N*,*N*-cyclised *mono*-adduct **±15** (247.2 mg, 52%) as a glassy, intense-blue solid. Spectral and physical characteristics (excepting optical rotation) were identical to those reported above for the *R*-isomer.

Fraction A was condensed and assessed by analytical RP-HPLC (80%-0% H_2O:MeCN; 1.0 mL/min), which showed the presence of small amounts of **11**, though this was not quantified.

Reaction of indigo with *N*-Tosyl-2-phenylaziridine (18)

(±)-*N*-tosyl-2-phenylaziridine (**18**)†

To a solution of chloramine-T trihydrate (8.46 g, 30 mmol) and iodine (381 mg, 1.50 mmol, 5 mol%) in dry acetonitrile (90 mL) was added styrene (3.6 mL, 31.6 mmol) with stirring under nitrogen at RT. The flask was wrapped in aluminium foil, and stirred vigorously under static nitrogen for 2 days. The resulting orange mixture was filtered through a thin pad of celite, eluted with acetonitrile (3×20 mL), and the filtrate extracted with pet. spirit (3×50 mL) to remove unreacted styrene. The solvent was removed *in vacuo*, then the residue dissolved in ethyl acetate (100 mL) and washed with sat. $Na_2S_2O_3$ solution (2×40 mL) and brine (50 mL), dried ($MgSO_4$) and the solvent removed. The resulting crystalline solid was triturated in hexane (50 mL) and collected by vacuum filtration to give phenylaziridine **18** (7.66 g, 93%) as off-white crystals. Spectral and physical characteristics were identical to those reported previously.[4]

(*E*)-*N*-(2-(3,3'-dioxo-[2,2'-biindolinylidene]-1-yl)-1-phenylethyl)-4-methylbenzenesulfonamide (19)

Prepared following ***General Procedure A***, using phenylaziridine **18** (1.363 g, 4.99 mmol) and a 5 min reaction time. The crude residue was fractionated on silica (60 g) by sequential elution with 1: 10% pet. spirit:CH_2Cl_2, 2: CH_2Cl_2, and 3: 10% EtOAc:CH_2Cl_2 to afford two major fractions. The solvent was removed from **Fraction 1** to give a dark blue residue, which was re-dissolved in a minimum of hot $CHCl_3$, and precipitated with a tenfold volume of pet. spirit. The resulting solid was collected on a Hirsch funnel, and dried *in vacuo* to give the terminally ring-opened aziridine adduct **19** (333.4 mg, 62%) as a dark teal powder, mp 211-213 ˚C R_F (10% Pet Spirit:CH_2Cl_2) 0.43. UV-Vis (CH_2Cl_2) λ_{max}/nm (ε, $M^{-1}cm^{-1}$) 294 (24346), 341 (9142), 645 (13774). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.65 (s, 1H), 7.72 (d, *J* = 7.6 Hz, 1H), 7.64 (d, *J* = 7.5 Hz, 1H), 7.57 (t, *J* = 5.9 Hz, 3H), 7.50 (t, *J* = 7.4 Hz, 1H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.32 (t,

* Longer reaction times typically led to extensive polymerisation

† Adapted from a literature procedure, with modifications made for easier scalability. ^{1}H and ^{13}C NMR spectra are reported in the SI.

J = 7.3 Hz, 1H), 7.28 – 7.19 (m, 2H), 7.06 (t, *J* = 7.4 Hz, 1H), 7.04 – 6.94 (m, 3H), 6.72 (d, *J* = 7.9 Hz, 2H), 5.23 – 5.11 (m, 1H), 4.96 – 4.85 (m, 1H), 3.91 (s, 1H), 2.22 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 189.0, 188.8, 151.8, 151.3, 142.7, 139.7, 138.1, 137.0, 136.2, 129.2, 128.3, 126.8, 126.1, 125.5, 125.0, 124.1, 121.4, 120.9, 120.8, 120.0, 112.0, 110.2, 77.2, 55.6, 51.9, 21.6. IR (neat) 3288 (w), 1630 (m), 1607 (s), 1026 (s). HRESI-MS calcd. for $C_{31}H_{26}N_3O_4S^+$ = 536.1644, found 536.1664.

(*E*)-*N*-(2-(3,3'-dioxo-[2,2'-biindolinylidene]-1-yl)-2-phenylethyl)-4-methylbenzenesulfonamide (**20**)

Fraction 2 was condensed to give a dark blue-green residue, which was dissolved in a minimum of hot $CHCl_3$, then diluted with a tenfold volume of hot pet. spirit. The solution was chilled, and the resulting precipitate collected by vacuum filtration and dried *in vacuo* to give the title compound **20** (191.9 mg, 36%) as a dark blue powder, mp 194-196 ˚C. X-ray quality crystals were grown by gradual diffusion of pet spirit into a saturated solution in 1:1 $CHCl_3$:MeOH in a sealed vapour chamber at room temperature. R_F (10% pet spirit:CH_2Cl_2) 0.31. UV-Vis (CH_2Cl_2) λ_{max}/nm (ε, $M^{-1}cm^{-1}$) 292 (23907), 340 (7457), 637 (9167). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.74 (s, 1H, N1'H), 7.64 (dd, *J* = 15.8, 7.6 Hz, 2H, H4, H4'), 7.50 (t, *J* = 8.2 Hz, 3H, 2×TsH, 1×ArH), 7.29 – 7.16 (m, 7H, 7×ArH), 7.02 (d, *J* = 8.0 Hz, 1H, ArH), 6.94 (dt, *J* = 14.6, 8.0 Hz, 5H, 3×ArH, 2×TsH), 6.57 (dd, *J* = 17.5, 8.1 Hz, 2H, ArH, H2''), 5.89 (s, 1H, C1''NH), 4.11 (dd, *J* = 17.1, 11.2 Hz, 2H, C1''H_2), 2.26 (s, 3H, TsCH_3). ^{13}C NMR (126 MHz, $CDCl_3$) δ 189.3, 188.2, 151.8, 150.0, 143.1, 137.2, 136.9, 136.7, 135.4, 129.5, 129.0, 128.0, 126.7, 126.7, 125.9, 125.3, 124.2, 122.4, 121.3, 121.1, 120.1, 114.1, 112.1, 77.2, 60.0, 43.4, 21.6. IR (neat) 3254 (w), 1628 (m), 1605 (s), 1069 (s). HRESI-MS calcd. for $C_{31}H_{25}N_3O_4SNa^+$ = 558.1463, found 558.1481.

Reaction of indigo with 2,2'-biaziridine

4-methyl-*N*-(((6*R*,7*R*)-7-((4-methylphenyl)sulfonamido)-14,15-dioxo-7,8,14,15-tetrahydro-6*H*-[1,4]diazepino[1,2-*a*:4,3-*a*']diindol-6-yl)methyl)benzenesulfonamide (**21**)

Prepared following ***General Procedure A*** using (2*R*,2'*R*)-*N,N'*-ditosyl-2,2'-biaziridine (512 mg, 1.30 mmol) in DMF (3 mL) over a 20 min reaction time. The crude residue was dissolved in a minimum of hot $CHCl_3$, and gradual dilution with a tenfold volume of hot pet. spirit precipitated an intense dark blue powder. The suspension was cooled to 0 ˚C overnight, and the fine dark blue precipitate was collected on a Hirsch funnel and dried *in vacuo* to give the 1,3-*di*-ring-opened biaziridine adduct **21** (610 mg, 93%) as an intense dark blue-black powder, mp 135-136˚ (dec.). R_F (10% EtOAc:CH_2Cl_2) 0.29. UV-Vis (CH_2Cl_2) λ_{max}/nm (ε, $M^{-1}cm^{-1}$) 573 (6647). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (d, *J* = 8.1 Hz, 2H, 2×TsH), 7.56 (t, *J* = 7.6 Hz, 1H, H10), 7.48 (t, *J* = 8.4 Hz, 3H, 2×TsH, 1×ArH), 7.34 (d, *J* = 8.2 Hz, 3H, 2×TsH, 1×ArH), 7.18 (t, *J* = 8.6 Hz, 3H, 2×TsH, 1×ArH), 7.01 (dd, *J* = 14.8, 7.3 Hz, 1H, ArH), 6.94 (t, *J* = 9.1 Hz, 3H, 2×TsH, 1×ArH), 6.69 (d, *J* = 5.9 Hz, 1H, C7NH), 5.71 (s, 1H, C6aNH), 4.95 (t, *J* = 4.7 Hz, 1H, H6), 4.63 (d, *J* = 12.9 Hz, 1H, H8a), 3.62 (d, *J* = 8.1 Hz, 1H, H8b), 3.14 – 2.98 (m, 1H, C6aHa), 2.83 (d, *J* = 14.8 Hz, 1H, C6aHb),

2.35 (s, 3H, $TsCH_3$), 2.23 (s, 3H, $TsCH_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 180.9, 180.8, 149.9, 149.3, 144.1, 143.8, 136.0, 135.4, 130.1, 130.0, 129.7, 128.1, 127.2, 127.1, 126.8, 125.0, 124.5, 124.2, 123.0, 122.2, 121.8, 121.5, 110.3, 110.1, 54.3, 52.5, 42.4, 39.4, 21.7, 21.6. IR (neat) 3253 (w), 1741 (w), 1697 (w), 1609 (m), 1304 (m), 745 (s). HRESI-MS calcd. for $C_{34}H_{30}N_4O_6S_2Na^+$ = 677.1499, found 677.1513. $[\alpha]_D^{29}$ = -15.0 ˚ (*c* 0.04, $CHCl_3$)

Biological testing

The antiplasmodial activity of the experimental compounds was assessed against *Plasmodium falciparum* 3D7 (chloroquine sensitive) and Dd2 (drug resistant) parasite strains. Preliminary cytotoxicity assessment was carried out using Human Embryonic Kidney (HEK293) cells, sourced from the American Type Culture Collection (ATCC, Manassas, VA, USA). The compounds were tested in a 22-point concentration-response range of 80 µM – 0.01 nM (160 µM – 0.02 nM for Compound **10**). Artesunate, dihydroartemisinin (DHA), chloroquine, puromycin and pyrimethamine were used as reference compounds / drugs, and were tested in both experiments using 22-point concentration-response range of 40 µM – 0.01 nM (10 µM – 0.003 nM for DHA and artesunate). 0.4% DMSO and 5 µM puromycin were used as negative and positive in-plate controls, respectively.

Antimalarial assay. *Plasmodium falciparum* 3D7 (chloroquine sensitive) and Dd2 (drug resistant) parasite strains were maintained in RPMI 1640 supplemented with 25 mM HEPES, 5% AB human male serum, 2.5 mg/ml Albumax II, and 0.37 mM hypoxanthine.

Parasites were subjected to two rounds of sorbitol synchronization before undergoing compound treatment. Ring stage parasites at 2% parasitemia and 0.3% hematocrit were exposed to the experimental compounds in 384-wells imaging CellCarrier microplates (PerkinElmer), as previously described.[5] Plates were incubated for 72 h at 37 °C, 90% N_2, 5% CO_2, 5% O_2, parasites then stained with 2-(4-amidinophenyl)-1*H*-indole-6-carboxamidine (DAPI) in permeabilization buffer (PBS, 5mM EDTA, 0.5ug/ml DAPI, 0.01% Triton X-100, and 0.001% saponin), and imaged with an Opera QEHS micro-plate confocal imaging system (PerkinElmer) using 20x water-immersion objective and 405 nm excitation, 450/50 nm emission filters. Images were analysed using a custom Acapella spot detection script, to quantify DAPI-stained parasites in each well, as previously described.[5]

Cytotoxicity Assay. Human Embryonic Kidney cells (HEK293) were maintained in DMEM medium supplemented with 10% FBS. Cells were seeded at 2,000 cells/well in TC-treated 384-wells clear-bottom plates (Greiner), 24h before the addition of compounds. Compounds were added to the plates as described above, then the plates were incubated for 72h at 37 °C, 5% CO_2. At the end of the incubation, the media was removed from the wells and replaced with an equal volume of 44 µM resazurin. After an additional 5-

6 hours incubation at standard conditions, the total fluorescence (excitation/emission: 530 nm / 595 nm) was measured using an Envision plate reader (PerkinElmer).

Raw data from each assay was normalized using the in-plate positive and negative controls to obtain normalized % inhibition data, which was then used to calculate IC_{50} values, through a 4 parameter logistic curve fitting in Prism v 6.0 (GraphPad).

The experiments were carried out in two biological replicates, each consisting of two technical repeats.

NMR data for isolated compounds

(*S*)-6-(hydroxymethyl)-6,7-dihydropyrazino[1,2-*a*:4,3-*a*']diindole-13,14-dione (8)

Figure 1: ^{1}H spectrum of compound **8**

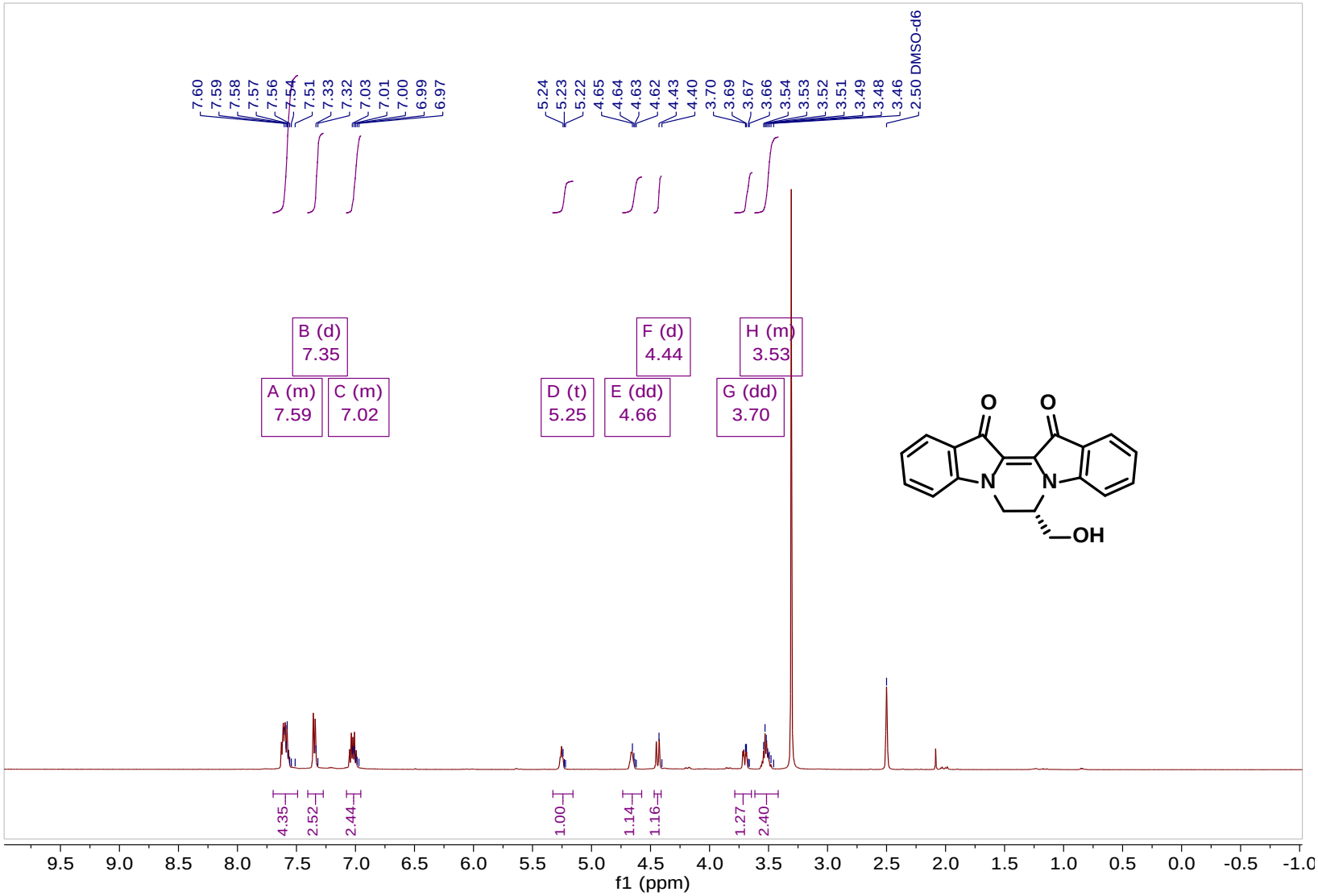


Figure 2: ^{13}C spectrum of compound **8**

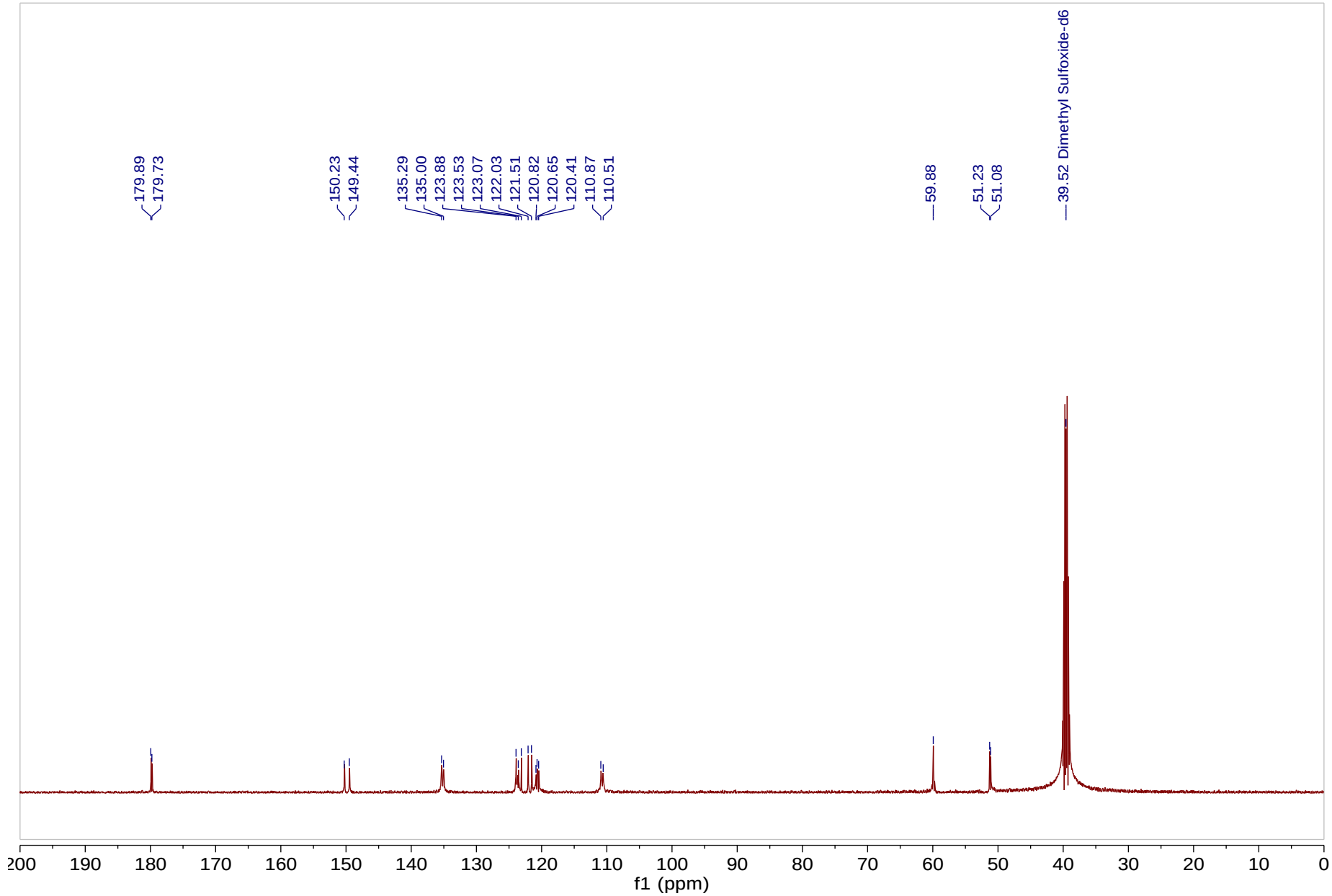


Figure 3: gCOSY spectrum of compound **8**

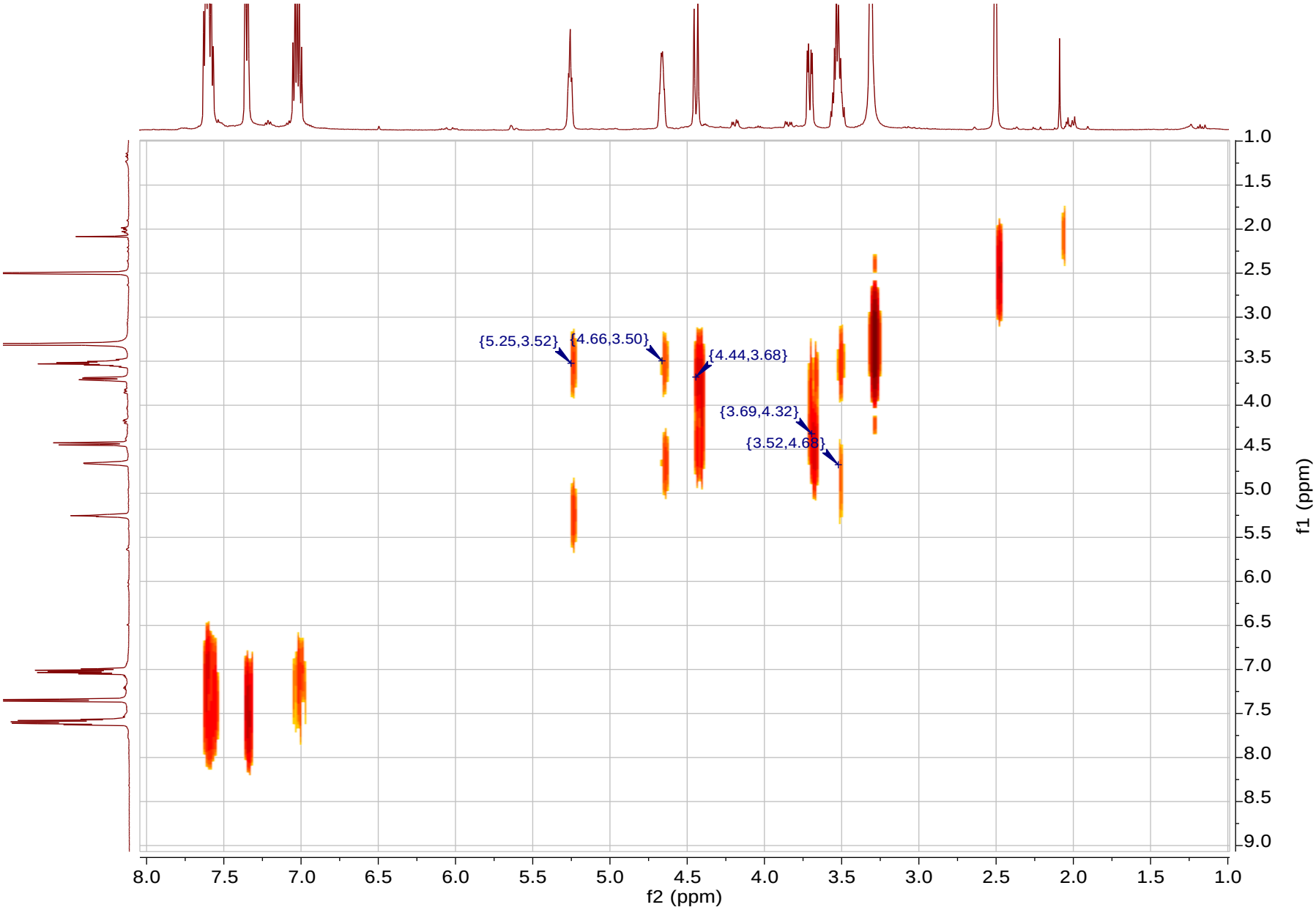


Figure 4: NOESY spectrum of compound **8**

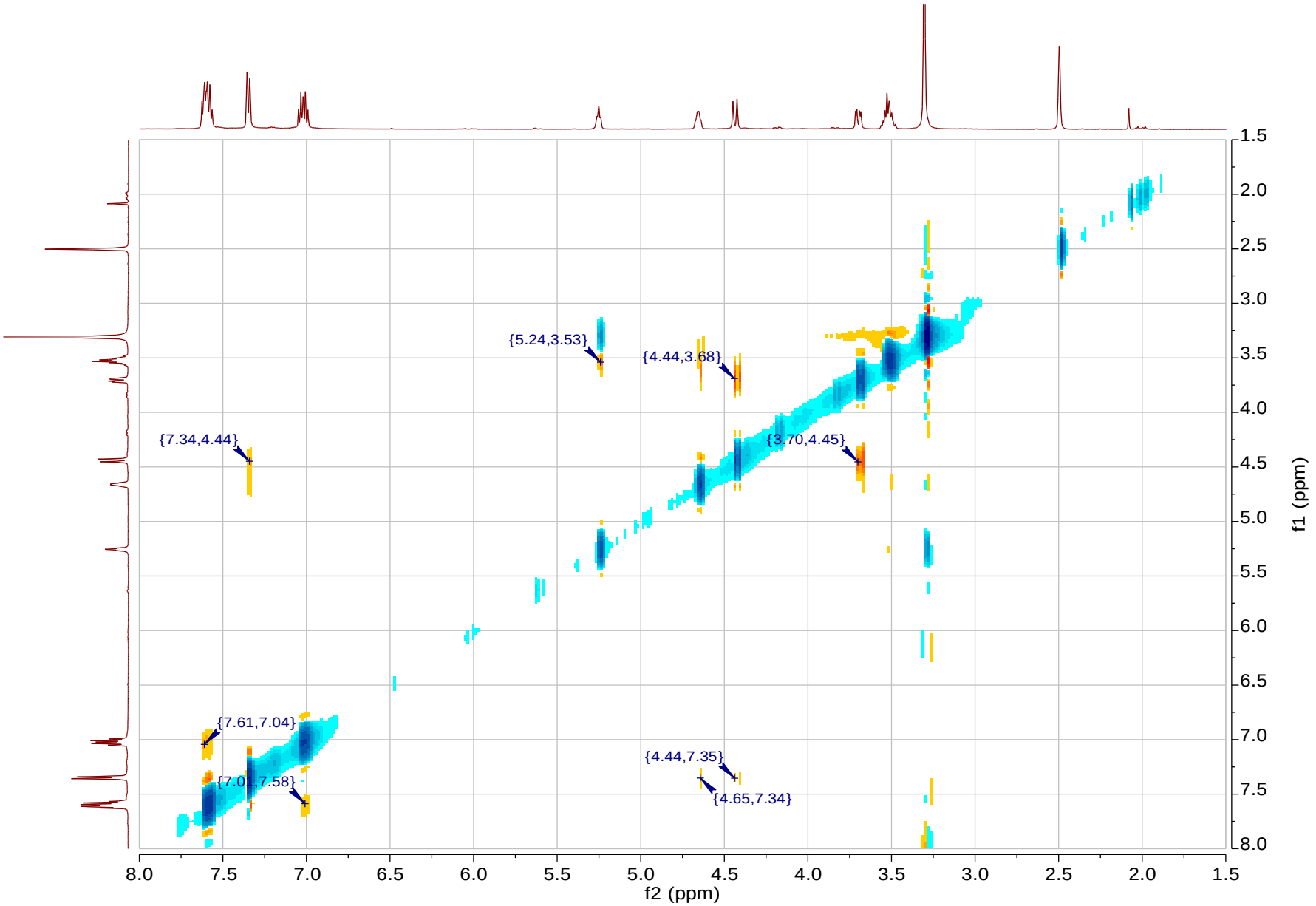
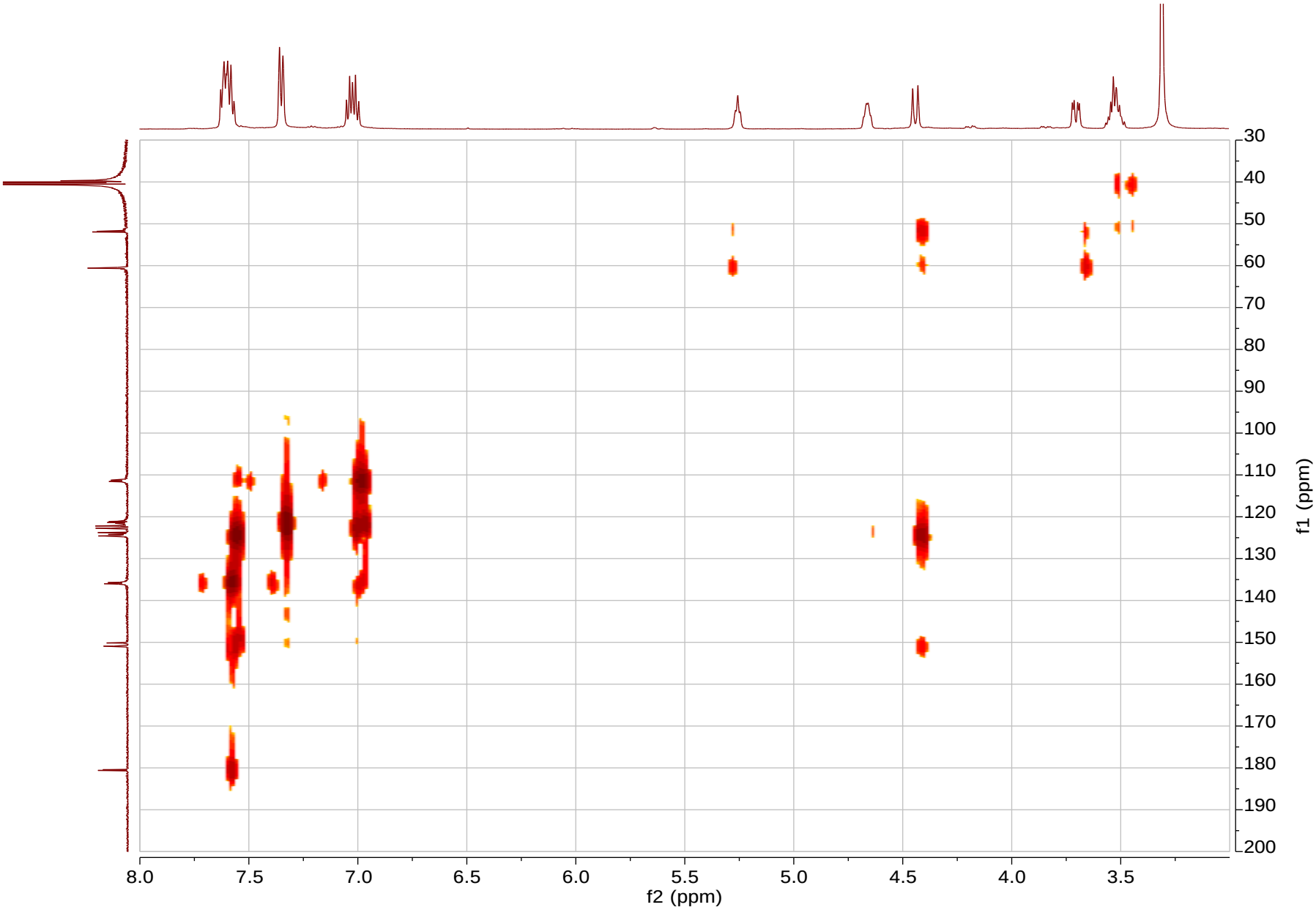


Figure 5: gHMBC spectrum of compound **8**



(7*S*,9a*R*)-7,8-dihydro-6*H*-7,9a-epoxy[1,5]oxazocino[5,4-*a*:3,2-*b*']diindol-15(14*H*)-one (9)

Figure 6: ^{1}H spectrum of compound **9**

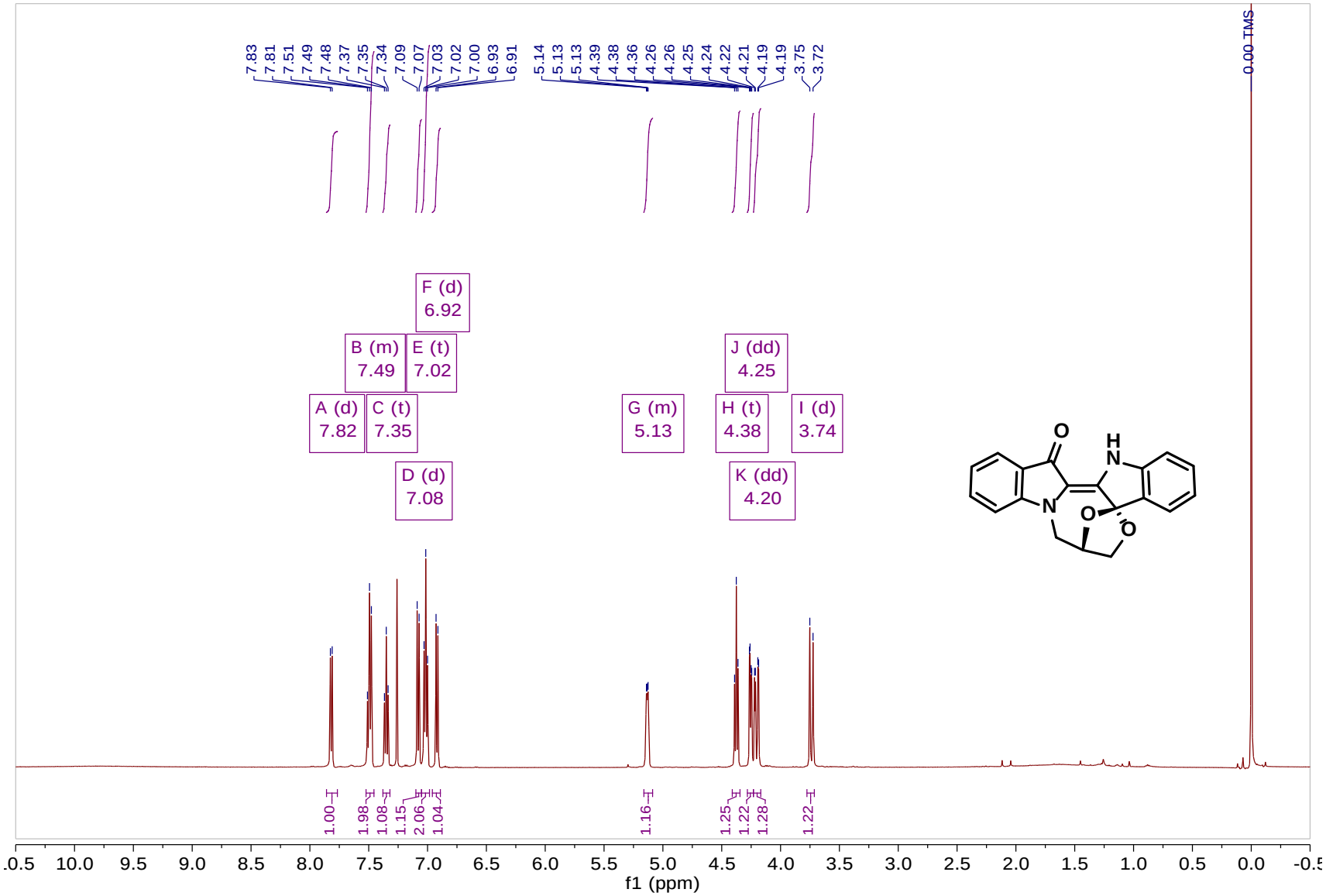


Figure 7: ^{13}C spectrum of compound **9**

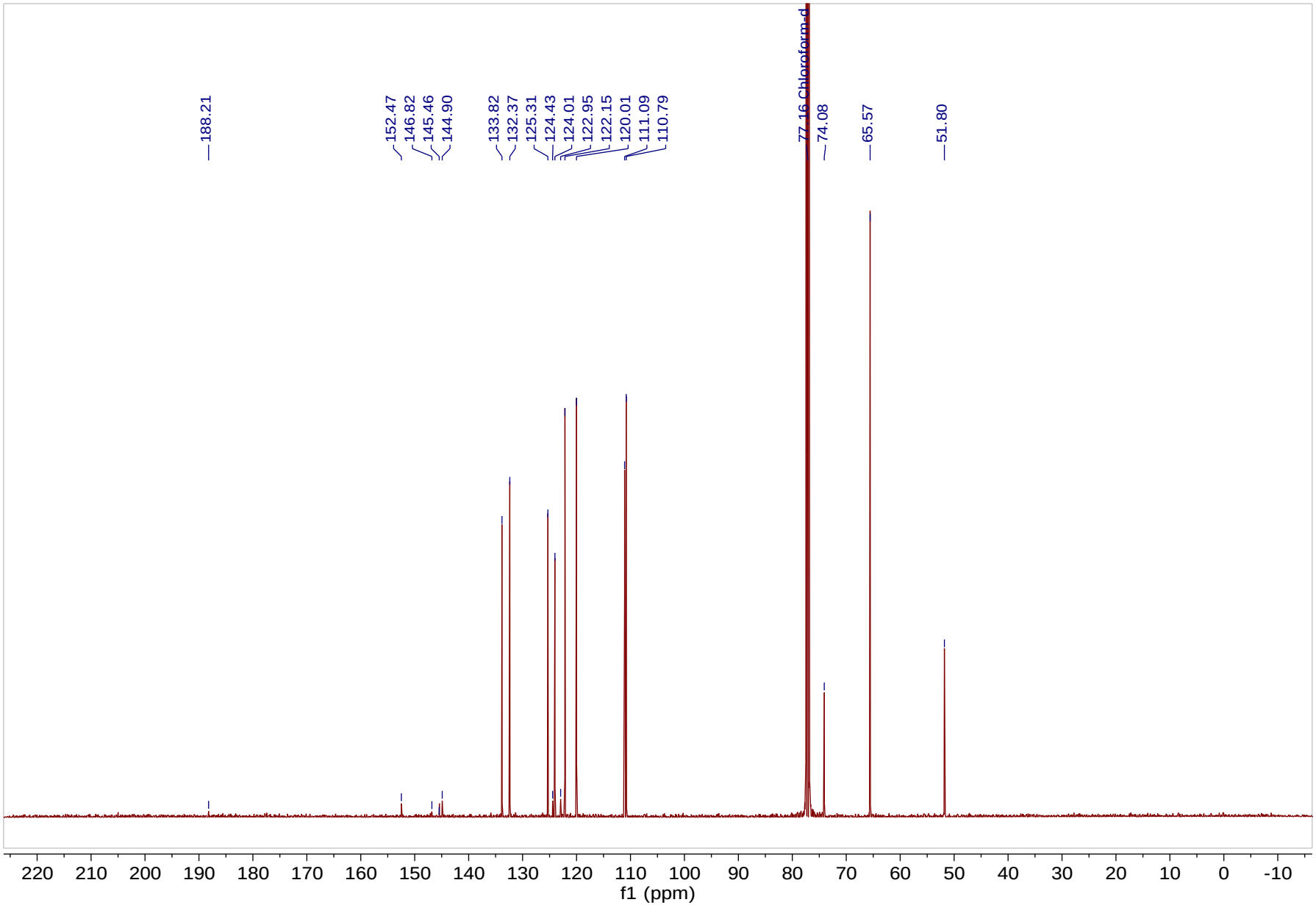


Figure 8: gCOSY spectrum of compound **9**

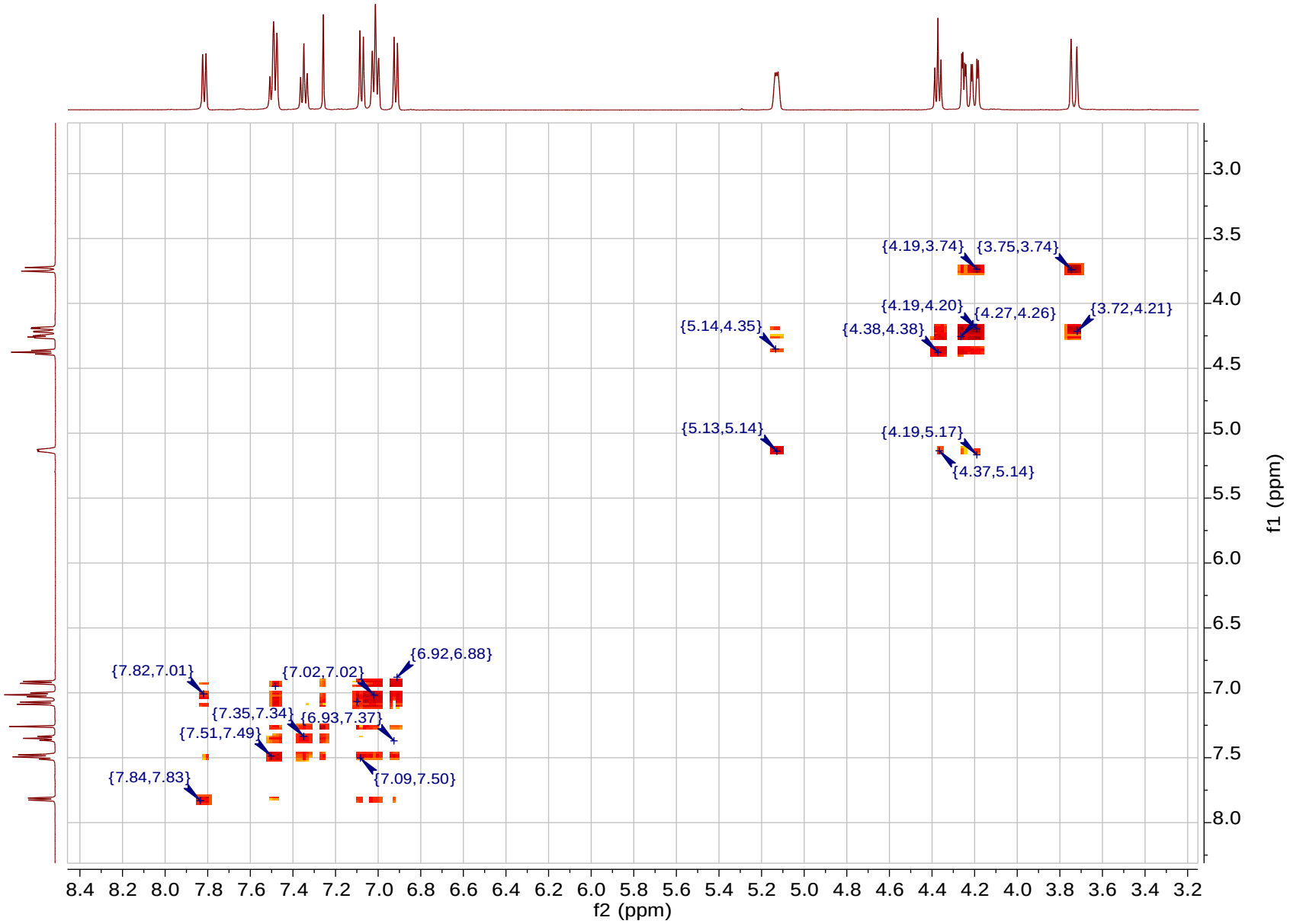


Figure 9: gHSQC spectrum of compound **9**

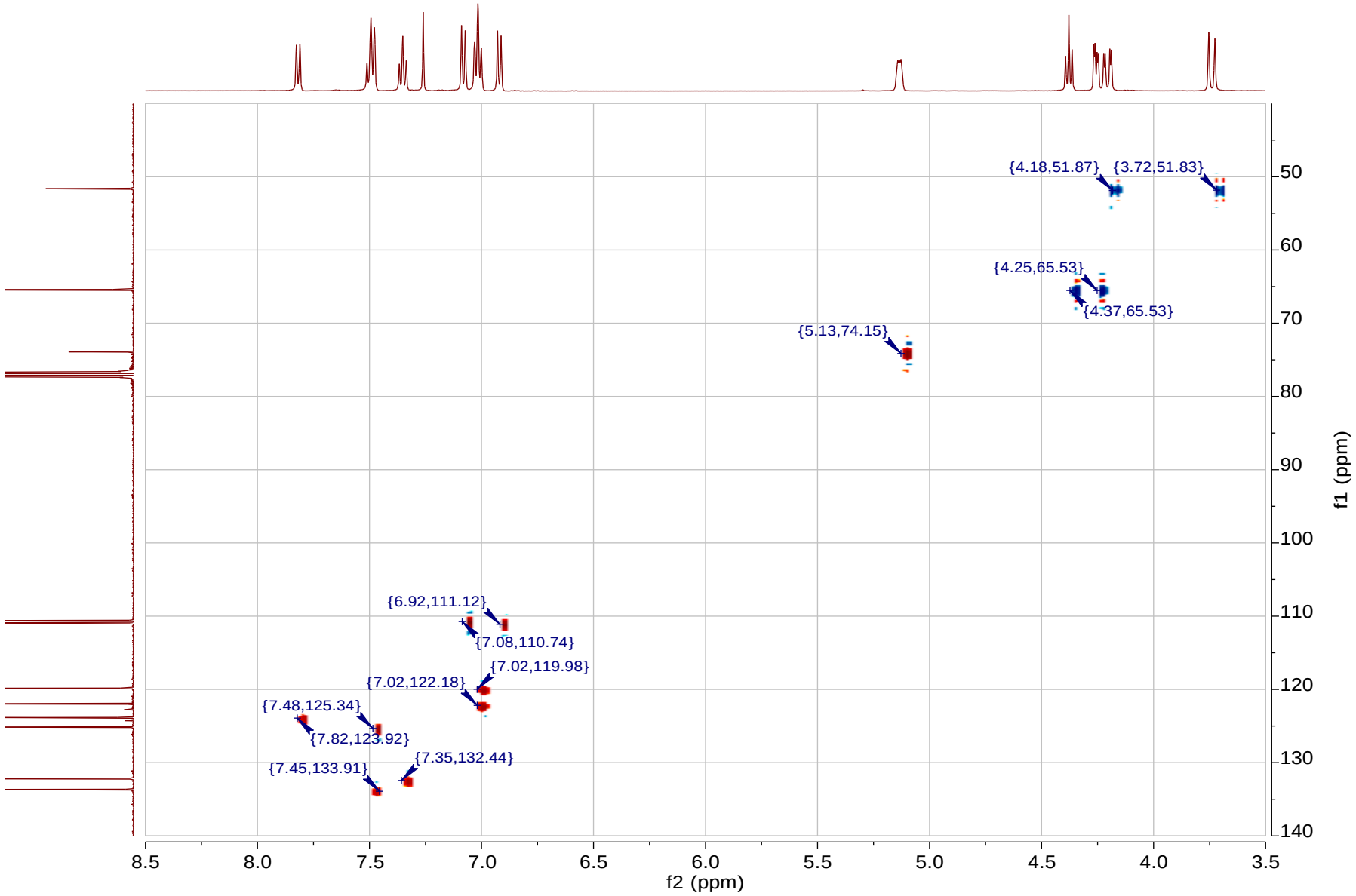


Figure 10: gHMBC spectrum of compound **9**

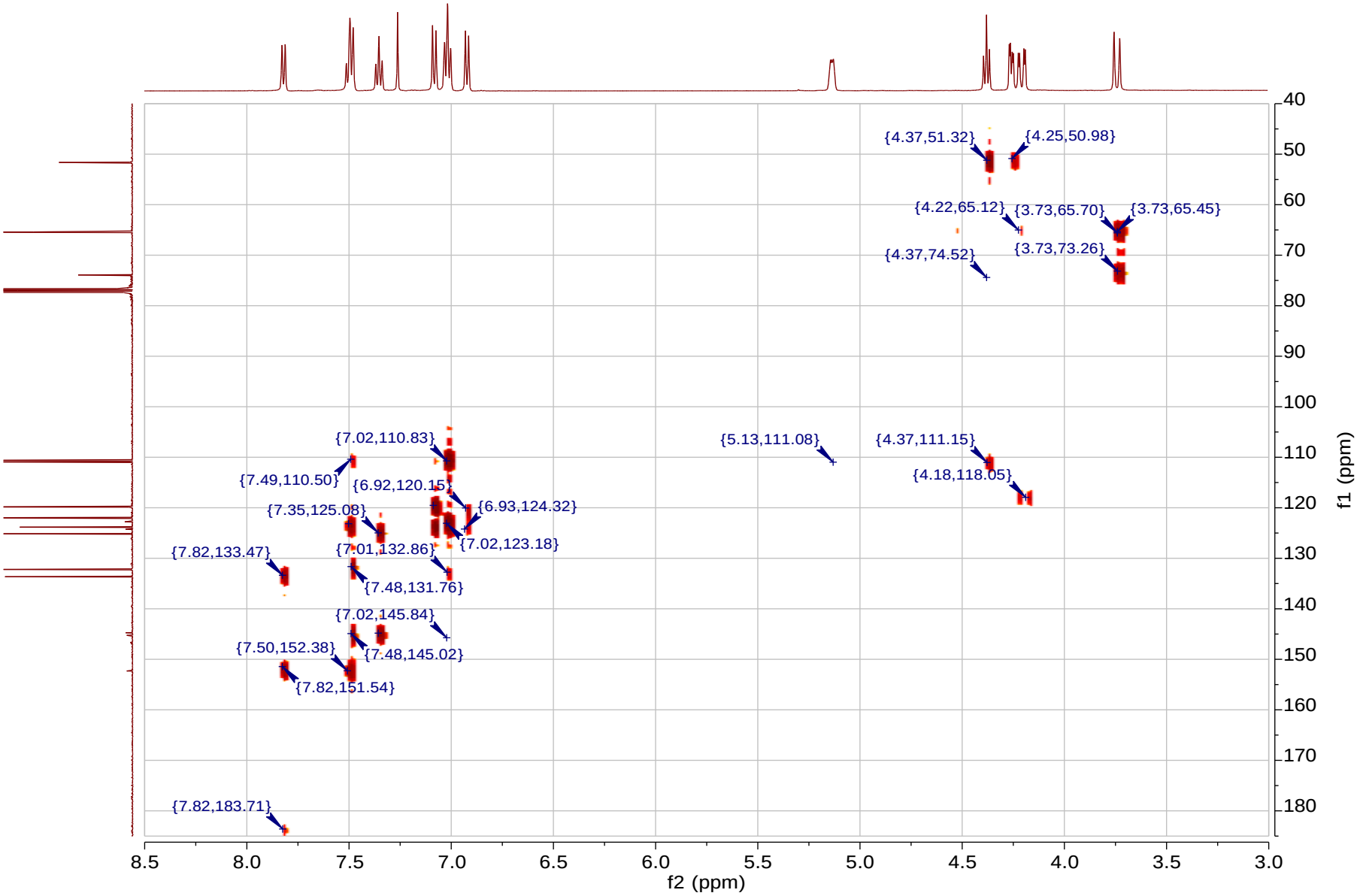
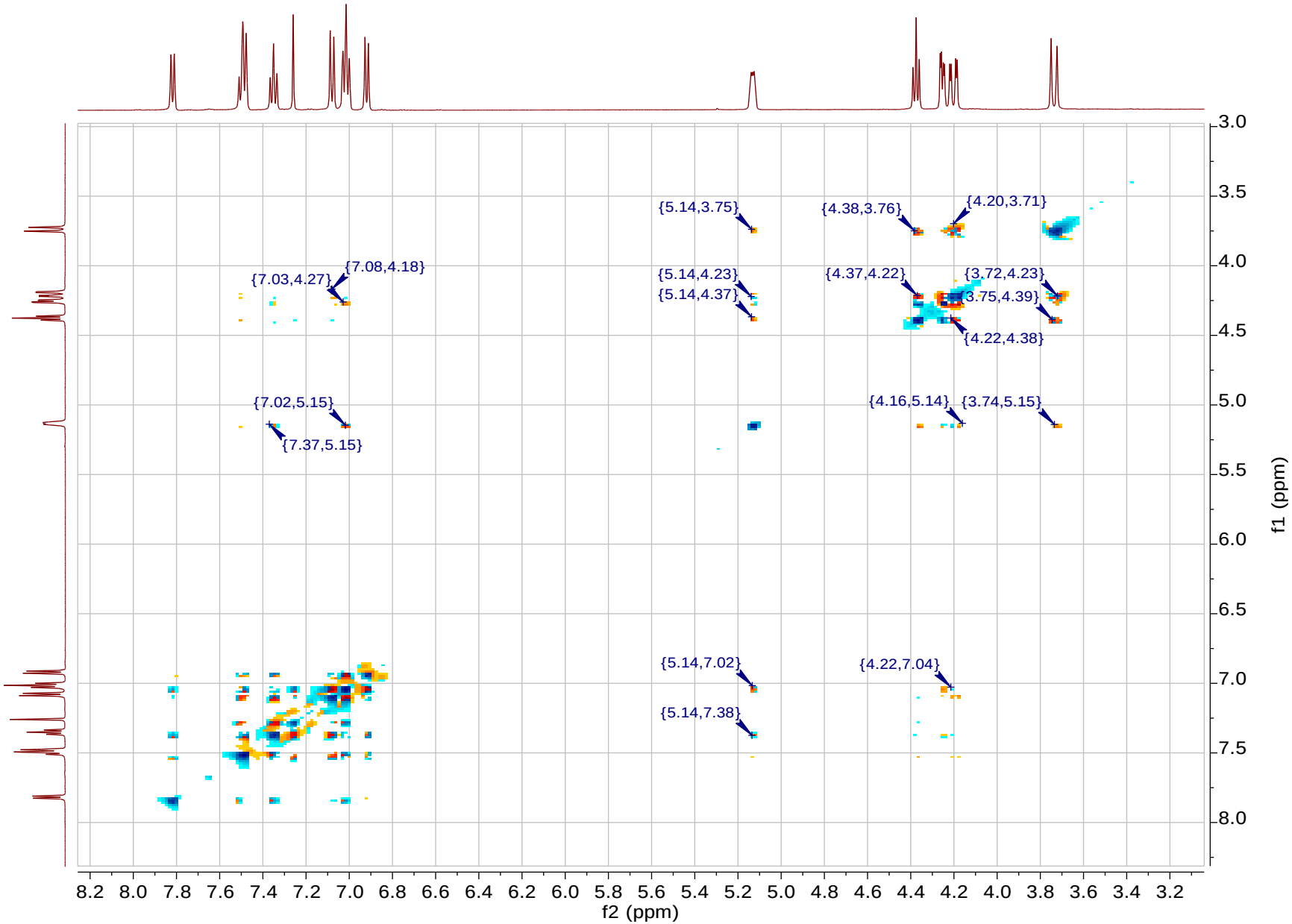


Figure 11: NOESY spectrum of compound **9**



(7*R*,9a*R*)-14-((*E*)-3-hydroxyprop-1-en-1-yl)-7,8-dihydro-6*H*-7,9a-epoxy[1,5]oxazocino[5,4-*a*:3,2-*b'*]diindol-15(14*H*)-one (10)

Figure 12: ^{1}H spectrum of compound **10.** The presence of the grease contaminant in the 1.0 – 2.0 ppm region of the spectrum could not be removed, despite repeated attempts.

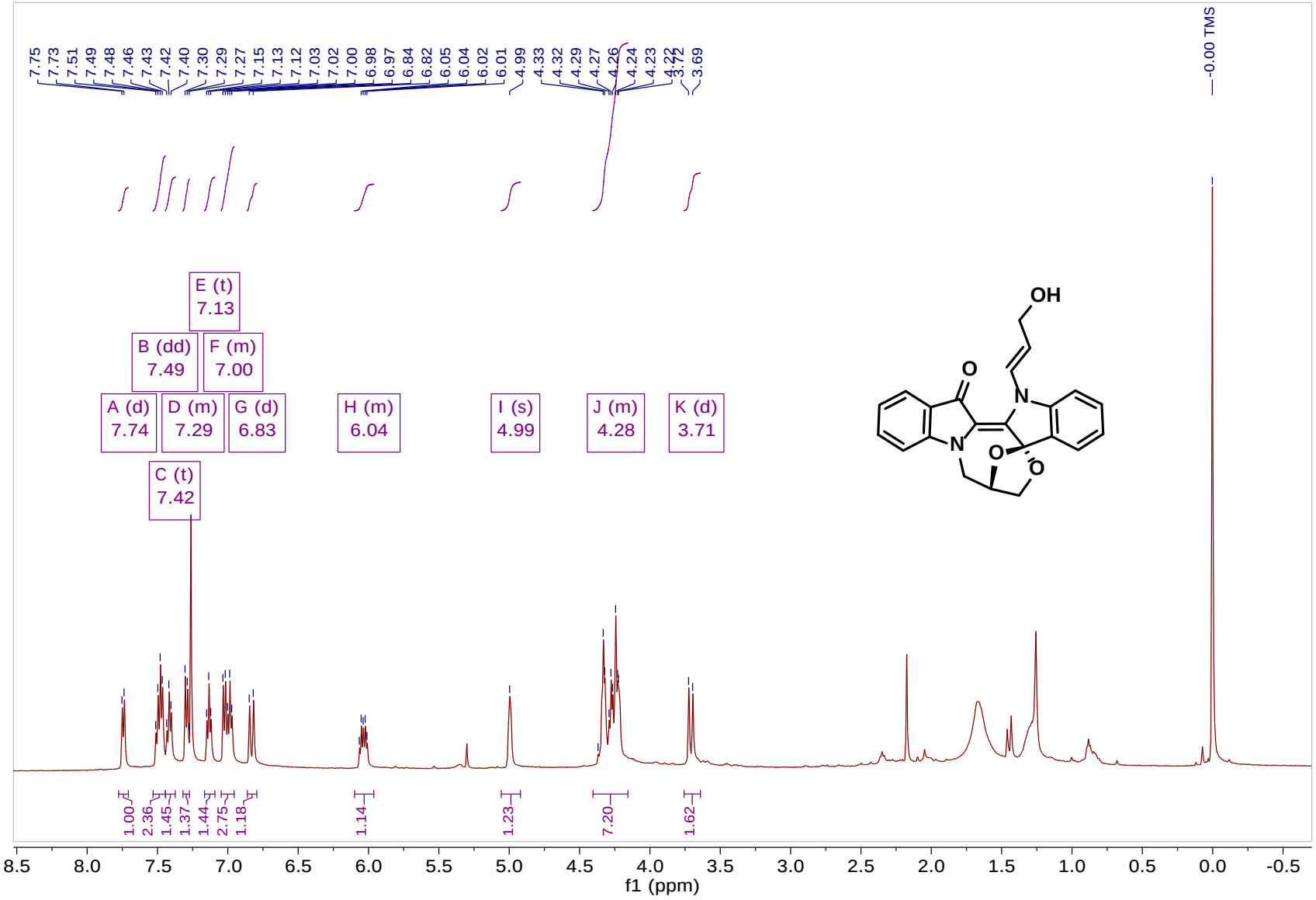


Figure 13: ^{13}C spectrum of compound **10**

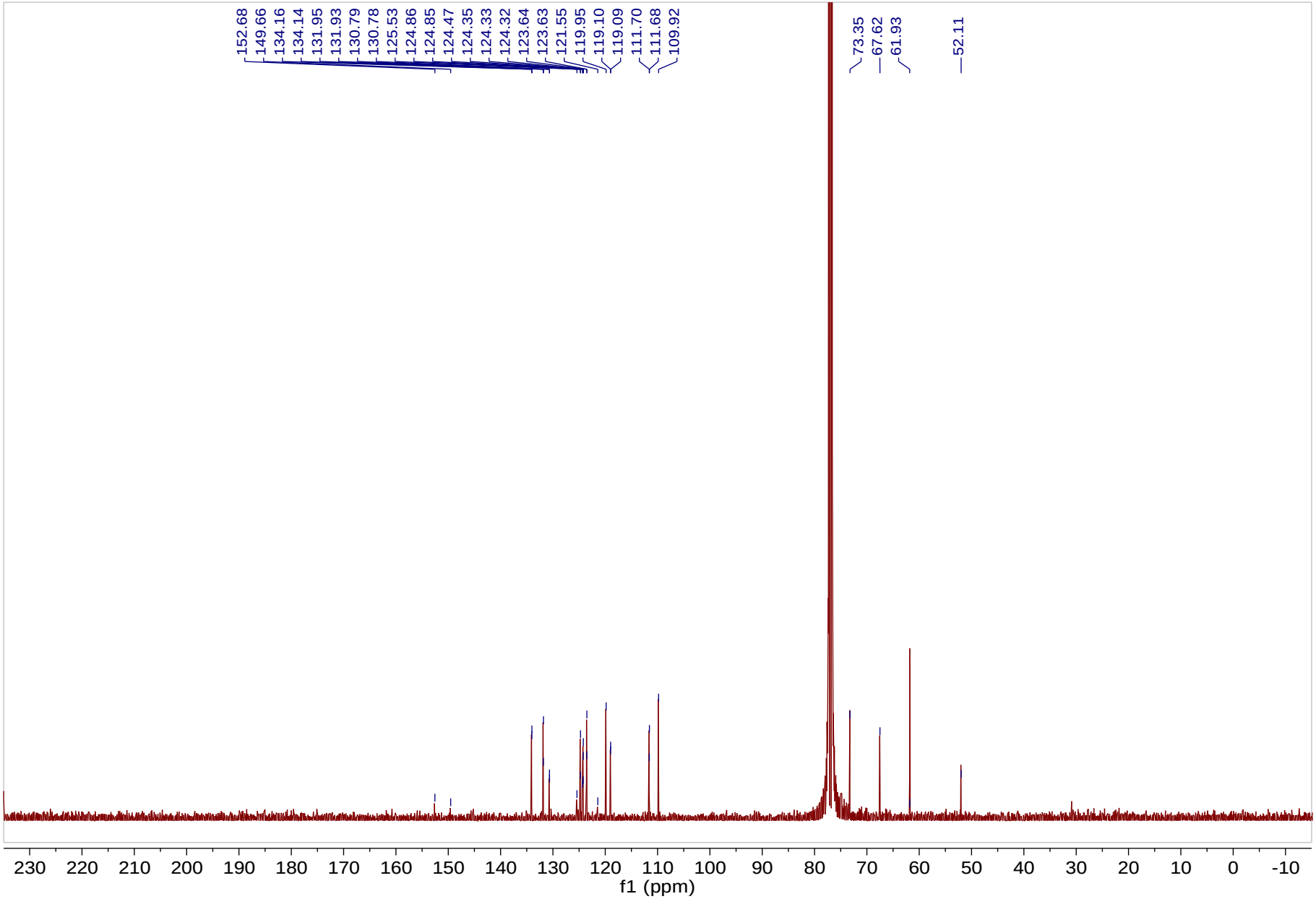


Figure 14: gCOSY spectrum of compound **10**

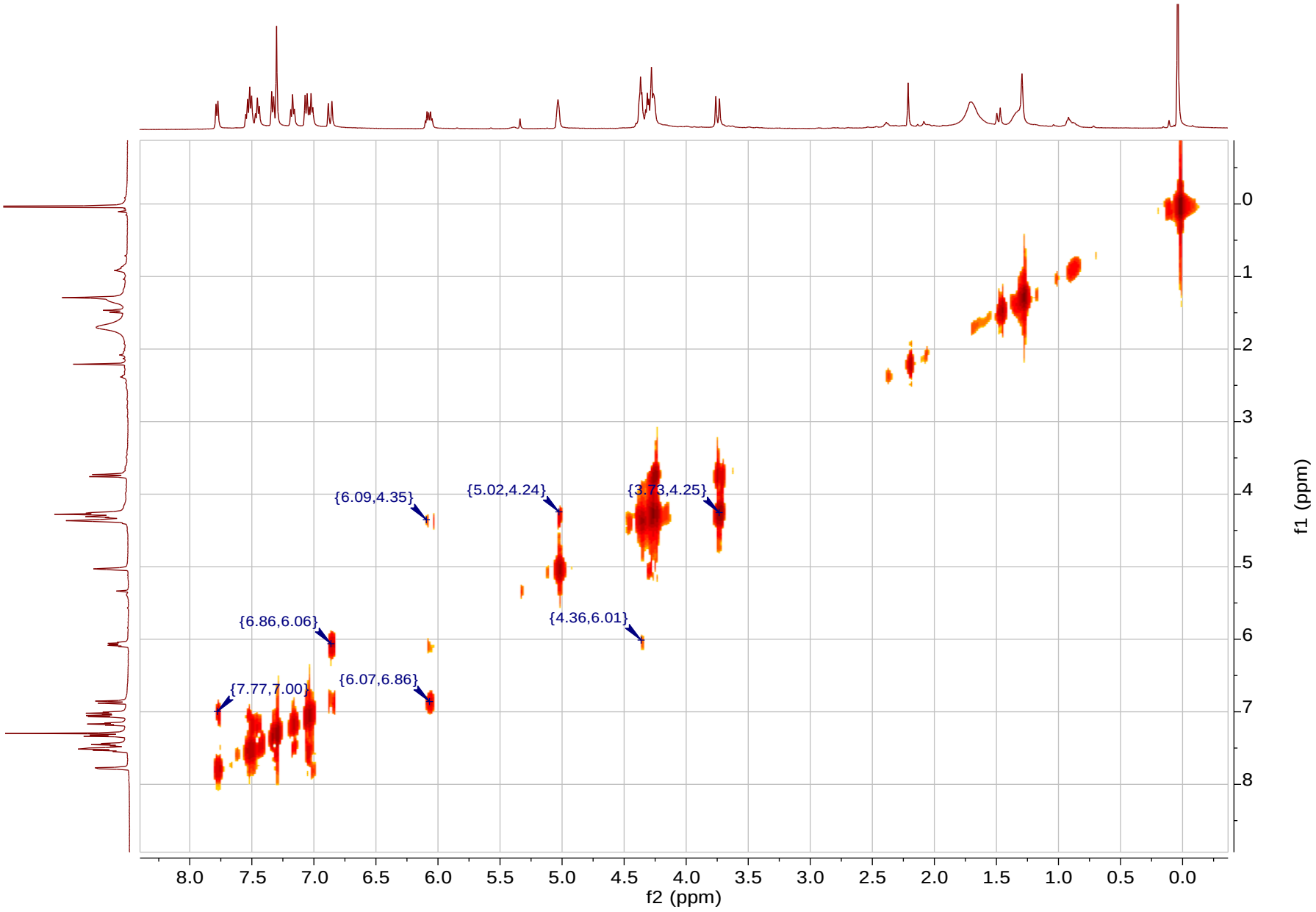


Figure 15: gHMBC spectrum of compound **10**

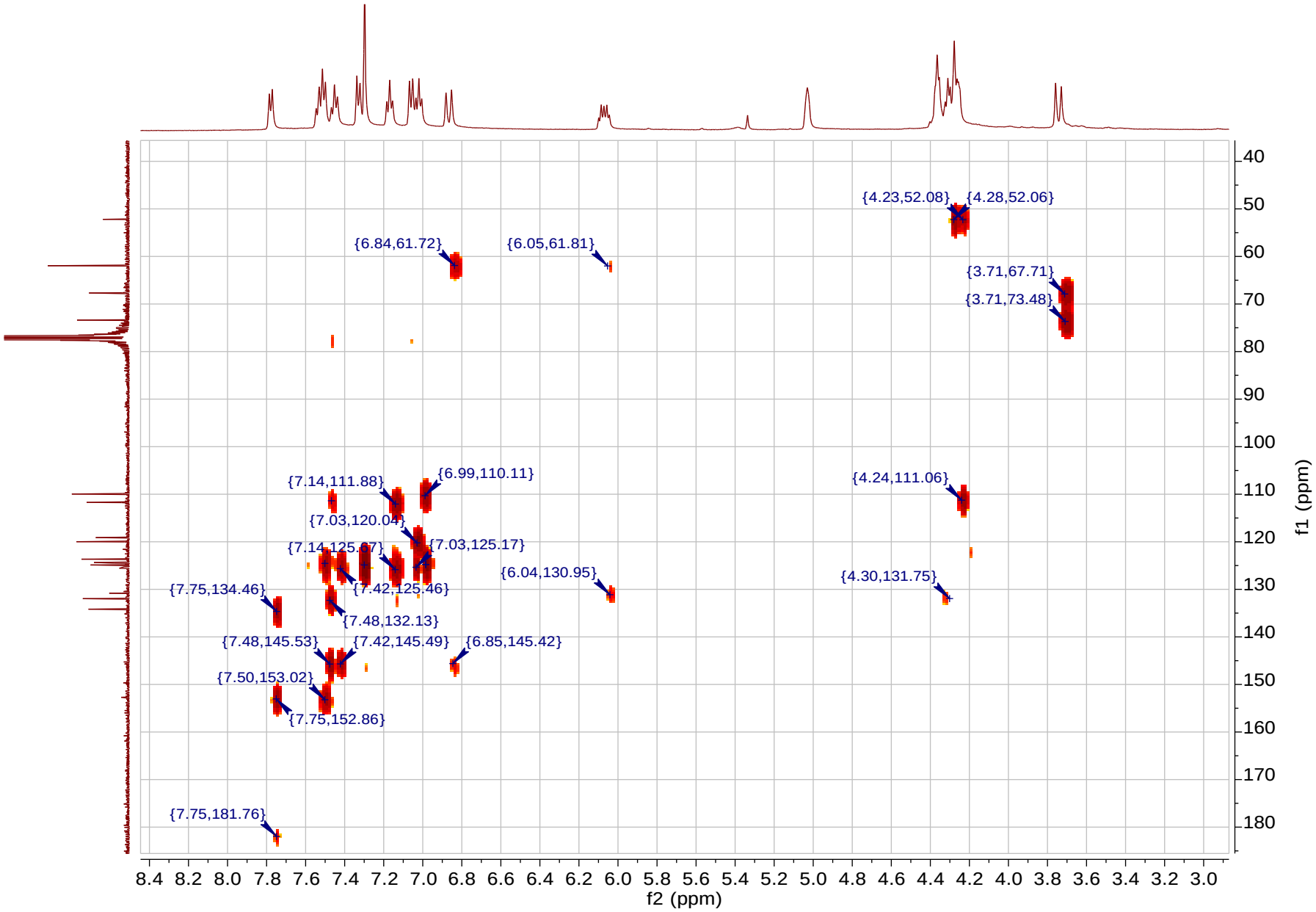


Figure 16: gHSQC spectrum of compound **10**

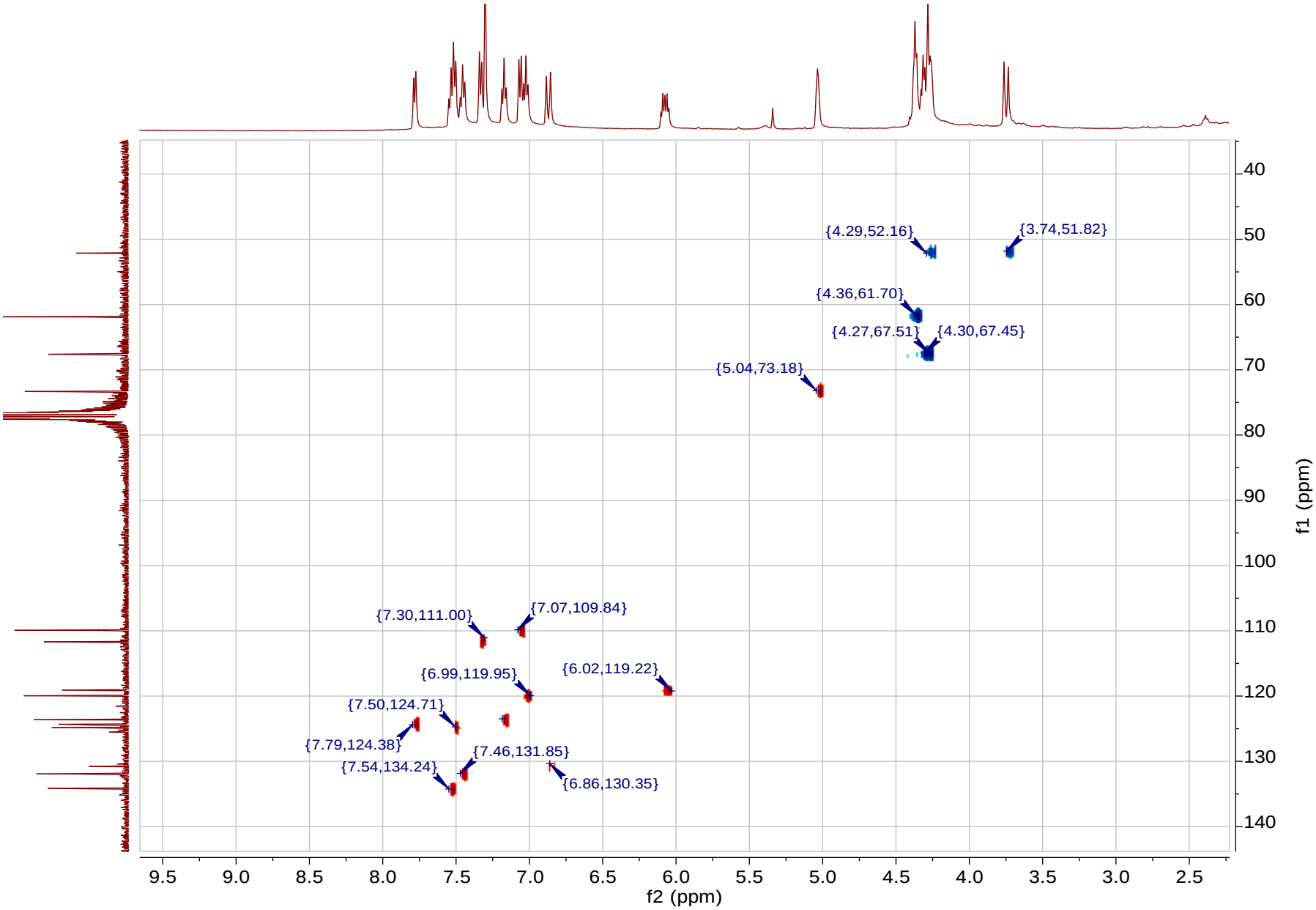


Figure 17: ROESY spectrum of compound **10**

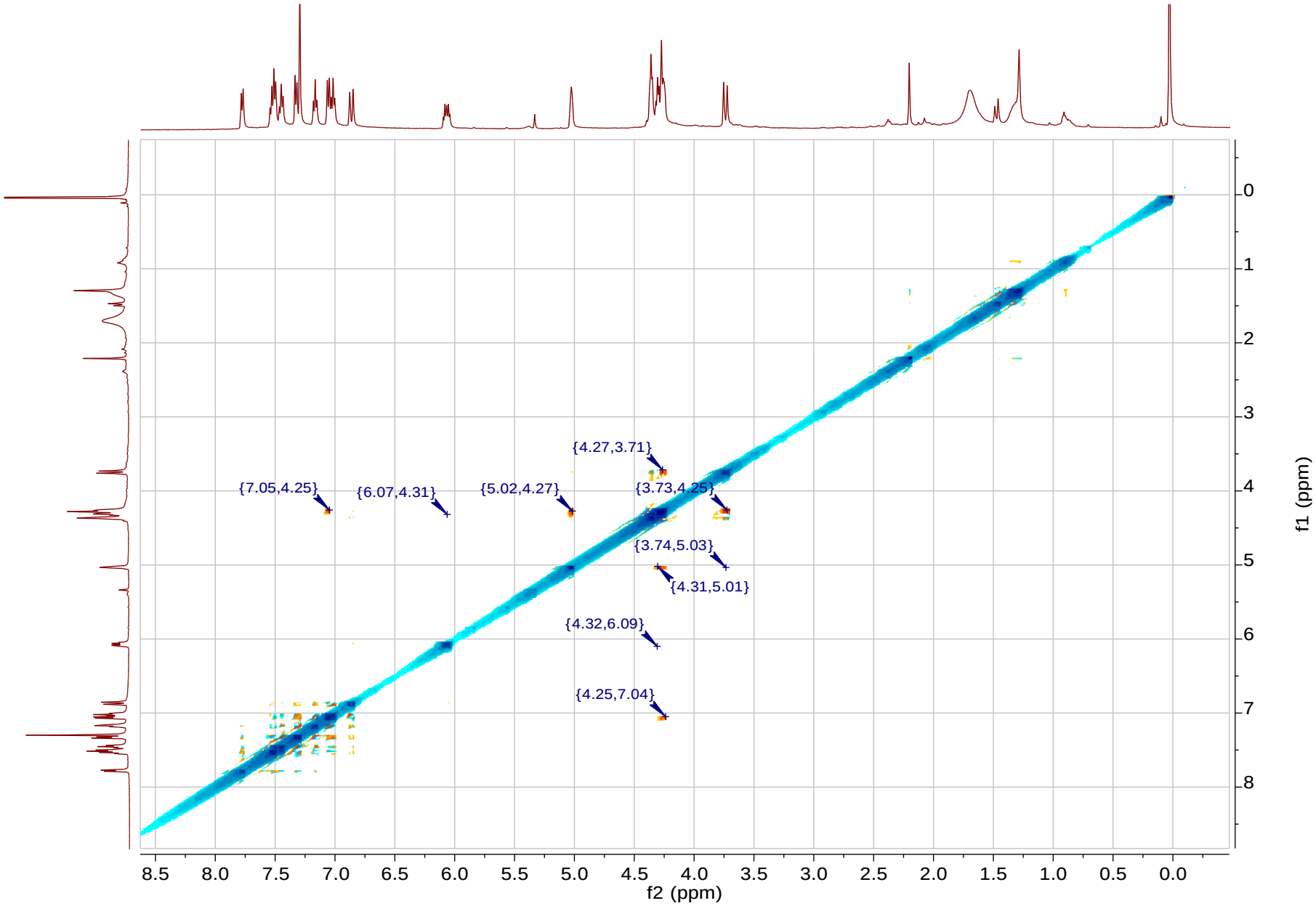
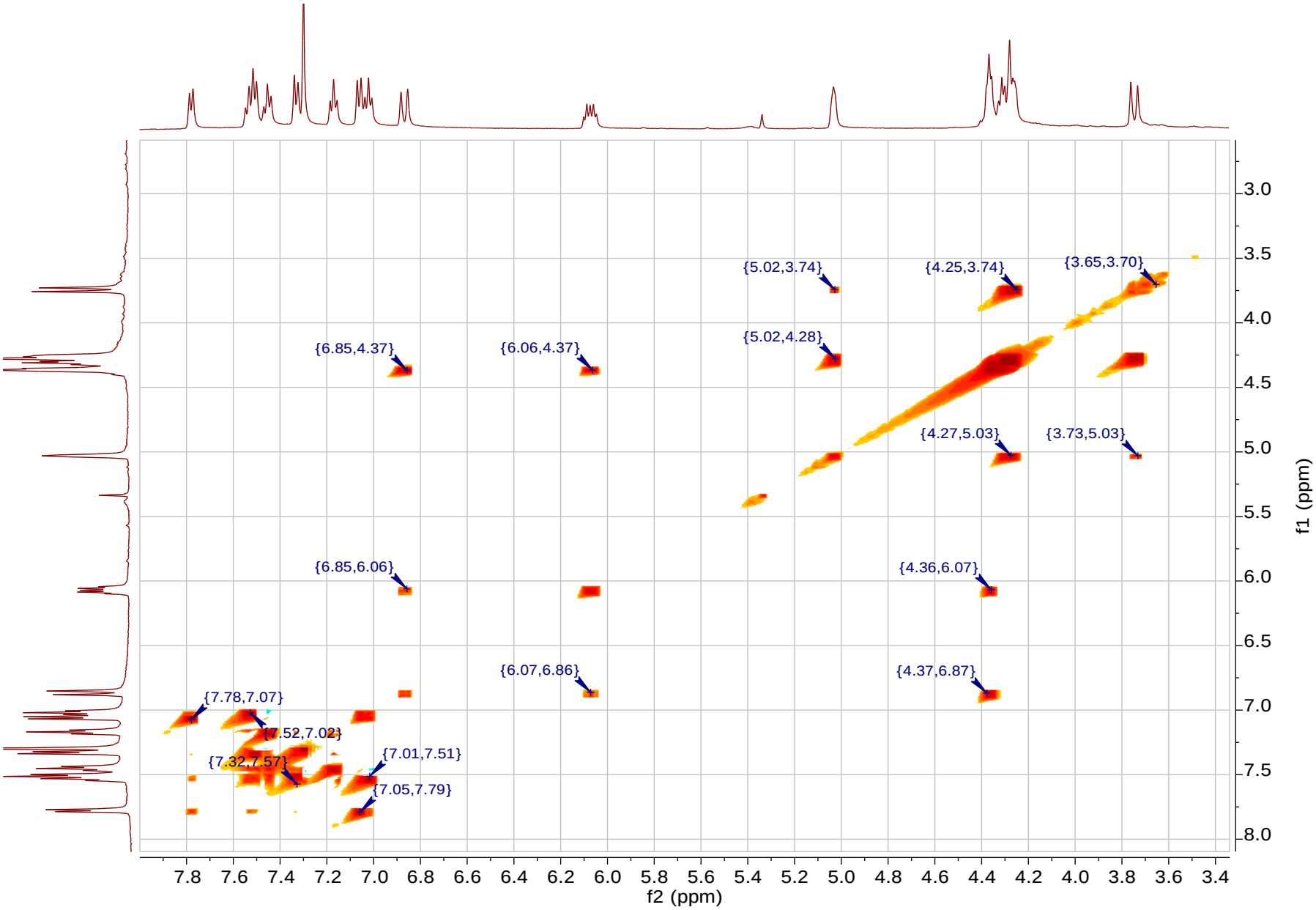


Figure 18: zTOCSY spectrum of compound **10**



(7*S*,9a*R*)-14-(((*S*)-2-oxo-1,3-dioxolan-4-yl)methyl)-7,8-dihydro-6*H*-7,9a-epoxy[1,5]oxazocino[5,4-*a*:3,2-*b'*]diindol-15(14*H*)-one (11)

Figure 19: ^{1}H spectrum of compound **11**

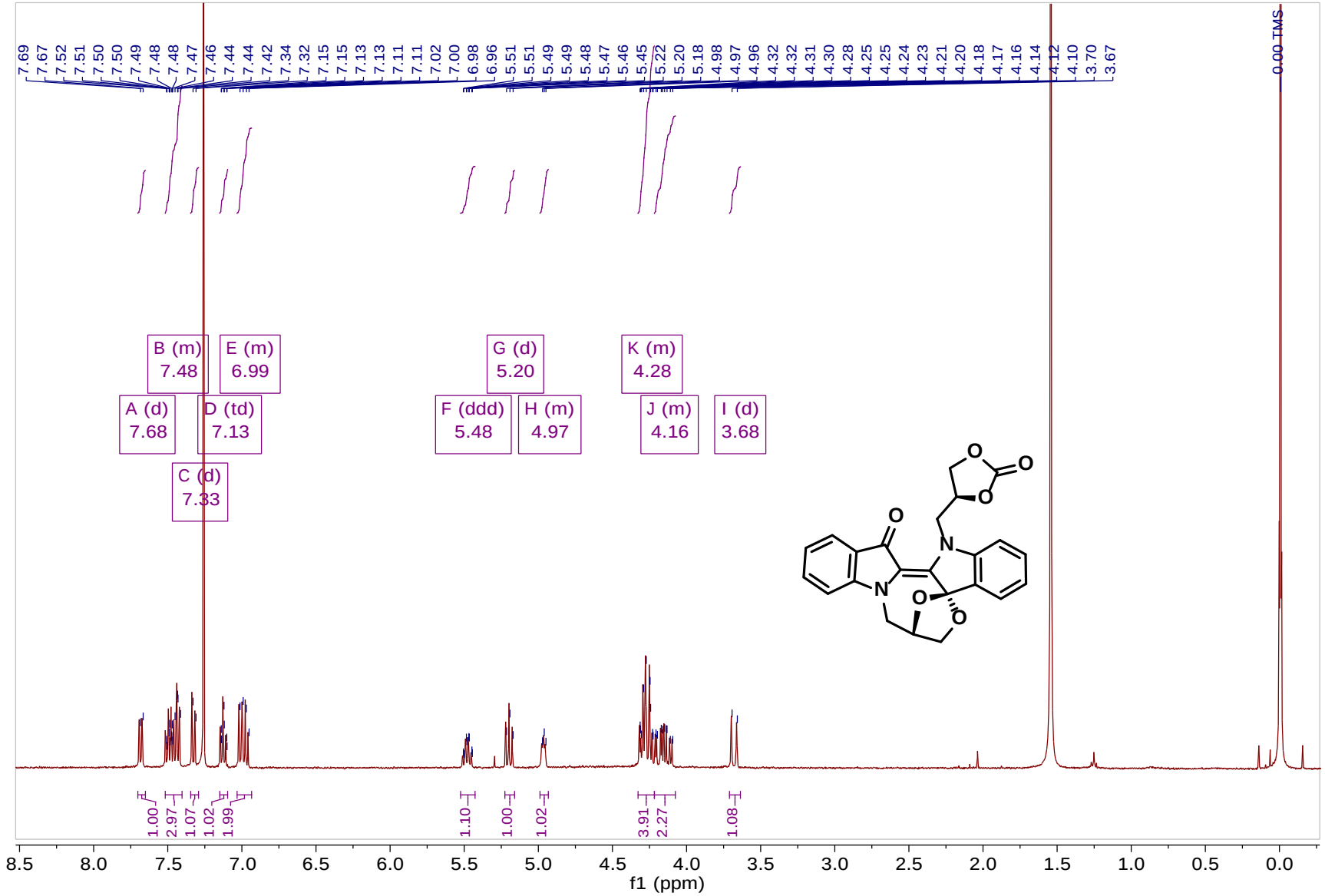


Figure 20: ^{13}C spectrum of compound **11**, with expansion of the region 185-105 ppm.

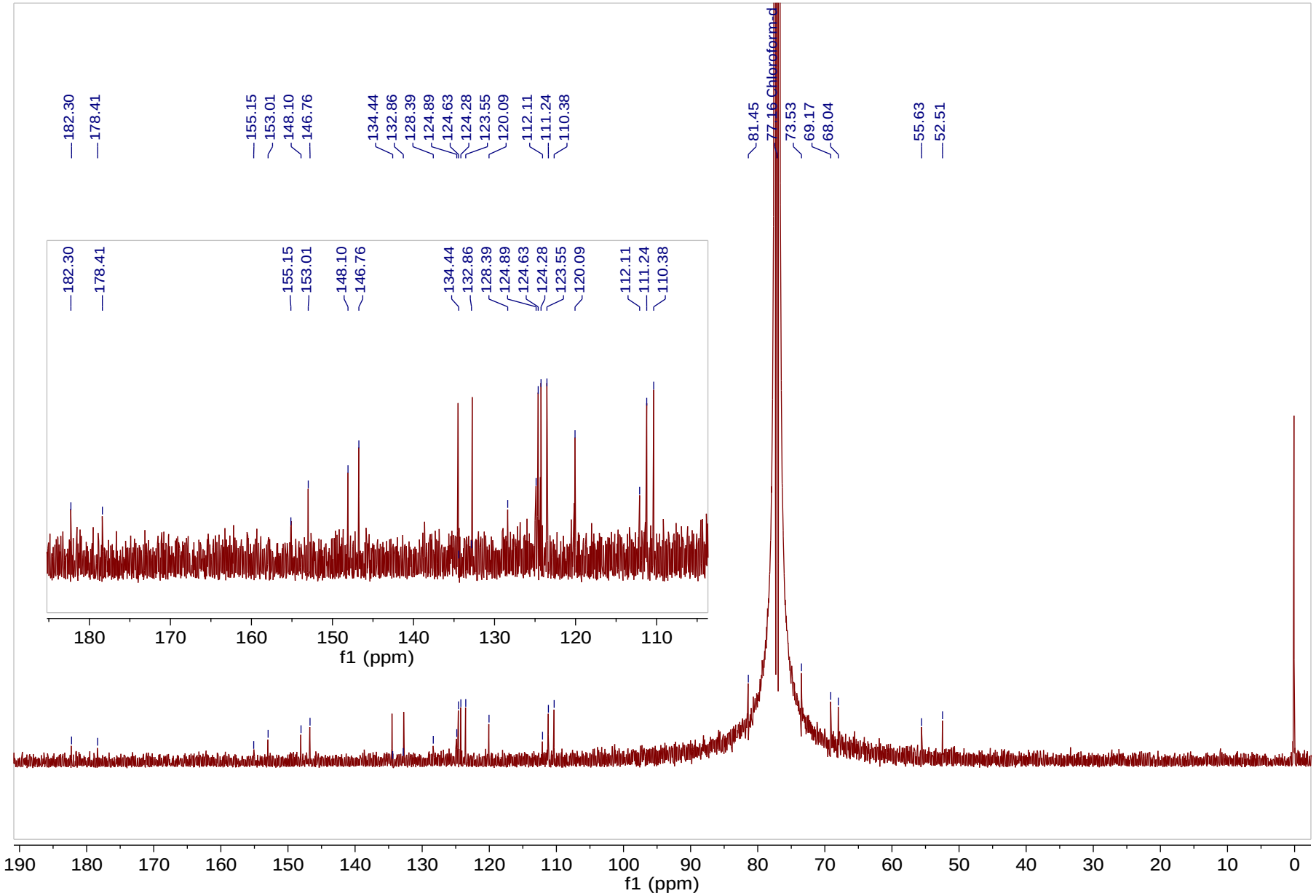


Figure 21: gCOSY spectrum of compound **11**

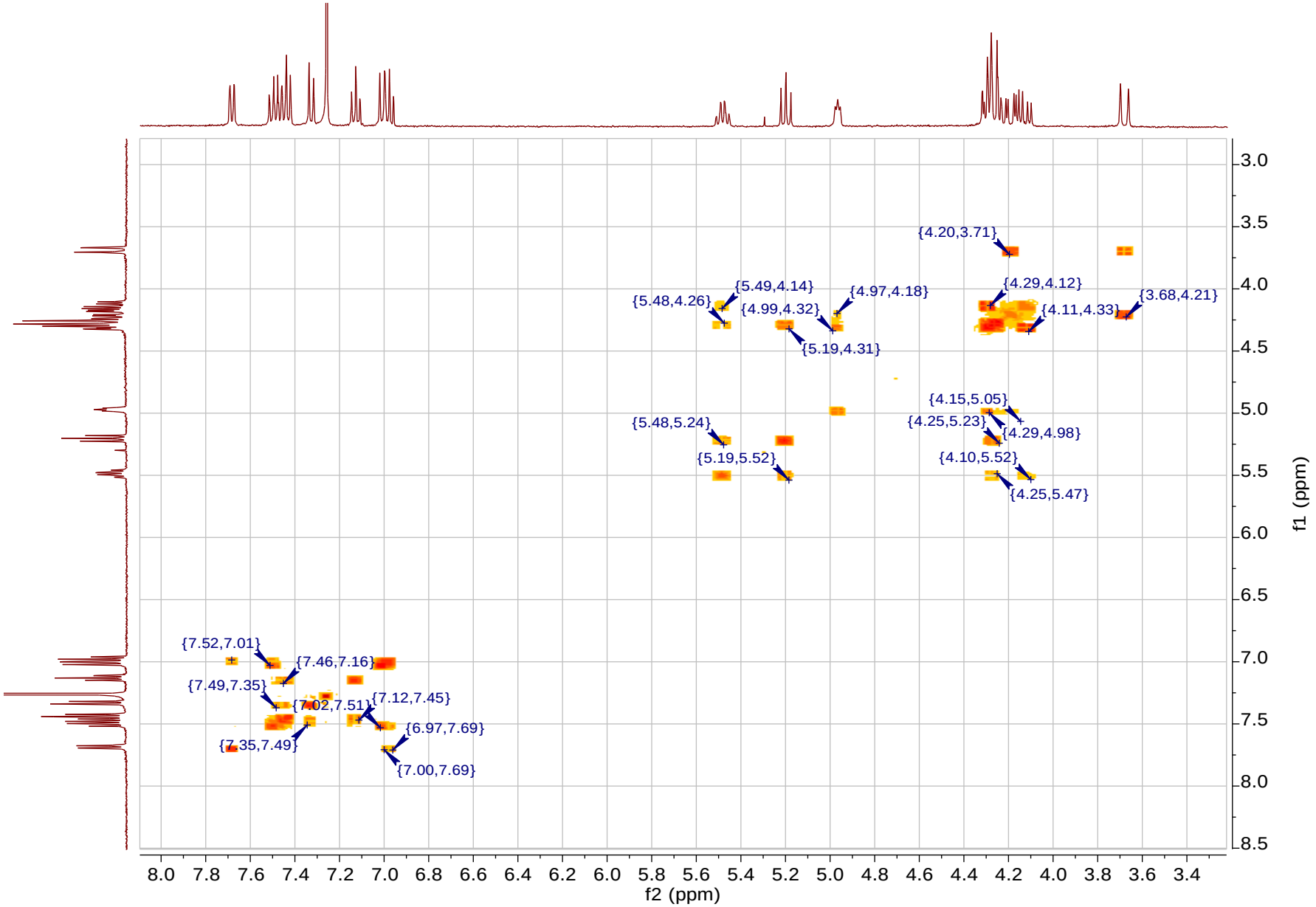


Figure 22: gHSQC spectrum of compound **11**

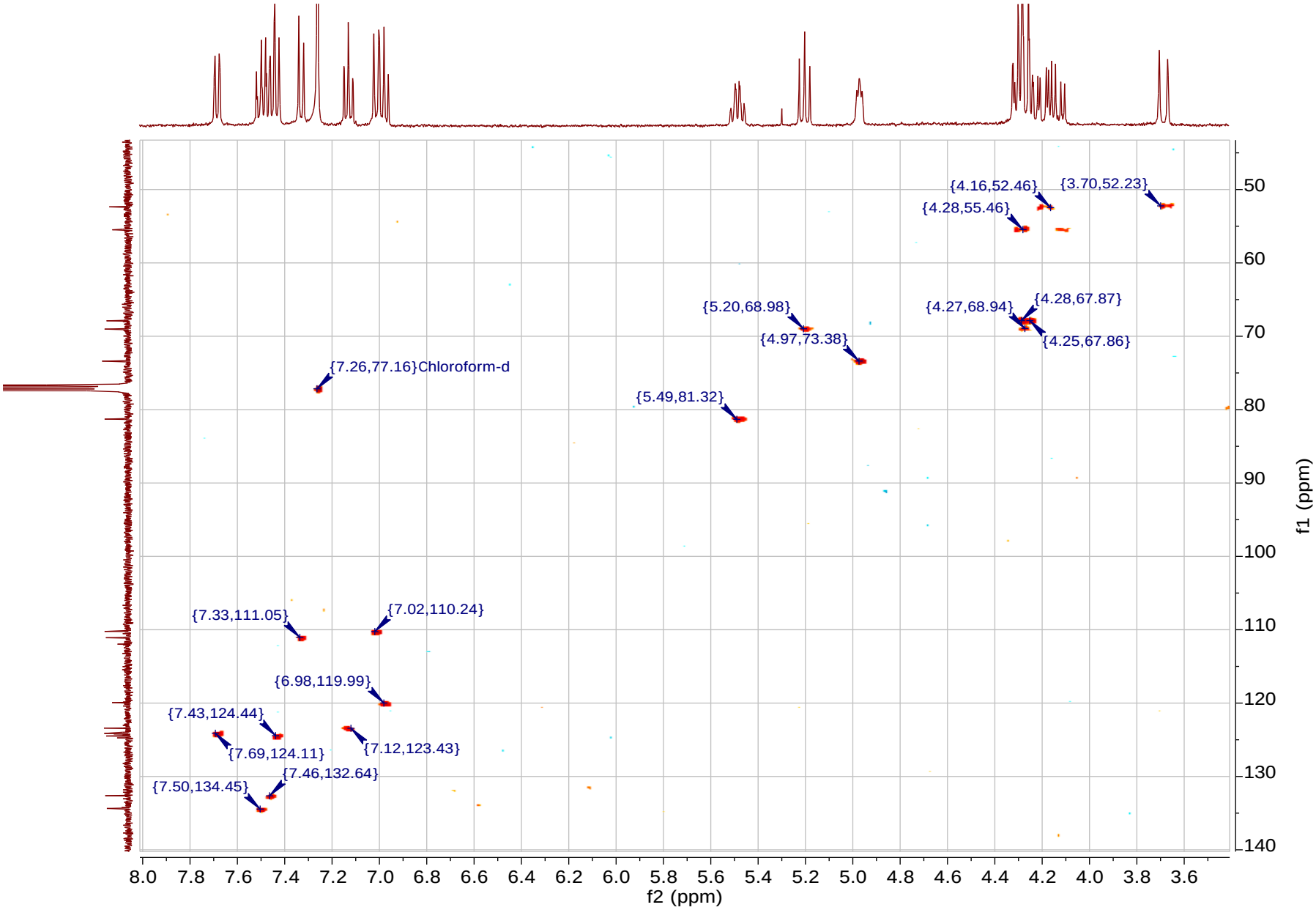
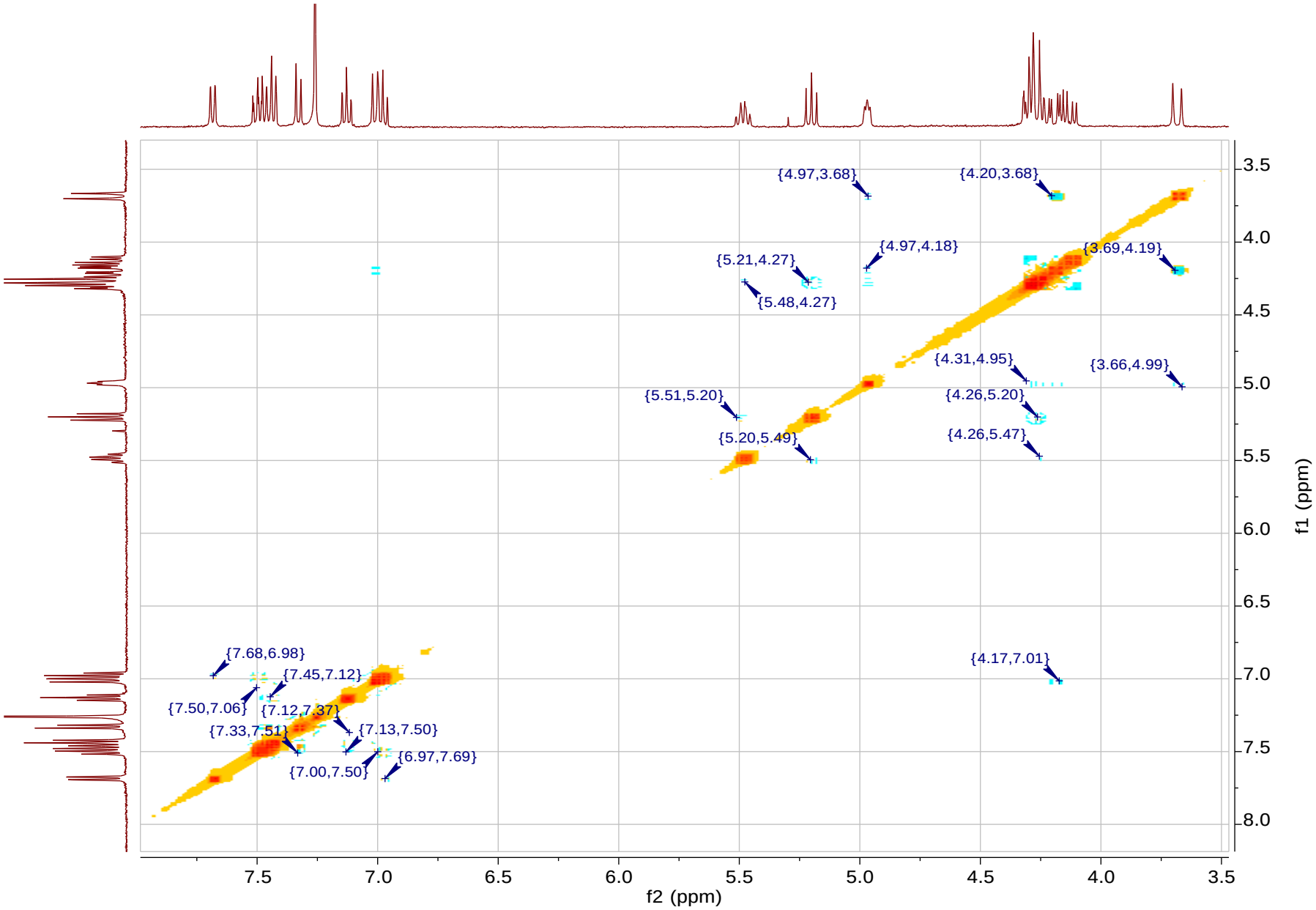


Figure 23: NOESY spectrum of compound **11**



(7*S*,8*R*,9a*S*)-8-(hydroxymethyl)-7,8-dihydro-6*H*-7,9a-epoxy[1,5]oxazocino[5,4-*a*:3,2-*b'*]diindol-15(14*H*)-one (12)

Figure 24: ^{1}H spectrum of compound **12**

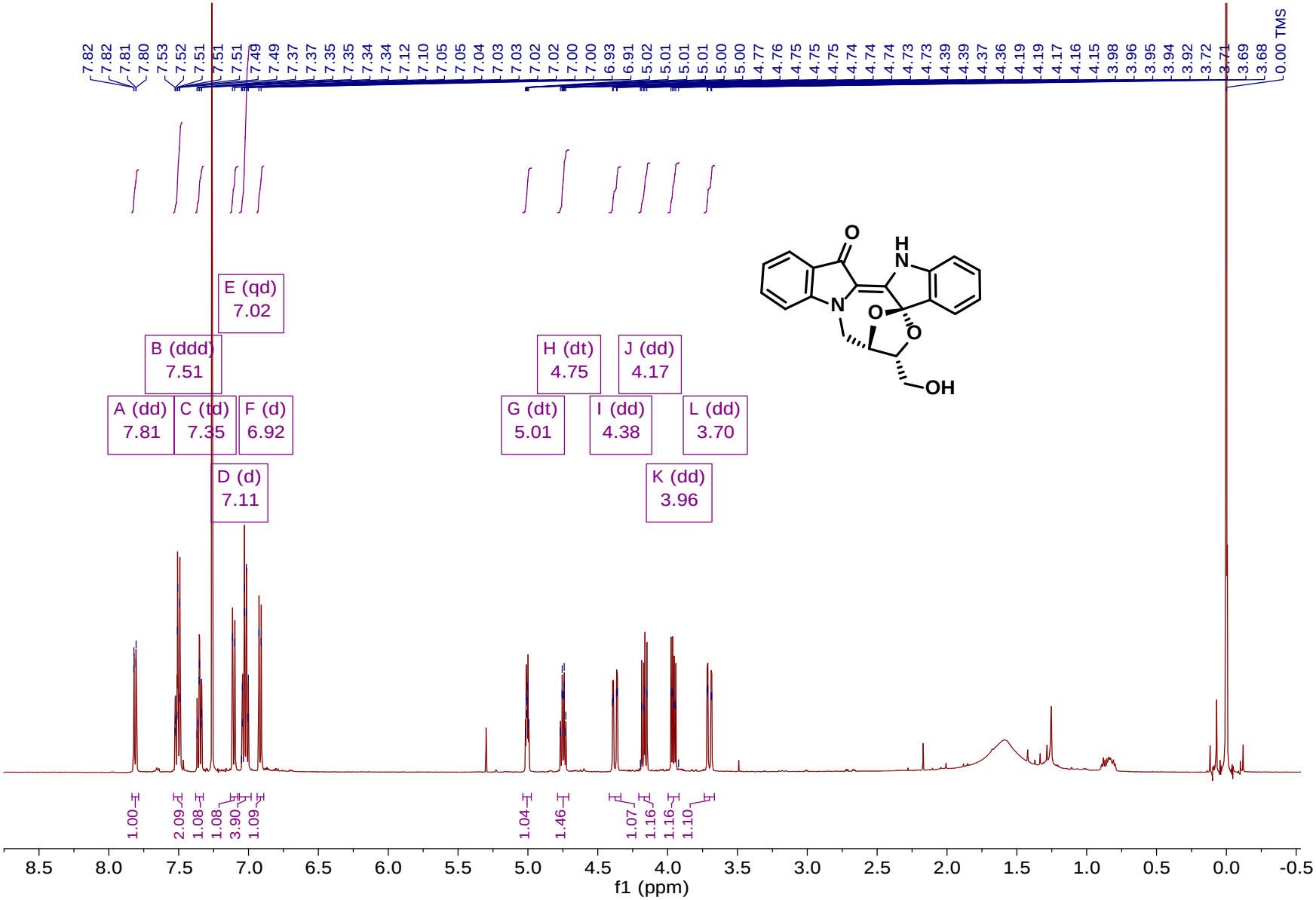


Figure 25: ^{13}C spectrum of compound **12**, and expansion of the region 81-73 ppm

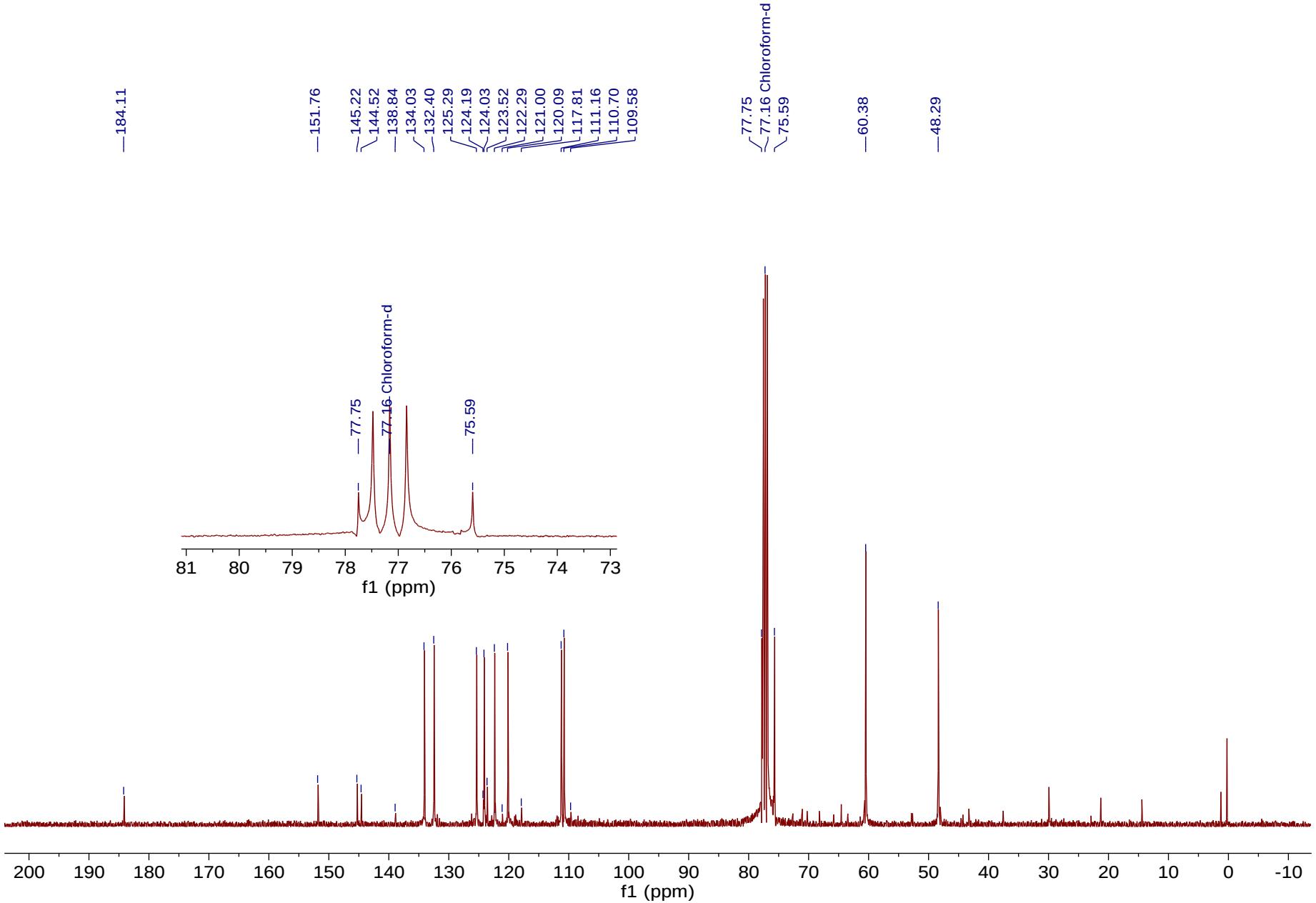


Figure 26: gCOSY spectrum of compound **12**

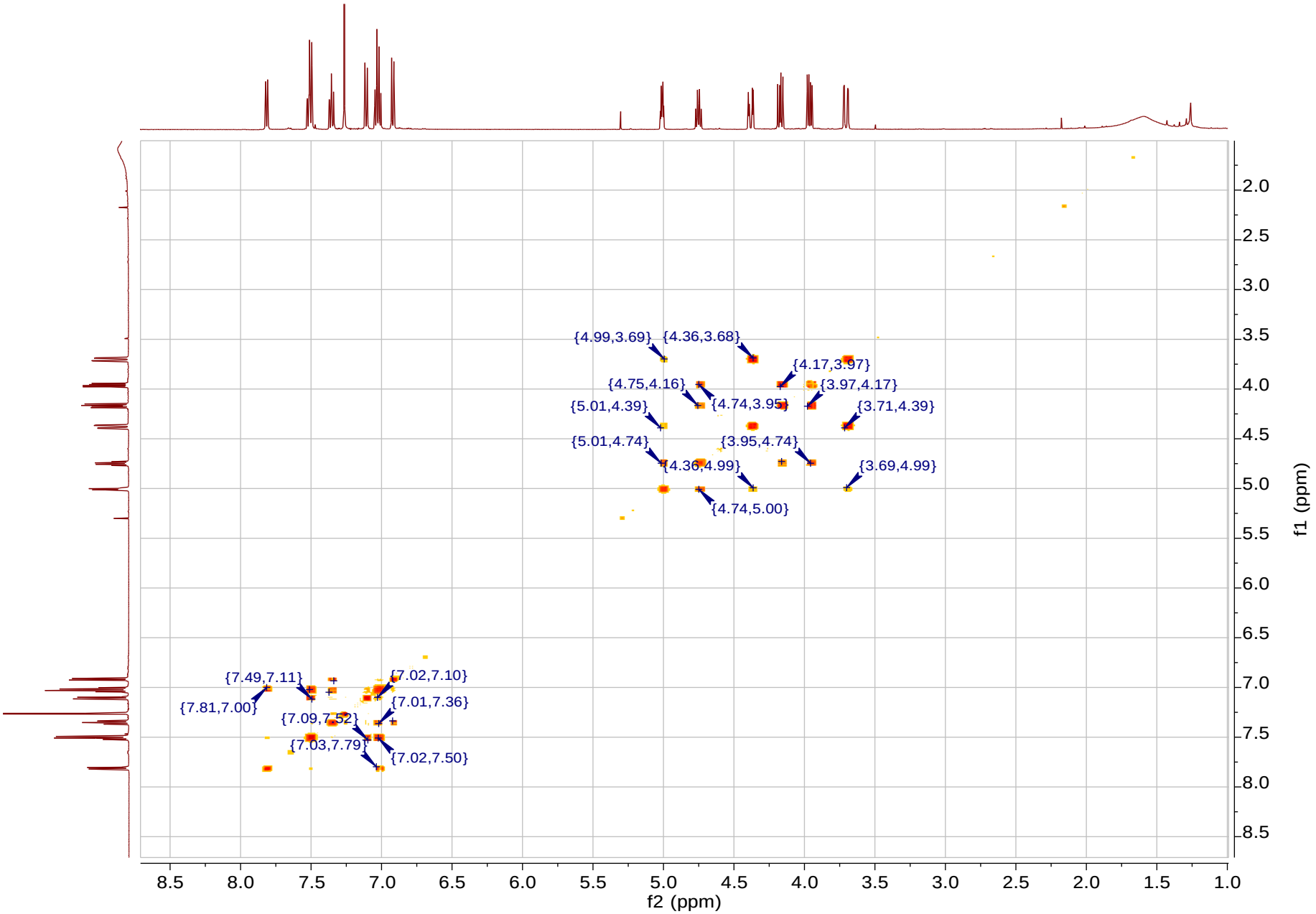


Figure 27: ROESY spectrum of compound **12**

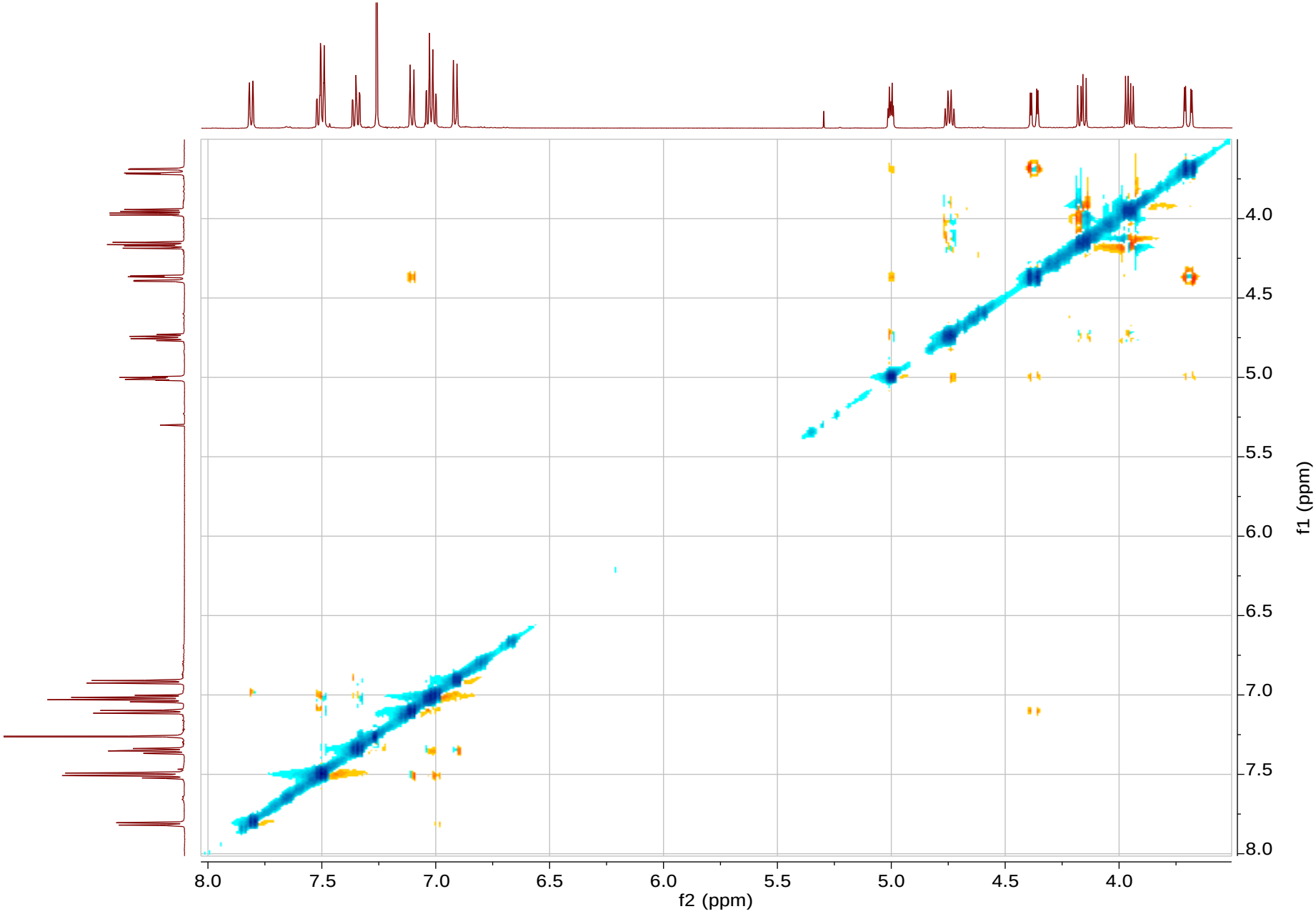


Figure 28: gHSQC spectrum of compound **12**

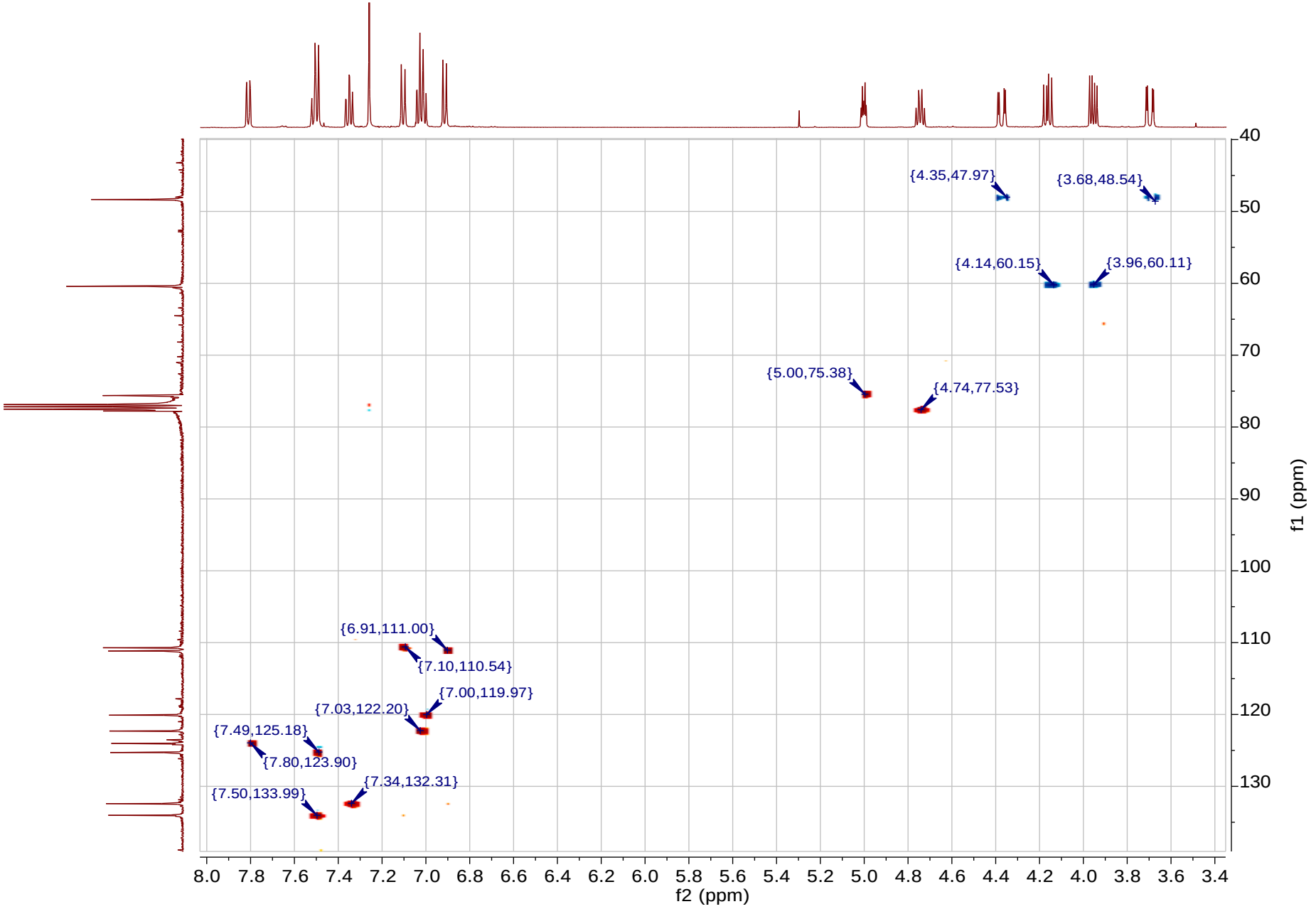
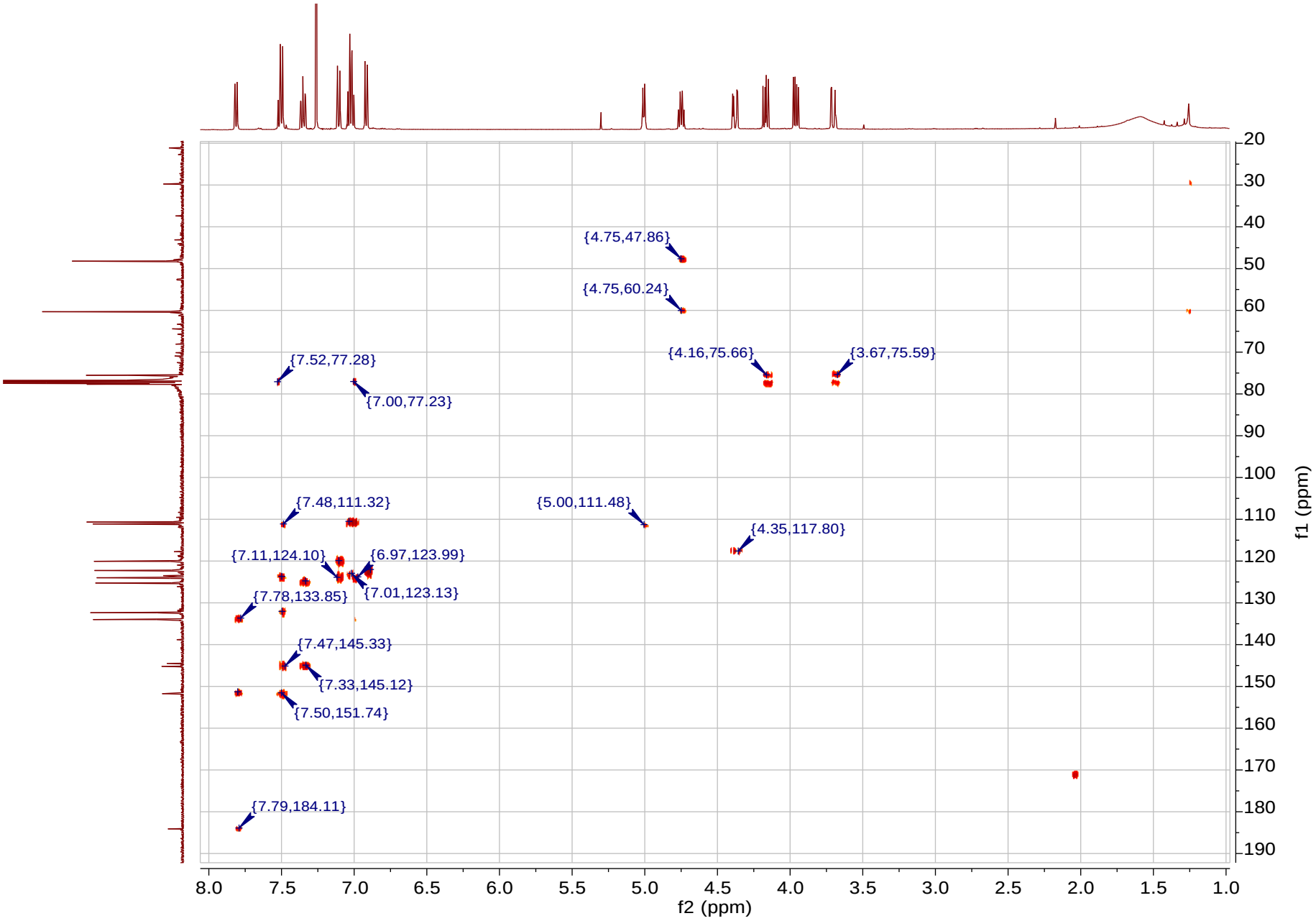


Figure 29: gHMBC spectrum of compound **12**



(7*S*,8*R*,9a*S*)-14-((*S*)-2-hydroxy-2-((*S*)-oxiran-2-yl)ethyl)-8-(hydroxymethyl)-7,8-dihydro-6*H*-7,9a-epoxy[1,5]oxazocino[5,4-*a*:3,2-*b*']diindol-15(14*H*)-one (13)

Figure 30: ^{1}H NMR spectrum of compound **13**

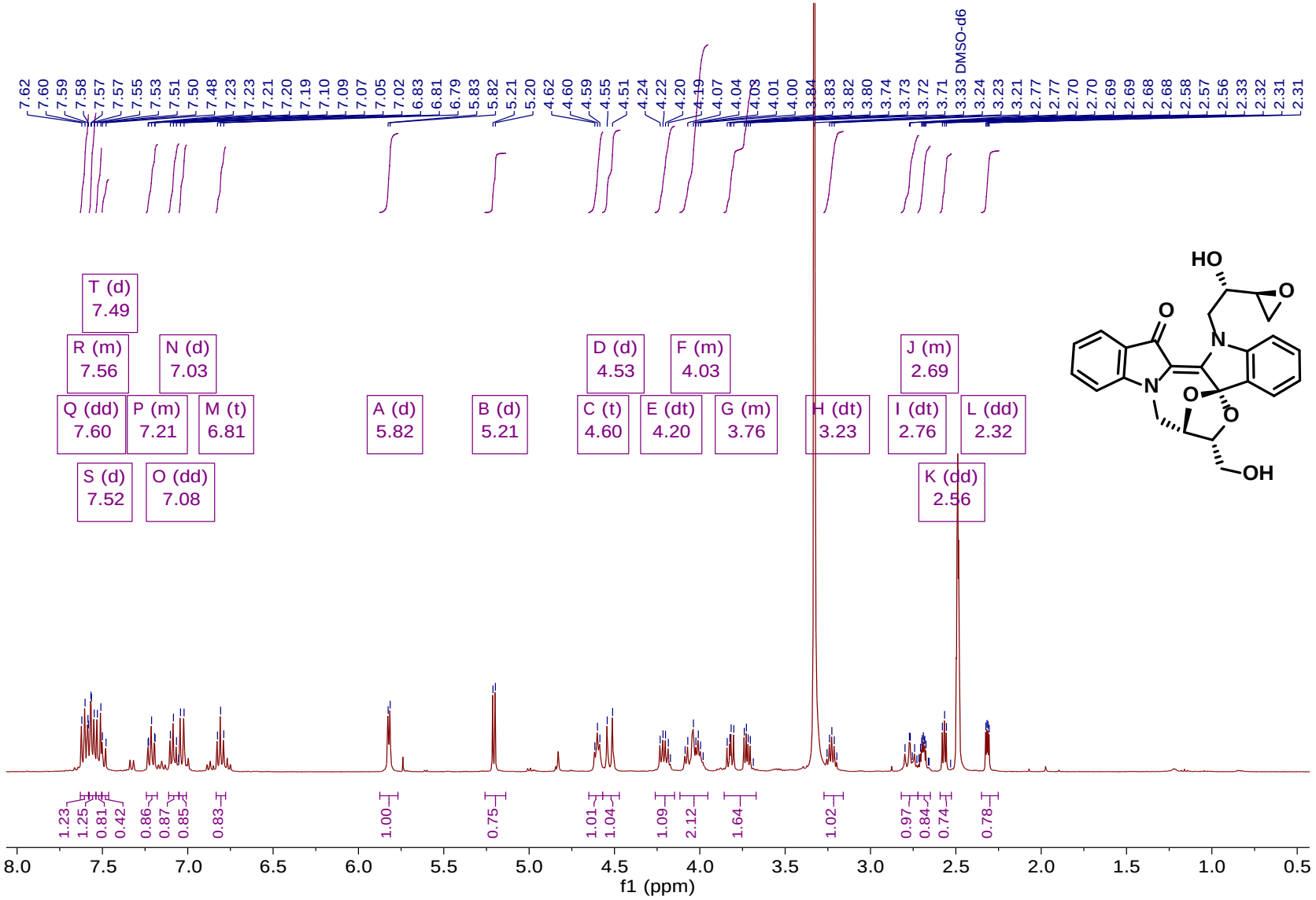


Figure 31: ^{13}C spectrum of compound **13**

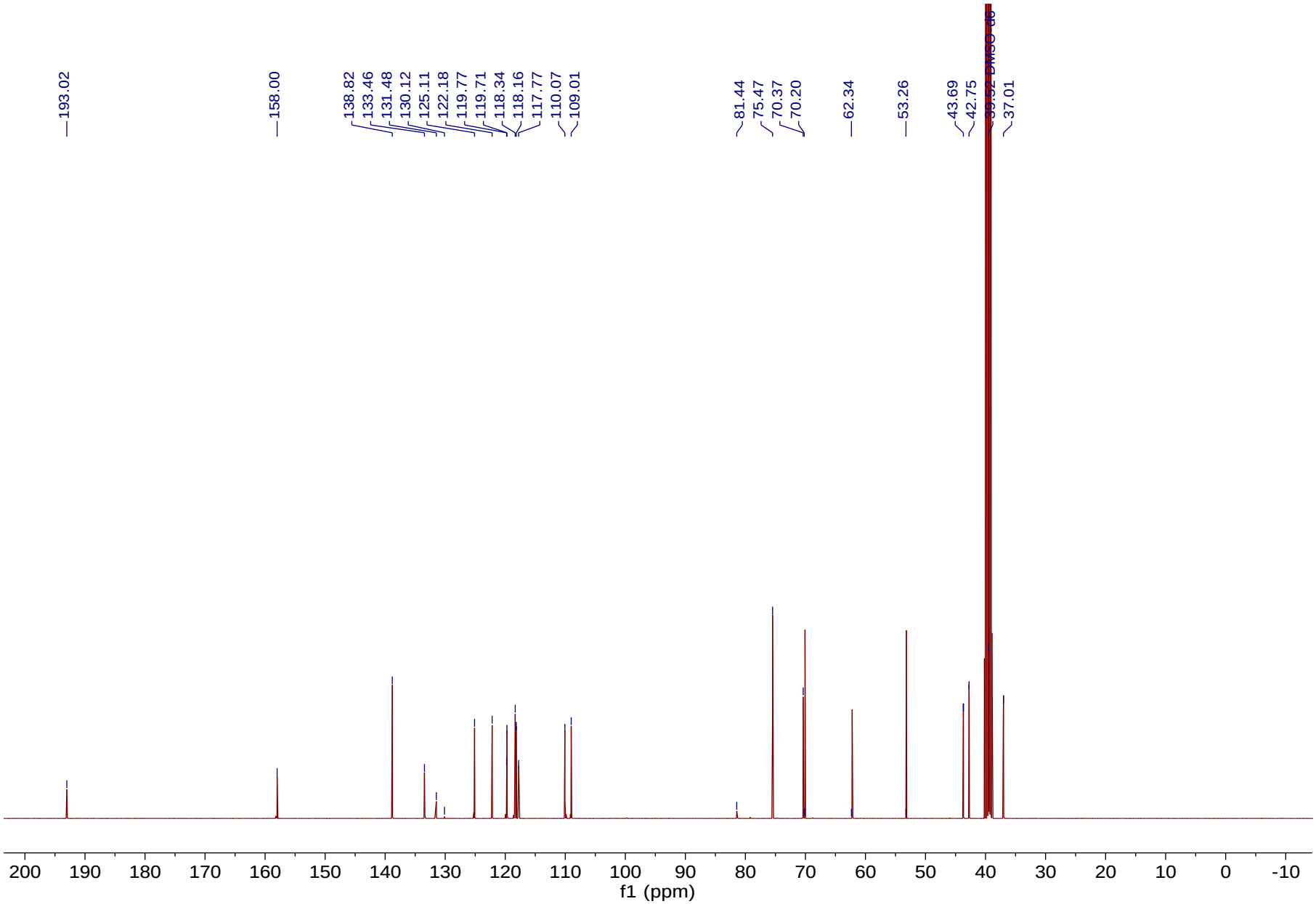


Figure 32: COSY spectrum of compound **13**

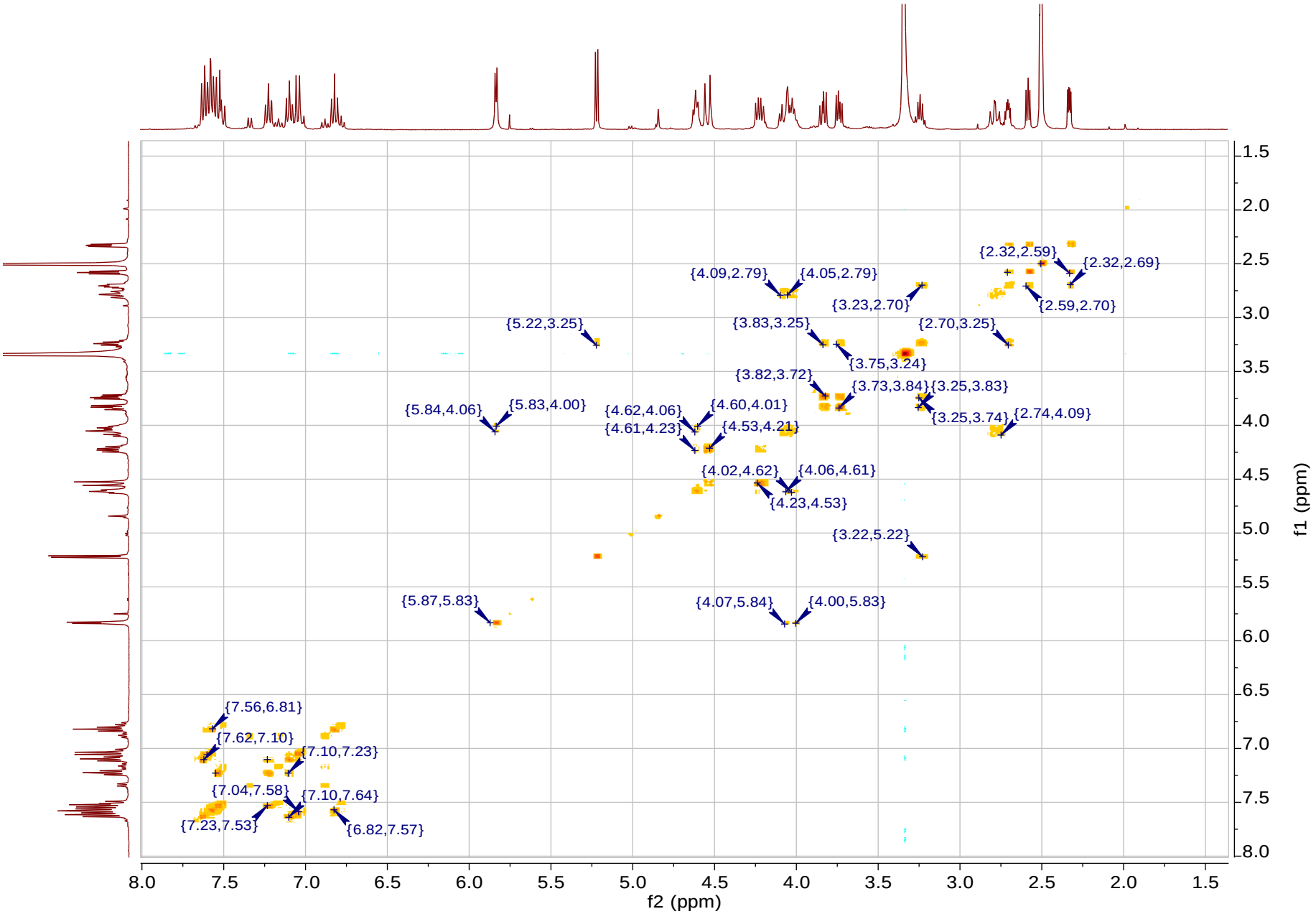


Figure 33: TOCSY spectrum of compound **13**

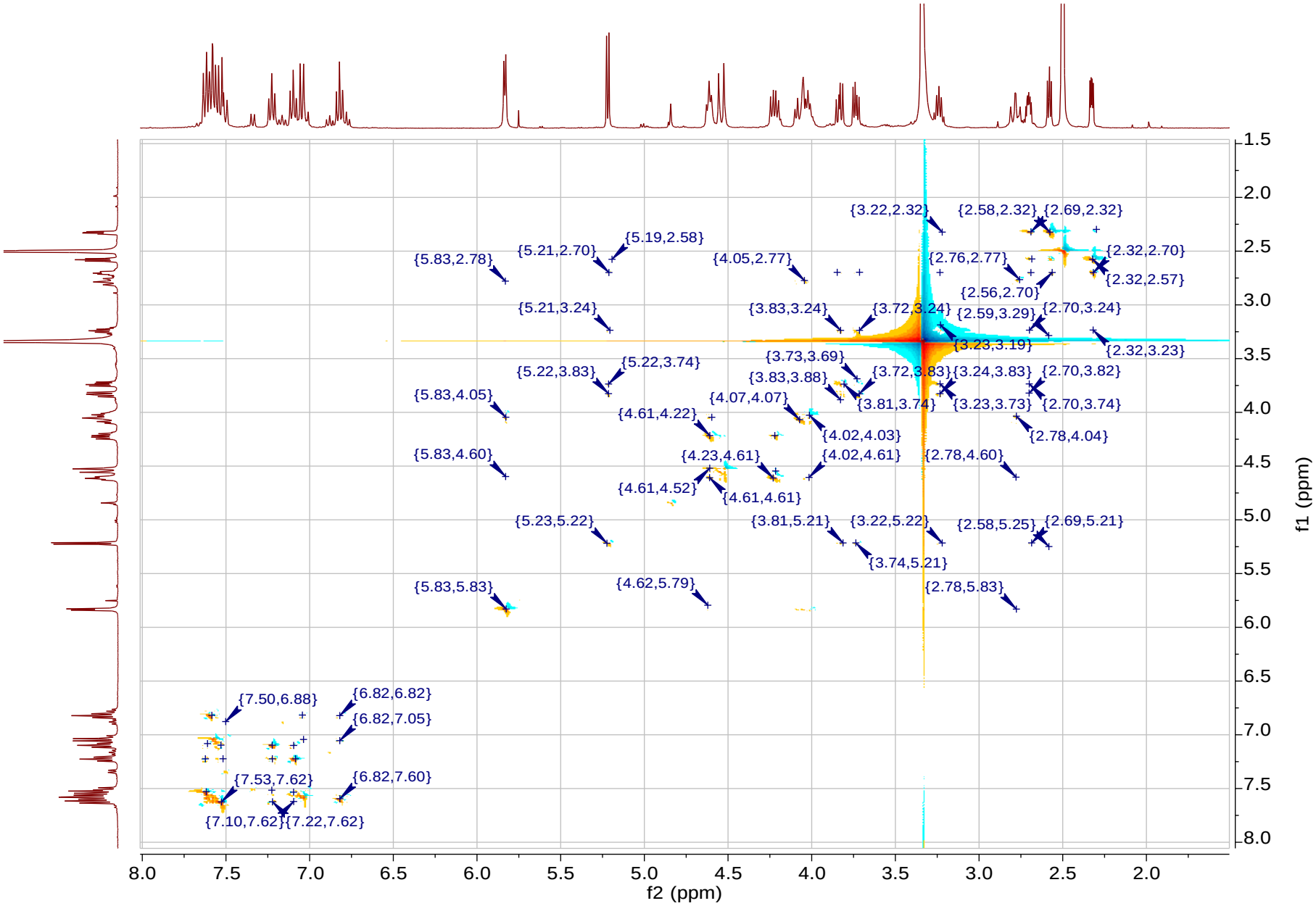


Figure 34: HMBC spectrum of compound **13**

Figure 35: HSQC spectrum of compound **13**

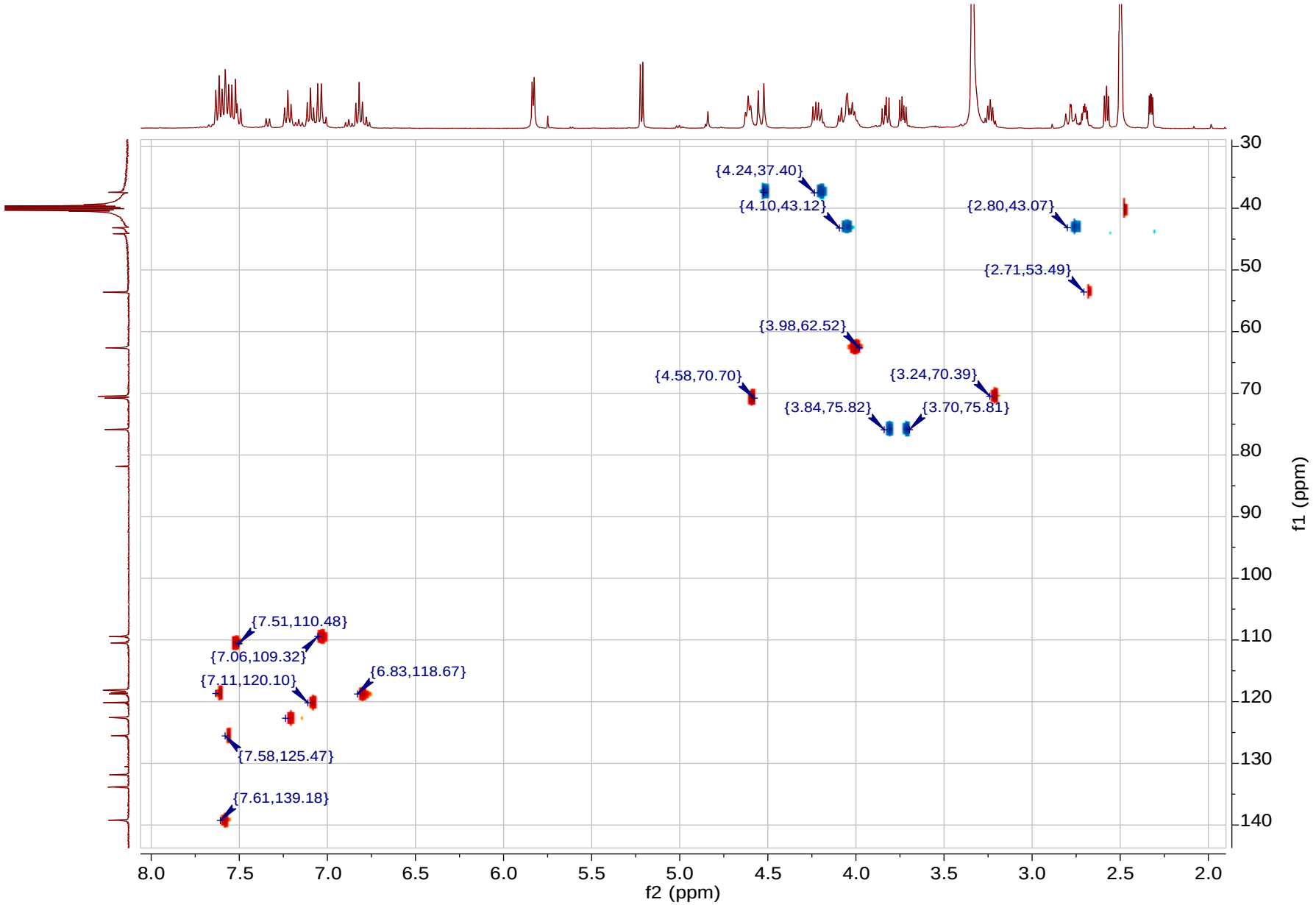
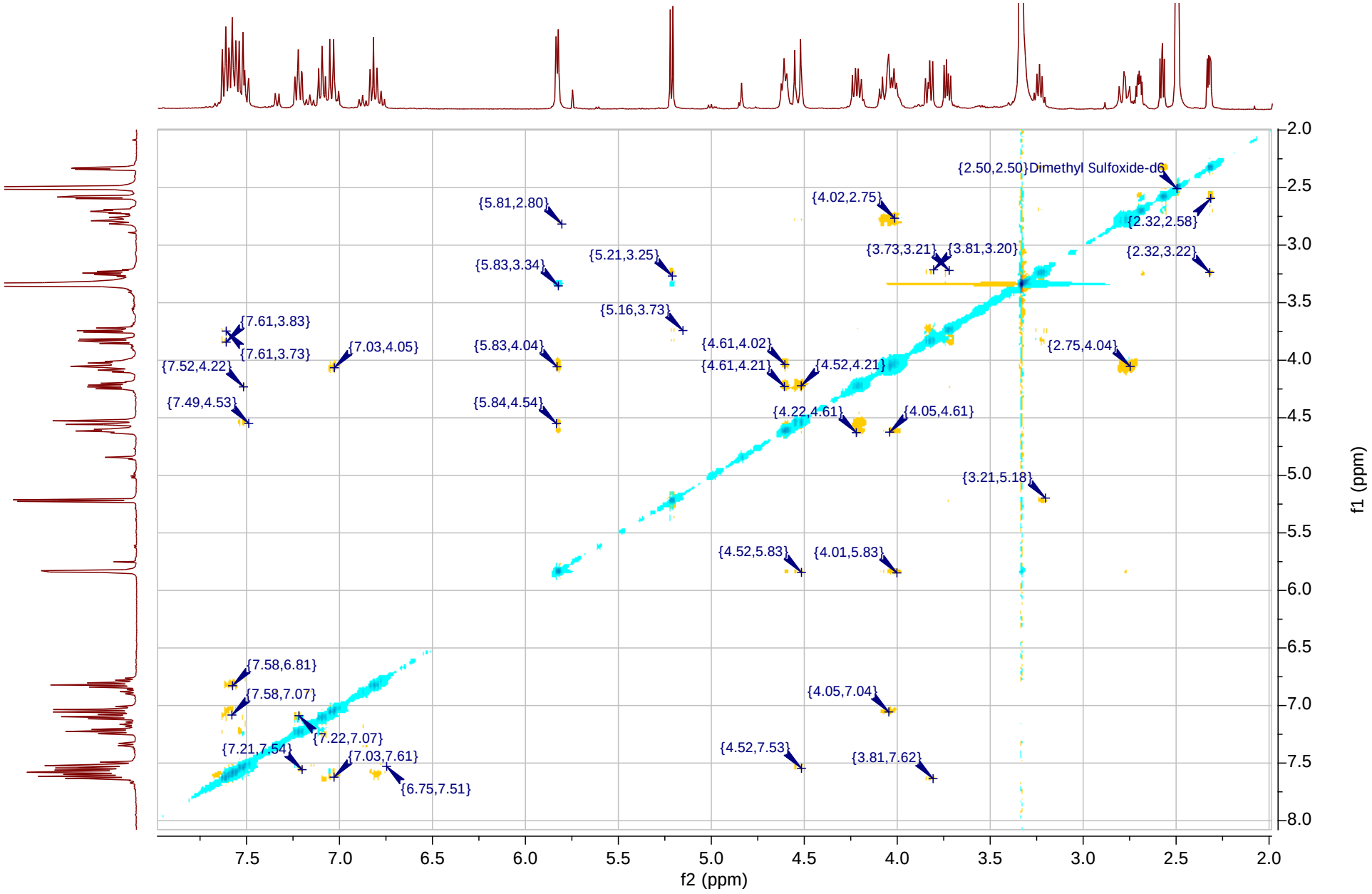


Figure 36: ROESY spectrum of compound **13**



(*S*)-*N*-(3-chloro-2-hydroxypropyl)-4-methylbenzenesulfonamide (23)

Figure 37: ^{1}H spectrum of compound **23**

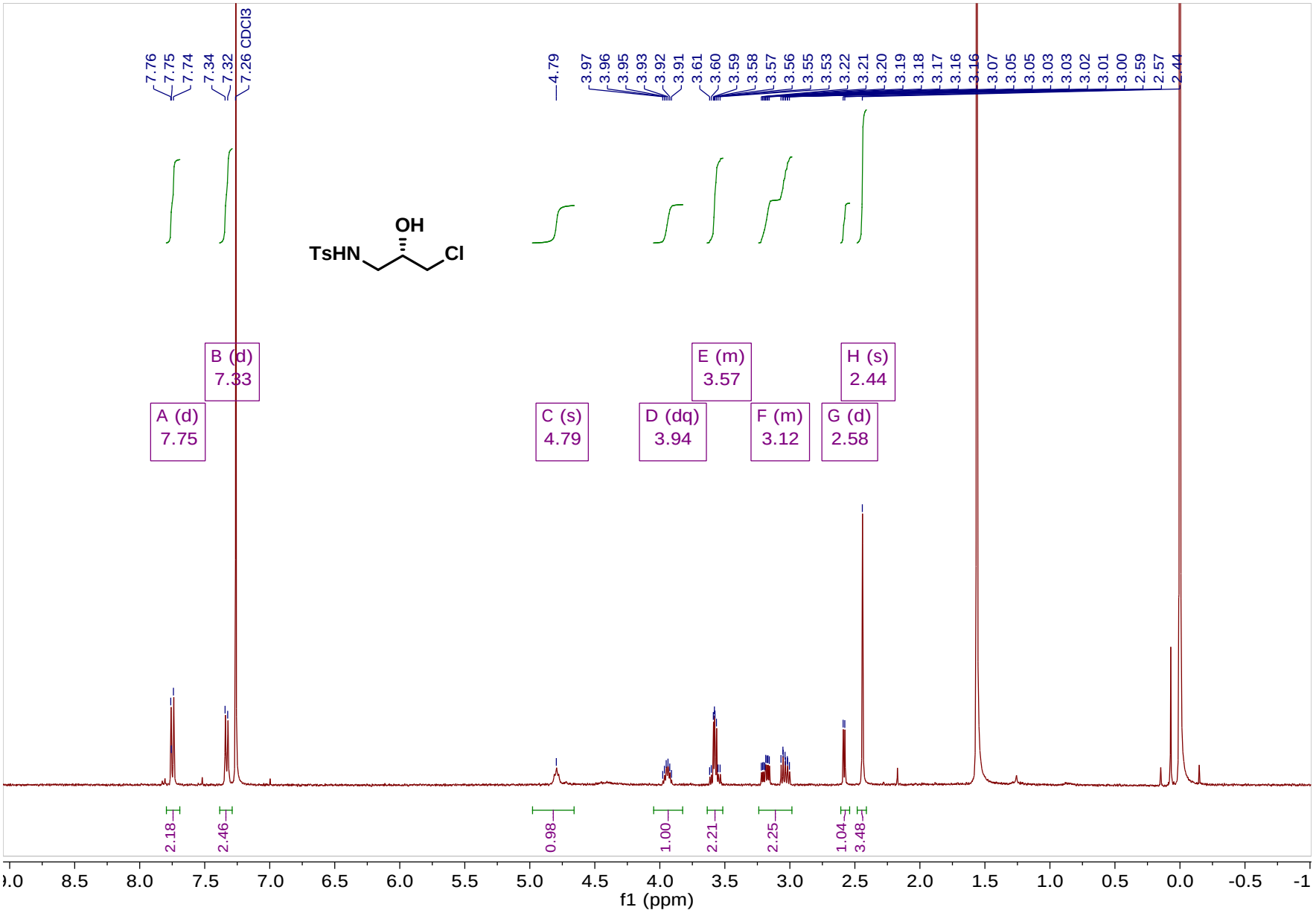


Figure 38: COSY spectrum of compound **23**

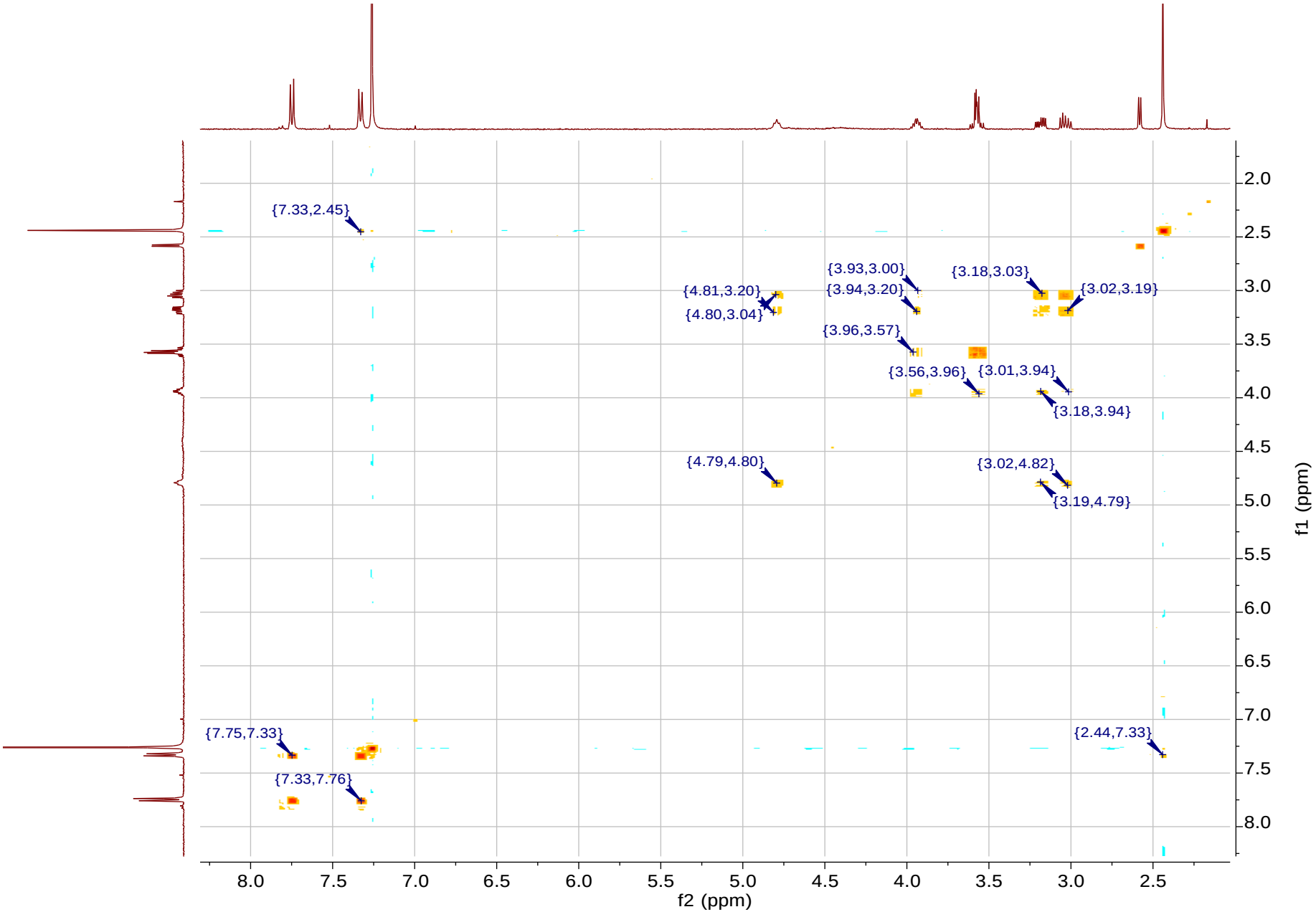


Figure 39: HSQC spectrum of compound **23**

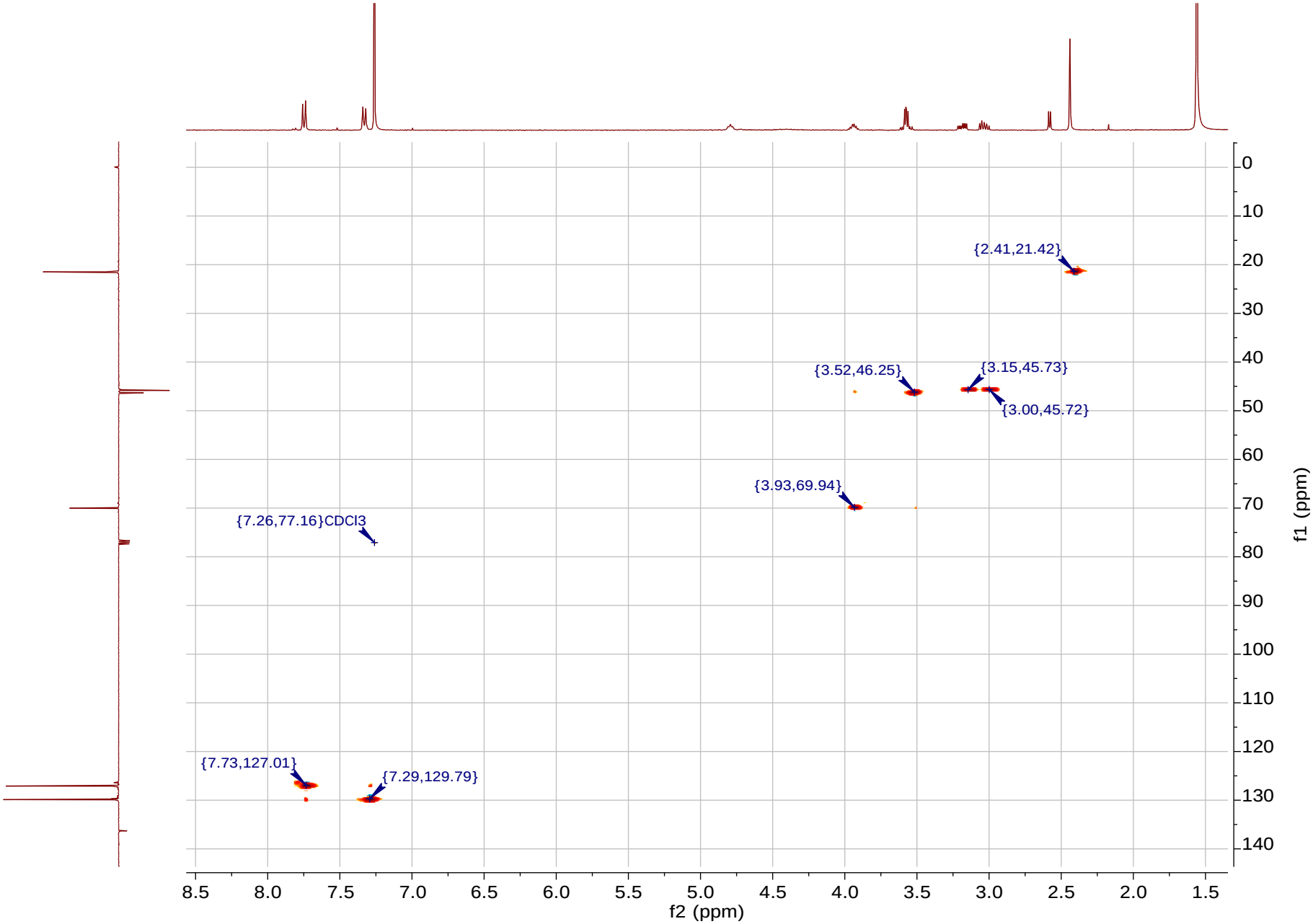
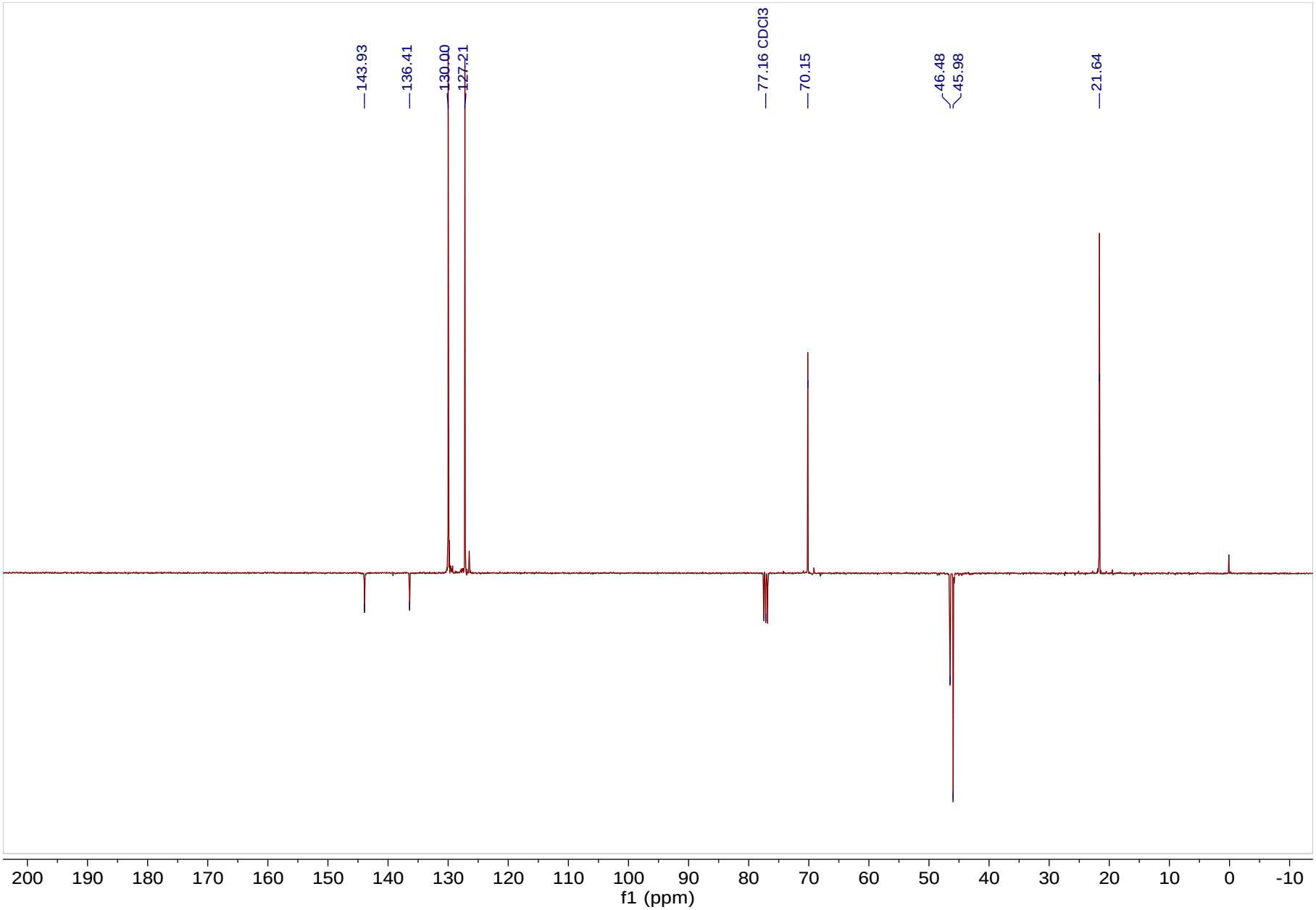


Figure 40: ^{13}C-DEPTQ spectrum of Compound **23**, with CH/CH_3 phased up, and CH_2/Cq phased down



(*S*)-1-chloro-3-((4-methylphenyl)sulfonamido)propan-2-yl methanesulfonate (24)

Figure 41: ^{1}H NMR spectrum of compound **24**

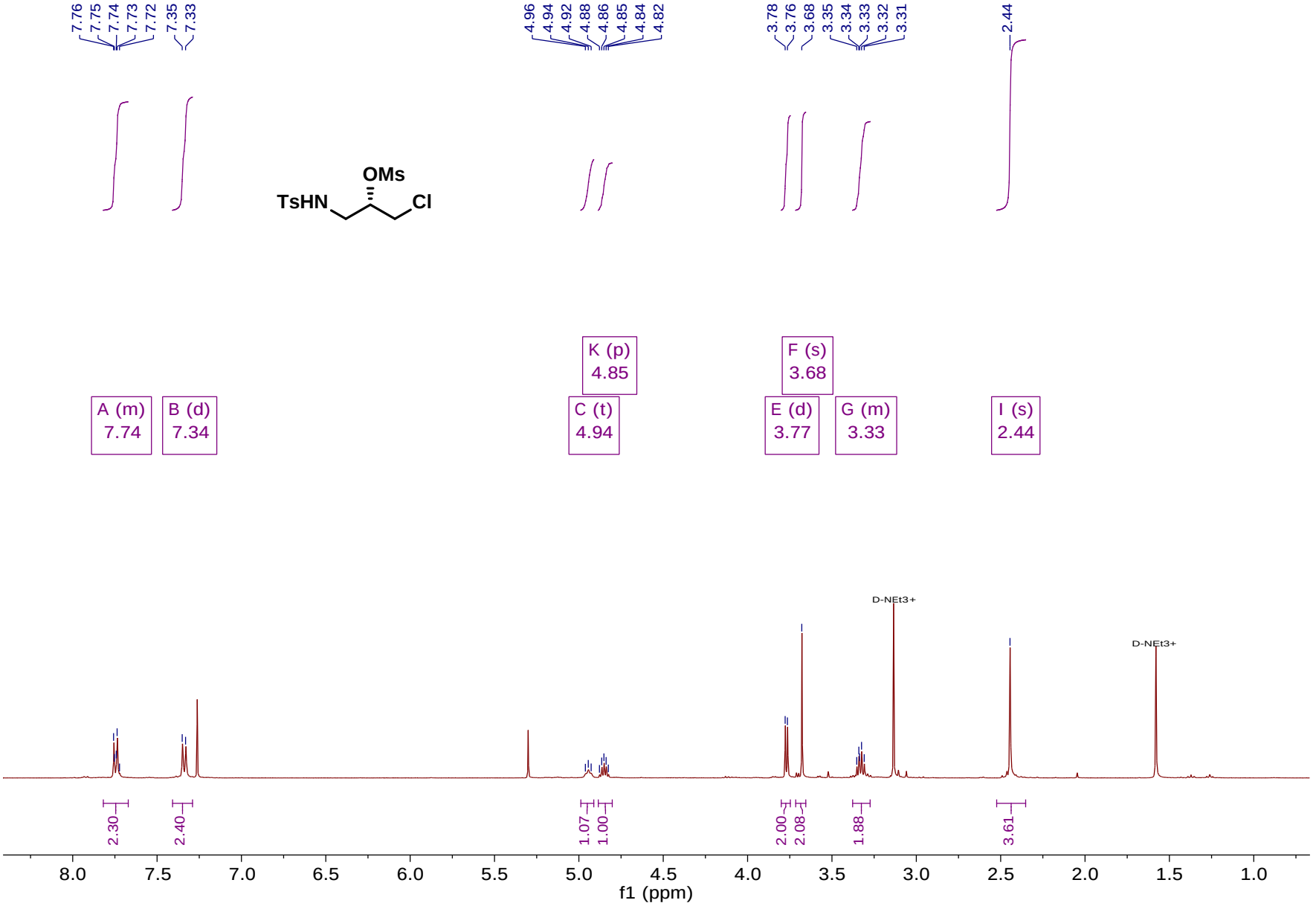


Figure 42: ^{13}C spectrum of compound **24**

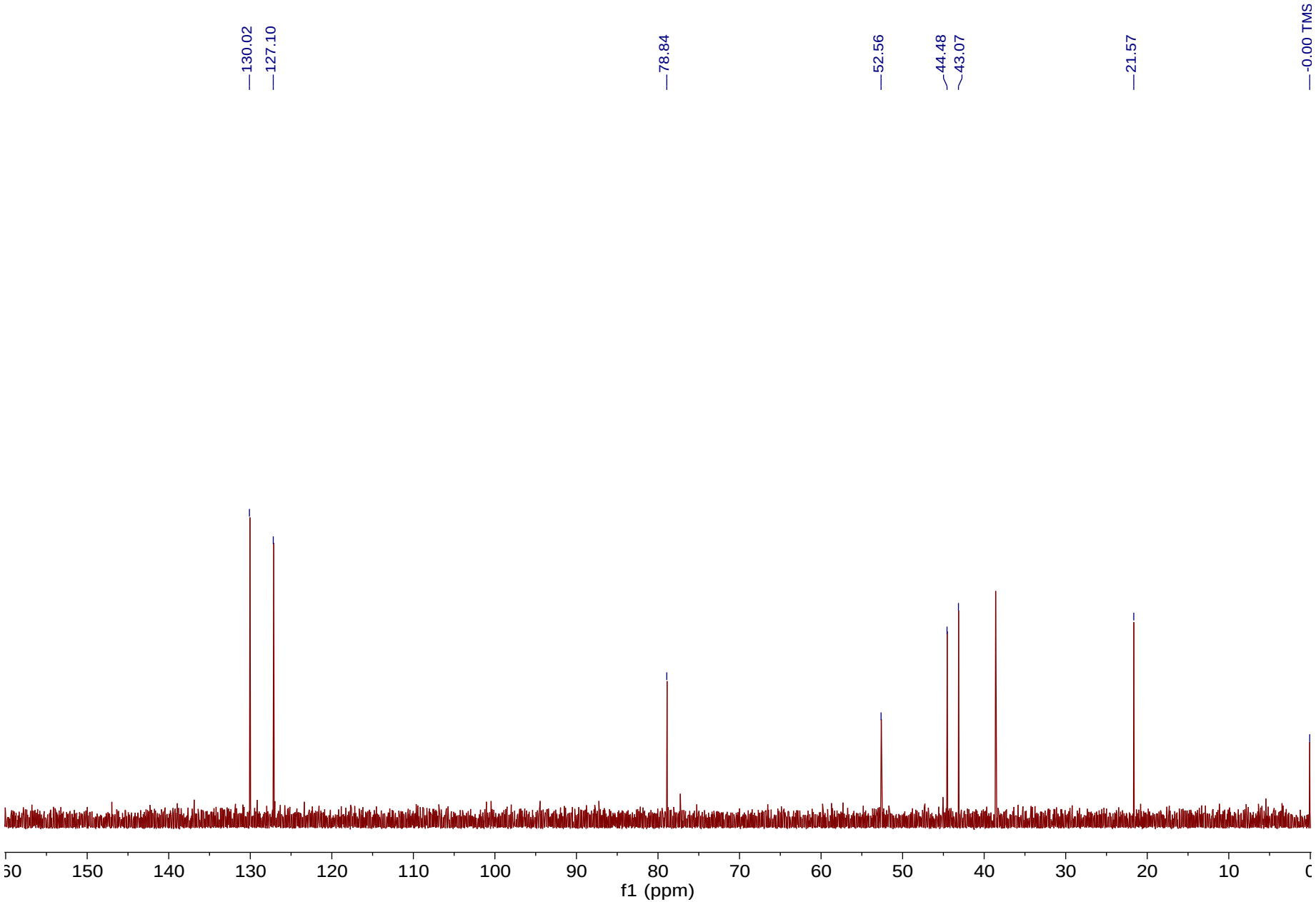


Figure 43: HMBC spectrum of compound **24**

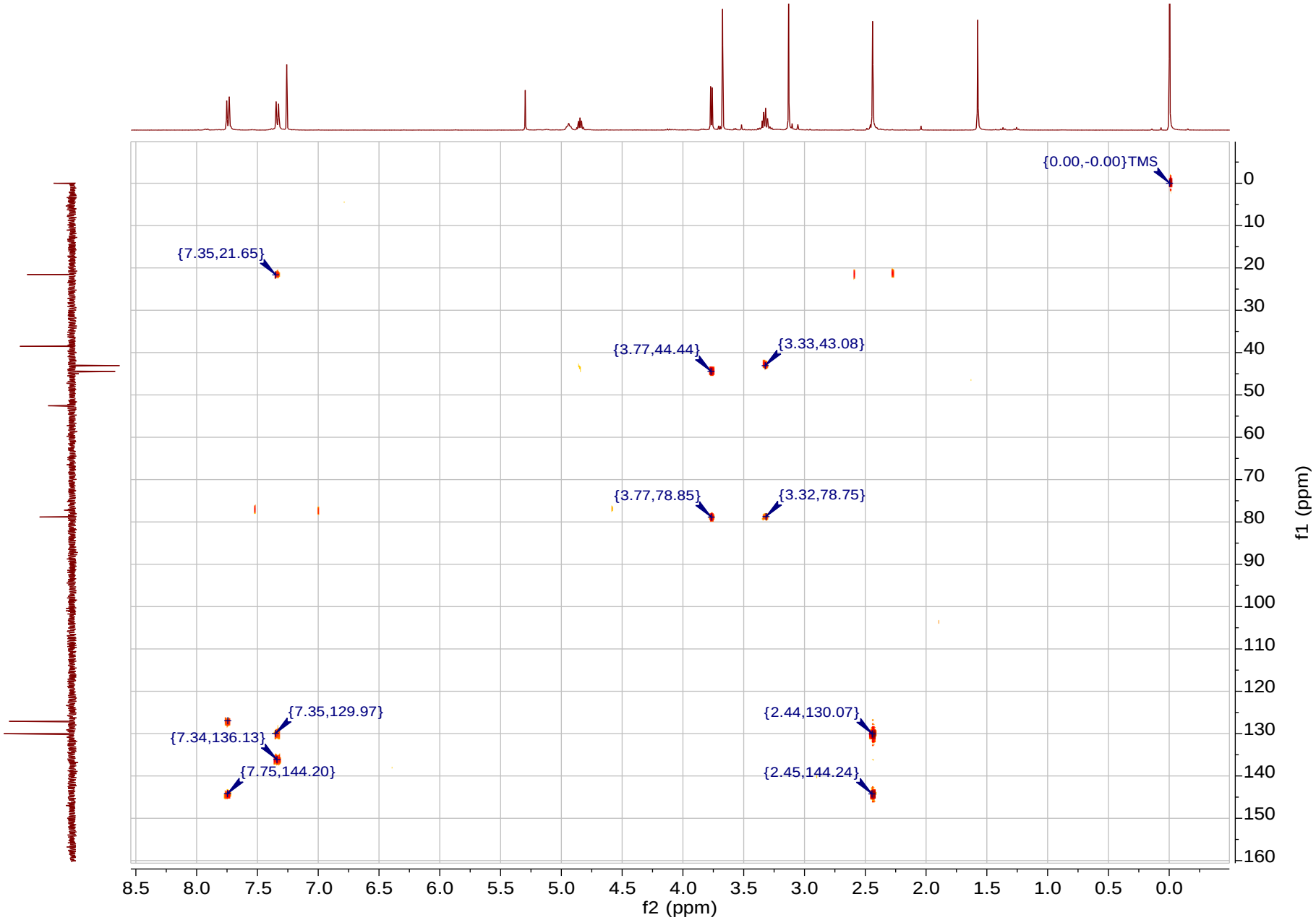
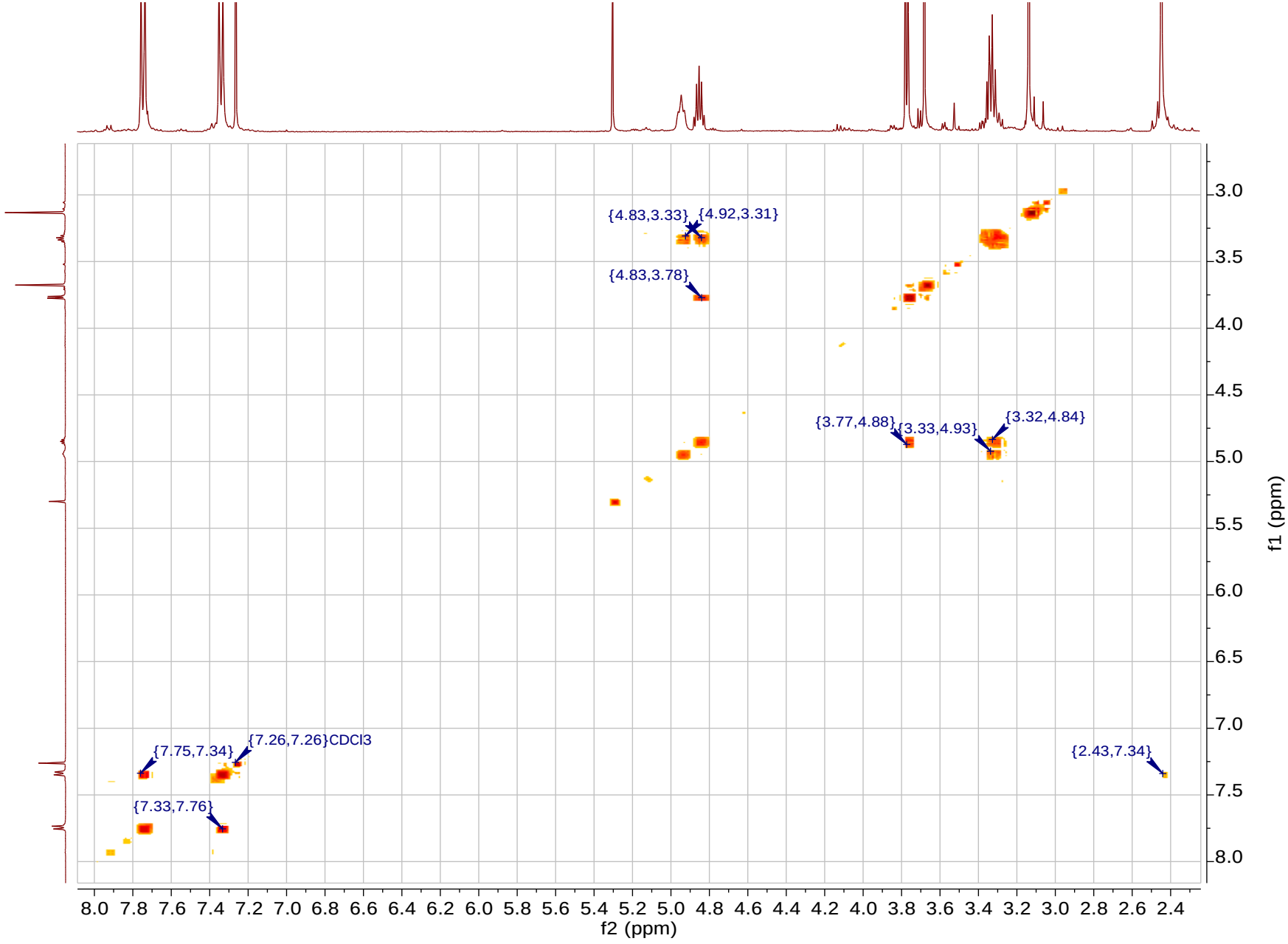


Figure 44: COSY spectrum of compound **24**



(*R*)-*N*-tosyl-2-chloromethylaziridine (14)

Figure 45: ^{1}H spectrum of compound **14**

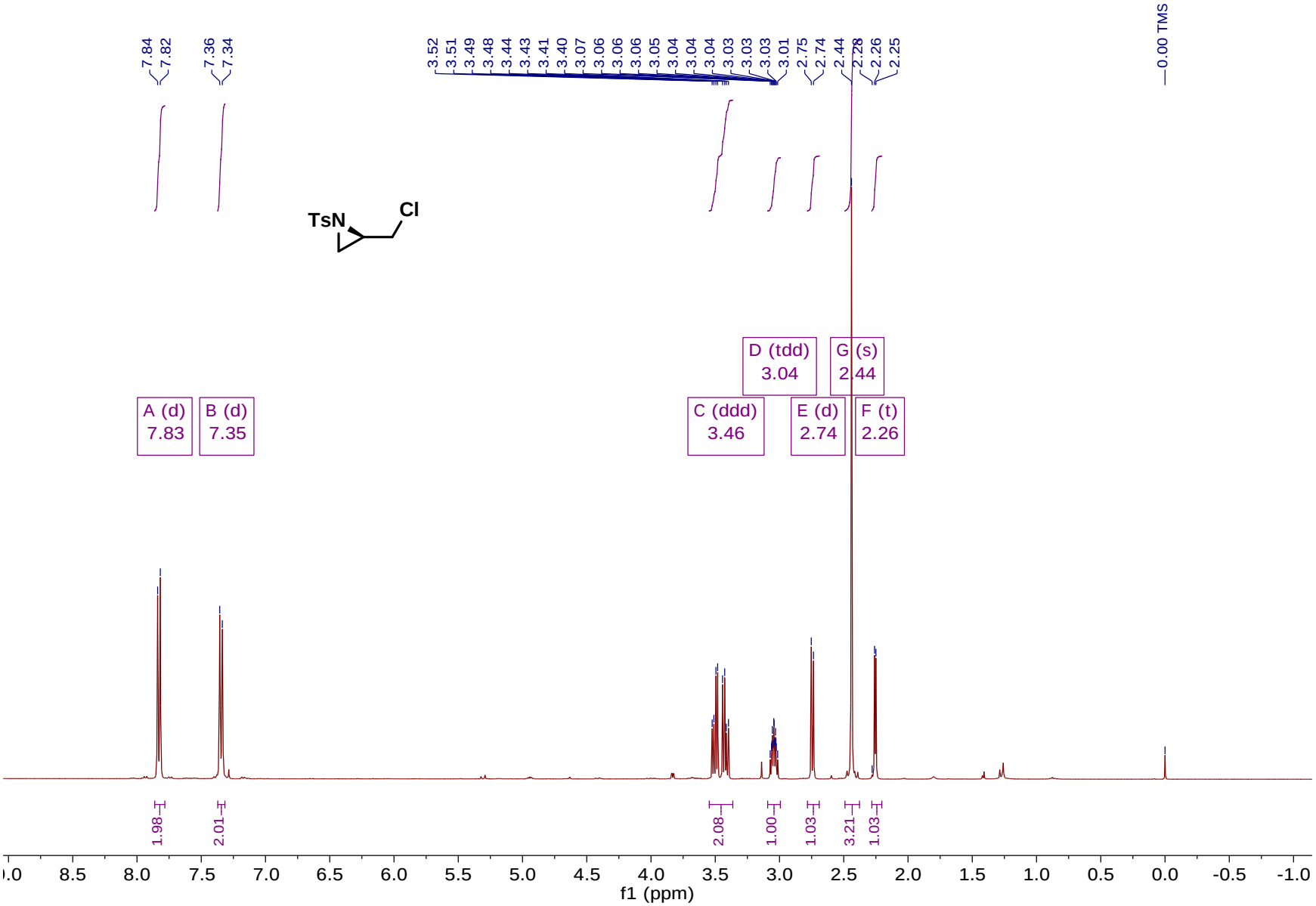


Figure 46: ^{13}C-DEPTQ spectrum of compound **14**, with CH/CH_3 phased up, and CH_2/Cq phased down.

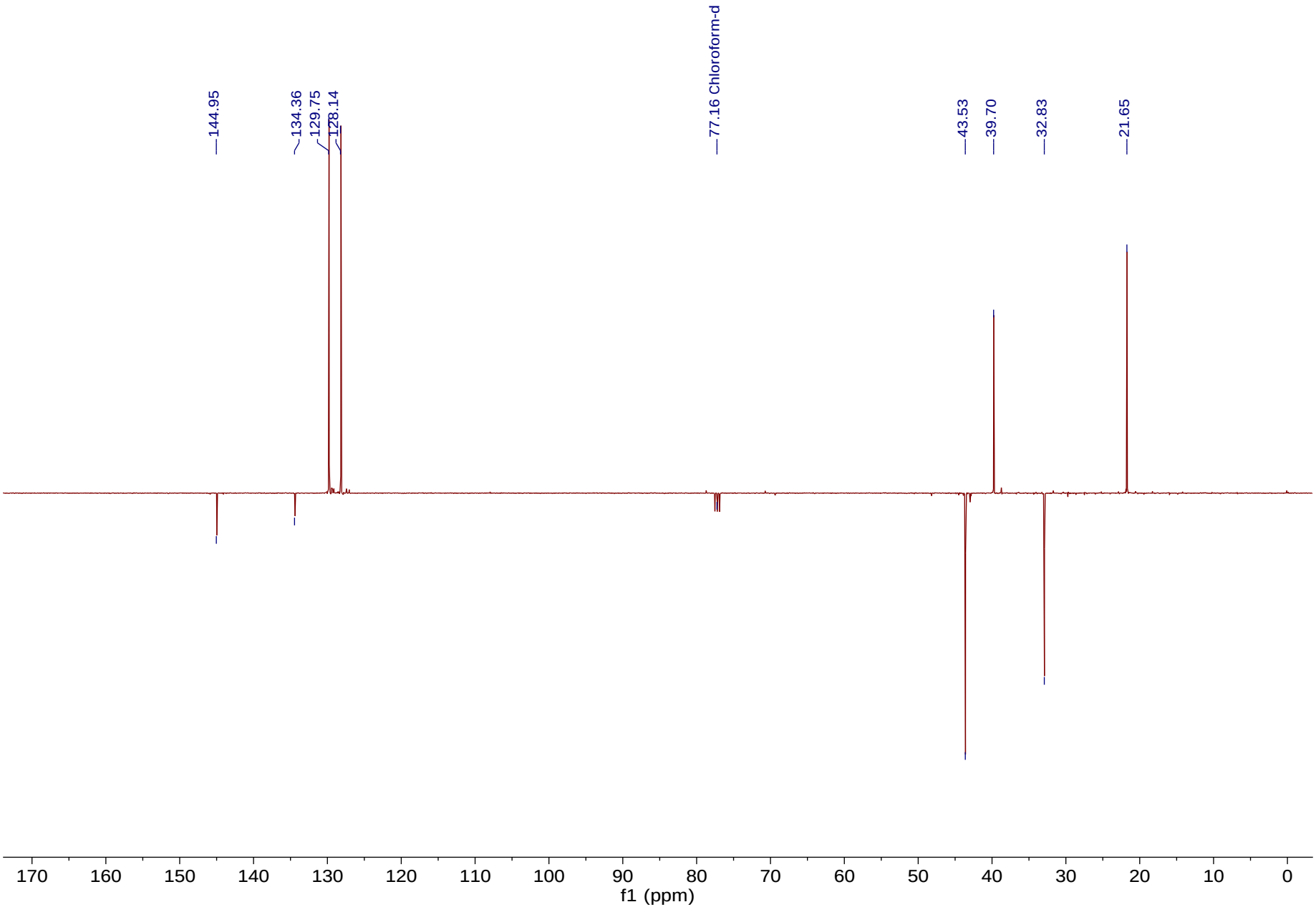


Figure 47: COSY spectrum of compound **14**.

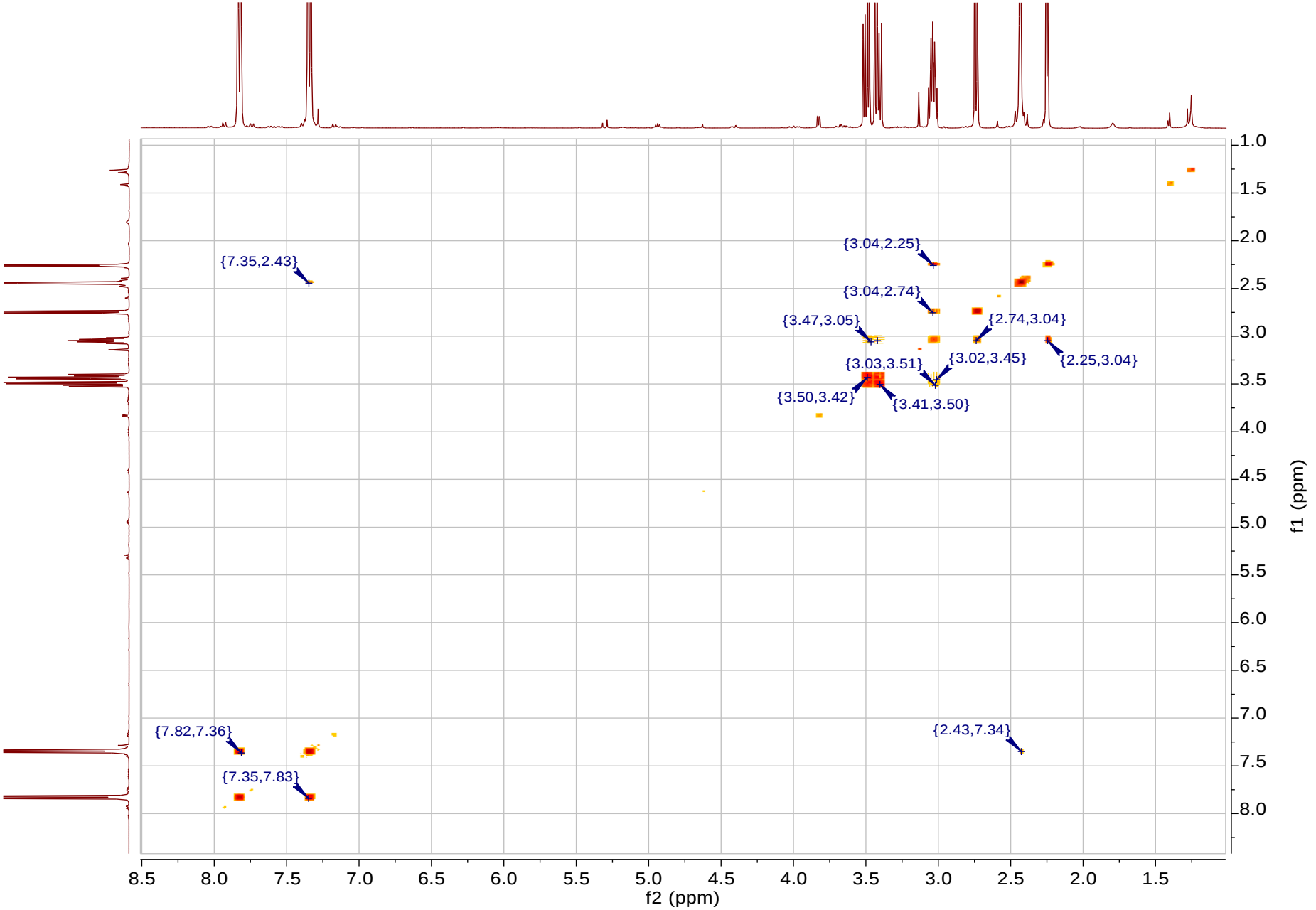
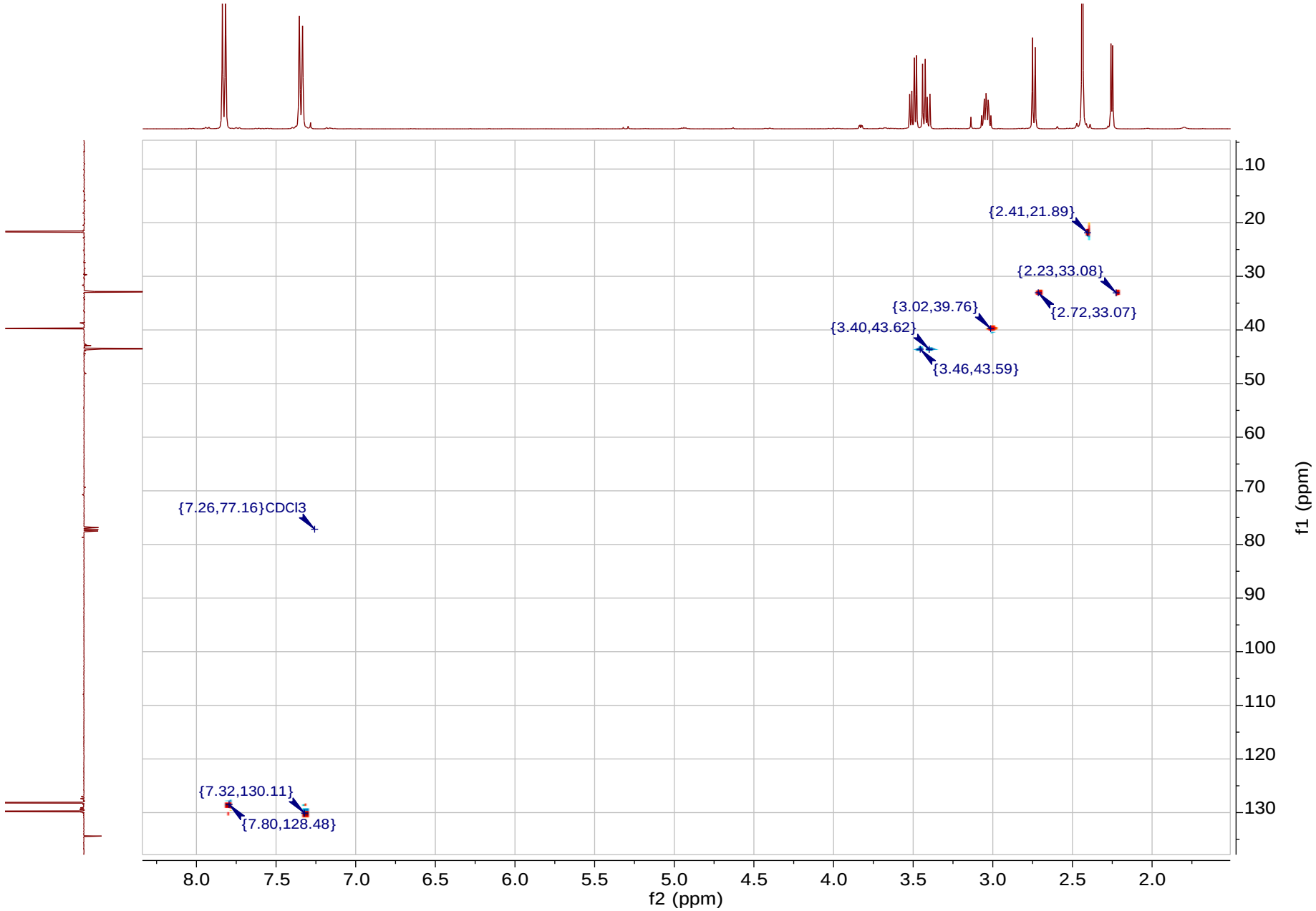


Figure 48: HSQC spectrum of compound **14**.



(*R*)-*N*-((13,14-dioxo-6,7,13,14-tetrahydropyrazino[1,2-*a*:4,3-*a'*]diindol-6-yl)methyl)-4-methylbenzenesulfonamide (15)

Figure 49: ^{1}H spectrum of compound **15**

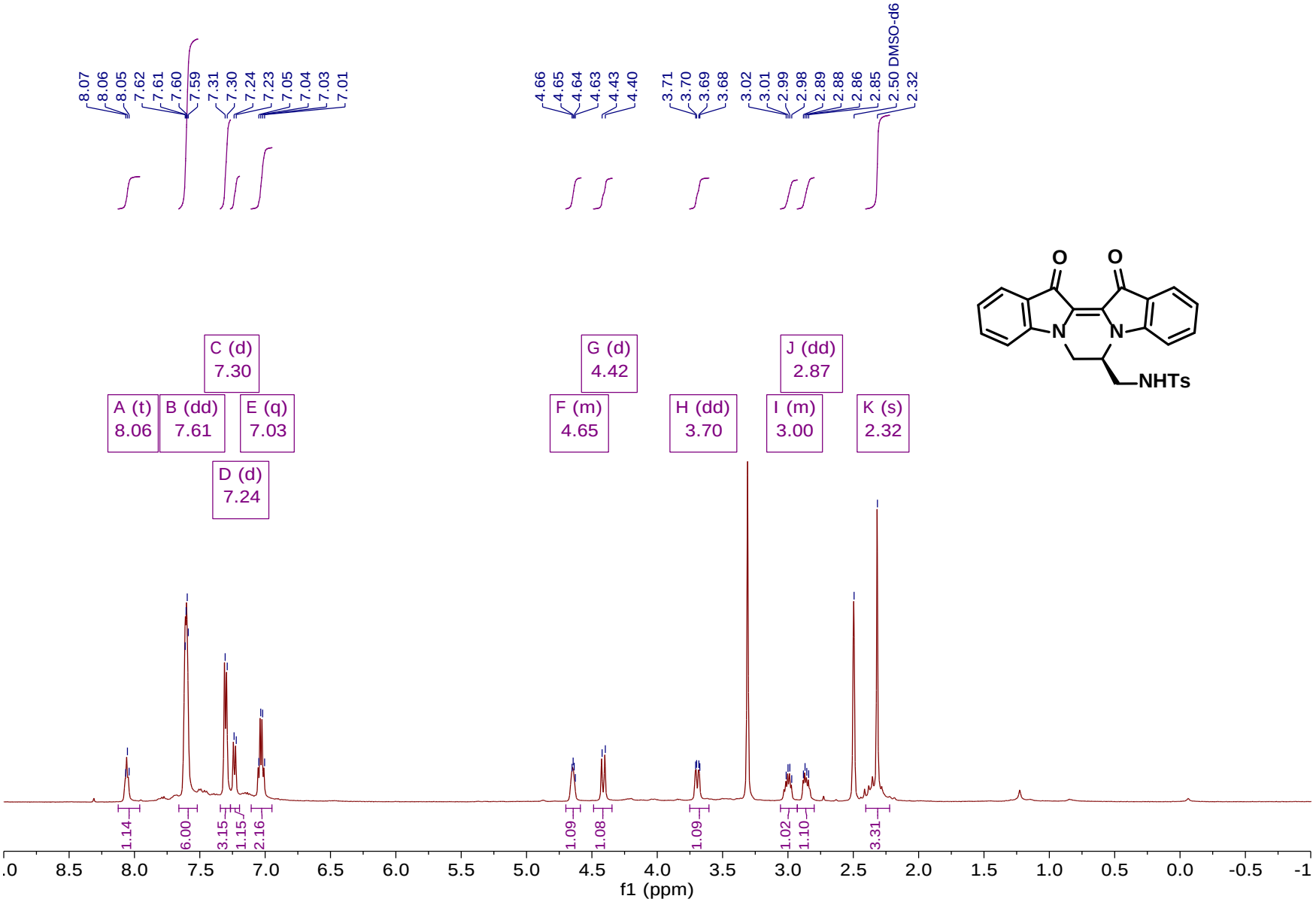


Figure 50: ^{13}C spectrum of compound **15**, with expansion of the region 155-100 ppm

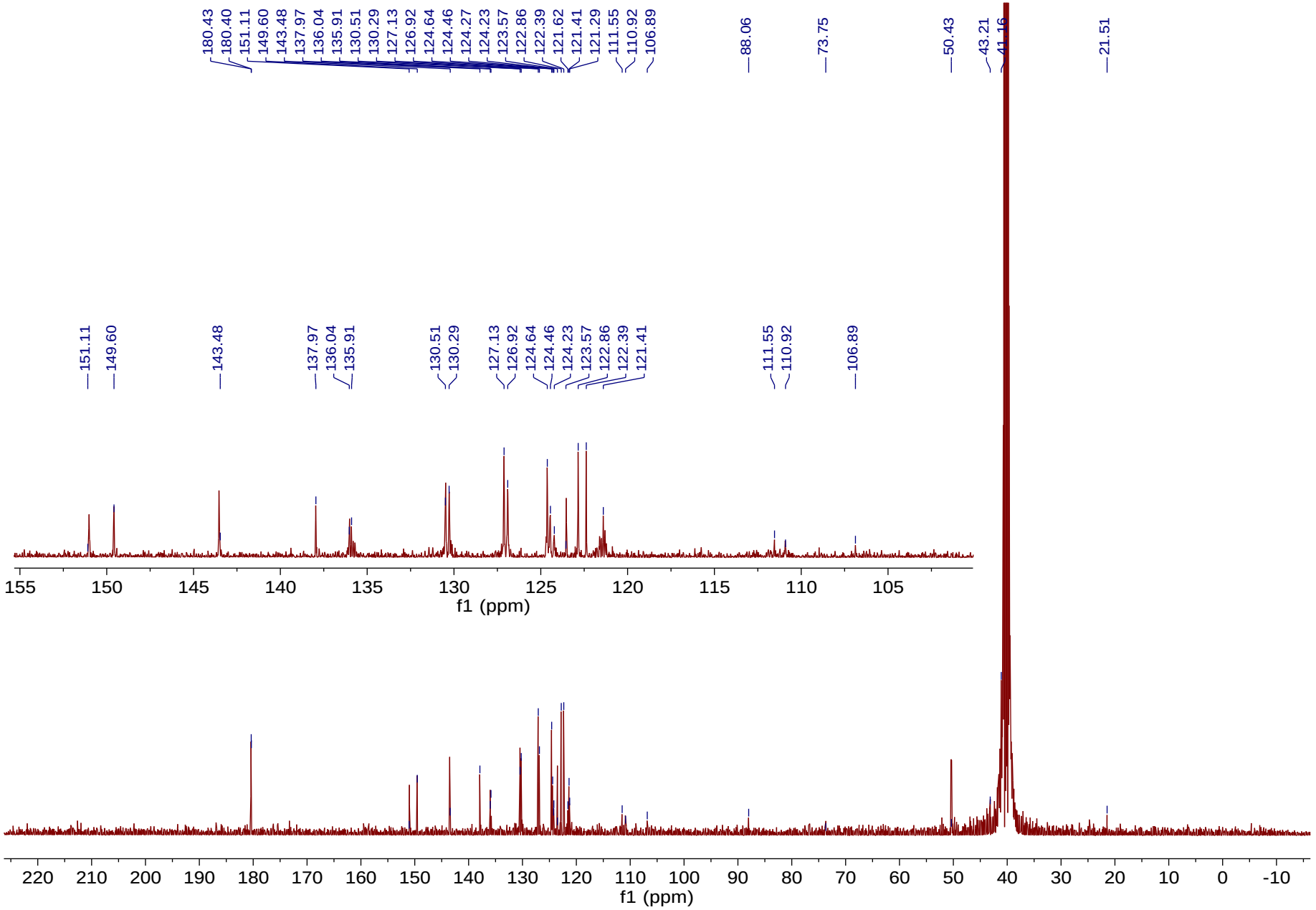


Figure 51: COSY spectrum of compound **15**

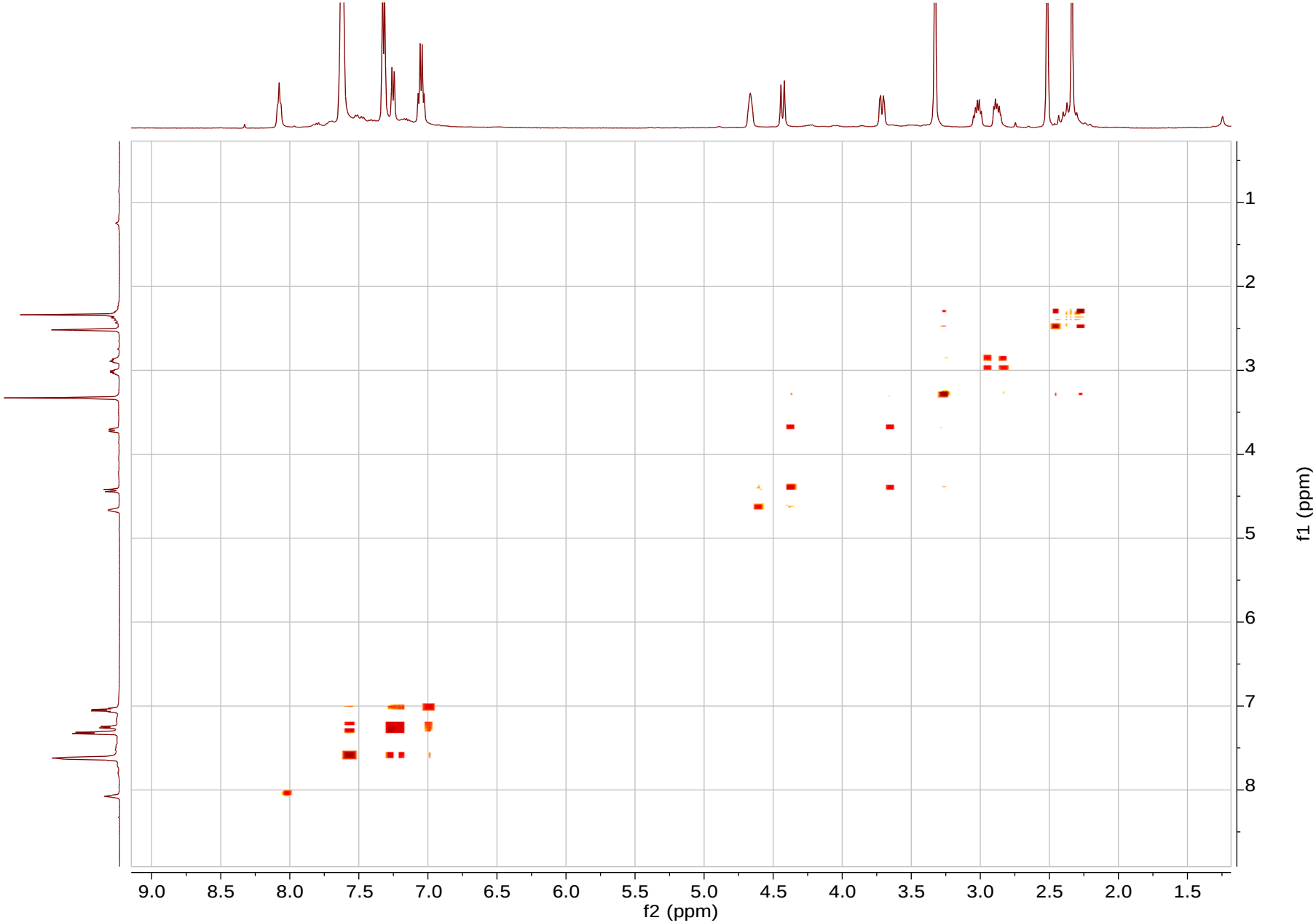


Figure 52: ROESY spectrum of compound **15**

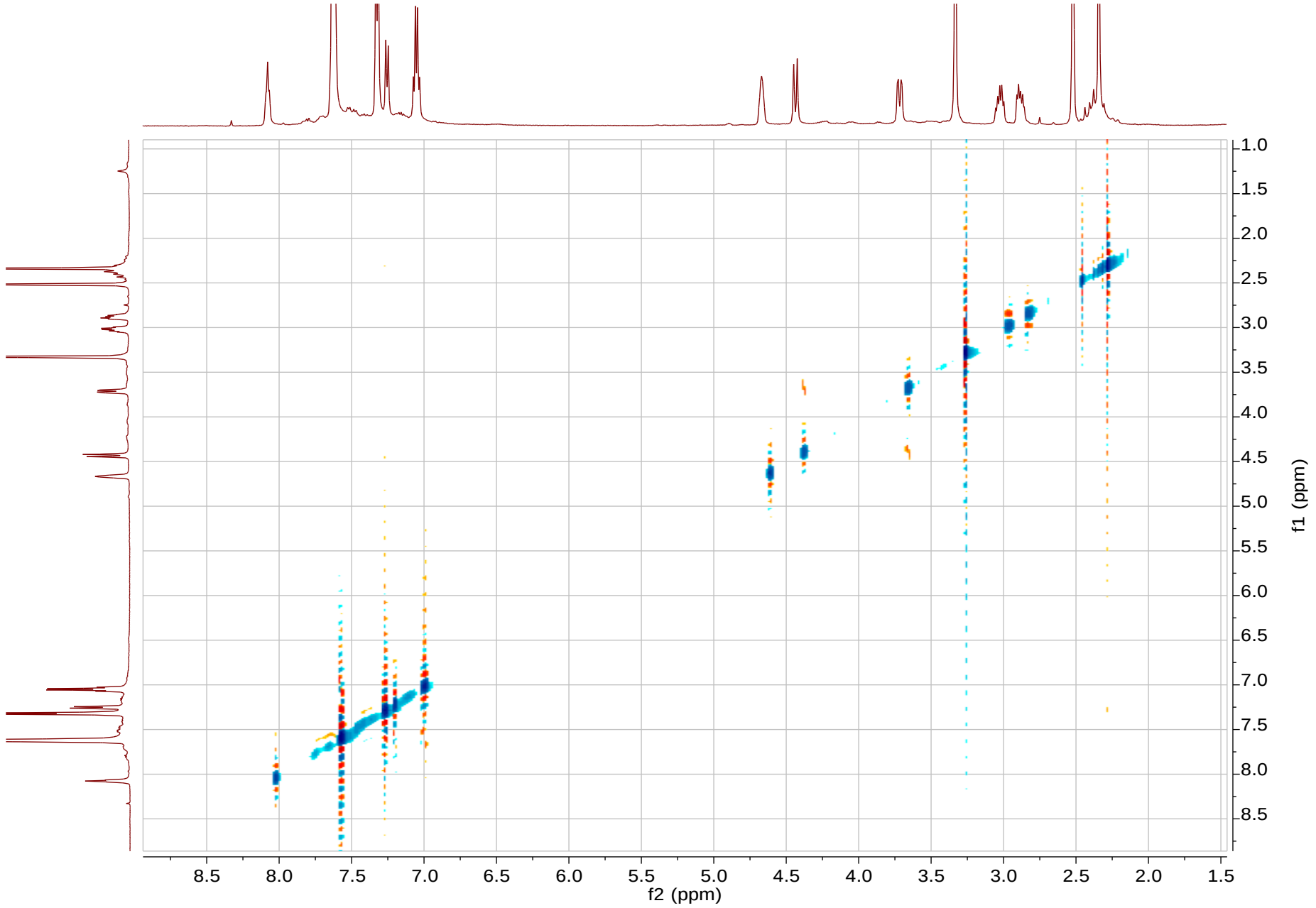


Figure 53: HSQC spectrum of compound **15**

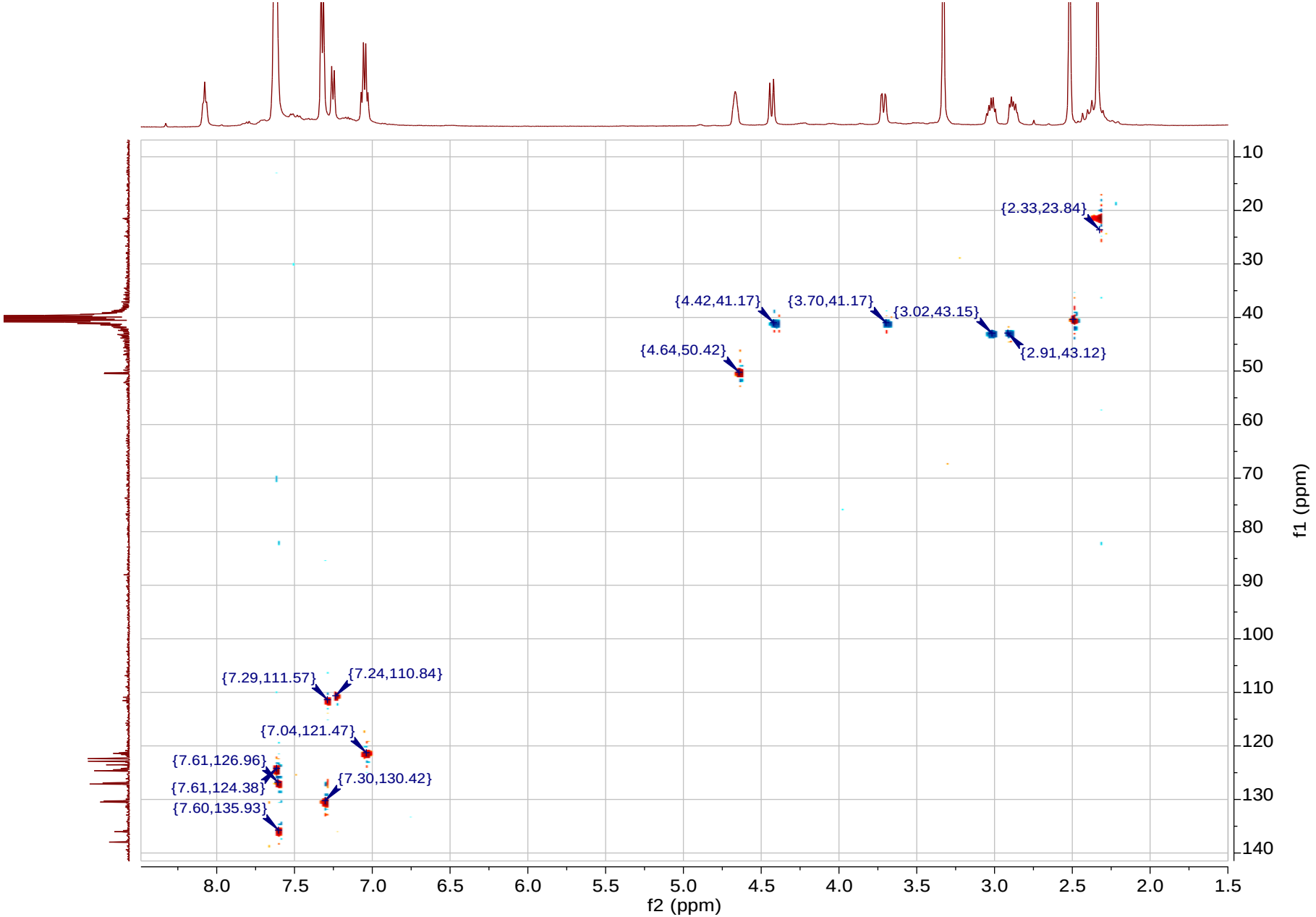
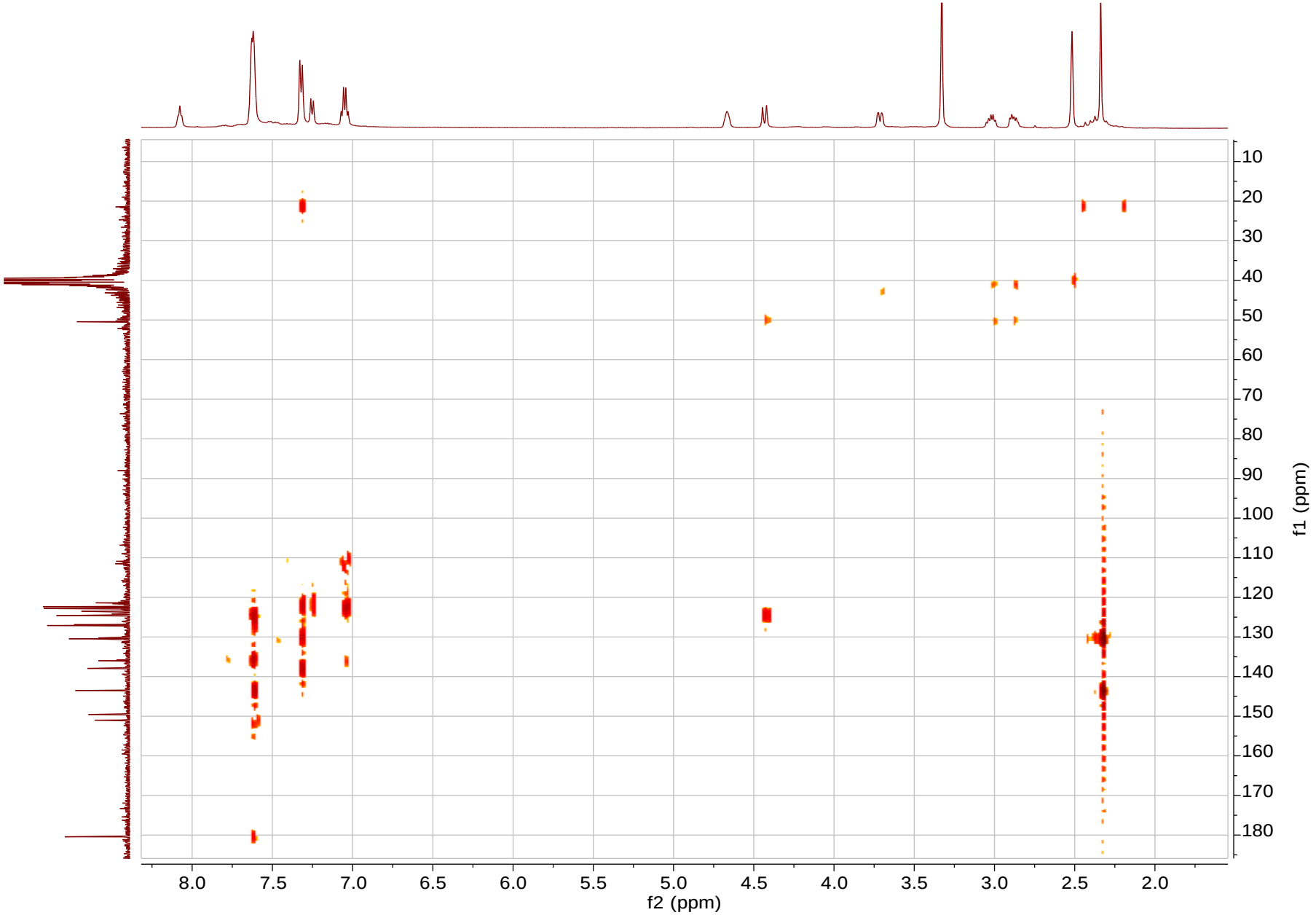


Figure 54: HMBC spectrum of compound **15**



_N_-(((_R_)-13,14-dioxo-6,7,13,14-tetrahydropyrazino[1,2-_a_:4,3-_a'_]diindol-6-yl)methyl)-4-methyl-_N_-(((_S_)-1-tosylaziridin-2-yl)methyl)benzenesulfonamide (16)

Figure 55: ^{1}H spectrum of compound **16** ($CDCl_3$). The impurities present at 1.8 – 2.0 ppm could not be removed despite repeated attempts. HPLC analysis indicated the presence of one compound only (Figure 87).

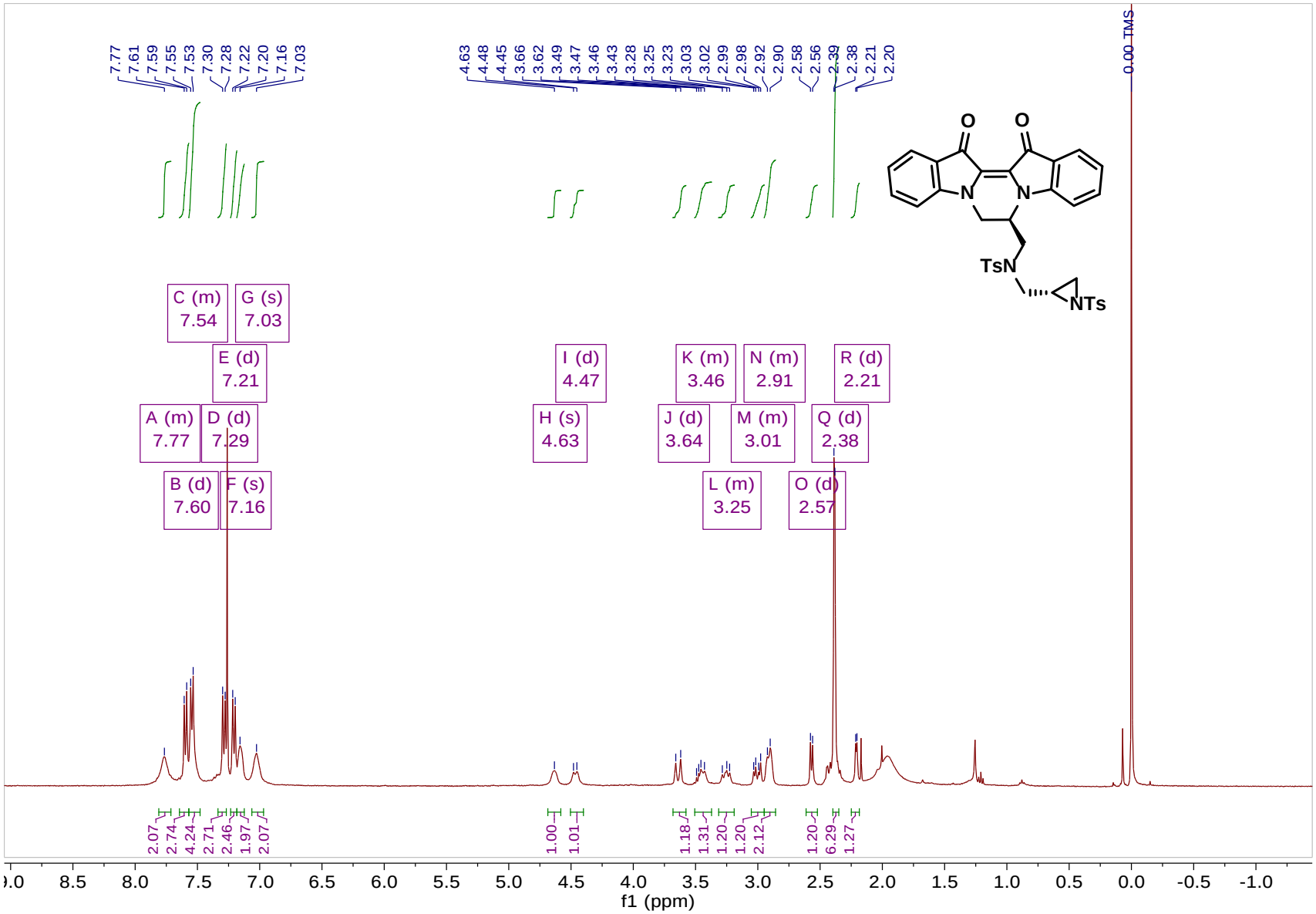


Figure 56: ^{13}C spectrum of compound **16** ($CDCl_3$)

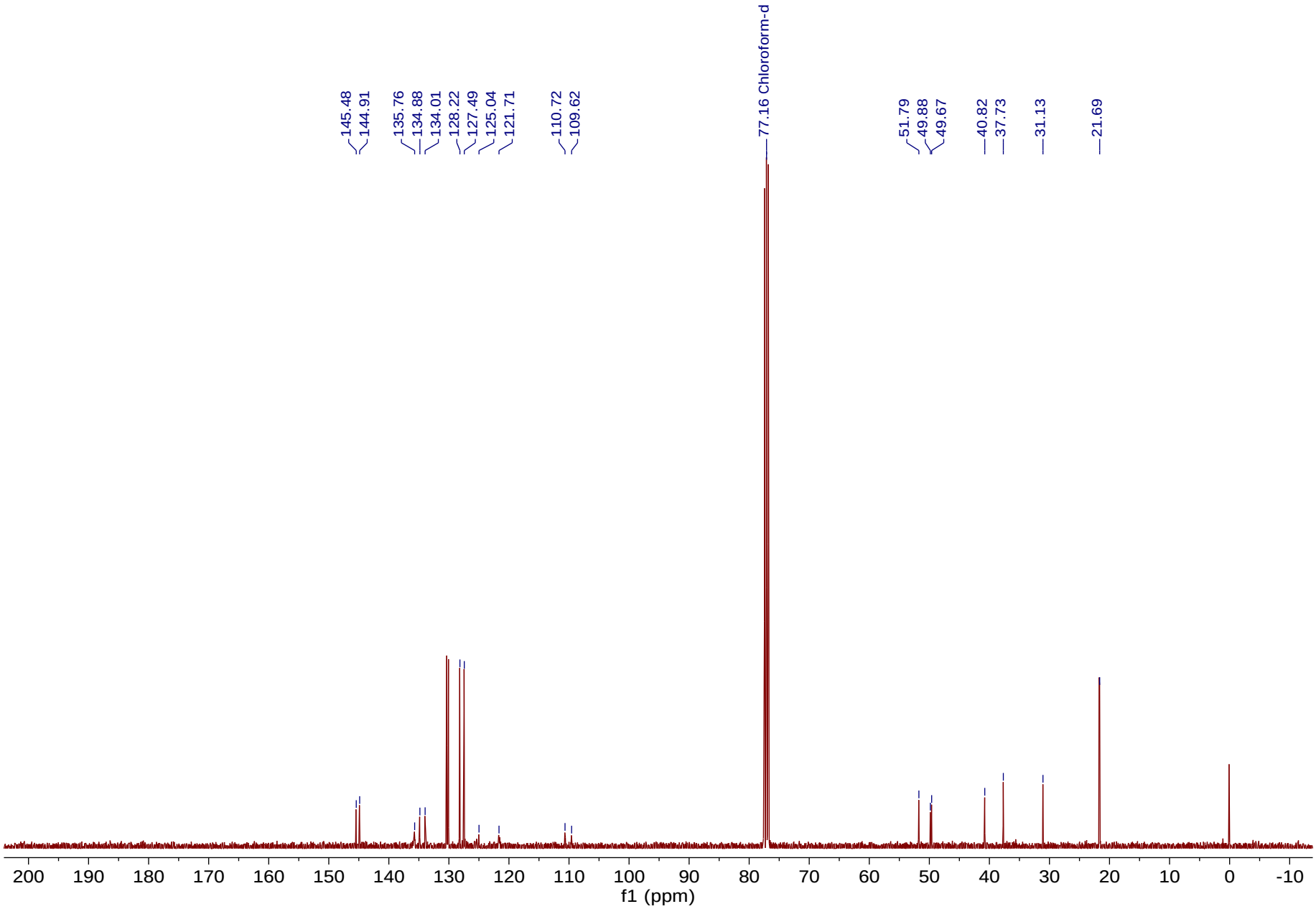


Figure 57: ^{13}C spectrum of compound **16 (**DMSO)

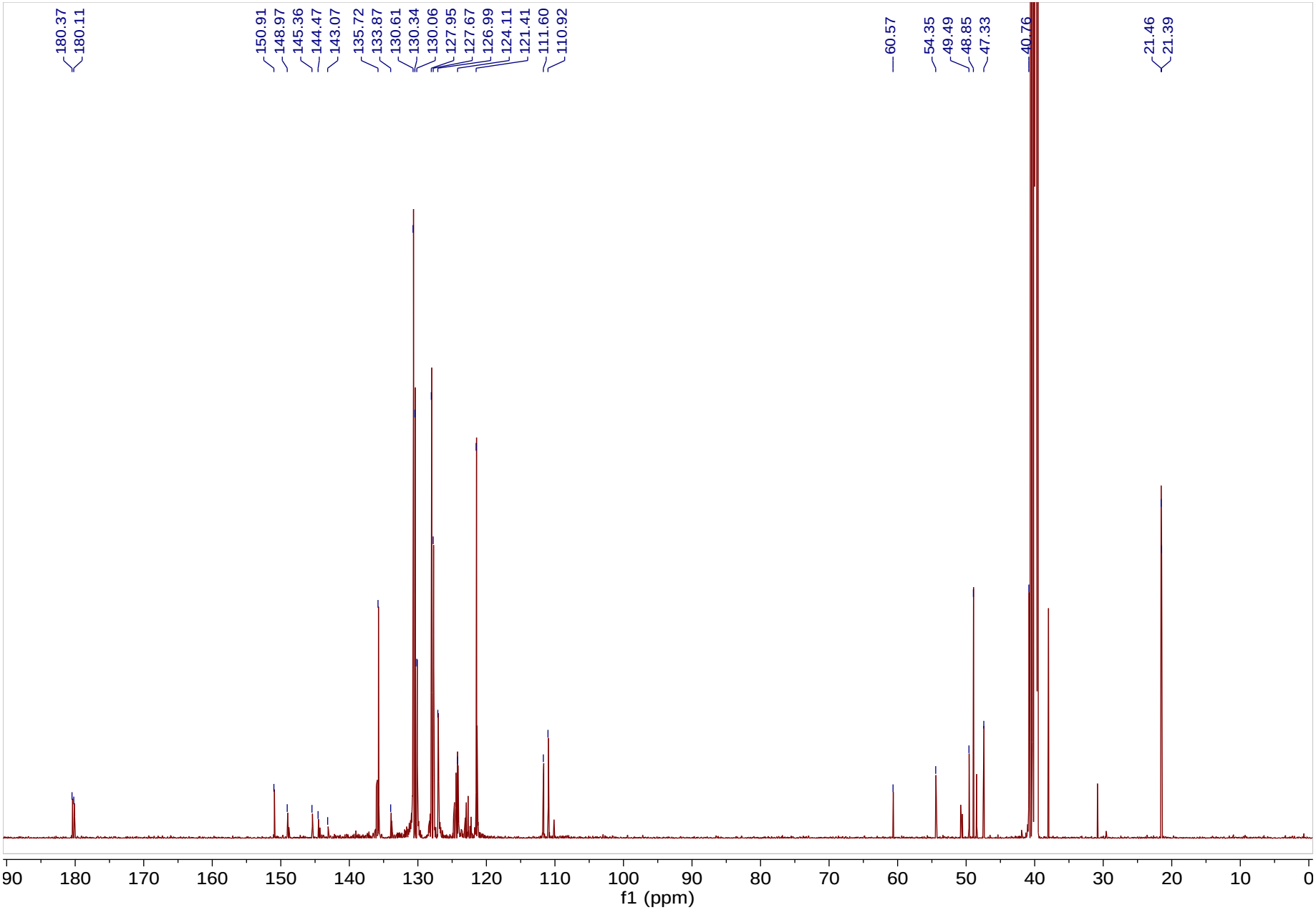


Figure 58: COSY spectrum of compound **16**

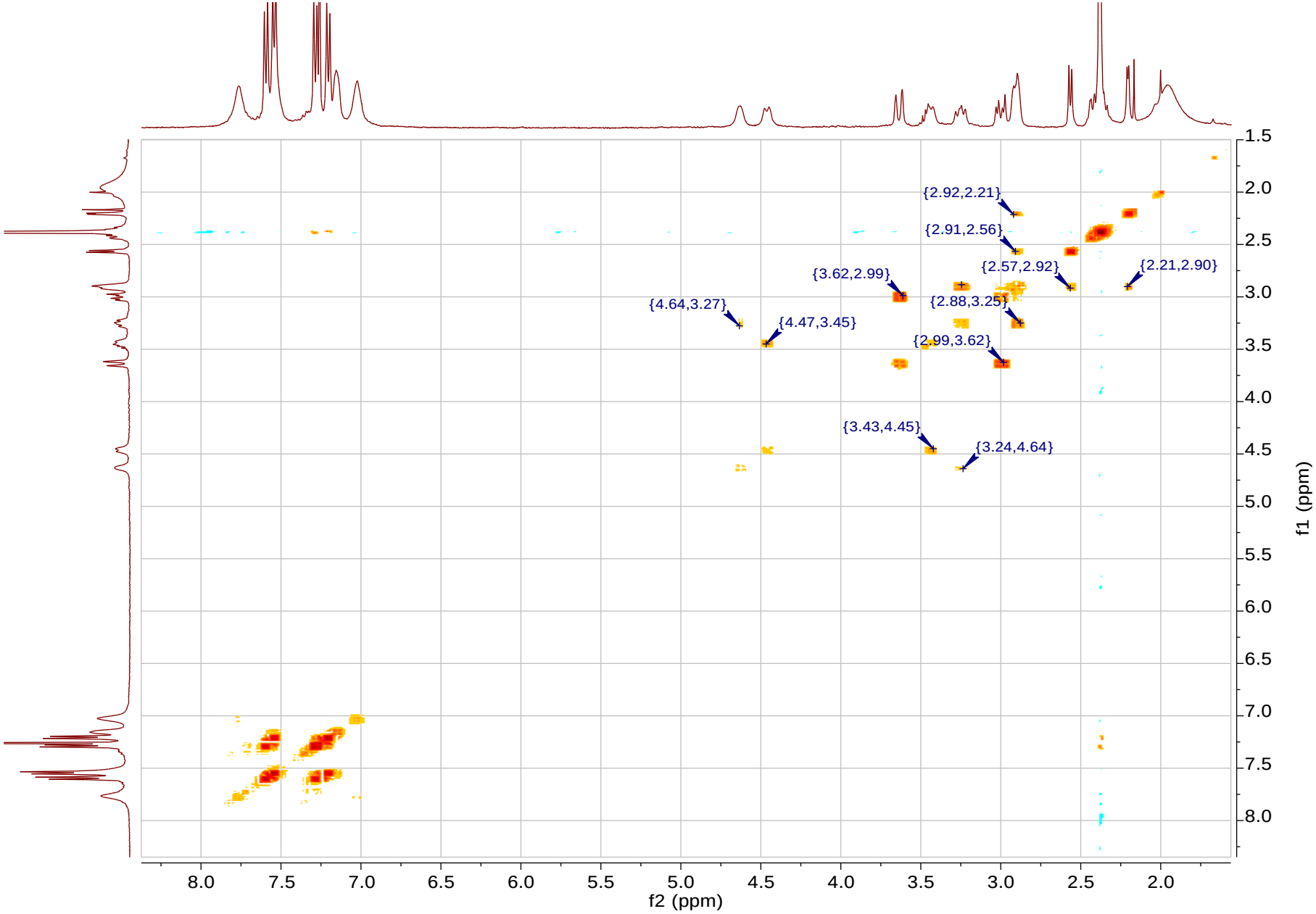


Figure 59: TOCSY spectrum of compound **16**

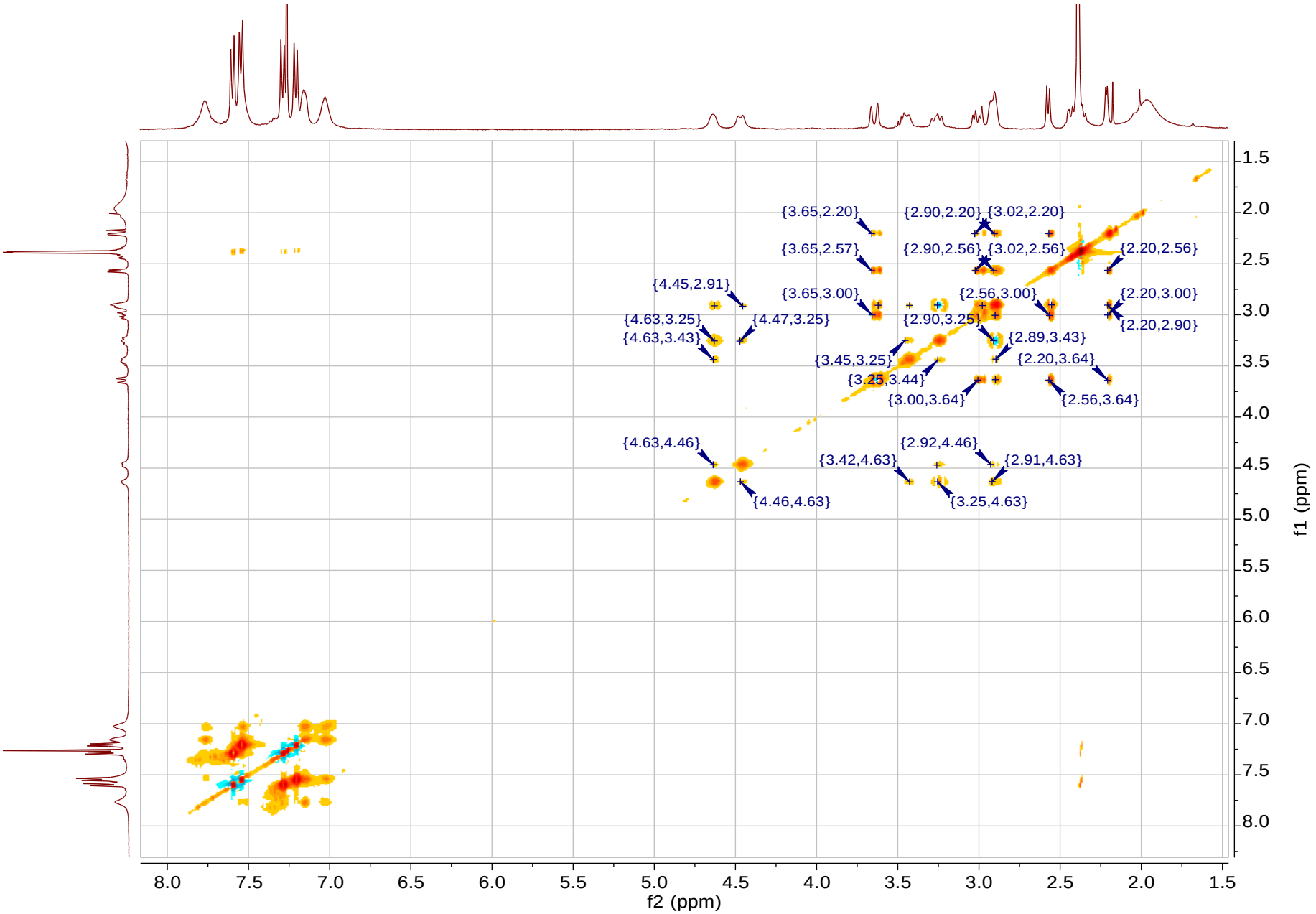
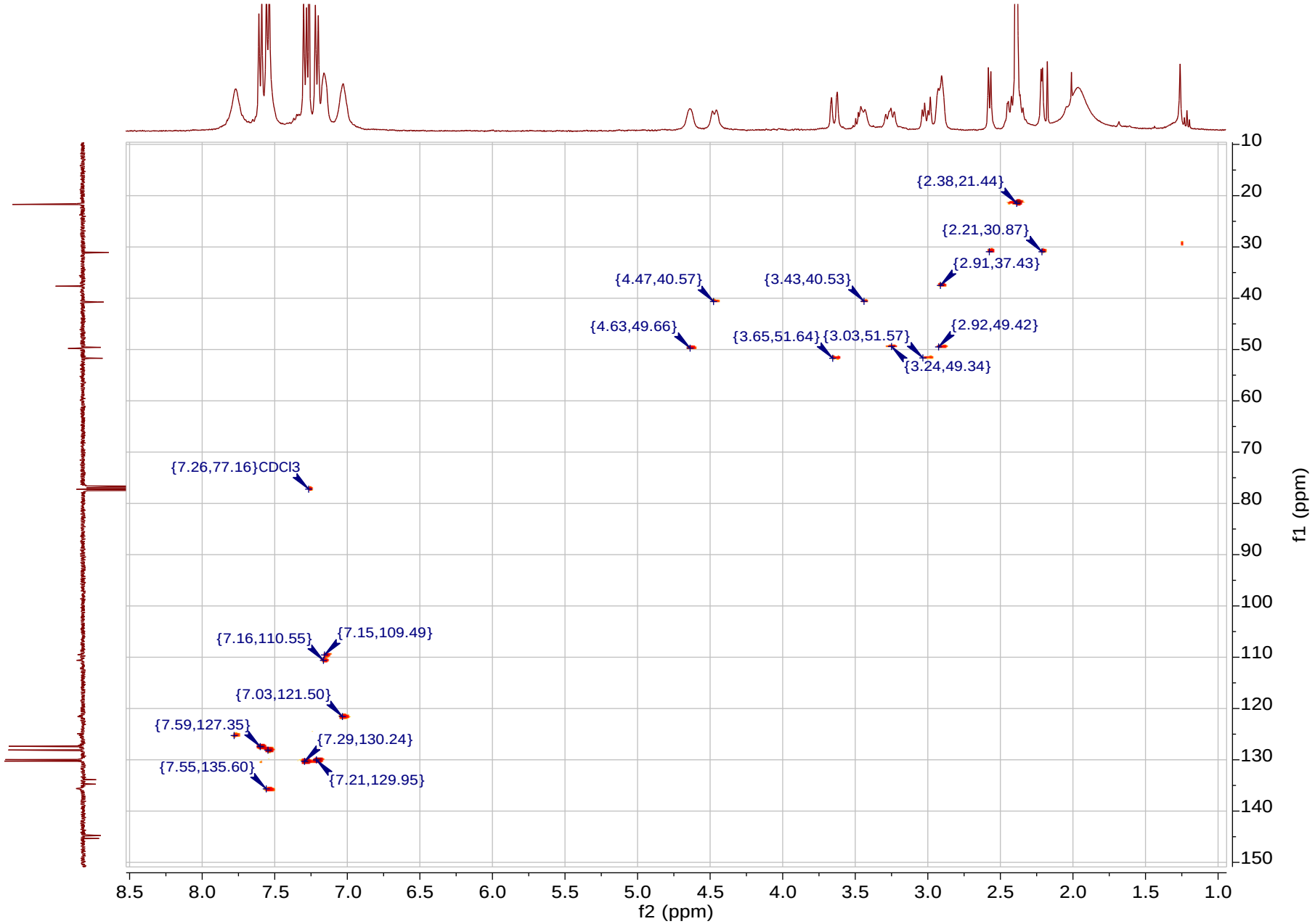


Figure 60: HSQC spectrum of Compound **16** ($CDCl_3$)



_N_-((_R_)-3-chloro-2-((4-methylphenyl)sulfonamido)propyl)-_N_-(((_R_)-13,14-dioxo-6,7,13,14-tetrahydropyrazino[1,2-_a_:4,3-_a'_]diindol-6-yl)methyl)-4-methylbenzenesulfonamide (17)

Figure 61: ^{1}H spectrum of compound **17**

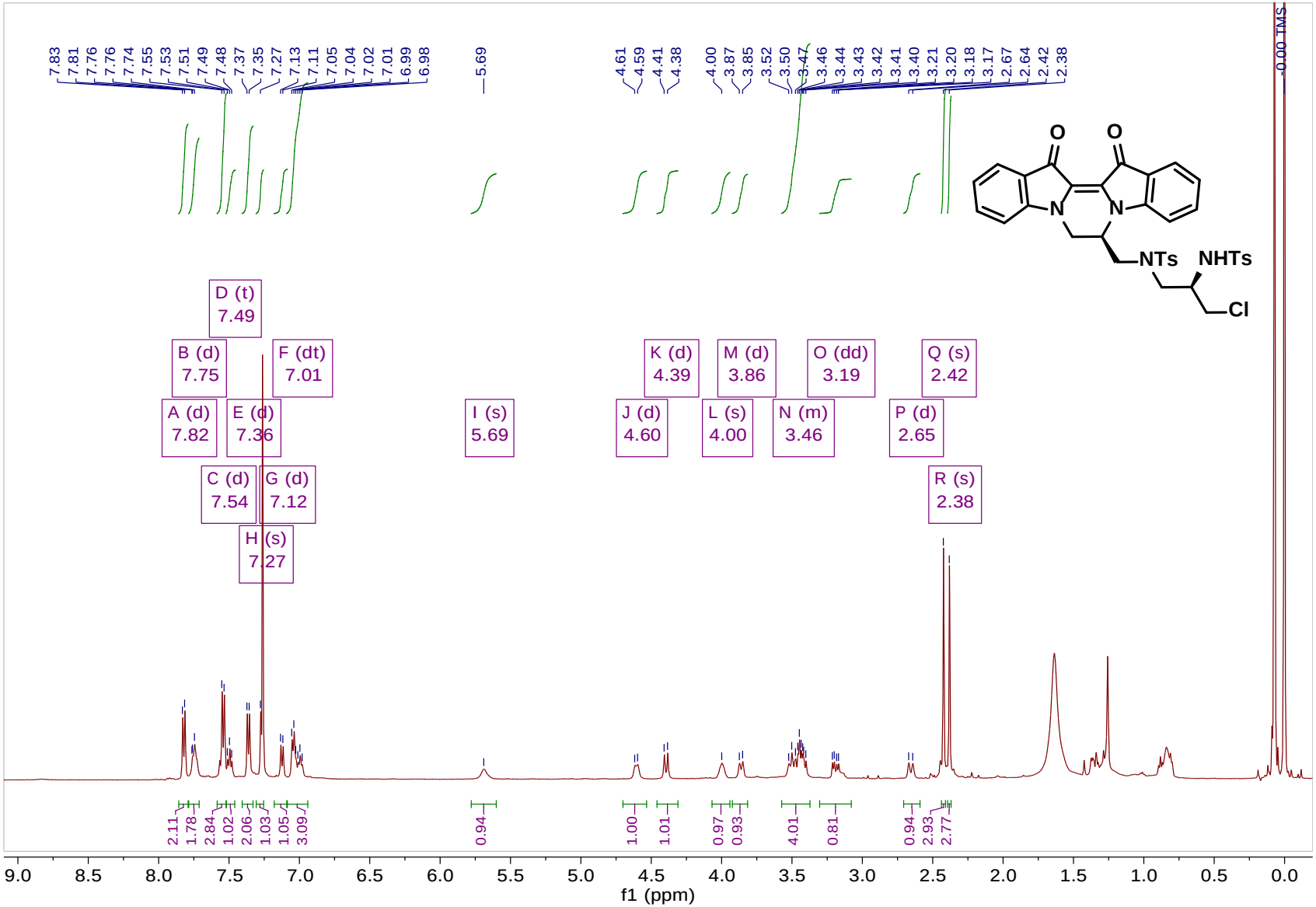


Figure 62: ^{13}C spectrum of compound **17**.

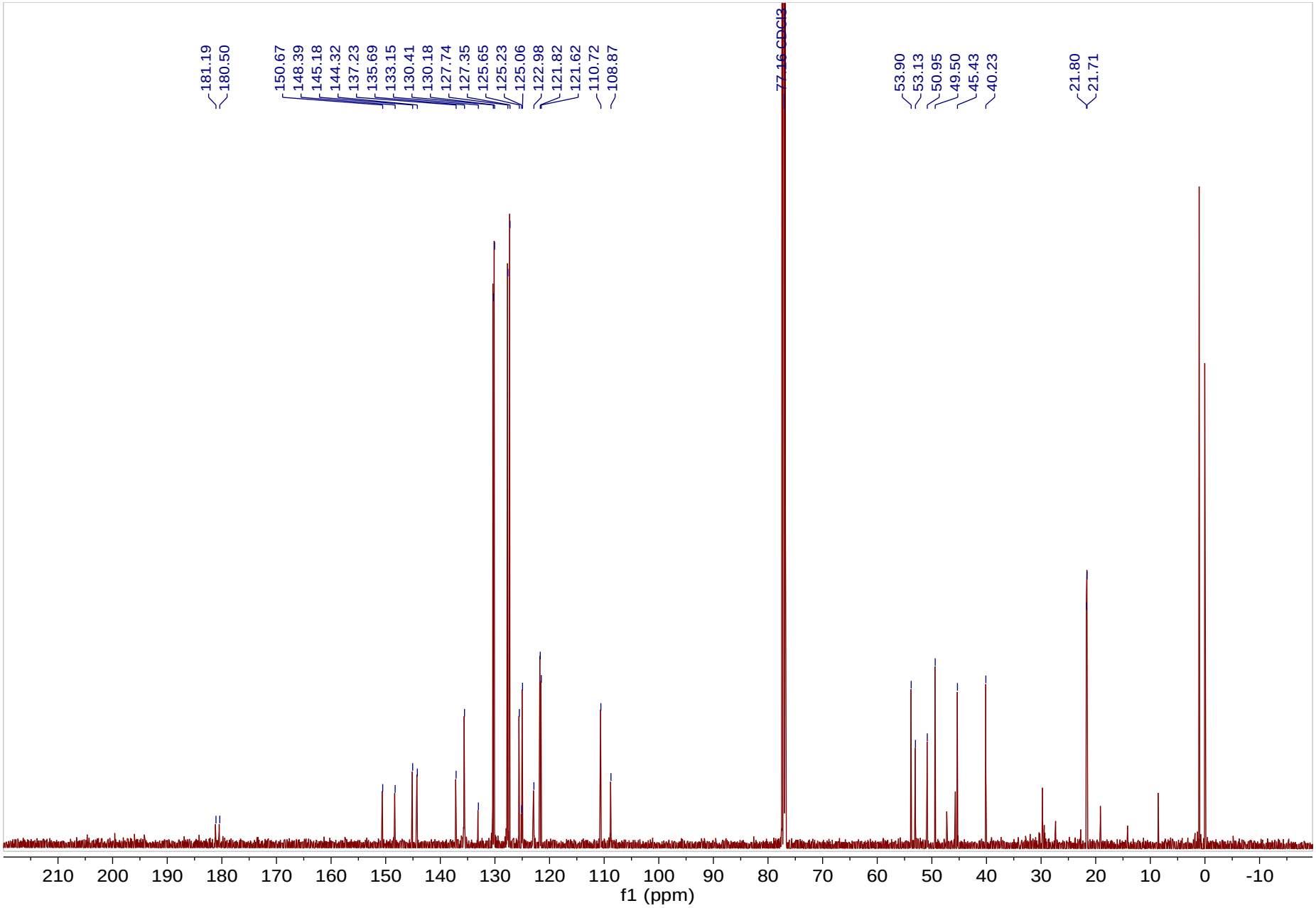


Figure 63: COSY spectrum of compound **17**.

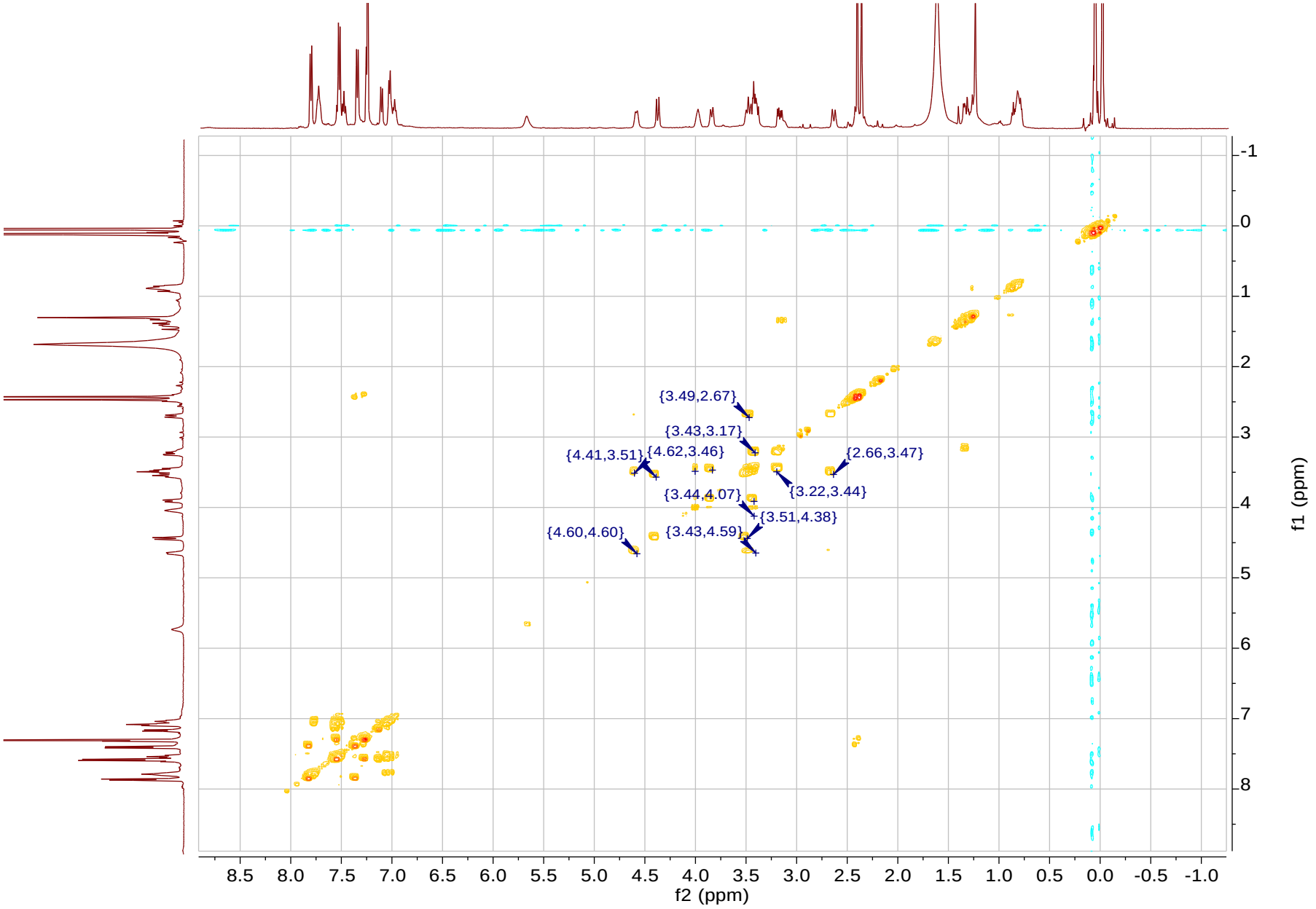


Figure 64: HMBC spectrum of compound **17**

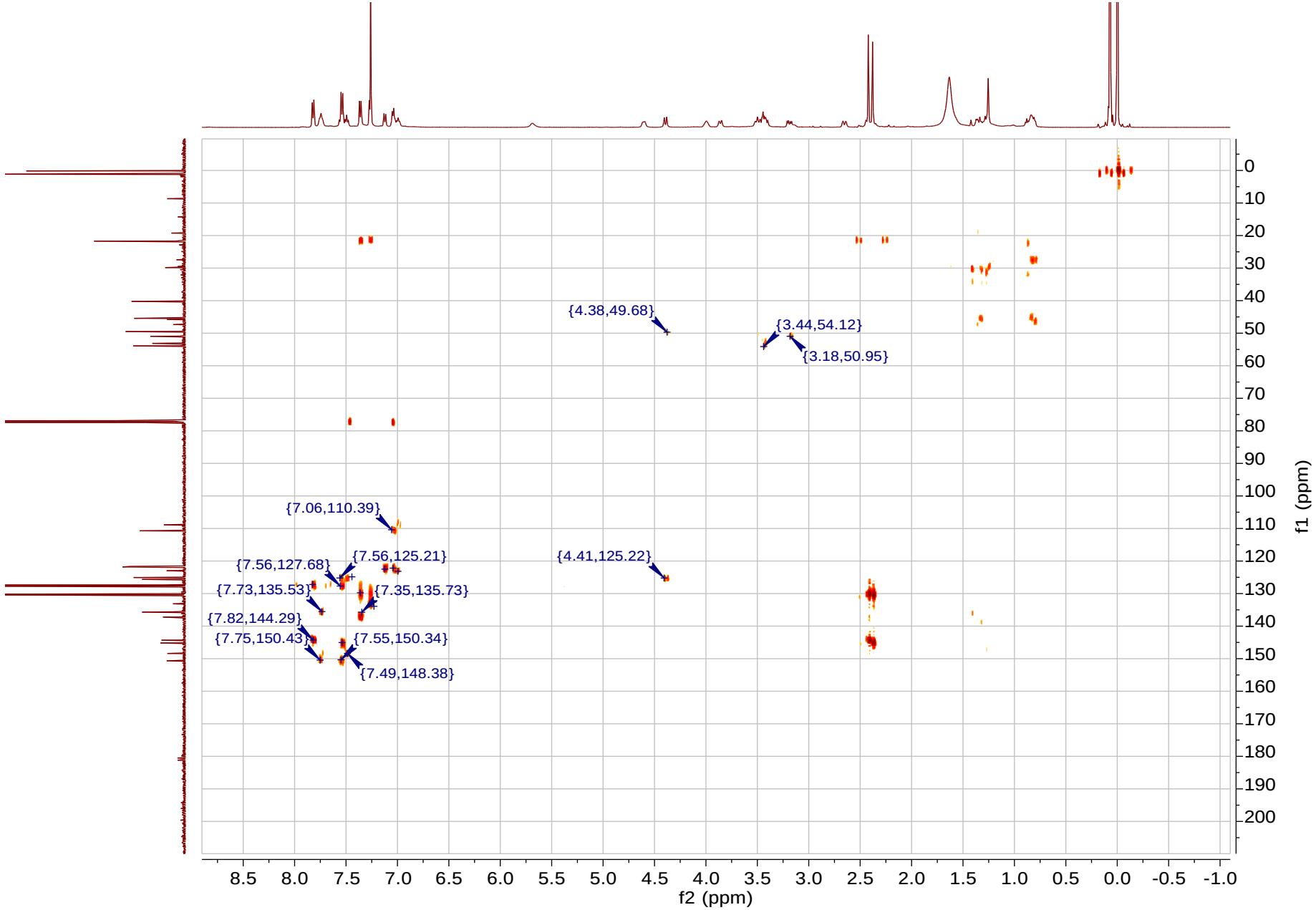


Figure 65: ROESY spectrum of compound **17**

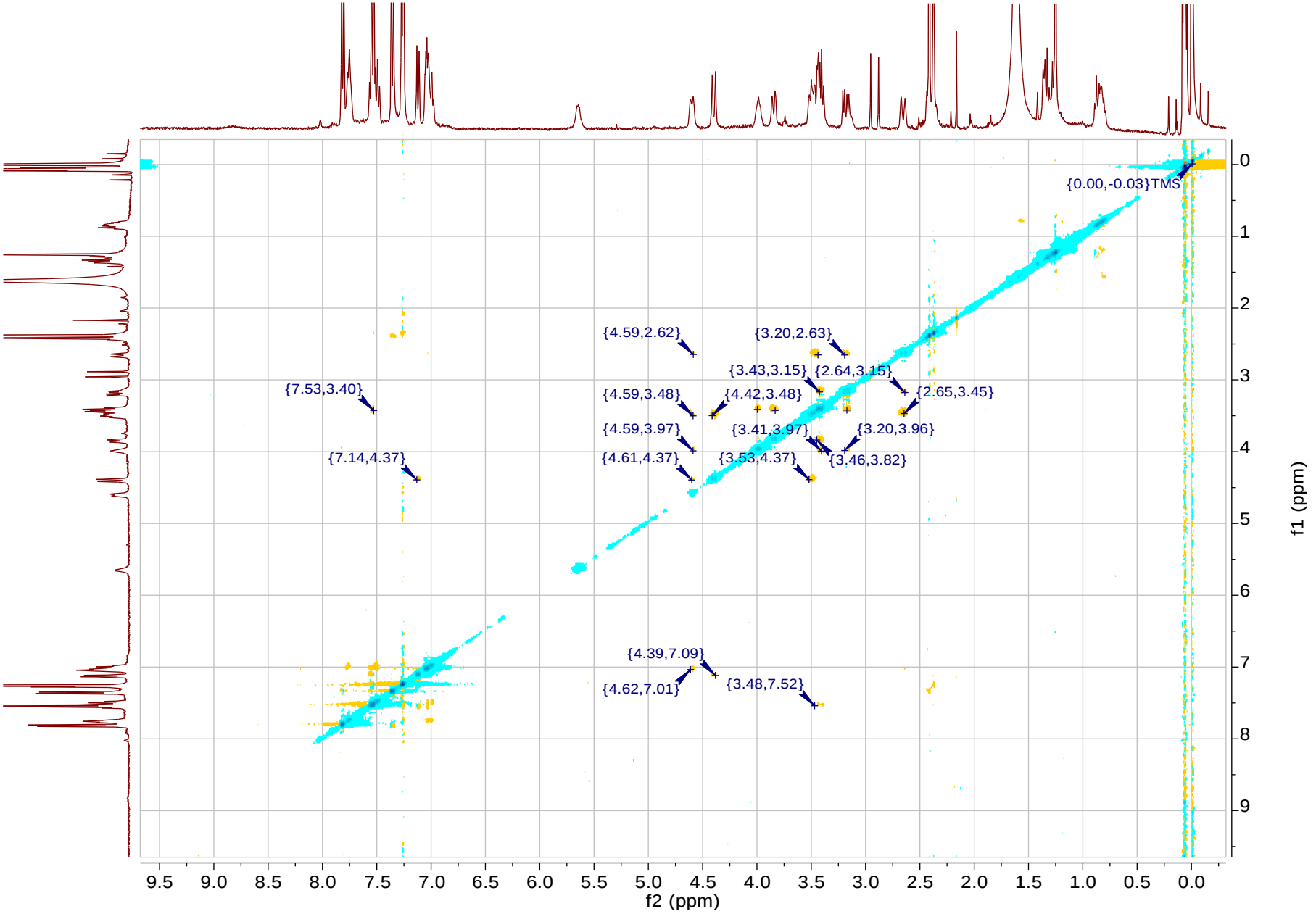


Figure 66: HSQC spectrum of compound **17**

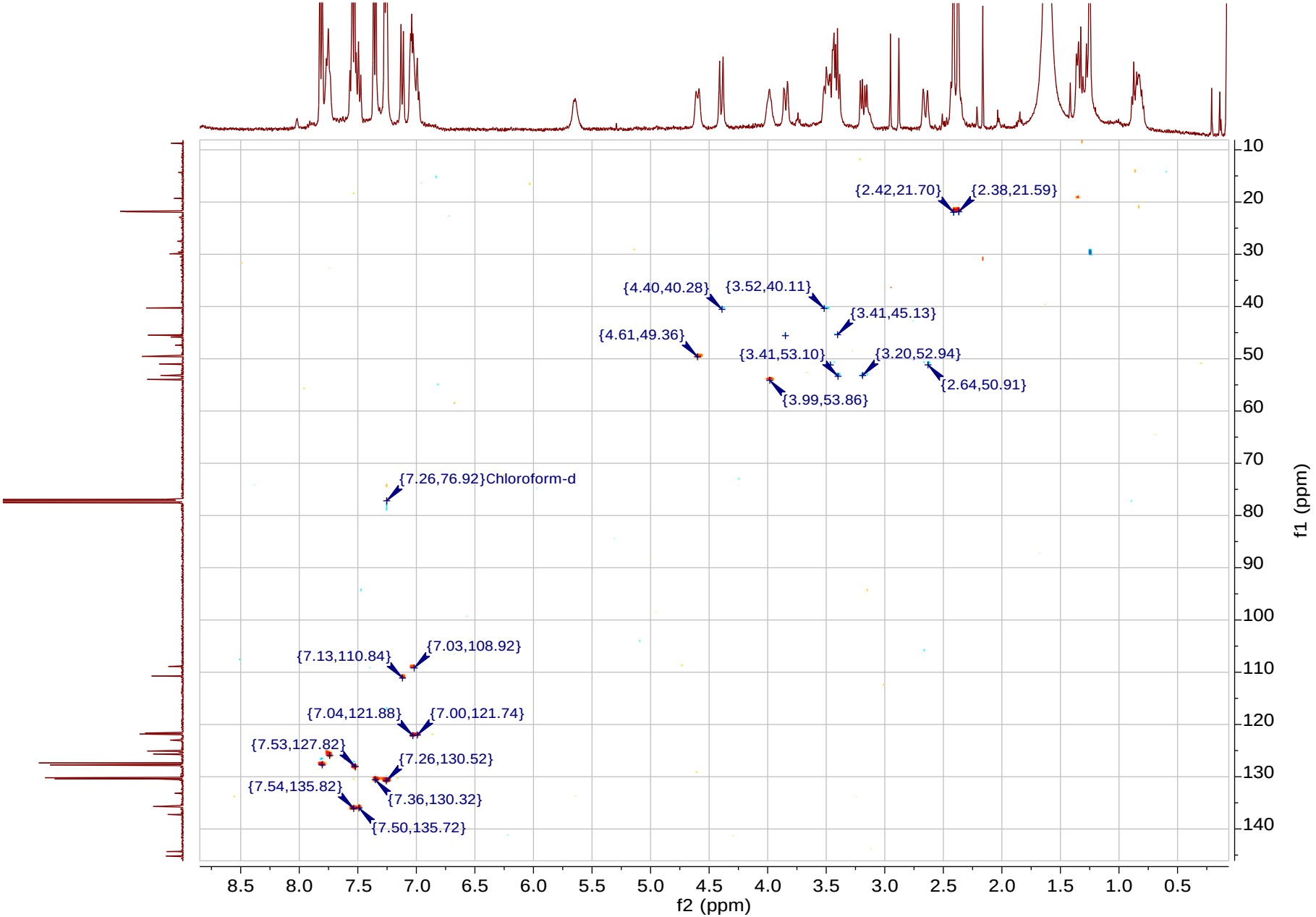
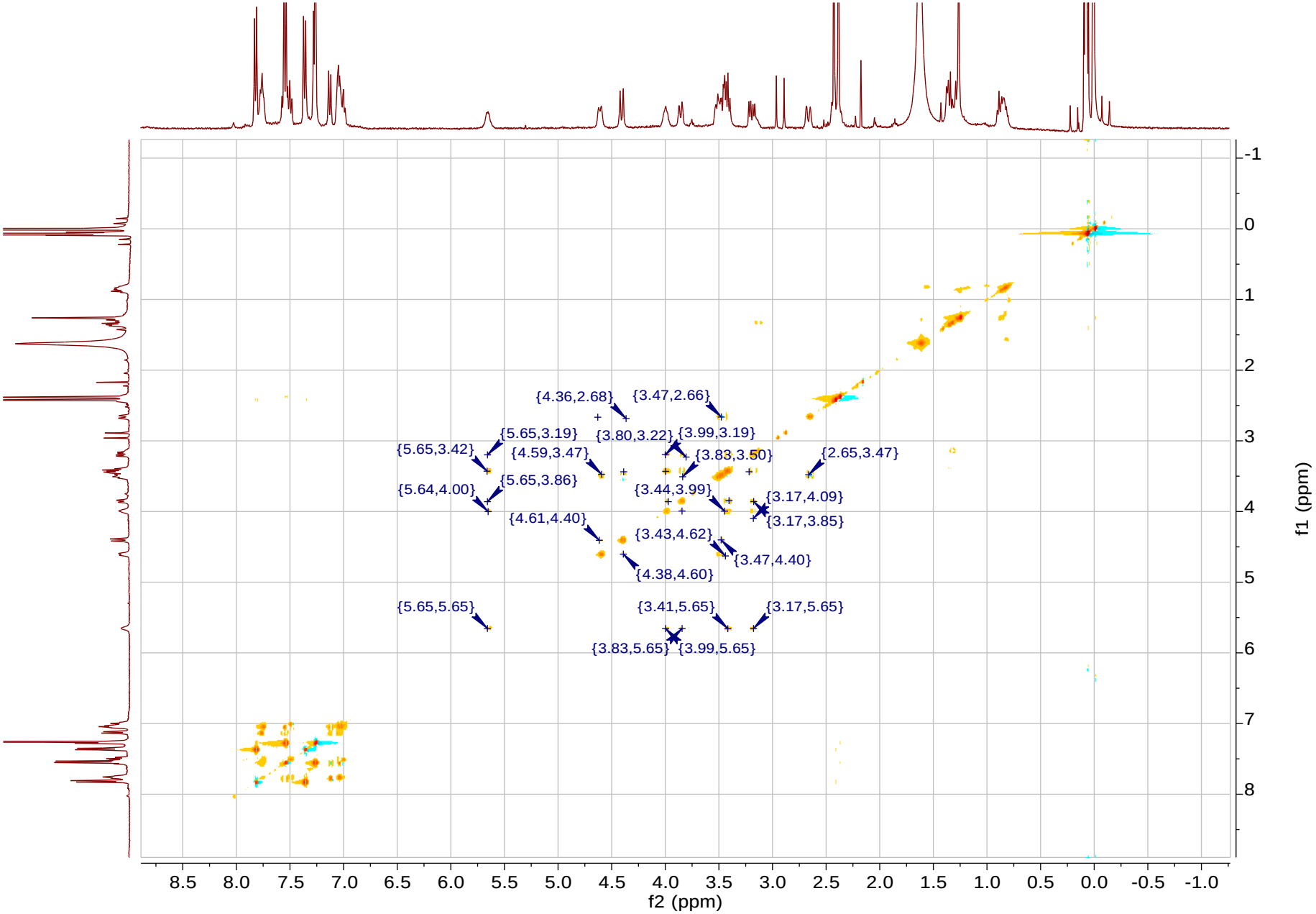
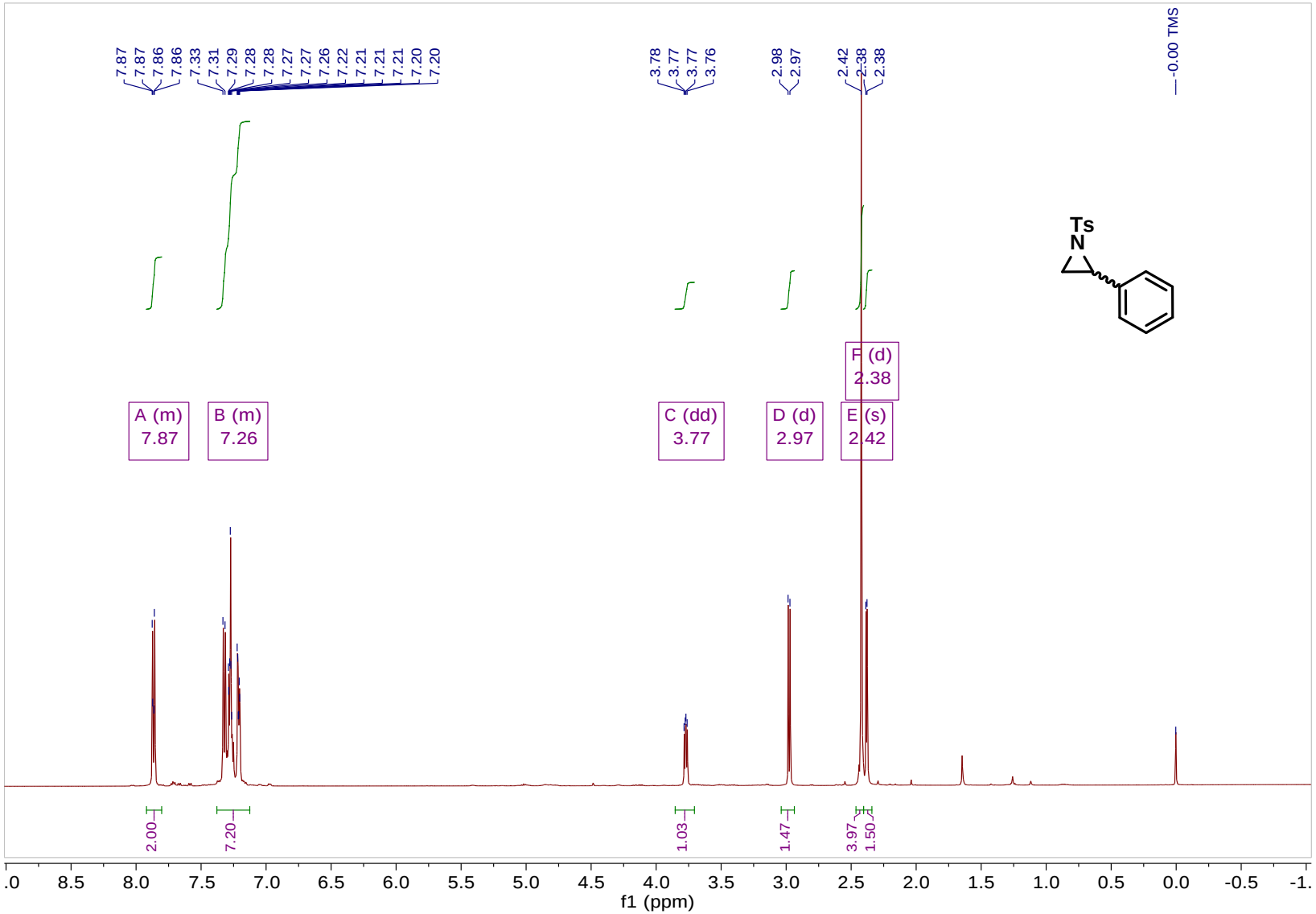


Figure 67: TOCSY spectrum of compound **17**



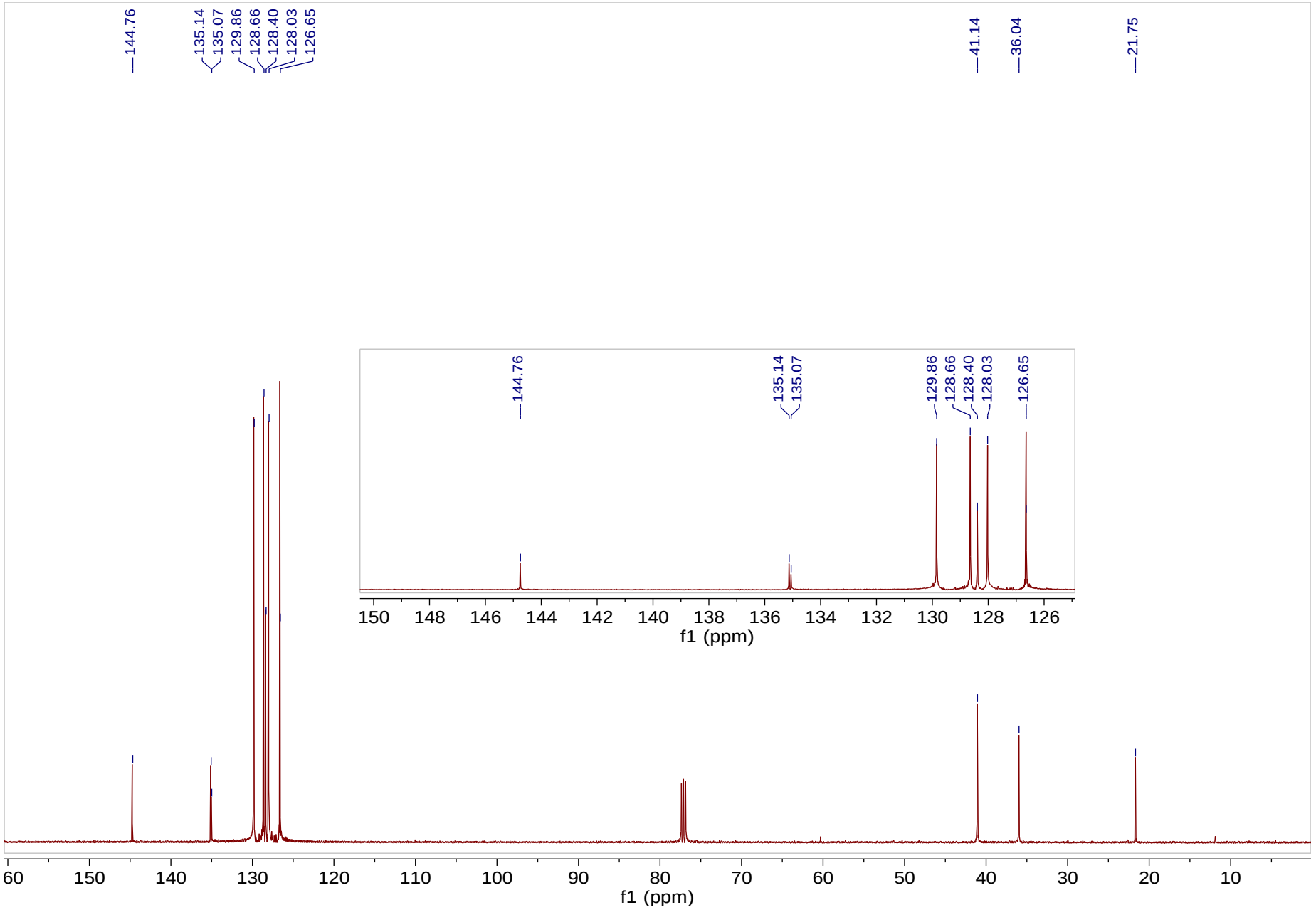
N-tosyl-2-phenylaziridine (18)[‡,4]

Figure 68: ^{1}H spectrum of compound **18**



[‡] Prepared by the method described in the main text, as a modification of the cited procedure.

Figure 69: ^{13}C spectrum of compound **18**, with expansion of the region downfield from 125 ppm



(*E*)-*N*-(2-(3,3'-dioxo-[2,2'-biindolinylidene]-1-yl)-1-phenylethyl)-4-methylbenzenesulfonamide (19)

Figure 70: ^{1}H spectrum of compound **19**

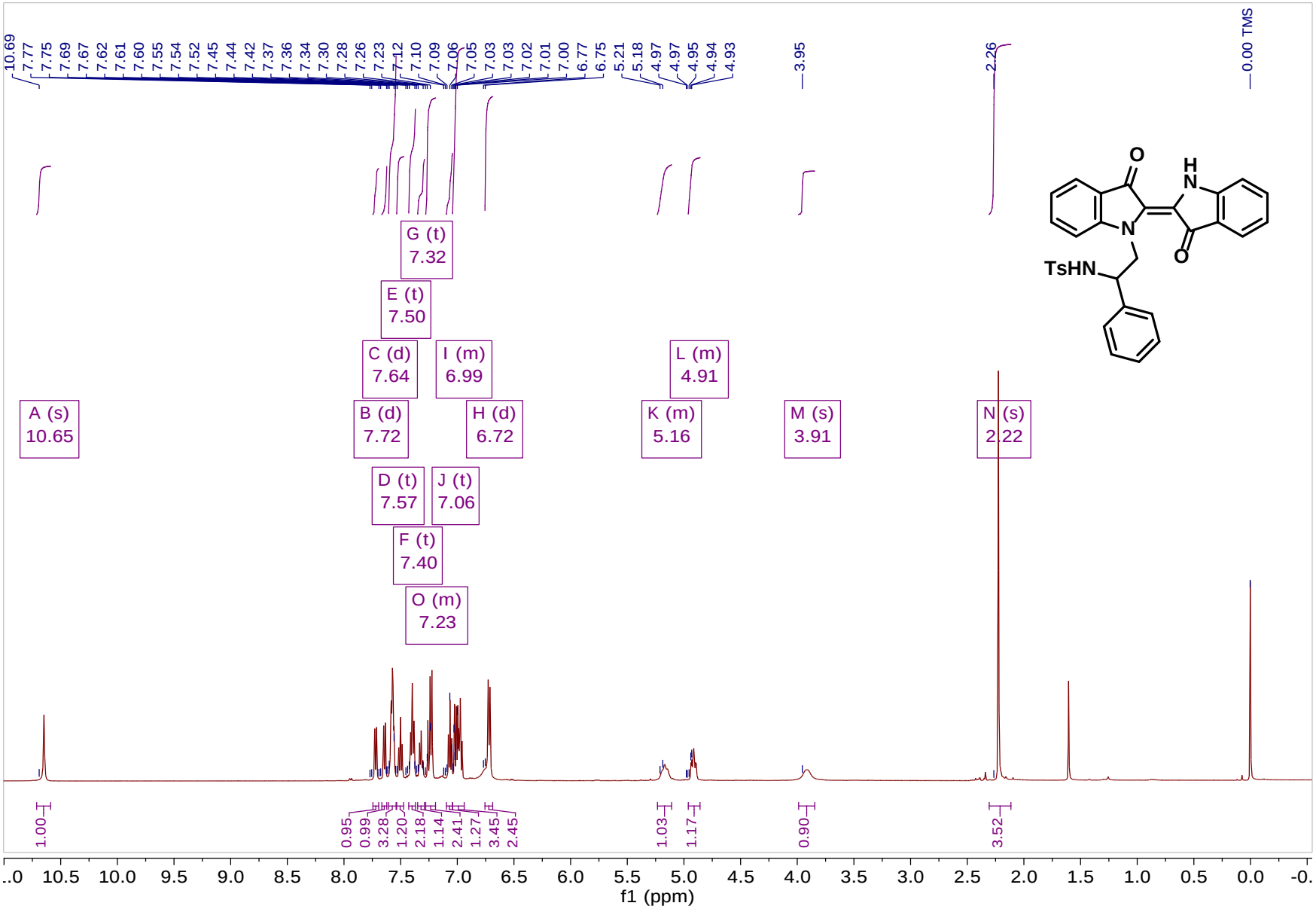


Figure 71: ^{13}C spectrum of compound **19**, with expansion of the region 155-105 ppm

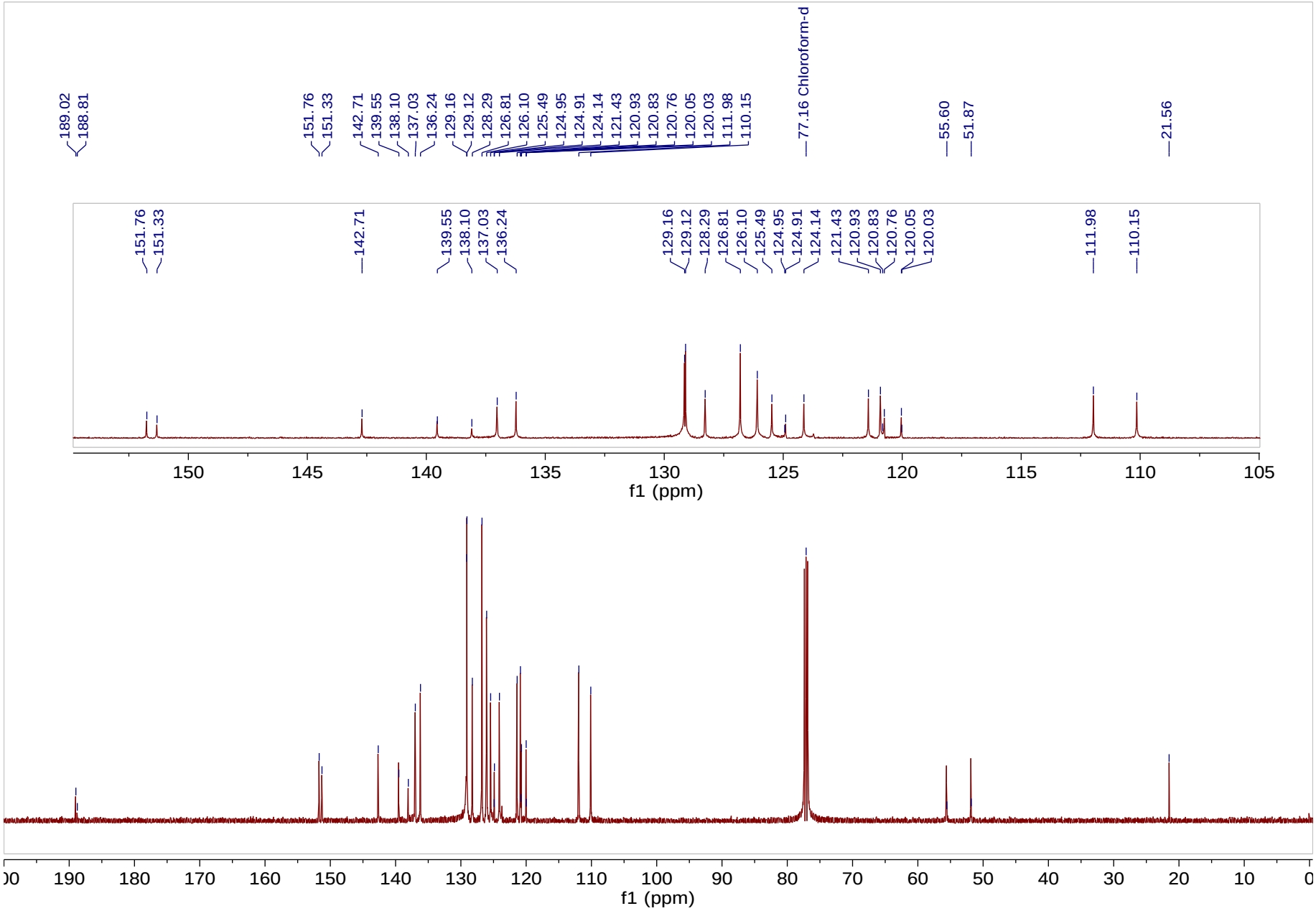


Figure 72: HSQC spectrum of compound **19**

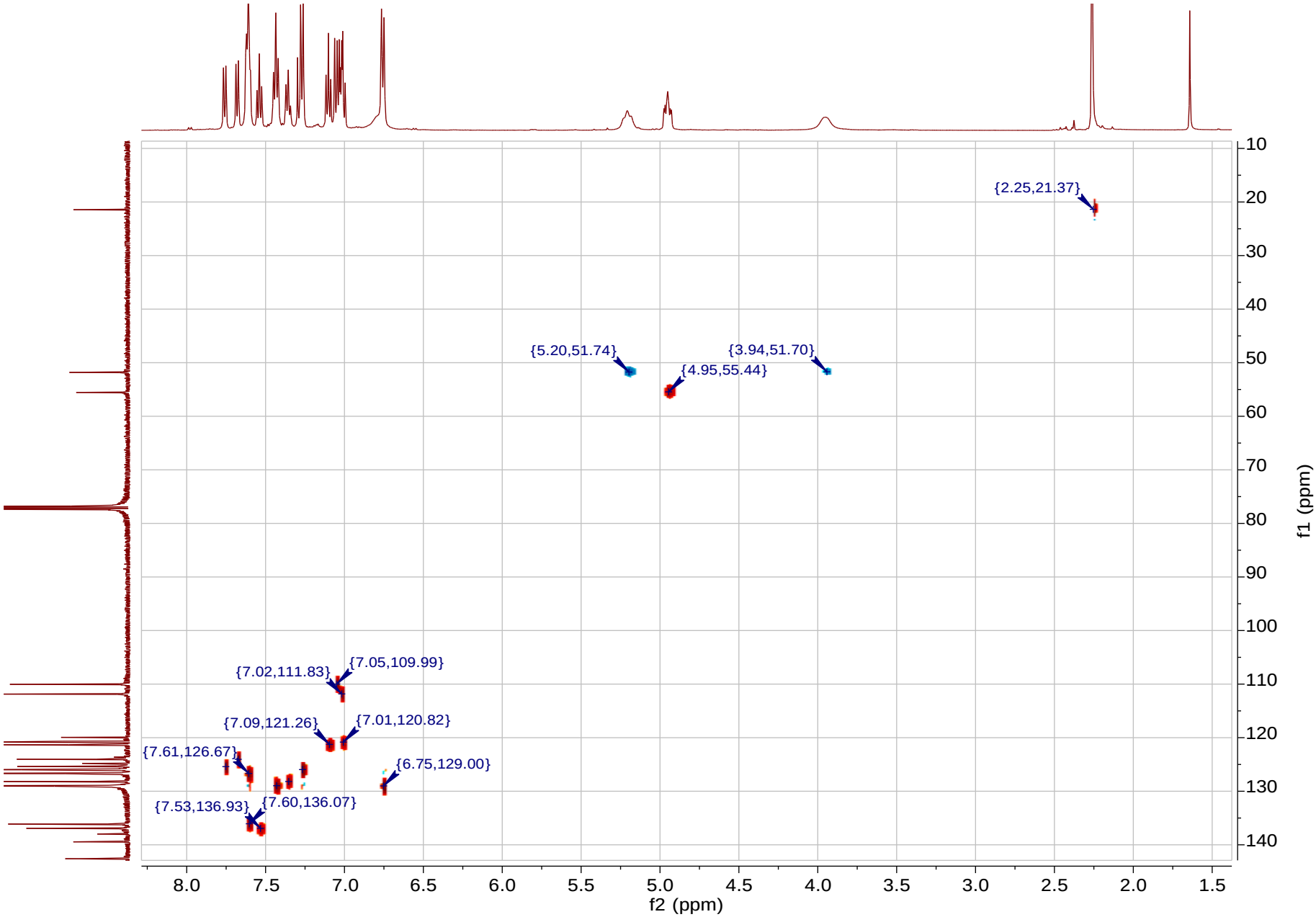


Figure 73: HMBC spectrum of compound **19**

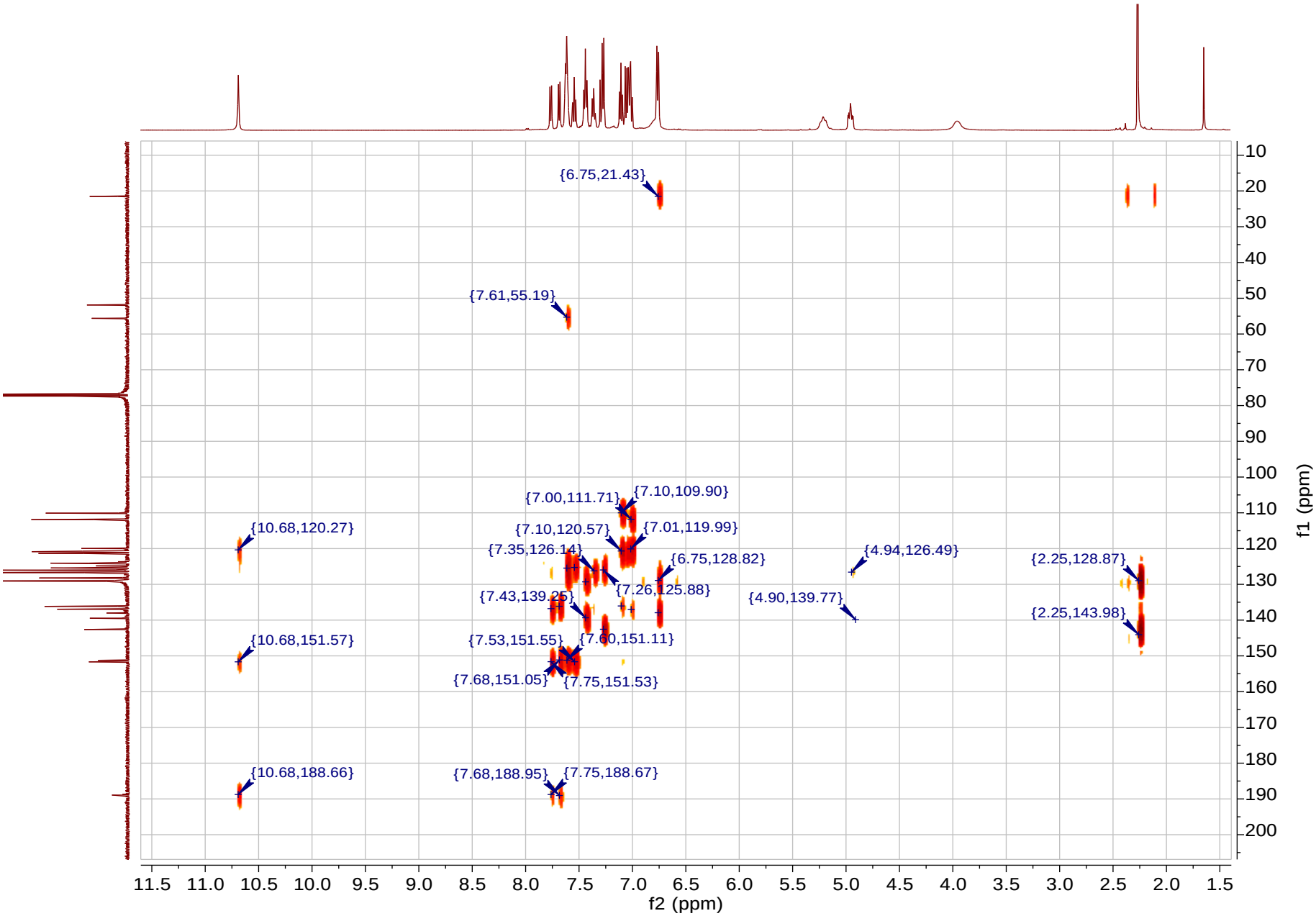
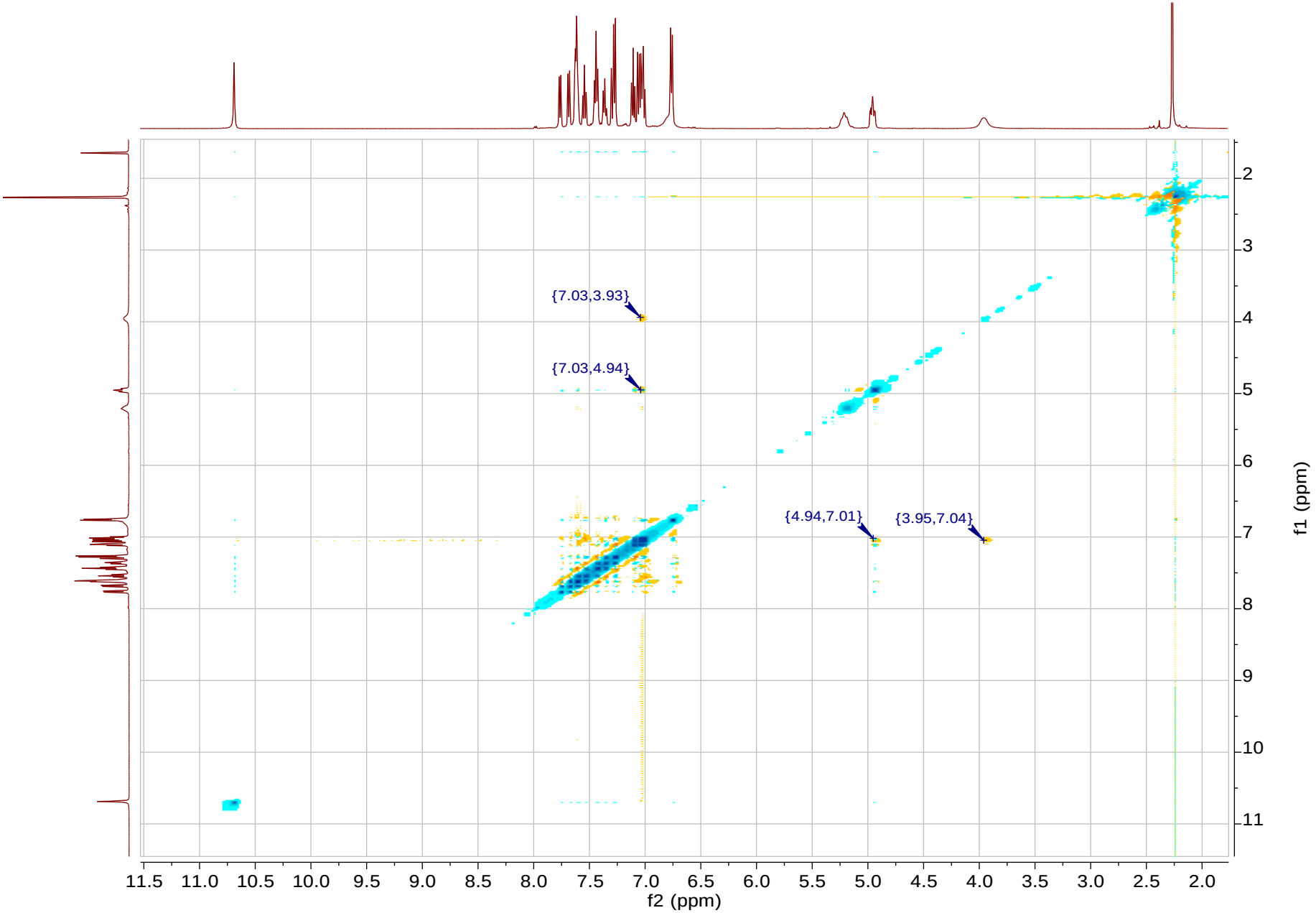


Figure 74: ROESY spectrum of compound **19**



(*E*)-*N*-(2-(3,3'-dioxo-[2,2'-biindolinylidene]-1-yl)-2-phenylethyl)-4-methylbenzenesulfonamide (20)

Figure 75: ^{1}H spectrum of compound **20**

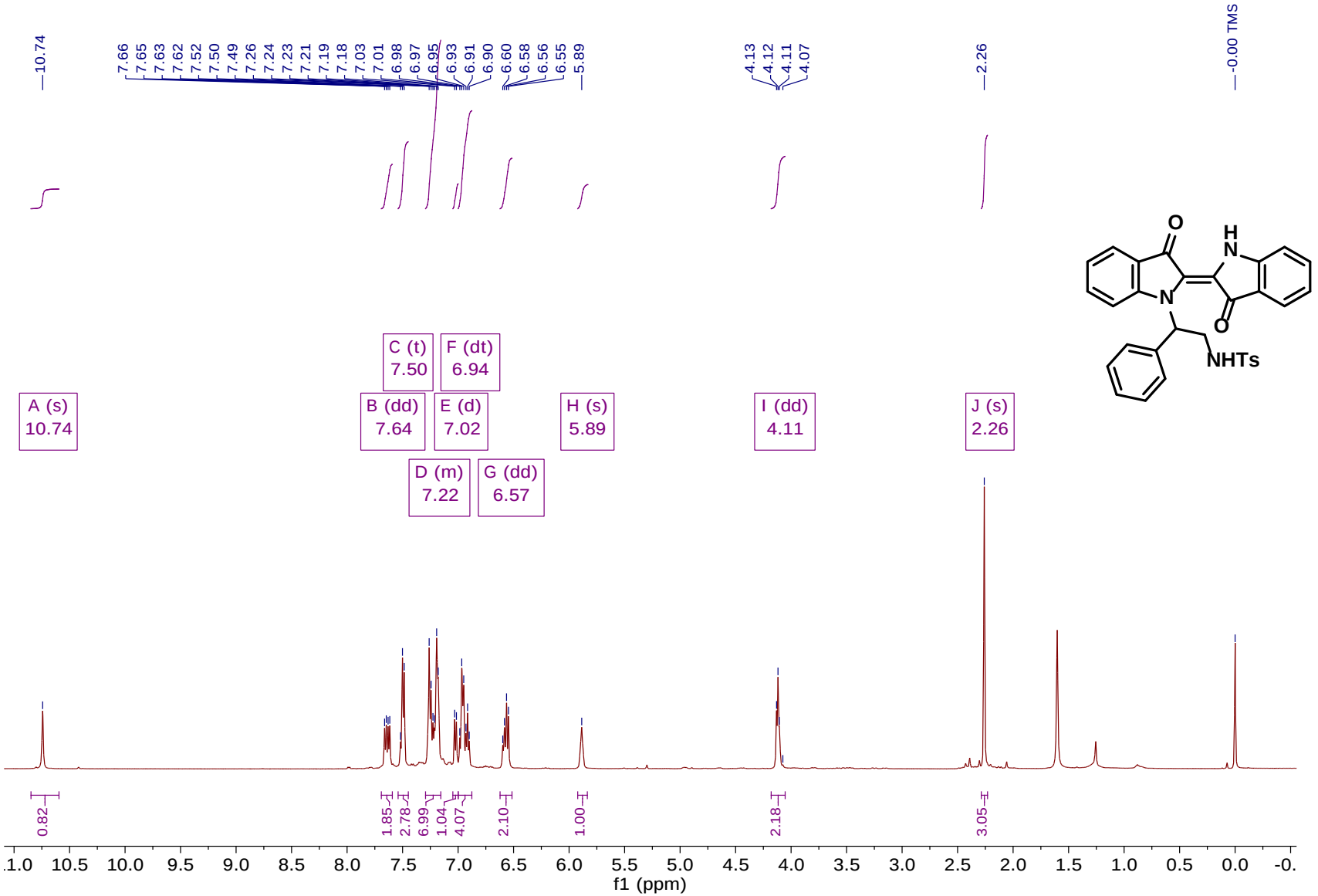


Figure 76: ^{13}C spectrum of compound **20**

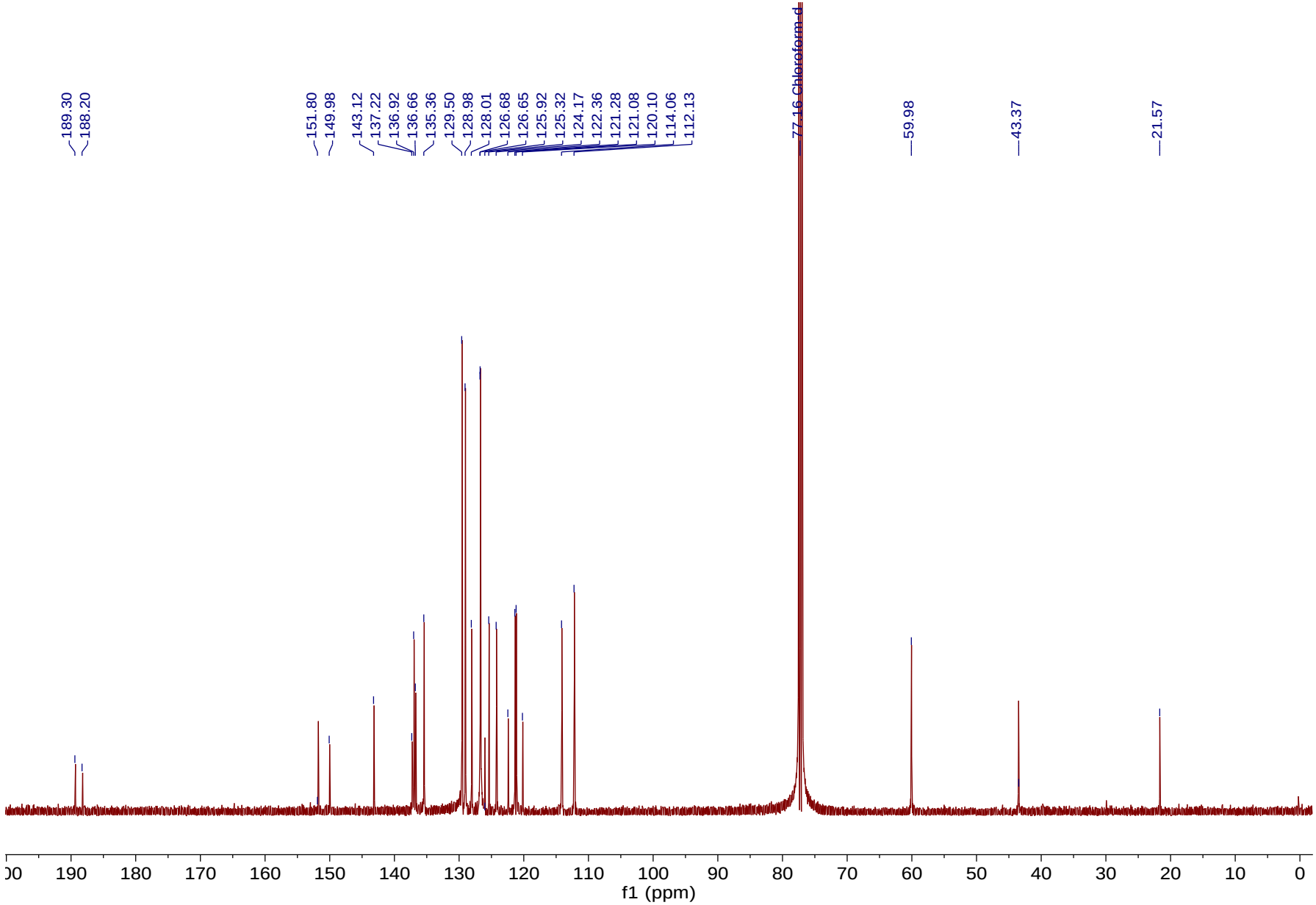


Figure 77: COSY spectrum of compound **20**

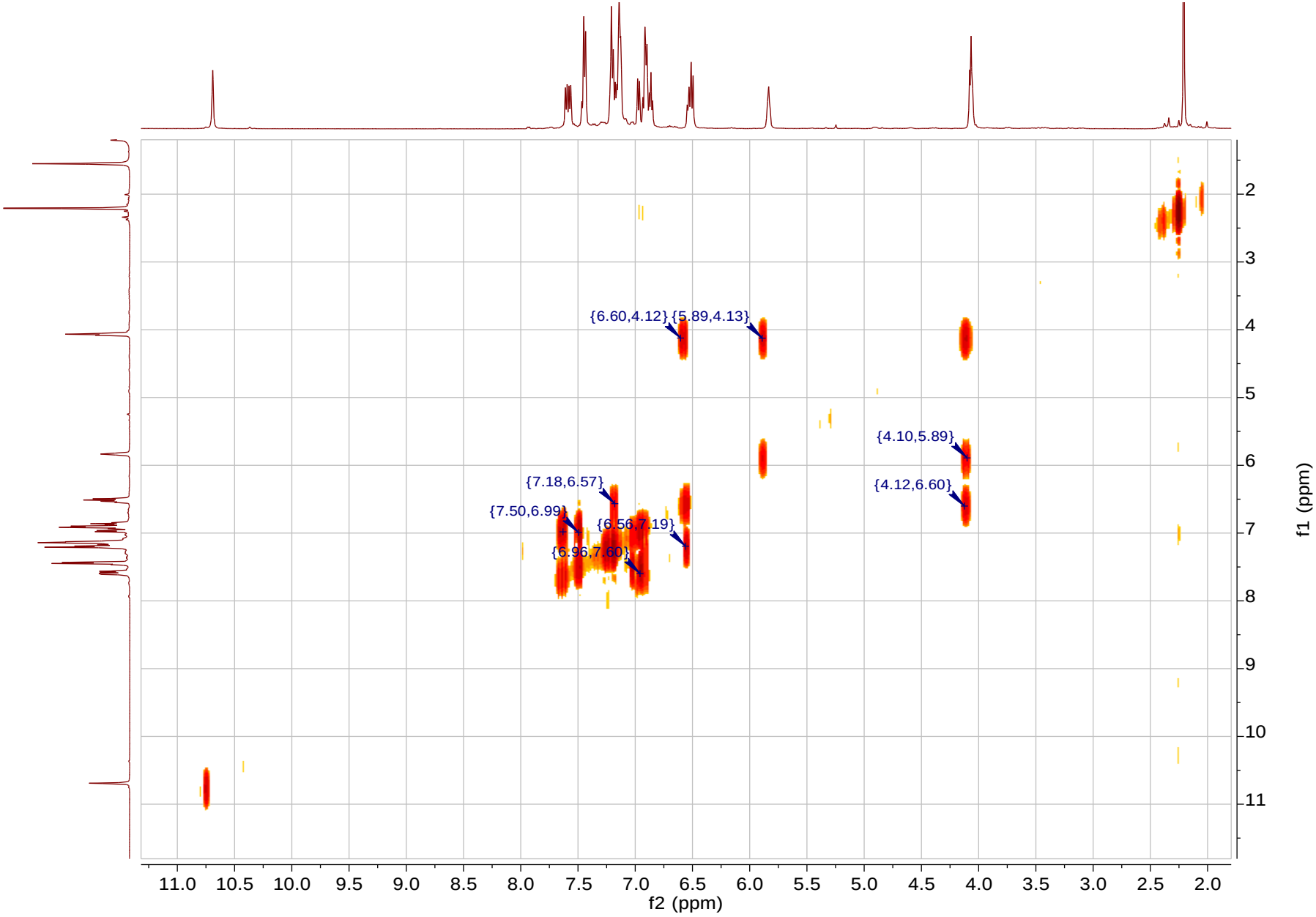


Figure 78: HMBC spectrum of compound **20**

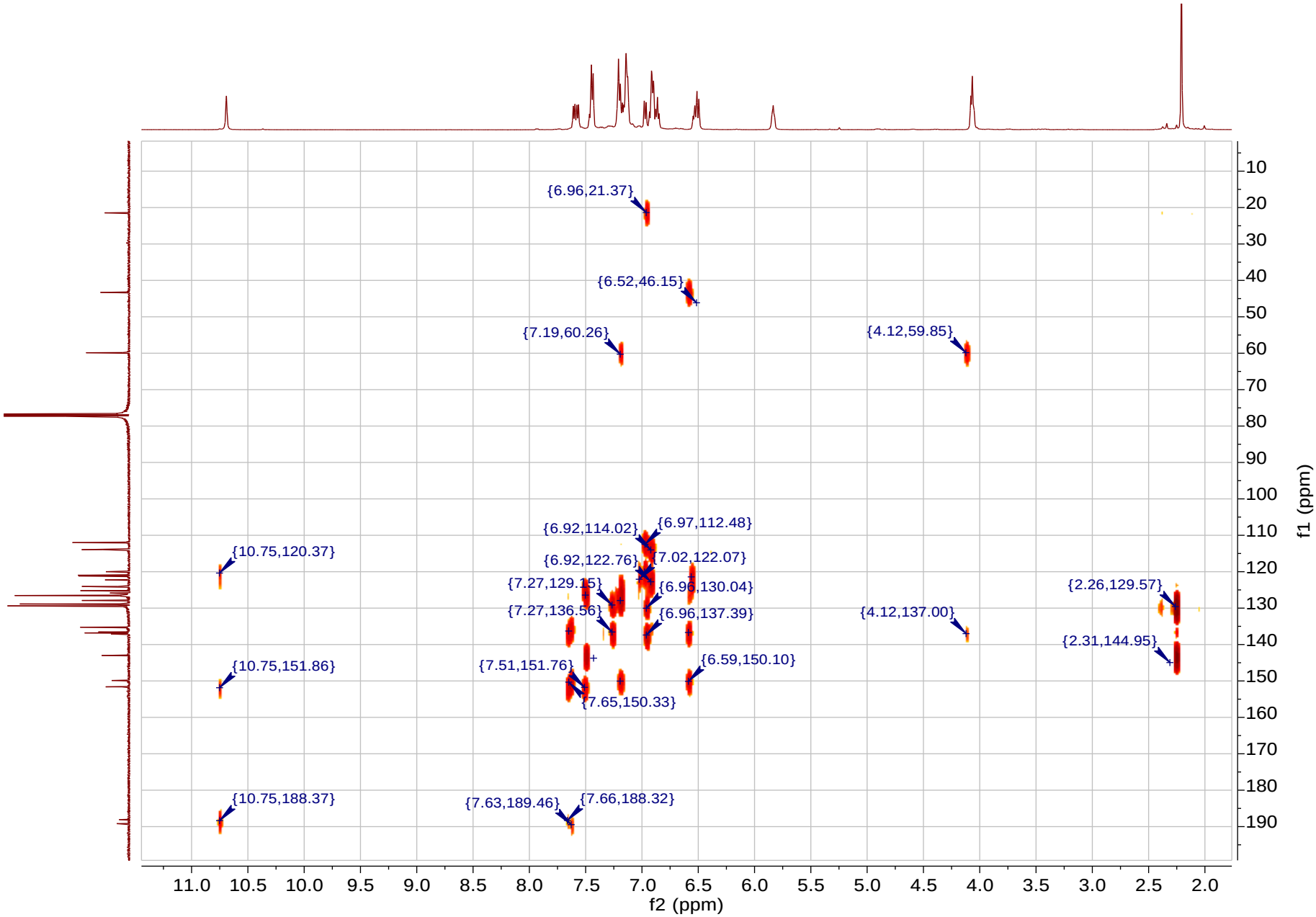
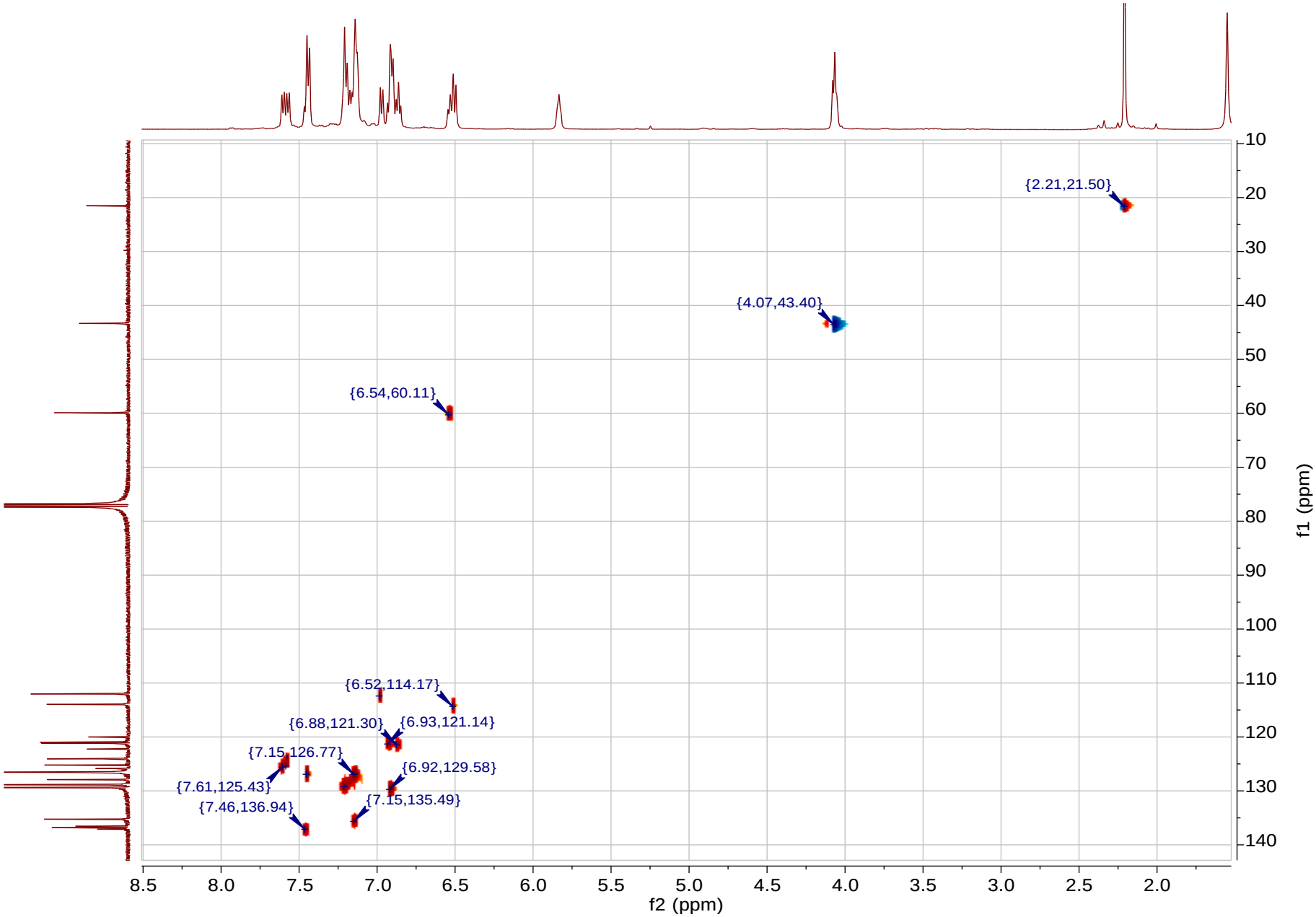


Figure 79: HSQC spectrum for compound **20**



4-methyl-*N*-(((6*R*,7*R*)-7-((4-methylphenyl)sulfonamido)-14,15-dioxo-7,8,14,15-tetrahydro-6*H*-[1,4]diazepino[1,2-*a*:4,3-*a*′]diindol-6-yl)methyl)benzenesulfonamide (21)

Figure 80: ^{1}H spectrum for compound **21**

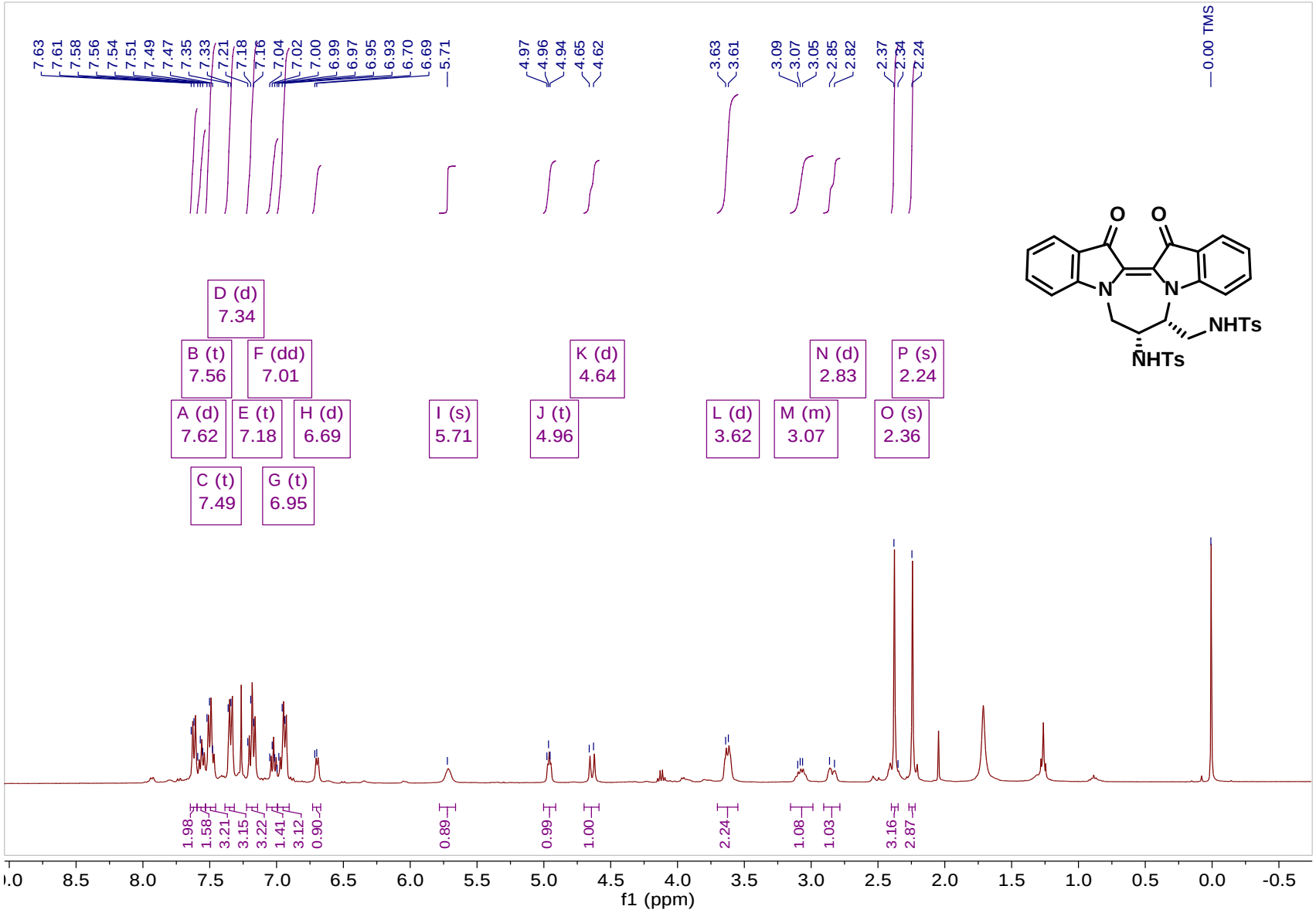


Figure 81: ^{13}C spectrum of compound **21**

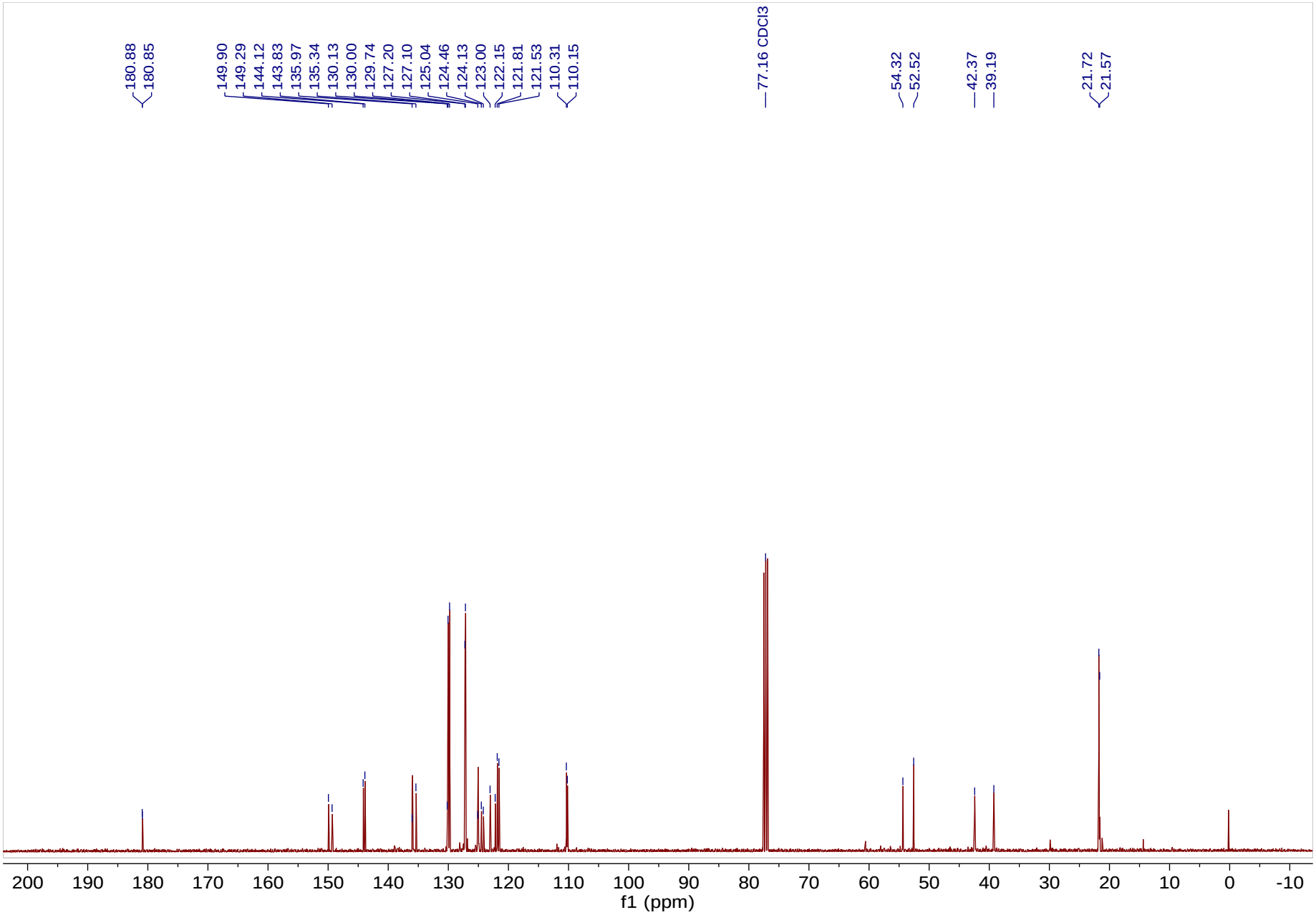


Figure 82: HSQC spectrum of compound **21**

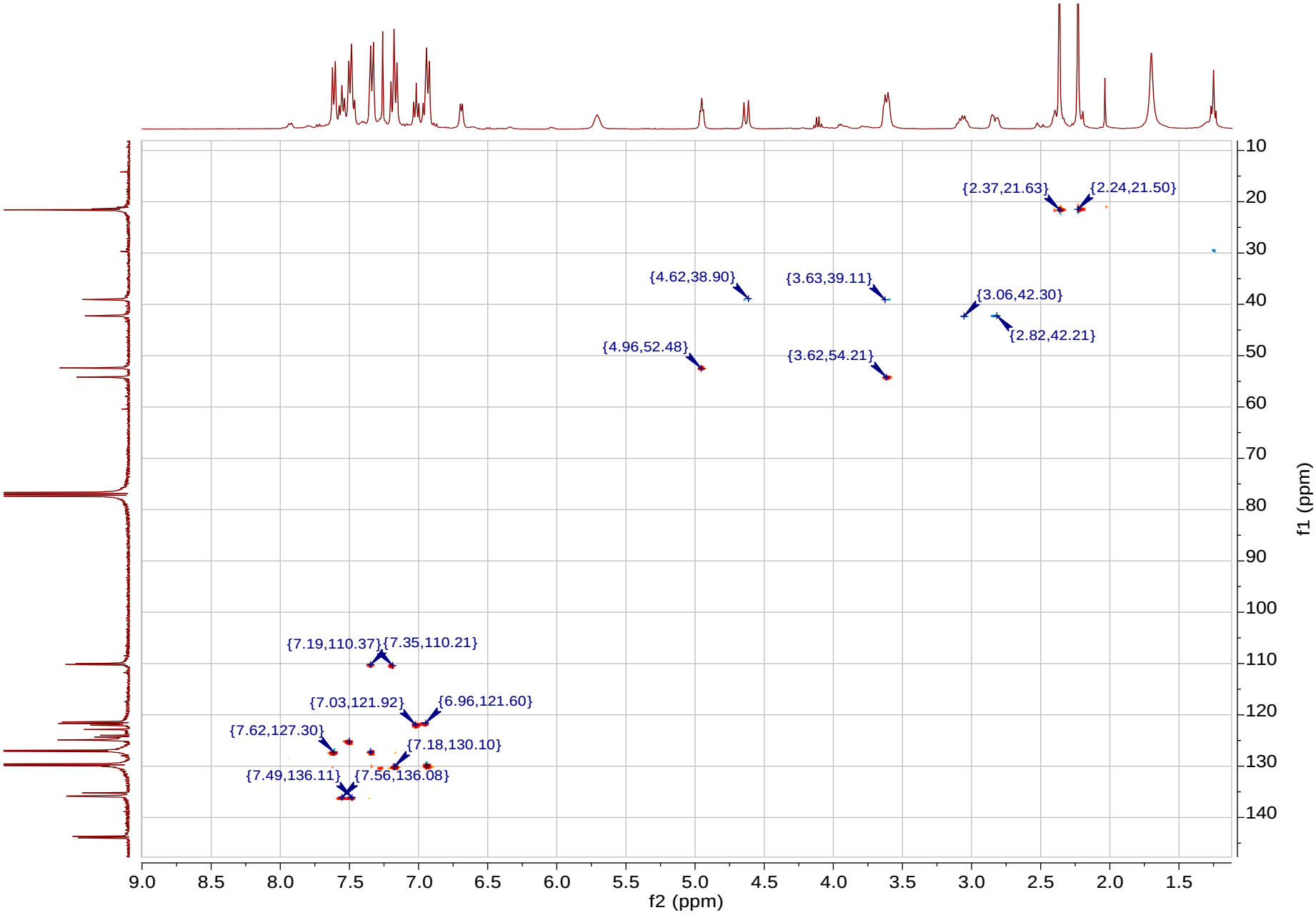


Figure 83: HMBC spectrum of compound **21**

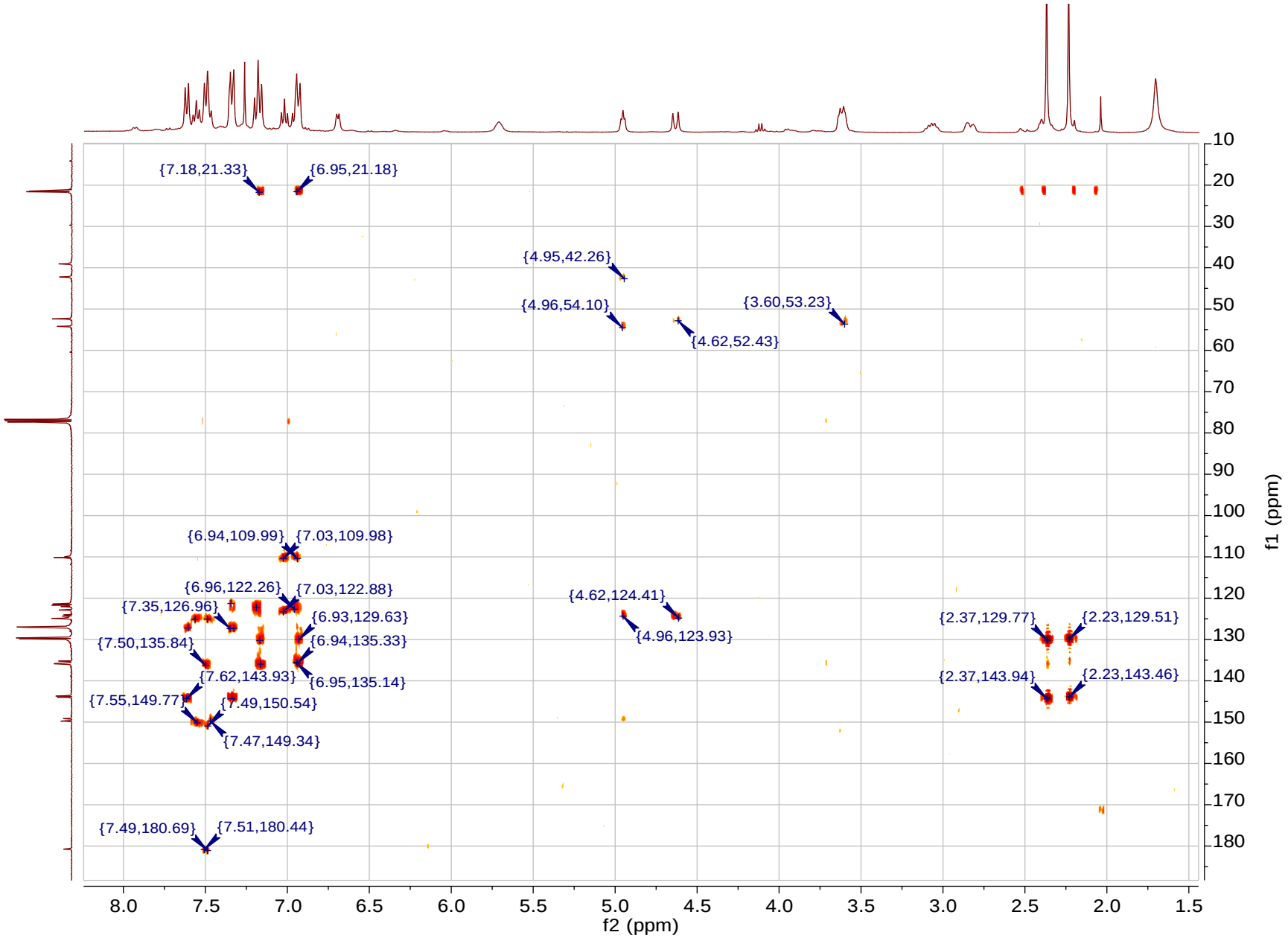


Figure 84: COSY spectrum of compound **21**

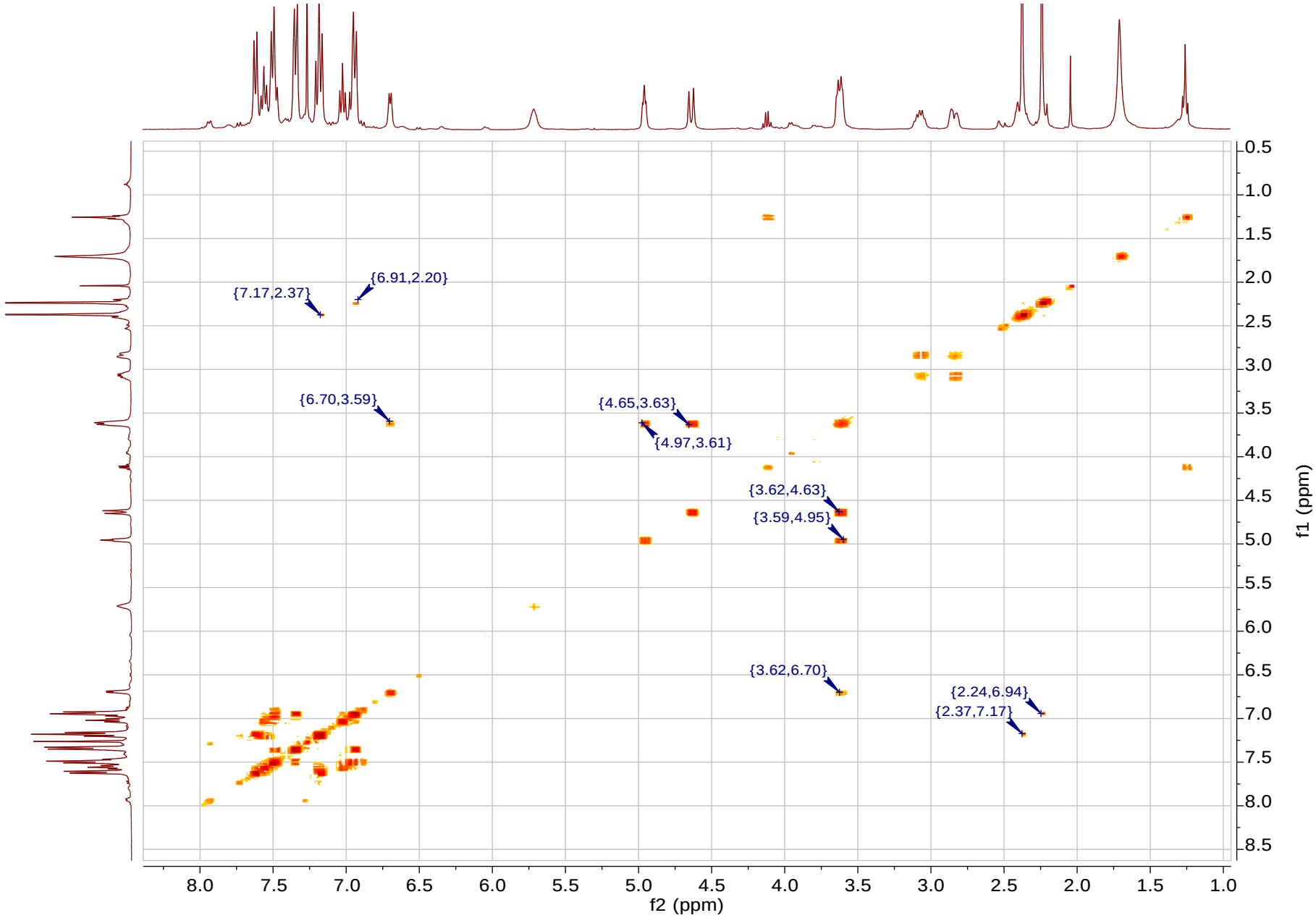
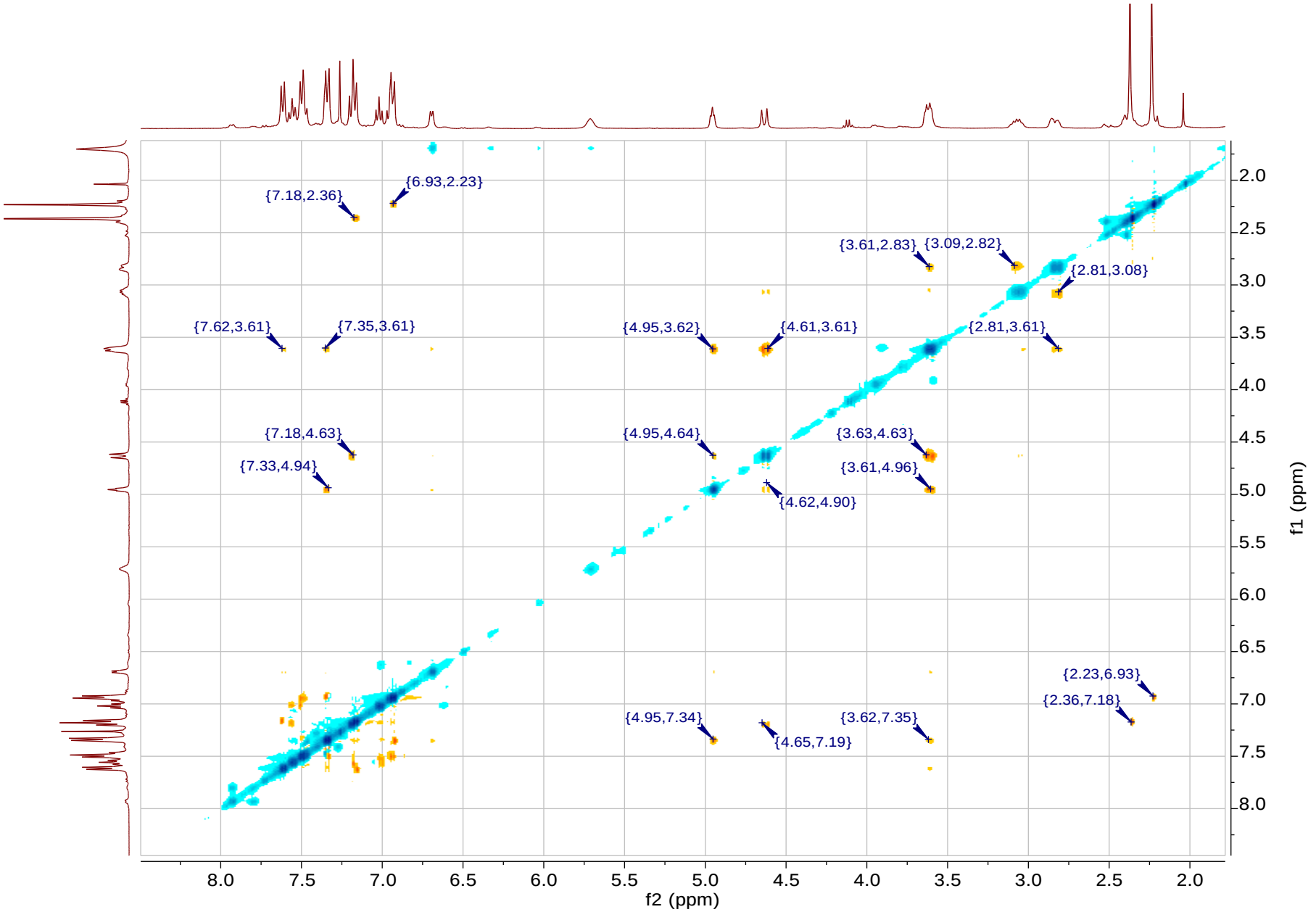


Figure 85: ROESY spectrum for compound **21**



RP-HPLC Traces for Compounds 16 and 17

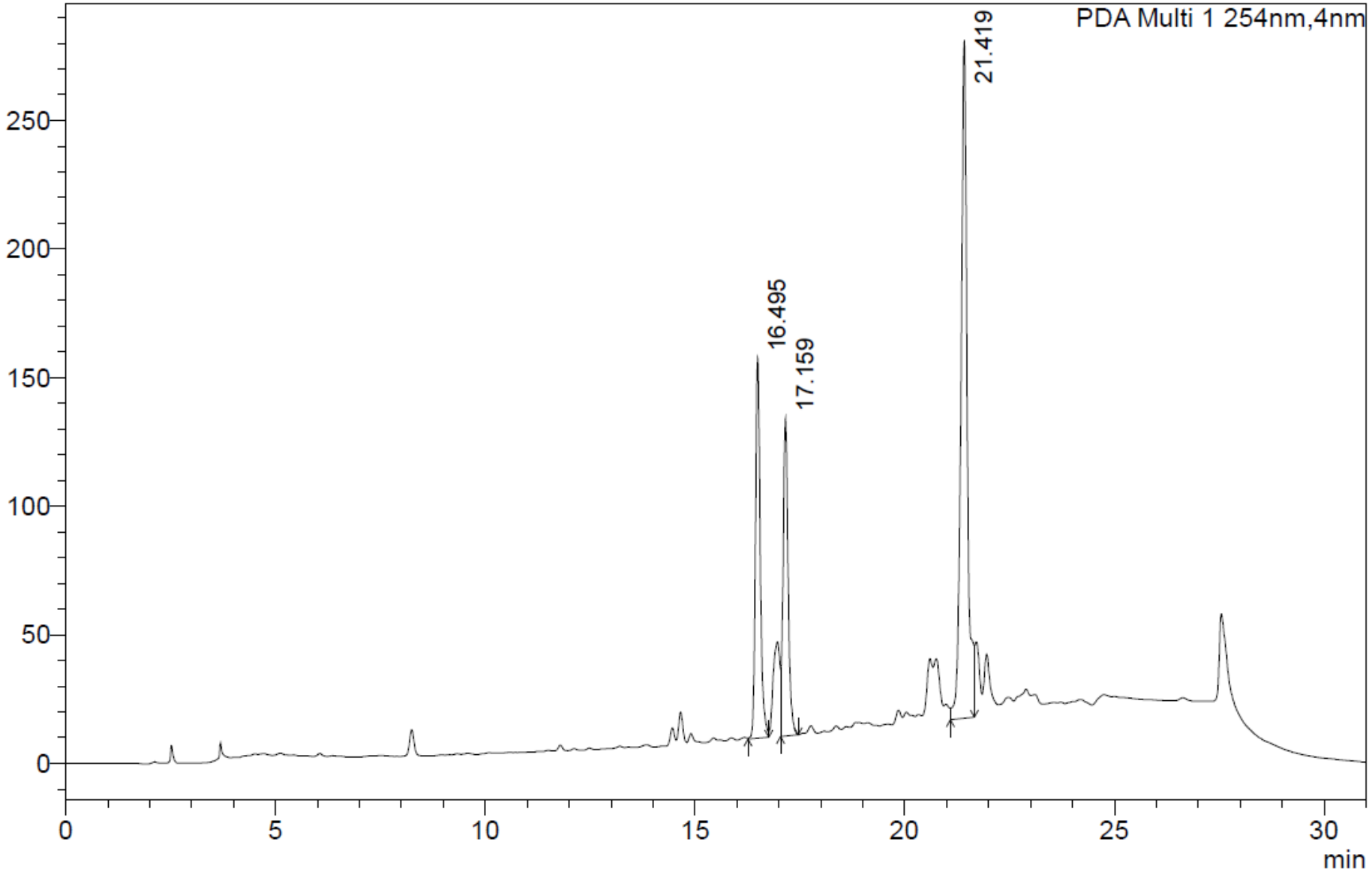


Figure 86: Analytical RP-HPLC chromatogram of the crude mixture prior to separation, visualised at 254 nm. Key peaks are highlighted at T_R = 16.5, 17.2, and 21.4 minutes, respectively.

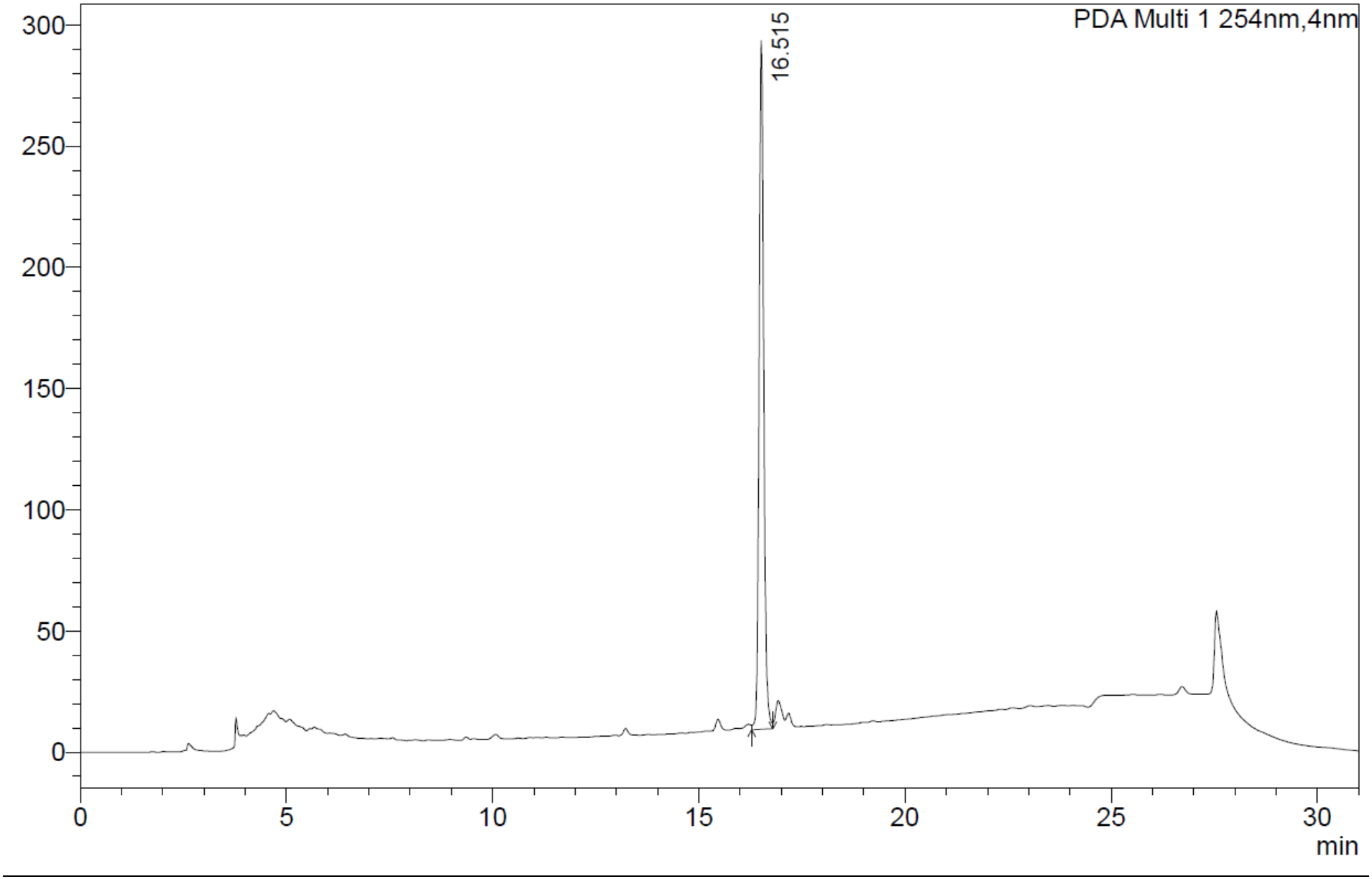


Figure 87: Analytical RP-HPLC chromatogram of Compound **16**, as collected from preparative RP-HPLC, visualised at 254 nm.

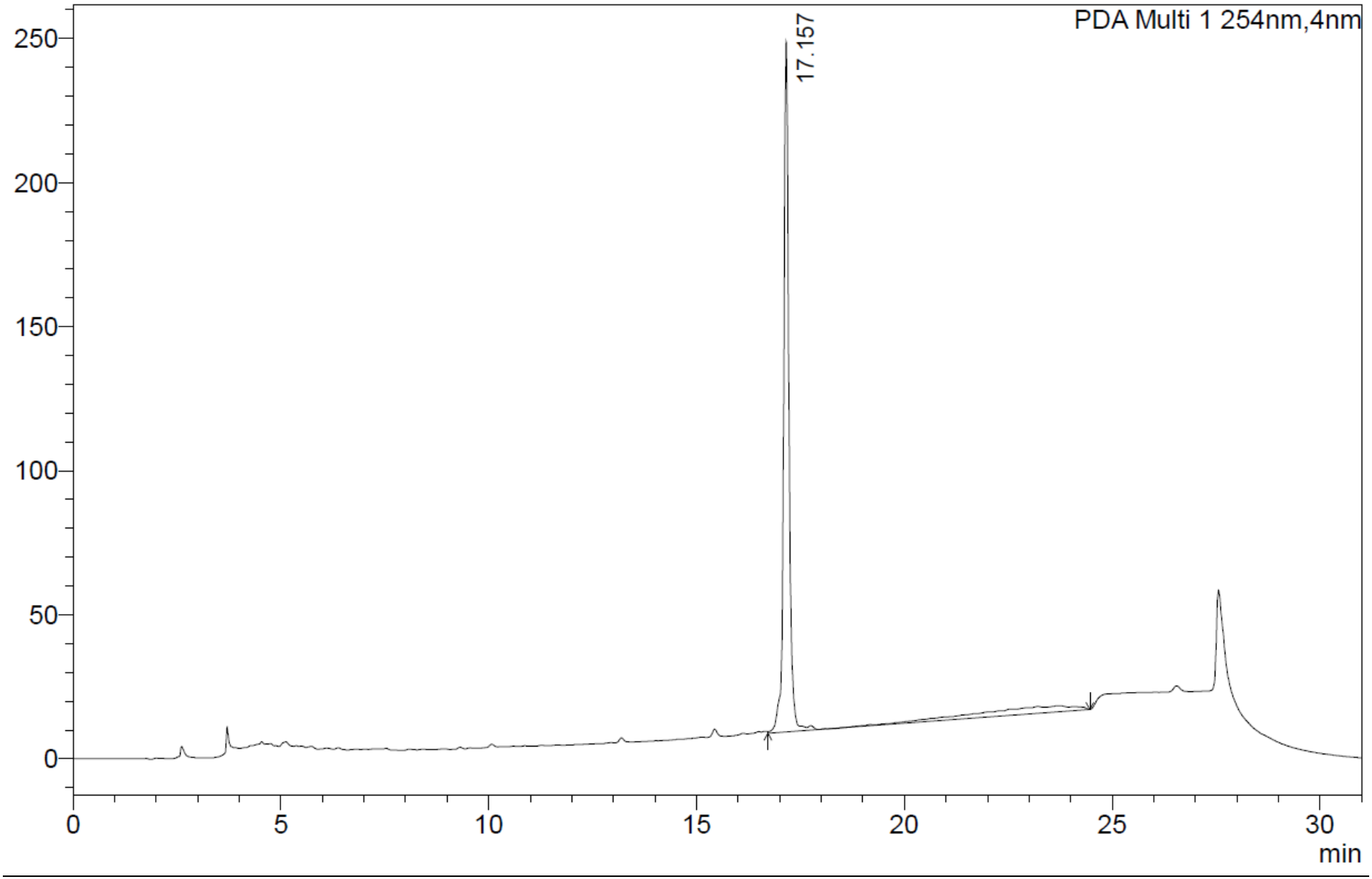


Figure 88: Analytical RP-HPLC chromatogram of Compound **17**, as collected from preparative RP-HPLC, visualised at 254 nm.

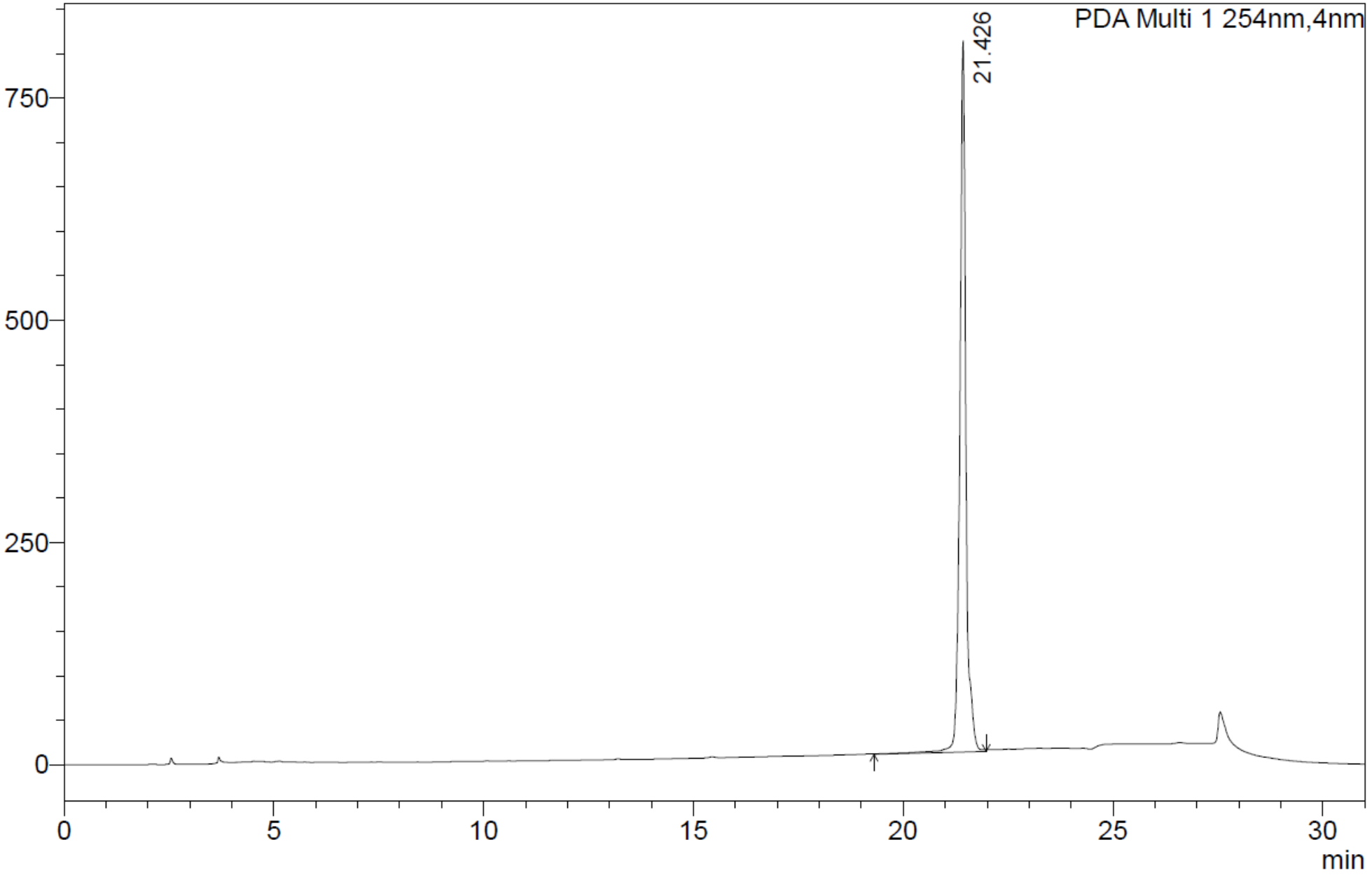
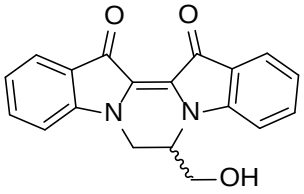
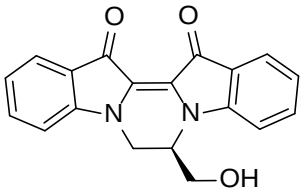
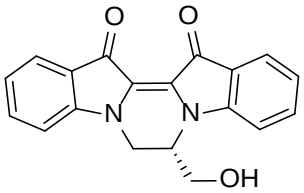
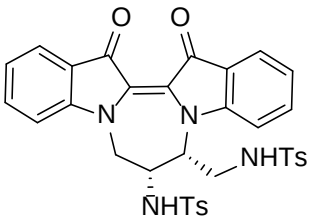
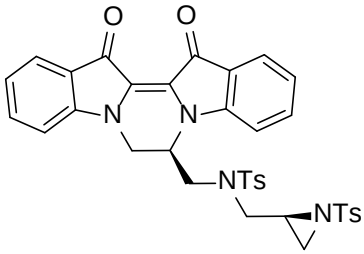
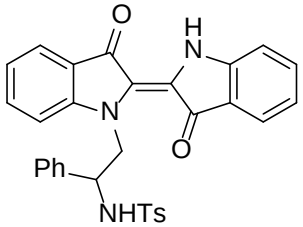
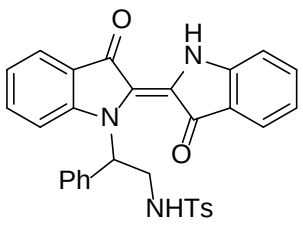
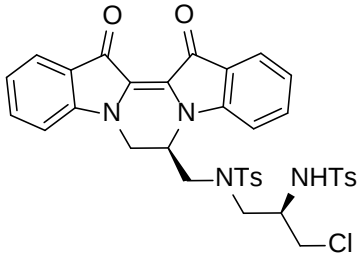
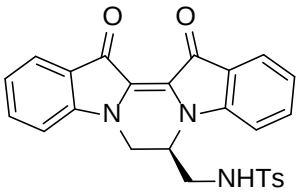
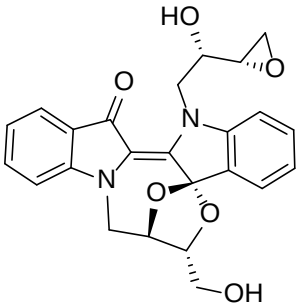
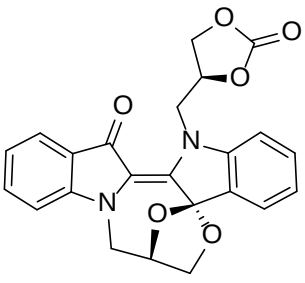
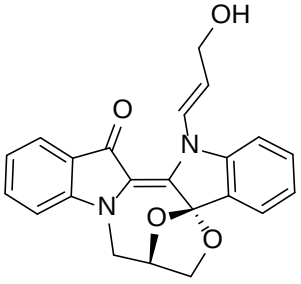
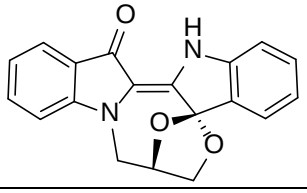


Figure 89: Analytical RP-HPLC chromatogram of the polymeric fraction collected from preparative RP-HPLC, visualised at 254 nm.

Structural Summary of Compounds Tested for Antiplasmodial Activity

ID	Compound Structure	IC_{50} 3D7 (nM)	IC_{50} Dd2 (nM)	IC_{50} HEK293 (nM)[a]	SI (3D7/HEK 293)
(±) 8		76.6 ± 4.0	201.7 ± 7.4	7726 ± 467	101
(*R*)8		88.6 ± 16.3	164.5 ± 32.2	10708 ± 1090	120
(*S*) 8		105.6 ± 37.4	195.5 ± 0.2	10361 ± 1028	98
21		2767 ± 731	4594 ± 1623	14677 ± 2000	5.3
16		3075 ± 600	3869 ± 2121	66% at 80 µM	> 11
19		3698 ± 731	6009 ± 2406	IA	> 21
20		3976 ± 801	8118 ± 55	IA	> 20

17		4653 ± 2472	8127 ± 5605	IA	> 17
15		5625 ± 1775	25209 ± 5459	IA	> 28
13		9411 ± 2082	16076 ± 4192	64% at 80 µM	> 2
(±) 11	 (±)	9922 ± 3476	21501 ± 5817	IA	> 8
10		-	-	-	-
9		*inactive*	-	-	-
	artesunate	1.09 ± 0.03	1.74 ± 0.50	71% at 10 µM	> 2000
	chloroquine	5.81 ± 0.13	94.6 ± 24.9	67% at 40 µM	> 3000
	dihydroartemisinin	0.40 ± 0.05	0.57 ± 0.08	62% at 10 µM	> 10000
	puromycin	141.9 ± 12.7	147.4 ± 23.3	1202 ± 3	8.47
	pyrimethamine	4.00 ± 0.05	IA	73% @ 40 µM	> 1250

[a]Percent inhibition at highest concentration is given if an IC50 value could not be calculated due to dose-response curve not reaching full inhibition plateau; IA = inactive (i.e. < 50% inhibition at the highest concentration).

Crystallographic data for Compounds 9-12, and 20

Crystallographic data for compound 9:

Crystal data. Compound 9. $C_{19}H_{14}N_2O_3$, M=318.33, T=150 K, orthorhombic, space group *Pccn*, Z=8, a=10.8798(1), b=18.4104(1), c=14.5081(1) Å, V=2905.99 (4) Å^3, D_x=1.455 Mg m^{-3}, Cu$K\alpha$ radiation, λ=1.54184 Å, 54126 reflections measured (θ = 5–72˚), merged to 2881 unique data, R=0.030 [for 2749 data with $I > 2\sigma(I)$], R_w= 0.075 [all data], S = 1.00

Structure determination of compound 9. Images were measured on an Agilent SuperNova diffractometer (Cu $K\alpha$ radiation, mirror monochromator, λ=1.54184 Å) and data extracted using the CrysAlis PRO package.[6] Structure solution was by direct methods (SIR92).[7] The structure was refined using the CRYSTALS program package.[8] Atomic coordinates, bond lengths and angles and displacement parameters have been deposited at the Cambridge Crystallographic Data Centre (CCDC no. 1590267). These data can be obtained free-of-charge via www.ccdc.cam.ac.uk/data_requerst/cif, by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

Crystallographic data for compound 10:

Crystal data. Compound 10. $C_{22}H_{18}N_2O_4$, M=374.40, T=150 K, orthorhombic, space group $P2_12_12_1$, Z=4, a=8.64640(7), b=11.41283(8), c=17.69376(13) Å, V=1746.02(2) Å^3, D_x=1.424 Mg m^{-3}, Cu$K\alpha$ radiation, λ=1.54184 Å, 28445 reflections measured (θ = 5–72˚), merged to 3528 unique data, R=0.030 [for 3486 data with $I > 2\sigma(I)$], R_w= 0.081 [all data], S = 1.00

Structure determination of compound 10. Images were measured on an Agilent SuperNova diffractometer (Cu $K\alpha$ radiation, mirror monochromator, λ=1.54184 Å) and data extracted using the CrysAlis PRO package.[6] Structure solution was by direct methods (SIR92).[7] The structure was refined using the CRYSTALS program package.[8] Atomic coordinates, bond lengths and angles and displacement parameters have been deposited at the Cambridge Crystallographic Data Centre (CCDC no. 1590268). These data can be obtained free-of-charge via www.ccdc.cam.ac.uk/data_requerst/cif, by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

Crystallographic data for compound 11:

Crystal data. Compound 11. $C_{23}H_{18}N_2O_6$, M=418.41, T=150 K, monoclinic, space group $C2/c$, Z=8, a=23.9584(7), b=9.6484(3), c=16.0090(6) Å, β = 90.165(3)˚, V=3700.6(2) Å^3, D_x=1.502 Mg m^{-3}, Mo$K\alpha$ radiation, λ=0.71073 Å, 39146 reflections measured (θ = 2–29˚), merged to 4736 unique data, R=0.044 [for 4495 data with $I > 2\sigma(I)$], R_w= 0.109 [all data], S = 1.03

Structure determination of compound 11. Images were measured on an Agilent SuperNova diffractometer (Mo $K\alpha$ radiation, mirror monochromator, λ=0.71073 Å) and data extracted using the CrysAlis PRO package.[6] Structure solution was by direct methods (SIR92).[7] The structure was refined using the CRYSTALS program package.[8] Atomic coordinates, bond lengths and angles and displacement parameters have been deposited at the Cambridge Crystallographic Data Centre (CCDC no. 1590269). These data can be obtained free-of-charge via www.ccdc.cam.ac.uk/data_requerst/cif, by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

Crystallographic data for compound 12:

Crystal data. Compound 12. $2(C_{20}H_{16}N_2O_4).1.85(CH_2Cl_2).0.15(CHCl_3)$, M=871.73, T=150 K, orthorhombic, space group $P2_12_12_1$, Z=4, a=10.5565(2), b=14.9406(2), c=25.9563(4) Å, V=4093.84(11) Å^3, D_x=1.414 Mg m^{-3}, Cu$K\alpha$ radiation, λ=1.54184 Å, 61022 reflections measured (θ = 3–64˚), merged to 7974 unique data, R=0.068 [for 6934 data with $I > 2\sigma(I)$], R_w= 0.151 [all data], S = 0.98

Structure determination of compound 12. Images were measured on an Agilent SuperNova diffractometer (Cu $K\alpha$ radiation, mirror monochromator, λ=1.54184 Å) and data extracted using the CrysAlis PRO package.[6] Structure solution was by direct methods (SIR92).[7] The structure was refined using the CRYSTALS program package.[8] Atomic coordinates, bond lengths and angles and displacement parameters have been deposited at the Cambridge Crystallographic Data Centre (CCDC no. 1590270). These data can be obtained free-of-charge via www.ccdc.cam.ac.uk/data_requerst/cif, by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

Crystallographic data for compound 20:

Crystal data. Compound 20. $C_{31}H_{25}N_3O_4S$, M=535.62, T=150 K, monoclinic, space group $P2_1/c$, Z=4, a=23.5193(13), b=11.6263(7), c=9.3856(5) Å, β = 90.966(5)˚, V=2566.1(3) Å^3, D_x=1.386 Mg m^{-3}, Cu$K\alpha$ radiation, λ=1.54184 Å, 16907 reflections measured (θ = 4–72˚), merged to 4953 unique data, R=0.071 [for 3162 data with $I > 2\sigma(I)$], R_w= 0.153 [all data], S = 1.01

Structure determination of compound 20. Images were measured on an Agilent SuperNova diffractometer (Cu $K\alpha$ radiation, mirror monochromator, λ=1.54184 Å) and data extracted using the CrysAlis PRO package.[6] Structure solution was by direct methods (SIR92).[7] The structure was refined using the CRYSTALS program package.[8] Atomic coordinates, bond lengths and angles and displacement parameters have been deposited at the Cambridge Crystallographic Data Centre (CCDC no. 1590271). These data can be obtained free-of-charge via www.ccdc.cam.ac.uk/data_requerst/cif, by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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