Supporting Information

Fluorine-18 Labeling of the MDM2 Inhibitor RG7388 for PET Imaging: Chemistry and Preliminary Evaluation

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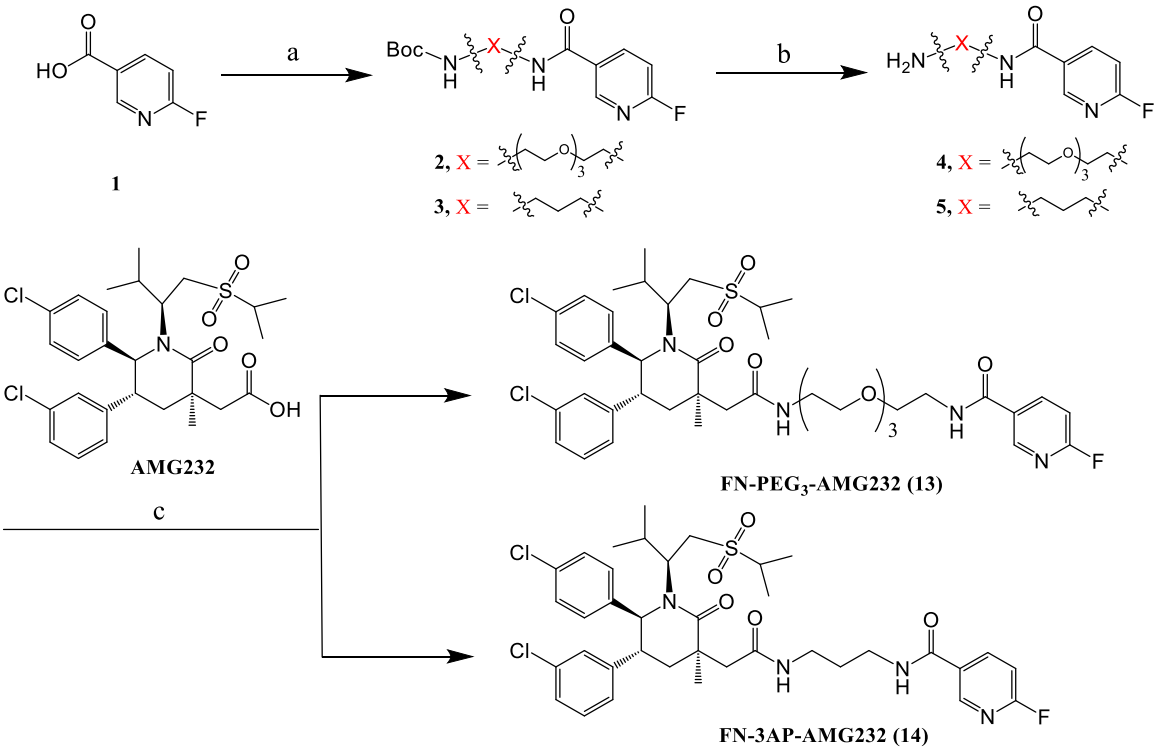
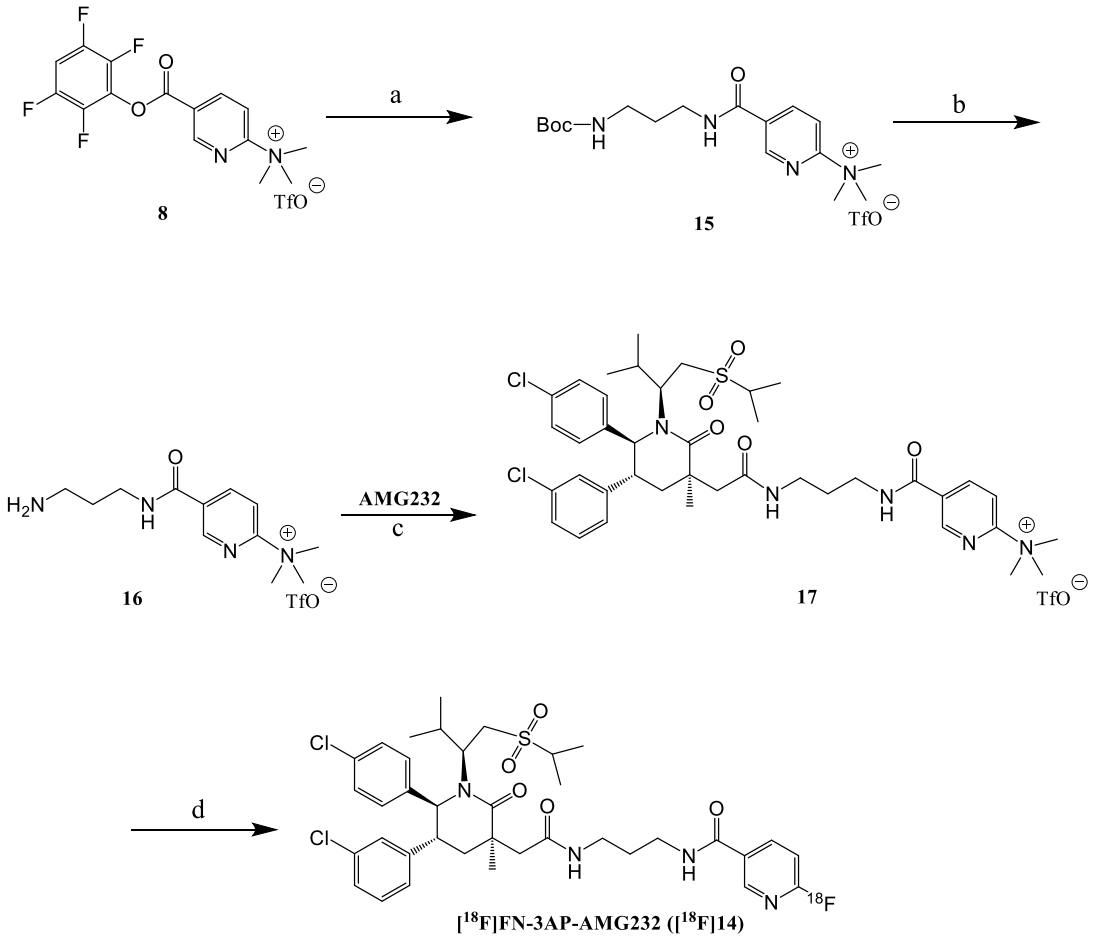


a) HATU, DIEA, DMF, Boc-NH-PEG_3-NH_2 or N-Boc-1,3-propanediamine b) TFA c) HATU, DIEA, DMF

Scheme S1. Synthesis of FN-X-AMG232



a) DIEA, DMF, N-Boc-1,3-propanediamine b) TFA c) HATU, DIEA, DMF d) [^{18}F]TEAF, CH_3CN

Scheme S2. Synthesis of [^{18}F]FN-3AP-AMG232

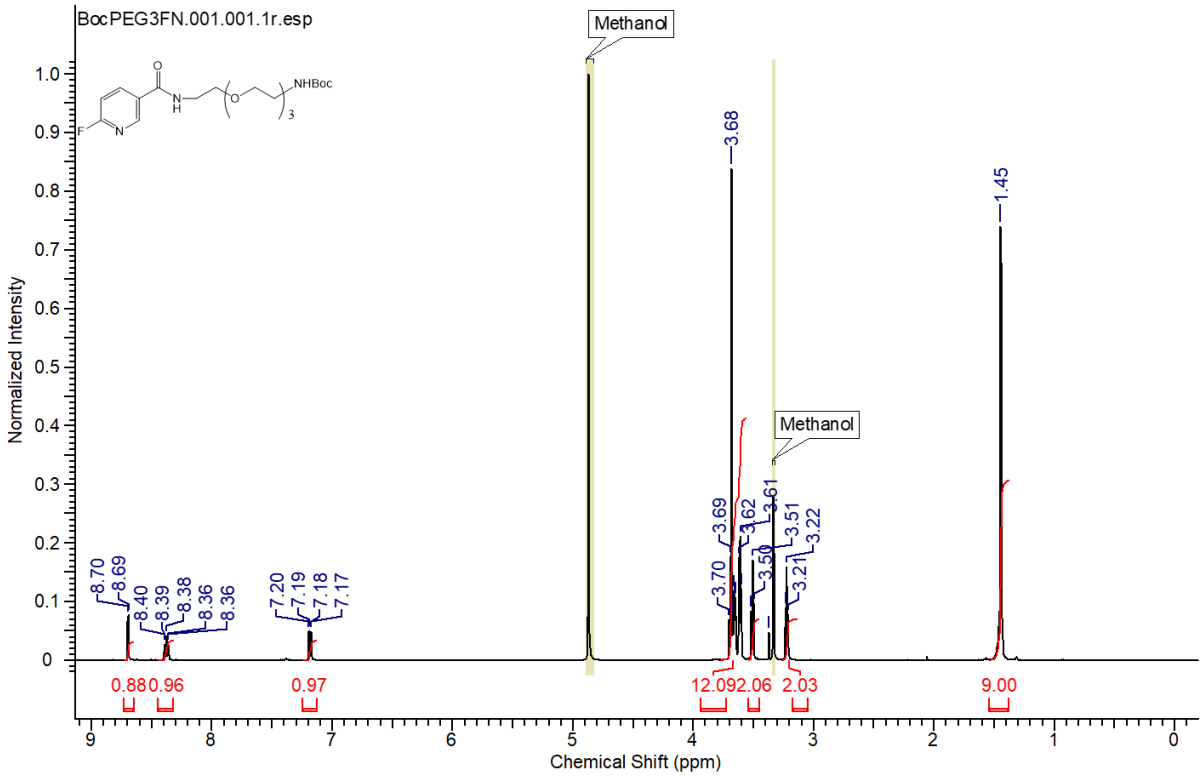


Figure S1. 1H-NMR (500MHz, CD_3OD) spectrum of compound **2**.

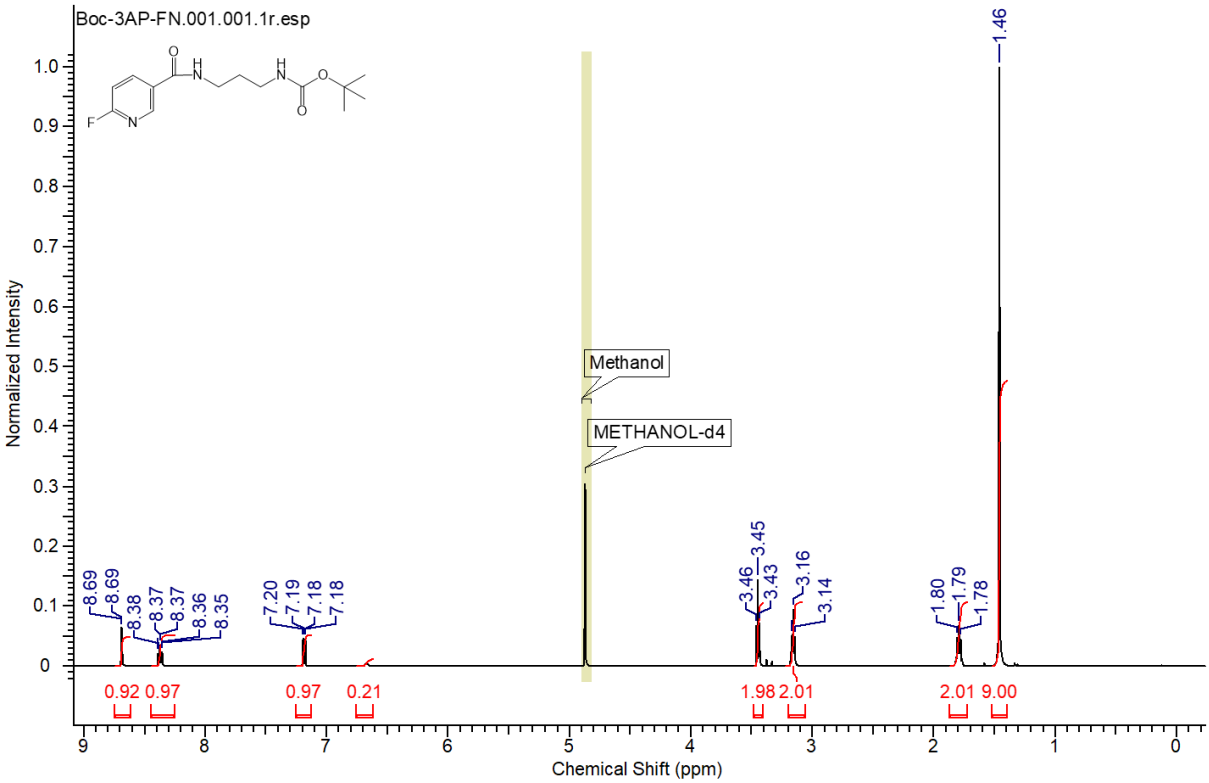


Figure S2. 1H-NMR (500MHz, D_2O) spectrum of compound **3**.

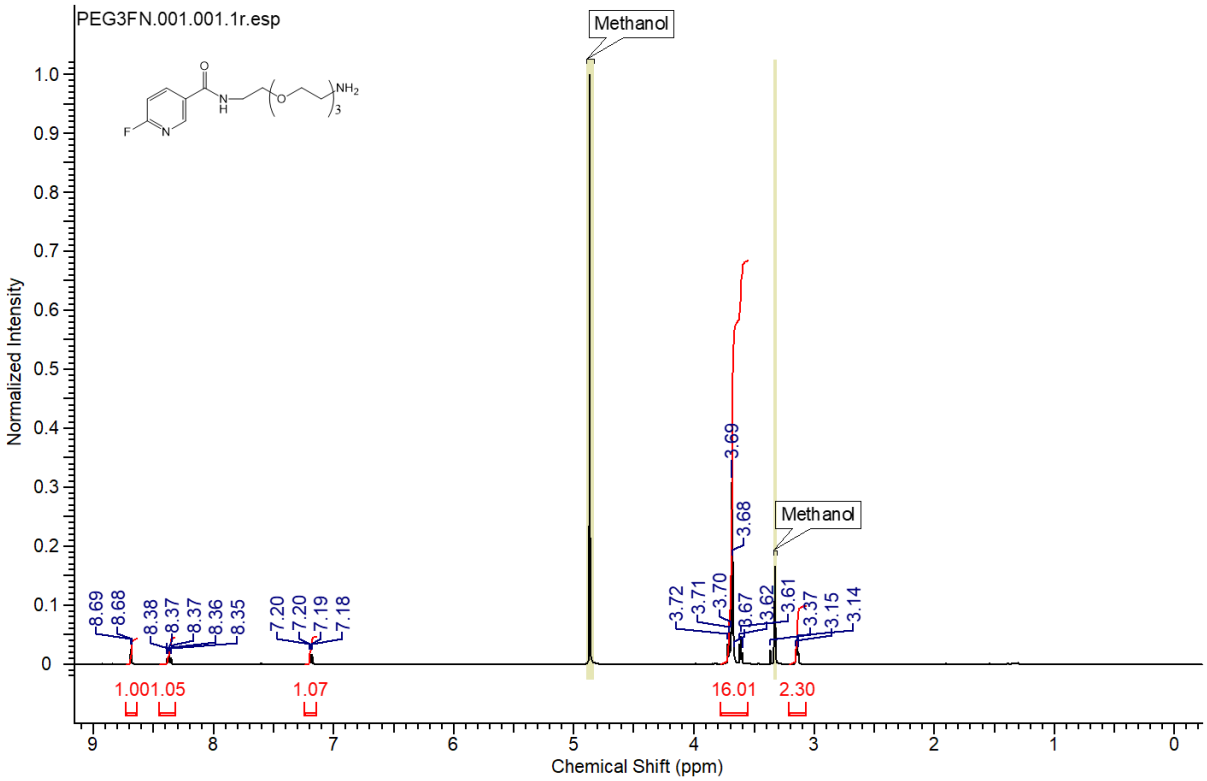


Figure S3. 1H-NMR (500MHz, D_2O) spectrum of compound **4**.

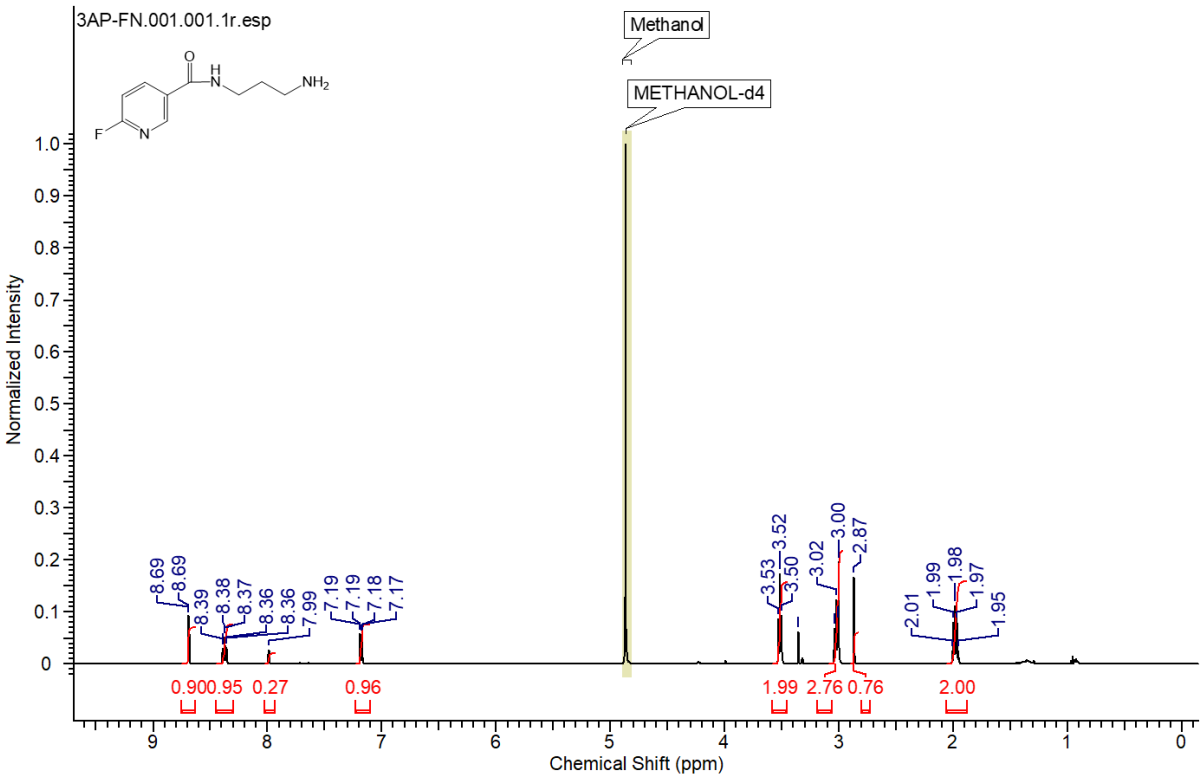


Figure S4. 1H-NMR (500MHz, D_2O) spectrum of compound **5**.

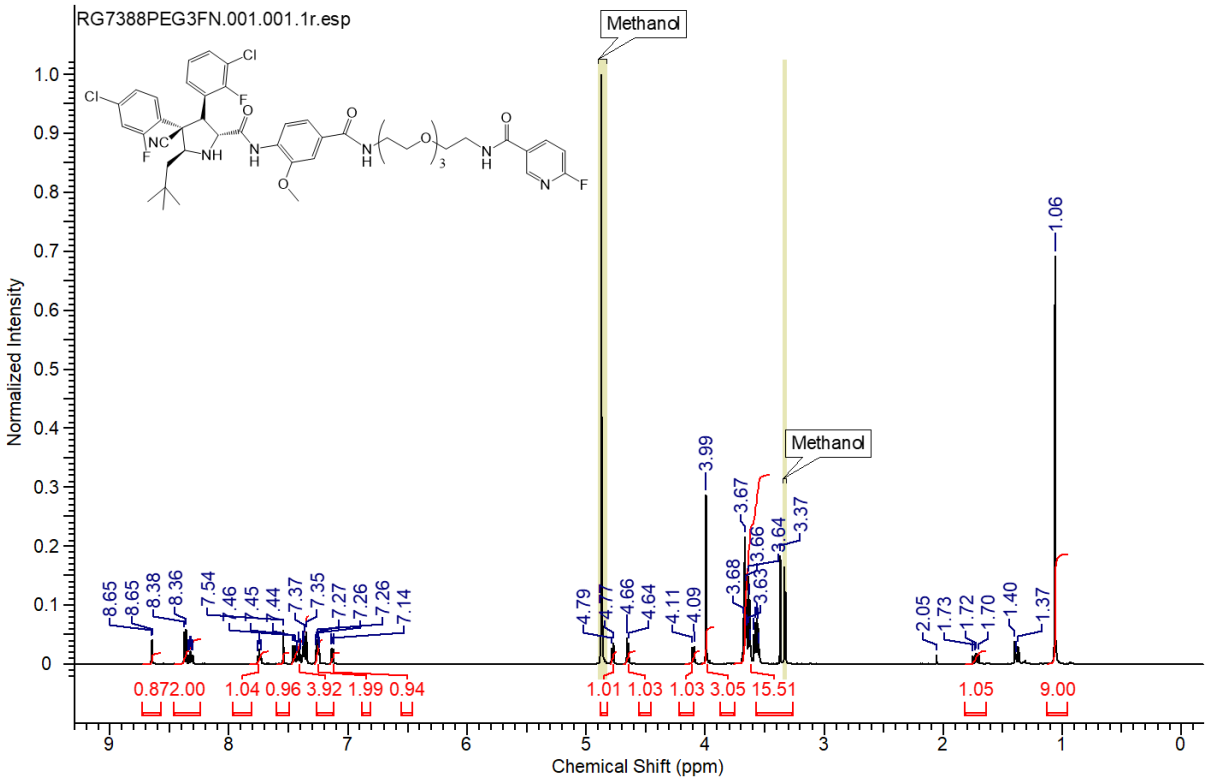


Figure S5. 1H-NMR (500MHz, CD_3OD) spectrum of compound **6**.

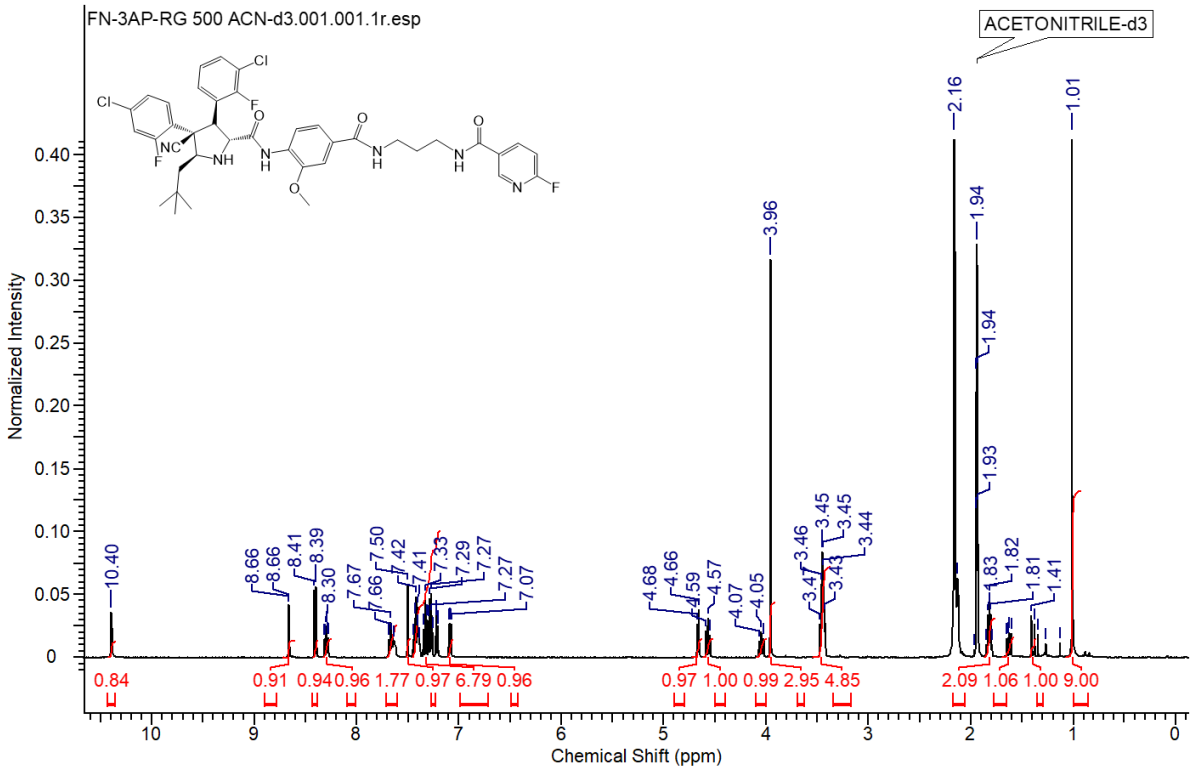


Figure S6. 1H-NMR (500MHz, CD_3CN) spectrum of compound **7**.

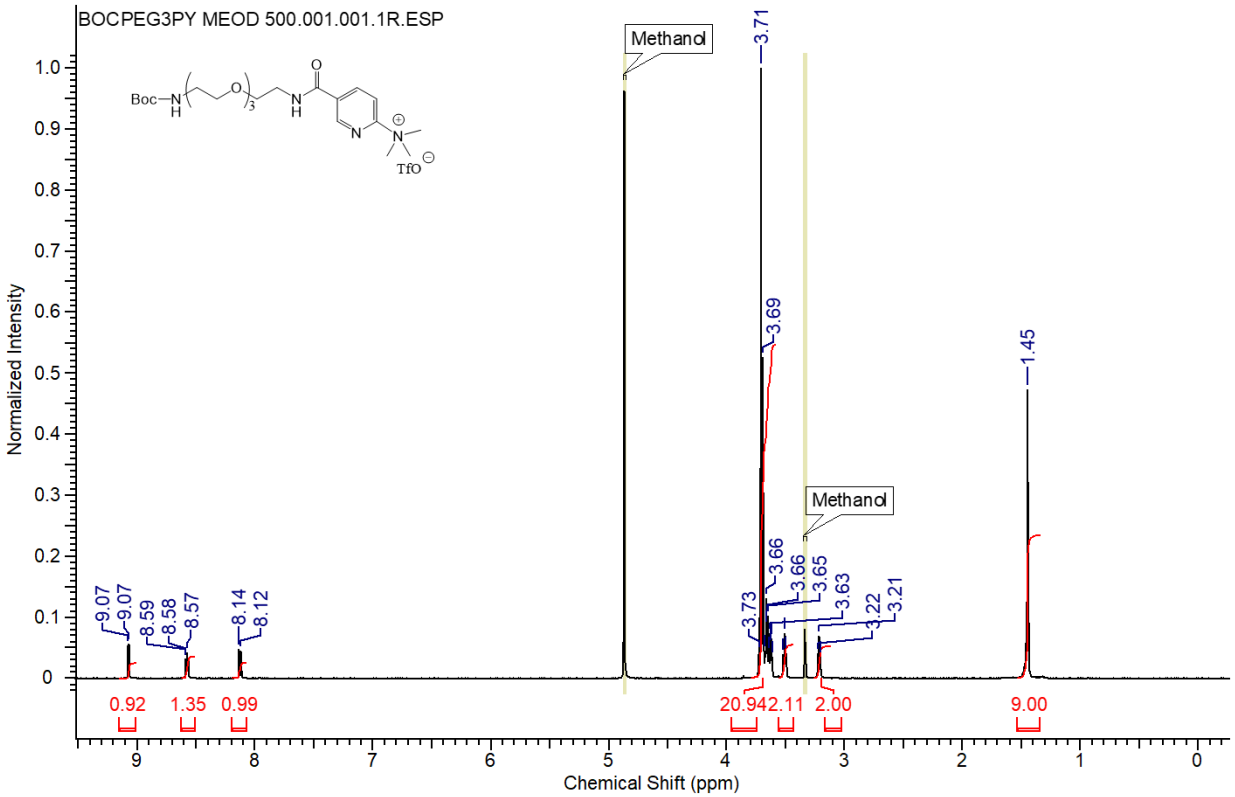


Figure S7. 1H-NMR (500MHz, CD_3OD) spectrum of compound **9**.

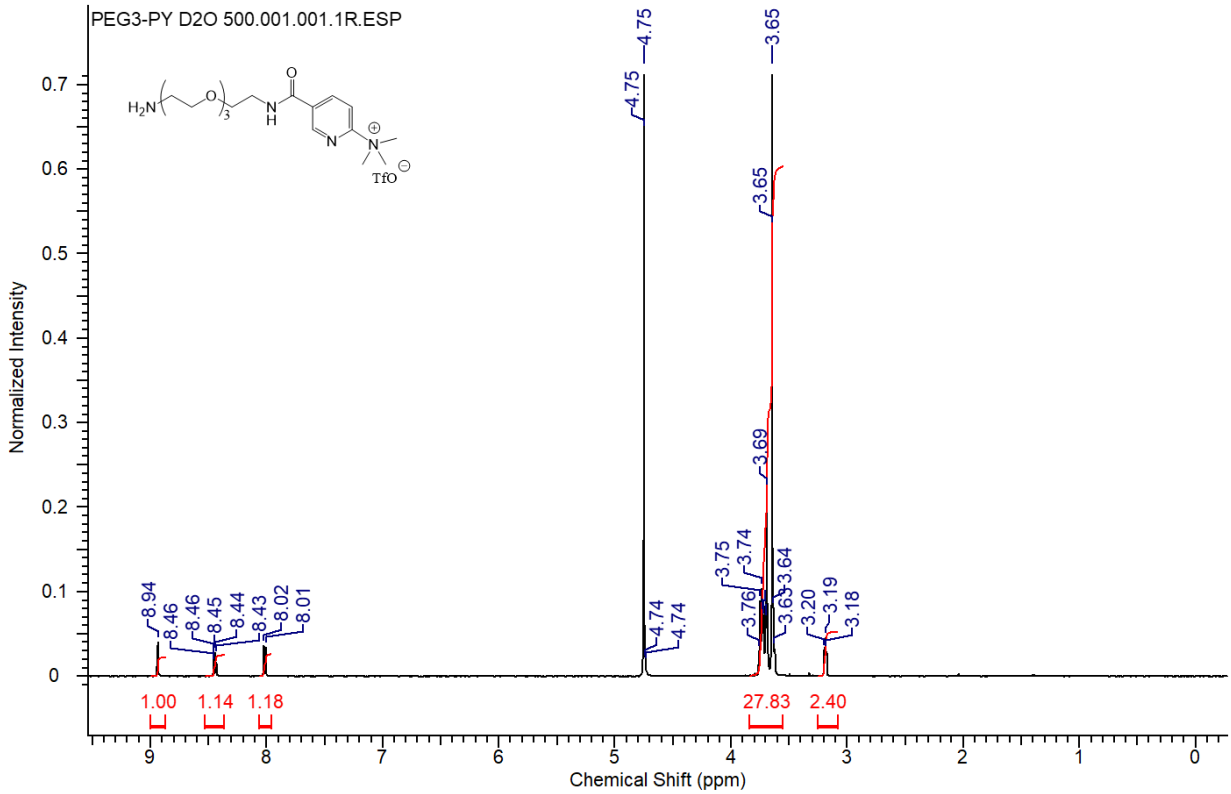


Figure S8. 1H-NMR (500MHz, D_2O) spectrum of compound **10**.

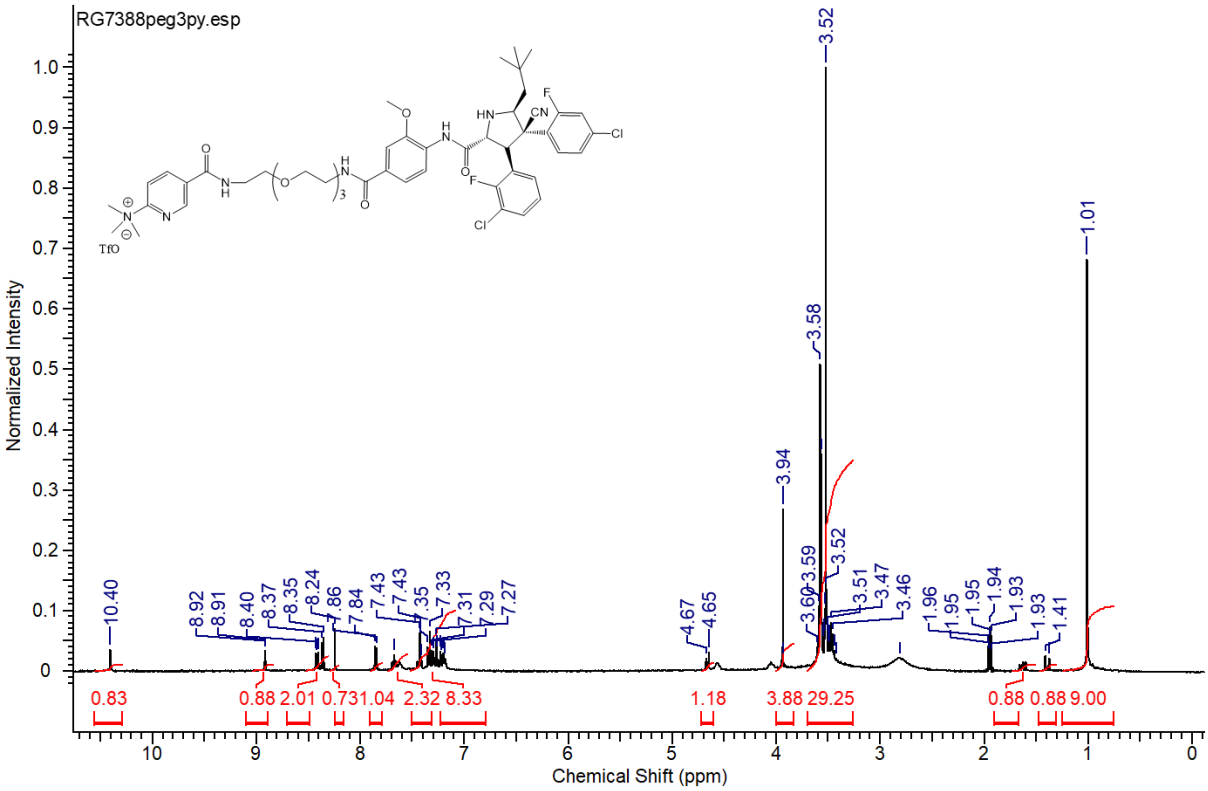


Figure S9. 1H-NMR (500MHz, CD_3CN) spectrum of compound **11**.

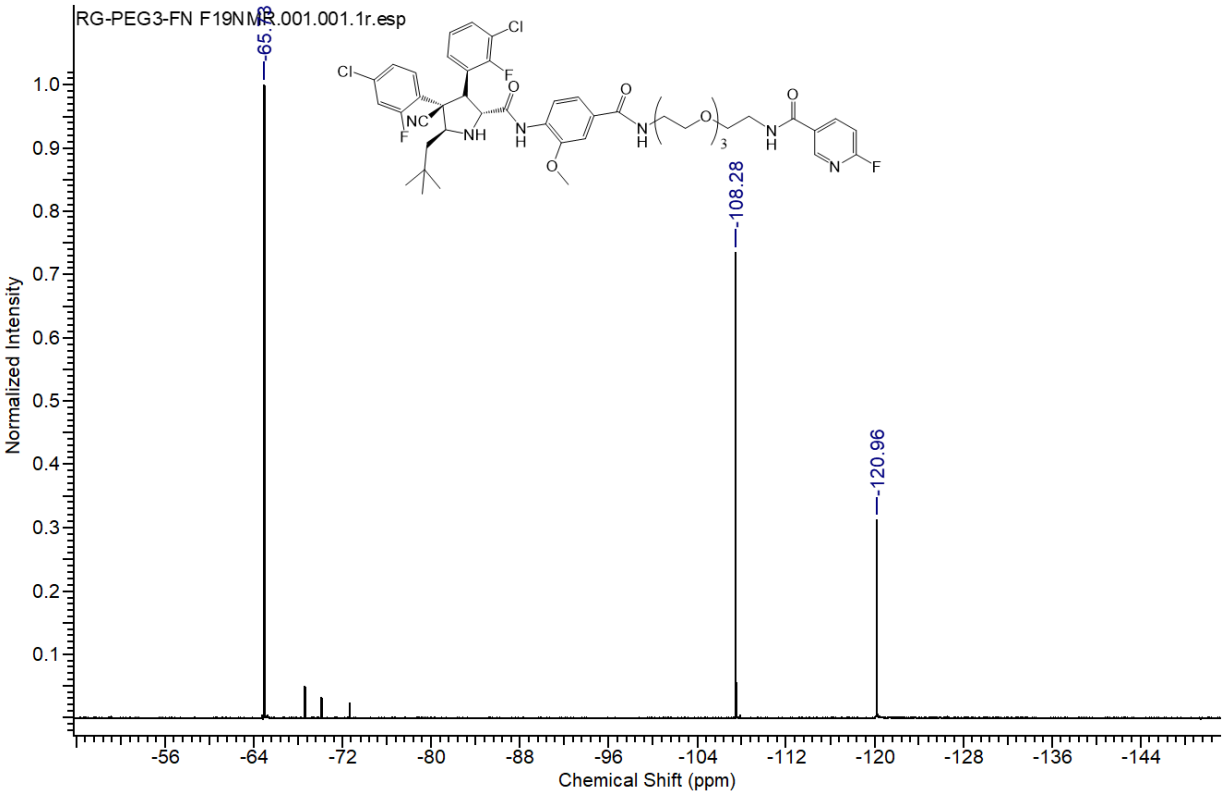


Figure S10. ^{19}F-NMR (471MHz, DMSO-d_6) spectrum of compound **6**.

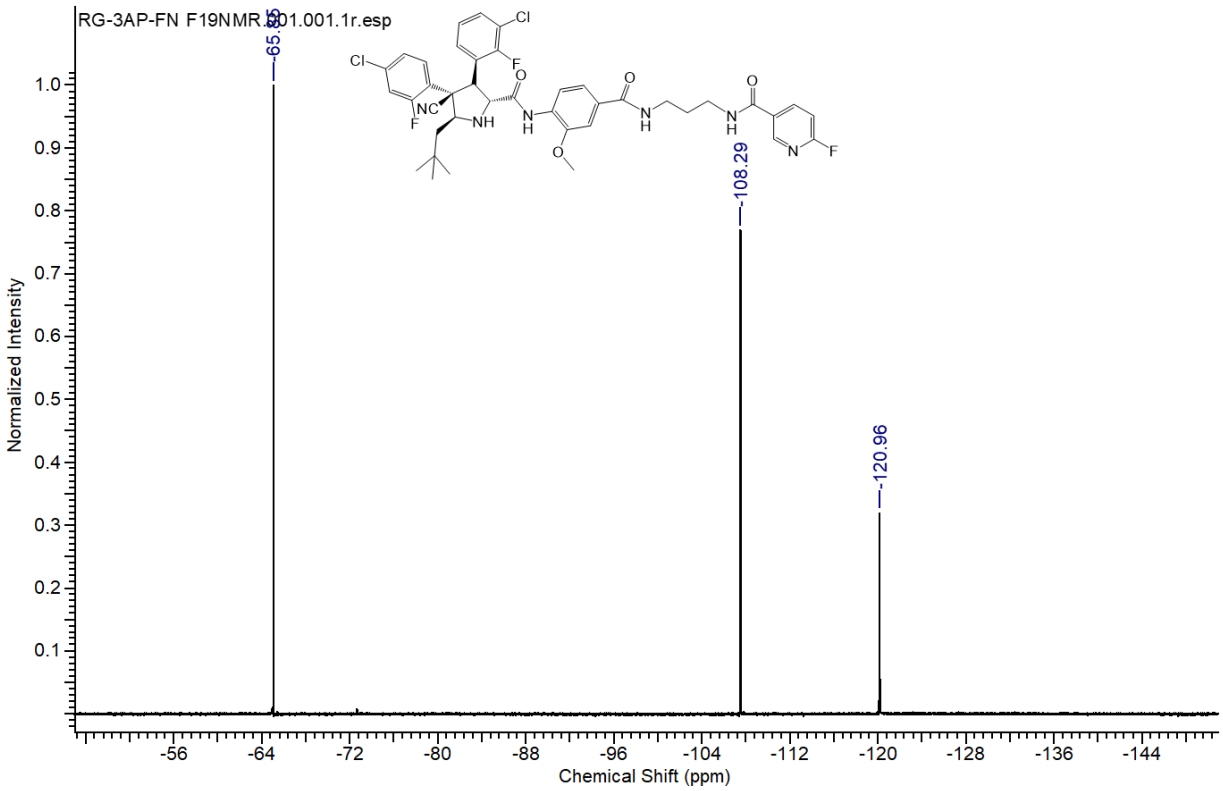


Figure S11. ^{19}F-NMR (471MHz, DMSO-d_6) spectrum of compound **7**.

Synthesis of FN-PEG$_3$-AMG232 (**13**)

Compound **4** (11.1 mg, 35.3 µmol), AMG232 (20 mg, 35.3 µmol), HATU (20 mg, 53.0 µmol) and *N,N*-diisopropylethylamine (8.7 mg, 70.6 µmol) were taken in 0.3 mL DMF and the mixture was stirred at RT (25°C) for 2 h. The crude mixture was then subjected to semi-preparative HPLC using an Agilent Zorbax SB-C18 (9.4 × 250 mm, 5 µm) reversed-phase column and eluted with a gradient consisting of 0.1% formic acid in both water (solvent A) and acetonitrile (solvent B) at a flow rate of 4 mL/min; the proportion of B was linearly increased from 25% to 70% over 15 min. Lyophilization of pooled HPLC fractions containing **13** (t_R = 13.7 min) rendered 25.7 mg (29.7 µmol, 84.2%) as a colorless oil: 1H NMR (CD_3CN, 500 MHz) δ_H = 0.46 (d, *J* = 6.87 Hz, 3H), 0.59 (d, *J* = 6.56 Hz, 3H), 1.26 (s, 3H), 1.35 (dd, *J* = 6.79, 1.14 Hz, 6H), 2.0-2.1 (m, 1H), 2.1-2.2 (m, 2H), 2.41 (d, *J* = 13.6 Hz, 2H), 2.75-2.85 (d, *J* = 13.6 Hz, 1H), 2.98 (d, *J* = 13.58 Hz, 1H), 3.10-3.25 (m, 2H), 3.3-3.4 (m, 2H), 3.4-3.7 (m, 15H), 3.90 (dd, *J* = 13.89, 10.83 Hz, 1H), 5.05 (d, *J* = 11.14 Hz, 1H), 6.88 (t, *J* = 5.26 Hz, 1H), 6.97 (d, *J* = 7.5 Hz 1H), 7.02 (s, 1H), 7.05-7.20 (m, 3.25), 8.27 (td, *J* = 8.16, 2.44 Hz, 1H), 8.63 (d, *J* = 2.29 Hz, 1H). ^{19}F-NMR (DMSO-d_6, 471 MHz) δ ppm = -65.71(s). LRMS (LC/MS-ESI) m/z: 865.4 $(M+H)^+$. HRMS (ESI, m/z): calcd for $C_{42}H_{55}Cl_2FN_4O_8S$ $(M+H)^+$: 865.3175; found: 865.3166.

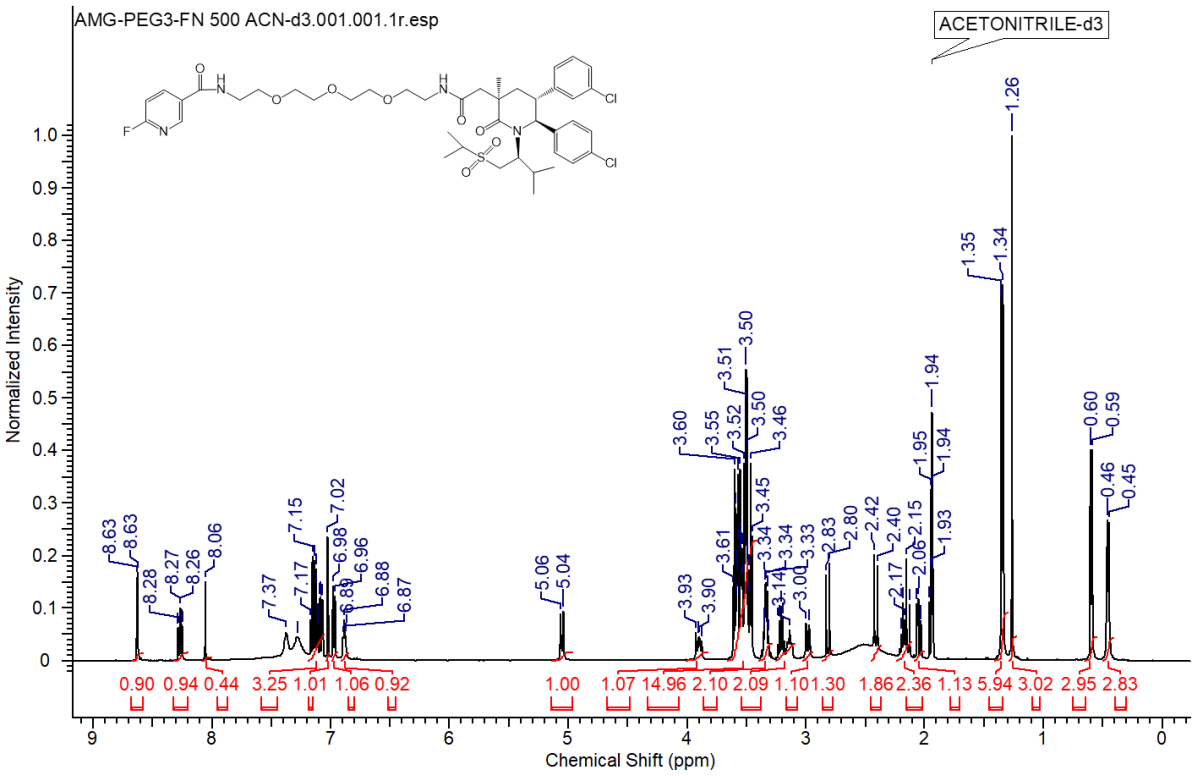


Figure S12. 1H-NMR (500MHz, CD_3CN) spectrum of compound **13**.

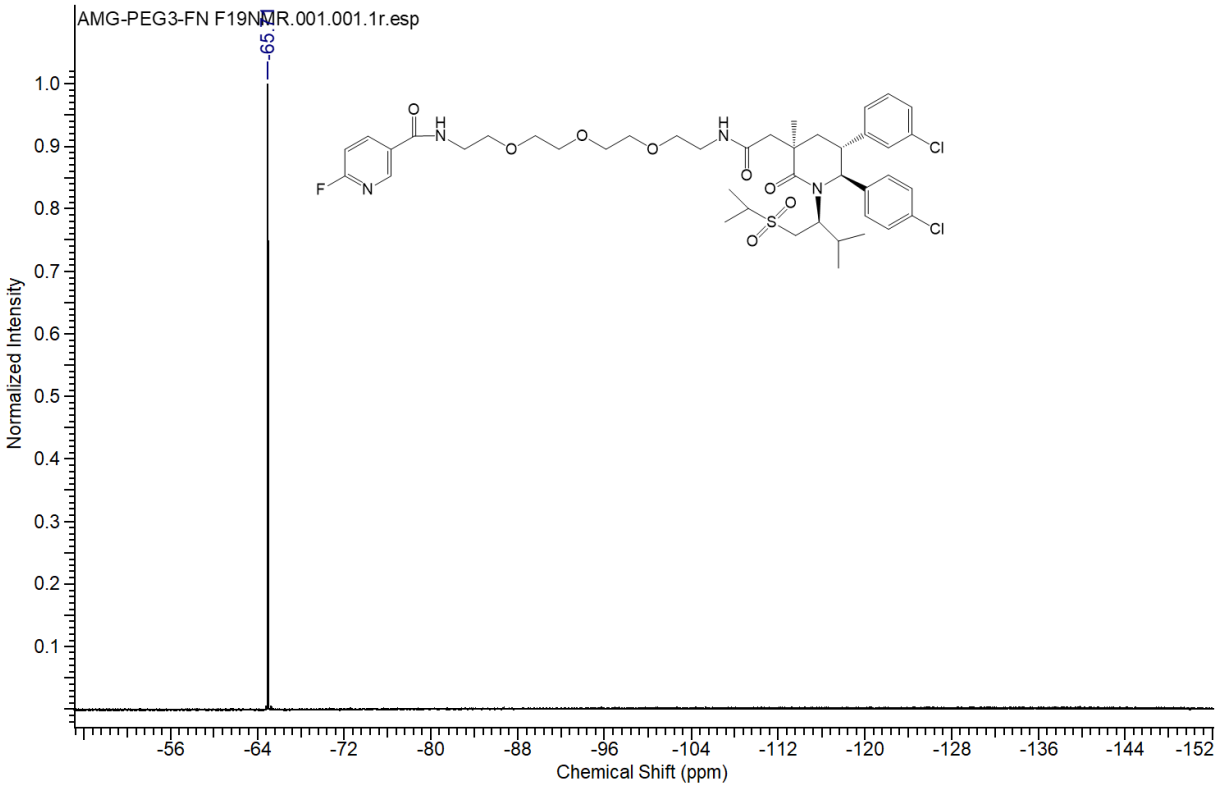


Figure S13. ^{19}F-NMR (471MHz, DMSO-d_6) spectrum of compound **13**.

Synthesis of FN-3AP-AM232 (**14**)

Compound **5** (3 mg, 10.0 µmol), RG7388 (5.6 mg, 10.0 µmol), HATU (6.5 mg, 17 µmol) and *N,N*-diisopropylethylamine (2.5 mg, 20 µmol) were taken in 0.2 mL DMF and the mixture was stirred at RT for 2 h. The crude mixture subsequently was purified by semi-preparative HPLC as described above, with a gradient consisting of 0.1% formic acid in both water (solvent A) and acetonitrile (solvent B); the proportion of B was linearly increased from 40% to 80% over 15 min. Lyophilization of pooled HPLC fractions containing **14** (t_R = 14.2 min) rendered 3.76 mg (5.0 µmol, 52.1%) as a colorless oil: 1H NMR (CD_3CN, 500 MHz) δ_H = 0.46 (d, *J* = 6.87 Hz 3H), 0.60 (d, *J* = 6.71 Hz, 3H), 1.2-1.4 (m, 10H), 1.6-1.8 (m, 2H), 2.43 (d, *J* = 13.58 Hz, 1H), 2.75-2.85 (m, 1H), 2.99 (d, *J* = 13.73 Hz, 1H), 3.10-3.60 (m, 7H), 3.8-4.0 (m, 1H), 5.05 (d, *J* = 10.99 Hz, 1H), 6.9-7.2 (m, 7H), 7.63 (s, 1H), 8.27 (td, *J* = 8.13, 2.52 Hz, 1H), 8.65 (d, *J* = 2.29 Hz, 1H). LRMS (LC/MS-ESI) m/z: 747.3 $(M+H)^+$. HRMS (ESI, m/z): calcd for $C_{37}H_{45}Cl_2FN_4O_5S$ $(M+H)^+$:747.2528; found:747.2562.

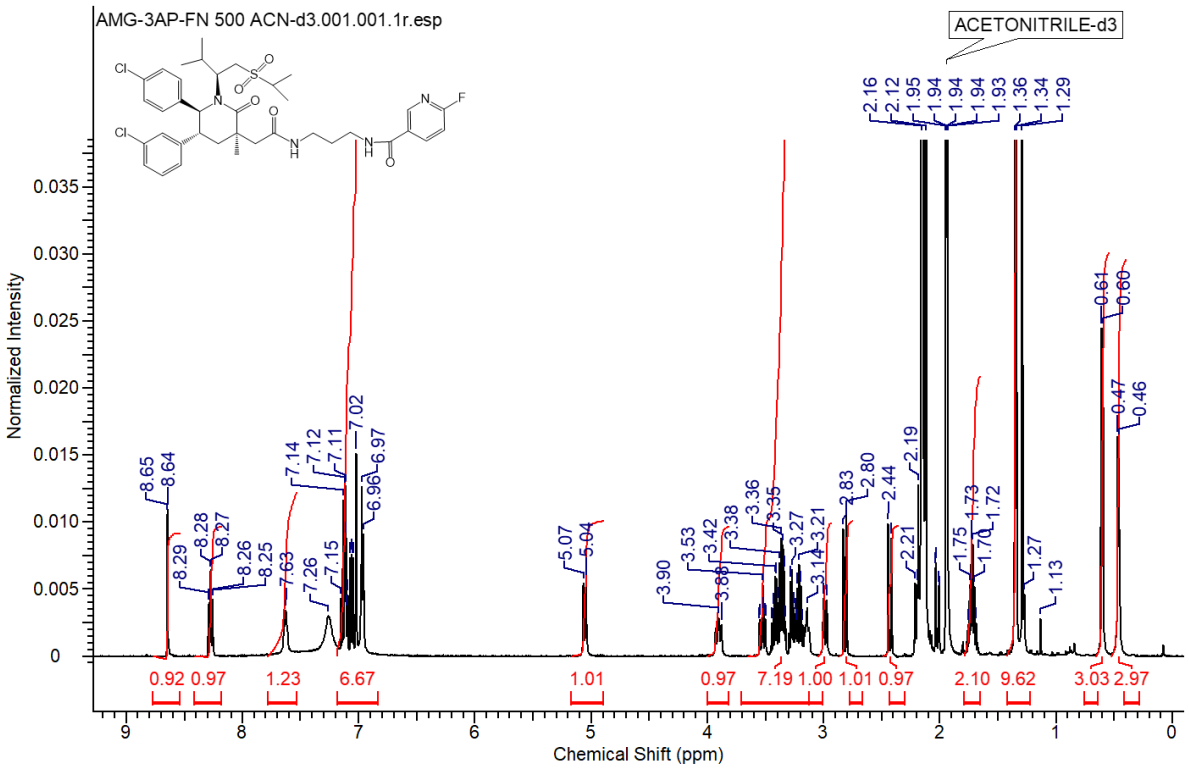


Figure S14. 1H-NMR (500MHz, CD_3CN) spectrum of compound **14**.

5-((3-((tert-butoxycarbonyl)amino)propyl)carbamoyl)-N,N,N-trimethylpyridin-2-aminium (**15**)

A mixture of compound **8** (12.0 mg, 25.3 µmol), *tert*-butyl (3-aminopropyl)carbamate (4.4 mg, 25.3 µmol) and *N,N*-diisopropylethylamine (6.5 mg, 50.6 µmol) in DMF (300 µL) was stirred at RT for 2 h. The product from this mixture was isolated by semi-preparative HPLC as described above, but the proportion of solvent B was linearly increased from 5% to 20% over 15 min. Under these conditions, the product eluted with a retention time (t_R) of 7.5 min. Pooled HPLC fractions containing the product were lyophilized to obtain 4.35 mg (9.0 µmol, 35.7%) of compound **15** as a colorless oil. LRMS (LC/MS-ESI) m/z: 337.3 (M^+). HRMS (ESI, m/z): calcd for $C_{17}H_{29}N_4O_3$ (M^+): 337.2225; found: 337.2248.

5-((3-aminopropyl)carbamoyl)-N,N,N-trimethylpyridin-2-aminium (**16**)

Compound **15** (4.35 mg, 9.0 µmol) was dissolved in 1 mL of TFA and stirred at RT for 30 min. Solvents were evaporated to yield 4.4 mg (98.7%, based on trifluoroacetate salt) of compound **16** as a colorless oil. LRMS (LC/MS-ESI) m/z: 237.2 (M^+). HRMS (ESI, m/z): calcd for $C_{12}H_{21}N_4O$ (M^+): 237.1715; found: 237.1752.

5-((3-(2-((3R,5R,6S)-5-(3-chlorophenyl)-6-(4-chlorophenyl)-1-((S)-1-(isopropylsulfonyl)-3-methylbutan-2-yl)-3-methyl-2-oxopiperidin-3-yl)acetamido)propyl)carbamoyl)-N,N,N-trimethylpyridin-2-aminium (**17**)

A mixture of compound **16** (4.4 mg, 9.0 µmol), AMG232 (5.1 mg, 9.0 µmol), HATU (5.1 mg, 13.5 µmol) and *N,N*-diisopropylethylamine (2.3 mg, 18.0 µmol) in DMF (0.5 mL) was stirred at RT for 1 h. The product was isolated from this mixture by semi-preparative HPLC as described above, but using a gradient wherein the proportion of solvent B was linearly increased from 15% to 70% over 15 min. Lyophilization of pooled HPLC fractions containing **17** (t_R = 11.5 min) yielded 6.28 mg (6.8 µmol, 76.0%) of a colorless oil: 1H NMR (CD_3CN, 500 MHz) δ_H = 0.47 (d, *J* = 6.71 Hz, 3H), 0.60 (d, *J* = 6.56 Hz, 3H), 1.25-1.40 (m, 10H), 1.70-1.75 (m, 2H), 2.45 (d, *J* = 13.43 Hz, 1H), 2.89 (d, *J* = 13.58 Hz, 1H), 2.99 (d, *J* = 13.89 Hz, 1H), 3.1-3.4 (m, 6H), 3.54 (s, 9H), 3.6-3.7 (m, 1H), 3.91 (dd, *J* = 13.43, 10.38 Hz, 1H), 5.04 (d, *J* = 10.99 Hz, 1H), 7.0-7.2 (m, 5H), 7.88 (d, *J* = 8.70 Hz, 1H), 8.0-8.1 (m, 1H), 8.68 (dd, *J* = 8.70, 2.14 Hz, 1H), 8.85-8.95 (m, 1H), 9.1-9.2 (m,1H). LRMS (LC/MS-ESI) m/z: 786.8 (M^+). HRMS (ESI, m/z): calcd for $C_{40}H_{54}Cl_2N_5O_5S$ (M+): 786.3217; found: 786.3240.

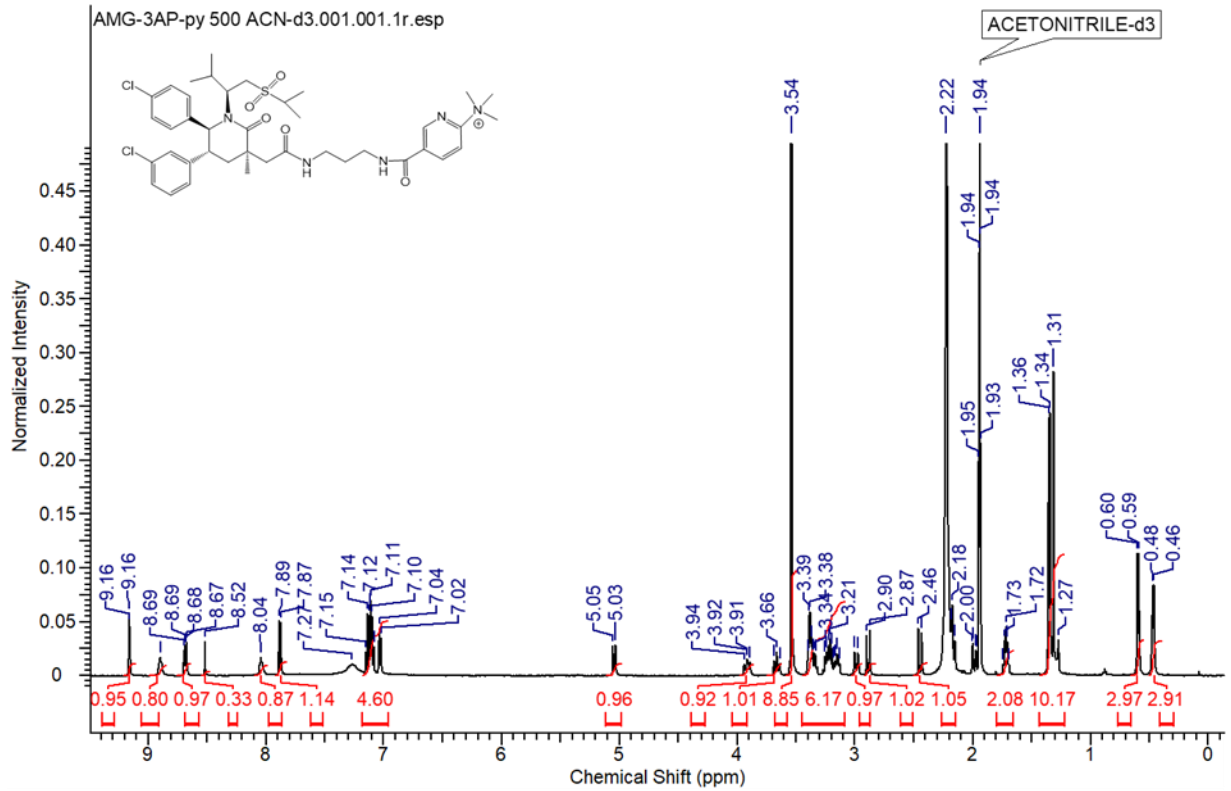


Figure S15. 1H-NMR (500MHz, CD_3CN) spectrum of compound **17**.

*Radiosynthesis of [^{18}F]FN-3AP-AMG232 ([^{18}F]**14**)*

Fluorine-18 trapped on a QMA cartridge (Waters Corp, Milford, MA) was obtained from PETNET Solutions (Durham, NC). The cartridge was eluted with 1 mL of tetraethylammonium bicarbonate (3 mg/mL) in 80% acetonitrile in water. The solvents from the eluate were evaporated at 100°C and the residual water was removed by azeotroping with acetonitrile (3 × 0.3 mL) at the same temperature. A solution of **17** (0.3 mg, 0.32 µmol) in 0.15 mL anhydrous acetonitrile was added to the dried ^{18}F activity (1.9-2.6 GBq; 51-70 mCi), and the mixture heated at 40°C for 15 min. The resultant solution containing [^{18}F]**14** was purified similar to [^{18}F]**6**, using 50% ethanol in sodium acetate buffer (0.05 M, pH 5.5) at a flow rate of 1.5 mL/min (t_R = 13 min).

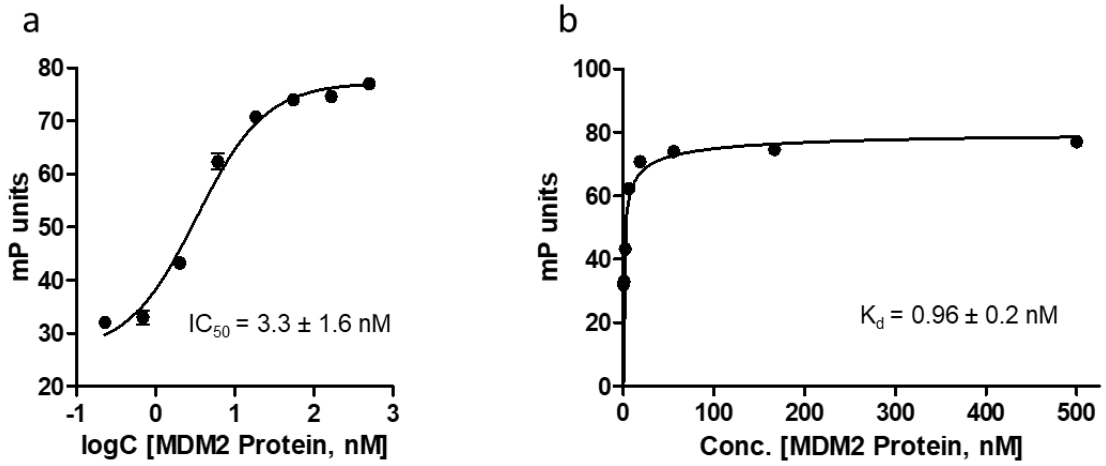


Figure S16. Half-maximal inhibitory concentration (IC_{50}, a) and saturation binding curves (b) of the fluorescent labeled peptide 5-FAM-PMDM6 (1 nM) against human recombinant MDM2 protein (Novus Biologicals).

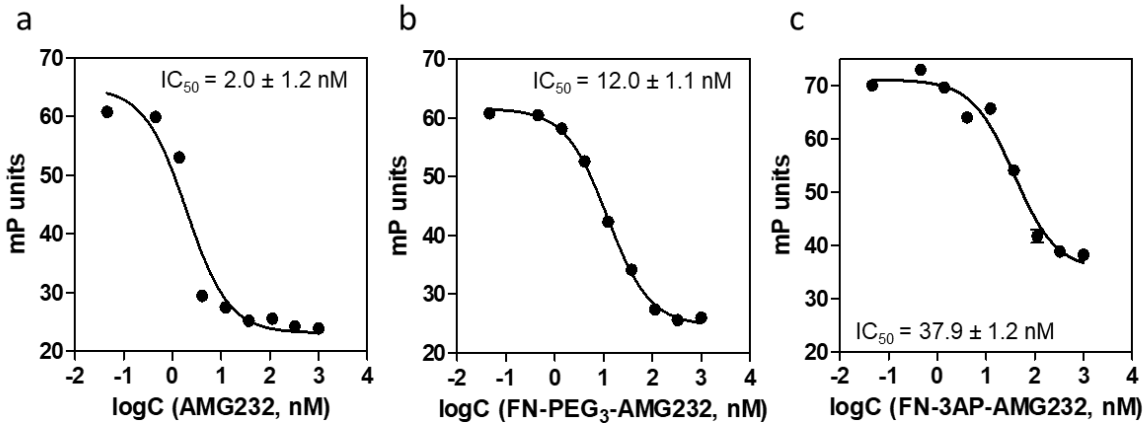


Figure S17. Determination of the inhibitory potency (IC_{50}) of AMG232 (a) and its nonradioactive fluorinated analogues, **13** (b) and **14** (c) by a fluorescence polarization (FP)-based competitive binding assay.

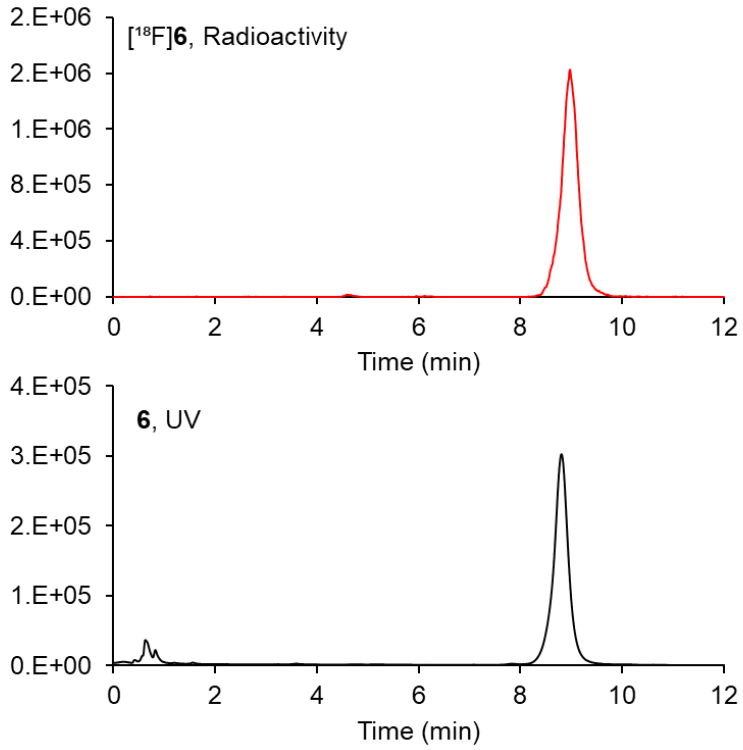


Figure S18. Identity confirmation of [^{18}F]**6** by comparing its retention time with the authenticated nonradioactive analogue **6** on the analytical HPLC system (Knauer, Germany) connected with a XBridge C18 column (3.5 µm, 3.0 × 100 mm; Waters) and eluted with acetonitrile and water (55:45; 0.1% TFA) at a flowrate of 1 mL per minute. Retention times: [^{18}F]**6** (Radioactivity) = 9.0 min, **6** (UV) = 8.8 min. Y-axis represents response in microvolts (µV).

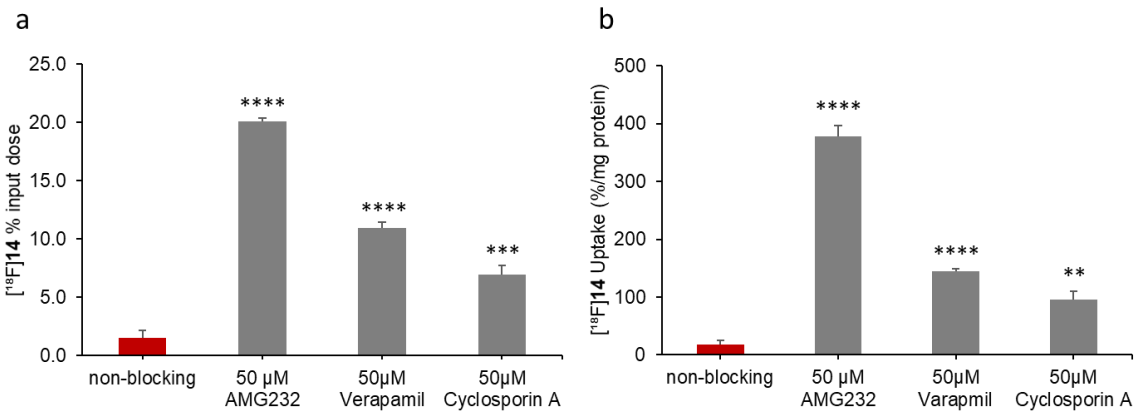


Figure S19. Uptake of [^{18}F]**14** in SJSA-1 tumor cells. Cells were incubated with [^{18}F]**14** alone (non-blocking) or with 50 µM of AMG232, Verapamil or Cyclosporin for 1 h. a) Cell uptake data presented as % input dose of [^{18}F]**14**; b) Cell uptake data presented as % uptake per mg protein. Data are shown as mean ± SD for triplicates. $^{**}p \leq 0.01$, $^{***}p \leq 0.001$, $^{***}p \leq 0.0001$ vs. no co-incubation (0 µM).

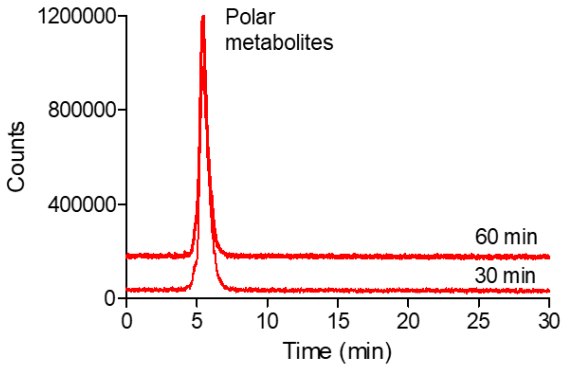


Figure S20. Radioactivity profiles (HPLC) of the urine samples collected from mice at 30 or 60 min post-injection (i.v.) of [^{18}F]**6**, showing polar labeled metabolite peaks (t_R = 5.5 min). No intact tracer (t_R = 20.4 min) was detected in the urine at either of the two time points.