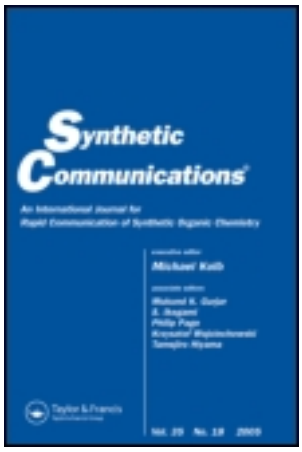

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[a] Department of Radiology, Indiana University School of Medicine, 1345 West 16th Street, L-3 Room 202, Indianapolis, Indiana, 46202-2111, USA

[b] Department of Urology, Indiana University School of Medicine, Indianapolis, Indiana, USA

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An Improved Total Synthesis of PET HSV-tk Gene Expression Imaging Agent 9-[(3-[^{18}F]Fluoro-1-hydroxy-2-propoxy)methyl]guanine ([^{18}F]FHPG)

Ji-Quan Wang,[1] Qi-Huang Zheng,[1,*] Xiangshu Fei,[1] Xuan Liu,[1] Thomas A. Gardner,[2] Chinghai Kao,[2] Sudhanshu P. Raikwar,[2] Barbara E. Glick-Wilson,[1] Michael L. Sullivan,[1] Bruce H. Mock,[1] and Gary D. Hutchins[1]

Departments of [1]Radiology and [2]Urology, Indiana University School of Medicine, Indianapolis, Indiana, USA

ABSTRACT

An improved total synthesis of [^{18}F]FHPG starting from 1,3-dibenzyloxy-2-propanol and guanine has been developed. [^{18}F]FHPG was prepared by nucleophilic substitution of the appropriate precursor with [^{18}F]KF/Kryptofix 2.2.2 followed by a quick deprotection reaction and purification with a simplified Silica Sep-Pak solid-phase extraction (SPE) method in 10–15% radiochemical yield, and 70 min synthesis time from end of bombardment (EOB).

*Correspondence: Qi-Huang Zheng, Ph.D., Department of Radiology, Indiana University School of Medicine, 1345 West 16th Street, L-3 Room 202, Indianapolis, IN 46202-2111, USA; Fax: (317) 278-9711; E-mail: qzheng@iupui.edu.

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Key Words: 9-[(3-[^{18}F]Fluoro-1-hydroxy-2-propoxy)methyl]guanine ([^{18}F]FHPG); Ganciclovir (GCV); Synthesis; Positron emission tomography (PET); Herpes simplex virus thymidine kinase (HSV-tk); Gene expression.

INTRODUCTION

Radiolabeled ganciclovir (GCV, 9-[(1,3-dihydroxy-2-propoxy)methyl]-guanine) and penciclovir (PCV, 9-[4-hydroxy-3(hydroxymethyl)butyl]guanine) analogs such as 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) (Fig. 1) have shown great potential as positron emission tomography (PET) imaging agents to detect herpes simplex virus thymidine kinase (HSV-tk) gene expression.[1–16] Considerable efforts have been devoted to the synthesis of these gene reporter probes and numerous improved synthesis were reported in the literature,[11,13,17–19] in which [^{18}F]FGCV and [^{18}F]FPCV were labeled with fluorine-18 at 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.

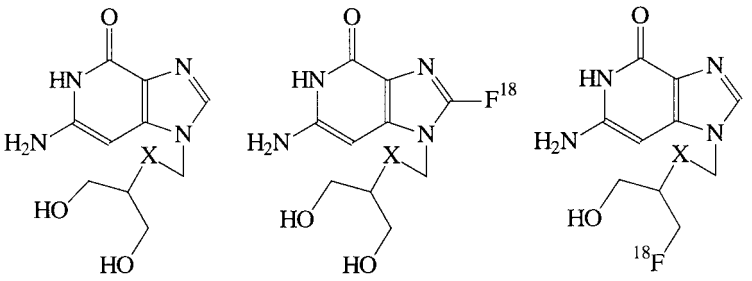

Figure 1. Chemical structures of GCV, PCV, [^{18}F]FGCV, [^{18}F]FPCV, [^{18}F]FHPG, [^{18}F]FHBG.

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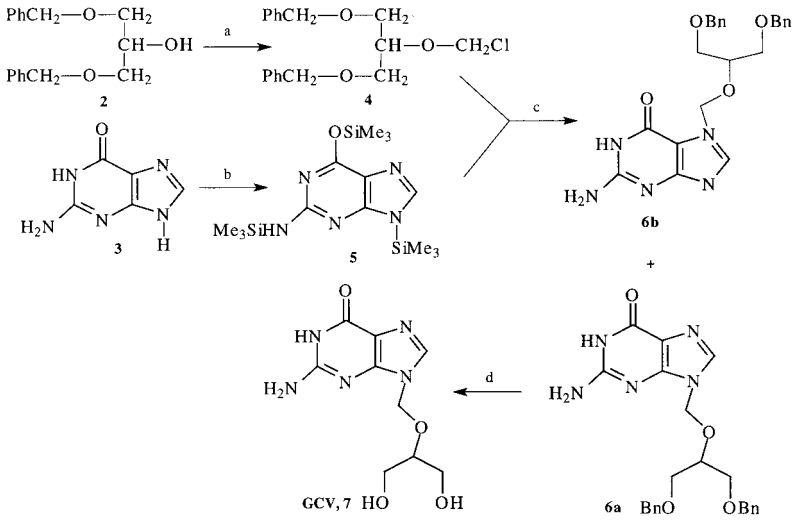
Wishing to study these compounds in this laboratory, we decided to make our own material by following the literature methods. Although several papers dealing with the synthesis of [^{18}F]FHPG from GCV have appeared, there are gaps in synthetic detail among them, and certain key steps gave poor yields or were difficult to repeat in our hands. Consequently, we investigated alternate approaches and modifications that eventually resulted in an improved total synthesis of [^{18}F]FHPG starting from very beginning materials 1,3-dibenzyloxy-2-propanol and guanine that was superior to previous works or addressed more synthetic details to reveal and explain technical tricks. In this paper we provide full experiment procedures, yields, analytical details and new findings for this improved [^{18}F]FHPG total synthesis, as well as key intermediate GCV and tosylated precursor.

RESULTS AND DISCUSSION

Synthesis of Ganciclovir

The synthesis of ganciclovir (GCV) as indicated in Sch. 1 was performed with the modifications according to procedures reported in the literature.[20,21]

The commercially available starting material 1,3-dibenzyloxy-2-propanol (**2**) was converted into its chloromethyl ether 1,3-dibenzyloxy-2-chloro-



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

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methoxypropane (**4**) through a chloromethylation reaction. Compound **4** was easy to decompose on silica gel column. Therefore, the purification of crude product **4** is impossible and unnecessary. The staring material guanine (**3**) was reacted with 1,1,1,3,3,3-hexamethyldisilazane (HMDS) to provide guanine derivative 2,6,9-tris(trimethylsilyl)guanine (**5**). The reaction went very slow, which took 4 days. The indication of reaction completion was that the initial suspension turned to be a clear solution. Compound **5** was sensitive to moisture, and it should be used for next step reaction immediately after removal of volatiles. The coupling reaction between **4** and **5** gave 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)[20] or tetrabutylammonium iodide (TBAI).[21] 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[20] 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 9-isomer from its mixture with 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, **7**. The reaction was catalyzed by either palladium chloride ($PdCl_2$)[20] or palladium black.[21] However, we found with $PdCl_2$[20] the chain of the reaction product GCV was cleaved; with 1 : 1 palladium black/**6a**[21] 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, which gave deprotective GCV, **7** in a yield of 87.7%.

The overall chemical yield of GCV from **2** and **3** was 15.6%.

Synthesis of FHPG (1) and Radiosynthesis of [^{18}F]FHPG (1)

The syntheses of standard sample 9-[(3-fluoro-1-hydroxy-2-propoxy)-methyl]guanine (FHPG, **1**) and target tracer [^{18}F]FHPG (**1**) as indicated in Sch. 2 were performed with the modifications according to procedures reported in the literature.[13,18,19,22]

The protection of the 2-amino group and one of the two hydroxyl groups of GCV, **7** was furnished by reacting with monomethoxytrityl chloride in the presence of 4-dimethylaminopyridine (DMAP) and triethylamine.[22] The

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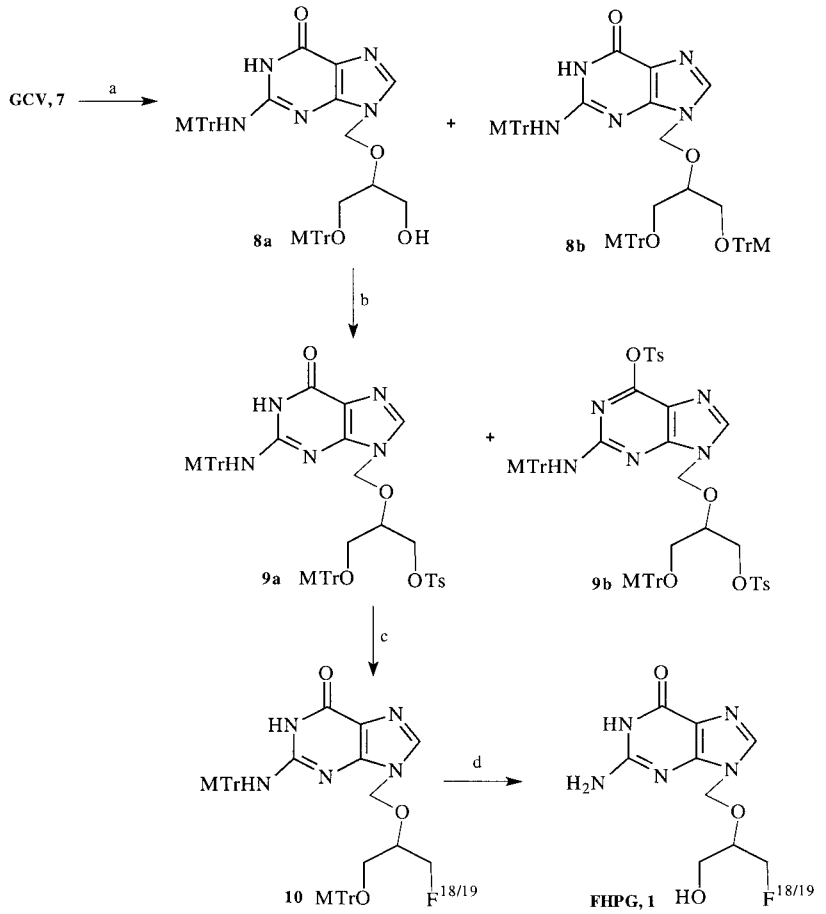

Scheme 2. Synthesis of FHPG and [^{18}F]FHPG. a. MTrCl, DMAP, Et_3N, DMF; b. TsCl, pyridine; c. K[$^{18/19}$F], Kryptofix 2.2.2, CH_3CN; d. 1 N HCl, MeOH.

bulky methoxytrityl group favored the desired product N^2-(*P*-anisyldiphenylmethyl)-9-[[1-(*P*-anisyldiphenylmethoxy)-3-hydroxy-2-propoxy]methyl]guanine (**8a**) in 36.2% chemical yield, 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**) is not avoidable, in 20.7% chemical yield. Compound **8a** reacted with *p*-tosyl chloride at room temperature afforded the desired precursor tosylate N^1-(*p*-anisyldiphenylmethyl)-9-[((1-anisyldiphenylmethoxy)-3-tosyl-2-propoxy)methyl]guanine (**9a**) in a

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yield of 50.8%, together with the undesired by-product 1-tosyl-N^2-(*p*-anisyldiphenylmethyl)-9-[((1-anisyldiphenylmethoxy)-3-tosyl-2-propoxy)-methyl]guanine (**9b**) in a yield of 17.5%. The tosylate **9a** was purified by recrystallization from ethanol in the literature.[22] We found it was convenient to perform flash column chromatography to produce pure and reliable precursor **9a**. The fluorination of the tosylate **9a** 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 (**10**) in a yield of 29.7%. This intermediate **10** was easily hydrolyzed by 1 N HCl aqueous solution to give the unlabeled standard sample FHPG, **1** in a yield of 87.3%.

The overall chemical yield of the tosylate precursor **9a** from GCV was 18.4%, and the overall chemical yield of the standard sample FHPG, **1** from GCV was 4.8%.

[^{18}F]FHPG (**1**) was synthesized by a modification of the procedures as reported in the literature.[13,18,19] The tosylated precursor **9a** was labeled by a conventional nucleophilic substitution with K[^{18}F]/Kryptofix 2.2.2 in CH_3CN at 120°C for 20 min to provide a radiolabeling intermediate **10**. The radiolabeling reaction was monitored by analytical radio-HPLC method, in which we employed a new HPLC system[23,24] by using a Prodigy (Phenomenex) 5 μm C-18 column, 4.6 × 250 mm; 3 : 1 : 1 CH_3CH: MeOH: 20 mM, pH 6.7 $KHPO_4^-$ 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**10** = 15.00 min, RTK[^{18}F] = 1.88 min. The radiolabeling mixture containing the intermediate **10** was passed through a Silica Sep-Pak to remove Kryptofix 2.2.2 and non-reacted K[^{18}F]. The large polarity difference between **10** and Kryptofix 2.2.2 and non-reacted K[^{18}F] permitted the use of a simple solid-phase extraction (SPE) technique[23–25] for fast isolation of **10** 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 residue **10**. The existence of the catalyst Kryptofix 2.2.2 and non-reacted K[^{18}F] would affect the deprotection reaction of **10**; therefore, they needed to be removed before **10** was deprotected to give the target labeled product **1**. The residue **10** was followed a quick deprotection with 1 N HCl for 10 min and neutralized with 6 N NaOH to provide **1**. 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.[19] 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 **1** and radioactive by-product permitted the use of SPE technique for fast purification of radiotracer **1** from radiolabeling mixture.

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The radiochemically pure compound **1** 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 **1** was 10–15%, and the synthesis time was ~70 min from 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**1** = 2.02 min. The chemical purities of precursor **9a** and standard sample **1** were >95%, the radiochemical purity of target radiotracer **1** was >99%, and the chemical purity of radiotracer **1** was ~93%. The average (n = 3–5) specific radioactivity of radiotracer **1** was 0.8–1.2 Ci/μmol at EOS.

In comparison with the results reported in the literature,[13,18,19,22] several improvements in the synthetic methodology for FHPG and [^{18}F]FHPG have been made. They include increased radiochemical yield and specific activity, enhanced radiochemical purity, shortened synthesis time, new Sep-Pak techniques for fast and efficient preparative separation of [^{18}F]FHPG from precursors, and new HPLC systems for the quality control (QC) method of target tracer [^{18}F]FHPG.

Compounds **6b**, **8b**, and **9b** are new compounds.

In conclusion, an improved total synthesis of [^{18}F]FHPG has been developed. Several improvements and new findings in the synthetic methodology, radiolabeling, preparative separation and analytical details for GCV, FHPG, precursor and [^{18}F]FHPG have been made and addressed. This improved method is 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 analogs for PET imaging of HSV-tk gene expression.

EXPERIMENTAL SECTION

All commercial reagents and solvents were used without further purification unless otherwise specified. Tetrahydrofuran (THF) solvent was distilled from $LiAlH_4$ immediately prior to use. Melting points were determined on a MEL-TEMP II capillary tube apparatus and were uncorrected. ^{1}H NMR spectra were recorded on a Bruker QE 300 NMR spectrometer using tetramethylsilane (TMS)as an internal standard. Chemical shift data for the proton resonances were reported in parts per million (δ) relative to internal standard TMS (δ 0.0). The low resolution mass spectra were obtained using a Bruker Biflex III MALDI-Tof mass spectrometer, and the high resolution mass measurements were obtained using a Kratos MS80 mass spectrometer, in the Department of Chemistry at Indiana University. Chromatographic solvent

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proportions are expressed on a volume : volume basis. Thin layer chromatography was run using Analtech silica gel GF uniplates ($5 \times 10\,cm^2$). Plates were visualized by UV light. Normal phase flash chromatography was carried out on EM Science silica gel 60 (230–400 mesh) with a forced flow of the indicated solvent system in the proportions described below. All moisture-sensitive reactions were performed under a positive pressure of nitrogen maintained by a direct line from a nitrogen source.

Analytical HPLC was performed using a Prodigy (Phenomenex) 5 μm C-18 column, 4.6 × 250 mm; 3 : 1 : 1 CH_3CN: MeOH: 20 mM, pH 6.7 $KHPO_4^-$ mobile phase, 1.5 mL/min flow rate, and UV (240 nm) and γ-ray (NaI) flow detectors. Semi-preparative C-18 SiO_2 Sep-Pak type cartridge was obtained from Waters Corporate Headquarters, Milford, MA. Sterile vented Millex-GS 0.22 μm vented filter unit was obtained from Millipore Corporation, Bedford, MA.

1,3-Dibenzyloxy-2-chloromethoxypropane (4)

The mixture of 1,3-dibenzyloxy-2-propanol **2** (9.1 mL, 36.7 mmol) and paraformaldehyde (2.39 g, 79.6 mmol) in anhydrous 1,2-dichloroethane (100 mL) was cooled to 0°C. Dry HCl gas was bubbled through the suspension at 0°C for 6 h. Anhydrous Na_2SO_4 was added to the mixture. After stirring the solvent was removed under vacuum to give a viscous liquid residue **4** (∼100%). The crude product was used for the next step reaction without further purification.

2,6,9-Tris(trimethylsilyl)guanine (5)

The mixture of guanine **3** (5.50 g, 36.4 mmol), ammonium sulfate (0.70 g, 132.1 mmol) and 1,1,1,3,3,3-hexamethyldisilazane (250 mL) was refluxed under nitrogen for 4 days until it became clear. Volatiles were removed under reduced pressure to give viscous syrup **5** (∼100%). The crude product was used for the next step reaction without further purification.

9-[(1,3-Dibenzyloxy-2-propoxy)methyl]guanine (6a) and 7-[(1,3-Dibenzyloxy-2-propoxy)methyl]guanine (6b)

Compounds **2** and **4** as obtained above were combined through the aid of dry THF (200 mL). The mixture was stirred at room temperature (r.t.). Tetrabutylammonium fluoride (TBAF, 0.40 g) dried by azeotroping

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distillation in anhydrous benzene (40 mL) was added to the mixture. The resulting solution was then refluxed for 22 h. After removal of solvent, the yellowish residue was transferred to a separation funnel with the aid of ethyl acetate, and then more ethyl acetate and water were added. Large amount of yellowish precipitate formed when shaking. The solid was filtered, washed with water and ethyl acetate, and dried under vacuum. The organic layer of the filtrate was washed twice with water. After removal of solvent the yellow viscous residue was subject to column chromatography on silica gel eluted with 9 : 1 EtOAc/MeOH to give a yellow solid. Both of solids were a mixture (9.33 g, 58.9%) of undesired by-product 7-isomer **6b** and desired 9-isomer **6a**, which could not be separated by TLC and gave the same R_f value ($R_f = 0.29$, 9 : 1 EtOAc/MeOH) on TLC. The NMR spectrum showed the ratio of **6a**/**6b** was about 2 : 1. They were separated and purified by recrystallization in ethanol. Crude product (~7.0 g) was stirred with ethyl acetate (200 mL) at r.t., and then filtered. The solid was transferred to a 1000 mL flask containing ethanol (500 mL). The suspension was heated to reflux, and filtered through a frit (fine). The clear yellowish solution was left at 4°C overnight to give the first precipitate that was mostly 7-isomer **6b**. ^{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. The filtrate was then left at −16°C overnight to give the second precipitate that was pure 9-isomer **6a**. The solution was concentrated, and left at −16°C to give third, forth and more portions of pure 9-isomer **6a**. The purity was monitored by NMR spectrum. The pure product **6a** (2.81 g, 17.8%) was obtained. **6a**, ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.63 (s, 1H, 1-NH), 7.81 (s, 1H, 8-CH), 7.15–7.35 (m, 10H, Ph), 6.49 (s, 2H, 2-NH_2), 5.45 (s, 2H, 1′-CH_2), 4.40 (s, 4H, PhCH_2), 4.02 (q, 1H, 3′-CH, $J = 4.78$ Hz), 3.30–3.50 (m, 4H, 4′-CH_2).

9-[(1,3-Dihydroxy-2-propoxy)methyl]guanine (Ganciclovir, GCV, 7)

Compound **6a** (0.50 g, 1.15 mmol) was dissolved in hot ethanol (30 mL), and then palladium black (1.50 g) and cyclohexene (20 mL) were added. The mixture was refluxed under nitrogen for 24 h until TLC showed starting material was gone. The catalyst was filtered through celite while the mixture was hot, washed with ethanol and hot DMF until the filtrate on TLC plate

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showed no spot under UV. The ethanol and DMF filtrates were evaporated to dryness separately. The solid residues were combined with 1 : 1 EtOH/H_2O, heated to dissolve, and filtered through celite topped with activate charcoal. After removal of solvents the colorless solid was recrystallized with 4 : 1 EtOH/H_2O to afford a white crystal solid **7** (0.26 g, 87.7%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.63 (s, 1H, 1-NH, D_2O exchangeable), 7.79 (s, 1H, 8-CH), 6.48 (s, 2H, 2-NH_2, D_2O exchangeable), 5.42 (s, 2H, 1′-CH_2), 4.60 (t, 2H, OH, $J = 5.15$ Hz, D_2O exchangeable), 3.20–3.65 (m, 5H, 3′-CH and 4′-CH_2).

N^2-(*p*-Anisyldiphenylmethyl)-9-[[1-(*p*-anisyldiphenylmethoxy)-3-hydroxy-2-propoxy]methyl]guanine (8a) and N^2-(*p*-Anisyldiphenylmethyl)-9-[[1,3-*bis*(*p*-anisyldiphenylmethoxy)-2-propoxy]methyl]guanine (8b)

GCV 7 (1.00 g, 3.98 mmol), monomethoxytrityl chloride (2.65 g, 8.58 mmol), 4-dimethylaminopyridine (DMAP, 0.030 g, 0.25 mmol), and triethylamine (2.5 mL) were dissolved in dry DMF (50 mL). The mixture was stirred at 55°C for 6 h. Methanol (10 mL) was added to quench the reaction, and solvents were evaporated. The residue was transferred to a separation funnel through the aid of ethyl acetate, washed with aqueous $NaHCO_3$ solution and water, dried over $MgSO_4$. After evaporation the resulting solid was dissolved in 4% MeOH/CH_2Cl_2, absorbed by a small amount of silica gel, and dried under vacuum. This silica gel was then transferred to the top of a column, and eluted with 2.5–3.0% MeOH/CH_2Cl_2. Two components were collected. The first component was undesired by-product **8b** as a white solid (0.87 g, 20.7%), 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. The second component was the desired product **8a** as a white solid (1.13 g, 36.2%), $R_f = 0.15$ (5% MeOH/CH_2Cl_2). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.67 (s, 1H, 1-NH, D_2O exchangeable), 7.78 (s, 1H, 8-CH), 7.68 (s, 1H, 2-NH, D_2O exchangeable), 6.66–7.33 (m, 28H, Ph), 4.97 (dd, 2H, 1′-CH_2, $J_1 = 36.03$ Hz, $J_2 = 11.03$ Hz), 4.44 (t, 1H, OH, $J = 5.14$ Hz, D_2O exchangeable), 3.74 (s, 3H, OCH_3), 3.56 (s, 3H, OCH_3), 3.40–3.50 (m, 1H, 3′-CH), 2.80–3.07 (m, 2H, 5′-CH_2), 2.51–2.80 (m, 2H, 4′-CH_2).

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N^2-(*p*-Anisyldiphenylmethyl)-9-[((1-anisyldiphenylmethoxy)-3-tosyl-2-propoxy)methyl]guanine (9a) and 1-Tosyl-N^2-(*p*-anisyldiphenylmethyl)-9-[((1-anisyldiphenylmethoxy)-3-tosyl-2-propoxy)methyl]guanine (9b)

Compound **8a** (0.28 g, 0.35 mmol) and *p*-tosyl chloride (0.26 g, 1.35 mmol) were dissolved in dry pyridine (5 mL). The mixture was stirred at r.t. for 2 days. To this brown solution was added water (1 mL) to quench the reaction, and then solvents were evaporated. The residue was dissolved in ethyl acetate, washed with water, dried over Na_2SO_4. After removal of solvent the solid was dissolved in a small volume of CH_2Cl_2, and transferred to a silica gel column, then eluted with 1% $MeOH/CH_2Cl_2$ to afford undesired by-product **9b** as a light brown solid (0.067 g, 17.5%), 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. Then the column was eluted with 3% $MeOH/CH_2Cl_2$ to give the desired product **9a** as a light brown solid (0.17 g, 50.8%), $R_f = 0.15$ (3% $MeOH/CH_2Cl_2$). ^{1}H NMR (300 MHZ, DMSO-d_6): δ 10.67 (s, 1H, 1-NH), 7.77 (s, 1H, 8-CH), 7.73 (s, 1H, 2-NH), 6.62–7.67 (m, 32H, Ph), 4.93 (dd, 2H, 1′-CH_2, $J_1 = 59.56$ Hz, $J_2 = 11.76$ Hz), 3.75 (s, 3H, OCH_3), 3.37–3.64 (m, 3H, 3′-CH and 4′-CH_2), 3.54 (s, 3H, OCH_3), 2.37–2.67 (m, 2H, 5′-CH_2), 2.43 (s, 3H, CH_3).

N^2-(*p*-Anisyldiphenylmethyl)-9-[[1-(*p*-anisyldiphenylmethyl)-3-fluoro-2-propoxy]methyl]guanine (10)

Tosylate **9a** (0.10 g, 0.11 mmol), potassium fluoride (0.14 g, 2.41 mmol) and Kryptofix 2.2.2. (0.24 g, 0.64 mmol) were dissolved in dry acetonitrile (8 mL). The mixture was heated to 115–120°C for 40 min. The solvent was removed, and the residue was transferred to the top of a silica gel column with the aid of CH_2Cl_2. The column was eluted with 2% $MeOH/CH_2Cl_2$ to give the desired compound **10** as a light brown solid (0.025 g, 29.7%), $R_f = 0.25$ (5% $MeOH/CH_2Cl_2$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.64 (brs, 1H, 1-NH), 7.80 (brs, 1H, 2-NH), 6.52–7.40 (m, 28H, Ph), 4.97 (s, 2H, 1′-CH_2), 3.89–4.29 (m, 2H, 5′-CH_2), 3.74 (s, 3H, OCH_3), 3.58 (s, 3H, OCH_3), 3.42–3.66 (m, 1H, 3′-CH), 2.73–2.93 (m, 2H, 4′-CH_2).

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9-[(3-Fluoro-1-hydroxy-2-propoxy)methyl]guanine (FHPG, 1)

The solution of compound **10** (0.025 g, 0.031 mmol) in methanol (2.5 mL) and 1 N aqueous HCl (0.5 mL) was refluxed for 15 min while TLC showed no starting material left. The solution was neutralized with 1 N aqueous NaOH to pH ~7–8. Silica gel was added to absorb the solution, and then the mixture was made to dry under vacuum and transferred to the top of a column. The column was eluted with 20 : 1 $MeCN/H_2O$ to give the target compound FHPG **1** as a light brown solid (7.0 mg, 87.3%), $R_f = 0.34$ (12 : 1 $MeCN/H_2O$). ^1H 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).

9-[(3-[^{18}F]Fluoro-1-hydroxy-2-propoxy)methyl]guanine ([^{18}F]FHPG, 1)

No-carrier-added (NCA) aqueous H[^{18}F] (0.5 mL) prepared by $^{18}O(p,n)^{18}F$ nuclear reaction in a RDS-112 cyclotron on an enriched $H_2^{18}O$ water (95 + %) target was added to a Pyrex vessel which contains K_2CO_3 (4 mg, in 0.2 mL H_2O) and Kryptofix 2.2.2 (10 mg, in 0.5 mL CH_3CN). Azeotropic distillation at 115°C with HPLC grade CH_3CN (3 × 1 mL) under a nitrogen steam efficiently removed the target H_2O. The tosylated precursor **9a** (2–3 mg, dissolved in 0.5 mL CH_3CN) was introduced to the anhydrous potassium [^{18}F]fluoride-Kryptofix 2.2.2 complex. The reaction mixture was sealed and heated at 120°C for 20 min and was subsequently allowed to cool down, at which time the crude product was passed through a Silica Sep-Pak cartridge to remove Kryptofix 2.2.2 and non-reacted K[^{18}F]. The Sep-Pak was eluted with 15% $MeOH/CH_2Cl_2$ (3.5 mL), and then passed onto a rotatory evaporator. The solvent was removed by evaporation under high vacuum. The residue was acidified with 1 N HCl (0.6 mL) and heated for 10 min at 80°C. The contents were neutralized with 6 N NaOH (0.1 mL), diluted with ethanol (3 mL), and evaporated in vacuo. The crude product was passed through they second Silica Sep-Pak cartridge through the aid of ethanol. The Sep-Pak was eluted with EtOH to remove radioactive by-product. The radiochemically pure product [^{18}F]FHPG, **1** was eluted from the Sep-Pak with 90 : 8 : 2 H_2O/EtOH/HOAc and adjusted pH to 5.5–7.0 with 2 M NaOH and 150 mM NaH_2PO_4 mixed solution, whose volume was dependent upon the use of the labeled product in tissue biodistribution studies (~3 mL, 3 × 1 mL) or in micro-PET imaging studies (1.5 mL, 3 × 0.5 mL) of HSV-tk prostate cancer tumors in athymic mice, and sterile-filtered through a 0.22 μm cellulose acetate

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membrane and collected into a sterile vial. The radiochemical yield of [^{18}F]FHPG was 10–15%, and the synthesis time was ~70 min from end of bombardment (EOB). Retention times in the analytical HPLC system were: RT**9a** = 14.03 min, RT**1** = 2.02 min. The chemical purities of precursor **9a** and standard sample **1** were >95%, the radiochemical purity of target radiotracer **1** was >99%, and the chemical purity of radiotracer **1** was ~93%. The average (n = 3–5) specific radioactivity of radiotracer **1** was 0.8–1.2 Ci/μmol at end-of-synthesis (EOS).

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