Supporting Information

New phenyl-glycinamide derivatives with hybrid structure as candidates on new effective anticonvulsants

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Table S1. Anticonvulsant activity screening data for compounds **45–66** and **BCTC** in mice *i.p.*

Cmpd	X	R	MES[a]	6 Hz (32 mA)[b]	*sc*PTZ[c]
45	C	H	3/4	3/4	0/4
46	C	2-Cl	0/4	2/4	-
47	C	3-Cl	0/4	3/4	-
48	C	4-Cl	1/4	3/4	-
49	C	3,4-diCl	0/4	1/4	-
50	C	3,5-diCl	0/1	1/4	-
51	C	3-Cl,5-CF$_3$	0/4	1/4	-
52	C	2-CF$_3$	0/4	2/4	-
53	C	3-CF$_3$	3/4	4/4	1/4
54	C	4-CF$_3$	0/4	2/4	-
55	C	3,5-diCF$_3$	0/4	1/4	-
56	C	3-CH$_3$	0/4	1/4	-
57	C	3-CHF$_2$	0/4	1/4	-
58	C	3-C$_6$H$_5$	0/4	2/4	-
59	C	3-OCH$_3$	0/4	-	-
60	C	3-OCF$_3$	3/4	4/4	1/4
61	C	3-OC$_6$H$_5$	0/4	1/4	-
62	C	3-SCF$_3$	3/4	4/4	0/4
63	N	3-CF$_3$	0/4	-	-
64	N	4-CF$_3$	0/4	1/4	-
65	N	5-CF$_3$	3/4	3/4	0/4
66	N	6-CF$_3$	0/4	2/4	-
BCTC[d]	-	-	0/4	0/4	0/4

Data indicate number of mice protected/number of mice tested. Dose of 100 mg/kg was administered *i.p.* The animals were examined at 0.5 h. The most potent compounds are marked in blue. A dash indicates not tested. [a] MES – maximal electroshock seizure test; [b] 6 Hz – psychomotor seizure test, 32 mA; [c] *sc*PTZ – subcutaneous pentylenetetrazole seizure test. [d] BCTC – model TRPV1 antagonist

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Table S2. Radioligand binding and functional assays.

Binding studies	
Na$^+$ channel (site 2)	[1]
Calcium Ca$_{v1.2}$ channels (dihydropyridine site, antagonist radioligand)	[2]
NMDA (antagonist radioligand)	[3]
N-type Ca^{2+} (antagonist radioligand)	[4]
GABA transporter (antagonist radioligand)	[5]
GABA$_A$ ion channel [^{3}H]GABA (agonist radioligand)	[6]
Potassium channel (hERG)	[7]
Functional studies	
TRPV1 (VR1) (h) (antagonist effect)	[8]
Ca$_{v1.2}$ (L-type) (h) calcium ion channel cell-based antagonist calcium flux assay	[9,10]

Assays were performed commercially in Eurofins Laboratories (Poitiers, France) or Eurofins Panlabs Discovery Services Taiwan, Ltd. (New Taipei City, Taiwan).

Table S3. *In vitro* TRPV1 channel antagonist activity for compounds **45–66** (concentration of 100 μM).

TRPV1 (VR1) (*h*) (antagonist effect)*			
Cmpd	% Inhibition of control agonist response[a]	Cmpd	% Inhibition of control agonist response[a]
45	36.1	**56**	57.4
46	116.3	**57**	43.7
47	125.2	**58**	92.0
48	126.9	**59**	20.1
49	135.1	**60**	109.7
50	128.0	**61**	52.5
51	-2.5	**62**	135.1
52	90.9	**63**	-2.8
53	128.5	**64**	39.7
54	128.5	**65**	8.5
55	-14.0	**66**	3.7

*Source: human recombinant CHO cells. [a] **Results showing activity higher than 50% are considered to represent significant effects of the test compounds**; results showing an inhibition between 25% and 50% are indicative of weak effect; results showing an inhibition lower than 25% are not considered significant and mostly attributable to variability of the sign al around the control level. Assays were performed commercially in Eurofins Laboratories (Poitiers, France).

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ADME-Tox in vitro - metabolic stability of compounds **53**, **60**, and **62**.


53   **60**   **62**
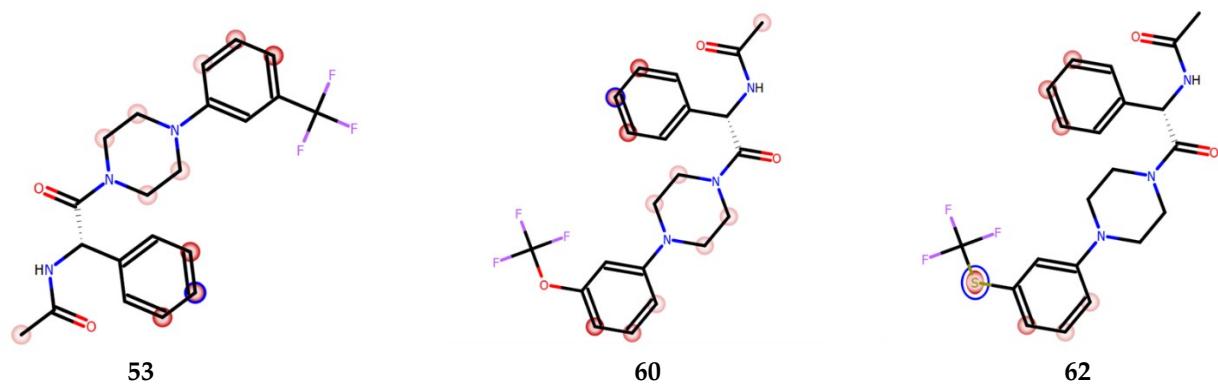
Figure S1. The MetaSite 6.0.1. software prediction of the most probably sites of tested compounds metabolism. The darker red color - the higher probability to be involved in the metabolism pathway. The blue circle marked the site of compound with the highest probability of metabolic bioconversion.

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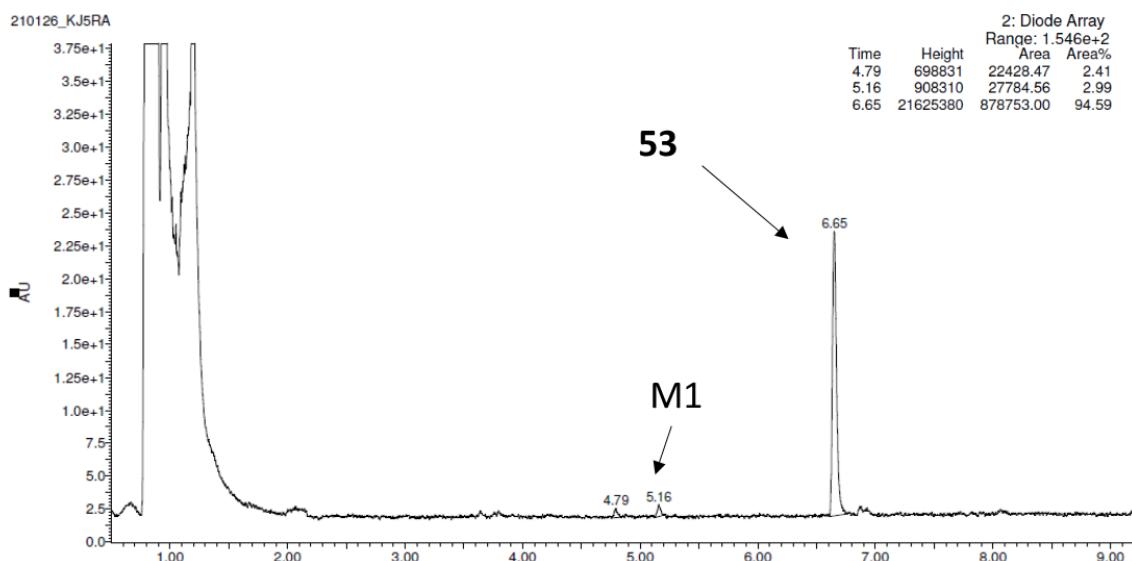
Figure S2. UPLC spectra after 120 min incubation of compound **53** with human liver microsomes **i**n TRIS buffer pH=7.4 at 37ºC.
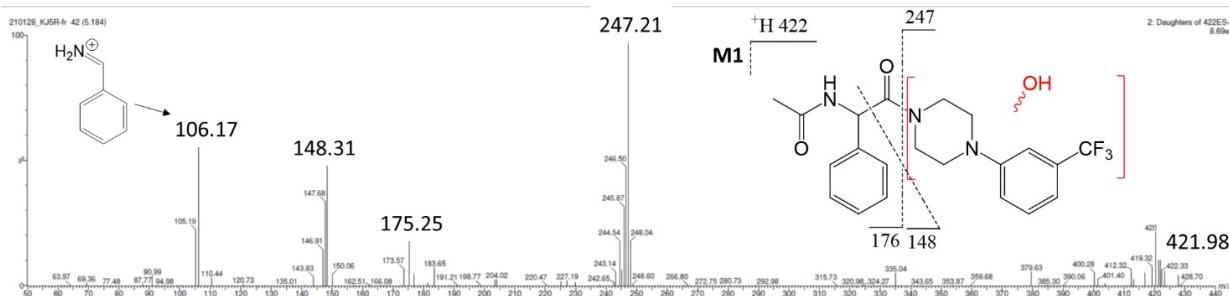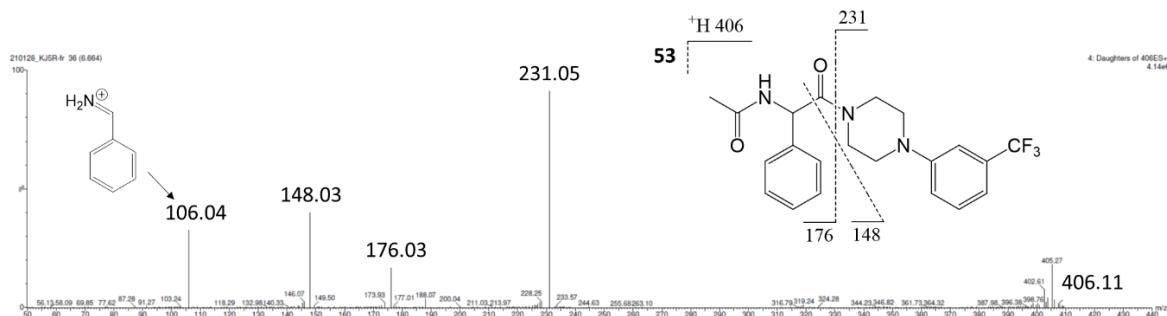
Figure S3. MS ion fragment analyses and the most probable structure of **53** metabolite **M1**.

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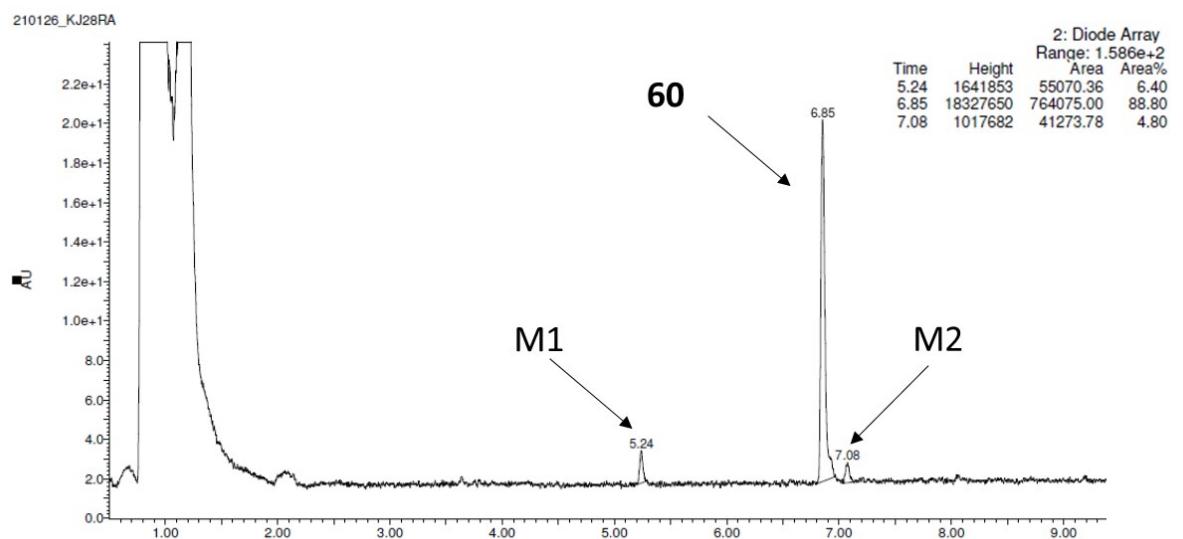
Figure S4. UPLC spectra after 120 min incubation of compound **60** with human liver microsomes in TRIS buffer pH=7.4 at 37ºC.
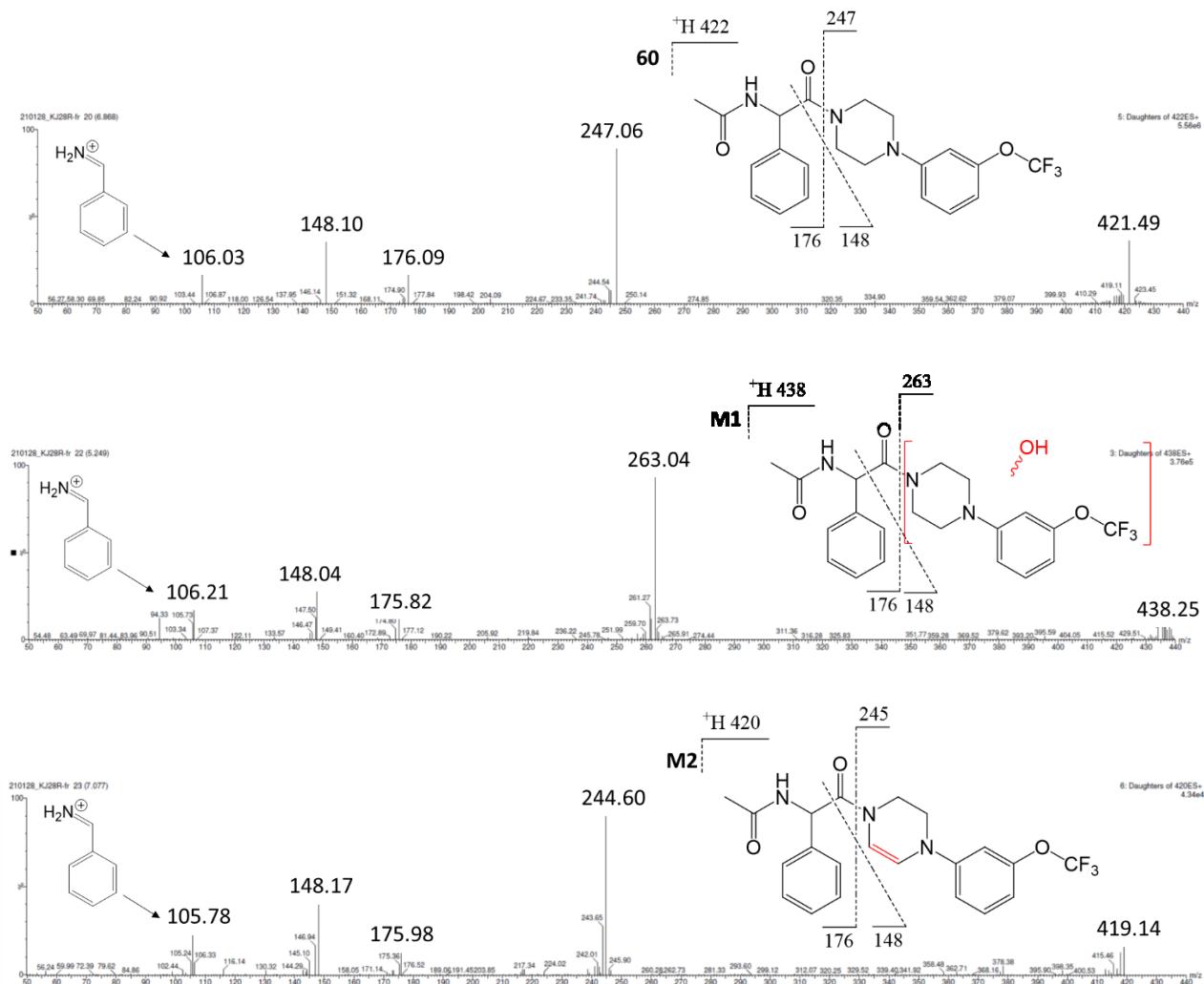
Figure S5. MS ion fragment analyses and the most probable structure of **60** metabolites **M1–M2**.

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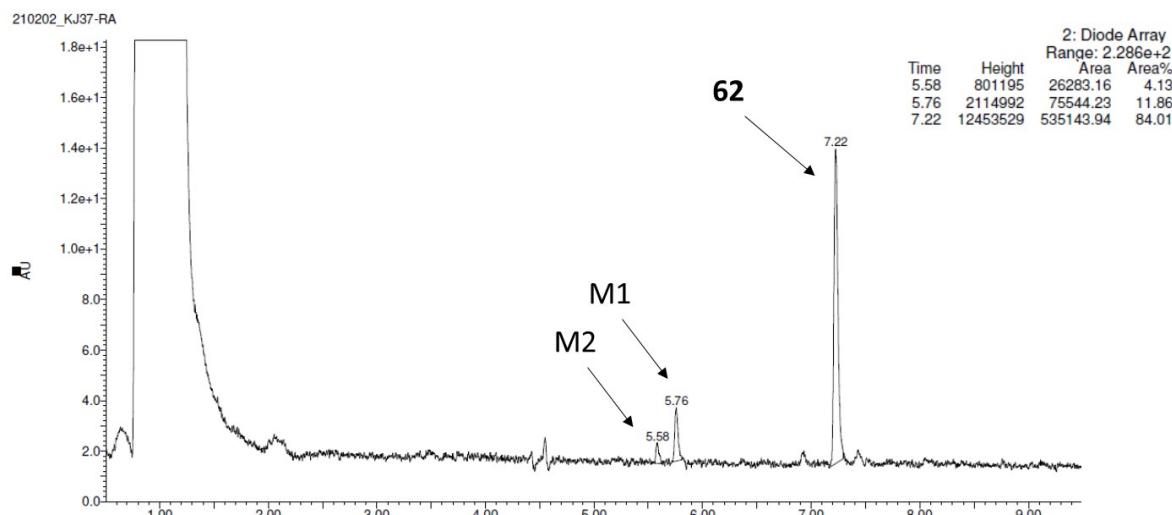
Figure S6. UPLC spectra after 120 min incubation of compound **62** with human liver microsomes in TRIS buffer pH=7.4 at 37ºC.
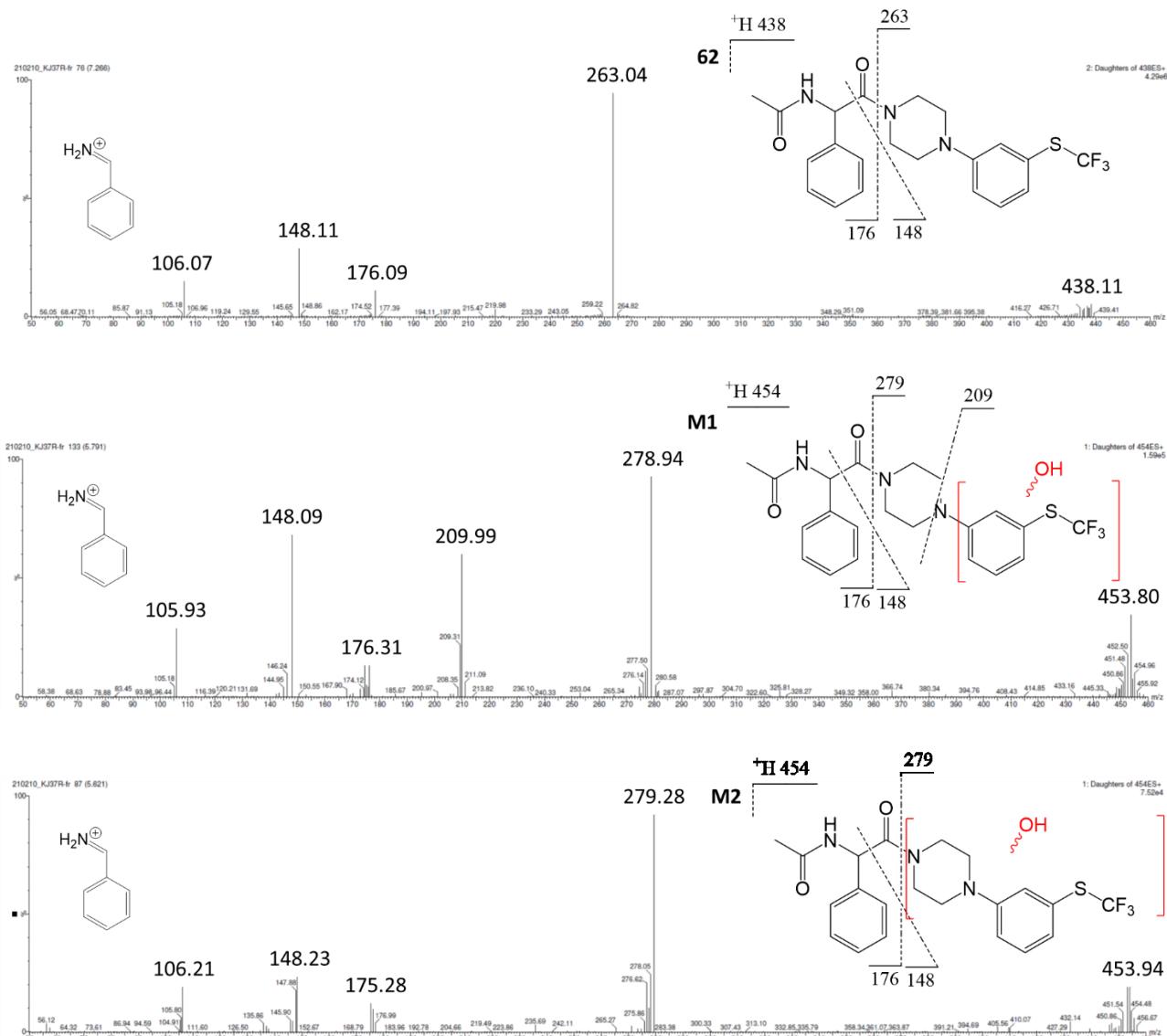
Figure S7. MS ion fragment analyses and the most probable structure of **62** metabolites **M1–M2**.

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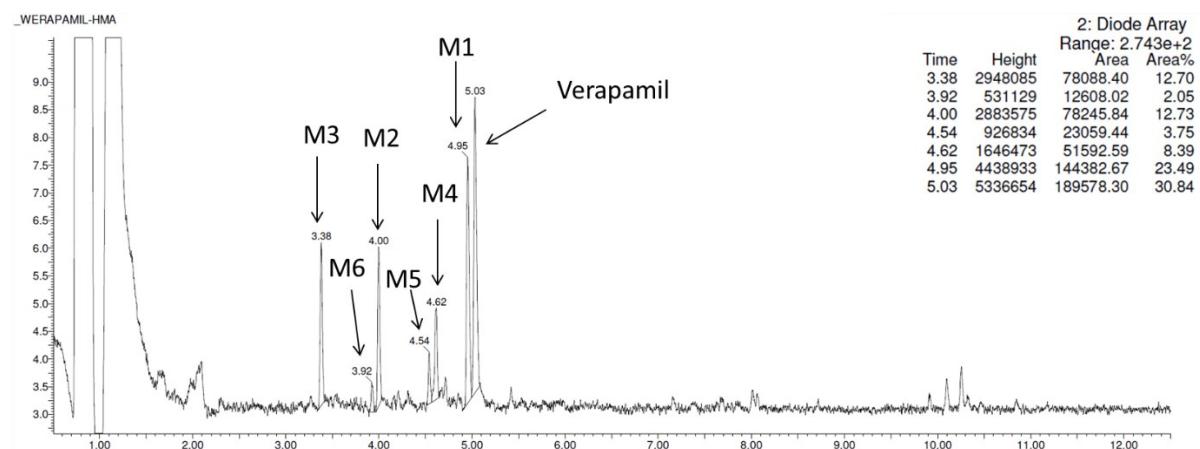
Figure S8. UPLC spectra after 120 min incubation of the reference drug verapamil with human liver microsomes in TRIS buffer pH=7.4 at 37ºC [11–13]

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¹H NMR and ¹³C NMR spectra for the final compounds (45–66)

N-(2-oxo-1-phenyl-2-(4-phenylpiperazin-1-yl)ethyl)acetamide (45) – ¹H NMR
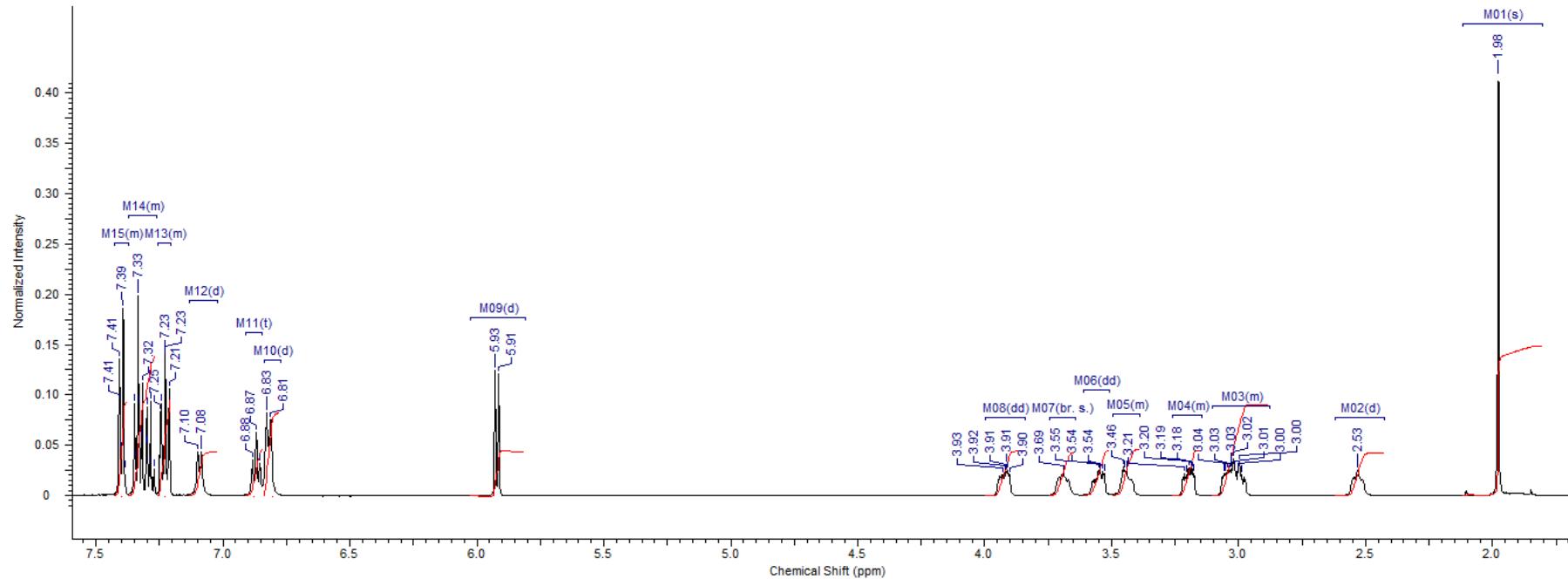
**N-(2-oxo-1-phenyl-2-(4-phenylpiperazin-1-yl)ethyl)acetamide (45) – ^{13}C NMR**

**N-(2-(4-(2-chlorophenyl)piperazin-1-yl)-2-oxo-1-phenylethyl)acetamide (46) – ^{1}H NMR**

*N*-(2-(4-(2-chlorophenyl)piperazin-1-yl)-2-oxo-1-phenylethyl)acetamide (46) – ¹³C NMR

N-(2-(4-(3-chlorophenyl)piperazin-1-yl)-2-oxo-1-phenylethyl)acetamide (47) – ¹H NMR

N-(2-(4-(3-chlorophenyl)piperazin-1-yl)-2-oxo-1-phenylethyl)acetamide (47) – ¹³C NMR

*N*-(2-(4-(4-chlorophenyl)piperazin-1-yl)-2-oxo-1-phenylethyl)acetamide (48) – ¹H NMR
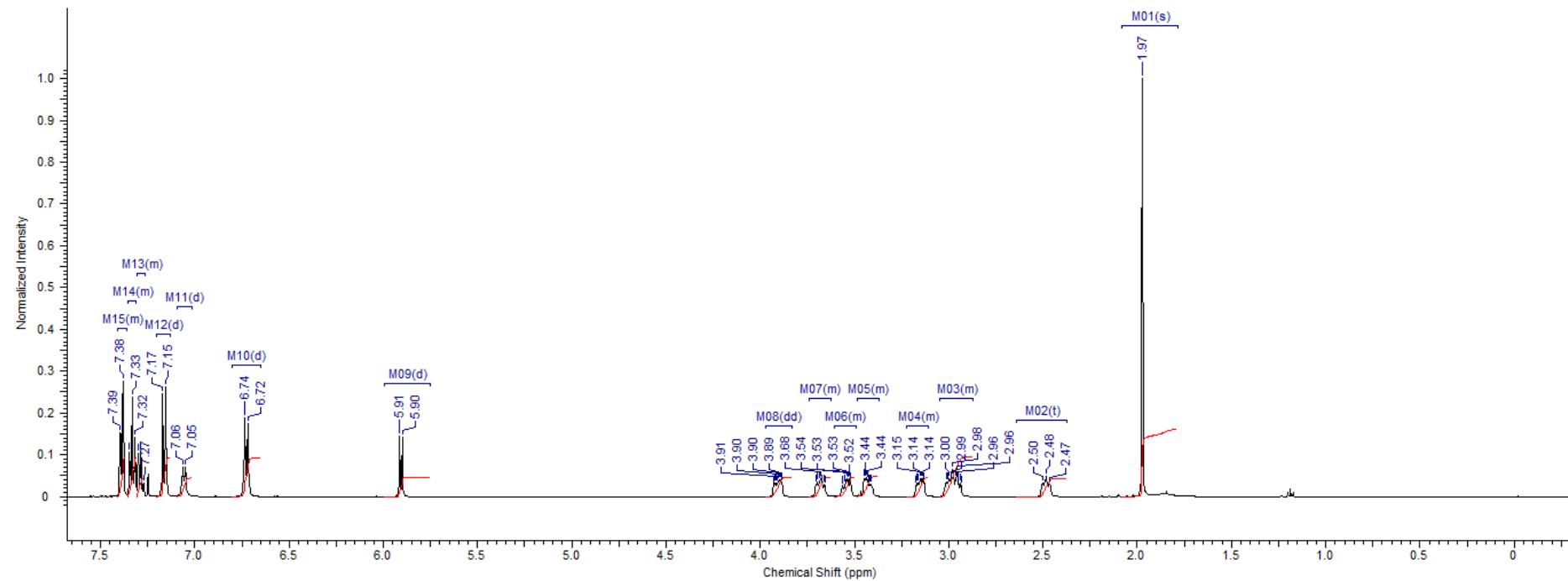
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N-(2-(4-(4-chlorophenyl)piperazin-1-yl)-2-oxo-1-phenylethyl)acetamide (48) – ¹³C NMR
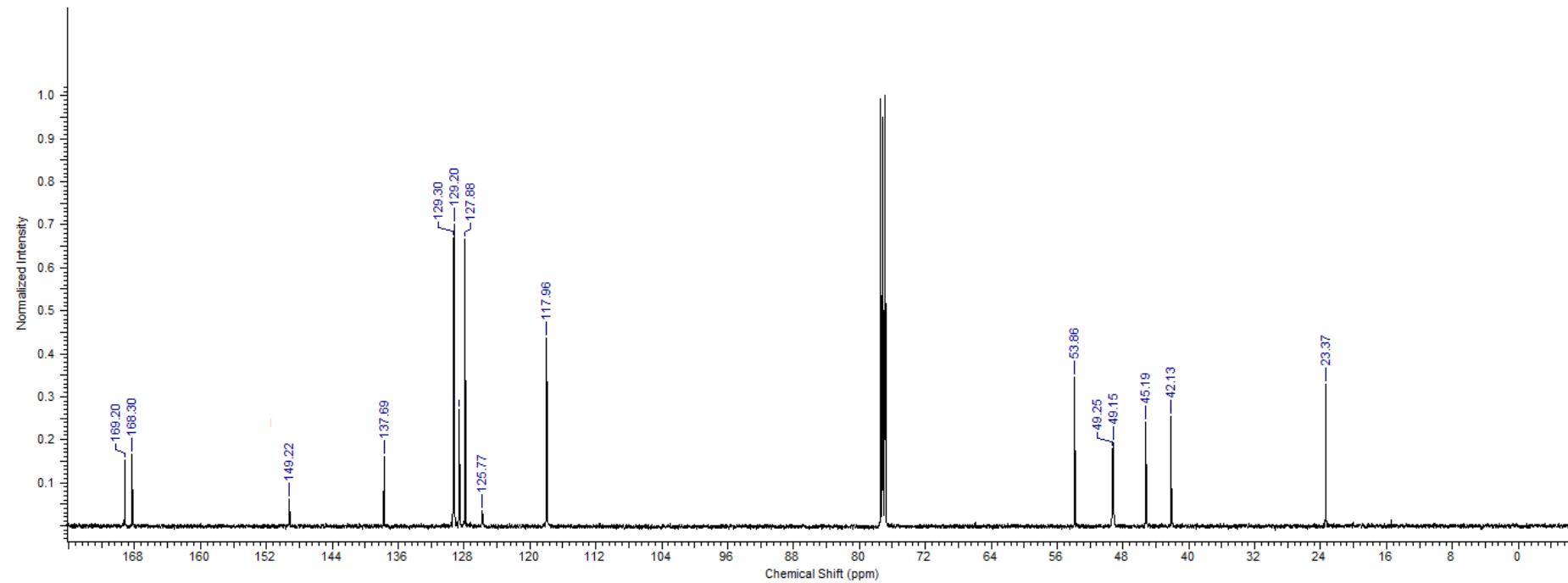
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N-(2-(4-(3,4-dichlorophenyl)piperazin-1-yl)-2-oxo-1-phenylethyl)acetamide (49) – ¹H NMR

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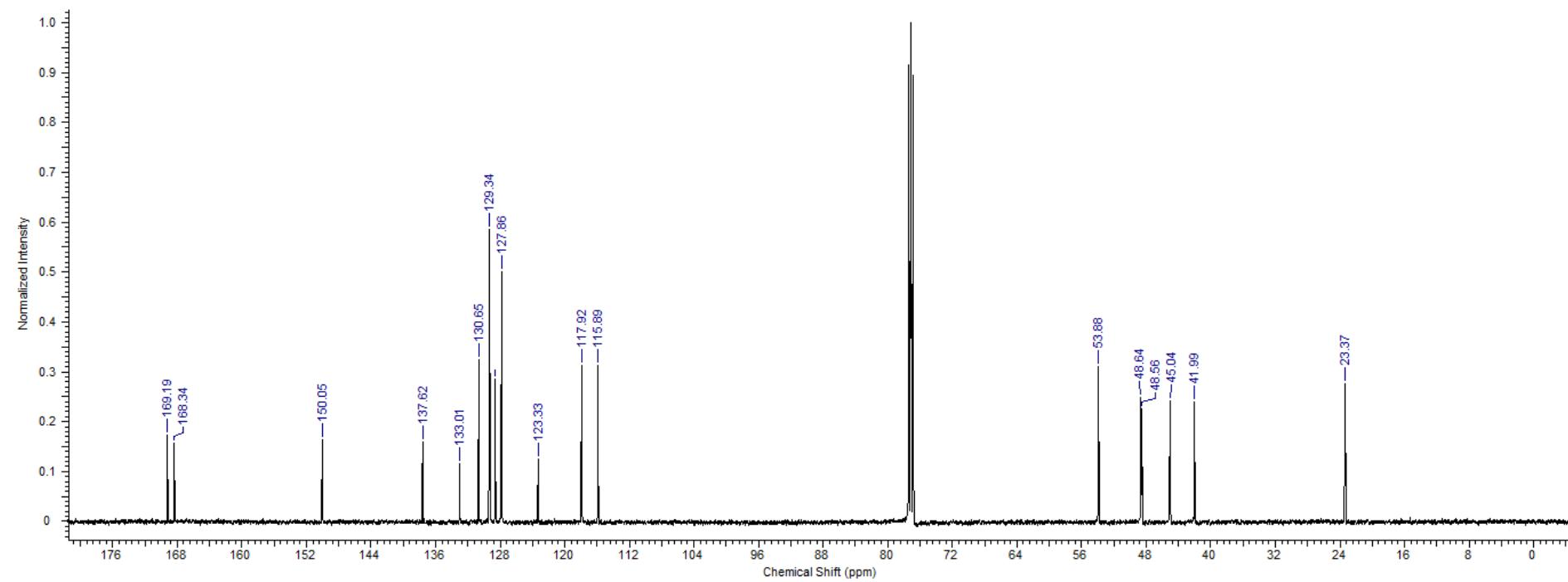
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*N-(2-(4-(3,5-dichlorophenyl)piperazin-1-yl)-2-oxo-1-phenylethyl)acetamide (50) – ¹H NMR*

N-(2-(4-(3,5-dichlorophenyl)piperazin-1-yl)-2-oxo-1-phenylethyl)acetamide (50) – ^{13}C NMR
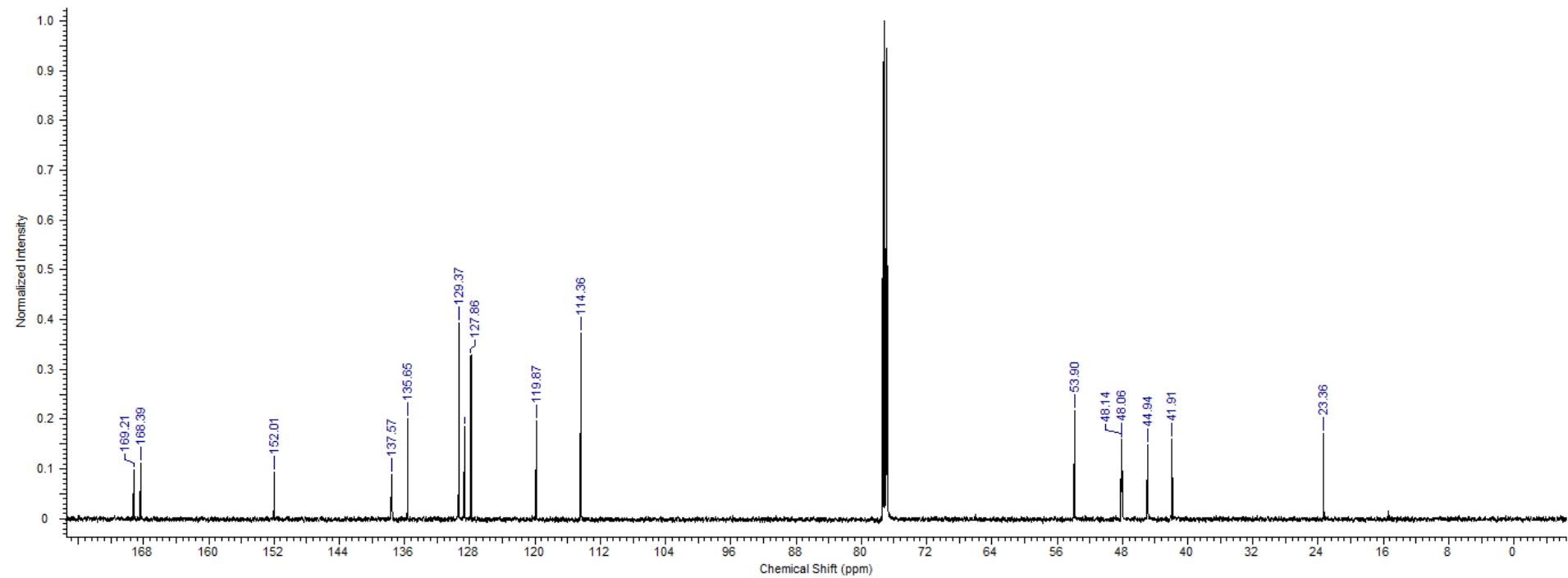
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**N-(2-(4-(3-chloro-5-(trifluoromethyl)phenyl)piperazin-1-yl)-2-oxo-1-phenylethyl) acetamide (51) – ¹H NMR**

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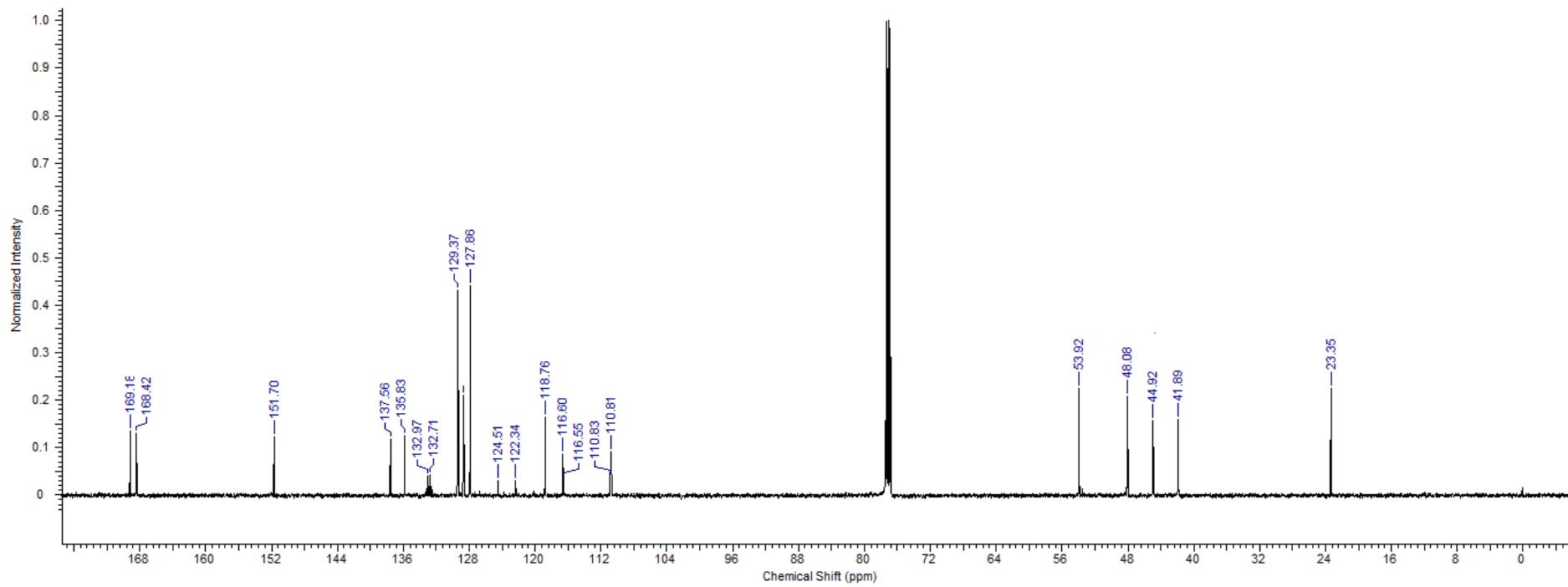
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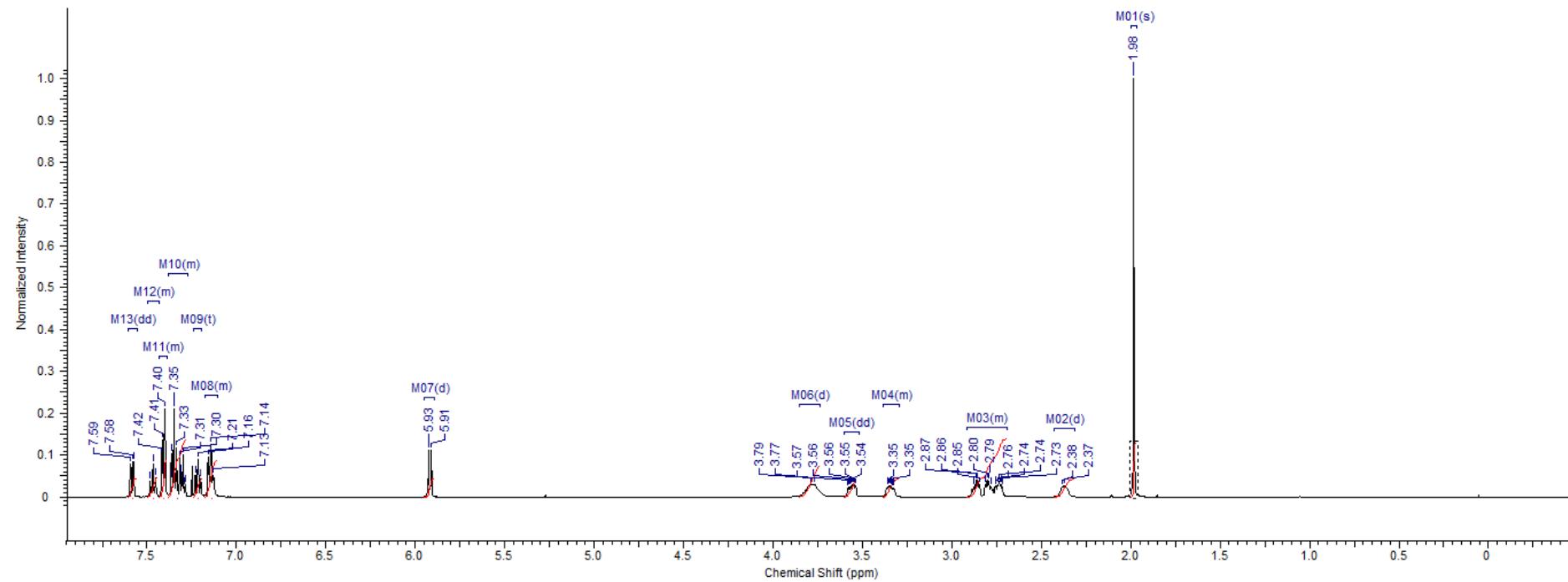
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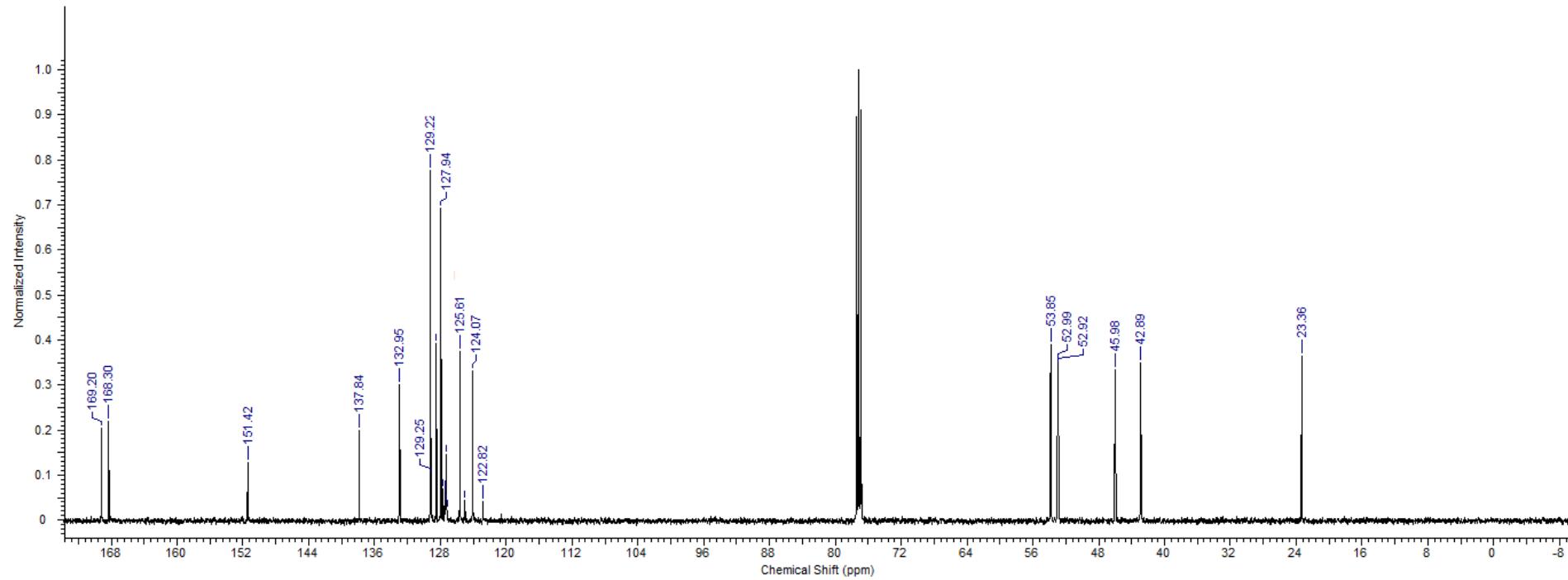
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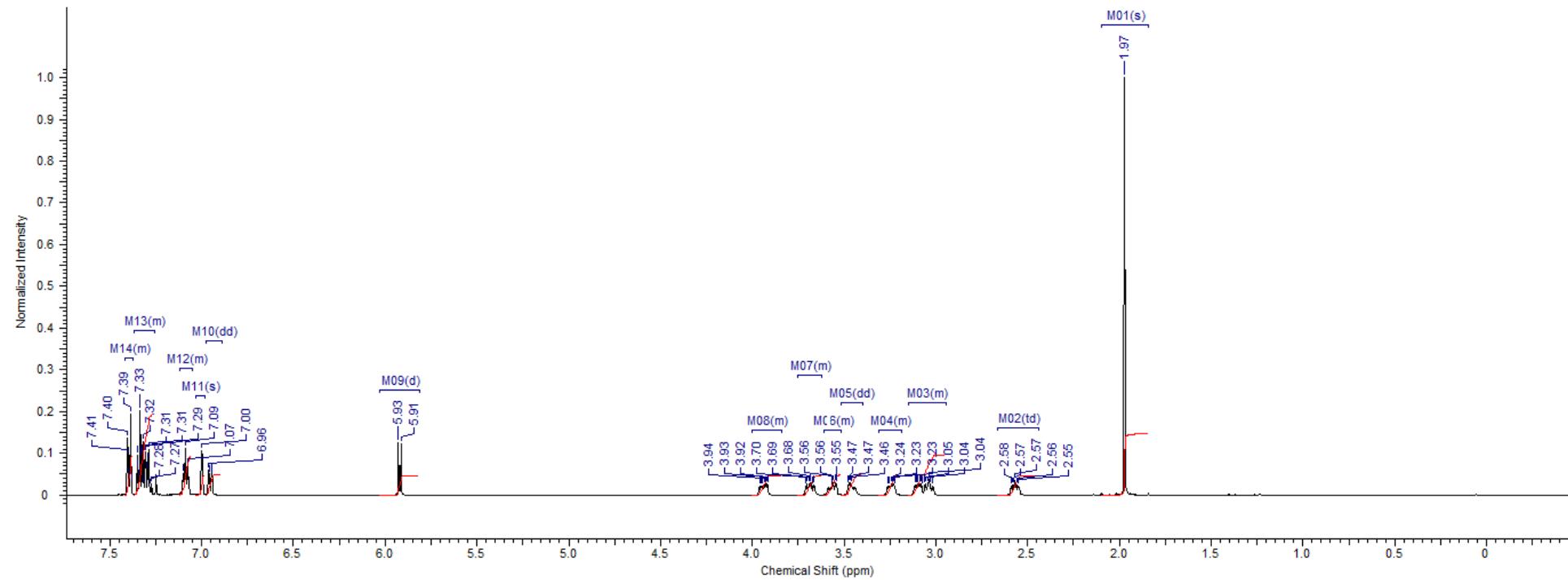
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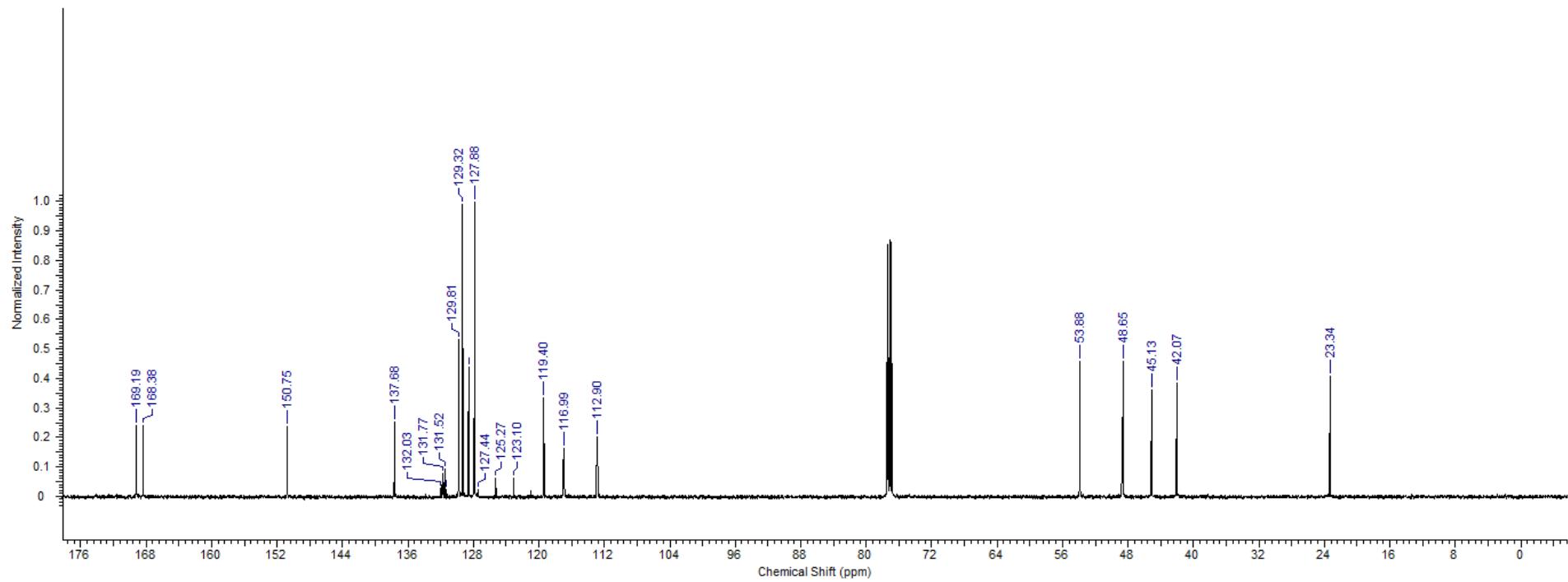
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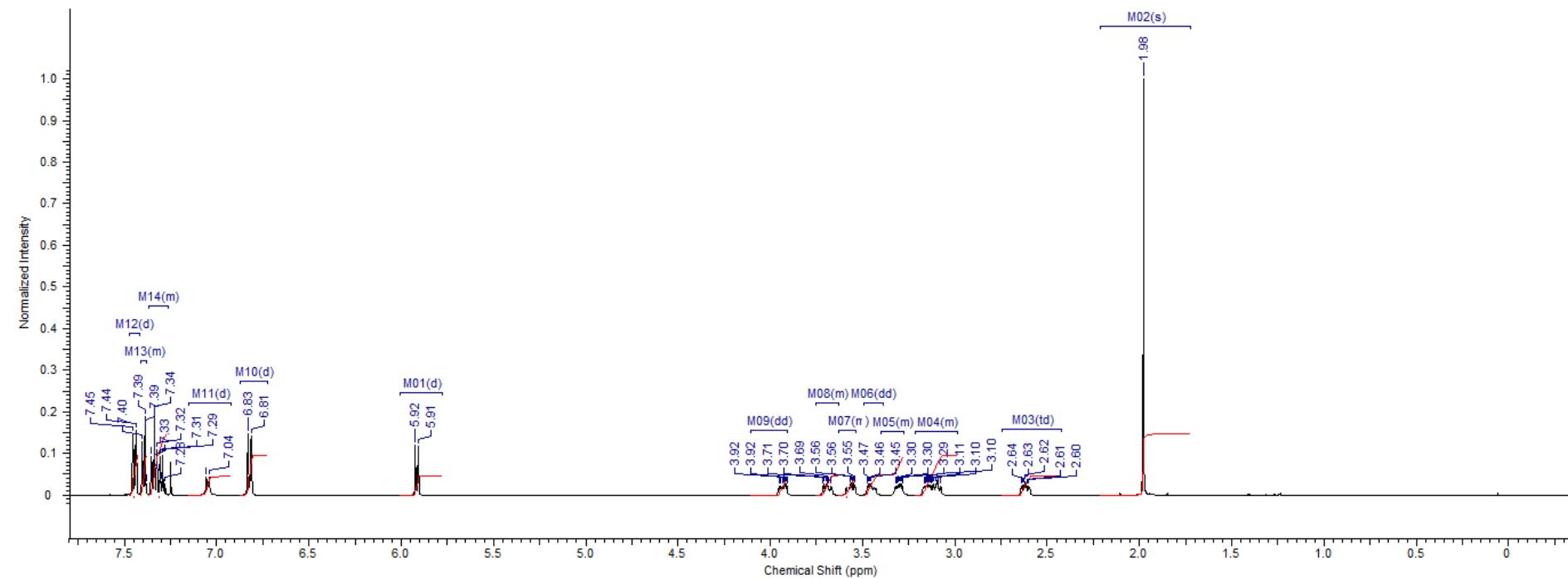
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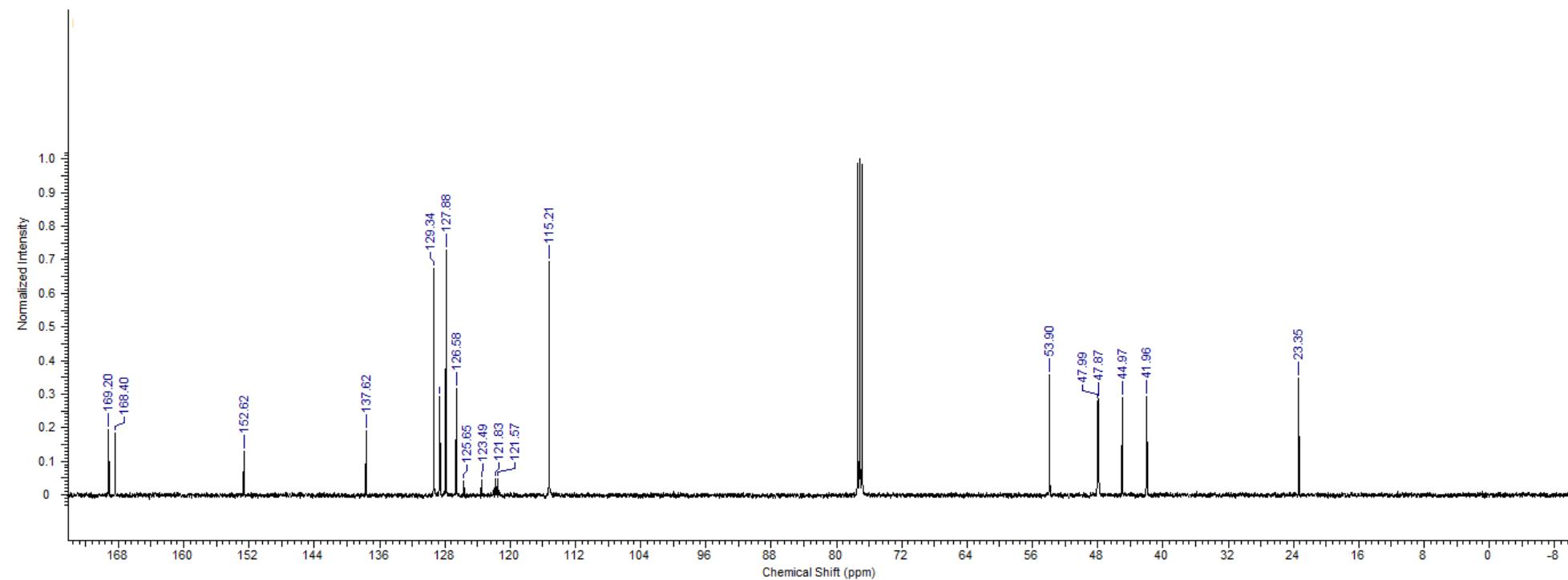
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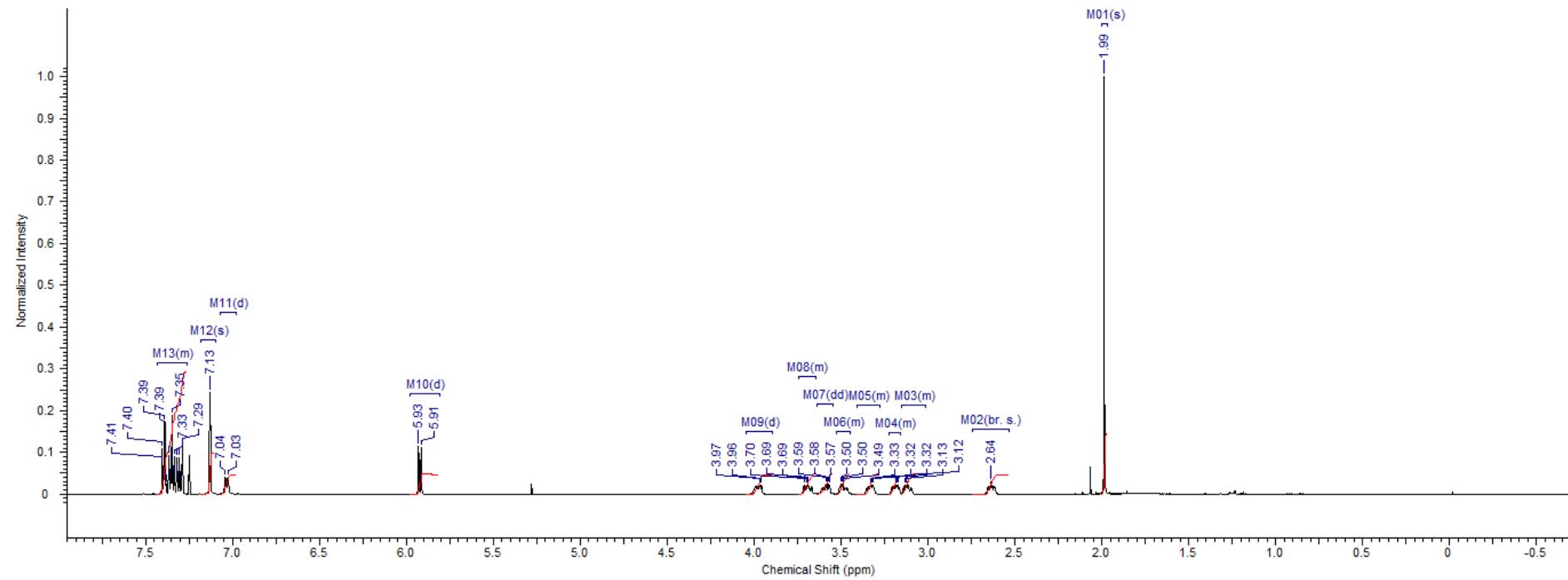
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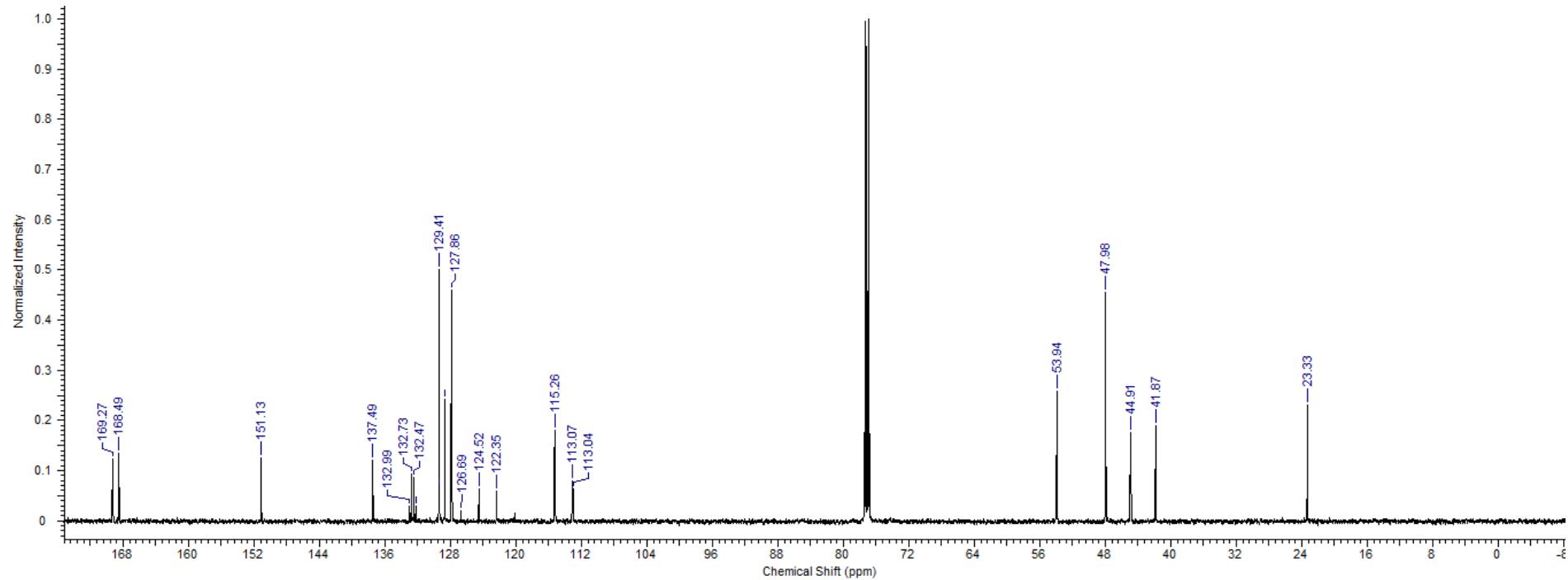
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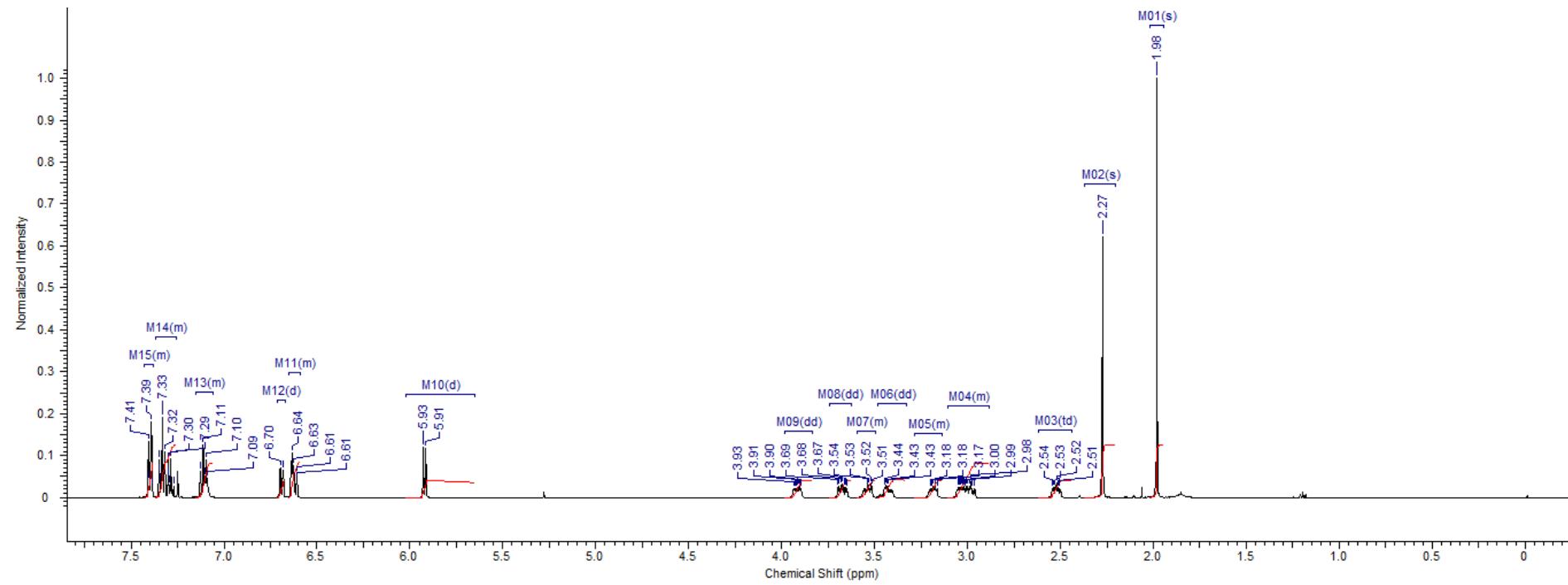
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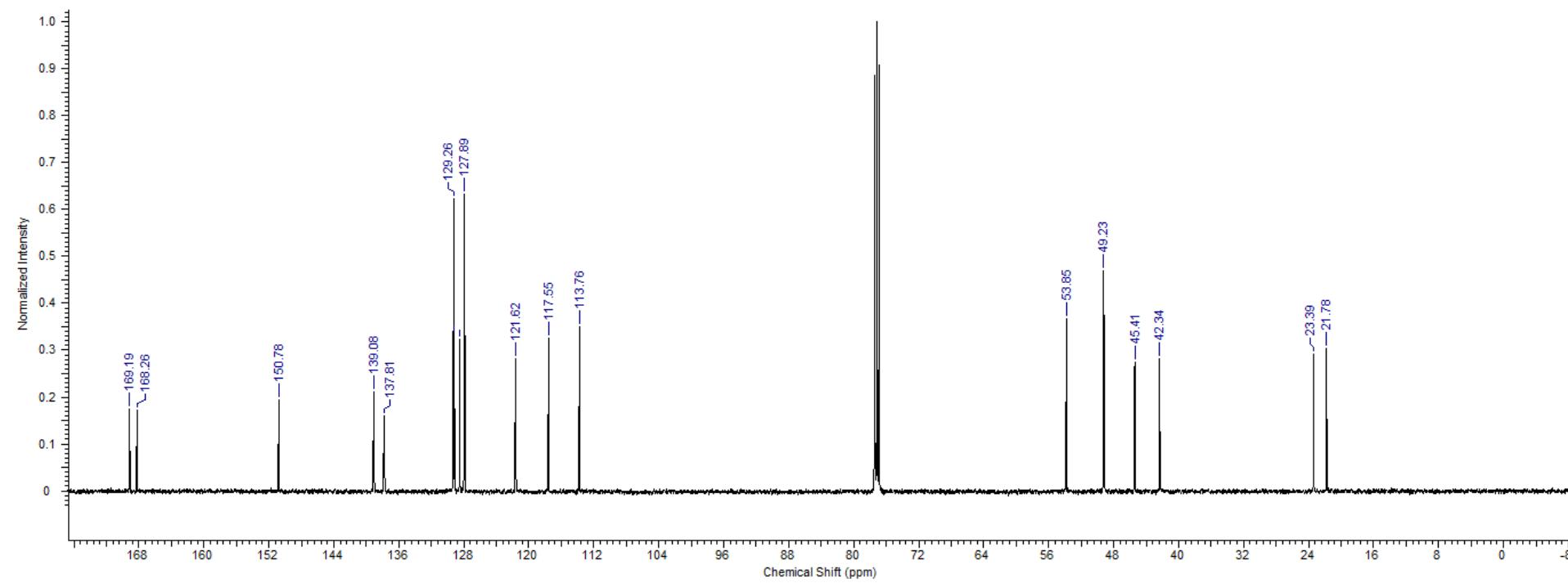
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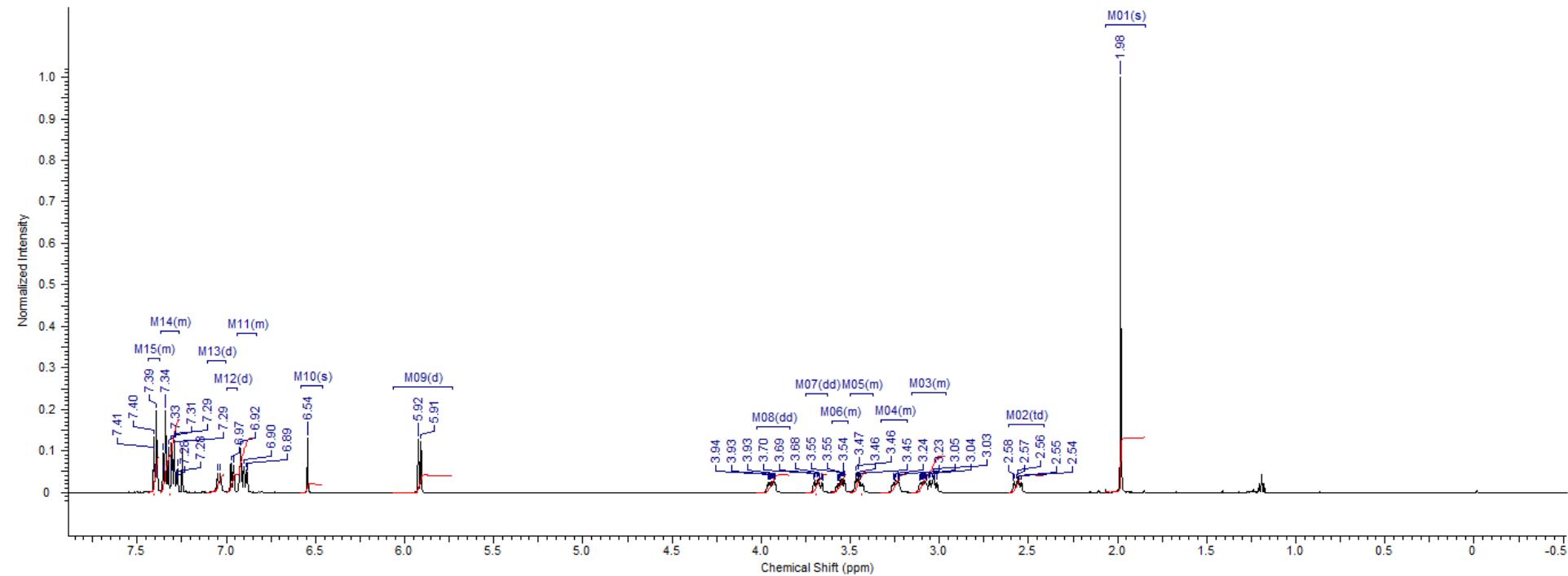
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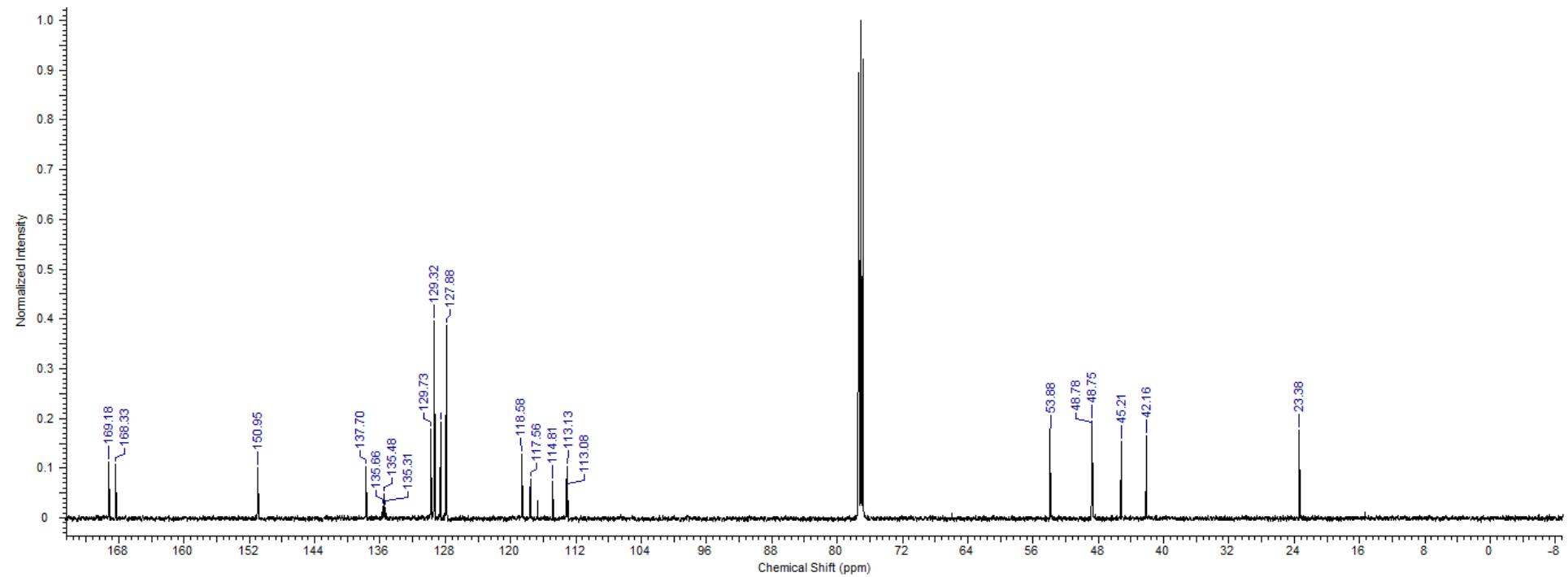
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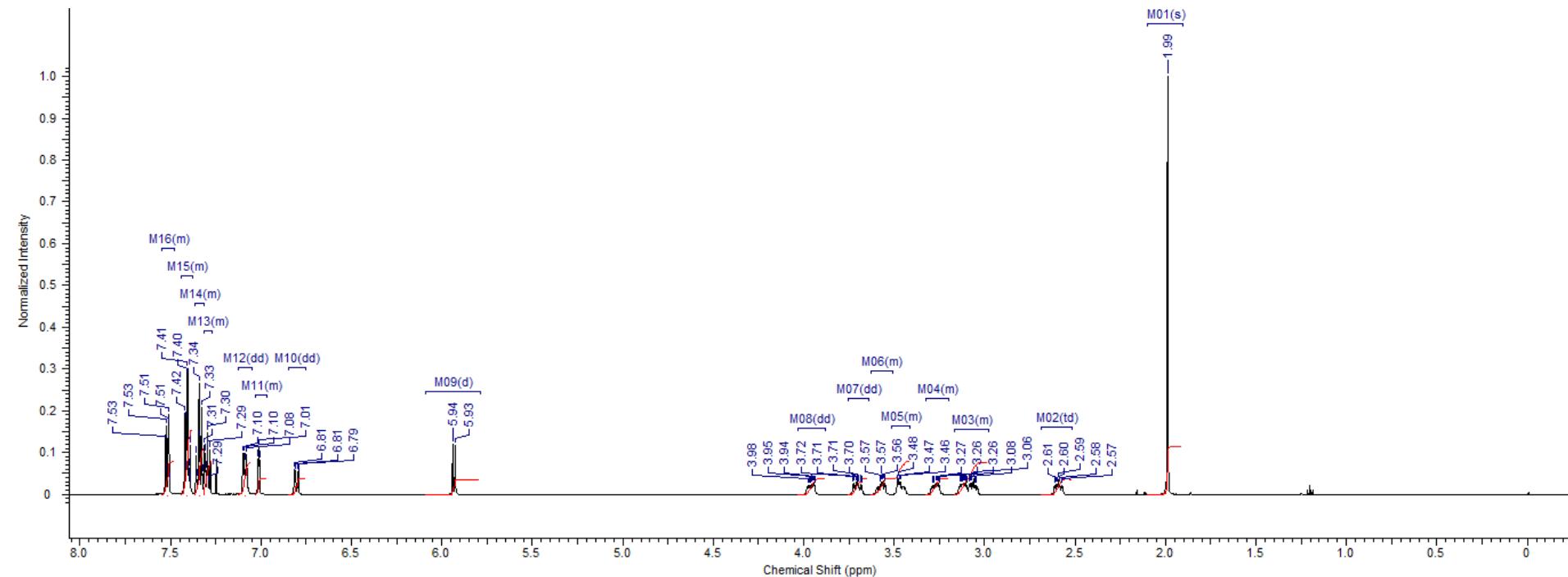
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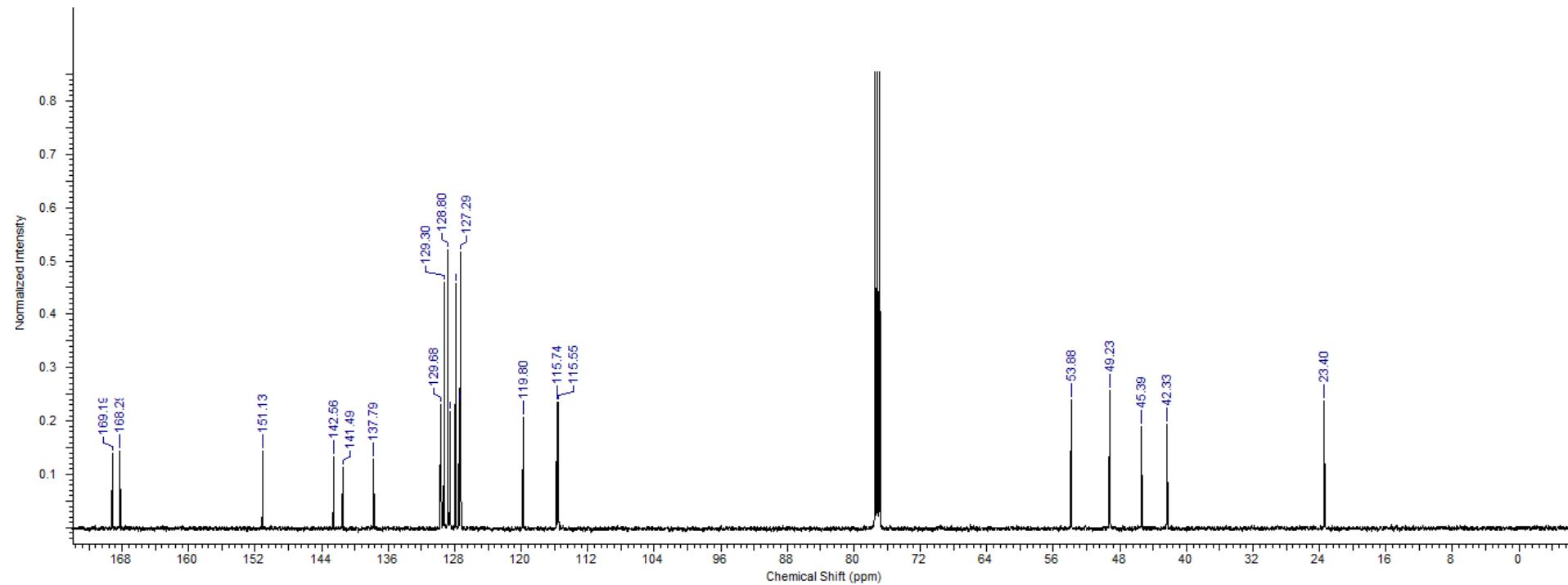
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N-(2-(4-(3-methoxyphenyl)piperazin-1-yl)-2-oxo-1-phenylethyl)acetamide (59) – ^{13}C NMR
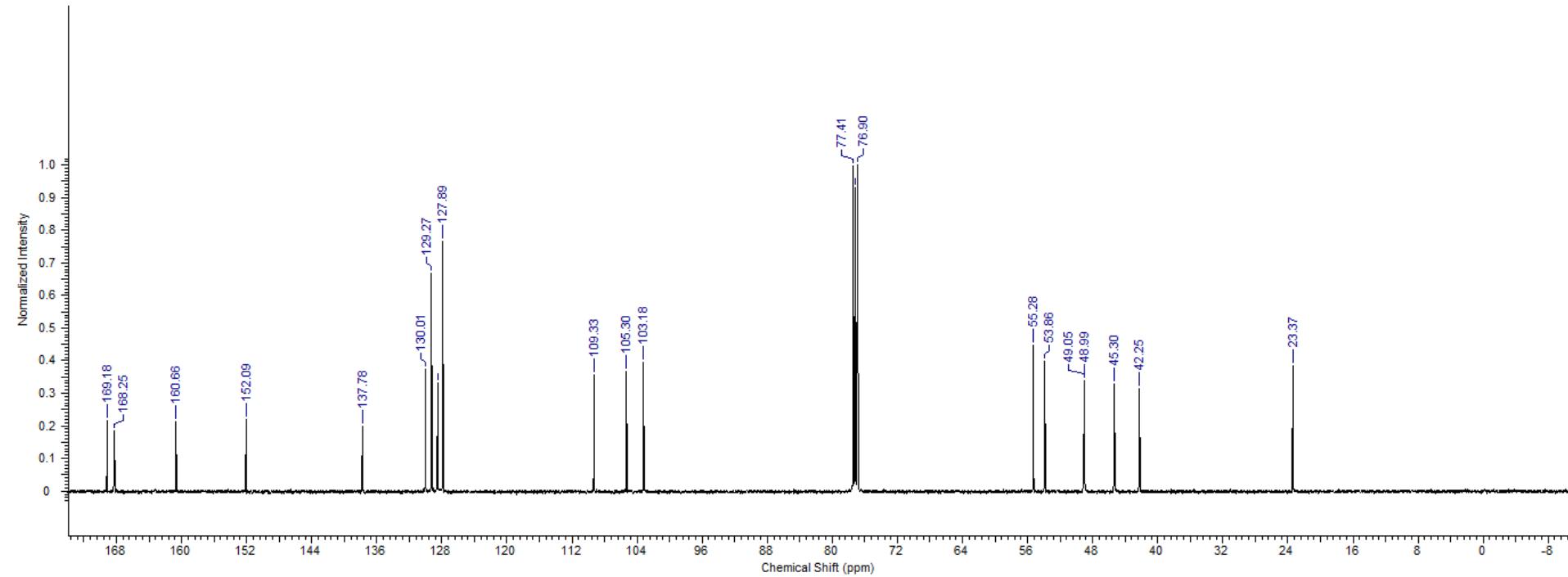
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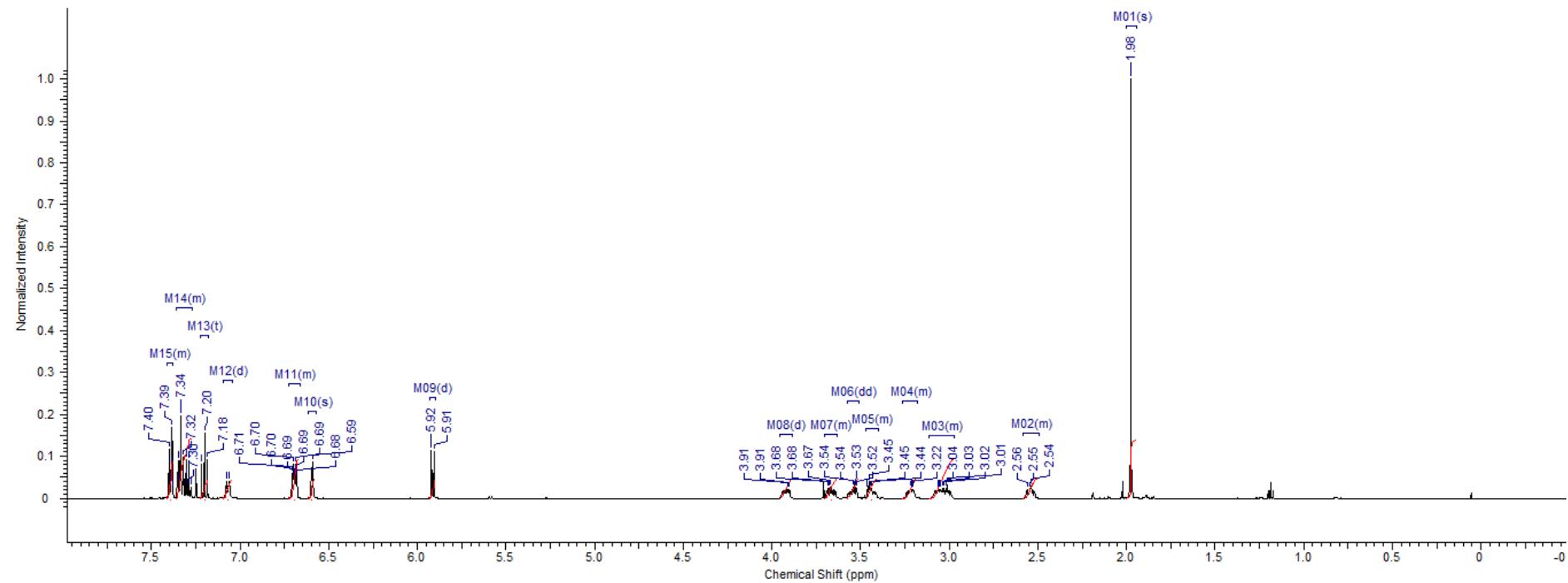
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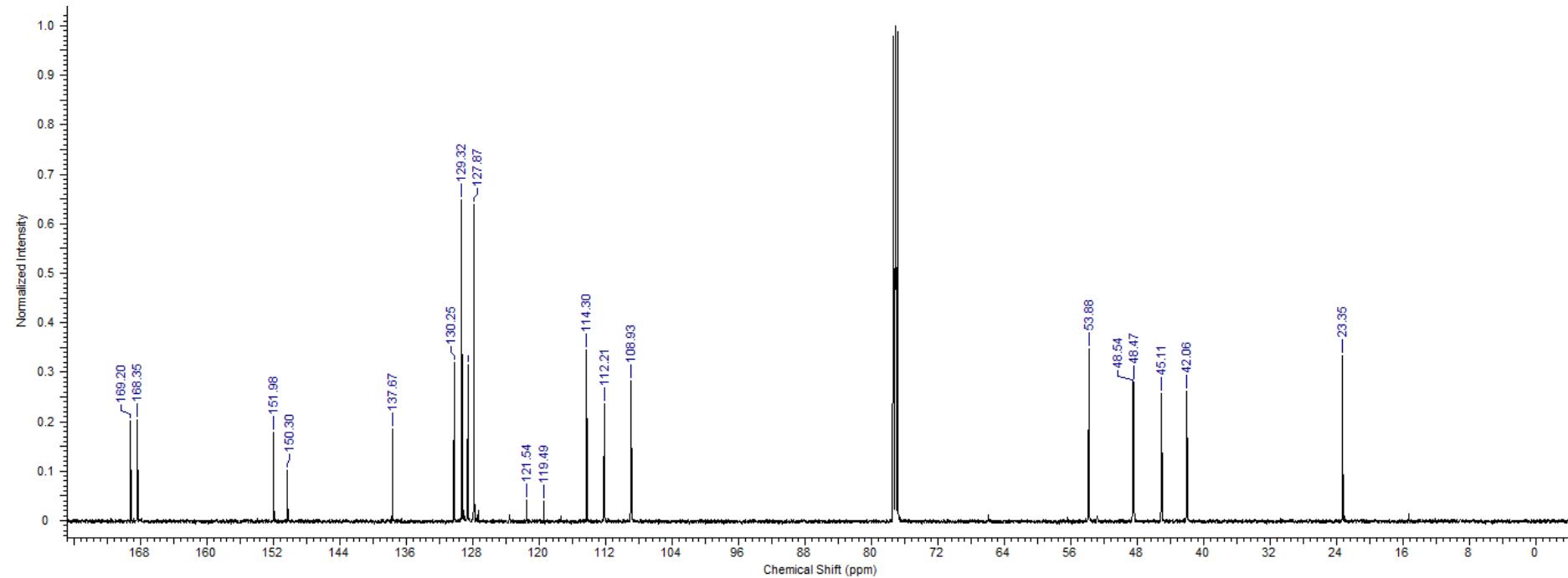
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*N*-(2-oxo-2-(4-(3-phenoxyphenyl)piperazin-1-yl)-1-phenylethyl)acetamide (61) – ^{1}H NMR

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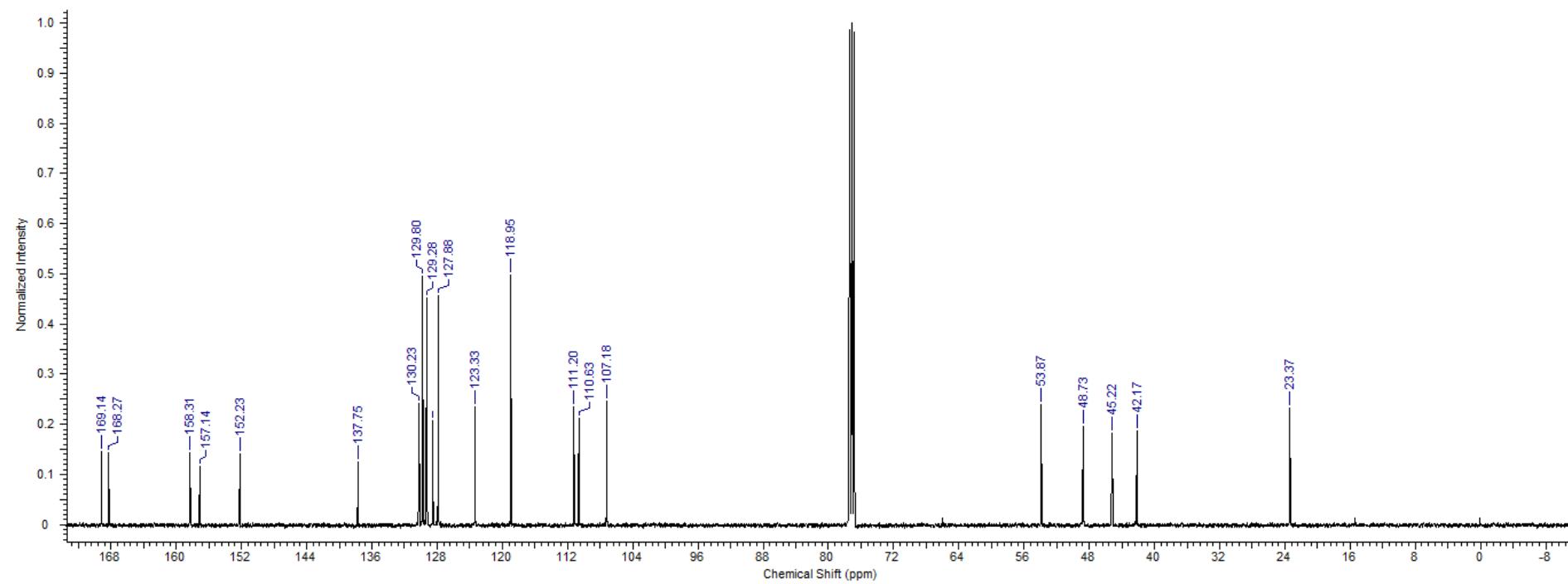
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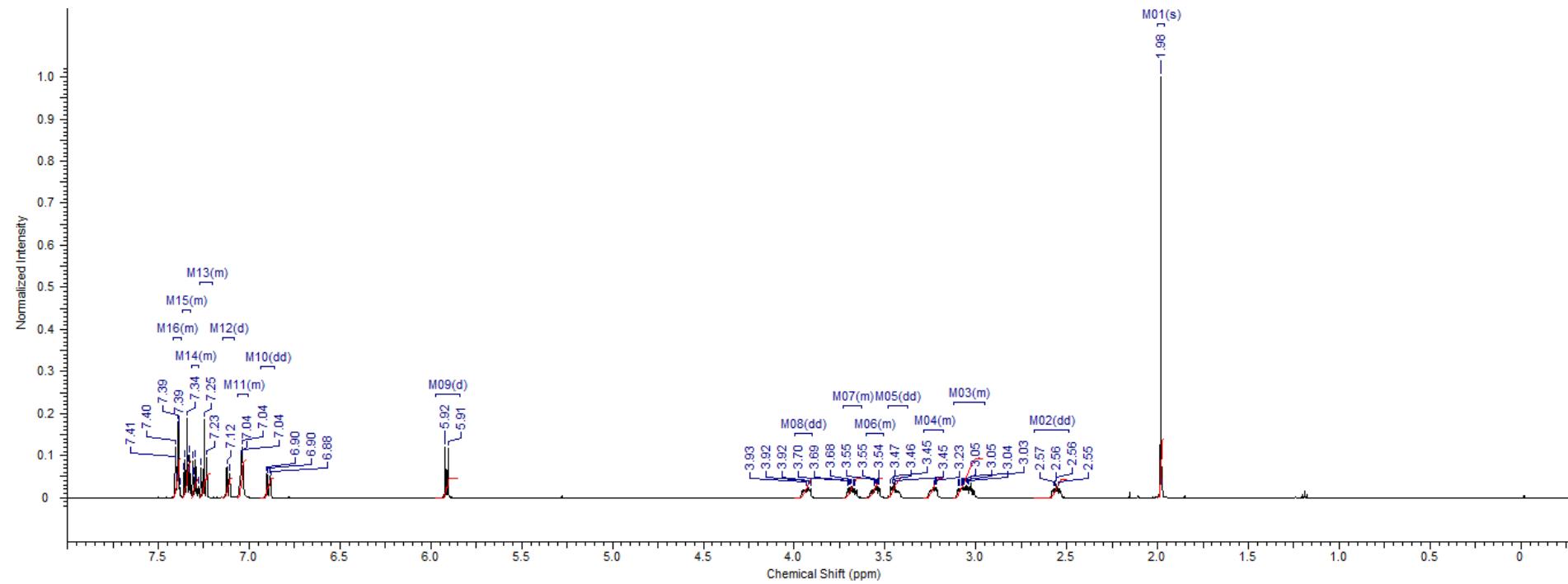
*N*-(2-oxo-1-phenyl-2-(4-(3-((trifluoromethyl)thio)phenyl)piperazin-1-yl)ethyl)acetamide (62) – ^{13}C NMR
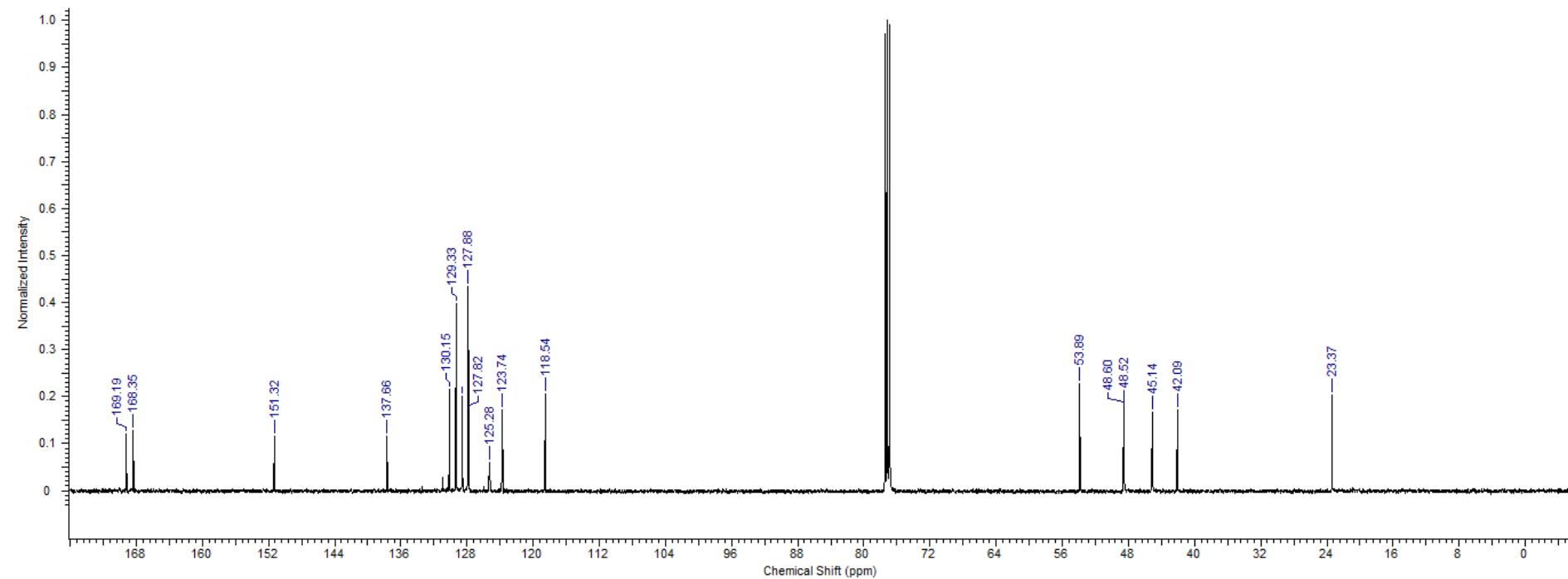
N-(2-oxo-1-phenyl-2-(4-(3-(trifluoromethyl)pyridin-2-yl)piperazin-1-yl)ethyl)acetamide (63) – ¹H NMR
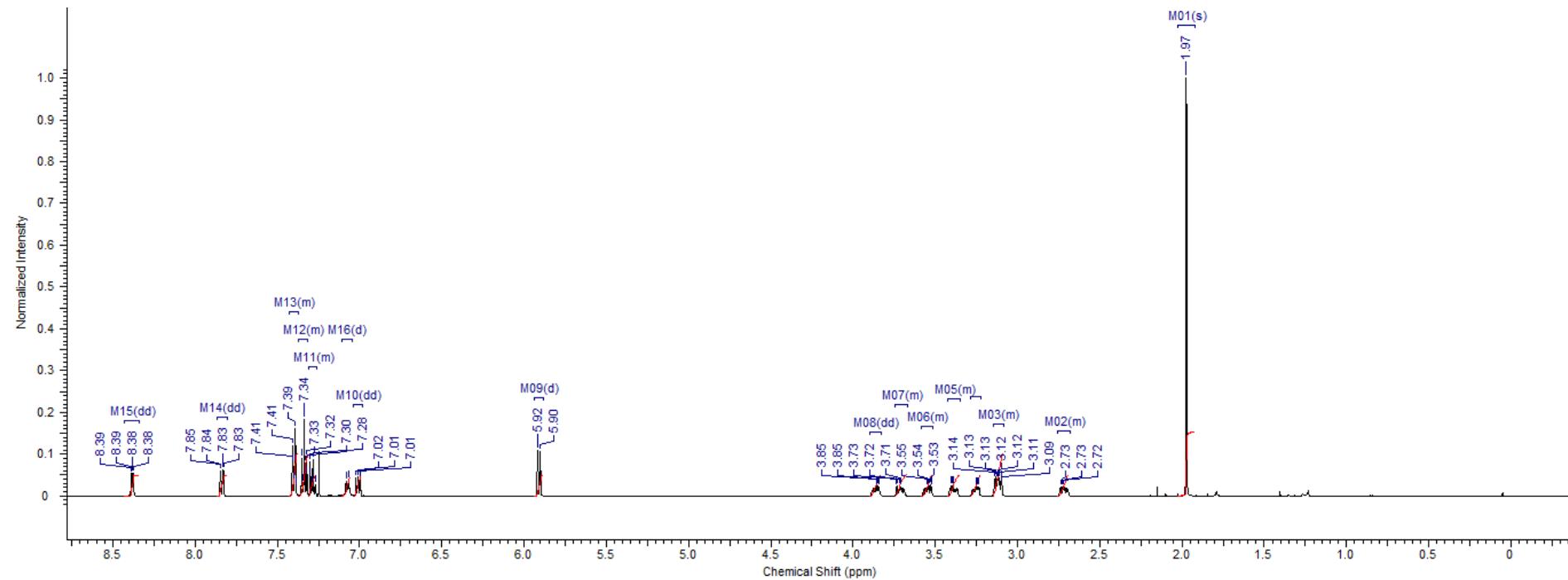
Supporting Information

*N*-(2-oxo-1-phenyl-2-(4-(3-(trifluoromethyl)pyridin-2-yl)piperazin-1-yl)ethyl)acetamide (63) – ^{13}C NMR
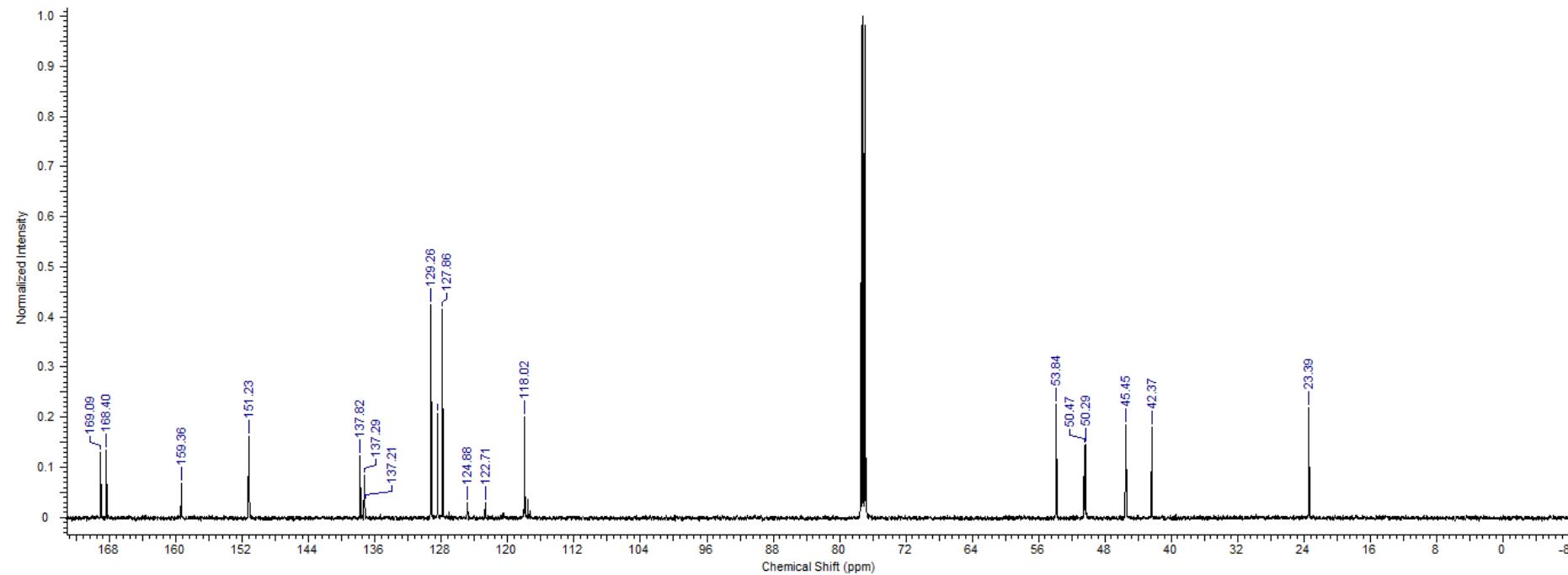
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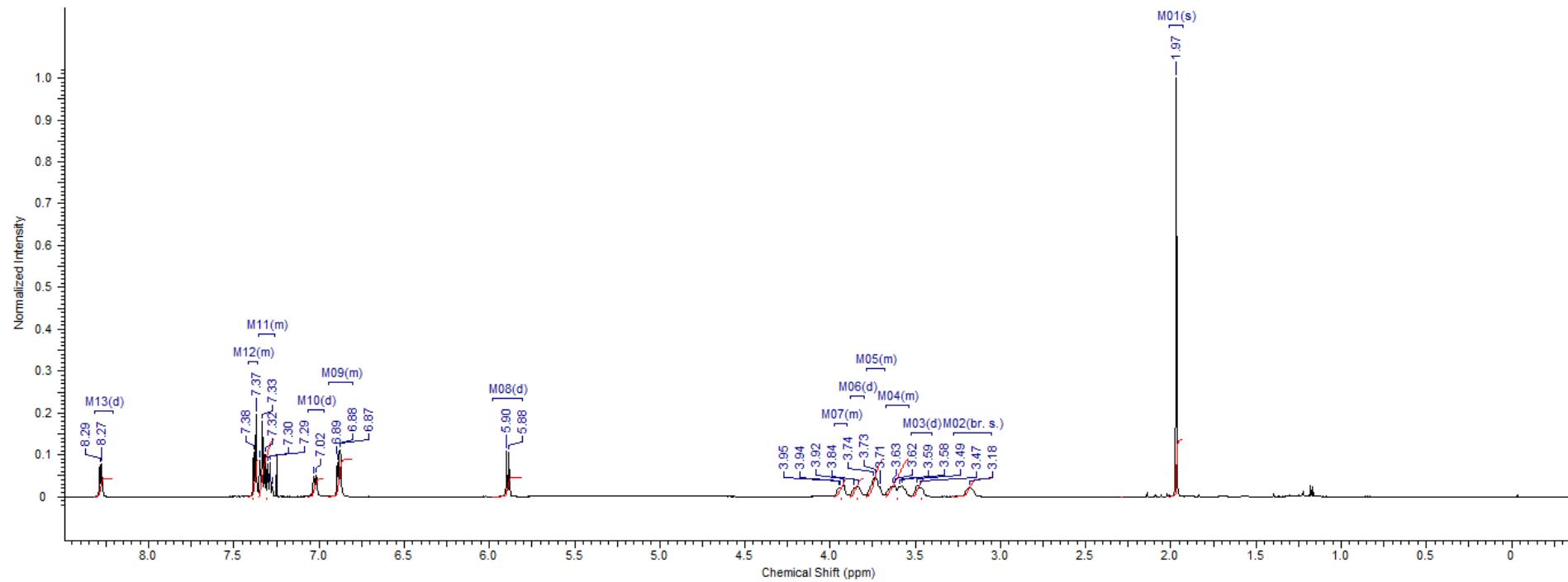
N-(2-oxo-1-phenyl-2-(4-(4-(trifluoromethyl)pyridin-2-yl)piperazin-1-yl)ethyl)acetamide (64) – ^{13}C NMR
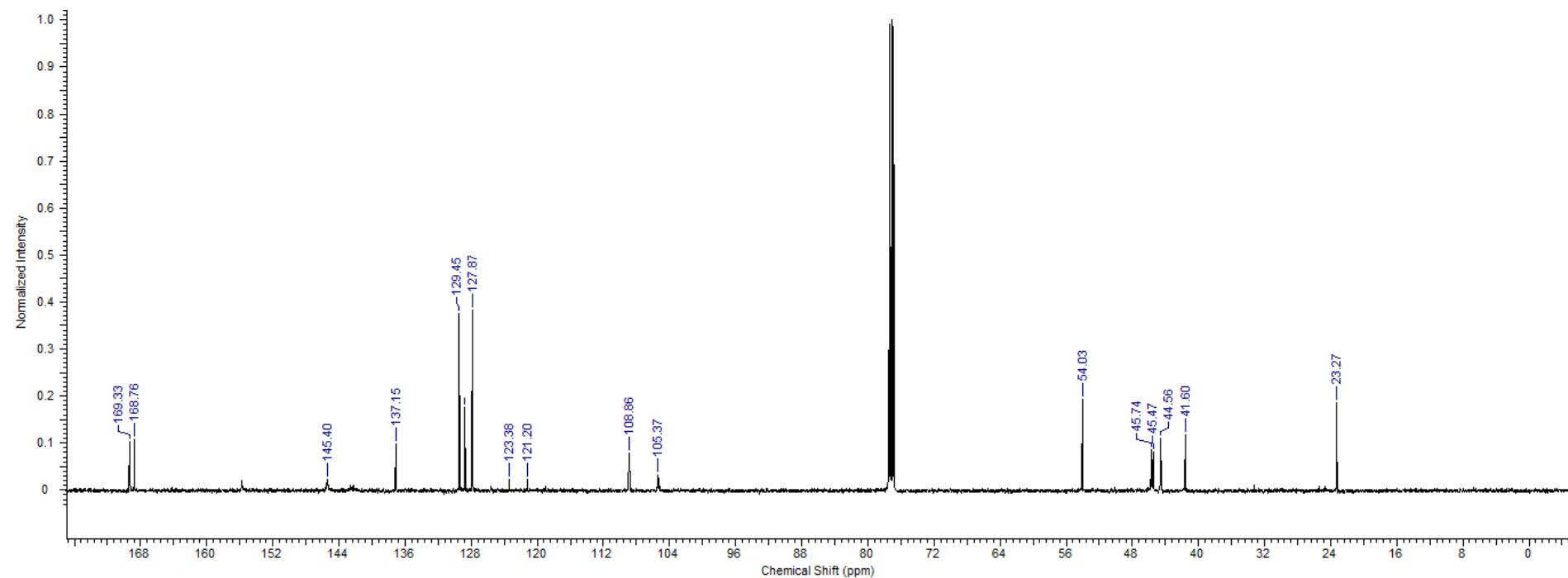
N-(2-oxo-1-phenyl-2-(4-(5-(trifluoromethyl)pyridin-2-yl)piperazin-1-yl)ethyl)acetamide (65) – ^{1}H NMR
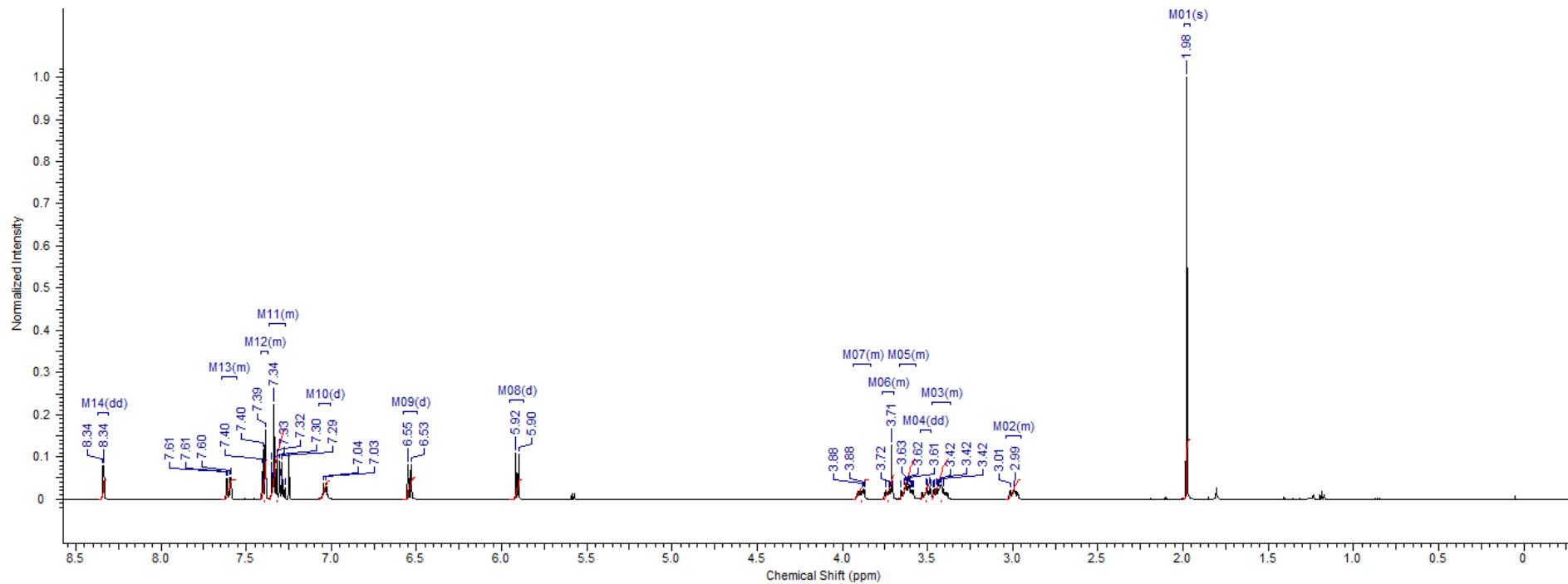
N-(2-oxo-1-phenyl-2-(4-(5-(trifluoromethyl)pyridin-2-yl)piperazin-1-yl)ethyl)acetamide (65) – ^{13}C NMR
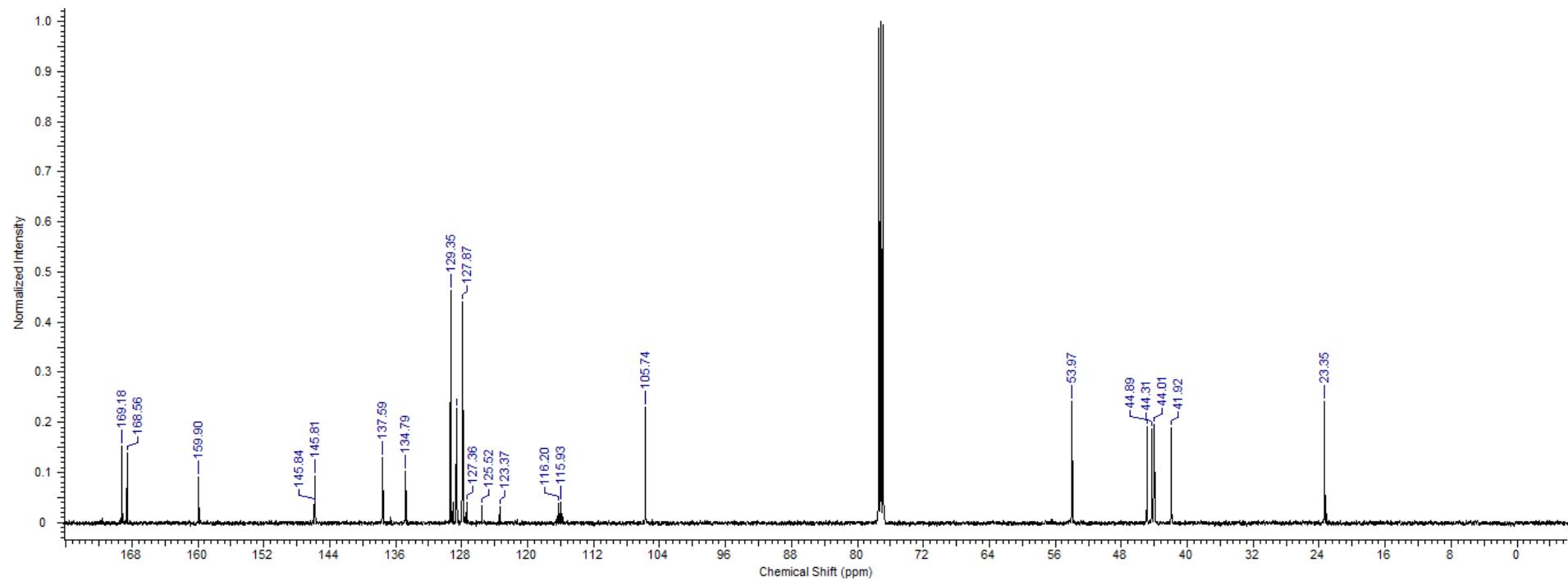
Supporting Information

N-(2-oxo-1-phenyl-2-(4-(6-(trifluoromethyl)pyridin-2-yl)piperazin-1-yl)ethyl)acetamide (66) – ¹H NMR

Supporting Information

N-(2-oxo-1-phenyl-2-(4-(6-(trifluoromethyl)pyridin-2-yl)piperazin-1-yl)ethyl)acetamide (66) – ^{13}C NMR