Supporting information

Intramolecular Povarov reactions for the Synthesis of Chromenopyridine fused 2-Pyridone Polyheterocycles Binding to α-Synuclein and Amyloid-β fibrils.

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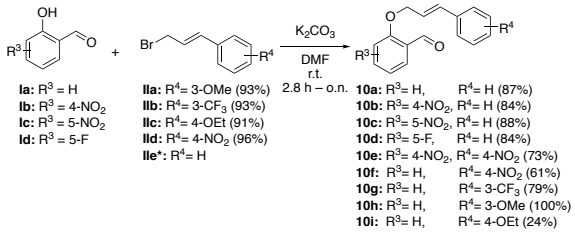
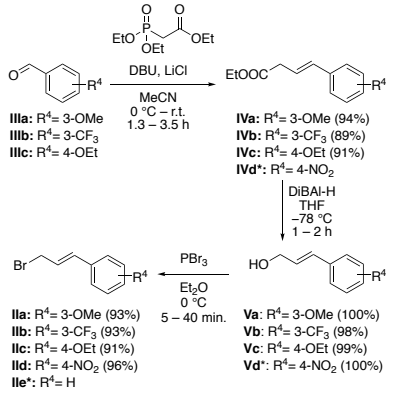
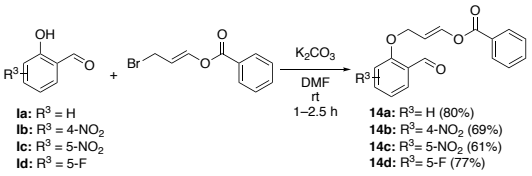


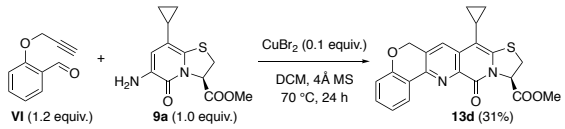
Scheme S1. Synthesis of *O*-alkylated salicylaldehydes **10a–i** for use in the intramolecular Povarov reaction. Tethering of the aldehyde and alkene components renders the reaction intramolecular. The substituted cinnamyl moiety was incorporated through alkylation of salicylaldehyde **Ia–d** with the corresponding cinnamyl bromide **IIa–e**, prepared in three steps from benzaldehydes **IIIa–c** or obtained commercially (**Scheme S2**). *This intermediate was obtained commercially.



Scheme S2. Synthesis of cinnamyl bromides **IIa–d** from the corresponding benzaldehydes **IIIa–c** via Horner-Wadsworth-Emmons olefination, reduction with DiBAl-H and bromination with PBr_3.[1] *This intermediate was obtained commercially.



Scheme S3. Preparation of *O*-propenyl benzoate salicylaldehydes **14a–d** from salicylaldehydes **Ia–d** and 3-bromopropenyl benzoate.[2]

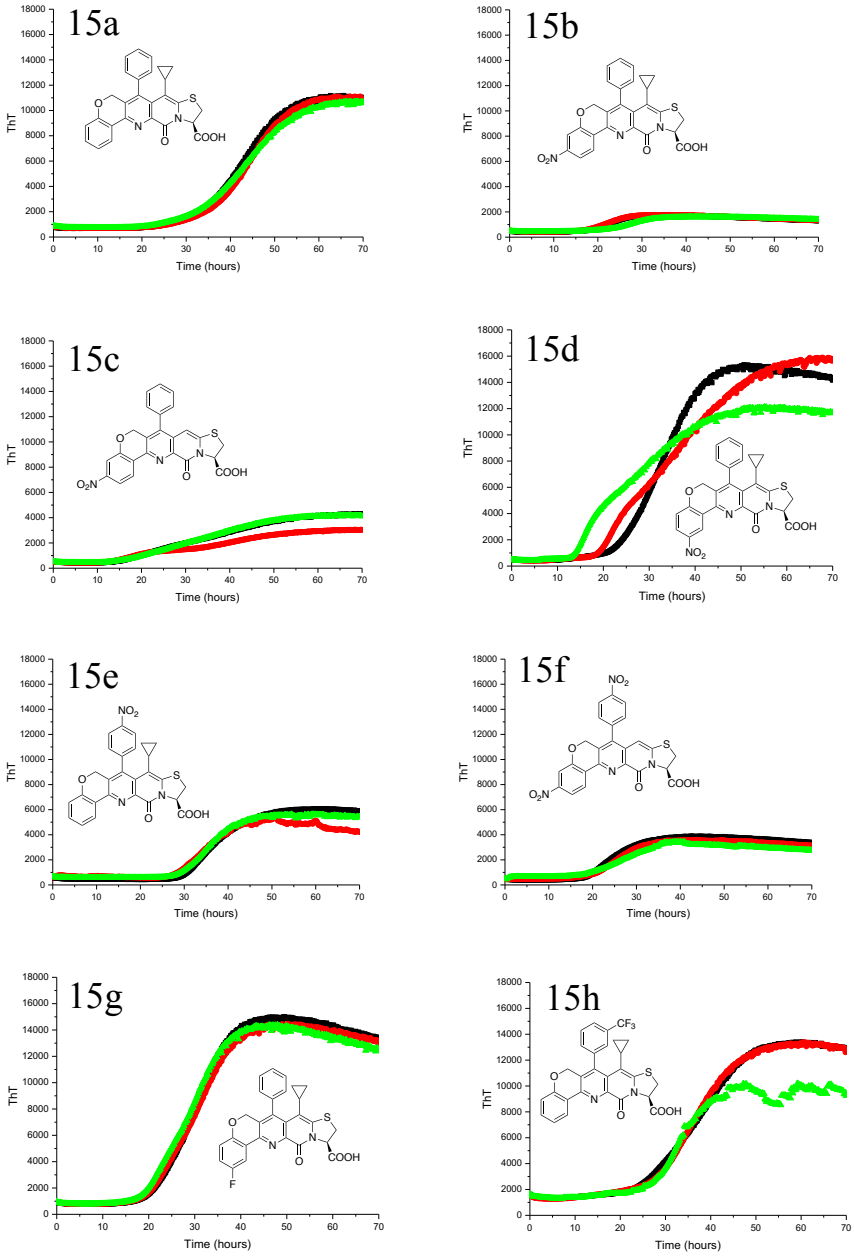


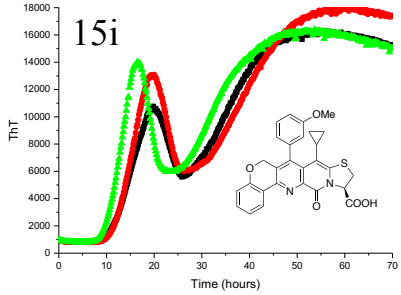
Scheme S4. Synthesis of **13d** from *O*-propargyl salicylaldehyde **VI** and thiazolino 2-pyridone **9a**. The low to moderate yields with *O*-propenyl benzoate salicylaldehydes **14a–d** motivated us to try Lewis acid catalyzed Povarov reaction with *O*-propargyl salicylaldehyde **VI**. Among TFA, $SnCl_4$, $TiCl_4$, $FeCl_3$, $Y(OTf)_3$, $Yb(OTf)_3$, $La(OTf)_3$, $Dy(OTf)_3$, $CuCl_2$, $Cu(OAc)_2$, $Cu(OTf)_2$, Cu(II)TMEDA, CuI and $CuBr_2$, the latter proved to be the best catalyst. Of, DCM, THF and MeCN, DCM was still the solvent in which the reaction provided the highest yield of **13d.** The reaction with *O*-propargyl salicylaldehyde was faster and provided **13d** directly, without the need for any auxiliary oxidant. Alas, the isolated yield was still poor and the product contained traces of impurities. Raising the temperature to 90 °C increased the amounts of by-products even further. At 50 °C, the reaction proceeded much slower, without providing cleaner conversion. Increasing the catalyst loading (0.3 equiv.) provided more by-products and lowered the isolated yield (21%) of **13d**. Decreasing catalyst loading to 0.03 equiv. slowed down the reaction, which was far from completion after two days.

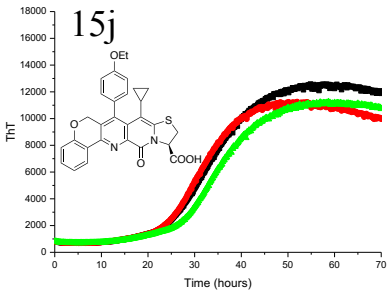
Figure S1:

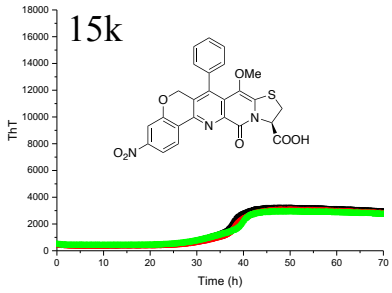
Evaluation of α-synuclein fibrillation modulating properties and screening for fibril binding.

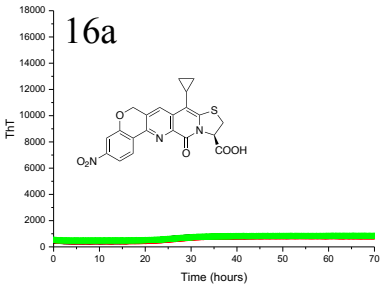
Human wild-type α-synuclein was expressed and purified as described previously.[3] A 96-well plate (Corning 3650, Sigma-Aldrich) was loaded with samples containing wild-type α-synuclein (70 μM) and compound (100 μM) solubilized in PBS (10 mM) and DMSO (100 μM), followed by addition of ThT (20 μM) and a 2 mm glass bead. The plate was incubated at 37 °C using orbital averaging, 500 cycles, and a cycle time of 600 seconds, during 70 hours. Each experiment was performed in triplicate. The formation of amyloid fibers was followed by ThT fluorescence (λ_{ex} = 440 nm, λ_{em} = 480 nm).[4]

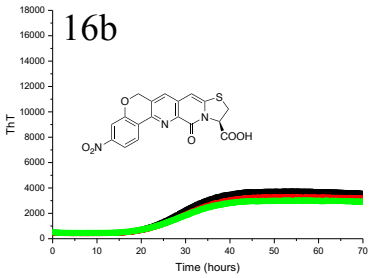


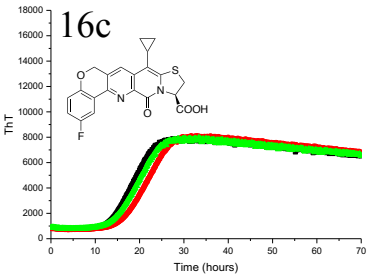

15i
ThT
Time (hours)
OMe
COOH

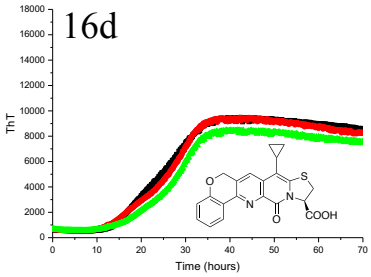

15j
ThT
Time (hours)
OEt
COOH

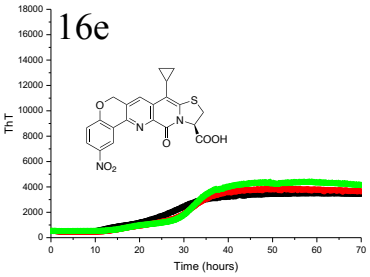

15k
ThT
Time (h)
OMe
O_2N
COOH

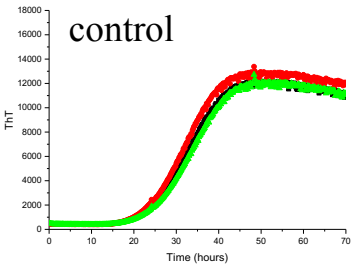

16a
ThT
Time (hours)
O_2N
COOH

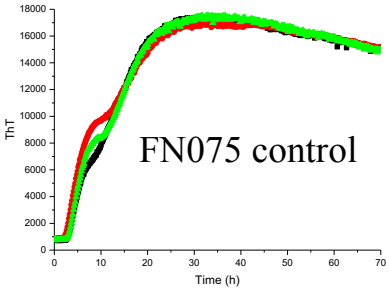

16b
ThT
Time (hours)
O_2N
COOH

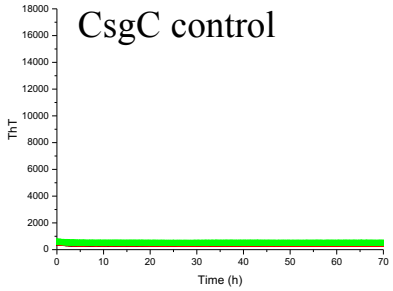

16c
ThT
Time (hours)
COOH
F


16d
ThT
Time (hours)
COOH


16e
ThT
Time (hours)
COOH
NO_2


control
ThT
Time (hours)


FN075 control
ThT
Time (h)


CsgC control
ThT
Time (h)

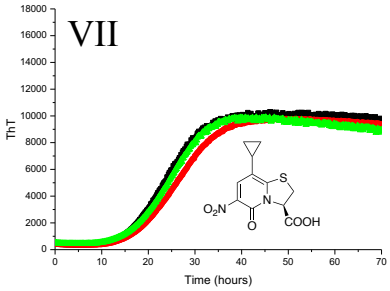
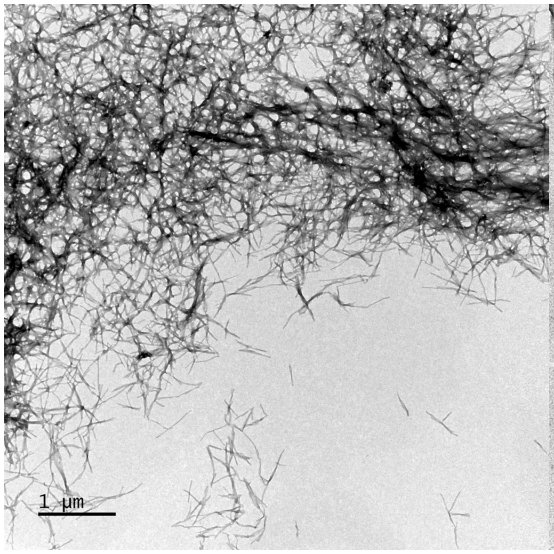
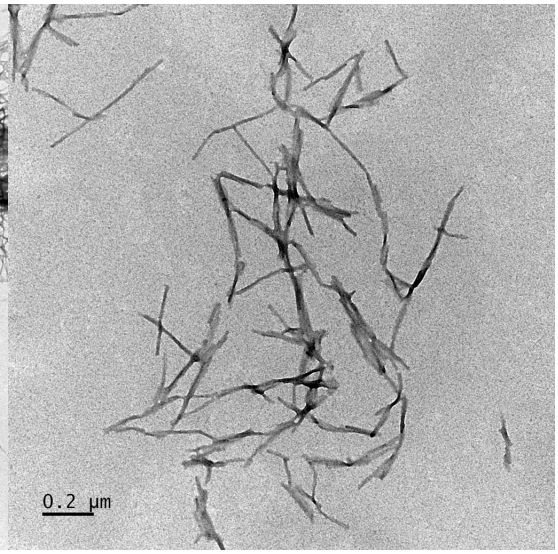

VII
ThT
Time (hours)
S
N
O2N
O
COOH

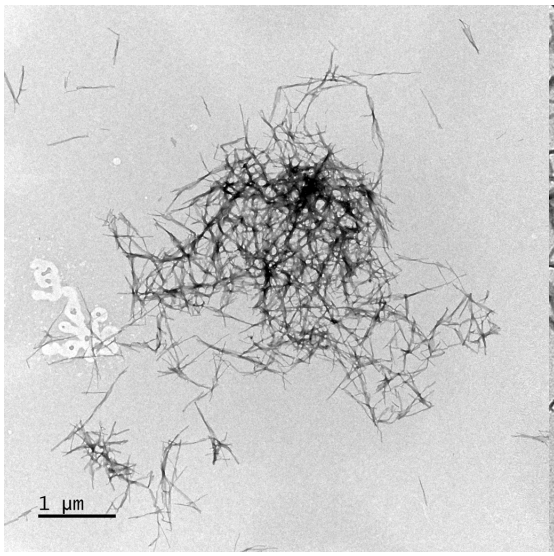
Figure S2:
Preparation of samples for TEM visualization of amyloid fibrils.
At the end point of the fibrilization experiments described in Figure S1, samples (3.5µL) were applied to glow discharged formvar and carbon coated Cu-grids. The grids were washed and then negatively stained in 1.5% uranyl acetate for 2 x 15 s. A Talos 120C microscope (FEI, Eindhoven, The Netherlands) was used for sample examination, operating at 120kV. Micrographs were acquired with a Ceta 16M CCD camera (FEI, Eindhoven, The Netherlands) using TEM Image & Analysis software ver. 4.17 (FEI, Eindhoven, The Netherlands). Pictures were taken at 12 000 X and 40 000 X magnification, and are shown below. Fibers formed in the presence of compound **16a** and in the absence of compound showed no visual differences (control).



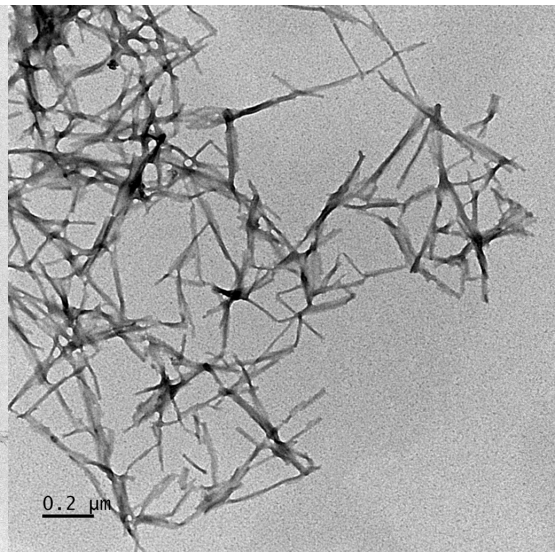
Compound 16a (12 000X)



Compound 16a (40 000X)



Control (12 000X)

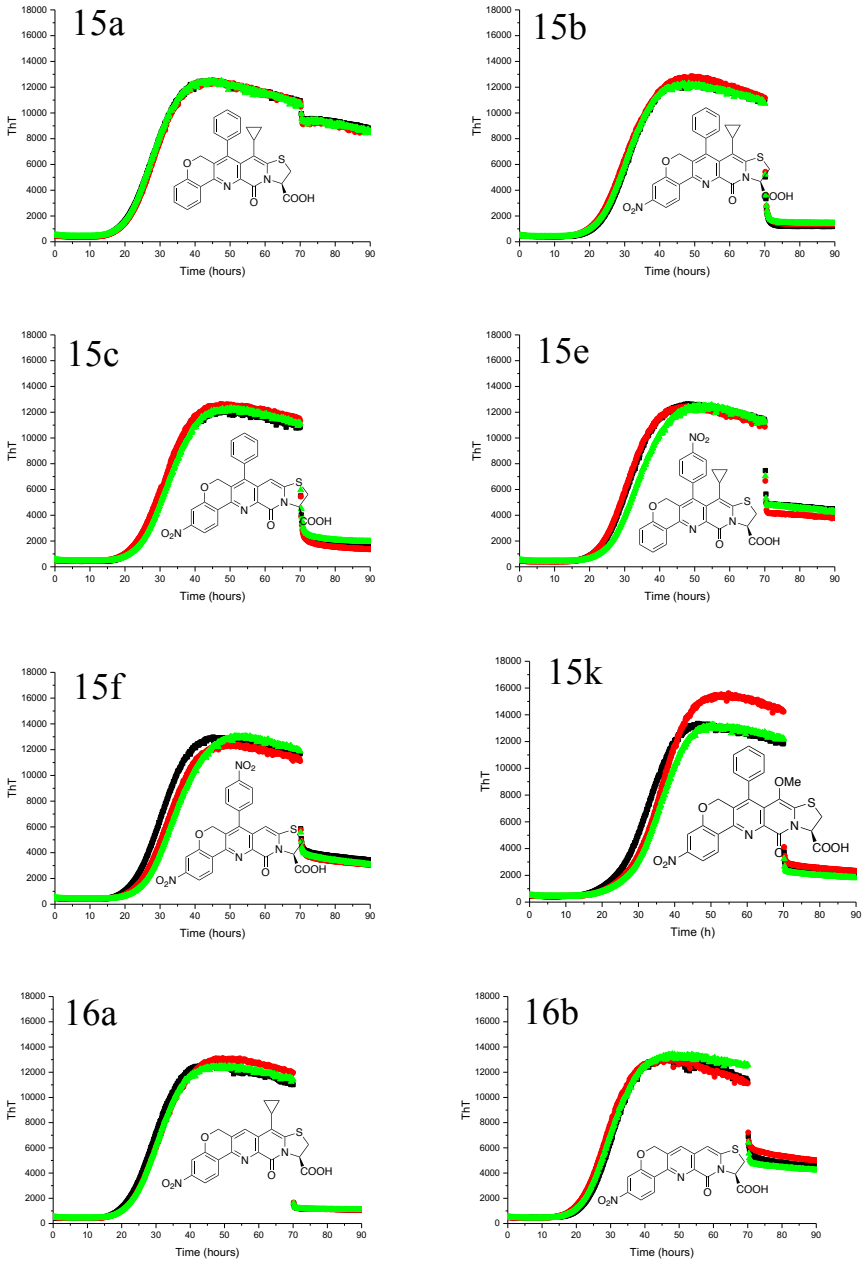


Control (40 000X)

Figure S3:

Addition of compounds to pre-formed α-Synuclein fibers to displace bound ThT.

α-Synuclein fibrilization experiments were set up as described in Figure S1, but without compounds. Displacement of ThT was monitored after 70 hours, when fibers were fully formed, by adding 100 µM of compound and incubated for 20 hours further. The loss in ThT signal was compared before (70 hours) and after addition (75 hours), when the signals had reached a steady plateau. The retained ThT signal for each tested compound is plotted in Figure 2B.



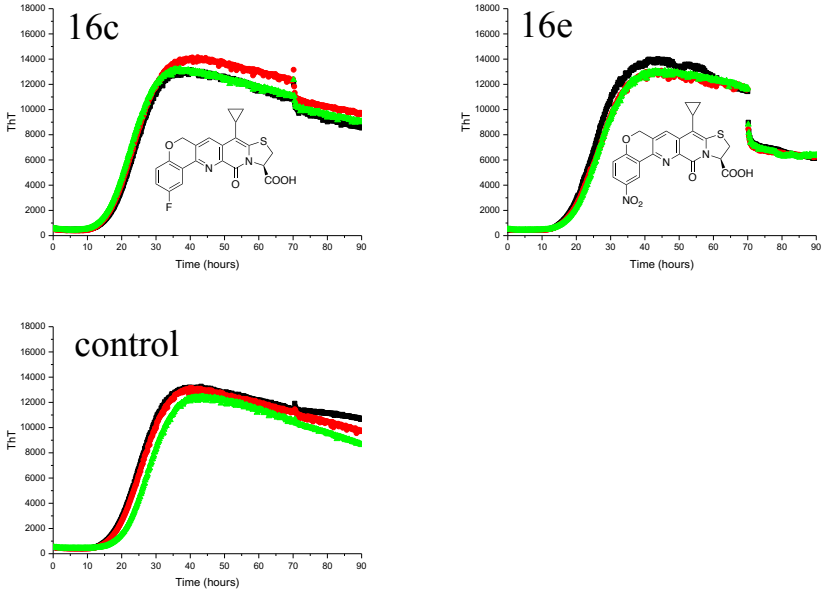


Figure S4:
Retained fluorescence after addition of 0, 10, 30, 50 and 100 mM of selected compound.

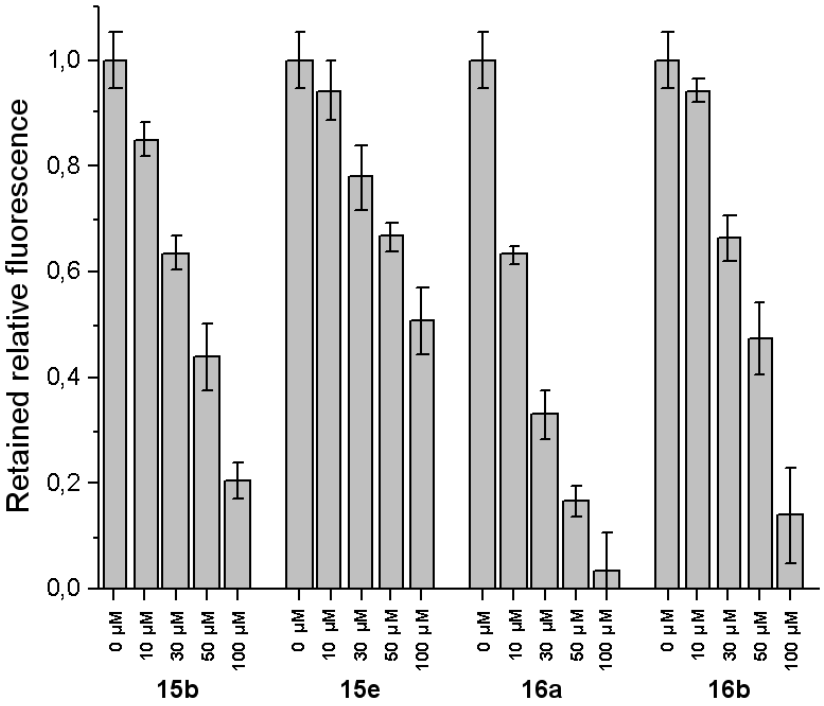
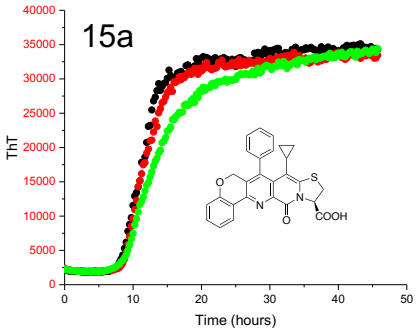
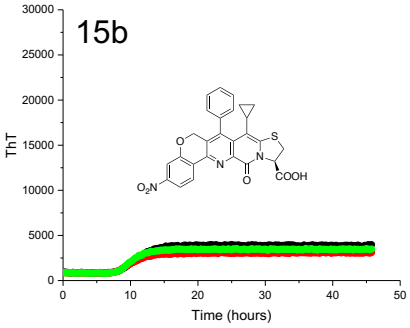
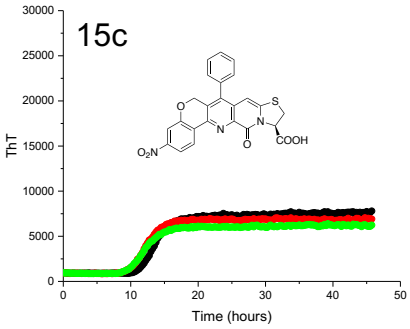
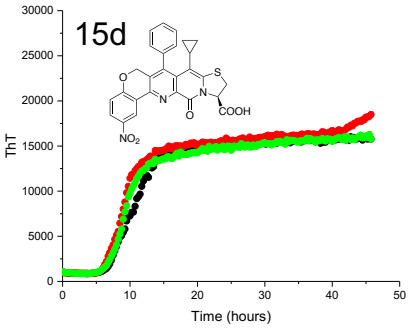


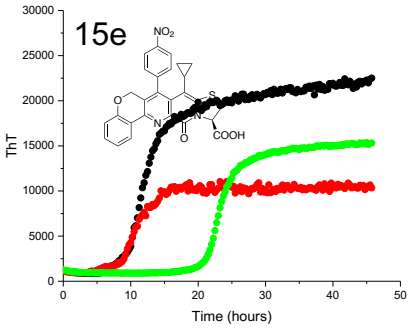
Figure S5:
Evaluation of Amyloid β 1–40 fibrilization modulating properties and screening for fibril binding.
Expressed and purified Aβ40 was supplied by Alexotech AB. A 96-well plate (Corning 3650, Sigma-Aldrich) was loaded with monomeric Aβ40 (5 µM) and compound (20 µM) solubilized in PBS (pH 7.4) with EDTA (1 mM), DMSO (1%) and NaN_3 (0.02%), followed by addition of ThT (40 µM). The plate was incubated at 37 °C during 46 h. ThT fluoresecence (λ_{ex} = 430 nm, λ_{em} = 485 nm) was measured every 30 min. The plate was agitated for 3 s. before each measurement. Each experiment was performed in triplicate. Notice for compound **15a**, the different scaling of the y-axis. **15g** was excluded from this evaluation assay.

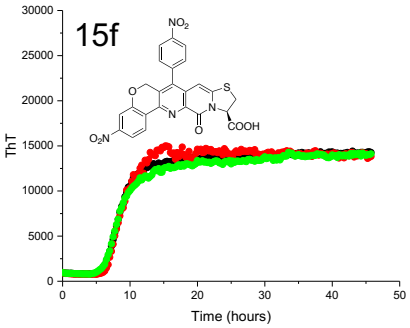

15a
ThT
Time (hours)

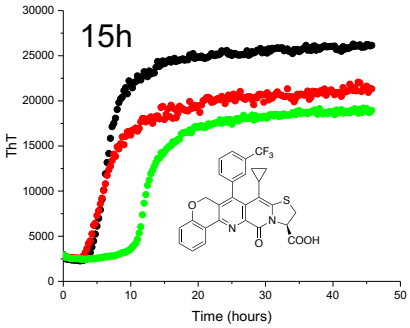

15b
ThT
Time (hours)

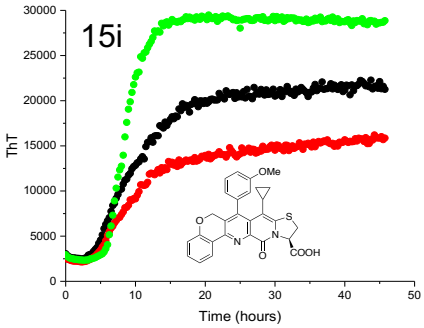

15c
ThT
Time (hours)


15d
ThT
Time (hours)


15e
ThT
Time (hours)


15f
ThT
Time (hours)


15h
ThT
Time (hours)


15i
ThT
Time (hours)

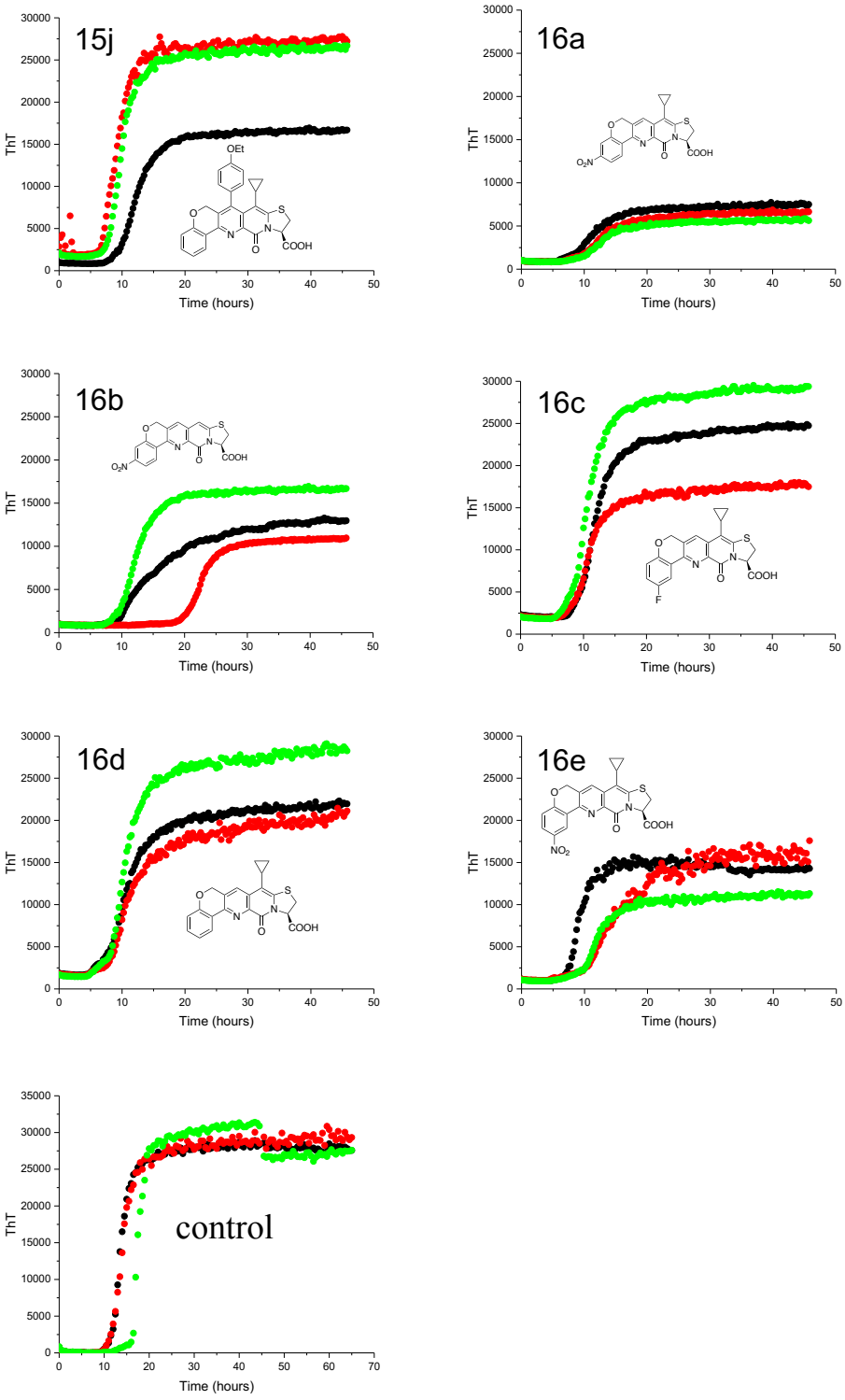
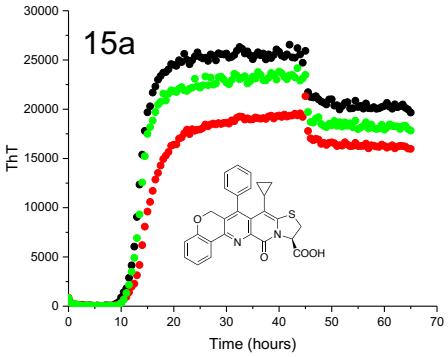
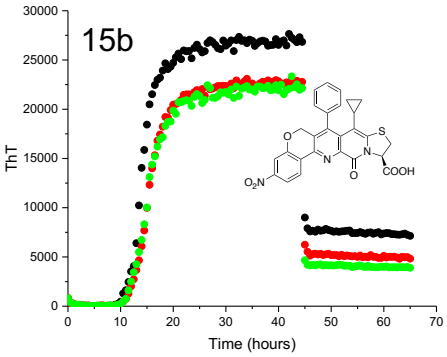


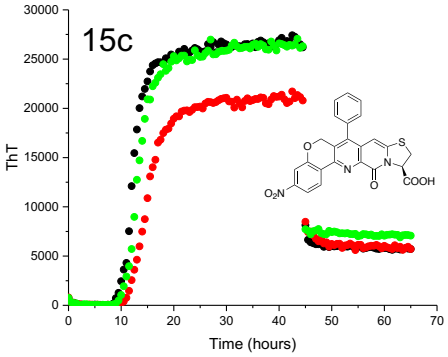
Figure S6:

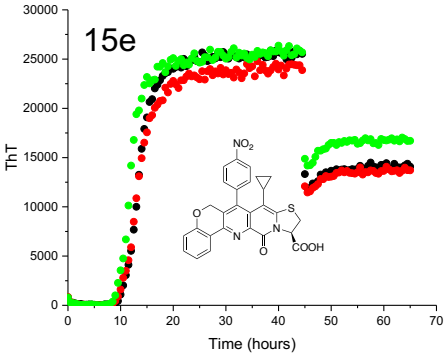
Addition of compounds to pre-formed Aβ40 fibers to displace bound ThT.

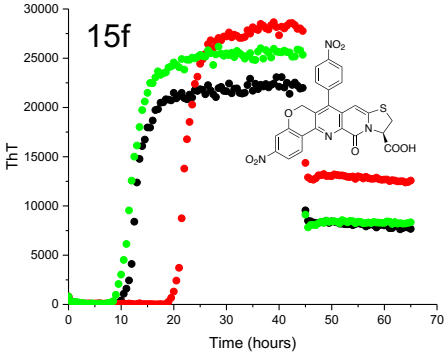
The experiments were performed as described in Figure S4, but without compounds added. Displacement of ThT from mature Aβ40 fibrils was monitored after 45 h, when the experiments had reached the plateau phase of ThT fluorescence. Compound (20 μM) was added and ThT fluorescence was recorded for 20 h more. The level of ThT fluorescence was compared before (44.5 h) and after (65 h) addition. The normalized retained ThT signal for each compound (average of tripicates) is presented in Figure 3.

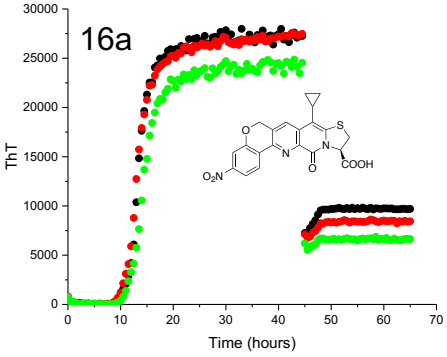

15a
ThT
Time (hours)
COOH

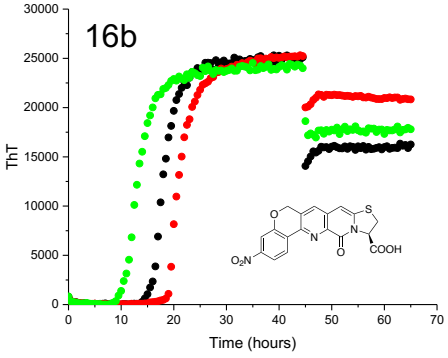

15b
ThT
Time (hours)
O_2N
COOH

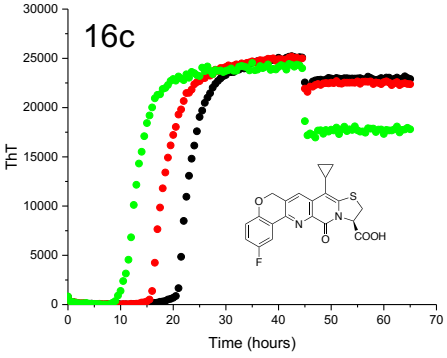

15c
ThT
Time (hours)
O_2N
COOH


15e
ThT
Time (hours)
NO_2
COOH


15f
ThT
Time (hours)
NO_2
O_2N
COOH


16a
ThT
Time (hours)
O_2N
COOH


16b
ThT
Time (hours)
O_2N
COOH


16c
ThT
Time (hours)
F
COOH

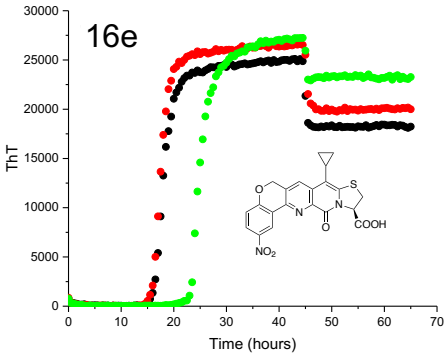


Figure S7:
Bar chart representing retained fluorescence after addition of 0, 0.5, 5, 10, 20, 30, 40 and 50 mM of selected compound.

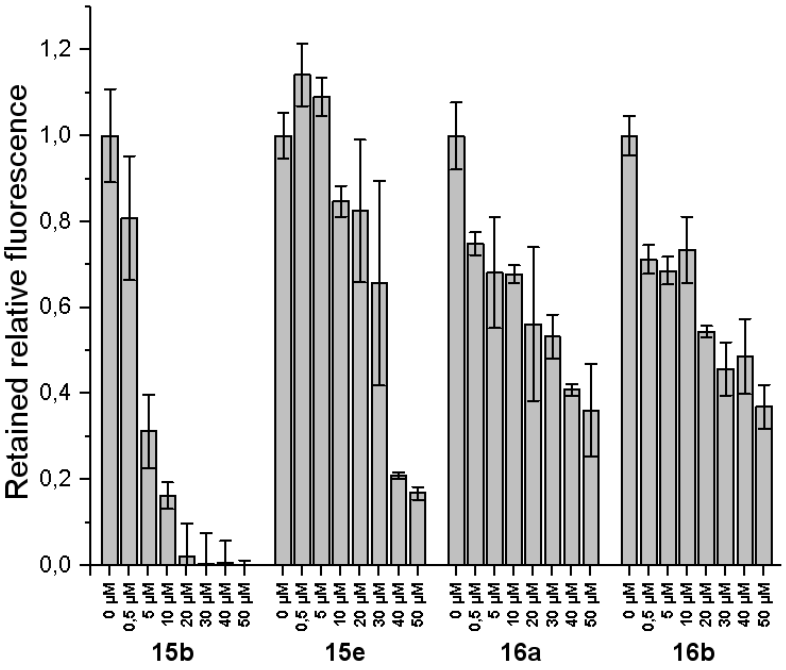
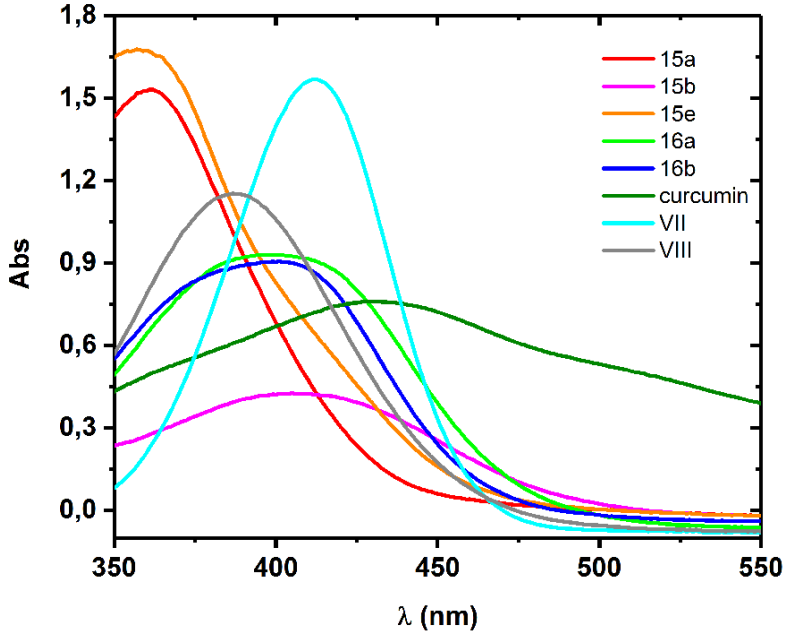


Figure S8:

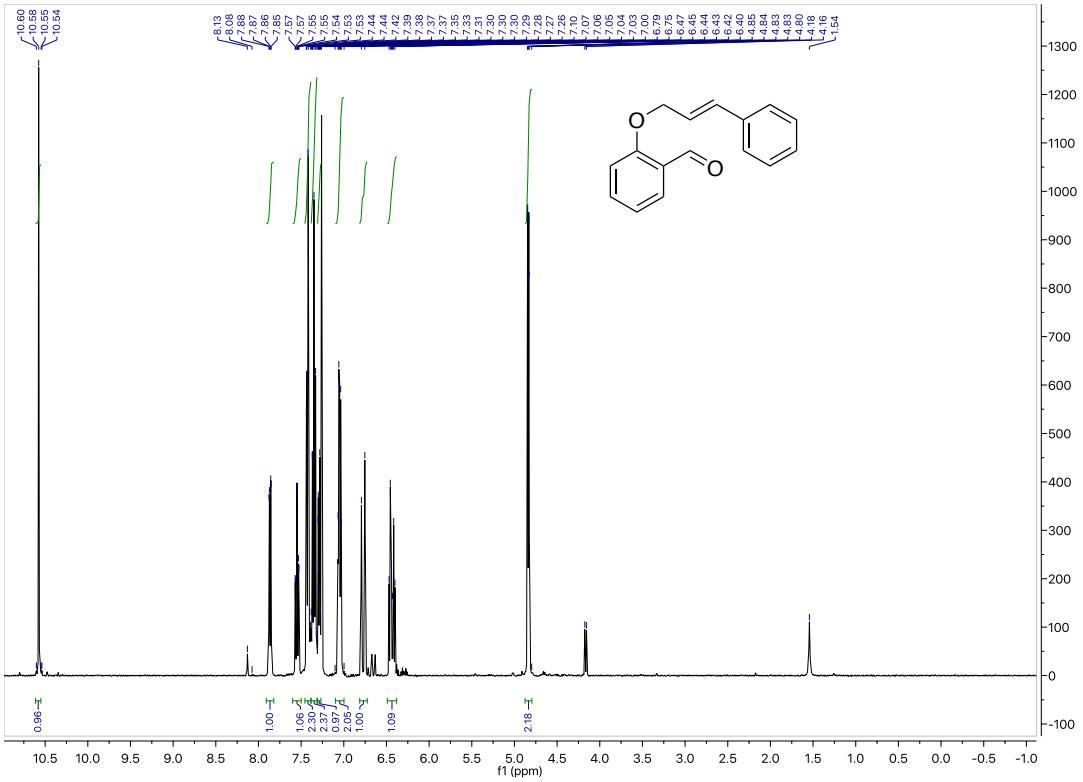
Absorbance spectra of selected compounds.

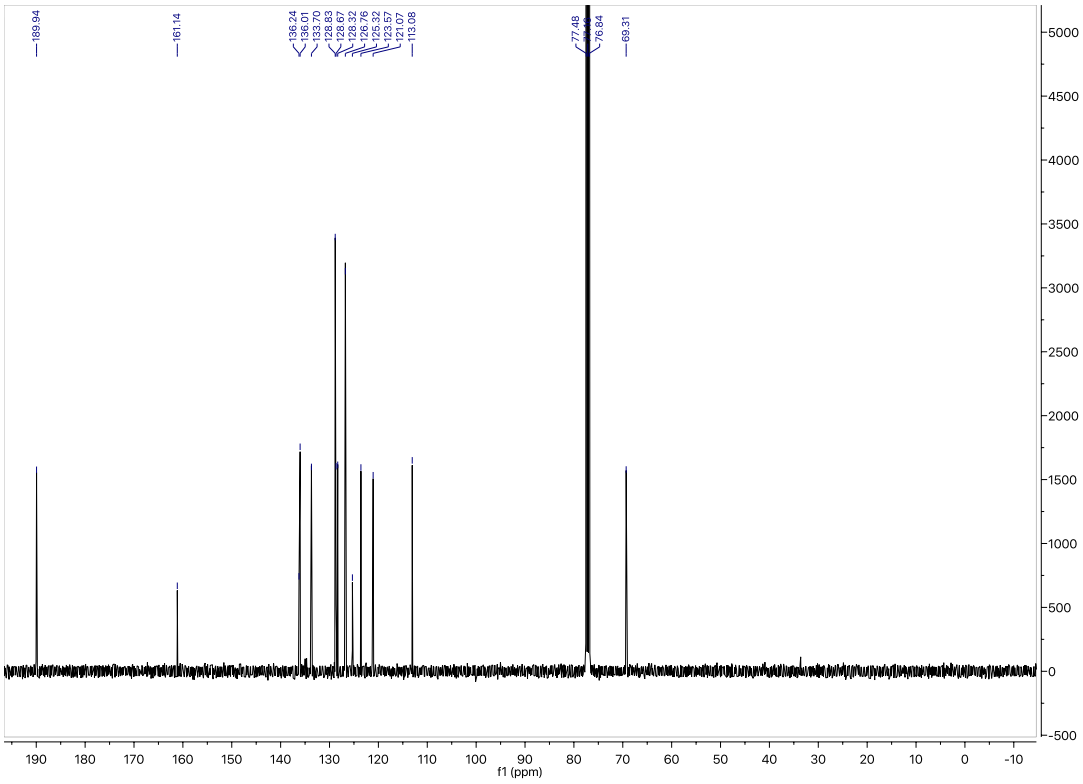
The more active fibril binding compounds **15b**, **15e**, **16a** and **16b** do have a higher absorptivity at 440 nm (the same wavelength used to excite ThT) than the inactive compound **15a** does. However, compound **VII** with even higher absorptivity at 440 nm does not lead to a reduced ThT fluorescence (see plot in Figure S1). Neither does compound **VIII**, which does not bind α-Syn fibrils, lead to a significantly reduced ThT fluorescence by absorbing the exciting light, although its absorptivity at 440 nm is similar to that of active fibril binding compounds.[3b] Thus, any interference by compounds absorbing the light used to excite ThT is not sufficient to explain the reduced ThT fluorescence upon compound addition to mature fibrils. For comparison the absorbance spectrum of Curcumin is included.



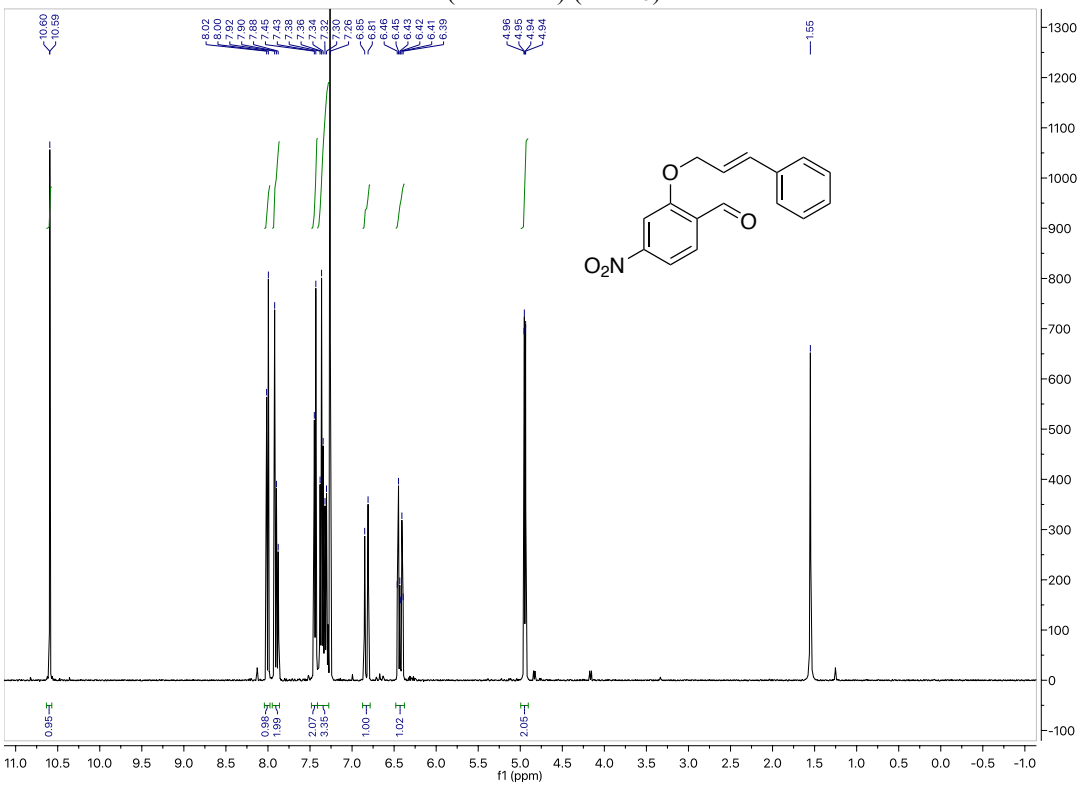
Copies of NMR-spectra:

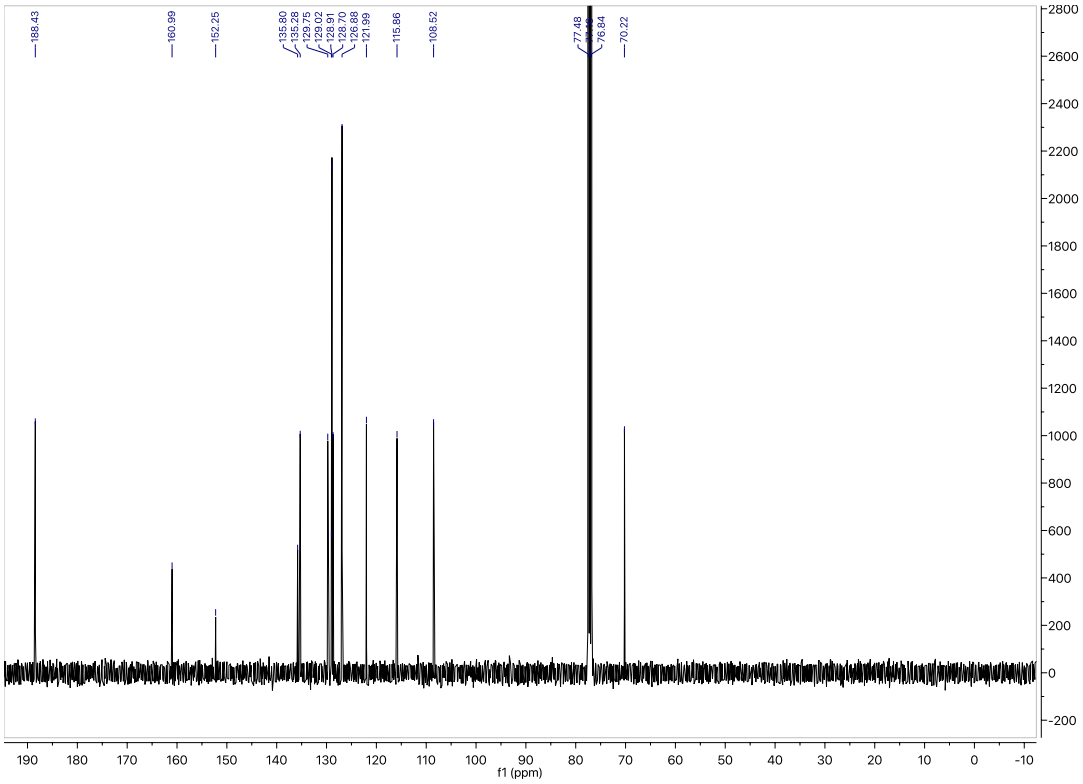
2-(Cinnamyloxy)benzaldehyde (Compound **10a**): ^{1}H-NMR (400 MHz) and ^{13}C{^{1}H}-NMR (100 MHz) ($CDCl_3$).



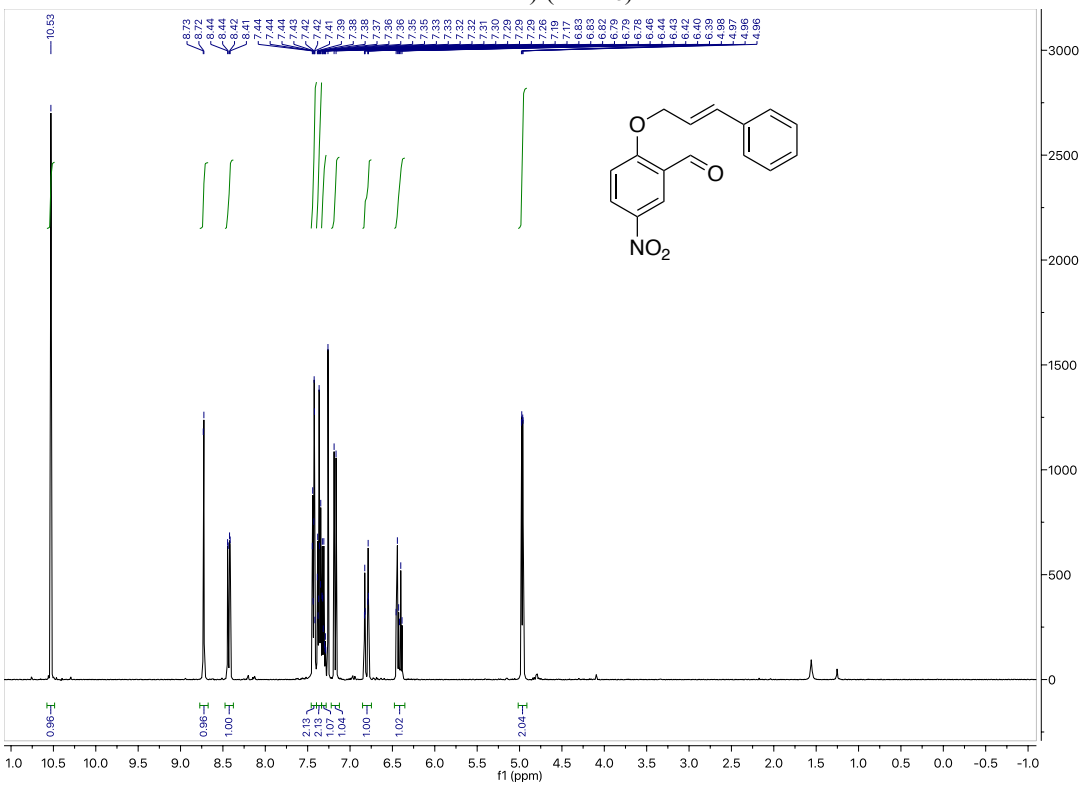


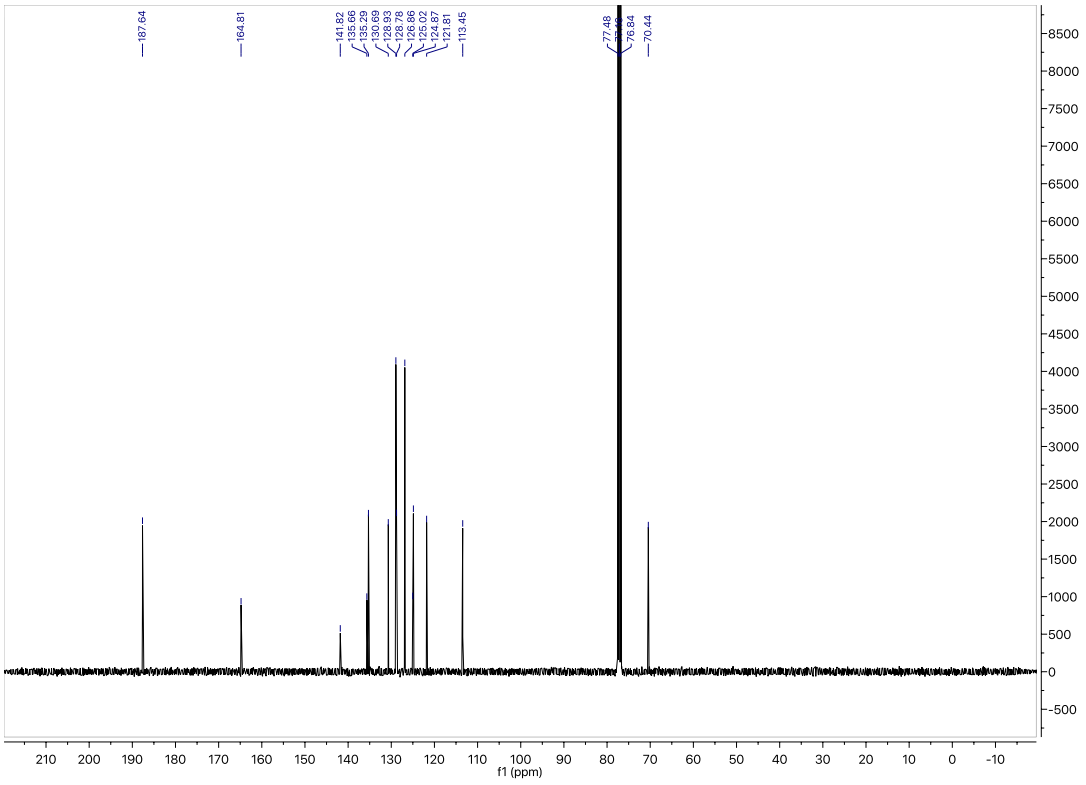
2-(Cinnamyloxy)-4-nitrobenzaldehyde (Compound **10b**): ^{1}H-NMR (400 MHz) and ^{13}C{^{1}H}-NMR (100 MHz) ($CDCl_3$).



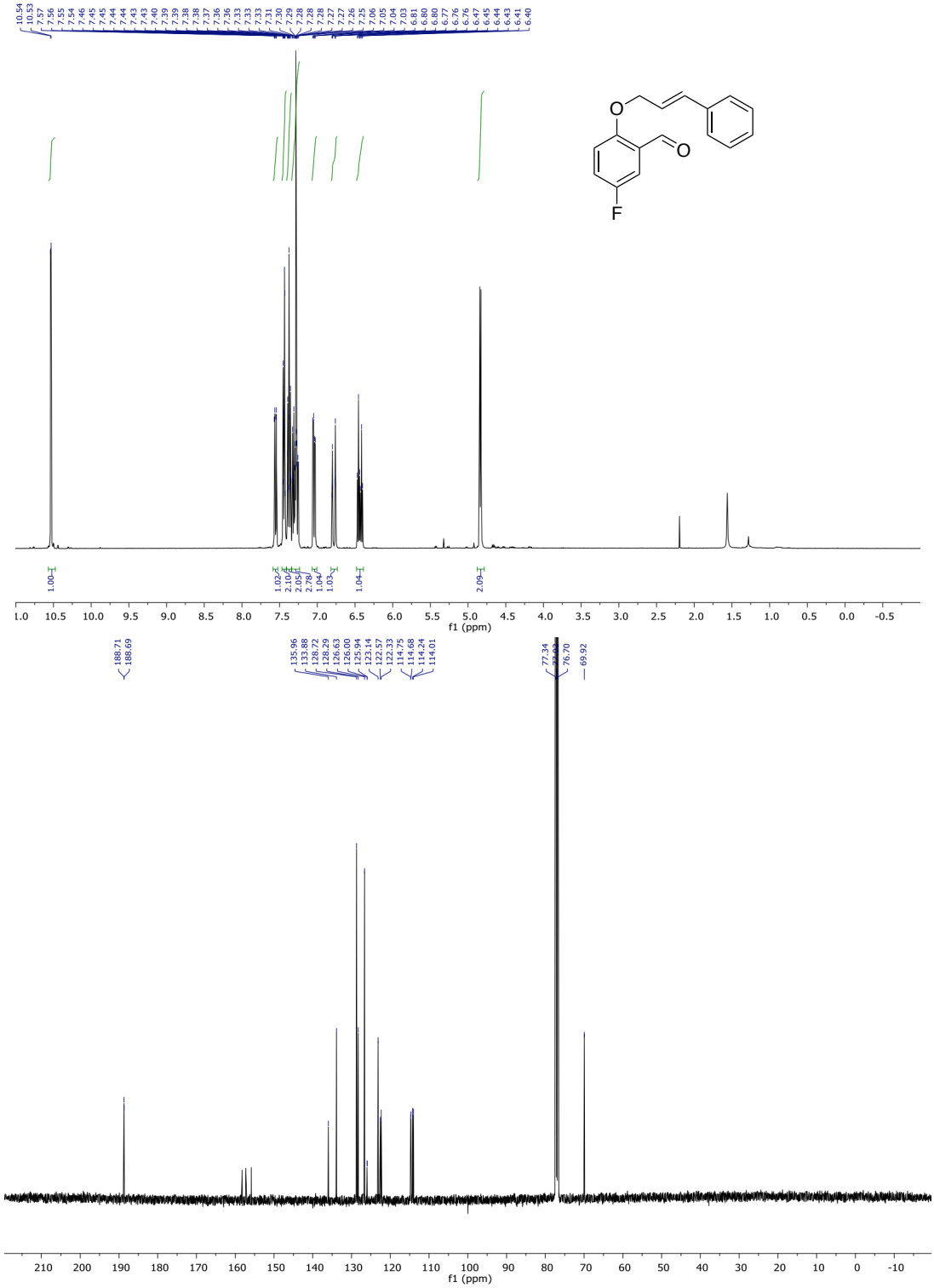


2-(Cinnamyloxy)-5-nitrobenzaldehyde (Compound **10c**): ^{1}H-NMR (400 MHz) and ^{13}C{^{1}H}-NMR (100 MHz) ($CDCl_3$).

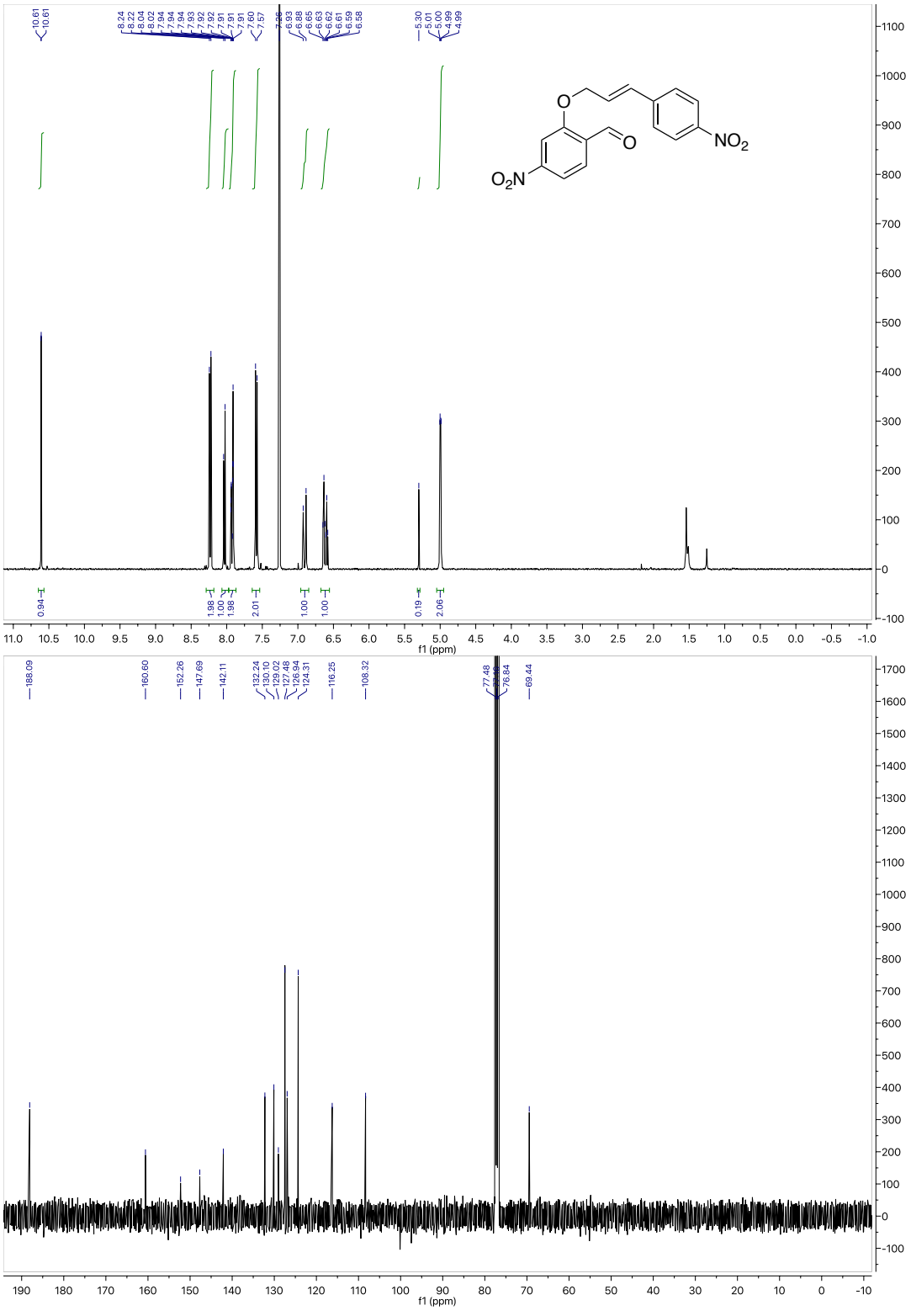




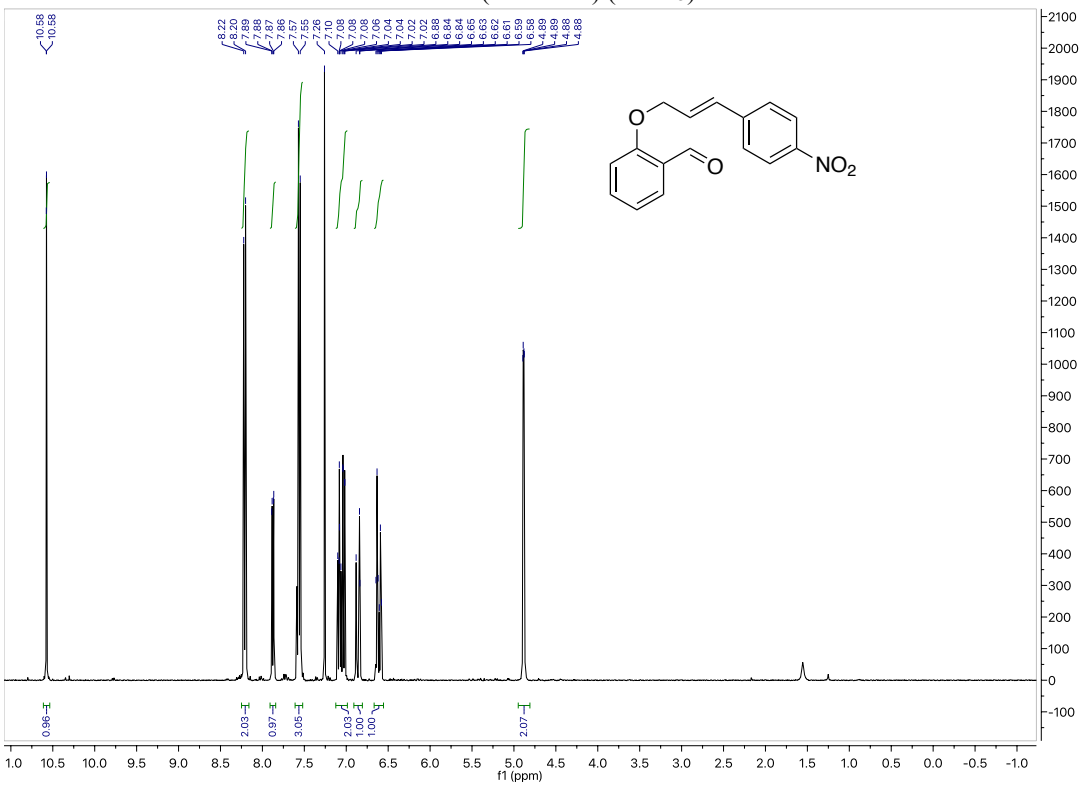
2-(Cinnamyloxy)-5-fluorobenzaldehyde (Compound **10d**): ^{1}H-NMR (400 MHz) and $^{13}C\{^{1}H\}$-NMR (100 MHz) ($CDCl_3$).

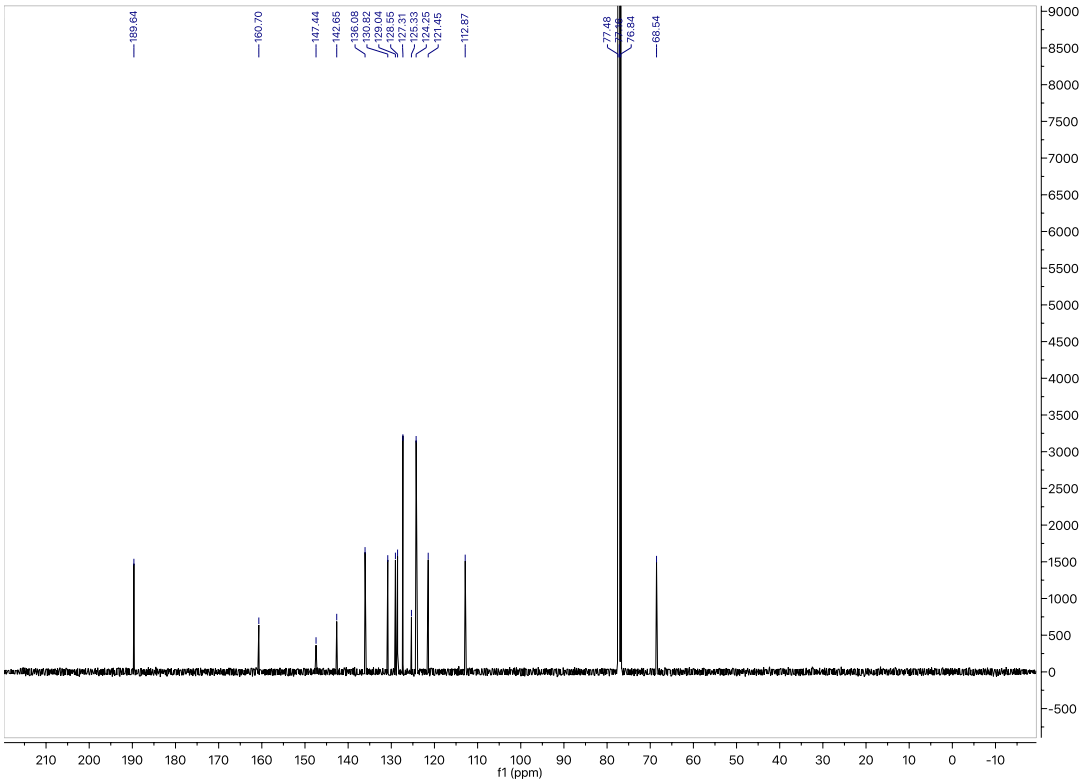


(*E*)-4-Nitro-2-((3-(4-nitrophenyl)allyl)oxy)benzaldehyde (Compound **10e**): ^{1}H-NMR (400 MHz) and $^{13}C\{^{1}H\}$-NMR (100 MHz) ($CDCl_3$).

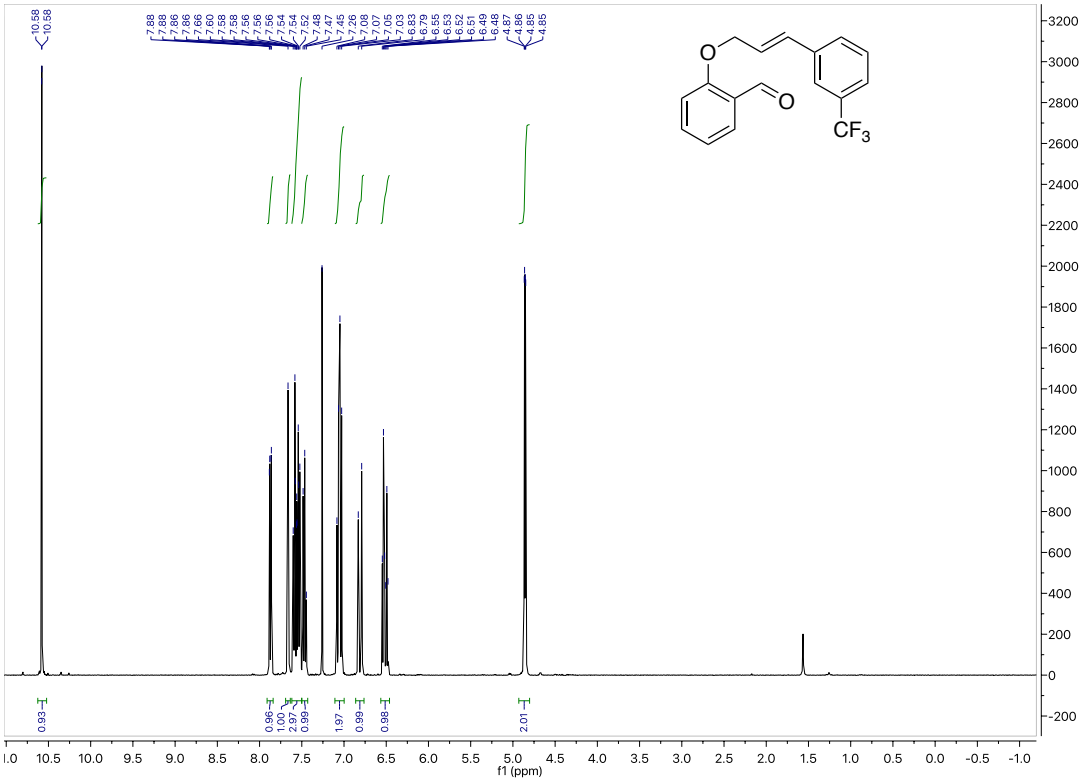


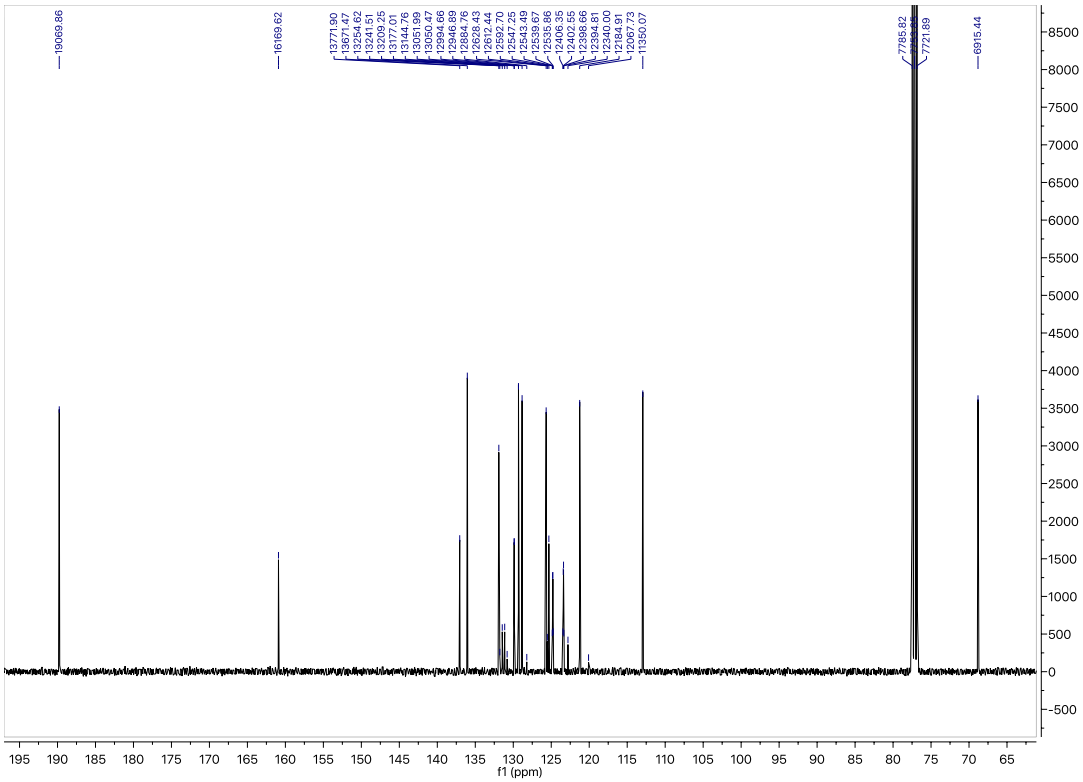
(*E*)-2-((3-(4-Nitrophenyl)allyl)oxy)benzaldehyde (Compound **10f**): ^{1}H-NMR (400 MHz) and ^{13}C{^{1}H}-NMR (100 MHz) ($CDCl_3$).

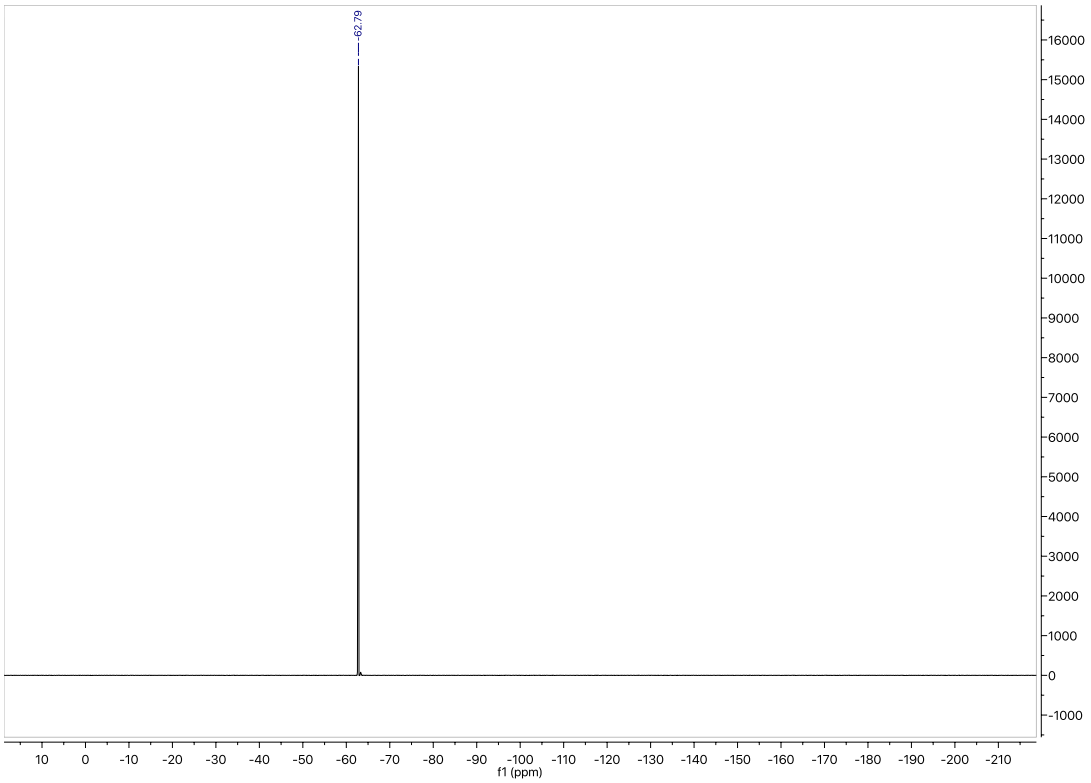




(*E*)-2-((3-(3-(Trifluoromethyl)phenyl)allyl)oxy)benzaldehyde (Compound **10g**): ^{1}H-NMR (400 MHz), ^{13}C{^{1}H}-NMR (100 MHz) and ^{19}F-NMR (376 MHz) ($CDCl_3$).



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4000
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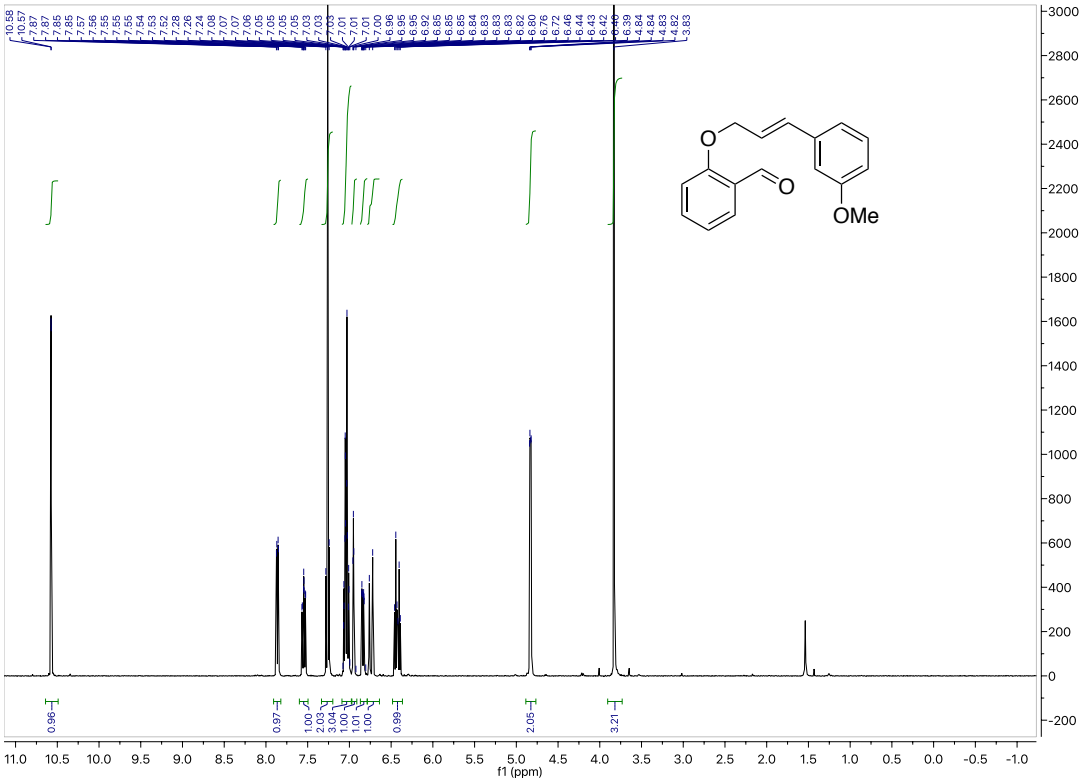
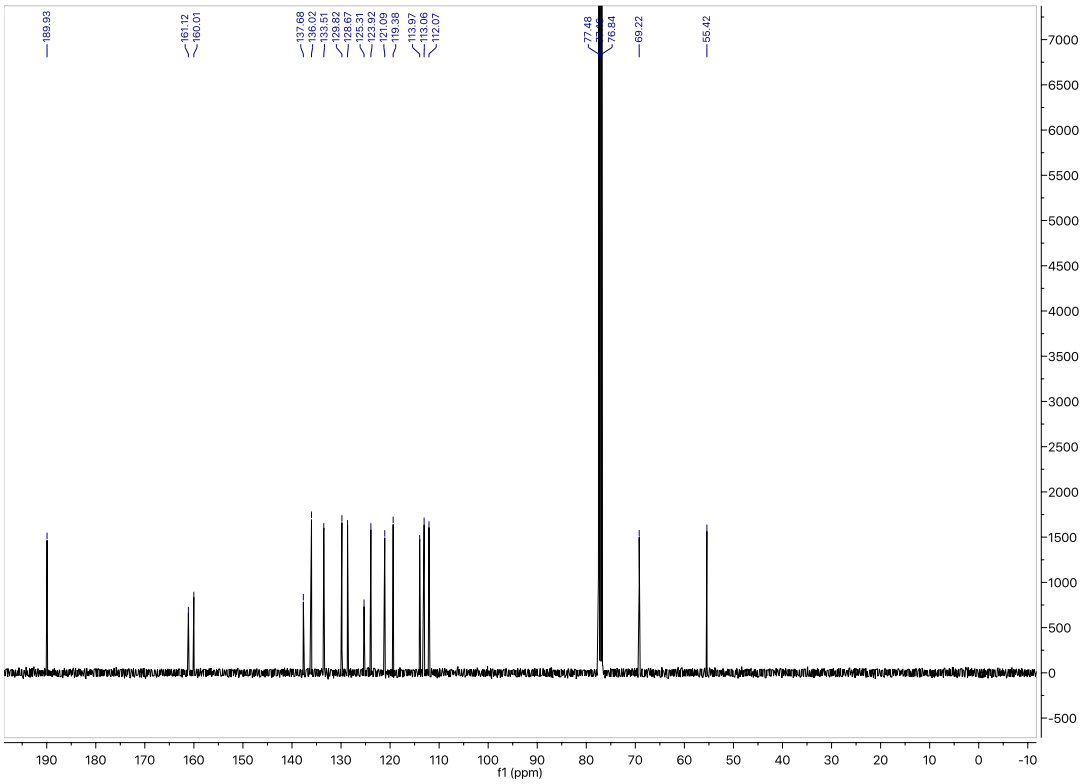
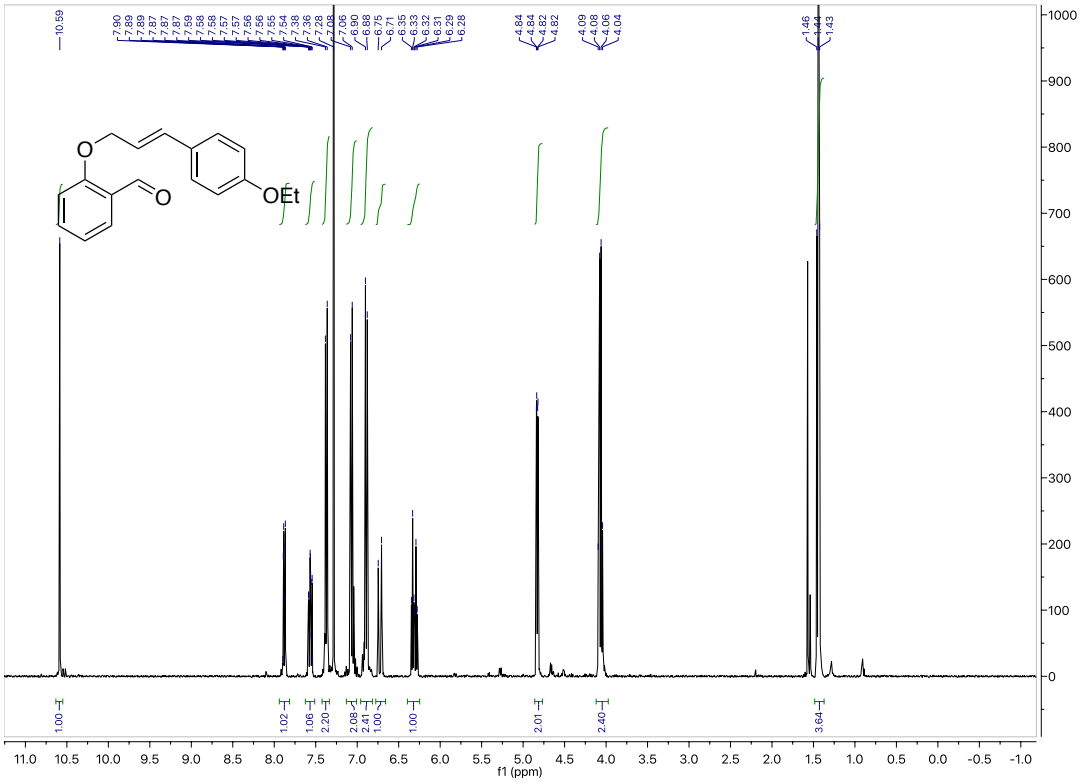
2000
1000
0
-1000
10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210
f1 (ppm)

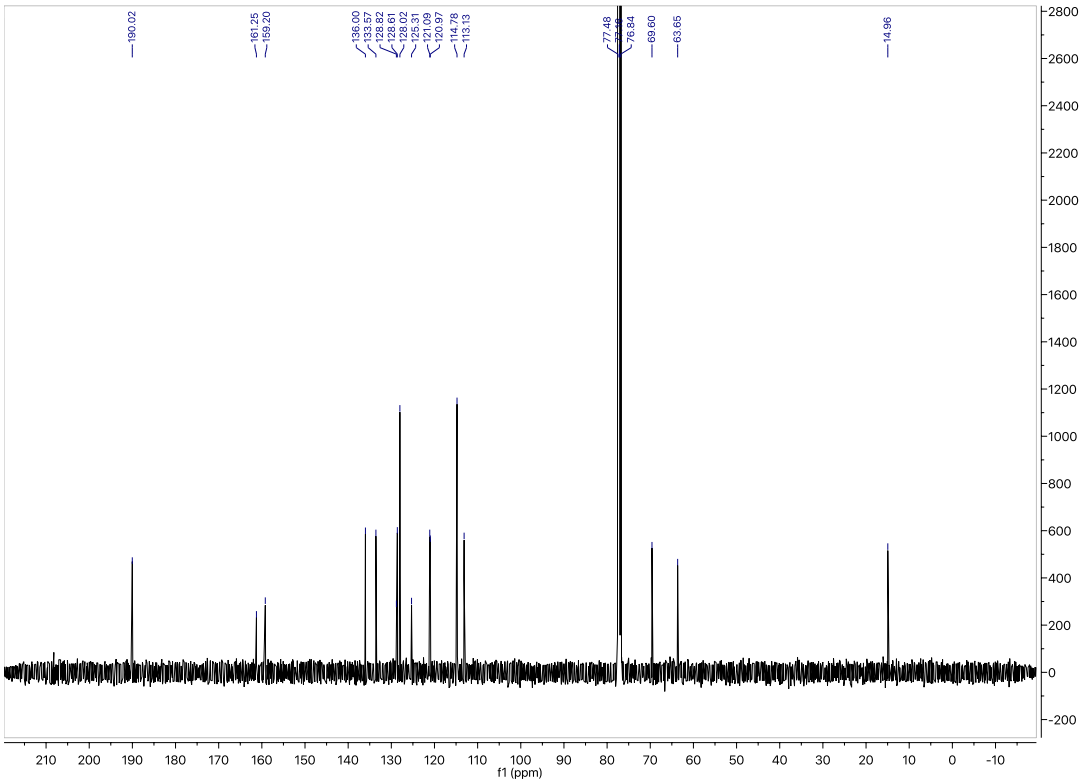
(*E*)-2-((3-(3-Methoxyphenyl)allyl)oxy)benzaldehyde (Compound **10h**): ^{1}H-NMR (400 MHz) and ^{13}C{^{1}H}-NMR (100 MHz) ($CDCl_3$).



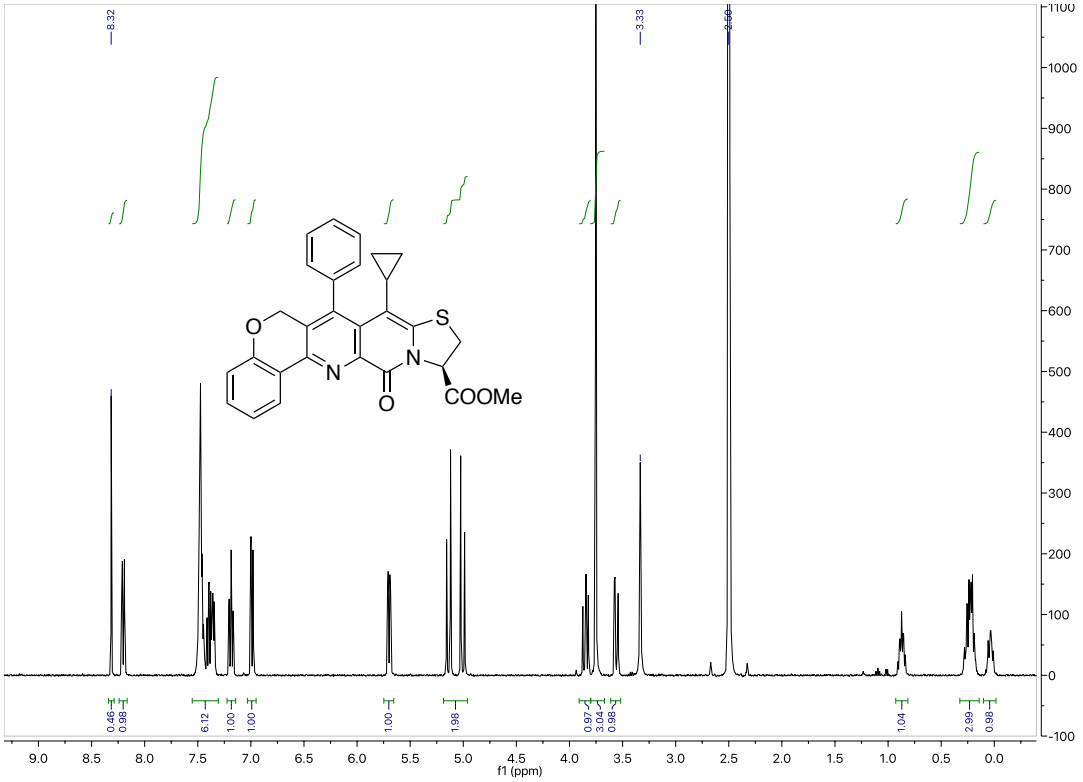


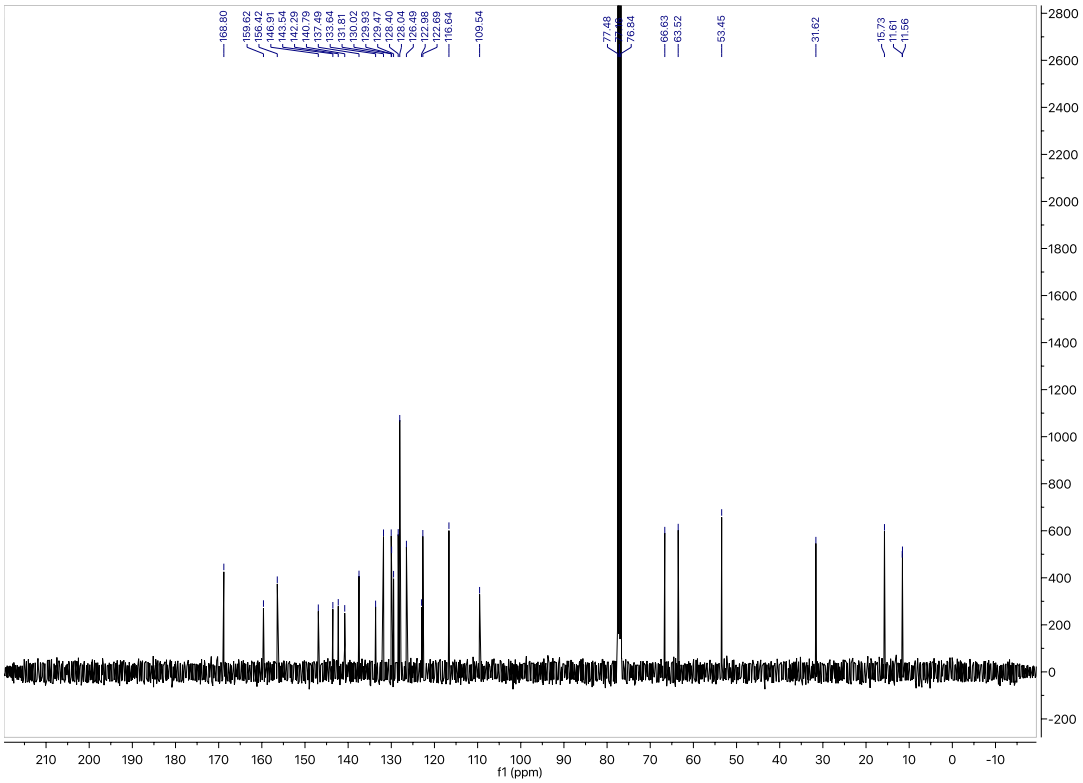
(*E*)-2-((3-(4-Ethoxyphenyl)allyl)oxy)benzaldehyde (Compound **10i**): ^{1}H-NMR (400 MHz) and $^{13}C\{^{1}H\}$-NMR (100 MHz) ($CDCl_3$).



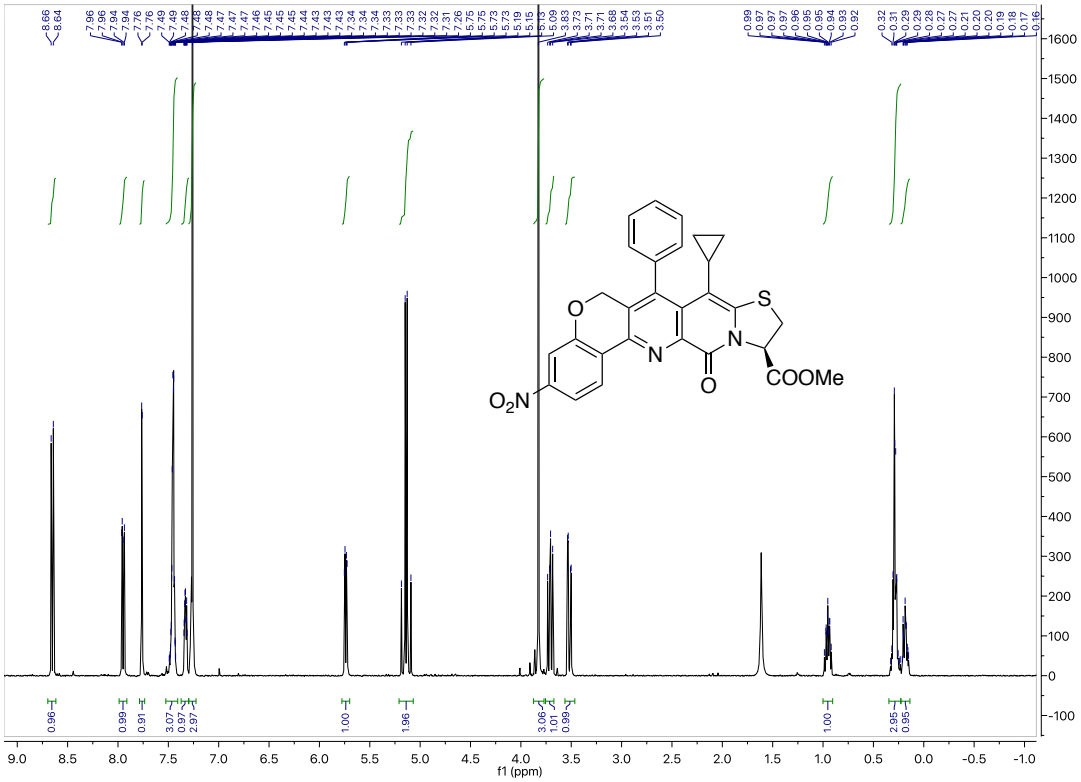


Methyl (*R*)-8-cyclopropyl-13-oxo-7-phenyl-6,10,11,13-tetrahydrochromeno[4,3-*b*]thiazolo[2,3-*g*][1,7]naphthyridine-11-carboxylate (Compound **11a**): ^{1}H-NMR [400 MHz. $(CD_3)_2SO$] and $^{13}C\{^{1}H\}$-NMR (100 MHz, $CDCl_3$).

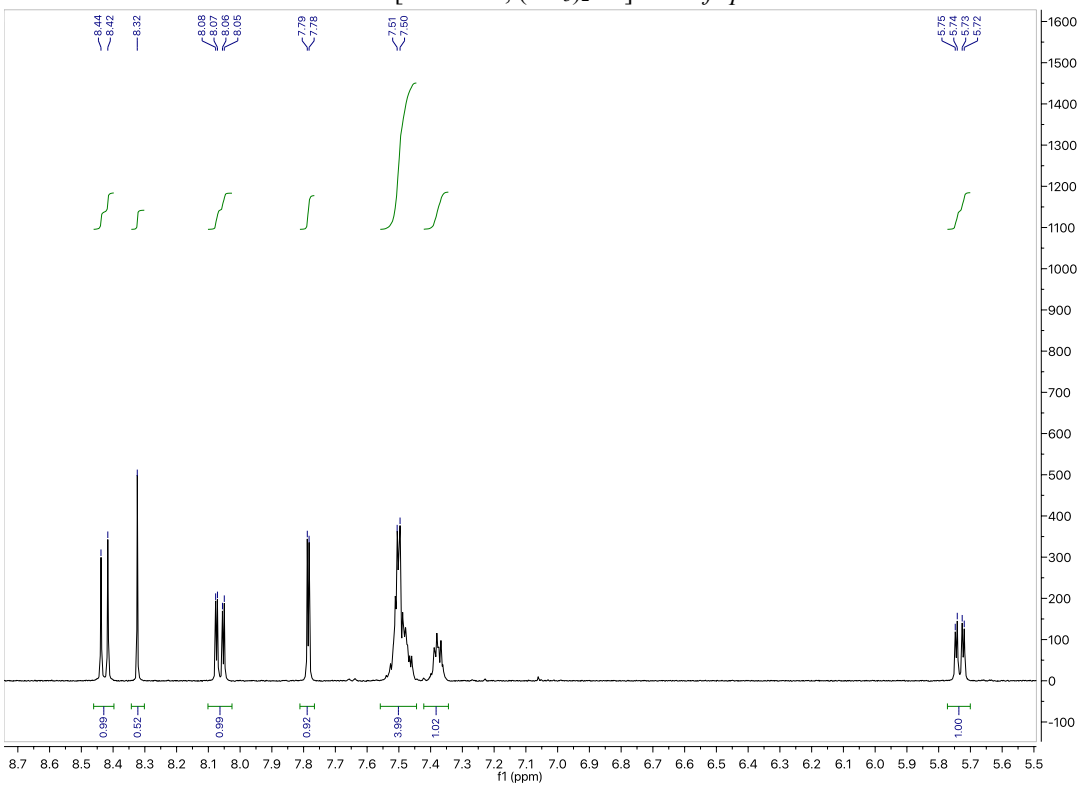




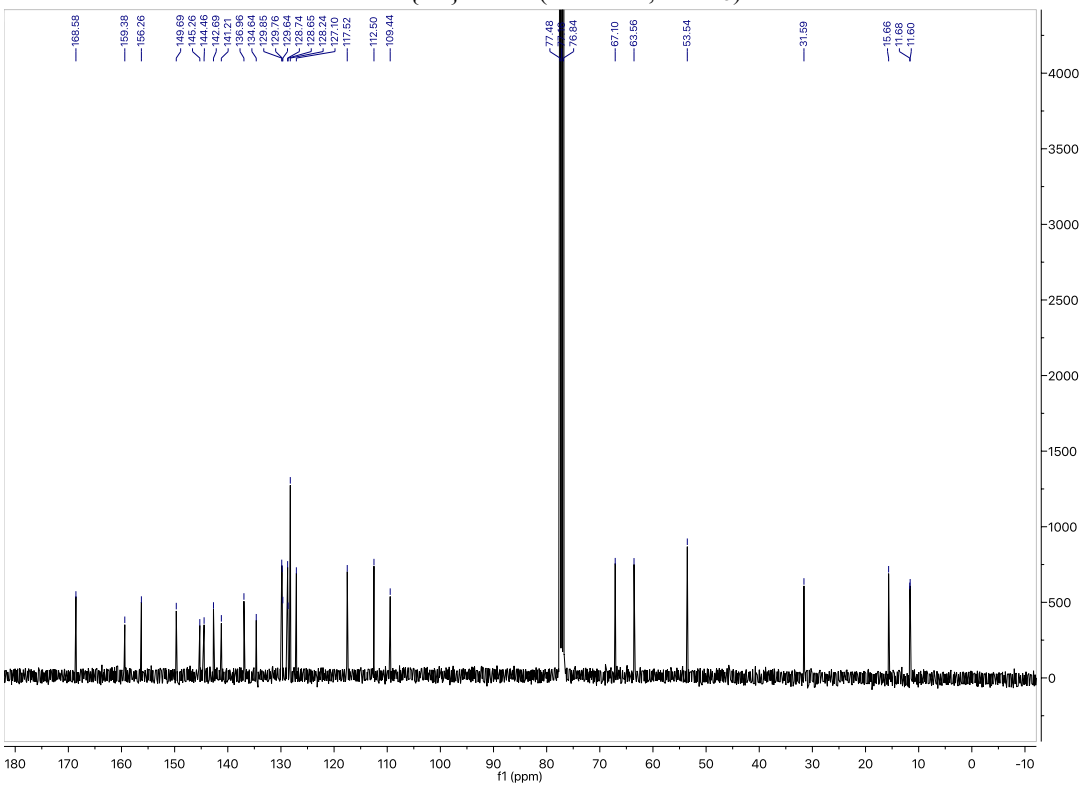
Methyl 8-cyclopropyl-3-nitro-13-oxo-7-phenyl-6,10,11,13- tetrahydrochromeno[4,3-*b*]thiazolo[2,3-*g*][1,7]naphthyridine-11-carboxylate (Compound **11b**): ^{1}H-NMR (400 MHz, $CDCl_3$).



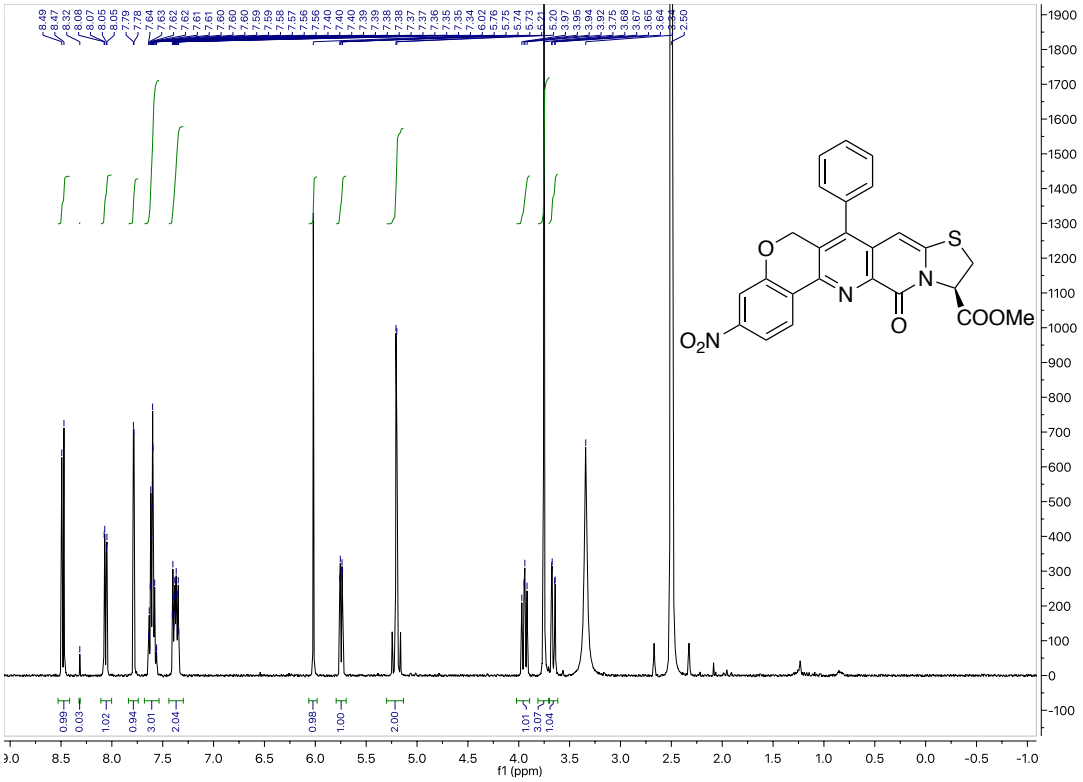
^{1}H-NMR [400 MHz, $(CD_3)_2SO$] *Part of spectrum.*

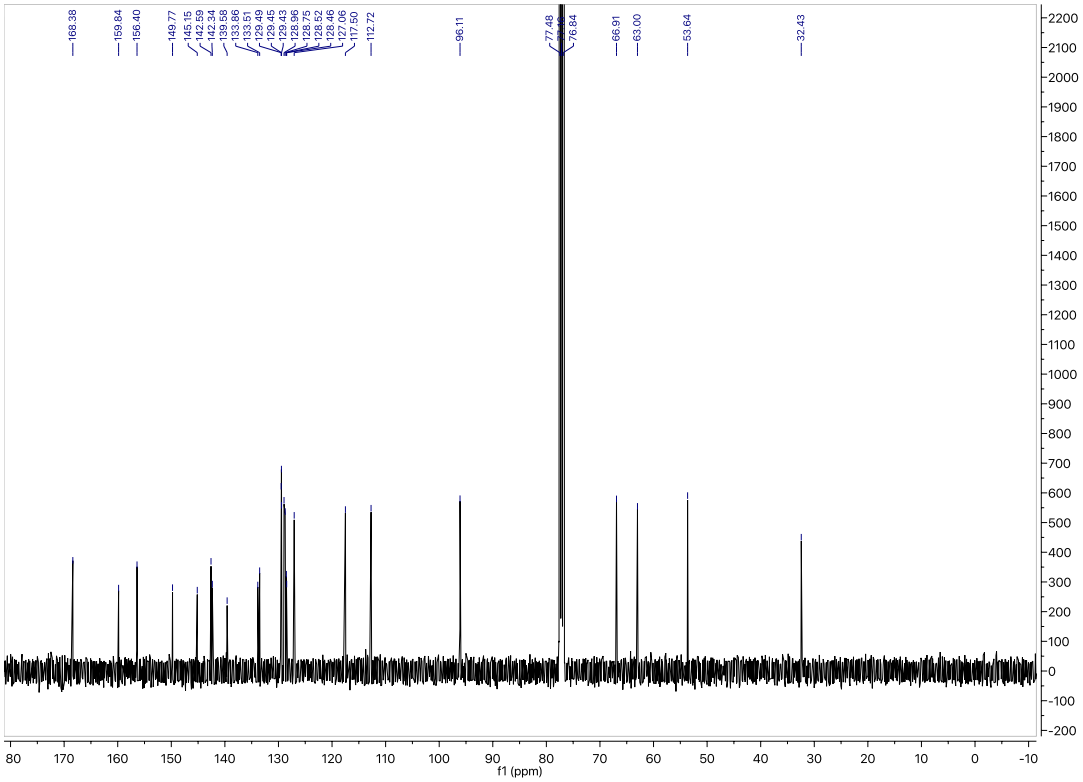


$^{13}C\{^{1}H\}$-NMR (100 MHz, $CDCl_3$)

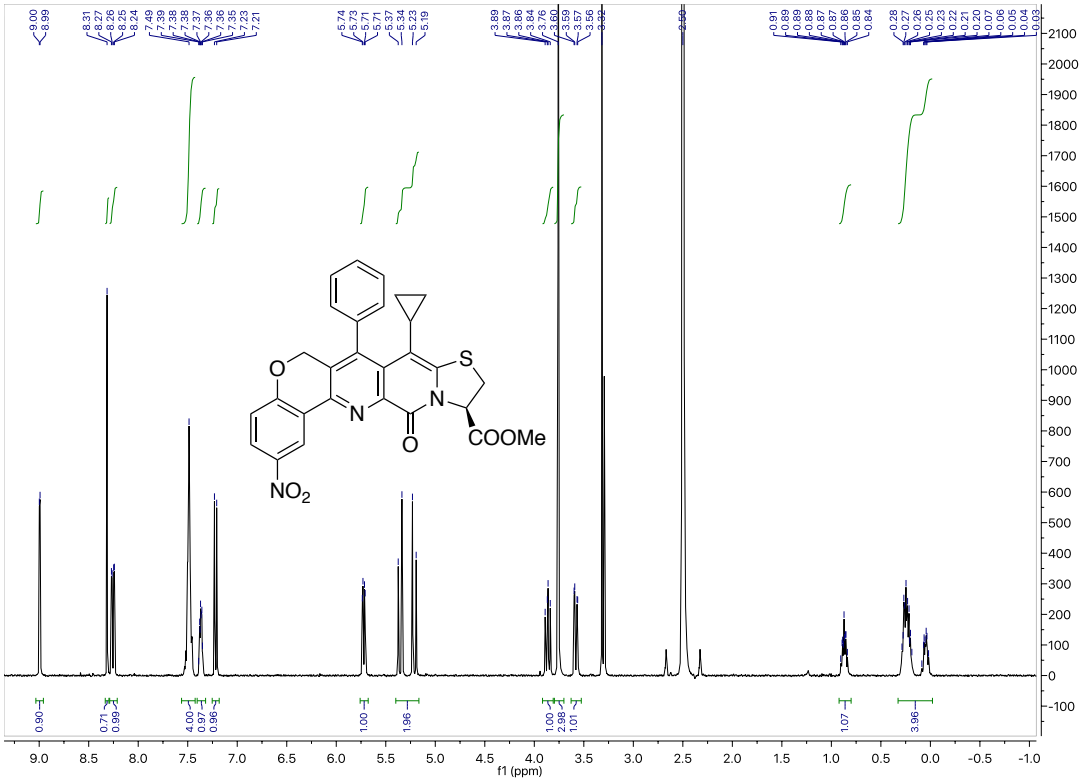


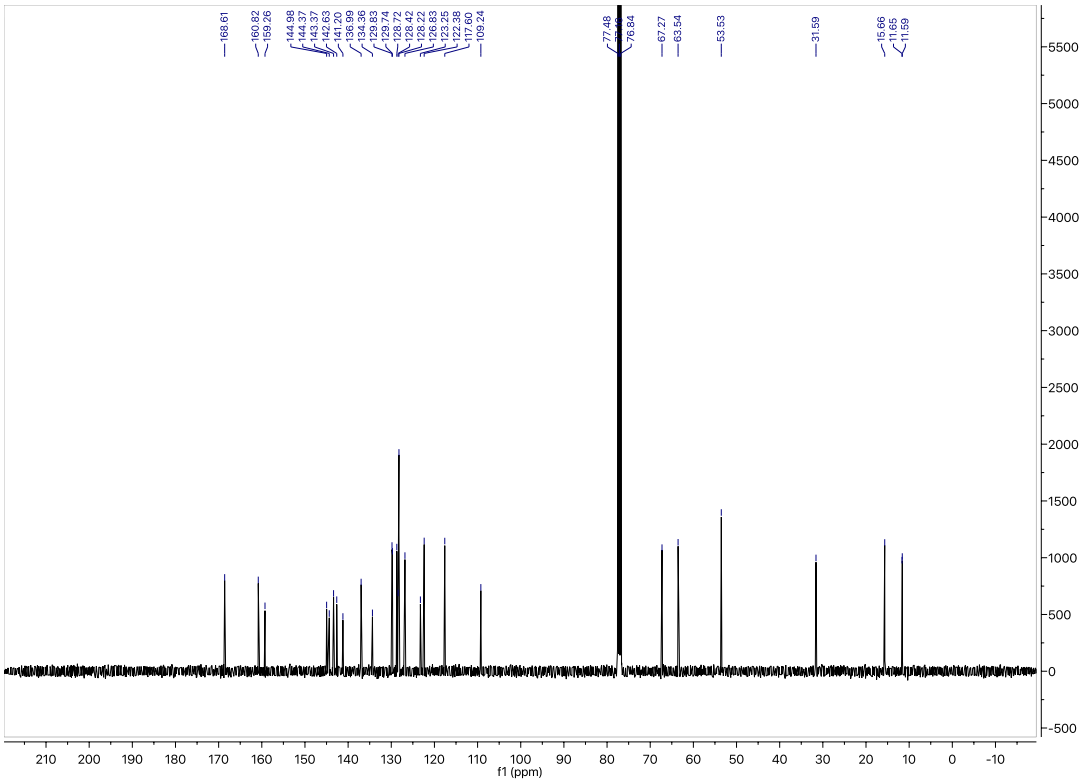
Methyl (*R*)-3-nitro-13-oxo-7-phenyl-6,10,11,13-tetrahydrochromeno[4,3-*b*]thiazolo[2,3-*g*][1,7]naphthyridine-11-carboxylate (Compound **11c**): ^{1}H-NMR [400 MHz, $(CD_3)_2SO$] and $^{13}C\{^{1}H\}$-NMR (100 MHz, $CDCl_3$)



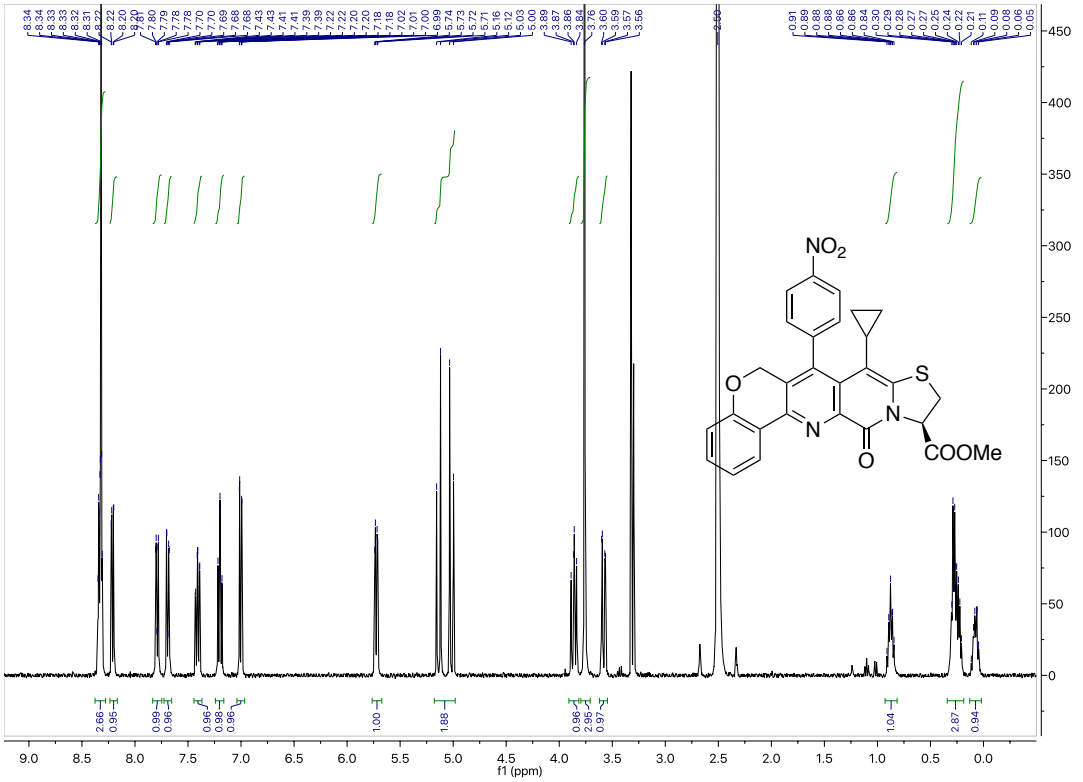


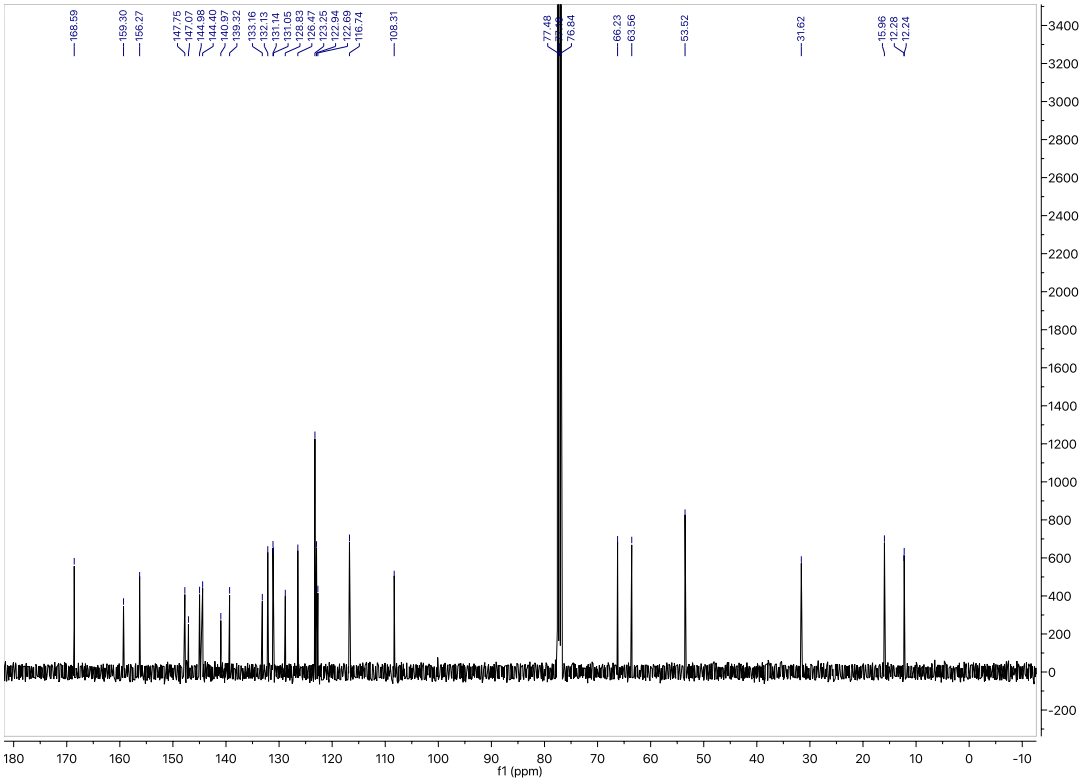
Methyl (*R*)-8-cyclopropyl-2-nitro-13-oxo-7-phenyl-6,10,11,13-tetrahydrochromeno[4,3-*b*]thiazolo[2,3-*g*][1,7]naphthyridine-11-carboxylate (Compound **11d**): ^{1}H-NMR [400 MHz. $(CD_3)_2SO$] and $^{13}C\{^{1}H\}$-NMR (100 MHz, $CDCl_3$).



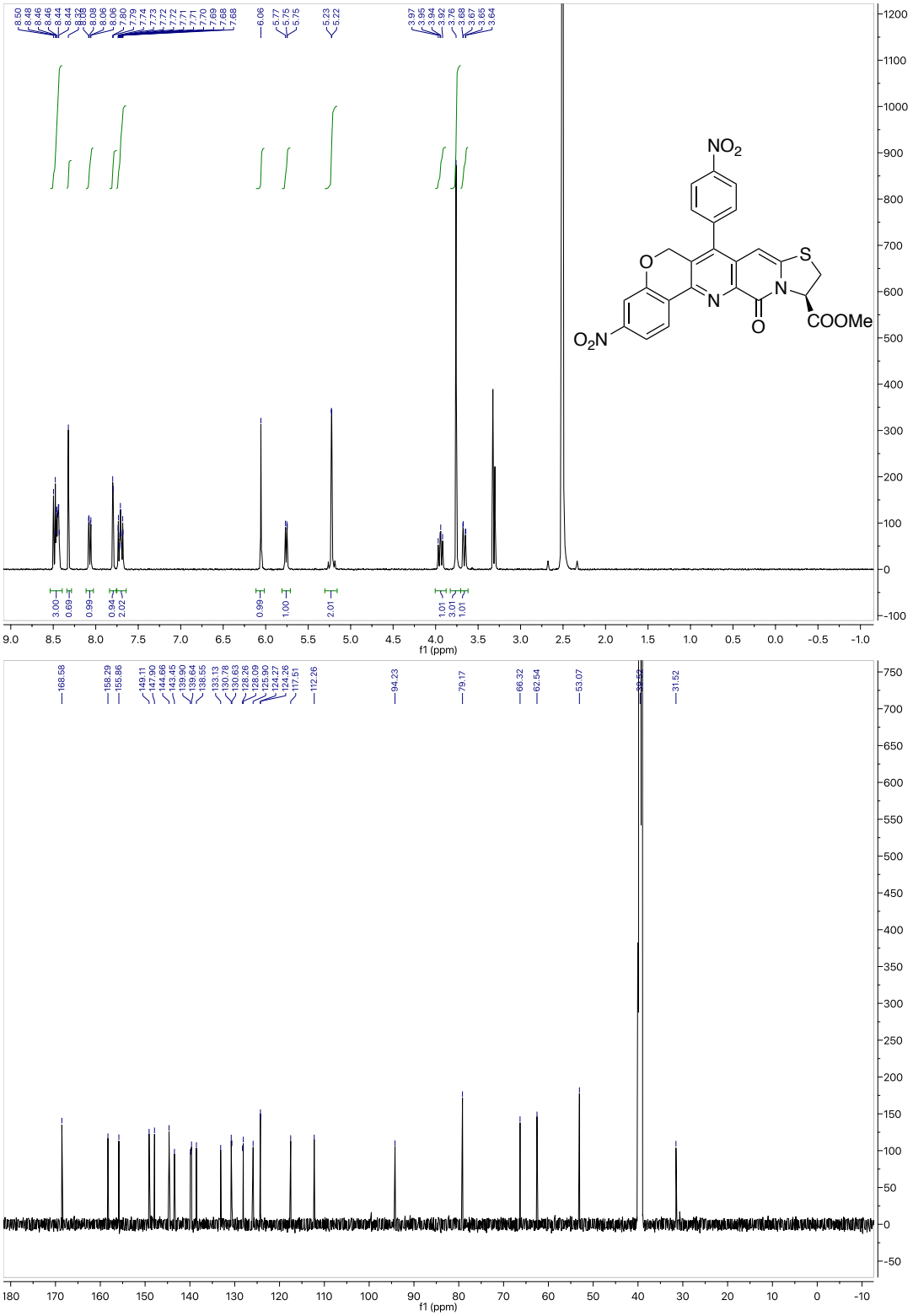


Methyl (*R*)-8-cyclopropyl-7-(4-nitrophenyl)-13-oxo-6,10,11,13-tetrahydrochromeno[4,3-*b*]thiazolo[2,3-*g*][1,7]naphthyridine-11-carboxylate (Compound **11e**): ^{1}H-NMR [400 MHz, $(CD_3)_2SO$] and $^{13}C\{^{1}H\}$-NMR (100 MHz, $CDCl_3$).

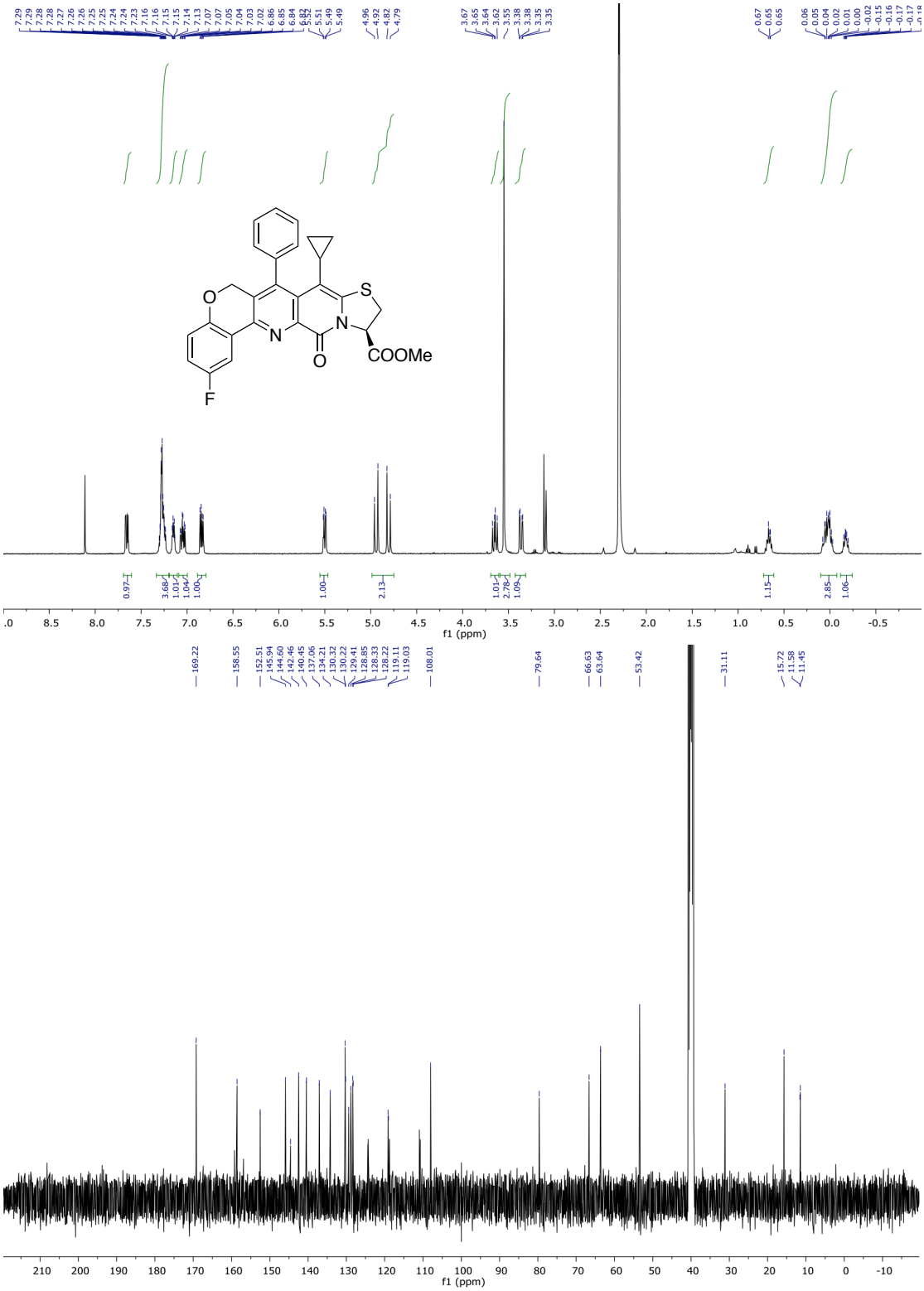


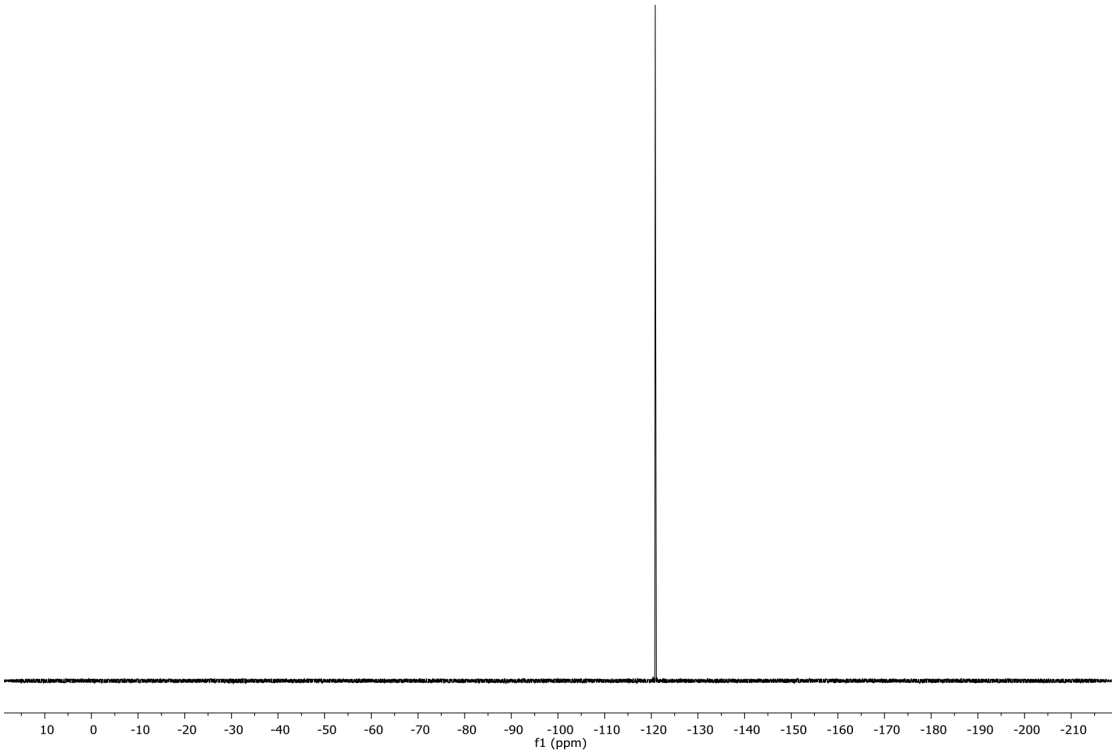


Methyl (*R*)-3-nitro-7-(4-nitrophenyl)-13-oxo-6,10,11,13-tetrahydrochromeno[4,3-*b*]thiazolo[2,3-*g*][1,7]naphthyridine-11-carboxylate (Compound **11f**): ^{1}H-NMR (400 MHz) and ^{13}C{^{1}H}-NMR (151 MHz) [$(CD_3)_2SO$].



Methyl (*R*)-8-cyclopropyl-2-fluoro-13-oxo-7-phenyl-6,10,11,13-tetrahydrochromeno[4,3-*b*]thiazolo[2,3-*g*][1,7]naphthyridine-11-carboxylate (Compound **11g**): ^{1}H-NMR (400 MHz), ^{13}C{^{1}H}-NMR (100 MHz) and ^{19}F{^{1}H}-NMR (376 MHz) [$(CD_3)_2SO$].

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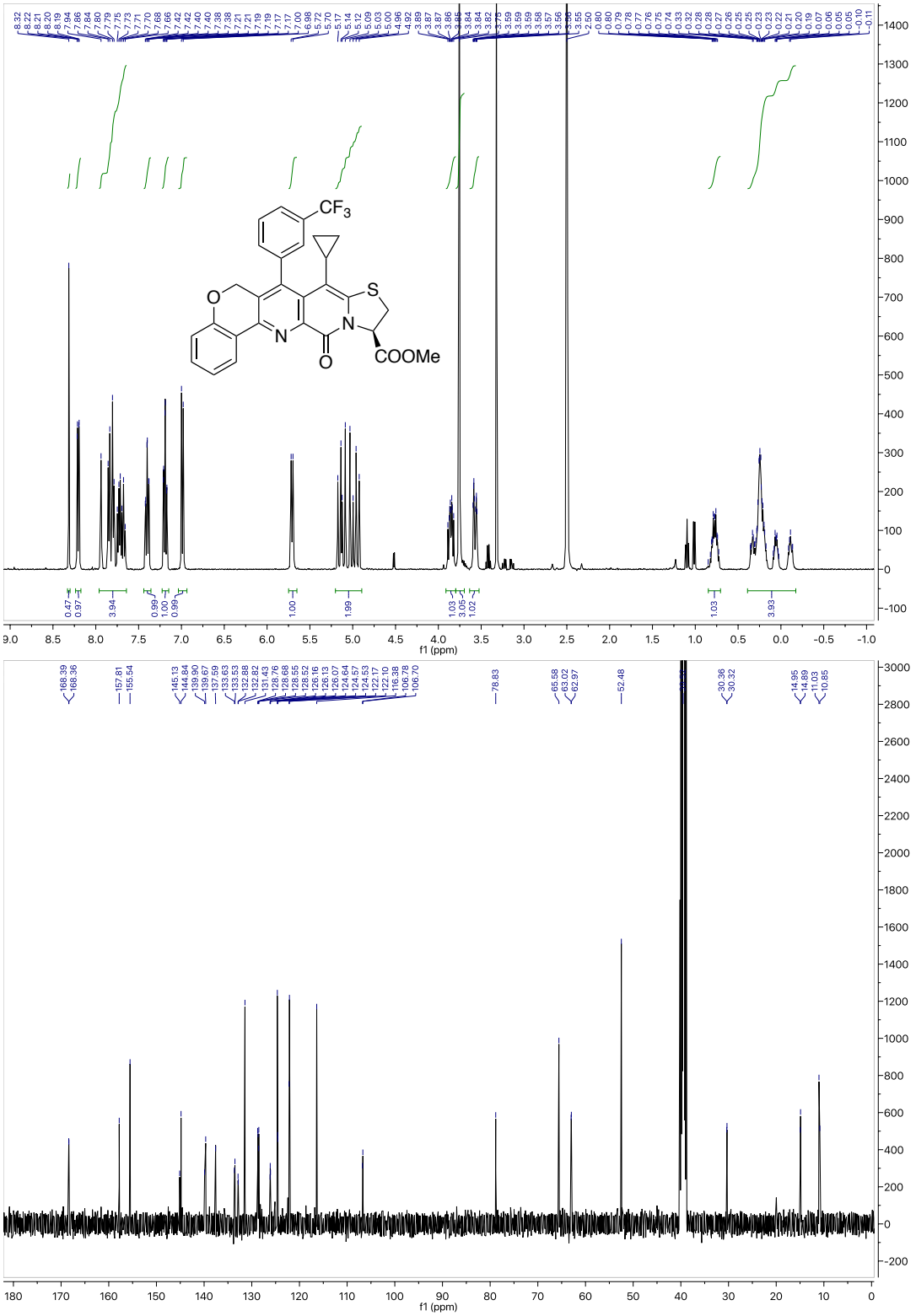
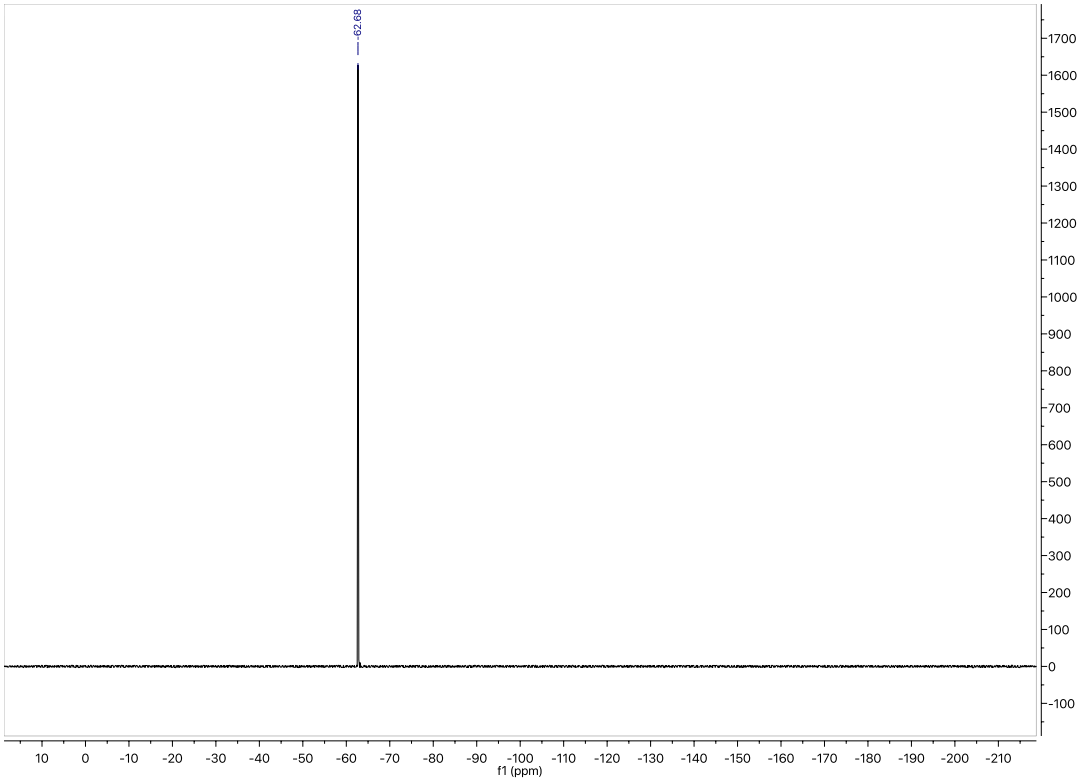
-120
-130
-140
-150
-160
-170
-180
-190
-200
-210
f1 (ppm)

Methyl (*R*)-8-cyclopropyl-13-oxo-7-(3-(trifluoromethyl)phenyl)-6,10,11,13-tetrahydrochromeno[4,3-*b*]thiazolo[2,3-*g*][1,7]naphthyridine-11-carboxylate (Compound **11h**): ^{1}H-NMR [400 MHz $(CD_3)_2SO$], $^{13}C\{^{1}H\}$-NMR [100 MHz, $(CD_3)_2SO$, 343 K] and ^{19}F-NMR (376 MHz, $CDCl_3$)

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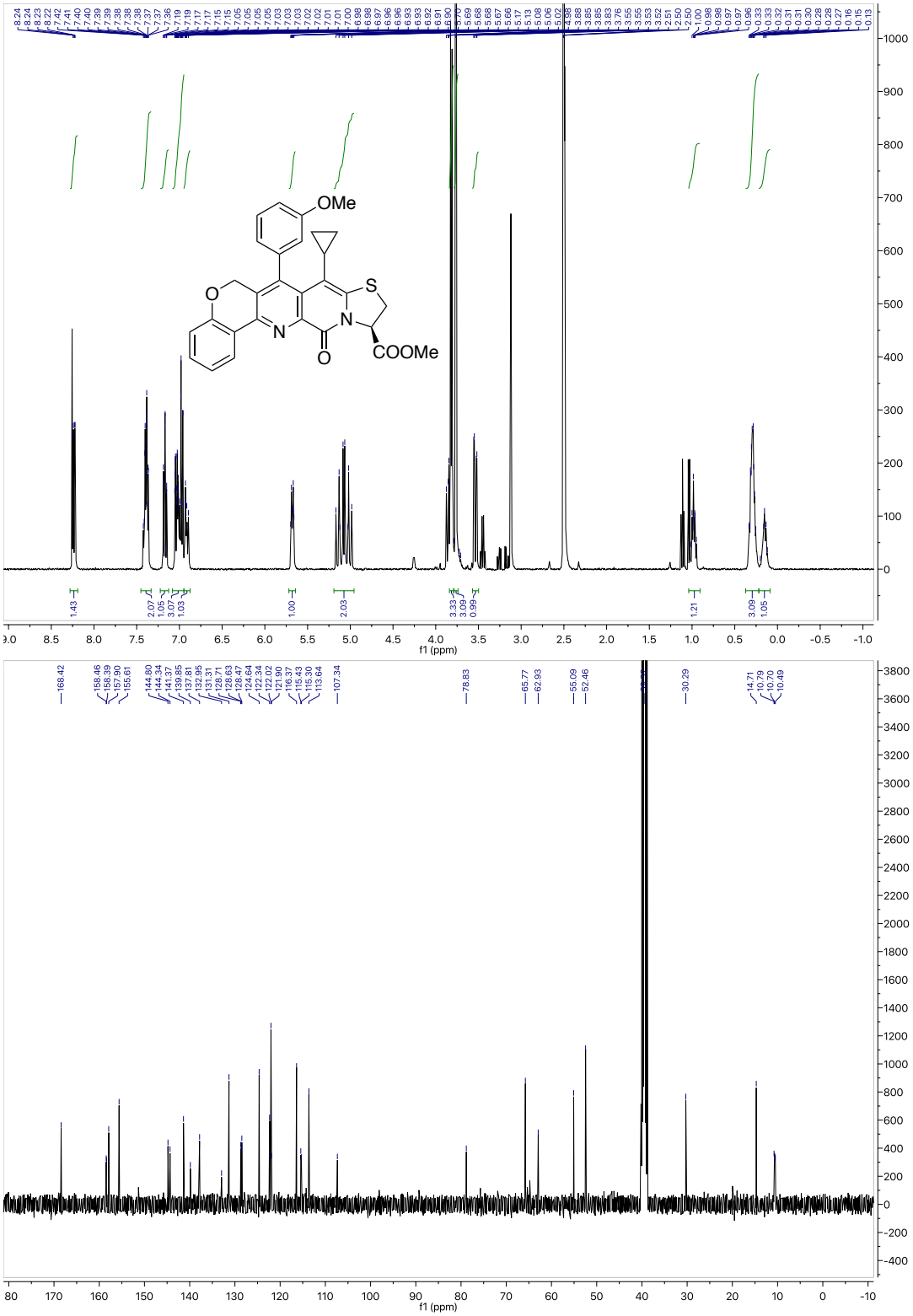
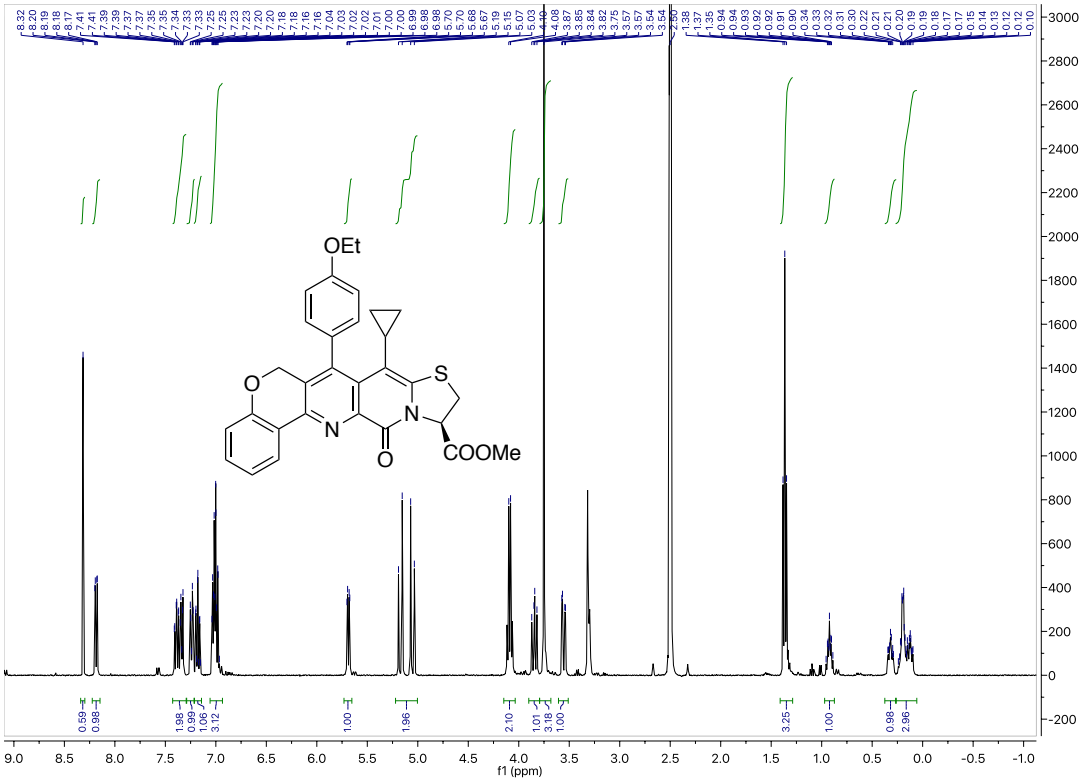
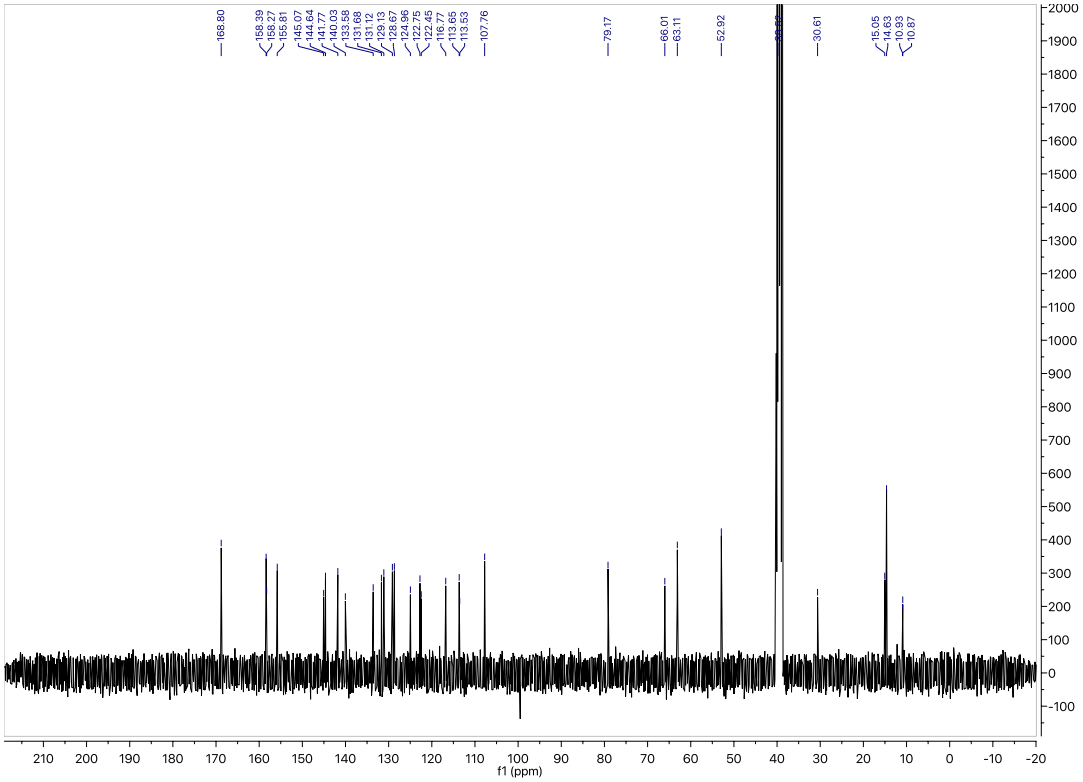
f1 (ppm)

Methyl (*R*)-8-cyclopropyl-7-(3-methoxyphenyl)-13-oxo-6,10,11,13-tetrahydrochromeno[4,3-*b*]thiazolo[2,3-*g*][1,7]naphthyridine-11-carboxylate (Compound **11i**): ^{1}H-NMR (400 MHz) and ^{13}C{^{1}H}-NMR (100 MHz) [$(CD_3)_2SO$, 343 K].

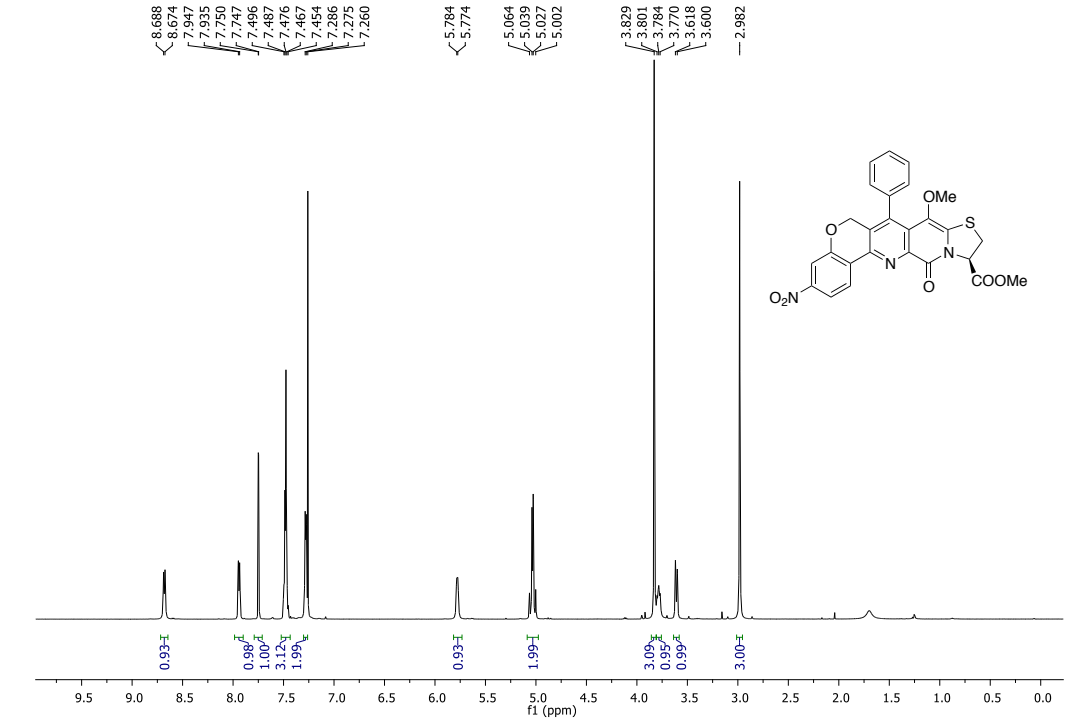


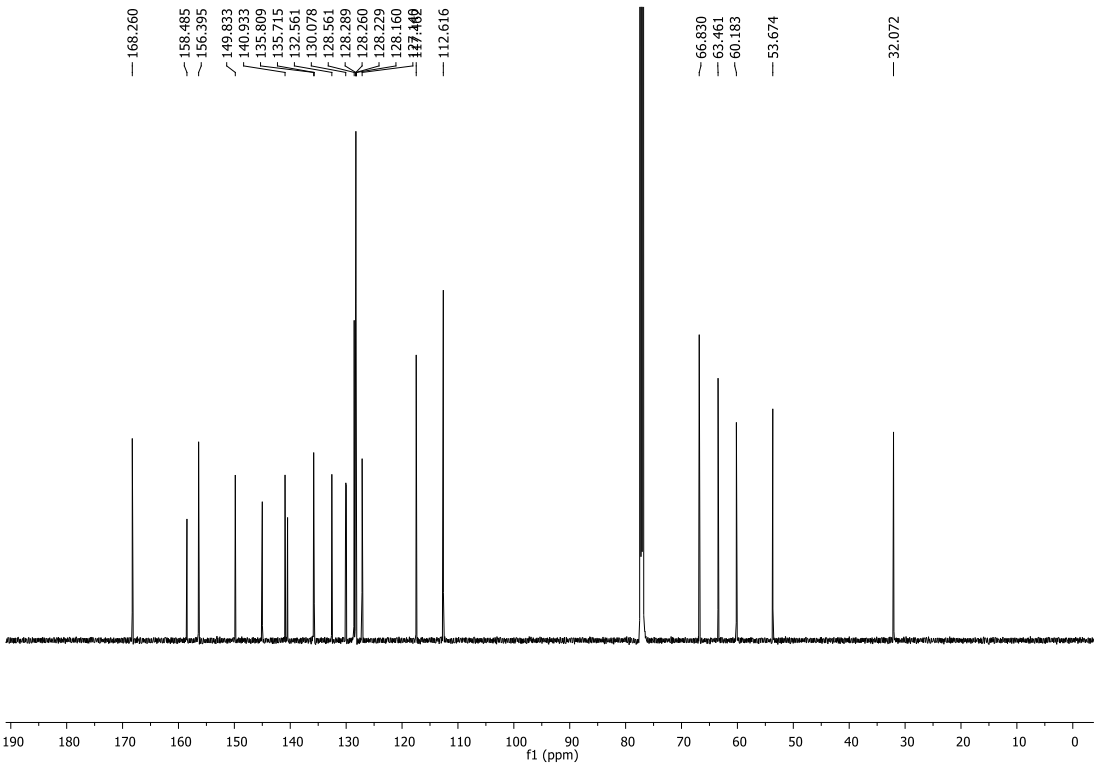
Methyl (*R*)-8-cyclopropyl-7-(4-ethoxyphenyl)-13-oxo-6,10,11,13-tetrahydrochromeno[4,3-*b*]thiazolo[2,3-*g*][1,7]naphthyridine-11-carboxylate (Compound **11j**): ^{1}H-NMR (400 MHz) and ^{13}C{^{1}H}-NMR (100 MHz) [$(CD_3)_2SO$].



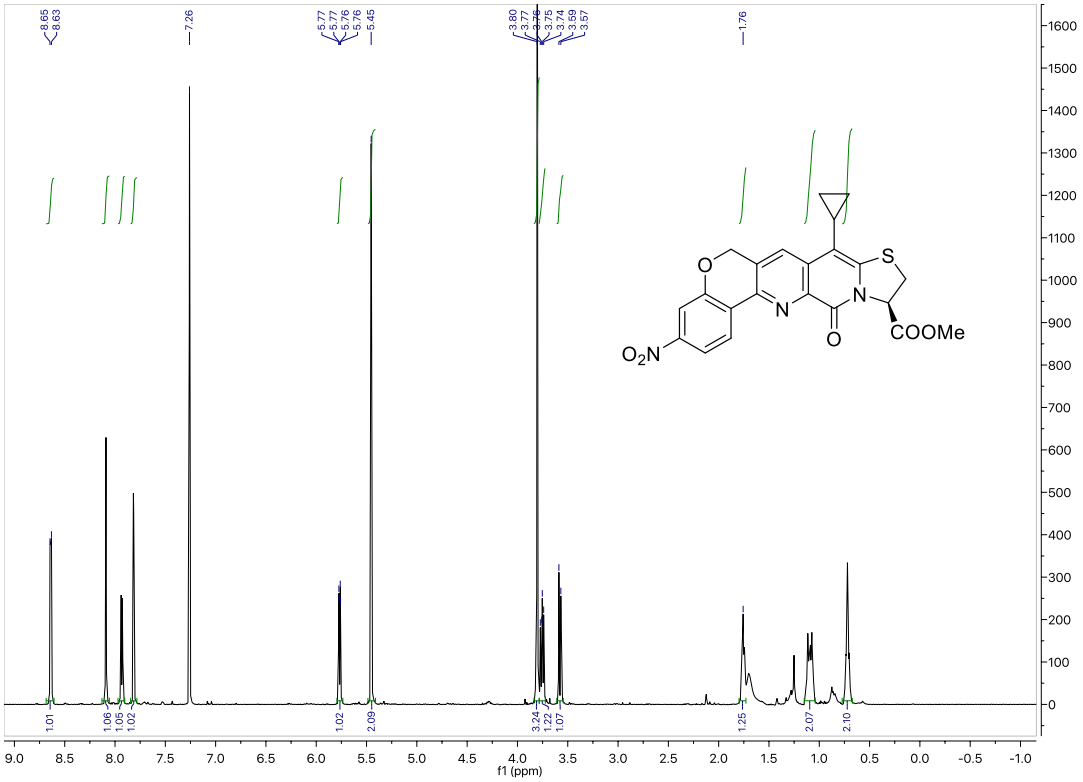


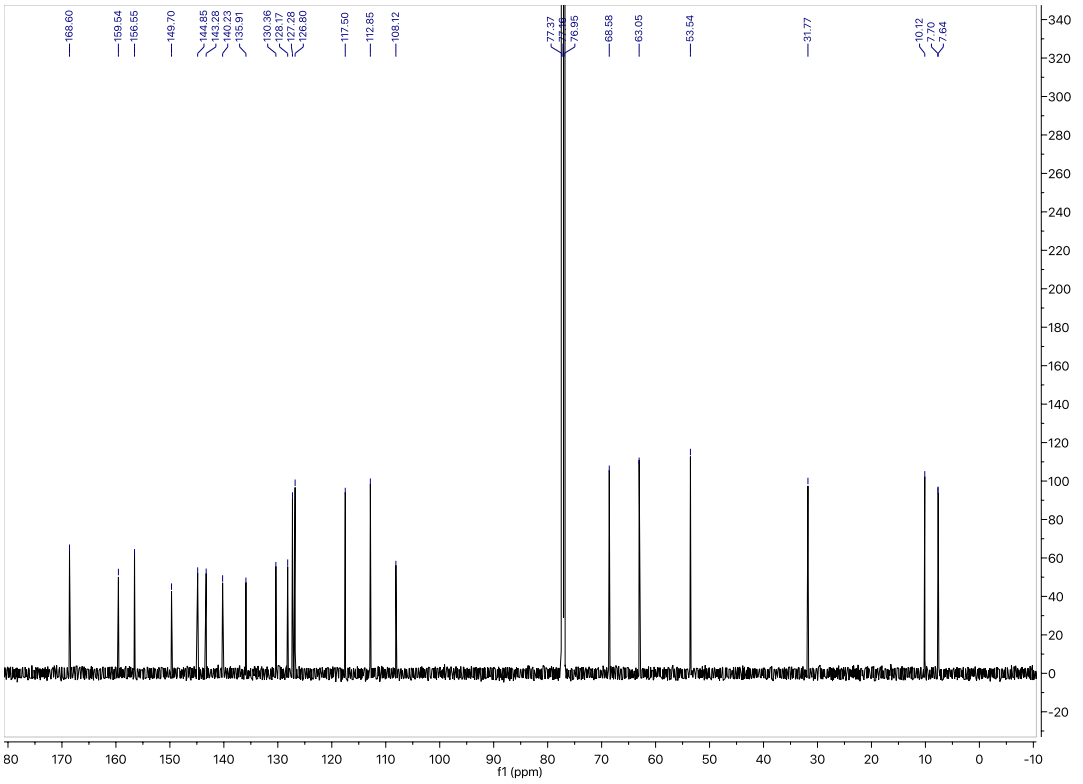
(R)-Methyl 8-methoxy-3-nitro-13-oxo-7-phenyl-6,10,11,13-tetrahydrochromeno[4,3-*b*]thiazolo[2,3-*g*][1,7]naphthyridine-11-carboxylate (Compound **11k**): ^{1}H-NMR (600 MHz) and ^{13}C{^{1}H}-NMR (151 MHz) ($CDCl_3$).



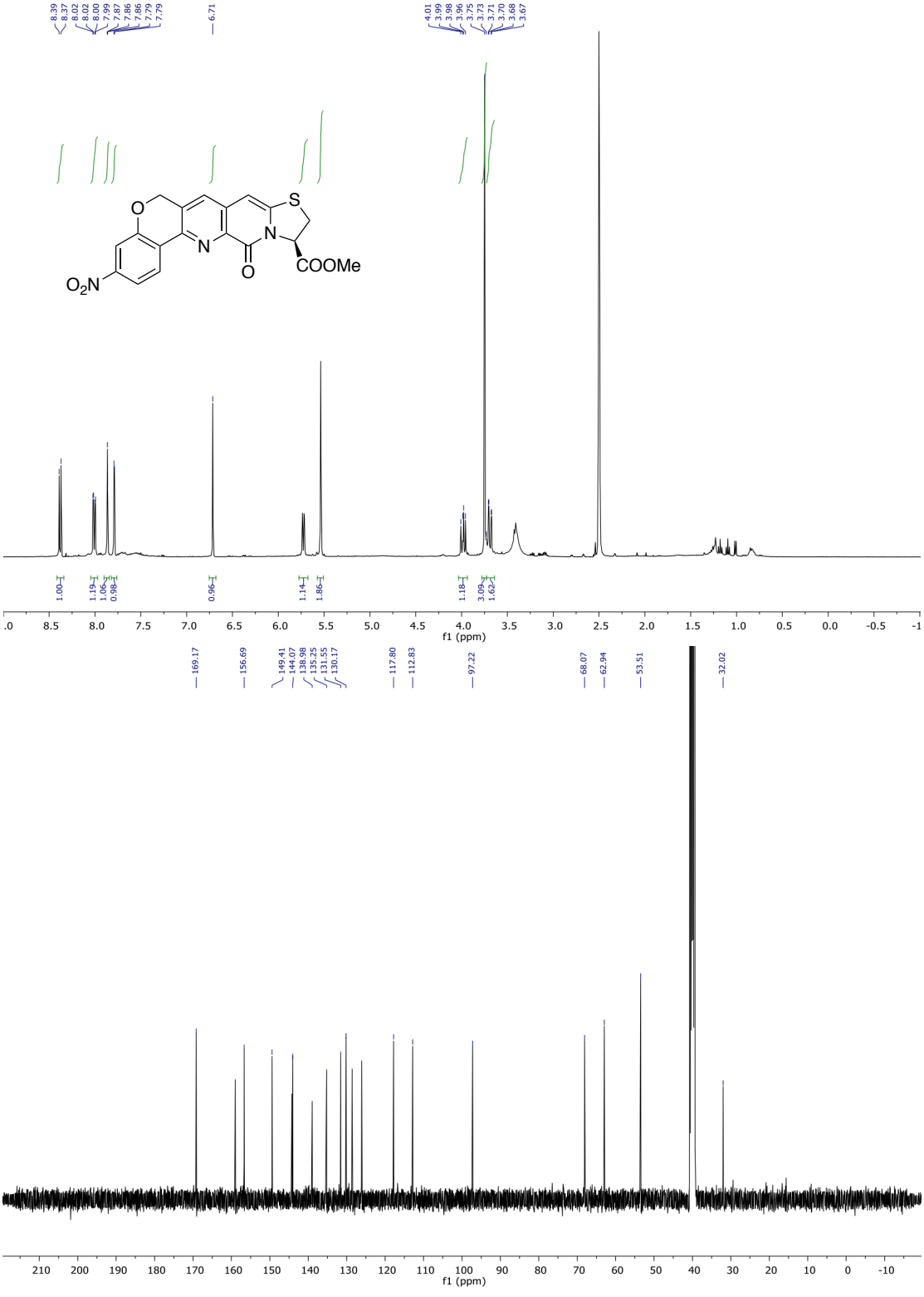


Methyl 8-cyclopropyl-3-nitro-13-oxo-6,10,11,13- tetrahydrochromeno[4,3-*b*]thiazolo[2,3-*g*][1,7]naphthyridine-11-carboxylate (Compound **13a**): ^{1}H-NMR (600 MHz) and ^{13}C{^{1}H}-NMR (151 MHz) ($CDCl_3$).

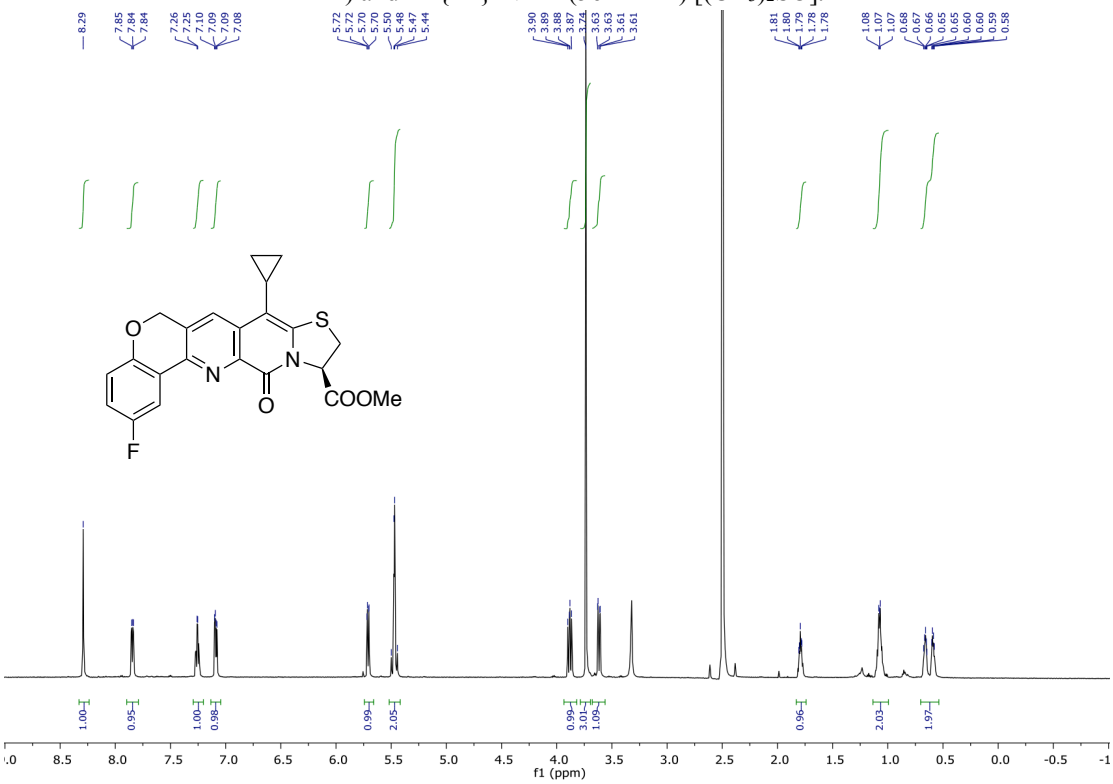


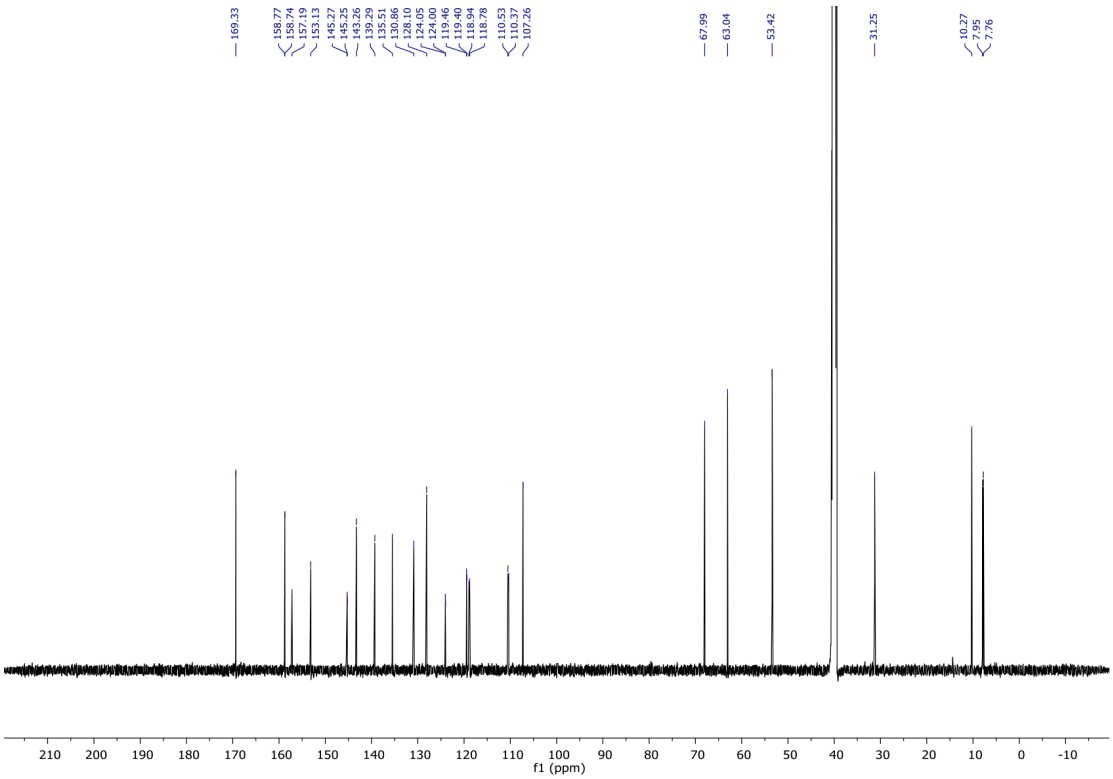


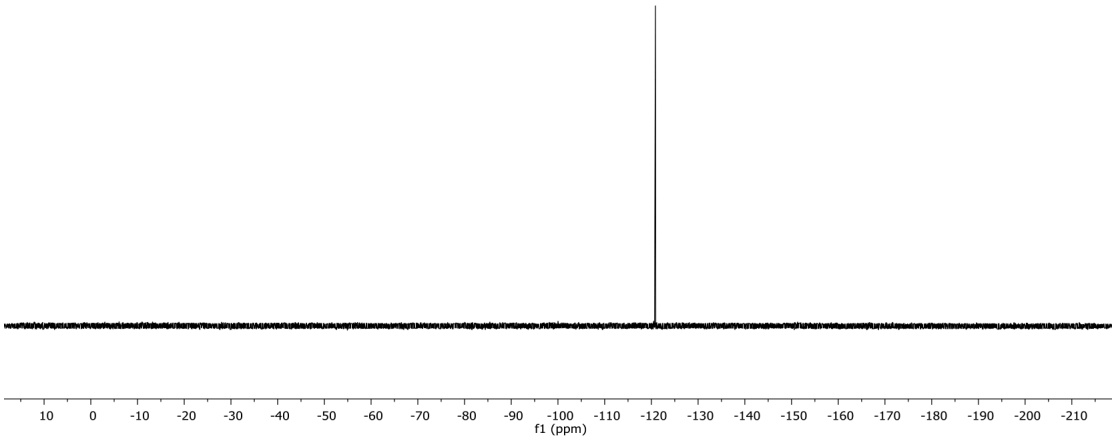
Methyl (*R*)-3-nitro-13-oxo-6,10,11,13-tetrahydrochromeno[4,3-*b*]thiazolo[2,3-*g*][1,7]naphthyridine-11-carboxylate (Compound **13b**): ^{1}H-NMR (400 MHz) and ^{13}C{^{1}H}-NMR (100 MHz) [$(CD_3)_2SO$].



Methyl (*R*)-8-cyclopropyl-2-fluoro-13-oxo-6,10,11,13-tetrahydrochromeno[4,3-*b*]thiazolo[2,3-*g*][1,7]naphthyridine-11-carboxylate (Compound **13c**): ^{1}H-NMR (600 MHz), ^{13}C{^{1}H}-NMR (151 MHz) and ^{19}F{^{1}H}-NMR (564 MHz) [$(CD_3)_2SO$].



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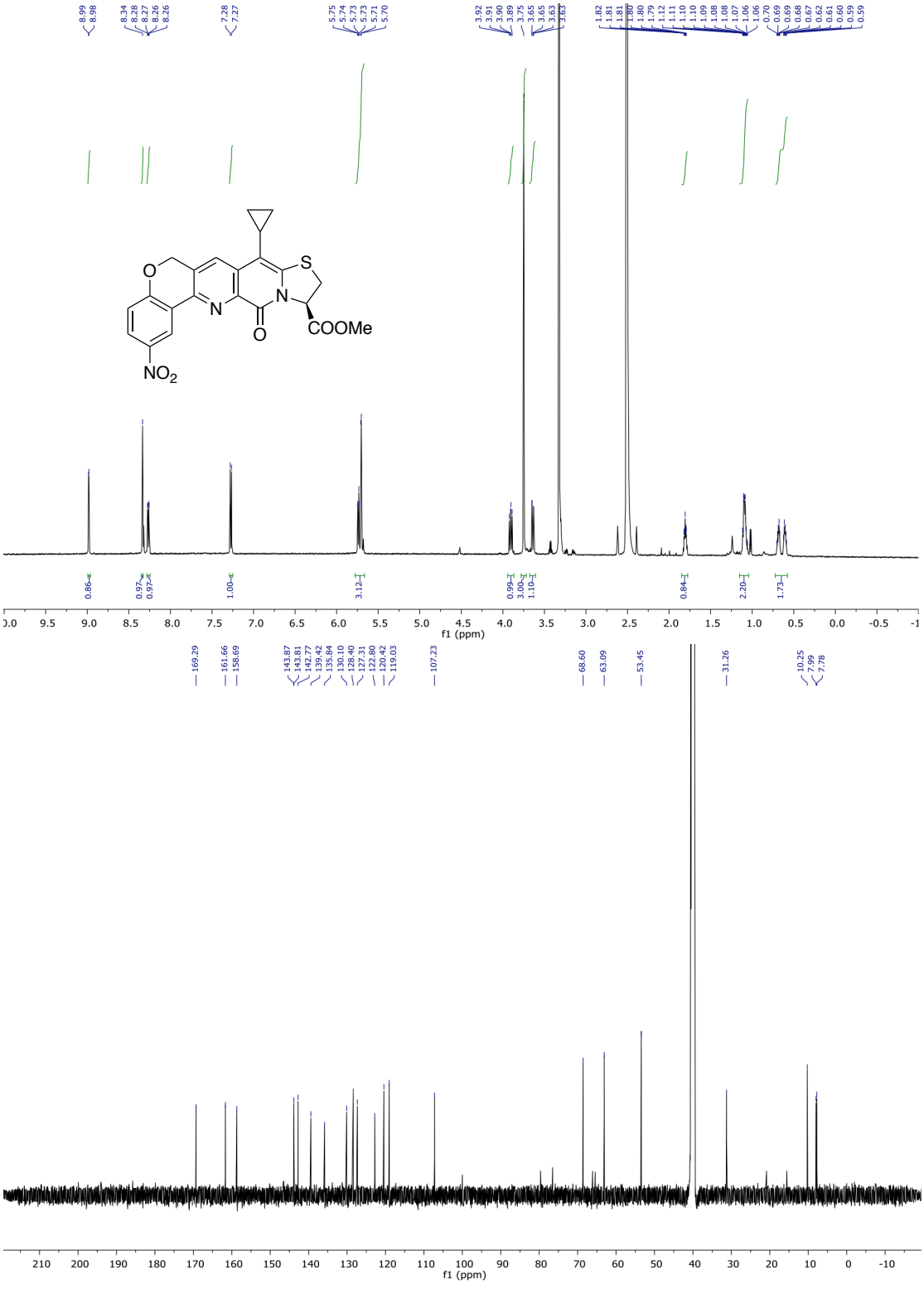
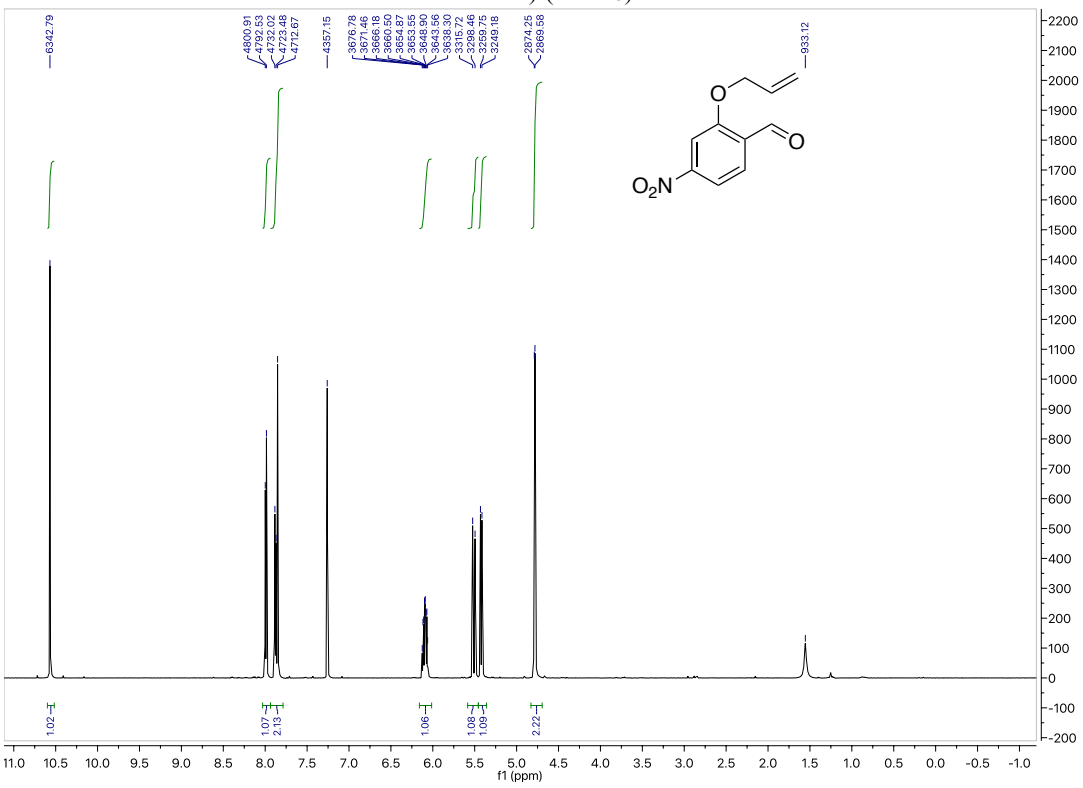
f1 (ppm)

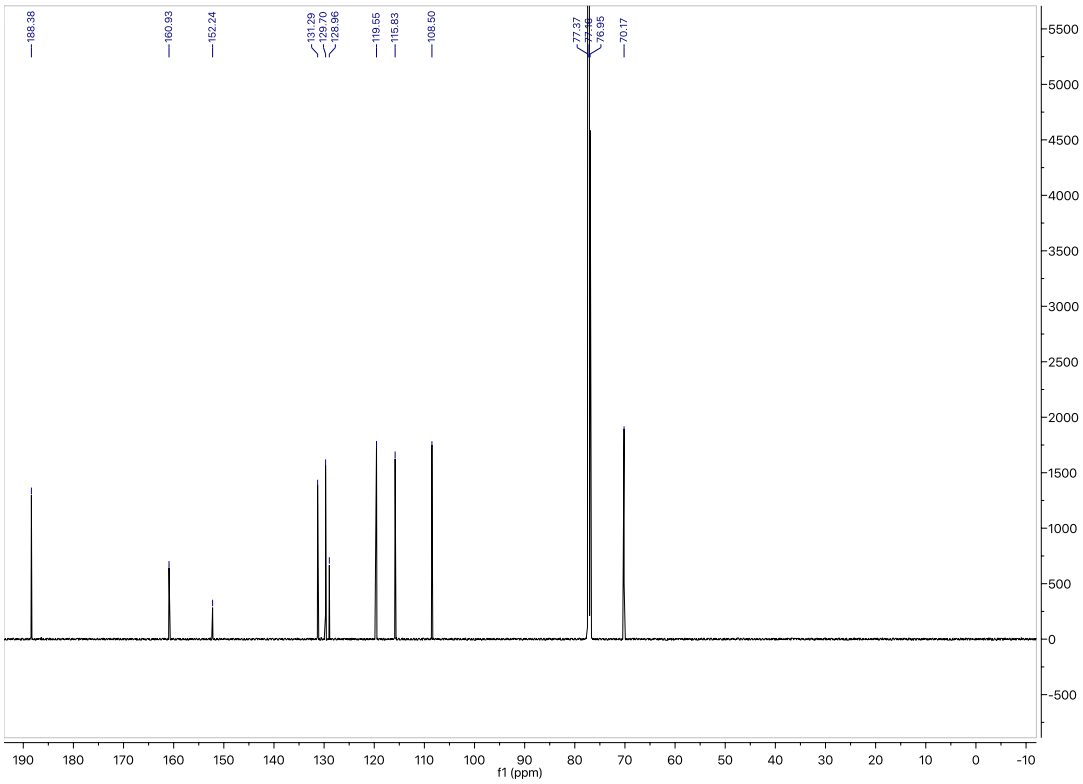
Methyl (*R*)-8-cyclopropyl-13-oxo-6,10,11,13-tetrahydrochromeno[4,3-*b*]thiazolo[2,3-*g*][1,7]naphthyridine-11-carboxylate (Compound **13d**): ^{1}H-NMR (600 MHz) and ^{13}C{^{1}H}-NMR (151 MHz) [$(CD_3)_2SO$].

Methyl (*R*)-8-cyclopropyl-2-nitro-13-oxo-6,10,11,13-tetrahydrochromeno[4,3-*b*]thiazolo[2,3-*g*][1,7]naphthyridine-11-carboxylate (Compound **13e**): ^{1}H-NMR (600 MHz) and ^{13}C{^{1}H}-NMR (151 MHz) [$(CD_3)_2SO$].

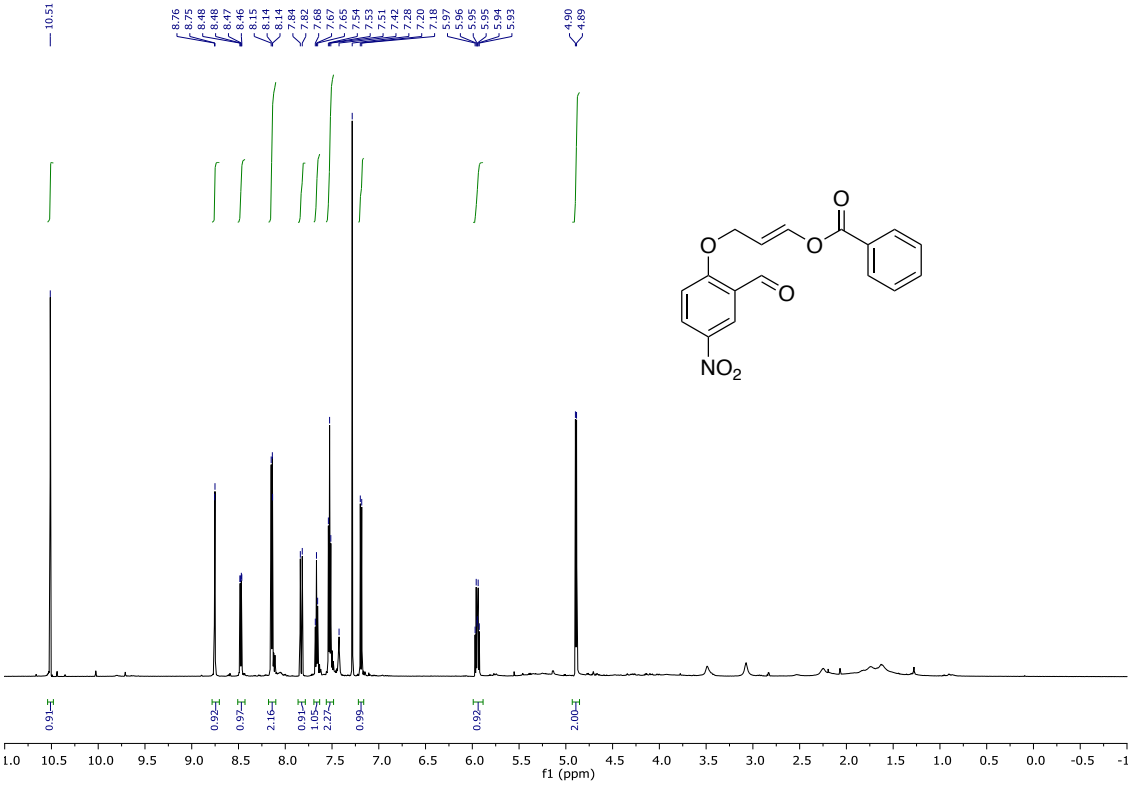


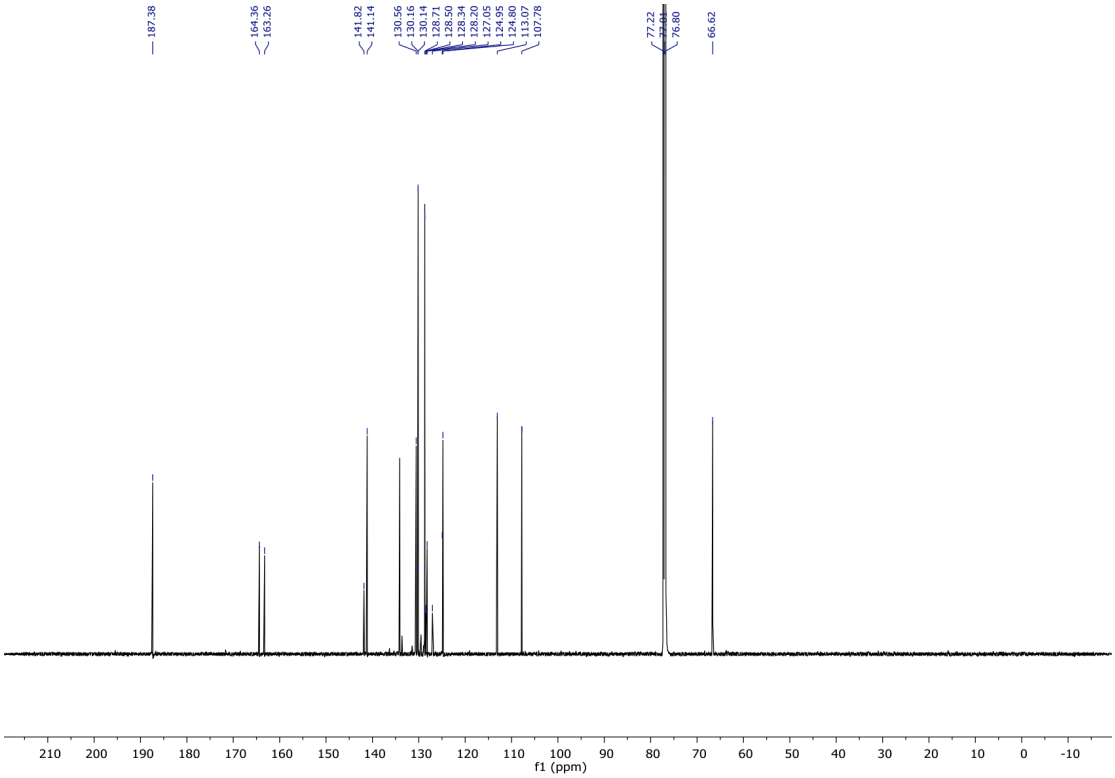
2-(Allyloxy)-4-nitrobenzaldehyde (Compound **12**): ^{1}H-NMR (600 MHz) and ^{13}C{^{1}H}-NMR (151 MHz) ($CDCl_3$).



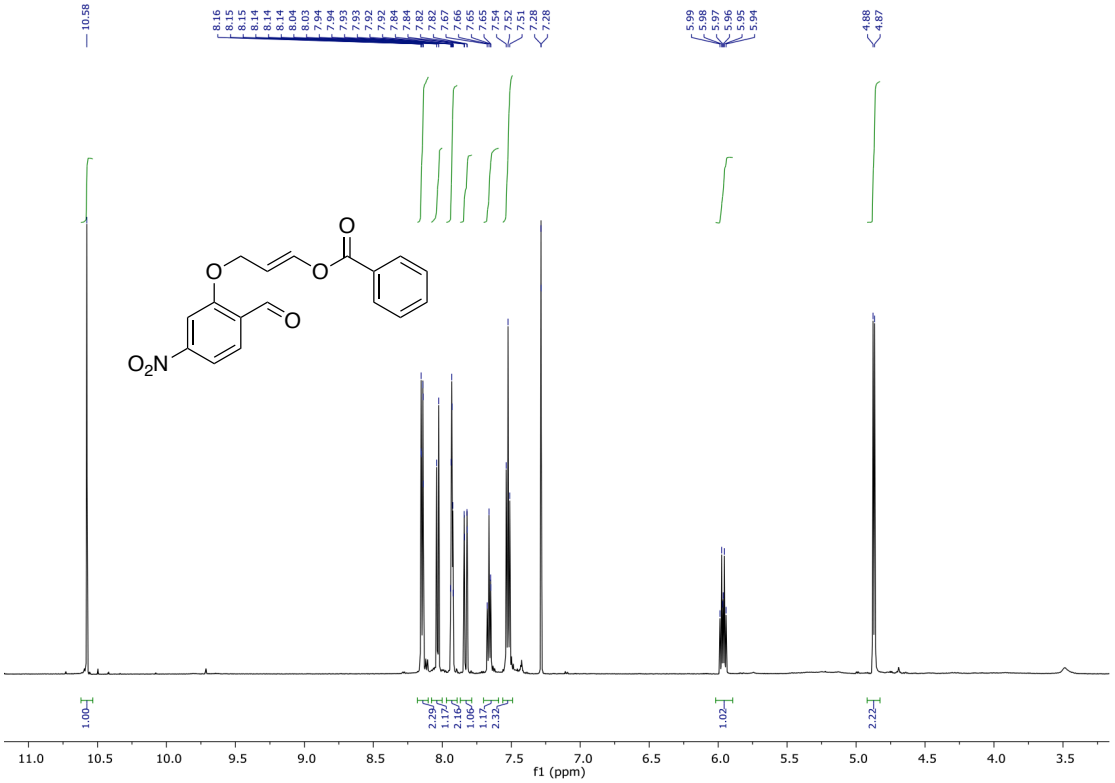


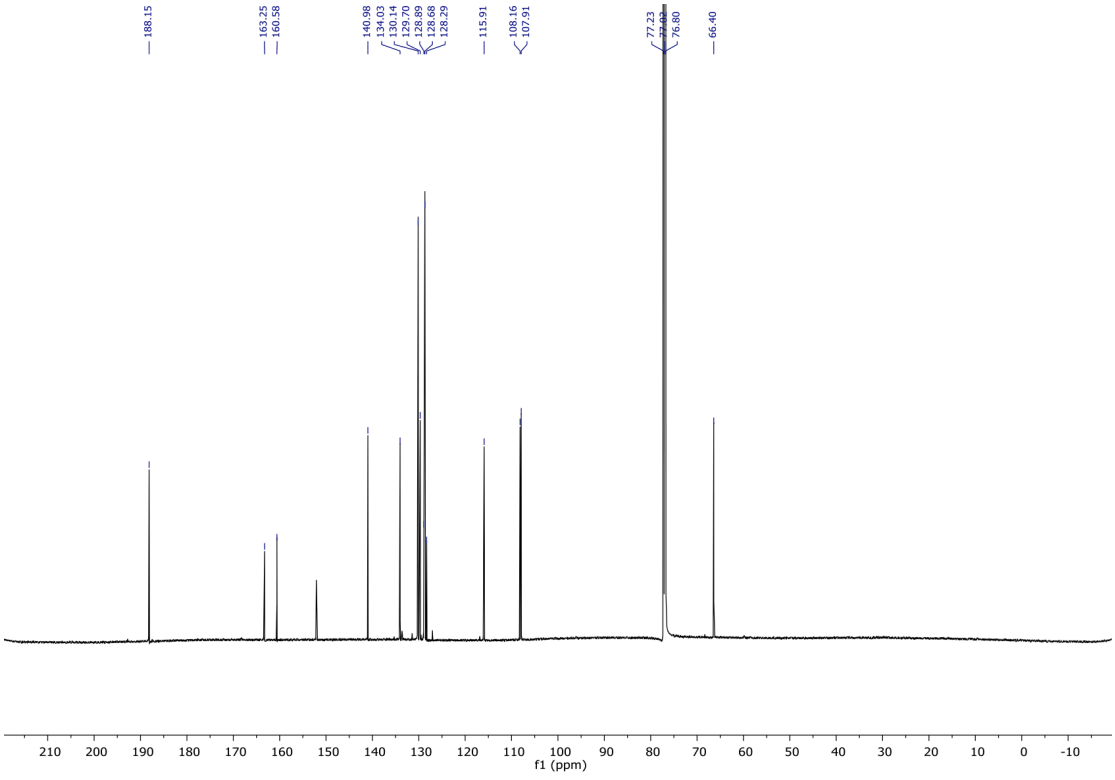
(E)-3-(2-Formyl-4-nitrophenoxy)prop-1-en-1-yl benzoate: (Compound **14a**) ^{1}H-NMR (600 MHz) and ^{13}C{^{1}H}-NMR (151 MHz) ($CDCl_3$).



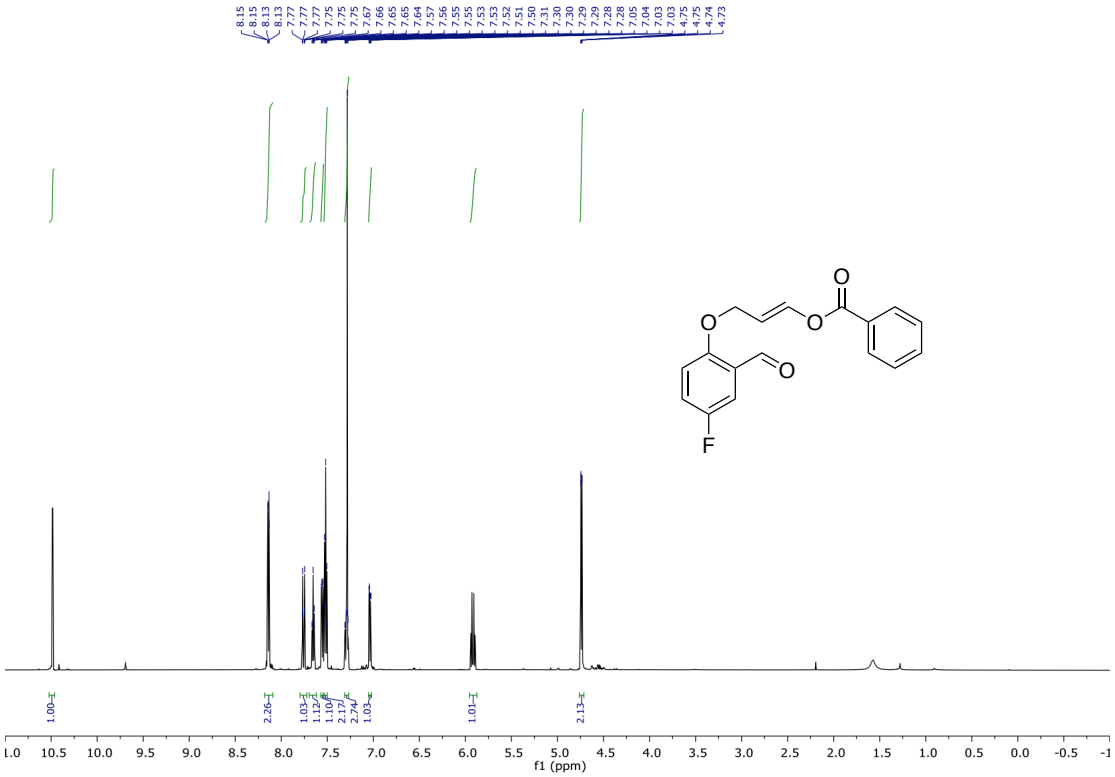


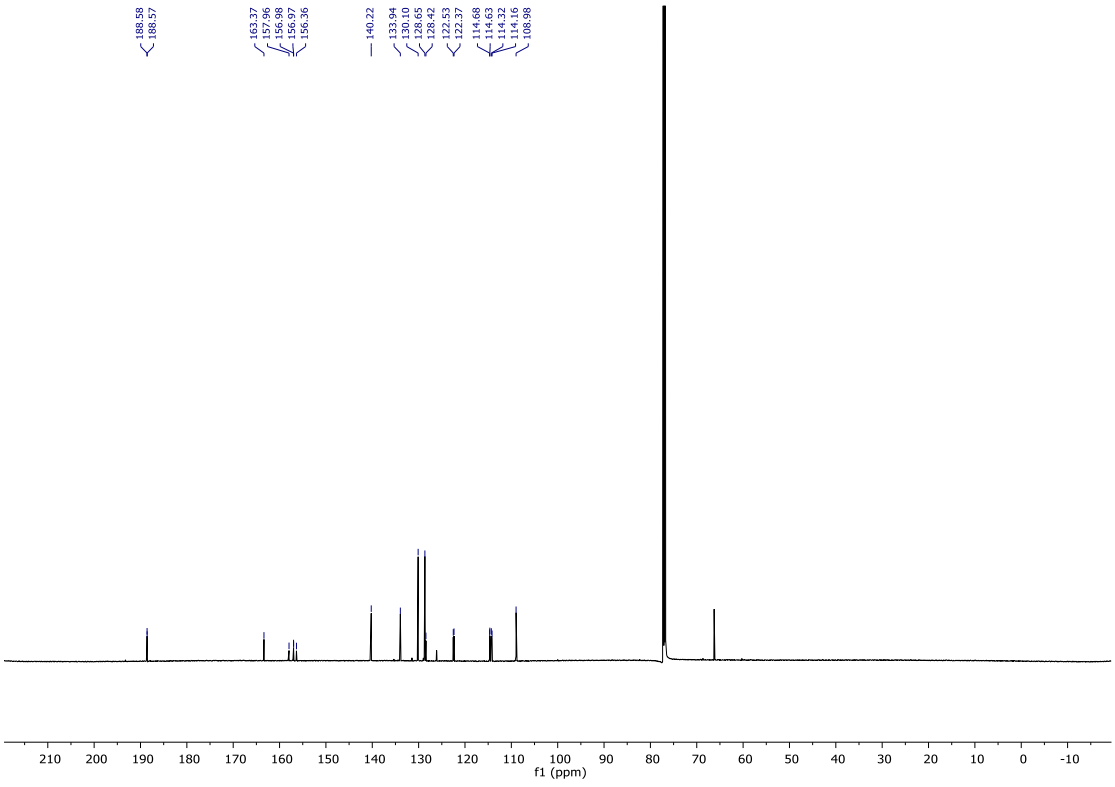
(E)-3-(2-Formyl-5-nitrophenoxy)prop-1-en-1-yl benzoate: Compound **14b**) ^{1}H-NMR (600 MHz) and ^{13}C{^{1}H}-NMR (151 MHz) ($CDCl_3$).

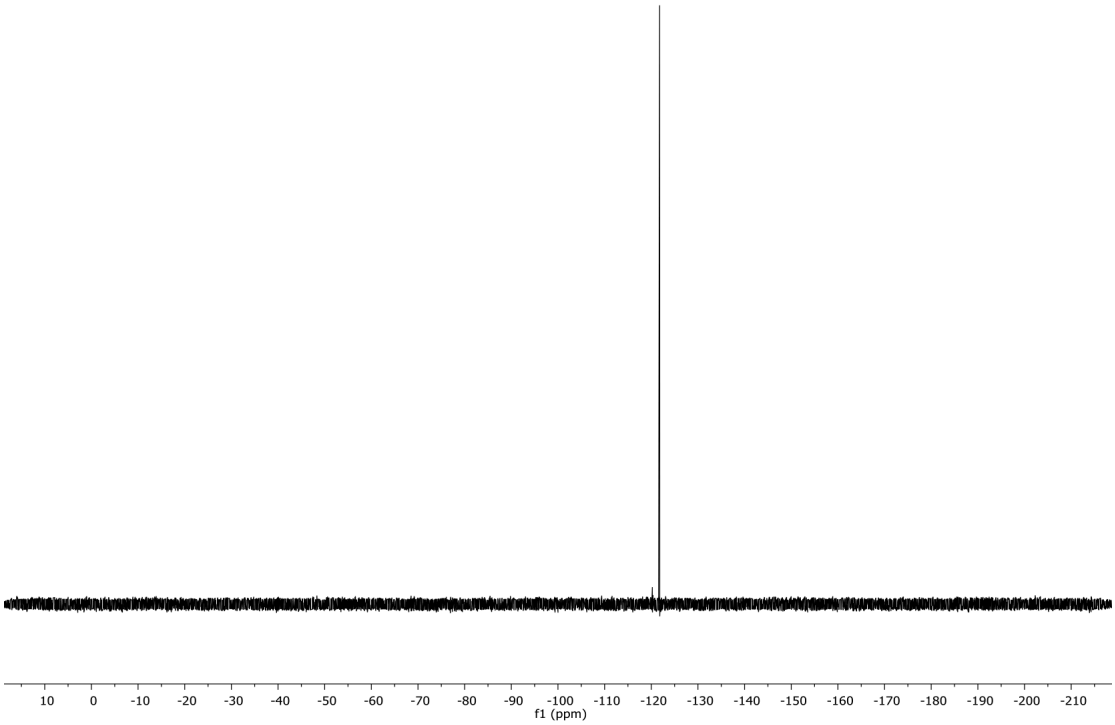




(E)-3-(4-Fluoro-2-formylphenoxy)prop-1-en-1-yl benzoate: Compound **14c**) ^{1}H-NMR (600 MHz), ^{13}C{^{1}H}-NMR (151 MHz) and ^{19}F{^{1}H}-NMR (376 MHz) ($CDCl_3$).



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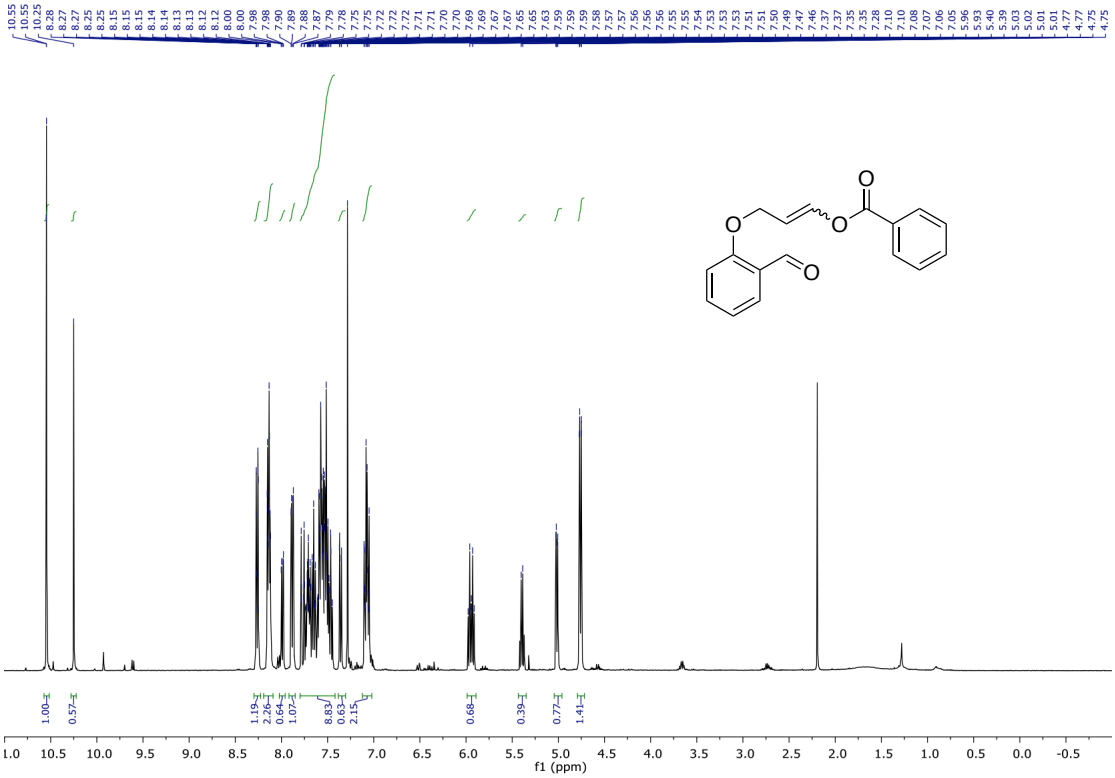
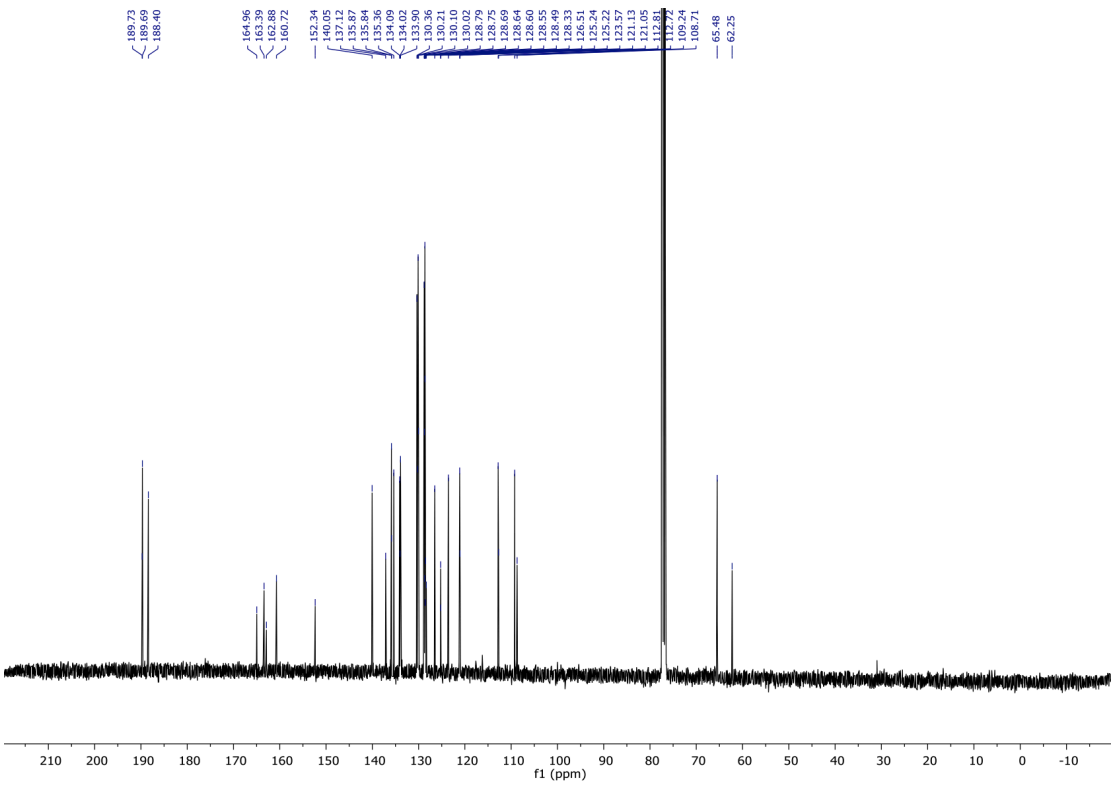
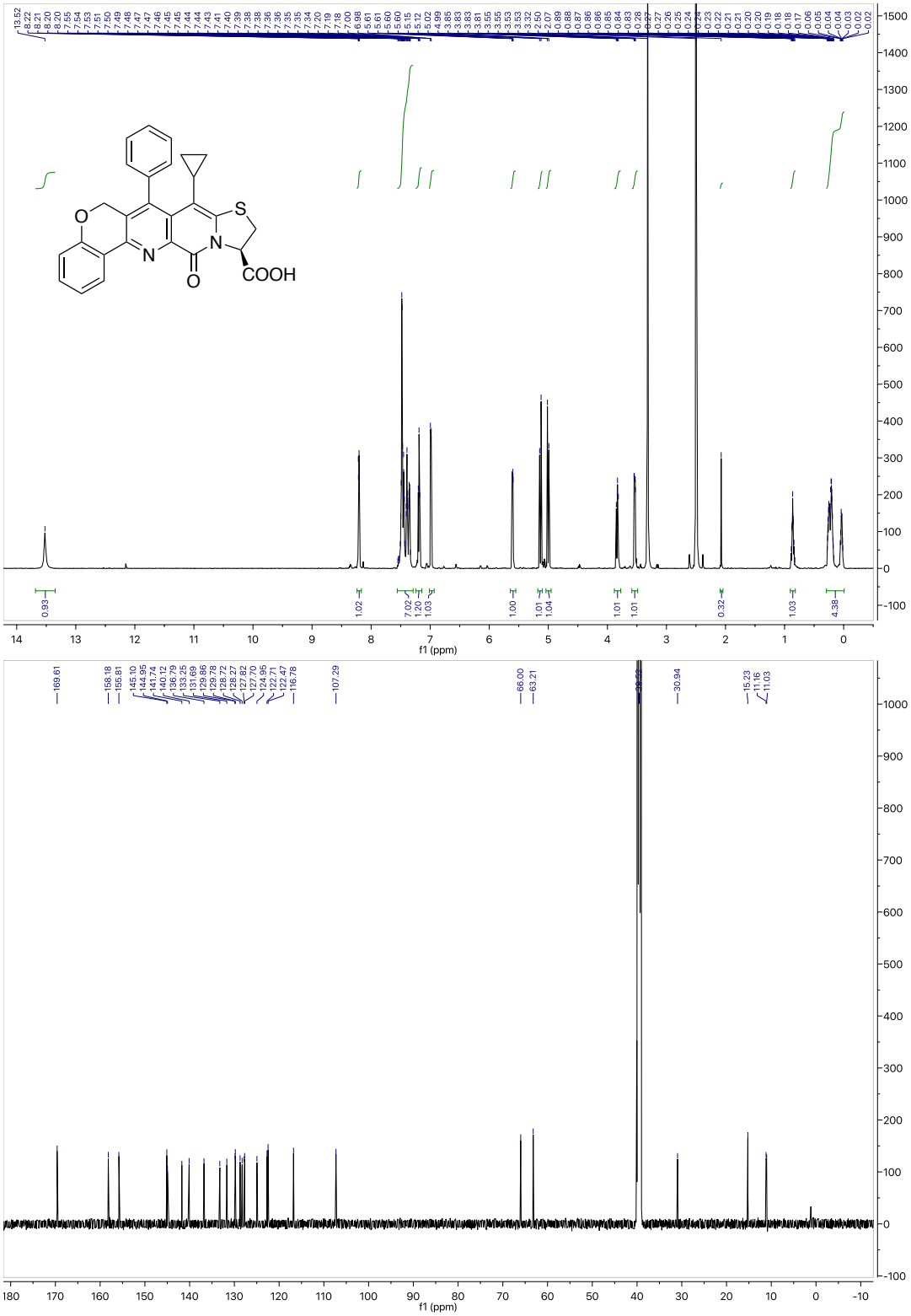
-190
-200
-210
f1 (ppm)

3-(2-Formylphenoxy)prop-1-en-1-yl benzoate: (Compound **14d**) ^{1}H-NMR (400 MHz) and $^{13}C\{^{1}H\}$-NMR (100 MHz) ($CDCl_3$).

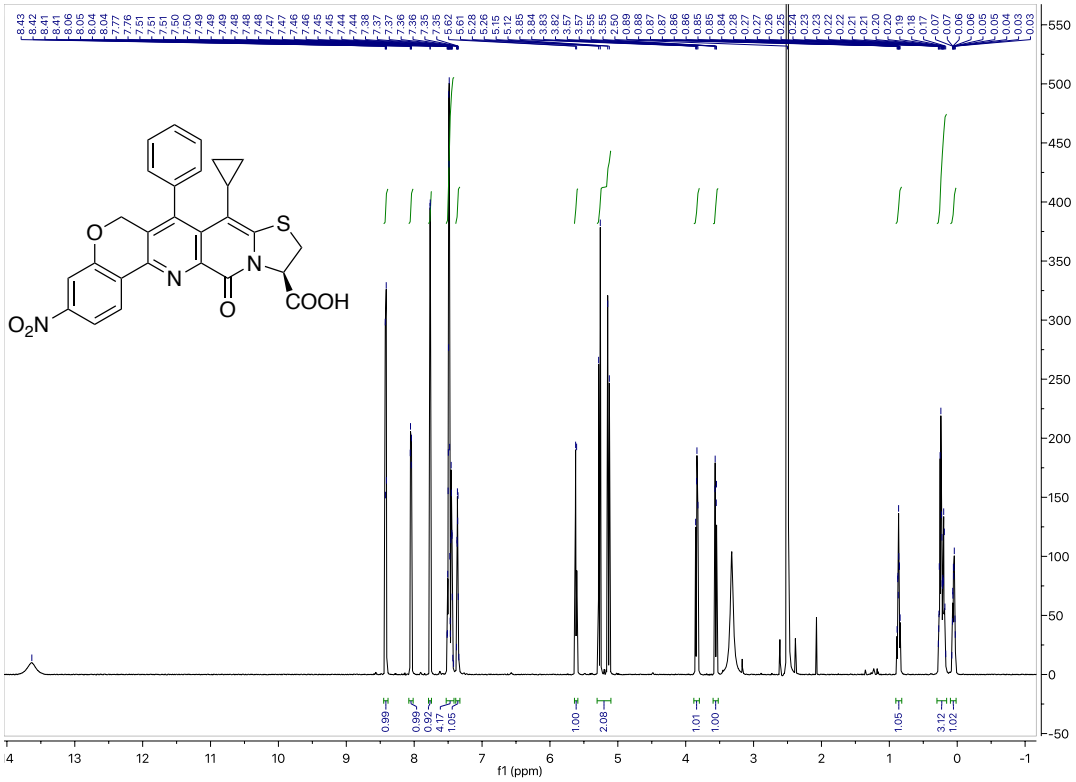


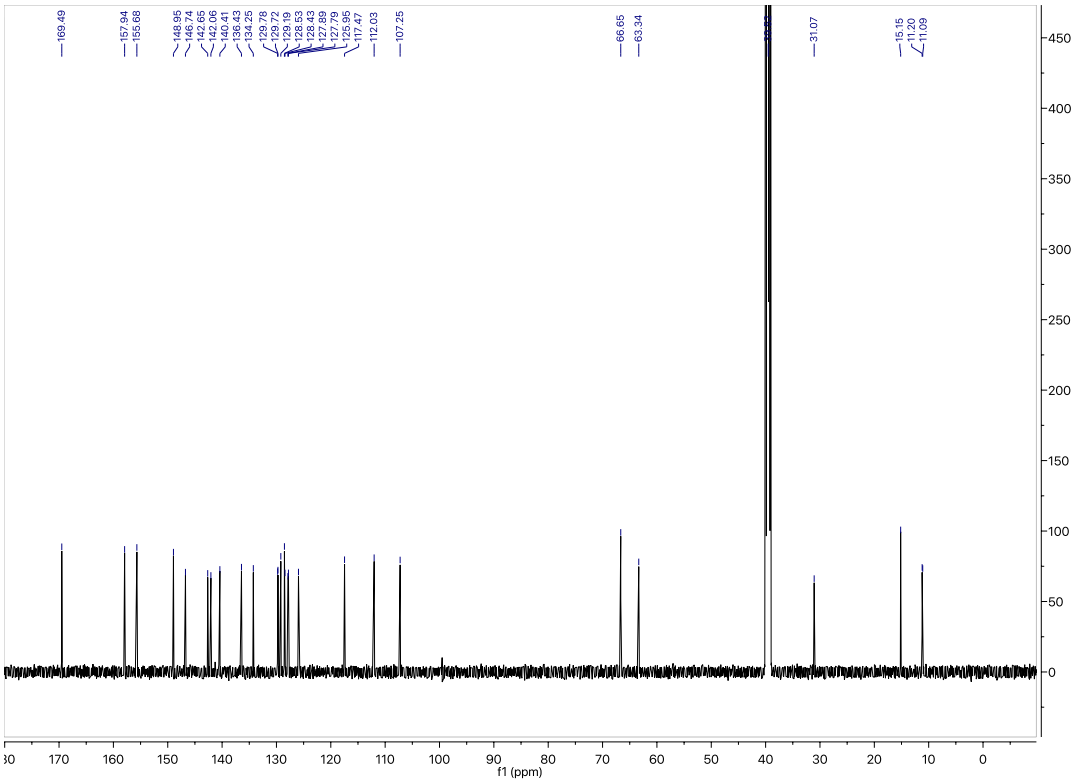


(*R*)-8-Cyclopropyl-13-oxo-7-phenyl-6,10,11,13-tetrahydrochromeno[4,3-*b*]thiazolo[2,3-*g*][1,7]naphthyridine-11-carboxylic acid (Compound **15a**): ^{1}H-NMR (600 MHz) and ^{13}C{^{1}H}-NMR (151 MHz) [$(CD_3)_2SO$].

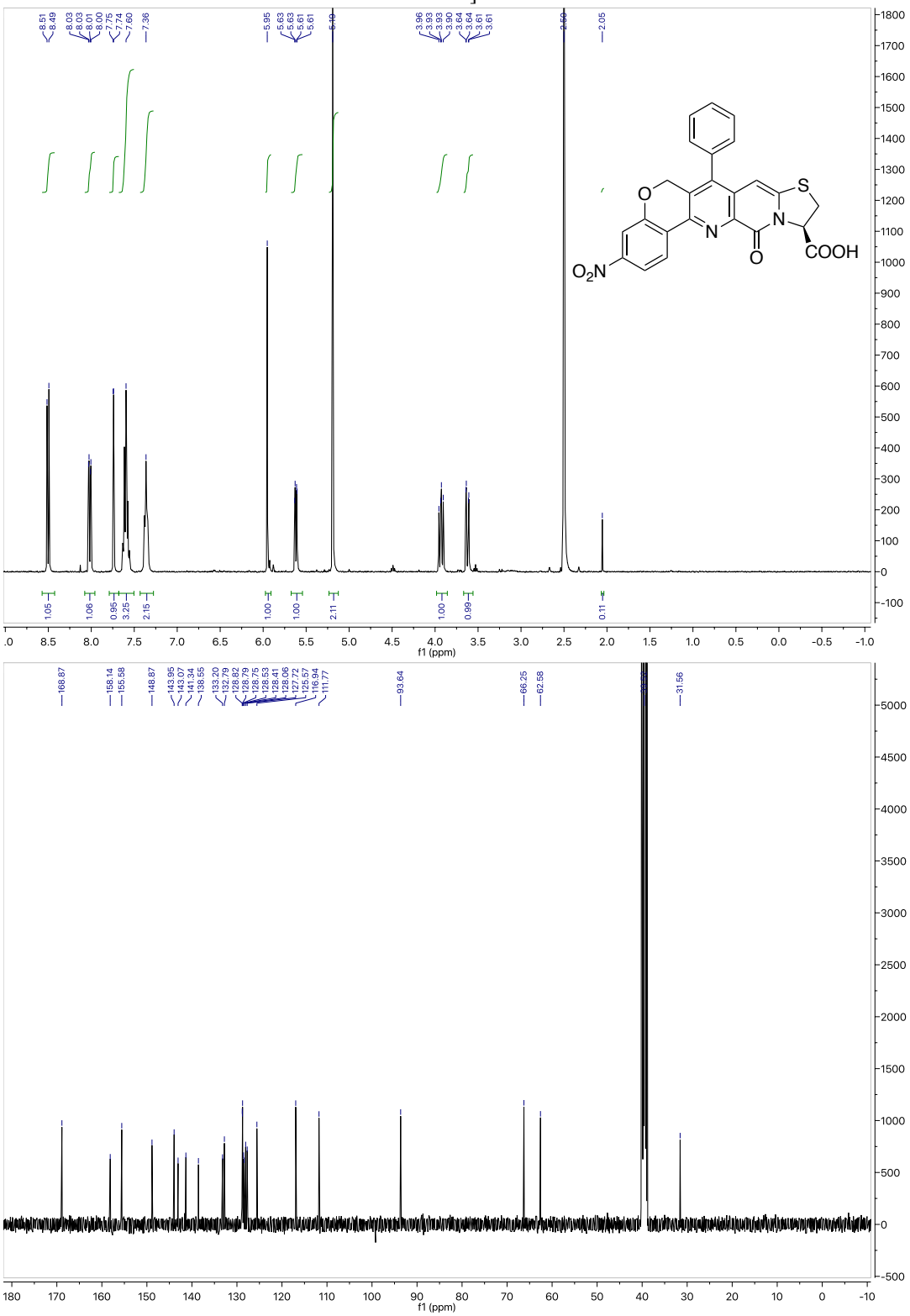


8-Cyclopropyl-3-nitro-13-oxo-7-phenyl-6,10,11,13- tetrahydrochromeno[4,3-*b*]thiazolo[2,3-*g*][1,7]naphthyridine-11-carboxylic acid (Compound **15b**): ^{1}H-NMR (600 MHz) and ^{13}C{^{1}H}-NMR (151 MHz) [$(CD_3)_2SO$].

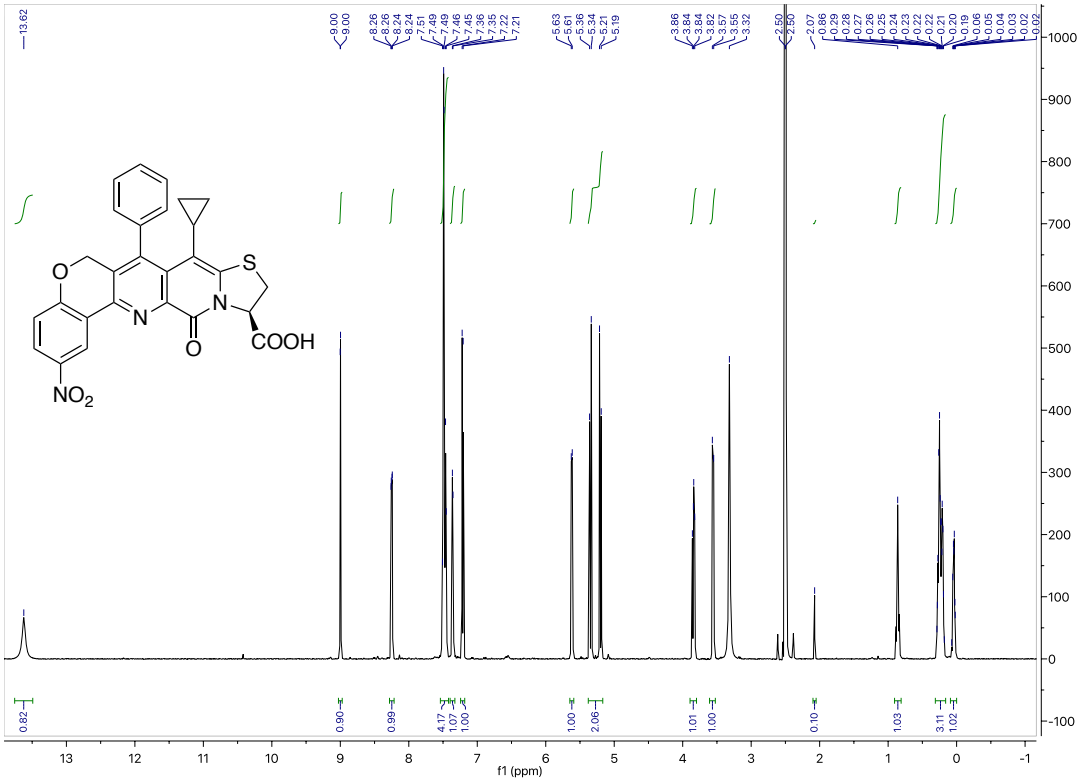


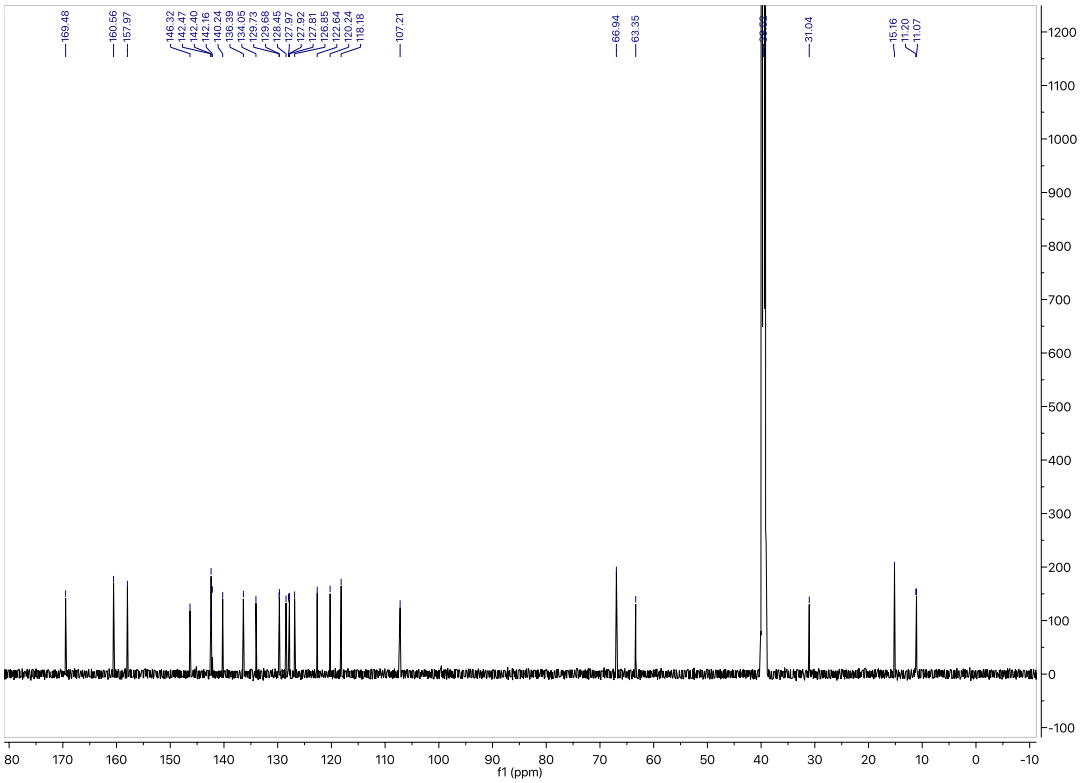


(*R*)-3-Nitro-13-oxo-7-phenyl-6,10,11,13-tetrahydrochromeno[4,3-*b*]thiazolo[2,3-*g*][1,7]naphthyridine-11-carboxylic acid (Compound **15c**): ^{1}H-NMR (400 MHz) and ^{13}C{^{1}H}-NMR (100 MHz) [$(CD_3)_2SO$, 343 K].

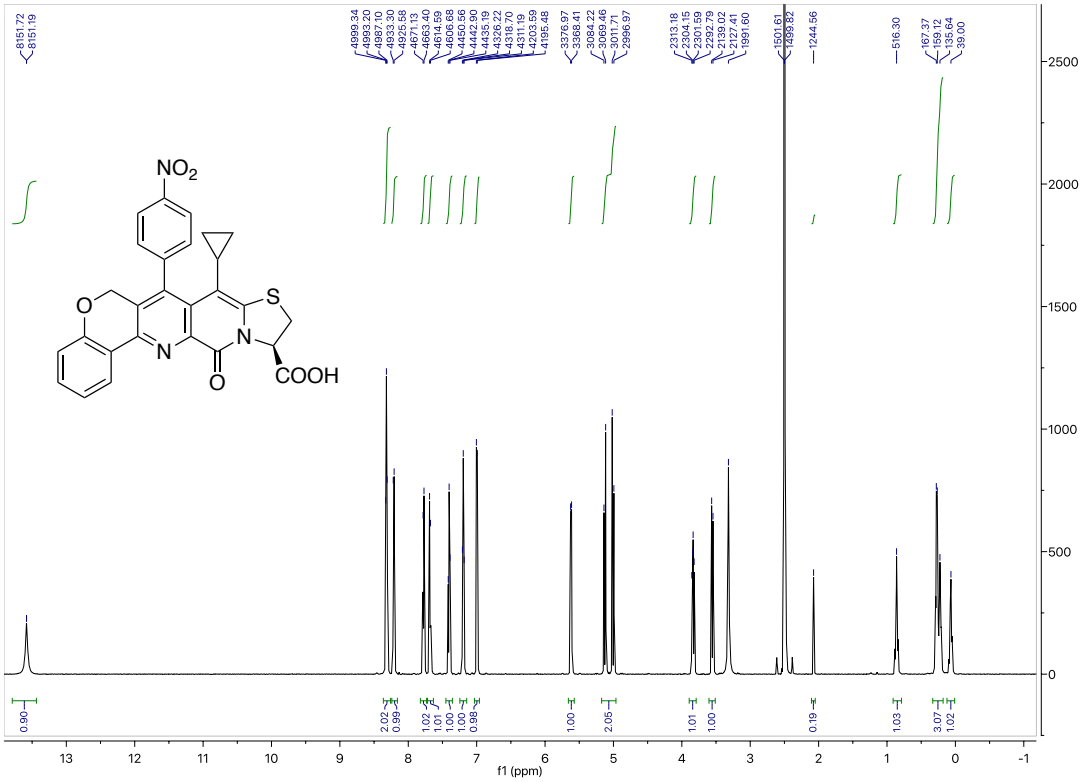


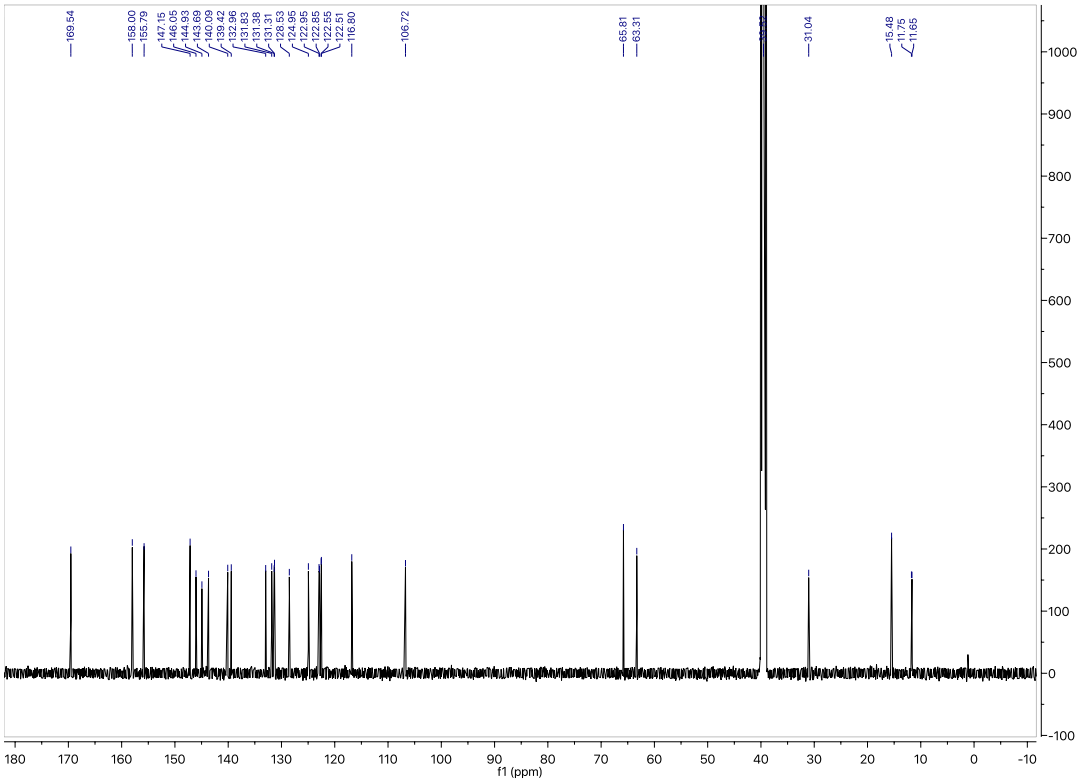
(*R*)-8-Cyclopropyl-2-nitro-13-oxo-7-phenyl-6,10,11,13-tetrahydrochromeno[4,3-*b*]thiazolo[2,3-*g*][1,7]naphthyridine-11-carboxylic acid (Compound **15d**): ^{1}H-NMR (600 MHz) and ^{13}C{^{1}H}-NMR (151 MHz) [$(CD_3)_2SO$].



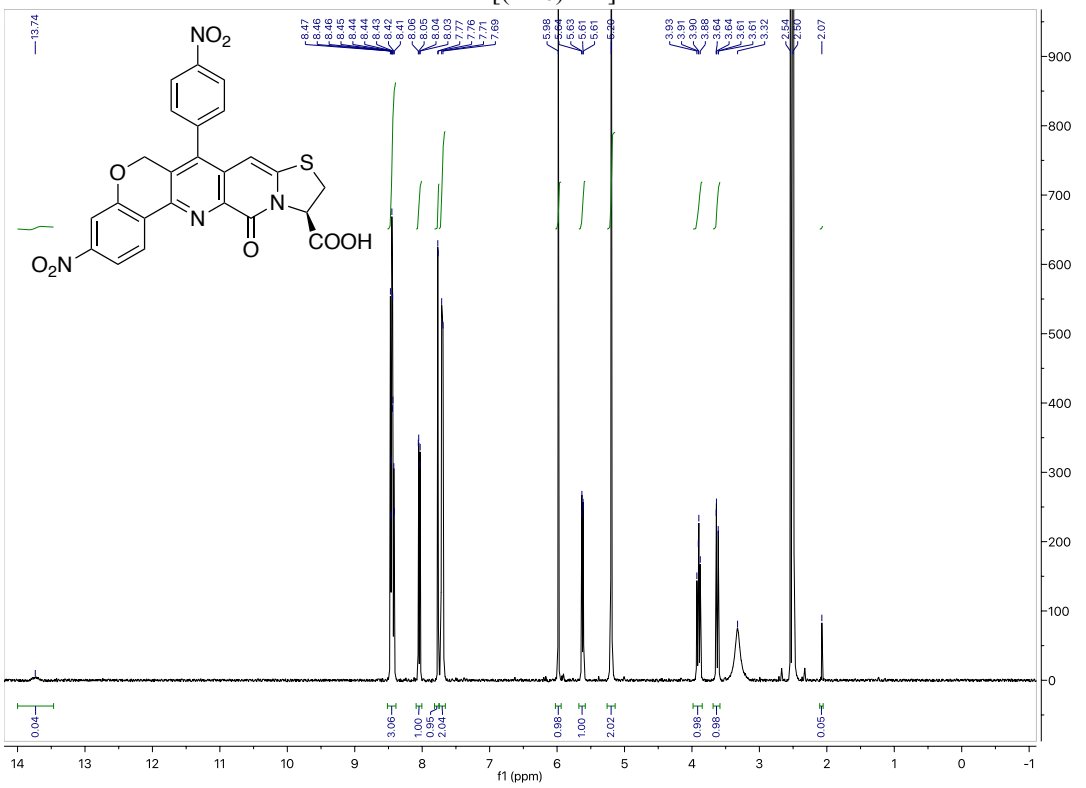


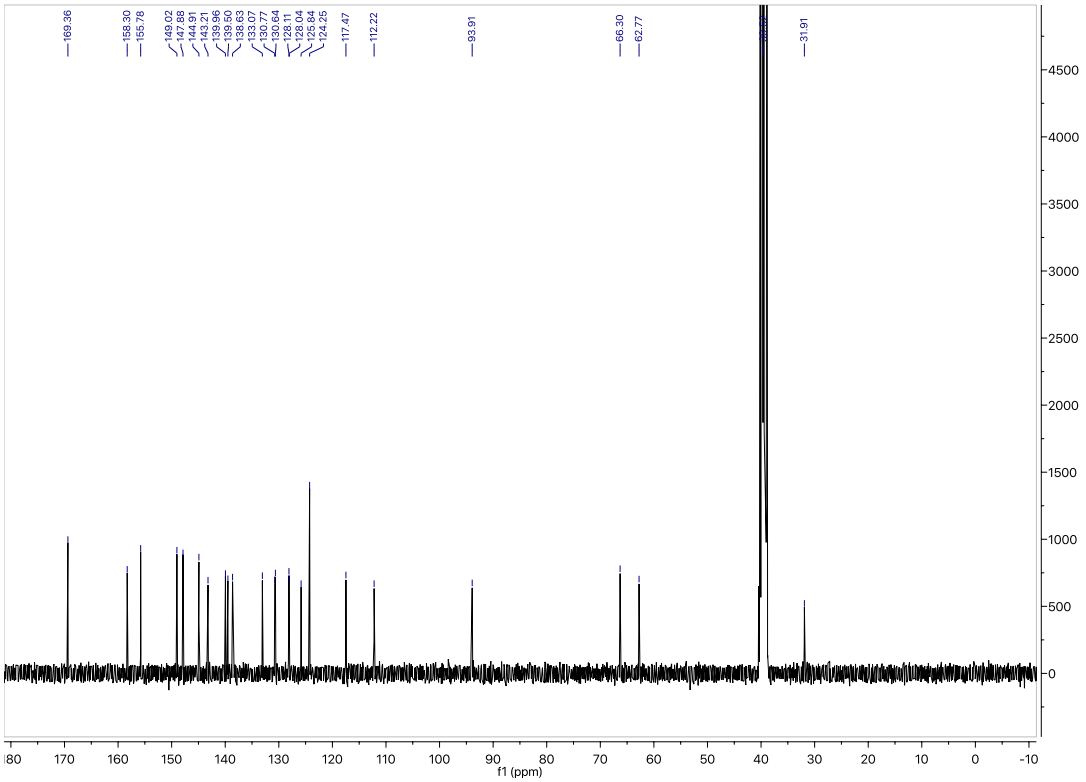
(*R*)-8-Cyclopropyl-7-(4-nitrophenyl)-13-oxo-6,10,11,13-tetrahydrochromeno[4,3-*b*]thiazolo[2,3-*g*][1,7]naphthyridine-11-carboxylic acid (Compound **15e**): ^{1}H-NMR (600 MHz) and ^{13}C{^{1}H}-NMR (151 MHz) [$(CD_3)_2SO$].



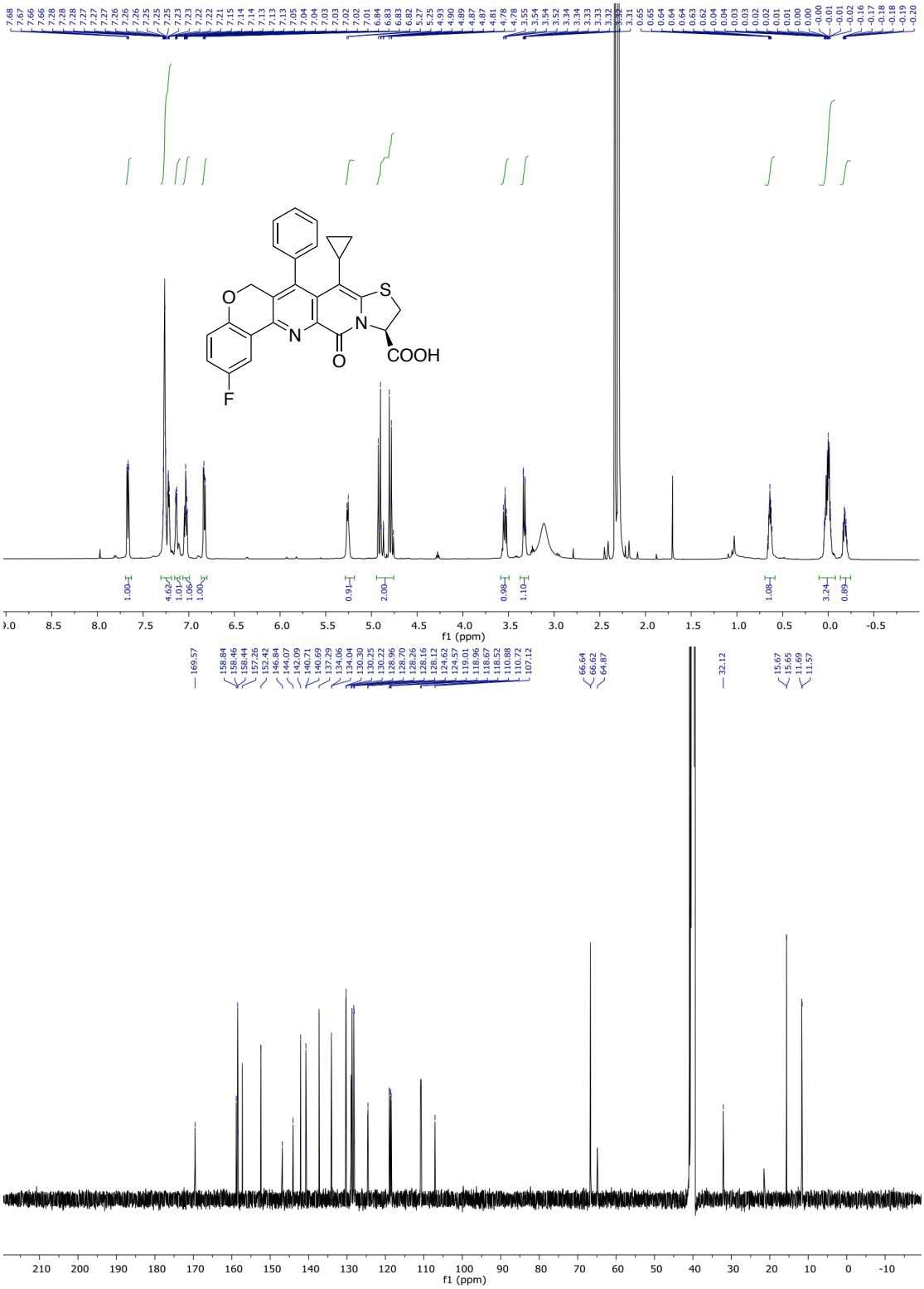


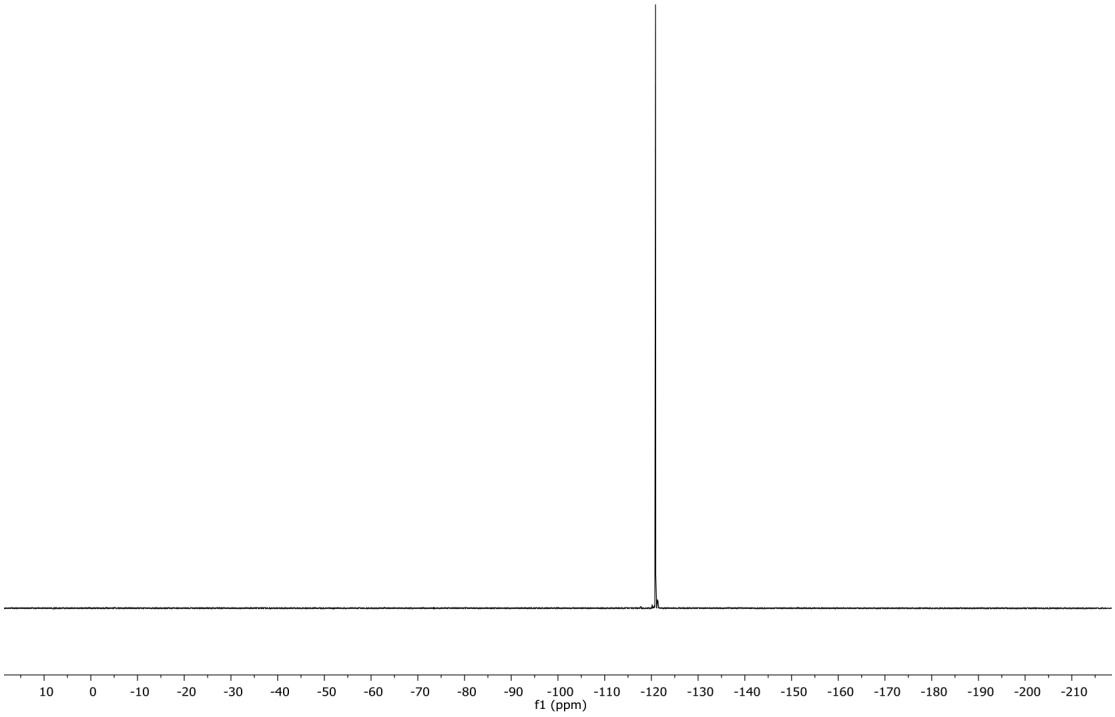
(*R*)-3-Nitro-7-(4-nitrophenyl)-13-oxo-6,10,11,13-tetrahydrochromeno[4,3-*b*]thiazolo[2,3-*g*][1,7]naphthyridine-11-carboxylic acid (Compound **15f**): ^{1}H-NMR (400 MHz) and ^{13}C{^{1}H}-NMR (100 MHz)
[$(CD_3)_2SO$].





(*R*)-8-Cyclopropyl-2-fluoro-13-oxo-7-phenyl-6,10,11,13-tetrahydrochromeno[4,3-*b*]thiazolo[2,3-*g*][1,7]naphthyridine-11-carboxylic acid (Compound **15g**): ^{1}H-NMR (600 MHz), ^{13}C{^{1}H}-NMR (151 MHz) and ^{19}F{^{1}H}-NMR (564 MHz) [$(CD_3)_2SO$].

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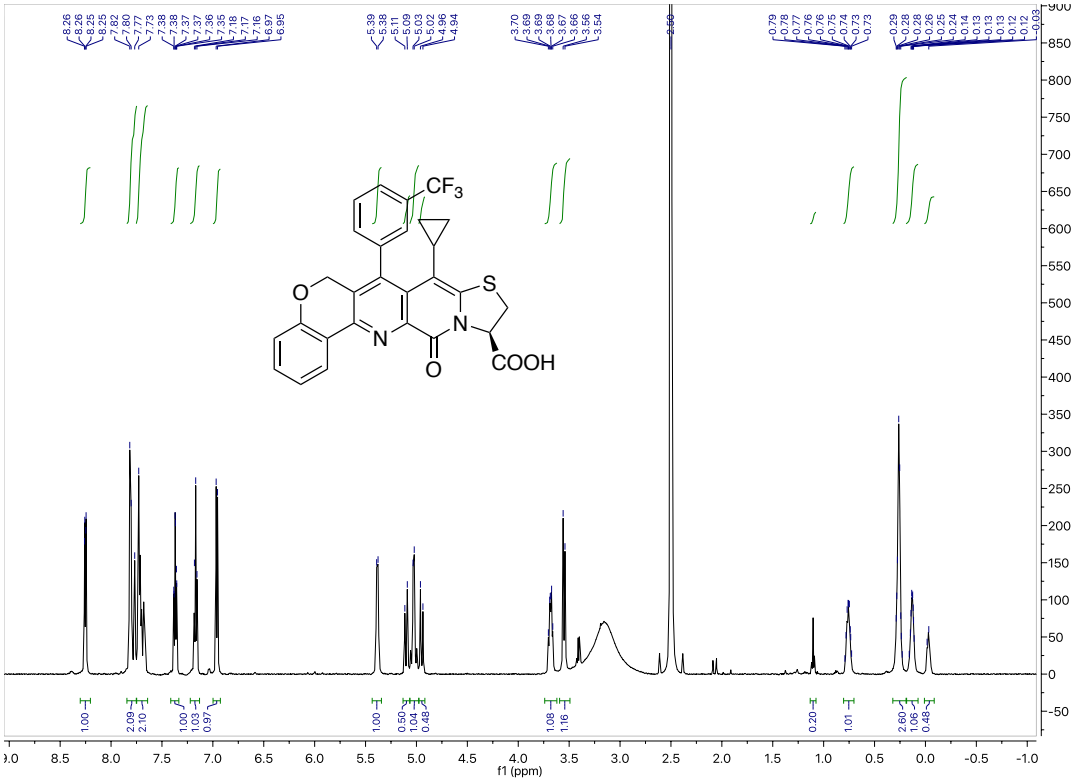
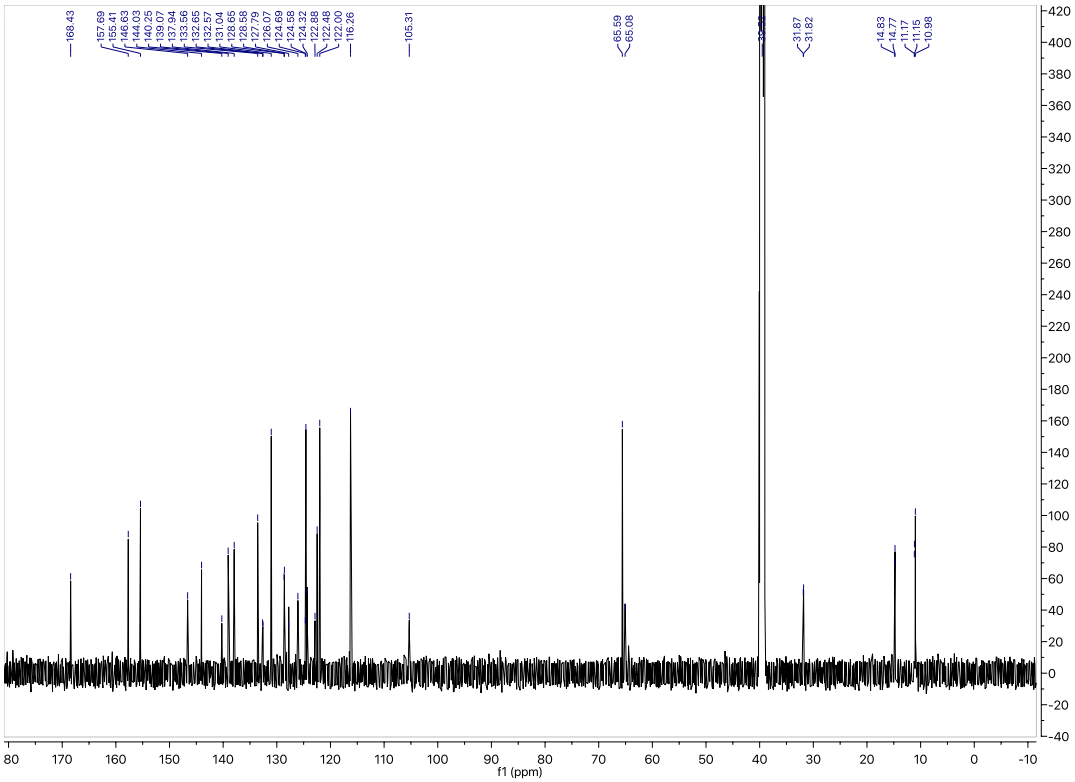
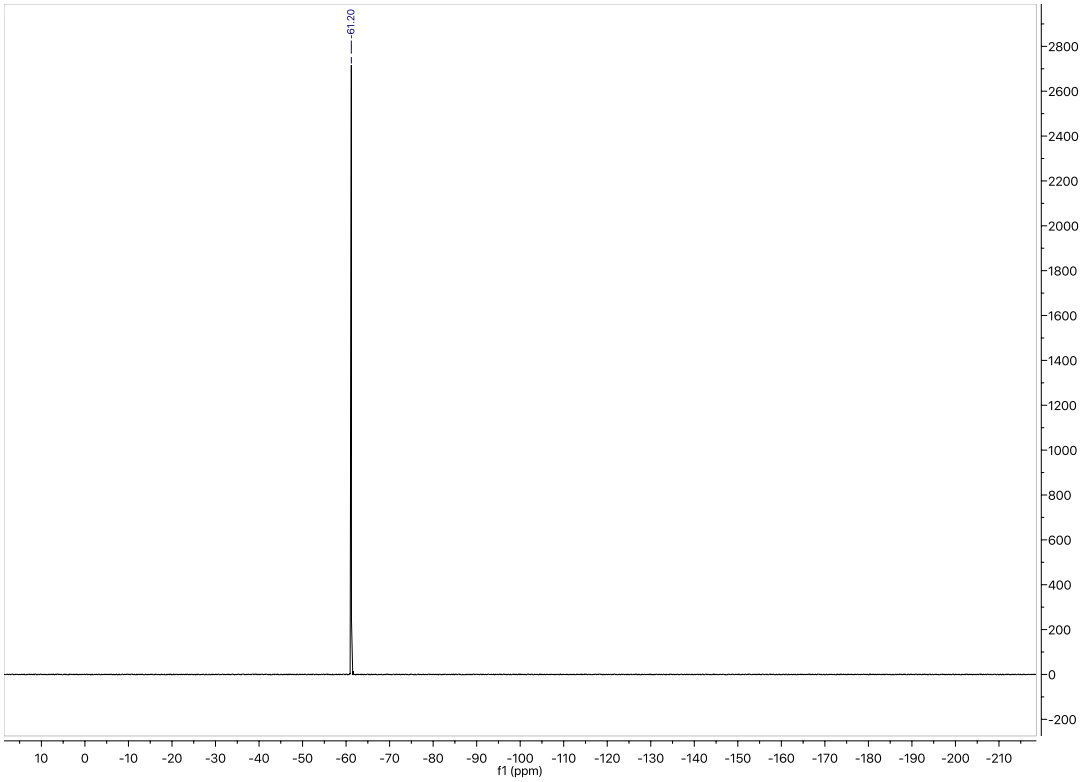
f1 (ppm)

(*R*)-8-Cyclopropyl-13-oxo-7-(3-(trifluoromethyl)phenyl)-6,10,11,13-tetrahydrochromeno[4,3-*b*]thiazolo[2,3-*g*][1,7]naphthyridine-11-carboxylic acid (Compound **15h**): ^{1}H-NMR (600 MHz), ^{13}C{^{1}H}-NMR (151 MHz) and ^{19}F-NMR (564 MHz) [$(CD_3)_2SO$, 343 K].



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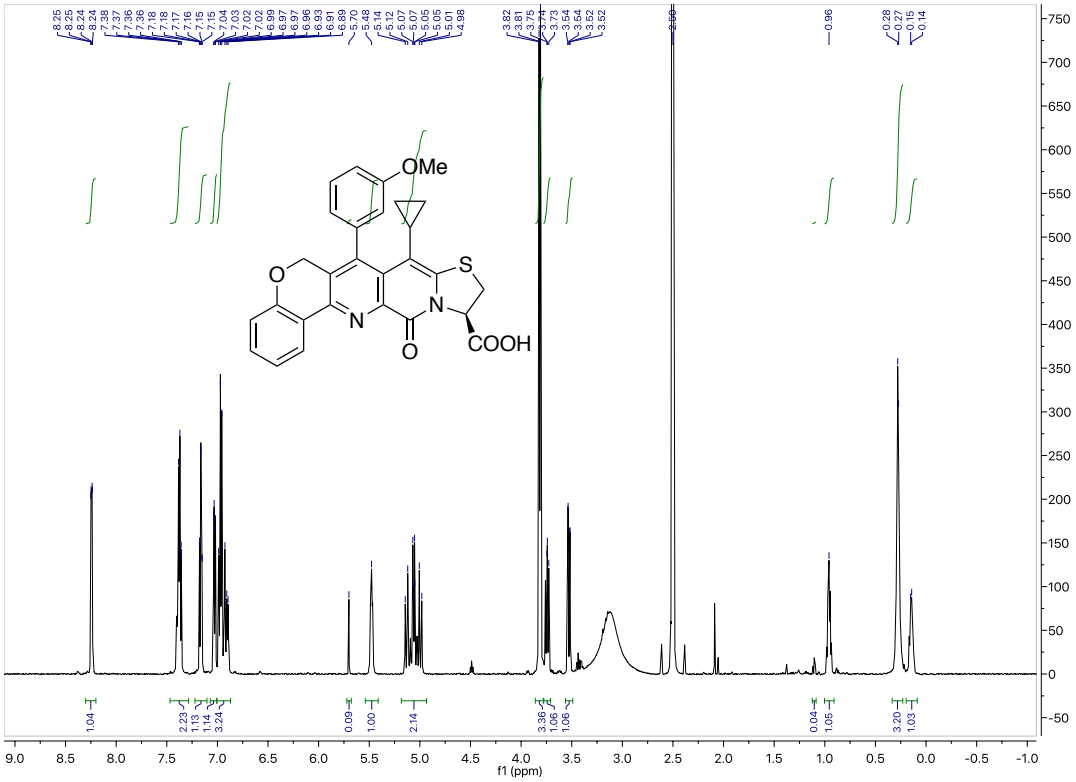
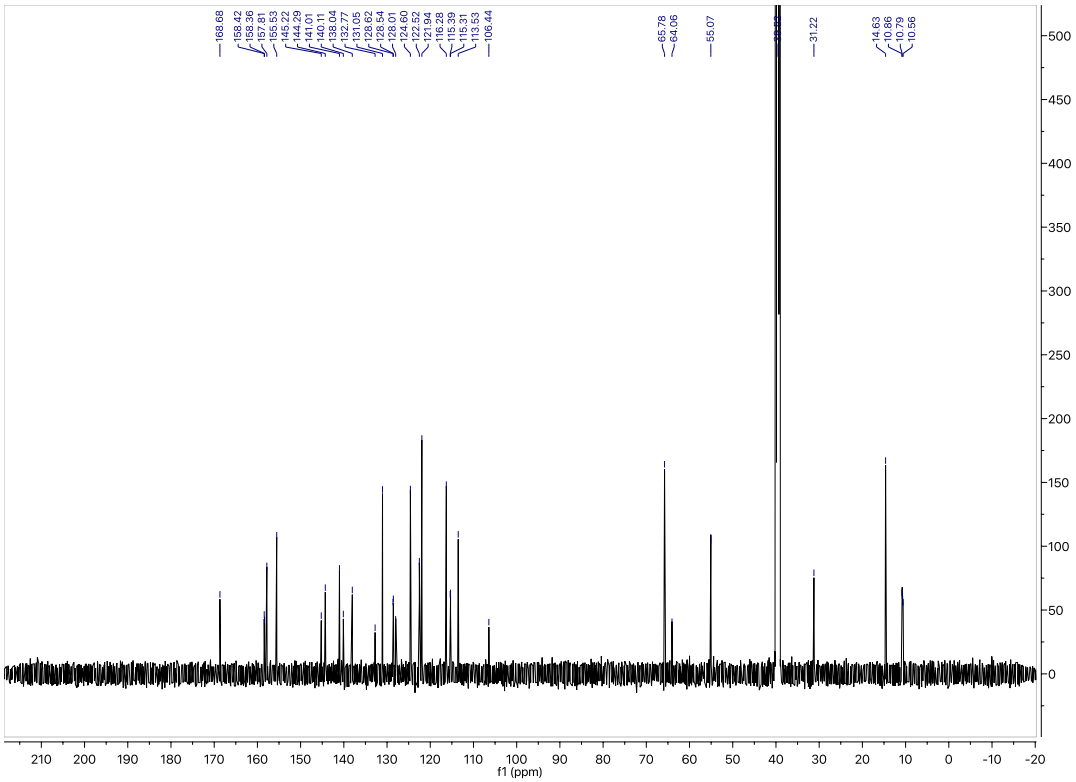
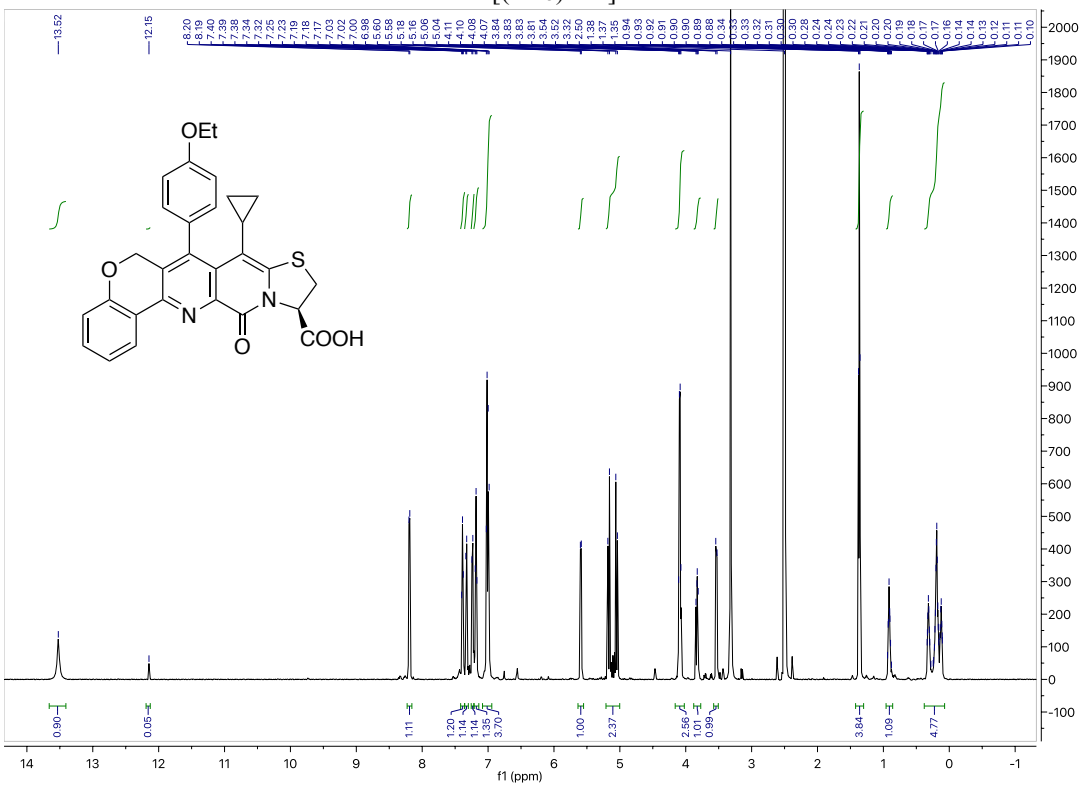
f1 (ppm)

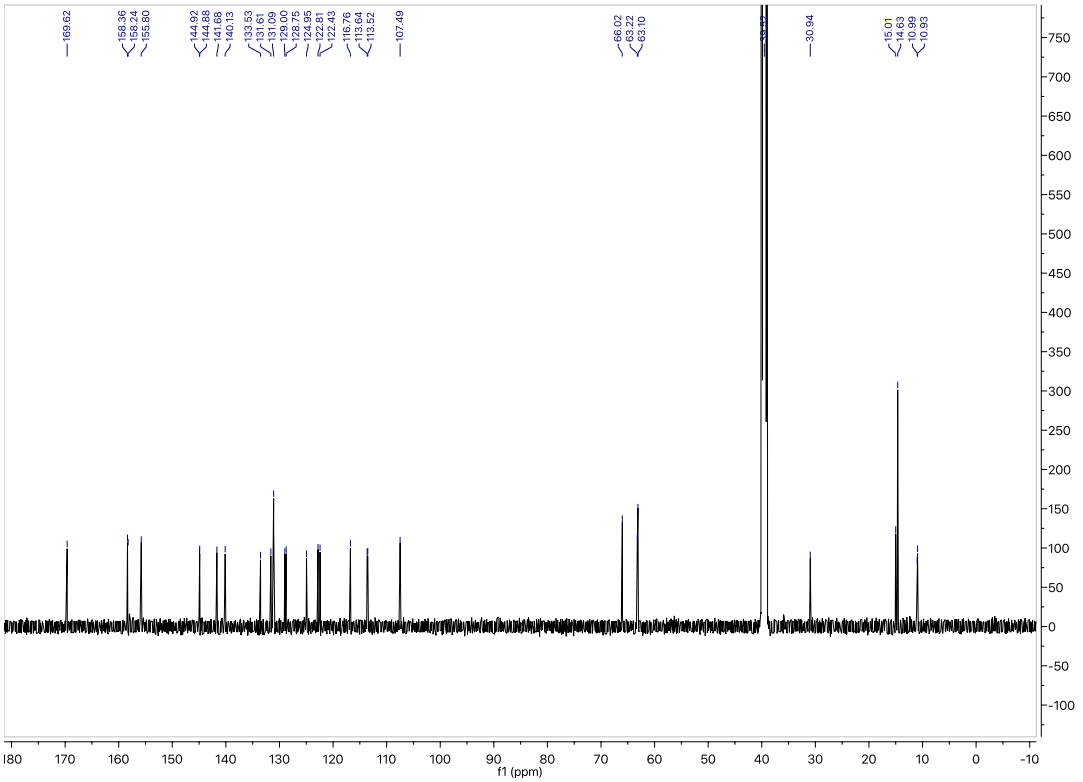
(*R*)-8-Cyclopropyl-7-(3-methoxyphenyl)-13-oxo-6,10,11,13-tetrahydrochromeno[4,3-*b*]thiazolo[2,3-*g*][1,7]naphthyridine-11-carboxylic acid (Compound **15i**): ^{1}H-NMR (600 MHz) and ^{13}C{^{1}H}-NMR (151 MHz) [$(CD_3)_2SO$, 343 K].



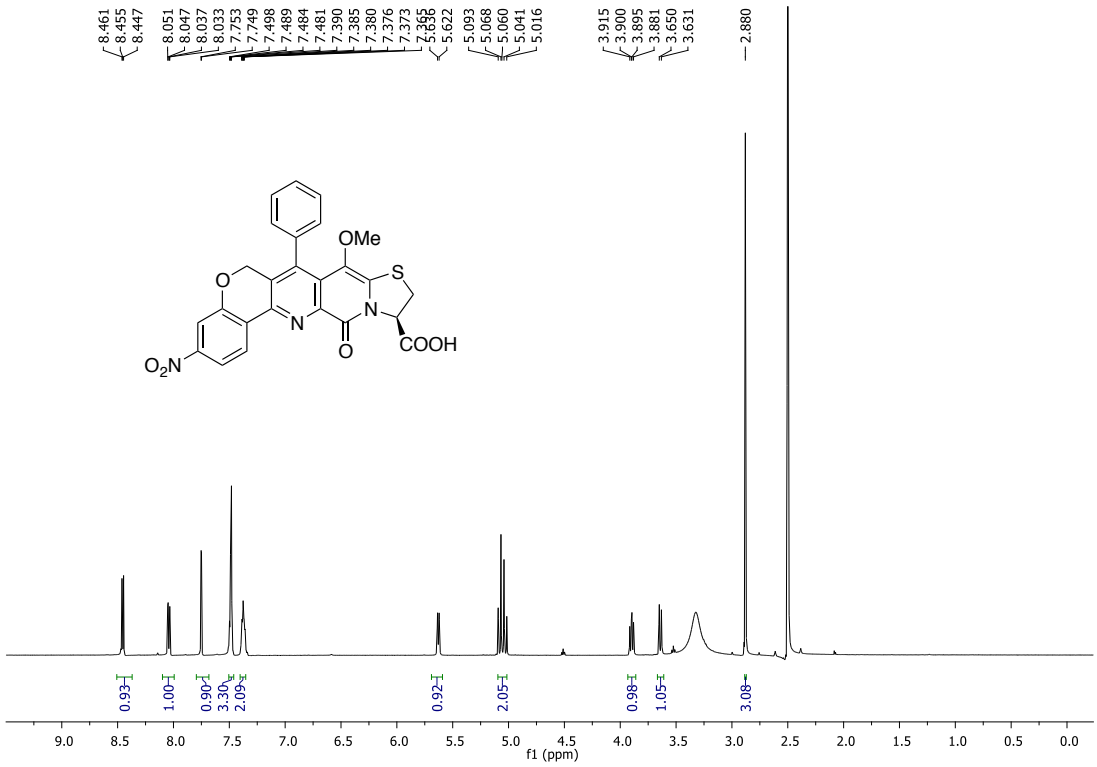


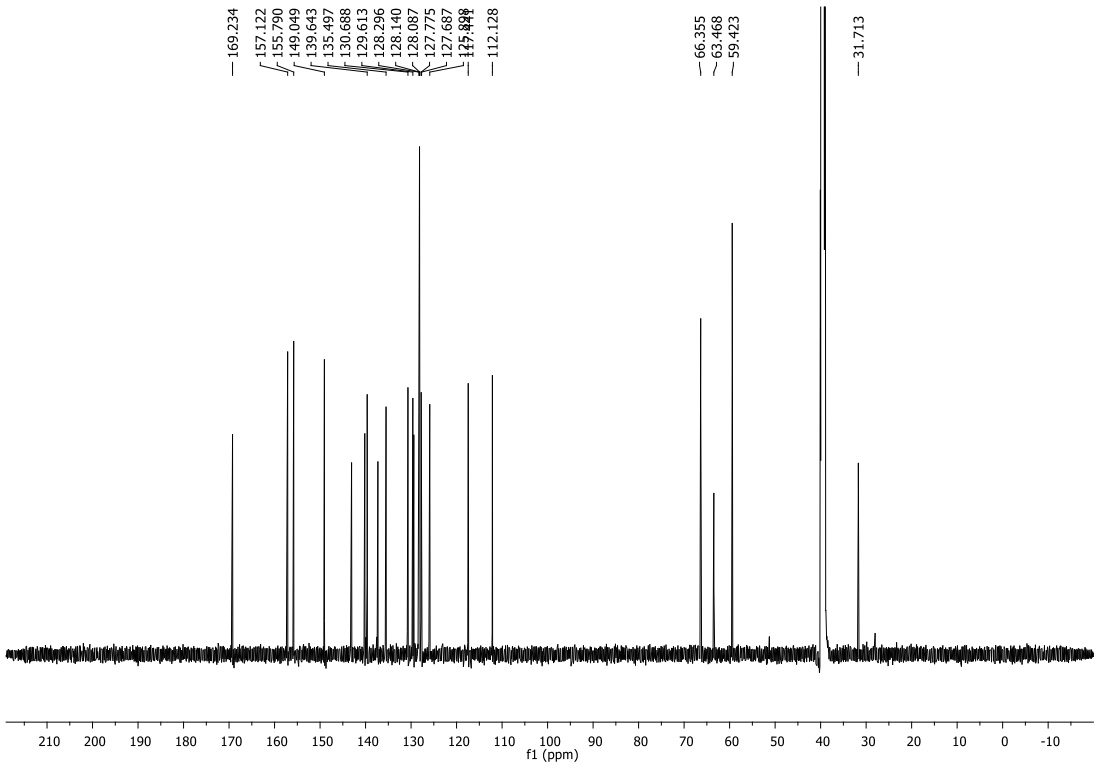
(*R*)-8-Cyclopropyl-7-(4-ethoxyphenyl)-13-oxo-6,10,11,13-tetrahydrochromeno[4,3-*b*]thiazolo[2,3-*g*][1,7]naphthyridine-11-carboxylic acid (Compound **15j**): ^{1}H-NMR (600 MHz) and ^{13}C{^{1}H}-NMR (151 MHz)
[$(CD_3)_2SO$].



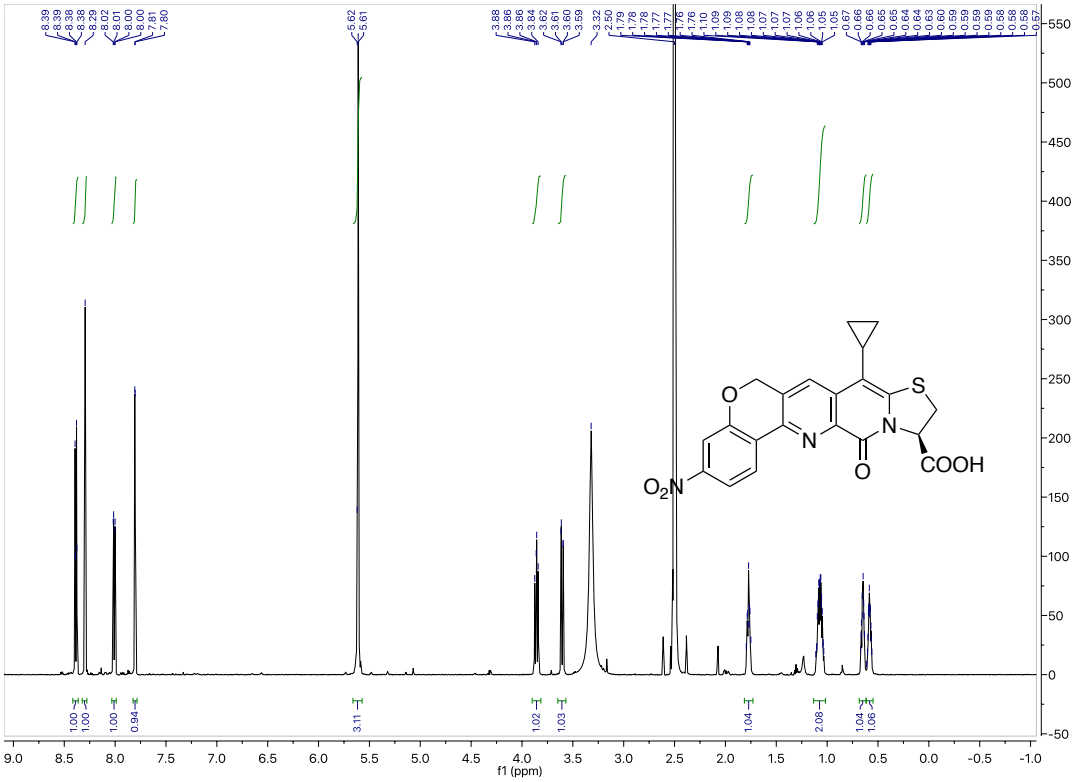


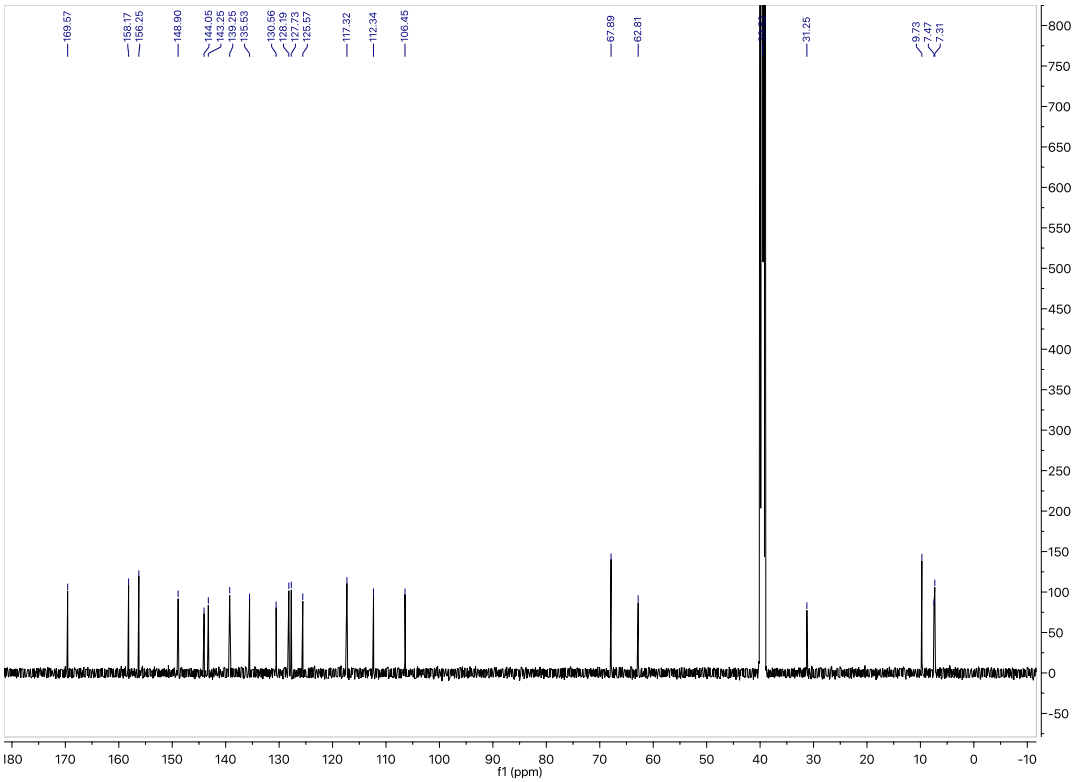
(*R*)-8-Methoxy-3-nitro-13-oxo-7-phenyl-6,10,11,13-tetrahydrochromeno[4,3-*b*]thiazolo[2,3-*g*][1,7]naphthyridine-11-carboxylic acid: (Compound **15k**): ^{1}H-NMR (600 MHz) and ^{13}C{^{1}H}-NMR (151 MHz) [$(CD_3)_2SO$]



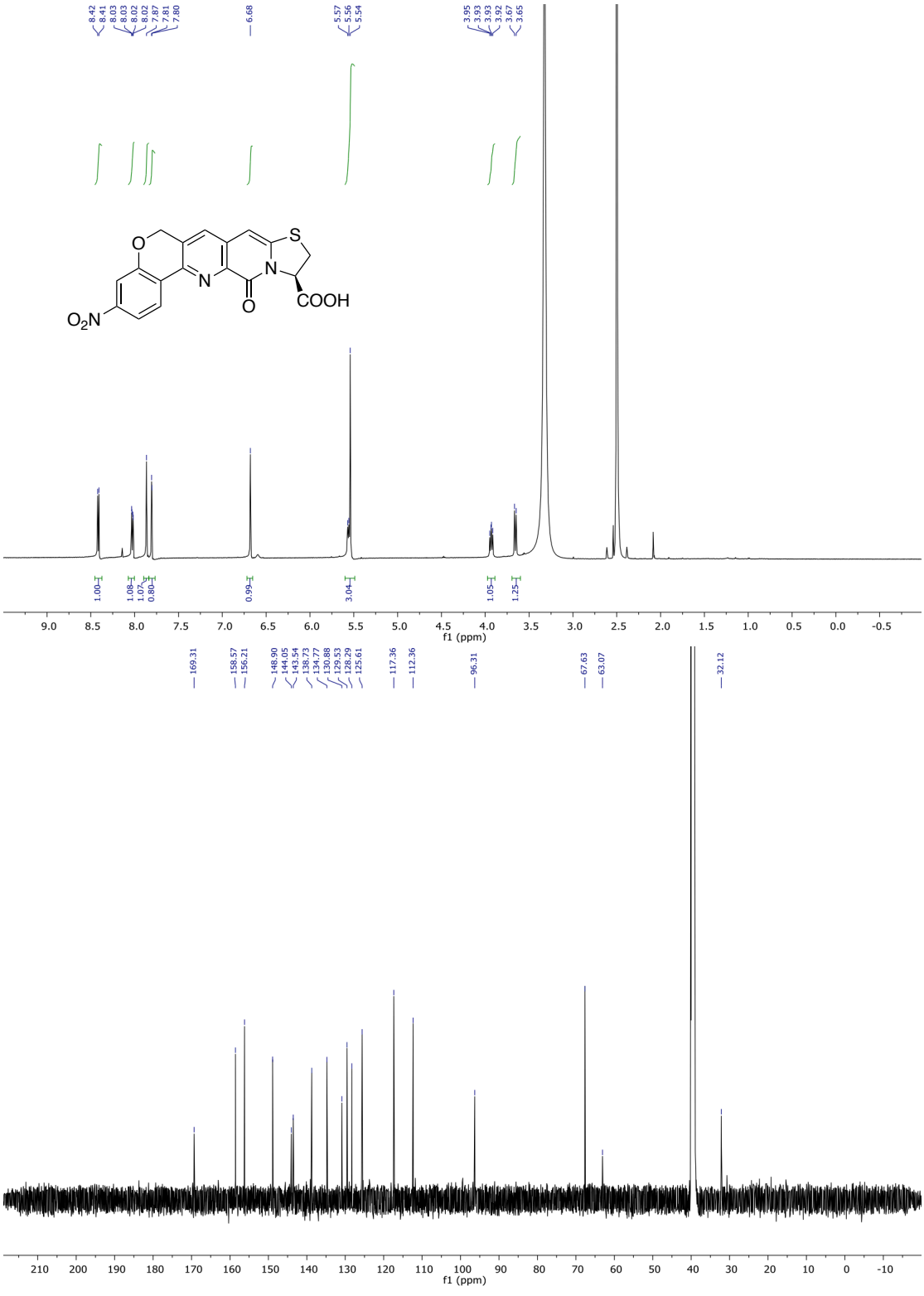


8-Cyclopropyl-3-nitro-13-oxo-6,10,11,13-tetrahydrochromeno[4,3- *b*]thiazolo[2,3-*g*][1,7]naphthyridine-11-carboxylic acid (Compound **16a**) ^{1}H-NMR (600 MHz) and ^{13}C{^{1}H}-NMR (151 MHz) [$(CD_3)_2SO$].

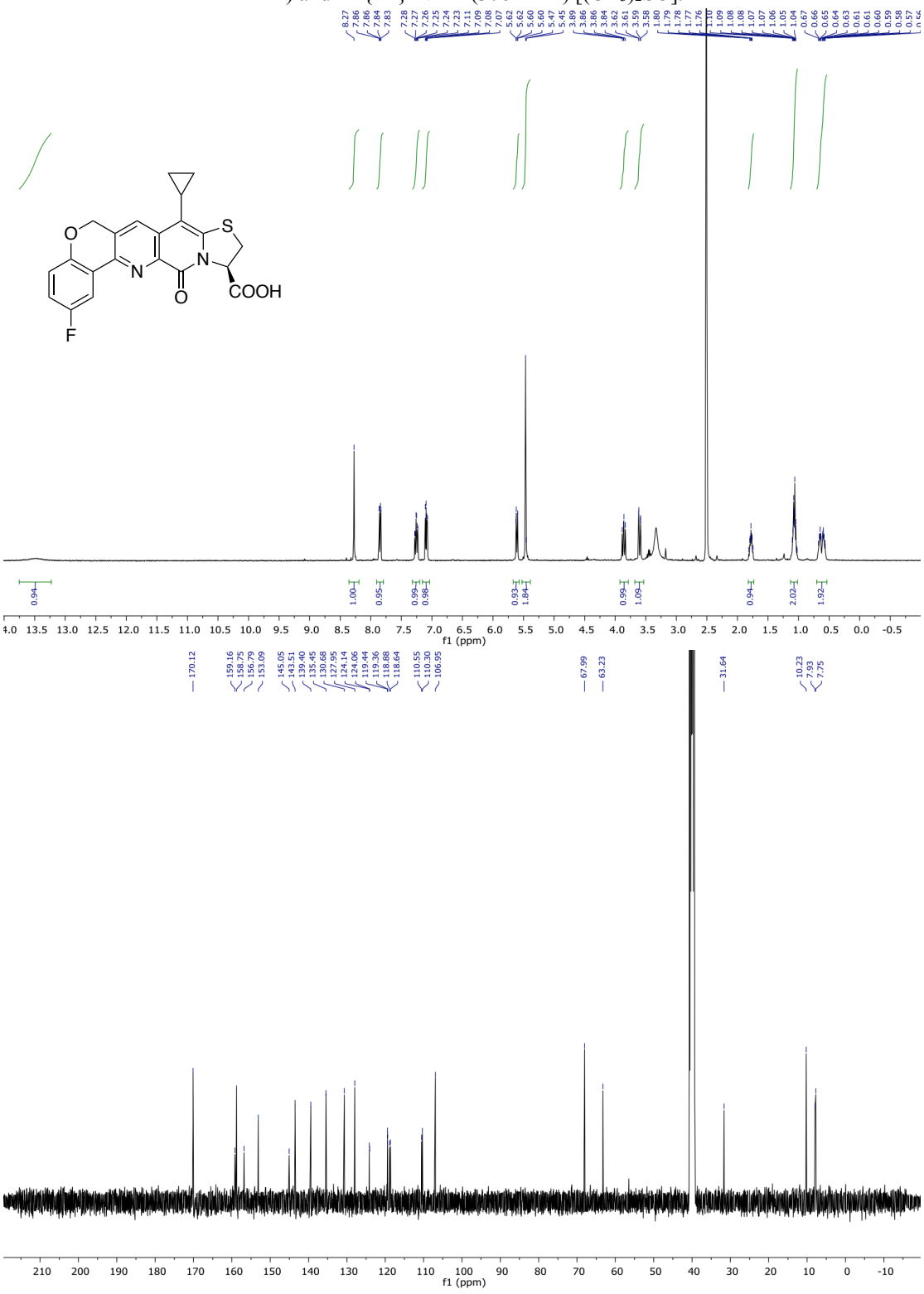


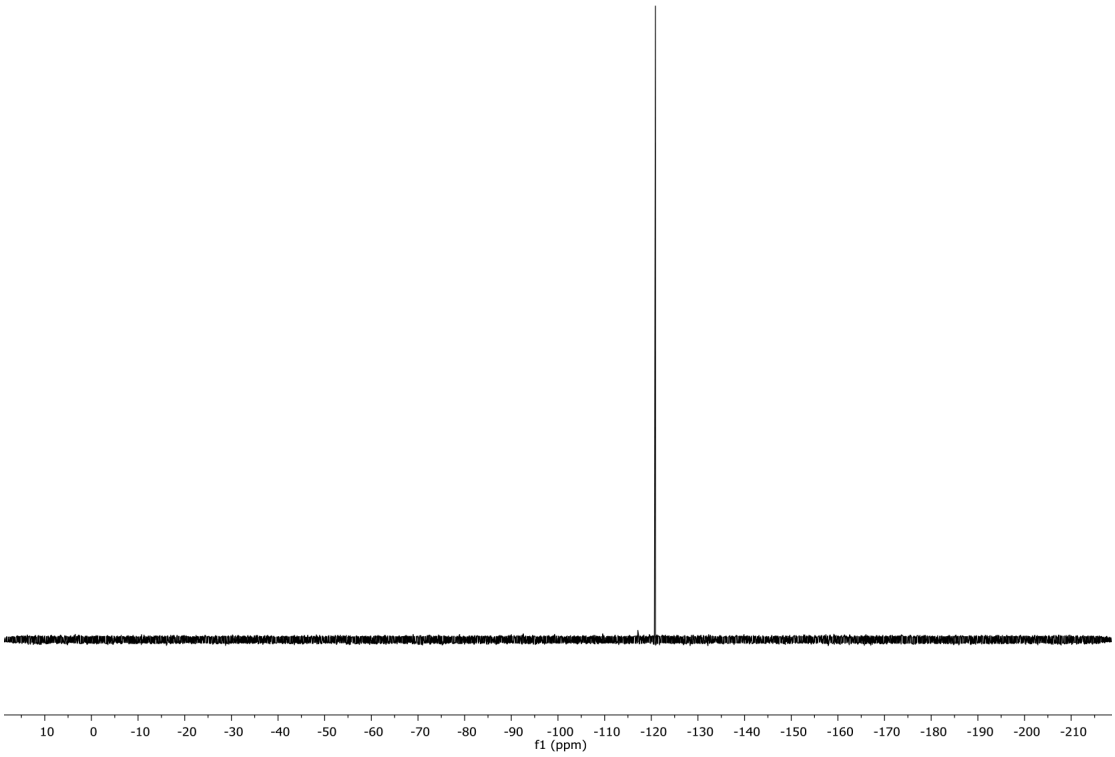


(*R*)-3-Nitro-13-oxo-6,10,11,13-tetrahydrochromeno[4,3-*b*]thiazolo[2,3-*g*][1,7]naphthyridine-11-carboxylic acid (Compound **16b**): ^{1}H-NMR (600 MHz) and ^{13}C{^{1}H}-NMR (151 MHz) [$(CD_3)_2SO$].



(*R*)-8-Cyclopropyl-2-fluoro-13-oxo-6,10,11,13-tetrahydrochromeno[4,3-*b*]thiazolo[2,3-*g*][1,7]naphthyridine-11-carboxylic acid (Compound **16c**): ^{1}H-NMR (400 MHz), ^{13}C{^{1}H}-NMR (100 MHz) and ^{19}F{^{1}H}-NMR (376 MHz) [$(CD_3)_2SO$].

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-190
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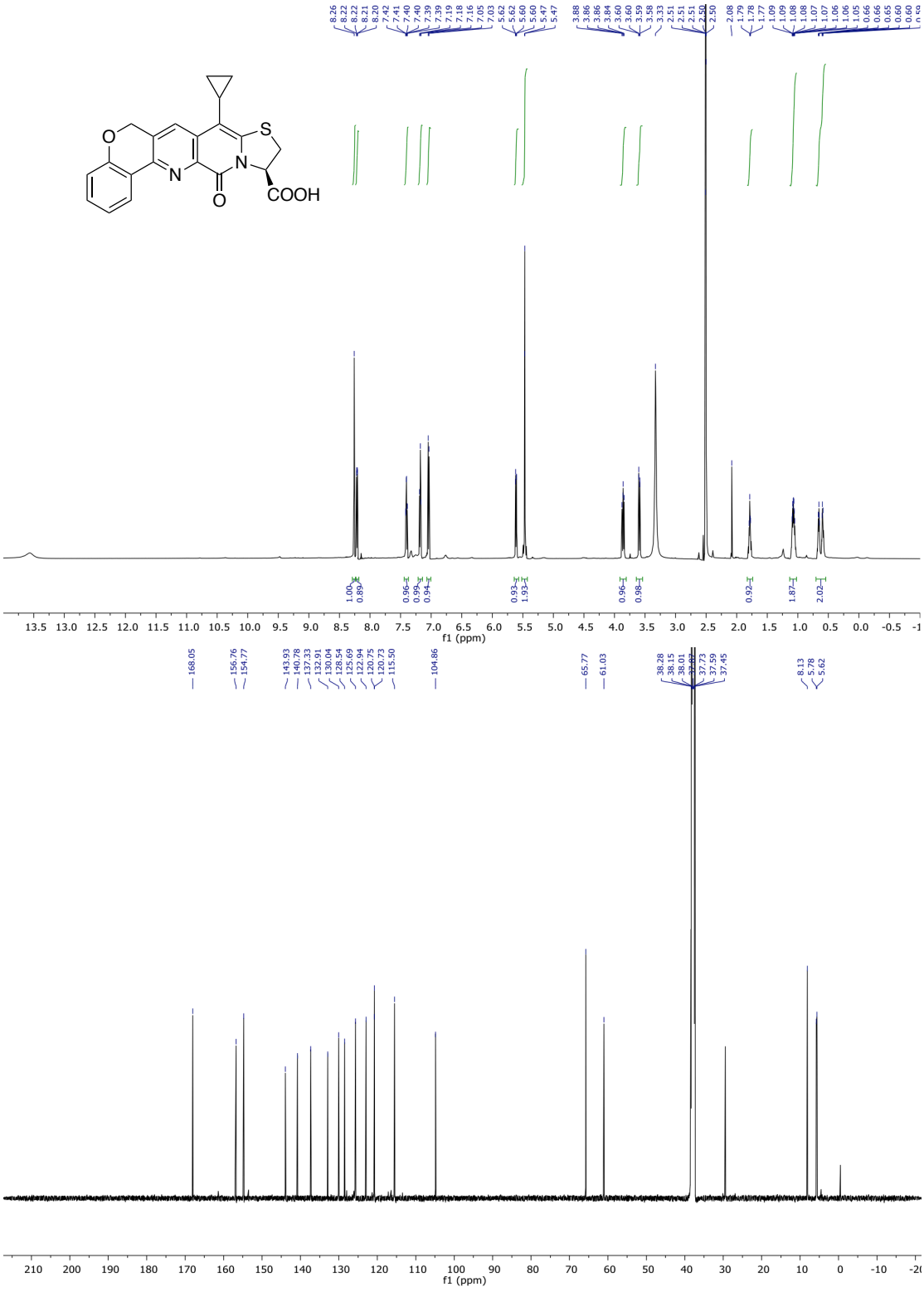
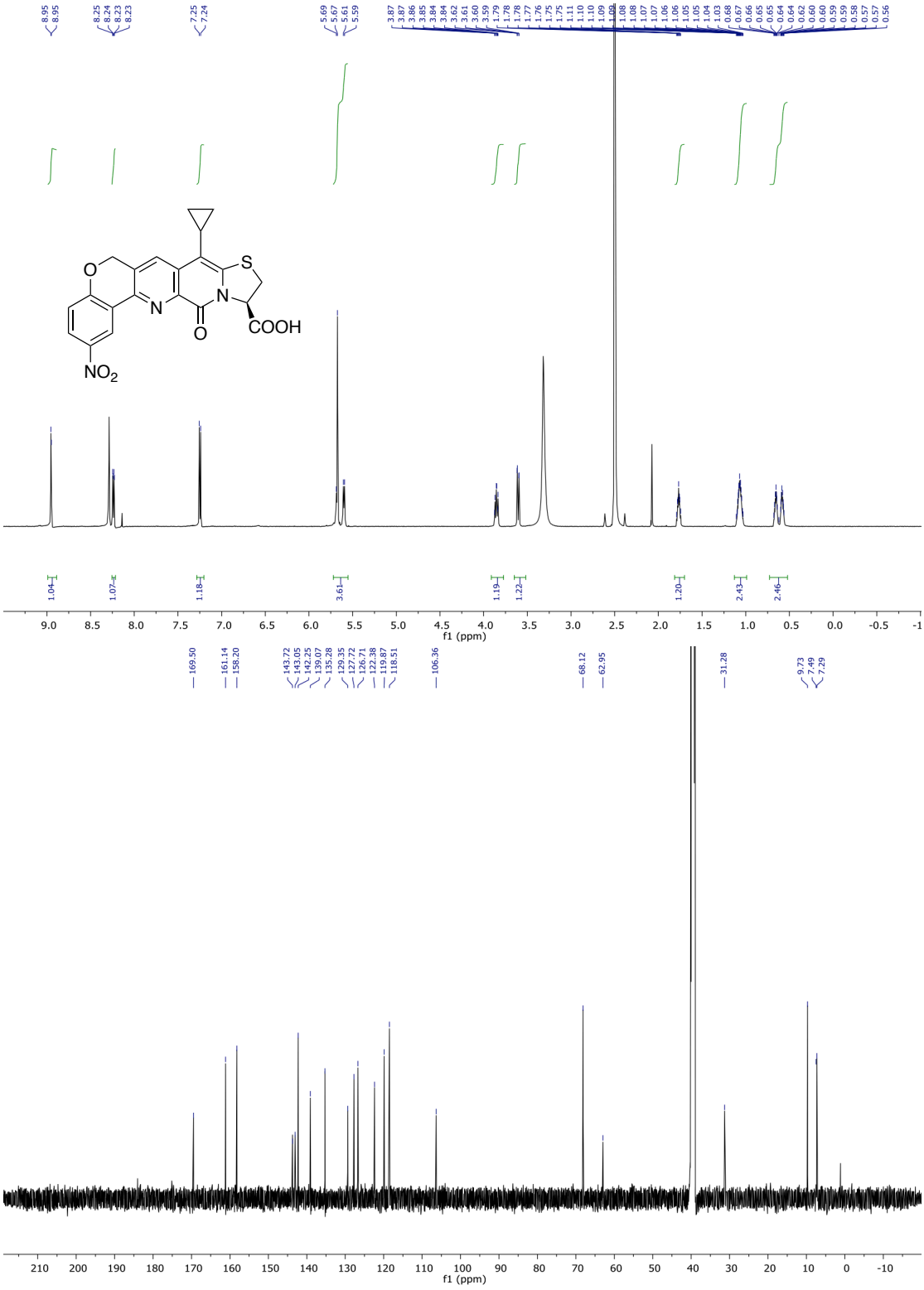
-210
f1 (ppm)

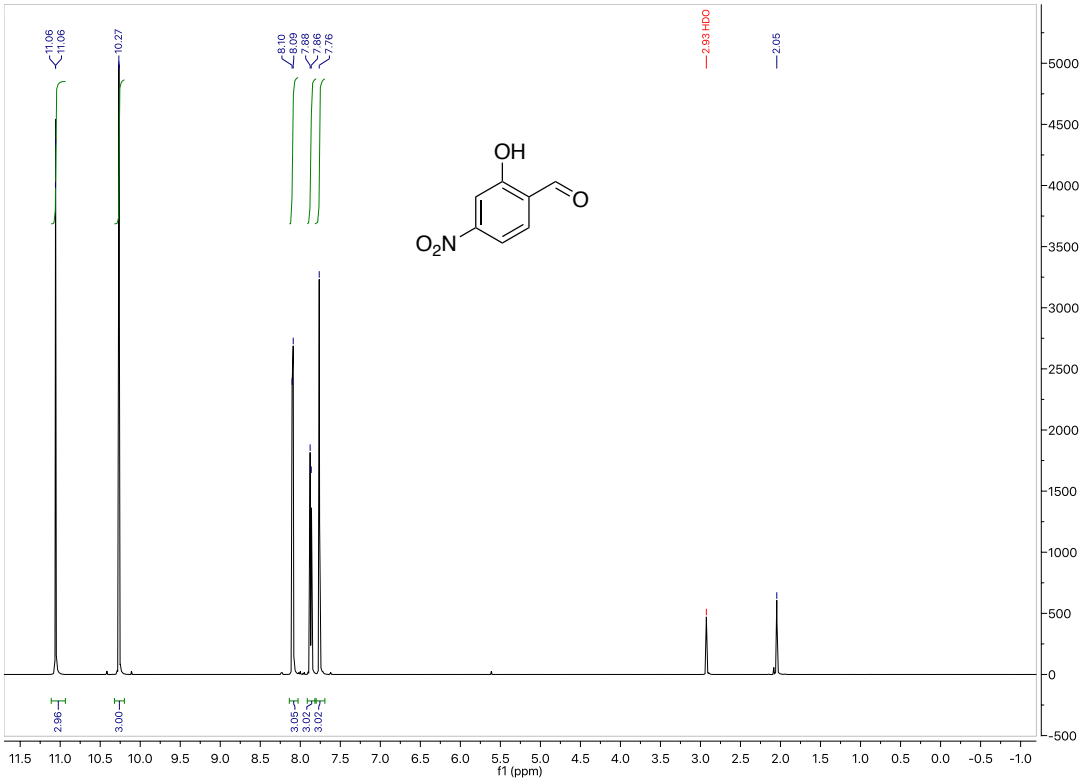
(*R*)-8-Cyclopropyl-13-oxo-6,10,11,13-tetrahydrochromeno[4,3-*b*]thiazolo[2,3-*g*][1,7]naphthyridine-11-carboxylic acid (Compound **16d**): ^{1}H-NMR (600 MHz) and ^{13}C{^{1}H}-NMR (151 MHz) [$(CD_3)_2SO$].



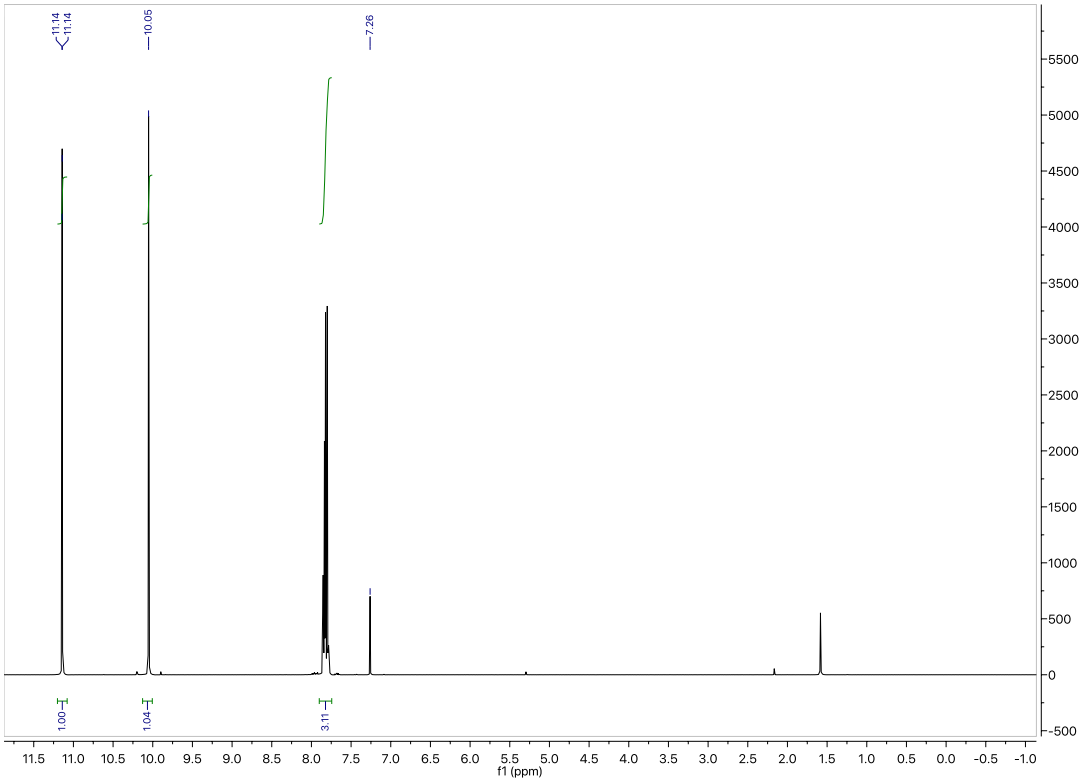
(*R*)-8-Cyclopropyl-2-nitro-13-oxo-6,10,11,13-tetrahydrochromeno[4,3-*b*]thiazolo[2,3-*g*][1,7]naphthyridine-11-carboxylic acid (Compound **16e**): ^{1}H-NMR (600 MHz) and ^{13}C{^{1}H}-NMR (151 MHz) [$(CD_3)_2SO$].



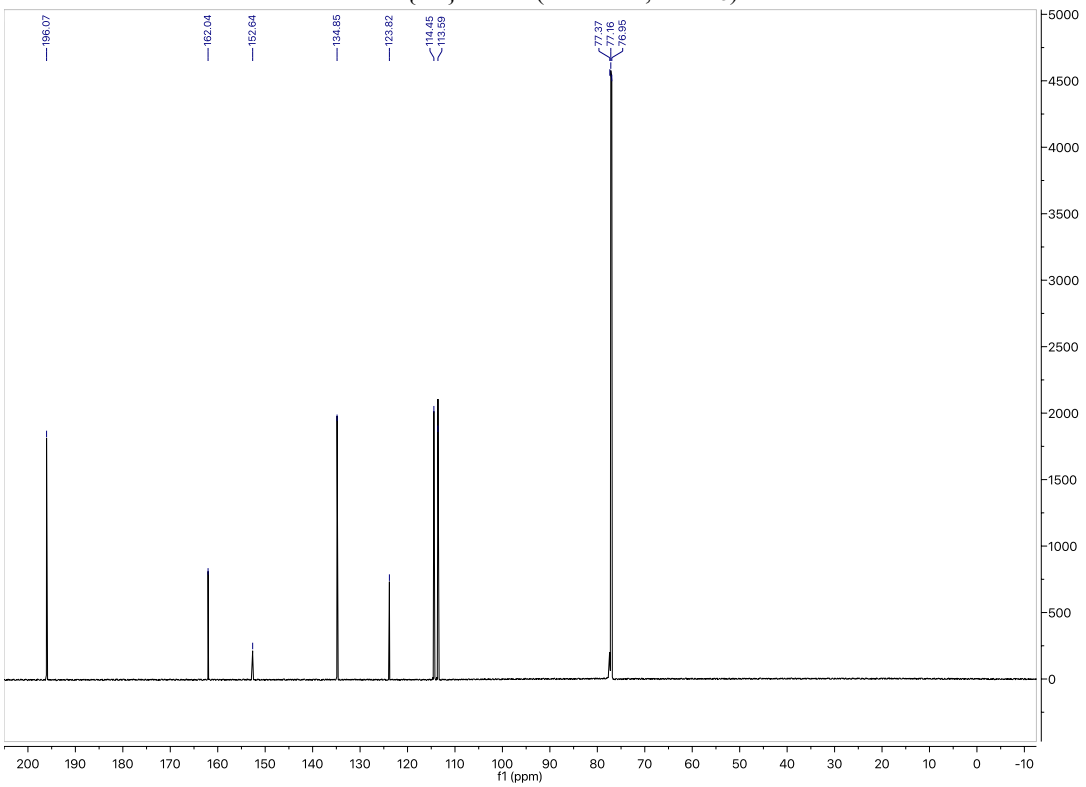
2-Hydroxy-4-nitrobenzaldehyde (Compound **Ib**): ^{1}H-NMR [600 MHz, $(CD_3)_2CO$].



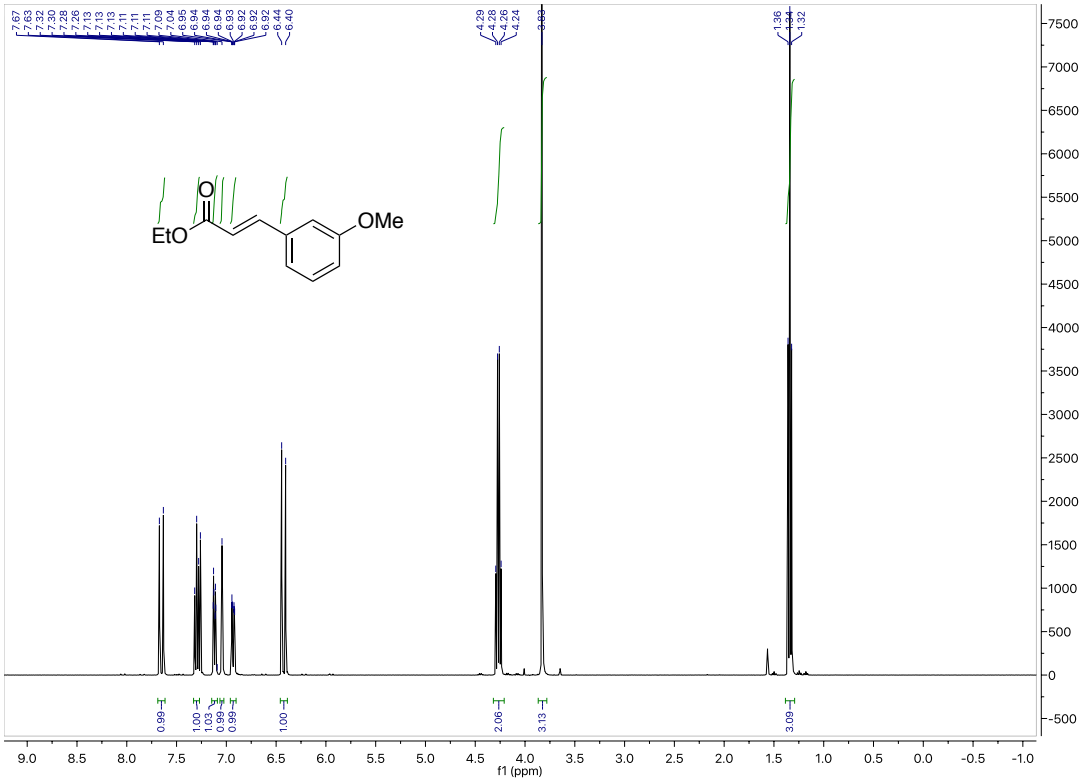
^{1}H-NMR (600 MHz, $CDCl_3$).

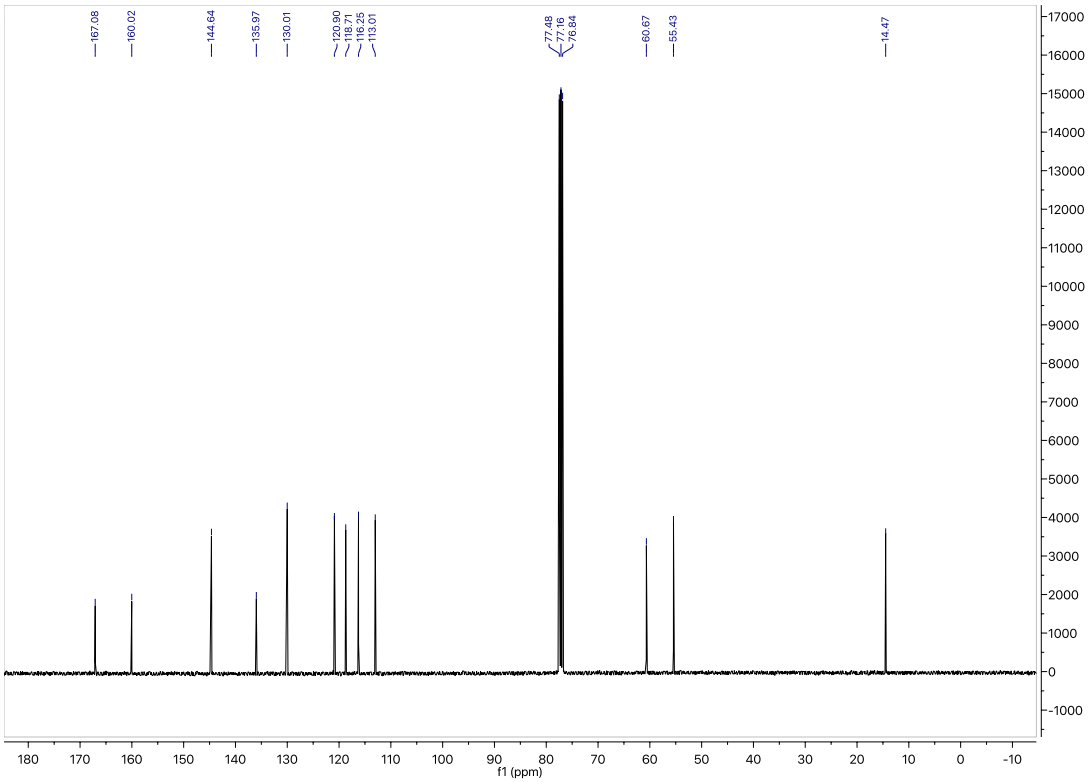


$^{13}C\{^{1}H\}$-NMR (151 MHz, $CDCl_3$).

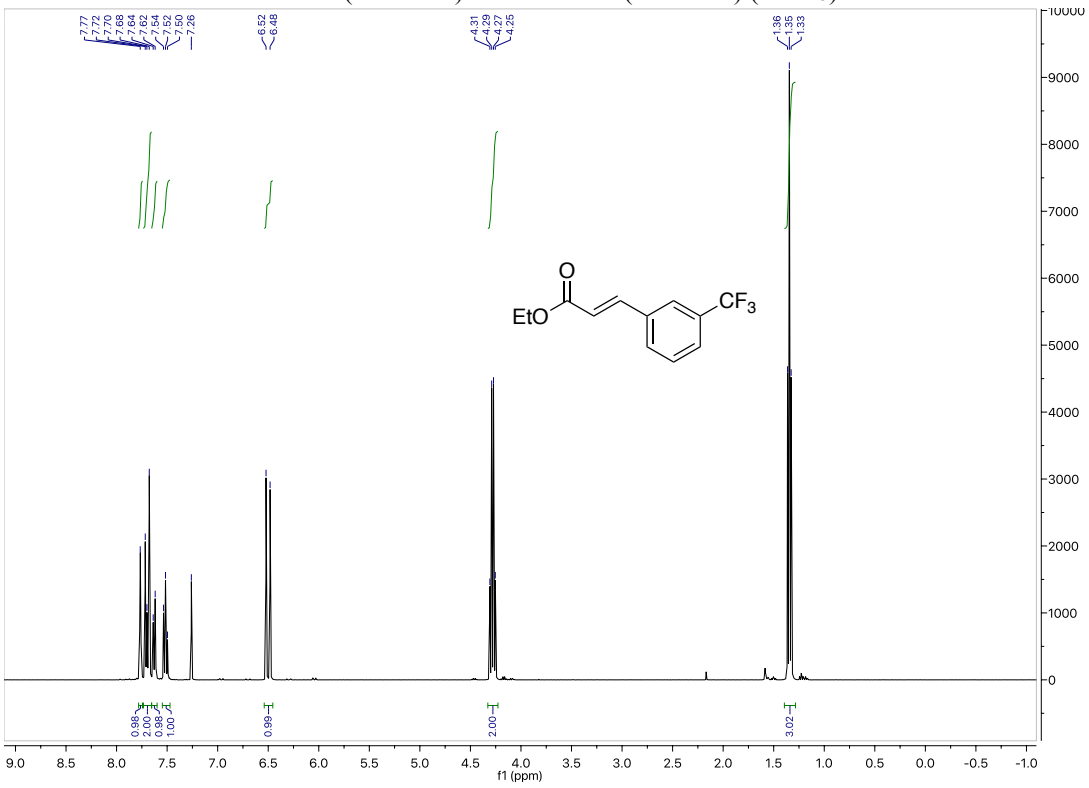


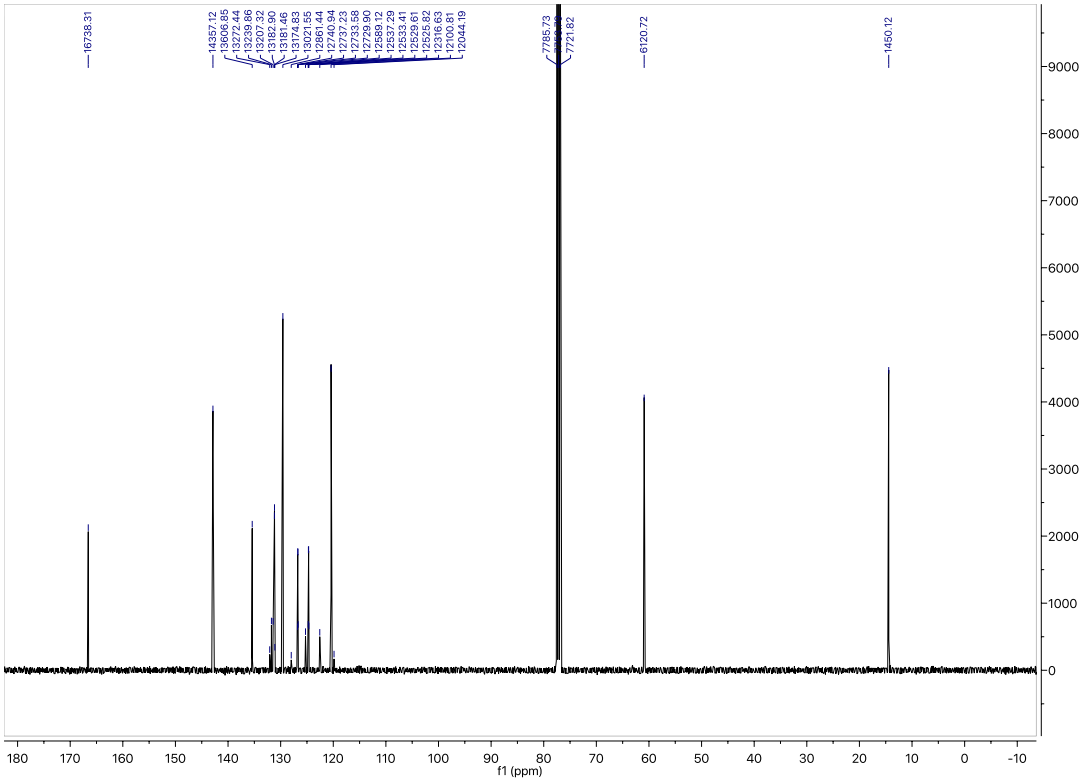
Ethyl (*E*)-3-(3-methoxyphenyl)acrylate (Compound **IVa**): ^{1}H-NMR (400 MHz) and ^{13}C{^{1}H}-NMR (100 MHz)
($CDCl_3$).

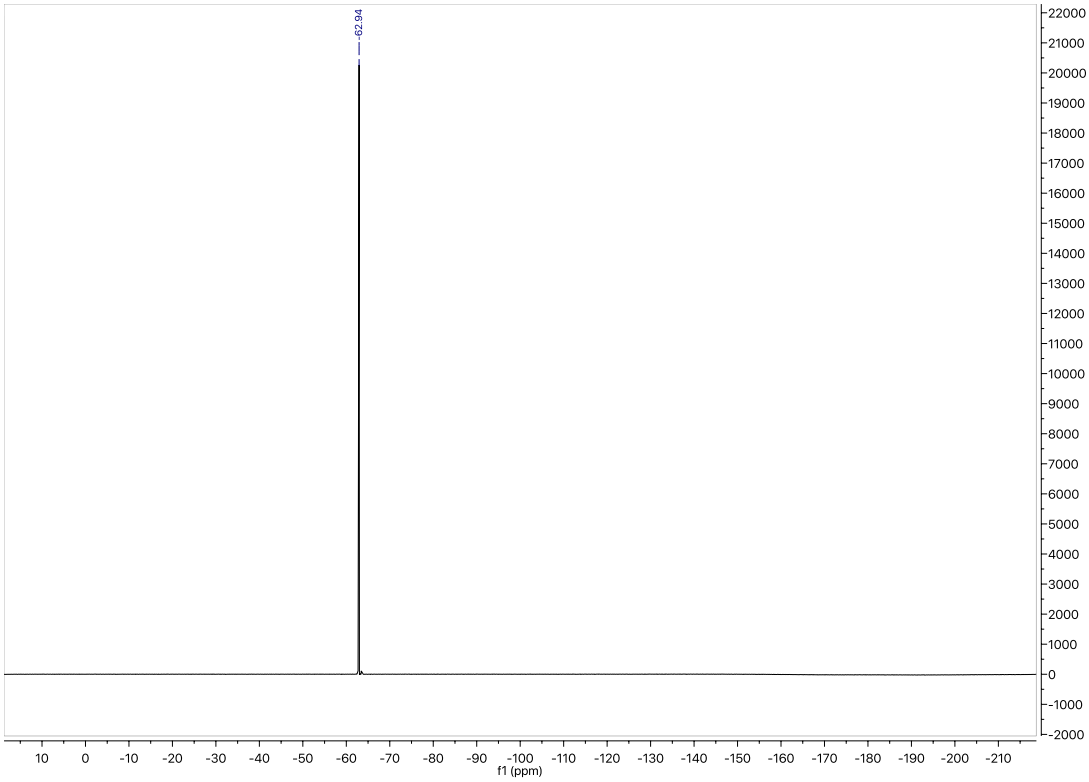




Ethyl (*E*)-3-(3-(trifluoromethyl)phenyl)acrylate (Compound **IVb**): ^{1}H-NMR (400 MHz), ^{13}C{^{1}H}-NMR (100 MHz) and ^{19}F-NMR (376 MHz) ($CDCl_3$)






-62.94
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21000
20000
19000
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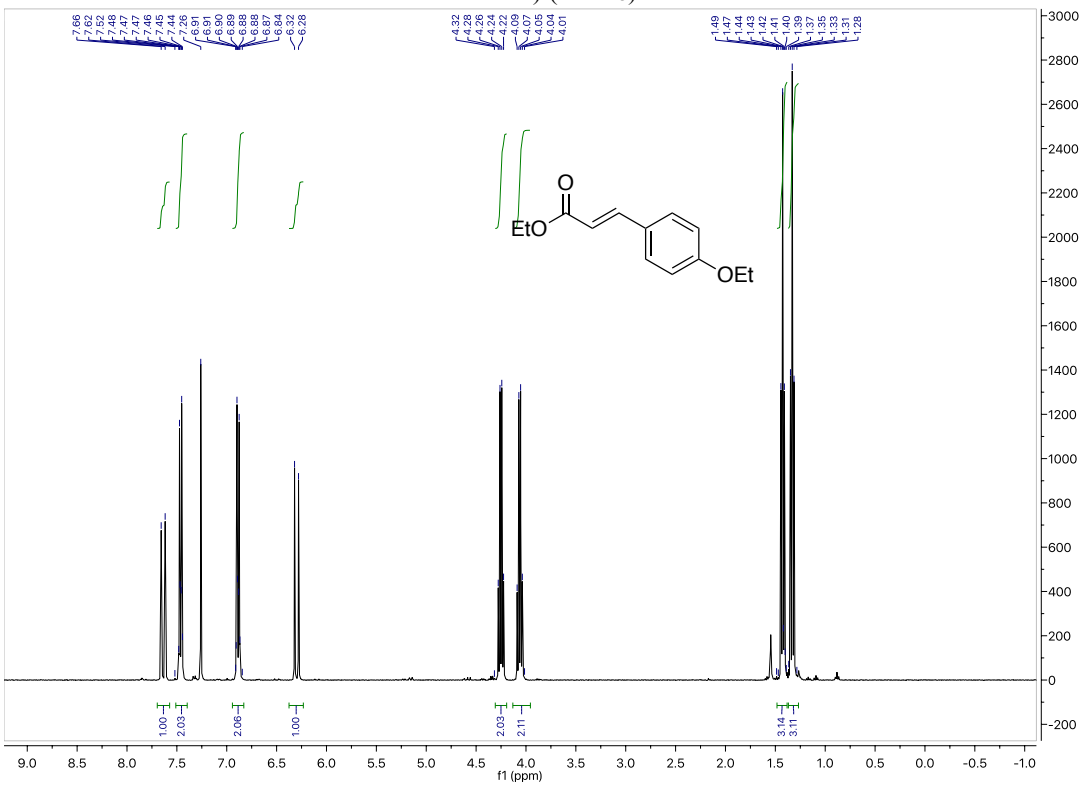
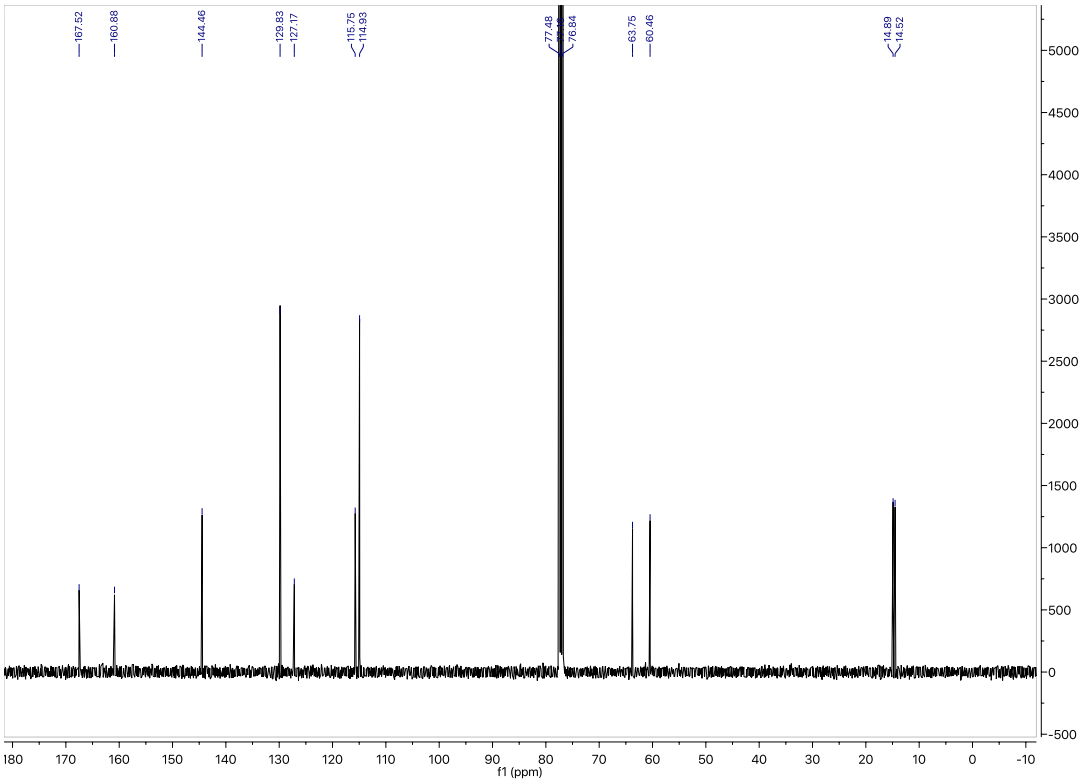
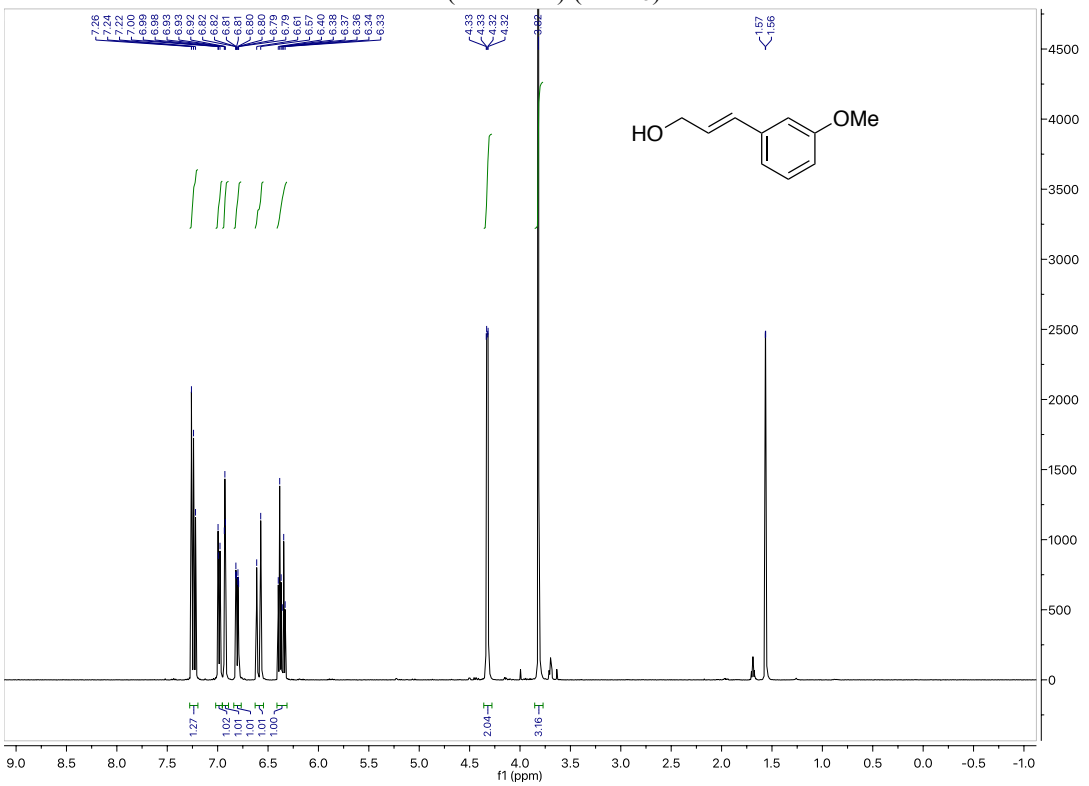
17000
16000
15000
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
-2000
10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210
f1 (ppm)

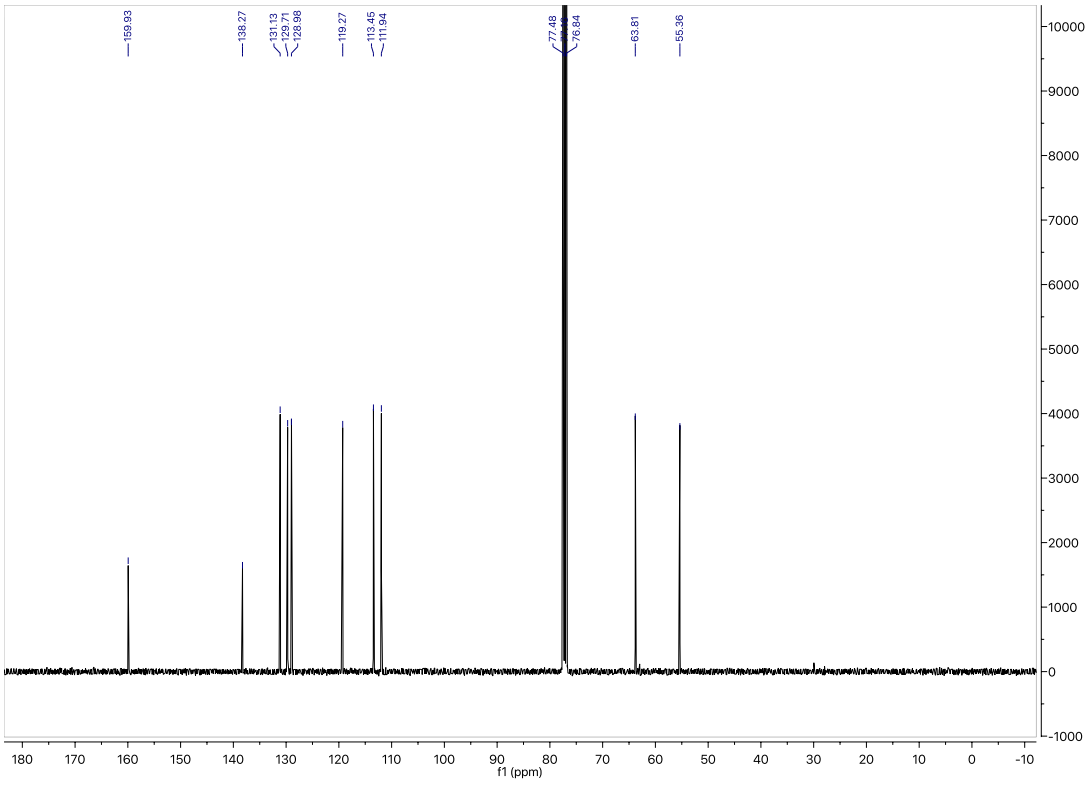
Ethyl (*E*)-3-(4-ethoxyphenyl)acrylate (Compound **IVc**) ^{1}H-NMR (400 MHz) and ^{13}C{^{1}H}-NMR (100 MHz) ($CDCl_3$)



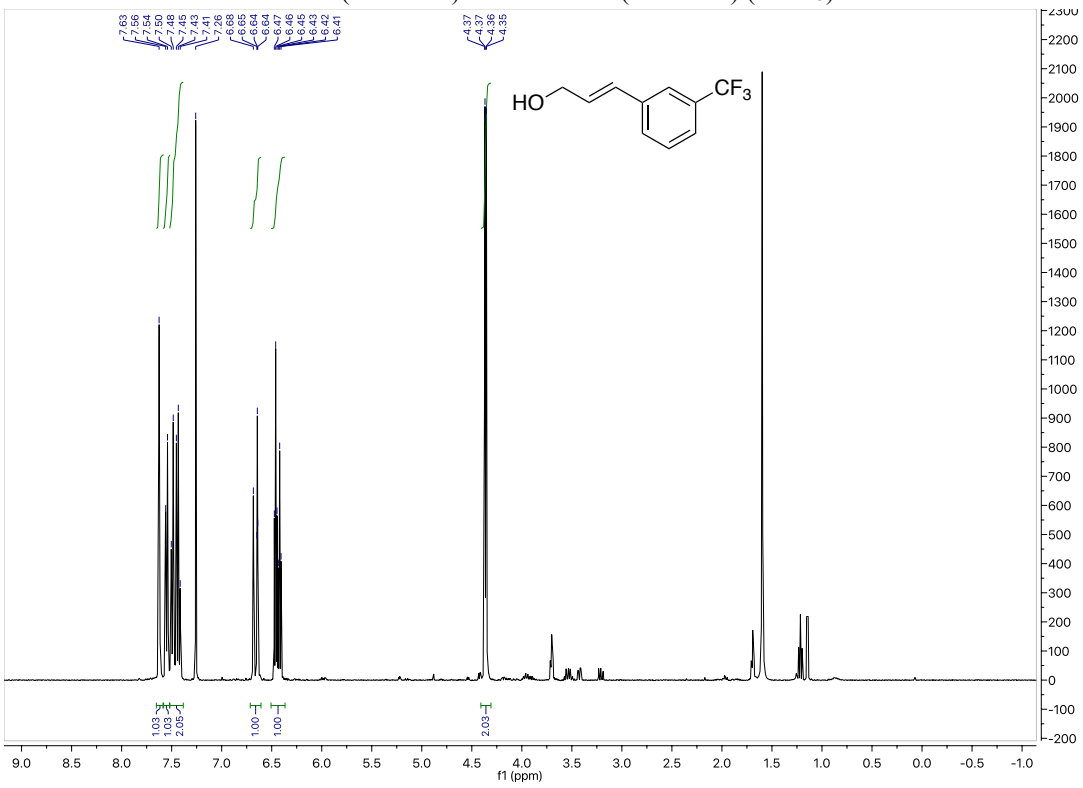


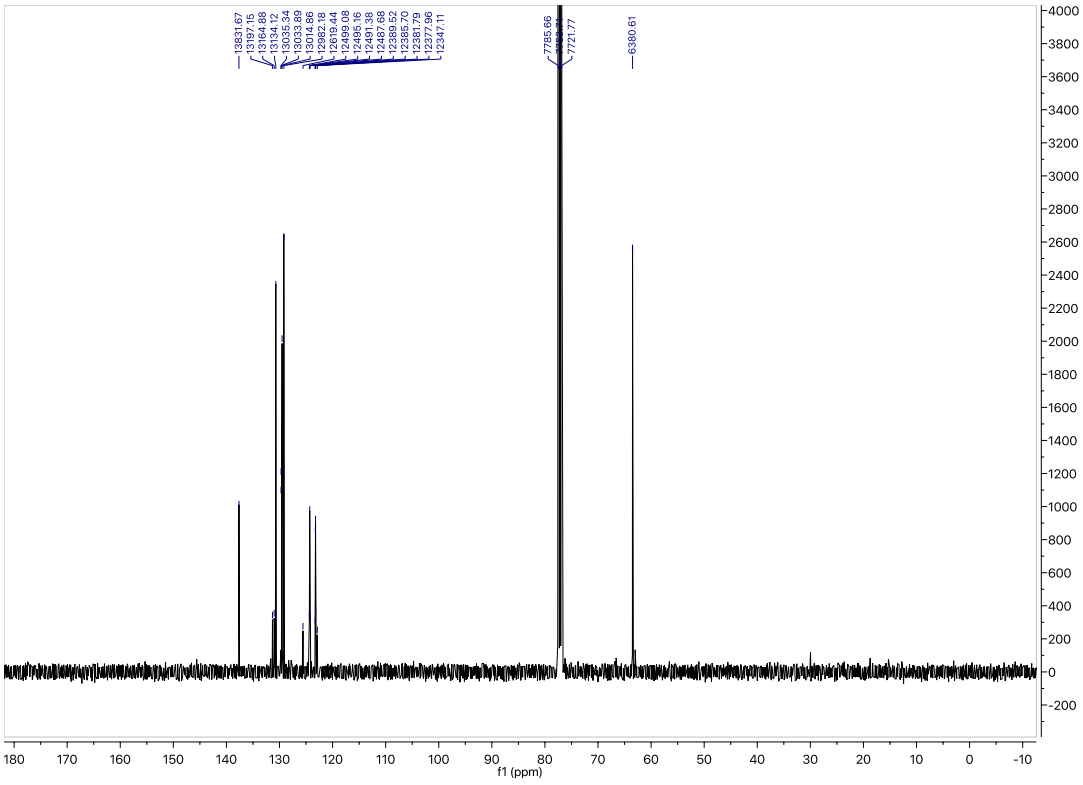
(*E*)-3-(3-Methoxyphenyl)prop-2-en-1-ol (Compound **Va**): ^{1}H-NMR (400 MHz) and ^{13}C{^{1}H}-NMR (100 MHz) ($CDCl_3$).

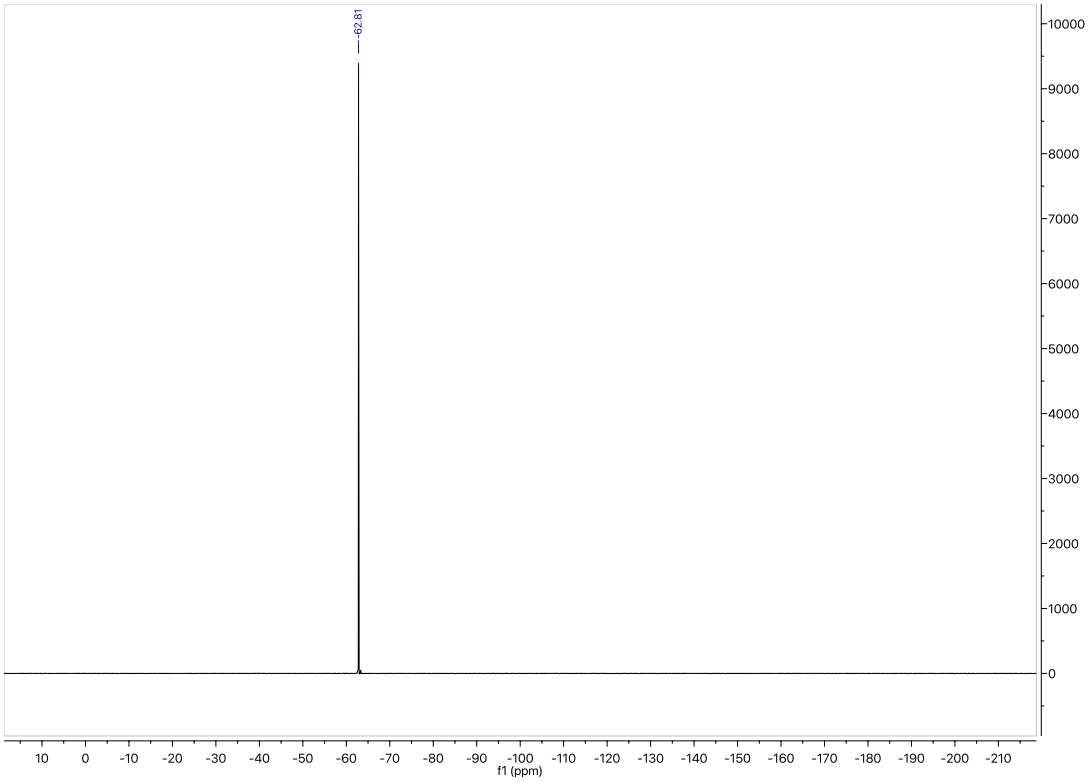




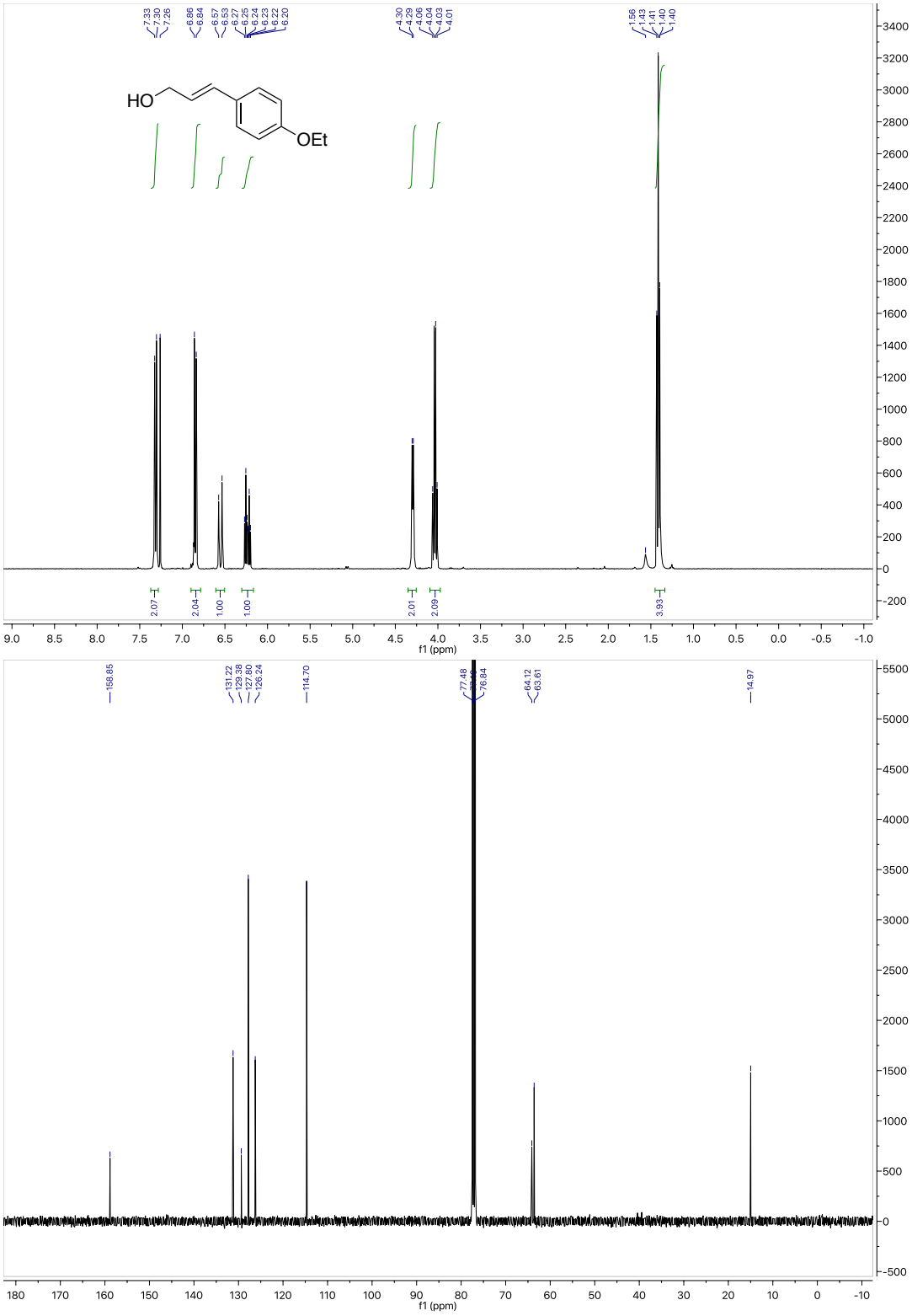
(*E*)-3-(3-(Trifluoromethyl)phenyl)prop-2-en-1-ol (Compound **Vb**): ^{1}H-NMR (400 MHz), ^{13}C{^{1}H}-NMR (100 MHz) and ^{19}F-NMR (376 MHz) ($CDCl_3$).



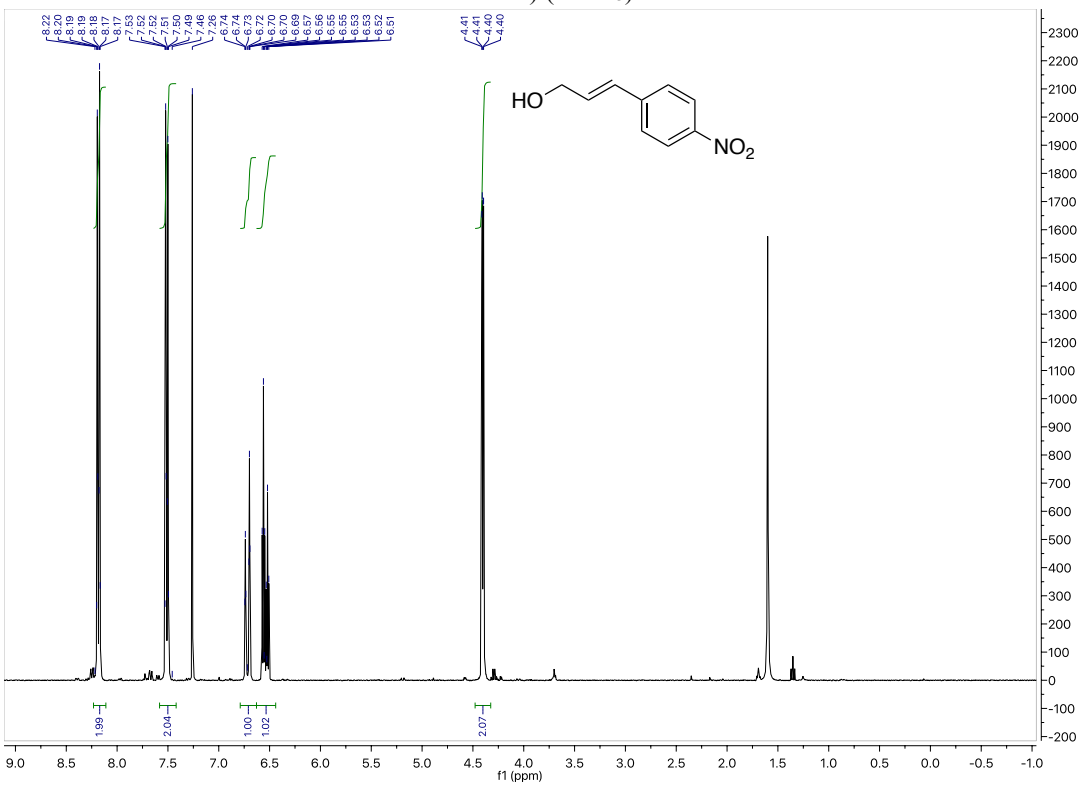


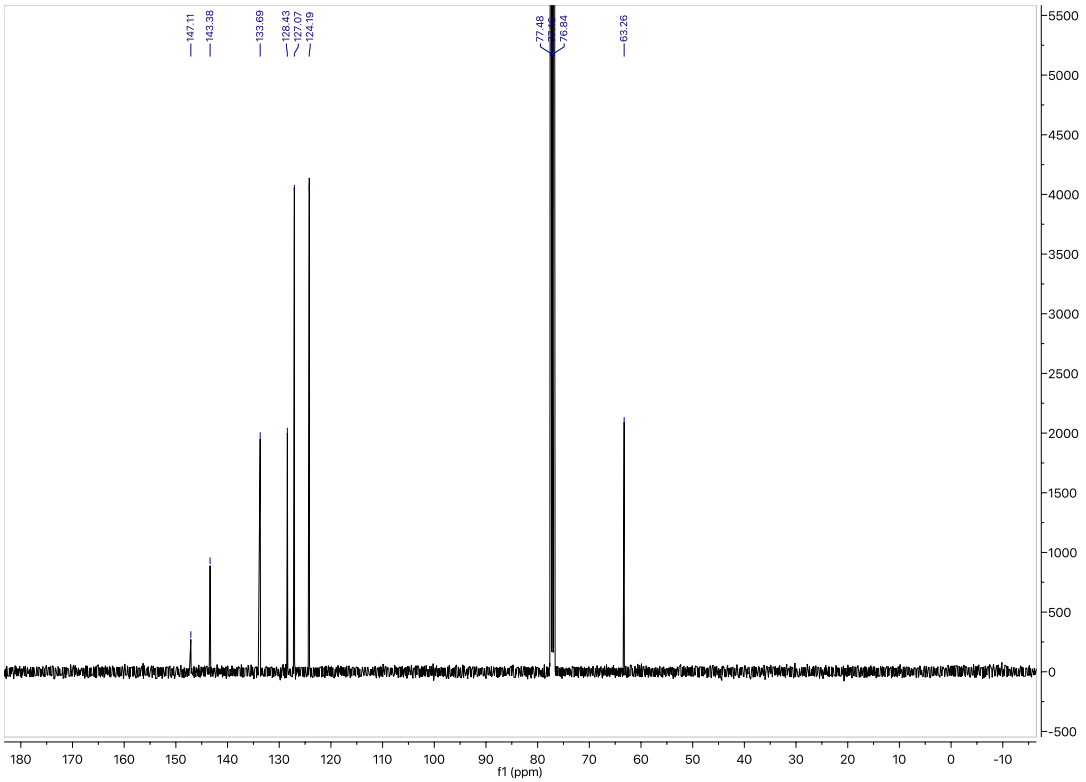

-62.81
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
10 0 -10 -20 -30 -40 -50 -60 -70 -80 -90 -100 -110 -120 -130 -140 -150 -160 -170 -180 -190 -200 -210
f1 (ppm)

(*E*)-3-(4-Ethoxyphenyl)prop-2-en-1-ol (Compound **Vc**): ^{1}H-NMR (400 MHz) and ^{13}C{^{1}H}-NMR (100 MHz) ($CDCl_3$).

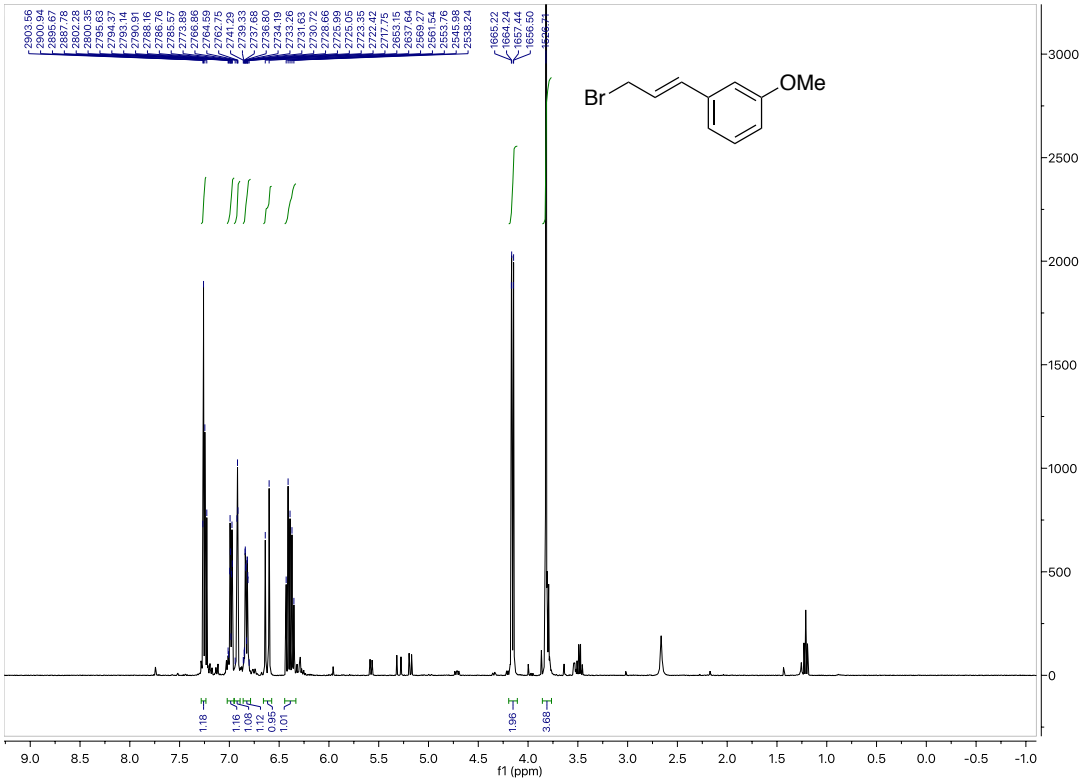


(*E*)-3-(4-Nitrophenyl)prop-2-en-1-ol (Compound **Vd**): ^{1}H-NMR (400 MHz) and ^{13}C{^{1}H}-NMR (100 MHz) ($CDCl_3$).

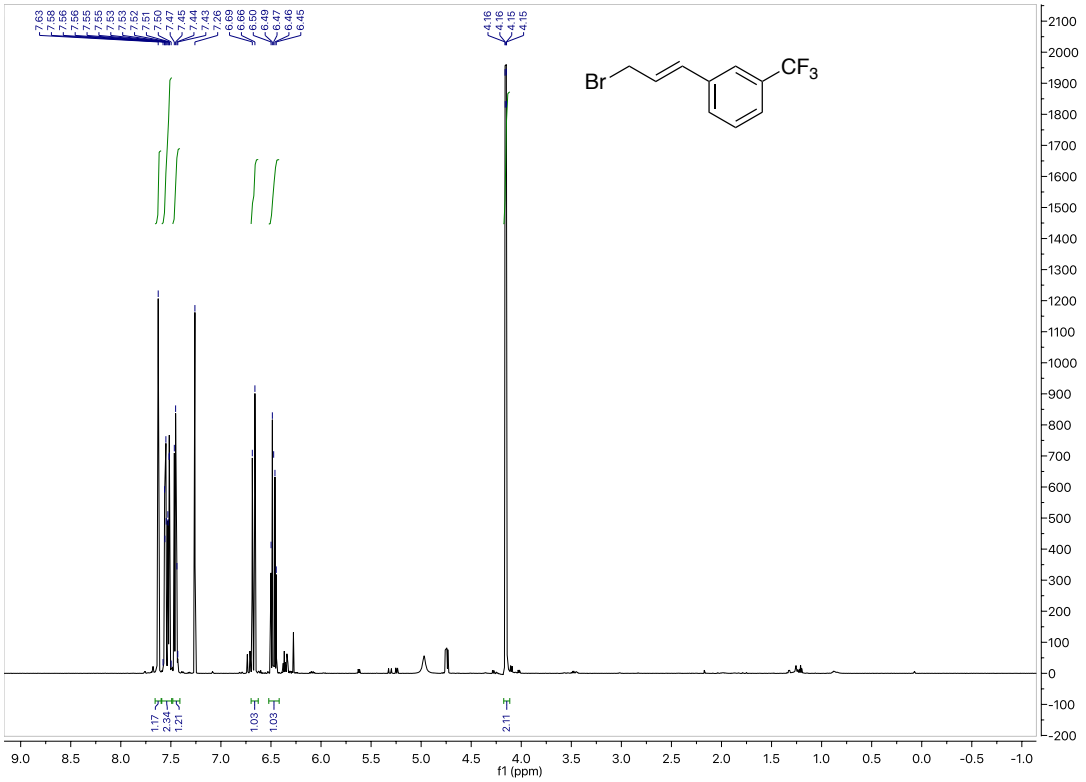




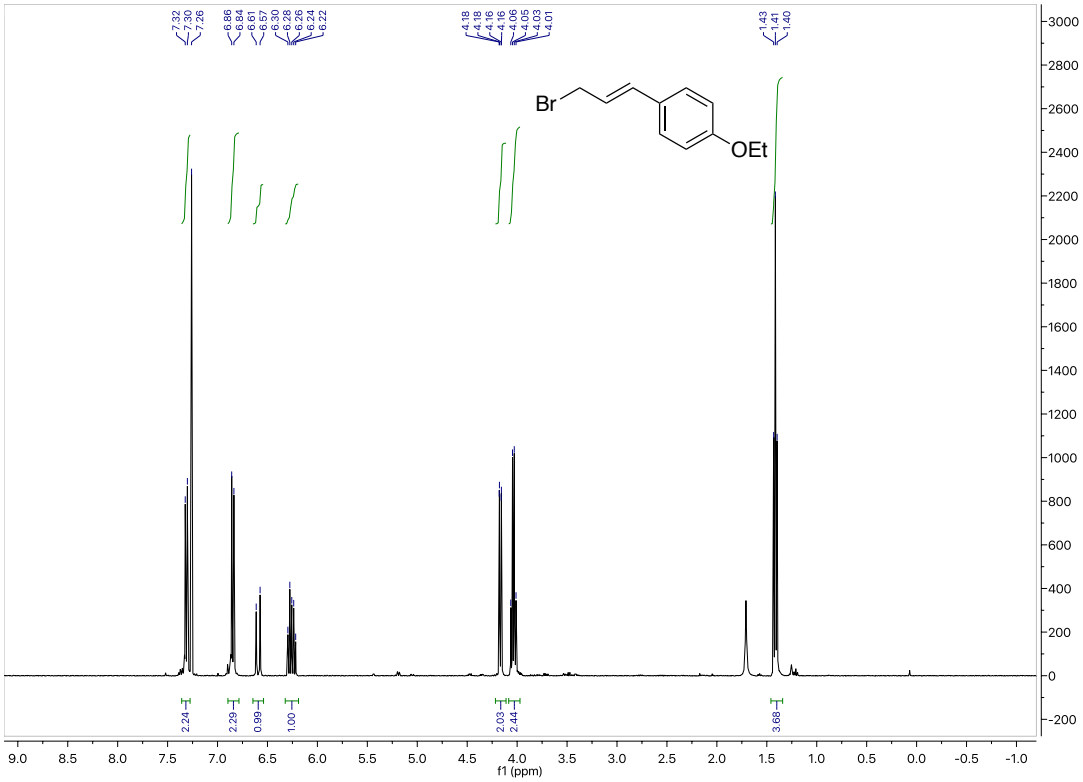
(*E*)-1-(3-Bromoprop-1-en-1-yl)-3-methoxybenzene (Compound **IIa**): ^{1}H-NMR (400 MHz) ($CDCl_3$).



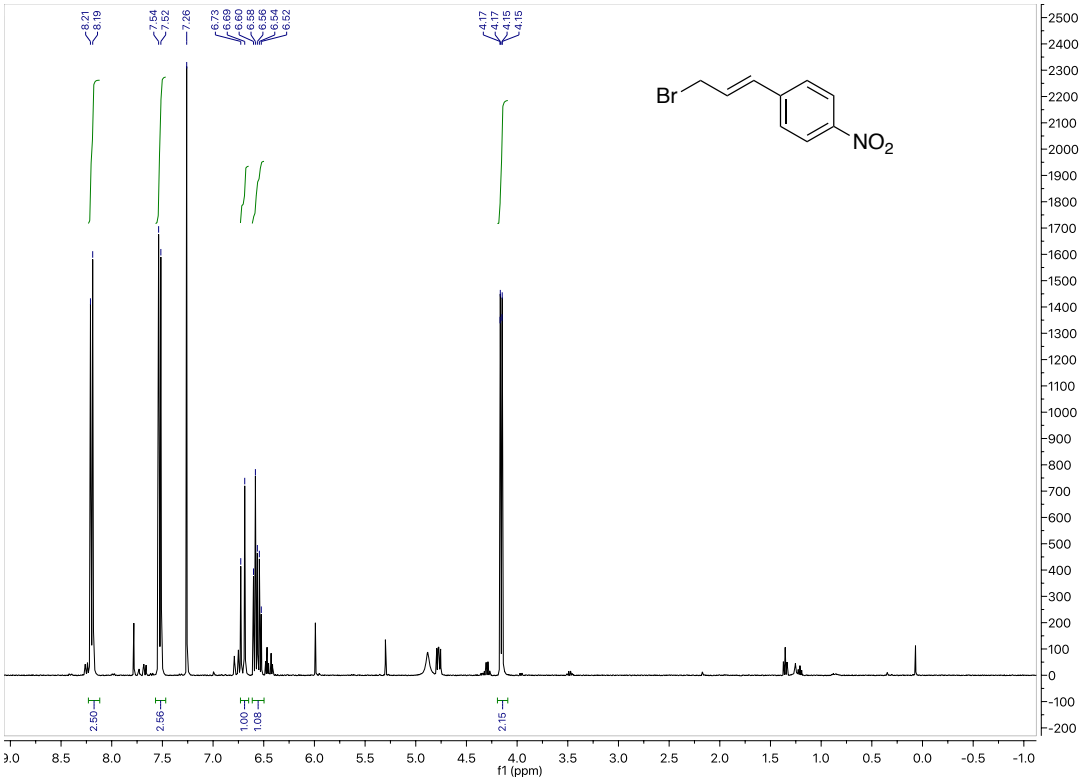
(*E*)-1-(3-Bromoprop-1-en-1-yl)-3-(trifluoromethyl)benzene (Compound **IIb**): ^{1}H-NMR (600 MHz) ($CDCl_3$).

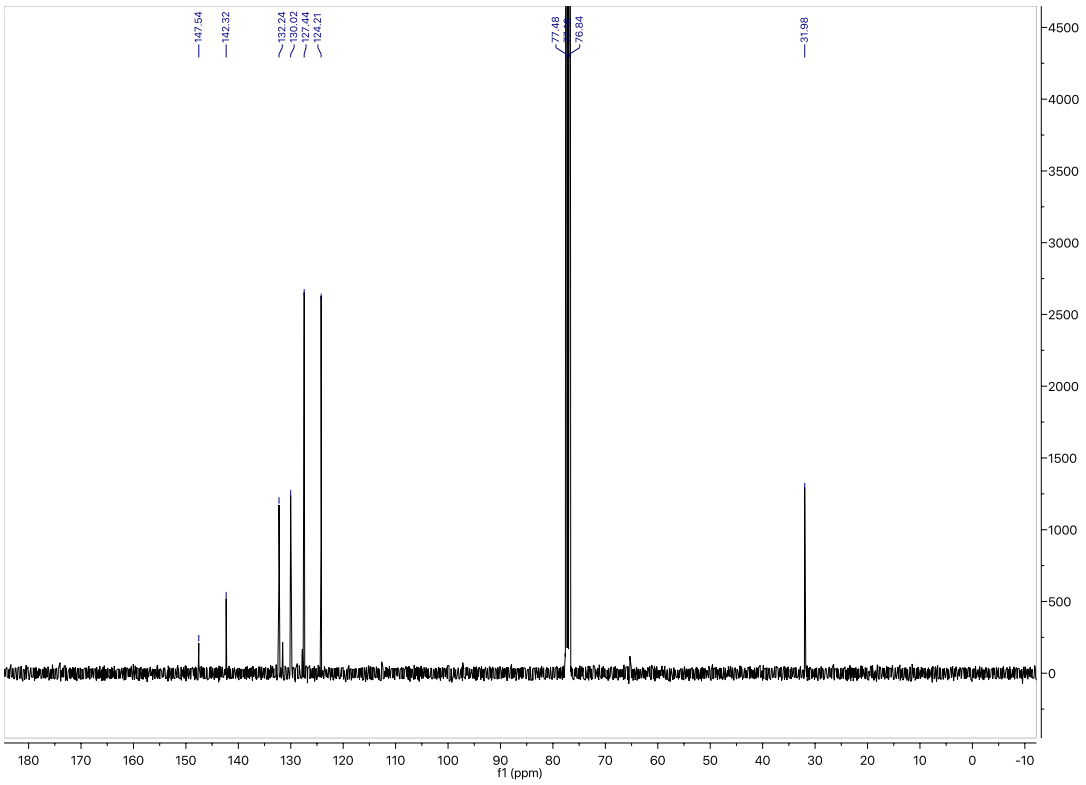


(*E*)-1-(3-Bromoprop-1-en-1-yl)-4-ethoxybenzene (Compound **IIc**): ^{1}H-NMR (400 MHz) ($CDCl_3$)

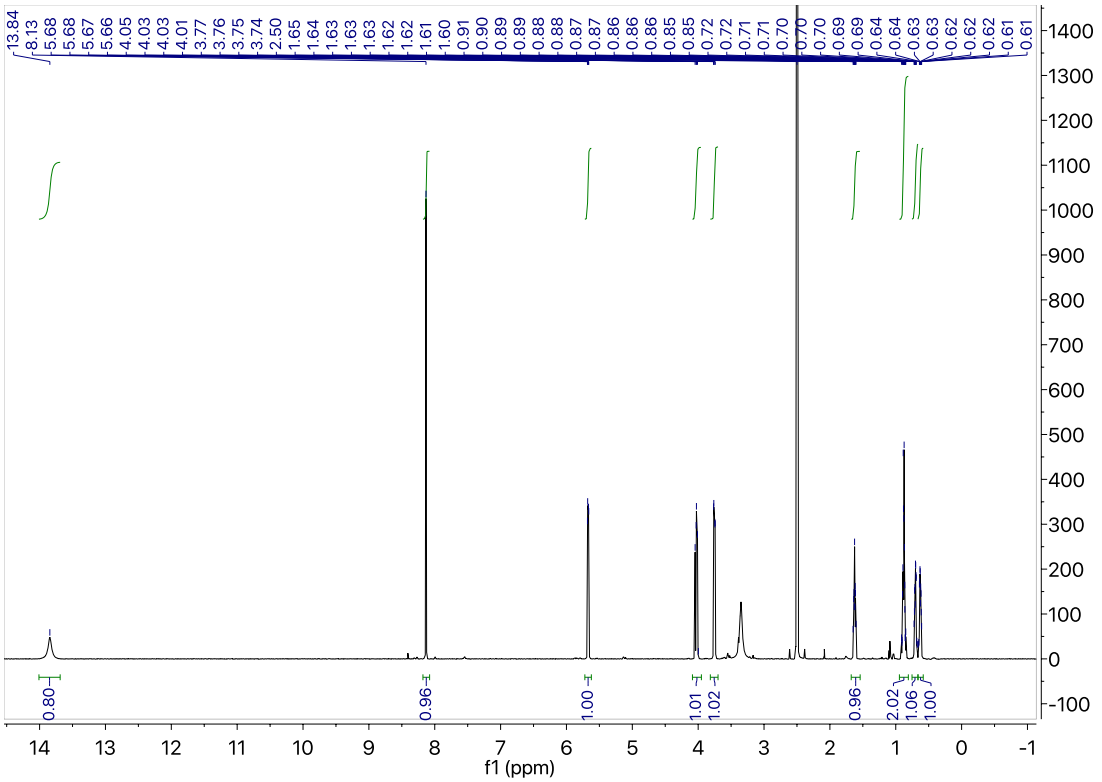


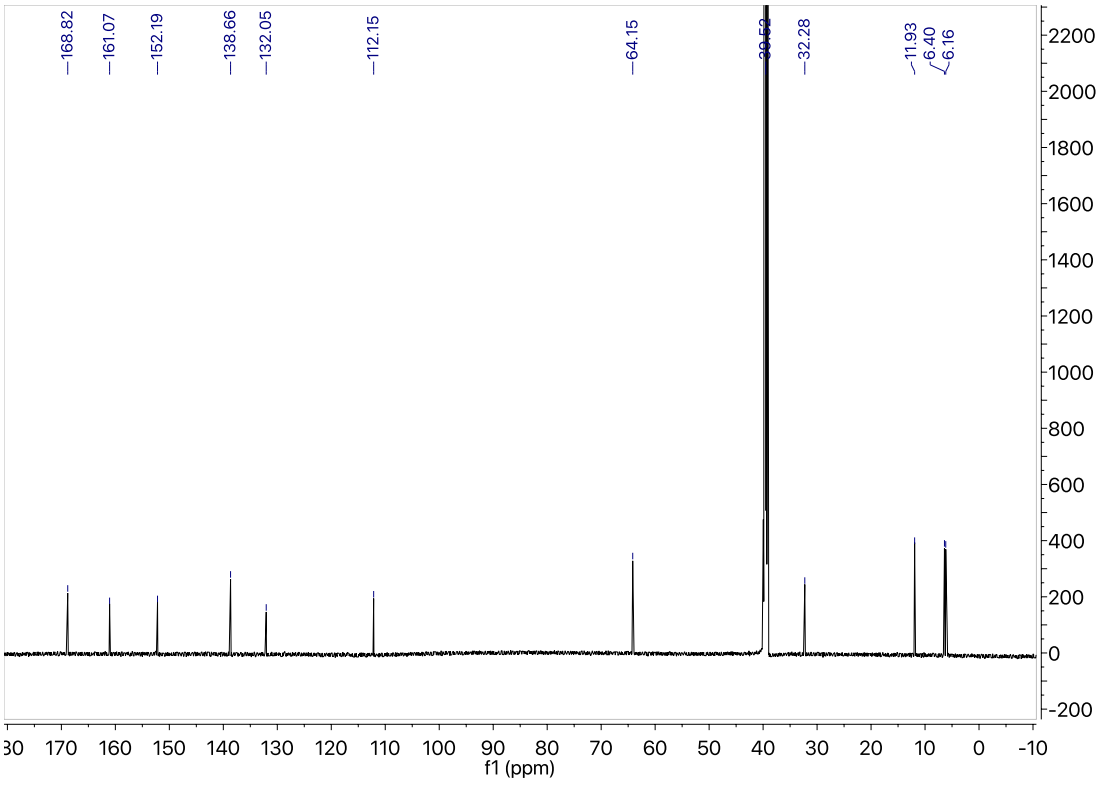
(*E*)-1-(3-Bromoprop-1-en-1-yl)-4-nitrobenzene (Compound **IId**): ^{1}H-NMR (400 MHz) and ^{13}C{^{1}H}-NMR (100 MHz) ($CDCl_3$).



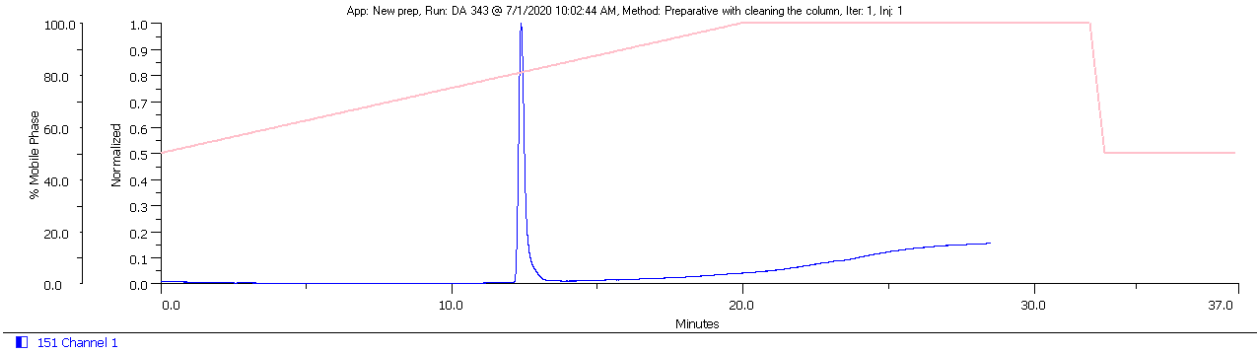


(R)-8-Cyclopropyl-6-nitro-5-oxo-2,3-dihydro-5H-thiazolo[3,2-a]pyridine-3-carboxylic acid (Compound **VII**): ^{1}H-NMR (600 MHz) and ^{13}C{^{1}H}-NMR (151 MHz) [$(CD_3)_2SO$].



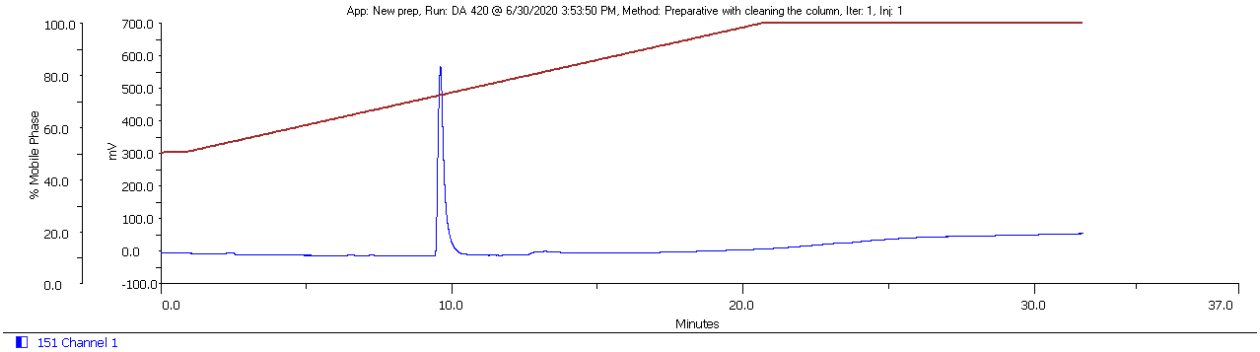


HPLC data for compound **15b**



Ret. Time(min)	Area	Area %
2.479	17734.17	0.17395
2.772	41760	0.409613
3.591	1185	0.011623
4.303	411.667	0.004038
5.809	630	0.00618
7.403	9000	0.088279
8.355	2015	0.019765
9.049	2810.417	0.027567
11.844	2088.333	0.020484
12.387	10086278	98.93365
15.382	9070.833	0.088973
16.603	22009.17	0.215882

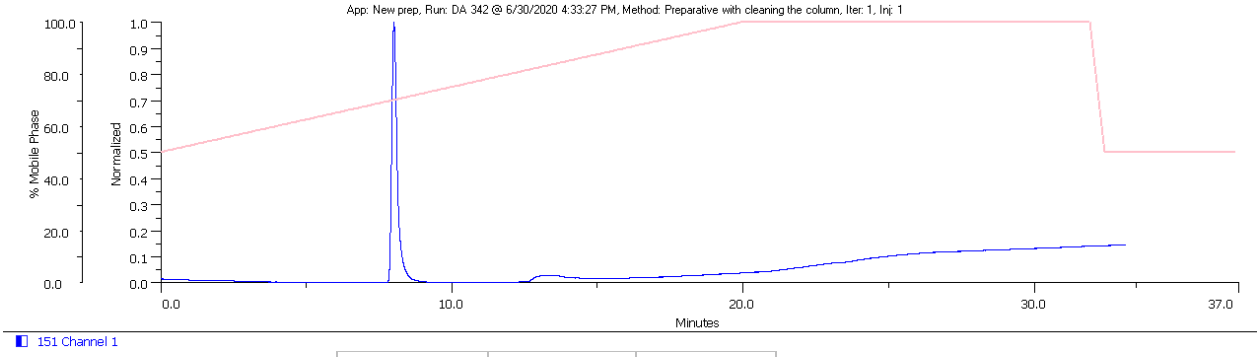
HPLC data for compound **15e**



Ret.	Area	Area %

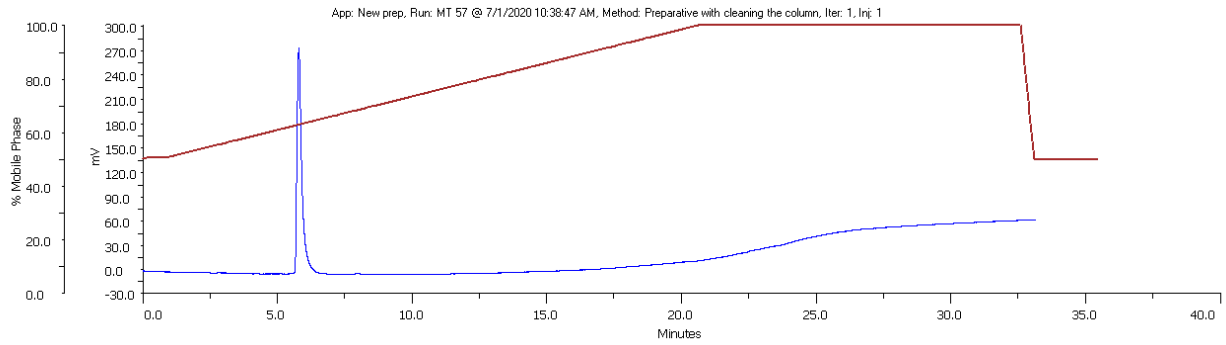
Time		
2.289	9693.75	0.065393
2.523	6031.667	0.040689
2.918	36418.333	0.245673
3.913	6071.667	0.040959
5.839	2346.667	0.01583
6.194	3534.167	0.023841
6.534	34844.167	0.235054
7.256	25950	0.175055
8.151	4461.667	0.030098
8.793	6309.167	0.042561
9.613	14382513.75	97.02232
10.896	793.75	0.005355

HPLC data for compound **16a**



Ret. Time(min)	Area	Area %
2.366	2704.167	0.026024
2.499	4878.333	0.046947
2.788	38218.33	0.367795
4.871	1071.667	0.010313
7.154	2206.667	0.021236
7.403	2003.333	0.019279
8.017	10092832	97.12848
10.476	2097.917	0.020189
11.206	1867.083	0.017968
12.901	161919.6	1.558235
13.627	31625	0.304344
14.32	691.667	0.006656
15.947	2652.5	0.025526
13.363	46449.58	0.447008

HPLC data for compound **16b**



Ret. Time	Area	Area %
0.588	2163.333	0.037186
2.798	28230.42	0.485255
5.802	5762691	99.05545
7.138	2863.333	0.049218
7.971	21207.92	0.364545
13.909	485.417	0.008344

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