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SYNTHESIS OF THE TETRASACCHARIDE REPEATING UNIT OF THE CRYOPROTECTANT CAPSULAR POLYSACCHARIDE FROM FROM *COLWELLIA PSYCHRERYTHRAEA* 34H

Supporting Information

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Synthetic procedures for the obtainment of monosaccharide and Thr building blocks:

Ethyl 6-*O*-*tert*-butyldimethylsilyl-3,4-*O*-isopropylidene-2-*O*-(2-naphthylmethyl)-1-thio-β-D-galactopyranoside (3). A solution of **7**[S1] (137.7 mg, 0.521 mmol) in pyridine (2 mL) was cooled to 0°C and TBDMSCl (99.1 mg, 0.656 mmol) was added. After ten minutes, the reaction mixture was gradually warmed up to rt. After four hours stirring at rt, the reaction was quenched by addition of CH_3OH (250 μL). After a further ten minutes, the reaction mixture was worked up by dilution with CH_2Cl_2 (30 mL). The solution was washed firstly with 0.2 M HCl (30 mL) and then with 1M $NaHCO_3$ (50 mL). The organic phase was collected, dried over anhydrous Na_2SO_4, filtered, concentrated and co-evaporated two times with toluene (20 mL each). The obtained residue was dissolved in DMF (1.5 mL), and then NAPBr (148.0 mg, 0.534 mmol) was added. The organic phase was collected dried over anhydrous Na_2SO_4, filtered, concentrated and co-evaporated two times with toluene (20 mL each). The obtained residue was dissolved in DMF (1.5 mL), and then NAPBr (148.0 mg, 0.534 mmol) was added. The mixture was cooled to 0°C and treated with NaH (60% w/w dispersion in mineral oil, 42.7 mg, 1.07 mmol). After five minutes stirring at 0°C, the reaction mixture was gradually warmed up to rt. After two hours stirring at rt, the reaction mixture was worked up by dilution with CH_2Cl_2 (30 mL). The solution was washed with H_2O (30 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, concentrated and co-evaporated two times with toluene (20 mL each). The residue was subjected to a column chromatography (15:1 to 12:1 v/v *n*-hexane-ethyl acetate) to afford **3** (154.7 mg, 57%) as a colourless oil. $[\alpha]_D^{20}$ +3 (c 0.5, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.86-7.44 (m, 7H, H-Ar), 5.01 (d, 1H, *J* 11.5 Hz, OC*H*HNAP), 4.93 (d, 1H, *J* 11.5 Hz, OC*H*HNAP), 4.44 (d, 1H, *J* 9.8 Hz, H-1), 4.25 (m, 2H, H-3, H-4), 3.86-3.76 (m, 3H, H-5, H-6a, H-6b), 3.49 (dd, 1H, *J* 9.8, 5.9 Hz, H-2), 2.82-2.66 (m, 2H, SC*H*$_2$CH$_3$), 1.40 (s, 3H, CCH_3), 1.35 (s, 3H, CCH_3), 1.31 (t, 3H, *J* 7.4 Hz, SCH_2CH_3), 0.90 (s, 9H, $SiC(CH_3)$), 0.08 (s, 6H, $Si(CH_3)_2$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 135.3-125.7 (C-Ar), 109.8 ($O_2C(CH_3)_2$), 83.6 (C-1), 79.7, 79.1, 76.9, 73.5, 73.4, 62.1 (C-2, C-3, C-4, C-5, C-6, O*C*H$_2$NAP), 27.9, 26.3 ($O_2C(CH_3)_2$), 26.7 ($SiC(CH_3)_3$), 24.4 (SCH_2CH_3), 18.2 ($SiC(CH_3)_3$), 14.9 (SCH_2CH_3), -5.4, -5.6 ($Si(CH_3)_2$). HRMS *m/z* $[M + Na]^+$ Calcd for $C_{28}H_{42}O_5SSiNa$ 541.2414, found 541.2394.

Allyl 4,6-*O*-benzylidene-2-deoxy-3-methoxycarbonyl-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranoside (9). A solution of compound **8**[S2] (700.9 mg, 1.457 mmol) in CH_2Cl_2 (10 mL) was treated at 0°C with TMEDA (331 μL, 2.19 mmol) and then with methyl chloroformate (226 μL, 2.92 mmol). The formation of a white precipitate was observed. After 1 hour stirring at 0°C, the reaction mixture was diluted with CH_2Cl_2 (80 mL) and washed with H_2O (80 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated to afford product **9** as a white foam (783.3 mg, 100%). $[\alpha]_D^{20}$ +40 (c 0.4, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.46-7.35 (m, 5H, H-Ar), 5.88 (m, 1H, $OCH_2CH=CH_2$), 5.53 (s, 1H, OC*H*Ph), 5.40 (d, 1H, *J* 9.8 Hz, NH), 5.32 (d, 1H, *J* 17.2 Hz, *trans* $OCH_2CH=CHH$), 5.25 (d, 1H, *J* 10,7 Hz, *cis* $OCH_2CH=CHH$), 5.17 (t, 1H, *J* 10.0 Hz, H-3), 4.93 (d, 1H, *J* 3.4 Hz, H-1), 4.83 (d, 1H, *J* 12.0 Hz, OC*H*$HCCl_3$), 4.64 (d, 1H, *J* 12.0 Hz, OCH*H*CCl_3), 4.31-3.92 (m, 5H, H-2, H-5, H-6a, OC*H*$_2$CH=CH$_2$) 3.81-3.73 (m, 5H, H-4, H-6b, CO_2CH_3,). ^{13}C NMR (100 MHz, $CDCl_3$): δ 154.2 ($NHCO_2CH_2CCl_3$, OCO_2CH_3), 136.8, 132.9, 129.1, 128.2, 126.2 118.6 (C-Ar, $OCH_2CH=CH_2$), 101.6, 96.9 (C-1, $PhCO_2$), 78.9, 74.5, 74.0, 68.9, 68.7, 62.9, 55.2, 54.5 (C-2, C-3, C-4, C-5, C-6, O*C*H$_2$CH=CH$_2$, O*C*H$_2$CCl$_3$, OCH$_3$). HRMS *m/z* $[M + Na]^+$ Calcd for $C_{21}H_{24}Cl_3NO_9Na$ 562.0409, found 562.0388.

4,6-*O*-benzylidene-2-deoxy-3-methoxycarbonyl-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl trichloroacetimidate (4). A solution of compound **9** (816.8 mg, 1.460 mmol) in ethyl acetate (33 mL) was treated with NaOAc (688 mg, 8.39 mmol), 9:1 v/v AcOH-H_2O (33 mL), and finally with $PdCl_2$ (388 mg, 2.19 mmol). After 21 hours stirring at rt, the reaction mixture was worked up by filtration on a Celite pad. The filtrate was diluted with ethyl acetate (150 mL) and washed with H_2O (150 mL). The organic phase was washed again with 1 M aqueous $NaHCO_3$ (150 mL), dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was purified through a rapid column chromatography (4:1-2:1 v/v *n*-hexane-ethyl acetate) and then dissolved in CH_2Cl_2 (40 ml). The solution was treated with Cl_3CCN (1.33 mL, 13.3 mmol) and with a 1.1 M solution of DBU in CH_2Cl_2 (300 μL, 330 μmol). The formation of a slightly yellow colour was observed. After two hours stirring at rt, the reaction was quenched by dilution with toluene; the solution was concentrated by rotoevaporation and the residue was immediately subjected to column chromatography (8:1:0.001-5:1:0.001 v/v *n*-hexane-ethyl acetate-triethylamine). Product **4** was obtained as a white foam (453.1 mg, 48%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.78 (s, 1H, $OC(NH)CCl_3$), 7.47-7.36 (m, 5H, H-Ar), 6.41 (d, 1H, *J* 3.6 Hz, H-1), 5.56 (s, 1H, OC*H*Ph), 5.41 (d, 1H, *J* 9.1 Hz, N*H*$CO_2CH_2CCl_3$), 5.26 (t, 1H, *J* 10.1 Hz, H-3), 4.75 (d, 1H, *J* 12.1 Hz, OC*H*$HCCl_3$), 4.71 (d, 1H, *J* 12.0 Hz, OCH*H*CCl_3), 4.38-4.30 (m, 2H, H-5, H-6a), 4.09-4.04 (dt, 1H, *J* 9.9, 3.6 Hz, H-2), 3.88 (t, 1H, *J* 9.6 Hz, H-4), 3.83-3.77 (m, 4H, H-6b, OCO_2CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.6 (O*C*(NH)CCl_3), 155.9, 154.2 (O*C*O_2CH_3, NH*C*$O_2CH_2CCl_3$), 136.5, 129.2, 128.3, 126.2 (C-Ar), 101.7, 95.2, 95.0, 90.6 (C-1, Ph*C*O_2, OCH_2CCl_3, OC(NH)*C*Cl_3,), 78.2, 74.6, 73.2, 68.4, 65.3, 55.4, 54.4 (C-2, C-3, C-4, C-5, C-6, O*C*H$_2$CCl$_3$, OCH$_3$). HRMS *m/z* $[M - CNCCl_3 + Na]^+$ Calcd for $C_{18}H_{20}Cl_3NO_9Na$ 522.0096, found 522.0070.

Ethyl 2,3-di-*O*-benzoyl-4,6-*O*-(2-naphthylidene)-1-thio-β-D-glucopyranoside (12). A solution of **11**[S3] (3.452 g, 9.536 mmol) in 3:1 v/v CH_2Cl_2-pyridine (22 mL) was cooled to 0°C and then treated with BzCl (3.1 mL, 26.7 mmol). After 10 min stirring at 0°C, a white precipitate was observed. The reaction mixture was gradually warmed up to rt under stirring. After 90 min the reaction mixture was diluted with CH_2Cl_2 (150 mL) and washed with H_2O (150 mL). The organic phase was

dried over anhydrous Na_2SO_4, filtered, concentrated and co-evaporated two times with toluene (30 mL each). The residue was subjected to column chromatography (5:1-0:1 v/v *n*-hexane-ethyl acetate, then 80:20 v/v dichloromethane-methanol). Product **12** was obtained as a slightly yellow amorphous solid (4.461 g, 82%). $[\alpha]_D^{20}$ –33 (c 0.5, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.98-7.35 (m, 17H, C-Ar), 5.84 (t, 1H, *J* 9.4 Hz, H-2), 5.71 (s, 1H, OC*H*NAP), 5.54 (t, 1H, *J* 9.8 Hz, H-3), 4.84 (d, 1H, *J* 9.9 Hz, H-1), 4.50 (dd, 1H, *J* 10.4, 4.8 Hz, H-6a), 4.02-3.90 (m, 2H, H-4, H-6b), 3.81 (m, 1H, H-5), 2.77 (m, 2H, SCH_2CH$_3$), 1.28 (t, 3H, *J* 7.4 Hz, SCH$_2$CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.6, 165.3 (2 *C*OPh), 134.1-123.6 (C-Ar), 101.7 (NAP*C*O$_2$), 84.5 (C-1), 78.9, 73.3, 71.1, 71.0, 68.7 (C-2, C-3, C-4, C-5, C-6), 24.4 (S*C*H$_2$CH$_3$), 14.8 (SCH$_2$*C*H$_3$). HRMS *m/z* [M + Na]$^+$ Calcd for $C_{33}H_{30}O_7SNa$ 593.1604, found 593.1588.

Ethyl 2,3-di-*O*-benzoyl-4-*O*-(2-naphthylmethyl)-1-thio-β-D-glucopyranoside (13). Compound **12** (4.464 g, 7.832 mmol) was coevaporated three times with dry toluene (15 mL each). The residue was dried under vacuum and then 1.0M solution of BH_3•THF in THF (38.8 mL, 39.2 mmol) was added *via* cannula under argon atmosphere in the presence of 3Å molecular sieves. The yellow mixture was then treated with TMSOTf (707 μL, 3.92 mmol). After 3 hours stirring at rt, the reaction mixture was neutralized with Et_3N and then filtered on a Celite pad. The filtrate was concentrated and coevaporated two times with methanol (40 mL each). The residue was subjected to column chromatography (4:1-1:1 v/v *n*-hexane-ethyl acetate) to afford product **13** as a white foam (3.07 g, 68%). $[\alpha]_D^{20}$ +25 (c 0.6, CH_2Cl_2). ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.93-7.24 (m, 17H, H-Ar), 5.76 (t, 1H, *J* 9.0 Hz, H-2), 5.36 (t, 1H, *J* 9.6 Hz, H-3), 4.79 (d, 1H, *J* 11.4 Hz, OC*H*HNAP), 4.74 (d, 1H, *J* 3.6 Hz, H-1), 4.73 (d, 1H, *J* 11.4 Hz, OC*H*HNAP), 4.02-3.98 (m, 2H, H-4, H-6a), 3.86 (m, 1H, H-5), 3.65 (dt, 1H, *J* 9.6, 2.7 Hz, H-6b), 2.74 (m, 2H, SCH_2CH$_3$), 2.02 (m, 1H, OH), 1.26 (t, 3H, *J* 7.2 Hz, SCH$_2$CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.7 (*C*OPh), 165.4 (*C*OPh), 134.6-125.9 (C-Ar), 83.9 (C-1), 79.8, 76.2, 75.6, 74.9, 70.9, 61.8 (C-2, C-3, C-4, C-5, C-6, O*C*H$_2$NAP), 24.5 (S*C*H$_2$CH$_3$), 14.9 (SCH$_2$*C*H$_3$). HRMS *m/z* [M + Na]$^+$ Calcd for $C_{33}H_{32}O_7SNa$ 595.1761, found 595.1744.

2,3-di-*O*-benzoyl-4-*O*-(2-naphthylmethyl)-β-D-glucopyranosylurono-γ-lactone (14) — A solution of **13** (250.0 mg, 437.1 μmol) in CH_2Cl_2 (9.7 mL) was cooled to 0°C and then treated with H_2O (1.6 mL), 1 M aqueous NaBr (243 μL), 1 M aqueous Bu_4NBr (486 μL), TEMPO (20.5 mg, 0.131 mmol), saturated aqueous $NaHCO_3$ (1.21 mL) and finally with an aqueous solution of NaOCl (1.46 mL, minimum 4% chlorine content). After 20 min stirring at 0°C, the orange biphasic mixture was warmed up to rt. After 2 hours stirring at rt, the reaction was quenched by neutralization with 1 N HCl (972 μL). Then *t*-BuOH (6.75 mL), a 2 M solution of 2-methyl-2-butene in THF (13.6 mL) and a 1.96 mL aliquot of a solution obtained by dissolving $NaClO_2$ (625 mg, 6.91 mmol) and NaH_2PO_4 (500 mg, 4.17 mmol) in H_2O (2.5 mL) were added. After 7 hours stirring at rt, the reaction mixture was worked up by dilution with a saturated aqueous solution of NaH_2PO_4 (48 mL) and extracted with ethyl acetate (100 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was subjected to column chromatography (8:1-4:1 v/v *n*-hexane-ethyl acetate). Product **14** was obtained as a white amorphous solid (101.6 mg, 44%). $[\alpha]_D^{20}$ –2 (c 0.4, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06-7.30 (m, 17H, H-Ar), 6.14 (s, 1H, H-1), 5.61 (s, 1H, H-3), 5.23 (s, 1H, H-2), 5.06 (d, 1H, *J* 11.7 Hz, OC*H*HNAP), 4.97 (d, 1H, *J* 11.7 Hz, OCH*H*NAP), 4.70 (s, 1H, H-4), 3.83 (s, 1H, H-5). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.2, 165.0, 164.5 (C-6, 2 *C*OPh), 134.0-125.6 (C-Ar), 100.6 (C-1), 72.4, 71.9, 71.5, 68.5, 66.3 (C-2, C-3, C-4, C-5, O*C*H$_2$NAP). HRMS *m/z* [M + H]$^+$ Calcd for $C_{31}H_{24}O_8Na$ 524.1471, found 524.1450.

Benzyl 2,3-di-*O*-benzoyl-4-*O*-(2-naphthylmethyl)-1-ethylthio-β-D-glucopyranosyluronate (5). A solution of compound **13** (533.8 mg, 933.2 μmol) in CH_2Cl_2 (3.5 mL) was treated with H_2O (1.7 mL), and then TEMPO (29.2 mg, 187 μmol) and BAIB (751.5 mg, 2.333 mmol) were added. After 2 hours stirring at rt, the orange mixture was worked up by addition of 10% $Na_2S_2O_3$ solution (35 mL) and ethyl acetate (35 mL). The organic phase was collected, dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was dissolved in freshly dried DMF (14 mL), then treated with Cs_2CO_3 (1.177 g, 3.612 mmol) and BnBr (286 μL, 2.41 mmol). After 90 min stirring at rt, the brown reaction mixture was worked up by dilution with ethyl acetate (150 mL). The solution was washed with H_2O (150 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, concentrated and co-evaporated four times with toluene (20 mL each). The residue was subjected to column chromatography (12:1-6:1 v/v *n*-hexane-ethyl acetate) to afford product **5** as a slightly yellow amorphous solid (436.7 mg, 69%). $[\alpha]_D^{20}$ –32 (c 0.3, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.92-7.07 (m, 22H, H-Ar), 5.74 (m, 1H, H-3), 5.43 (t, 1H, *J* 9.6 Hz, H-2), 5.23 (s, 2H, OCH_2Ph), 4.75 (d, 1H, *J* 9.9 Hz, H-1), 4.61 (d, 1H, *J* 11.3 Hz, OC*H*HNAP), 4.53 (d, 1H, *J* 11.2 Hz, OCH*H*NAP), 4.22-4-20 (m, 2H, H-4, H-5), 2.75 (m, 2H, SCH_2CH$_3$), 1.23 (t, 3H, *J* 7.1 Hz, SCH$_2$CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.6, 165.4, 165.2 (C-6, 2 *C*OPh), 134.4-125.7 (C-Ar), 84.2, 78.5, 77.4, 75.3, 74.7, 70.2, 67.5 (C-1, C-2, C-3, C-4, C-5, O*C*H$_2$NAP, O*C*H$_2$Ph), 24.2 (S*C*H$_2$CH$_3$), 14.7 (SCH$_2$*C*H$_3$). HRMS *m/z* [M + Na]$^+$ Calcd for $C_{40}H_{36}O_8SNa$ 699.2023, found 699.2000.

1,2''-anhydro-1'-(6-*O*-*tert*-butyldimethylsilyl-3,4-di-*O*-isopropylidene-α-D-galactopyranosyl)-2'-naphthylmethanol (15) and 1,2''-anhydro-1'-(3,4-di-*O*-isopropylidene-α-D-galactopyranosyl)-2'-naphthylmethanol (16). Glycosyl acceptor **2**[S4] (22.1 mg, 66.4 μmol) and **3** (51.6 mg, 99.5 μmol) were mixed and co-evaporated three times with dry toluene (1 mL each). The residue was dried under vacuum and then mixed to NIS (28.0 mg, 124 μmol) under Ar atmosphere in the presence of acid-washed molecular sieves AW-300 4Å-MS. The mixture was cooled to -40°C and then treated with freshly

dried CH_2Cl_2 (1 mL). After ten minutes stirring at -40°C, a 0.66 M solution of trifluoromethanesulfonic acid in freshly dried CH_2Cl_2 (39.7 μL, 26.2 μmol) was added. The gradual formation of a brown colour was observed. After three hours stirring at -40°C, the reaction mixture was worked up by dilution with CH_2Cl_2 (40 mL). The solution was washed with 1:1 v/v 1M $NaHCO_3$ – 10% $Na_2S_2O_3$ (50 mL). The organic phase was dried over anhydrous sodium sulfate, filtered, and concentrated. The residue was subjected to a column chromatography (6:1-0:1 v/v *n*-hexane-ethyl acetate) to afford, as first eluted fraction, **15** (21.6 mg, 48% from **3**) as a colourless oil. Product **16** (9.8 mg, 29% from **3**) was obtained as second eluted fraction as a colourless oil. Compound **15**: $[\alpha]_D^{20}$ +24.2 (c 1.6, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.32-7.10 (m, 6H, H-Ar), 5.39 (d, 1H, *J* 2.9 Hz, H-1), 4.98 (d, 1H, *J* 15.2 Hz, OC*H*HNAP), 4.89 (d, 1H, *J* 15.2 Hz, OC*H*HNAP), 4.64 (dd, 1H, *J* 7.7, 2.6 Hz, H-3), 4.48 (dd, 1H, *J* 7.8, 1.0 Hz, H-4), 4.15 (dt, 1H, *J* 6.3, 0.7 Hz, H-5), 4.07 (t, 1H, *J* 2.9 Hz, H-2), 3.78 (dd, 1H, *J* 9.7, 7.5 Hz, H-6a), 3.62 (dd, 1H, *J* 9.7, 6.0 Hz, H-6b), 1.66 (s, 3H, CCH_3), 1.46 (s, 3H, CCH_3), 0.76 (s, 9H, $SiC(CH_3)$), -0.01 (s, 3H, $SiCH_3$), -0.14 (s, 3H, $SiCH_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 132.9-121.9 (C-Ar), 109.7 ($O_2C(CH_3)_2$), 73.1, 72.9, 72.1, 70.1, 68.1, 62.4, 62.3 (C-1, C-2, C-3, C-4, C-5, C-6, OCH_2NAP), 26.6, 24.8 ($O_2C(CH_3)_2$), 25.7 ($SiC(CH_3)_3$), 18.1 ($SiC(CH_3)_3$), -5.5, -5.6 ($Si(CH_3)_2$). HRMS *m/z* [M + Na]$^+$ Calcd for $C_{26}H_{36}O_5SiNa$ 479.2224, found 479.2202. Compound **16**: $[\alpha]_D^{20}$ +147 (c 0.1, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.35-7.11 (m, 6H, H-Ar), 5.43 (d, 1H, *J* 3.2 Hz, H-1), 4.96 (d, 1H, *J* 15.2 Hz, OC*H*HNAP), 4.87 (d, 1H, *J* 15.2 Hz, OC*H*HNAP), 4.69 (dd, 1H, *J* 7.7, 2.9 Hz, H-3), 4.39 (dd, 1H, *J* 7.7, 1.5 Hz, H-4), 4.18 (m, 2H, H-2, H-6a), 3.79 (dd, 1H, *J* 11.4, 7.7 Hz, H-6b), 3.63 (m, 1H, H-5), 1.67 (s, 3H, CCH_3), 1.45 (s, 3H, CCH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 132.7-121.9 (C-Ar), 110.3 ($O_2C(CH_3)_2$), 73.3, 73.3, 72.3, 70.1, 68.1, 63.0, 62.9 (C-1, C-2, C-3, C-4, C-5, C-6, OCH_2NAP), 26.5, 24.9 ($O_2C(CH_3)_2$). HRMS *m/z* [M + Na]$^+$ Calcd for $C_{20}H_{22}O_5Na$ 365.1359, found 365.1343.

Ethyl 6-*O*-*tert*-butyldiphenylsilyl-3,4-*O*-isopropylidene-1-thio-β-D-galactopyranoside (18). To a solution of compound **7**[S1] (2.658 g, 10.18 mmol) in CH_2Cl_2 (18 mL), TBDPSCl (3.18 mL), Et_3N (6 mL) and DMAP (68.20 mg, 560.0 μmol) were consecutively added. The formation of a white precipitate was observed. After two hours stirring at rt, the reaction mixture was diluted with CH_2Cl_2 (150 mL) and washed with 0.2M HCl (150 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, and concentrated to give a residue that was purified by column chromatography (10:1 to 4:1 v/v *n*-hexane-ethyl acetate). Product **18** (4.936 g, 97%) was obtained as a colourless oil. $[\alpha]_D^{20}$ –1 (c 0.7, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73-7.36 (m, 10H, H-Ar), 4.34 (d, 1H, *J* 5.3 Hz, H-4), 4.26 (d, 1H, *J* 10.2 Hz, H-1), 4.09 (t, 1H, *J* 6.3 Hz, H-3), 3.99-3.88 (m, 3H, H-5, H-6a, H-6b), 3.57 (t, 1H, *J* 9.0 Hz, H-2), 2.72 (m, 3H, SCH_2CH_3, OH), 1.53 (s, 3H, CCH_3), 1.38 (s, 3H, CCH_3), 1.30 (t, 3H, *J* 7.4 Hz, SCH_2CH_3), 1.07 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 135.5-127.5 (C-Ar), 109.9 ($C(CH_3)_2$), 85.2 (C-1), 78.9, 76.9, 73.2, 72.2, 62.6 (C-2, C-3, C-4, C-5, C-6), 28.2, 26.2 ($C(CH_3)_2$), 26.6 ($SiC(CH_3)_3$), 24.2 (SCH_2CH_3), 19.1 ($SiC(CH_3)_3$), 15.2 (SCH_2CH_3). HRMS *m/z* [M + Na]$^+$ Calcd for $C_{27}H_{38}O_5SSiNa$ 525.2101, found 525.2088.

Ethyl 6-*O*-*tert*-butyldiphenylsilyl-3,4-*O*-isopropylidene-2-*O*-(2-naphthylmethyl)-1-thio-β-D-galactopyranoside (19). To a solution of compound **18** (4.937 g, 9.834 mmol) in toluene (48 mL), TBAB (950.0 mg, 2.947 mmol), 33% NaOH (24 mL), and NAPBr (3.260 g, 14.74 mmol) were consecutively added. After four hours stirring at rt, the biphasic mixture was diluted with diethyl ether (200 mL) and H_2O (200 mL). The organic phase was collected, dried over anhydrous Na_2SO_4, filtered, and concentrated to give a residue that was purified by chromatography (20:1 to 17:1 v/v *n*-hexane-ethyl acetate). Product **19** (5.999 g, 95%) was obtained as a yellow oil. $[\alpha]_D^{20}$ +26 (c 0.5, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.93-7.34 (m, 17H, H-Ar), 5.01 (d, 1H, *J* 11.6 Hz, OC*H*HNAP), 4.92 (d, 1H, *J* 11.6 Hz, OC*H*HNAP), 4.42 (d, 1H, *J* 9.6 Hz, H-1), 4.31 (dd, 1H, *J* 5.6, 2.0 Hz, H-4), 4.25 (t, 1H, *J* 6.4 Hz, H-3), 3.91 (d, 2H, *J* 6.4 Hz, H-6a, H-6b), 3.82 (dt, 1H, *J* 6.4, 2.0 Hz, H-5), 3.49 (dd, 1H, *J* 9.8, 6.4 Hz, H-2), 2.76 (dq, 1H, *J* 12.4, 7.4 Hz, $SCHHCH_3$), 2.67 (dq, 1H, *J* 12.4, 7.4 Hz, $SCHHCH_3$), 1.39 (s, 3H, CCH_3), 1.35 (s, 3H, CCH_3), 1.28 (t, 3H, *J* 7.4 Hz, SCH_2CH_3), 1.05 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 135.6-125.8 (C-Ar), 109.8 ($C(CH_3)_2$), 83.5 (C-1), 79.7, 79.2, 77.3, 73.5, 62.8 (C-2, C-3, C-4, C-5, C-6, OCH_2NAP), 27.9, 26.3 ($C(CH_3)_2$), 26.7 ($SiC(CH_3)_3$), 24.3 (SCH_2CH_3), 19.2 ($SiC(CH_3)_3$), 14.8 (SCH_2CH_3). HRMS *m/z* [M + Na]$^+$ Calcd for $C_{38}H_{46}O_5SSiNa$ 665.2727, found 665.2701.

Ethyl 3,4-di-*O*-benzoyl-6-*O*-*tert*-butyldiphenylsilyl-2-*O*-(2-naphthylmethyl)-1-thio-β-D-galactopyranoside (17). A solution of compound **19** (5.999 g, 8.877 mmol) in CH_2Cl_2 (100 mL) was cooled to 0°C, and 98:2 v/v trifluoroacetic acid/H_2O (8 mL) was added. After two hours stirring at 0°C the reaction mixture was quenched by addition of a 1M $NaHCO_3$ solution (100 mL). After twenty minutes, it was worked up by dilution with CH_2Cl_2 (100 mL) and then heating to rt. The organic phase was collected, washed with H_2O (50 mL), dried over anhydrous sodium sulfate, filtered, and concentrated. The obtained residue was dissolved in CH_2Cl_2 (30 mL) and treated with pyridine (10 mL) and benzoyl chloride (3.28 mL, 28.4 mmol). After two hours stirring at rt, the reaction mixture was worked up by addition of CH_3OH (10 mL) and, after few minutes, by dilution with CH_2Cl_2 (150 mL). The solution was washed with 0.1M HCl (150 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, concentrated and co-evaporated thrice with toluene (25 mL each). The residue was subjected to a column chromatography (20:1 to 10:1 v/v *n*-hexane-ethyl acetate). Product **17** (4.313 g, 60%) was obtained as a white foam. $[\alpha]_D^{20}$ +90 (c 0.4, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.98-7.08 (m, 27H, H-Ar), 5.98 (dd, 1H, *J* 3.4, 0.8 Hz, H-4), 5.50 (dd, 1H, *J* 9.6, 3.4 Hz, H-3), 4.98 (d, 1H, *J* 11.0 Hz, OC*H*HNAP), 4.74 (d, 1H, *J* 11.0 Hz, OCH*H*NAP), 4.69 (d, 1H, *J* 9.6 Hz, H-1), 3.98-3.91 (m, 2H, H-2, H-5), 3.80 (dd, 1H, *J* 10.2, 5.9 Hz, H-6a), 3.73 (dd, 1H, *J* 10.2, 8.0 Hz, H-6b), 2.89-2.73 (m, 2H, SCH_2CH_3), 1.35 (t, 3H, *J* 7.4 Hz, SCH_2CH_3), 0.98 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.5 (*C*OPh), 165.3

(*C*OPh) 135.6-125.7 (C-Ar), 85.5 (C-1), 77.2, 77.1, 75.6, 74.8, 68.5, 61.4 (C-2, C-3, C-4, C-5, C-6, O*C*H_2NAP), 26.6 (SiC(CH_3)$_3$), 25.0 (S*C*H_2CH_3), 18.9 (Si*C*(CH_3)$_3$), 15.0 (SCH$_2$*C*H_3). HRMS *m/z* [M + Na]$^+$ Calcd for $C_{49}H_{50}O_7SSiNa$ 833.2939, found 833.2900.

4,6-*O*-benzylidene-2-deoxy-3-(9-*O*-fluorenylmethyloxycarbonyl)-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranose (27). Crabtree's catalyst (95.8 mg, 113 μmol) was suspended in dry THF (11.5 mL) under argon atmosphere. The suspension was degassed and then H_2 was bubbled inside for 5 min. The red suspension turned into a yellow solution, that was treated *via* cannula under Ar atmosphere with a solution of **26**[S5] (1.592 g, 2.265 mmol) in dry THF (11.5 mL). The reaction mixture was stirred at rt overnight, after that a solution of I_2 (1.150 g, 4.530 mmol) in 4:1 v/v THF-H_2O (12.5 mL) was added. The brown solution was stirred at rt for 1 hour, and then treated with a 10% $Na_2S_2O_3$ solution (50 mL). The biphasic mixture was concentrated by rotoevaporation to approximately 40 mL and then diluted with ethyl acetate (200 mL). The organic phase was washed with 10% $Na_2S_2O_3$ solution (200 mL), dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was subjected to column chromatography (6:1-1:1 v/v *n*-hexane-ethyl acetate) to afford **27** as a slightly yellow foam (1.312 g, 87%, α/β 8:1). α-anomer: ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.76-7.18 (m, 13H, H-Ar), 5.59 (d, 1H, *J* 9.6 Hz, N*H*$CO_2CH_2CCl_3$), 5.58 (s, 1H, OC*H*Ph), 5.34 (t, 1H, *J* 3.5 Hz, H-1), 5.28 (t, 1H, *J* 9.6 Hz, H-3), 4.61 (d, 1H, *J* 12.0 Hz, OC*H*$HCCl_3$), 4.54 (d, 1H, *J* 12.0 Hz, OCH*H*CCl_3), 4.42-4.16 (m, 6H, H-2, H-6a, H-6b, O*C*H_2C*H* Fmoc), 3.86-3.78 (m, 2H, H-4, H-5), 3.29 (d, 1H, J 2.3 Hz, OH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.2, 154.3 (NH*C*$O_2CH_2CCl_3$, O*C*O_2 Fmoc), 143.2-120.0 (C-Ar), 101.7, 95.2, 92.7 (C-1, Ph*C*O_2, OCH$_2$*C*Cl_3), 79.0, 74.6, 74.0, 70.5, 68.8, 62.9, 54.9, 46.5 (C-2, C-3, C-4, C-5, C-6, O*C*H_2*C*H Fmoc, O*C*H_2CCl_3). HRMS *m/z* [M + Na]$^+$ Calcd for $C_{31}H_{28}Cl_3NO_9Na$ 686.0722, found 686.0699.

4,6-*O*-benzylidene-2-deoxy-3-(9-*O*-fluorenylmethyloxycarbonyl)-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranosyl trichloroacetimidate (25). Compound **27** (1.182 g, 1.783 mmol) was dissolved in 4:1 v/v Cl_3CCN-CH_2Cl_2 (25 mL). The mixture was cooled to 0°C and treated with NaH (60% w/w dispersion in mineral oil, 14.3 mg, 356 μmol). After a few minutes a clear yellow solution was obtained. After 1 hour stirring at 0°C, the solution was worked up by neutralization with silica powder and concentrated by rotoevaporation. The residue was immediately subjected to column chromatography (8:1-5:2 v/v *n*-hexane-ethyl acetate). Product **25** was obtained as a white foam (1.072 g, 75%, β/α 6:5). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.78 (s, 1H, OC(N*H*)CCl_3-β), 8.73 (s, 1H, OC(N*H*)CCl_3-α), 7.76-7.20 (m, 26H, H-Ar), 6.43 (d, 1H, *J* 3.6 Hz, H-1-β) 6.04 (d, 1H, *J* 9.0 Hz, H-1-α), 5.62 (s, 1H, OC*H*Ph-β), 5.58 (s, 1H, OC*H*Ph-α), 5.39 (d, 1H, *J* 9.0 Hz, N*H*$CO_2CH_2CCl_3$-β), 5.34-5.26 (m, 3H, N*H*$CO_2CH_2CCl_3$-α, H-3-α, H-3-β), 4.71 (d, 1H, *J* 12.6 Hz, OC*H*$HCCl_3$-β), 4.54 (s, 2H, OCH_2CCl_3-α), 4.52 (d, 1H, *J* 12.6 Hz, OC*H*$HCCl_3$-β), 4.47-4.07 (m, 12H, H-2-α, H-2-β, H-6a-α, H-6b-α, H-6a-β, H-6b-β, O*C*H_2C*H*Fmoc-α, O*C*H_2C*H*Fmoc-β), 3.98-3.83 (m, 3H, H-4-α, H-4-β, H-5-α), 3.73 (m, 1H, H-5-β). ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.1, 160.6 (O*C*(NH)CCl_3-α and -β), 155.3, 155.2, 154.1, 154.0 (NH*C*$O_2CH_2CCl_3$-α and –β, O*C*O_2 Fmoc–α and -β), 143.1-120.0 (C-Ar), 101.6, 101.4, 96.3, 95.0 (Ph*C*O_2-α and -β, C-1-α, C-1-β), 90.5, 90.3 (OCH$_2$*C*Cl_3-α and -β), 78.2, 78.1, 75.4, 74.6, 74.4, 73.4, 70.7, 70.6, 68.3, 68.1, 66.9, 65.3, 55.9, 54.5, 46.4, 46.3 (C-2-α, C-2-β, C-3-α, C-3-β, C-4-α, C-4-β, C-5-α, C-5-β, C-6-α, C-6-β, O*C*H_2C*H* Fmoc-α and β, O*C*H_2CCl_3-α and β). HRMS *m/z* [M – C$NCCl_3$ + Na]$^+$ Calcd for $C_{31}H_{28}Cl_3NO_9Na$ 686.0722, found 686.0705.

Ethyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2-naphthylmethyl)-1-thio-β-D-glucopyranoside (28). A solution of **13** (200.2 mg, 350.0 μmol) in 3:1 v/v CH_2Cl_2-pyridine (1.5 mL) was treated with BzCl (56.9 μL, 490 μmol). After a few minutes stirring at 0°C, the formation of a precipitate was observed. The reaction mixture was gradually warmed up to rt. After 3 hours stirring at rt, a second aliquot of pyridine (200 μL) and BzCl (28.9 L, μ245 mmol) was added. After 20 hours stirring at rt, the reaction mixture was diluted with CH_2Cl_2 (30 mL) and washed with H_2O (30 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered, concentrated and co-evaporated three times with toluene (15 mL). The residue was subjected to column chromatography (10:1-8:1 v/v *n*-hexane-ethyl acetate) to afford product **28** as a white powder (164.2 mg, 69%). $[\alpha]_D^{20}$ +50 (c 0.4, CH_2Cl_2). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.03-7.21 (m, 22H, H-Ar), 5.82 (t, 1H, *J* 9.3 Hz, H-2), 5.43 (t, 1H, *J* 9.8 Hz, H-3), 4.77-4.69 (m, 4H, H-1, H-6a, O*C*H_2NAP), 4.61 (dd, 1H, *J* 12.1, 4.2 Hz, H-6b), 4.01 (t, 1H, *J* 9.5 Hz, H-4), 3.92 (bd, 1H, *J* 9.6 Hz, H-5), 2.73 (m, 2H, S*C*H_2CH_3), 1.22 (t, 3H, *J* 7.4 Hz, SCH$_2$*C*H_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.1, 165.7, 165.4 (3 *C*OPh), 134.1-126.0 (C-Ar), 83.7 (C-1), 77.5, 76.3, 75.6, 74.8, 70.8, 63.3 (C-2, C-3, C-4, C-5, C-6, O*C*H_2NAP), 24.3 (S*C*H_2CH_3), 14.9 (SCH$_2$*C*H_3). HRMS *m/z* [M + Na]$^+$ Calcd for $C_{40}H_{36}O_8SNa$ 699.2023, found 699.1997.

Benzyl 2,3-di-*O*-benzoyl-4-*O*-(2-naphthylmethyl)-D-glucopyranosyluronate (33). A solution of **5** (527.6 mg, 780.5 μmol) in CH_2Cl_2 (9 mL) was treated with H_2O (1 mL). The mixture was cooled to 0°C and then treated with NIS (280.7 mg, 1.248 mmol) and a 2.2 M solution of TFA in CH_2Cl_2 (573 μL, 1.25 mmol). A dark red solution was obtained. After three hours stirring at 0°C, the reaction was worked up by neutralization with Et_3N, then the mixture was diluted with CH_2Cl_2 (100 mL) and washed with 1:1 v/v 1 M $NaHCO_3$ – 10% $Na_2S_2O_3$ (100 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered and concentrated. The residue was subjected to column chromatography (10:1-2:1 v/v *n*-hexane-ethyl acetate). Product **33** was obtained as a slightly yellow amorphous solid (323.2 mg, 63%, α/β 8:1). α anomer: ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.96-7.12 (m, 22H, H-Ar), 6.09 (t, 1H, *J* 9.5 Hz, H-3), 5.72 (d, 1H, *J* 2.5 Hz, H-1), 5.23-5.19 (m, 3H, H-2, O*C*H_2Ph), 4.76 (d, 1H, *J* 9.5 Hz, H-5), 4.67 (d, 1H, *J* 11.3 Hz, OC*H*HNAP), 4.60 (d, 1H, *J* 11.3 Hz, OCH*H*NAP), 4.19 (t, 1H, *J* 9.6 Hz, H-4), 3.10 (bs, 1H, OH). HRMS *m/z* [M + Na]$^+$ Calcd for $C_{38}H_{32}O_9Na$ 655.1939, found 655.1918.

Benzyl 2,3-di-*O*-benzoyl-4-*O*-(2-naphthylmethyl)-1-*O*-trichloroacetimidoyl-α-D-glucopyranuronate (32). A solution of compound **33** (323.2 mg, 493.4 μmol) in CH_2Cl_2 (5 mL) was treated with Cl_3CCN (247 μL, 2.47 mmol) and then with a 0.61 M solution of DBU in CH_2Cl_2 (243 μL, 148 μmol). The gradual formation of a brown colour was observed. After one hour stirring at rt, the reaction was worked up by dilution with toluene (5 mL) and then the mixture was concentrated by rotoevaporation. The residue was subjected to column chromatography (13:1:0.001-6:1:0.001 v/v *n*-hexane-ethyl acetate-triethylamine) to afford product **32** as a white foam (294.9 mg, 77%). $[\alpha]_D^{20}$ +24 (c 0.6, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.61 (s, 1H, OC(NH)*C*Cl_3), 7.92-7.06 (m, 22H, H-Ar), 6.79 (d, 1H, *J* 3.6 Hz, H-1), 6.14 (t, 1H, *J* 9.8 Hz, H-3), 5.47 (dd, 1H, *J* 10.2, 3.6 Hz, H-2), 5.28 (d, 1H, *J* 12.2, OC*H*HPh), 5.18 (d, 1H, *J* 12.2 Hz, OCH*H*Ph), 4.70 (d, 1H, *J* 9.9 Hz, H-5), 4.61 (d, 1H, *J* 11.2 Hz, OC*H*HNAP), 4.54 (d, 1H, *J* 11.2 Hz, OCH*H*NAP), 4.26 (t, 1H, *J* 9.7 Hz, H-4). ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.0, 165.4, 165.3, 160.5 (C-6, O*C*(NH)CCl_3, 2 *C*OPh), 134.7-125.9 (C-Ar), 93.2 (C-1), 90.5 (OC(NH)*C*Cl_3), 77.4, 75.0, 72.8, 71.2, 70.5, 67.7 (C-2, C-3, C-4, C-5, O*C*H_2Ph, O*C*H_2NAP). HRMS *m/z* $[M - CNCCl_3 + Na]^+$ Calcd for $C_{38}H_{32}O_9Na$ 655.1939, found 655.1911.

***N*-(*tert*-Butoxycarbonyl)-*O*-benzyl-L-threonine benzyl ester (38).** To a 0°C solution of commercially available **37** (502.2 mg, 1.623 mmol) in freshly dried DMF (7.5 mL), triethylamine (236 μL, 1.71 mmol) and benzyl bromide (202 μL, 1.71 mmol) were added. The reaction mixture was stirred at rt for five hours, then diluted with ethyl acetate (70 mL) and washed successively with 1 M citric acid aqueous solution (70 mL), saturated $NaHCO_3$ aqueous solution (70 mL) and brine (70 mL). The organic phase was collected, dried over anhydrous Na_2SO_4, filtered, concentrated and coevaporated four times with toluene (10 mL each). The residue was subjected to column chromatography (15:1-10:1 v/v *n*-hexane-ethyl acetate) to afford **38** as a colourless oil (407.7 mg, 63%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.29-7.16 (m, 10H, H-Ar), 5.30 (bd, 1H, *J* 9.7 Hz, N*H*Boc), 5.12 (s, 2H, CO_2C*H*$_2$Ph), 4.47 (d, 1H, *J* 11.6 Hz, OC*H*HPh), 4.34 (dd, 1H, *J* 9.7, 2.2 Hz, H-α), 4.26 (d, 1H, *J* 11.6 Hz, OC*H*HPh), 4.14 (dq, 1H, *J* 6.3, 2.2 Hz, H-β), 1.45 (s, 9H, C(CH_3)$_3$ Boc), 1.25 (d, 3H, *J* 6.4 Hz, γ-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.8 (*C*O_2Bn), 156.0 (NHCO), 137.7-127.4 (C-Ar), 79.6, 74.4, 70.7, 66.9, 58.2 (α-CH, β-CH, 2 *C*H_2Ph, *C*(CH_3)$_3$ Boc), 28.1 (C(*C*H_3)$_3$ Boc), 16.1 (γ-CH_3). HRMS *m/z* $[M + Na]^+$ Calcd for $C_{23}H_{29}NO_5$ 399.2046, found 422.1922.

***O*-Benzyl-L-threonine benzyl ester trifluoroacetate (39).** Compound **38** (407.7 mg, 1.021 mmol) was dissolved in trifluoroacetic acid (3 mL) at 0°C and the solution was warmed up to rt under stirring. After one hour, the reaction mixture was concentrated and dried under vacuum to afford pure **39** as a colourless oil (530.0 mg, >99%). $[\alpha]_D^{20}$ –31.2 (c 1.0, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34-7.13 (m, 10H, H-Ar), 5.14 (d, 1H, *J* 11.9 Hz, CO_2C*H*HPh), 5.09 (d, 1H, *J* 11.9 Hz, CO_2CH*H*Ph), 4.54 (d, 1H, *J* 11.7 Hz, OC*H*HPh), 4.28 (d, 1H, *J* 11.8 Hz, OCH*H*Ph), 4.15 (dq, 1H, *J* 6.4, 2.8 Hz, H-β), 4.08 (d, 1H, *J* 2.9 Hz, H-α), 1.35 (d, 3H, *J* 6.5 Hz, γ-C*H*$_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.5 (*C*O_2Bn), 161.4 (q, $J_{C,F}$ 38.6 Hz, *C*O_2CF_3), 136.6, 133.9, 128.9, 128.7, 128.5, 128.1, 127.9 (C-Ar), 71.2, 70.7, 68.7, 58.0 (α-CH, β-CH, 2 *C*H_2Ph), 15.9 (γ-CH_3). HRMS *m/z* $[M - CF_3O_2]^+$ Calcd for $C_{18}H_{22}NO_3$ 300.1594, found 300.1606.

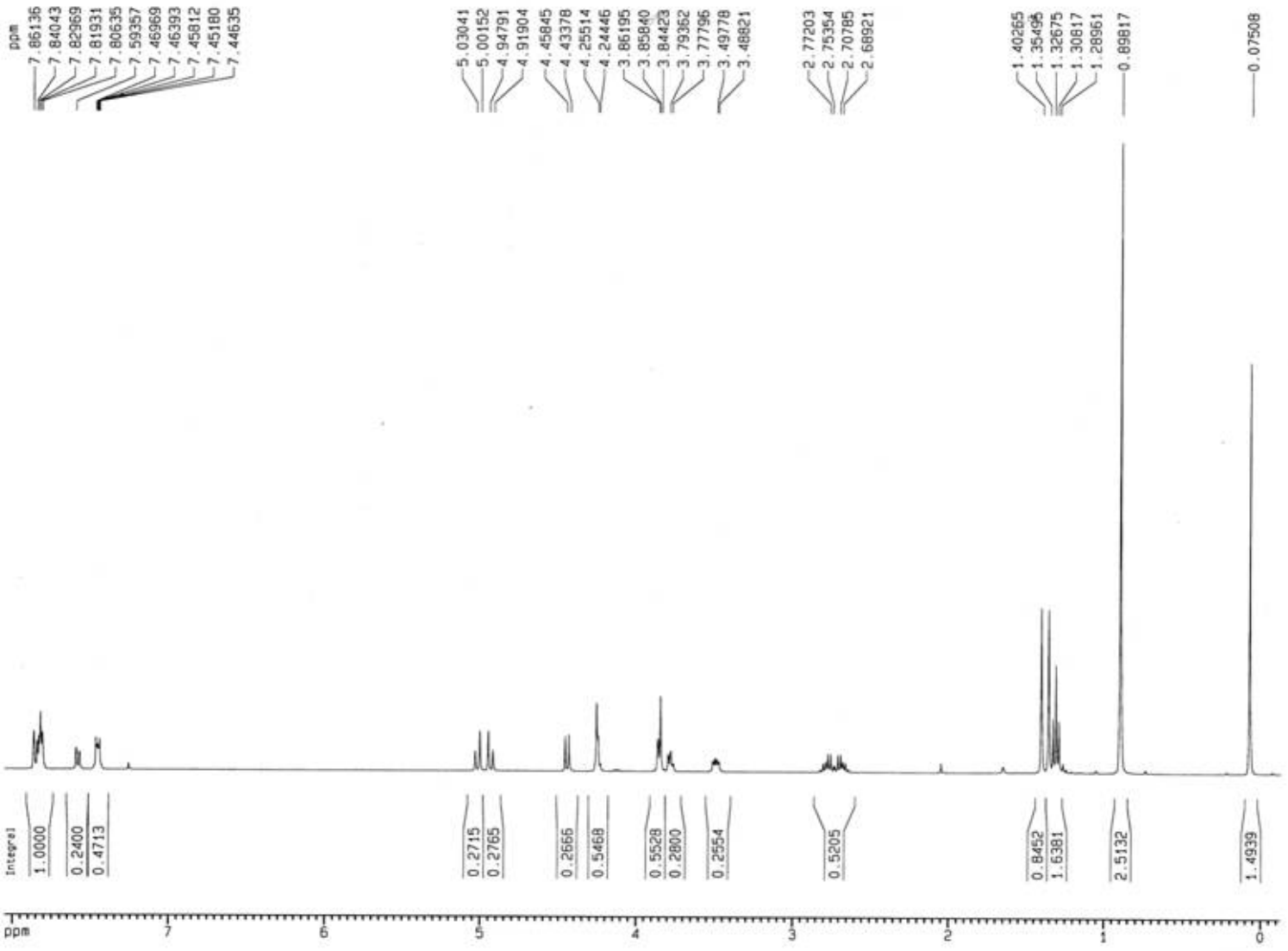

Figure S1: ^{1}H NMR spectrum of **3** (400 MHz, $CDCl_3$, 298K)

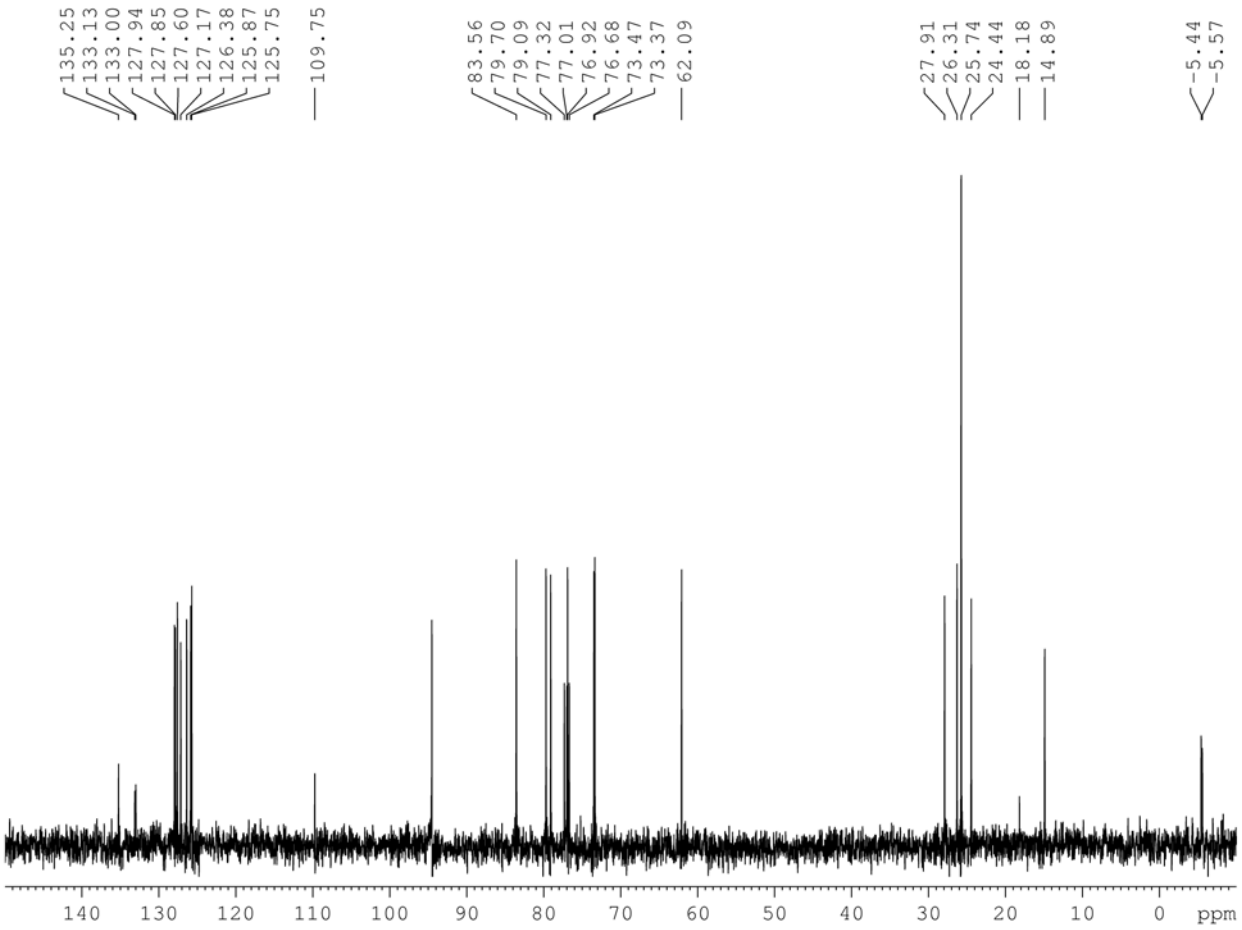

Figure S2: ^{13}C NMR spectrum of **3** (100 MHz, $CDCl_3$, 298K)

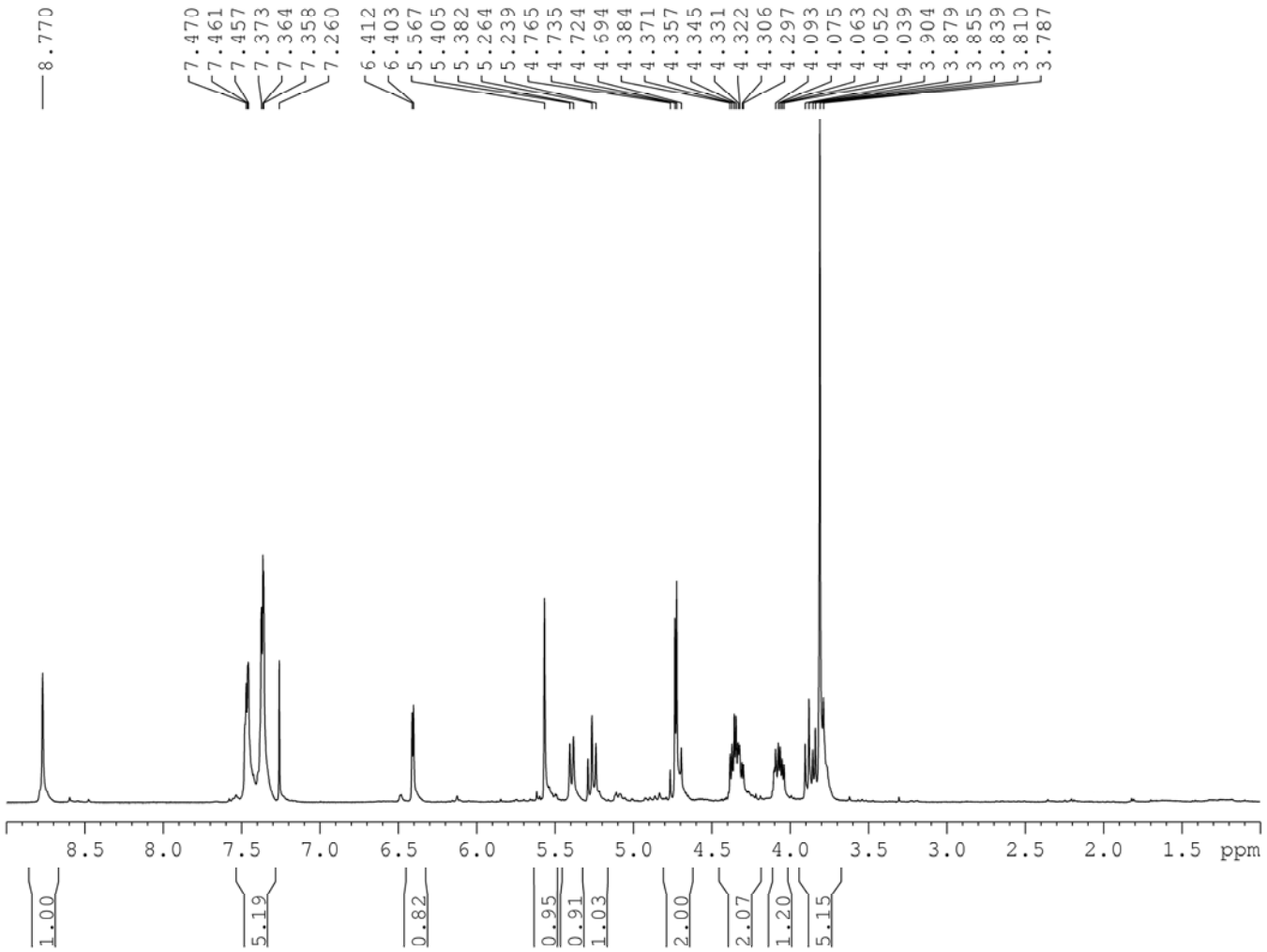

Figure S3: ^{1}H NMR spectrum of **4** (400 MHz, $CDCl_3$, 298K)

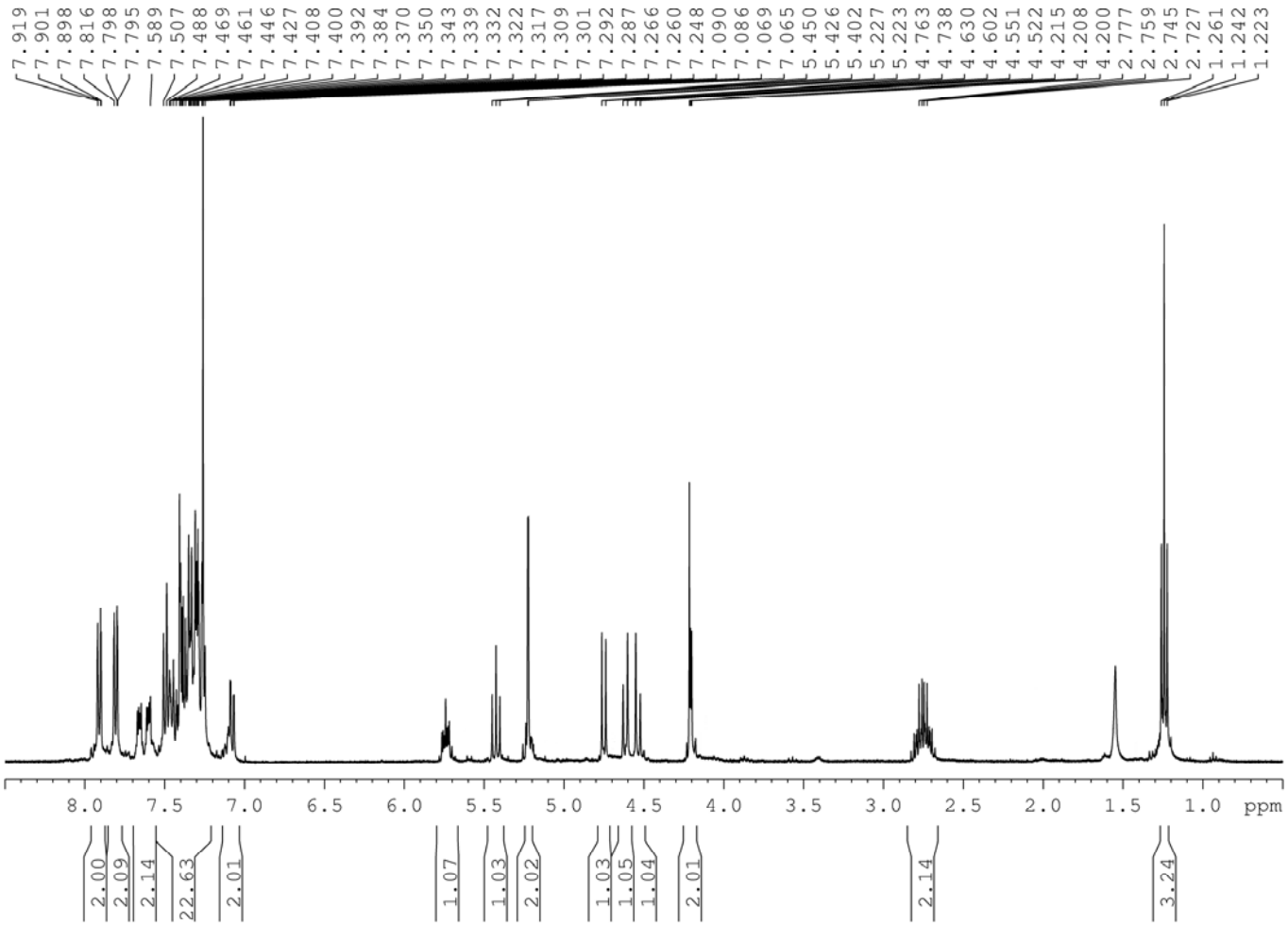

Figure S4: ^{1}H NMR spectrum of **5** (400 MHz, $CDCl_3$, 298K)

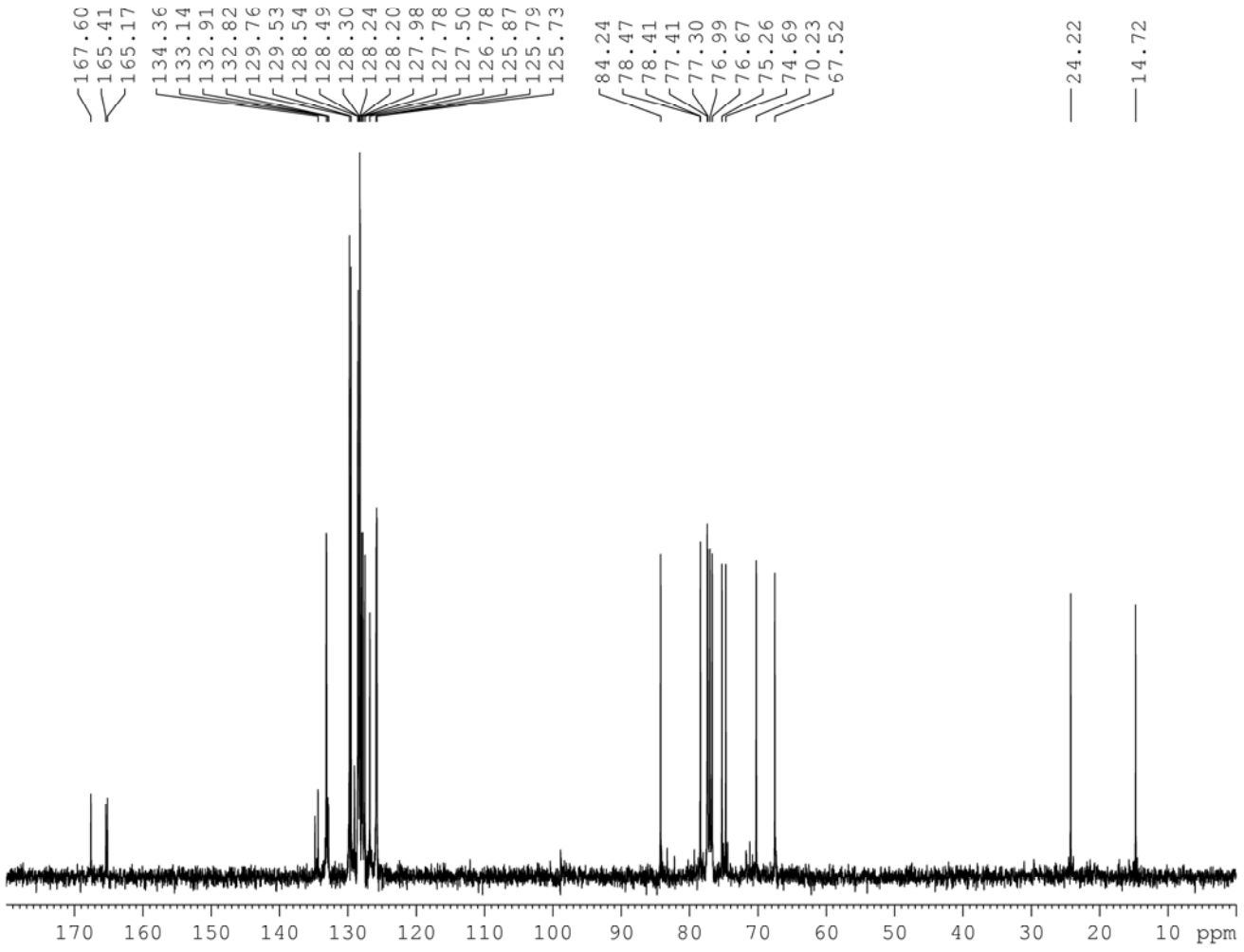

Figure S5: ^{13}C NMR spectrum of **5** (100 MHz, $CDCl_3$, 298K)

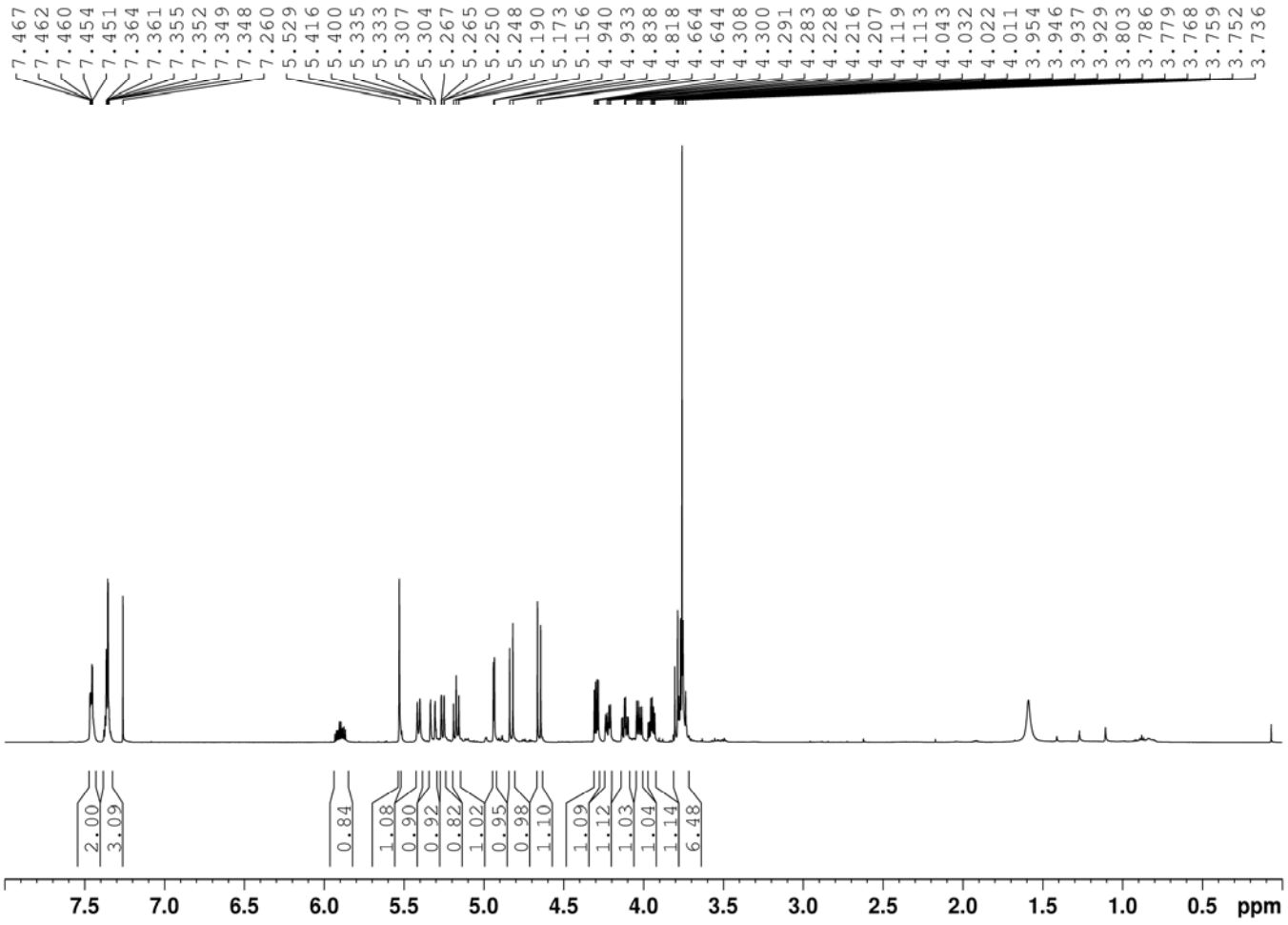

Figure S6: ^{1}H NMR spectrum of **9** (600 MHz, $CDCl_3$, 298K)

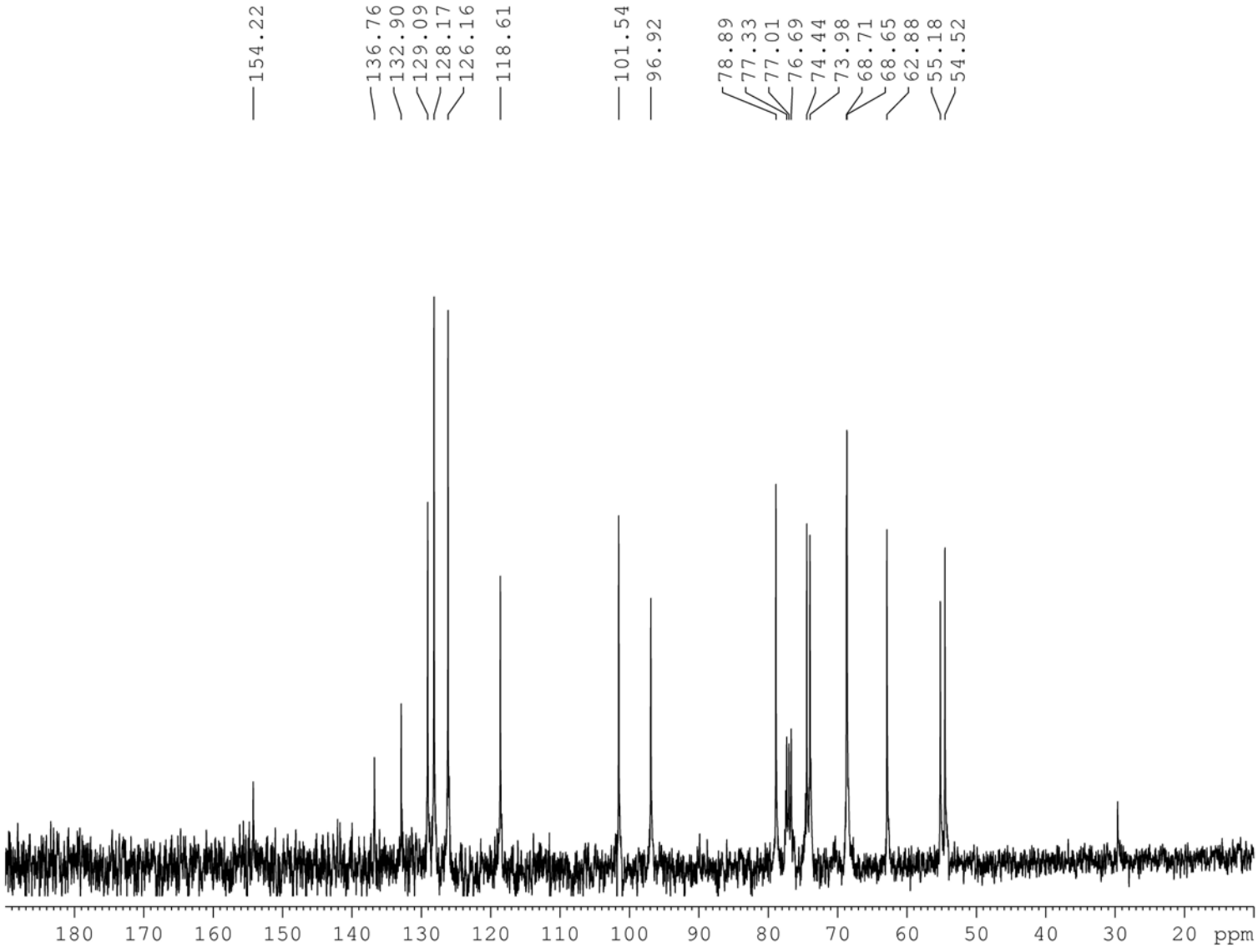

Figure S7: ^{13}C NMR spectrum of **9** (100 MHz, $CDCl_3$, 298K)

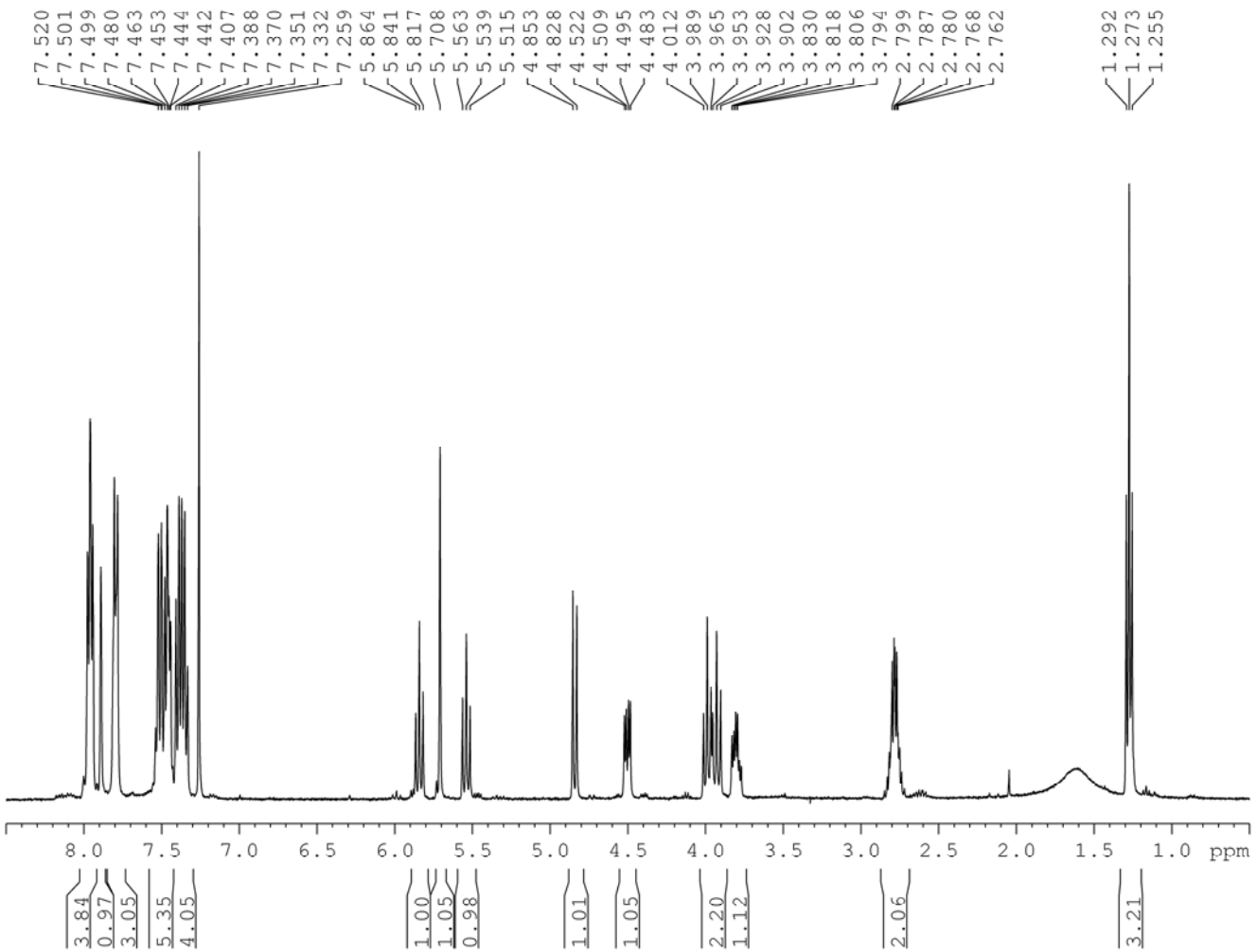

Figure S8: ^{1}H NMR spectrum of **12** (400 MHz, $CDCl_3$, 298K)

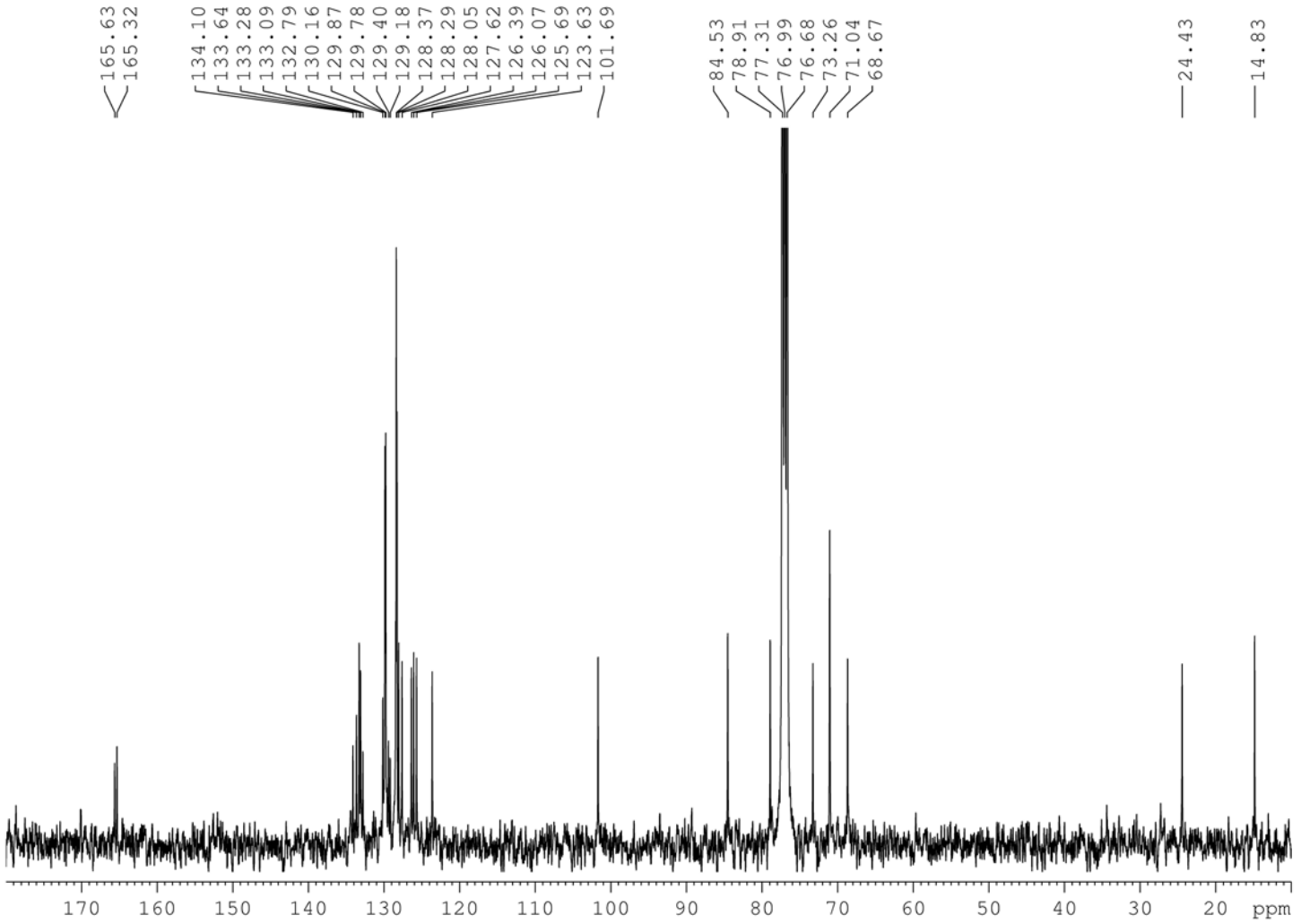

Figure S9: ^{13}C NMR spectrum of **12** (100 MHz, $CDCl_3$, 298K)

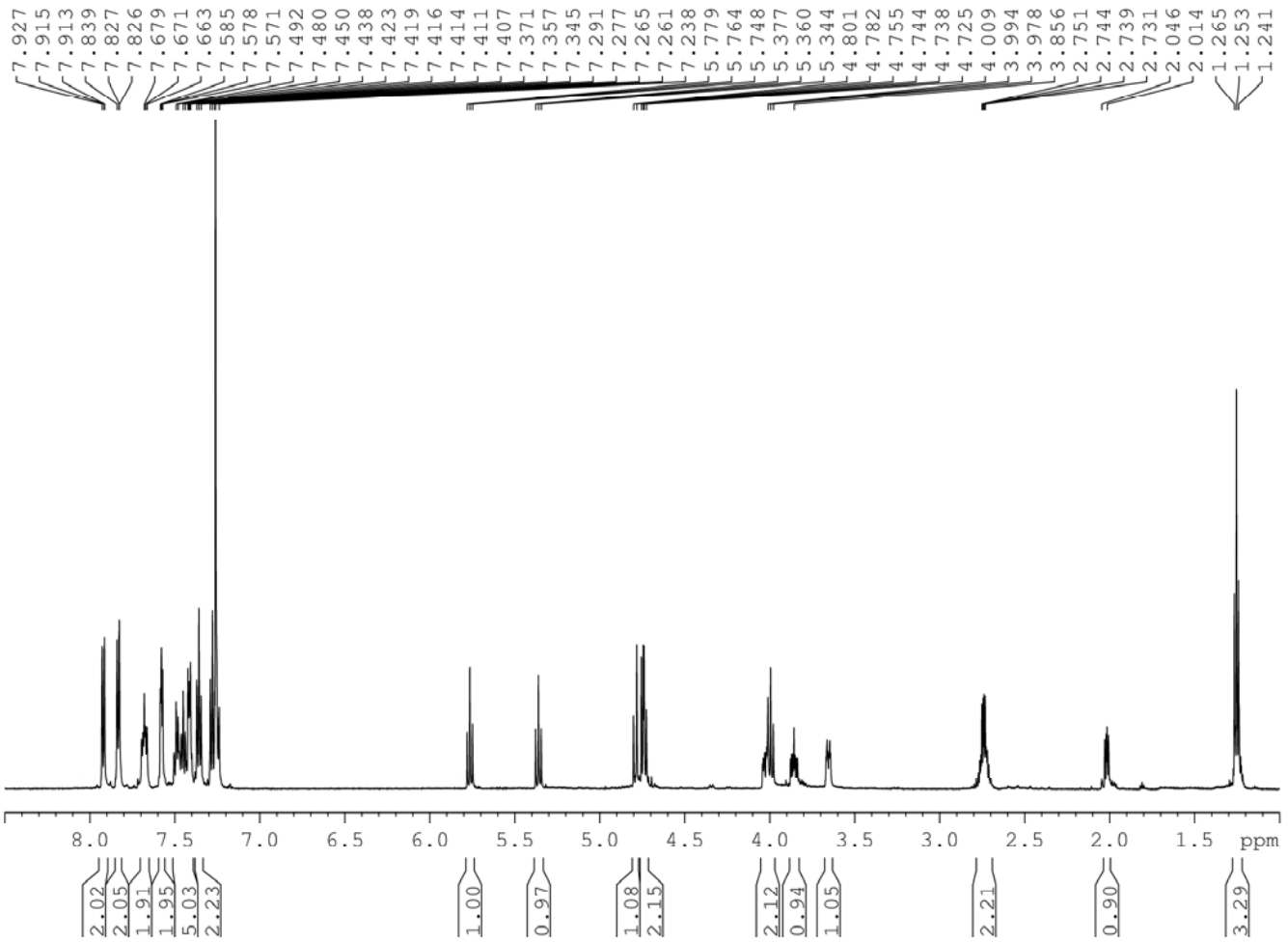

Figure S10: ^{1}H NMR spectrum of **13** (600 MHz, $CDCl_3$, 298K)

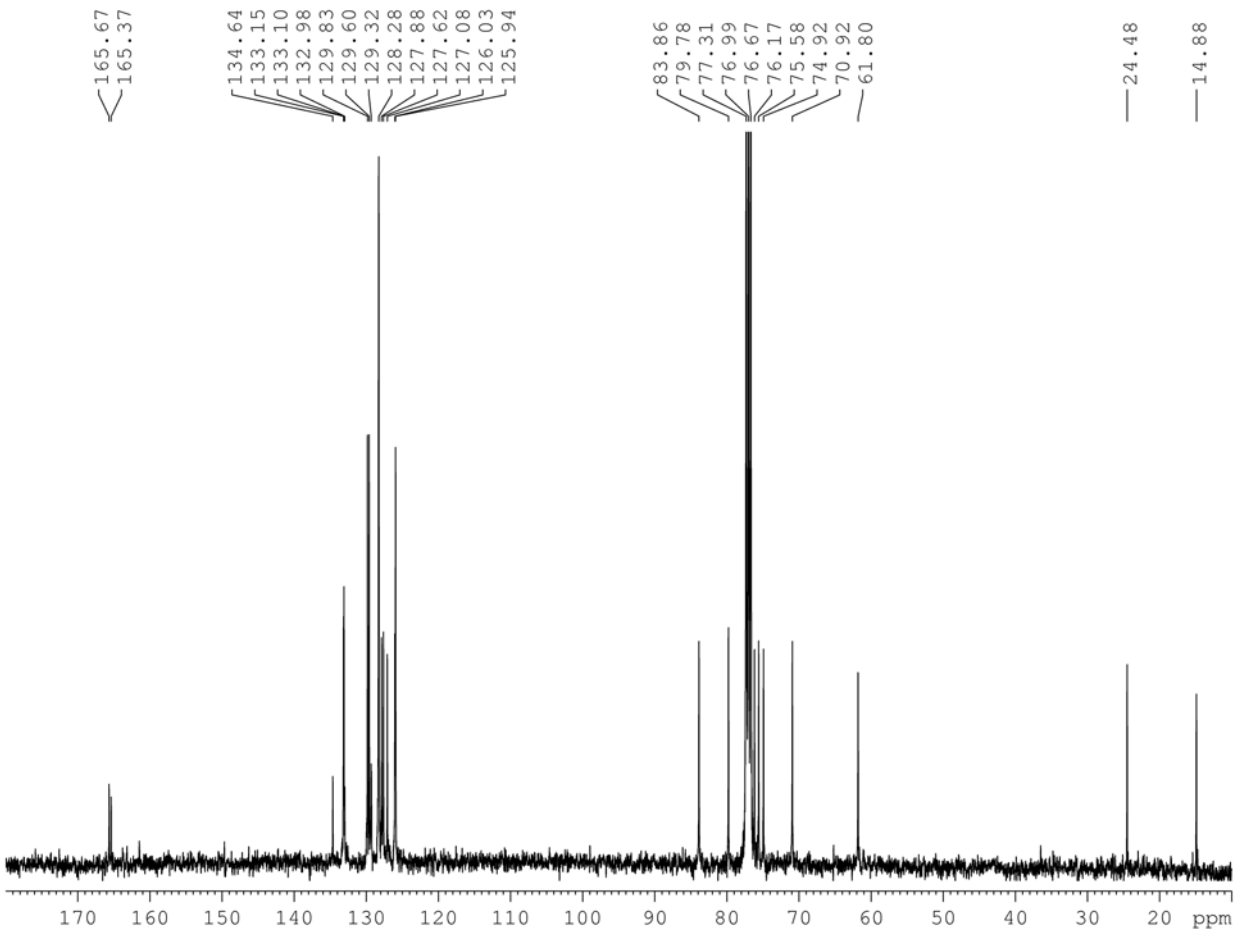

Figure S11: ^{13}C NMR spectrum of **13** (100 MHz, $CDCl_3$, 298K)

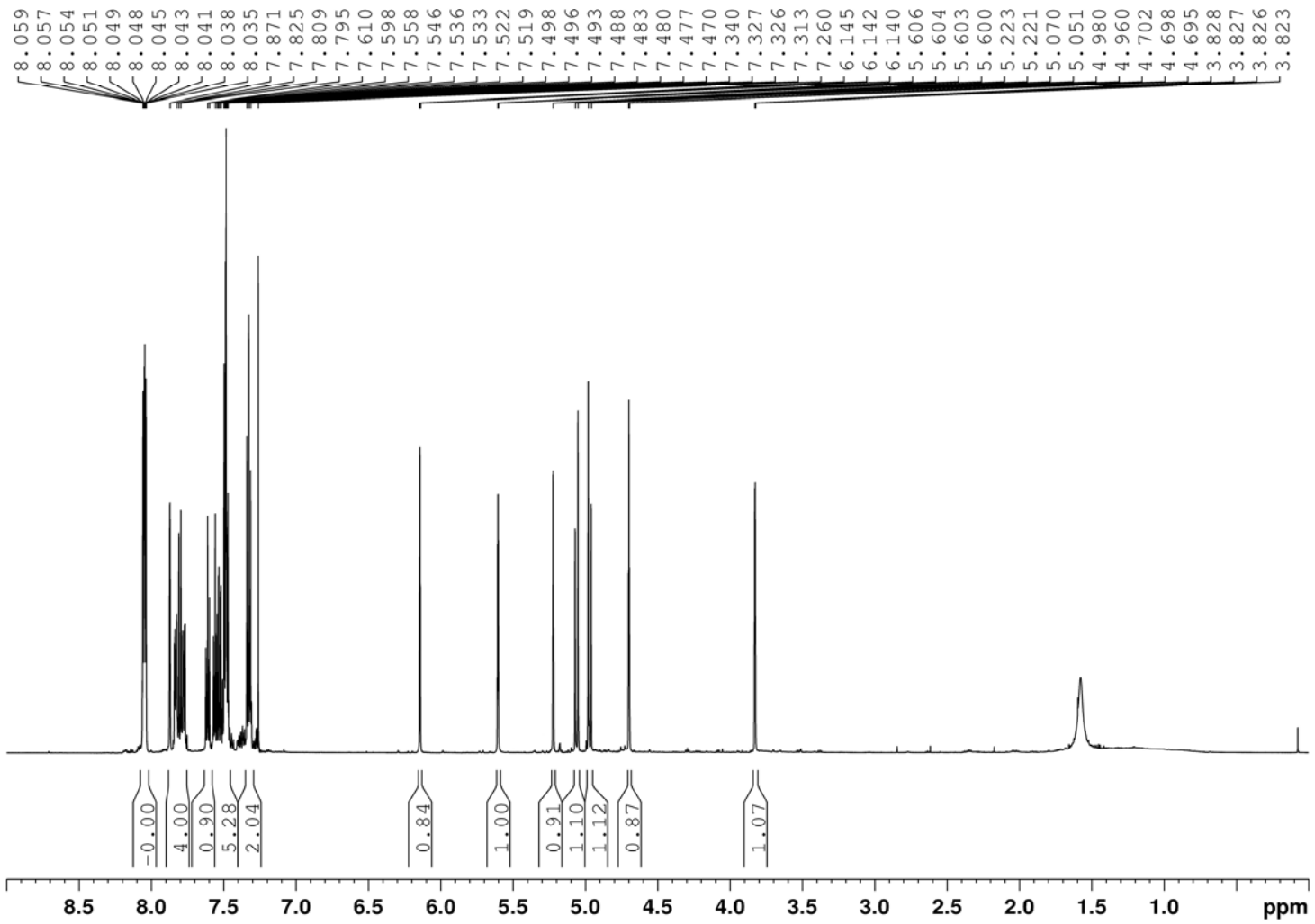

Figure S12: ^{1}H NMR spectrum of **14** (600 MHz, $CDCl_3$, 298K)

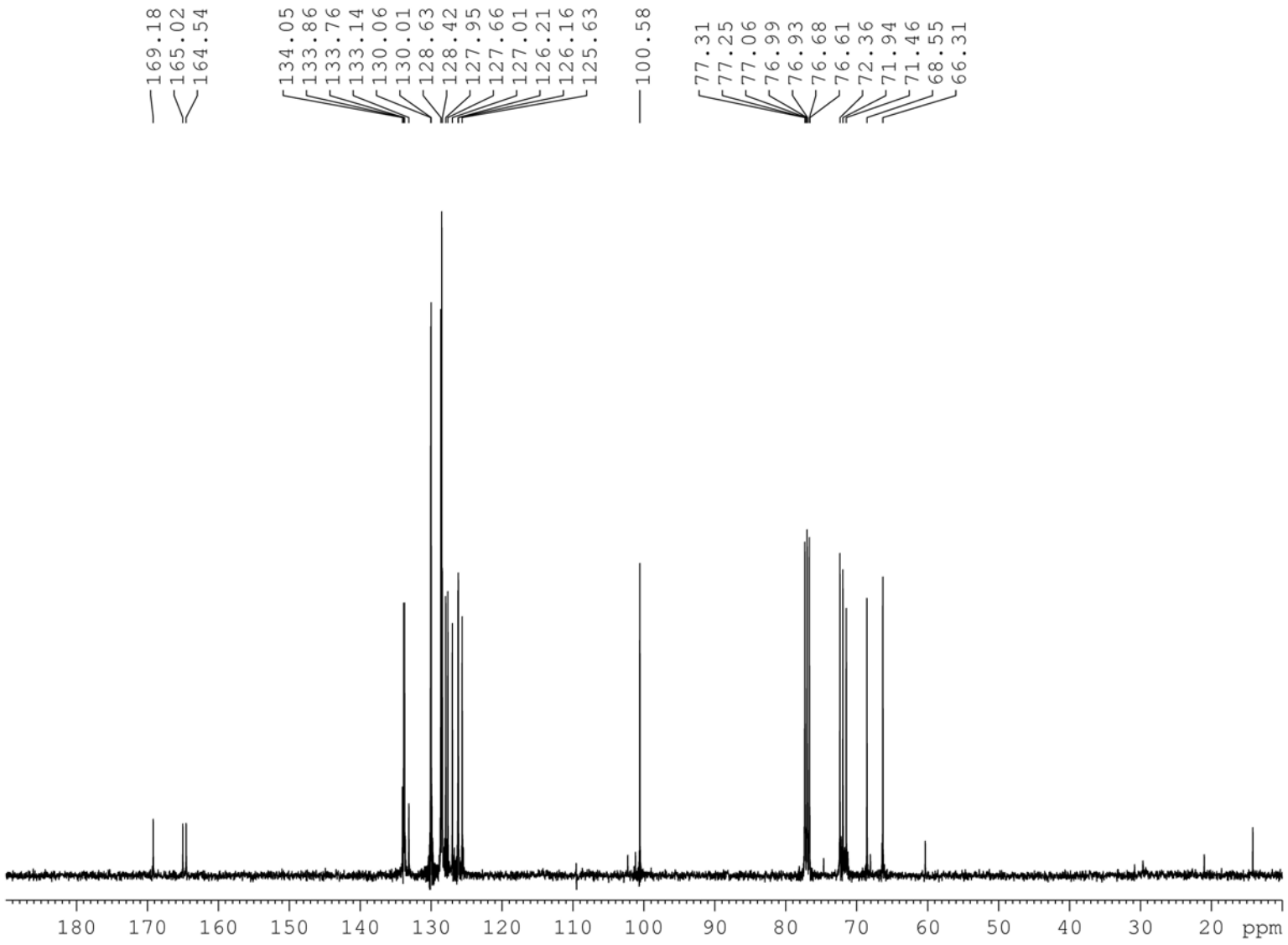

Figure S13: ^{13}C NMR spectrum of **14** (100 MHz, $CDCl_3$, 298K)

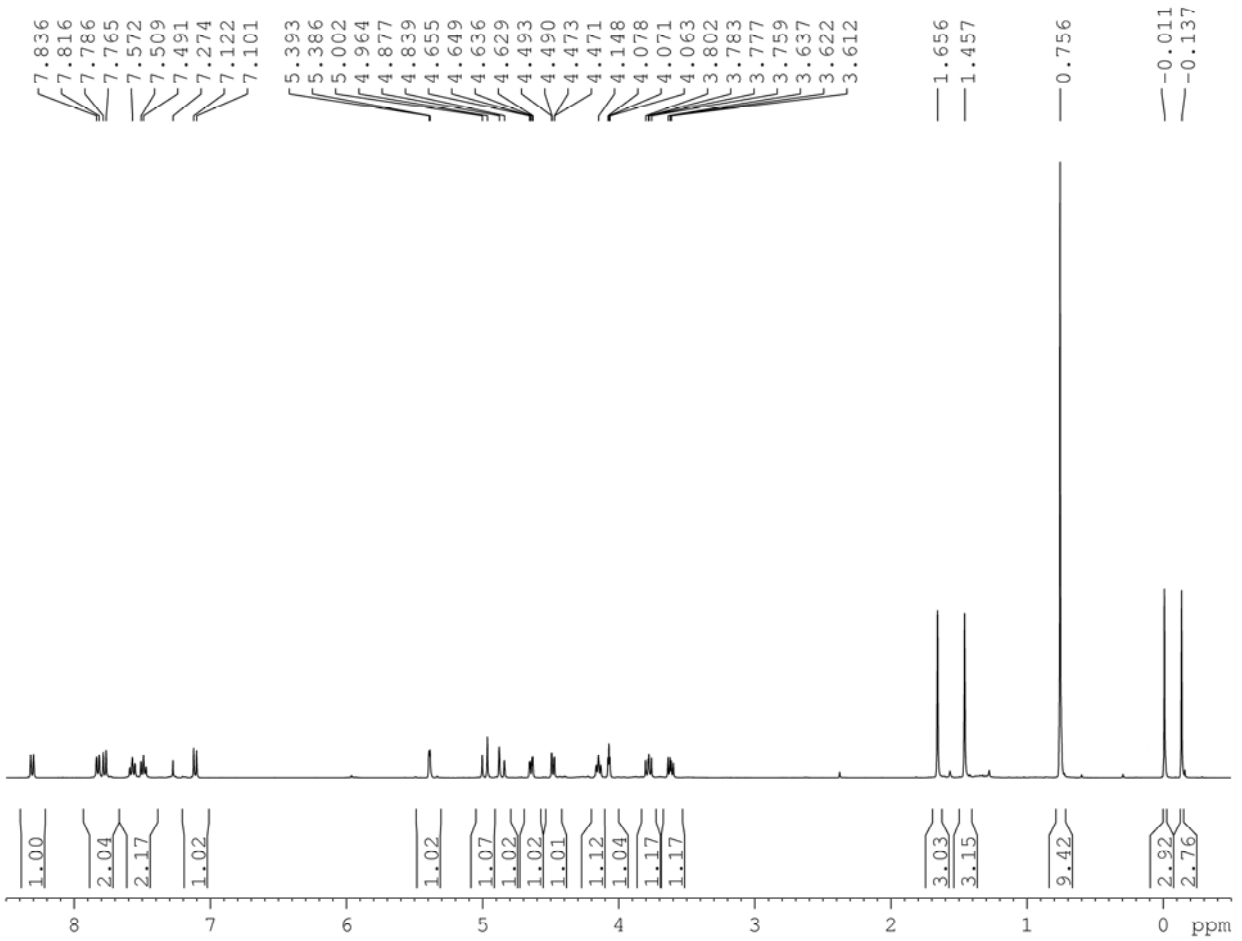

Figure S14: ^{1}H NMR spectrum of **15** (400 MHz, $CDCl_3$, 298K)

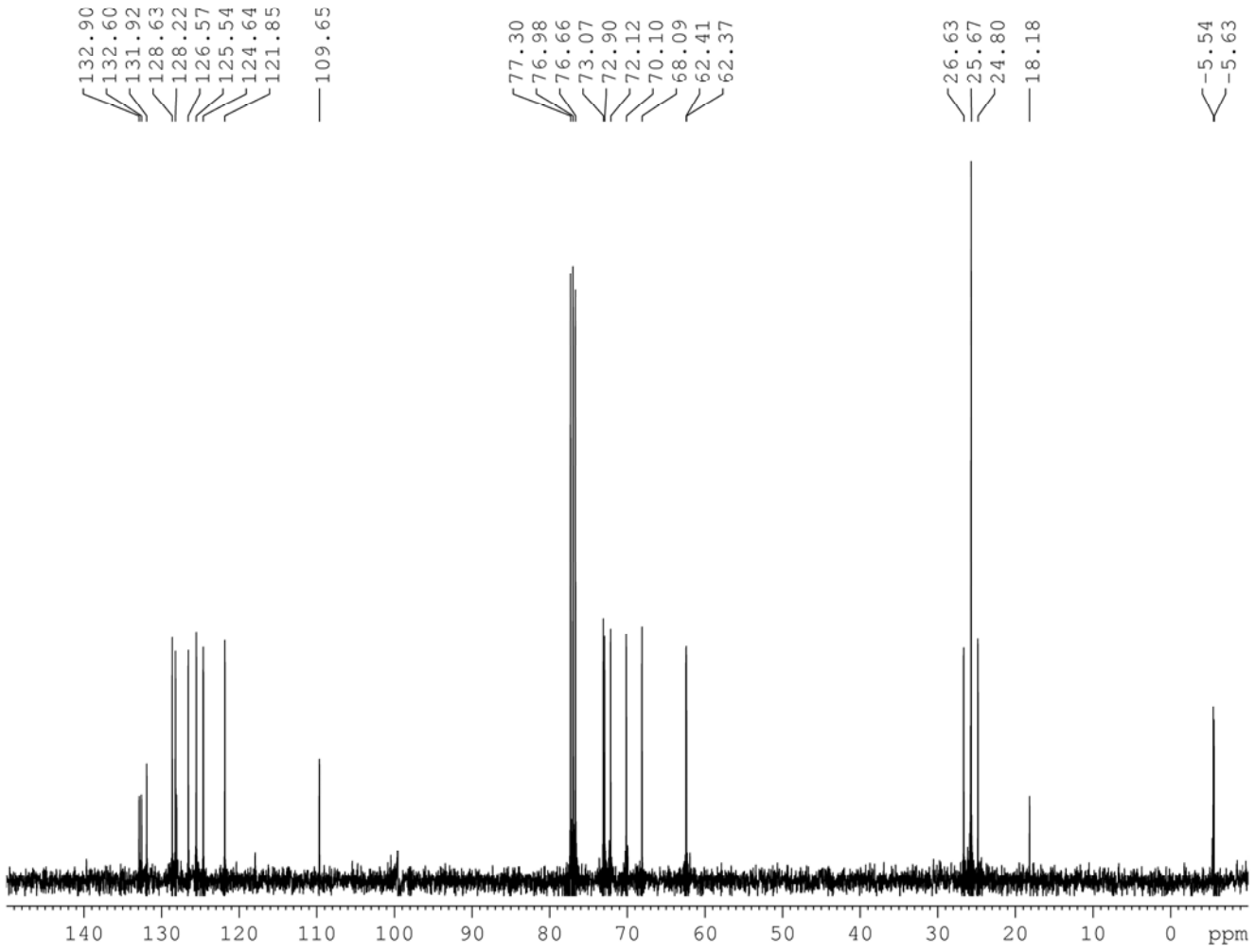

Figure S15: ^{13}C NMR spectrum of **15** (100 MHz, $CDCl_3$, 298K)

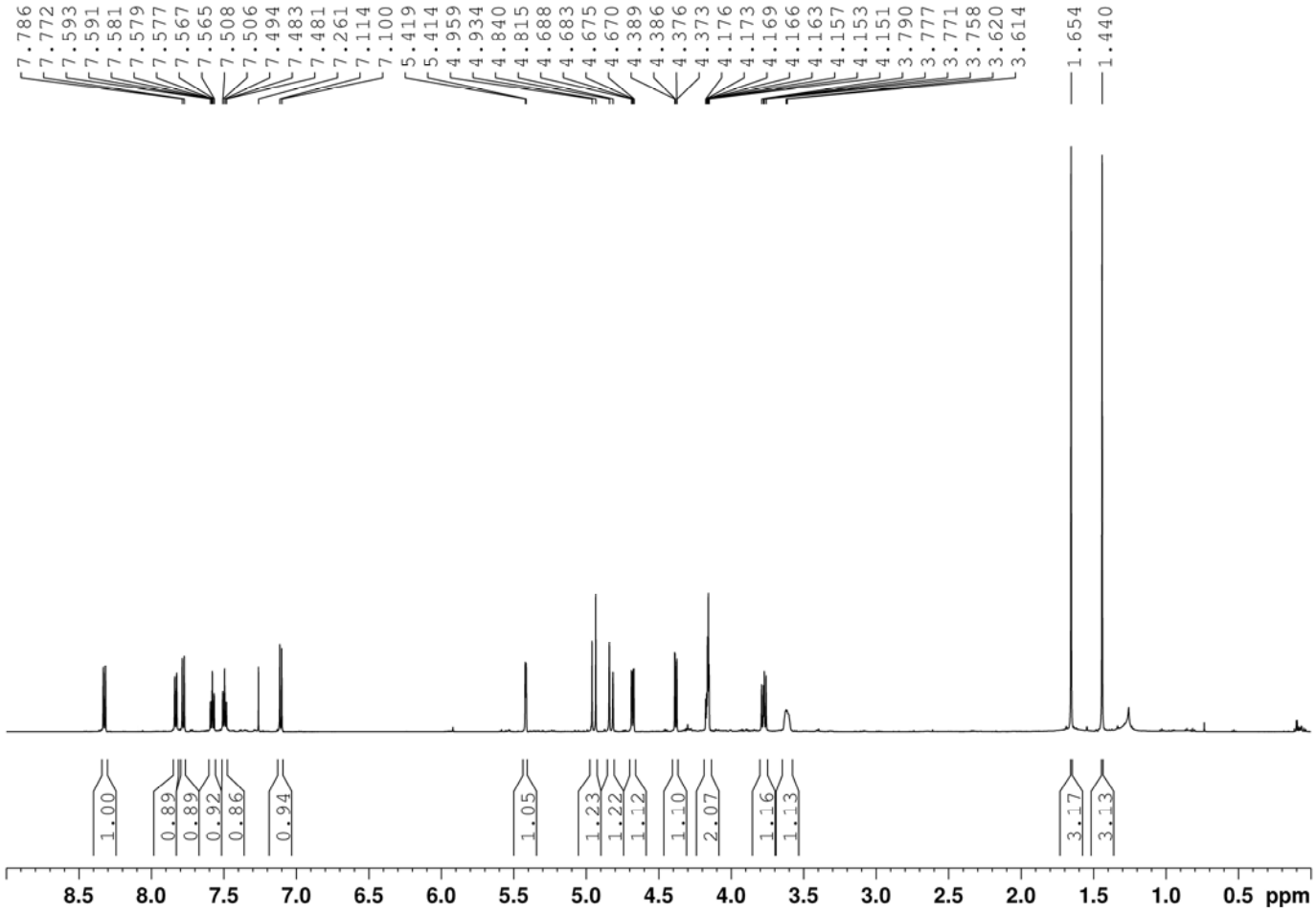

Figure S16: ^{1}H NMR spectrum of **16** (600 MHz, $CDCl_3$, 298K)

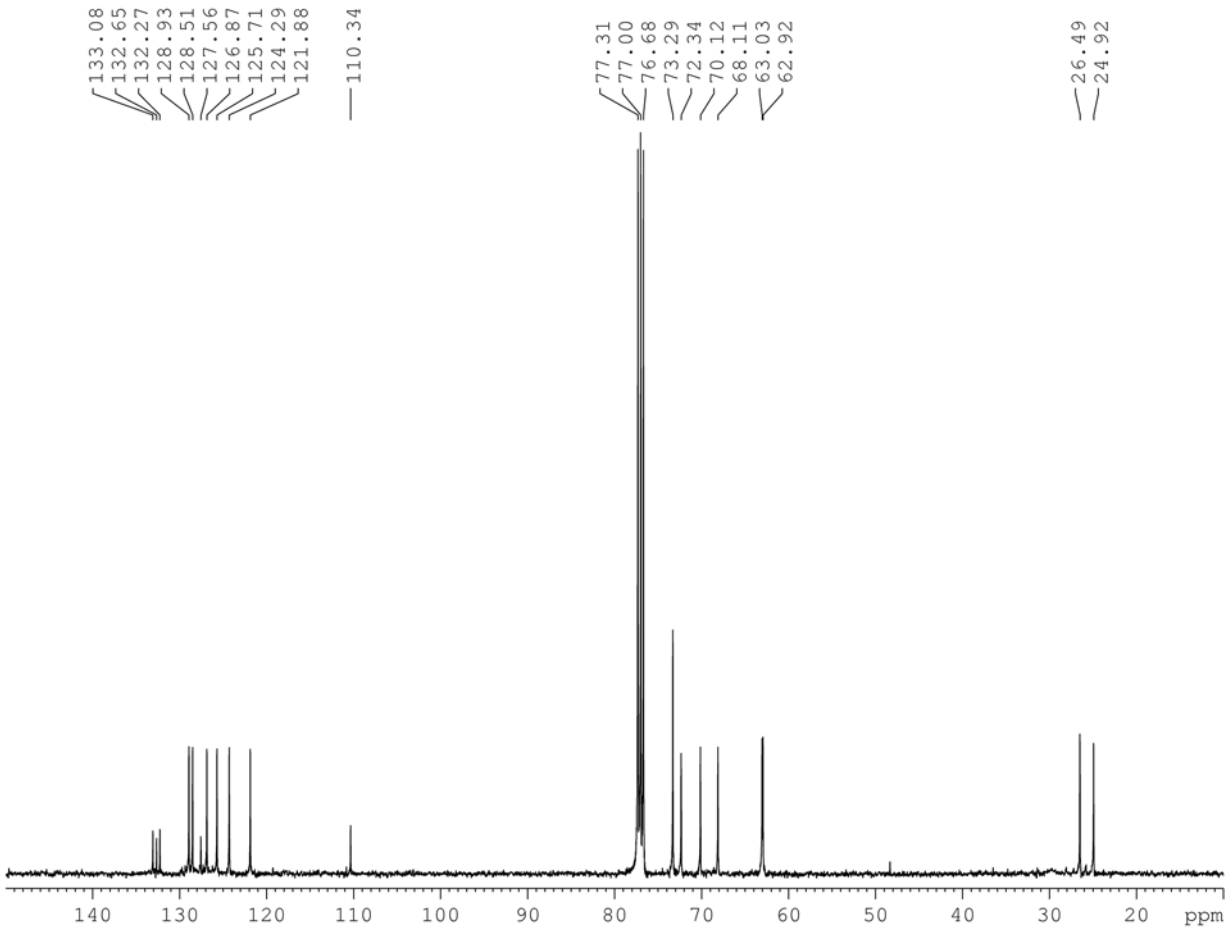

Figure S17: ^{13}C NMR spectrum of **16** (100 MHz, $CDCl_3$, 298K)

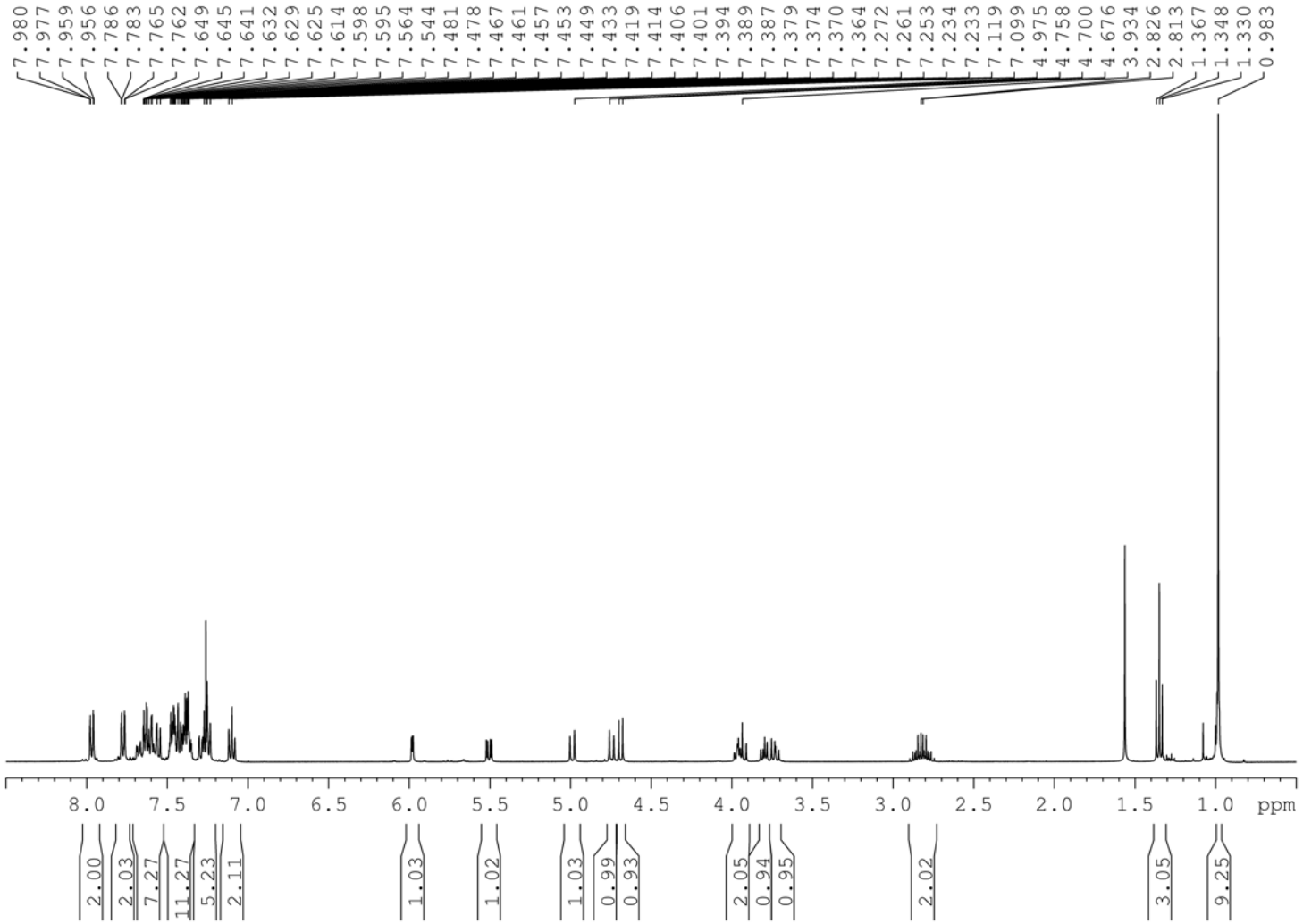

Figure S18: ^{1}H NMR spectrum of **17** (400 MHz, $CDCl_3$, 298K)

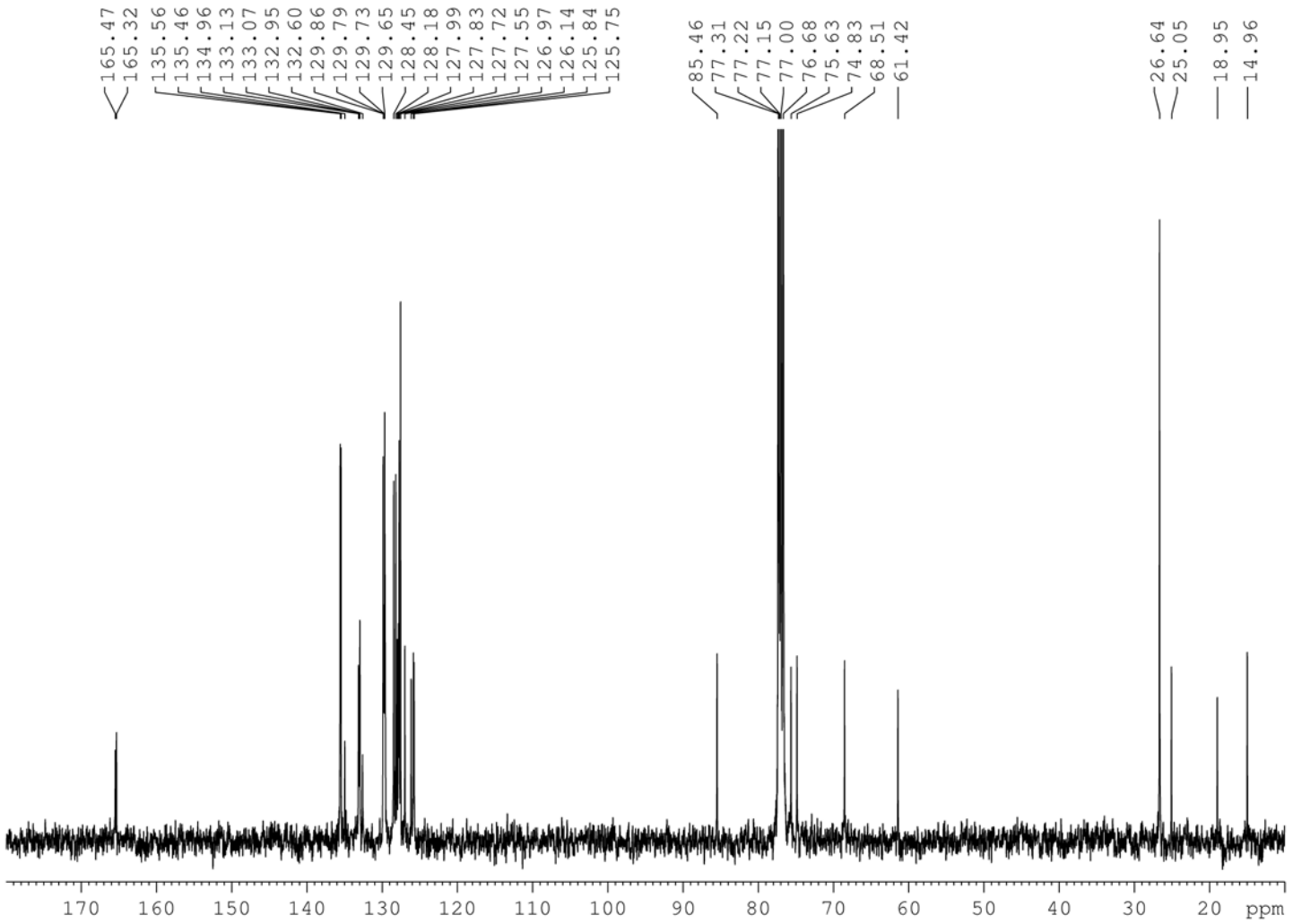

Figure S19: ^{13}C NMR spectrum of **17** (100 MHz, $CDCl_3$, 298K)

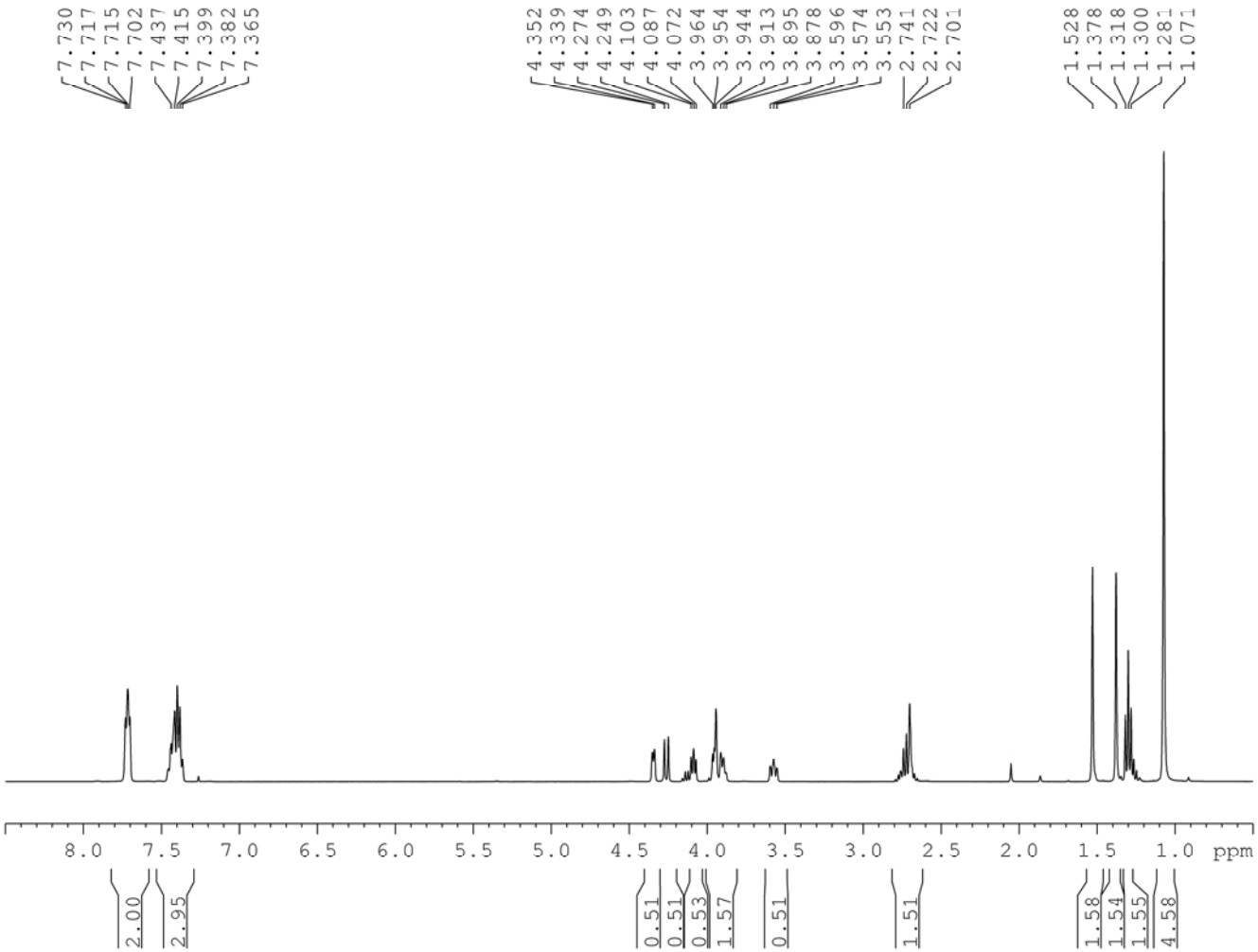

Figure S20: ^{1}H NMR spectrum of **18** (400 MHz, $CDCl_3$, 298K)

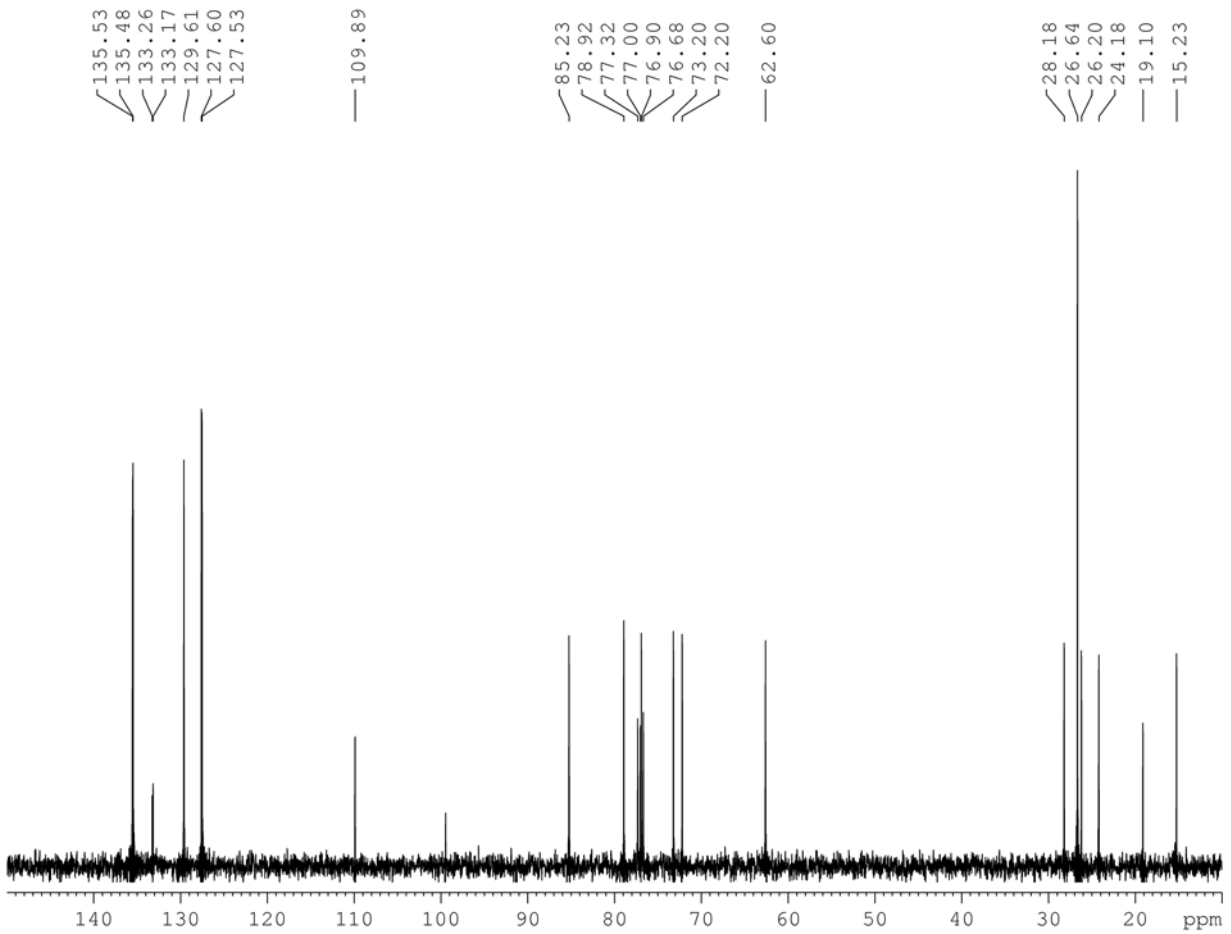

Figure S21: ^{13}C NMR spectrum of **18** (100 MHz, $CDCl_3$, 298K)

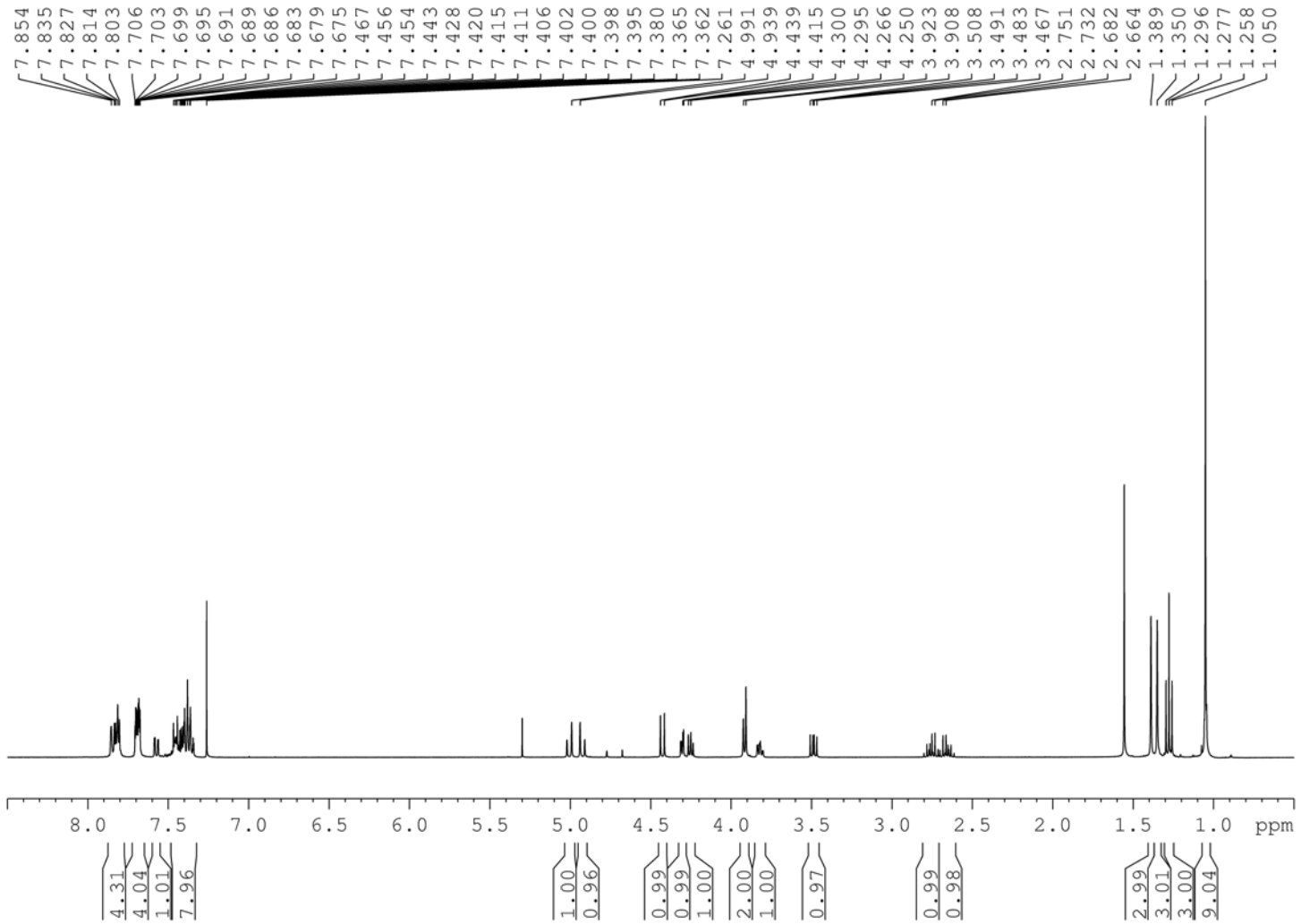

Figure S22: ^{1}H NMR spectrum of **19** (400 MHz, $CDCl_3$, 298K)

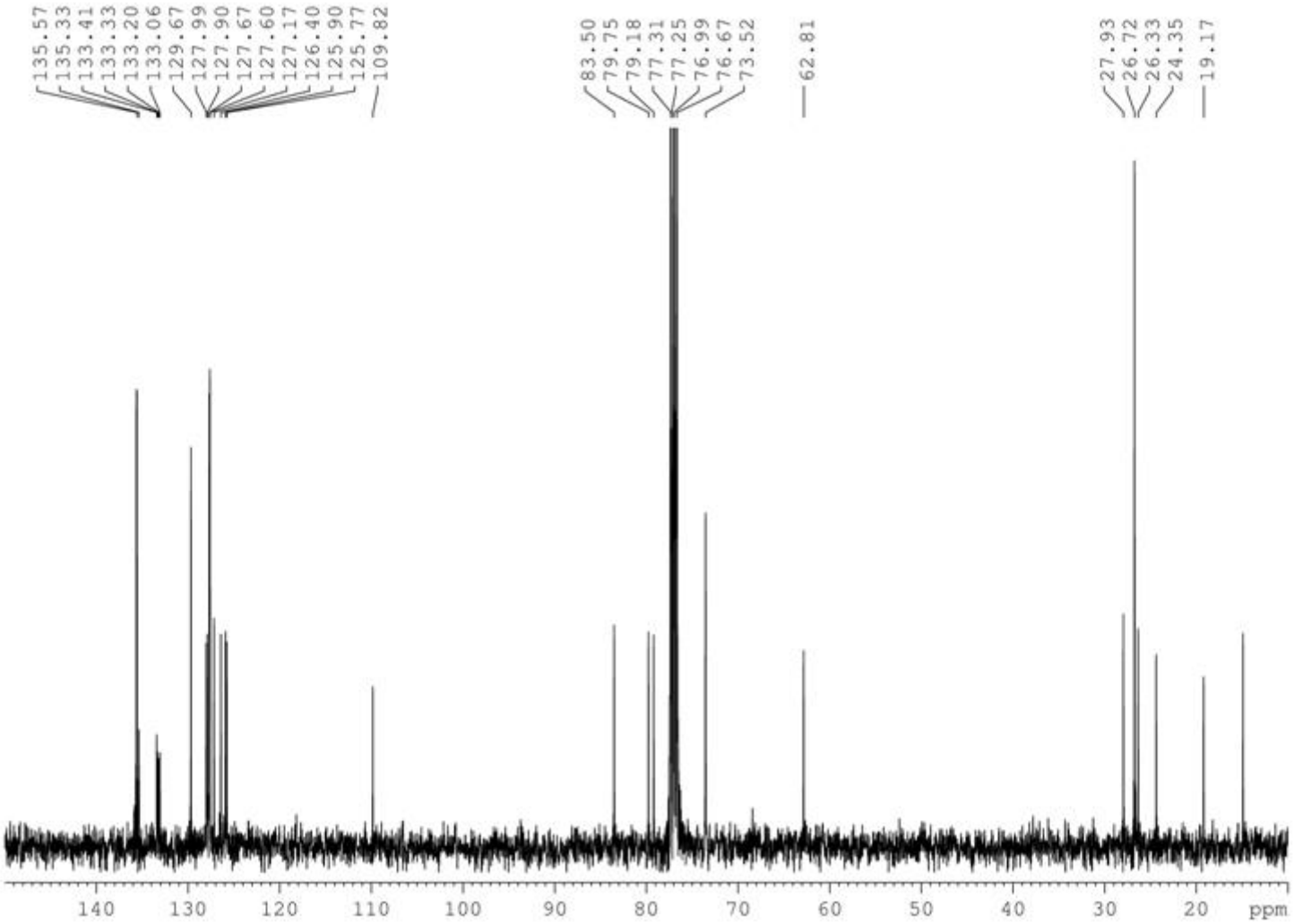

Figure S23: ^{13}C NMR spectrum of **19** (100 MHz, $CDCl_3$, 298K)

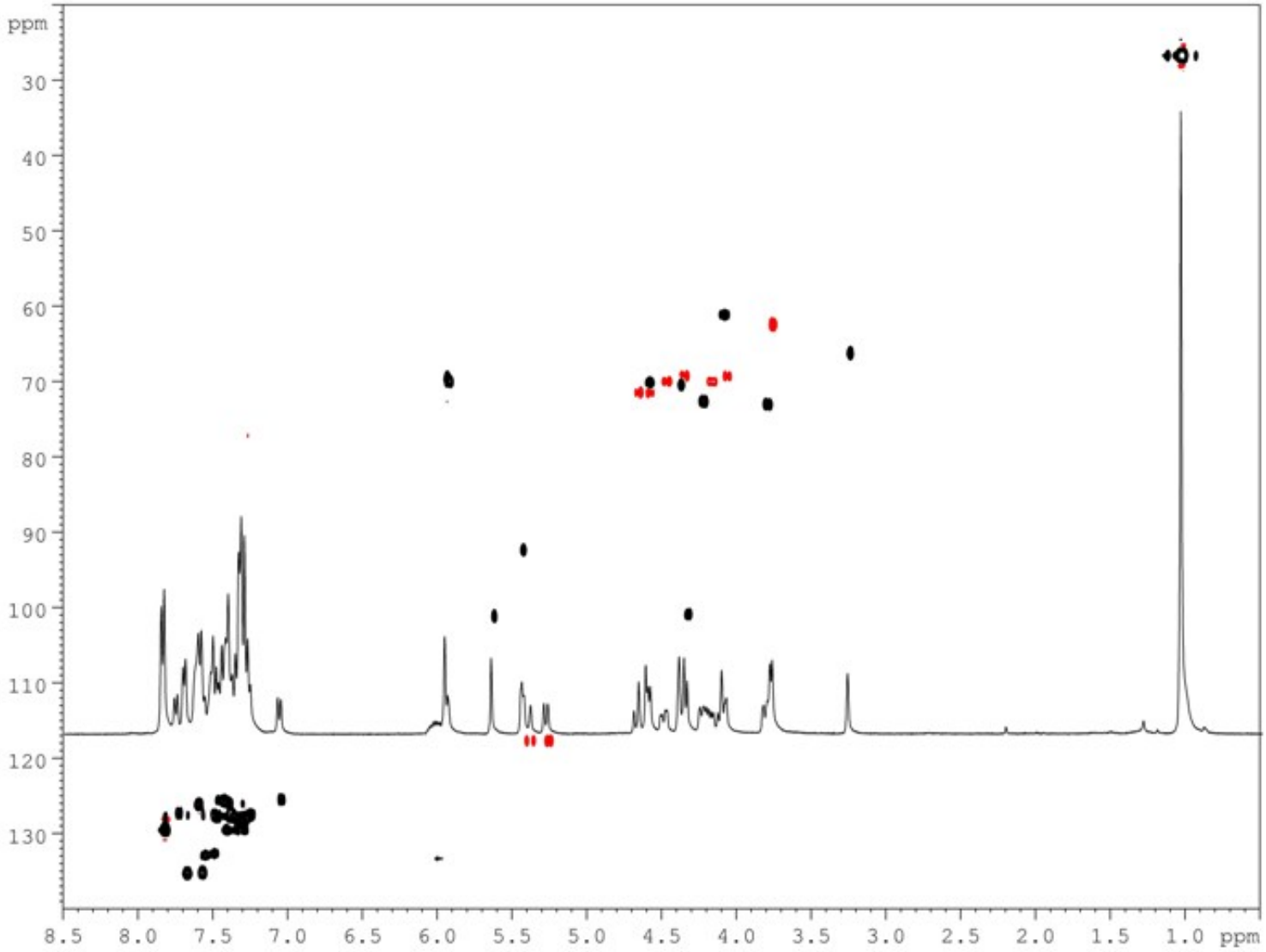

Figure S24: ^{1}H and DEPT-HSQC NMR spectra of **20** (400 MHz, $CDCl_3$, 298K)

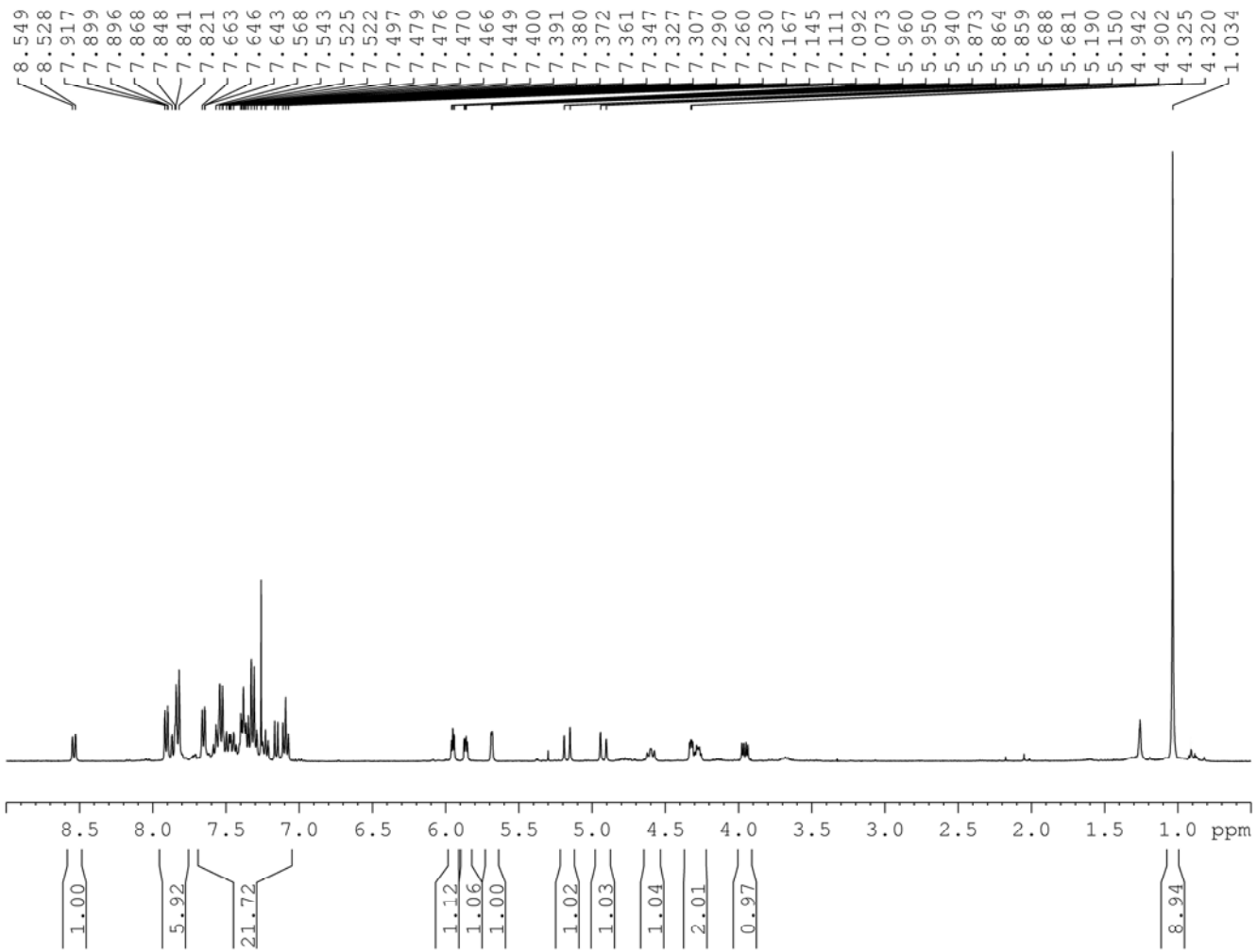

Figure S25: ^{1}H NMR spectrum of **21** (400 MHz, $CDCl_3$, 298K)

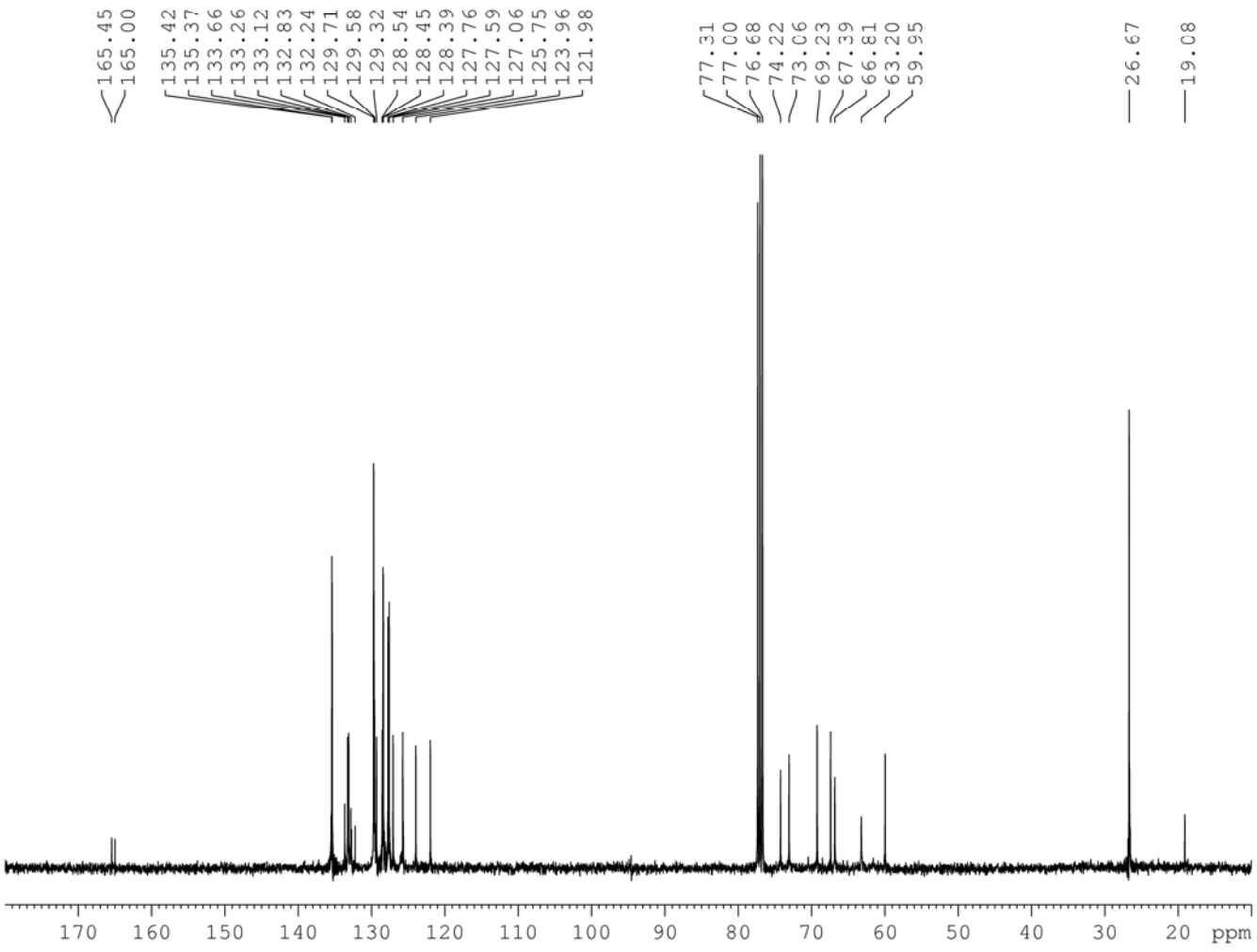

Figure S26: ^{13}C NMR spectrum of **21** (100 MHz, $CDCl_3$, 298K)

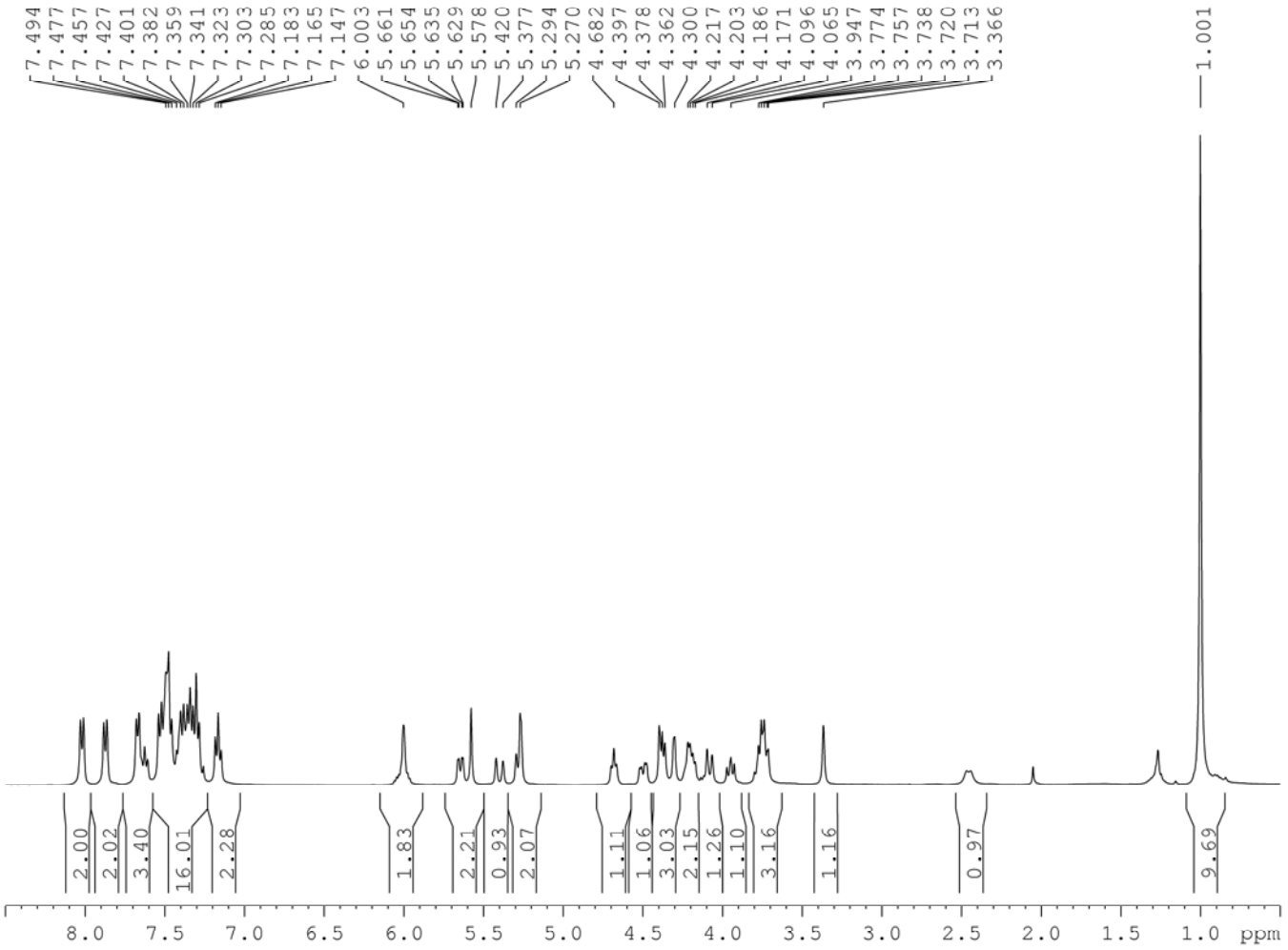

Figure S27: ^{1}H NMR spectrum of **22** (400 MHz, $CDCl_3$, 298K)

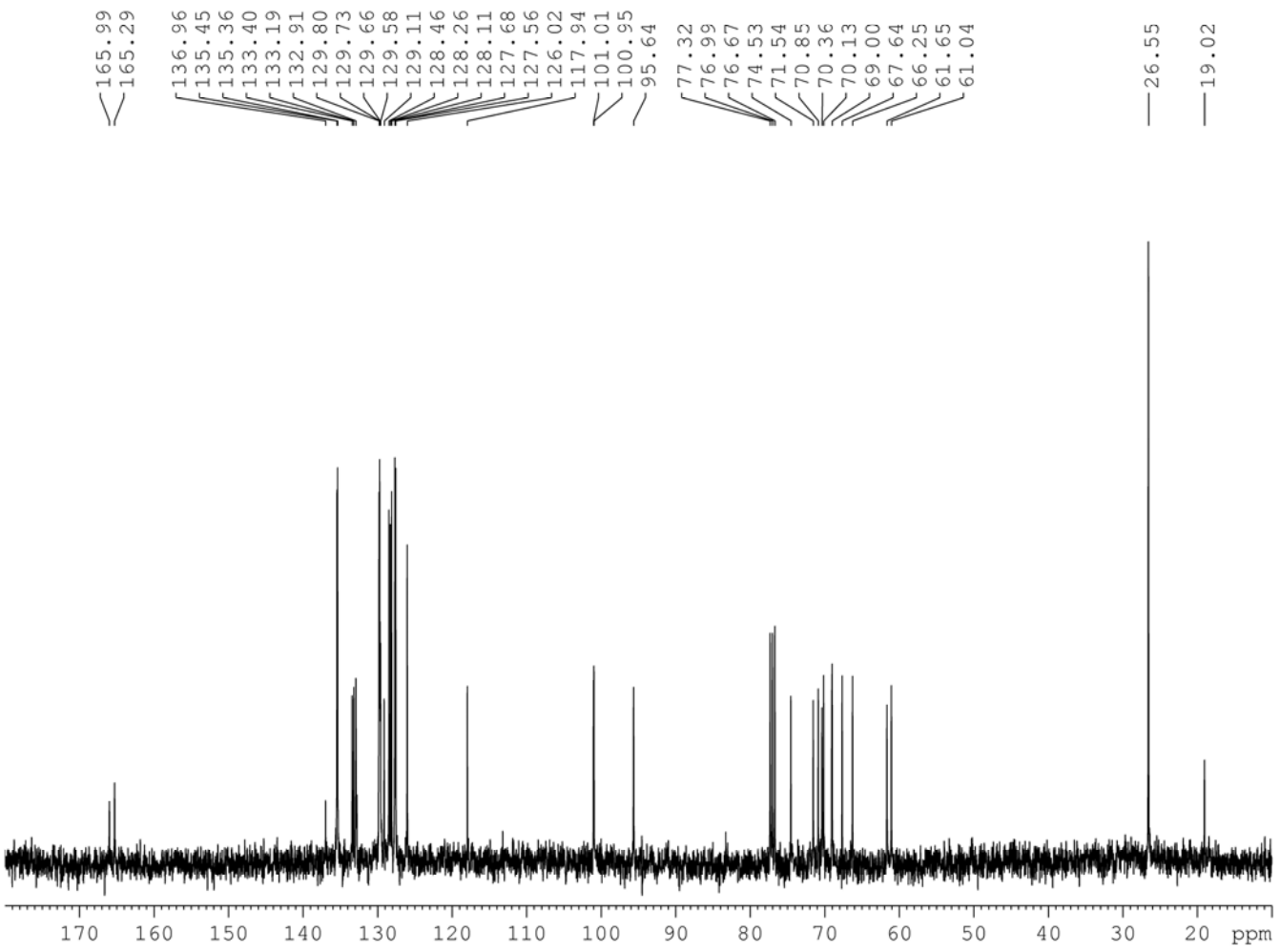

Figure S28: ^{13}C NMR spectrum of **22** (100 MHz, $CDCl_3$, 298K)

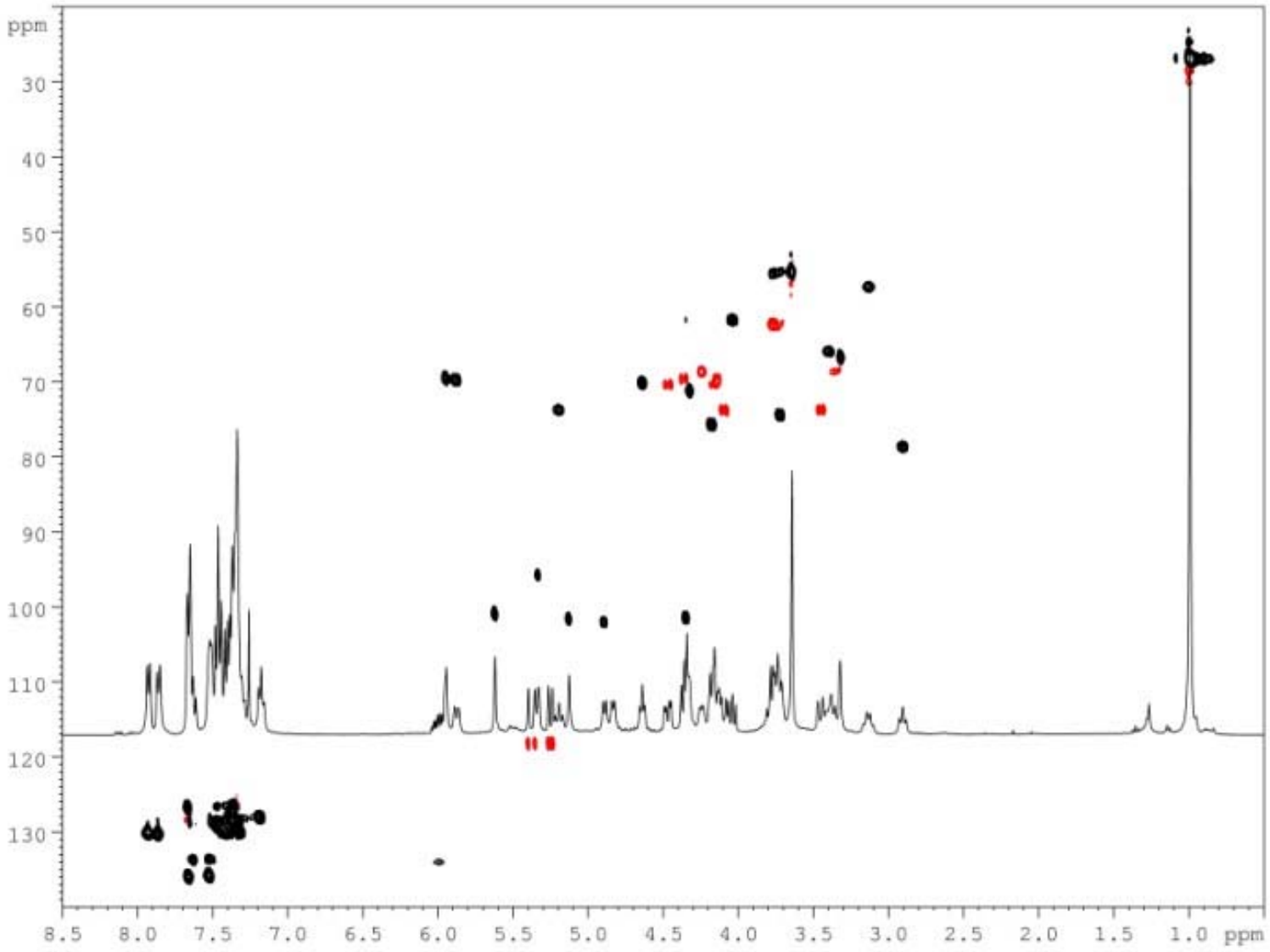

Figure S29: ^{1}H and DEPT-HSQC NMR spectra of **23** (400 MHz, $CDCl_3$, 298K)

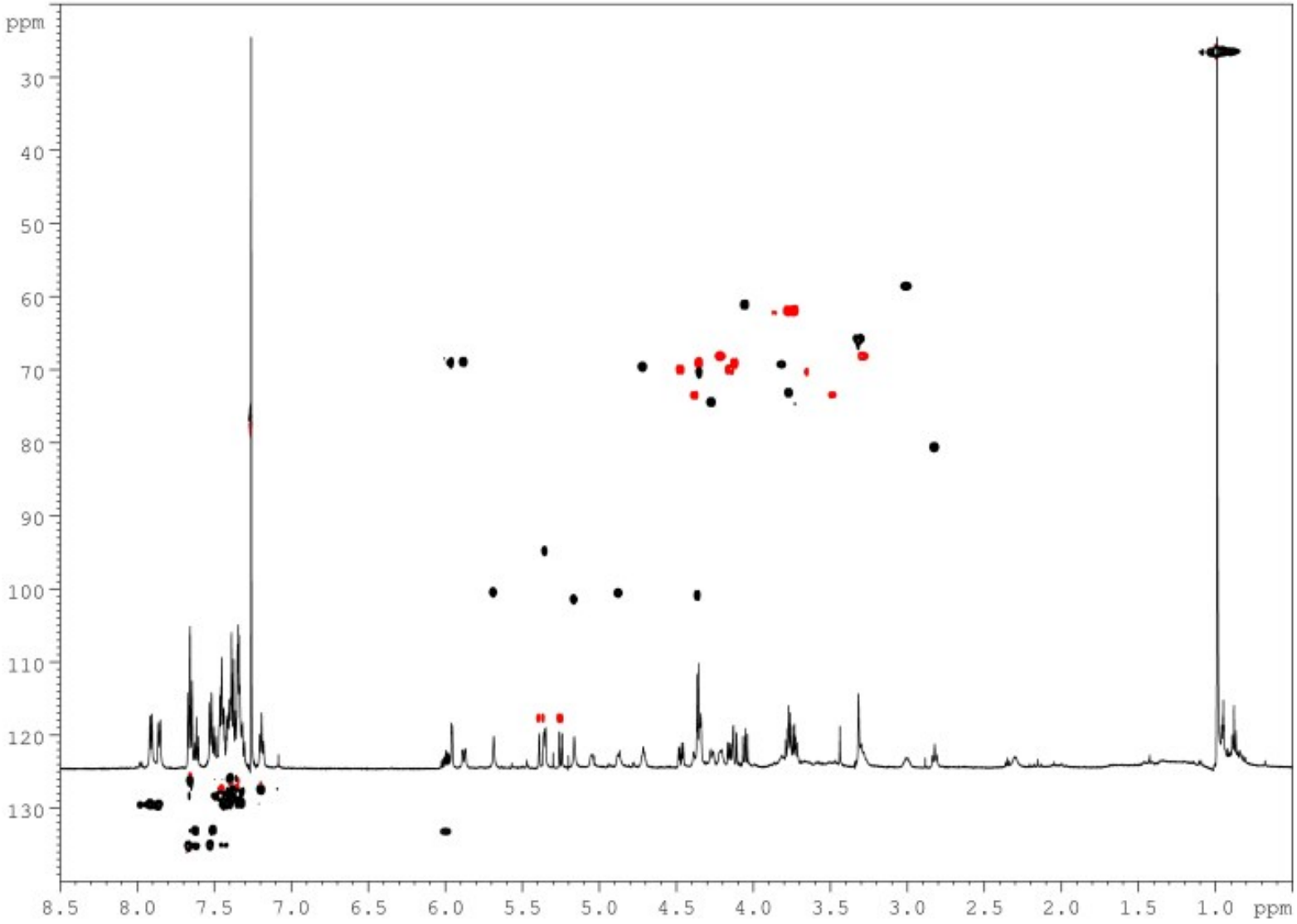

Figure S30: ^{1}H and DEPT-HSQC NMR spectra of **24** (600 MHz, $CDCl_3$, 298K)

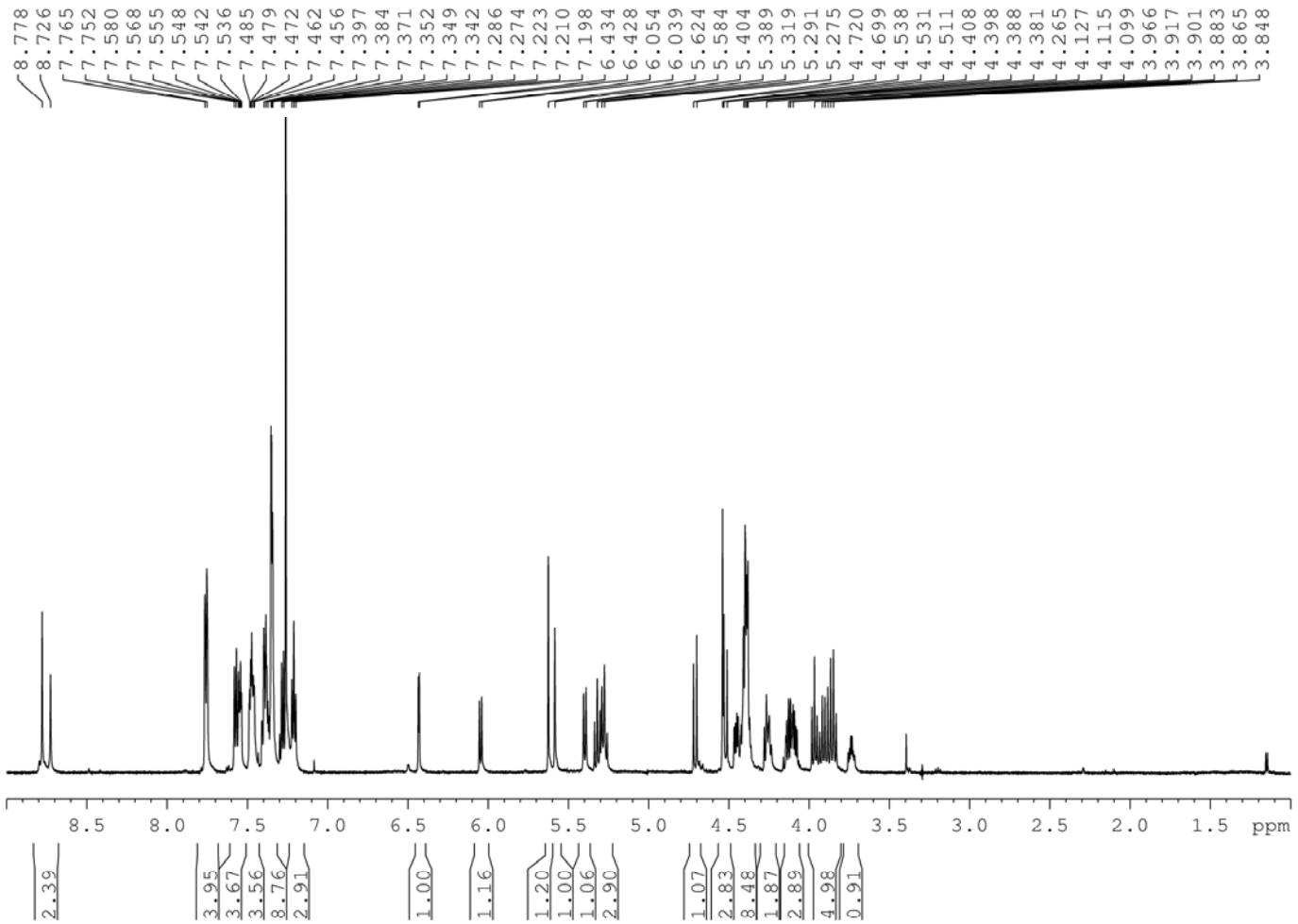

Figure S31: ^{1}H NMR spectrum of **25** (600 MHz, $CDCl_3$, 298K)

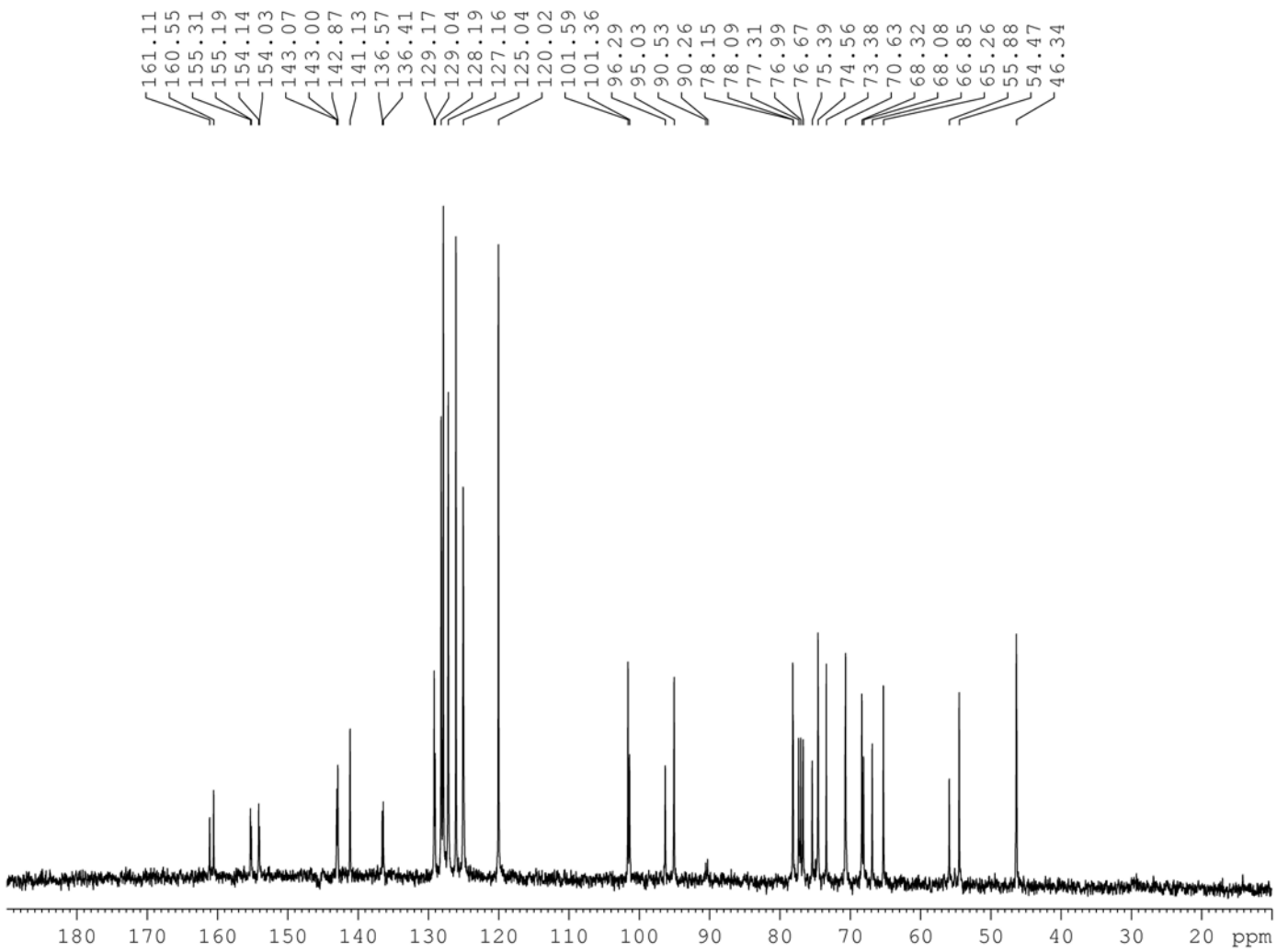

Figure S32: ^{13}C NMR spectrum of **25** (100 MHz, $CDCl_3$, 298K)

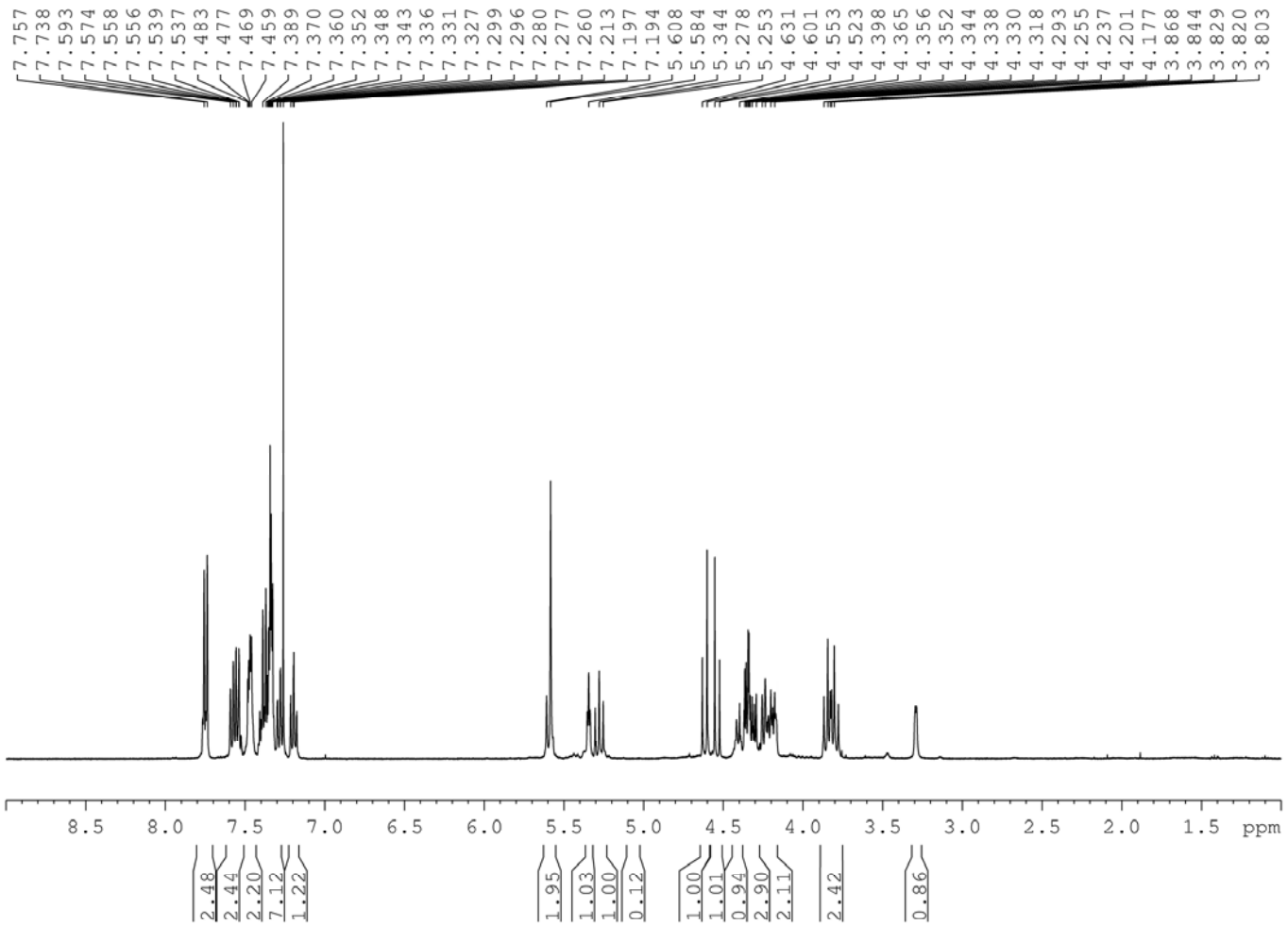

Figure S33: ^{1}H NMR spectrum of **27** (600 MHz, $CDCl_3$, 298K)

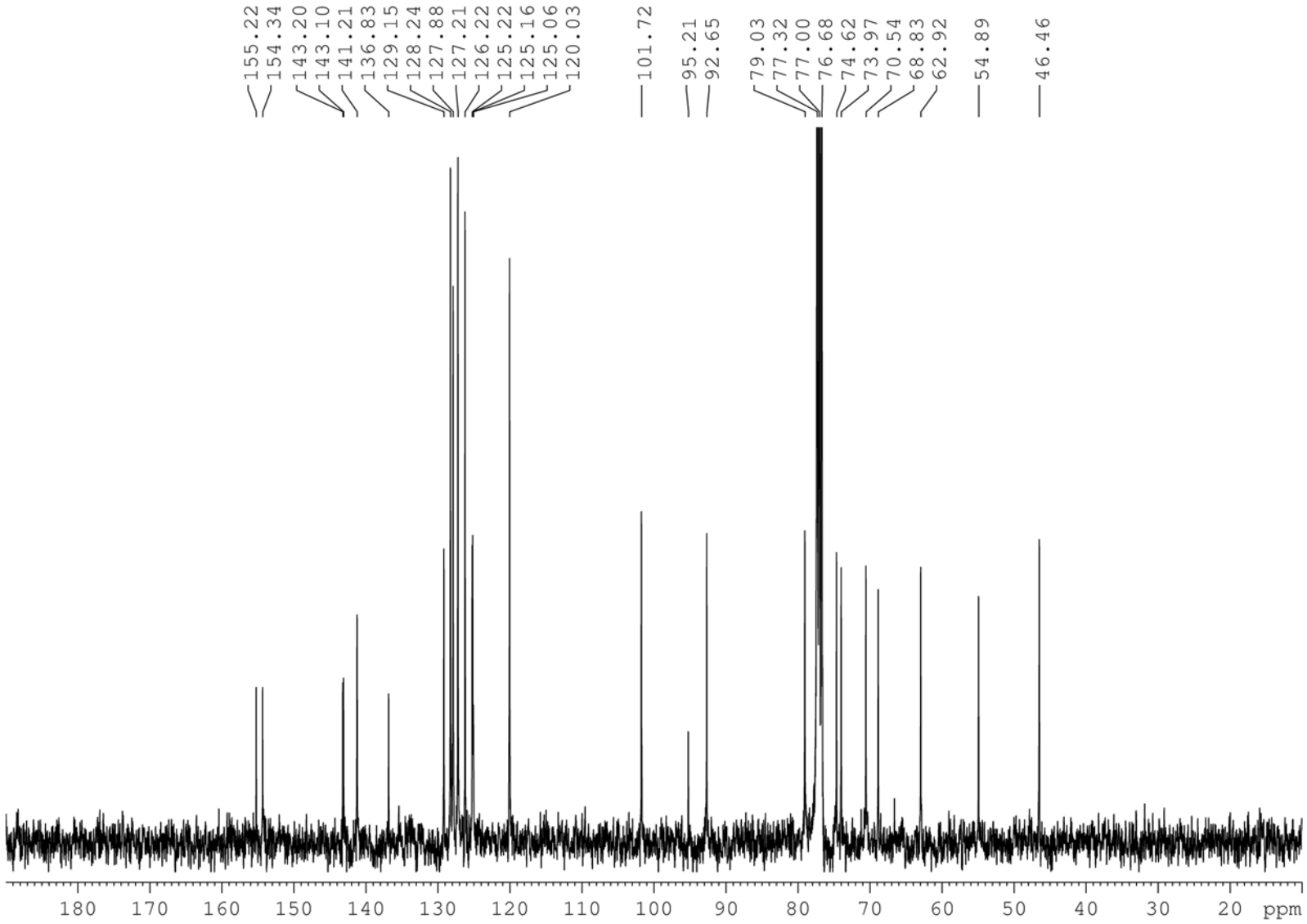

Figure S34: ^{13}C NMR spectrum of **27** (100 MHz, $CDCl_3$, 298K)

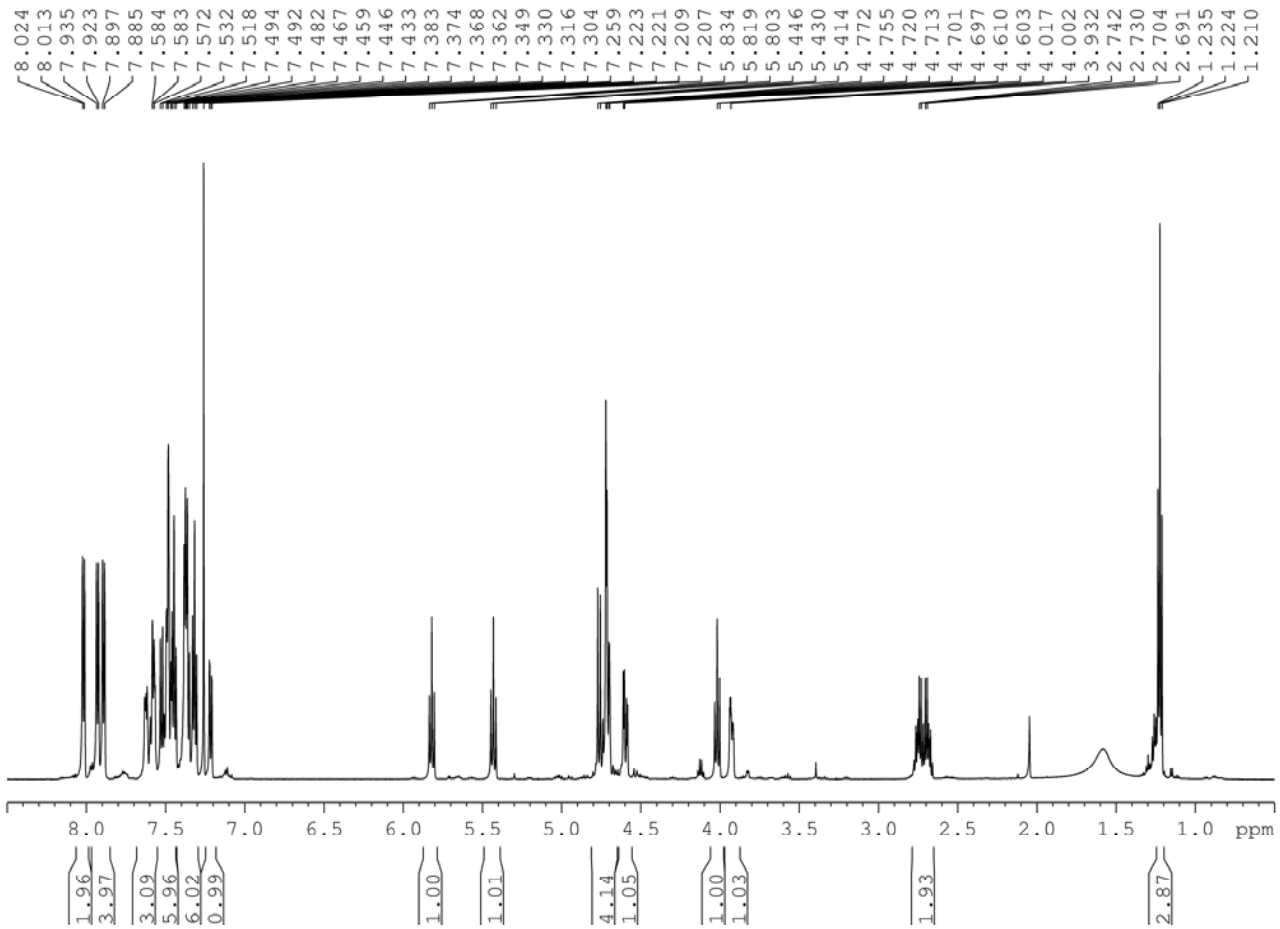

Figure S35: ^{1}H NMR spectrum of **28** (600 MHz, $CDCl_3$, 298K)

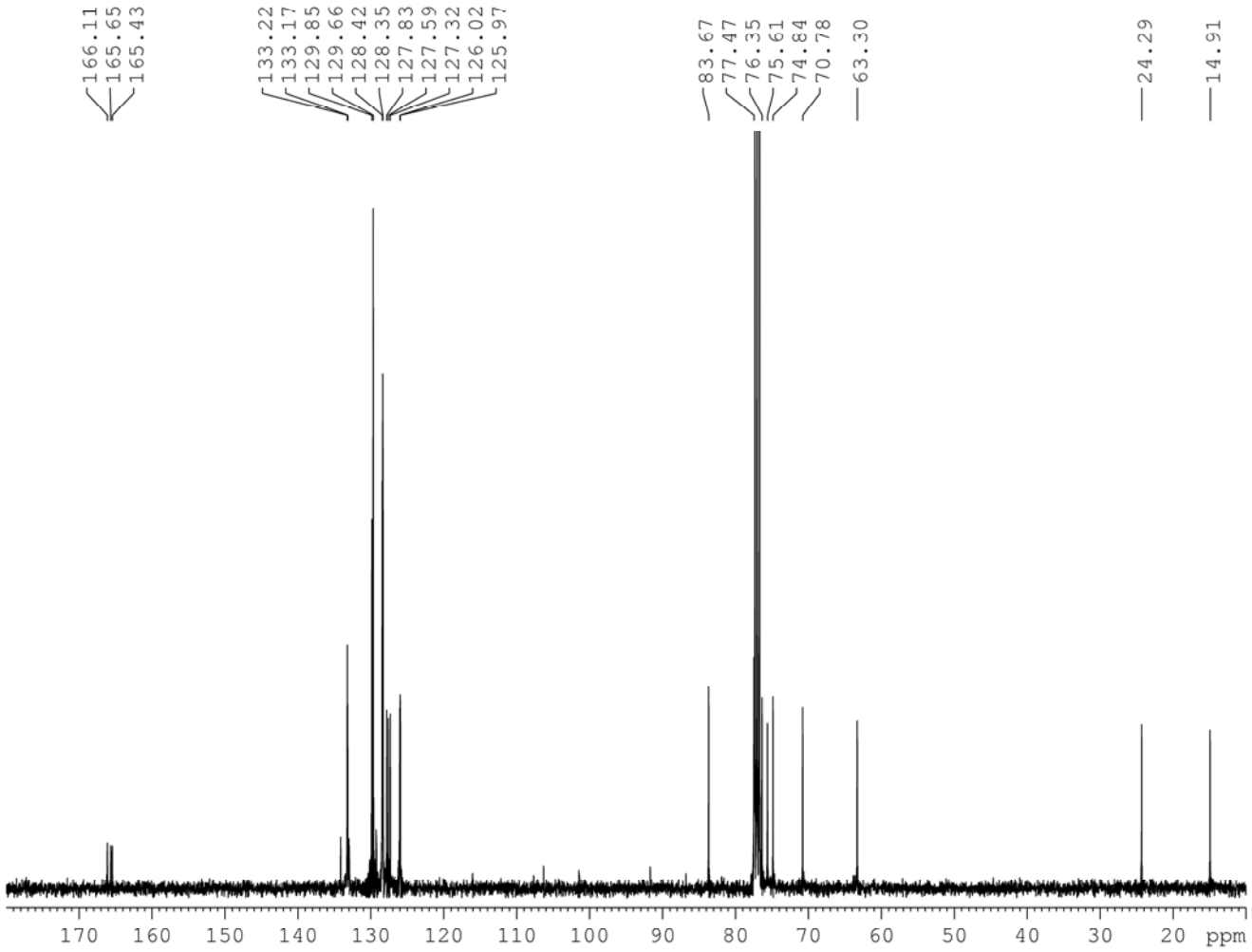

Figure S36: ^{13}C NMR spectrum of **28** (100 MHz, $CDCl_3$, 298K)

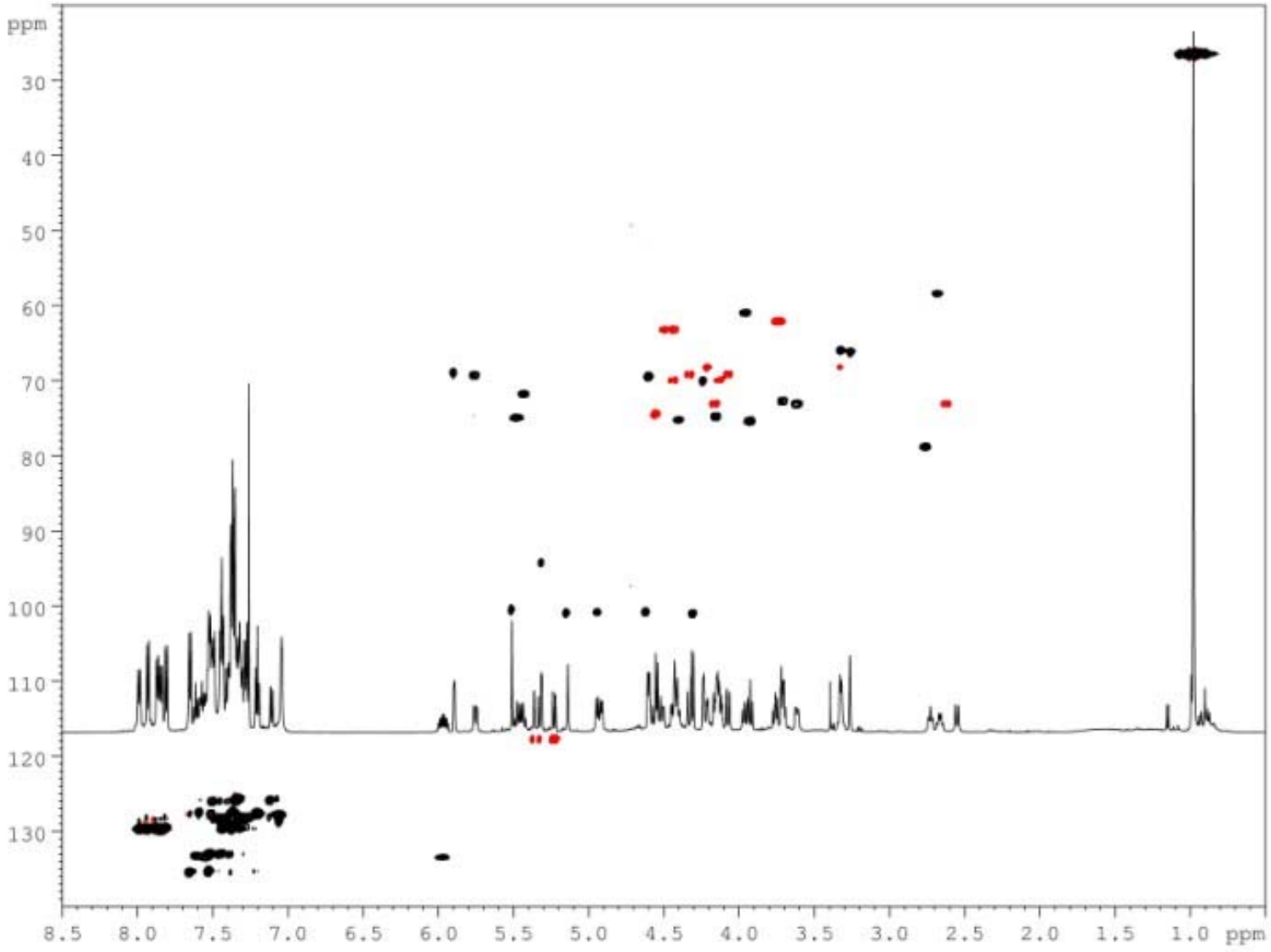

Figure S37: ^{1}H and DEPT-HSQC NMR spectra of **29** (600 MHz, $CDCl_3$, 298K)

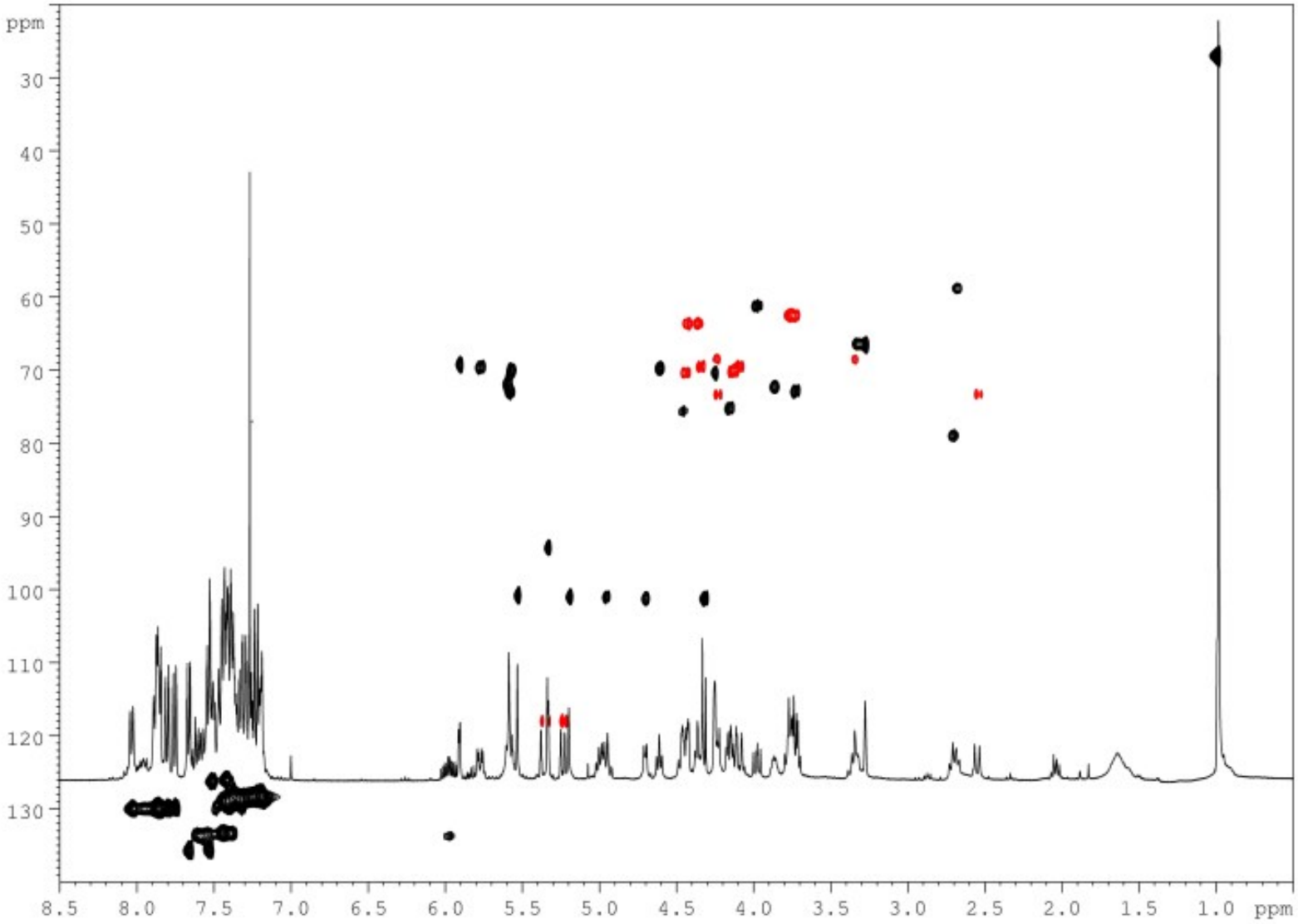

Figure S38: ^{1}H and DEPT-HSQC NMR spectra of **31** (400 MHz, $CDCl_3$, 298K)

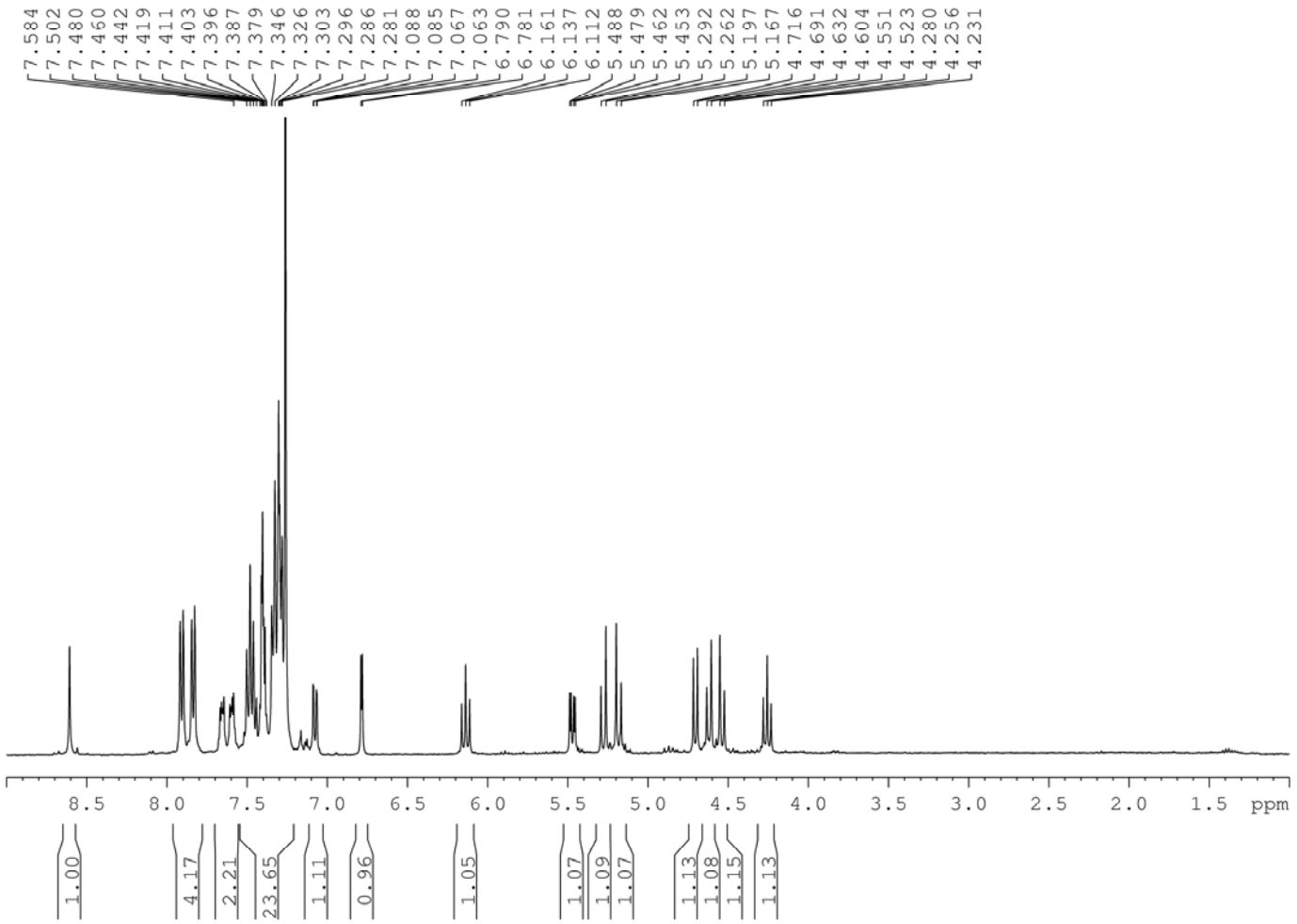

Figure S39: ^{1}H NMR spectrum of **32** (400 MHz, $CDCl_3$, 298K)

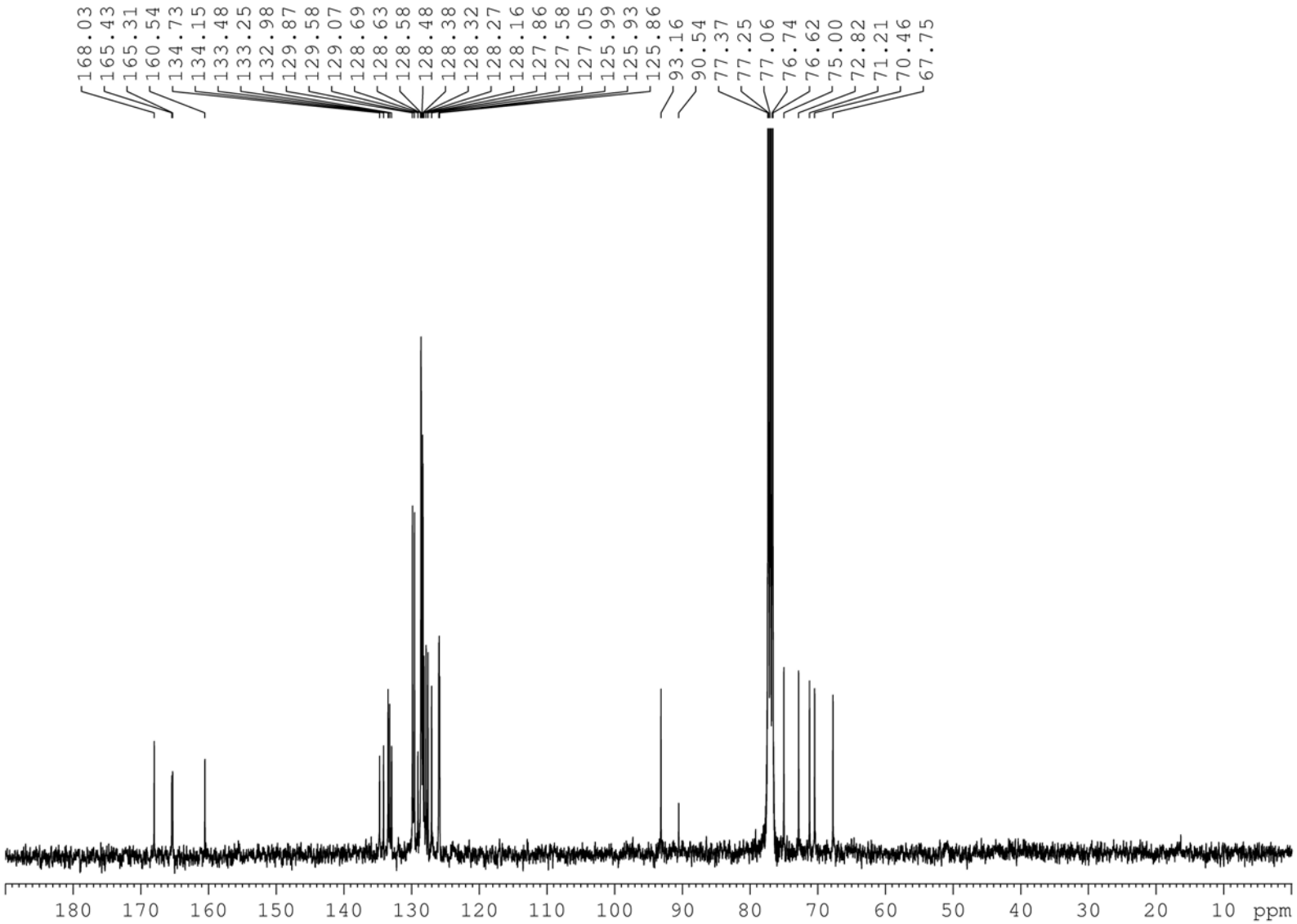

Figure S40: ^{13}C NMR spectrum of **32** (100 MHz, $CDCl_3$, 298K)

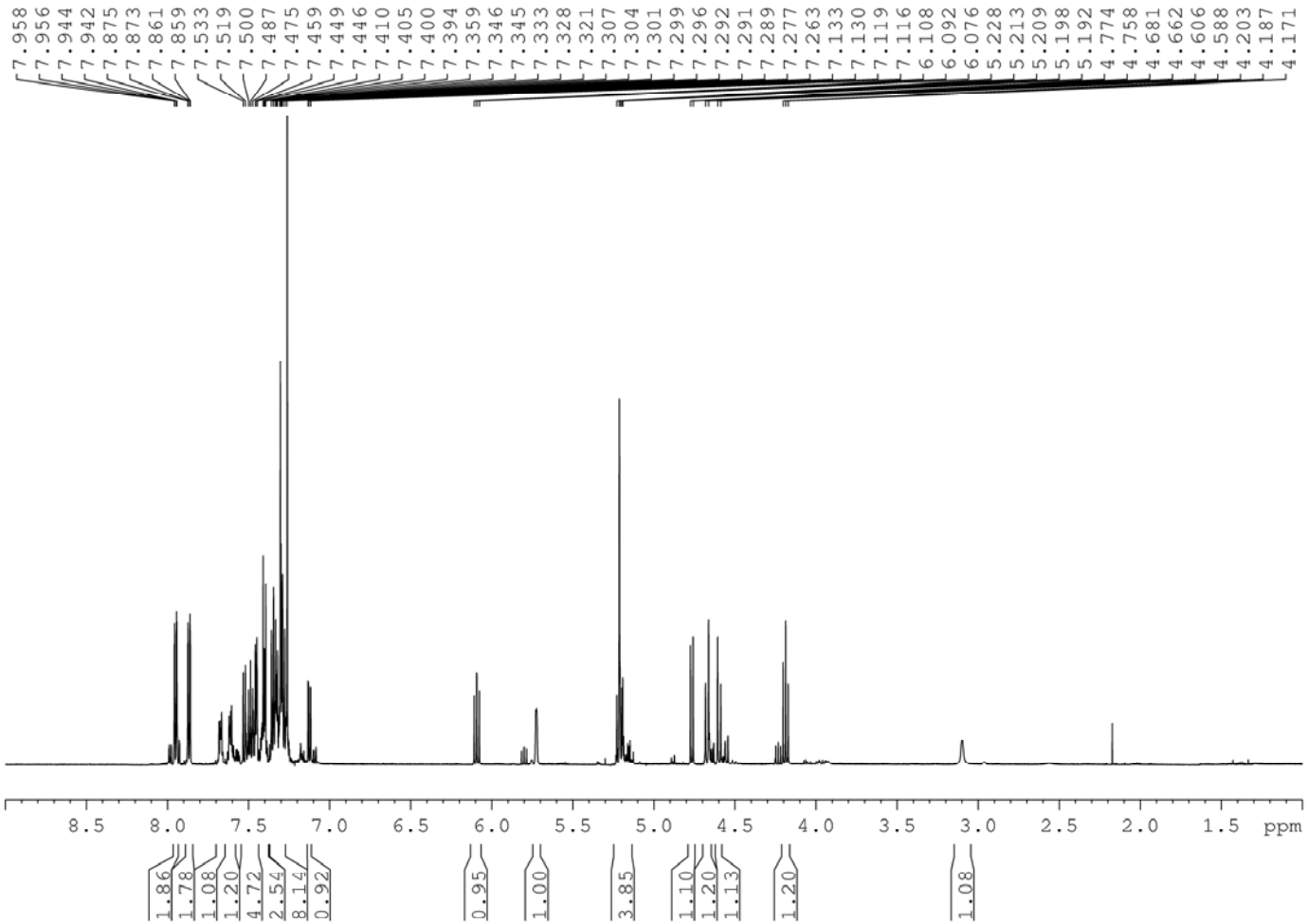

Figure S41: ^{1}H NMR spectrum of **33** (600 MHz, $CDCl_3$, 298K)

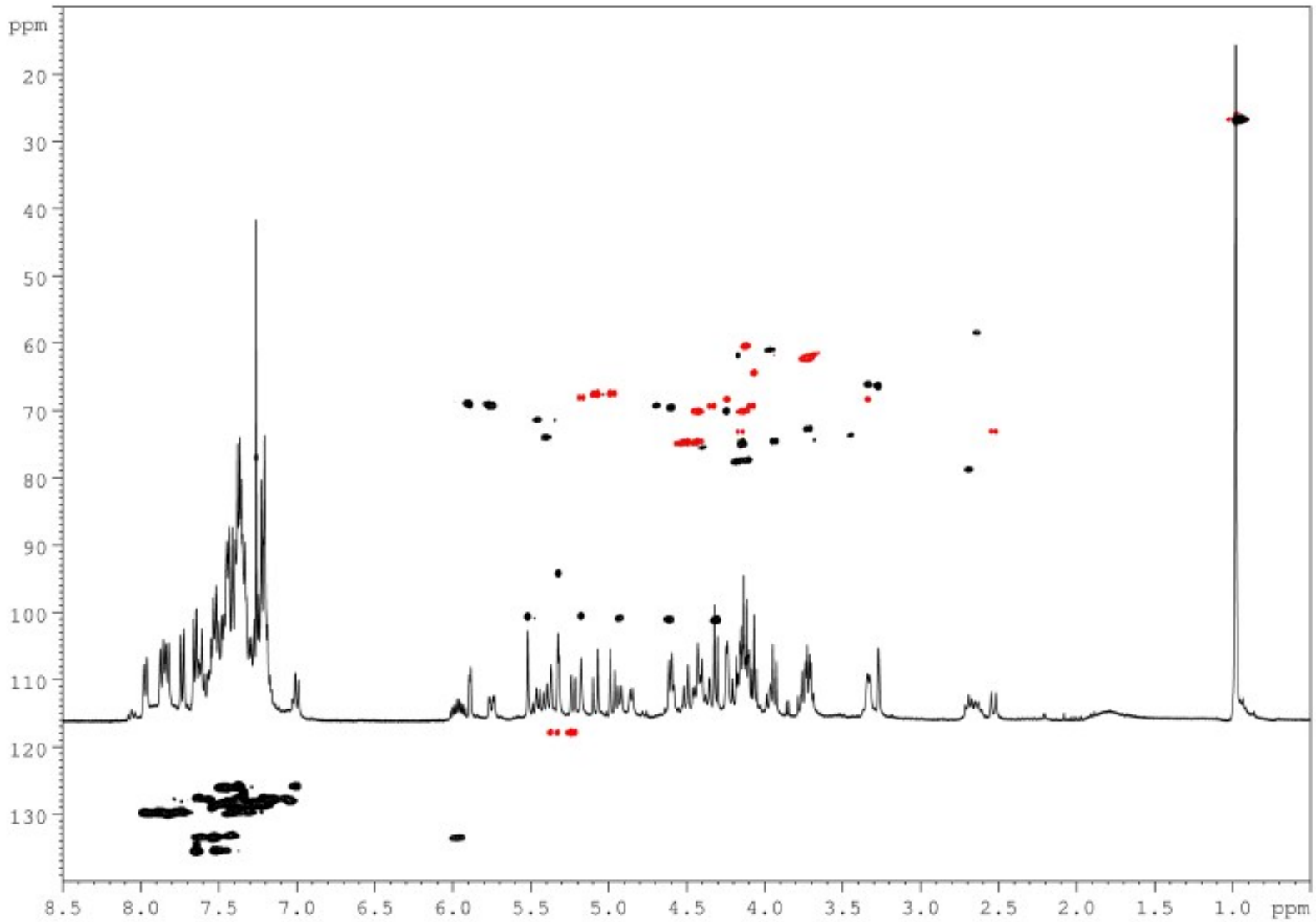

Figure S42: ^{1}H and DEPT-HSQC NMR spectra of **34** (400 MHz, $CDCl_3$, 298K)

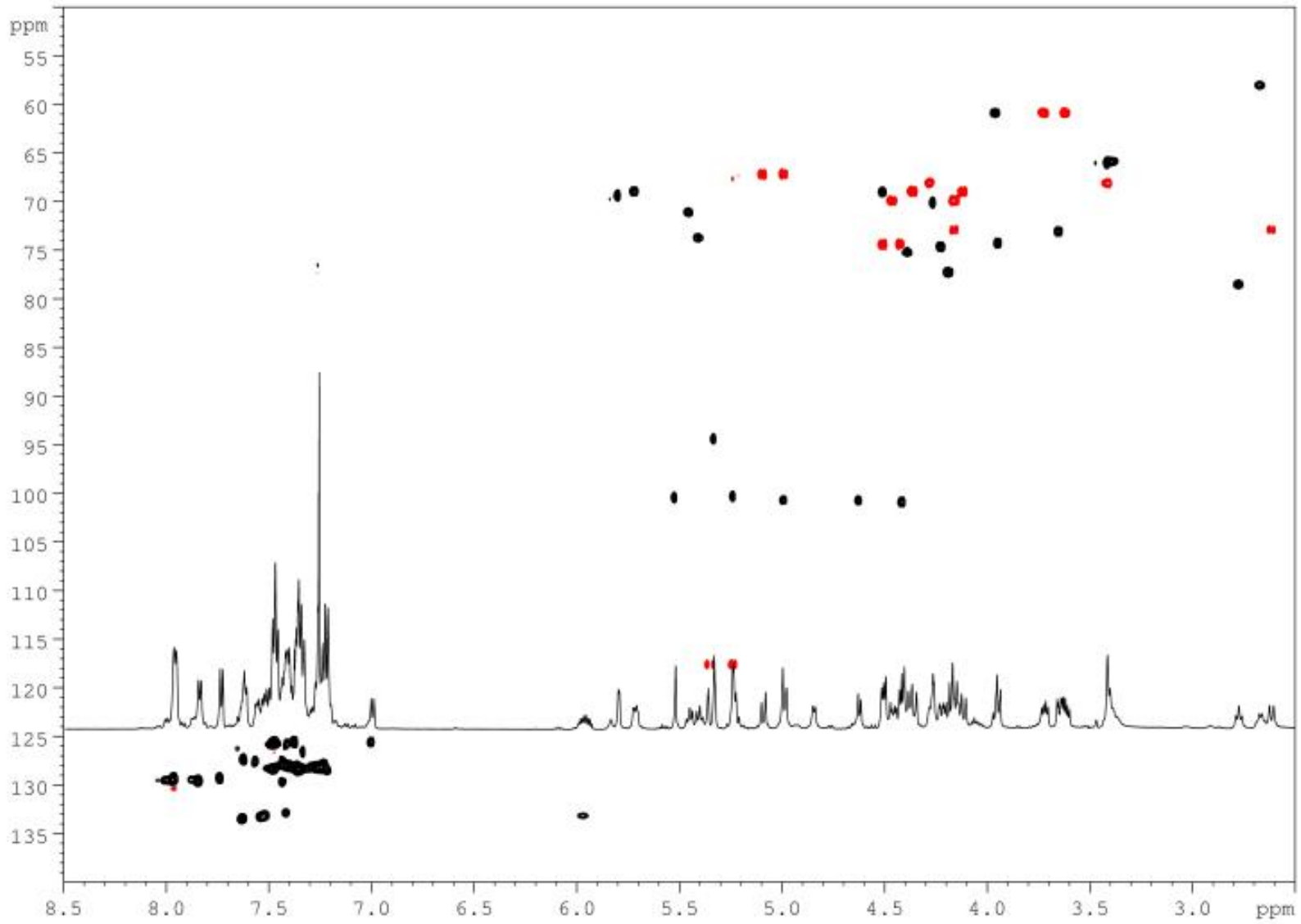

Figure S43: ^{1}H and DEPT-HSQC NMR spectra of **35** (600 MHz, $CDCl_3$, 298K)

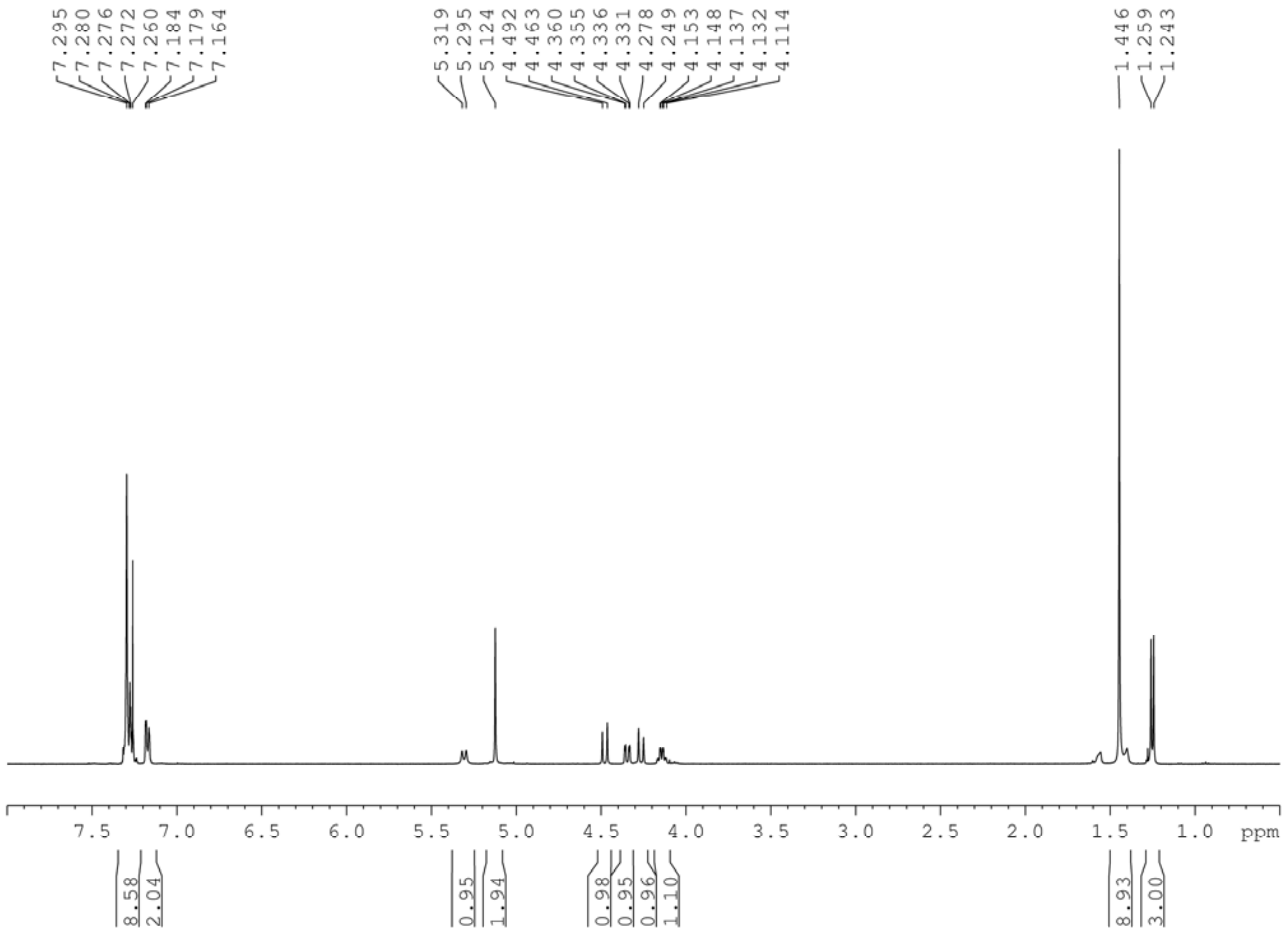

Figure S44: ^{1}H NMR spectrum of **38** (400 MHz, $CDCl_3$, 298K)

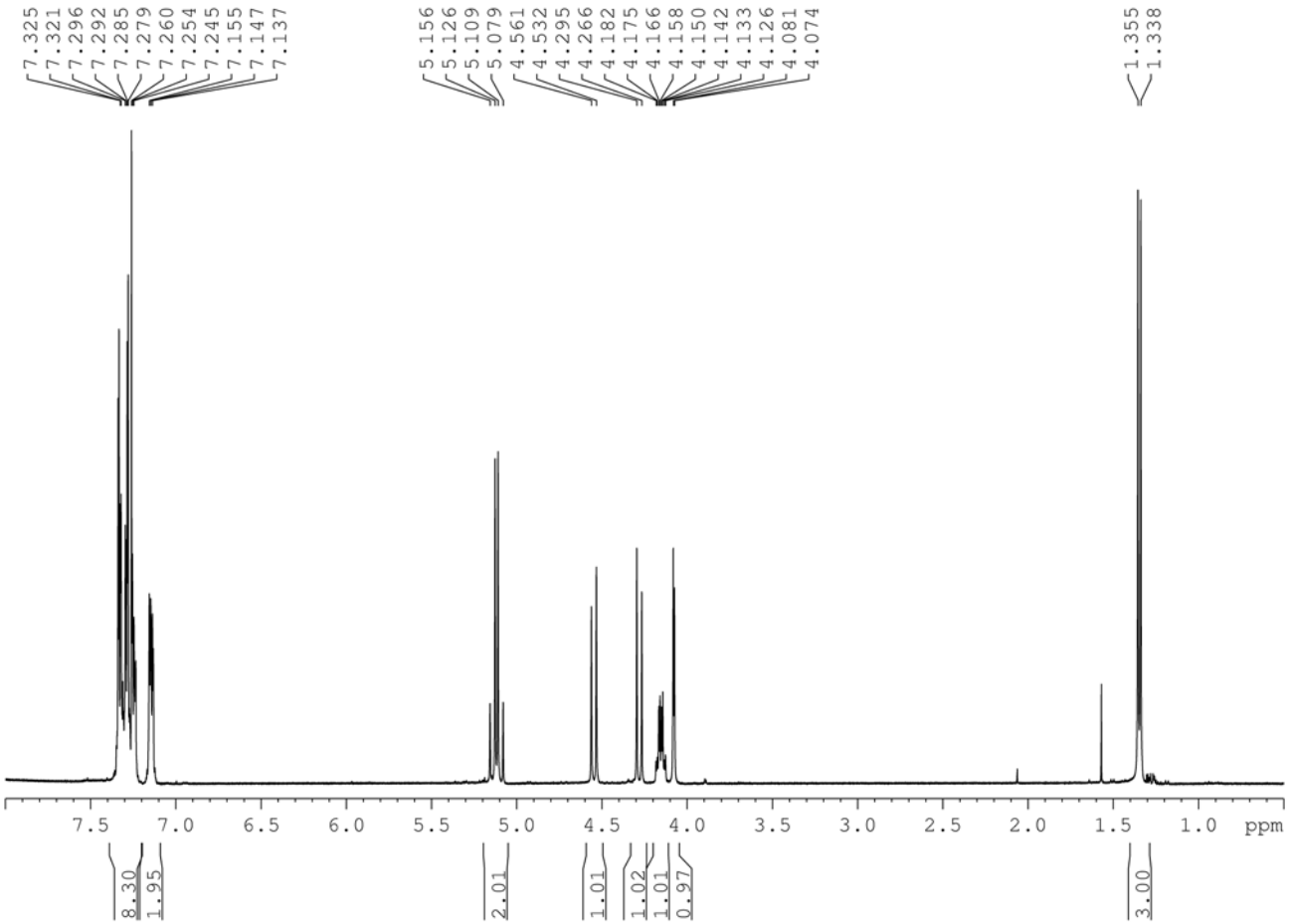

Figure S45: ^{1}H NMR spectrum of **39** (400 MHz, $CDCl_3$, 298K)

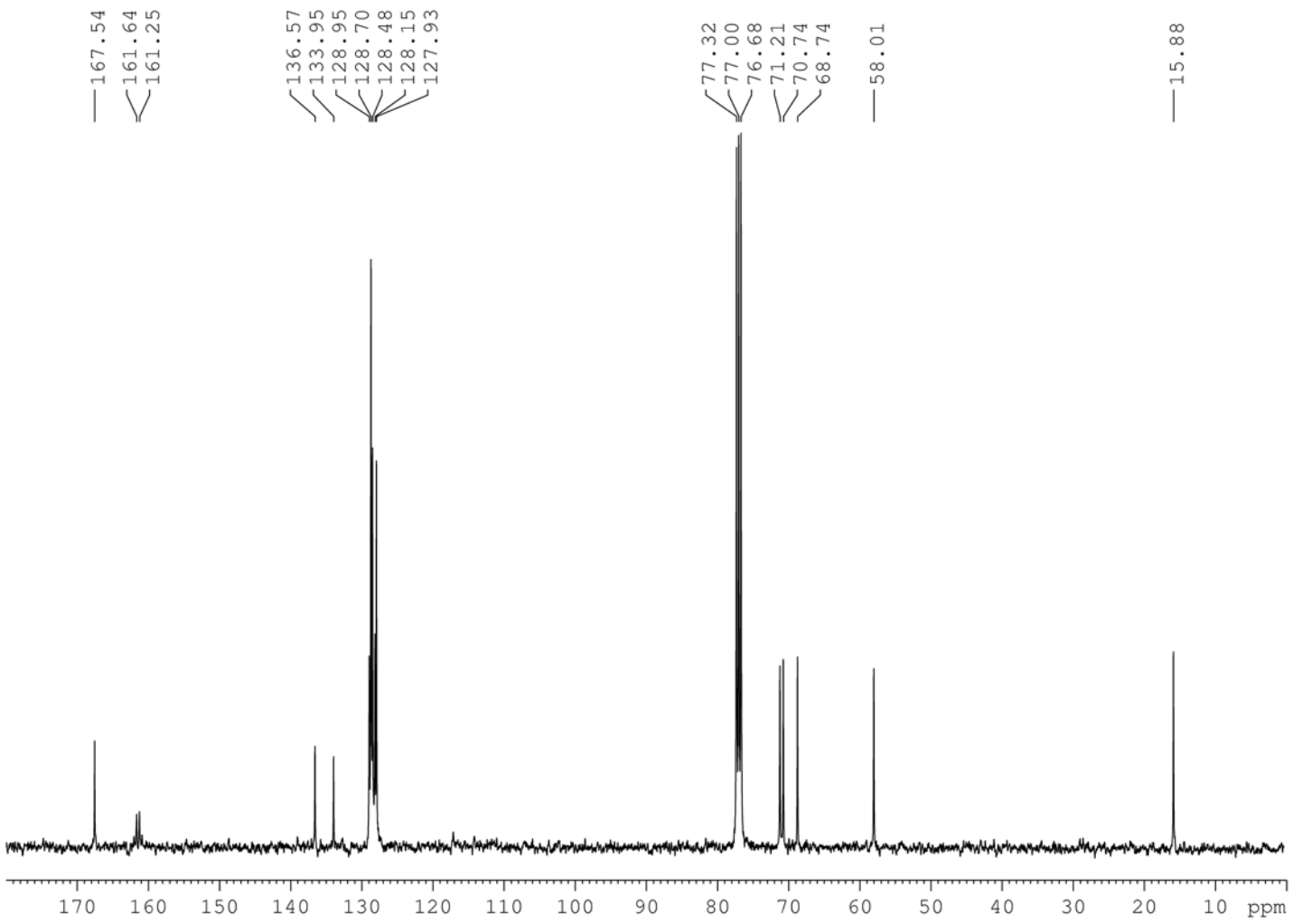

Figure S46: ^{13}C NMR spectrum of **39** (100 MHz, $CDCl_3$, 298K)

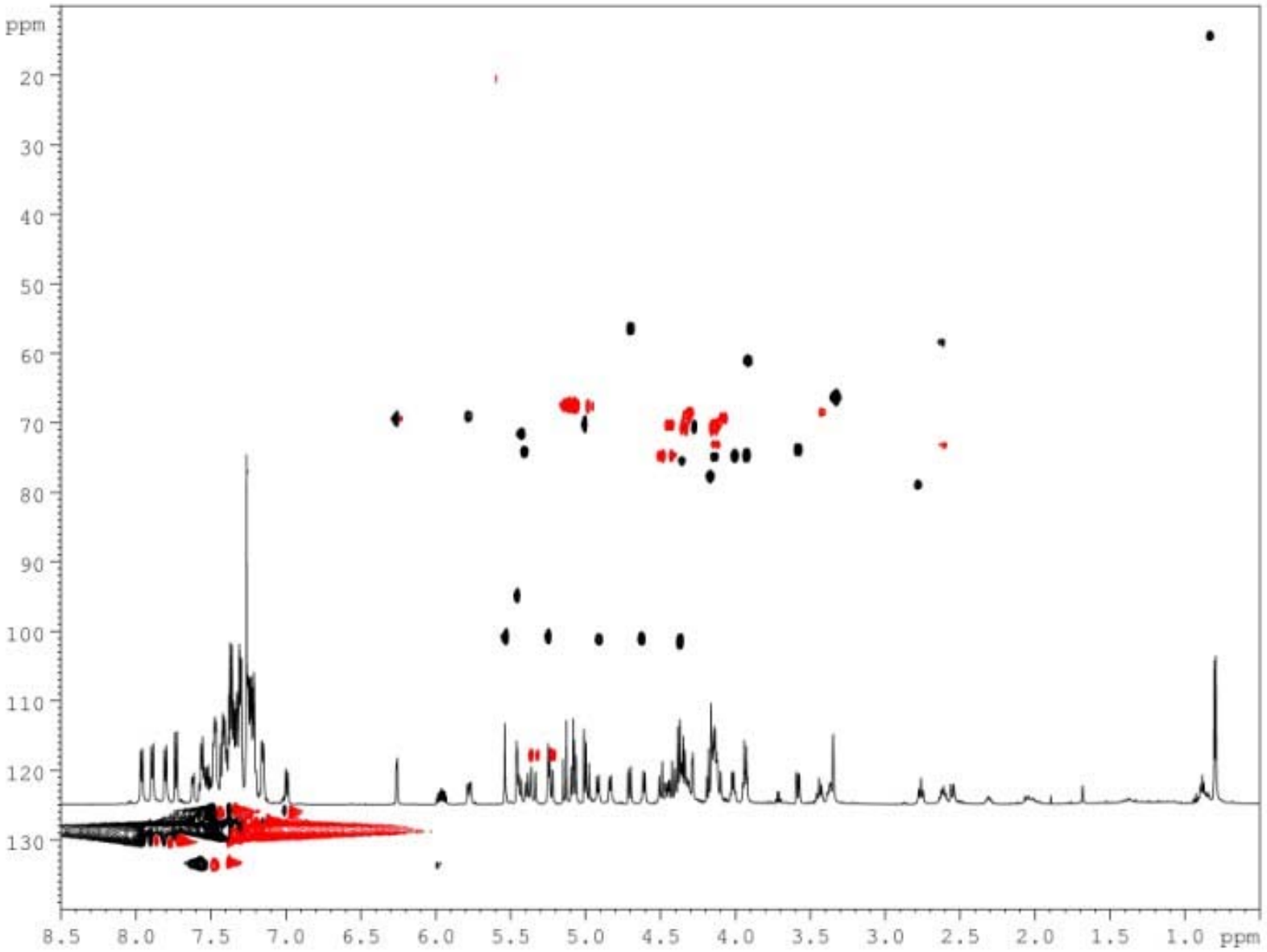

Figure S47: ^{1}H and DEPT-HSQC NMR spectra of **40** (600 MHz, $CDCl_3$, 298K)

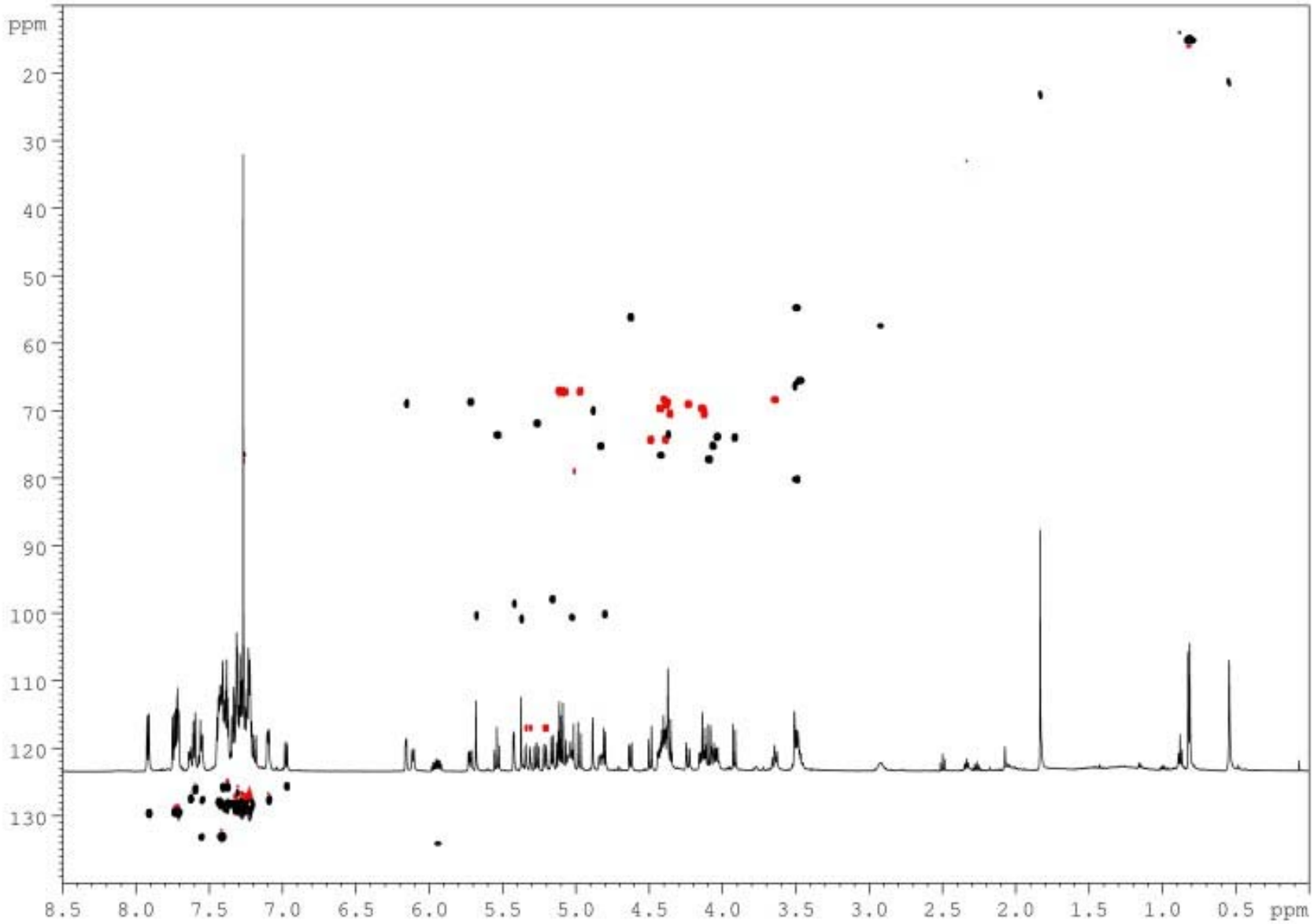

Figure S48: ^{1}H and DEPT-HSQC NMR spectra of **41** (600 MHz, $CDCl_3$, 298K)

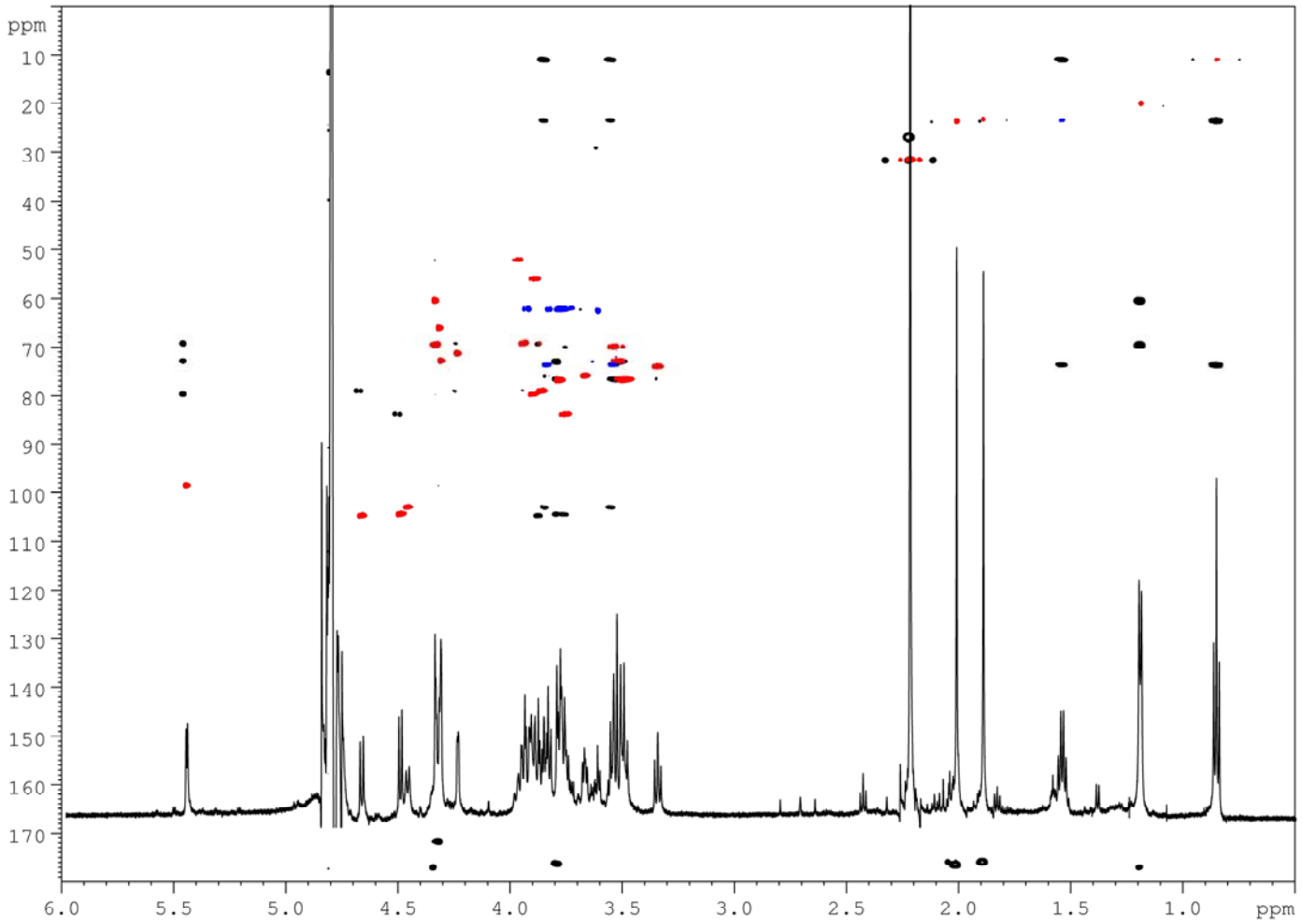

Figure S49: ^{1}H, DEPT-HSQC (red and blue) and HMBC (black) NMR spectra of tetrasaccharide **1** (600 MHz, D_2O, 298K)

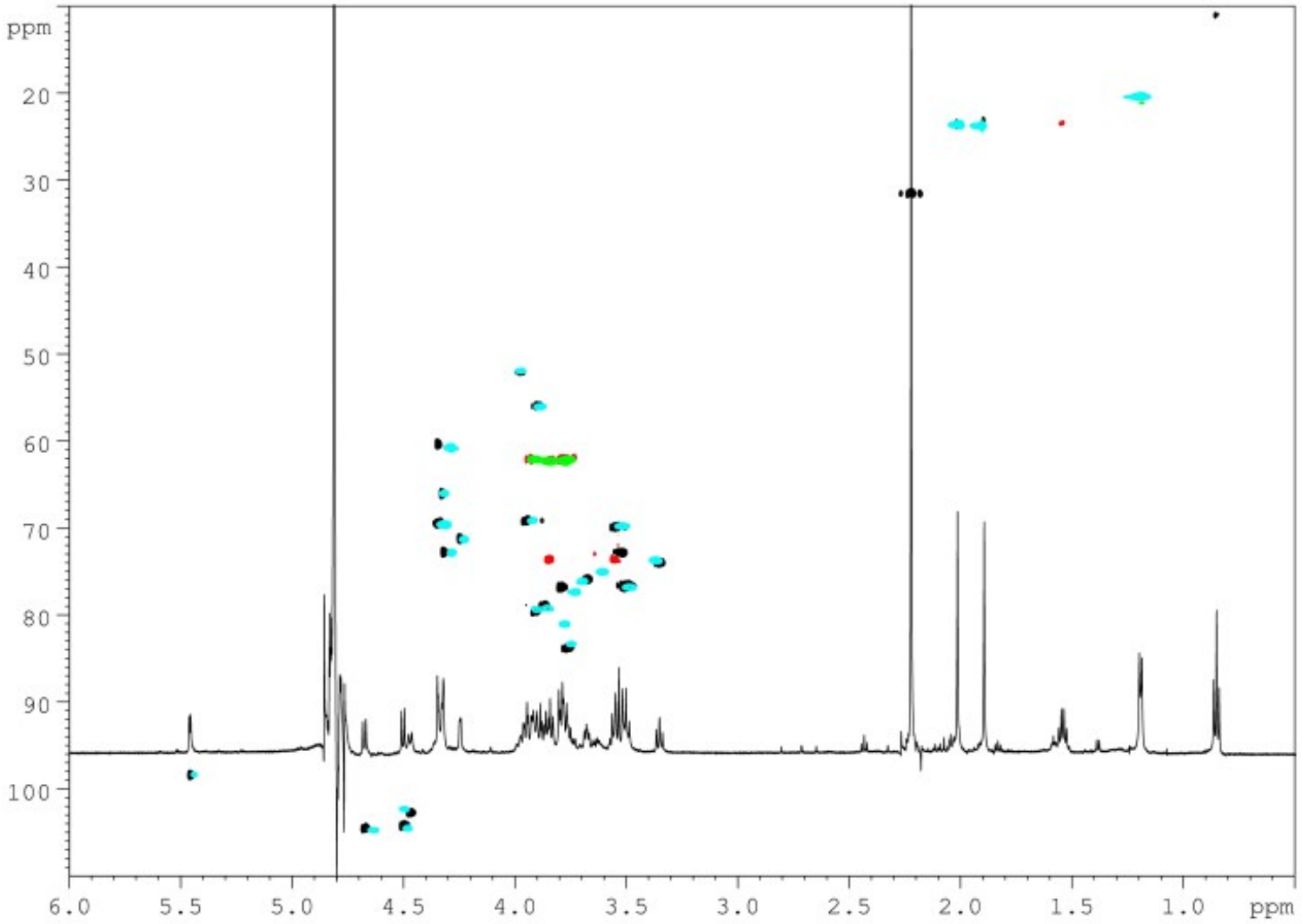

Figure S50: DEPT-HSQC NMR spectrum of CPS from *C. psychrerythraea* 34H (600 MHz, D_2O, 298K, in green and light blue) superimposed on ^1H and DEPT-HSQC NMR spectra of tetrasaccharide **1** (600 MHz, D_2O, 298K, in black and red)

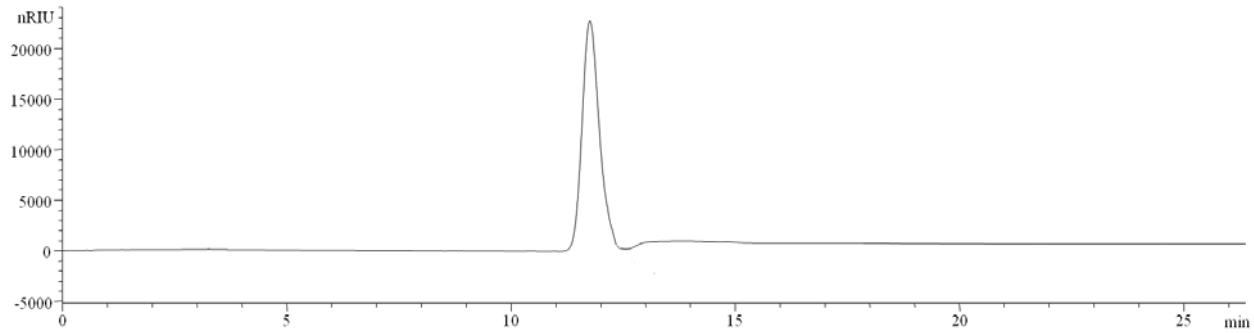

Figure S51: HPLC trace of tetrasaccharide **1**

(HPLC instrument: Agilent 1200 Series System; size exclusion column: Tosoh Bioscience, TSKgel G3000PWXL, 7.8 x 300 mm; detection: refractive index; elution: 50 mM aq. NH_4HCO_3 at a flow rate of 0.8 mL/min; sample: 100 µL of a 1 mg/mL solution).

References:

S1 A. Marinier, A. Martel, C. Bachand, S. Plamondon, B. Turmel, J.-P. Daris, J. Banville, P. Lapointe, C. Ouellet, P. Dextraze, M. Menard, J. K. Wright, J. Alford, D. Lee, P. Stanley, X. Nair, G. Todderud and K. M. Tramposch, *Bioorg. Med. Chem.* 2001, **9**, 1395.

S2 G. Schüle and T. Ziegler, *Liebigs Ann.* 1996, 1599.

S3 S. Traboni, E. Bedini, M. Giordano and A. Iadonisi, *Adv. Synth. Cat.* 2015, **357**, 3562.

S4 E. Bedini, L. Cirillo, M. Parrilli, *Carbohydr. Res.* 2012, **349**, 24.

S5 J. D. Lewicky, M. Ulanova and Z.-H. Jiang, *Carbohydr. Res.* 2011, **346**, 1705.