Supporting Information for

Amidinoquinoxaline *N*-oxides: Synthesis and activity against anaerobic bacteria

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Table S1: *In vitro* activity of amidinoquinoxaline *N*-oxides **1**, **2** against clinical isolates of relevant anaerobic species (complete version)

Organism (nº of isolates) and compounds	MIC (µg/mL)		
	Range	MIC_{50}	MIC_{90}
Gram negative bacilli			
Bacteroides fragilis			
1a (19)	0.125 - >32	0,5	1
1b (19)	0.5 - >32	1	2
1c (19)	≤0.06 - 0.25	≤0.06	≤0.06
1d (19)	0.5 - >32	1	2
1e (16)	0.125 – 0.5	0.25	0.5
1f (16)	≤0.06 - 0.125	≤0.06	≤0.06
1g (16)	0.125 - 1	0.25	0.5
1h (16)	0.5 - 4	1	4
1i (16)	≤0.06 - 0.125	≤0.06	≤0.06
1j (16)	≤0.06 - 0.125	≤0.06	0.125
1k (16)	≤0.06 - 0.125	≤0.06	≤0.06
1l (16)	≤0.06 - 0.125	≤0.06	≤0.06
1m (19)	0.5 - 2	1	2
2a (16)	0.5 - 4	2	2
2b (16)	1-8	4	8
2c (16)	0.125 - 2	0.5	1
Mtz (16)	0.25 - 1	0.5	1
Other *Bacteroides* spp. and *Parabacteroides*[a]			
1a (13)	0.25 - 1	0.5	1
1b (13)	0.5 - 2	1	1
1c (13)	≤0.06 - 0.125	≤0.06	≤0.06
1d (13)	0.5 - 2	1	2
1e (13)	0.125 - 1	0.25	0.5
1f (13)	≤0.06 - 0.125	≤0.06	≤0.06
1g (13)	0.25 - 1	0.25	0.5
1h (13)	1 - 4	1	4
1i (13)	≤0.06 - ≤0.06	≤0.06	≤0.06
1j (13)	≤0.06 – 0.125	≤0.06	0.125
1k (13)	≤0.06 - ≤0.06	≤0.06	≤0.06
1l (13)	≤0.06 – 0.125	≤0.06	≤0.06
1m (13)	0.5 - 2	1	2
2a (13)	1-4	2	4

Organism (nº of isolates) and compounds	MIC (μg/mL)		
	Range	MIC_{50}	MIC_{90}
2b (13)	2 - 8	4	8
2c (13)	0.25 - 2	0.25	1
Mtz (13)	0.25 - 1	1	1
Prevotella spp.			
1a (21)[b]	≤0.06 - 1	0.25	1
1b (21)[b]	≤0.06 - 1	0.5	1
1c (21)[b]	≤0.06 – 0.125	≤0.06	0.125
1d (21)[b]	0.125 - 2	0.5	1
1e (14)[c]	0.125-0.5	0.25	0.5
1f (14)[c]	≤0.06 – 0.125	≤0.06	0.125
1g (14)[c]	≤0.06 – 1	0.125	0.5
1h (14)[c]	0.5-4	1	4
1i (14)[c]	≤0.06-≤0.06	≤0.06	≤0.06
1j (14)[c]	≤0.06-≤0.06	≤0.06	≤0.06
1k (14)[c]	≤0.06-≤0.06	≤0.06	≤0.06
1l (14)[c]	≤0.06-0,25	≤0.06	≤0.06
1m (21)[b]	0.125-0.5	0.5	0.5
2a (14)[c]	1-8	4	8
2b (14)[c]	4-16	4	8
2c (14)[c]	≤0.06-2	0.5	1
Mtz (21)[b]	0.25-2	0.5	1
Fusobacterium nucleatum			
1a (7)	0.125-1	0.25	1
1b (7)	0.25-1	0.25	1
1c (7)	≤0.06 – 0.125	≤0.06	≤0.06
1d (7)	0.5-2	0.5	2
1e (7)	0.25-1	0.25	0.25
1f (7)	≤0.06 – 0.25	0.125	0.125
1g (7)	≤0.06 – 1	0.25	0.5
1h (7)	0.5-4	1	4
1i (7)	≤0.06 – 0.125	≤0.06	0.125
1j (7)	≤0.06 – 0.125	≤0.06	0.125
1k (7)	≤0.06 -≤0,06	≤0.06	≤0.06
1l (7)	≤0.06 – 0.125	≤0.06	≤0.06
1m (7)	0.25-0.5	0.5	0.5
2a (7)	2-16	4	4
2b (7)	4-16	4	8
2c (7)	0.25-1	0.25	1
Mtz (7)	≤0.06 – 0.25	≤0.06	0.25

Organism (nº of isolates) and compounds	MIC (µg/mL)		
	Range	MIC_{50}	MIC_{90}
Gram positive bacilli			
Clostridium difficile			
1a (9)	2 - 4	2	4
1b (9)	8 - 16	8	16
1c (9)	≤0.06 - 1	0.25	0.5
1d (9)	0.25 - 8	4	8
1e (14)	1 - 8	2	8
1f (14)	0.25 - 1	0.5	0.5
1g (14)	2 - 4	2	2
1h (14)	8 - 32	16	16
1i (14)	0.125 - 1	0.5	0.5
1j (14)	0.125 - 0.25	0.25	0.25
1k (14)	≤0.06 - ≤0.06	≤0.06	≤0.06
1l (14)	0.125 - 1	0.25	0.5
1m (9)	0.25 - 4	2	4
2a (14)	8 - 64	16	32
2b (14)	4 - 32	32	32
2c (14)	0.25 - 1	0.25	0.5
Mtz (14)	≤0.06 - 0.5	0.25	0.25
Clostridium perfringens			
1a (10)	4 - 32	8	16
1b (10)	16 - 64	32	64
1c (10)	0.25 - 2	0.5	2
1d (10)	4 - 64	16	32
1e (10)	1-16	4	8
1f (10)	0.25 - 1	0.5	1
1g (10)	2-8	4	8
1h (10)	32 - >32	>32	>32
1i (10)	0.5 - 1	0.5	1
1j (10)	0.25 - 1	0.5	1
1k (10)	≤0.06 – 0.25	≤0.06	0.125
1l (10)	0.25 - 1	0.5	1
1m (10)	4 - 64	16	32
2a (10)	16-32	32	32
2b (10)	16 - >32	32	32
2c (10)	0.125 - 2	0.25	1
Mtz (10)	0.5 - 2	0.5	1

[a] Includes 3 isolates of *Parabacteroides distasonis* and 10 corresponding to species of *Bacteroides*: 6 *Bacteroides thetaiotaomicron/ovatus*, 2 *Bacteroides uniformis*, 1 *Bacteroides vulgatus* and 1 *Bacteroides caccae.*

[b] Includes 16 isolates of *Prevotella intermedia/nigrescens*, 2 of *Prevotella oralis group*, 2 of *Prevotella buccae* and 1 of *Prevotella bivia.*

[c] Includes 5 isolates of *Prevotella intermedia/nigrescens*, 1 of *Prevotella corporis*, 1 of *Prevotella oralis group*, 1 of *Prevotella oris*, 1 of *Prevotella baroniae*, 2 of *Prevotella buccae*, 2 of *Prevotella bivia* and 1 of *Prevotella dentalis.*

MTZ = metronidazole.

Copies of ^{1}H and ^{13}C NMR spectra

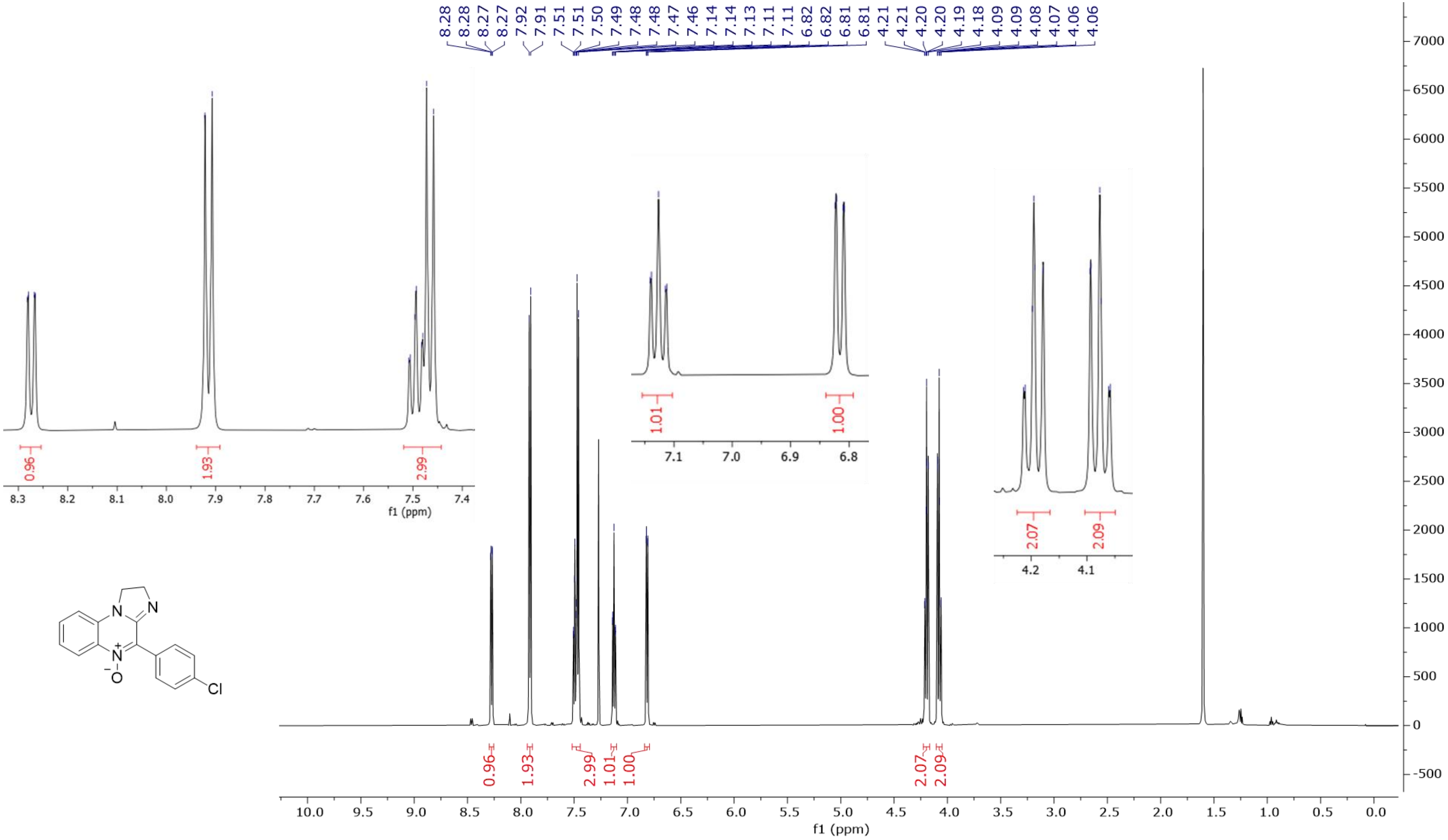


Figure S1: ^{1}H NMR spectrum of compound **1j** (600 MHz, $CDCl_3$)

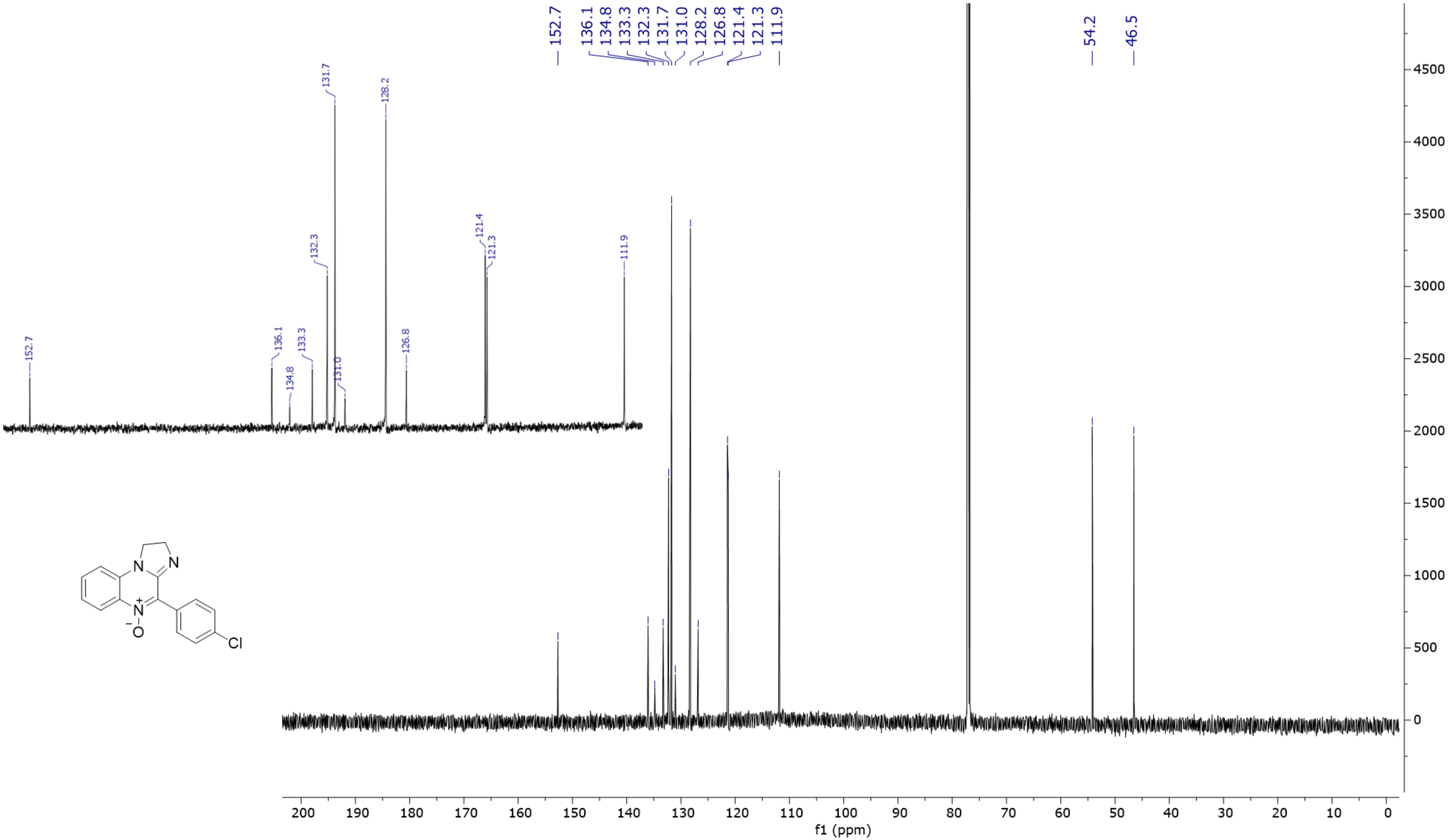


Figure S2: ^{13}C NMR spectrum of compound **1j** (151 MHz, $CDCl_3$)

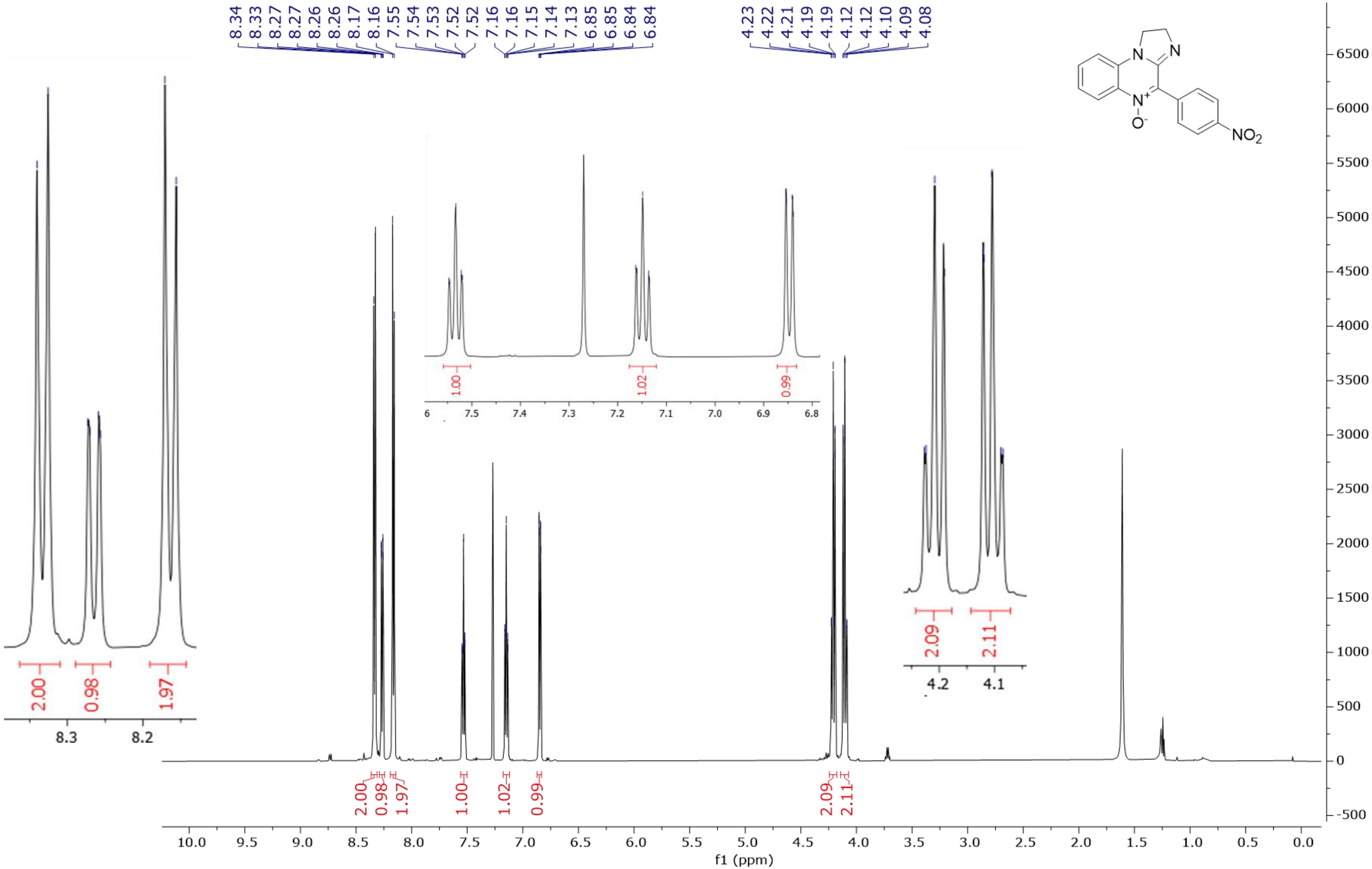


Figure S3: ^{1}H NMR spectrum of compound **1k** (600 MHz, $CDCl_3$)

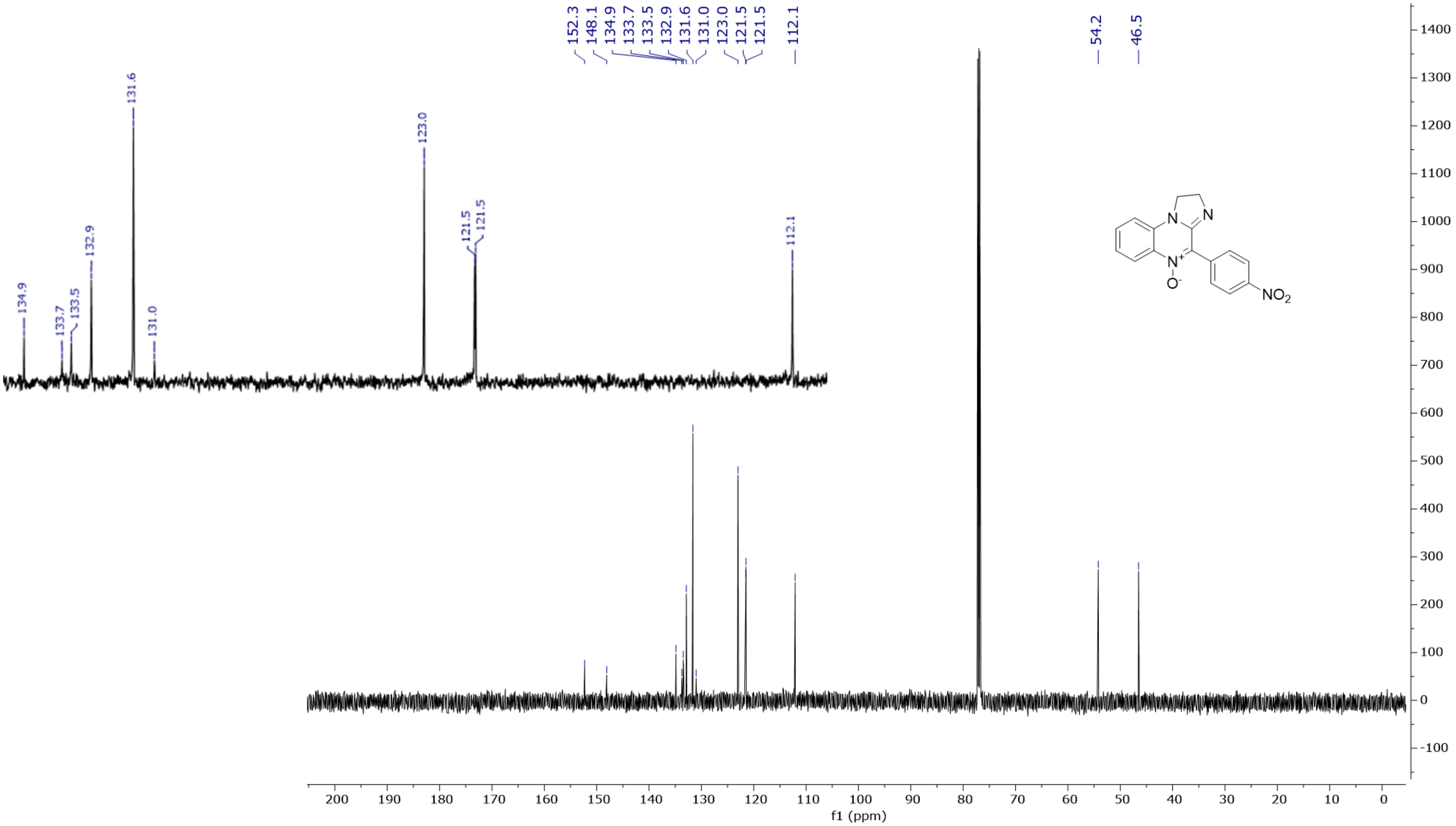


Figure S4: ^{13}C NMR spectrum of compound **1k** (151 MHz, $CDCl_3$)

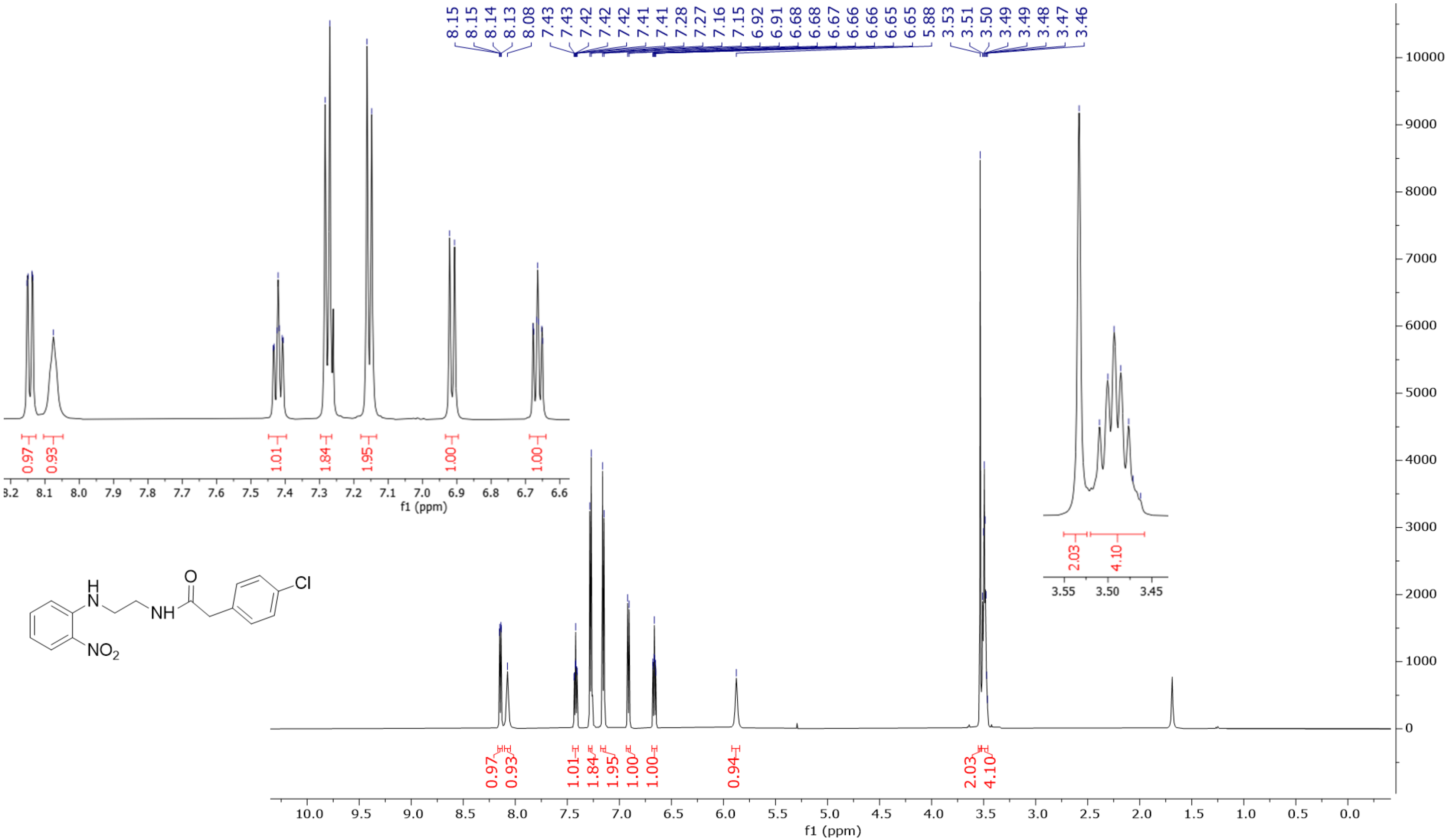


Figure S5:[1]H NMR spectrum of compound **3j** (600 MHz, $CDCl_3$)

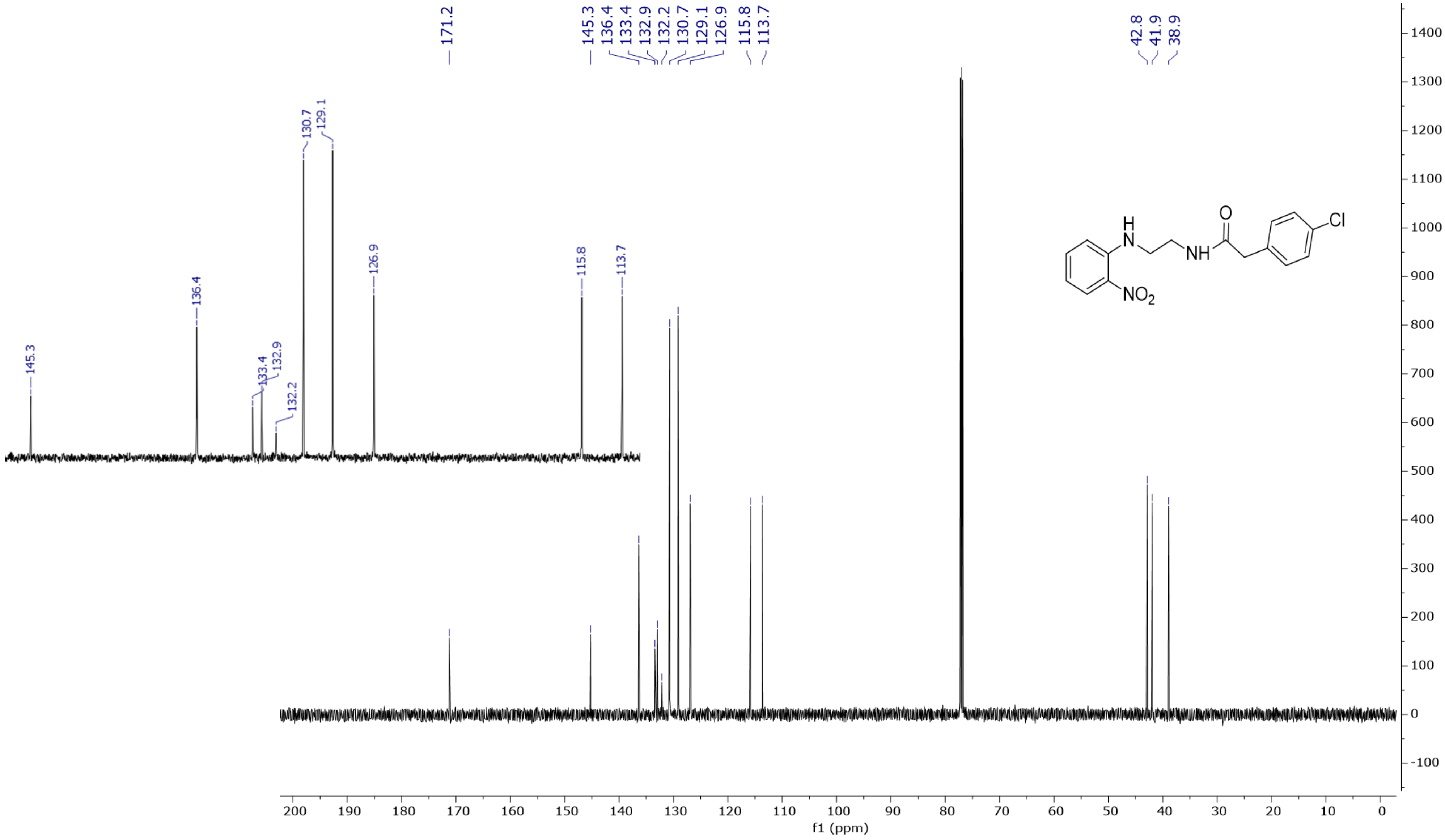


Figure S6: ^{13}C NMR spectrum of compound **3j** (151 MHz, $CDCl_3$)

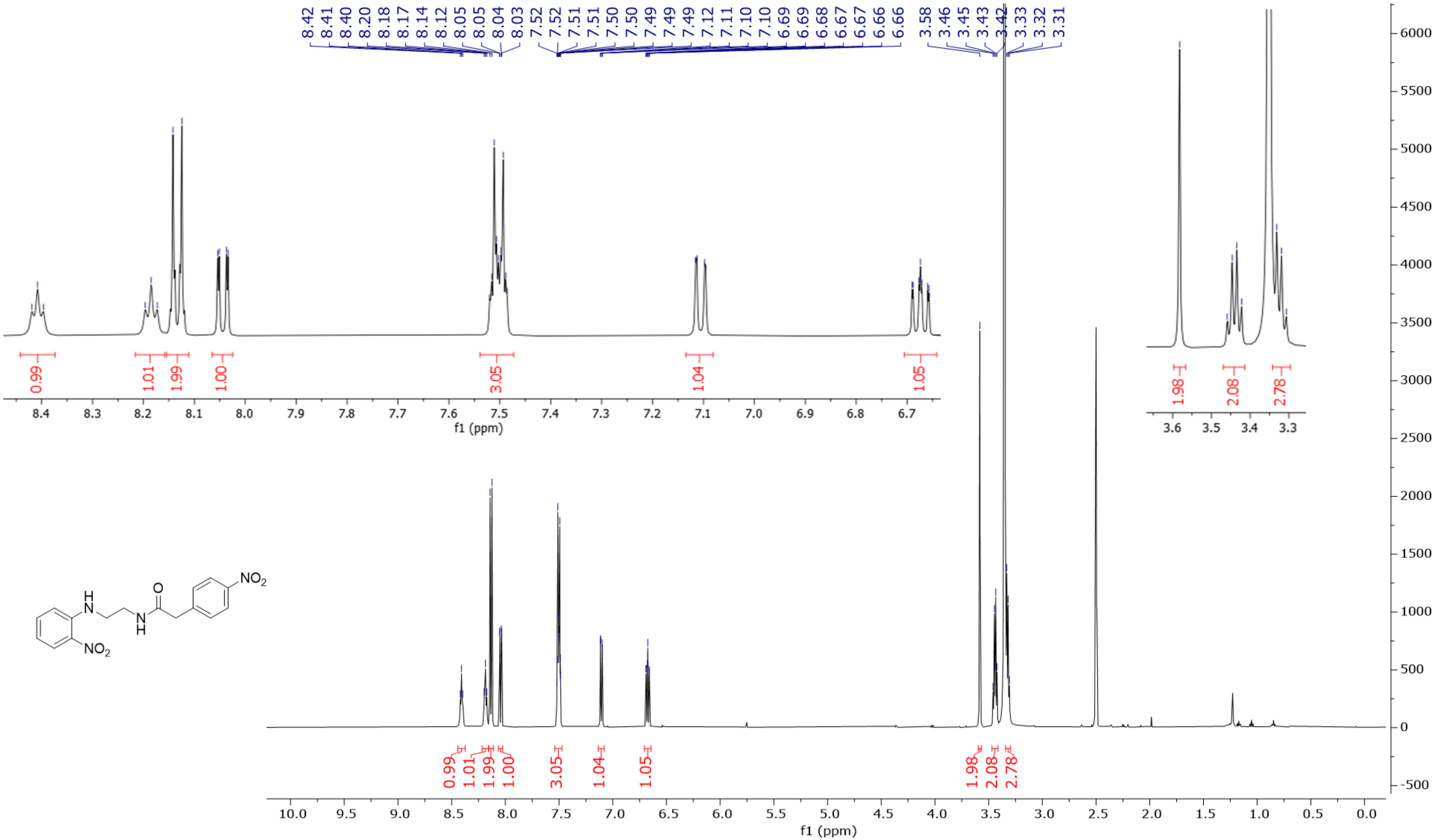


Figure S7: ^{1}H NMR spectrum of compound **3k** (500 MHz, DMSO-*d6*)

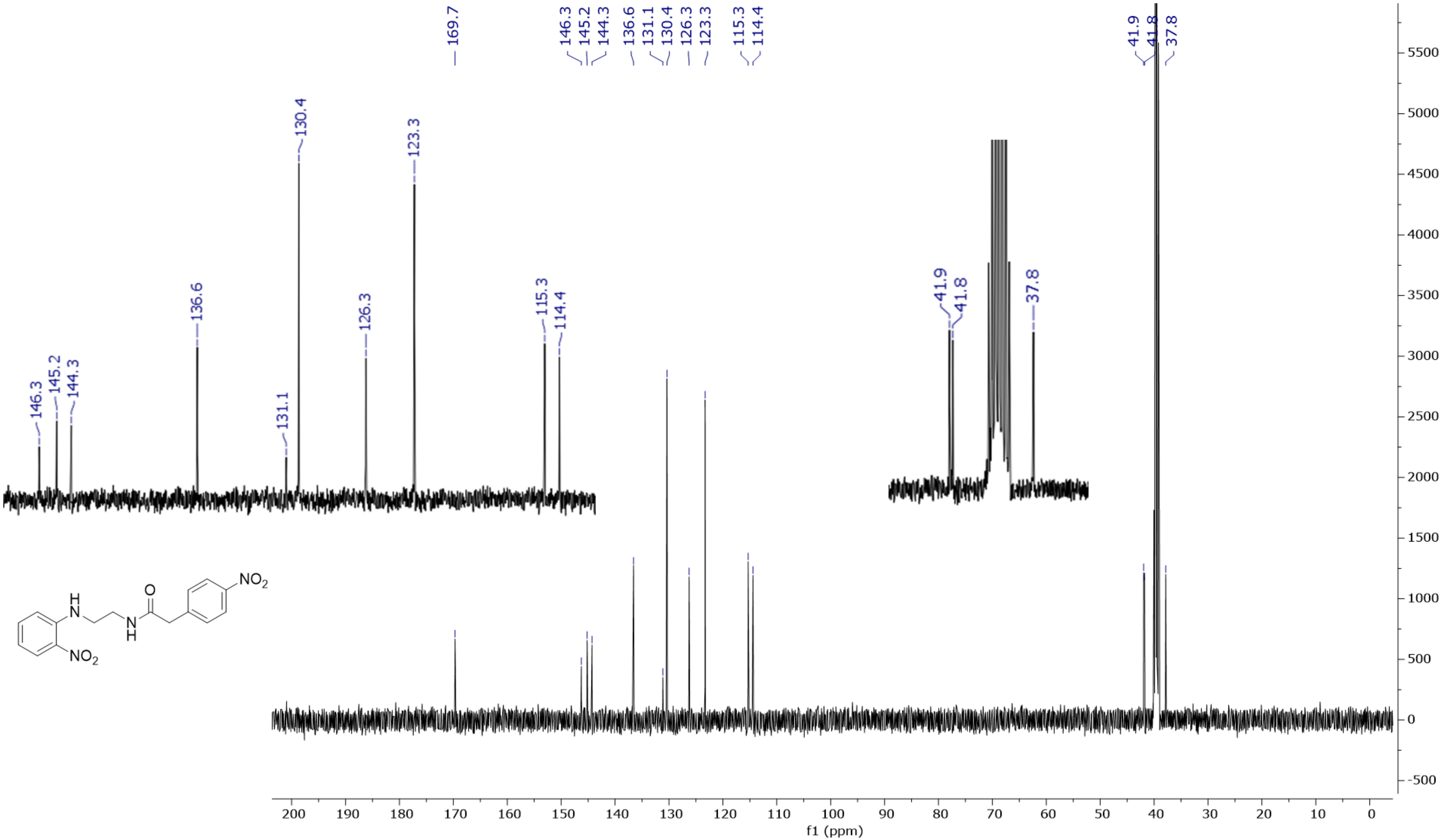


Figure S8: ^{13}C NMR spectrum of compound **3k** (126 MHz, DMSO-*d*6)

Spectra of compounds **4j-l** contain signals due to spontaneous rearrangement to the corresponding *N*-oxides **1**.

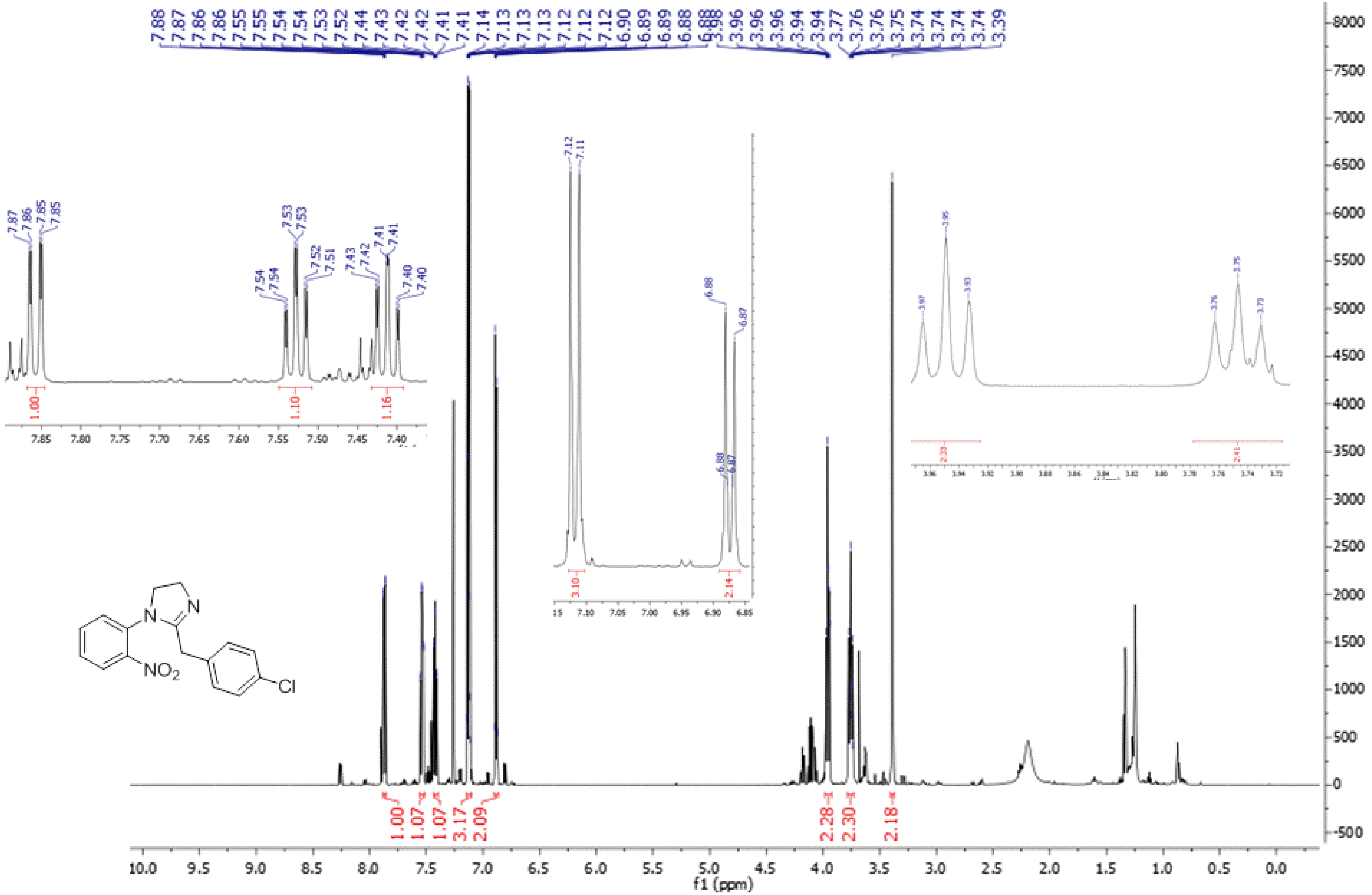


Figure S9: ^{1}H NMR spectrum of compound **4j** (600 MHz, $CDCl_3$)

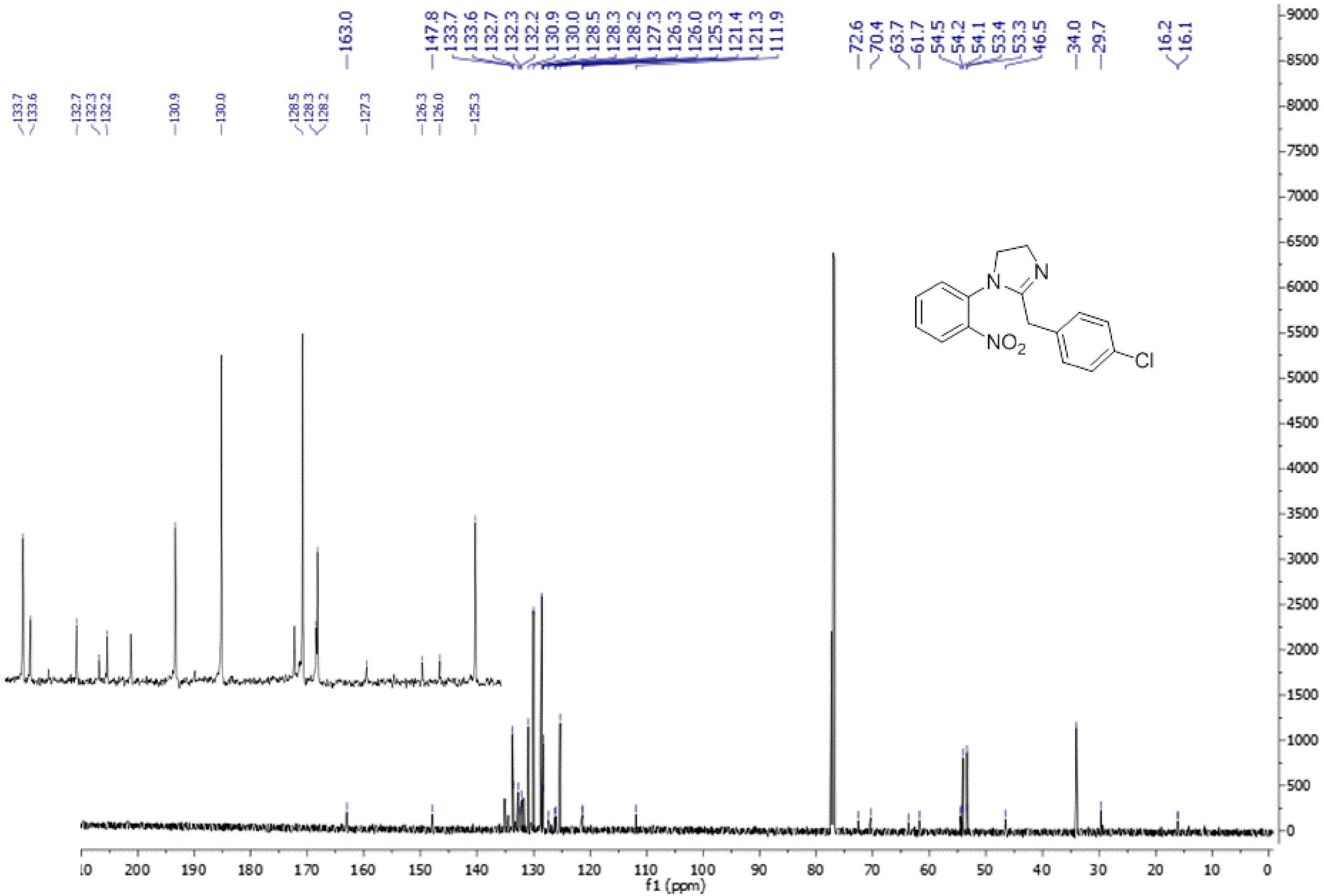


Figure S10: ^{13}C NMR spectrum of compound **4j** (151 MHz, $CDCl_3$)

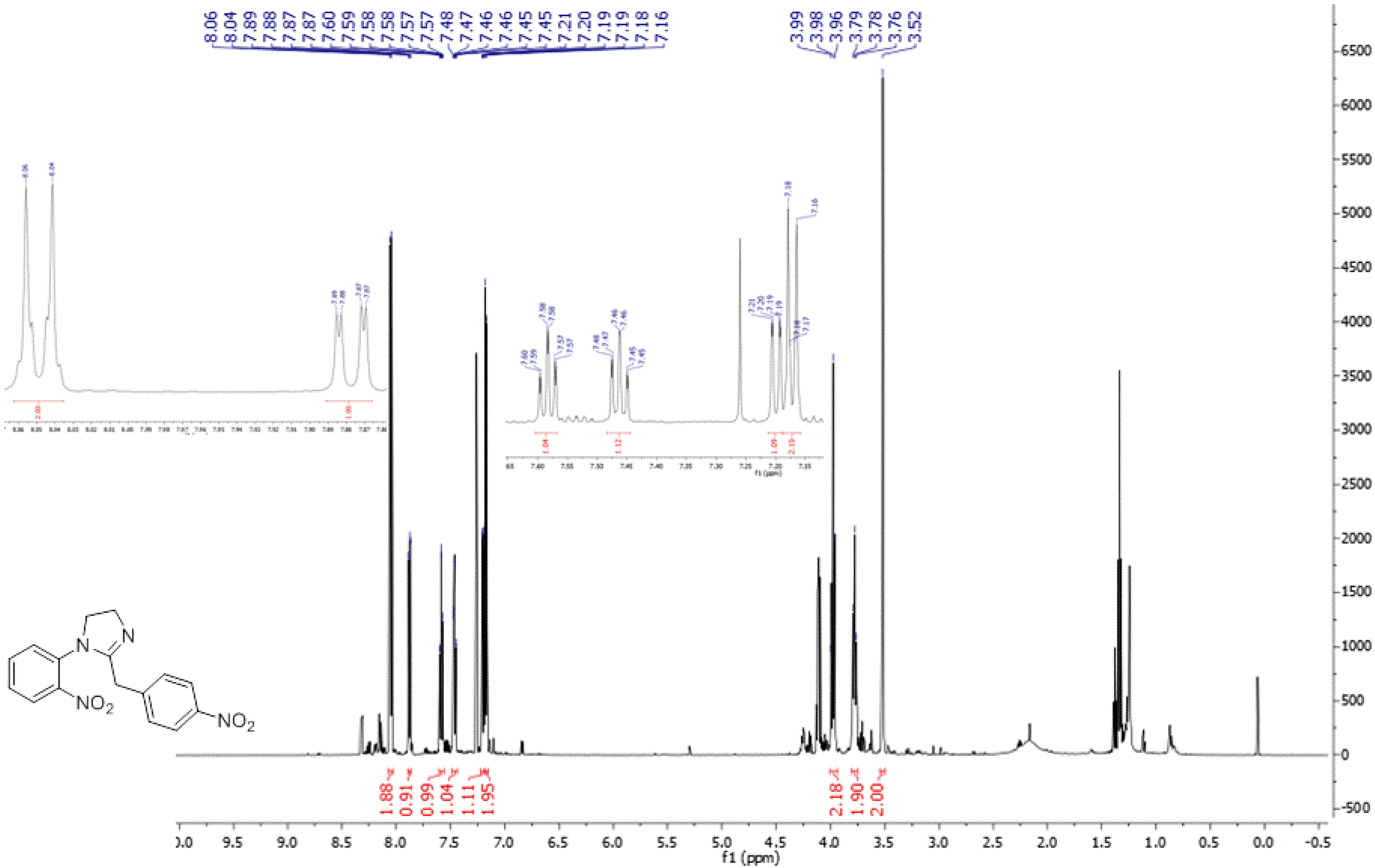


Figure S11: ^{1}H NMR spectrum of compound **4k** (600 MHz, $CDCl_3$)

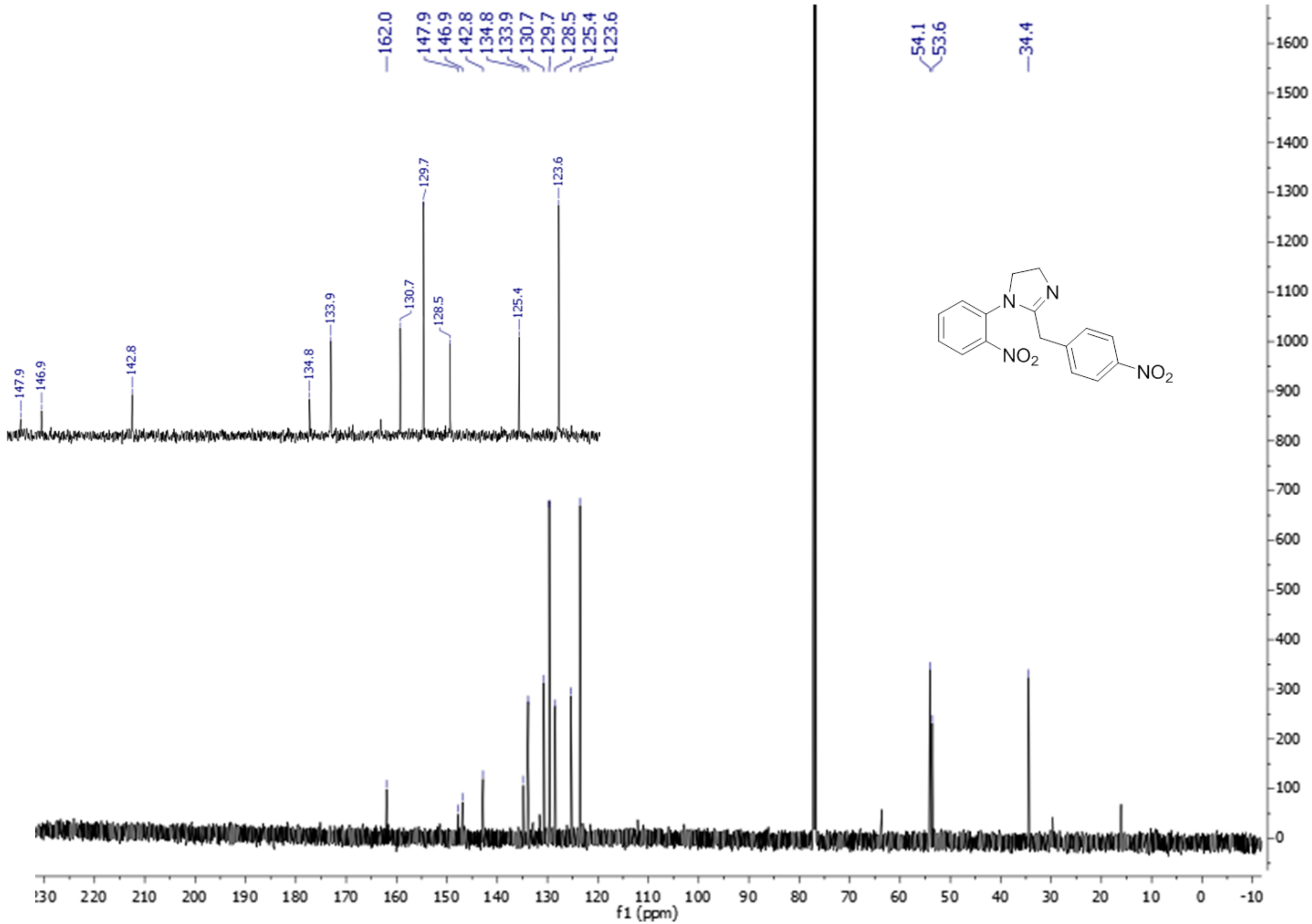


Figure S12: ^{13}C NMR spectrum of compound **4k** (151 MHz, $CDCl_3$)

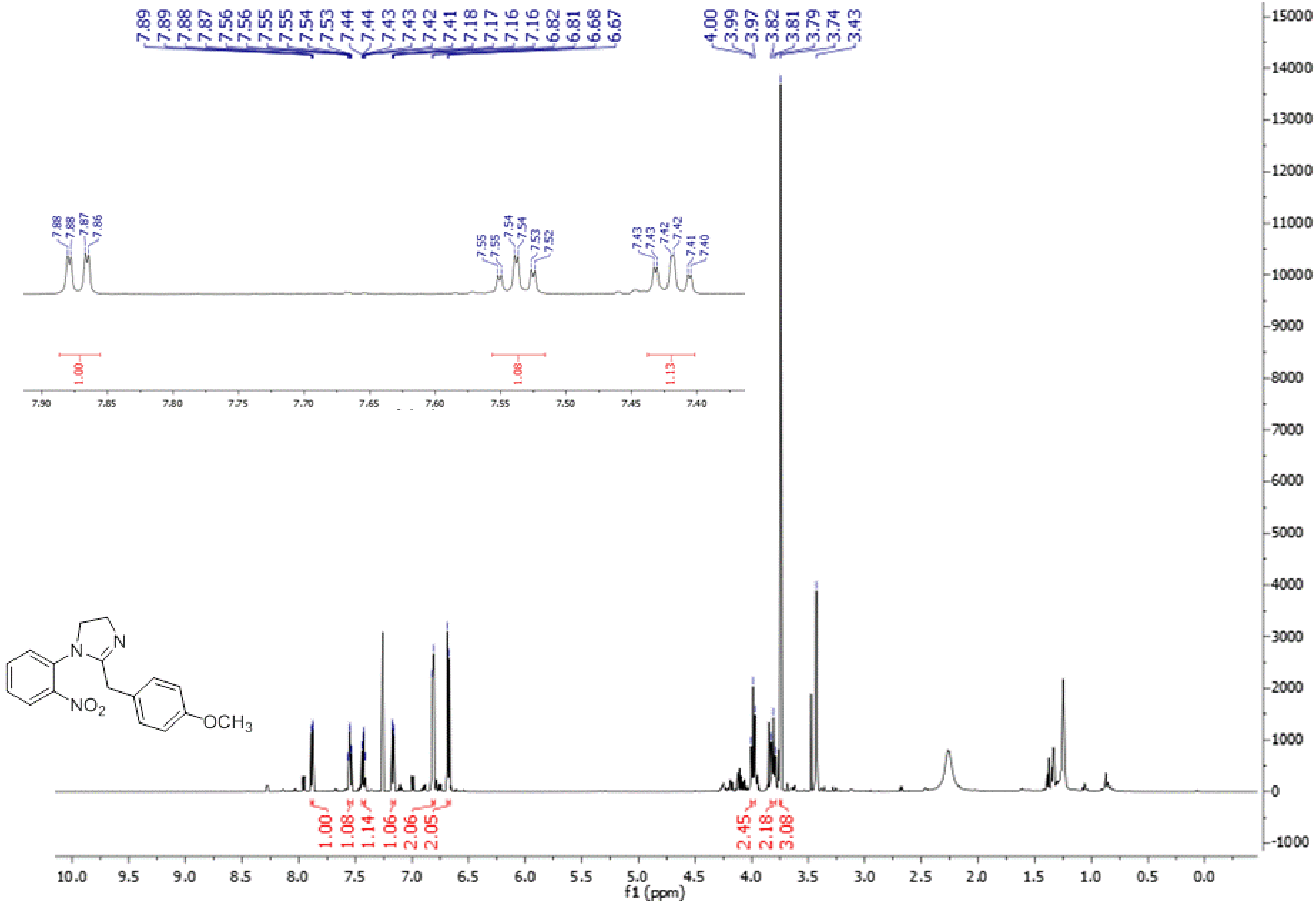


Figure S13: ^{1}H NMR spectrum of compound **4l** (600 MHz, $CDCl_3$)

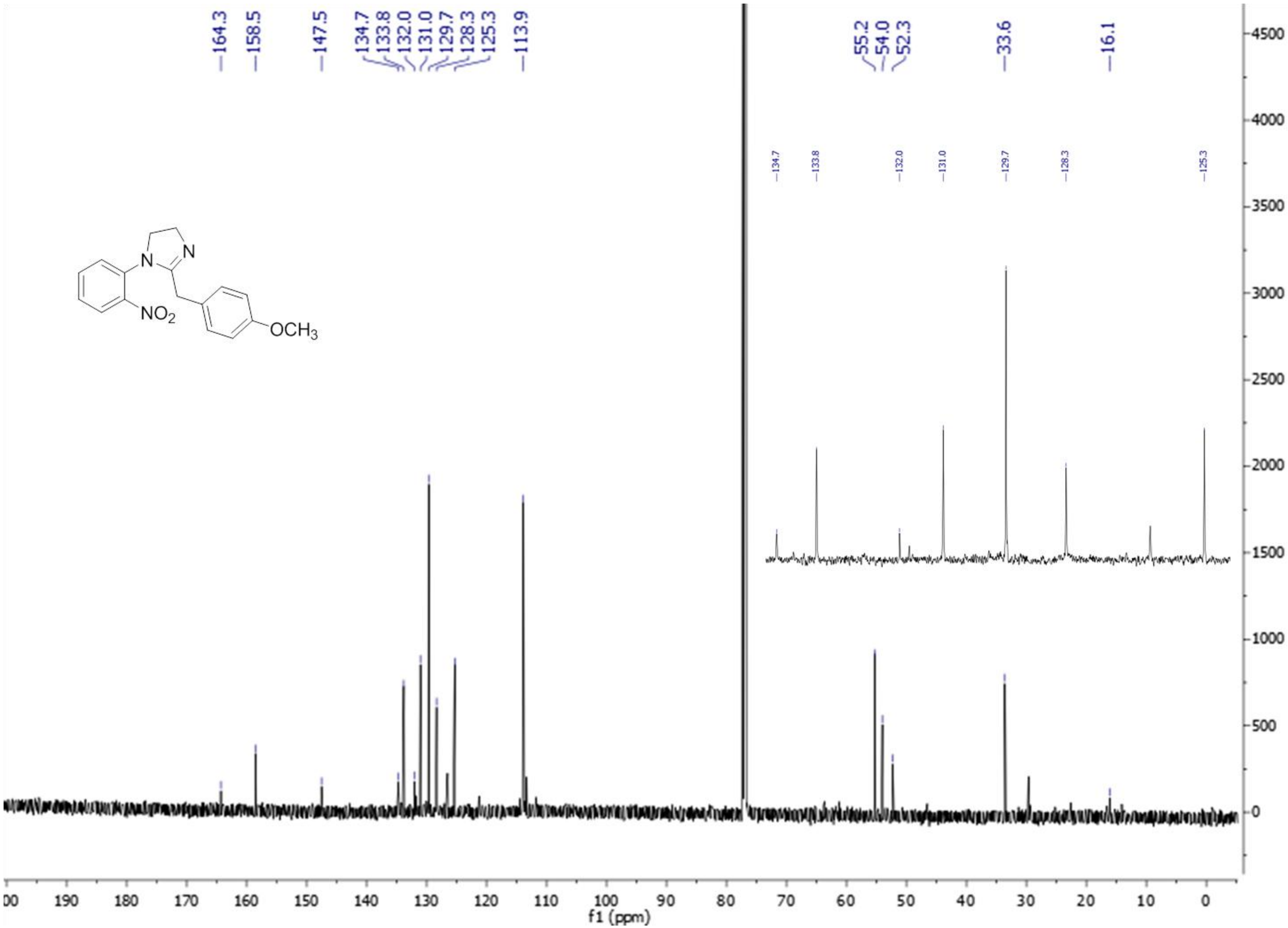


Figure S14: ^{13}C NMR spectrum of compound **4l** (151 MHz, $CDCl_3$)

Copies of IR Spectra

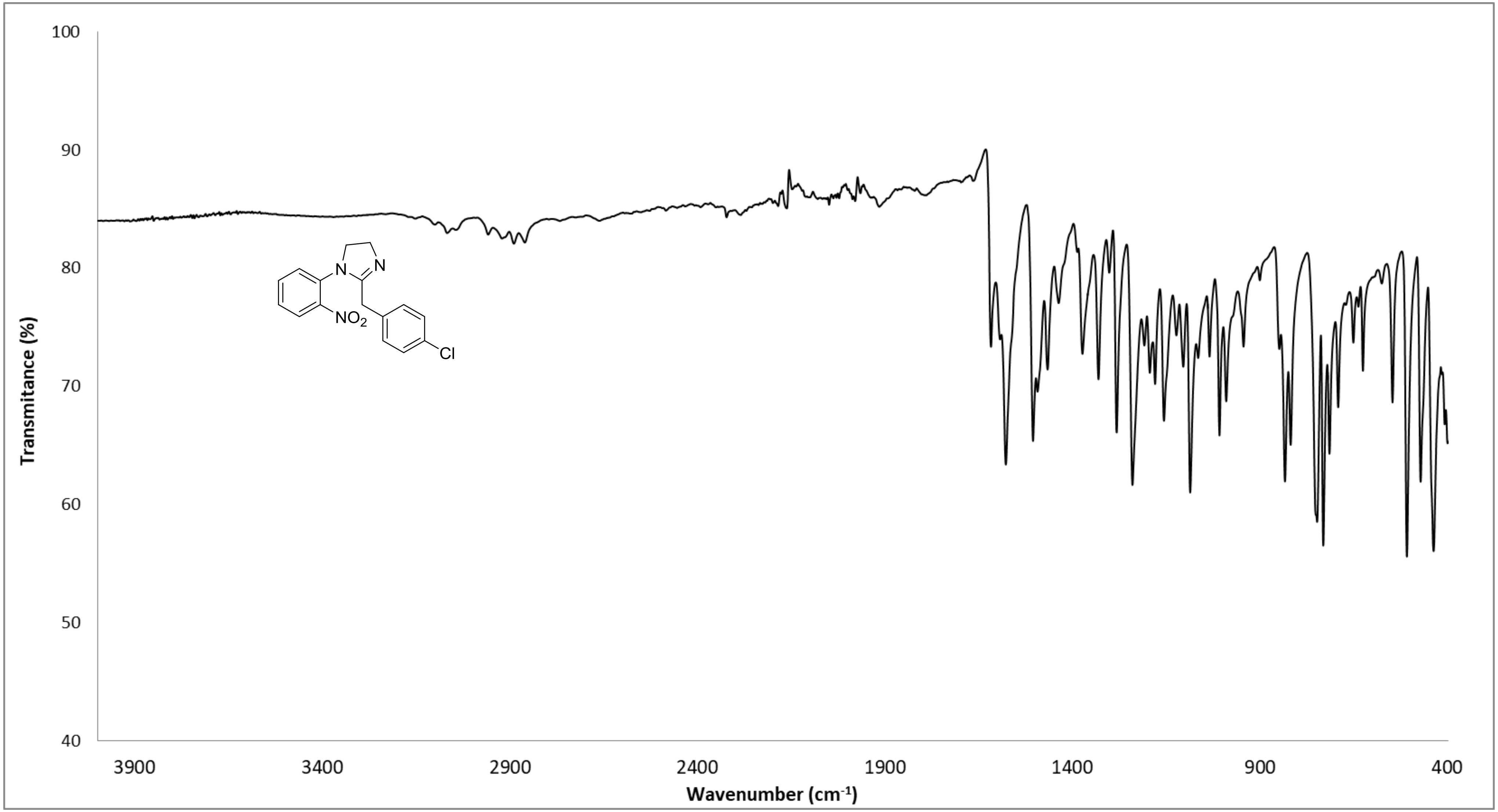


Figure S15: Diamond ATR-FTIR spectrum of **1j**

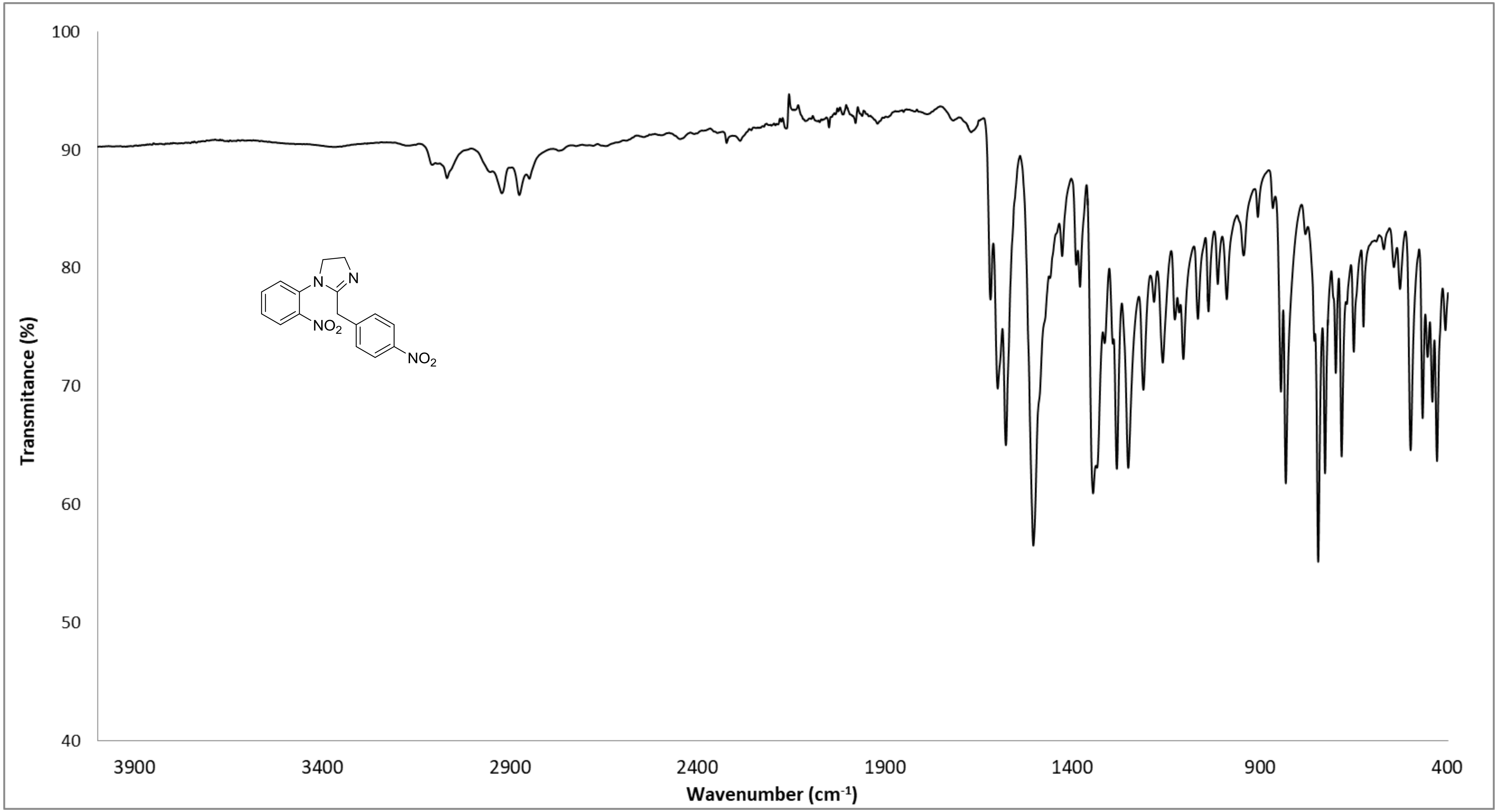


Figure S16: Diamond ATR-FTIR spectrum of **1k**

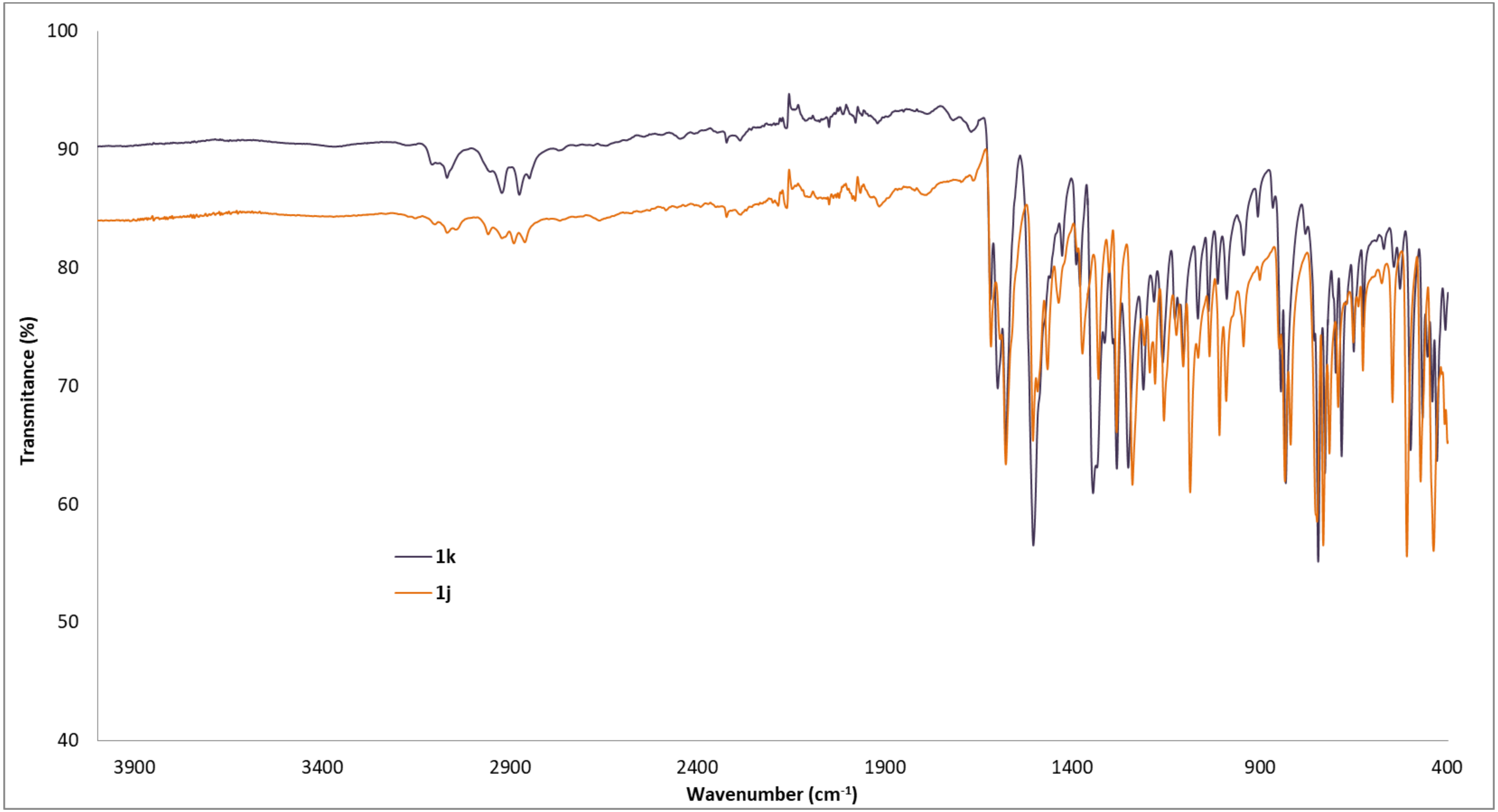
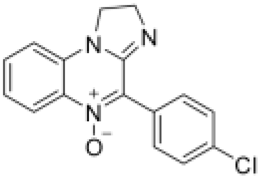


Figure S17: Overlapping Diamond ATR-FTIR spectrum of **1j** and **1k**

Copies of HRMS spectra



m/z experimental	Especie iónica detectada	Fórmula molecular de M	*m/z*	Error (mDa)	Error (ppm)
298,0747	$[M+H]^+$	$C_{16}H_{12}ClN_3O$	298,0743	0,4	1

Espectros de Masas

Especie iónica $[M+H]^+$

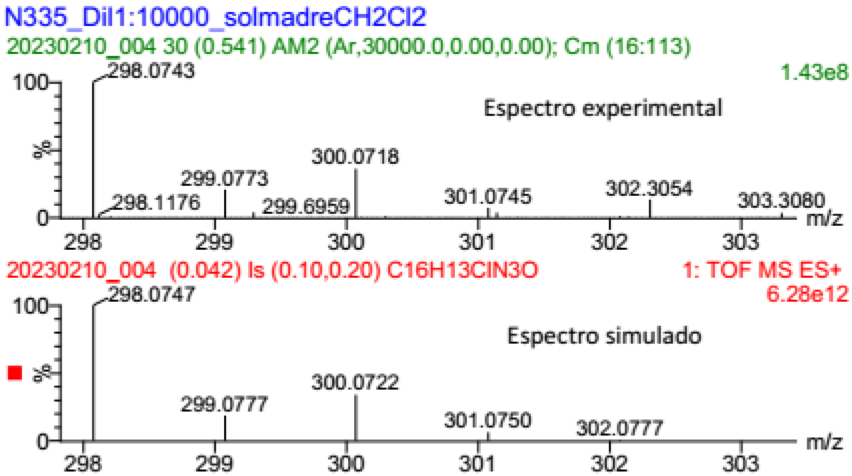
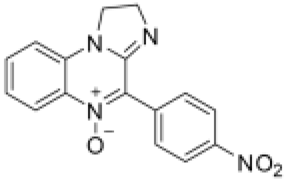


Figure S18: HRMS spectrum of compound **1j**



m/z experimental	Especie iónica detectada	Fórmula molecular de M	*m/z*	Error (mDa)	Error (ppm)
309,0985	$[M+H]^+$	$C_{16}H_{12}N_4O_3$	309,0988	0,3	1

Espectros de Masas

Especie iónica $[M+H]^+$

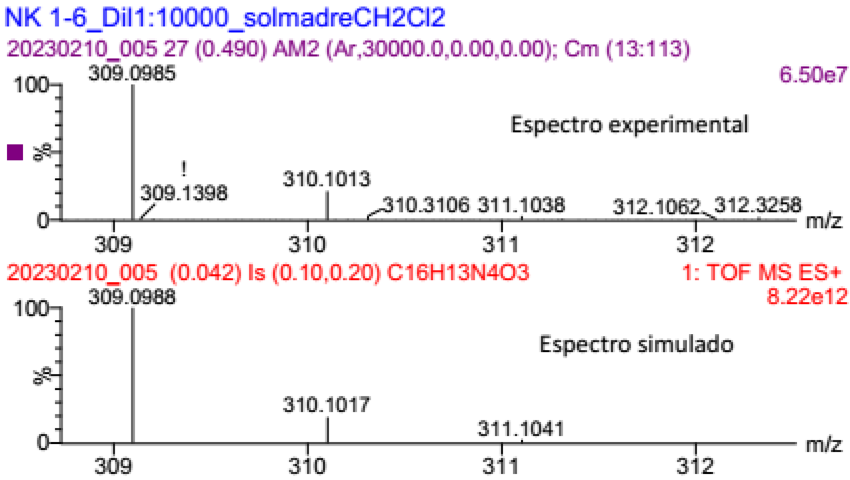
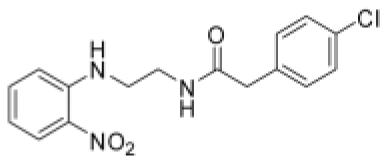


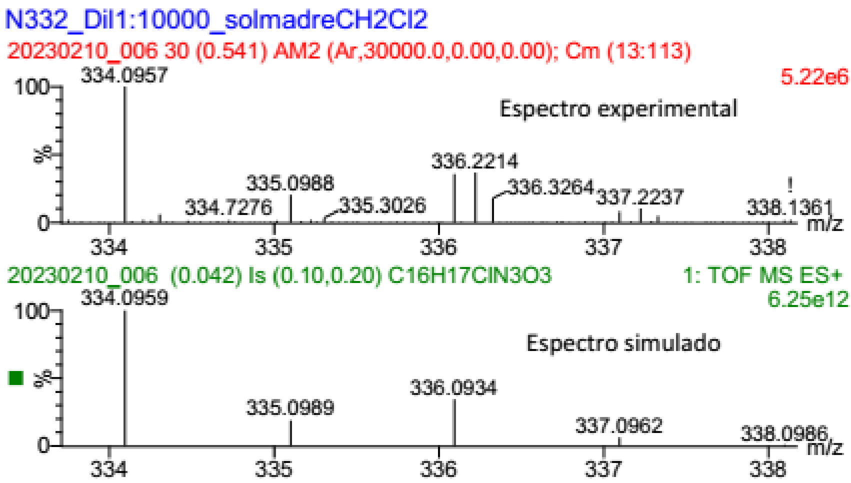
Figure S19: HRMS spectrum of compound **1k**



m/z experimental	Especie iónica detectada	Fórmula molecular de M	*m/z*	Error (mDa)	Error (ppm)
334,0957	$[M+H]^+$	$C_{16}H_{16}ClN_3O_3$	334,0959	0,2	1
356,0776	$[M+Na]^+$		334,0778	0,2	1

Espectros de Masas

Especie iónica $[M+H]^+$



Especie iónica $[M+Na]^+$

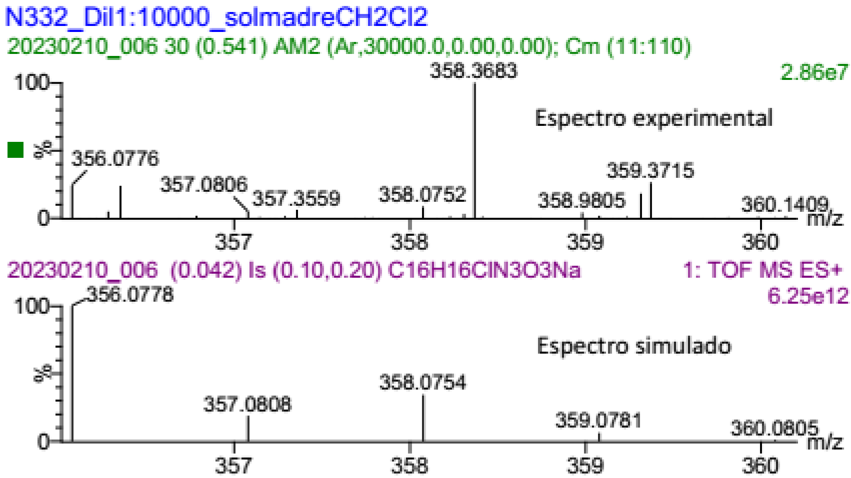
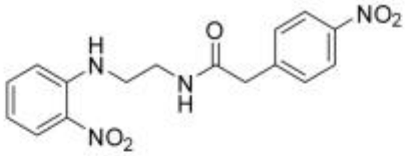


Figure S20: HRMS spectrum of compound **3j**



m/z experimental	Especie iónica detectada	Fórmula molecular de M	*m/z*	Error (mDa)	Error (ppm)
345,1204	$[M+H]^+$	$C_{16}H_{16}N_4O_5$	345,1199	0,5	1

Espectros de Masas

Especie iónica $[M+H]^+$

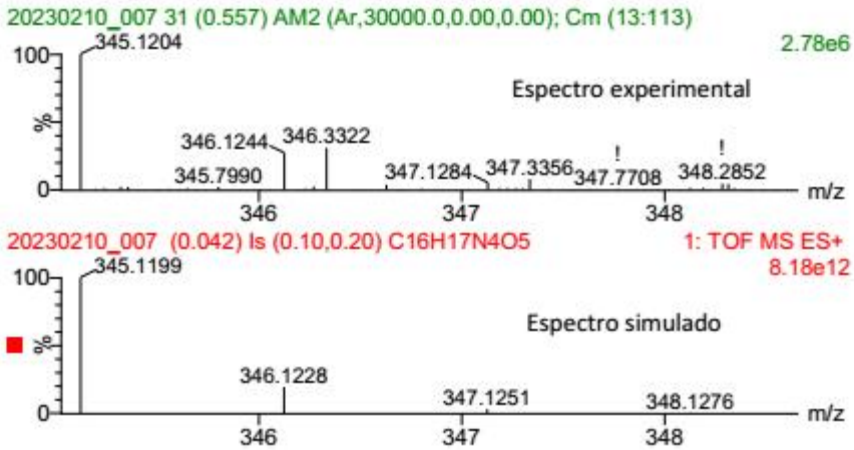


Figure S21: HRMS spectrum of compound **3k**

Elemental analysis report of compounds **4j** (sample N377)**, k** (sample N379).

CONICET UBA

I N Q U I M A E

Instituto de Química Física de los Materiales, Medio Ambiente y Energía

Servicio de Análisis Elemental

MA-20230719-172/II02

Análisis de C, H, N y S sobre muestras sólidas solicitado por, y rotuladas por:

Dra. Liliana Orelli
(Nadia Gruber)

Sustancia Patrón utilizada para la determinación de C, H, N y S: Sulfanilamida
Estandar Muestra utilizada en la determinación de C, H, N y S: Acido sulfanílico

# INQUIMAE	MUESTRA	N (%)	C (%)	H (%)	S (%)
#10139	**N377**	14,1	64,8	4,1	ND
#10140	**N379**	17,7	61,4	3,9	ND
	Patrón de Acido sulfanílico	8,09	41,61	4,07	18,56
	Ensayo de Control de Acido sulfanílico	8,0	41,8	4,3	18,4

ND: No detectado.
NOTA: El error típico para CHNS es ±0,2%.

El Análisis Elemental de CHNS se realizó en un equipo Carlo Erba EA 1108. Para el análisis se produce la combustión de la muestra en un tubo reactor donde los elementos a analizar son convertidos en CO_2, H_2O, N_2 y SO_2. La separación de los gases resultantes se realiza por cromatografía gaseosa con columna de porapac de longitud variable y para la detección se utiliza un detector de conductividad térmica. El método requiere una calibración previa con sustancia patrón de composición conocida.

Buenos Aires, 19 de Julio de 2023.