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Synthesis of 2-amino-6-(4-[^{11}C]methoxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine bis(2,2,2-trifluoroethyl) ester as a novel potential PET gene reporter probe for HBV and HSV-tk in cancers

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Abstract—Acyclic nucleoside 2-amino-6-(4-methoxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine bis(2,2,2-trifluoroethyl) ester (ABE, **1**) is a new hepatitis B virus (HBV) specific antiviral reagent and shows high anti-HBV activity. Carbon-11 labeled ABE may serve as a novel reporter probe for positron emission tomography (PET) to image HBV and herpes simplex virus thymidine kinase (HSV-tk) in cancers. The radiolabeling precursors 2-amino-6-(4-hydroxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine bis(2,2,2-trifluoroethyl) ester (**10**) and 2-*N*-Boc protected analogue 2-*N*-bis(Boc)amino-6-(4-hydroxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine bis(2,2,2-trifluoroethyl) ester (**12**), and the reference standard ABE were synthesized from bis(trifluoroethyl) (2-iodoethoxy)methylphosphonate (**5**), guanine (**6**), and 2-amino-6-chloropurine (**8**). The target radiotracer 2-amino-6-(4-[^{11}C]methoxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine bis(2,2,2-trifluoroethyl) ester ([^{11}C]ABE, [^{11}C]**1**) was prepared by *O*-[^{11}C]methylation of the unprotected HO-precursor **10**, or 2-*N*-Boc protected HO-precursor **12** with [^{11}C]methyl triflate followed by a quick deprotection reaction, and isolated by solid-phase extraction (SPE) purification in 40–55% radiochemical yields.

1. Introduction

Hepatitis B virus (HBV) infection is responsible for both acute and chronic hepatitis. Chronic HBV infection dramatically increases risks for development of liver cancer and cirrhosis.[1–3] The World Health Organization (WHO) estimates about 400 million chronic carriers worldwide, with roughly 4 million deaths annually from the resulting cirrhosis and hepatocellular carcinoma. Treatment of HBV infection constitutes one of the therapeutic challenges in virology, and only a few drugs are currently available for the clinical treatment of HBV such as interferon α and lamivudine.[4] Several nucleoside analogues like 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA) and its analogues,[5–8] ganciclovir (GCV), penciclovir (PCV), and 5-substituted analogue of thymidine 1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-iodouracil (FIAU) have also been investigated as potent chemotherapeutic agents against viruses, for example, HBV and human immunodeficiency virus (HIV), and certain forms of cancer.[1–3] Radiolabeled 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 have been synthesized as gene reporter probes for biomedical imaging techniques positron emission tomography (PET) or single photon emission computed tomography (SPECT) to image herpes simplex virus thymidine kinase (HSV-tk) gene expression,[9–22] and we have also developed new carbon-11 labeled GCV and PCV analogues 8-[^{11}C]methoxyganciclovir ([^{11}C]MeOGCV) and

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8-[^{11}C]methoxypenciclovir ([^{11}C]MeOPCV) as novel HSV-tk gene reporter probes (Fig. 1).[23,24] 2-Amino-6-(4-methoxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]-purine bis(2,2,2-trifluoroethyl) ester (ABE, **1**) is a novel HBV-specific antiviral reagent and shows high anti-HBV activity in vitro, and its active metabolite was highly detected in the liver.[4] Compound ABE might be suitable for hepatitis B chemotherapy. Carbon-11 labeled antiviral nucleoside analogue 2-amino-6-(4-[^{11}C]methoxyphenylthio)-9-[2-(phosphonomethoxy)-ethyl]purine bis(2,2,2-trifluoroethyl) ester ([^{11}C]ABE, [^{11}C]**1**) may serve as a novel reporter probe for PET to image HBV and HSV-tk in cancers. In this study, we report the synthesis of [^{11}C]ABE, for the first time.

2. Results and discussion

2.1. Chemistry

The synthesis of the unprotected HO-precursor 2-amino-6-(4-hydroxyphenylthio)-9-[2-(phosphonomethoxy)-ethyl]purine bis(2,2,2-trifluoroethyl) ester (**10**) and reference standard ABE, **1** as indicated in Scheme 1 was performed with the modifications according to procedures reported in the literature.[4] 2-Chloroethyl chloromethyl ether (**2**) was treated with tris(2,2,2-trifluoroethyl)phosphite (**3**) to quantitatively give bis(trifluoroethyl) (2-chloroethoxy)methylphosphonate (**4**). Compound **4** was converted to bis(trifluoroethyl) (2-iodoethoxy)methylphosphonate (**5**) with sodium iodide through halogen exchange reaction in 80% yield. Following the procedures reported in the literature,[25] guanine (**6**) was treated first with trifluoroacetic anhydride and then with 4-methoxybenzenethiol and eventually with ethanolic methylamine to afford 2-amino-6-[(4-methoxyphenyl)sulfanyl]purine (**7**) in 66% yield. Compound **7** was treated first with 1,8-diazabicyclo-[5,4,0]undec-7-ene (DBU) and then with compound **5** to yield the reference standard **1** in 42% yield. 2-Amino-6-chloropurine (**8**) was treated first with DBU and then with compound **5** to provide desired 9-isomer N^9-substituted product 2-amino-6-chloro-9-[2-(phosphonomethoxy)ethyl]purine bis(2,2,2-trifluoroethyl) ester (**9**) as the major product in 33% yield, and undesired 7-isomer N^7-substituted product as the minor byproduct.[18–20] Compound **9** was then reacted with 4-hydroxythiophenol to give the unprotected precursor **10** in 55% yield.

The synthesis of compound **1** has been reported in the reference,[4] in which it was synthesized from compound **9** with 4-methoxybenzenethiol. In this paper, an improved synthetic approach[25] through the reaction of compound **7** with compound **5** was used for the synthesis of compound **1**. The purpose of this modification is to increase the yield of compound **1** through the avoidance of the formation and separation of undesired N^7-substituted product, the 7-isomer of compound **9**. However, the improved method[25] did not work well for the synthesis of compound **10**, since the hydroxyl group in 4-hydroxythiophenol will be also reacted with compound **5**, therefore, the reported method[4] through compound **9** appears to be the only approach to prepare compound **10**.

The 2-amino group in purine ring may affect the *O*-[^{11}C]methylation of the unprotected HO-precursor **10**, therefore, we also designed and synthesized a 2-*N*-Boc protected HO-precursor 2-*N*-bis(Boc)amino-6-(4-hydroxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]-purine bis(2,2,2-trifluoroethyl) ester (**12**).[26,27] The synthesis of the 2-*N*-Boc protected precursor **12** as outlined in Scheme 2 was performed with the modifications according to procedures indicated in the Scheme 1. Compound **9** was reacted with *t*-butoxycarbonyl anhydride (Boc_2O) to afford 2-*N*-bis(Boc)amino-6-chloro-9-[2-(phosphonomethoxy)ethyl]purine bis(2,2,2-trifluoroethyl) ester (**11**) in 77% yield. Compound **11**

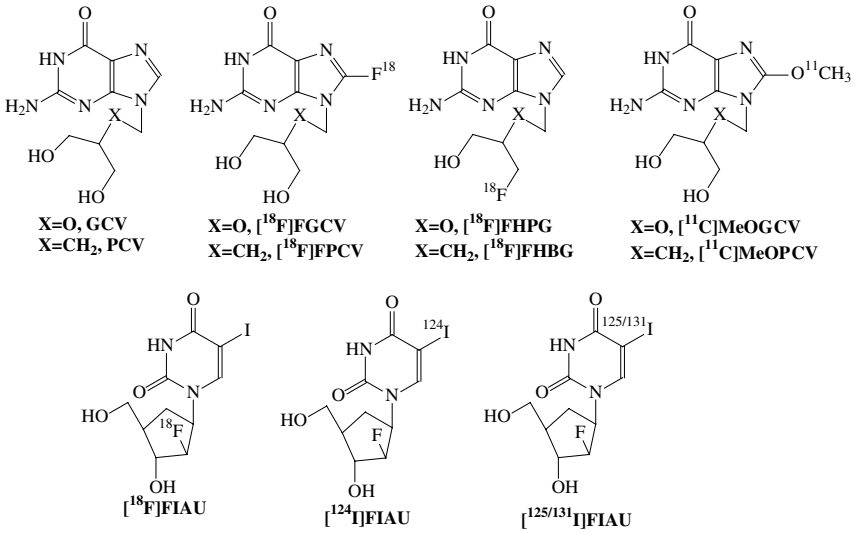
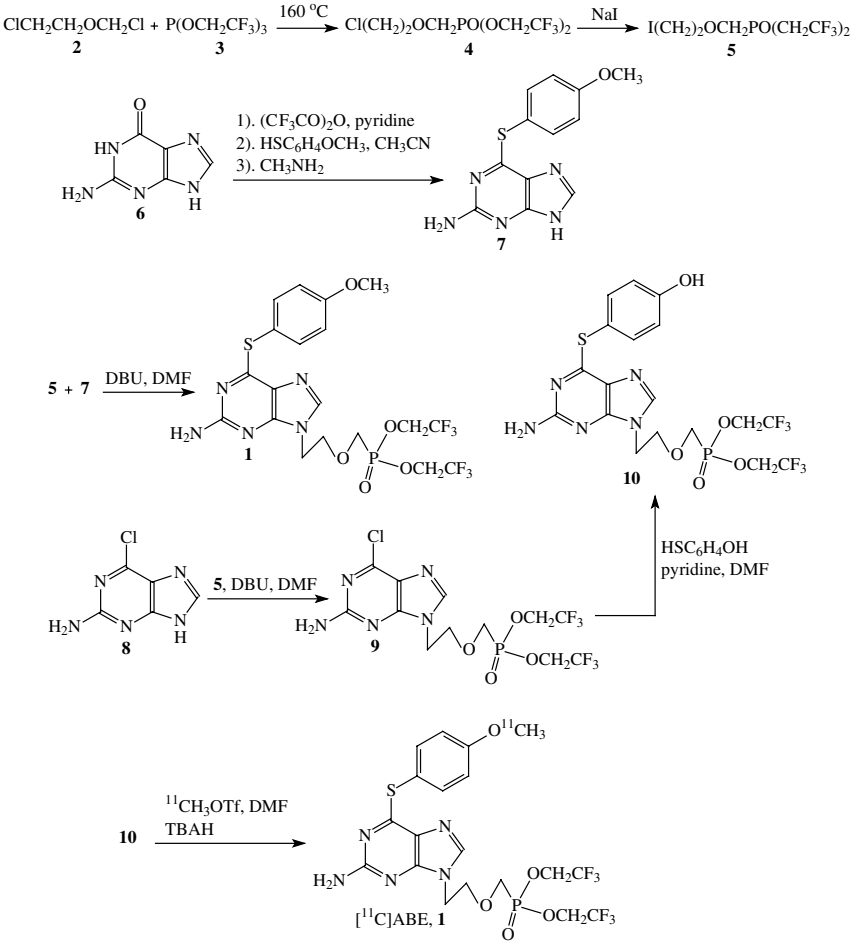


Figure 1. Chemical structures of GCV, PCV; [^{18}F]FGCV, [^{18}F]FPCV; [^{18}F]FHPG, [^{18}F]FHBG; [^{11}C]MeOGCV, [^{11}C]MeOPCV; [^{18}F]FIAU, [^{124}I]FIAU, and [$^{125/131}$I]FIAU.



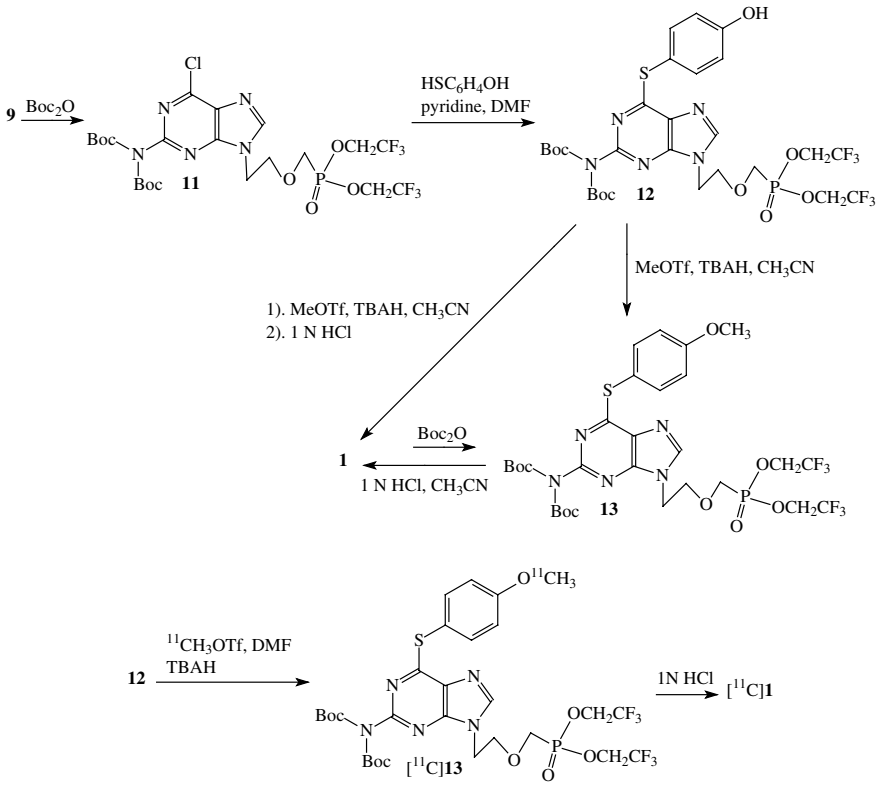
Scheme 1. Synthesis of 2-amino-6-(4-[^{11}C]methoxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine bis(2,2,2-trifluoroethyl) ester ([^{11}C]ABE, [^{11}C]**1**) using unprotected HO-precursor **10**.

was then reacted with 4-hydroxythiophenol to give the 2-*N*-Boc protected precursor **12** in 31% yield. The direct methylation of compound **12** with methyl triflate (CH_3OTf) gave the *O*-methylated product 2-*N*-bis(Boc)-amino-6-(4-methoxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine bis(2,2,2-trifluoroethyl) ester (**13**) in very low yield, in which compound **13** will serve as a reference intermediate for the radiolabeling of 2-*N*-Boc protected precursor **12**. Thus, the small-scale synthesis of compound **13** was carried out, in which the reference standard **1** was directly reacted with Boc_2O to provide compound **13** in 45% yield. The deprotection of compound **13** with 1 N HCl in acetonitrile gave the reference standard **1**. The methylation of 2-*N*-Boc protected precursor **12** with CH_3OTf under basic conditions using tetrabutylammonium hydroxide (TBAH) followed by a quick deprotection with 1 N HCl to remove the 2-*N*-Boc groups also gave the reference standard **1**.

2-Amino-6-(3-methoxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine bis(2,2,2-trifluoroethyl) ester (*meta*-ABE, IC_{50} 0.04 μM) and 2-amino-6-(2-methoxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine bis(2,2,2-trifluoroethyl) ester (*ortho*-ABE, IC_{50} 0.08 μM) have similar in vitro anti-HBV activity to the target compound **1** (*para*-ABE, IC_{50} 0.03 μM).[4] The potentiation effect order of 6-arylthio analogues is *para* > *meta* > *ortho*, which is consistent with the potentiation effect order of O^6-benzylguanine (O^6-BG) analogues,[28–31] most likely because the steric effect at the *ortho*-position plays a more important role than the electronic effect. Therefore, *para*-ABE was designed as the target compound for radiolabeling.

2.2. Radiochemistry

The synthesis of the target tracer [^{11}C]ABE, [^{11}C]**1** using both unprotected precursor **10** and protected precursor **12** are outlined in Schemes 1 and 2. The unprotected HO-precursor **10** was labeled by [^{11}C]methyl triflate ($^{11}CH_3OTf$)[32,33] through *O*-[^{11}C]methylation of hydroxyphenyl position under basic conditions using TBAH.[34–38] The tracer was isolated by solid-phase extraction (SPE) purification[34,39,40] to produce pure target compound radiolabeled [^{11}C]**1** with 50–55% radiochemical yields, based on $^{11}CO_2$, decay corrected to end of bombardment (EOB). The large polarity difference between the HO-precursor and the labeled methylated product permitted the use of SPE technique for purification of labeled product from radiolabeling



Scheme 2. Synthesis of 2-amino-6-(4-[^{11}C]methoxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine bis(2,2,2-trifluoroethyl) ester ([^{11}C]ABE, [^{11}C]**1**) using 2-*N*-Boc protected HO-precursor **12**.

reaction mixture. The reaction mixture was diluted with $NaHCO_3$ and loaded onto C-18 cartridge by gas pressure. The cartridge column was washed with water to remove unreacted $^{11}CH_3OTf$ and precursor and reaction solvent, and then final labeled product was eluted with ethanol. Similarly, 2-*N*-Boc protected HO-precursor **12** was labeled with $^{11}CH_3OTf$ followed by a quick deprotection reaction with 1 N HCl and isolated by SPE purification technique to produce [^{11}C]**1** in 40–55% radiochemical yields. Comparing the radiochemistry of unprotected precursor **10** and protected precursor **12**, no distinct difference was found. It is likely the concern that 2-amino group in purine ring may affect the *O*-[^{11}C]methylation reaction of HO-precursor is unnecessary. The *N*-[^{11}C]methylation at 2-amino group in the purine ring is a potential competing reaction in comparison with the *O*-[^{11}C]methylation at 4-hydroxyl group in the 6-(4-hydroxyphenylthio) ring. However, we do not have any evidence for labeling of the 2-amino group in the purine ring, it is consistent with our previous works on the synthesis of the radiolabeled O^6-BG analogues.[28,29,31] It is also consistent with the theoretical explanation that the deprotonization at the 4-hydroxyl group is easier than at the 2-amino group since the acidity of HO– is greater than the acidity of H_2N–, and the [^{11}C]methylation with [^{11}C]methyl triflate will prefer to occur at the oxygen position rather than at the nitrogen position.[38] The identity of the labeled product was determined by analytical high pressure liquid chromatography (HPLC) method, and the evidence provided by the exact same HPLC retention time data of the labeled product [^{11}C]**1** and reference standard **1** shows [^{11}C]methyl triflate does not label the 2-amino group and confirms the theoretical explanation. Chemical purity, radiochemical purity and specific radioactivity were determined by analytical HPLC method. The chemical purity of precursors **10**, **12**, and reference standard **1** was >97%. The radiochemical purity of target tracer [^{11}C]**1** was >95%, and the chemical purity of target tracer [^{11}C]**1** was >93%. The average ($n = 3$–5) specific radioactivity of target tracer [^{11}C]**1** was 0.6–0.8 Ci/μmol at the end-of-synthesis (EOS).

3. Conclusion

An efficient and convenient chemical and radiochemical synthesis of the unprotected precursor and 2-*N*-Boc protected precursor, reference intermediate and standard, and target tracer of ABE has been developed. Radiosynthesis produced [^{11}C]ABE in amounts and purity suitable for the preclinical application of [^{11}C]ABE in animal studies by PET imaging techniques. Labeled product suitable for injection, with the specific radioactivities in a range of 0.6–0.8 Ci/μmol at EOS, can be obtained in 20–25 min from EOB, including SPE purification and formulation. These chemistry results provide the foundation for further biological evaluation of [^{11}C]ABE as a novel potential PET cancer imaging agent for hepatitis B virus and herpes simplex virus thymidine kinase in vivo.

4. Experimental

4.1. General

All commercial reagents and solvents were used without further purification unless otherwise specified. 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 400 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 (LRMS) were obtained using a Bruker Biflex III MALDI-Tof mass spectrometer, and the high resolution mass spectra (HRMS) measurements were obtained using a Kratos MS80 mass spectrometer, in the Department of Chemistry at Indiana University. Chromatographic solvent 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; 3:1:3 CH_3CN–MeOH–20 mM, pH 6.7 $KHPO_4^-$ mobile phase, 1.5 mL/min flow rate, UV (240 nm) and γ-ray (NaI) flow detectors. Semi-prep C-18 guard cartridge column 1×1 cm was obtained from E. S. Industries, Berlin, NJ, and part number 300121-C18-BD 10 μm. Sterile vented Millex-GS 0.22 μm vented filter unit was obtained from Millipore Corporation, Bedford, MA.

4.2. Bis(trifluoroethyl) (2-chloroethoxy)methylphosphonate (4)

Compound **4** was prepared by the reaction[4] of 2-chloroethyl chloromethyl ether (**2**) with tris(2,2,2-trifluoroethyl)phosphite (**3**) as a colorless liquid in a quantitative yield, $R_f = 0.58$ (1:1 hexane/EtOAc).

4.3. Bis(trifluoroethyl) (2-iodoethoxy)methylphosphonate (5)

Compound **5** was prepared by the reaction[4] of compound **4** with sodium iodide as a light brown liquid in 80% yield, $R_f = 0.31$ (CH_2Cl_2). ^{1}H NMR ($CDCl_3$, ppm): δ 4.40–4.55 (m, 4H, CH_2CF_3), 4.01 (d, 2H, $J = 8.08$ Hz, OCH_2P), 3.86 (t, 2H, $J = 6.61$ Hz, ICH_2-CH_2O), 3.27 (t, 2H, $J = 6.62$ Hz, ICH_2CH_2O). LRMS (FAB, *m/z*): 453 [(M+Na)$^+$, 100%]. HRMS (FAB, *m/z*): calcd for $C_7H_{10}F_6INaO_4P$ 452.9163. Found: 452.9163.

4.4. 2-Amino-6-[(4-methoxyphenyl)sulfanyl]purine (7)

To a 250 mL two-necked flask containing guanine (**6**, 5.00 g, 31.1 mmol) and dry pyridine (50 mL) was added trifluoroacetic anhydride (17.0 mL, 120 mmol) dropwise over a period of 10 min at 0 °C. After 30 min, a solution of 4-methoxybenzenethiol (10 mL, 81.3 mmol) in dry acetonitrile (10 mL) was added dropwise over a period of 15 min. The reaction was allowed to warm up to rt and was stirred at rt overnight. Then ethanolic methylamine (16 mL) was added and the solution was stirred at rt for 2 h. After concentration under reduced pressure, the residue obtained was triturated with petroleum ether (bp 40–60 °C) and was then collected by filtration. The solid was suspended in water (100 mL), stirred, and filtered. Crystallization of the resultant solid from ethanol give a colorless crystalline solid **7** (5.58 g, 66%), mp 259–260 °C. ^{1}H NMR (DMSO-d_6, ppm): δ 12.54 (s, 1H, 9-NH), 7.93 (s, 1H, 8-CH), 7.49 (d, 2H, $J = 8.82$ Hz, Ph), 7.00 (d, 2H, $J = 8.82$ Hz, Ph), 6.13 (s, 2H, 2-NH_2), 3.79 (s, 3H, OCH_3). LRMS (FAB, *m/z*): 154 (100%), 296 [(M+Na)$^+$, 9.6%]. HRMS (FAB, *m/z*): calcd for $C_{12}H_{11}N_5NaOS$ 296.0582. Found 296.0582.

4.5. 2-Amino-6-(4-methoxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine bis(2,2,2-trifluoroethyl) ester (ABE, 1)

To a 100 mL two-necked flask were charged with compound **7** (1.00 g, 3.66 mmol), dry DMF (20 mL) and DBU (0.6 mL, 4.01 mmol). The solution was heated at 80 °C for 1 h. Then compound **5** (1.74 g, 4.04 mmol) was added to the reaction mixture and the mixture was heated at 100 °C overnight. Solvent was removed by rotatory evaporation, and the residue was dissolved in CH_2Cl_2 and transferred to the top of a silica gel column. The column was eluted with 50:1 CH_2Cl_2/MeOH to afford a white solid **1** (0.88 g, 42%), mp 84–85 °C, $R_f = 0.36$ (20:1 CH_2Cl_2/MeOH). The chemical purity (CP) of compound **1** was determined by analytical HPLC method, t_R**1** = 6.95 min, CP**1** = 99.4%. The analysis of the chemical purity of compound **1** was also confirmed by the ^{1}H NMR method. ^{1}H NMR ($CDCl_3$, ppm): δ 7.70 (s, 1H, 8-CH), 7.52 (d, 2H, $J = 8.09$ Hz, Ph), 6.93 (d, 2H, $J = 8.83$ Hz, Ph), 4.90 (s, 2H, NH_2, D_2O exchangeable), 4.30–4.50 (m, 4H, OCH_2CF_3), 4.10–4.30 (m, 2H, NCH_2CH_2O), 3.85–4.01 (m, 4H, OCH_2P and NCH_2CH_2O), 3.82 (s, 3H, OCH_3).

4.6. 2-Amino-6-chloro-9-[2-(phosphonomethoxy)ethyl]-purine bis(2,2,2-trifluoroethyl) ester (9)

Compound **9** was prepared by the reaction[4] of 2-amino-6-chloropurine (**8**) with compound **5** in dry DMF and DBU as a white solid in 33% yield, mp 103–104 °C, $R_f = 0.26$ (25:1 CH_2Cl_2/MeOH). ^{1}H NMR ($CDCl_3$, ppm): δ 7.83 (s, 1H, 8-CH), 5.08 (s, 2H, 2-NH_2), 4.25–4.45 (m, 6H, NCH_2CH_2O and CH_2CF_3), 3.88–3.98 (m, 4H, OCH_2P and NCH_2CH_2O). LRMS (EI, *m/z*): 212 (100%), 471 (M$^+$, 42%). HRMS (EI, *m/z*): calcd for $C_{12}H_{13}ClF_6N_5O_4P$ 471.0298. Found 471.0289.

4.7. 2-Amino-6-(4-hydroxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine bis(2,2,2-trifluoroethyl) ester (10)

Compound **10** was prepared by the reaction[4] of compound **9** with 4-hydroxythiophenol in dry pyridine

and dry DMF as a white solid in 55% yield, mp 55 °C (dec.), R_f = 0.20 (25:1 CH_2Cl_2/MeOH). The chemical purity (CP) of compound **10** was determined by analytical HPLC method, t_R**10** = 4.10 min, CP**10** = 98.7%. The analysis of the chemical purity of compound **10** was also confirmed by the ^{1}H NMR method. ^{1}H NMR (DMSO-d_6, ppm): δ 9.85 (s, 1H, OH, D_2O exchangeable), 7.88 (s, 1H, 8-CH), 7.36 (d, 2H, J = 8.09 Hz, Ph), 6.83 (d, 2H, J = 8.83 Hz, Ph), 6.26 (s, 2H, 2-NH_2, D_2O exchangeable), 4.55–4.73 (m, 4H, CH_2CF_3), 4.20 (t, 2H, J = 5.14 Hz, N$CH_2$$CH_2$O), 4.13 (d, 2H, J = 8.09 Hz, OCH_2P), 3.86 (t, 2H, J = 5.15 Hz, NCH_2CH_2O). LRMS (EI, *m/z*): 561 (M^+, 100%). HRMS (EI, *m/z*): calcd for $C_{18}H_{18}F_6N_5O_5PS$ 561.0670. Found 561.0665.

4.8. 2-*N*-Bis(Boc)amino-6-chloro-9-[2-(phosphonomethoxy)ethyl]purine bis(2,2,2-trifluoroethyl) ester (11)

To a 25 mL two-necked flask equipped with a stir-bar were charged with compound **9** (0.50 g, 1.06 mmol), 4-dimethylaminopyridine (DMAP, 0.010 g, 0.082 mmol), dry THF (10 mL), and Boc_2O (0.7 mL, 3.05 mmol). The reaction mixture was stirred at rt overnight. After removal of solvent, the residue was dissolved in CH_2Cl_2 and transferred to a silica gel column. The column was eluted with 50:1 CH_2Cl_2/MeOH to give a viscous solid **11** (0.55 g, 77%), R_f = 0.18 (50:1 CH_2Cl_2/MeOH). ^{1}H NMR ($CDCl_3$, ppm): δ 8.22 (s, 1H, 8-CH), 4.32–4.52 (m, 6H, N$CH_2$$CH_2$O and CH_2CF_3), 3.98 (t, 2H, J = 5.15 Hz, NCH_2CH_2O), 3.94 (d, 2H, J = 7.35 Hz, OCH_2P), 1.47 (s, 18H, Boc). LRMS (EI, *m/z*): 471 (100%), 671 (M^+, 2.1%). HRMS (EI, *m/z*): calcd for $C_{22}H_{29}ClF_6N_5O_8P$ 671.1346. Found 671.1338.

4.9. 2-*N*-Bis(Boc)amino-6-(4-hydroxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine bis(2,2,2-trifluoroethyl) ester (12)

To a 25 mL two-necked flask fitted with a condenser were charged with compound **11** (0.52 g, 0.77 mmol), 4-hydroxythiophenol (0.12 g, 0.95 mmol), pyridine (0.2 mL), and DMF (5 mL). The mixture was heated at 60 °C for 5 h. After removal of solvent at 45–50 °C, the residue was dissolved in CH_2Cl_2 and transferred to the top of a silica gel column. The column was eluted with 50:1 CH_2Cl_2/MeOH to give a white foam solid **12** (0.18 g, 31%), R_f = 0.15 (40:1 CH_2Cl_2/MeOH). The chemical purity (CP) of compound **12** was determined by analytical HPLC method, t_R**12** = 2.68 min, CP**12** = 98.3%. The analysis of the chemical purity of compound **12** was also confirmed by the ^{1}H NMR method. ^{1}H NMR ($CDCl_3$, ppm): δ 8.07 (s, 1H, 8-CH), 7.46 (d, 2H, J = 8.83 Hz, Ph), 6.83 (d, 2H, J = 8.83 Hz, Ph), 5.73 (s, 1H, OH), 4.31–4.48 (m, 6H, N$CH_2$$CH_2$O and CH_2CF_3), 3.86–4.00 (m, 4H, NCH_2CH_2O and OCH_2P), 1.36 (s, 18H, Boc). LRMS (EI, *m/z*): 561 (100%), 761 (M^+, 4%). HRMS (EI, *m/z*): calcd for $C_{28}H_{34}F_6N_5O_9PS$ 761.1719. Found 761.1721.

4.10. 2-*N*-Bis(Boc)amino-6-(4-methoxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine bis(2,2,2-trifluoroethyl) ester (13)

To a 100 mL two-necked flask containing compound **1** (0.10 g, 0.17 mmol), DMAP (0.001 g), and dry THF (30 mL) was added Boc_2O (0.16 mL, 0.70 mmol). The solution was stirred at rt overnight. Solvent was removed at reduced pressure. The residue was dissolved in CH_2Cl_2, and transferred to the top of a silica gel column. The column was eluted first with CH_2Cl_2, then 50:1 CH_2Cl_2/MeOH to afford a viscous solid **13** (0.060 g, 45%), R_f = 0.18 (50:1 CH_2Cl_2/MeOH). The chemical purity (CP) of compound **13** was determined by analytical HPLC method, t_R**13** = 4.34 min, CP**13** = 97.8%. The analysis of the chemical purity of compound **13** was also confirmed by the ^{1}H NMR method. ^{1}H NMR ($CDCl_3$, ppm): δ 8.06 (s, 1H, 8-H), 7.52 (d, 2H, J = 8.82 Hz, Ph), 6.92 (d, 2H, J = 8.82 Hz, Ph), 4.30–4.50 (m, 6H, N$CH_2$$CH_2$O and CH_2CF_3), 3.87–4.00 (m, 4H, $NCH_2CH_2OCH_2$P), 3.84 (s, 3H, OCH_3), 1.35 (s, 18H, Boc). LRMS (EI, *m/z*): 121 (100%), 775 (M^+, 12.5%). HRMS (EI, *m/z*): calcd for $C_{29}H_{36}F_6N_5O_9PS$ 775.1876. Found 775.1879.

4.11. 2-Amino-6-(4-[^{11}C]methoxyphenylthio)-9-[2-(phosphonomethoxy)ethyl]purine bis(2,2,2-trifluoroethyl) ester ([^{11}C]ABE, [^{11}C]1)

4.11.1. Starting from unprotected precursor 10. $^{11}CO_2$ was produced by the $^{14}N(p,\alpha)^{11}C$ nuclear reaction in small volume (12.3 cm^3) aluminum gas target (CTI) from 11 MeV proton cyclotron on research purity nitrogen (+3% O_2) in a Siemens radionuclide delivery system (RDS-112). The precursor **10** (0.6–1.0 mg) was dissolved in CH_3CN (300 μL). To this solution was added TBAH (2–3 μL, 1 M solution in methanol). The mixture was transferred to a small volume, three-neck reaction tube. $^{11}CH_3OTf$ that was produced by the gas-phase production method[32] from $^{11}CO_2$ through $^{11}CH_4$ and $^{11}CH_3Br$ was passed into the air-cooled reaction tube at −15 to −20 °C, which was generated by a Venturi cooling device powered with 100 psi compressed air, until radioactivity reached a maximum (~3 min), then the reaction tube was heated at 70–80 °C for 3 min. The contents of the reaction tube were diluted with $NaHCO_3$ (1 mL, 0.1 M). This solution was passed onto a C-18 cartridge by gas pressure. The cartridge was washed with H_2O (2 × 3 mL), and the aqueous washing was discarded. The product was eluted from the column with EtOH (2 × 3 mL), and then passed onto a rotatory evaporator. The solvent was removed by evaporation under high vacuum. The labeled product [^{11}C]**1** was formulated with NaH_2PO_4 (50 mM), whose volume was dependent upon the use of the labeled product **1** in tissue biodistribution studies (~6 mL, 3 × 2 mL) or in micro-PET imaging studies (1–3 mL) of tumor-bearing athymic mice[39], sterile-filtered through a sterile vented Millex-GS 0.22 μm cellulose acetate membrane and collected into a sterile vial. Total radioactivity was assayed and total volume was noted. The overall synthesis, purification and formulation time was ~20 min from EOB. The decay corrected yield, from $^{11}CO_2$, was 50–55%. Retention times in the analytical HPLC system were: t_R**10** = 4.10 min, t_R[^{11}C]**1** = 6.95 min.

4.11.2. Starting from protected precursor 12. The solution of the precursor **12** (0.6–1.0 mg) in CH_3CN (300 μL) and TBAH (2–3 μL, 1 M solution in methanol) was reacted with $^{11}CH_3OTf$. The radiolabeling mixture

containing [^{11}C]**13** in reaction tube was added with 1 N HCl (0.5 mL) and heated at 70–80 °C for another 3 min. The contents of the reaction tube were diluted with $NaHCO_3$ (0.5 mL, 0.1 M). The target tracer [^{11}C]**1** was isolated from the radiolabeling mixture by a C-18 cartridge through SPE purification in ~25 min with 40–55% radiochemical yield. Retention times in the analytical HPLC system were t_R**12** = 2.68 min, t_R[^{11}C]**13** = 4.34 min, t_R[^{11}C]**1** = 6.95 min.

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