Supporting information

Synthesis and evaluation of isothiazolo[4,5-*b*]pyridines as cyclin G-associated kinase (GAK) inhibitors

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1. 2D NMR discussion of compounds 9 and 18c

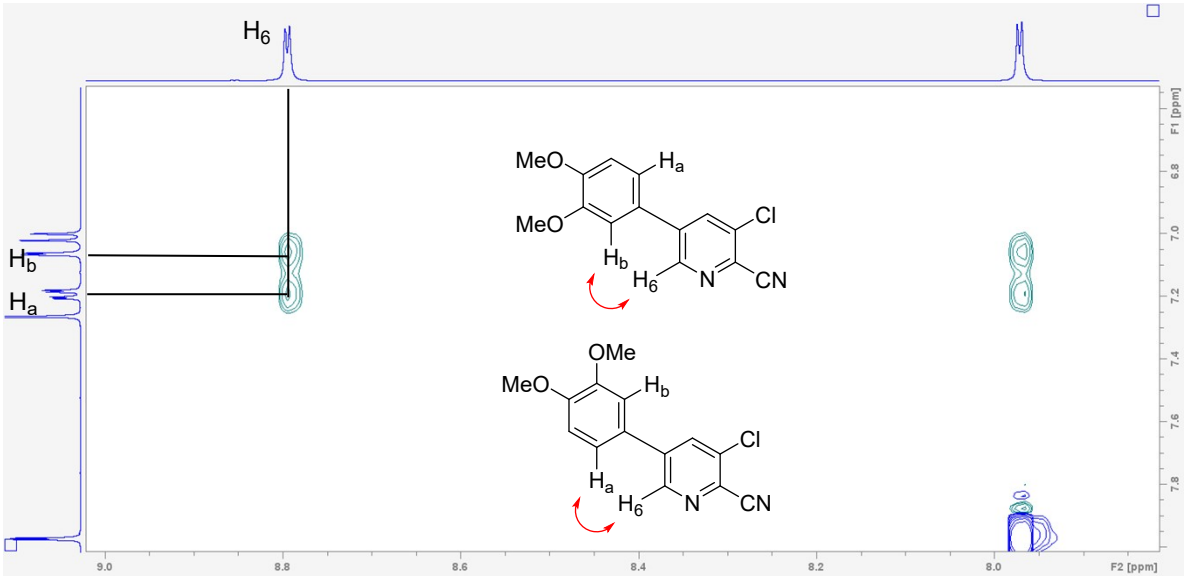


Fig. S1. NOESY spectrum of compound **9**. Red arrows represent the spatial interaction between protons of the rotational isomers.

The regioselectivity of the Suzuki reaction leading to the compound **9** was elucidated by a 2D NMR NOESY experiment (Fig.S1). The clear NOE correlations between H_6 and H_a/H_b confirmed that the 3,4-dimethoxyphenyl moiety is attached to position 5 of the pyridine scaffold. No NOE correlation would be observed in case coupling occurred at position 3.

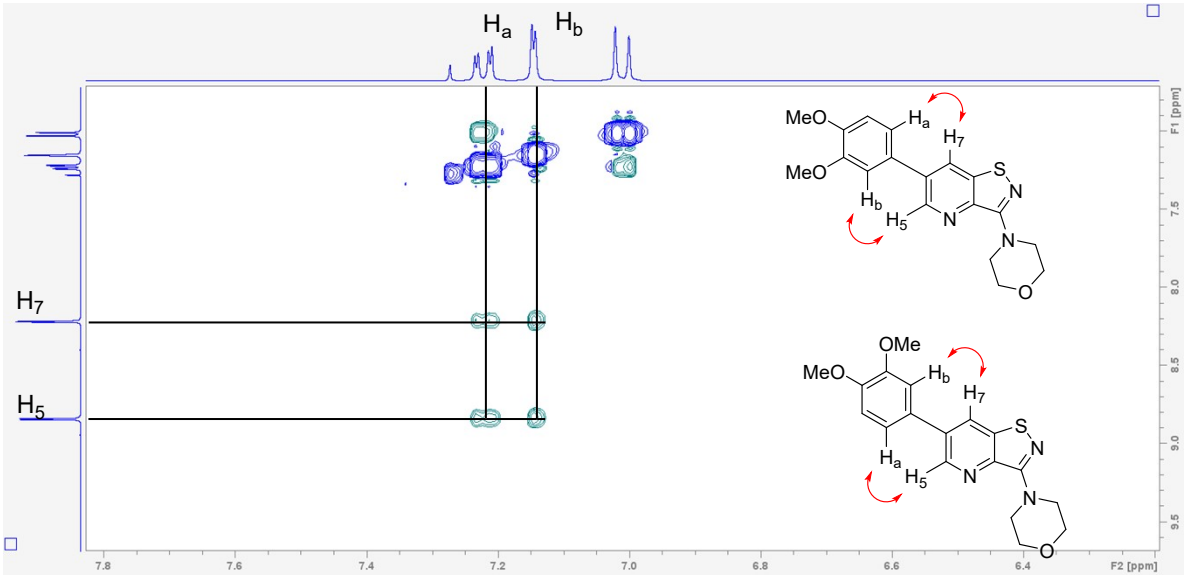


Fig. S2. NOESY spectrum of compound **18c**. Red arrows represent the spatial interaction between protons of the rotational isomers.

Similarly, the regioselectivity of nucleophilic substitution followed by Suzuki coupling leading to the library of 3,6- and 3,5-substituted isothiazolo[4,5-*b*]pyridine analogues was elucidated by a 2D NMR NOESY experiment (Fig.S2). For this purpose compound **18c** was chosen as an example of the prepared series. The clear NOE correlations between H_a and H_5/H_7 and H_b and H_5/H_7 proved that nucleophilic substitution occurs at position 3, while 3,4-dimethoxyphenyl ring is attached to position 6 of isothiazolo[4,5-*b*]pyridine scaffold.

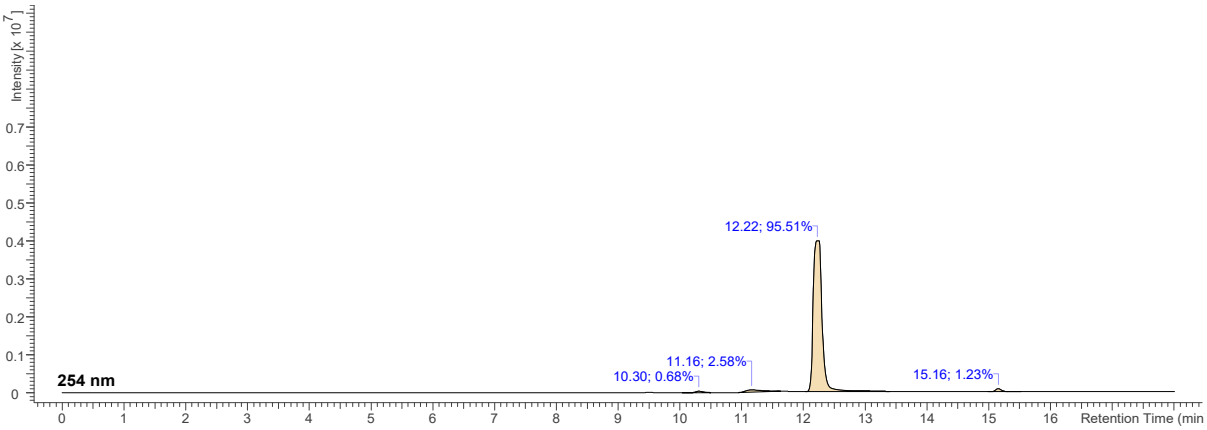
2. HPLC purity of compounds 18c-f and 23a-b

Table S1. HPLC purity

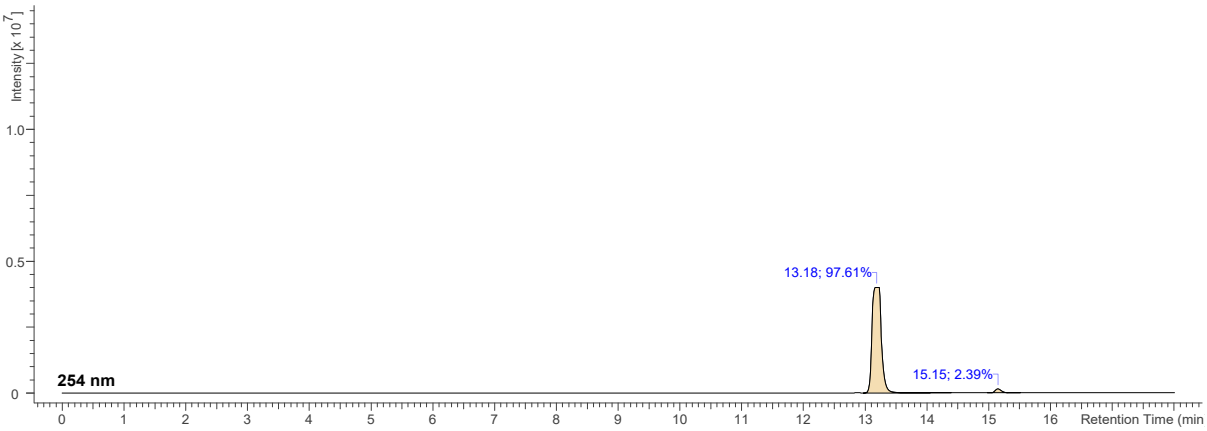
Compound	Retention time (min)	% Purity Area
18c	**12.22**	**95.5**
18d	**13.18**	**97.6**
18e	**12.45**	**95.3**
18f	**12.43**	**97.4**
23a	**12.34**	**96.2**
23b	**13.37**	**95.5**

UV detection wavelength 254 nm, Shimadzu Prominence-i LC-2030C 3D Plus instrument, Shim-pack GISS-HP column, 3µm C18, 2.1x100mm; method: 0 (0% D/ 100% A)→ 100% D (100% D/ 0% A) within 12 min, 100% D for 6 min (A: water + 0.1% formic acid, D: MeOH + 0.1% formic acid). The intensity of the ions (arbitrary units) is shown on the y-axis, and the retention time (in min) of the ions is shown on the x-axis.

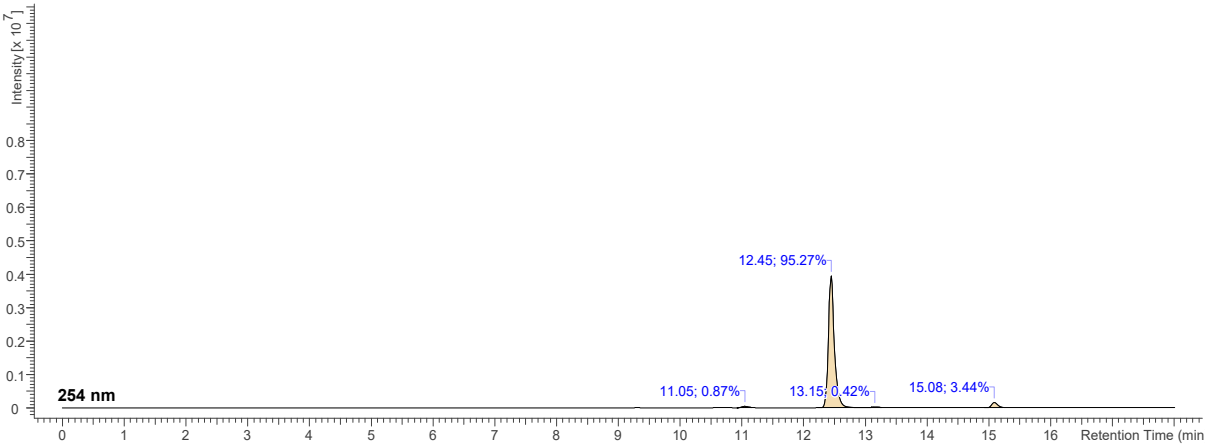
4-(6-(3,4-Dimethoxyphenyl)isothiazolo[4,5-*b*]pyridin-3-yl)morpholine (18c)



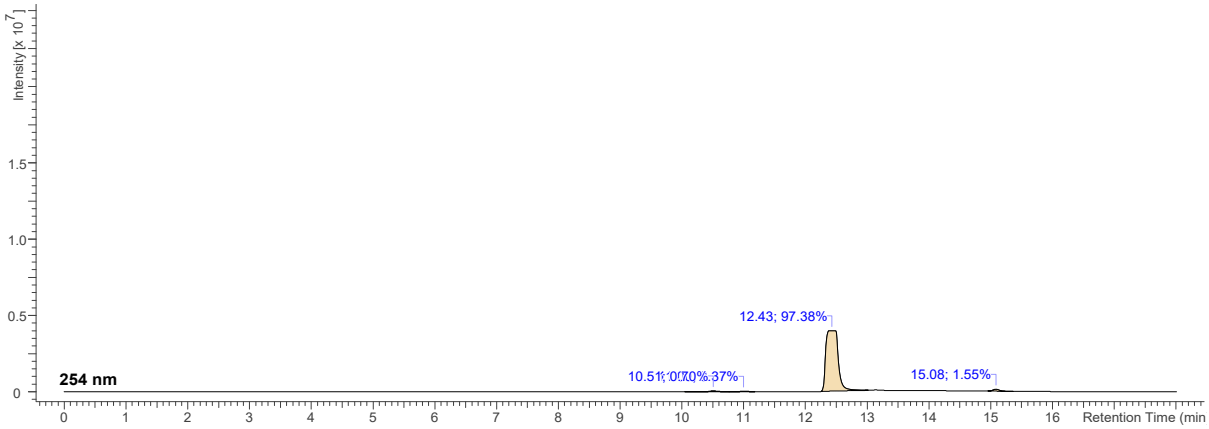
(2S,6R)-4-(6-(3,4-Dimethoxyphenyl)isothiazolo[4,5-*b*]pyridin-3-yl)-2,6-dimethylmorpholine (18d)



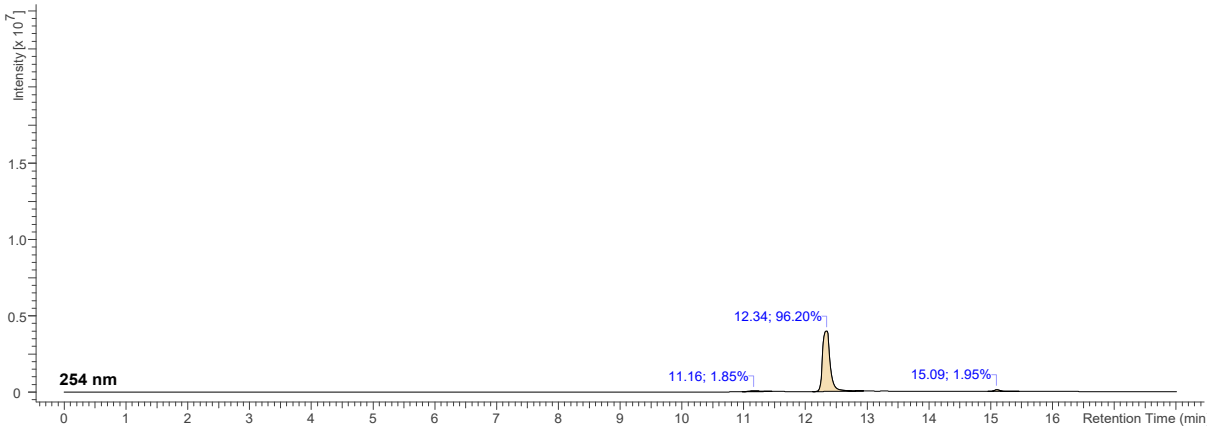
N-((1-(6-(3,4-dimethoxyphenyl)isothiazolo[4,5-*b*]pyridin-3-yl)piperidin-3-yl)methyl)cyclopropanecarboxamide (18e)



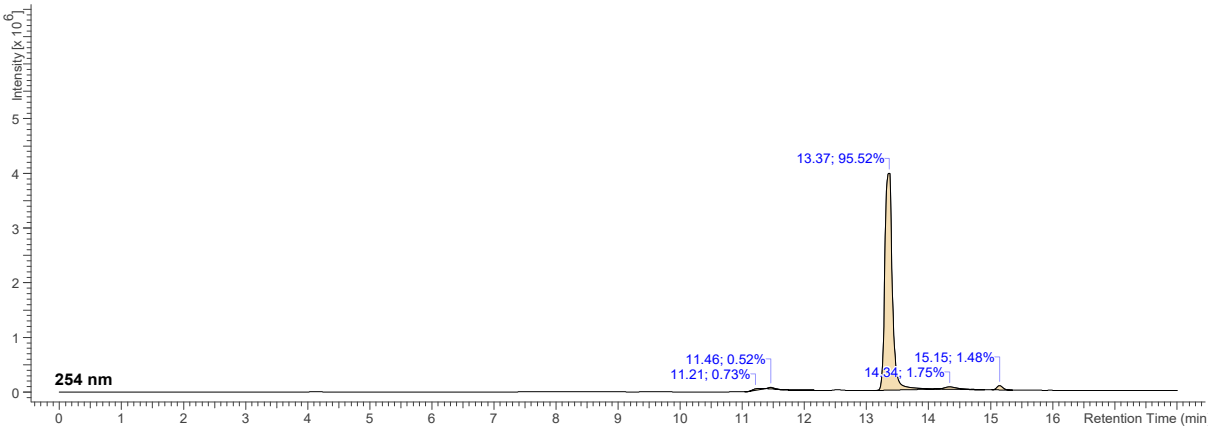
6-(3,4-Dimethoxyphenyl)-3-isopropoxyisothiazolo[4,5-*b*]pyridine (18f)



4-(5-(3,4-Dimethoxyphenyl)isothiazolo[4,5-*b*]pyridin-3-yl)morpholine (23a)



(2S,6R)-4-(5-(3,4-Dimethoxyphenyl)isothiazolo[4,5-*b*]pyridin-3-yl)-2,6-dimethylmorpholine (23b)



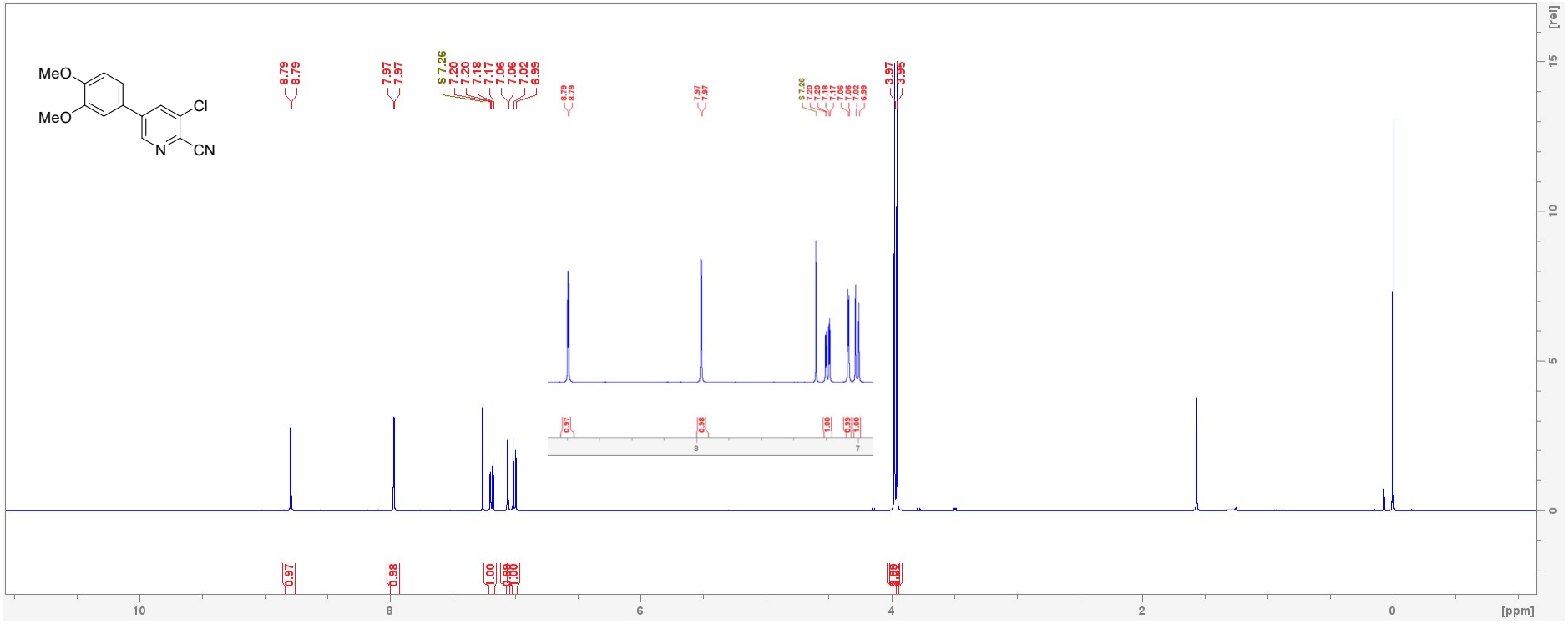
3. Crystal structure determination of 16

Table S2. Crystal data, data collection and structure refinement details for **16**.

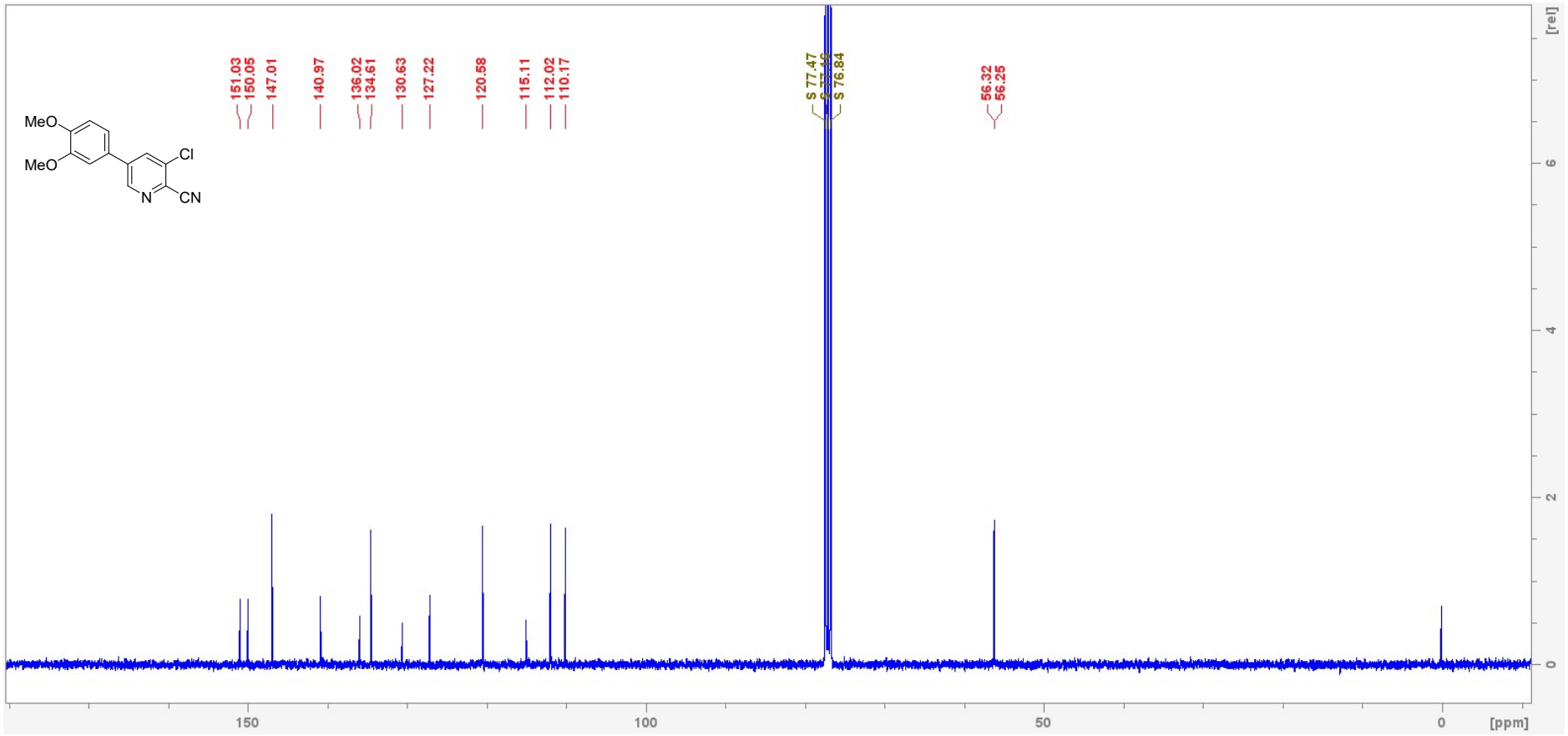
Compound	**16**
Molecular formula	$C_6H_2Br_2N_2S$
M (g.mol^{-1})	293.98
Crystal system	monoclinic
Space group	$P2_1/n$
a (Å)	4.0454(3)
b (Å)	10.5096(8)
c (Å)	19.0866(16)
α (°)	90
β (°)	93.419(7)
γ (°)	90
Volume (Å^3)	810.03(11)
Z	4
ρ_{calc} (g.cm^{-3})	2.411
μ (mm^{-1})	10.193
F(000)	552.0
Crystal size (mm^3)	0.3 × 0.25 × 0.15
Reflections collected	8601
Independent reflections	1638 [R_{int} = 0.0322, R_{sigma} = 0.0260]
Data/restraints/parameters	1638/0/100
Final R indexes [I ≥ 2σ(I)]	1.082
Final R indexes [all data]	R_1 = 0.0316, wR_2 = 0.0582
Goodness-of-fit on F^2	R_1 = 0.0499, wR_2 = 0.0645
Largest diff. peak/hole (e. Å^{-3})	0.52/-0.47

4. NMR spectra

3-Chloro-5-(3,4-dimethoxyphenyl)pyridine-2-carbonitrile (9)

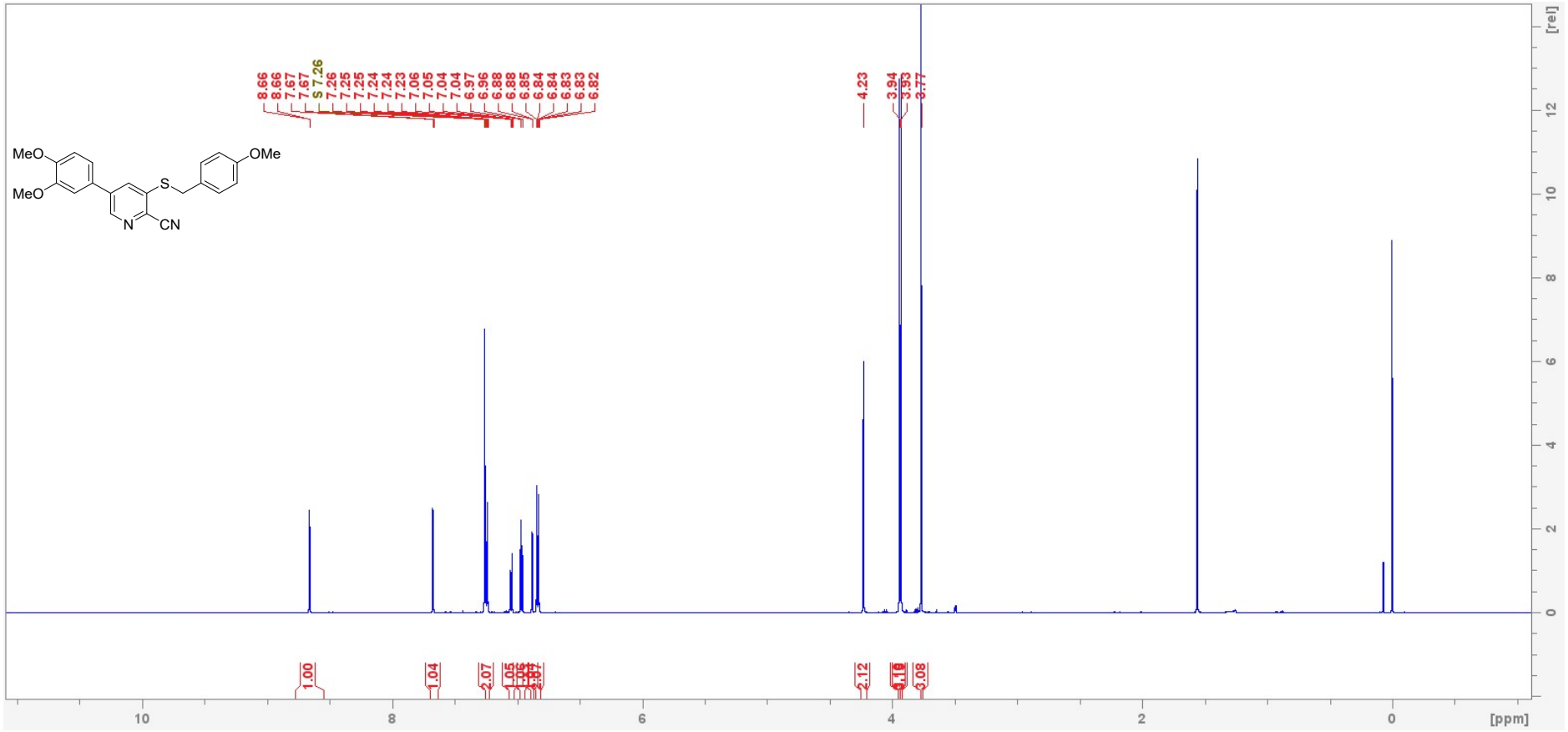


^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **9**



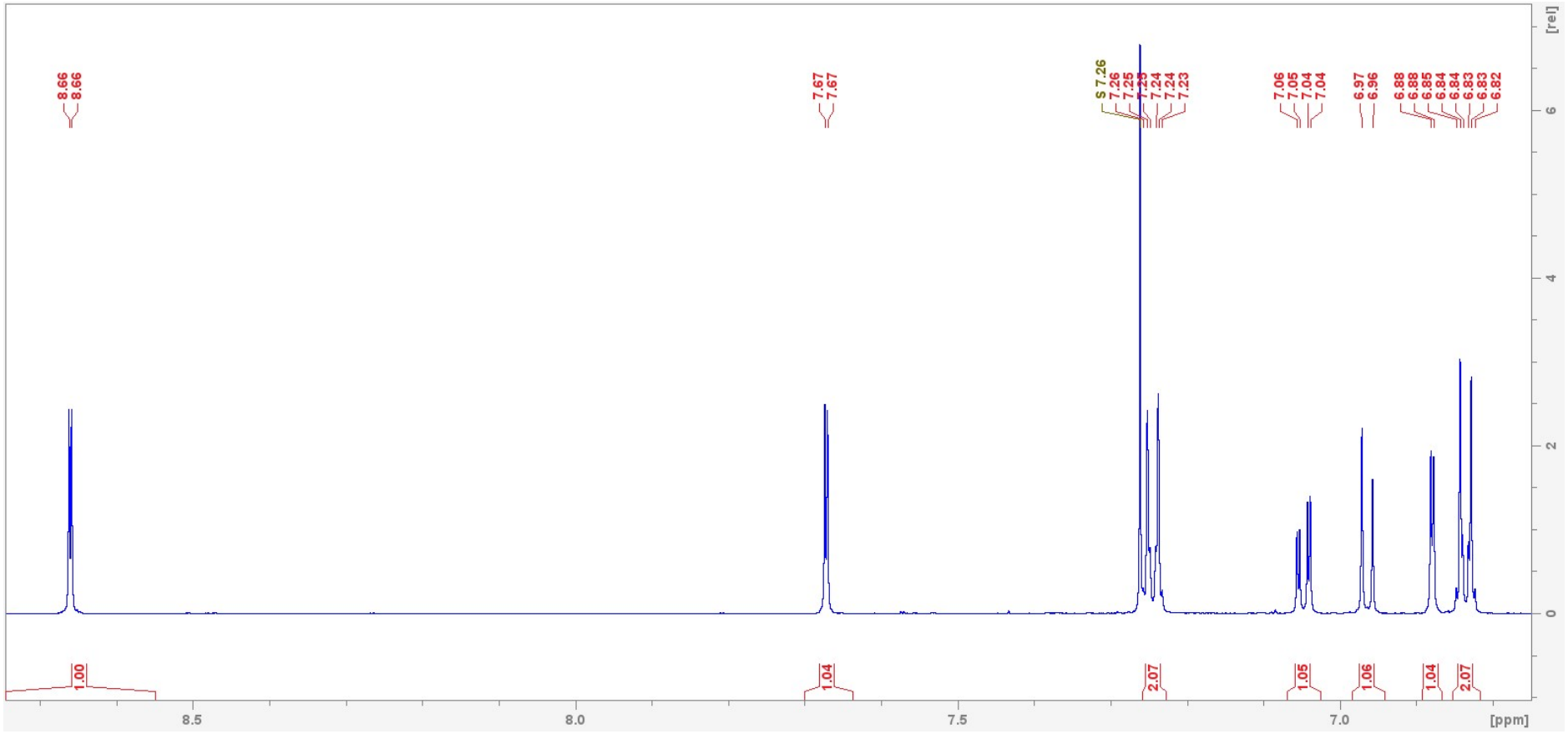
^{13}C NMR spectrum (151 MHz, $CDCl_3$) of **9**

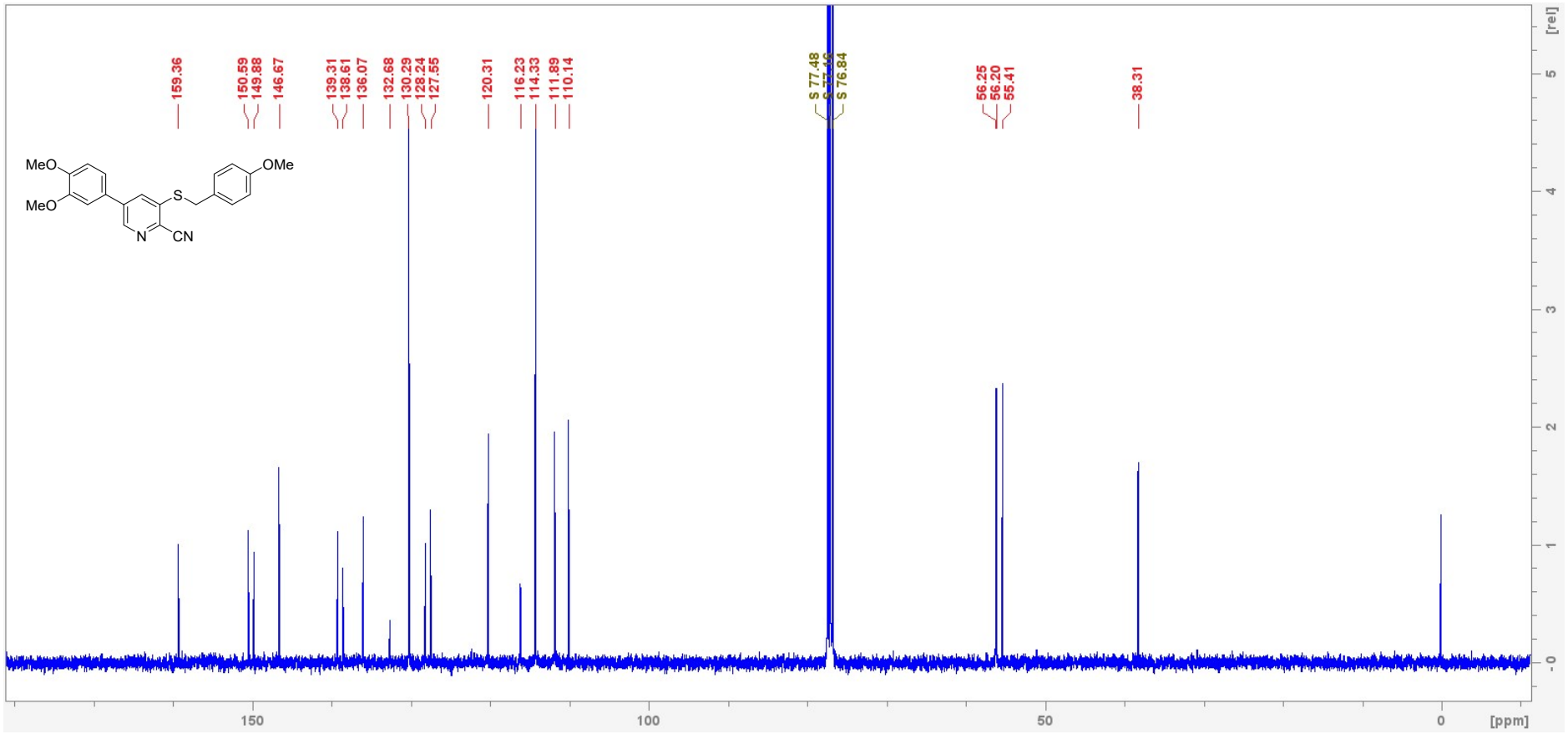
5-(3,4-Dimethoxyphenyl)-3-((4-methoxybenzyl)thio)picolinonitrile (11)



^{1}H NMR spectrum (600 MHz, $CDCl_3$) of **11**

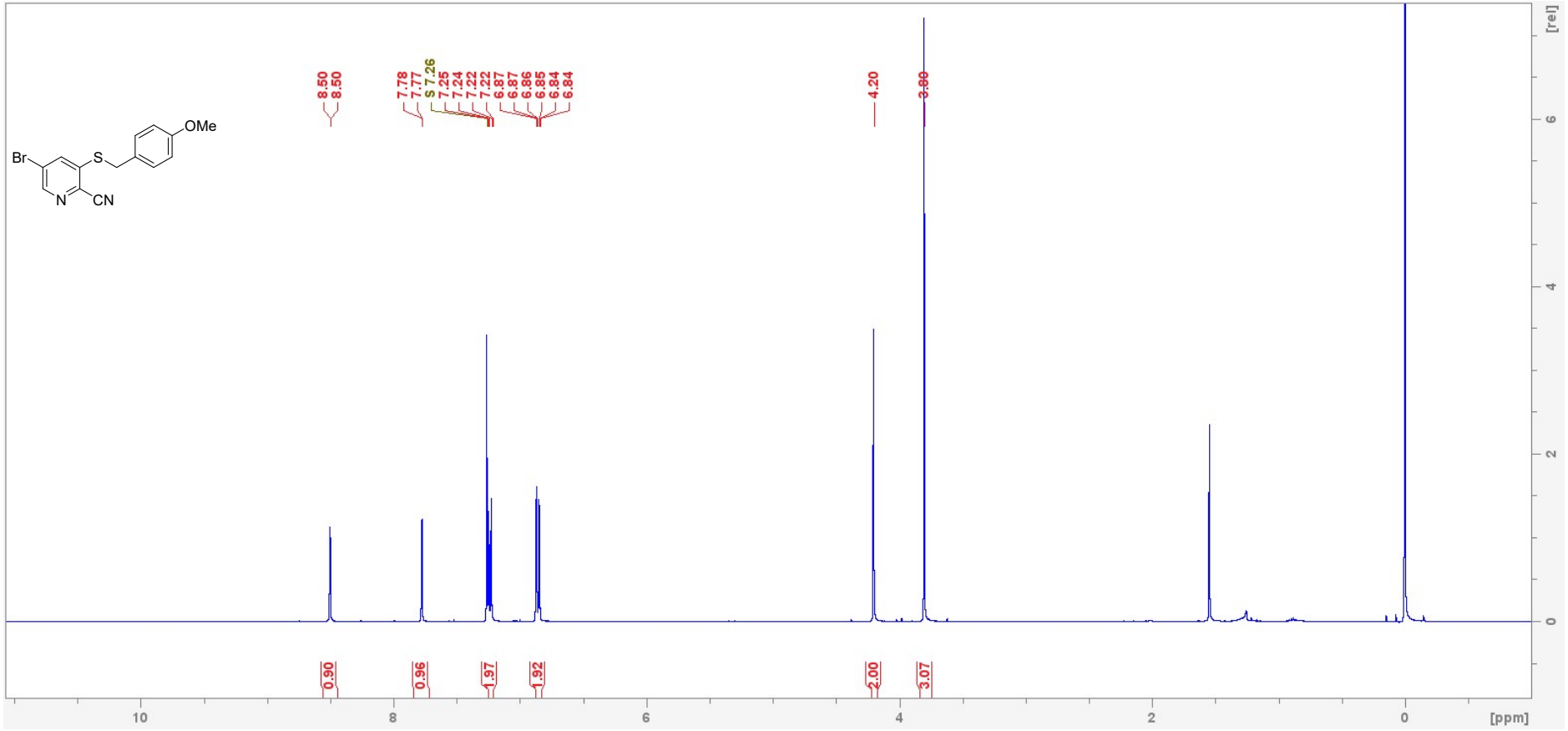
Zoom-in on the aromatic area:



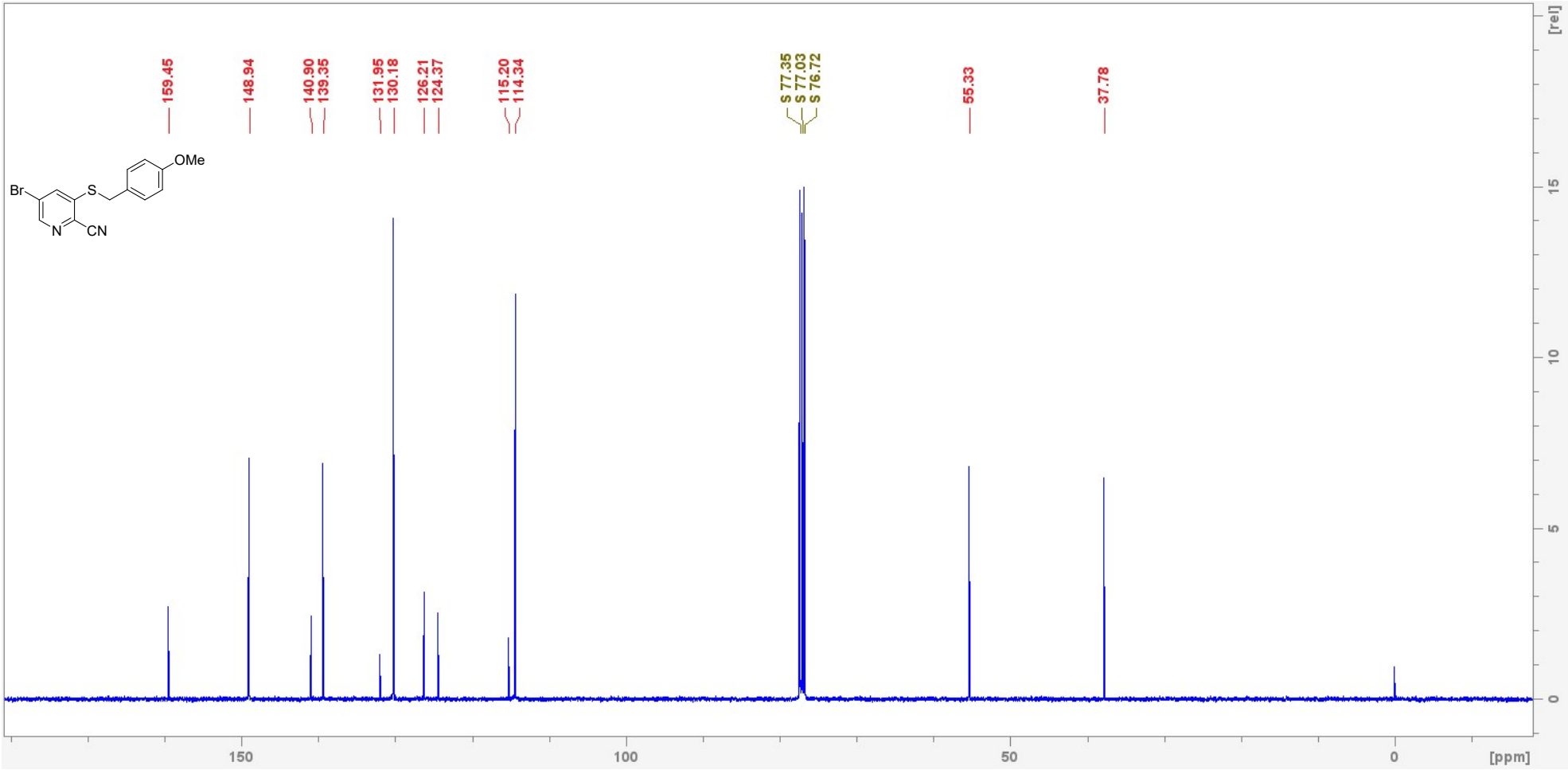


^{13}C NMR spectrum (101 MHz, $CDCl_3$) of **11**

5-Bromo-3-((4-methoxybenzyl)thio)picolinonitrile (15)

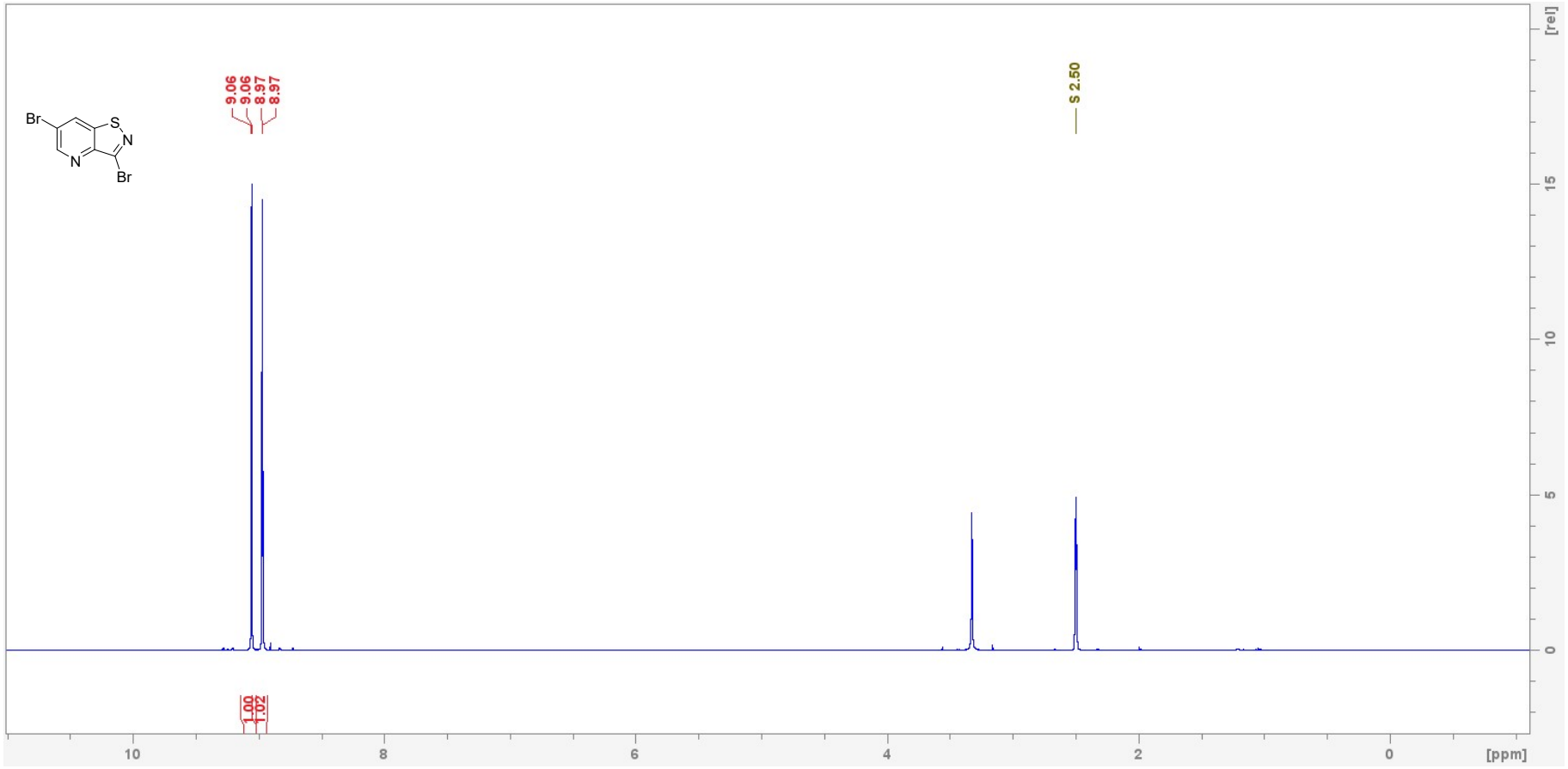


^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **15**

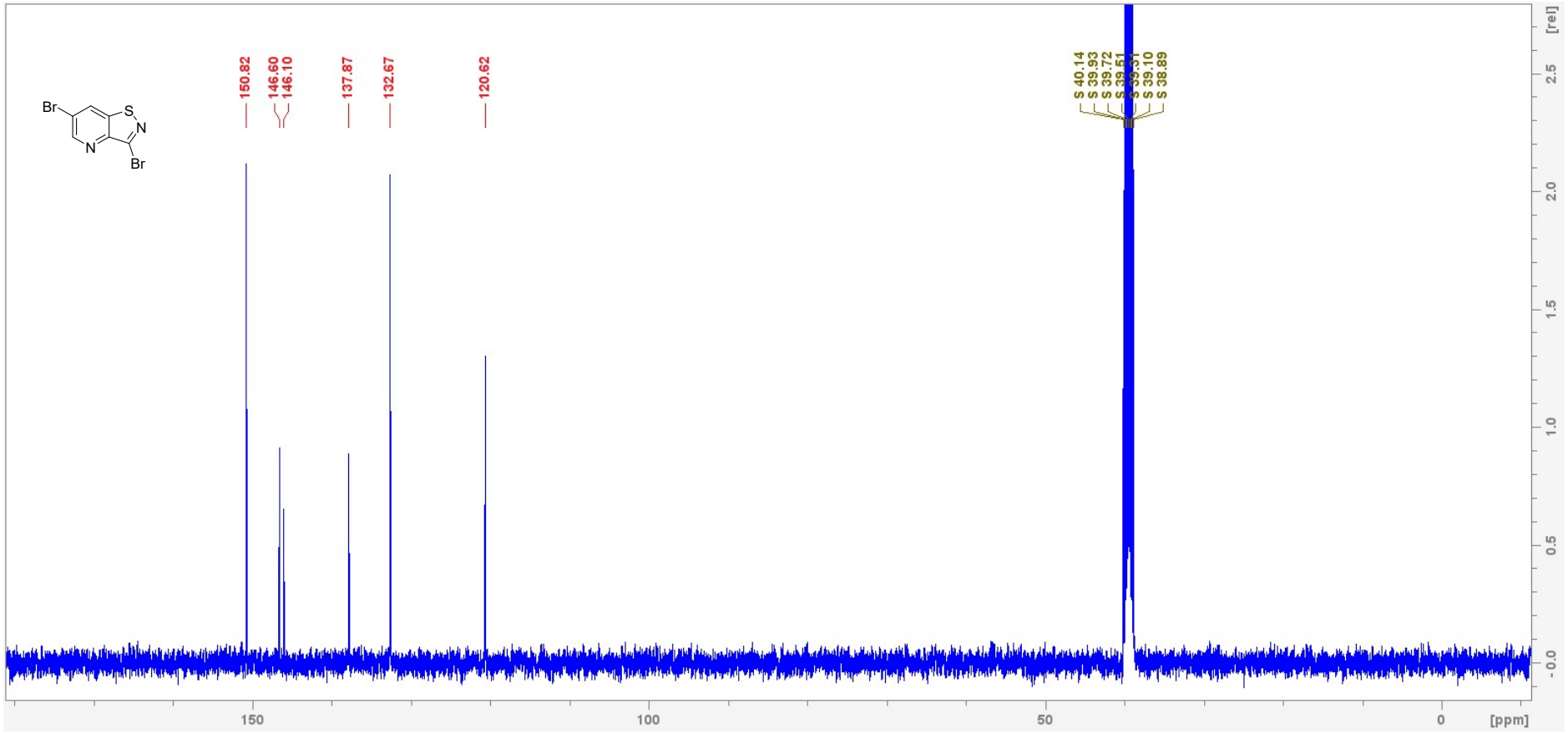


^{13}C NMR spectrum (101 MHz, $CDCl_3$) of **15**

3,6-Dibromoisothiazolo[4,5-*b*]pyridine (16)

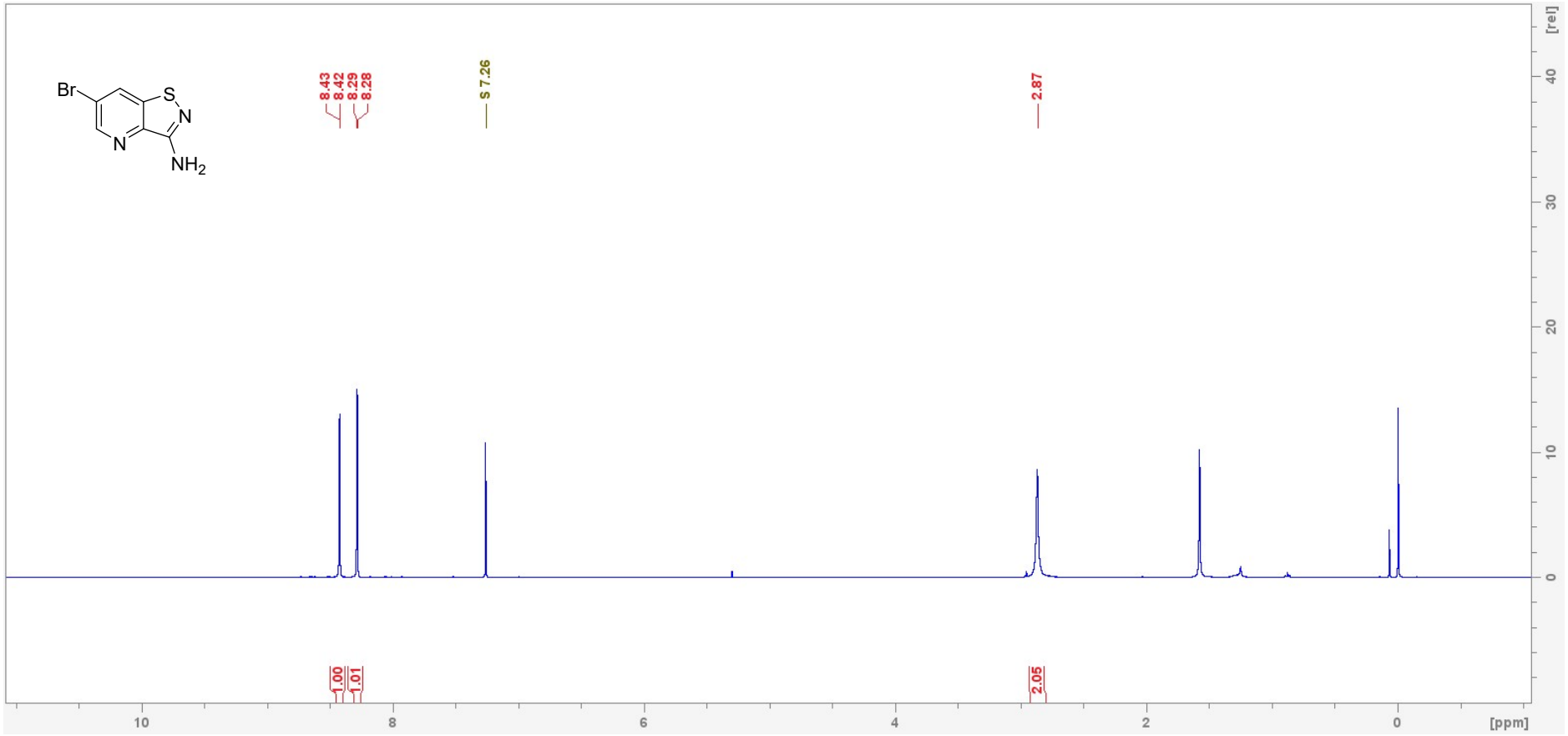


^{1}H NMR spectrum (400 MHz, DMSO-d_6) of **16**

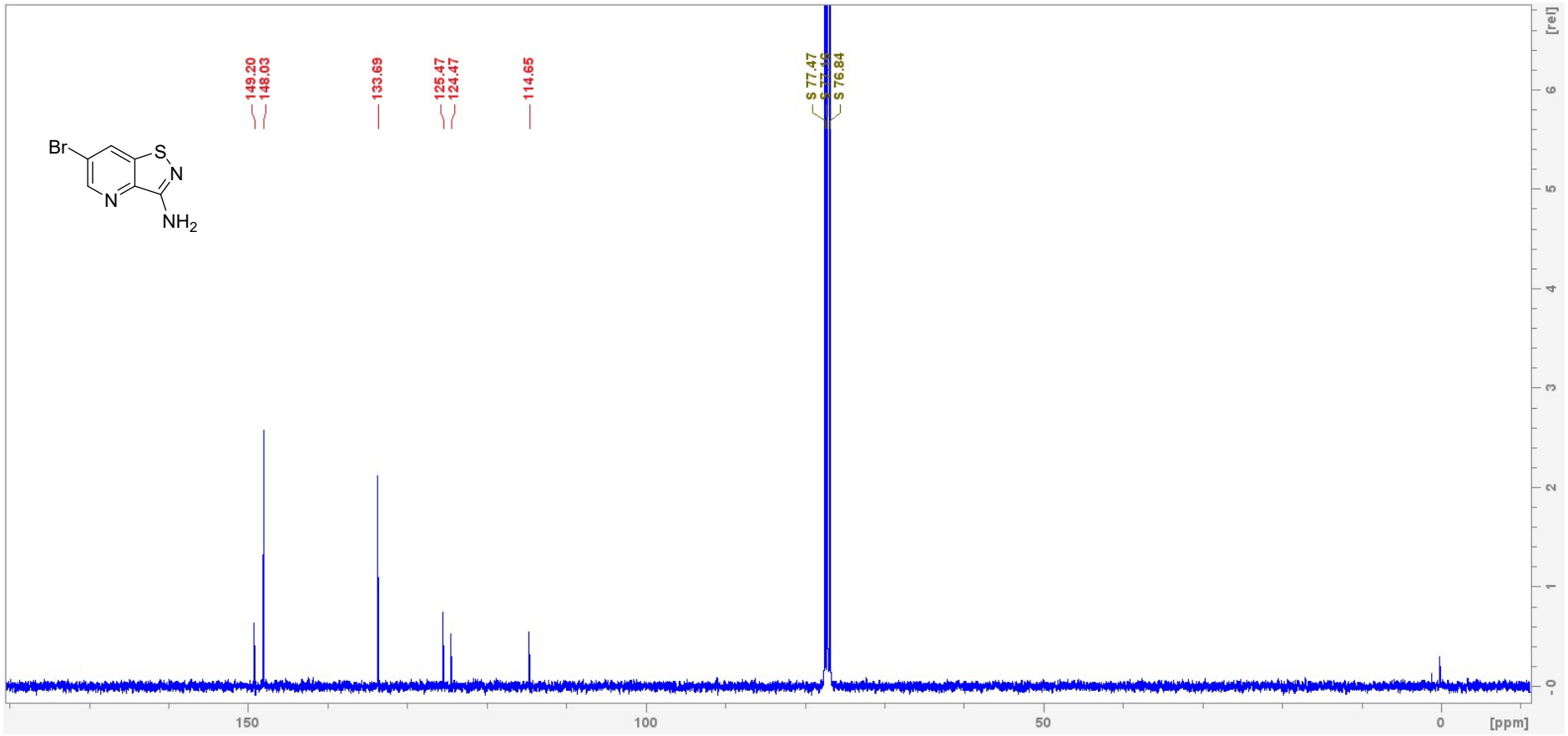


^{13}C NMR spectrum (101 MHz, DMSO-d_6) of **16**

6-Bromoisothiazolo[4,5-*b*]pyridin-3-amine (17a)

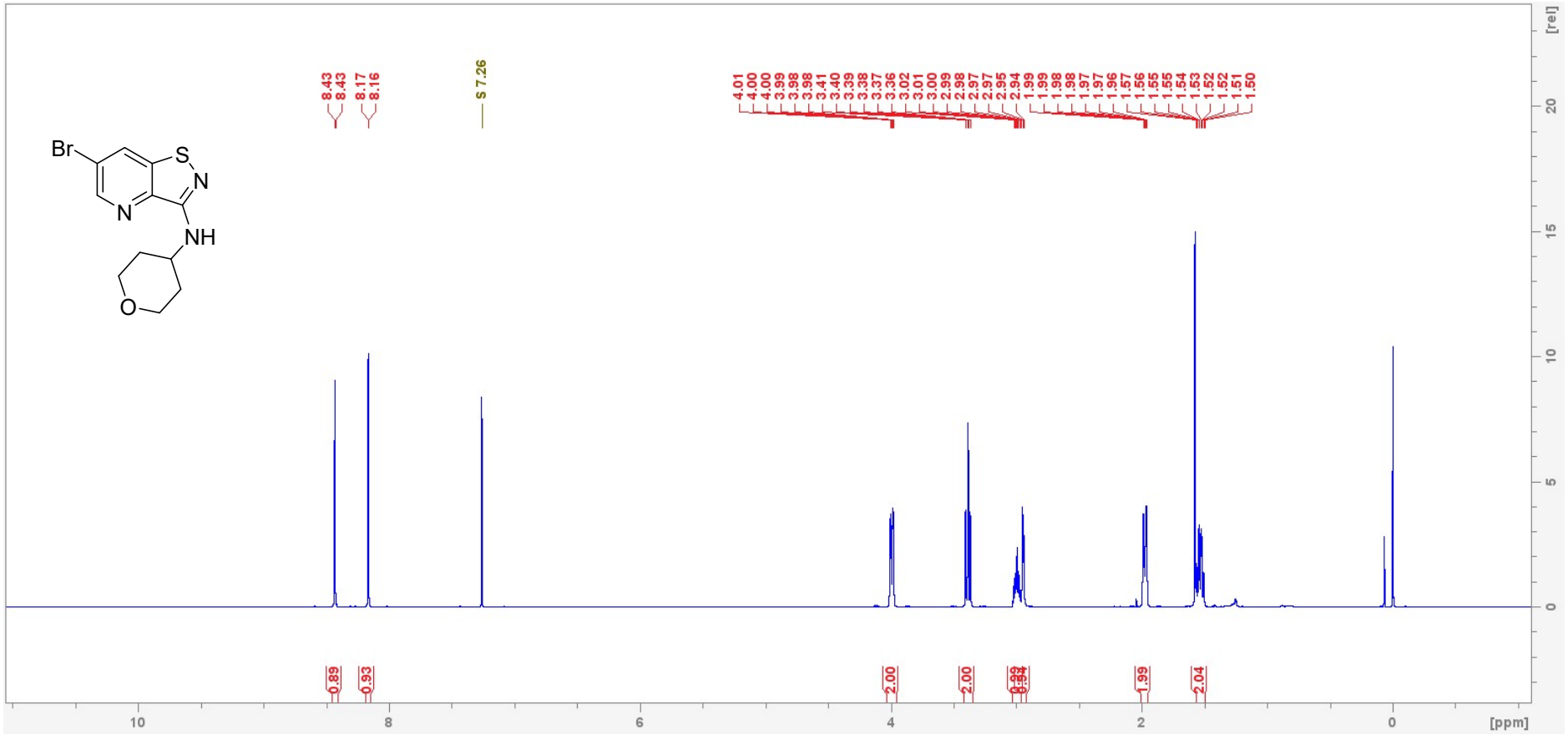


^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **17a**



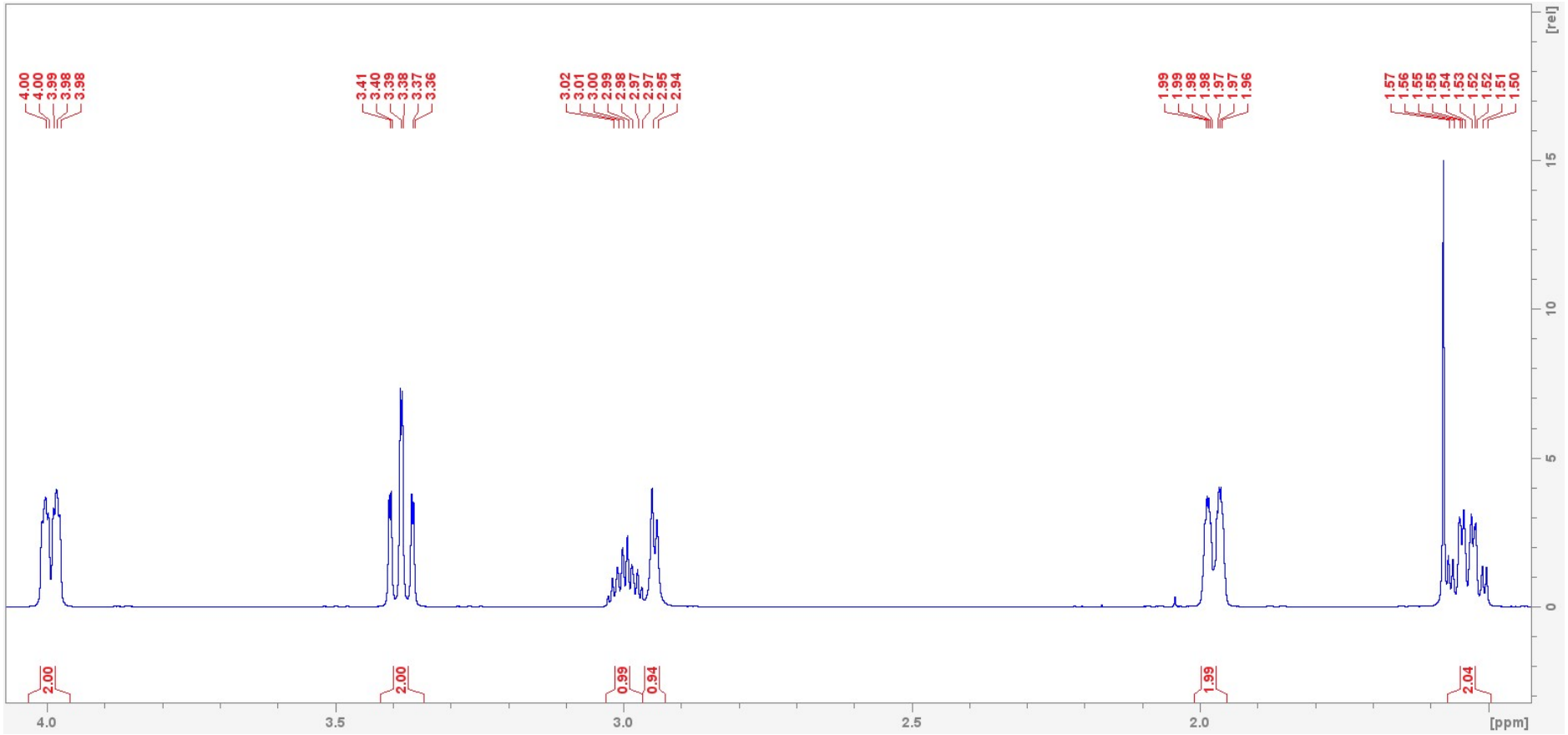
^{13}C NMR spectrum (101 MHz, $CDCl_3$) of **17a**

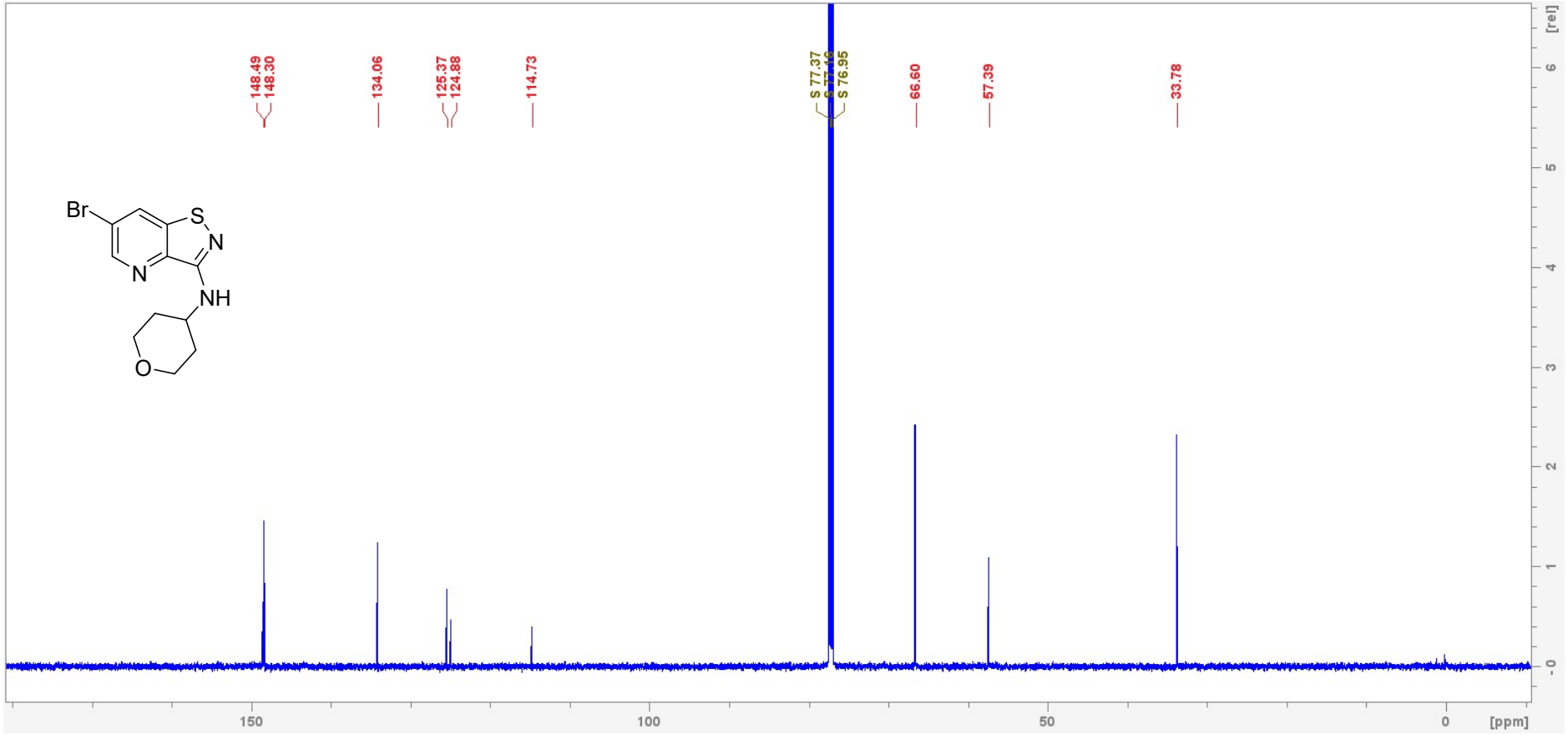
6-Bromo-*N*-(tetrahydro-2*H*-pyran-4-yl)isothiazolo[4,5-*b*]pyridin-3-amine (17b)



^{1}H NMR spectrum (600 MHz, $CDCl_3$) of **17b**

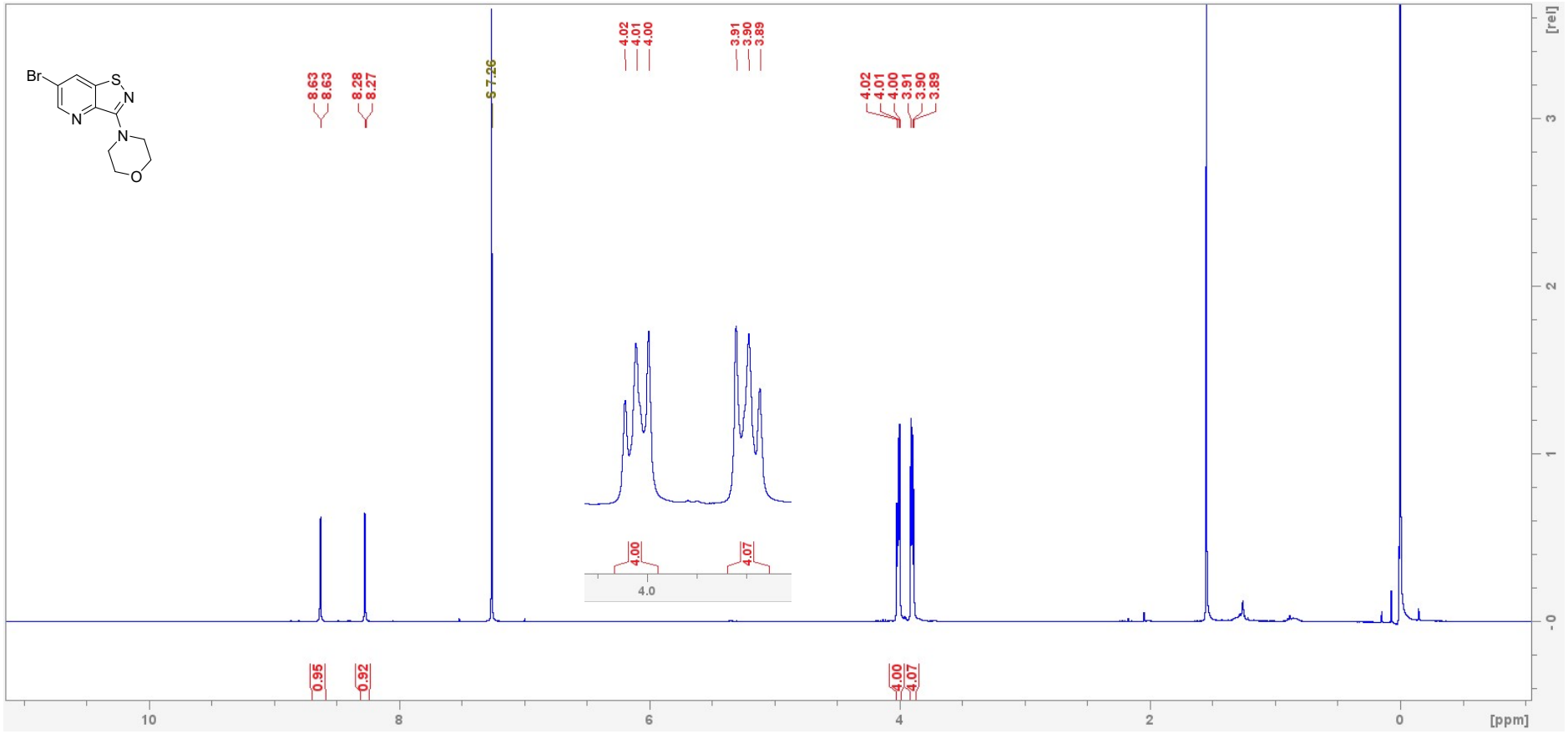
Zoom-in on the aliphatic area:



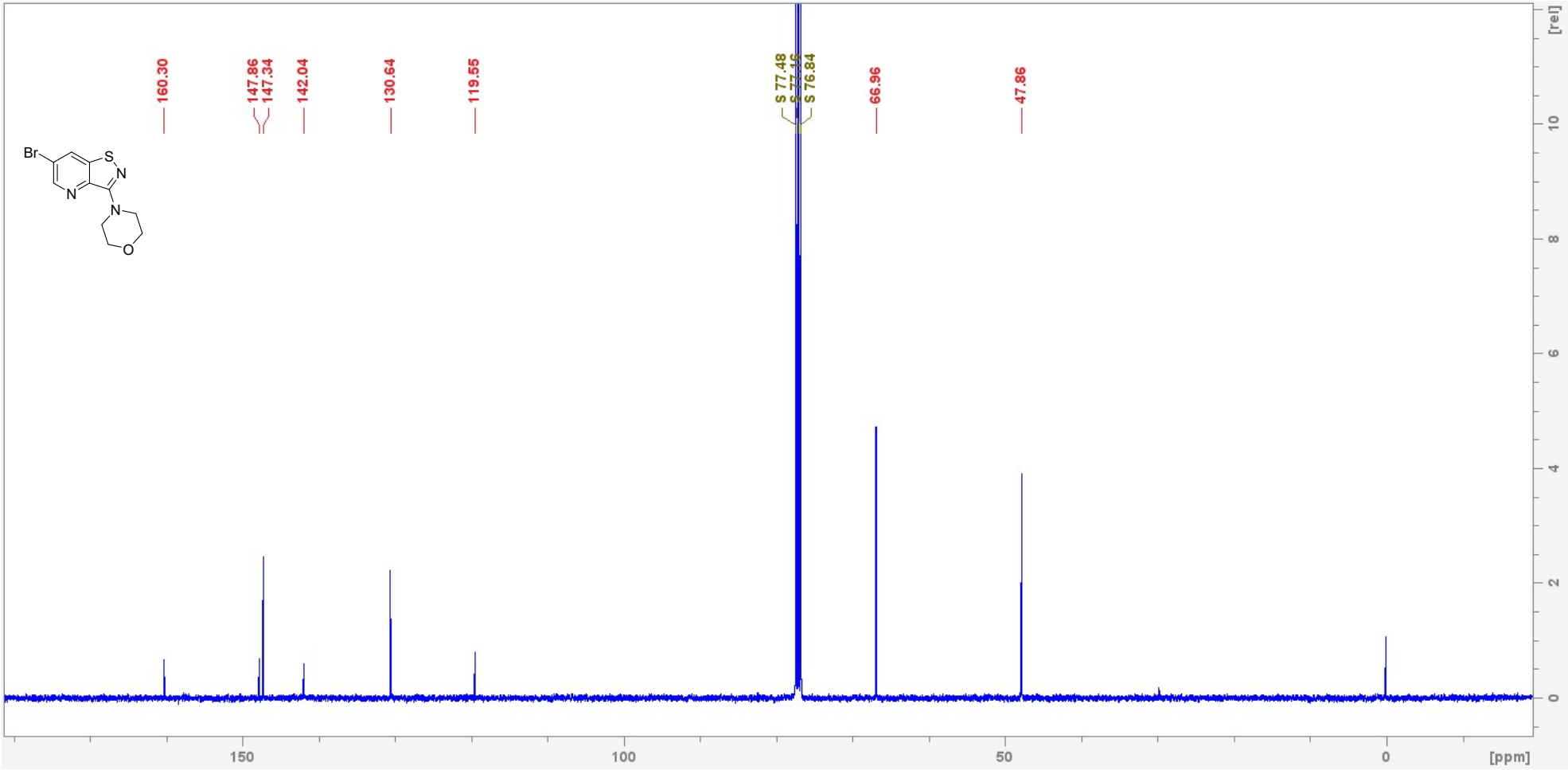


^{13}C NMR spectrum (151 MHz, $CDCl_3$) of **17b**

4-(6-Bromoisothiazolo[4,5-*b*]pyridin-3-yl)morpholine (17c)

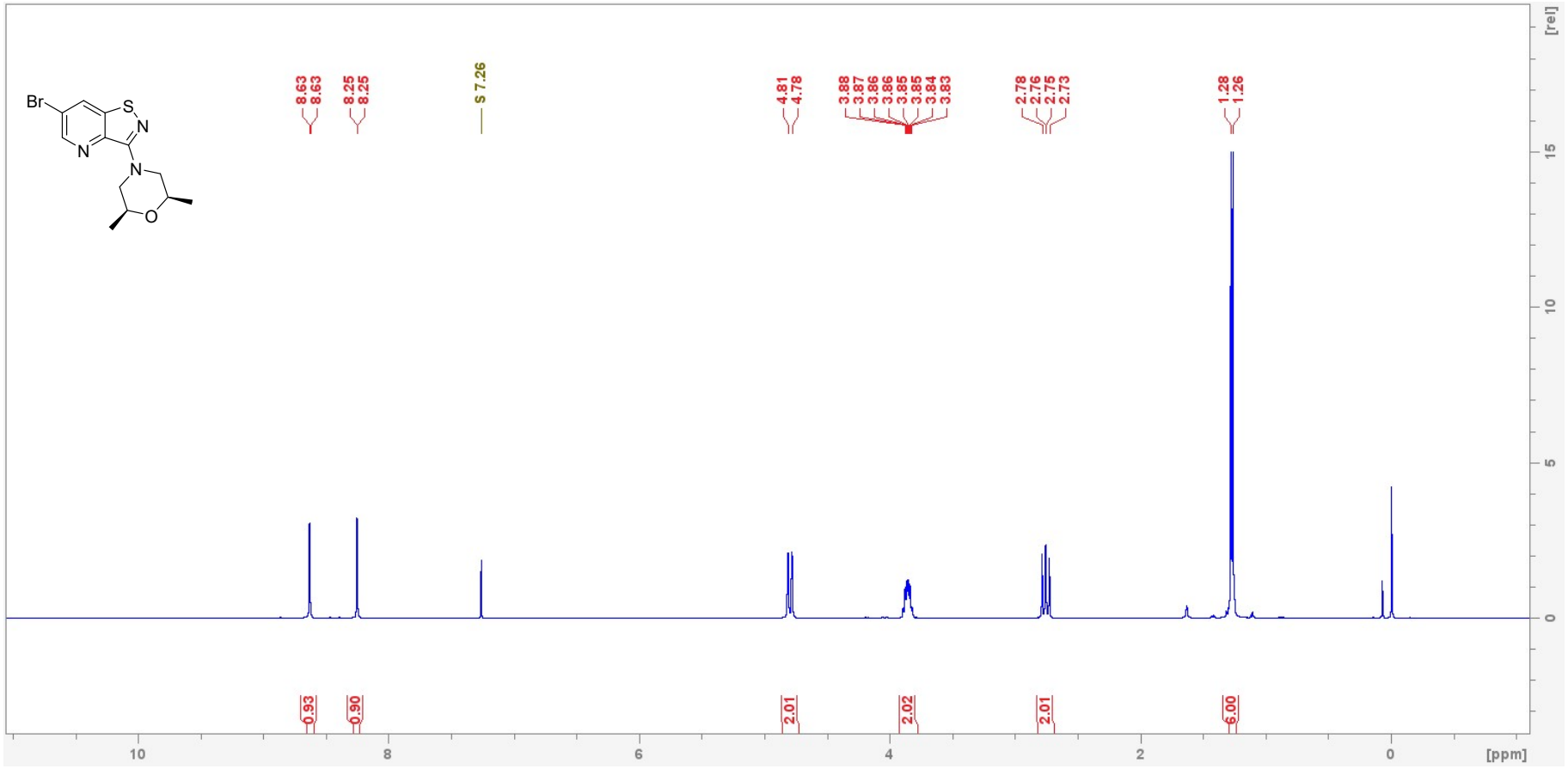


^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **17c**

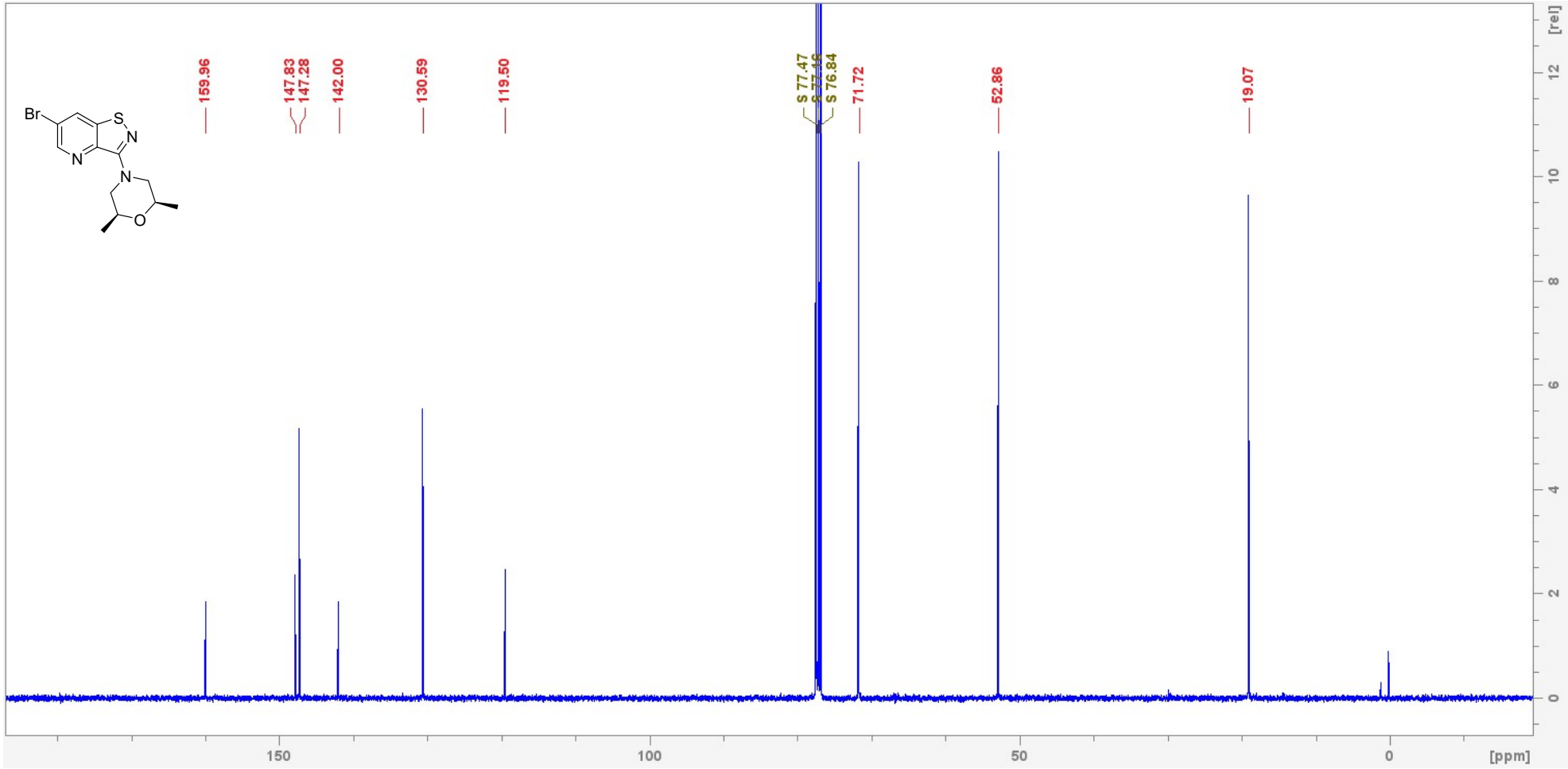


^{13}C NMR spectrum (101 MHz, $CDCl_3$) of **17c**

(2S,6R)-4-(6-Bromoisothiazolo[4,5-*b*]pyridin-3-yl)-2,6-dimethylmorpholine (17d)

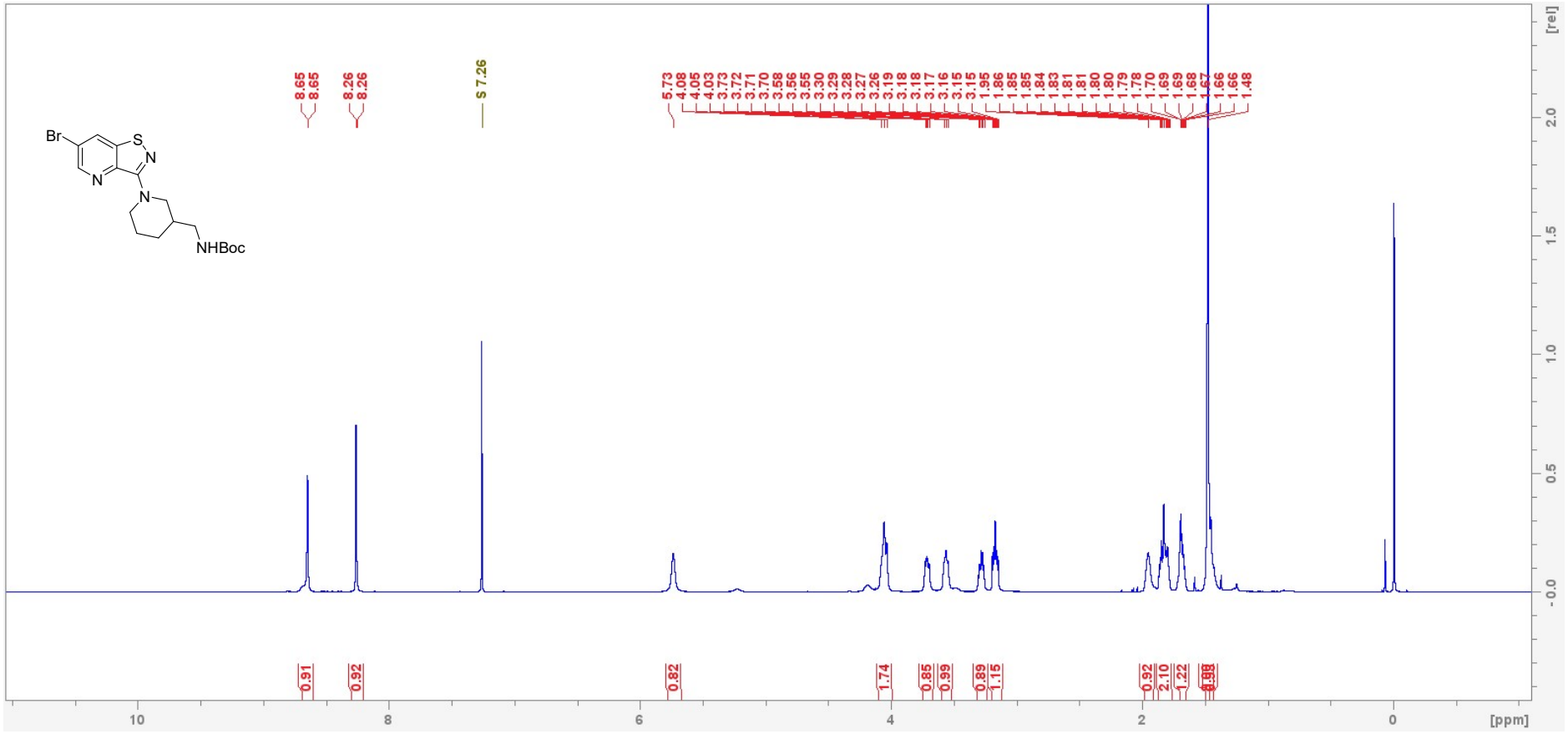


^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **17d**

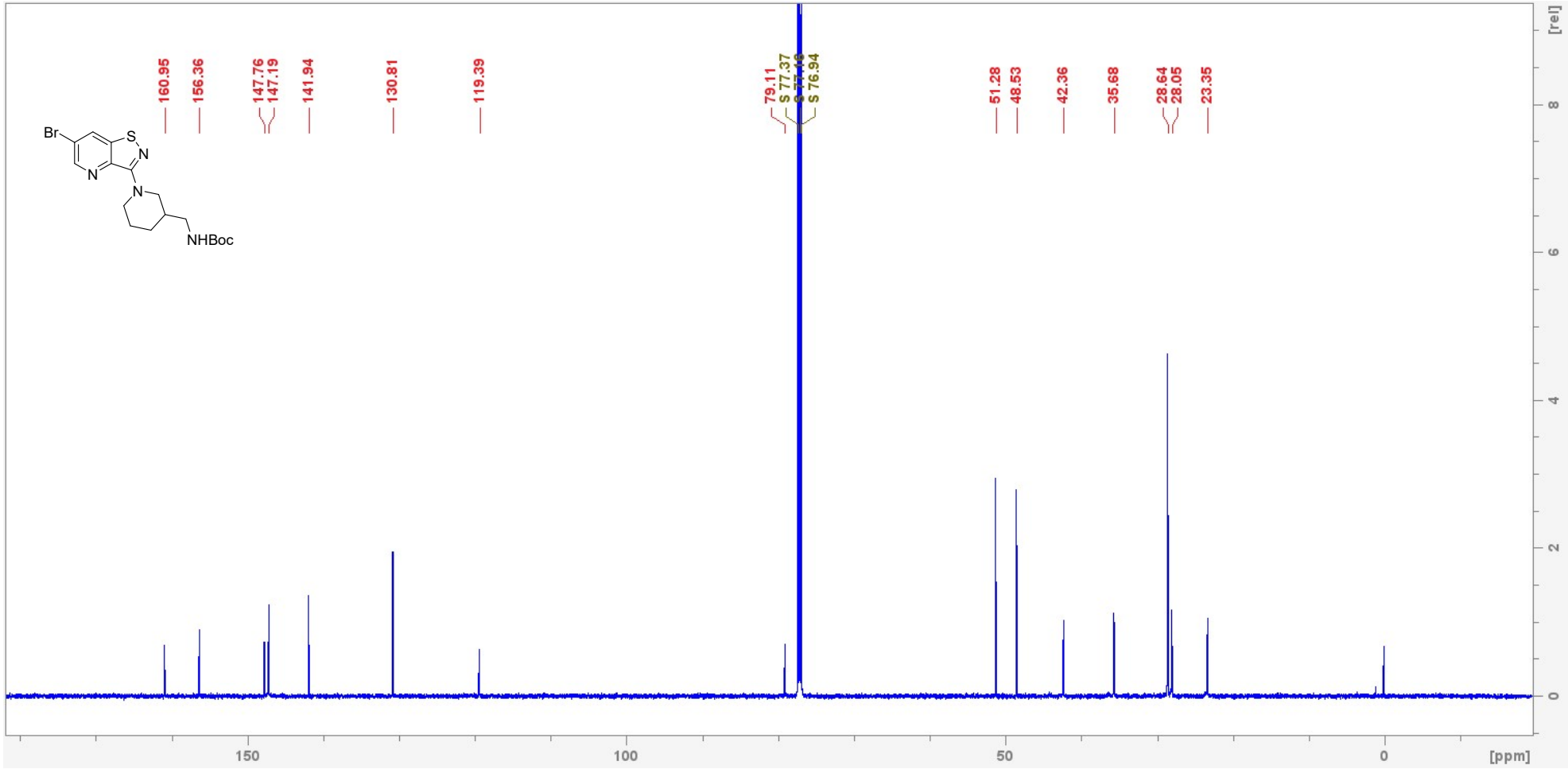


^{13}C NMR spectrum (101 MHz, $CDCl_3$) of **17d**

Tert-butyl ((1-(6-bromoisothiazolo[4,5-*b*]pyridin-3-yl)piperidin-3-yl)methyl)carbamate (17ea)

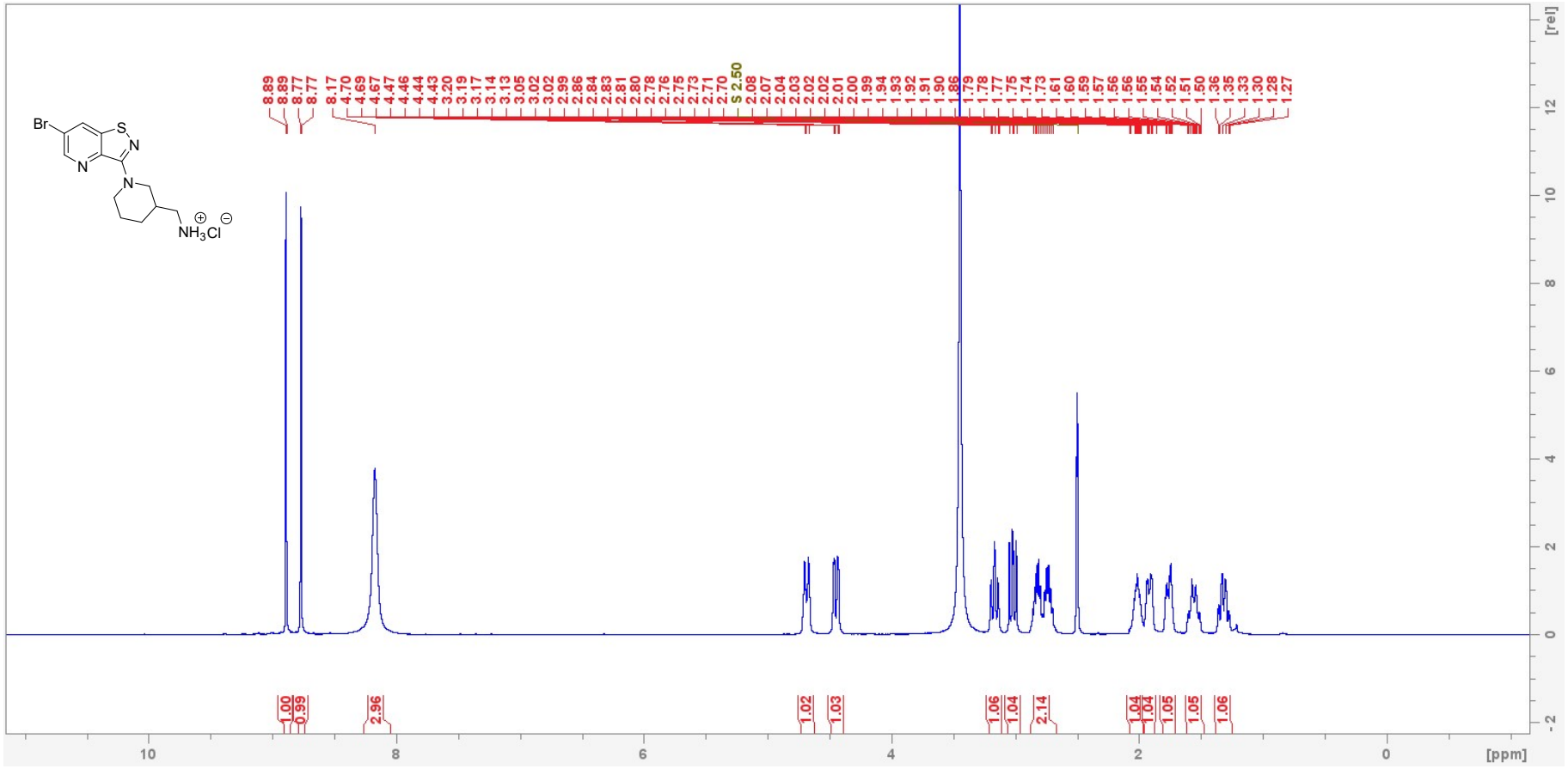


^{1}H NMR spectrum (600 MHz, $CDCl_3$) of **17ea**

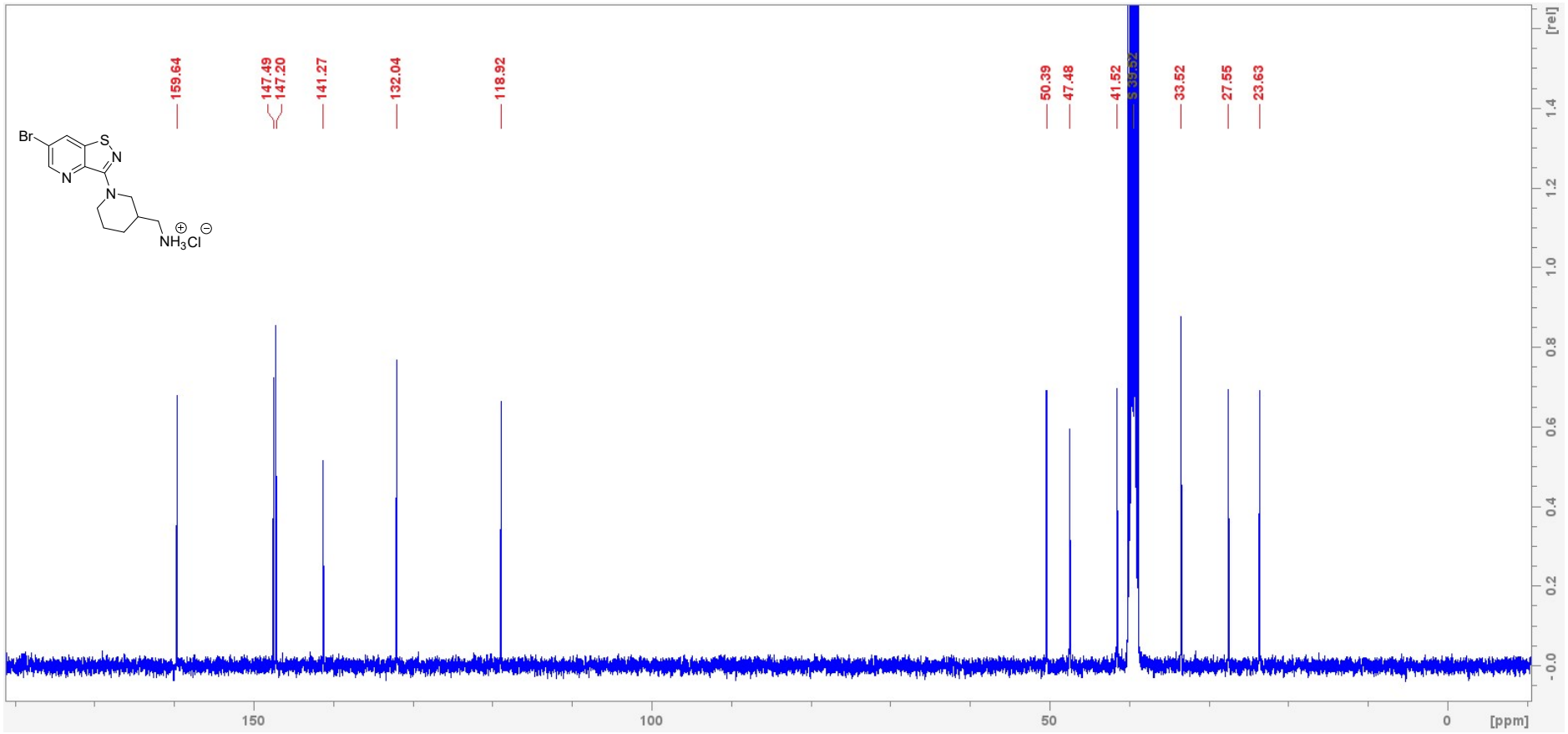


^{13}C NMR spectrum (151 MHz, $CDCl_3$) of **17ea**

(1-(6-Bromoisothiazolo[4,5-*b*]pyridin-3-yl)piperidin-3-yl)methanaminium chloride (17eb)

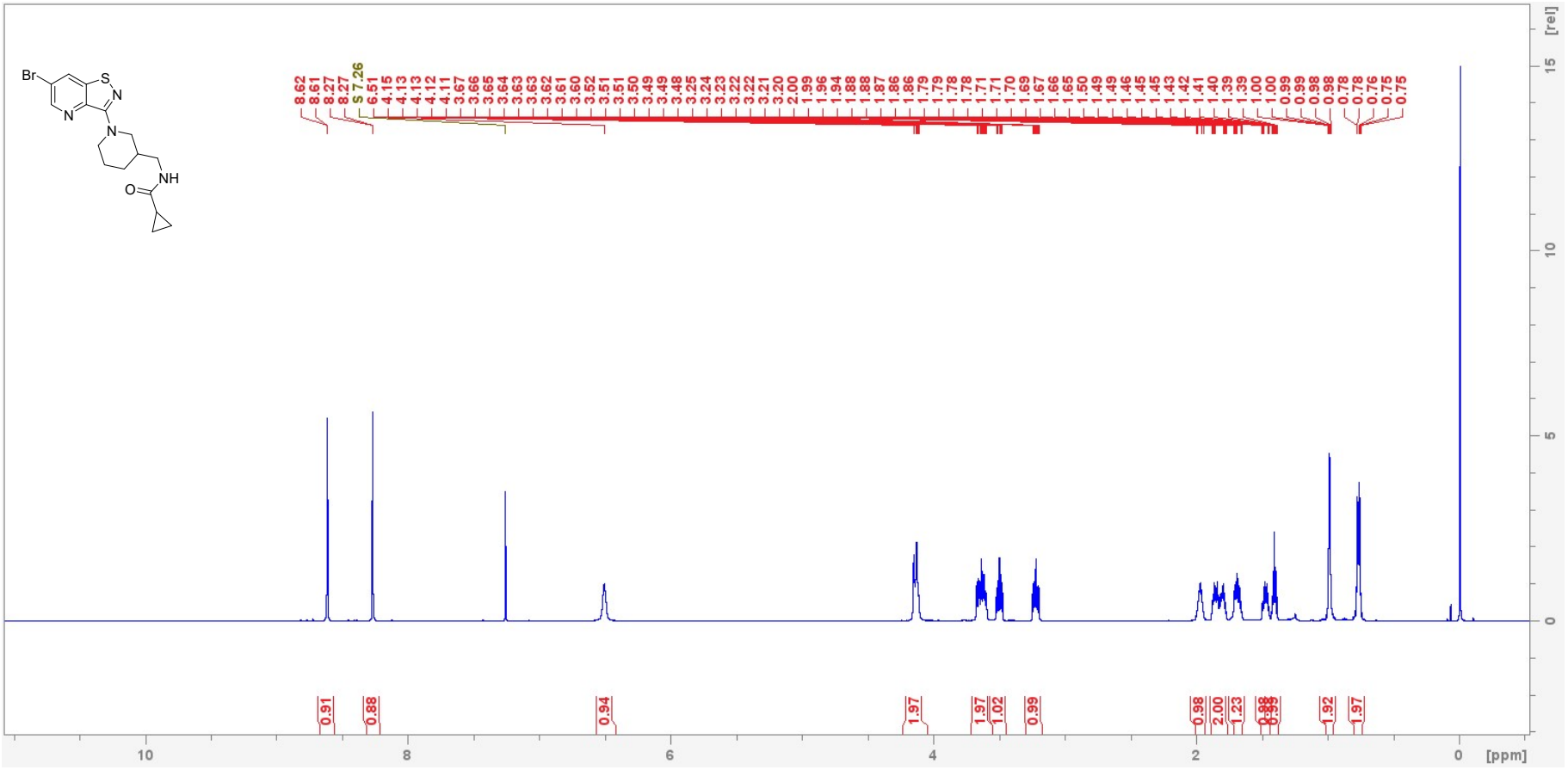


^{1}H NMR spectrum (400 MHz, DMSO-d_6) of **17eb**

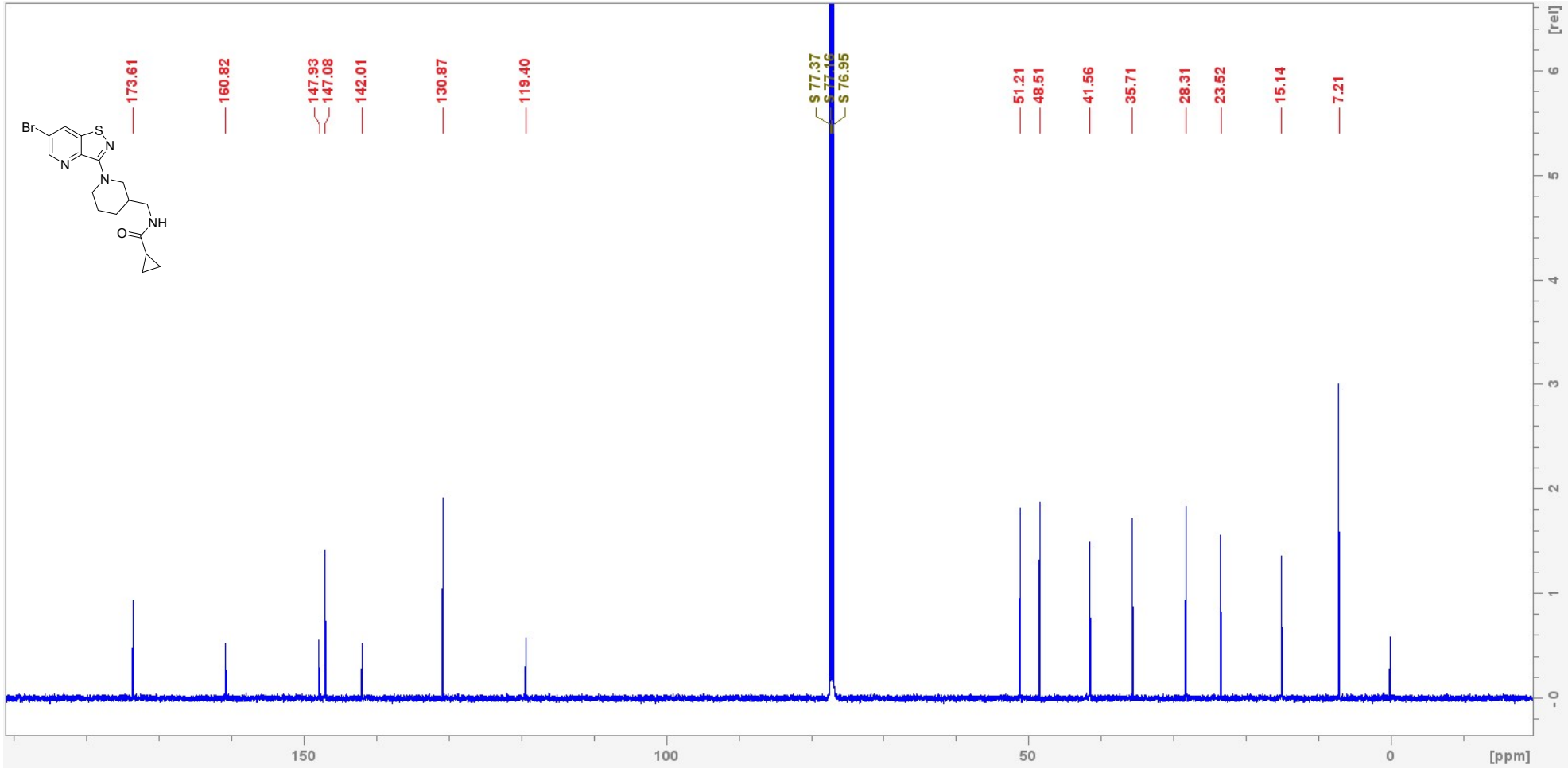


^{13}C NMR spectrum (101 MHz, DMSO-d_6) of **17eb**

N-((1-(6-bromoisothiazolo[4,5-*b*]pyridin-3-yl)piperidin-3-yl)methyl) cyclopropanecarboxamide (17ec)

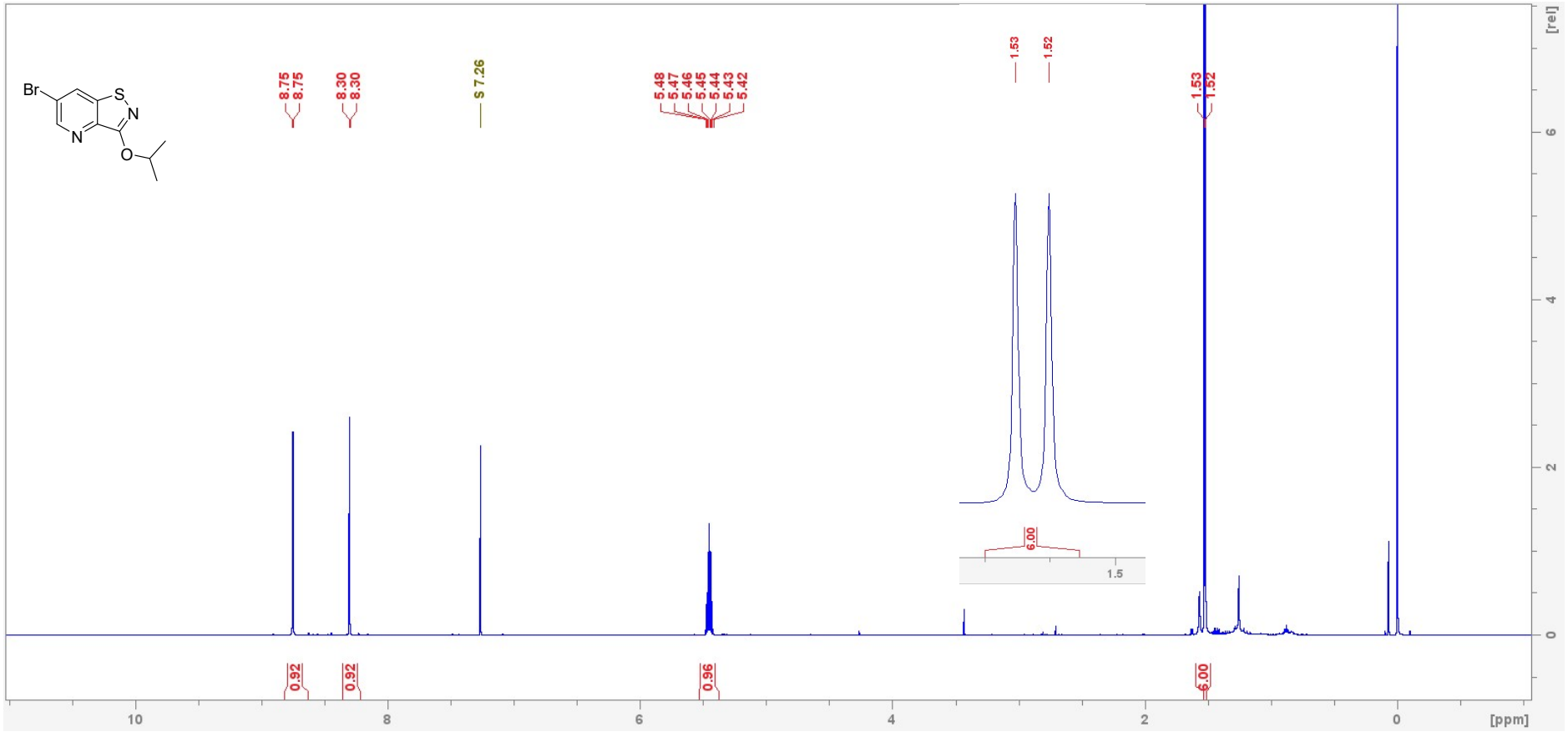


^{1}H NMR spectrum (600 MHz, $CDCl_3$) of **17ec**

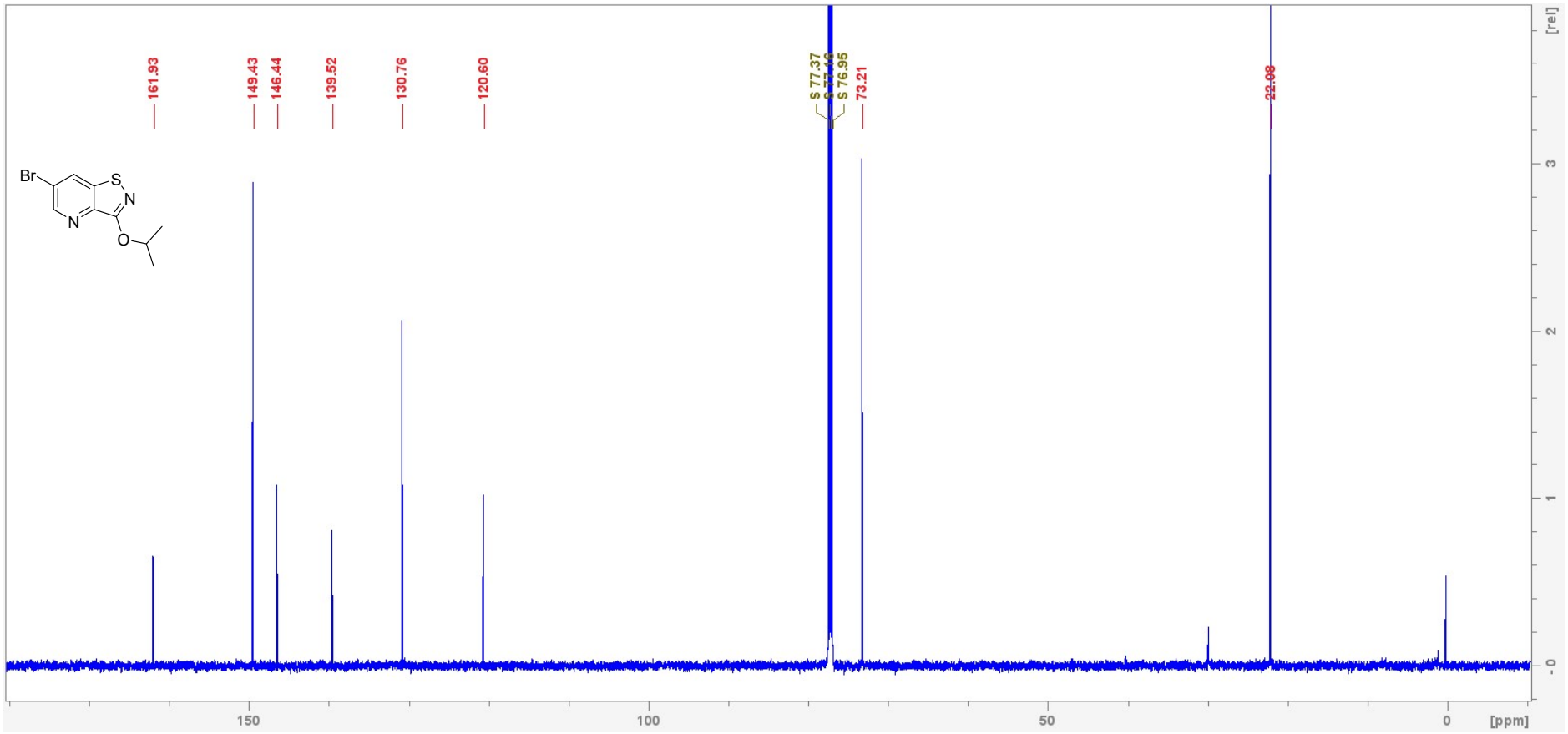


^{13}C NMR spectrum (151 MHz, $CDCl_3$) of **17ec**

6-Bromo-3-isopropoxyisothiazolo[4,5-*b*]pyridine (17f)

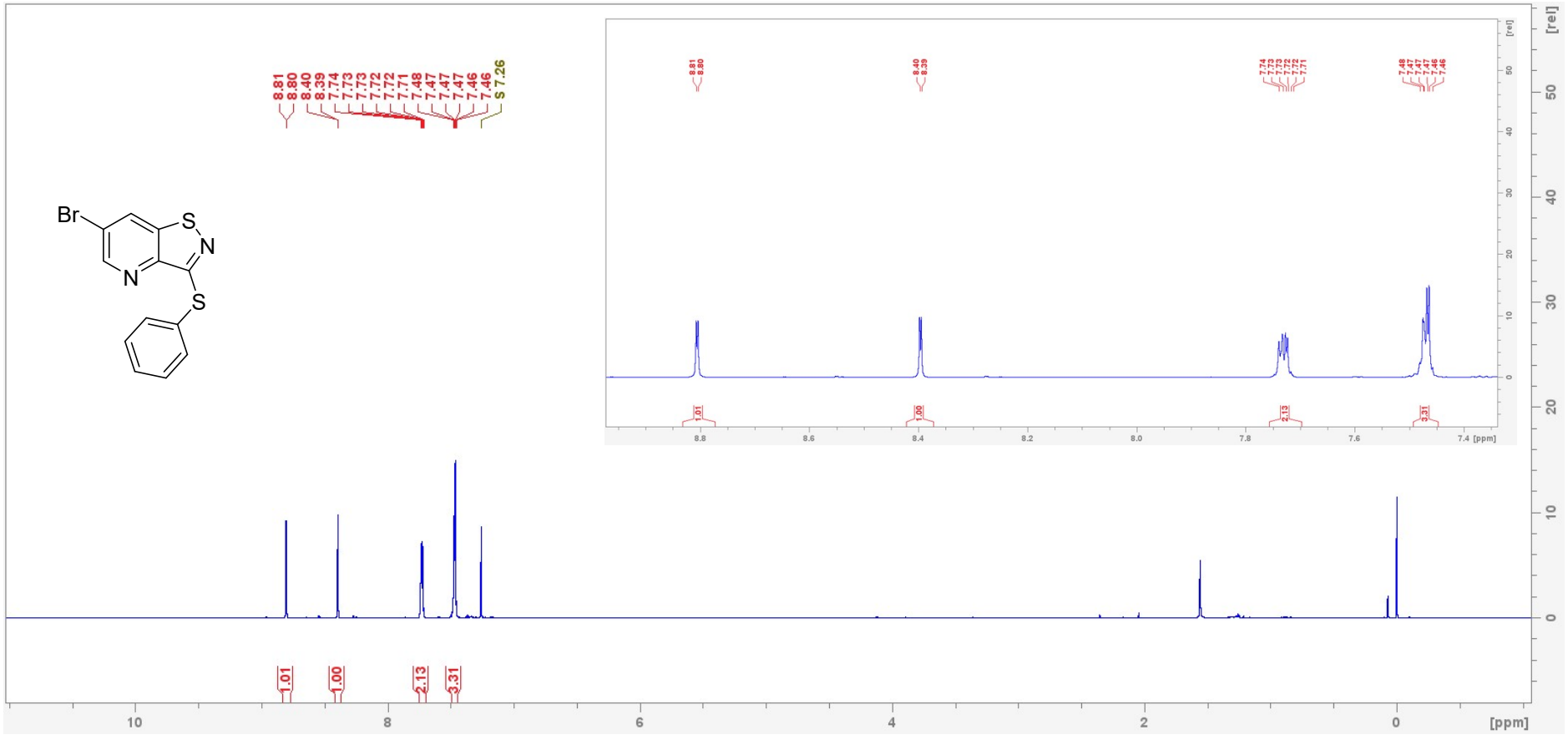


^{1}H NMR spectrum (600 MHz, $CDCl_3$) of **17f**

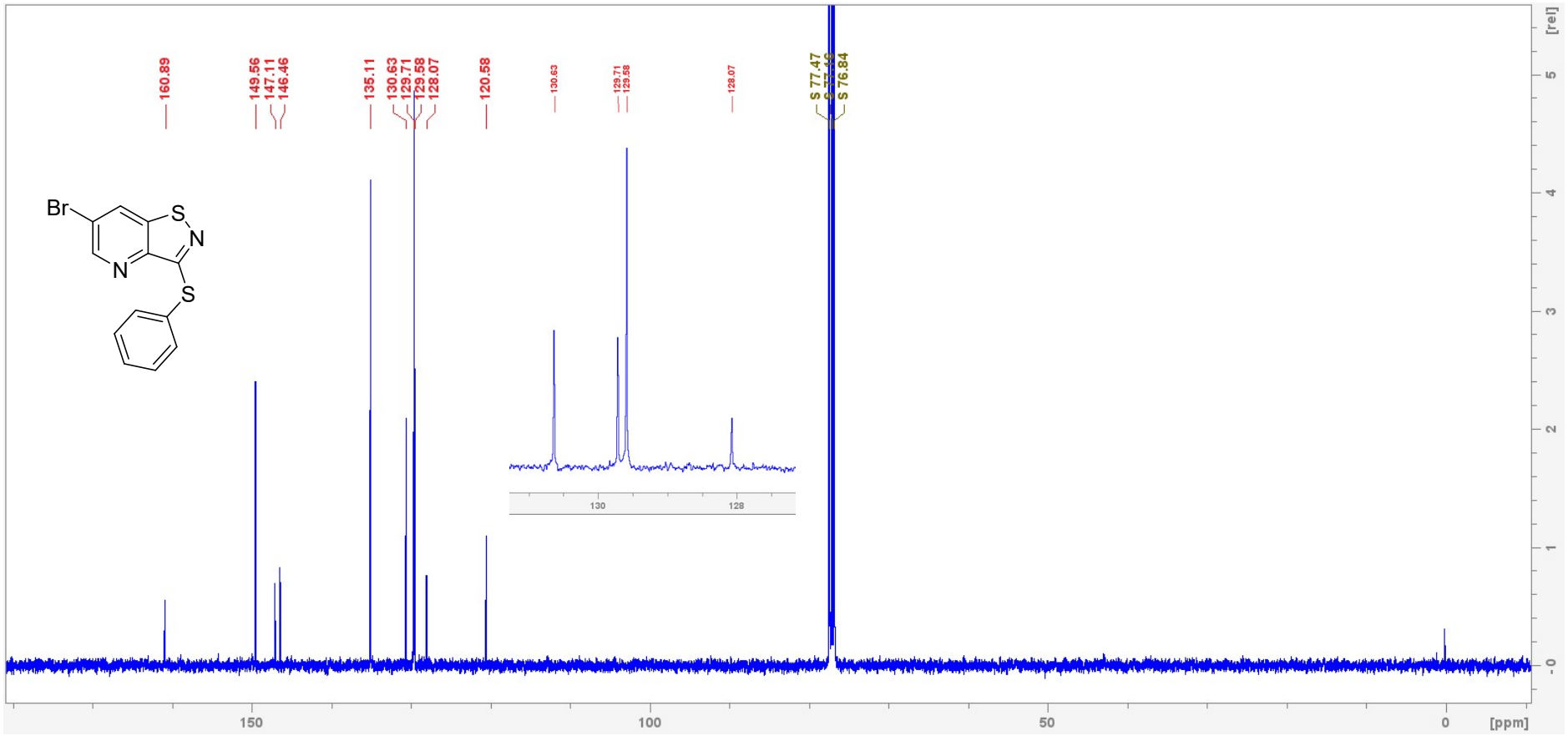


^{13}C NMR spectrum (151 MHz, $CDCl_3$) of **17f**

6-Bromo-3-(phenylthio)isothiazolo[4,5-*b*]pyridine (17g)

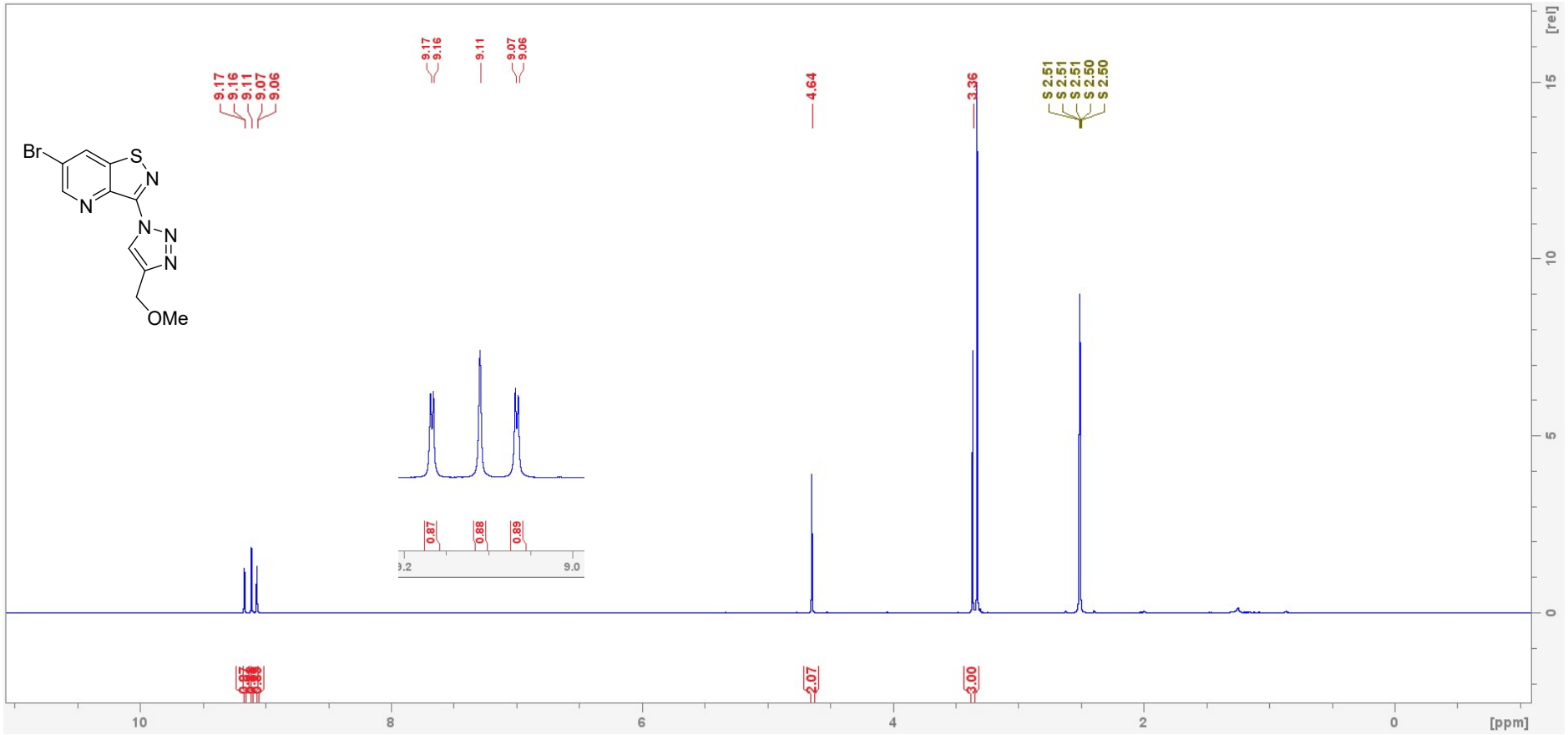


^{1}H NMR spectrum (600 MHz, $CDCl_3$) of **17g**

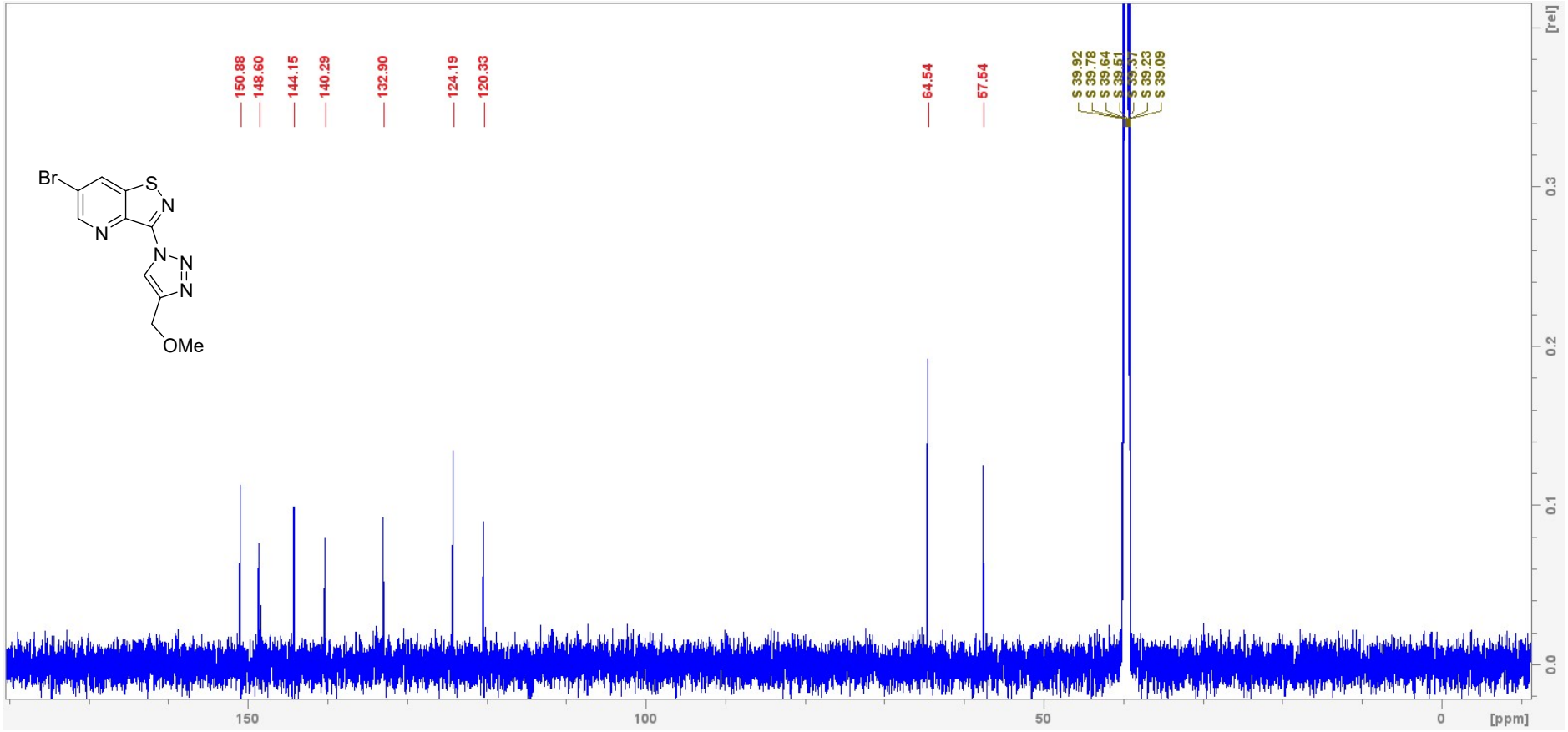


^{13}C NMR spectrum (101 MHz, $CDCl_3$) of **17g**

6-Bromo-3-(4-(methoxymethyl)-1*H*-1,2,3-triazol-1-yl)isothiazolo[4,5-*b*]pyridine (17h)

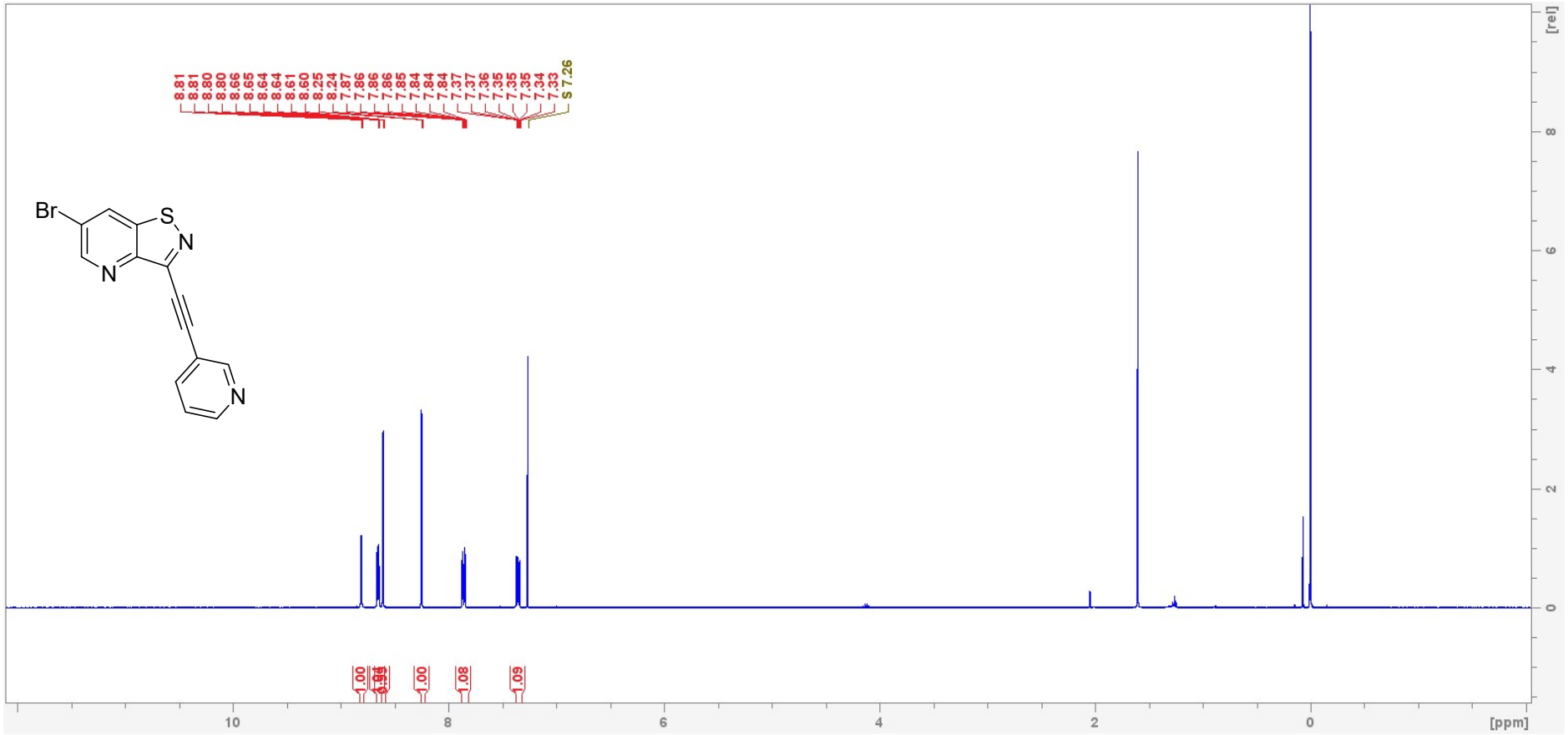


^{1}H NMR spectrum (600 MHz, DMSO-d_6) of **17h**



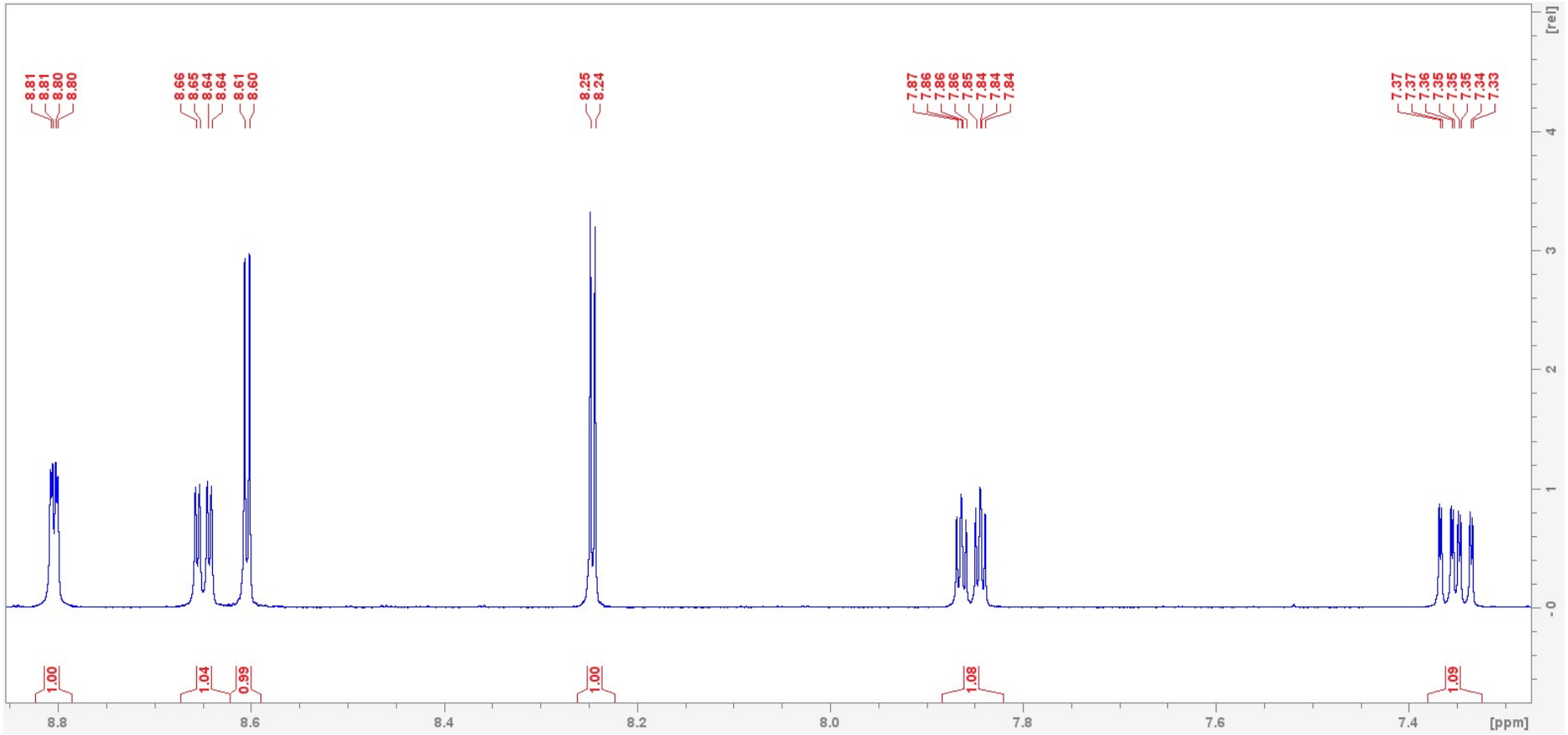
^{13}C NMR spectrum (151 MHz, DMSO-d_6) of **17h** (low concentration due to reduced solubility of **17h**)

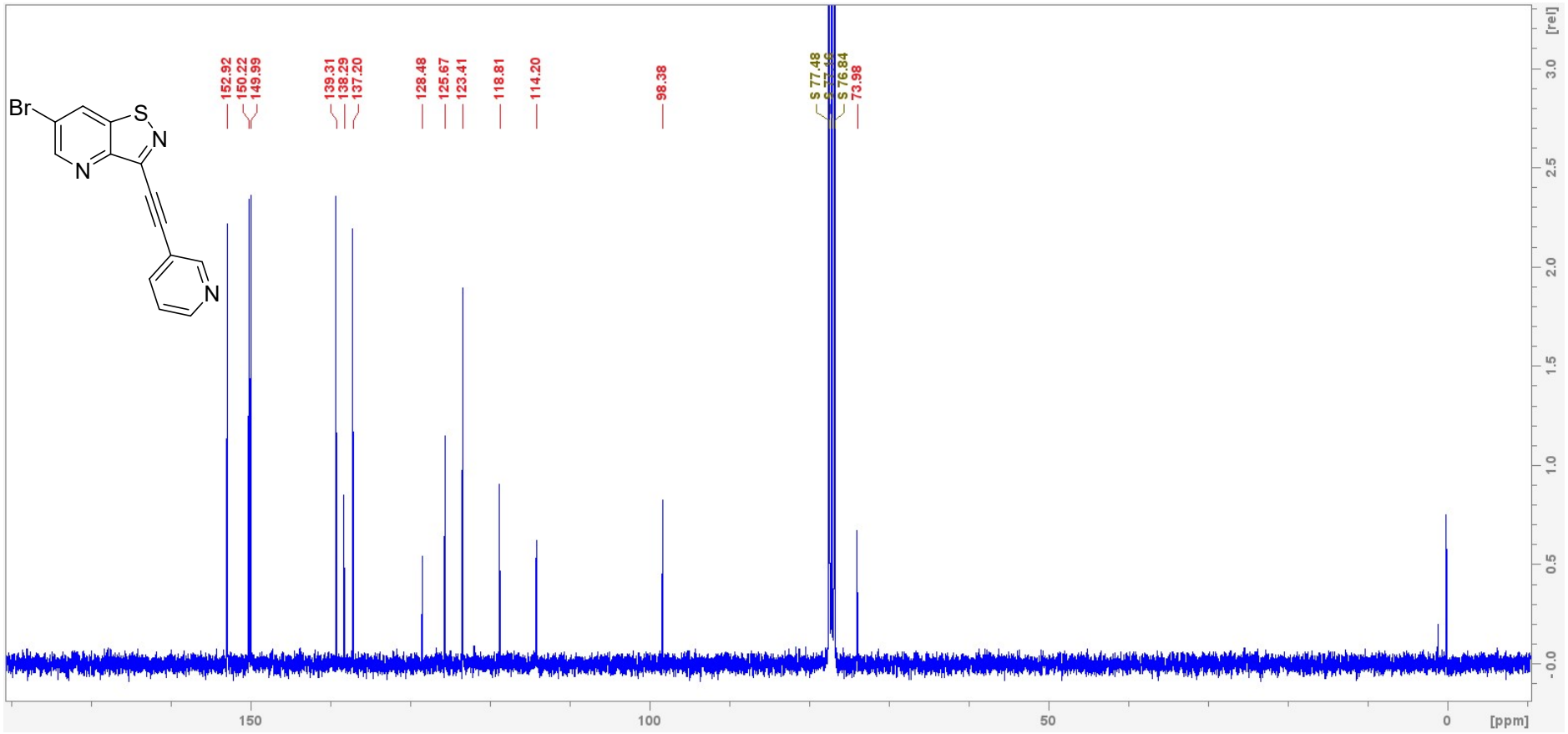
6-Bromo-3-(pyridin-3-ylethynyl)isothiazolo[4,5-*b*]pyridine (17i)



^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **17i**

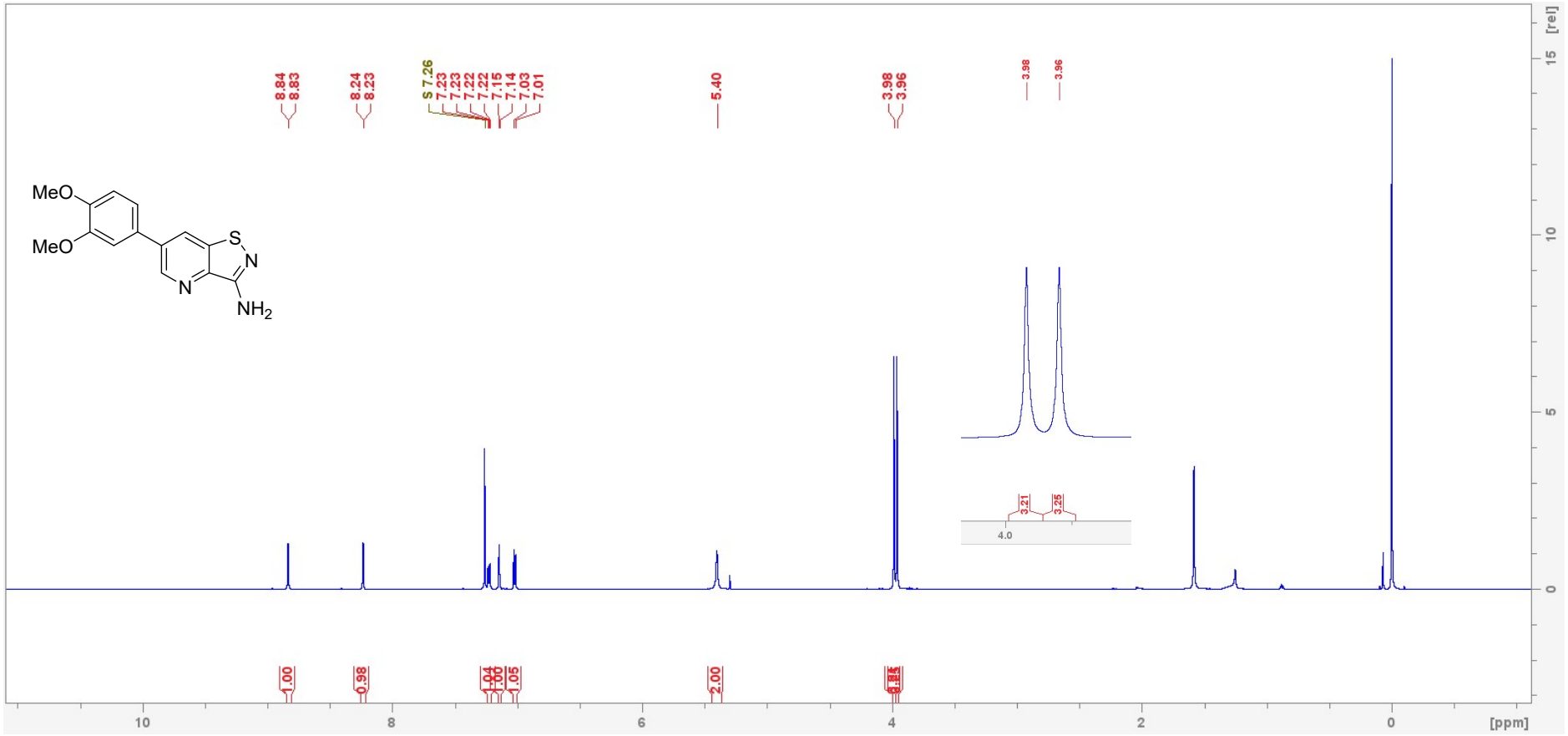
Zoom-in on the aromatic area:



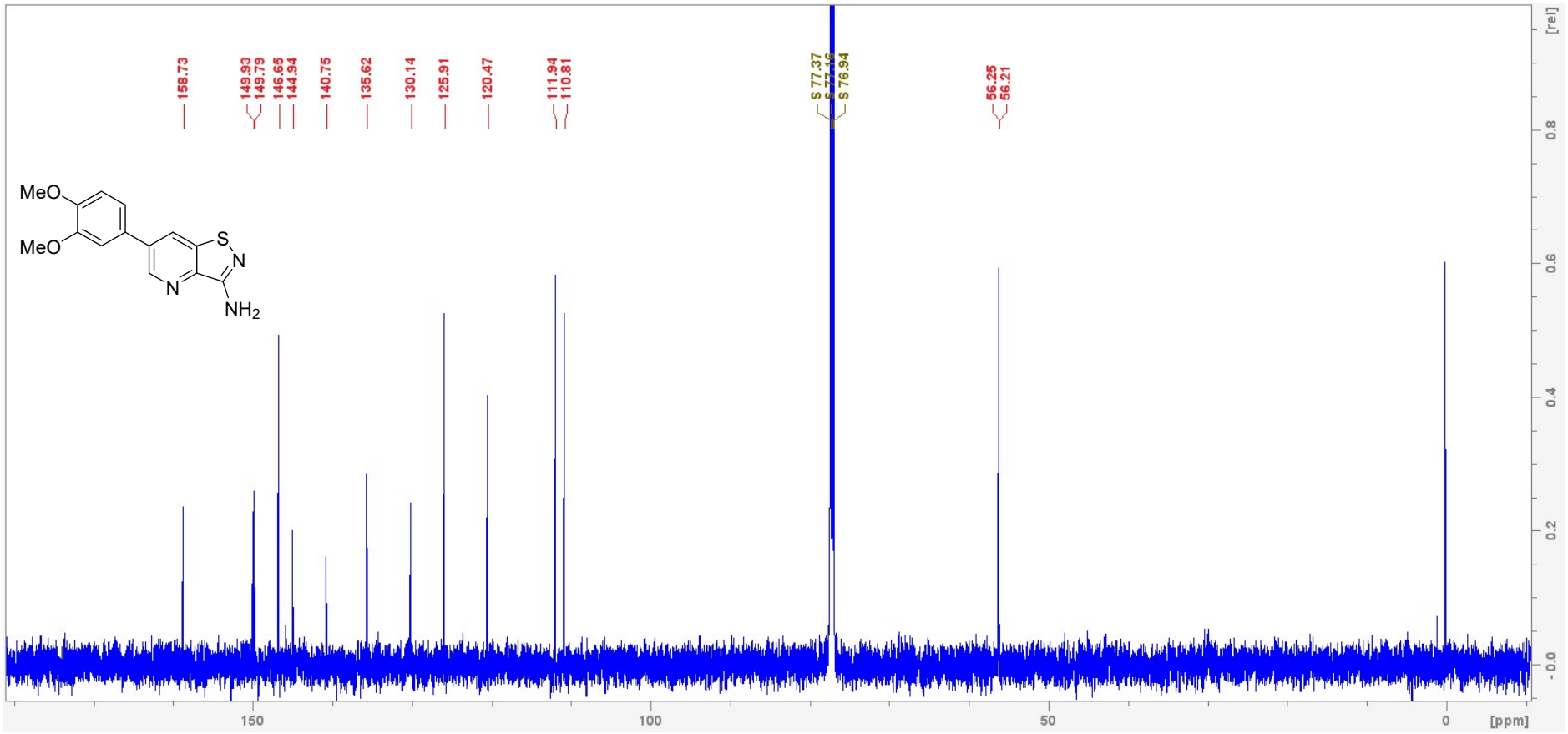


^{13}C NMR spectrum (101 MHz, $CDCl_3$) of **17i**

6-(3,4-Dimethoxyphenyl)isothiazolo[4,5-*b*]pyridin-3-amine (18a)

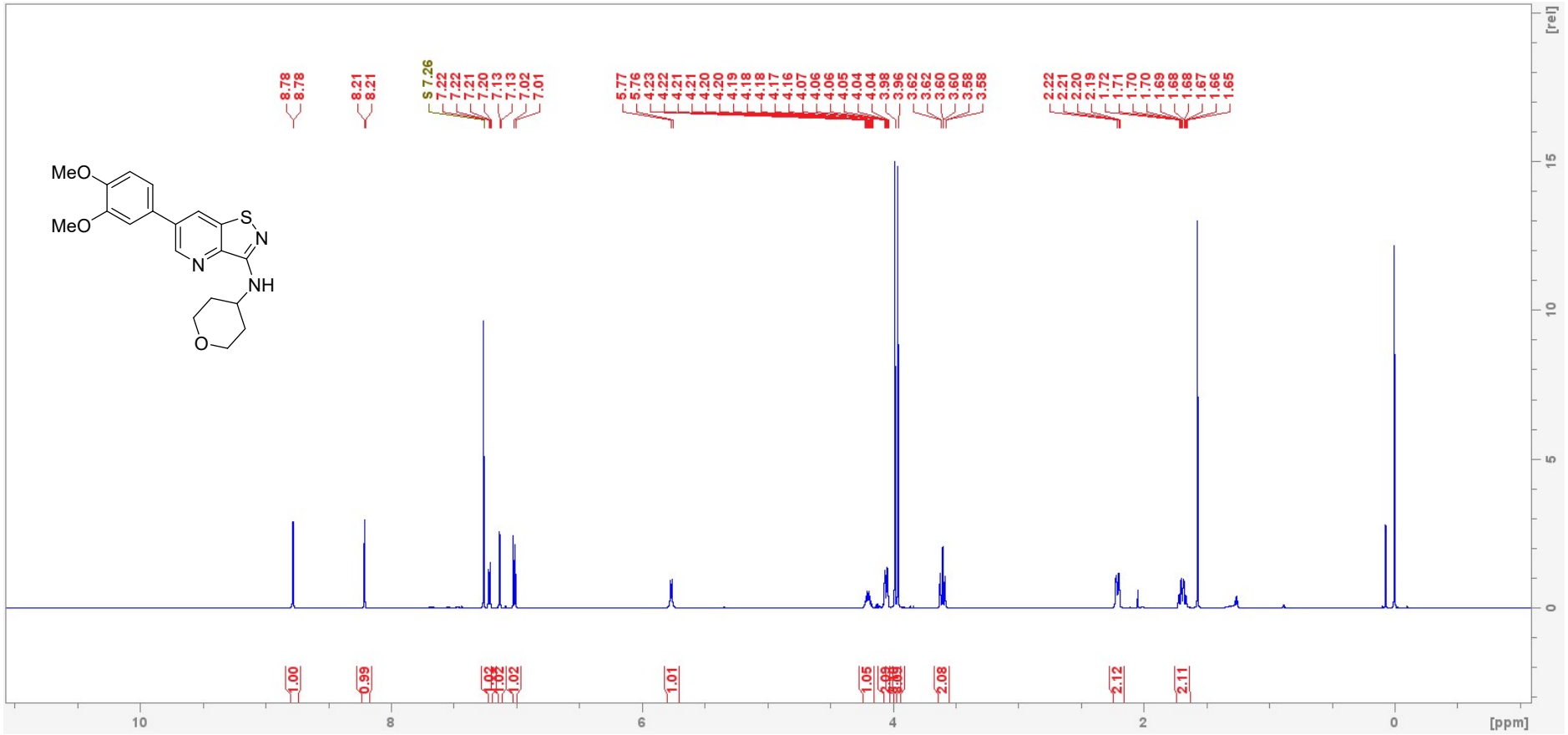


^{1}H NMR spectrum (600 MHz, $CDCl_3$) of **18a**



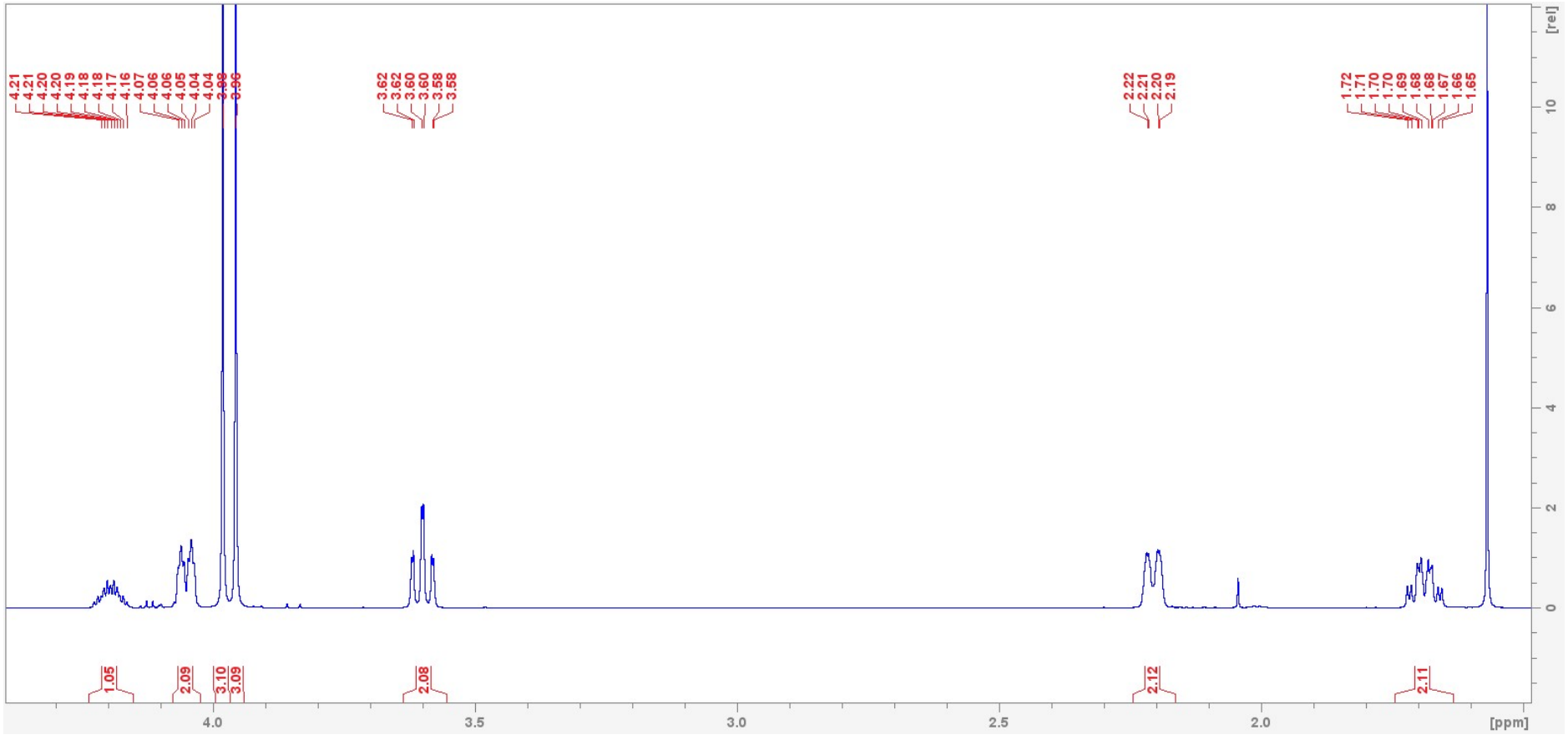
^{13}C NMR spectrum (151 MHz, $CDCl_3$) of **18a**

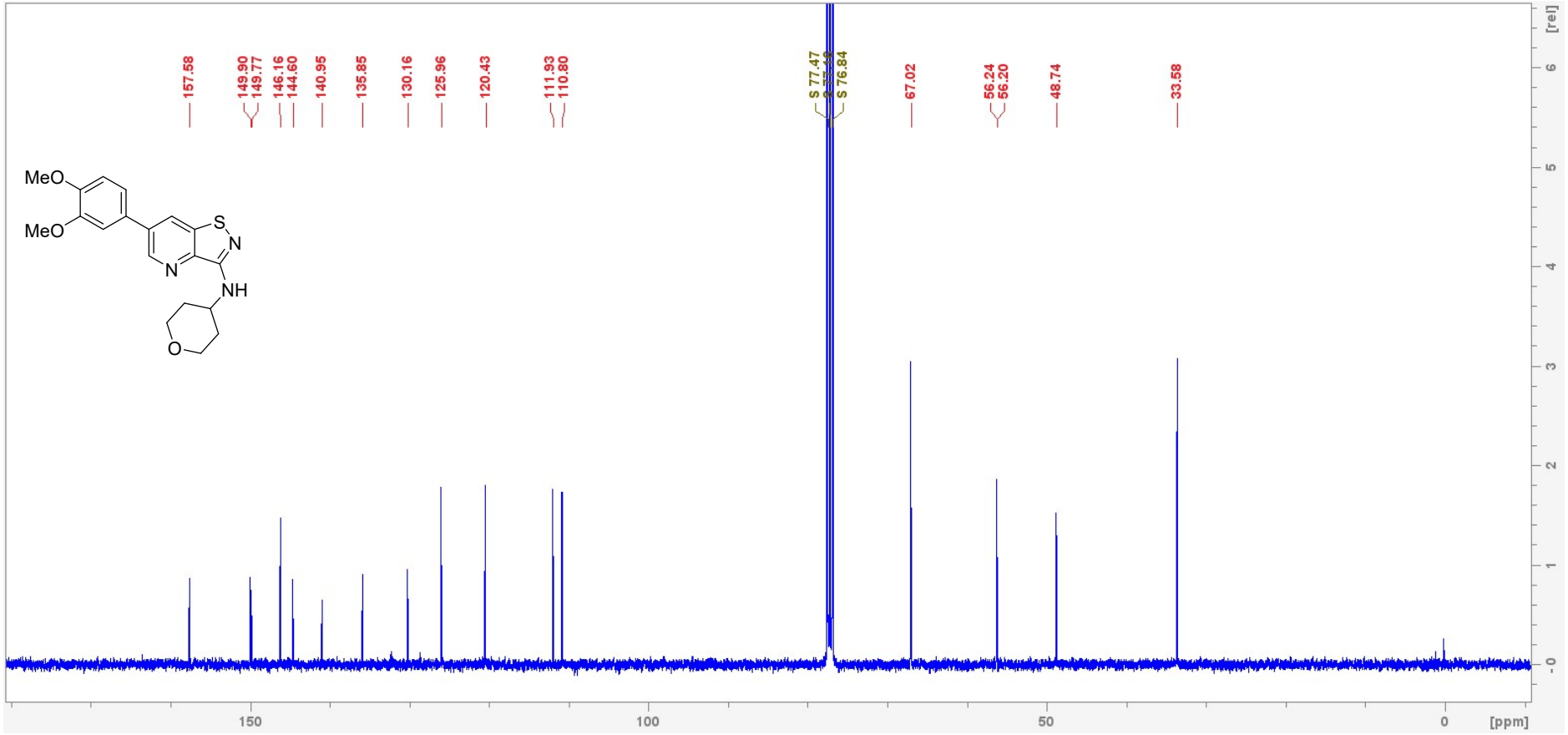
6-(3,4-Dimethoxyphenyl)-*N*-(tetrahydro-2*H*-pyran-4-yl)isothiazolo[4,5-*b*]pyridin-3-amine (18b)



^{1}H NMR spectrum (600 MHz, $CDCl_3$) of **18b**

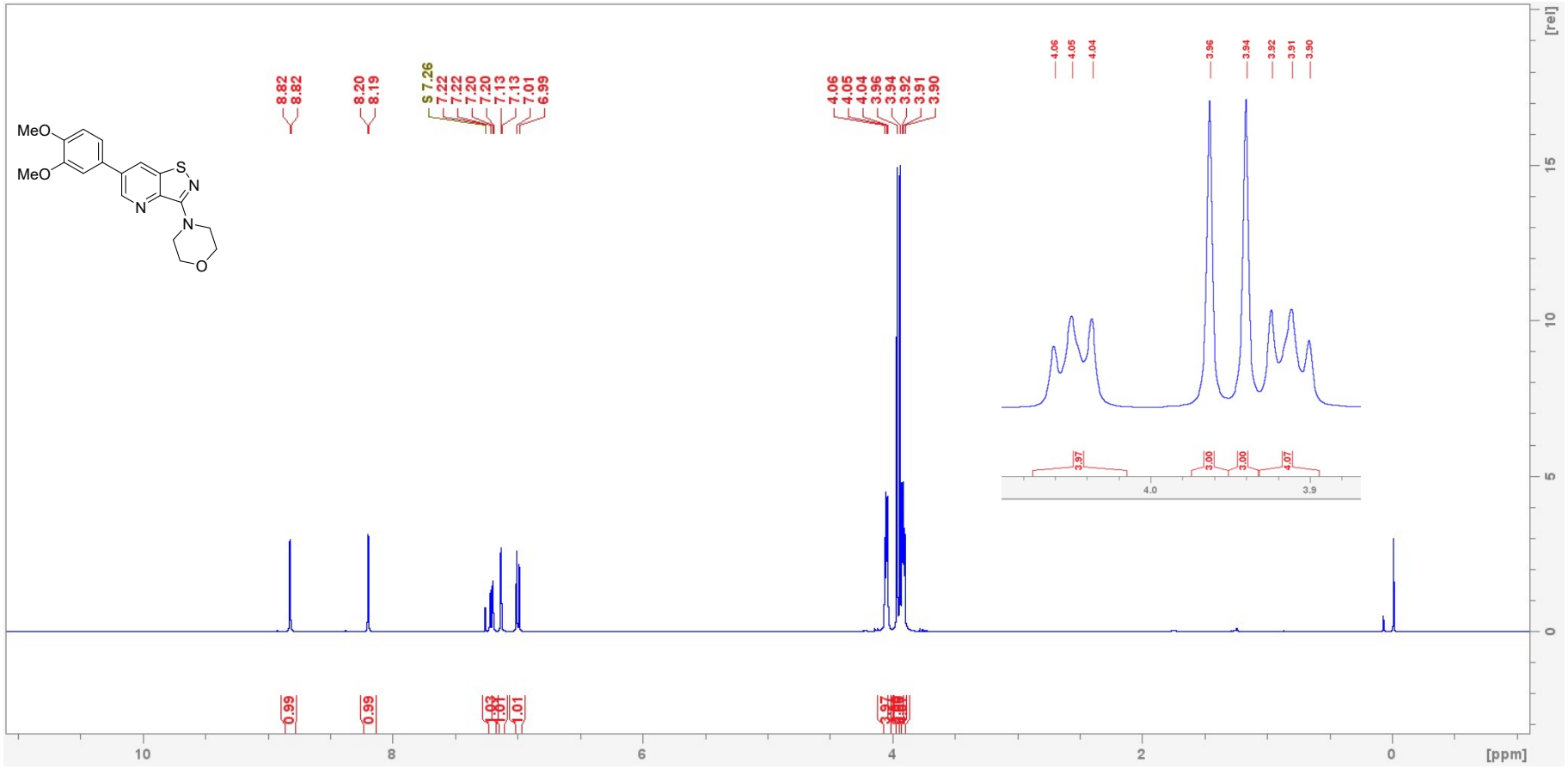
Zoom-in on the aliphatic area:



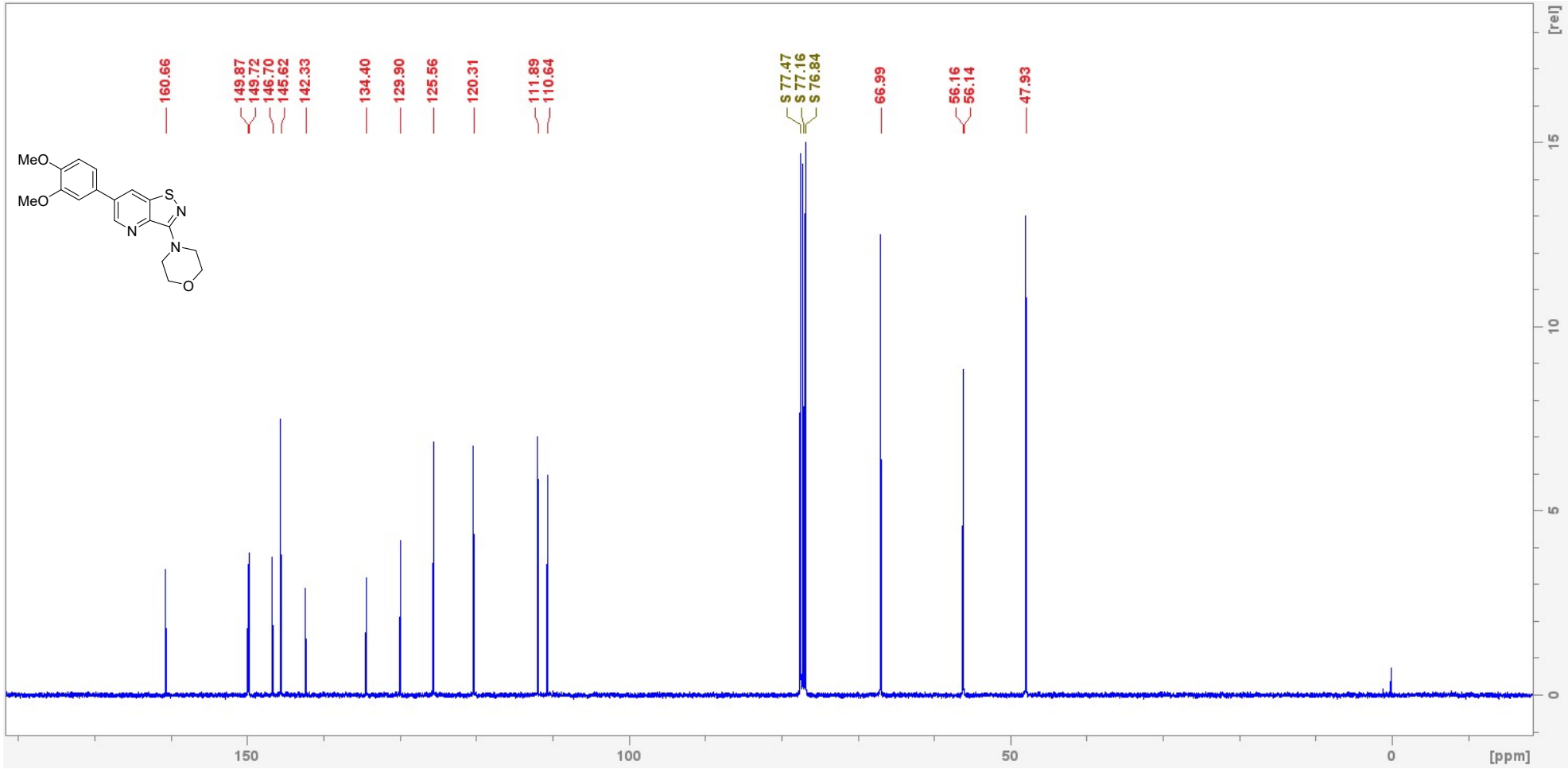


^{13}C NMR spectrum (101 MHz, $CDCl_3$) of **18b**

4-(6-(3,4-Dimethoxyphenyl)isothiazolo[4,5-*b*]pyridin-3-yl)morpholine (18c)

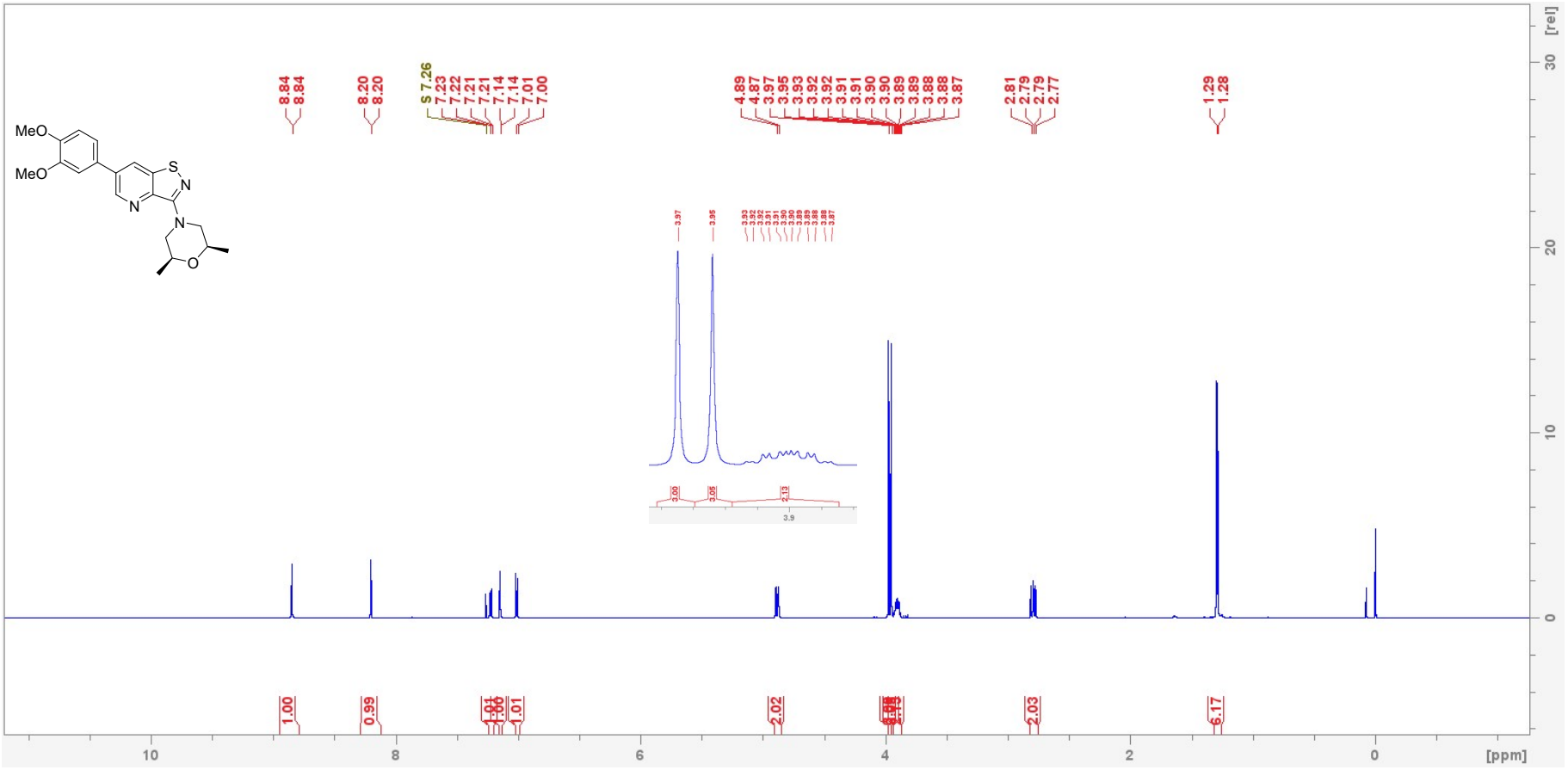


^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **18c**

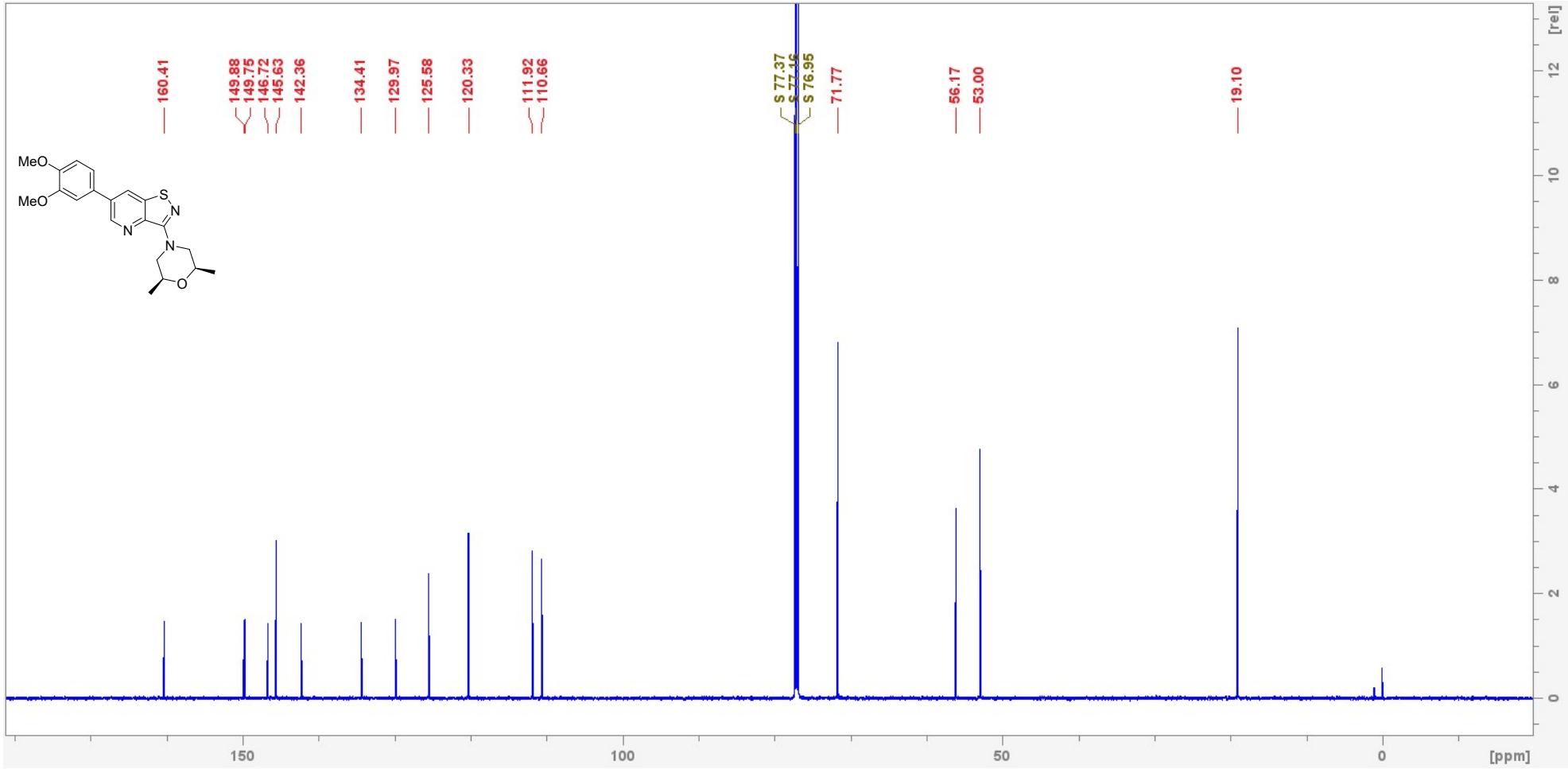


^{13}C NMR spectrum (101 MHz, $CDCl_3$) of **18c**

(2S,6R)-4-(6-(3,4-Dimethoxyphenyl)isothiazolo[4,5-*b*]pyridin-3-yl)-2,6-dimethylmorpholine (18d)

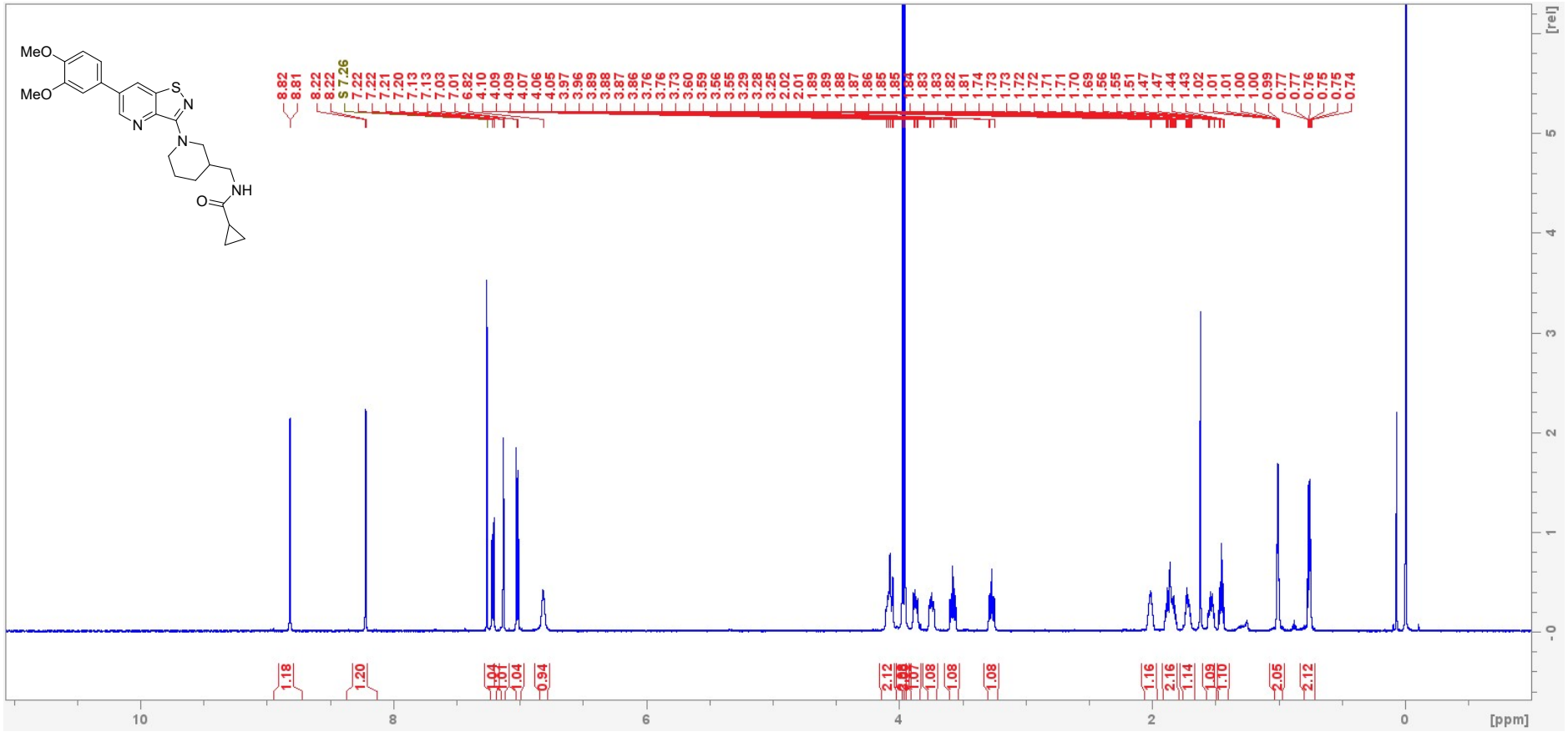


^{1}H NMR spectrum (600 MHz, $CDCl_3$) of **18d**



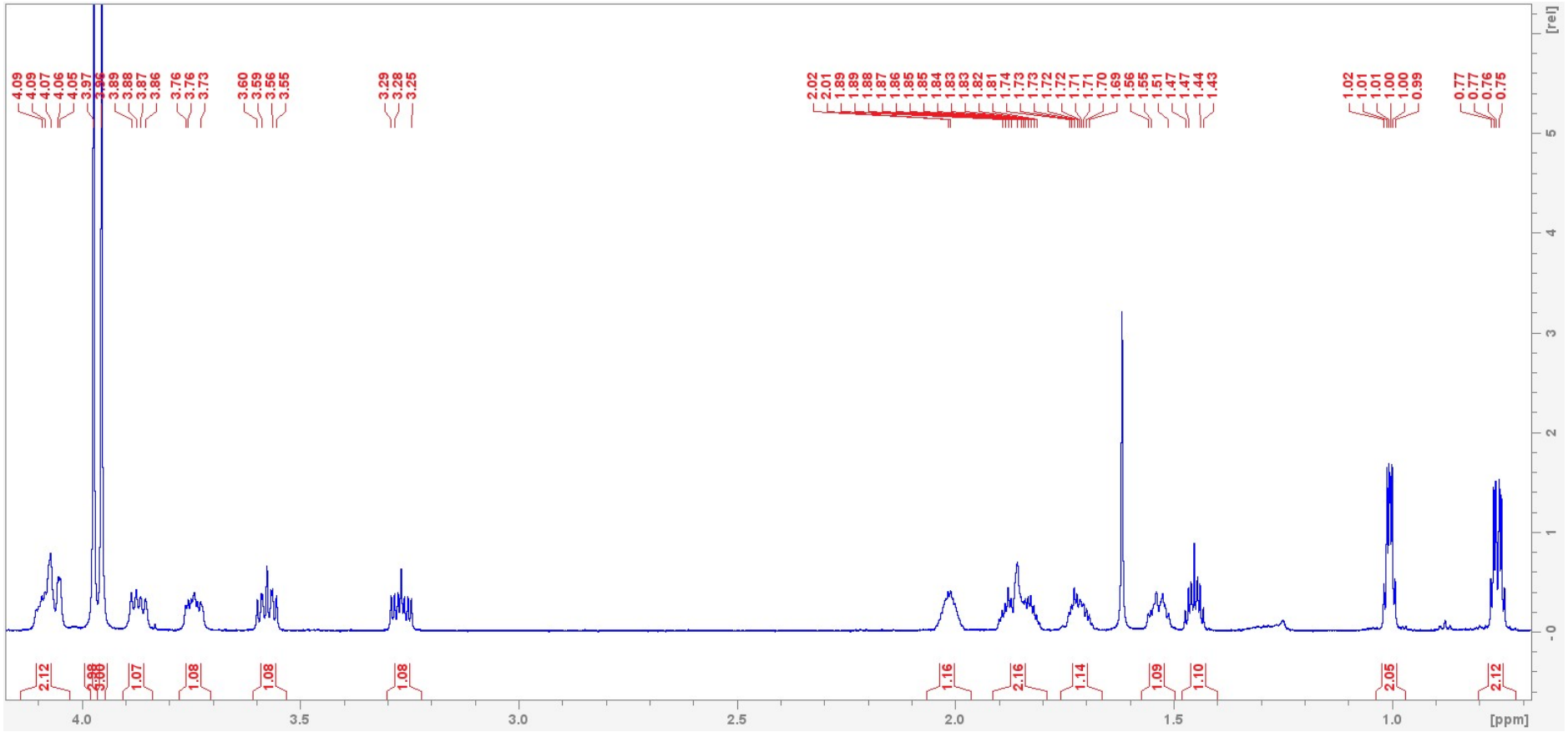
^{13}C NMR spectrum (151 MHz, $CDCl_3$) of **18d**

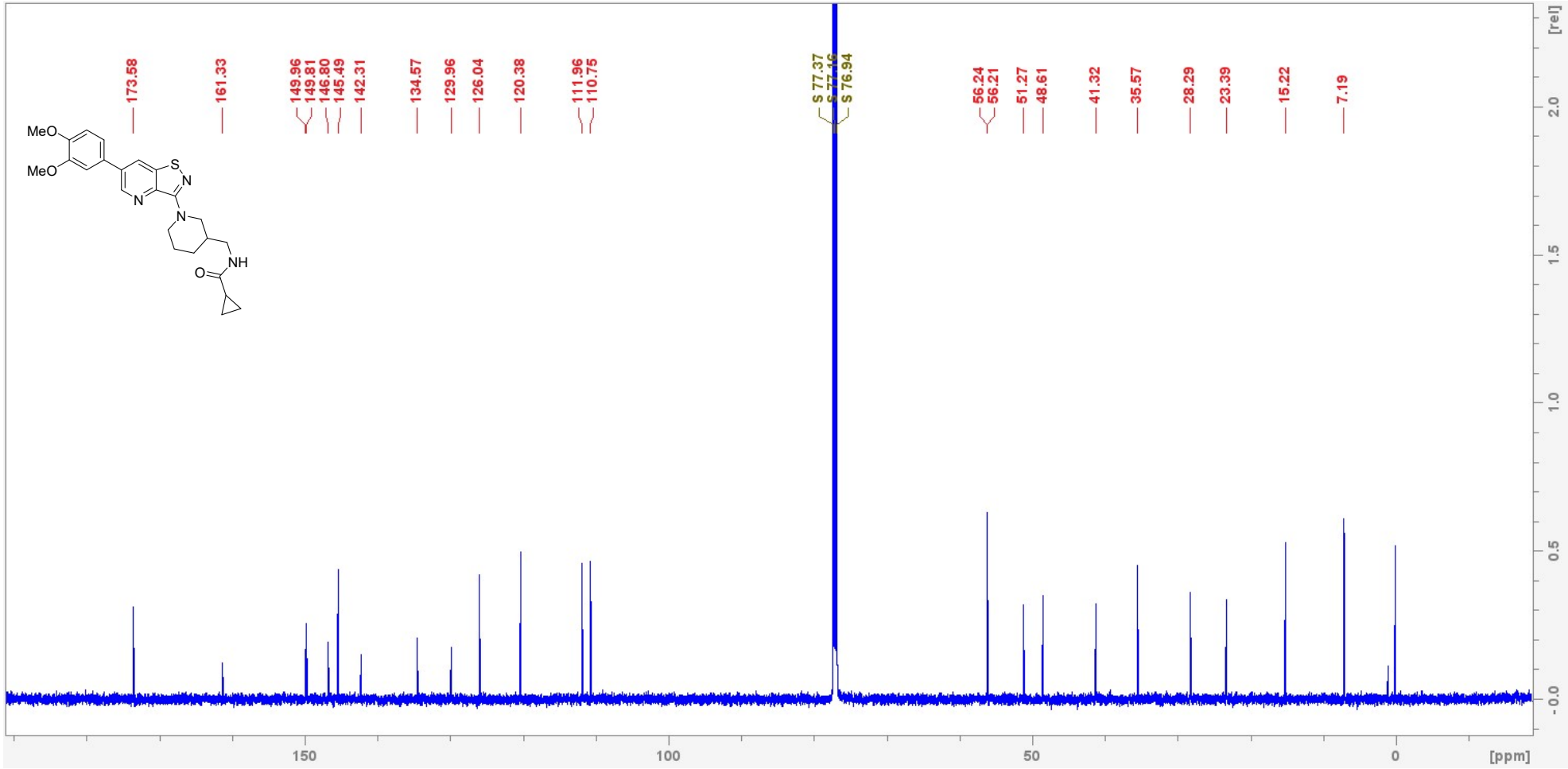
N-((1-(6-(3,4-dimethoxyphenyl)isothiazolo[4,5-*b*]pyridin-3-yl)piperidin-3-yl)methyl)cyclopropanecarboxamide (18e)



^{1}H NMR spectrum (600 MHz, $CDCl_3$) of **18e**

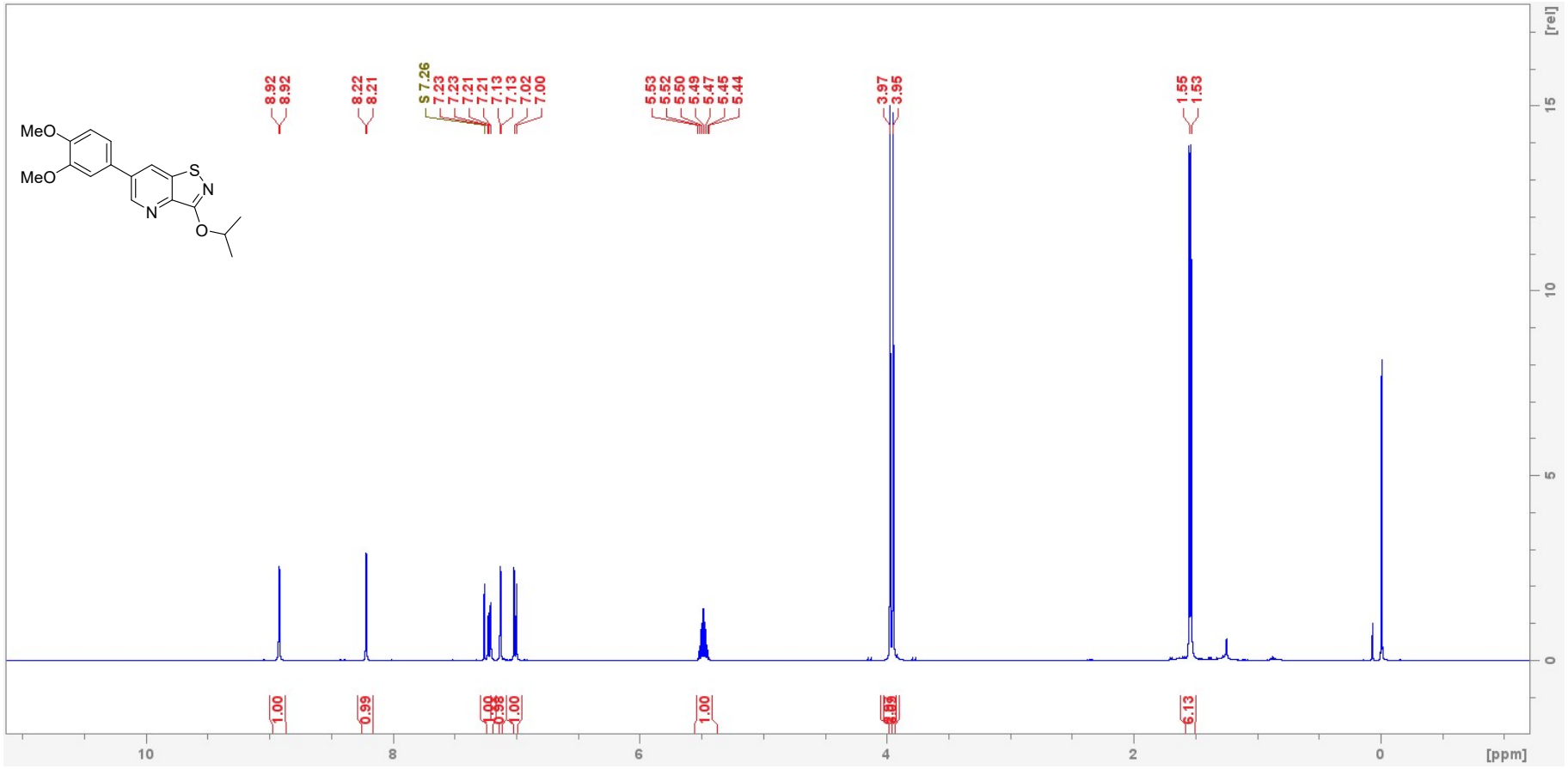
Zoom-in on the aliphatic area:



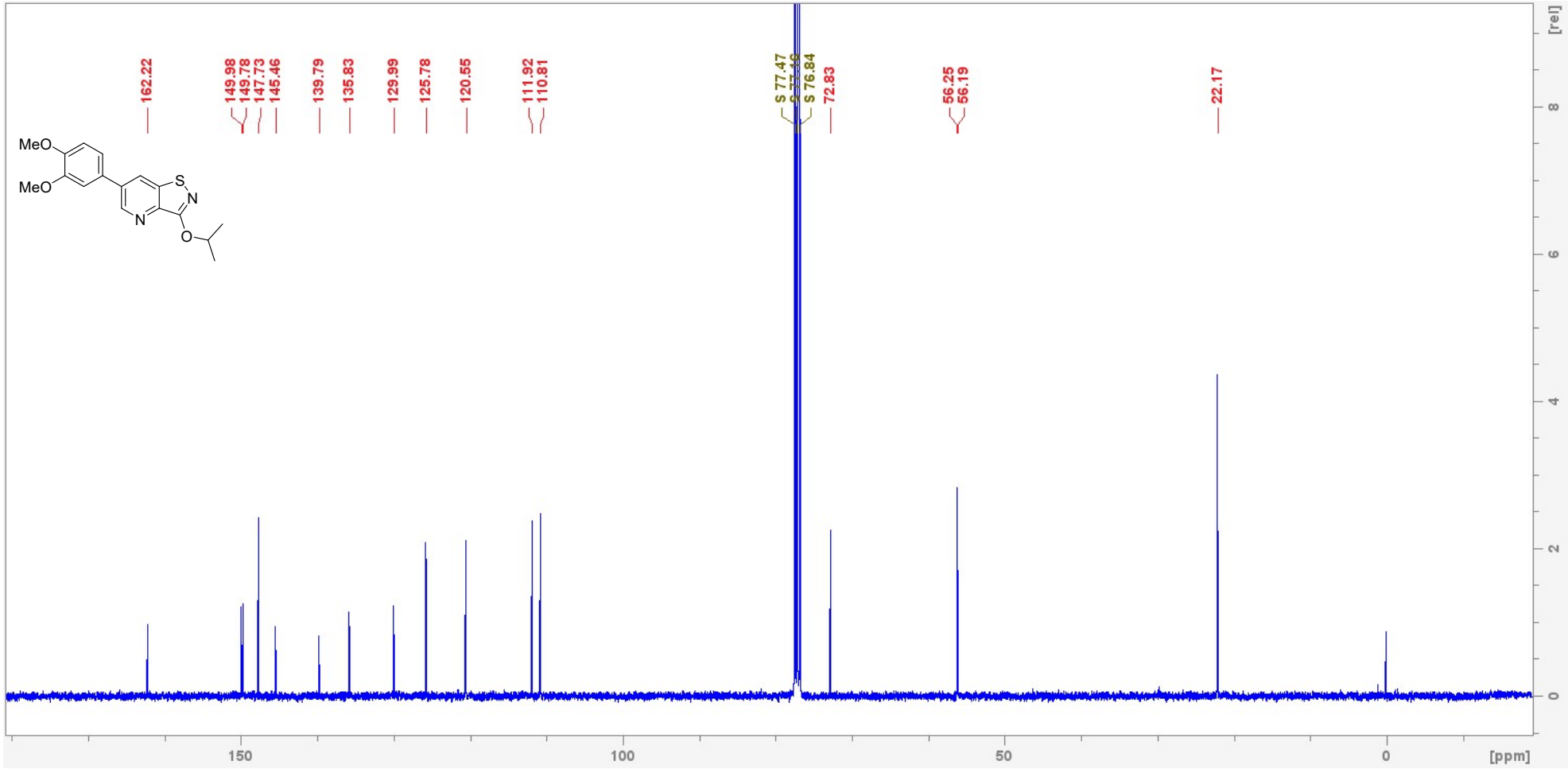


^{13}C NMR spectrum (151 MHz, $CDCl_3$) of **18e**

6-(3,4-Dimethoxyphenyl)-3-isopropoxyisothiazolo[4,5-*b*]pyridine (18f)

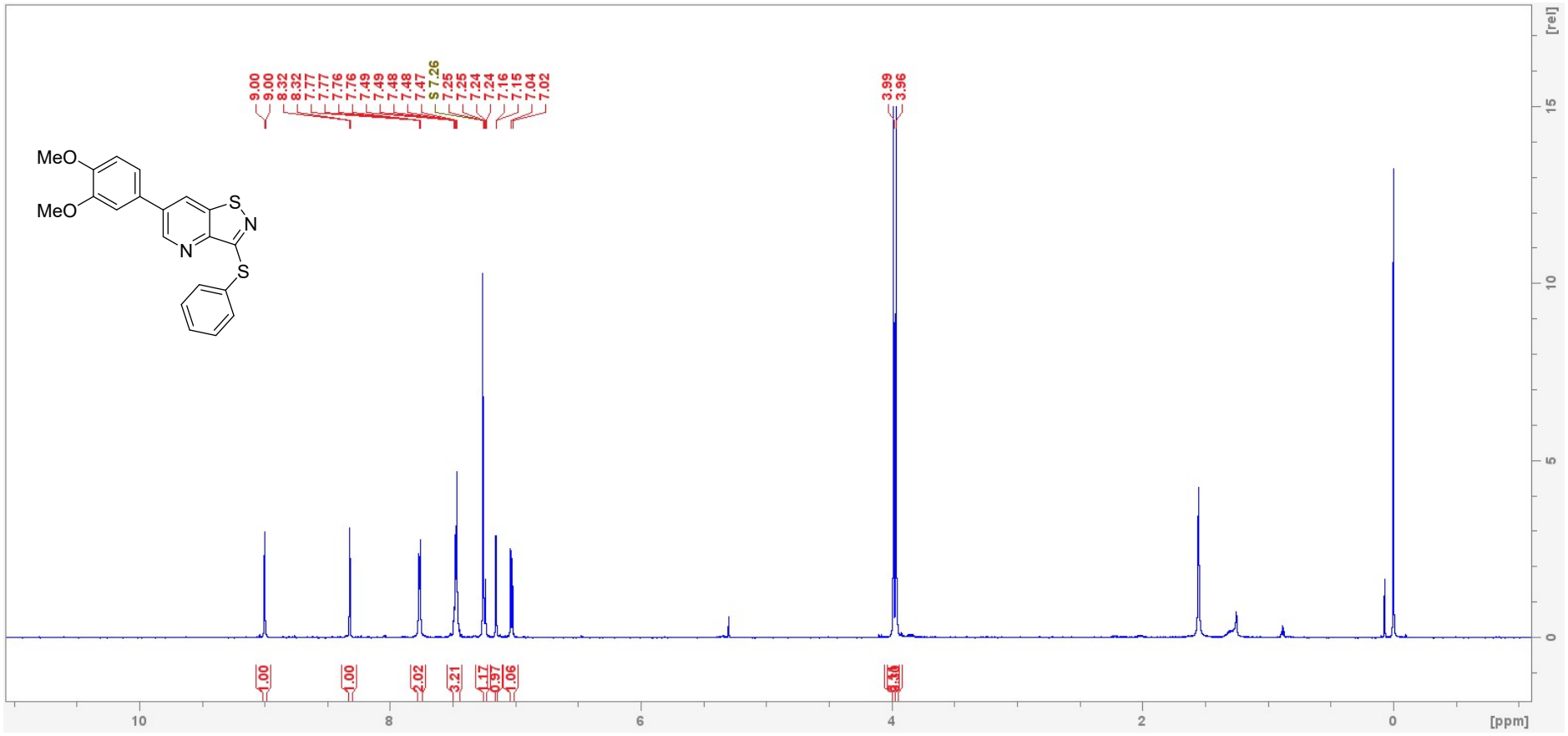


^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **18f**



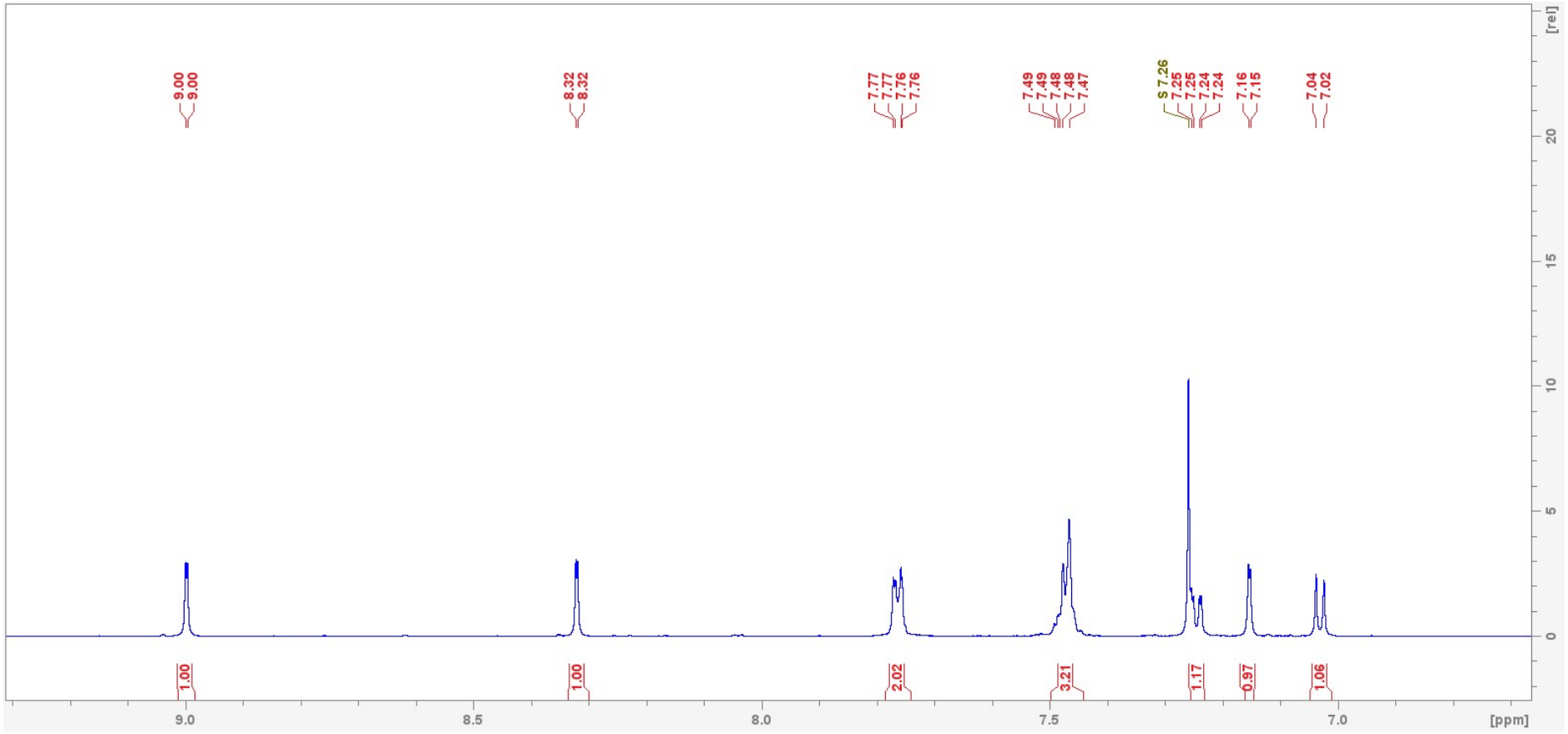
^{13}C NMR spectrum (101 MHz, $CDCl_3$) of **18f**

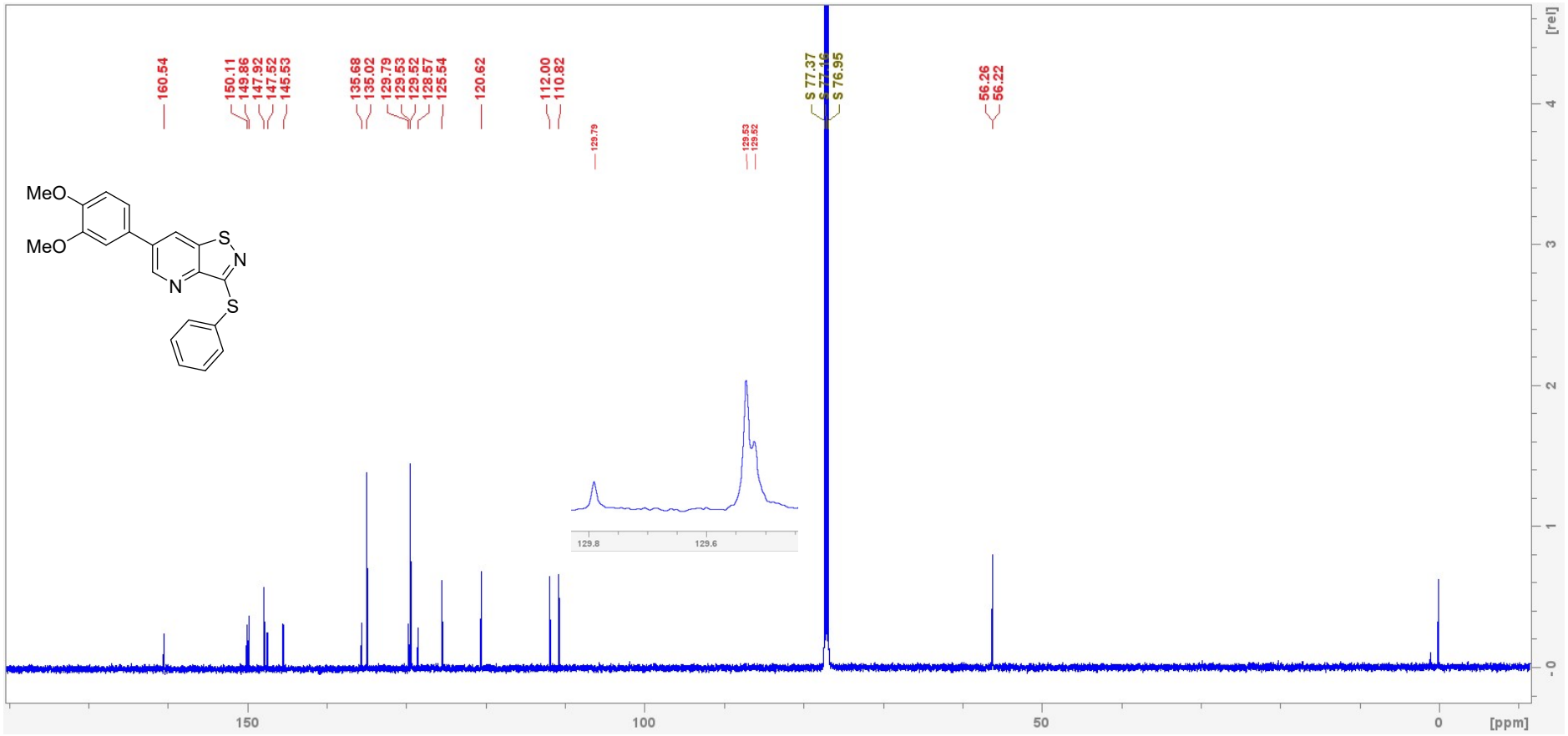
6-(3,4-Dimethoxyphenyl)-3-(phenylthio)isothiazolo[4,5-*b*]pyridine (18g)



^{1}H NMR spectrum (600 MHz, $CDCl_3$) of **18g**

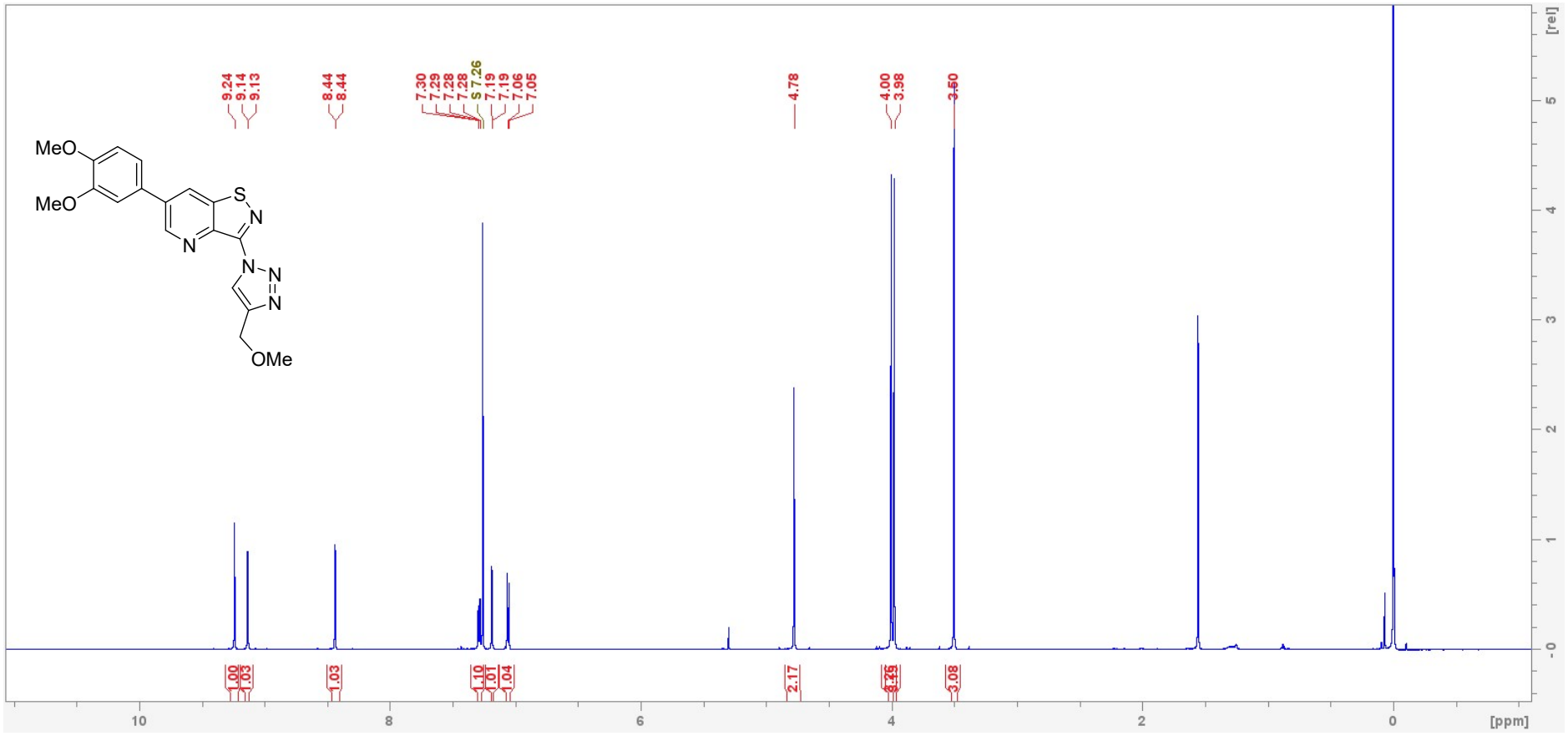
Zoom-in on the aromatic area:





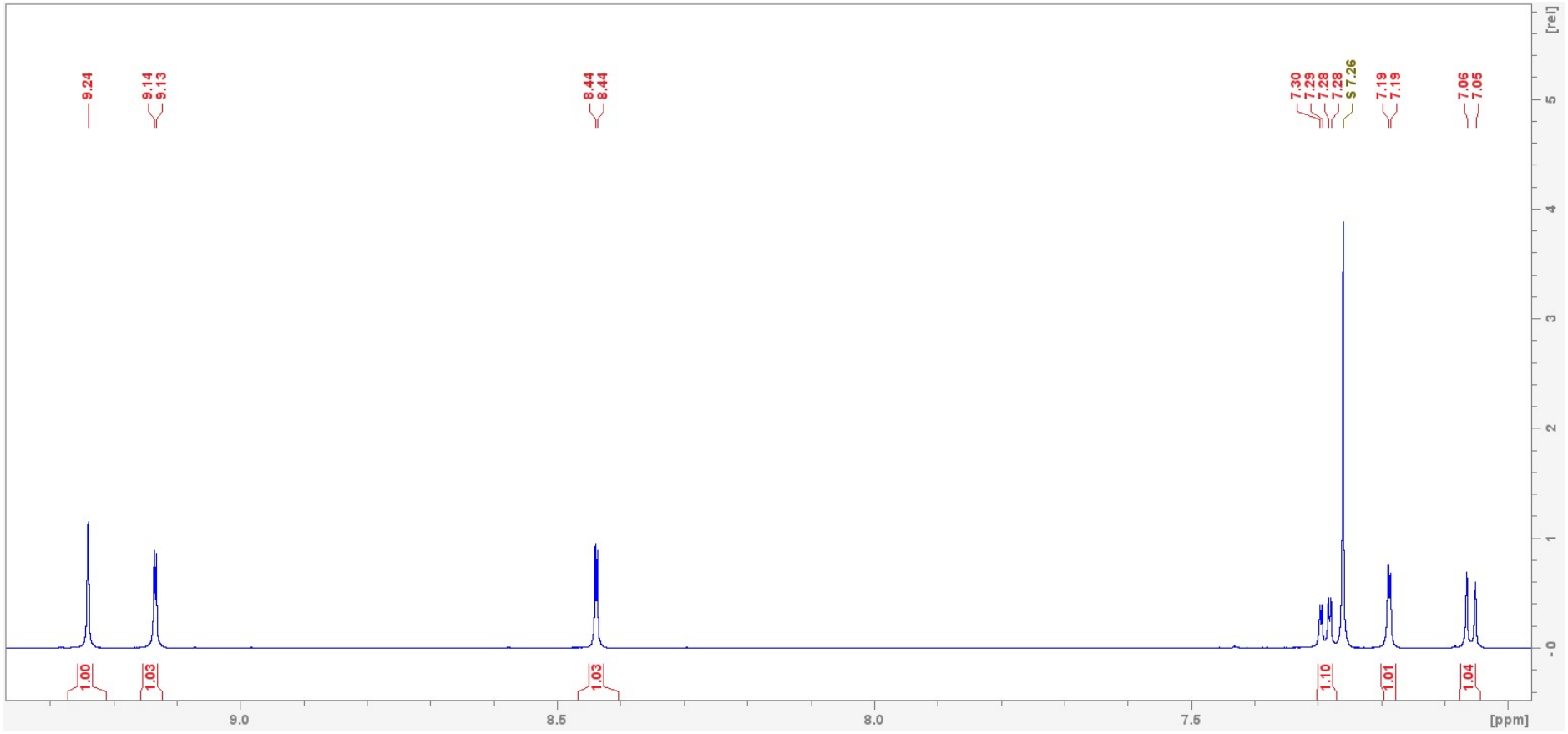
^{13}C NMR spectrum (151 MHz, $CDCl_3$) of **18g**

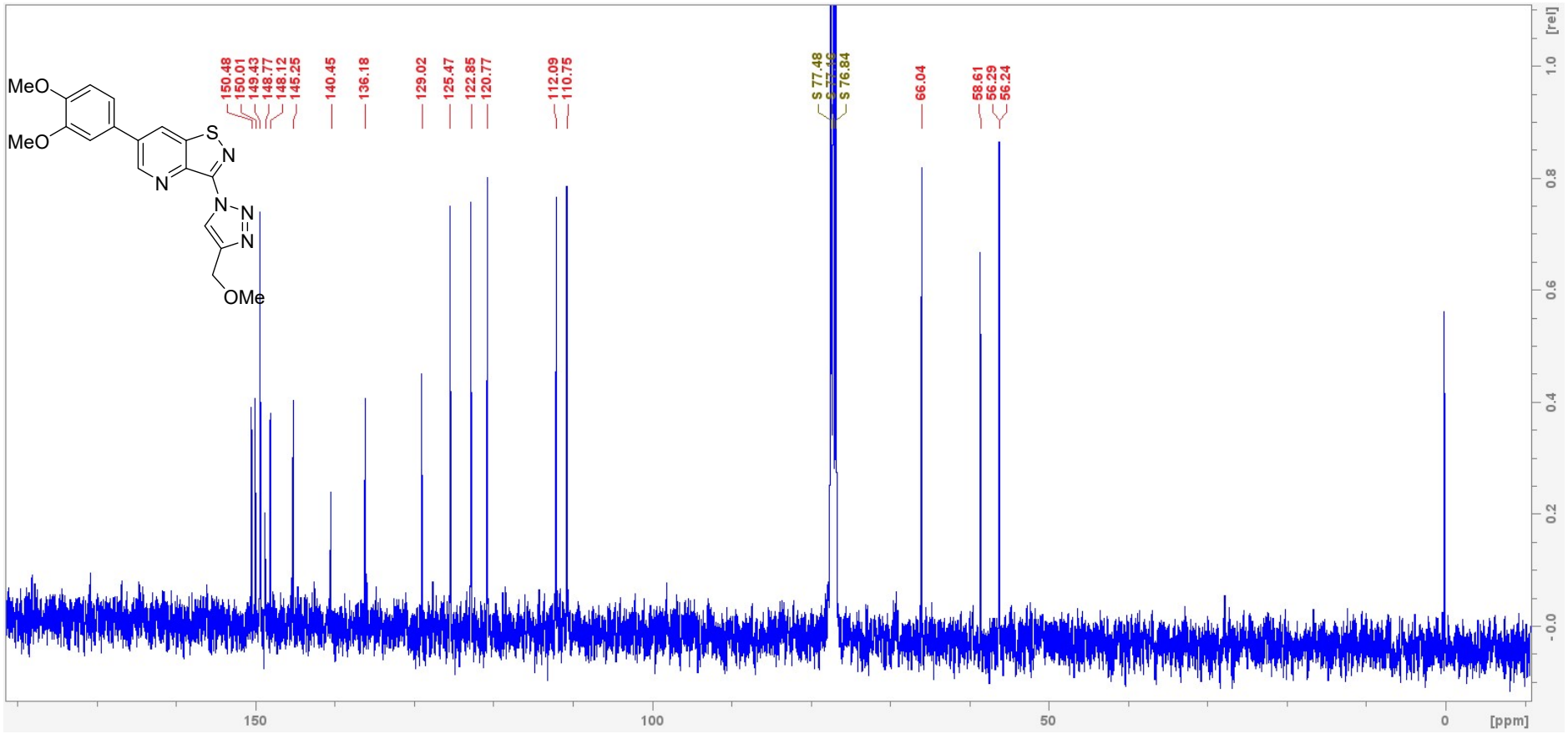
6-(3,4-Dimethoxyphenyl)-3-(4-(methoxymethyl)-1*H*-1,2,3-triazol-1-yl)isothiazolo[4,5-*b*]pyridine (18h)



^{1}H NMR spectrum (600 MHz, $CDCl_3$) of **18h**

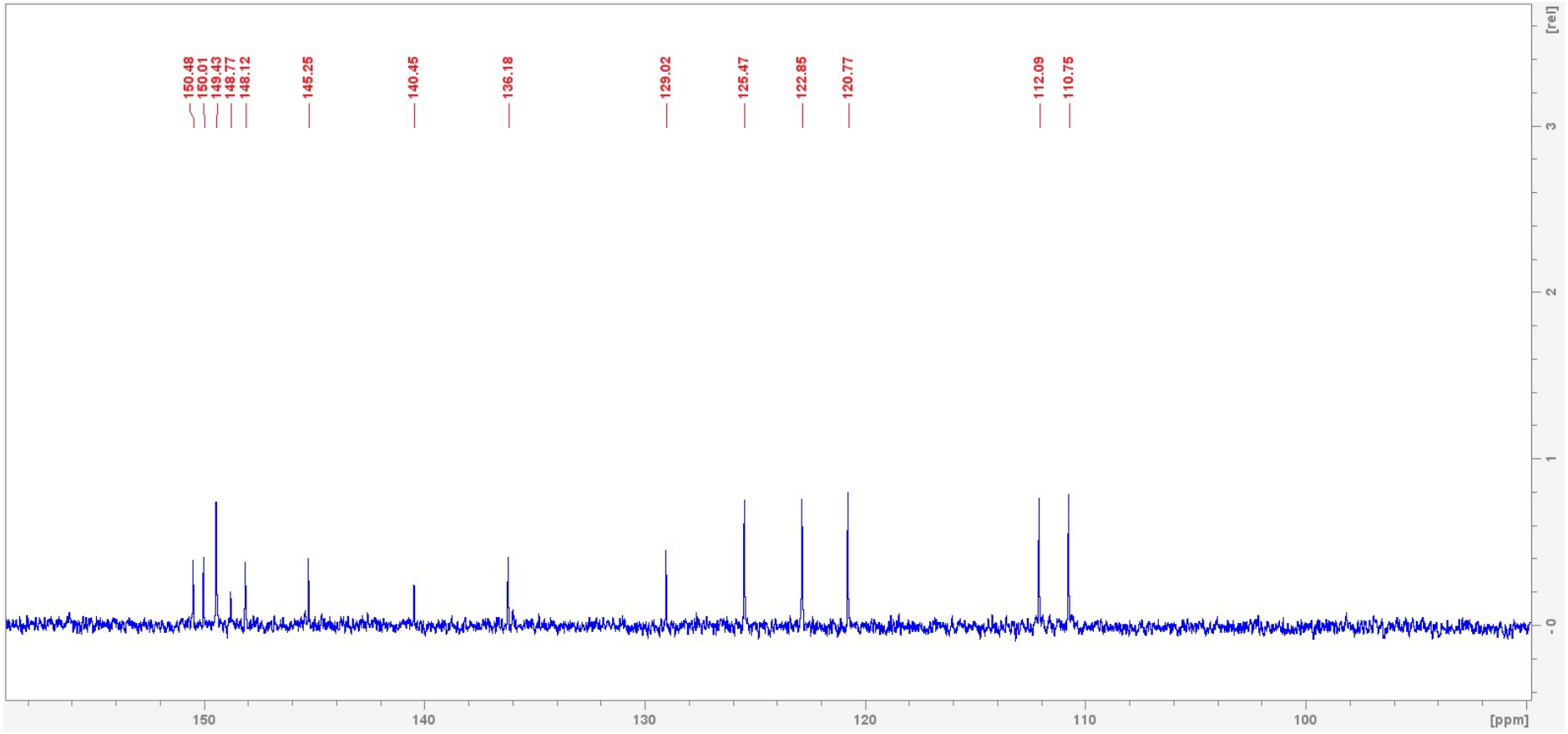
Zoom-in on the aromatic area:



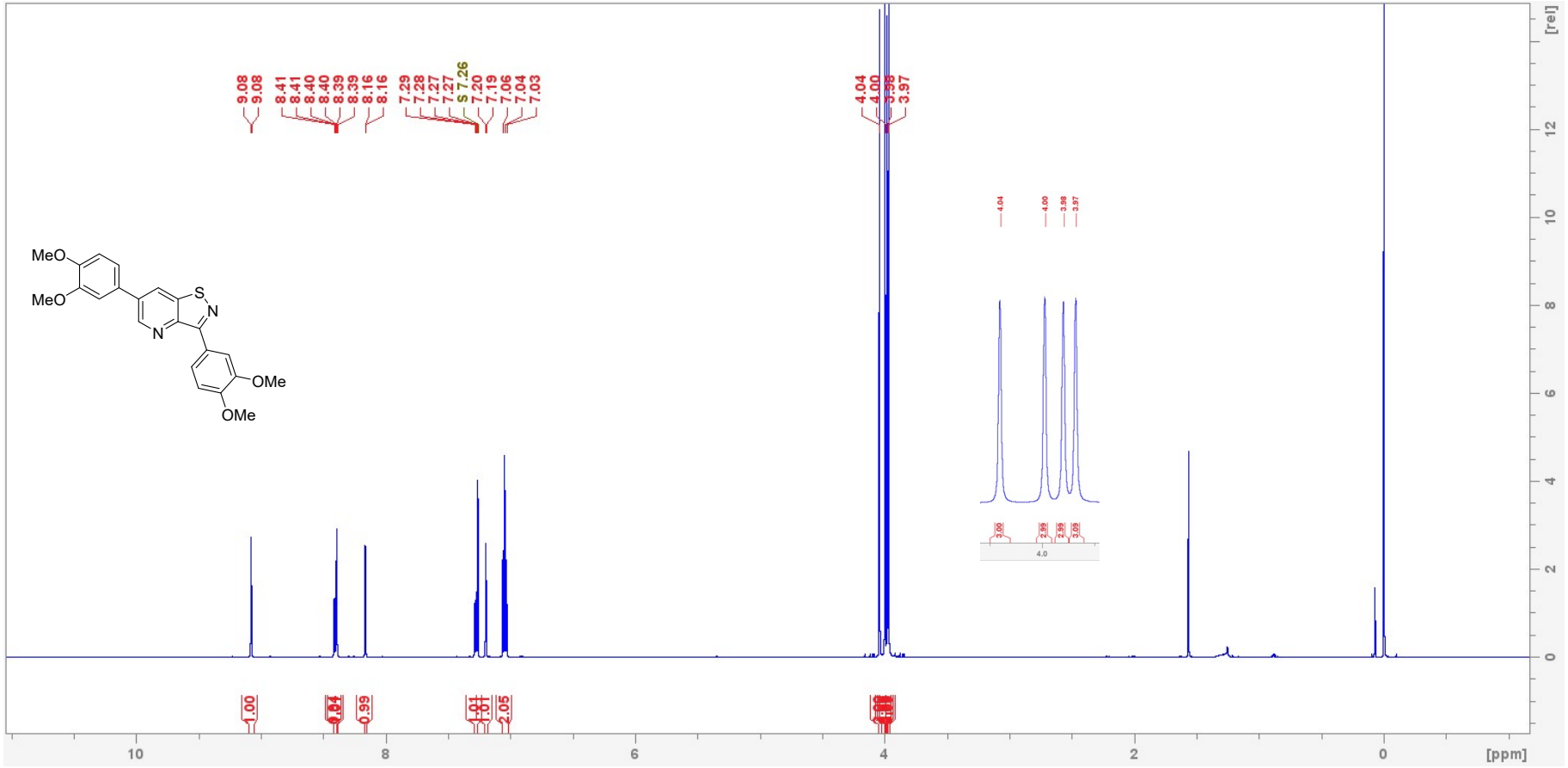


^{13}C NMR spectrum (101 MHz, $CDCl_3$) of **18h**

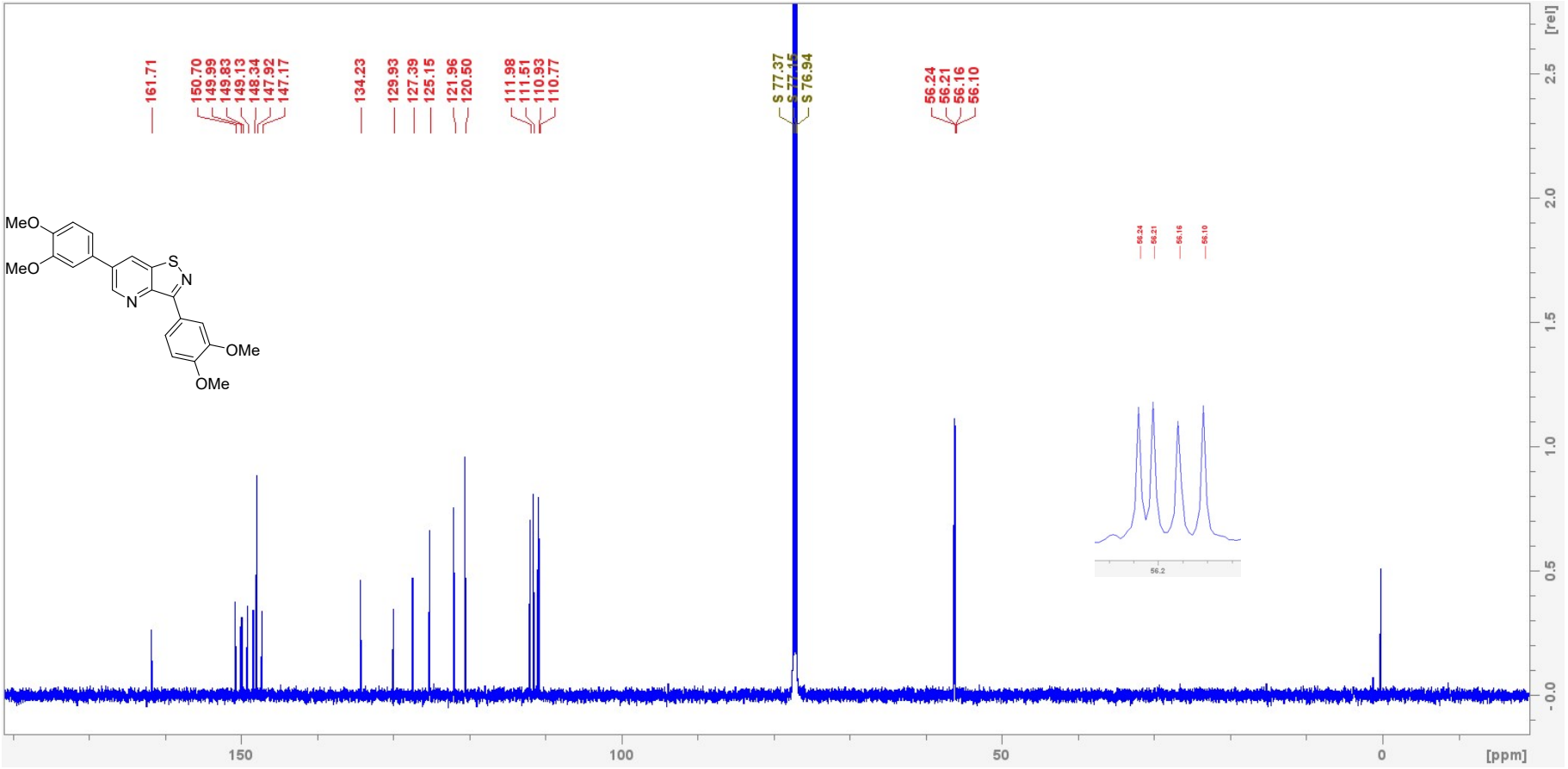
Zoom-in on the aromatic area:



3,6-Bis(3,4-dimethoxyphenyl)isothiazolo[4,5-*b*]pyridine (18j)

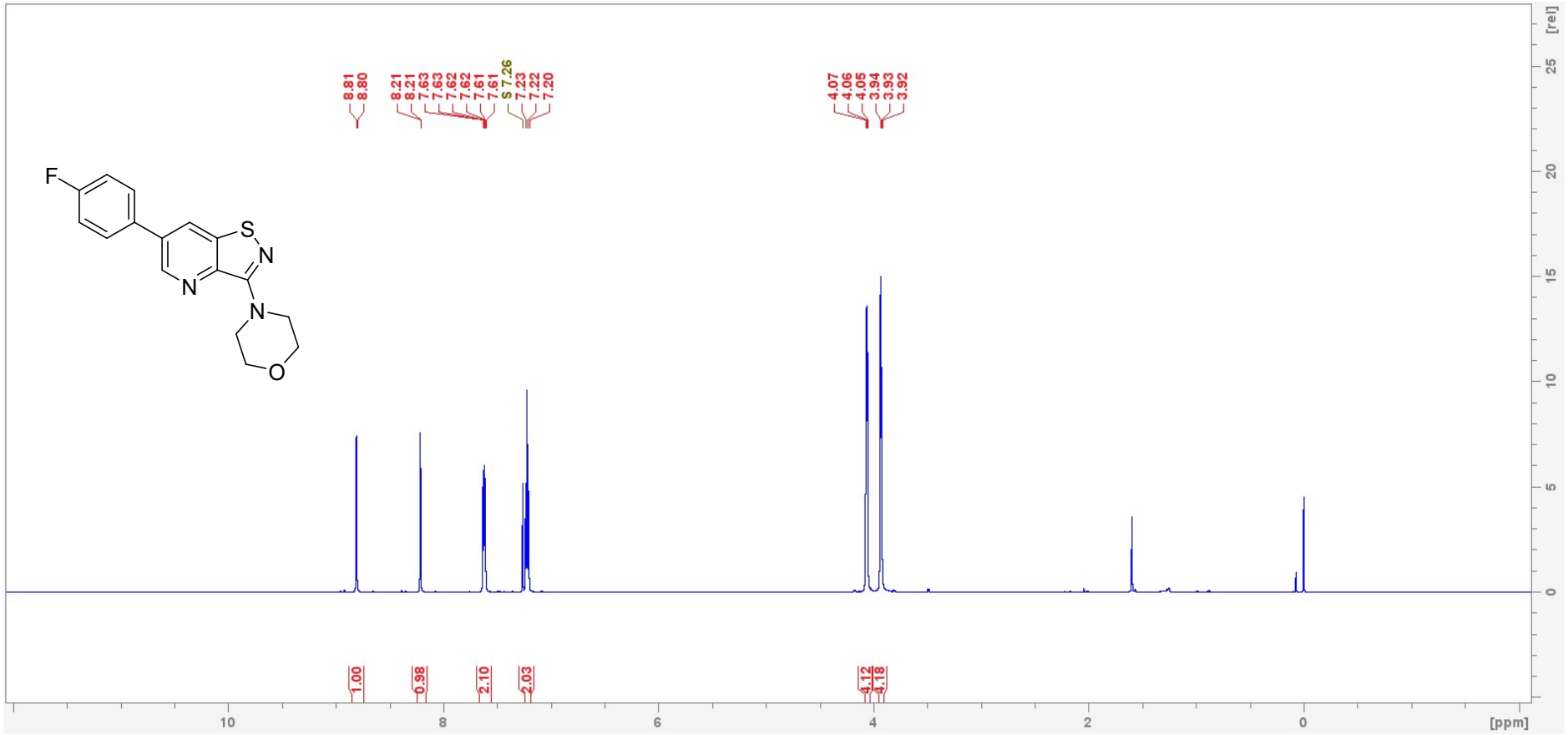


^{1}H NMR spectrum (600 MHz, $CDCl_3$) of **18j**

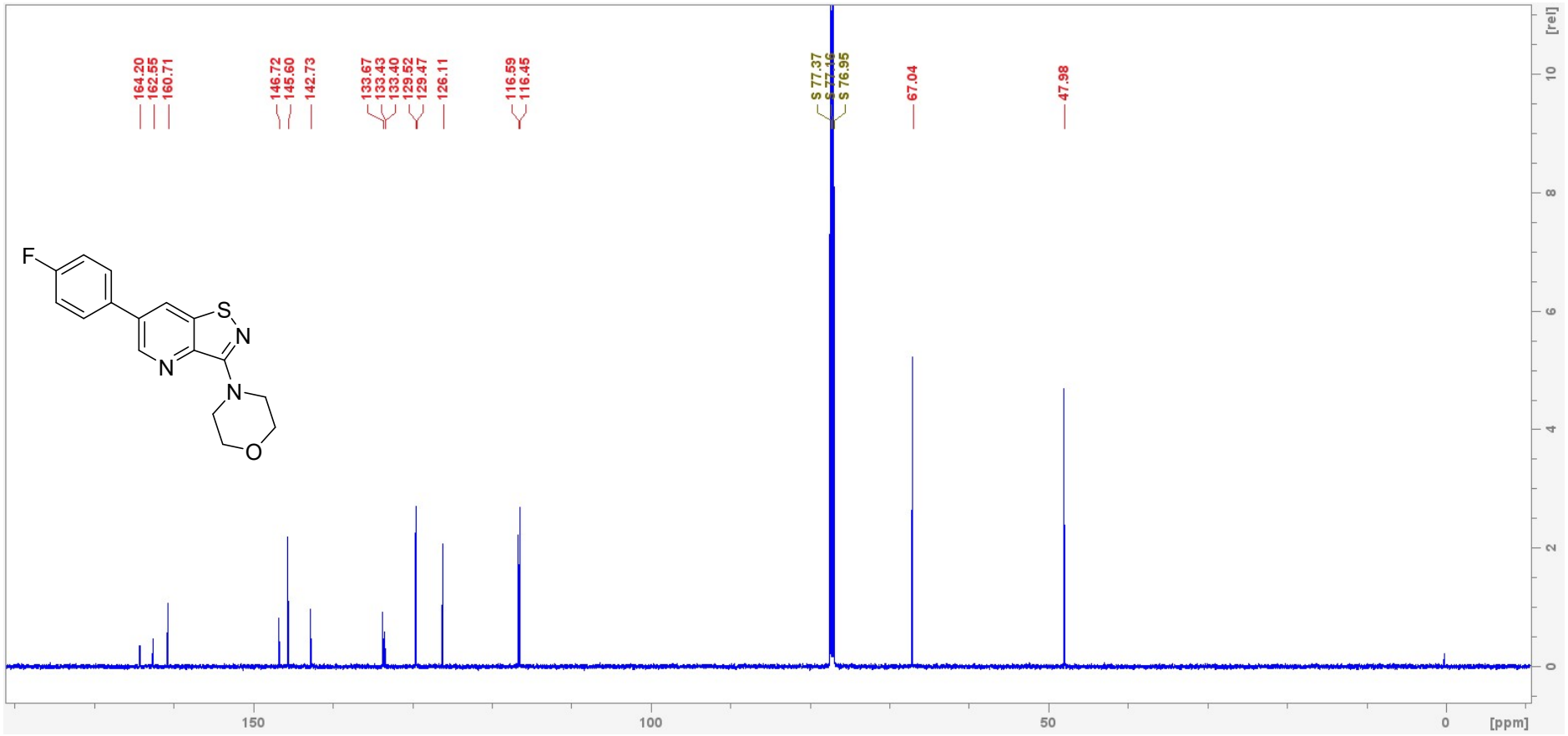


^{13}C NMR spectrum (151 MHz, $CDCl_3$) of **18j**

4-(6-(4-Fluorophenyl)isothiazolo[4,5-*b*]pyridin-3-yl)morpholine (18k)

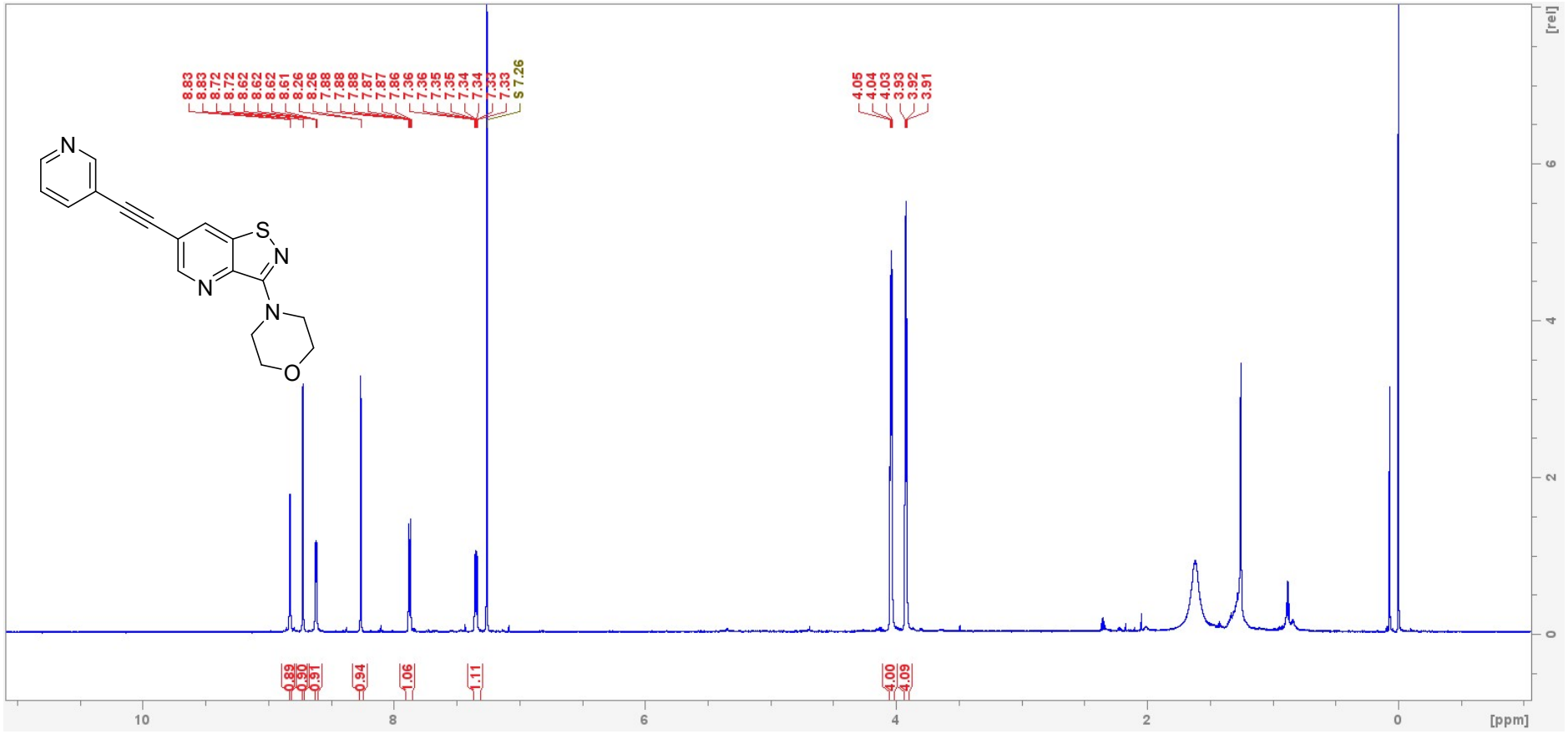


^{1}H NMR spectrum (600 MHz, $CDCl_3$) of **18k**

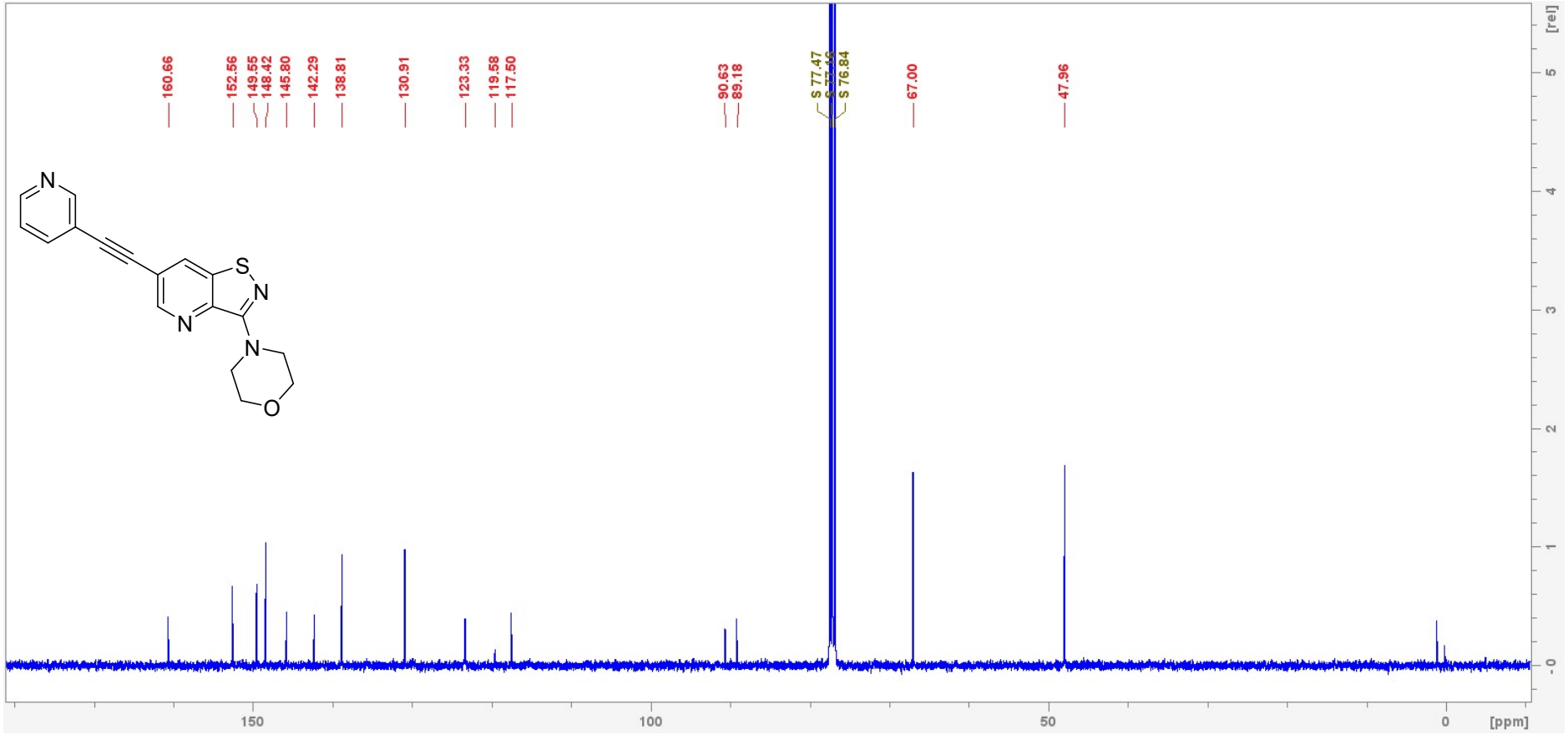


^{13}C NMR spectrum (151 MHz, $CDCl_3$) of **18k**

4-(6-(Pyridin-3-ylethynyl)isothiazolo[4,5-*b*]pyridin-3-yl)morpholine (18l)

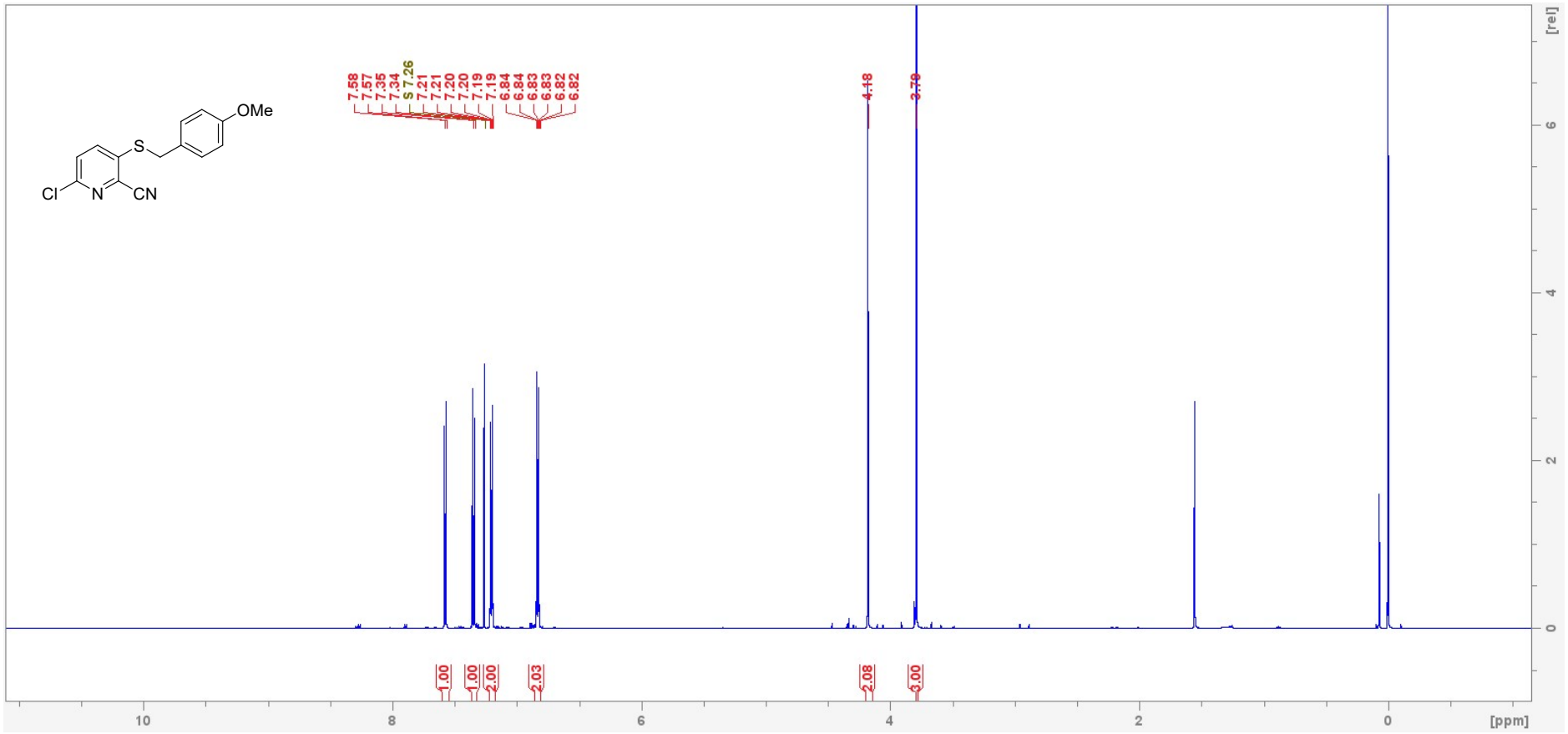


^{1}H NMR spectrum (600 MHz, $CDCl_3$) of **18l**



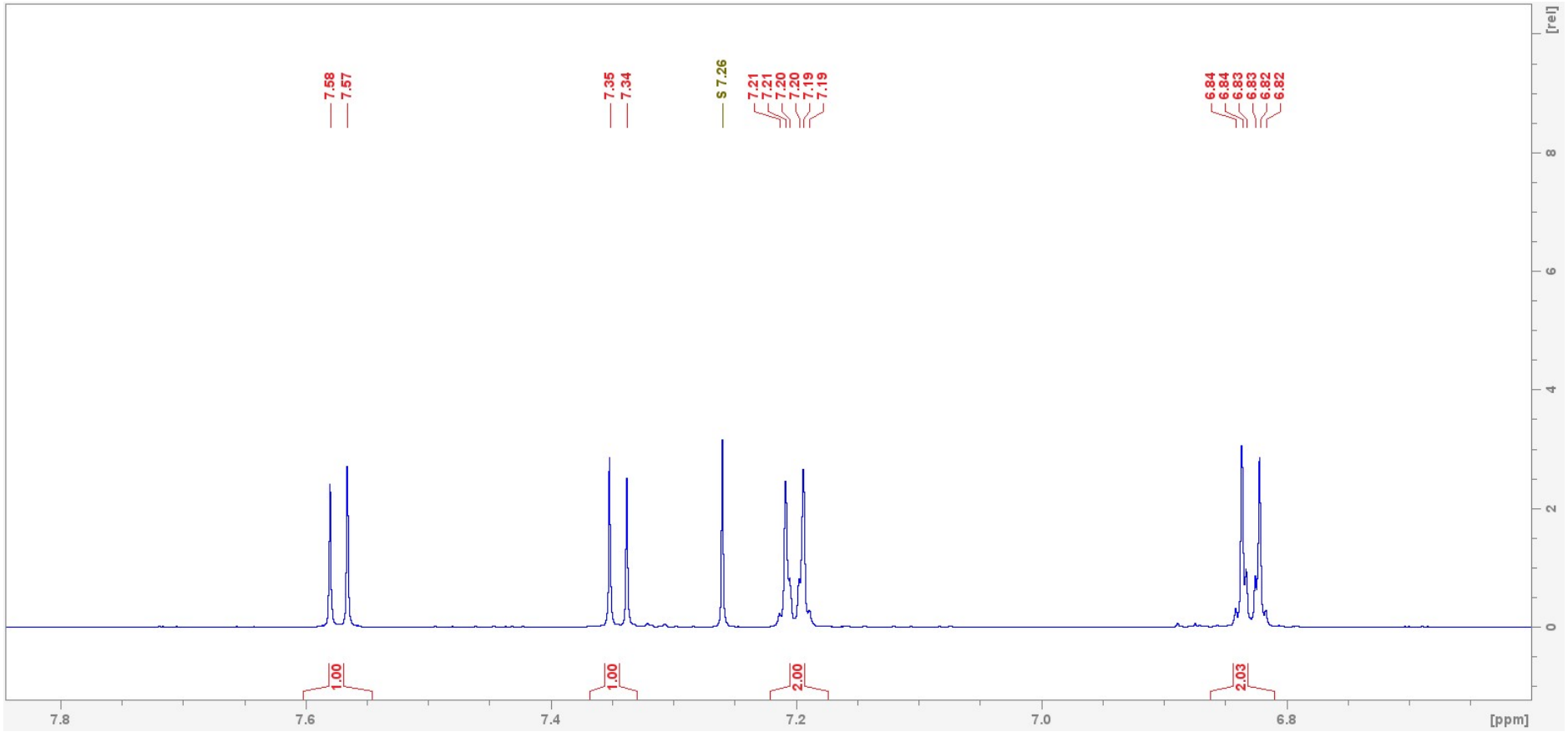
^{13}C NMR spectrum (101 MHz, $CDCl_3$) of **18l**

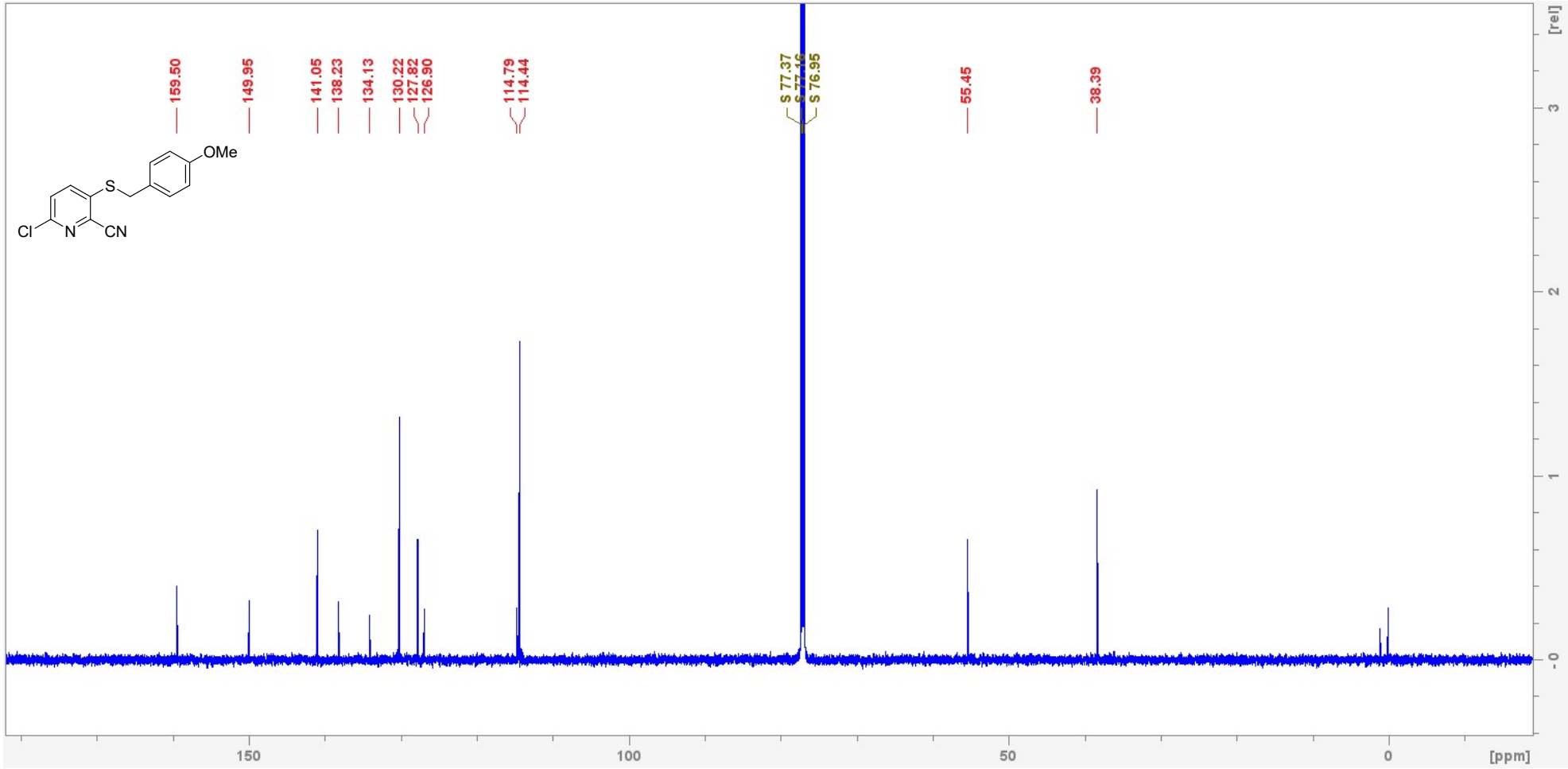
6-Chloro-3-((4-methoxybenzyl)thio)picolinonitrile (20)



^{1}H NMR spectrum (600 MHz, $CDCl_3$) of **20**

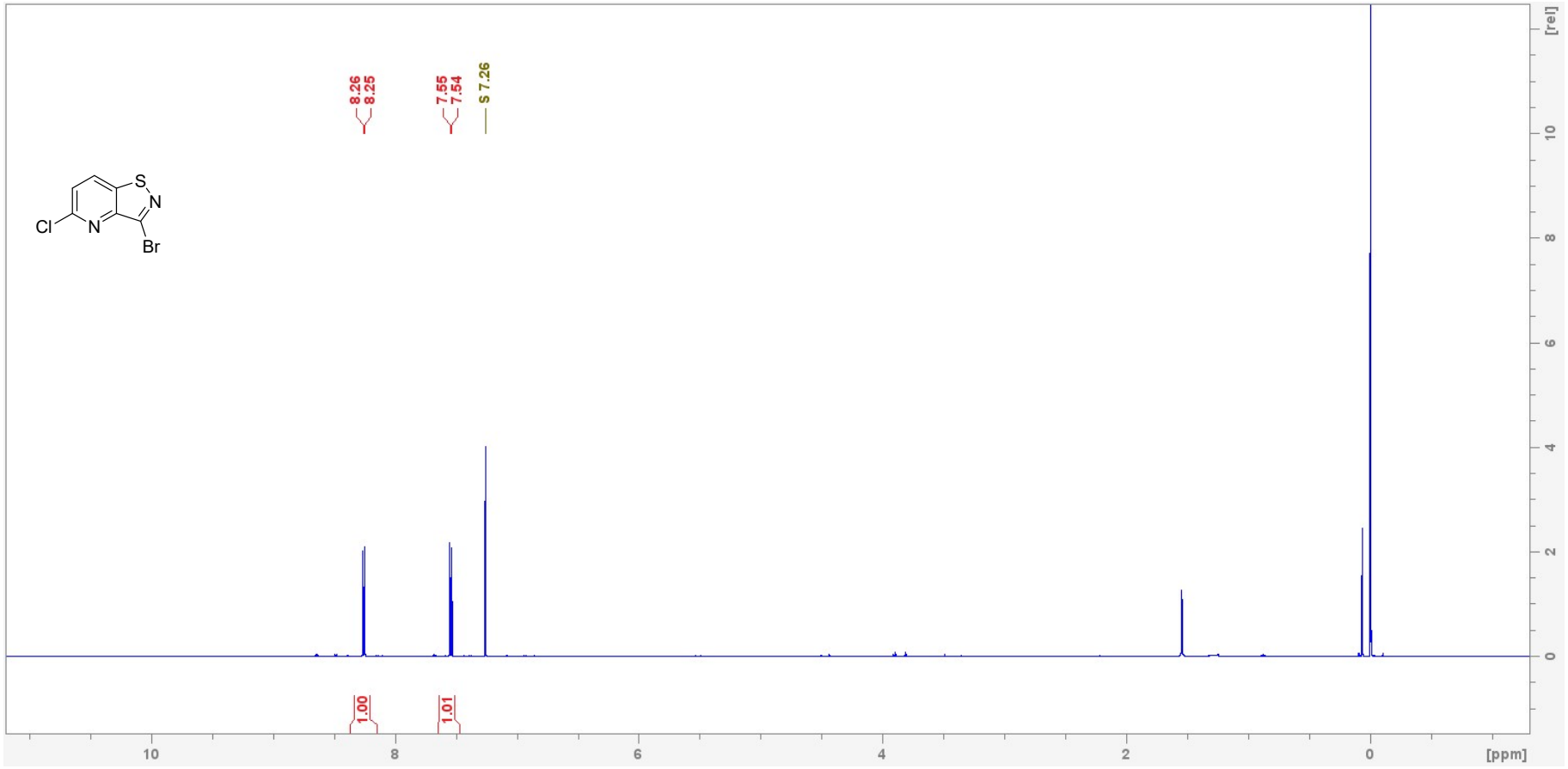
Zoom-in on the aromatic area:



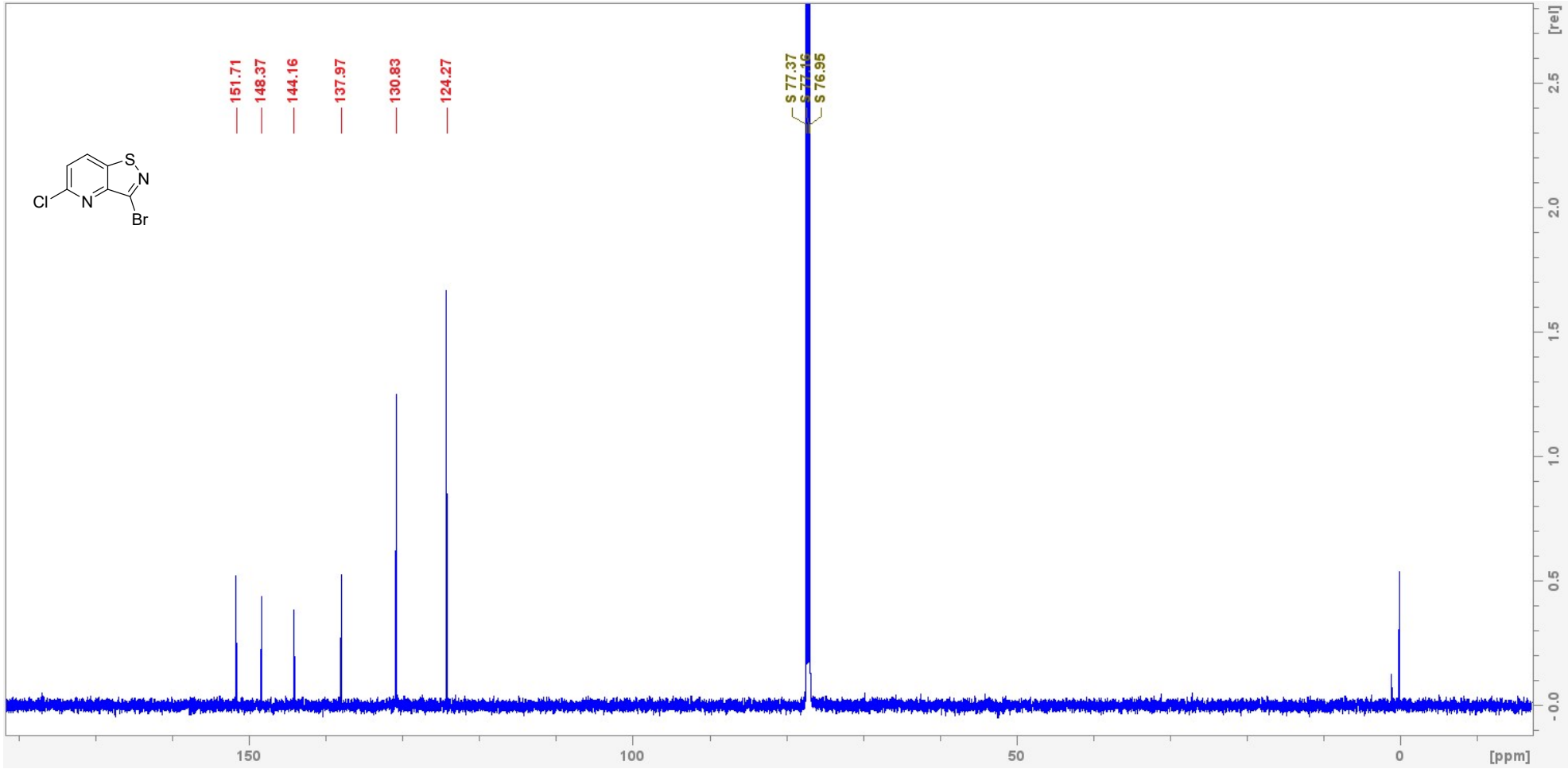


^{13}C NMR spectrum (151 MHz, $CDCl_3$) of **20**

3-Bromo-5-chloroisothiazolo[4,5-*b*]pyridine (21)

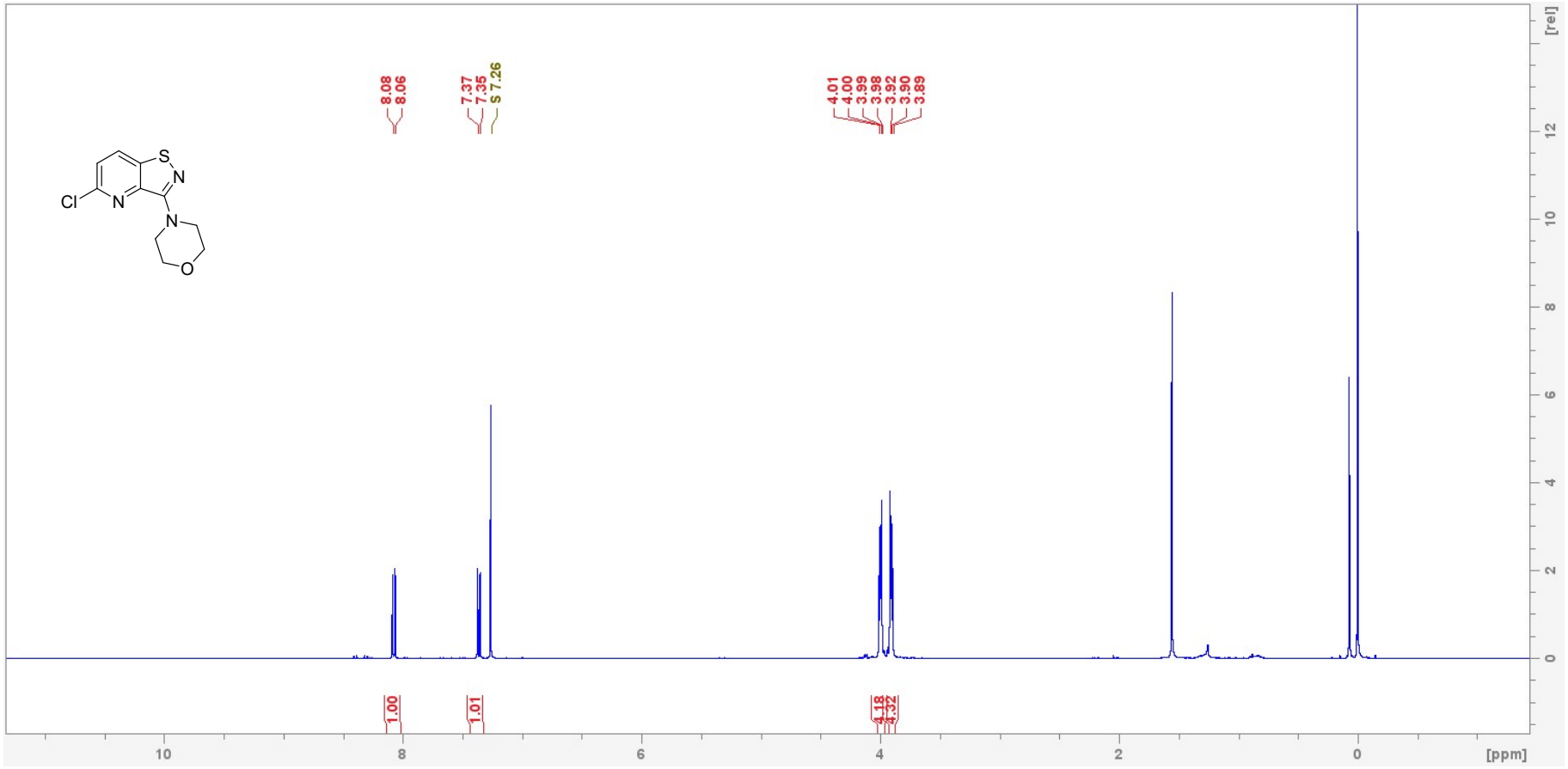


^{1}H NMR spectrum (600 MHz, $CDCl_3$) of **21**

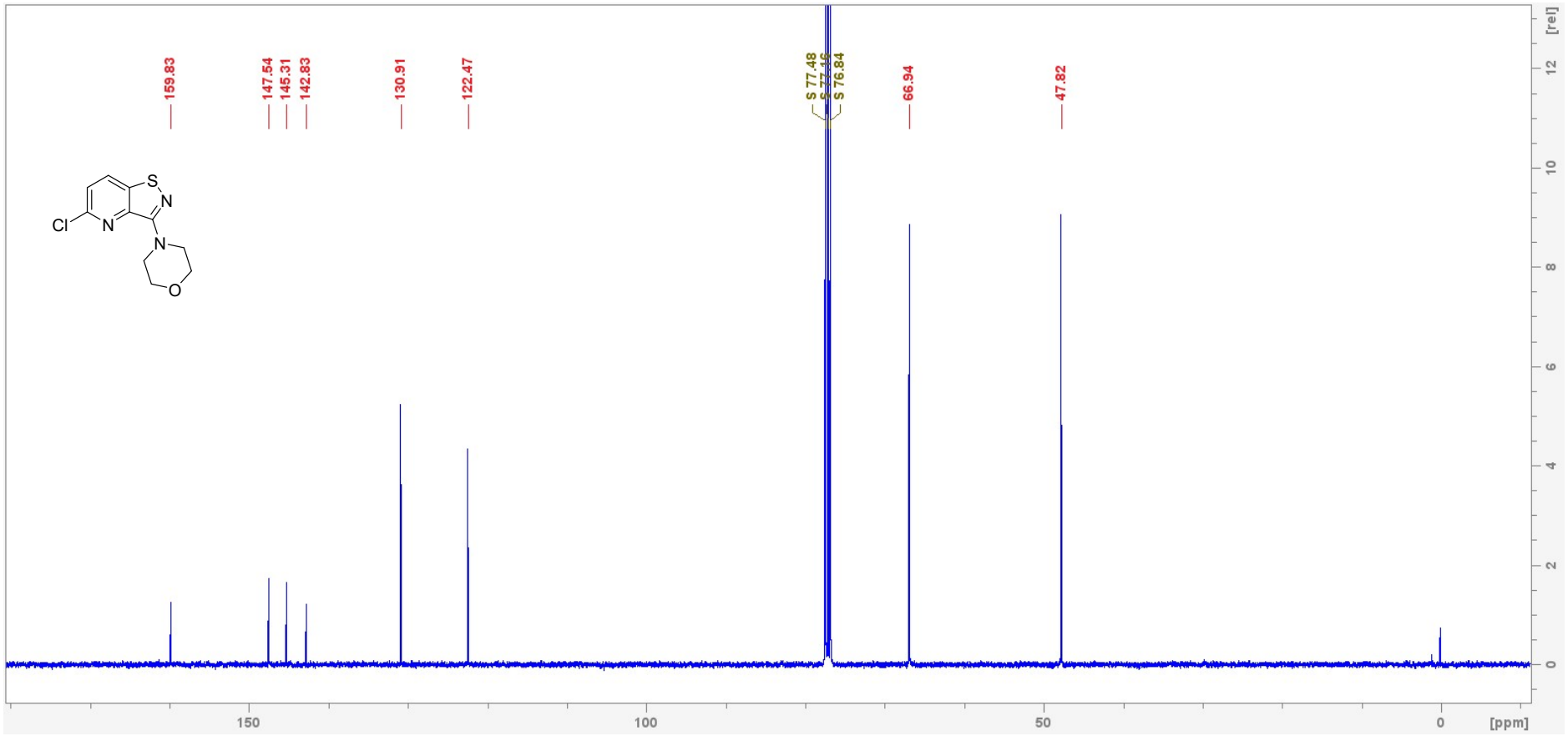


^{13}C NMR spectrum (151 MHz, $CDCl_3$) of **21**

4-(5-Chloroisothiazolo[4,5-*b*]pyridin-3-yl)morpholine (22a)

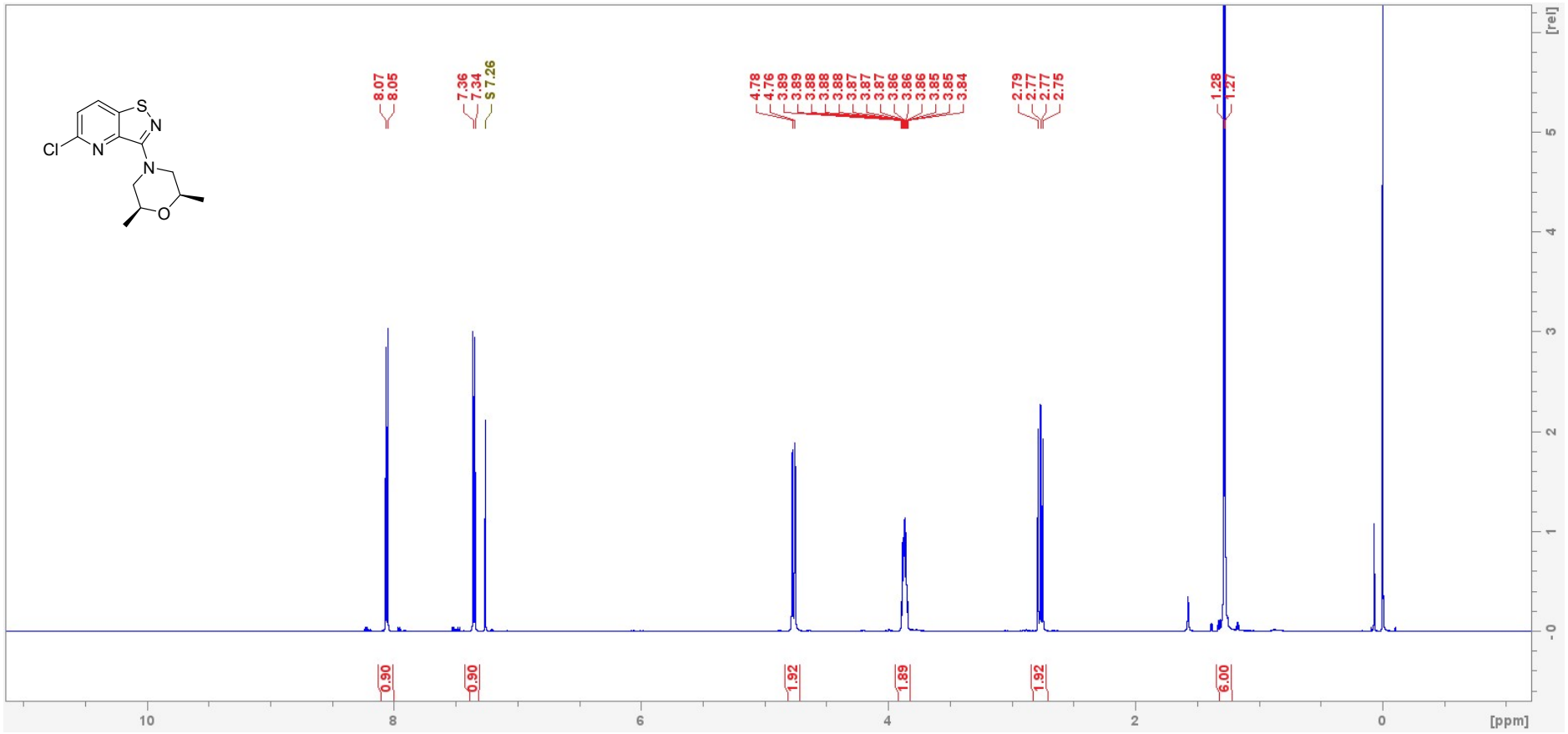


^{1}H NMR spectrum (400 MHz, $CDCl_3$) of **22a**

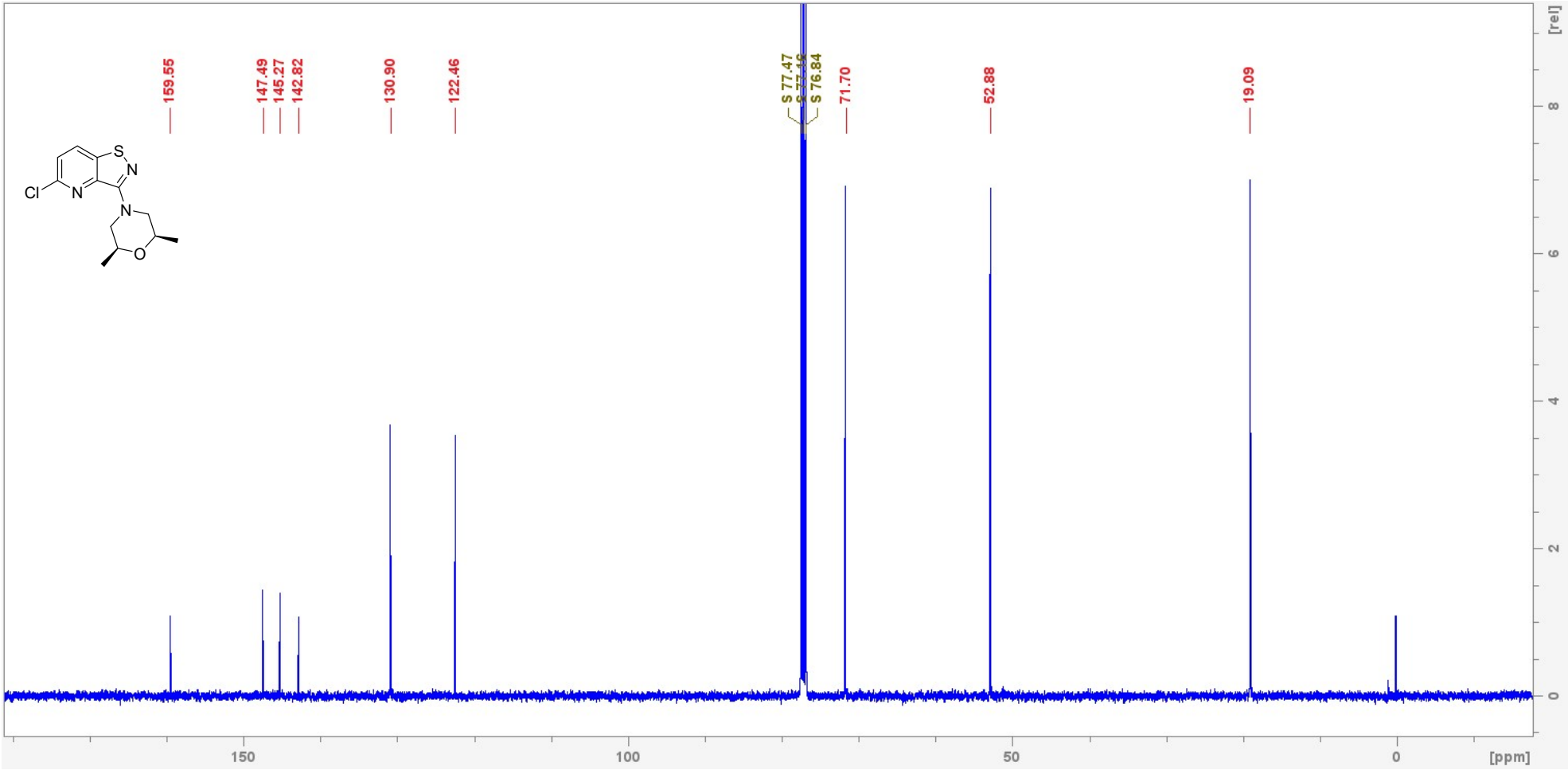


^{13}C NMR spectrum (151 MHz, $CDCl_3$) of **22a**

(2S,6R)-4-(5-Chloroisothiazolo[4,5-*b*]pyridin-3-yl)-2,6-dimethylmorpholine (22b)

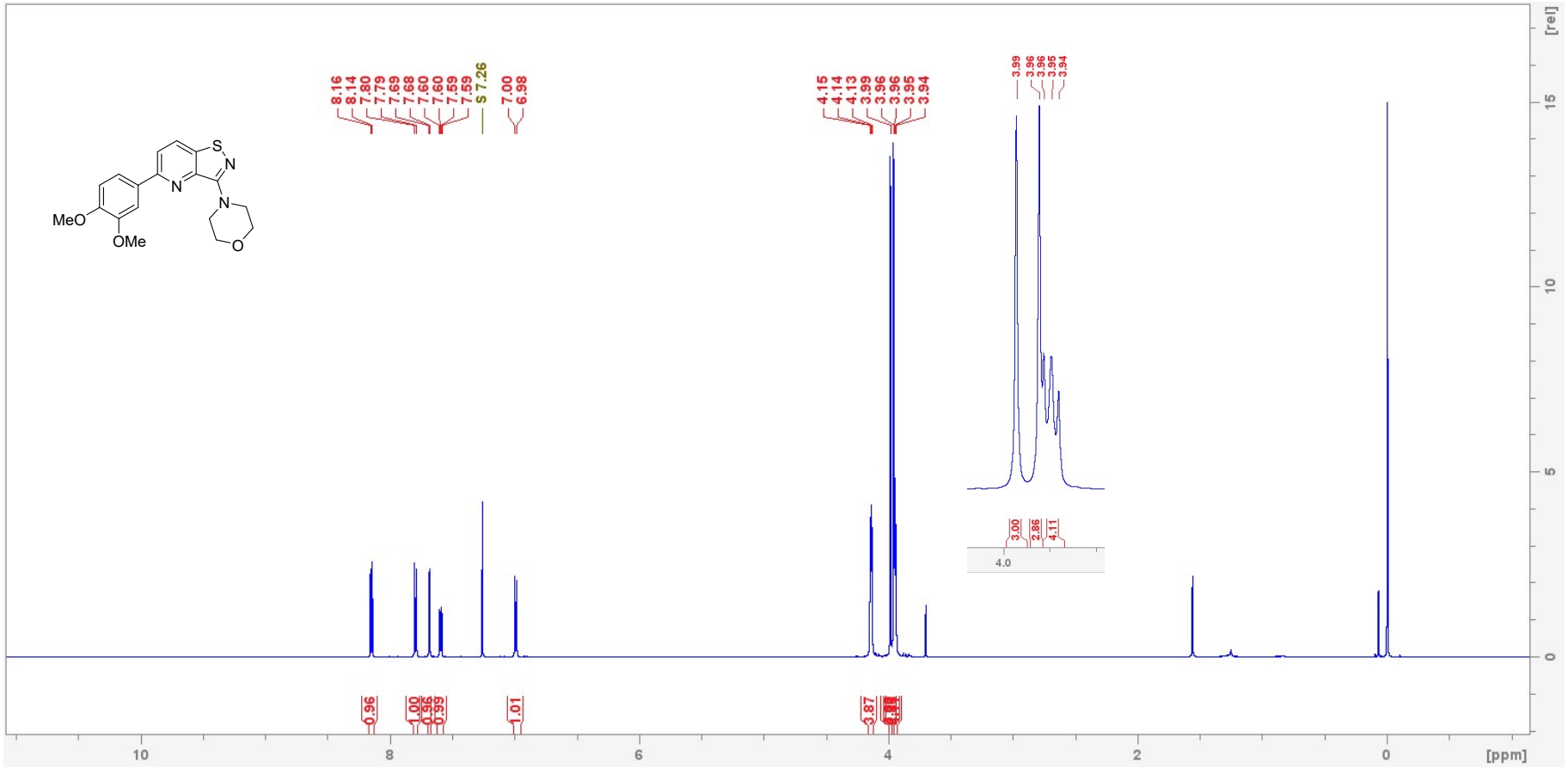


^{1}H NMR spectrum (600 MHz, $CDCl_3$) of **22b**

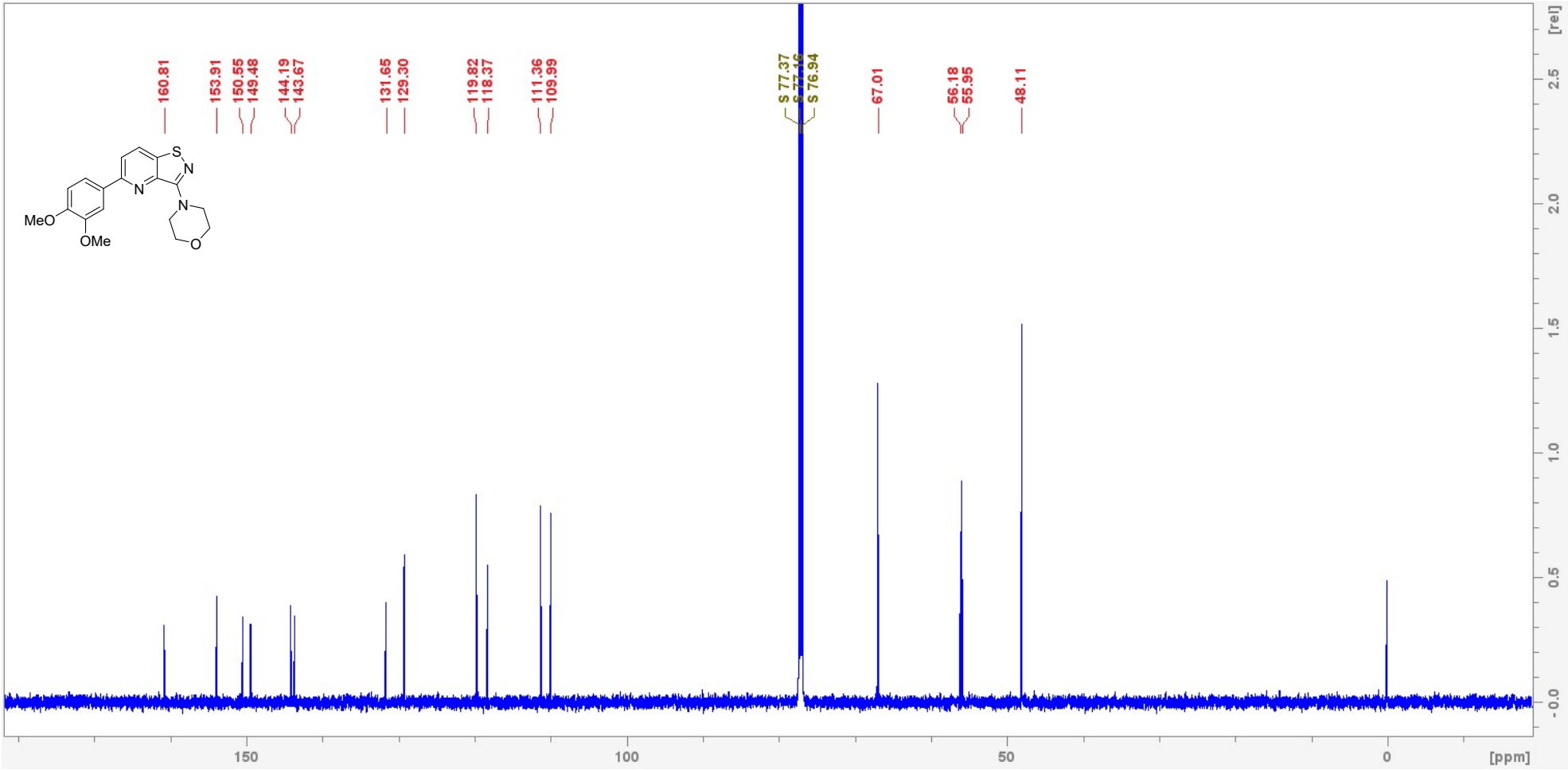


^{13}C NMR spectrum (101 MHz, $CDCl_3$) of **22b**

4-(5-(3,4-Dimethoxyphenyl)isothiazolo[4,5-*b*]pyridin-3-yl)morpholine (23a)

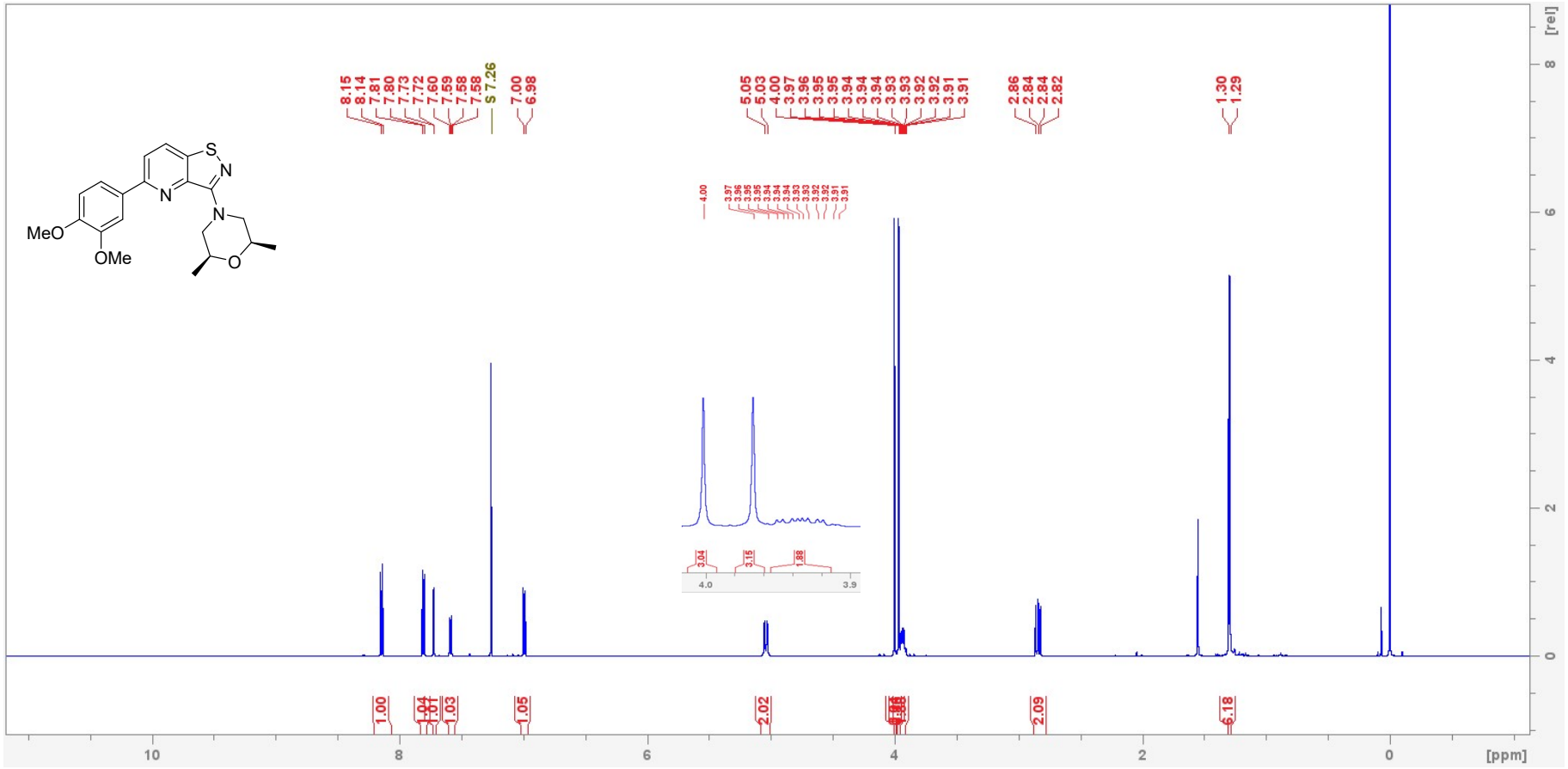


^{1}H NMR spectrum (600 MHz, $CDCl_3$) of **23a**

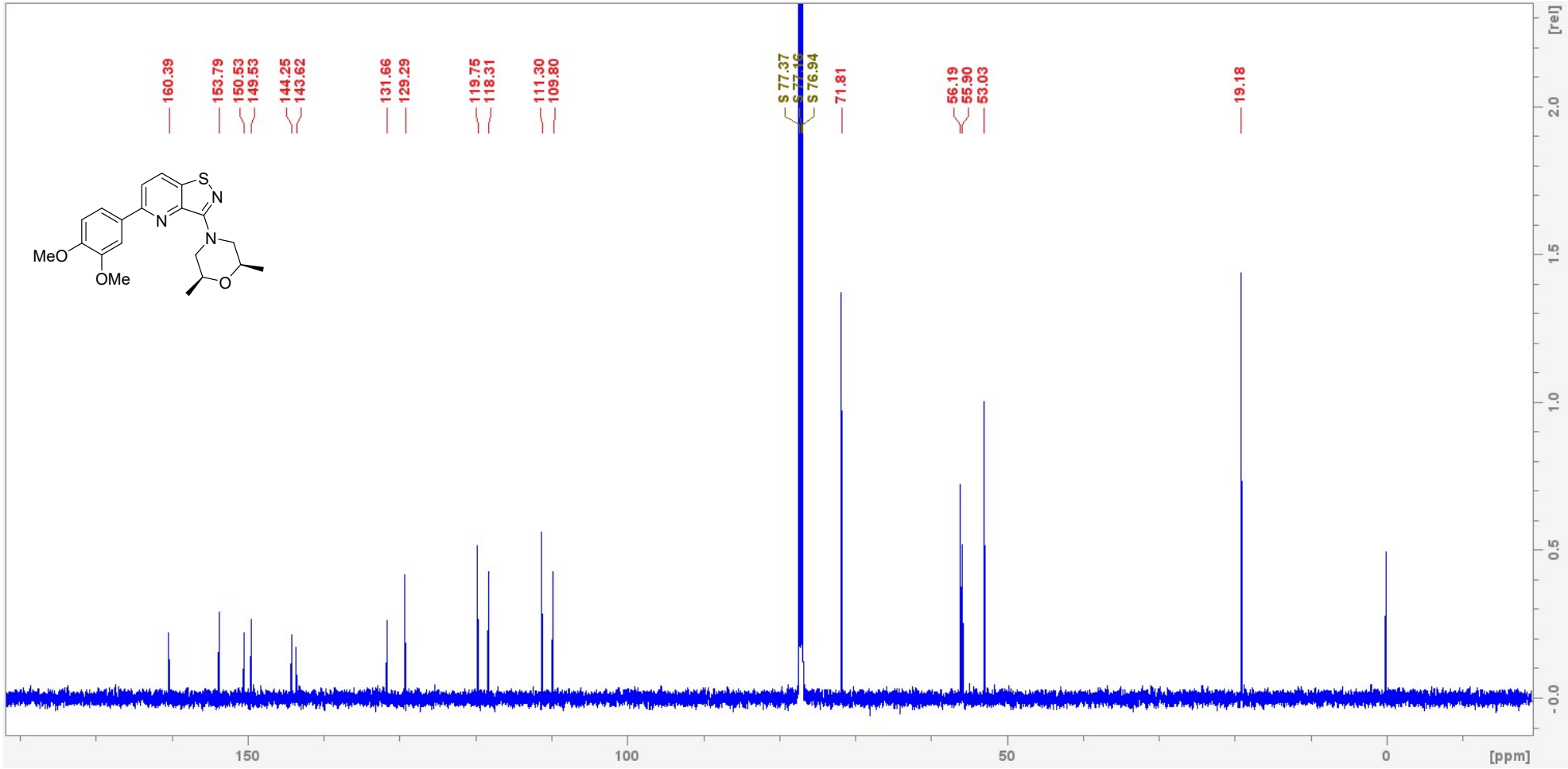


^{13}C NMR spectrum (151 MHz, $CDCl_3$) of **23a**

(2S,6R)-4-(5-(3,4-Dimethoxyphenyl)isothiazolo[4,5-*b*]pyridin-3-yl)-2,6-dimethylmorpholine (23b)



^{1}H NMR spectrum (600 MHz, $CDCl_3$) of **23b**



^{13}C NMR spectrum (151 MHz, $CDCl_3$) of **23b**