pharmaceuticals

MDPI

Supplementary Materials

Development and biological evaluation of the first highly potent and selective benzamide-based radiotracer [^{18}F]BA3 for imaging of histone deacetylases 1 and 2 in brain

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Figure S1: Scheme of the synthesis module TRACERlab FX2 N for the radiosynthesis of [^{18}F]**BA3**

Figure S2: Representative (**A**) UV- and (**B**) radio-RP-HPLC chromatograms of formulated [^{18}F]**BA3**

Figure S3: Representative radio-MLC chromatograms of *in vivo* metabolism studies

Figure S4: Baseline time-activity curves (TACs) of CD-1 mice brain region after injection of [^{18}F]**BA3**

Figure S5: Biodistribution of [^{18}F]**BA3** at different time points derived from PET imaging

Table S1: Tissue biodistribution of radioactivity at different time point after i.v. injection of [^{18}F]**BA3** in CD-1 mice

Figure S6: Representative maximal intensity projection map of [^{18}F]**BA3**

Figure S7 - **Figure S46**: ^{1}H-, ^{13}C- and ^{19}F-NMR spectra and LC-MS chromatograms for compounds **BA1**-**BA10**

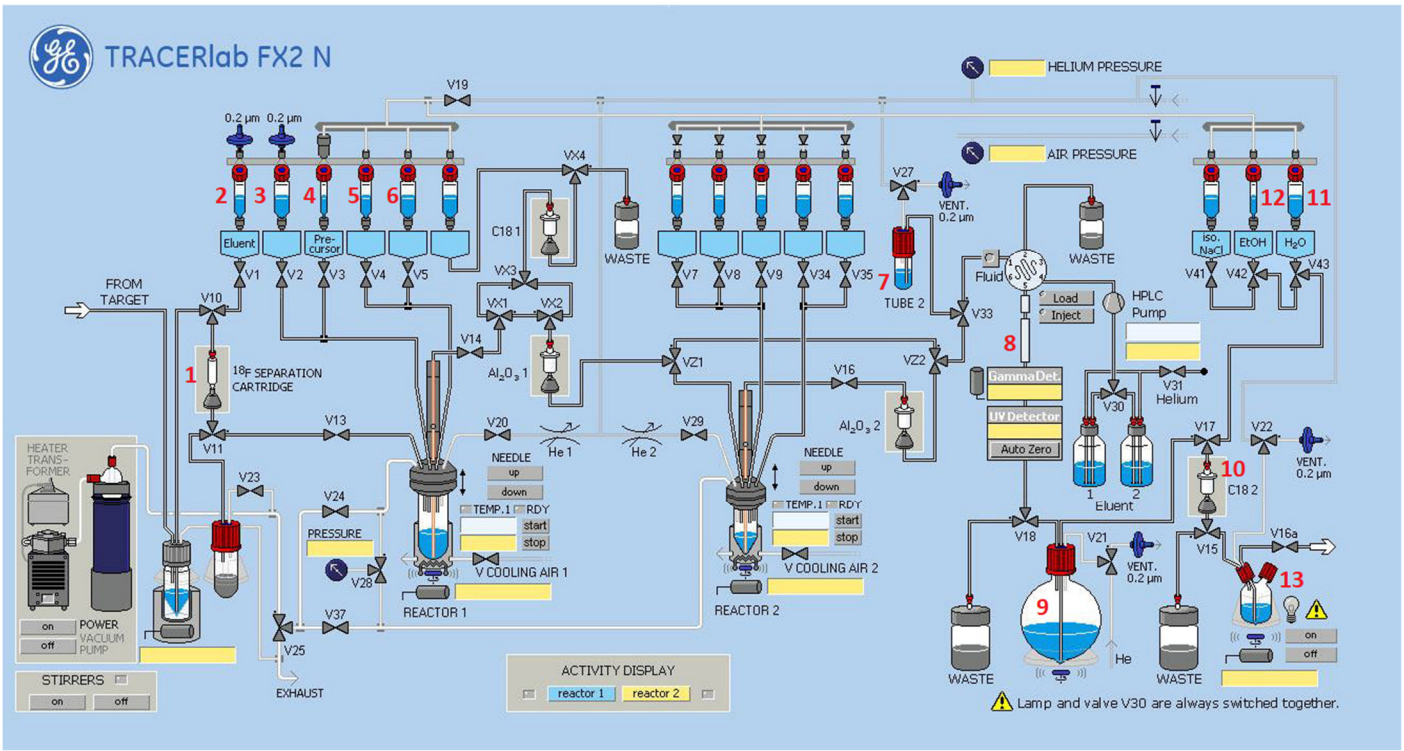


Figure S1. Scheme of the synthesis module TRACERlab FX2 N for the radiosynthesis of [^{18}F]**BA3**. (**1**) Sep-Pak® Accell QMA light cartridge, (**2**) 150 µL $TBAHCO_3$ (0.075 M) in 300 µL H_2O and 600 µL MeCN, (**3**) 2 mL MeCN, (**4**) precursor (4 mg of **9** in 800 µL MeCN), (**5**) 800 µL 2M HCl_{aq}, (**6**) 1.6 mL 1M $NaHCO_{3,aq}$ and 1.8 mL 100mM phosphate buffer (pH

= 6), (**7**) injection vial, (**8**) Reprosil-Pur C18-AQ (40% ACN/20mM NH_4OAc_{aq}, flow 4.0 mL/min), (**9**) 40 mL water, (**10**) Sep-Pak® C18 light, (**11**) 2 mL H_2O, (**12**) 1.2 mL EtOH, (**13**) product vial.

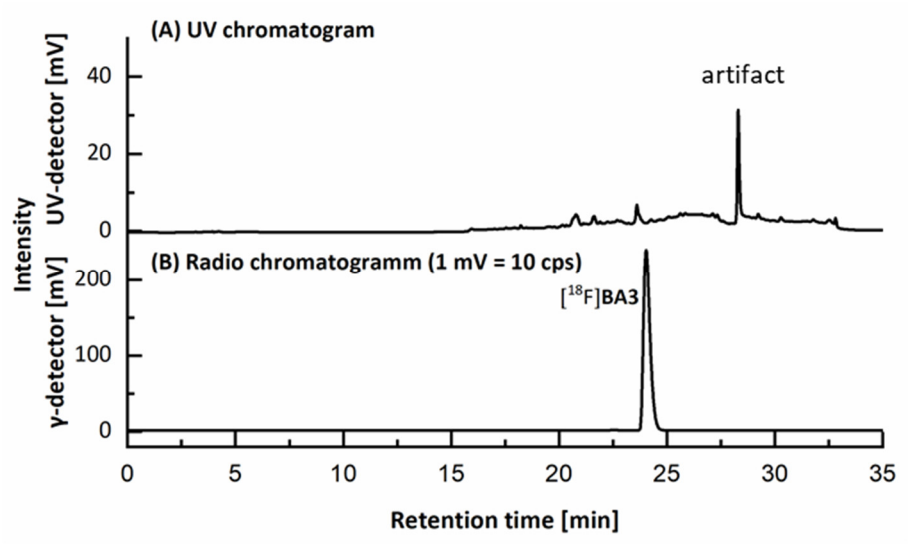


Figure S2. Representative (**A**) UV- and (**B**) radio-RP-HPLC chromatograms of formulated [^{18}F]**BA3** (ReproSil-Pur 120 C18-AQ column (250 x 4.6 mm, 5 µm), Gradient MeCN/20 mM $NH_4OAc_{aq.}$ (see quality control), flow rate: 1 mL/min).

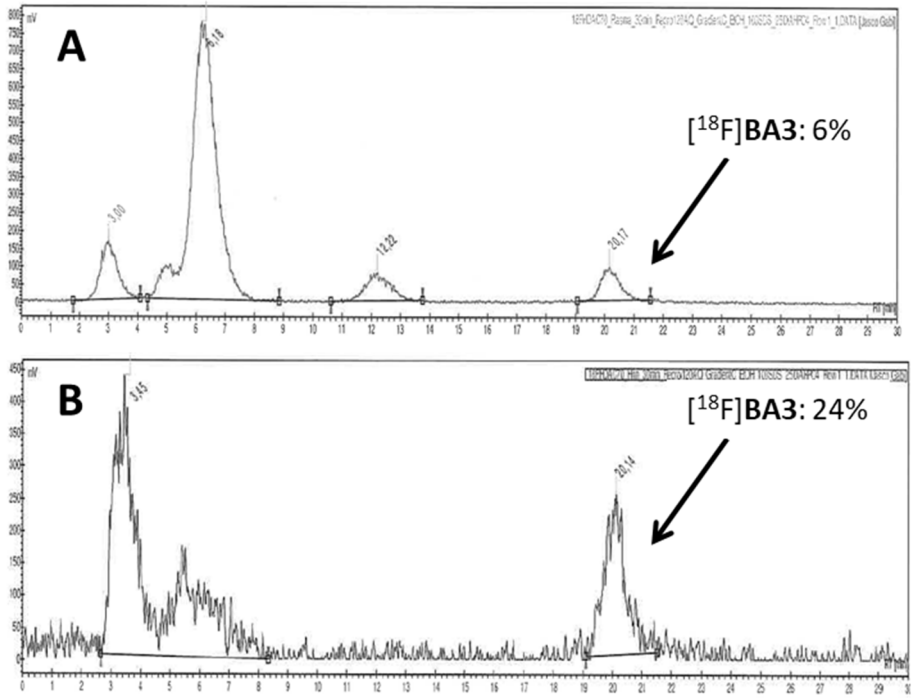


Figure S3. Representative radio-MLC chromatograms of *in vivo* metabolism studies from plasma (**A**) and brain (**B**) samples obtained 30 min p.i. of [^{18}F]**BA3** in a CD-1 mouse (gamma-detection via radio-RP-HPLC: ReproSil-Pur 120 C18-AQ column (250 x 4.6 mm, 10 µm + 10 mm pre-column), Gradient EtOH/100 mM SDS_{aq}/25 mM $(NH_4)_2HPO_4$ (see section 3.5.1.), flow: 1 mL/min).

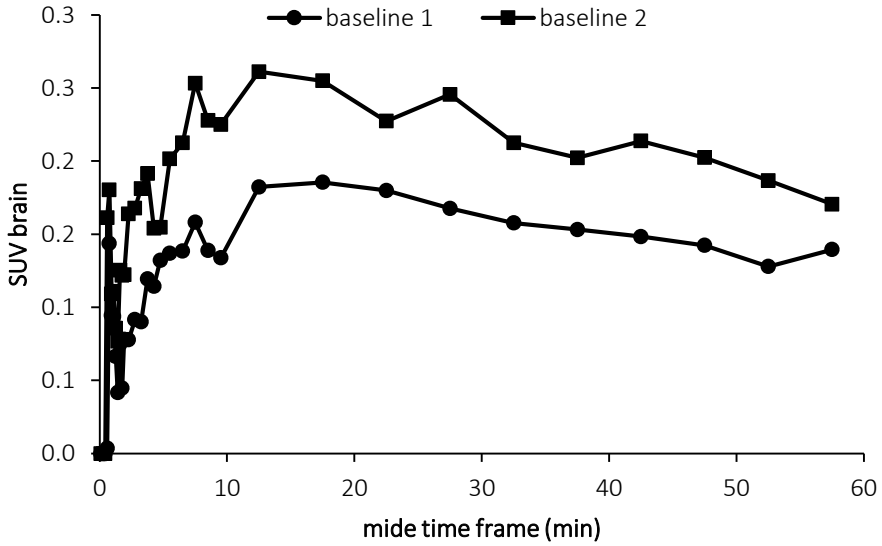


Figure S4. Baseline time-activity curves (TACs) of CD-1 mice brain region after injection of [^{18}F]**BA3** (n = 2).

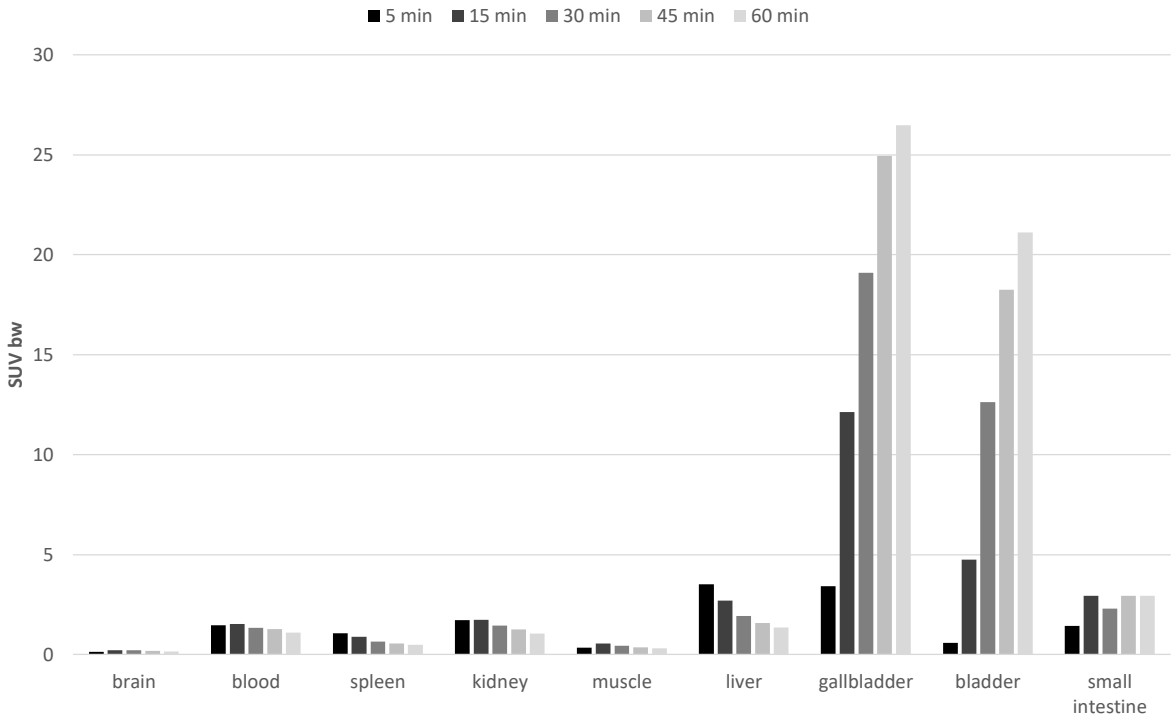


Figure S5. Biodistribution of [^{18}F]**BA3** at different time points derived from PET imaging uncorrected for metabolites (n = 2, SUV_{mean})

Table S1. Tissue biodistribution of radioactivity at different time point after i.v. injection of [^{18}F]**BA3** in CD-1 mice based on PET data uncorrected for metabolites (n = 2).

	Uptake (SUV)				
	5 min	**15 min**	**30 min**	**45 min**	**60 min**
brain	0.1 / 0.2	0.2 / 0.3	0.2 / 0.3	0.2 / 0.2	0.1 / 0.2
blood	1.7 / 1.2	1.8 / 1.2	1.6 / 1.0	1.6 / 0.9	1.4 / 0.9
spleen	1.2 / 0.9	1.0 / 0.8	0.7 / 0.6	0.6 / 0.5	0.5 / 0.5

kidney	1.7 / 1.7	1.7 / 1.8	1.4 / 1.6	1.1 / 1.4	0.9 / 1.2
muscle	0.3 / 0.4	0.6 / 0.6	0.5 / 0.4	0.4 / 0.4	0.3 / 0.3
liver	4.1 / 3.0	3.1 / 2.4	2.2 / 1.7	1.8 / 1.4	1.6 / 1.2
gallbladder	5.0 / 1.9	19.1 / 5.2	23.1 / 15.1	26.3 / 23.5	21.3 / 31.6
bladder	0.7 / 0.5	5.9 / 3.6	13.4 / 11.8	19.0 / 17.5	19.3 / 23.0
small intestine	1.7 / 1.2	3.7 / 2.2	2.4 / 2.2	2.5 / 3.4	3.0 / 2.9

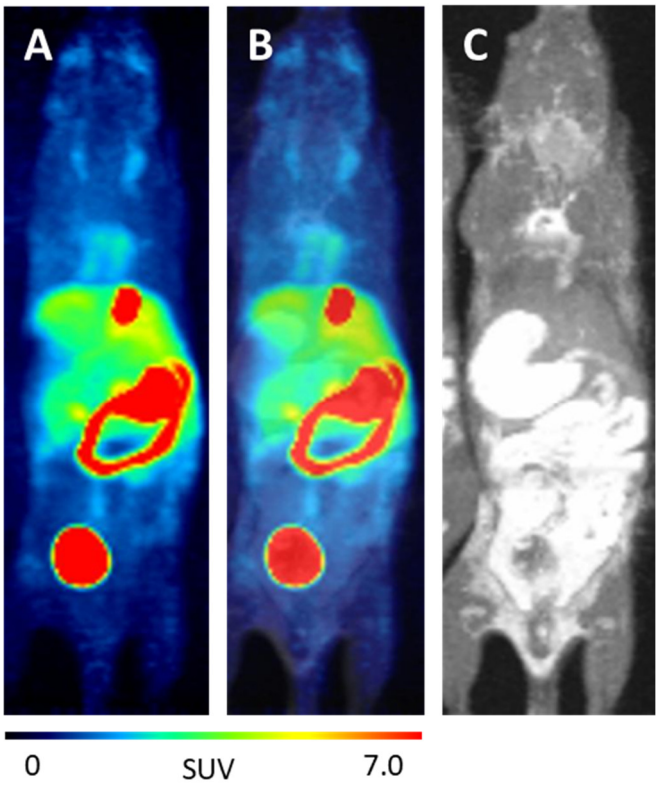


Figure S6. Representative maximal intensity projection map of [^{18}F]**BA3** of (**A**) PET modality or (**B**) merged PET and MR modalities and (**C**) MR modality.

***N*-[2-amino-5-(thiophen-2-yl)phenyl]-4-[(2-fluoropropanamido)methyl]benzamide (BA1)**

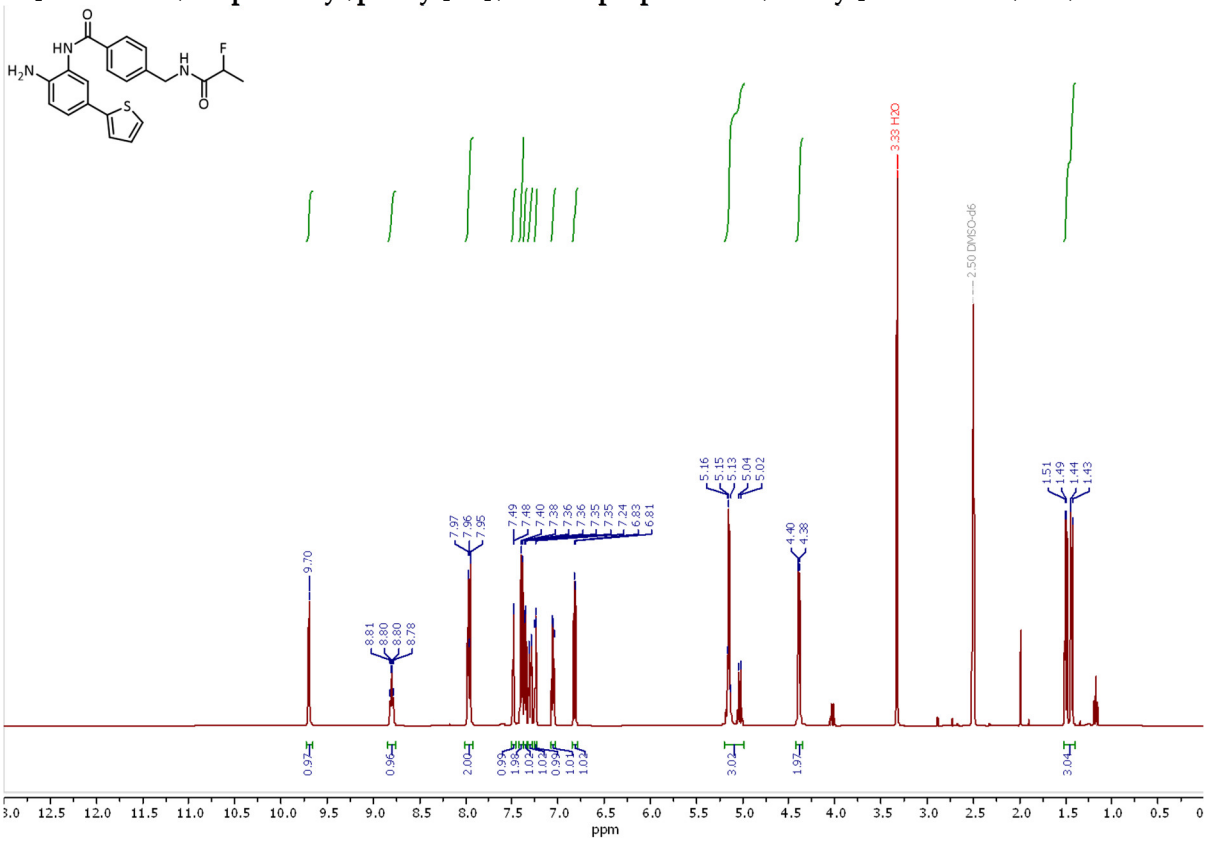


Figure S7. [1]H-NMR of **BA1**

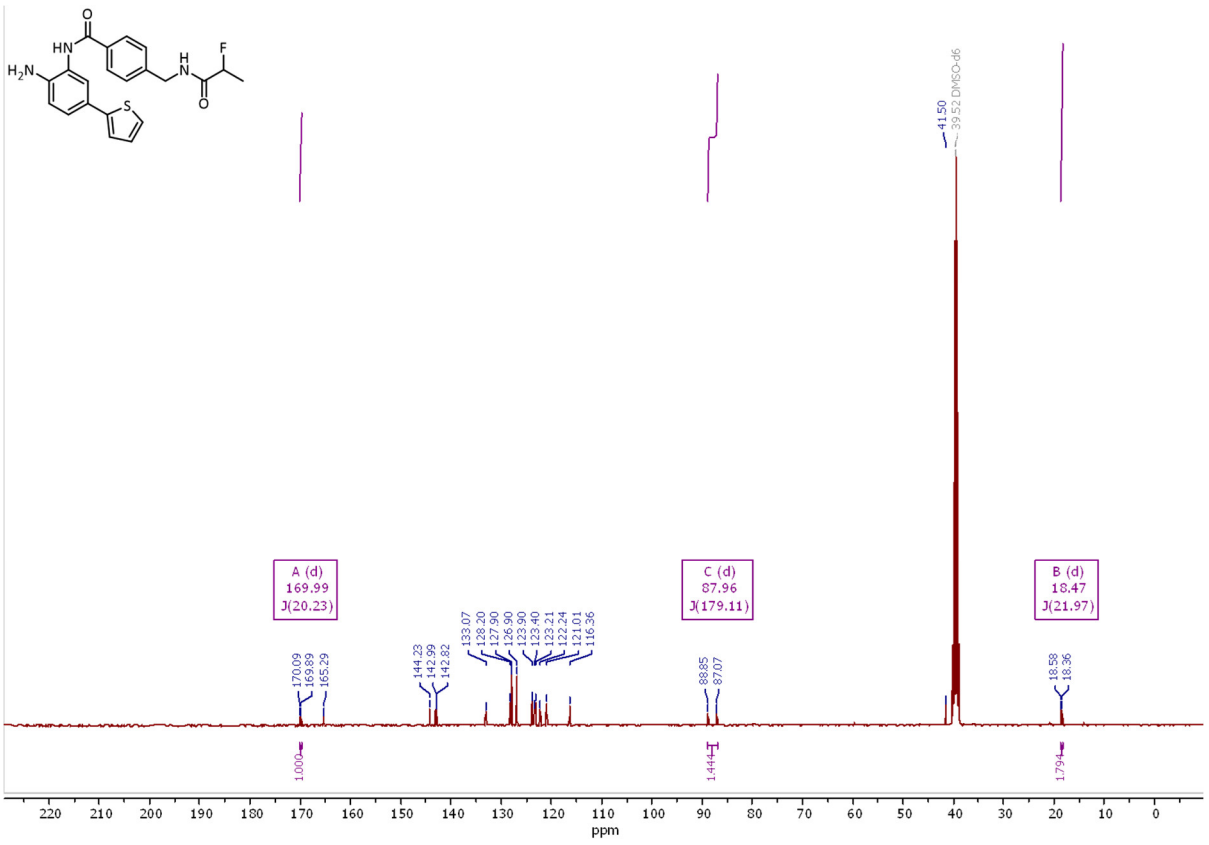


Figure S8. [13]C-NMR of **BA1**

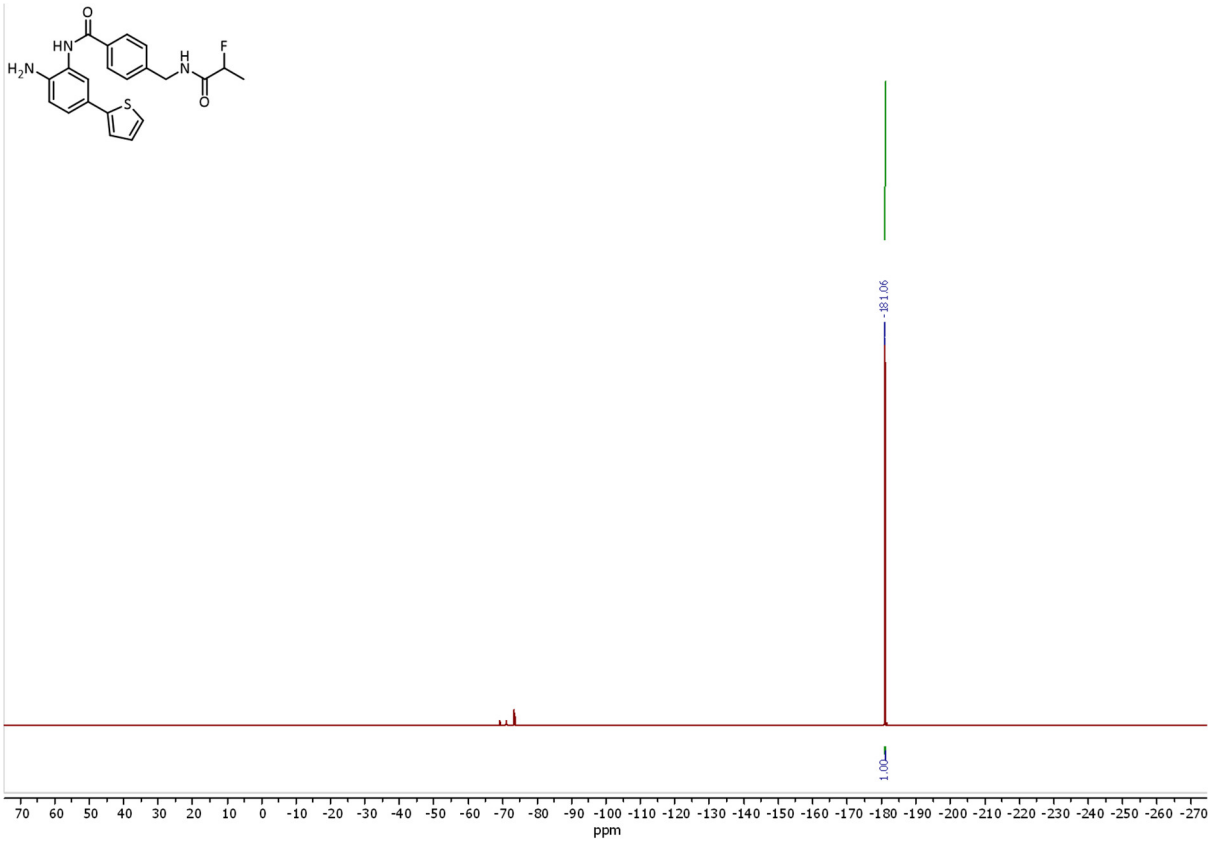


Figure S9. ^{19}F-NMR of **BA1**

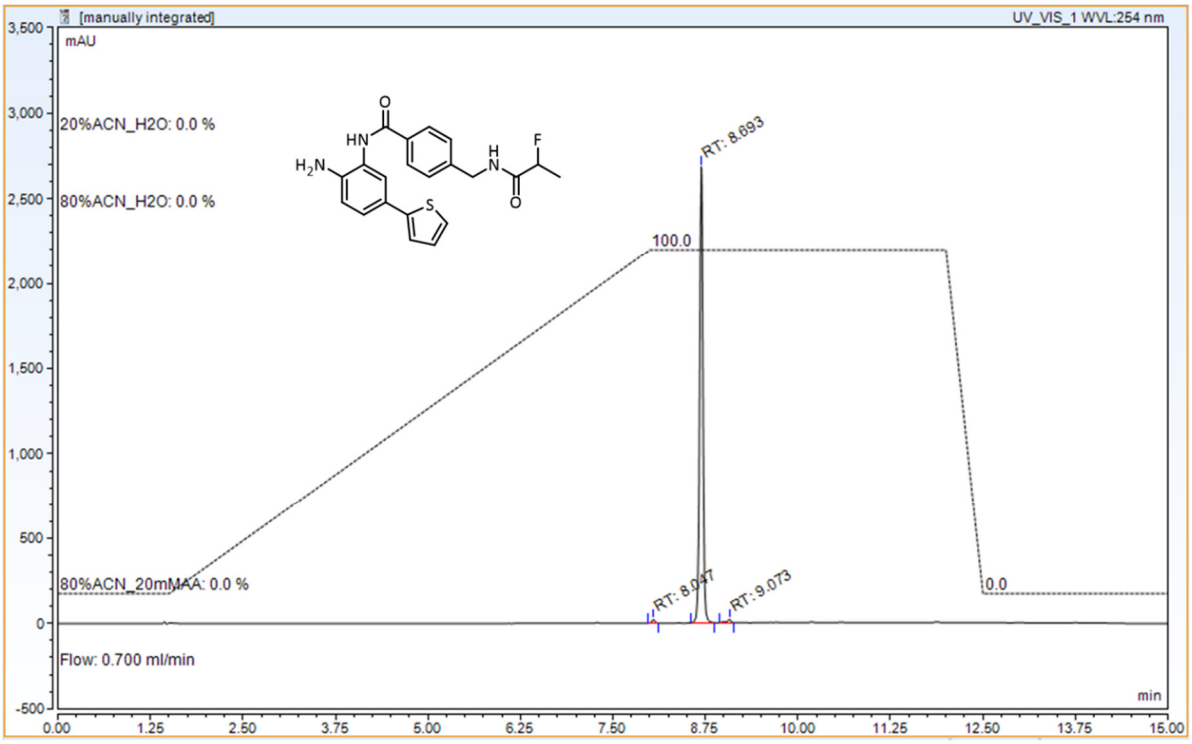


Figure S10. LC-MS chromatogram of **BA1**

***N*-[2-amino-5-(thiophen-2-yl)phenyl]-4-(2-fluoropropanamido)benzamide (BA2)**

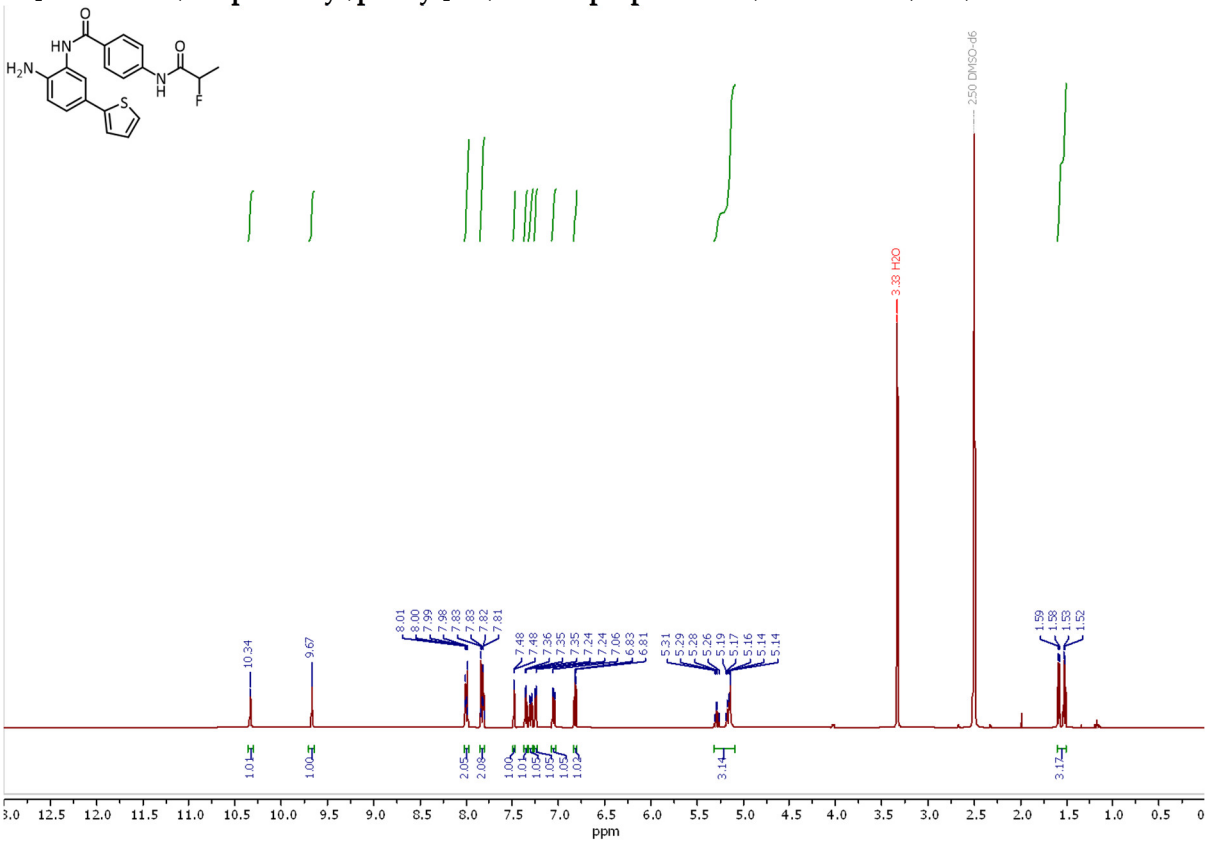


Figure S11. [1]H-NMR of **BA2**

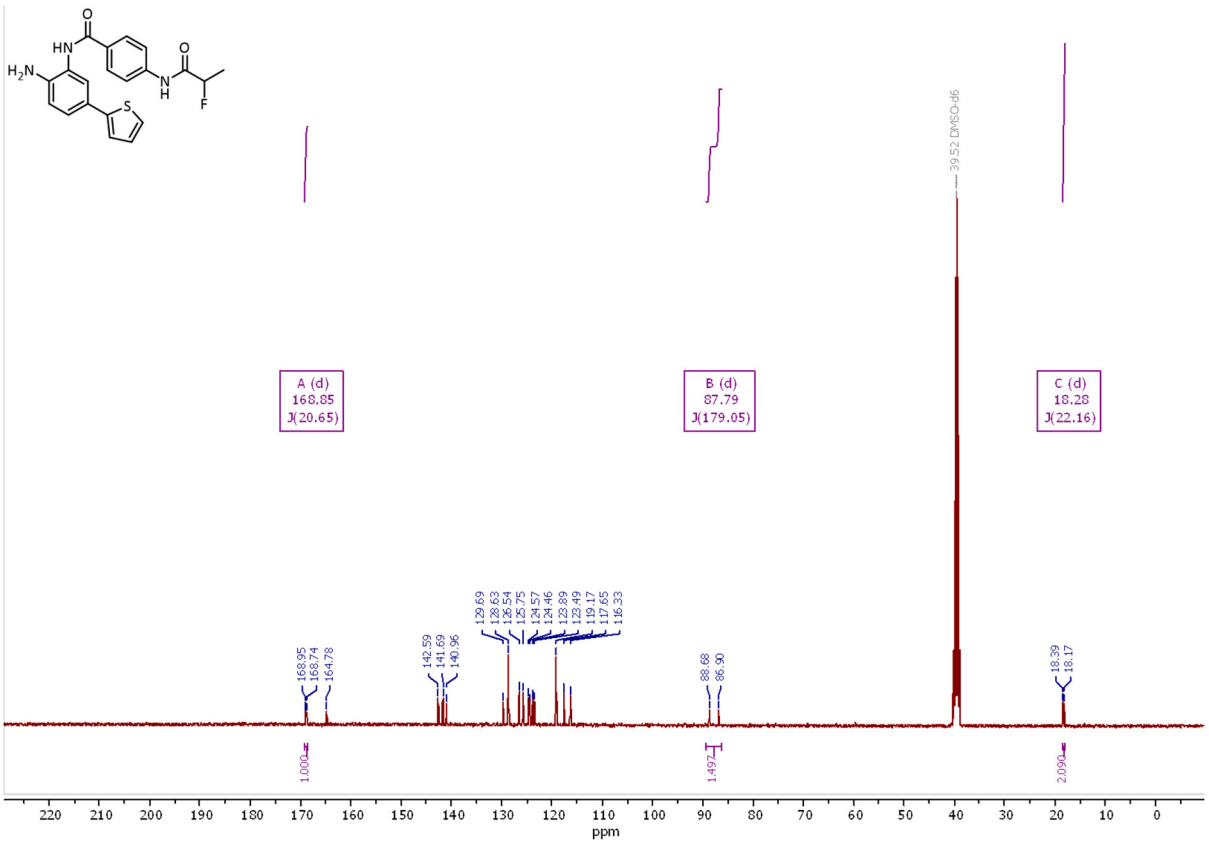


Figure S12. [13]C-NMR of **BA2**

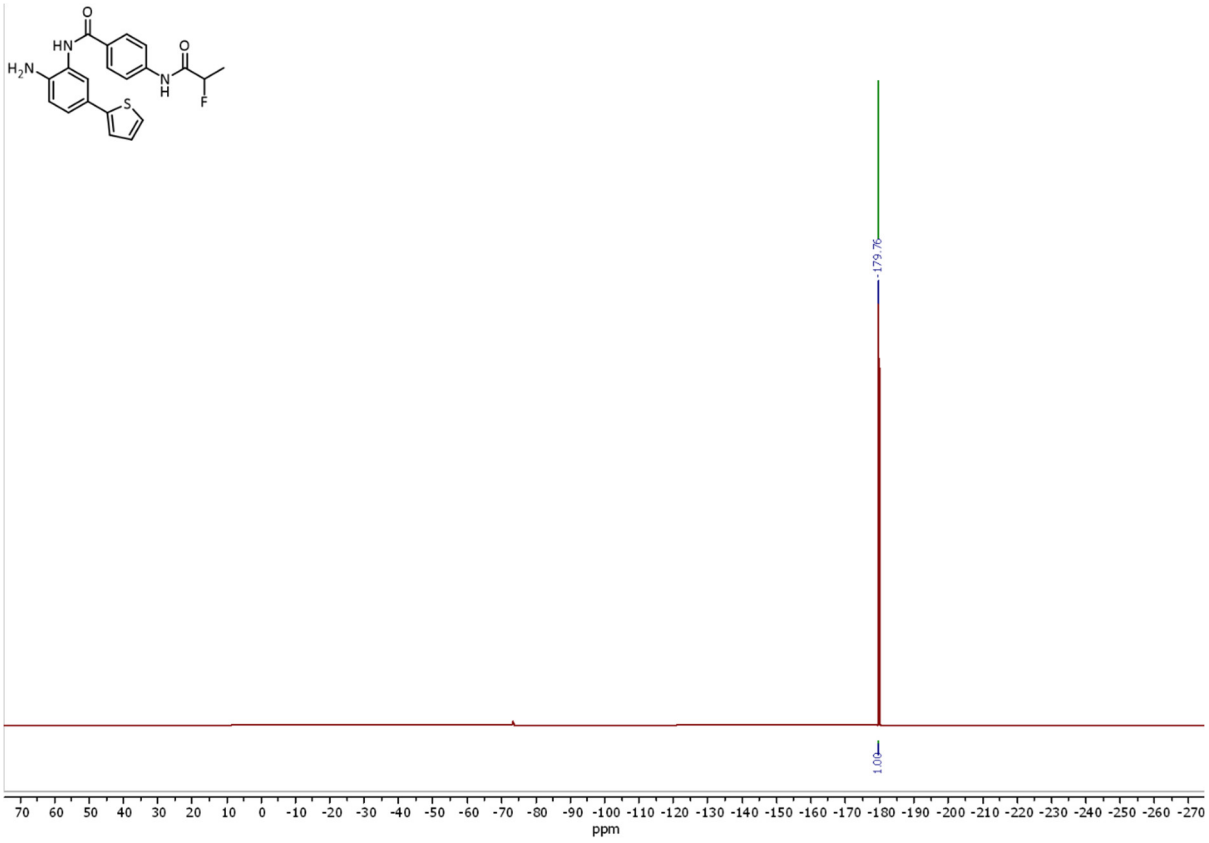


Figure S13. ^{19}F-NMR of **BA2**

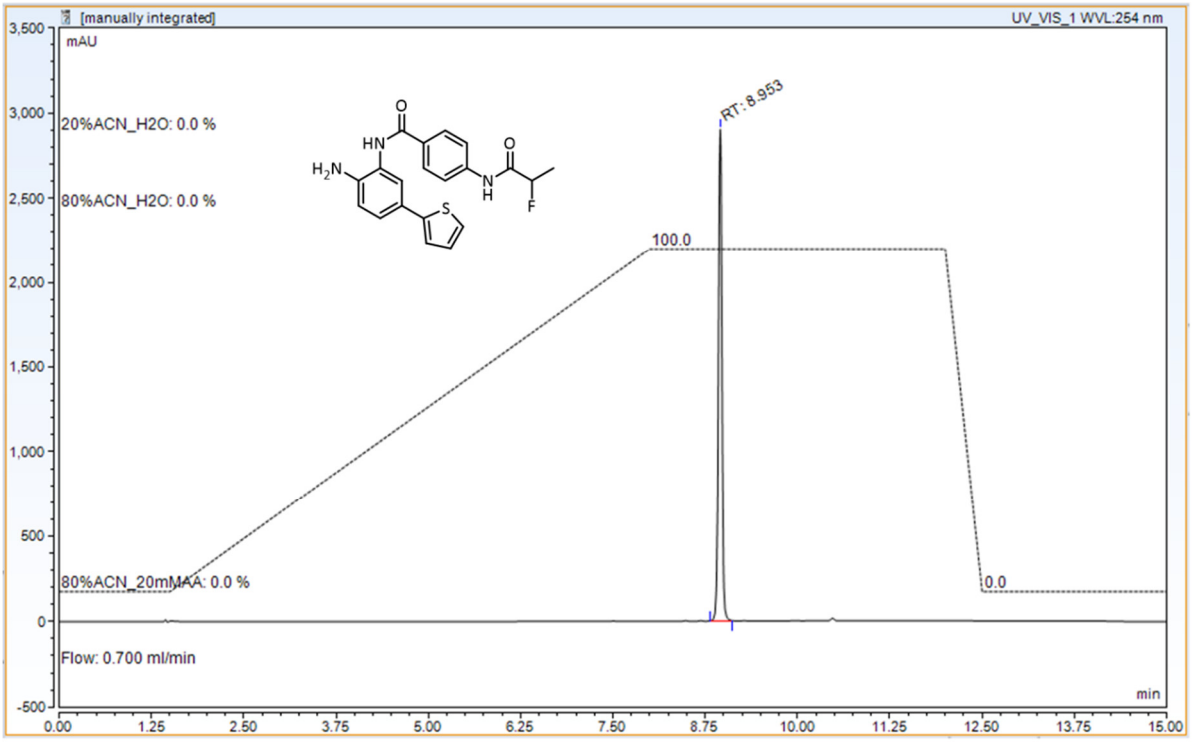


Figure S14. LC-MS chromatogram of **BA2**

***N*-[2-amino-5-(thiophen-3-yl)phenyl]-4-[(2-fluoropropanamido)methyl]benzamide (BA3)**

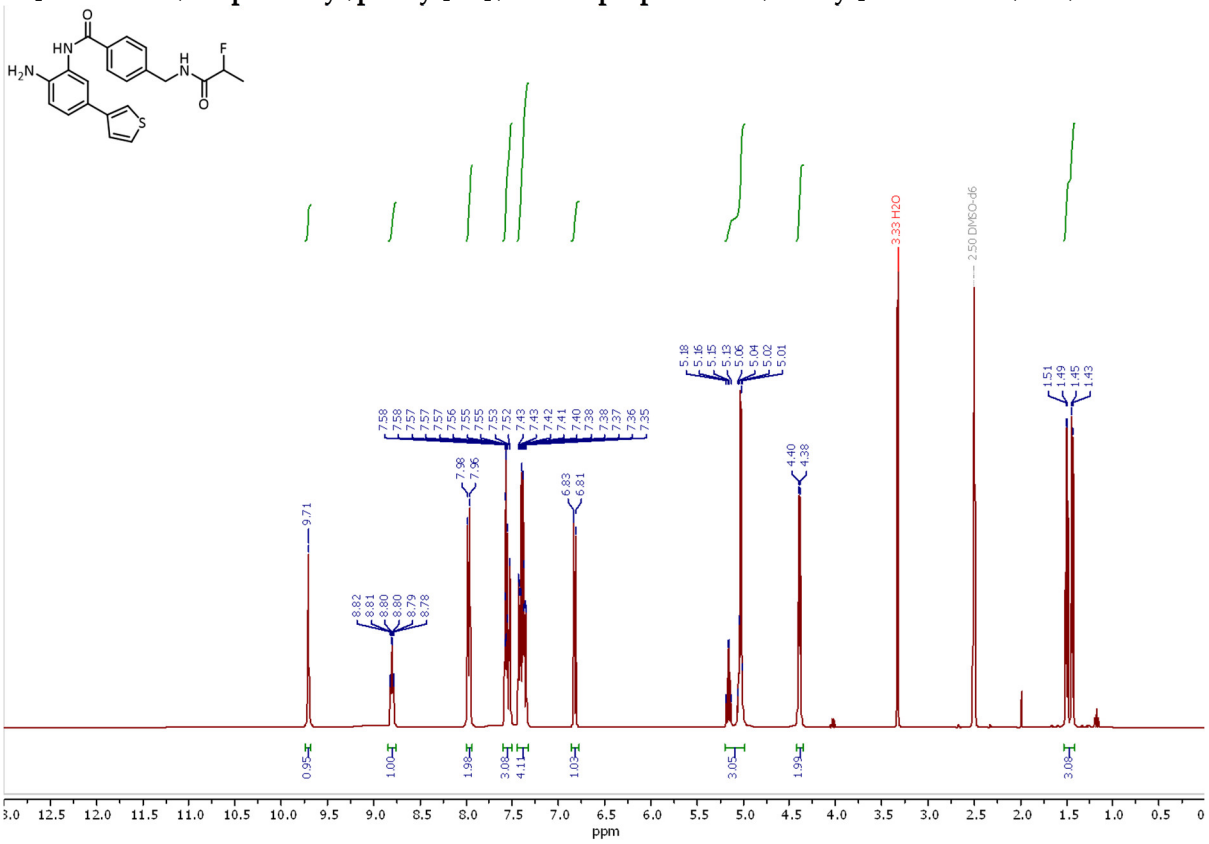


Figure S15. [1]H-NMR of **BA3**

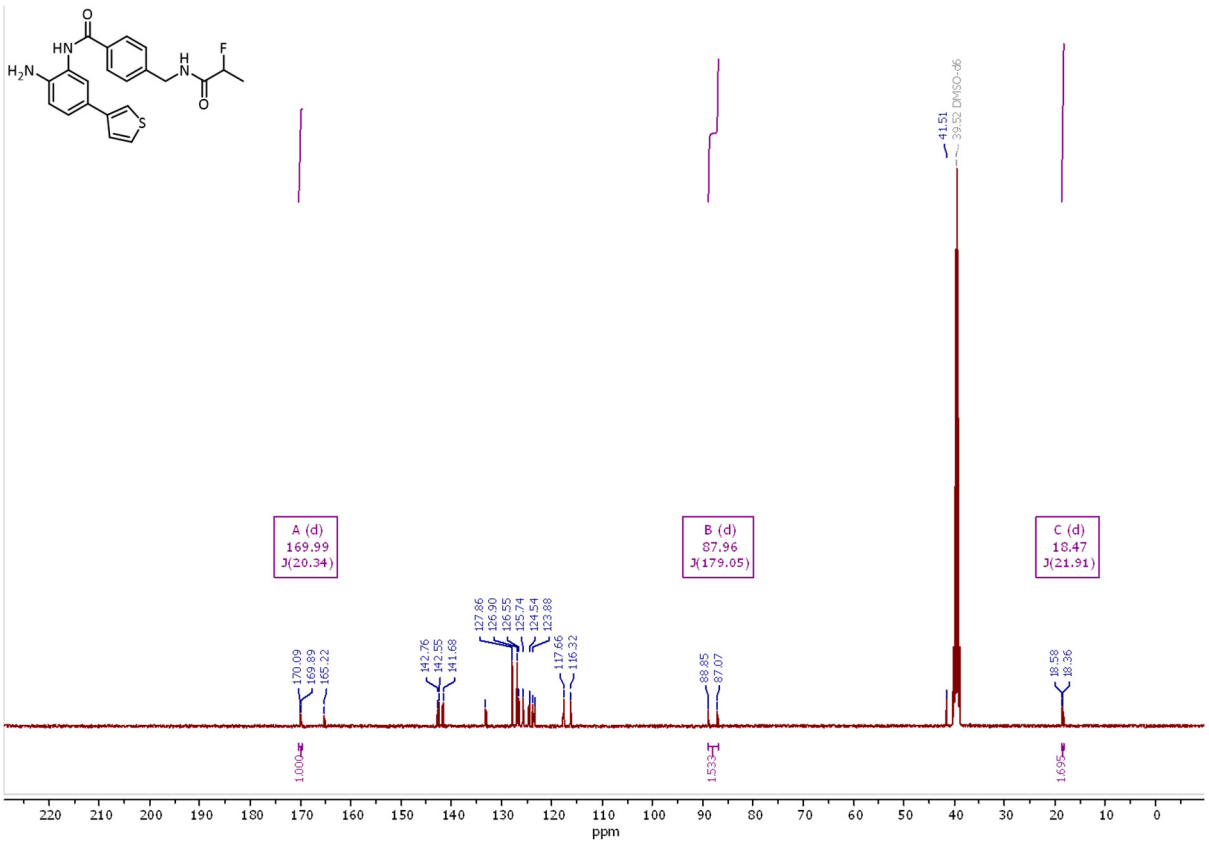


Figure S16. [13]C-NMR of **BA3**

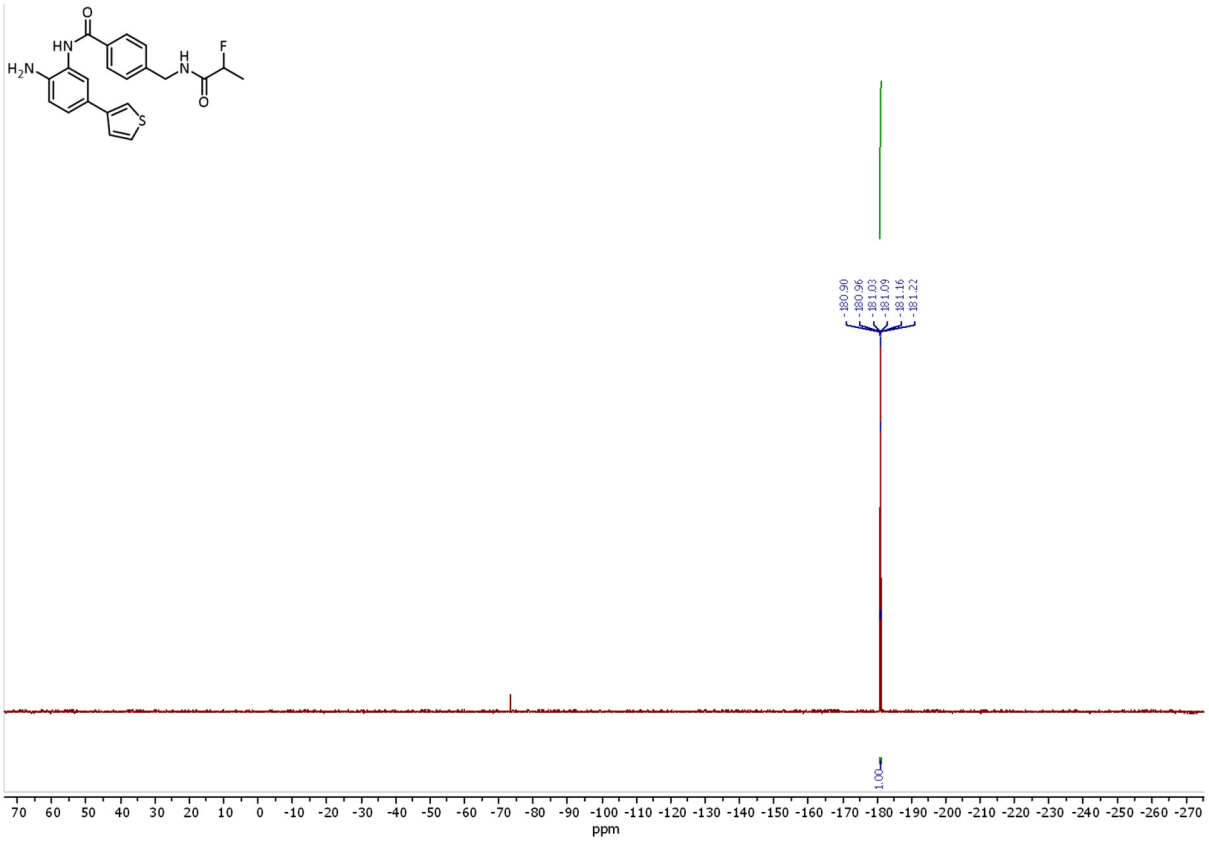


Figure S17. ^{19}F-NMR of **BA3**

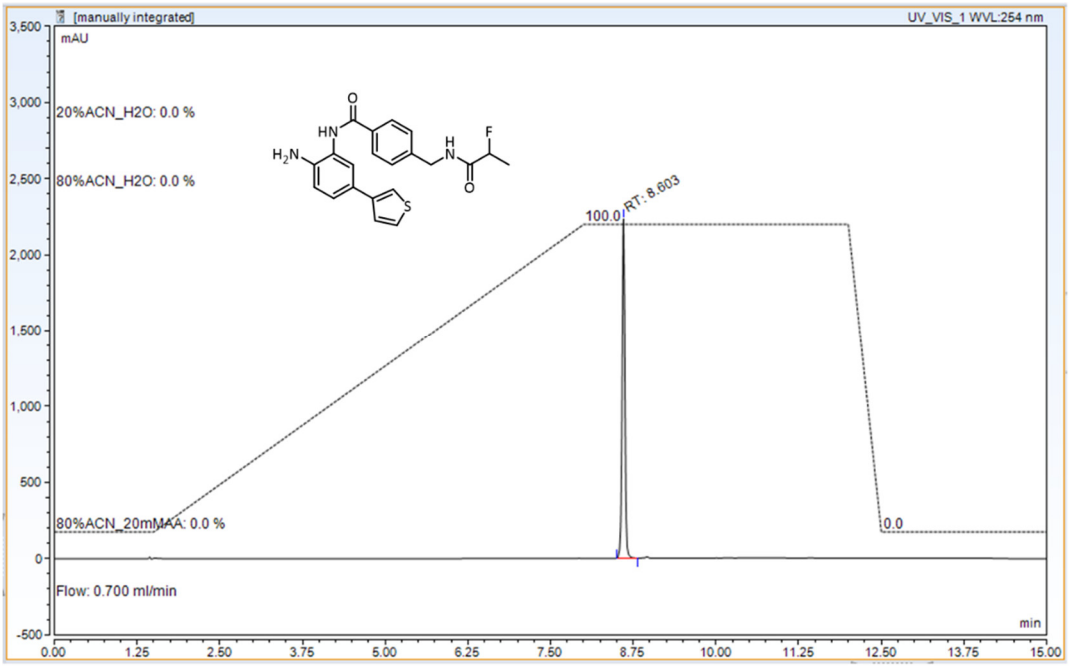


Figure S18. LC-MS chromatogram of **BA3**

***N*-[2-amino-5-(thiophen-3-yl)phenyl]-4-(2-fluoropropanamido)benzamide (BA4)**

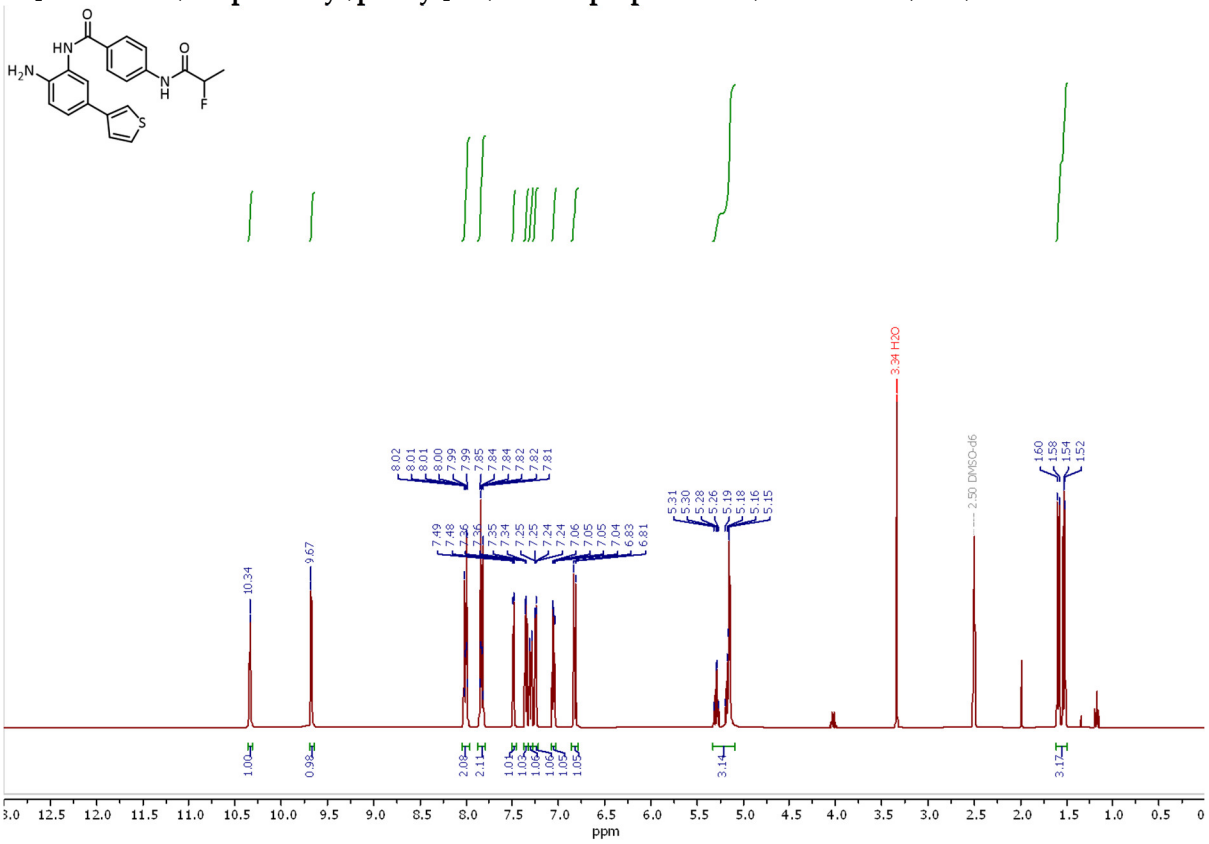


Figure S19. [1]H-NMR of **BA4**

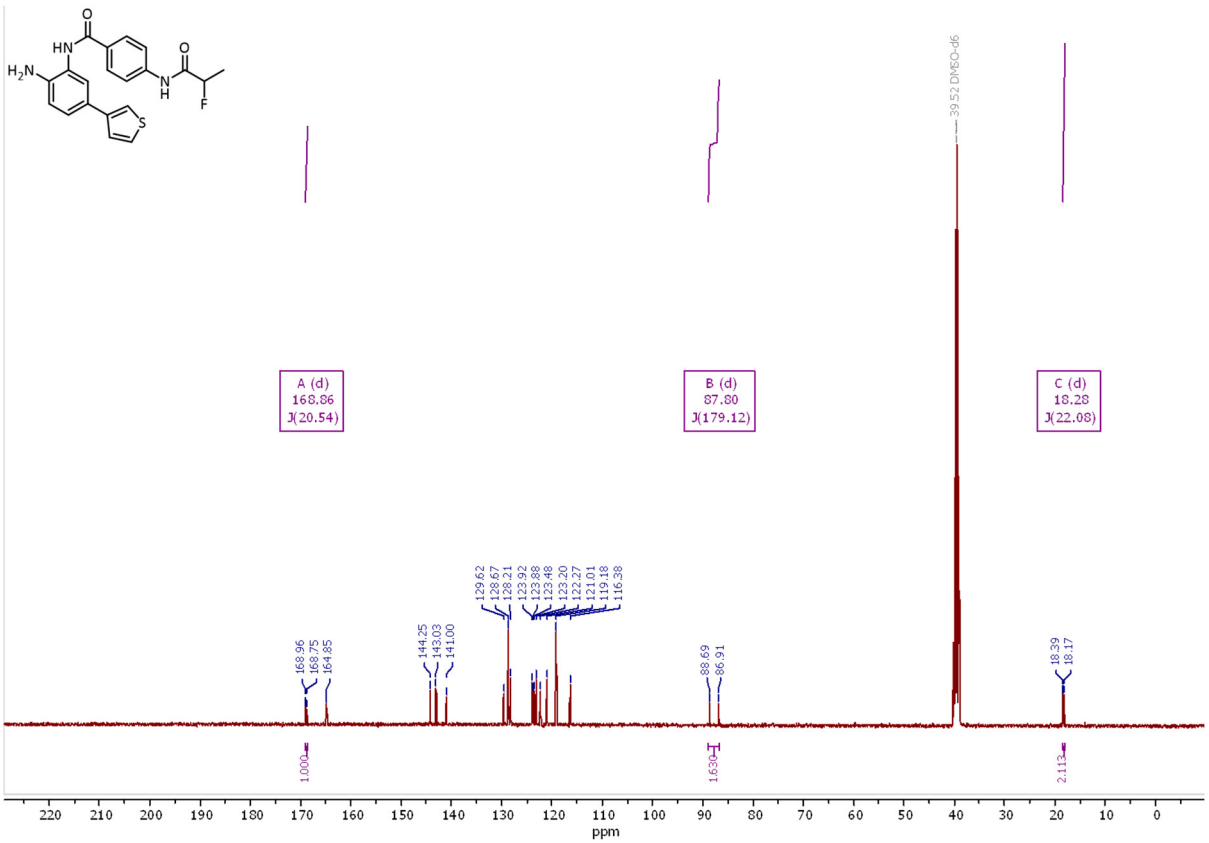


Figure S20. [13]C-NMR of **BA4**

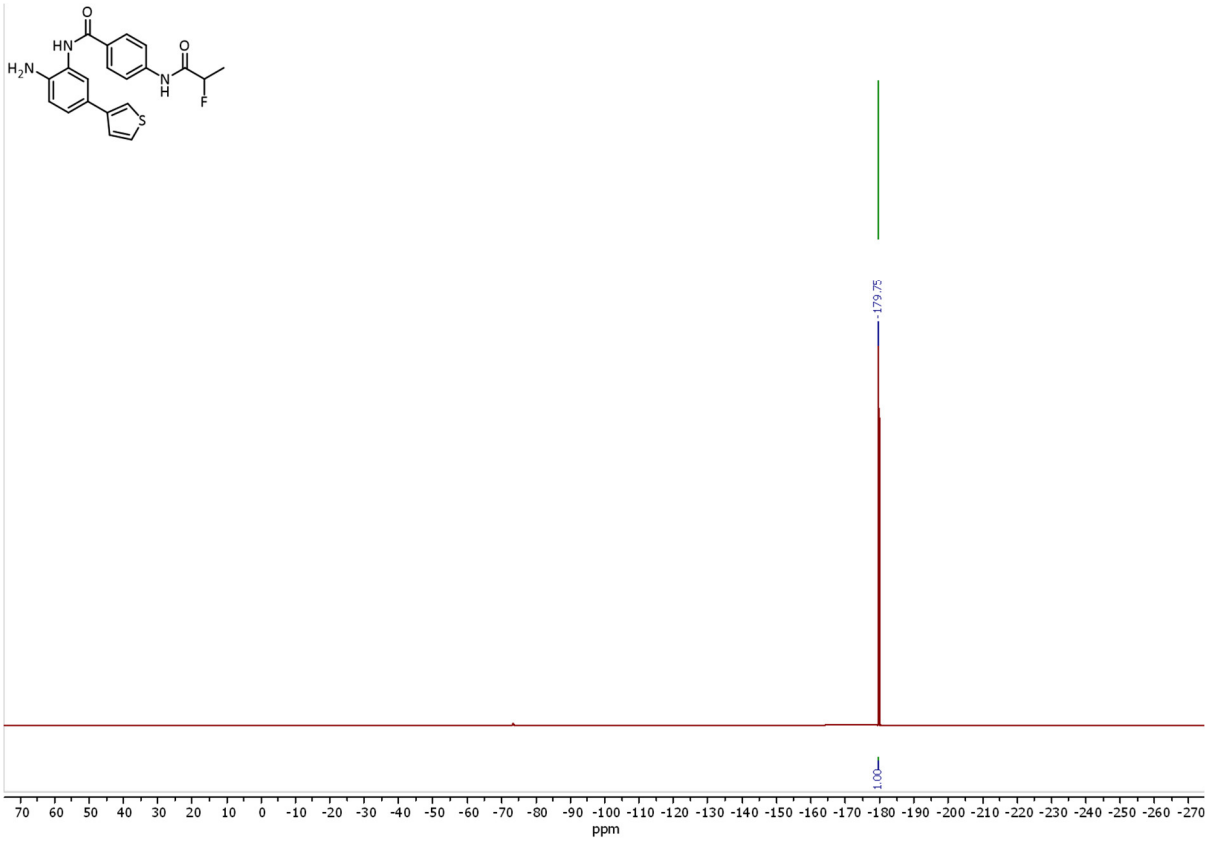


Figure S21. ^{19}F-NMR of **BA4**

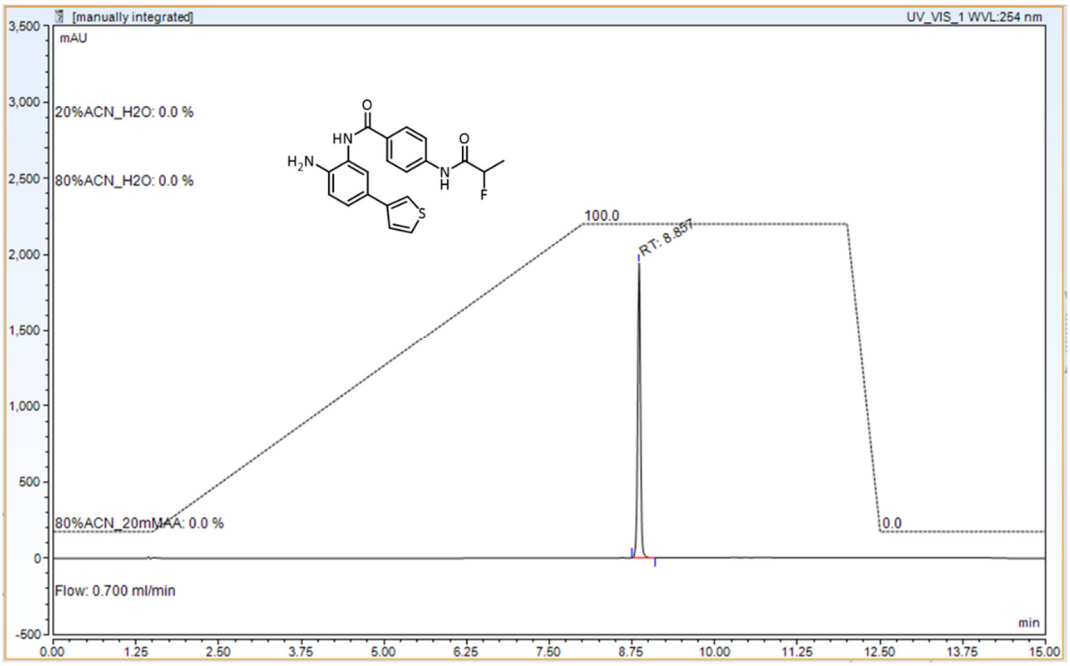


Figure S22. LC-MS chromatogram of **BA4**

***N*-(4-amino-4'-fluoro-[1,1'-biphenyl]-3-yl)-4-[(2-fluoropropanamido)methyl]benzamide (BA5)**

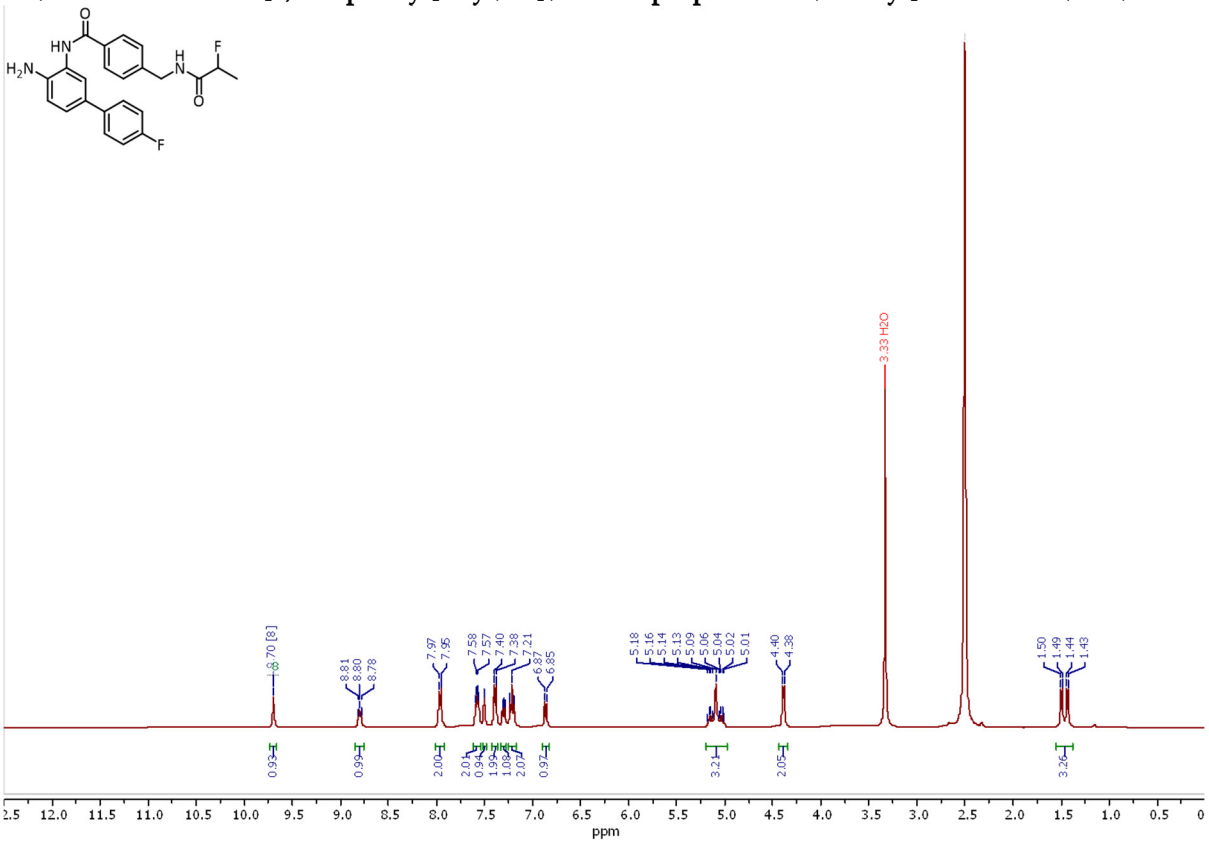


Figure S23. ^{1}H-NMR of **BA5**

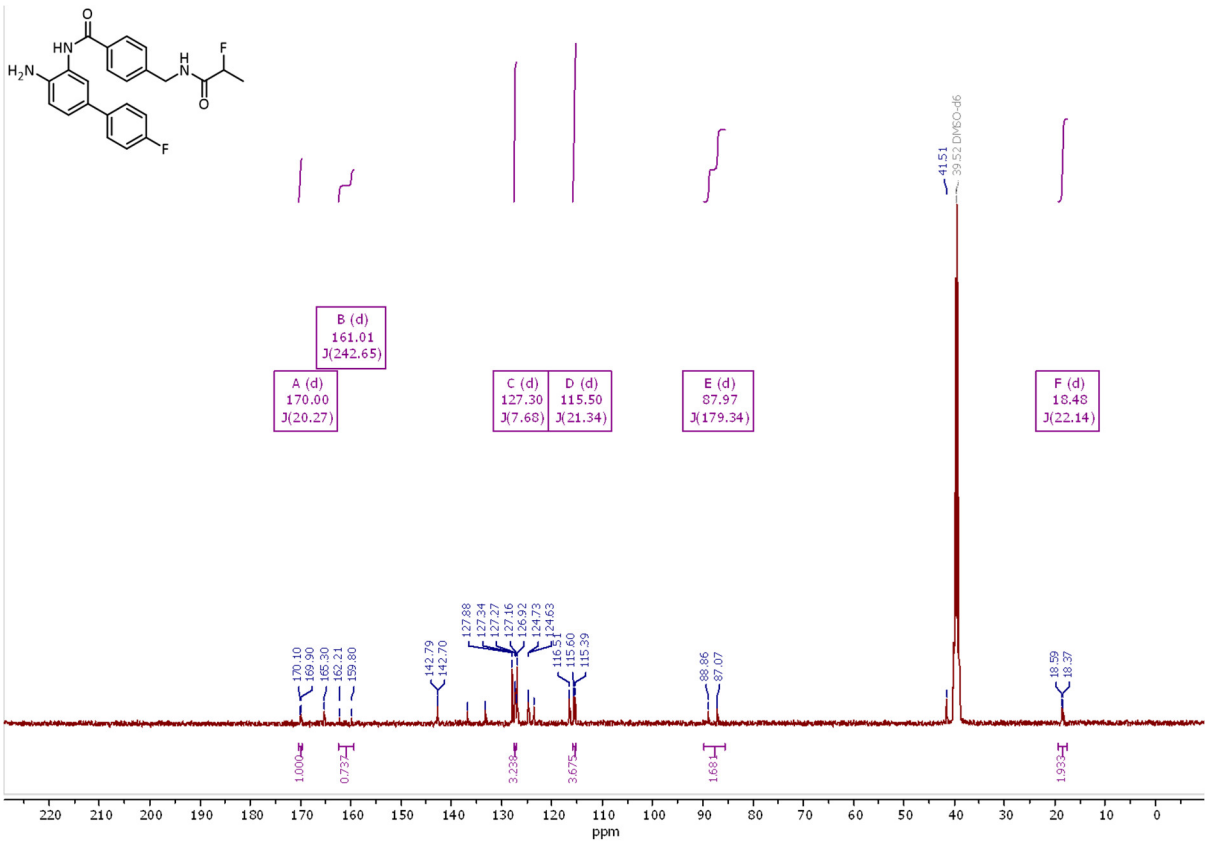


Figure S24. ^{13}C-NMR of **BA5**

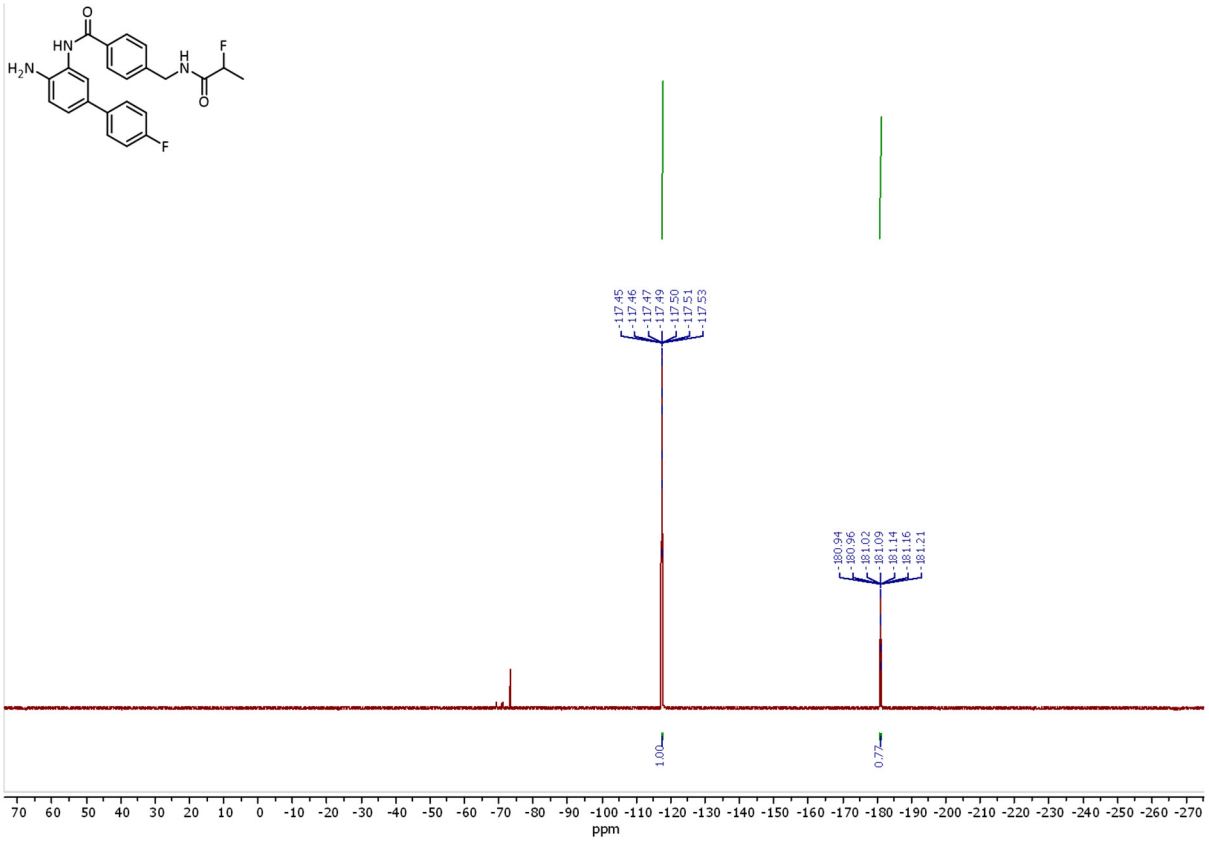


Figure S25. ^{19}F-NMR of **BA5**

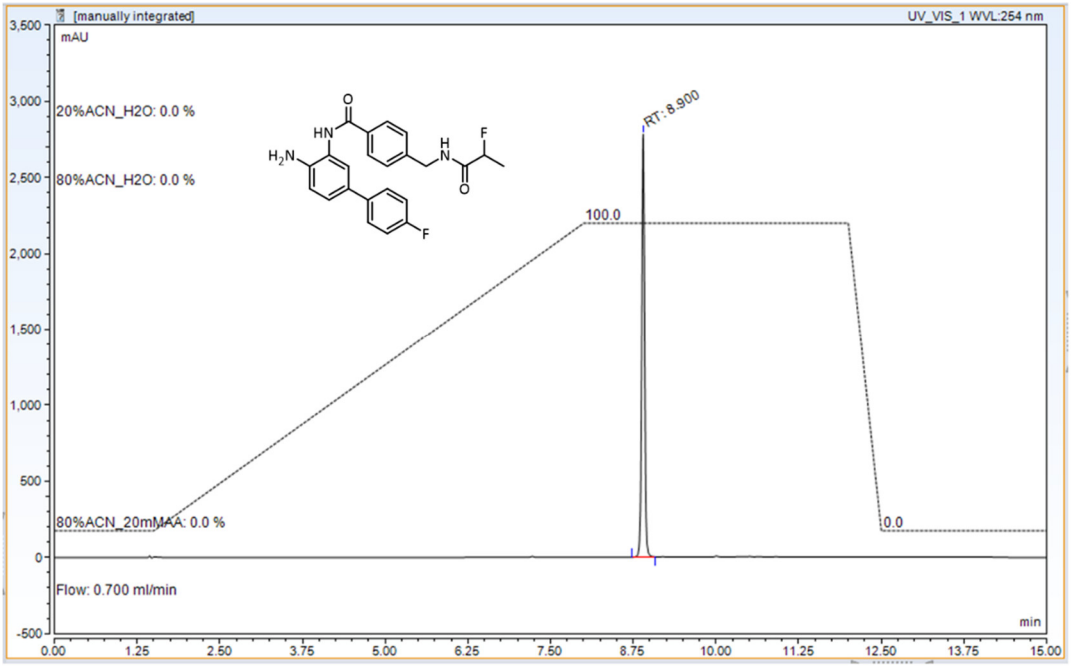


Figure S26. LC-MS chromatogram of **BA5**

***N*-(4-amino-4'-fluoro-[1,1'-biphenyl]-3-yl)-4-(2-fluoropropanamido)benzamide (BA6)**

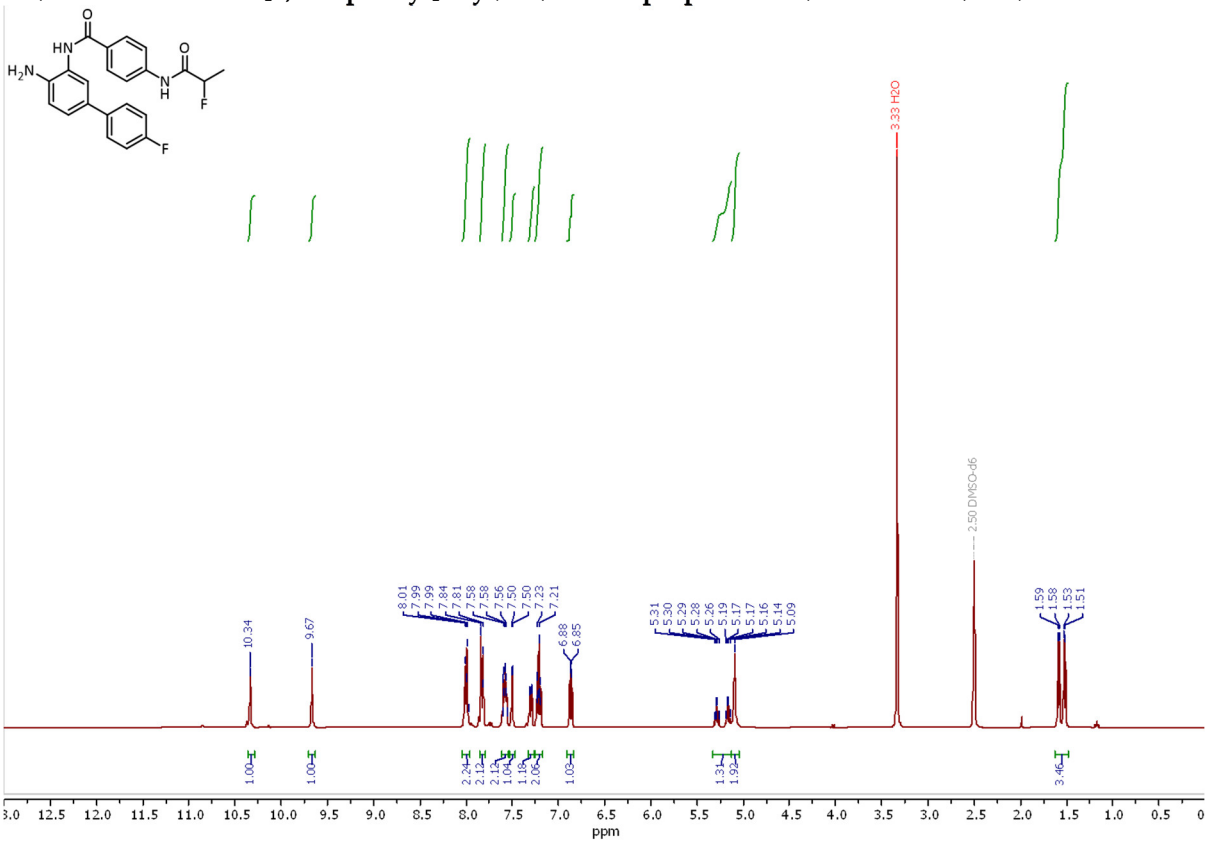


Figure S27. [1]H-NMR of **BA6**

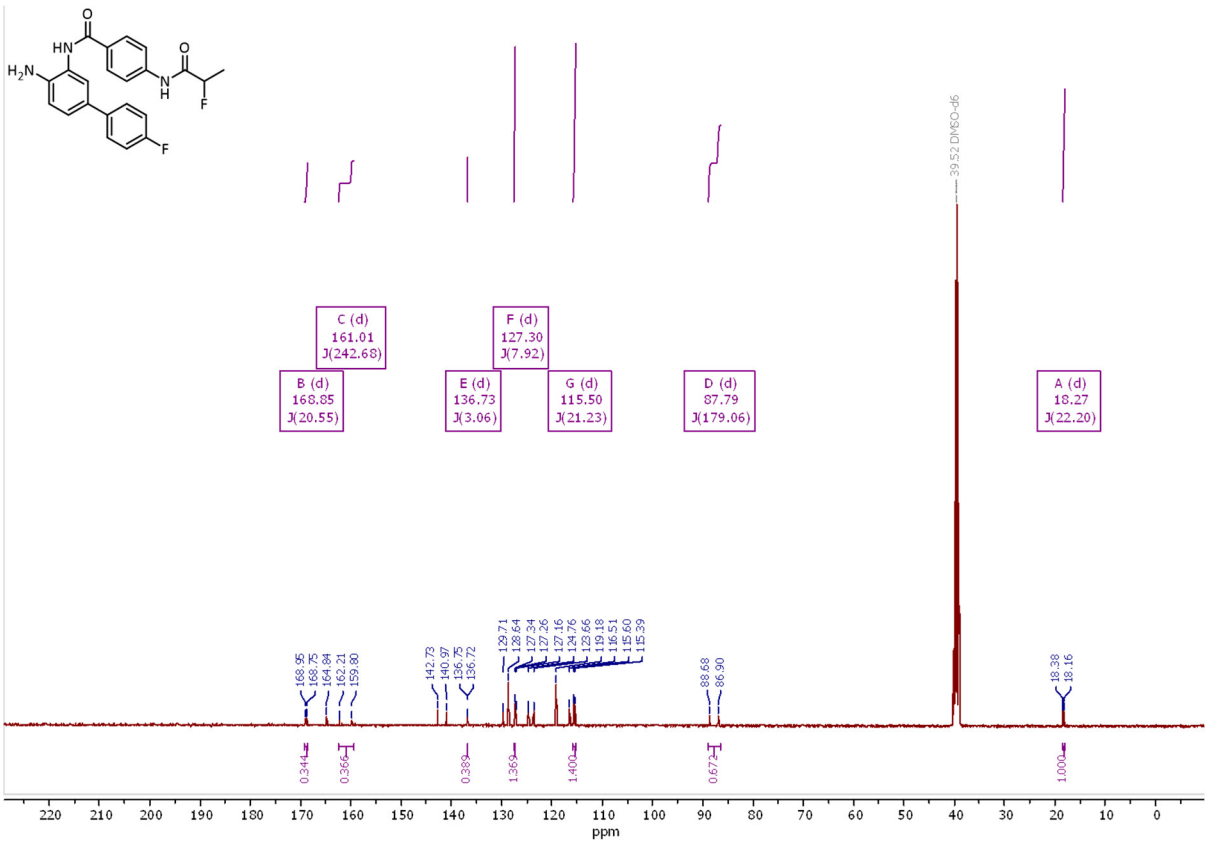


Figure S28. [13]C-NMR of **BA6**

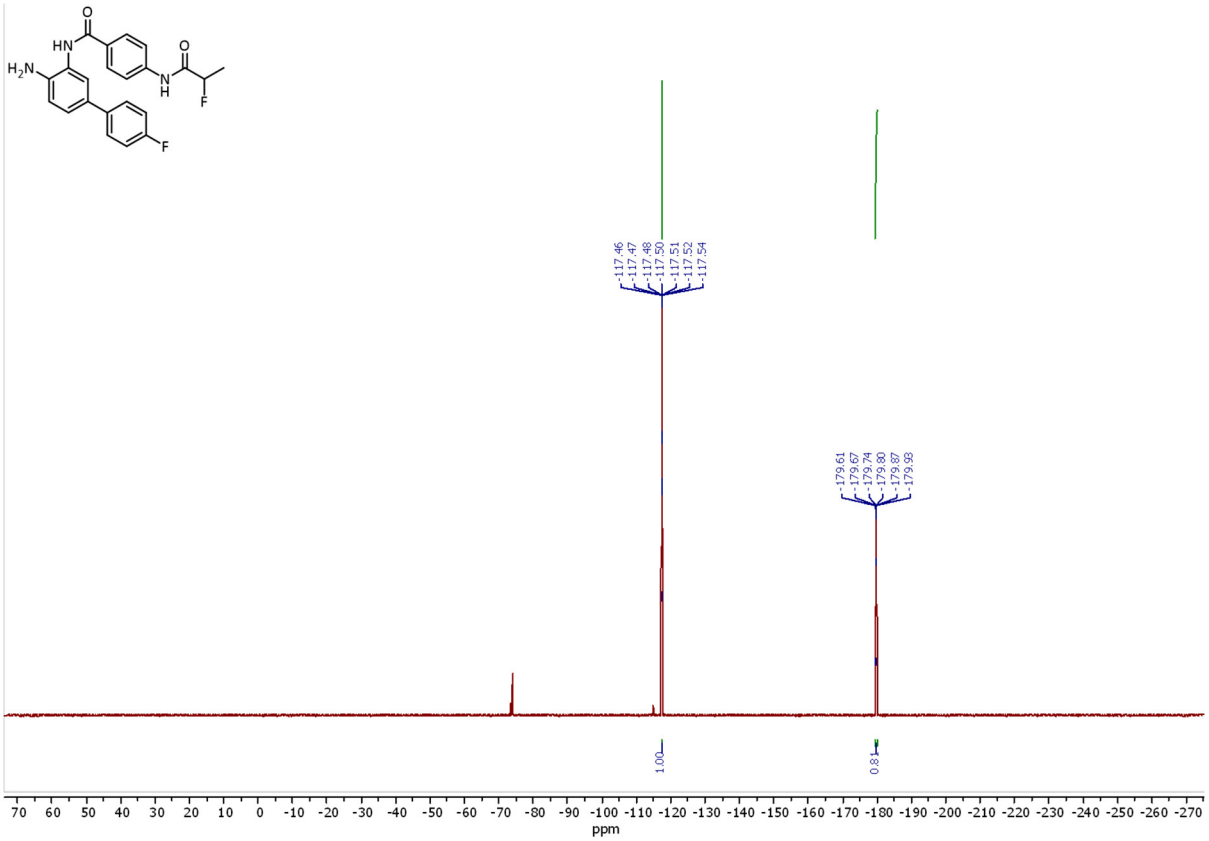


Figure S29. ^{19}F-NMR of **BA6**

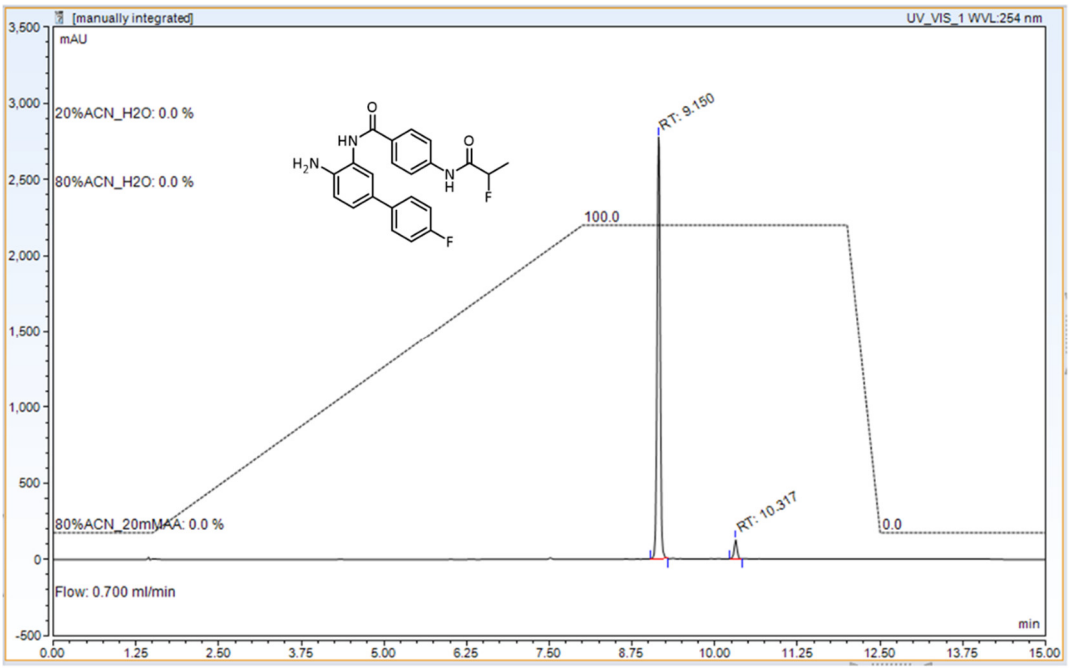


Figure S30. LC-MS chromatogram of **BA6**

***N*-[2-amino-5-(furan-2-yl)phenyl]-4-[(2-fluoropropanamido)methyl]benzamide (BA7)**

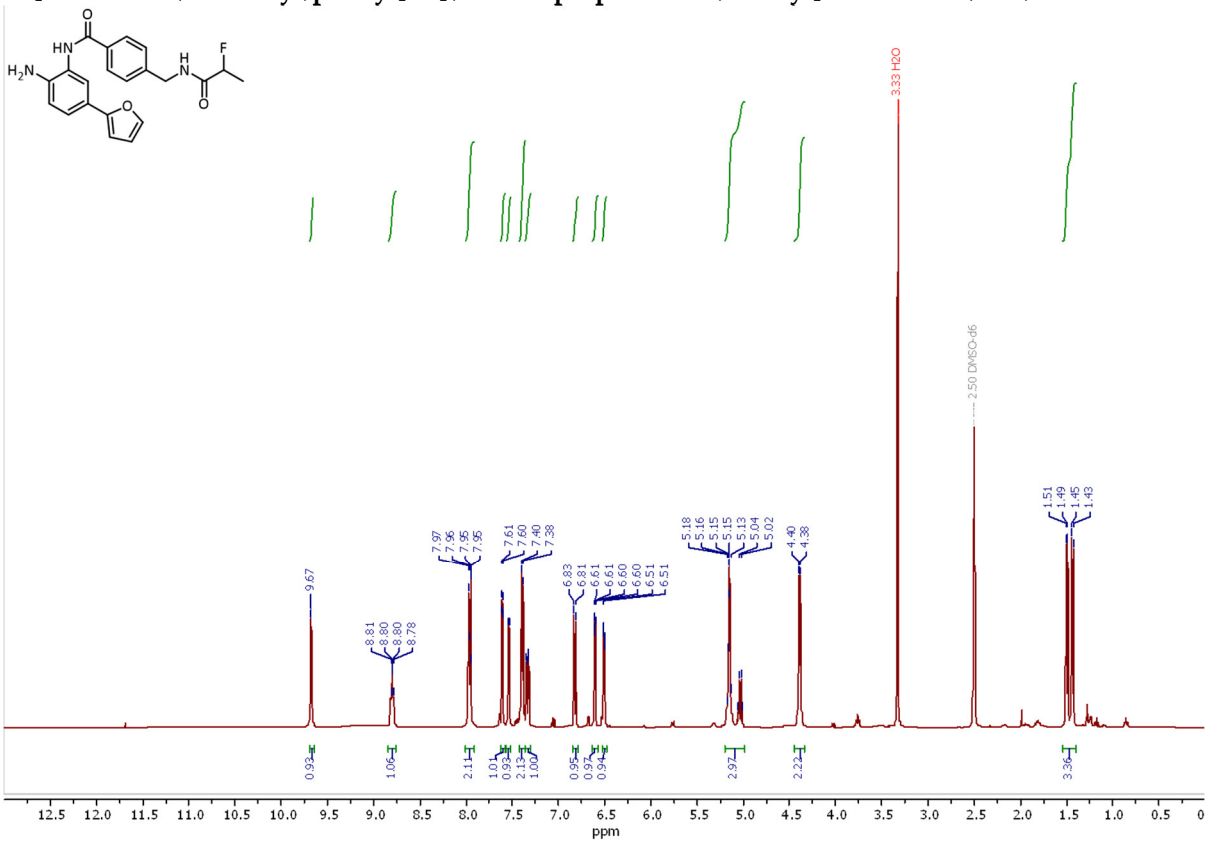


Figure S31. [1]H-NMR of **BA7**

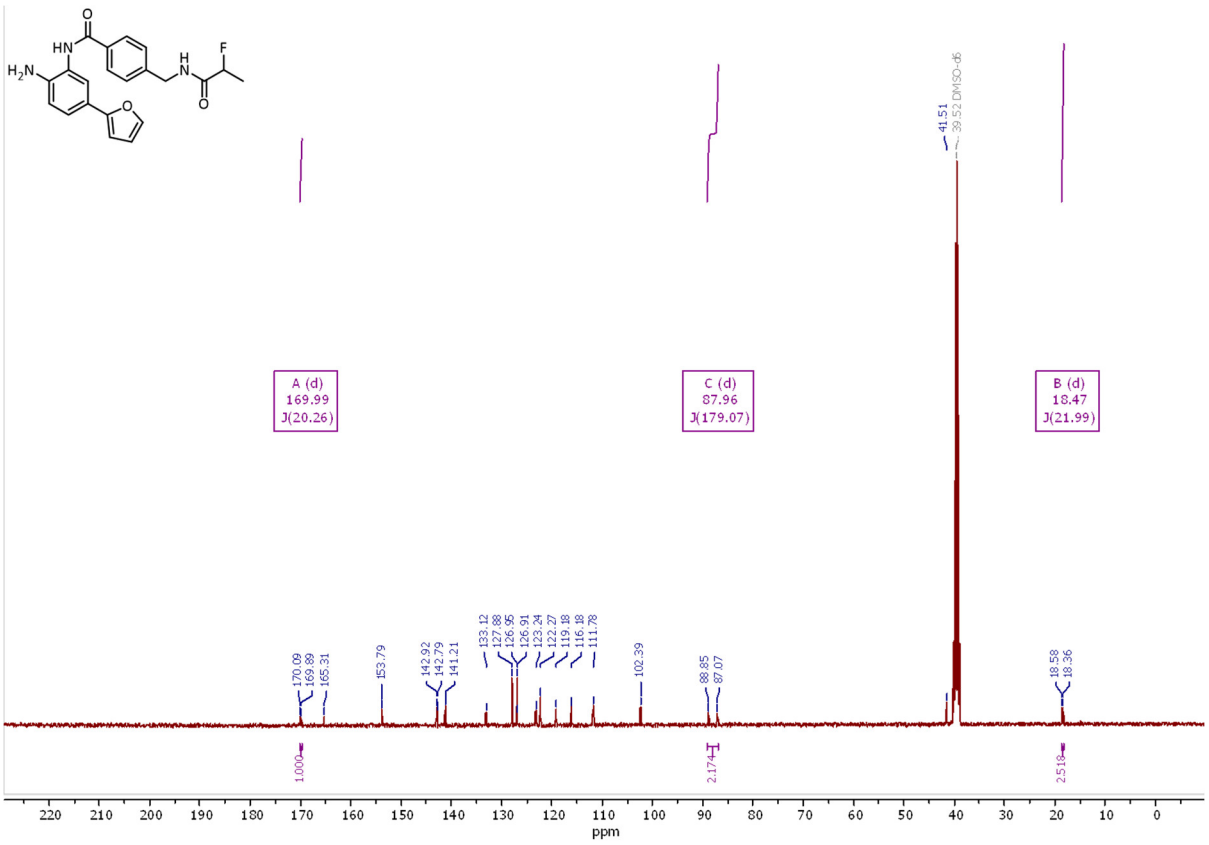


Figure S32. [13]C-NMR of **BA7**

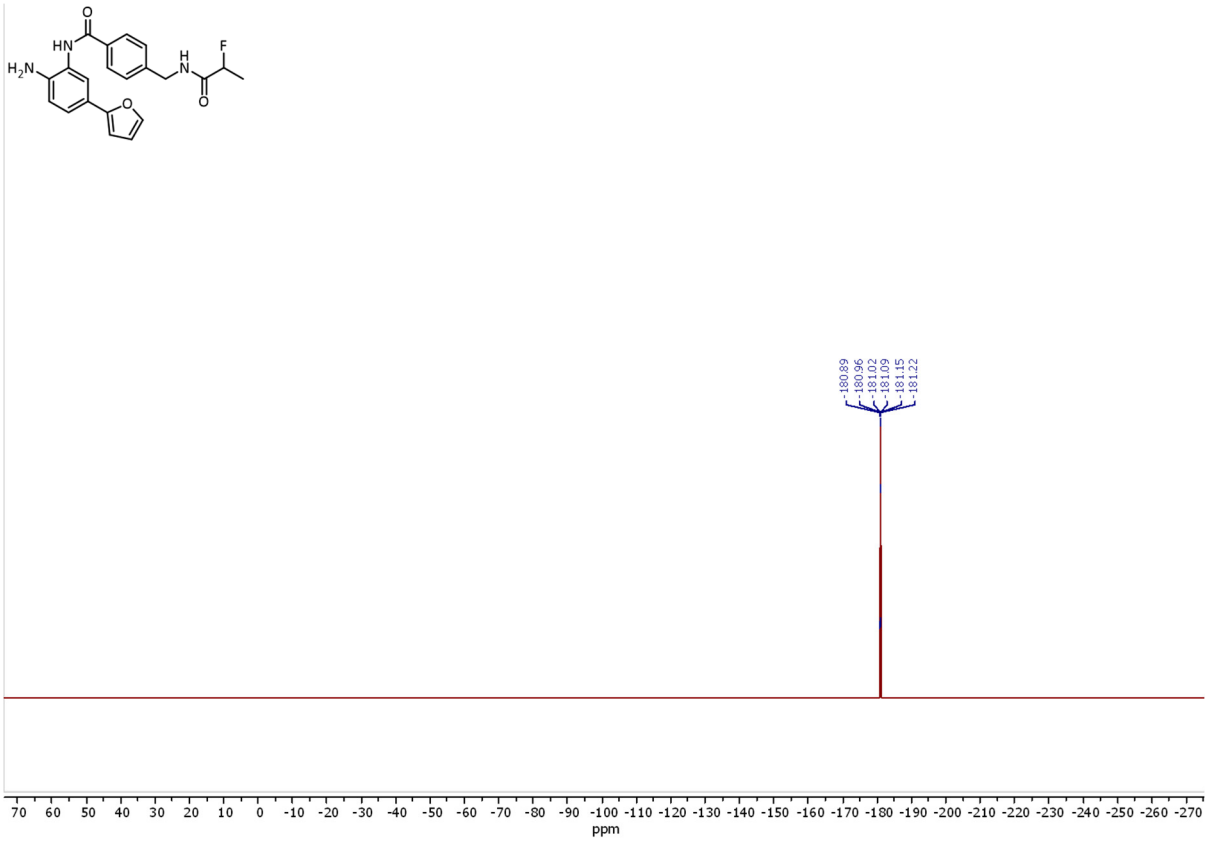


Figure S33. ^{19}F-NMR of **BA7**

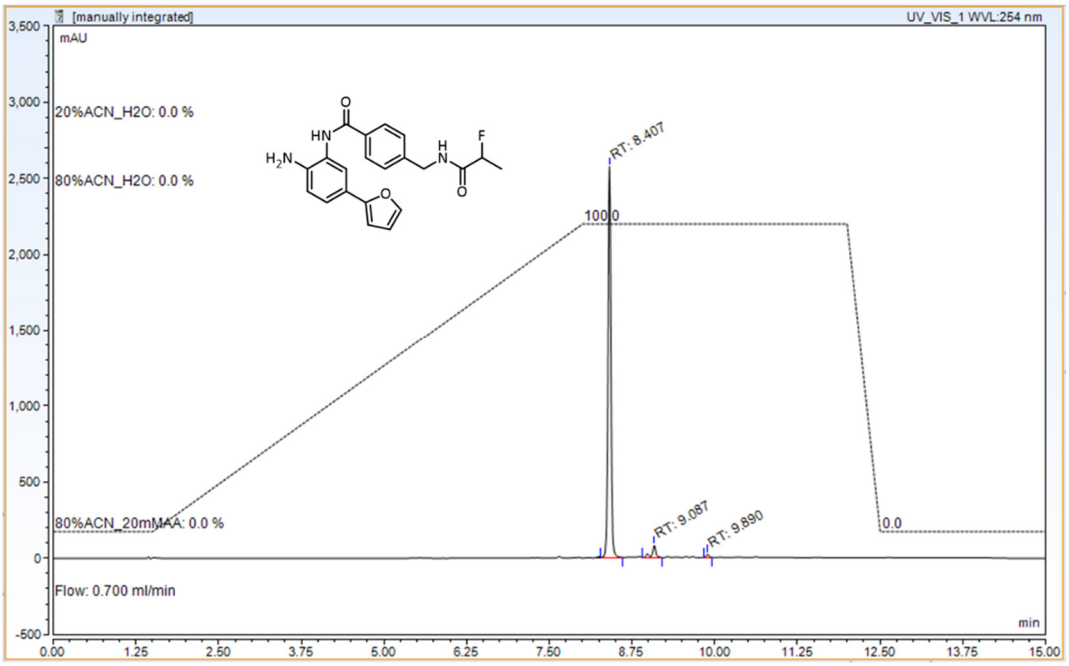


Figure S34. LC-MS chromatogram of **BA7**

***N*-[2-amino-5-(furan-2-yl)phenyl]-4-(2-fluoropropanamido)benzamide (BA8)**

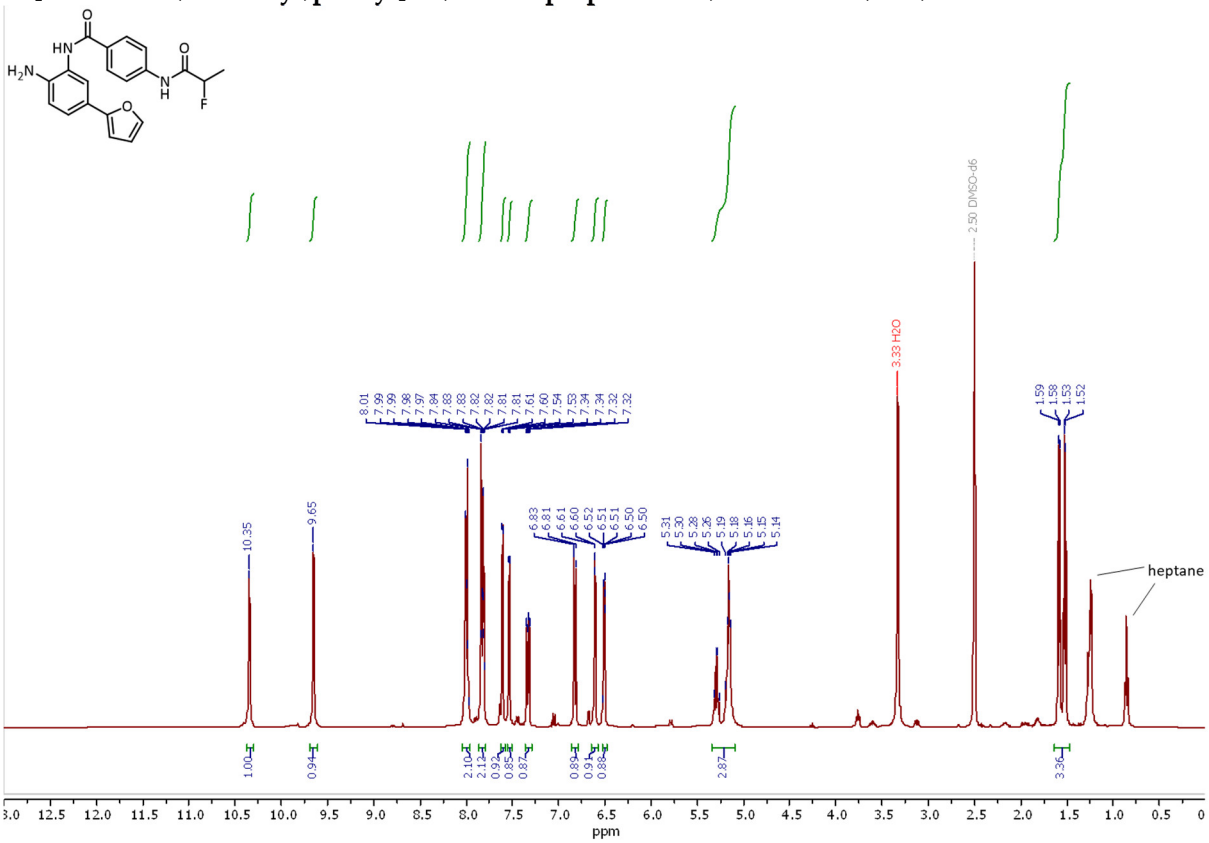


Figure S35. [1]H-NMR of **BA8**

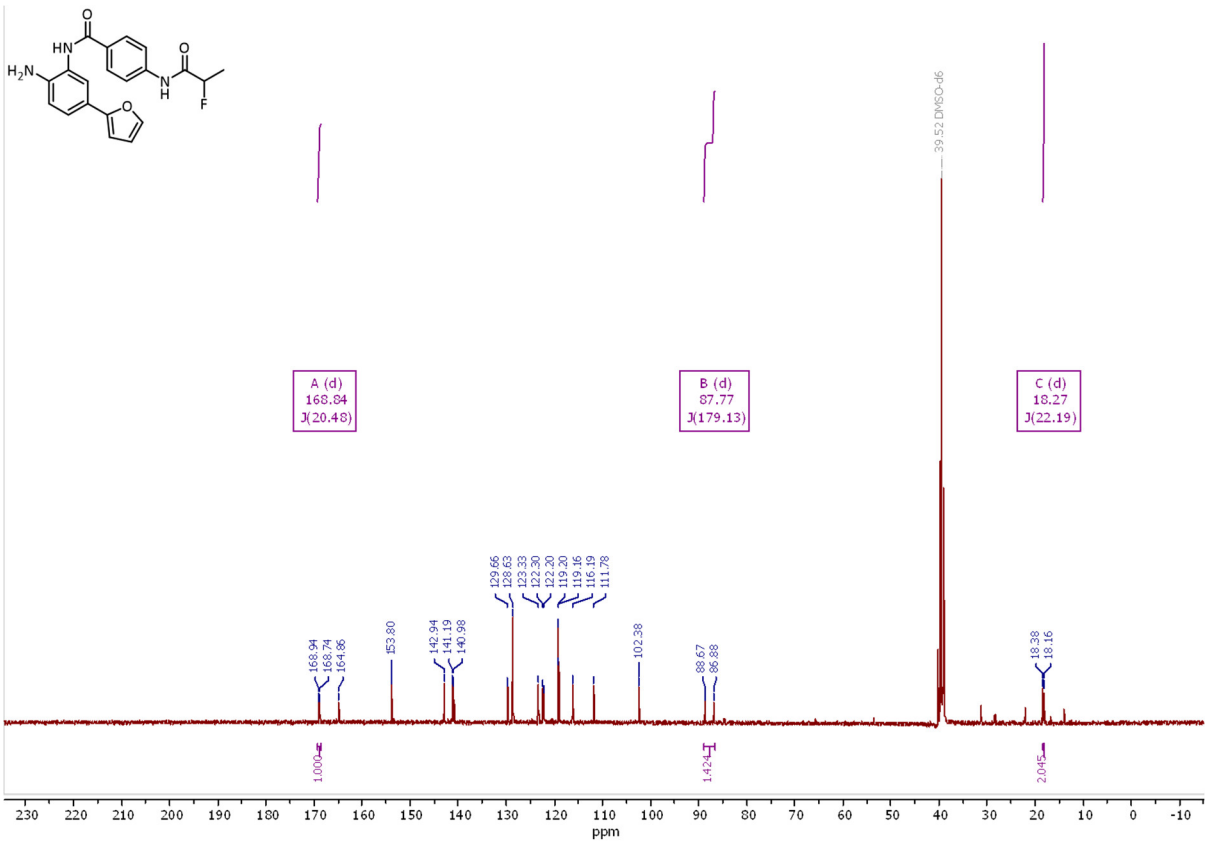


Figure S36. [13]C-NMR of **BA8**

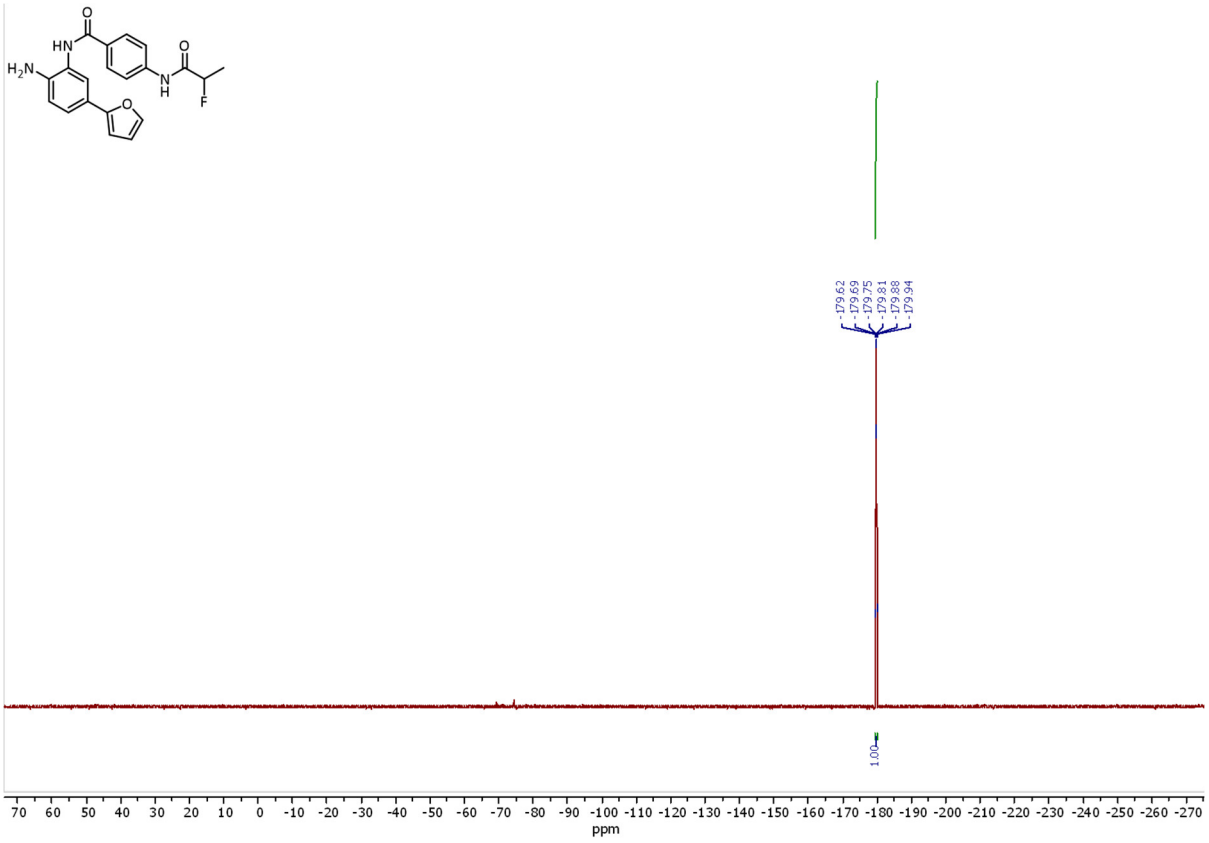


Figure S37. ^{19}F-NMR of **BA8**

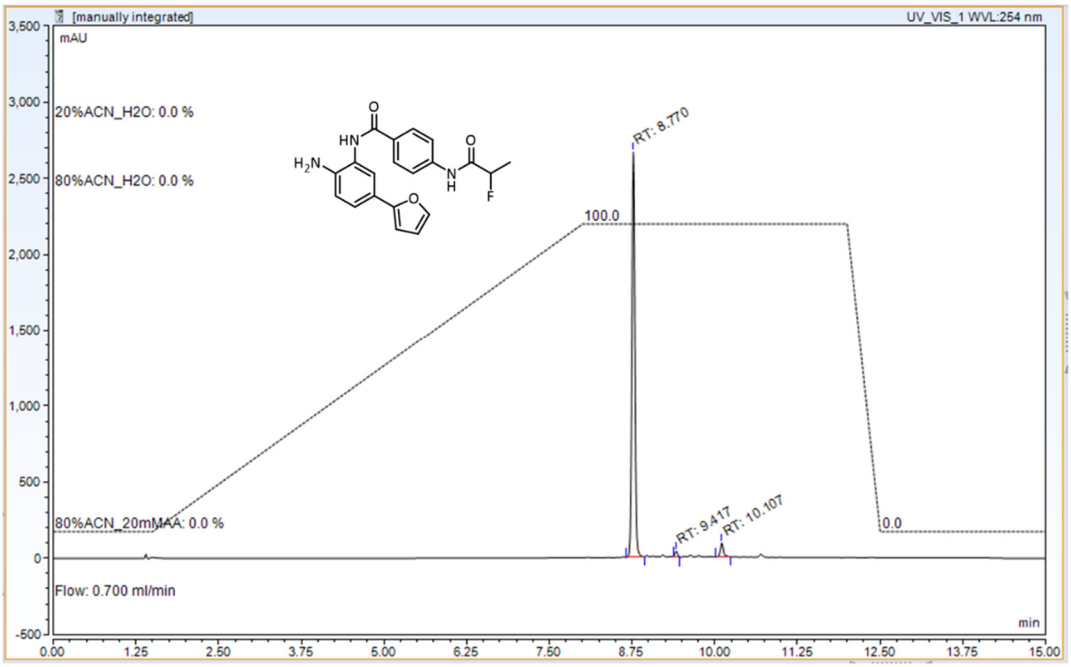


Figure S38. LC-MS chromatogram of **BA8**

***N*-[2-amino-5-(furan-3-yl)phenyl]-4-[(2-fluoropropanamido)methyl]benzamide (BA9)**

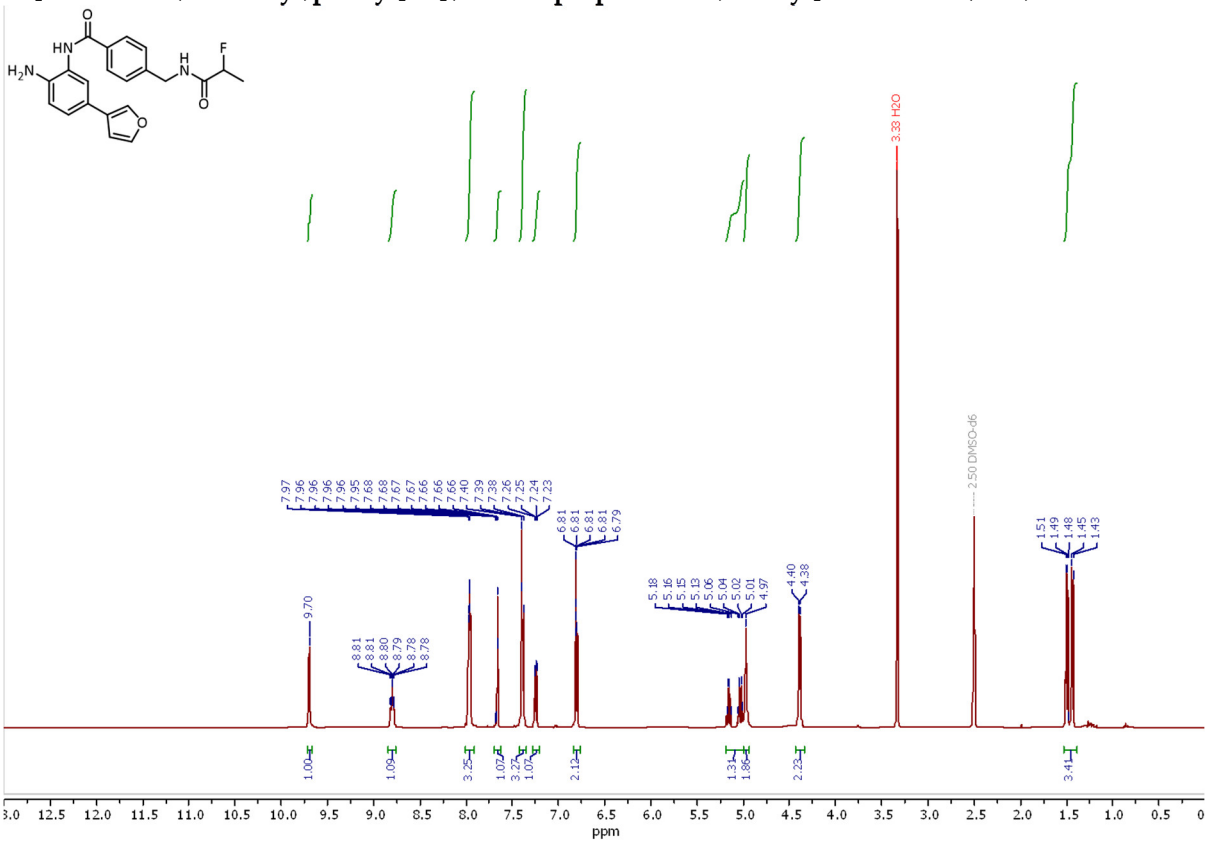


Figure S39. ^{1}H-NMR of **BA9**

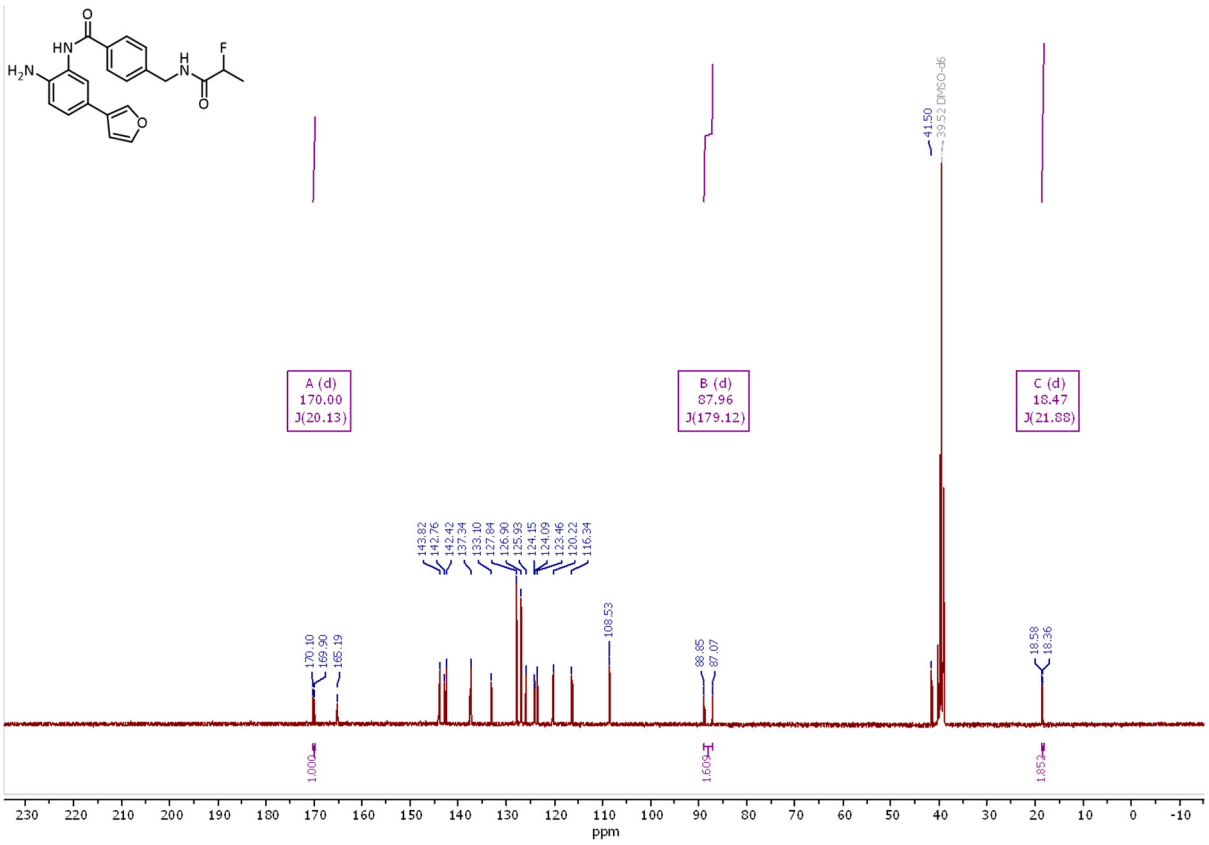


Figure S40. ^{13}C-NMR of **BA9**

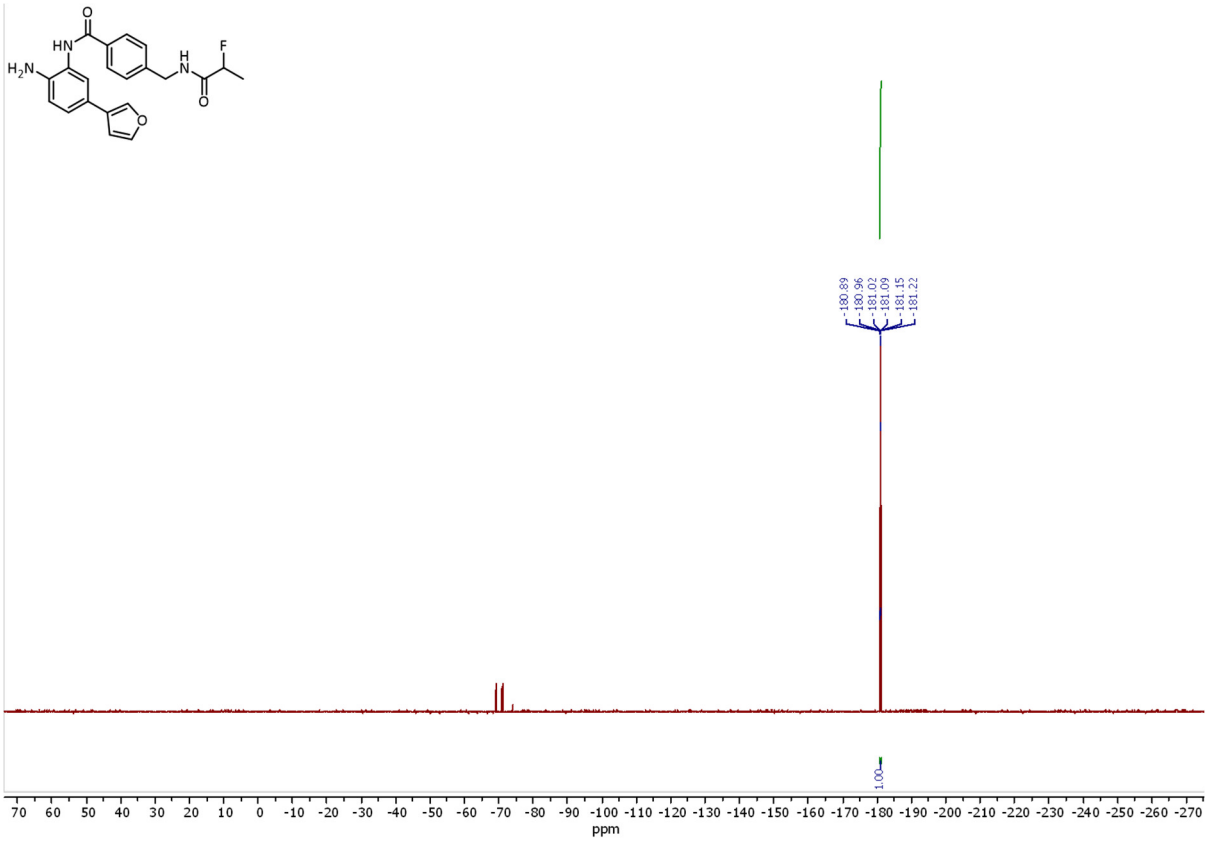


Figure S41. ^{19}F-NMR of **BA9**

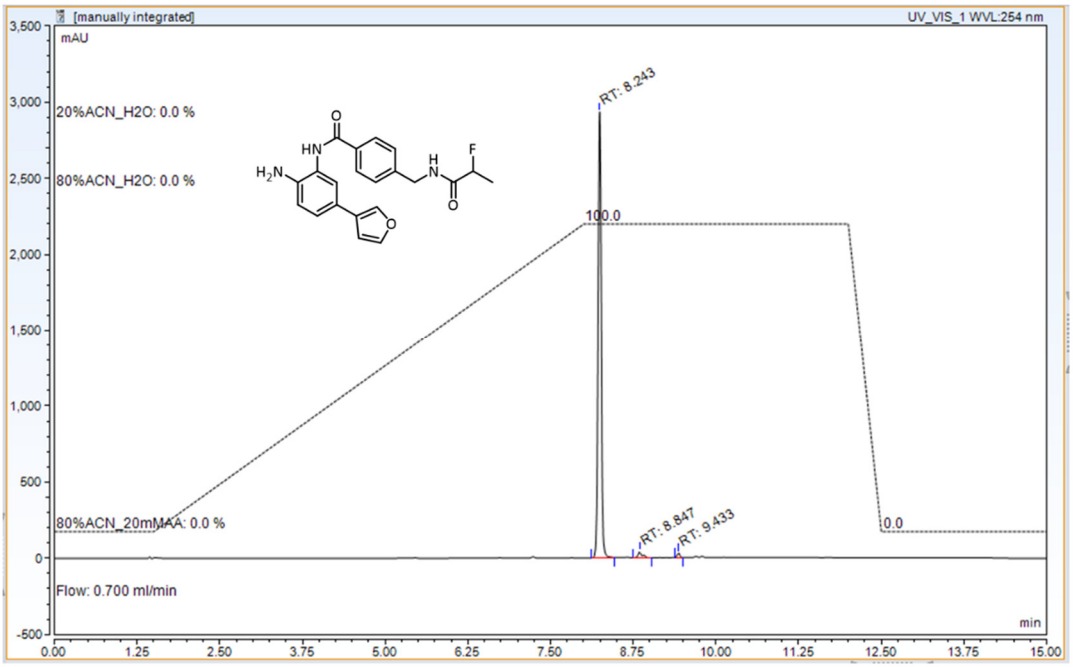


Figure S42. LC-MS chromatogram of **BA9**

***N*-[2-amino-5-(furan-3-yl)phenyl]-4-(2-fluoropropanamido)benzamide (BA10)**

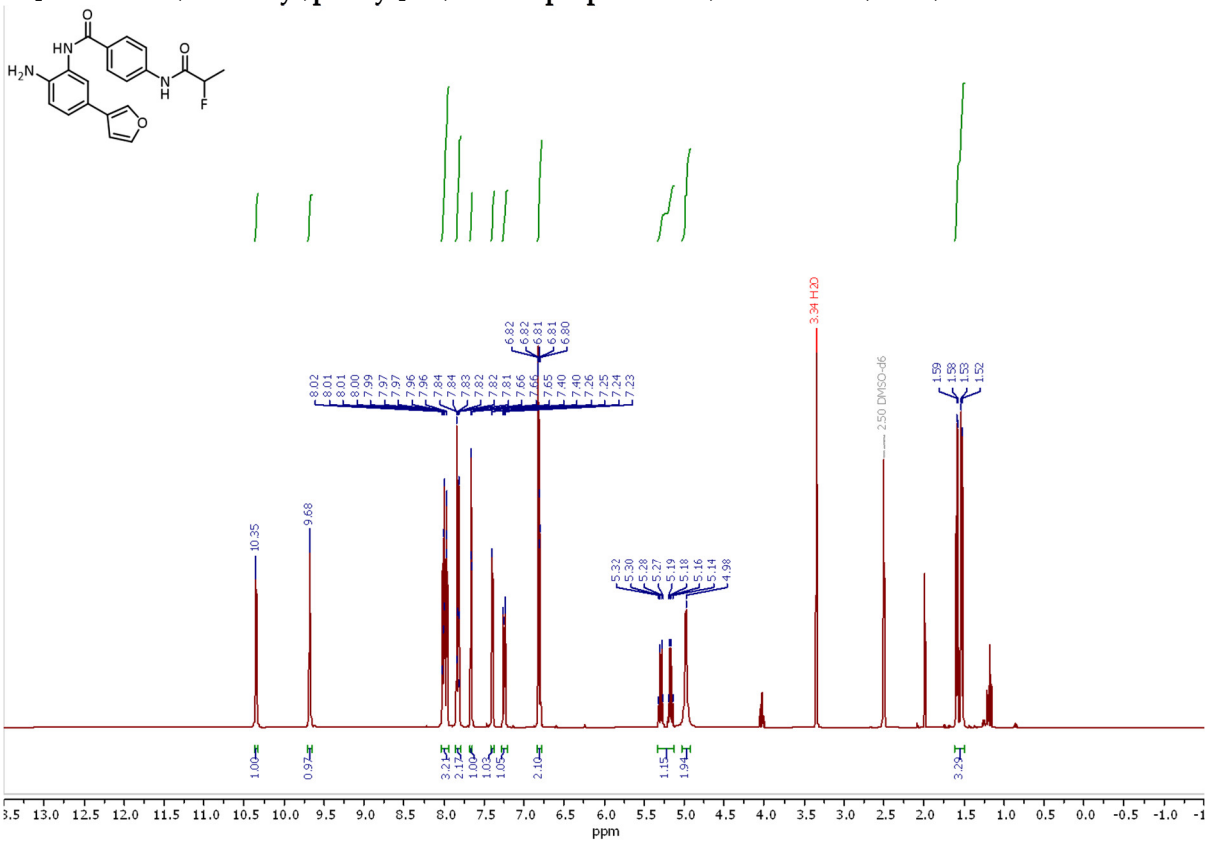


Figure S43. [1]H-NMR of **BA10**

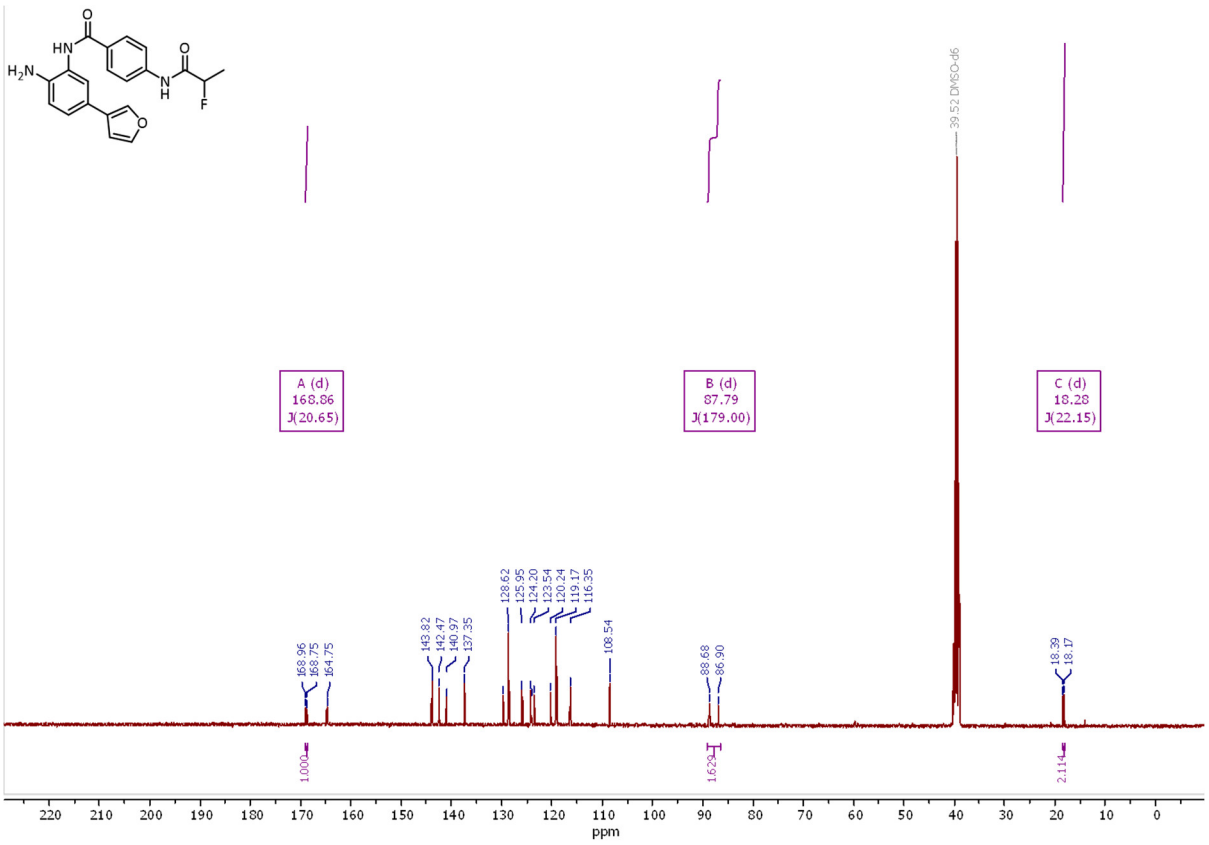


Figure S44. [13]C-NMR of **BA10**

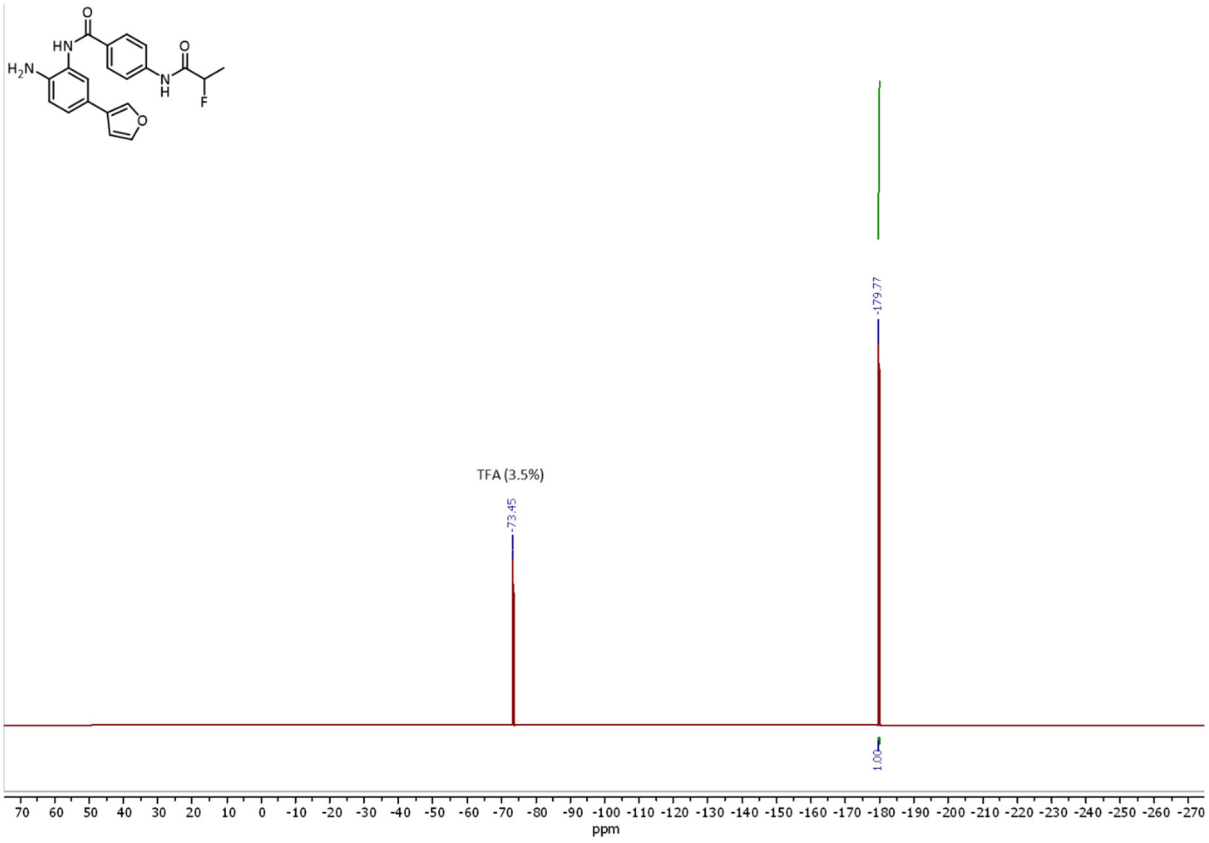


Figure S45. ^{19}F-NMR of **BA10**

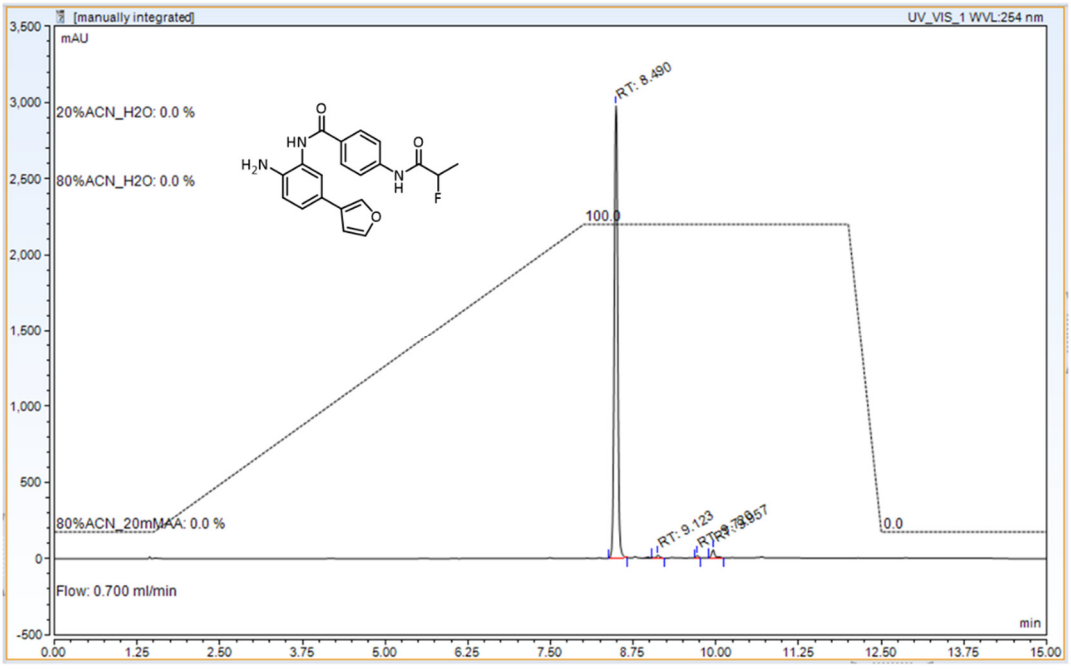


Figure S46. LC-MS chromatogram of **BA10**