SUPPLEMENTAL DATA FILE:

Structure-guided engineering of human thymidine kinase 2 as a positron emission tomography reporter gene for enhanced phosphorylation of a non-natural thymidine analog reporter probe*

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***Running title**: Improved TK2 PET reporter gene

TABLE OF CONTENTS:

Supplemental Methods……………………………………………………..S-2

Synthesis of L-^{18}F-FMAU………………………………………………..S-2

References…………………………………………………………………...S-3

Supplemental Table Legends……………………………..…..………..S-4

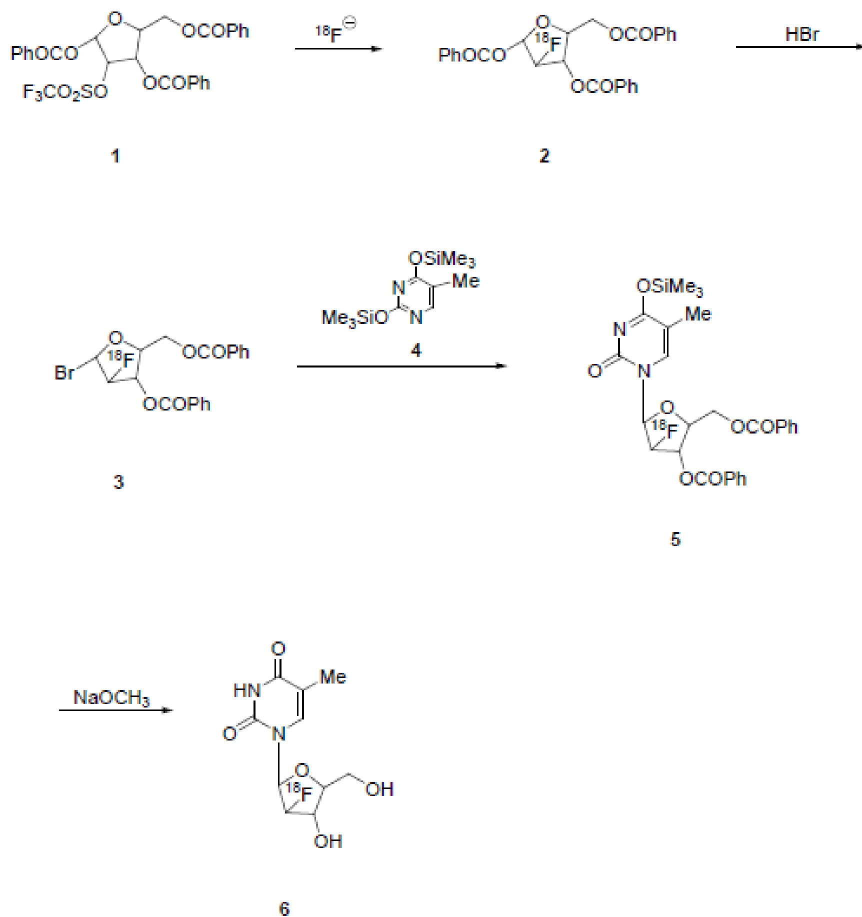
Supplemental Tables…………………………………………………..…S-5

SUPPLEMENTAL METHODS:

Synthesis of L-^{18}F-FMAU:

L-2-O-[(Trifluoromethyl)sulfonyl]-1,3,5-tri-O-benzoyl-α-D-ribofuranose (**1**) was prepared based on the procedure reported for the corresponding D-isomer(ref. 1). The synthesis of the ^{18}F-fluoro analog **2** was carried out by a modification of the method reported by Mangner et al (ref 2.) for the corresponding D-isomer. No-carrier-added [^{18}F]-fluoride ion was produced by 11 MeV proton bombardment of 98 % enriched [^{18}O]water in a tantalum target body using a RDS-112 cyclotron. The aqueous [^{18}F]fluoride ion was passed through a small cartridge of BioRad MP-1 anion exchange resin (10 mg, bicarbonate form) to trap the [^{18}F]fluoride ion. The [^{18}F]fluoride ion was subsequently released from the cartridge with a solution of K_2CO_3 (1 mg in 0.4 mL of water) and mixed with a solution of Kryptofix 2.2.2 (10 mg) dissolved in water (0.04 mL) and acetonitrile (0.75 mL) mixture. The solution was evaporated at 115 ^{0}C with a stream of nitrogen gas. The residue was dried by the azeotropic distillation with acetonitrile (3 X 0.5 mL). To the dry residue, a solution of the triflate **1** (10 mg) in 0.7 mL of acetonitrile was added and the reaction mixture was heated at 165 ^{0}C for 15 min in a sealed vessel. The solution was cooled to room temperature and passed through a Waters silica gel Sep-Pak. The product was eluted from the cartridge with 5 mL of ethyl acetate. The ethyl acetate solution was evaporated to dryness and 0.1 mL of a solution of 30 % HBr in acetic acid was added followed by 0.4 mL of dichloroethane. This new reaction mixture was heated at 80 ^{0}C in a sealed vessel for 10 min and the solution was concentrated to ~ 50 % of the initial volume. Toluene (0.7 mL) was then added and the solution was evaporated at 110 ^{0}C to give the bromo compound **3**. A solution of the disilyl derivative of 5-methyluracil (**4**, 20 mg, Aldrich Chemical Company) was dissolved in 1 mL of dichloroethane and added to the bromo compound **3**. The condensation reaction was carried out at 160 ^{0}C in a sealed vessel for 30 min. The reaction mixture was cooled to room temperature and then passed through a Waters silica gel Sep-Pak. The product was eluted off the column using 5 mL of a solution mixture of 10% methanol and 90 % dichloromethane. This solution was evaporated to dryness at 100 ^{0}C and then treated with 0.5 mL of a solution of 0.5 M sodium methoxide in methanol. The reaction mixture was heated at 100 ^{0}C for 5 min in a sealed vessel. The basic reaction mixture was neutralized with 0.25 mL of 1M HCl in water. This reaction mixture was diluted to a total volume of 3 mL with a mixture of 4% acetonitrile and 96% 50 mM ammonium acetate in water and injected into a semi-preparative HPLC column (Phenomenex Gemini C-18 column; 25 cm X 1 cm). The HPLC column was eluted with a solvent mixture of 4% acetonitrile and 96% 50 mM ammonium acetate at a flow rate of 5.0 mL/min. The effluent from the HPLC column was monitored with a 254 nm UV detector followed by a gamma radioactive detector. The chemically and radiochemically pure L-[^{18}F] FMAU (**6**) that eluted off the column with a retention time of ~ 24 min was collected and the solvents were evaporated in a rotary evaporator. One mL of ethanol was added to the residue and evaporated to remove the last traces of acetonitrile. This was followed by an addition of one mL sterile water and evaporation to remove the ethanol. The product was finally dissolved in 5 mL

of sterile water and made isotonic with saline and sterilized by passing through a Millipore filter (0.22 µm) into a sterile multi-dose vial.



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SUPPLEMENTAL TABLE LEGENDS:

Supplemental Table 1: Calculated accumulation of PET reporter probes in tissues of C57/BL6 mice 3 hours post-injection. All values are %ID/cc +/- SEM

Supplemental Table 2: Kinetics of the phosphorylation of D and L-nucleosides by the TK2 mutants. Kinase assays were performed with recombinant protein with 200 µM of the listed substrate

Supplemental Table 3: Human Biodistribution of the PET reporter genes. Values are calculated at 2 hours post injection. All values are Standard Uptake Value (SUV) +/- SEM

Supplemental Table 1

	L-^{18}F-FMAU	^{18}F-FHBG
TISSUES	**%ID/g +/- SEM**	**%ID/g +/- SEM**
Bone Marrow	0.45 +/- 0.11	0.24 +/- 0.10
Gallbladder	1.68 +/- 0.46	7.45 +/- 5.31
GI tract	10.47 +/- 2.51	32.37 +/- 13.39
Liver	0.81 +/- 0.70	0.19 +/- 0.11
Muscle	0.56 +/- 0.15	0.03 +/- 0.01

Supplemental Table 2

		WT	N93D	L109F	N93D-L109F
Nucleoside					
dC	k_{obs}/sec	0.134	0.026	0.860	0.083
dT	k_{obs}/sec	0.116	0.024	0.455	0.061
L-dT	k_{obs}/sec	0.283	0.194	1.078	0.538
L-FMAU	k_{obs}/sec	0.410	0.336	0.938	0.756

Supplemental Table 3

	L-^{18}F-FMAU	^{18}F-FHBG
TISSUES	SUV +/- SEM	SUV +/- SEM
Bladder	24.33 +/- 9.74	50.90
GI Tract	1.83 +/- 0.31	3.12
Brain	0.12 +/- 0.04	0.07
Left Kidney	4.65 +/- 0.37	1.38
Right Lung	0.67 +/- 0.05	0.09
Myocardium	6.45 +/- 1.84	0.21
Leg Muscle	0.73 +/- 0.07	0.43
Bone Marrow	0.70 +/- 0.11	0.11
Gall Bladder	12.42 +/- 2.54	11.31
Liver	14.61 +/- 1.77	2.36
Vertebrae + Spinal Cord	1.75 +/- 0.13	0.22
Bone	0.68 +/- 0.36	0.29