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Supporting Information

Bridged Tetrahydroisoquinolines as selective NADPH oxidase 2 (Nox2) inhibitors

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EXPERIMENTAL METHODS

General Chemistry Methods. All moisture sensitive reactions were performed using syringe-septum techniques under an atmosphere of either dry N_2 or dry argon unless otherwise noted. All glassware used in moisture sensitive reactions was flame-dried or oven dried at 136 ºC overnight and cooled in a desiccator prior to use. All chemicals were purchased from commercial suppliers and unless otherwise noted were used as received. All the microwave reactions were performed using a Biotage® Initiator microwave reactor. Tetrahydrofuran (THF) was dried by distillation over sodium/benzophenone under an argon atmosphere. Dry dichloromethane (CH_2Cl_2), dichloroethane ($C_2H_4Cl_2$), and ethyl acetate (EtOAc) were obtained by distillation over calcium hydride under an argon atmosphere. Deuterated chloroform ($CDCl_3$) was filtered through an alumina plug prior to use. Reactions were monitored by TLC analysis (pre-coated silica gel 60 F_{254} plates, 250 μm layer thickness) and visualized by using UV lamp (254 nm) and/or a potassium permanganate solution (3 g of $KMnO_4$ and 4 g of K_2CO_3 in 4 mL of 5% NaOH solution and 200 mL of H_2O). Flash column chromatography was performed with 40-63 μm silica gel (SiO_2, Silicycle). Infrared spectra were recorded on a Smiths IdentifyIR ATR spectrometer or a Perkin Elmer Spectrum 100 FT-IR spectrometer using the Universal ATR Sampling Accessory. All NMR data was collected at room temperature in $CDCl_3$ on a 300, 400 or 500 MHz Bruker instrument. Chemical shifts (δ) are reported in parts per million (ppm) with internal $CHCl_3$ (δ 7.26 ppm for ^1H and 77.23 ppm for ^{13}C). ^1H NMR data are reported as follows: chemical shift, multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bm = broad multiplet, dd = doublet of doublets, dt = doublet of triplets, td = triplet of doublets, qd = quartet of doublets, sep = septet, app t = apparent triplet), integration, and coupling constant(s) (*J*) in Hertz (Hz). ^{13}C NMR spectra were measured using a proton-decoupled pulse sequence. Final products were >95 % purity as analyzed by reverse-phase HPLC (ACCELA PDA detector, 1250 pump, Waters XTerra® MS 3.5 μm C-18 50 x 2 mm column, 0.5 mL/ min, CH_3CN, H_2O and 0.1 % formic acid) with UV (210, 220 and 254 nm), ELSD (Agilent Technologies 385-ELSD, nebulizer 45 ^oC, evaporator 45 ^oC, N_2 flow 1.80 SLM), and MS detection using a Thermo Scientific Exactive Orbitrap LC-MS (HESI positive).

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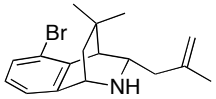
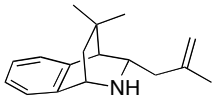


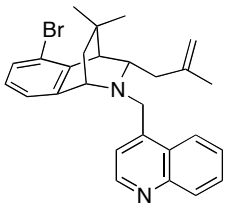
(±)-(1*S*,4*R*,9*S*)-5-Bromo-3,3-dimethyl-9-(2-methylallyl)-1,2,3,4-tetrahydro-1,4-(epimino-methano)-naphthalene (7). To a stirred solution of 5-bromoisoquinoline (0.1 g, 0.5 mmol) in anhydrous THF (0.2 mL) was added methallylzinc bromide in THF (2 mL, 1.0 M) dropwise at 0 ^{o}C and the resulting solution was stirred for 10 min. The reaction mixture was warmed to room temperature, heated to 70 ^{o}C for 4.5 h, cooled to room temperature and further cooled to -10 ^{o}C. The reaction was quenched by dropwise addition of MeOH (0.1 mL). The reaction mixture was poured onto ice water (20 mL) and extracted with Et_2O (2 x 15 mL). The combined organic portion was dried (Na_2SO_4), concentrated, and the crude product was purified by chromatography on neutral Al_2O_3 (1:1, hexanes:EtOAc followed by 7:93, CH_3OH:CH_2Cl_2) to give **7** (105 mg, 68%, 97% purity by ELSD) as a colorless oil: IR (neat) 3073, 2934, 1647, 1564, 1451, 1369, 1330, 1310, 1273, 1177, 1142, 1117, 1073, 965, 891, 822, 767, 742, 702, 688 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 (dd, *J* = 1.3, 7.8 Hz, 1 H), 7.10-7.00 (m, 2 H), 4.73 (s, 1 H), 4.55 (s, 1 H), 3.85 (t, *J* = 2.9 Hz, 1 H), 3.74 (ddd, *J* = 1.6, 5.2, 8.7 Hz, 1 H), 2.94 (s, 1 H), 1.86-1.79 (m, 2 H), 1.71 (s, 3 H), 1.34-1.18 (m, 5 H), 0.62 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 144.4, 142.2, 139.3, 129.9, 127.7, 123.7, 120.9, 113.1, 52.7, 49.7, 48.8, 42.5, 32.4, 30.9, 28.8, 22.4; HRMS (ESI) *m/z* calcd for $C_{17}H_{23}BrN$ $(M+H)^+$ 320.1008, found 320.1001.



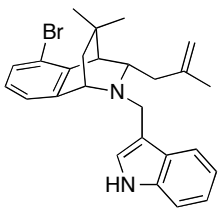
(±)-(1*S*,4*R*,9*S*)-3,3-Dimethyl-9-(2-methylallyl)-1,2,3,4-tetrahydro-1,4-(epiminomethano) -naphthalene (8). Isoquinoline (0.1 g, 0.8 mmol) was charged with a solution of methallylzinc bromide (2.50 mL, 1.12 M in THF, 2.47 mmol) at 0 ^{o}C and stirred for 10 min. After warming the reaction mixture to room temperature, it was heated to 70 ^{o}C for 1 h, then cooled to room temperature and further cooled to -10 ^{o}C. The reaction was quenched by dropwise addition of MeOH (0.2 mL). To this solution was added 6 N NaOH (20 mL) and the organic layer was separated, dried (Na_2SO_4), and purified by chromatography on neutral Al_2O_3 (1:1, hexanes:EtOAc

followed by 7:93, CH_3OH:CH_2Cl_2) to give **8** (50 mg, 27%) as a colorless oil. The ^{1}H NMR matched that of the previously reported compound.[1]

General Method A: Reductive amination.



(±)-(1*S*,4*R*,9*S*)-5-Bromo-3,3-dimethyl-9-(2-methylallyl)-10-(quinolin-4-ylmethyl)-1,2,3,4-tetra-hydro-1,4-(epiminomethano)naphthalene (11b). To a stirred solution of **7** (0.04 g, 0.1 mmol), 4-quinoline carboxaldehyde (22 mg, 0.14 mmol) and acetic acid (7.0 mL, 0.12 mmol) in dichloroethane (1.2 mL) was added sodium triacetoxyborohydride (53 mg, 0.25 mmol). The reaction mixture was stirred for 48 h under argon, diluted with CH_2Cl_2 (10 mL), and treated with a saturated solution of $NaHCO_3$ (10 mL). The organic layer was washed with H_2O (10 mL) and the combined organic layer was dried (Na_2SO_4) and concentrated. The crude product was purified by chromatography on SiO_2 (95:5, hexanes:EtOAc) to give **11b** (0.04 g, 69%, 99% purity by ELSD) as a colorless oil: IR (neat) 3068, 2958, 1590, 1567, 1506, 1452, 1357, 1189, 908, 760, 731 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.85 (d, *J* = 4.3 Hz, 1 H), 8.21 (dd, *J* = 1.1, 8.5 Hz, 1 H), 8.12 (dd, *J* = 0.9, 8.5 Hz, 1 H), 7.69 (ddd, *J* = 1.4, 6.9, 8.3 Hz, 1 H), 7.52-7.48 (m, 2 H), 7.43 (dd, *J* = 0.9, 8.1 Hz, 1 H), 7.04 (app t, *J* = 7.6 Hz, 1 H), 6.88 (d, *J* = 7.2 Hz, 1 H), 4.78 (d, *J* = 4.8 Hz, 1 H), 4.46 (d, *J* = 14.9 Hz, 1 H), 4.23 (d, *J* = 15.0 Hz, 1 H), 3.54 (t, *J* = 2.5 Hz, 1 H), 3.38 (ddd, *J* = 1.7, 4.4, 9.0 Hz, 1 H), 3.05 (d, *J* = 1.5 Hz, 1 H), 2.12 (dd, *J* = 2.6, 13.7 Hz, 1 H), 1.79 (dd, *J* = 4.3, 14.3 Hz, 1 H), 1.82-1.67 (m, 1 H), 1.73 (s, 3 H), 1.41 (s, 3 H), 1.00 (dd, *J* = 2.5, 13.6 Hz, 1 H), 0.6 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.6, 148.4, 145.9, 144.1, 142.5, 139.9, 130.3, 130.2, 129.3, 127.7, 127.6, 126.5, 124.0, 123.3, 121.3, 121.0, 113.2, 57.5, 55.8, 53.3, 49.4, 44.4, 35.2, 33.0, 31.0, 28.5, 22.9; HRMS (ESI) *m/z* calcd for $C_{27}H_{30}O_2BrN_2$ $(M+H)^+$ 461.1587, found 461.1581.



[1] Y. N. Bubnov, F. V. Pastukhov, Z. A. Starikova and A. V. Ignatenko, *Russ. Chem, Bull.*, 2001, **50**, 2172-

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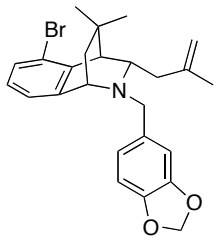
(±)-(1*S*,4*R*,9*S*)-10-((1*H*-Indol-3-yl)methyl)-5-bromo-3,3-dimethyl-9-(2-methylallyl)-1,2,3,4-tetrahydro-1,4-(epiminomethano)naphthalene (11c). According to General Method A, **7** (0.04 g, 0.1 mmol) was converted to pale yellow oily **11c** (15 mg, 27%, 98% purity by ELSD): IR (neat) 3411, 2926, 1727, 1567, 1455, 1353, 1264, 1189, 1091, 1044, 1010, 930, 893, 807, 772, 734 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.04 (s, 1 H), 7.71 (d, *J* = 8.0 Hz, 1 H), 7.37 (dd, *J* = 8.2, 12.8 Hz, 2 H), 7.21–7.15 (m, 2 H), 7.07 (t, *J* = 7.5 Hz, 1 H), 6.99 (t, *J* = 7.7 Hz, 1 H), 6.83 (d, *J* = 7.0 Hz, 1 H), 4.81 (d, *J* = 8.1 Hz, 2 H), 4.09 (s, 2 H), 3.75 (t, *J* = 2.7 Hz, 1 H), 3.26 (dd, *J* = 3.5, 9.9 Hz, 1 H), 3.0 (s, 1 H), 2.15 (dd, *J* = 2.4, 13.6 Hz, 1 H), 1.89 (dd, *J* = 3.5, 14.4 Hz, 1 H), 1.75-1.65 (m, 1 H), 1.69 (s, 3 H), 1.37 (s, 3 H), 0.92 (dd, *J* = 2.3, 13.6 Hz, 1 H), 0.57 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.8, 143.1, 140.3, 136.6, 130.0, 127.8, 127.5, 123.4, 123.2, 122.3, 121.6, 120.2, 119.6, 114.6, 113.0, 111.1, 57.1, 55.1, 49.3, 47.5, 44.5, 34.7, 33.0, 31.1, 29.9, 28.4, 22.9; HRMS (ESI) *m/z* calcd for $C_{26}H_{30}BrN_2$ $(M+H)^+$ 449.1587, found 449.1575.



(±)-(1*S*,4*R*,9*S*)-10-(Benzo[*d*][1,3]dioxol-5-ylmethyl)-5-bromo-3,3-dimethyl-9-(2-methylallyl)-1,2,3,4-tetrahydro-1,4-(epiminomethano)naphthalene (11d). According to General Method A, **7** (0.04 g, 0.1 mmol) was converted to colorless oily **11d** (40.1 mg, 70%, 99% purity by ELSD): IR (neat) 2958, 2867, 1500, 1485, 1439, 1361, 1238, 1187, 1038, 928, 908, 771, 732 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.40 (dd, *J* = 1.2, 8.1 Hz, 1 H), 7.03 (dd, *J* = 7.3, 8.0 Hz, 1 H), 6.91 (d, *J* = 7.2 Hz, 1 H), 6.87 (d, *J* = 1.5 Hz, 1 H), 6.79 (dd, *J* = 1.5, 7.9 Hz, 1 H), 6.73 (d, *J* = 7.0 Hz, 1 H), 5.93 (dd, *J* = 1.4, 5.1 Hz, 2 H), 4.80 (s, 2 H), 3.78 (AB q, *J* = 13.4 Hz, 2 H), 3.57 (t, *J* = 2.5 Hz, 1 H), 3.19 (ddd, *J* = 1.7, 4.3, 9.2 Hz, 1 H), 2.98 (d, *J* = 1.7 Hz, 1 H), 2.07 (dd, *J* = 2.6, 13.5 Hz, 1 H), 1.78 (dd, *J* = 4.3, 14.2 Hz, 1 H), 1.71 (s, 3 H), 1.67-1.61 (m, 1 H), 1.33 (s, 3 H), 0.93 (dd, *J* = 2.5, 13.6 Hz, 1 H), 0.57 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.7, 146.5 , 144.4, 142.7, 140.0, 134.1, 129.9, 127.5, 123.1, 121.6, 121.2, 112.8, 109.2, 107.8, 100.9, 56.5, 55.9, 54.8, 49.2, 44.4, 34.6, 32.8, 30.9, 28.2, 22.7; HRMS (ESI) *m/z* calcd for $C_{25}H_{29}O_2BrN$ $(M+H)^+$ 454.1376, found 454.1370.

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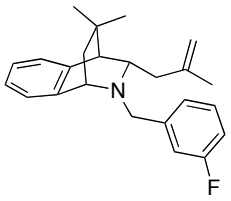
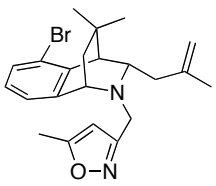
General Method B: Reductive amination.



(±)-(1*S*,4*R*,9*S*)-10-(3-Fluorobenzyl)-3,3-dimethyl-9-(2-methylallyl)-1,2,3,4-tetrahydro-1,4-(epiminomethano)naphthalene (11a). To a stirred solution of **8**[1] (16 mg, 0.070 mmol), 3-fluorobenzaldehyde (8.0 mL, 0.077 mmol) and acetic acid (4 mL, 0.07 mmol) in dichloroethane (0.7 mL) was added sodium triacetoxyborohydride (29 mg, 0.14 mmol). The reaction mixture was stirred overnight under argon and then diluted with CH_2Cl_2 (10 mL). The solution was treated with saturated solution of $NaHCO_3$ (10 mL), and the organic layer was washed with H_2O (10 mL) and concentrated under reduced pressure. The crude product was dissolved in THF-H_2O (10:1, 1 mL) and treated with $NaBH_4$ (10 mg). The solution was stirred for 3 h at room temperature, quenched with AcOH (1 drop) and diluted with EtOAc (10 mL). The solution was neutralized with a saturated solution of $NaHCO_3$ (5 mL) and washed with H_2O (5 mL). The combined organic layers were dried (Na_2SO_4), and the crude product was purified by chromatography on SiO_2 (9:1, hexanes:EtOAc) to give **11a** (15 mg, 63%, 99 % purity by ELSD) as a colorless oil: IR (neat) 3073, 2927, 1647, 1615, 1590, 1485, 1446, 1359, 1253, 1127, 1069, 928, 890, 783, 754, 714, 684 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.30-7.20 (m, 3 H), 7.19 -7.11 (m, 3 H), 7.04-7.01 (m, 1 H), 6.95 (dt, *J* = 2.6, 8.4 Hz, 1 H), 4.78 (s, 1 H), 4.66 (s, 1 H), 3.92 (AB q, *J* = 13.8 Hz, 2 H), 3.60 (t, *J* = 2.5 Hz, 1 H), 3.23 (ddd, *J* = 1.7, 4.3, 10.0 Hz, 1 H), 2.51 (br s, 1 H), 2.13 (dd, *J* = 2.6, 13.3 Hz, 1 H), 1.79 (dd, *J* = 4.2, 13.7 Hz, 1 H), 1.69 (s, 3 H), 1.63-1.57 (m, 2 H), 1.34 (s, 3 H), 1.01 (dd, *J* = 2.3, 13.3 Hz, 1 H), 0.57 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.2, 161.8, 143.5, 143.4, 143.2, 142.0, 139.7, 129.6, 129.5, 127.2, 126.1, 126.0, 124.1, 121.9, 115.5, 115.3, 113.8, 113.6, 112.4, 56.1, 56.0, 55.1, 49.6, 44.3, 35.1, 32.0, 31.9, 28.4, 22.4; HRMS (ESI) *m/z* calcd for $C_{24}H_{29}FN$ $(M)^+$ 350.2279, found 350.2266.



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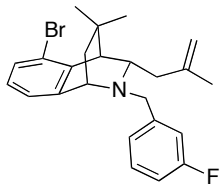
(±)-3-(1*S*,4*R*,9*S*)-5-Bromo-3,3-dimethyl-9-(2-methylallyl)-1,2,3,4-tetrahydro-1,4-(epimino-methano) naphthalen-10-yl)methyl)-5-methylisoxazole (11e). According to General Method B, **7** (25 mg, 0.12 mmol) was converted to pale yellow oily **11e** (15 mg, 27%, 98% purity by ELSD): IR (neat) 3073, 2928, 1647, 1606, 1568, 1453, 1358, 1258, 1190, 1139, 1107, 1002, 957, 888, 797, 773, 716, 689 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 (d, *J* = 7.9 Hz, 1 H), 7.03 (t, *J* = 7.5 Hz, 1 H), 6.94 (d, *J* = 7.0 Hz, 1 H), 5.96 (s, 1 H), 4.79 (s, 2 H), 3.97 (d, *J* = 13.8 Hz, 1 H), 3.74 (d, *J* = 13.8 Hz, 1 H), 3.58 (t, *J* = 2.4 Hz, 1 H), 3.23-3.19 (m, 1 H), 2.97 (s, 1 H), 2.38 (s, 3 H), 2.10 (dd, *J* = 2.6, 13.8 Hz, 1 H), 1.77-1.62 (m, 2 H), 1.72 (s, 3 H), 1.31 (s, 3 H), 0.95 (dd, *J* = 2.2, 13.8 Hz, 1 H), 0.56 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.5, 163.5, 144.0, 142.6, 139.9, 130.3, 127.7, 123.3, 121.5, 113.2, 101.9, 56.9, 55.9, 49.4, 47.4, 44.7, 34.7, 32.9, 31.0, 28.3, 22.8, 12.5; HRMS (ESI) *m/z* calcd for $C_{22}H_{28}BrON_2$ $(M+H)^+$ 415.1380, found. 415.1373.



(±)-(1*S*,4*R*,9*S*)-5-Bromo-10-(3-fluorobenzyl)-3,3-dimethyl-9-(2-methylallyl)-1,2,3,4-tetrahydro-1,4-(epiminomethano)naphthalene (11f). According to General Method B, **7** (25 mg, 0.080 mmol) was converted to colorless oily **11f** (13.2 mg, 39%, 98% purity by ELSD): IR (neat) 3074, 2928, 1648, 1614, 1590, 1568, 1485, 1449, 1358, 1253, 1189, 1139, 1123, 1070, 958, 924, 890, 772, 749, 713, 684 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 (dd, *J* = 1.0, 8.0 Hz, 1 H), 7.31-7.24 (m, 1 H), 7.15-7.04 (m, 3 H), 6.99-6.92 (m, 2 H), 4.82 (s, 2 H), 3.89 (s, 2 H), 3.56 (t, *J* = 2.5 Hz, 1 H), 3.24 (ddd, *J* = 1.7, 4.4, 8.9 Hz, 1 H), 3.02 (d, *J* = 1.6 Hz, 1 H), 2.10 (dd, *J* = 2.6, 13.5 Hz, 1 H), 1.80 (dd, *J* = 4.4, 14.1 Hz, 1 H), 1.74-1.65 (m, 1 H), 1.74 (s, 3 H), 1.37 (s, 3 H), 0.97 (dd, *J* = 2.4, 13.6 Hz, 1 H), 0.60 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.3, 161.8, 144.2, 143.0, 142.5, 139.9, 130.0, 129.7, 129.6, 127.5, 124.0, 123.1, 121.2, 115.4, 115.2, 113.9, 113.7, 112.9, 56.6, 55.6, 54.9, 49.2, 44.4, 34.6, 32.8, 30.8, 28.2, 22.7; HRMS (ESI) *m/z* calcd for $C_{24}H_{28}BrFN$ $(M+H)^+$ 428.1384, found 428.1371.

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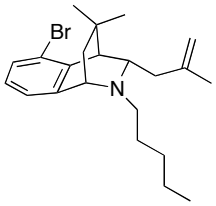
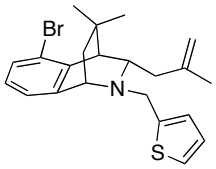


(±)-(1*S*,4*R*,9*S*)-5-Bromo-3,3-dimethyl-9-(2-methylallyl-10-pentyl-1,2,3,4-tetrahydro-1,4-(epiminomethano)naphthalene (11g). According to General Method B, **7** (0.04 g, 0.1 mmol) was converted to colorless oily **11g** (35 mg, 72%, 99% purity by ELSD): IR (neat) 2928, 2860, 1648, 1568, 1453, 1360, 1275, 1189, 1138, 1102, 1060, 997, 958, 932, 891, 822, 768, 689 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 (dd, *J* = 2.8, 6.3 Hz, 1 H), 7.04-6.99 (m, 2 H), 4.79 (s, 2 H), 3.73 (t, *J* = 2.6 Hz, 1 H), 3.03-2.98 (m, 1 H), 2.92 (s, 1 H), 2.70-2.60 (m, 2 H), 2.01 (dd, *J* = 2.6, 13.6 Hz, 1 H), 1.86-1.82 (m, 1 H), 1.72 (s, 3 H), 1.61-1.43 (m, 3 H), 1.34-1.22 (m, 7 H), 0.93-0.85 (m, 1 H), 0.88 (t, *J* = 7.0 Hz, 3 H), 0.52 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.3, 142.9, 140.3, 130.3, 127.7, 123.3, 121.7, 112.8, 57.6, 56.4, 53.5, 48.8, 44.7, 34.7, 32.9, 31.1, 30.0, 29.3, 28.2, 23.0, 22.9, 14.3; HRMS (ESI) *m/z* calcd for $C_{22}H_{33}BrN$ $(M+H)^+$ 390.1791, found 390.1783.



(±)-(1*S*,4*R*,9*S*)-5-Bromo-3,3-dimethyl-9-(2-methylallyl)-10-(thiophen-2-ylmethyl)-1,2,3,4-tetrahydro-1,4-(epiminomethano)naphthalene (11h). According to General Method B, **7** (0.03 g, 0.09 mmol) was converted to colorless oily **11h** (26.3 mg, 67%, 99% purity by ELSD): IR (neat) 3074, 2927, 1647, 1567, 1453, 1360, 1310, 1276, 1189, 1138, 1106, 957, 892, 823, 772, 753, 696 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (dd, *J* = 1.0, 8.0 Hz, 1 H), 7.21 (dd, *J* = 1.5, 4.7 Hz, 1 H), 7.04 (dd, *J* = 7.3, 8.0 Hz, 1 H), 6.97-6.93 (m, 3 H), 4.81 (d, *J* = 5.2 Hz, 2 H), 4.15 (d, *J* = 14.0 Hz, 1 H), 3.97 (d, *J* = 14.0 Hz, 1 H), 3.71 (t, *J* = 2.6 Hz, 1 H), 3.22 (ddd, *J* = 1.8, 4.5, 8.7 Hz, 1 H), 2.99 (d, *J* = 1.7 Hz, 1 H), 2.05 (dd, *J* = 2.6, 13.6 Hz, 1 H), 1.81 (dd, *J* = 4.6, 14.0 Hz, 1 H), 1.73 (s, 3 H), 1.68 (dd, *J* = 8.7, 14.1 Hz, 1 H), 1.33 (s, 3 H), 0.95 (dd, *J* = 2.5, 13.7 Hz, 1 H), 0.58 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.0, 144.3, 142.7, 140.1, 130.2, 127.7, 126.5, 125.0, 123.2, 121.5, 113.1, 56.8, 55.0, 50.9, 49.5, 44.6, 34.8, 32.9, 31.0, 28.4, 23.0; HRMS (ESI) *m/z* calcd for $C_{22}H_{26}BrNS$ $(M)^+$ 416.1042, found 416.1036.

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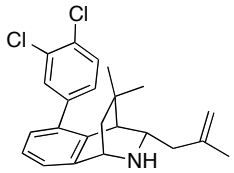
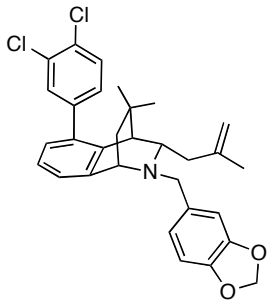
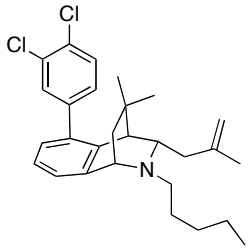


(±)-(1*S*,4*R*,9*S*)-5-(3,4-Dichlorophenyl)-3,3-dimethyl-9-(2-methylallyl)-1,2,3,4-tetrahydro-1,4-(epiminomethano)naphthalene (12a). A solution of **7** (0.05 g, 0.2 mmol), 3,4-dichlorophenylboronic acid (59.5 mg, 0.310 mmol) and $Pd(PPh_3)_2Cl_2$ (11 mg, 0.015 mmol) in dioxane (0.7 mL) and 2 M aqueous Na_2CO_3 (2.2 mL) was heated to 120 ^oC for 1 h in the microwave. After addition of EtOAc (20 mL) the mixture was washed with H_2O (10 mL) and brine (10 mL). The combined organic layers were dried (Na_2SO_4) and the crude residue was purified by chromatography on Al_2O_3 (1:1 to 1:2, hexanes:EtOAc) to give **12a** (45 mg, 74%, 95% purity by ELSD) as a yellow oil: IR (neat) 2929, 1647, 1548, 1458, 1372, 1274, 1195, 1133, 1078, 1031, 888, 816, 772, 694 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.72-7.65 (m, 1 H), 7.52-7.47 (m, 1 H), 7.43 (s, 1 H), 7.31-7.26 (m, 1 H), 7.19-7.15 (m, 2 H), 4.71 (s, 1 H), 4.45 (s, 1 H), 3.97 (s, 1 H), 3.72 (dd, *J* = 4.4, 9.5 Hz, 1 H), 2.57 (s, 1 H), 1.88 (dd, *J* = 2.8, 12.9 Hz, 1 H), 1.80 (dd, *J* = 4.2, 13.6 Hz, 1 H), 1.71 (s, 3 H), 1.32-1.26 (m, 5 H), 0.61 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.5, 142.3, 141.0, 138.5, 136.9, 132.2, 131.5, 131.3, 130.1, 129.1, 127.4, 126.2, 121.8, 112.9, 52.8, 48.5, 47.2, 45.3, 42.6, 32.6, 32.0, 29.0, 22.3; HRMS (ESI) *m/z* calcd for $C_{23}H_{26}NCl_2$ $(M+H)^+$ 346.1437, found 346.1428.



(±)-(1*S*,4*R*,9*S*)-10-(Benzo[*d*][1,3]dioxol-5-ylmethyl)-5-(3,4-dichlorophenyl)-3,3-dimethyl-9-(2-methylallyl)-1,2,3,4-tetrahydro-1,4-(epiminomethano)naphthalene (12b). A solution of **11d** (0.04 g, 0.09 mmol), 3,4-dichlorophenylboronic acid (33.5 mg, 0.170 mmol), $Pd(PPh_3)_2Cl_2$ (6 mg, 0.009 mmol) and anhydrous sodium carbonate (27 mg, 0.25 mmol) in degassed dioxane (0.5 mL) and H_2O (0.15 mL) was heated to 120 ^oC for 1 h in the microwave and diluted with EtOAc (5 mL). The organic layer was washed with H_2O (10 mL), brine (10 mL), dried (Na_2SO_4), and concentrated. The crude product was purified by chromatography on SiO_2 (95:5, hexanes:EtOAc)

to give **12b** (35.1 mg, 77%, 99% purity by ELSD) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (d, *J* = 8.3 Hz, 1 H), 7.42 (d, *J* = 2.0 Hz, 1 H), 7.25 (t, *J* = 7.3 Hz, 1 H), 7.18 (dd, *J* = 2.0, 8.2 Hz, 1 H), 7.15 (dd, *J* = 1.3, 7.8 Hz, 1 H), 7.04 (dd, *J* = 1.1, 7.2 Hz, 1 H), 6.93 (d, *J* = 1.4 Hz, 1 H), 6.84-6.77 (m, 2 H), 5.98, 5.97 (AB q, *J* = 1.5 Hz, 2 H), 4.69 (s, 1 H), 4.44 (s, 1 H), 3.81, 3.80 (AB q, *J* = 13.4 Hz, 2 H), 3.66 (t, *J* = 2.6 Hz, 1 H), 3.24 (ddd, *J* = 1.7, 4.5, 8.2 Hz, 1 H), 2.71 (d, *J* = 1.4 Hz, 1 H), 2.14 (dd, *J* = 2.5, 13.5 Hz, 1 H), 1.85 (dd, *J* = 4.4, 15.4 Hz, 1 H), 1.71-1.64 (m, 1 H), 1.69 (s, 3 H), 1.31 (s, 3 H), 0.99 (dd, *J* = 2.5, 13.6 Hz, 1 H), 0.56 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.8, 146.6, 143.2, 143.0, 141.2, 138.5, 137.7, 134.3, 132.2, 131.5, 131.2, 130.0, 129.2, 127.6, 126.2, 122.4, 121.7, 111.6, 109.3, 108.0, 101.0, 56.3, 56.0, 54.6, 46.7, 44.3, 34.7, 33.2, 32.0, 28.6, 23.5; HRMS (ESI) *m/z* calcd for $C_{31}H_{32}O_2NCl_2$ $(M+H)^+$ 520.1805, found 520.1788.



(±)-(1*S*,4*R*,9*S*)-5-(3,4-Dichlorophenyl)-3,3-dimethyl-9-(2-methylallyl)-10-pentyl-1,2,3,4-tetrahydro-1,4-(epiminomethano)naphthalene (12c). A solution of **11g** (34 mg, 0.090 mmol), 3,4-dichlorophenylboronic acid (33.2 mg, 0.170 mmol), $Pd(PPh_3)_2Cl_2$ (6 mg, 0.009 mmol), and anhydrous sodium carbonate (26.7 mg, 0.250 mmol) in degassed dioxane (0.5 mL) and H_2O (0.15 mL) was heated to 120 ^{o}C for 1 h in the microwave and diluted with EtOAc (5 mL). The organic layer was washed with H_2O (10 mL), brine (10 mL), dried (Na_2SO_4), and concentrated. The crude product was purified by chromatography on SiO_2 (95:5, hexanes:EtOAc) to give **12c** (21.2 mg, 53%, 99% purity by ELSD) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (d, *J* = 8.3 Hz, 1 H), 7.39 (d, *J* = 2.0 Hz, 1 H), 7.29-7.25 (m, 1 H), 7.22-7.13 (m, 3 H), 4.68 (s, 1 H), 4.35 (s, 1 H), 3.87 (t, *J* = 2.5 Hz, 1 H), 3.09-3.05 (m, 1 H), 2.80-2.62 (m, 3 H), 2.10 (dd, *J* = 2.6, 13.5 Hz, 1 H), 1.90 (d, *J* = 15.0 Hz, 1 H), 1.70-1.50 (m, 3 H), 1.68 (s, 3 H), 1.40-1.25 (m, 4 H), 1.26 (s, 3 H), 1.01 (dd, *J* = 2.6, 13.5 Hz, 1 H), 0.93 (t, *J* = 6.9 Hz, 1 H), 0.54 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 143.2, 142.8, 141.2, 138.6, 137.7, 132.1, 131.5, 131.1, 130.0, 129.2, 127.7, 126.3, 122.7, 111.0, 57.2, 56.1, 53.2, 46.1, 43.9, 34.8, 33.2, 32.1, 30.0, 29.2, 28.3, 23.7, 22.8, 14.3; HRMS (ESI) *m/z* calcd for $C_{28}H_{36}NCl_2$ $(M+H)^+$ 456.2219, found 456.2205.

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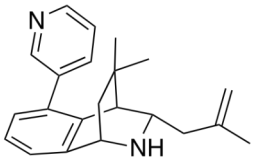
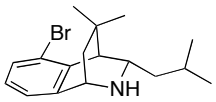


(±)-(1*S*,4*R*,9*S*)-3,3-Dimethyl-9-(2-methylallyl)-5-(pyridin-3-yl)-1,2,3,4-tetrahydro-1,4-(epimino-methano)naphthalene (12d). A solution of **7** (81 mg, 0.25 mmol), pyridine-3-boronic acid (62 mg, 0.50 mmol), and $Pd(PPh_3)_2Cl_2$ (18 mg, 0.025 mmol) in degassed dioxane (1.1 mL) was treated with a 2 M aqueous solution of Na_2CO_3 (0.4 mL) and heated to 120 ^oC for 1 h in a microwave. The reaction mixture was diluted with EtOAc (10 mL) and washed with H_2O (10 mL) and brine (10 mL). The combined organic layers were dried (Na_2SO_4), and the crude residue was purified by chromatography on SiO_2 (5:95, MeOH:CH_2Cl_2 with 0.1% Et_3N) to give **12d** (19.5 mg, 24%, 97% purity by LC-MS) as a wax: IR (neat) 2928, 1463, 1437,1405, 1191, 1176, 773, 747, 715 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.63 (d, *J* = 1.2 Hz, 2 H), 7.68-7.65 (m, 1 H), 7.37 (dd, *J* = 7.2, 5.5 Hz, 1 H), 7.34-7.30 (m, 1 H), 7.28 (d, *J* = 1.3 Hz, 1 H), 7.22-7.17 (m, 2 H), 4.67 (s, 1 H), 4.40 (s, 1 H), 3.98 (s, 1 H), 3.72 (dd, *J* = 9.7, 4.3 Hz, 1 H), 2.57 (s, 1 H), 1.91-1.86 (m, 1 H), 1.79 (dd, *J* = 13.7, 4.2 Hz, 1 H), 1.67 (s, 3 H), 1.35-1.25 (m, 2 H), 1.27 (s, 3 H), 0.63 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.3, 148.4, 142.5, 142.3, 137.3, 137.2, 137.0, 136.6, 127.8, 126.3, 123.2, 122.0, 113.0, 52.8, 48.6, 47.2, 45.3, 42.6, 32.6, 32.0, 29.0, 23.0; HRMS (ESI) *m/z* calcd for $C_{22}H_{27}N_2$ $(M+H)^+$ 319.2169, found 319.2161.



(±)-(1*S*,4*R*,9*S*)-5-Bromo-3,3-dimethyl-9-(2-methylallyl)-1,2,3,4-tetrahydro-1,4-(epimino-methano)-naphthalene (13). A solution of **7** (37 mg, 0.11 mmol) in dry EtOAc (0.4 mL) was treated with 5% rhodium on carbon (37 mg) and stirred under a hydrogen atmosphere for 24 h. The catalyst was filtered through Celite and the solvent was removed in vacuo. The crude product was purified by chromatography on SiO_2 (100% EtOAc) to give **13** (34 mg, 91%, 99% purity by ELSD) as a yellow oil: IR (neat) 2954, 1564, 1452, 1385, 1366, 1326, 1180, 1116, 1066, 968, 758, 707, 687 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.43 (dd, *J* = 1.7, 7.3 Hz, 1 H), 7.09-7.01 (m, 2 H), 3.89 (br t, *J* = 2.9 Hz, 1 H), 3.62 (t, *J* = 7.0 Hz, 1 H), 2.95 (s, 1 H), 1.82 (dd, *J* = 2.7, 13.2 Hz, 1 H), 1.77-1.65 (m, 1 H), 1.30 (s, 3 H), 1.23 (dd, *J* = 3.0, 13.3 Hz, 1 H), 0.79 (d, *J* = 6.6 Hz, 3 H), 0.78 (d, *J* = 6.6 Hz, 3 H), 0.77 (dd, *J* = 6.8, 13.7 Hz, 1 H), 0.70-0.50 (m, 1 H), 0.58 (s, 3 H); ^{13}C NMR (75

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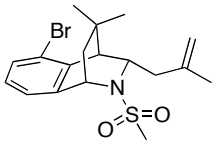
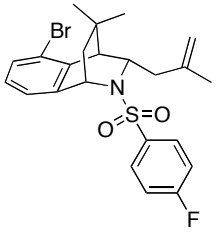
MHz, $CDCl_3$) δ 143.8, 139.3, 130.0, 127.7, 123.6, 121.2, 52.8, 52.7, 49.3, 45.6, 42.1, 32.3, 31.0, 28.7, 24.8, 23.2, 22.7; HRMS (ESI) *m/z* calcd for $C_{17}H_{25}BrN$ $(M+H)^+$ 322.1165, found 322.1159.



(±)-(1*S*,4*R*,9*S*)-5-Bromo-3,3-dimethyl-9-(2-methylallyl)-10-(methylsulfonyl)-1,2,3,4-tetrahydro-1,4-(epiminomethano)naphthalene (14a). A solution of **76** (25 mg, 0.078 mmol) in dry CH_2Cl_2 (0.8 mL) was treated at 0 °C with triethylamine (22 µL, 0.16 mmol) and methanesulfonyl chloride (7 µL, 0.08 mmol), stirred for 24 h at room temperature, diluted with CH_2Cl_2 (3 mL), washed with H_2O (2 x 5 mL), dried (Na_2SO_4), and concentrated. The crude product was purified by chromatography on SiO_2 (10-20% EtOAc:hexanes) to give **14a** (28.5 mg, 92%, 99% purity by ELSD) as a colorless wax: IR (neat) 3075, 2959, 1650, 1568, 1454, 1319, 1189, 1146, 1121, 1073, 1053, 963, 949, 927, 909, 891, 800, 769, 687, 662 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.53 (t, *J* = 4.7 Hz, 1 H), 7.16 (d, *J* = 4.4 Hz, 1 H), 4.89 (s, 1 H), 4.79 (s, 1 H), 4.74 (s, 1 H), 4.53 (d, *J* = 10.4 Hz, 1 H), 3.25 (s, 1 H), 2.92 (s, 3 H), 2.26 (d, *J* = 13.8 Hz, 1 H), 2.15 (dd, *J* = 3.1, 13.4 Hz, 1 H), 1.79 (s, 3 H), 1.51 (dd, *J* = 10.7, 13.7 Hz, 1 H), 1.38 (s, 3 H), 1.19 (d, *J* = 13.7 Hz, 1 H), 0.71 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 141.0, 140.7, 139.6, 131.6, 128.4, 123.9, 121.8, 114.3, 54.6, 53.2, 49.3, 43.3, 42.7, 42.3, 31.6, 31.1, 28.3, 22.4; HRMS (ESI) *m/z* calcd for $C_{10}H_9NO_2SBr$ $(M-C_8H_{15})^+$ 285.9532, found 285.9526.



(±)-(1*S*,4*R*,9*S*)-5-Bromo-10-((4-fluorophenyl)sulfonyl)-3,3-dimethyl-9-(2-methylallyl)-1,2,3,4-tetrahydro-1,4-(epiminomethano)naphthalene (14b). A solution of **7** (25 mg, 0.078 mmol) in dry CH_2Cl_2 (0.8 mL) was treated at 0 °C with triethylamine (22 µL, 0.16 mmol) and 4-fluorobenzenesulfonyl chloride (16 mg, 0.085 mmol), stirred overnight under argon at room temperature, diluted with CH_2Cl_2 (5 mL), washed with H_2O (10 mL), brine (10 mL), dried (Na_2SO_4), and concentrated. The crude product was purified by chromatography on SiO_2 (4:1,

hexanes:EtOAc) to give **14b** (25 mg, 67%, 99% purity by ELSD) as a colorless wax: IR (neat) 3075, 2962, 1648, 1592, 1493, 1455, 1349, 1234, 1153, 1090, 946, 908, 839, 769, 732, 682 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.89-7.85 (m, 2 H), 7.51 (dd, *J* = 1.2, 8.0 Hz, 1 H), 7.21-7.16 (m, 2 H), 7.13 (t, *J* = 7.5 Hz, 1 H), 7.09 (d, *J* = 7.3 Hz, 1 H), 4.88 (br s, 1 H), 4.83 (app t, *J* = 2.5 Hz, 1 H), 4.79 (s, 1 H), 4.37 (dt, *J* = 2.6, 11.0 Hz, 1 H), 3.23 (d, *J* = 2.3 Hz, 1 H), 2.33 (d, *J* = 14.2 Hz, 1 H), 1.95 (dd, *J* = 3.3, 13.4 Hz, 1 H), 1.78 (s, 3 H), 1.46 (dd, *J* = 11.1, 14.2 Hz, 1 H), 1.11-1.06 (m, 1 H), 1.09 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.2, 164.2, 141.2, 140.7, 139.5, 137.8, 131.7, 130.0, 129.9, 128.4, 123.4, 121.9, 116.5, 116.4, 113.9, 54.9, 53.4, 49.2, 42.3, 41.4, 31.6, 31.1, 27.6, 22.6; HRMS (ESI) *m/z* calcd for $C_{15}H_{10}NO_2SFBr$ $(M-C_8H_{15})^+$ 365.9594, found 365.9588.

General Biology Methods

Cell lines

All cell culture reagents were obtained from Invitrogen, unless indicated. COS-22 (COS-7 cells stably expressing human $p22^{phox}$) and COS-Nox2 (a.k.a. COS-phox) cells (COS-7 cells stably expressing human $p22^{phox}$, Nox2, $p47^{phox}$ and $p67^{phox}$) were kindly provided by Dr. Mary C. Dinauer (Indiana University, School of Medicine).[2] COS-22 cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) with 4.5 g/L glucose, L-glutamine and sodium pyruvate containing 10% heat-inactivated fetal bovine serum (FBS), 100 units/ml penicillin and 100 µg/ml streptomycin (complete media) supplemented with 1.8 mg/ml G418 (Calbiochem/EMB Bioscience, Gibbstown, NJ). COS-Nox2 cells were maintained in complete media supplemented with 1.8 mg/mL G418, 1 µg/mL puromycin (Sigma, St Louis, MO) and 0.2 mg/mL hygromycin B (Invitrogen, Carlsbad, CA). HEK-Nox5 cells were kindly provided by Dr. David Fulton (Georgia Health Sciences University) and maintained in complete media.

Plasmid preparation, amplification, and purification

Plasmids encoding full-length human cDNAs for Nox1 (pcDNA3.1-hNox1), NOXO1 (pcDNA3.1-hNOXO1), NOXA1 (pCMVsport 6-hNOXA1), p47phox (pCMV-Tag4A-hp47) and Nox4 (pcDNA3-hNox4) were kindly provided by Dr. David Lambeth (Emory University, GA). Plasmids encoding Nox1, NOXO1, or NOXA1 were transformed and amplified into Escherichia coli strain TOP10 (Invitrogen, Carlsbad, CA). Plasmids were purified using a QIAfilter plasmid purification kit (QIAGEN Inc., Valencia, CA.). For human Nox4 expression, the BglII/NotI restriction fragment

[2] M. O. Price, L. C. McPhail, J. D. Lambeth, C. H. Han, U. G. Knaus and M. C. Dinauer, *Blood*, 2002, **99**, 2653-2661.

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from the pcDNA3-hNox4 was subcloned into the plasmid pcDNA3.1/Hygro(−) (Invitrogen, Carlsbad, CA) to generate pcDNA3.1/Hygro-hNox4. The fragment sequence, in-frame insertion and orientation were validated by DNA sequencing after PCR amplification. pcDNA3.1/Hygro-hNox4 was amplified into Escherichia coli strain TOP10 and purified with a QIAfilter plasmid purification kit.

Transfection

Cell transfection was carried out using Lipofectamine LTX and Plus reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions. COS-22 cells were transiently co-transfected with pcDNA 3.1-hNox1, pCMVsport 6-hNOXA1 and pcDNA3.1-hNoxO1 (COS-Nox1/NOXO1/NOXA1 cells) or with pcDNA3.1/Hygro-hNox4 (COS-Nox4 cells). Cells were used 24 hr after transfection. Adherent cells were harvested by incubating with 0.05% trypsin/EDTA for 5 min at 37 °C. Following addition of DMEM/10%FBS to neutralize the trypsin, the cells were pelleted by centrifugation at 1100 × g for 5 min at 4 °C and used for the experiments.

Measurements of ROS generation in whole cells

L-012 chemiluminescence

COS-Nox2, COS-Nox1/NOXO1/NOXA1, HEK-Nox5 and COS-22 cells were re-plated in OPTIMEM into 384-well white micro-plates (Greiner-Bio One GmbH, Germany) at a density of 2.5 x 10^3 cells/well for COS-Nox2 and 5 × 10^4 cells/well for all others. The cells were incubated at 37 °C in PBS containing 400 µm L-012 for 10 min. Reaction was initiated by addition of 5 µM PMA in the case of Nox2 or 1 µM PMA/ 0.1 µM Ionomycin in the case of Nox5. Since Nox1 was constitutively active, Nox1-derived $O_2^{\bullet-}$ was assessed by comparison to non-transfected cells (Cos22). Luminescence was quantified over time using a Biotek Synergy 4 Hybrid Multi-Mode Microplate Reader. The specificity of L-012 for $O_2^{\bullet-}$ was confirmed by the addition of SOD (150 U/mL).

Hydrogen peroxide (H_2O_2)-generating activity

H_2O_2-production was quantified in intact COS-Nox4 cells by Amplex® Red. Briefly, COS-Nox4 cells (5 × 10^4 cells/ml) were re-plated into 384-well plate in assay buffer (25 mM Hepes, pH 7.4, containing 0.12 M NaCl, 3 mM KCl, 1 mM $MgCl_2$). The cells were incubated in the presence or

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absence of drugs at 37 °C in assay buffer supplemented with 0.1 mM Amplex® Red, and 0.32 U/ml of horse radish peroxidase (HRP) for 15 min. Fluorescence measurements were made using a Biotek Synergy 4 Hybrid Multi-Mode Microplate Reader with a 530/25-excitation and a 590/35-emission filter. The reaction was monitored at 37 ^{o}C for 1 h. A standard curve of known H_2O_2 concentrations was included on each plate. Nox4 activity was obtained by calculating the rate of H_2O_2 production as RFU/min and then subtracting the equivalent value given by non-transfected COS-22 cells. Similarly, H_2O_2 production by Nox2 cells was also measured by Amplex ® Red as described above but in the presence of 150 U /mL of SOD and the reaction was initiated by addition of 5 µM PMA. The rate of H_2O_2 production in this case was calculated as RFU/min after the subtraction of the equivalent value given by non-stimulated cells. Data are expressed as % of vehicle control.

Measurements of superoxide generation in cell-free preparations

Cytochrome C assay

COS-Nox2, COS-Nox1/NOXO1/NOXA1 and COS-22 cells were suspended to a concentration of 5 × 10^7 cells/ml in ice-cold disruption buffer (8 mM potassium, sodium phosphate buffer pH 7.0, 131 mM NaCl, 340 mM sucrose, 2 mM NaN_3, 5 mM $MgCl_2$, 1mM EGTA, 1 mM EDTA and protease inhibitor cocktail).[3,4] The cells were lysed by freeze/thaw cycles (5 cycles), and passed through a 30-gauge needle 5 times to further lyse the cells. Cell disruption was confirmed by phase contrast microscopy. The cell lysate was centrifuged at 1000 × g for 10 min at 4 °C to remove unbroken cells, nuclei and debris. Throughout all procedures, extreme care was taken to maintain the lysate at a temperature close to 0 °C. Superoxide ($O_2^{\bullet-}$) production was calculated from the initial linear rate (over 10 min) of SOD-inhibitable cytochrome c reduction quantified at 550 nm using the extinction coefficient of 21.1 mM^{-1} cm^{-1} (Biotek Synergy 4 Hybrid Multi-Mode Microplate Reader). The oxidase assay buffer consisted of 65 mM sodium phosphate buffer (pH 7.0), 1 mM EGTA, 10 µM FAD, 1 mM $MgCl_2$, 2 mM NaN_3 and 0.2 mM cytochrome C.[3] The components of the cell-free system were added in the following order: oxidase assay buffer, cell lysate (5 × 10^5 cell equivalents/well) and drugs at a final concentration as shown on individual

[3] M. O. Price, L. C. McPhail, J. D. Lambeth, C. H. Han, U. G. Knaus and M. C. Dinauer, *Blood*, 2002, **99**, 2653-2661

[4] S. Molshanski-Mor, A. Mizrahi, Y. Ugolev, I. Dahan, Y. Berdichevsky and E. Pick, *Methods Mol. Biol.*, 2007, **412**, 385-428.

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graphs. The plates were placed on an orbital shaker to mix contents for 5 min at120 movements/min at room temperature. LiDS, an established lipid activator of phagocyte cell-free system, was added at a concentration of 130 µM and $O_2^{\bullet -}$ production was initiated by the addition of 180 µM NADPH

Xanthine oxidase assay

Bovine milk XO (grade 1, ammonium sulfate suspension; Sigma–Aldrich) was first desalted using PD-10 columns (Sephadex G-25, GE Healthcare Biosciences , Piscataway, NJ) equilibrated in PBS. XO activity was assayed by measuring H_2O_2 production using the Amplex® Red assay. Reaction mixture contained: 0.02 U/mL XO, 25 mM Hepes, pH 7.4, containing 0.12 M NaCl, 3 mM KCl, 1 mM $MgCl_2$, 0.1 mM Amplex® Red, and 0.32 U/mL of horse radish peroxidase (HRP), reaction was initiated by addition of 1 mM hypoxanthine. Fluorescence was quantified over time using a Biotek Synergy 4 Hybrid Multi-Mode Microplate Reader. Data are expressed as % of vehicle control.

Cytotoxicity assay

The cytotoxic effect of compounds was tested using CytoTox-Glo kit (Promega, Madison, WI). This assay uses a luminogenic peptide substrate, the AAF-Glo™ Substrate, to measure dead-cell protease activity, which is released from cells that have lost membrane integrity. Cells were plated in 384-well plate at 2.5 x 10^3 cells/well in OPTIMEM, incubated for 15 min with the compounds at various concentrations and then carried out dead cell measurements according to manufacturer protocol. Fluorescence signals before and after the addition of digitonin was used to calculate the % of dead cells in each independent well. Data are expressed as % of live cells in each independent well (=100-%dead cells).

Statistical analysis

All data are expressed as means ± SEM. IC_{50} values were determined using the GraphPad Prism software for non-linear regression three-parameters, with constrain values of 100 and 0 for top and bottom, respectively, and assumes a Hill slope of 1.

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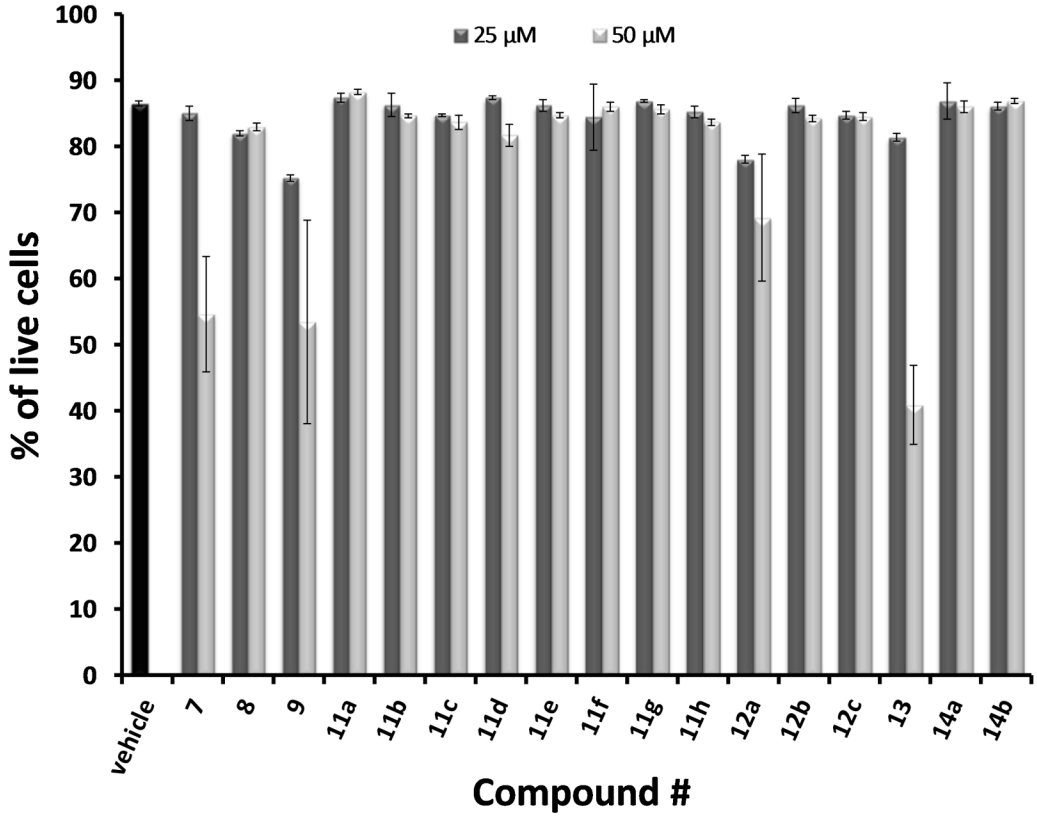
Cytotoxicity assessment of **7** and analogs



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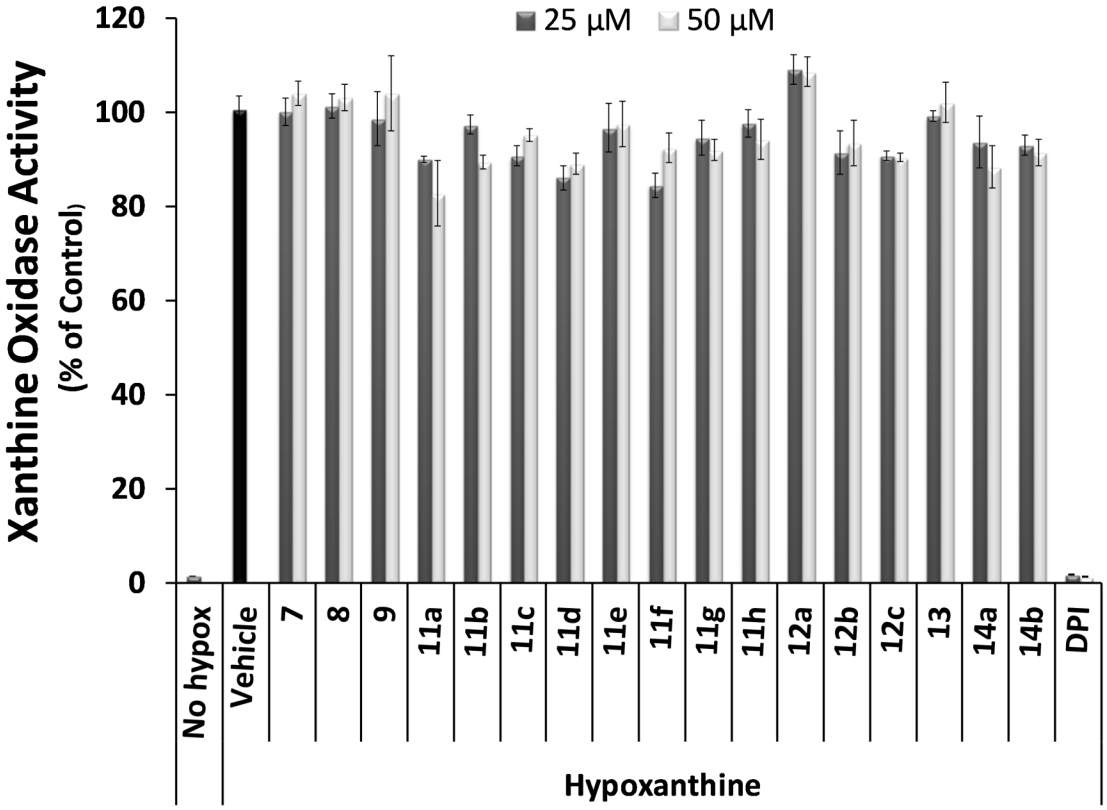
Xanthine oxidase (XO) assay data



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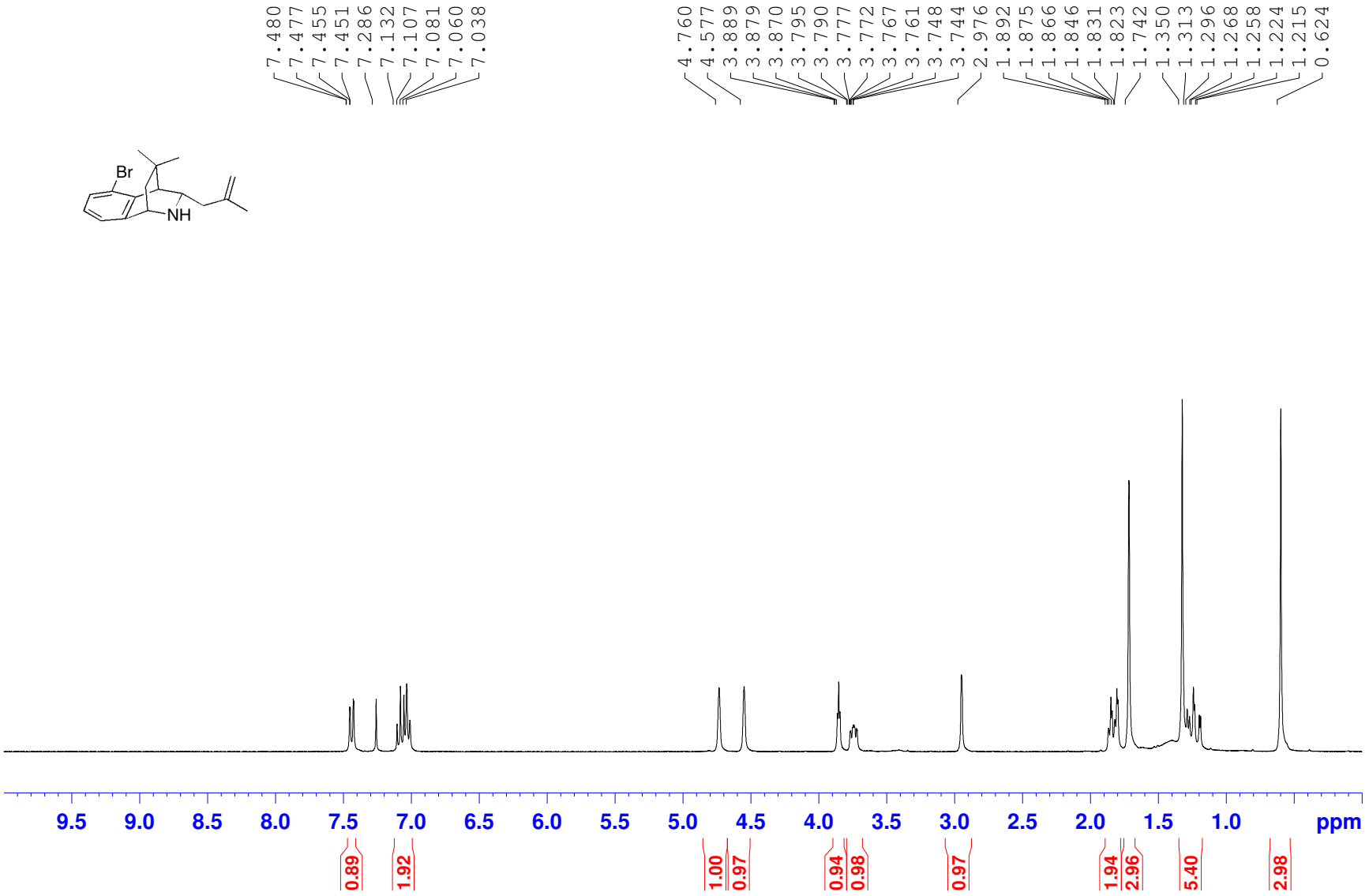

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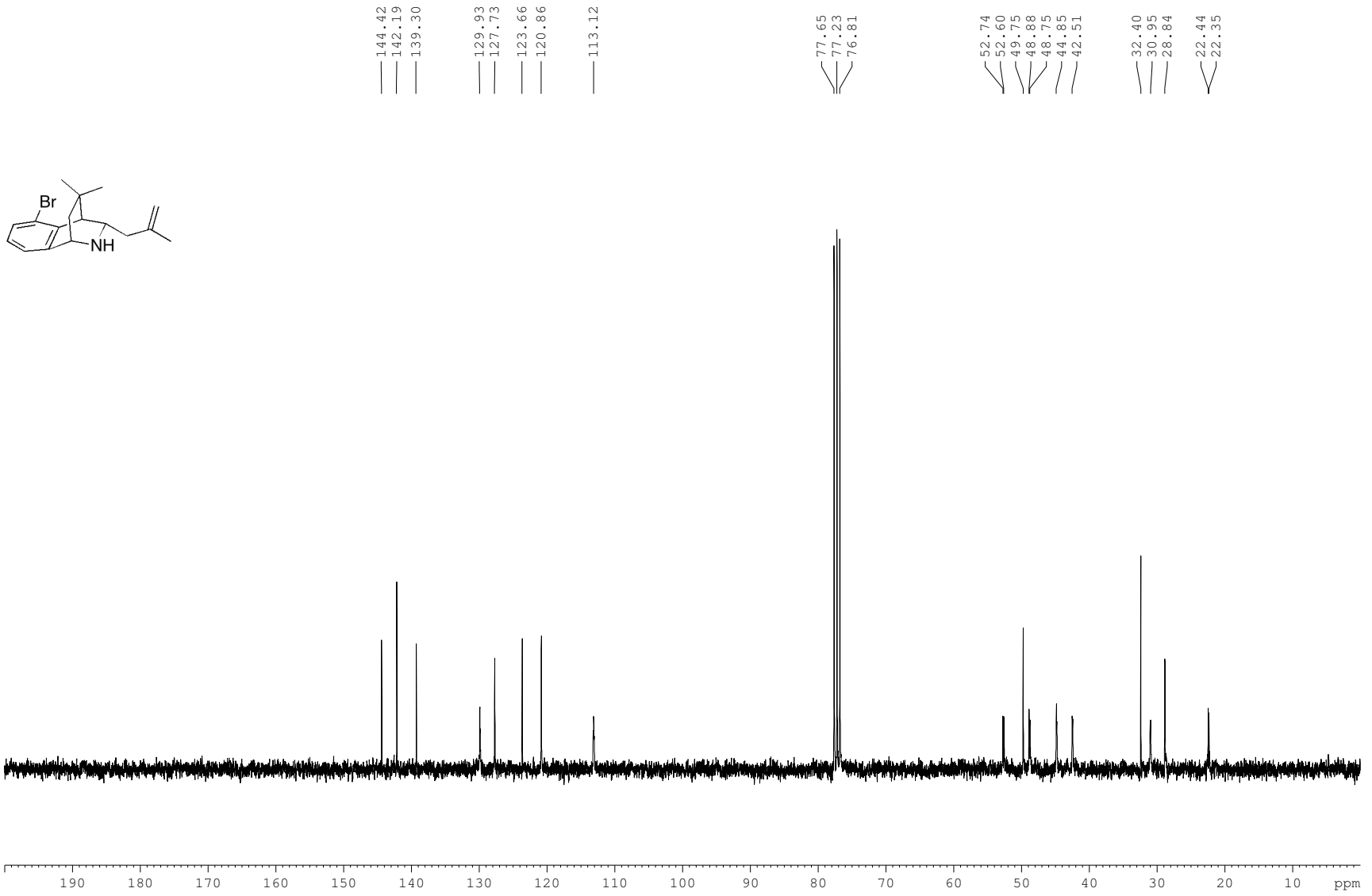

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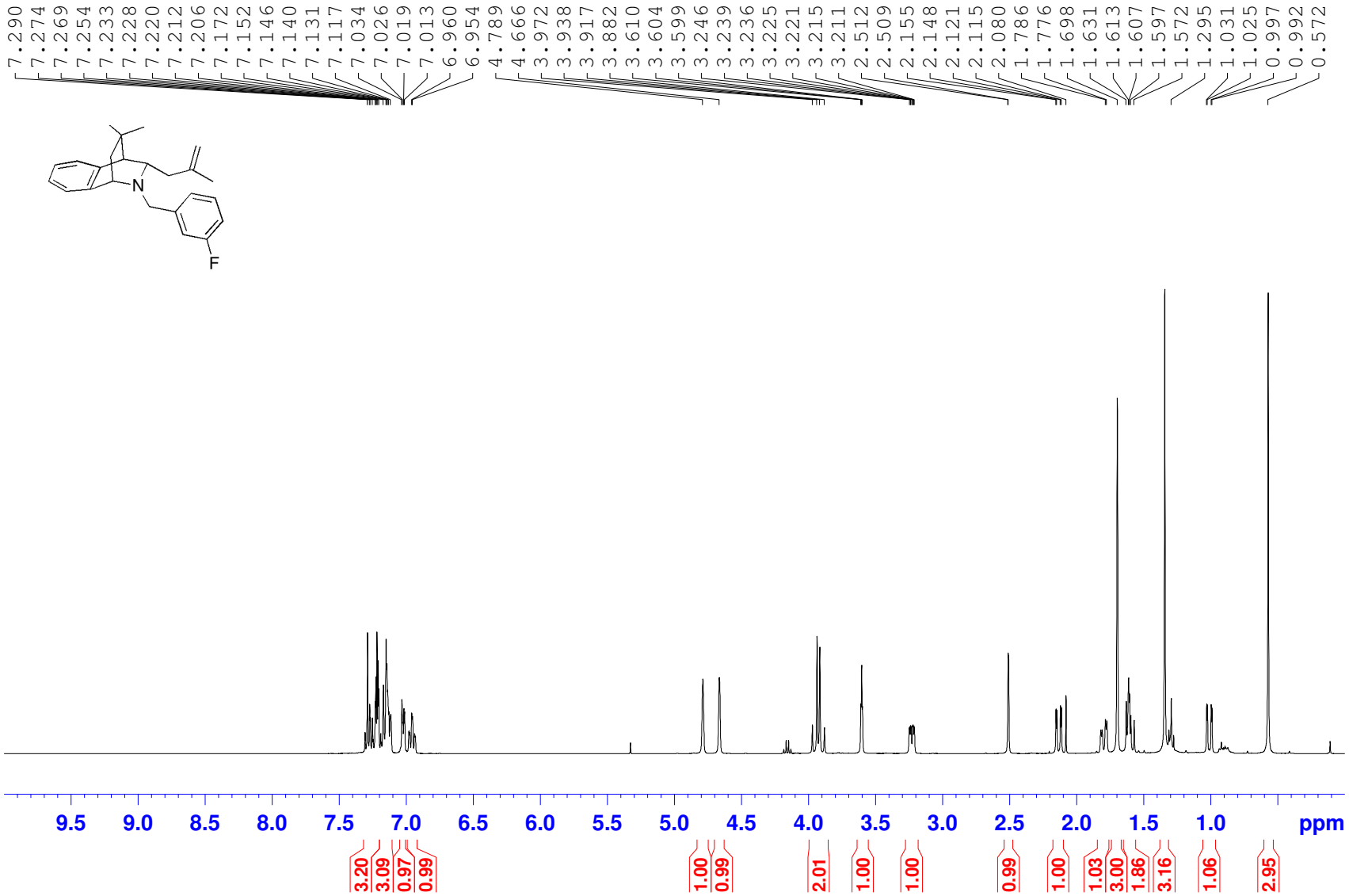

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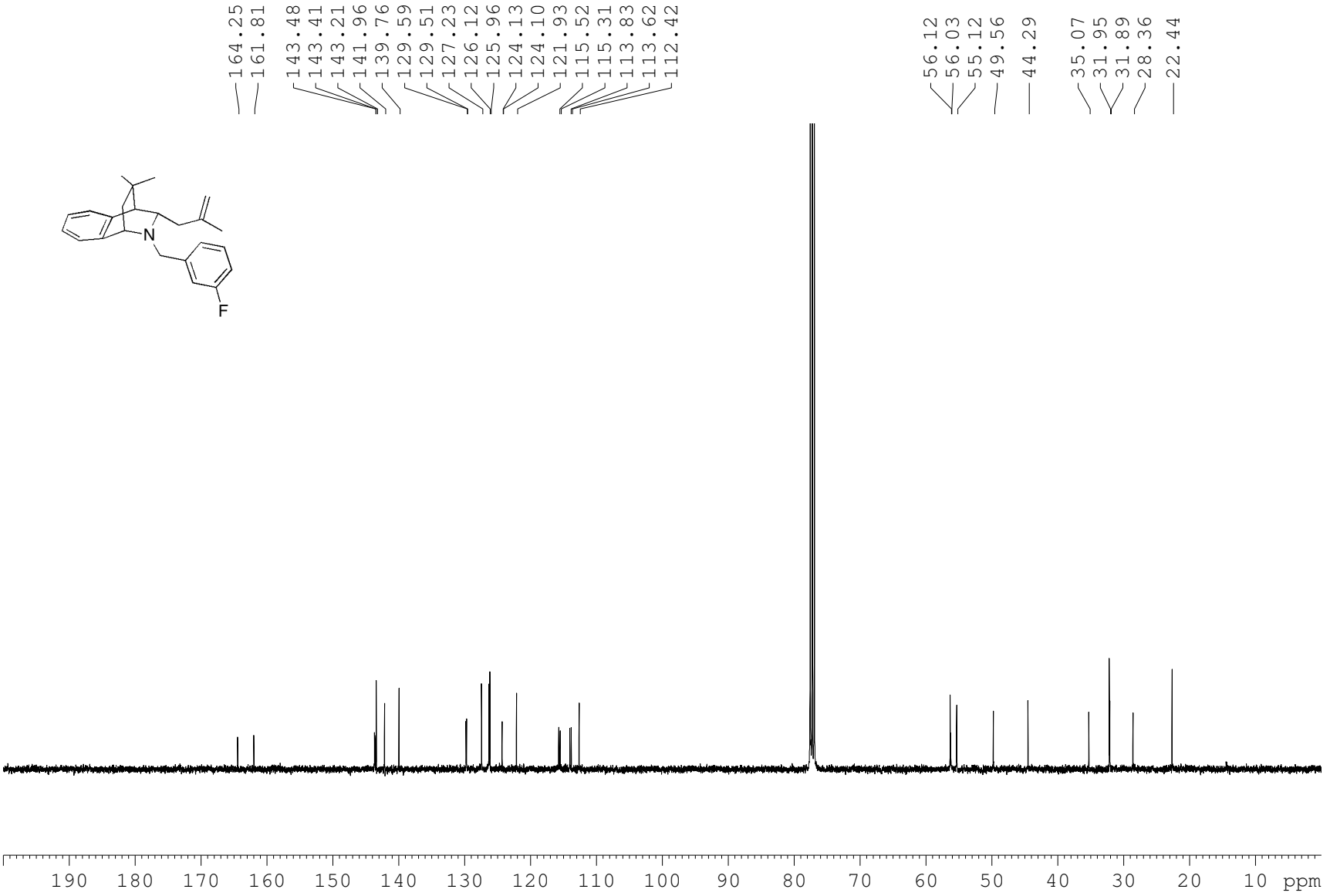

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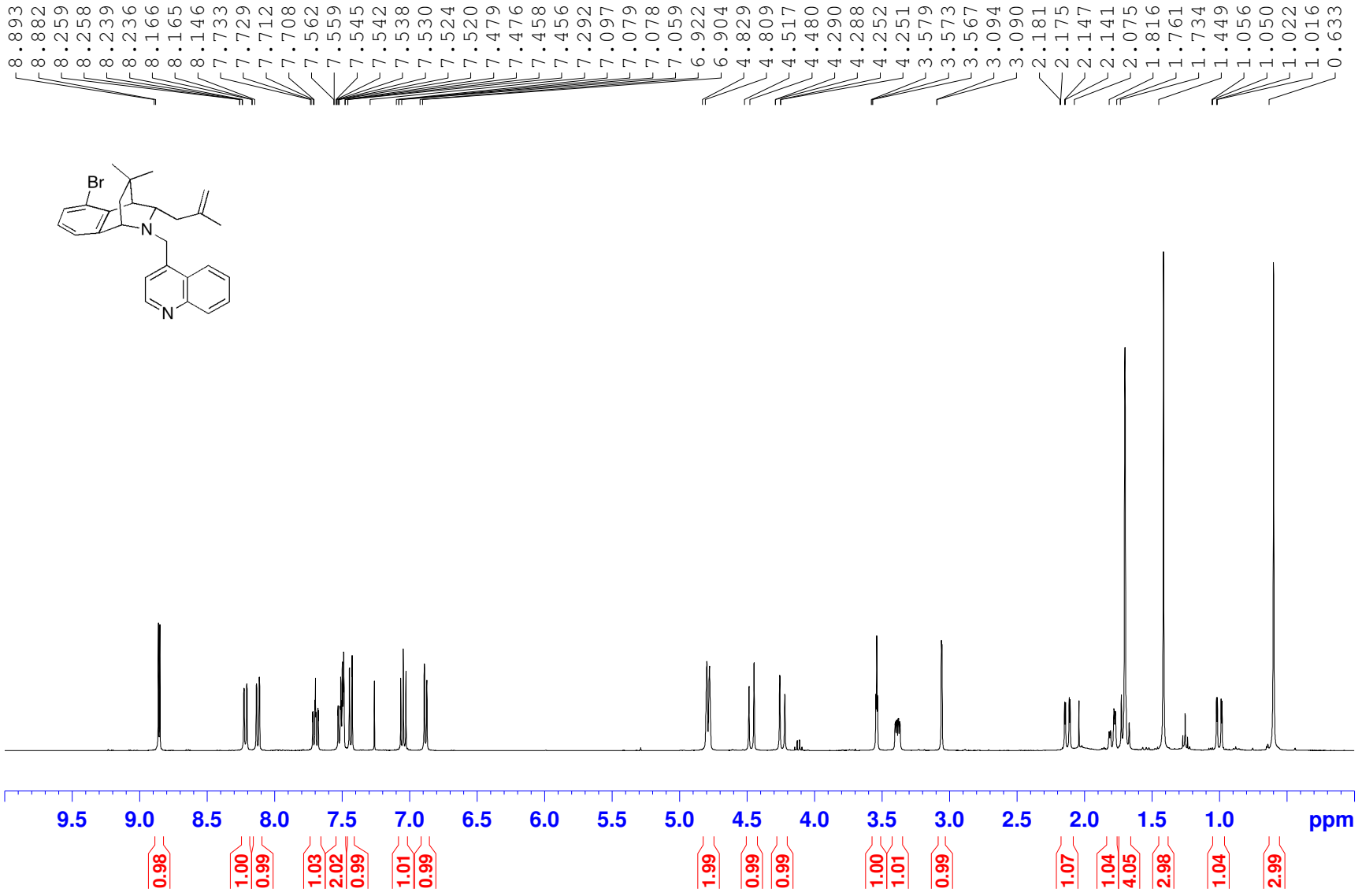

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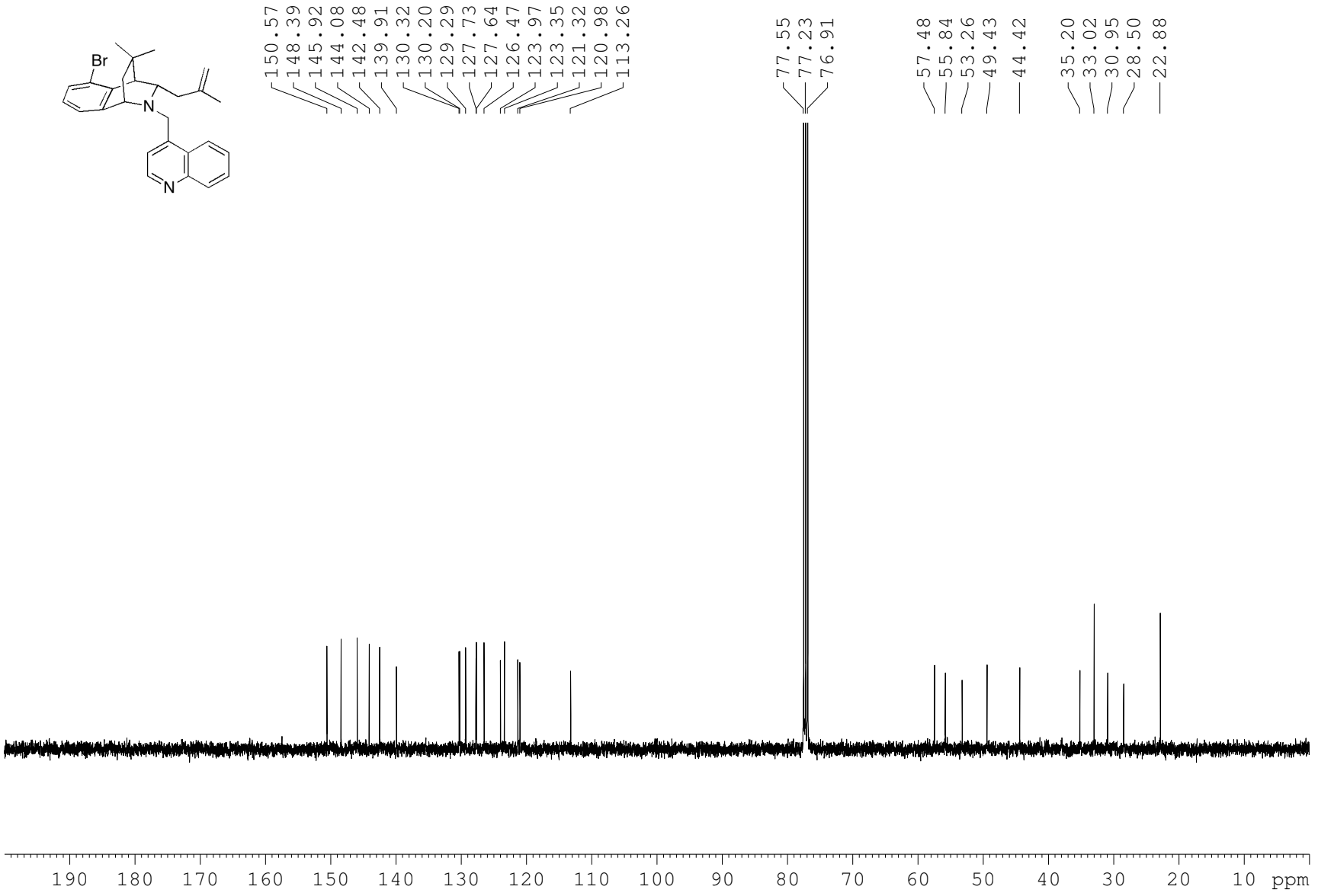

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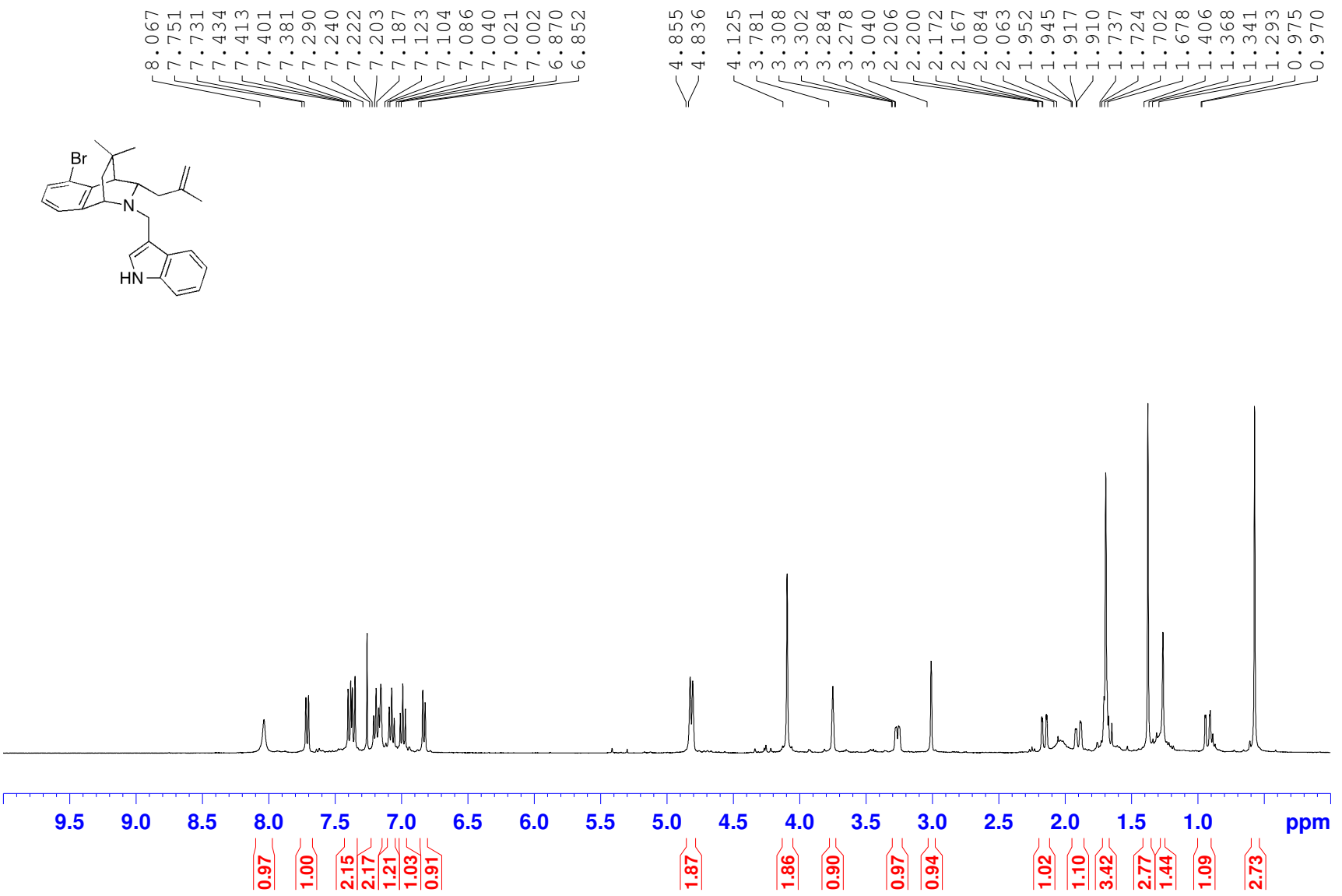

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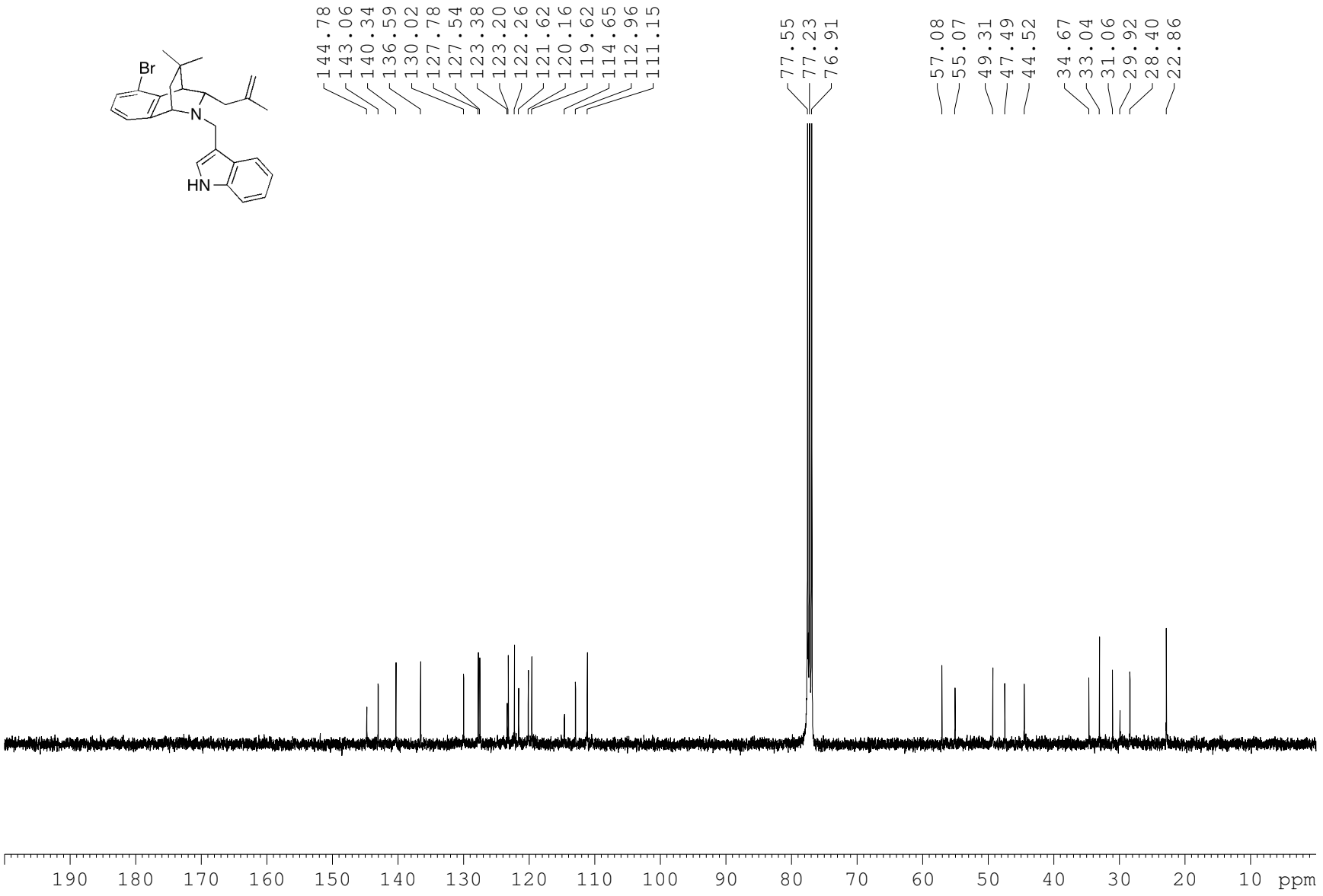

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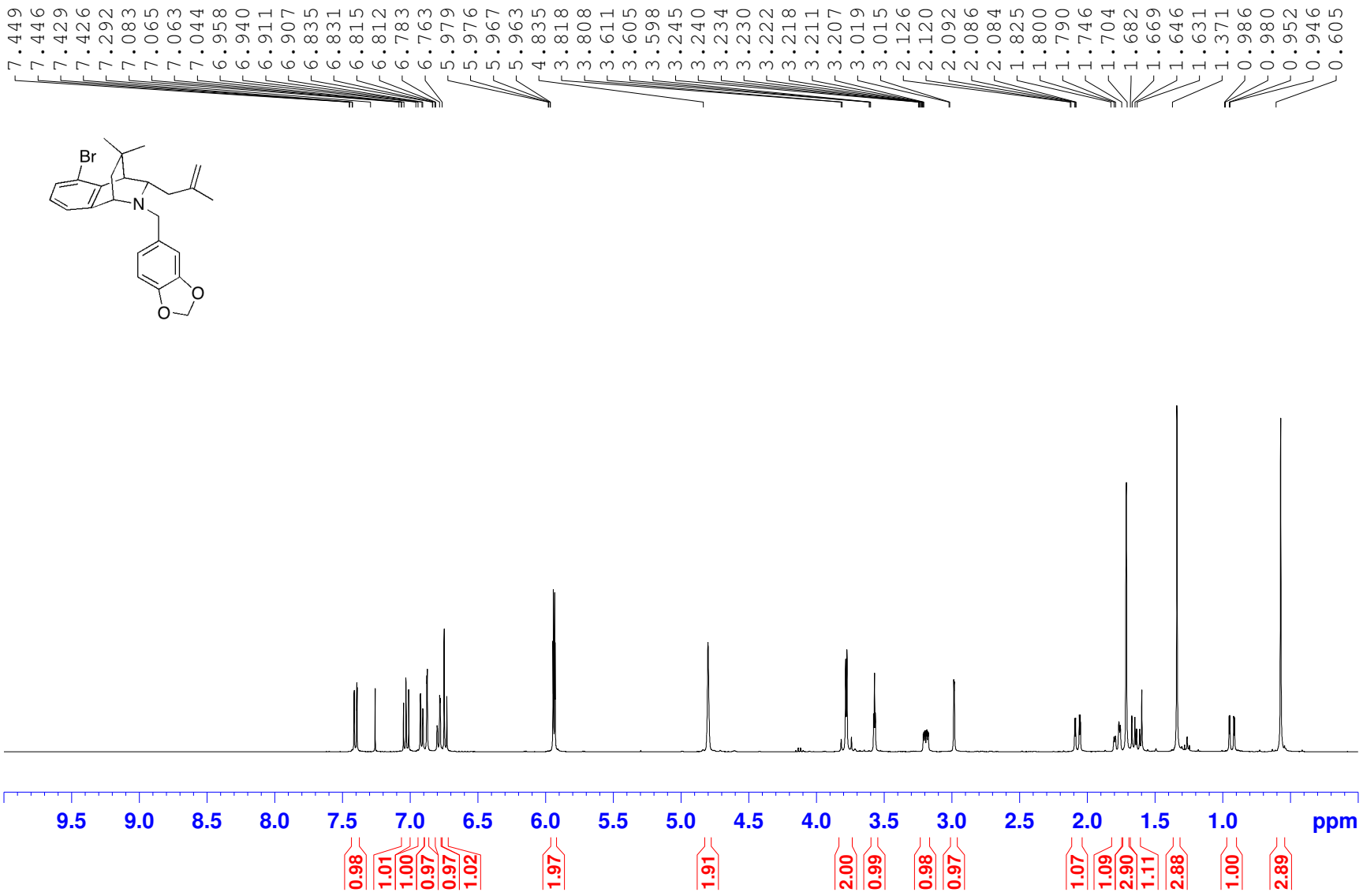

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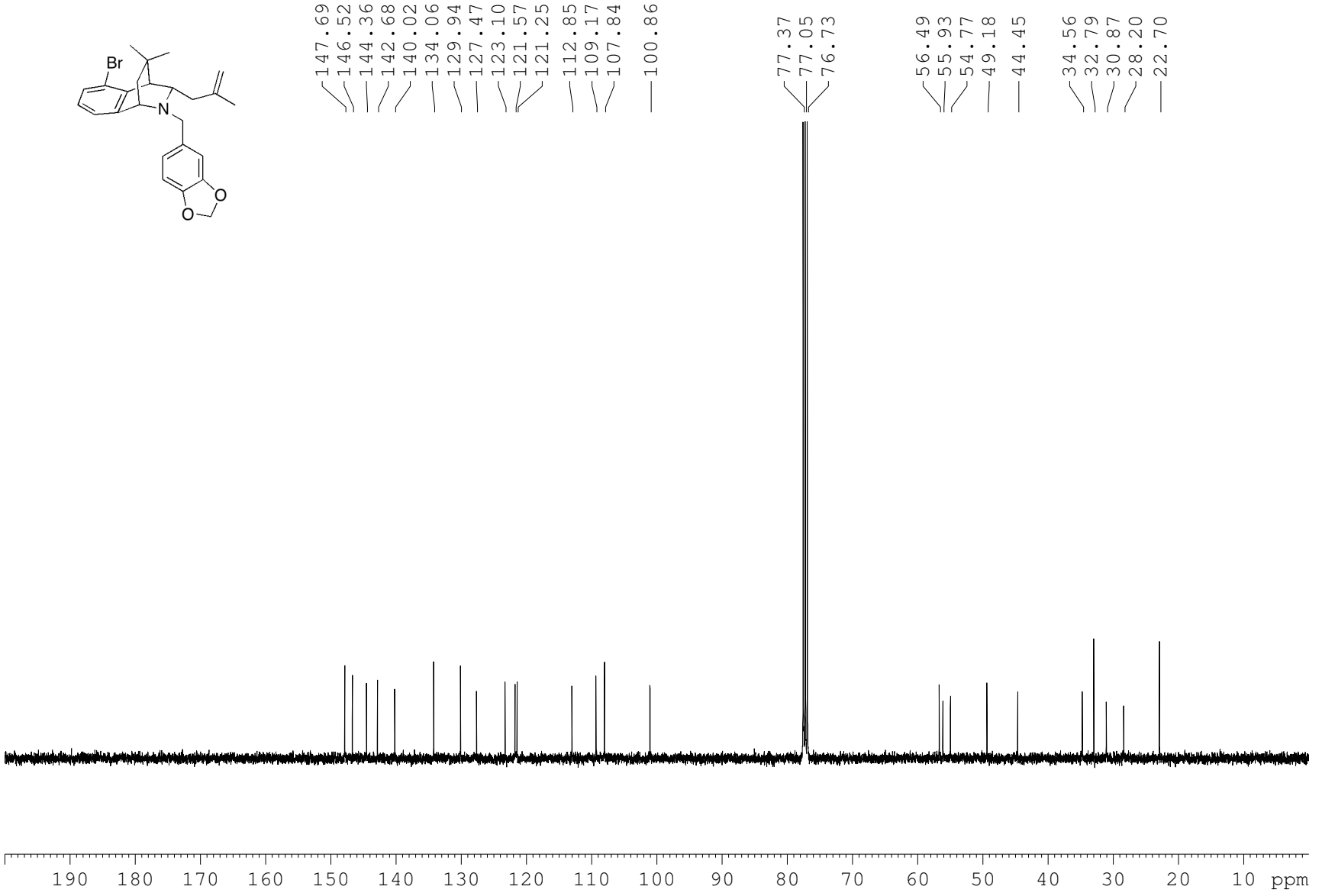

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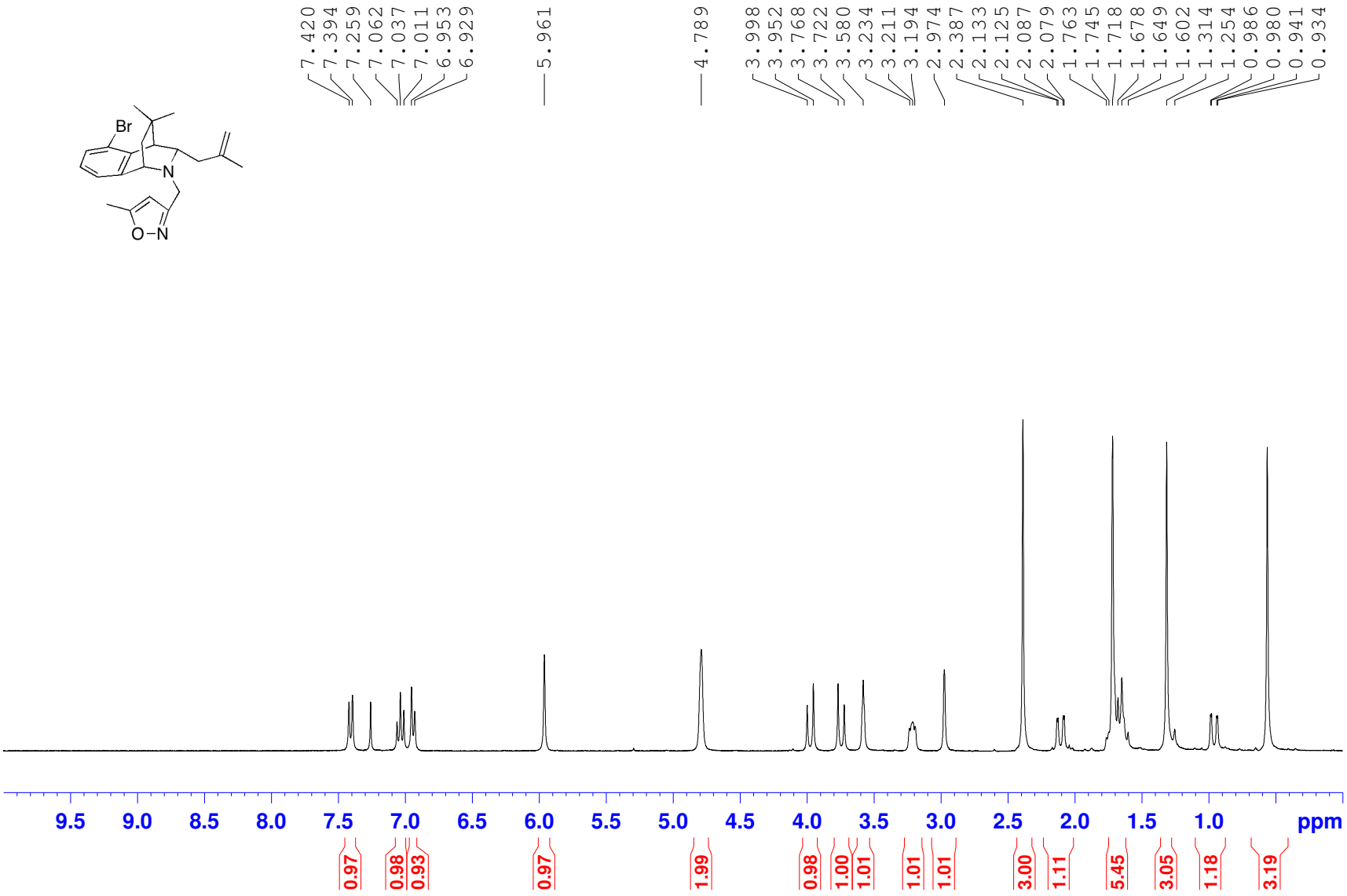

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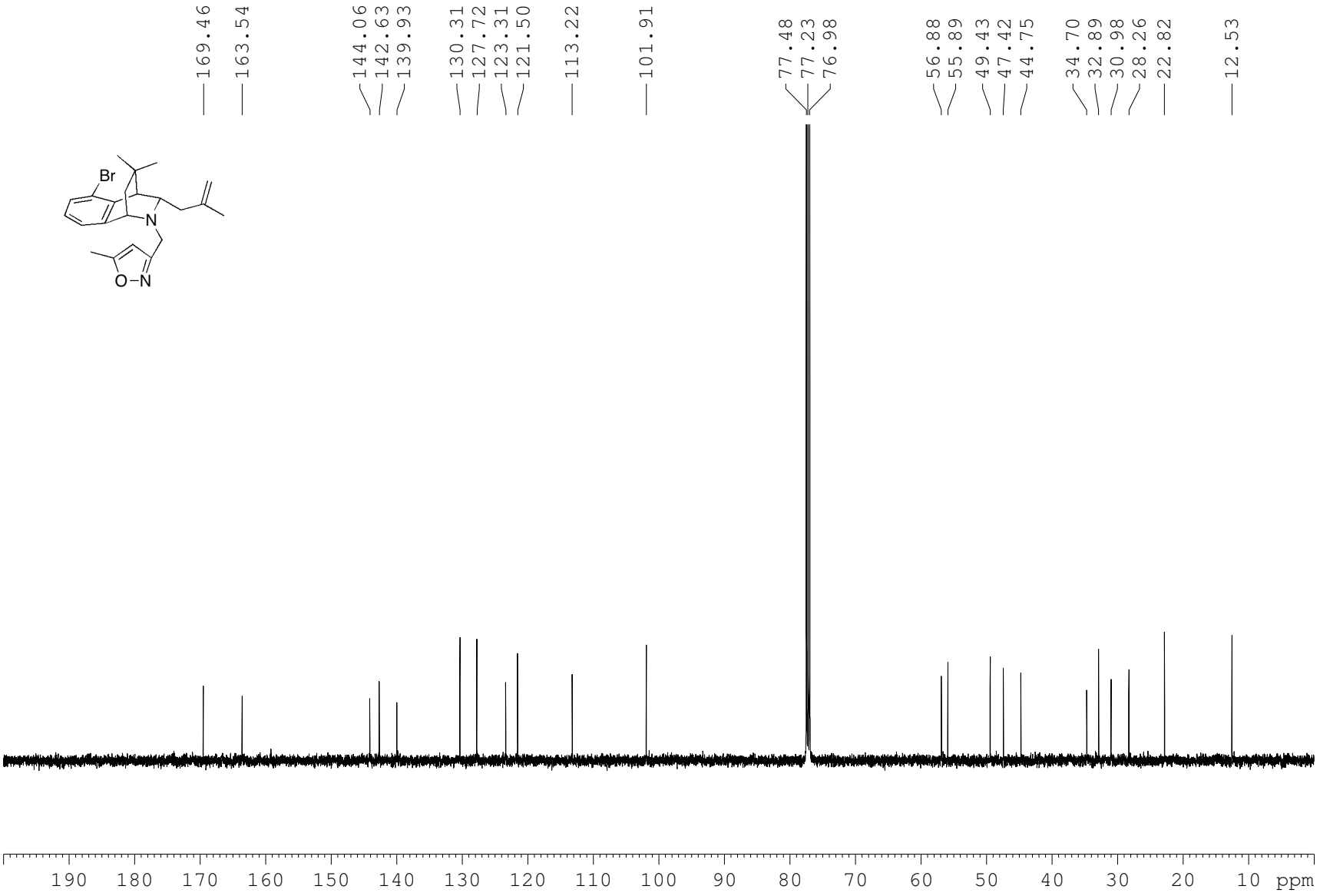

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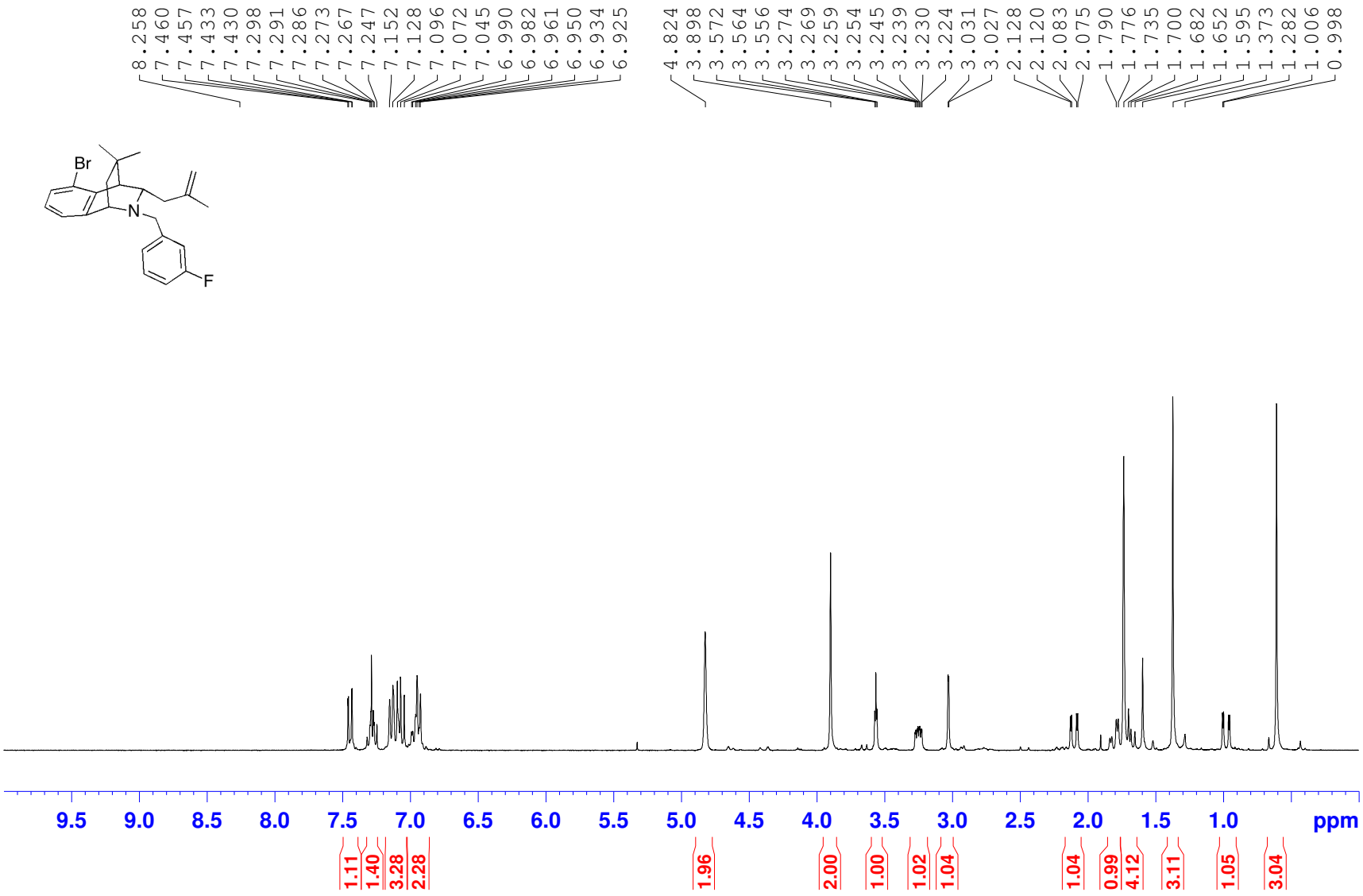

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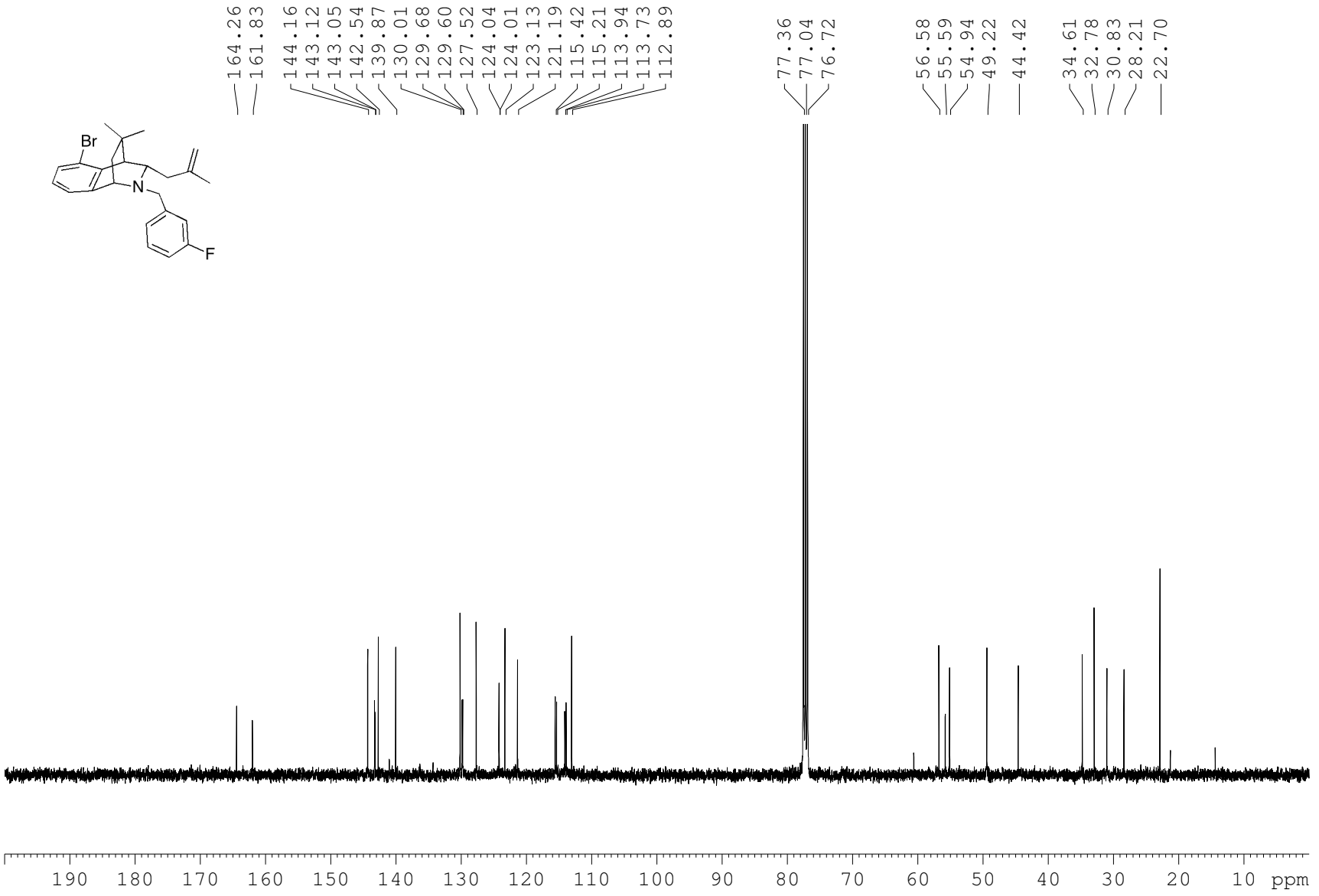

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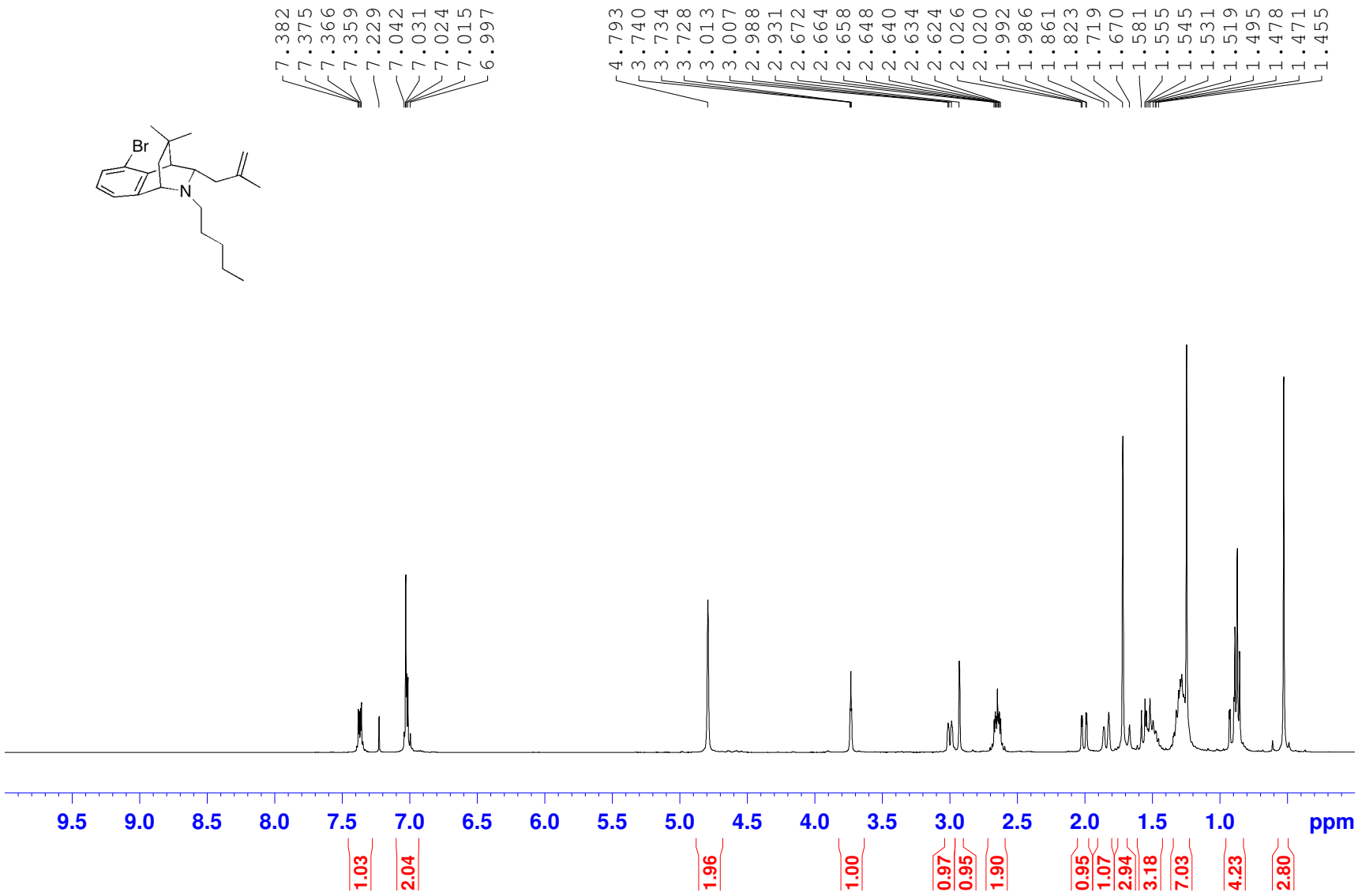

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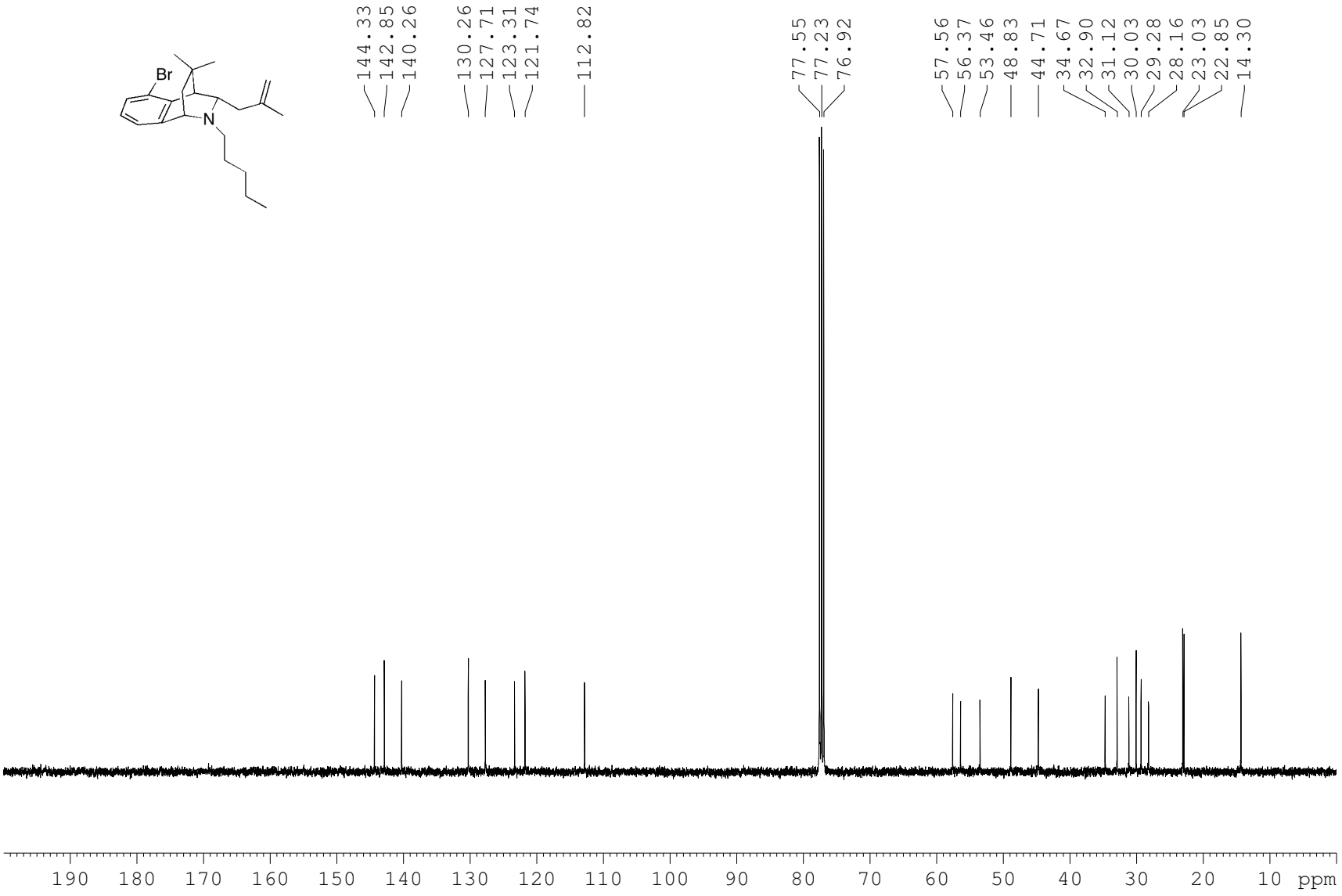

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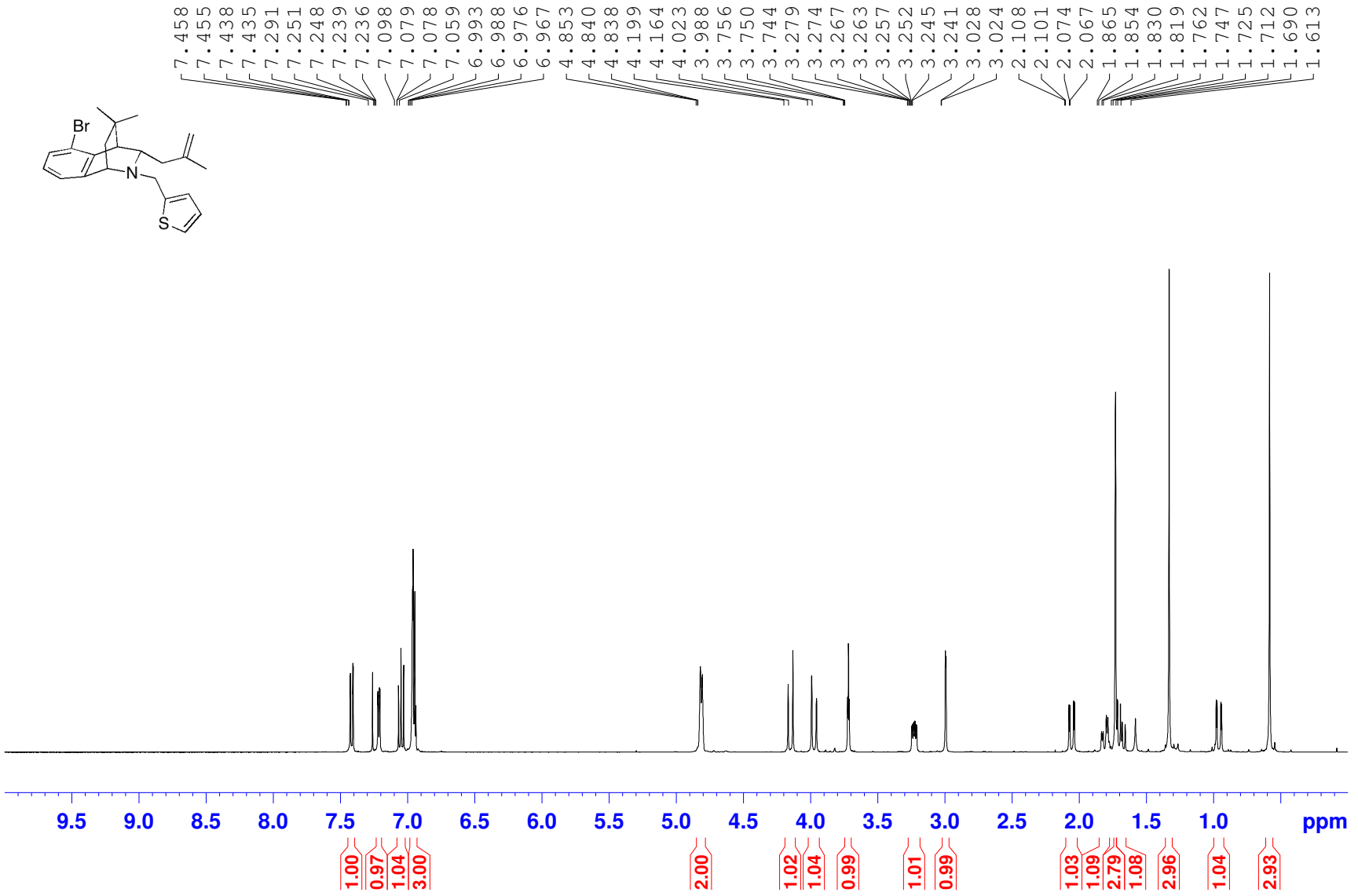

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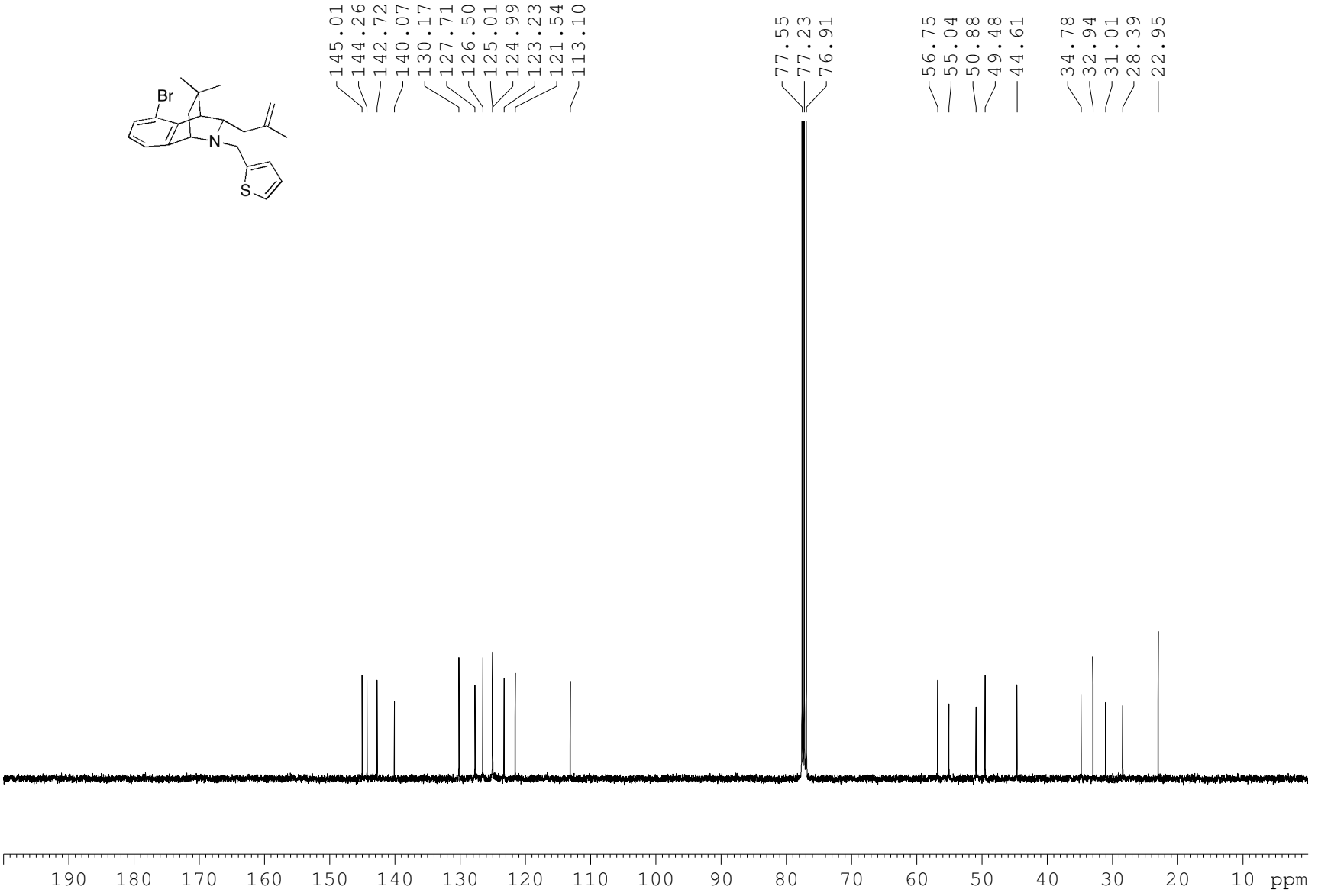

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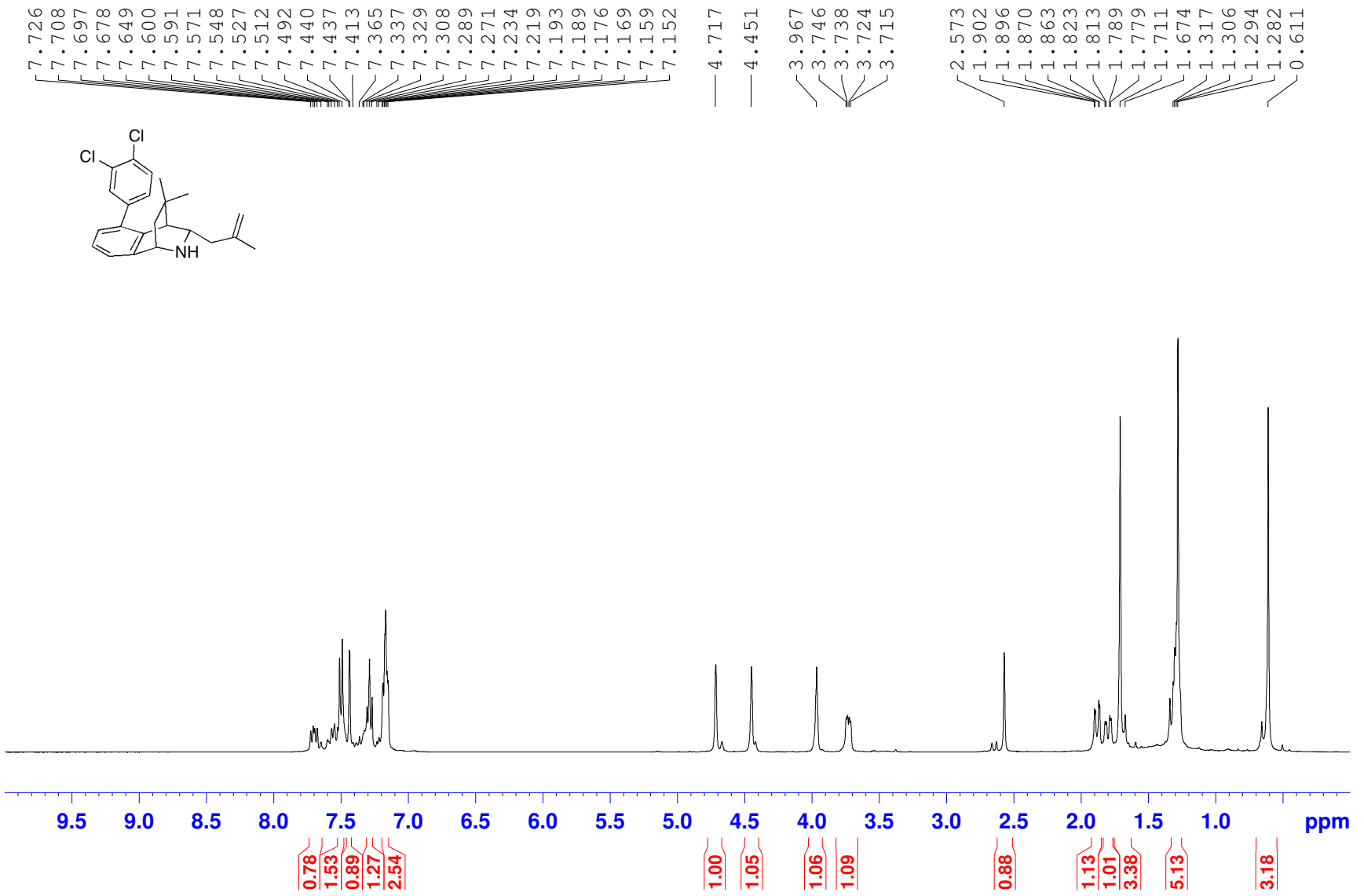

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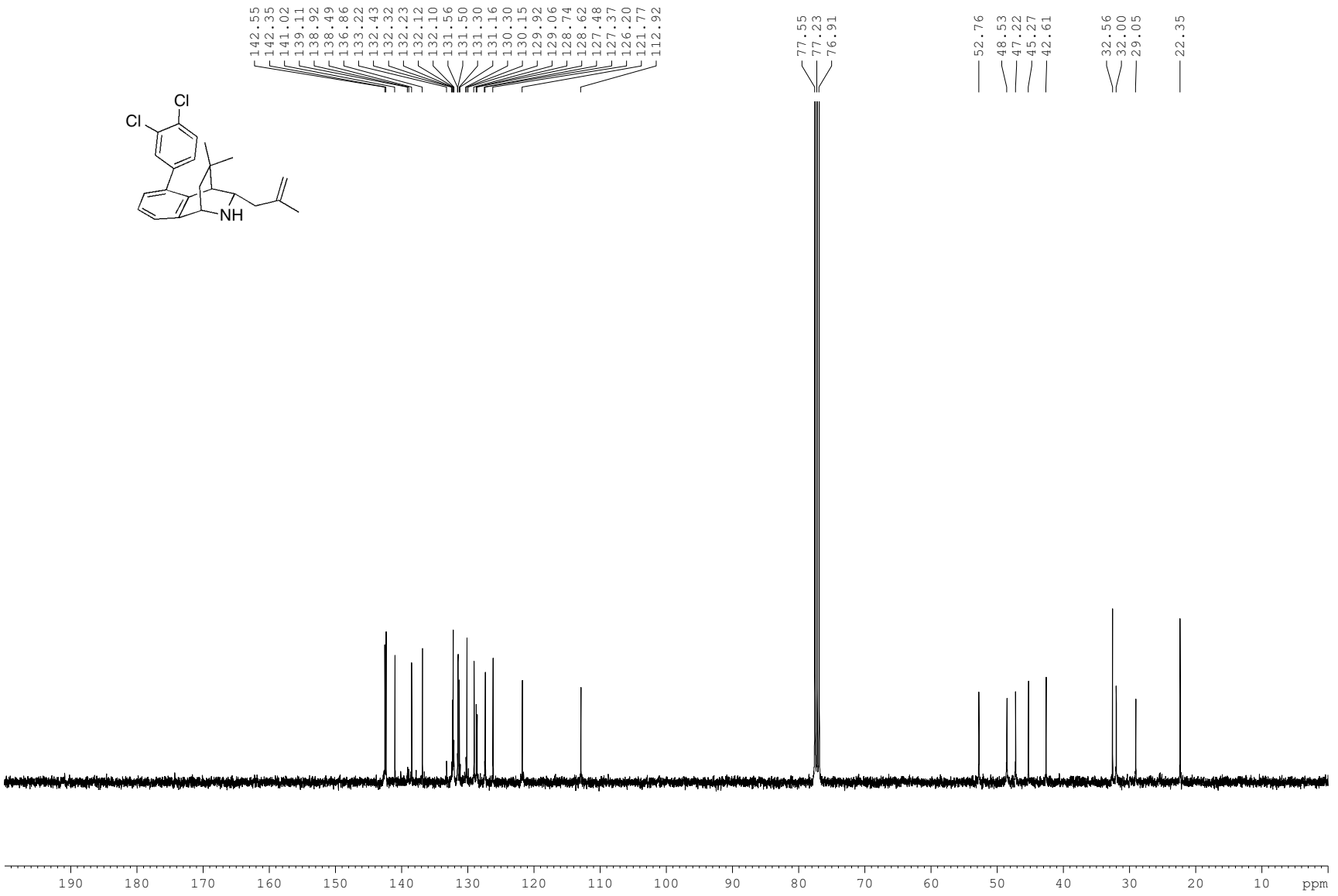

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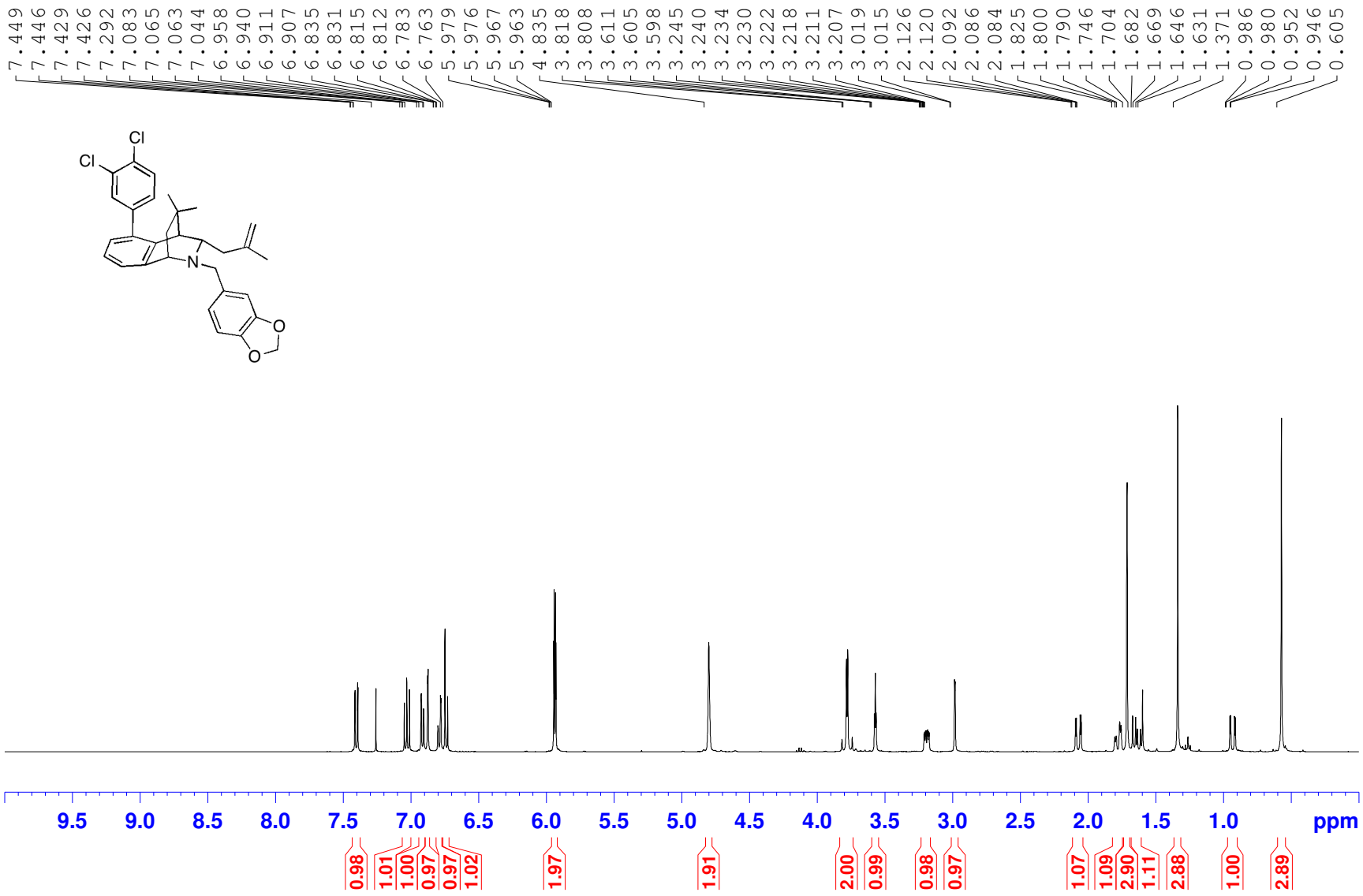

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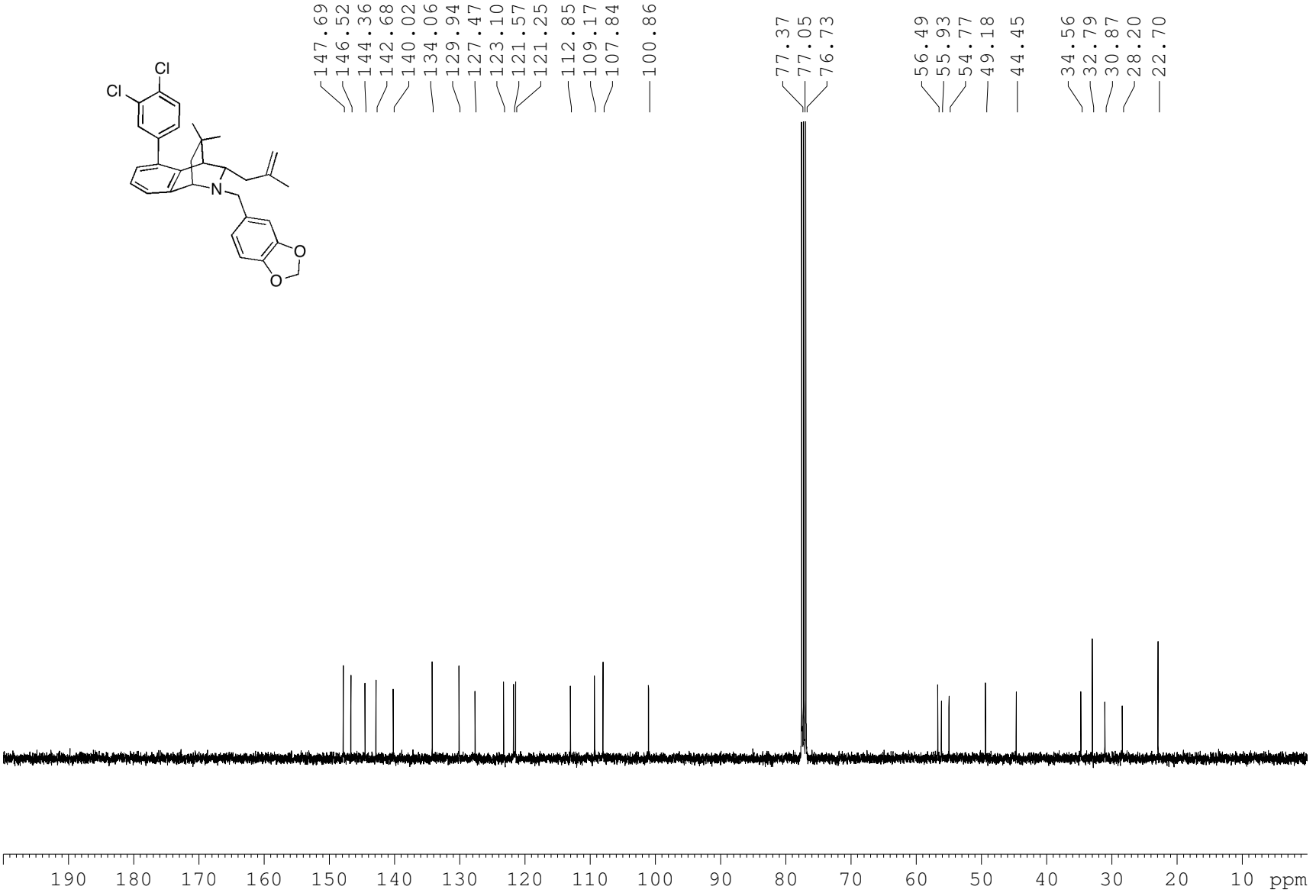

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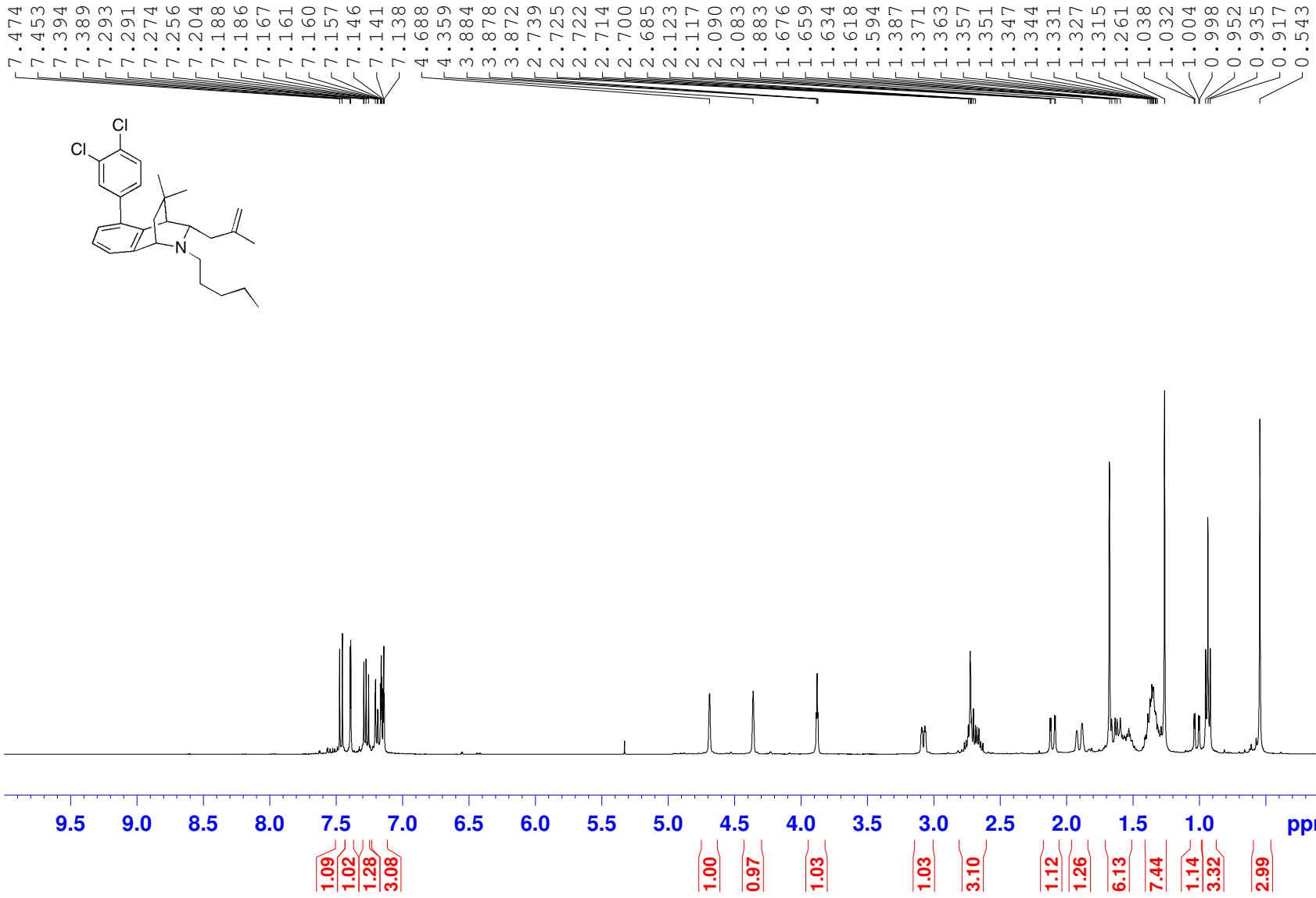

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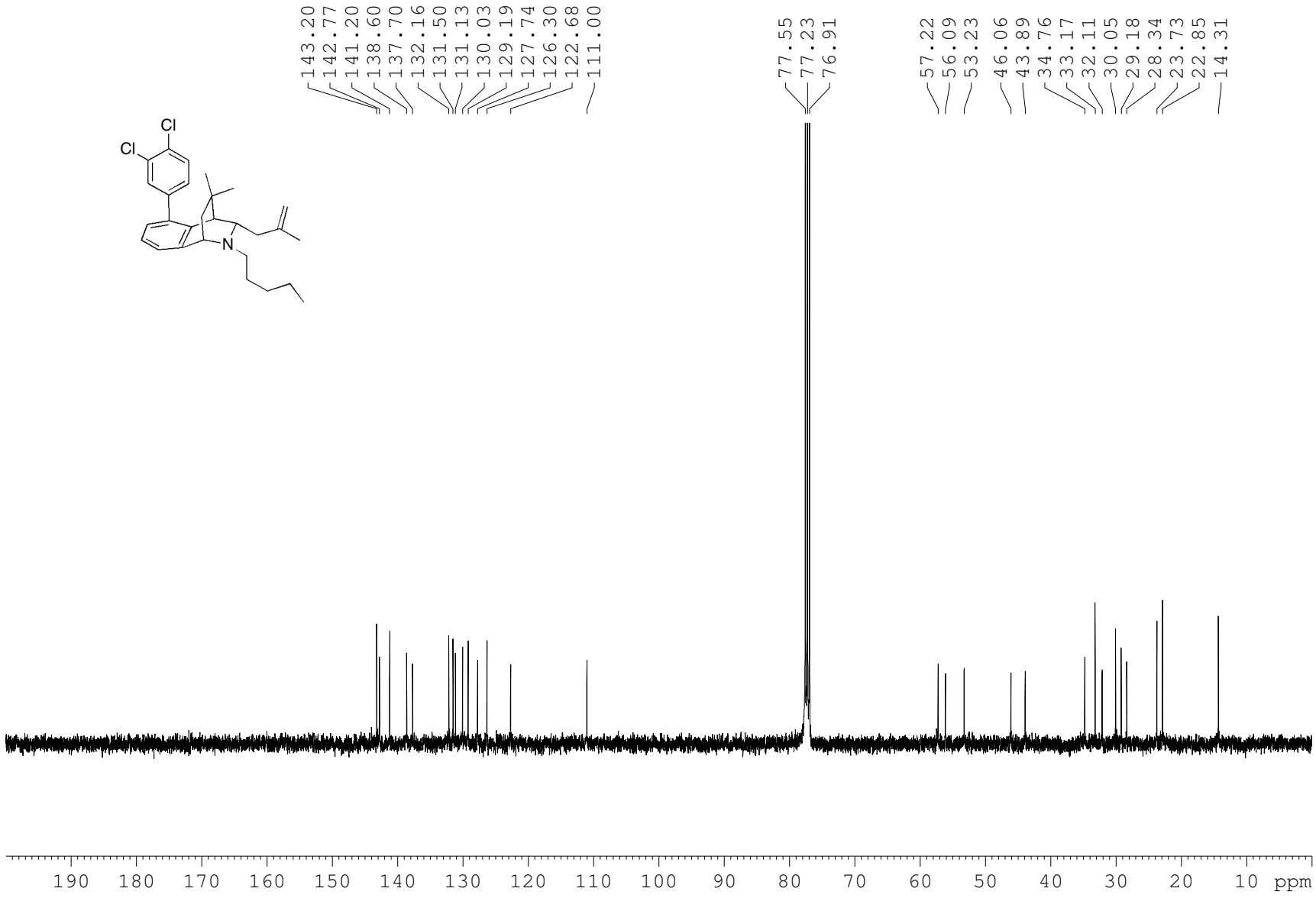

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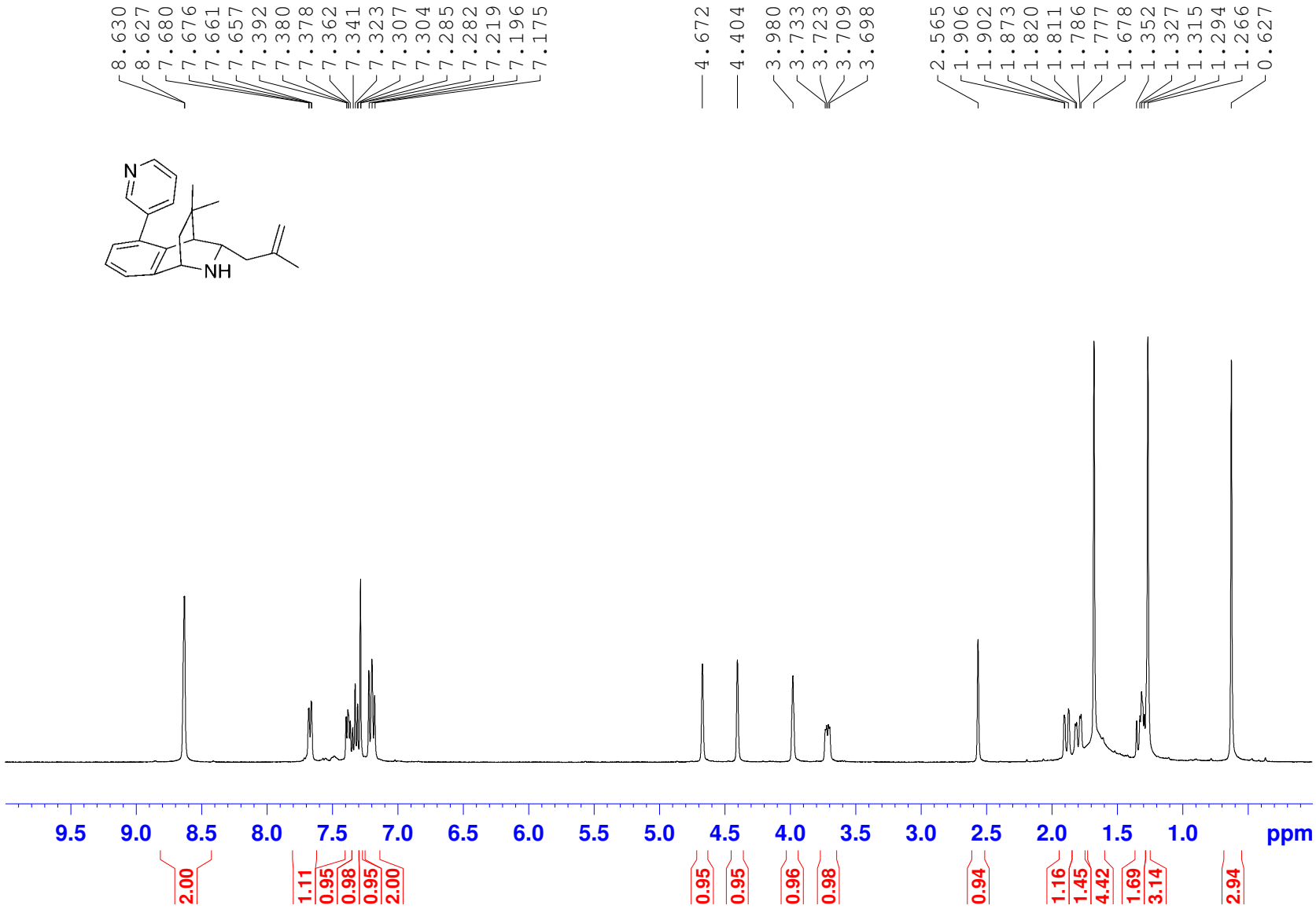

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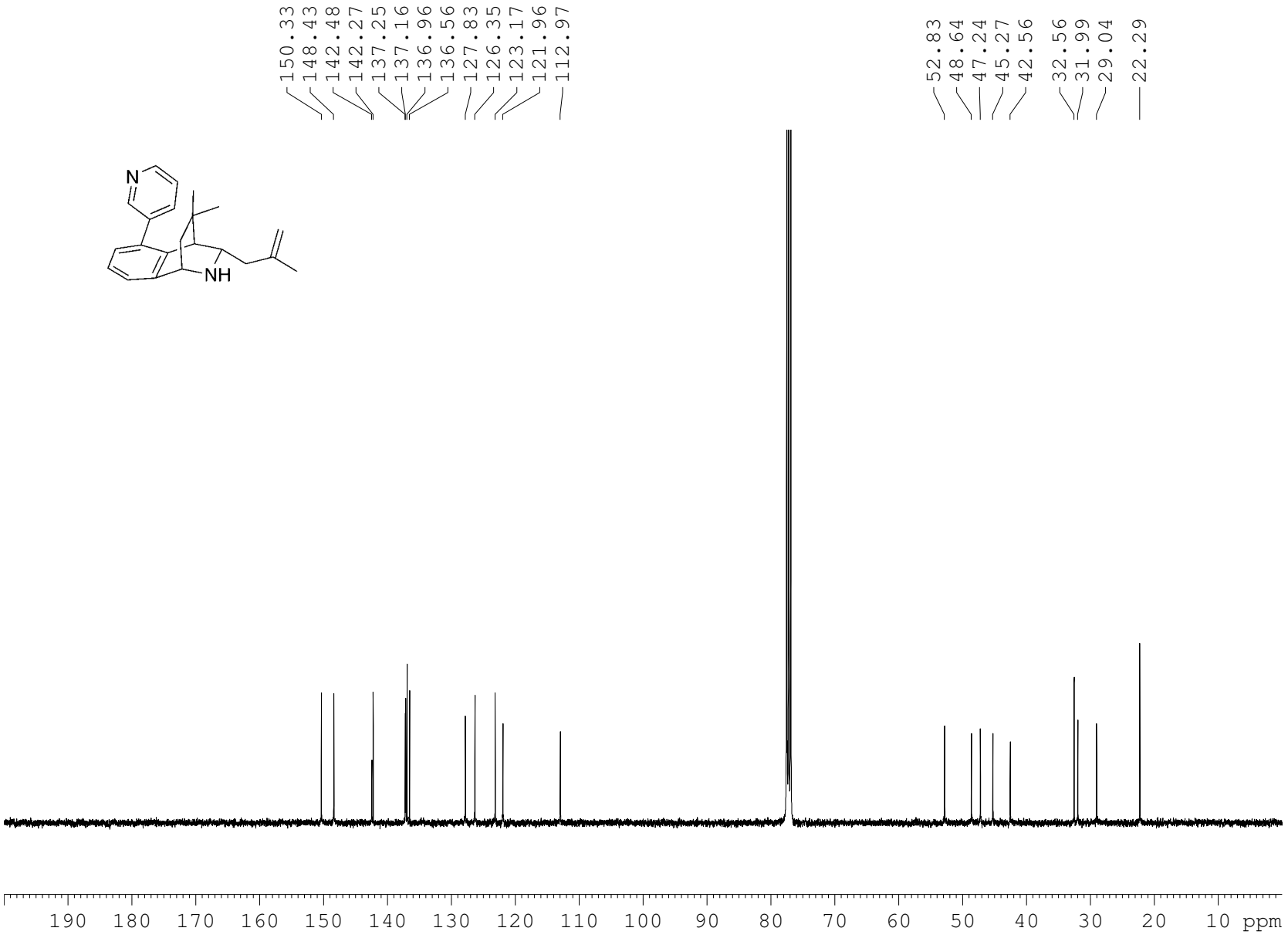

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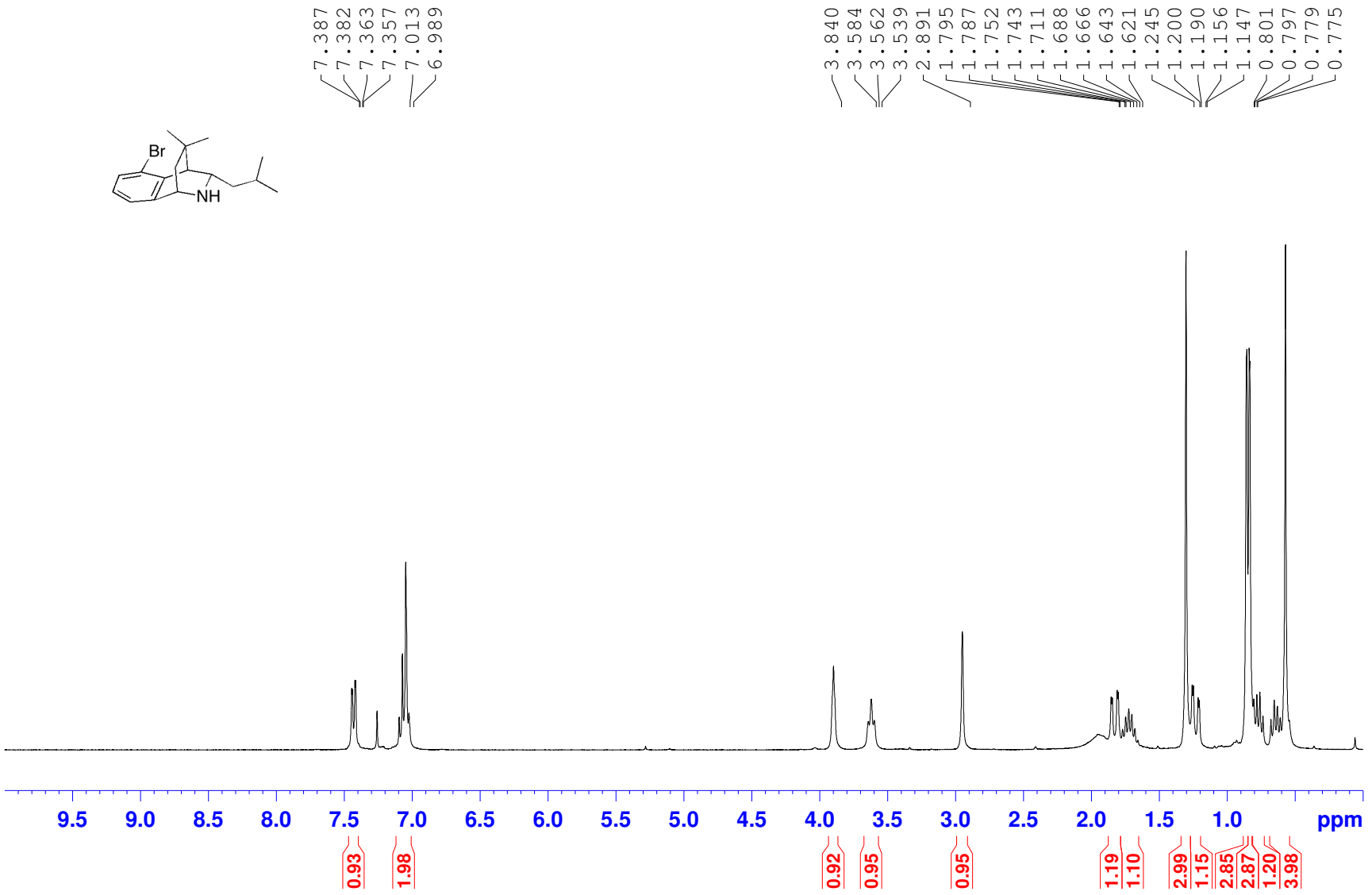

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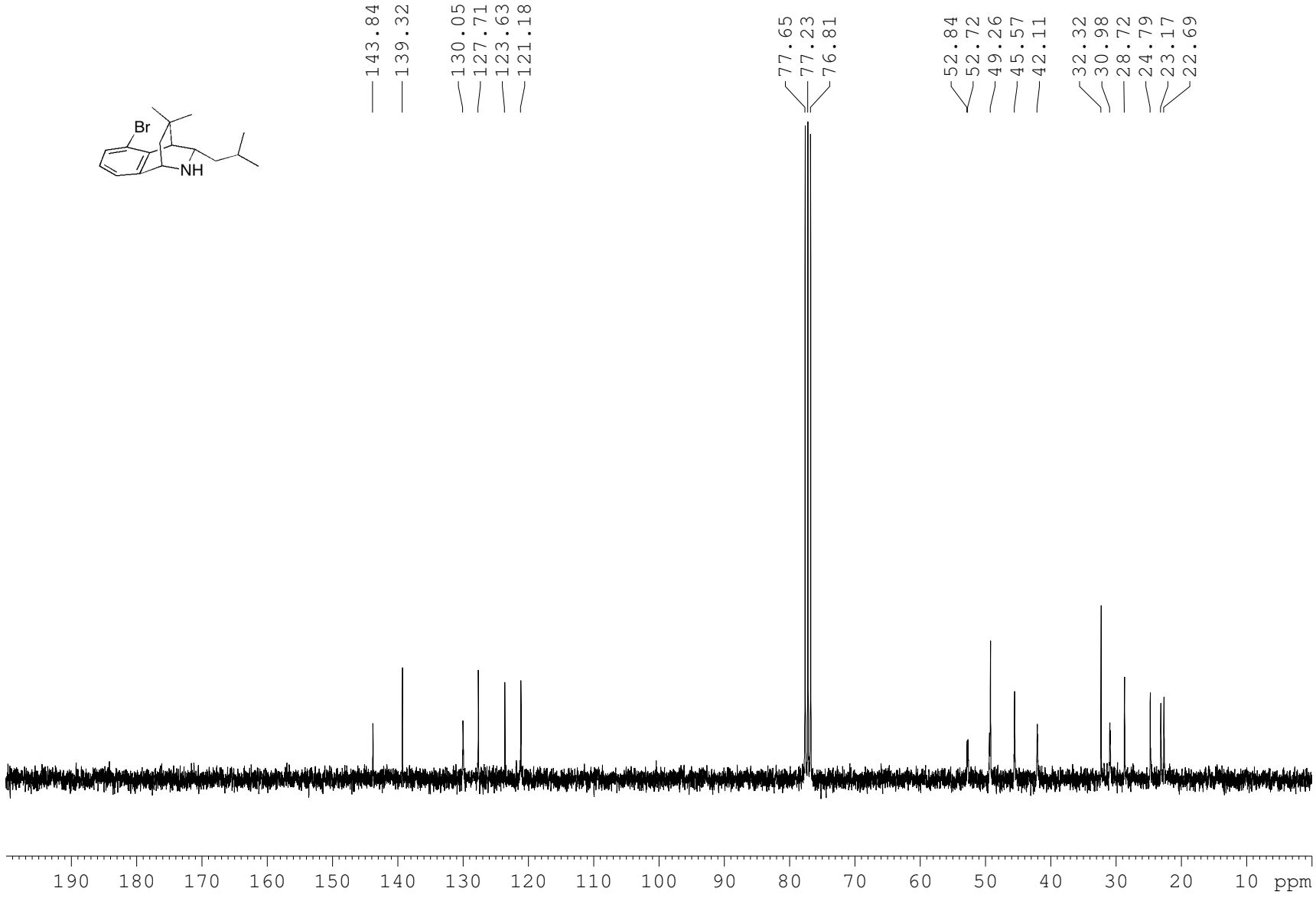

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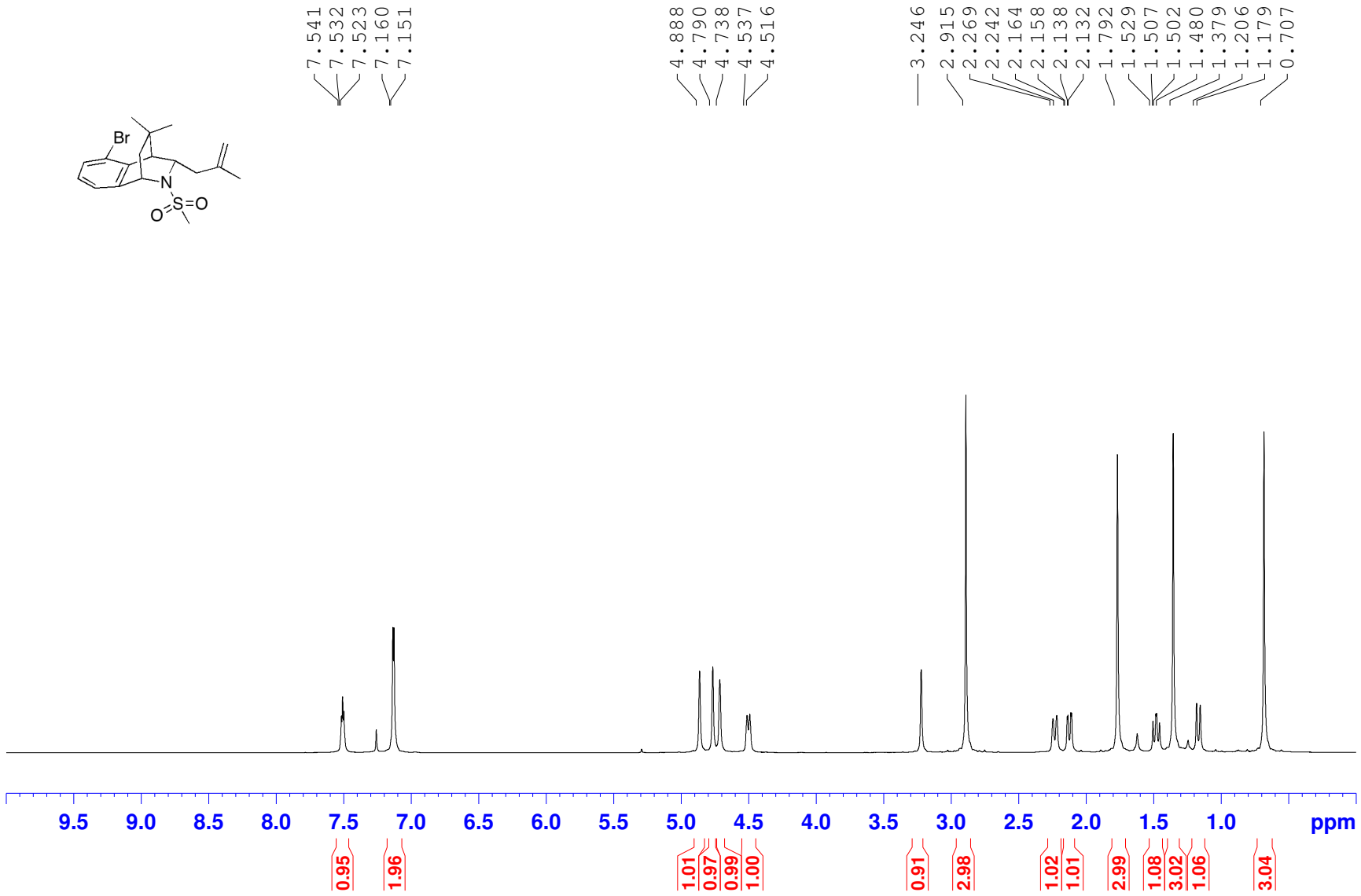

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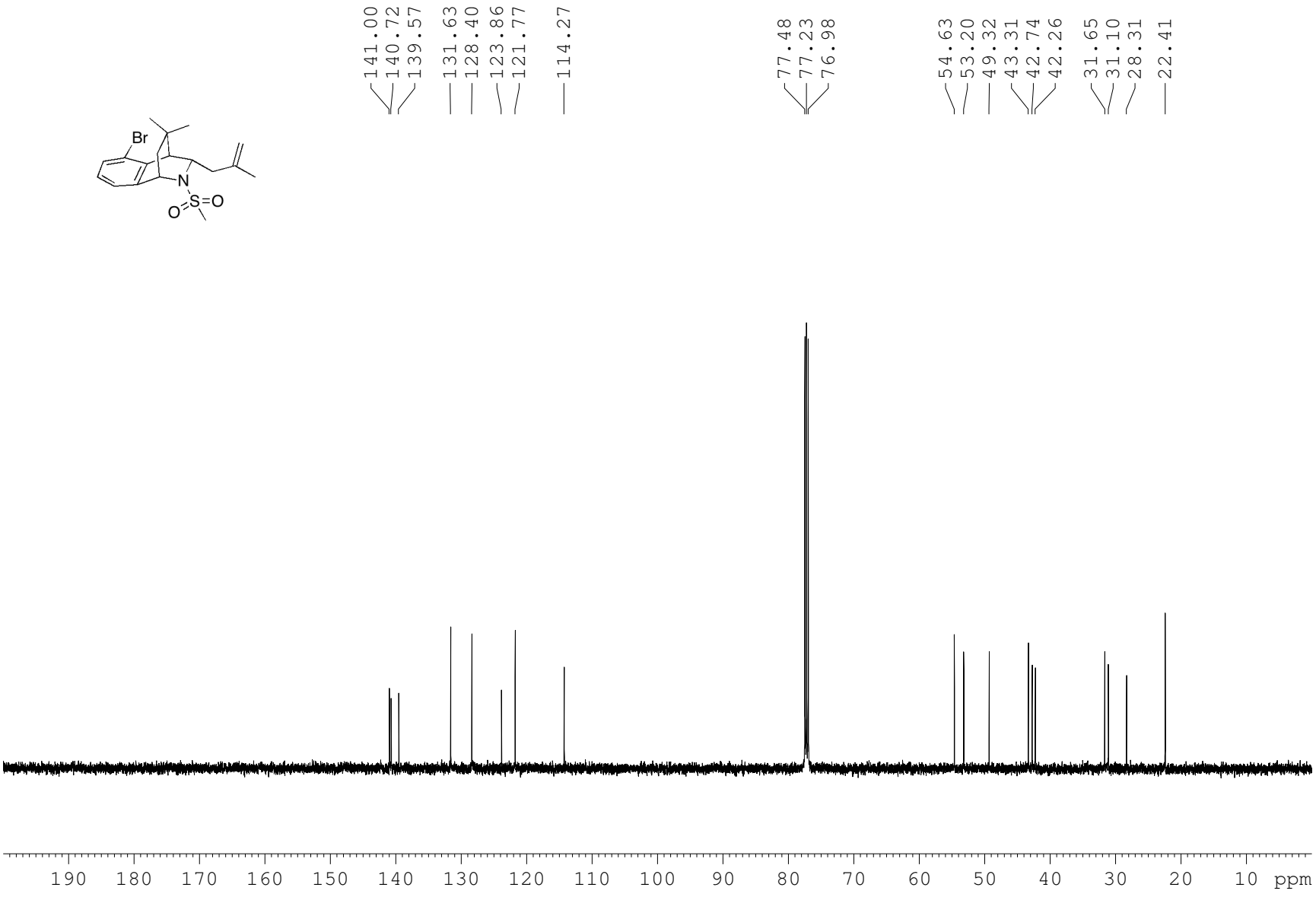

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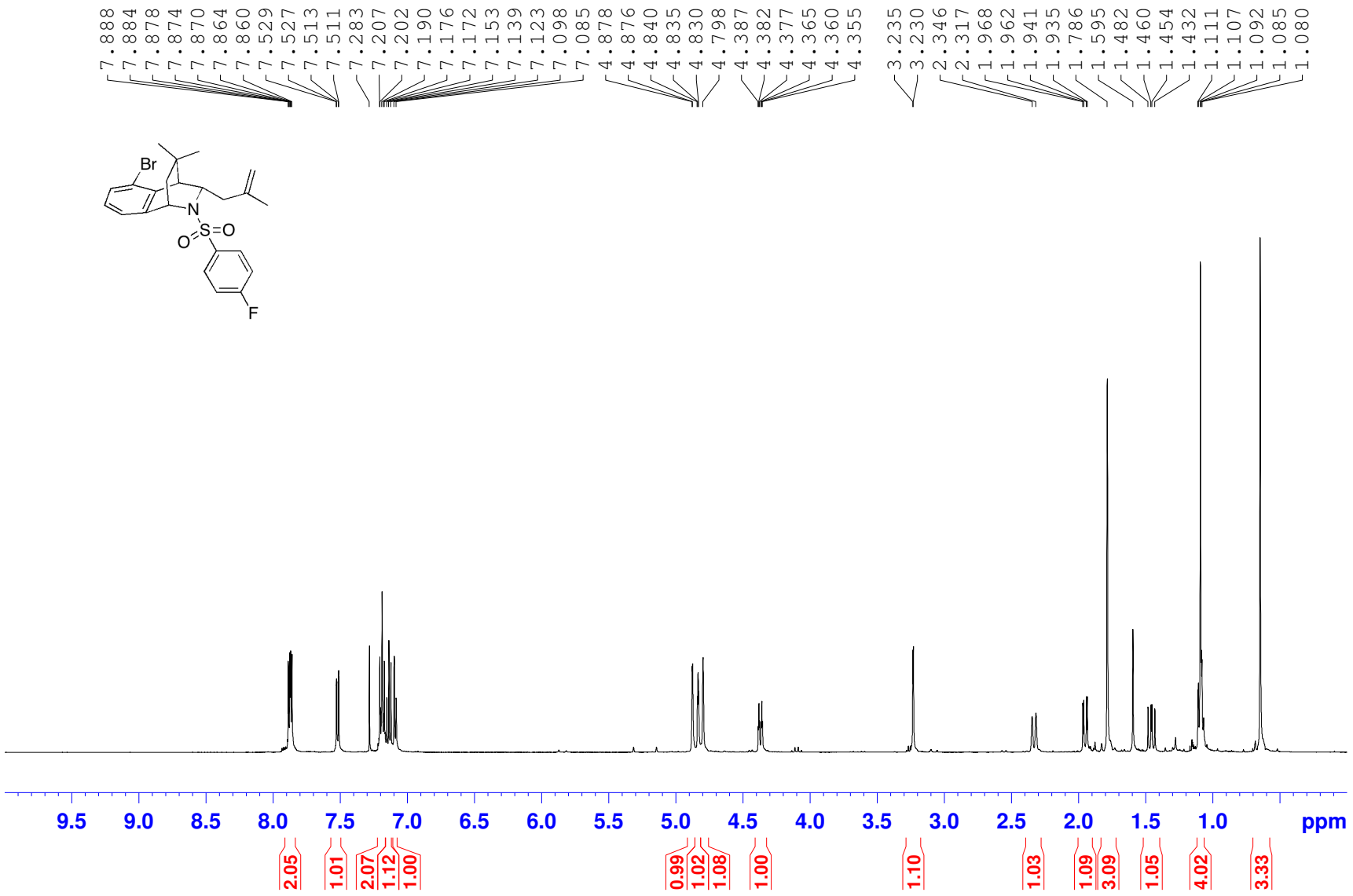

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