Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Supporting Information for:

Towards Kit-Like ^{18}F-Labeling of Marimastat, a Reversible MMPI, for *in vivo* PET Imaging of Cancer Associated Matrix Metalloproteases

Ying Li[1], Richard Ting[1], Curtis W. Harwig[1], Caroline L. Bellac[2], Philipp F. Lange[2], Ulrich auf dem Keller[2], James A. H. Inkster[3], Paul Schaffer[3], Michael J. Adam[1,3], Thomas J. Ruth[3], Christopher M. Overall[2], David M. Perrin[1]*

Table of Contents

Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Visual Schematic of Specific Activity Tripling:

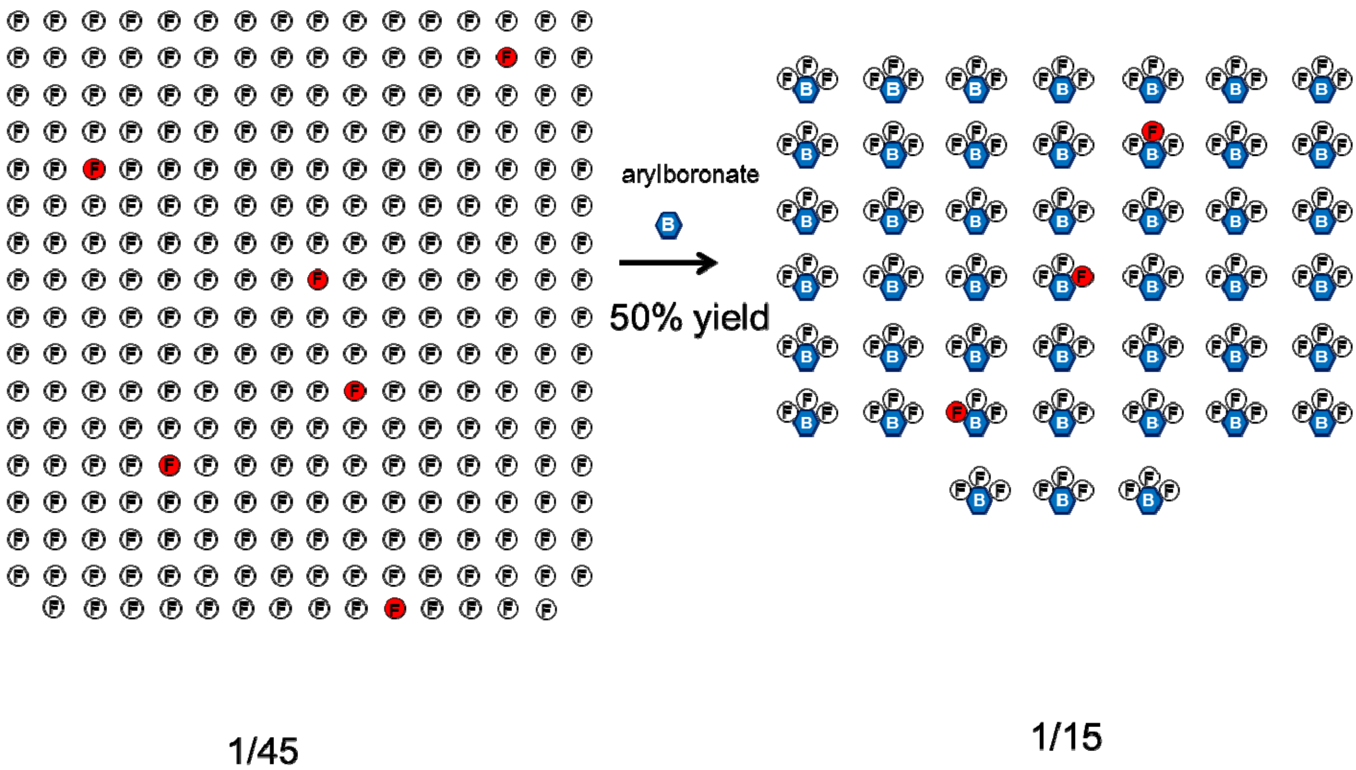
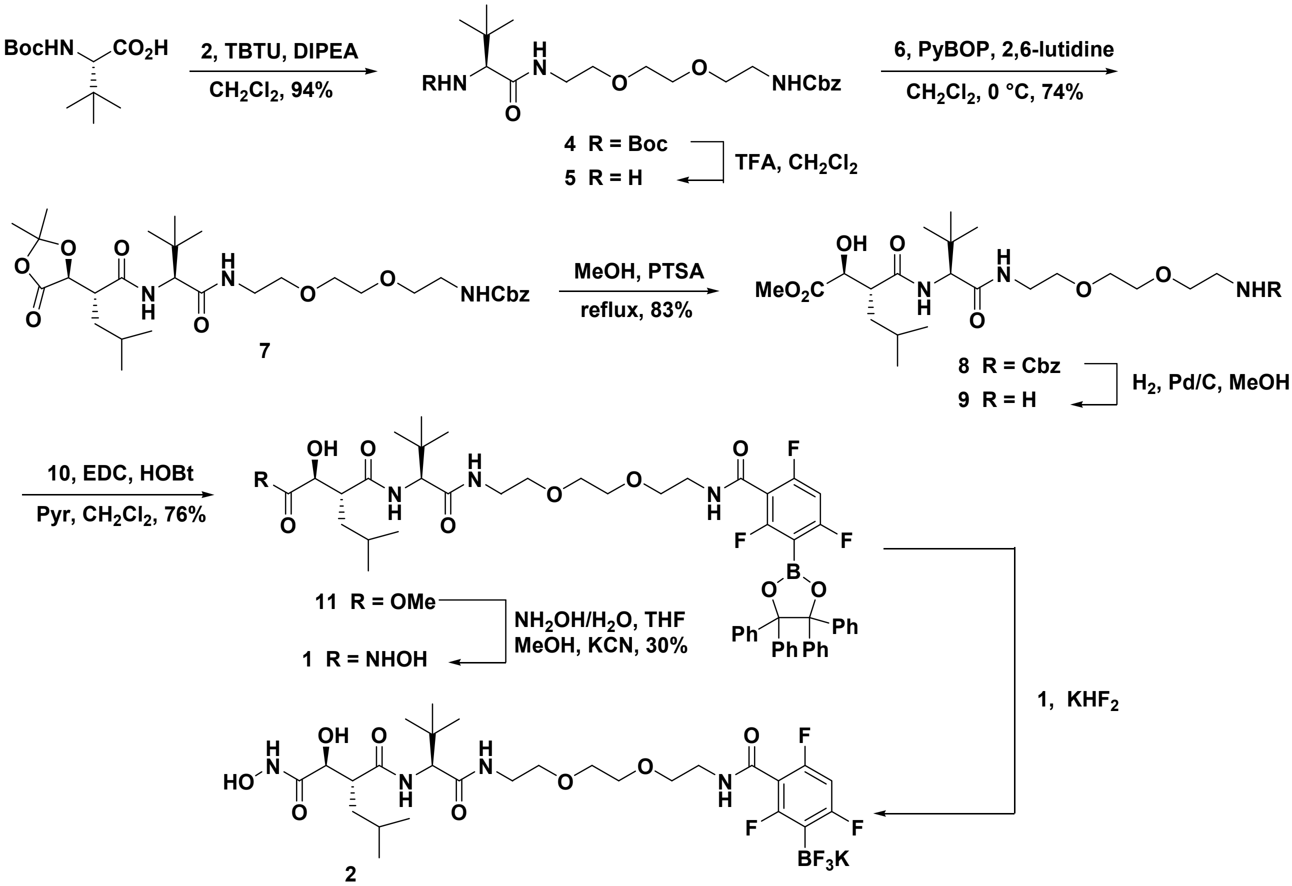


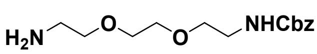
Figure Legend: Above, 1 in 45 atoms of fluoride is radioactive, (6 of 270 total atoms), where the red atom indicates an atom of ^{18}F. Based on a 50% radiochemical yield, 135 atoms of fluoride condense with 45 atoms of boron in a 3-to-1 stoichiometry to give 45 aryltrifluoroborate molecules, of which 3 are radioactive, or where 1 in 15 is radioactive. The radioactivity is contained on 1/3 as many aryltrifluoroborate molecules. Therefore the specific activity has *tripled*.

Nevertheless, there is only 1 radioactive fluoride atom per molecule of $ArBF_3$. While there exists a slight chance that two atoms of [^{18}F]-fluoride atoms condense with one boron atom, the probability of such an event is insignificant: approximately (1/45) x (5/269) or 0.04% in *this* case, or more generally $(1/45)^2$ if one is not limited to exactly 270 fluoride ions. The above case where 1-in-45 fluoride atoms is radioactive provides a specific activity of 38 Ci/µmol.

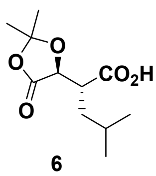
Because in practice the $^{18}F/^{19}F$ fluoride ratio is ~ 1:100 (17 Ci/micromole), the chance of producing a doubly labeled $ArBF_3$ is even lower i.e. 0.01%.

Synthetic Scheme:

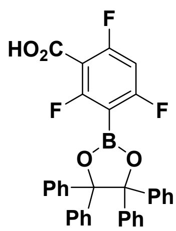




3



6



10

HPLC purification of 1:

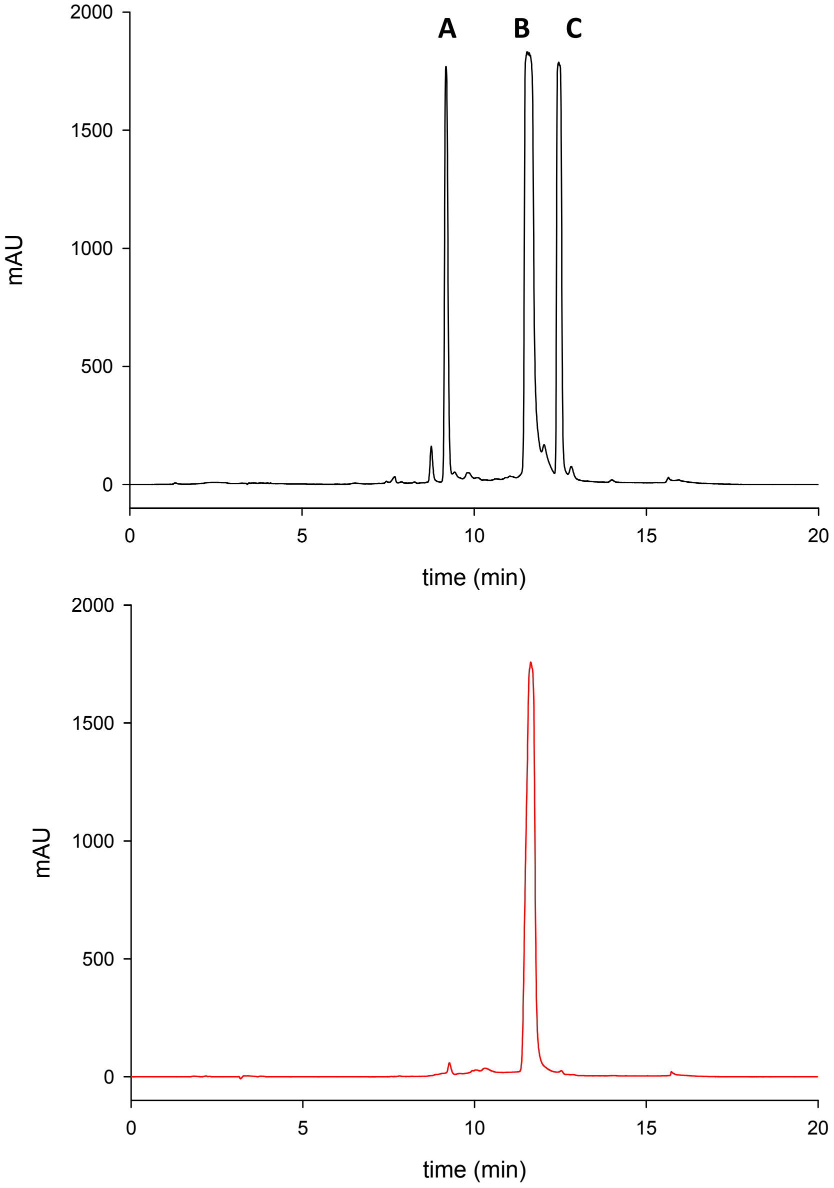
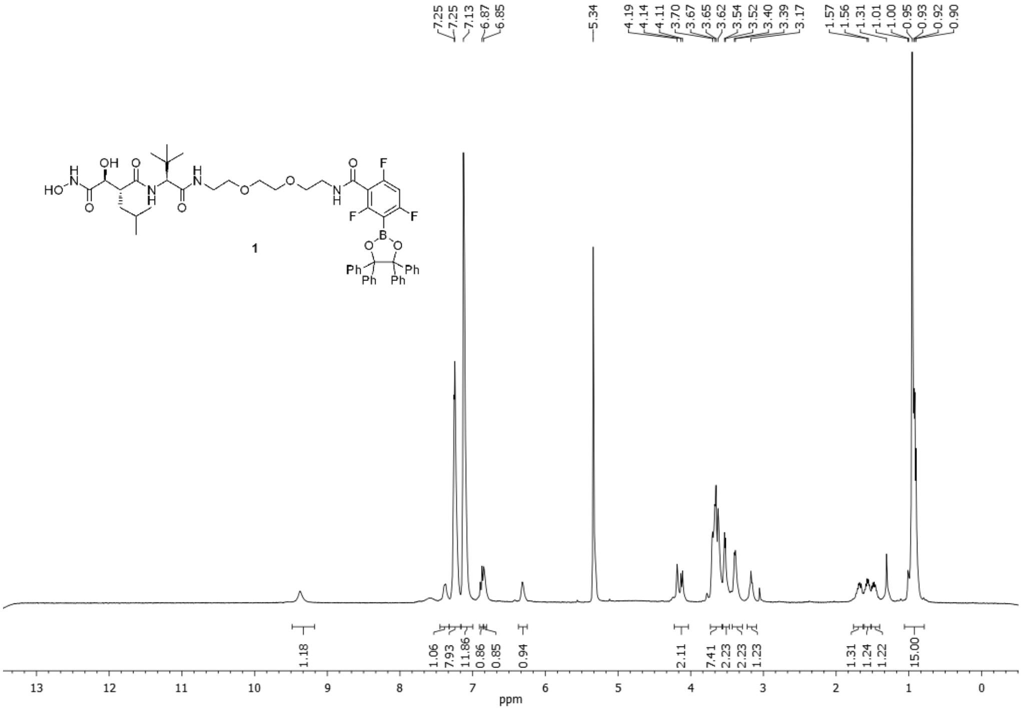
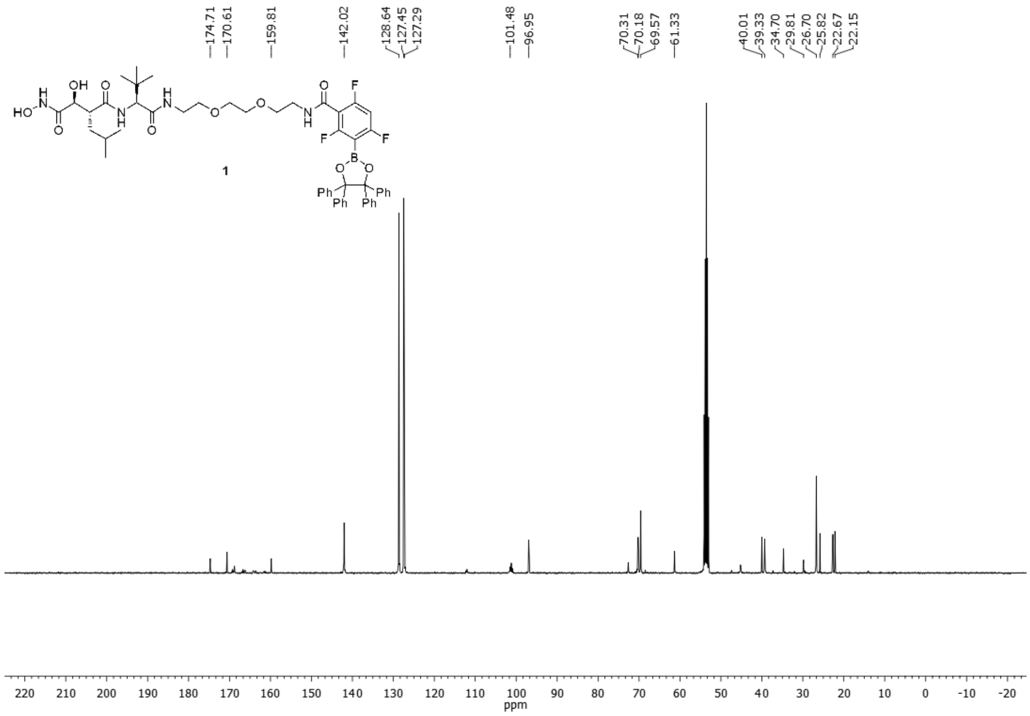


Figure Legend: Above: HPLC Purification of (**1**): detection at 229 nm, peak-**A**: deboronated-(**1**); peak-**B**: desired compound (**1**); peak-**C**: tetraphenylpinacol. ESI-MS was used to identify composition of matter in each peak, Below: reinjection of HPLC purified **1** taken from peak **B** above.

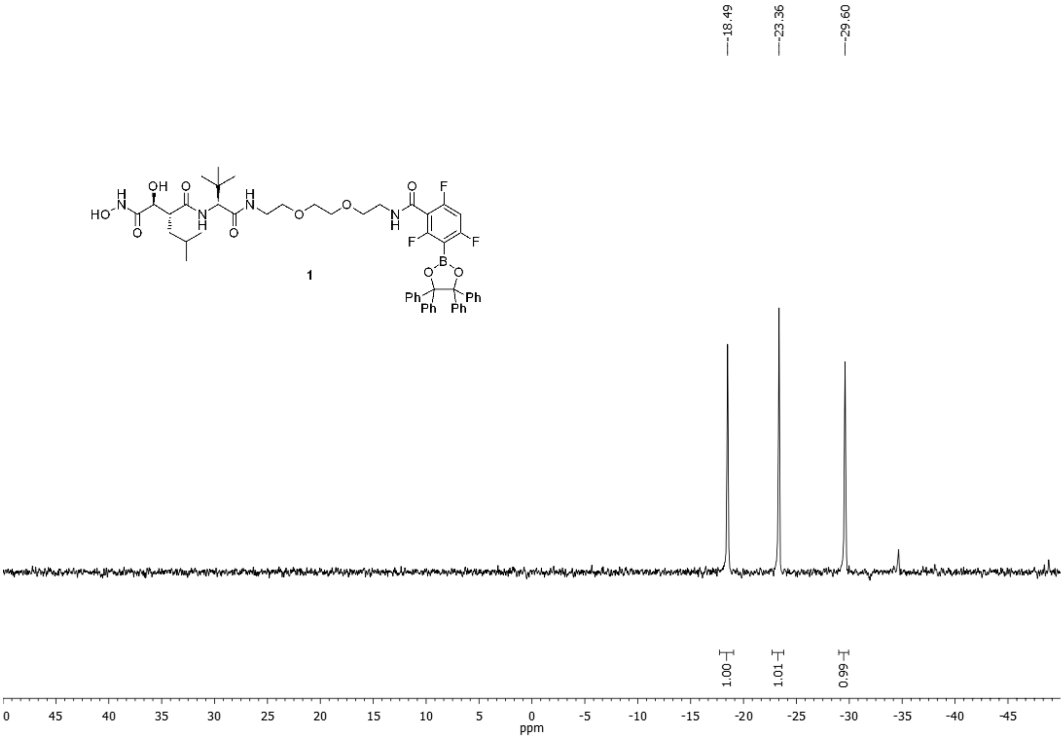
^{1}H NMR of 1:



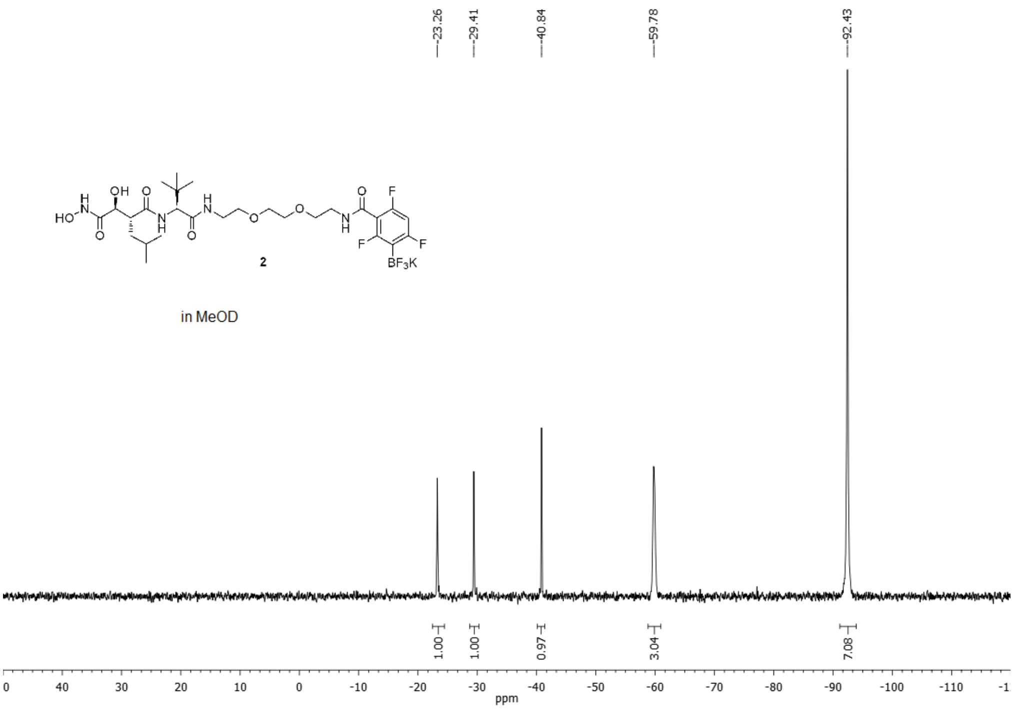
^{13}C NMR of 1:



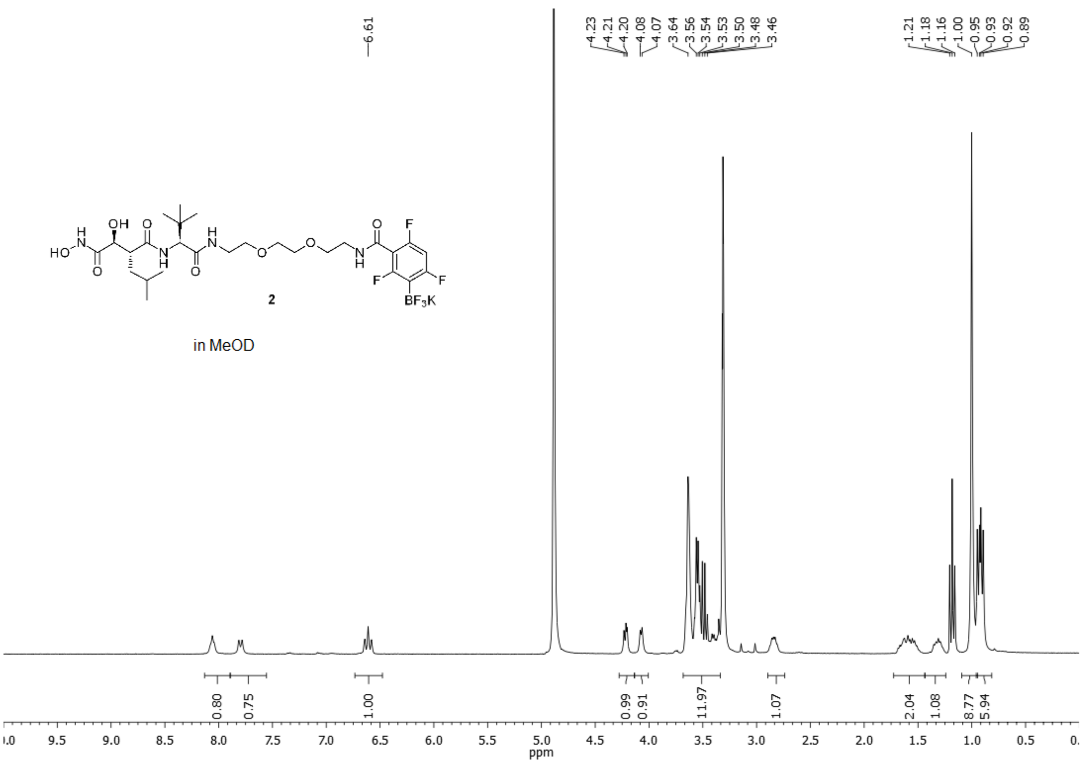
[19]F NMR of 1:


-18.49
-23.36
-29.60
1.00
1.01
0.99
0
45
40
35
30
25
20
15
10
5
0
ppm
-5
-10
-15
-20
-25
-30
-35
-40
-45

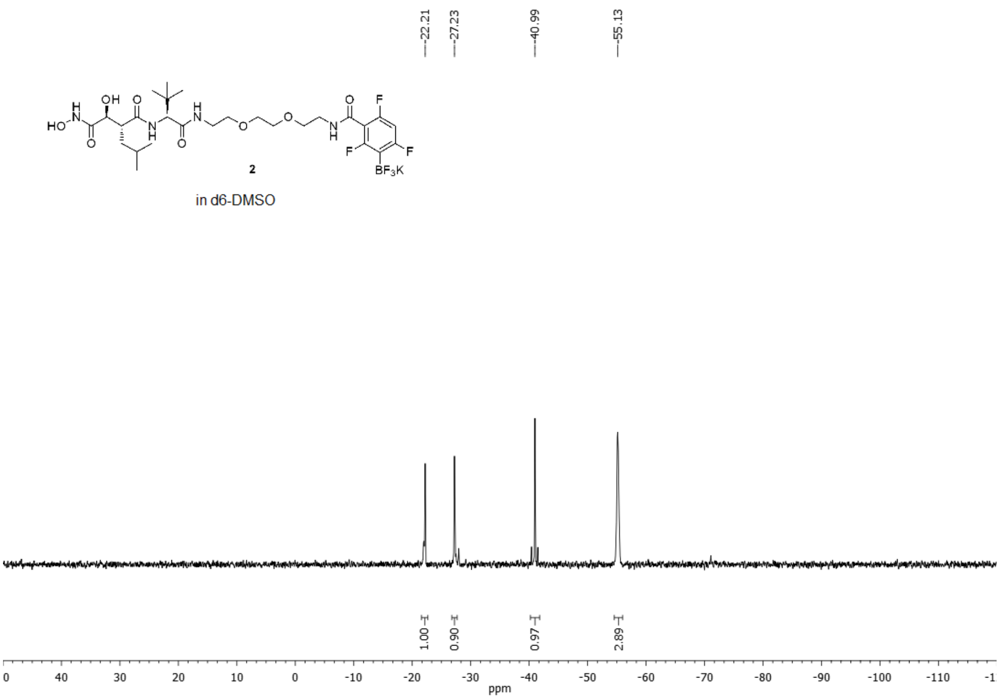
^{19}F NMR (d_3-MeOD) of 2 with some free fluoride present:



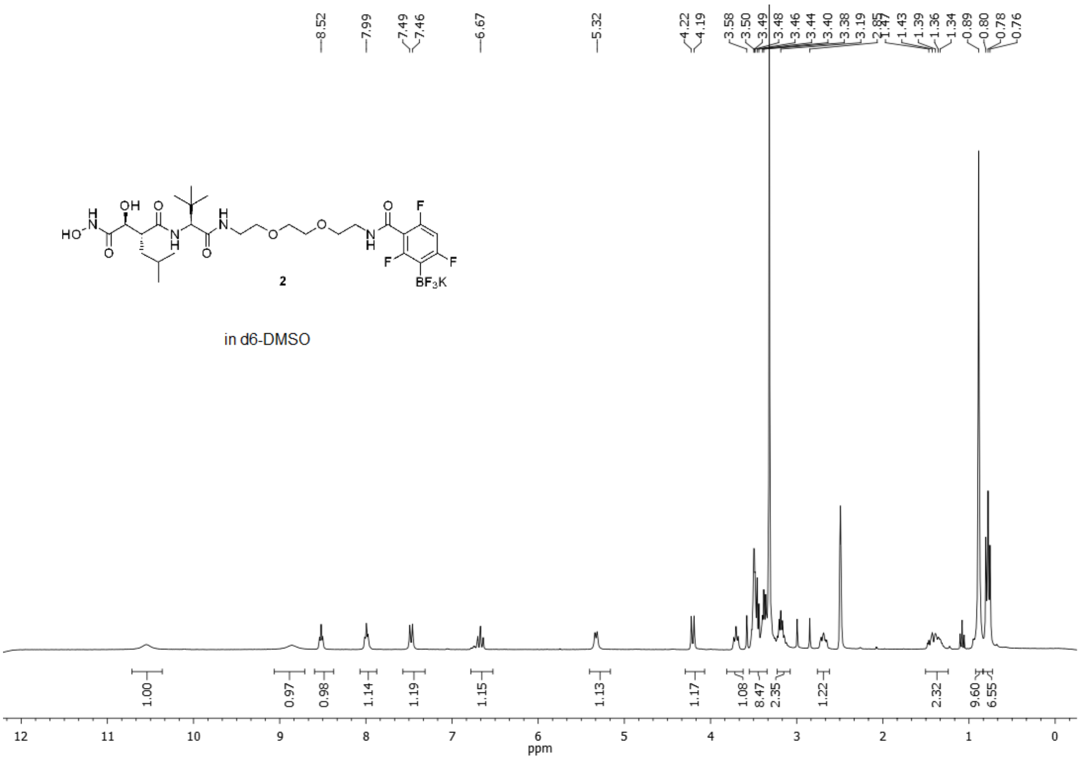
1H NMR (d_3-MeOD) of 2 with some free fluoride present:



^{19}F NMR (d_6-DMSO)of 2 without free fluoride present:



^{1}H NMR (d_6-DMSO)of 2 without free fluoride:



UV-vis and Radiochromatograms of ^{18}F-labeled 2, Run-1:

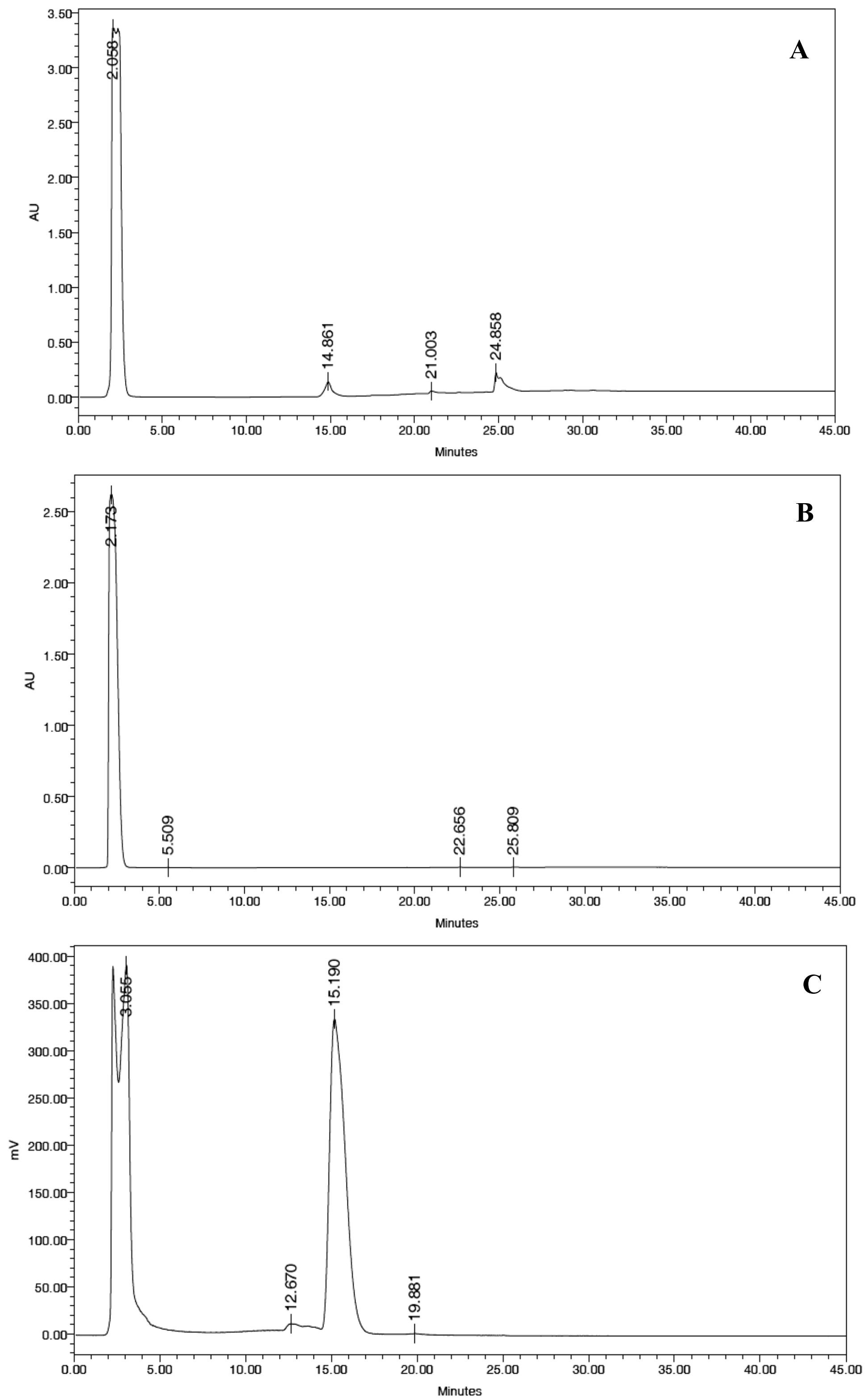


Figure Legend: Crude reaction from Run-1, injected onto analytical column: **A)** UV-trace at 229 nm, **B)** UV-trace at 260 nm, **C)** rad detector at high attenuation.

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Analytical Trace of ^{18}F-labeled 2, Run-1:

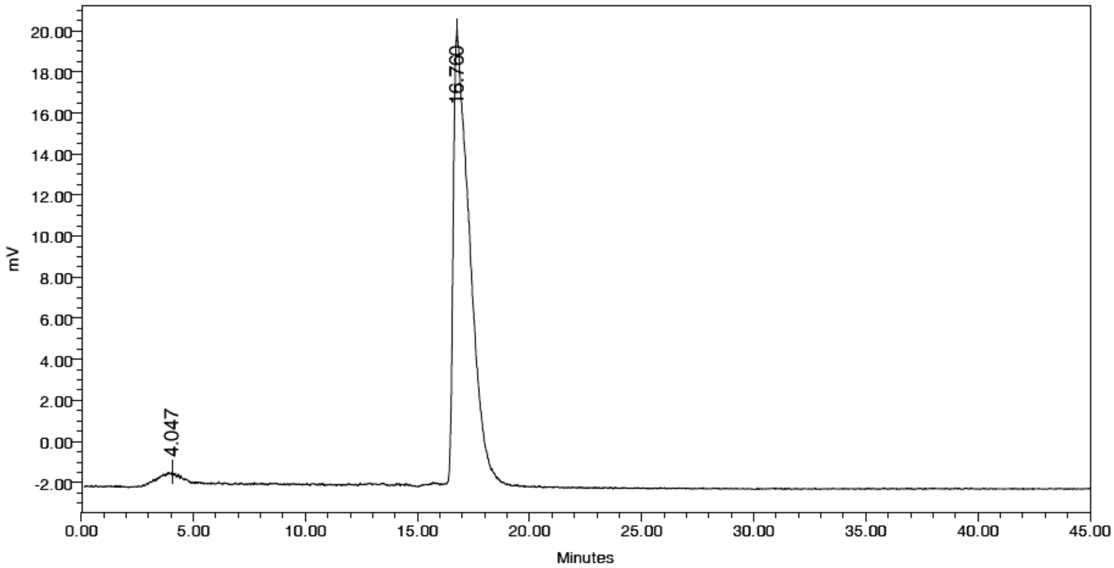

Figure Legend: Analytical trace of purified **2**, shows ~3% free ^{18}F-fluoride at high attenuation (no meaningful UV absorbance was detected – not shown).

IC_{50} Analysis of ^{18}F-labeled 2, Run-1:

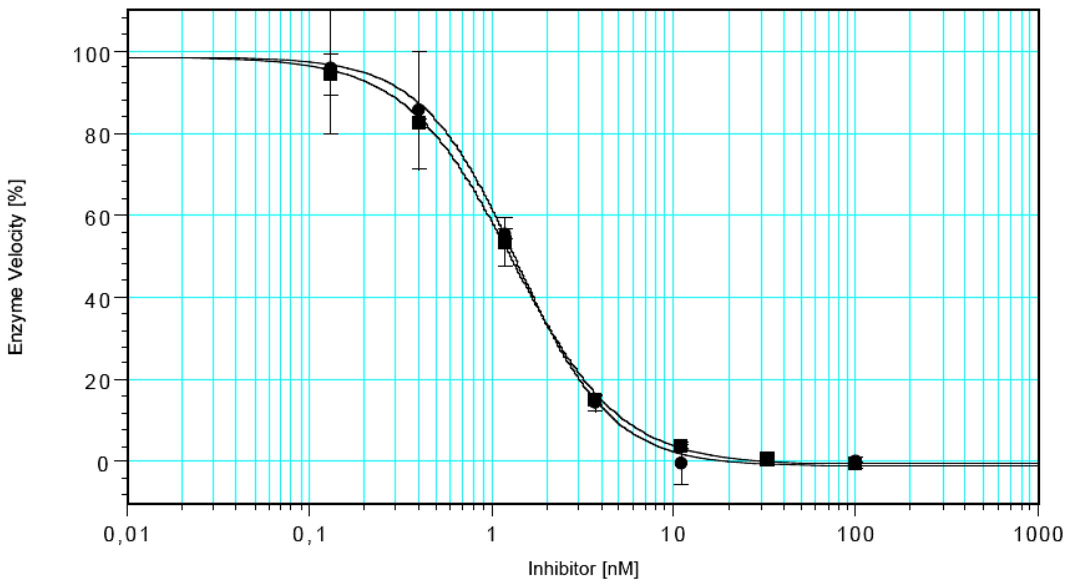

Figure Legend: MMP-2 IC_{50} Inhibition Assay on Marimastat standard (circles): IC_{50}: 1.32 nM; and on ^{18}F-labeled **2** (squares), IC_{50}: 1.28 nM

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UV-vis and Radiochromatograms of ^{18}F-labeled 2, Run-2:

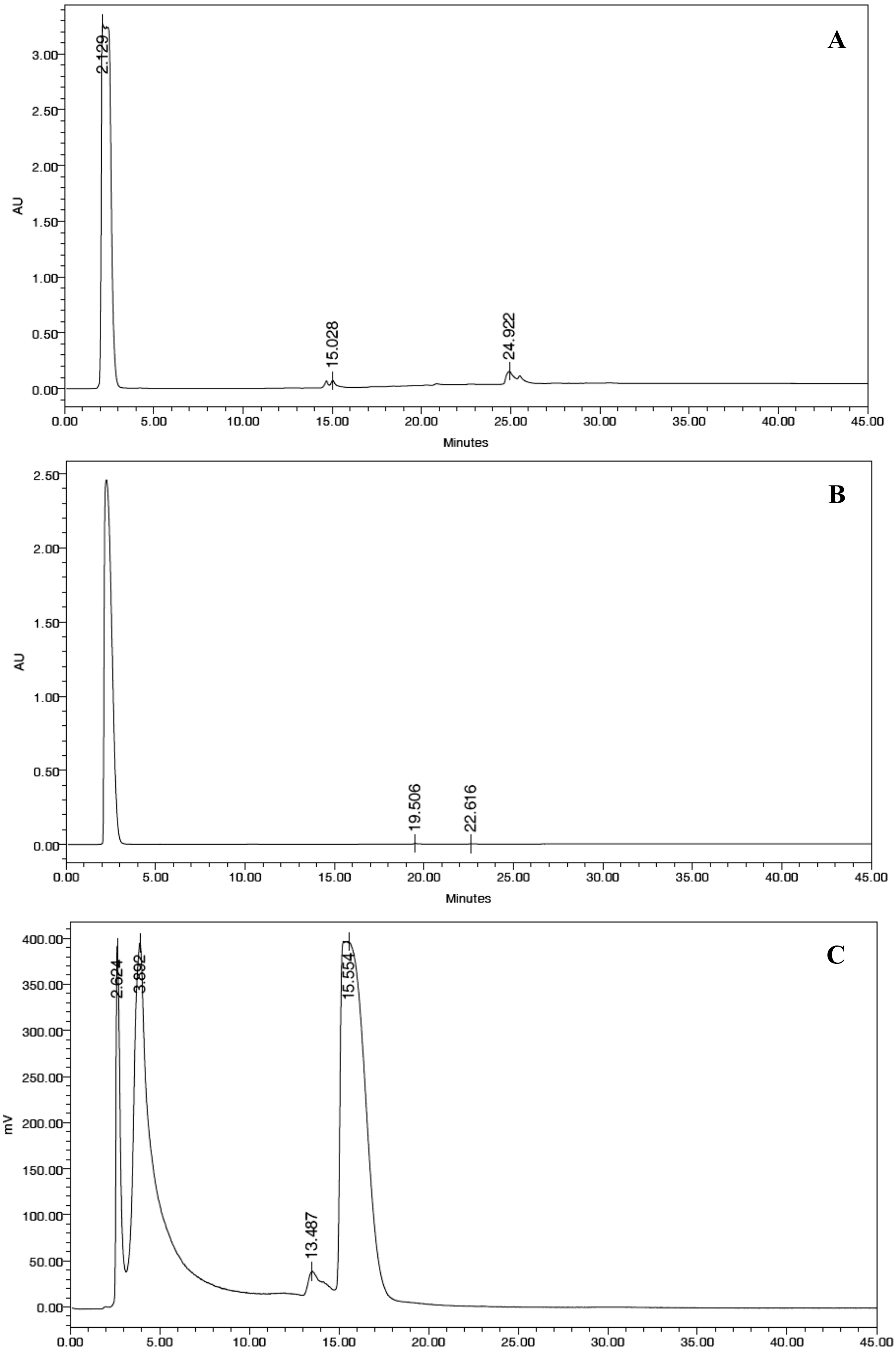

Figure Legend: Crude reaction from Run-2, injected onto analytical column: **A)** UV-trace at 229 nm, **B)** UV-trace at 260 nm, **C)** rad detector at high attenuation.

Analytical Trace of ^{18}F-labeled 2, Run-2:

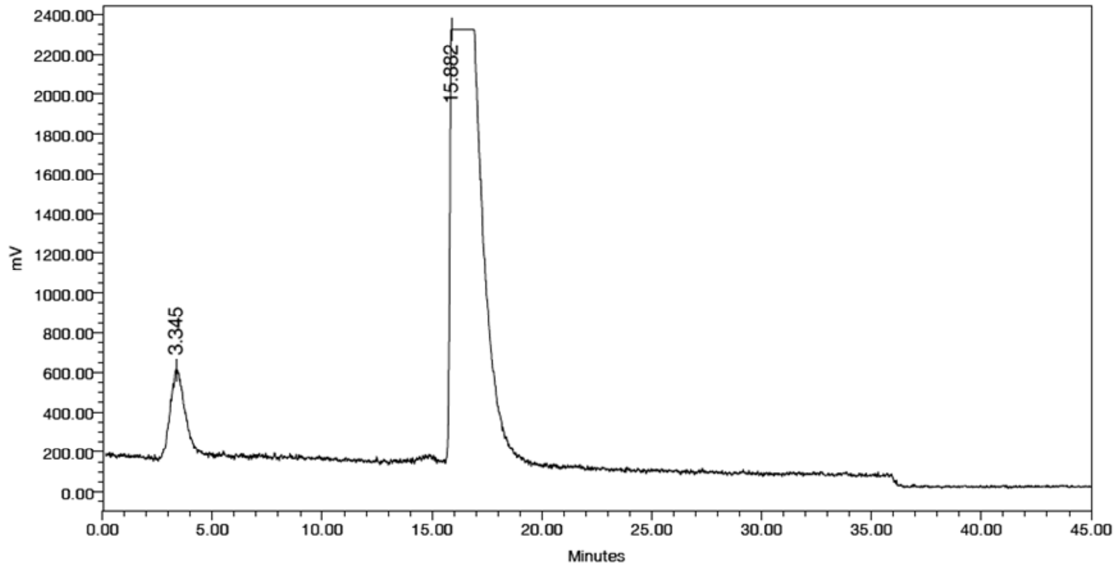

Figure Legend: Analytical trace of purified **2** (Run-2), at low attenuation shows an estimated ~5% free ^{18}F-fluoride (no meaningful UV absorbance was detected – not shown).

IC$_{50}$ Analysis of ^{18}F-labeled 2, Run-2: (reproduced from Cancer Research)

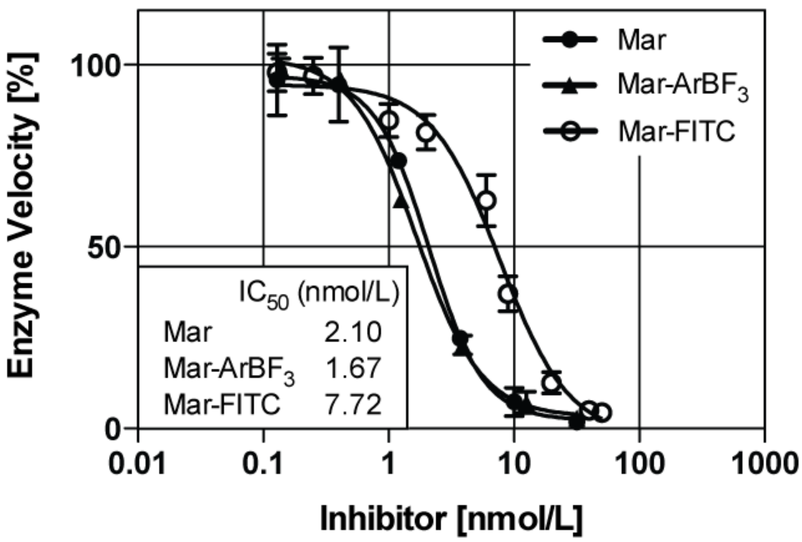

Figure Legend:MMP2 Inhibition assay with marimastat (circles) IC$_{50}$ = 2.10 nM) or ^{18}F-labeled **2** (post decay) (triangles) IC$_{50}$ = 1.67 nM, and marimastat-linked to fluoresceine (Mar-FITC) (reproduced from Cancer Research).

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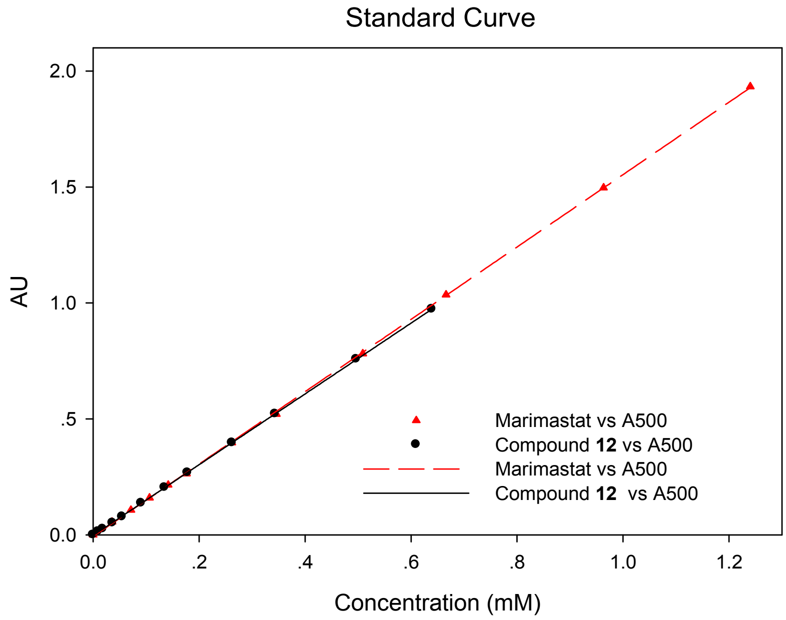
Standard Curves for Ferroin Test for Marimastat and for Compound 12



Ferroin Assay of Compound **12**		Ferroin Assay of Marimastat	
Concentration of 12 (mM)[a]	A (500 nm)	Concentration of Marimastat (mM)[a]	A (500 nm)
0.0000	0.0000	0.0000	3.0000×10^{-4}
9.2545×10^{-3}	.0138	.0358	.0561
.0185	.0254	.0714	.1068
.0368	.0514	.1066	.1600
.0550	.0780	.1416	.2157
.0909	.1368	.1763	.2638
.1350	.2041	.2619	.3982
.1783	.2683	.3458	.5207
.2624	.3968	.5090	.7813
.3434	.5212	.6661	1.0350
.4968	.7574	.9634	1.4972
.6395	.9730	1.2403	1.9330

NOTE: [a] the concentration is that of the compound in the assay mixture.
In the table, the UV absorbance at 500 nm was summarized for the Ferroin assay of both compound **12** and marimastat. Then a standard curve was fitted with a linear equation and the slope of each stands for the extinction coefficient of the complex of Fe^{3+} and the compound: 1526 ± 2.7 $M^{-1}cm^{-1}$ (R^2=1) for that of compound **12**; 1560 ± 4.5 $M^{-1}cm^{-1}$ (R^2=0.9999) for that of marimastat.

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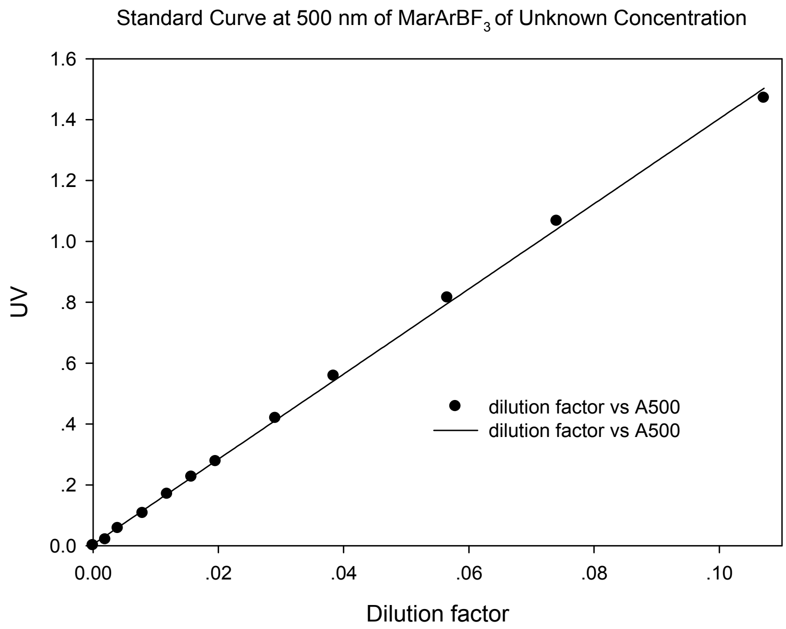
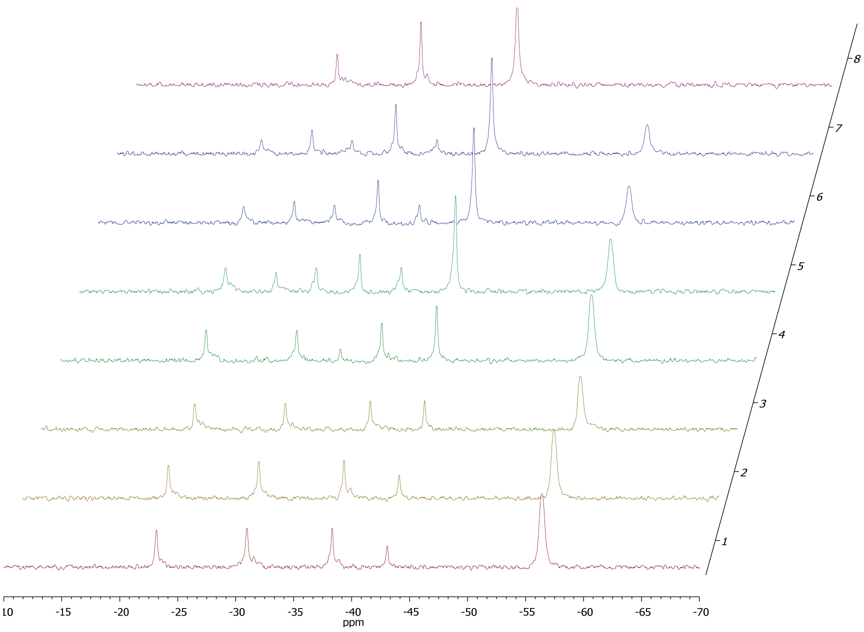
Standard Curve for Ferroin Test for ^{19}F-labeled 2



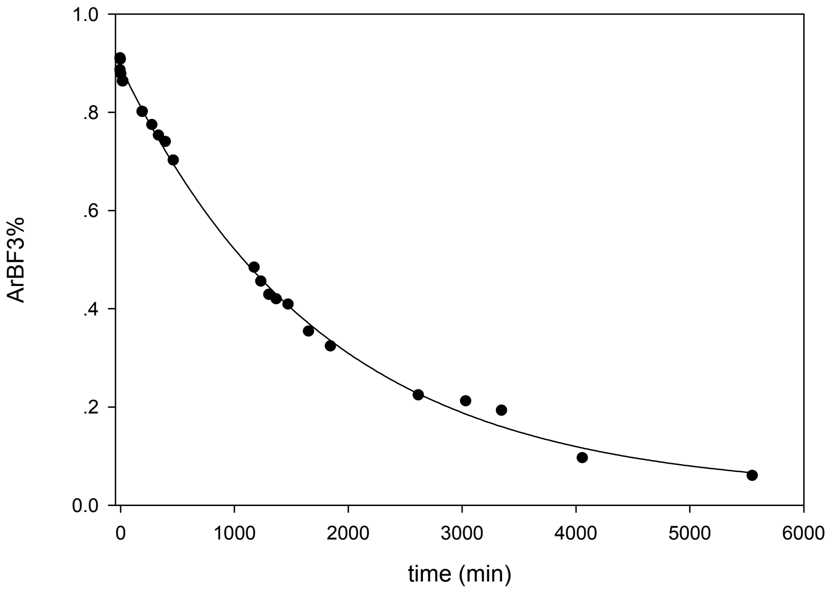
Dilution factor of $ArBF_3$[a]	A (500 nm)
0.0000	6.0000×10^{-4}
$1.9960e\times10^{-3}$	.0197
3.9841×10^{-3}	.0569
7.9365×10^{-3}	.1063
.0119	.1691
.0157	.2255
.0196	.2764
.0291	.4185
.0385	.5576
.0566	.8143
.0741	1.0660
.1071	1.4705

NOTE: [a] the dilution factor is calculated by the added volume of the stock solution of ^{19}F-labeled **2** of unknown concentration divided by the total volume in the final mixture. The table gives the UV absorbance at 500 nm of Ferroin assay for ^{19}F-labeled **2**, and the data were fitted linearly and the slope, 13.99 ± 0.146 (R^2=0.9989) is related to both the extinction coefficient and the concentration of the stock solution. By using the extinction coefficient compound **12** (1526 ± 2.7 $M^{-1}cm^{-1}$), the concentration of the stock solution of ^{19}F-labeled **2** is 9.07± 9.46×10^{-2} mM.

Visualization of Defluorination by ^{19}F-NMR and Rate Calculation



(A)



(B)

Figure Legend: The solvolytic hydrolysis of the ^{19}F-labeled **2** in 1× PBS (pH 7.4). (**A**) The time points from bottom spectrum to top spectrum is 2, 25, 196, 400, 1180, 1479, 1851, 5555 min.. (**B**) For each spectrum, -55.8 ppm (BF_3) and -42.5 ppm (free F^-) were integrated and the percentages of the $ArBF_3$ were plotted against time for the kinetics and fitted with the equation of $y = y0 + a*e^{-bx}$ where $b = 5.61\times10^{-4} \pm 0.23 \times10^{-4}$ (where R^2=0.9973). $t_{1/2}$=1236 ± 17 min.