Deracemisation of Benzylisoquinoline Alkaloids Employing Monoamine Oxidase Variants

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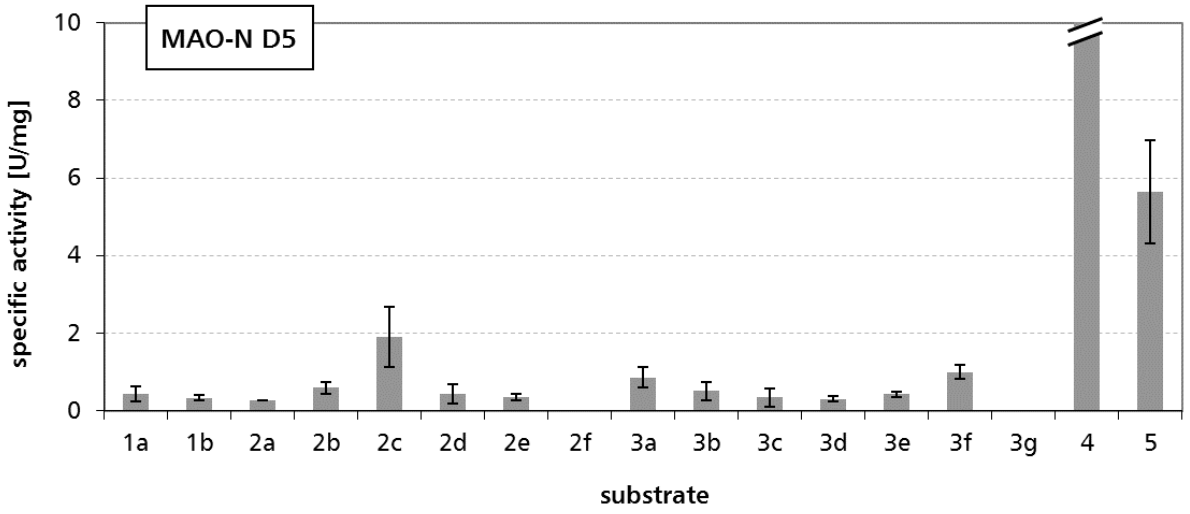
Electronic Supplementary Information

Contents:

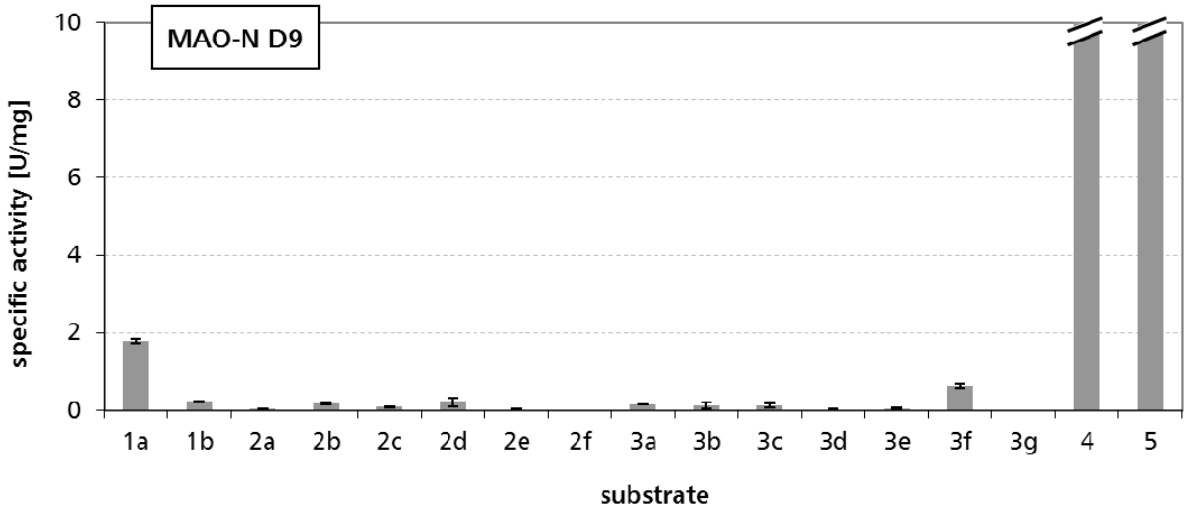
Supplementary Data

Substrate screening of MAO-N variants

The results obtained with MAO-N variants D5 and D9 in the colorimetric screening employing substrates **1a**, **1b**, **2a–f** and **3a–g** are presented in Supplementary Figure S1 and Supplementary Figure S2, respectively.



Supplementary Figure S1. Results of substrate screening with MAO-N variant D5. Activity (criterion: mean > 5 standard deviations) was only found for the positive controls *rac*-1-phenylethylamine **4** and *rac*-crispine A **5**. Error ranges represent standard deviations of triplicate experiments.



Supplementary Figure S2. Results of substrate screening with MAO-N variant D9. Activity (criterion: mean > 5 standard deviations) was found for substrates **1a**, **1b**, **2b**, **3a**, and **3f** as well as the positive controls *rac*-1-phenylethylamine **4** and *rac*-crispine A **5**. Error ranges represent standard deviations of triplicate experiments.

Improvement of Heterologous Protein Expression

Since the *maoN* gene has never been codon-optimised for heterologous expression in *E. coli*, rare codons in the nucleotide sequence may be a limiting factor, especially when using *E. coli* BL21 (DE3) as host. *E. coli* C43 (DE3) has been reported to be more suitable for expression of genes with rare codons,[1] and we therefore chose to investigate this strain along with BL21 (DE3), which has been used in previous studies. Both strains were transformed with the MAO-N expression plasmid and transformants were grown in LB and TB medium with and without IPTG induction. In addition, expression under auto-inducing conditions[2,3] was investigated. Strikingly, IPTG induction of the BL21 (DE3) cultures did not lead to any improvement in activity, and even appeared to be detrimental to growth of the bacterial culture, as indicated by the low cell yield (Supplementary Table S1).[4]

		Relative specific activity[a]		
medium	**cell yield [g/L]**	**wet cells**	**lyophilised cells**	**cell-free extract**
LB	6.2	1.0	0.9	0.4
LB + IPTG	3.6	1.0	0.9	0.5
TB	15.5	1.8	1.9	0.9
TB + IPTG	7.9	1.5	1.3	0.8
auto-induction	26.6	0.9	1.0	0.5

Supplementary Table S1. Cell yields and relative specific activities of MAO-N-expressing *E. coli* BL21 (DE3) cells from cultivation under different conditions. [a] Corrected for weight loss due to cell disruption and/or lyophilisation. 1 mg of wet cells with relative specific activity 1.0 oxidised 0.062 μmol of substrate **1a** per minute at 37 °C.

In contrast, IPTG induction of the C43(DE3) cultures resulted in a cell yield comparable to the one obtained under non-inducing conditions, and a higher specific activity (Supplementary Table S2). Growth in auto-inducing medium (4ZY-LAC-SUC)[3] allowed to achieve high cell mass and high specific activity, and offered the advantage that biocatalyst preparation could be carried out conveniently over the weekend.

		relative specific activity[a]		
medium	**cell yield [g/L]**	**wet cells**	**lyophilised cells**	**cell-free extract**
LB	6.3	1.0	0.9	0.4
LB + IPTG	6.6	7.2	7.2	5.4
TB	19.5	6.5	6.6	4.7
TB + IPTG	17.9	7.7	8.5	5.7
auto-induction	28.9	11.2	12.5	8.3

Supplementary Table S2. Cell yields and relative specific activities of MAO-N-expressing *E. coli* C43 (DE3) cells from cultivation under different conditions. [a] Corrected for weight loss due to cell disruption and/or lyophilisation. 1 mg of wet cells with relative specific activity 1.0 oxidised 0.016 μmol of substrate **1a** per minute at 37 °C.

Supplementary Methods

Synthesis of Substrates

1-Benzyl-2-methyl-1,2,3,4-tetrahydroisoquinoline (1a).

***t*-Butyl 1-benzyl-3,4-dihydro-2(1*H*)-isoquinolinecarboxylate.** A solution of *t*-butyl 3,4-dihydro-2(1*H*)-carboxylate[5] (2.33 g, 10.0 mmol) and tetramethylethylenediamine (1.22 g, 10.5 mmol) in anhydrous THF under argon atmosphere was cooled to –78 °C. *t*-Butyl lithium solution (1.7 M in pentane; 6.2 mL, 10.5 mmol) was added dropwise over 30 min, resulting in a deep-red solution which was stirred at –78 °C for 30 min. A solution of benzyl bromide (1.79 g, 10.5 mmol) in anhydrous THF (10 mL) was added dropwise over 30 min. The mixture was then stirred for 3 h, during which time the temperature was allowed to rise to –50 °C. The resulting yellow suspension was quenched with saturated aq. NH_4Cl solution (10 mL). Water (30 mL) was added, the phases were separated and the aqueous phase was extracted with *t*-butyl methyl ether (2 × 20 mL). The combined organic phases were dried over Na_2SO_4 and the solvent evaporated under reduced pressure to give 3.61 g of an orange liquid. Flash chromatography (silica; petrol ether/EtOAc = 95/5) afforded *t*-butyl 1-benzyl-3,4-dihydro-2(1*H*)-isoquinolinecarboxylate (1.79 g, 55%) as a colourless liquid. TLC (petrol ether/EtOAc = 3/1): R_f = 0.72. MS (EI, 70 eV): *m/z* = 266 (M^+–^{t}Bu, <1), 250 (3), 232 (9), 176 (43), 132 (100), 117 (9), 91 (10), 57 (13) . HRMS calcd for $C_{17}H_{16}NO_2$ (M^+–^{t}Bu): 266.1181; found: 266.1183.

NMR spectroscopy revealed that the product is a mixture of isomers (ratio *cis/trans* = 2/1). Based on the peak intensities as well as the HSQC spectrum, the NMR signals were assigned to the isomers as follows:

cis-t-Butyl 1-benzyl-3,4-dihydro-2(1*H*)-isoquinolinecarboxylate. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 1.14 (9H, s, CH_3), 2.51–3.02 (4H, m, CH_2), 3.22 (1H, ddd, J_1 = 13.3 Hz, J_2 = 10.8 Hz, J_3 = 4.3 Hz, CH_2), 4.14 (1H, ddd, J_1 = 13.1 Hz, J_2 = 5.7 Hz, J_3 = 3.3 Hz, CH_2), 5.15 (1H, dd, J_1 = 8.2 Hz, J_2 = 5.8 Hz, CH), 6.97–7.23 (9H, m, Ar). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ = 28.1, 28.5, 36.9, 43.0, 56.8, 79.5, 125.9, 126.3, 126.6, 127.2, 128.3, 129.1, 129.7, 134.7, 137.1, 138.6, 154.5.

trans-t-Butyl 1-benzyl-3,4-dihydro-2(1*H*)-isoquinolinecarboxylate. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 1.33 (9H, s, CH_3), 2.51–3.02 (4H, m, CH_2), 3.22–3.32 (1H, m, CH_2), 3.68–3.76 (1H, m, CH_2), 5.29 (1H, t, J = 6.8 Hz, CH), 6.97–7.32 (9H, m, Ar). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ = 28.1, 28.4, 39.3, 42.7, 55.7, 79.4, 126.2, 126.4, 126.5, 127.5, 128.0, 128.4, 129.7, 134.5, 137.0, 138.2, 154.9.

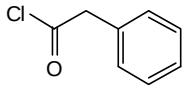
The ^{1}H-NMR data are in accordance with literature values.[5] No ^{13}C-NMR reference data were available for this compound.

1-Benzyl-2-methyl-1,2,3,4-tetrahydroisoquinoline (1a): A solution of *t*-butyl 1-benzyl-3,4-dihydro-2(1*H*)-isoquinolinecarboxylate (1.70 g, 5.25 mmol) in anhydrous THF (40 mL) under argon atmosphere was cooled to 0 °C on an ice bath. $LiAlH_4$ (0.99 g, 26.1 mmol) was added in portions to the stirred solution; afterwards the ice bath was removed and the mixture was refluxed for 16 h. The suspension was diluted with THF (30 mL) and cooled to 0 °C on an ice bath. To the vigorously stirred mixture were added water (990 μL), 15% aq. NaOH solution (990 μL) and water (2.97 mL), the ice bath was removed and stirring continued for 1 h at room temperature. The resulting suspension was filtered through Celite, washed thoroughly with THF (100 mL), dried over Na_2SO_4 and evaporated under reduced pressure to give 1.14 g of a yellowish liquid. Flash chromatography (silica; CH_2Cl_2/MeOH/NH_3(aq) = 96/3/1) afforded 1-benzyl-2-methyl-1,2,3,4-tetrahydroisoquinoline (0.75 g, 61%) as a yellowish liquid. TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.55. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 2.40 (3H, s, NCH_3), 2.56 (1H, dt, J_1 = 15.8 Hz, J_2 = 4.8 Hz,

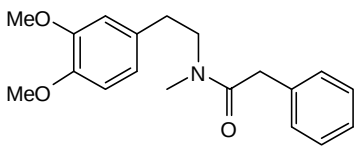
CH_2), 2.66 (1H, dt, J_1 = 12.3 Hz, J_2 = 5.0 Hz, CH_2), 2.73−2.84 (2H, m, CH_2), 3.73 (1H, t, J = 6.2 Hz, CH), 6.67 (1H, d, J = 7.6 Hz, Ar), 6.90−7.18 (8H, m, Ar). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ = 26.1, 41.5, 42.9, 47.2, 65.2, 125.3, 125.9, 126.0, 128.0, 128.1, 128.8, 129.7, 134.4, 137.9, 140.1. MS (EI, 70 eV): m/z = 236 [$(M–H)^+$, <1], 146 (100), 131 (8), 91 (5). HRMS calcd for $C_{17}H_{18}N$ (M^+–H): 236.1439; found: 236.1463. The ^{1}H-NMR data are in accordance with literature values.[6] No ^{13}C-NMR reference data were available for this compound.

1-Benzyl-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (2a).

Phenylacetyl chloride. A solution of phenylacetic acid (2.00 g, 14.7 mmol), oxalyl chloride (2.81 g, 22.1 mmol) and one drop of DMF in dry toluene (40 mL) was stirred at room temperature under argon for 1 h. The solvent was evaporated under reduced pressure to give 2.27 g (quant.) of phenylacetyl chloride as a brownish liquid, which was used in the following transformation without further purification. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 4.17 (2H, s, CH_2-COCl), 7.28–7.43 (5H, m, Ar). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ = 53.1, 128.2, 129.0, 129.4, 129.5, 131.2, 171.9. The NMR data are in accordance with literature values.[7]



***N*-(3,4-Dimethoxyphenethyl)-*N*-methyl-2-phenylacetamide.** *N*-Methylhomoveratrylamine (3.11 g, 15.9 mmol) was dissolved in $CHCl_3$ (30 mL). 3% aq. NaOH solution (150 mL) was added and the mixture was cooled to 0 °C on an ice bath. A solution of phenylacetyl chloride (2.25 g, 14.6 mmol) in chloroform (30 mL) was added dropwise over 1 h to the vigorously stirred mixture. The ice bath was removed and stirring was continued overnight at room temperature. The phases were separated and the aqueous phase was extracted with $CHCl_3$ (50 mL). The combined organic phases were washed with 1 M aq. HCl solution (100 mL), then water (100 mL) and dried over Na_2SO_4. Evaporation of the solvent under reduced pressure yielded 4.67 g of a highly viscous yellow liquid. Flash chromatography (silica; petrol ether/EtOAc = 1/1) afforded *N*-(3,4-dimethoxyphenethyl)-*N*-methyl-2-phenylacetamide (3.93 g, 85%) as a yellowish liquid. TLC (petrol ether/EtOAc = 1/1): R_f = 0.25. MS (EI, 70 eV): m/z = 313 (M^+, 3), 164 (100), 151 (12), 91 (30). HRMS calcd for $C_{19}H_{23}NO_3$: 313.1678; found: 313.1703.



NMR spectroscopy revealed that the product is a mixture of isomers (ratio *trans/cis* = 1.25/1). Based on the peak intensities as well as the DEPT, COSY and HMQC spectra, the NMR signals were assigned to the isomers as follows:

trans-N-(3,4-Dimethoxyphenethyl)-*N*-methyl-2-phenylacetamide. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 2.71 (2H, t, J = 7.5 Hz, CH_2-CH_2-N), 2.79 (3H, s, N-CH_3), 3.51 (2H, t, J = 7.5 Hz, CH_2-CH_2-N), 3.60 (2H, s, CH_2-CO), 3.73 (3H, s, OCH_3), 3.77 (3H, s, OCH_3), 6.60–6.69 (3H, m, Ar), 7.06–7.25 (5H, m, Ar). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ = 33.2 (CH_2), 36.5 (CH_3), 41.3 (CH_2), 50.1 (CH_2), 55.8 (CH_3), 55.9 (CH_3), 111.3 (CH), 112.0 (CH), 120.7 (CH), 126.7 (CH), 128.6 (CH), 128.7 (CH), 131.6 (C), 135.0 (C), 147.5 (C), 148.9 (C), 170.8 (C). No NMR reference data were available for this compound.

cis-N-(3,4-Dimethoxyphenethyl)-*N*-methyl-2-phenylacetamide. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 2.57 (2H, t, J = 7.2 Hz, CH_2-CH_2-N), 2.90 (3H, s, N-CH_3), 3.37–3.42 (4H, t + s overlap, CH_2-CH_2-N + CH_2-CO), 3.76 (3H, s, OCH_3), 3.77 (3H, s, OCH_3), 6.48 (1H, d, J = 2.0 Hz, Ar-2), 6.54 (1H, dd, J_1 = 8.1 Hz, J_2 = 2.0 Hz, Ar-6), 6.72 (1H, d, J = 8.1 Hz, Ar-5), 7.06–7.25 (5H, m, Ar). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ = 33.6 (CH_3), 34.3 (CH_2), 40.7 (CH_2), 52.2 (CH_2), 55.8 (CH_3), 55.9 (CH_3), 111.5 (CH), 112.0 (CH), 120.8 (CH), 126.7 (CH), 128.6 (CH), 128.7 (CH), 130.7 (C), 135.3 (C), 147.9 (C), 149.1 (C), 171.0 (C). No NMR reference data were available for this compound.

1-Benzyl-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (2a). A solution of *N*-(3,4-dimethoxyphenethyl)-*N*-methyl-2-phenylacetamide (3.93 g, 12.6 mmol) and $POCl_3$ (5.88 g, 38.4 mmol) in dry acetonitrile (50 mL) was refluxed for 4 h under argon atmosphere. The solvent and excess $POCl_3$ were evaporated under reduced pressure and the residue was dissolved in dry methanol (50 mL), put under argon and cooled to 0 °C on an ice bath. $NaBH_4$ (2.38 g, 62.9 mmol) was added in portions to the stirred mixture. The ice bath was then removed and stirring continued for 16 h at room temperature. The solvent was evaporated and the residue was treated with half-saturated aq. Na_2CO_3 solution (100 mL). The product was extracted with CH_2Cl_2 (3 × 30 mL), the combined organic phases were dried over Na_2SO_4 and evaporated under reduced pressure to give 3.71 g of a yellow liquid. Flash chromatography (silica; CH_2Cl_2/MeOH/NH_3(aq) = 95/4/1) afforded 1-benzyl-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (3.34 g, 89%) as a yellowish liquid that crystallised upon standing in the fridge to an off-white solid. mp: 70–71 °C (ref.[8] 79–81 °C). TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.59. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 2.57 (3H, s, NCH_3), 2.61–2.68 (1H, m CH_2), 2.78–2.88 (3H, m, CH_2), 3.23–3.31 (2H, m, CH_2), 3.51 (3H, s, OCH_3), 3.74–3.79 (1H, m, CH), 3.85 (3H, s, OCH_3), 5.94 (1H, s, Ar), 6.58 (1H, s, Ar), 7.11–7.30 (5H, m, Ar). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ = 25.3, 41.2, 42.5, 46.6, 55.4, 55.7, 64.9, 111.0, 111.1, 125.5, 126.0, 128.2, 128.9, 129.9, 129.9, 146.2, 147.3. MS (EI, 70 eV): *m/z* = 296 (M^+–H, <1), 206 (100), 190 (15), 162 (5). HRMS calcd for $C_{19}H_{22}NO_2$ (M^+–H): 296.1650; found: 296.1683. The NMR data are in accordance with literature values.[9]

6,7-Dimethoxy-1-(3-methoxybenzyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline (2c).

***N*-(3,4-Dimethoxyphenethyl)-2-(3-methoxyphenyl)-*N*-methylacetamide.** *N*-Methylhomoveratrylamine (1.90 g, 9.74 mmol) was dissolved in $CHCl_3$ (20 mL). 3% aq. NaOH solution (150 mL) was added and the mixture was cooled to 0 °C on an ice bath. A solution of 3-methoxyphenylacetyl chloride[10] (1.86 g, 10.1 mmol) in chloroform (20 mL) was added dropwise over 1 h to the vigorously stirred mixture. The ice bath was removed and stirring was continued for 2 h at room temperature. The phases were separated and the aqueous phase was extracted with $CHCl_3$ (50 mL). The combined organic phases were washed with 1 M aq. HCl solution (100 mL), then water (50 mL) and dried over Na_2SO_4. Evaporation of the solvent under reduced pressure yielded 3.58 g of a highly viscous yellow liquid. Flash chromatography (silica; petrol ether/EtOAc = 1/1) afforded *N*-(3,4-dimethoxyphenethyl)-2-(3-methoxyphenyl)-*N*-methylacetamide (2.81 g, 84%) as a yellowish liquid. TLC (petrol ether/EtOAc): R_f = 0.37. MS (EI, 70 eV): *m/z* = 343 (M^+, 8), 164 (100), 151 (11), 121 (23). HRMS calcd for $C_{20}H_{25}NO_4$: 343.1783; found: 343.1801.

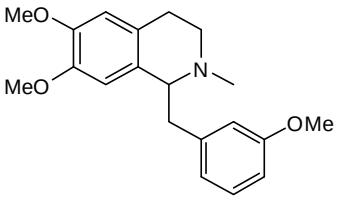
NMR spectroscopy revealed that the product is a mixture of isomers (ratio *trans/cis* = 1.16/1). Based on the peak intensities as well as the DEPT, COSY and HMQC spectra, the NMR signals were assigned to the isomers as follows:

trans-*N*-(3,4-Dimethoxyphenethyl)-2-(3-methoxyphenyl)-*N*-methylacetamide.
^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 2.77 (2H, t, *J* = 7.5 Hz, CH_2-CH_2-N), 2.86 (3H, s, NCH_3), 3.57 (2H, t, *J* = 7.2 Hz, CH_2-CH_2-N), 3.64 (2H, s, CO-CH_2-Ar), 3.74–3.84 (9H, m, 3 × OCH_3), 6.67–6.80 (6H, m, Ar), 7.20 (1H, td, J_1 = 7.8 Hz, J_2 = 3.0 Hz, Ar). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ = 33.2, 36.5, 41.3, 50.2, 55.8, 111.2, 112.0, 112.2, 114.3, 120.7, 121.1, 129.6, 131.6, 136.5, 147.5, 148.8, 159.8, 170.6. No NMR reference data were available for this compound.

cis-*N*-(3,4-Dimethoxyphenethyl)-2-(3-methoxyphenyl)-*N*-methylacetamide.
^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 2.63 (2H, t, *J* = 7.2 Hz, CH_2-CH_2-N), 2.96 (3H, s, NCH_3), 3.43 (2H, s, CO-CH_2-Ar), 3.45 (2H, t, *J* = 7.2, CH_2-CH_2-N), 3.74–3.84 (9H, m, 3 × OCH_3), 6.56 (1H, s, Ar), 6.61 (1H, d, *J* = 8.2 Hz, Ar), 6.67–6.80 (5H, m, Ar). ^{13}C-NMR ($CDCl_3$, 75

MHz): δ = 33.6, 34.2, 40.8, 52.1, 55.1, 111.4, 111.9, 112.2, 114.3, 120.8, 121.0, 129.6, 130.7, 136.8, 147.8, 149.1, 159.8, 170.8. No NMR reference data were available for this compound.

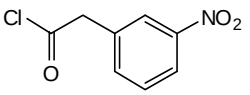
6,7-Dimethoxy-1-(3-methoxybenzyl)-2-ethyl-1,2,3,4-tetrahydroisoquinoline (2c).



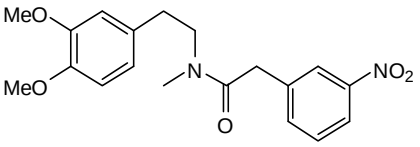
A solution of *N*-(3,4-dimethoxyphenethyl)-2-(3-methoxy-phenyl)-*N*-methylacetamide (2.72 g, 7.93 mmol) and $POCl_3$ (3.70 g, 24.1 mmol) in dry acetonitrile (50 mL) was refluxed for 3 h under argon atmosphere. The solvent and excess $POCl_3$ were evaporated under reduced pressure and the residue was dissolved in dry methanol (40 mL), put under argon and cooled to 0 °C on an ice bath. $NaBH_4$ (3.00 g, 79.3 mmol) was added in portions to the stirred mixture. The ice bath was then removed and stirring continued for 16 h at room temperature. The solvent was evaporated and the residue was treated with half-saturated aq. Na_2CO_3 solution (50 mL). The product was extracted with CH_2Cl_2 (3 × 15 mL), the combined organic phases were dried over Na_2SO_4 and evaporated under reduced pressure to give 2.53 g of a yellow liquid. Flash chromatography (silica; CH_2Cl_2/MeOH/NH_3(aq) = 98/1/1) afforded 1-(3-benzyloxybenzyl)-6,7-dimethoxy-2-ethyl-1,2,3,4-tetrahydroisoquinoline (1.96 g, 76%) as a yellowish liquid that solidified upon standing in the fridge to an off-white solid. mp: 61–62 °C. TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.56. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 2.54 (3H, s, NCH_3), 2.57–2.64 (1H, m, CH_2), 2.73–2.90 (3H, m, CH_2), 3.14–3.21 (2H, m, CH_2), 3.54 (3H, s, OCH_3), 3.70–3.74 (1H, m, CH), 3.74 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 6.01 (1H, s, Ar), 6.56 (1H, s, Ar), 6.67 (1H, s, Ar), 6.71–6.75 (2H, m, Ar), 7.17 (1H, t, J = 7.8 Hz , Ar). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ = 25.5, 41.3, 42.7, 46.8, 55.1, 55.4, 55.7, 111.0, 111.1, 111.3, 115.5, 122.3, 125.8, 129.1, 129.3, 141.7, 146.3, 147.2, 159.5. MS (EI, 70 eV): m/z = 326 (M^+–H, <1), 206 (100), 190 (19). HRMS calcd for $C_{20}H_{24}NO_3$ (M^+–H): 326.1756; found: 326.1738. No NMR reference data were available for this compound.

1-(3-Aminobenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (2d).

3-Nitrophenylacetyl chloride. A solution of 3-nitrophenylacetic acid (1.45 g, 8.0 mmol), oxalyl chloride (1.32 g, 10.0 mmol) and one drop of DMF in dry toluene (30 mL) was stirred at room temperature under argon for 1 h. The solvent was evaporated under reduced pressure to give 1.60 g (quant.) of 3-nitrophenylacetyl chloride as a yellow solid, which was used in the following transformation without further purification. mp: 68–69 °C (ref.[11] 72–73 °C). ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 4.31 (2H, s, CH_2-COCl), 7.56–7.66 (2H, m, Ar), 8.17–8.24 (2H, m, Ar). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ = 52.2, 123.3, 124.6, 130.0, 133.1, 135.7, 148.5, 171.2. No NMR reference data were available for this compound.



***N*-(3,4-Dimethoxyphenethyl)-2-(3-nitrophenyl)-*N*-methylacetamide.**



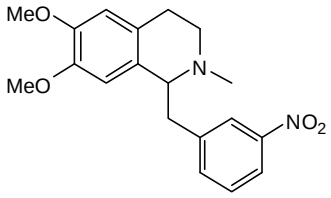
N-Methylhomoveratrylamine (2.05 g, 10.5 mmol) was dissolved in $CHCl_3$ (30 mL). 3% aq. NaOH solution (150 mL) was added and the mixture was cooled to 0 °C on an ice bath. A solution of 3-nitrophenylacetyl chloride (2.20 g, 11.0 mmol) in chloroform (30 mL) was added dropwise over 1 h to the vigorously stirred mixture. The ice bath was removed and stirring was continued overnight at room temperature. The phases were separated and the aqueous phase was extracted with $CHCl_3$ (50 mL). The combined organic phases were washed with 1 M aq. HCl solution (100 mL), then water (100 mL) and dried over Na_2SO_4. Evaporation of the solvent under reduced pressure yielded 4.11 g of a highly viscous yellow liquid. Flash chromatography (silica; petrol ether/EtOAc = 1/1) afforded *N*-(3,4-dimethoxyphenethyl)-2-(3-nitrophenyl)-*N*-methylacetamide (3.24 g, 81%) as a yellow liquid. TLC (petrol ether/EtOAc = 1/1): R_f = 0.50. MS (EI, 70 eV): m/z = 358 (M^+, 5), 164 (100), 151 (28), 136 (16), 90 (18). HRMS calcd for $C_{19}H_{22}N_2O_5$: 358.1529; found: 358.1538.

NMR spectroscopy revealed that the product is a mixture of isomers (ratio *trans/cis* = 1.07/1). Based on the peak intensities as well as the DEPT, COSY and HMQC spectra, the NMR signals were assigned to the isomers as follows:

trans-N-(3,4-Dimethoxyphenethyl)-2-(3-nitrophenyl)-*N*-methylacetamide. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 2.79–2.84 (2H, m, C*H*$_2$-CH_2-N), 2.99 (3H, s, N-CH_3), 3.56–3.66 (2H, m, CH_2-C*H*$_2$-N), 3.78 (2H, s, CH_2-CO), 3.85 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 6.65–6.79 (3H, m, Ar), 7.38–7.57 (2H, m, Ar), 8.07–8.15 (2H, m, Ar). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ = 33.2, 36.3, 39.2, 50.1, 55.9, 111.2, 111.9, 120.7, 121.9, 124.2, 129.4, 131.2, 135.6, 137.1, 148.1, 148.3, 148.9, 169.4. No NMR reference data were available for this compound.

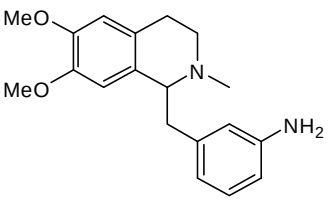
cis-N-(3,4-Dimethoxyphenethyl)-2-(3-nitrophenyl)-*N*-methylacetamide. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 2.79–2.84 (2H, m, C*H*$_2$-CH_2-N), 3.03 (3H, s, N-CH_3), 3.39 (2H, s, CH_2-CO), 3.56–3.66 (2H, m, CH_2-C*H*$_2$-N), 3.87 (3H, s, OCH_3), 3.89 (3H, s, OCH_3), 6.65–6.79 (2H, m, Ar), 6.86 (1H, d, J = 8.1 Hz, Ar), 7.38–7.57 (2H, m, Ar), 7.90–7.91 (1H, m, Ar), 8.07–8.15 (1H, m, Ar). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ = 33.7, 34.1, 40.2, 52.0, 55.9, 111.5, 111.9, 120.9, 121.8, 124.2, 129.2, 130.4, 135.6, 137.2, 147.6, 148.3, 149.2, 169.7. No NMR reference data were available for this compound.

6,7-Dimethoxy-2-methyl-1-(3-nitrobenzyl)-1,2,3,4-tetrahydroisoquinoline.



A solution of *N*-(3,4-dimethoxyphenethyl)-2-(3-nitrophenyl)-*N*-methylacetamid (3.24 g, 9.0 mmol) and $POCl_3$ (4.16 g, 27.1 mmol) in dry acetonitrile (50 mL) was refluxed for 4 h under argon atmosphere. The solvent and excess $POCl_3$ were evaporated under reduced pressure and the residue was dissolved in dry methanol (50 mL), put under argon and cooled to 0 °C on an ice bath. $NaBH_4$ (3.40 g, 89.9 mmol) was added in portions to the stirred mixture. The ice bath was then removed and stirring continued for 16 h at room temperature. The solvent was evaporated and the residue was treated with half-saturated aq. Na_2CO_3 solution (100 mL). The product was extracted with CH_2Cl_2 (3 × 30 mL), the combined organic phases were dried over Na_2SO_4 and evaporated under reduced pressure to give 3.01 g of a yellow liquid. Flash chromatography (silica; CH_2Cl_2/MeOH/NH_3(aq) = 96/3/1) afforded 6,7-dimethoxy-2-ethyl-1-(3-nitrobenzyl)-1,2,3,4-tetrahydroisoquinoline (2.74 g, 88%) as a yellow solid. mp: 82–83 °C. TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.61. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 2.49–2.55 (1H, m, CH_2), 2.53 (3H, s, NCH_3), 2.73–2.83 (2H, m, CH_2), 3.07 (1H, dd, J_1 = 14.0 Hz, J_2 = 6.0 Hz, CH_2), 3.16–3.27 (2H, m, CH_2), 3.71 (3H, s, OCH_3), 3.77–3.81 (1H, m, CH), 3.85 (3H, s, OCH_3), 6.28 (1H, s, Ar), 6.56 (1H, s, Ar), 7.38–7.40 (2H, m, Ar), 8.04–8.06 (2H, m, Ar). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ = 25.2, 40.8, 42.6, 47.1, 55.8, 64.3, 110.5, 111.4, 121.1, 124.6, 126.6, 128.0, 128.6, 136.1, 141.7, 147.0, 147.6, 148.0. MS (EI, 70 eV): m/z = 341 (M^+–H, <1), 206 (100), 190 (20), 162 (8). HRMS calcd for $C_{19}H_{21}N_2O_4$ (M^+–H): 341.1501; found: 341.1504. No NMR reference data were available for this compound.

1-(3-Aminobenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (2d).

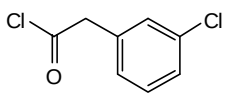


A mixture of 6,7-dimethoxy-2-ethyl-1-(3-nitrobenzyl)-1,2,3,4-tetrahydroisoquinoline (2.74 g, 8.0 mmol), Pd 10% on activated charcoal (0.60 g), acetic acid (2.0 g, 33.3 mmol) and dry methanol (100 mL) was stirred under H_2 atmosphere (1 atm) for 16 h. The mixture was filtered through Celite, washed with methanol (100 mL) and evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 (70 mL) and washed with half-saturated aq. Na_2CO_3 solution (50 mL). The organic phase was dried over Na_2SO_4 and evaporated under reduced pressure to give 2.97 g of a yellowish liquid. Flash chromatography (silica; CH_2Cl_2/MeOH/NH_3(aq) = 96/3/1) afforded 1-(3-aminobenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (2.74 g, 88%) as a yellowish liquid that crystallised upon standing in the fridge to an off-white solid. mp: 52–53 °C. TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.11. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 2.58 (3H, s, NCH_3), 2.63–2.72 (2H, m, CH_2), 2.84–2.96 (2H, m, CH_2), 3.22–3.29

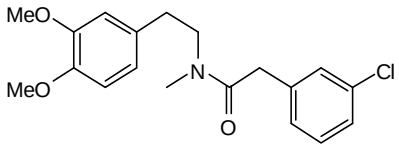
(2H, m, CH_2), 3.53 (3H, s, OCH_3), 3.79–3.84 (4H, s + m overlap, OCH_3 + CH), 4.38 (2H, br s, NH_2), 5.97 (1H, s, Ar), 6.46–6.55 (3H, m, Ar), 6.57 (1H, s, Ar), 7.05 (1H, t, J = 7.7 Hz, Ar). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ = 24.8, 41.2, 41.9, 46.1, 55.4, 55.7, 64.6, 111.0, 111.2, 113.0, 116.6, 120.2, 124.7, 128.2, 129.1, 140.6, 146.3, 146.4, 147.4. MS (EI, 70 eV): m/z = 311 (M^+–H, <1), 206 (100), 190 (19), 162 (7). HRMS calcd for $C_{19}H_{23}N_2O_2$ (M^+–H): 311.1760; found: 311.1745. No NMR reference data were available for this compound.

1-(3-Chlorobenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (2e).

3-Chlorophenylacetyl chloride. A solution of 3-chlorophenylacetic acid (1.71 g, 10.0 mmol), oxalyl chloride (1.53 g, 12.1 mmol) and one drop of DMF in dry toluene (40 mL) was stirred at room temperature under argon for 1 h. The solvent was evaporated under reduced pressure to give 1.89 g (quant.) of 3-chlorophenylacetyl chloride as a red liquid, which was used in the following transformation without further purification. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 4.14 (2H, s, CH_2-COCl), 7.16–7.19 (1H, m, Ar), 7.28–7.30 (1H, m, Ar), 7.32–7.35 (2H, m, Ar). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ = 52.4, 127.7, 128.5, 129.7, 130.2, 133.0, 134.8, 171.4. The NMR data are in accordance with literature values.[12]



2-(3-Chlorophenyl)-*N*-(3,4-dimethoxyphenethyl)-*N*-methylacetamide. *N*-Methylhomoveratrylamine (1.96 g, 10.0 mmol) was dissolved in $CHCl_3$ (30 mL). 3% aq. NaOH solution (150 mL) was added and the mixture was cooled to 0 °C on an ice bath. A solution of 3-chlorophenylacetyl chloride (1.84 g, 9.7 mmol) in chloroform (30 mL) was added dropwise over 1 h to the vigorously stirred mixture. The ice bath was removed and stirring was continued overnight at room temperature. The phases were separated and the aqueous phase was extracted with $CHCl_3$ (50 mL). The combined organic phases were washed with 1 M aq. HCl solution (100 mL), then water (100 mL) and dried over Na_2SO_4. Evaporation of the solvent under reduced pressure yielded 3.91 g of a highly viscous yellow liquid. Flash chromatography (silica; petrol ether/EtOAc = 2/1) afforded 2-(3-chlorophenyl)-*N*-(3,4-dimethoxyphenethyl)-*N*-methylacetamide (2.68 g, 77%) as a yellowish liquid. TLC (EtOAc): R_f = 0.82. MS (EI, 70 eV): m/z = 347 (M^+, 4), 164 (100), 151 (13), 149 (6), 125 (11). HRMS calcd for $C_{19}H_{22}NO_3Cl$: 347.1288; found: 347.1294.

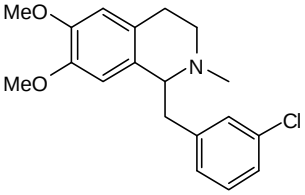


NMR spectroscopy revealed that the product is a mixture of isomers (ratio *trans/cis* = 1.21/1). Based on the peak intensities as well as the DEPT, COSY and HMQC spectra, the NMR signals were assigned to the isomers as follows:

trans-2-(3-Chlorophenyl)-*N*-(3,4-dimethoxyphenethyl)-*N*-methylacetamide.
^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 2.74 (2H, t, J = 7.5 Hz, CH_2-CH_2-N), 2.83 (3H, s, NCH_3), 3.55 (2H, t, J = 7.5 Hz, CH_2-CH_2-N), 3.58 (2H, s, CH_2-CO), 3.77 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 6.56–6.68 (1H, m, Ar), 6.71 (1H, d, J = 8.1 Hz, Ar), 7.04–7.06 (1H, m, Ar), 7.13–7.19 (3H, m, Ar). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ = 33.5, 34.2, 40.5, 51.9, 55.8, 111.3, 112.0, 120.7, 126.8, 127.1, 129.0, 129.8, 131.4, 134.2, 137.1, 147.5, 148.9, 169.9. No NMR reference data were available for this compound.

cis-2-(3-Chlorophenyl)-*N*-(3,4-dimethoxyphenethyl)-*N*-methylacetamide.
^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 2.66 (2H, t, J = 7.0 Hz, CH_2-CH_2-N), 2.93 (3H, s, NCH_3), 3.30 (2H, s, CH_2-CO), 3,44 (2H, t, J = 7.0 Hz, CH_2-CH_2-N), 3.80 (6H, s, 2 × OCH_3), 6.56–6.68 (1H, m, Ar), 6.77 (1H, d, J = 8.1 Hz, Ar), 6.93–6.96 (1H, m, Ar), 7.04–7.06 (1H, m, Ar), 7.13–7.19 (2H, m, Ar). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ = 33.2, 36.4, 39.8, 50.0, 55.8, 111.6, 112.0, 120.9, 126.8, 127.1, 129.0, 129.7, 130.6, 134.1, 137.3, 148.0, 149.2, 170.2. No NMR reference data were available for this compound.

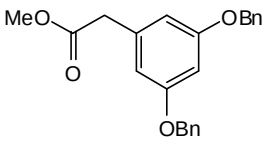
1-(3-Chlorobenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (2e).



A solution of *N*-(3,4-dimethoxyphenethyl)-2-(3-nitrophenyl)-*N*-methylacetamide (2.34 g, 6.7 mmol) and $POCl_3$ (3.07 g, 20.0 mmol) in dry acetonitrile (50 mL) was refluxed for 4 h under argon atmosphere. The solvent and excess $POCl_3$ were evaporated under reduced pressure and the residue was dissolved in dry methanol (50 mL), put under argon and cooled to 0 °C on an ice bath. $NaBH_4$ (2.54 g, 67.1 mmol) was added in portions to the stirred mixture. The ice bath was then removed and stirring continued for 16 h at room temperature. The solvent was evaporated and the residue was treated with half-saturated aq. Na_2CO_3 solution (100 mL). The product was extracted with CH_2Cl_2 (3 × 30 mL), the combined organic phases were dried over Na_2SO_4 and evaporated under reduced pressure to give 2.40 g of a yellow liquid. Flash chromatography (silica; CH_2Cl_2/MeOH/NH_3(aq) = 98/1/1) afforded 1-(3-chlorobenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (2.02 g, 91%) as a yellow liquid. TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.59. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 2.51 (3H, s, NCH_3), 2.51–2.61 (1H, m, CH_2), 2.71–2.87 (3H, m, CH_2), 3.09-3.18 (2H, m, CH_2), 3.61 (3H, s, OCH_3), 3.70 (1H, t, J = 6.4 Hz, CH), 3.83 (3H, s, OCH_3), 6.09 (1H, s, Ar), 6.56 (1H, s, Ar), 6.96–6.99 (1H, m, Ar), 7.13–7.17 (3H, m, Ar). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ = 25.4, 40.9, 42.7, 46.9, 55.6, 55.8, 64.6, 110.9, 111.4, 126.0, 126.2, 128.0, 129.0, 129.3, 129.8, 133.8, 142.2, 146.5, 147.4. MS (EI, 70 eV): m/z = 328 (M^+–3H, <1), 206 (100), 190 (13). HRMS calcd for $C_{19}H_{19}NO_2Cl$ (M^+–3H): 328.1104; found: 328.1124. No NMR reference data were available for this compound.

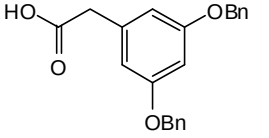
6,7-Dimethoxy-1-(3,5-dihydroxybenzyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline (2f).

Methyl (3,5-bisbenzyloxy)phenylacetate: A mixture of methyl 3,5-dihydroxyphenyl-



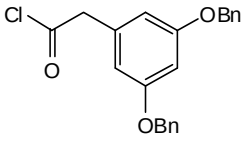
acetate (2.50 g, 13.7 mmol), benzyl bromide (6.30 g, 36.2 mmol), K_2CO_3 (5.00 g, 36.2 mmol) and KI (0.1 g, 0.6 mmol) in acetone (50 mL) was refluxed for 16 h. The solvent was evaporated under reduced pressure and the residue was dissolved in water (100 mL). The product was extracted with EtOAc (2 × 50 mL), the combined organic phases were dried over Na_2SO_4 and evaporated under reduced pressure to give 6.13 g of a yellow liquid. Flash chromatography (silica; petrol ether/EtOAc = 9/1) afforded methyl (3,5-bisbenzyloxy)phenylacetate (3.93 g, 79%) as a yellowish liquid. TLC (petrol ether/EtOAc = 1/1): R_f = 0.80. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 3.59 (2H, s, CH_2-$COOCH_3$), 3.72 (3H, s, OCH_3), 5.05 (4H, s, Ph-CH_2-O), 6.58 (3H, s, Ar), 7.15–7.44 (10H, m, Ar). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ = 41.5, 52.1, 70.1, 100.9, 108.5, 127.6, 128.0, 128.6, 136.1 136.8, 160.1, 171.8. The NMR data are in accordance with literature values.[13]

(3,5-Bisbenzyloxy)phenylacetic acid: A suspension of methyl (3,5-bisbenzyloxy)phenyl-

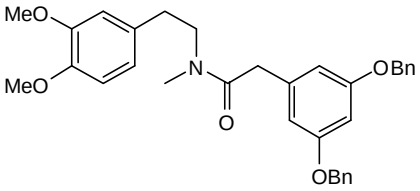


acetate (3.90 g, 10.8 mmol) in 2 M aq. NaOH solution (50 mL) was refluxed for 16 h. The mixture was cooled on an ice bath and acidified by addition of conc. hydrochloric acid. The product was extracted with EtOAc (2 × 50 mL), the combined organic phases were dried over Na_2SO_4 and evaporated under reduced pressure to give 3.72 g of a yellowish solid. Recrystallisation from petrol ether/acetone afforded (3,5-bisbenzyloxy)-phenylacetic acid (3.11 g, 82%) as an off-white solid. mp: 101–102 °C (ref.[14] 108 °C). TLC (petrol ether/EtOAc = 1/1 + 1 drop AcOH): R_f = 0.51. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 3.62 (2H, s, CH_2-COOH), 5.05 (4H, s, Ph-CH_2-O), 6.59 (3H, s, Ar), 7.28–7.46 (10H, m, Ar). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ = 41.3, 70.1, 101.0, 108.7, 127.6, 128.0, 128.6, 135.4, 136.8, 160.0, 177.4. The NMR data are in accordance with literature values.[13]

(3,5-Bisbenzyloxy)phenylacetyl chloride. A solution of (3,5-bisbenzyloxy)phenylacetic acid (3.08 g, 8.85 mmol), oxalyl chloride (1.35 g, 10.6 mmol) and one drop of DMF in dry toluene (40 mL) was stirred at room temperature under argon for 1 h. The solvent was evaporated under reduced pressure to give 3.25 g (quant.) of (3,5-bisbenzyloxy)phenylacetyl chloride as a brownish liquid, which was used in the following transformation without further purification. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 4.08 (2H, s, CH_2-COCl), 5.05 (4H, s, Ph-CH_2-O), 6.54 (2H, d, J = 2.1 Hz, Ar), 6.62 (H, t, J = 1.8 Hz, Ar), 7.28–7.46 (10H, m, Ar). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ = 53.2, 70.2, 101.7, 108.8, 127.6, 128.1, 128.7, 133.2, 136.6, 160.3, 171.7. No NMR reference data were available for this compound.



2-(3,5-Bisbenzyloxyphenyl)-*N*-(3,4-dimethoxyphenethyl)-*N*-methylacetamide. *N*-Methylhomoveratrylamine (1.66 g, 8.51 mmol) was dissolved in $CHCl_3$ (20 mL). 3% aq. NaOH solution (100 mL) was added and the mixture was cooled to 0 °C on an ice bath. A solution of (3,5-bisbenzyloxy)phenylacetyl chloride (3.23 g, 8.80 mmol) in chloroform (20 mL) was added dropwise over 1 h to the vigorously stirred mixture. The ice bath was removed and stirring was continued for 2 h at room temperature. The phases were separated and the aqueous phase was extracted with $CHCl_3$ (50 mL). The combined organic phases were washed with 1 M aq. HCl solution (100 mL), then water (50 mL) and dried over Na_2SO_4. Evaporation of the solvent under reduced pressure yielded 4.39 g of a highly viscous yellow liquid. Flash chromatography (silica; petrol ether/EtOAc = 1/1) afforded 2-(3,5-bisbenzyloxy-phenyl)-*N*-(3,4-dimethoxyphenethyl)-*N*-methylacetamide (3.05 g, 66%) as a yellowish liquid. TLC (petrol ether/EtOAc = 1/1): R_f = 0.21. MS (EI, 70 eV): m/z = 525 (M^+, 6), 270 (6), 164 (100), 151 (7), 91 (50). HRMS calcd for $C_{33}H_{35}NO_5$: 525.2515; found: 525.2532.

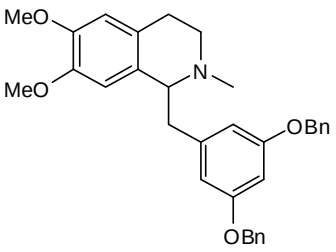


NMR spectroscopy revealed that the product is a mixture of isomers (ratio *trans/cis* = 1.08/1). Based on the peak intensities as well as the DEPT, COSY and HMQC spectra, the NMR signals were assigned to the isomers as follows:

trans-2-(3,5-Bisbenzyloxyphenyl)-*N*-(3,4-dimethoxyphenethyl)-*N*-methylacetamide. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 2.76 (2H, t, J = 7.5 Hz, CH_2-CH_2-N), 2.92 (3H, s, N-CH_3), 3.41 (2H, s, CO-CH_2-Ar), 3.55 (2H, t, J = 7.5 Hz, CH_2-N), 3.82 (6H, s, 2 × OCH_3), 5.01 (4H, s, Ar-CH_2-O), 6.43–6.77 (6H, m, Ar), 7.25–7.42 (10H, m, Ar). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ = 33.3, 33.6, 41.2, 52.2, 55.9, 70.0, 100.5, 107.9, 111.2, 112.0, 120.7, 127.6, 128.0, 128.6, 131.7, 136.8, 137.2, 147.9, 149.1, 160.1, 170.7. No NMR reference data were available for this compound.

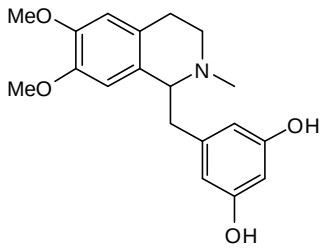
cis-2-(3,5-Bisbenzyloxyphenyl)-*N*-(3,4-dimethoxyphenethyl)-*N*-methylacetamide. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 2.59 (2H, t, J = 7.4 Hz, CH_2-CH_2-N), 2.95 (3H, s, N-CH_3), 3.42 (2H, t , J = 7.2, CH_2-N), 3.62 (2H, s, CO-C$H2$-Ar), 3.82 (6H, s, 2 × OCH_3), 4.99 (4H, s, Ar-CH_2-O), 6.43–6.77 (6H, m, Ar), 7.25–7.42 (10H, m, Ar). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ = 34.2, 36.7, 41.7, 50.4, 55.9, 70.0, 100.5, 107.8, 111.4, 111.9, 120.8, 127.6, 128.0, 128.6, 130.7, 136.8, 137.6, 147.5, 148.9, 160.1, 170.5. No NMR reference data were available for this compound.

1-(3,5-Bisbenzyloxy)benzyl-6,7-dimethoxy-2-ethyl-1,2,3,4-tetrahydroisoquinoline. A solution of 2-(3,5-bisbenzyloxyphenyl)-*N*-(3,4-dimethoxyphenethyl)-*N*-methylacetamide (3.00 g, 5.71 mmol) and $POCl_3$ (2.66 g, 17.4 mmol) in dry acetonitrile (50 mL) was refluxed for 3 h under argon atmosphere. The solvent and excess $POCl_3$ were evaporated under reduced pressure and the residue was dissolved in dry methanol (40 mL), put under argon and cooled to 0 °C on an ice bath. $NaBH_4$ (2.11 g, 55.8 mmol) was added in portions to the stirred mixture. The ice bath was then removed and stirring continued for 16 h at room temperature. The solvent was evaporated and the residue was



treated with half-saturated aq. Na_2CO_3 solution (50 mL). The product was extracted with CH_2Cl_2 (3 × 15 mL), the combined organic phases were dried over Na_2SO_4 and evaporated under reduced pressure to give 2.84 g of a yellow liquid. Flash chromatography (silica; CH_2Cl_2/MeOH/NH_3(aq) = 96/3/1) afforded 1-(3,5-bisbenzyloxy)benzyl-6,7-dimethoxy-2-ethyl-1,2,3,4-tetrahydroisoquinoline (2.37 g, 82%) as a yellowish liquid. TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.63. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 2.54 (3H, s, NCH_3), 2.58–2.92 (4H, m, CH_2), 3.13–3.22 (2H, m, CH_2), 3.59 (3H, s, OCH_3), 3.73 (1H, dd, J_1 = 7.4 Hz, J_2 = 3.7 Hz, CH), 3.86 (3H, s, OCH_3), 5.00 (4H, s, $PhCH_2O$), 6.10 (1H, s, Ar), 6.42 (2H, s, Ar), 6.50 (1H, s, Ar), 6.58 (1H, s, Ar), 7.31–7.44 (10H, m, Ar). ^{13}C-NMR ($CDCl_3$, 75 MHz): δ = 25.5, 41.6, 42.7, 46.8, 55.5, 55.8, 64.6, 70.0, 99.8, 109.0, 111.0, 111.2, 125.8, 127.6, 128.0, 128.6, 129.4, 137.0, 142.6, 146.4, 147.3, 159.7. MS (EI, 70 eV): *m/z* = 508 (M^+–H, <1), 206 (100), 190 (9), 91 (13). HRMS calcd for $C_{33}H_{34}NO_4$ (M^+–H): 508.2488; found: 508.2511. No NMR reference data were available for this compound.

1-(3,5-Dihydroxybenzyl)-6,7-dimethoxy-2-ethyl-1,2,3,4-tetrahydroisoquinoline (2f).



A mixture of 1-(3,5-bisbenzyloxy)benzyl-6,7-dimethoxy-2-ethyl-1,2,3,4-tetrahydroisoquinoline (2.36 g, 4.63 mmol), Pd 10% on activated charcoal (0.25 g), acetic acid (0.80 g, 13.3 mmol) and dry methanol (50 mL) was stirred under H_2 atmosphere (1 atm) for 16 h. The mixture was filtered through Celite, washed with methanol (50 mL) and evaporated under reduced pressure. The residue was dissolved in EtOAc (50 mL) and washed with half-saturated aq. $NaHCO_3$ solution (40 mL). [**NOTE:** *In halogenated solvents such as dichloromethane and chloroform, the substance will oligomerise, as indicated by heavily broadened NMR signals.*] The organic phase was dried over Na_2SO_4 and evaporated under reduced pressure to afford 1-(3,5-dihydroxybenzyl)-6,7-dimethoxy-2-ethyl-1,2,3,4-tetrahydroisoquinoline (1.22 g, 80%) as an off-white solid. mp: 108–110 °C. TLC (CH_2Cl_2/MeOH/NH_3(aq) = 90/9/1): R_f = 0.38. ^{1}H-NMR (acetone-d_6, 300 MHz): δ = 2.45 (3H, s, NCH_3), 2.52–2.81 (4H, m, CH_2), 3.01 (1H, dd, J_1 = 13.6 Hz, J_2 = 5.7 Hz, CH_2), 3.09–3.17 (1H, m, CH_2), 3.59 (3H, s, OCH_3), 3.67 (1H, t, J = 6.2 Hz, CH), 3.75 (3H, s, OCH_3), 6.20 (3H, s, Ar), 6.35 (1H, s, Ar), 6.61 (1H, s, Ar). ^{13}C-NMR (acetone-d_6, 75 MHz): δ = 25.4, 40.9, 42.2, 54.8, 55.0, 64.6, 100.1, 108.3, 111.6, 111.7, 126.1, 129.9, 142.8, 147.0, 147.7, 158.1. MS (EI, 70 eV): *m/z* = 328 (M^+–H, <1), 206 (100), 190 (13). HRMS calcd for $C_{19}H_{22}NO_4$ (M^+–H): 328.1549; found: 328.1558. No NMR reference data were available for this compound.

Protein Expression and Purification

Cultivation of *E. coli* strains expressing MAO-N (for protein purification). A single colony of *E. coli* BL21 (DE3) harbouring the [pET16b MAO-N] plasmid was used to inoculate 8 mL of LB medium (containing 100 μg/mL ampicillin) and the culture was grown to an OD_{600} of 0.6–1.0 at 30 °C and 250 rpm. From this culture, 6 mL were used to inoculate 600 mL of LB medium (containing 100 μg/mL ampicillin) and the new culture was grown at 30 °C and 250 rpm for 24 h. The cells were harvested by centrifugation (5500 rpm, 20 min), resuspended in potassium phosphate buffer (100 mM, pH 7.7) and centrifuged again (4000 rpm, 15 min). The cell pellets were stored at –20 °C until use.

Purification of MAO-N. Protein purification was performed on an ÄKTAexplorer 900 system (*GE Healthcare*) at 10 °C according to the following protocol: 5 g of cell pellet prepared as described above were resuspended in 25 mL of potassium phosphate buffer (100 mM, pH 7.7, 300 mM NaCl, containing 1 mg/mL of lysozyme) and incubated at 30 °C for 30 min. The suspension was cooled to 4 °C and cells were disrupted by ultrasonication (*MSE* Soniprep 150; 30 s pulse, 30 s pause; 15 cycles). The cell debris was removed by centrifugation (20000 rpm, 40 min), the supernatant was filtered (0.45 μm syringe microfilter) and loaded onto a HisTrap Ni-NTA-sepharose column (1 mL, *GE Healthcare*) pre-

equilibrated with buffer A (100 mM potassium phosphate, pH 7.7, 300 mM NaCl). Proteins were step-eluted using buffer A (10 mL), buffer A / buffer B (100 mM potassium phosphate, pH 7.7, 300 mM NaCl, 1 M imidazole) = 80/20 (10 mL) and buffer A / buffer B = 65/35 (10–30 mL); collecting 1 mL fractions. The MAO-containing fractions (from the 35% buffer B step) were pooled and concentrated using the *Sartorius* Vivaspin 6 system (30 kDa mass cut-off), the volume was adjusted to 2.5 mL, and the protein solution was desalted using a PD-10 column (*GE Healthcare*) and MAO reaction buffer (100 mM potassium phosphate, pH 7.7) for elution. The protein solution thus prepared was directly used for the activity assay. Protein concentration was determined using the Pierce BCA protein assay (*Thermo Scientific*), preparing all samples (MAO and BSA standard) in triplicate.

Improvement of Heterologous Protein Expression

Cultivation in LB medium without induction. LB medium (50 mL; containing 100 μg/mL ampicillin) was inoculated from a glycerol stock (20 μL) of *E. coli* BL21 (DE3) or *E. coli* C43 (DE3) harbouring the [pET16b MAO-N (D11)] plasmid and the culture was grown to an OD_{600} >2 at 30 °C and 120 rpm (approx. 16 h). From this culture, 6 mL were used to inoculate LB medium (600 mL; containing 100 μg/mL ampicillin) and the new culture was grown at 30 °C and 175 rpm for 24 h. The cells were harvested by centrifugation (8000 rpm, 20 min), resuspended in potassium phosphate buffer (100 mM, pH 7.7) and centrifuged again (4000 rpm, 20 min). The cell pellets were stored at –20 °C.

Cultivation in LB medium with IPTG induction. LB medium (50 mL; containing 100 μg/mL ampicillin) was inoculated from a glycerol stock (20 μL) of *E. coli* BL21 (DE3) or *E. coli* C43 (DE3) harbouring the [pET16b MAO-N (D11)] plasmid and the culture was grown to an OD_{600} >2 at 30 °C and 120 rpm (approx. 16 h). From this culture, 6 mL were used to inoculate LB medium (600 mL; containing 100 μg/mL ampicillin) and the new culture was grown at 30 °C and 175 rpm until an OD_{600} of 0.5–0.6 was reached. At this stage, IPTG was added to a final concentration of 1 mM and the culture was shaken at 20 °C and 120 rpm for 20 h. The cells were harvested by centrifugation (8000 rpm, 20 min), resuspended in potassium phosphate buffer (100 mM, pH 7.7) and centrifuged again (4000 rpm, 20 min). The cell pellets were stored at –20 °C.

Cultivation in TB medium without induction. LB medium (50 mL; containing 100 μg/mL ampicillin) was inoculated from a glycerol stock (20 μL) of *E. coli* BL21 (DE3) or *E. coli* C43 (DE3) harbouring the [pET16b MAO-N (D11)] plasmid and the culture was grown to an OD_{600} >2 at 30 °C and 120 rpm (approx. 16 h). From this culture, 6 mL were used to inoculate of TB medium (600 mL; containing 100 μg/mL ampicillin) and the new culture was grown at 30 °C and 175 rpm for 24 h. The cells were harvested by centrifugation (8000 rpm, 20 min), resuspended in potassium phosphate buffer (100 mM, pH 7.7) and centrifuged again (4000 rpm, 20 min). The cell pellets were stored at –20 °C.

Cultivation in TB medium with IPTG induction. LB medium (50 mL; containing 100 μg/mL ampicillin) was inoculated from a glycerol stock (20 μL) of *E. coli* BL21 (DE3) or *E. coli* C43 (DE3) harbouring the [pET16b MAO-N (D11)] plasmid and the culture was grown to an OD_{600} >2 at 30 °C and 120 rpm (approx. 16 h). From this culture, 6 mL were used to inoculate TB medium (600 mL; containing 100 μg/mL ampicillin) and the new culture was grown at 30 °C and 175 rpm until an OD_{600} of 0.7–0.8 was reached. At this stage, IPTG was added to a final concentration of 1 mM and the culture was shaken at 20 °C and 120 rpm for 20 h. The cells were harvested by centrifugation (8000 rpm, 20 min), resuspended in potassium phosphate buffer (100 mM, pH 7.7) and centrifuged again (4000 rpm, 20 min). The cell pellets were stored at –20 °C.

Cultivation under auto-inducing conditions. LB medium (50 mL; containing 100 μg/mL ampicillin) was inoculated from a glycerol stock (20 μL) of *E. coli* BL21 (DE3) or *E. coli* C43 (DE3) harbouring the [pET16b MAO-N (D11)] plasmid and the culture was grown to an

OD_{600} >2 at 30 °C and 120 rpm (approx. 16 h). From this culture, 6 mL were used to inoculate autoinduction medium (600 mL, 4ZY-LAC-SUC; containing 100 μg/mL ampicillin) and the new culture was grown at 25 °C and 150 rpm for 72 h. The cells were harvested by centrifugation (8000 rpm, 20 min), resuspended in potassium phosphate buffer (100 mM, pH 7.7) and centrifuged again (4000 rpm, 20 min). The cell pellets were stored at –20 °C.

Cell lyophilisation. Cell pellets (approx. 2 g) prepared as described above were resuspended in 10 mL of potassium phosphate buffer (100 mM, pH 7.7). The cell suspension was transferred to a round-bottom flask and flash-frozen by submerging the rotating flask into a bath of liquid nitrogen. The resulting frozen cell suspension was lyophilised overnight.

Preparation of cell-free extract. Cell pellets (approx. 4 g) prepared as described above were resuspended in potassium phosphate buffer (20 mL; 100 mM, pH 7.7; containing 1 mg/mL of lysozyme) and incubated at 30 °C for 30 min. The suspension was cooled to 4 °C and cells were disrupted by ultrasonication (*Branson* Digital Sonifier 250; 2 s pulse, 4 s pause, amplitude 20%; total pulse time 2 min 30 s = 75 cycles). Cell debris was removed by centrifugation (16000 rpm, 30 min), the supernatant was transferred to a round-bottom flask and flash-frozen by submerging the rotating flask into a bath of liquid nitrogen. The resulting frozen lysate was lyophilised overnight.

Activity assay. Frozen cells of *E. coli* BL21 (DE3) or C43 (DE3) [pET16b MAO-N (D11)] (50 mg) were thawed and resuspended in 450 μL of potassium phosphate buffer (100 mM, pH 7.7). Substrate **1a** (1.2 mg, final concentration 10 mM) was dissolved in DMSO (50 μL) and added to the resuspended cells. The reaction mixture was shaken at 37 °C and 150 rpm for 2 h. The samples were basified by addition of 2 M NaOH solution (100 μL), extracted with EtOAc (2 × 500 μL) and dried over Na_2SO_4. Conversion was determined by GC–FID analysis.

Media and media components:

LB medium: 10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl

TB medium: 12 g/L tryptone, 24 g/L yeast extract, 4 mL/L glycerol, 100 mL/L 1 M potassium phosphate buffer pH 7.5

4ZY-LAC-SUC: 20 mL/L 50x LAC, 50 mL/L 20x NPSC, 1 mL/L 1000x trace element solution, 2 mL/L 500x $MgSO_4$ stock, 50 mL/L 20x SUC stock, 410 mL/L 8x ZY, 470 mL/L H_2O dest.

50x LAC: 25% w/v glycerol, 2.5% w/v glucose, 10% w/v □-lactose monohydrate

20x NPSC: 1 M NH_4Cl, 0.1 M Na_2SO_4, 0.5 M KH_2PO_4, 0.5 M Na_2HPO_4

1000x trace element solution: 50 mM $FeCl_3$, 20 mM $CaCl_2$, 10 mM $MnCl_2$, 10 mM $ZnSO_4$, 2 mM $CoCl_2$, 2 mM $CuCl_2$, 2 mM $NiCl_2$, 2 mM Na_2MoO_4, 2 mM Na_2SeO_3, 2 mM H_3BO_4

500x $MgSO_4$ stock: 1 M $MgSO_4$

20x SUC: 0.5 M sodium succinate

8x ZY: 80 g/L tryptone, 40 g/L yeast extract

Analytical Methods

GC–FID analysis. GC–FID analysis was carried out on an *Agilent* 7890a GC system using a (5%-phenyl)methylpolysiloxane stationary phase (*J&W* HP-5, 30 m × 0.32 mm × 0.25 μm) and helium as carrier gas.

Method A. Oven program: 120 °C for 1 min, 15 °C/min to 300 °C; column flow rate: 1 mL/min; split ratio: 15/1.

Method B. Oven program: 200 °C for 0.5 min, 10 °C/min to 300 °C, keep for 4 min; column flow rate: 2 mL/min; split ratio: 15/1.

The retention times of all investigated substrates and their oxidation products are given in Supplementary Table S3.

substrate	method	t_r [min]	t_r(ox) [min]
1a	A	9.7	11.1
1b	B	5.2	*na*
2a	B	6.0	7.6
2b	B	8.0	*na*
2c	B	7.4	9.1
3a	B	8.1	*na*
3c	B	6.9	*na*
3f	B	9.1	*na*

Supplementary Table S3. GC–FID retention times of substrates and their oxidation products. *na* … not applicable since oxidation product could not be extracted.

HPLC analysis (achiral stationary phase). HPLC analysis was carried out on a *Shimadzu* HPLC system using a C18 stationary phase (*Phenomenex* LUNA-C18(2), 250 mm × 4.6 mm) and diode array detection. Eluent: buffer (30 mM $HCOONH_4$, pH 2.8)/methanol/acetonitrile = 67/18/15; column temperature: 20 °C; flow rate: 0.5 mL/min; integration wavelength: 280 nm. 2,3-Dimethoxy-9-hydroxyberbine[10] was used as internal standard. The retention times of all investigated substrates and the internal standard are given in Supplementary Table S4.

substrate	t_r [min]
1b	17.7
2a	24.8
2b	12.6
2c	27.1
3a	8.9
3c	21.6
3f	9.3
ISTD	15.9

Supplementary Table S4. HPLC retention times of substrates and the internal standard 2,3-dimethoxy-9-hydroxyberbine (ISTD).

HPLC analysis (chiral stationary phase). HPLC analysis was carried out on a *Shimadzu* HPLC system using a stationary phase composed of cellulose-tris-(4-methylbenzoate) on silica gel (*Daicel* Chiralcel OJ, 250 mm × 4.6 mm) and diode array detection.

Method A. Eluent: *n*-heptane/2-propanol = 90/10 (+0.1% TFA); flow rate: 0.35 mL/min; column temperature: 40 °C; integration wavelength: 280 nm.

Method B. Eluent: *n*-heptane/2-propanol = 90/10 (+0.1% TFA); flow rate: 0.75 mL/min; column temperature: 40 °C; integration wavelength: 280 nm.

Method C. Eluent: *n*-heptane/2-propanol = 80/20 (+0.1% TFA); flow rate: 0.35 mL/min; column temperature: 40 °C; integration wavelength: 280 nm.

Method D. Eluent: *n*-heptane/2-propanol = 80/20 (+0.1% TFA); flow rate: 0.5 mL/min; column temperature: 40 °C; integration wavelength: 280 nm.

Method E. Eluent: *n*-heptane/2-propanol = 70/30 (+0.1% formic acid); flow rate: 0.35 mL/min; column temperature: 18 °C; integration wavelength: 280 nm.

Method F. Eluent: *n*-heptane/2-propanol = 70/30 (+0.1% formic acid); flow rate: 0.5 mL/min; column temperature: 18 °C; integration wavelength: 280 nm.

The retention times of all investigated substrates are given in Supplementary Table S5.

substrate	method	t_r(*S*) [min]	t_r(*R*) [min]
1a	A	30.5	33.8
1b	B	21.9	25.7
2a	C	22.3	27.3
2b	D	17.3	24.5
2c	C	30.8	38.9
3a	E	32.9	40.0
3c	C	31.7	34.6
3f	F	28.6	32.6

Supplementary Table S5. HPLC retention times of substrate enantiomers.

Determination of absolute configuration. Absolute configurations of substrates **1b**, **2b**, **3a**, **3c**, and **3f** were assigned based on optical rotation, circular dichroism and HPLC elution order analogies as previously described.[10] Absolute configurations of substrates **1a**, **2a**, and **2c** were assumed solely based on HPLC elution order analogy.

NMR and MS Spectra

NMR and MS spectra of substrates **1b**, **2b**, and **3a–f** and their synthetic intermediates have been previously reported.[10,15,16] The spectra of all other compounds are provided in this section of the Electronic Supplementary Information.

Substrate 1a and its Synthetic Intermediates

Provided Material:

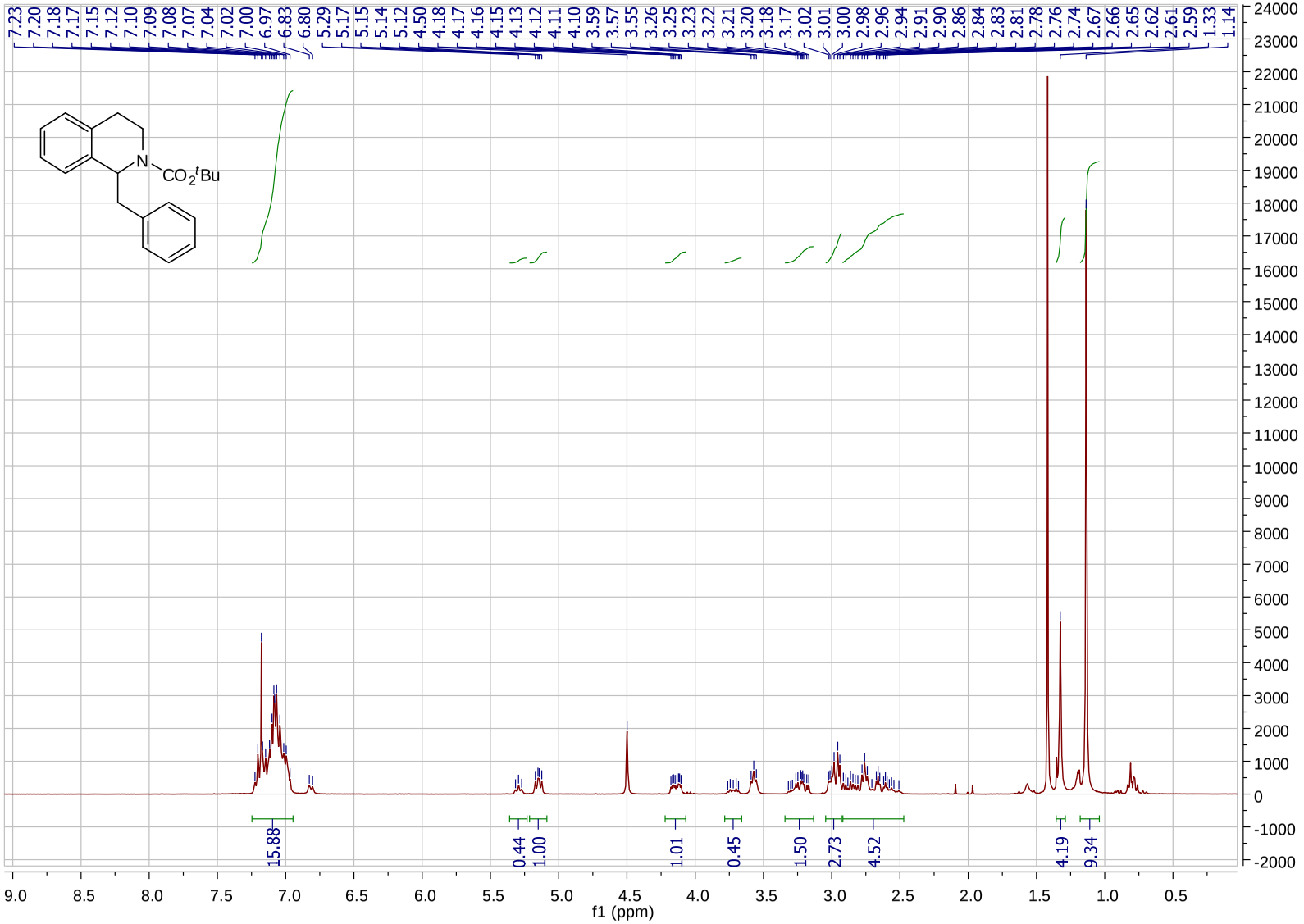
***t*-Butyl 1-benzyl-3,4-dihydro-2(1*H*)-isoquinolinecarboxylate:**

^{1}H-NMR spectrum, ^{13}C-NMR spectrum, HSQC spectrum, MS spectrum, HRMS results

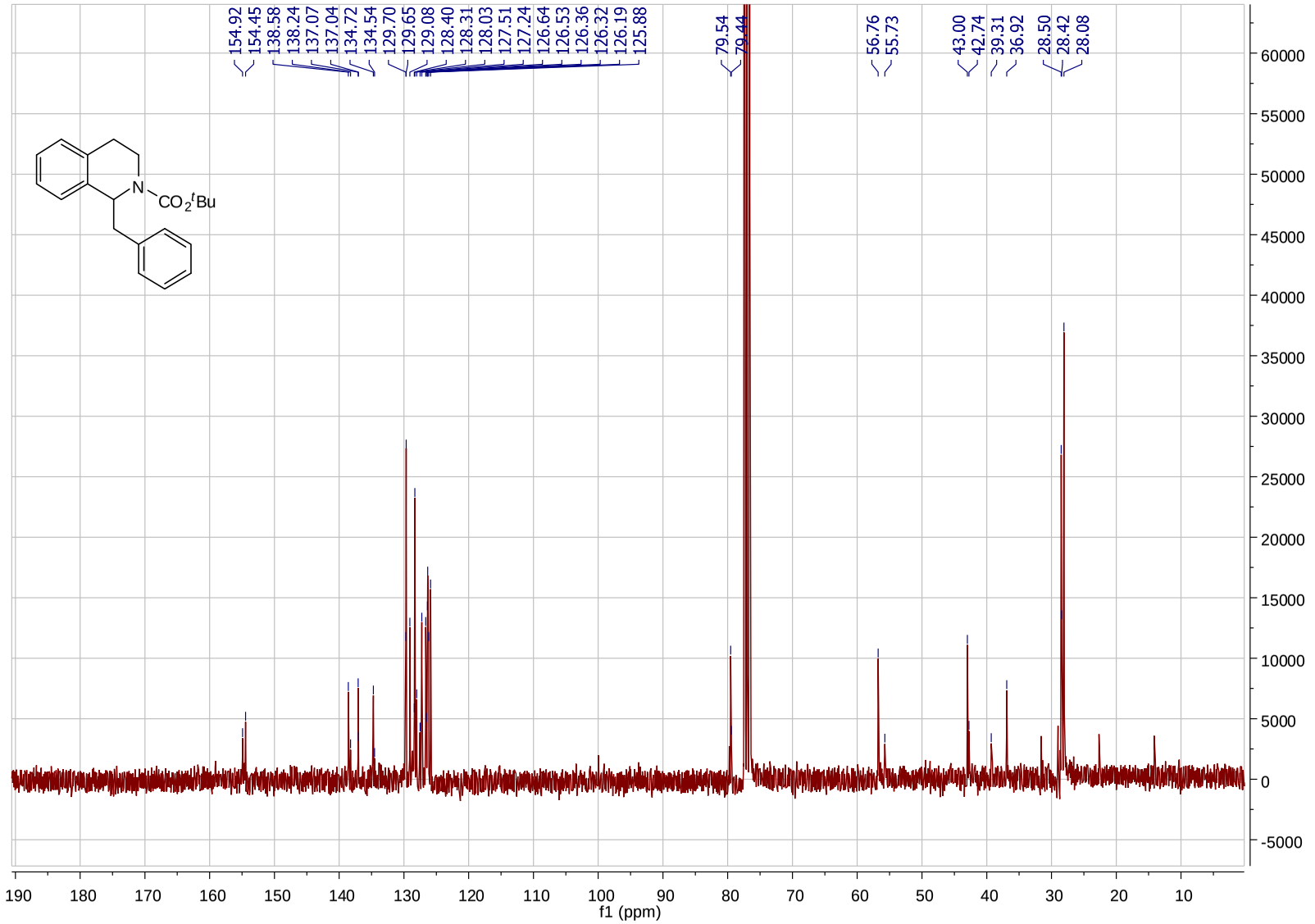
1-Benzyl-2-methyl-1,2,3,4-tetrahydroisoquinoline (1a):

^{1}H-NMR spectrum, ^{13}C-NMR spectrum, COSY spectrum, HSQC spectrum, MS spectrum, HRMS results

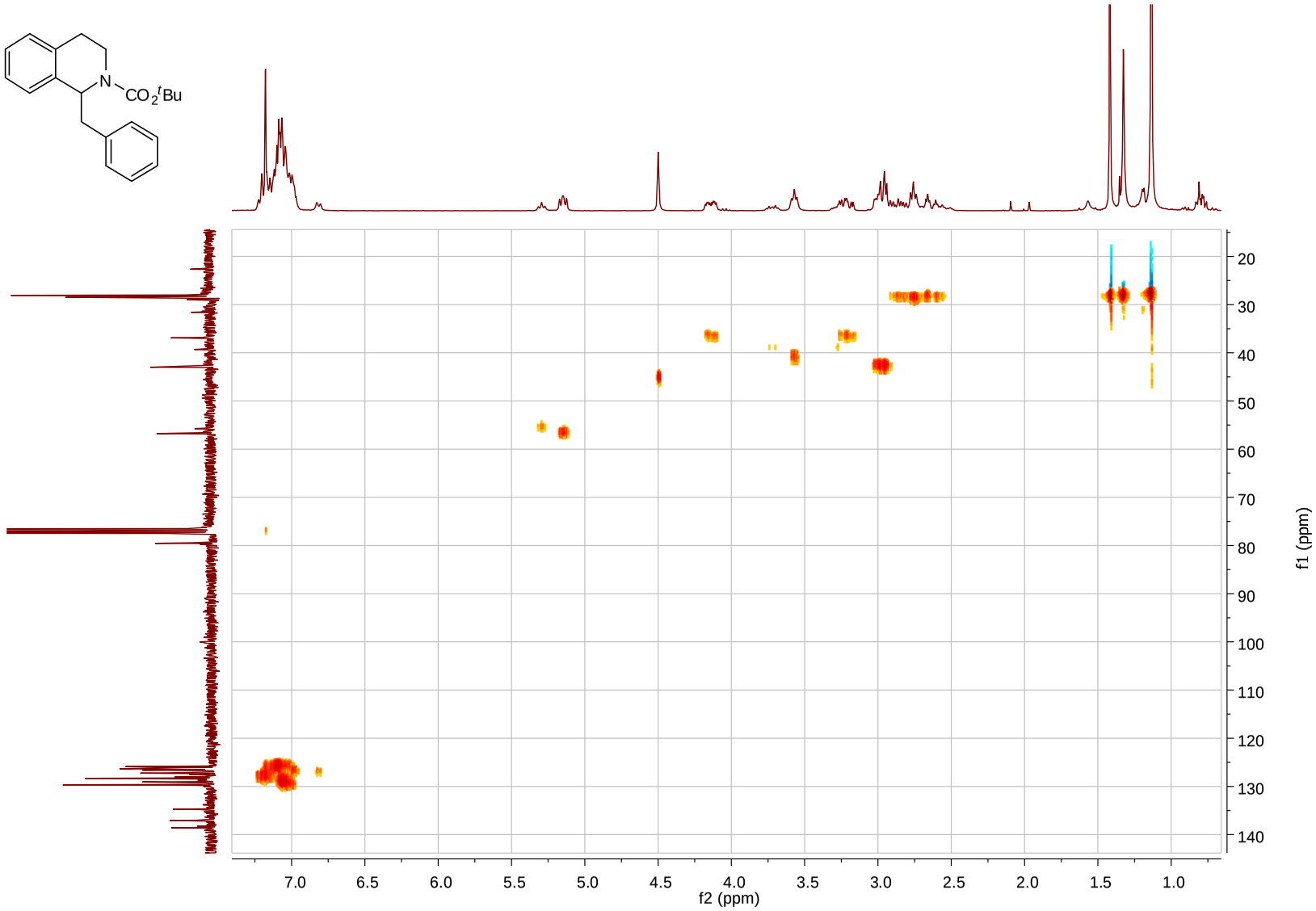
_t_-Butyl 1-benzyl-3,4-dihydro-2(1*H*)-isoquinolinecarboxylate: ^{1}H-NMR spectrum



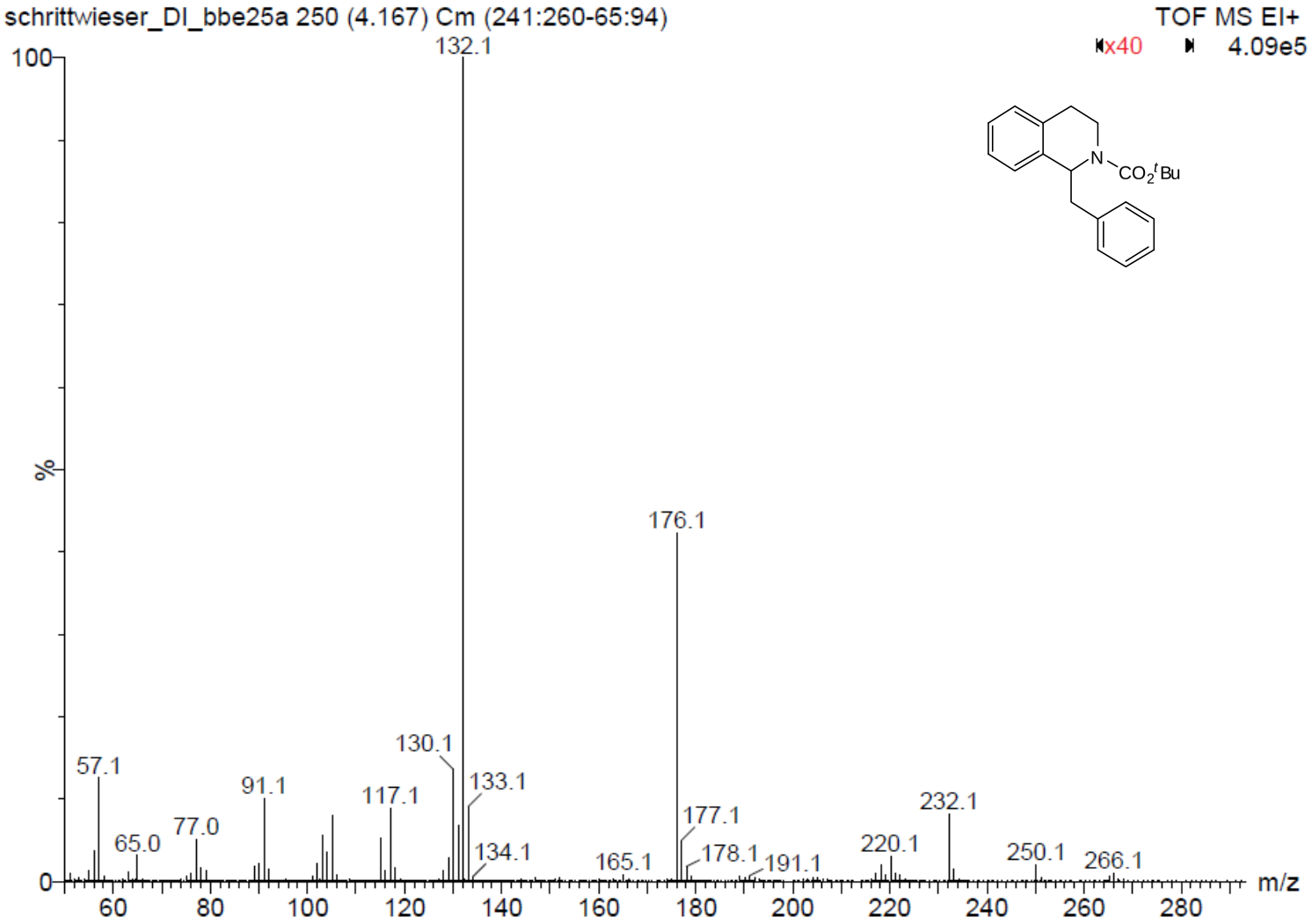
_t_-Butyl 1-benzyl-3,4-dihydro-2(1_H_)-isoquinolinecarboxylate: ^{13}C-NMR spectrum



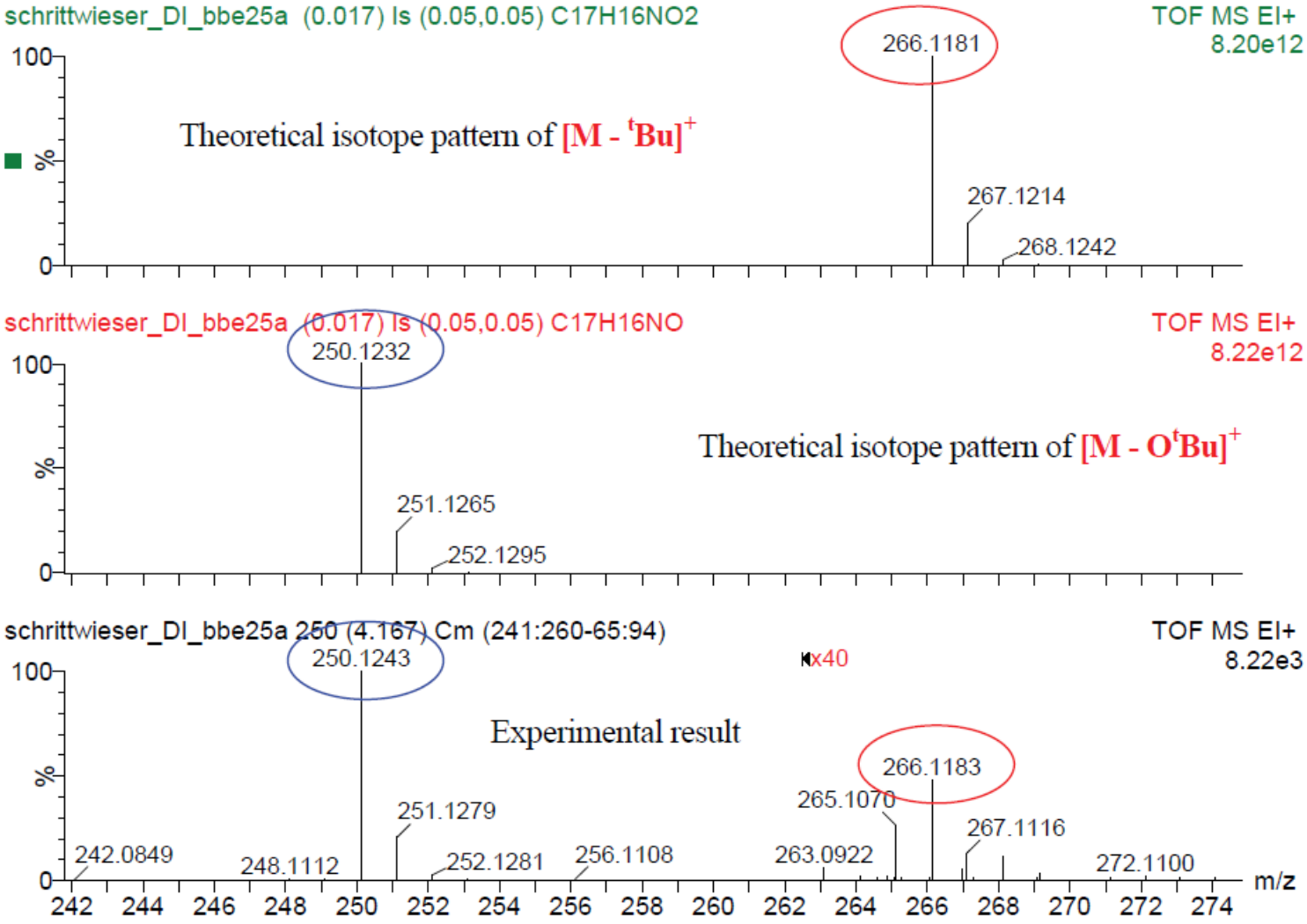
_t_-Butyl 1-benzyl-3,4-dihydro-2(1*H*)-isoquinolinecarboxylate: HSQC spectrum



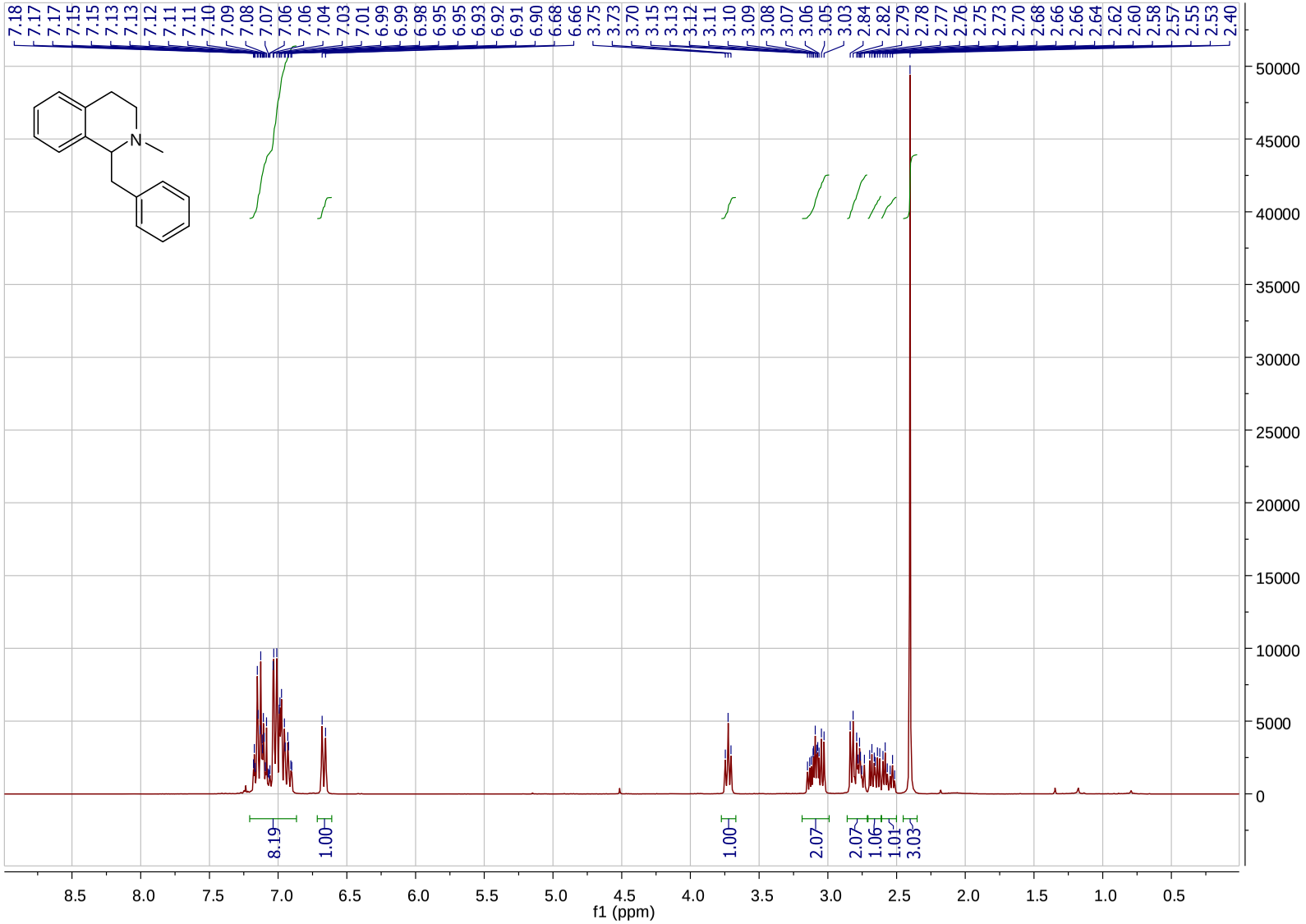
***t*-Butyl 1-benzyl-3,4-dihydro-2(1*H*)-isoquinolinecarboxylate:** MS spectrum



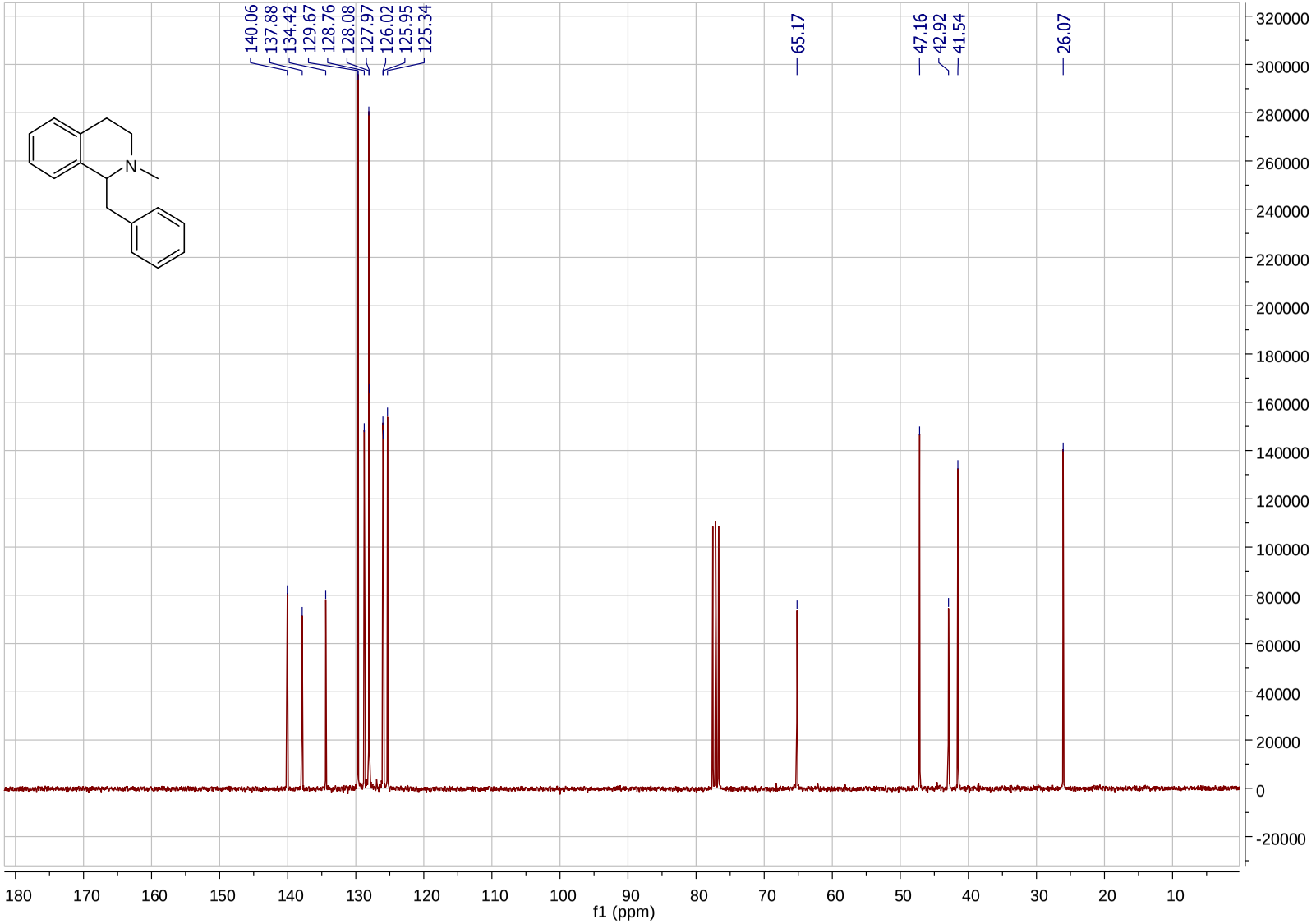
***t*-Butyl 1-benzyl-3,4-dihydro-2(1*H*)-isoquinolinecarboxylate:** HRMS results



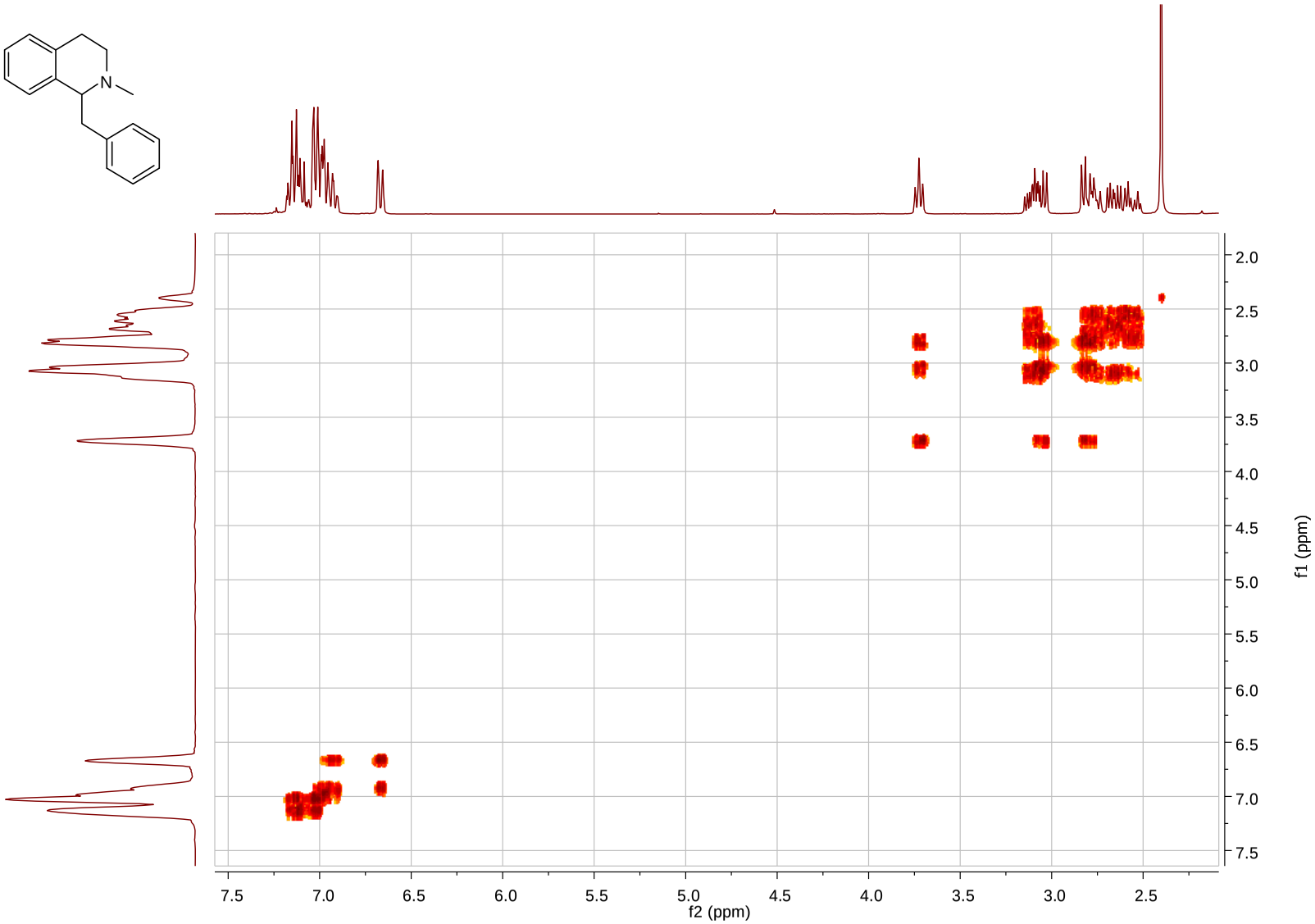
1-Benzyl-2-methyl-1,2,3,4-tetrahydroisoquinoline (1a): ^{1}H-NMR spectrum



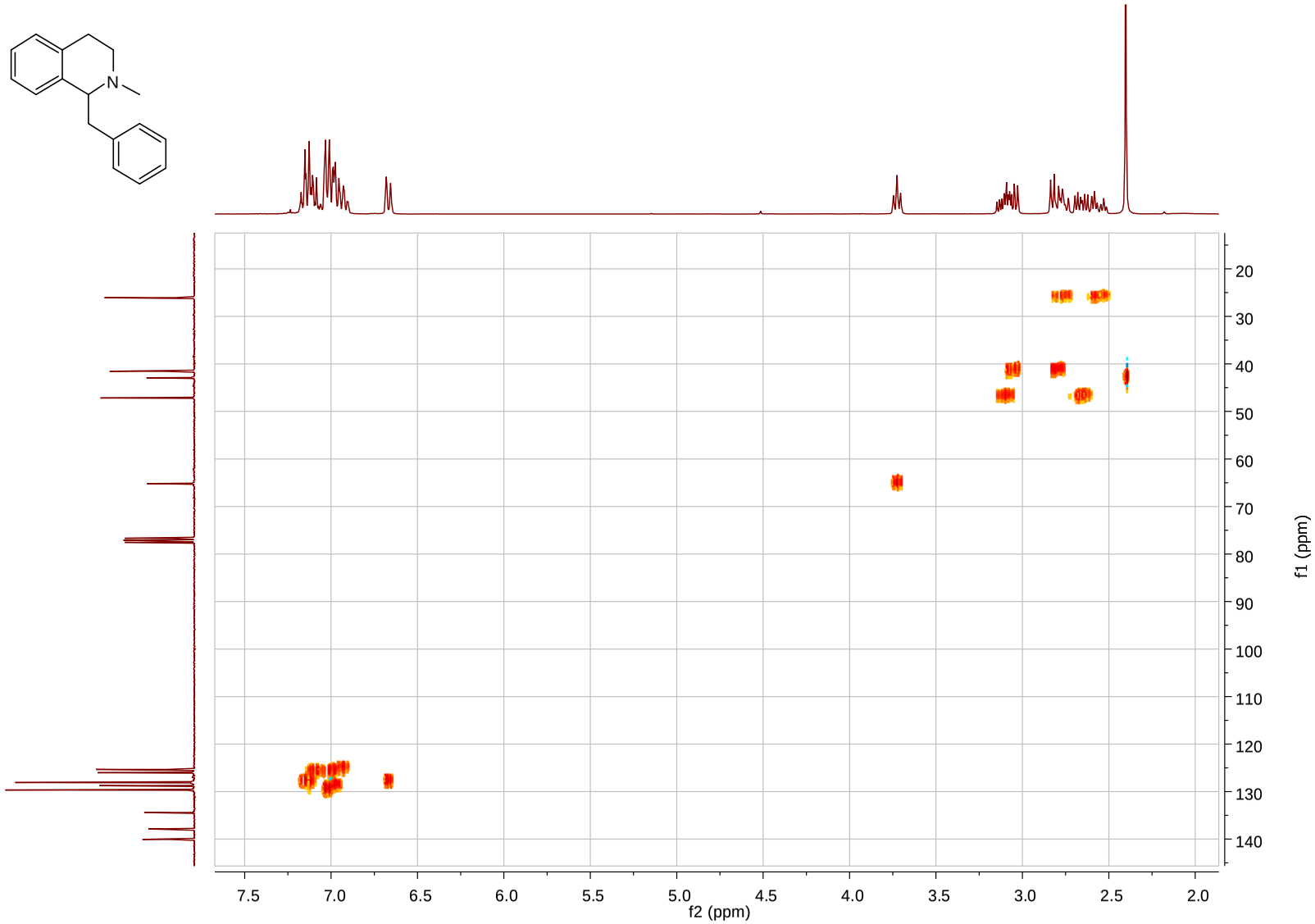
1-Benzyl-2-methyl-1,2,3,4-tetrahydroisoquinoline (1a): ^{13}C-NMR spectrum



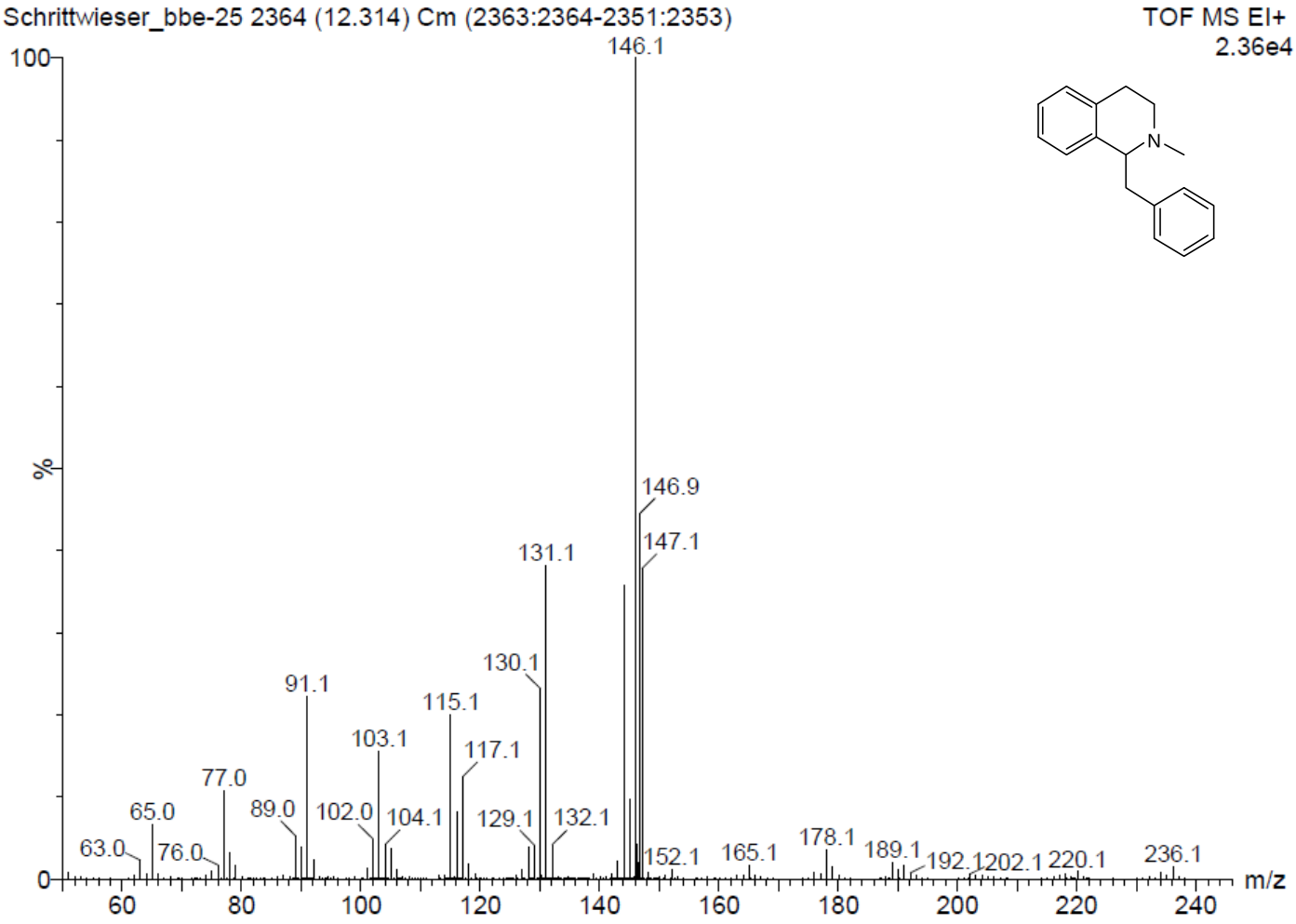
1-Benzyl-2-methyl-1,2,3,4-tetrahydroisoquinoline (1a): COSY spectrum



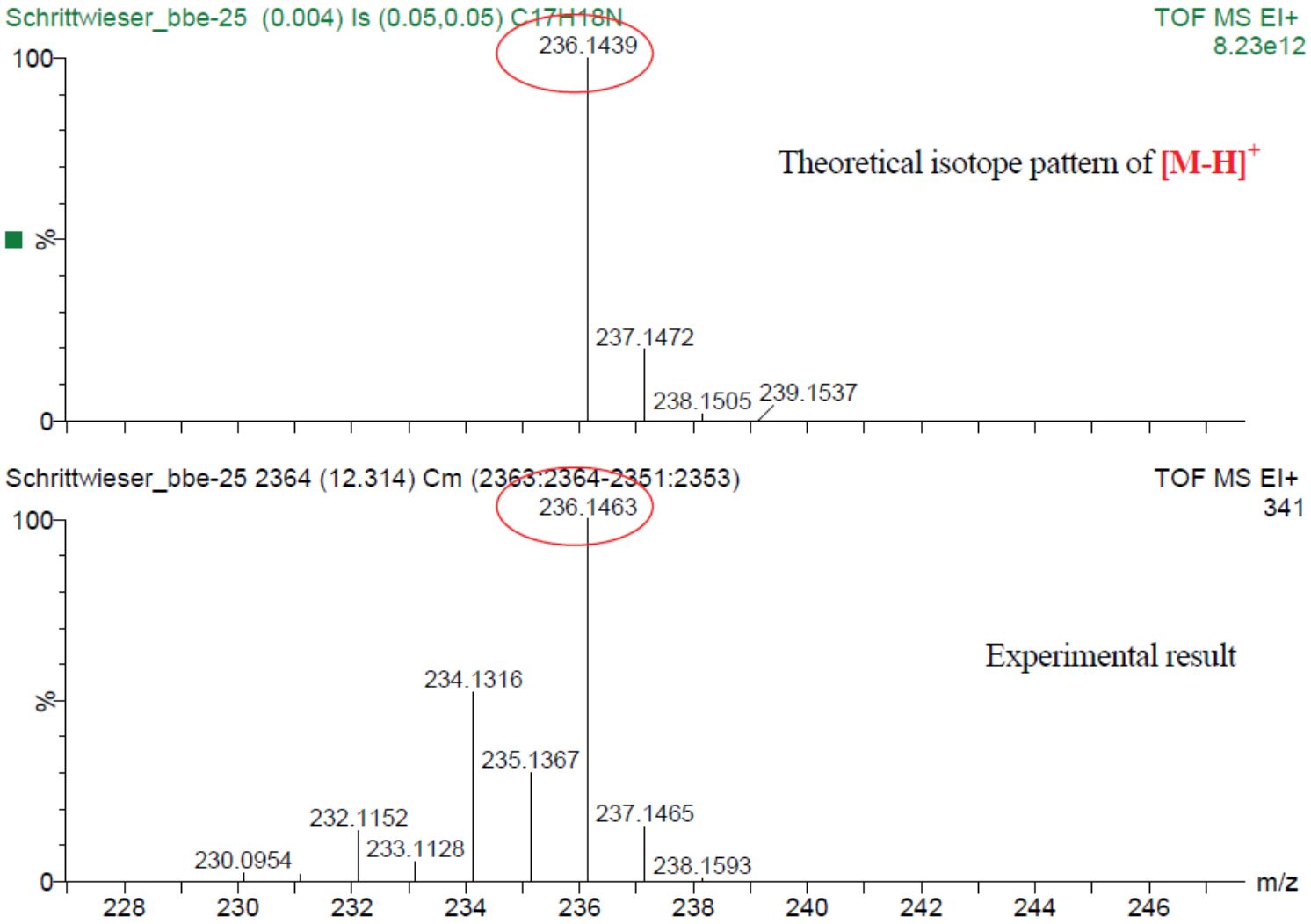
1-Benzyl-2-methyl-1,2,3,4-tetrahydroisoquinoline (1a): HSQC spectrum



1-Benzyl-2-methyl-1,2,3,4-tetrahydroisoquinoline (1a): MS spectrum



1-Benzyl-2-methyl-1,2,3,4-tetrahydroisoquinoline (1a): HRMS results



Substrate 2a and its Synthetic Intermediates

Provided Material:

Phenylacetyl chloride:

^{1}H-NMR spectrum, ^{13}C-NMR spectrum

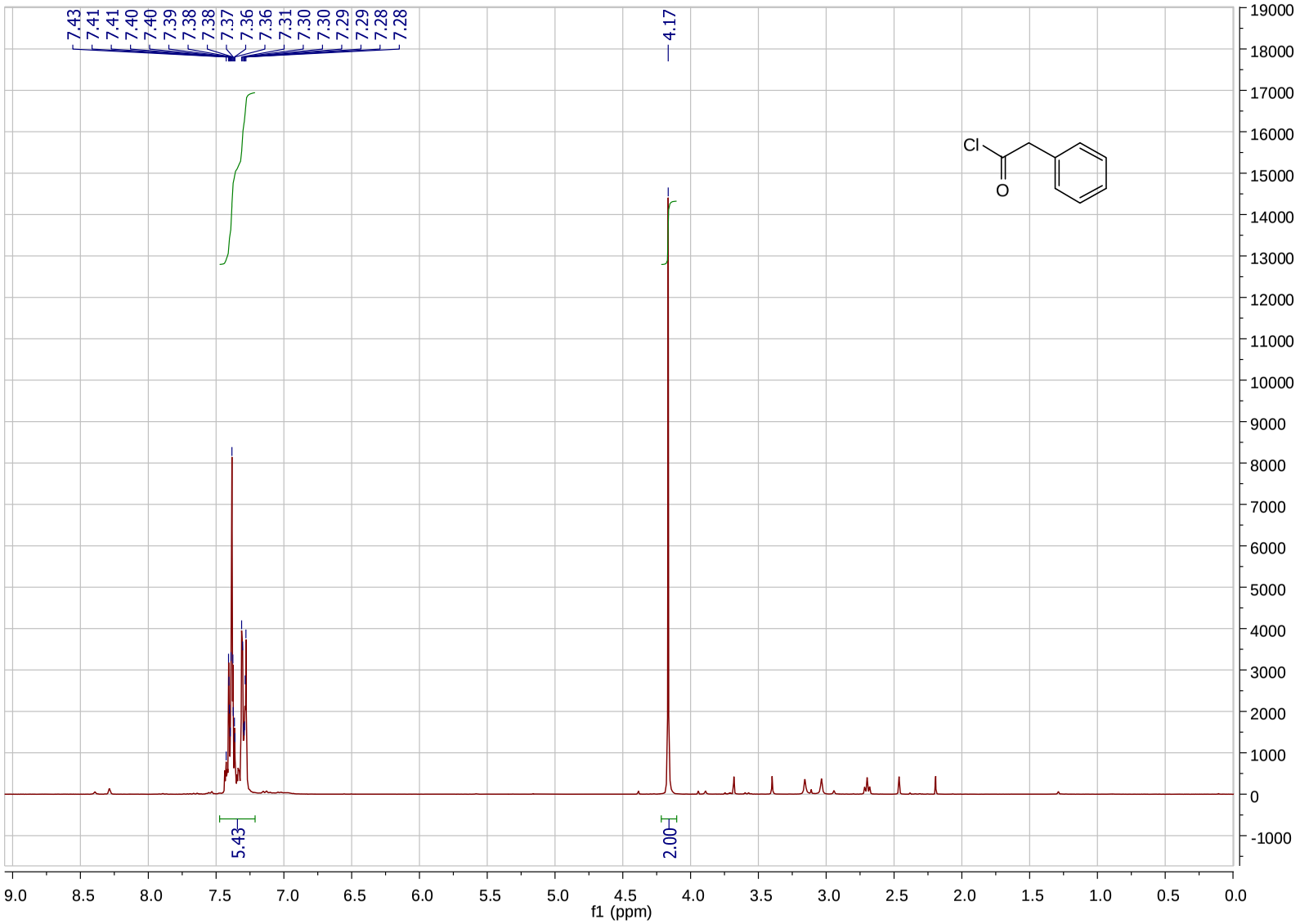
***N*-(3,4-Dimethoxyphenethyl)-*N*-methyl-2-phenylacetamide:**

^{1}H-NMR spectrum, ^{13}C-NMR spectrum, ^{13}C-NMR DEPT135 spectrum, ^{13}C-NMR DEPT90 spectrum, COSY spectrum, HSQC spectrum, MS spectrum, HRMS results

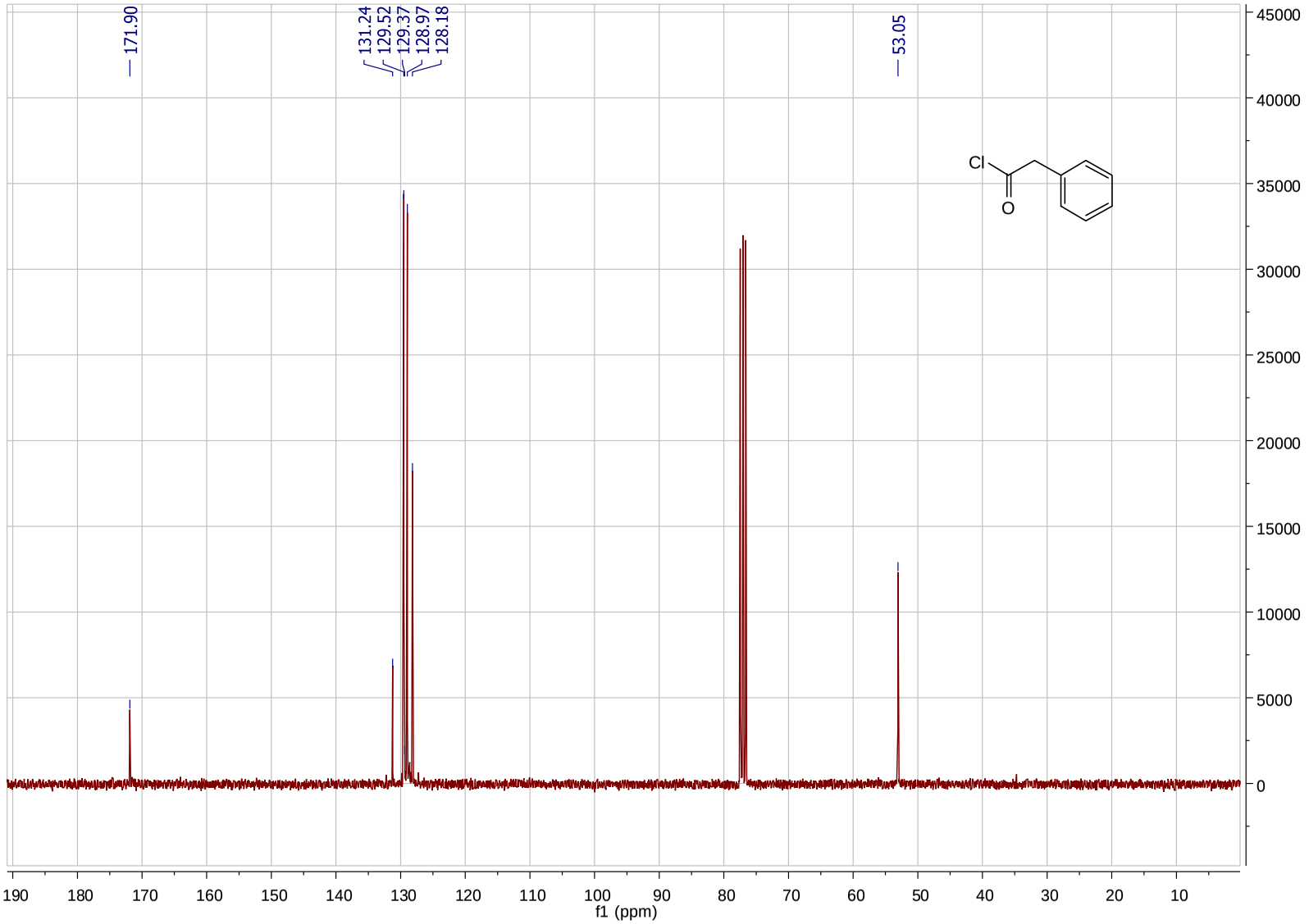
1-Benzyl-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (2a):

^{1}H-NMR spectrum, ^{13}C-NMR spectrum, COSY spectrum, HSQC spectrum, MS spectrum, HRMS results

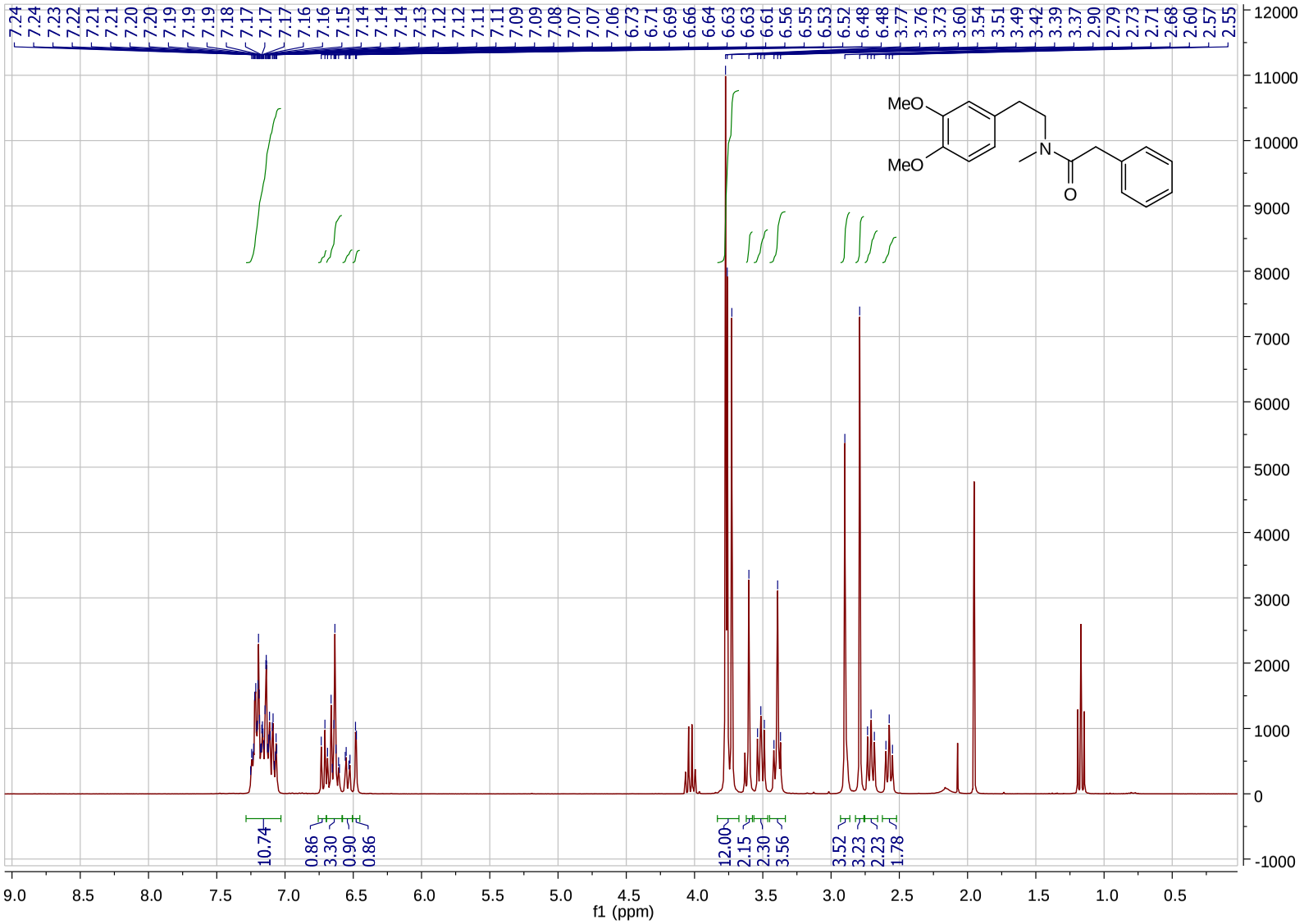
Phenylacetyl chloride: ^{1}H-NMR spectrum



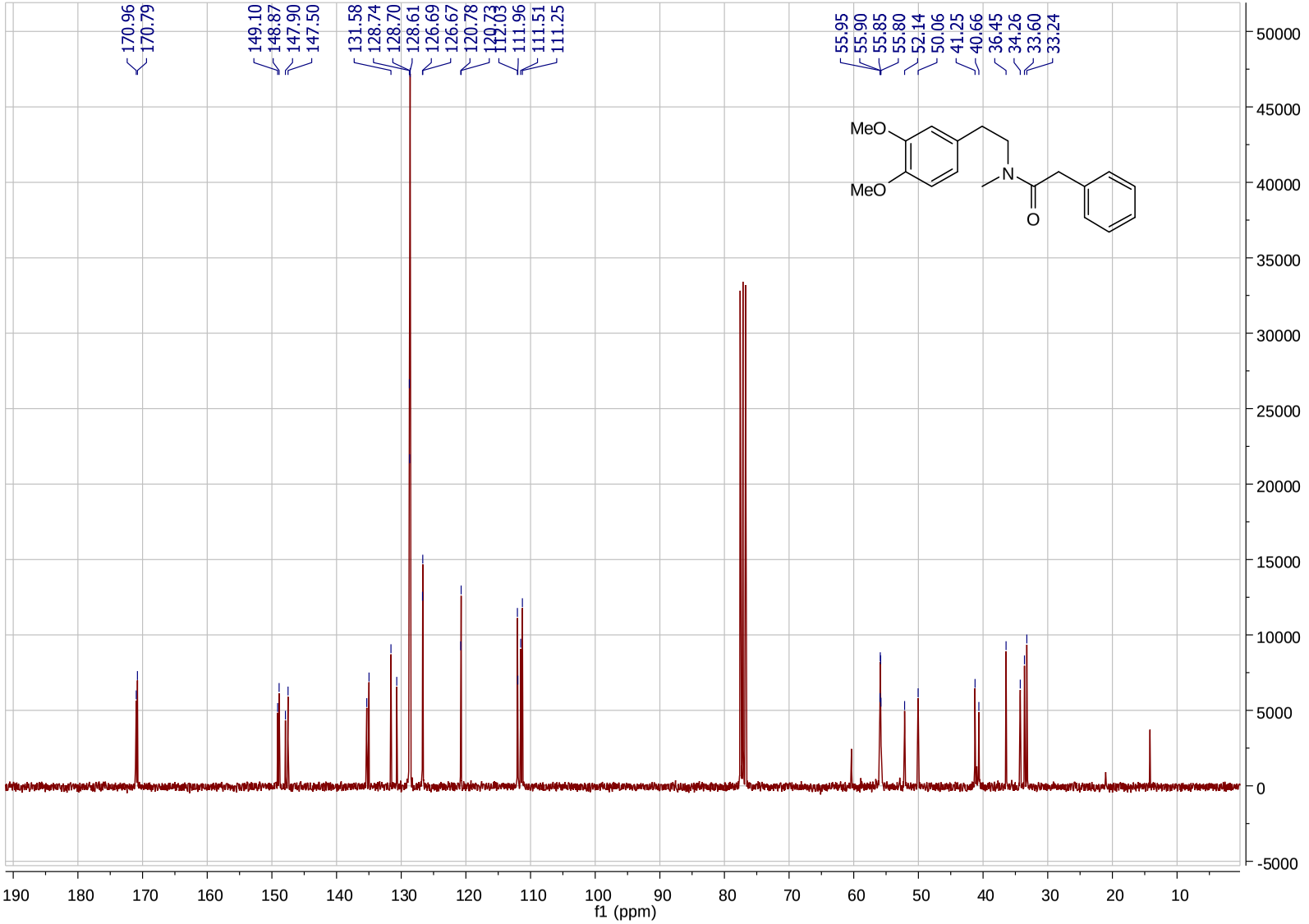
Phenylacetyl chloride: ^{13}C-NMR spectrum



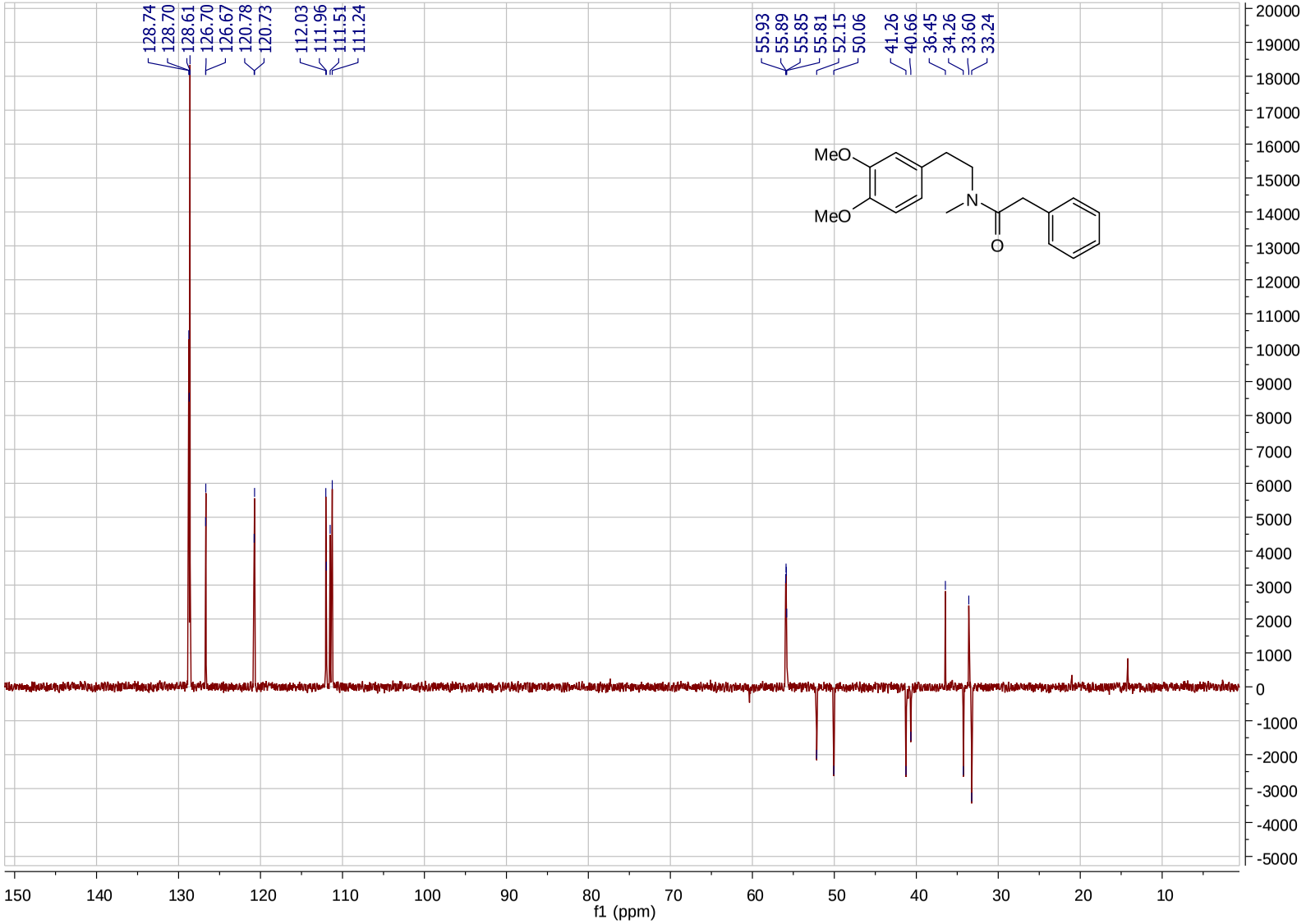
_N_-(3,4-Dimethoxyphenethyl)-_N_-methyl-2-phenylacetamide: ^{1}H-NMR spectrum



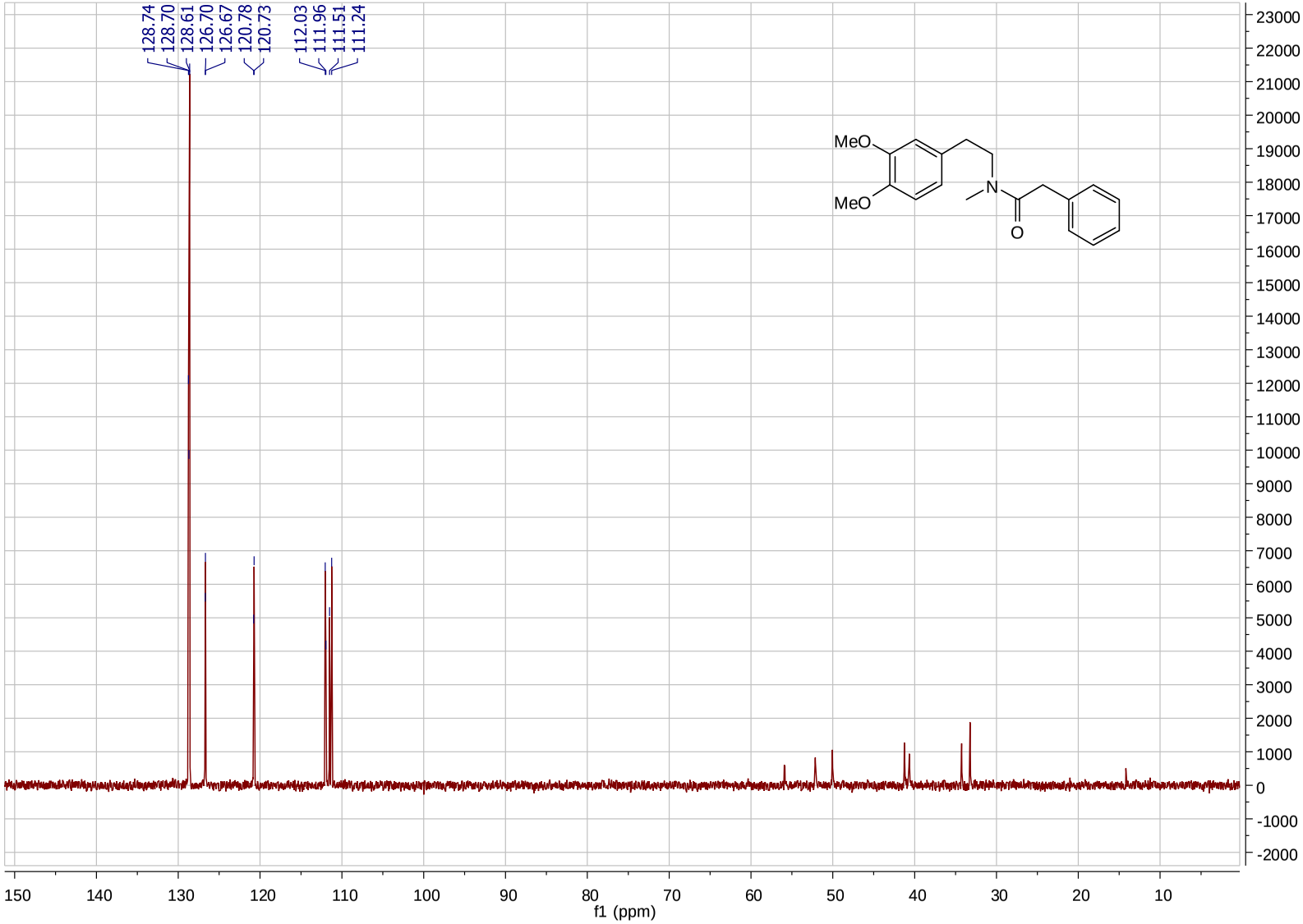
_N_-(3,4-Dimethoxyphenethyl)-_N_-methyl-2-phenylacetamide: ^{13}C-NMR spectrum



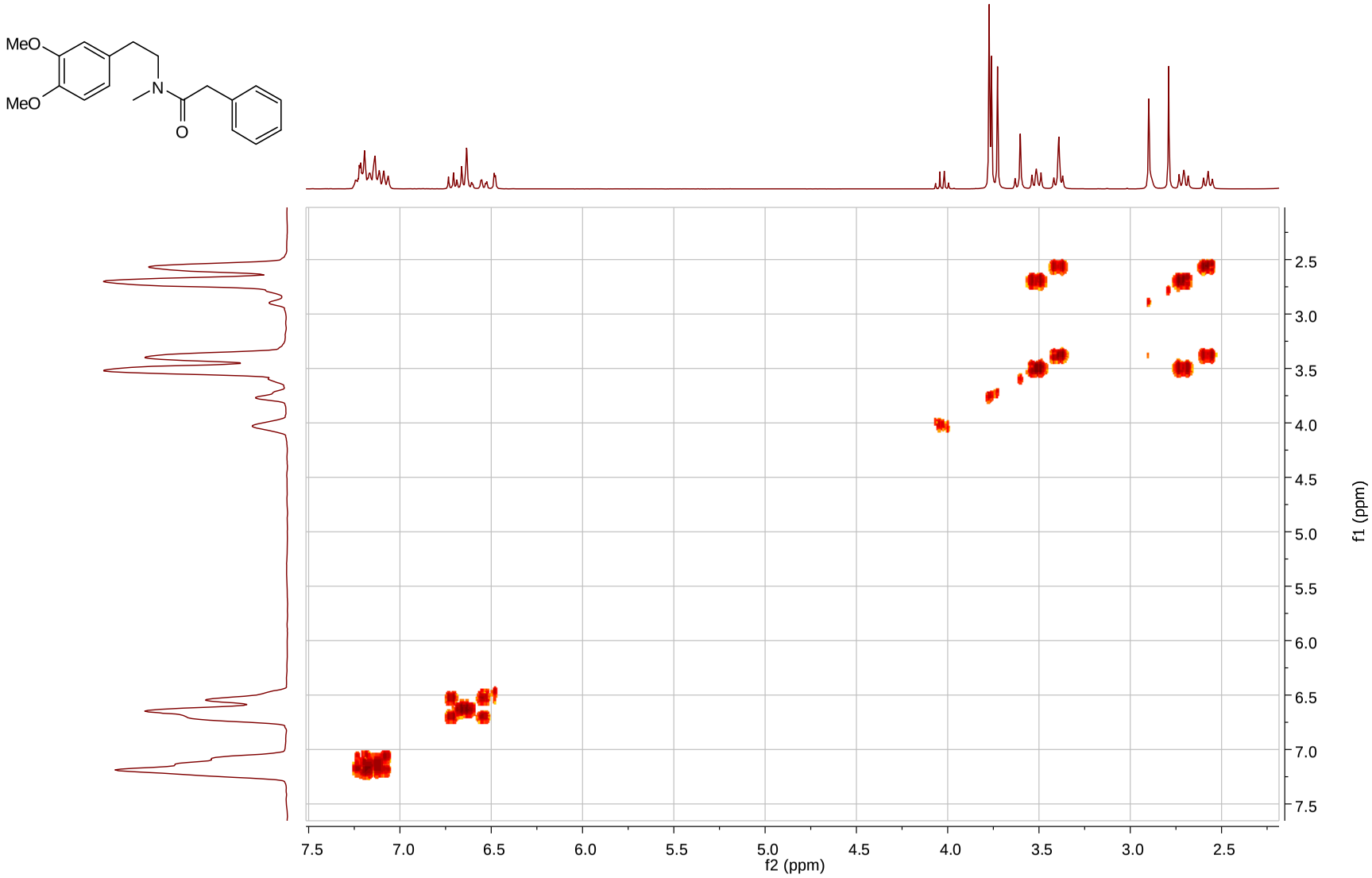
_N_-(3,4-Dimethoxyphenethyl)-_N_-methyl-2-phenylacetamide: ^{13}C-NMR DEPT135 spectrum



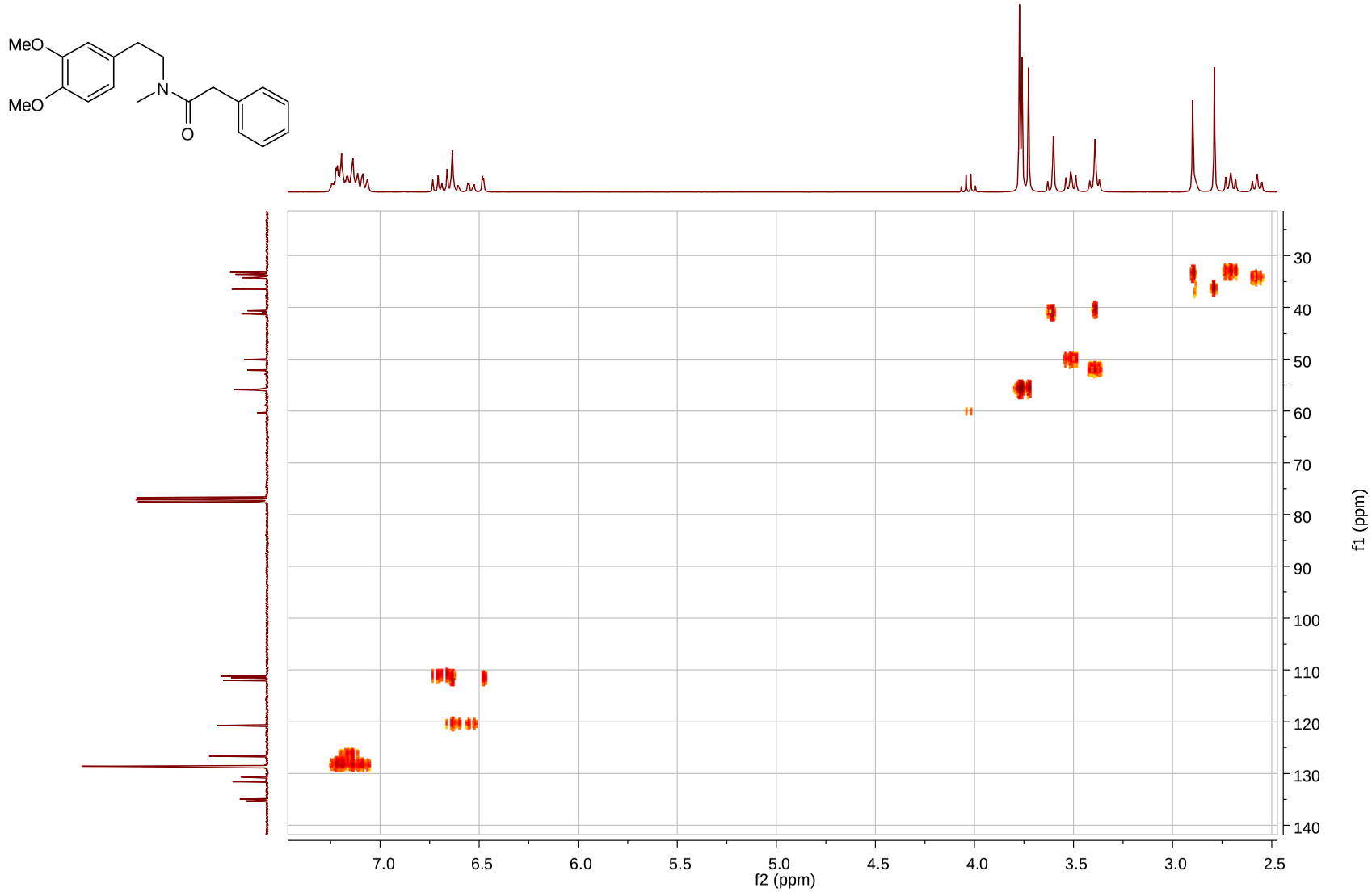
_N_-(3,4-Dimethoxyphenethyl)-_N_-methyl-2-phenylacetamide: ^{13}C-NMR DEPT90 spectrum



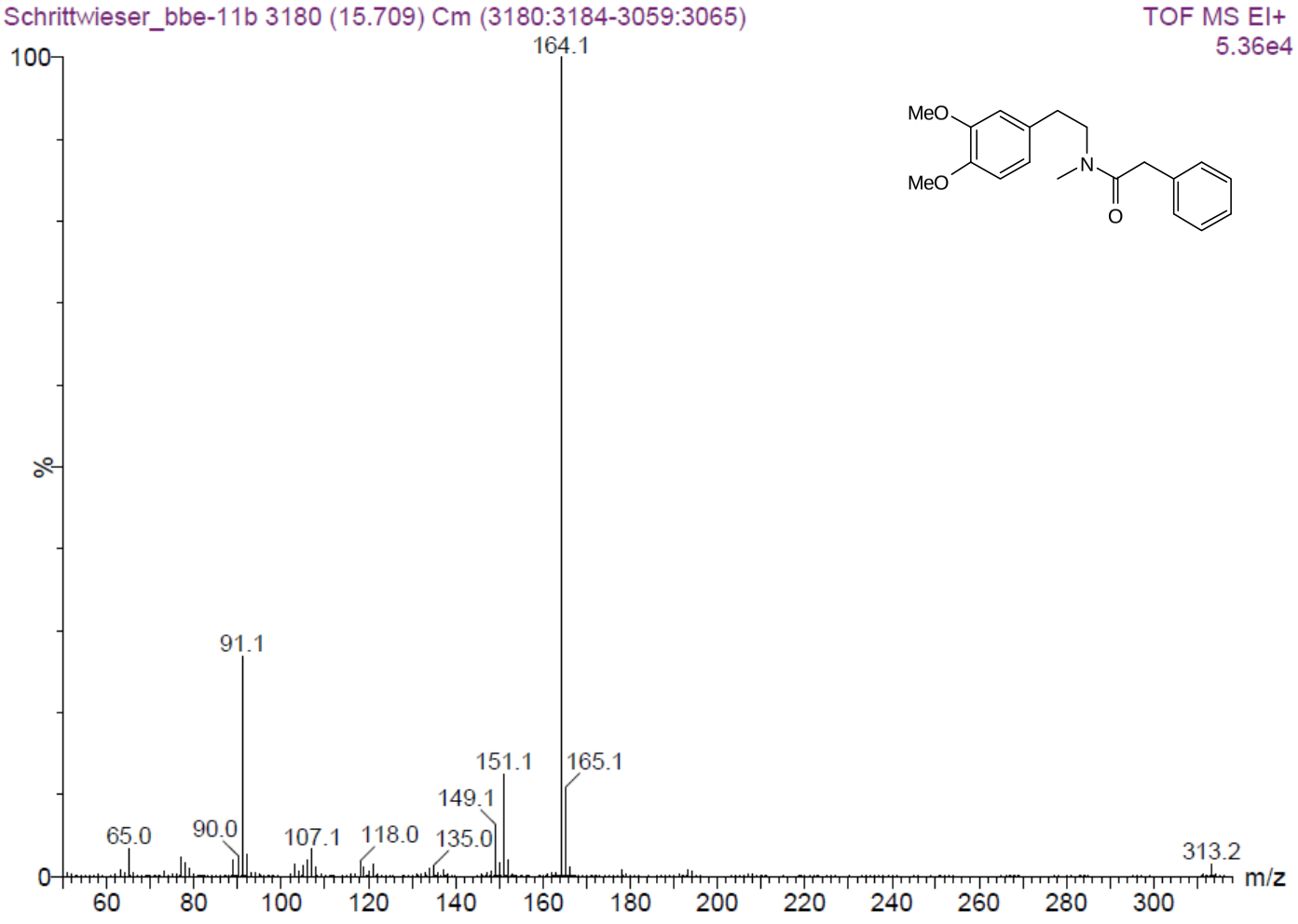
_N_-(3,4-Dimethoxyphenethyl)-_N_-methyl-2-phenylacetamide: COSY spectrum



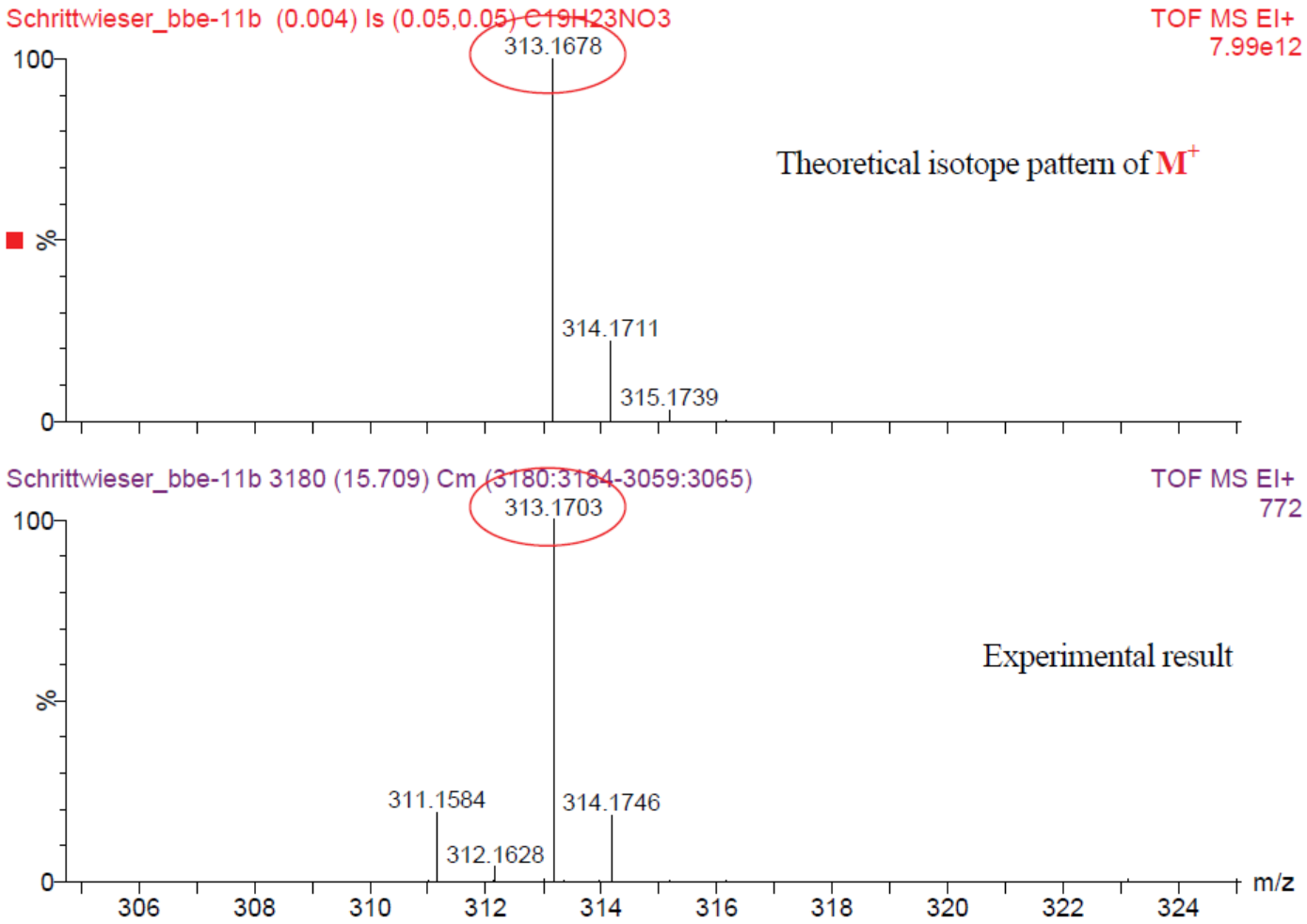
_N_-(3,4-Dimethoxyphenethyl)-_N_-methyl-2-phenylacetamide: HSQC spectrum



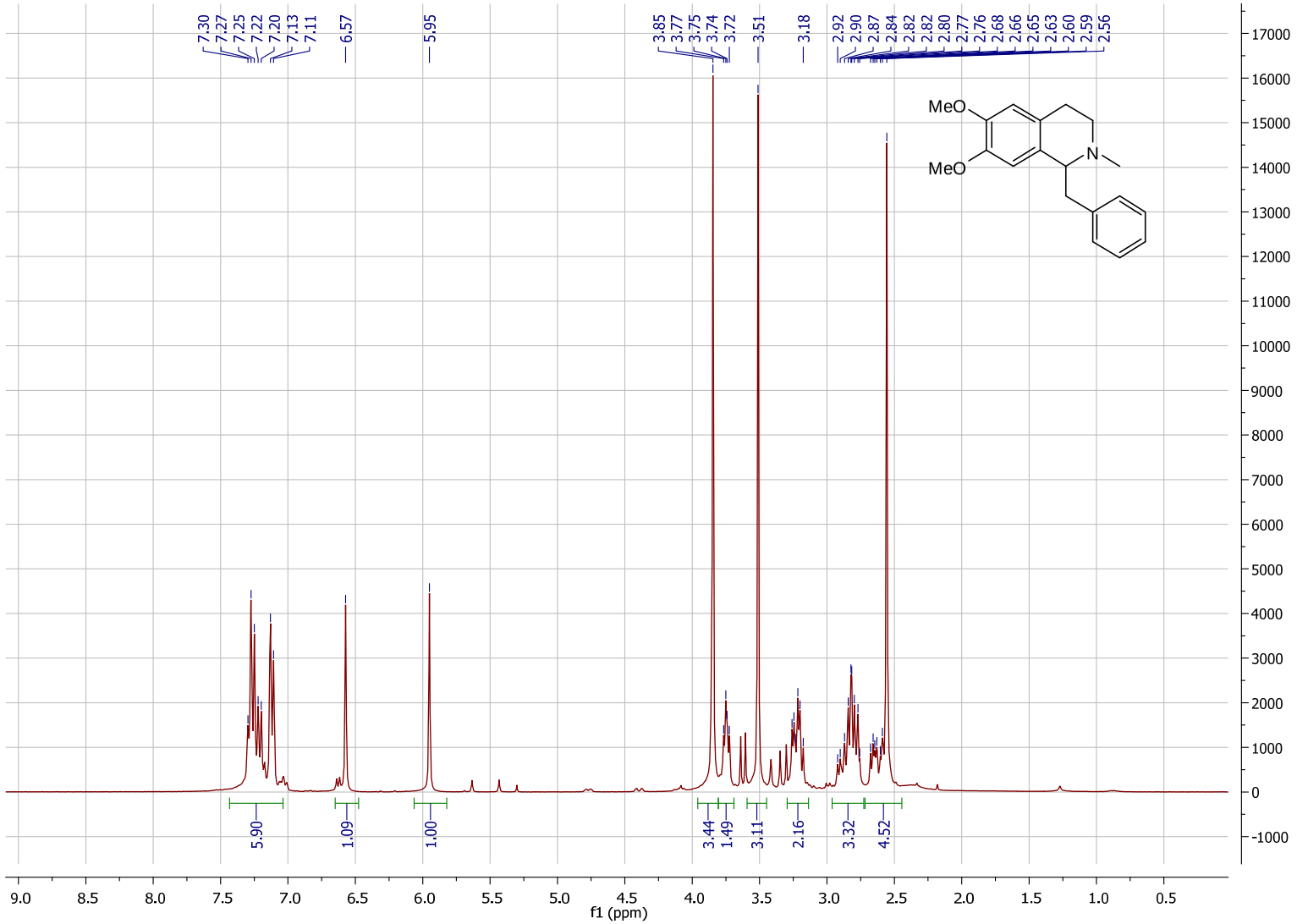
***N*-(3,4-Dimethoxyphenethyl)-*N*-methyl-2-phenylacetamide:** MS spectrum



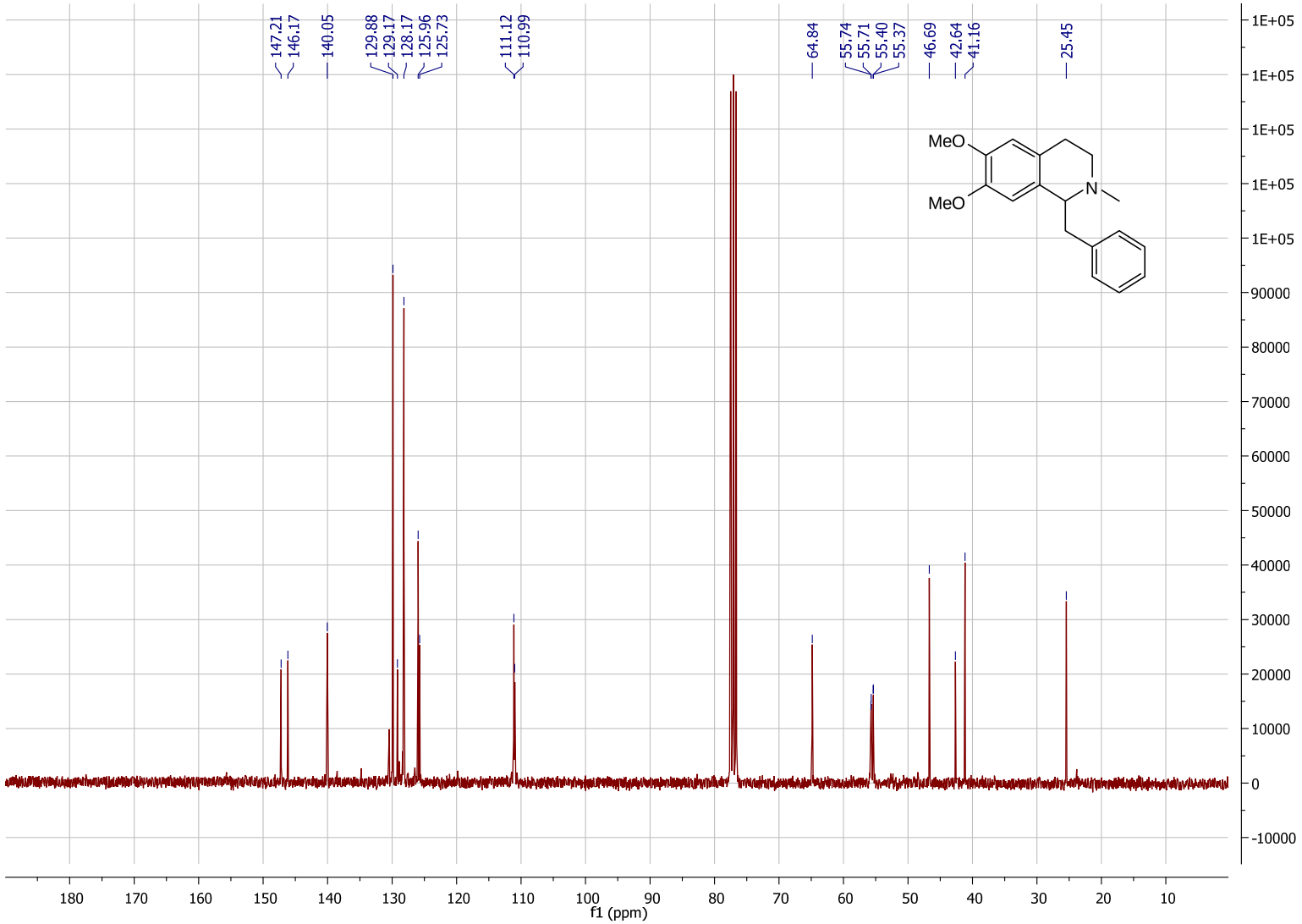
***N*-(3,4-Dimethoxyphenethyl)-*N*-methyl-2-phenylacetamide:** HRMS results



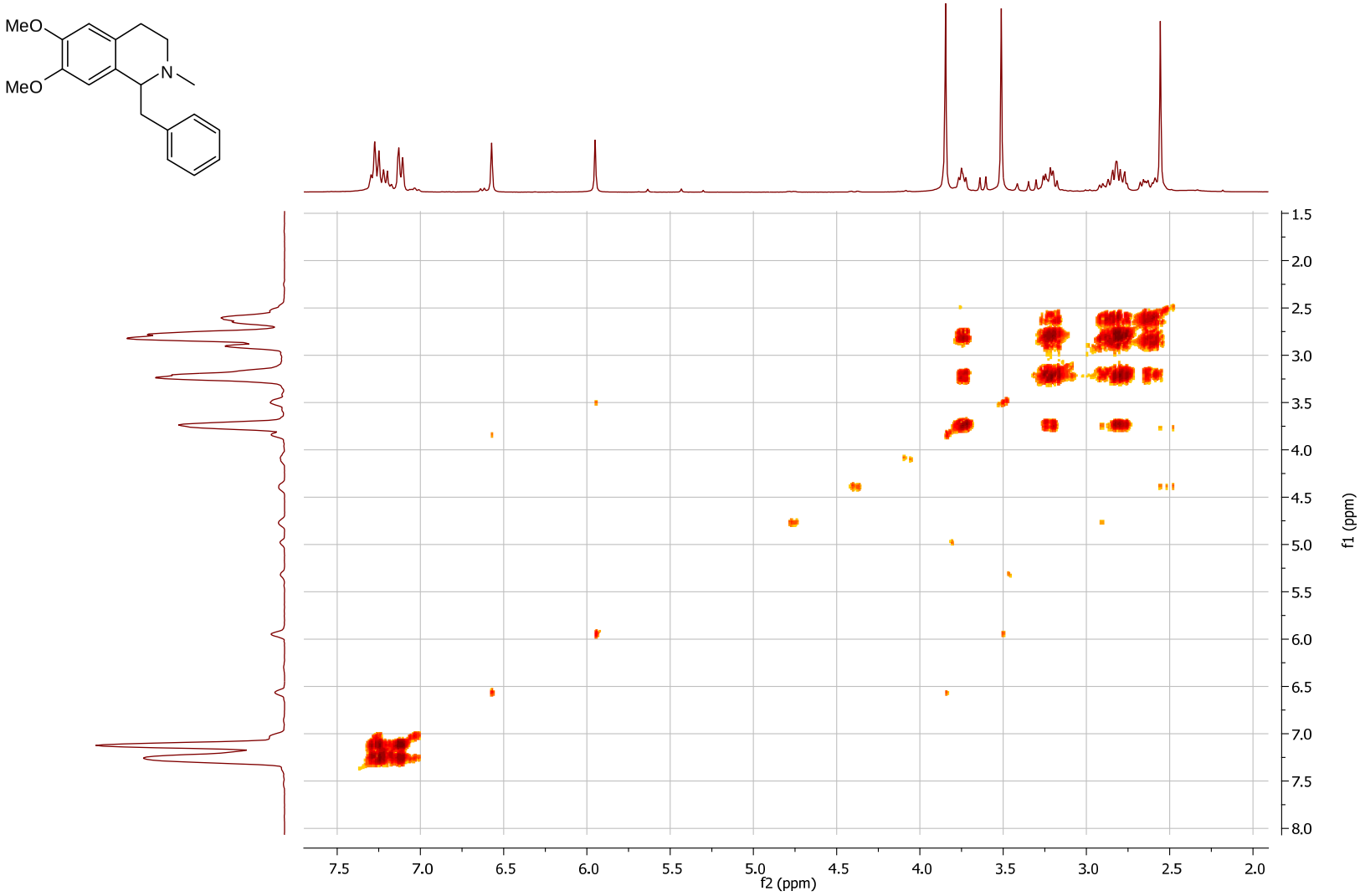
1-Benzyl-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (2a): ^{1}H-NMR spectrum



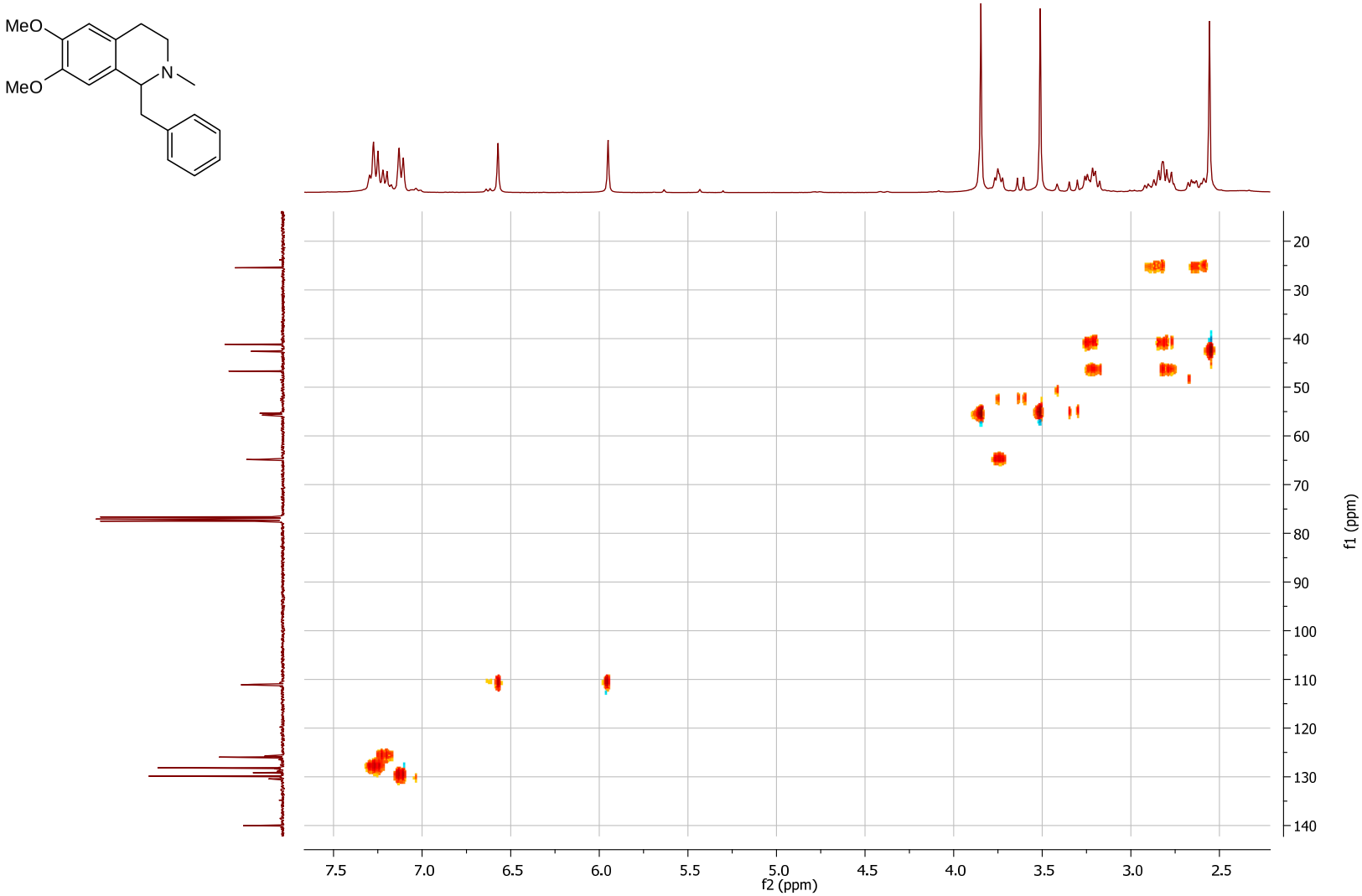
1-Benzyl-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (2a): ^{13}C-NMR spectrum



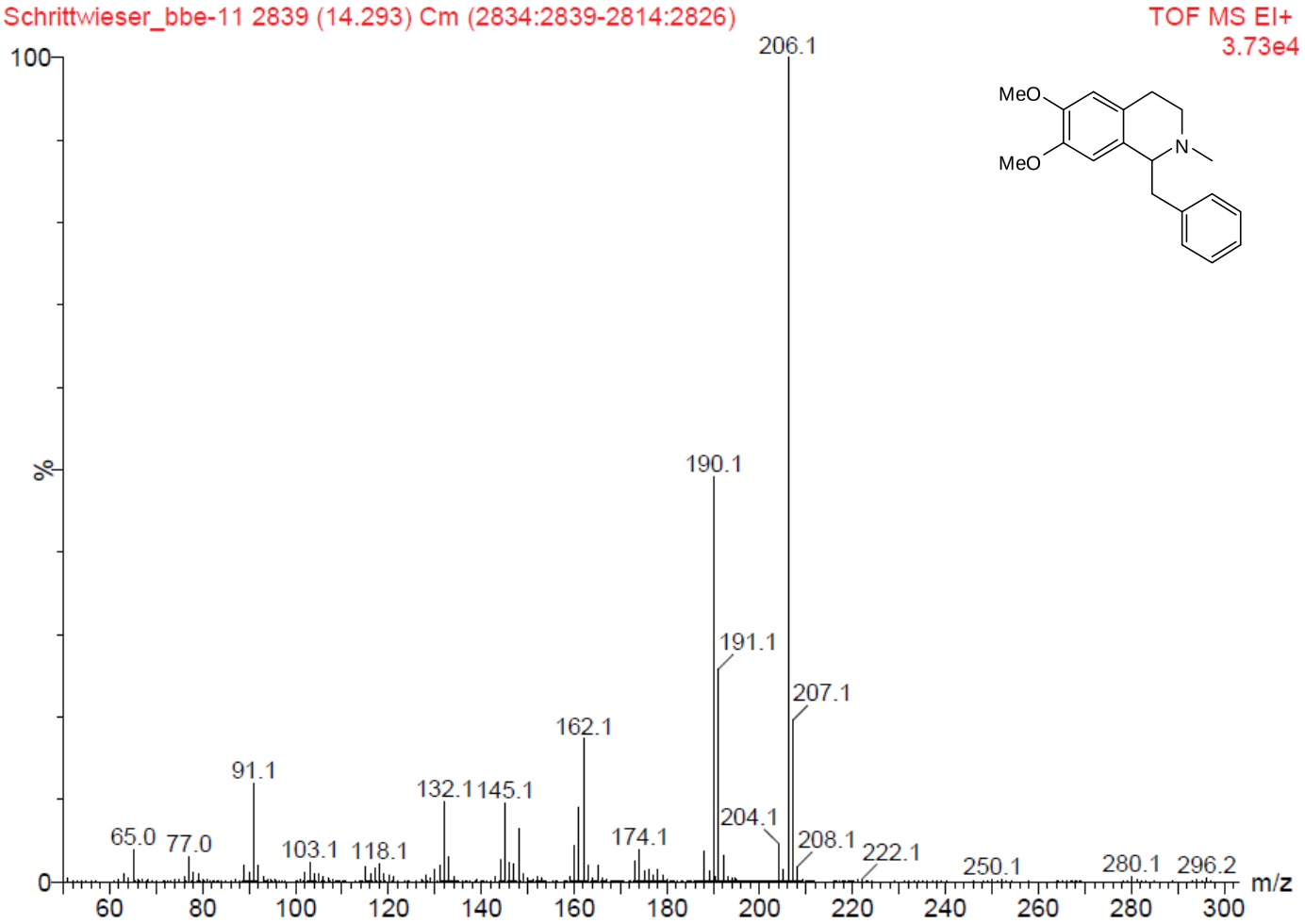
1-Benzyl-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (2a): COSY spectrum



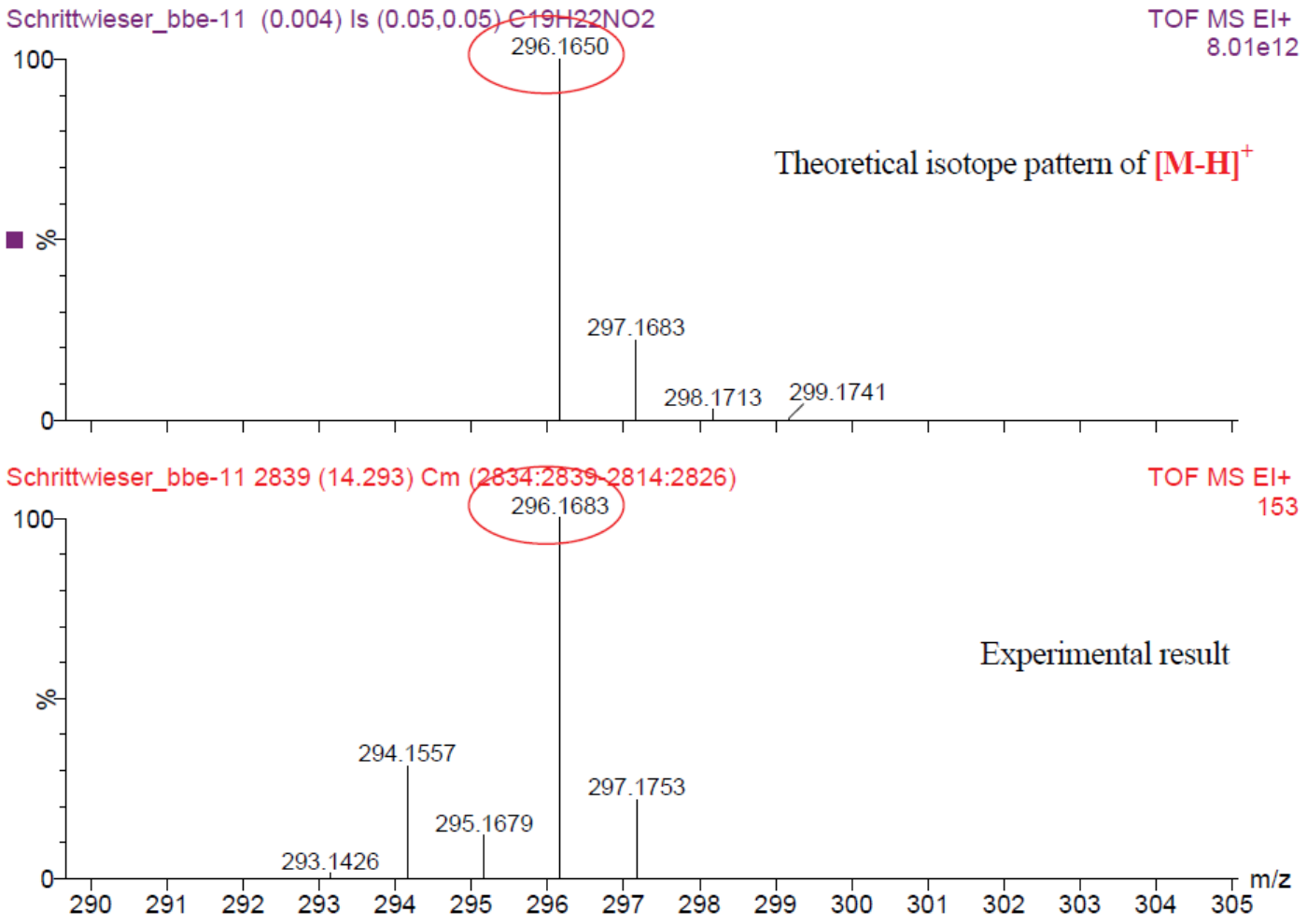
1-Benzyl-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (2a): HSQC spectrum



1-Benzyl-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (2a): MS spectrum



1-Benzyl-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (2a): HRMS results



Substrate 2c and its Synthetic Intermediates

Provided Material:

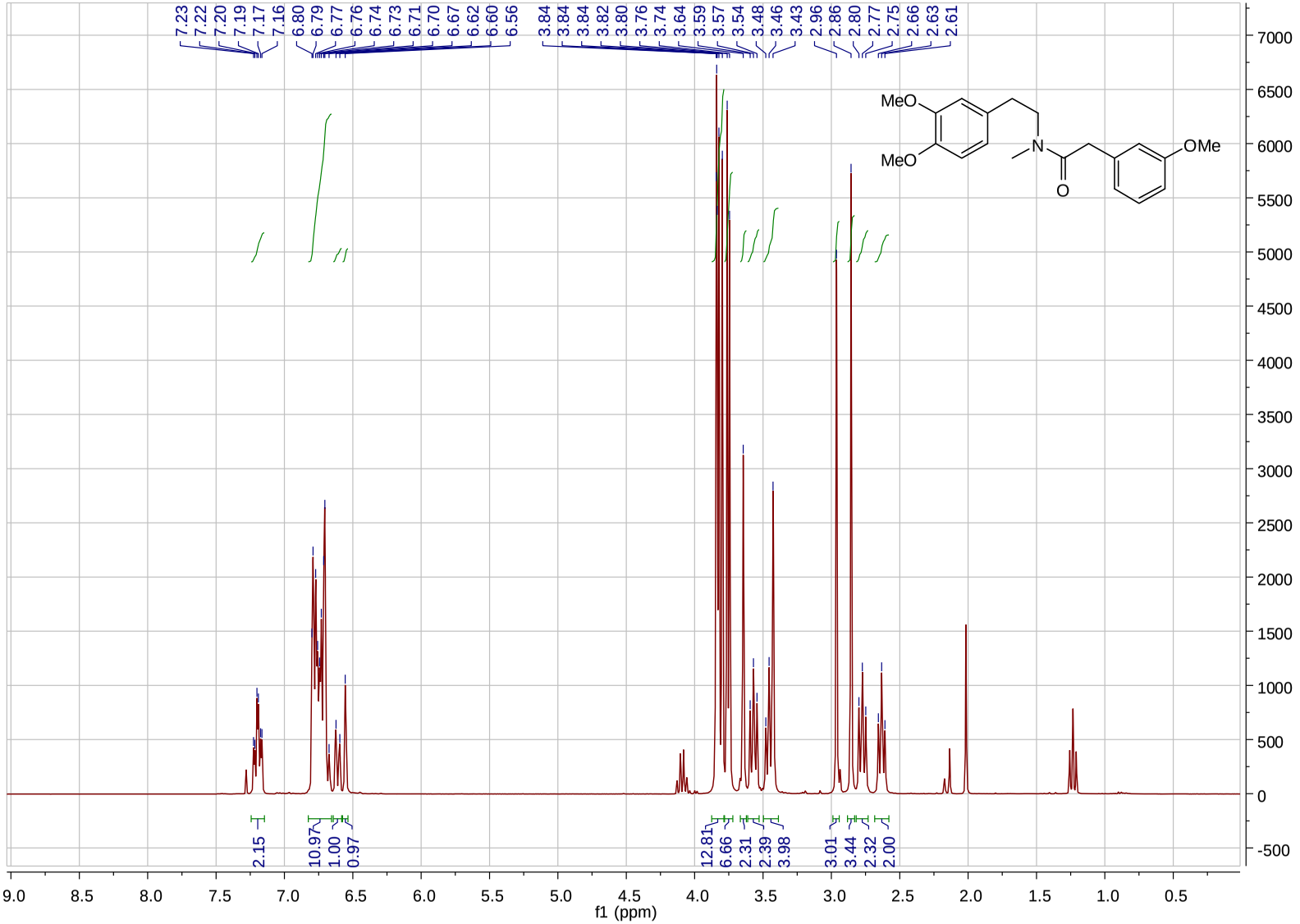
***N*-(3,4-Dimethoxyphenethyl)-2-(3-methoxyphenyl)-*N*-methylacetamide:**

^{1}H-NMR spectrum, ^{13}C-NMR spectrum, ^{13}C-NMR DEPT135 spectrum, ^{13}C-NMR DEPT90 spectrum, COSY spectrum, HSQC spectrum, MS spectrum, HRMS results

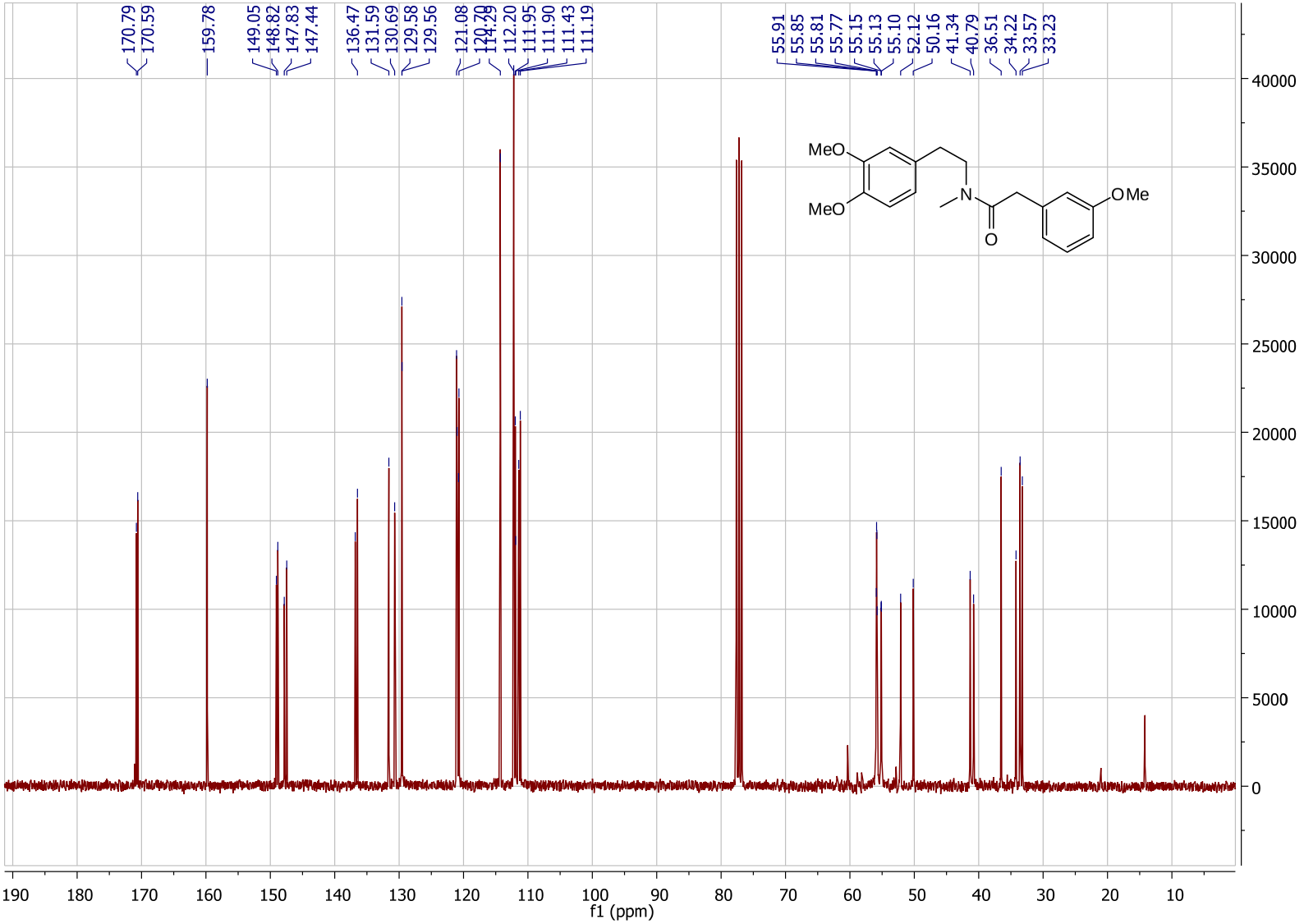
6,7-Dimethoxy-1-(3-methoxybenzyl)-2-ethyl-1,2,3,4-tetrahydroisoquinoline (2c):

^{1}H-NMR spectrum, ^{13}C-NMR spectrum, MS spectrum, HRMS results

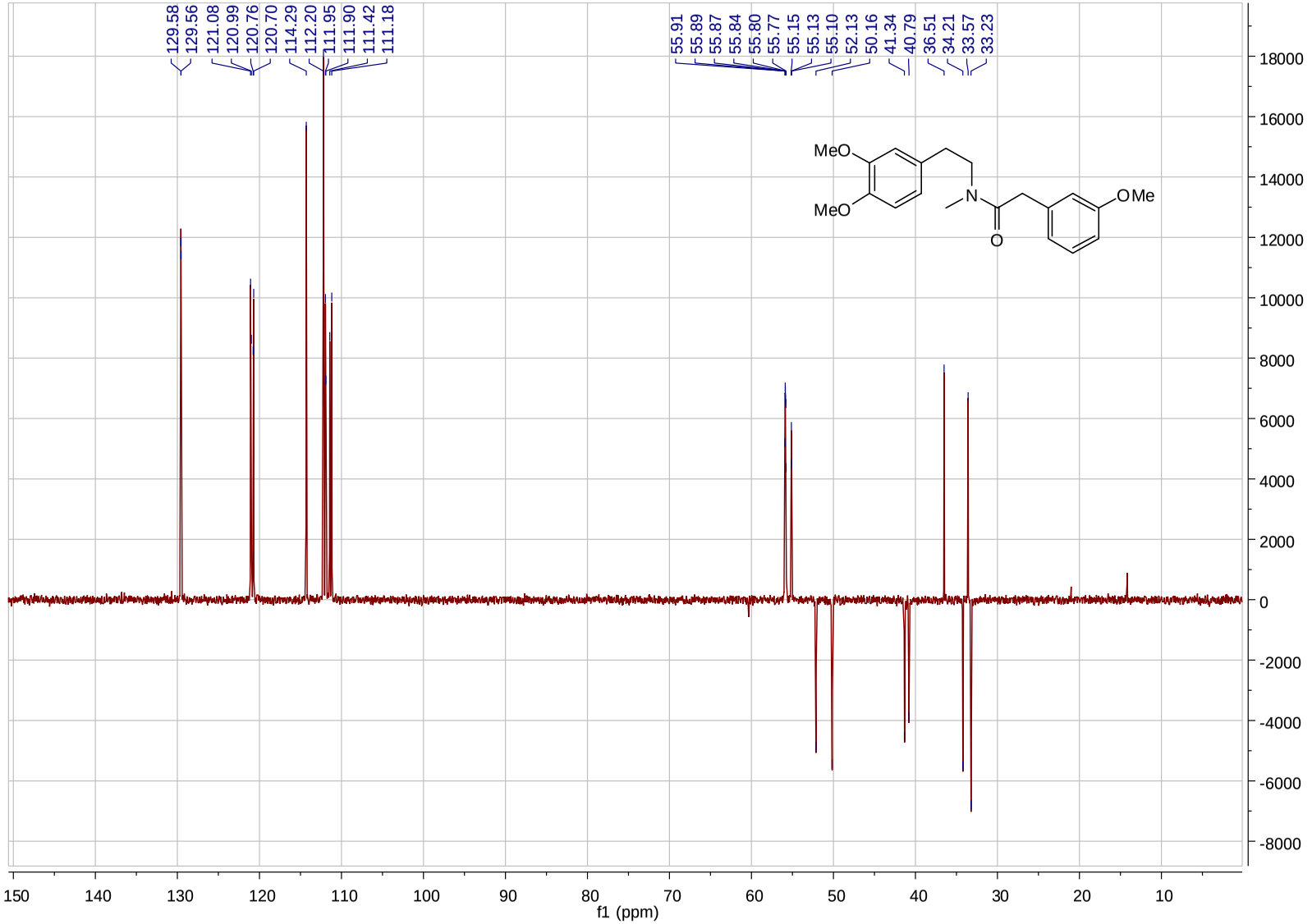
_N_-(3,4-Dimethoxyphenethyl)-2-(3-methoxyphenyl)-_N_-methylacetamide: ^{1}H-NMR spectrum



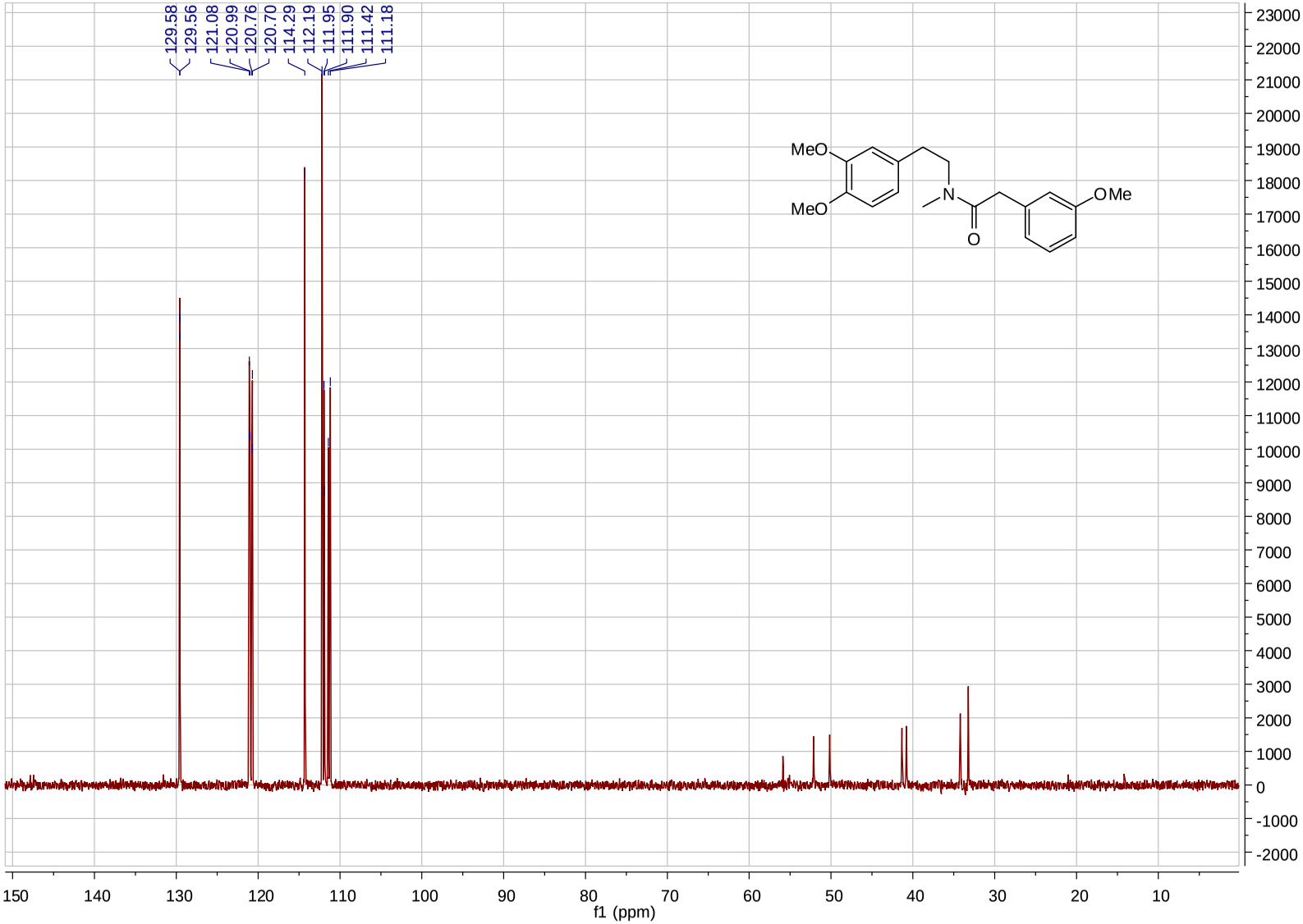
***N*-(3,4-Dimethoxyphenethyl)-2-(3-methoxyphenyl)-*N*-methylacetamide:** ^{13}C-NMR spectrum



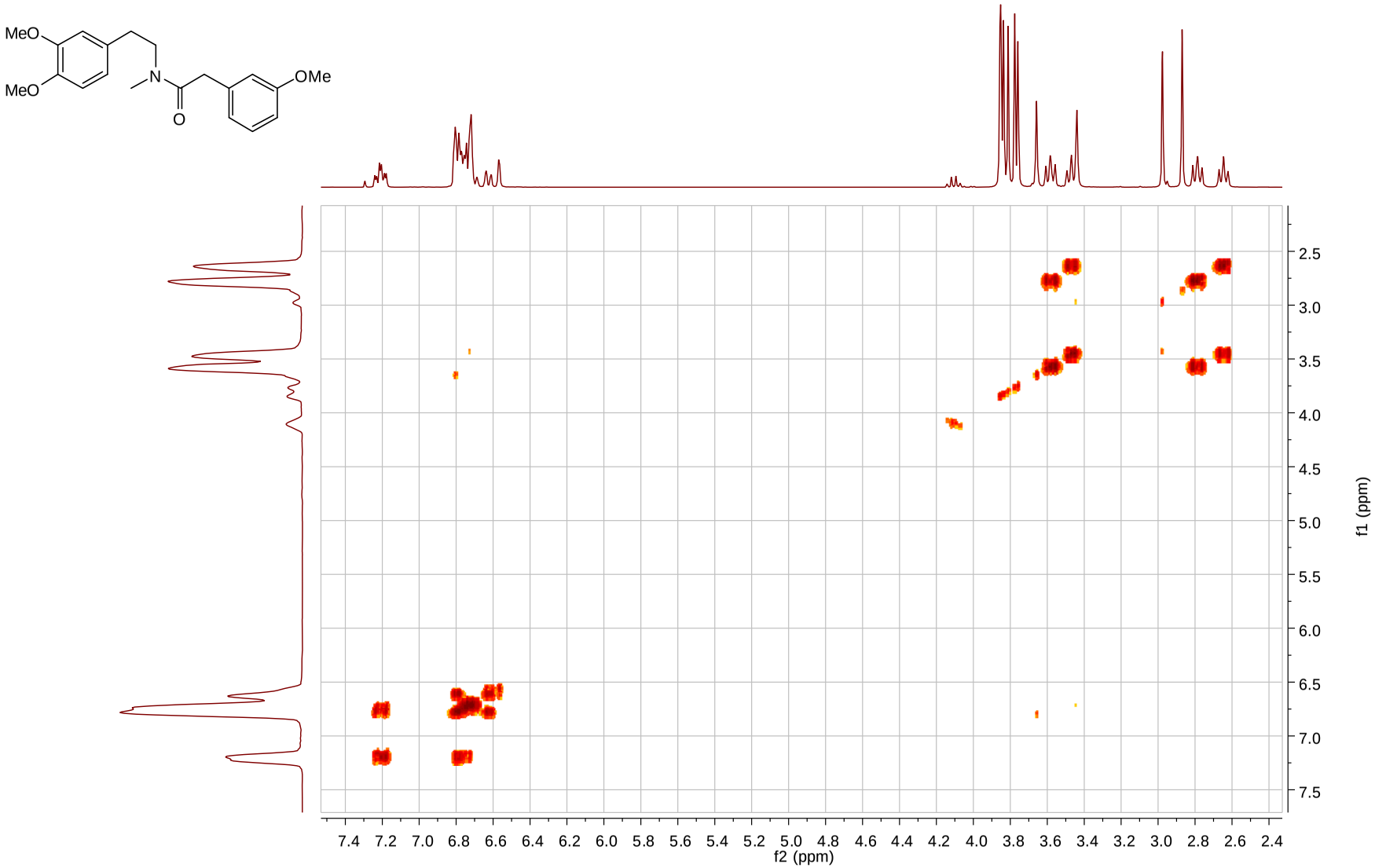
_N_-(3,4-Dimethoxyphenethyl)-2-(3-methoxyphenyl)-_N_-methylacetamide: ^{13}C-NMR DEPT135 spectrum



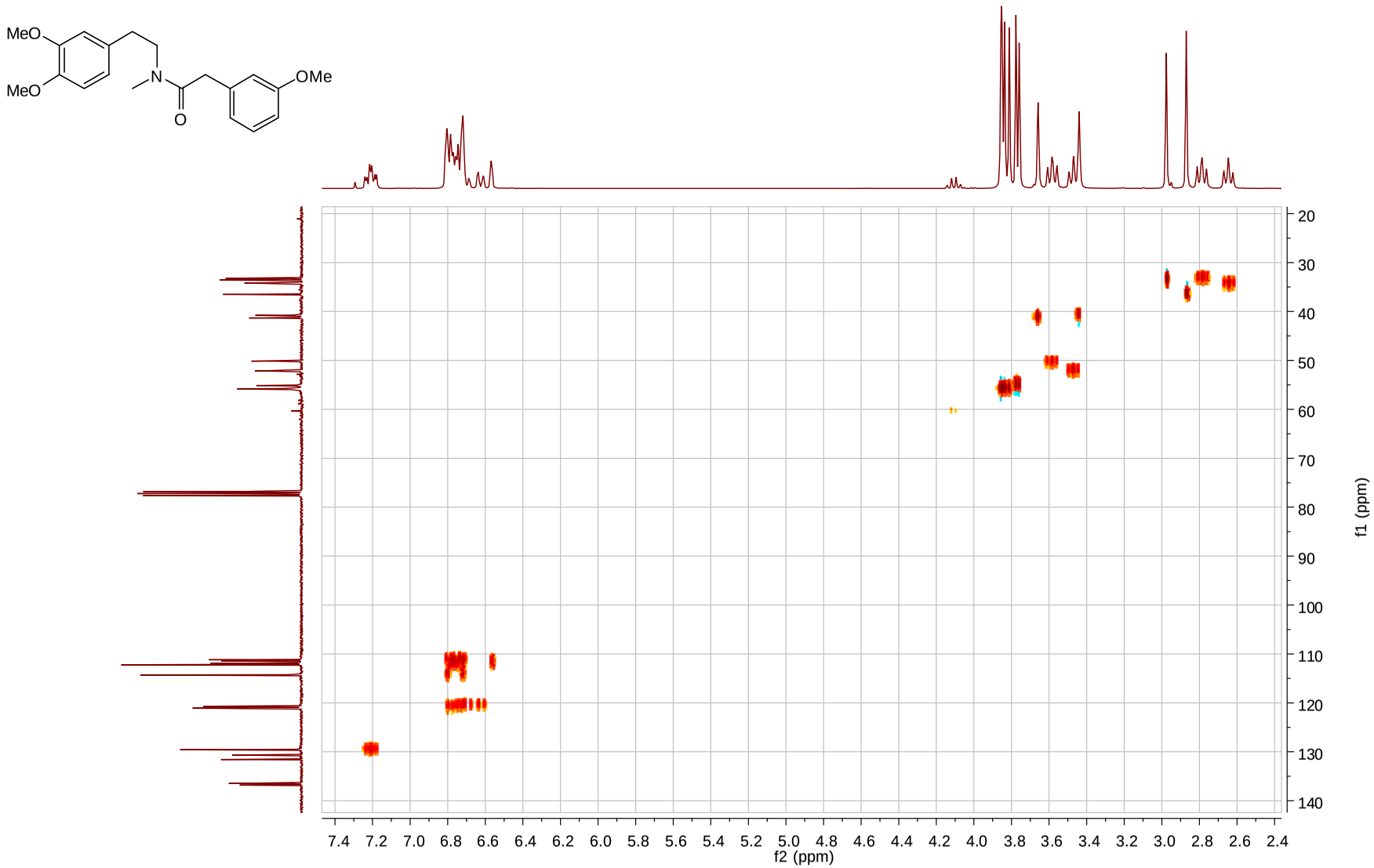
_N_-(3,4-Dimethoxyphenethyl)-2-(3-methoxyphenyl)-_N_-methylacetamide: ^{13}C-NMR DEPT90 spectrum



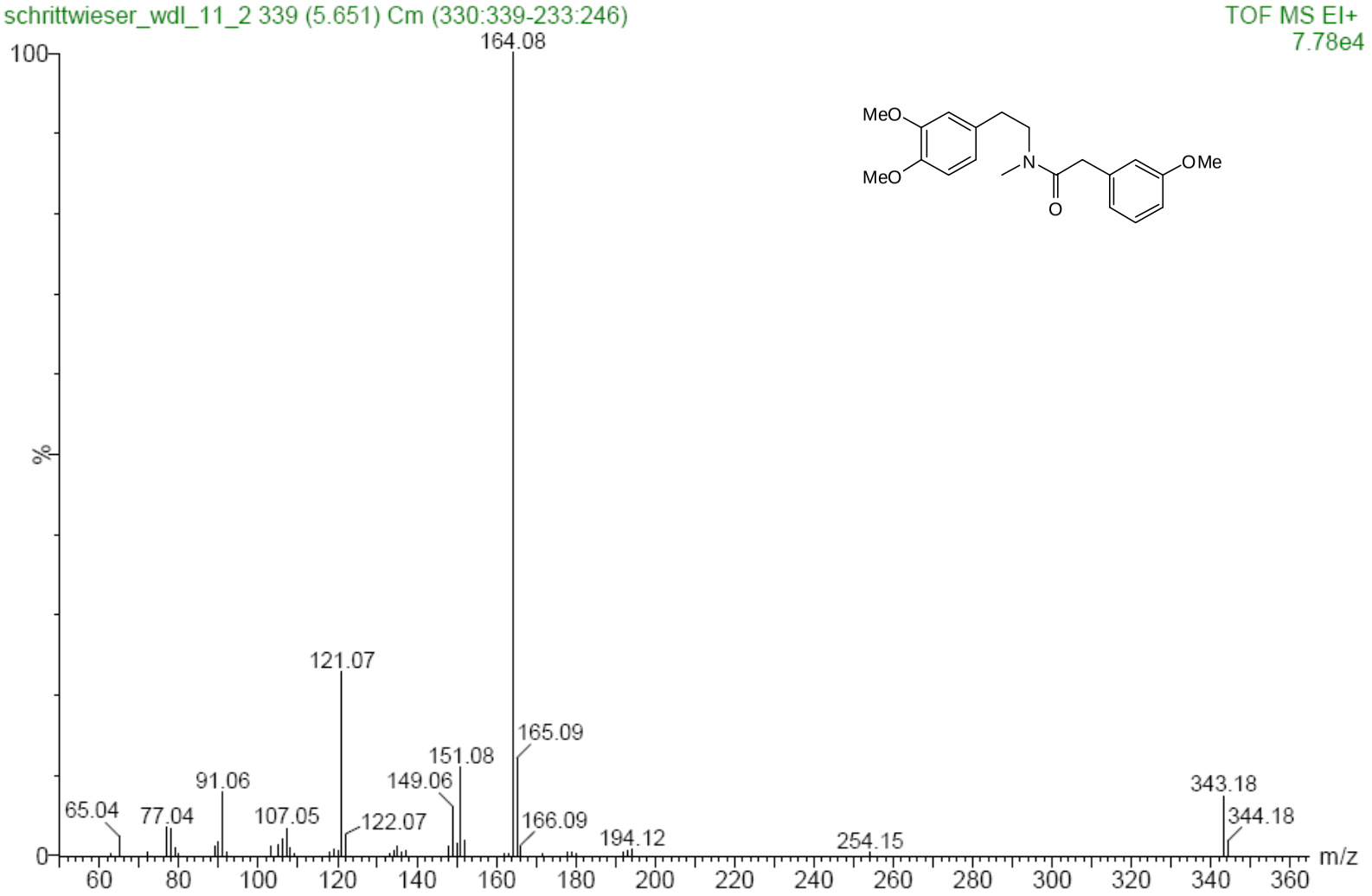
_N_-(3,4-Dimethoxyphenethyl)-2-(3-methoxyphenyl)-_N_-methylacetamide: COSY spectrum



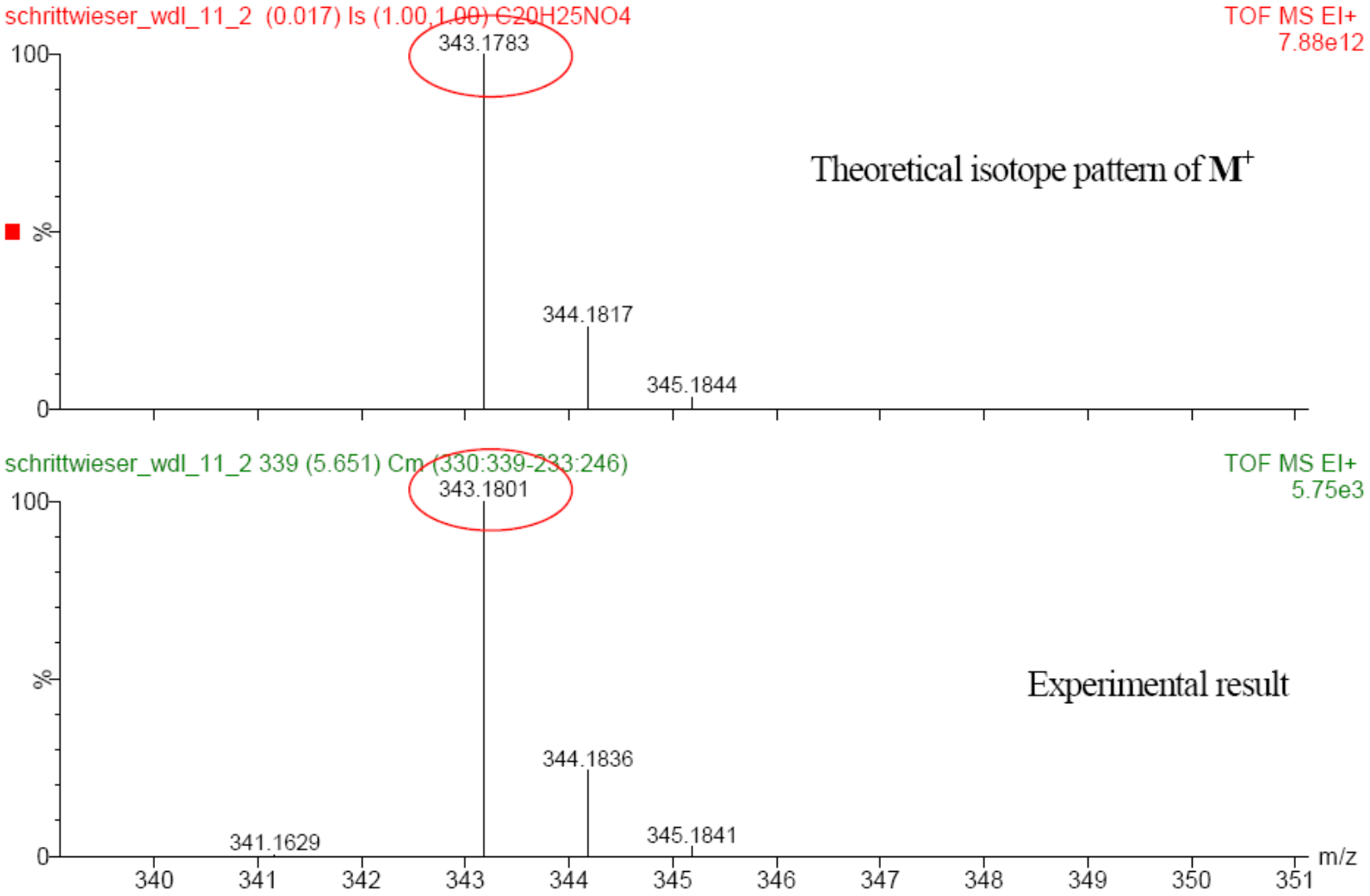
_N_-(3,4-Dimethoxyphenethyl)-2-(3-methoxyphenyl)-_N_-methylacetamide: HSQC spectrum



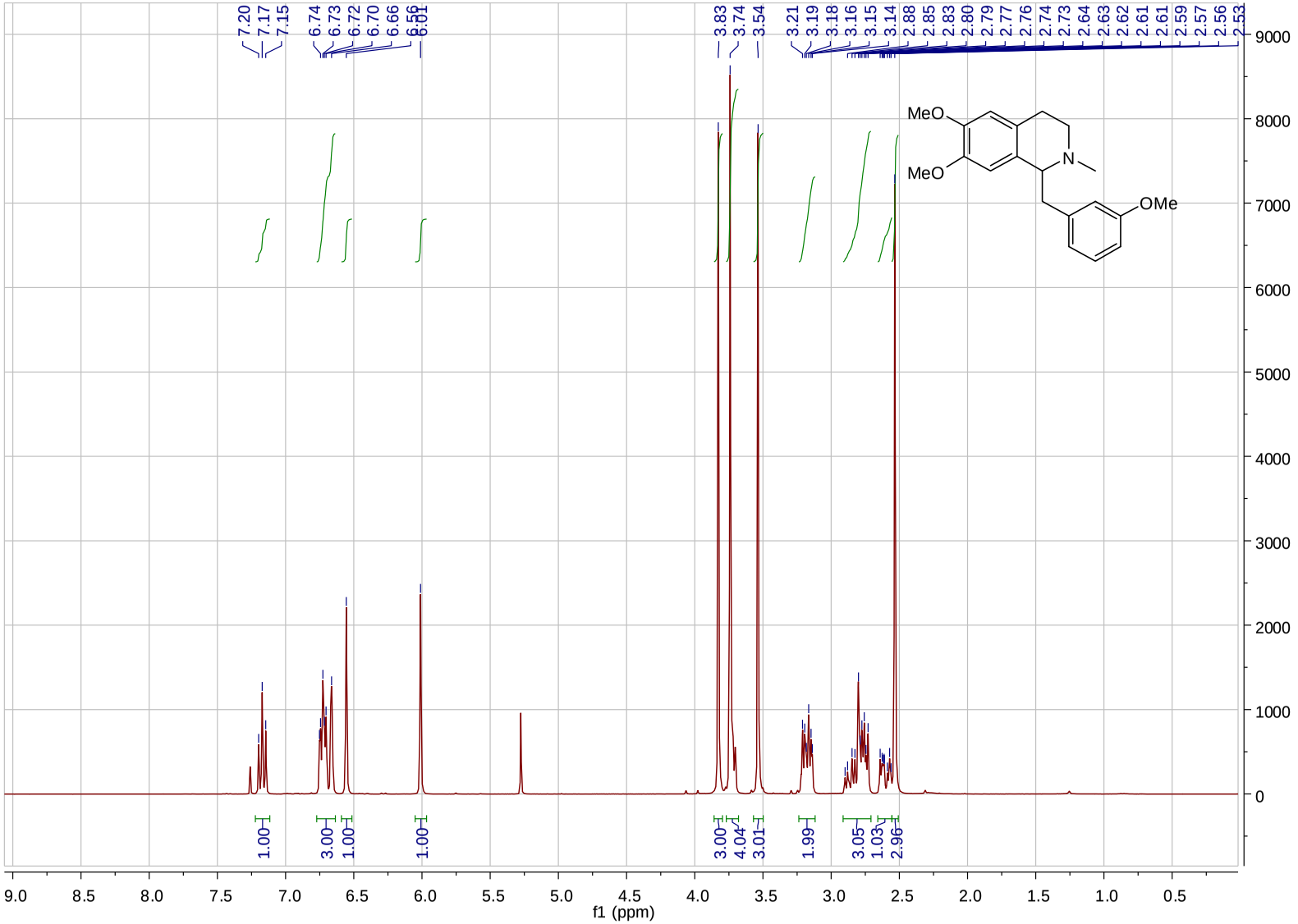
***N*-(3,4-Dimethoxyphenethyl)-2-(3-methoxyphenyl)-*N*-methylacetamide:** MS spectrum



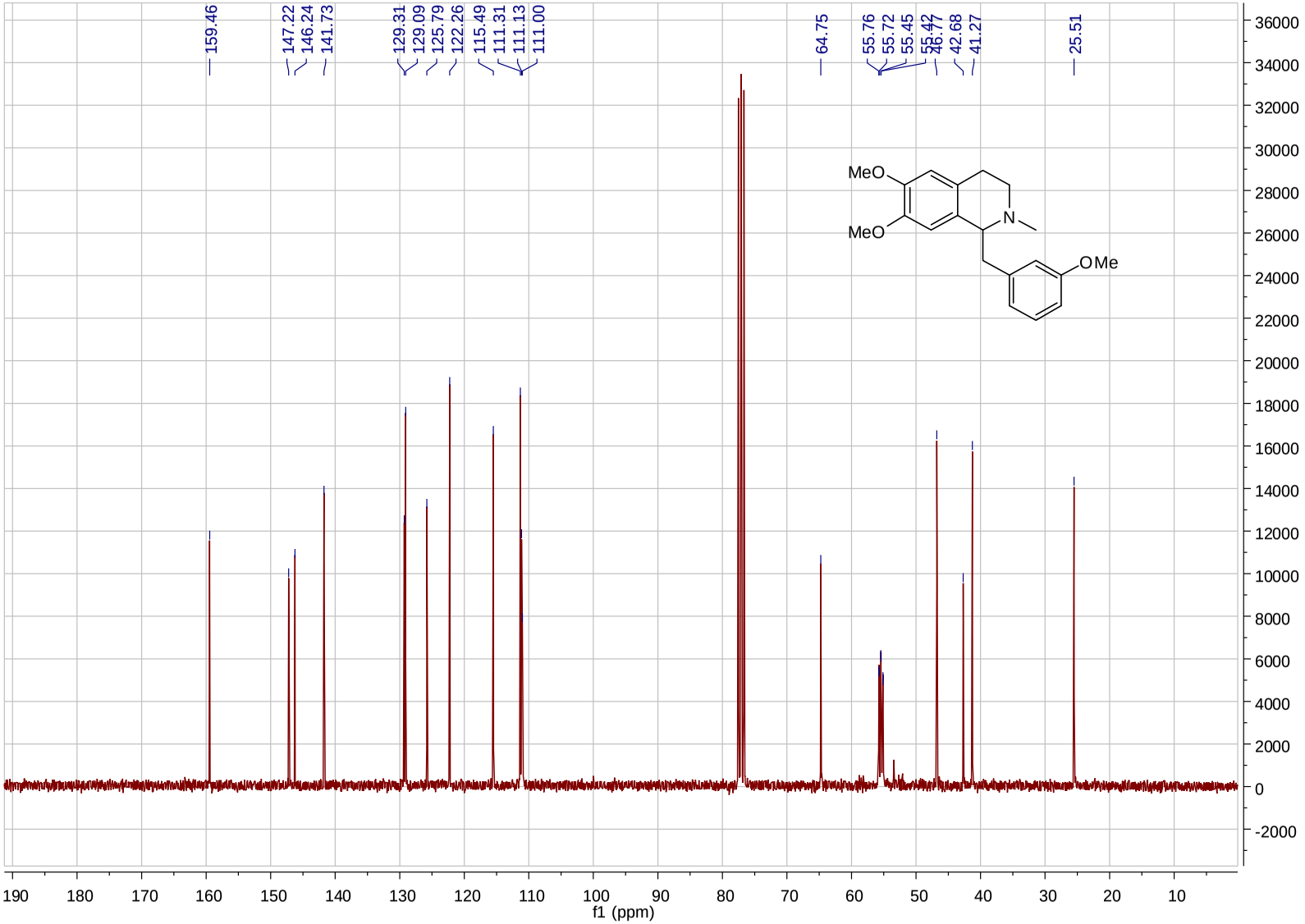
***N*-(3,4-Dimethoxyphenethyl)-2-(3-methoxyphenyl)-*N*-methylacetamide:** HRMS results



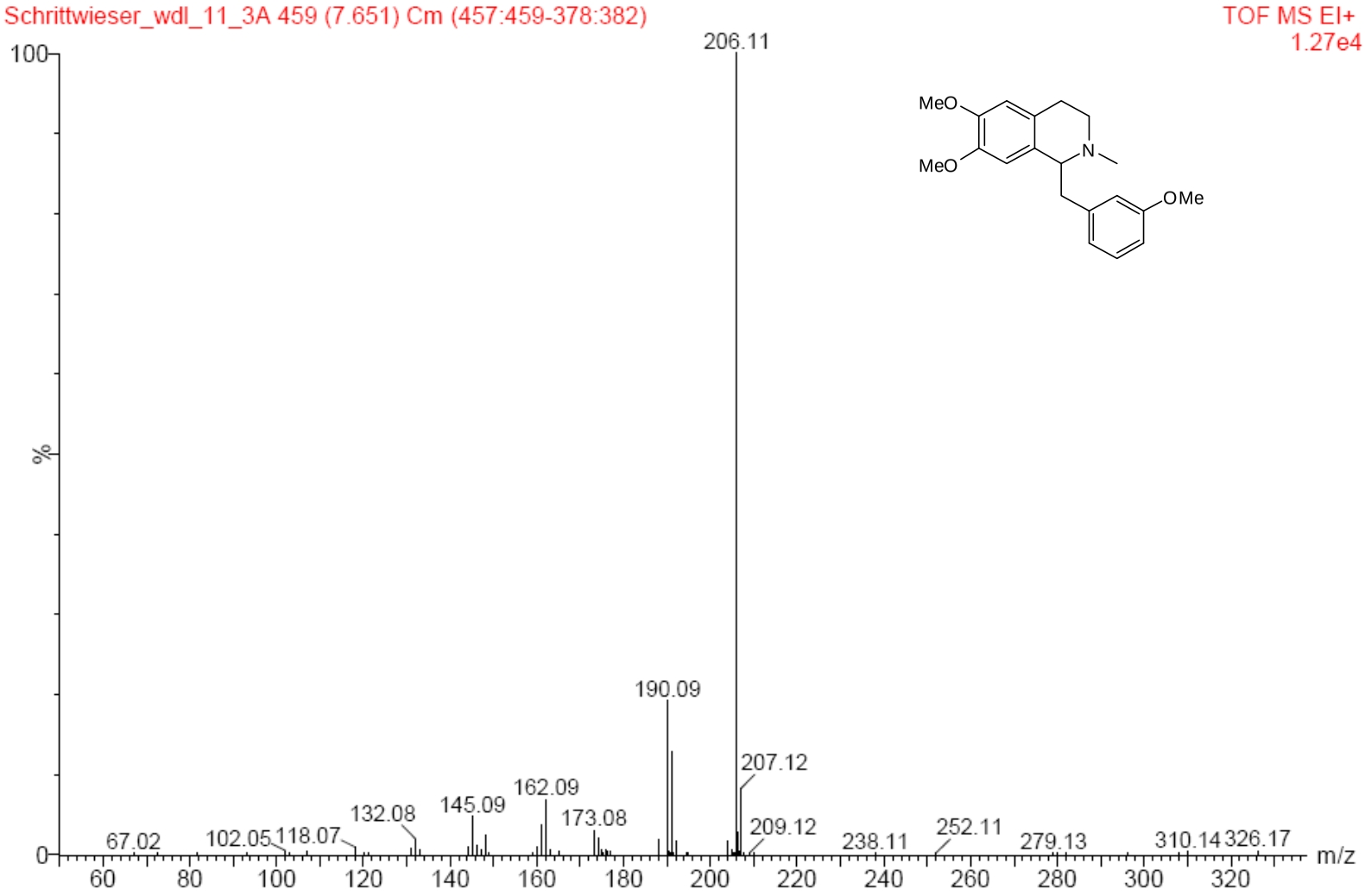
6,7-Dimethoxy-1-(3-methoxybenzyl)-2-ethyl-1,2,3,4-tetrahydroisoquinoline (2c): ^{1}H-NMR spectrum



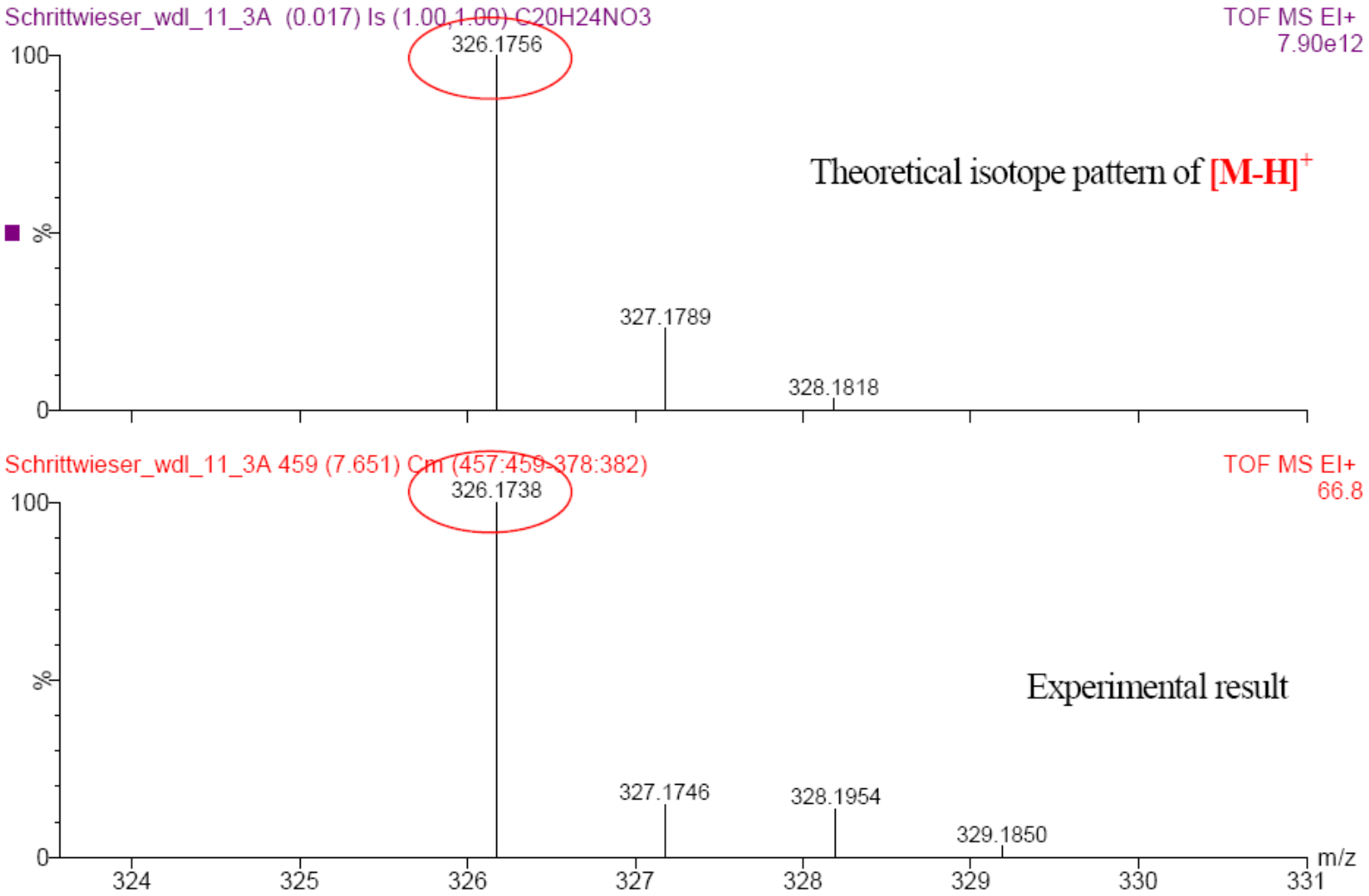
6,7-Dimethoxy-1-(3-methoxybenzyl)-2-ethyl-1,2,3,4-tetrahydroisoquinoline (2c): ^{13}C-NMR spectrum



6,7-Dimethoxy-1-(3-methoxybenzyl)-2-ethyl-1,2,3,4-tetrahydroisoquinoline (2c): MS spectrum



6,7-Dimethoxy-1-(3-methoxybenzyl)-2-ethyl-1,2,3,4-tetrahydroisoquinoline (2c): HRMS results



Substrate 2d and its Synthetic Intermediates

Provided Material:

3-Nitrophenylacetyl chloride:

^{1}H-NMR spectrum, ^{13}C-NMR spectrum

***N*-(3,4-Dimethoxyphenethyl)-2-(3-nitrophenyl)-*N*-methylacetamide:**

^{1}H-NMR spectrum, ^{13}C-NMR spectrum, COSY spectrum, HSQC spectrum, MS spectrum, HRMS results

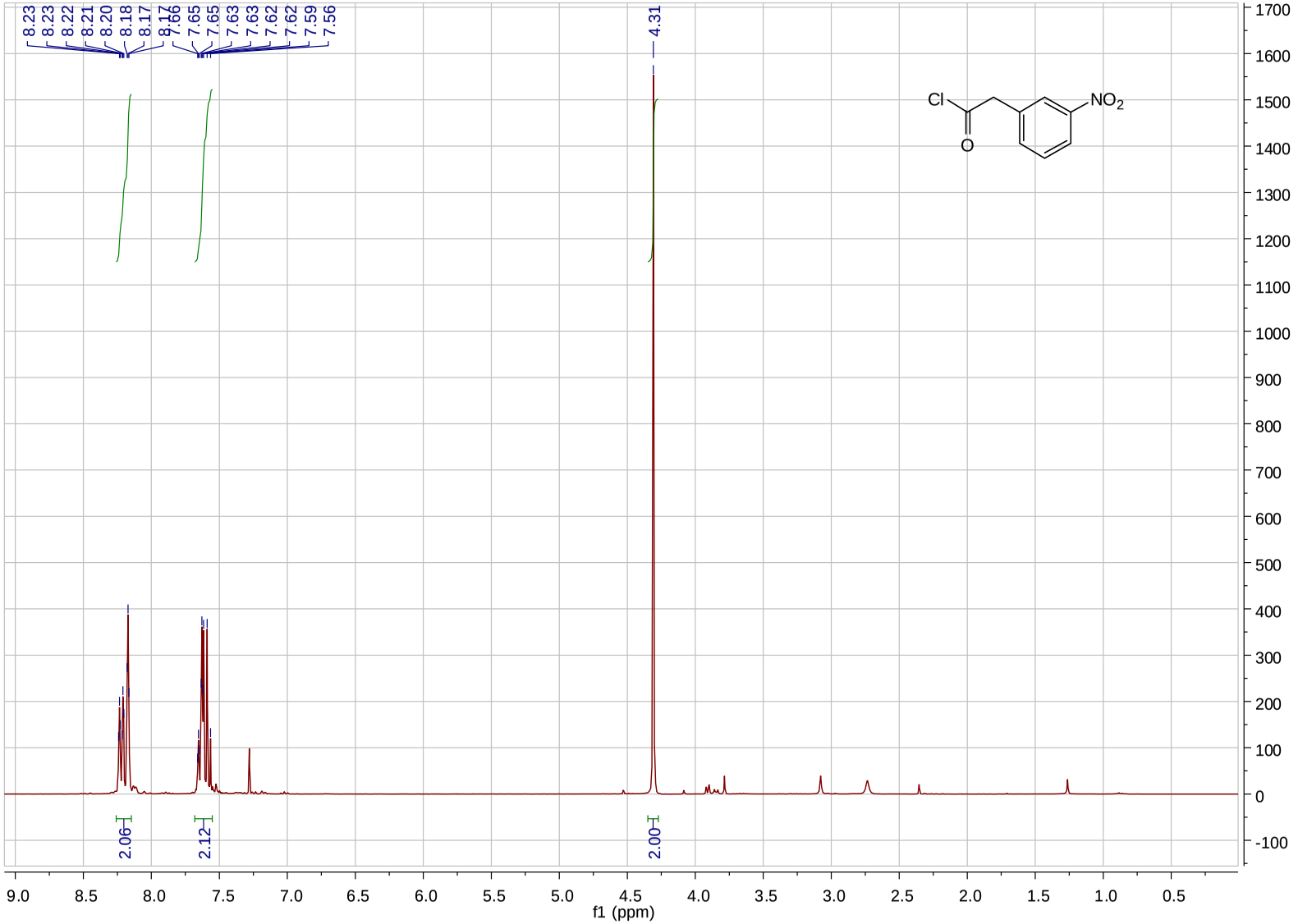
6,7-Dimethoxy-2-methyl-1-(3-nitrobenzyl)-1,2,3,4-tetrahydroisoquinoline:

^{1}H-NMR spectrum, ^{13}C-NMR spectrum, COSY spectrum, HSQC spectrum, MS spectrum, HRMS results

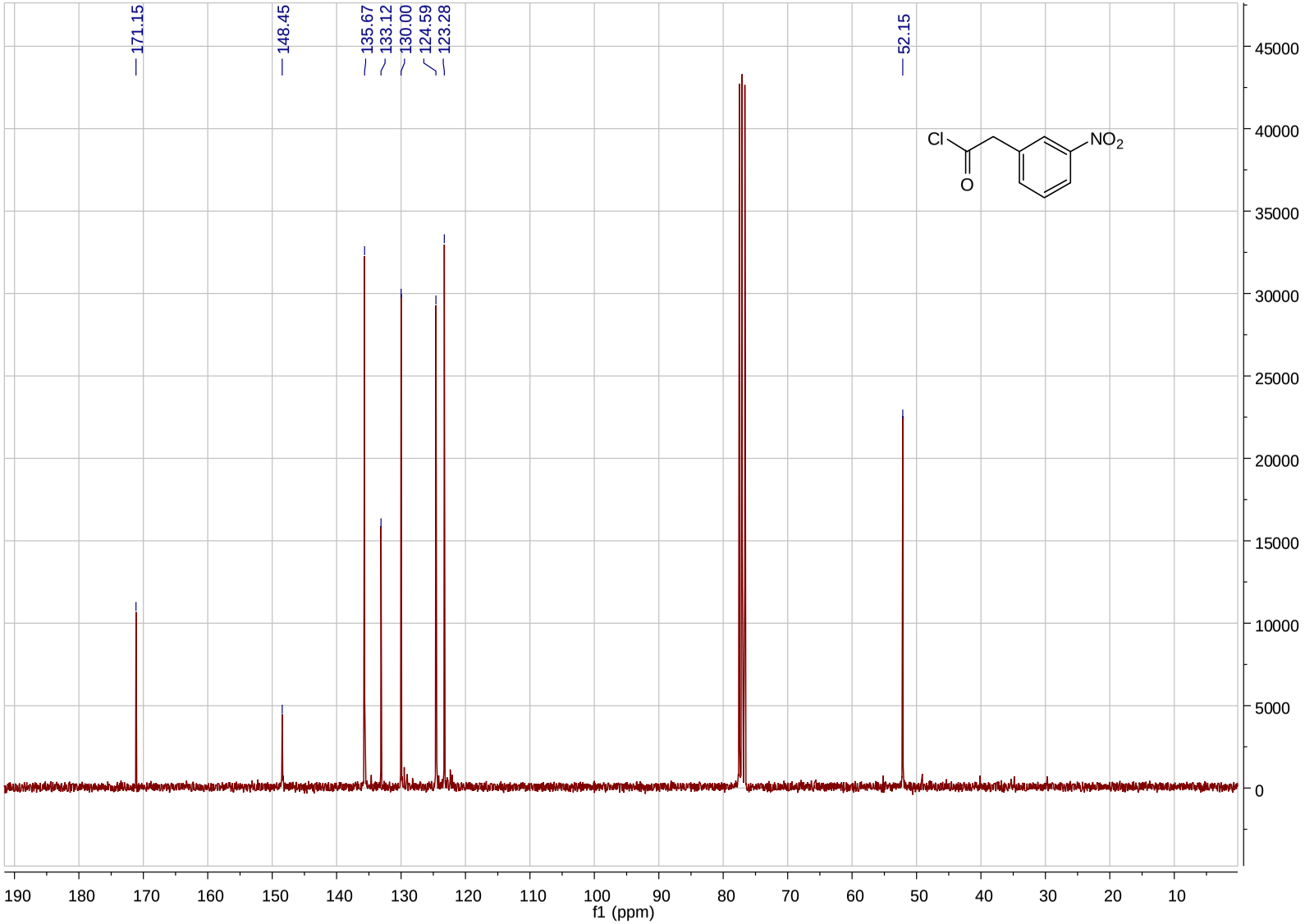
1-(3-Aminobenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (2d):

^{1}H-NMR spectrum, ^{13}C-NMR spectrum, COSY spectrum, HSQC spectrum, MS spectrum, HRMS results

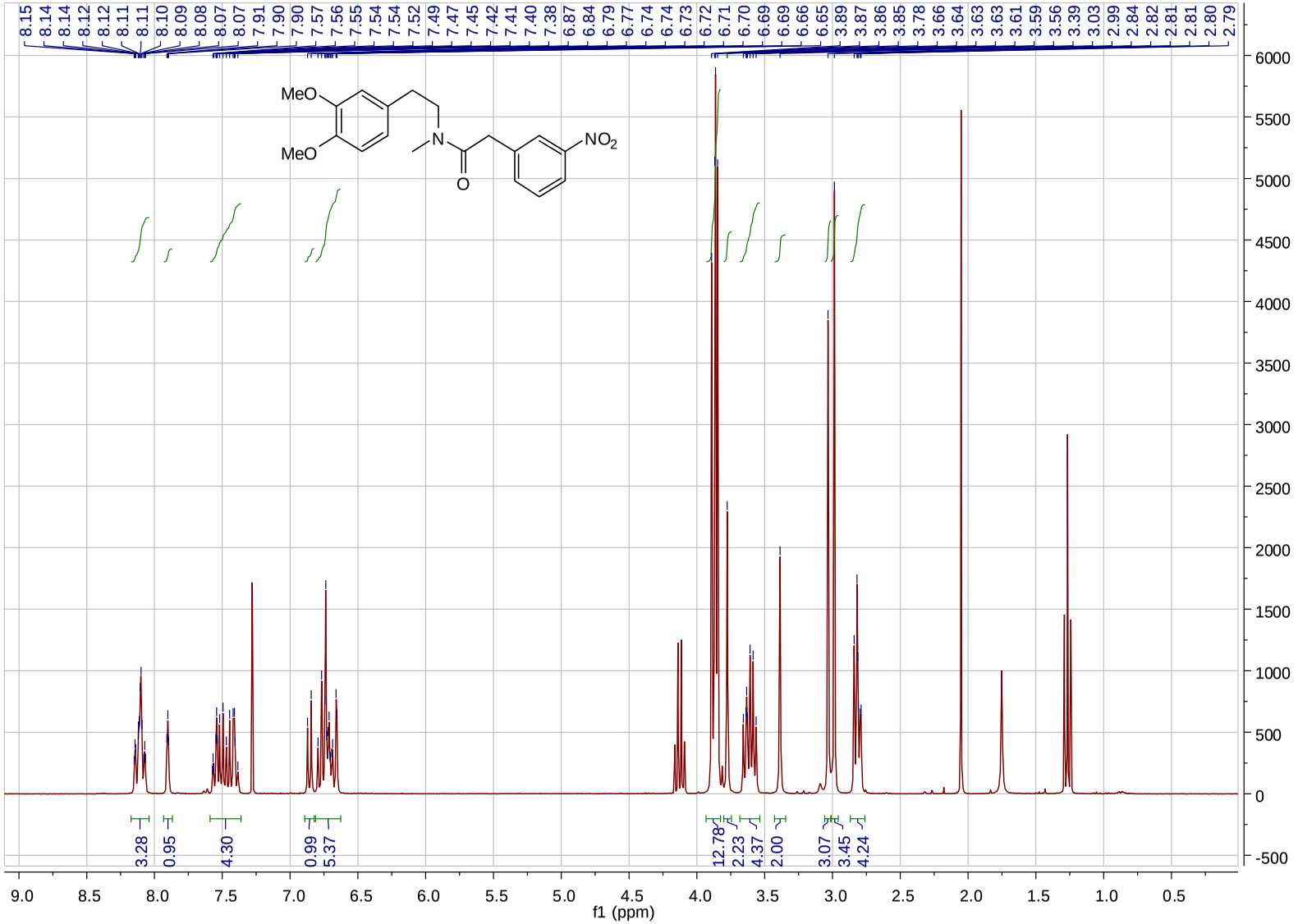
3-Nitrophenylacetyl chloride: ^{1}H-NMR spectrum



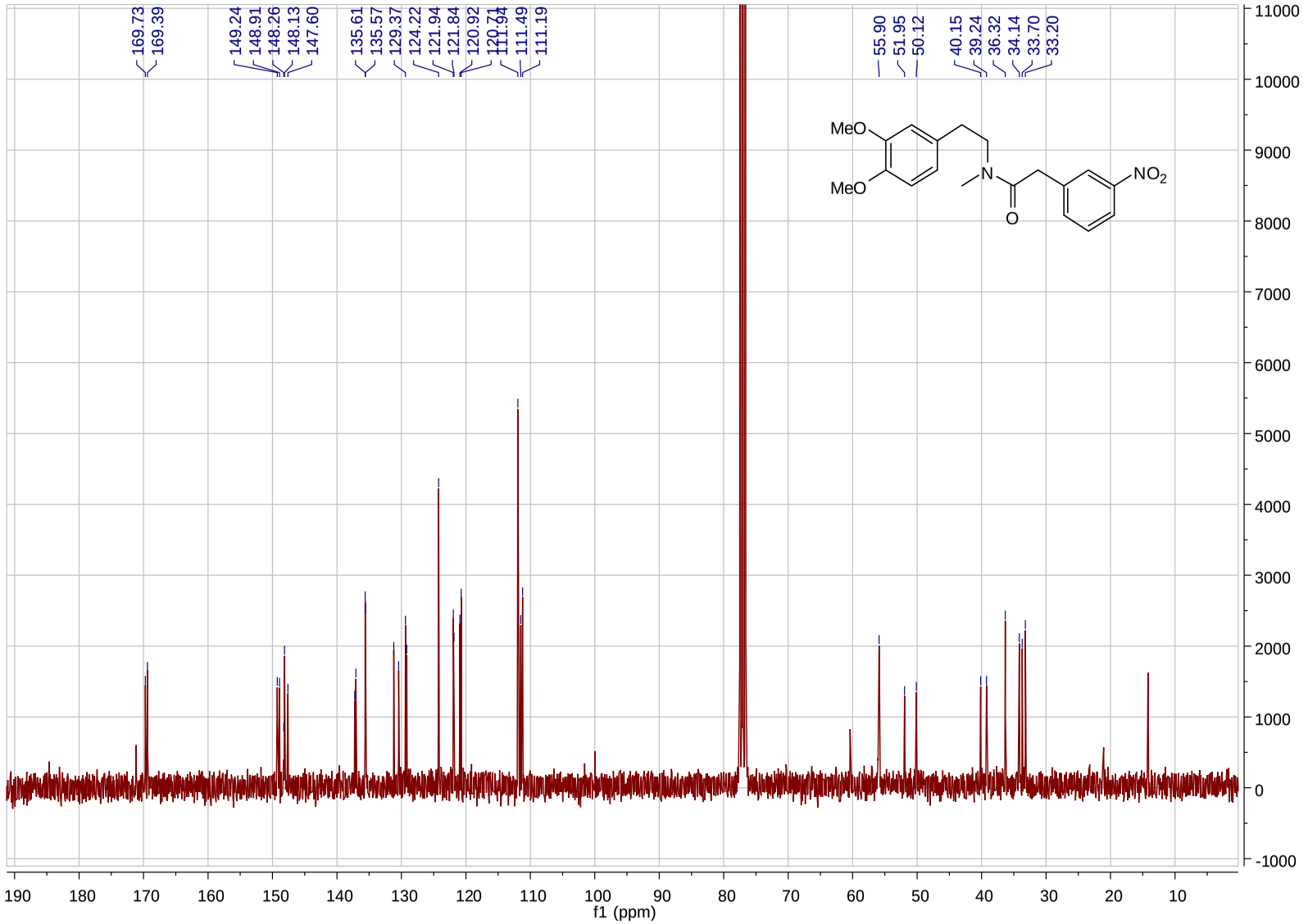
3-Nitrophenylacetyl chloride: ^{13}C-NMR spectrum



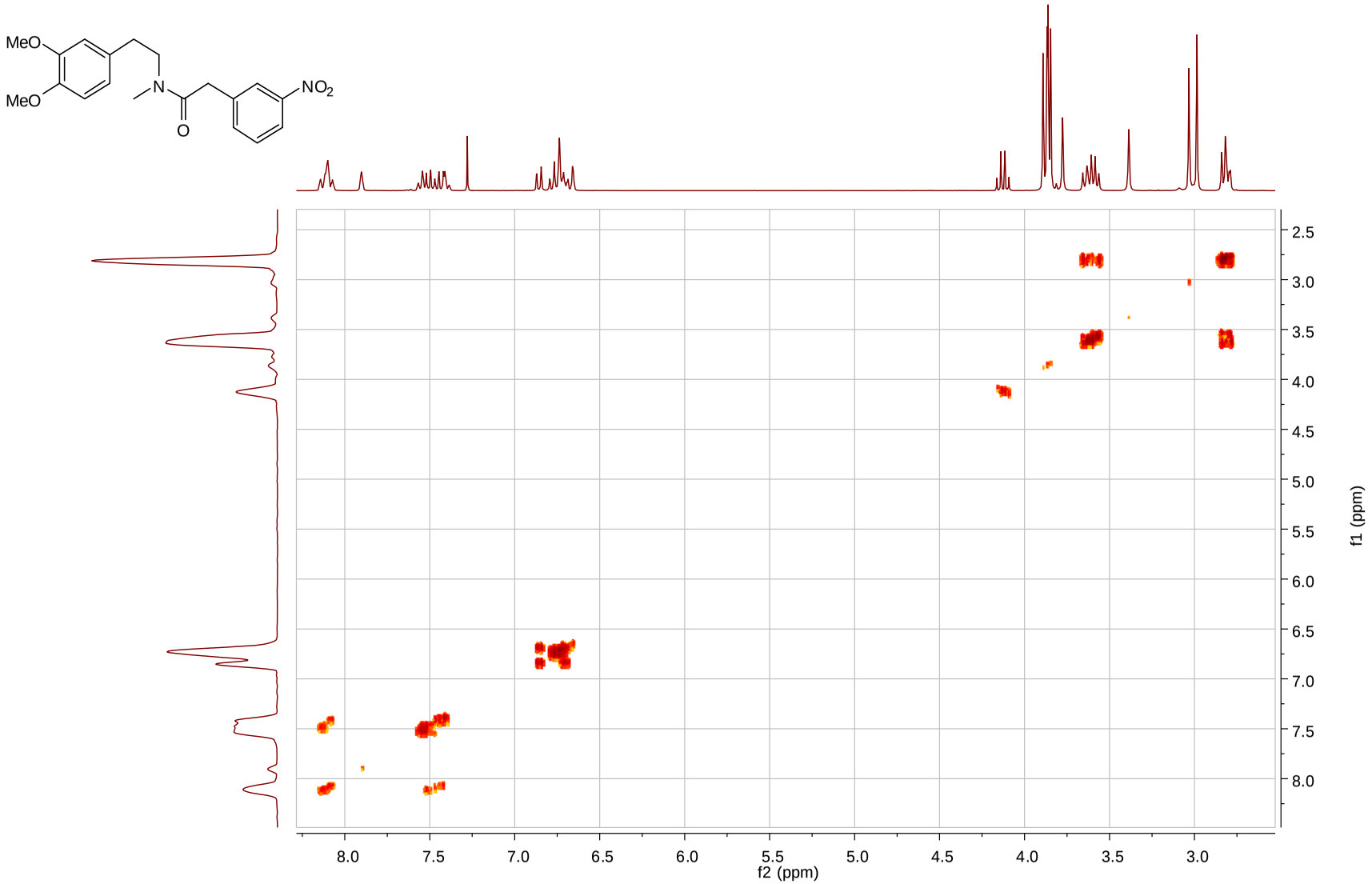
_N_-(3,4-Dimethoxyphenethyl)-2-(3-nitrophenyl)-_N_-methylacetamide: ^{1}H-NMR spectrum



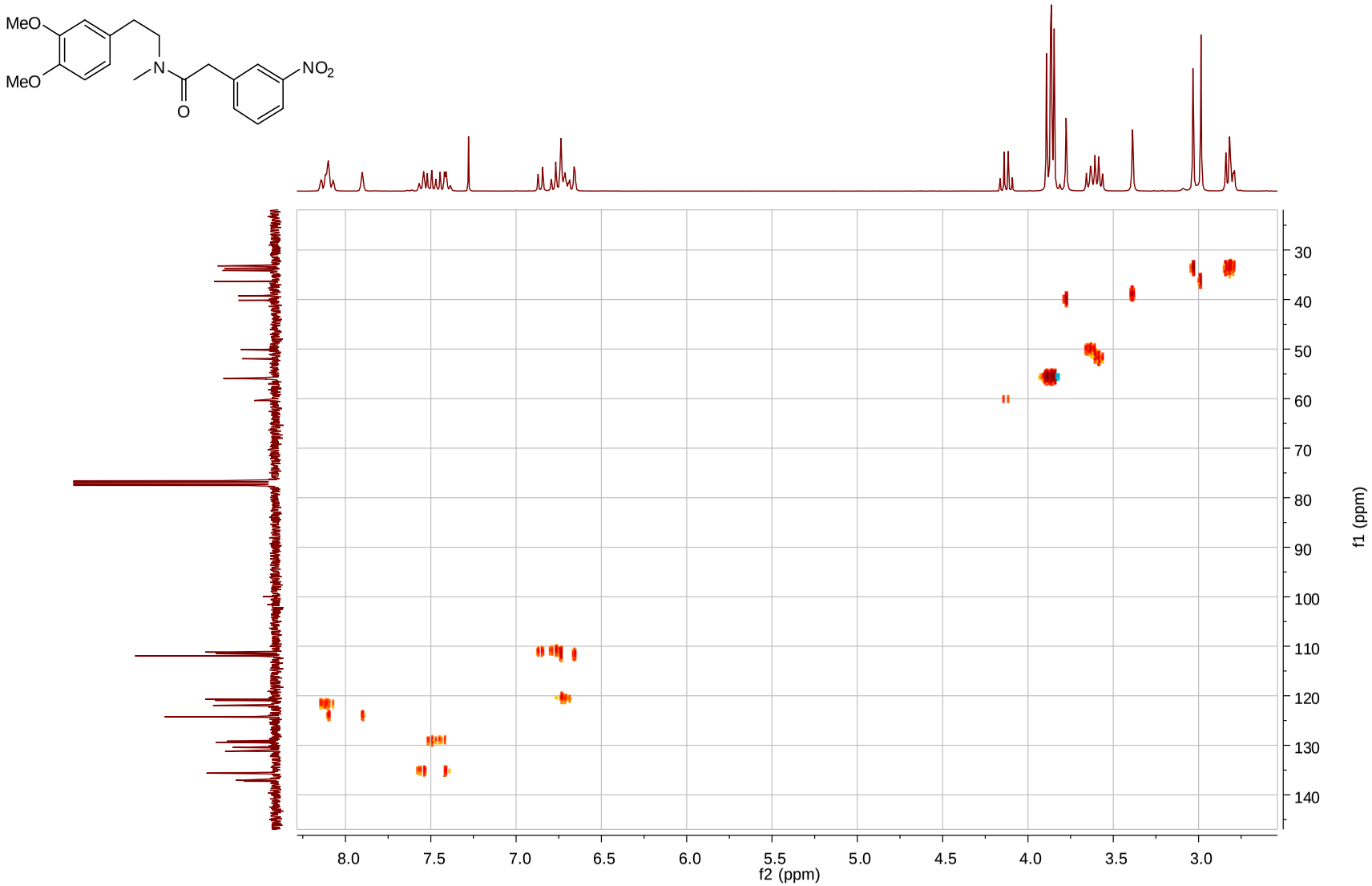
_N_-(3,4-Dimethoxyphenethyl)-2-(3-nitrophenyl)-_N_-methylacetamide: ^{13}C-NMR spectrum



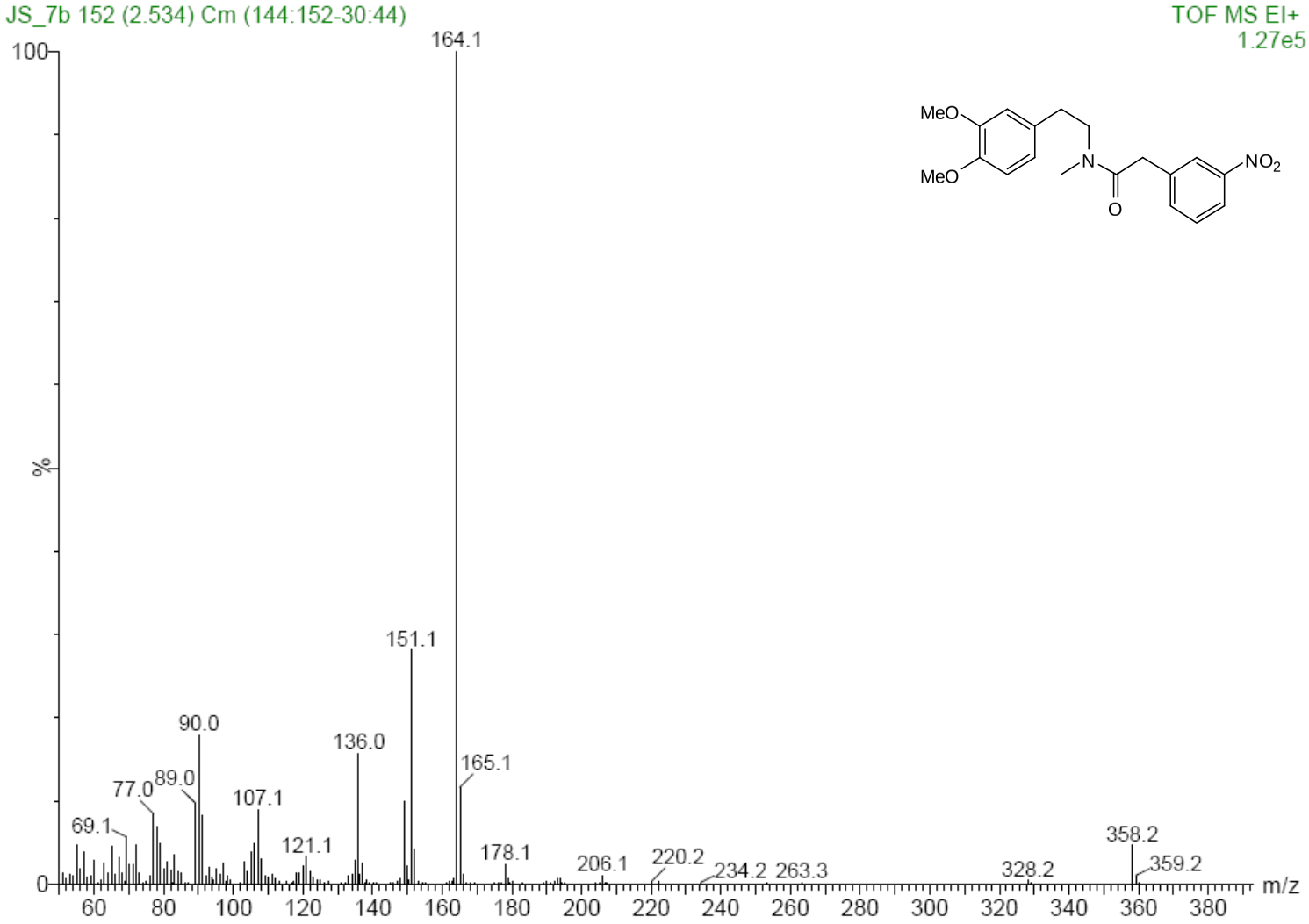
_N_-(3,4-Dimethoxyphenethyl)-2-(3-nitrophenyl)-_N_-methylacetamide: COSY spectrum



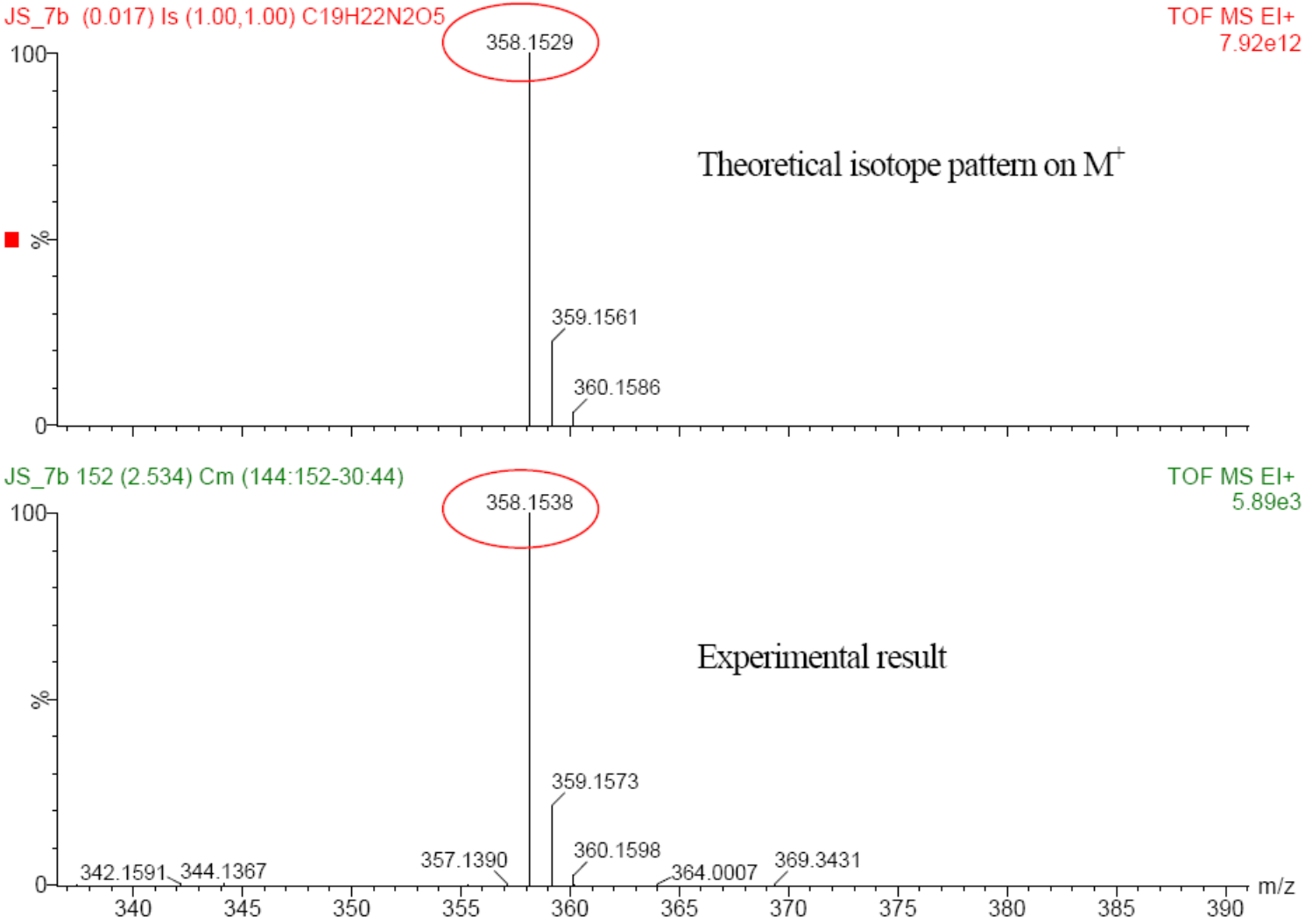
_N_-(3,4-Dimethoxyphenethyl)-2-(3-nitrophenyl)-_N_-methylacetamide: HSQC spectrum



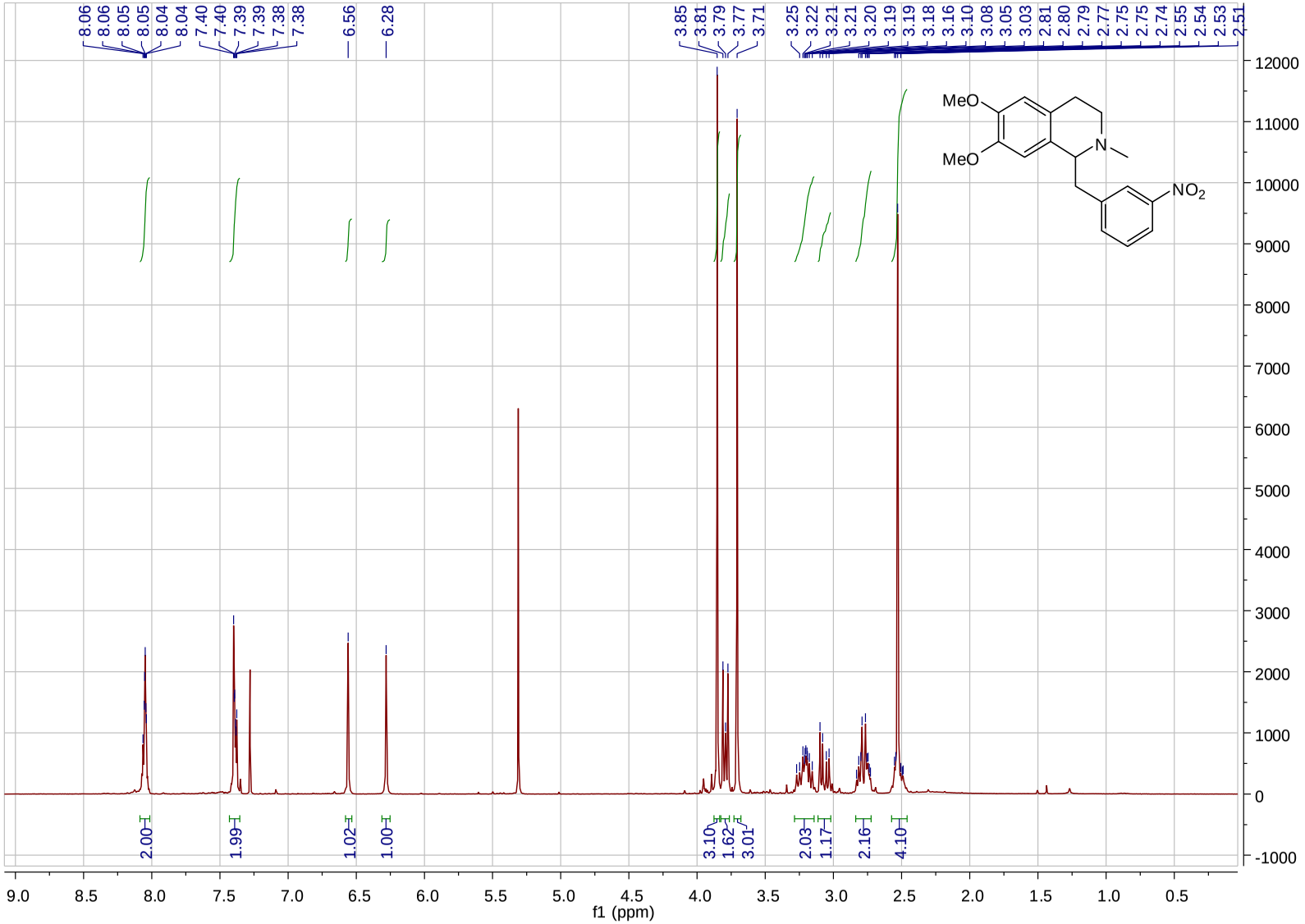
_N_-(3,4-Dimethoxyphenethyl)-2-(3-nitrophenyl)-_N_-methylacetamide: MS spectrum



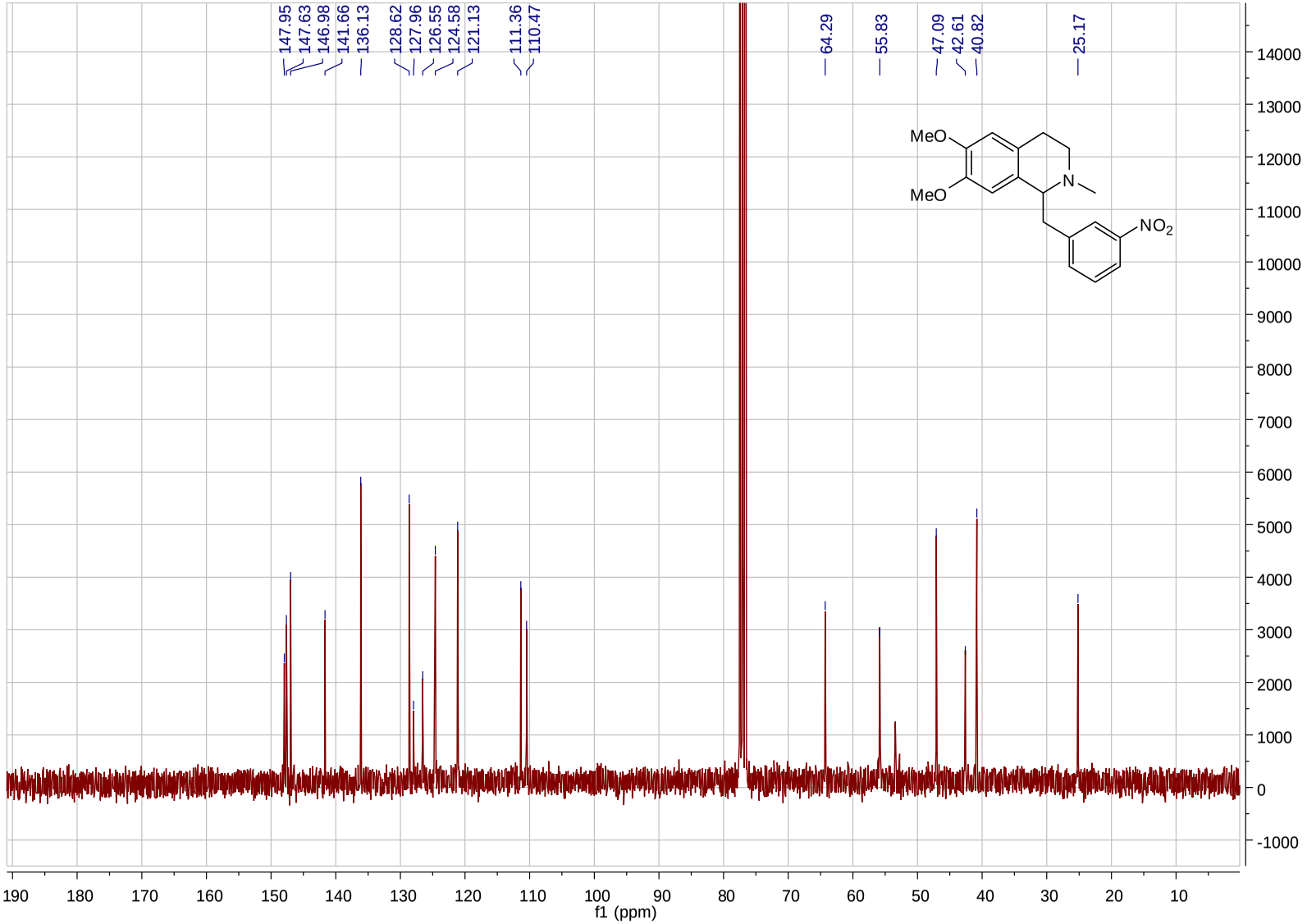
***N*-(3,4-Dimethoxyphenethyl)-2-(3-nitrophenyl)-*N*-methylacetamide:** HRMS results



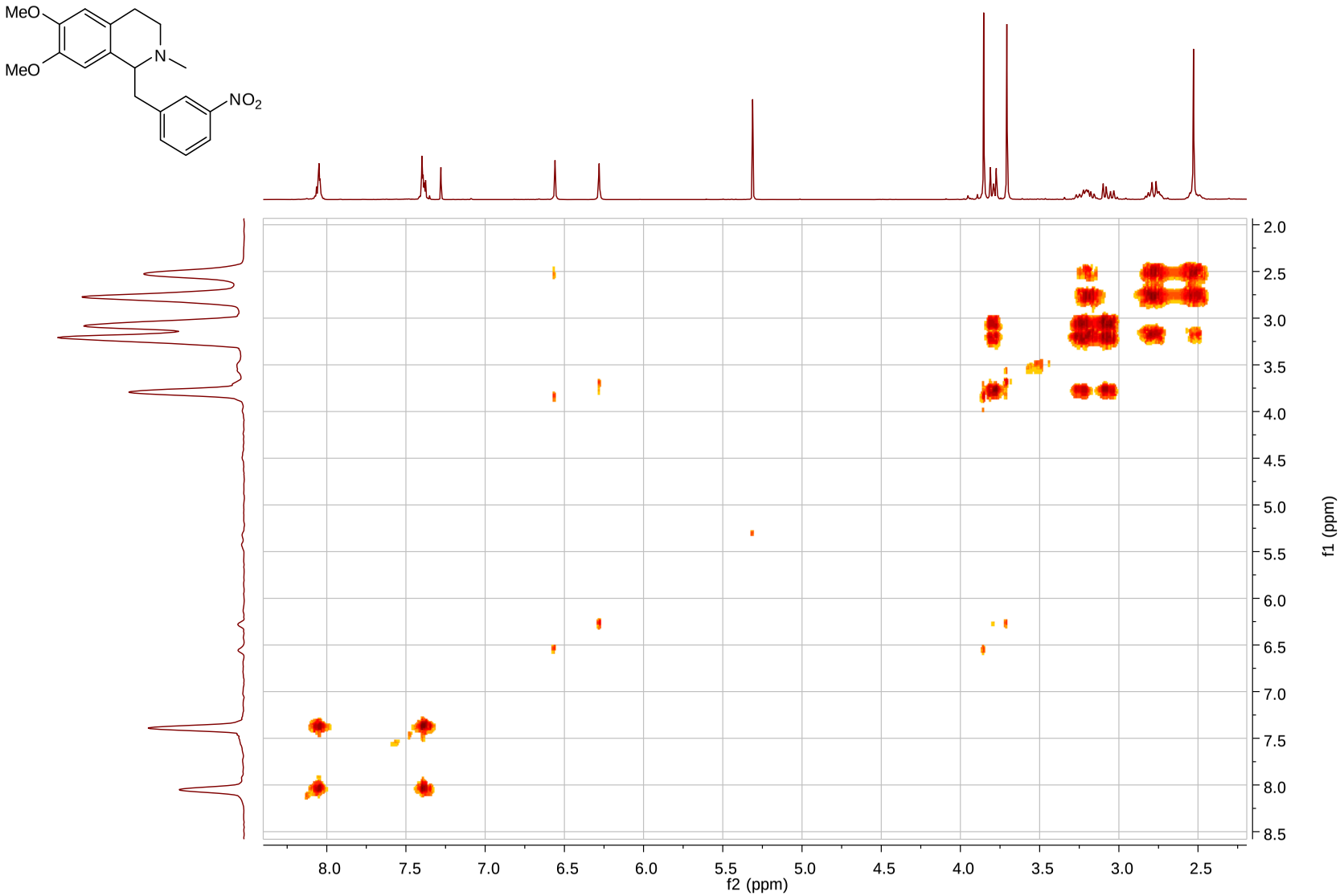
6,7-Dimethoxy-2-methyl-1-(3-nitrobenzyl)-1,2,3,4-tetrahydroisoquinoline: ^{1}H-NMR spectrum



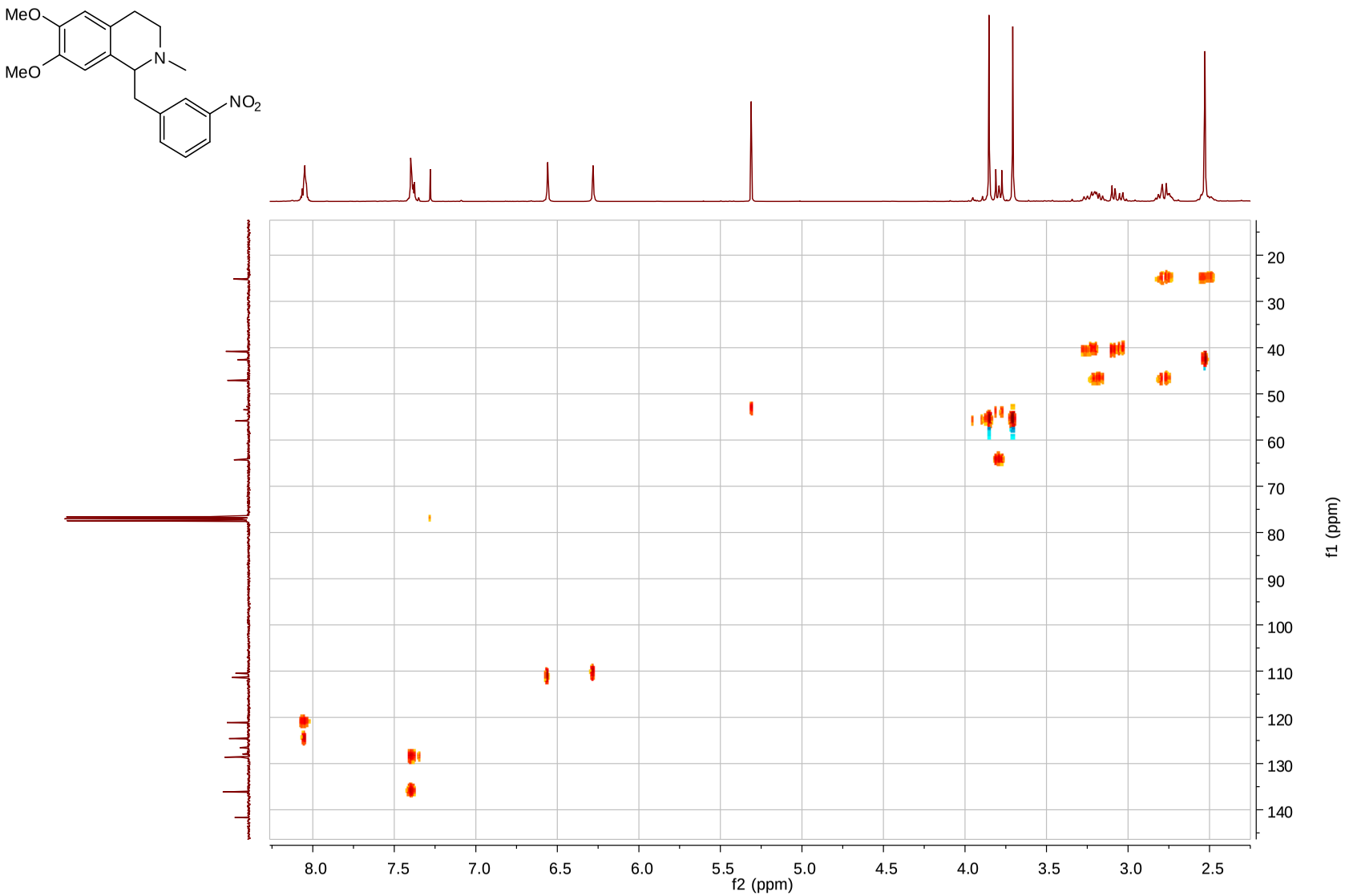
6,7-Dimethoxy-2-methyl-1-(3-nitrobenzyl)-1,2,3,4-tetrahydroisoquinoline: ^{13}C-NMR spectrum



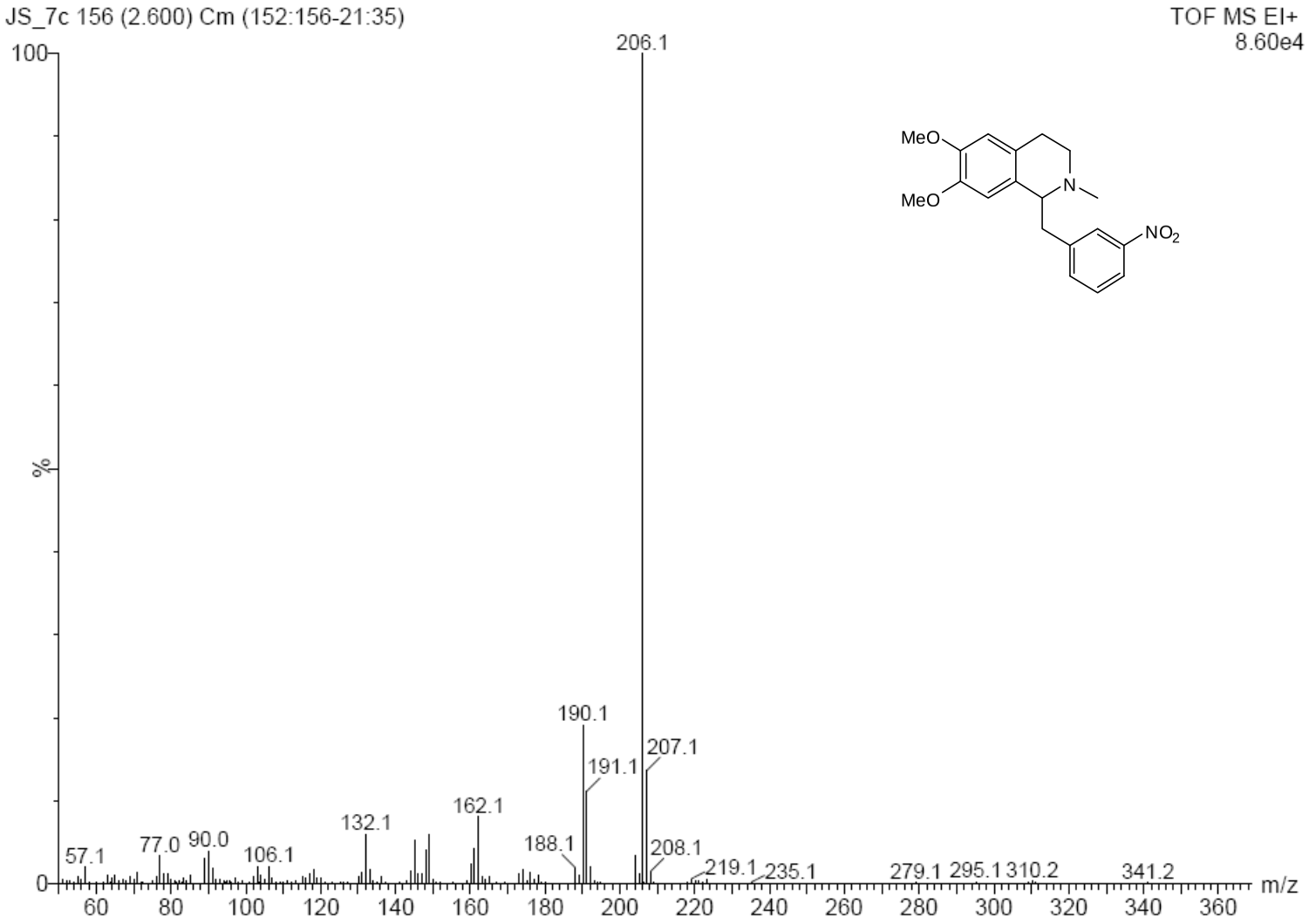
6,7-Dimethoxy-2-methyl-1-(3-nitrobenzyl)-1,2,3,4-tetrahydroisoquinoline: COSY spectrum



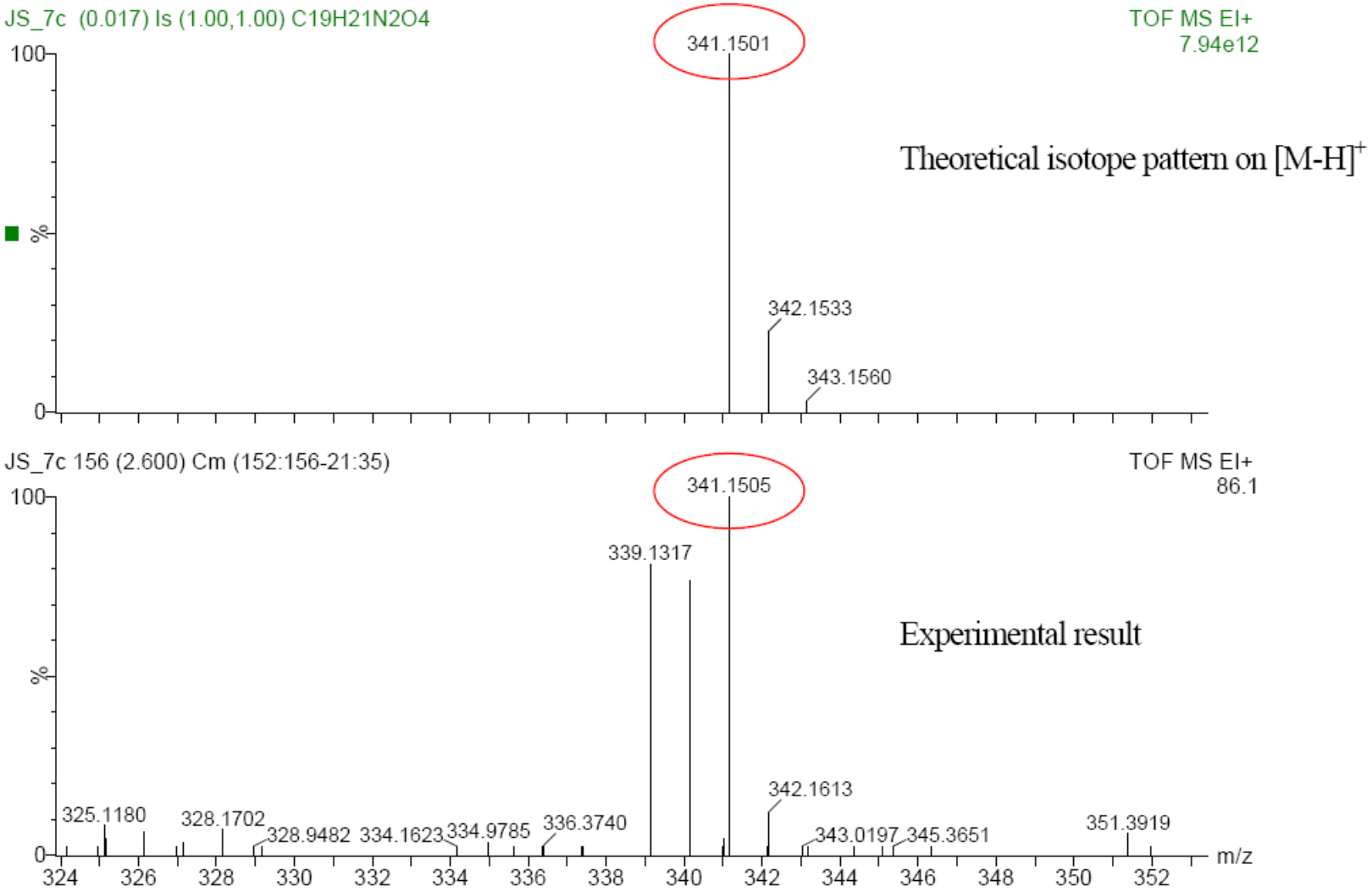
6,7-Dimethoxy-2-methyl-1-(3-nitrobenzyl)-1,2,3,4-tetrahydroisoquinoline: HSQC spectrum



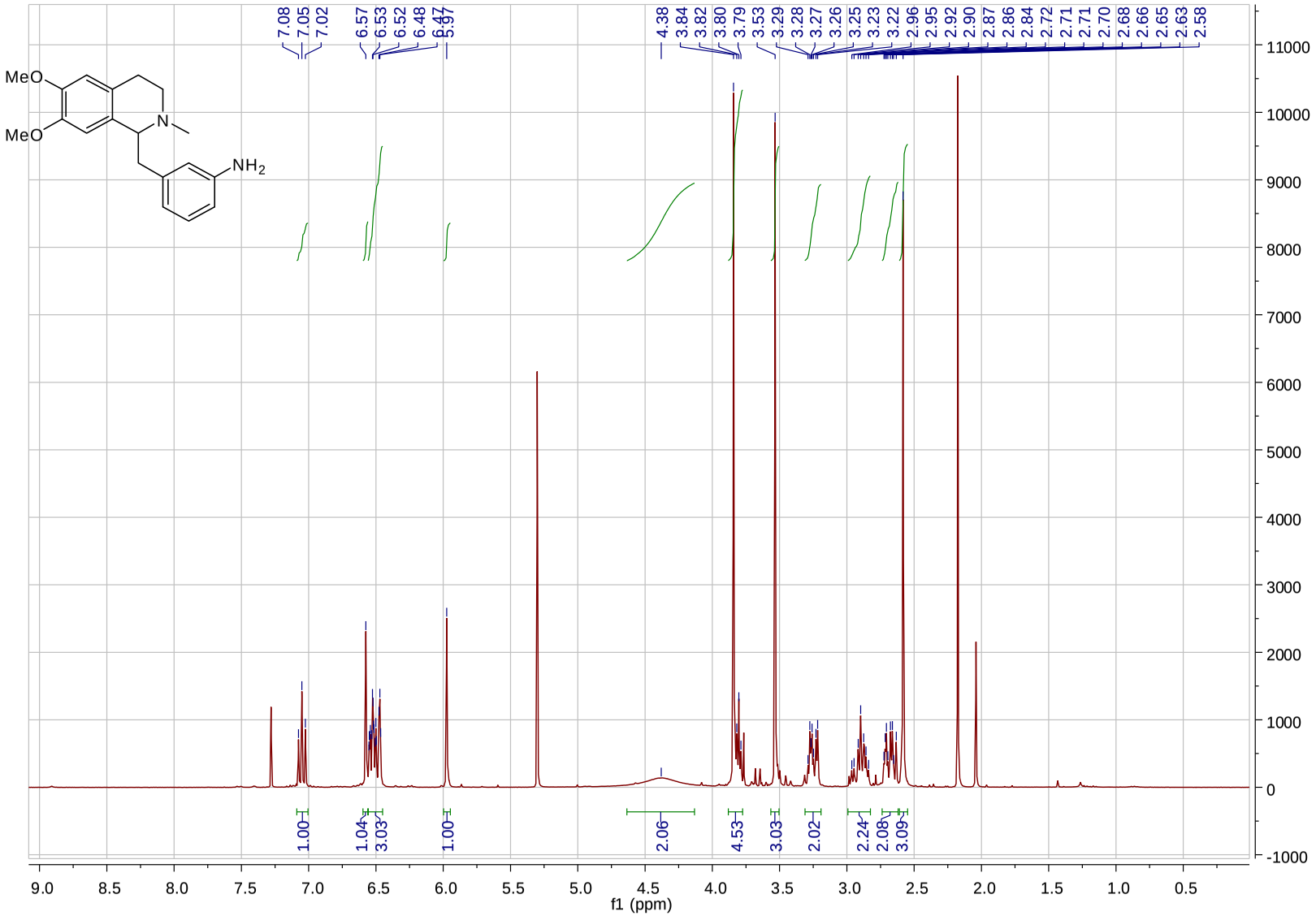
6,7-Dimethoxy-2-methyl-1-(3-nitrobenzyl)-1,2,3,4-tetrahydroisoquinoline: MS spectrum



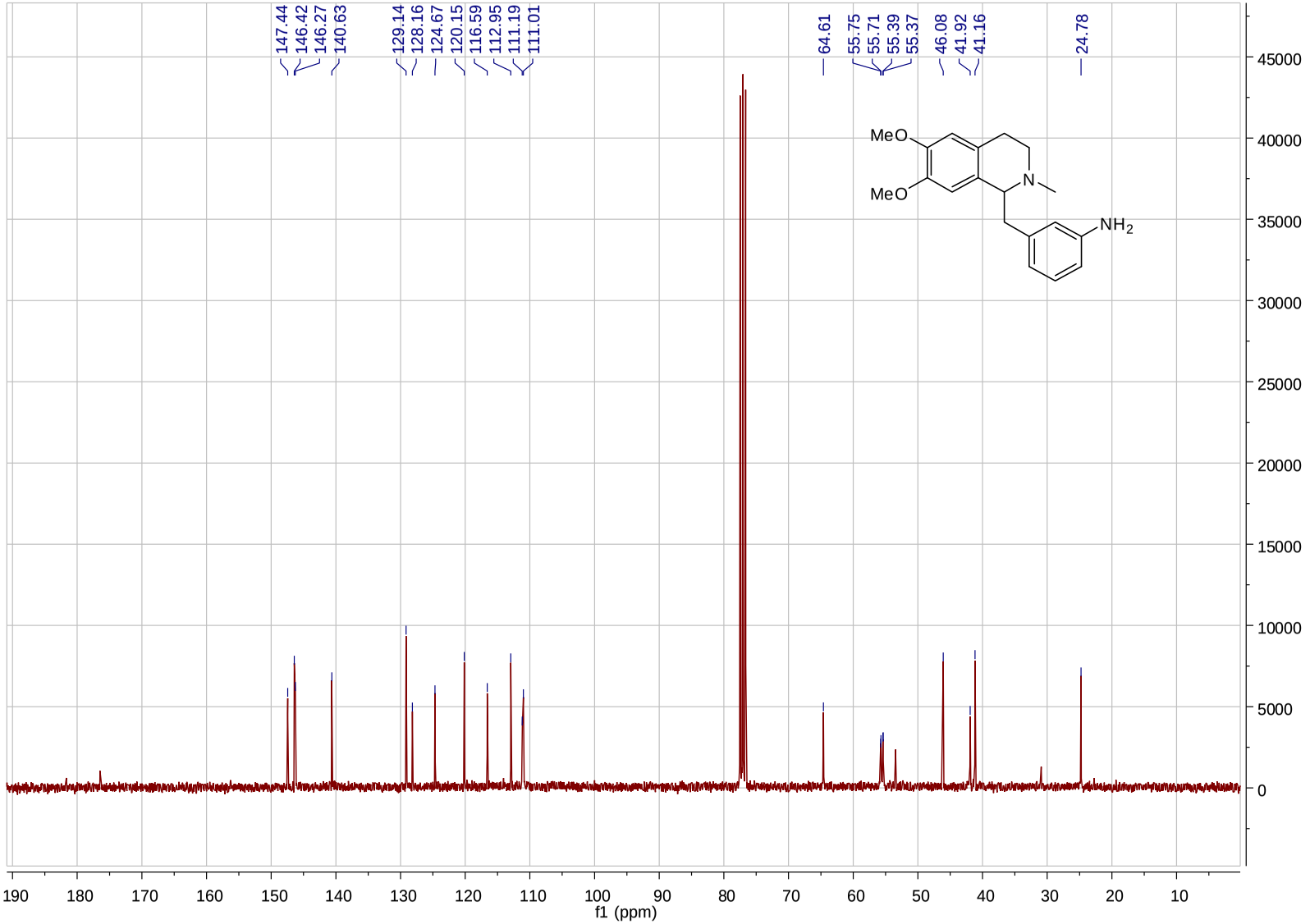
6,7-Dimethoxy-2-methyl-1-(3-nitrobenzyl)-1,2,3,4-tetrahydroisoquinoline: HRMS results



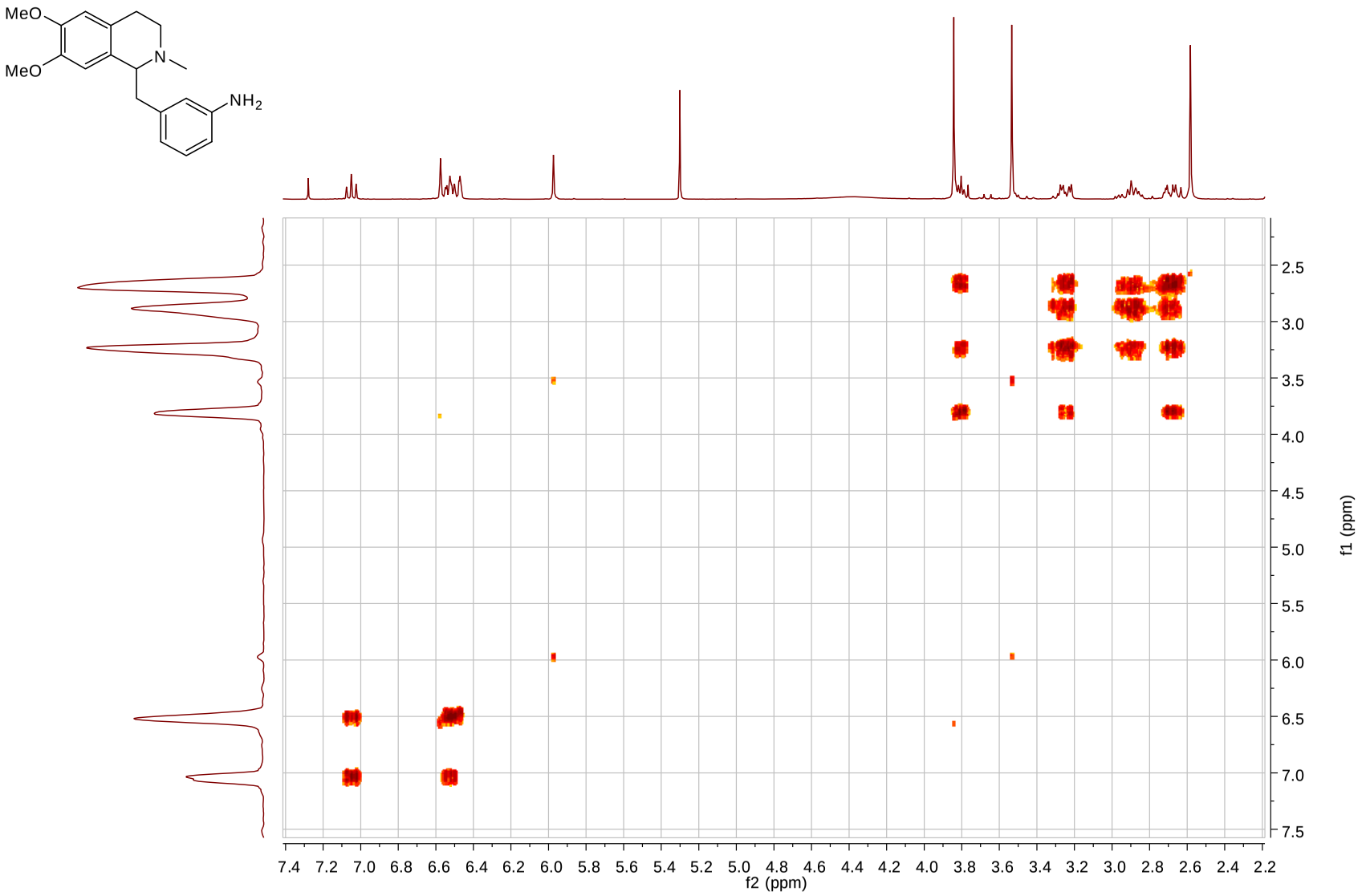
1-(3-Aminobenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (2d): ^{1}H-NMR spectrum



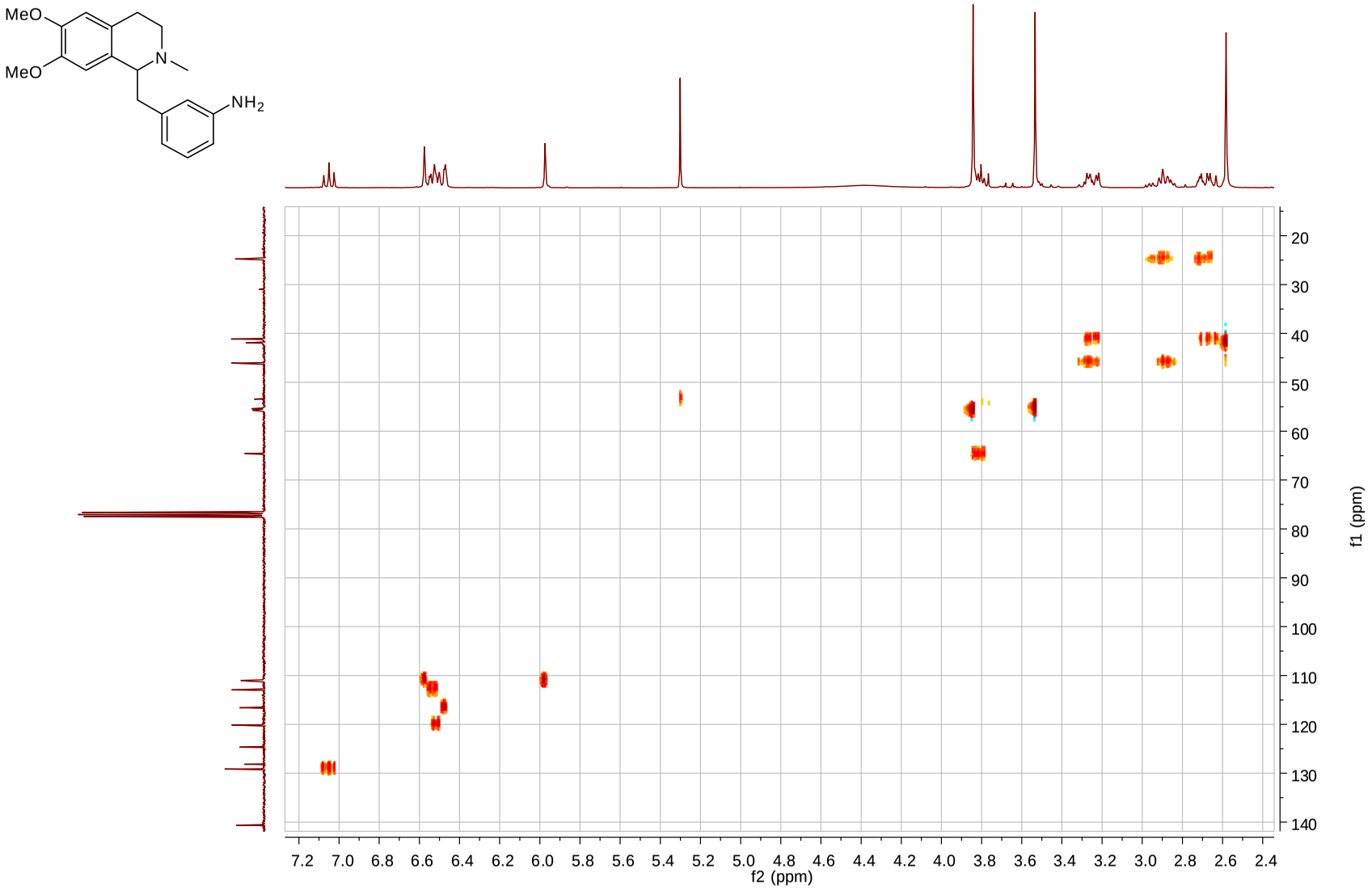
1-(3-Aminobenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (2d): ^{13}C-NMR spectrum



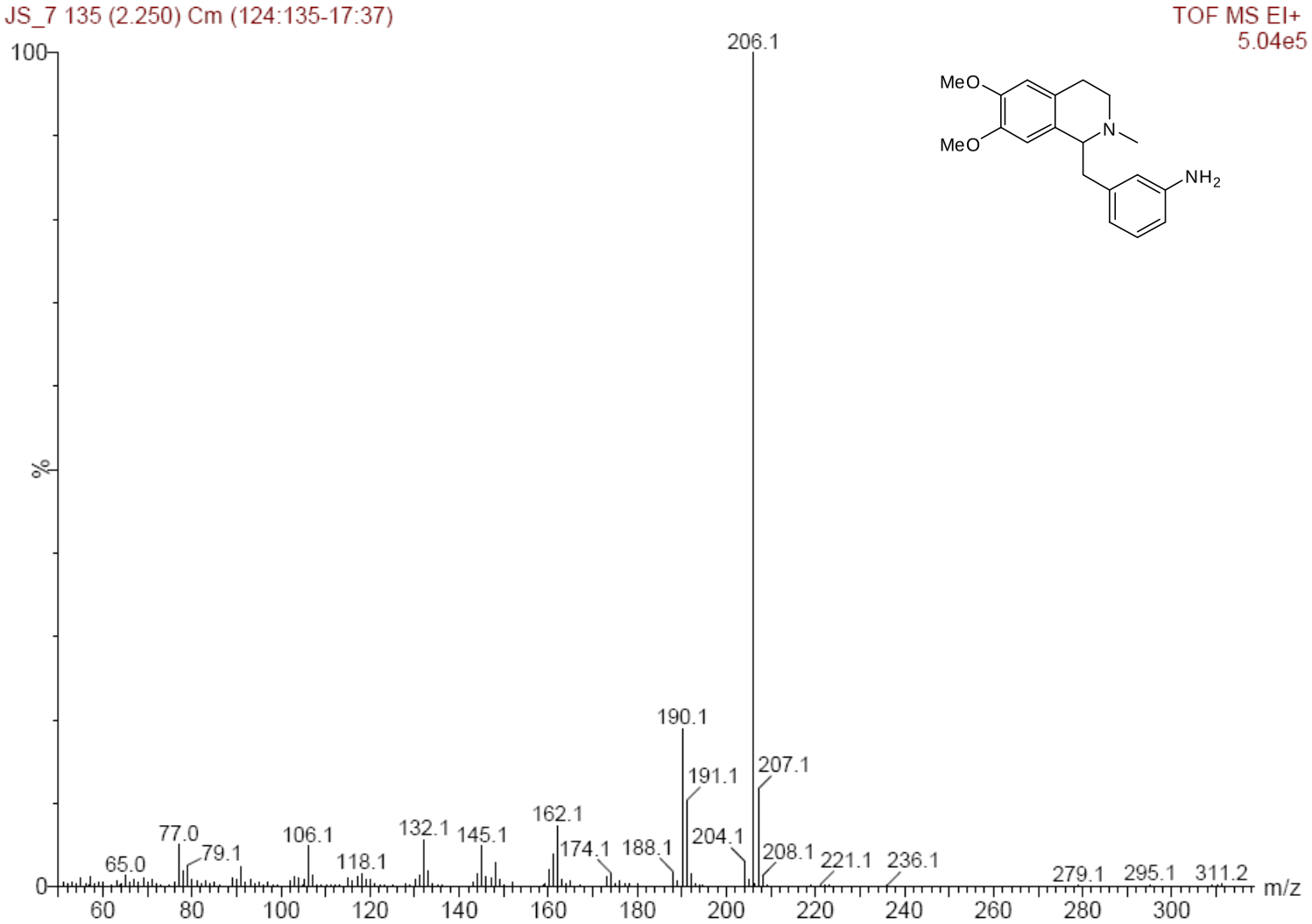
1-(3-Aminobenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (2d): COSY spectrum



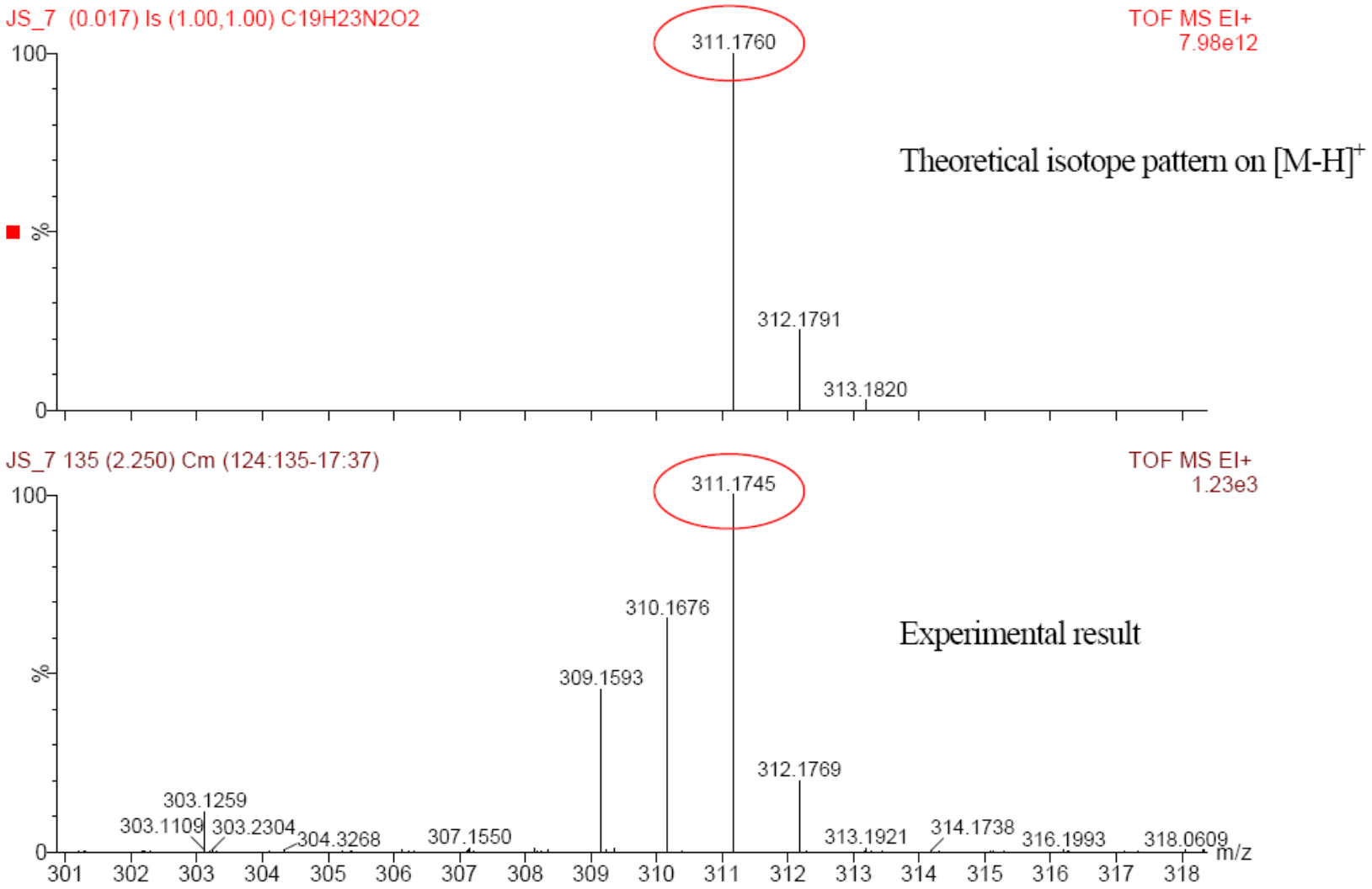
1-(3-Aminobenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (2d): HSQC spectrum



1-(3-Aminobenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (2d): MS spectrum



1-(3-Aminobenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (2d): HRMS results



Substrate 2e and its Synthetic Intermediates

Provided Material:

3-Chlorophenylacetyl chloride:

^{1}H-NMR spectrum, ^{13}C-NMR spectrum

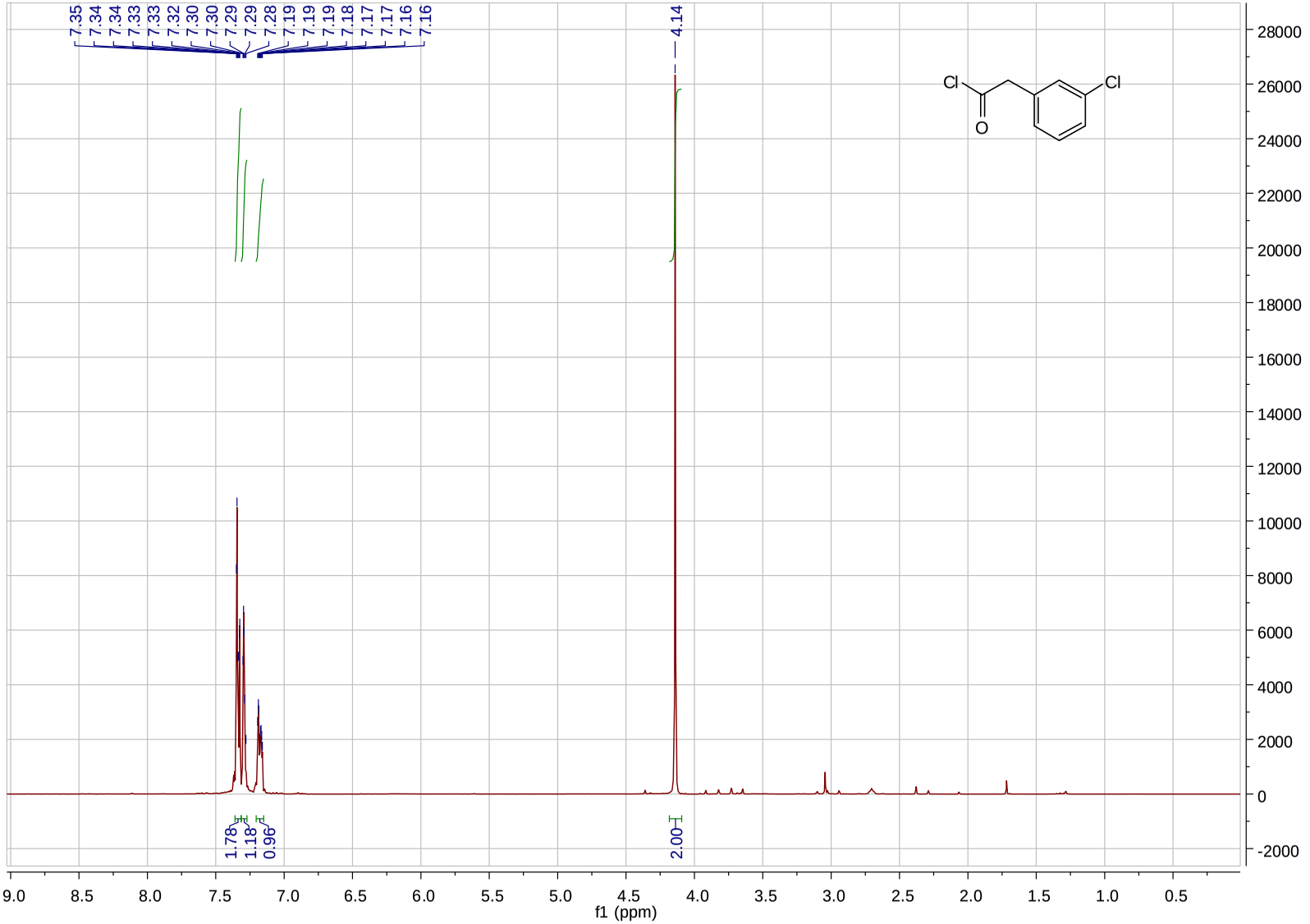
2-(3-Chlorophenyl)-*N*-(3,4-dimethoxyphenethyl)-*N*-methylacetamide:

^{1}H-NMR spectrum, ^{13}C-NMR spectrum, ^{13}C-NMR DEPT135 spectrum, COSY spectrum, HSQC spectrum, MS spectrum, HRMS results

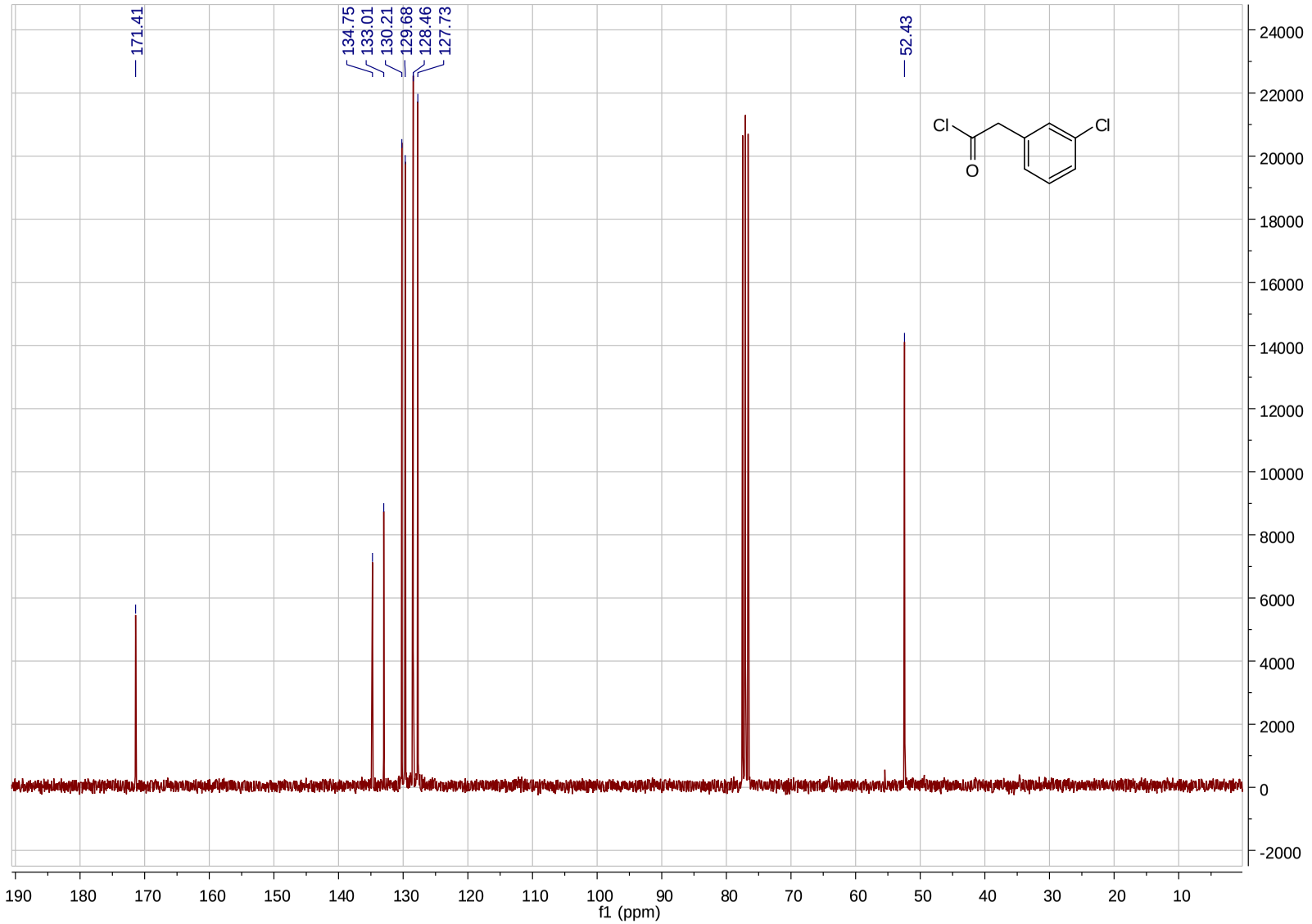
1-(3-Chlorobenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (2e):

^{1}H-NMR spectrum, ^{13}C-NMR spectrum, MS spectrum, HRMS results

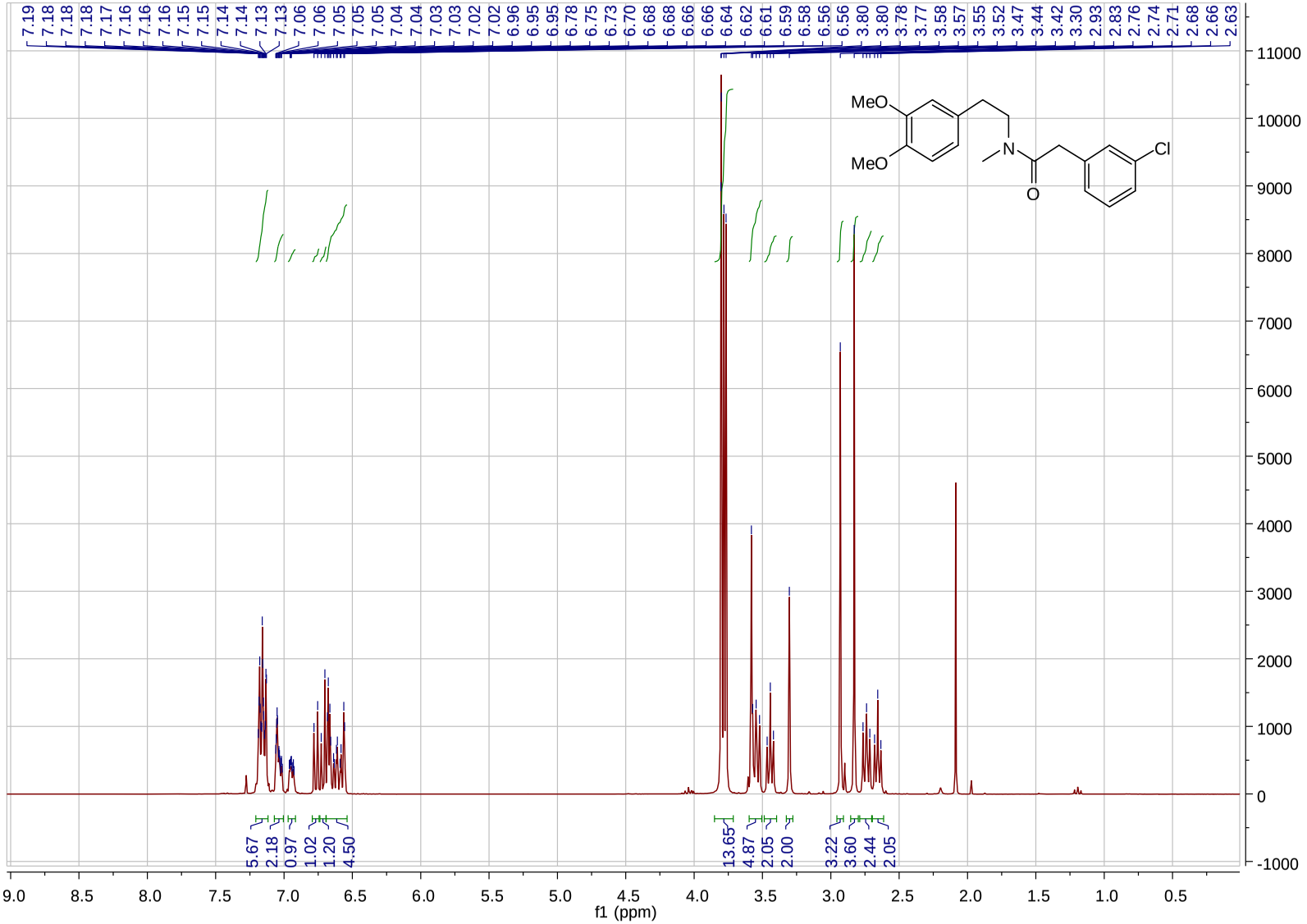
3-Chlorophenylacetyl chloride: ^{1}H-NMR spectrum



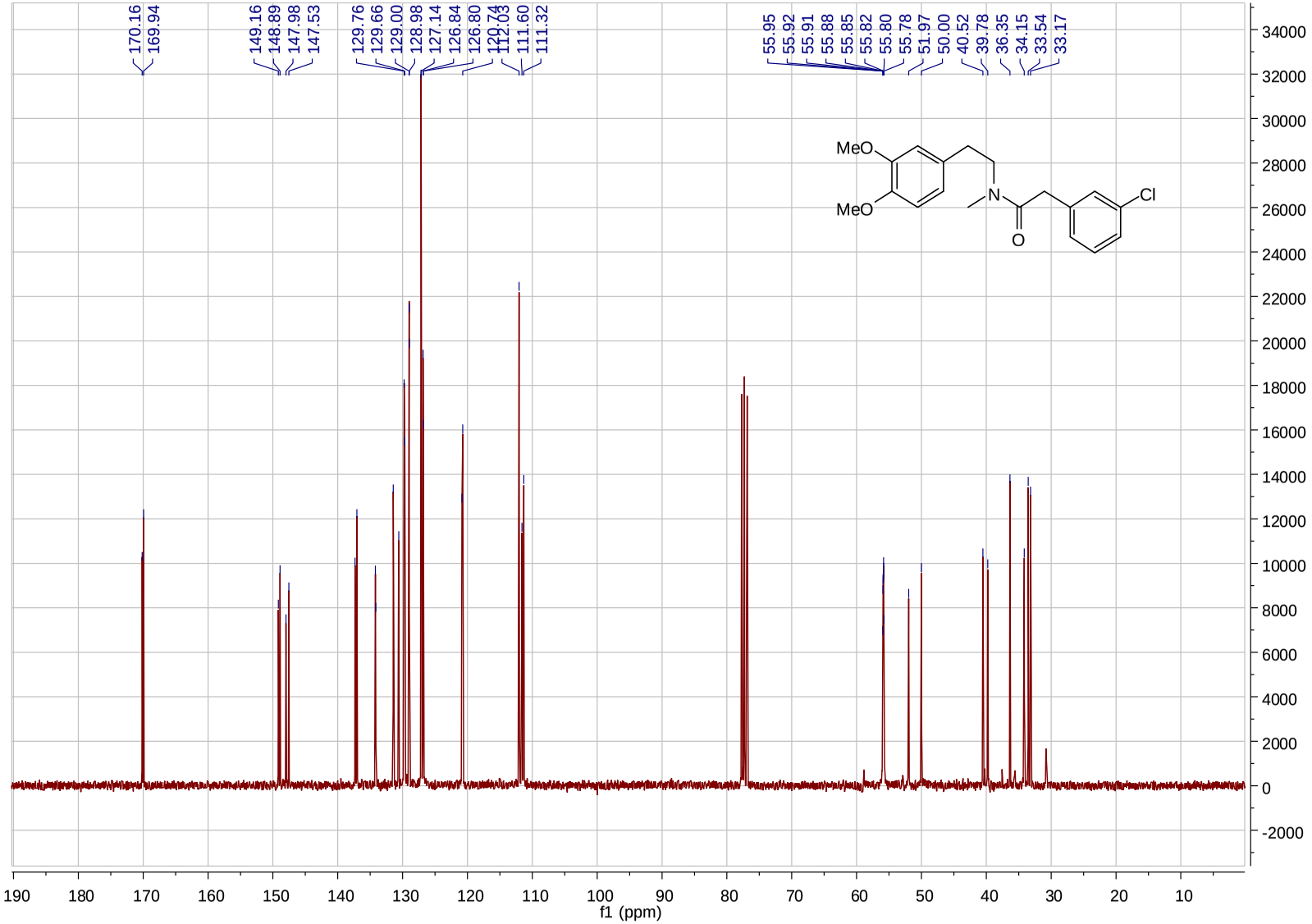
3-Chlorophenylacetyl chloride: ^{13}C-NMR spectrum



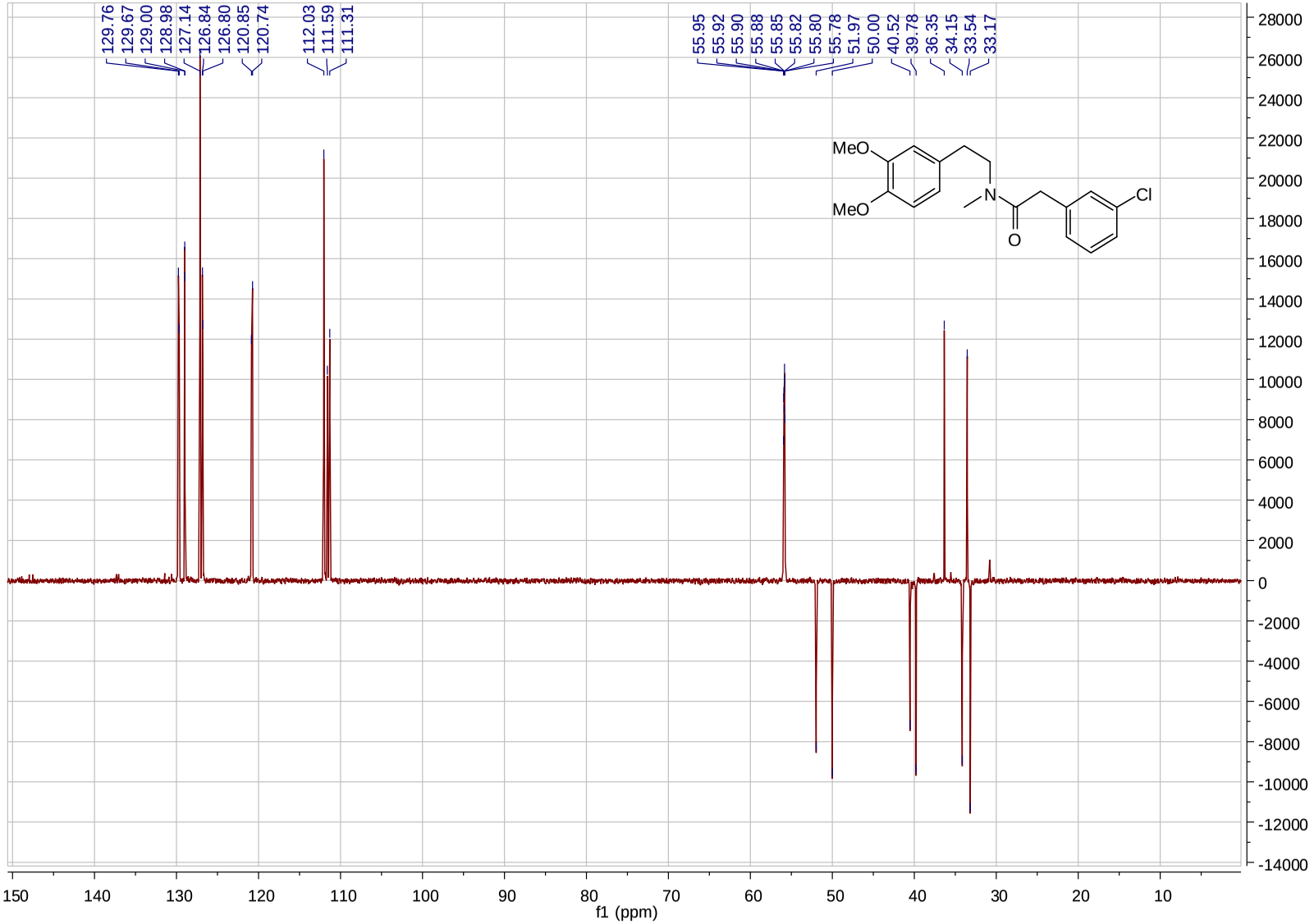
2-(3-Chlorophenyl)-*N*-(3,4-dimethoxyphenethyl)-*N*-methylacetamide: ^{1}H-NMR spectrum



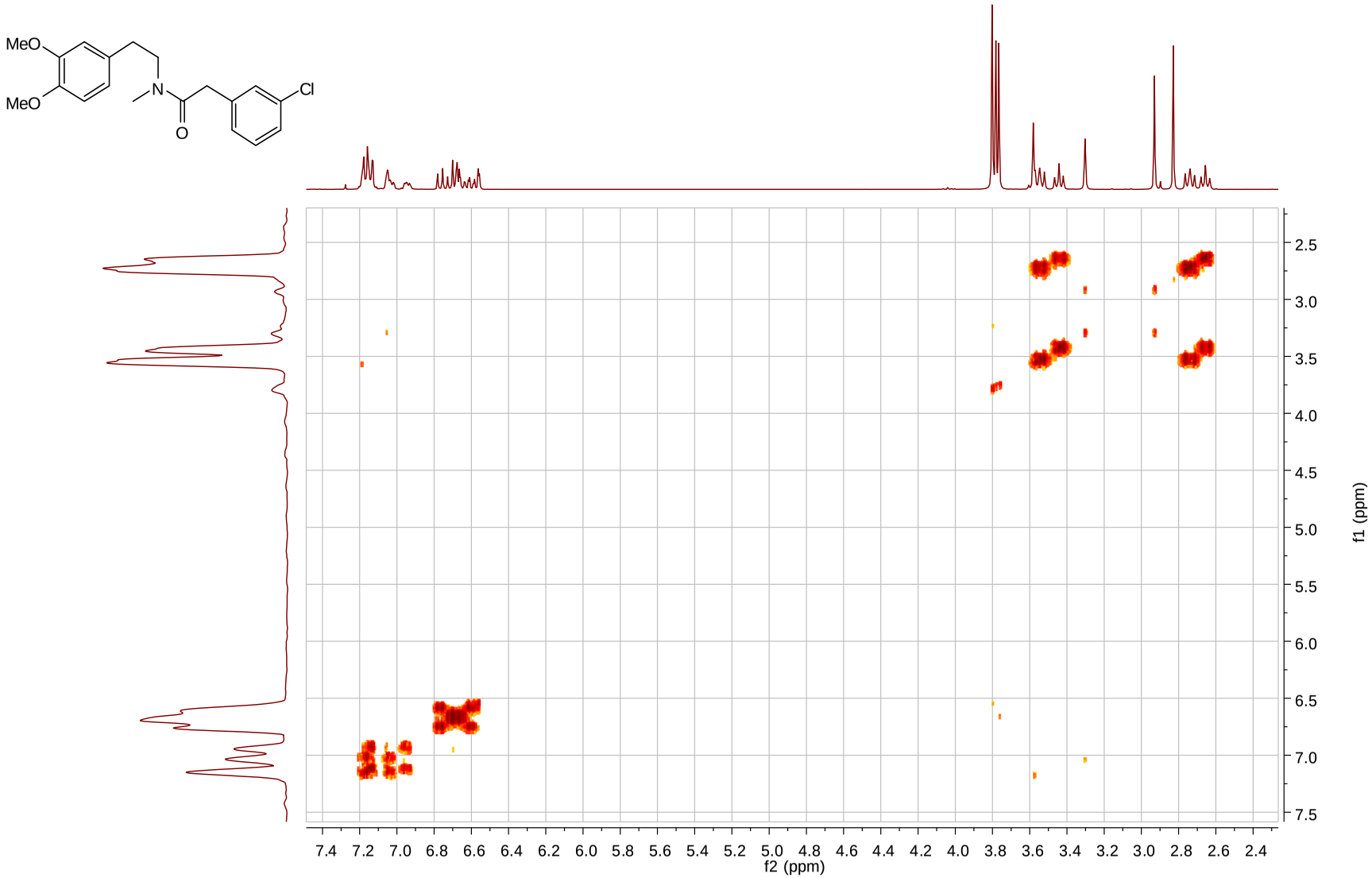
2-(3-Chlorophenyl)-*N*-(3,4-dimethoxyphenethyl)-*N*-methylacetamide: ^{13}C-NMR spectrum



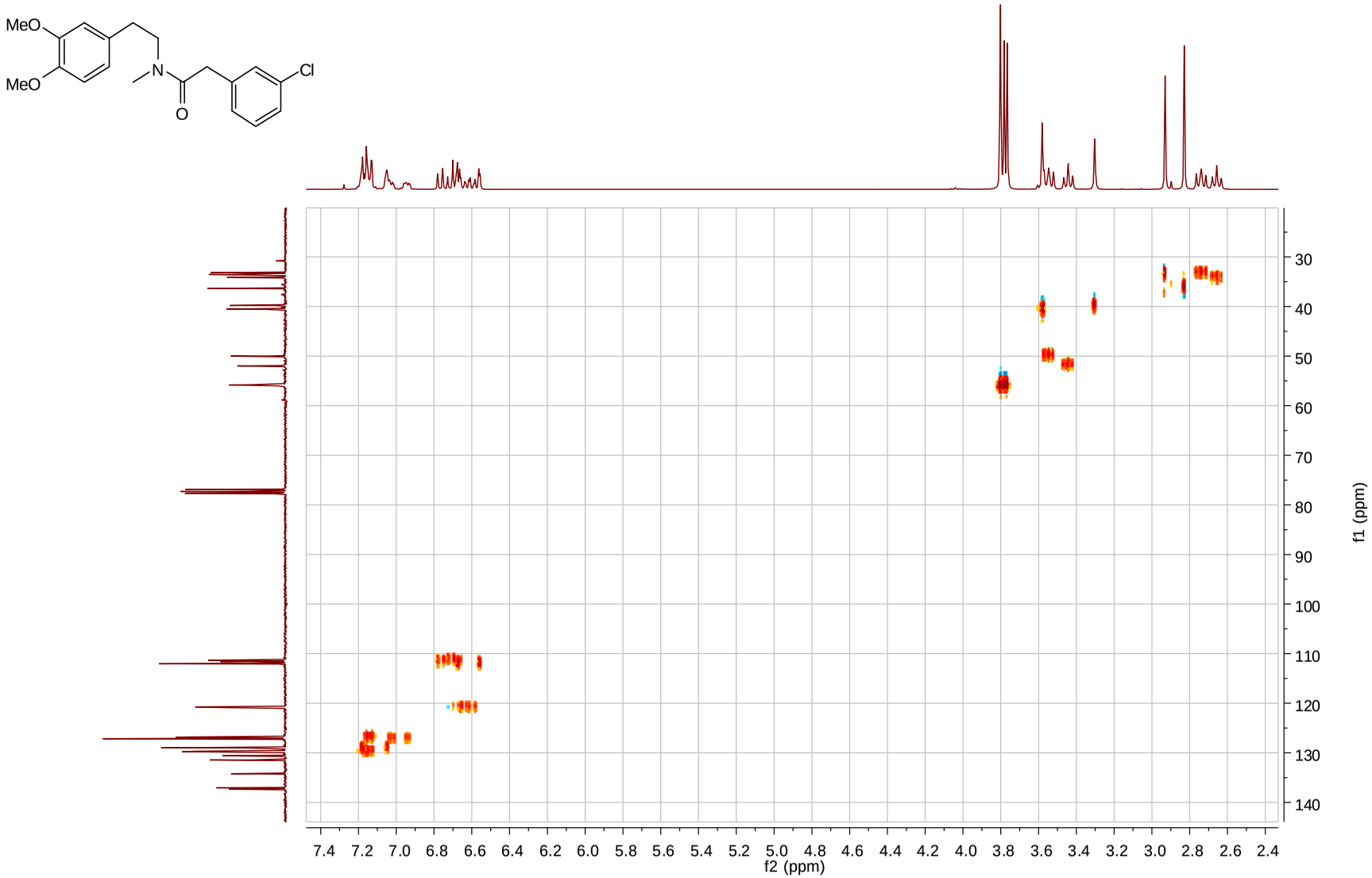
2-(3-Chlorophenyl)-*N*-(3,4-dimethoxyphenethyl)-*N*-methylacetamide: ^{13}C-NMR DEPT135 spectrum



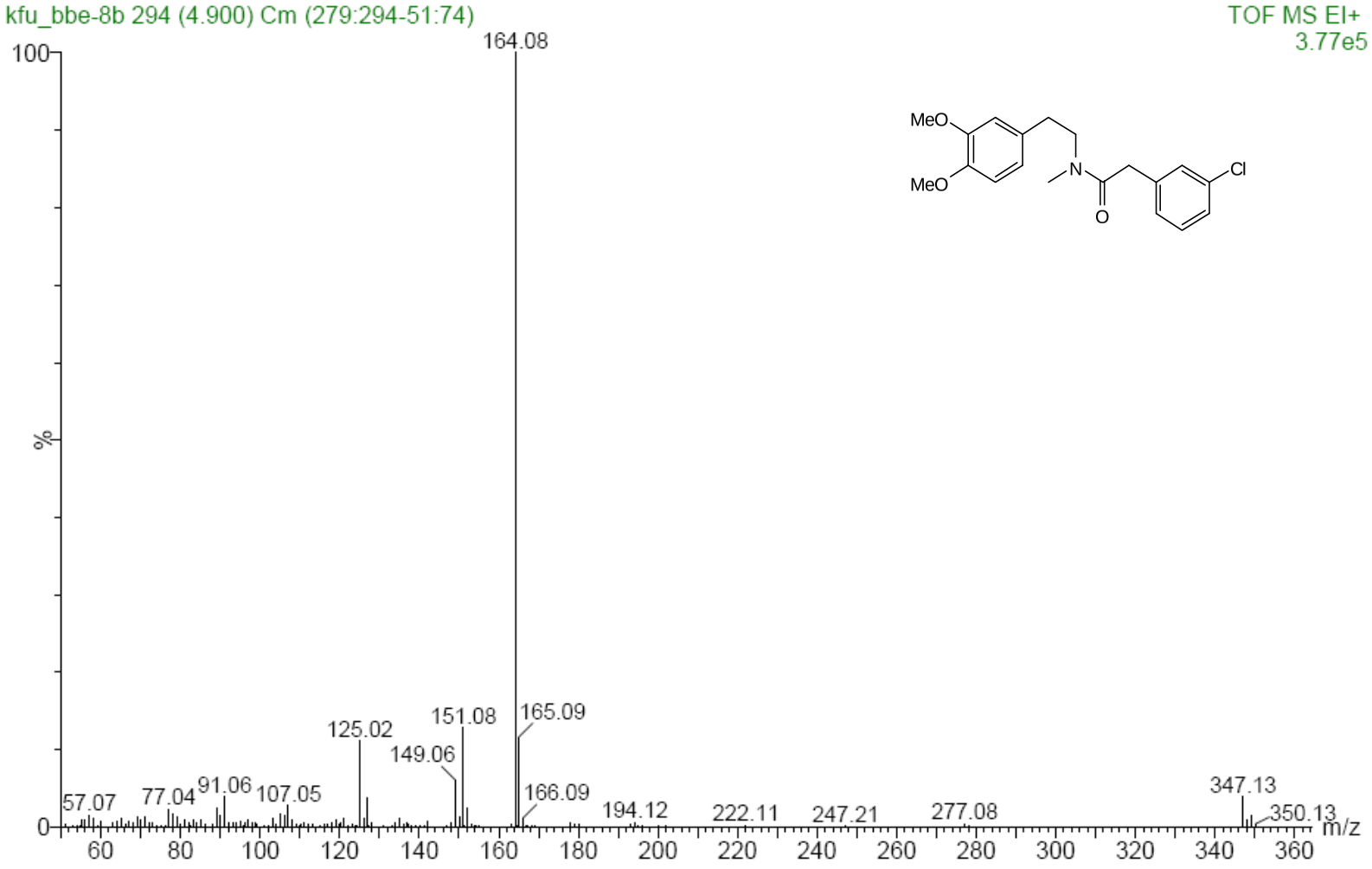
2-(3-Chlorophenyl)-*N*-(3,4-dimethoxyphenethyl)-*N*-methylacetamide: COSY spectrum



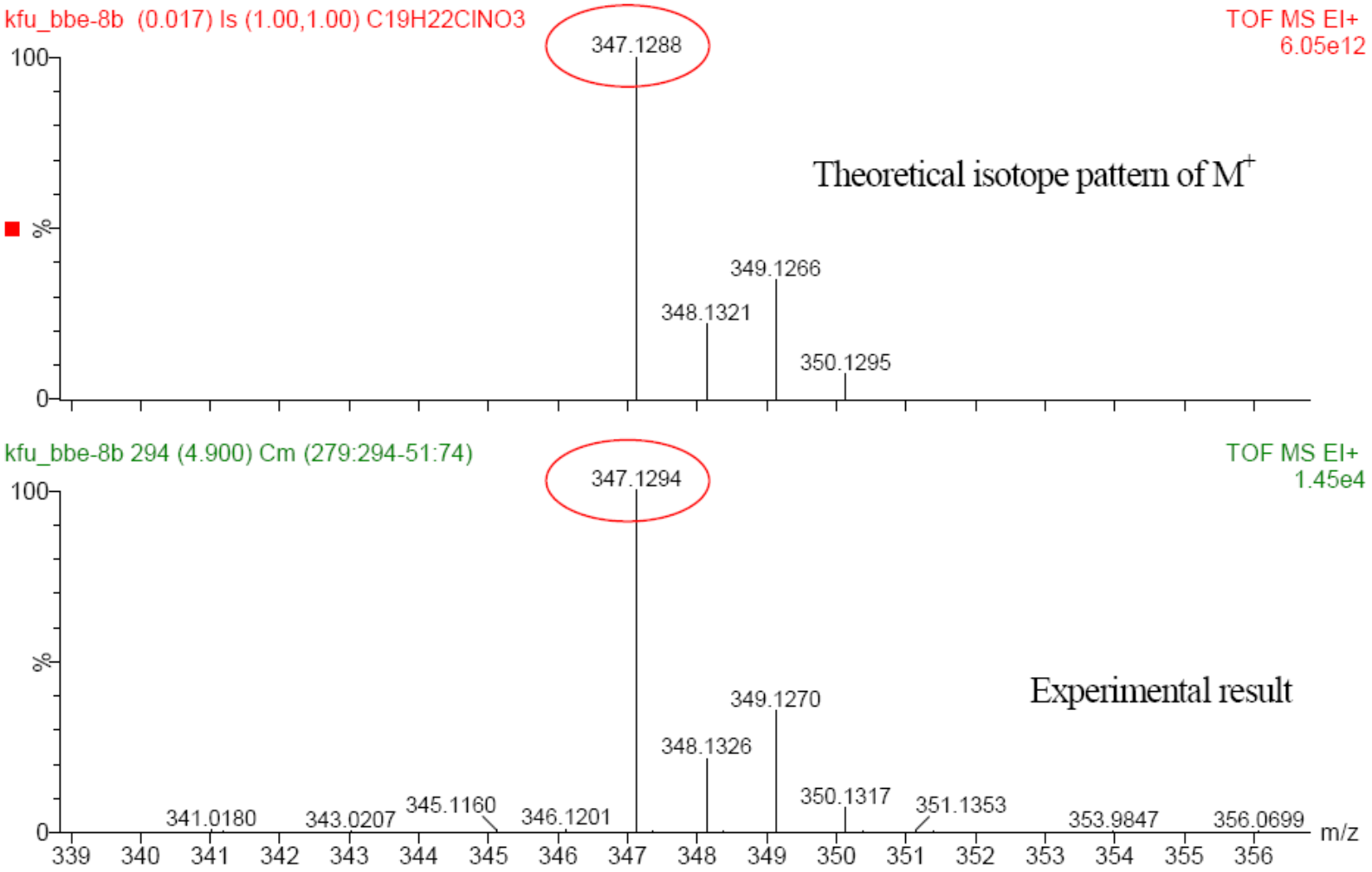
2-(3-Chlorophenyl)-*N*-(3,4-dimethoxyphenethyl)-*N*-methylacetamide: HSQC spectrum



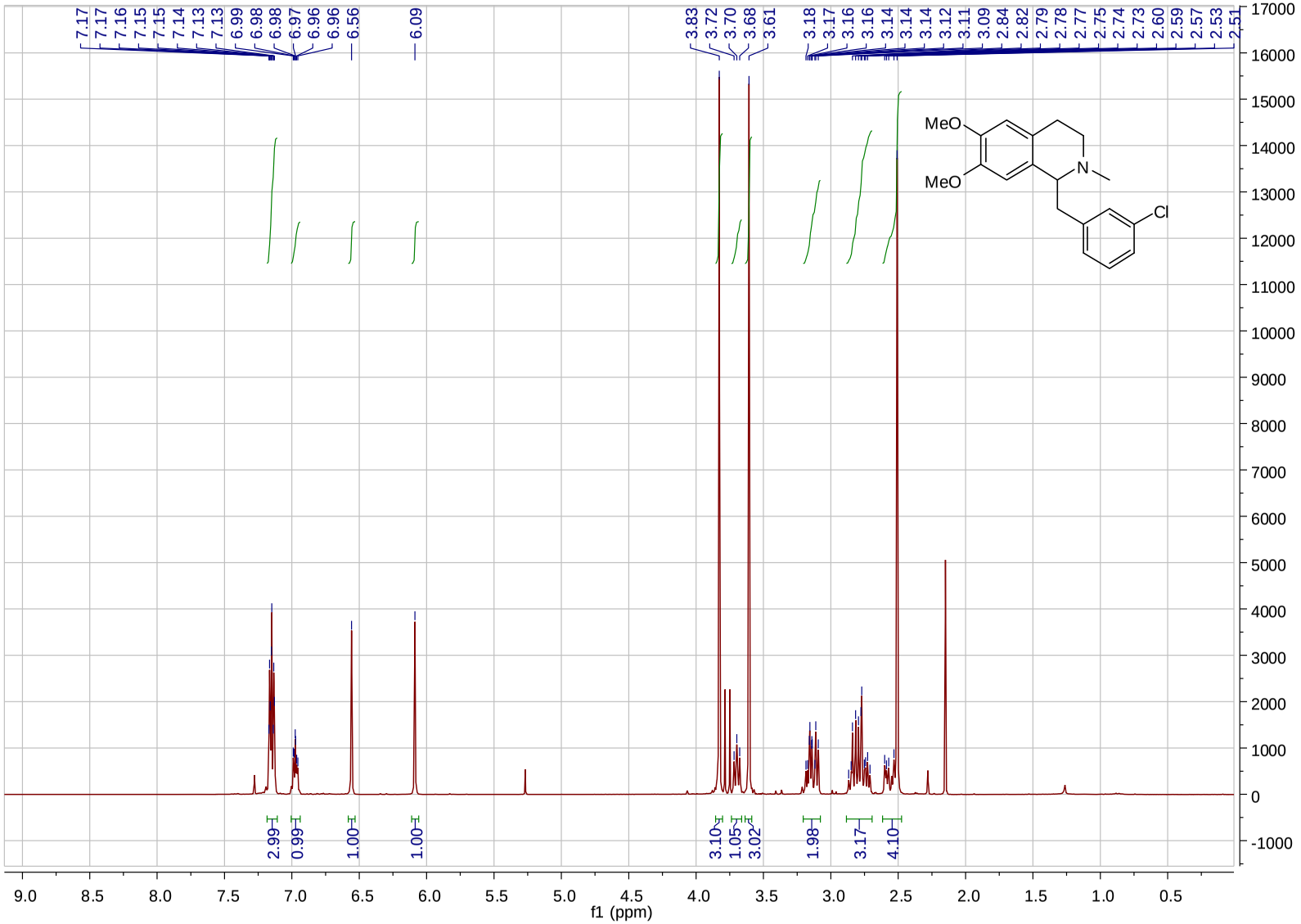
2-(3-Chlorophenyl)-*N*-(3,4-dimethoxyphenethyl)-*N*-methylacetamide: MS spectrum



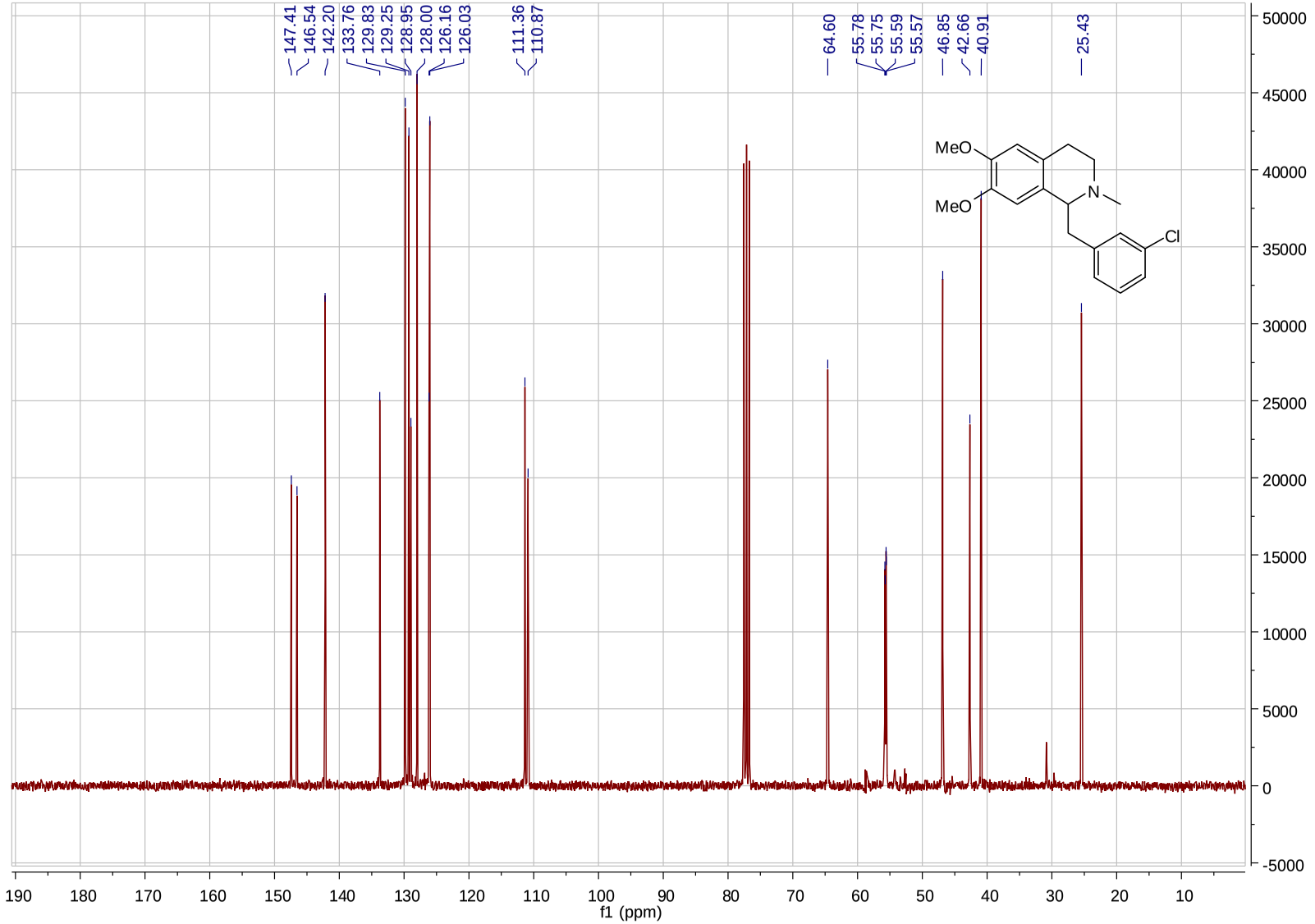
2-(3-Chlorophenyl)-*N*-(3,4-dimethoxyphenethyl)-*N*-methylacetamide: HRMS results



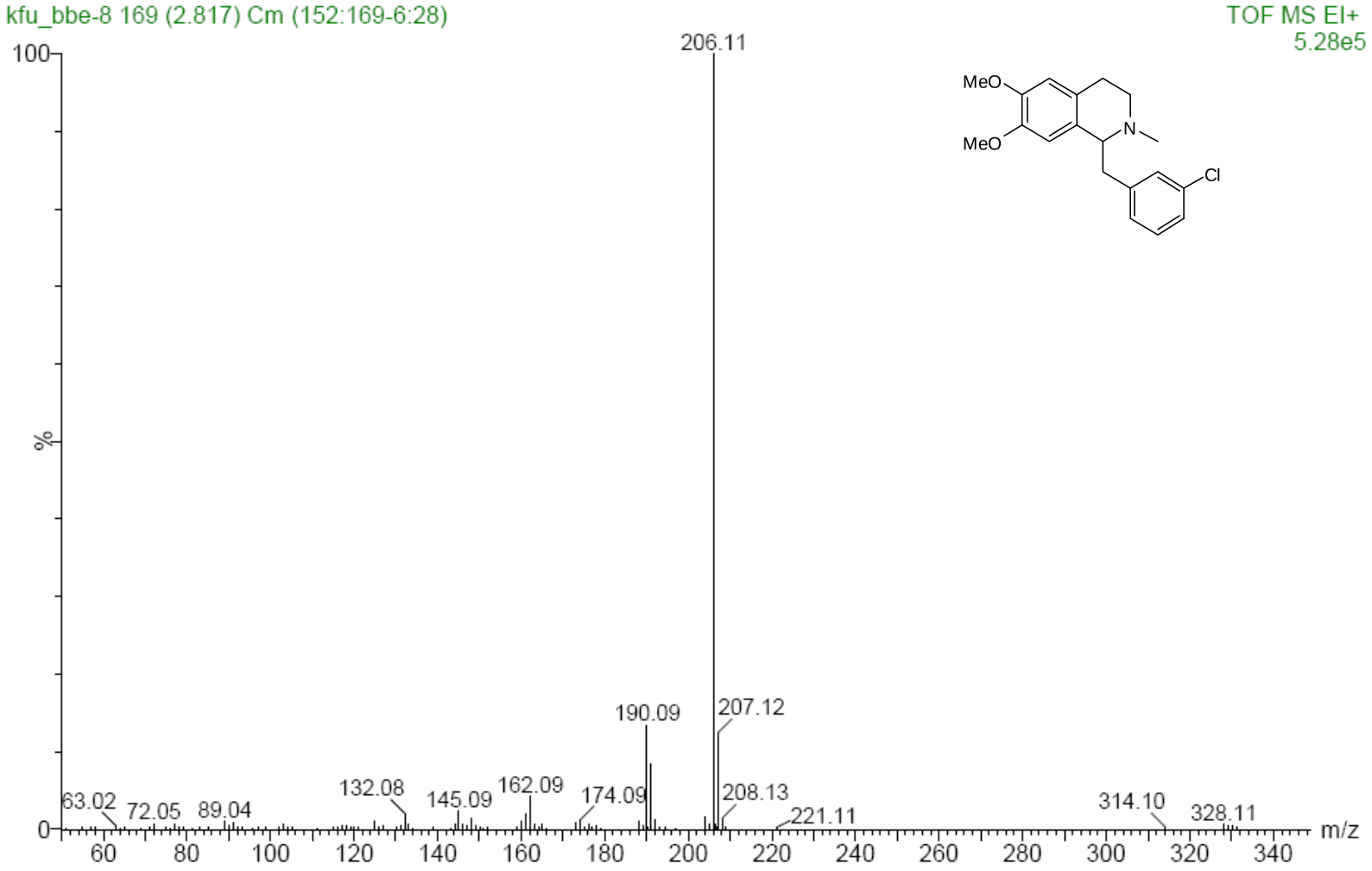
1-(3-Chlorobenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (2e): ^{1}H-NMR spectrum



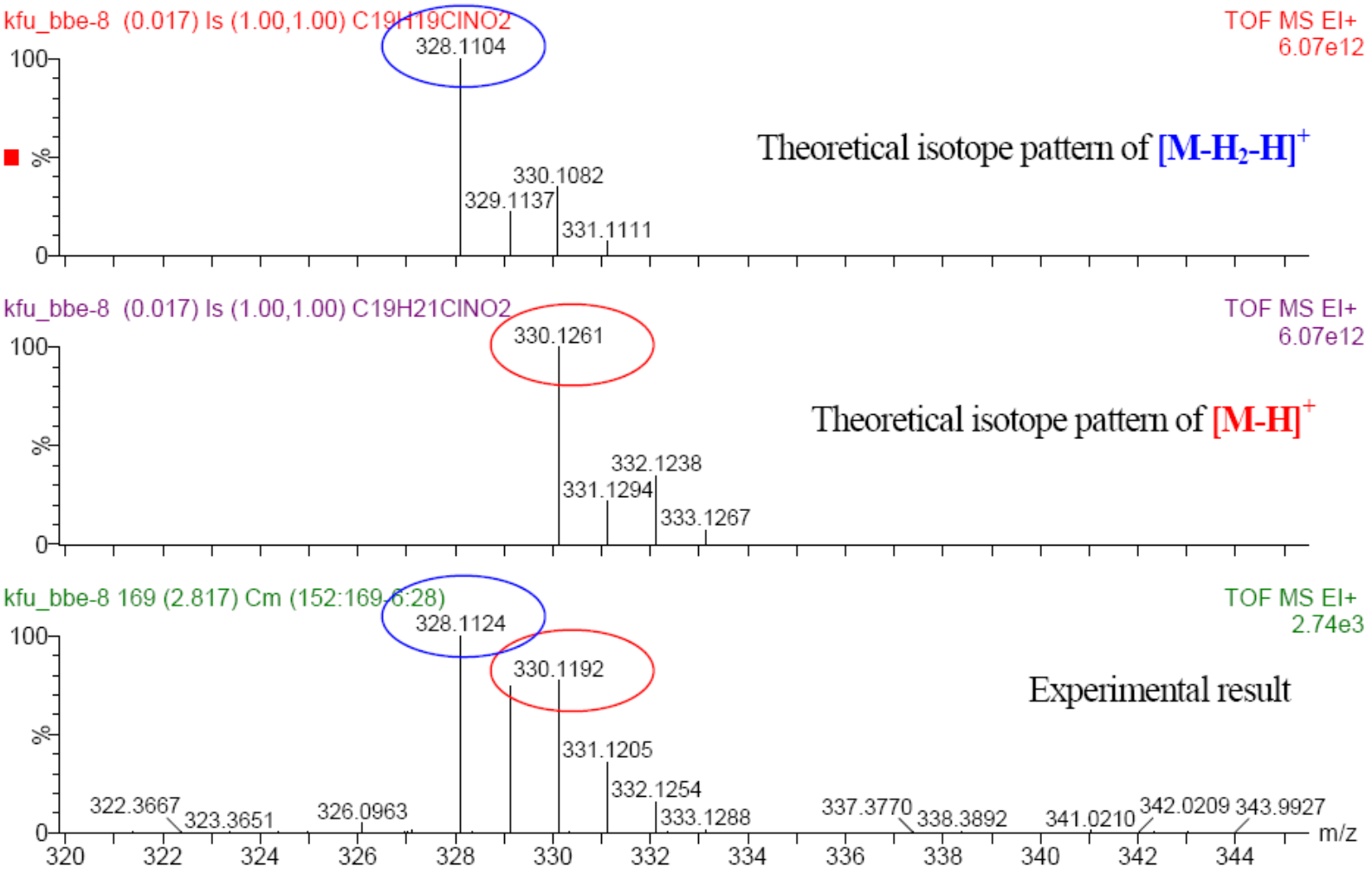
1-(3-Chlorobenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (2e): ^{13}C-NMR spectrum



1-(3-Chlorobenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (2e): MS spectrum



1-(3-Chlorobenzyl)-6,7-dimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (2e): HRMS results



Substrate 2f and its Synthetic Intermediates

Provided Material:

Methyl (3,5-bisbenzyloxy)phenylacetate:

^{1}H-NMR spectrum, ^{13}C-NMR spectrum

(3,5-Bisbenzyloxy)phenylacetic acid:

^{1}H-NMR spectrum, ^{13}C-NMR spectrum

(3,5-Bisbenzyloxy)phenylacetyl chloride:

^{1}H-NMR spectrum, ^{13}C-NMR spectrum

2-(3,5-Bisbenzyloxyphenyl)-*N*-(3,4-dimethoxyphenethyl)-*N*-methylacetamide:

^{1}H-NMR spectrum, ^{13}C-NMR spectrum, ^{13}C-NMR DEPT135 spectrum, ^{13}C-NMR DEPT90 spectrum, COSY spectrum, HSQC spectrum, MS spectrum, HRMS results

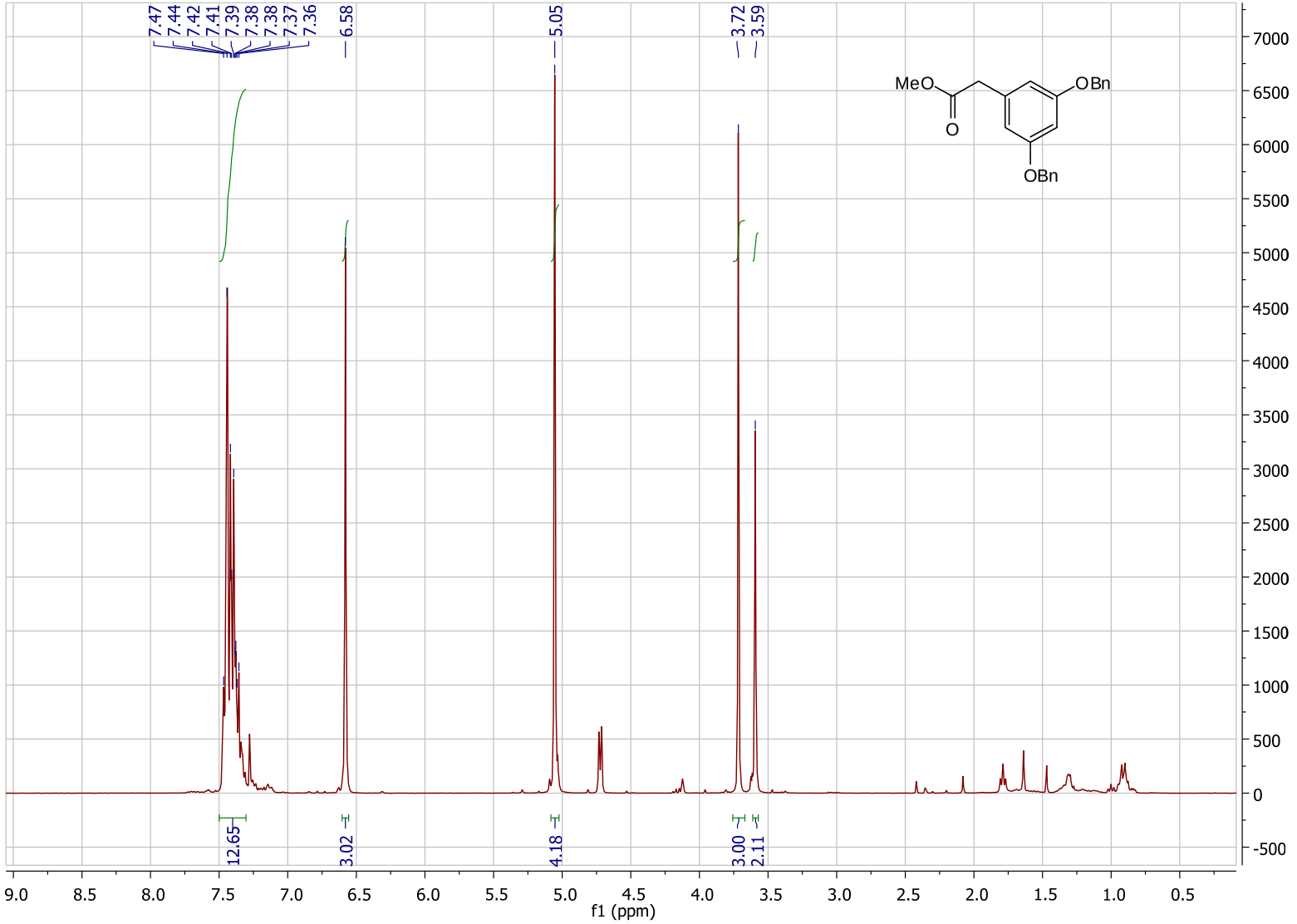
1-(3,5-Bisbenzyloxy)benzyl-6,7-dimethoxy-2-ethyl-1,2,3,4-tetrahydroisoquinoline:

^{1}H-NMR spectrum, ^{13}C-NMR spectrum, MS spectrum, HRMS results

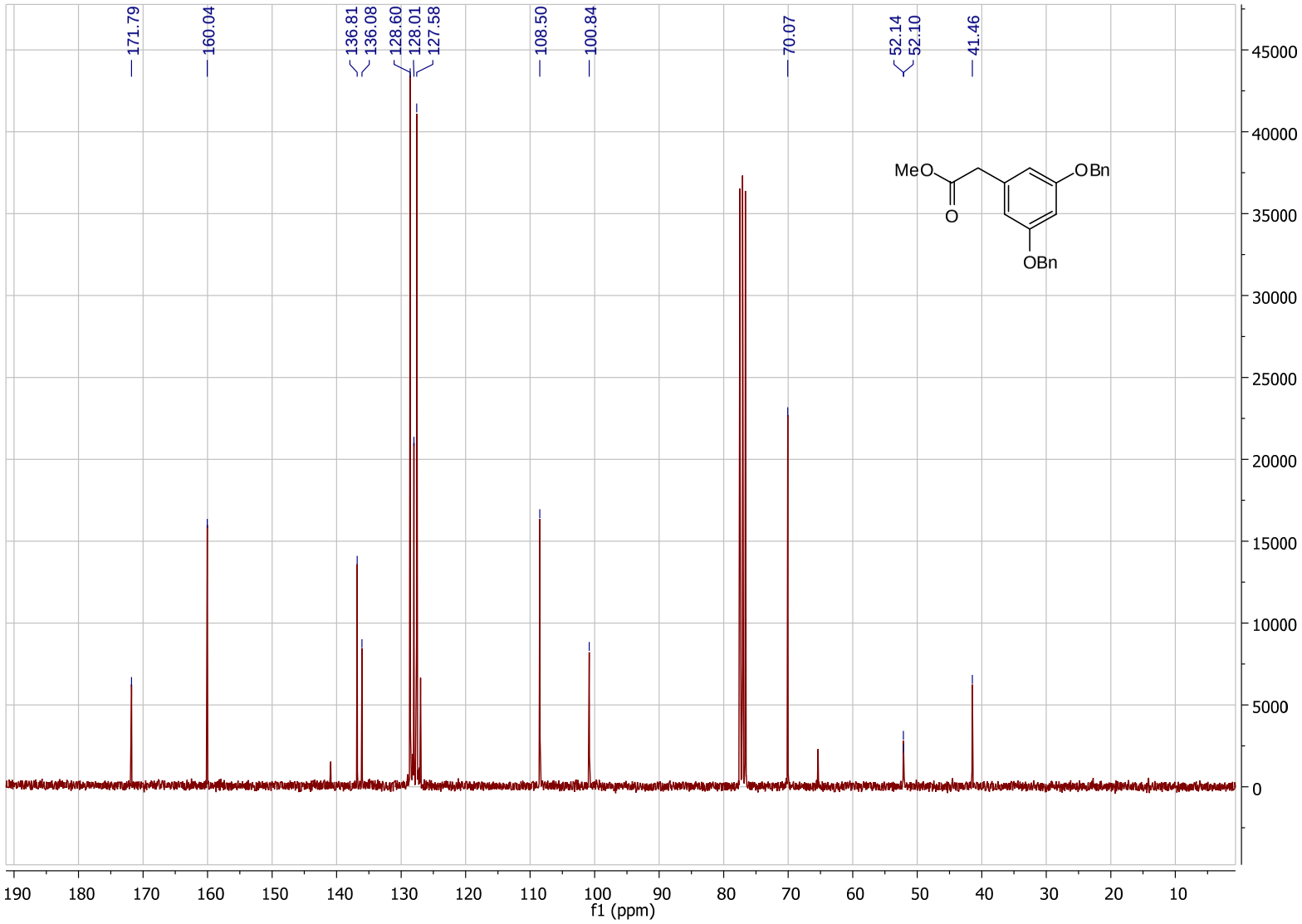
1-(3,5-Dihydroxybenzyl)-6,7-dimethoxy-2-ethyl-1,2,3,4-tetrahydroisoquinoline (2f):

^{1}H-NMR spectrum, ^{13}C-NMR spectrum, MS spectrum, HRMS results

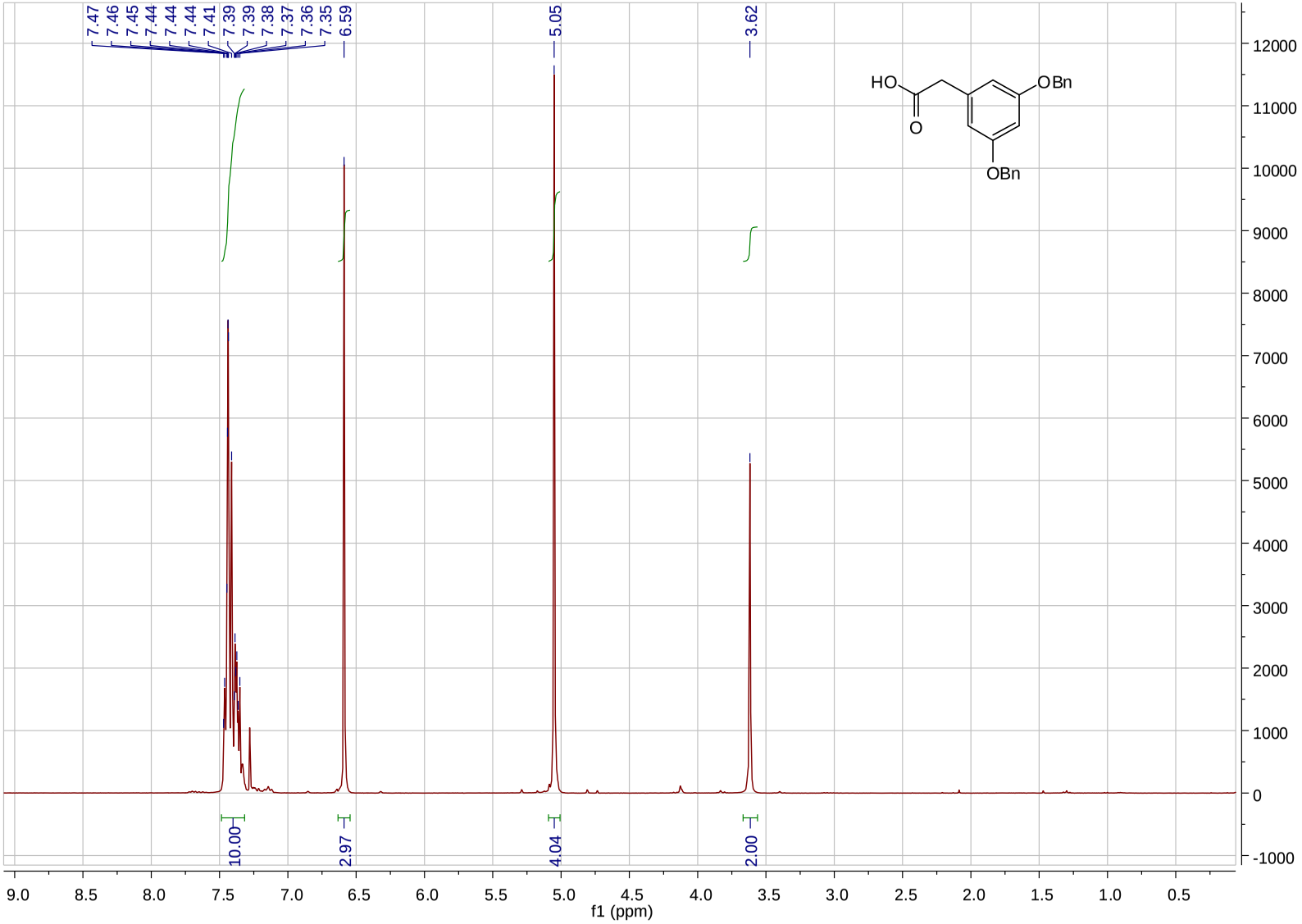
Methyl (3,5-bisbenzyloxy)phenylacetate: ^{1}H-NMR spectrum



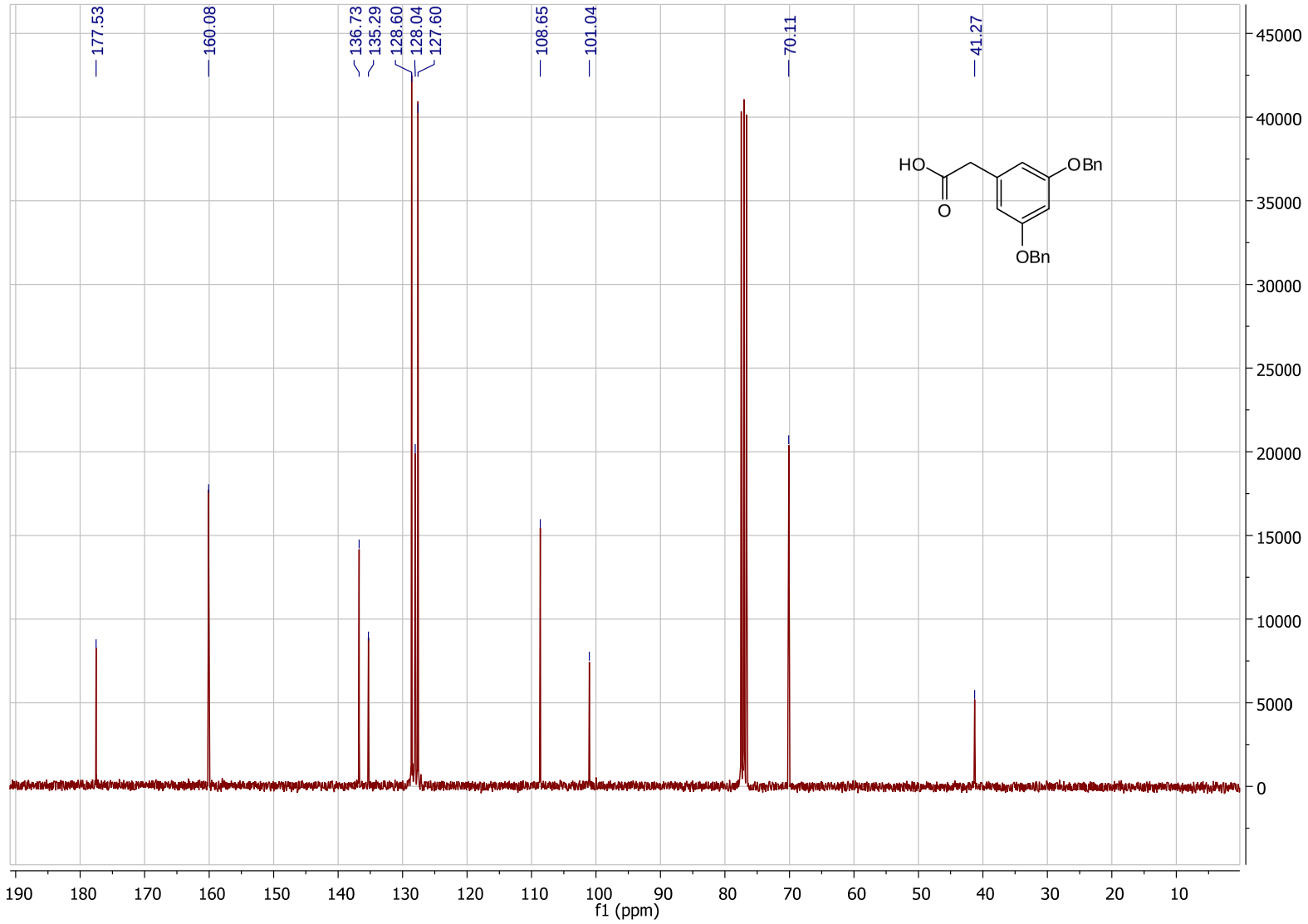
Methyl (3,5-bisbenzyloxy)phenylacetate: ^{13}C-NMR spectrum



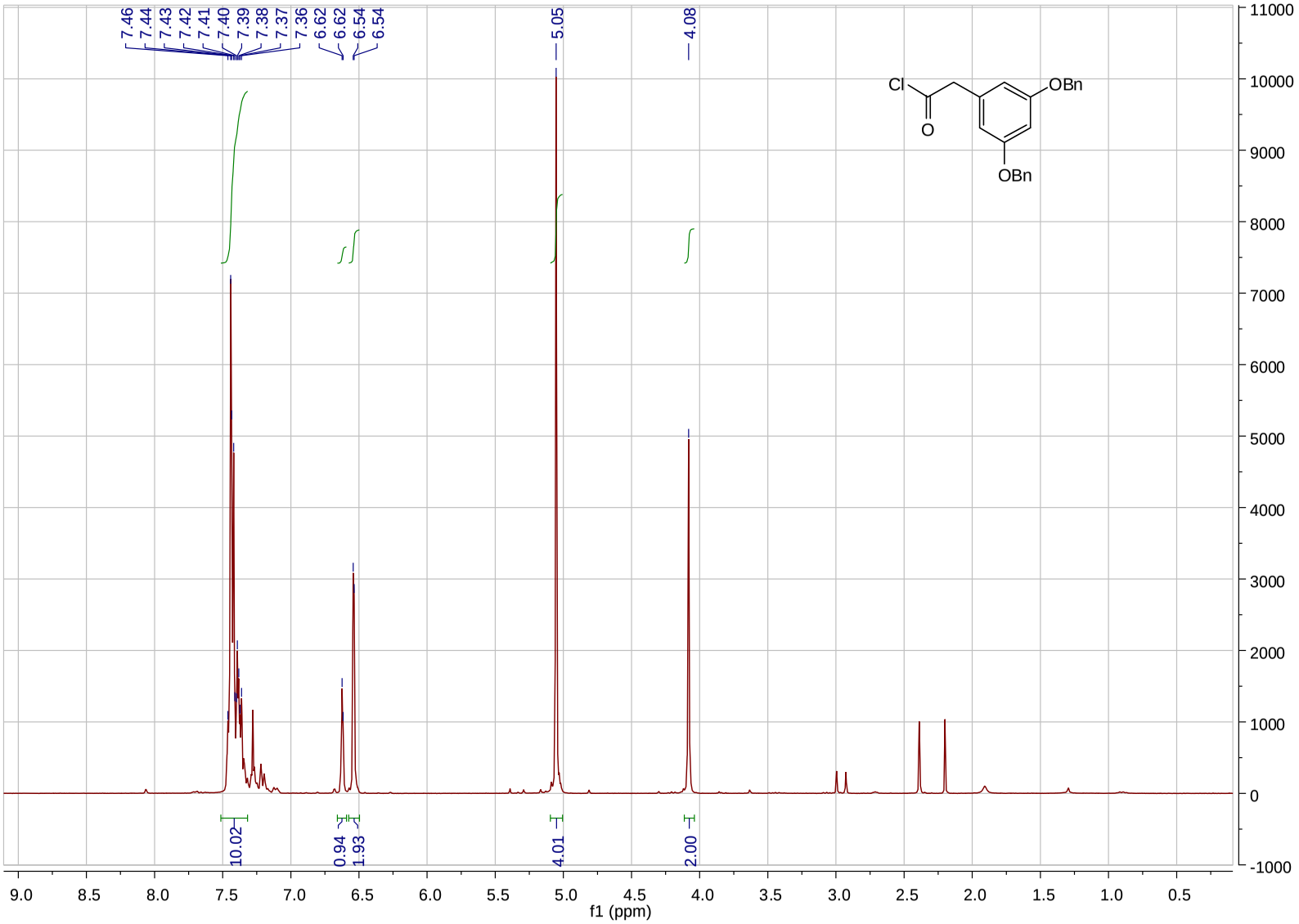
(3,5-Bisbenzyloxy)phenylacetic acid: ^{1}H-NMR spectrum



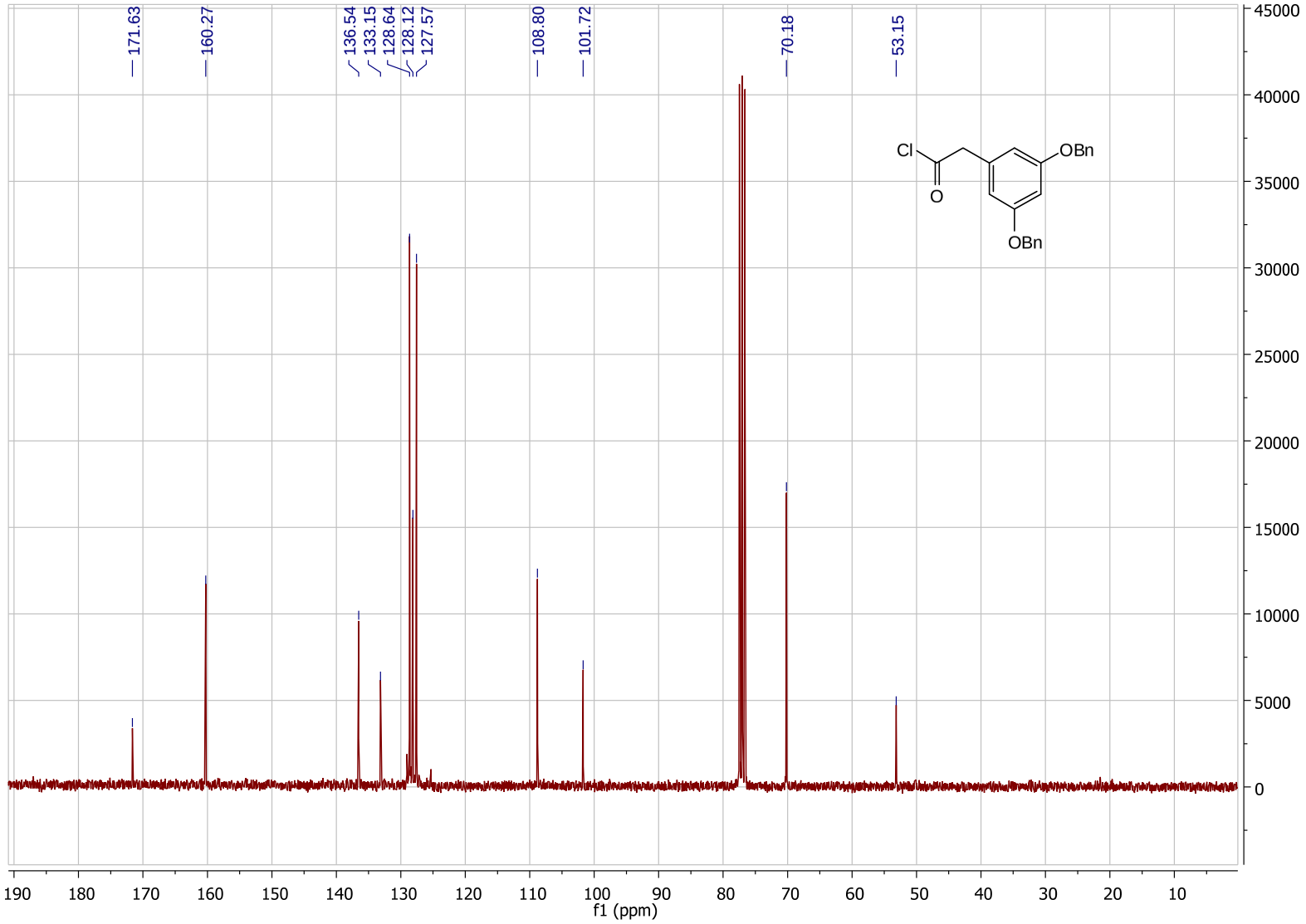
(3,5-Bisbenzyloxy)phenylacetic acid: ^{13}C-NMR spectrum



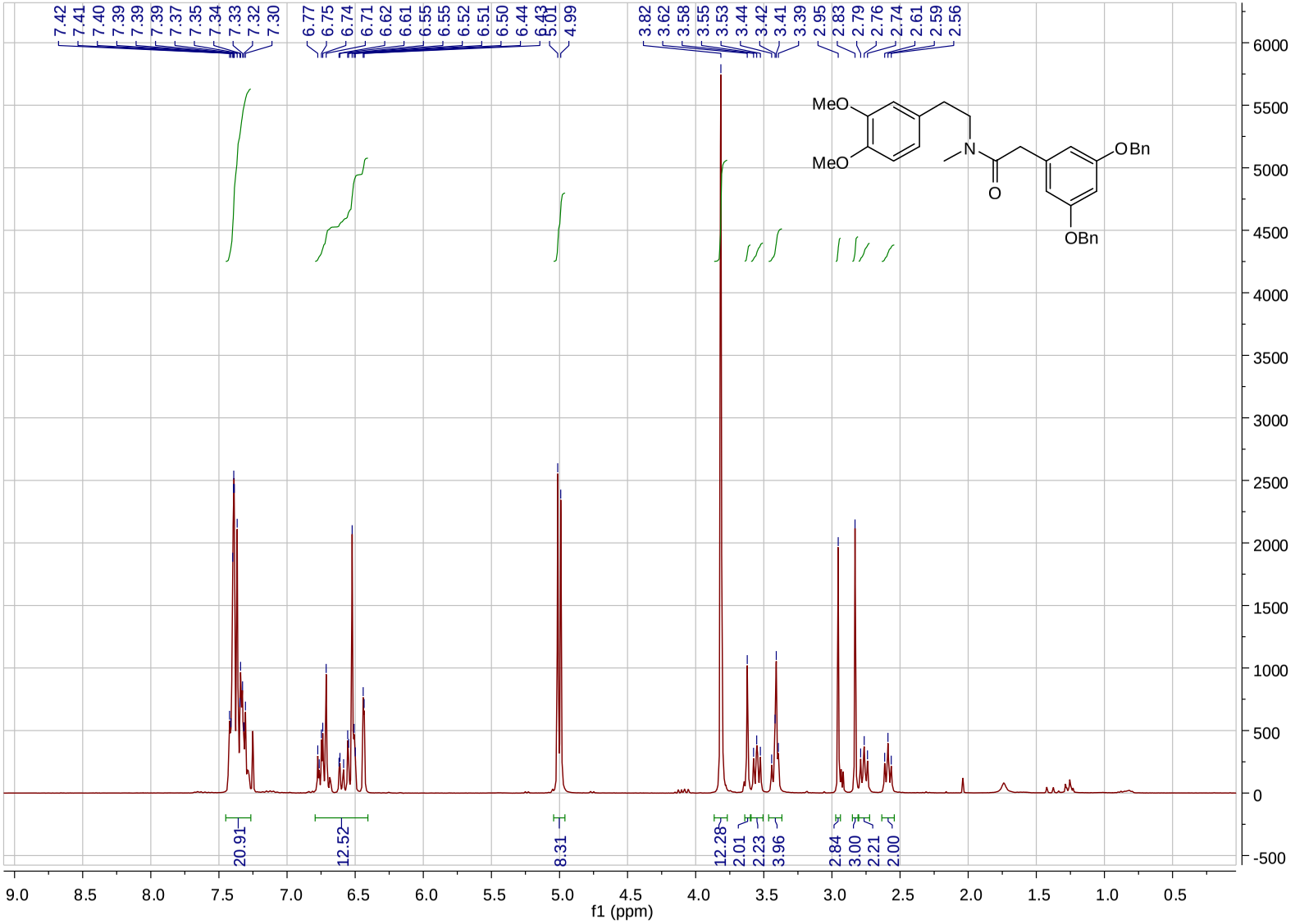
(3,5-Bisbenzyloxy)phenylacetyl chloride: ^{1}H-NMR spectrum



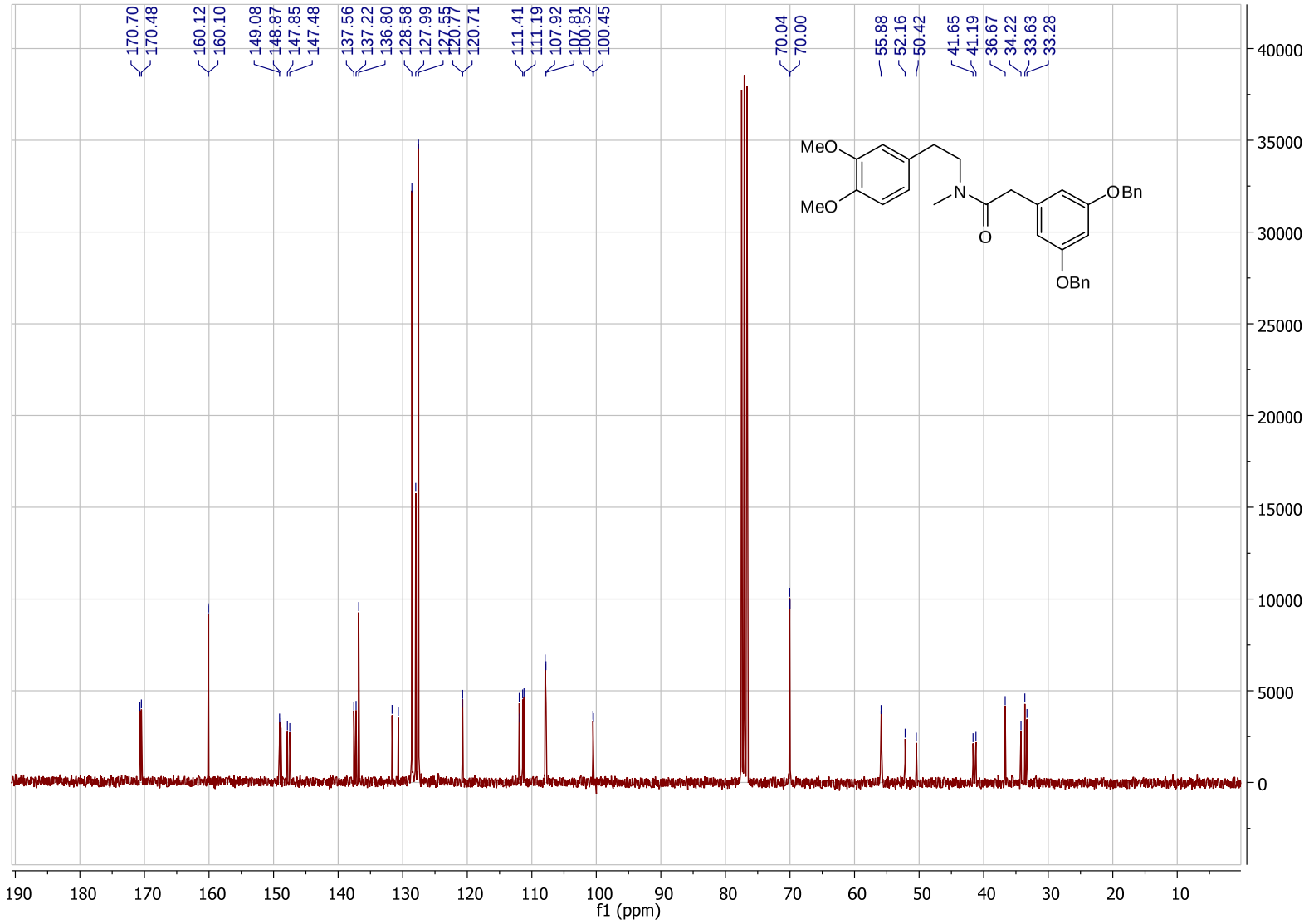
(3,5-Bisbenzyloxy)phenylacetyl chloride: ^{13}C-NMR spectrum



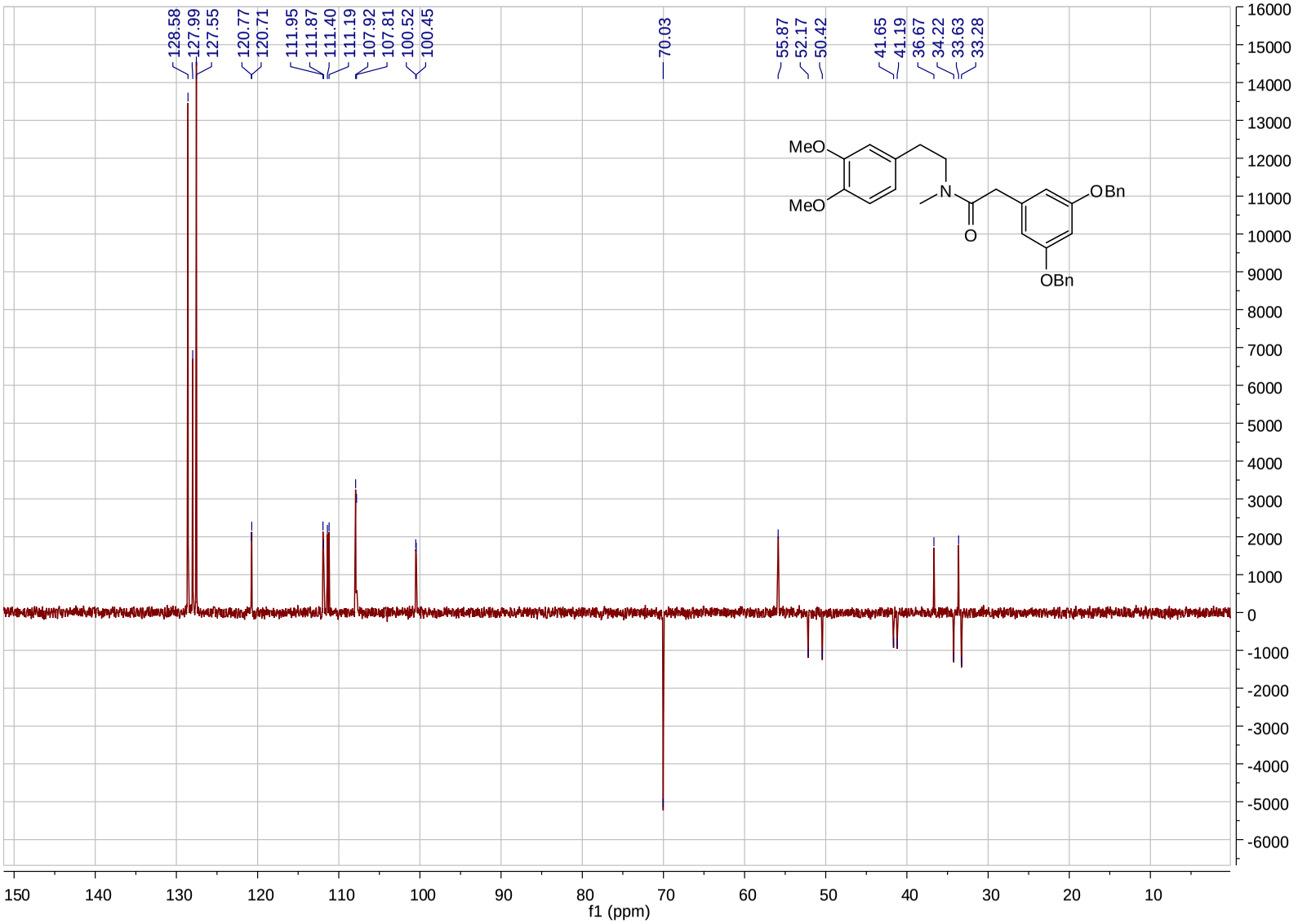
2-(3,5-Bisbenzyloxyphenyl)-*N*-(3,4-dimethoxyphenethyl)-*N*-methylacetamide: ^{1}H-NMR spectrum



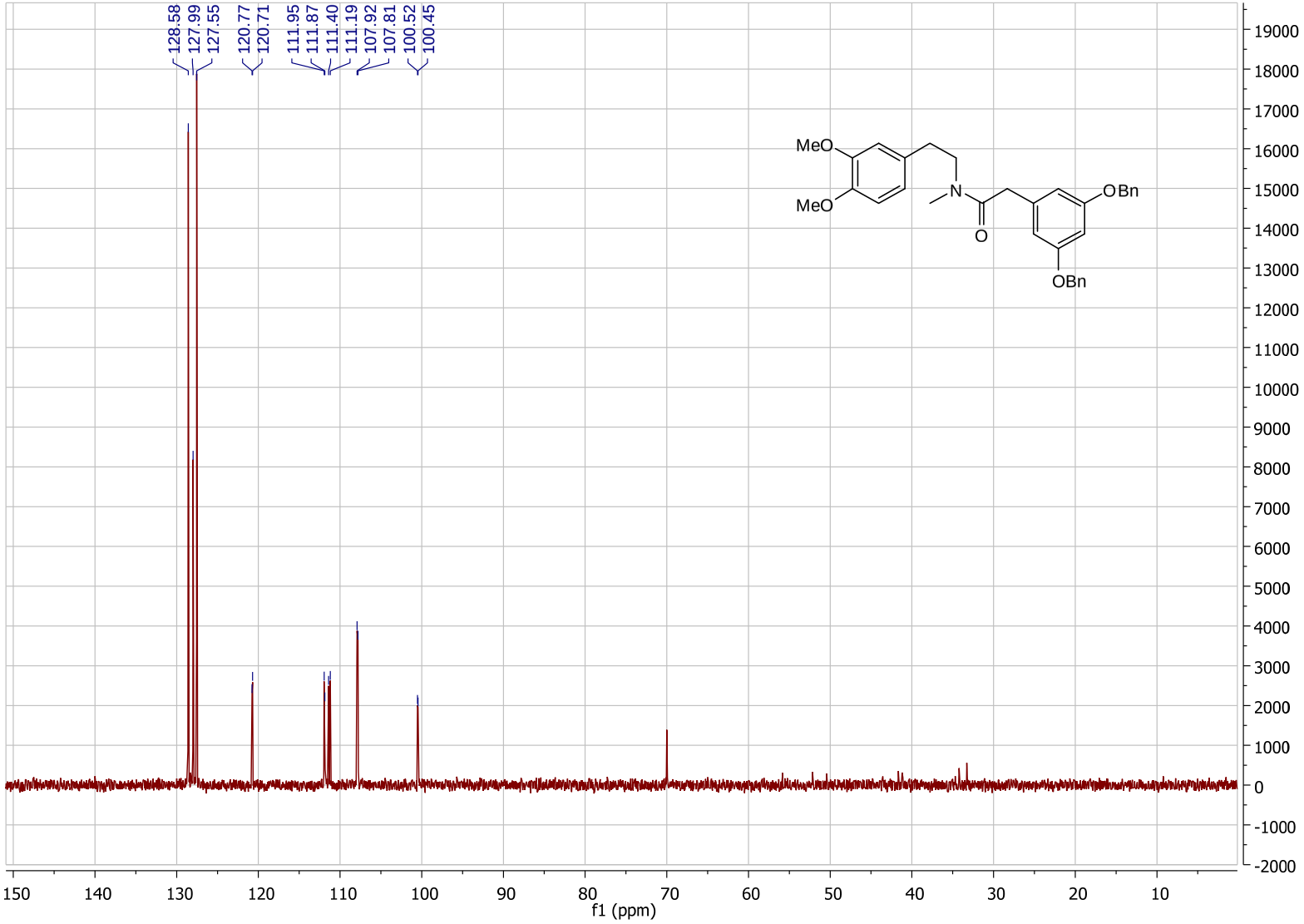
2-(3,5-Bisbenzyloxyphenyl)-*N*-(3,4-dimethoxyphenethyl)-*N*-methylacetamide: ^{13}C-NMR spectrum



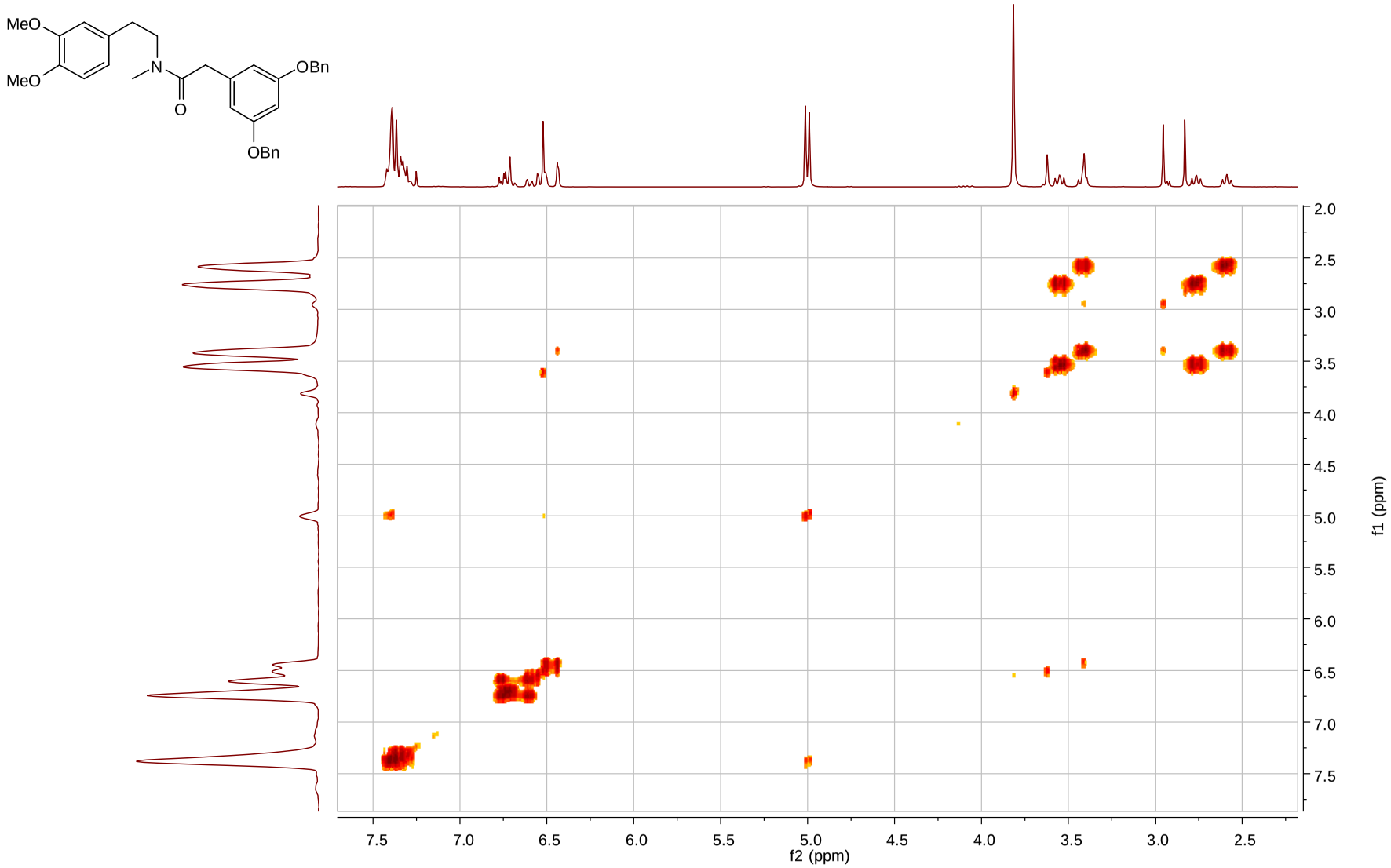
2-(3,5-Bisbenzyloxyphenyl)-*N*-(3,4-dimethoxyphenethyl)-*N*-methylacetamide: ^{13}C-NMR DEPT135 spectrum



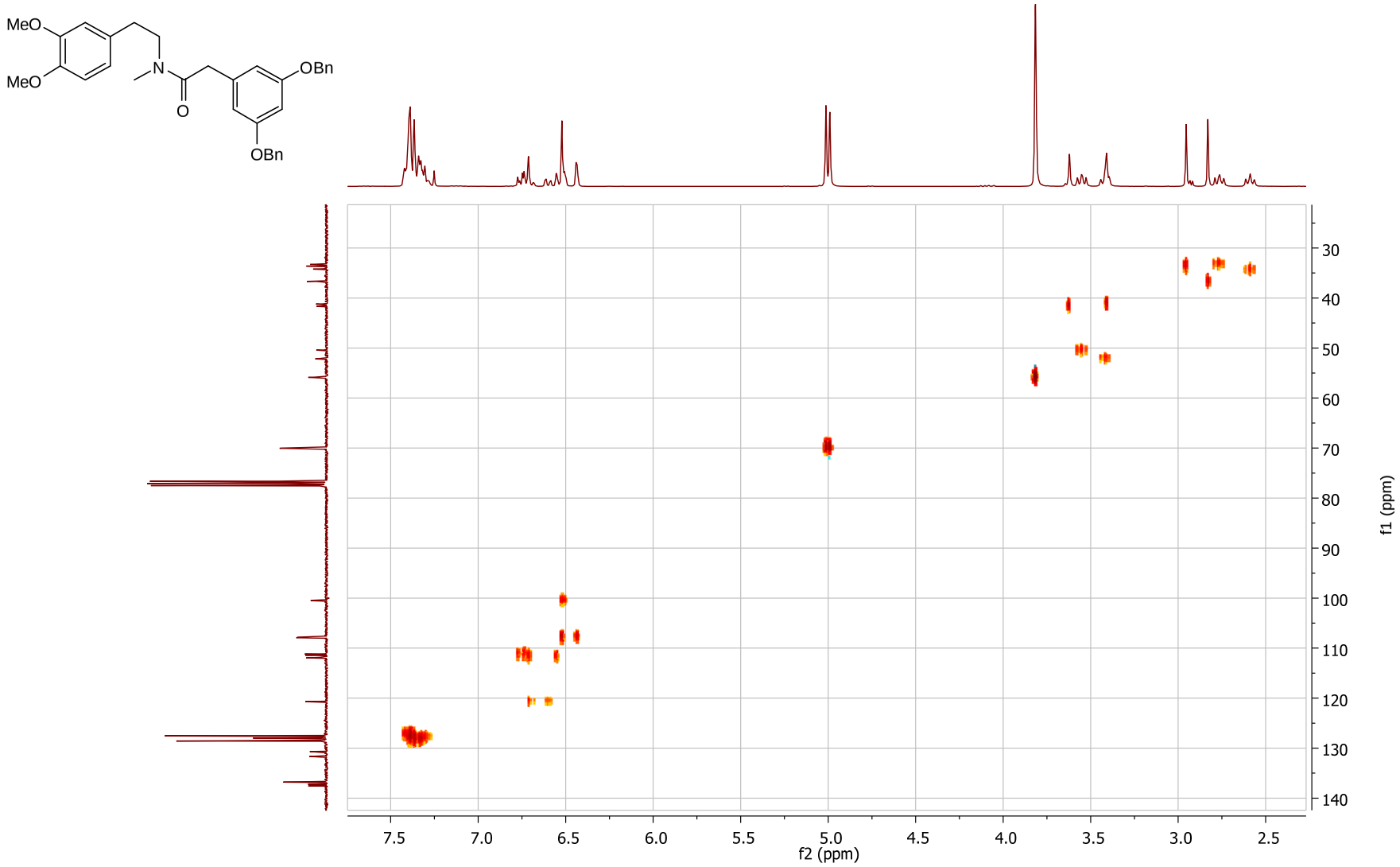
2-(3,5-Bisbenzyloxyphenyl)-*N*-(3,4-dimethoxyphenethyl)-*N*-methylacetamide: ^{13}C-NMR DEPT90 spectrum



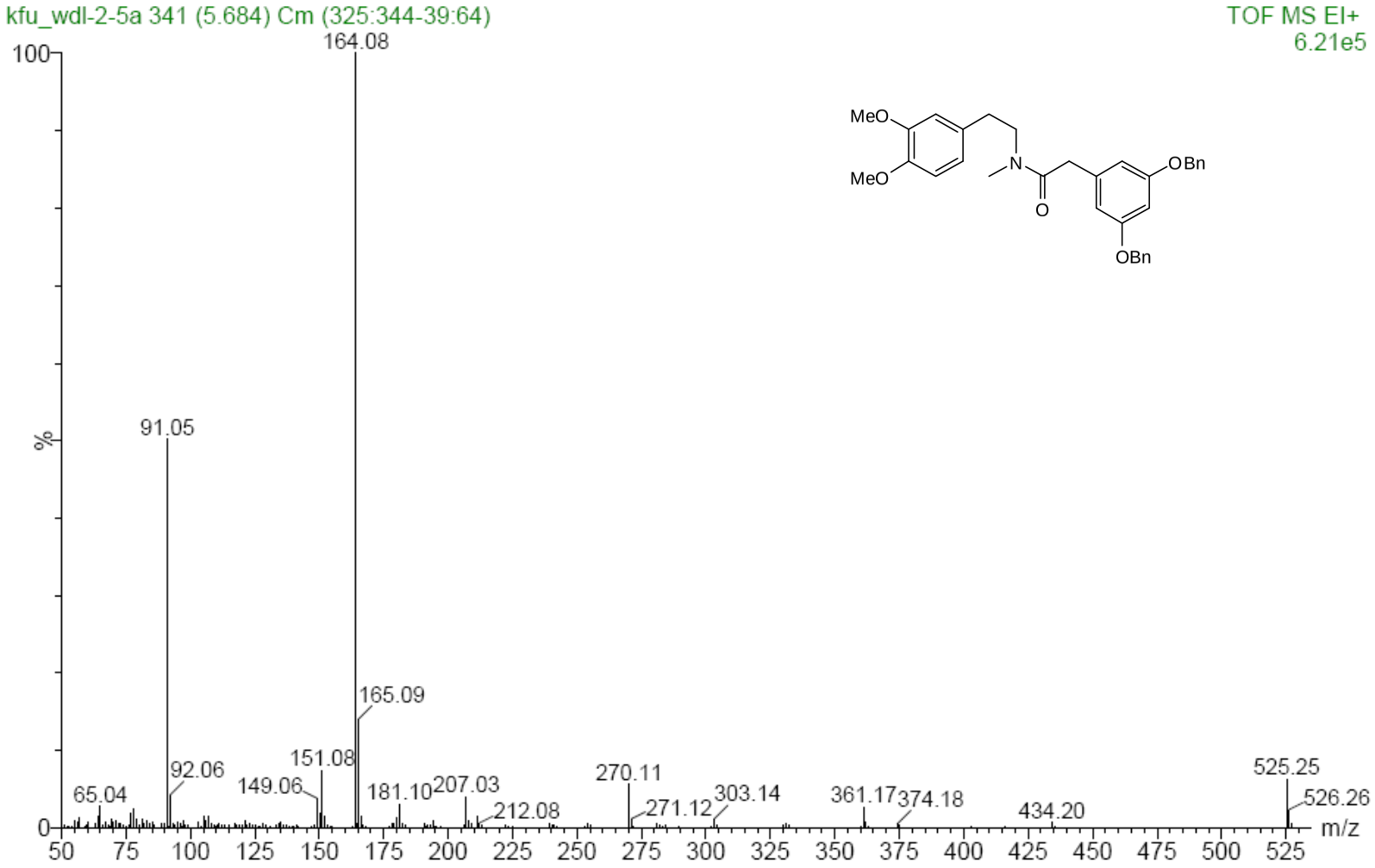
2-(3,5-Bisbenzyloxyphenyl)-*N*-(3,4-dimethoxyphenethyl)-*N*-methylacetamide: COSY spectrum



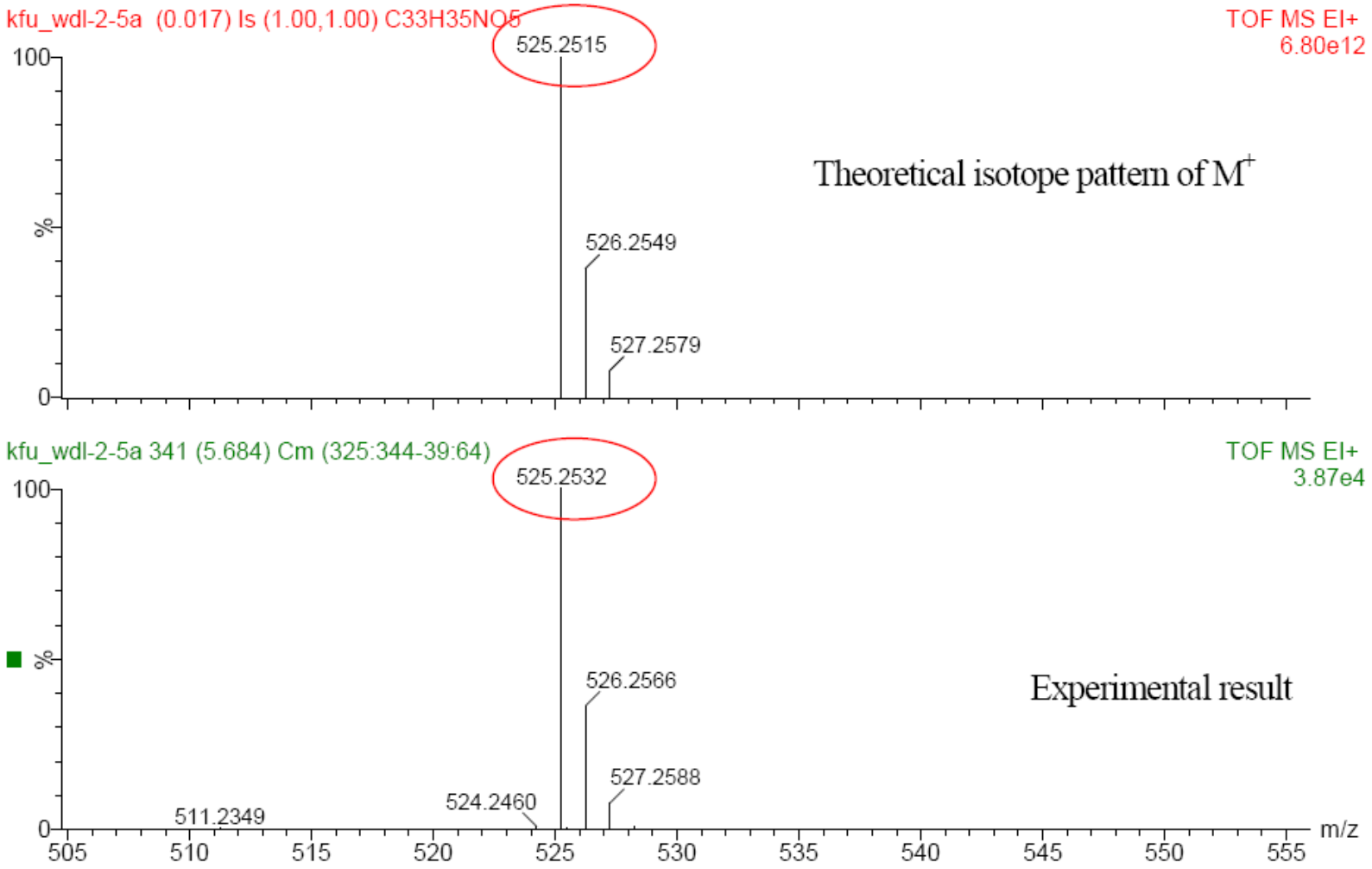
2-(3,5-Bisbenzyloxyphenyl)-*N*-(3,4-dimethoxyphenethyl)-*N*-methylacetamide: HSQC spectrum



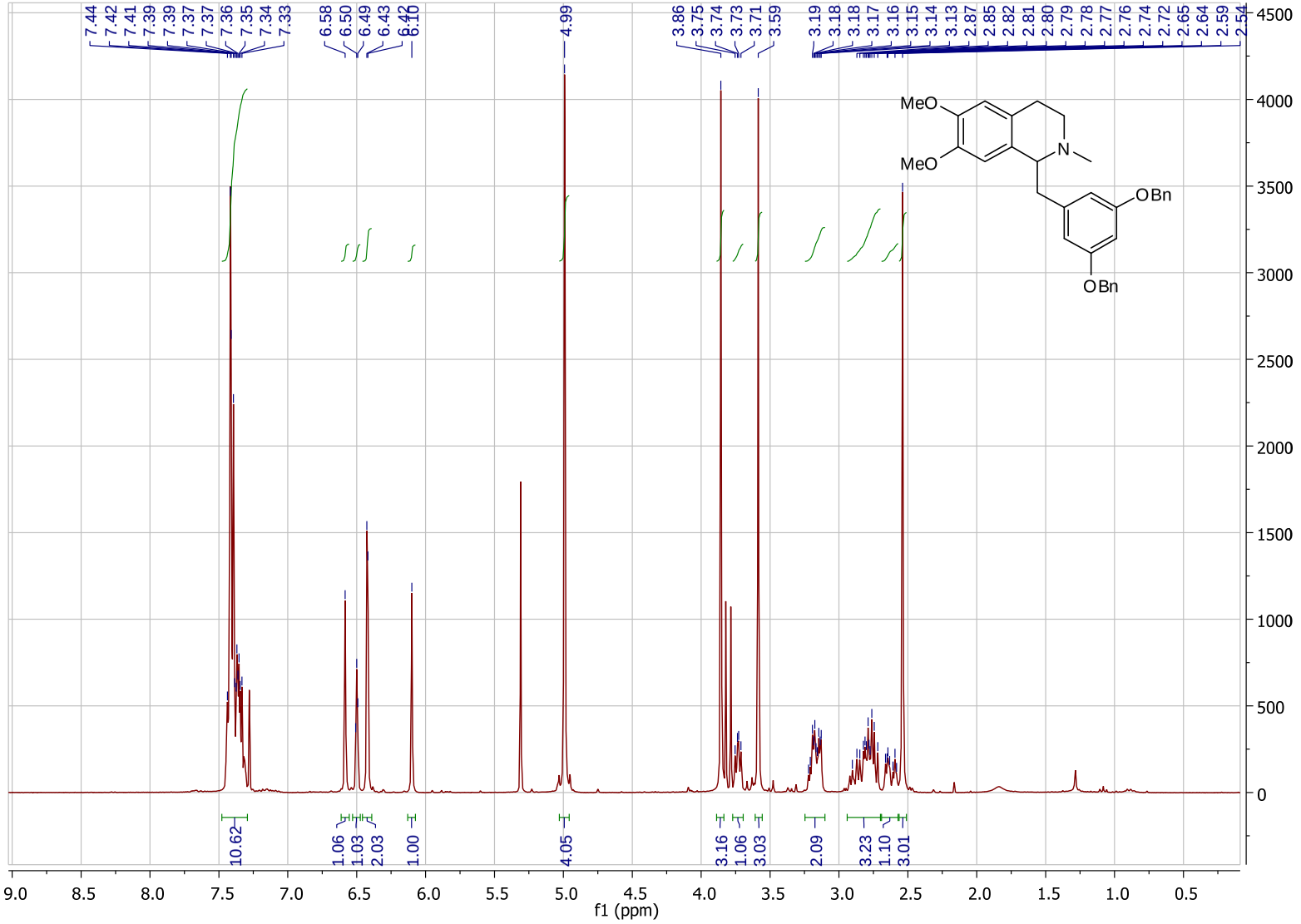
2-(3,5-Bisbenzyloxyphenyl)-*N*-(3,4-dimethoxyphenethyl)-*N*-methylacetamide: MS spectrum



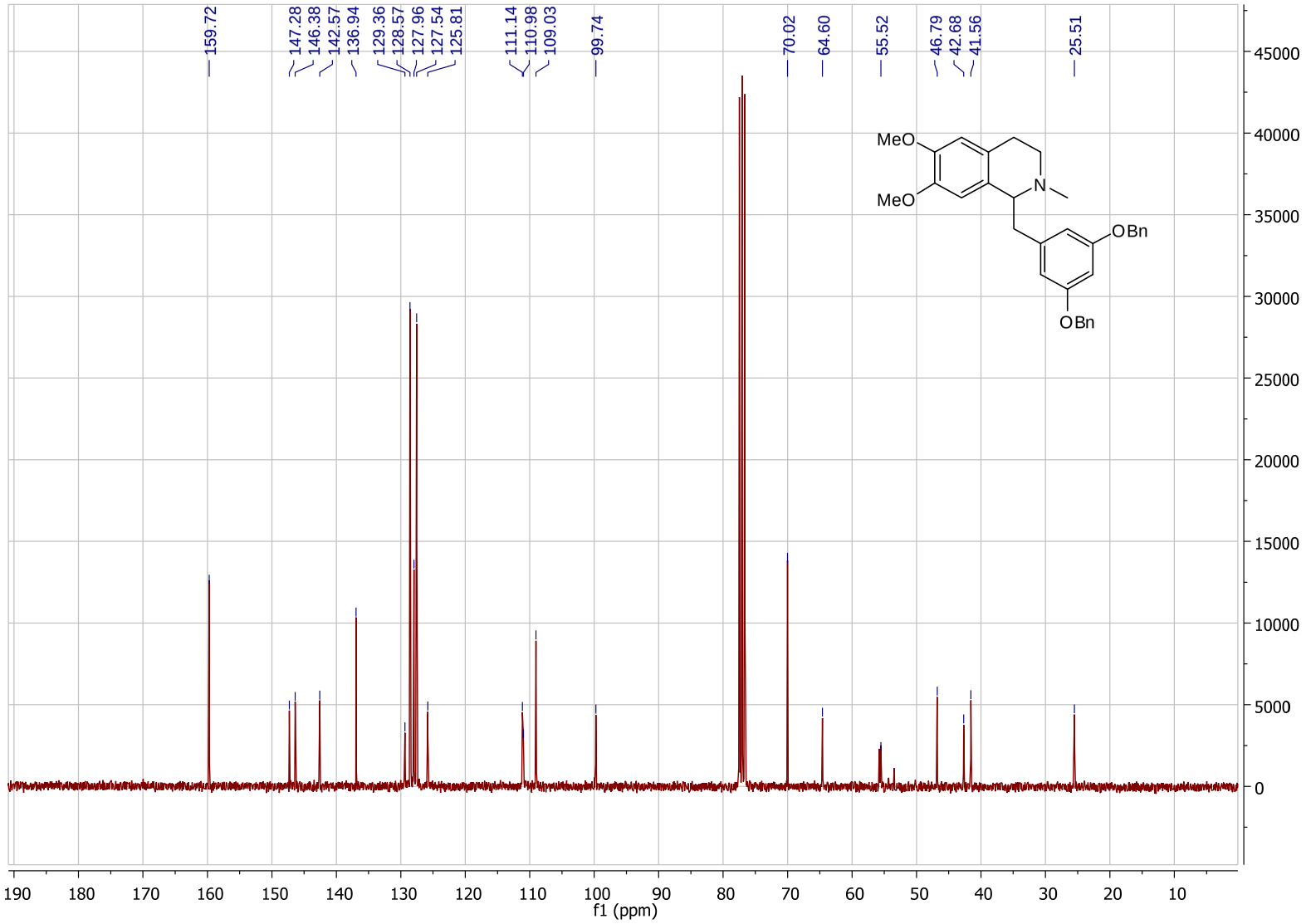
2-(3,5-Bisbenzyloxyphenyl)-*N*-(3,4-dimethoxyphenethyl)-*N*-methylacetamide: HRMS results



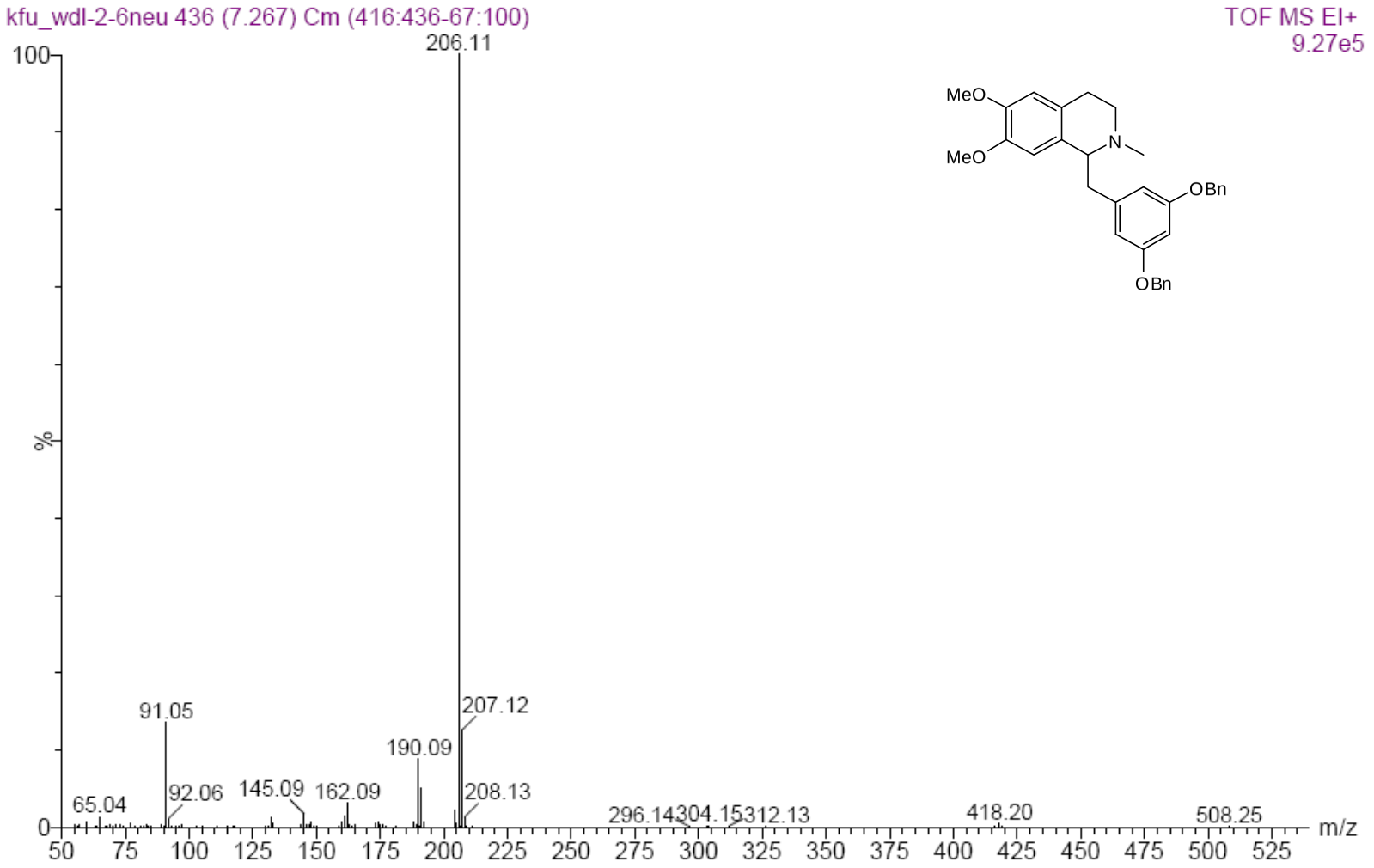
1-(3,5-Bisbenzyloxy)benzyl-6,7-dimethoxy-2-ethyl-1,2,3,4-tetrahydroisoquinoline: ^{1}H-NMR spectrum



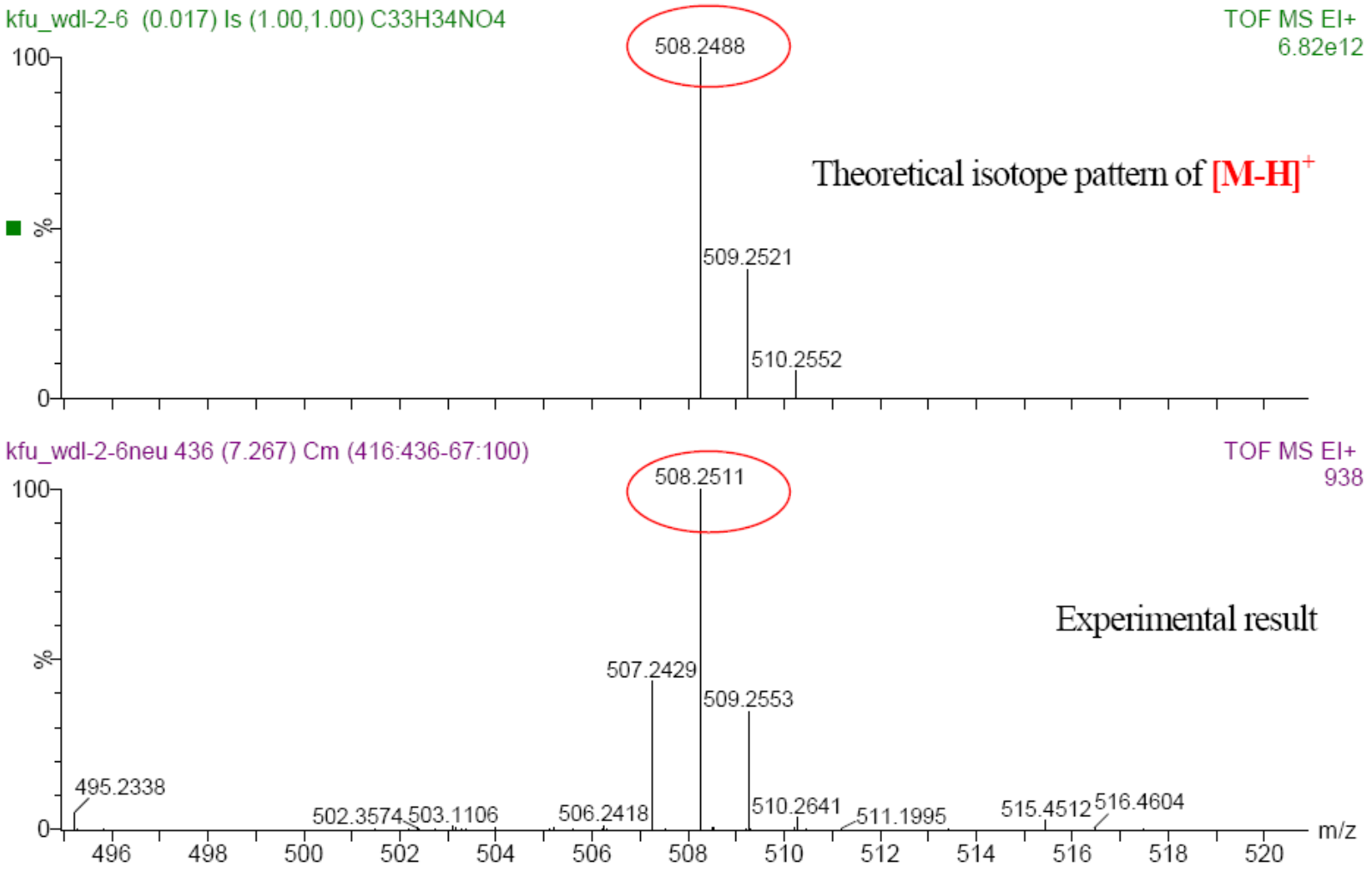
1-(3,5-Bisbenzyloxy)benzyl-6,7-dimethoxy-2-ethyl-1,2,3,4-tetrahydroisoquinoline: ^{13}C-NMR spectrum



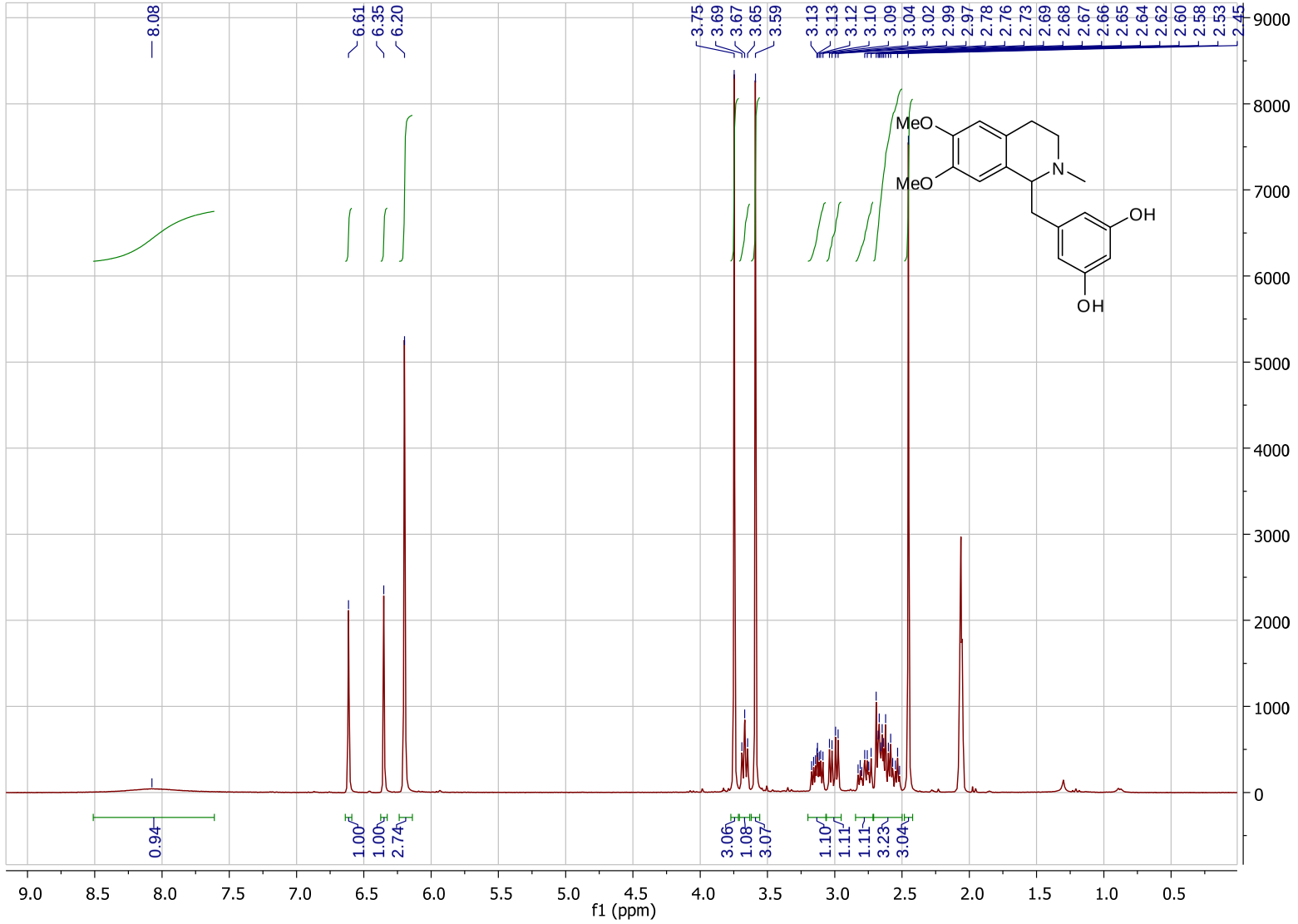
1-(3,5-Bisbenzyloxy)benzyl-6,7-dimethoxy-2-ethyl-1,2,3,4-tetrahydroisoquinoline: MS spectrum



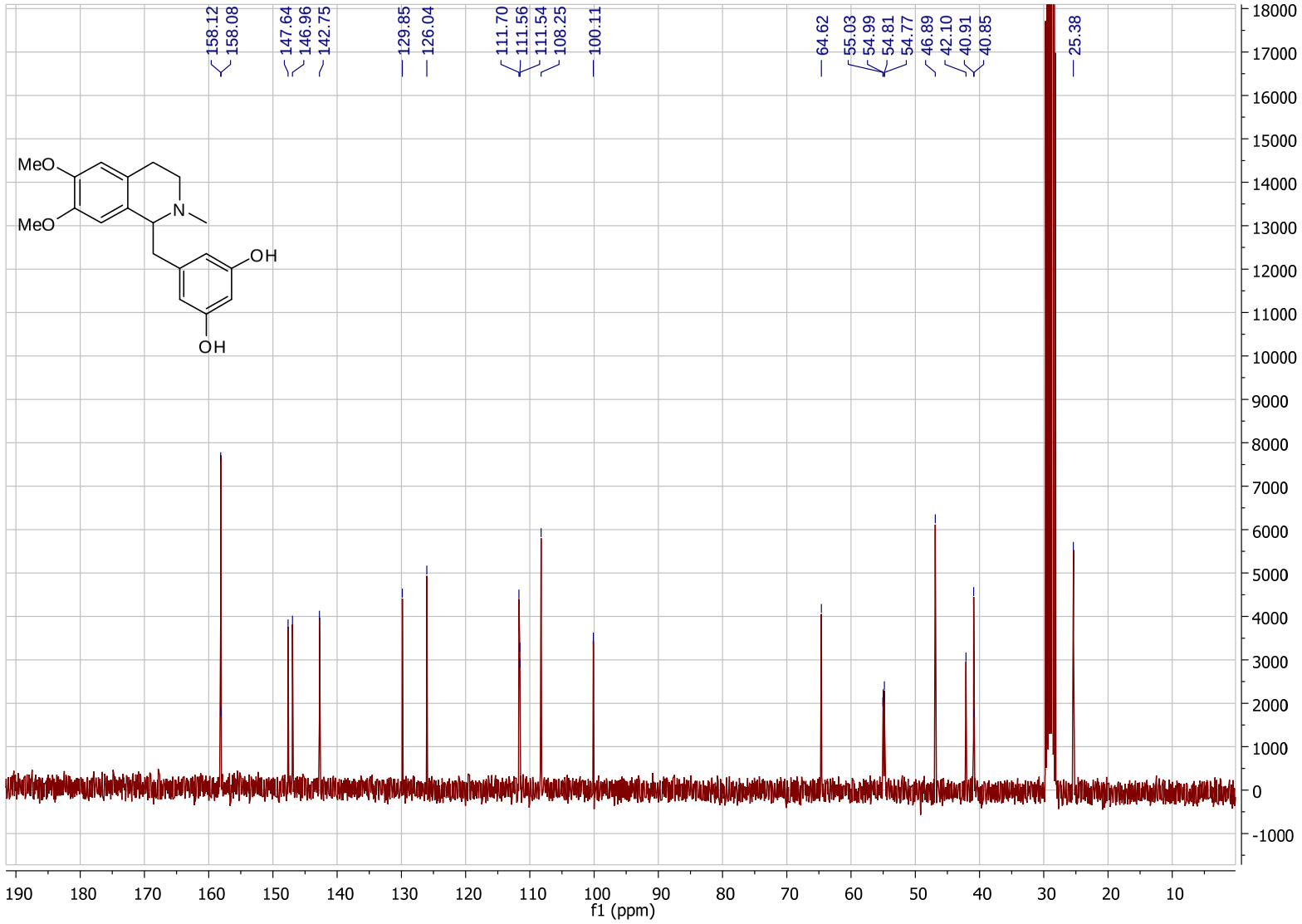
1-(3,5-Bisbenzyloxy)benzyl-6,7-dimethoxy-2-ethyl-1,2,3,4-tetrahydroisoquinoline: HRMS results



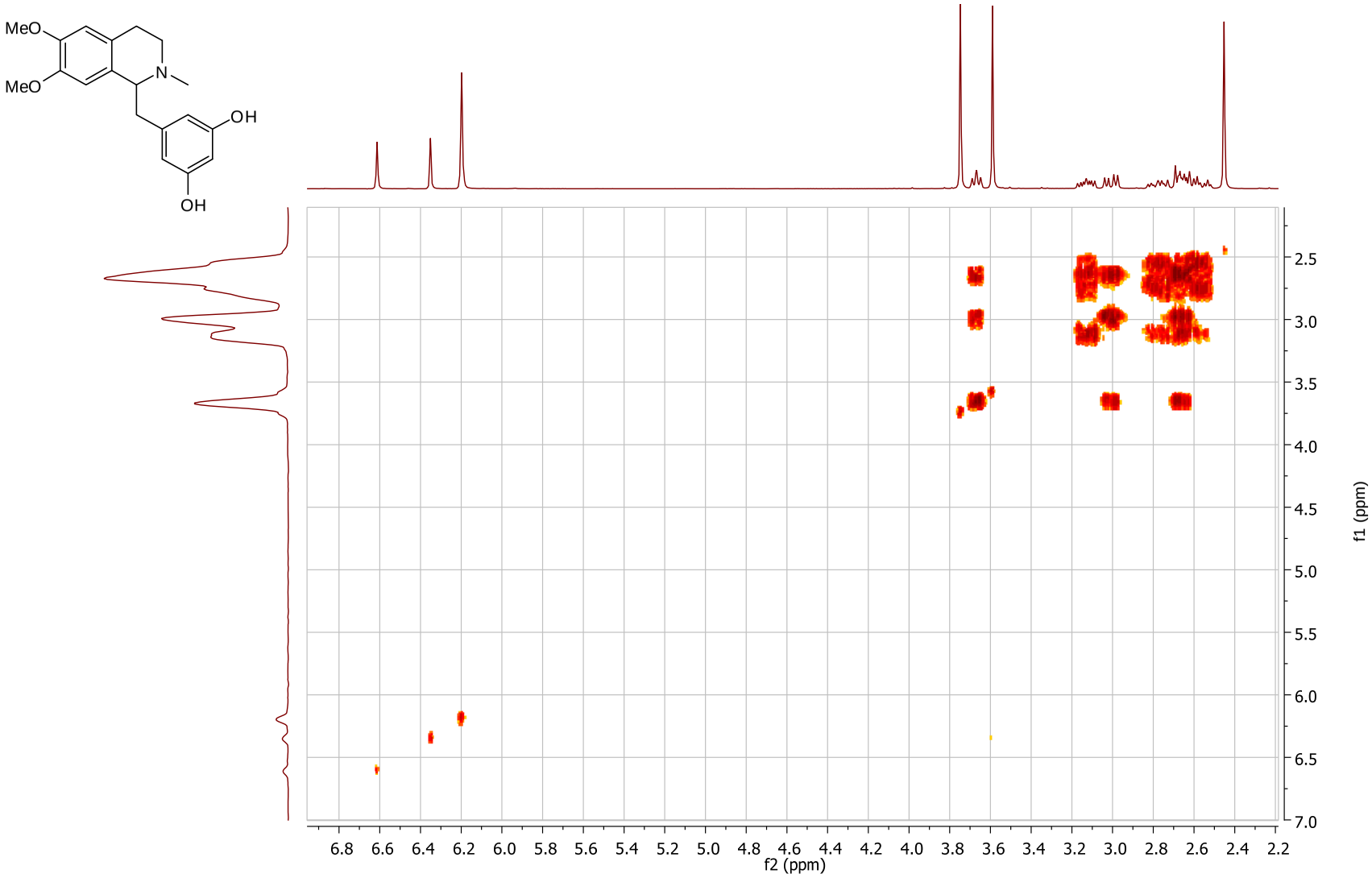
1-(3,5-Dihydroxybenzyl)-6,7-dimethoxy-2-ethyl-1,2,3,4-tetrahydroisoquinoline (2f): ^{1}H-NMR spectrum



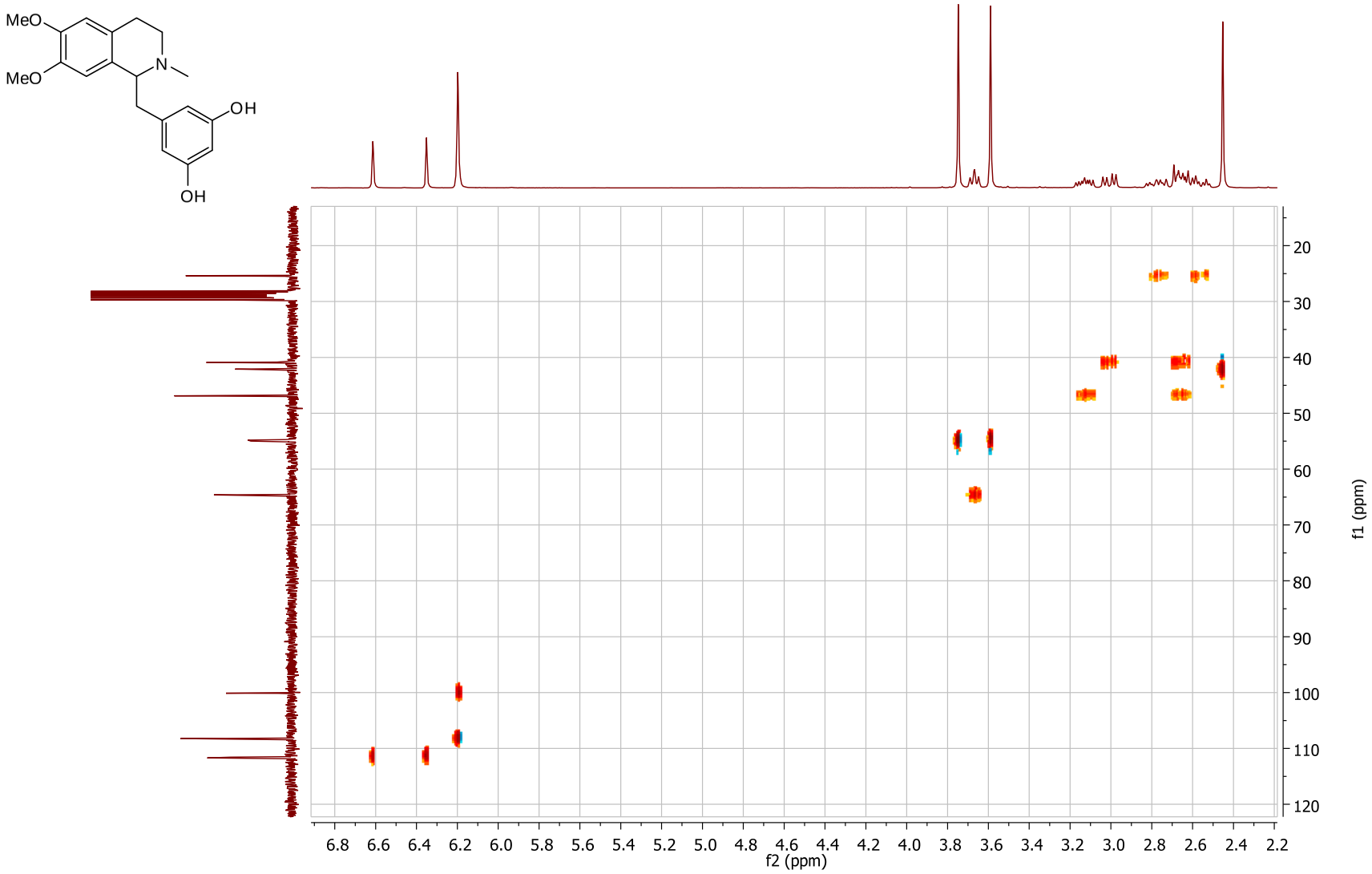
1-(3,5-Dihydroxybenzyl)-6,7-dimethoxy-2-ethyl-1,2,3,4-tetrahydroisoquinoline (2f): ^{13}C-NMR spectrum



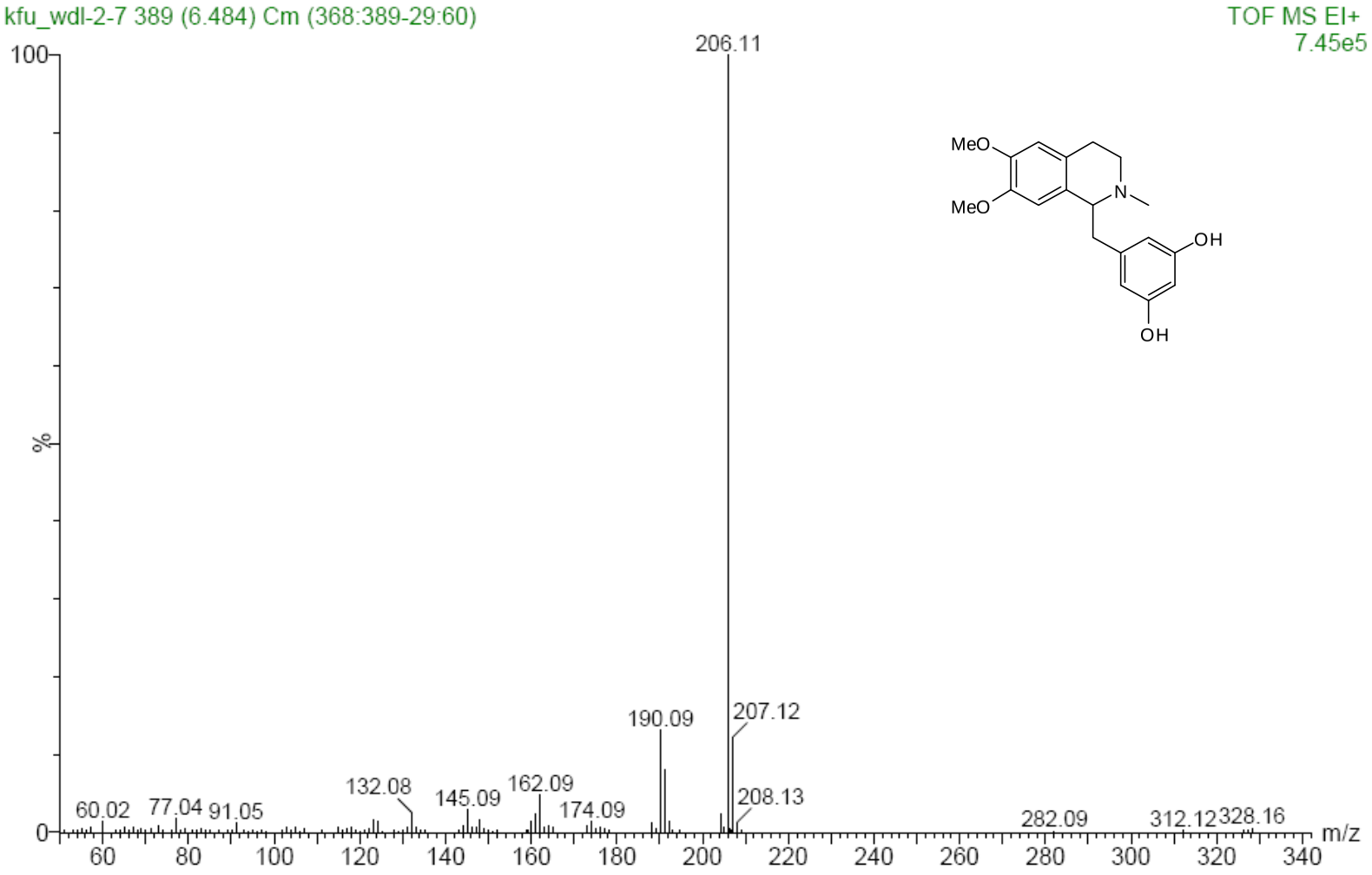
1-(3,5-Dihydroxybenzyl)-6,7-dimethoxy-2-ethyl-1,2,3,4-tetrahydroisoquinoline (2f): COSY spectrum



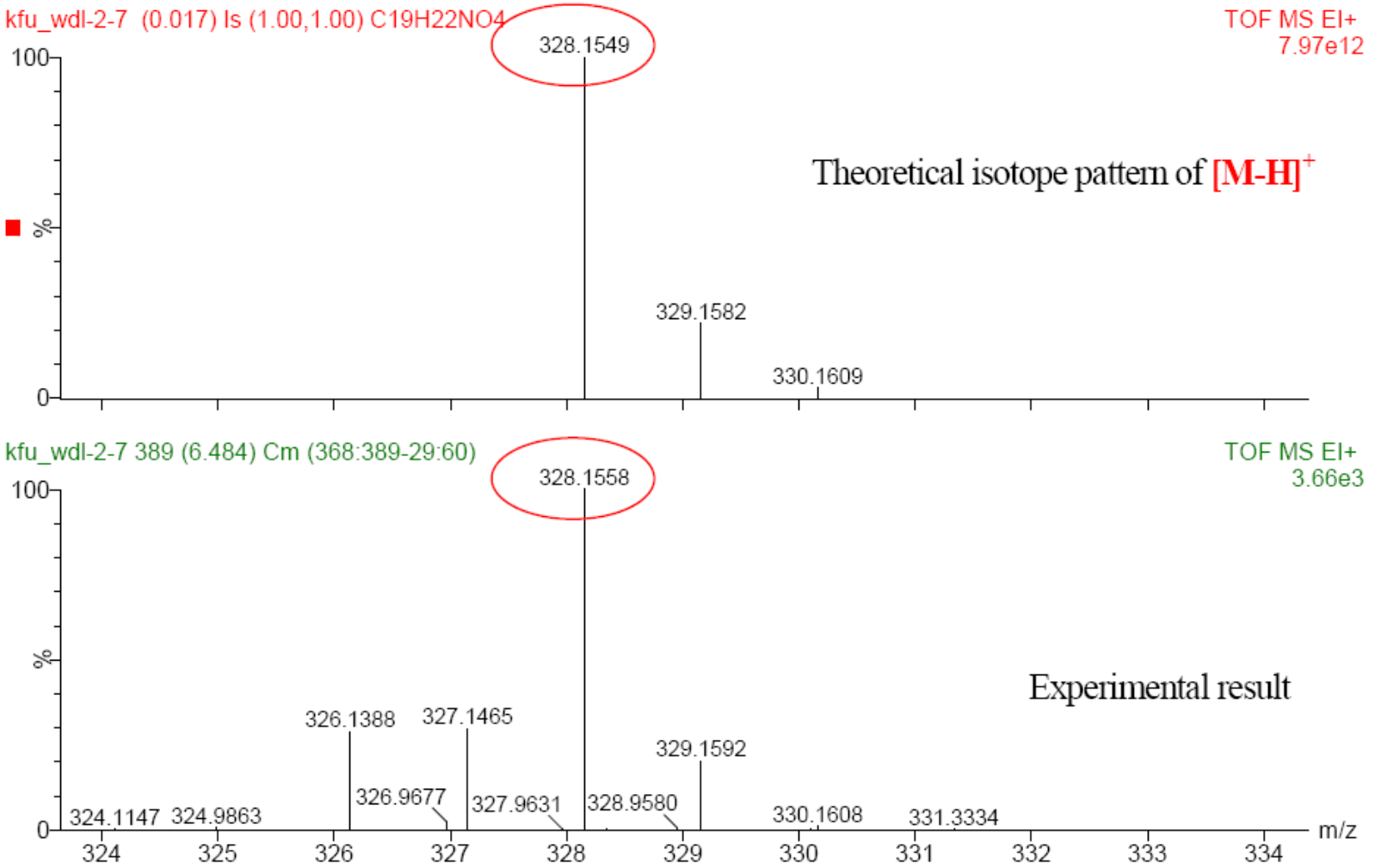
1-(3,5-Dihydroxybenzyl)-6,7-dimethoxy-2-ethyl-1,2,3,4-tetrahydroisoquinoline (2f): HSQC spectrum



1-(3,5-Dihydroxybenzyl)-6,7-dimethoxy-2-ethyl-1,2,3,4-tetrahydroisoquinoline (2f): MS spectrum



1-(3,5-Dihydroxybenzyl)-6,7-dimethoxy-2-ethyl-1,2,3,4-tetrahydroisoquinoline (2f): HRMS results



Products of Enzymatic Conversions

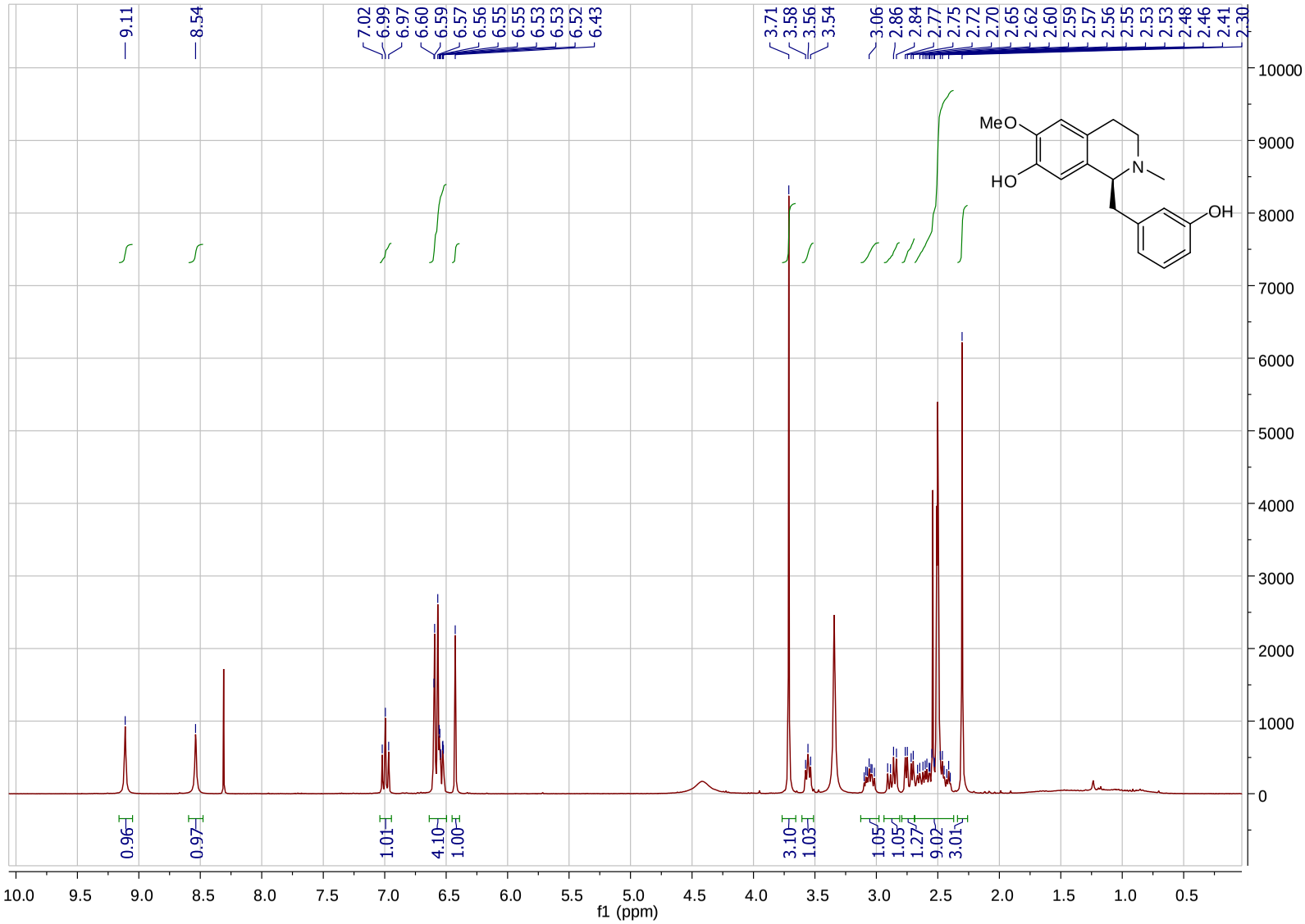
Provided Material:

(*S*)-1-(3-Hydroxybenzyl)-7-hydroxy-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (*S*)-3a:
^{1}H-NMR spectrum, ^{13}C-NMR spectrum, HSQC spectrum, MS spectrum, chiral-phase HPLC chromatogram

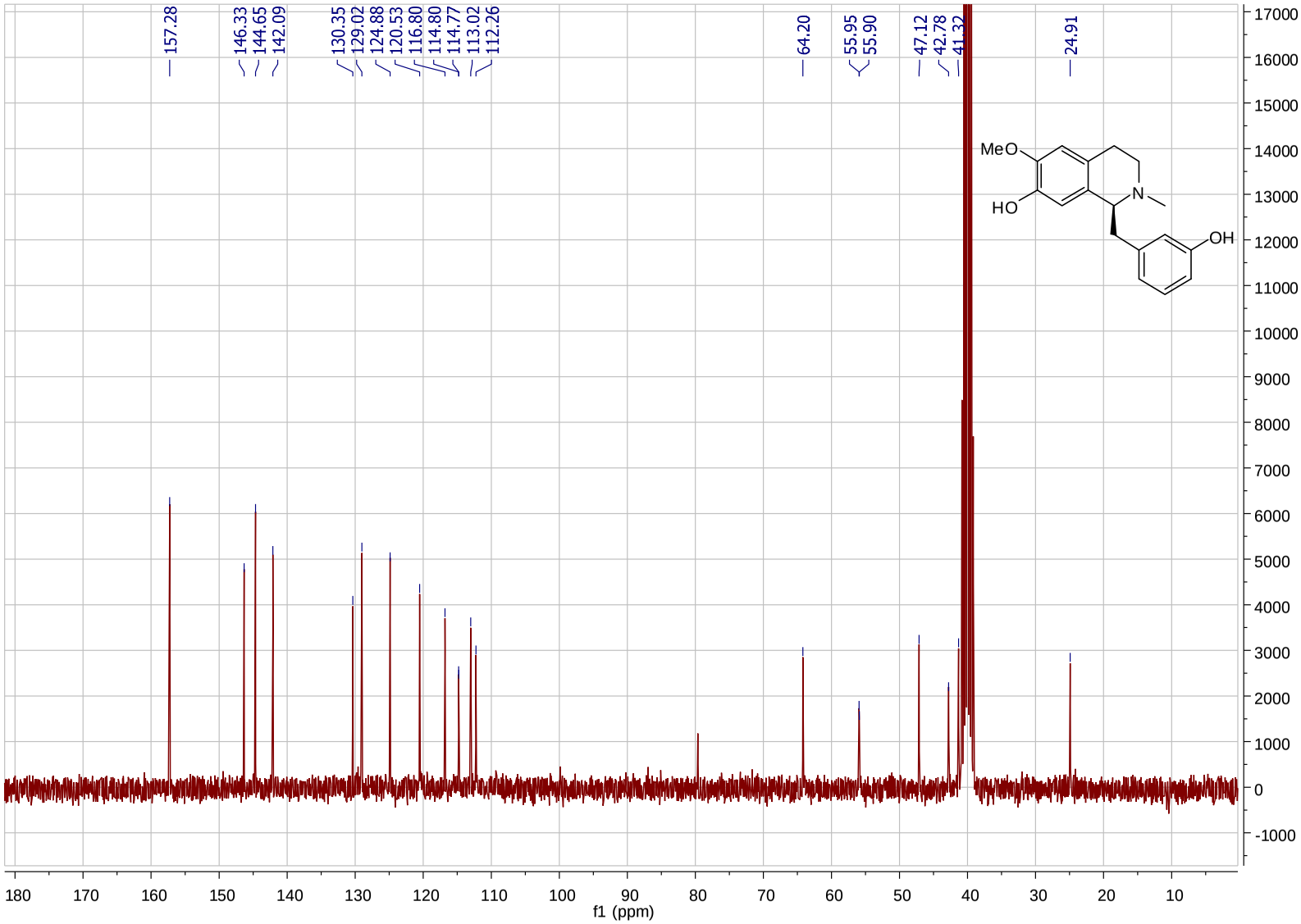
(*S*)-1-(3-Hydroxybenzyl)-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (*S*)-3c:
^{1}H-NMR spectrum, ^{13}C-NMR spectrum, HSQC spectrum, MS spectrum, chiral-phase HPLC chromatogram

(*S*)-1-(3-Hydroxy-4-methoxybenzyl)-7-hydroxy-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (*S*)-3f:
^{1}H-NMR spectrum, ^{13}C-NMR spectrum, HSQC spectrum, MS spectrum, chiral-phase HPLC chromatogram

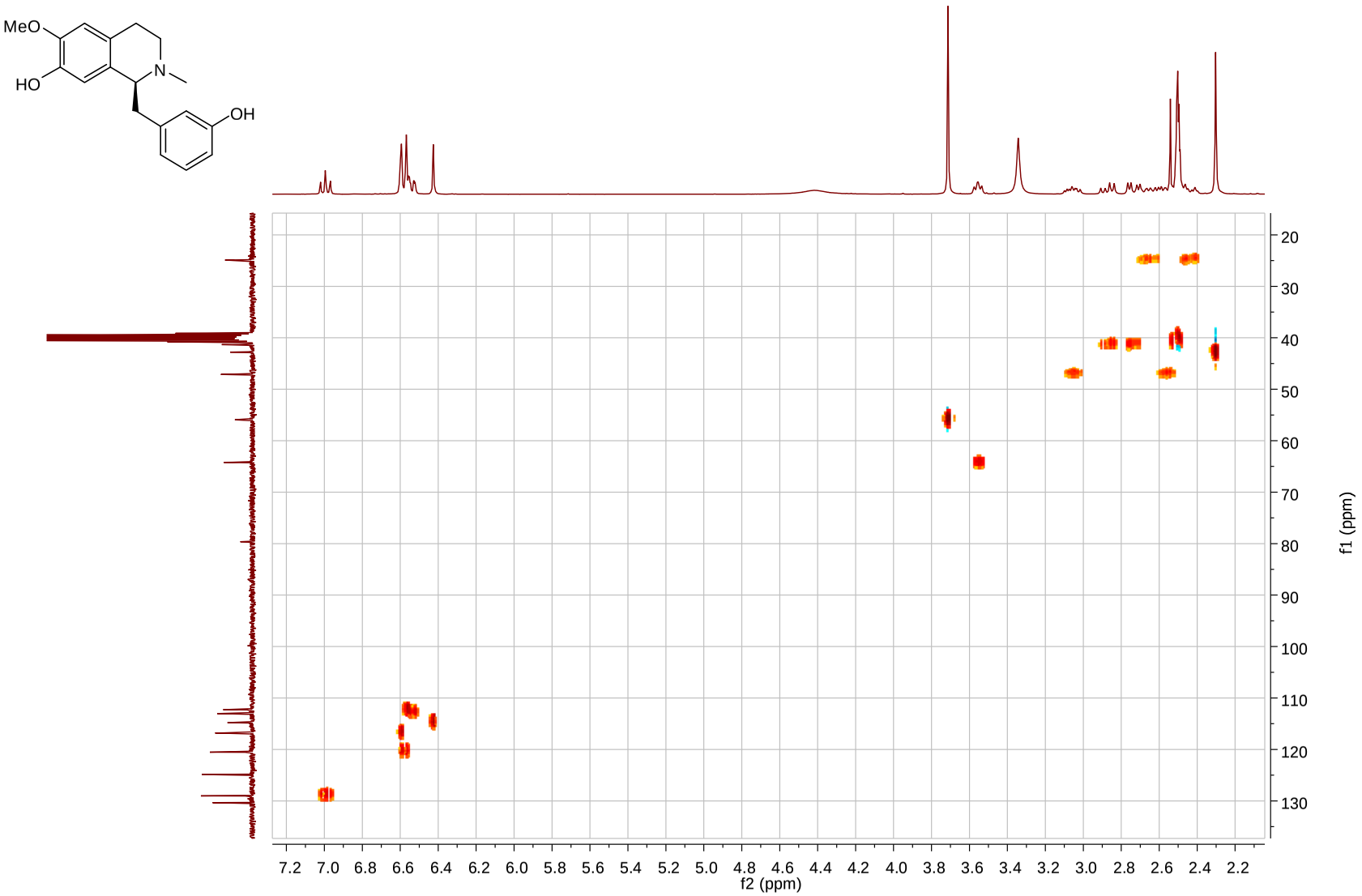
(*S*)-1-(3-Hydroxybenzyl)-7-hydroxy-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (*S*)-3a: ^{1}H-NMR spectrum



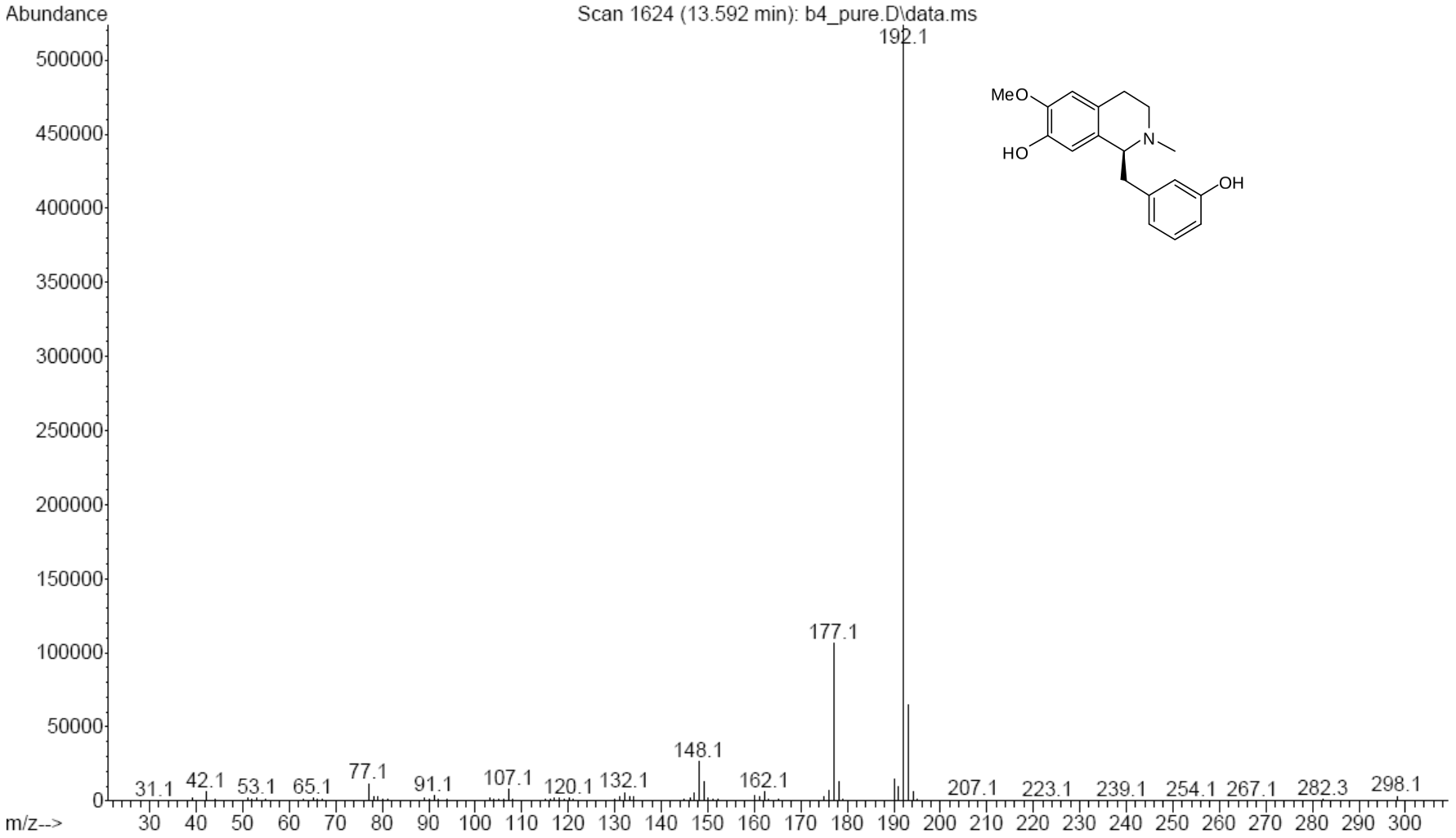
(*S*)-1-(3-Hydroxybenzyl)-7-hydroxy-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (*S*)-3a: ^{13}C-NMR spectrum



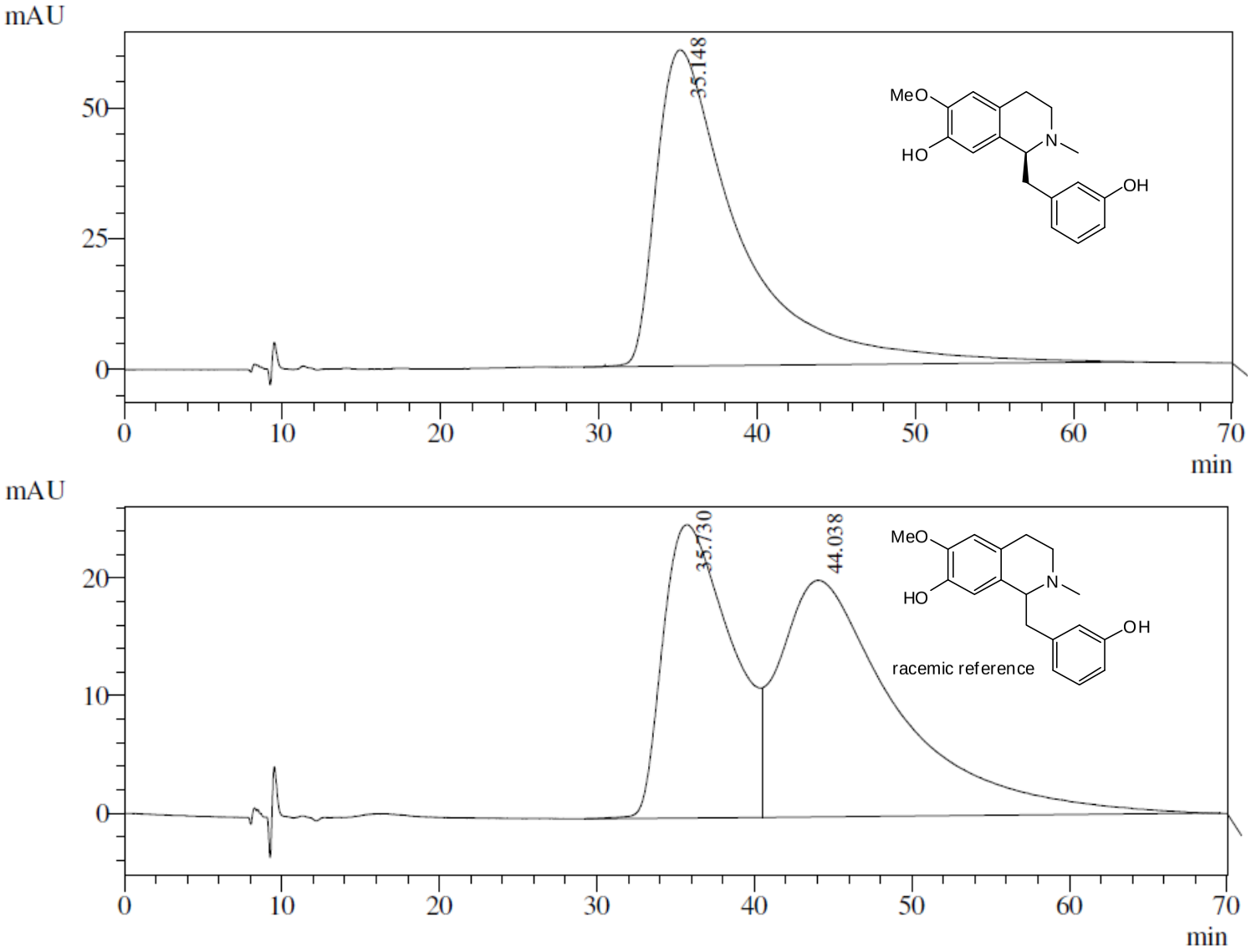
(*S*)-1-(3-Hydroxybenzyl)-7-hydroxy-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (*S*)-3a: HSQC spectrum



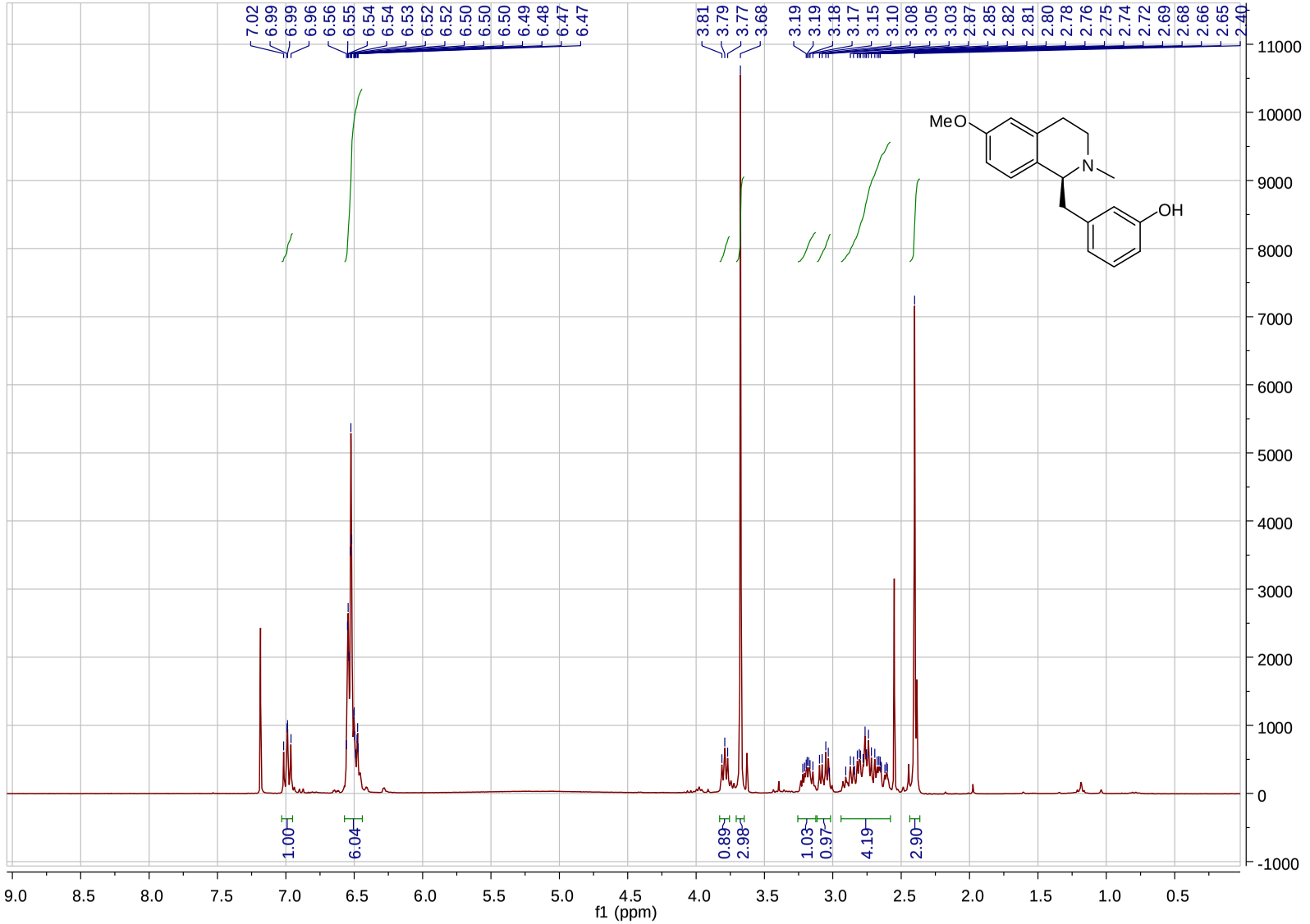
(*S*)-1-(3-Hydroxybenzyl)-7-hydroxy-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (*S*)-3a: MS spectrum



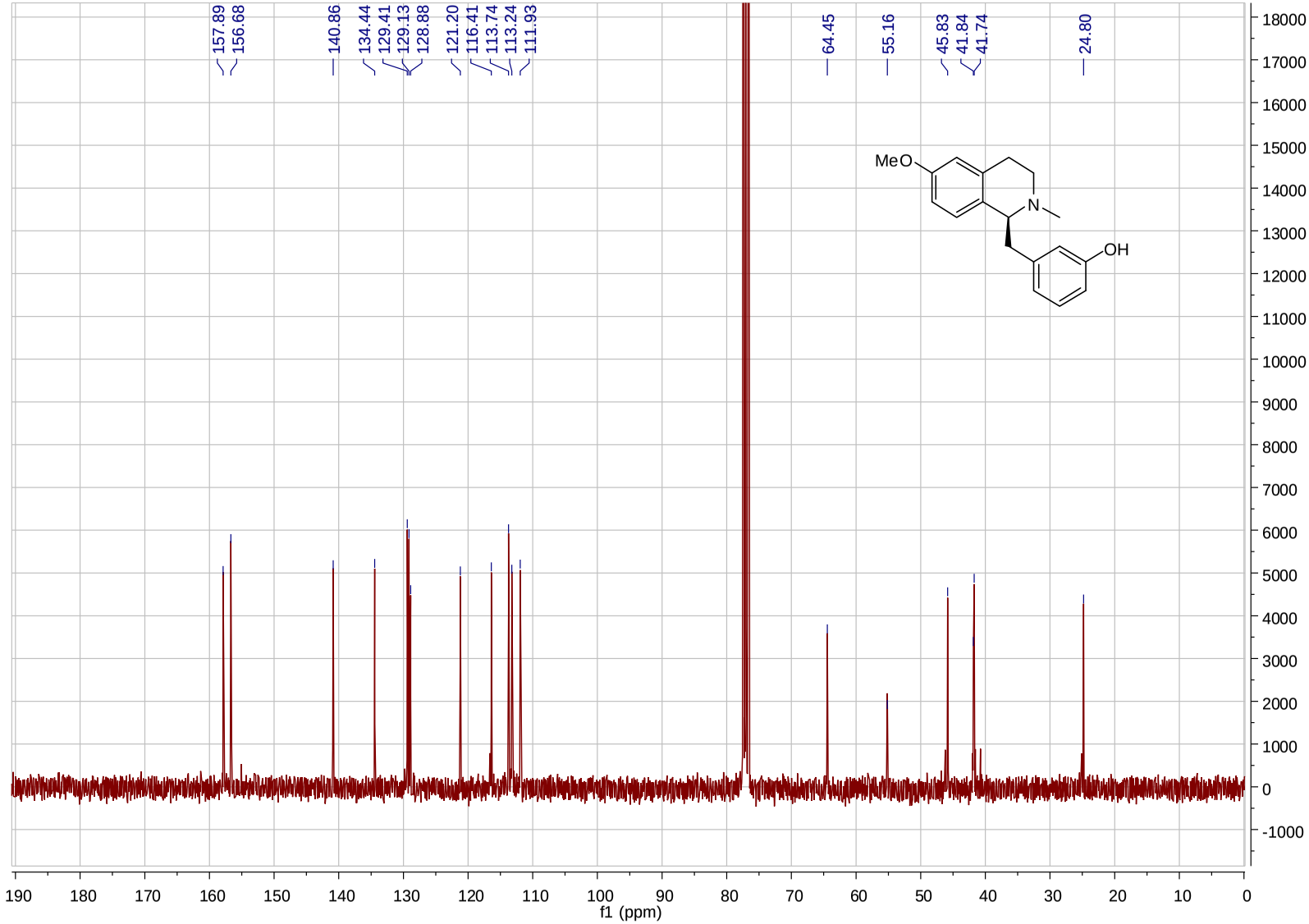
(*S*)-1-(3-Hydroxybenzyl)-7-hydroxy-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (*S*)-3a: Chiral-phase HPLC chromatogram



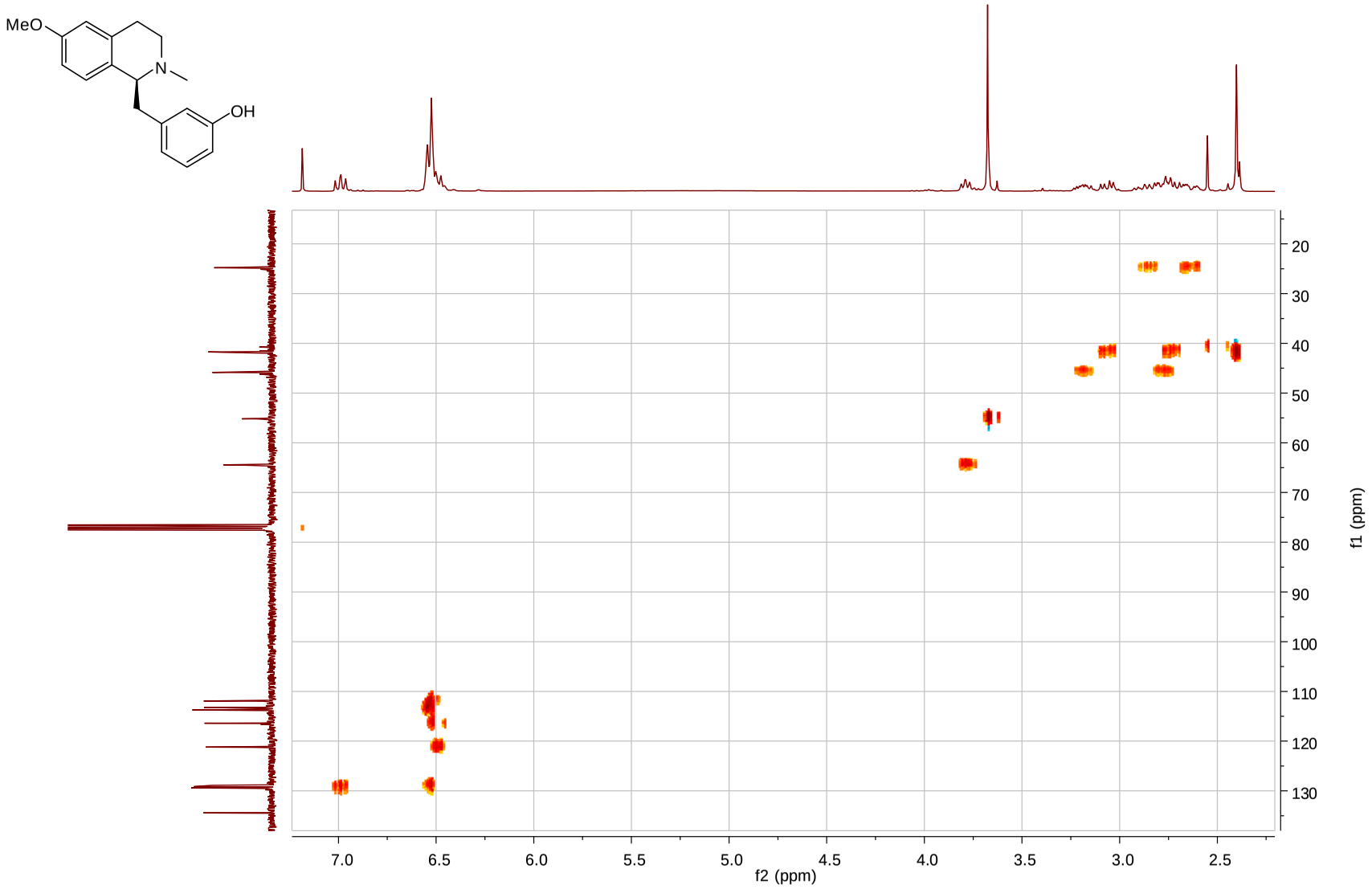
(*S*)-1-(3-Hydroxybenzyl)-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (*S*)-3c: ^{1}H-NMR spectrum



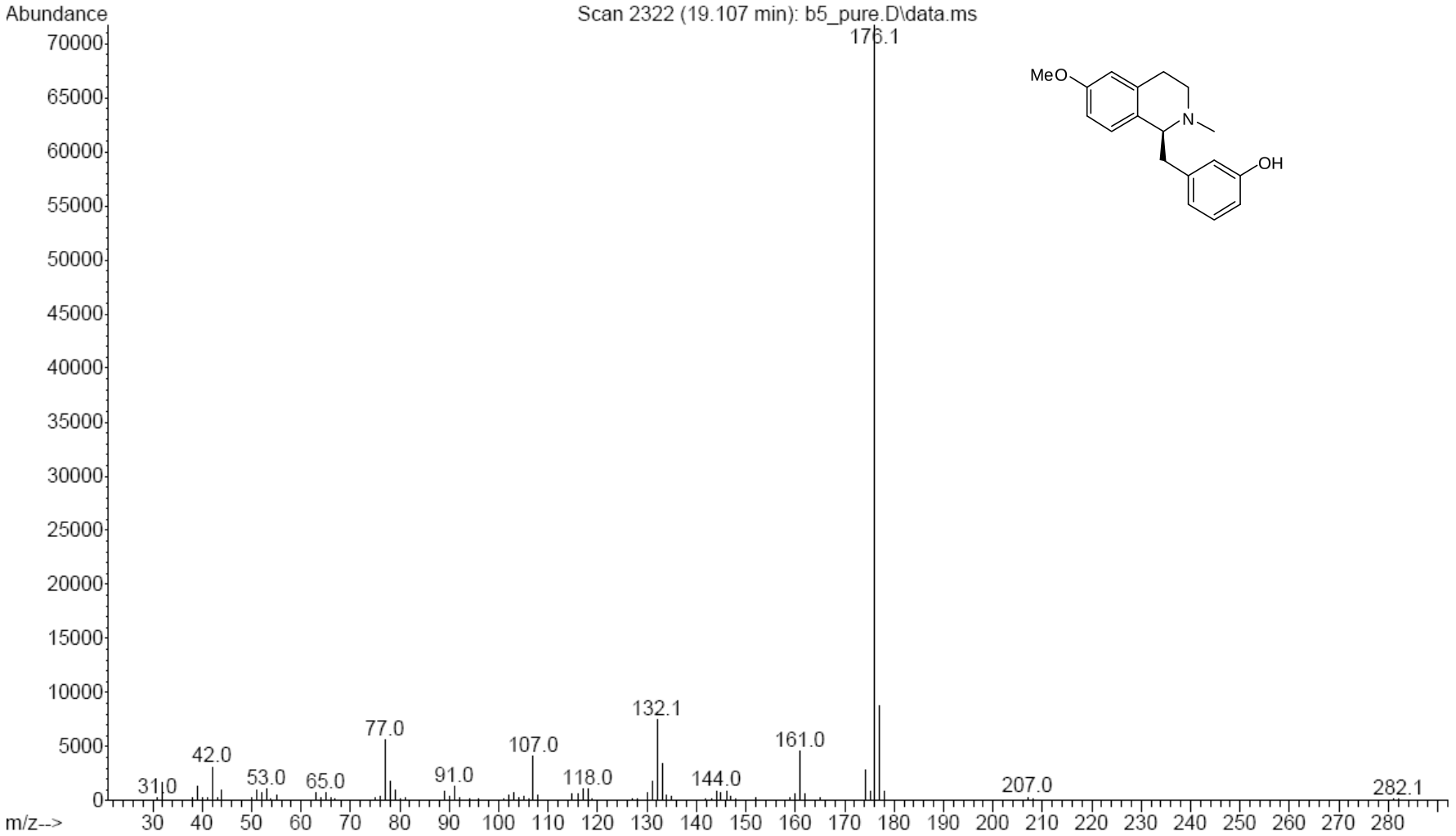
(*S*)-1-(3-Hydroxybenzyl)-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (*S*)-3c: ^{13}C-NMR spectrum



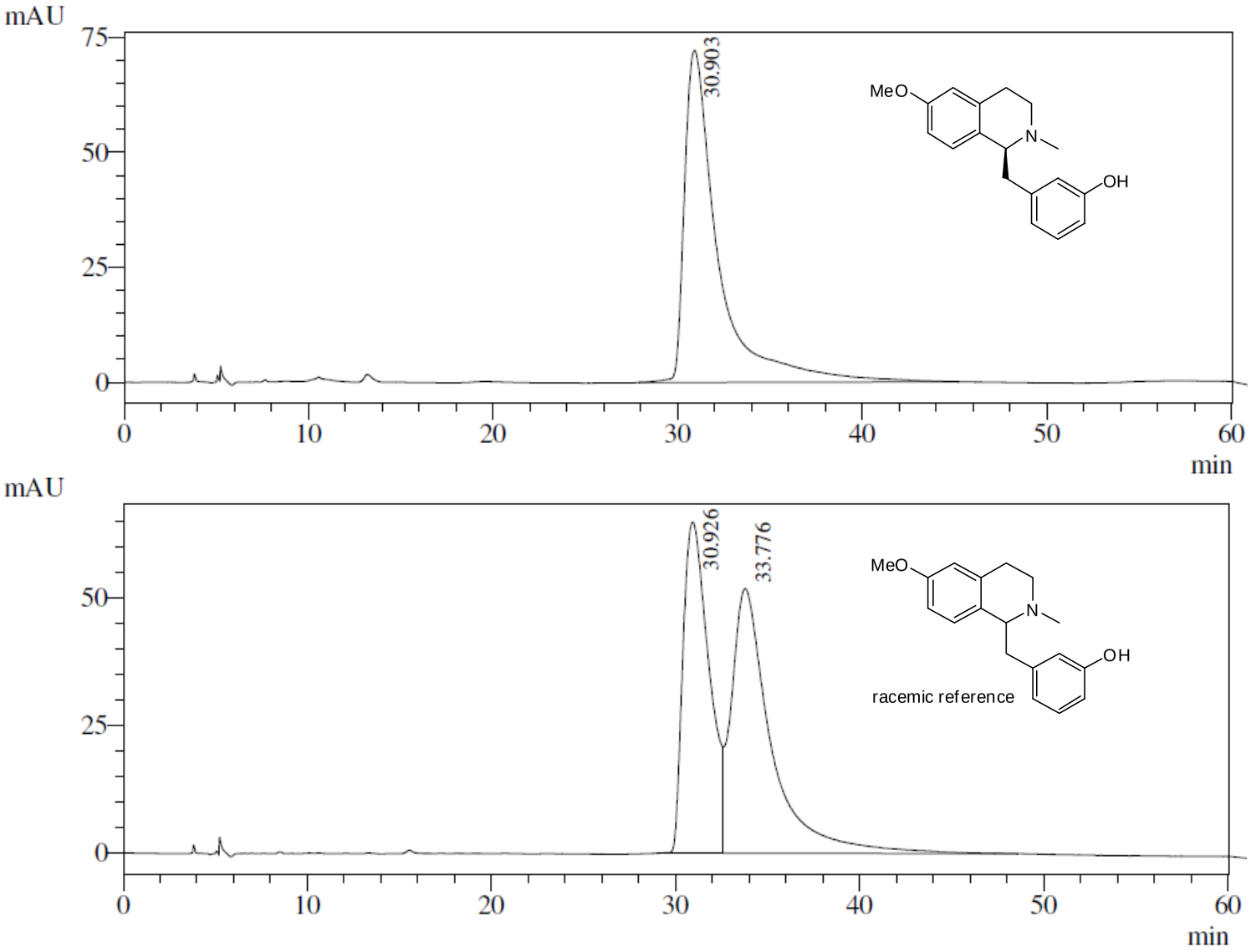
(*S*)-1-(3-Hydroxybenzyl)-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (*S*)-3c: HSQC spectrum



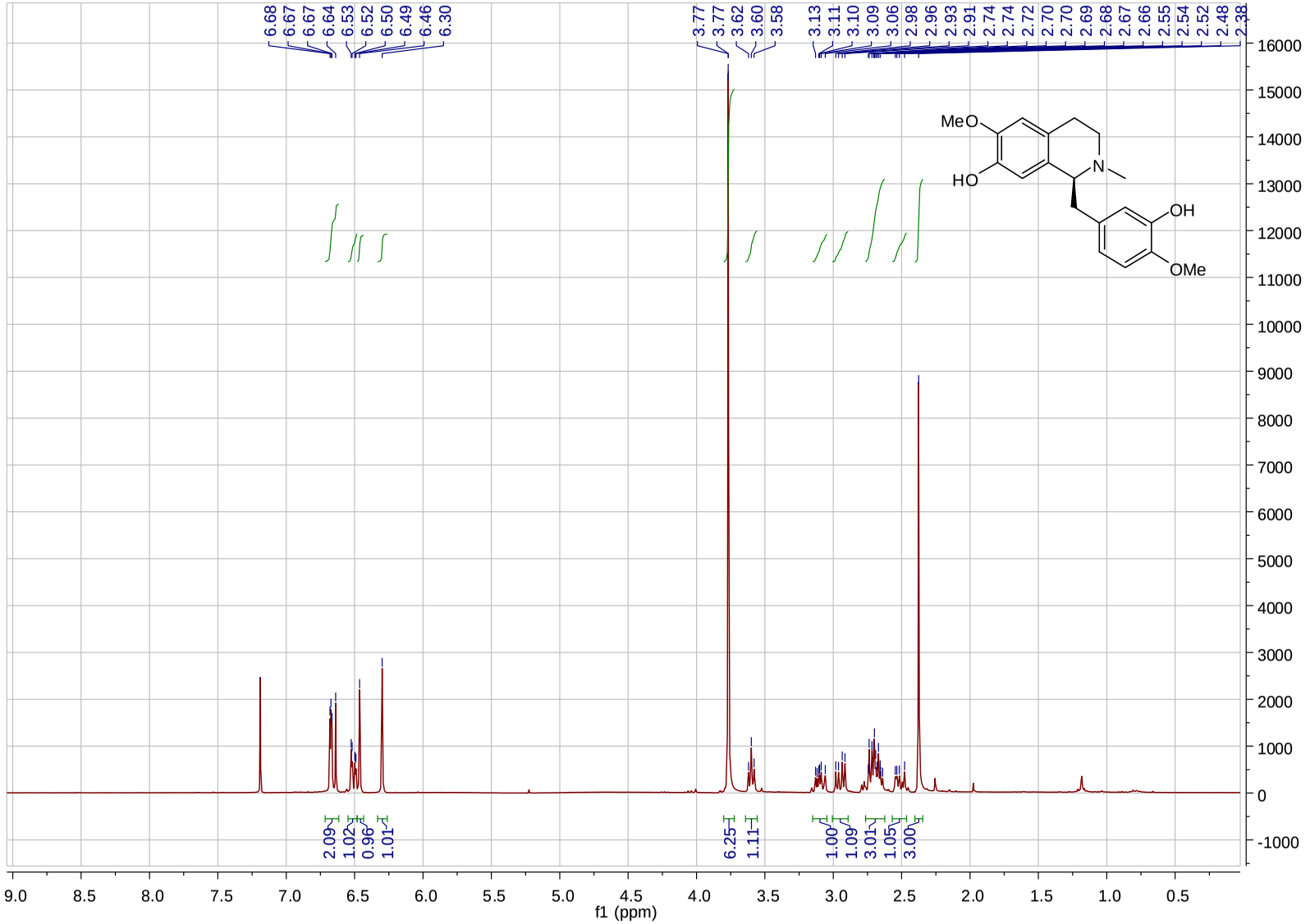
(*S*)-1-(3-Hydroxybenzyl)-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (*S*)-3c: MS spectrum



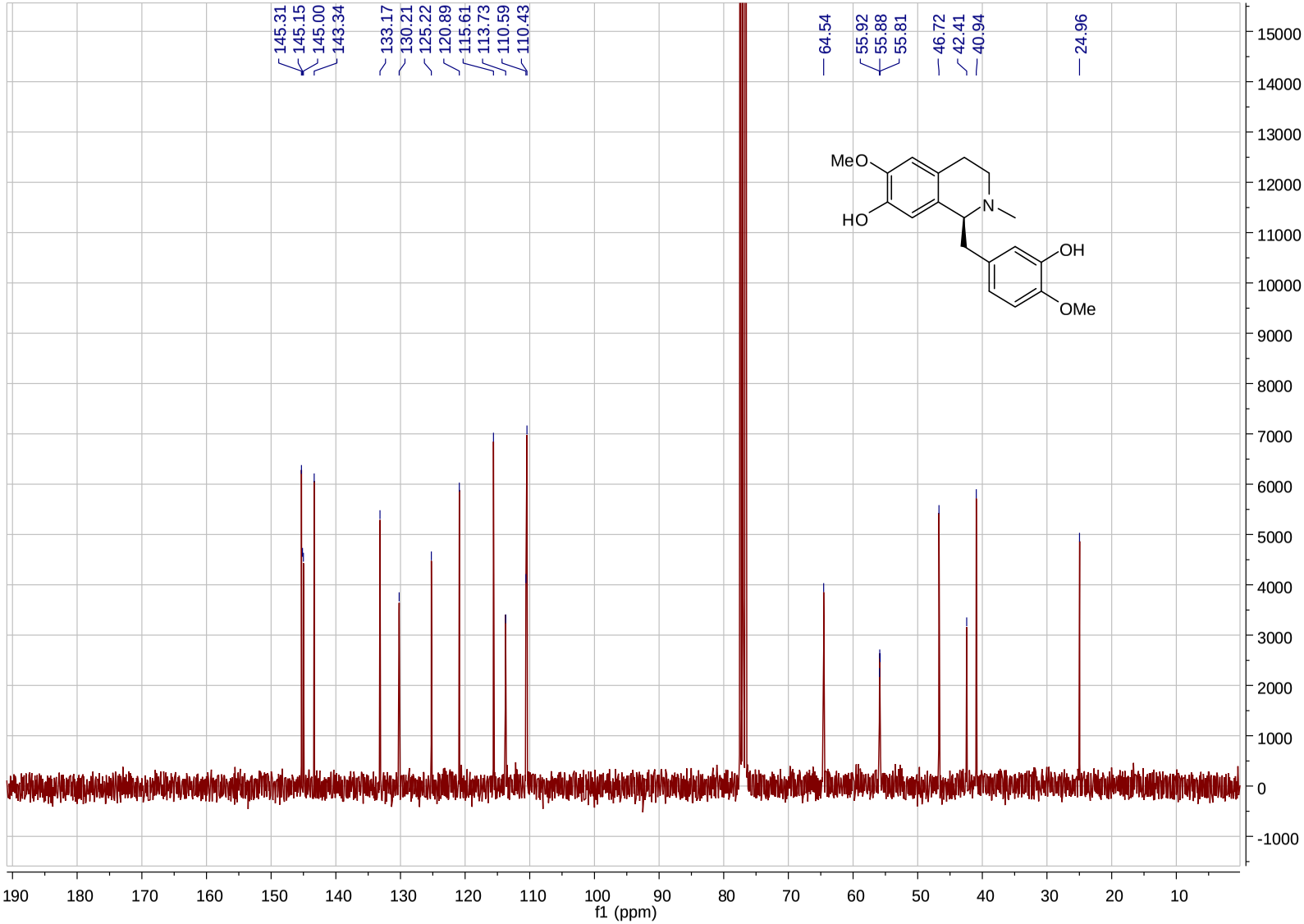
(*S*)-1-(3-Hydroxybenzyl)-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (*S*)-3c: Chiral-phase HPLC chromatogram



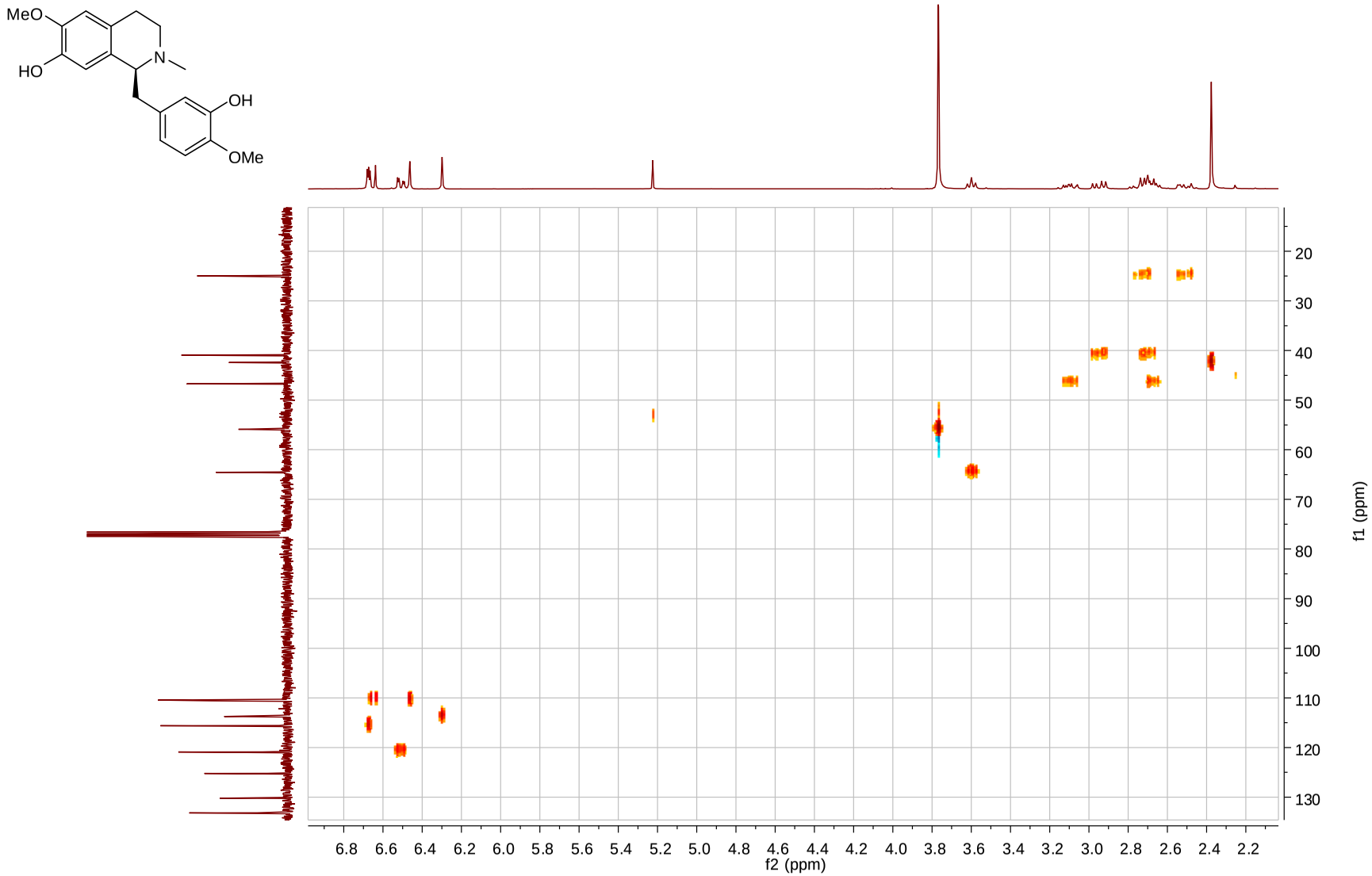
(*S*)-1-(3-Hydroxy-4-methoxybenzyl)-7-hydroxy-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (*S*)-3f: ^{1}H-NMR spectrum



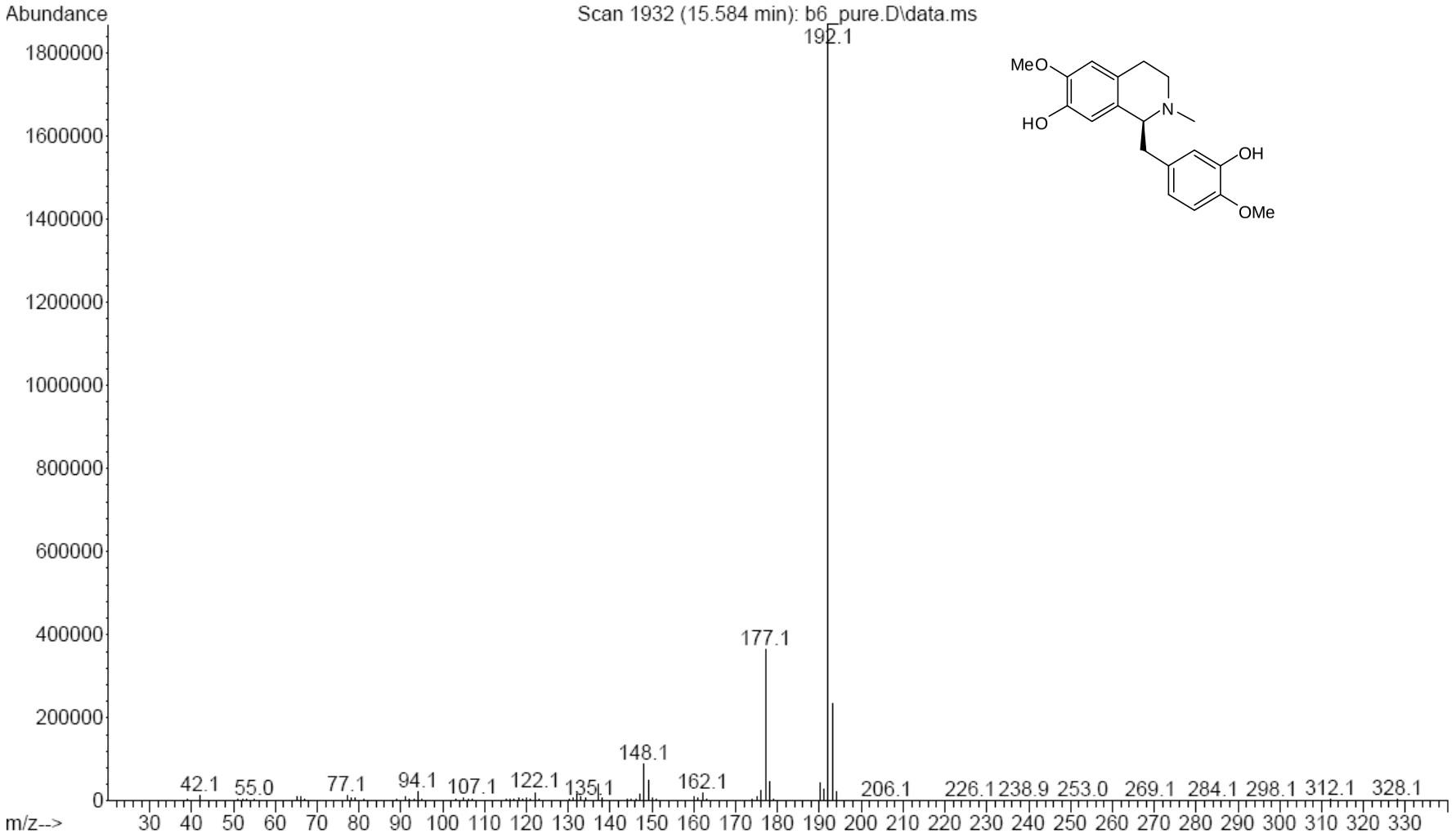
(*S*)-1-(3-Hydroxy-4-methoxybenzyl)-7-hydroxy-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (*S*)-3f: ^{13}C-NMR spectrum



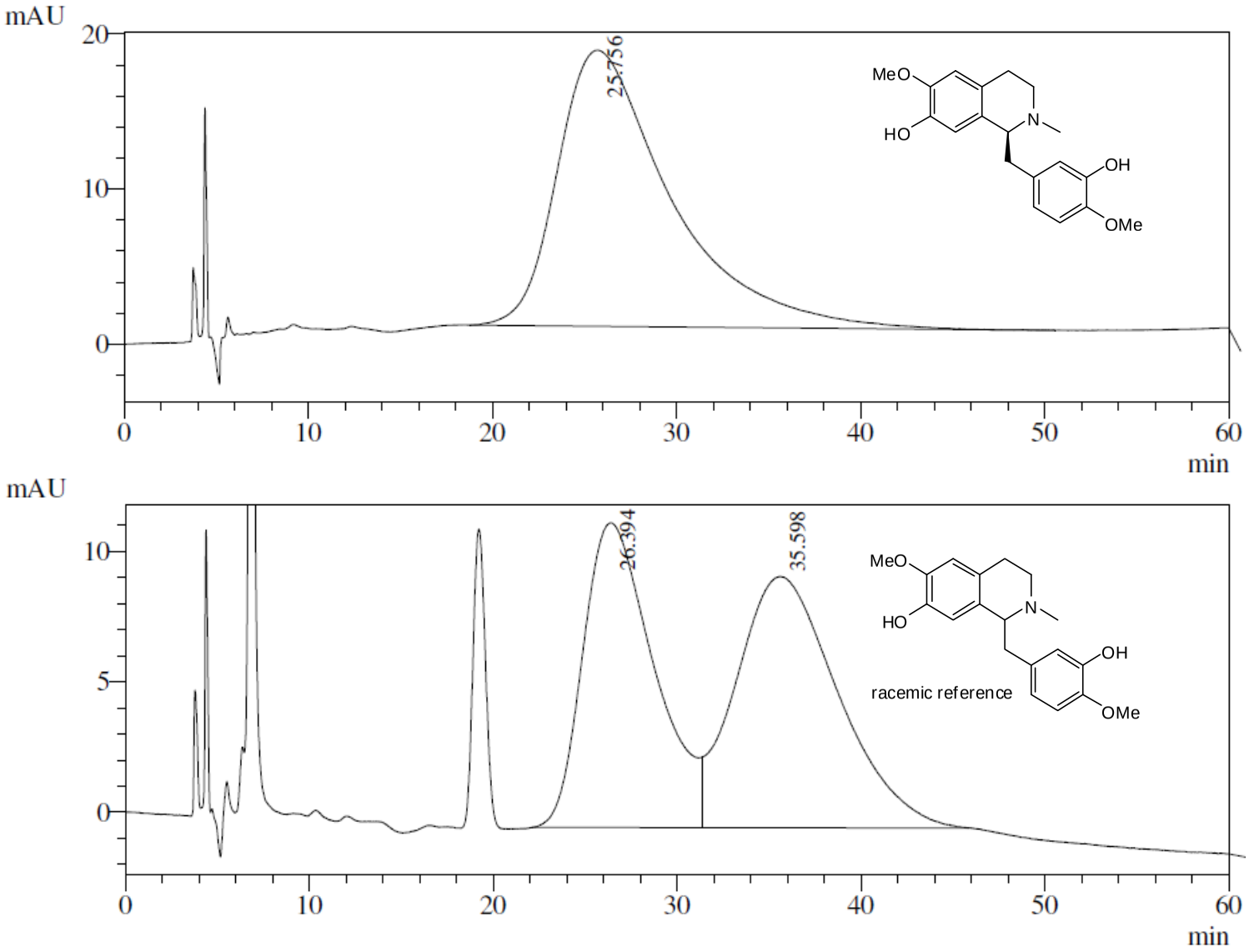
(*S*)-1-(3-Hydroxy-4-methoxybenzyl)-7-hydroxy-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (*S*)-3f: HSQC spectrum



(*S*)-1-(3-Hydroxy-4-methoxybenzyl)-7-hydroxy-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (*S*)-3f: MS spectrum



(*S*)-1-(3-Hydroxy-4-methoxybenzyl)-7-hydroxy-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (*S*)-3f:
Chiral-phase HPLC chromatogram



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