Chemoenzymatic asymmetric synthesis of 1,4-benzoxazine derivatives. Application in the synthesis of a Levofloxacin precursor

*María López-Iglesias,[a] Eduardo Busto,[b] Vicente Gotor[a] and Vicente Gotor-Fernández[a,]**

[a] *Organic and Inorganic Chemistry Department, Biotechnology Institute of Asturias (IUBA), University of Oviedo, Avenida Julián Clavería s/n, 33006 Oviedo, Spain.*

[b] *Department of Chemistry, Organic and Bioorganic Chemistry, University of Graz, Heinrichstrasse 28, 8010 Graz, Austria*

Corresponding author: vicgotfer@uniovi.es

Phone: +34 98 5103454. Fax: +34 98 5103456

SUPPORTING INFORMATION (page 1 of 129 pages)

Table of Contents

1. General information **S2**

2. Screening for the kinetic resolution of model substrate (±)-5a: amount and type of lipase, and reaction medium **S3**

3. HPLC separations **S4**

4. HPLC chromatograms of racemic and optically active compounds **S7**

5. NMR spectra **S34**

1. General information

Chemical reagents were purchased from different commercial sources and used without further purification. Solvents were distilled over an adequate desiccant under nitrogen.

Alcohol dehydrogenases from *Candida parapsilosis* (ADH CP, 30 U/mL) and *Lactobacillus brevis* (ADH-LB, 3.7 U/µL) were obtained from Codexis Inc. *Lactobacillus Kefir* (LK-ADH, 0.42 U/mg) was purchased from Fluka. Baker's yeast (type II from *Saccharomyces cerevisiae*) was obtained from Sigma. Alcohol dehydrogenase evo-1.1.200 (17.6 U/mg) was purchased from evocatal GmbH.

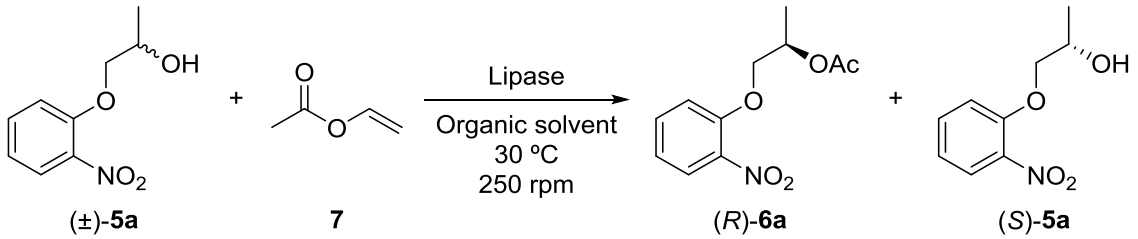
We especially thank Prof. Wolfgang Kroutil for the generous gift of ADH-A over expressed in *E. coli* BL21 cells: Cells were grown in LB-amp medium (1 L, 100 mg/L) at 30 ºC and 250 rpm for 24 h. Then, an additional amount of ampicillin (50 mg/L), $ZnCl_2$ (100 mg/L) and isopropyl thiogalactoside (IPTG, 450 mg/L) were added and shaken for 24 h at 20 ºC. The cells were centrifuged afterwards and the pellet was washed and suspended in phosphate buffer (pH 7.5, 50 mM), frozen with liquid nitrogen and lyophilized. The obtained cells were stored at 4 ºC.

Candida antarctica lipase type B (CAL-B, Novozyme 435, 7300 PLU/g) and *Rhizomucor miehei* lipase (RM-IM, 150 IUN/g) were a kind gift from Novozymes. *Pseudomonas cepacia* lipase currently known as *Burkhloderia cepacia* lipase (PSL-C I, 1638 U/g solid) was obtained from Sigma-Aldrich. *Candida antarctica* lipase type A (CAL-A, 2.6 U/mg solid) was purchased from Codexis Inc.

Column chromatographies were performed using silica gel 60 (230-240 mesh). Melting points were taken on samples in open capillary tubes and are uncorrected. IR spectra were recorded on using NaCl plates or KBr pellets. ^{1}H, ^{13}C NMR, DEPT and NMR-2D experiments (COSY, HMBC and HSQC) were obtained using different spectrometers (^{1}H, 300.13 MHz and ^{13}C, 75.5 MHz) or (^{1}H, 400.13 MHz and ^{13}C, 100.6 MHz). The chemical shifts are given in delta (δ) values and the coupling constants (*J*) in Hertz (Hz). High resolution mass spectra (HRMS) experiments were carried out by ESI^{+} or ESI^{-} using a Micro Tof Q spectrometer. Measurement of the optical rotation was done at 590 nm.

2. Screening for the kinetic resolution of model substrate (±)-5a: amount and type of lipase, and reaction medium

Table S1. Enzymatic kinetic resolution of racemic alcohol **5a** using 3 equivalents of vinyl acetate at 30 ºC.



Entry	Lipase[a]	Solvent	t (h)	ee_p (%)[b]	ee_s (%)[b]	c (%)[c]	E[d]
1	RML IM (1:1)	MTBE	2	96	63	40	104
2	RML IM (1:1)	MTBE	5	94	89	48	103
3	CAL-A (1:1)	MTBE	2.5	69	22	76	3
4	CAL-B (1:1)	MTBE	2	<1	<1	>99	n.d.
5	PSL-C I (1:1)	MTBE	3	13	>99	88	6
6	RML IM (1:4)	MTBE	51.5	95	86	48	108
7	RML IM (1:1)	Toluene	29.5	97	92	49	188
8	RML IM (1:1)	1,4-Dioxane	22.5	98	20	17	96
9	RML IM (1:1)	MeCN	22.5	>99	2	2	>200
10	RML IM (1:1)	THF	22.5	97	11	10	72
11	RML IM (1:1)	Et_2O	20	97	82	46	164

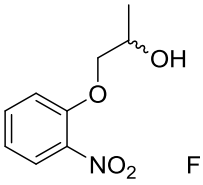
[a] Ratio lipase: substrate (w/w).
[b] Determined by HPLC.
[c] $c = ee_s/(ee_s + ee_p)$.
[d] $E = \ln[(1-c)(1-ee_p)]/\ln[(1-c)(1+ee_p)]$.[1]

[1] Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *102*, 7294-7299.

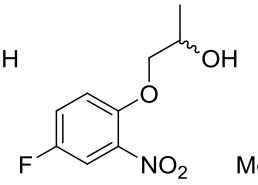
3. HPLC separations

High performance liquid chromatography (HPLC) analyses were carried out at 210 nm using a Chiralcel OJ-H (25 cm × 4.6 mm I.D.), Chiralpak IC (25 cm × 4.6 mm I.D.) and Chiralpak IA column (25 cm × 4.6 mm I.D.). HPLC conditions and retention times are given in Tables S2-S6.

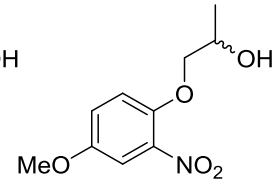
Table S2. Analytical separation by HPLC for nitro alcohols (±)-**5a-e**.



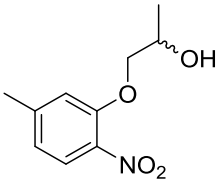
(±)-**5a**



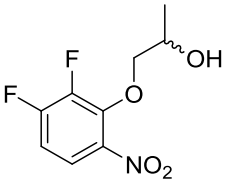
(±)-**5b**



(±)-**5c**



(±)-**5d**

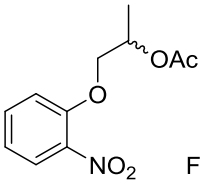


(±)-**5e**

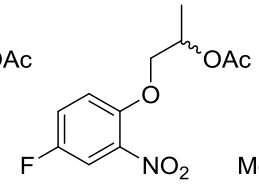
Compound	Column	T (°C)	Eluent (*n*-hexane/2-propanol)[a]	Time (min)	*Rs*[b]
(±)-**5a**	IA	30	90:10	14.7 (*R*) and 19.1 (*S*)	3.8
(±)-**5b**	IA	30	90:10	14.8 (*R*) and 17.3 (*S*)	2.6
(±)-**5c**	IA	30	90:10	18.3 (*R*) and 22.2 (*S*)	3.0
(±)-**5d**	IA	30	90:10	13.1 (*R*) and 19.0 (*S*)	5.1
(±)-**5e**	IA	30	98:2	64.7 (*R*) and 84.8 (*S*)	4.8

[a] A 0.8 mL/min flow was used in all cases; [b] $R_s = 2x[t_R(S) - t_R(R)]/ w_{b1}(S) + w_{b2}(R)$.

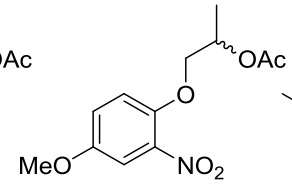
Table S3. Analytical separation by HPLC for acetates (±)-**6a-e**.



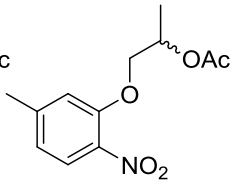
(±)-**6a**



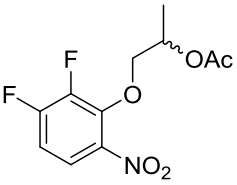
(±)-**6b**



(±)-**6c**



(±)-**6d**

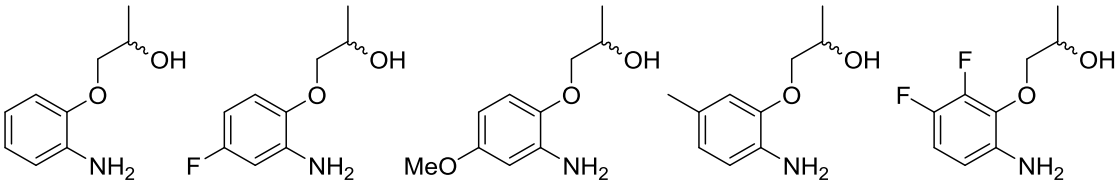


(±)-**6e**

Compound	Column	T (°C)	Eluent (*n*-hexane/2-propanol)[a]	Time (min)	*Rs*[b]
(±)-**6a**	IA	30	90:10	9.0 (*R*) and 9.6 (*S*)	1.0
(±)-**6b**	IA	30	90:10	8.6 (*R*) and 9.5 (*S*)	1.5
(±)-**6c**	IA	30	90:10	10.5 (*R*) and 11.2 (*S*)	1.1
(±)-**6d**	IA	30	97:3	13.7 (*R*) and 14.6 (*S*)	1.0
(±)-**6e**	IA	30	98:2	13.8 (*R*) and 15.3 (*S*)	1.8

[a] A 0.8 mL/min flow was used in all cases; [b] $R_s = 2x[t_R(S) - t_R(R)]/ w_{b1}(S) + w_{b2}(R)$.

Table S4. Analytical separation by HPLC for amino alcohols (±)-**8a-e**.

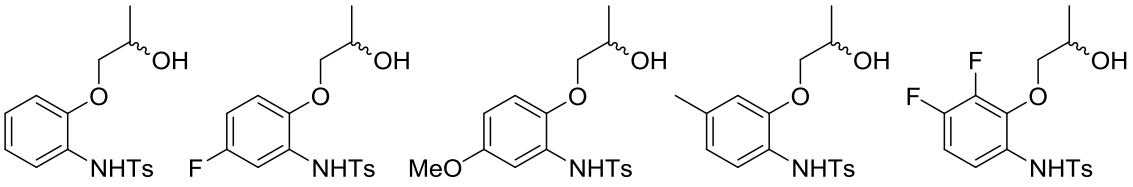


(±)-**8a** (±)-**8b** (±)-**8c** (±)-**8d** (±)-**8e**

Compound	Column	T (°C)	Eluent (n-hexane/2-propanol)[a]	Time (min)	*Rs*[b]
(±)-**8a**	IC	30	80:20	12.7 (*R*) and 15.0 (*S*)	3.0
(±)-**8b**	IA	30	90:10	25.7 (*R*) and 28.9 (*S*)	1.9
(±)-**8c**	IC	30	80:20	26.9 (*R*) and 33.2 (*S*)	3.5
(±)-**8d**	IC	30	80:20	14.3 (*R*) and 17.7 (*S*)	3.6
(±)-**8e**	OJ-H	30	90:10	15.9 (*S*) and 21.1 (*R*)	4.2

[a] A 0.8 mL/min flow was used in all cases; [b] $R_s = 2x[t_R(2) - t_R(1)]/ w_{b1}(2) + w_{b2}(1)$.

Table S5. Analytical separation by HPLC for monotosylated amino alcohols (±)-**9a-e**.

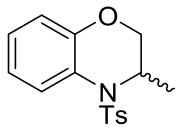


(±)-**8a** (±)-**8b** (±)-**8c** (±)-**8d** (±)-**8e**

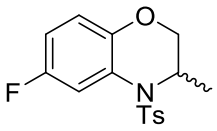
Compound	Column	T (°C)	Eluent (n-hexane/2-propanol)[a]	Time (min)	*Rs*[b]
(±)-**9a**	IC	30	70:30	16.7 (*S*) and 21.9 (*R*)	4.3
(±)-**9b**	IC	30	80:20	16.8 (*S*) and 20.8 (*R*)	3.0
(±)-**9c**	IC	30	80:20	38.4 (*S*) and 44.9 (*R*)	2.3
(±)-**9d**	IC	30	80:20	32.3 (*S*) and 46.3 (*R*)	5.2
(±)-**9e**	IC	30	90:10	23.2 (*S*) and 25.4 (*R*)	1.5

[a] A 0.8 mL/min flow was used in all cases; [b] $R_s = 2x[t_R(S) - t_R(R)]/ w_{b1}(S) + w_{b2}(R)$.

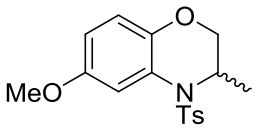
Table S6. Analytical separation by HPLC for tosylated benzoxazines (±)-**10a-e** and benzoxazines (±)-**4a** and (±)-**4e**.



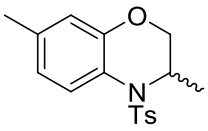
(±)-**10a**



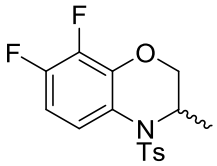
(±)**10b**



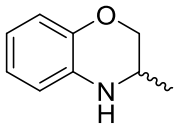
(±)-**10c**



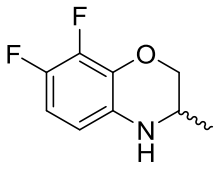
(±)-**10d**



(±)-**10e**



(±)-**4a**



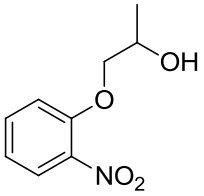
(±)-**4e**

Compound	Column	*t* (°C)	Eluent (n-hexane/2-propanol)[a]	Time (min)	*Rs*[b]
(±)-**10a**	OJ-H	30	90:10	16.6 (*R*) and 19.8 (*S*)	2.1
(±)-**10b**	OJ-H	30	90:10	15.7(*S*) and 20.7 (*R*)	3.7
(±)-**10c**	OJ-H	30	90:10	20.7 (*R*) and 26.2 (*S*)	2.8
(±)-**10d**	OJ-H	30	90:10	14.9 (*R*) and 17.9 (*S*)	1.5
(±)-**10e**	OJ-H	30	90:10	17.1 (*R*) and 20.5 (*S*)	1.6
(±)-**4a**	OJ-H	30	90:10	18.2 (*S*) and 22.6 (*R*)	3.5
(±)-**4e**	OJ-H	30	95:5	24.4 (*S*) and 26.7 (*R*)	1.1

[a] A 0.8 mL/min flow was used in all cases; [b] $R_s = 2x[t_R(2) - t_R(1)]/ w_{b1}(2) + w_{b2}(1)$.

4. HPLC chromatograms of racemic and optically active compounds

1-(2-Nitrophenoxy)propan-2-ol (5a)

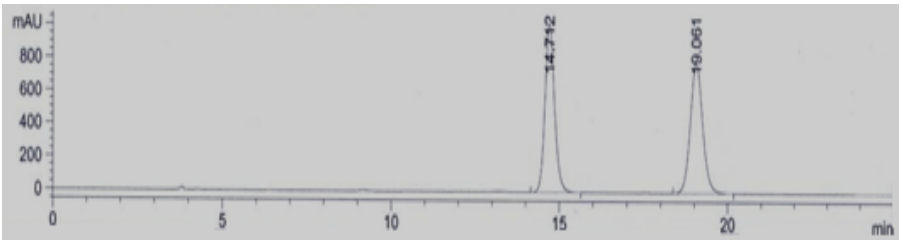


Analytical data for alcohol 5a

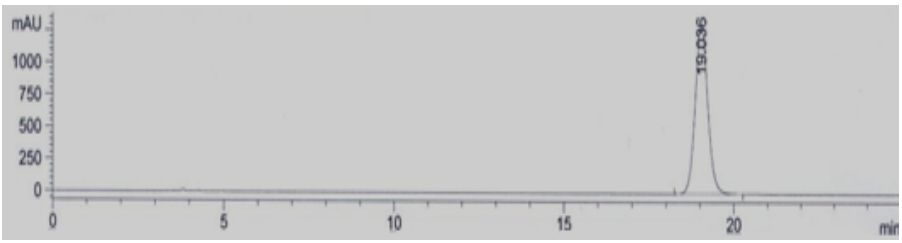
Column: Chiralcel IA
Flow: 0.8 mL/min
Retention times: t_R (*R*) = 14.7 min, t_R (*S*) = 19.1 min

Eluent: *n*-hexane/2-propanol 90:10
Temperature: 30 ºC
Rs: 3.8

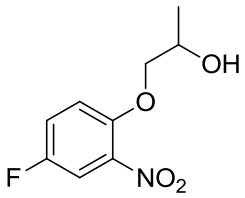
HPLC separation for both enantiomers of 5a



(*S*)-5a in >99% *ee*



1-(4-Fluoro-2-nitrophenoxy)propan-2-ol (5b)

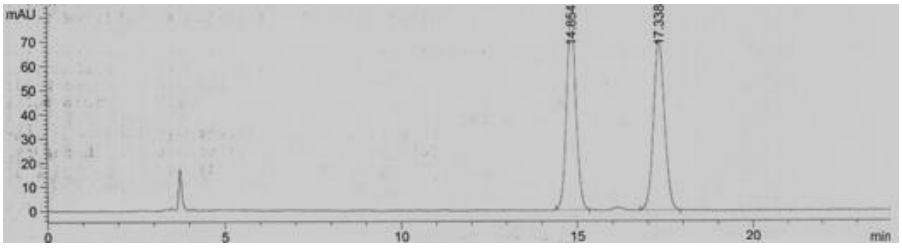


Analytical data for alcohol 5b

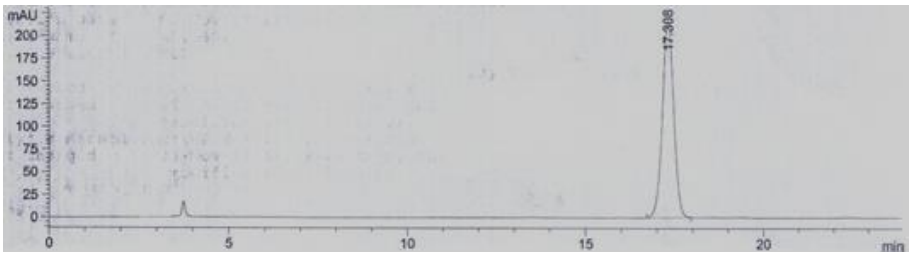
Column: Chiralcel IA
Flow: 0.8 mL/min
Retention times: t_R (*R*) = 14.8 min, t_R (*S*) = 17.3 min

Eluent: *n*-hexane/2-propanol 90:10
Temperature: 30 ºC
Rs: 2.6

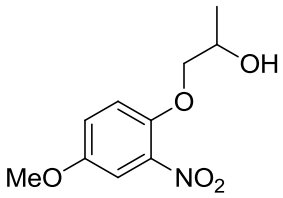
HPLC separation for both enantiomers of 5b



(*S*)-5b in >99% *ee*



1-(4-Methoxy-2-nitrophenoxy)propan-2-ol (5c)

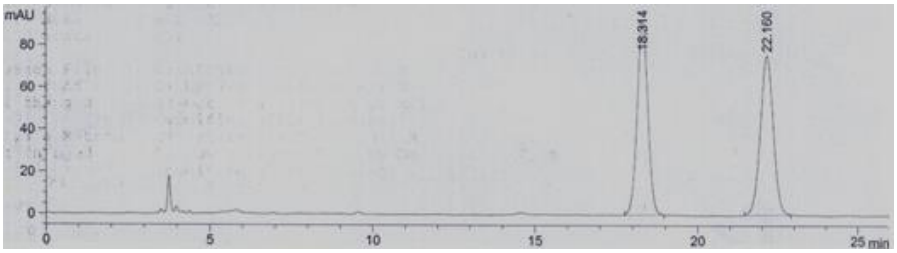


Analytical data for alcohol 5c

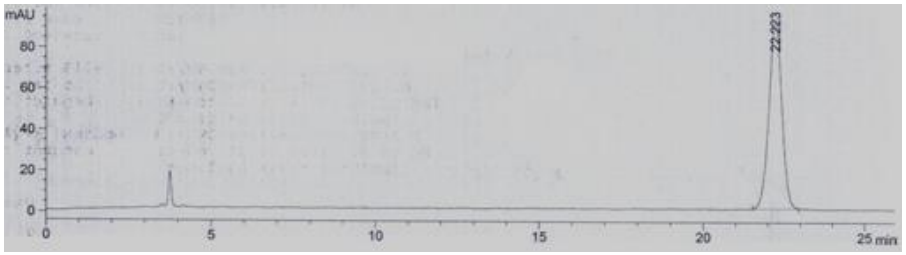
Column: Chiralcel IA
Flow: 0.8 mL/min
Retention times: t_R (*R*) = 18.3 min, t_R (*S*) = 22.2 min

Eluent: *n*-hexane/2-propanol 90:10
Temperature: 30 ºC
Rs: 3.0

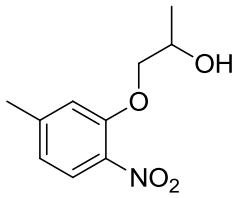
HPLC separation for both enantiomers of 5c



(*S*)-5c in >99% *ee*



1-(5-Methyl-2-nitrophenoxy)propan-2-ol (5d)

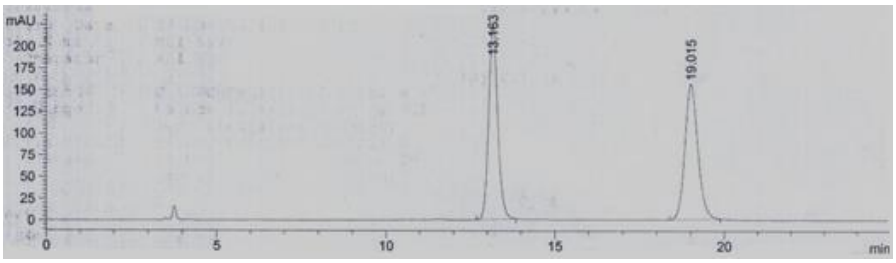


Analytical data for alcohol 5d

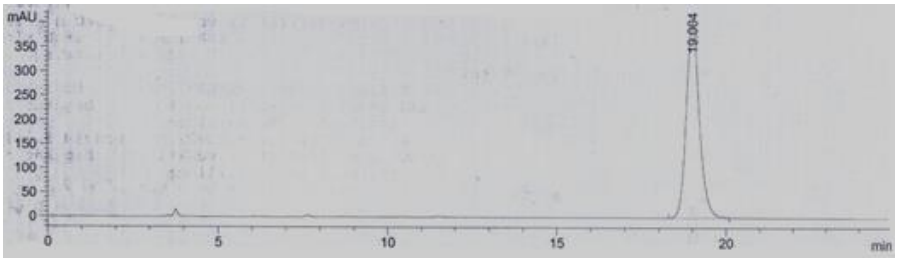
Column: Chiralcel IA
Flow: 0.8 mL/min
Retention times: t_R (*R*) = 13.1 min, t_R (*S*) = 19.0 min

Eluent: *n*-hexane/2-propanol 90:10
Temperature: 30 ºC
Rs: 5.1

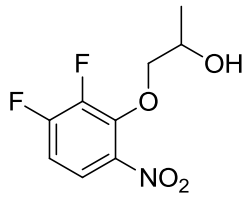
HPLC separation for both enantiomers of 5d



(*S*)-5d in >99% *ee*



1-(2,3-Difluoro-6-nitrophenoxy)propan-2-ol (5e)

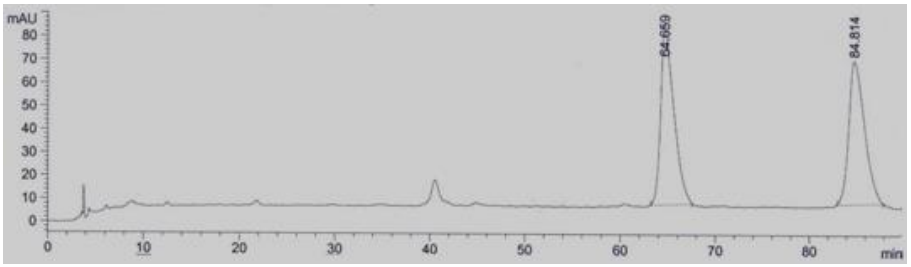


Analytical data for alcohol 5e

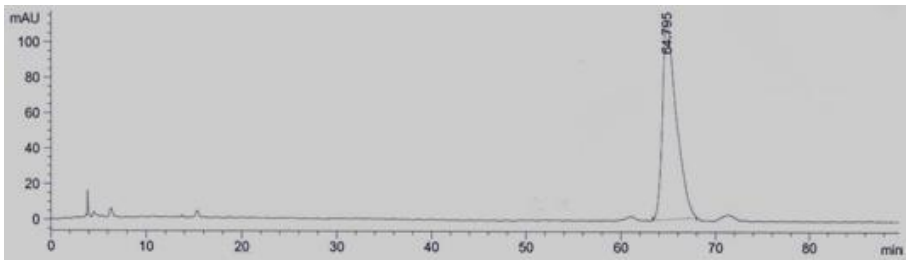
Column: Chiralcel IA
Flow: 0.8 mL/min
Retention times: t_R (*R*) = 64.7 min, t_R (*S*) = 84.8 min

Eluent: *n*-hexane/2-propanol 98:2
Temperature: 30 ºC
Rs: 4.8

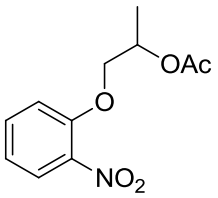
HPLC separation for both enantiomers of 5e



(*R*)-5e in >99% *ee*



1-(2-Nitrophenoxy)propan-2-yl acetate (6a)

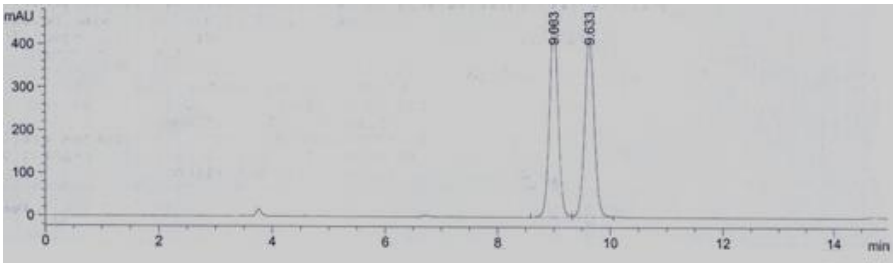


Analytical data for acetate 6a

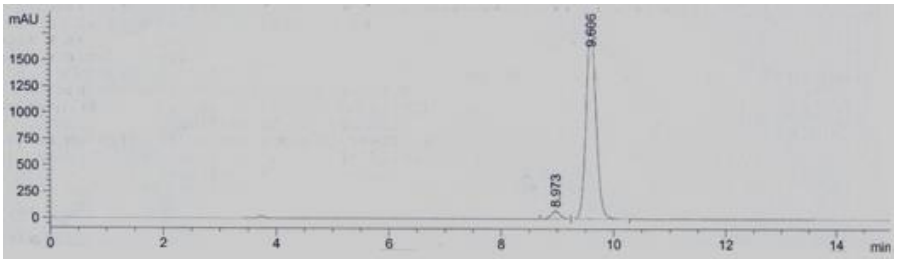
Column: Chiralcel IA
Flow: 0.8 mL/min
Retention times: t_R (*R*) = 9.0 min, t_R (*S*) = 9.6 min

Eluent: *n*-hexane/2-propanol 90:10
Temperature: 30 ºC
Rs: 1.0

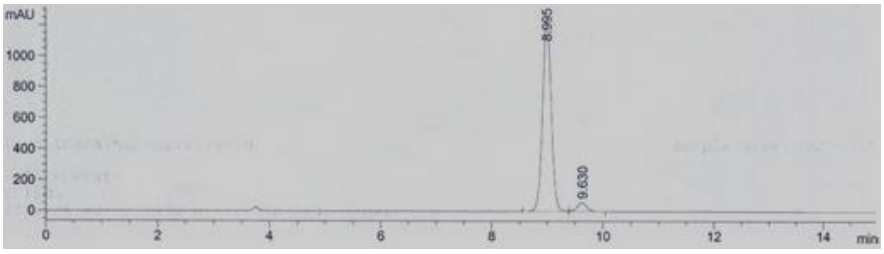
HPLC separation for both enantiomers of 6a



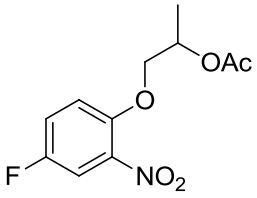
(*S*)-6a in 93% *ee*



(*R*)-6a in 90% *ee*



1-(4-Fluoro-2-nitrophenoxy)propan-2-yl acetate (6b)

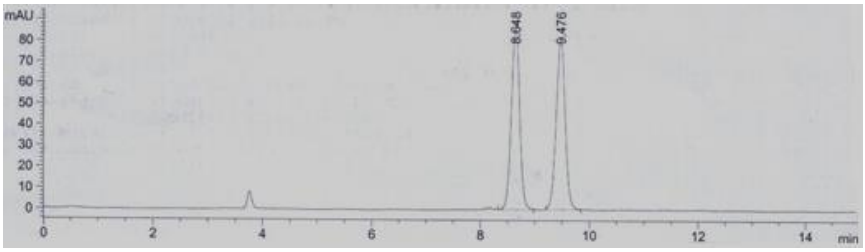


Analytical data for acetate 6b

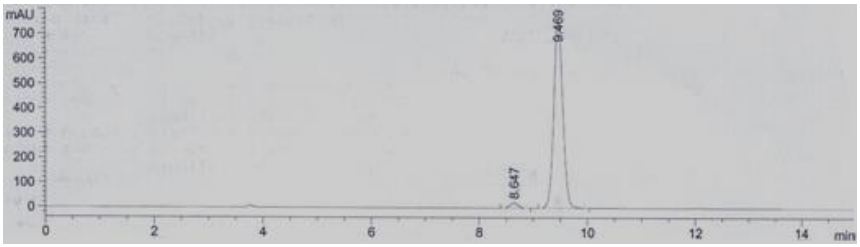
Column: Chiralcel IA
Flow: 0.8 mL/min
Retention times: t_R (*R*) = 8.6 min, t_R (*S*) = 9.5 min

Eluent: *n*-hexane/2-propanol 90:10
Temperature: 30 ºC
Rs: 1.5

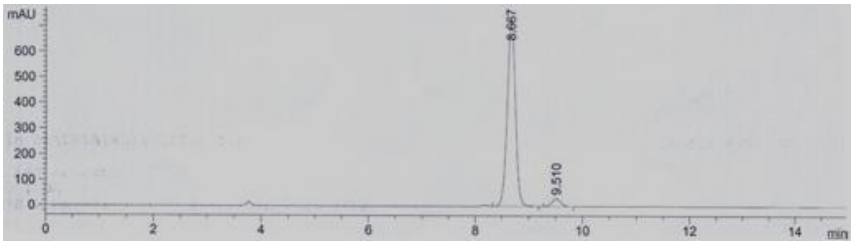
HPLC separation for both enantiomers of 6b



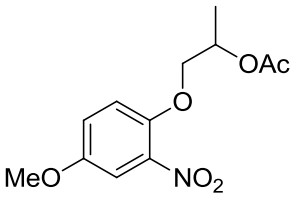
(*S*)-6b in 92% *ee*



(*R*)-6b in 91% *ee*



1-(4-Methoxy-2-nitrophenoxy)propan-2-yl acetate (6c)

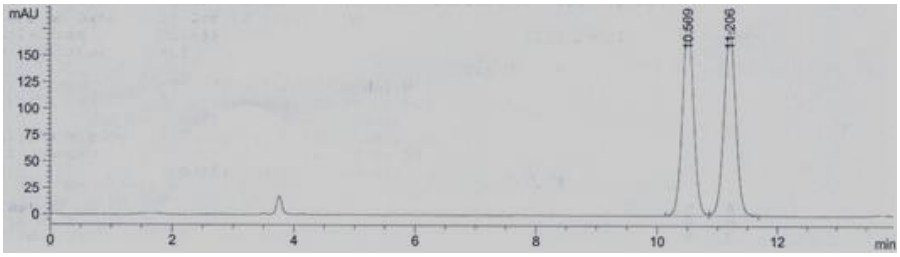


Analytical data for acetate 6c

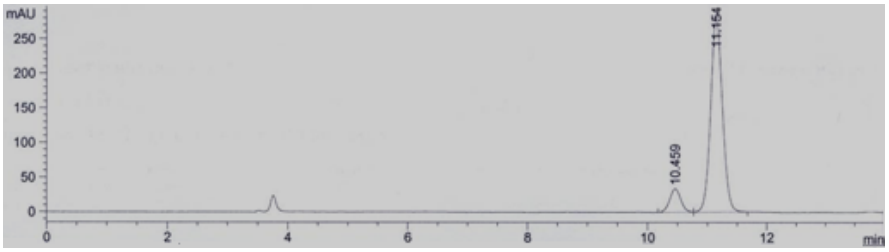
Column: Chiralcel IA
Flow: 0.8 mL/min
Retention times: t_R (*R*) = 10.5 min, t_R (*S*) = 11.2 min

Eluent: *n*-hexane/2-propanol 90:10
Temperature: 30 ºC
Rs: 1.1

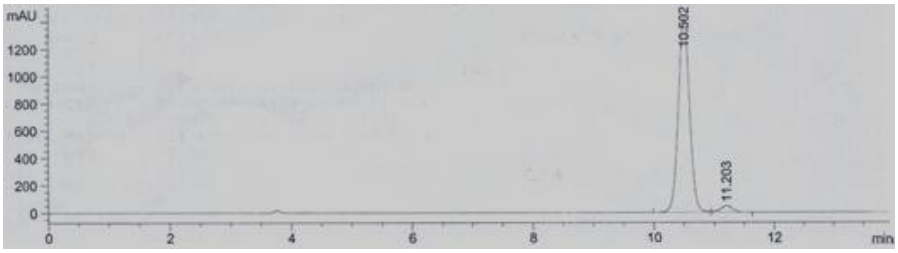
HPLC separation for both enantiomers of 6c



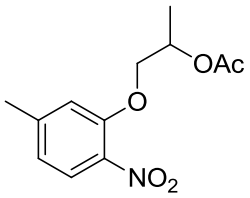
(*S*)-6c in 80% *ee*



(*R*)-6c in 93% *ee*



1-(5-Methyl-2-nitrophenoxy)propan-2-yl acetate (6d)

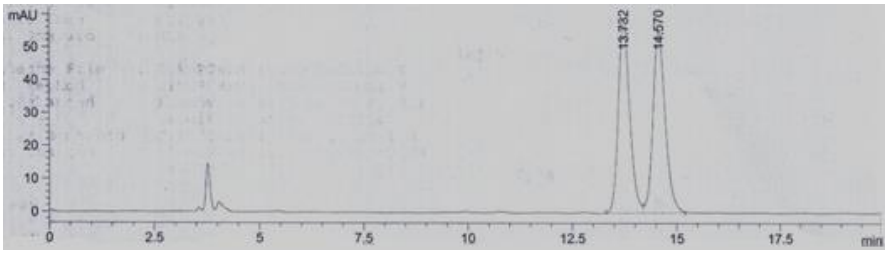


Analytical data for acetate 6d

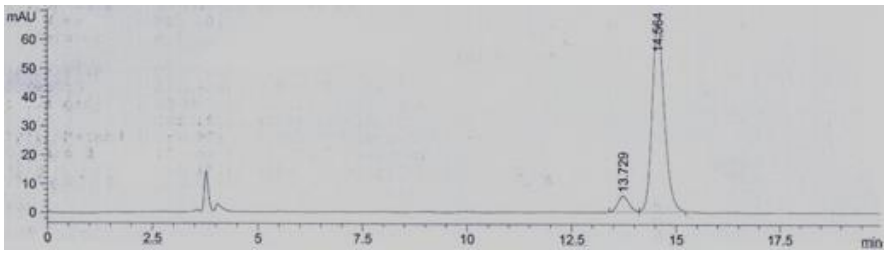
Column: Chiralcel IA
Flow: 0.8 mL/min
Retention times: t_R (*R*) = 13.7 min, t_R (*S*) = 14.6 min

Eluent: *n*-hexane/2-propanol 97:3
Temperature: 30 ºC
Rs: 1.0

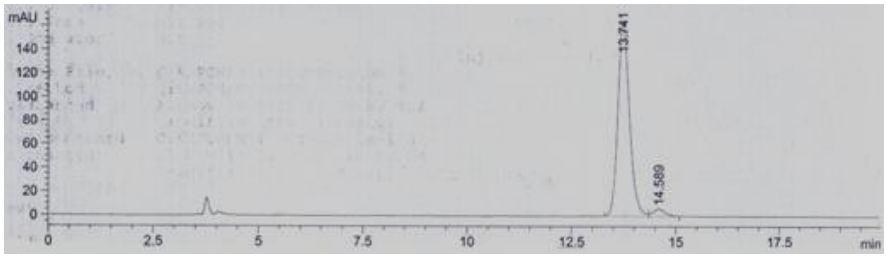
HPLC separation for both enantiomers of 6d



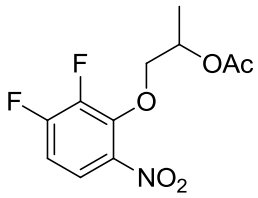
(*S*)-6d in 87% *ee*



(*R*)-6d in 93% *ee*



1-(2,3-Difluoro-6-nitrophenoxy)propan-2-yl acetate (6e)

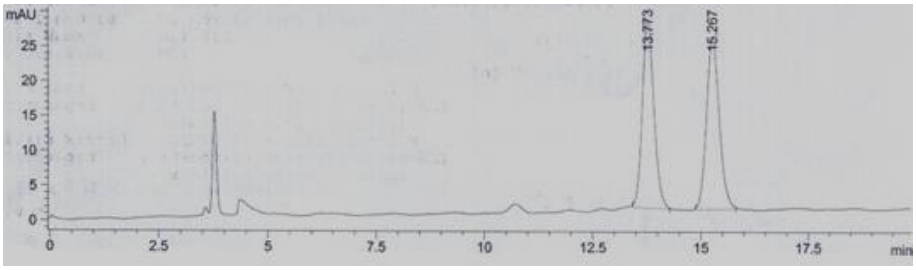


Analytical data for acetate 6e

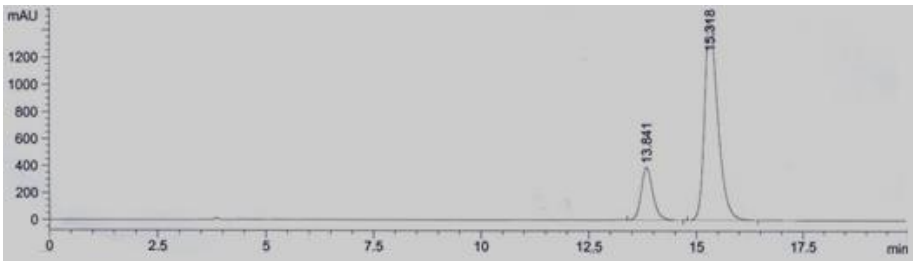
Column: Chiralcel IA
Flow: 0.8 mL/min
Retention times: t_R (*R*) = 13.8 min, t_R (*S*) = 15.3 min

Eluent: *n*-hexane/2-propanol 98:2
Temperature: 30 ºC
Rs: 1.8

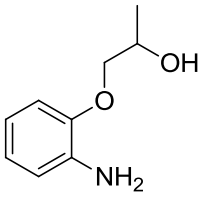
HPLC separation for both enantiomers of 6e



(*S*)-6e in 63% *ee*



1-(2-Aminophenoxy)propan-2-ol (8a)

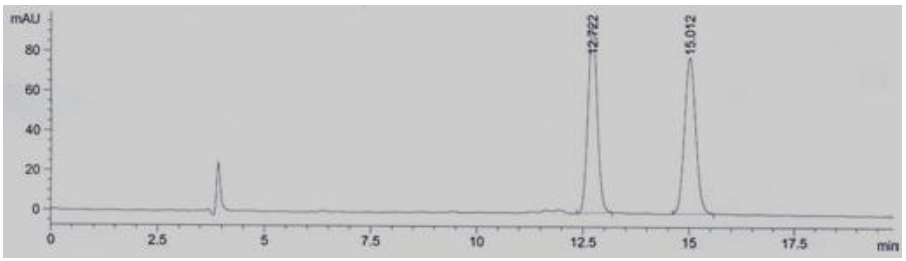


Analytical data for alcohol 8a

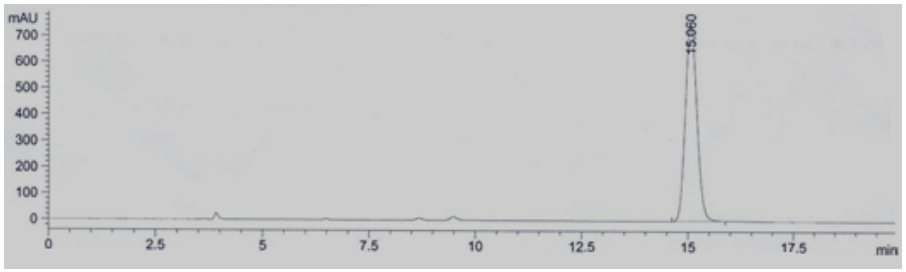
Column: Chiralcel IC
Flow: 0.8 mL/min
Retention times: t_R (*R*) = 12.7 min, t_R (*S*) = 15.0 min

Eluent: *n*-hexane/2-propanol 80:20
Temperature: 30 ºC
Rs: 3.0

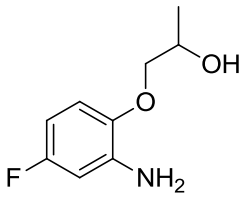
HPLC separation for both enantiomers of 8a



(*S*)-8a in >99% *ee*



1-(2-Amino-4-fluorophenoxy)propan-2-ol (8b)

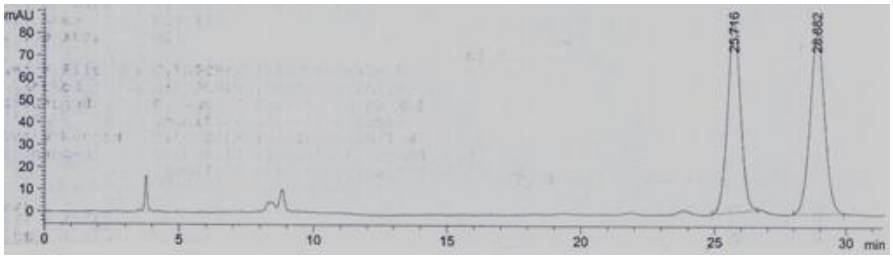


Analytical data for alcohol 8b

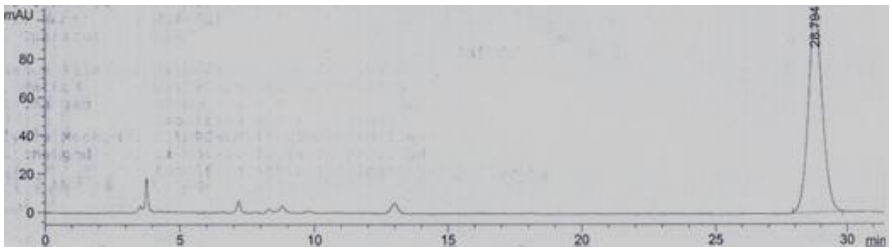
Column: Chiralcel IA
Flow: 0.8 mL/min
Retention times: t_R (*R*) = 25.7 min, t_R (*S*) = 28.9 min

Eluent: *n*-hexane/2-propanol 90:10
Temperature: 30 ºC
Rs: 1.9

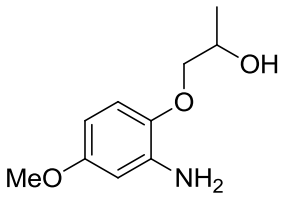
HPLC separation for both enantiomers of 8b



(*S*)-8b in >99% *ee*



1-(2-Amino-4-methoxyphenoxy)propan-2-ol (8c)

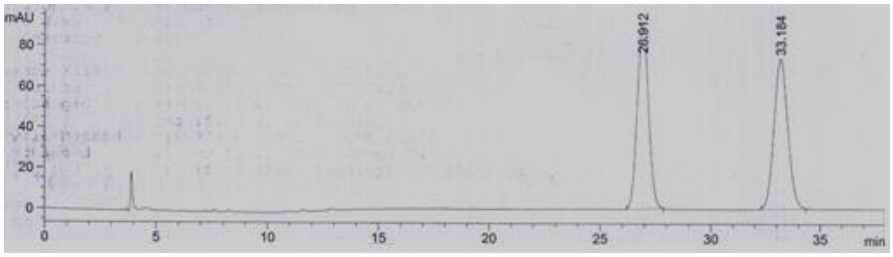


Analytical data for alcohol 8c

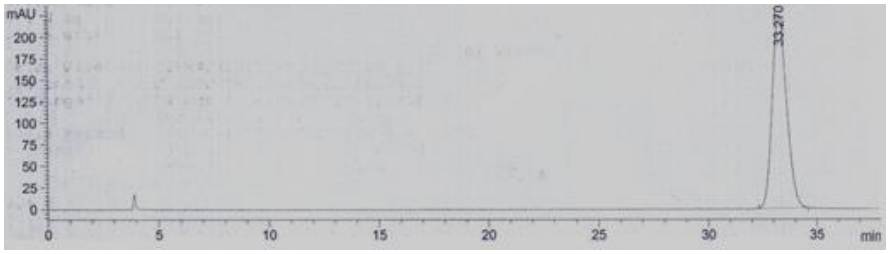
Column: Chiralcel IC
Flow: 0.8 mL/min
Retention times: t_R (*R*) = 26.9 min, t_R (*S*) = 33.2 min

Eluent: *n*-hexane/2-propanol 80:20
Temperature: 30 ºC
Rs: 3.5

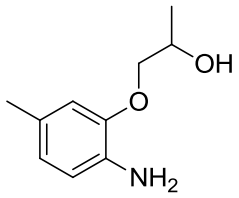
HPLC separation for both enantiomers of 8c



(*S*)-8c in >99% *ee*



1-(2-Amino-5-methylphenoxy)propan-2-ol (8d)

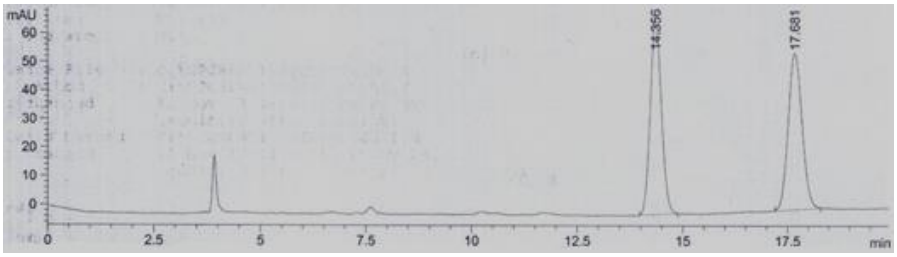


Analytical data for alcohol 8d

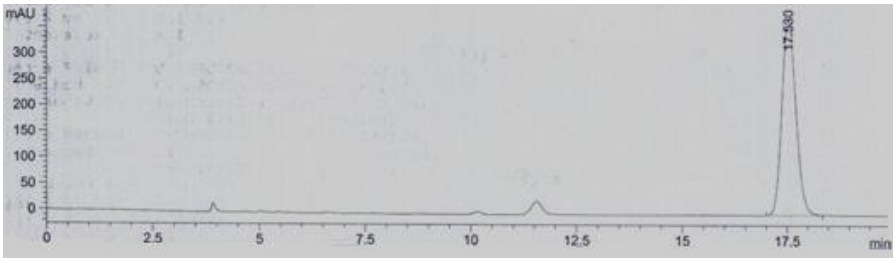
Column: Chiralcel IC
Flow: 0.8 mL/min
Retention times: t_R (*R*) = 14.3 min, t_R (*S*) = 17.7 min

Eluent: *n*-hexane/2-propanol 80:20
Temperature: 30 ºC
Rs: 3.6

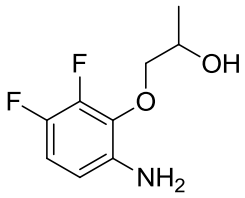
HPLC separation for both enantiomers of 8d



(*S*)-8d in >99% *ee*



1-(6-Amino-2,3-difluorophenoxy)propan-2-ol (8e)

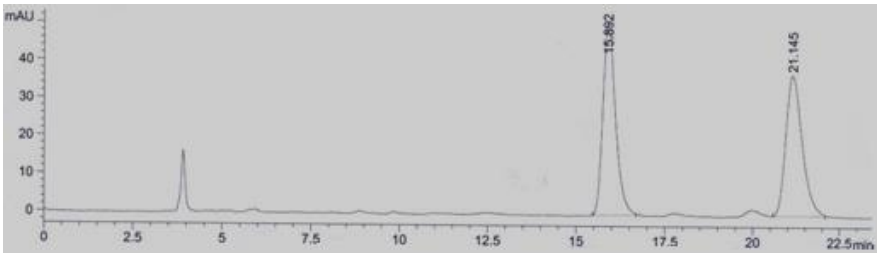


Analytical data for alcohol 8e

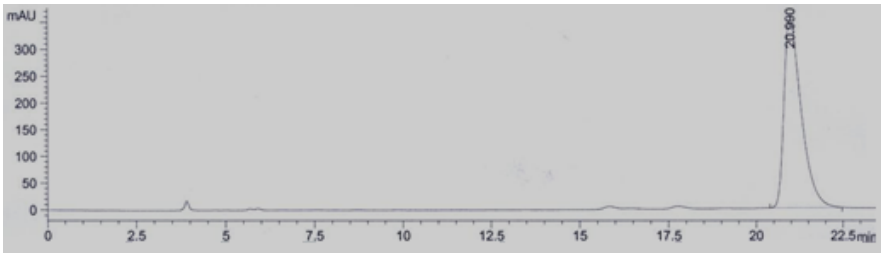
Column: Chiralcel OJ-H
Flow: 0.8 mL/min
Retention times: t_R (*S*) = 15.9 min, t_R (*R*) = 21.1 min

Eluent: *n*-hexane/2-propanol 90:10
Temperature: 30 ºC
Rs: 3.0

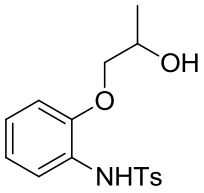
HPLC separation for both enantiomers of 8e



(*R*)-8e in >99% *ee*



***N*-(2-(2-Hydroxypropoxy)phenyl)-4-methylbenzenesulfonamide (9a)**

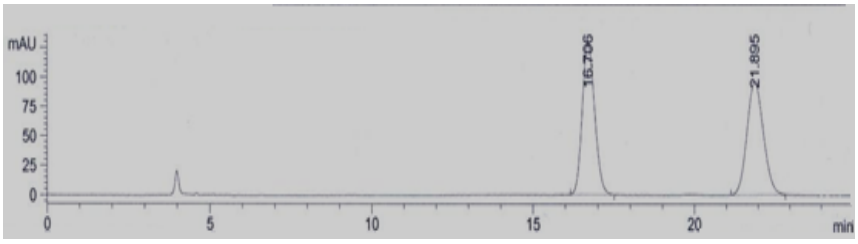


Analytical data for sulfonamide 9a

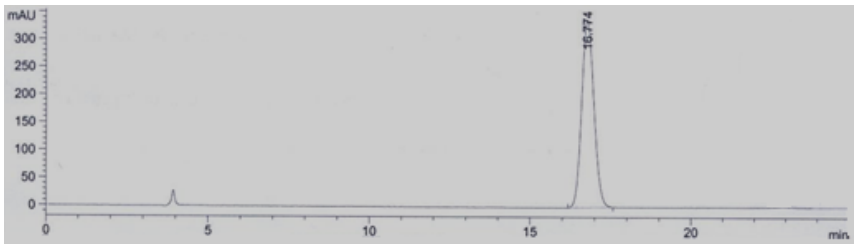
Column: Chiralcel IC
Flow: 0.8 mL/min
Retention times: t_R (*S*) = 16.7 min, t_R (*R*) = 21.9 min

Eluent: *n*-hexane/2-propanol 70:30
Temperature: 30 ºC
Rs: 4.3

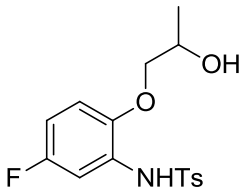
HPLC separation for both enantiomers of 9a



(*S*)-9a in >99% *ee*



***N*-(5-Fluoro-2-(2-hydroxypropoxy)phenyl)-4-methylbenzenesulfonamide (9b)**

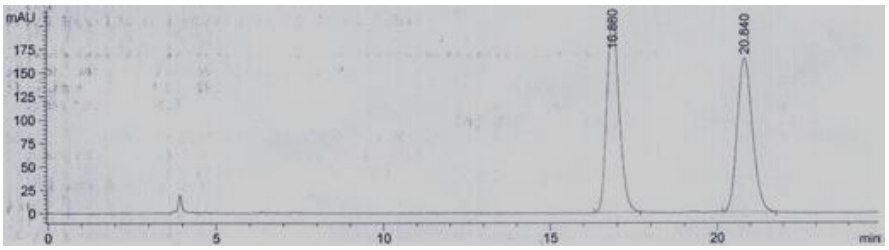


Analytical data for sulfonamide 9b

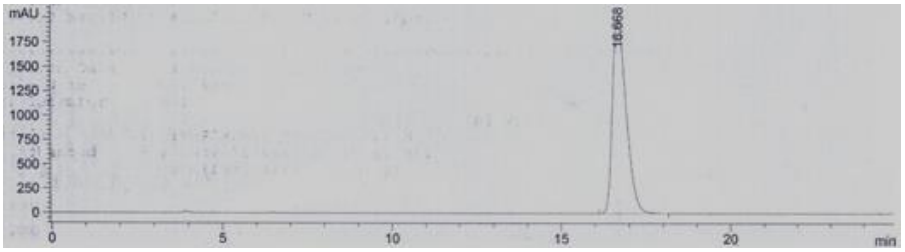
Column: Chiralcel IC
Flow: 0.8 mL/min
Retention times: t_R (*S*) = 16.8 min, t_R (*R*) = 20.8 min

Eluent: *n*-hexane/2-propanol 80:20
Temperature: 30 ºC
Rs: 3.0

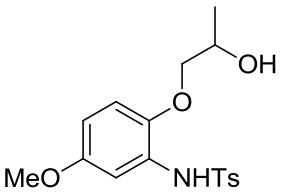
HPLC separation for both enantiomers of 9b



(*S*)-9b in >99% *ee*



***N*-(2-(2-Hydroxypropoxy)-5-methoxyphenyl)-4-methylbenzenesulfonamide (9c)**

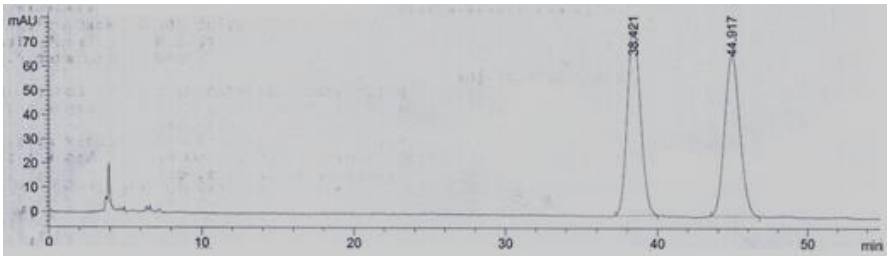


Analytical data for sulfonamide 9c

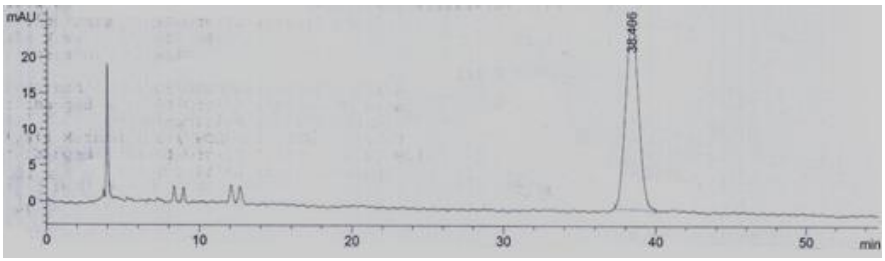
Column: Chiralcel IC
Flow: 0.8 mL/min
Retention times: t_R (*S*) = 38.4 min, t_R (*R*) = 44.9 min

Eluent: *n*-hexane/2-propanol 80:20
Temperature: 30 ºC
Rs: 2.3

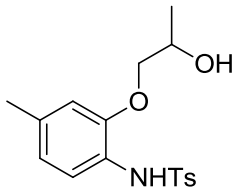
HPLC separation for both enantiomers of 9c



(*S*)-9c in >99% *ee*



***N*-(2-(2-Hydroxypropoxy)-4-methylphenyl)-4-methylbenzenesulfonamide (9d)**

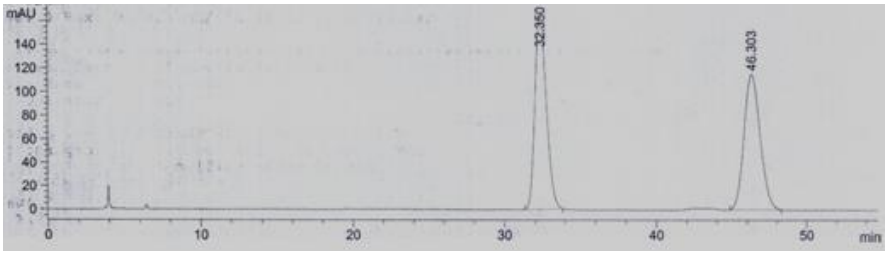


Analytical data for sulfonamide 9d

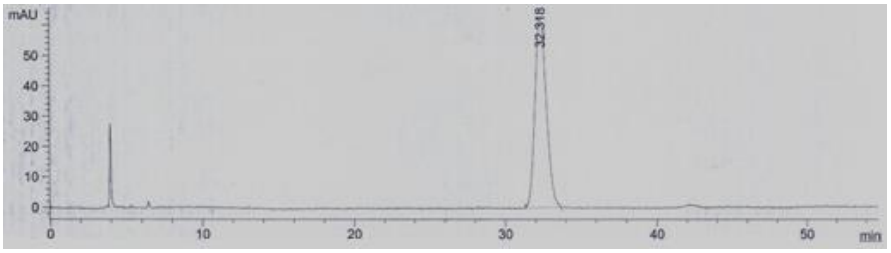
Column: Chiralcel IC
Flow: 0.8 mL/min
Retention times: t_R (*S*) = 32.3 min, t_R (*R*) = 46.3 min

Eluent: *n*-hexane/2-propanol 80:20
Temperature: 30 ºC
Rs: 5.2

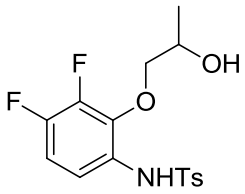
HPLC separation for both enantiomers of 9d



(*S*)-9d in >99% *ee*



***N*-(3,4-Difluoro-2-(2-hydroxypropoxy)phenyl)-4-methylbenzenesulfonamide (9e)**

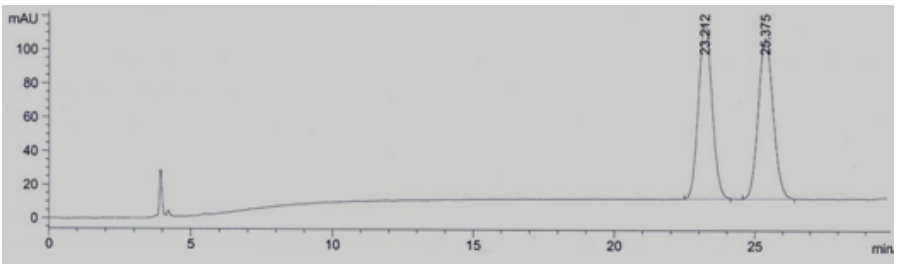


Analytical data for sulfonamide 9e

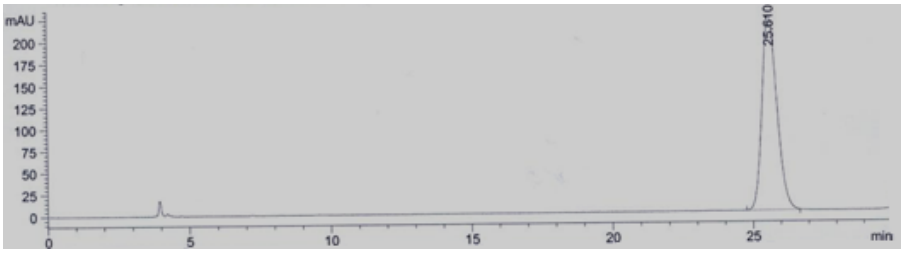
Column: Chiralcel IC
Flow: 0.8 mL/min
Retention times: t_R (*S*) = 23.2 min, t_R (*R*) = 25.4 min

Eluent: *n*-hexane/2-propanol 90:10
Temperature: 30 ºC
Rs: 5.2

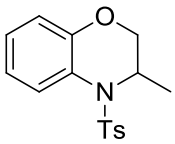
HPLC separation for both enantiomers of 9e



(*R*)-9e in >99% *ee*



3-Methyl-4-tosyl-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine (10a)

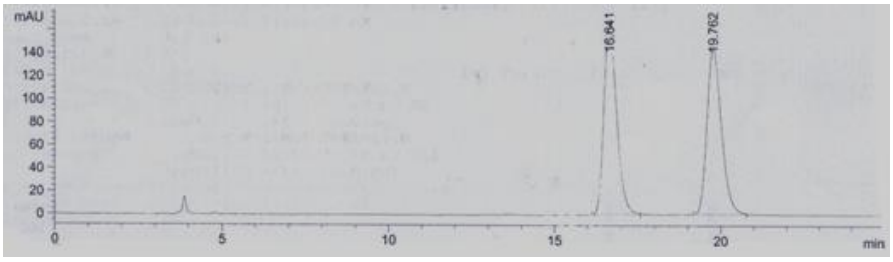


Analytical data for tosylated benzoxazine 10a

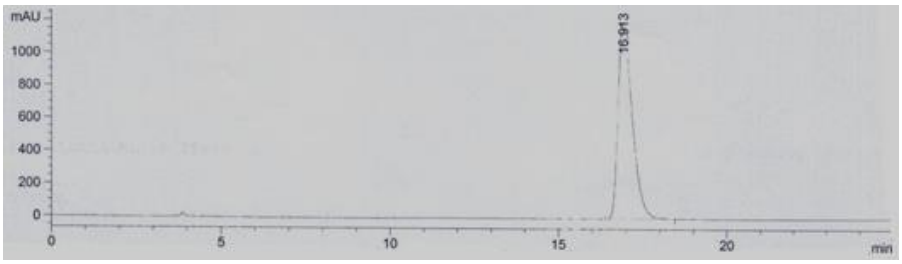
Column: Chiralcel OJ-H
Flow: 0.8 mL/min
Retention times: t_R (*R*) = 16.6 min, t_R (*S*) = 19.8 min

Eluent: *n*-hexane/2-propanol 90:10
Temperature: 30 ºC
Rs: 2.1

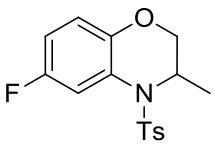
HPLC separation for both enantiomers of 10a



(*R*)-10a in >99% *ee*



6-Fluoro-3-methyl-4-tosyl-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine (10b)

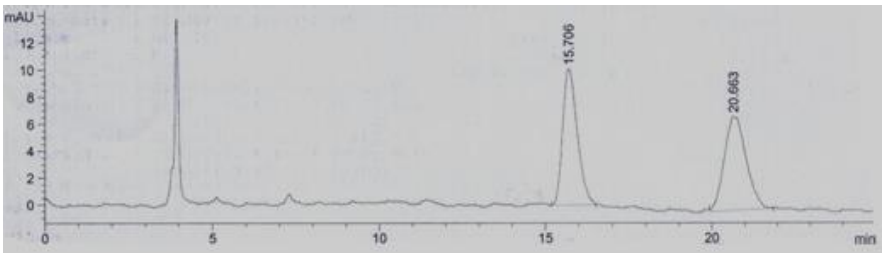


Analytical data for tosylated benzoxazine 10b

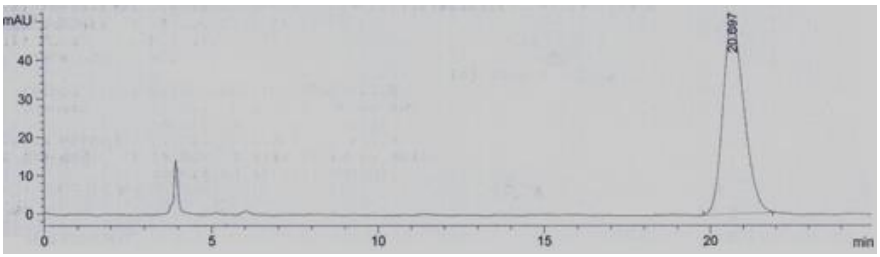
Column: Chiralcel OJ-H
Flow: 0.8 mL/min
Retention times: t_R (*S*) = 15.7 min, t_R (*R*) = 20.7 min

Eluent: *n*-hexane/2-propanol 90:10
Temperature: 30 ºC
Rs: 3.7

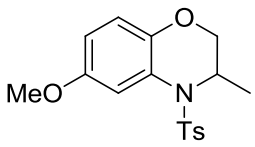
HPLC separation for both enantiomers of 10b



(*R*)-10b in >99% *ee*



6-Methoxy-3-methyl-4-tosyl-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine (10c)

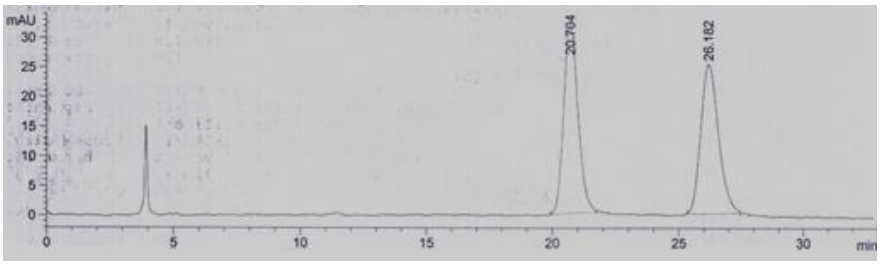


Analytical data for tosylated benzoxazine 10c

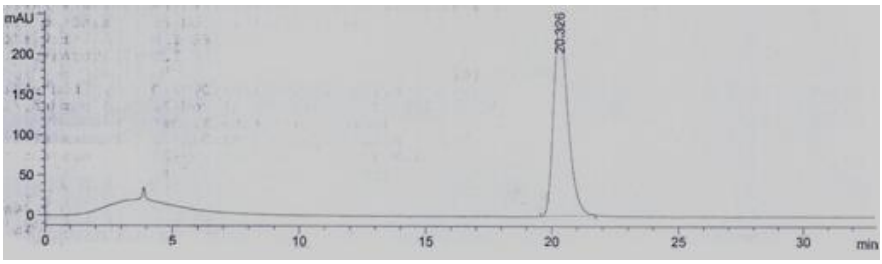
Column: Chiralcel OJ-H
Flow: 0.8 mL/min
Retention times: t_R (*R*) = 20.7 min, t_R (*S*) = 26.2 min

Eluent: *n*-hexane/2-propanol 90:10
Temperature: 30 ºC
Rs: 2.8

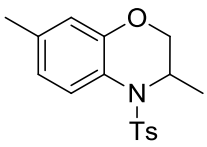
HPLC separation for both enantiomers of 10c



(*R*)-10c in >99% *ee*



3,7-Dimethyl-4-tosyl-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine (10d)

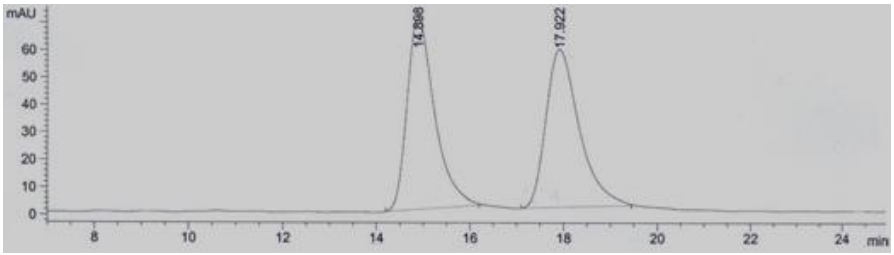


Analytical data for tosylated benzoxazine 10d

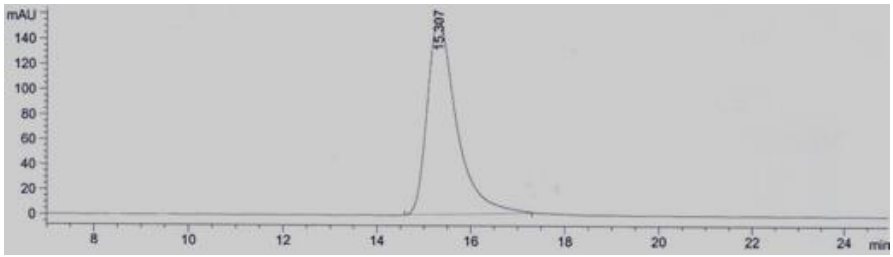
Column: Chiralcel OJ-H
Flow: 0.8 mL/min
Retention times: t_R (*R*) = 14.9 min, t_R (*S*) = 17.9 min

Eluent: *n*-hexane/2-propanol 90:10
Temperature: 30 ºC
Rs: 1.5

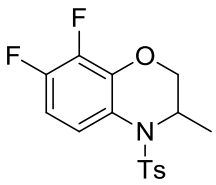
HPLC separation for both enantiomers of 10d



(*R*)-10d in >99% *ee*



7,8-difluoro-3-methyl-4-tosyl-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine (10e)

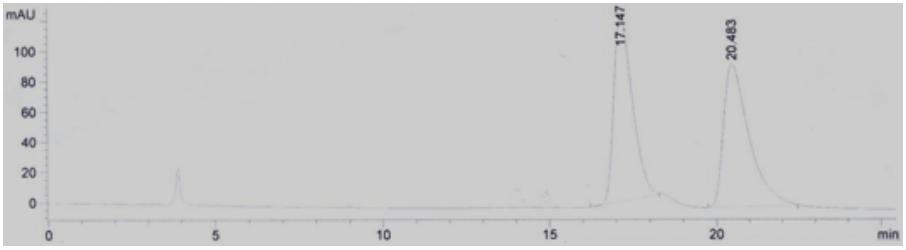


Analytical data for tosylated benzoxazine 10e

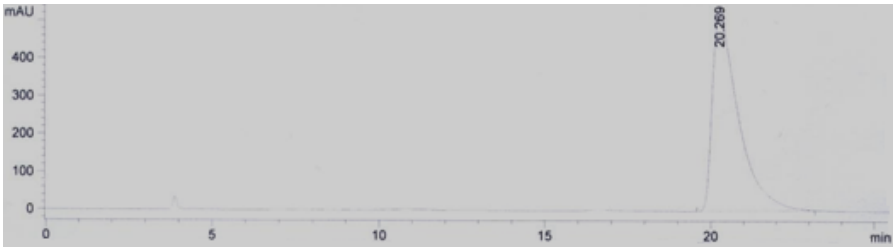
Column: Chiralcel OJ-H
Flow: 0.8 mL/min
Retention times: t_R (*R*) = 17.1 min, t_R (*S*) = 20.5 min

Eluent: *n*-hexane/2-propanol 90:10
Temperature: 30 ºC
Rs: 1.6

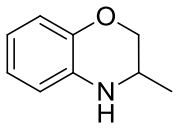
HPLC separation for both enantiomers of 10e



(*S*)-10e in >99% *ee*



3-Methyl-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine (4a)

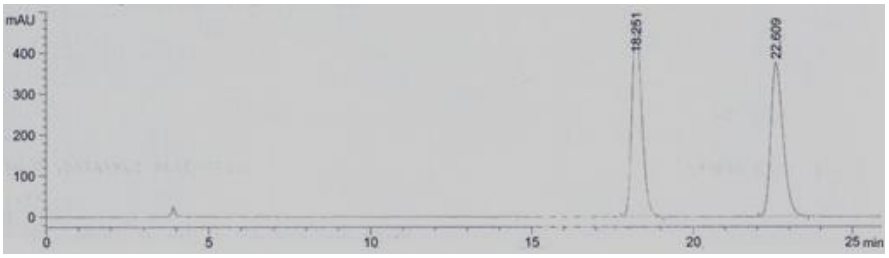


Analytical data for benzoxazine 4a

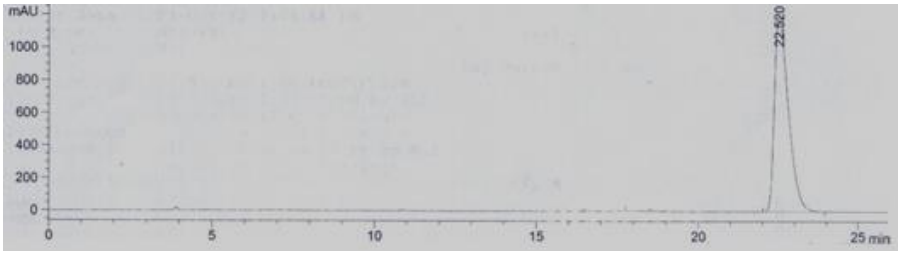
Column: Chiralcel OJ-H
Flow: 0.8 mL/min
Retention times: t_R (*R*) = 18.2 min, t_R (*S*) = 22.6 min

Eluent: *n*-hexane/2-propanol 90:10
Temperature: 30 ºC
Rs: 3.5

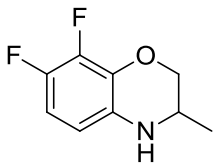
HPLC separation for both enantiomers of 4a



(*R*)-4a in >99% *ee*



7,8-Difluoro-3-methyl-3,4-dihydro-2*H*-benzo[*b*][1,4]oxazine (4e)

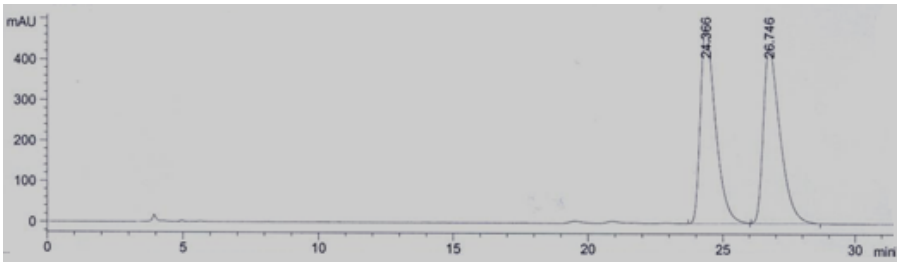


Analytical data for benzoxazine 4e

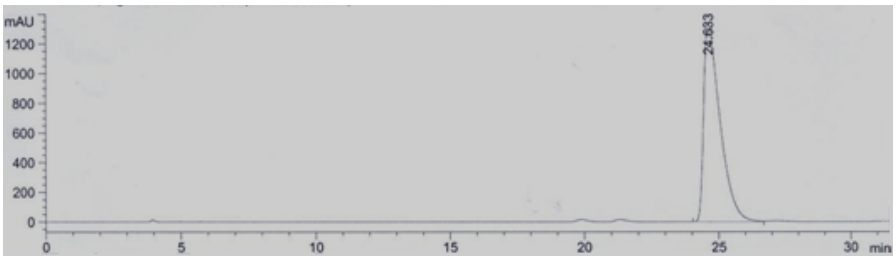
Column: Chiralcel OJ-H
Flow: 0.8 mL/min
Retention times: t_R (*S*) = 24.4 min, t_R (*R*) = 26.7 min

Eluent: *n*-hexane/2-propanol 95:5
Temperature: 30 ºC
Rs: 1.1

HPLC separation for both enantiomers of 4e

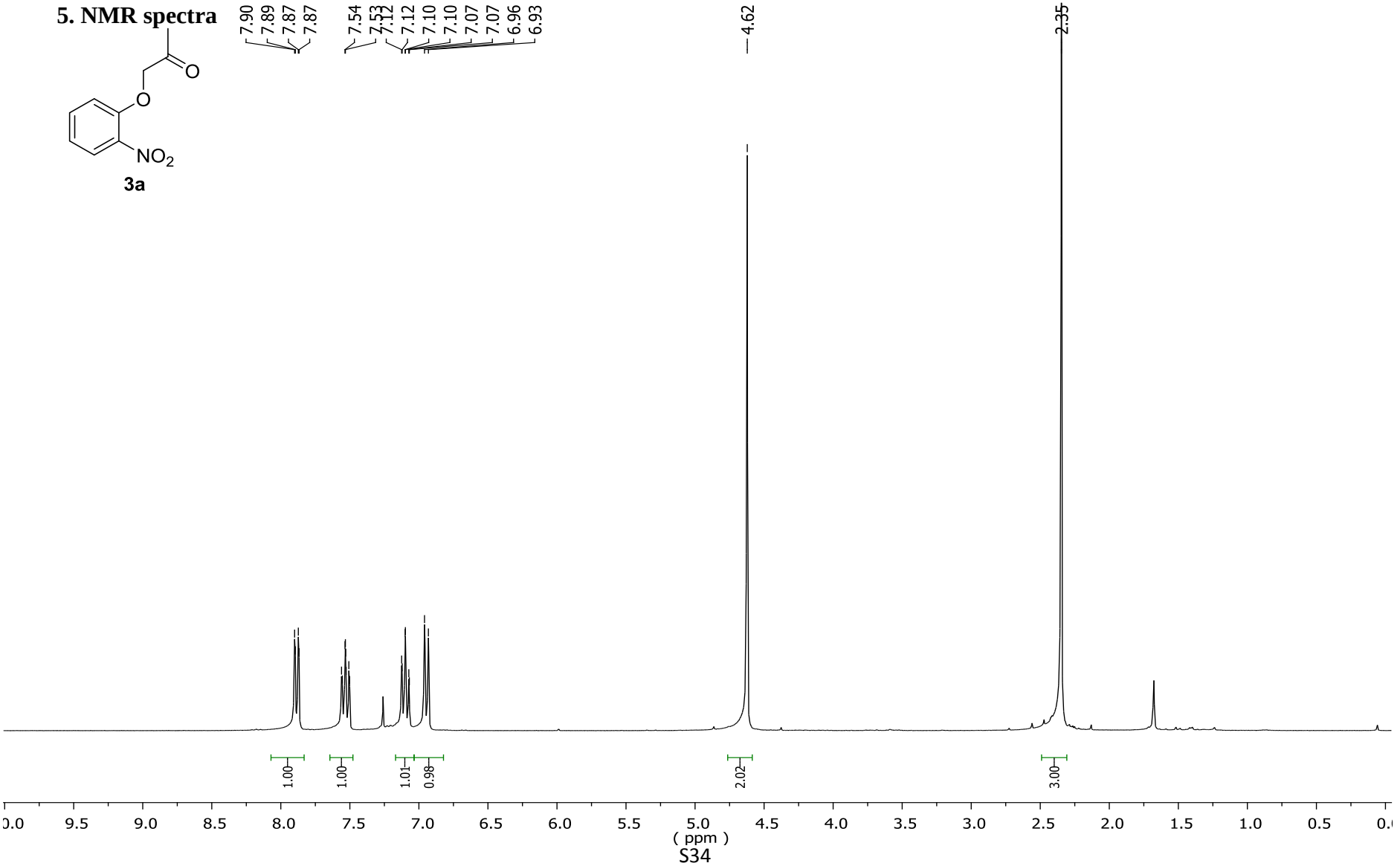


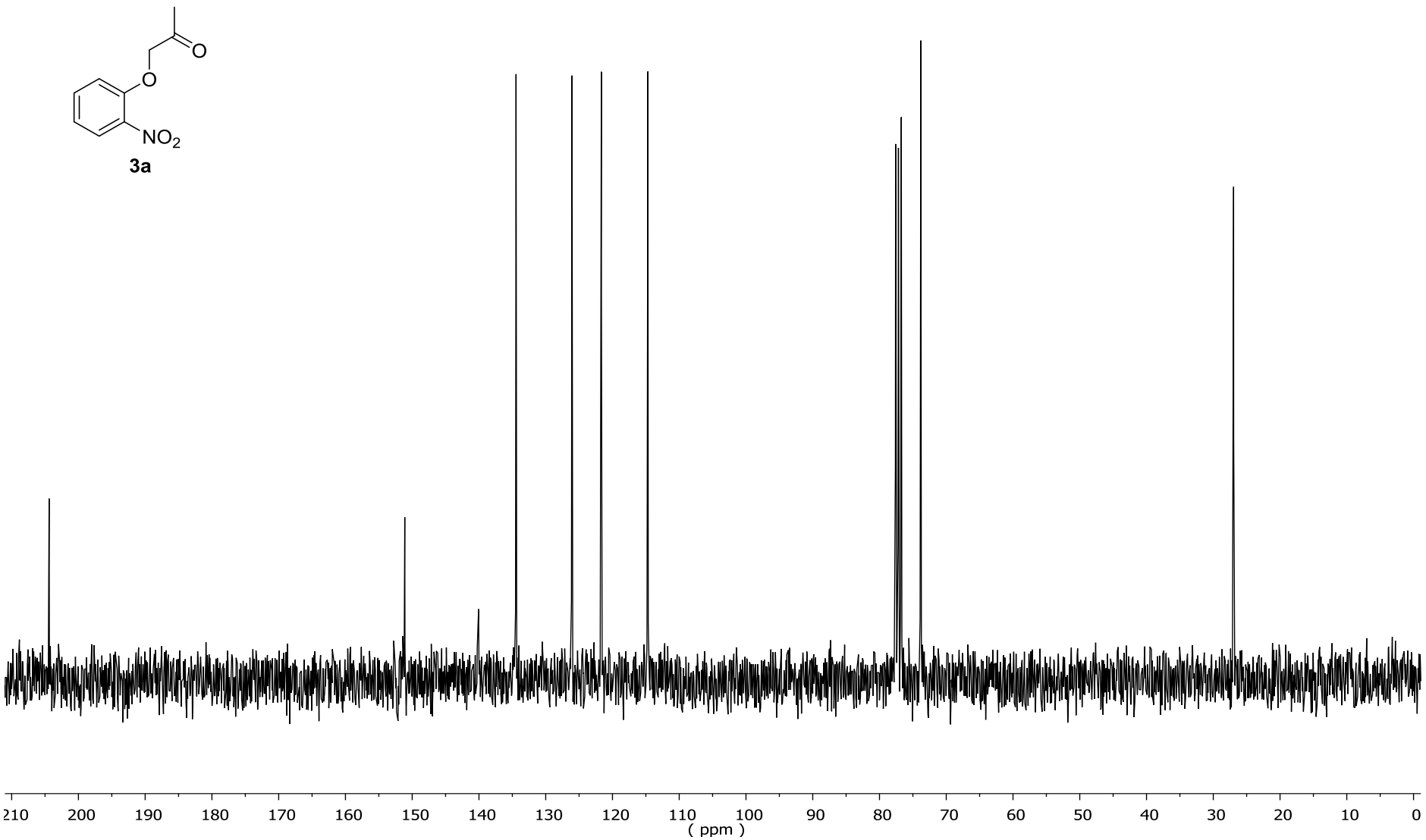
(*S*)-4e in >99% *ee*



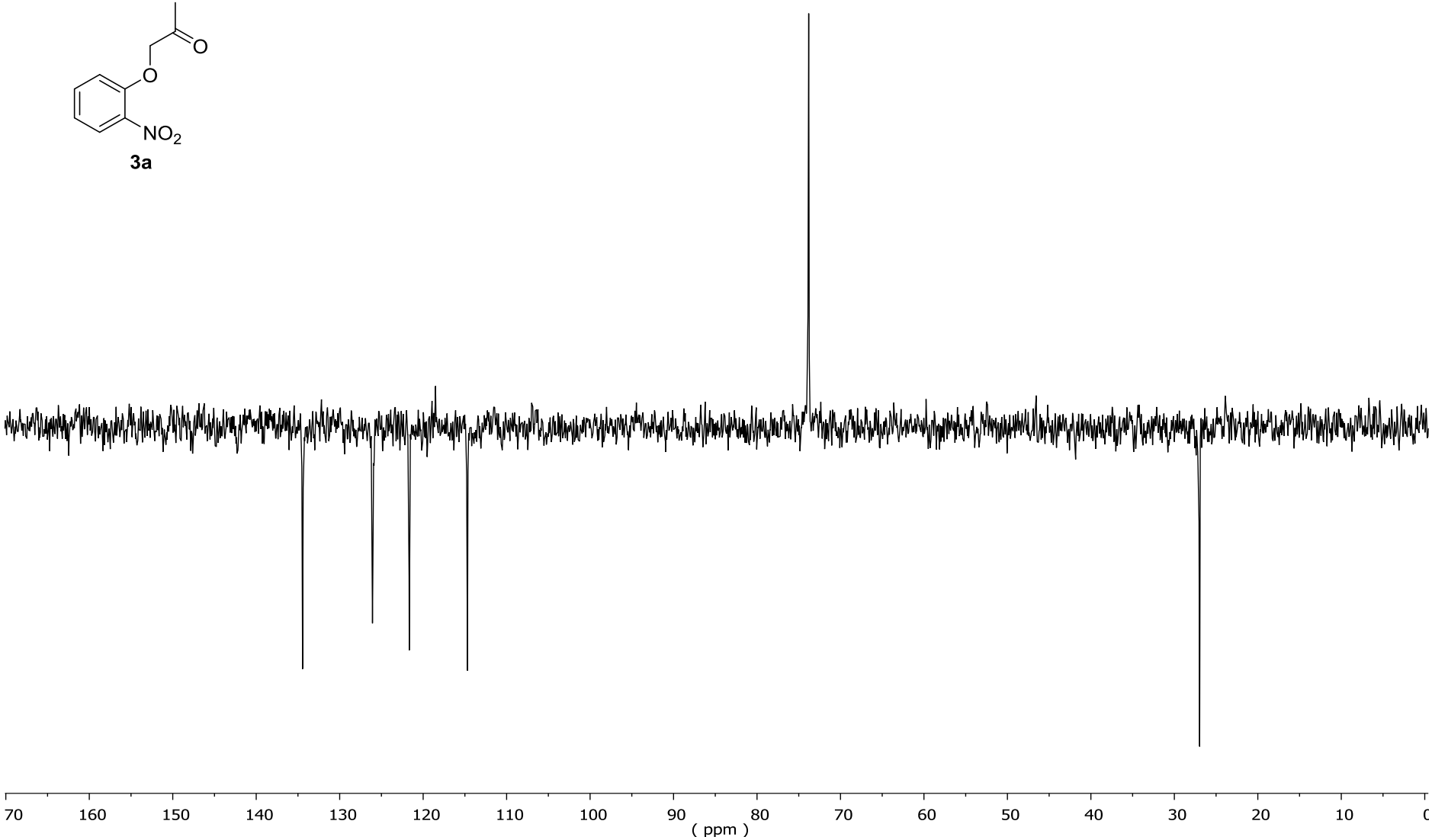
5. NMR spectra

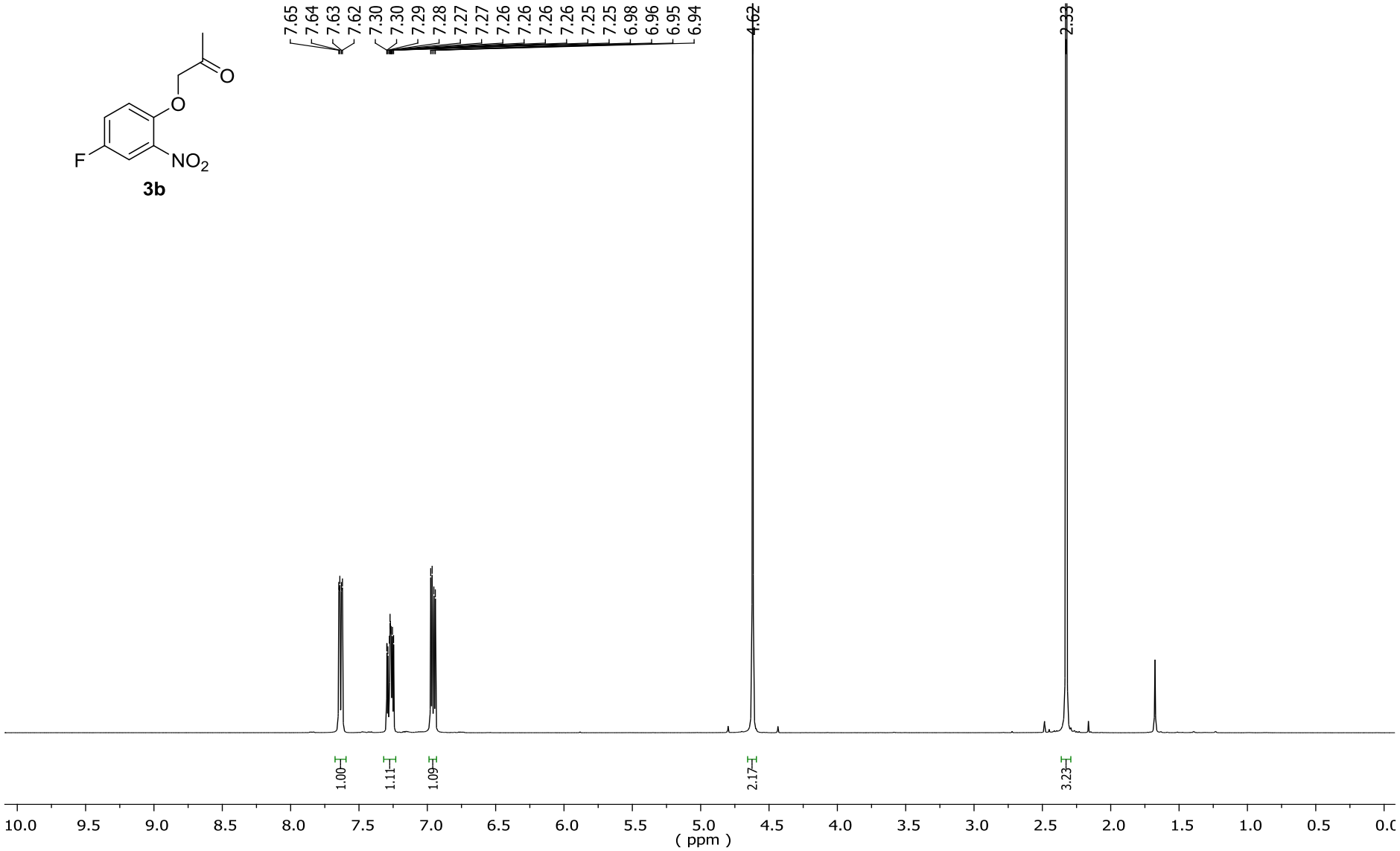
3a




O
O
NO2
3a
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)

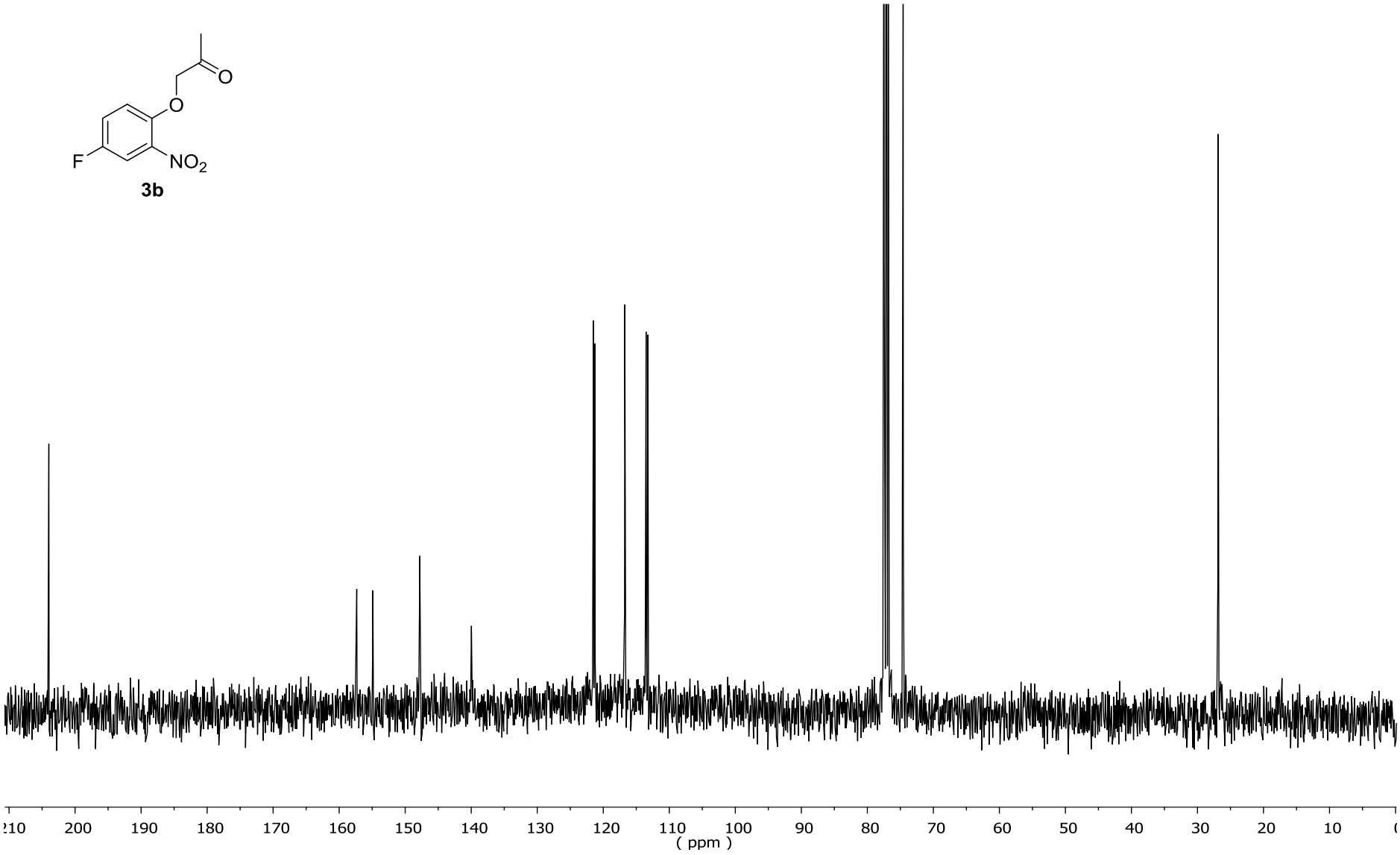
3a




7.65
7.64
7.63
7.62
7.30
7.30
7.29
7.28
7.27
7.27
7.26
7.26
7.26
7.25
7.25
6.98
6.96
6.95
6.94
4.62
2.33
O
O
F
NO2
3b
1.00
1.11
1.09
2.17
3.23
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
(ppm)

O
O
F
NO2

3b


210
200
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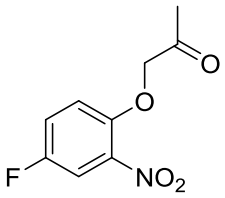
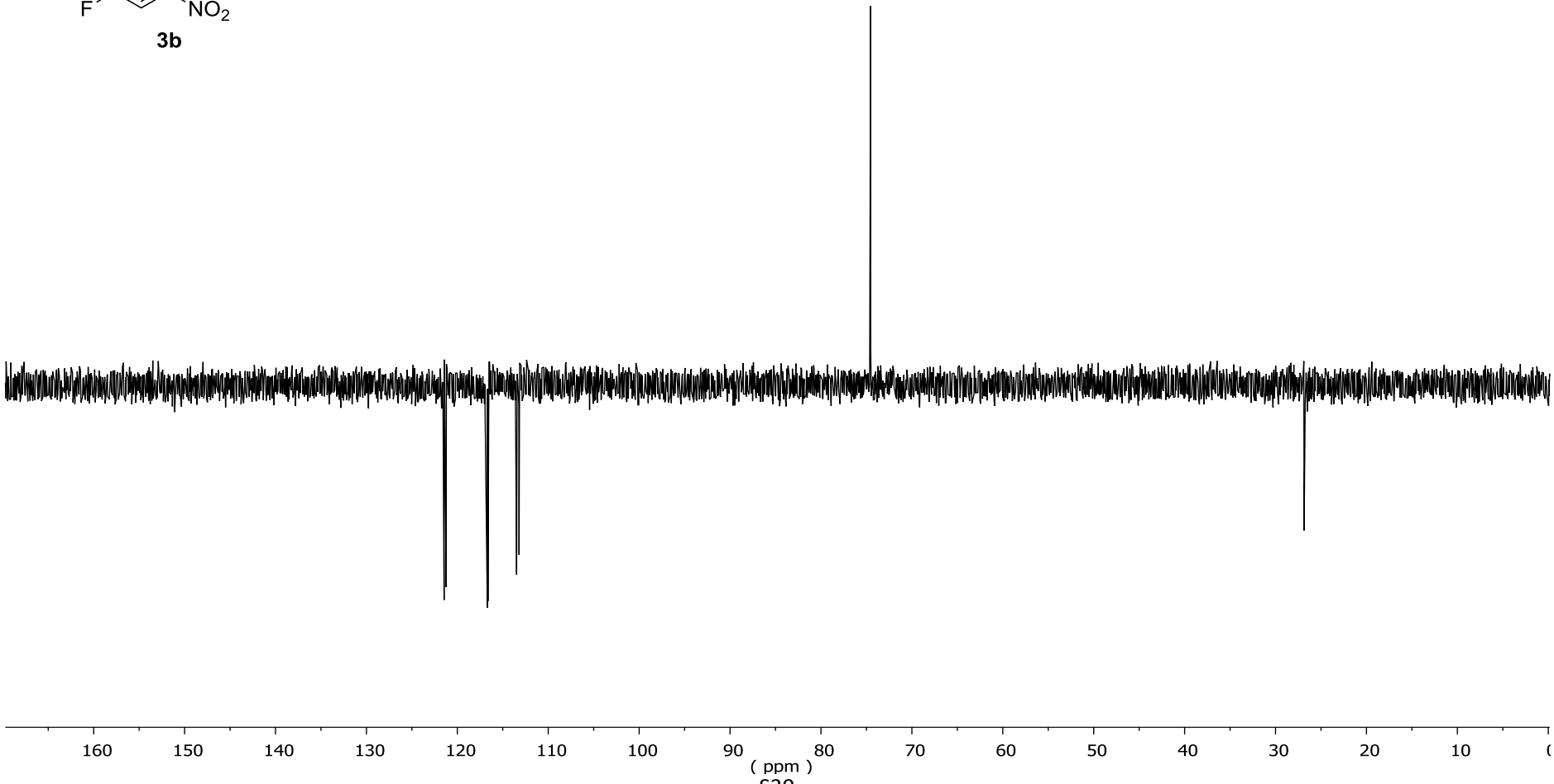
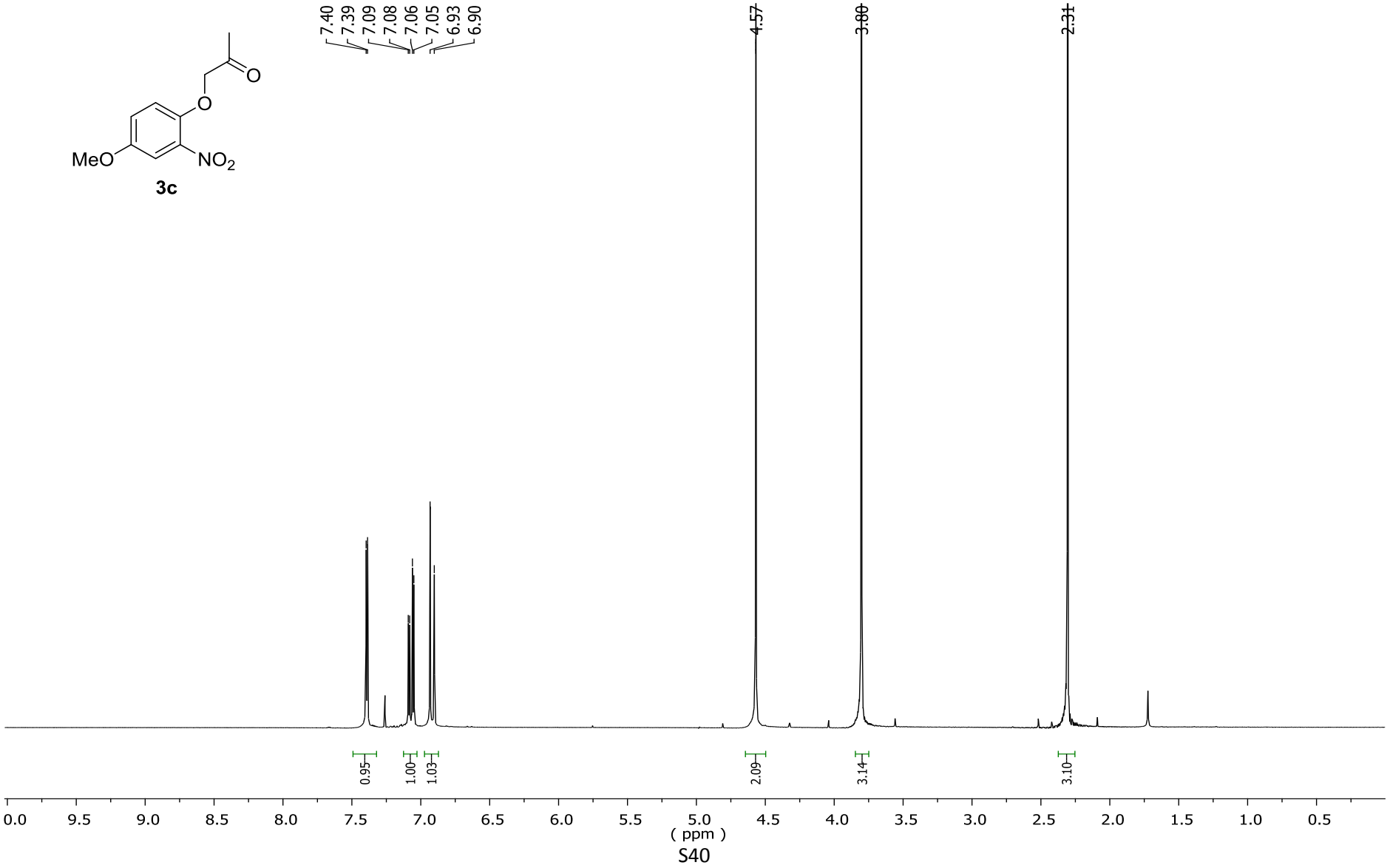
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)



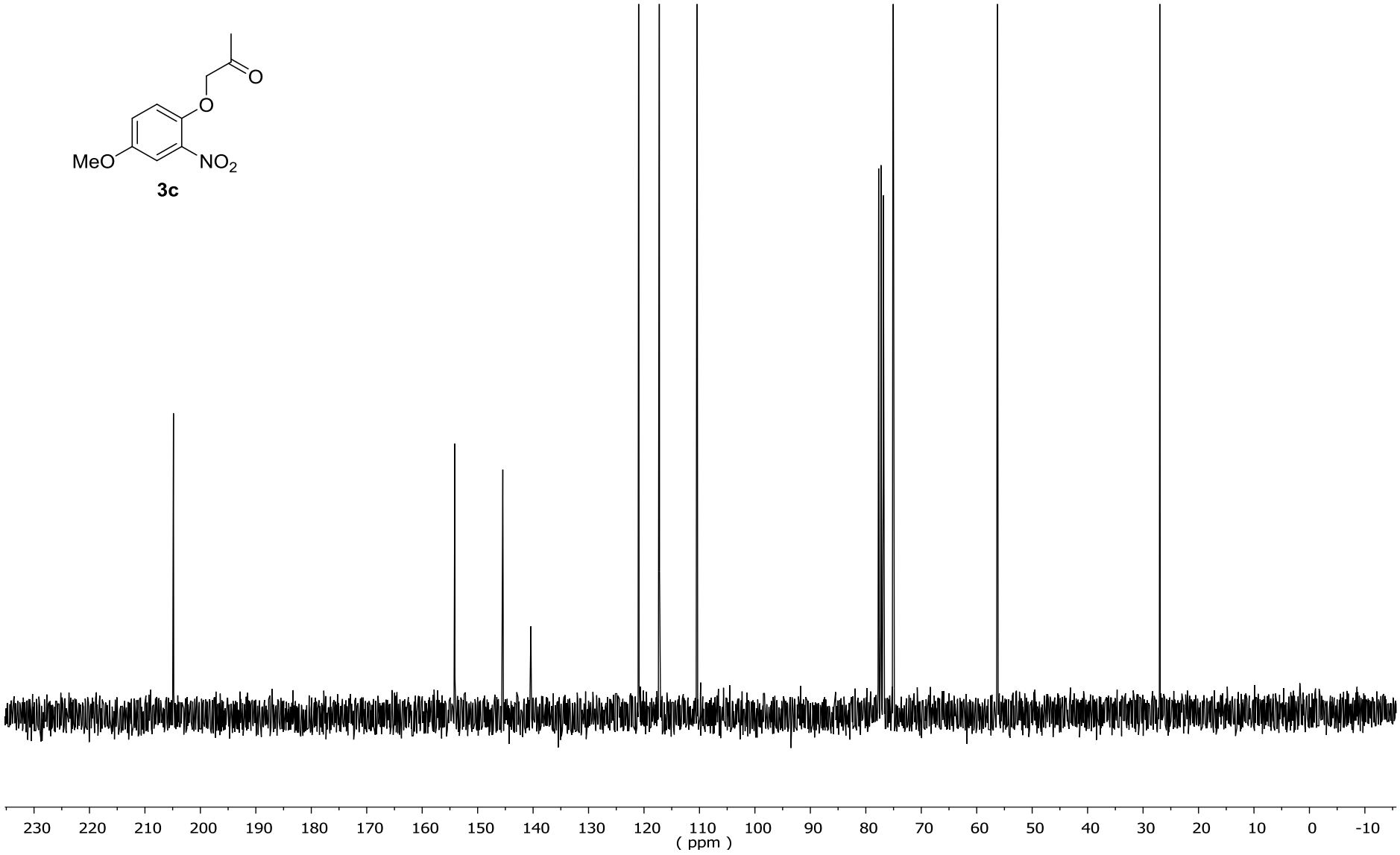
3b




7.40
7.39
7.09
7.08
7.06
7.05
6.93
6.90
4.57
3.80
2.31
O
O
MeO
NO2
3c
0.95
1.00
1.03
2.09
3.14
3.10
0.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
(ppm)

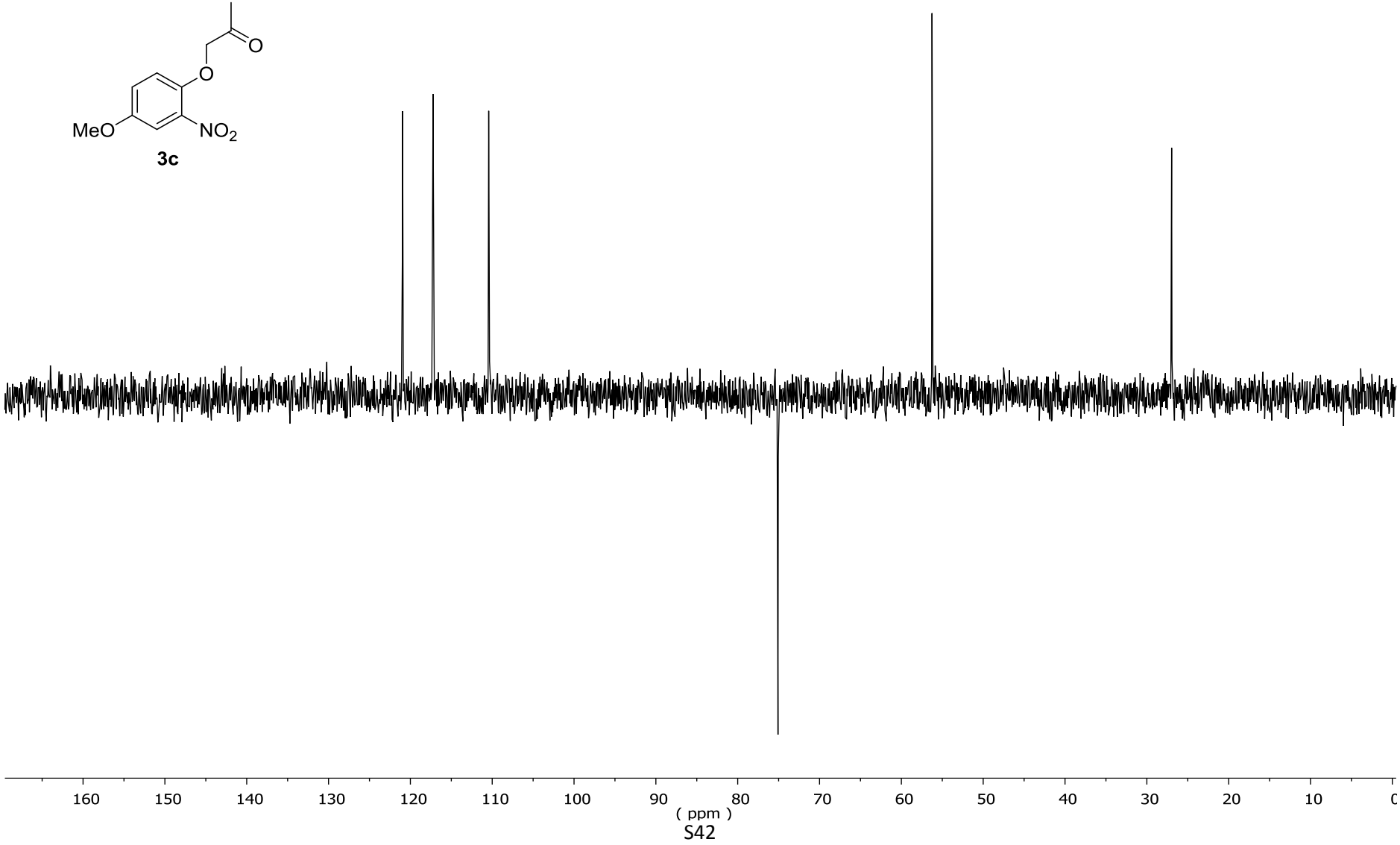
MeO
NO2
O
O

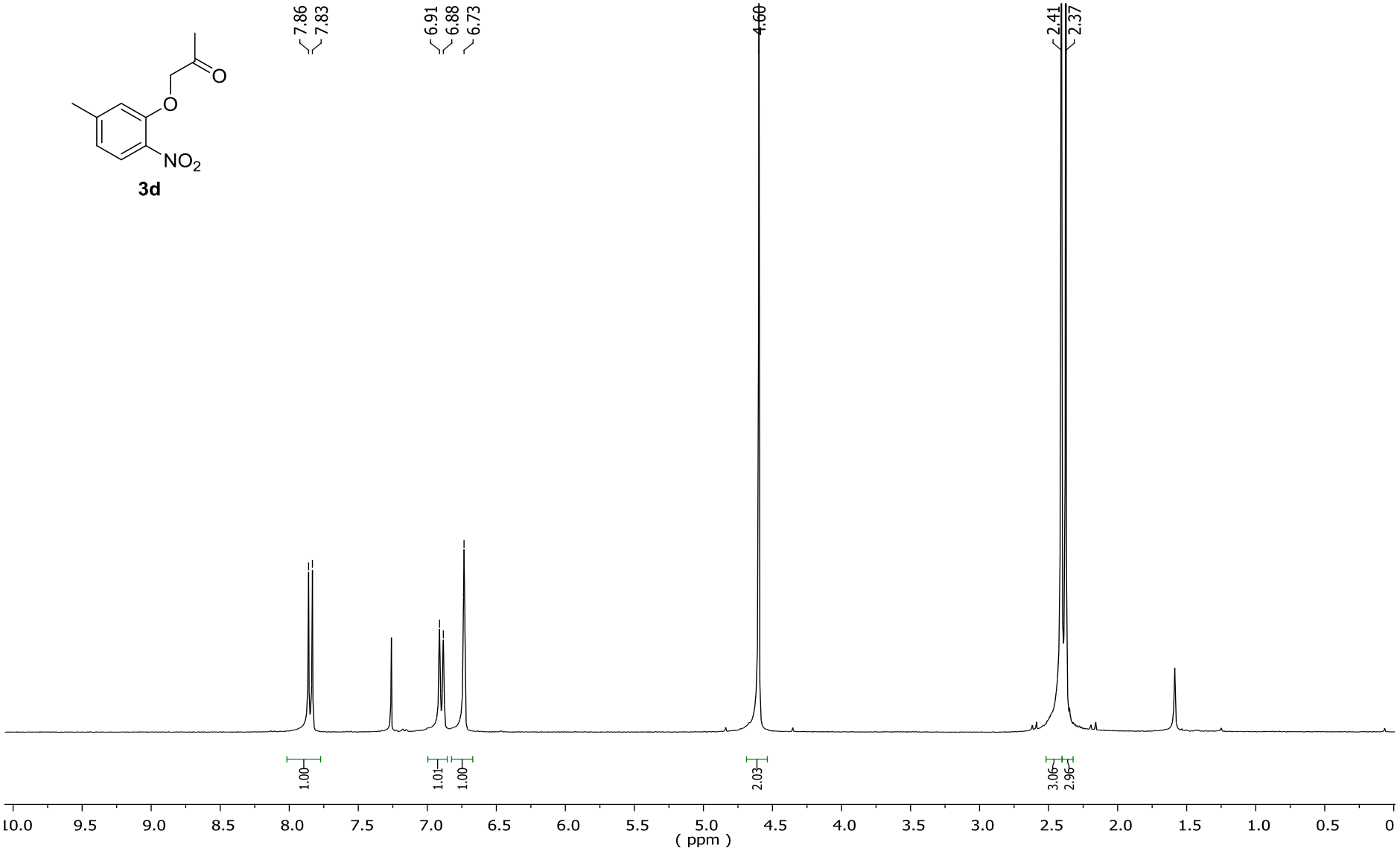
3c


230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
(ppm)

O
O
MeO
NO2

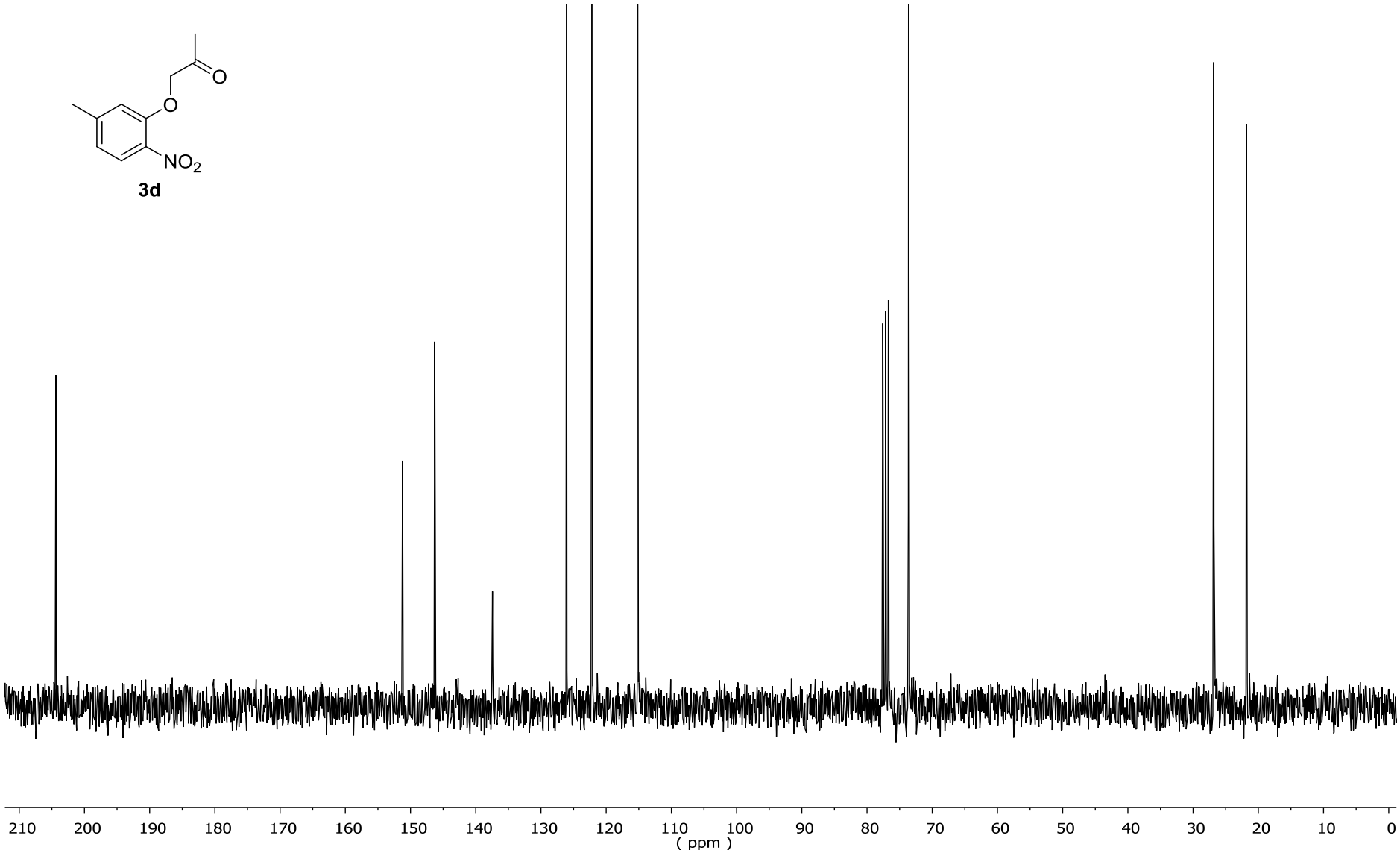
3c


160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)


7.86
7.83
6.91
6.88
6.73
4.60
2.41
2.37
O
O
NO2
3d
1.00
1.01
1.00
2.03
3.06
2.96
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
(ppm)

O
O
NO2

3d


210
200
190
180
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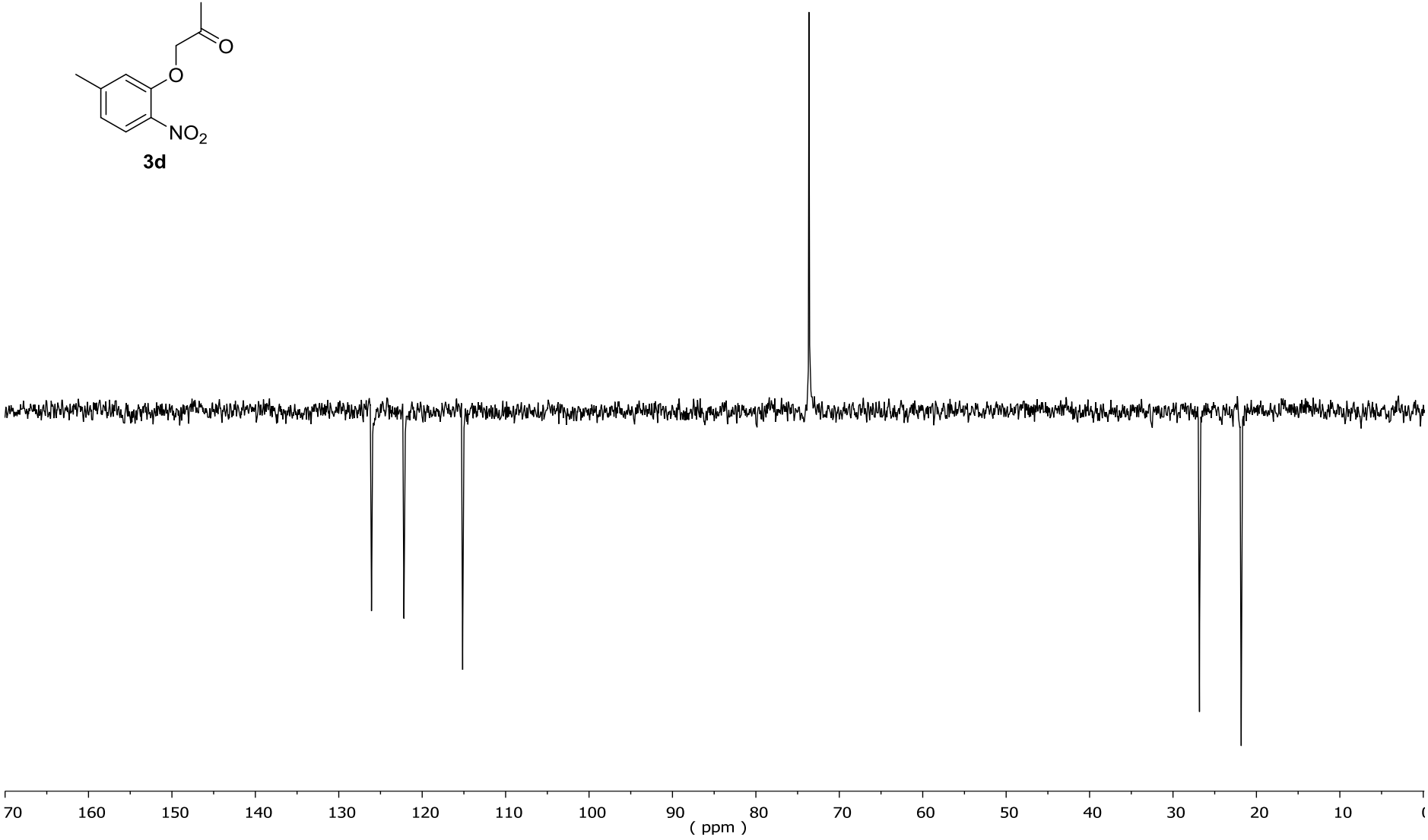
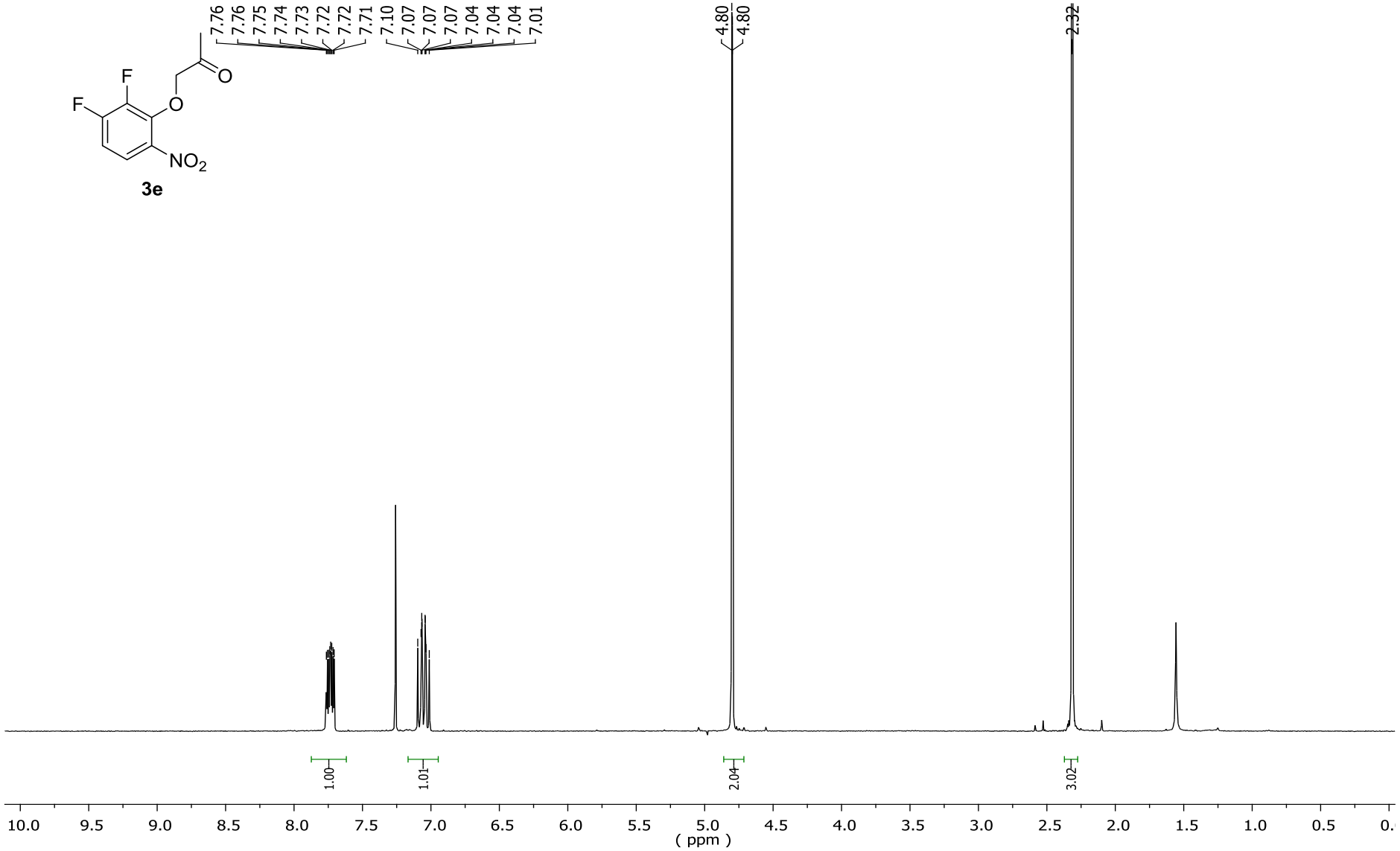
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)

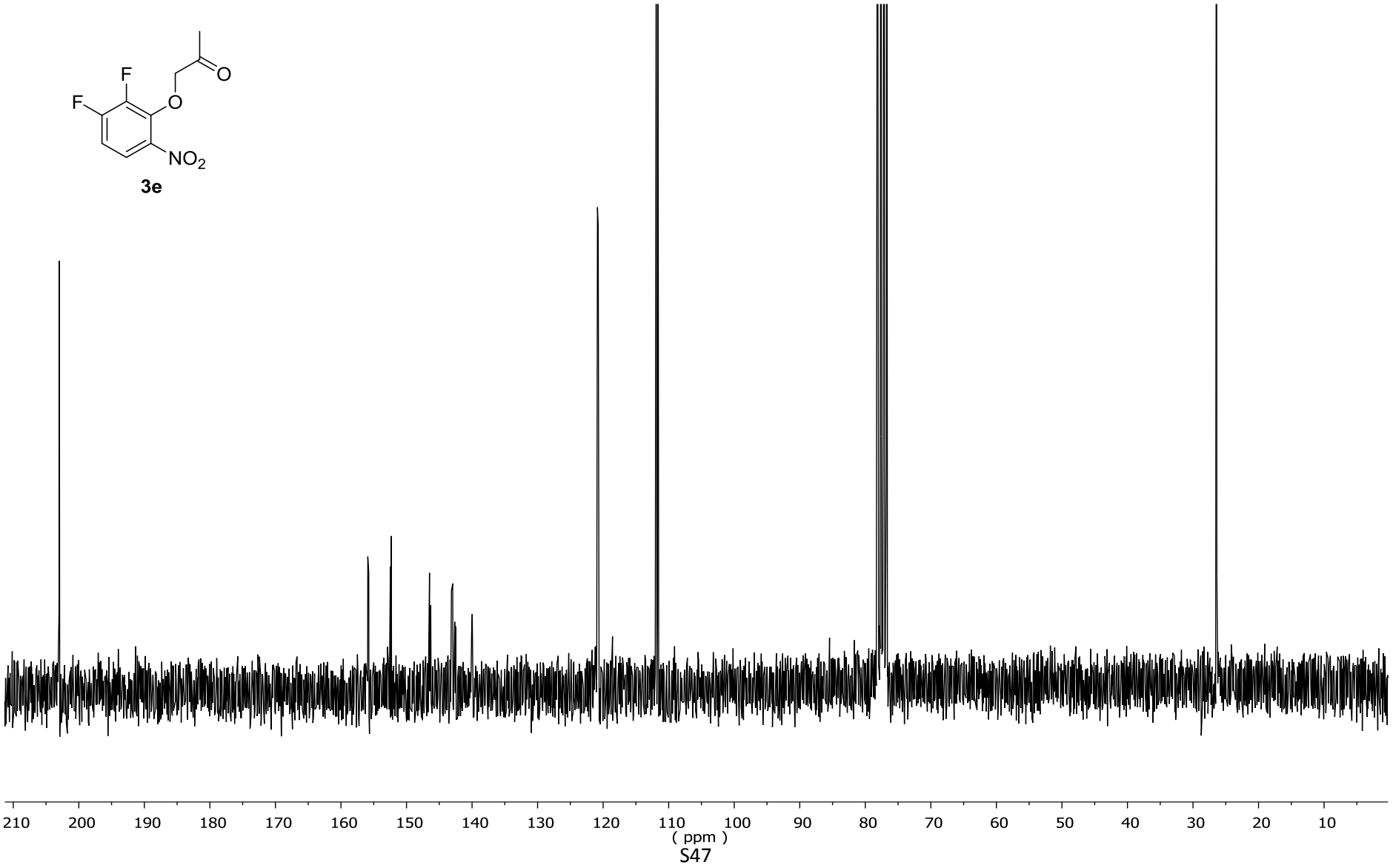
3d



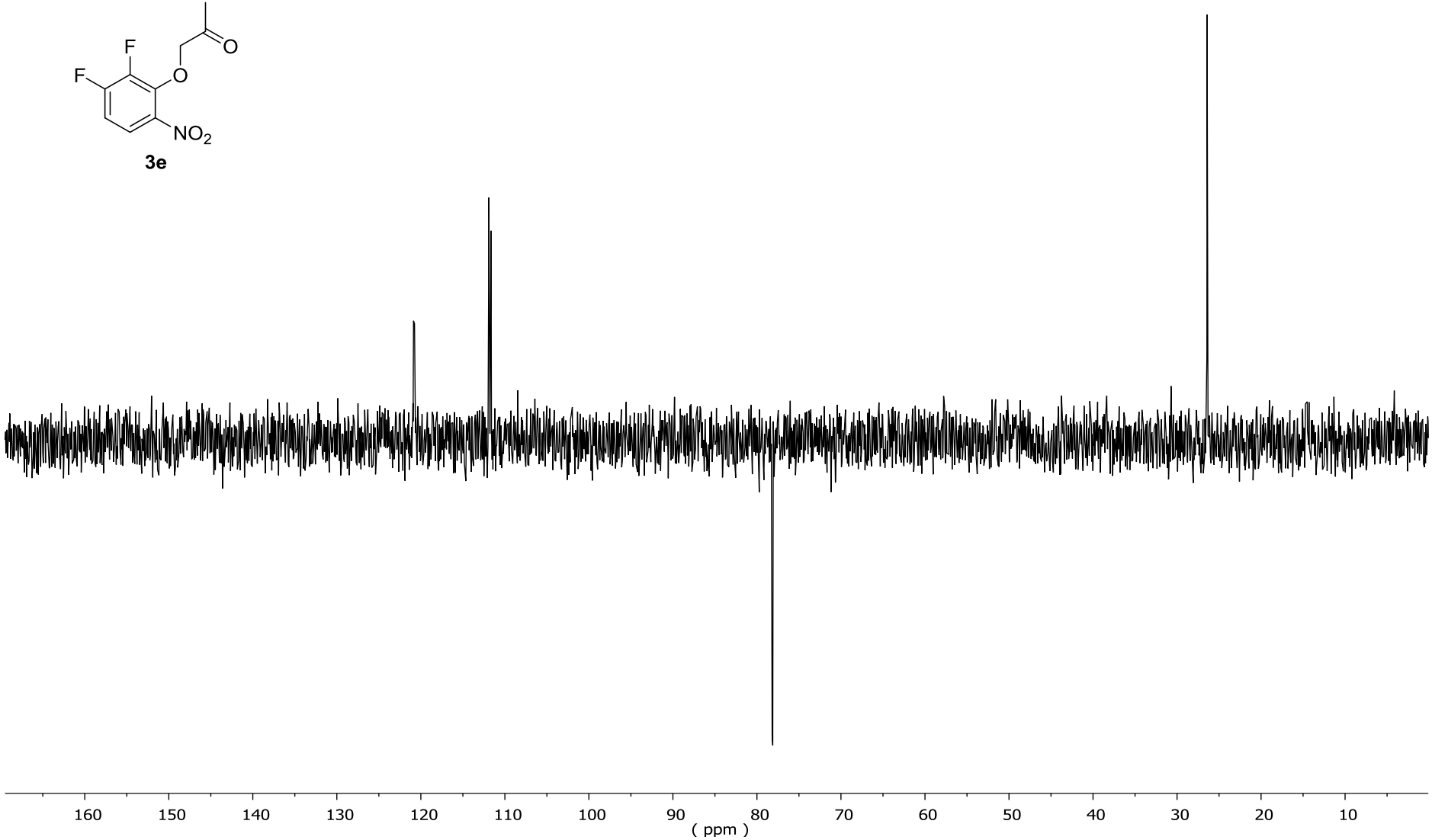

7.76
7.76
7.75
7.74
7.73
7.72
7.72
7.71
7.10
7.07
7.07
7.07
7.04
7.04
7.04
7.01
4.80
4.80
2.32
F
F
O
O
NO2
3e
1.00
1.01
2.04
3.02
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.
(ppm)

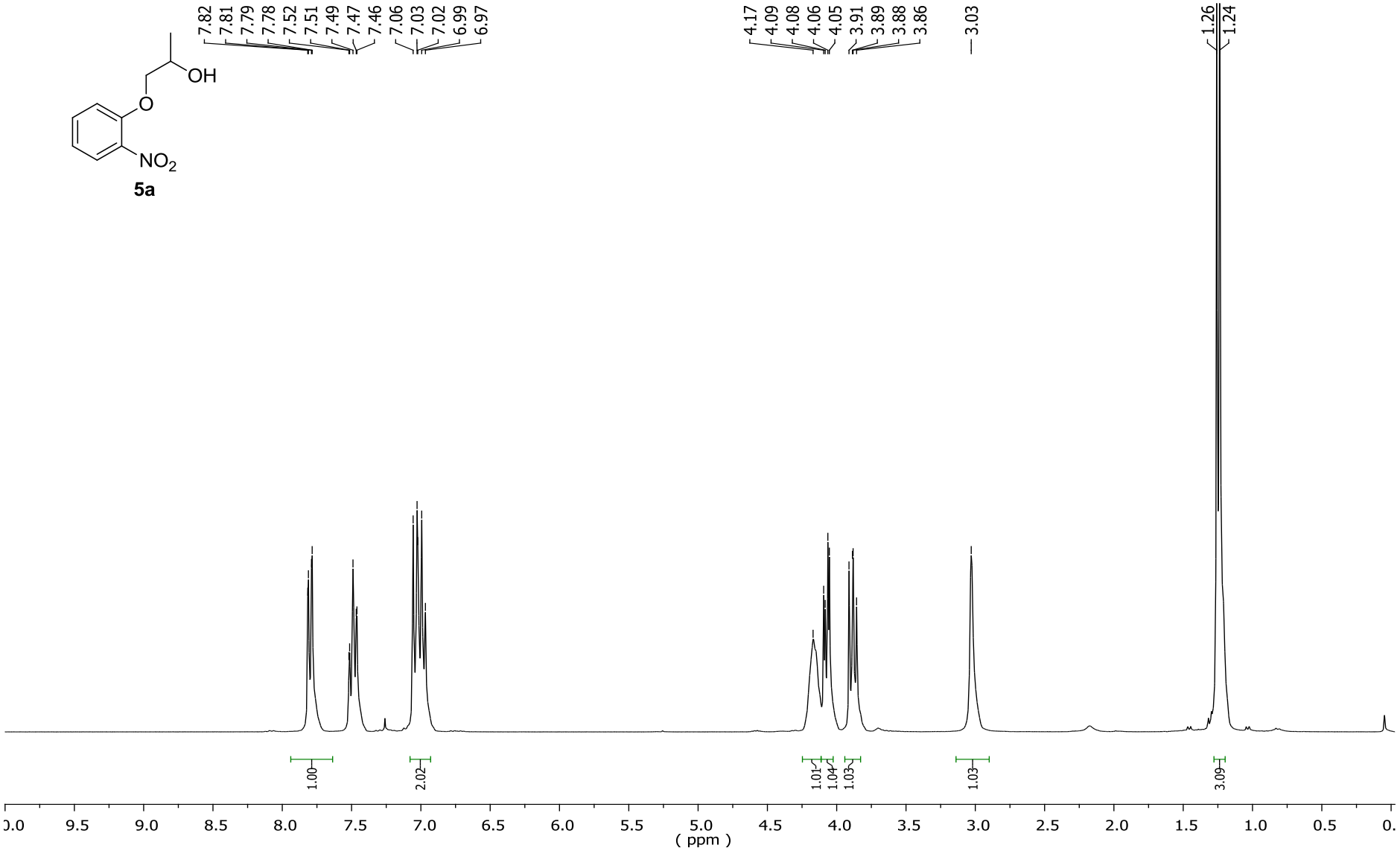
F
O
F
O
NO2

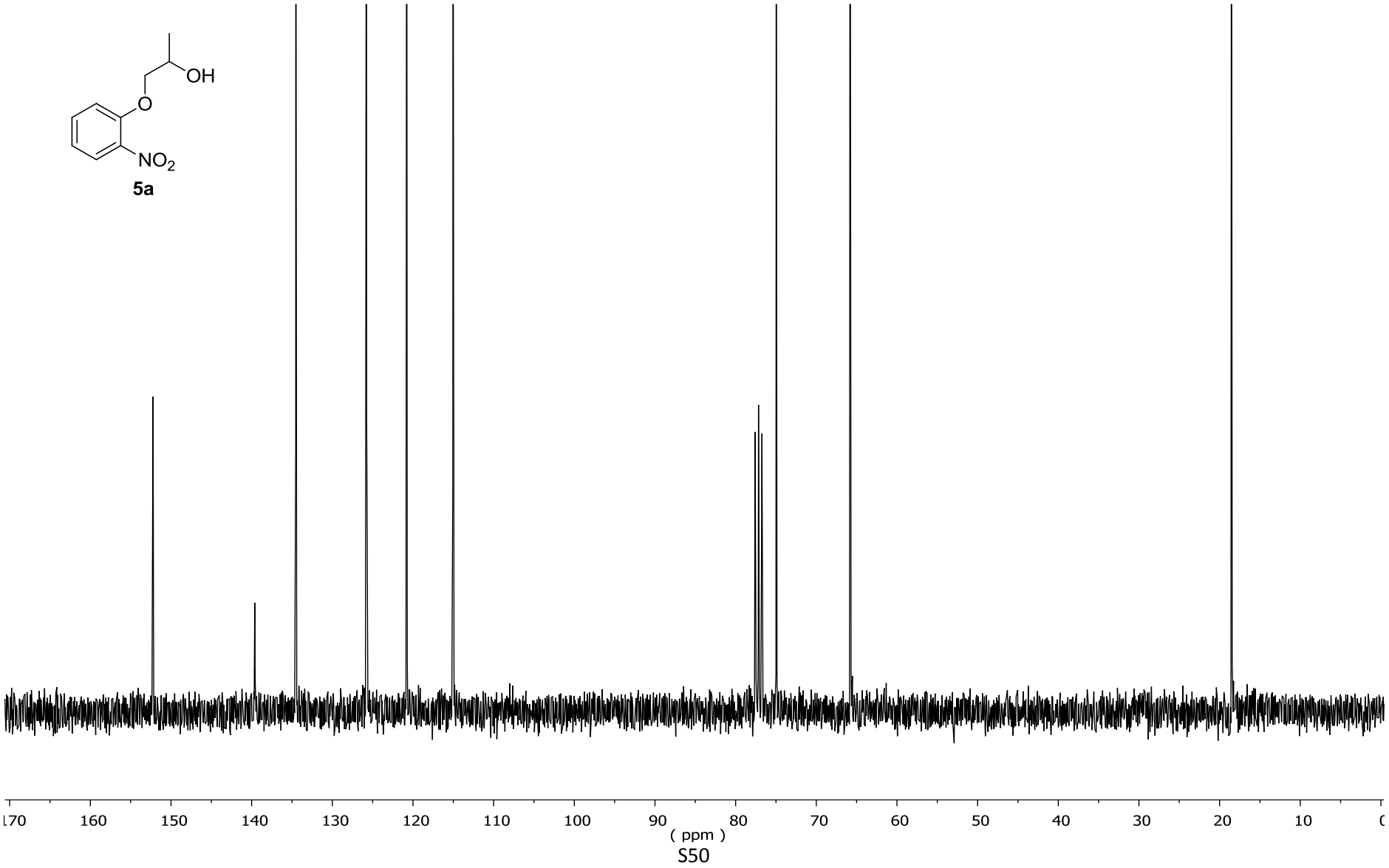
3e


210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)

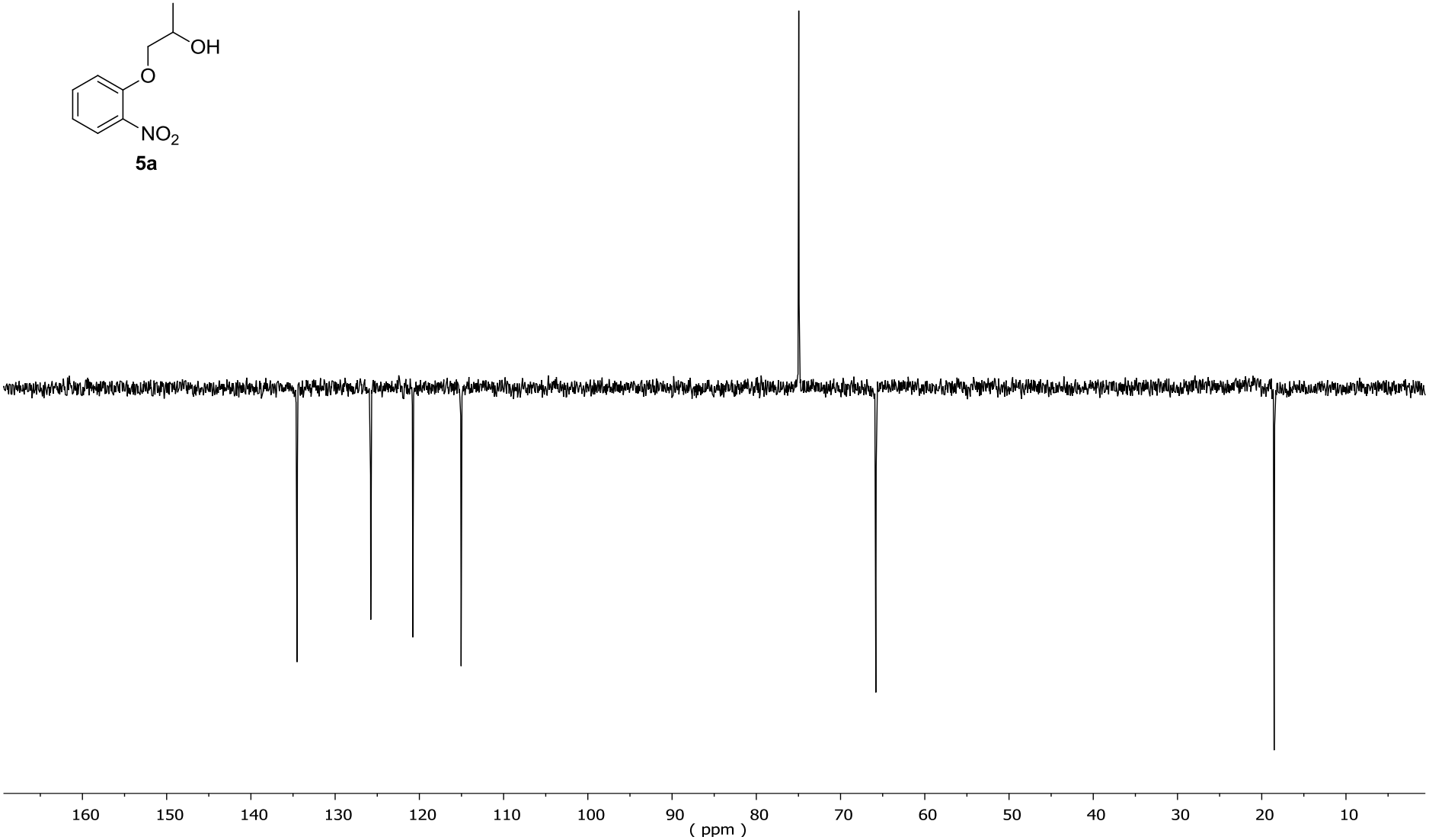
3e

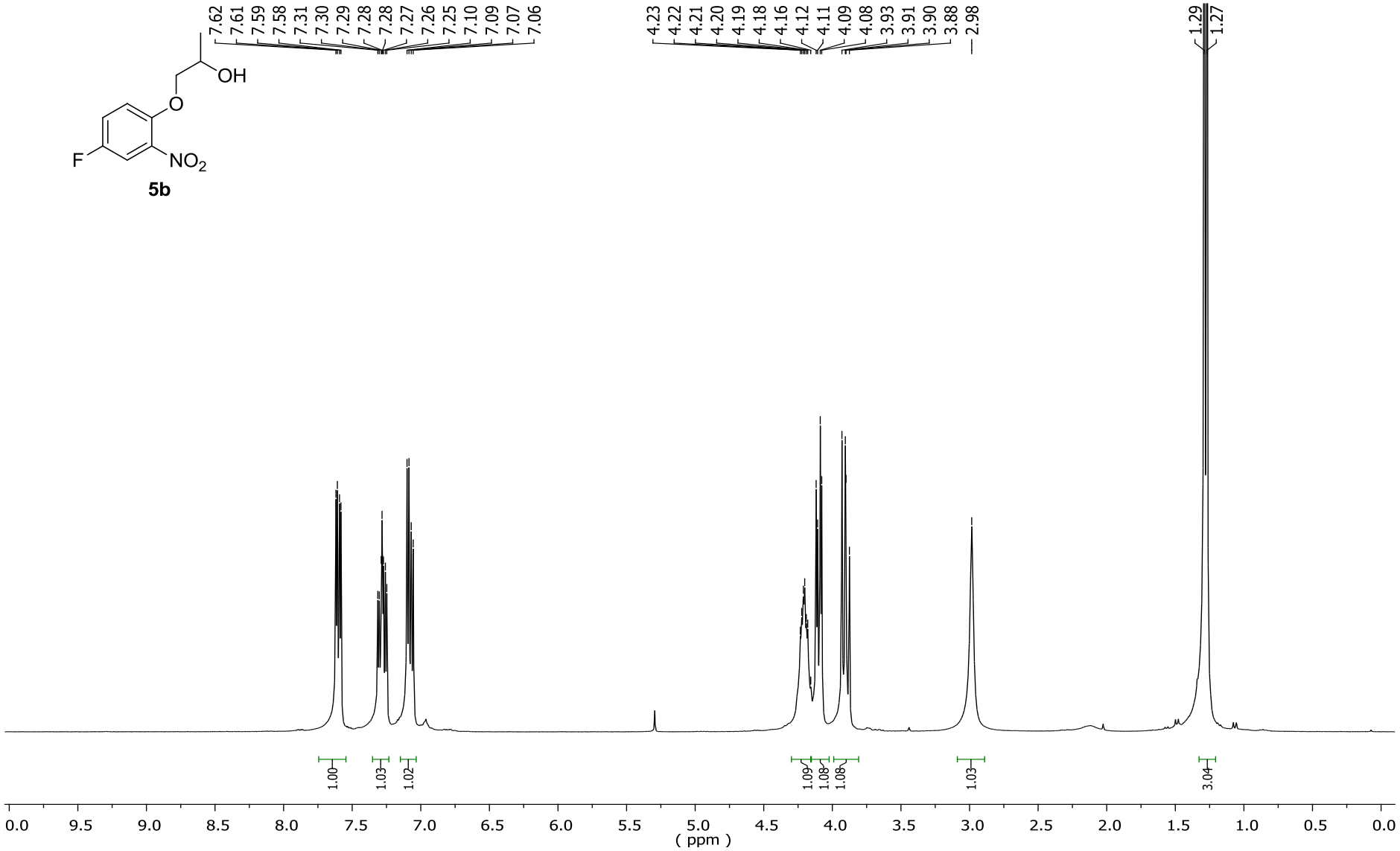



7.82
7.81
7.79
7.78
7.52
7.51
7.49
7.47
7.46
7.06
7.03
7.02
6.99
6.97
4.17
4.09
4.08
4.06
4.05
3.91
3.89
3.88
3.86
3.03
1.26
1.24
OH
O
NO2
5a
1.00
2.02
1.01
1.04
1.03
1.03
3.09
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
(ppm)


OH
O
NO2
5a
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)

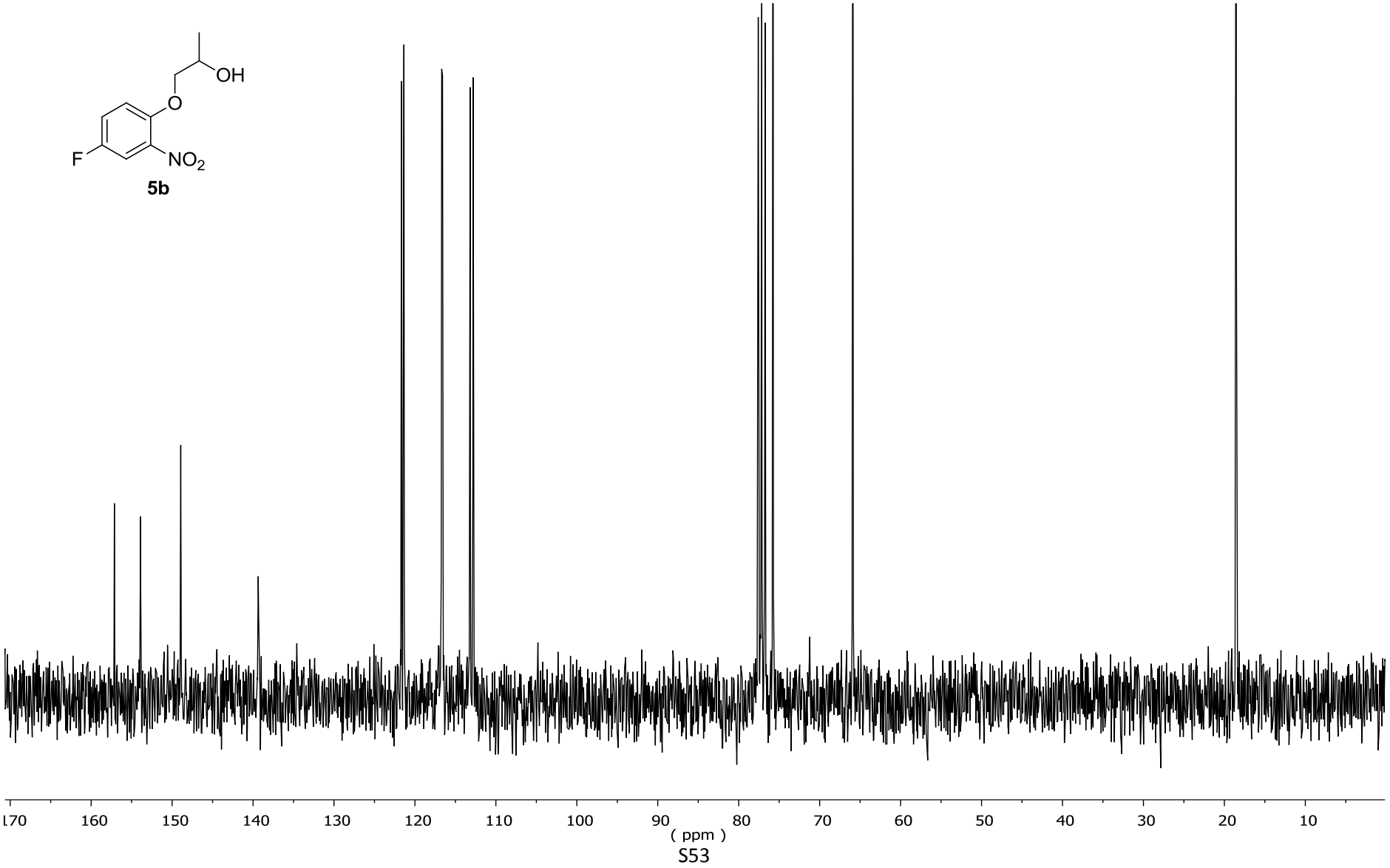
5a




7.62
7.61
7.59
7.58
7.31
7.30
7.29
7.28
7.28
7.27
7.26
7.25
7.10
7.09
7.07
7.06
4.23
4.22
4.21
4.20
4.19
4.18
4.16
4.12
4.11
4.09
4.08
3.93
3.91
3.90
3.88
2.98
1.29
1.27
OH
O
F
NO2
5b
1.00
1.03
1.02
1.09
1.08
1.08
1.03
3.04
0.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
(ppm)

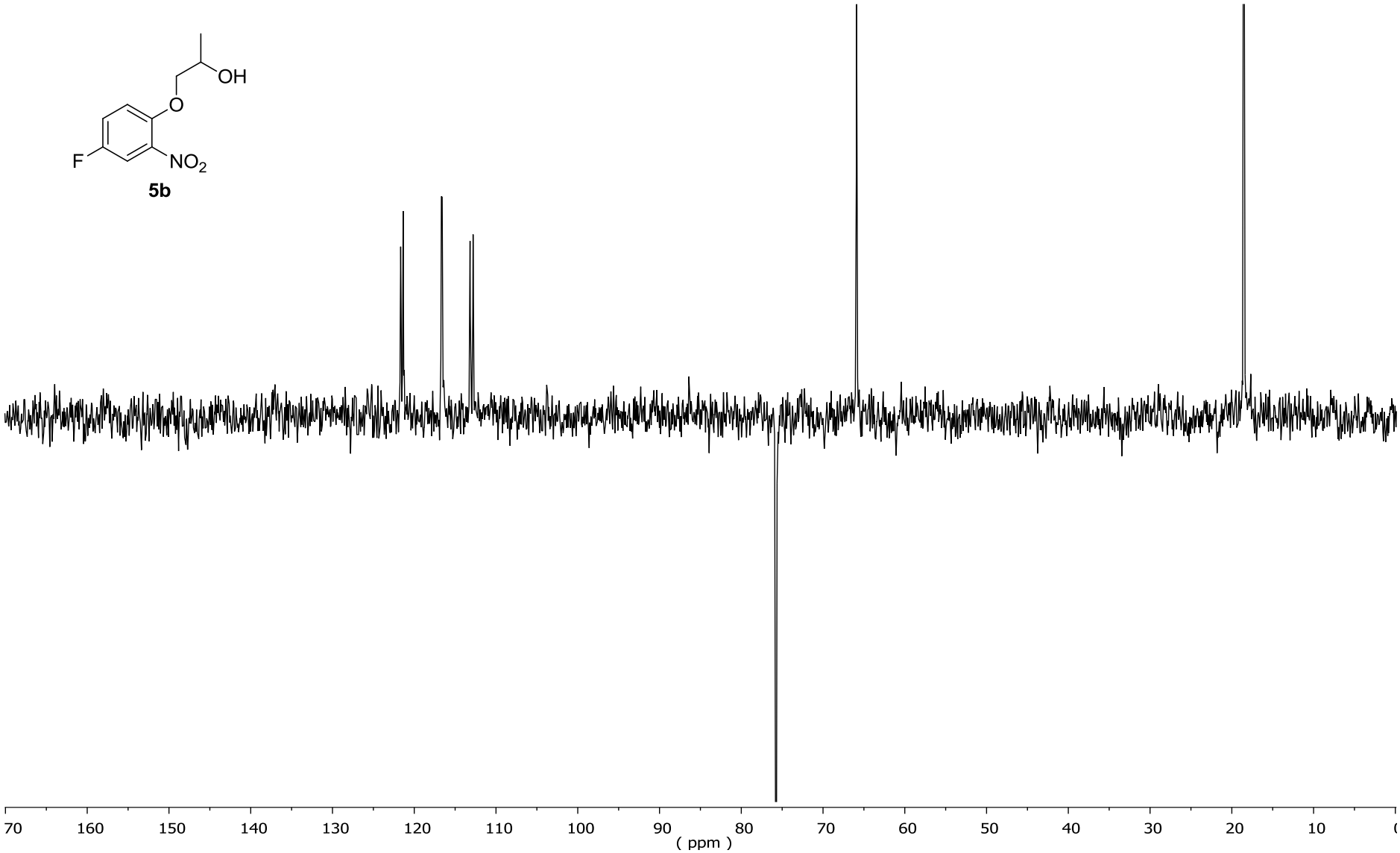
OH
O
F
NO2

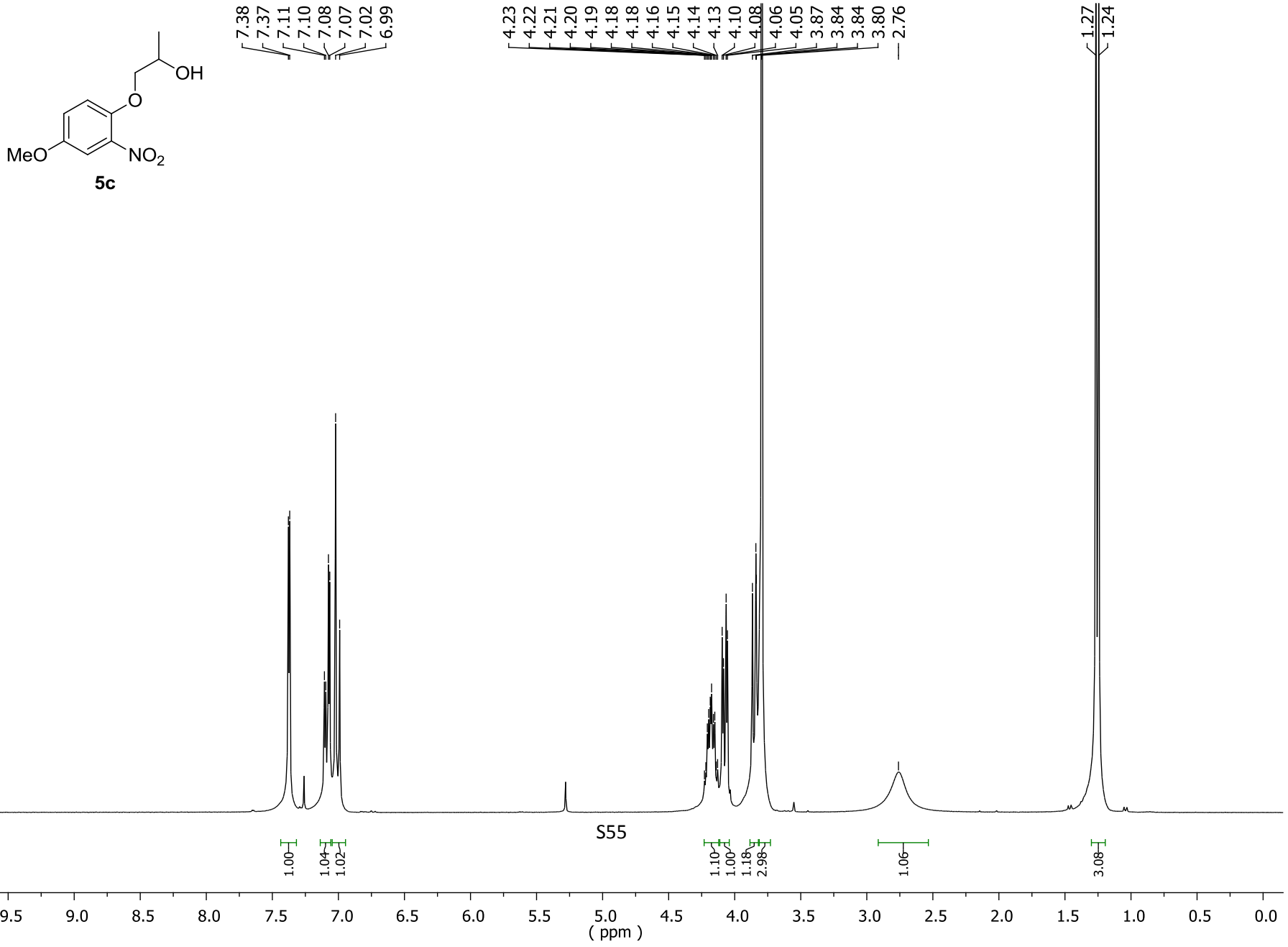
5b

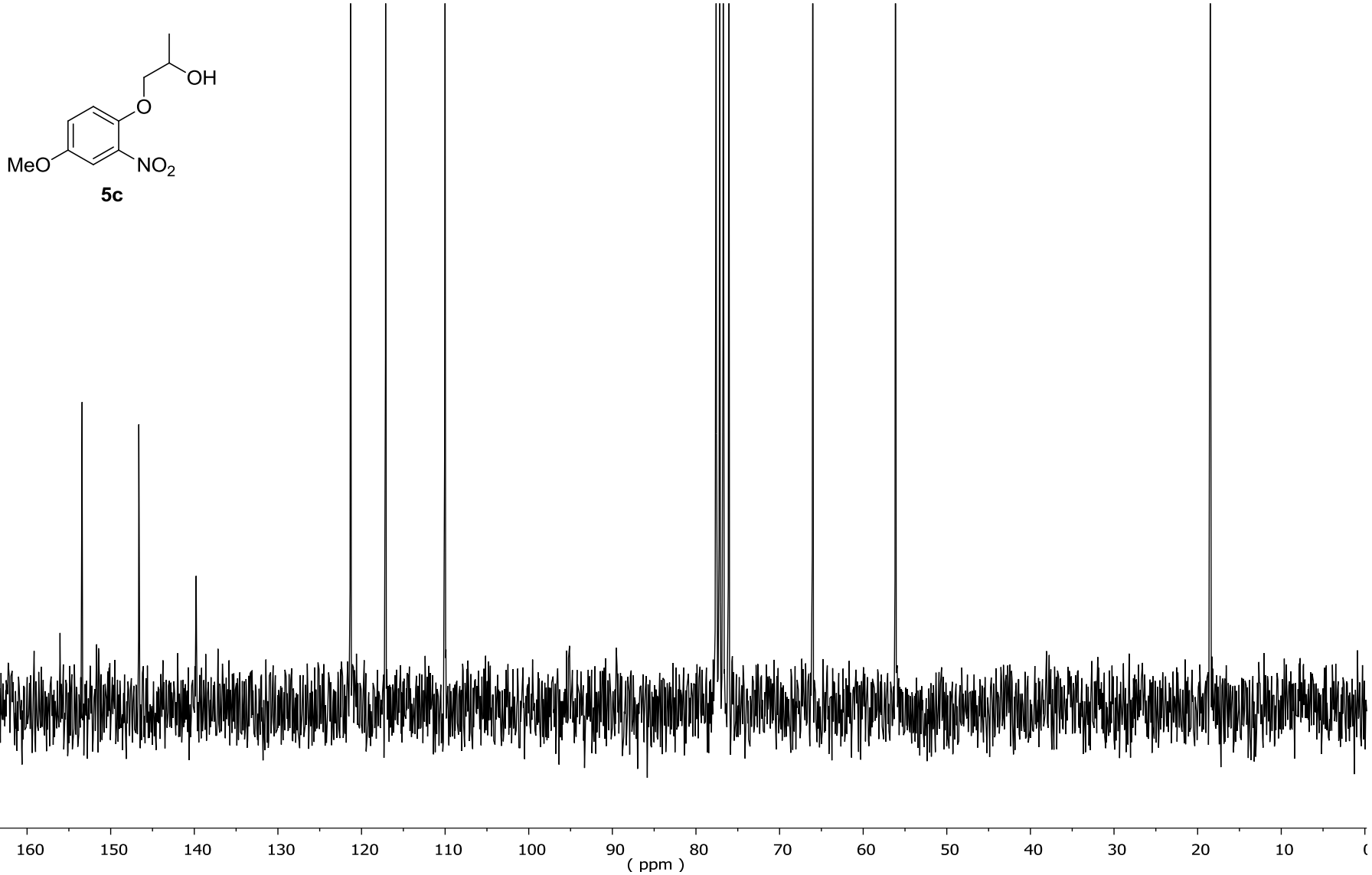

170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)

OH
O
F
NO2

5b

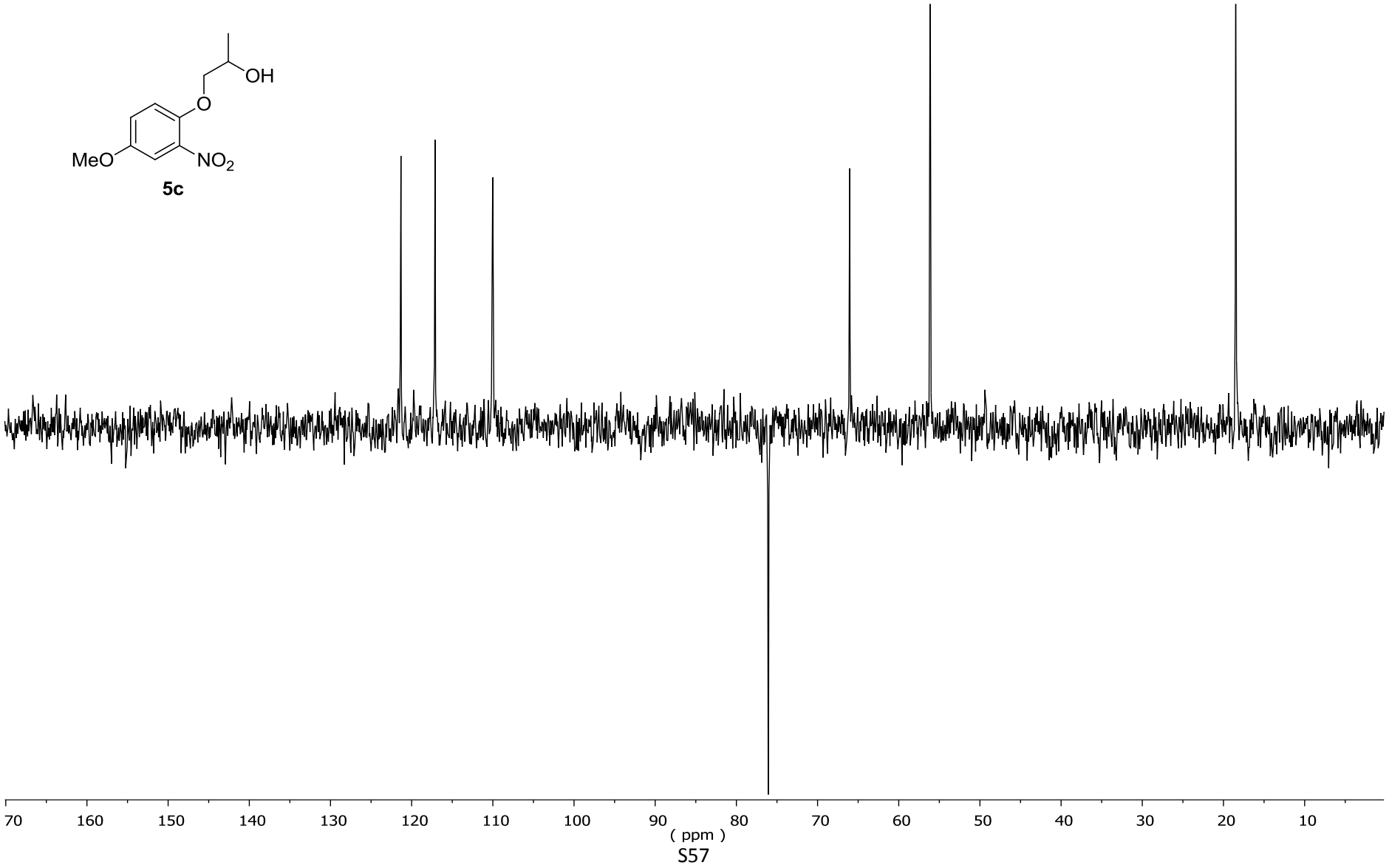

70
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)


OH
O
MeO
NO2
5c
7.38
7.37
7.11
7.10
7.08
7.07
7.02
6.99
4.23
4.22
4.21
4.20
4.19
4.18
4.18
4.16
4.15
4.14
4.13
4.10
4.08
4.06
4.05
3.87
3.84
3.84
3.80
2.76
1.27
1.24
1.00
1.04
1.02
1.10
1.00
1.18
2.98
1.06
3.08
(ppm)


OH
O
MeO
NO2
5c
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)

OH
O
MeO
NO2

5c


70
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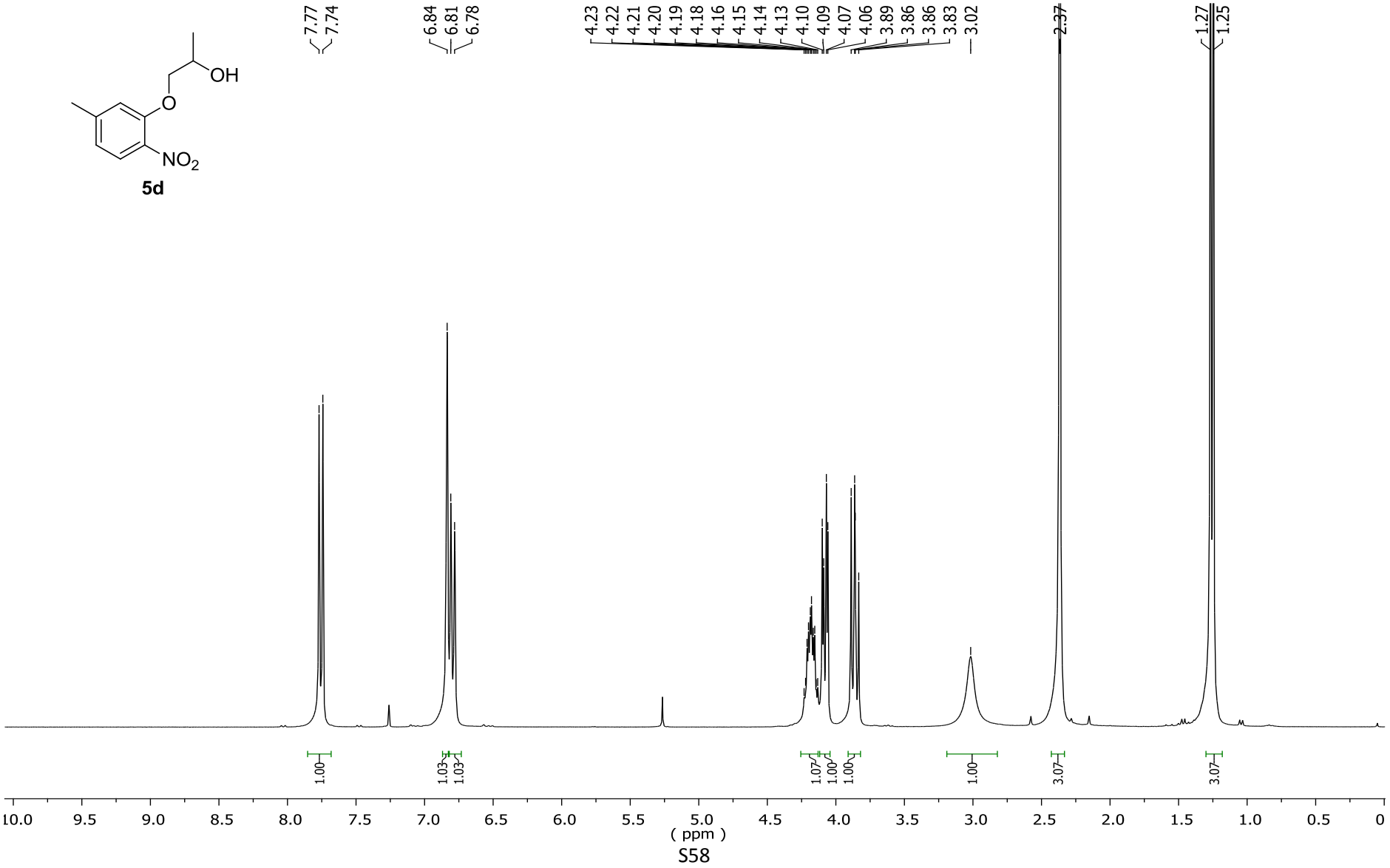
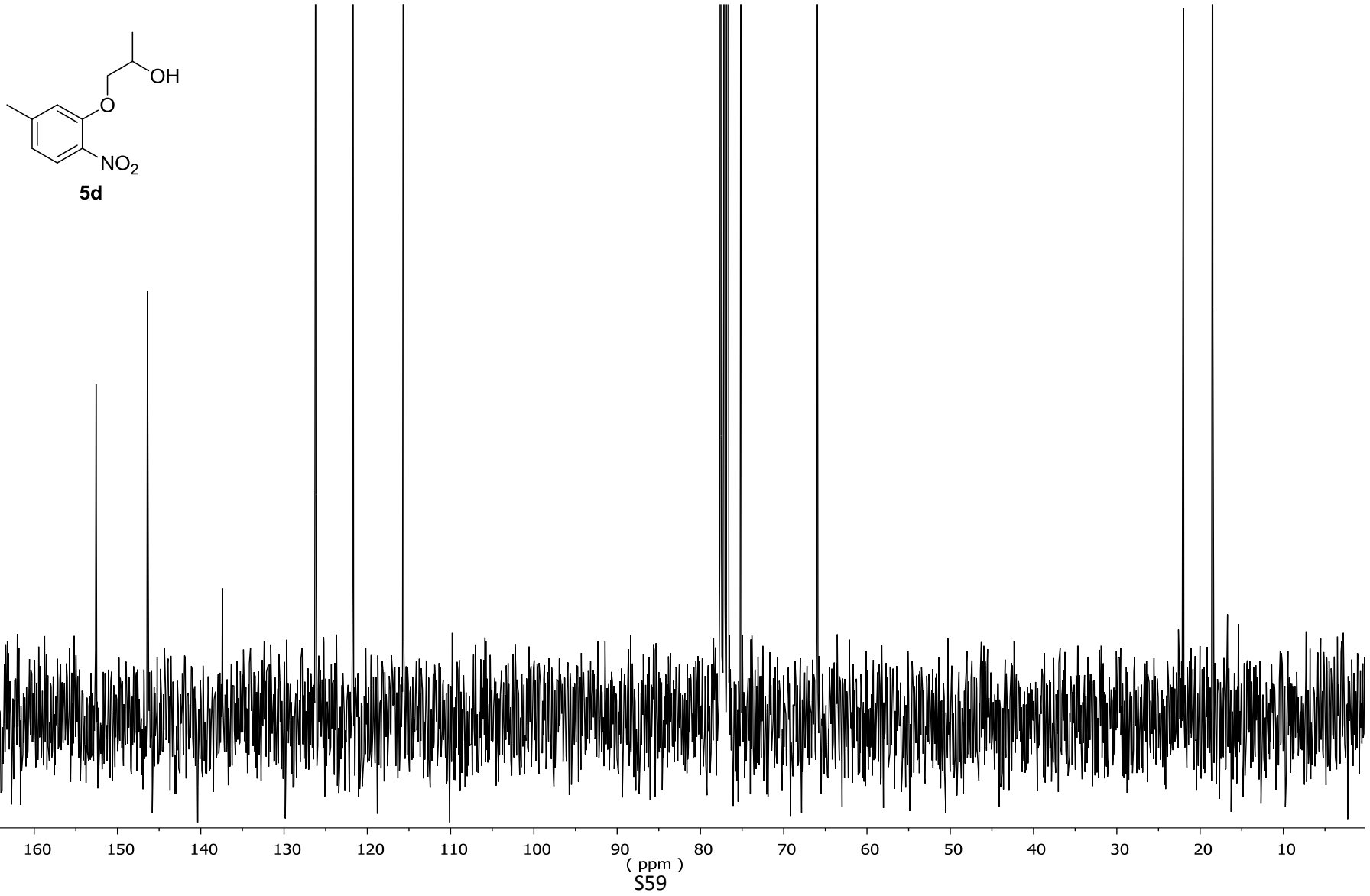
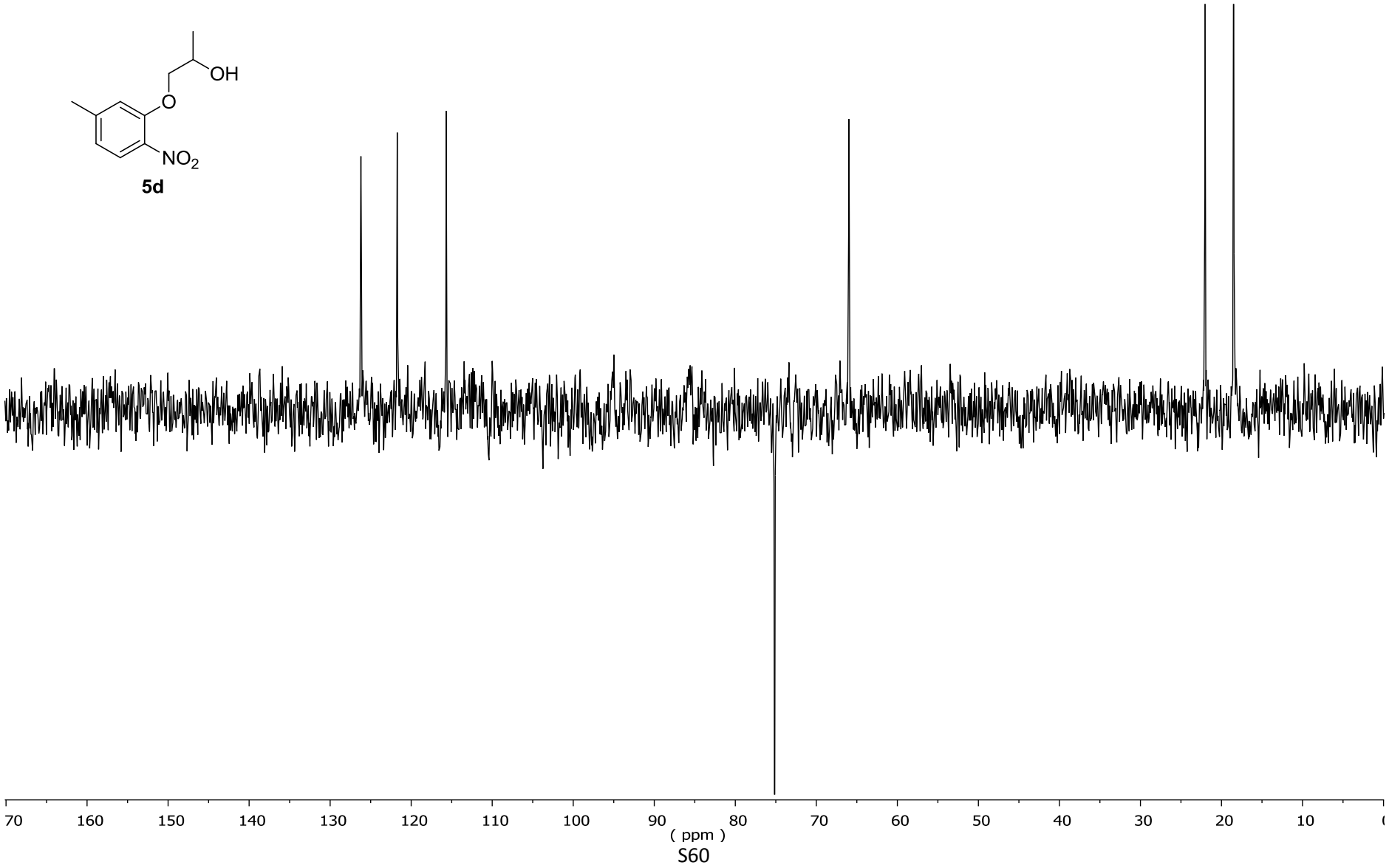
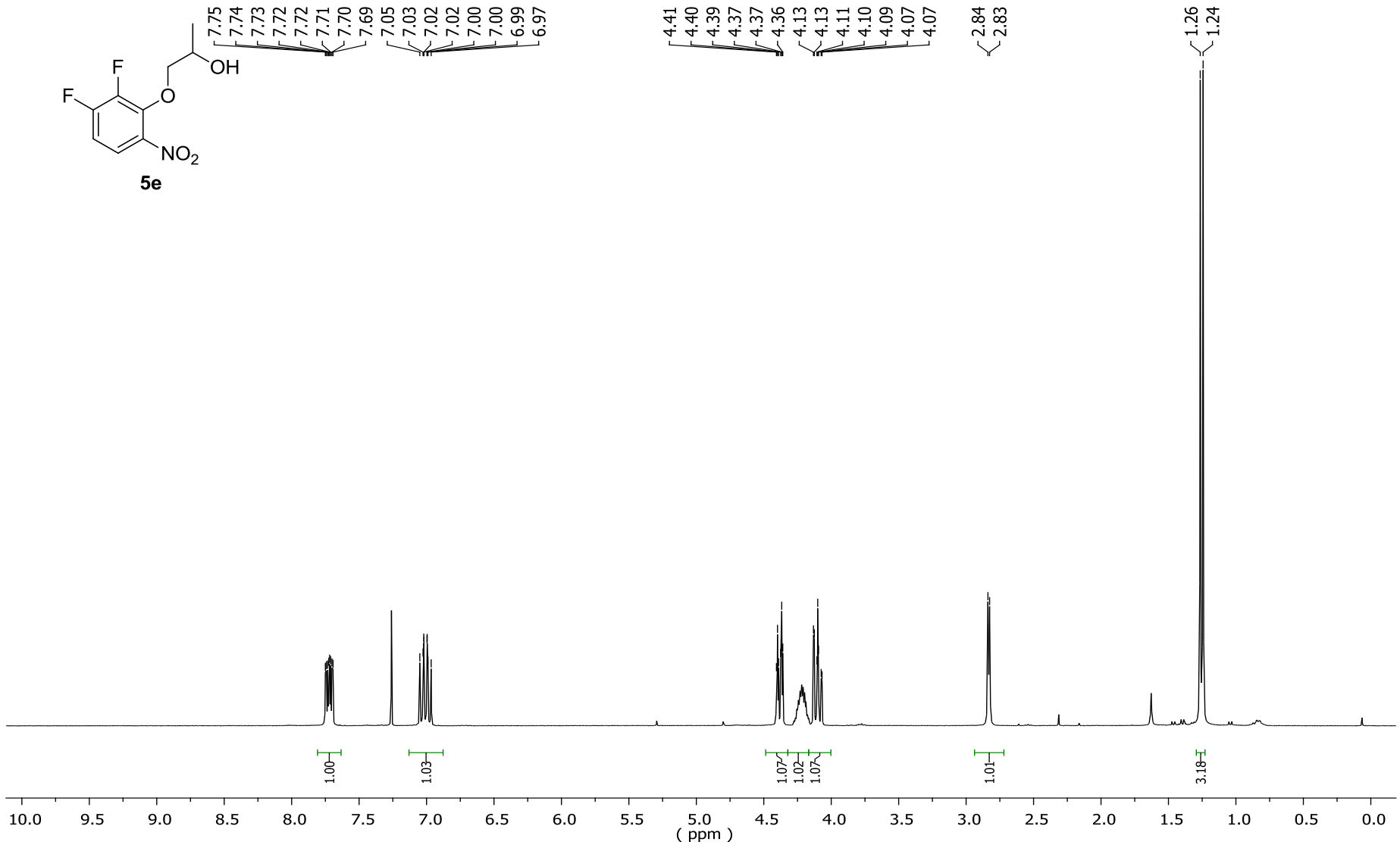
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)


OH
O
NO2
5d
7.77
7.74
6.84
6.81
6.78
4.23
4.22
4.21
4.20
4.19
4.18
4.16
4.15
4.14
4.13
4.10
4.09
4.07
4.06
3.89
3.86
3.86
3.83
3.02
2.37
1.27
1.25
1.00
1.03
1.03
1.07
1.00
1.00
1.00
3.07
3.07
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
(ppm)


OH
O
NO2
5d
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)


OH
O
NO2
5d
70
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)


7.75
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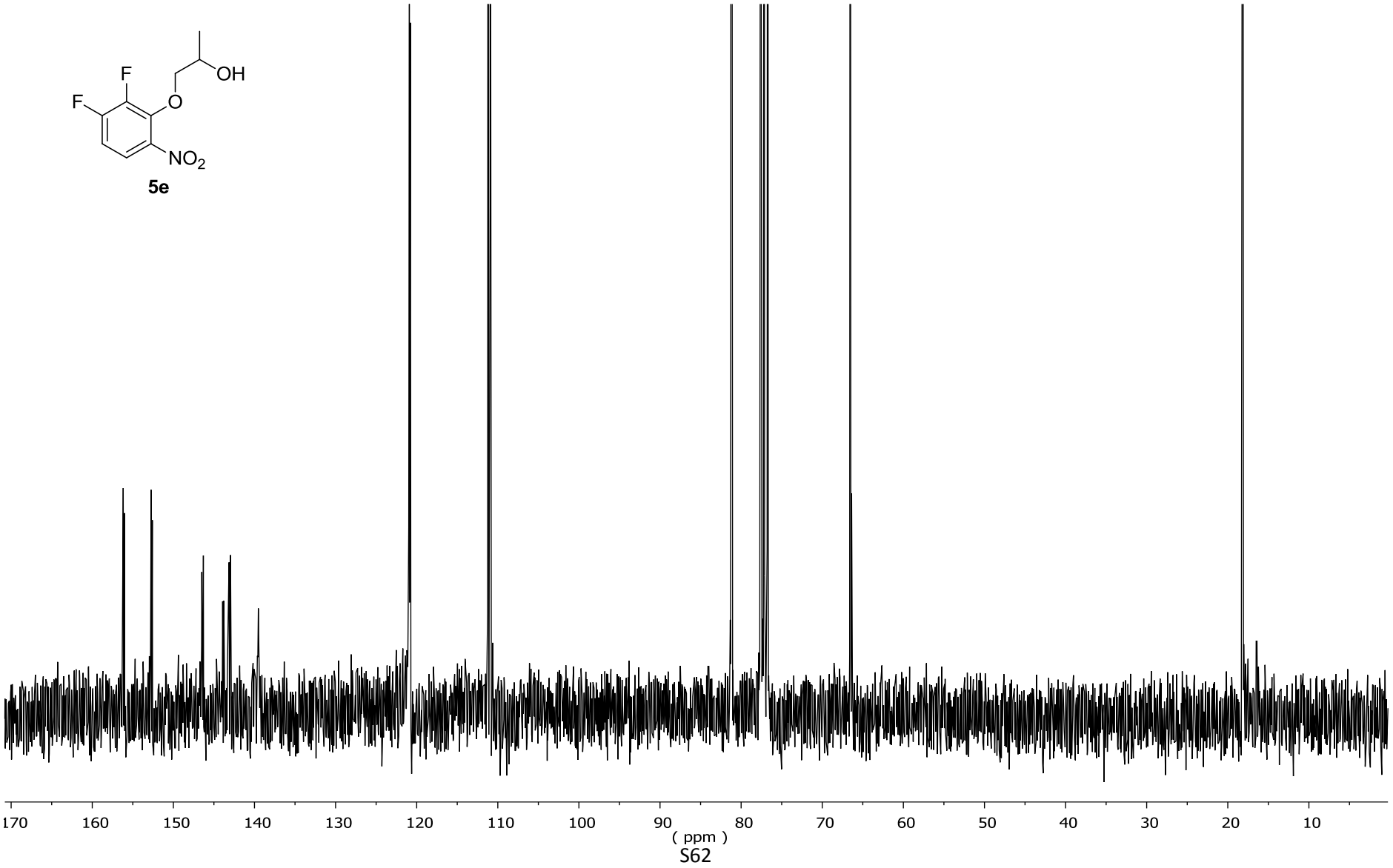
7.73
7.72
7.72
7.71
7.70
7.69
7.05
7.03
7.02
7.02
7.00
7.00
6.99
6.97
4.41
4.40
4.39
4.37
4.37
4.36
4.13
4.13
4.11
4.10
4.09
4.07
4.07
2.84
2.83
1.26
1.24
F
F
O
OH
NO2
5e
1.00
1.03
1.07
1.02
1.07
1.01
3.18
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
(ppm)

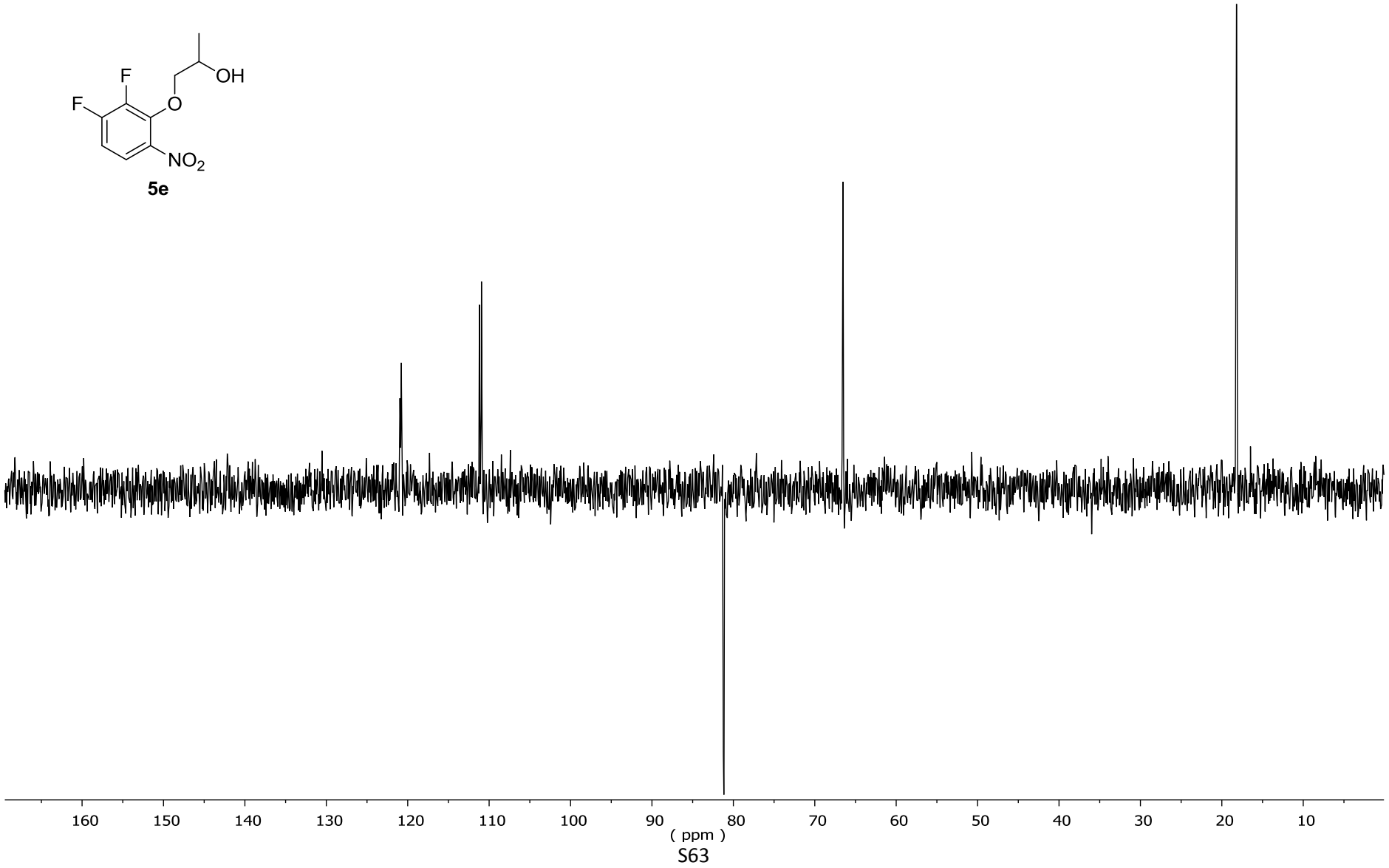
F
OH
F
O
NO2

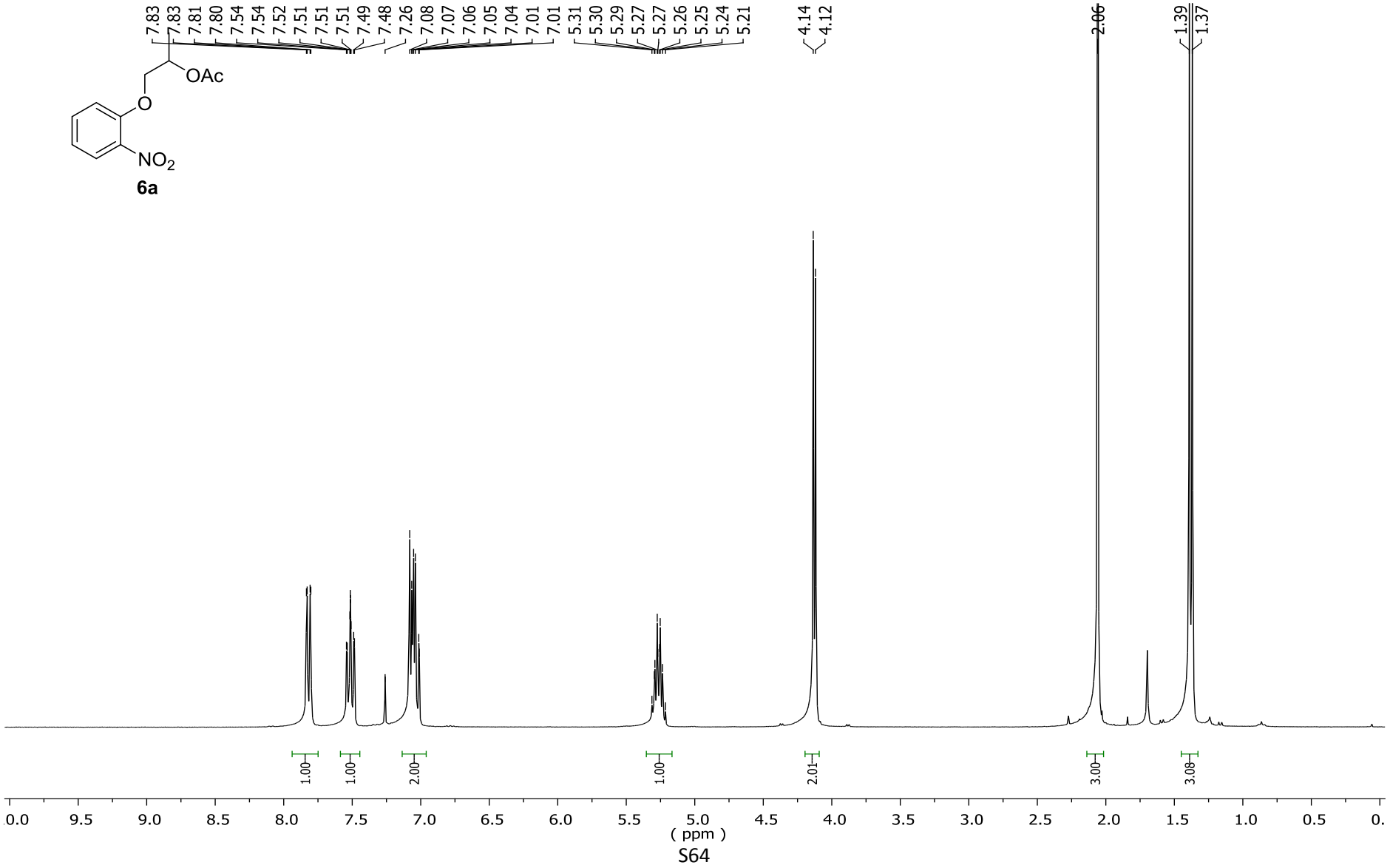
5e


170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)

F
OH
F
O
NO2

5e


160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)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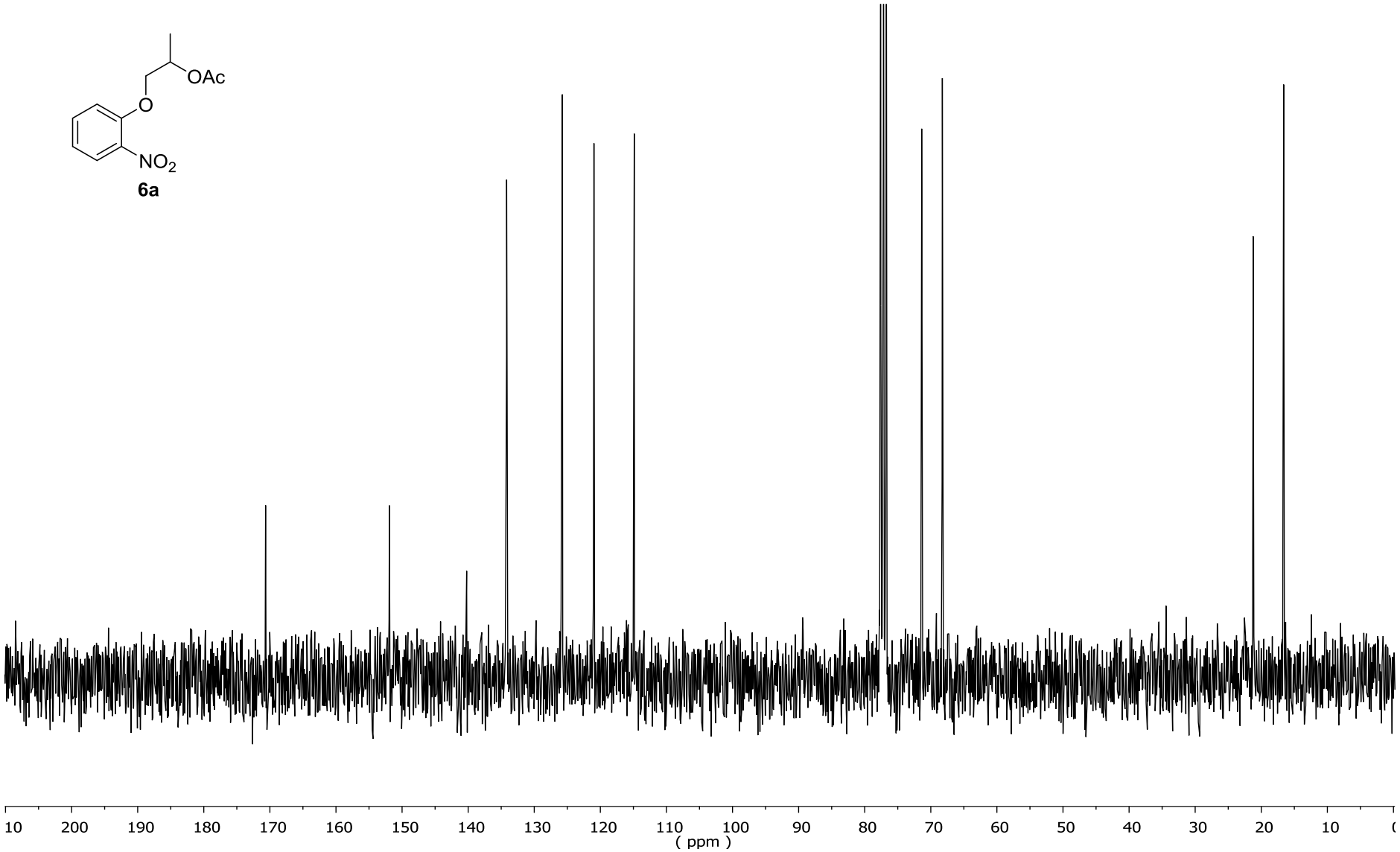
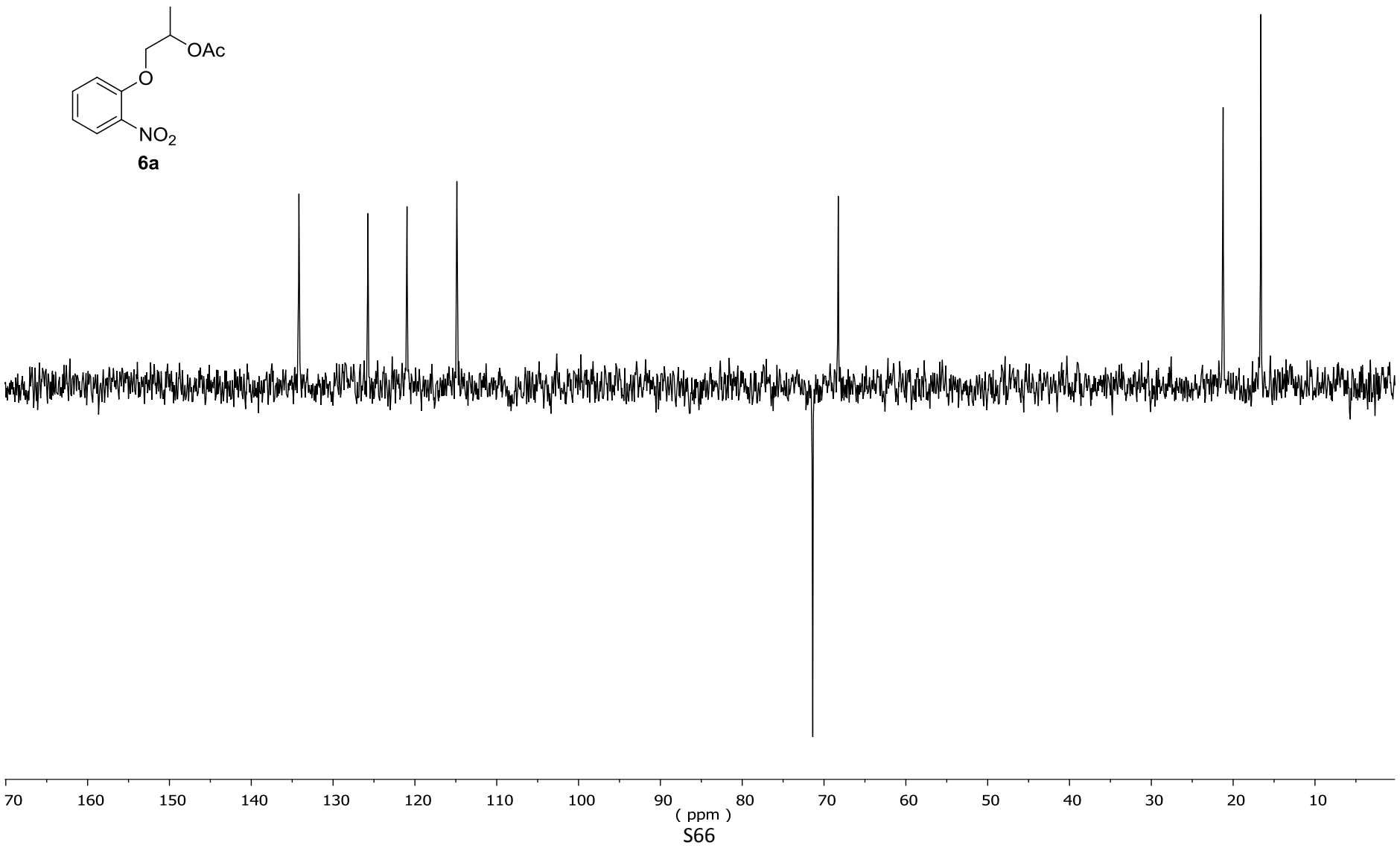
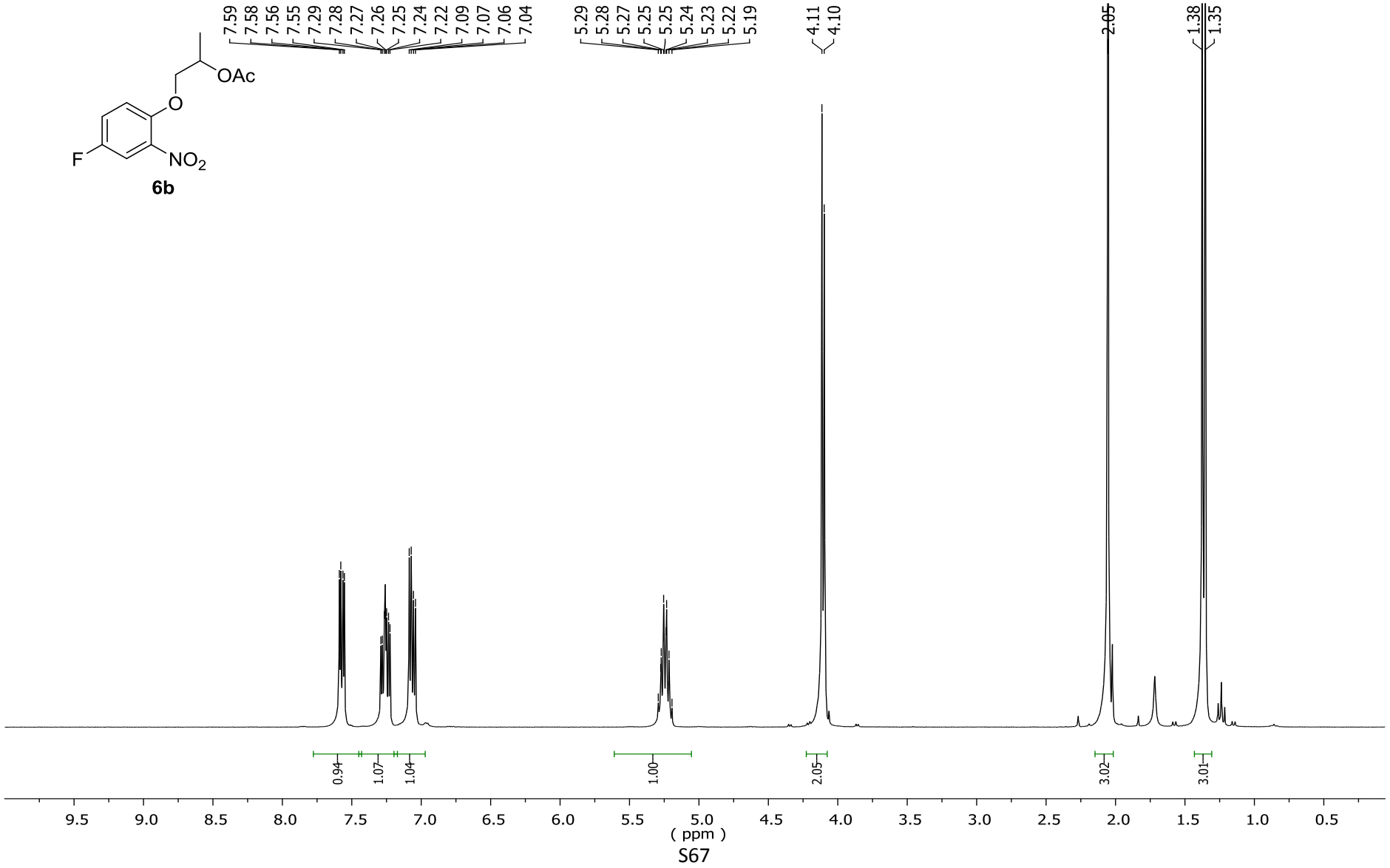
7.83
7.81
7.80
7.54
7.54
7.52
7.51
7.51
7.51
7.49
7.48
7.26
7.08
7.07
7.06
7.05
7.04
7.01
7.01
5.31
5.30
5.29
5.27
5.27
5.26
5.25
5.24
5.21
4.14
4.12
2.06
1.39
1.37
OAc
O
NO2
6a
1.00
1.00
2.00
1.00
2.01
3.00
3.08
0.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.
(ppm)

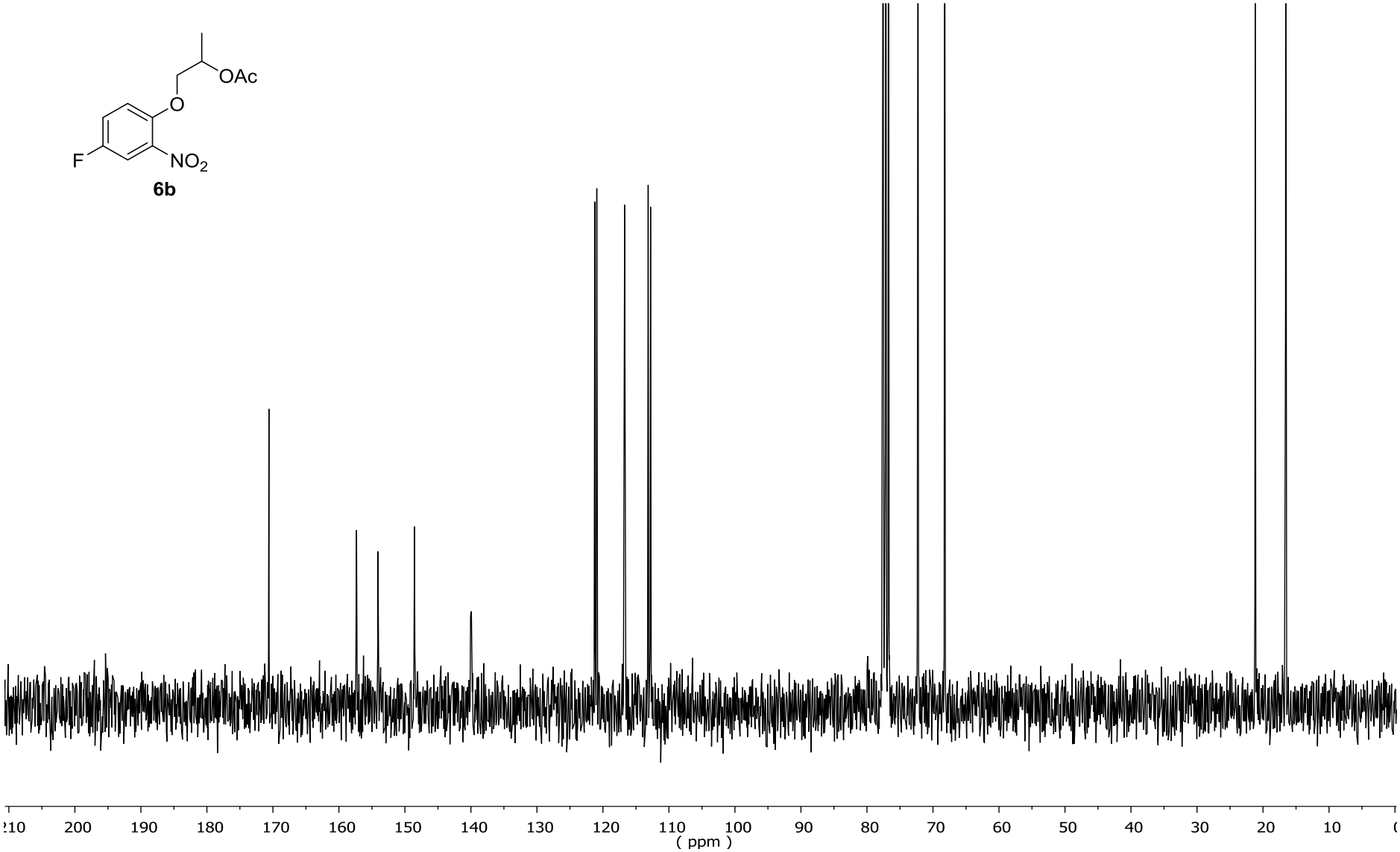

OAc
O
NO2
6a
10
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)


OAc
O
NO2
6a
70
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)


7.59
7.58
7.56
7.55
7.29
7.28
7.27
7.26
7.25
7.24
7.22
7.09
7.07
7.06
7.04
5.29
5.28
5.27
5.25
5.25
5.24
5.23
5.22
5.19
4.11
4.10
2.05
1.38
1.35
OAc
O
F
NO2
6b
0.94
1.07
1.04
1.00
2.05
3.02
3.01
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
(ppm)

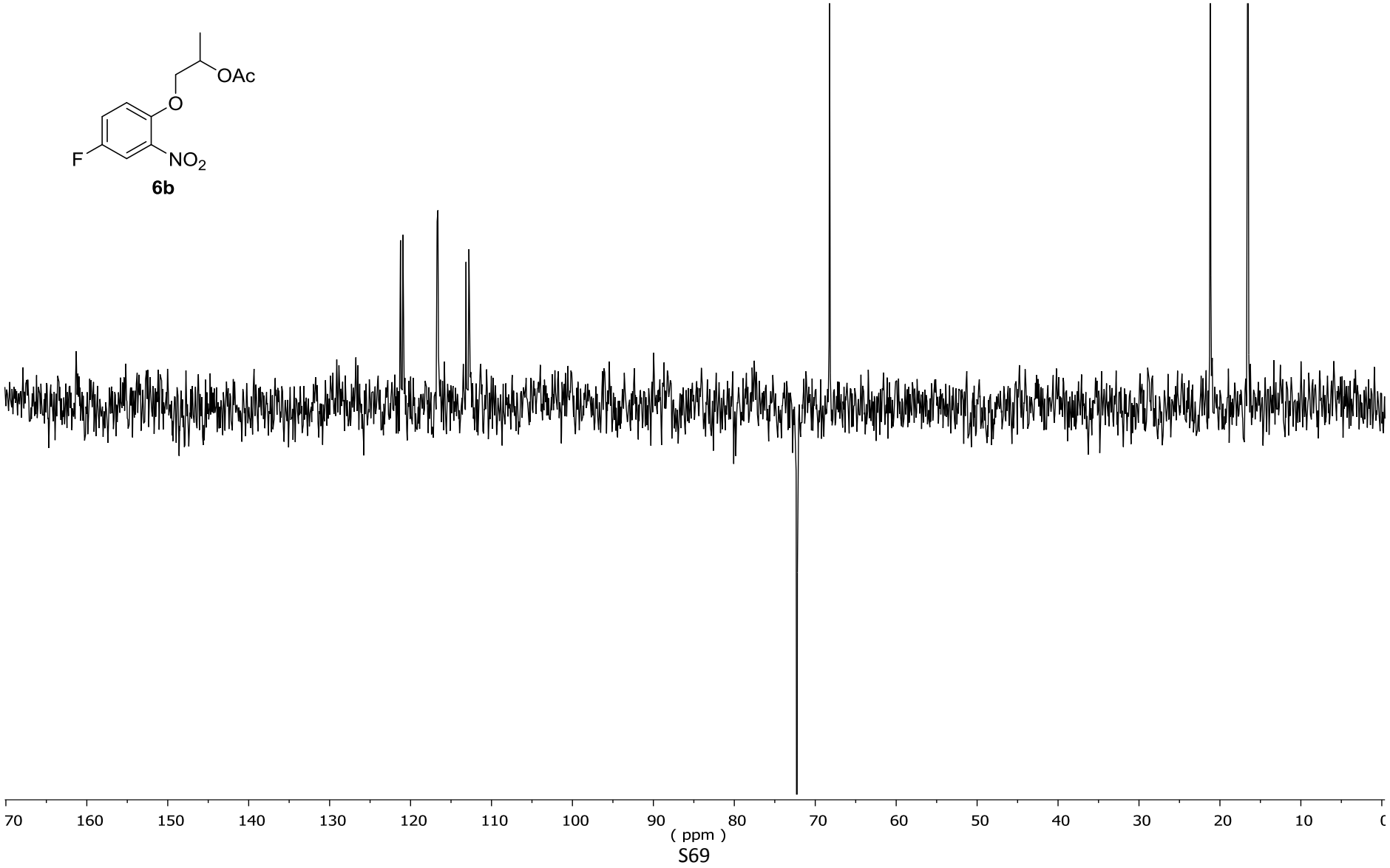
OAc
O
F
NO2

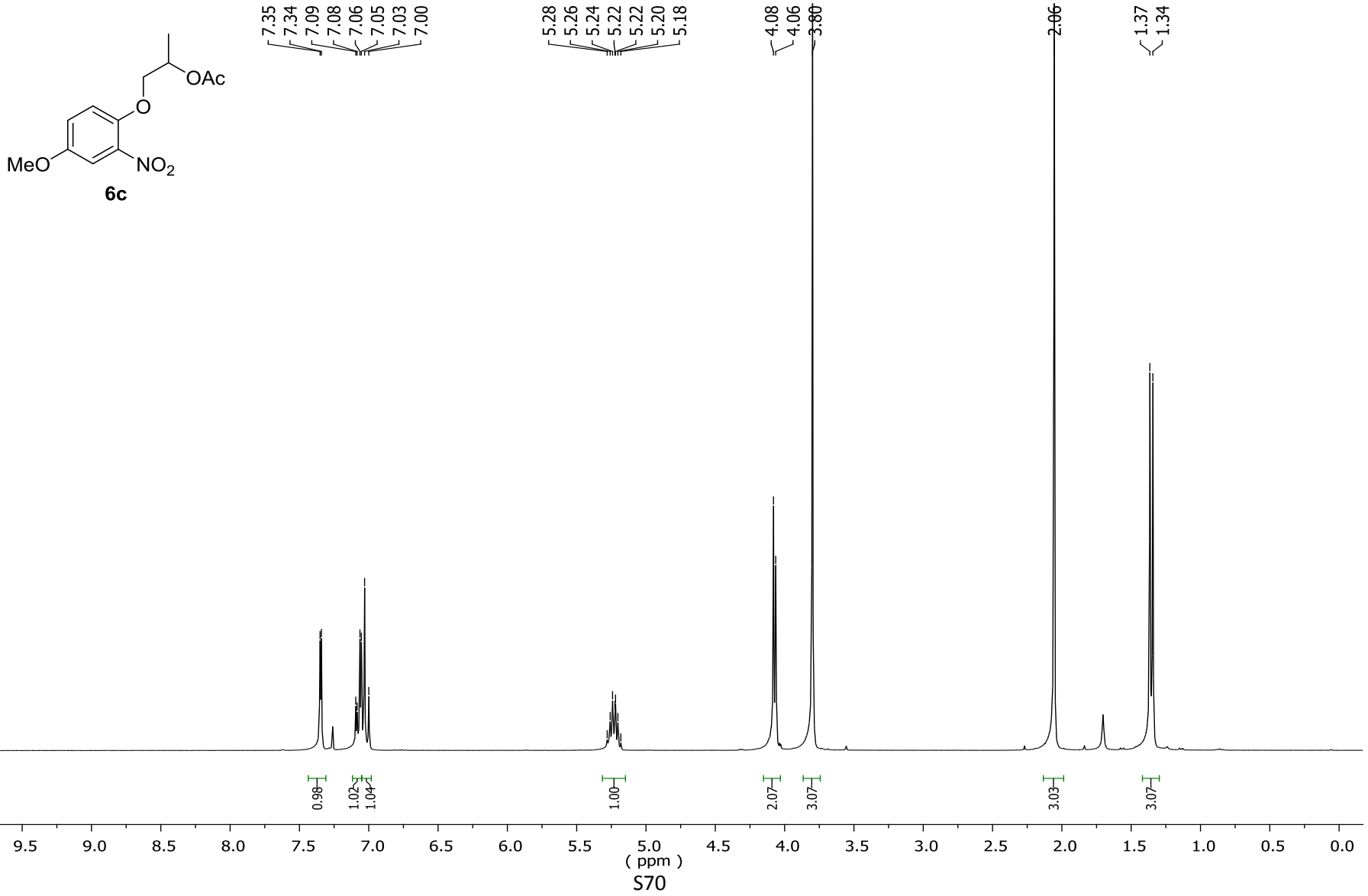
6b

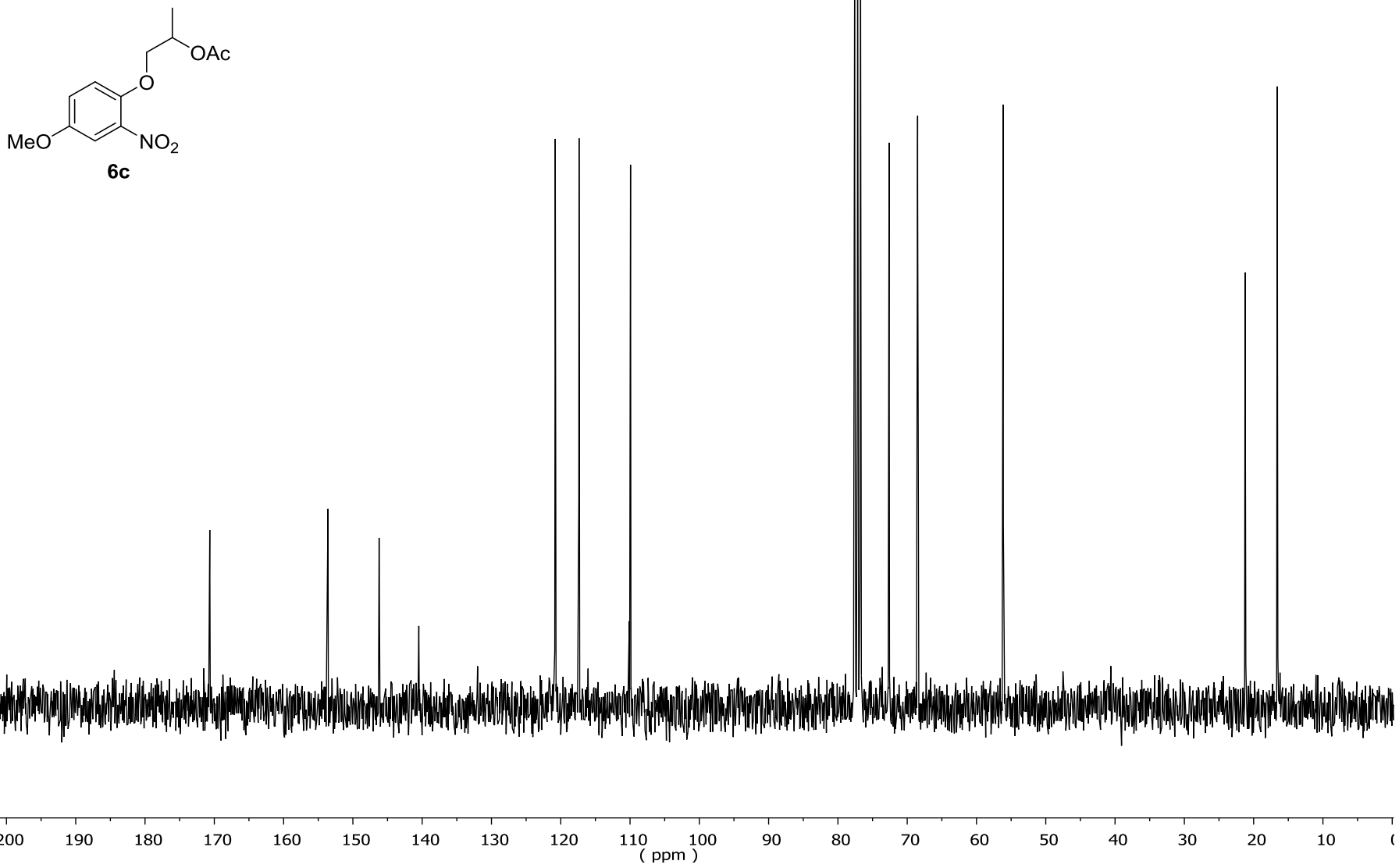

210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)

OAc
O
F
NO2

6b

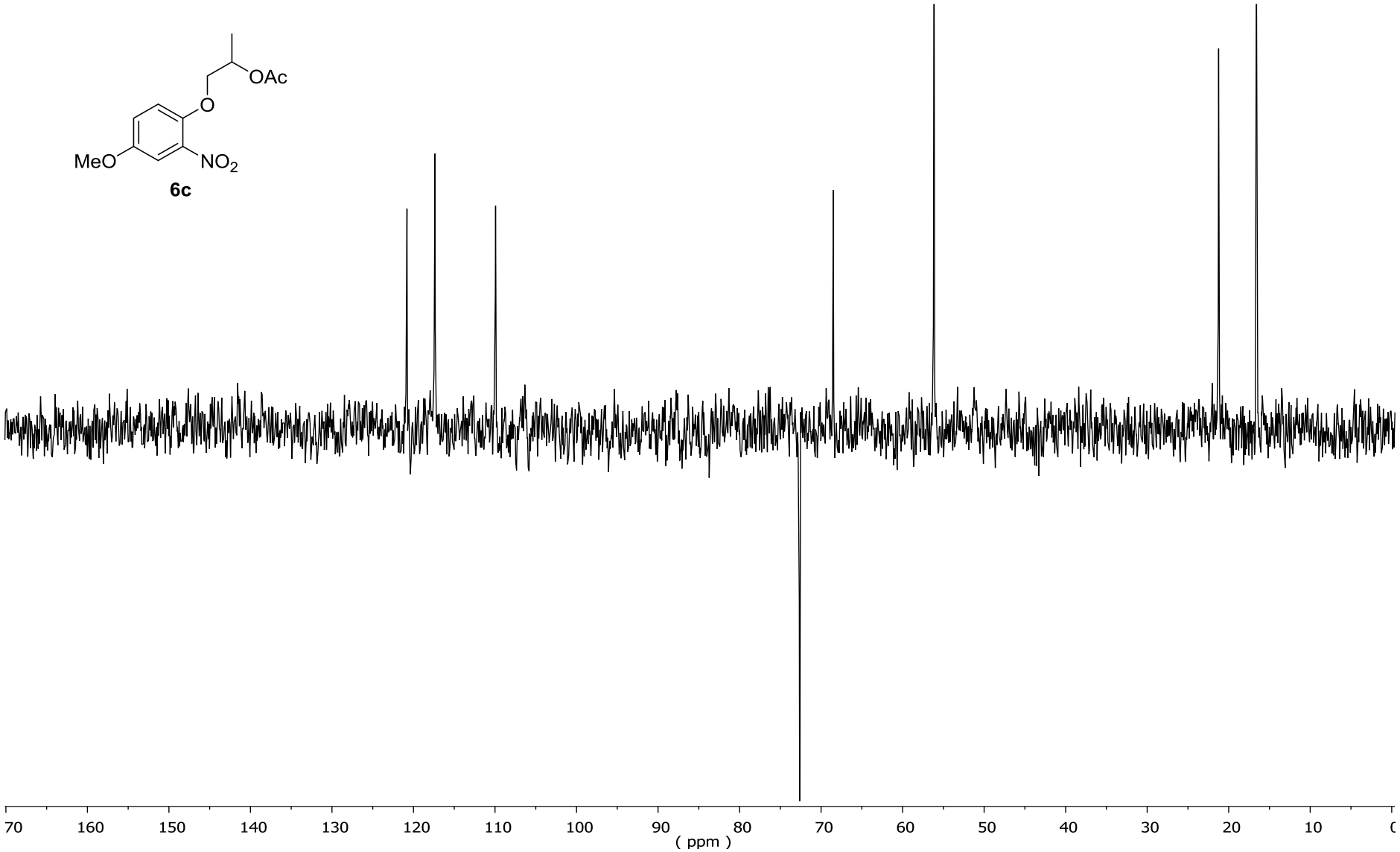

70
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)


OAc
O
MeO
NO2
6c
7.35
7.34
7.09
7.08
7.06
7.05
7.03
7.00
5.28
5.26
5.24
5.22
5.22
5.20
5.18
4.08
4.06
3.80
2.06
1.37
1.34
0.98
1.02
1.04
1.00
2.07
3.07
3.03
3.07
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
(ppm)


OAc
O
MeO
NO2
6c
10
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)

OAc
O
MeO
NO2

6c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110
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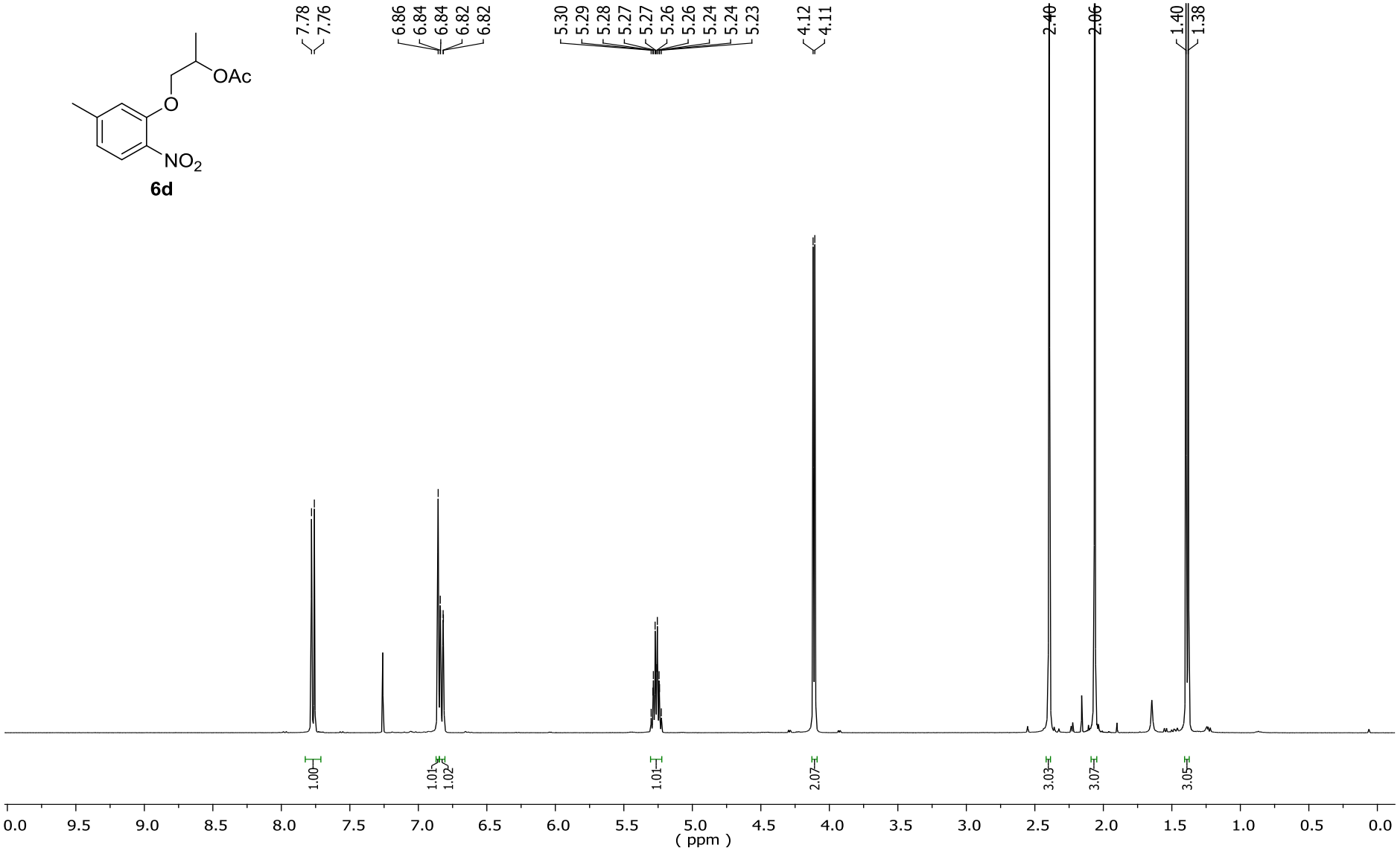
10
(ppm)


OAc
O
NO2
6d
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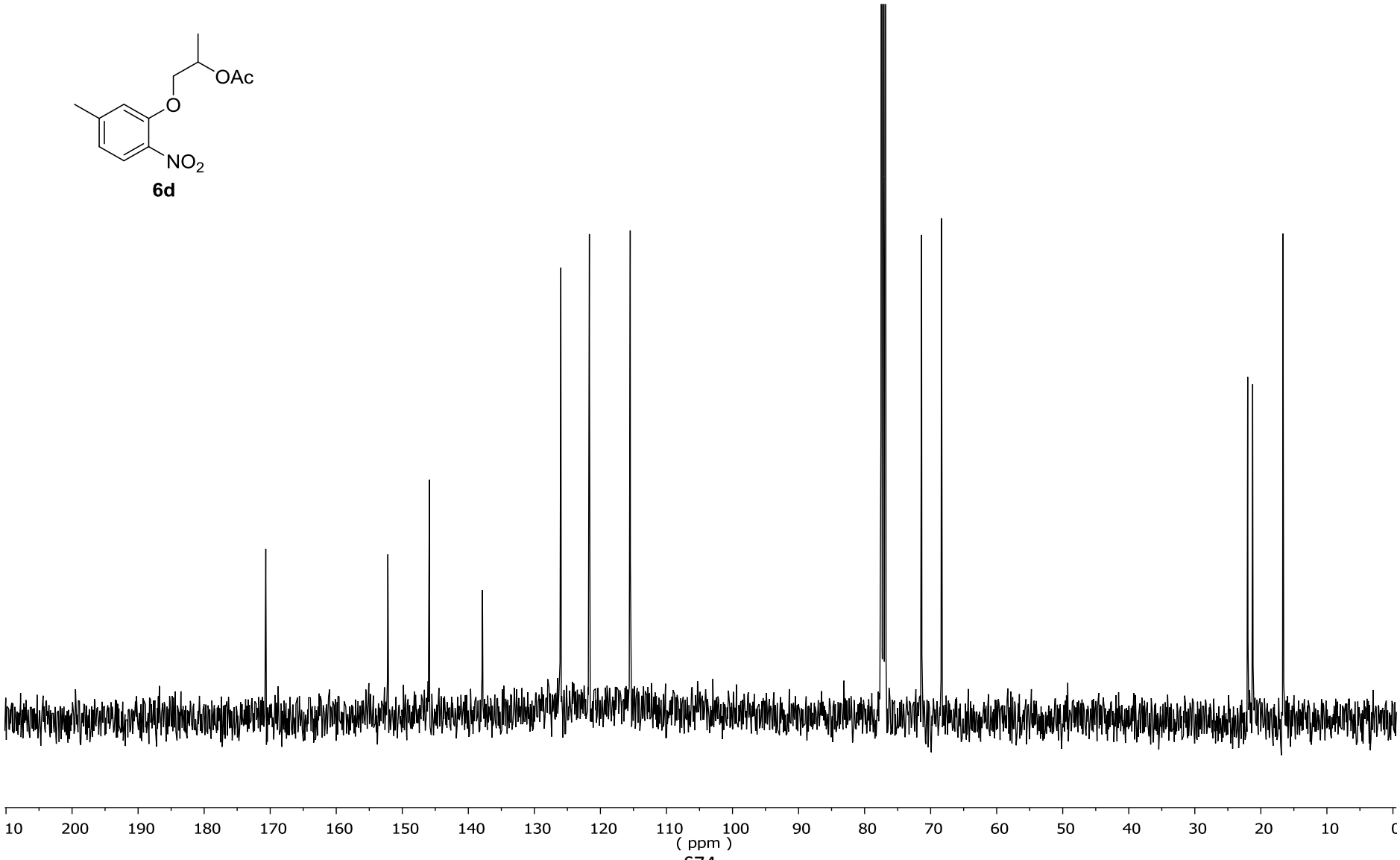
7.76
6.86
6.84
6.84
6.82
6.82
5.30
5.29
5.28
5.27
5.27
5.26
5.26
5.24
5.24
5.23
4.12
4.11
2.40
2.06
1.40
1.38
1.00
1.01
1.02
1.01
2.07
3.03
3.07
3.05
0.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
(ppm)

OAc
O
NO2

6d


10
200
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170
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150
140
130
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110
100
90
80
70
60
50
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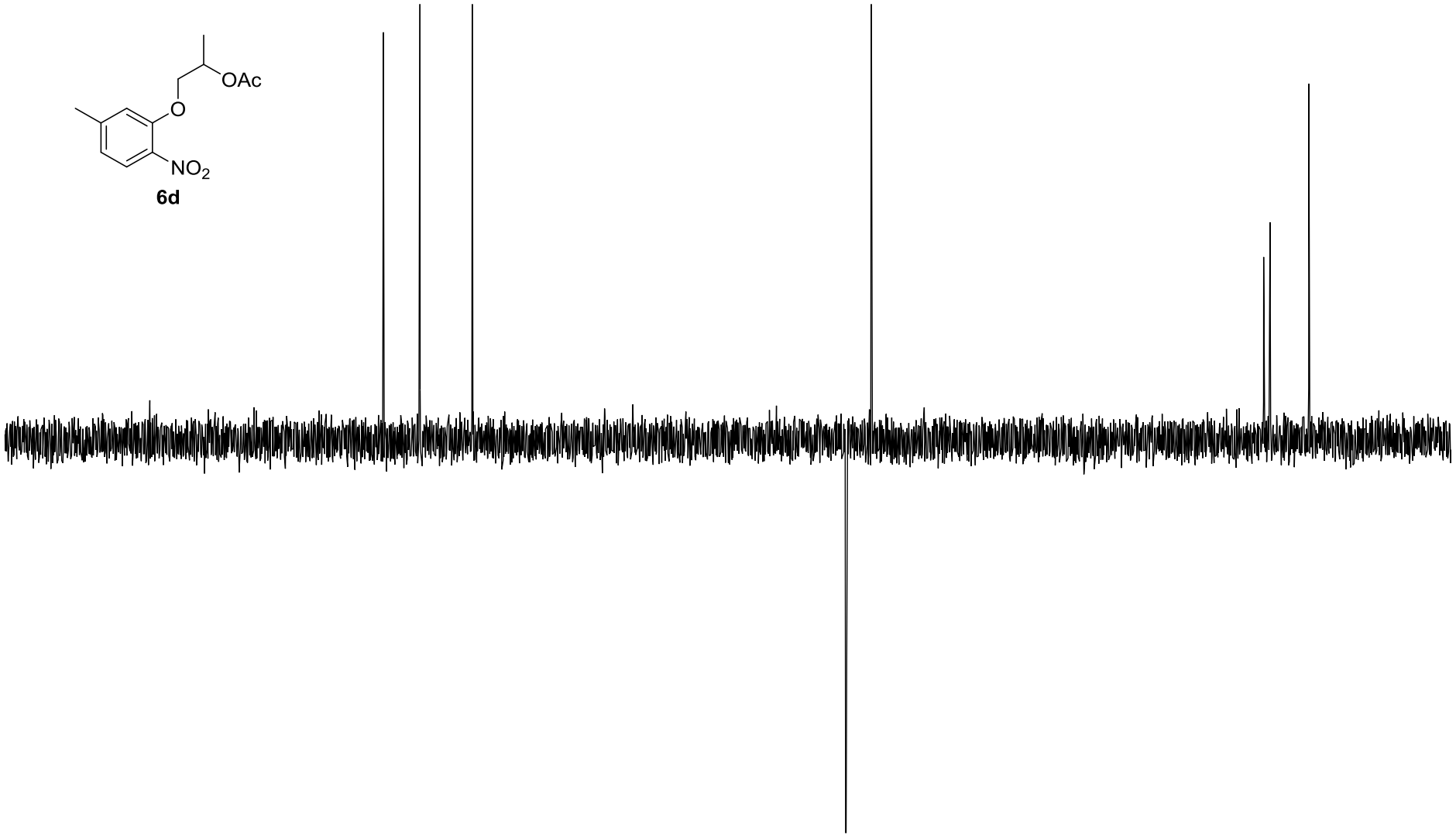
20
10
(ppm)


OAc
O
NO2
6d
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140
130
120
110
100
90
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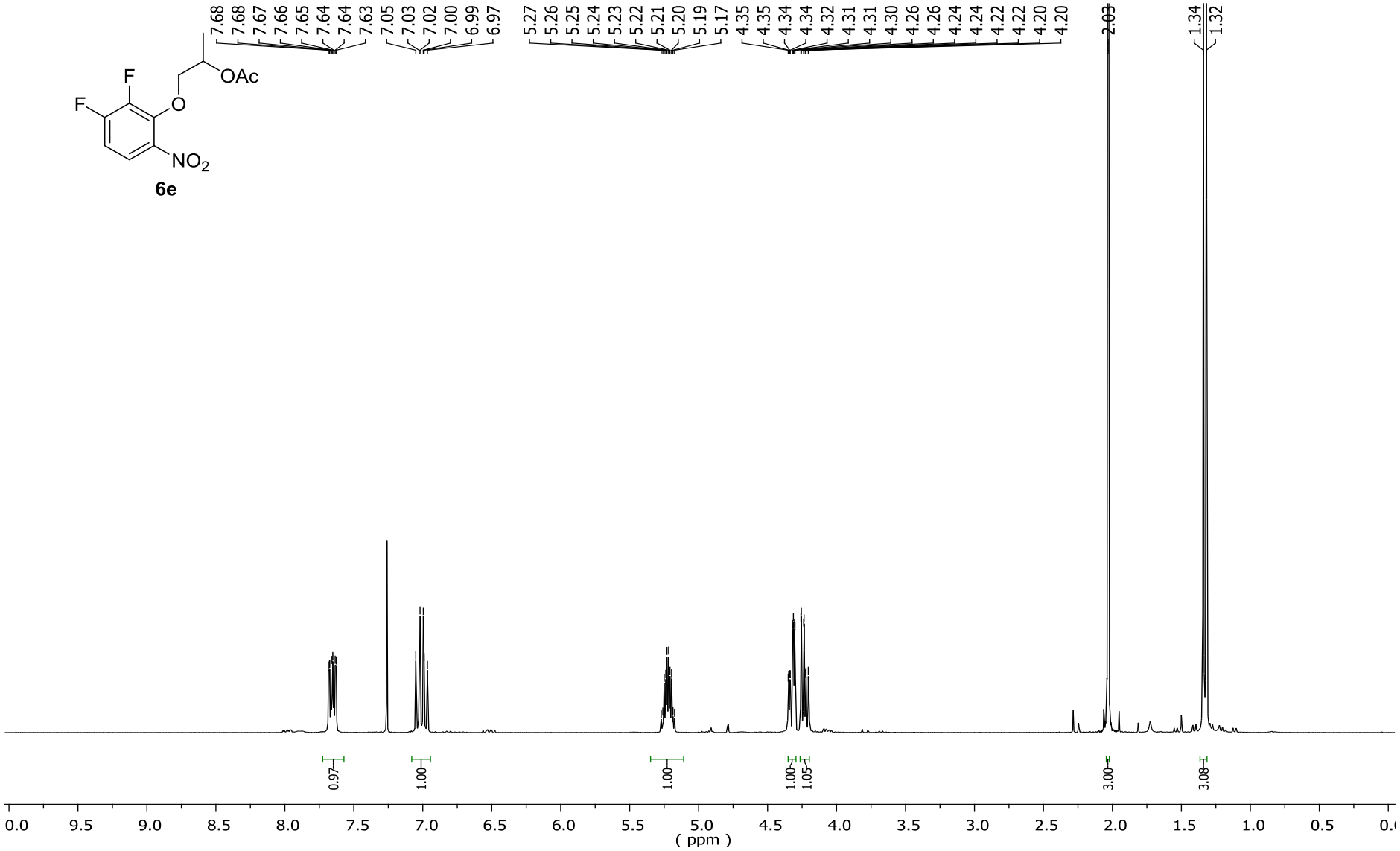
70
60
50
40
30
20
10
(ppm)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7.68
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7.64
7.64
7.63
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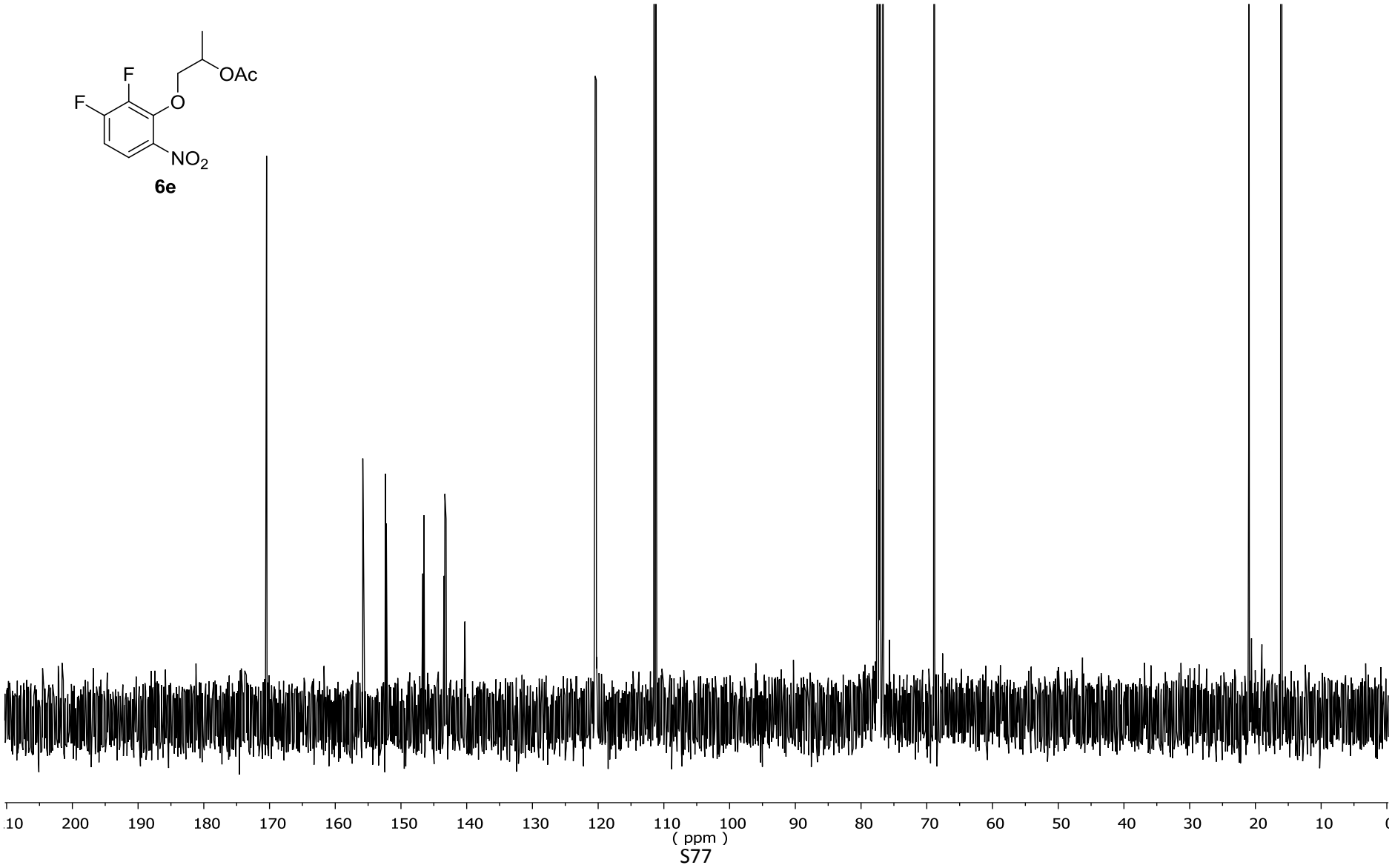
7.03
7.02
7.00
6.99
6.97
5.27
5.26
5.25
5.24
5.23
5.22
5.21
5.20
5.19
5.17
4.35
4.35
4.34
4.34
4.32
4.31
4.31
4.30
4.26
4.26
4.24
4.24
4.22
4.22
4.20
4.20
2.03
1.34
1.32
F
F
O
OAc
NO2
6e
0.97
1.00
1.00
1.00
1.05
3.00
3.08
0.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
(ppm)

F
OAc
F
O
NO2

6e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170
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150
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130
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80
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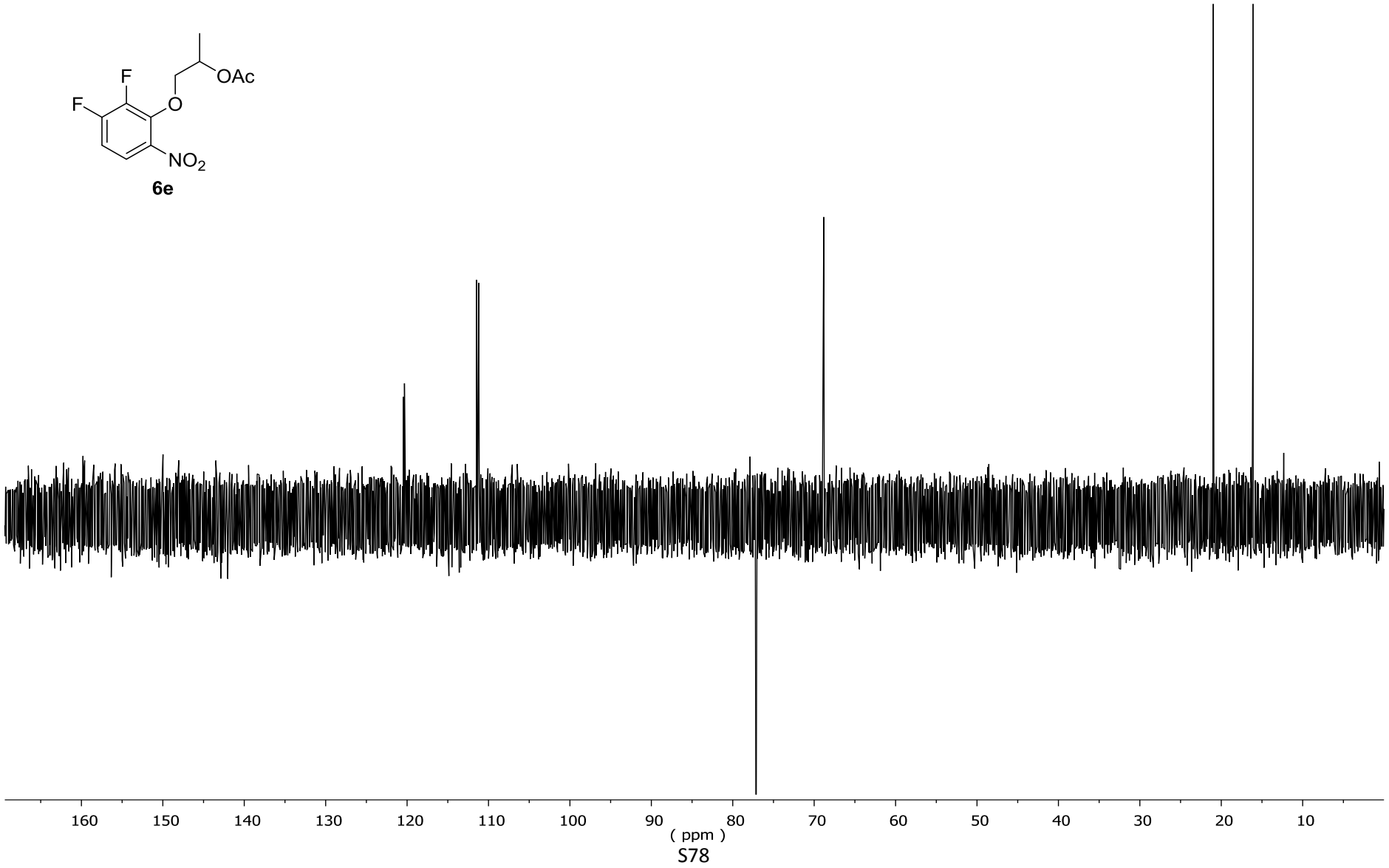
60
50
40
30
20
10
(ppm)

F
OAc
F
O
NO2

6e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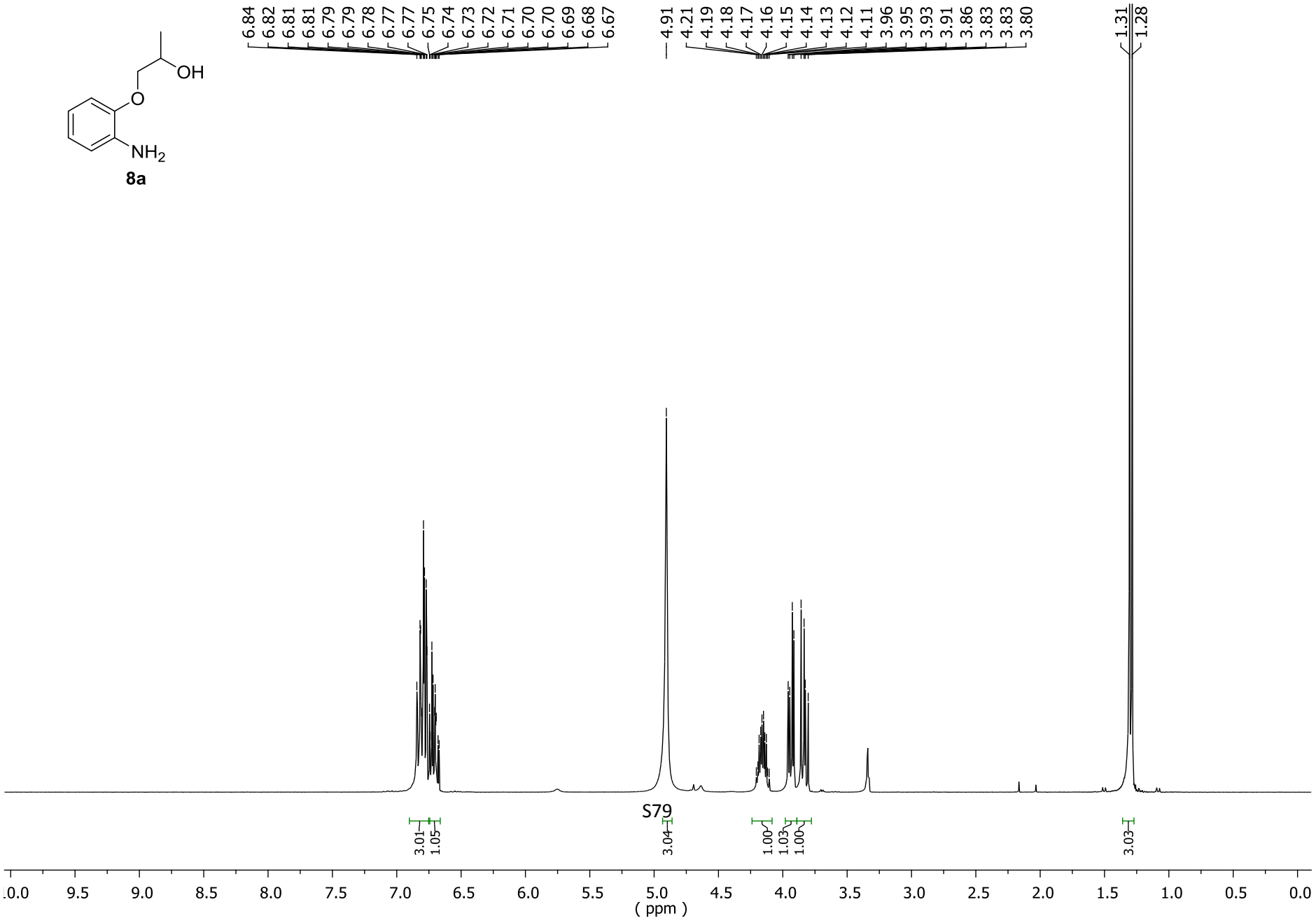
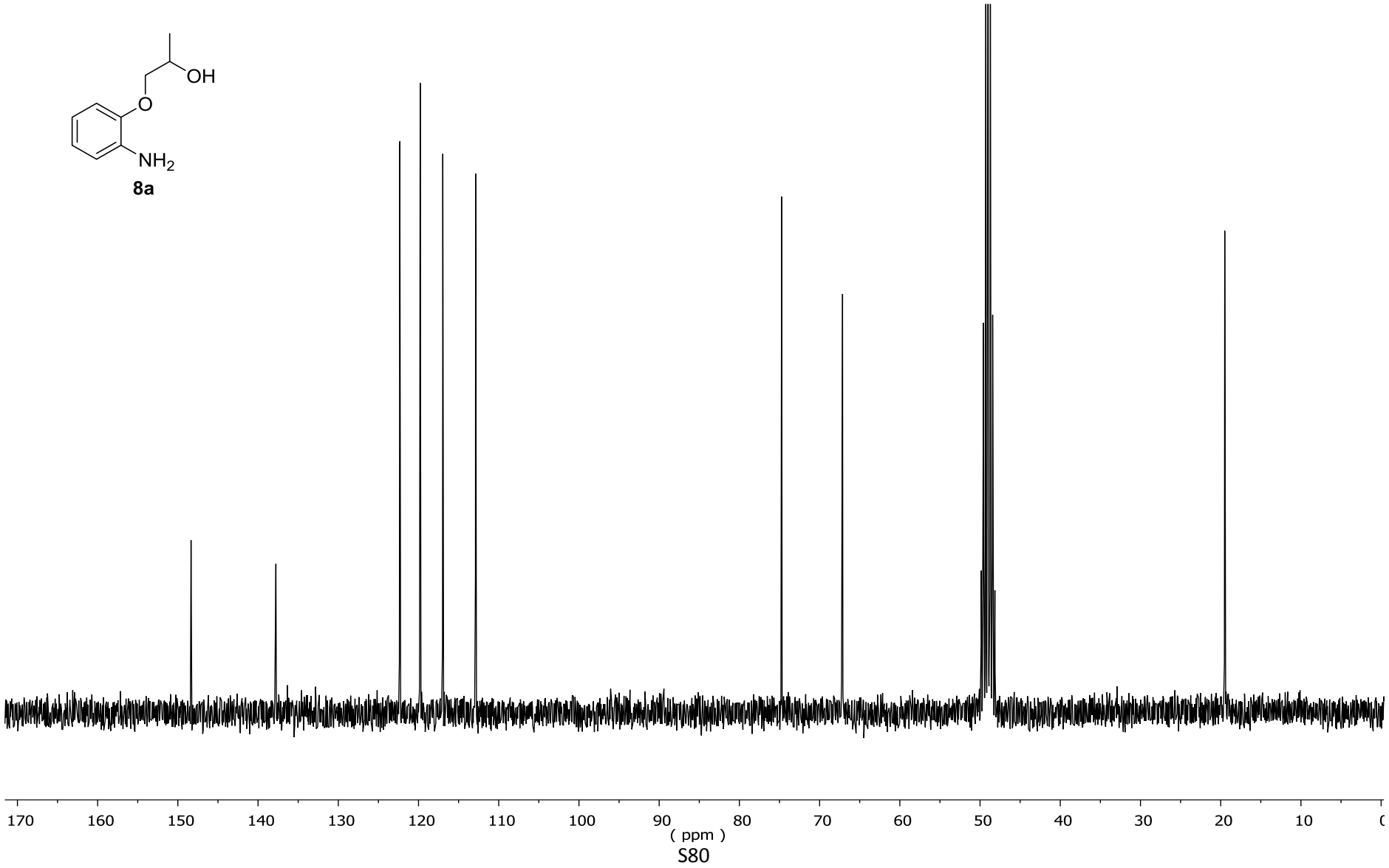
100
90
80
70
60
50
40
30
20
10
(ppm)


OH
O
NH2
8a
6.84
6.82
6.81
6.81
6.79
6.79
6.78
6.77
6.77
6.75
6.74
6.73
6.72
6.71
6.70
6.70
6.69
6.68
6.67
4.91
4.21
4.19
4.18
4.17
4.16
4.15
4.14
4.13
4.12
4.11
3.96
3.95
3.93
3.91
3.86
3.83
3.83
3.80
1.31
1.28
3.01
1.05
3.04
1.00
1.03
1.00
3.03
(ppm)


OH
O
NH2
8a
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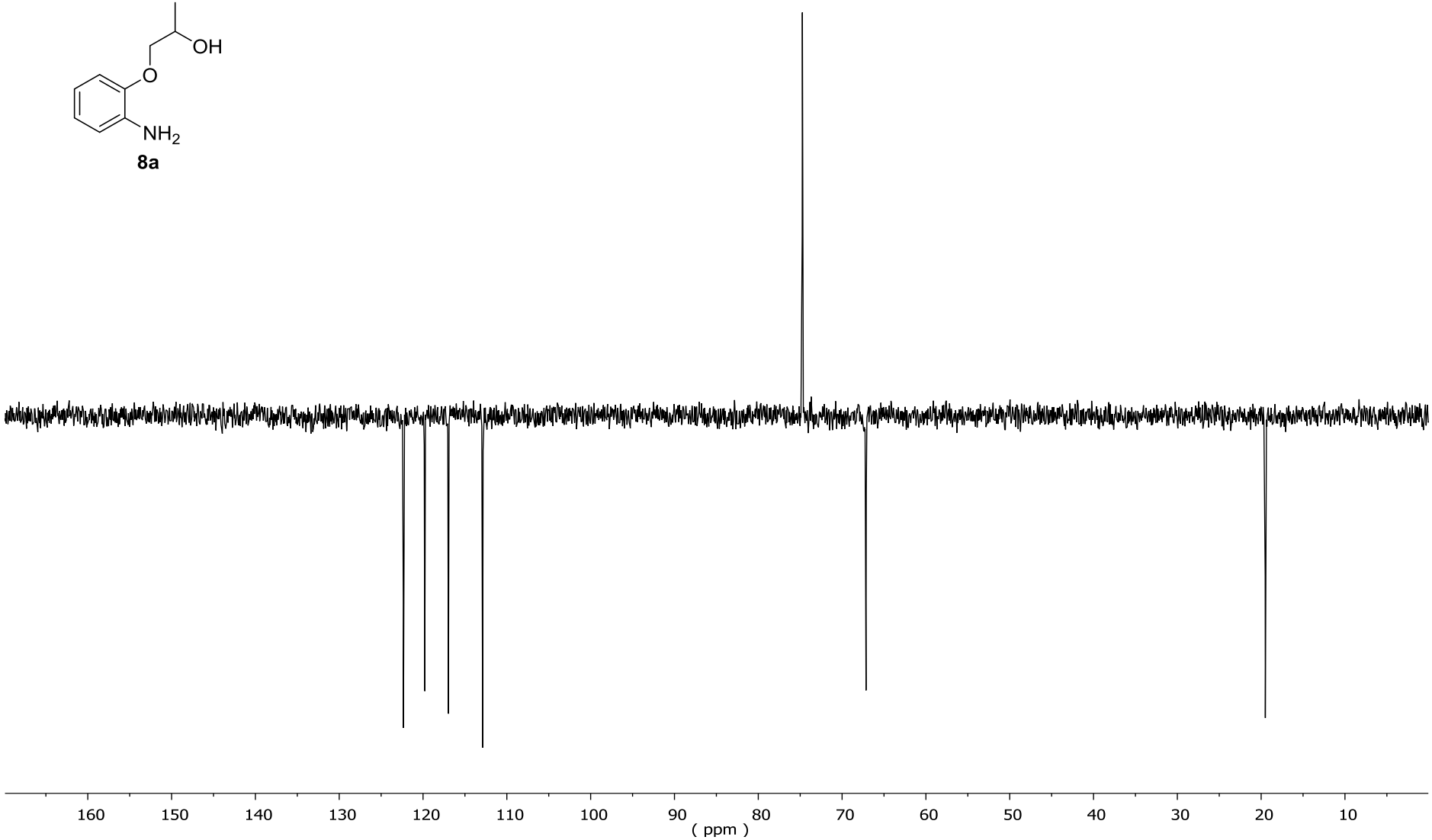
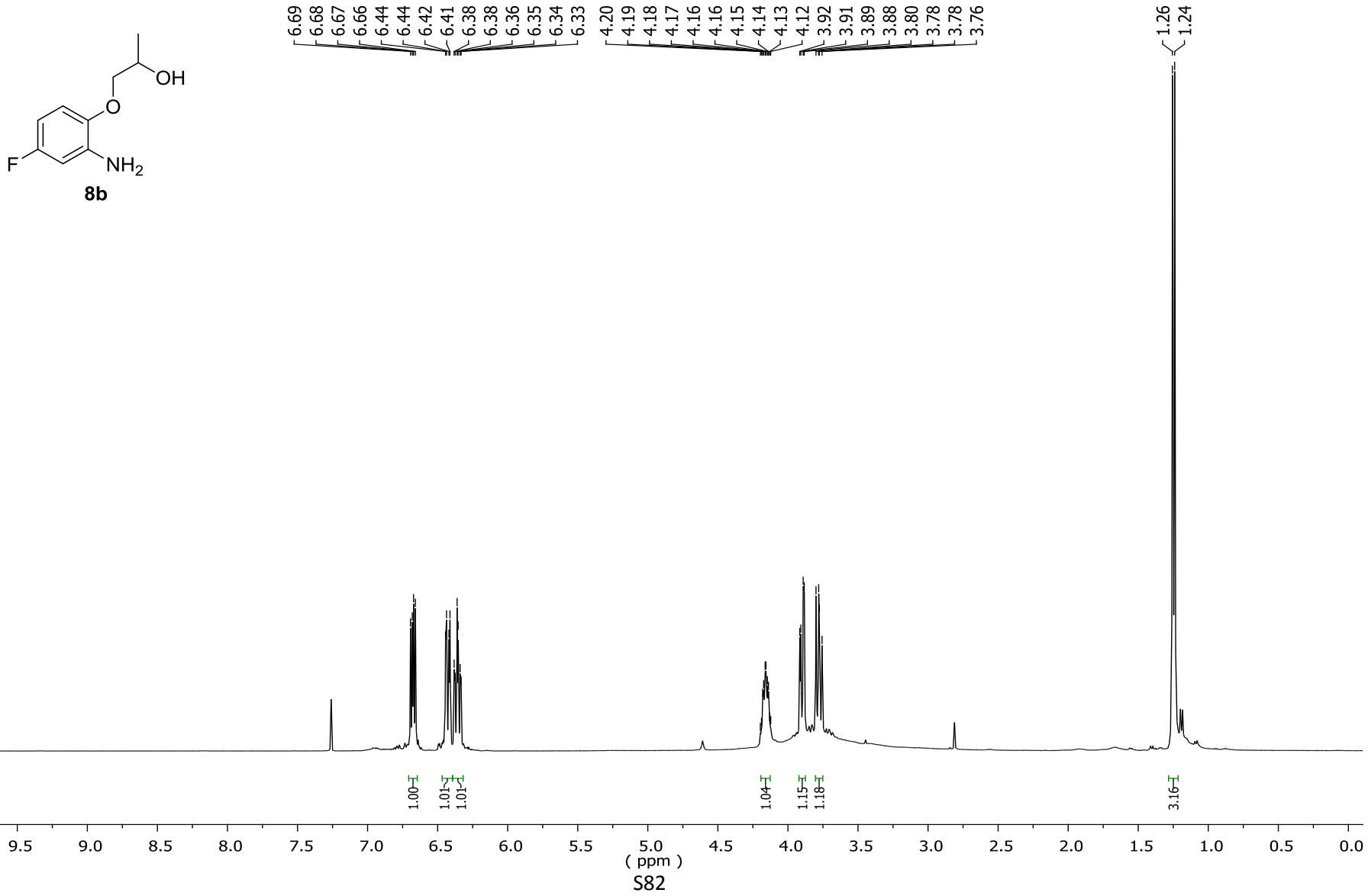
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)

8a

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6.67
6.66
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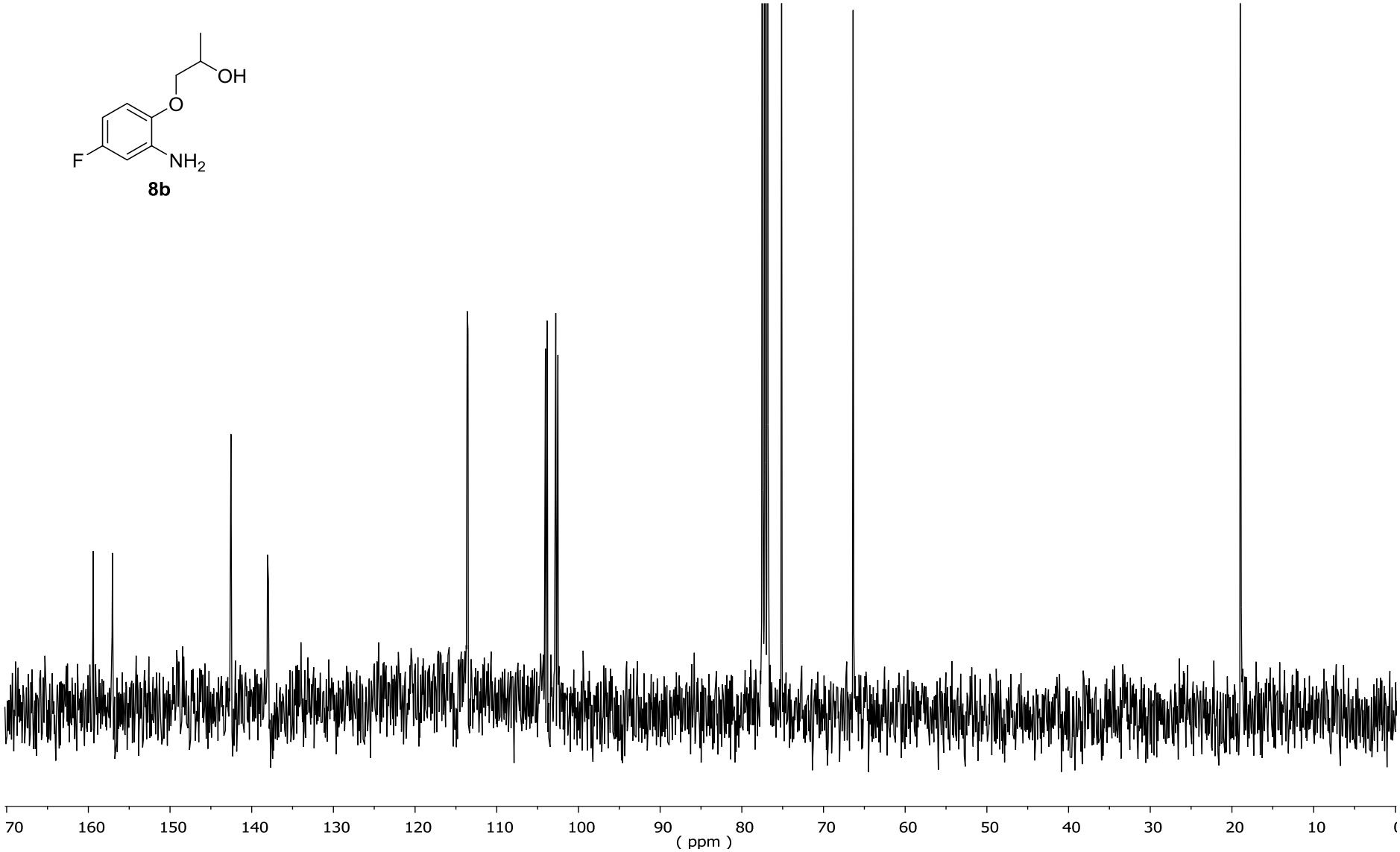
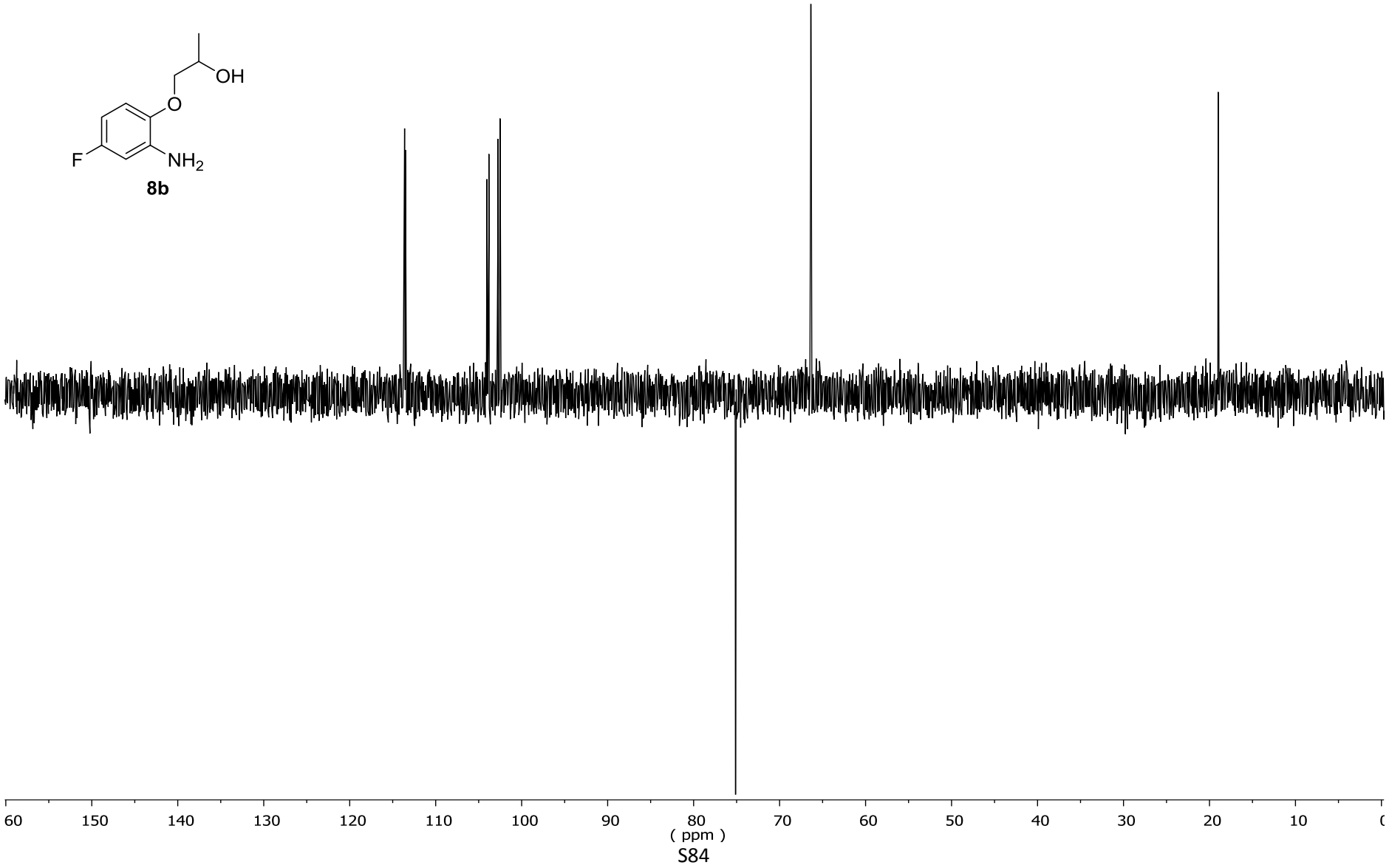
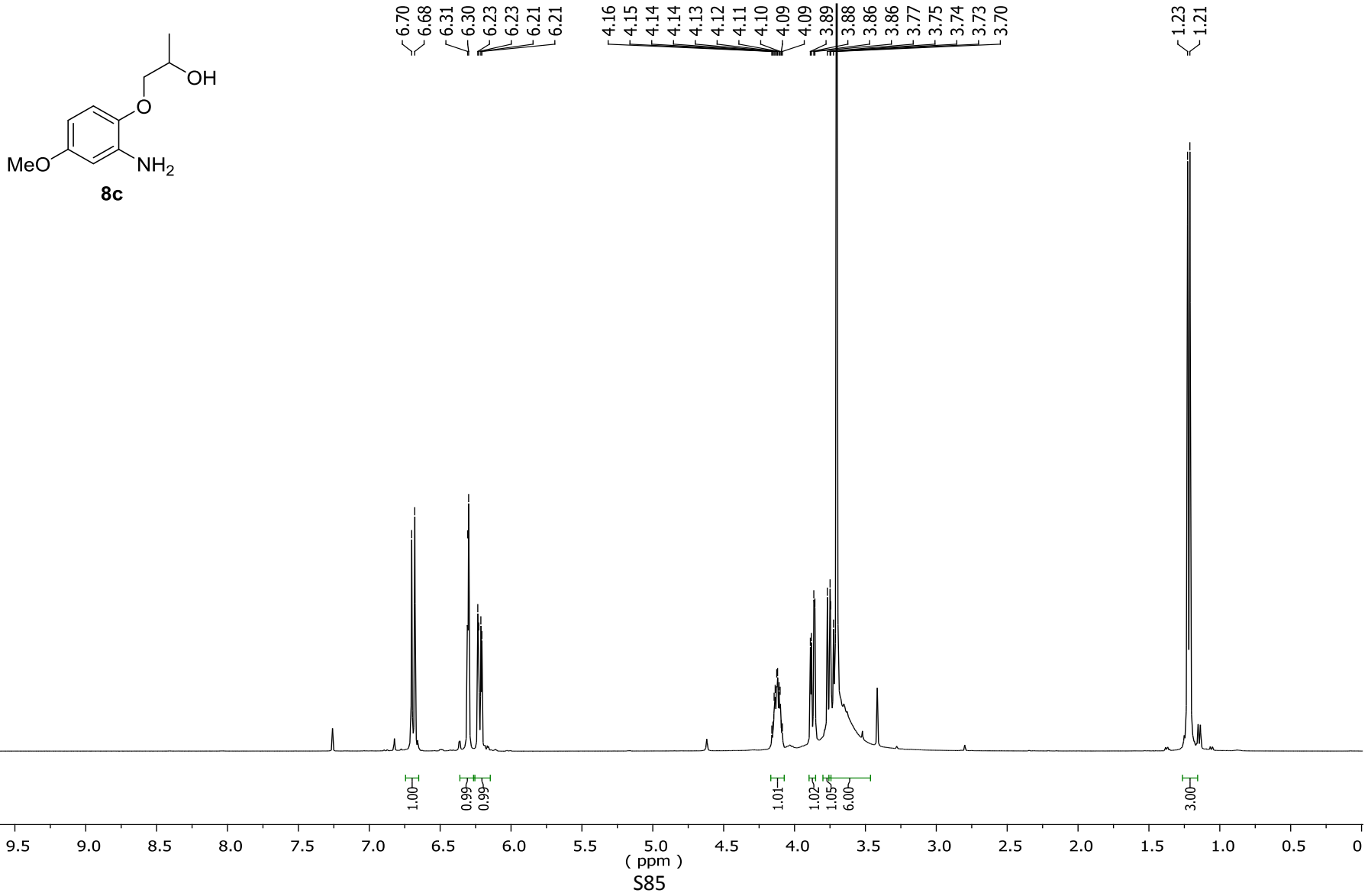
6.44
6.42
6.41
6.38
6.38
6.36
6.35
6.34
6.33
4.20
4.19
4.18
4.17
4.16
4.16
4.15
4.14
4.13
4.12
3.92
3.91
3.89
3.88
3.80
3.78
3.78
3.76
1.26
1.24
OH
O
F
NH2
8b
1.00
1.01
1.01
1.04
1.15
1.18
3.16
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
(ppm)

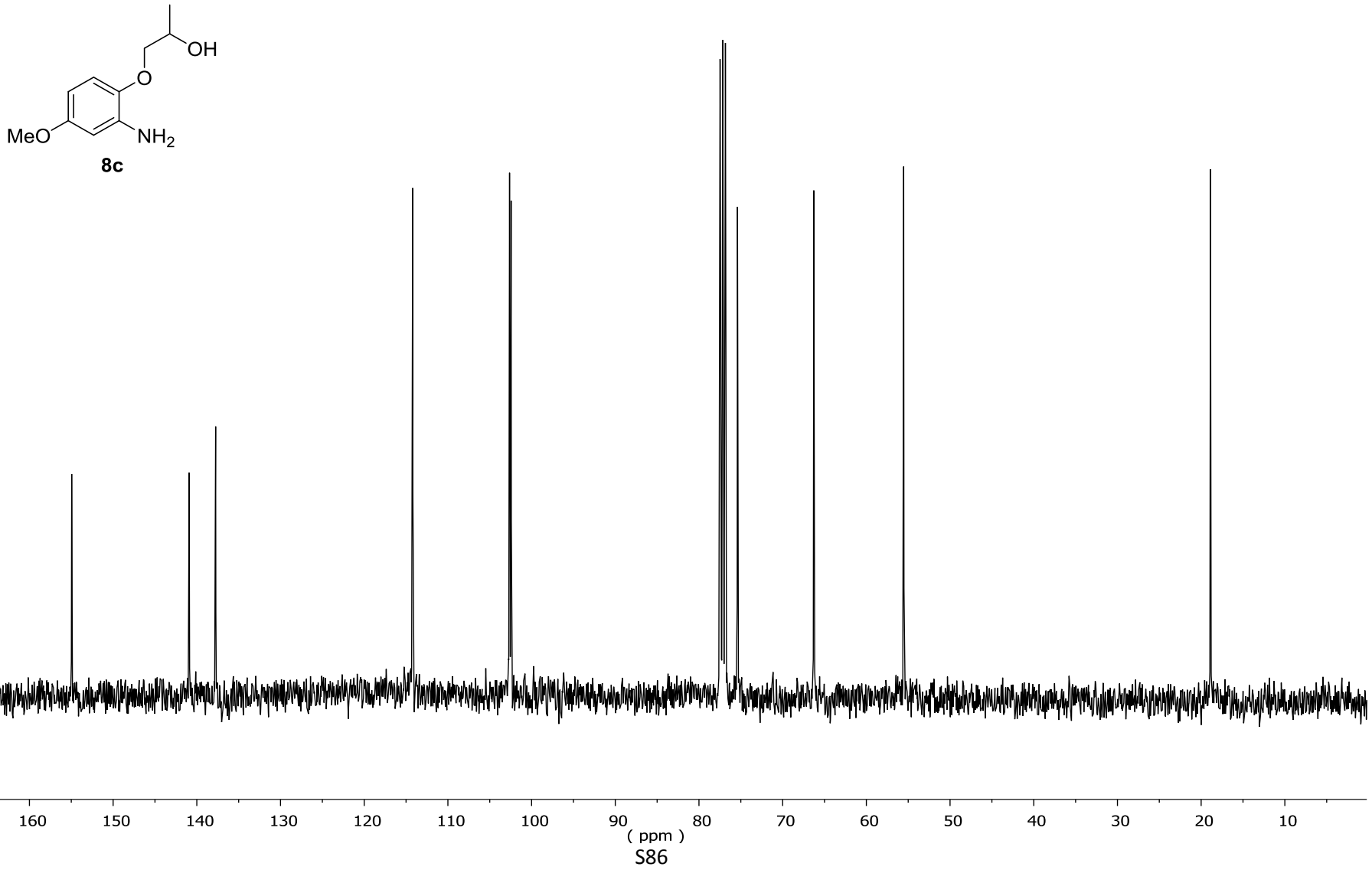
OH
O
F
NH2

8b


70
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)

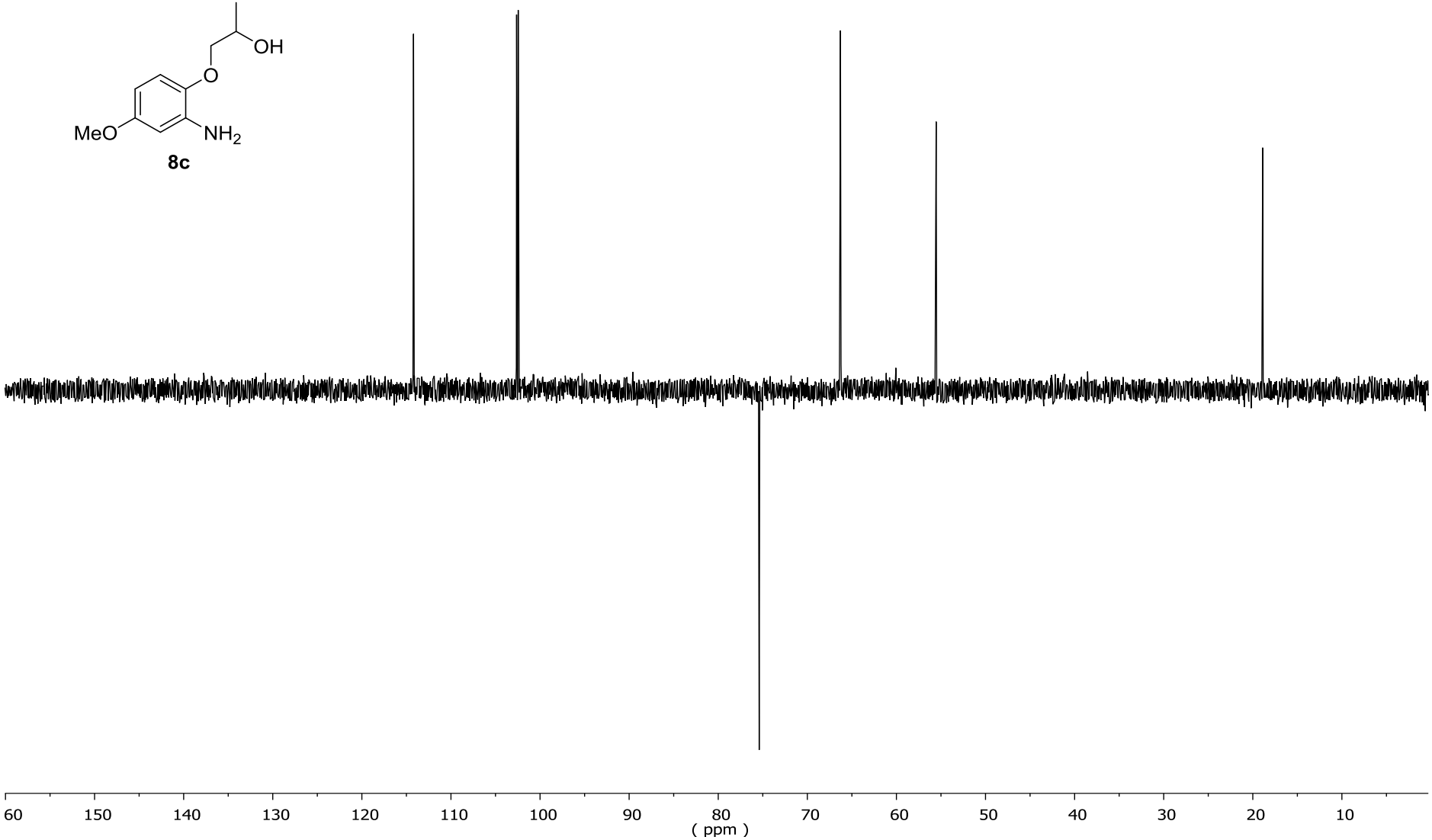

OH
O
F
NH2
8b
60
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)


OH
O
MeO
NH2
8c
6.70
6.68
6.31
6.30
6.23
6.23
6.21
6.21
4.16
4.15
4.14
4.14
4.13
4.12
4.11
4.10
4.09
4.09
3.89
3.88
3.86
3.86
3.77
3.75
3.74
3.73
3.70
1.23
1.21
1.00
0.99
0.99
1.01
1.02
1.05
6.00
3.00
(ppm)


OH
O
MeO
NH2
8c
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)

OH
O
MeO
NH2

8c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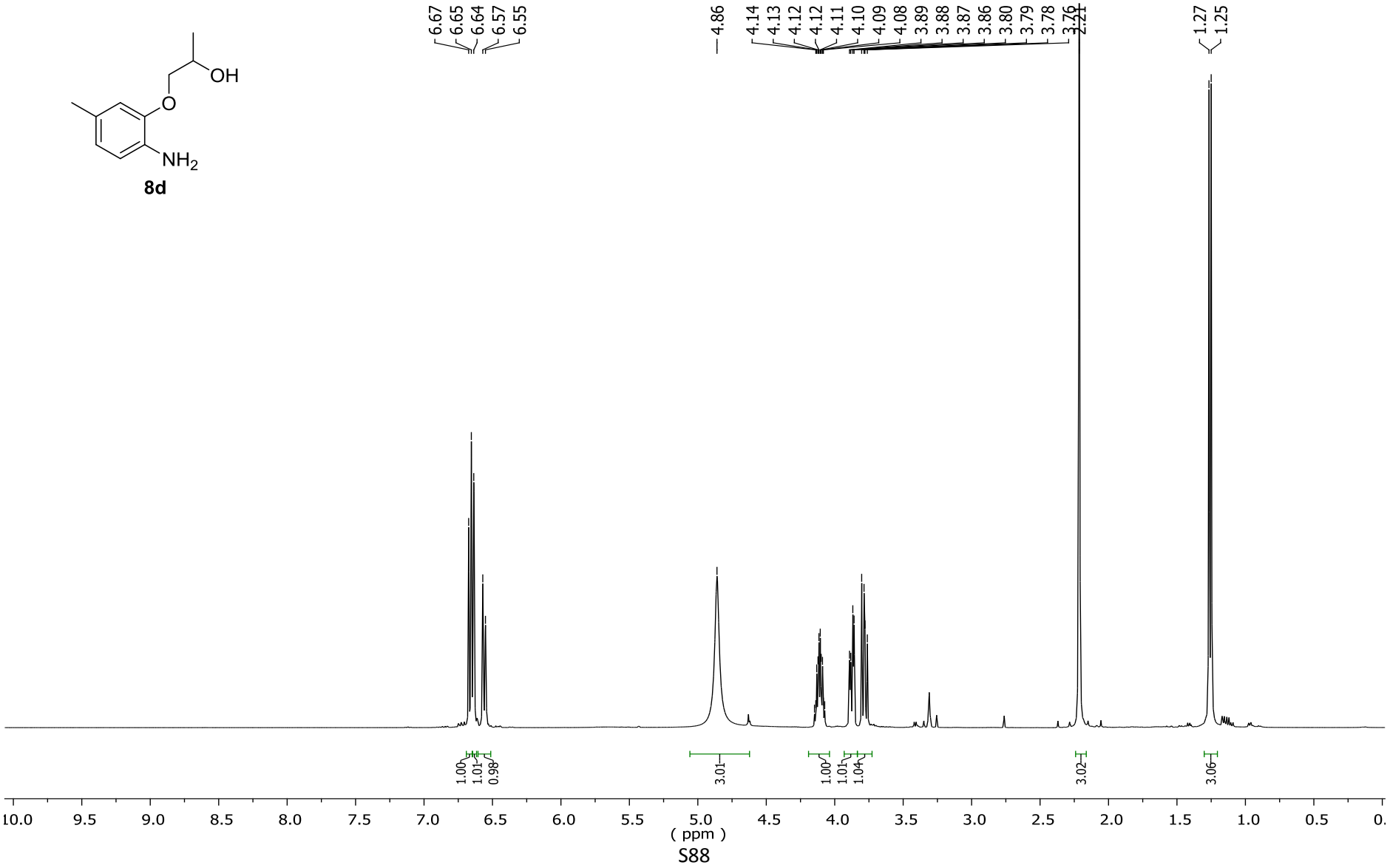
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)


OH
O
NH2
8d
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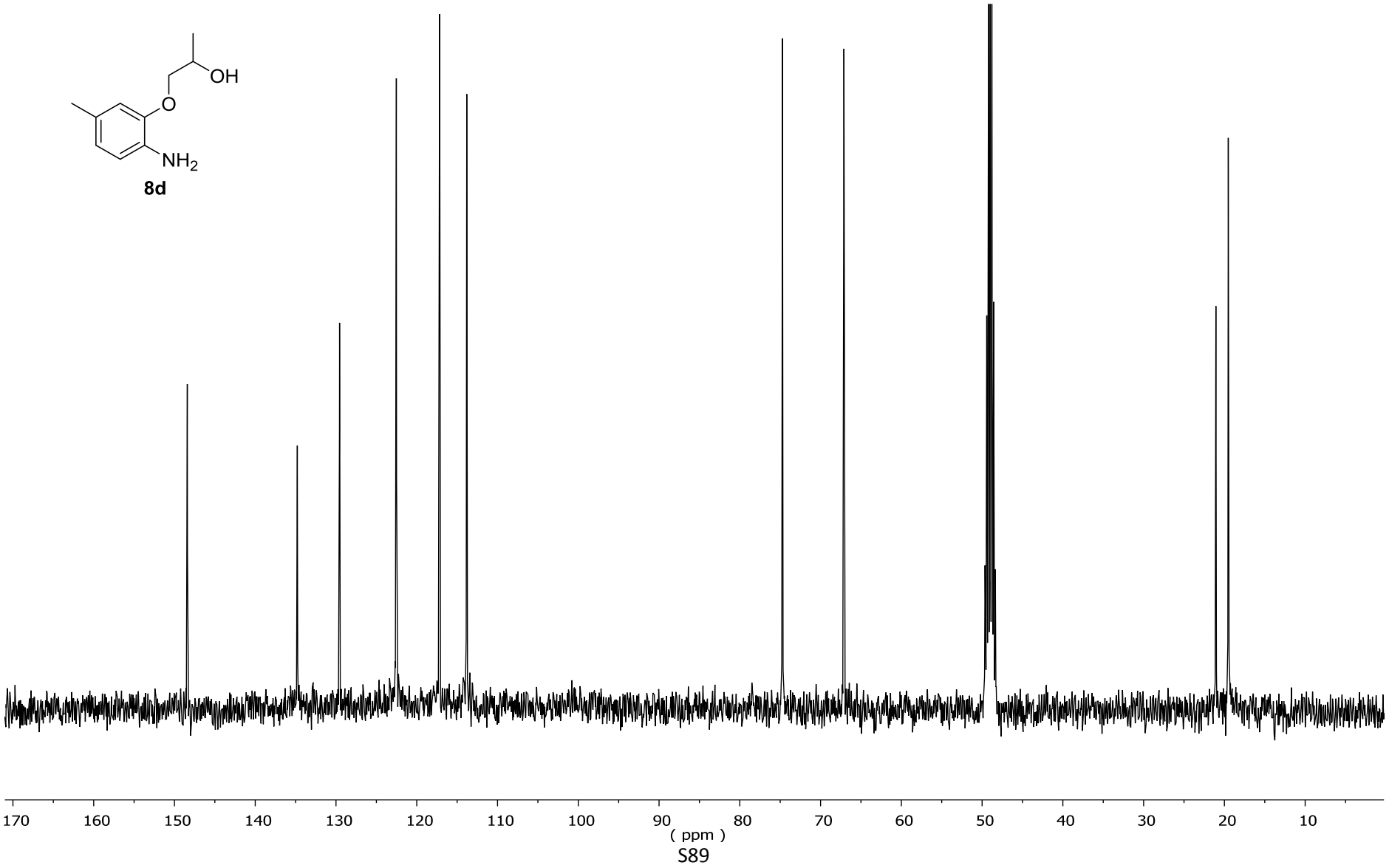
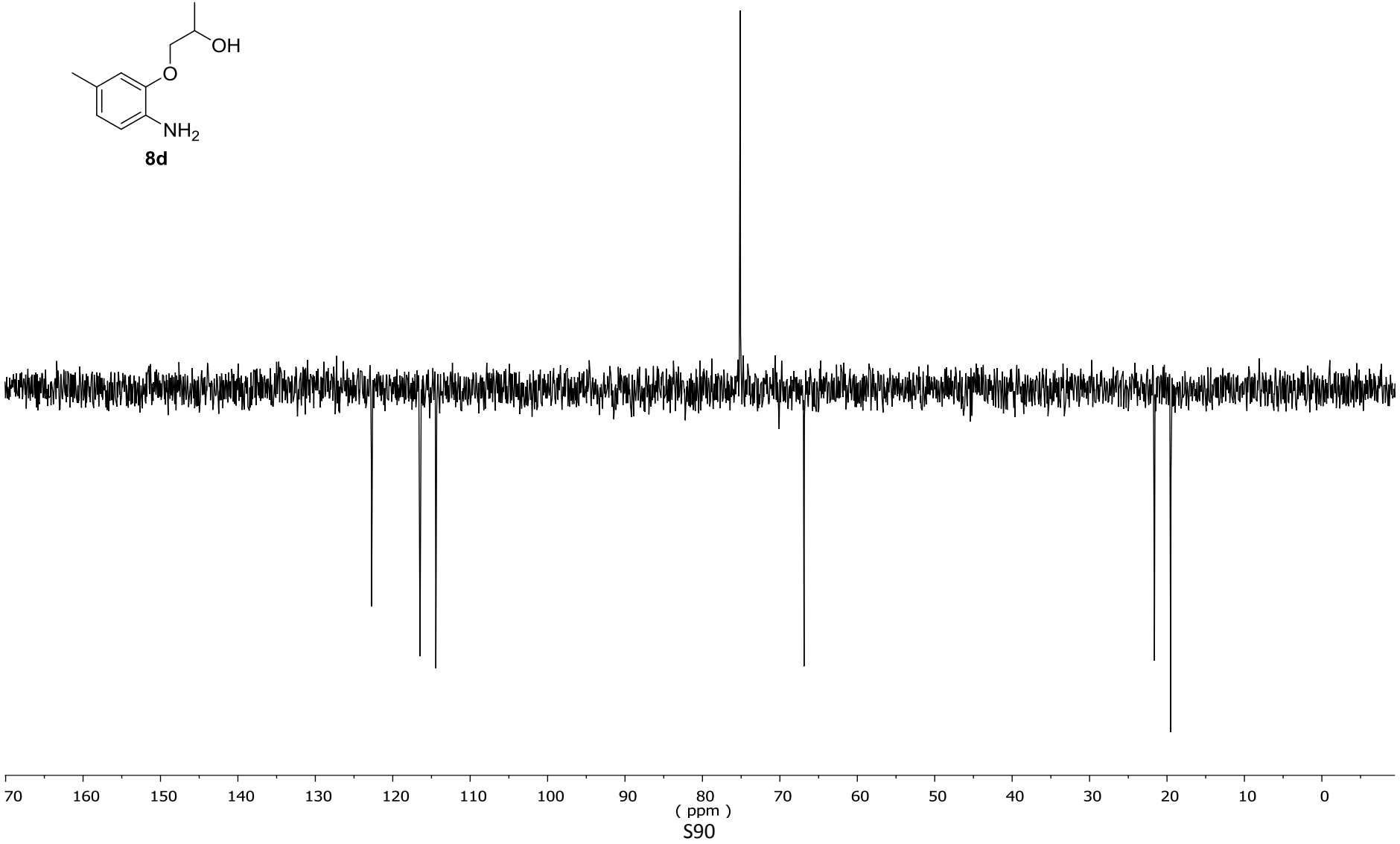
6.65
6.64
6.57
6.55
4.86
4.14
4.13
4.12
4.12
4.11
4.10
4.09
4.08
3.89
3.88
3.87
3.86
3.80
3.79
3.78
3.76
2.21
1.27
1.25
1.00
1.01
0.98
3.01
1.00
1.01
1.04
3.02
3.06
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.
(ppm)


OH
O
NH2
8d
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)

OH
O
NH2

8d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160
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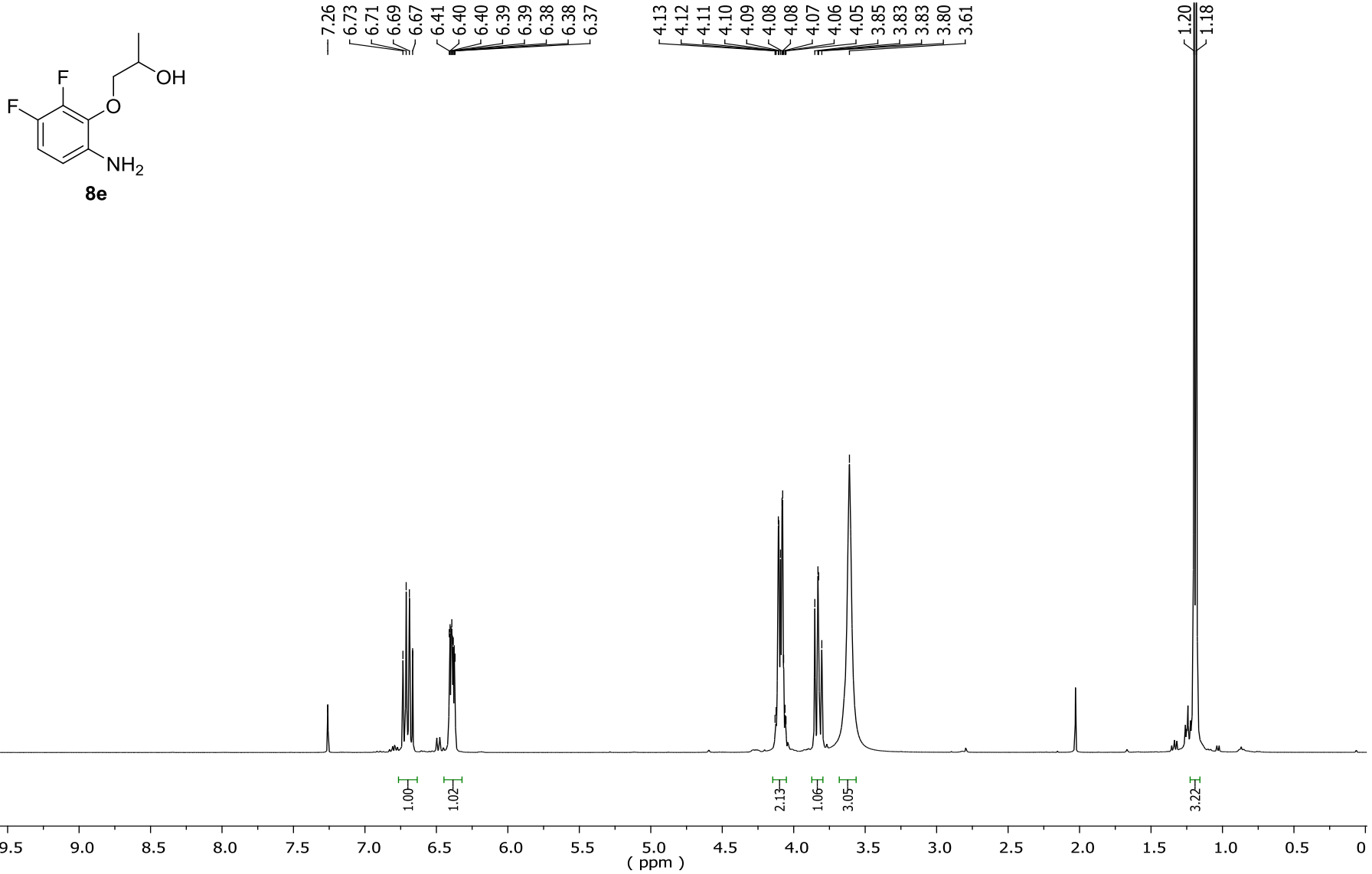
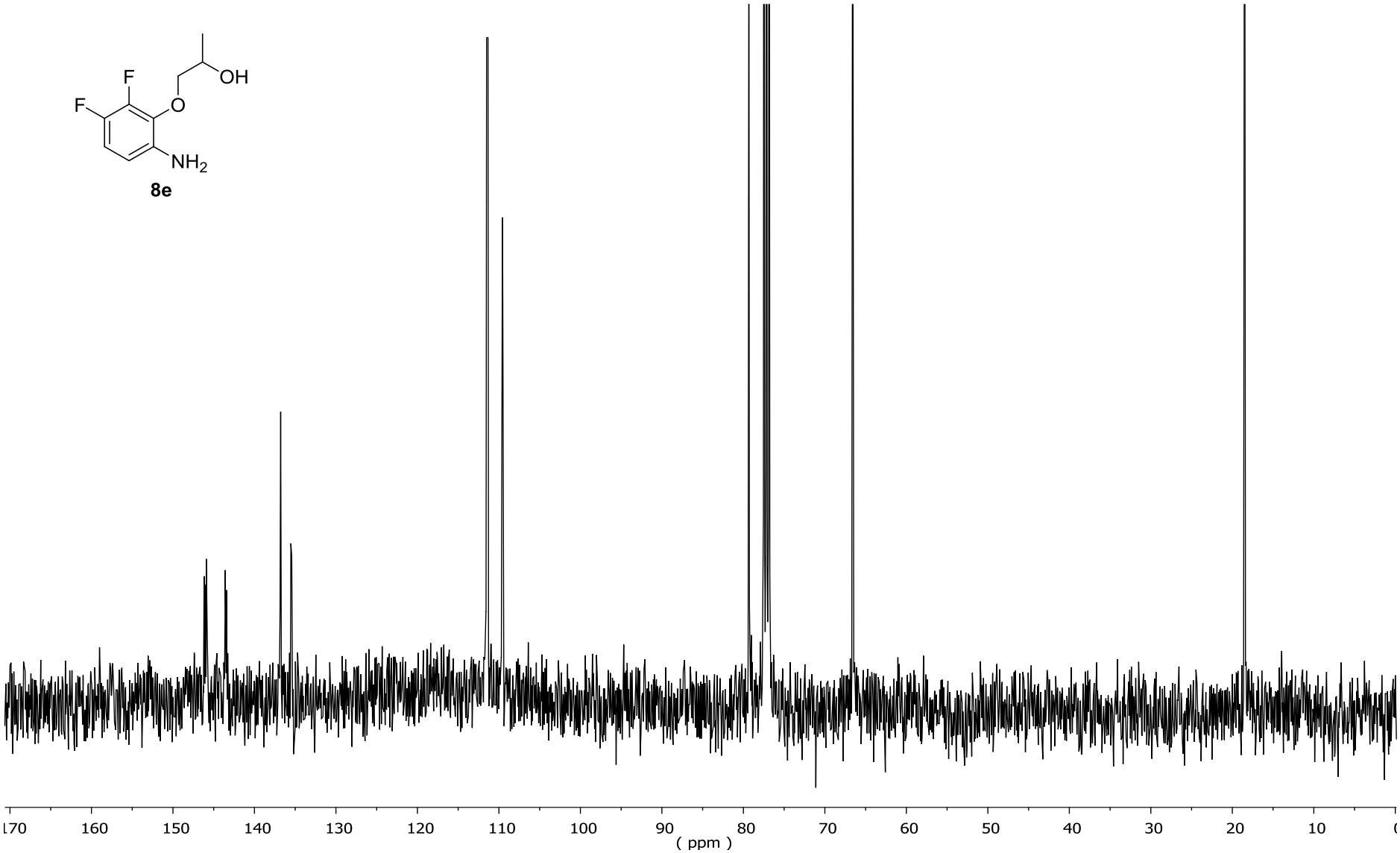
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)


F
OH
F
O
NH2
8e
7.26
6.73
6.71
6.69
6.67
6.41
6.40
6.40
6.39
6.39
6.38
6.38
6.37
4.13
4.12
4.11
4.10
4.09
4.08
4.08
4.07
4.06
4.05
3.85
3.83
3.83
3.80
3.61
1.20
1.18
1.00
1.02
2.13
1.06
3.05
3.22
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
(ppm)

F
OH
F
O
NH2

8e


170
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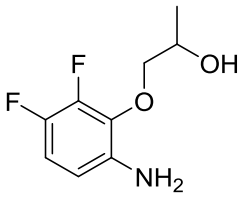
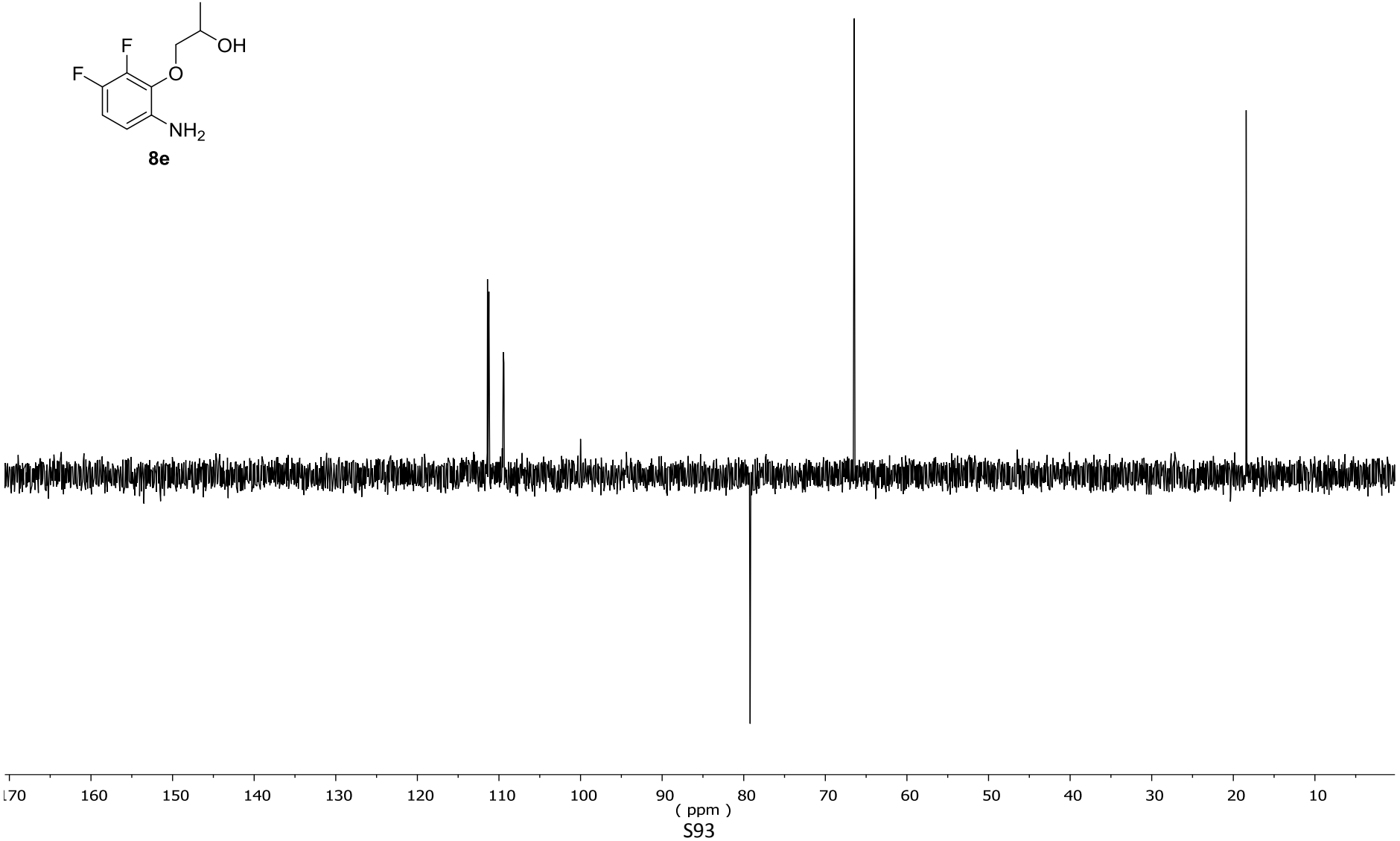
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)


F
OH
F
O
NH2

8e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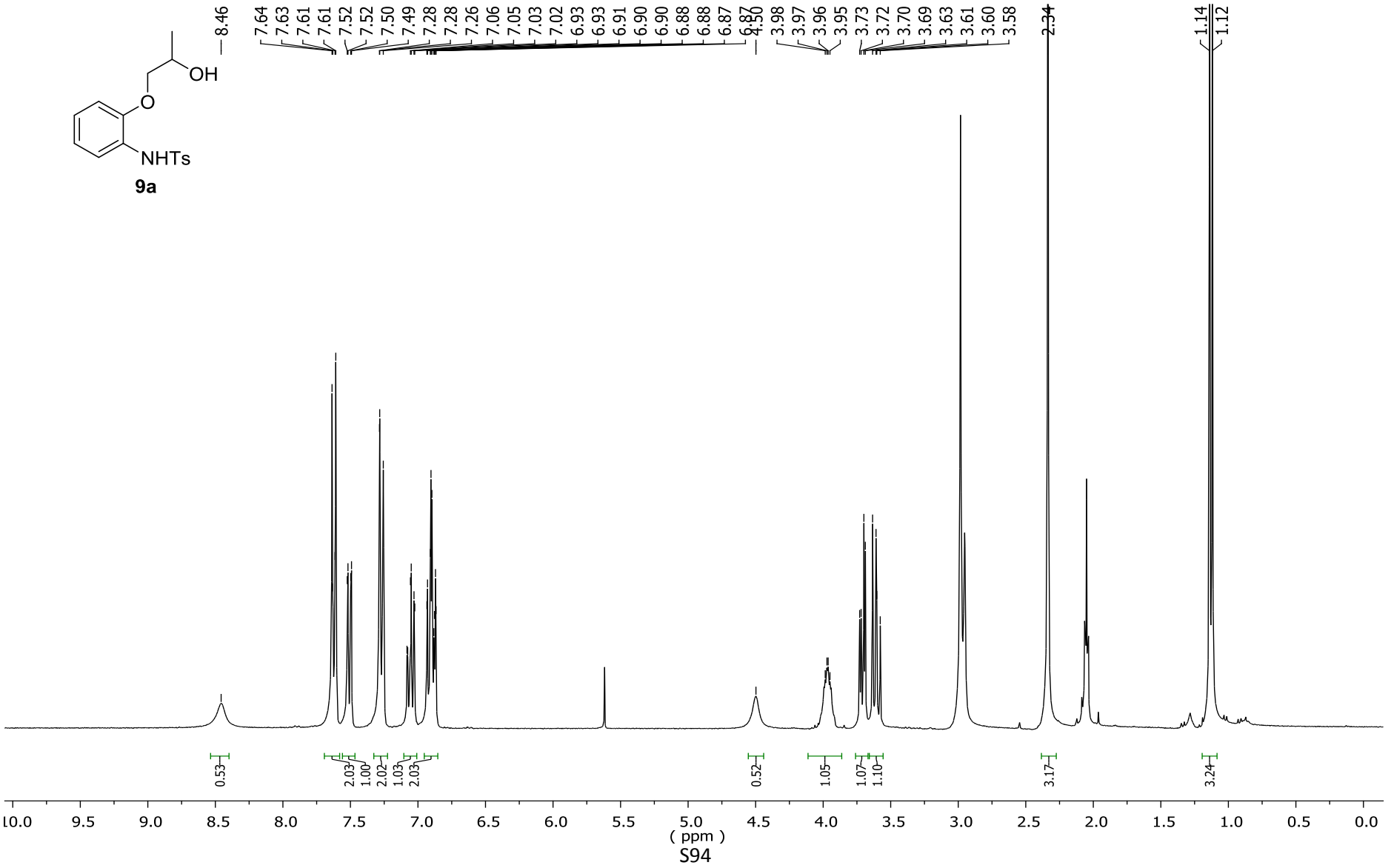
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)


8.46
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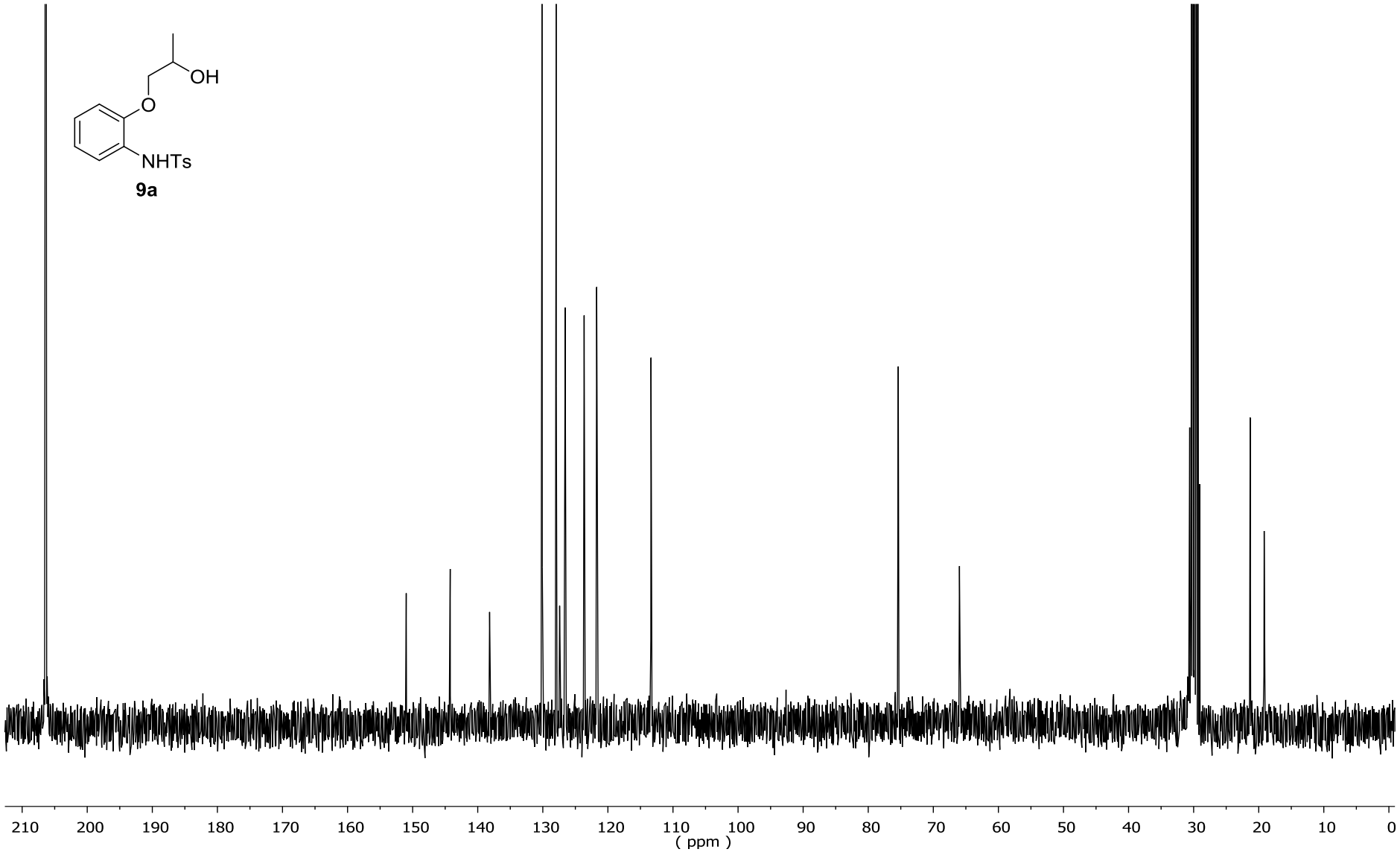
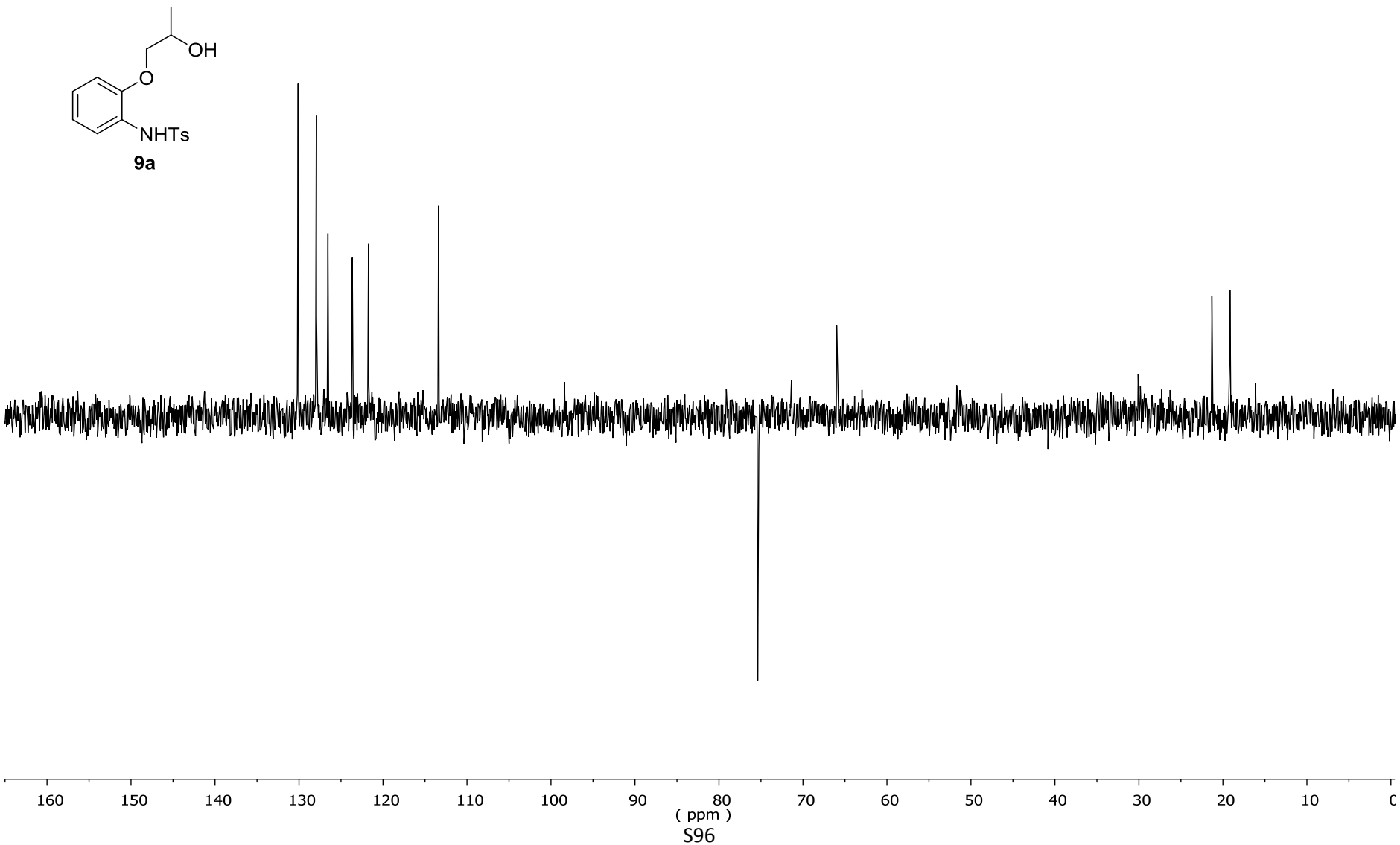
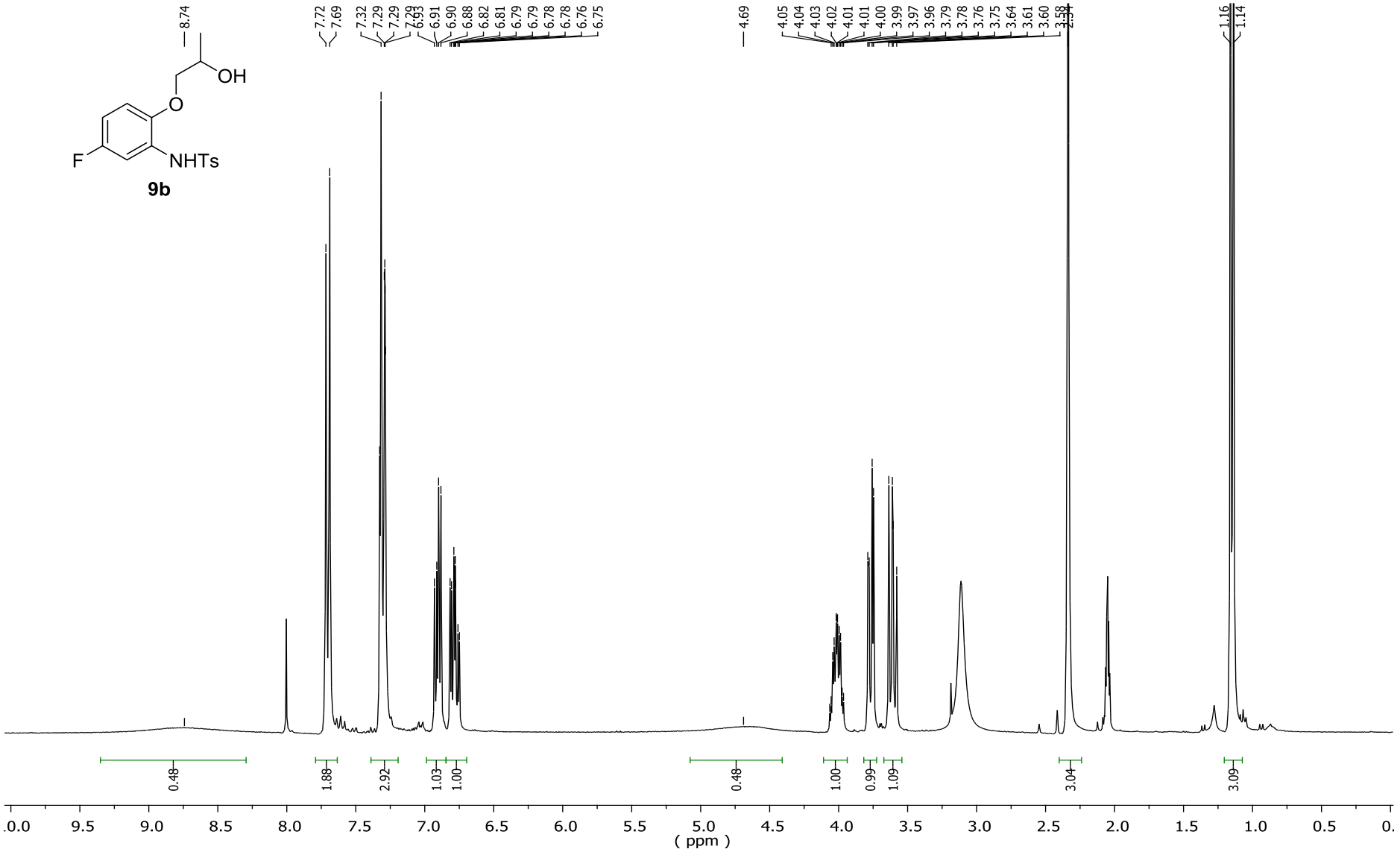
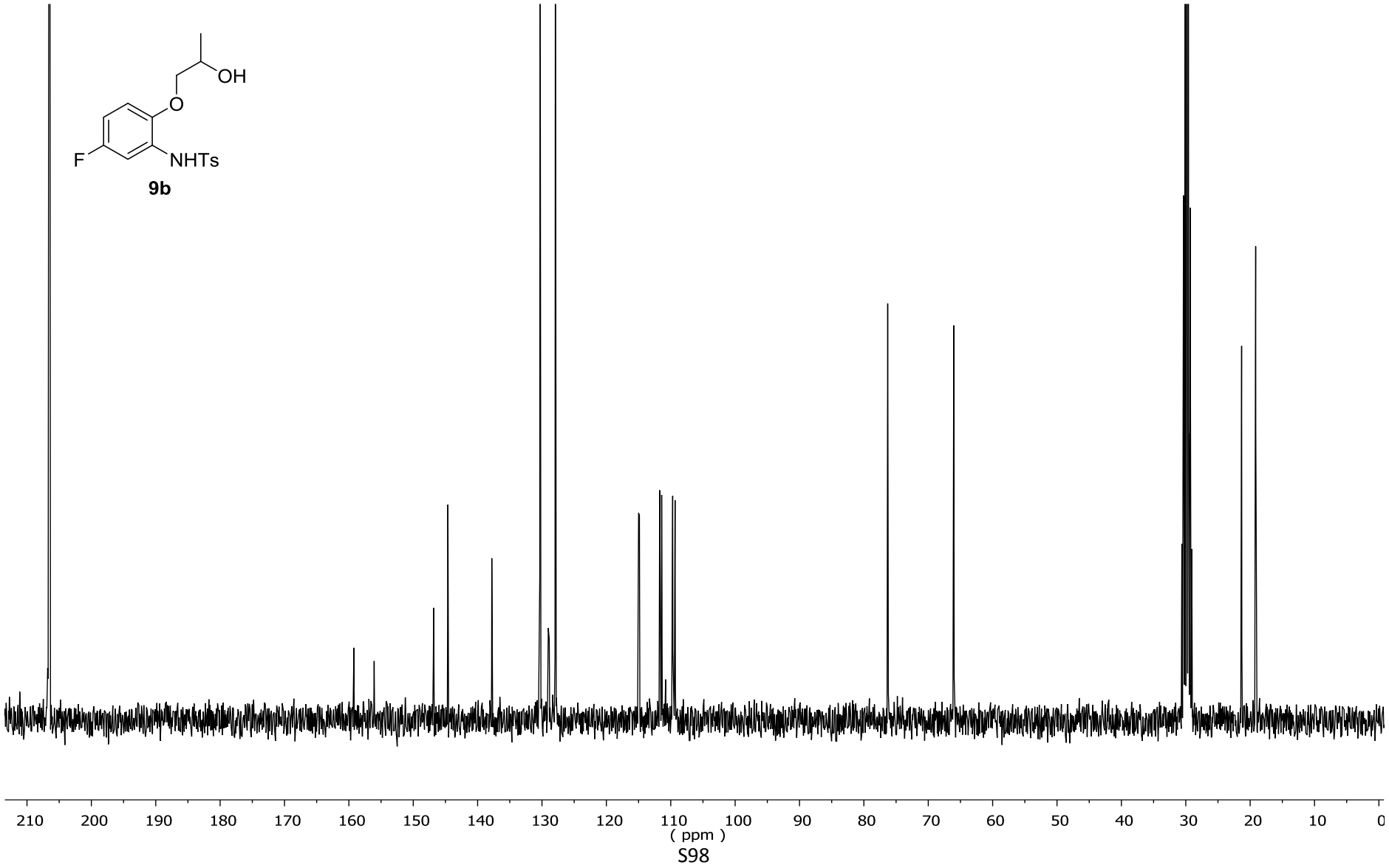
7.63
7.61
7.61
7.52
7.52
7.50
7.49
7.28
7.28
7.26
7.06
7.05
7.03
7.02
6.93
6.93
6.91
6.90
6.90
6.88
6.88
6.87
6.87
4.50
3.98
3.97
3.96
3.95
3.73
3.72
3.70
3.69
3.63
3.61
3.60
3.58
2.34
1.14
1.12
OH
O
NHTs
9a
0.53
2.03
1.00
2.02
1.03
2.03
0.52
1.05
1.07
1.10
3.17
3.24
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
(ppm)


OH
O
NHTs
9a
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)

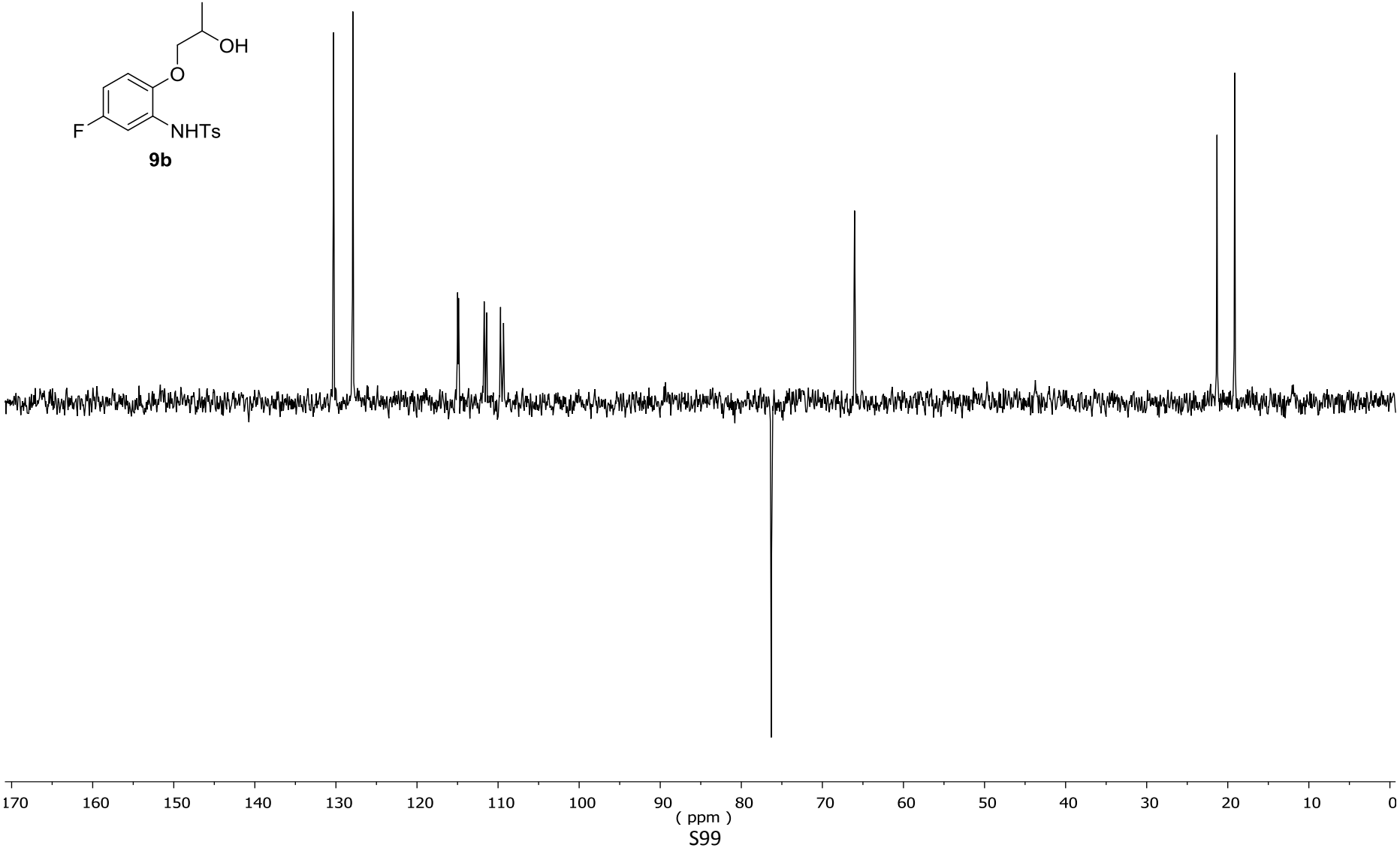

OH
O
NHTs
9a
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)

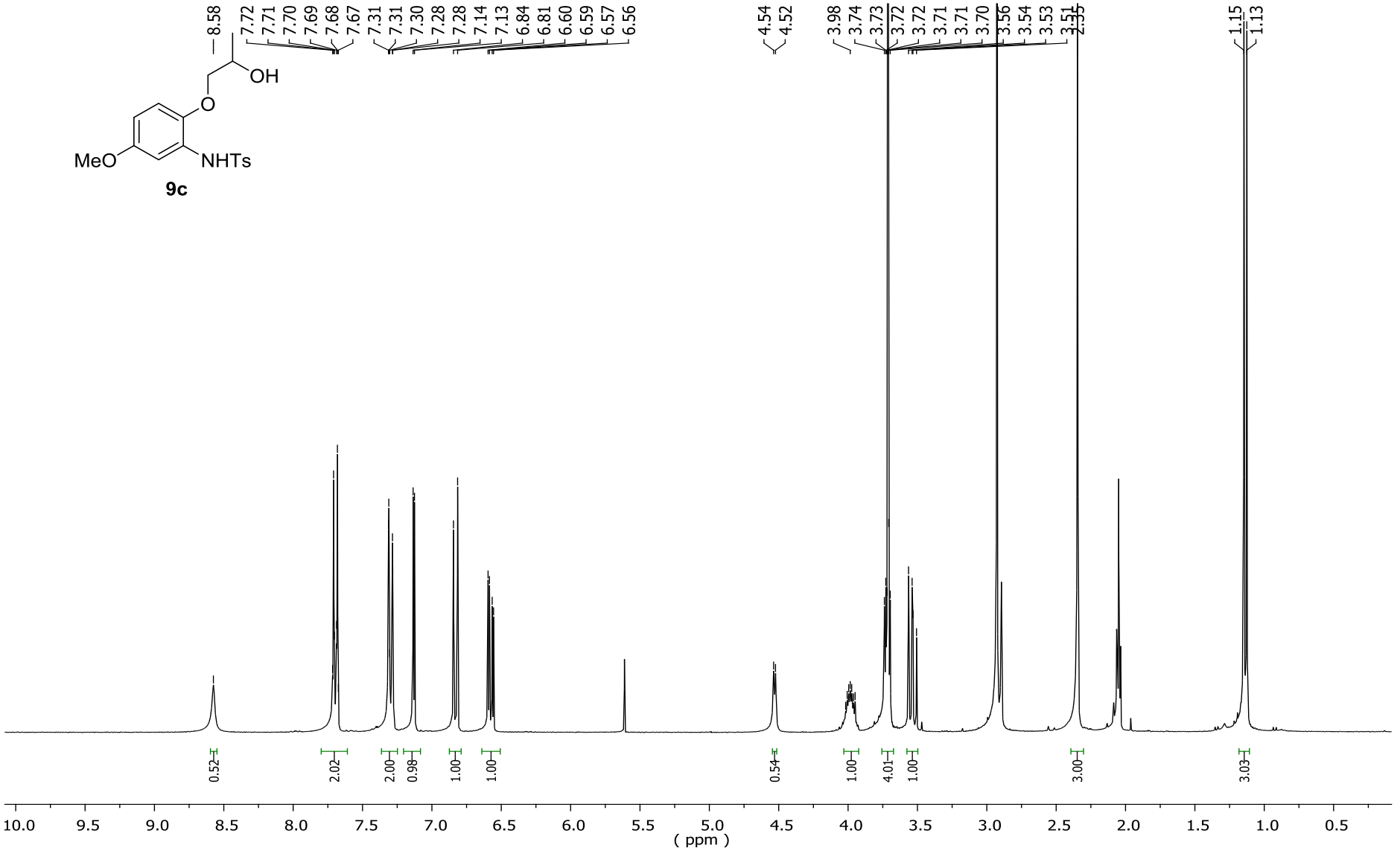

8.74
7.72
7.69
7.32
7.29
7.29
6.91
6.90
6.88
6.82
6.81
6.79
6.79
6.78
6.78
6.76
6.75
4.69
4.05
4.04
4.03
4.02
4.01
4.01
4.00
3.99
3.97
3.96
3.79
3.78
3.76
3.75
3.64
3.61
3.60
3.58
2.32
1.16
1.14
OH
O
F
NHTs
9b
0.48
1.88
2.92
1.03
1.00
0.48
1.00
0.99
1.09
3.04
3.09
(ppm)


OH
O
F
NHTs
9b
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)

OH
O
F
NHTs

9b


170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)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7.72
7.71
7.70
7.69
7.68
7.67
7.31
7.31
7.30
7.28
7.28
7.14
7.13
6.84
6.81
6.60
6.59
6.57
6.56
4.54
4.52
3.98
3.74
3.73
3.72
3.72
3.71
3.71
3.70
3.56
3.54
3.53
3.51
2.35
1.15
1.13
OH
O
MeO
NHTs
9c
0.52
2.02
2.00
0.98
1.00
1.00
0.54
1.00
4.01
1.00
3.00
3.03
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
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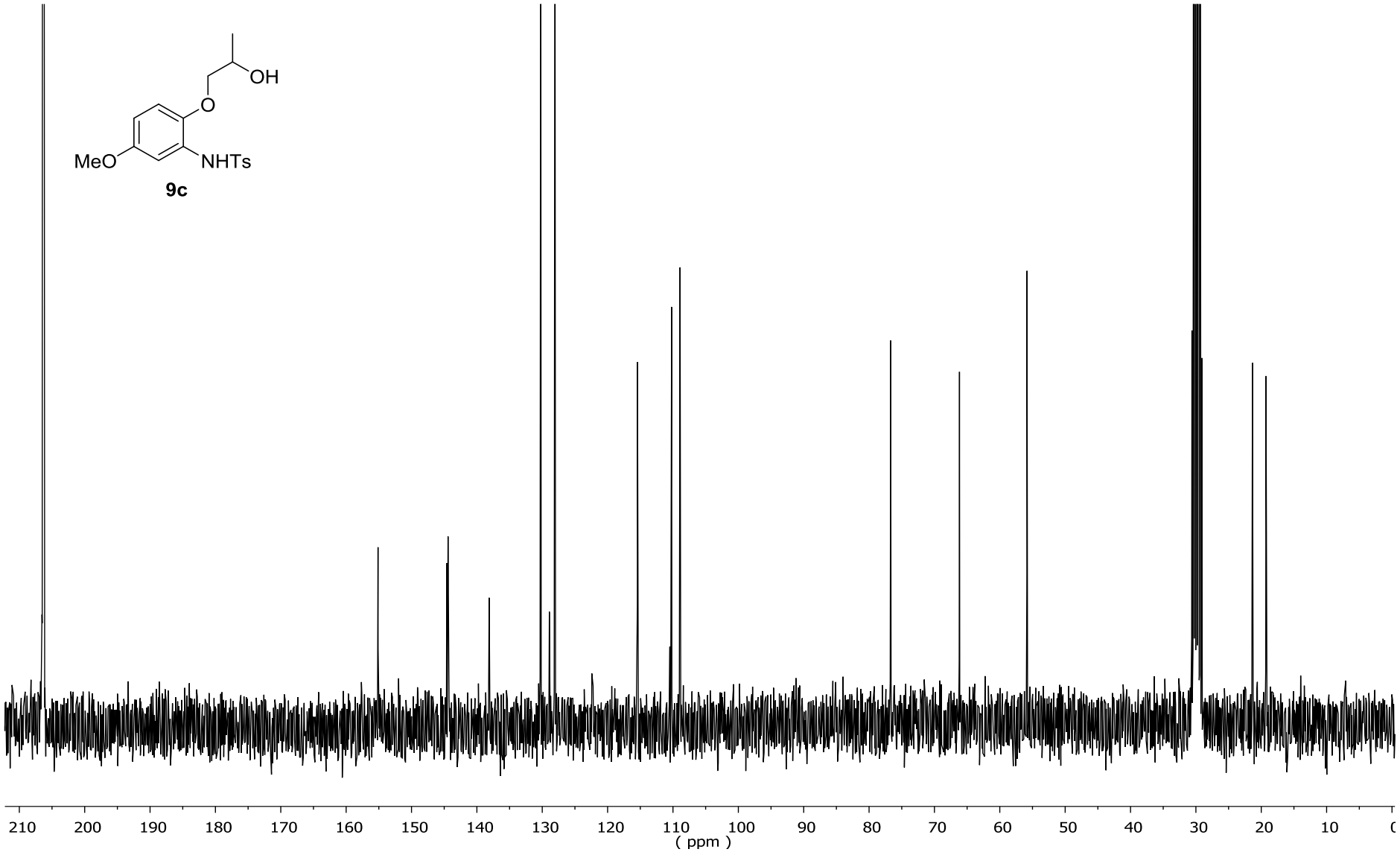
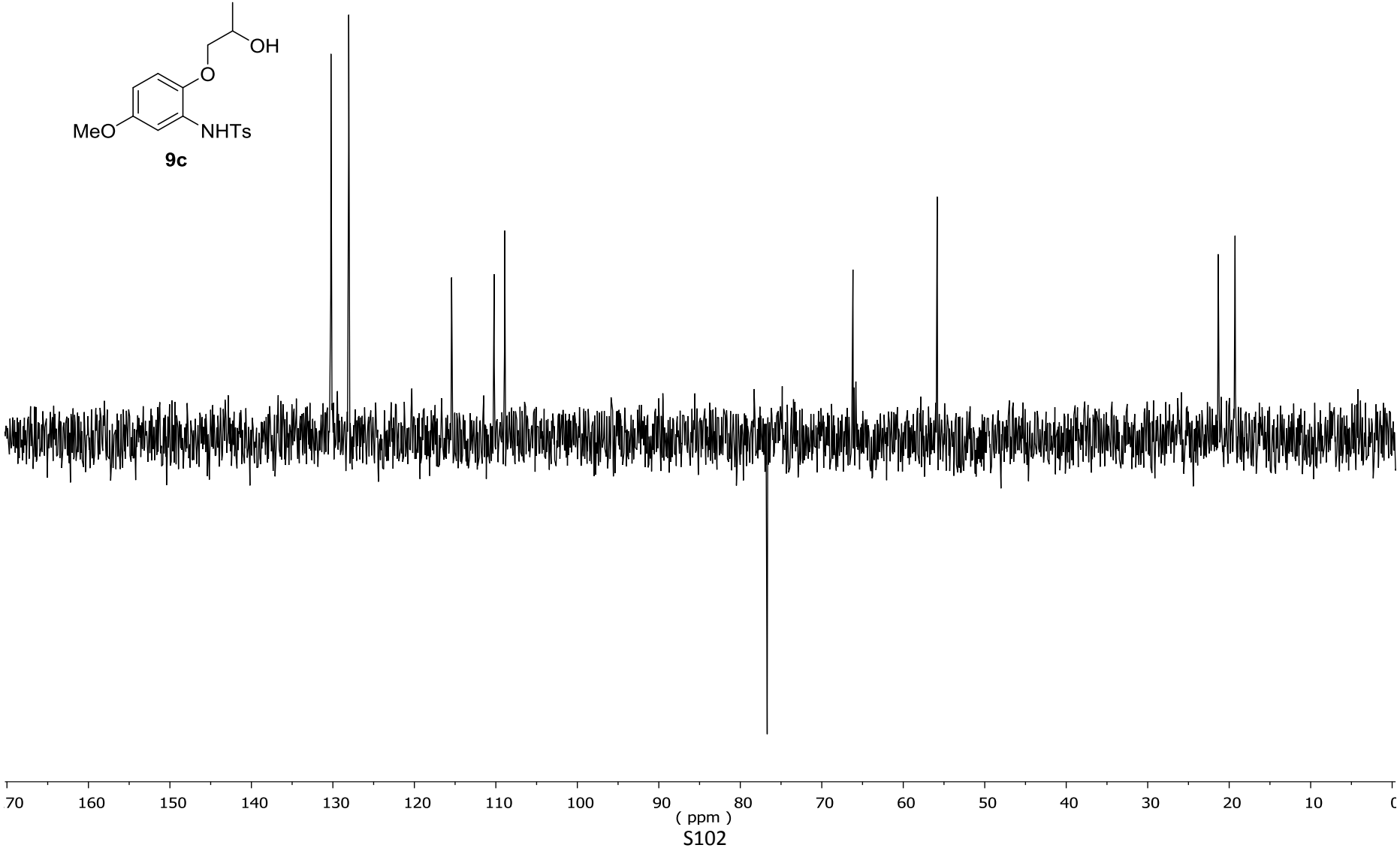
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
(ppm)


OH
O
MeO
NHTs
9c
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)

OH
O
MeO
NHTs

9c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160
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140
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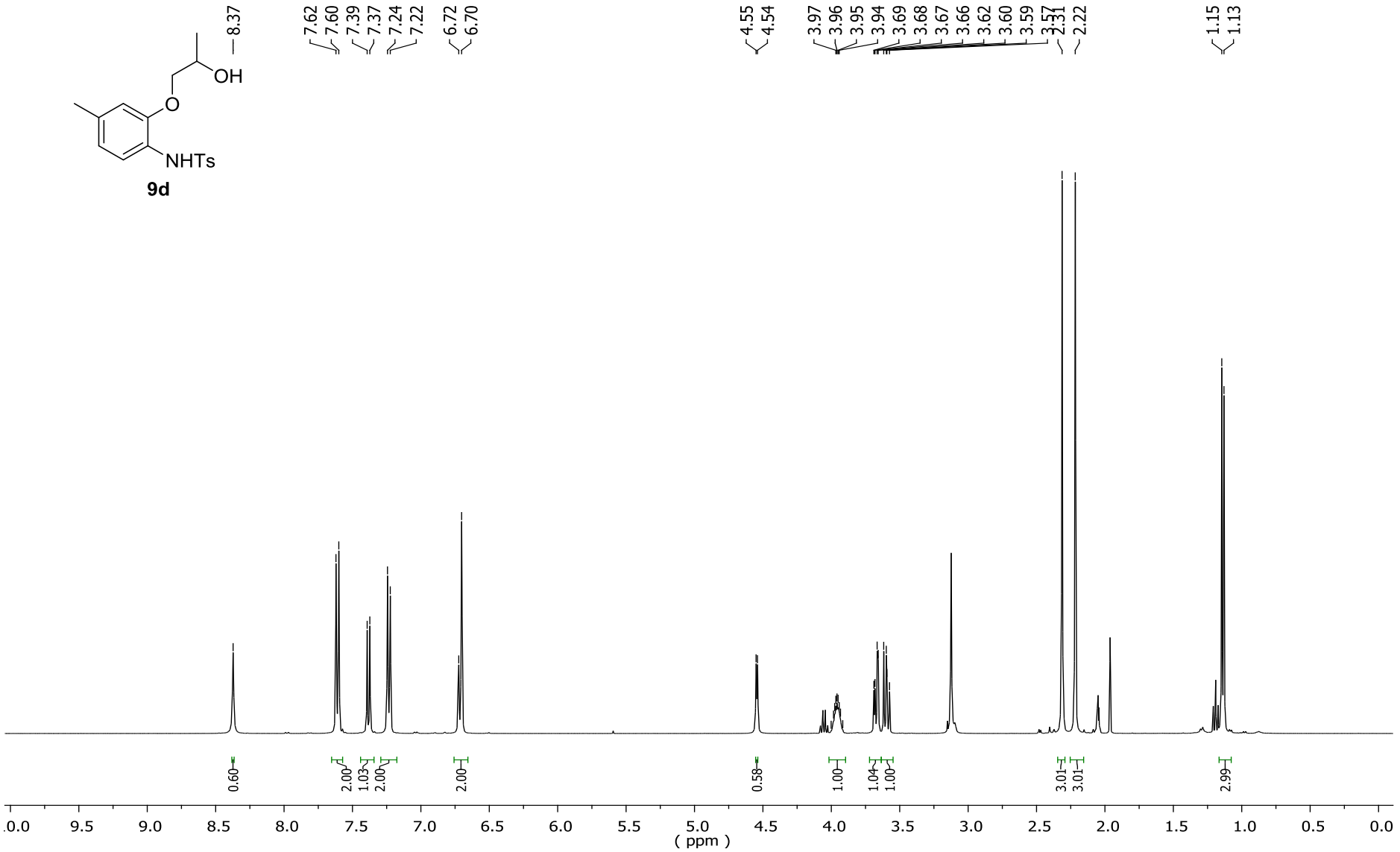
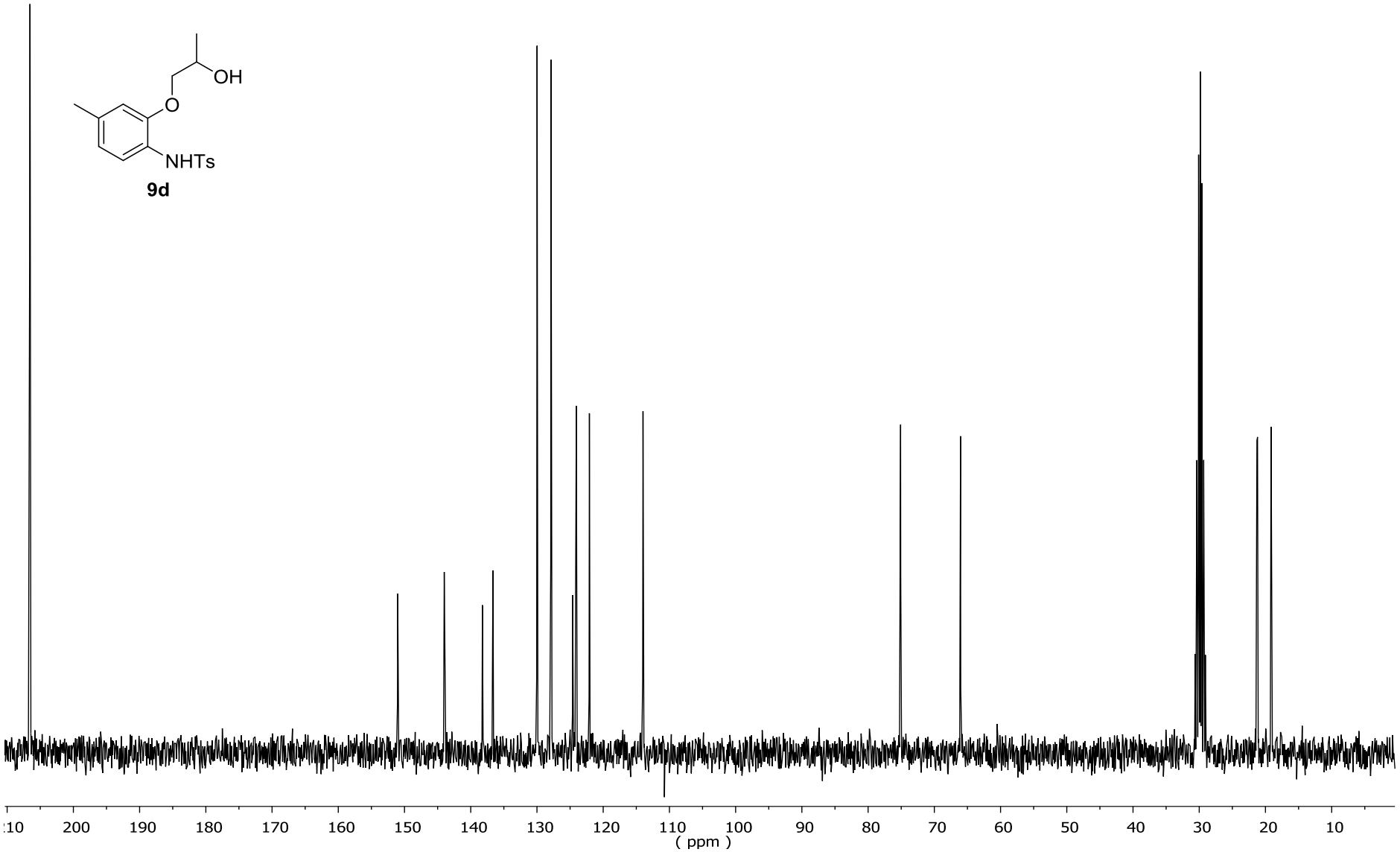
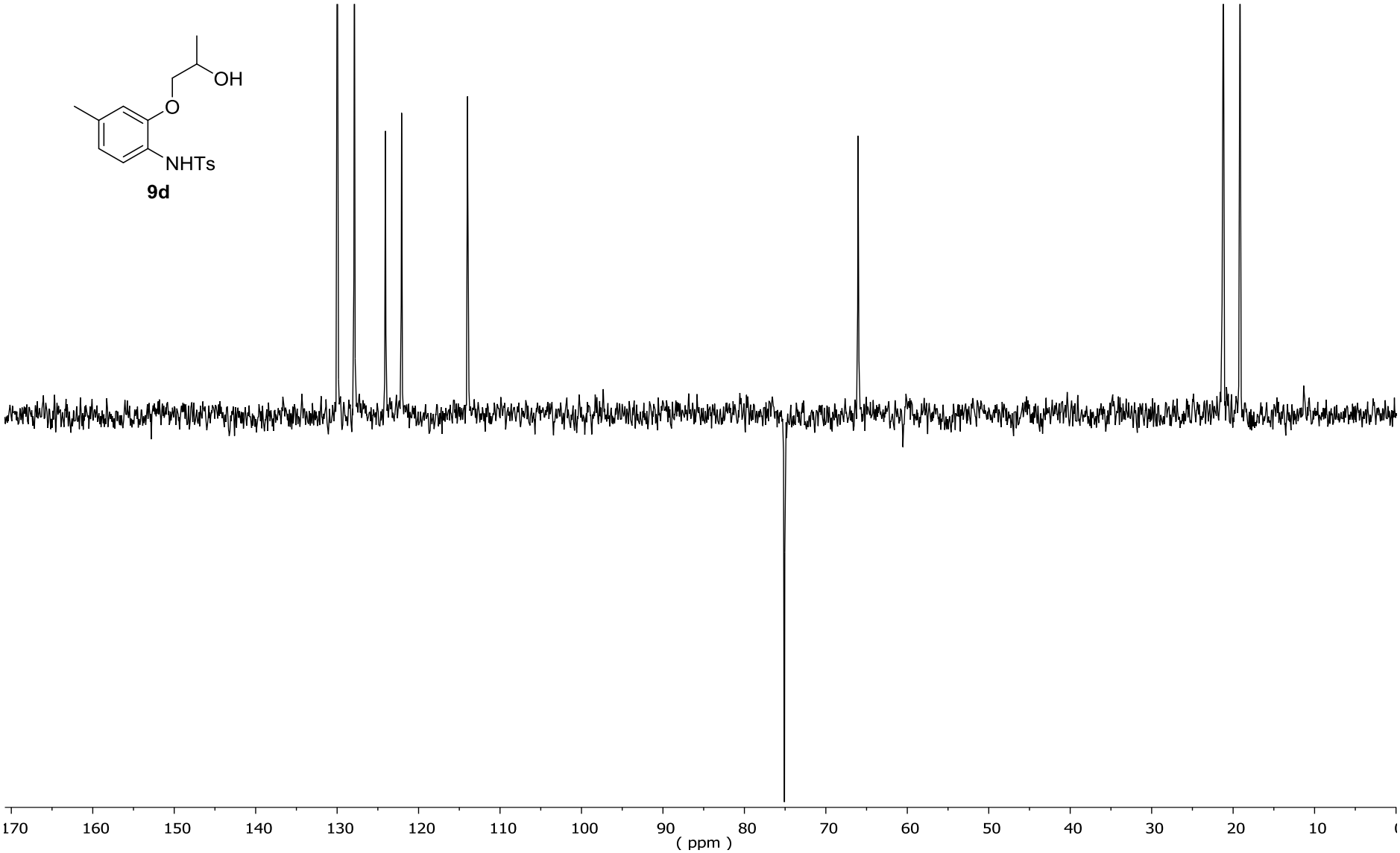
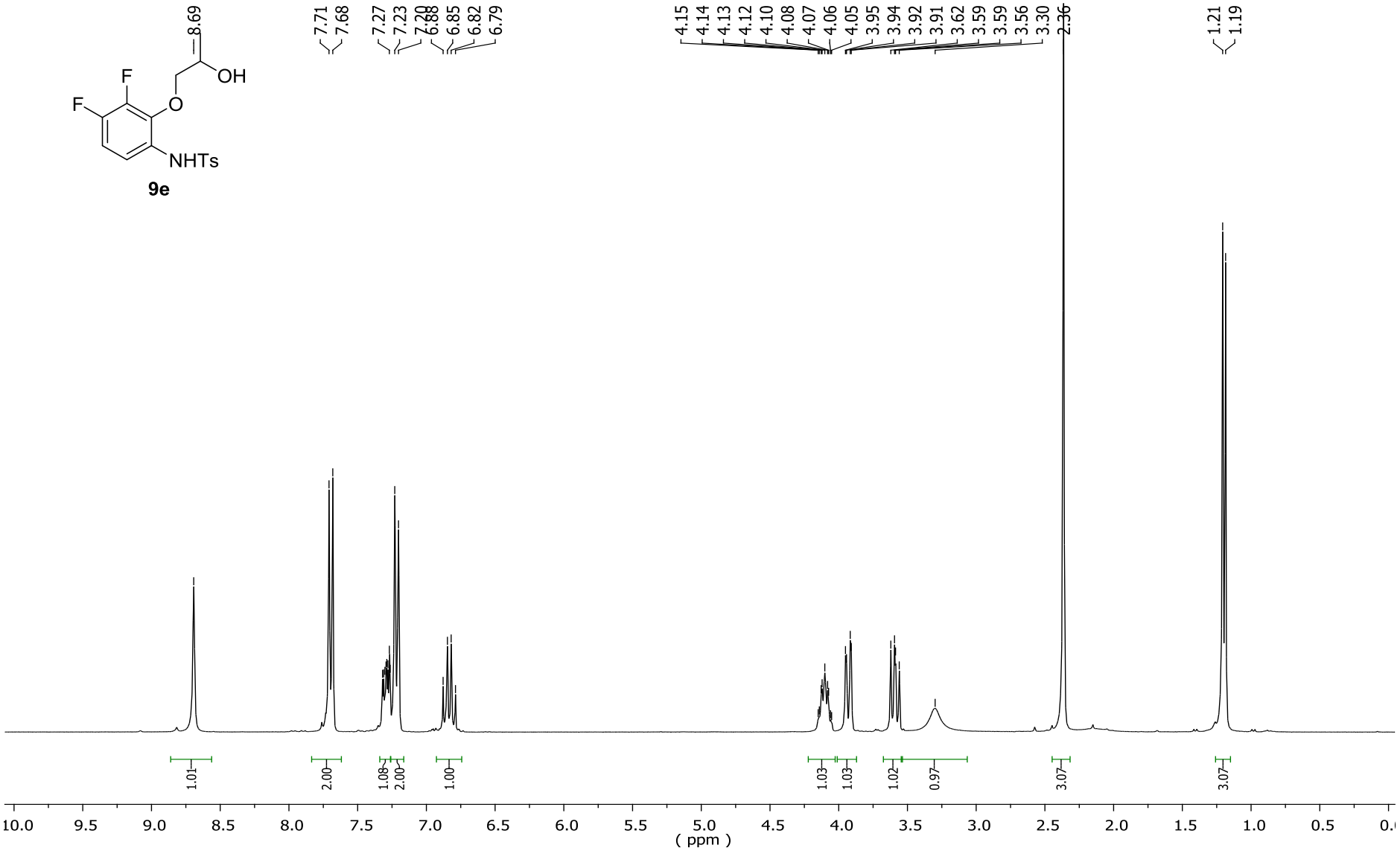
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)


8.37
7.62
7.60
7.39
7.37
7.24
7.22
6.72
6.70
4.55
4.54
3.97
3.96
3.95
3.94
3.69
3.68
3.67
3.66
3.62
3.60
3.59
3.57
2.31
2.22
1.15
1.13
OH
O
NHTs
9d
0.60
2.00
1.03
2.00
2.00
0.58
1.00
1.04
1.00
3.01
3.01
2.99
(ppm)

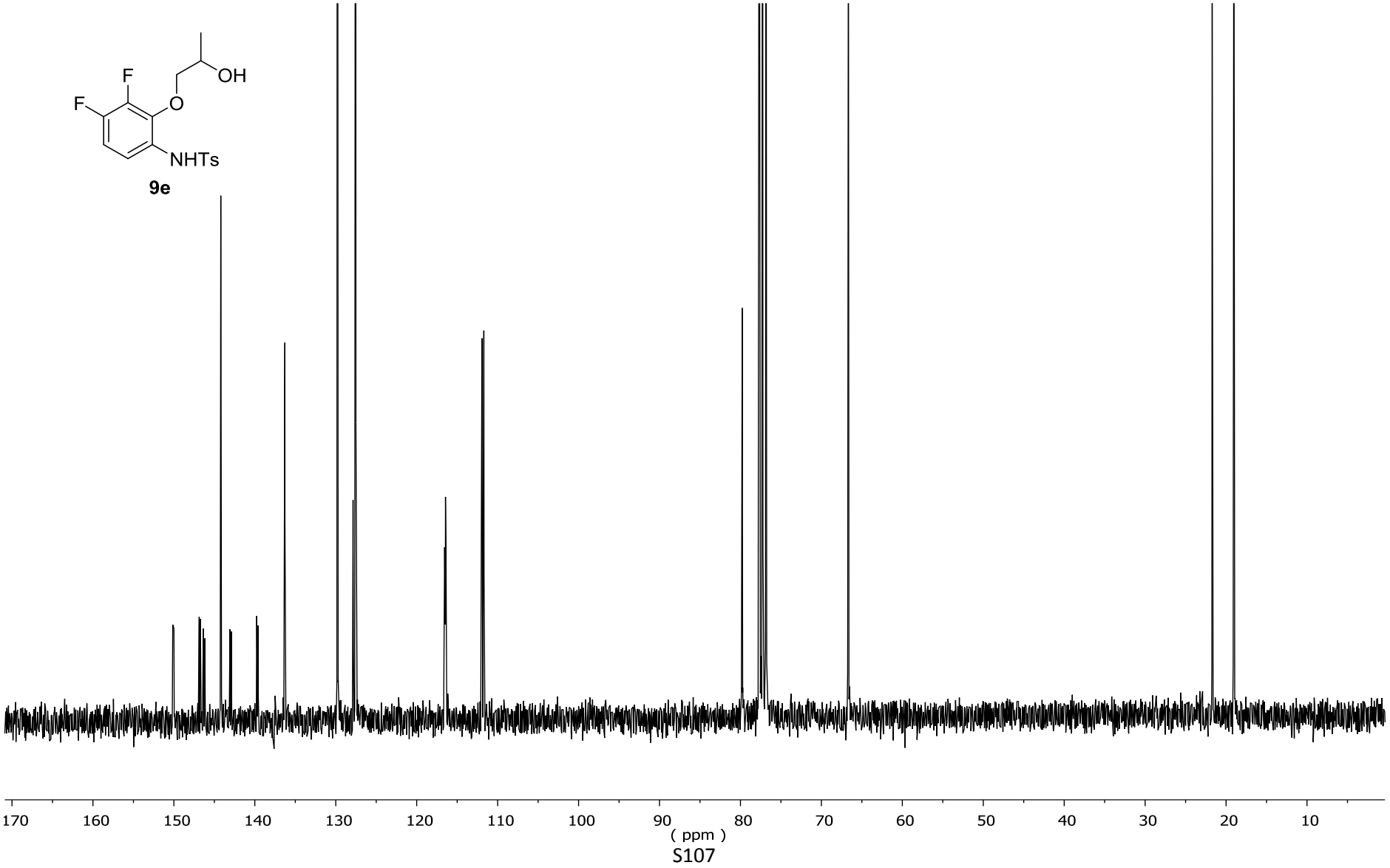

OH
O
NHTs
9d
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)


OH
O
NHTs
9d
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)


F
F
O
OH
NHTs
9e
8.69
7.71
7.68
7.27
7.23
7.20
6.88
6.85
6.82
6.79
4.15
4.14
4.13
4.12
4.10
4.08
4.07
4.06
4.05
3.95
3.94
3.92
3.91
3.62
3.59
3.59
3.56
3.30
2.36
1.21
1.19
1.01
2.00
1.08
2.00
1.00
1.03
1.03
1.02
0.97
3.07
3.07
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
(ppm)

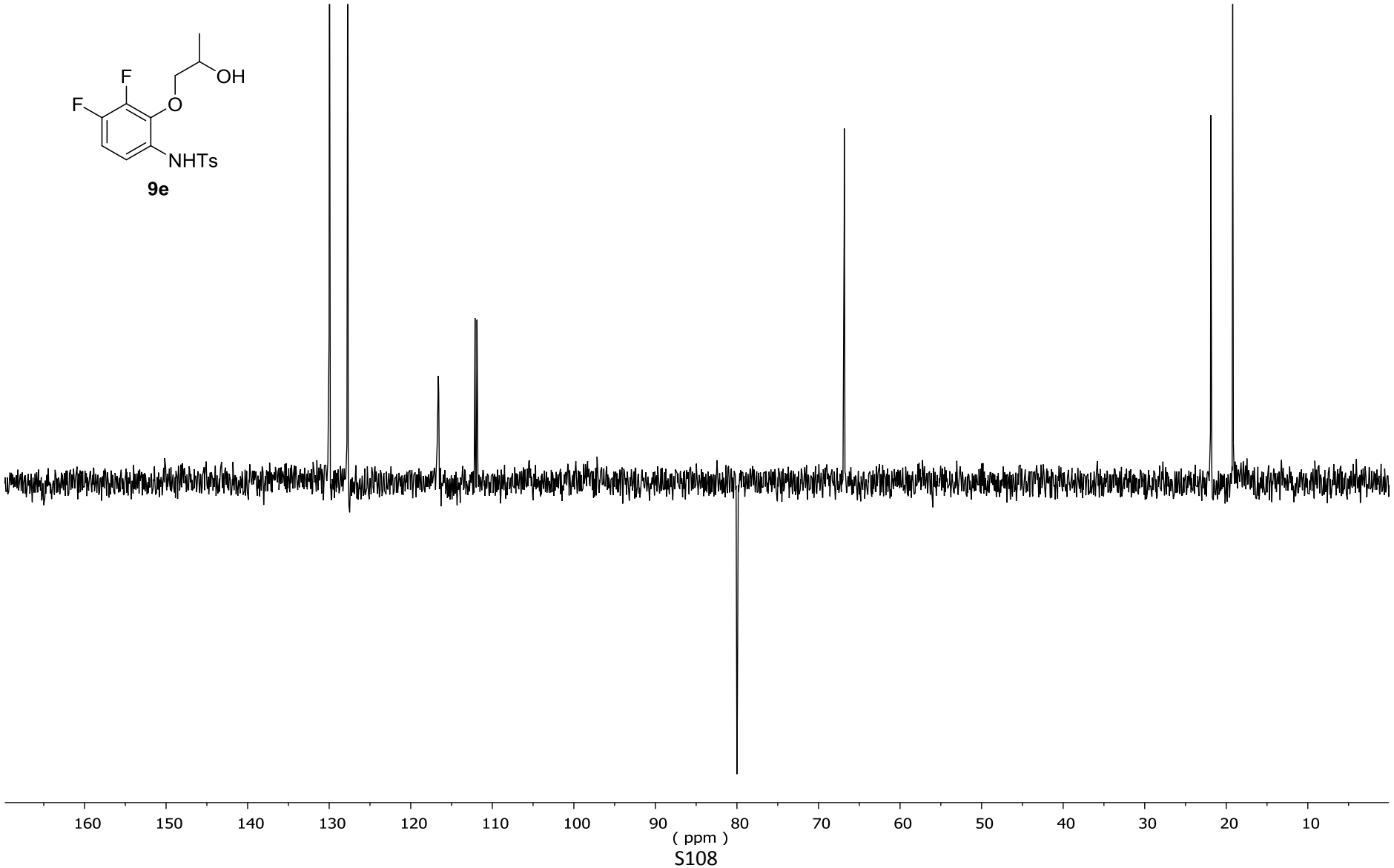
F
OH
F
O
NHTs

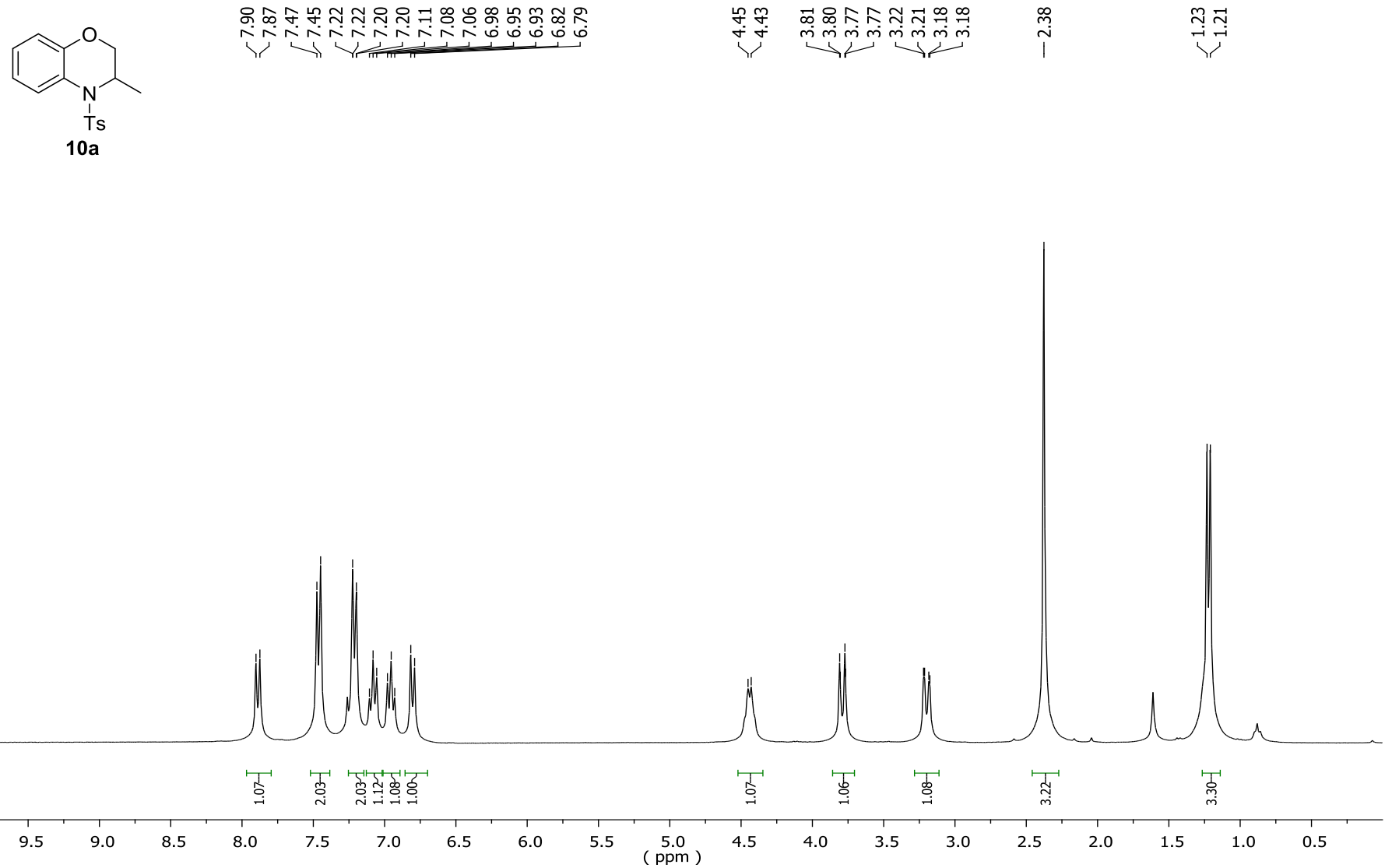
9e


170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)

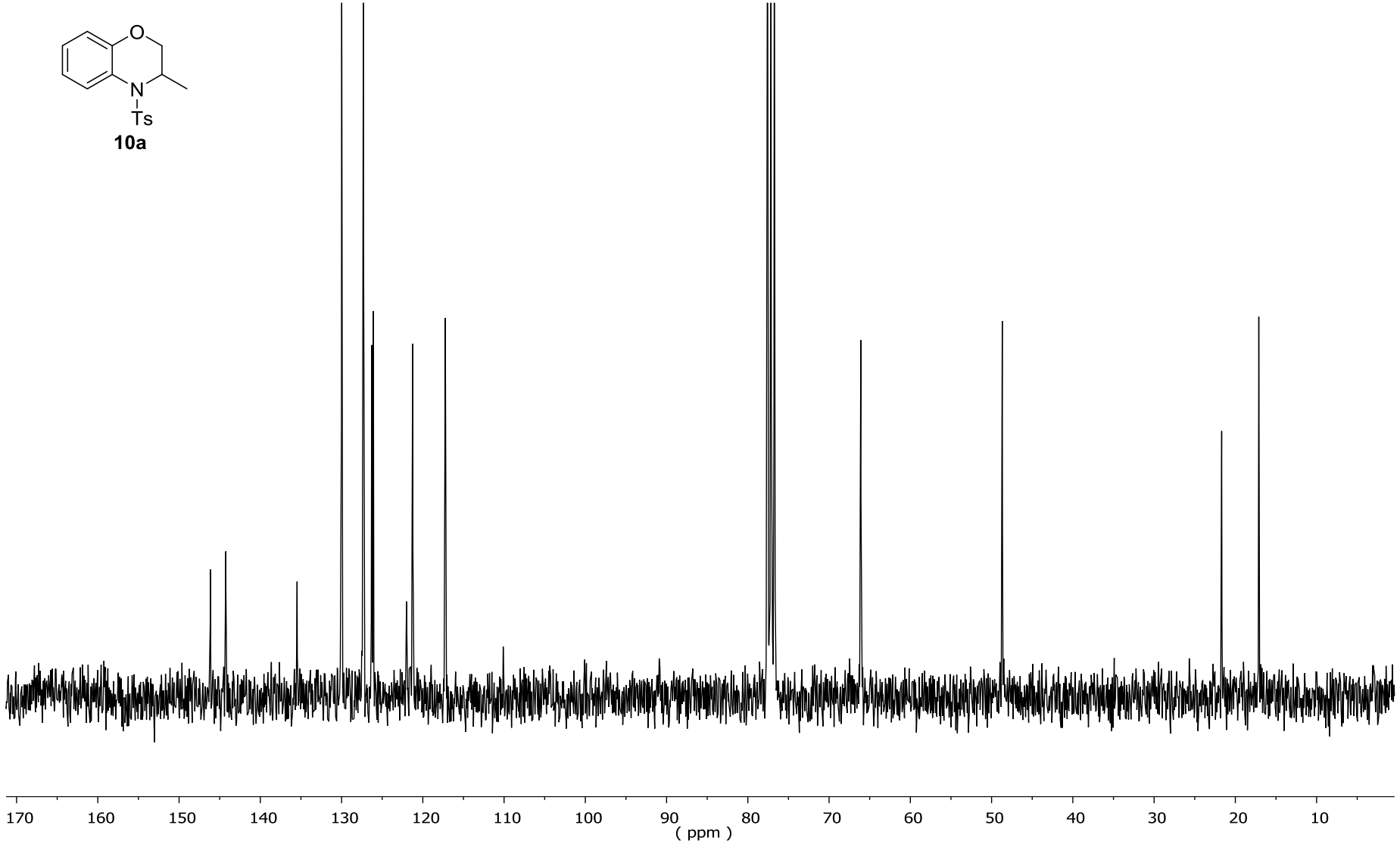
F
OH
F
O
NHTs

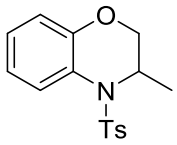
9e


160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)

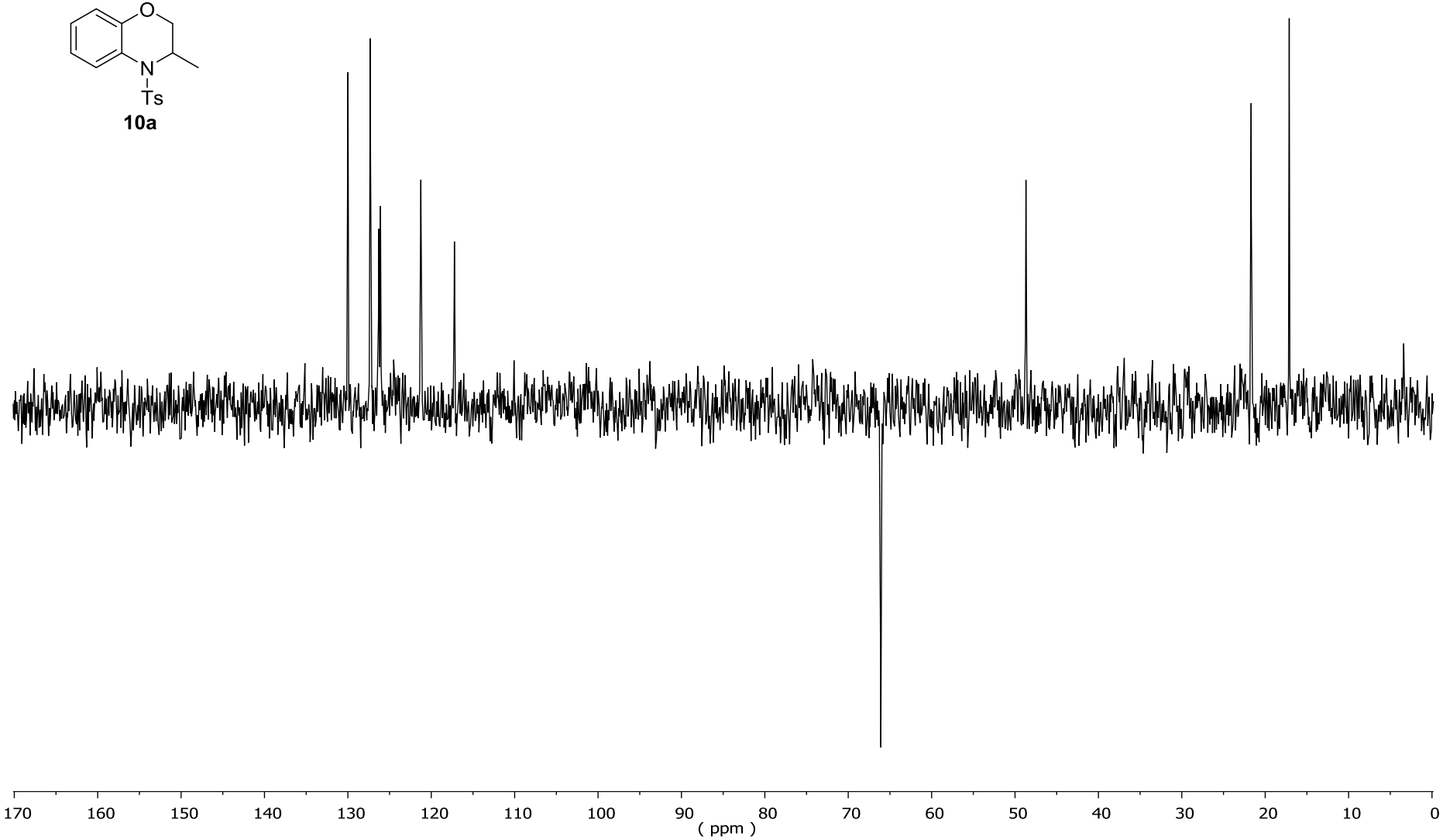

O
N
Ts
10a
7.90
7.87
7.47
7.45
7.22
7.22
7.20
7.20
7.11
7.08
7.06
6.98
6.95
6.93
6.82
6.79
4.45
4.43
3.81
3.80
3.77
3.77
3.22
3.21
3.18
3.18
2.38
1.23
1.21
1.07
2.03
2.03
1.12
1.08
1.00
1.07
1.06
1.08
3.22
3.30
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
(ppm)

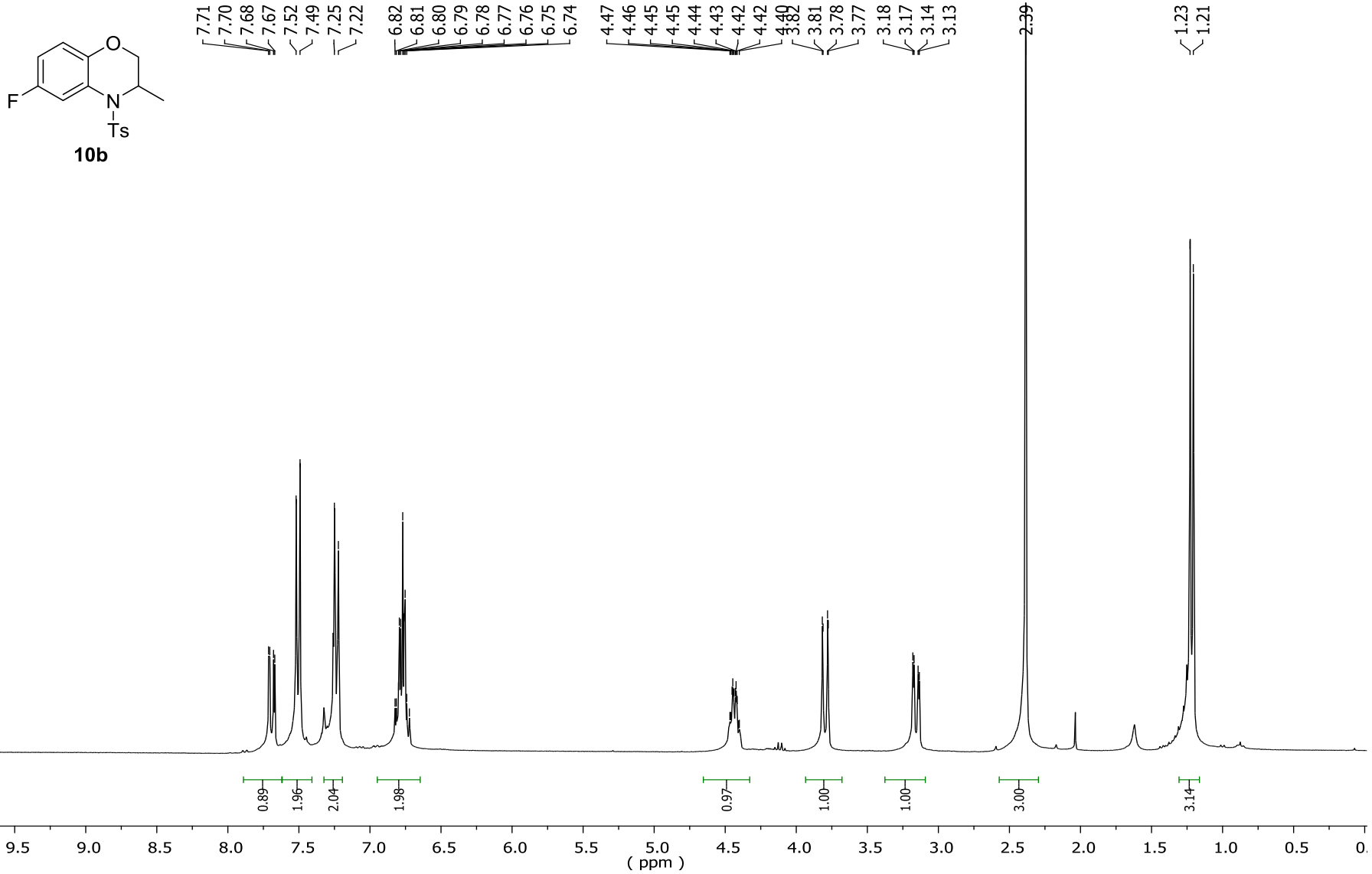
10a

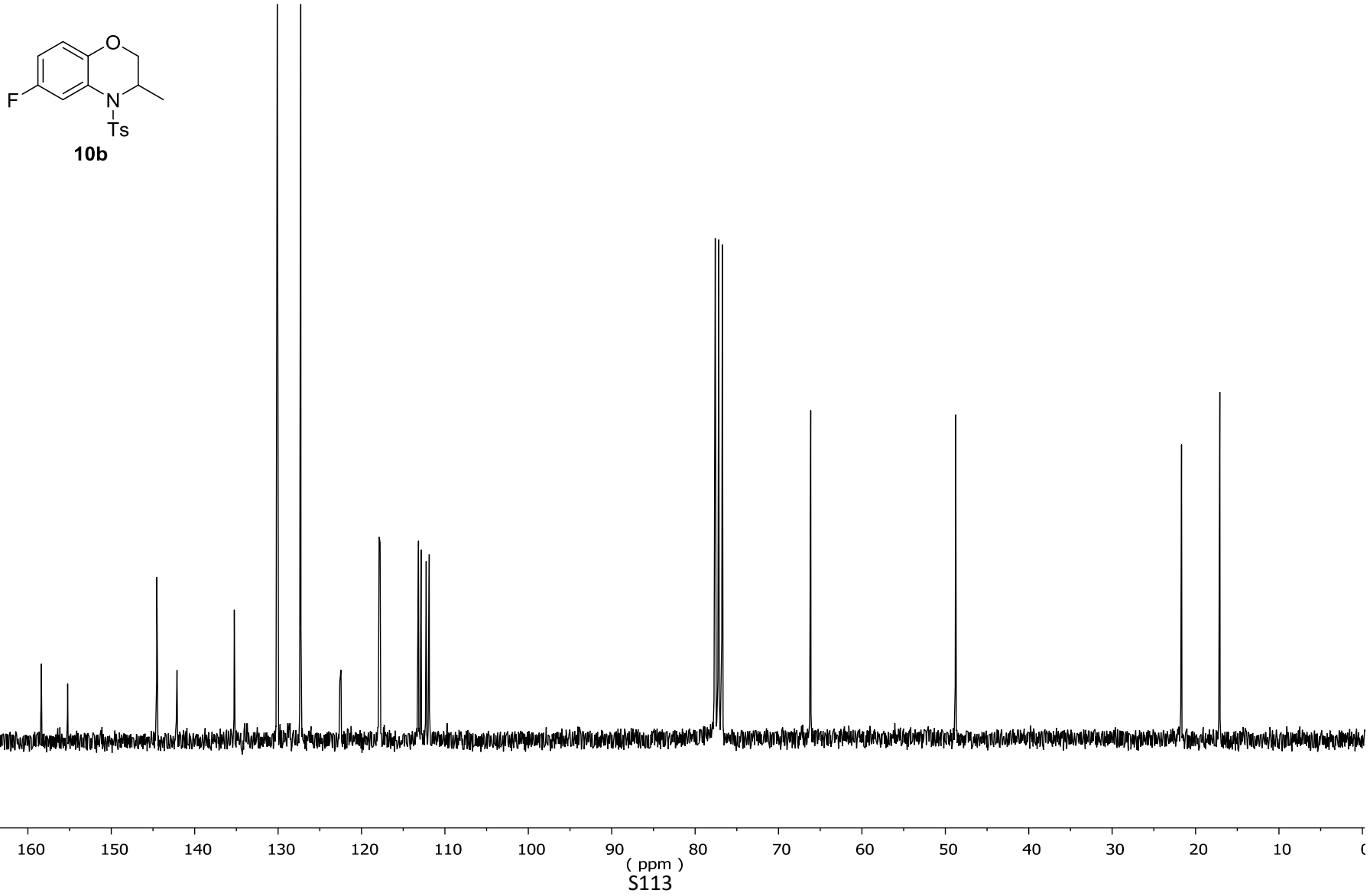


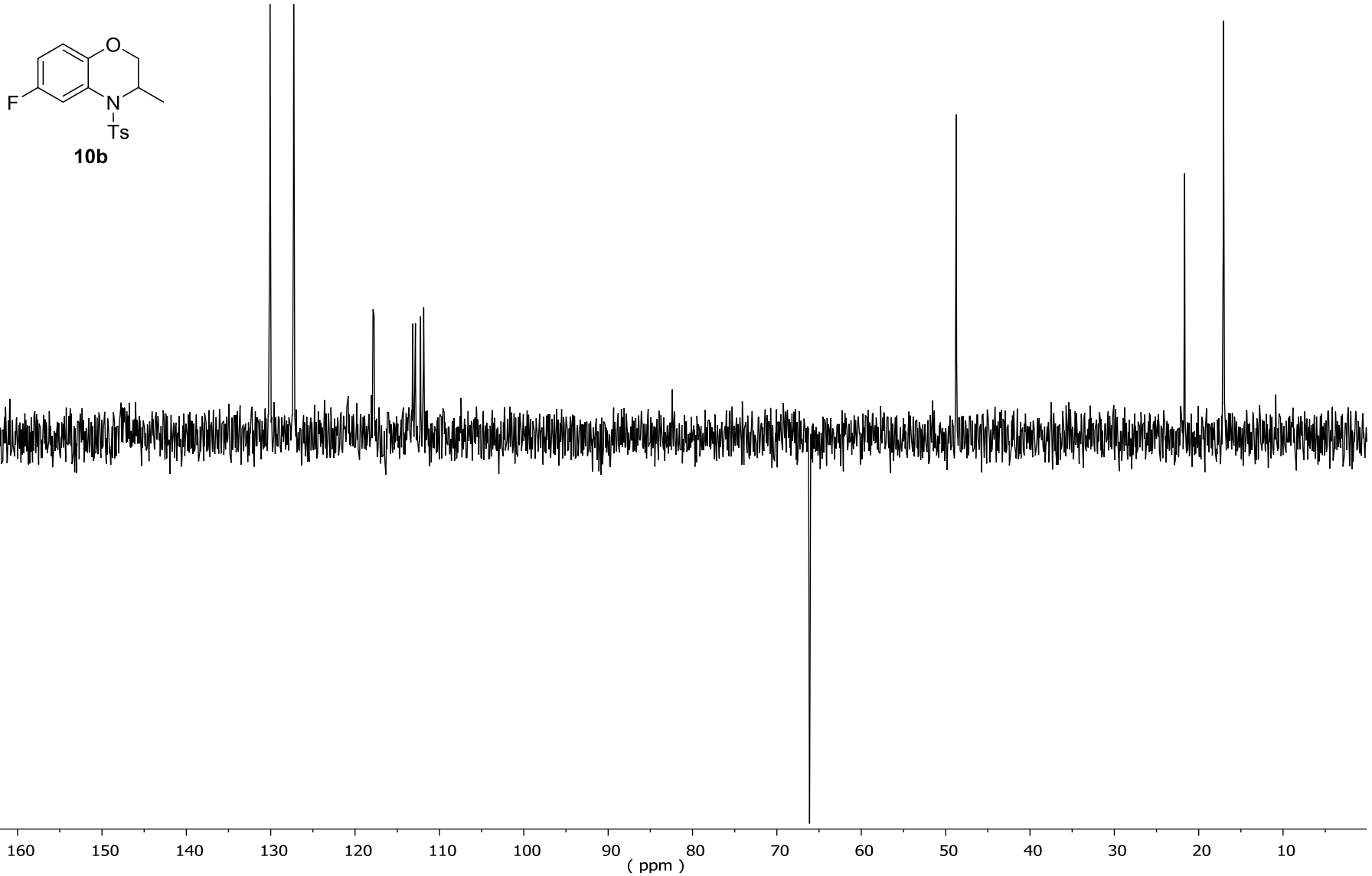

O
N
Ts

10a

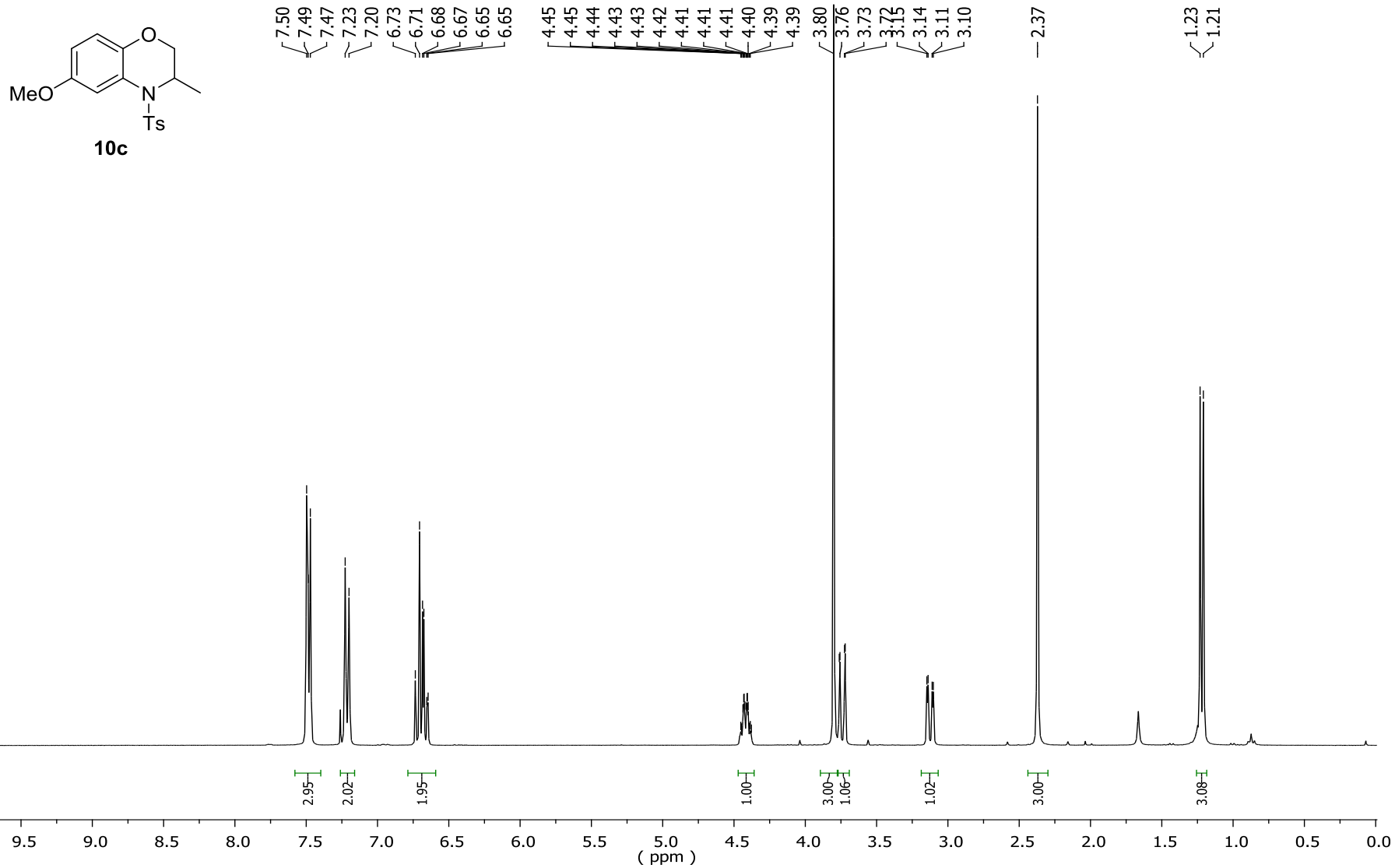

170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)


F
O
N
Ts
10b
7.71
7.70
7.68
7.67
7.52
7.49
7.25
7.22
6.82
6.81
6.80
6.79
6.78
6.77
6.76
6.75
6.74
4.47
4.46
4.45
4.45
4.44
4.43
4.42
4.42
4.40
3.82
3.81
3.78
3.77
3.18
3.17
3.14
3.13
2.39
1.23
1.21
0.89
1.96
2.04
1.98
0.97
1.00
1.00
3.00
3.14
(ppm)


O
F
N
Ts
10b
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)



10b


MeO
O
N
Ts
10c
7.50
7.49
7.47
7.23
7.20
6.73
6.71
6.68
6.67
6.65
6.65
4.45
4.45
4.44
4.43
4.43
4.42
4.41
4.41
4.41
4.40
4.39
4.39
3.80
3.76
3.73
3.15
3.14
3.11
3.10
2.37
1.23
1.21
2.95
2.02
1.95
1.00
3.00
1.06
1.02
3.00
3.08
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
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4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
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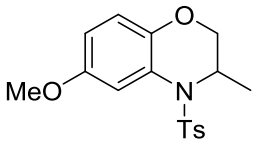
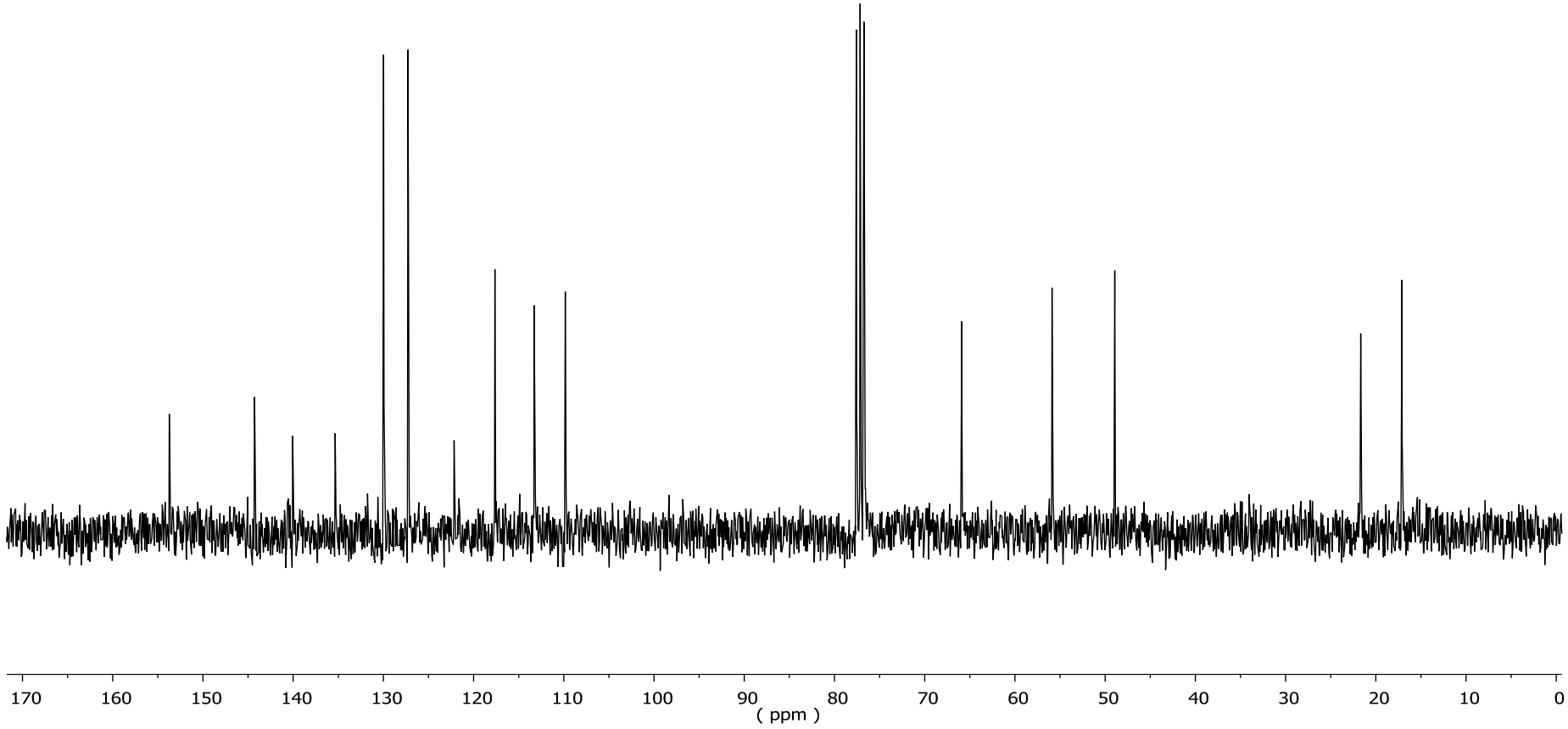
0.0
(ppm)


O
MeO
N
Ts

10c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160
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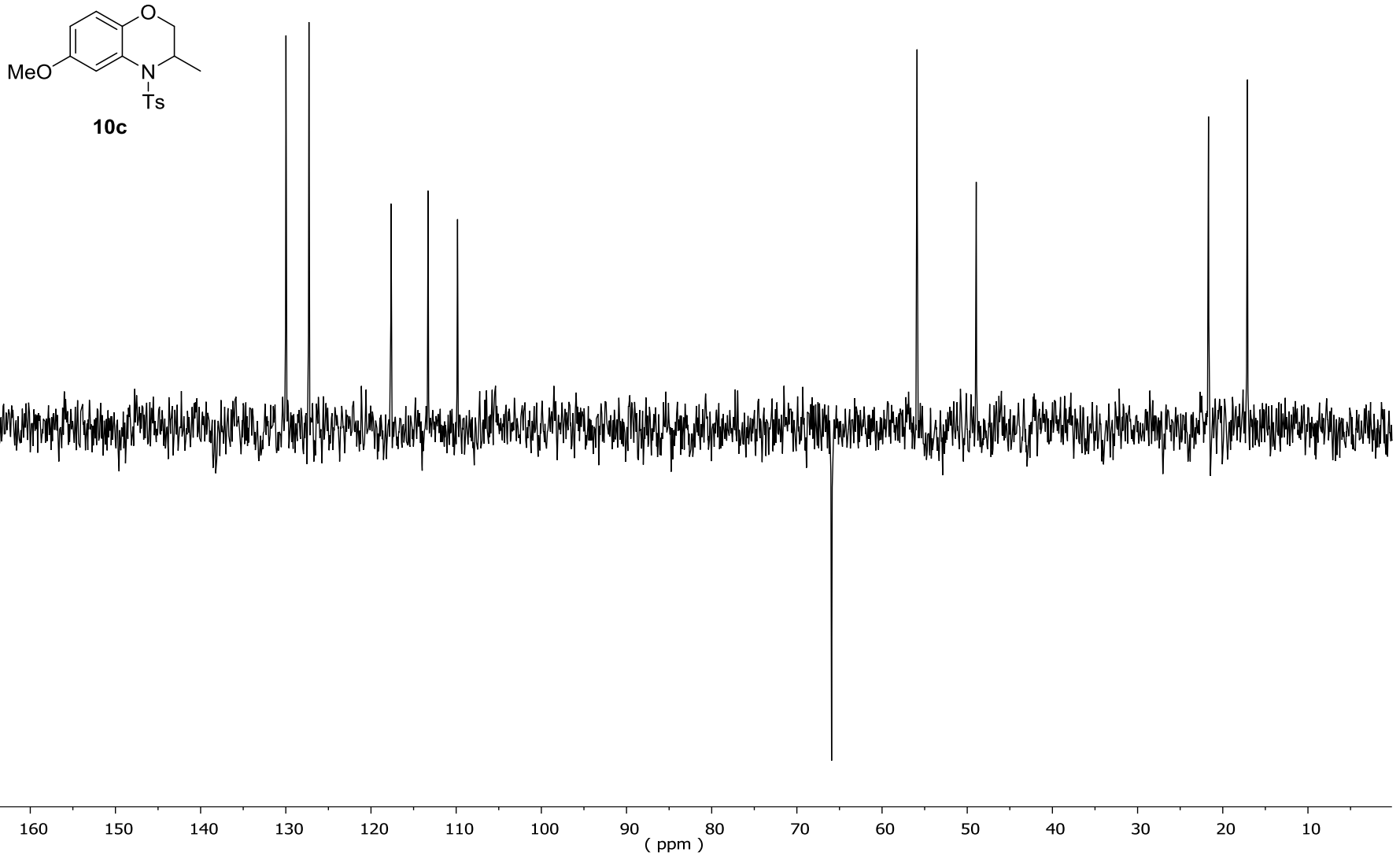
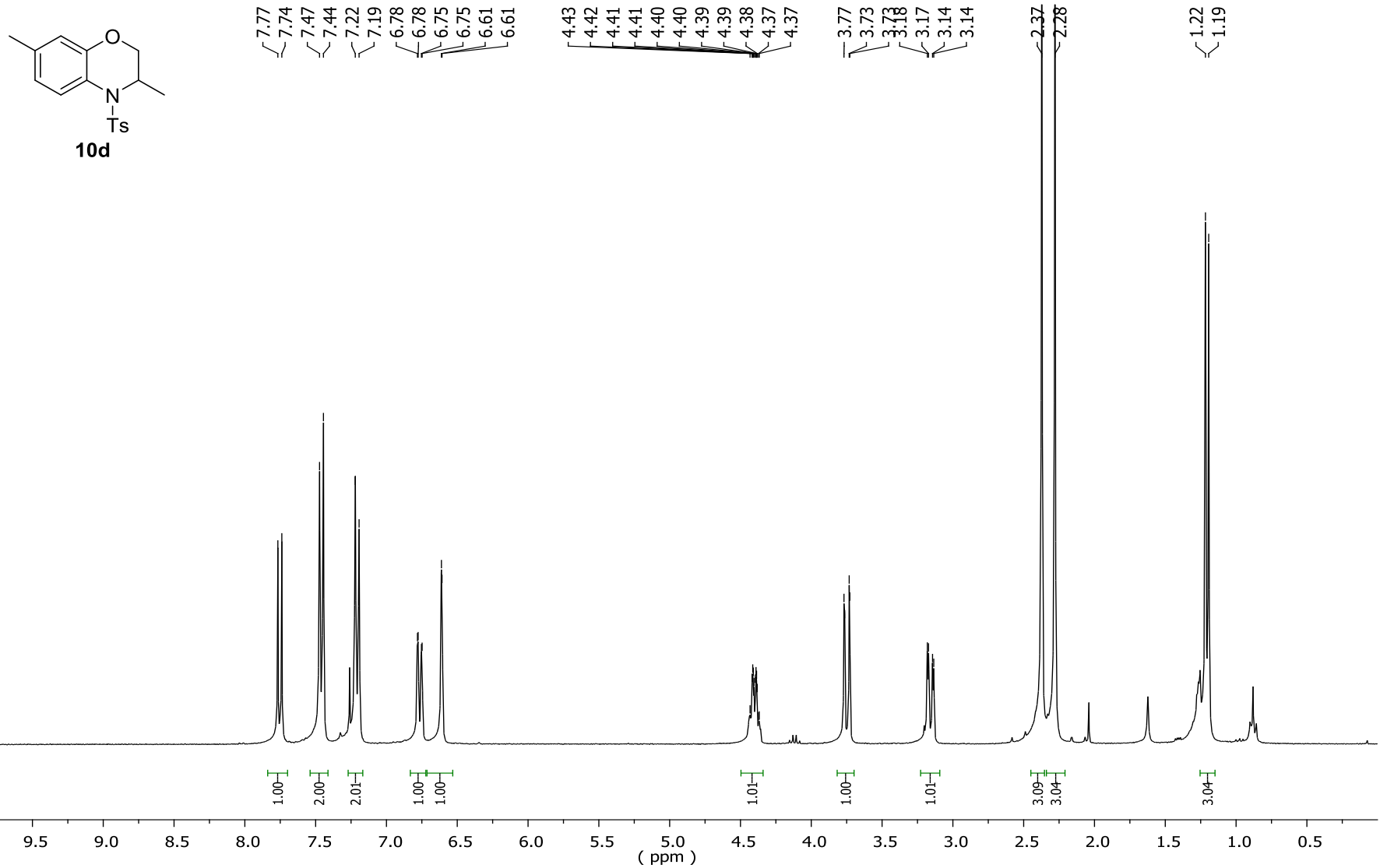
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)



10c


O
N
Ts
10d
7.77
7.74
7.47
7.44
7.22
7.19
6.78
6.78
6.75
6.75
6.61
6.61
4.43
4.42
4.41
4.41
4.40
4.40
4.39
4.39
4.38
4.37
4.37
3.77
3.73
3.73
3.18
3.17
3.14
3.14
2.37
2.28
1.22
1.19
1.00
2.00
2.01
1.00
1.00
1.01
1.00
1.01
3.09
3.04
3.04
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
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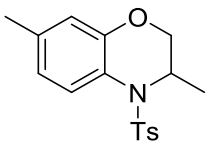
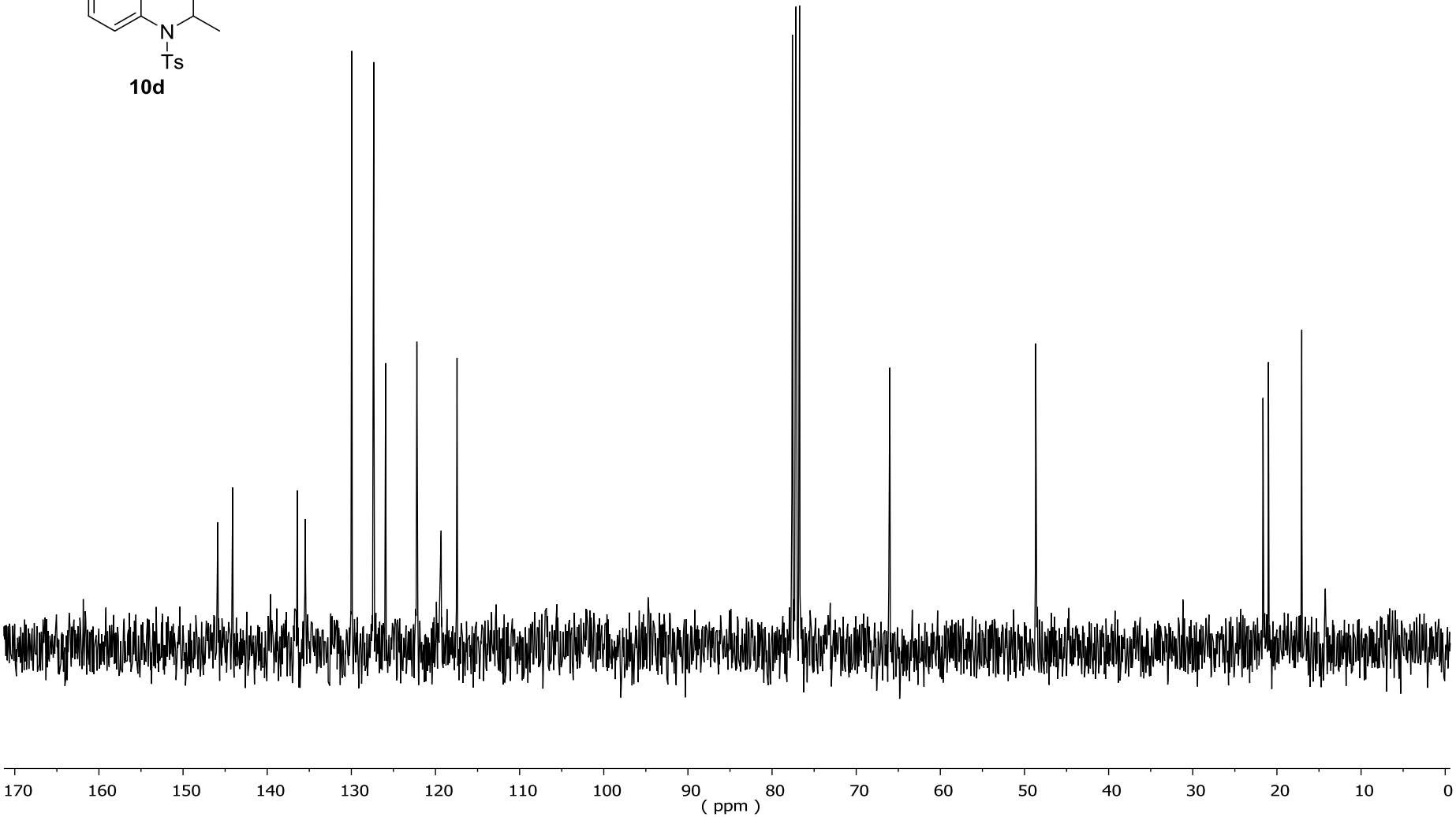
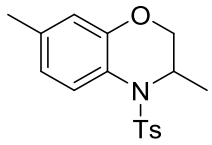
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
(ppm)

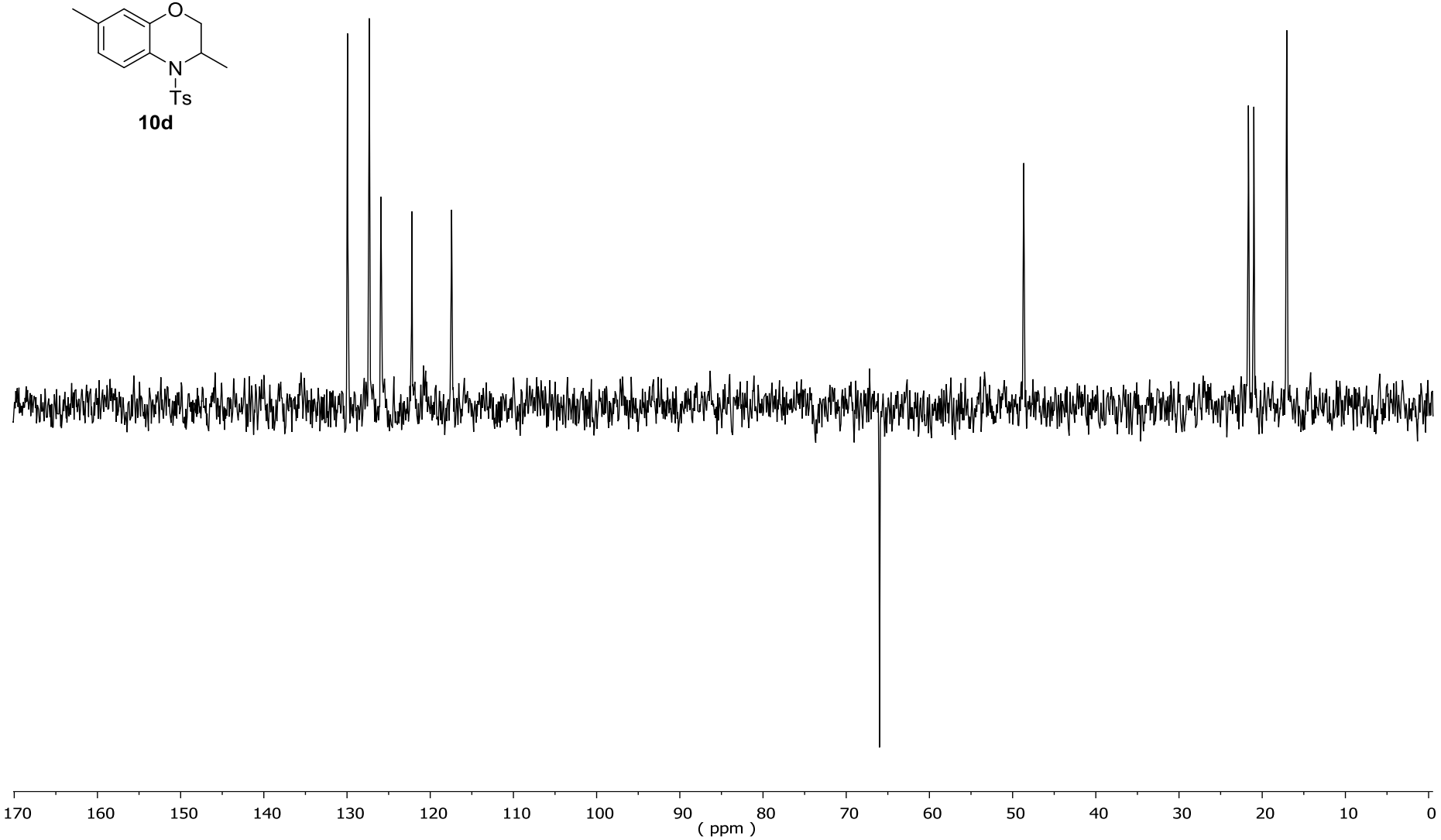

O
N
Ts

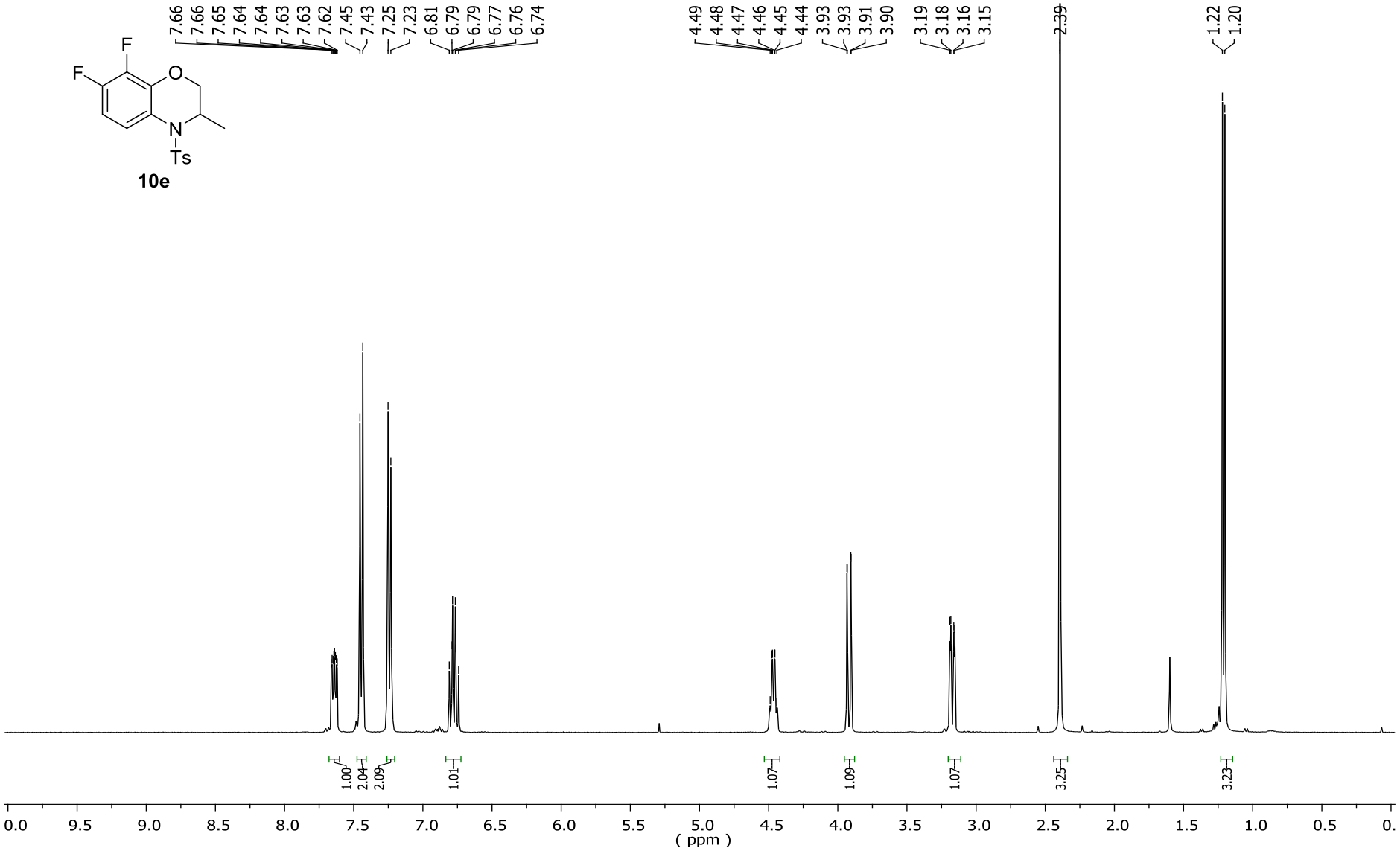
10d


170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)


O
N
Ts

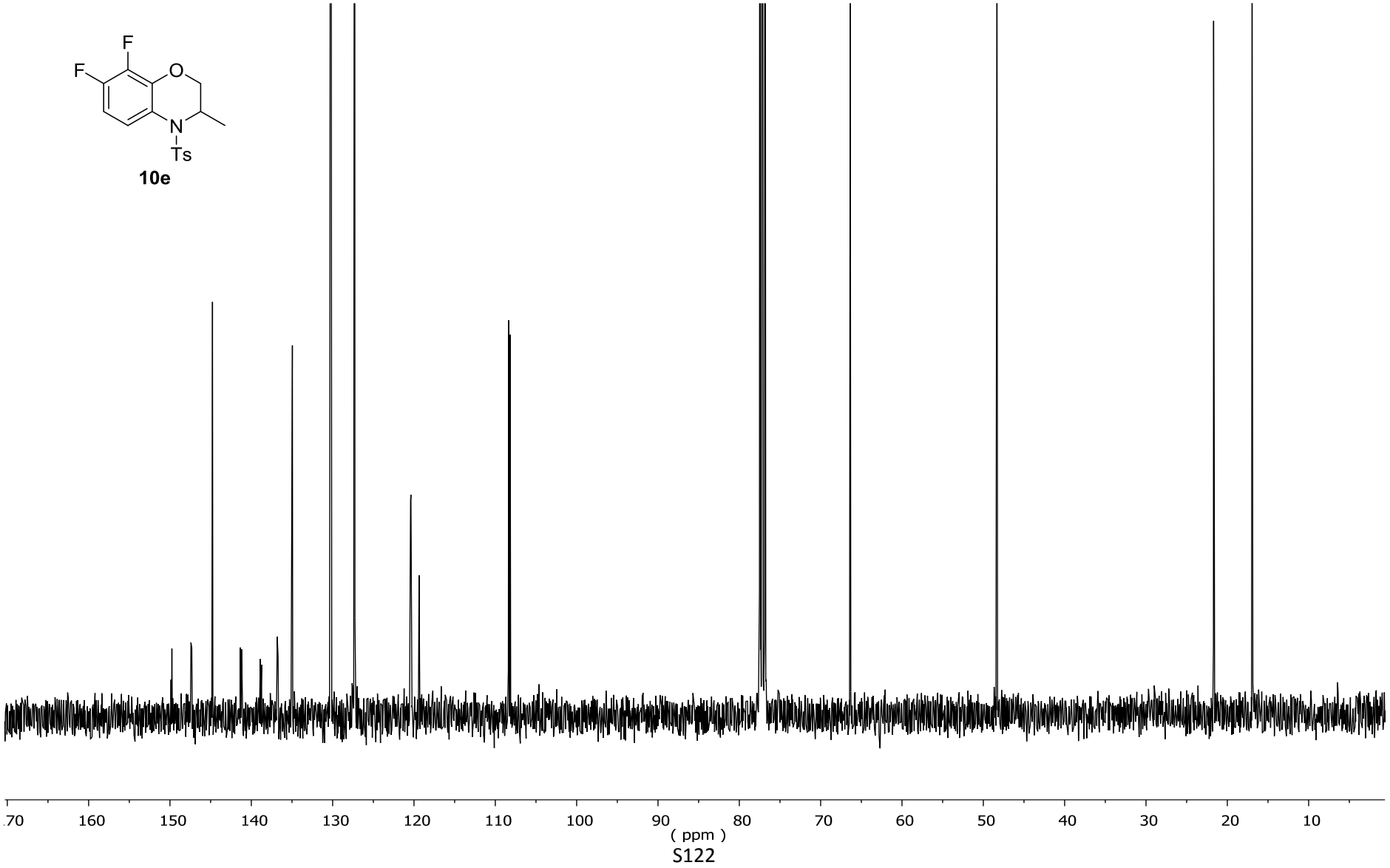
10d


170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
(ppm)

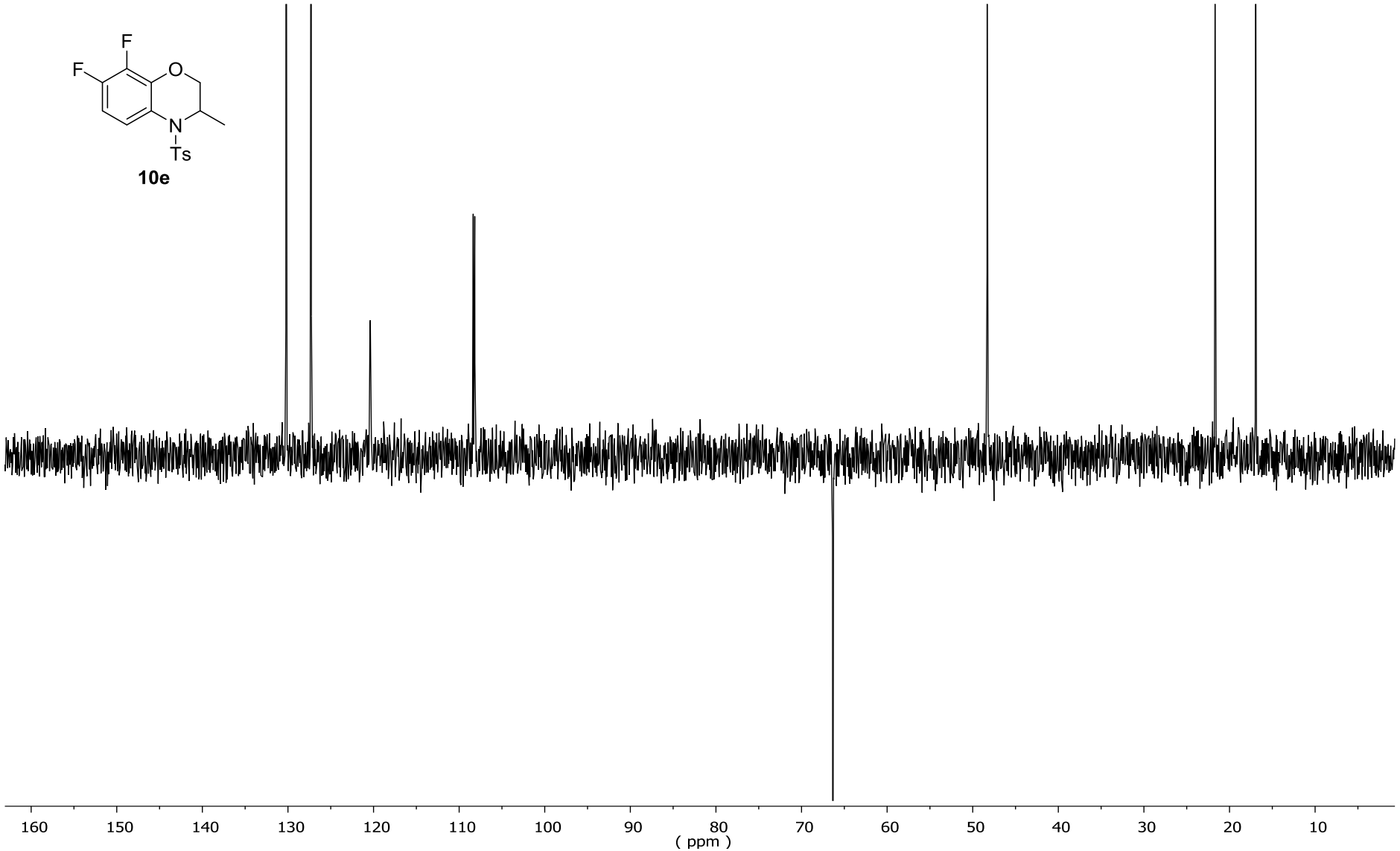

7.66
7.66
7.65
7.64
7.64
7.63
7.63
7.62
7.45
7.43
7.25
7.23
6.81
6.79
6.79
6.77
6.76
6.74
4.49
4.48
4.47
4.46
4.45
4.44
3.93
3.93
3.91
3.90
3.19
3.18
3.16
3.15
2.39
1.22
1.20
F
F
O
N
Ts
10e
1.00
2.04
2.09
1.01
1.07
1.09
1.07
3.25
3.23
0.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.
(ppm)

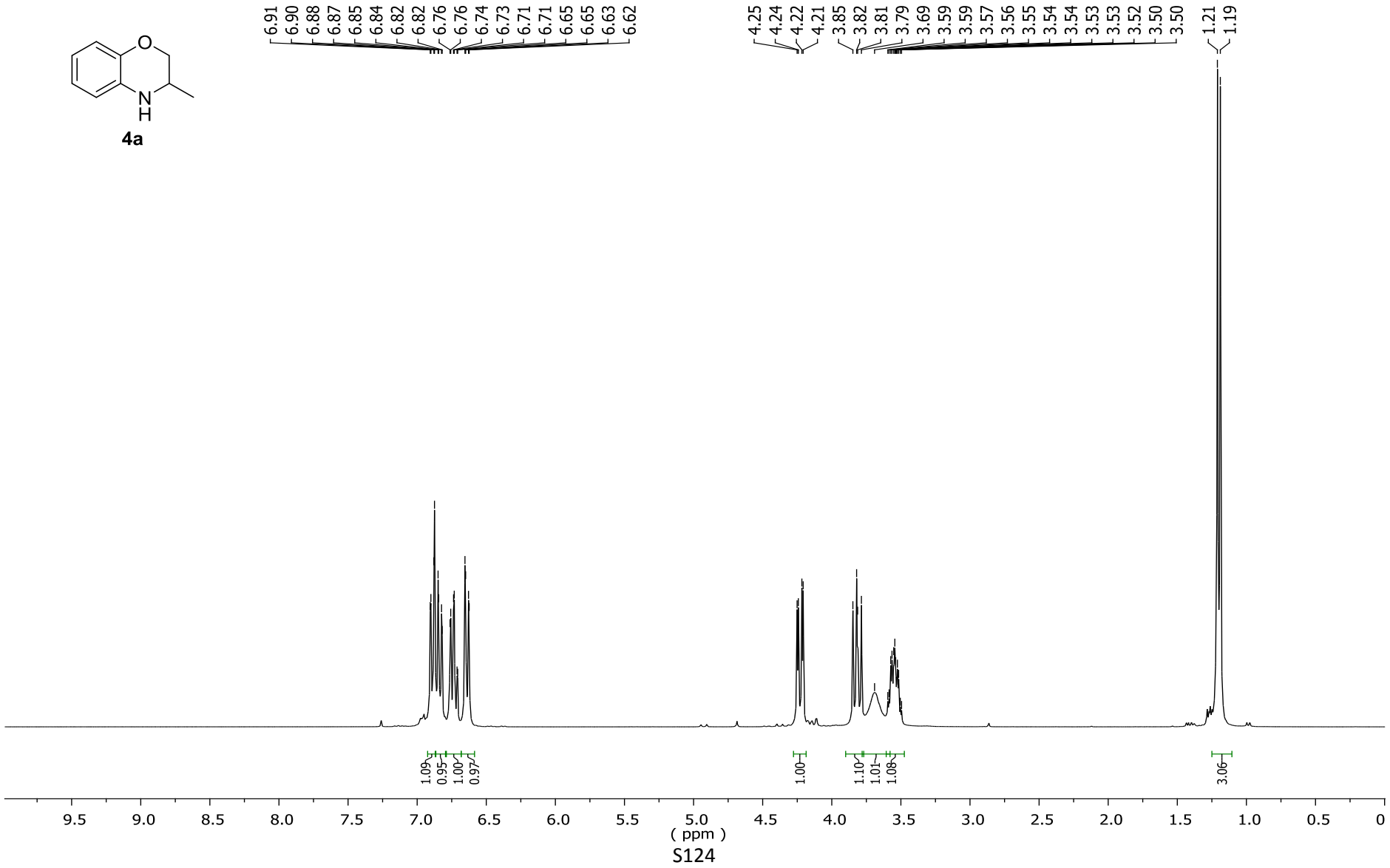
F
F
O
N
Ts

10e


70
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)

10e




6.91
6.90
6.88
6.87
6.85
6.84
6.82
6.82
6.76
6.76
6.74
6.73
6.71
6.71
6.65
6.65
6.63
6.62
4.25
4.24
4.22
4.21
3.85
3.82
3.81
3.79
3.69
3.59
3.59
3.57
3.56
3.55
3.54
3.54
3.53
3.53
3.52
3.50
3.50
1.21
1.19
O
N
H
4a
1.09
0.95
1.00
0.97
1.00
1.10
1.01
1.08
3.06
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
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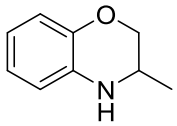
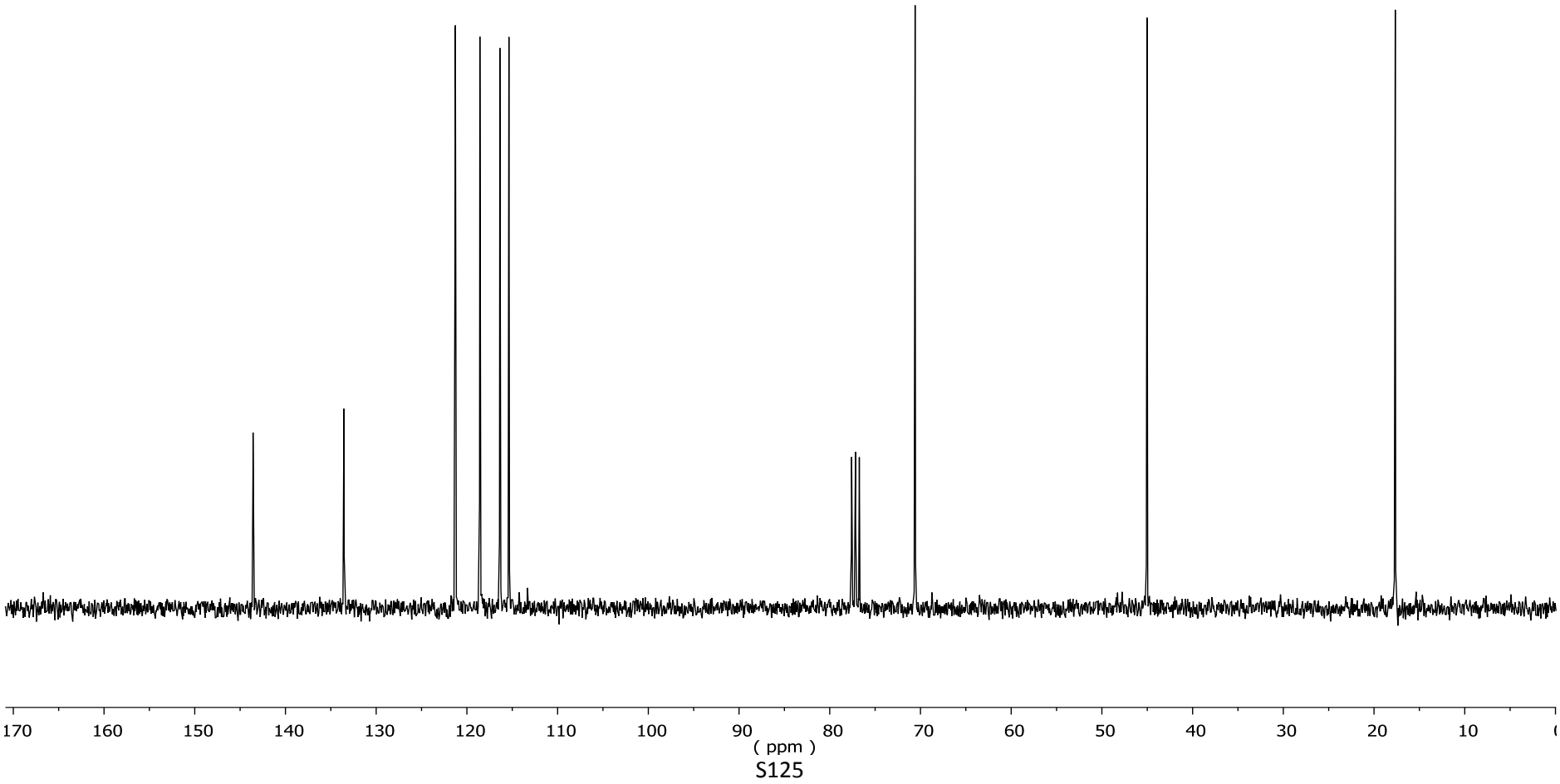
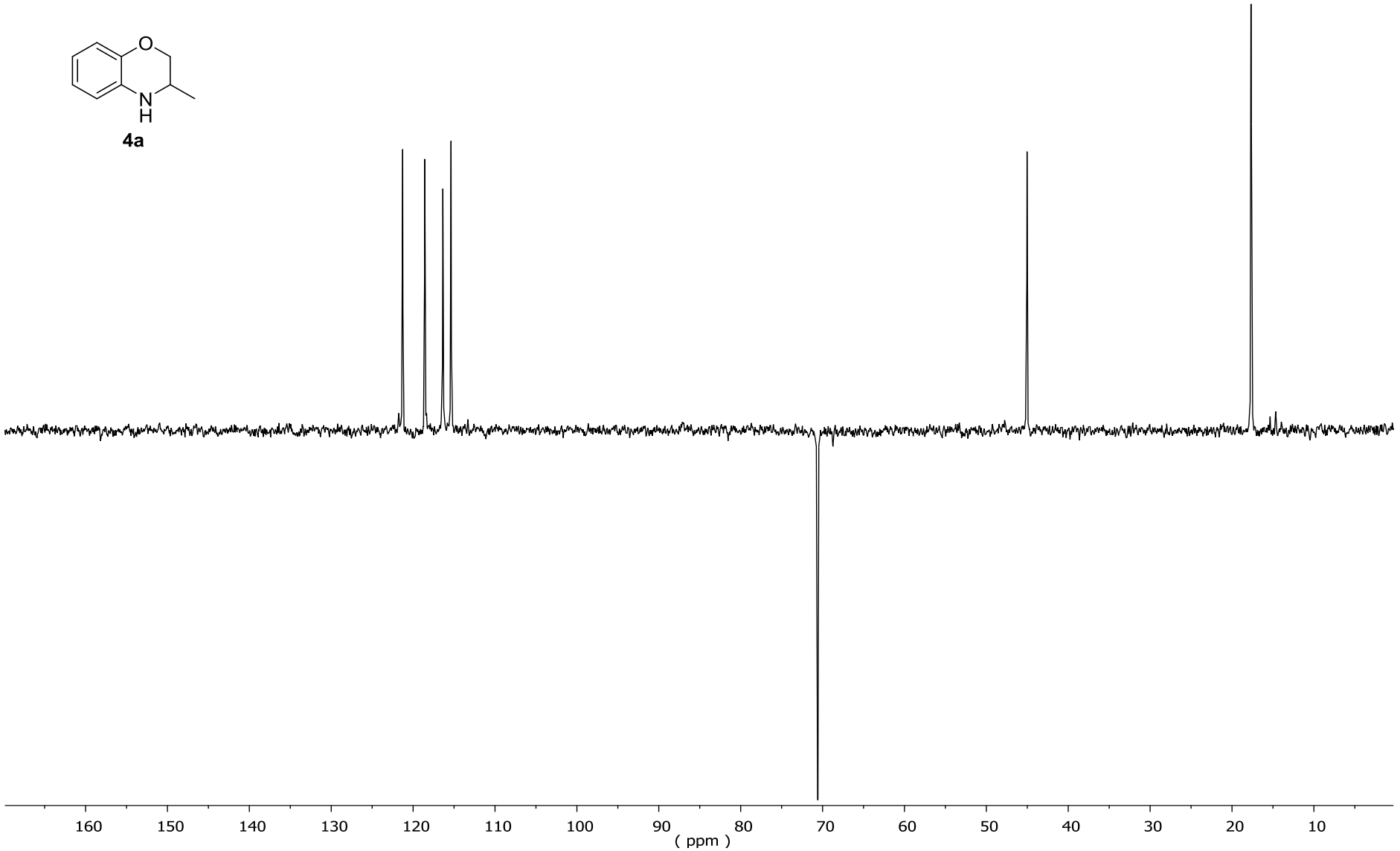
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
(ppm)



4a




O
N
H
4a
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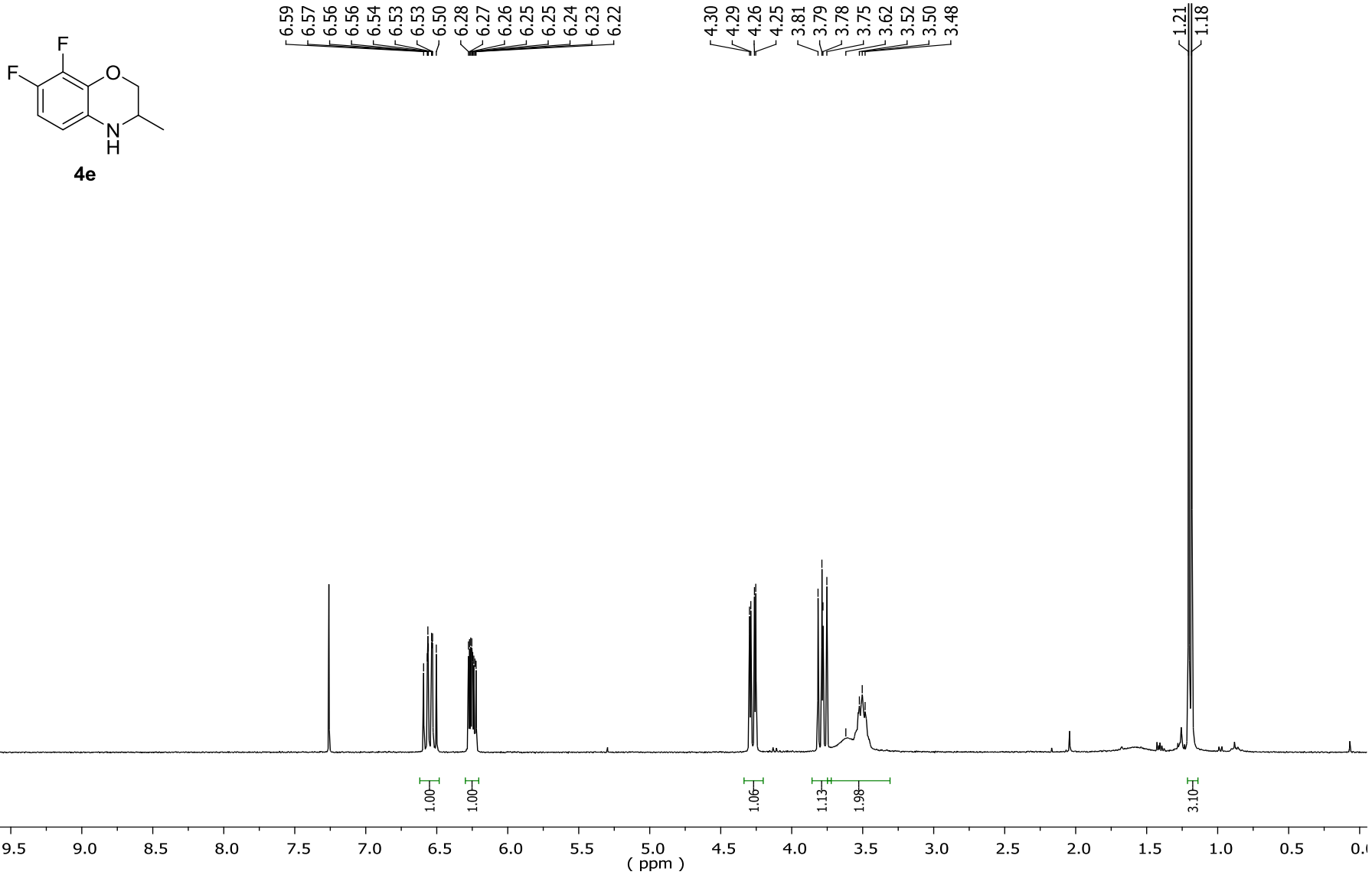
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
(ppm)


F
F
O
N
H
4e
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6.57
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6.56
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6.53
6.50
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6.27
6.26
6.25
6.25
6.24
6.23
6.22
4.30
4.29
4.26
4.25
3.81
3.79
3.78
3.75
3.62
3.52
3.50
3.48
1.21
1.18
1.00
1.00
1.06
1.13
1.98
3.10
0.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
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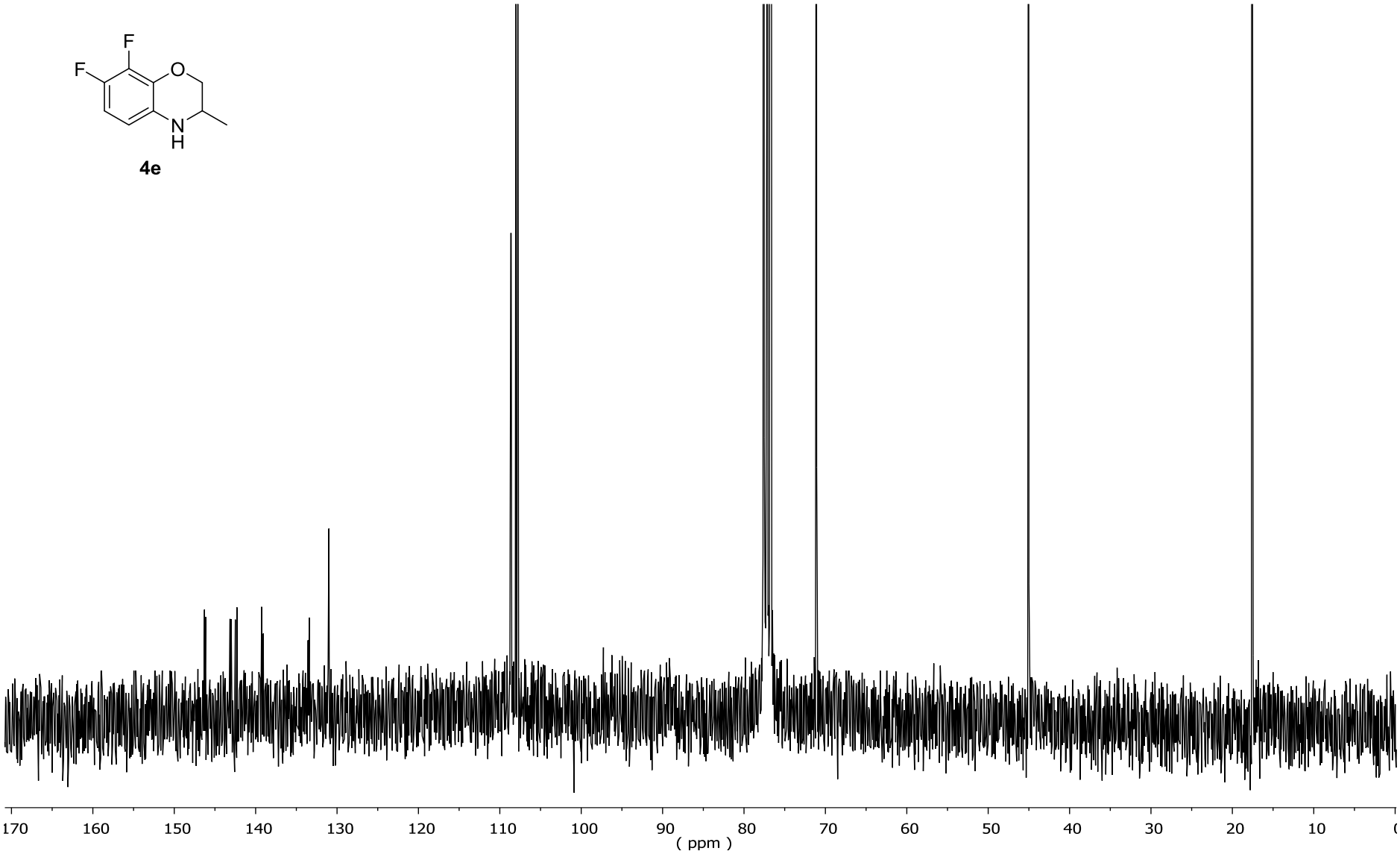
0.5
(ppm)

F
F
O
N
H

4e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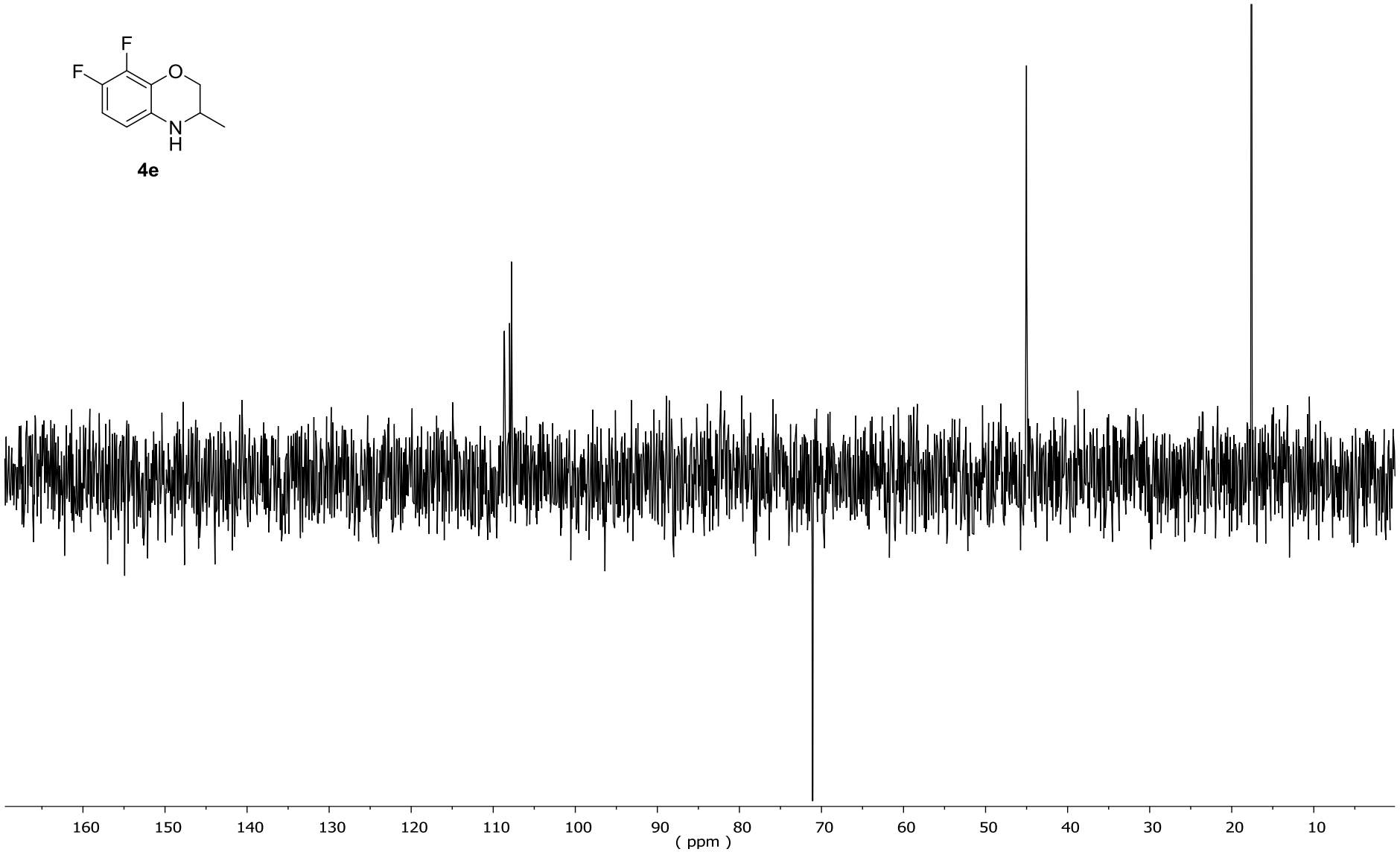
4e