

Investigation of the 2,5-dimethoxy motif in phenethylamine Serotonin 2A receptor agonists

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Head Twitch Response data

Table S1. Summary of the head twitch response (HTR) data.

Compound	ANOVA	Time (min)	Dose (mg/kg)	N	HTR Counts (Mean \pm SEM)	ED ₅₀ mg/kg (95% CI)	ED ₅₀ μ mol/kg (95% CI)
2C-B ^a		30	0	6	11.8 \pm 2.8	0.72 (0.48-1.09)	2.43 (1.62-3.66)
			0.1	5	23.8 \pm 6.5		
			0.3	5	21.6 \pm 4.9		
			1	5	54.6 \pm 5.9**		
			3	5	79.2 \pm 9.3**		
			10	5	55.0 \pm 7.2**		
2H-2C-B	$F(5,24)=7.40$, $p=0.0003$	30	0	5	6.0 \pm 1.4	16.3 (8.5-31.1)	61.1 (32.0-116.5)
			1	5	7.8 \pm 0.9		
			3	5	6.8 \pm 1.7		
			10	5	8.0 \pm 1.1		
			30	5	29.4 \pm 6.9**		
			100	5	6.6 \pm 3.7		
5H-2C-B	$F(5,24)=3.73$, $p=0.0123$	30	0	5	9.2 \pm 2.5	<i>Not Determined</i>	<i>Not Determined</i>
			1	5	9.8 \pm 2.2		
			3	5	7.8 \pm 0.5		
			10	5	8.6 \pm 0.7		
			30	5	4.8 \pm 1.6		
			100	5	2.0 \pm 0.6*		
DOB ^a		30	0	7	9.1 \pm 1.3	0.23 (0.17-0.32)	0.75 (0.55-1.03)
			0.03	5	16.4 \pm 4.1		
			0.1	5	29.4 \pm 3.5		
			0.3	5	75.8 \pm 8.6**		
			1	6	118.8 \pm 15.4**		

			3	5	85.4 ± 8.2**		
2H-DOB	$F(5,24)=12.30,$ $p<0.0001$	30	0	5	9.4 ± 0.9	0.64 (0.42- 0.97)	2.26 (1.48-3.46)
			0.1	5	10.0 ± 2.5		
			0.3	5	16.0 ± 1.7		
			1	5	34.2 ± 1.6**		
			3	5	42.8 ± 7.3**		
			10	5	24.0 ± 4.8		
5H-DOB	$F(3,19)=7.19,$ $p=0.002$	30	0	5	8.6 ± 1.7	0.72 (0.35- 1.45)	2.55 (1.26-5.17)
			1	6	31.8 ± 3.8*		
			3	6	41.3 ± 7.3**		
			10	6	21.7 ± 5.0		

* $p<0.05$, ** $p<0.01$, significant difference from the vehicle control group (Tukey's test)

^aData previously reported⁴⁸

Experimental Procedures

General Information

Purchased chemicals were utilized without purification. Solvents were of chromatography grade or dried either by an SG Water solvent purification system (DCM, DMF, THF) or with 3Å molecular sieves (DMSO, toluene, MeCN, Et₂O, EtOH and MeOH). Anhydrous (anh.) reactions were run in flame- or oven-dried (150 °C) glassware under N₂ or argon. Purification by column chromatography and dry column vacuum chromatography (DCVC) was done following standard procedures using silica gel 60 (40-63 µm and 15-40 µm mesh, respectively).

Melting point

Melting points were measured for recrystallized compounds on a Stanford Research System OptiMelt capillary melting point apparatus with visual inspection and the values are reported in a range rounded to the nearest 0.5 °C.

HRMS

Performed by matrix-assisted laser ionisation time-of-flight mass spectrometry (MALDI-TOF) at Department of Pharmacy, University of Copenhagen. Analysis was performed in positive ion mode with MALDI ionization on a Thermo QExactive Orbitrap mass spectrometer (Thermo Scientific, Bremen, Germany) equipped with an AP-SMALDI 10 ion source (TransmitMIT, Giessen, Germany) and operated with mass resolving power 140,000 at m/z 200. 2,5-Dihydroxybenzoic acid was used as matrix and lock-mass for internal mass calibration, providing a mass accuracy of 3 ppm or better. Samples were prepared using 2,5-dihydroxybenzoic acid as the matrix.

High Performance Liquid Chromatography (HPLC)

HPLC retention times (t_R) are reported in minutes (min) and recorded on a Thermo Scientific Dionex 3000 UltiMate instrument connected to a Thermo Scientific Dionex 3000 Diode Array Detector by a Gemini-NX 3 µm C18 110A (250×4.6 mm) column with UV detection at 205 and 280 nm. MP A: 0.1% TFA in H₂O (v/v). MP B: 0.1% TFA, 10% H₂O in MeCN (v/v/v). Flow rate: 1.0 mL/min. Gradient: 0-30 min: 0-100% MP B.

Nuclear Magnetic Resonance Spectroscopy (NMR)

NMR experiments were performed on a 400 or 600 MHz Bruker instrument. The obtained spectra were analysed using MestReNova 11.0 software typically using Whittaker smoother baseline correction. Chemical shifts are reported in ppm (δ) with reference to the deuterated solvent used. Coupling constants (J) are reported in Hertz (Hz). Multiplet patterns are designated the following abbreviations or combinations thereof: br (broad), m (multiplet), s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), sex (sextet) and hep (heptet).

General Synthetic Procedures (GP)

GP1: Henry reaction

To a stirred solution of benzaldehyde (1 eq.) in Isopropanol (IPA) (1M), heated to 75°C, was added Nitromethan or Nitroethane (2 eq.) followed by Acetic acid (0.55 eq.) and ethylene diamine (0.1 eq). The reaction mixture was stirred at 75°C for 1h then allowed to cool to ambient temp. before being cooled to 4°C overnight. The resulting solids were isolated by filtration and washed with cold IPA giving the desired nitrostyrene as a tanned amorphous solid. The product was used in subsequent reduction without further purification

GP2A: Diisobutylaluminiumhydride reduction

To a stirred solution of Diisobutylaluminiumhydride in anhydrous THF (0.5M, 7 eq.) under argon flow, was added nitrostyrene in anhydrous THF (0.6M). The reaction mixture was brought to reflux for 2h then cooled to 0°C before addition of diethylether (Et₂O) (100 ml). The reaction was subsequently quenched by addition of H₂O (1.7 ml), 15% aqueous NaOH solution (4 ml) then additional H₂O (4 ml) followed by 15% aqueous NaOH solution (5 ml). An additional 50 ml of Et₂O was added to facilitate stirring of the suspension. After 20 minutes 30 g of anhydrous Na₂SO₄ was added and the stirring continued for an additional 20 minutes. The suspension was filtered and concentrated *invacou*. The crude freebase was dissolved in MeOH (5 ml) and treated with a stoichiometric amount of 4M HCl in dioxane. The volatiles were evaporated and the crude solid recrystallized from boiling acetone and isolated by filtration, giving the desired primary amine hydrochloride, as a white amorphous solid.

GP2B: Diisobutylaluminiumhydride reduction

To a stirred solution of Diisobutylaluminiumhydride in anhydrous THF (0.5M, 7 eq.) under argon flow, was added nitrostyrene in anhydrous THF (0.6M). The reaction mixture was brought to reflux for 2h then cooled to -78°C and quenched by addition of H₂O (2 ml) then neutralized with 2M NaOH aqueous solution. Ethylacetate (EtOAc)(40 ml) was added followed by saturated aqueous Potassium sodium tartrate solution (50 ml) and brine (20 ml) giving a thick slurry which was stirred overnight eventually giving two separate phases. Phases were separated and the aqueous phase further extracted with EtOAc (2x 40ml). Combined organic phases were washed with H₂O (40 ml) and Brine (40 ml) and dried over MgSO₄. Filtration followed by concentration *invacou* gave the crude product as an amorphous solid. Major impurities were removed by flash column chromatography [MeOH in DCM+25% Ammonia solution (2:98+0.4%)] then [MeOH in DCM+25% Ammonia solution (1:9+0.4%)]. The pure freebase was treated with stoichiometric amounts of 4M dioxane HCl, generating the desired hydrochloride which was recrystallized from boiling IPA and Et₂O.

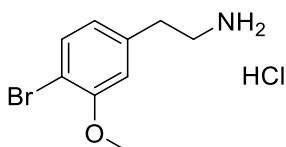
2-(4-bromo-2,5-dimethoxyphenyl)ethan-1-amine hydrochloride(1)

Synthesized as previously reported²⁶. Analytical data consistent with reported literature values.

1-(4-bromo-2,5-dimethoxyphenyl)propan-2-amine hydrochloride(2)

Synthesized as previously reported⁴². Analytical data consistent with reported literature values.

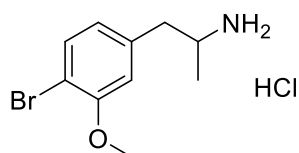
2-(4-bromo-3-methoxyphenyl)ethan-1-amine hydrochloride (3)



Synthesized from nitrostyrene **20** using GP2A. The desired hydrochloride was isolated as a white solid (205 mg, 55%)

MP: 173-174°C; $^1\text{H NMR}$ (600 MHz, MeOD) δ 7.49 (d, $J = 8.0$ Hz, 1H), 6.98 (d, $J = 1.9$ Hz, 1H), 6.79 (dd, $J = 8.0, 1.9$ Hz, 1H), 3.90 (s, 3H), 3.20 (t, $J = 7.7$ Hz, 2H), 2.95 (t, $J = 7.7$ Hz, 2H); $^{13}\text{C NMR}$ (151 MHz, MeOD) δ 156.31, 137.61, 133.18, 121.70, 112.51, 109.88, 55.31, 40.26, 32.91; **HRMS** m/z calculated for $[\text{C}_9\text{H}_{12}\text{BrNO}]^+$ ($M_{\text{free base}} + \text{H}$) 229.0102, found 214.9892 [$M_{\text{free base}} + \text{H} - \text{CH}_3$]; **HPLC** $t_R = 16.39$ min

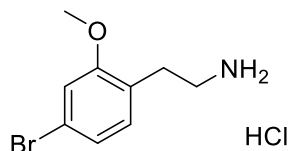
1-(4-bromo-3-methoxyphenyl)propan-2-amine hydrochloride (4)



Synthesized from nitrostyrene **21** using GP2A. The desired hydrochloride was isolated as a white amorphous solid (299 mg, 71%).

MP: 151-152°C; $^1\text{H NMR}$ (600 MHz, $\text{DMSO}-d_6$) δ 7.97 (s, 3H), 7.51 (d, $J = 8.0$ Hz, 1H), 7.02 (d, $J = 1.9$ Hz, 1H), 6.77 (dd, $J = 8.0, 1.9$ Hz, 1H), 3.85 (s, 3H), 3.45 (dd, $J = 12.6, 6.6$ Hz, 1H), 2.94 – 2.91 (m, 1H), 2.73 – 2.69 (m, 1H), 1.14 (d, $J = 6.5$ Hz, 3H); $^{13}\text{C NMR}$ (151 MHz, $\text{DMSO}-d_6$) δ 155.33, 137.94, 132.80, 122.82, 113.79, 108.96, 56.12, 47.72, 40.06, 17.82; **HRMS** m/z calculated for $[\text{C}_{10}\text{H}_{14}\text{BrNO}]^+$ ($M_{\text{free base}} + \text{H}$) 243.0259, found 229.0046 [$M_{\text{free base}} + \text{H} - \text{CH}_3$]; **HPLC** $t_R = 17.24$ min.

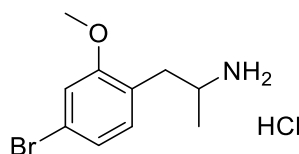
2-(4-bromo-2-methoxyphenyl)ethan-1-amine hydrochloride (5)



Synthesized from nitrostyrene **22** (1.6g, 6.2 mmol) using GP2A. The desired hydrochloride was isolated as a fluffy white solid (1.22g, 74%)

MP 172-173°C; $^1\text{H NMR}$ (600 MHz, $\text{DMSO}-d_6$) δ 8.00 (s, 3H), 7.18 (s, 1H), 7.14 (d, $J = 8.0$ Hz, 1H), 7.11 (d, $J = 7.9$ Hz, 1H), 3.82 (s, 3H), 2.97 – 2.92 (m, 2H), 2.84 – 2.81 (m, 2H); $^{13}\text{C NMR}$ (151 MHz, $\text{DMSO}-d_6$) δ 158.12, 131.65, 124.57, 123.20, 120.66, 114.15, 55.90, 38.20, 27.52; **HRMS** m/z calculated for $[\text{C}_9\text{H}_{12}\text{BrNO}]^+$ ($M_{\text{free base}} + \text{H}$) 229.0102, found 214.9892 [$M_{\text{free base}} + \text{H} - \text{CH}_3$]; **HPLC** $t_R = 17.13$ min

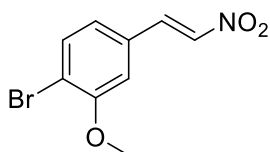
1-(4-bromo-2-methoxyphenyl)propan-2-amine hydrochloride (6)



Synthesized from nitrostyrene **23** using GP2B. The desired hydrochloride was isolated as a white amorphous solid (198 mg, 63%).

MP178-179°C; **¹H NMR** (600 MHz, DMSO-*d*₆) δ 8.08 (s, 3H), 7.18 (s, 1H), 7.13 (d, *J* = 8.0 Hz, 1H), 7.10 (d, *J* = 8.1 Hz, 1H), 3.81 (s, 3H), 3.39 – 3.35 (m, 1H), 2.91 (dd, *J* = 13.3, 5.7 Hz, 1H), 2.69 (dd, *J* = 13.3, 8.5 Hz, 1H), 1.09 (d, *J* = 6.5 Hz, 3H); **¹³C NMR** (151 MHz, DMSO-*d*₆) δ 158.24, 132.49, 124.03, 123.15, 120.78, 114.24, 55.89, 46.43, 34.40, 17.78; **HRMS** *m/z* calculated for [C₁₀H₁₄BrNO]⁺ (*M*_{free base} + H) 243.0259, found 229.0046 [*M*_{free base} + H - CH₃]; **HPLC** *t*_R = 17.74 min.

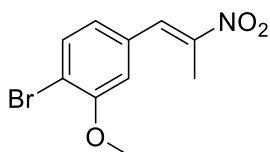
(E)-1-bromo-2-methoxy-4-(2-nitrovinyl)benzene (**23**)



Synthesized using 4-bromo-3-methoxybenzaldehyde and Nitromethane using GP1. Desired nitrostyrene isolated as a pale yellow amorphous solid (860 mg, 71%).

¹H NMR (400 MHz, CDCl₃) δ 7.95 (d, *J* = 13.7 Hz, 1H), 7.62 (d, *J* = 8.1 Hz, 1H), 7.59 (d, *J* = 13.7 Hz, 1H), 7.04 (dd, *J* = 8.1, 2.0 Hz, 1H), 6.99 (s, 1H), 3.95 (s, 3H); **¹³C NMR** (101 MHz, CDCl₃) δ 156.72, 138.32, 137.66, 134.43, 130.67, 122.70, 116.69, 111.56, 56.53.

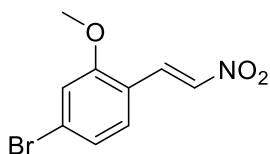
(E)-1-bromo-2-methoxy-4-(2-nitroprop-1-en-1-yl)benzene (**24**)



Synthesized from 4-bromo-3-methoxybenzaldehyde and Nitroethane using GP1. The desired nitrostyrene was isolated as a pale yellow solid (738 mg, 54%).

MP: 68-69°C; **¹H NMR** (600 MHz, CDCl₃) δ 8.02 (s, 1H), 7.62 (d, *J* = 8.0 Hz, 1H), 6.92 – 6.90 (m, 2H), 3.93 (s, 3H), 2.45 (s, 3H); **¹³C NMR** (151 MHz, CDCl₃) δ 156.34, 148.35, 133.93, 133.06, 132.78, 123.12, 114.09, 113.30, 56.48, 14.28.

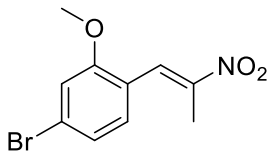
(E)-4-bromo-2-methoxy-1-(2-nitrovinyl)benzene (**25**)



Synthesized from 4-bromo-2-methoxybenzaldehyde and Nitromethane using GP1. The desired nitrostyrene was isolated as a tanned amorphous solid (877mg, 68%).

¹H NMR (400 MHz, CDCl₃) δ 8.06 (d, *J* = 13.6 Hz, 1H), 7.84 (d, *J* = 13.7 Hz, 1H), 7.30 (d, *J* = 8.2 Hz, 1H), 7.18 (dd, *J* = 8.2, 1.8 Hz, 1H), 7.13 (s, 1H), 3.96 (s, 3H); ¹³C NMR (101 MHz, CDCl₃) δ 159.82, 138.73, 134.51, 133.28, 127.66, 124.67, 118.43, 115.39, 56.23.

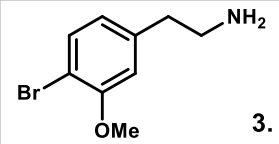
(E)-4-bromo-2-methoxy-1-(2-nitroprop-1-en-1-yl)benzene (26)



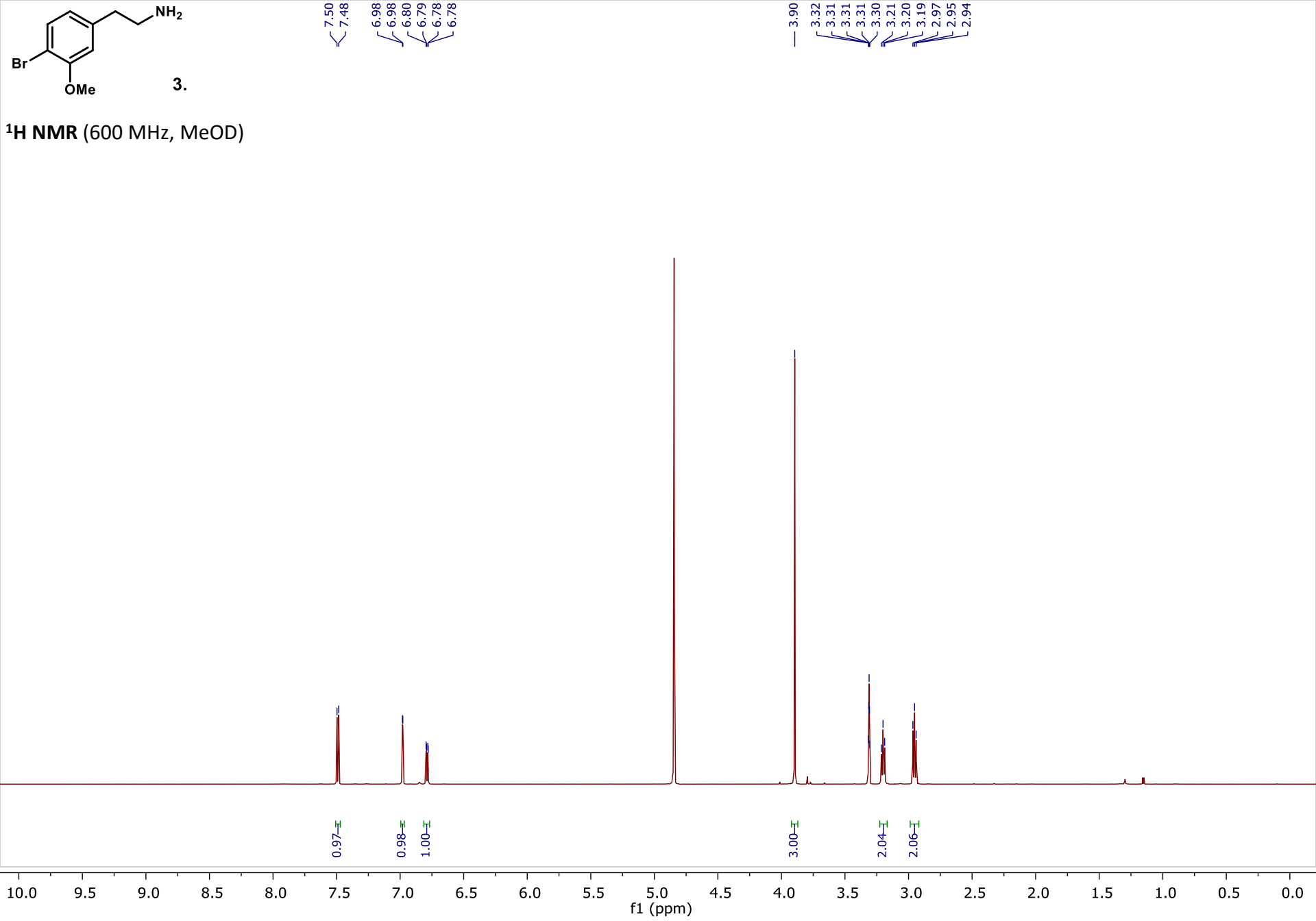
Synthesized from 4-bromo-2-methoxybenzaldehyde and Nitroethane using GP1. The desired nitrostyrene was isolated as a yellow crystalline solid (324mg, 59%).

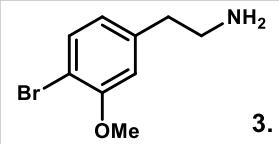
MP: 89-90°C; ¹H NMR (400 MHz, CDCl₃) δ 8.18 (s, 1H), 7.21 – 7.15 (m, 2H), 7.11 (d, *J* = 1.5 Hz, 1H), 3.90 (s, 3H), 2.38 (s, 3H); ¹³C NMR (101 MHz, CDCl₃) δ 158.69, 147.99, 130.99, 128.87, 125.35, 123.88, 120.74, 114.82, 56.10, 14.32.

^1H and ^{13}C NMR Spectra

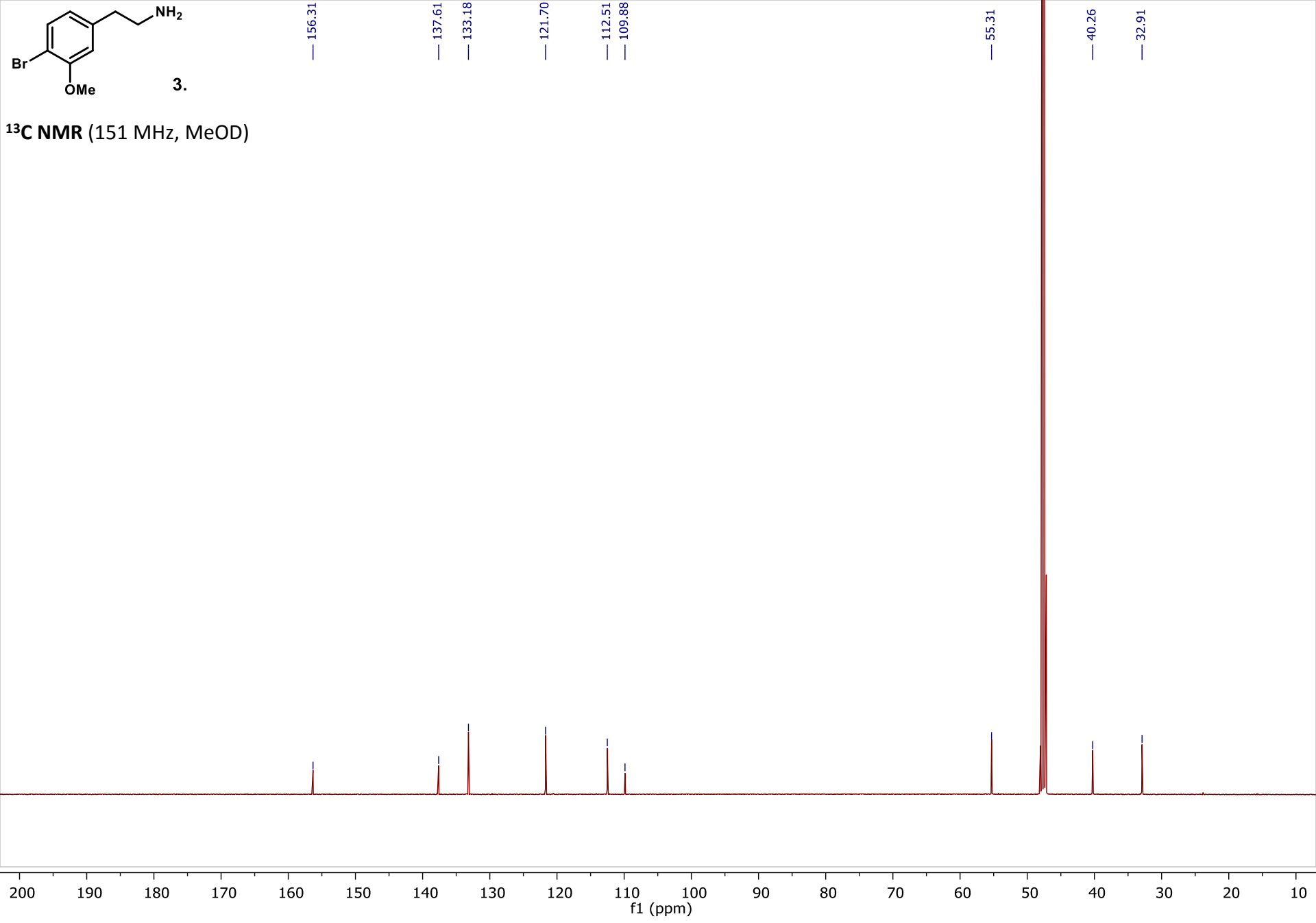


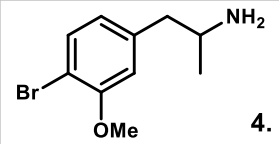
¹H NMR (600 MHz, MeOD)



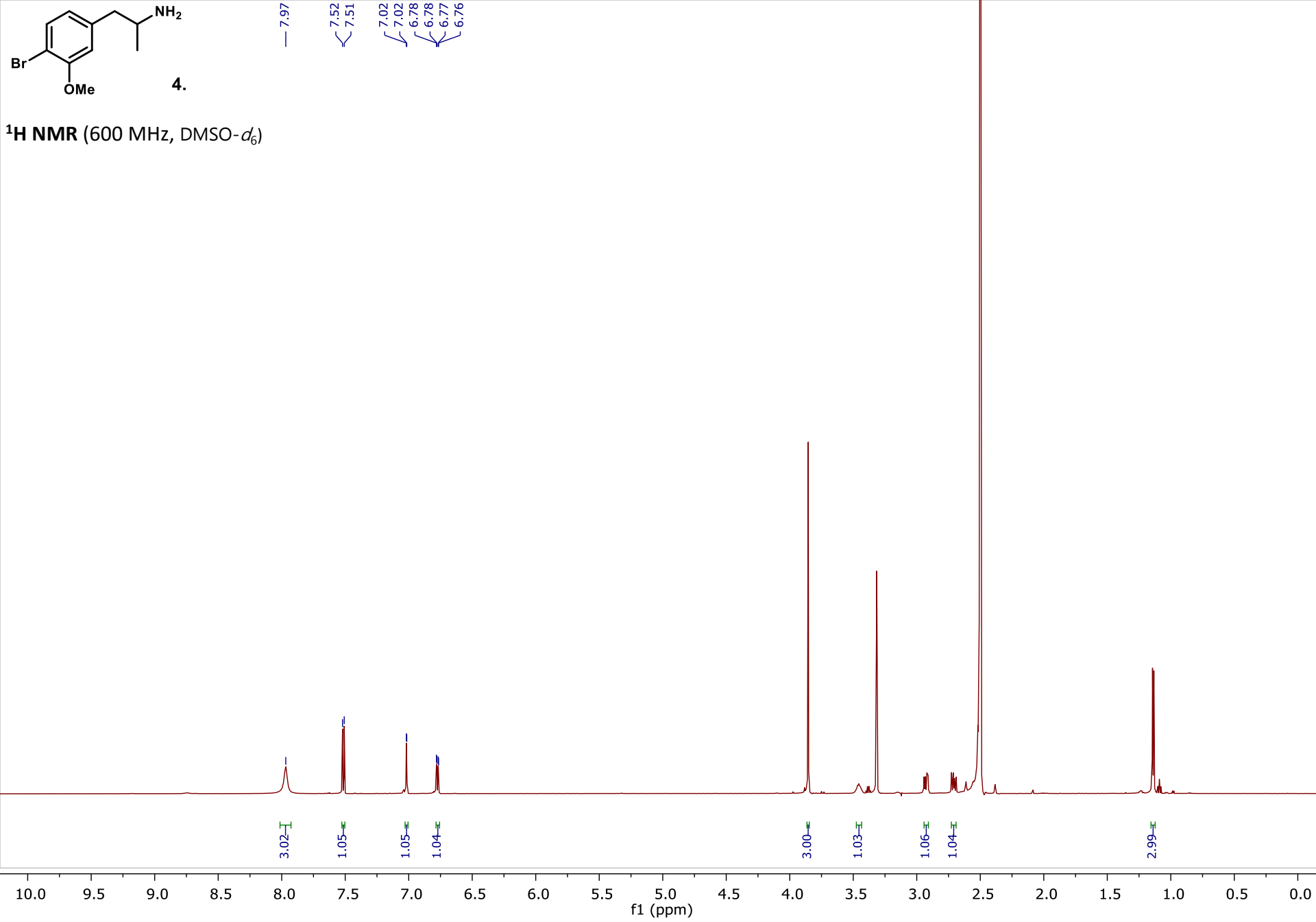


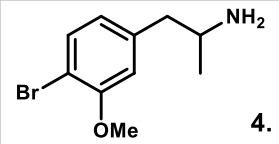
¹³C NMR (151 MHz, MeOD)



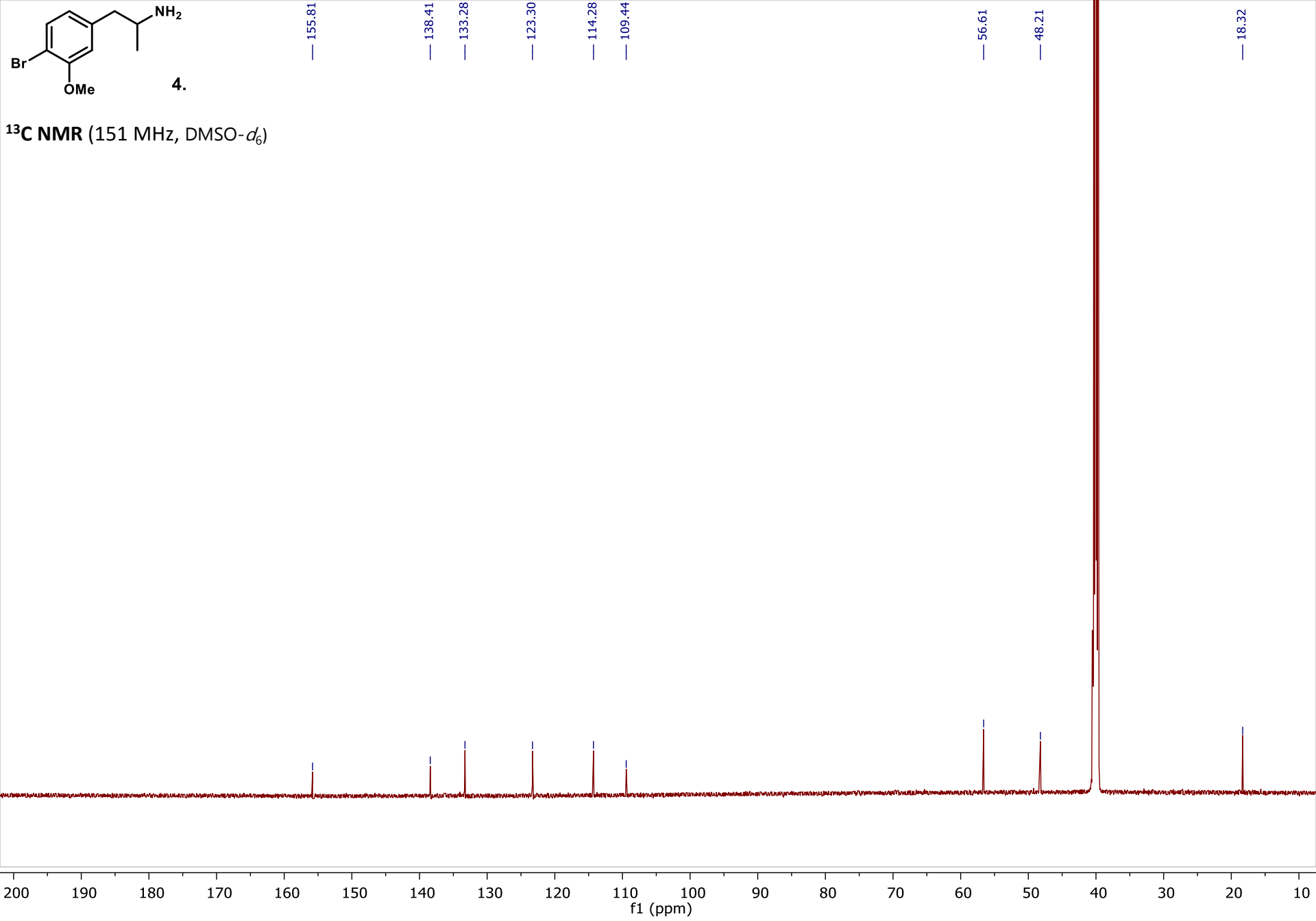


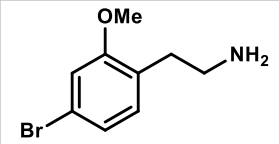
¹H NMR (600 MHz, DMSO-d₆)





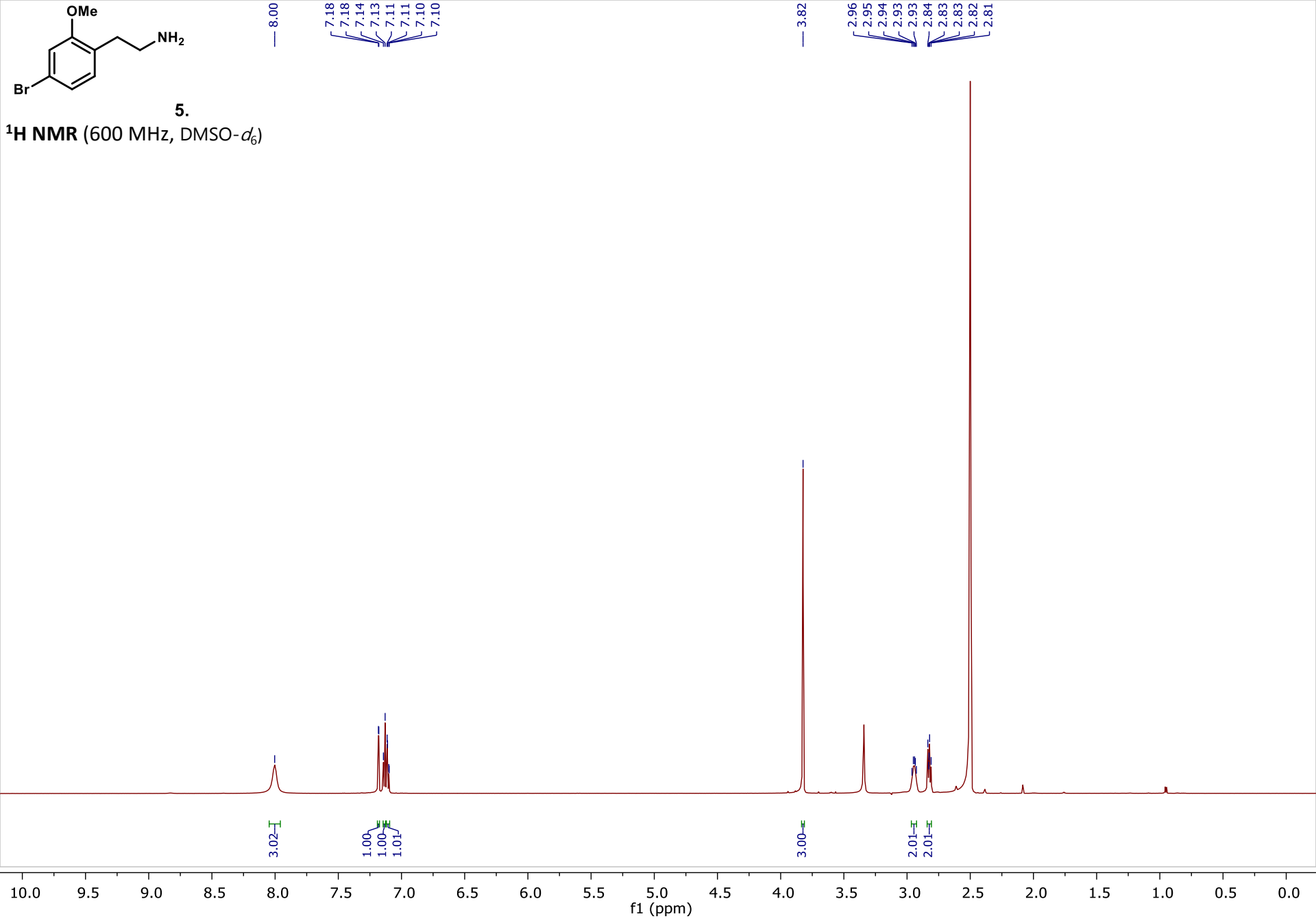
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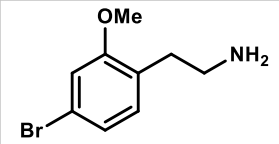




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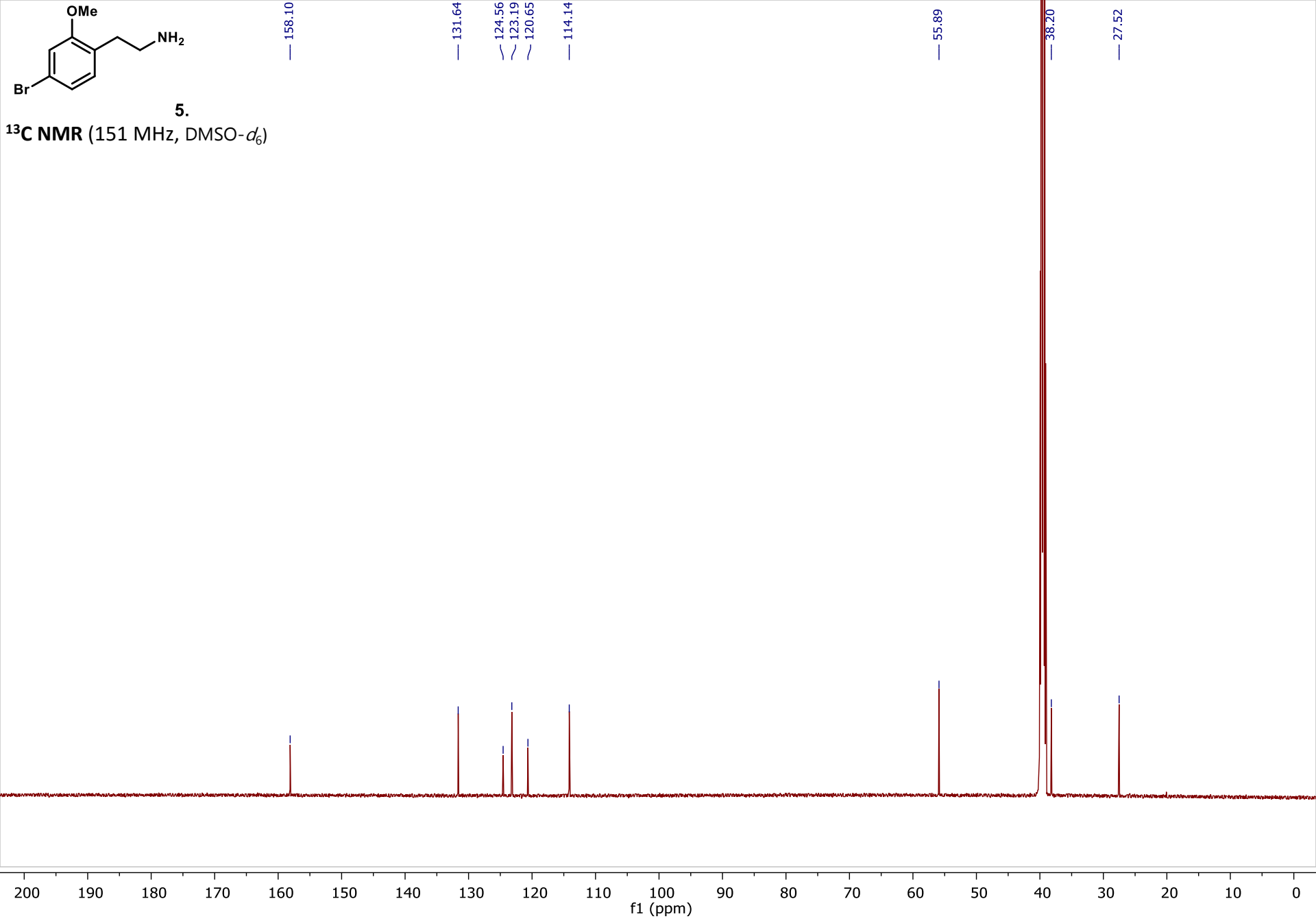
¹H NMR (600 MHz, DMSO-d₆)

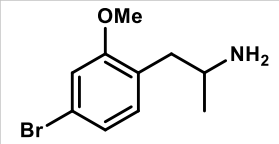




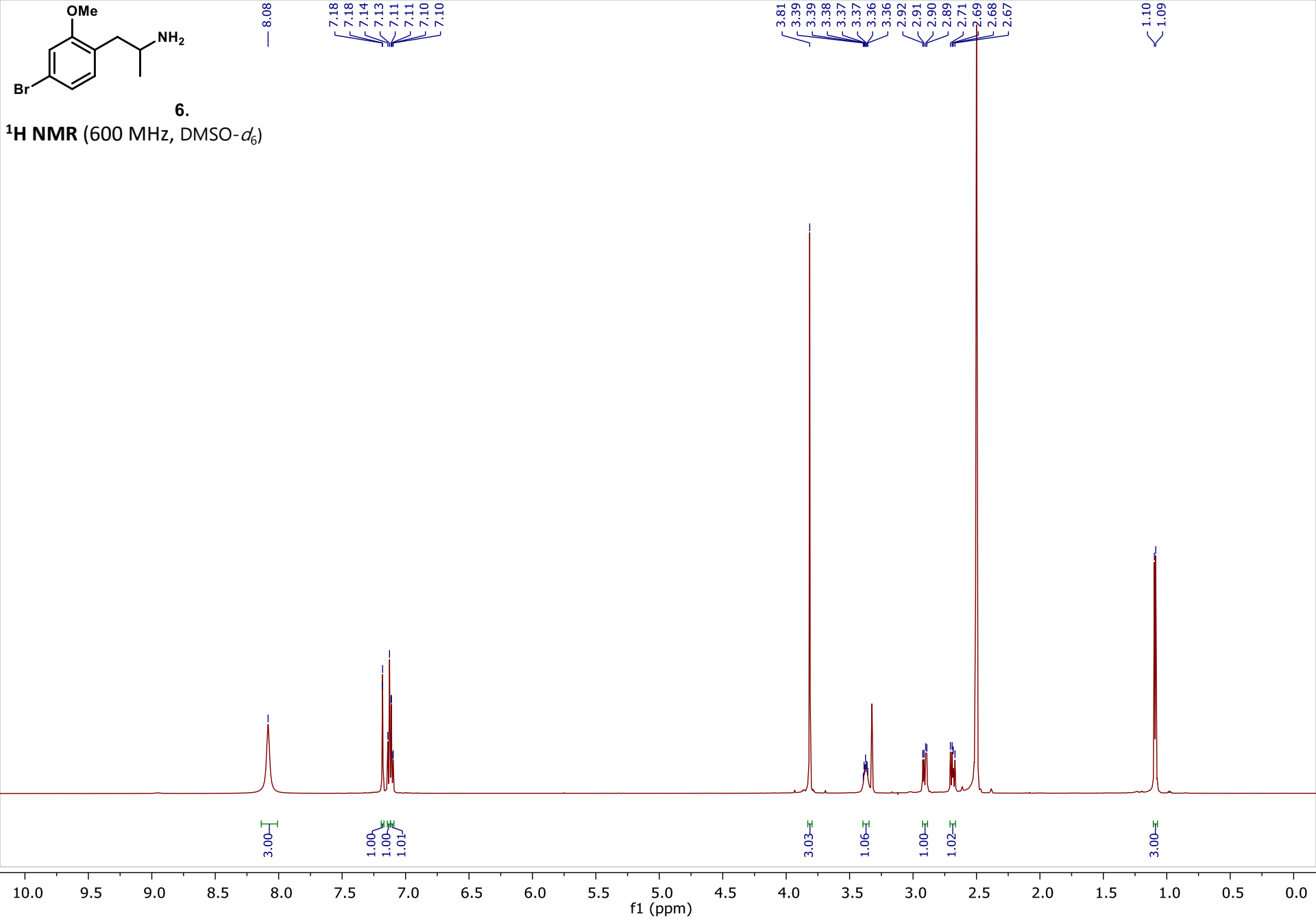
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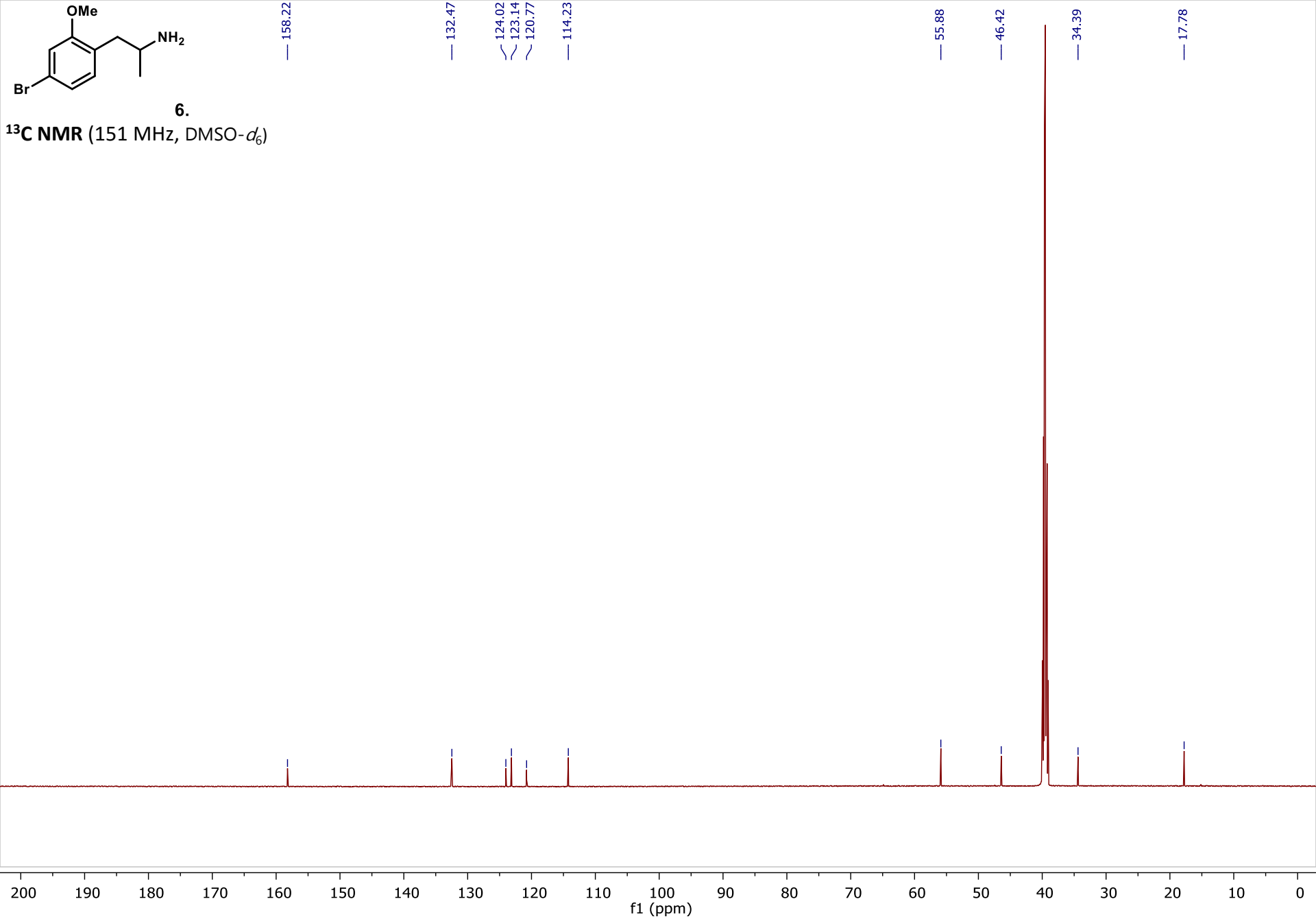
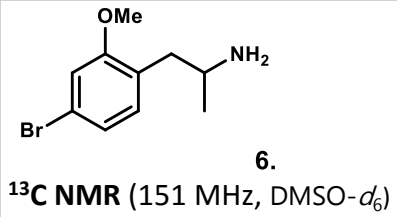
¹³C NMR (151 MHz, DMSO-*d*₆)

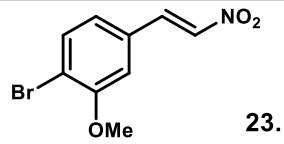




¹H NMR (600 MHz, DMSO-d₆)



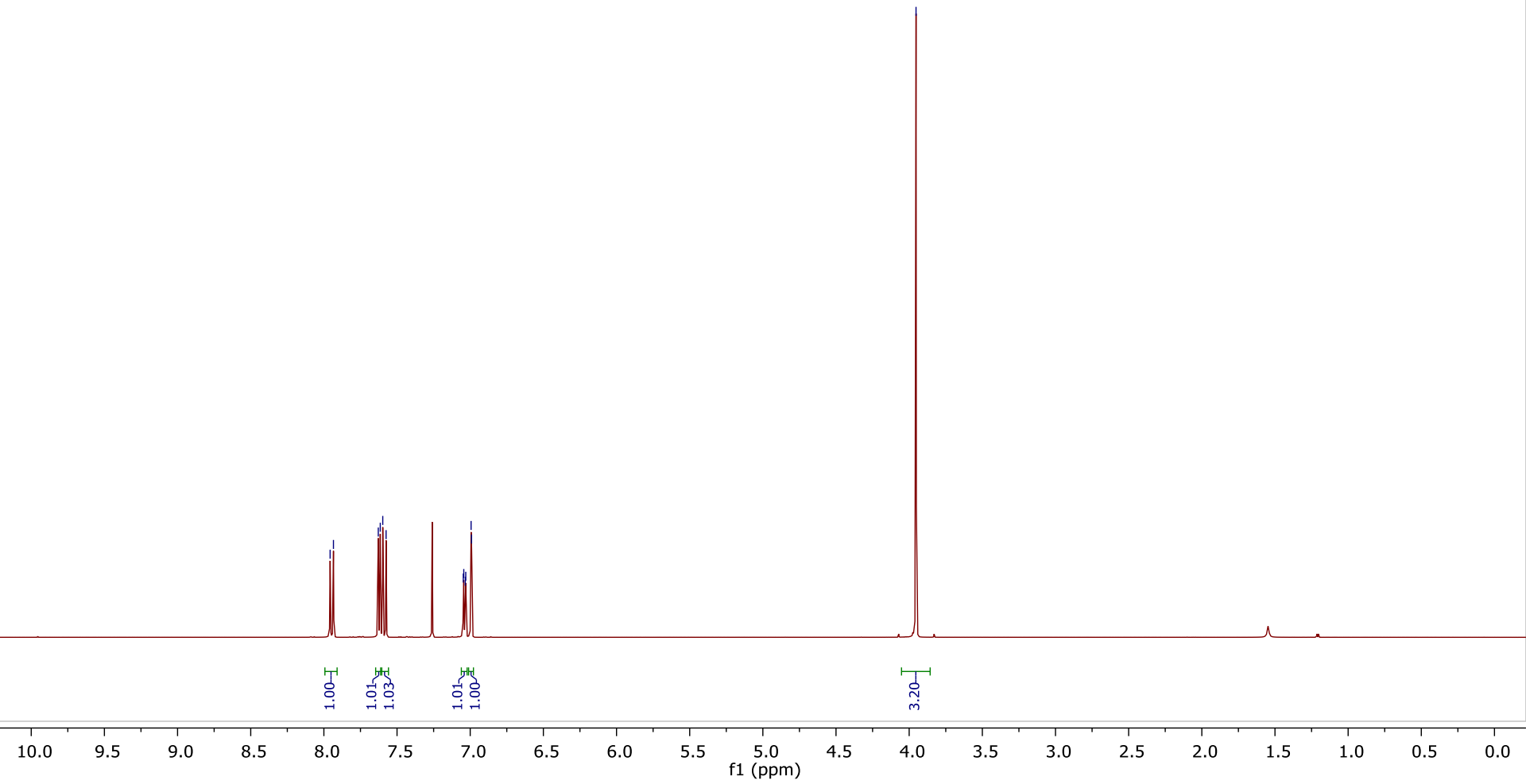


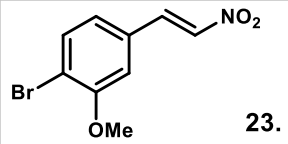


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7.04
7.03
7.03
6.99
6.99

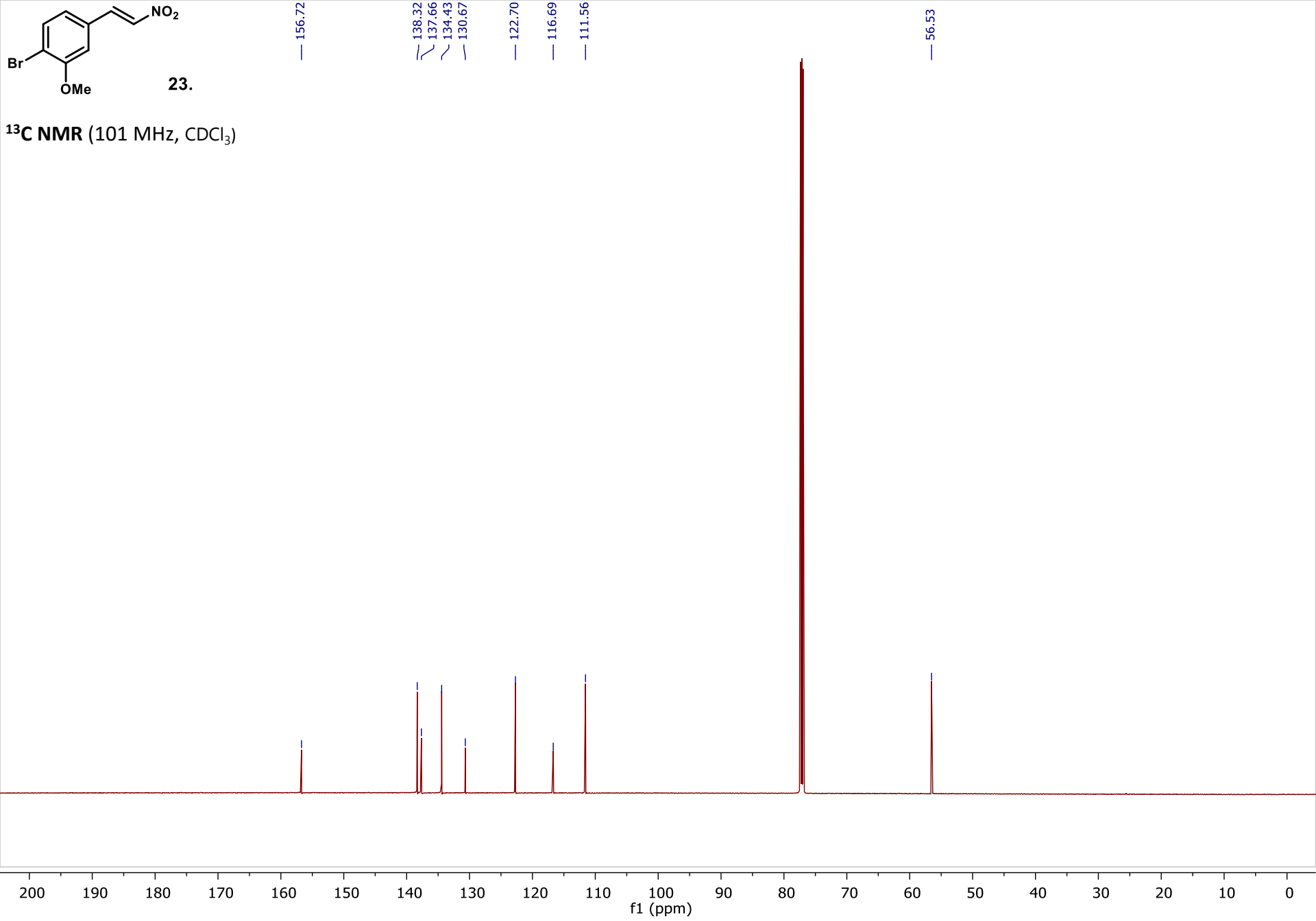
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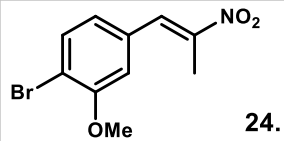
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¹³C NMR (101 MHz, CDCl₃)



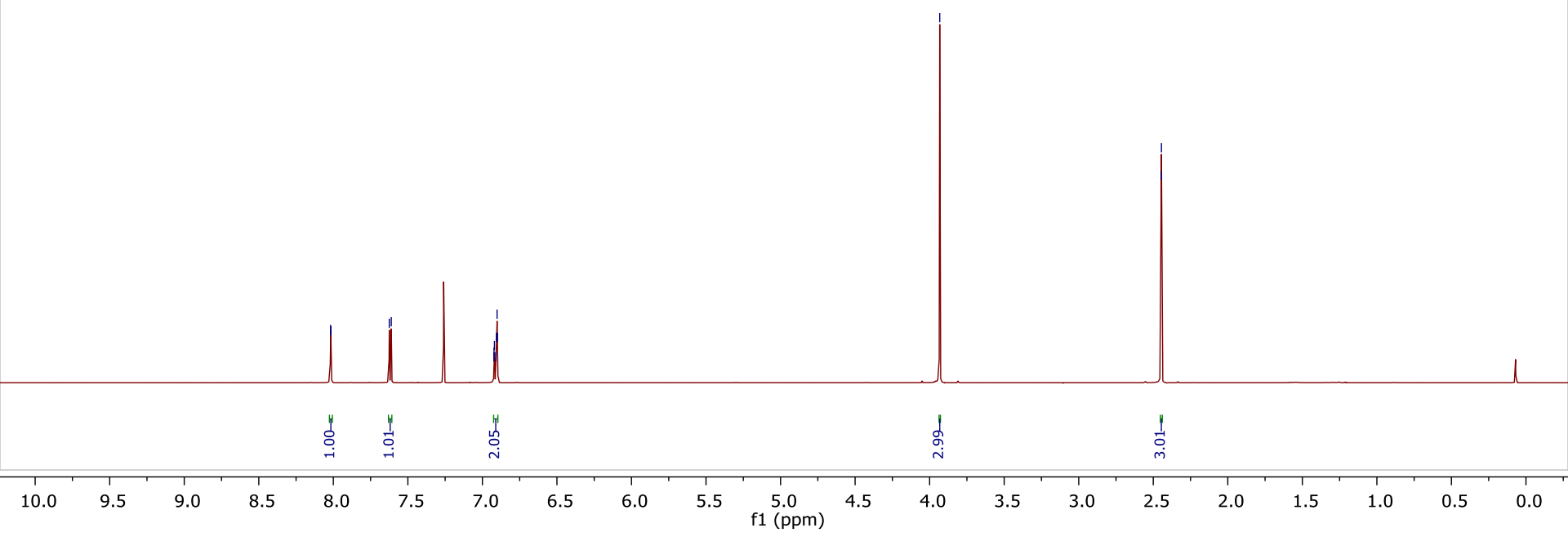


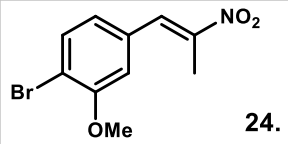
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6.91
6.90
6.90

3.93

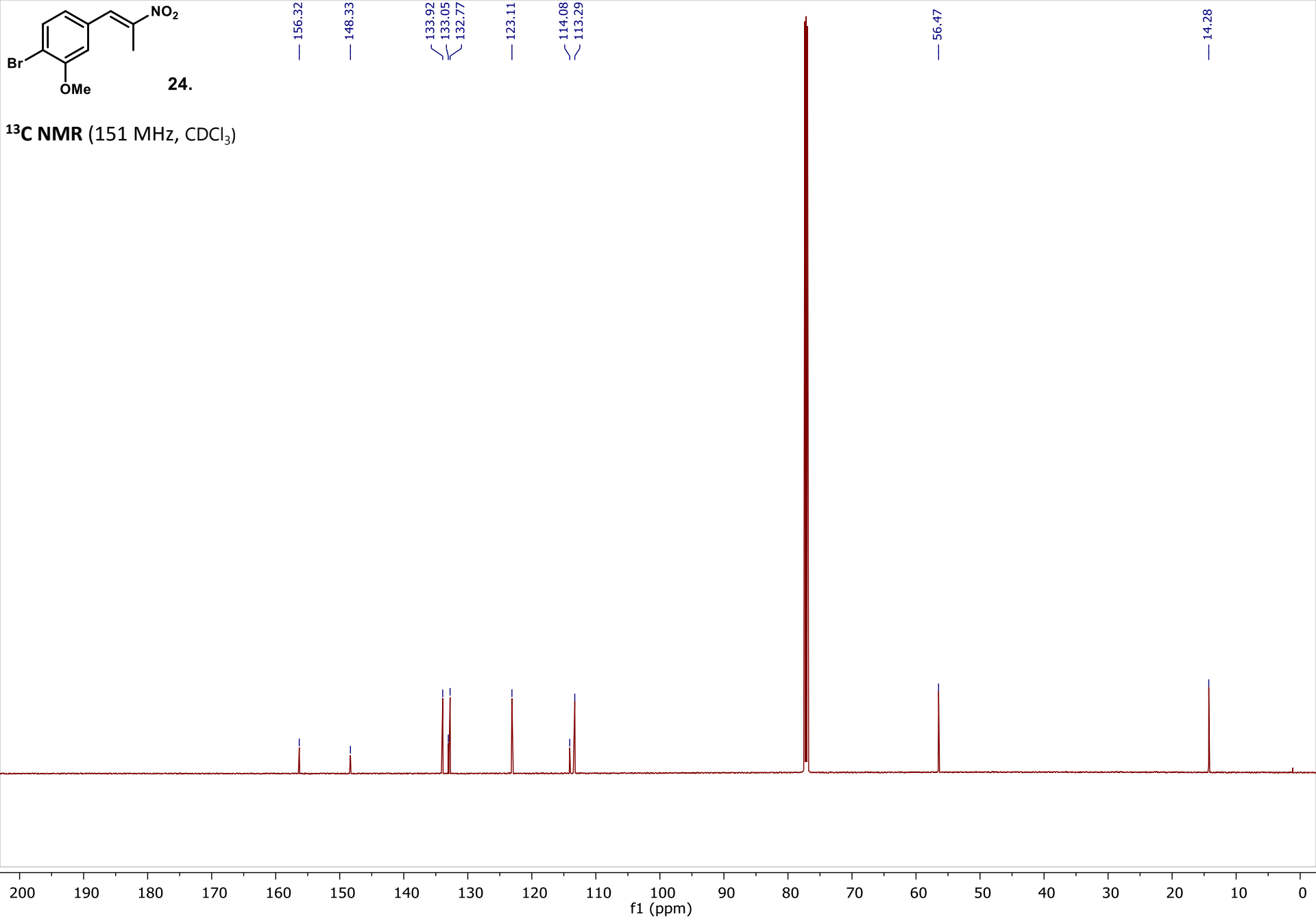
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2.45

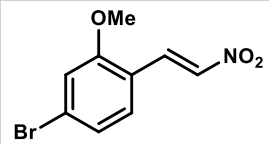
¹H NMR (600 MHz, CDCl₃)





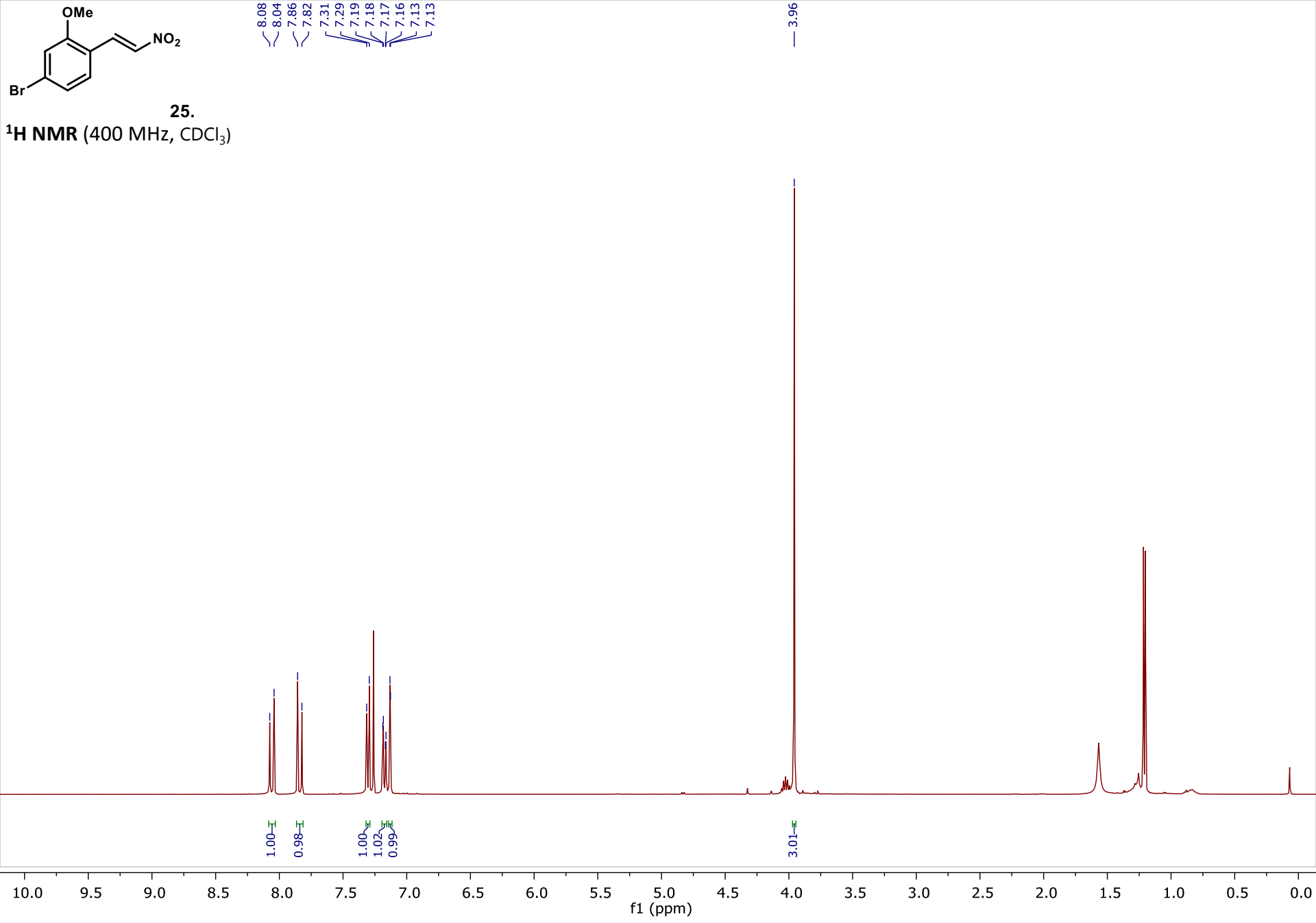
¹³C NMR (151 MHz, CDCl₃)

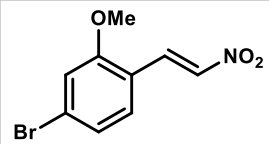




25.

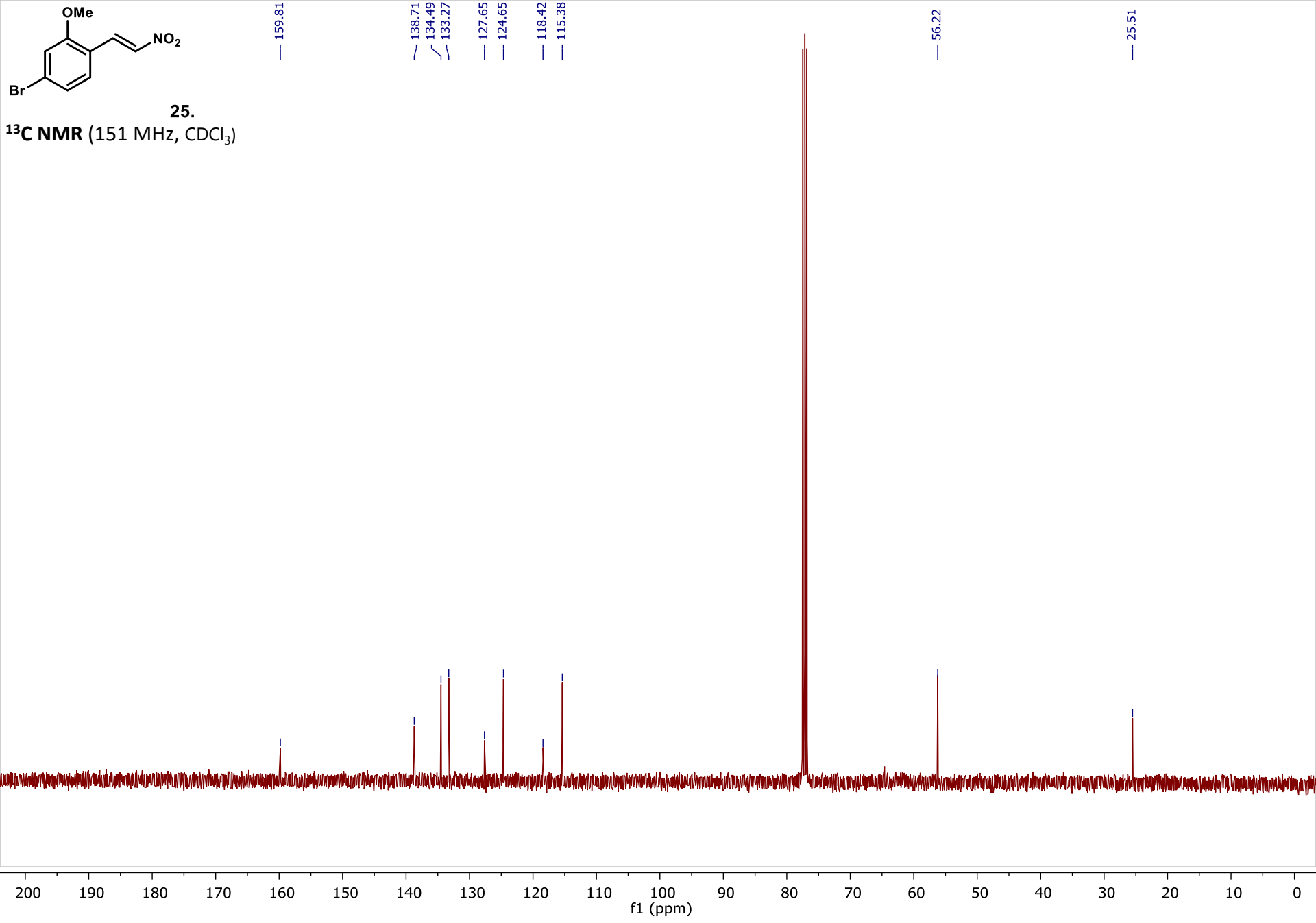
¹H NMR (400 MHz, CDCl₃)

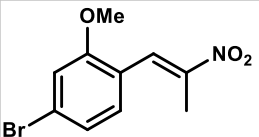




25.

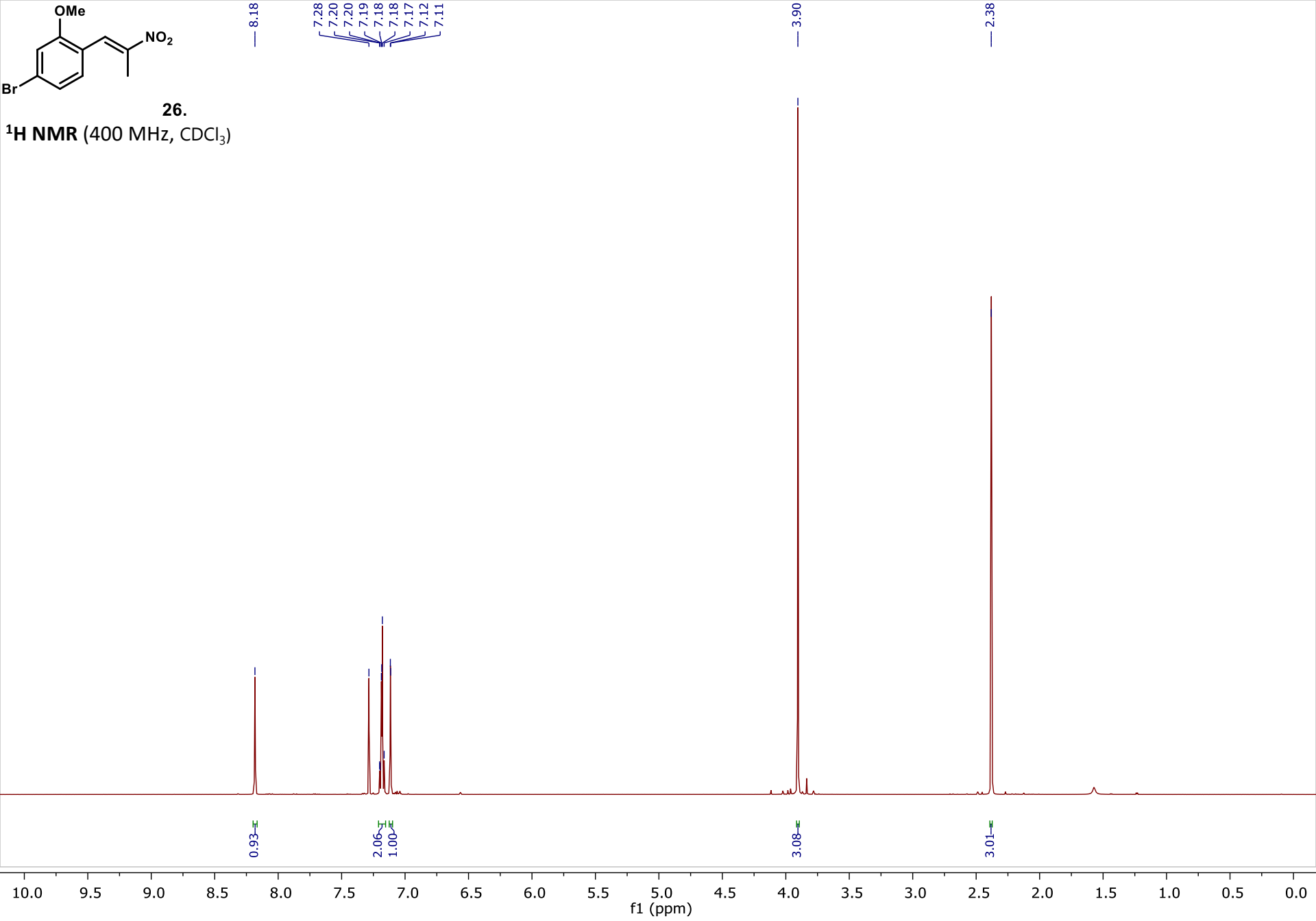
¹³C NMR (151 MHz, CDCl₃)

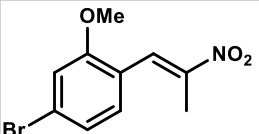




26.

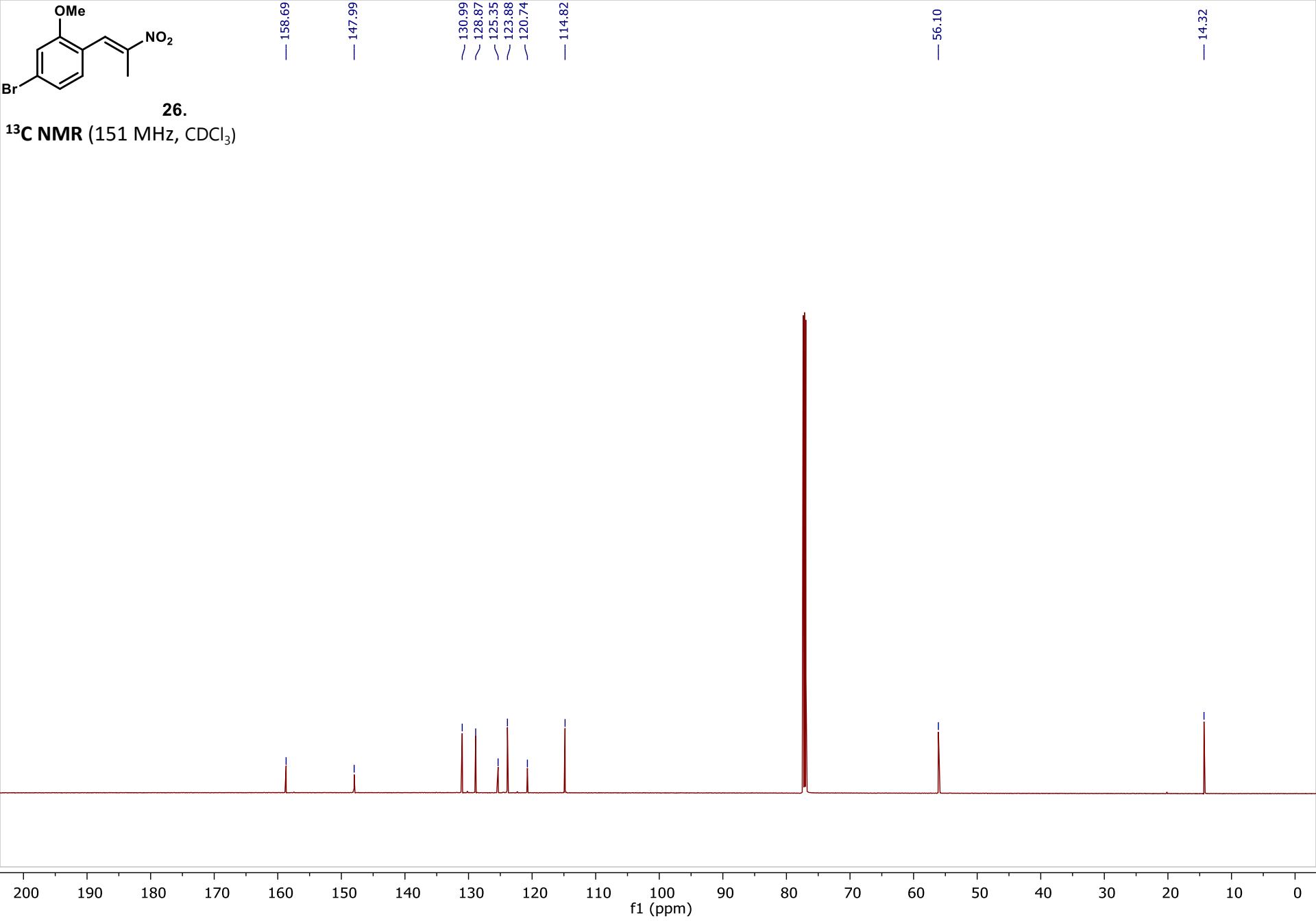
^1H NMR (400 MHz, CDCl_3)



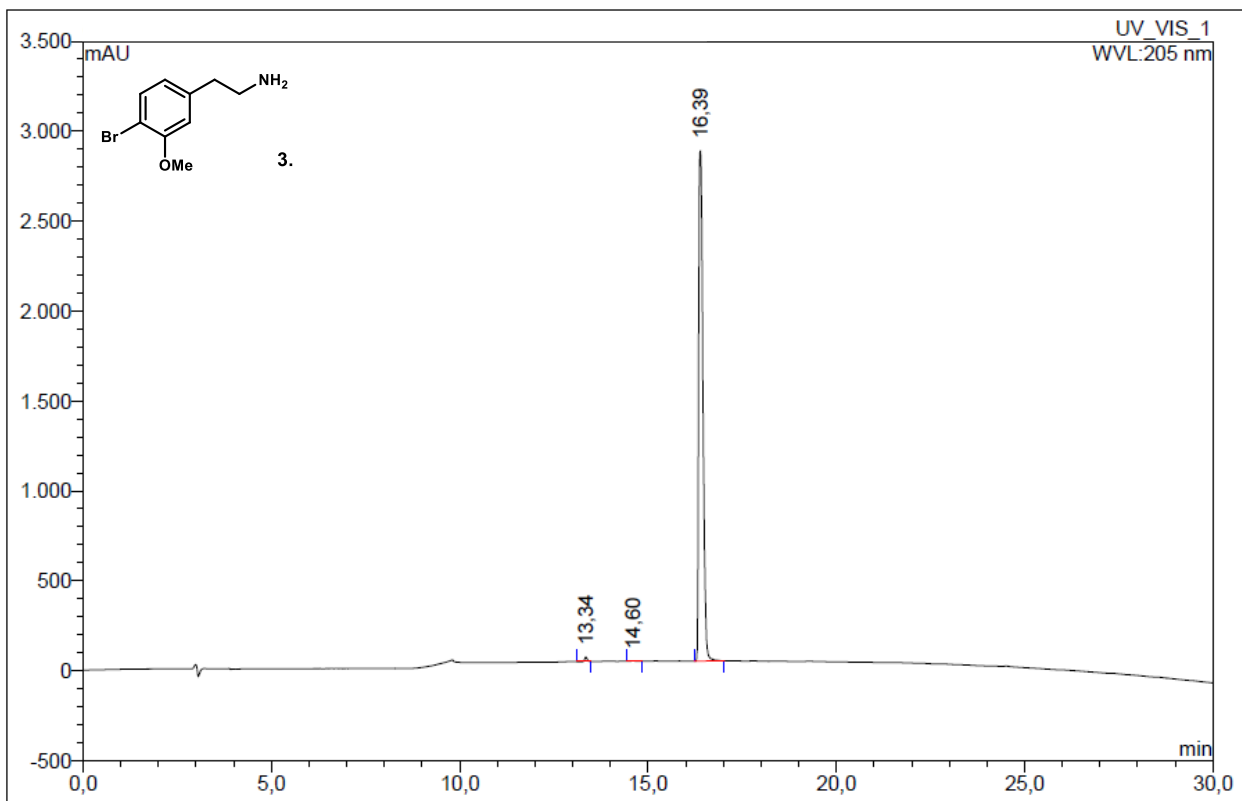


26.

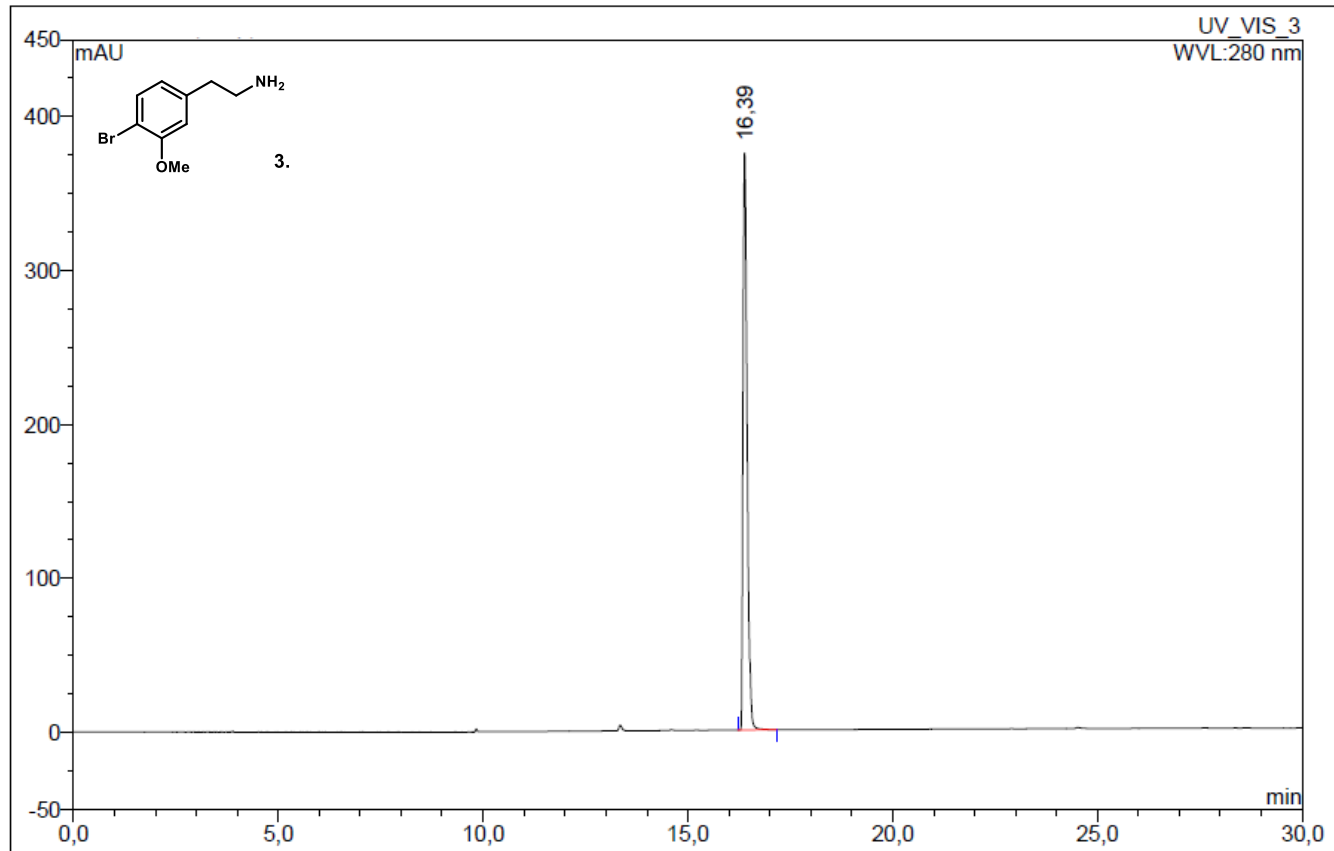
¹³C NMR (151 MHz, CDCl₃)



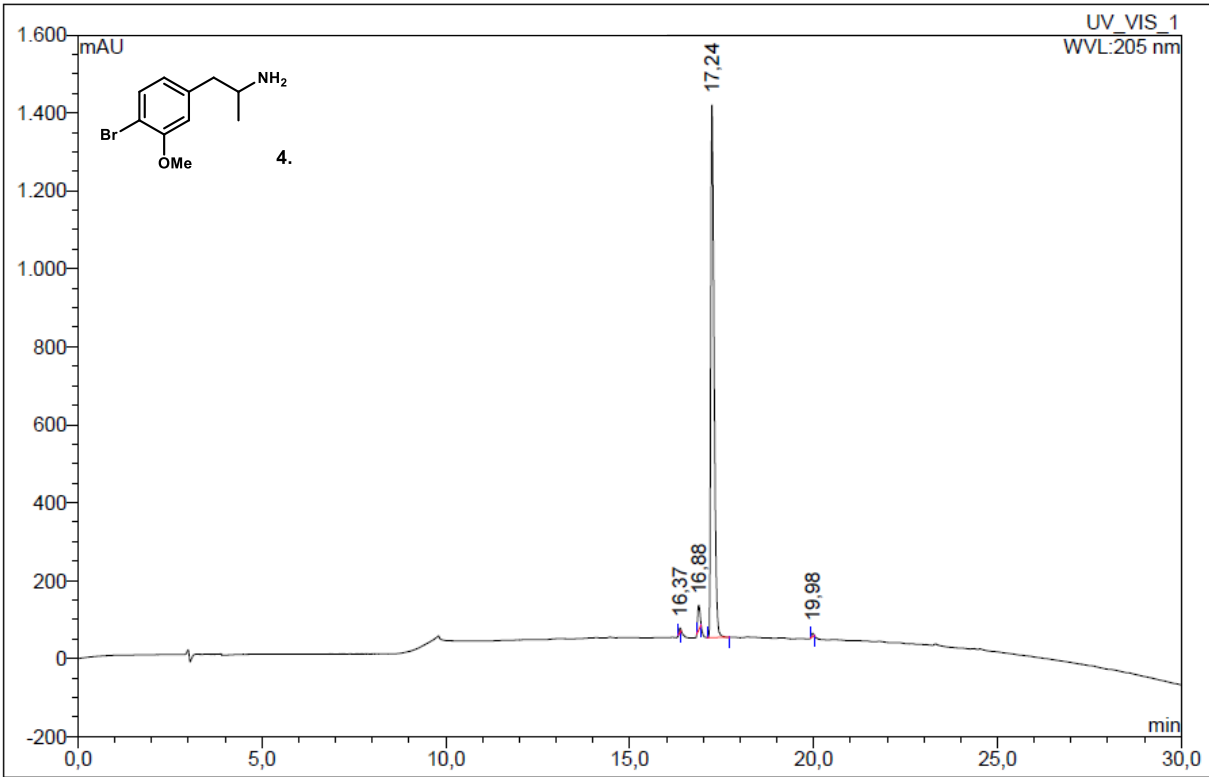
HPLC Chromatograms Compounds 3-6



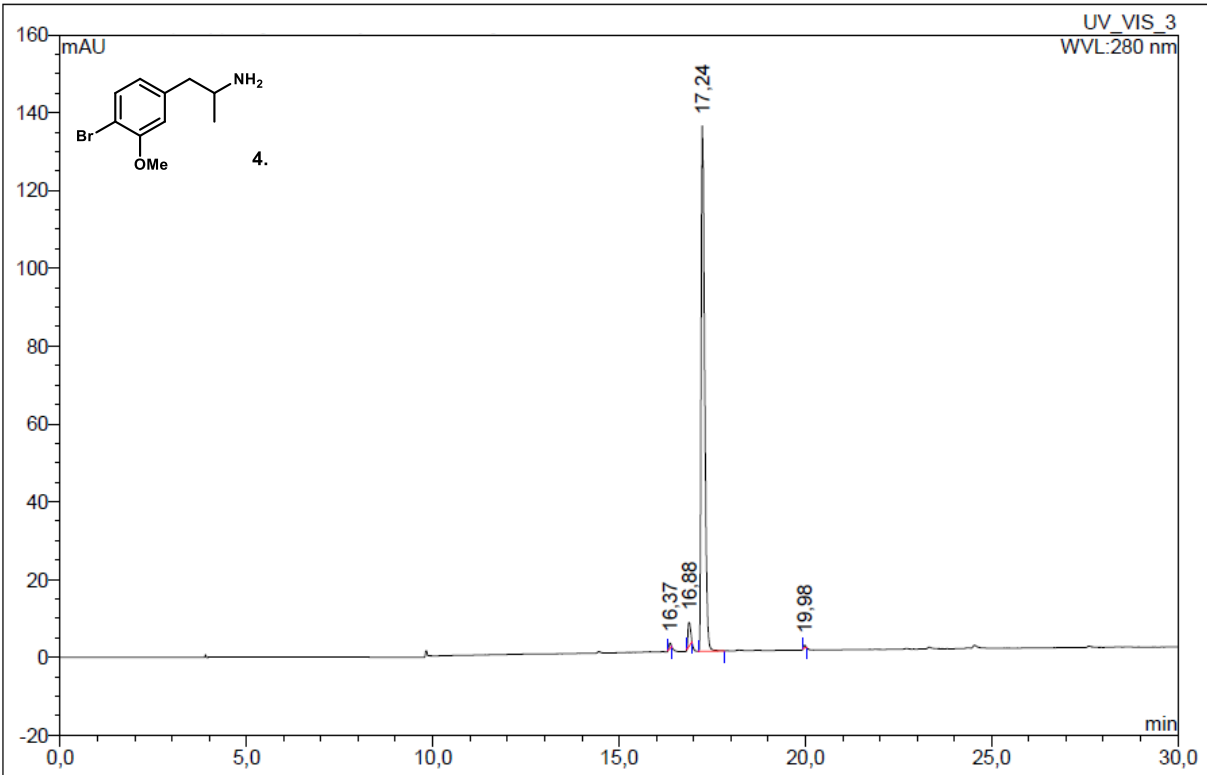
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Resolution(EP)
1	13,34	n.a.	24,385	1,872	0,48	n.a.	9,10
2	14,60	n.a.	2,779	0,316	0,08	n.a.	9,78
3	16,39	n.a.	2839,618	386,772	99,44	n.a.	n.a.
Total:			2866,781	388,960	100,00	0,000	



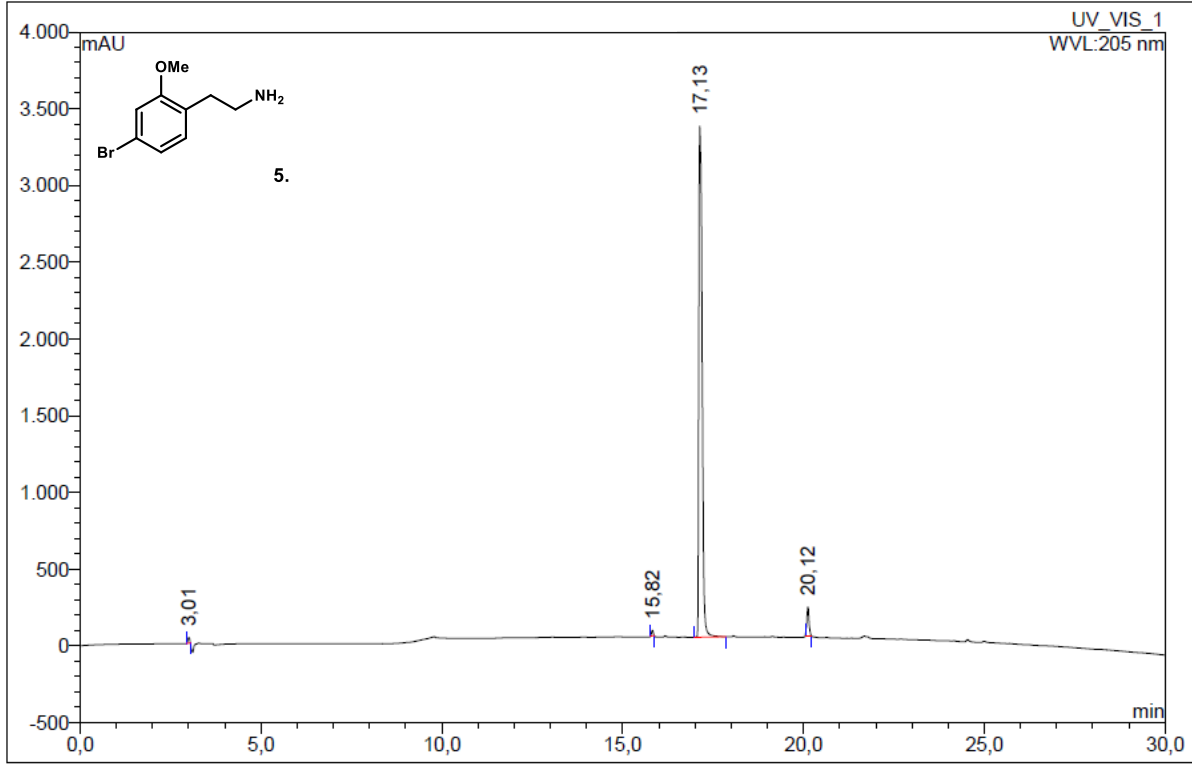
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Resolution(EP)
1	16,39	n.a.	374,967	44,714	100,00	n.a.	n.a.
Total:			374,967	44,714	100,00	0,000	



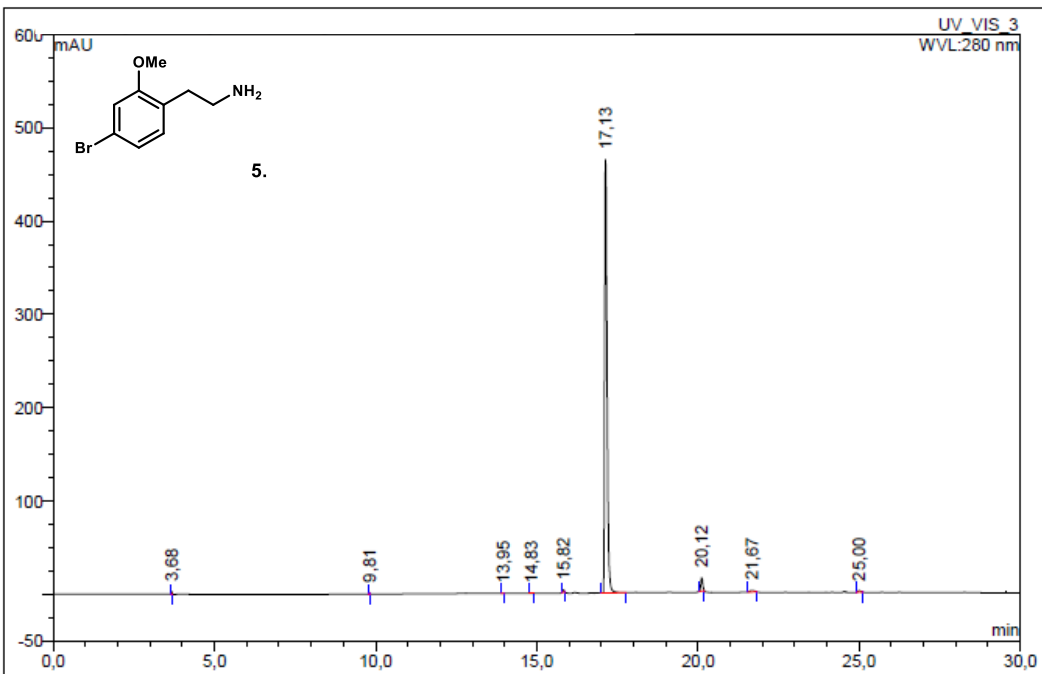
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Resolution(EP)
1	16,37	n.a.	11,105	0,559	0,36	n.a.	4,96
2	16,88	n.a.	63,709	4,505	2,92	n.a.	2,46
3	17,24	n.a.	1366,488	148,478	96,34	n.a.	19,90
4	19,98	n.a.	9,231	0,569	0,37	n.a.	n.a.
Total:			1450,533	154,112	100,00	0,000	



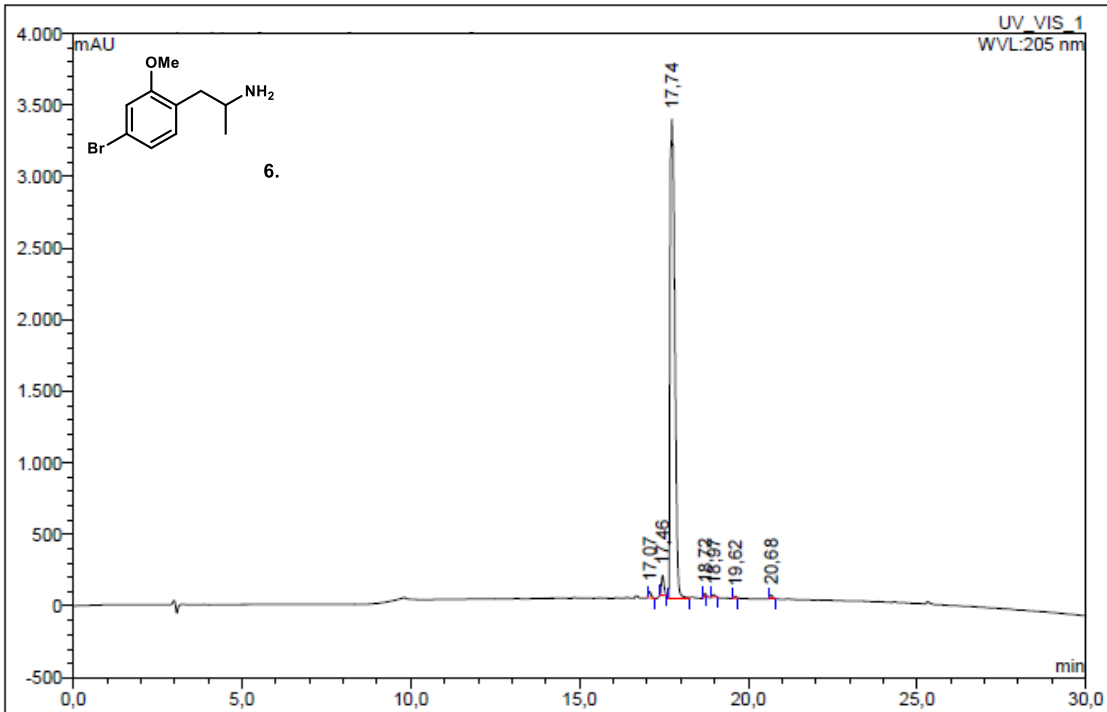
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Resolution(EP)
1	16,37	n.a.	1,394	0,086	0,58	n.a.	4,44
2	16,88	n.a.	6,041	0,448	3,03	n.a.	2,48
3	17,24	n.a.	135,088	14,220	96,13	n.a.	21,04
4	19,98	n.a.	0,690	0,039	0,26	n.a.	n.a.
Total:			143,213	14,793	100,00	0,000	



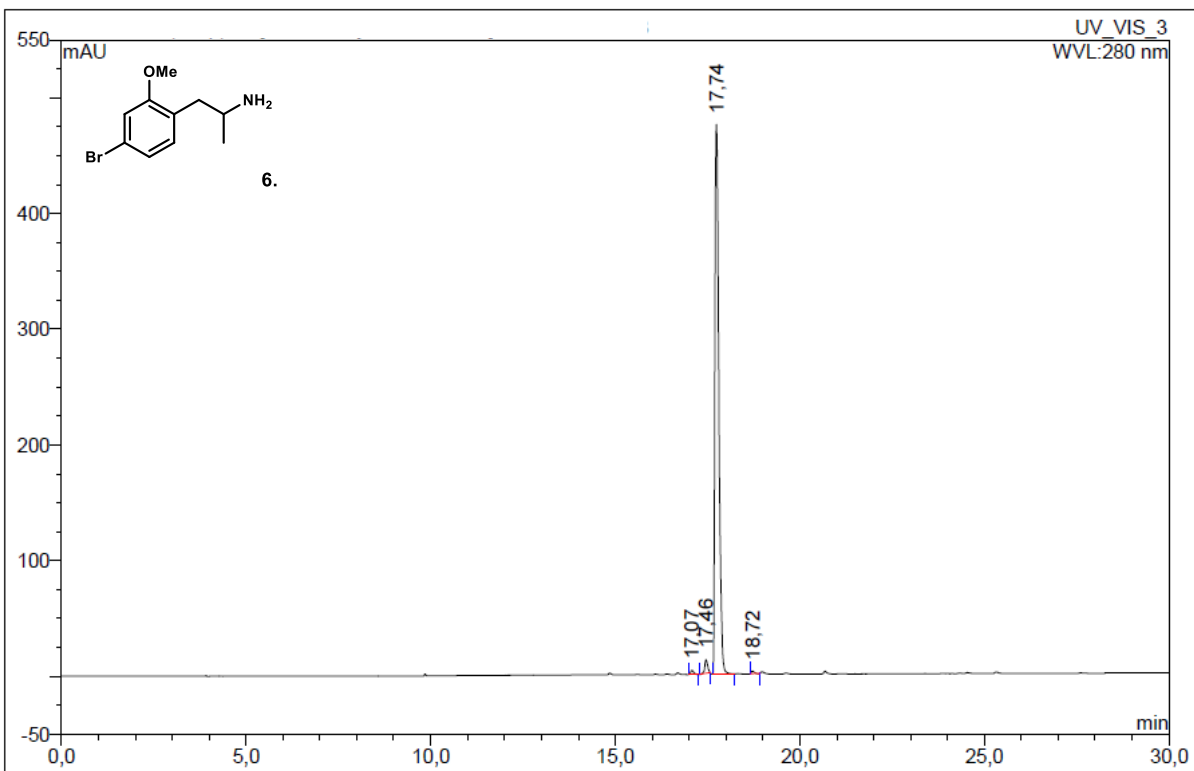
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Resolution(EP)
1	3,01	n.a.	36,626	1,948	0,51	n.a.	138,12
2	15,82	n.a.	36,544	2,014	0,52	n.a.	9,73
3	17,13	n.a.	3331,719	367,399	95,57	n.a.	20,59
4	20,12	n.a.	186,384	13,082	3,40	n.a.	n.a.
Total:			3591,273	384,444	100,00	0,000	



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Resolution(EP)
1	3,68	n.a.	2,349	0,051	0,12	n.a.	159,47
2	9,81	n.a.	1,811	0,043	0,10	n.a.	68,48
3	13,95	n.a.	0,143	0,007	0,02	n.a.	9,32
4	14,83	n.a.	0,203	0,014	0,03	n.a.	10,05
5	15,82	n.a.	2,728	0,144	0,34	n.a.	11,71
6	17,13	n.a.	464,701	40,340	96,16	n.a.	24,25
7	20,12	n.a.	14,422	0,992	2,37	n.a.	9,83
8	21,67	n.a.	1,574	0,209	0,50	n.a.	19,81
9	25,00	n.a.	1,854	0,152	0,36	n.a.	n.a.
Total:			489,784	41,952	100,00	0,000	



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Resolution(EP)
1	17,07	n.a.	40,992	3,584	0,65	n.a.	2,74
2	17,46	n.a.	138,218	11,804	2,15	n.a.	1,44
3	17,74	n.a.	3343,487	527,843	96,05	n.a.	5,35
4	18,72	n.a.	21,678	1,336	0,24	n.a.	1,82
5	18,97	n.a.	19,175	1,994	0,36	n.a.	4,17
6	19,62	n.a.	11,198	0,902	0,16	n.a.	7,78
7	20,68	n.a.	24,029	2,072	0,38	n.a.	n.a.
Total:			3598,776	549,534	100,00	0,000	



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Resolution(EP)
1	17,07	n.a.	3,447	0,323	0,52	n.a.	2,63
2	17,46	n.a.	11,703	1,105	1,78	n.a.	1,63
3	17,74	n.a.	475,049	60,352	97,44	n.a.	5,87
4	18,72	n.a.	2,154	0,158	0,26	n.a.	n.a.
Total:			492,353	61,938	100,00	0,000	