

Development and biological evaluation of the first highly potent and selective benzamide-based radiotracer [¹⁸F]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 [¹⁸F]BA3

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

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

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

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

Figure S6: Representative maximal intensity projection map of [¹⁸F]BA3

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

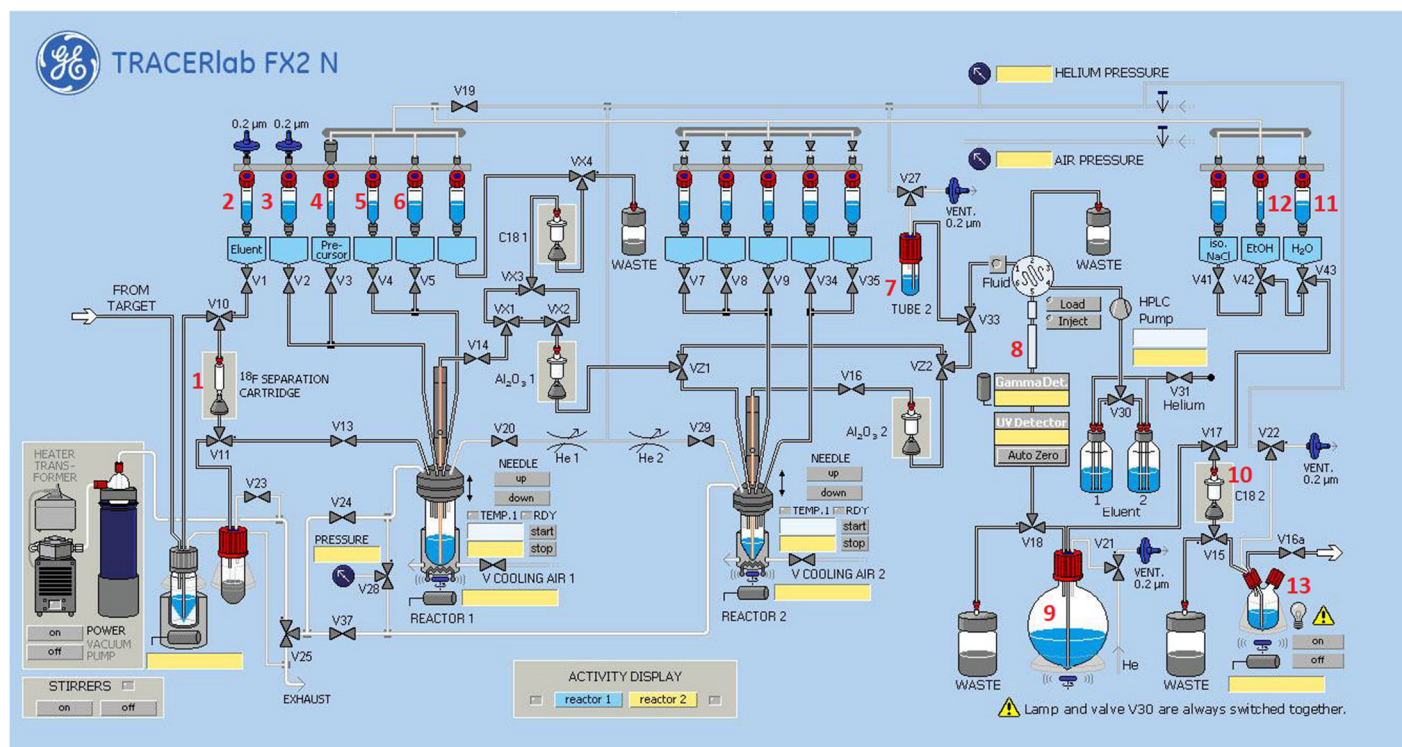


Figure S1. Scheme of the synthesis module TRACERlab FX2 N for the radiosynthesis of [¹⁸F]BA3. (1) Sep-Pak® Accell QMA light cartridge, (2) 150 µL TBAHCO₃ (0.075 M) in 300 µL H₂O and 600 µL MeCN, (3) 2 mL MeCN, (4) precursor (4 mg of 9 in 800 µL MeCN), (5) 800 µL 2M HCl_{aq}, (6) 1.6 mL 1M NaHCO_{3, aq} and 1.8 mL 100mM phosphate buffer (pH

= 6), (7) injection vial, (8) Reprosil-Pur C18-AQ (40% ACN/20mM NH₄OAc_{aq}, flow 4.0 mL/min), (9) 40 mL water, (10) Sep-Pak® C18 light, (11) 2 mL H₂O, (12) 1.2 mL EtOH, (13) product vial.

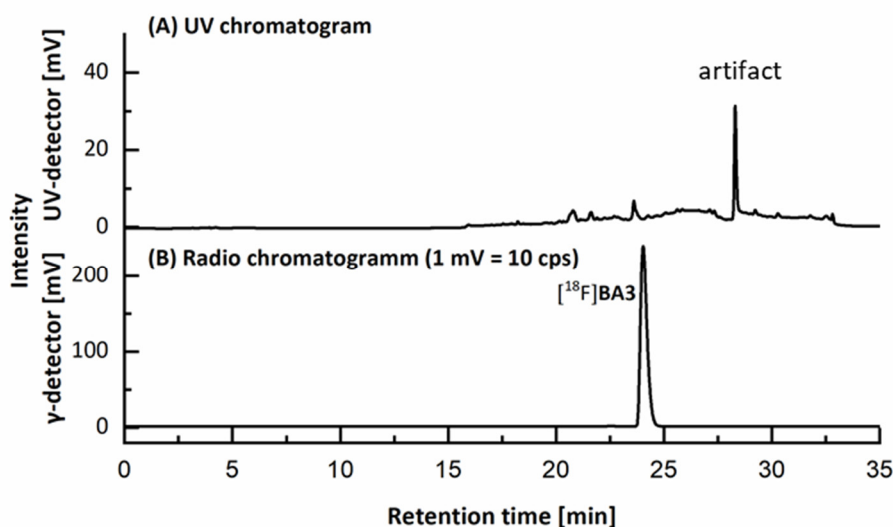


Figure S2. Representative (A) UV- and (B) radio-RP-HPLC chromatograms of formulated [¹⁸F]BA3 (ReproSil-Pur 120 C18-AQ column (250 × 4.6 mm, 5 μm), Gradient MeCN/20 mM NH₄OAc_{aq}, (see quality control), flow rate: 1 mL/min).

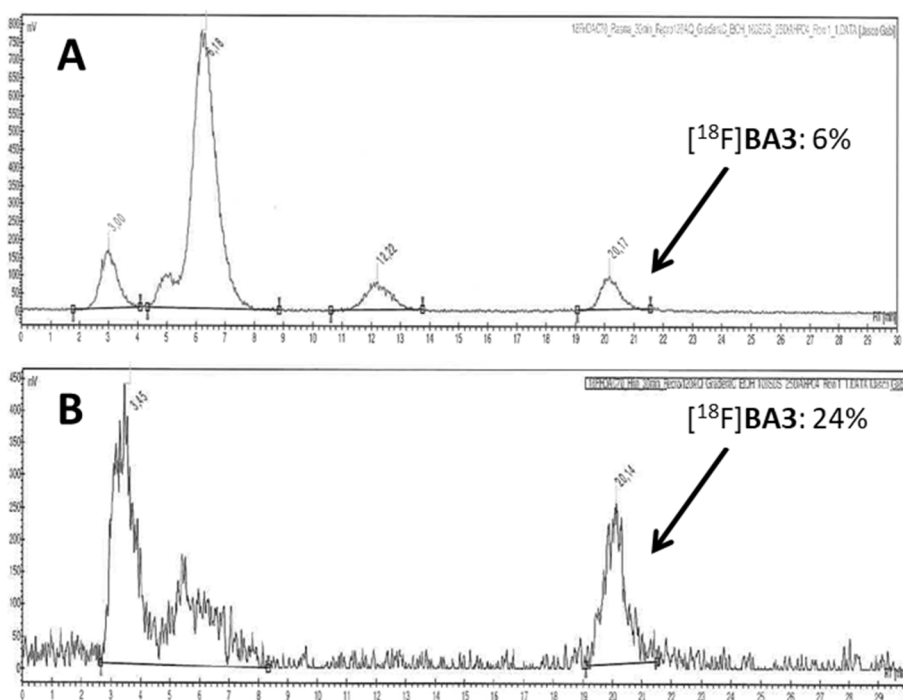


Figure S3. Representative radio-MLC chromatograms of *in vivo* metabolism studies from plasma (A) and brain (B) samples obtained 30 min p.i. of [¹⁸F]BA3 in a CD-1 mouse (gamma-detection via radio-RP-HPLC: Reprosil-Pur 120 C18-AQ column (250 × 4.6 mm, 10 μm + 10 mm pre-column), Gradient EtOH/100 mM SDS_{aq}/25 mM (NH₄)₂HPO₄ (see section 3.5.1.), flow: 1 mL/min).

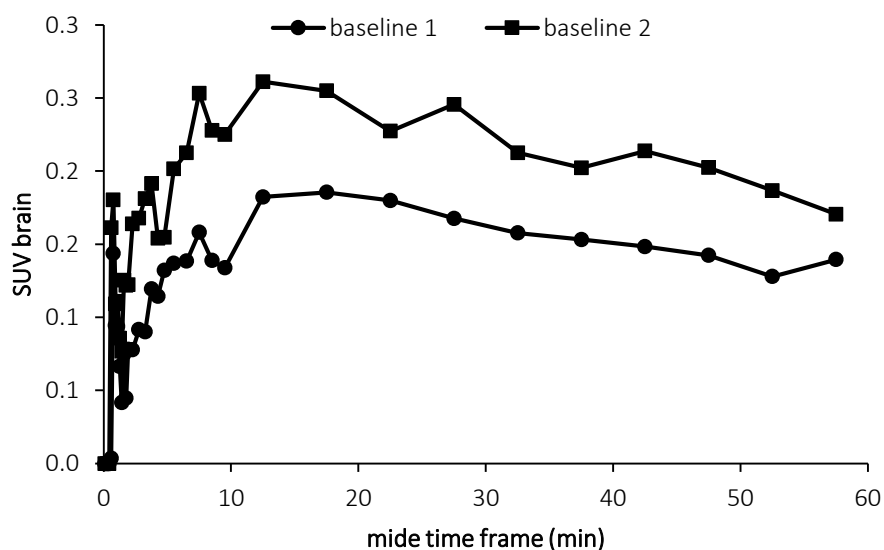


Figure S4. Baseline time-activity curves (TACs) of CD-1 mice brain region after injection of [¹⁸F]BA3 (n = 2).

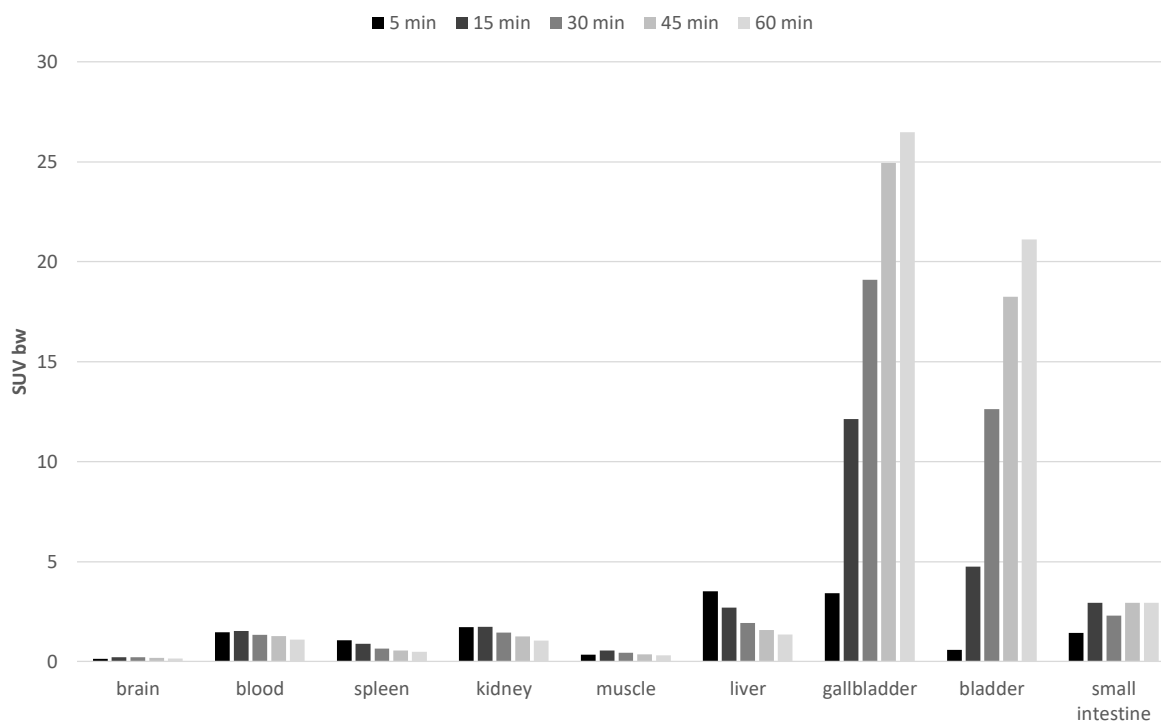


Figure S5. Biodistribution of [¹⁸F]BA3 at different time points derived from PET imaging uncorrected for metabolites (n = 2, SUV_{mean})

Table S1. Tissue biodistribution of radioactivity at different time point after i.v. injection of [¹⁸F]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

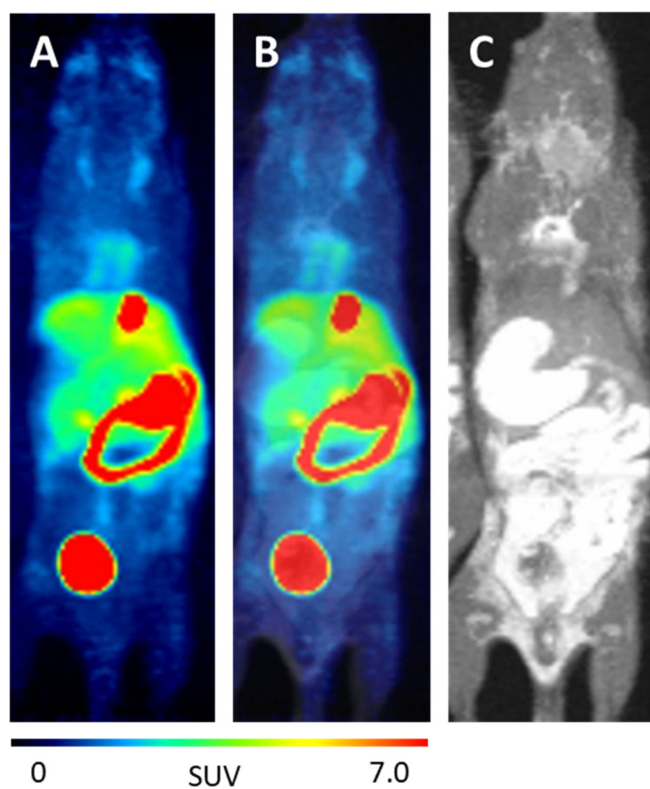
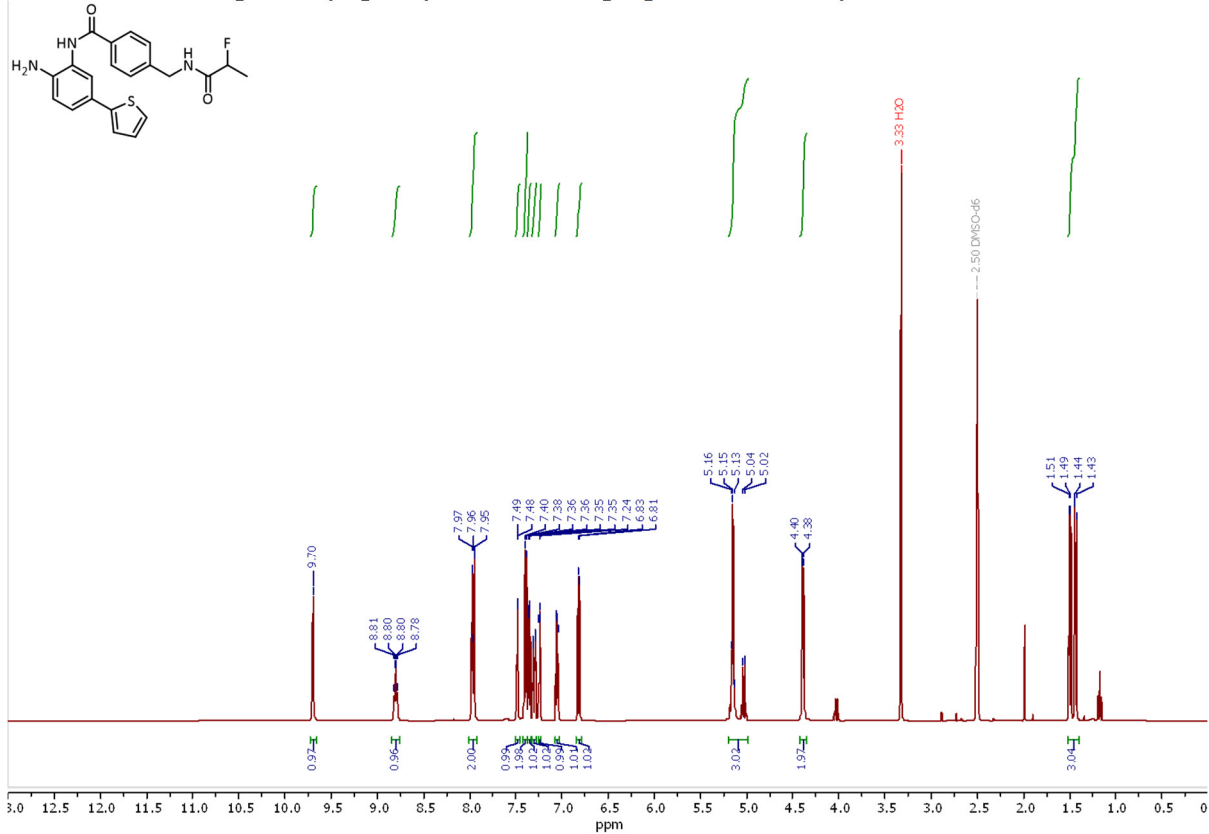
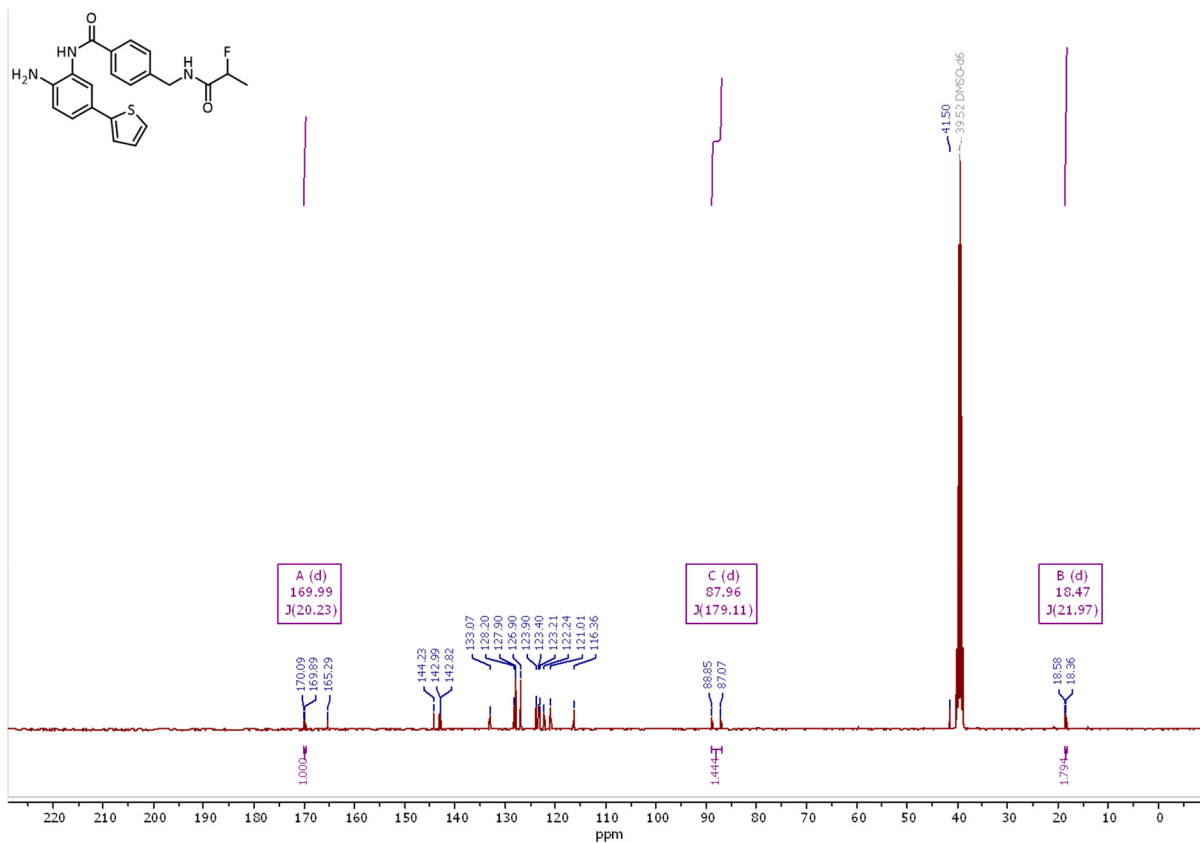


Figure S6. Representative maximal intensity projection map of $[^{18}\text{F}]\text{BA3}$ of (A) PET modality or (B) merged PET and MR modalities and (C) MR modality.

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

Figure S7. ¹H-NMR of BA1Figure S8. ¹³C-NMR of BA1

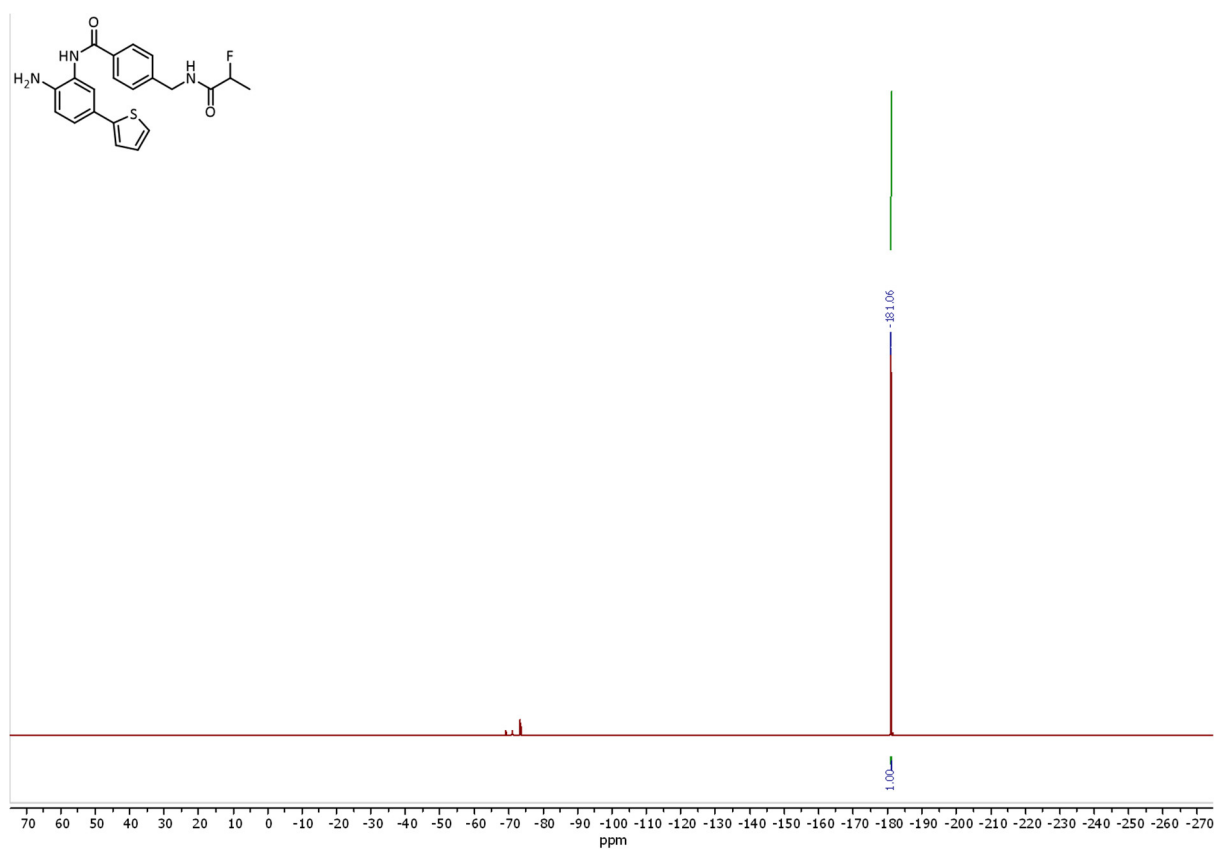
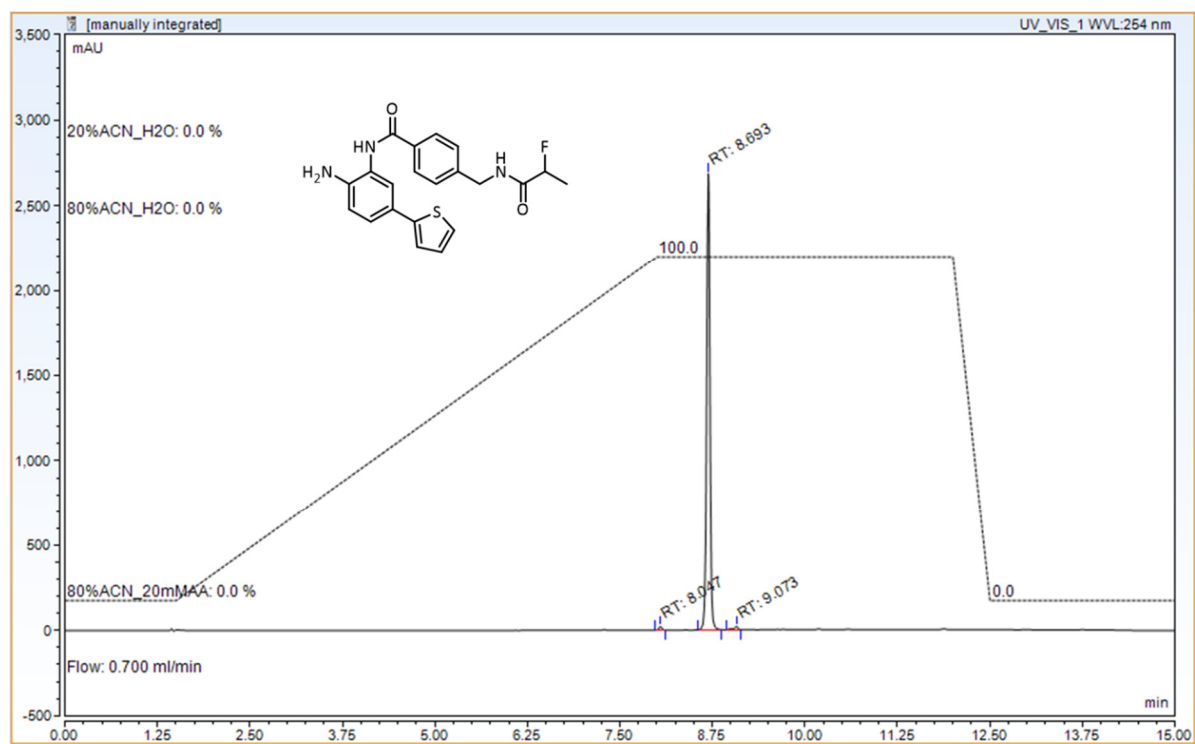
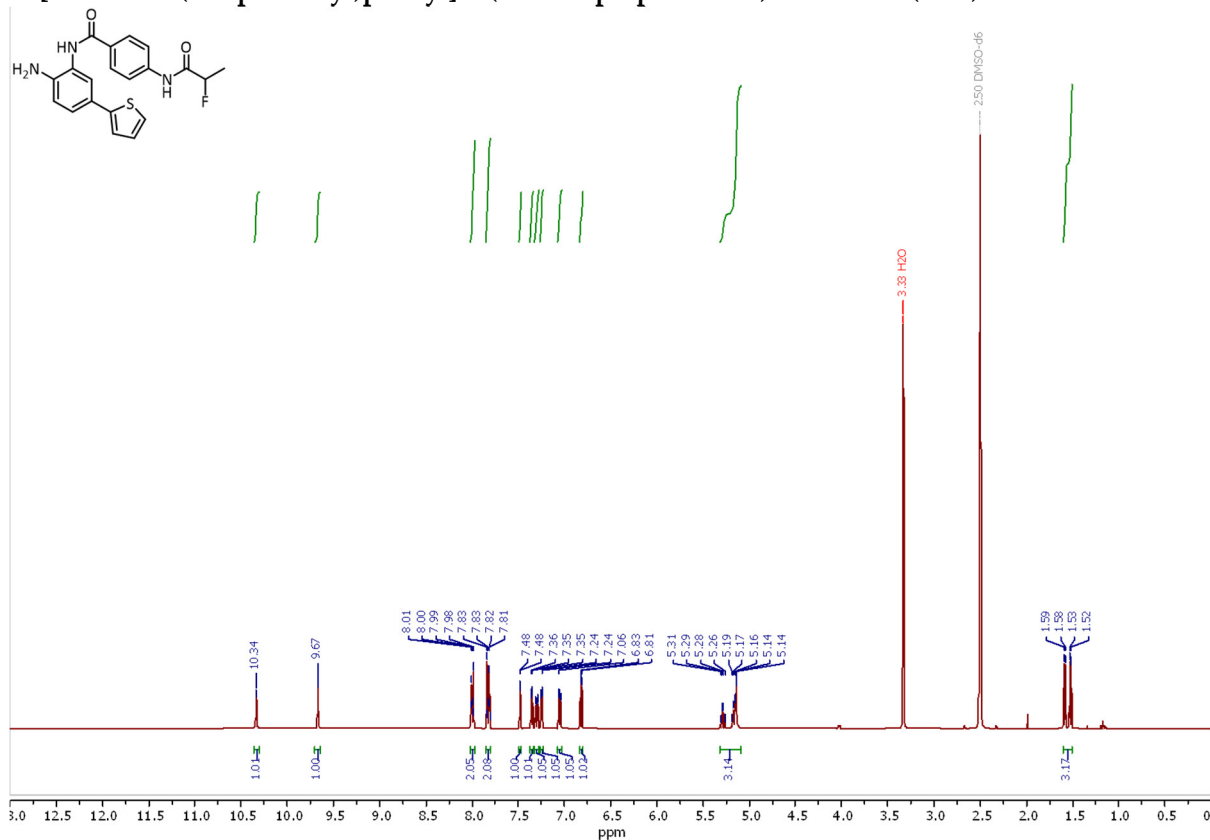
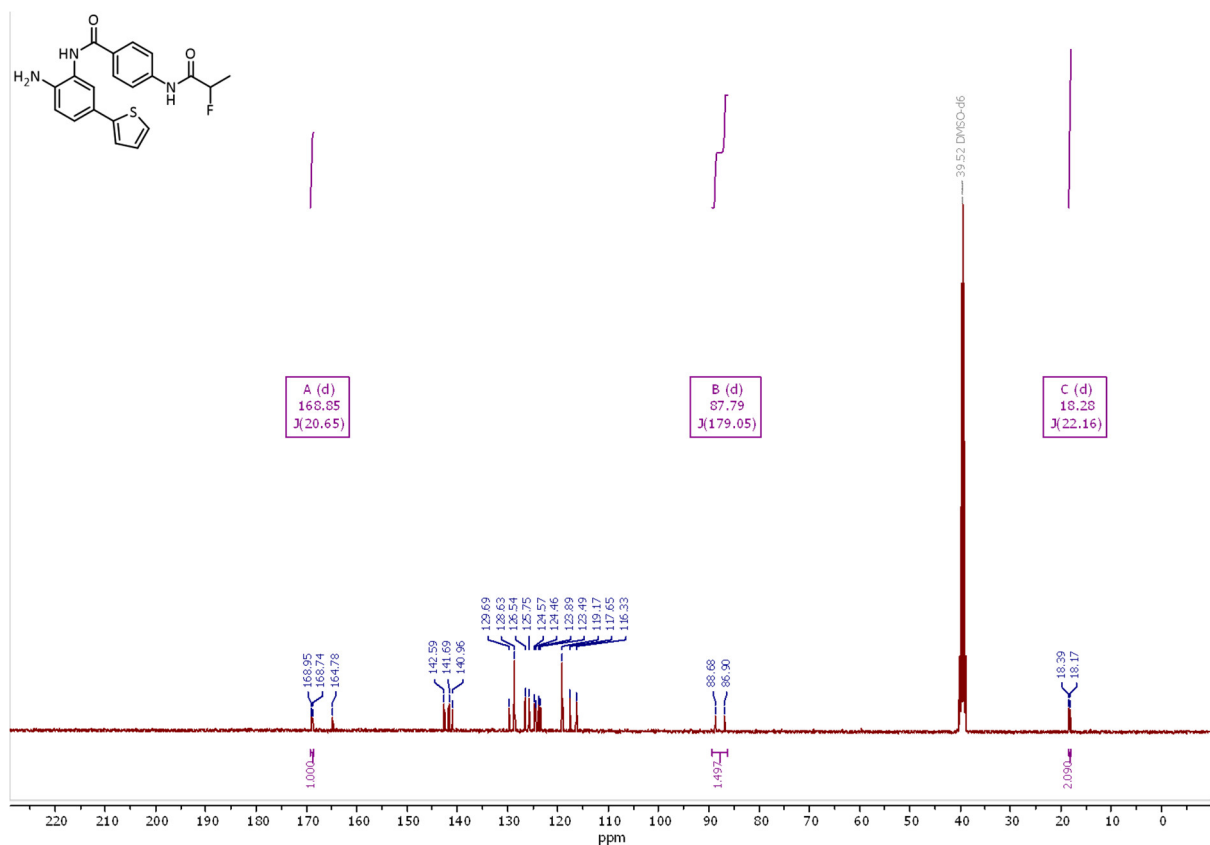
Figure S9. ^{19}F -NMR of BA1

Figure S10. LC-MS chromatogram of BA1

N-[2-amino-5-(thiophen-2-yl)phenyl]-4-(2-fluoropropanamido)benzamide (BA2)**Figure S11. ¹H-NMR of BA2****Figure S12. ¹³C-NMR of BA2**

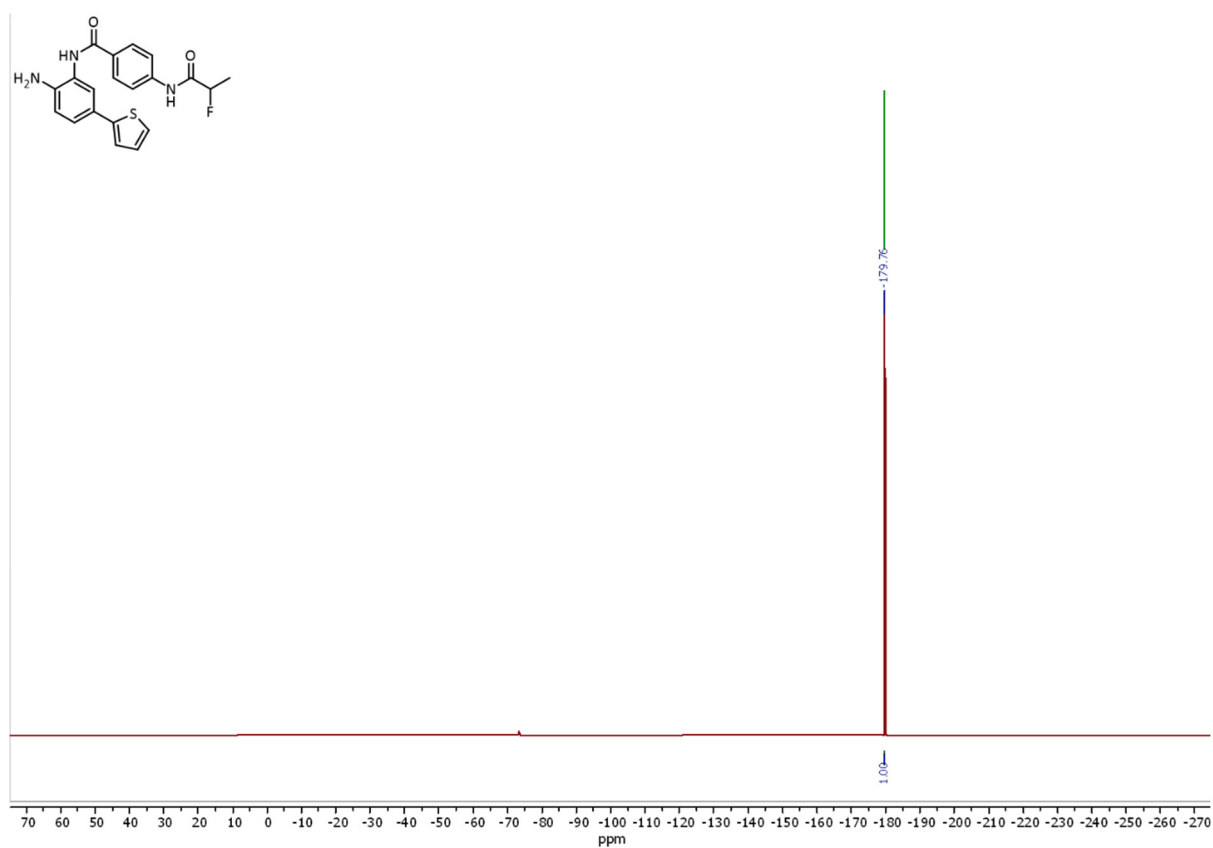
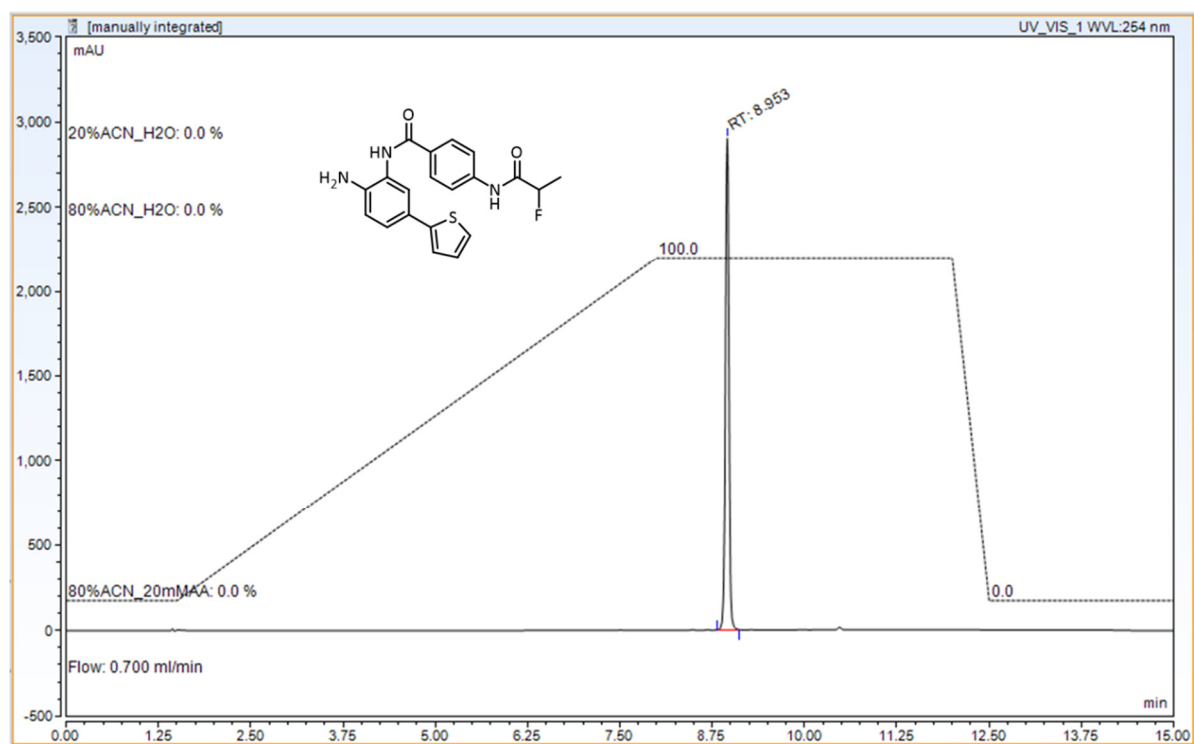
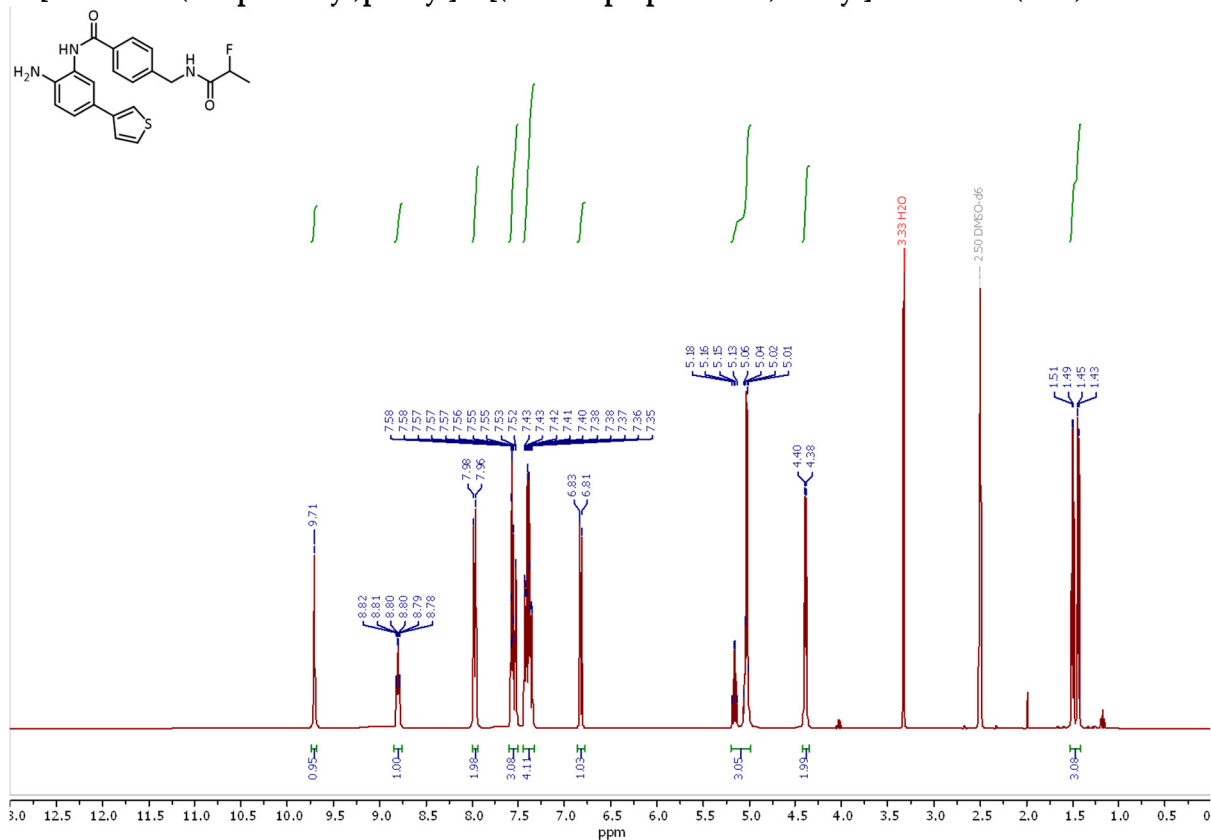
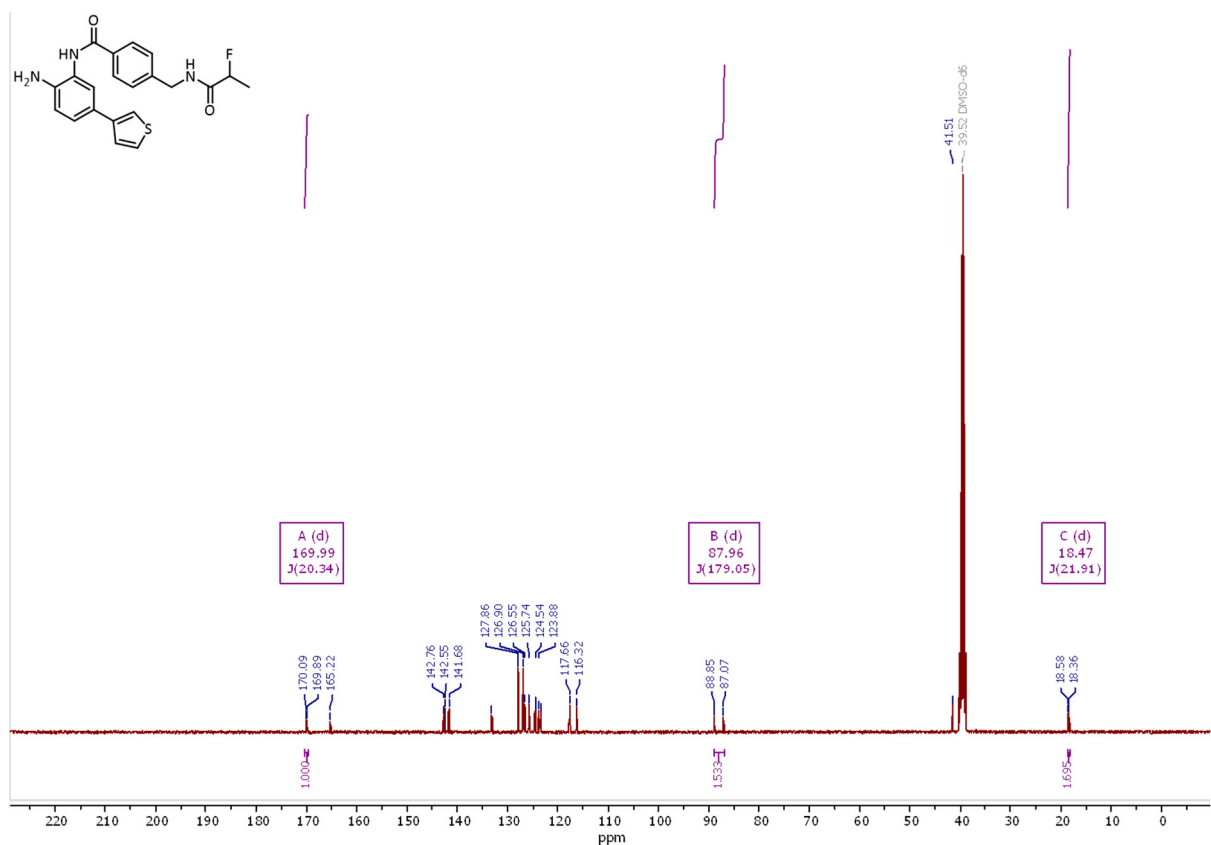
Figure S13. ^{19}F -NMR of BA2

Figure S14. LC-MS chromatogram of BA2

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

Figure S15. ¹H-NMR of BA3Figure S16. ¹³C-NMR of BA3

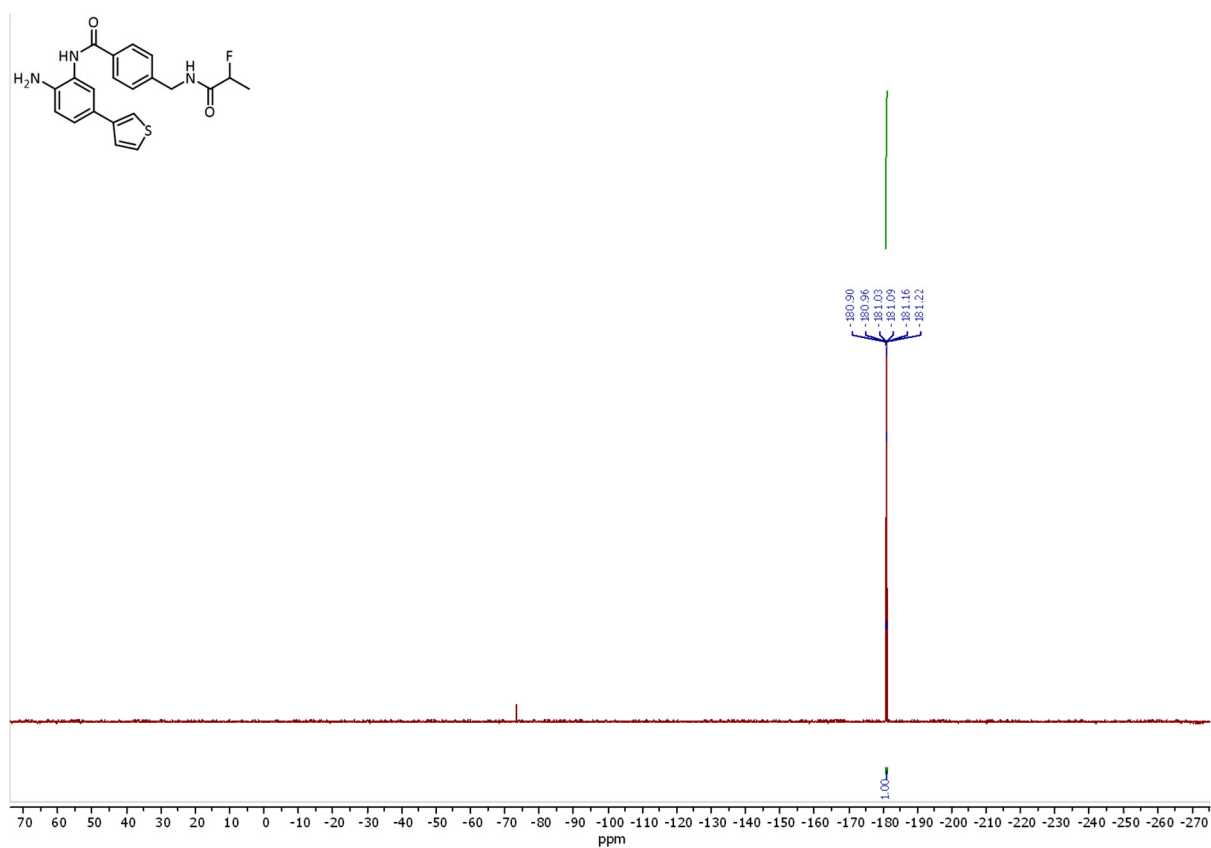
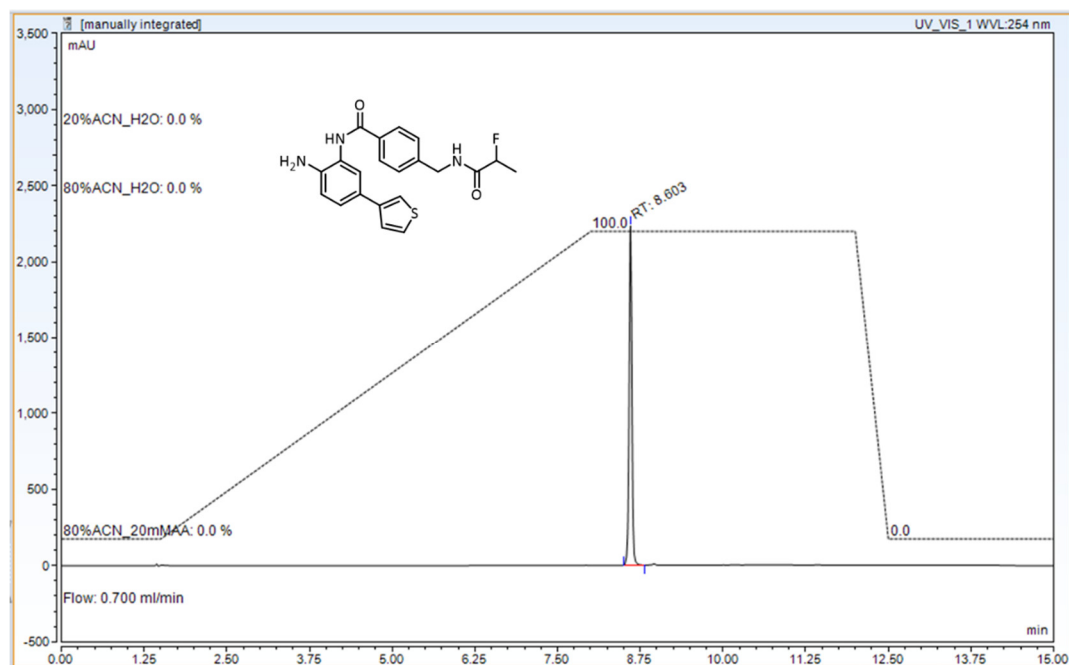
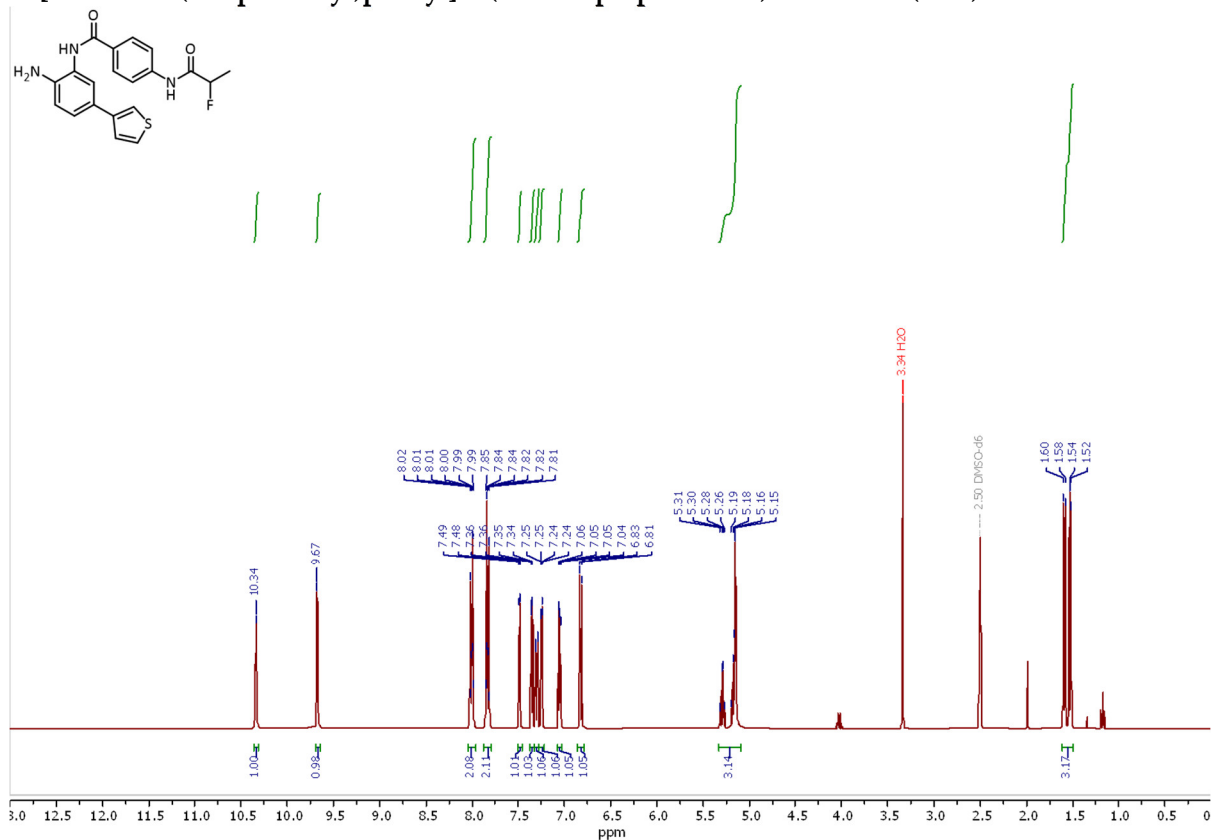
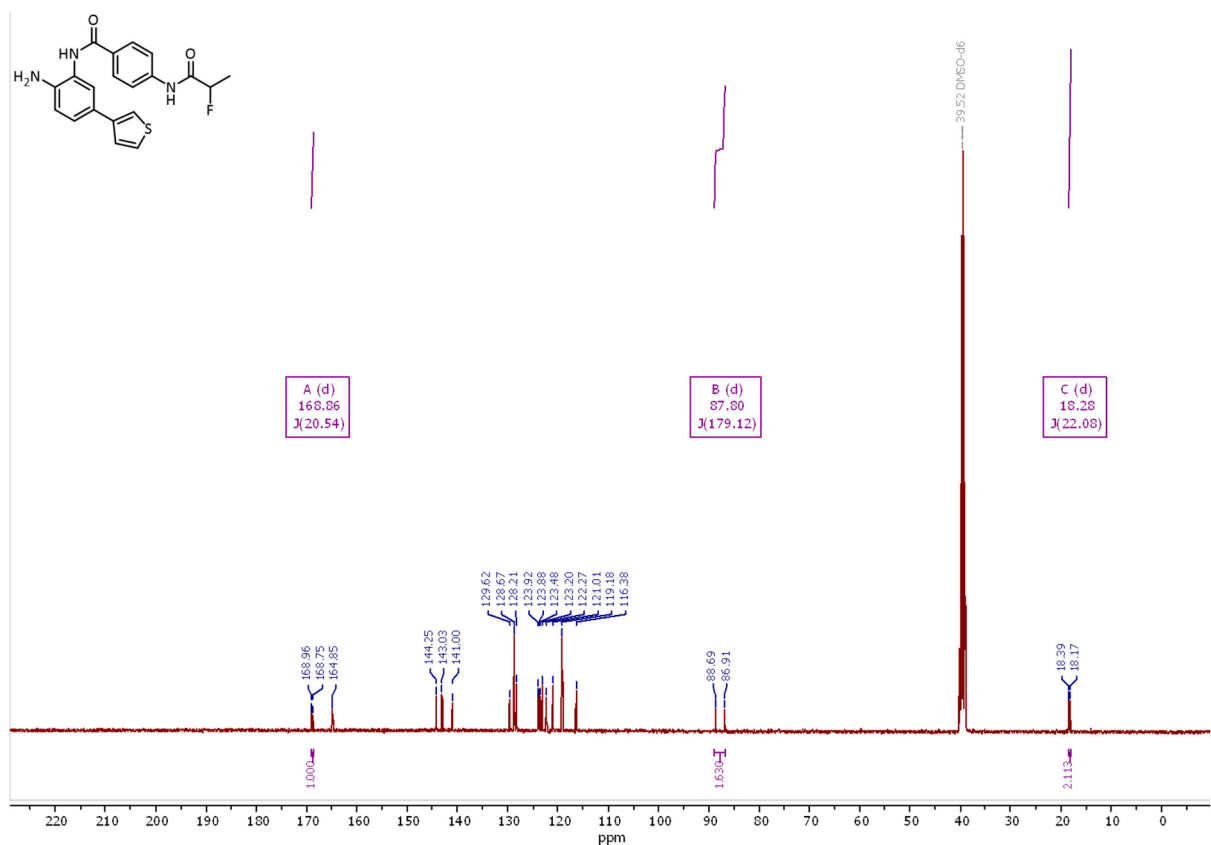
Figure S17. ¹⁹F-NMR of BA3

Figure S18. LC-MS chromatogram of BA3

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

Figure S19. ¹H-NMR of BA4Figure S20. ¹³C-NMR of BA4

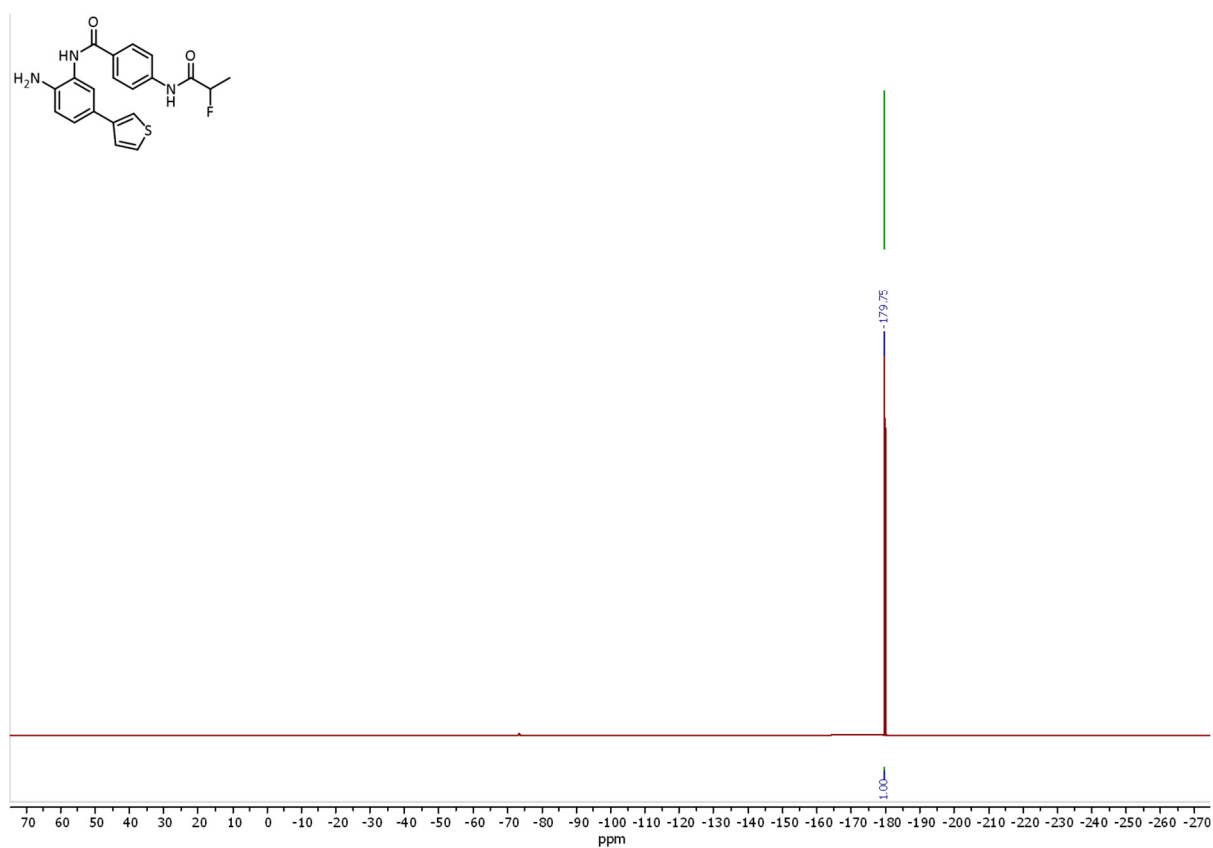
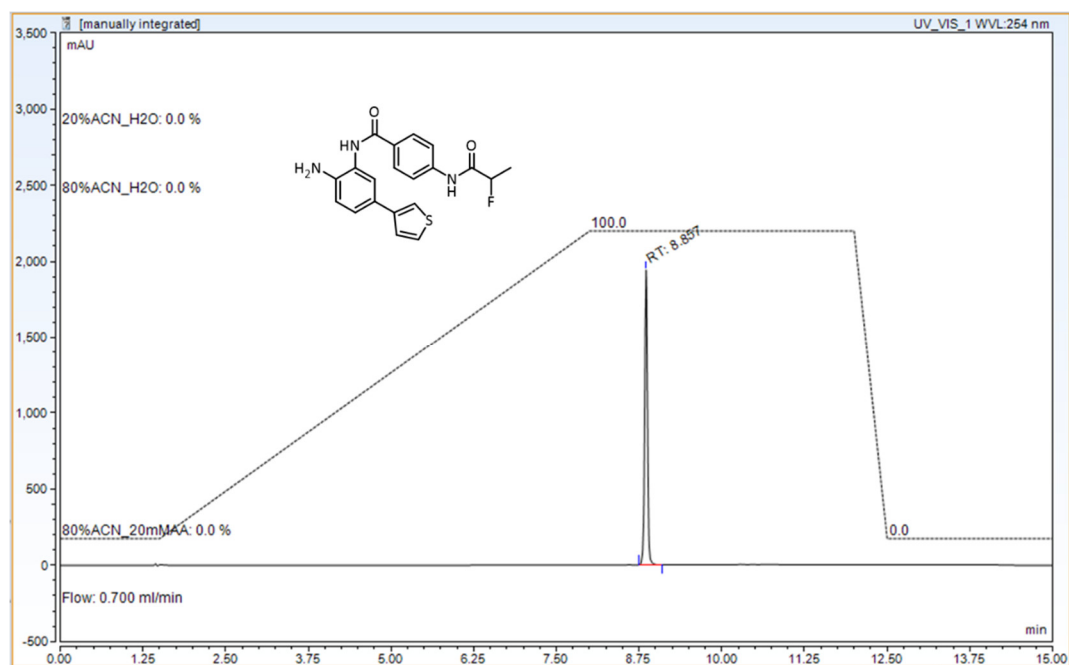
Figure S21. ¹⁹F-NMR of BA4

Figure S22. LC-MS chromatogram of BA4

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

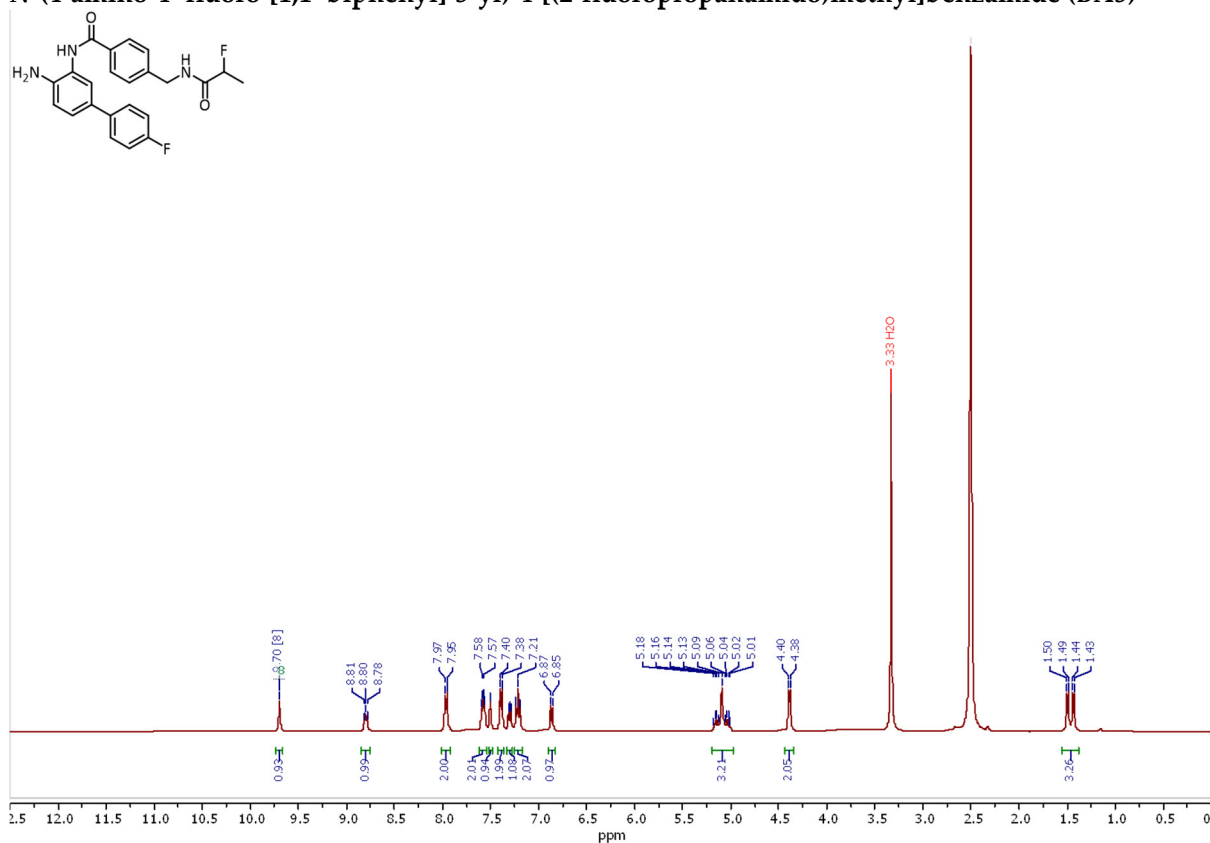


Figure S23. ¹H-NMR of BA5

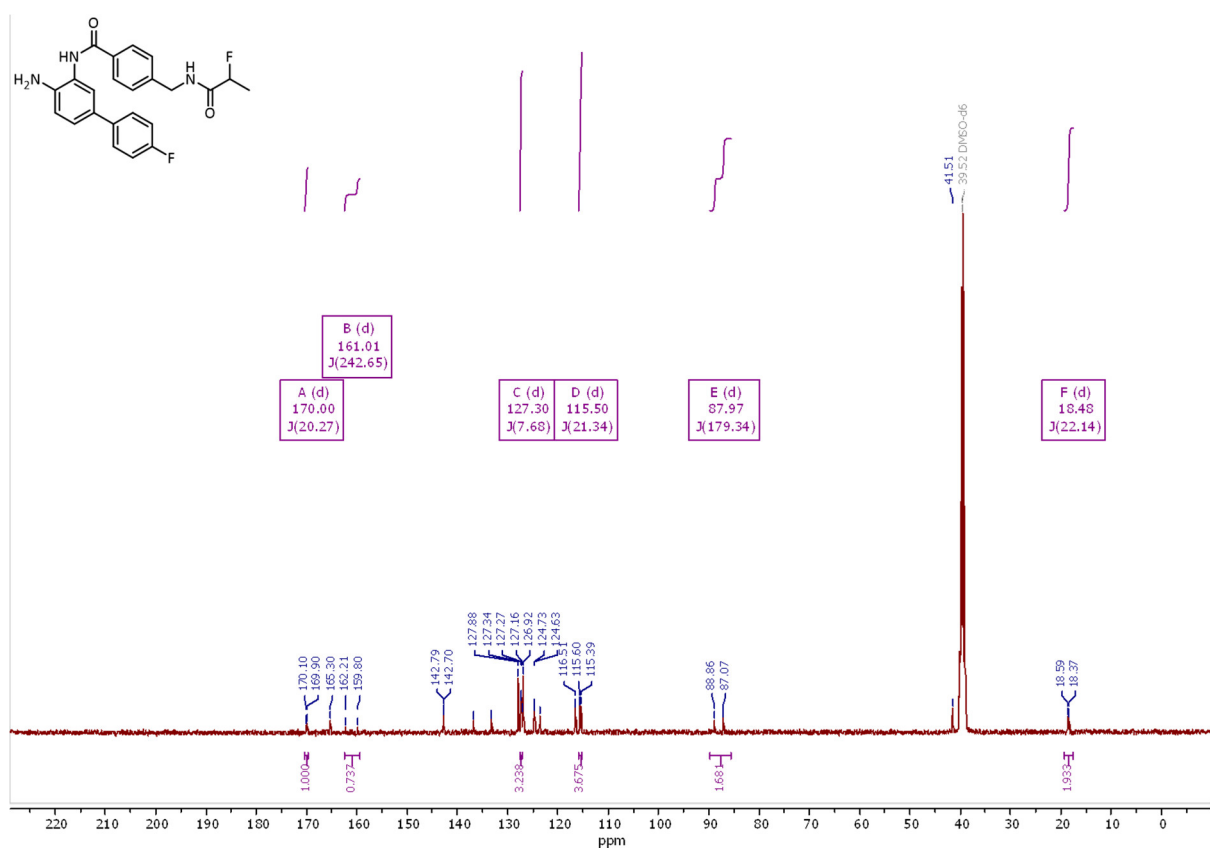


Figure S24. ¹³C-NMR of BA5

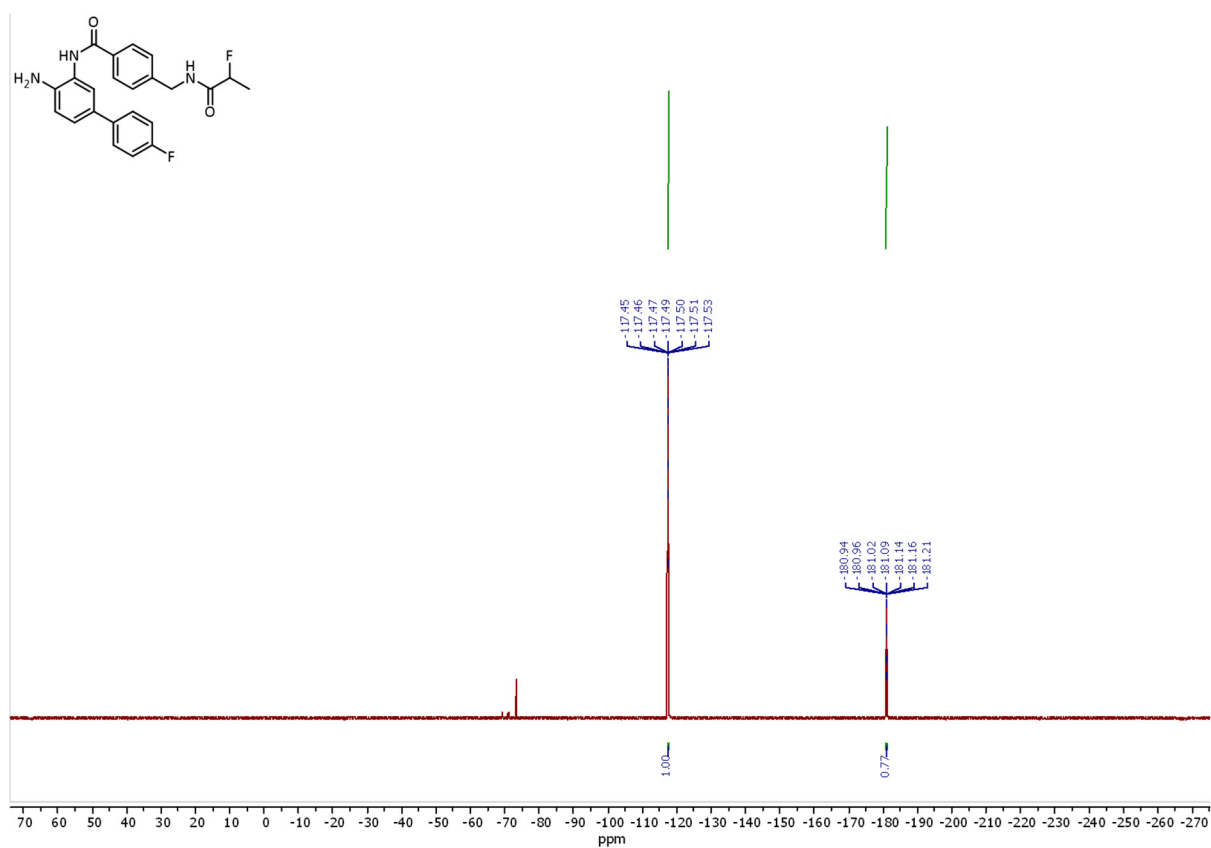
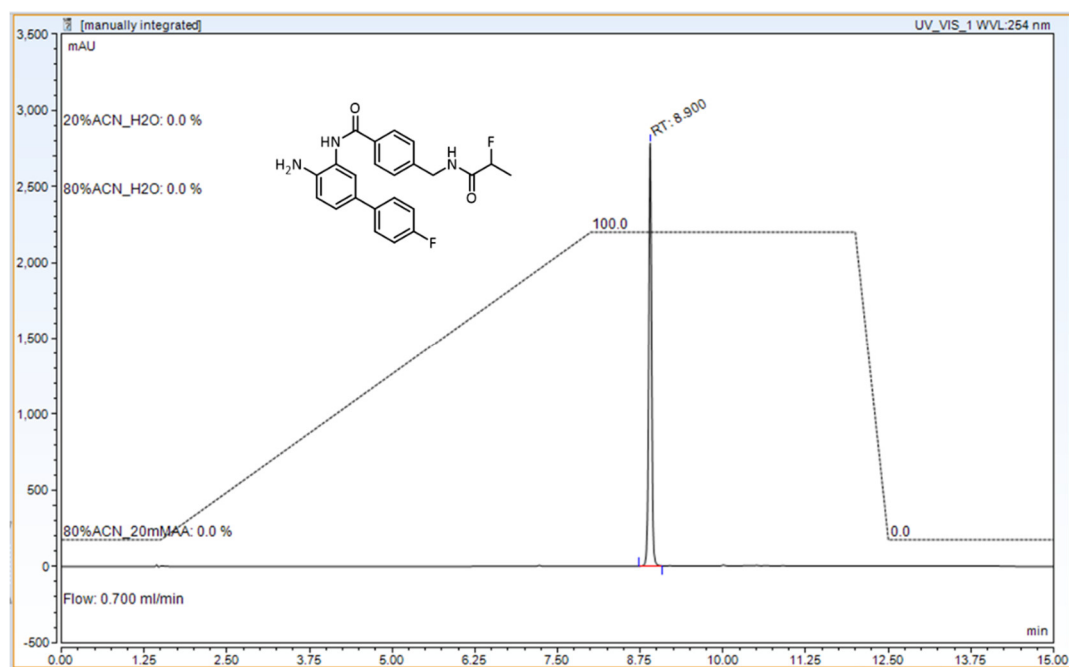
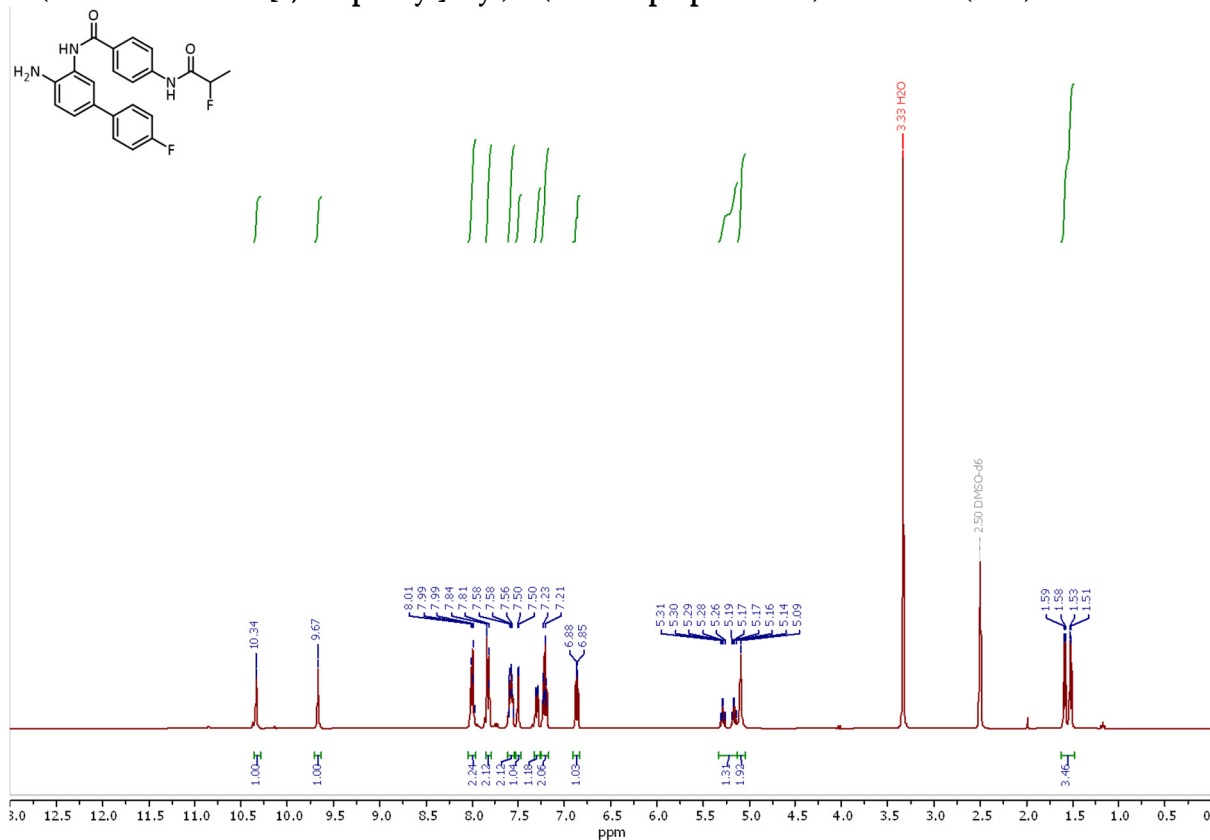
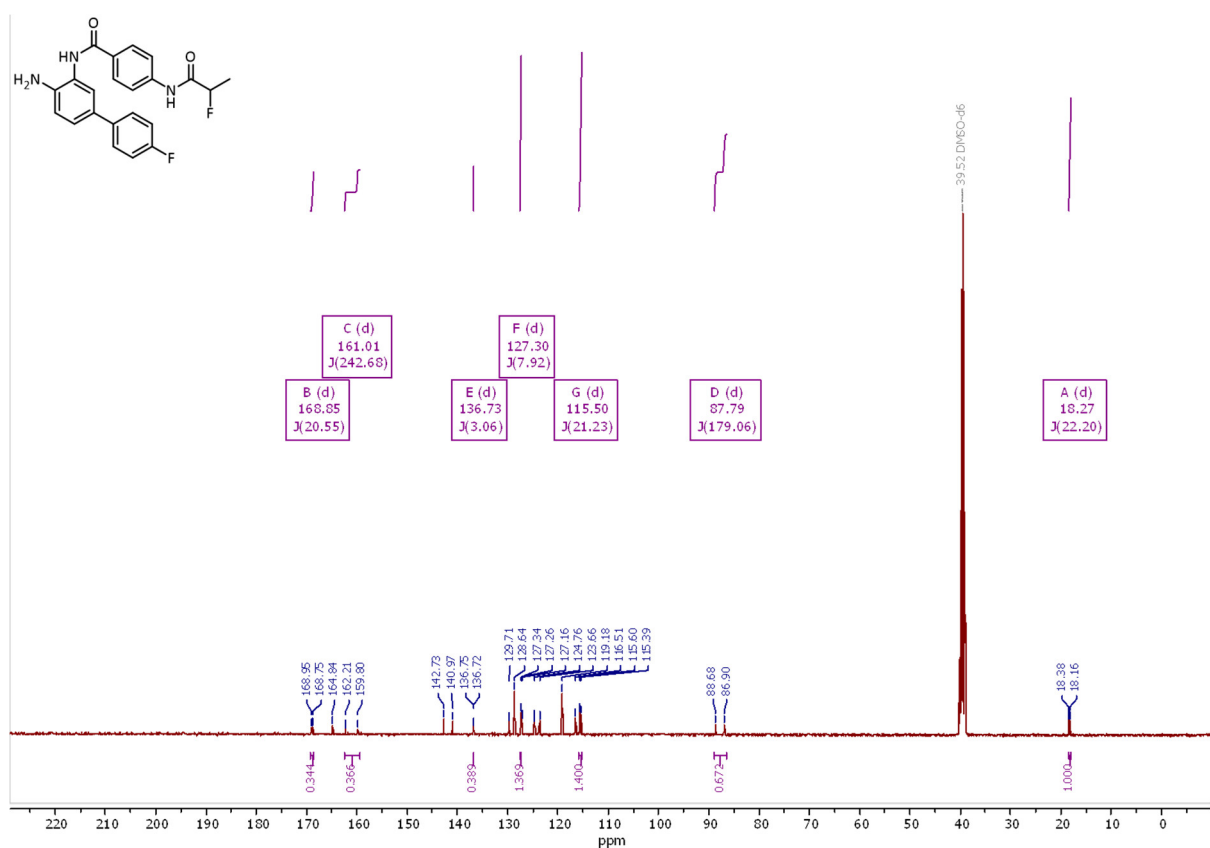
Figure S25. ¹⁹F-NMR of BA5

Figure S26. LC-MS chromatogram of BA5

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

Figure S27. ¹H-NMR of BA6Figure S28. ¹³C-NMR of BA6

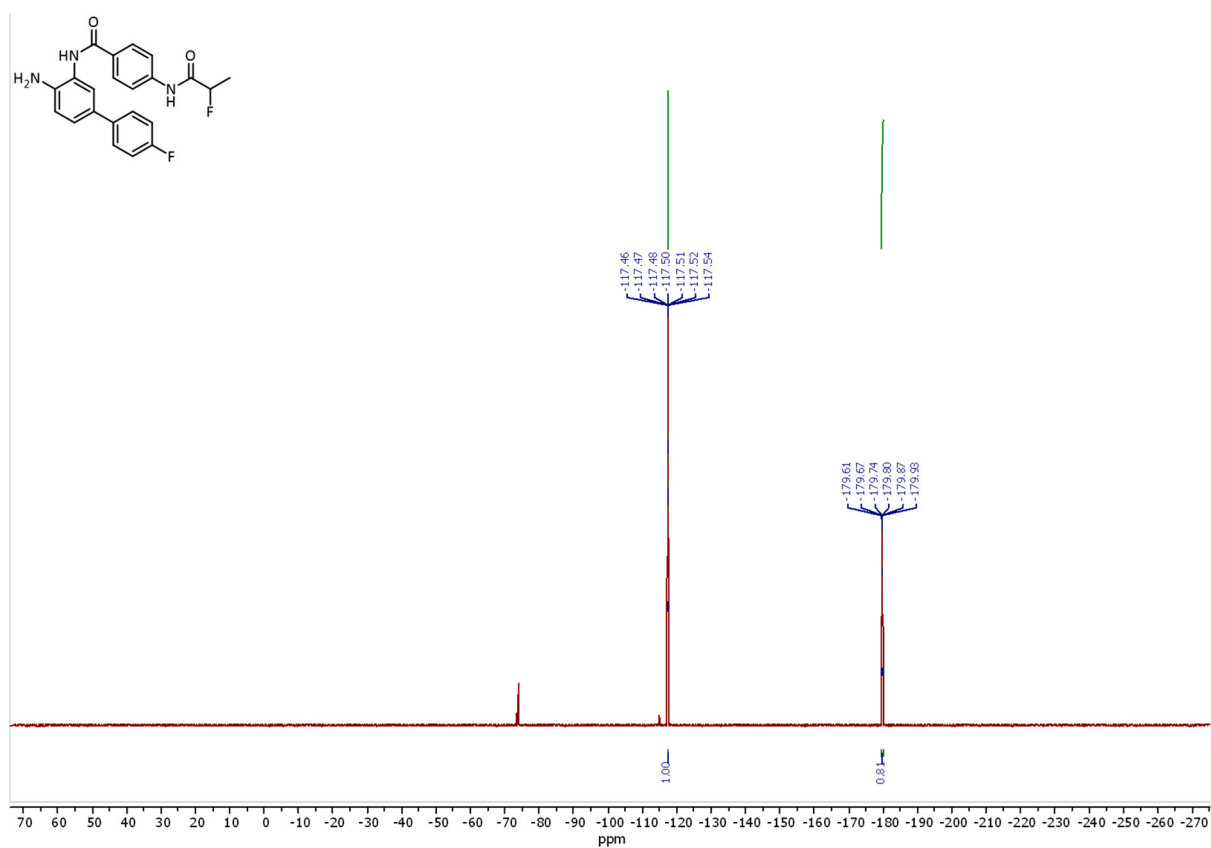
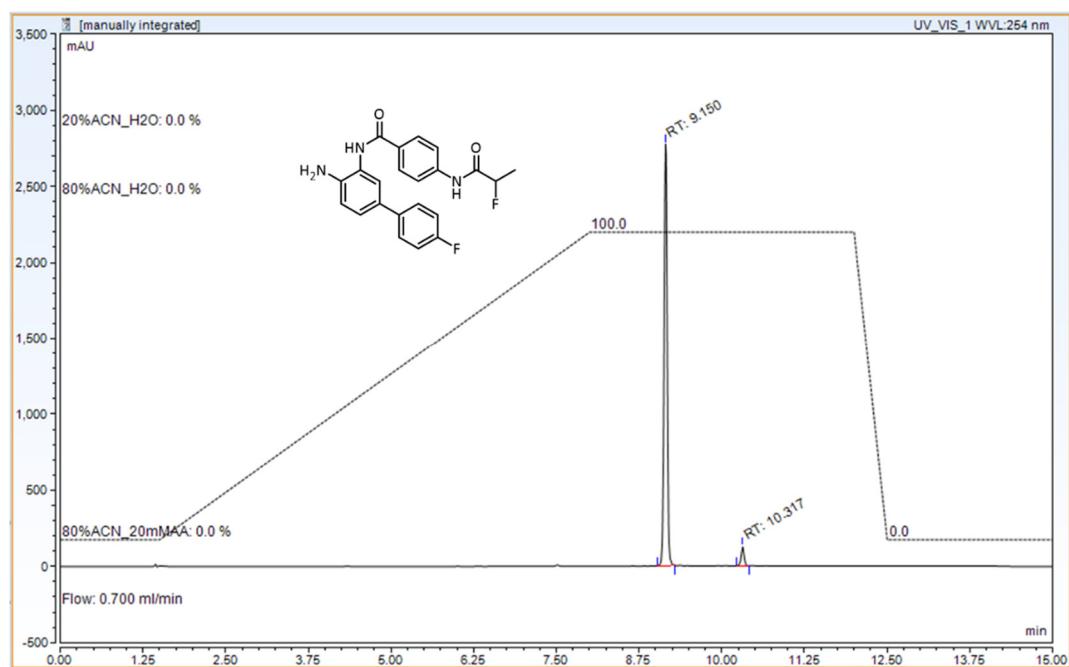
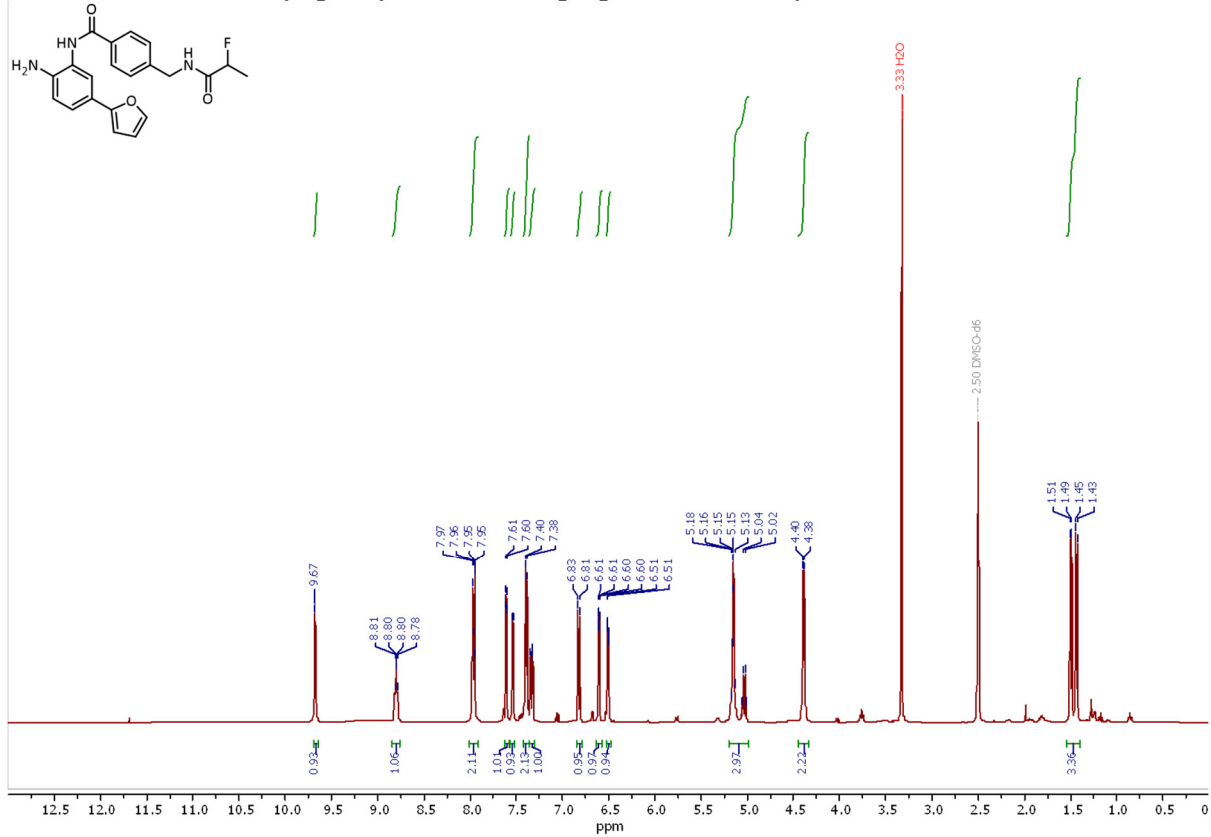
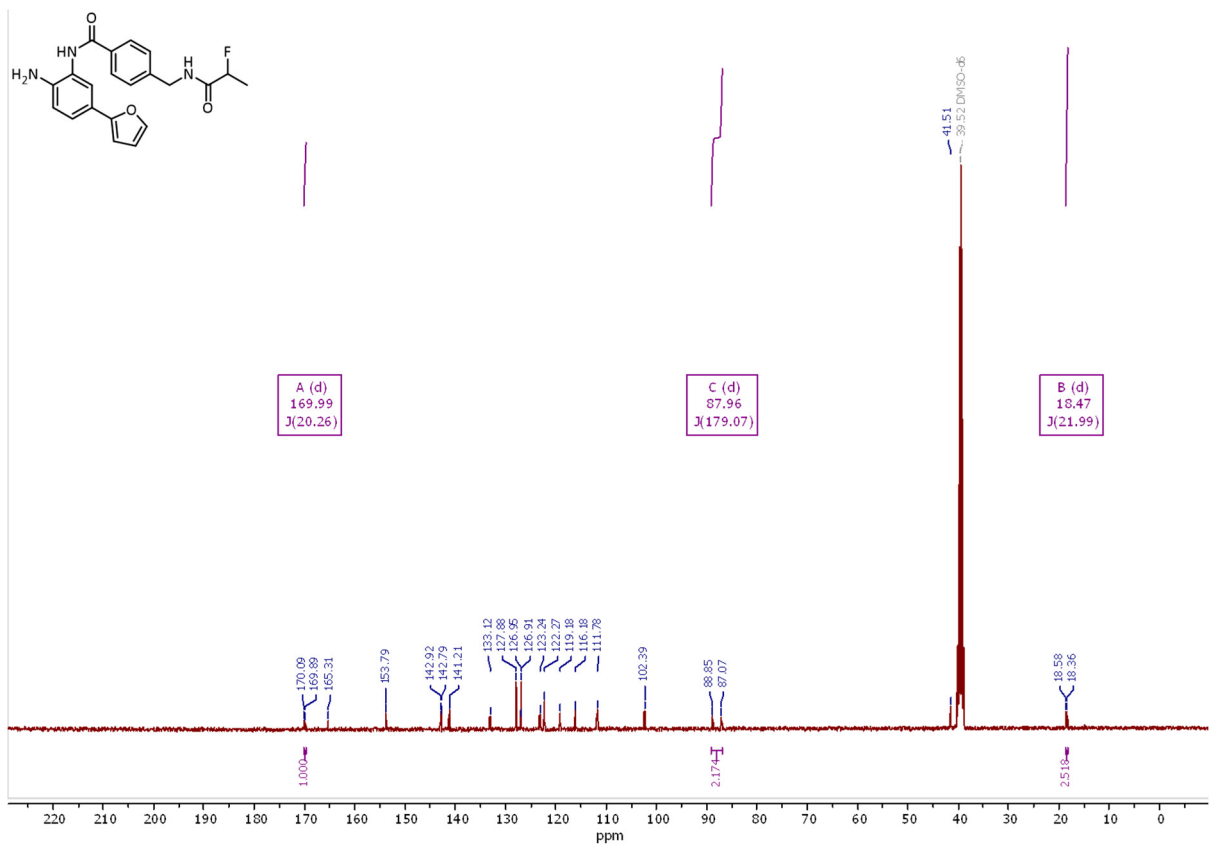
Figure S29. ¹⁹F-NMR of BA6

Figure S30. LC-MS chromatogram of BA6

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

Figure S31. ¹H-NMR of BA7Figure S32. ¹³C-NMR of BA7

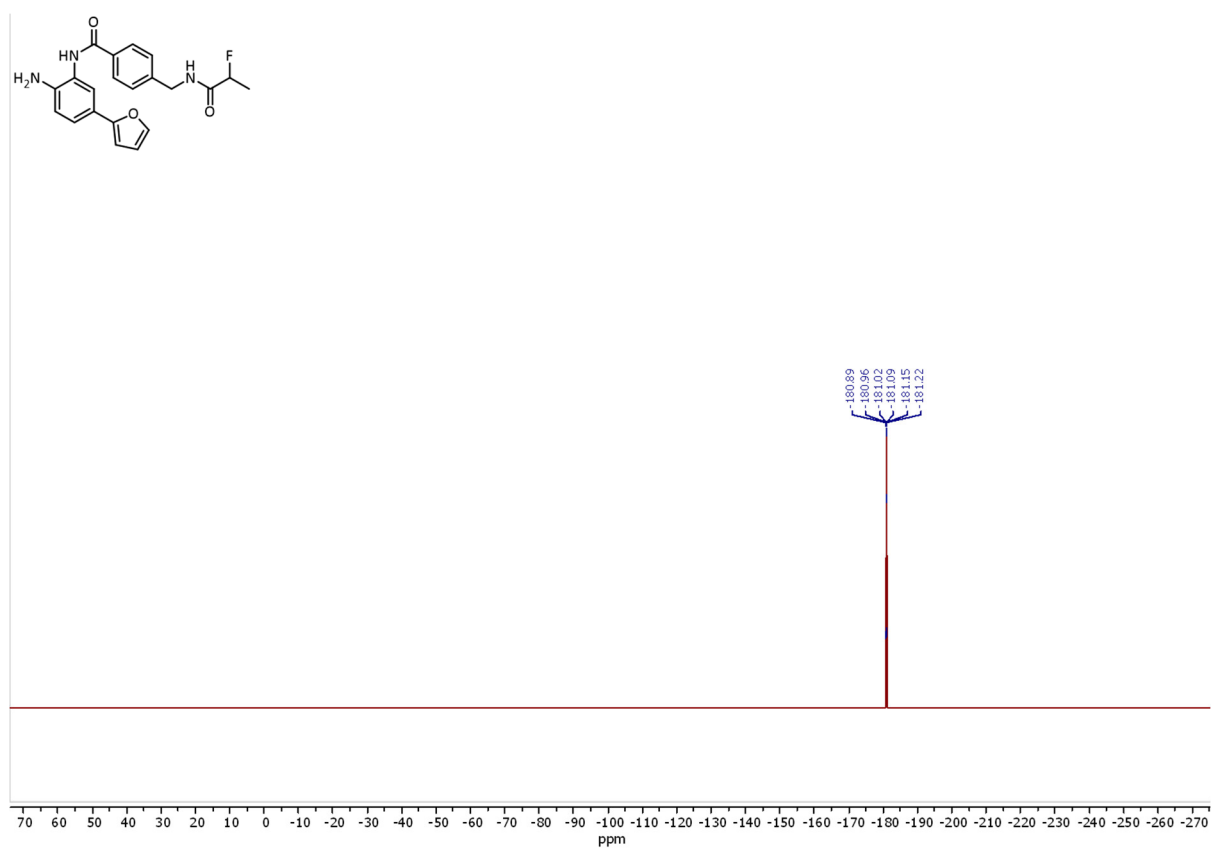
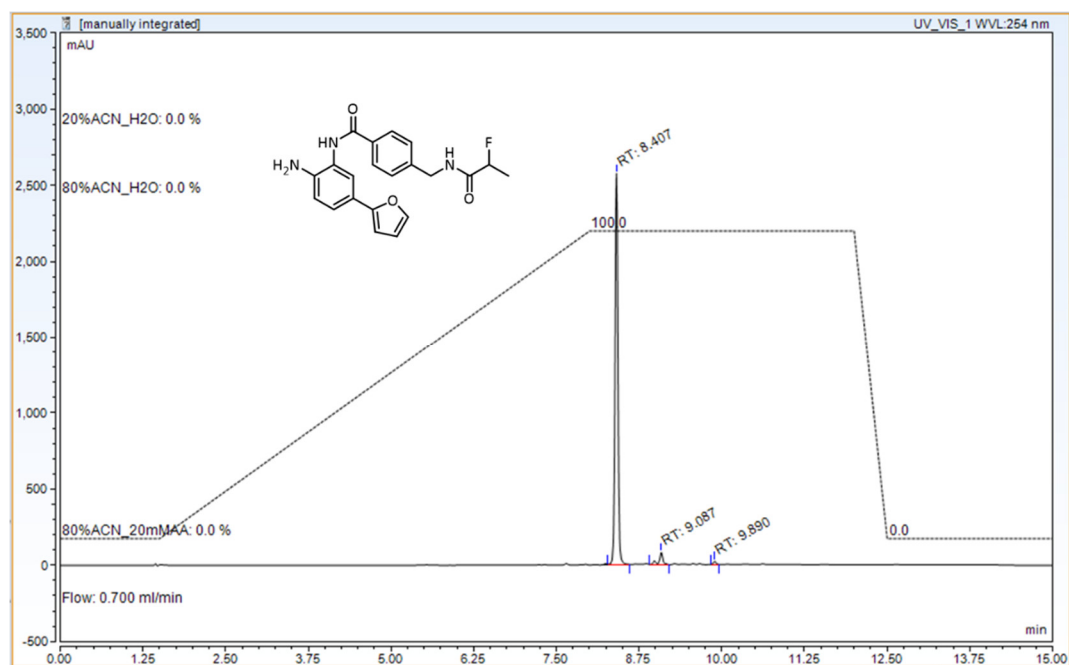
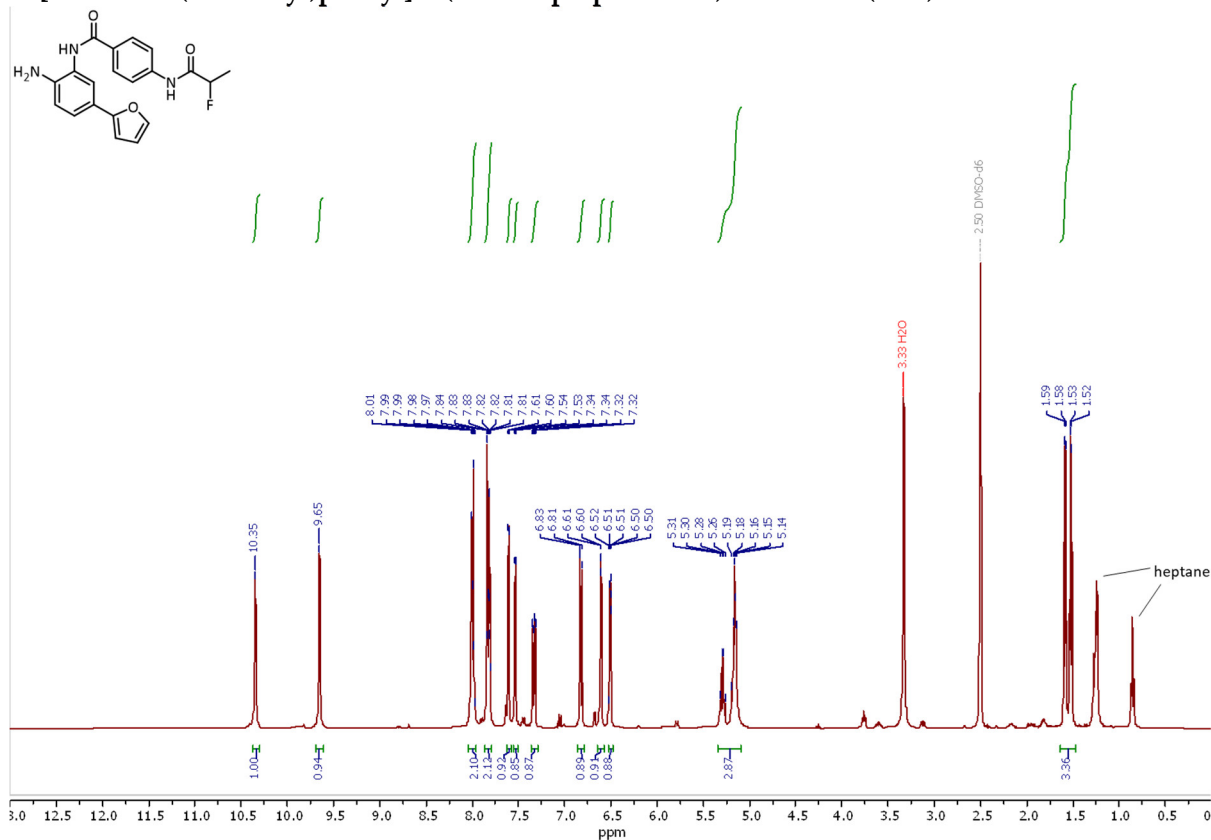
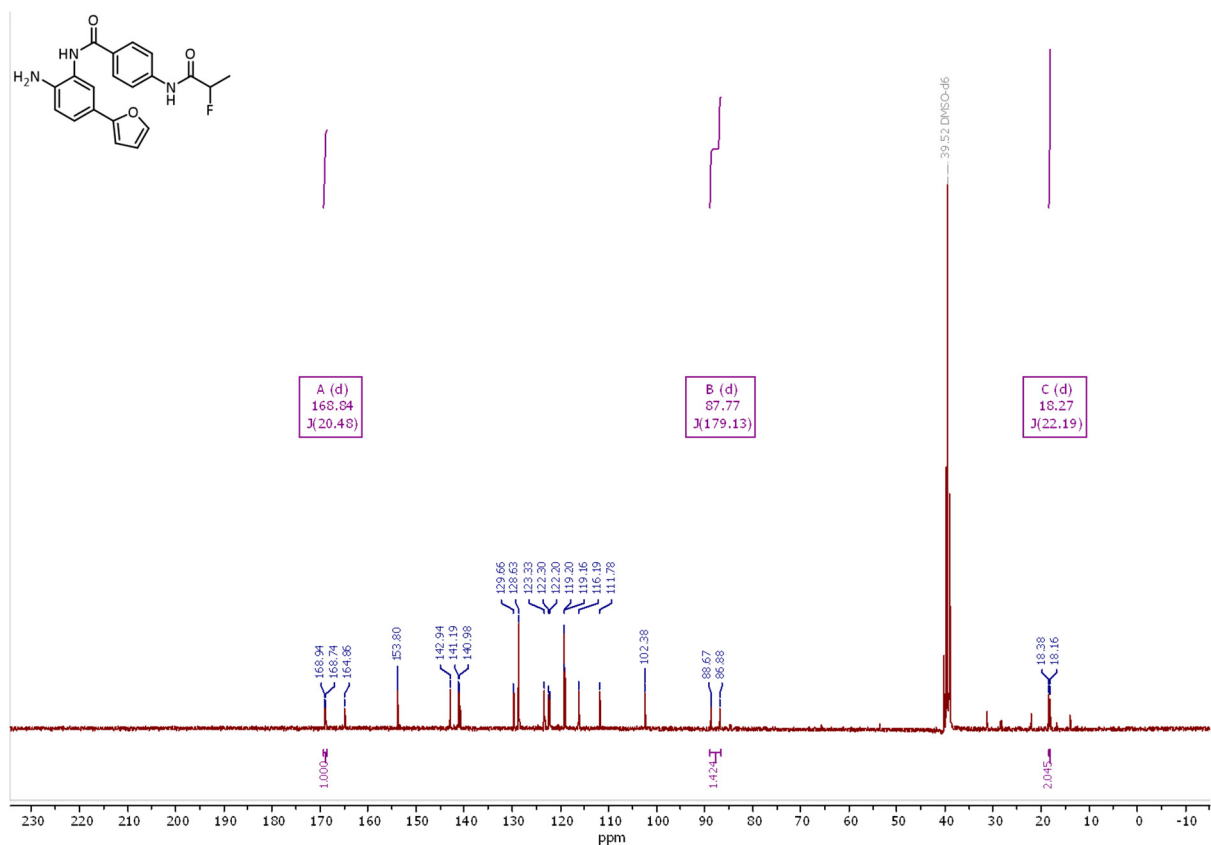
Figure S33. ¹⁹F-NMR of BA7

Figure S34. LC-MS chromatogram of BA7

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

Figure S35. ¹H-NMR of BA8Figure S36. ¹³C-NMR of BA8

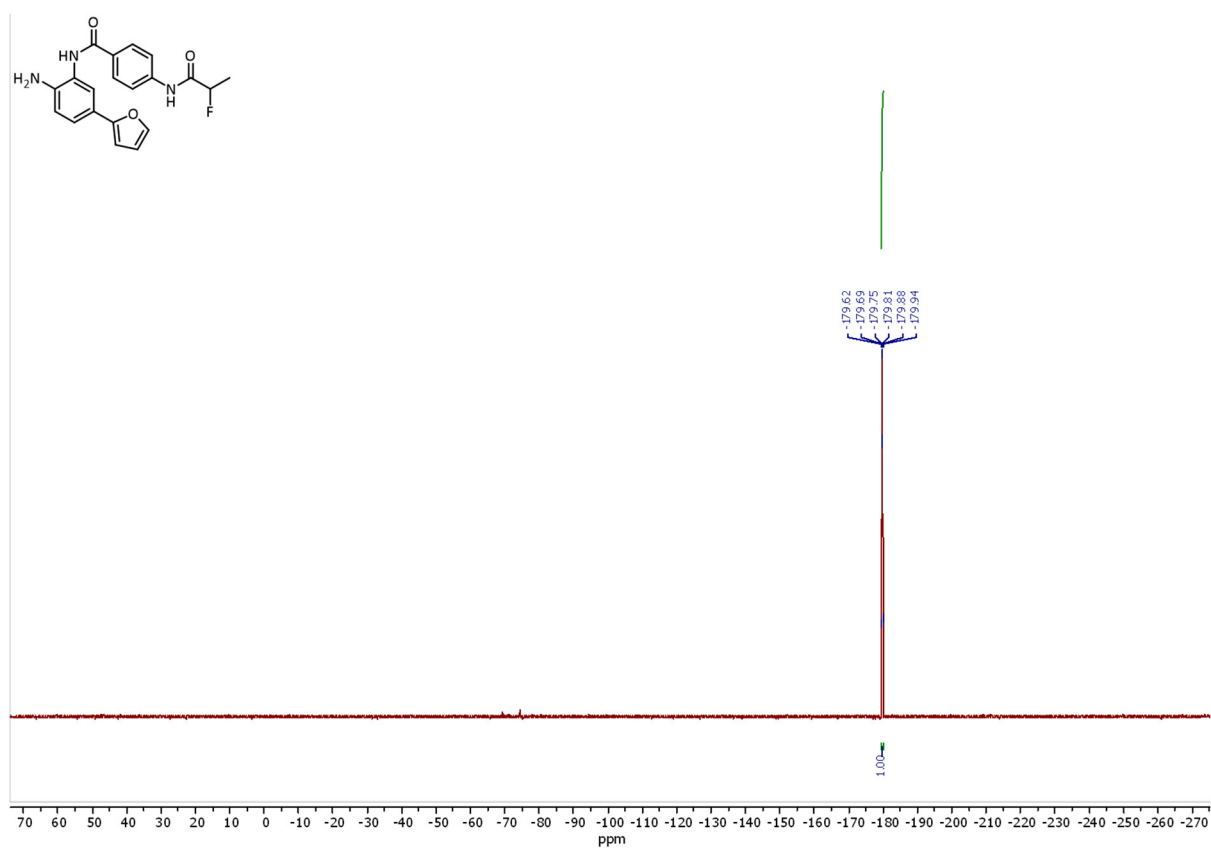
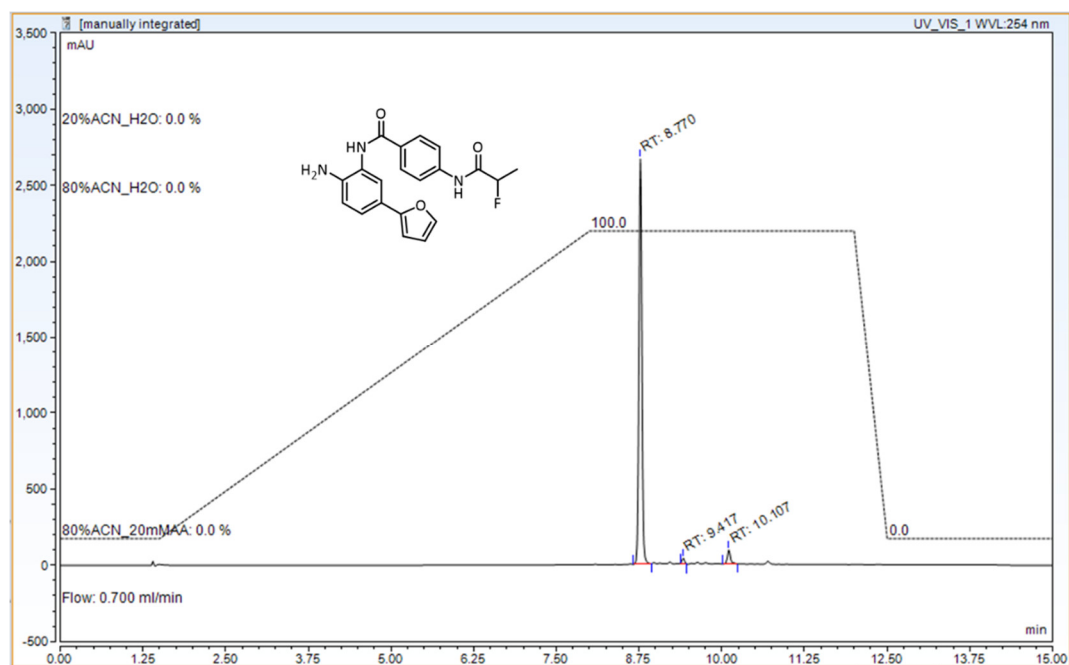
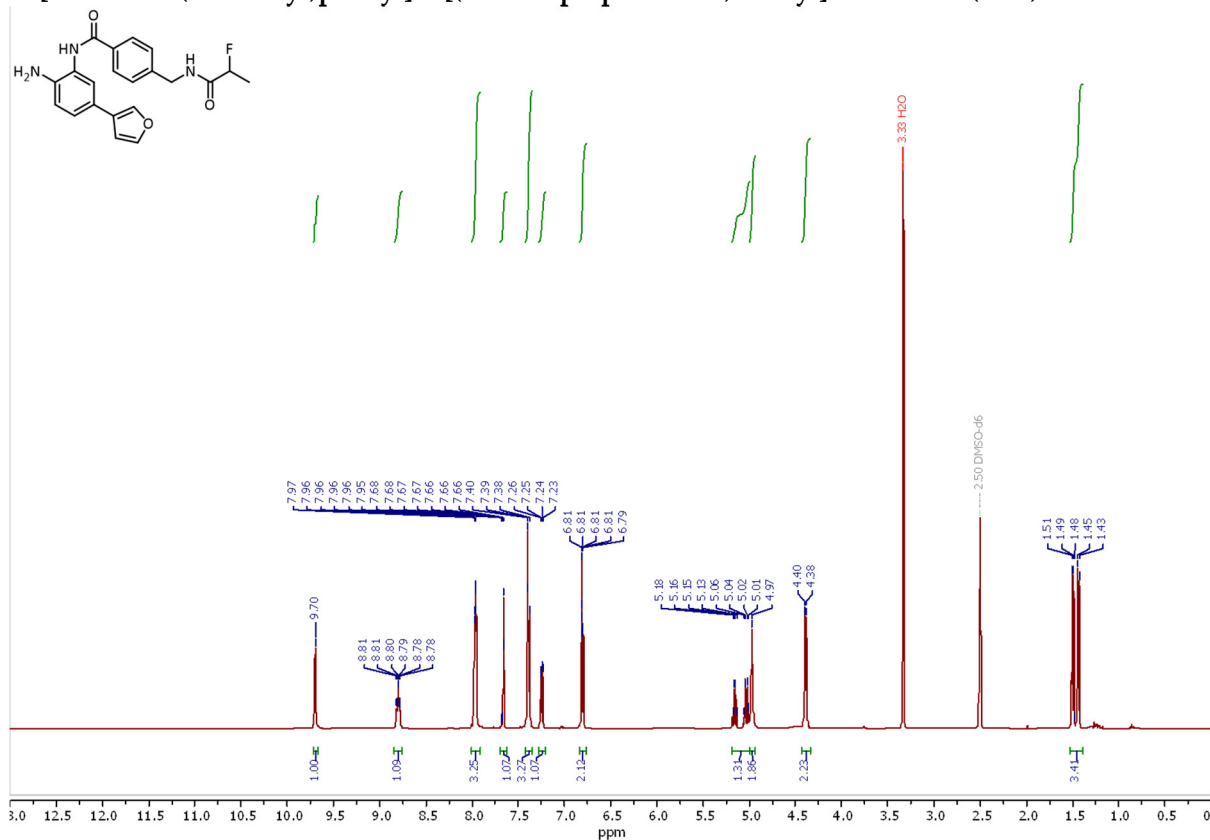
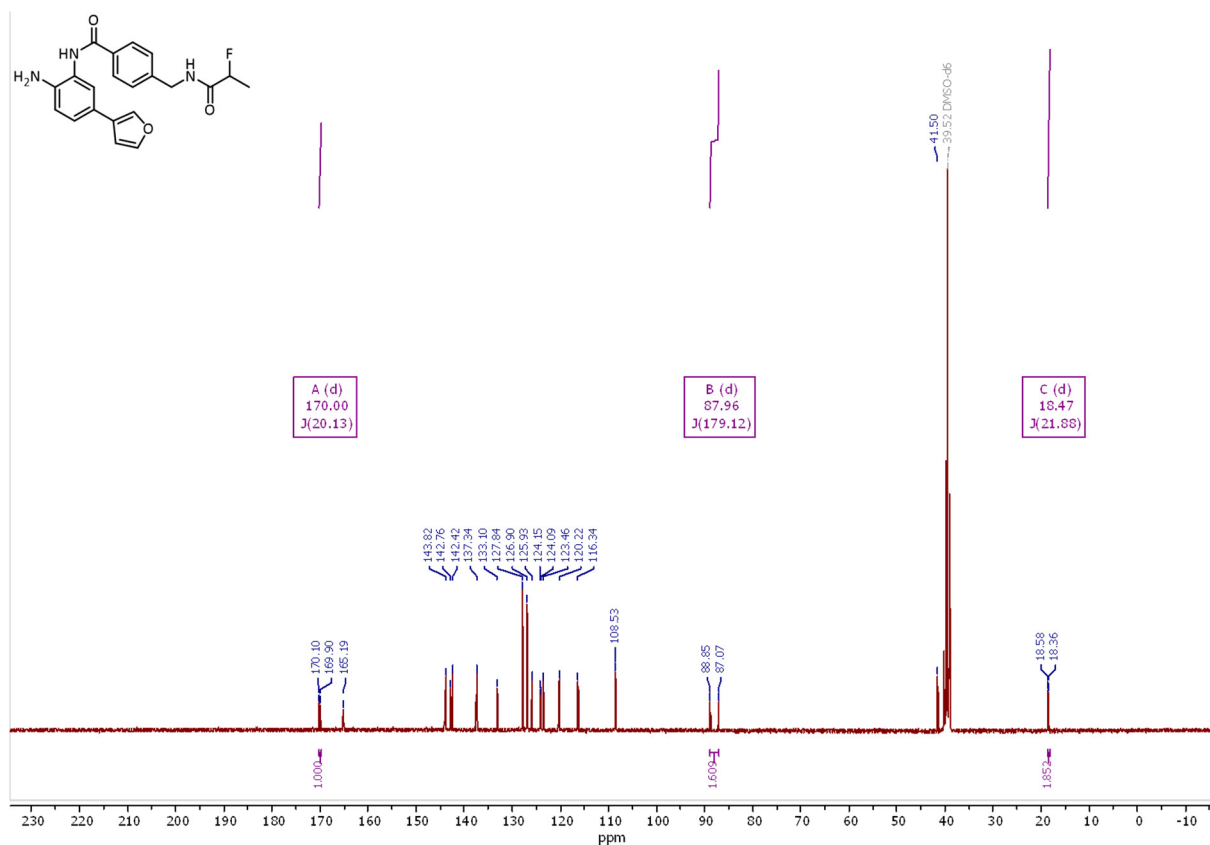
Figure S37. ^{19}F -NMR of BA8

Figure S38. LC-MS chromatogram of BA8

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

Figure S39. ¹H-NMR of BA9Figure S40. ¹³C-NMR of BA9

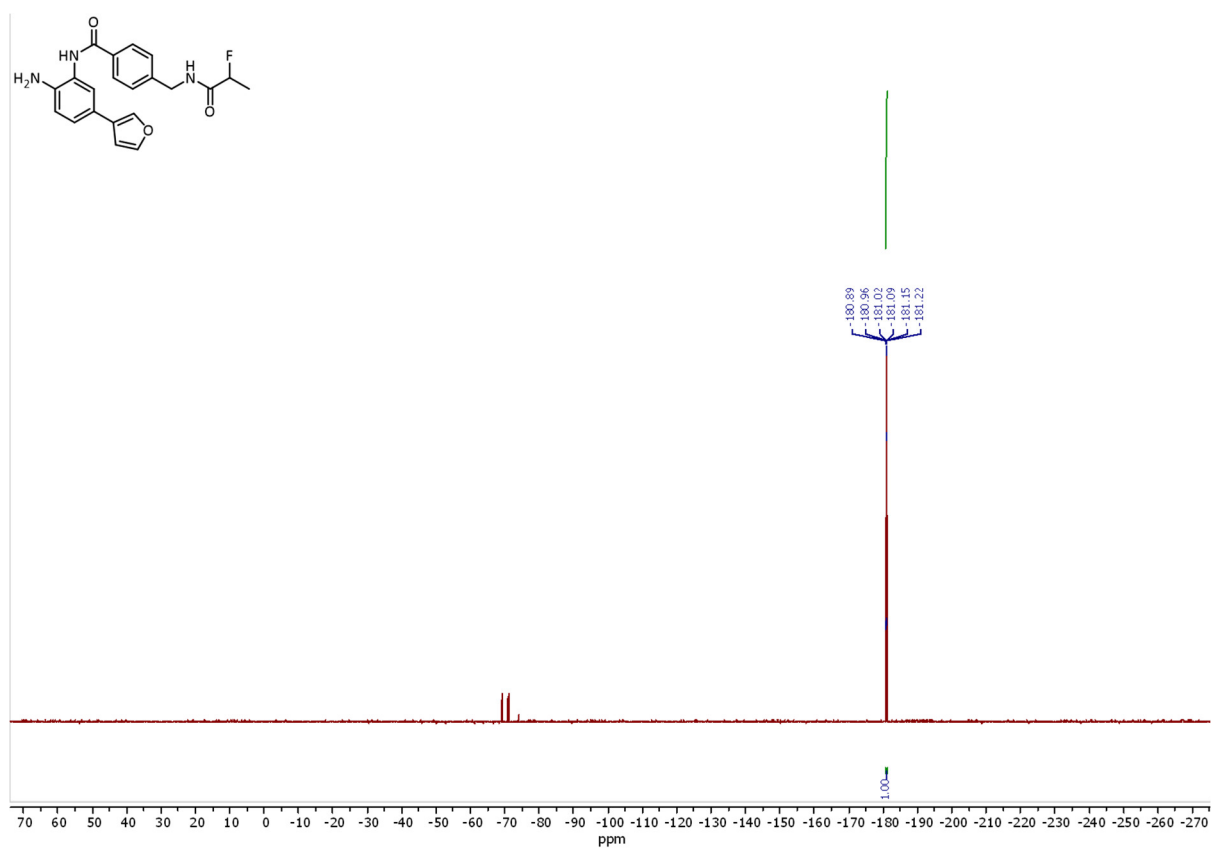
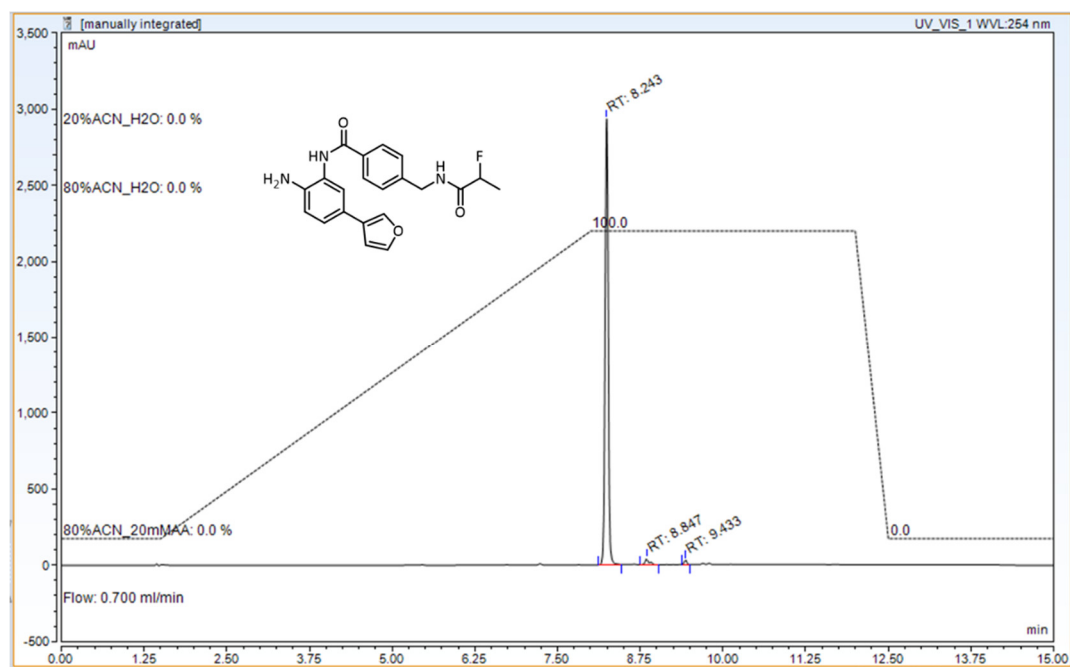
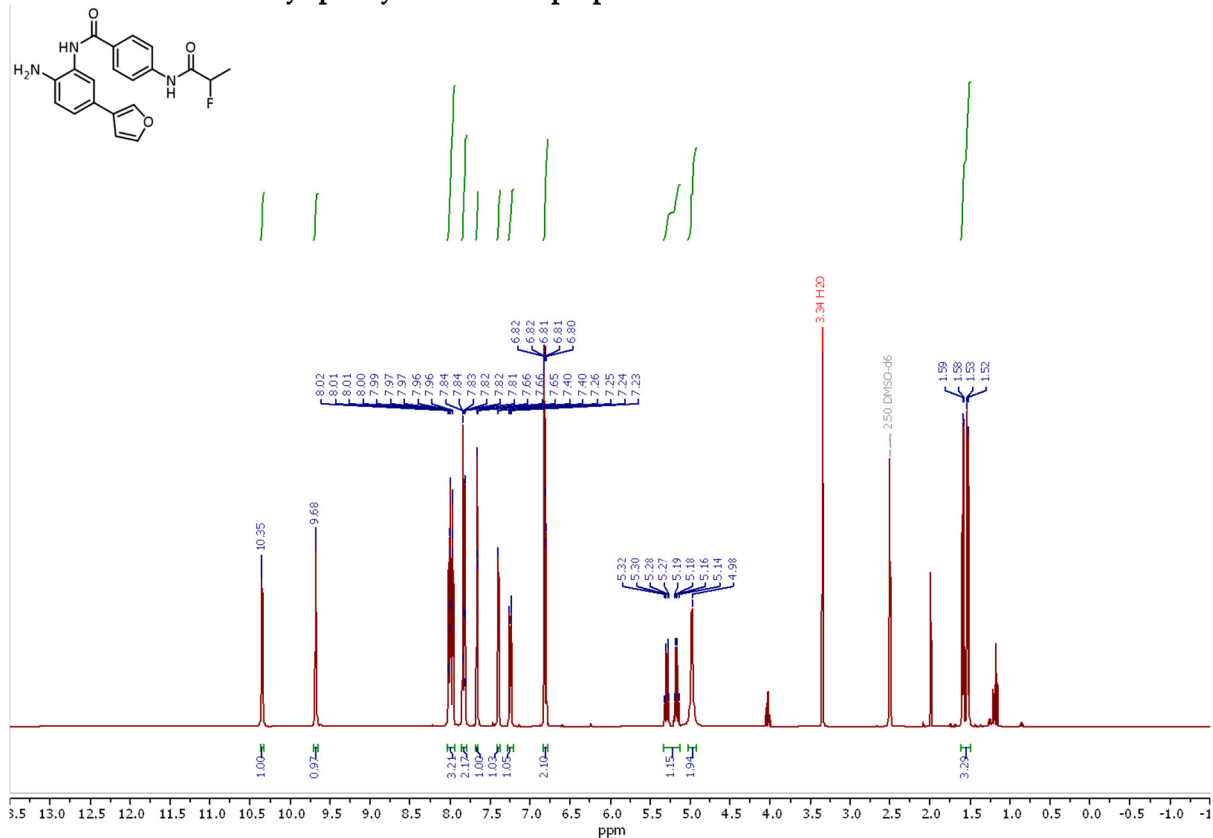
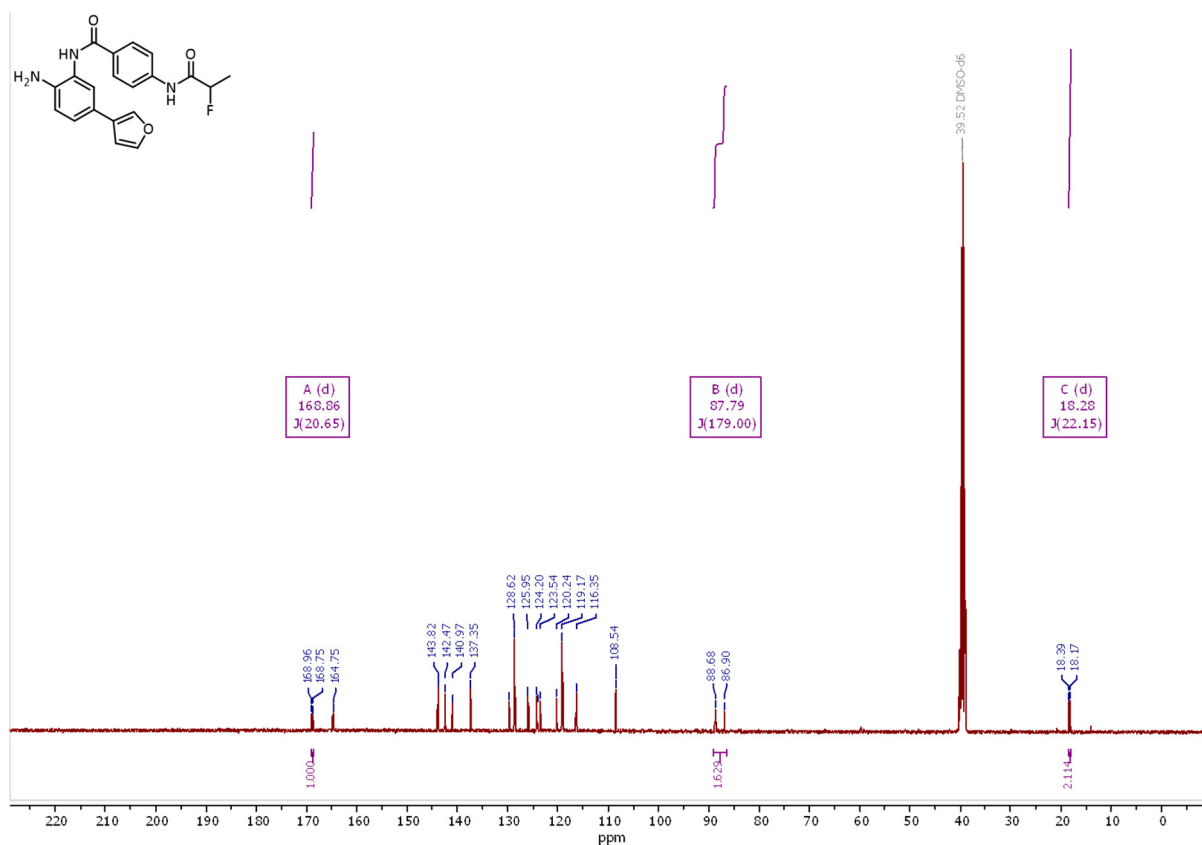
Figure S41. ^{19}F -NMR of BA9

Figure S42. LC-MS chromatogram of BA9

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

Figure S43. ¹H-NMR of BA10Figure S44. ¹³C-NMR of BA10

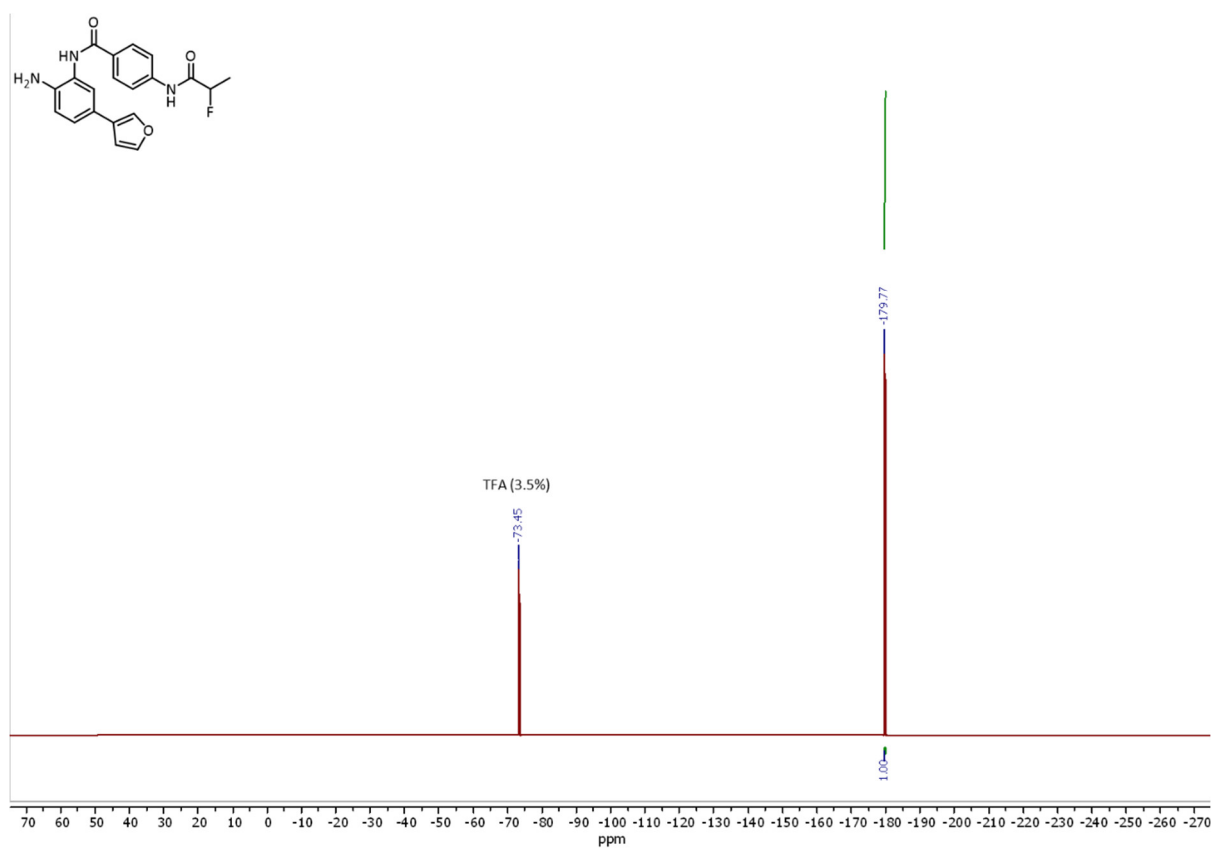
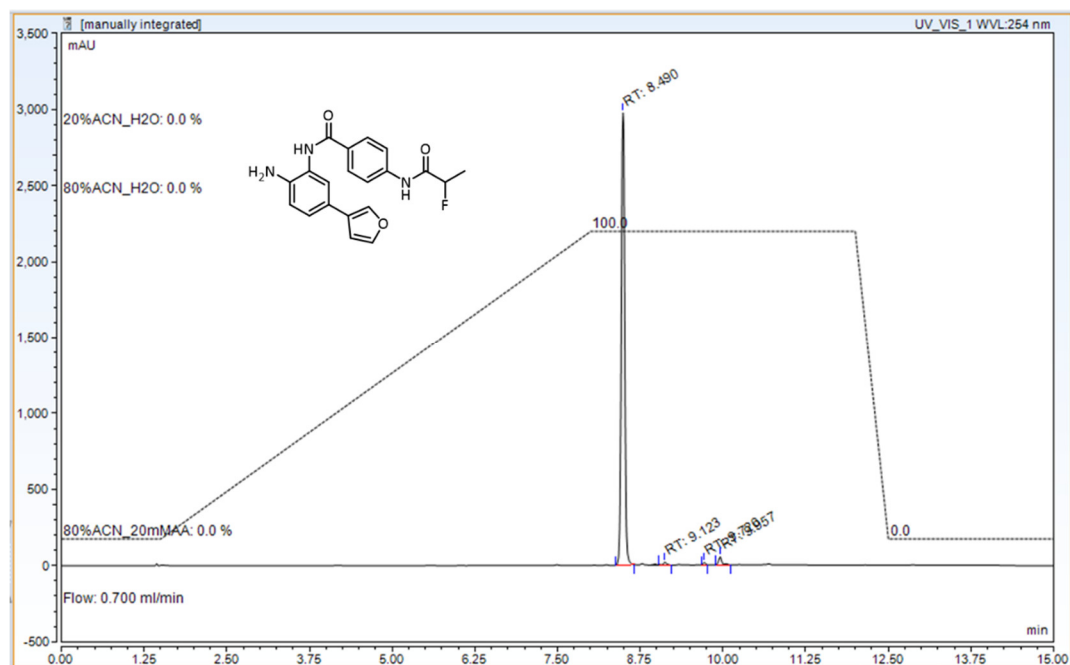
Figure S45. ¹⁹F-NMR of BA10

Figure S46. LC-MS chromatogram of BA10