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# Synthesis of androstane derivatives fused with polyheterocycles at the D ring

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## 1. Experimental Section

$^{1}$H, $^{13}$C NMR, $^{1}$H ROE NMR, 2D NMR HSQC, HMBC and ROESY spectra were recorded on Bruker AV-600 (600 and 151 MHz, respectively). The chemical shifts (δ) were expressed in ppm and referred to DMSO-$d_6$ (39.5 ppm) for $^{1}$H and $^{13}$C NMR, respectively. The coupling constants (*J*) are in Hertz. The assignment of the signals in the NMR spectra was based on the 2D NMR data. High-resolution mass spectra were obtained on a Bruker MicroTOF mass spectrometer by electrospray ionization (ESI) using Q-TOF detection. IR spectra were recorded on a Bruker Alpha spectrometer as KBr pellets, significant band (ν) reported in cm$^{-1}$. The melting points were determined on a Kofler hot stage apparatus and are uncorrected. TLC was performed using Silica gel 60 F254 plates. The chromatograms were visualized with an UV lamp (254 and 365 nm) and [$Ce(SO_4)_2/H_2SO_4$] developing solution. Column chromatography was carried out on silica gel 60 (0.063–0.200 mm, Merck). Commercial reagents were used without further purification. All reactions were carried out using freshly distilled and dry solvents. 16α,17α-epoxypregn-5-en-20-one was prepared according to published procedure [A. V. Komkov *et al*, *Chem. Heterocyclic Comp.*, 2023, **59**, 554.; A. V. Komkov *et al*, *Russ. Chem. Bull.*, 2018, **67**, 1088. https://doi.org/10.1007/s11172-018-2185-5].

**3β-Acetoxy-17*α*-hydroxy-16,17-dihydro-2′-thioxo-1,2,4-triazolo[2′,3′-1″,2″:1′,2′-*d*]pyrimido [6″,1″-*e*]androst-5-eno[17,16-*d*]pyrazole (4) and 3β-acetoxy-17*α*-hydroxy-16,17-dihydro-[1,2,4]triazolo[3′,4′-2″,1″:5′,4′-*b*]pyrimido[6″,1″-*c*]androst-5-eno[17,16-*e*]-1,3-thiazine (6)**. A mixture of steroid **1** (0.083 g, 0.215 mmol) and 3-amino-5-mercapto-1,2,4-triazole (0.075 g, 0.644 mmol) in AcOH (7 ml) was refluxed for 11 h, acetic acid was removed *in vacuo*, $CHCl_3$ (20 ml) was added, unreacted 3-amino-5-mercapto-1,2,4-triazole is filtered off, the solid was washed with $CHCl_3$ (10 ml), the solvent is removed from the filtrate *in vacuo*. The residue in $CHCl_3$ was placed onto a column with $SiO_2$ and chromatographed (eluted with a mixture of $CHCl_3$ and MeOH 100 : 1, 60 : 1, 30 : 1), the solvent was removed from the fractions *in vacuo*, the residue was crystallized with a mixture of benzene (2 ml) and petroleum ether (10 ml) to obtain 0.035 g (34%) of a mixture of substances **4** and **6** in a ratio of 4 : 1, yellow solid, m.p. 265-270°C (with decomposition), $R_f$ 0.34 ($CHCl_3$ : MeOH 25 : 1). IR spectrum, ν, cm$^{-1}$: 2942, 2902, 2868, 1732(CO), 1600, 1571, 1490, 1416, 1368, 1330, 1295, 1246, 1134, 1035, 823, 768, 730, 674, 609, 521, 460, 431.

Steroid **4.** $^{1}$H NMR (600 MHz, DMSO-$d_6$): δ 0.61 (s, 3H, 18-$CH_3$); 1.00 (s, 3H, 19-$CH_3$); 0.99—1.15 (m, 2H, 9-CH, 1-$CH_2$); 1.45 (q, *J* = 13.0, 1H, 11-$CH_2$); 1.58 (q, *J* = 13.2, 1H, 15-$CH_2$);1.63—1.73 (m, 4H, 8-CH, 2-$CH_2$, 7-$CH_2$, 11-$CH_2$); 1.78—1.82 (m, 1H, 15-$CH_2$); 1.84—1.93 (m, 3H, 1-$CH_2$, 2-$CH_2$, 14-CH); 1.99 (c, 3H, $CH_3COO$); 2.03—2.09 (m, 2H, 7-$CH_2$, 12-$CH_2$); 2.25—2.32 (m, 2H, 4-$CH_2$); 2.57—2.63 (m, 1H, 12-$CH_2$); 4.42—4.50 (m, 1H, 3-CH); 4.75 (dd, $J_1$ = $J_2$ = 7.8, 1H, 16-CH); 5.37—

5.41 (m, 1H, 6-CH); 6.76 (s, 1H, 17-OH); 7.30 (d, *J* = 4.9, 1H, 5′-CH); 8.86 (d, *J* = 4.9, 1H, 6′-CH). $^{13}$C NMR (150.9 MHz, DMSO-$d_6$), δ: 14.8 (18-$CH_3$); 18.9 (19-$CH_3$); 19.6 (11-$CH_2$); 21.0 ($\underline{C}H_3COO$); 27.3 (2-$CH_2$); 30.2 (15-$CH_2$); 30.9 (12-CH2); 31.2 (7-$CH_2$, 8-CH); 36.1 (10-C); 36.4 (1-$CH_2$); 37.6 (4-$CH_2$); 46.4 (13-C); 49.0 (9-CH); 51.7 (14-CH); 73.1 (3-CH); 74.3 (16-CH); 102.3 (17-C); 105.0 (5′-CH); 121.7 (6-CH); 139.4 (5-C); 144.1 (4a′-C); 150.8 (7a′-C); 160.4 (6′-CH); 169.7 (COO); 178.1 (C=S). HRMS (ESI) for $C_{26}H_{33}N_4O_3S$ ([M + H]$^+$): calcd 481.2268, found 481.2266.

Steroid **6**. $^1$H NMR (600 MHz, DMSO-$d_6$): δ 0.70 (s, 3H, 18-$CH_3$); 3.72 (dd, *J* = 8.5, 5.8, 1H, 16-CH); 6.22 (s, 1H, 17-OH); 7.10 (d, *J* = 4.3, 1H, 6′-CH); 8.77 (d, *J* = 4.3, 1H, 5′-CH), other signals overlap with those of steroid **4**. $^{13}$C NMR (150.9 MHz, DMSO-$d_6$), δ: 15.6 (18-$CH_3$); 19.4 (11-$CH_2$); 29.6 (12-$CH_2$); 31.6 (8-CH); 33.7 (15-$CH_2$); 42.5 (16-CH); 49.3 (9-CH); 50.0 (14-CH); 79.0 (17-C, HMBC); 107.2 (6′-CH); 154.7 (5′-CH), other signals either overlap with the signals of the main connection **4** or are not accumulated.

**3β,17*α*-Dihydroxy-16*α*-methoxy-1′-pyrimidin-2″-yl-2'-thioxo-2′,3′,16,17-tetrahydro-1′*H*-androst-5-eno[16,17-*d*]imidazole (7) and 3β,17*α*-dihydroxy-16,17-dihydro-[1,2,4]triazolo[3′,4′-2″,1″:5′,4′-*b*]pyrimido[6″,1″-*c*]androst-5-eno[17,16-*e*]-1,3-thiazine (8)**. Potassium carbonate (0.013 g, 0.095 mmol) and MeOH (8 ml) are added to a mixture of steroids **4** and **6** from the previous experiment (ratio 4 to 1) (30 mg, 0.063 mmol). The mixture was stirred at room temperature for 27 h, methanol was removed *in vacuo*, the residue in $CHCl_3$ was applied to a $SiO_2$ column and chromatographed (eluent $CHCl_3$ and MeOH 100 : 1), the solvent was removed from the fractions *in vacuo*, the residue was washed with $C_6H_6$ (1 ml) and petroleum ether (4 ml) to give 14 mg (57%) of steroid **7**, a white solid, m.p. 183-185°C, $R_f$ 0.46 ($CHCl_3$ : MeOH 25 : 1). IR spectrum, ν, cm$^{-1}$: 3178, 2931, 2901, 1568, 1494, 1420, 1297, 1259, 1184, 1137, 1060, 1045, 983, 840, 809, 793, 725, 651, 602.

$^1$H NMR (600 MHz, DMSO-$d_6$): δ 0.97 (s, 6H, 18-$CH_3$, 19-$CH_3$); 0.87—0.92 (m, 1H, 9-CH); 0.94—1.00 (m, 1H, 1-$CH_2$); 1.34—1.62 (m, 6H, 8-CH, 14-CH, 2-$CH_2$, 12-$CH_2$, 11-$CH_2$); 1.65—1.71 (m, 1H, 7-$CH_2$); 1.75—1.81 (m, 1H, 1-$CH_2$); 1.91—2.19 (m, 6H, 7-$CH_2$, 12-$CH_2$, 4-$CH_2$, 15-$CH_2$); 3.22—3.30 (m, 1H, 3-CH); 3.28 (s, 3H, $OCH_3$); 4.62 (d, *J* = 4.5, 1H, 3-OH); 5.26—5.28 (m, 1H, 6-CH); 5.85 (c, 1H, 17-OH); 7.36 (t, *J* = 4.9, 1H, 5″-CH); 8.80 (d, *J* = 4.9, 2H, 4″-CH, 6″-CH); 9.98 (s, 1H, NH). $^{13}$C NMR (150.9 MHz, DMSO-$d_6$), δ: 13.1 (18-$CH_3$); 19.2 (19-$CH_3$); 20.1 (11-$CH_2$); 30.4 (12-$CH_2$); 31.4 (8-CH, 7-$CH_2$, 2-$CH_2$); 35.6 (15-$CH_2$); 36.2 (10-C); 36.8 (1-$CH_2$); 42.2 (4-$CH_2$); 49.0 (14-CH); 49.4 (9-CH); 49.8 (13-C); 50.5 ($OCH_3$); 70.0 (3-CH); 95.2 (16-C); 103.7 (17-C); 119.0 (5″-CH); 120.1 (6-CH); 141.4 (5-C); 157.1 (2″-C); 158.2 (4″-CH, 6″-CH); 179.4 (C=S). HRMS (ESI) for $C_{25}H_{35}N_4O_3S$ ([M + H]$^+$): calcd 471.2424, found 471.2426.

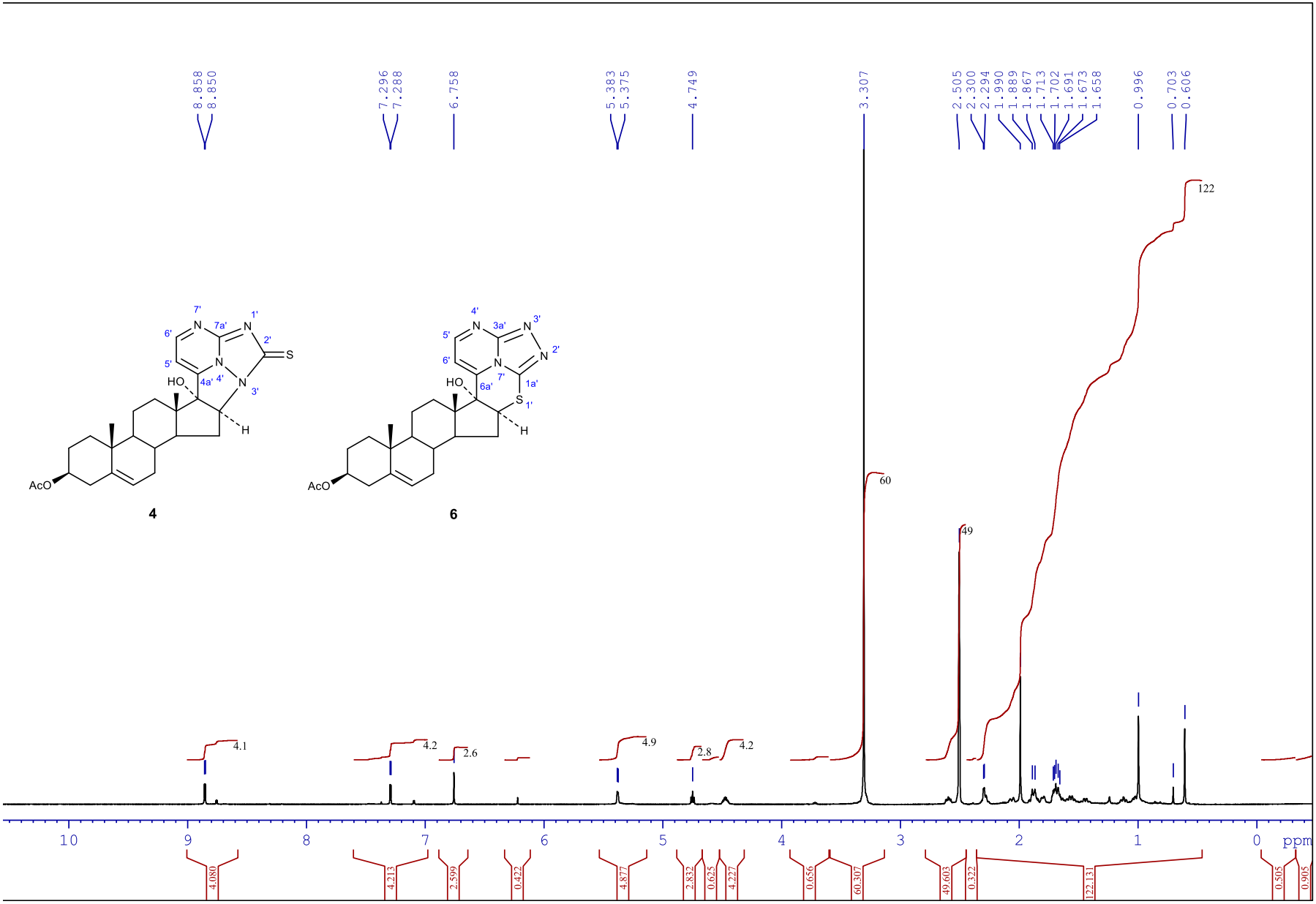
Then the column was eluted with a mixture of $CHCl_3$ and MeOH 20 : 1 and 15 : 1, the solvent was removed from the fraction *in vacuo* and 6 mg (95%) of steroid **8** was obtained, a yellow solid, mp. 310–

314°C (with decomposition), Rf 0.14 ($CHCl_3$ : MeOH 25 : 1), development at 365 nm. IR spectrum, ν, $cm^{-1}$: 3153, 2957, 2925, 2854, 1608, 1581, 1526, 1462, 1435, 1363, 1276, 1250, 1191, 1046, 832, 769, 730, 631. $^1$H NMR (600 MHz, DMSO-$d_6$): δ 0.69 (s, 3H, 18-$CH_3$); 0.95 (s, 3H, 19-$CH_3$); 0.96—1.06 (m, 2H, 9-CH, 1-$CH_2$); 1.32—1.71 (m, 8H, 7-$CH_2$, 8-CH, 12-$CH_2$, 15-$CH_2$, 2-$CH_2$, 11-$CH_2$); 1.75—1.80 (m, 1H, 1-$CH_2$); 1.93—2.02 (m, 2H, 7-$CH_2$, 12-$CH_2$); 2.05—2.18 (m, 3H, 14-CH, 4-$CH_2$); 2.53—2.60 (m, 1H, 15-$CH_2$); 3.23—3.30 (m, 1H, 3-CH); 3.71 (dd, *J* = 8.5, 5.8, 1H, 16-CH); 4.61 (d, *J* = 4.0, 1H, 3-OH); 5.27—5.32 (m, 1H, 6-CH); 6.23 (s, 1H, 17-OH); 7.08 (d, *J* = 4.3, 1H, 6′-CH); 8.75 (d, *J* = 4.3, 1H, 5′-CH). $^{13}$C NMR (150.9 MHz, DMSO-$d_6$), δ: 15.7 (18-$CH_3$); 19.1 (19-$CH_3$); 19.5 (11-$CH_2$); 29.7 (12-$CH_2$); 31.2 (2-$CH_2$); 31.4 (8-CH); 31.8 (7-$CH_2$); 33.8 (15-$CH_2$); 36.2 (10-C); 36.9 (1-$CH_2$); 42.2 (4-$CH_2$); 42.5 (16-CH); 49.0 (13-C); 49.6 (9-CH); 50.1 (14-CH); 70.0 (3-CH); 78.9 (17-C); 107.3 (6′-CH); 120.1 (6-CH); 136.3 (3a′-C); 141.4 (5-C); 147.5 (6a′-C); 153.4 (1a′-C); 154.8 (5′-CH). HRMS (ESI) for $C_{24}H_{31}N_4O_2S$ ($[M + H]^+$): calcd 439.2162, found 439.2164.

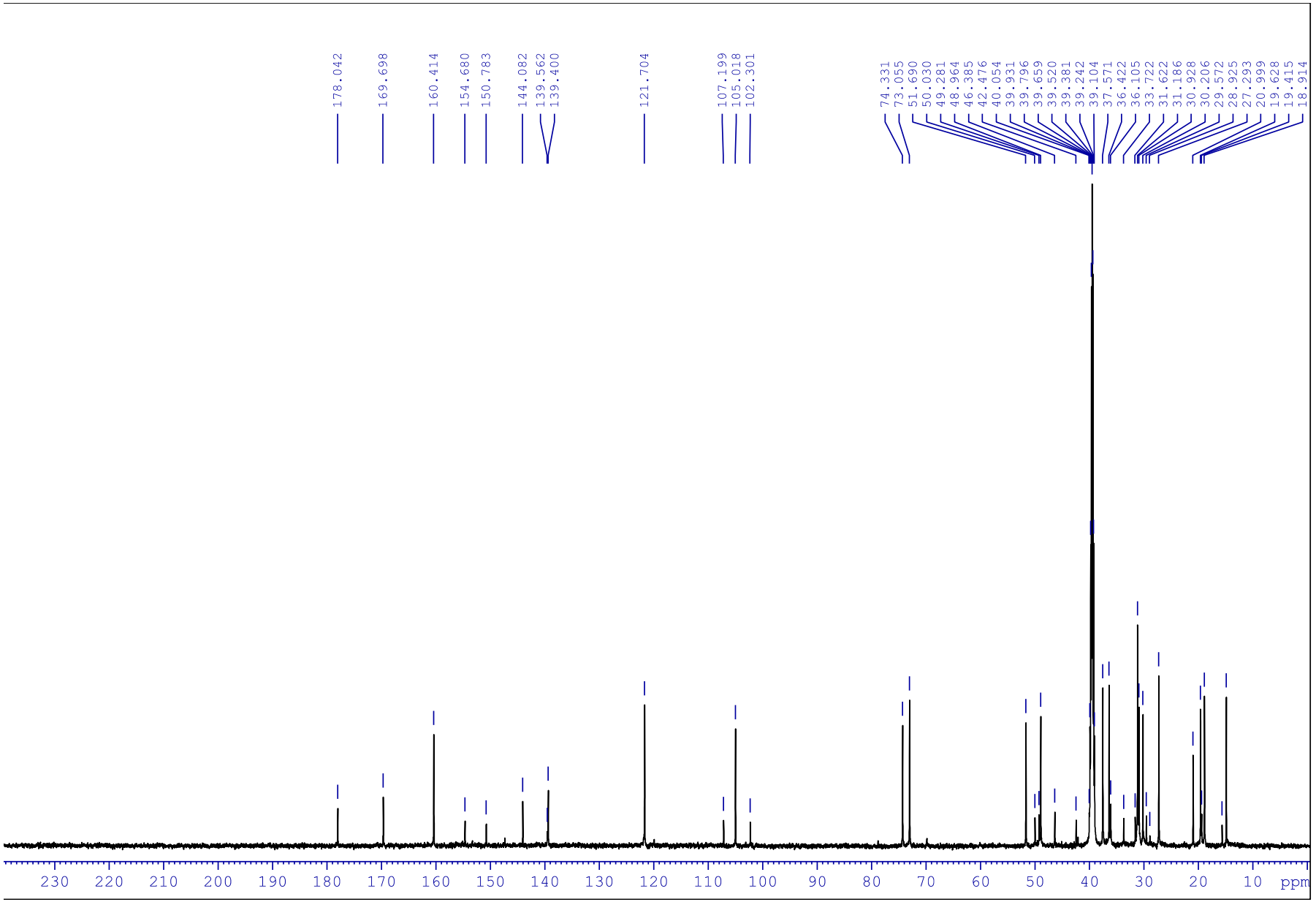
**3β,17α-Dihydroxy-16α-methoxy-1′-pyrimidin-2″-yl-2-methylsulfanyl-16,17-dihydro-1′*H*-androst-5-eno[16,17-*d*]imidazole (9)**. Potassium carbonate (0.028 g, 0.20 mmol) and MeOH (16 ml) are added to a mixture of steroids **4** and **6** from the previous experiment (ratio 4 to 1) (66 mg, 0.14 mmol). The reaction mixture was stirred for 27 h and then MeI (0.15 ml, 2.4 mmol) was added, and this was stirred for 3 h. Methanol was removed *in vacuo*, the residue was chromatographed on a column with $SiO_2$ (eluent mixture of $CHCl_3$ and MeOH 100 : 1), the solvent was removed from the fractions *in vacuo*, the residue was crystallized with petroleum ether (2 ml) to give 36 mg (68%) of steroid **9**, a white solid, m.p. 202-203°C, $R_f$ 0.61 ($CHCl_3$ : MeOH 25 : 1). IR spectrum, ν, $cm^{-1}$: 3440, 2945, 2925, 2891, 2856, 1569, 1546, 1442, 1381, 1298, 1281, 1243, 1217, 1177, 1129, 1092, 1020, 980, 855, 809, 790, 727, 638, 598, 509. $^1$H NMR (600 MHz, DMSO-$d_6$): δ 0.54 (s, 1H, 18-$CH_3$); 0.89—0.95 (m, 1H, 9-CH); 0.92 (s, 3H, 19-$CH_3$); 1.00 (t, *J* = 13.2, 1H, 1-$CH_2$); 1.30—1.62 (m, 7H, 2-$CH_2$, 15-$CH_2$, 8-CH, 7-$CH_2$, 14-CH, 11-$CH_2$); 1.69 (d, *J* = 11.3, 1H, 2-$CH_2$); 1.73—1.85 (m, 2H, 1-$CH_2$, 12-$CH_2$); 1.97 (d, *J* = 16.2, 1H, 7-$CH_2$); 2.05—2.20 (m, 4H, 12-$CH_2$, 15-$CH_2$, 4-$CH_2$); 2.35 (s, 3H, $SCH_3$); 3.23—3.30 (m, 1H, 3-CH); 3.22 (s, 3H, $OCH_3$); 4.58 (b.s., 1H, 3-OH); 5.26—5.28 (m, 1H, 6-CH); 5.59 (s, 1H, 17-OH); 7.08 (t, *J* = 4.7, 1H, 5″-CH); 8.59 (d, *J* = 4.7, 2H, 4″-CH, 6″-CH). $^{13}$C NMR (150.9 MHz, DMSO-$d_6$), δ: 13.3 (18-$CH_3$); 15.5 ($SCH_3$); 19.1 (19-$CH_3$); 20.4 (11-$CH_2$); 31.2 (8-CH); 31.4 (7-$CH_2$); 31.5 (2-$CH_2$); 32.8 (12-$CH_2$); 36.1 (10-C); 36.8 (1-$CH_2$); 38.3 (15-$CH_2$); 50.6 ($OCH_3$); 69.9 (3-CH); 102.3 (17-C); 104.4 (16-C); 115.3 (5″-CH); 120.2 (6-CH); 141.3 (5-C); 155.9 (2″-C); 157.7 (4″-CH, 6″-CH); 160.2 (2′-C). HRMS (ESI) for $C_{26}H_{37}N_4O_3S$ ($[M + H]^+$): calcd 485.2581, found 485.2580.

Further elution of the column with a mixture of $CHCl_3$ and MeOH 20 : 1 and 15 : 1 gave 92 mg (76%) of steroid **8**, identical to the previous experiment.

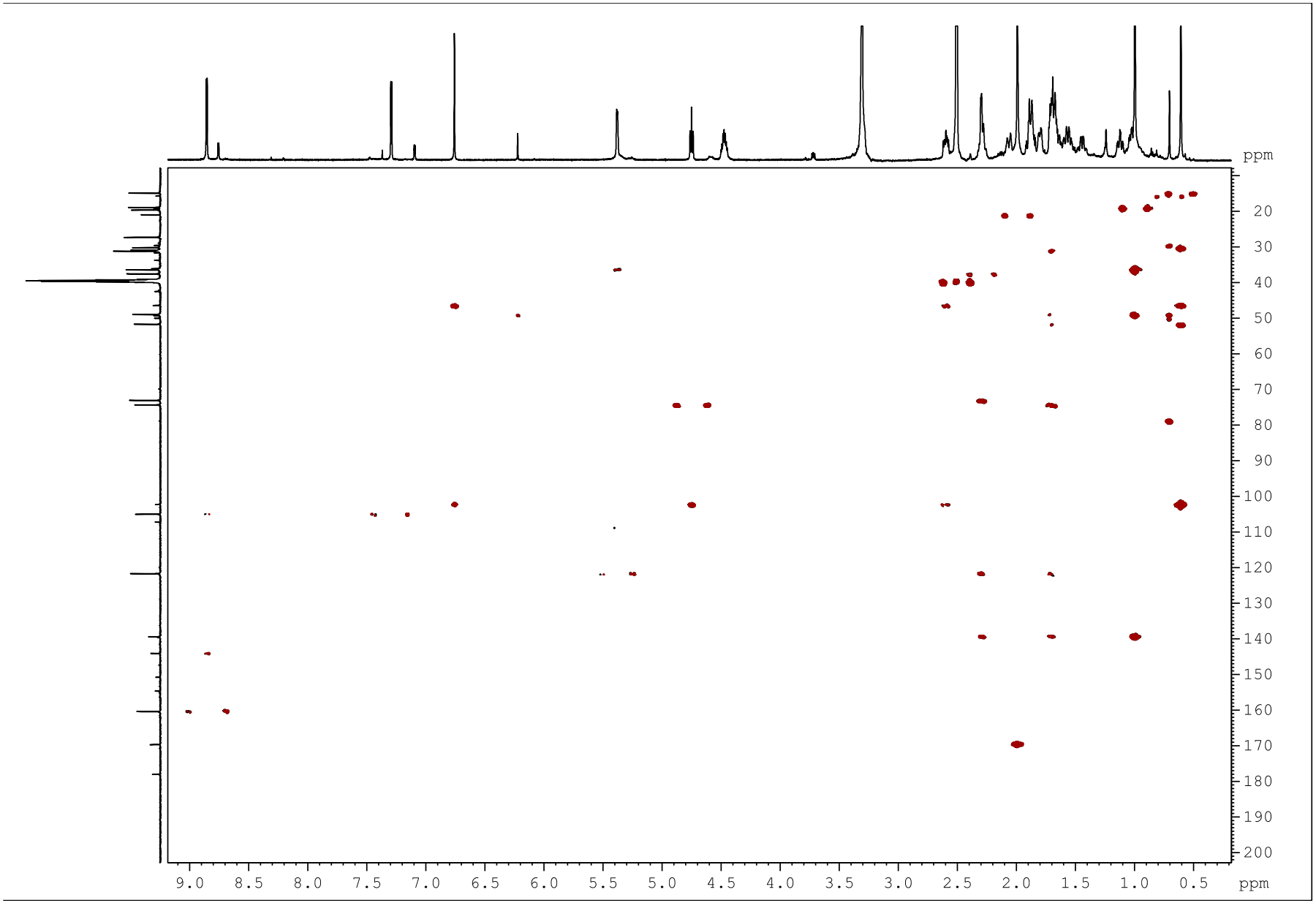
## 2. NMR spectra



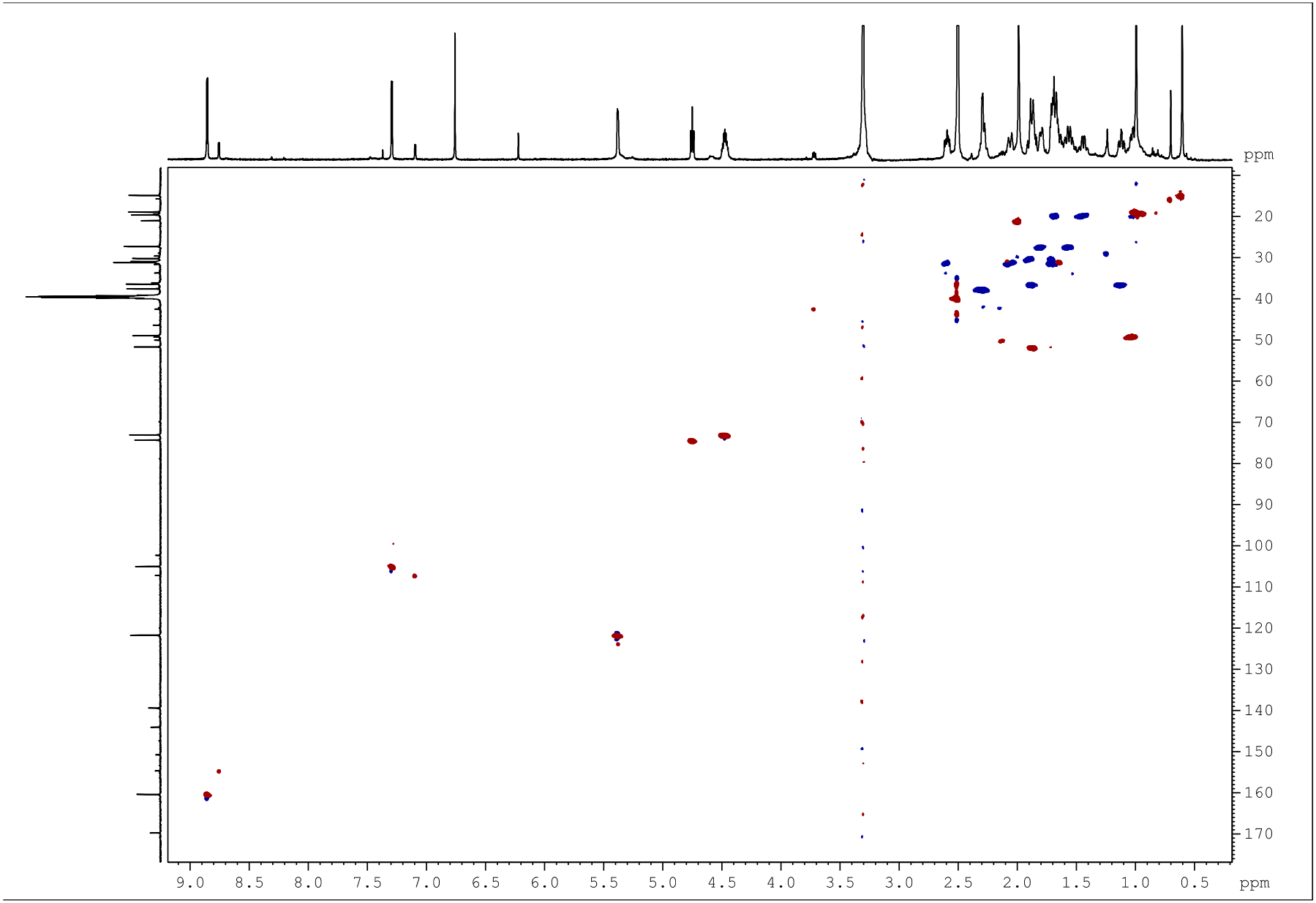

$^{1}$H NMR spectrum of mixture of **4** and **6**.



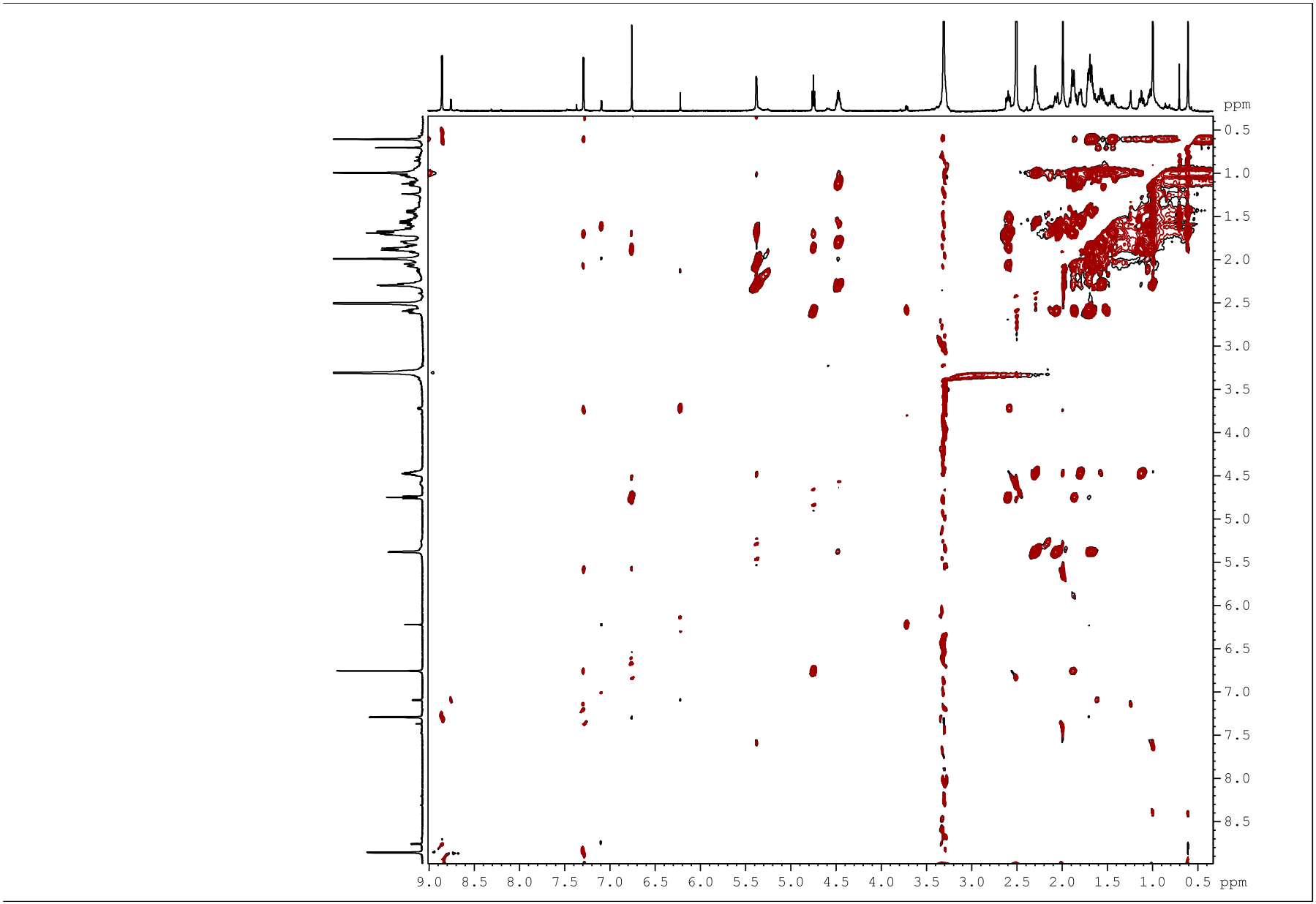

$^{13}$C NMR spectrum of mixture of **4** and **6**.



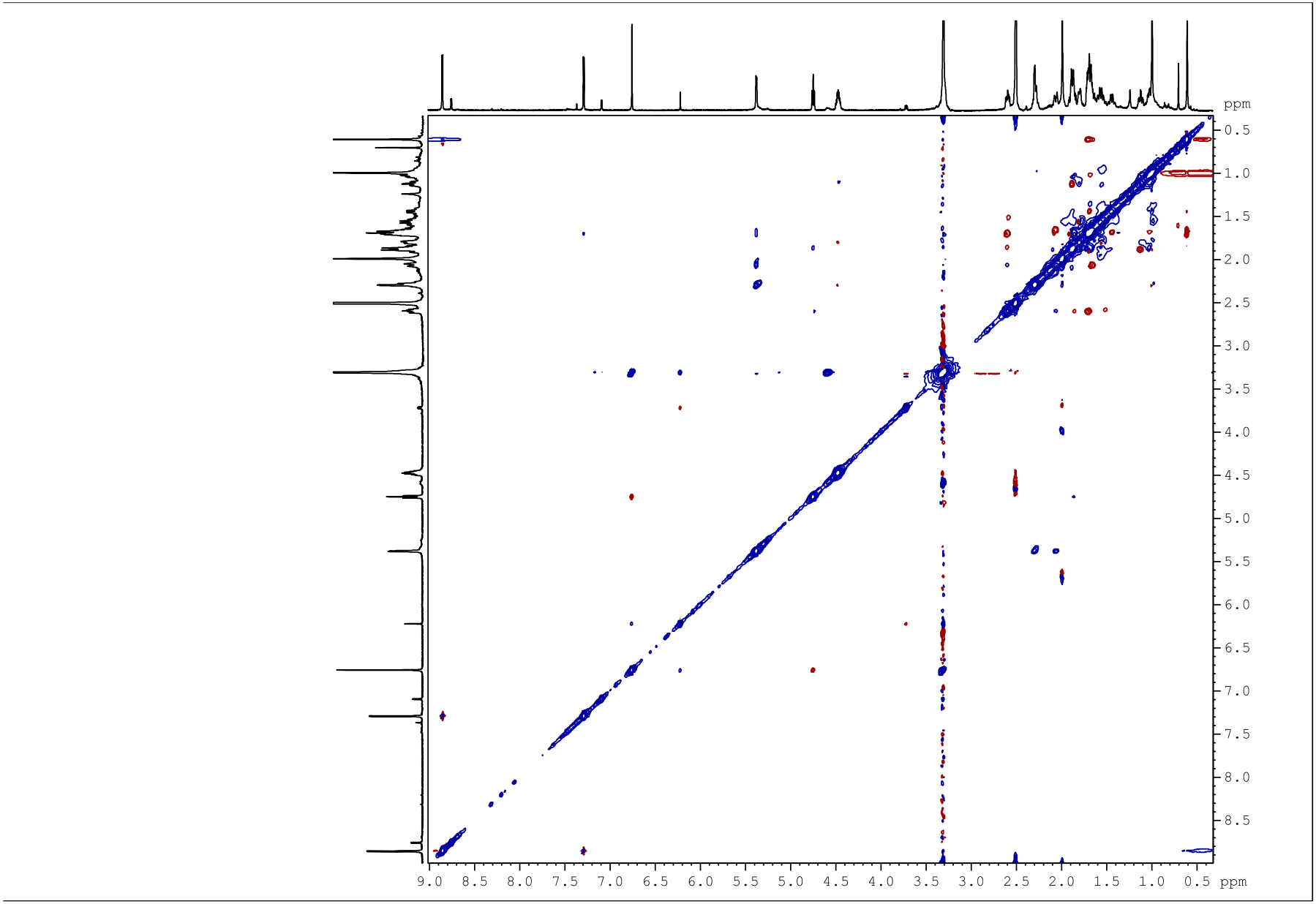

2D $^{1}$H-$^{13}$C HMBC NMR spectrum of mixture of **4** and **6**.



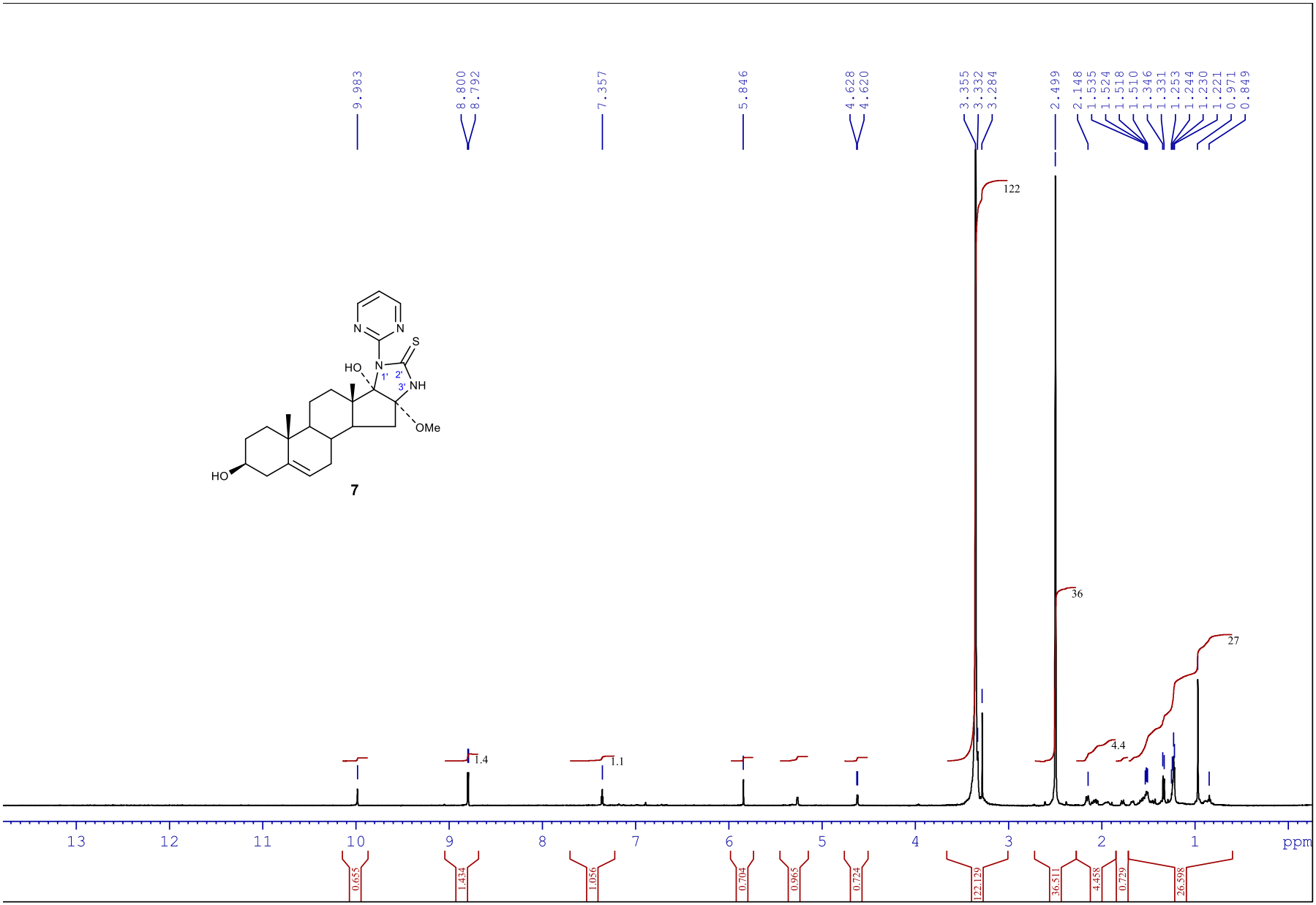

2D $^{1}H$-$^{13}C$ HSQC NMR spectrum of mixture of **4** and **6**.



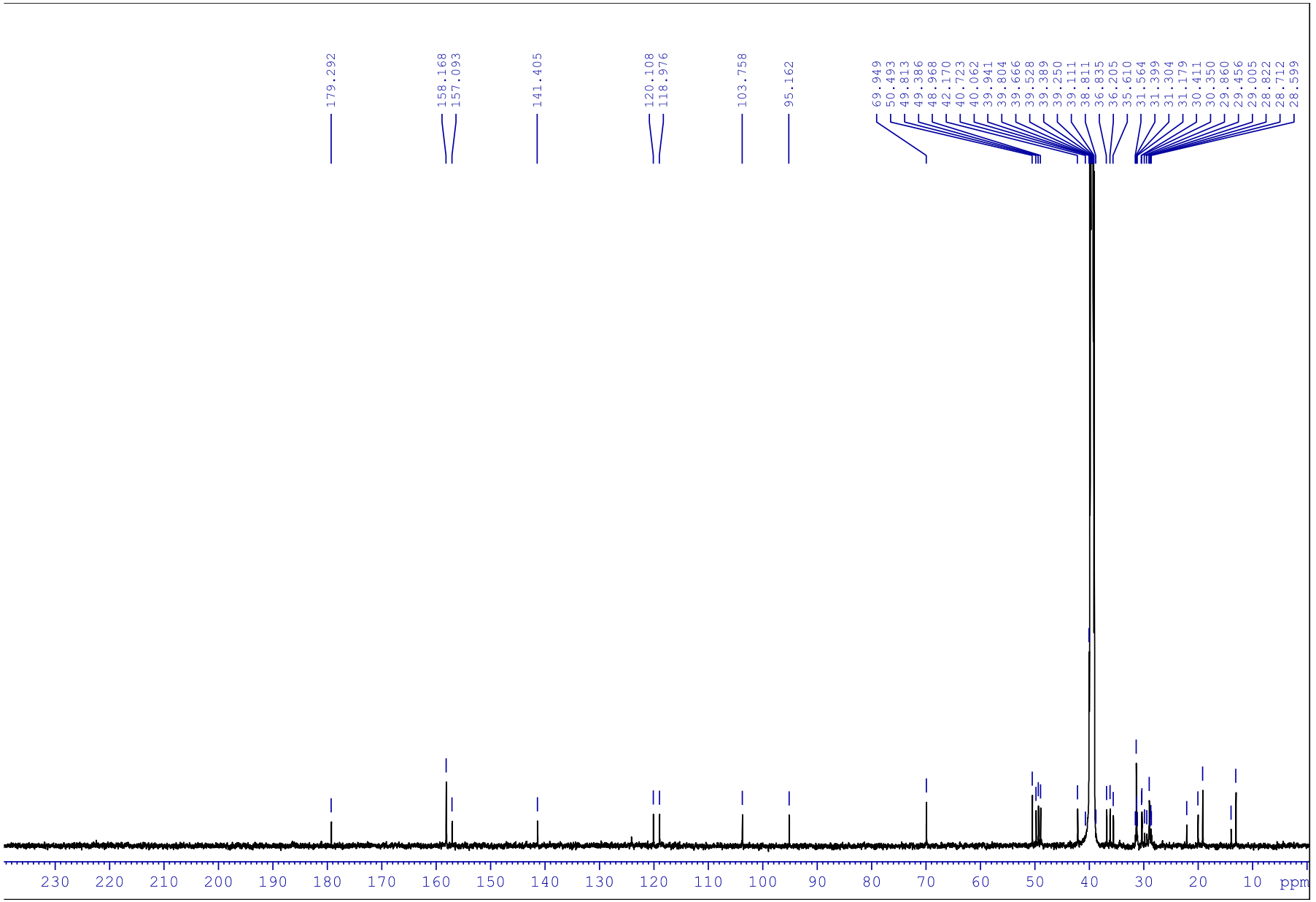

Selective gradient $^{1}$H-$^{1}$H ROESY (*selrogp*) NMR spectrum of mixture of **4** and **6**.



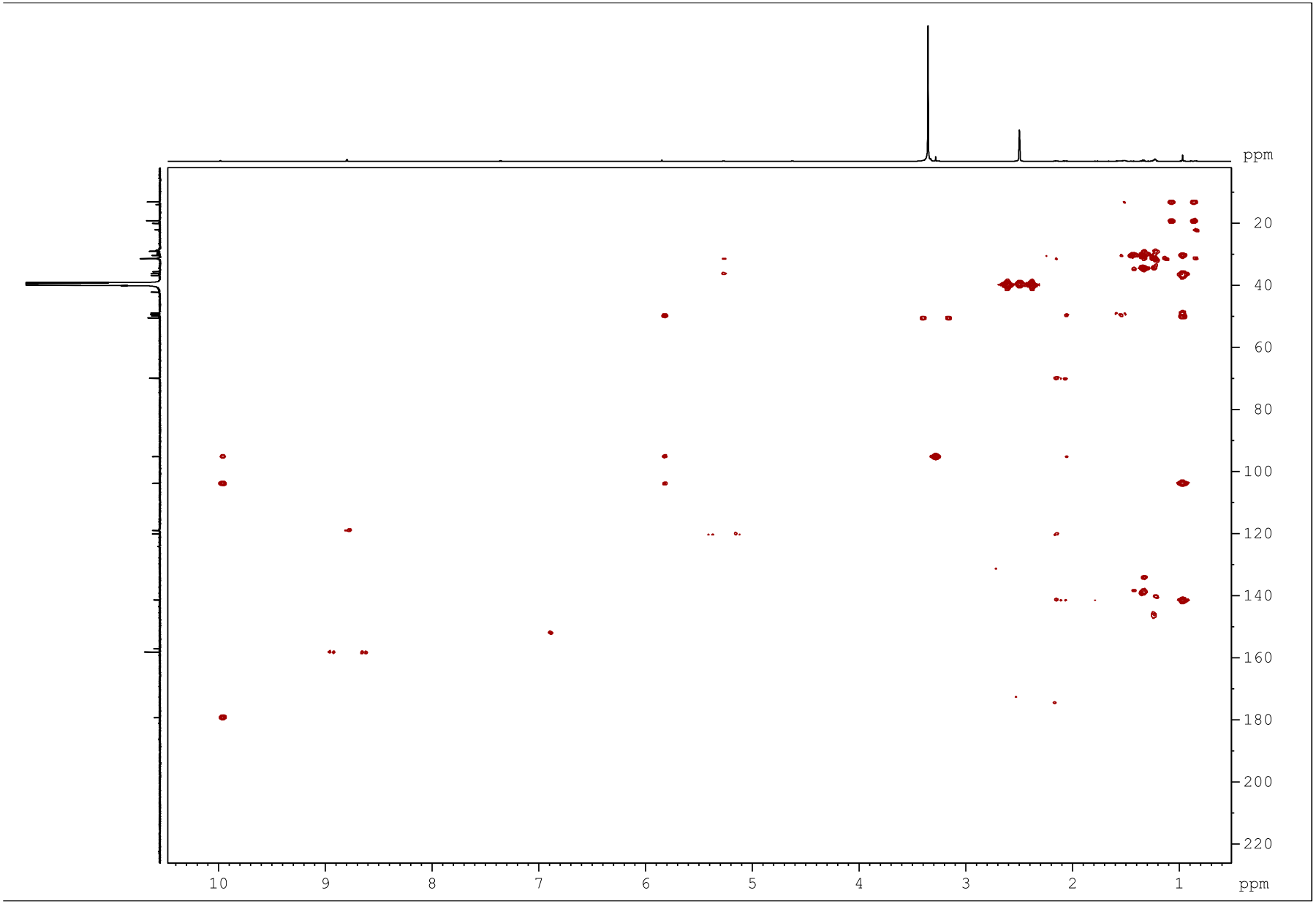

1D $^{1}$H NOESY (selnogp) NMR spectrum of mixture of **4** and **6**.



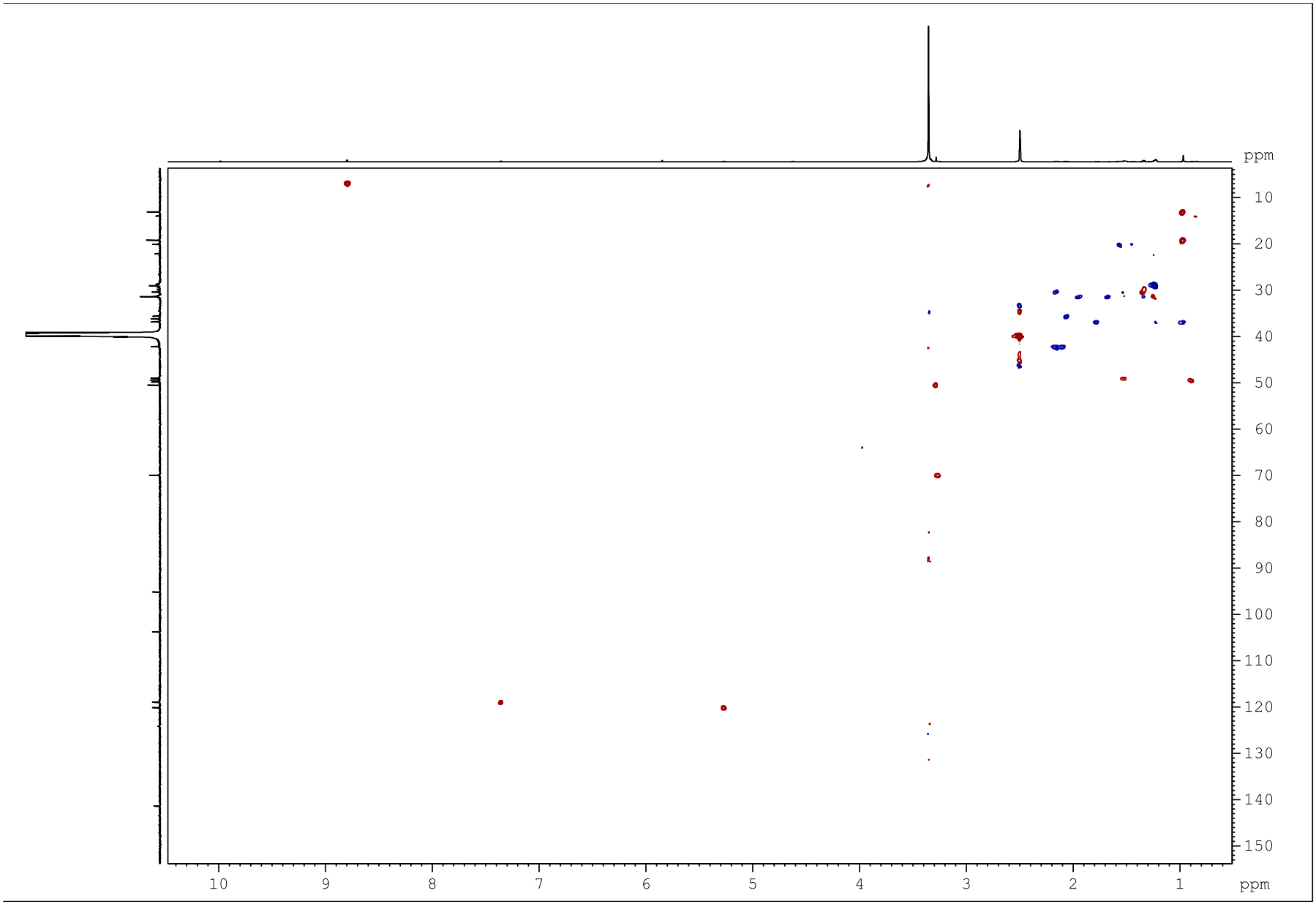

$^{1}$H NMR spectrum of **7**.



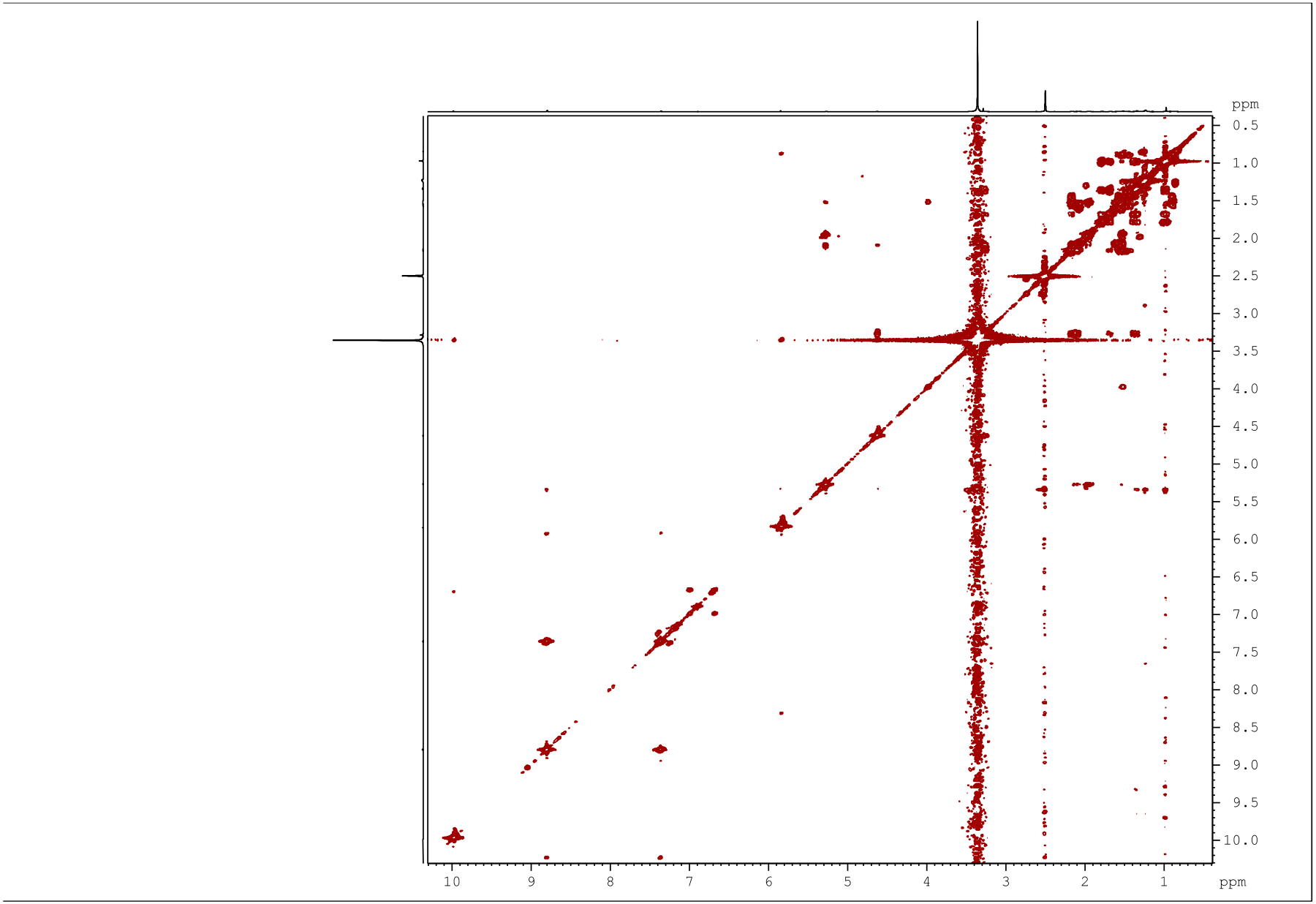

$^{13}$C NMR spectrum of **7**.



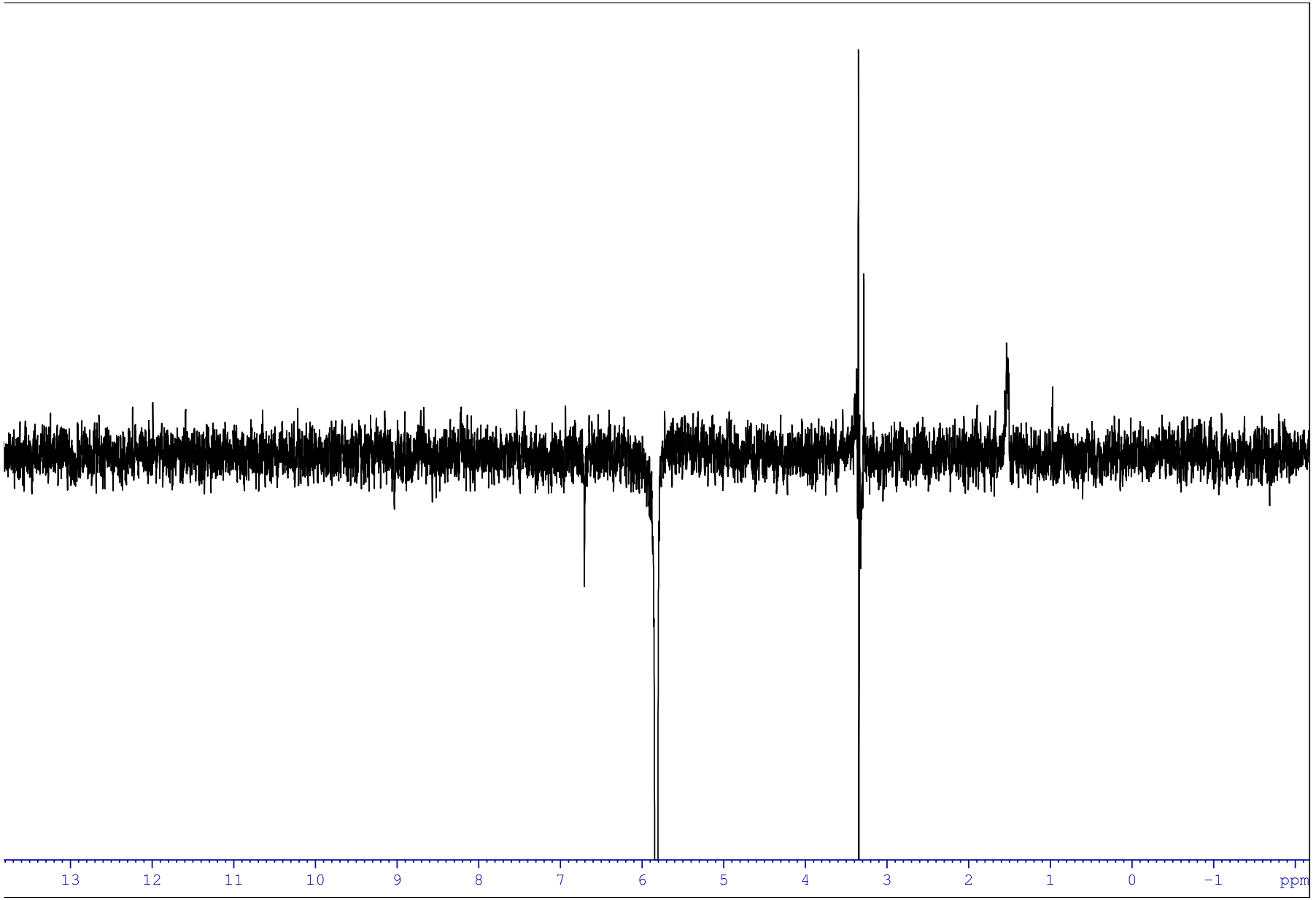

2D $^{1}$H-$^{13}$C HMBC NMR spectrum of **7**.



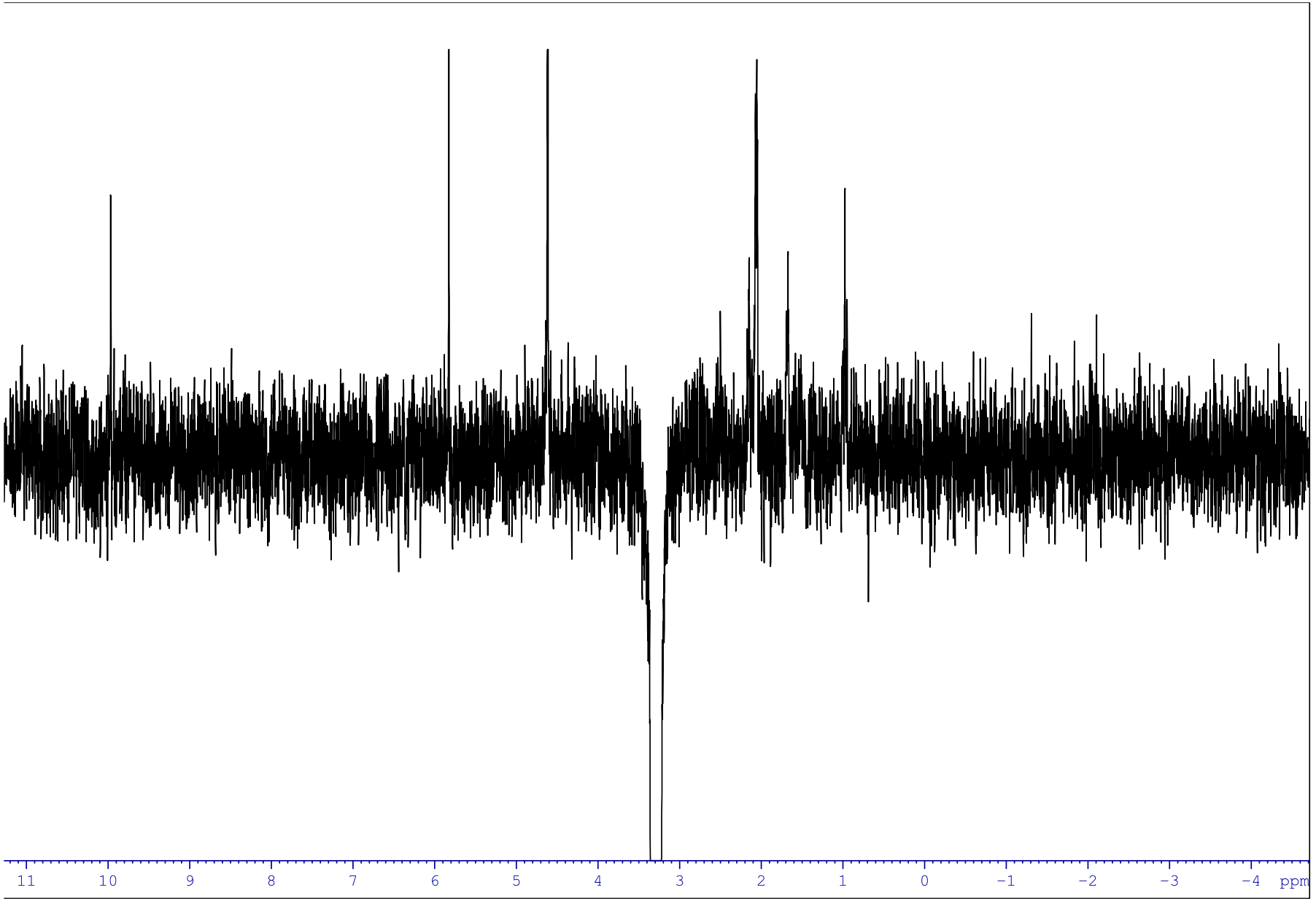

2D $^{1}$H-$^{13}$C HSQC NMR spectrum of **7**.



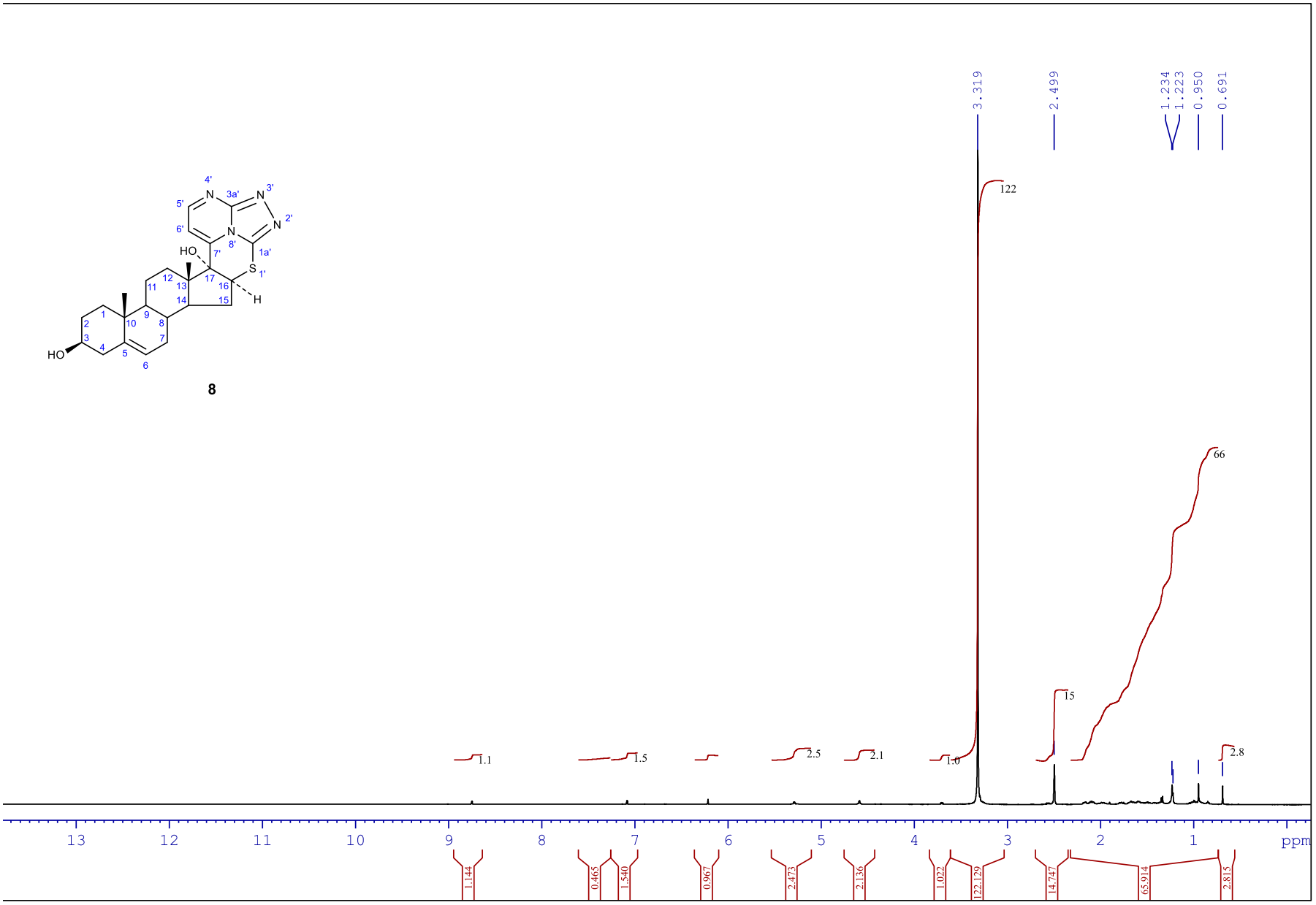

2D $^{1}$H-$^{1}$H COSY NMR spectrum of **7**.




$^{1}$H-$^{1}$H ROESY (5.85 ppm 17-OH) NMR spectrum of **7**.

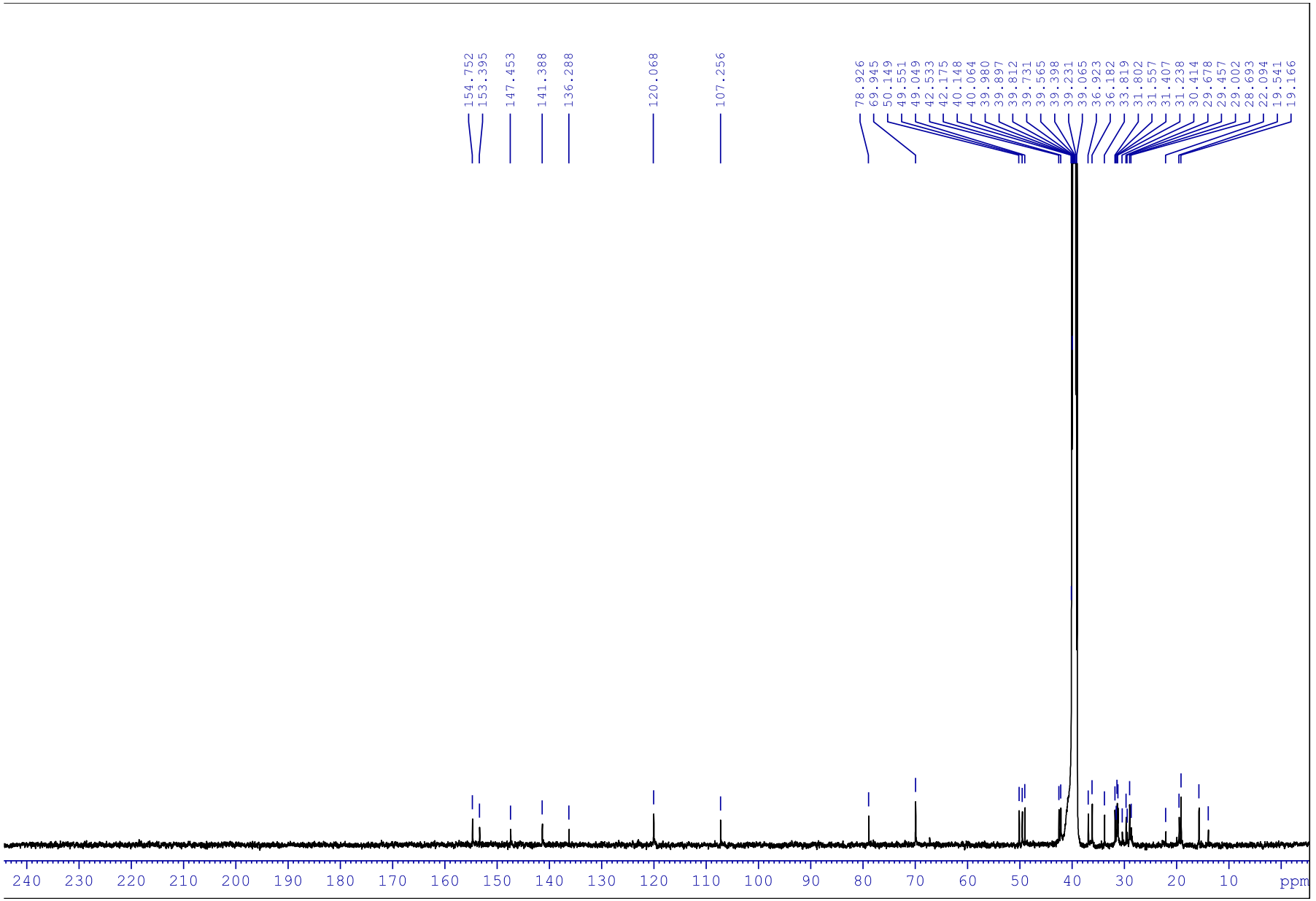



$^{1}$H-$^{1}$H ROESY (3.28 ppm OMe) NMR spectrum of **7**.

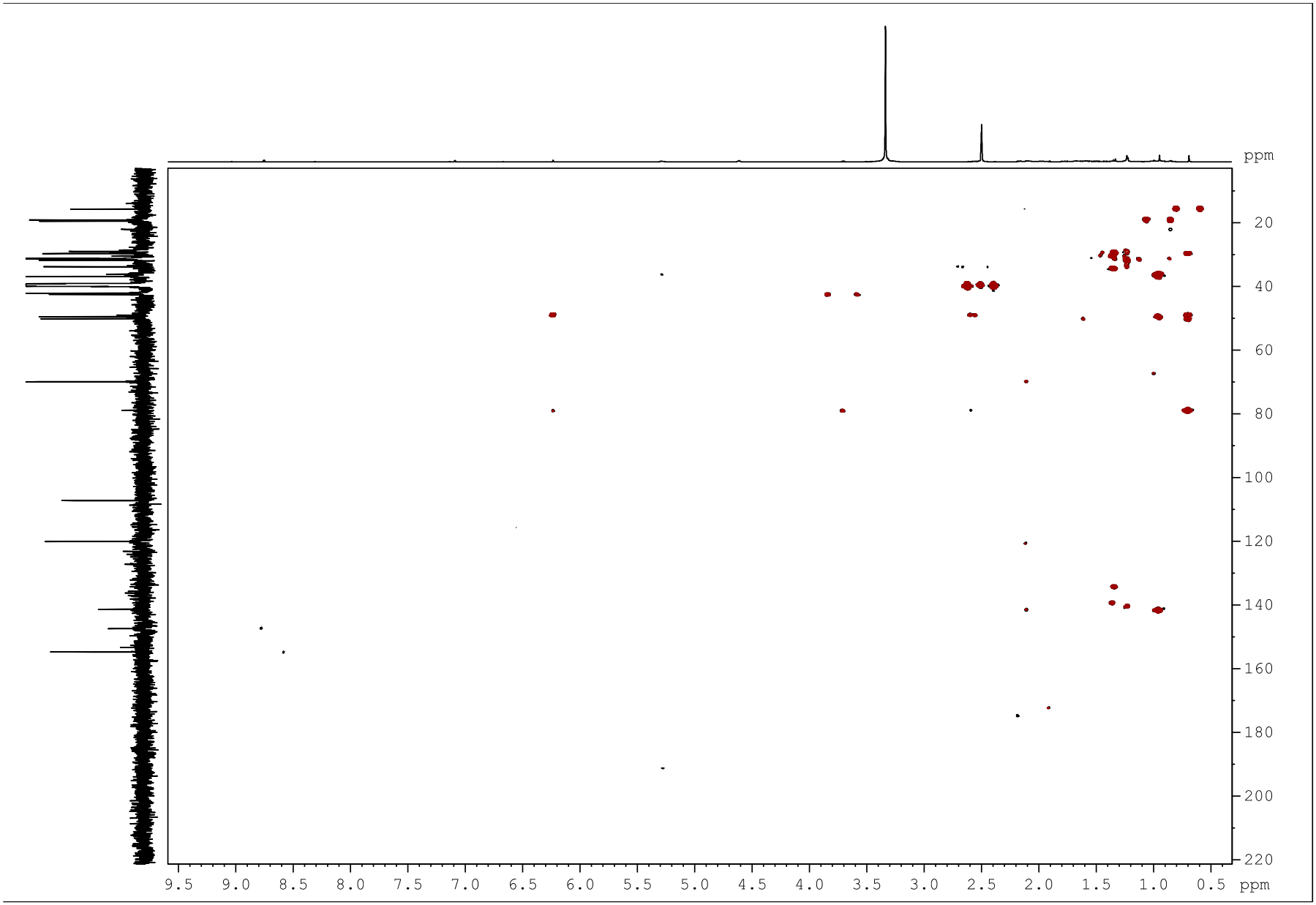



**8**

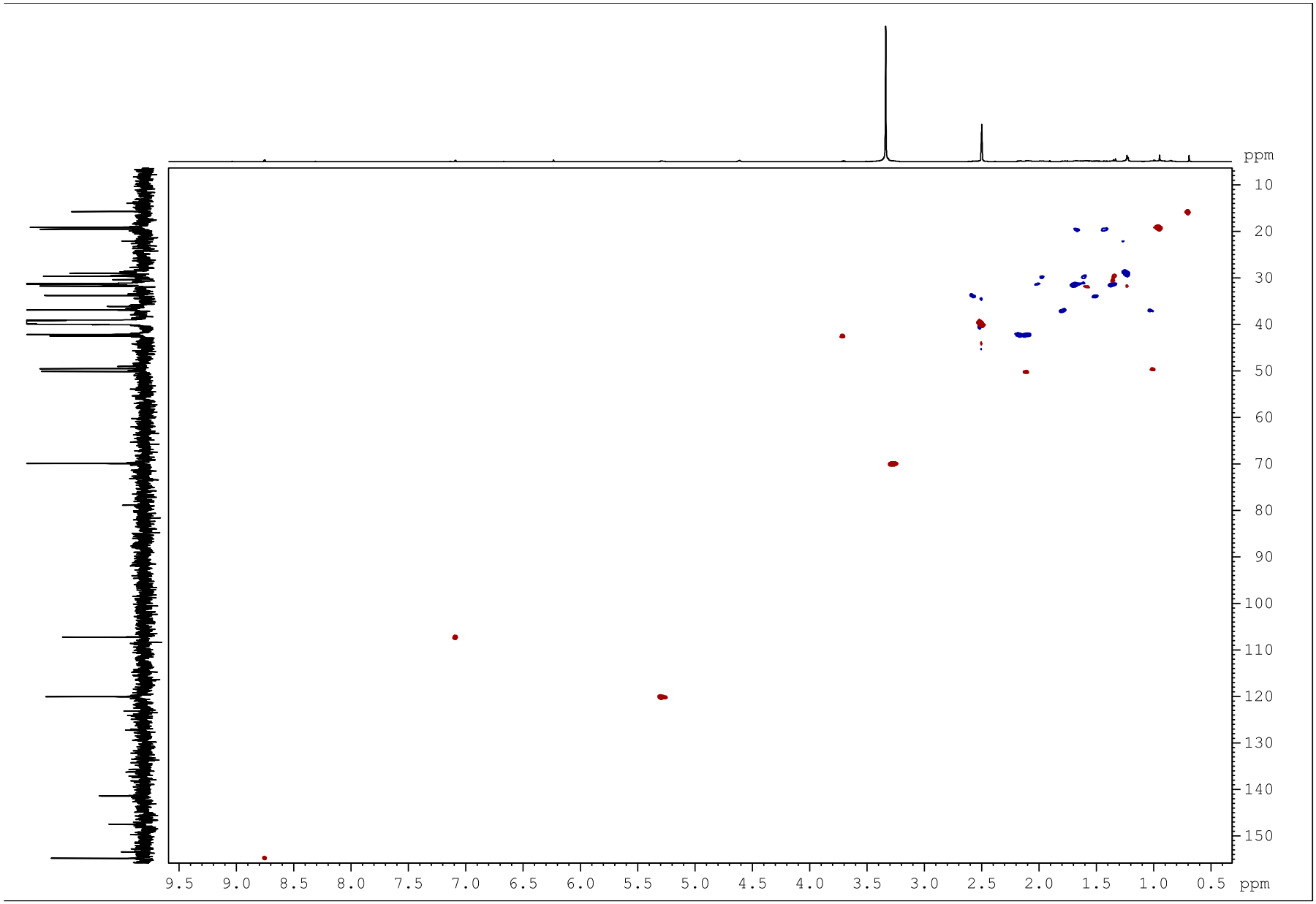
$^{1}$H NMR spectrum of **8**.



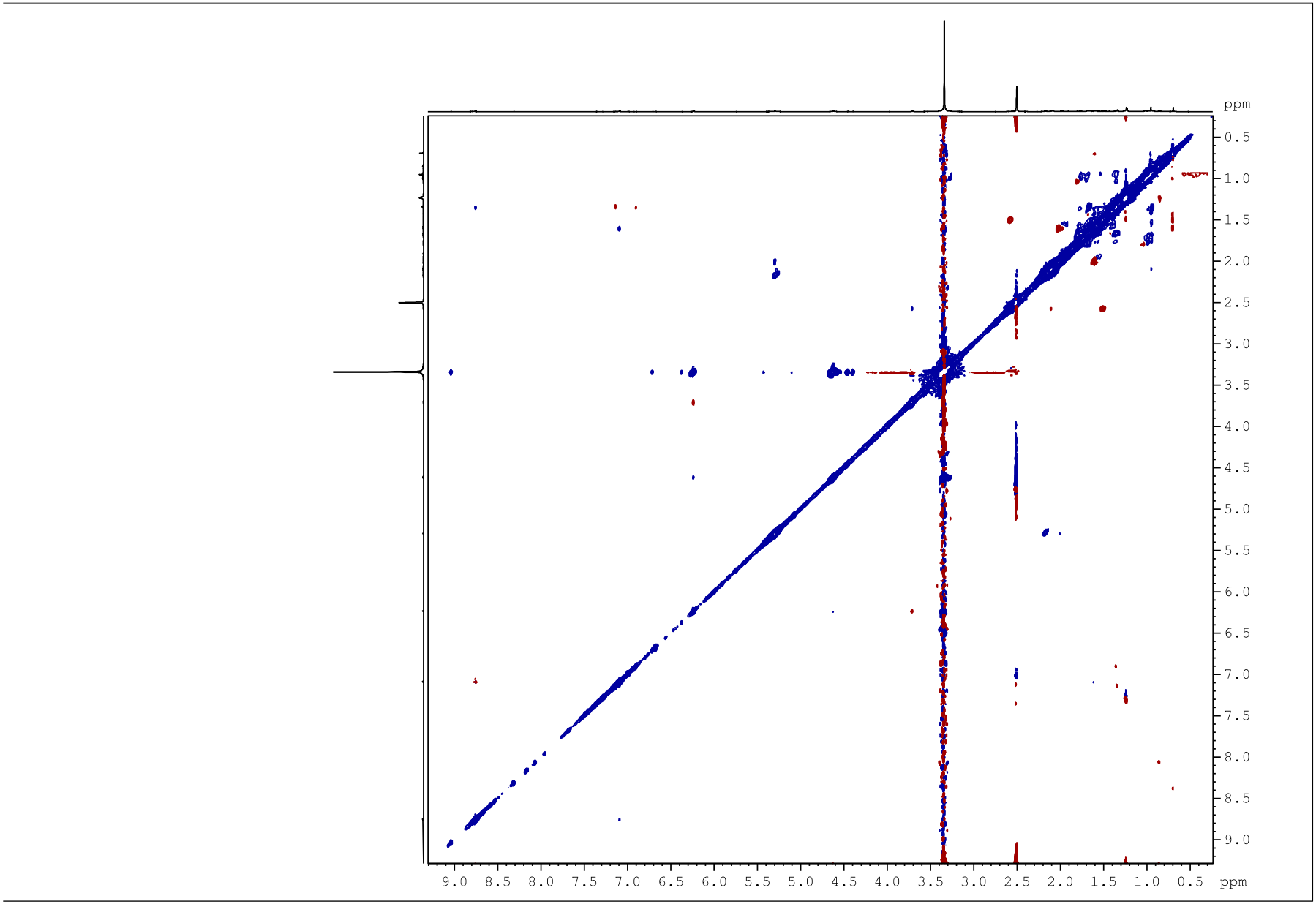

$^{13}$C NMR spectrum of **8**.



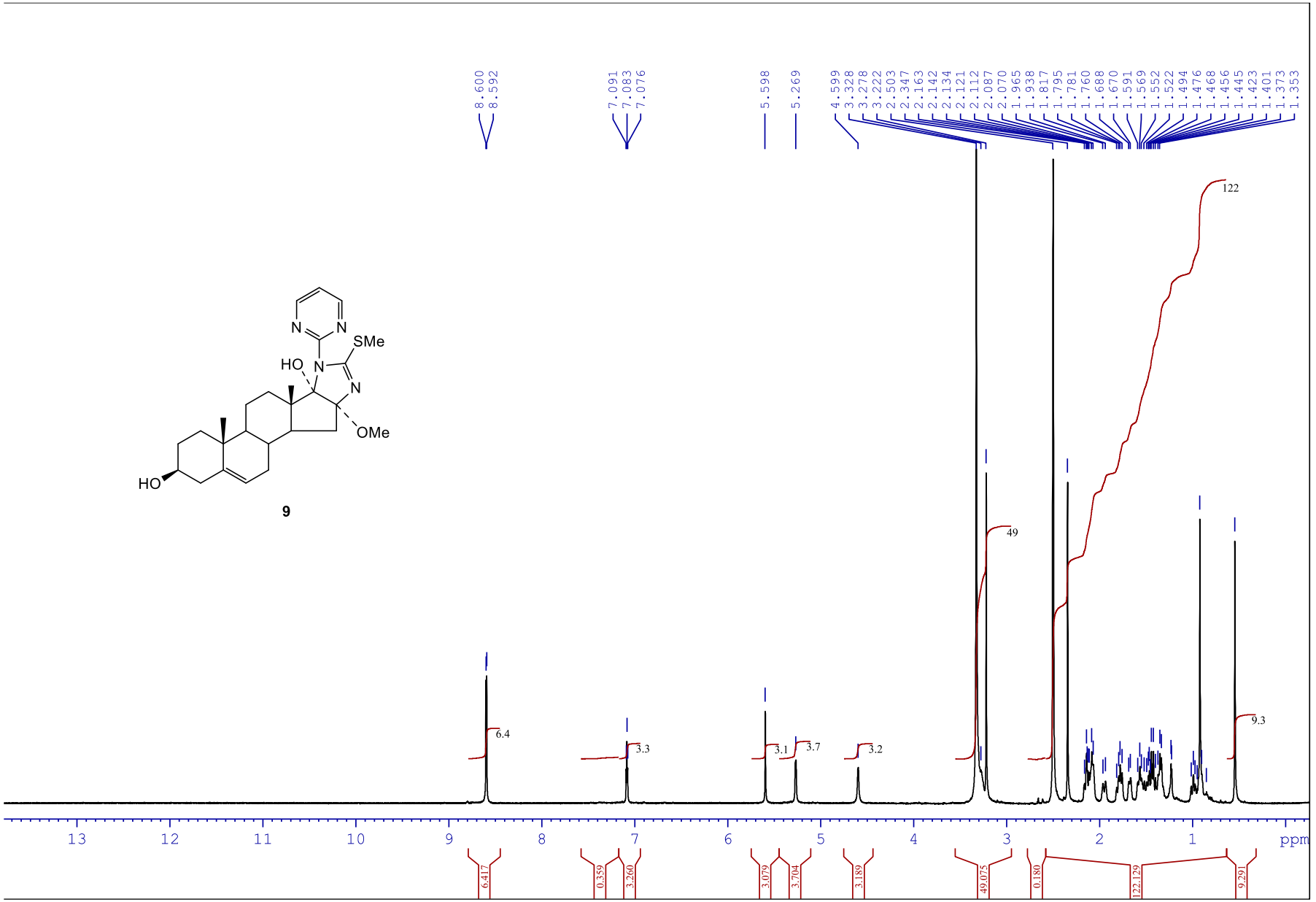

2D $^{1}$H-$^{13}$C HMBC NMR spectrum of **8**.




2D $^{1}$H-$^{13}$C HSQC NMR spectrum of **8**.

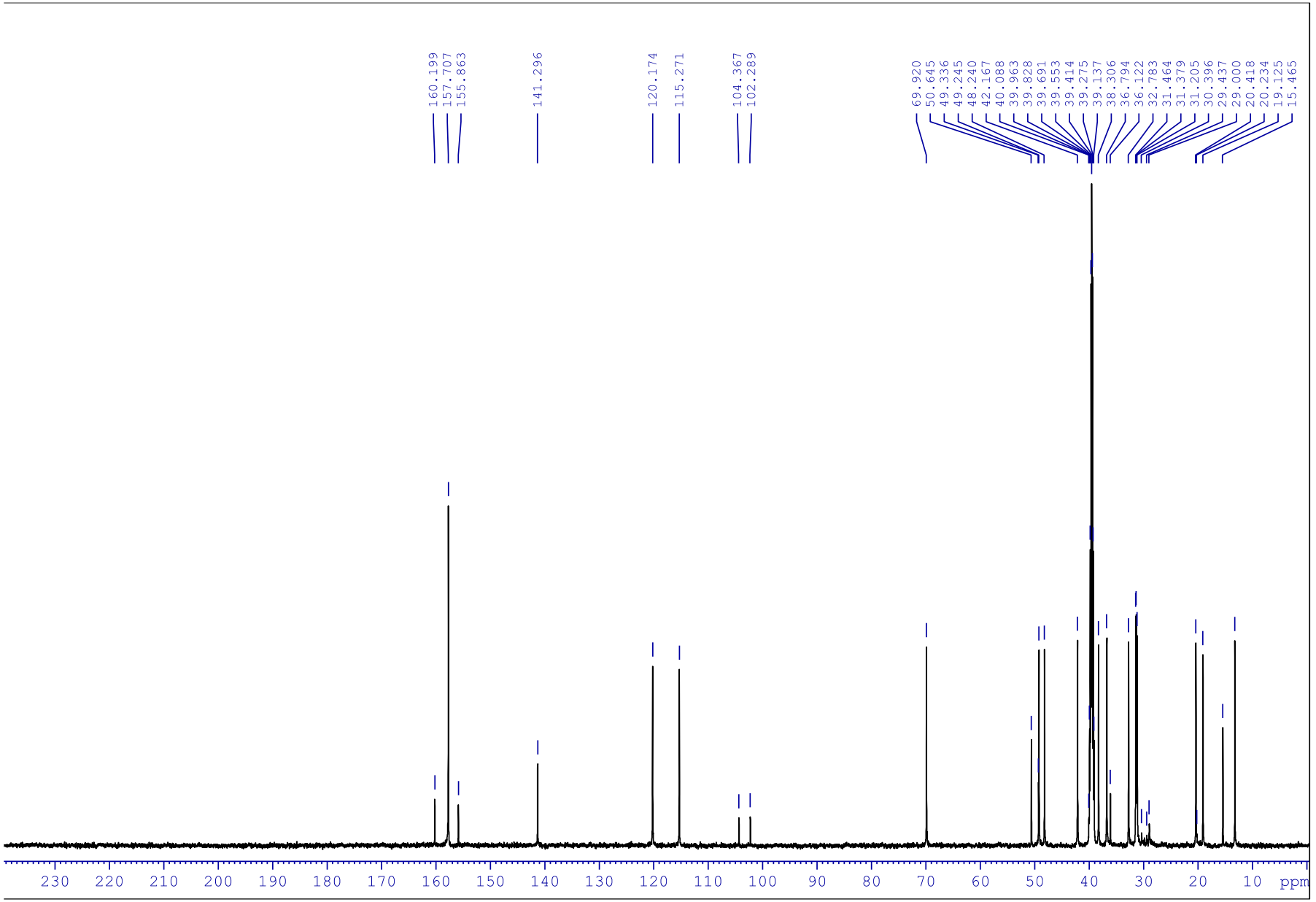



1D $^{1}$H NOESY (selnogp) NMR spectrum of **8**.

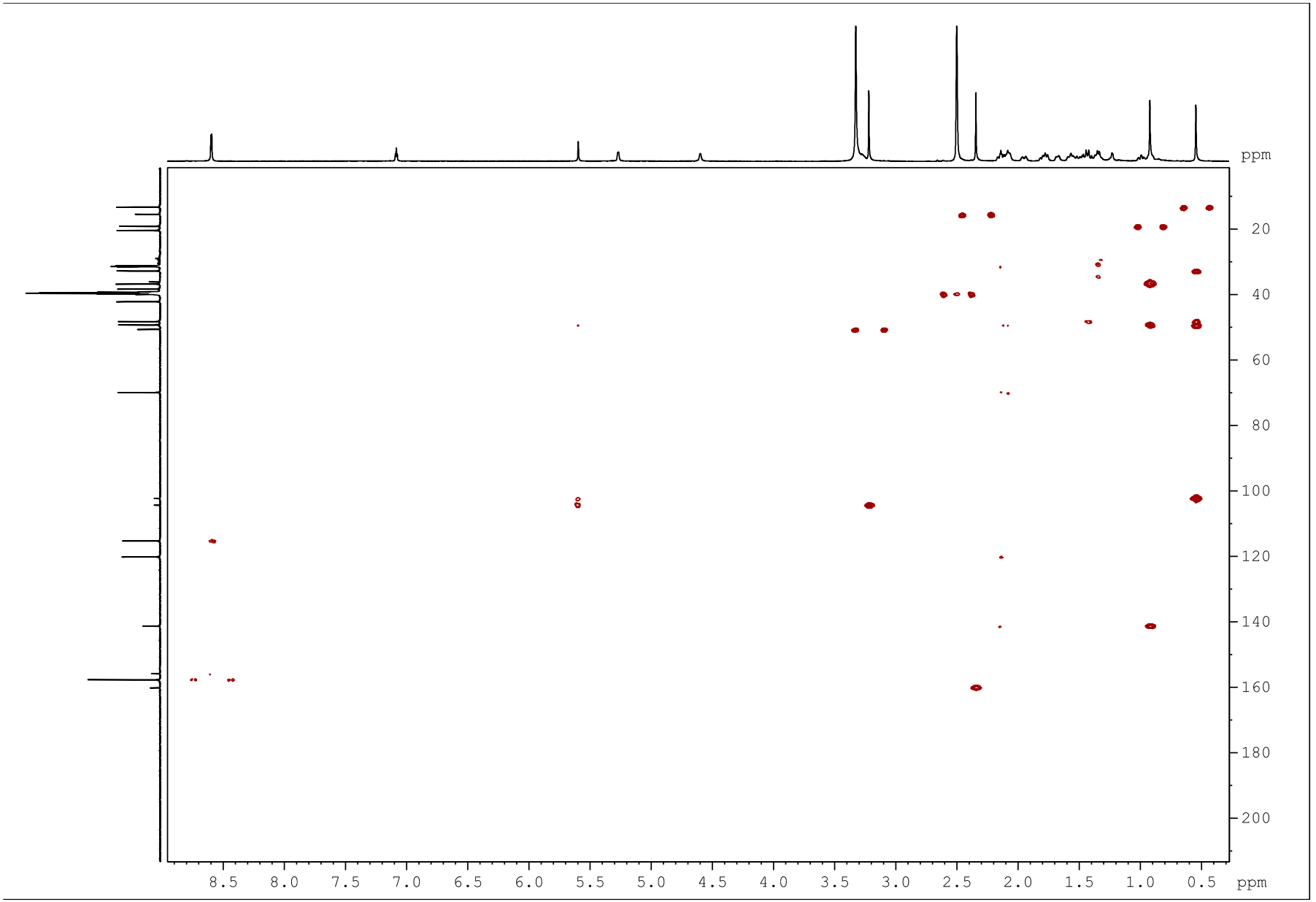



**9**

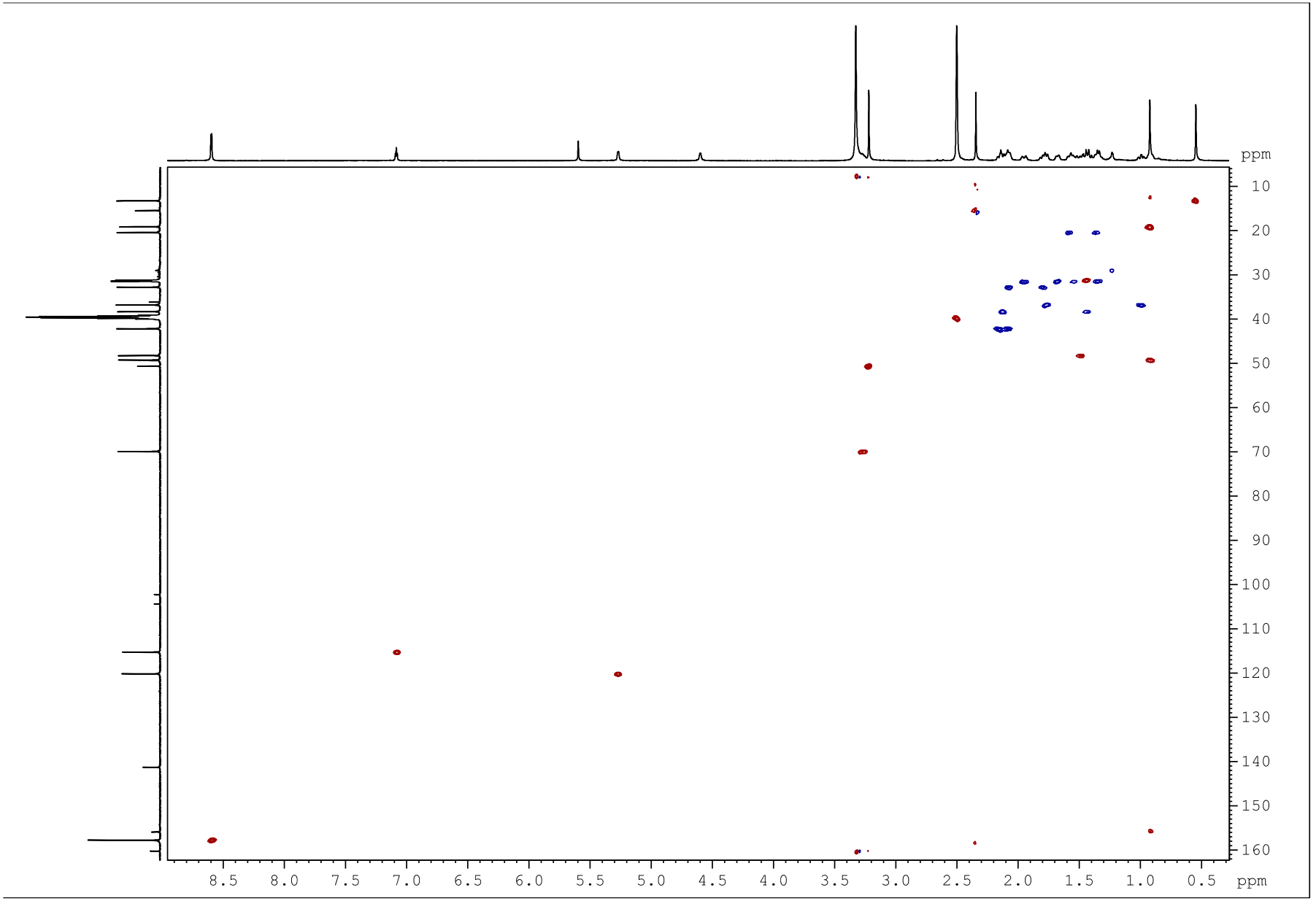
$^{1}$H NMR spectrum of **9**.



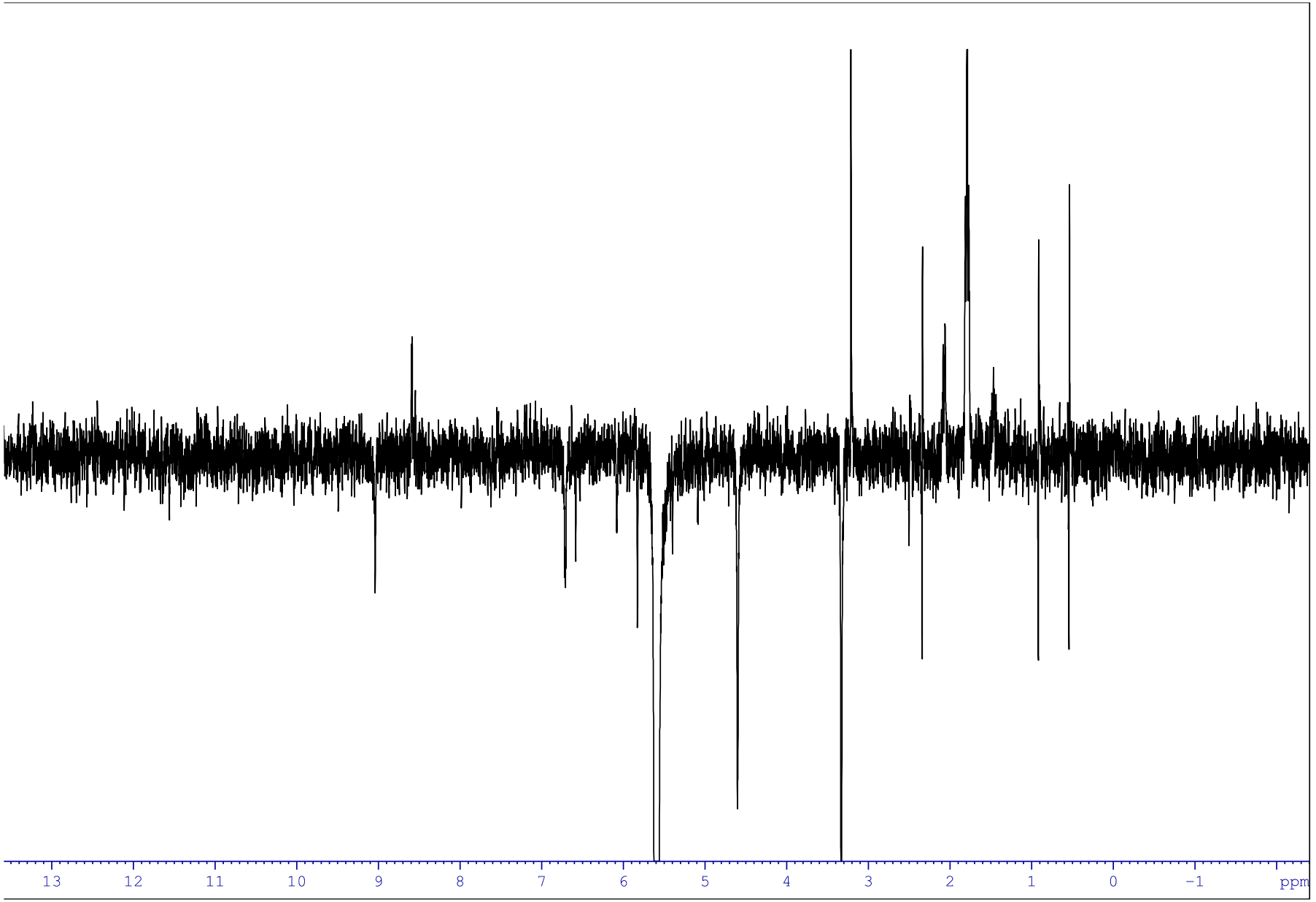

$^{13}$C NMR spectrum of **9**.



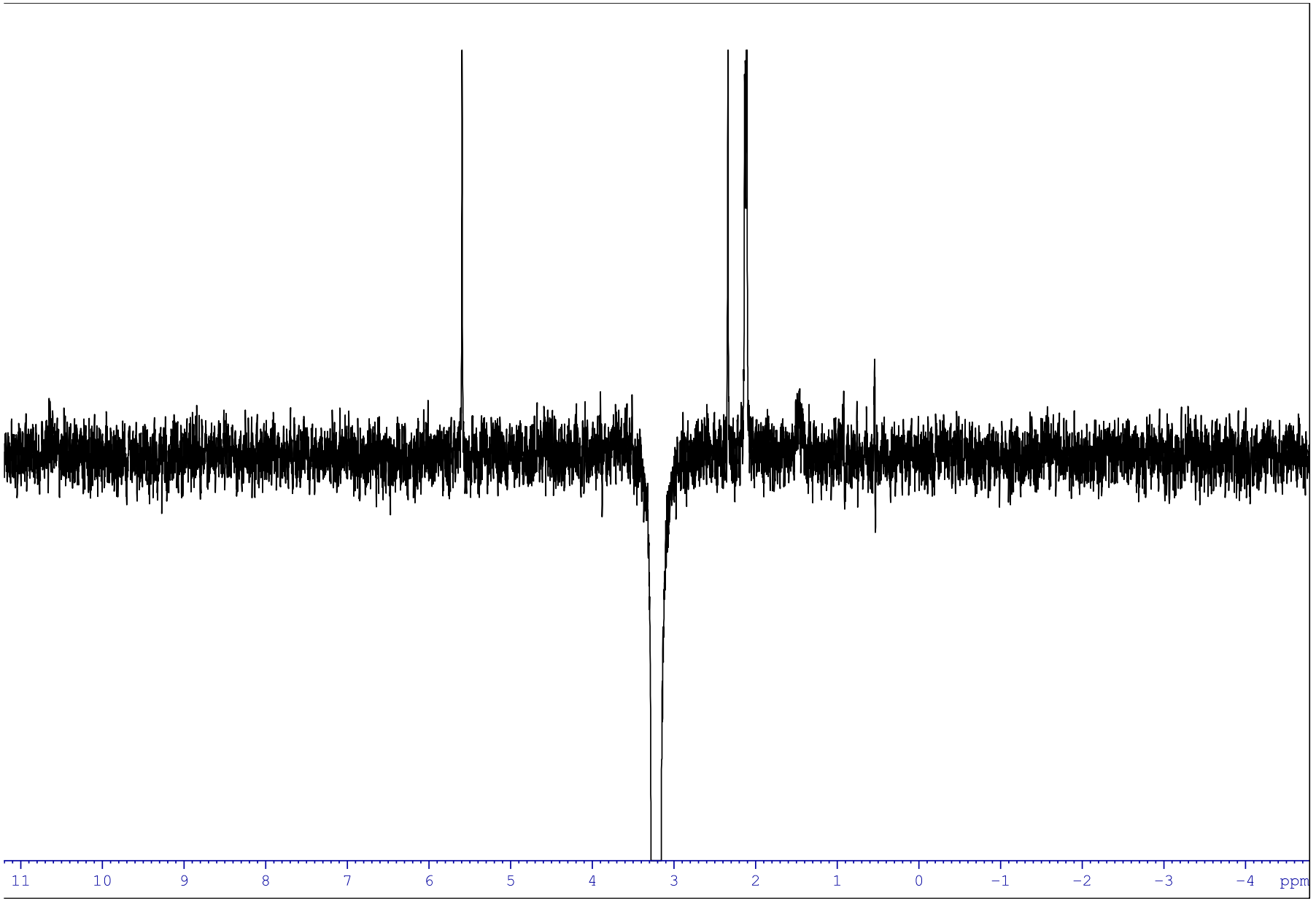

2D $^{1}$H-$^{13}$C HMBC NMR spectrum of **9**.



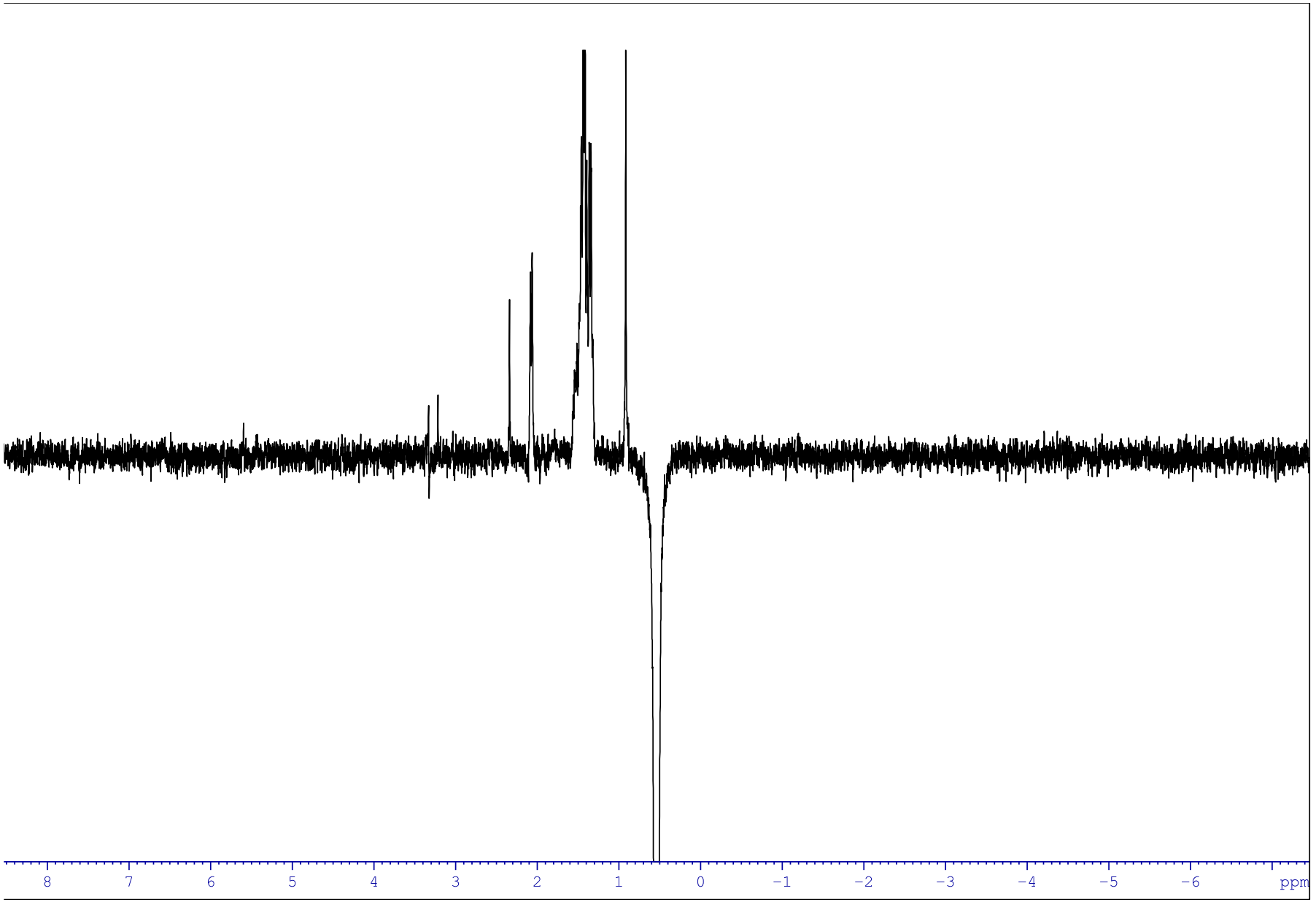

2D $^{1}H$-$^{13}C$ HSQC NMR spectrum of **9**.




$^{1}$H-$^{1}$H ROESY (5.57 ppm, 17OH) NMR spectrum of **9**.




$^{1}$H-$^{1}$H ROESY (3.22 ppm, OMe) NMR spectrum of **9**.




$^{1}$H-$^{1}$H ROESY (0.59 ppm, 18-Me) NMR spectrum of **9**.

## 3. Mass spectra

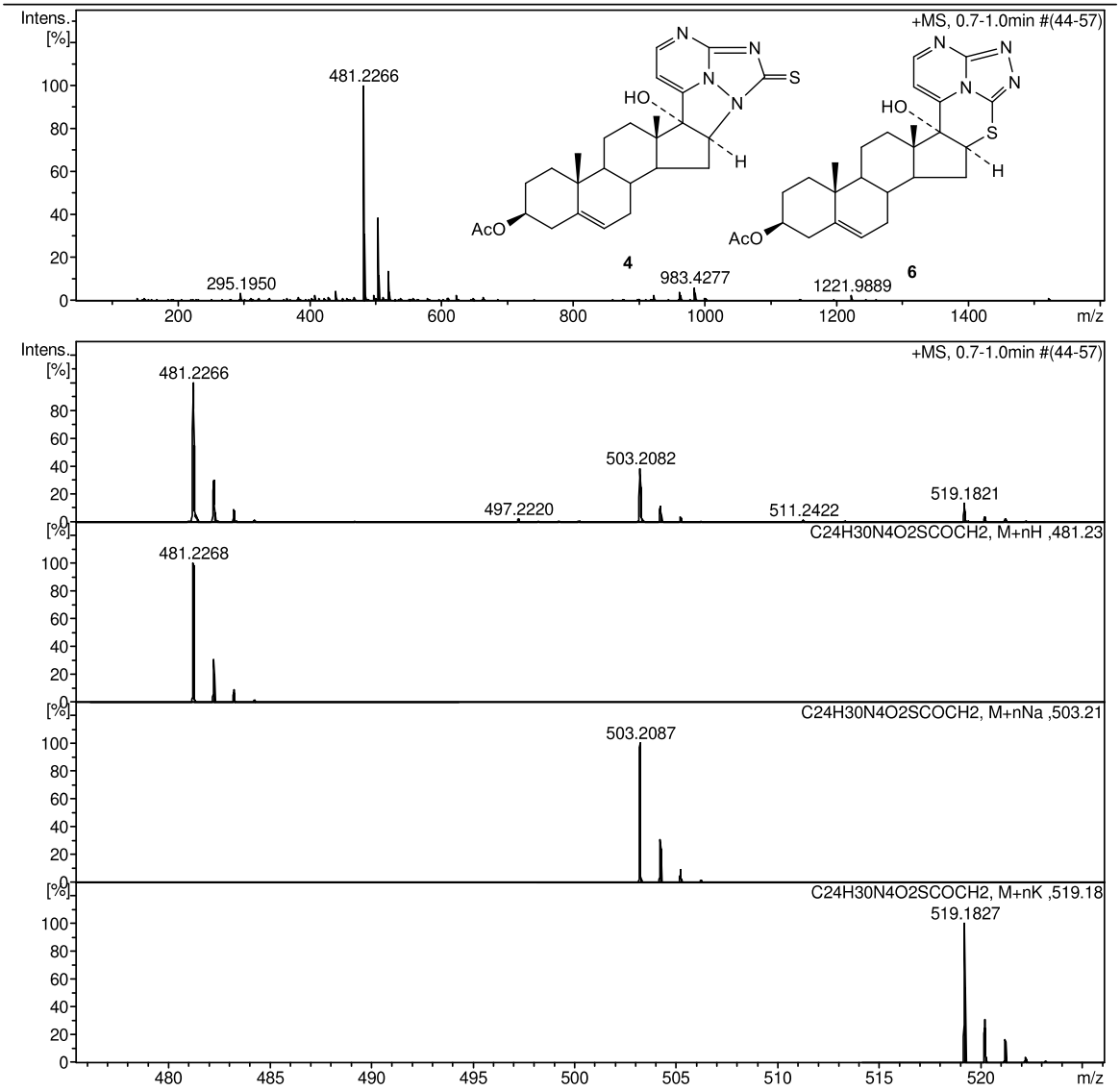
Display Report

**Analysis Info**

| | | | |
|---|---|---|---|
| Method | tune_50-1600_pos_15_12.m | | |
| | | Instrument / Ser# | micrOTOF 10248 |
| Comment | C24H30N4O2S mH 439.2162 calibrant added CH3OH | | |

**Acquisition Parameter**

| | | | | | |
|---|---|---|---|---|---|
| Source Type | ESI | Ion Polarity | Positive | Set Nebulizer | 0.4 Bar |
| Focus | Not active | | | Set Dry Heater | 180 °C |
| Scan Begin | 50 m/z | Set Capillary | 4500 V | Set Dry Gas | 4.0 l/min |
| Scan End | 1600 m/z | Set End Plate Offset | -500 V | Set Divert Valve | Waste |




Mass-spectra of mixture of **4** and **6**.

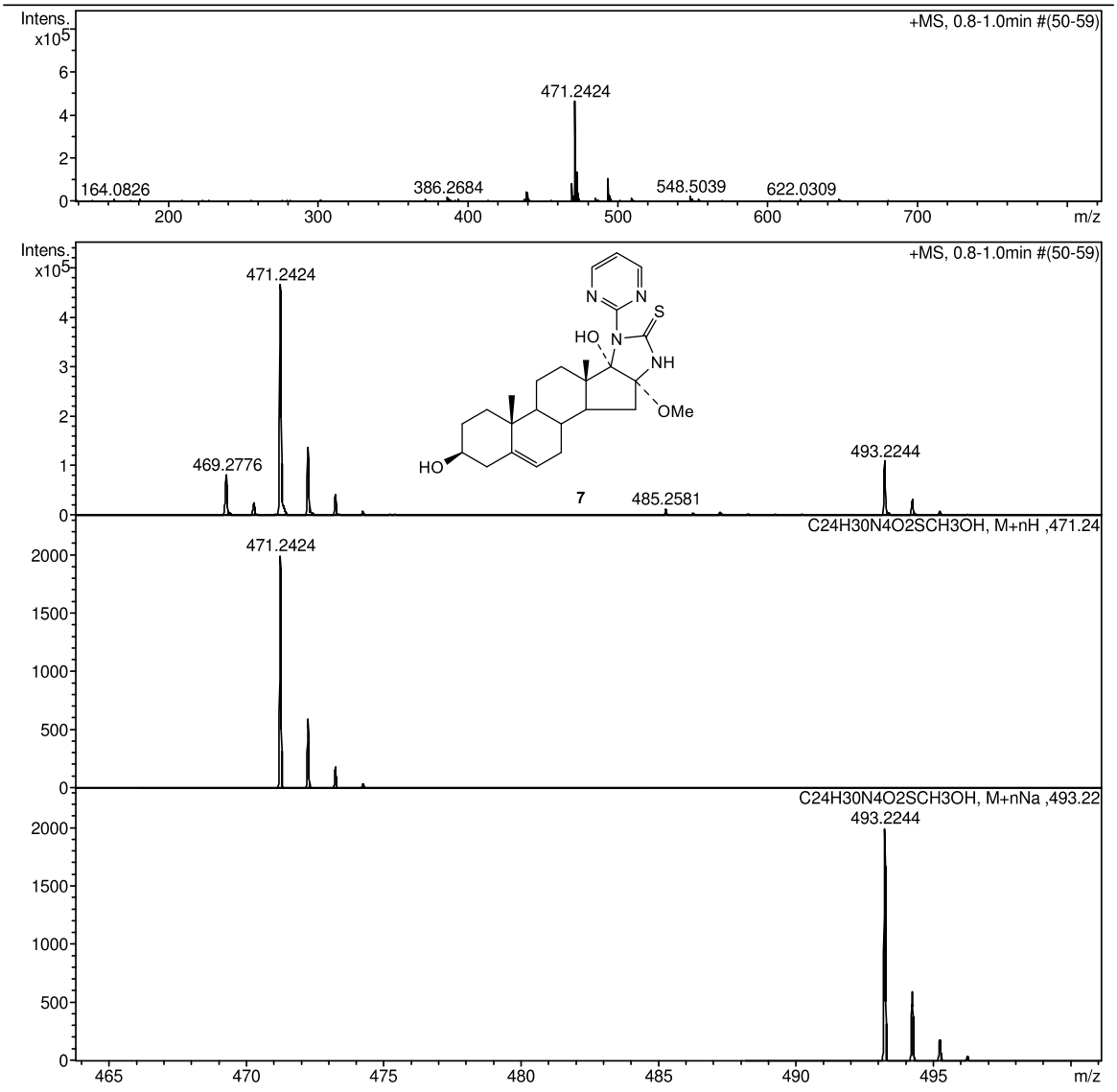
# Display Report

**Analysis Info**

| | | | |
|---|---|---|---|
| Method | tune_low.m | Operator | |
| | | Instrument / Ser# | micrOTOF 10248 |
| Comment | C5H2N4OS mH167.0022 calibrant added, CH3OH | | |

**Acquisition Parameter**

| | | | | | |
|---|---|---|---|---|---|
| Source Type | ESI | Ion Polarity | Positive | Set Nebulizer | 0.4 Bar |
| Focus | Not active | | | Set Dry Heater | 180 °C |
| Scan Begin | 50 m/z | Set Capillary | 4500 V | Set Dry Gas | 4.0 l/min |
| Scan End | 2500 m/z | Set End Plate Offset | -500 V | Set Divert Valve | Waste |




Mass-spectra of **7**.

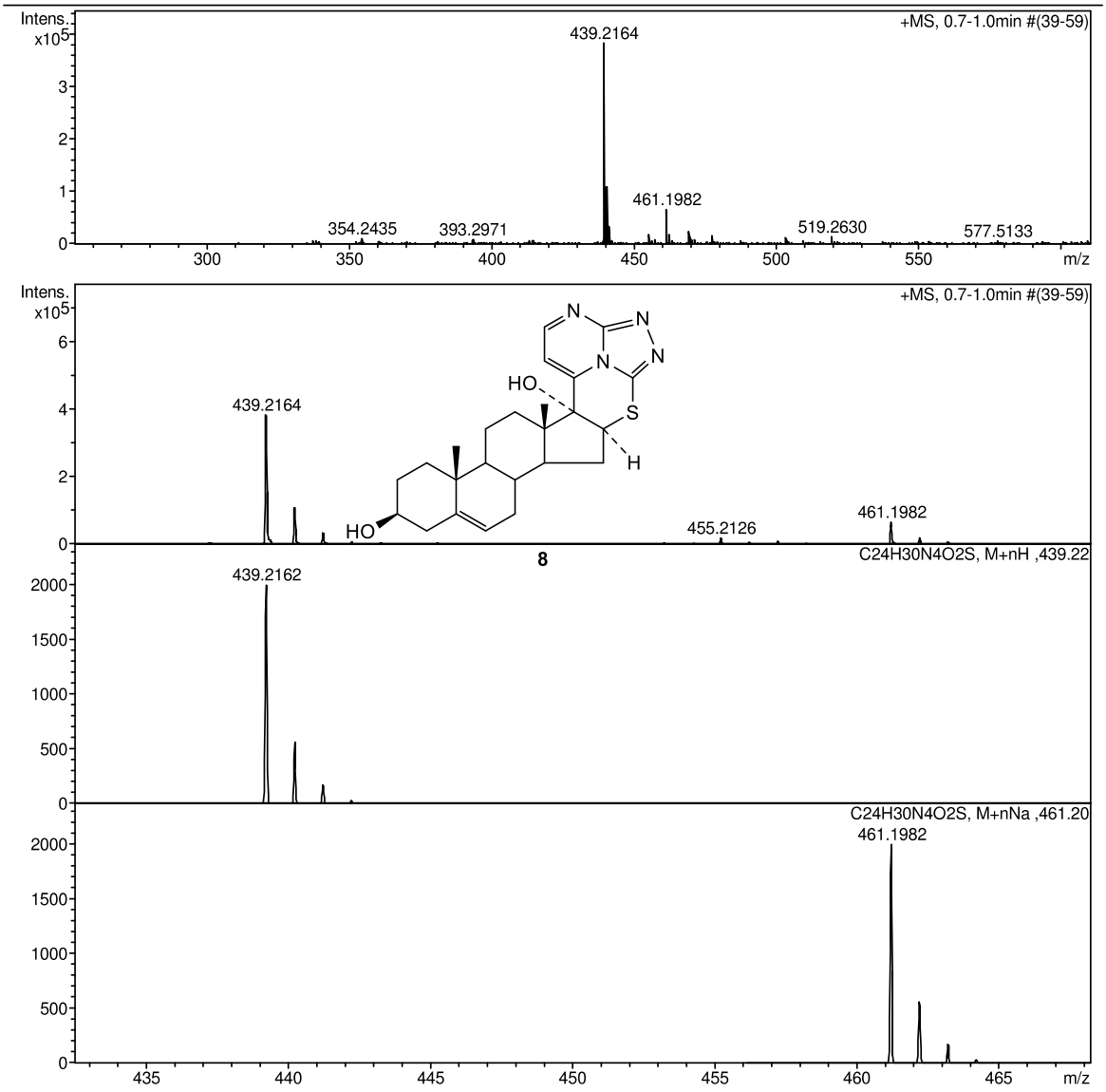
# Display Report

**Analysis Info**

| | | | |
|---|---|---|---|
| Method | tune_wide.m | Operator | |
| | | Instrument / Ser# | micrOTOF 10248 |
| Comment | C24H30N4O2S mH 439.2162 calibrant added CH3CN | | |

**Acquisition Parameter**

| | | | | | |
|---|---|---|---|---|---|
| Source Type | ESI | Ion Polarity | Positive | Set Nebulizer | 0.4 Bar |
| Focus | Not active | | | Set Dry Heater | 180 °C |
| Scan Begin | 50 m/z | Set Capillary | 4500 V | Set Dry Gas | 4.0 l/min |
| Scan End | 3000 m/z | Set End Plate Offset | -500 V | Set Divert Valve | Waste |




Mass-spectra of **8**.

# Display Report

**Analysis Info**

Acquisition Date 13.12.2022 13:18:33

Method tune_low.m

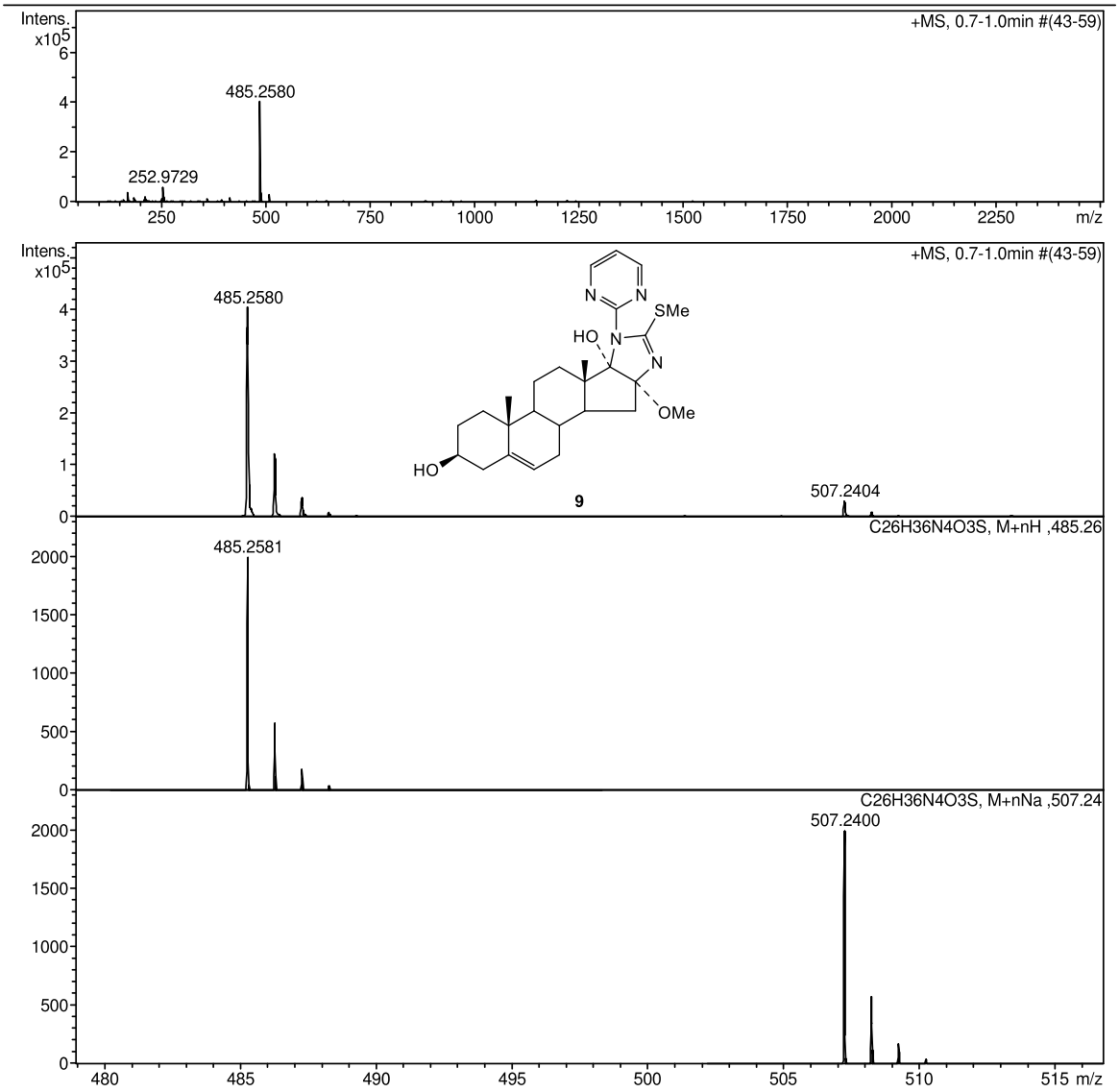
Operator

Instrument / Ser# micrOTOF 10248

Comment C26H36N4O3S mH 485.2580 calibrant added CH3OH

**Acquisition Parameter**

| | | | | | |
|---|---|---|---|---|---|
| Source Type | ESI | Ion Polarity | Positive | Set Nebulizer | 0.4 Bar |
| Focus | Not active | | | Set Dry Heater | 180 °C |
| Scan Begin | 50 m/z | Set Capillary | 4500 V | Set Dry Gas | 4.0 l/min |
| Scan End | 2500 m/z | Set End Plate Offset | -500 V | Set Divert Valve | Waste |




Mass-spectra of **9**.