Supporting Information for

Quinazoline-2-carboxamides as Selective PET Radiotracers for Matrix Metalloproteinase-13 Imaging in Atherosclerosis

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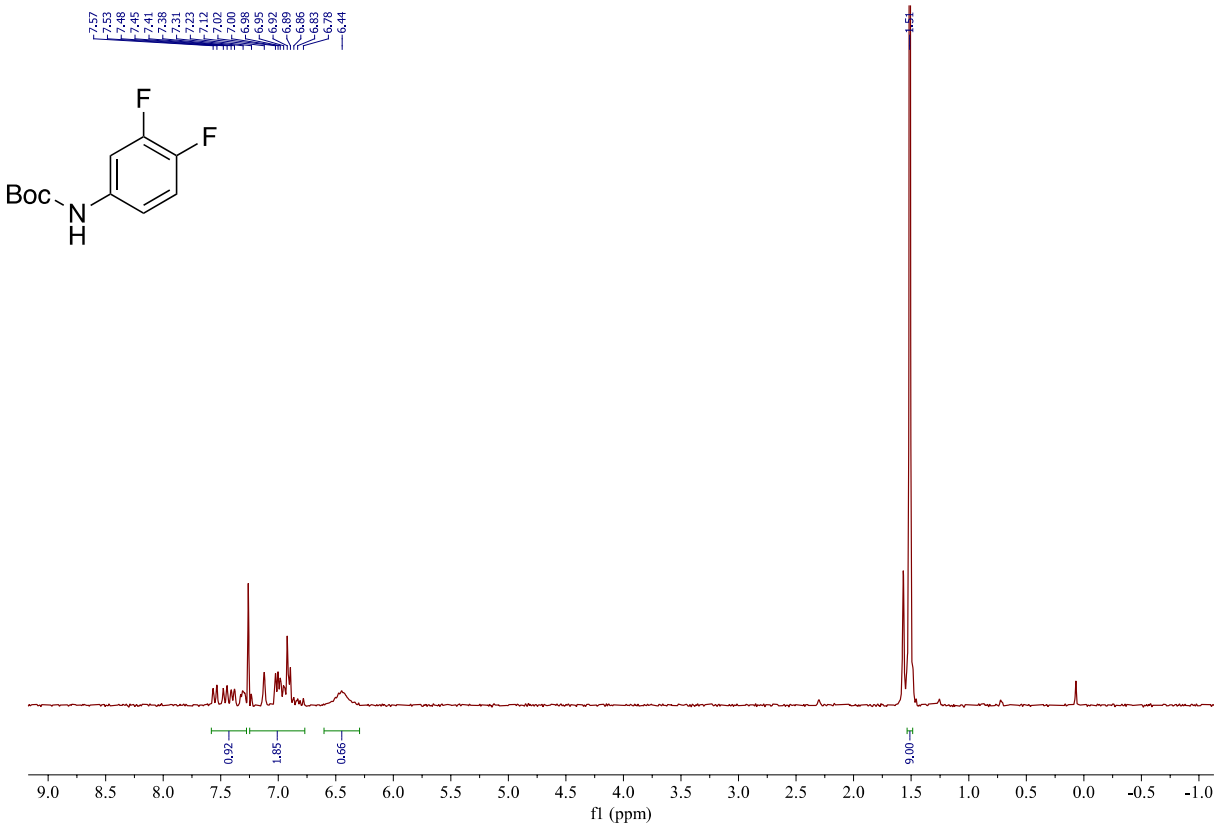
Contents

^{1}H NMR and ^{13}C NMR spectra for all synthesized compounds (1–10)

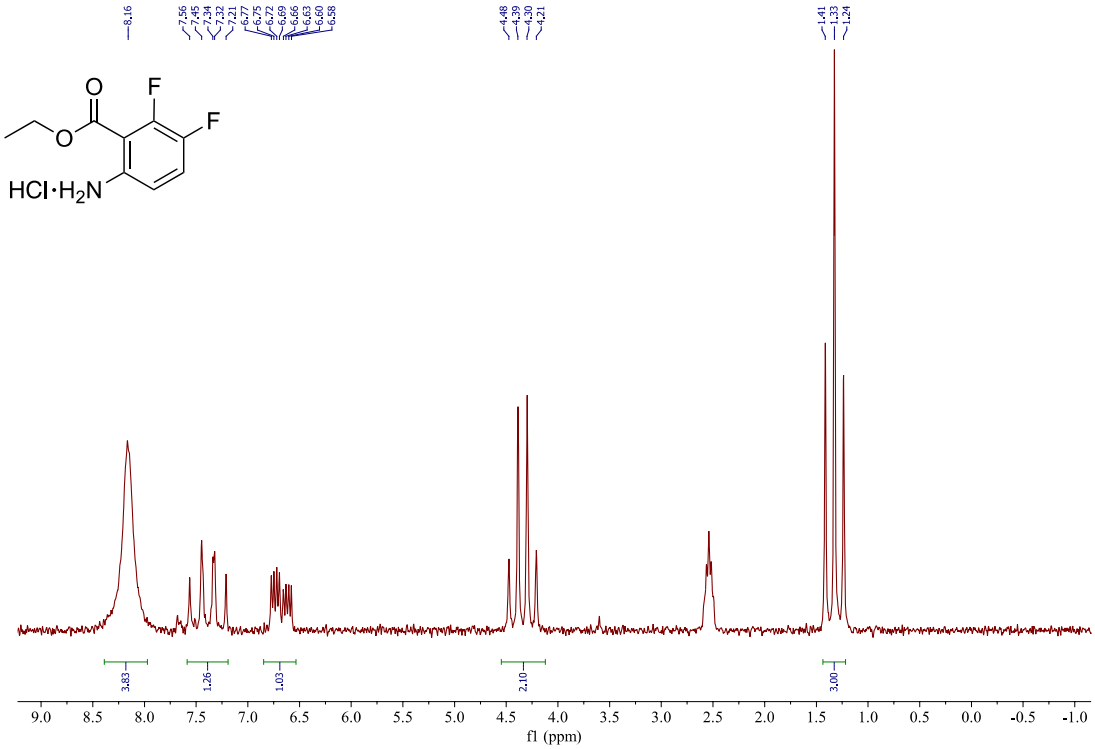
Compound 1

^{1}H-NMR (80 MHz, $CDCl_3$)



Compound 2

^{1}H-NMR (80 MHz, $(CD_3)_2SO$)



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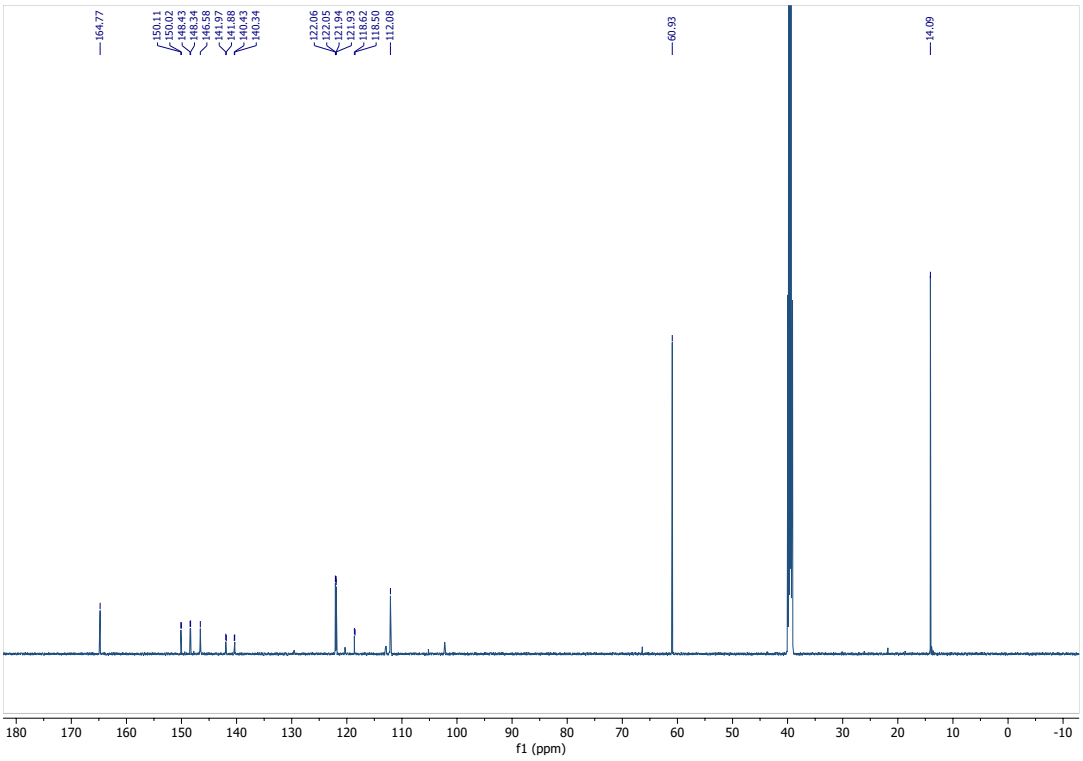

Compound 3

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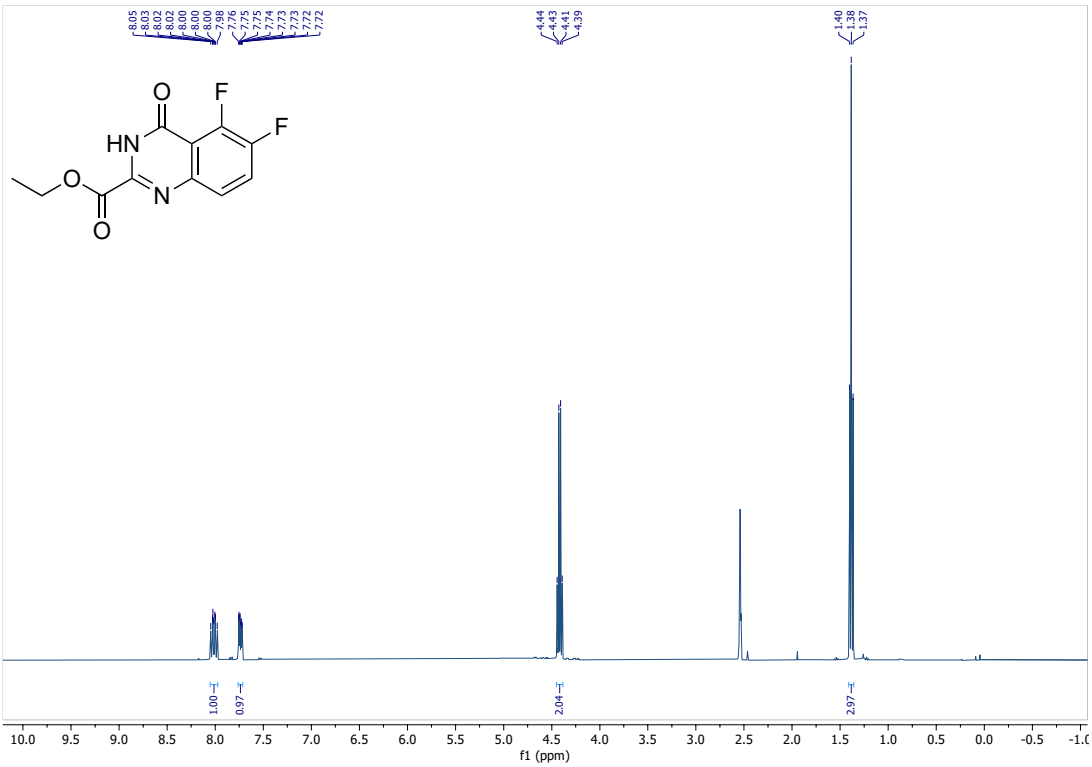

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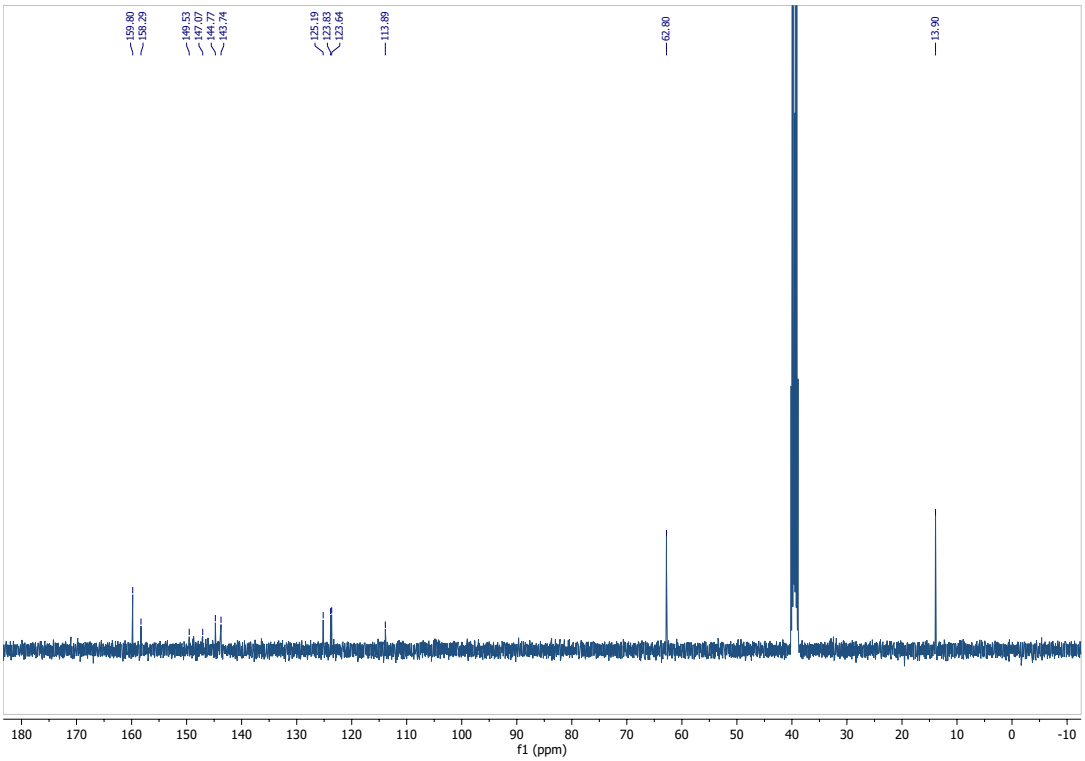
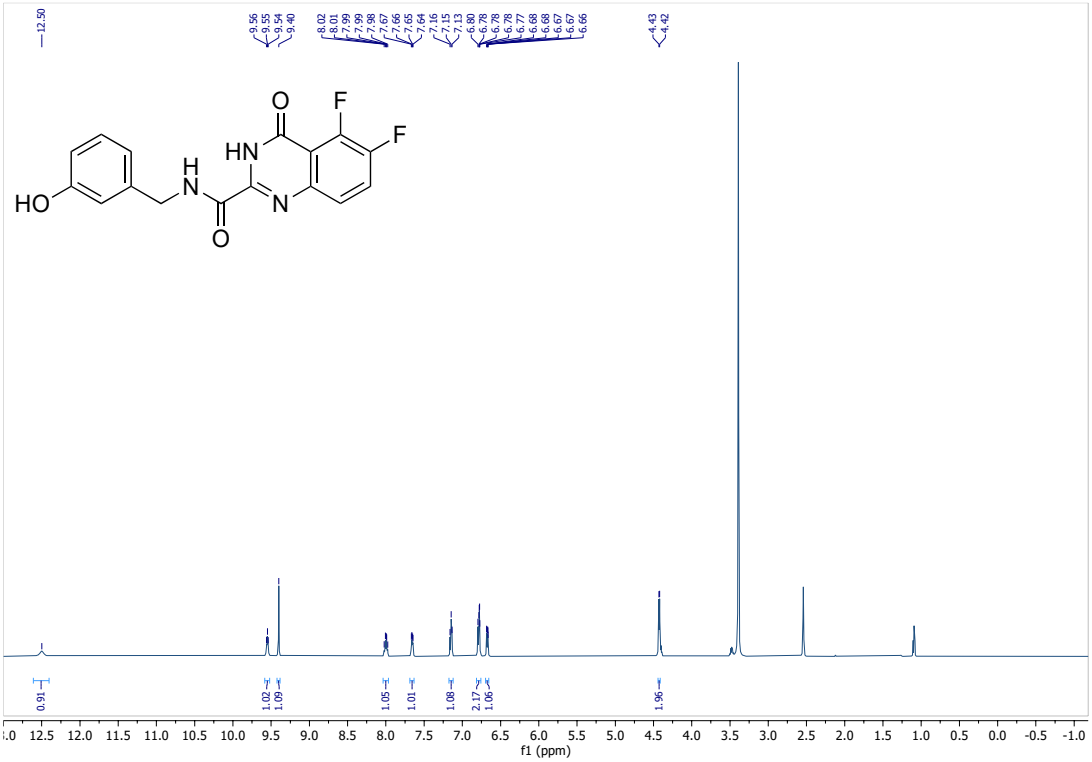


Compound 4a

^{1}H-NMR (600 MHz, $(CD_3)_2SO$)



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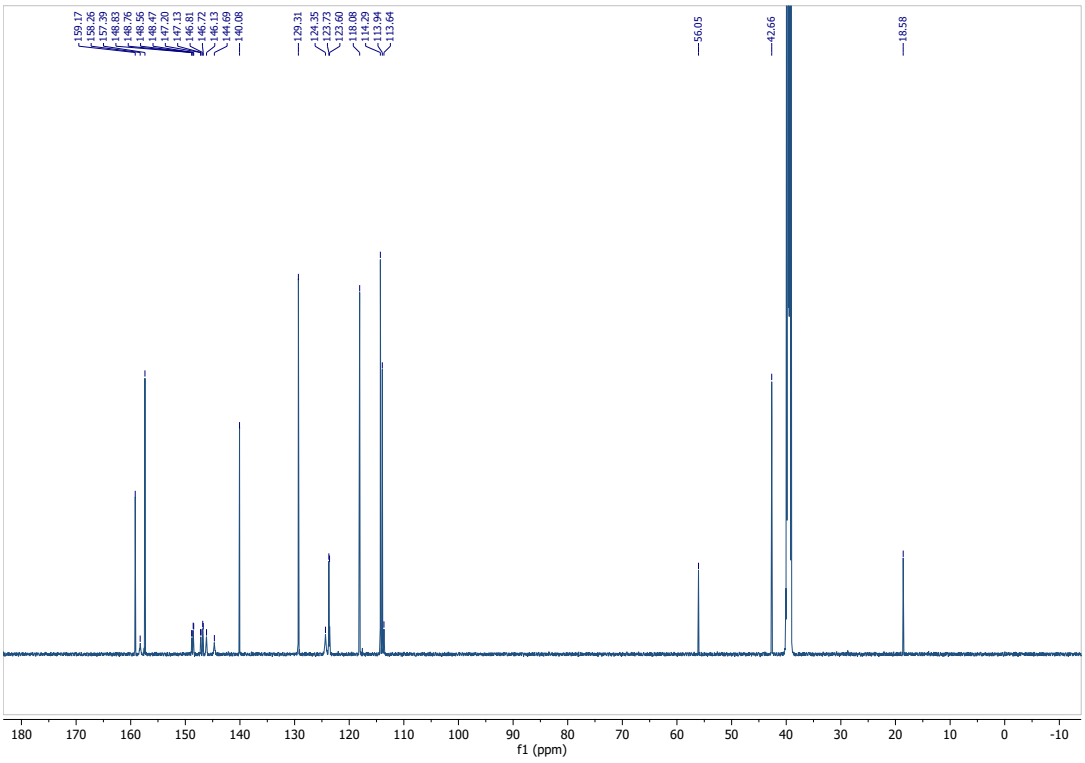
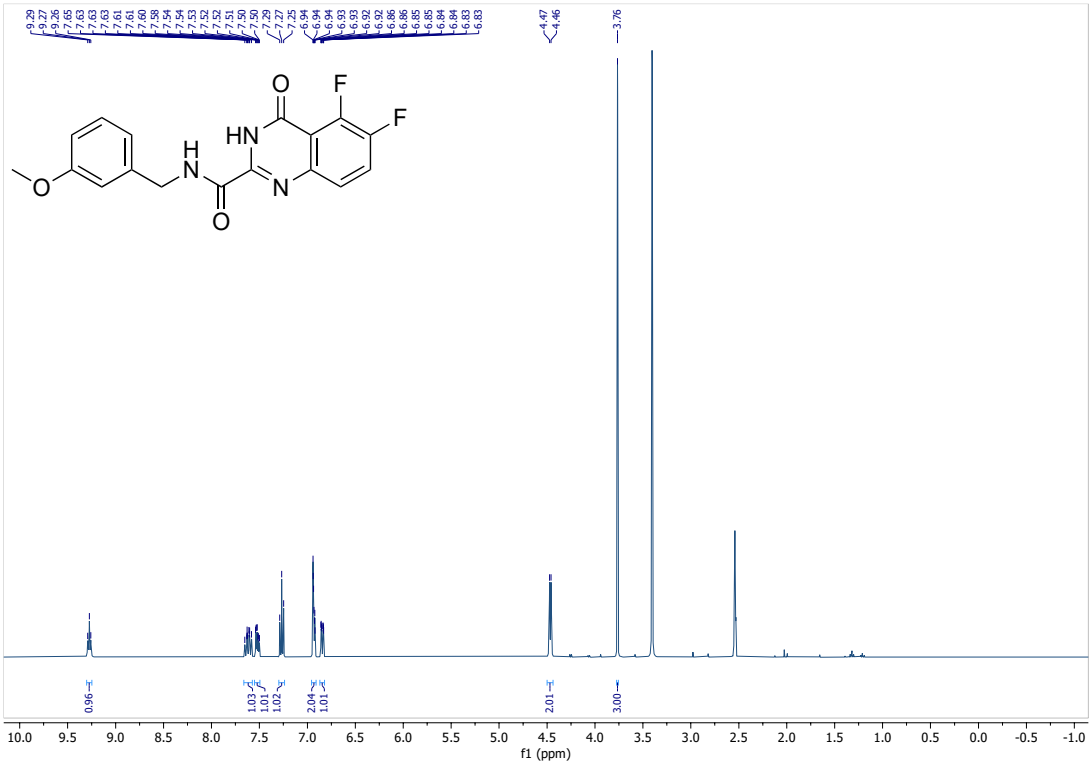


Compound 4b

^{1}H-NMR (600 MHz, $(CD_3)_2SO$)



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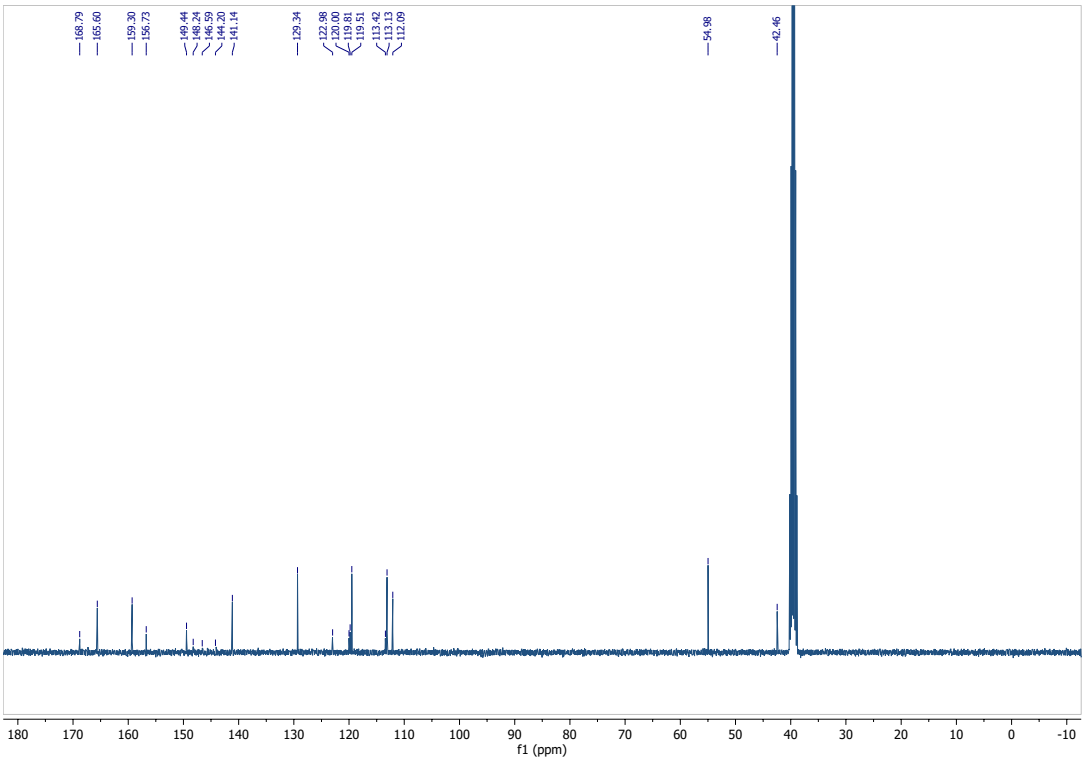

Compound 4c

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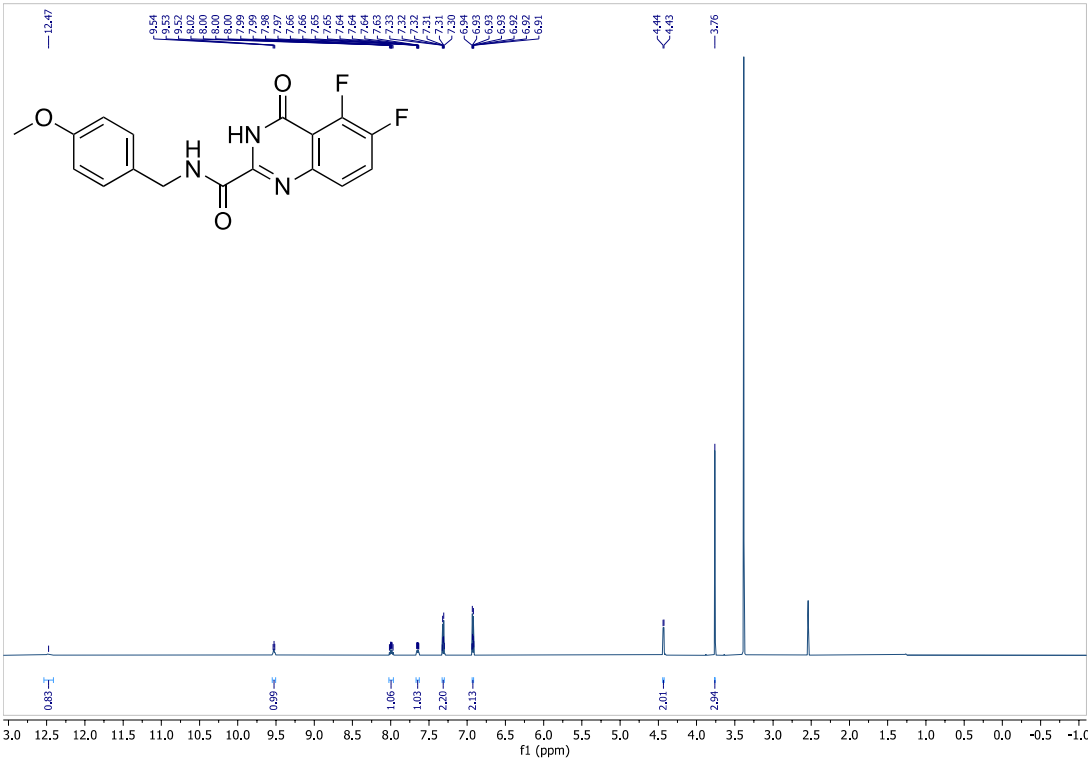

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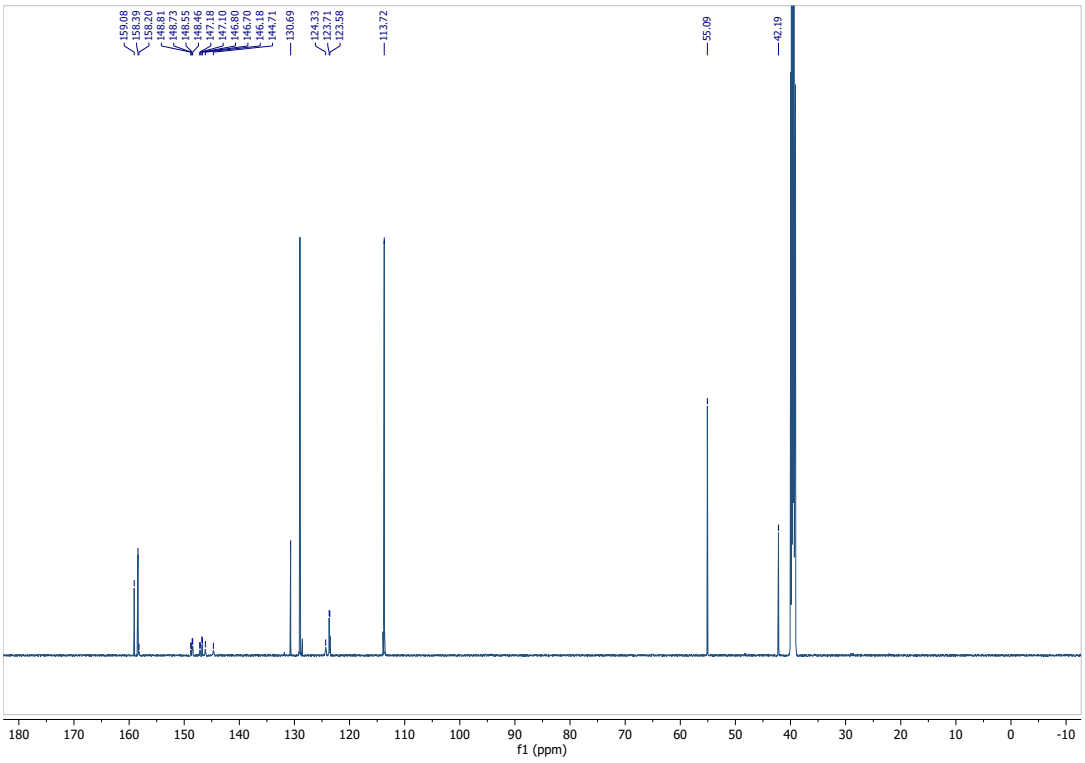

Compound 4d

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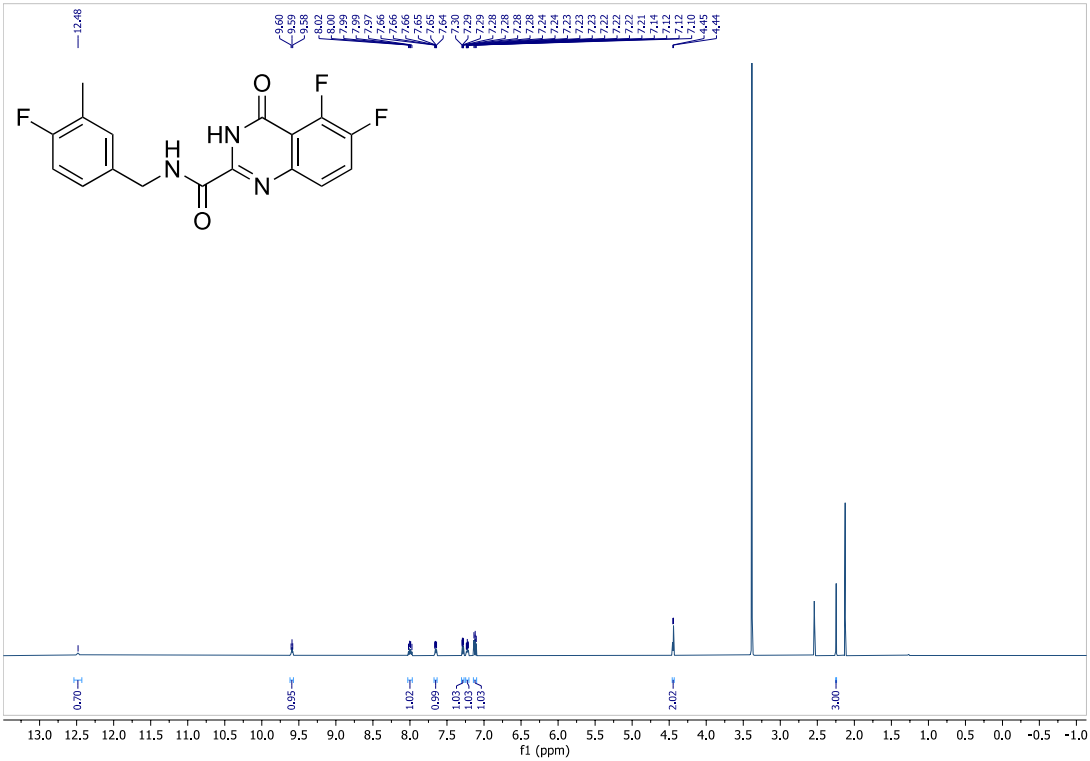

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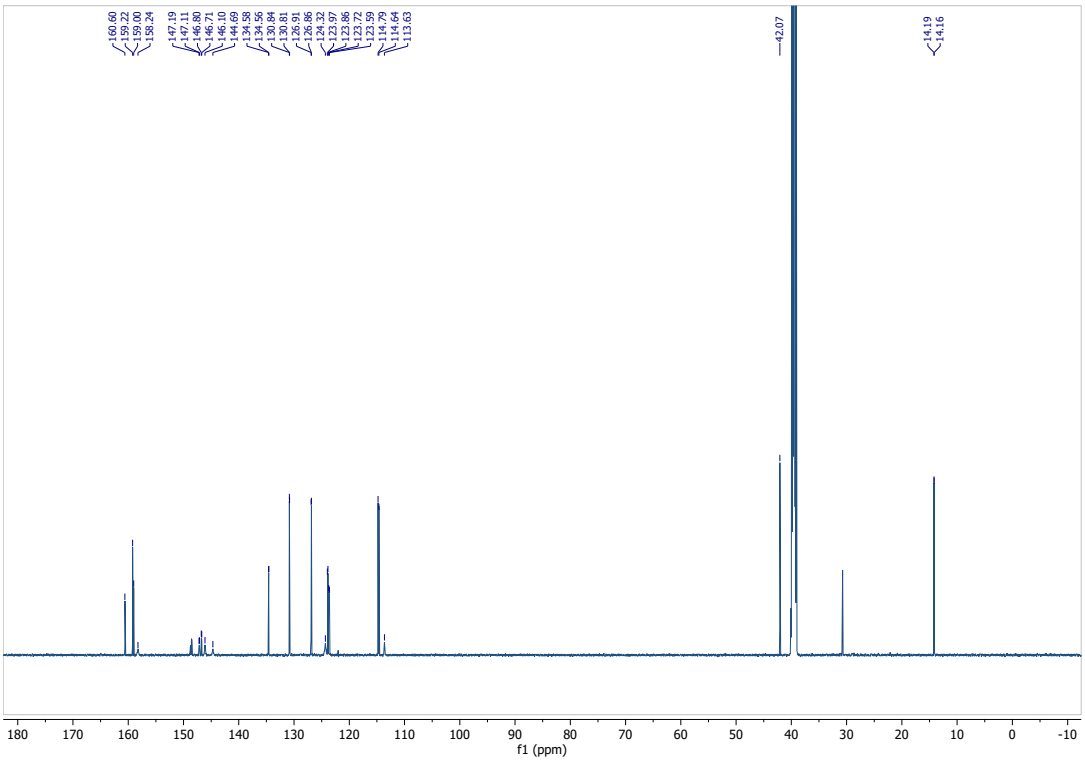

Compound 5a

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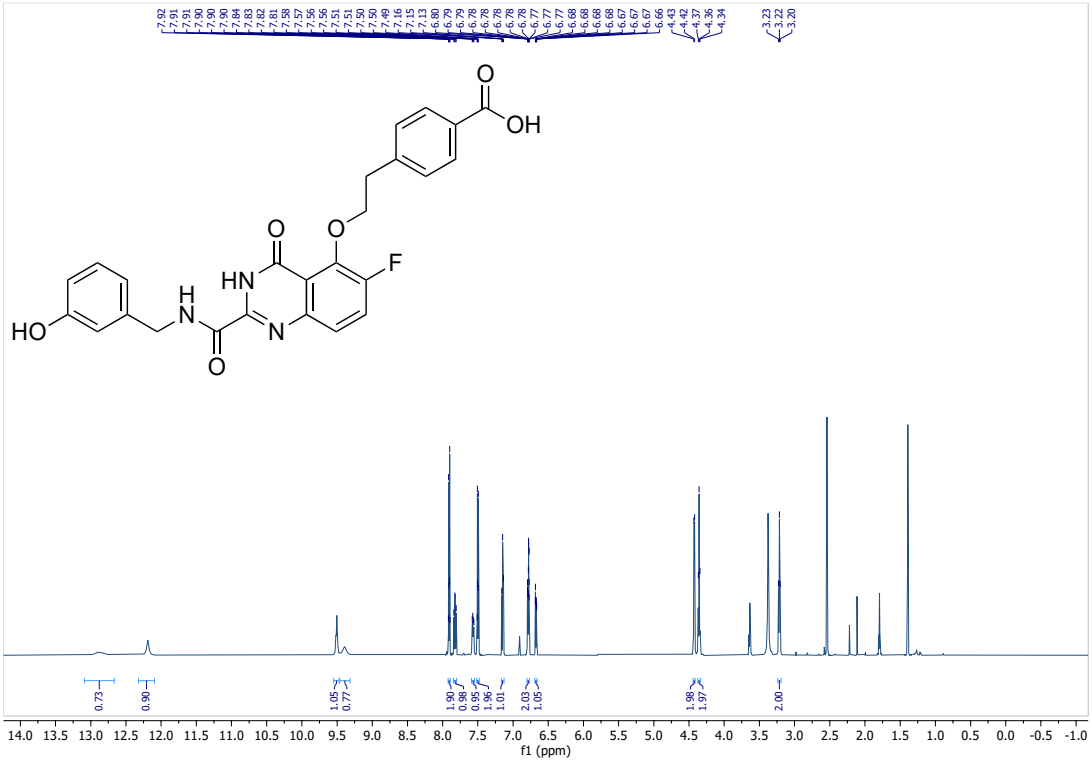

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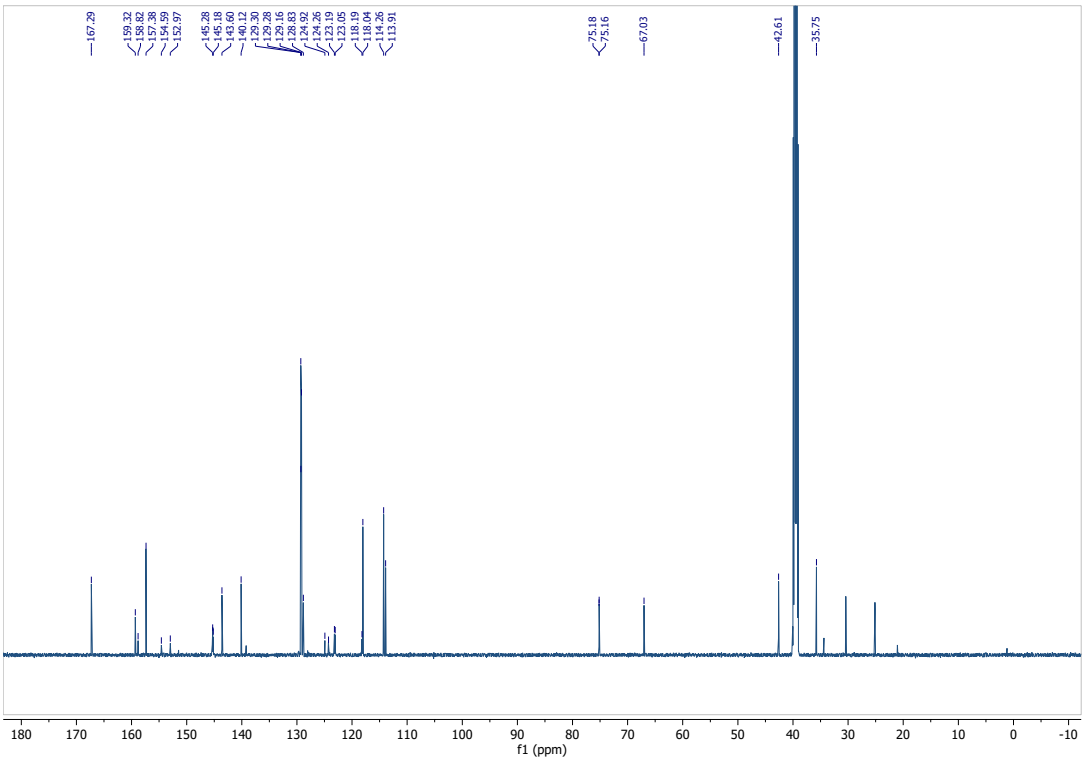

Compound 5b

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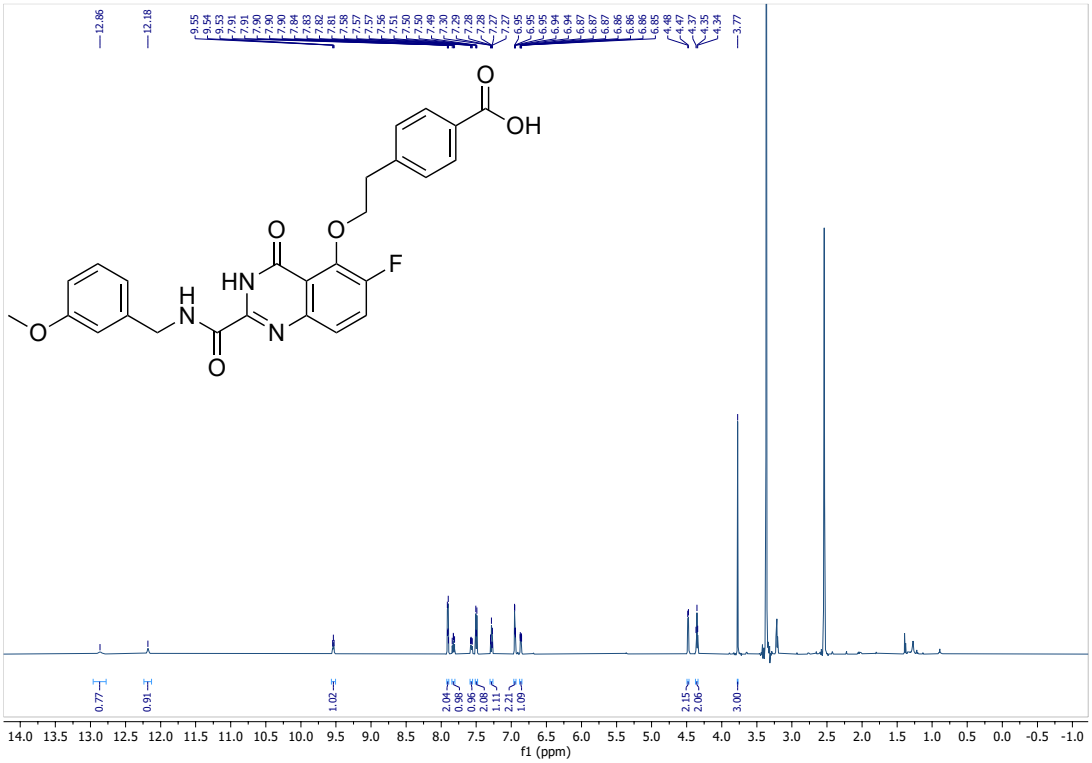

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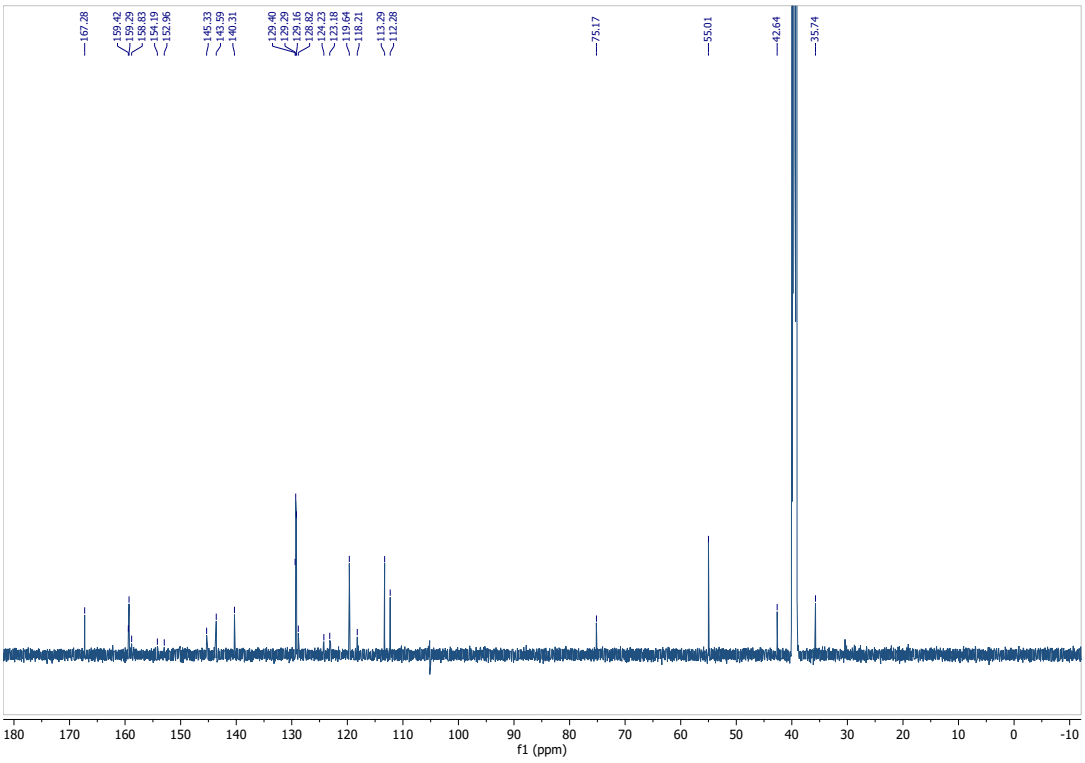
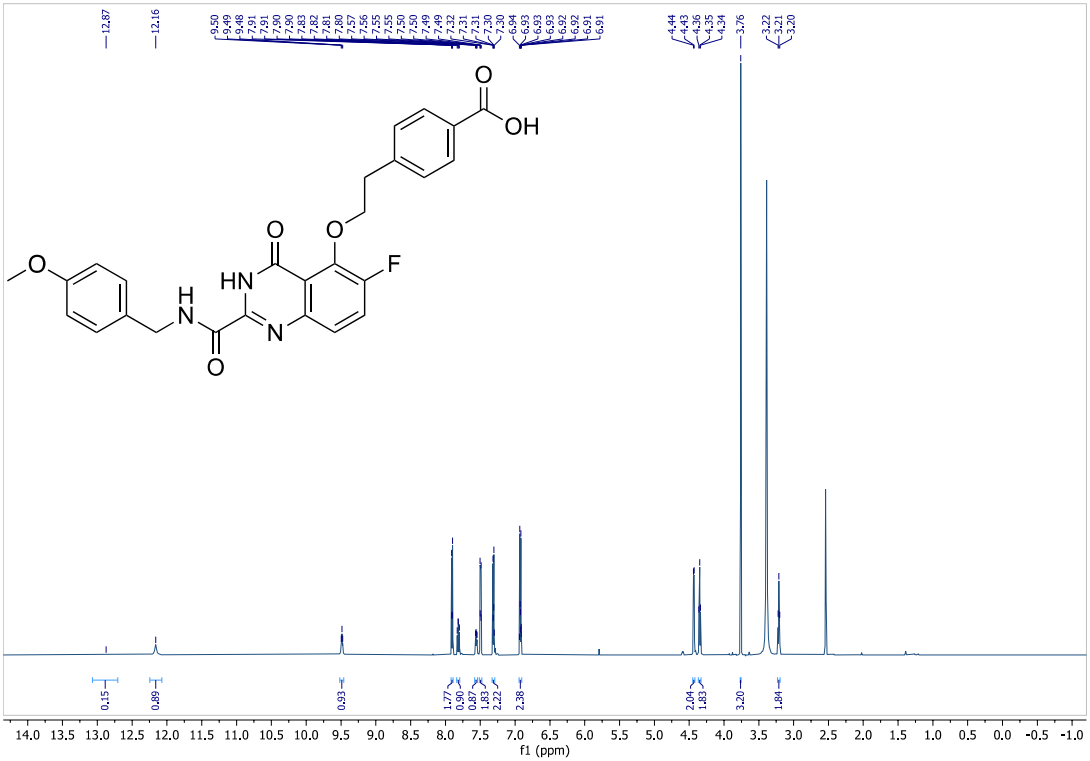


Compound 5c

^{1}H-NMR (600 MHz, $(CD_3)_2SO$)



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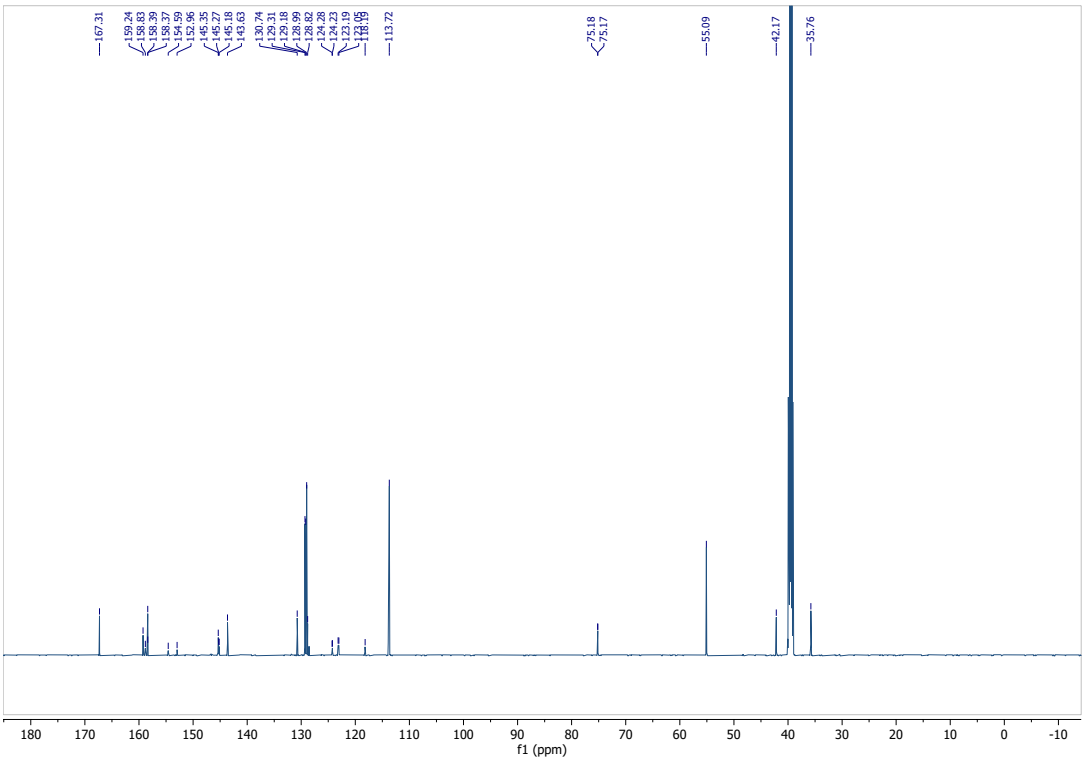

Compound 5d

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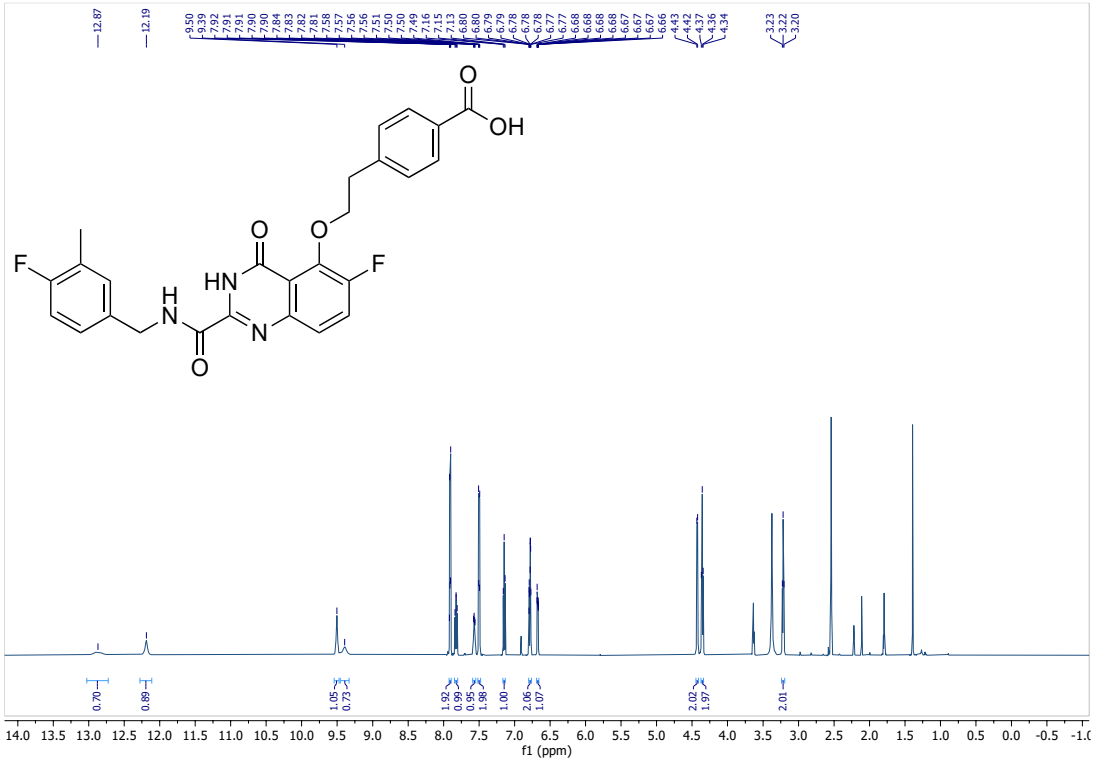

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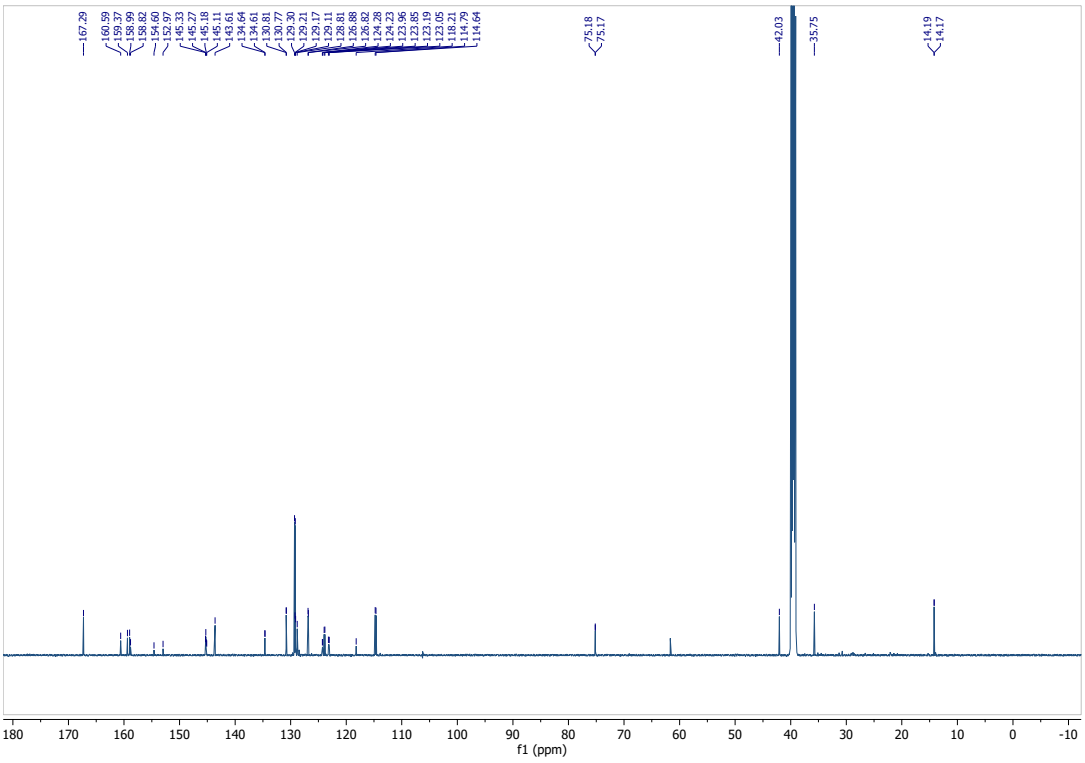
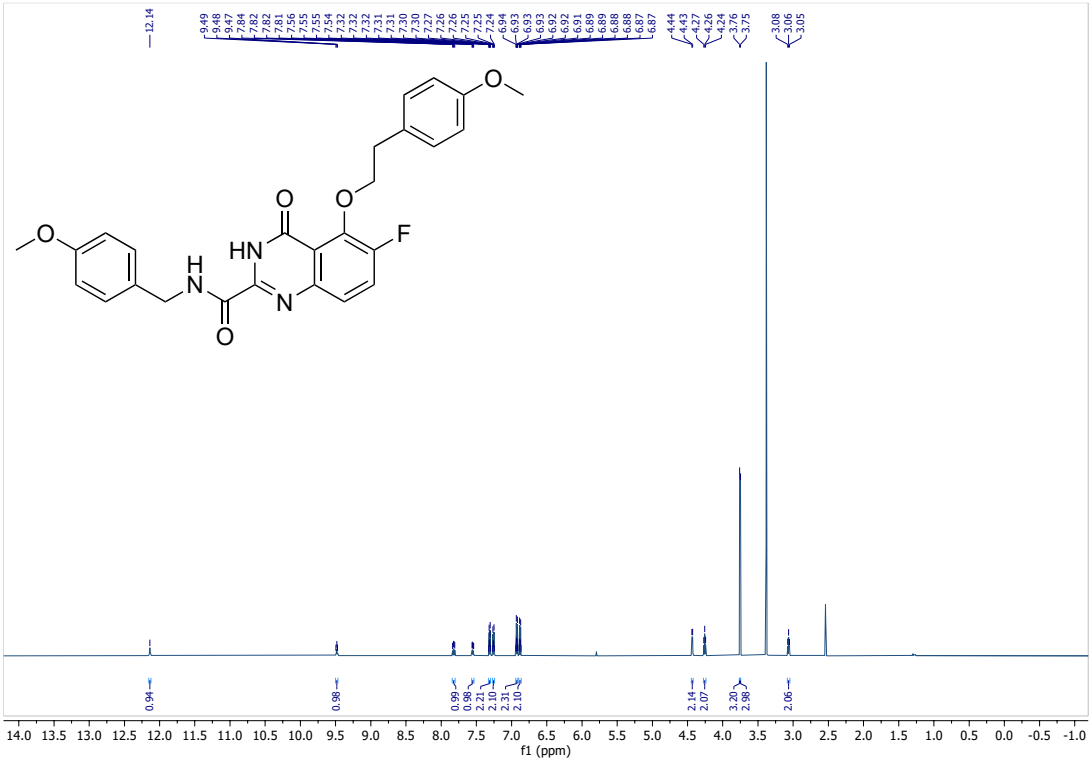


Compound 5e

^{1}H-NMR (600 MHz, $(CD_3)_2SO$)



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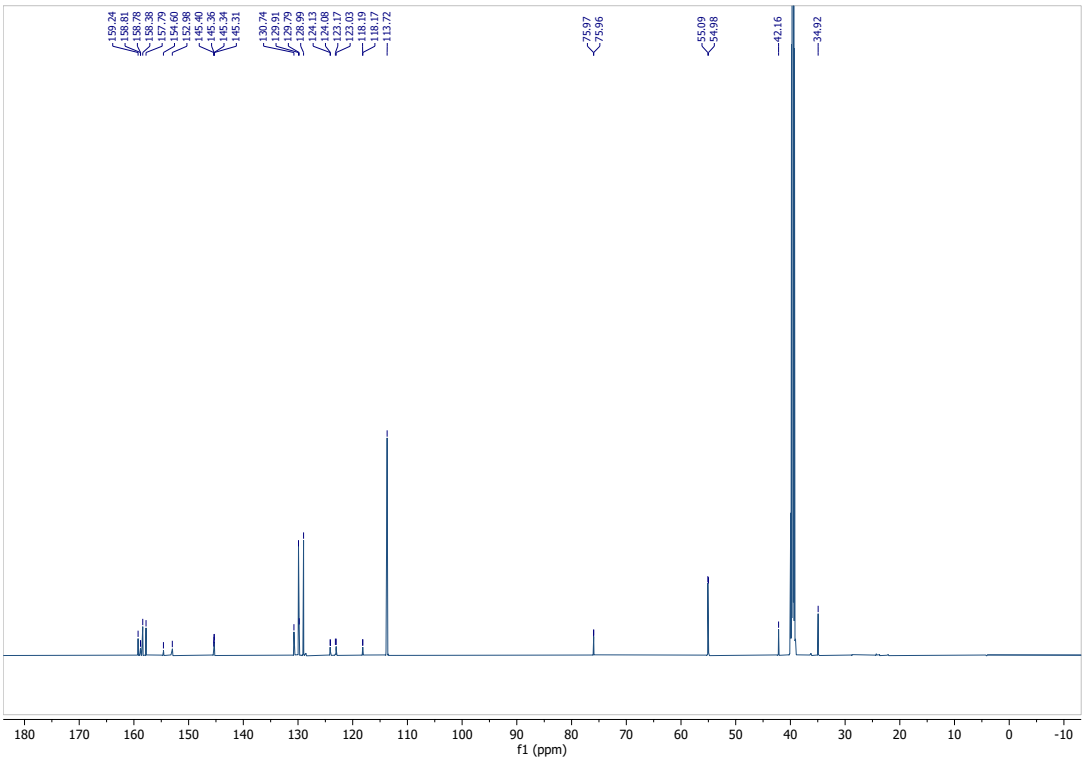
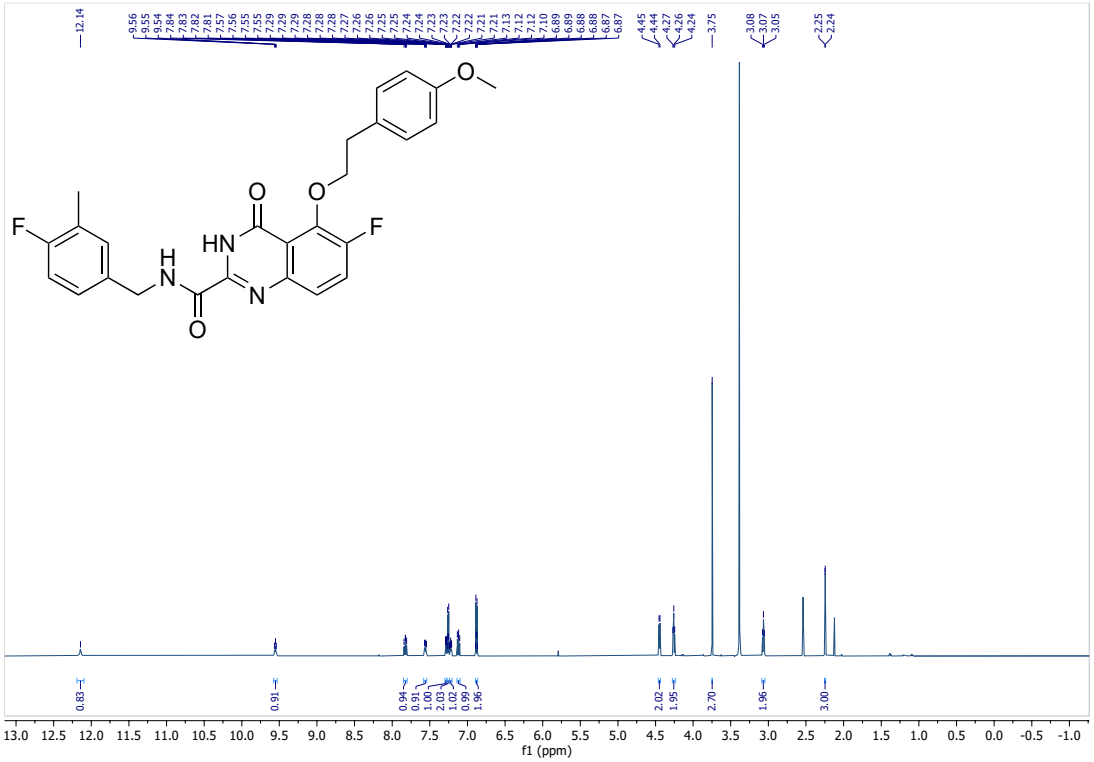


Compound 5f

^{1}H-NMR (600 MHz, $(CD_3)_2SO$)



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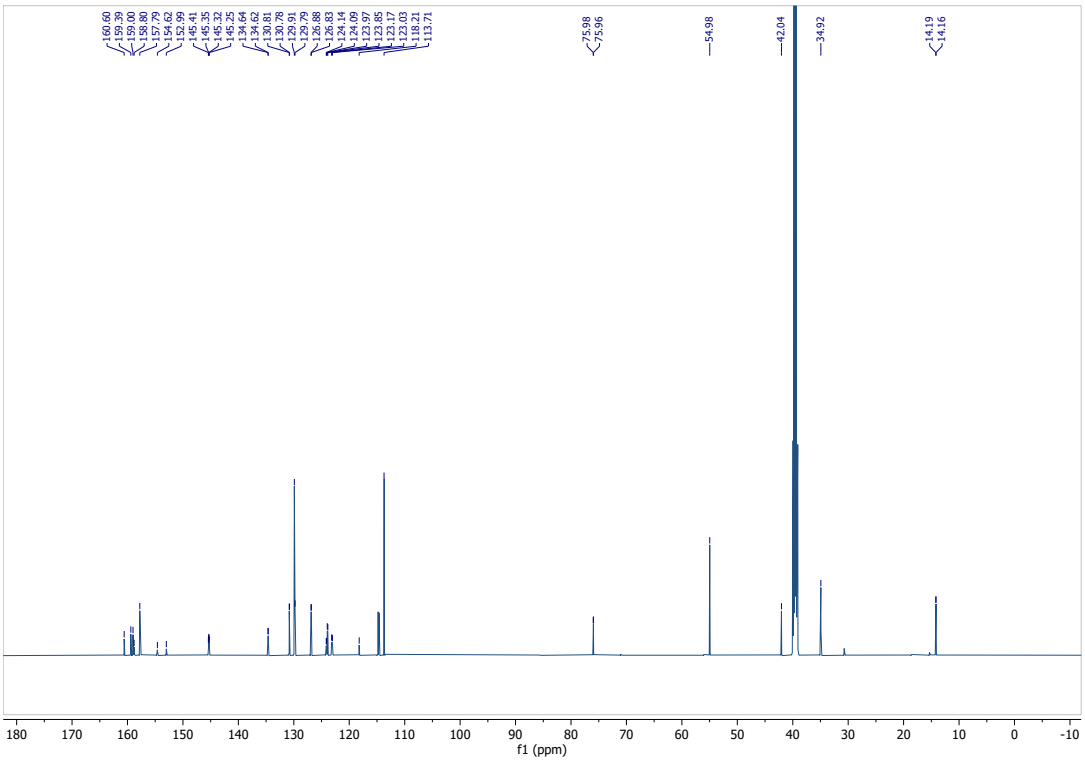
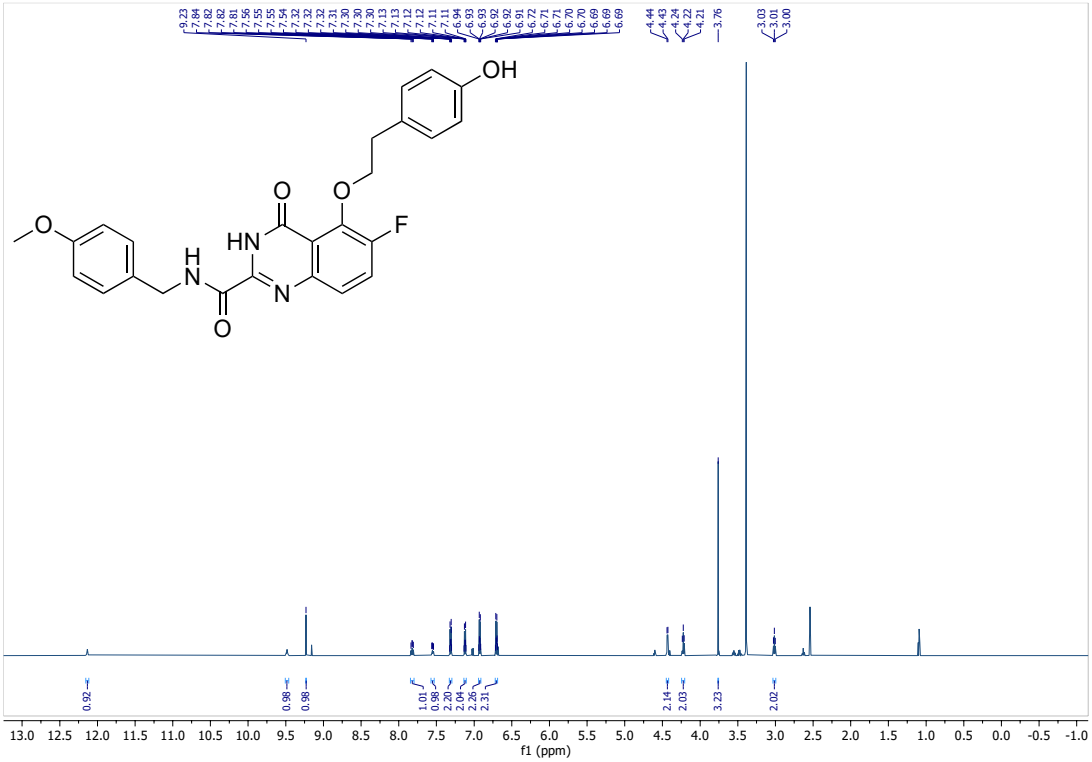
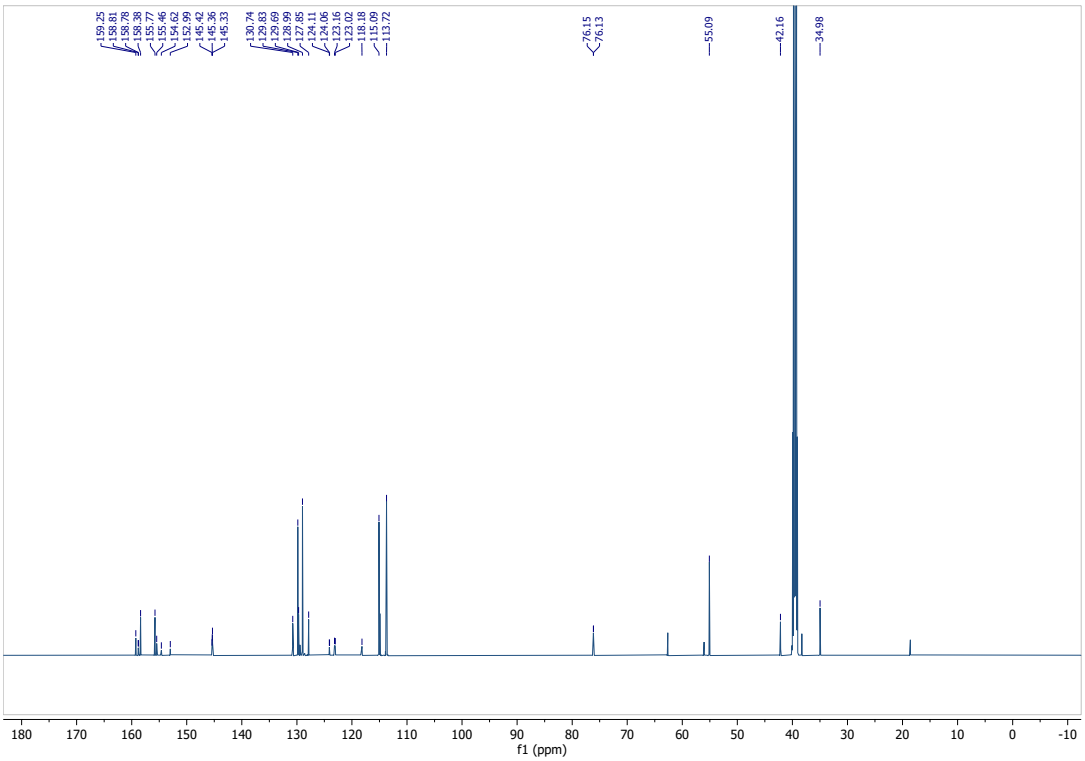


Compound 5g

^{1}H-NMR (600 MHz, $(CD_3)_2SO$)

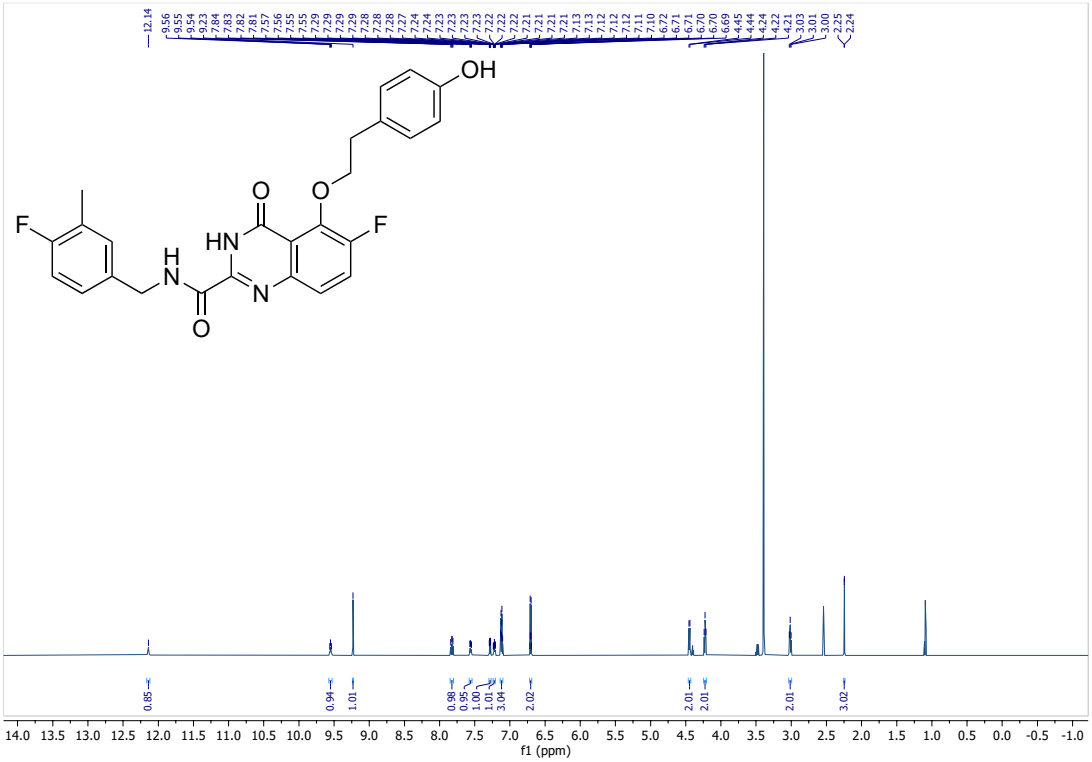


^{13}C-NMR (150 MHz, $(CD_3)_2SO$)

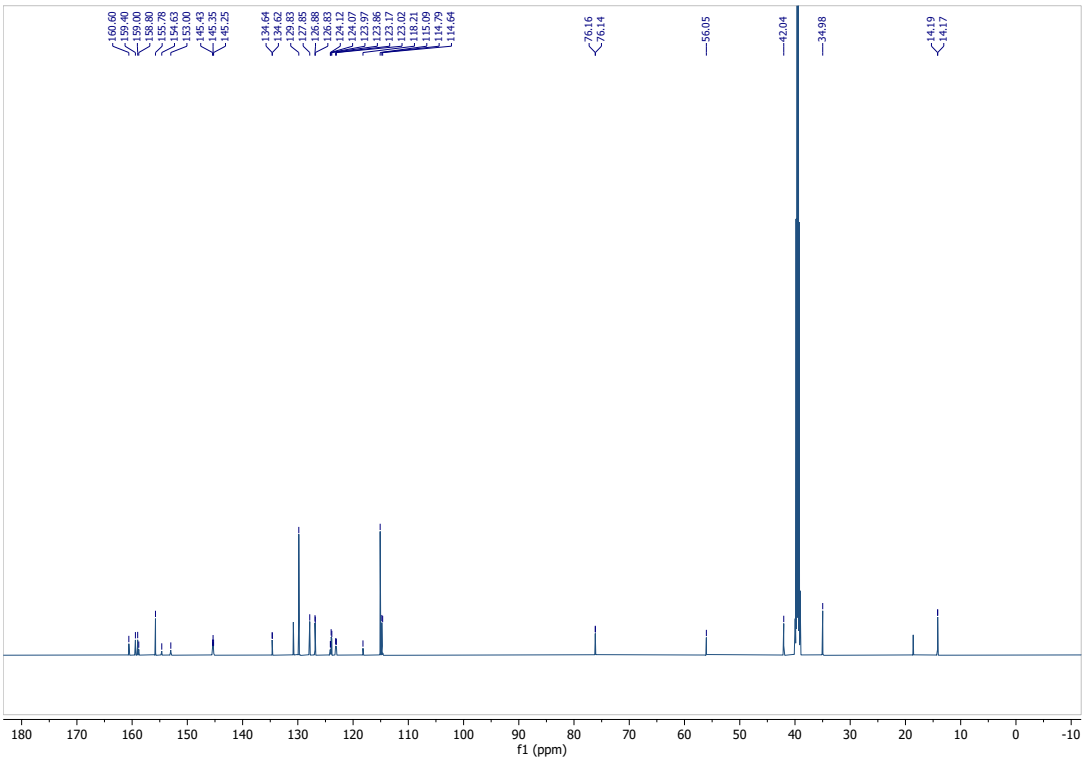


Compound 5h

^{1}H-NMR (600 MHz, $(CD_3)_2SO$)

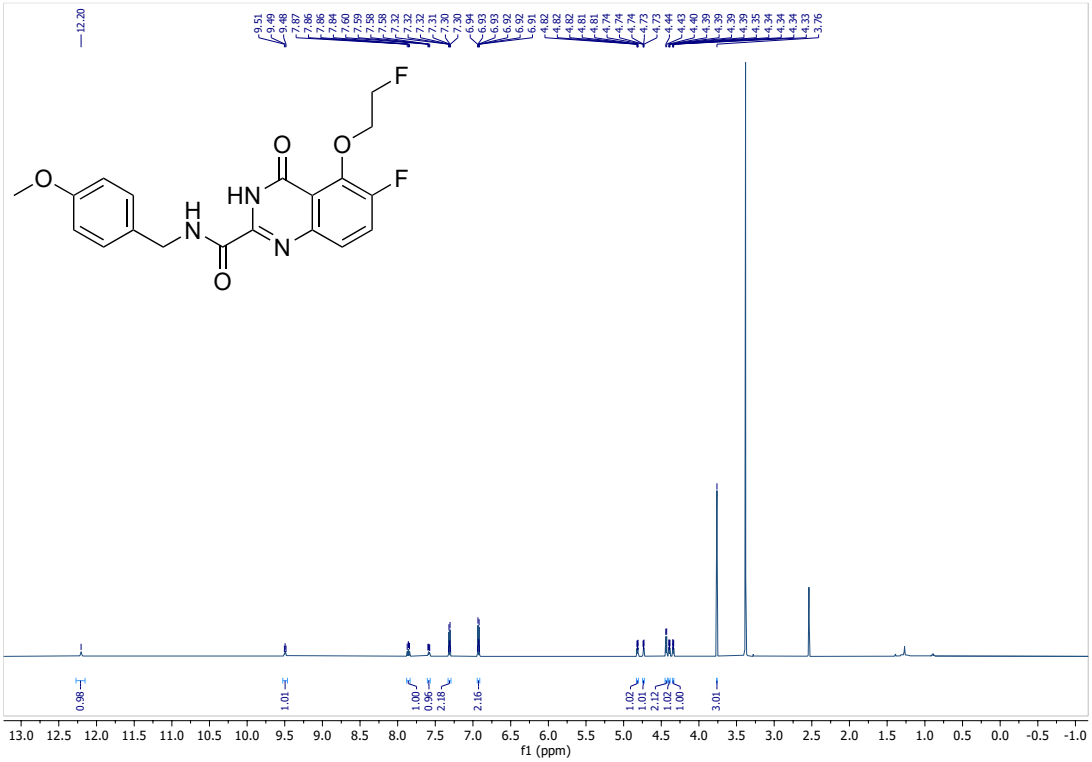


^{13}C-NMR (150 MHz, $(CD_3)_2SO$)

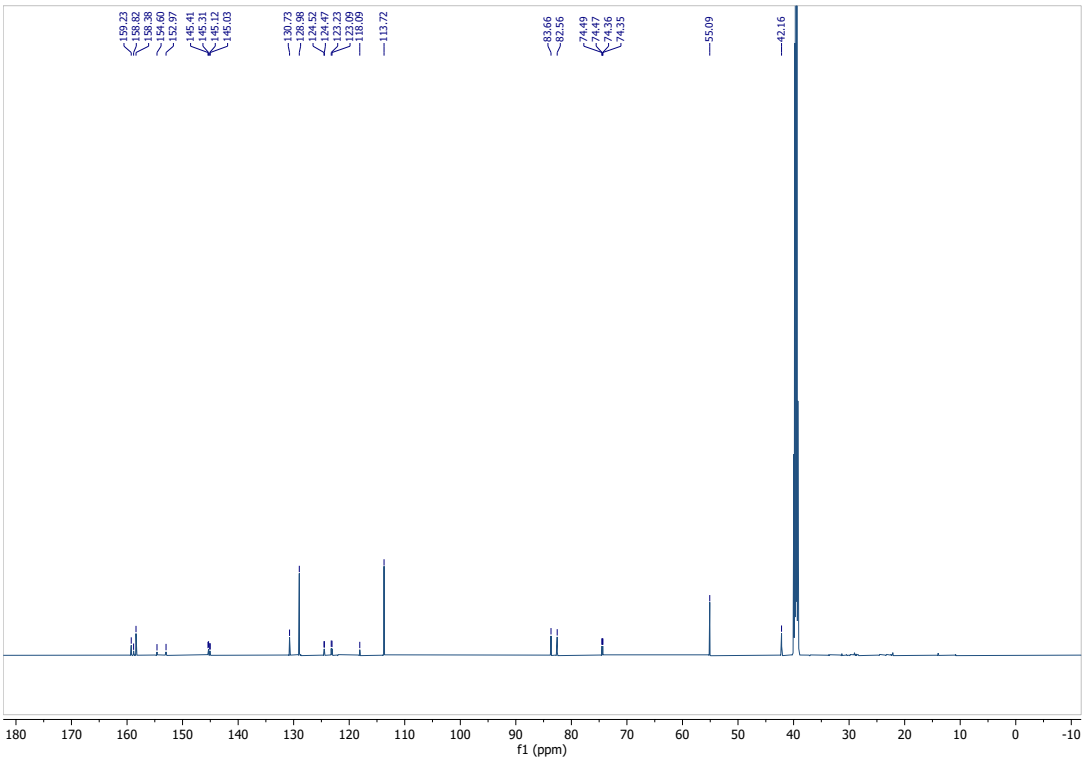


Compound 5i

^{1}H-NMR (600 MHz, $(CD_3)_2SO$)

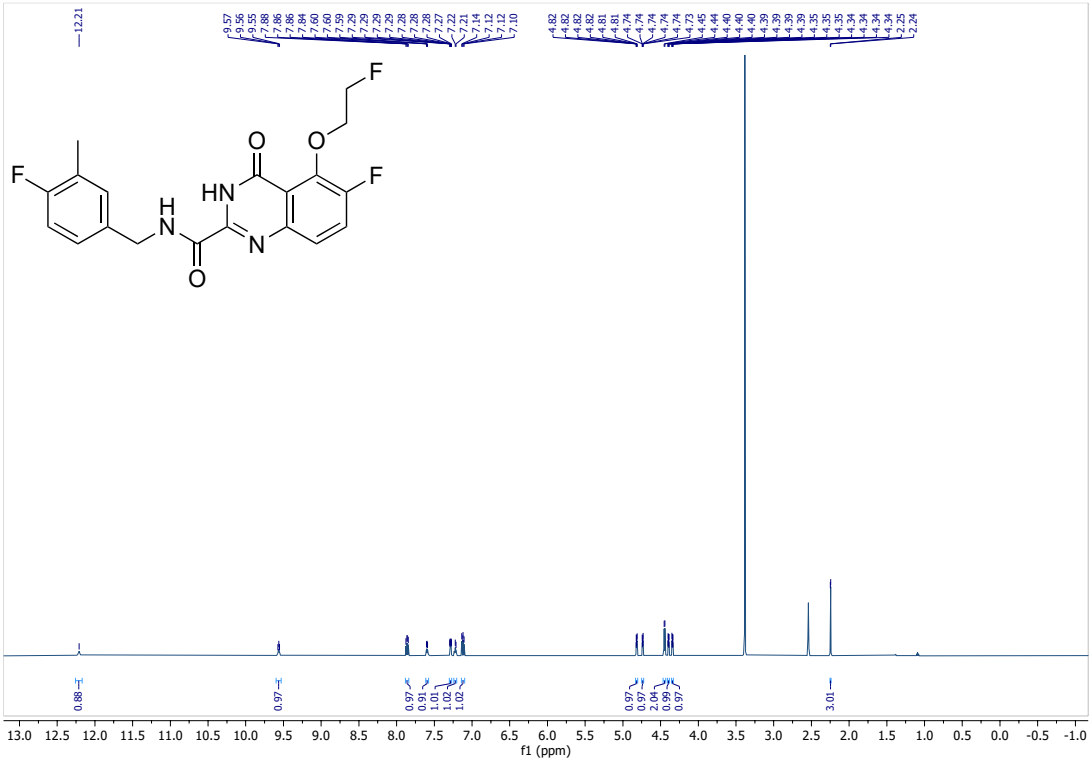


^{13}C-NMR (150 MHz, $(CD_3)_2SO$)

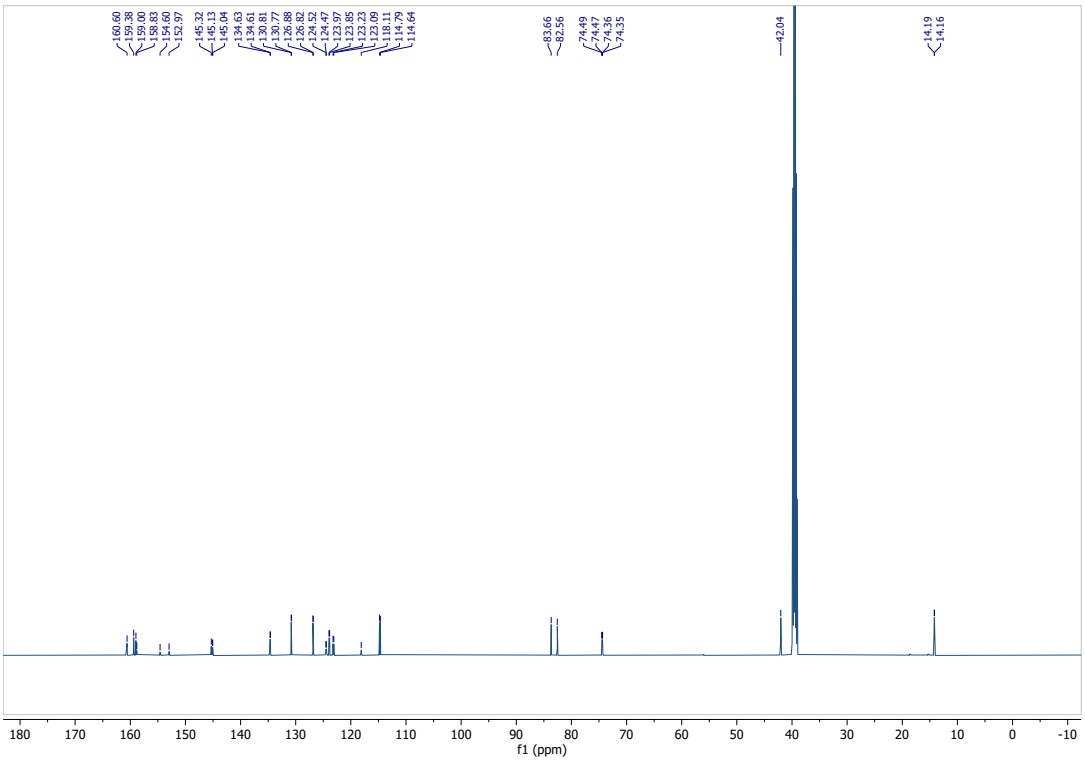


Compound 5j

^{1}H-NMR (600 MHz, $(CD_3)_2SO$)



^{13}C-NMR (150 MHz, $(CD_3)_2SO$)



Compound 6

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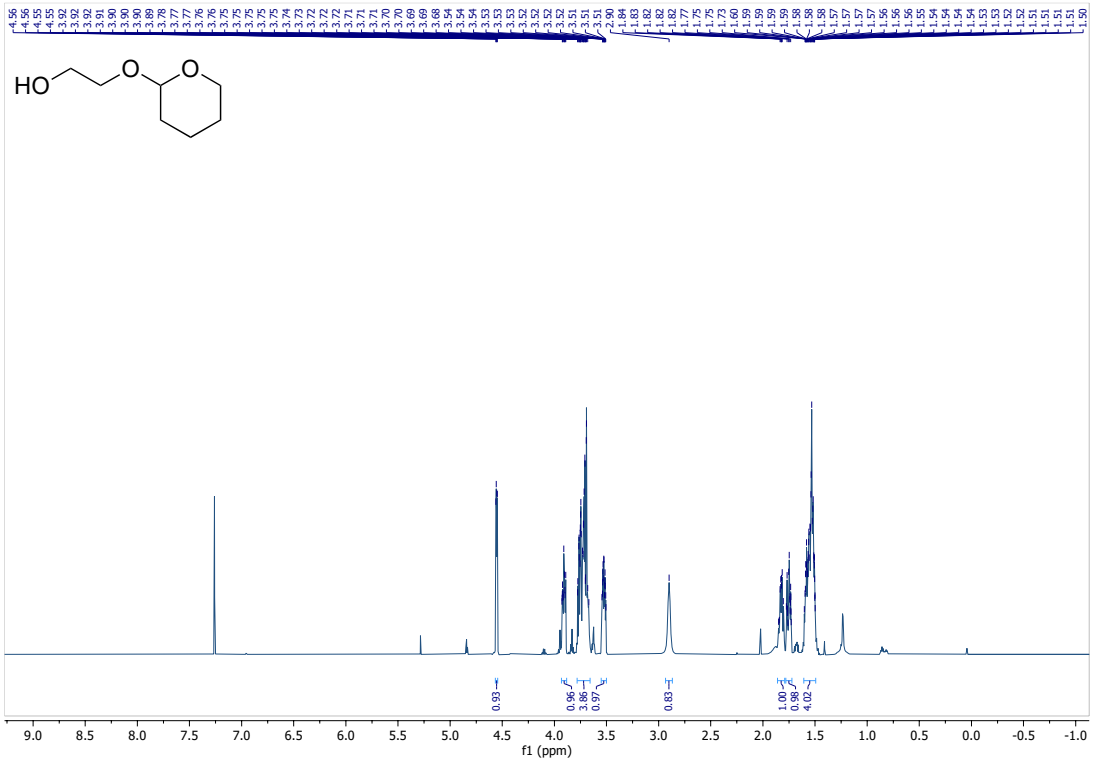

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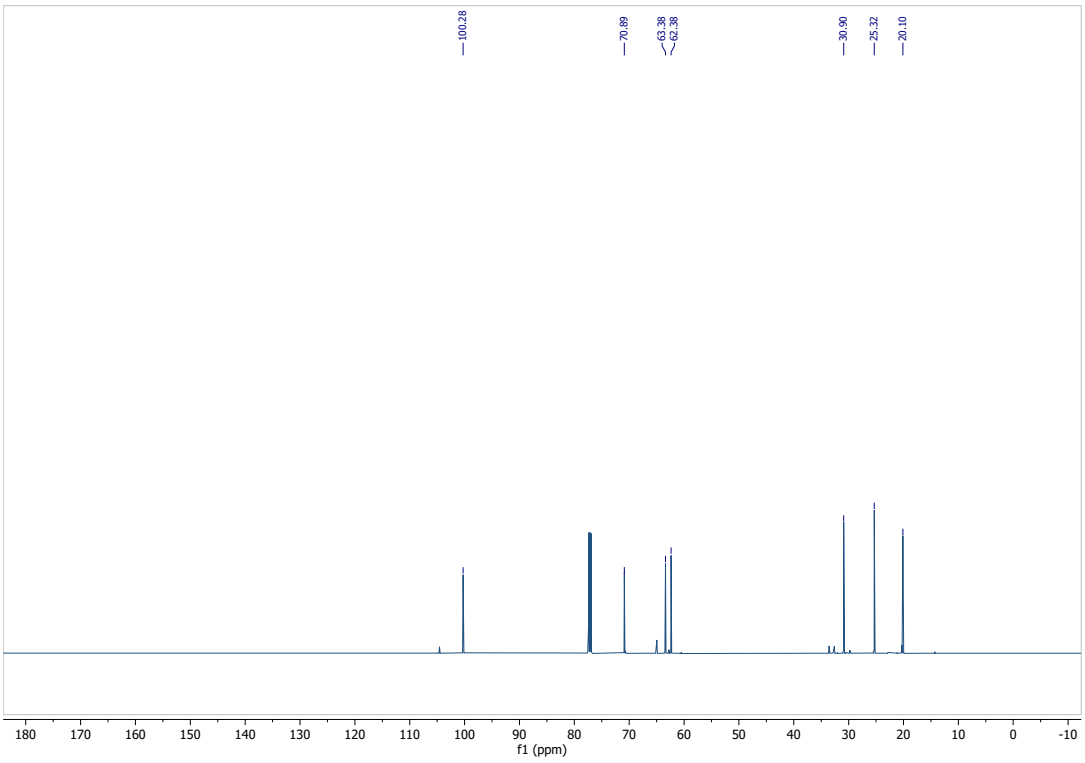

Compound 7

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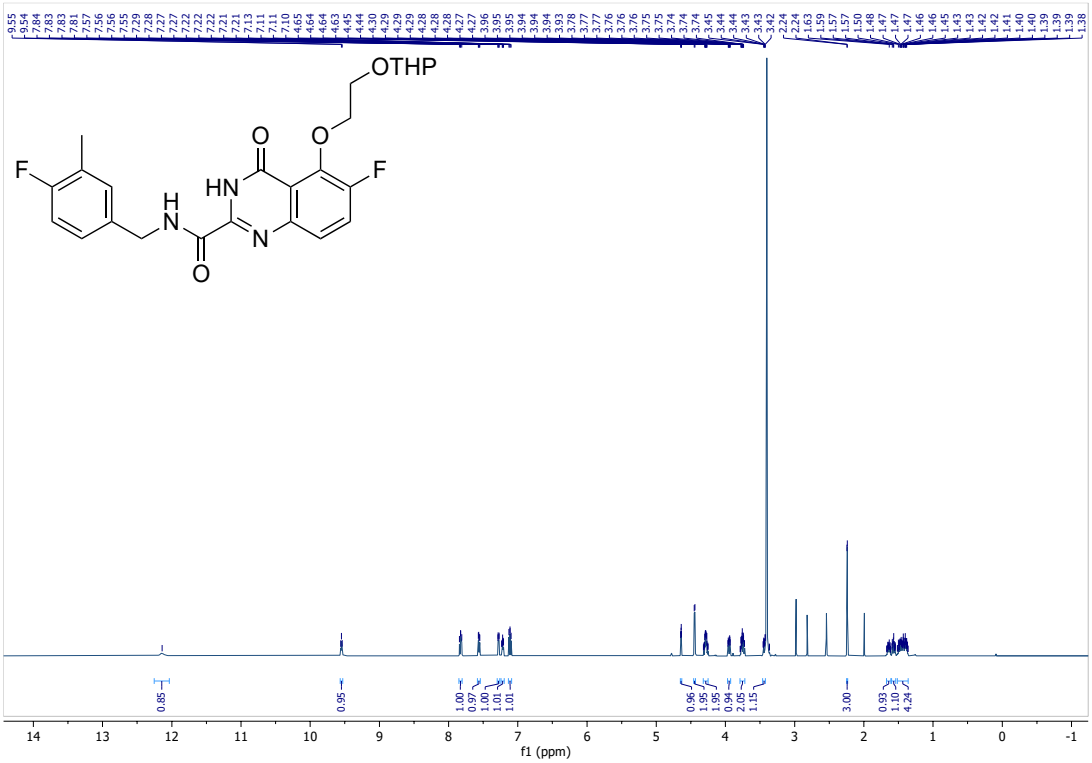

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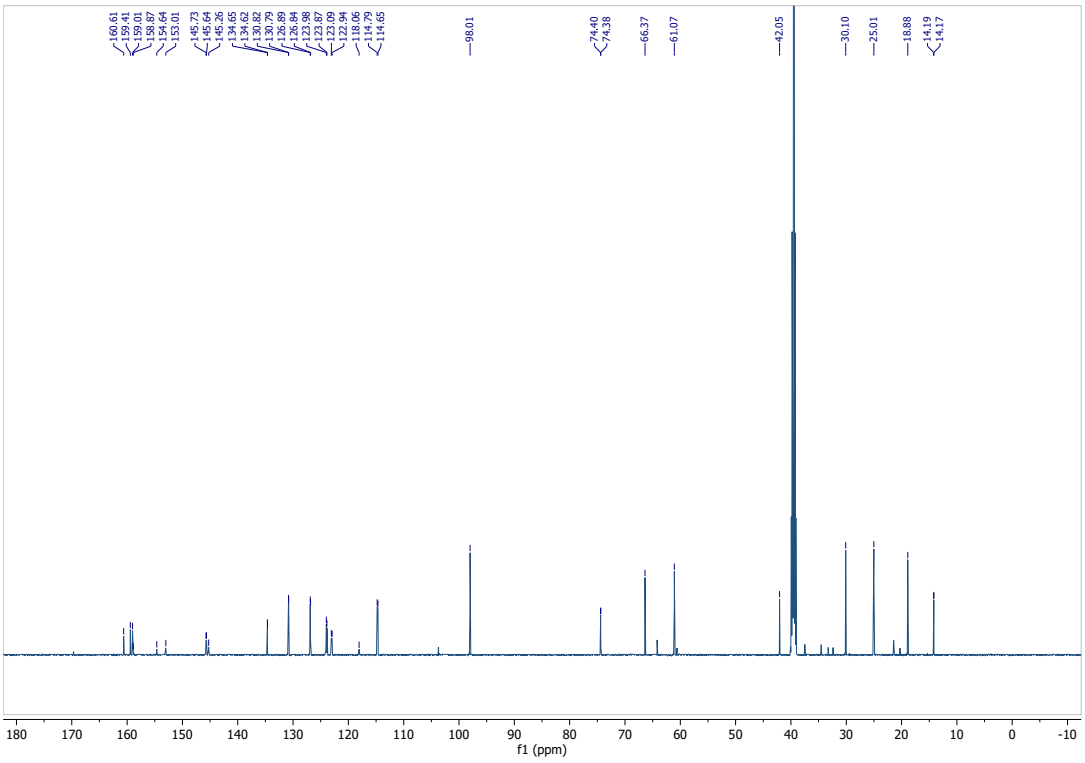

Compound 8

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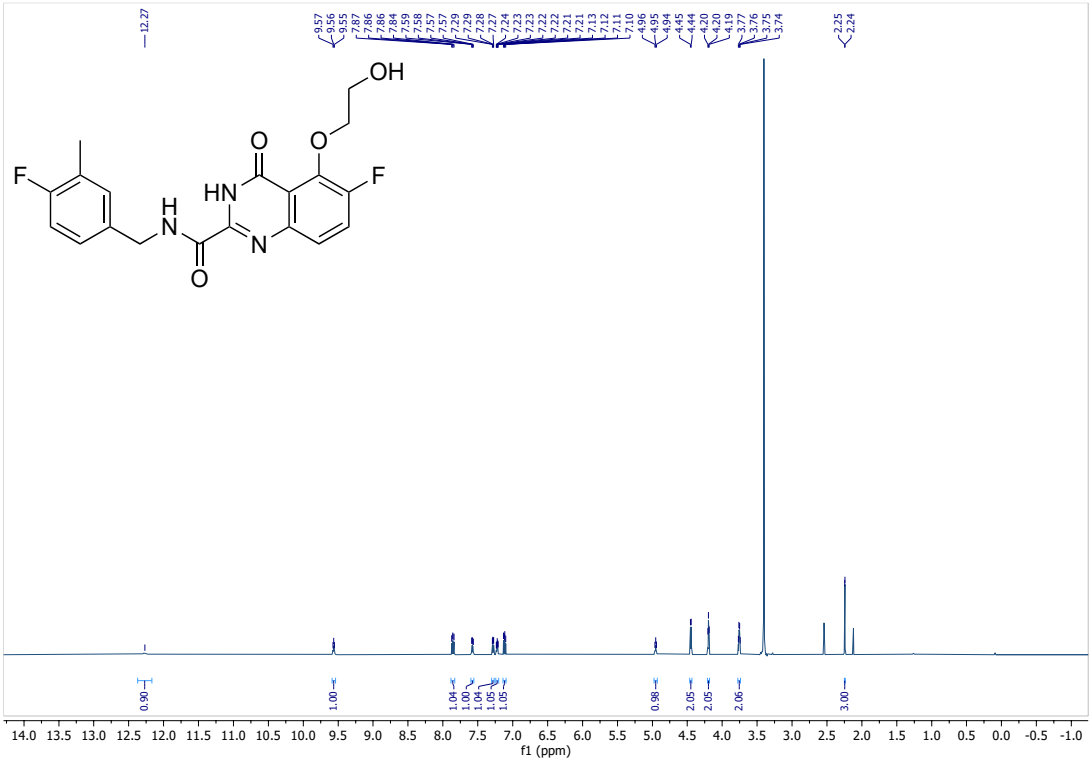

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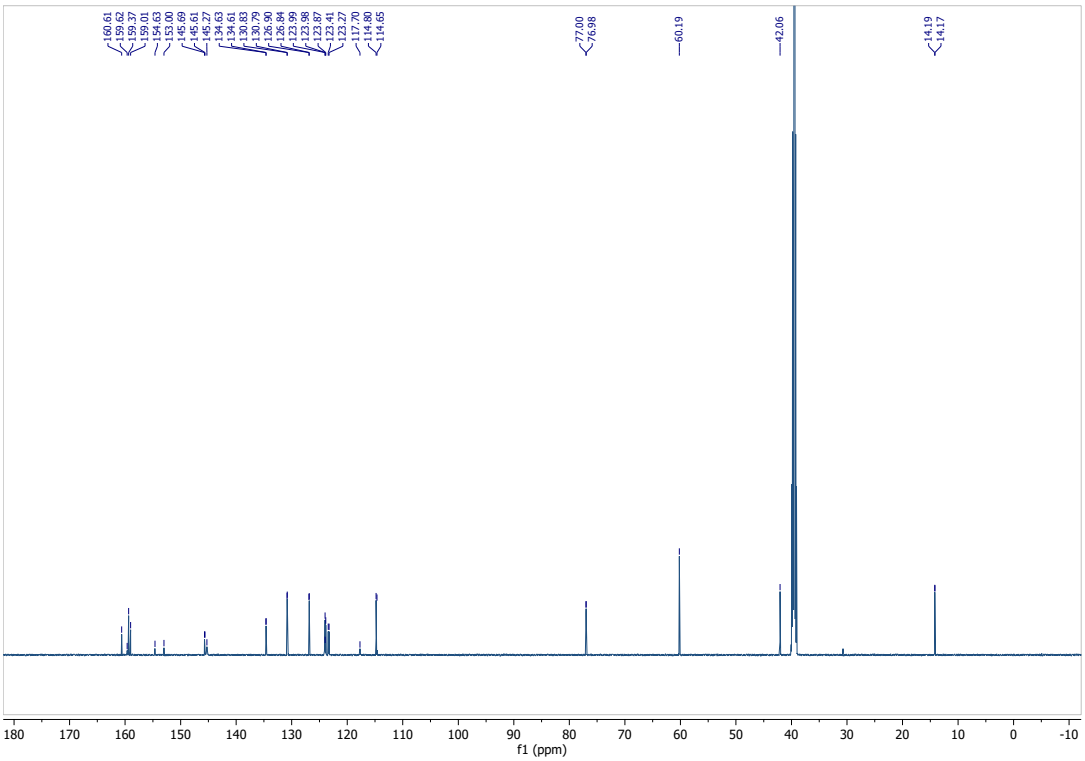

Compound 9

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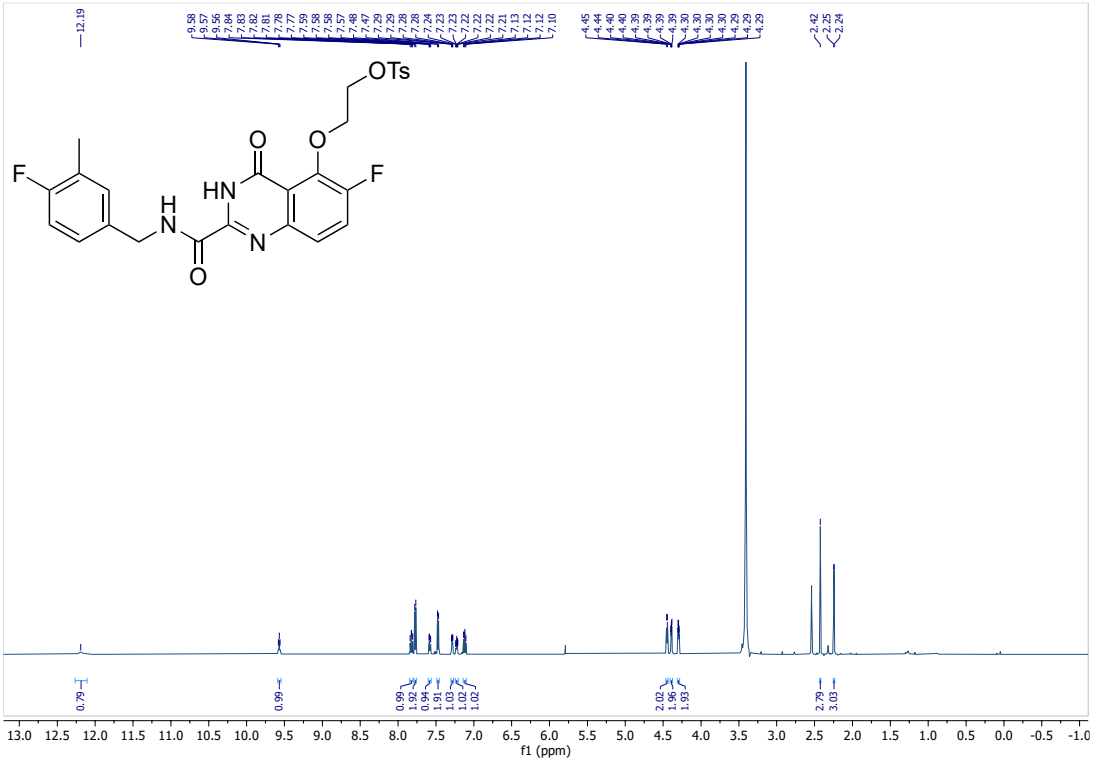
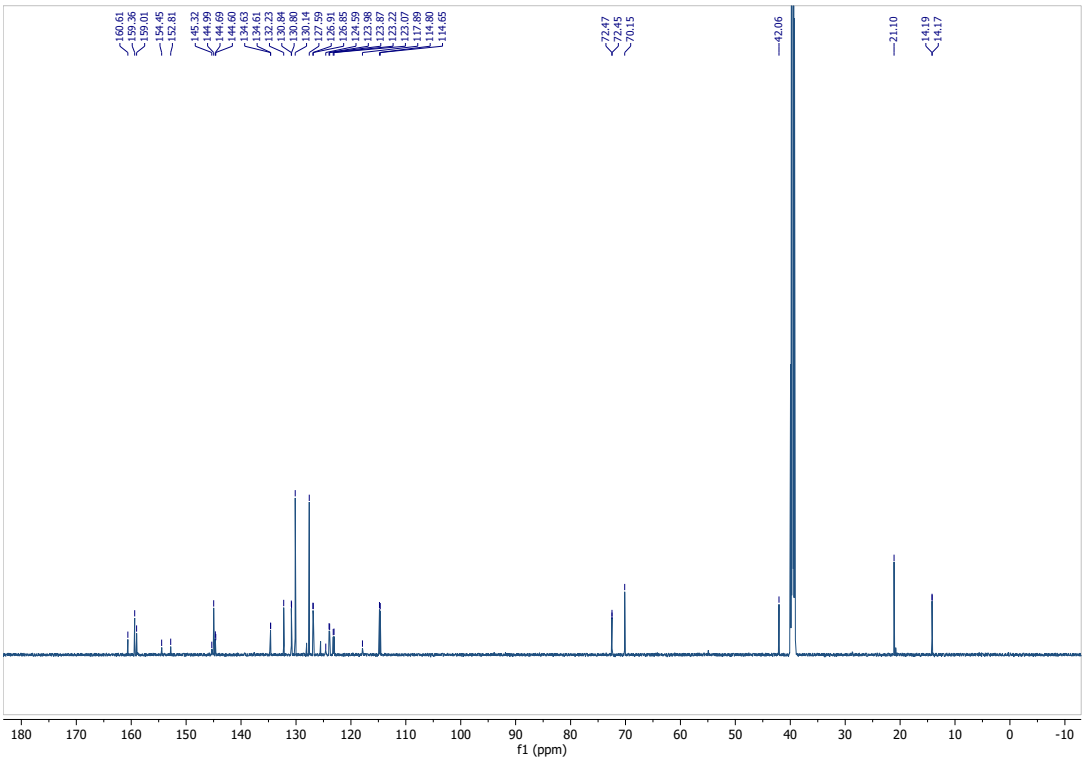


^{13}C-NMR (150 MHz, $(CD_3)_2SO$)



Compound 10

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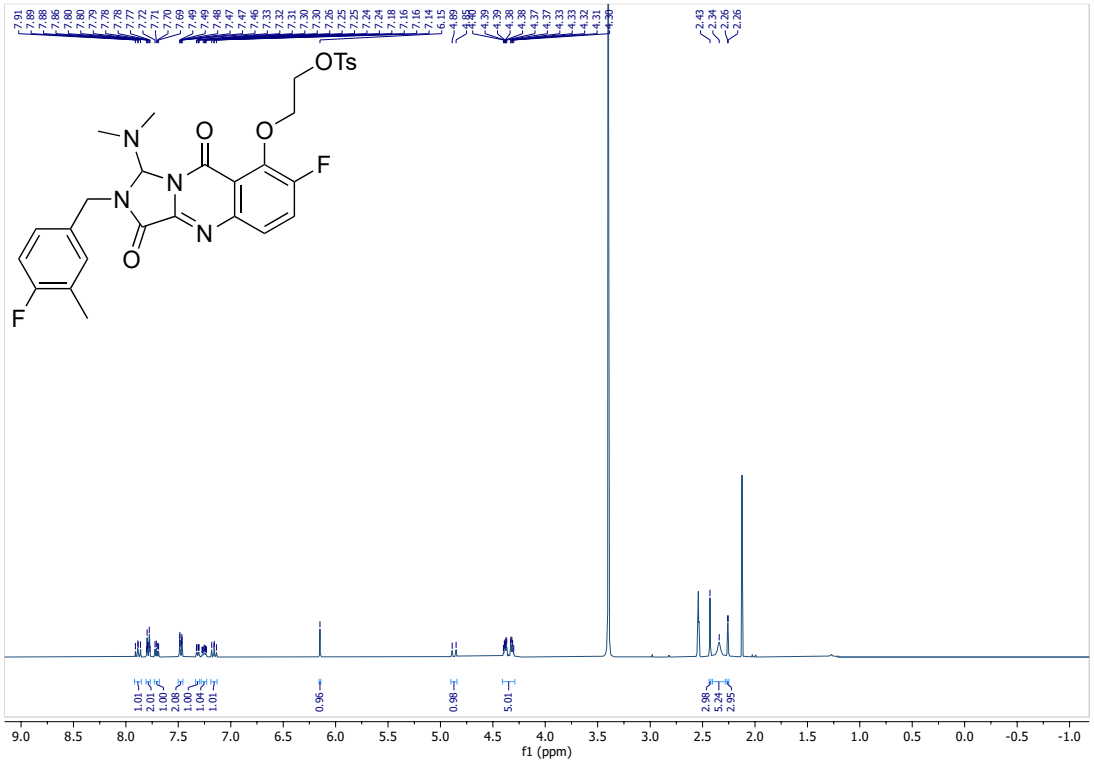
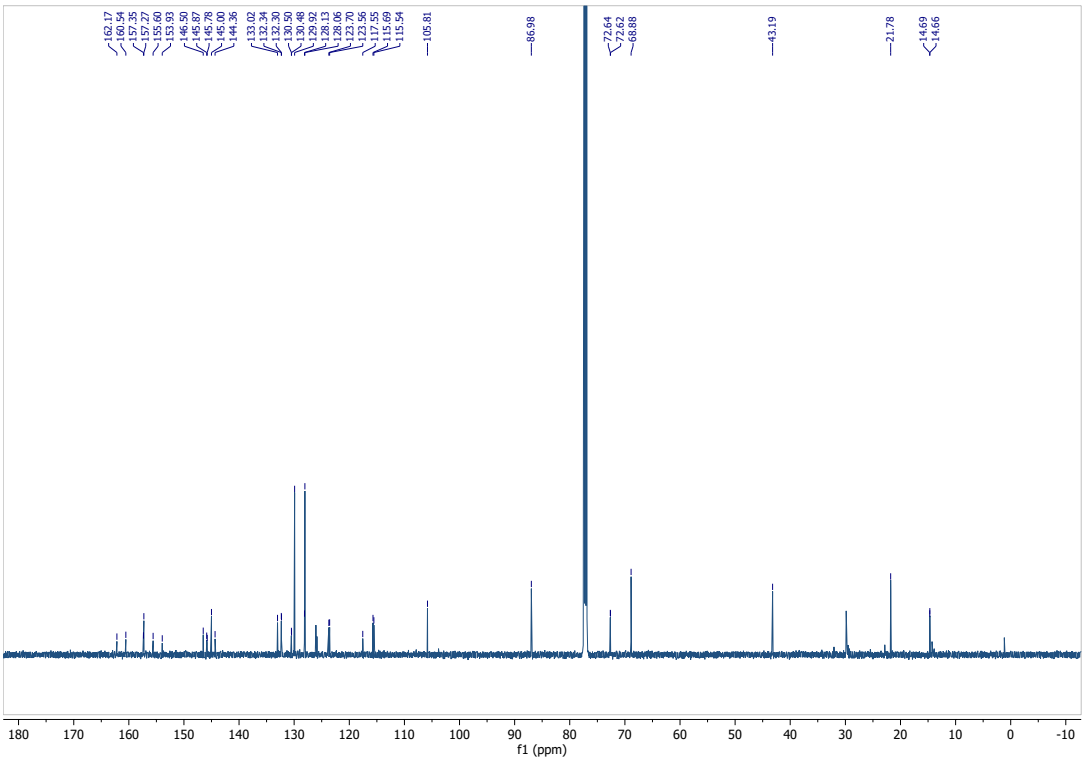


^{13}C-NMR (150 MHz, $CDCl_3$)



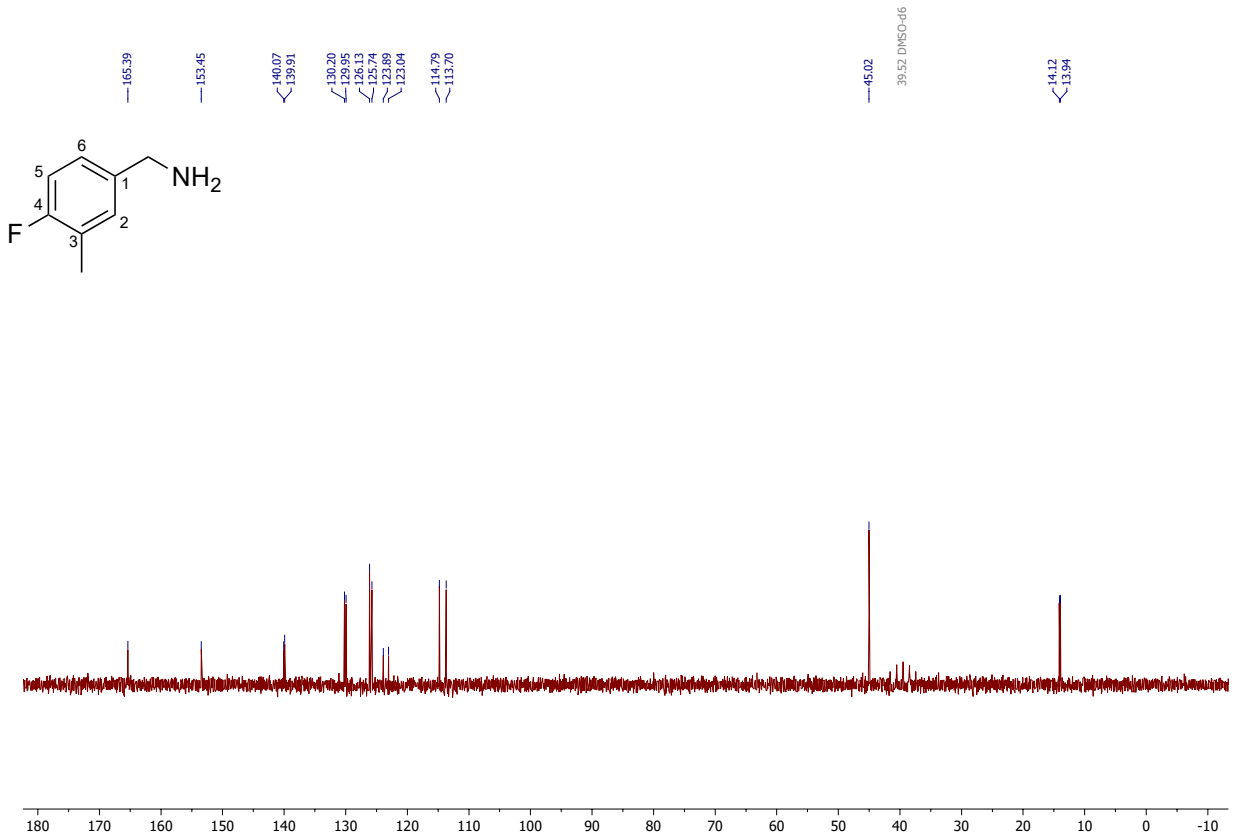


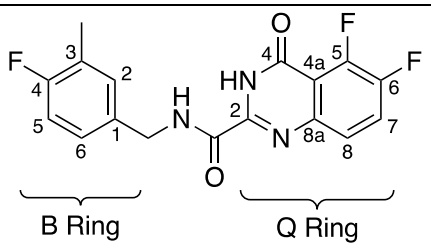
Figure S1. ^{13}C NMR spectrum of 4-fluoro-3-methylbenzylamine (20 MHz, $(CD_3)_2SO$))

Table S1. ^{13}C NMR assignments for 4-fluoro-3-methylbenzylamine.[a]

Carbon Position	δ Chemical Shift (ppm)	J Value (Hz)	C-F Coupling
1	140.0	3.3	*para*
2	125.9	7.7	*meta*
3	123.5	17	*ortho*
4	159.4	241	*ipso*
5	114.2	22	*ortho*
6	130.1	5.1	*meta*

[a] For C–F coupling constants of fluorobenzenes, please see reference 2.

Table S2. ^{13}C NMR assignments for compound **5d**.



Carbon Position	δ Chemical Shift (ppm)	J Value(s) (Hz)	C-F Coupling
B1	134.6	3.4	*para*
B2	126.9	8.1	*meta*
B3	123.9	17	*ortho*
B4	159.8	241	*ipso*
B5	114.7	22	*ortho*
B6	130.8	5.1	*meta*
Q2	146.1	-	-
Q4	158.2	-	-
Q4a	113.6	-	-
Q5	147.7	263, 14	*ipso*, *ortho*
Q6	148.0	245, 11	*ipso*, *ortho*
Q7	123.6	19	*ortho*
Q8	124.3	br	-
Q8a	144.7	-	-

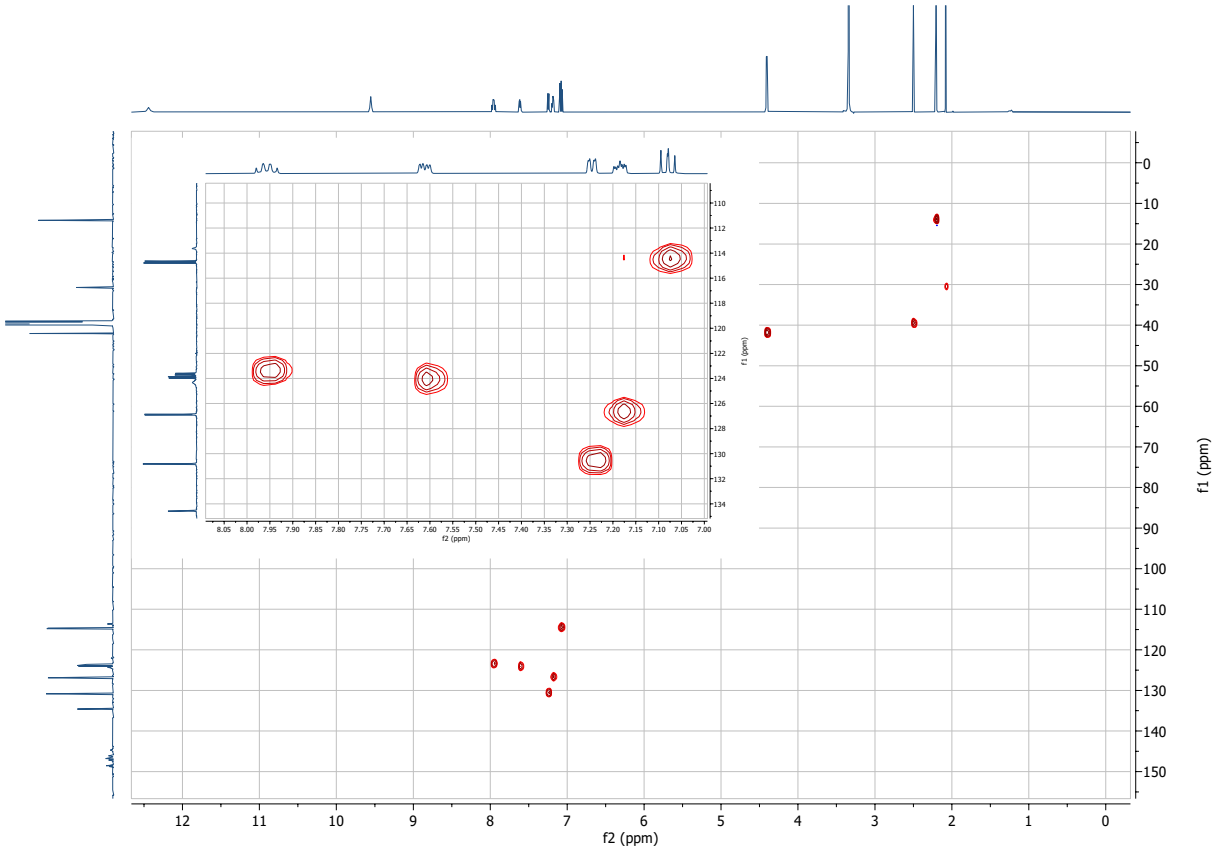
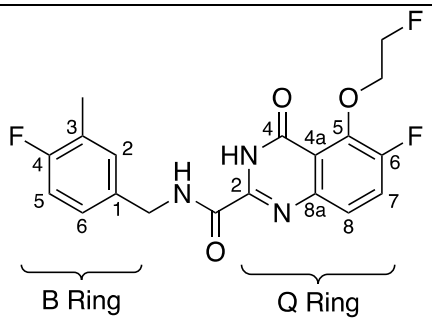


Figure S2. HSQC analysis of compound **5d** (^{1}H (600 MHz) vs ^{13}C (150 MHz), $(CD_3)_2SO$). ^{13}C NMR signals from the B ring are maintained. Remaining aromatic carbon signals which are HSQC positive correspond to Q7 and Q8 on the Q ring. Q7 (123.6, J = 19 Hz, C-F *ortho* coupling) corresponds to signal at 8.02–7.97 (1H, m). Q8 (124.3, br, C-F *meta* coupling) corresponds to signal at 7.66–7.64 (1H, m).

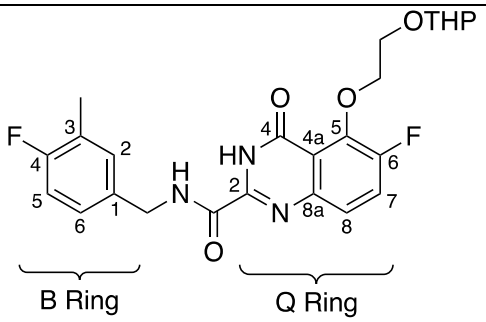
Table S3. ^{13}C NMR assignments for compound **5j**.



Carbon Position	δ Chemical Shift (ppm)	J Value(s) (Hz)	C-F Coupling
B1	134.6	3.3	*para*
B2	126.8	8.3	*meta*
B3	123.9	17	*ortho*
B4	159.8	240	*ipso*
B5	114.7	22	*ortho*
B6	130.8	4.7	*meta*
Q2	145.3	-	-
Q4	158.8	-	-
Q4a	118.1	-	-
Q5	145.0	14	*ortho*
Q6	153.8	245	*ipso*
Q7	123.1	21	*ortho*
Q8	124.5	6.5	*meta*
Q8a	145.2	-	-

Q7 and Q8 *J*-values are consistent with SNAr at the 5-position on the Q-ring as *ortho* and *meta* C-F coupling is maintained (J = 21 Hz and 6.5 Hz, respectively).

Table S4. ^{13}C NMR assignments for compound **7**.



Carbon Position	Chemical Shift (ppm)	J Value(s) (Hz)	C-F Coupling
B1	134.6	4.5	*para*
B2	126.9	7.5	*meta*
B3	123.9	17	*ortho*
B4	159.8	240	*ipso*
B5	114.7	21	*ortho*
B6	130.8	4.5	*meta*
Q2	145.3	-	-
Q4	158.9	-	-
Q4a	118.1	-	-
Q5	145.7	14	*ortho*
Q6	153.8	245	*ipso*
Q7	123.0	23	*ortho*
Q8	124.1	br	-
Q8a	145.2	-	-

Q7 *J*-value is consistent with S_NAr at 5-position on the Q ring as *ortho* C-F coupling is maintained (J = 23 Hz).

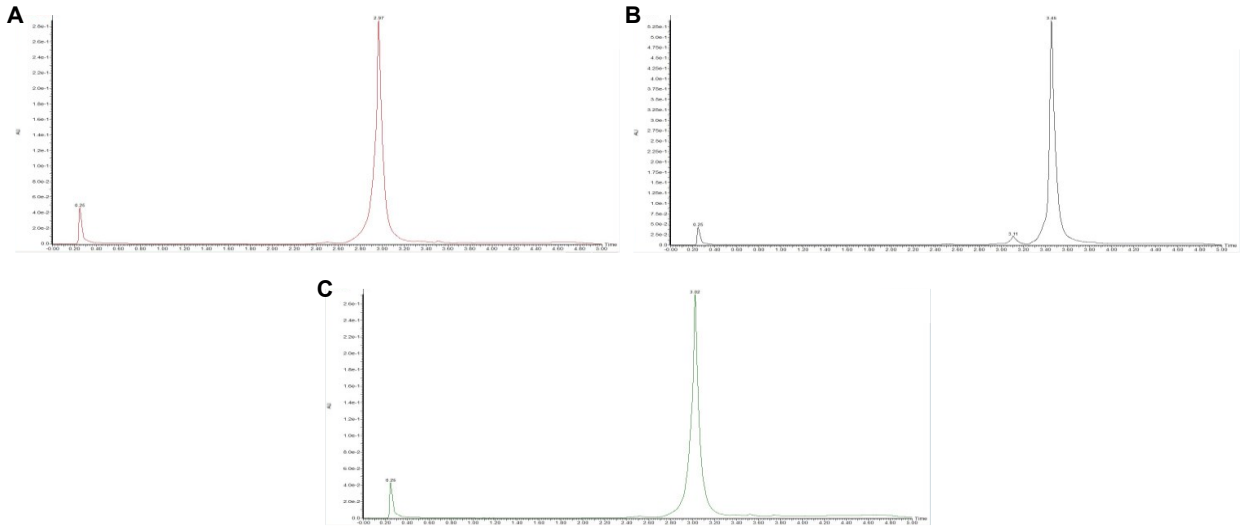


Figure S3. UPLC chromatograms for compounds (A) **5b**, (B) **5f** and (C) **5j** (254 nm). Solvent A: water, solvent B: ACN, solvent C: 5% formic acid, flowrate: 0.5 mL·min^{-1}. Method: 0-0.5 min 93% A 5% B 2% C, 0.5-3.5 min gradient to 5% A 93% B 2% C, 3.5-4.0 min 5% A 93% B 2%, 4.0-4.2 min gradient to 93% A 5% B 2% C, 4.2-5.0 min 93% A 5% B 2% C.

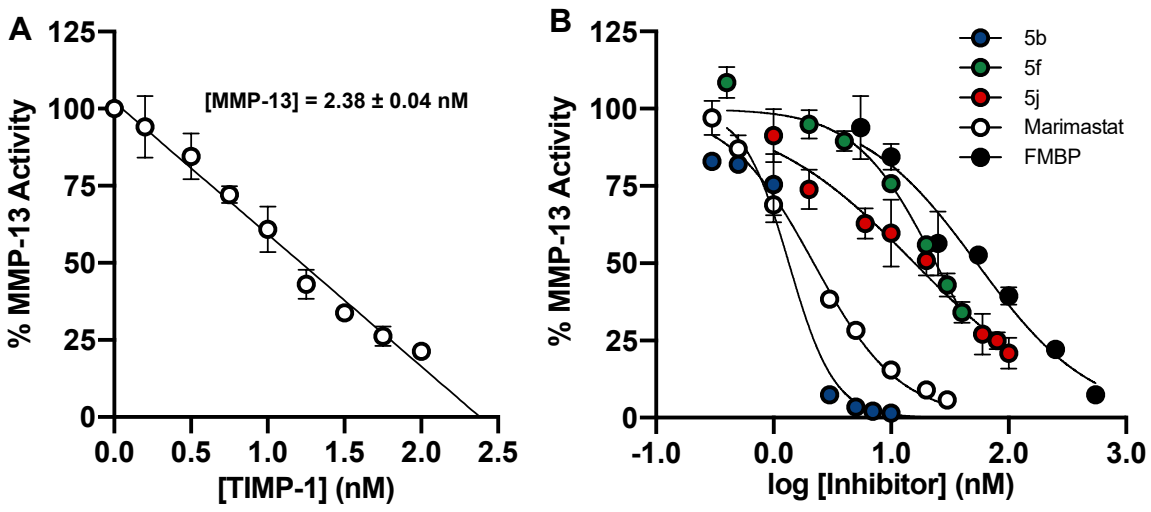


Figure S4. MMP-13 inhibition assays. (A) MMP-13 active site titration with TIMP-1 (R&D Systems).[1] (B) Representative MMP-13 IC_{50} curves.

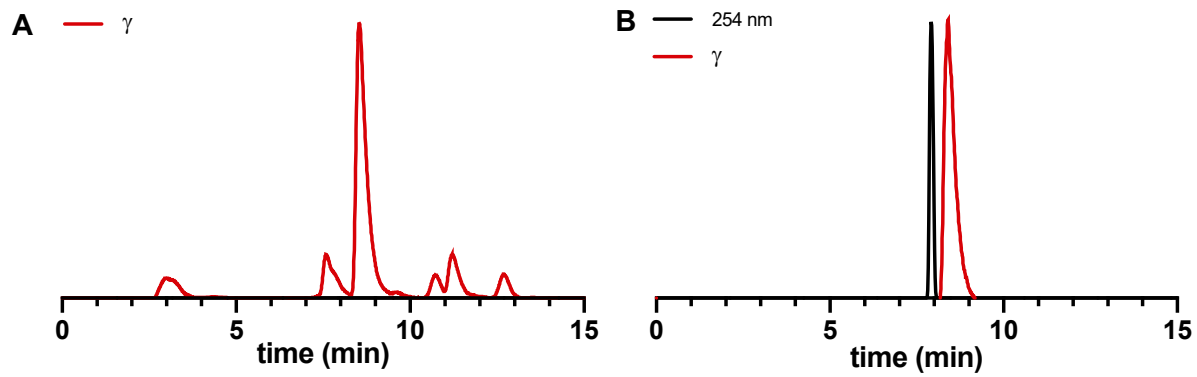


Figure S5. RadioHPLC chromatograms for synthesis of **[^{11}C]5b**. (A) Crude [^{11}C]methylation of precursor **5a** and (B) Purified **[^{11}C]5b** with co-injection of **5b**.

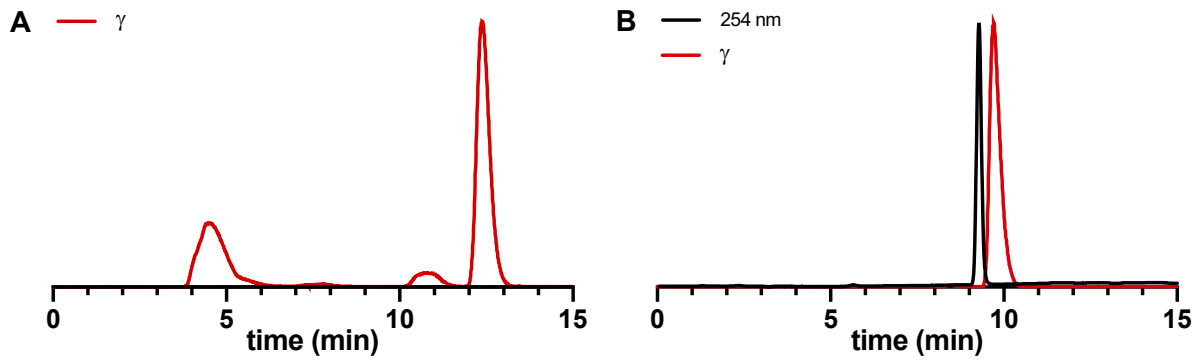


Figure S6. RadioHPLC chromatograms for synthesis of **[^{11}C]5f**. (A) Crude [^{11}C]methylation of precursor **5h** and (B) Purified **[^{11}C]5f** with co-injection of **5f**. *Note: the retention time of the product shifted following HPLC maintenance.

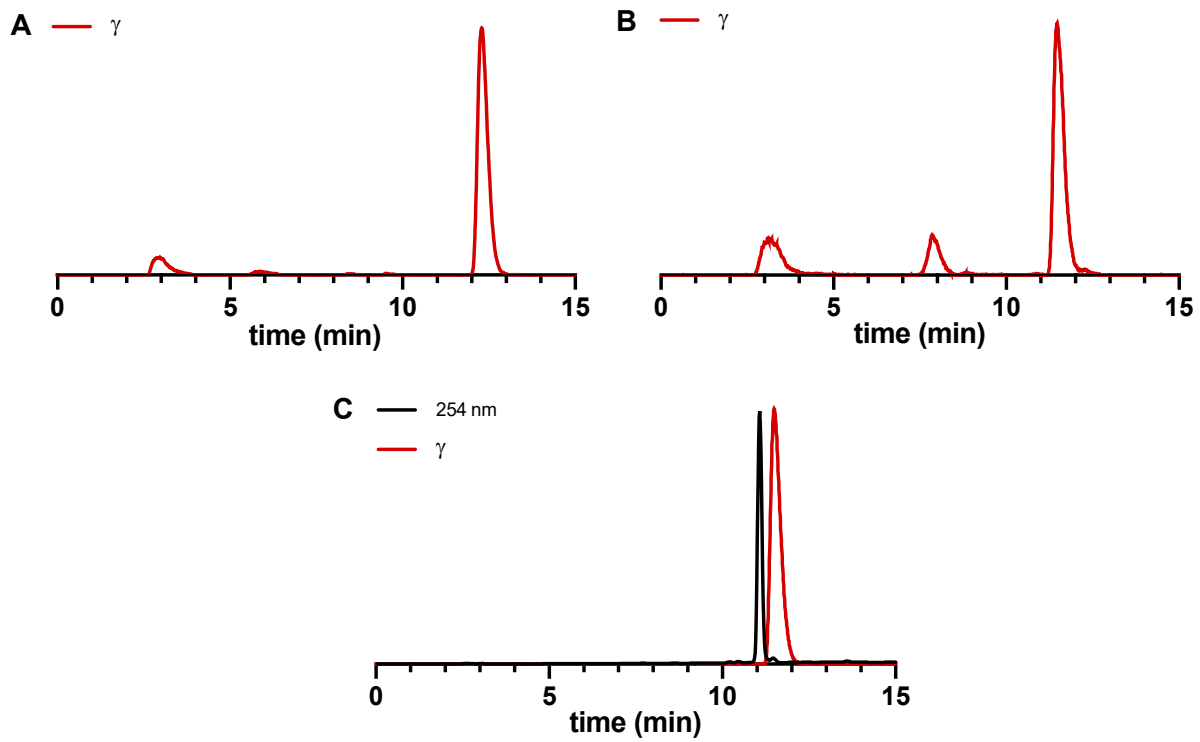


Figure S7. RadioHPLC chromatograms for synthesis of **[^{18}F]5j**. (A) Crude radiofluorination of precursor **10** (B) Crude deprotection and (C) Purified **[^{18}F]5j** with co-injection of **5j**.

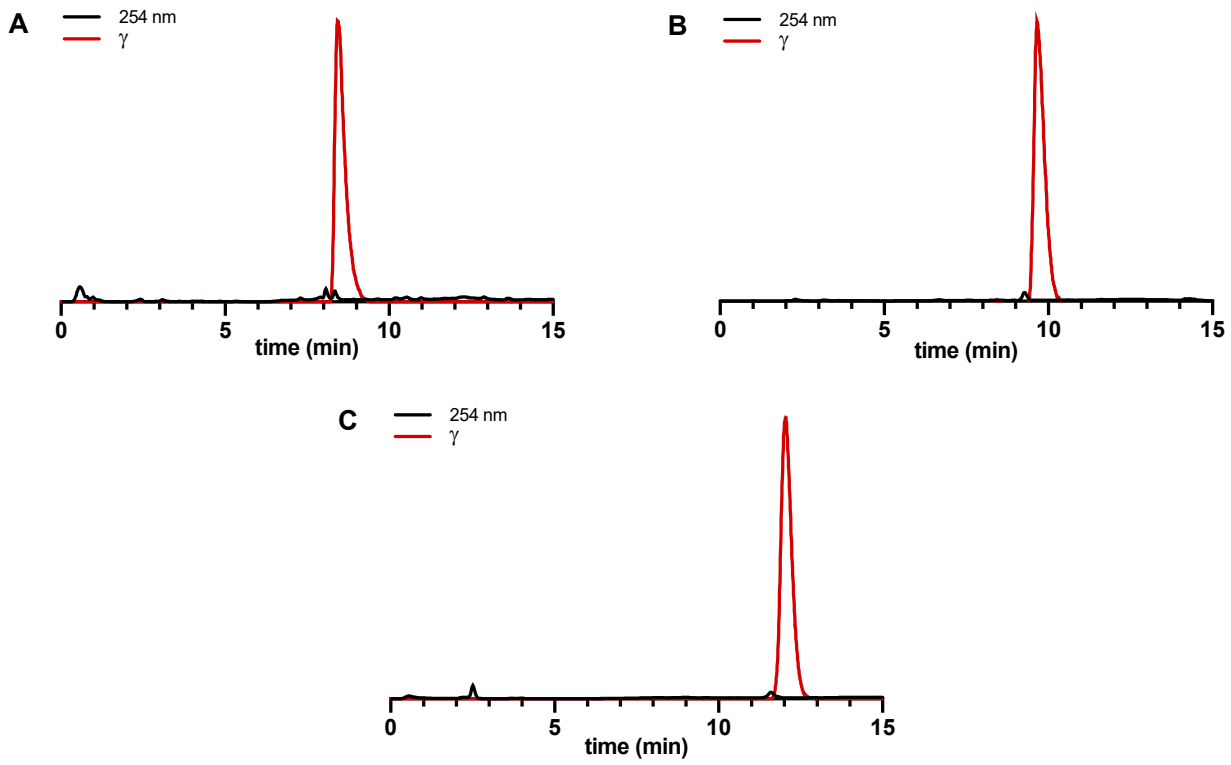


Figure S8. RadioHPLC chromatograms without coinjection. (A) **[^{11}C]5b**. (B) **[^{11}C]5f**. (C) **[^{18}F]5j**.

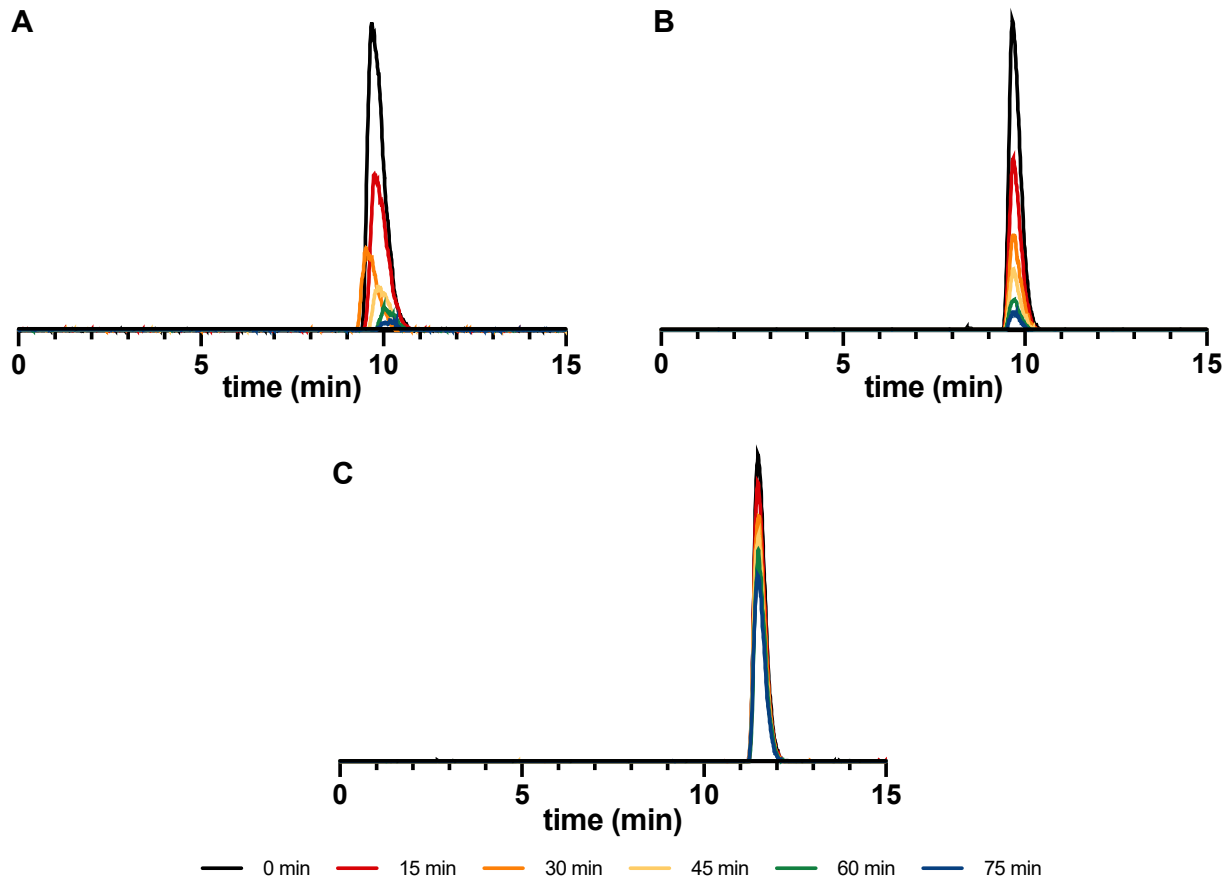


Figure S9. Radiotracer stability following reformulation in 10% EtOH/saline (0.9%). (A) **[^{11}C]5b**. (B) **[^{11}C]5f**. (C) **[^{18}F]5j**.

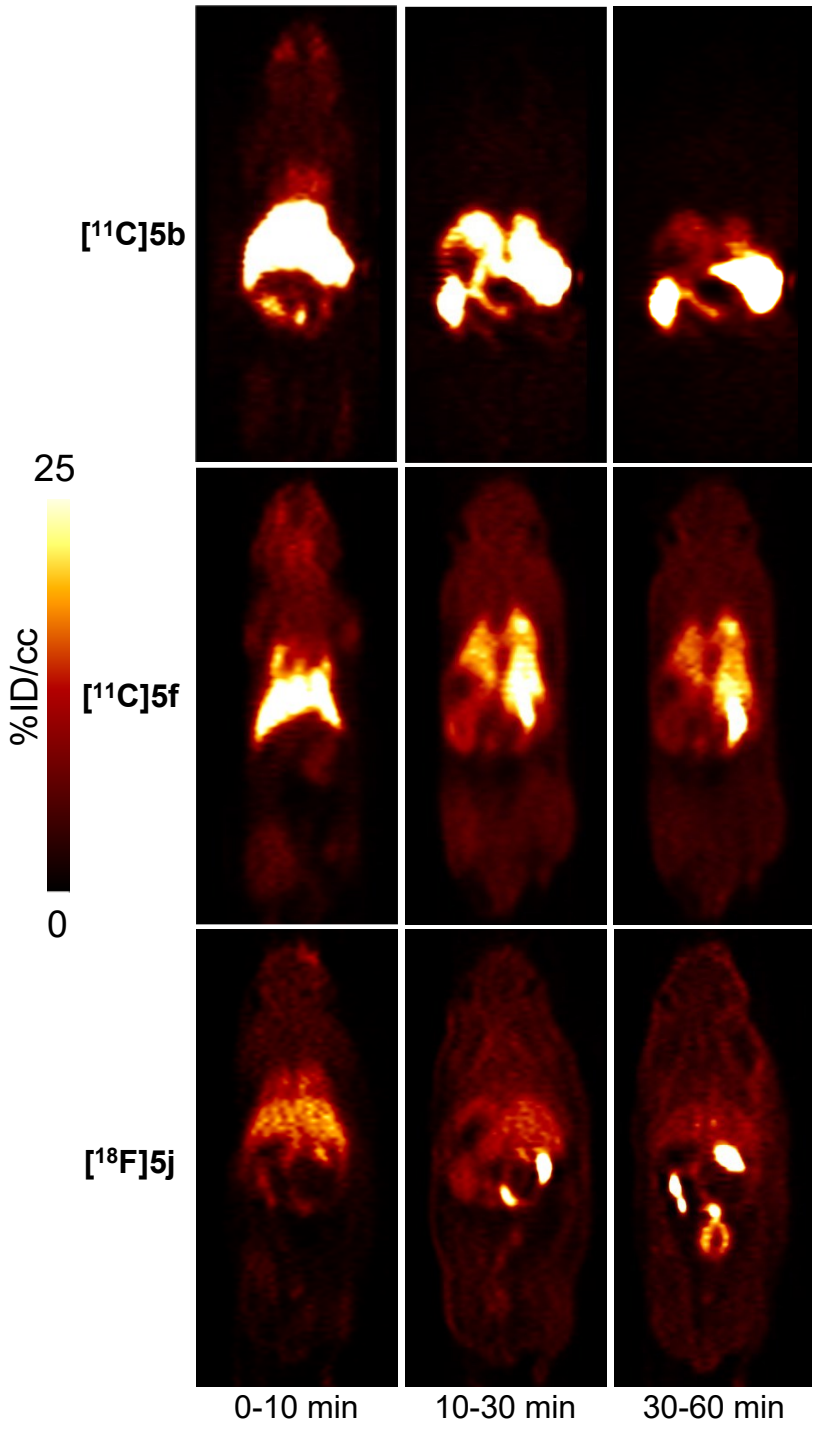


Figure S10. Representative summed coronal whole-body PET images in *ApoE*$^{-/-}$ mice

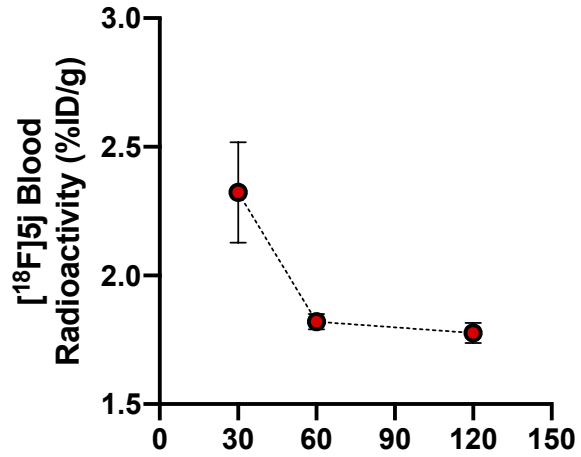


Figure S11. [^{18}F]5j blood radioactivity time-course. Anesthetized *ApoE*$^{-/-}$ mice were intravenously administered with **[^{18}F]5j (**15 ± 1 MBq) via the lateral tail vein and immediately returned to their cages. Mice were sacrificed by CO_2 asphyxiation at the indicated time (30, 60 or 120 min) and blood was collected by cardiac puncture. Samples were weighed and counted for radioactivity using a gamma counter (*n* = 2–3 per group).

References

(1) Knight, C. G.; Willenbrock, F.; Murphy, G. A Novel Coumarin-Labelled Peptide for Sensitive Continuous Assays of the Matrix Metalloproteinases. *FEBS Lett.* **1992**, *296* (3), 263–266. https://doi.org/10.1016/0014-5793(92)80300-6.

(2) Roberts, J. D.; Weigert, F. J. ^{13}C Nuclear Magnetic Resonance Spectroscopy. Determination of Carbon-Fluorine Couplings. *J. Am. Chem. Soc.* **1971**, *93* (10), 2361–2369. https://doi.org/10.1021/ja00739a001.