

Radiosynthesis of 9-(4-[¹⁸F]-fluoro-3-hydroxymethylbutyl)-guanine ([¹⁸F]FHBG)

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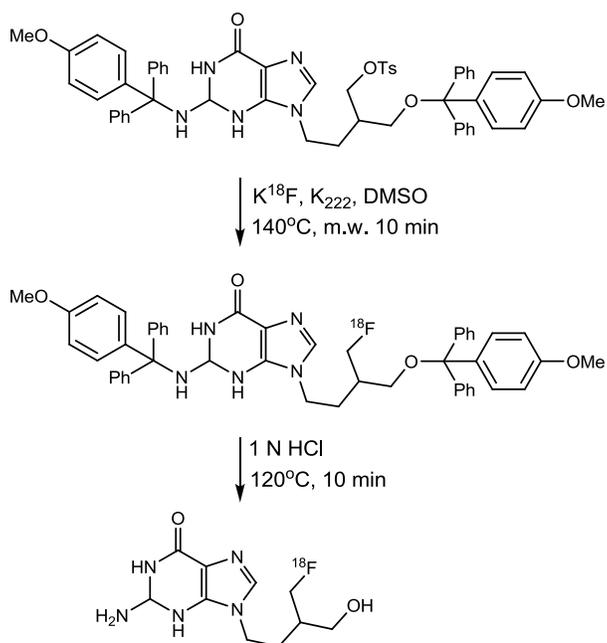
Background

Molecular imaging has been extensively used in research, diagnosis and treatment of cancer and neurology studies. In virology, functional molecular imaging could be useful to track *in vivo* molecular and cellular processes during viral infections. Thymidine kinase (TK) of Herpes Simplex virus 1 (HSV-1) is a reporter enzyme currently being used for *in vivo* imaging of gene delivery and expression in humans.¹ Radiolabeled probes of analogous nucleoside substrates of *HSV1-tk gene* are phosphorylated and ‘trapped’ inside the *HSV1-tk* infected cells. The *HSV1-tk* positive cells are thus differentiated from normal tissues by the radioactive signal. To evaluate the feasibility of *HSV1-tk* as reporter gene for monitoring viruses *in vivo*, *HSV1-tk* substrate 9-(4-¹⁸F-fluoro-3-[hydroxymethyl] butyl)guanine (¹⁸F-FHBG) was chosen as a probe²⁻⁵. The progress of disease in animals infected by the *HSV1-tk* gene can be monitored by PET/CT over time by injecting ¹⁸F-FHBG at different time points after infection. Here, the radiosynthesis of ¹⁸F-FHBG is described.

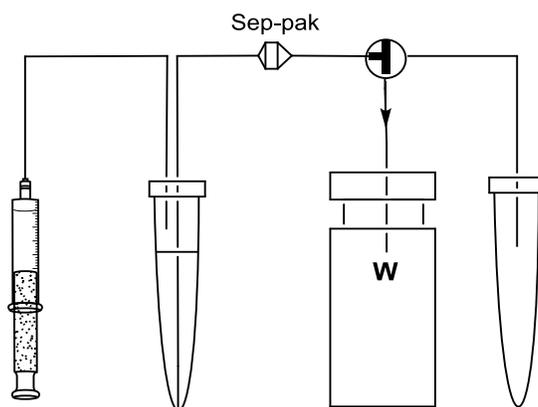
Chemistry

[¹⁸F]FHBG is synthesized by a modification of the method of Yaghoubi *et al*². The radiolabeling was carried out manually with the assistance of simple semiautomatic devices and microwave heating.

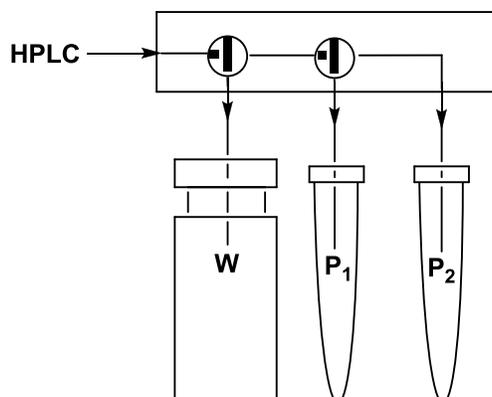
Scheme 1. Radiolabeling of [^{18}F]FHBG



Scheme 2. C18 Sep-Pak trapping of the triarylmethyl protected [^{18}F]FHBG from the reaction mixture.



Scheme 3. Collection of the HPLC fractions of [^{18}F]FHBG using three-way valve control device.



General

TsO-FHBG precursor was purchased from ABX (Radeberg, Germany). No-carrier-added [^{18}F] fluoride solution was provided by PETNET (North Wales, PA). K_2CO_3 , K_{222} , anhydrous DMSO and anhydrous acetonitrile were purchased from Sigma-Aldrich (Milwaukee, WI). Millex[®]-GV syringe driven filter units (0.22 μm) were purchased from Millipore (Billerica, MA), Microwave reaction vials and stir bars were purchased from Biotage (Charlotte, NC). C18 Plus Sep-Pak cartridges were purchased from Waters (Milford, MA). Dose calibration was performed on a CAPINTEC CRC-15R Dose Calibrator (Ramsey, NJ). Product purification and quality control were performed on a Beckman Coulter HPLC system equipped with a System Gold 126 UV detector, a Bioscan Flow-count radioactive detector and an Eclipse-SB C18 (250 \times 9.8mm, 5 μm) column. HPLC analysis was carried out by using a solvent mixture of 2 % ethanol, 8 % acetonitrile and 90 % ammonium acetate (50 mM), at a flow rate of 3 mL/min and UV detection at 254 nm.

Radiosynthesis of [^{18}F]FHBG. No-carrier-added [^{18}F] fluoride ion solution was eluted from the QMA cartridge into a microwave reaction vial (Biotage 0.5-2 mL size, with a stir bar) with 0.6 mL of K_2CO_3 solution (7 mg/mL, 0.05M) and K_{222} solution (20 mg in 1 mL of acetonitrile). The solution was evaporated at 120 $^\circ\text{C}$ by bubbling nitrogen gas through it, and the residue was dried by azeotropic distillation with acetonitrile (3 \times 0.5 mL). To this anhydrous residue was added a solution of N^2 - (p-anisyl)diphenylmethyl)-9-[4-tosyl]-3-p-

anisylidiphenylmethoxymethylbutyl]guanine (2 mg) in dry dimethyl sulfoxide (0.5 mL). The reaction mixture was heated at 130 °C for 10 min in an oil bath. The solution was cooled, diluted with 4 mL water, and passed through a C18 Sep-Pak cartridge activated previously by flushing 5 mL CH₃OH followed by 10 mL deionized water. The cartridge was first eluted with water (3 x 4 mL) and then the triarylmethyl protected compound was eluted from the C-18 cartridge with 3 mL of methanol into a reaction vial containing a stir bar. The methanolic solution was acidified with 0.5 mL 1N HCl and concentrated to about 0.5 mL at 100 °C with a stream of nitrogen gas. The solution was cooled, partially neutralized with 0.4 mL 1 N NaOH, and diluted with the HPLC mobile phase (1 mL). The crude product was injected onto a C-18 semi-preparative HPLC column [Eclipse-SB C18 (250 x 9.8 mm, 5 µm), λ=254 nm, 3 mL/min flow rate, with a mobile phase of 50 mM NH₄OAc: CH₃CN:C₂H₅OH: 90:8:2]. [¹⁸F]FHBG was eluted from the column at a retention time of 11 min. The product fraction was eluted from the cartridge with 3 mL ethanol into a reaction vial containing a stir bar. The vial was heated in an oil bath (110 °C) and taken to dryness under nitrogen flow. The product was reconstituted with injection buffer and then filtered through a 0.22 µm membrane syringe filter unit into a sterile multidose vial. The product was found to be 99% radiochemically and chemically pure as determined by HPLC. Radiochemical yield ranged from 10% to 40 % (decay corrected).

References

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