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Novel Radiosynthesis of PET HSV-tk Gene Reporter Probes [^{18}F]FHPG and [^{18}F]FHBG Employing Dual Sep-Pak SPE Techniques

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Abstract—Positron emission tomography (PET) herpes simplex virus thymidine kinase (HSV-tk) gene reporter probes 9-[(3-[^{18}F]fluoro-1-hydroxy-2-propoxy)methyl]guanine ([^{18}F]FHPG) and 9-(4-[^{18}F]fluoro-3-hydroxymethylbutyl)guanine ([^{18}F]FHBG) were prepared by nucleophilic substitution of the appropriate tosylated precursors with [^{18}F]KF/Kryptofix 2.2.2 followed by a quick deprotection reaction and purification with a simplified dual Silica Sep-Pak solid-phase extraction (SPE) method in 15–30% radiochemical yield.

Gene transfer technology has shown significant potential in treating several common cancers using a variety of viral and non-viral vectors.[1–7] Among these, herpes simplex virus thymidine kinase (HSV-tk) has been used as a key prodrug-converting enzyme for a number of anticancer gene therapy approaches.[8,9] The enzyme has a broad substrate specificity and can convert less toxic ganciclovir (GCV, 9-[(1,3-dihydroxy-2-propoxy)methyl]-guanine) or penciclovir (PCV, 9-[4-hydroxy-3(hydroxy-methyl)butyl]guanine) into toxic compounds that result in cell death.[10] The 5-substituted analogue of thymidine 1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-iodouracil (FIAU) is the substrate for thymidine kinase and is incorporated into DNA.[11–14] Imaging of HSV-tk expression is reliant on the use of enzyme imaging agents, fluorinated (fluorine-18) or iodinated (iodine-124, 125 and 131) prodrugs such as fluorinated GCV and PCV analogues 8-[^{18}F]fluoroganciclovir ([^{18}F]FGCV), 9-[(3-[^{18}F]fluoro-1-hydroxy-2-propoxy)methyl]guanine ([^{18}F]FHPG), 8-[^{18}F]fluoropenciclovir ([^{18}F]FPCV), 9-(4-[^{18}F]fluoro-3-hydroxymethylbutyl)guanine ([^{18}F]-FHBG); and fluorinated and iodinated FIAU analogues [^{18}F]FIAU, [^{124}I]FIAU and [$^{125/131}$I]FIAU, coupled with biomedical imaging technique positron emission tomography (PET) or single photon emission computed tomography (SPECT) (Fig. 1).[15–37] Several recent reports[13,14,34] suggest that FIAU is superior to FHPG and FHBG, however, [^{18}F]FIAU is still under developing;[11] ^{124}I target and [^{124}I]FIAU are not available for most PET centers; and [$^{125/131}$I]FIAU is for SPECT imaging. Our aim is to develop and synthesize PET HSV-tk gene reporter probes.

Considerable efforts have been devoted to the synthesis of these gene reporter probes and numerous improved synthesis were reported in the literature,[15,16,29,33,34] in which [^{18}F]FGCV and [^{18}F]FPCV were labeled with fluorine-18 at the 8-position of guanine ring of GCV and PCV; [^{18}F]FHPG and [^{18}F]FHBG were labeled with fluorine-18 at the side chain of GCV and PCV. The potential importance of these compounds as gene therapy imaging tools is great, and broader research investigation to fully explore and validate their utility is important. However, the limited commercial availability, complicated synthetic procedure and high costs of starting materials GCV and PCV can present an obstacle to more widespread evaluation of these intriguing agents. Wishing to study these compounds in this laboratory, we decided to make our own material by following the literature methods. Although several papers dealing with the synthesis of [^{18}F]FHPG and [^{18}F]FHBG from GCV and PCV have appeared, there are gaps in synthetic detail among them, and certain key steps gave poor yields or were difficult to repeat in our hands. Consequently, we investigated alternate approaches and modifications that eventually resulted in an

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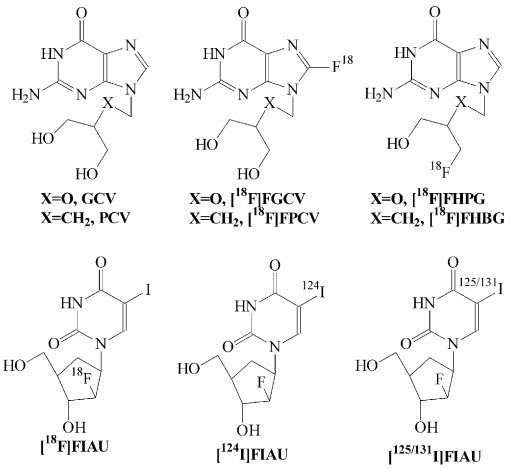
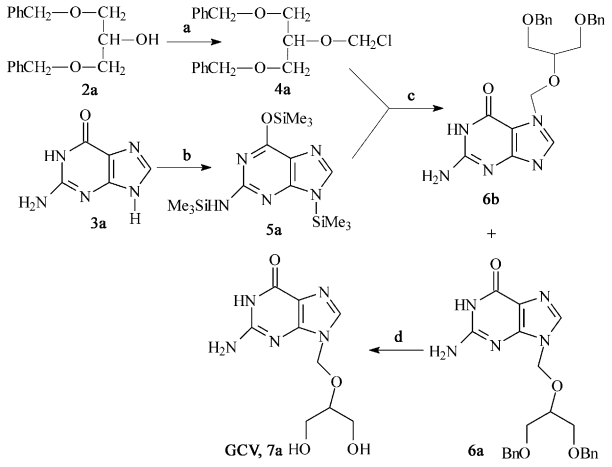


Figure 1. Chemical structures of GCV, PCV, [^{18}F]FGCV, [^{18}F]FPCV, [^{18}F]FHPG, [^{18}F]FHBG, [^{18}F]FIAU, [^{124}I]FIAU, and [$^{125/131}$I]FIAU.



Scheme 1. Synthesis of GCV: (a) $(CH_2O)n$, HCl (gas), $ClCH_2CH_2Cl$, 0 °C; (b) $NH(SiMe_3)_2$, $(NH_4)_2SO_4$; (c) Bu_4NF, THF; (d) Pd black, cyclohexene, EtOH.

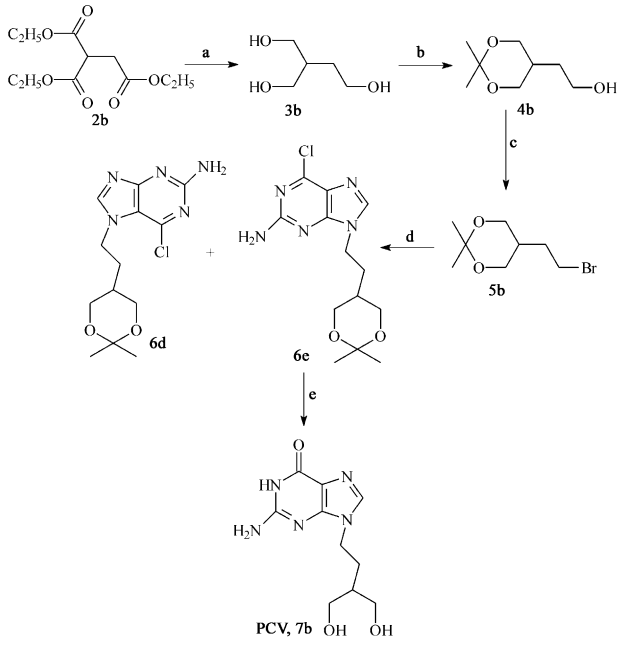
improved total synthesis of [^{18}F]FHPG and [^{18}F]FHBG starting from very beginning materials 1,3-dibenzyloxy-2-propanol and guanine, and triethyl-1,1,2-ethanetricarboxylate and 2-amino-6-chloropurine; and a novel radiosynthesis of [^{18}F]FHPG and [^{18}F]FHBG with a simplified dual Silica Sep-Pak solid-phase extraction (SPE) method that were superior to previous works or addressed more synthetic details to reveal and explain technical tricks.

The synthesis of GCV as indicated in Scheme 1 was performed with the modifications according to procedures reported in the literature.[24,31] The commercially available starting material 1,3-dibenzyloxy-2-propanol (**2a**) was converted into its chloromethyl ether 1,3-dibenzyloxy-2-chloromethoxypropane (**4a**) through a chloromethylation reaction. Compound **4a** was easy to decompose on silica gel column. Therefore, the purification of crude product **4a** was not attempted. The starting material guanine (**3a**) was reacted with 1,1,1,3,3,3-hexamethyldisilazane (HMDS) to provide guanine derivative 2,6,9-tris(trimethylsilyl)guanine (**5a**). The reaction went very slowly, requiring 4 days. The reaction was completed when the initial suspension turned to be a clear solution. Compound **5a** was sensitive to moisture, and it should be used for next step reaction immediately after removal of volatiles. The coupling reaction between **4a** and **5a** gave the desired product 9-isomer 9-[(1,3-dibenzyloxy-2-propoxy)methyl]-guanine (**6a**) and undesired by-product 7-isomer 7-[(1,3-dibenzyloxy-2-propoxy)methyl]guanine (**6b**). The reaction needed to be catalyzed by either tetrabutylammonium fluoride (TBAF)[24] or tetrabutylammonium iodide (TBAI).[31] However, there was not much difference observed in the reaction, and the ratio of products **6a**/**6b** was around 2:1 for both catalysts. The claimed high yield of 9-isomer with TBAF[24] was not achievable in our hands. The separation of the two isomers was challenging, since they gave the same R_f value on TLC. Thus, the preparative TLC and the flash column chromatography did not work for the purification of the 9-isomer from its mixture with the 7-isomer. Fortunately, repeated recrystallization of the mixture of the two isomers in ethanol could provide pure 9-isomer **6a** and 7-isomer **6b**. **6b** came out from their solution first, and then **6a**. The purities of **6a** and **6b** could be monitored by ^{1}H NMR spectrum, which was used to guide the recrystallization. The transfer hydrogenolysis reaction of compound **6a** to remove the protecting benzyl groups gave the key intermediate GCV, **7a**. The reaction was catalyzed by either palladium chloride ($PdCl_2$)[24] or palladium black.[31] However, we found with $PdCl_2$[24] the chain of the reaction product GCV was cleaved; with 1:1 (w/w) palladium black/**6a**[31] the deprotection reaction would not complete no matter how long the reaction was heated. The ratio of palladium black/**6a** we used was 3:1 (w/w), which gave deprotective GCV, **7a** in a yield of 87.7%. The overall chemical yield of GCV from **2a** and **3a** was 15.6%.

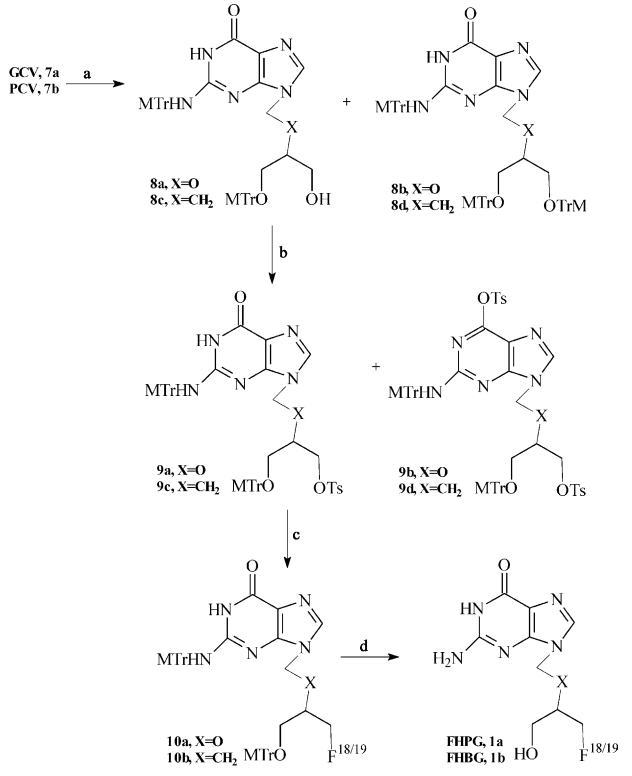
The synthesis of PCV as indicated in Scheme 2 was performed with the modifications according to procedures reported in the literature.[25] The commercially available starting material triethyl-1,1,2-ethanetricarboxylate (**2b**) was converted into 2-(hydroxymethyl)-butane-1,4-diol (**3b**) through reduction reaction with excessive sodium borohydride in *tert*-butyl alcohol in a yield of 66.4%. The crude product contained a polar contaminant, which was suspected to be boric acid generated from hydrolysis of sodium borohydride. Although the contaminant did not affect the further use of **3b**, it was convenient to purify it by column chromatography eluted with 10:1 EtOAc/MeOH. Another reagent lithium aluminum hydride (LAH) was ever tried to reduce the triester, which resulted in a mixture of two unidentified components. The ^{1}H NMR spectrum of pure **3b** (in D_2O) showed four multiple peaks corresponding to the four kinds of protons except hydroxyl groups of the molecule. The selective 1,3-protection of the triol **3b** was completed by the isopropylidene group through the reaction of **3b** with 2,2-dimethoxypropane catalyzed by *p*-toluenesulfonic acid. The reaction took 19 h to produce 5-(2-hydroxyethyl)-2,2-dimethyl-1,3-dioxane (**4b**) in a yield of 57.0%. A small amount of seven-membered ring by-product was also formed, which was very close to the desired 6-membered ring

product **4b** in TLC. The separation of both desired six-membered ring product and undesired seven-membered ring by-product proved to be successful by column chromatography eluted with 2:1 EtOAc/hexane. The ^{1}H NMR spectrum of **4b** [δ 3.95 (dd, 2H, H_{eq}, $J_1 = 11.77$ Hz, $J_2 = 4.42$ Hz), 3.71 (t, 2H, CH_2OH, $J = 6.62$ Hz), 3.64 (dd, 2H, H_{ax}, $J_1 = 11.77$ Hz, $J_2 = 8.09$ Hz), 1.88–2.02 (m, 2H, CH and OH), 1.52–1.59 (q, 2H, CH_2, $J = 6.60$ Hz), 1.43 (s, 3H, CH_3), 1.42 (s, 3H, CH_3)] showed that the molecule favors a chair configuration where the two protons of methylene groups in the ring and one of the two methyl groups of the acetonide would take either equatorial or axial positions, and the hydroxyl ethyl group would take equatorial position and the proton of the methane at the point of attachment would take axial position. There are not any experimental data to prove the interconversion of the two chair conformations, but we can predict that the true importance of the conformation is to require a chair conformation in which hydroxyl ethyl group is in equatorial position, since this chair configuration would be more stable. Bromination of compound **4b** with carbon tetrabromide and triphenylphosphine afforded the 5-(2-bromoethyl)-2,2-dimethyl-1,3-dioxane (**5b**). The reaction and the whole workup process should be kept at 0 °C, even for removal of solvent. Because compound **5b** decomposed readily at room temperature, the purification of **5b** was not attempted. Similarly, the ^{1}H NMR spectrum of **5b** [δ 3.96 (dd, 2H, H_{eq}, $J_1 = 11.77$ Hz, $J_2 = 3.68$ Hz), 3.61 (dd, 2H, H_{ax}, $J_1 = 12.13$ Hz, $J_2 = 6.62$ Hz), 3.44 (t, 2H, CH_2Br, $J = 6.62$ Hz), 1.88–2.02 (m, 3H, $CHCH_2CH_2$), 1.43 (s, 3H, CH_3), 1.41 (s, 3H, CH_3)] also showed that the two protons of methylene groups in the ring and one of the two methyl groups of acetonide would take either equatorial or axial positions, and the bromoethyl group would take equatorial position and the proton of the methane at the point of attachment would take axial position. Coupling of bromide **5b** with 2-amino-6-chloropurine under a basic media gave a mixture of undesired by-product 7-isomer 2-amino-6-chloro-7-[2-(2,2-dimethyl-1,3-dioxan-5-yl)ethyl]-purine (**6d**) and desired product 9-isomer 2-amino-6-chloro-9-[2-(2,2-dimethyl-1,3-dioxan-5-yl)ethyl]purine (**6c**) in yields of 3.5 and 53.6%, respectively. The TLC R_f values of both isomers in developing solvent ethyl acetate were very close, 0.32 and 0.31 for **6d** and **6c**, respectively. However, careful and repeated column chromatography eluted with ethyl acetate made the separation possible. The 7-isomer **6d** was fully characterized for the first time, and the ^{1}H NMR, LRMS, HRMS and elemental analysis data of **6d** are consistent with the assigned structure. Hydrolysis of 9-isomer **6c** with aqueous hydrochloric acid at reflux, followed by neutralization and filtration, afforded PCV, **7b** in a yield of 79.5%. The overall chemical yield of PCV from **2b** was 16.1%.

The syntheses of standard samples 9-[(3-fluoro-1-hydroxy-2-propoxy)methyl]guanine (FHPG, **1a**), 9-(4-fluoro-3-hydroxymethylbutyl)guanine (FHBG, **1b**) and target tracers [^{18}F]FHPG (**1a**), [^{18}F]FHBG (**1b**) as indicated in Scheme 3 were performed with the modifications according to procedures reported in the literature.[15,16,30,33,34] The protection of the 2-amino group and one of the two hydroxyl groups of GCV **7a** or PCV **7b** was furnished by reacting with monomethoxytrityl chloride in the presence of 4-dimethyl-



Scheme 2. Synthesis of PCV: (a) $NaBH_4$, *t*BuOH; (b) 2,2-dimethoxypropane, *p*-toluenesulfonic acid, THF; (c) CBr_4, PPh_3, THF, 0 °C; (d) 2-amino-6-chloropurine, K_2CO_3, DMF; (e) 2 M HCl.



Scheme 3. Synthesis of FHPG, FHBG, [^{18}F]FHPG, and [^{18}F]FHBG: (a) MTrCl, DMAP, Et_3N, DMF; (b) TsCl, pyridine; (c) K[$^{18/19}$F], Kryptofix 2.2.2, CH_3CN; (d) 1 N HCl, MeOH.

aminopyridine (DMAP) and triethylamine.[30] The bulky methoxytrityl group favored the desired product N^2-(*p*-anisyldiphenylmethyl)-9-[[1-(*p*-anisyldiphenylmethoxy)-3-hydroxy-2-propoxy]methyl]guanine (**8a**) or N^2-(*p*-Anisyldiphenylmethyl)-9-[(4-hydroxy)-3-*p*-anisyldiphenylmethoxymethylbutyl]guanine (**8c**) in yields of 36.2 and 52.6%, respectively, if the ratio of starting materials and reaction temperature are appropriate, although the formation of the undesired fully protected by-product N^2-(*p*-anisyldiphenylmethyl)-9-[[1,3-bis(*p*-anisyldiphenylmethoxy)-2-propoxy]methyl]guanine (**8b**) or N^2-(*p*-anisyldiphenylmethyl)-9-[(4-*p*-anisyldiphenylmethoxy)-3-*p*-anisyldiphenylmethoxymethylbutyl]guanine (**8d**) is not avoidable, in yields of 20.7 and 16.1%, respectively. Compound **8a** or **8c** reacted with *p*-tosyl chloride at room temperature afforded the desired precursor tosylate N^2-(*p*-anisyldiphenylmethyl)-9-[((1-anisyldiphenylmethoxy)-3-tosyl-2-propoxy)methyl]guanine (**9a**) or N^2-(*p*-Anisyldiphenylmethyl)-9-[(4-tosyl)-3-*p*-anisyldiphenylmethoxymethylbutyl]guanine (**9c**) in yields of 50.8 and 29.0%, respectively, together with the undesired by-product 1-tosyl-N^2-(*p*-anisyldiphenylmethyl)-9-[((1-anisyldiphenylmethoxy)-3-tosyl-2-propoxy)methyl]guanine (**9b**) or 1-tosyl-N^2-(*p*-anisyldiphenylmethyl)-9-[(4-tosyl)-3-*p*-anisyldiphenylmethoxymethylbutyl]guanine (**9d**) in yields of 17.5 and 21.9%, respectively. The tosylate **9a** was purified by recrystallization from ethanol in the literature.[30] We found it was convenient to perform flash column chromatography to produce pure and reliable precursor **9a**. The ^{1}H NMR data of **9c** with 1-NH and 2-NH assigned collected in this work contained more information than in the literature.[15,25,30,33,34] The fluorination of the tosylate **9a** or **9c** with anhydrous potassium fluoride in dry acetonitrile catalyzed by Kryptofix 2.2.2 gave the intermediate N^2-(*p*-anisyldiphenylmethyl)-9-[[1-(*p*-anisyldiphenylmethyl)-3-fluoro-2-propoxy]-methyl]guanine (**10a**) or N^2-(*p*-Anisyldiphenylmethyl)-9-[(4-fluoro)-3-*p*-anisyldiphenylmethoxymethylbutyl]-guanine (**10b**) in yields of 29.7 and 69.1%, respectively. The intermediate **10a** or **10b** was easily hydrolyzed by 1 N HCl aqueous solution to give the unlabeled standard sample FHPG, **1a** or FHBG, **1b** in yields of 87.3 and 97.0%, respectively. The overall chemical yields of the tosylate precursors **9a** from GCV and **9c** from PCV were 18.4 and 15.3%, respectively, and the overall chemical yields of the standard samples FHPG, **1a** from GCV and FHBG, **1b** from PCV were 4.8 and 10.2%, respectively.

[^{18}F]FHPG (**1a**) or [^{18}F]FHBG (**1b**) was synthesized by a modification of the procedures as reported in the literature.[15,16,33,34] The tosylated precursor **9a** or **9c** was labeled by a conventional nucleophilic substitution with K[^{18}F]/Kryptofix 2.2.2 in CH_3CN at 120 °C for 15 min to provide a radiolabeling intermediate **10a** or **10b**. The radiolabeling reaction was monitored by analytical radio-HPLC method, in which we employed a new HPLC system[14,38] by using a Prodigy (Phenomenex) 5 μm C-18 column, 4.6×250 mm; 3:1:1 CH_3CN/MeOH/20 mM, pH 6.7 buffer (120 mL 100 mM KH_2PO_4 and 80 mL 100 mM K_2HPO_4 in 800 mL Zyza-tech H_2O) mobile phase, 1.5 mL/min flow rate, and UV (240 nm) and γ-ray (NaI) flow detectors. Retention times in the analytical HPLC system were: RT**10a** = 15.00 min, RT**10b** = 17.50 min, RTK[^{18}F] = 1.88 min. The radiolabeling mixture containing the intermediate **10a** or **10b** was passed through a Silica Sep-Pak to remove Kryptofix 2.2.2 and non-reacted [^{18}F]fluoride. The large polarity difference between **10a** or **10b** and Kryptofix 2.2.2 and non-reacted [^{18}F]fluoride permitted the use of a simple solid-phase extraction (SPE) technique[14,38,39] for fast isolation of **10a** or **10b** from the radiolabeling reaction mixture. The key part in this technique is a SiO_2 Sep-Pak type cartridge, which contains ~0.5–2 g of adsorbent. The Sep-Pak was eluted with 15% MeOH/CH_2Cl_2 and the solvent was evaporated under high vacuum to give the compound **10a** or **10b**. The existence of the catalyst Kryptofix 2.2.2 and non-reacted [^{18}F]fluoride would affect the deprotection reaction of **10a** or **10b** and the quality control (QC)[40] of the labeled product **1a** or **1b**; therefore, they needed to be removed before **10a** or **10b** was deprotected to give the target labeled product **1a** or **1b**. The *p*-anisyldiphenylmethyl protecting groups of **10a** or **10b** were rapidly removed by treating with methanolic 1 N HCl for 10 min followed by neutralization with 6 N NaOH to provide **1a** or **1b,** respectively. To simplify the synthetic procedure, the final reaction mixture was purified with SPE method instead of HPLC method so that it will be amenable for automation.[33] The crude product was once again passed through the second Silica Sep-Pak to remove radioactive by-product by simple SPE with ethanol. The large polarity difference between **1a** or **1b** and radioactive by-product permitted the use of SPE technique for fast purification of radiotracer **1a** or **1b** from radiolabeling mixture. The radiochemically pure compound **1a** or **1b** was isolated with 90:8:2 H_2O/EtOH/HOAc from the Sep-Pak and adjusted pH to 5.5–7.0 with 2 M NaOH and 150 mM NaH_2PO_4 mixed solution. The radiochemical yield of **1a** or **1b** was 15–30%, and the synthesis time was 60–70 min from end of bombardment (EOB). Chemical purity, radiochemical purity, and specific radioactivity were determined by analytical HPLC method. Retention times in the analytical HPLC system were: RT**9a** = 14.03 min, RT**9c** = 19.80 min, RT**1a** = 2.02 min, RT**1b** = 1.80 min. The chemical purities of precursors **9a** and **9c**, and standard samples **1a** and **1b** were >95%, the radiochemical purities of target radiotracers **1a** and **1b** were >99%, and the chemical purities of radiotracers **1a** and **1b** were ~93%. The average (n = 3–5) specific radioactivities of radiotracers **1a** and **1b** were 0.8–1.2 Ci/μmol at end of synthesis (EOS).

The 'cold' synthesis (organic synthesis) of acyclic nucleosides reported in this paper is an improved synthesis.[41,42] The novelty in the synthesis is the discovery of several new compounds **6b**, **6d**; **8b**, **8d**; and **9b**, **9d** which have analytical data such as mp, ^{1}H NMR and MS in agreement with the indicated structures, and the analytical data with more information for the compounds **9c**, **1a**, and **1b** were also given.[43] The simplified 'hot' synthesis (radiosynthesis) of [^{18}F]FHPG and [^{18}F]FHBG is novel and employs new dual Sep-Pak techniques superior to other methods currently being used. This new method will shorten overall synthesis, purification and formulation time by at least 20 min,

reduce complicated HPLC manipulation, and improve the radiochemical yields.

In comparison with the results reported in the literature,[15,16,30,33,34] an improved total synthesis of FHPG, FHBG, [^{18}F]FHPG, and [^{18}F]FHBG, and a novel radiosynthesis of [^{18}F]FHPG and [^{18}F]FHBG employing simplified dual Silica Sep-Pak techniques have been developed, several improvements and new findings in the synthetic methodology, radiolabeling, preparative separation and analytical details for GCV, PCV, FHPG, FHBG, tosylate precursors, [^{18}F]FHPG, and [^{18}F]FHBG have been made and addressed. They include newly discovered compounds, increased radiochemical yields and specific activities, enhanced radiochemical purities, shortened synthesis time, new dual Sep-Pak techniques for fast and efficient preparative separation of [^{18}F]FHPG and [^{18}F]FHBG from precursors, and new HPLC systems for the QC method of target tracers [^{18}F]FHPG and [^{18}F]FHBG. These improved methods are efficient and convenient. It is anticipated that the approaches and improvements described here can be applied with advantage to the synthesis of other radiolabeled GCV and PCV analogues for PET imaging of HSV-tk gene expression.

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43. **6b**, R_f 0. 29 (9:1 EtOAc/MeOH). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.92 (s, 1H, 1-NH), 8.11 (s, 1H, 8-CH), 7.10–7.40 (m, 10H, Ph), 6.24 (s, 2H, 2-NH_2), 5.68 (s, 2H, 1′-CH_2), 4.40 (s, 4H, PhCH_2), 4.10 (q, 1H, 3′-CH, J = 4.78 Hz), 3.30–3.60 (m, 4H, 4′-CH_2). LRMS (EI, m/z) 91 (100%), 436 [$(M+H)^+$, 0.4%]. HRMS (FAB, m/z) calcd for $C_{23}H_{26}N_5O_4$ 436.4838, found 436.1993. **6d**, mp 130 °C (dec.), R_f 0.32 (EtOAc). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.08 (s, 1H, 8-H), 6.91 (s, 2H, 2-NH_2), 3.30–4.10 (m, 6H, 1′- and 4′-H), 2.12–2.30 (m, 2H, 2′-H), 1.35–1.52 (m, 1H, 3′-H), 1.24 (s, 3H, CH_3), 1.19 (s, 3H, CH_3). The analysis calculated for $C_{13}H_{18}ClN_5O_2$ was C, 50.08; H, 5.82. The values found were C, 50.13; H, 5.83. LRMS (EI, m/z): 154 (100%), 312 [$(M+H)^+$, 13.9%]. HRMS (FAB, m/z) calcd for $C_{13}H_{19}ClN_5O_2$ 312.1227, found 312.1234. **8b**, mp 115 °C (dec.), R_f 0.34 (5% MeOH/CH_2Cl_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.68 (s, 1H, 1-NH), 7.84 (s, 1H, 8-CH), 7.67 (s, 1H, 2-NH), 6.53–7.36 (m, 42H, Ph), 4.91 (s, 2H, 1′-CH_2), 3.75 (s, 6H, OCH_3), 3.57–3.67 (m, 1H, 3′-CH), 3.48 (s, 3H, OCH_3), 2.63 (brs, 4H, 4′-CH_2). LRMS (EI, m/z) 273 (100%), 1094 [$(M+Na)^+$, 1.6%]. HRMS (FAB, m/z) calcd for $C_{69}H_{61}NaN_5O_7$ 1094.4469, found 1094.4452. **8d**, mp 145 °C (dec.), R_f 0.26 (4% MeOH/CH_2Cl_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.48 (s, 1H, 1-NH), 7.55 (s, 1H, 2-NH), 6.66–7.32 (m, 43H, Ph and 8-CH), 3.72 (s, 6H, 9-OCH_3), 3.59 (s, 3H, 2-OCH_3), 3.24 (t, 2H, 1′-H, J = 6.62 Hz), 2.78–2.92 (m, 4H, 4′-H), 1.52–1.65 (m, 1H, 3′-H), 1.08–1.20 (m, 2H, 2′-H). The analysis calculated for $C_{70}H_{63}N_5O_6$ was C, 78.55; H, 5.93. The values found were C, 77.56; H, 5.92. LRMS (EI, m/z) 273 (100%), 1092 [$(M+Na)^+$, 3.9%). HRMS (FAB, m/z) calcd for $C_{70}H_{63}N_5NaO_6$ 1092.4676, found 1092.4684. **9b**, mp, 95 °C (dec.), R_f 0. 36 (1% MeOH/CH_2Cl_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.56–8.27 (m, 38H, Ph and 8-CH), 5.11 (dd, 2H, 1′-CH_2, J_1 = 44.85 Hz, J_2 = 11.03 Hz), 3.73 (s, 3H, OCH_3), 3.53 (s, 3H, OCH_3), 3.38–3.57 (m, 3H, 3′-CH and 4′-CH_2), 2.30–2.71 (m, 2H, 5′-CH_2), 2.41 (s, 6H, CH_3). LRMS (EI, m/z) 273 (100%), 1108 [$(M+H)^+$, 1.4%]. HRMS (FAB, m/z) calcd for $C_{63}H_{58}N_5O_{10}S_2$ 1108.3625, found 1108.3667. **9c**, R_f 0. 29 (4% MeOH/CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.63 (brs, 1H, 1-NH, D_2O exchangeable), 7.87 (brs, 1H, 2-NH, D_2O exchangeable), 7.75 (d, 2H, Ph, J = 7.36 Hz), 6.87–7.45 (m, 27H, Ph and 8-H), 6.81 (d, 2H, Ph, J = 8.09 Hz), 6.61 (d, 2H, Ph, J = 8.09 Hz), 3.75–4.05 (m, 2H, 4′-CH_2OTs), 3.76 (s, 3H, 9-OCH_3), 3.62 (s, 3H, 2-OCH_3), 3.15–3.35 (m, 2H, 1′-H), 2.76–3.04 (m, 2H, 5′-CH_2OTrM), 2.41 (s, 3H, CH_3), 1.51 (brs, 1H, 3′-H), 1.26 (br. s, 2H, 2′-H). **9d**, mp 95–98 °C, R_f 0.35 (4% MeOH/CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 (d, 2H, Ph, J = 7.35 Hz), 7.74 (d, 2H, Ph, J = 8.09 Hz), 6.95–7.45 (m, 29H, Ph and 2-NH), 6.80 (d, 2H, Ph, J = 8.09 Hz), 6.70 (d, 2H, Ph, J = 8.82 Hz), 6.37 (s, 1H, 8-CH), 3.78–4.03 (m, 2H, 4′-CH_2OTs), 3.77 (s, 3H, 9-OCH_3), 3.72 (s, 3H, 2-OCH_3), 3.28–3.47 (m, 2H, 1′-CH_2), 2.80–3.04 (m, 2H, 5′-CH_2OTrM), 2.42 (s, 6H, CH_3), 1.45 (brs, 1H, 3′-CH), 1.20–1.38 (m, 2H, 2′-CH_2). The analysis calculated for $C_{64}H_{59}N_5O_9S_2$: C, 69.48; H, 5.38. The values found were C, 68.88; H, 5.40. LRMS (EI, m/z) 273 (100%), 1106 [$(M+H)^+$, 2.6%]. HRMS (FAB, m/z) calcd for $C_{64}H_{60}N_5O_9S_2$ 1106.3833, found 1106.3861. **1a**, R_f 0.34 (12:1 MeCN/H_2O). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.89 (s, 1H, 1-NH, D_2O exchangeable), 7.81 (s, 1H, 8-CH), 6.78 (s, 2H, 2-NH_2, D_2O exchangeable), 5.42 (s, 2H, 1′-CH_2), 4.93 (t, 1H, OH, J = 5.88 Hz, D_2O exchangeable), 4.24–4.58 (m, 2H, 4′-CH_2F), 3.73–3.88 (m, 1H, 3′-CH), 3.28–3.43 (m, 2H, 5′-CH_2). **1b**, R_f 0.19 (4:1 MeCN/H_2O). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.53 (s, 1H, 8-CH), 6.74 (s, 2H, 2-NH_2, D_2O exchangeable), 5.06 (brs, 1H, OH, D_2O exchangeable), 4.30 (brs, 2H, 1′-CH_2), 4.13 (dd, 2H, 4′-CH_2, J = 47.06 Hz), 3.35 (d, 2H, 5′-CH_2, J = 52.94 Hz), 2.05–2.32 (m, 2H, 2′-CH_2), 1.59–1.74 (m, 1H, 3′-CH).