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Synthesis of a technetium-99m-labeled thymidine analog: a potential HSV1-TK substrate for non-invasive reporter gene expression imaging

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Abstract—{2-[5-(2′-Fluoro-2′-deoxyuridin-5-yl)pent-4(*E*)-enyl] [2-(2-mercaptoethyl)aminoethyl]aminoethanethiolato(3)-*N,N′,S,S′*}-oxo-[^{99m}Tc]technetium(V), a potential viral thymidine kinase substrate, was synthesized by coupling 2′-fluoro-2′-deoxyuridine analog with a N2S2 radiometal chelator, followed by [Tc-99m]technetium conjugation. The chemical structure of the radioactive probe was characterized by ^{1}H NMR and high resolution MS using Re-188 conjugated mimics. The radiochemical purity and yield were identified as 98% and 42%, respectively.

Non-invasive nuclear imaging of Herpes simplex virus type-1 thymidine kinase (HSV1-TK) expression has gained broad interests because of its potential in clinical application.[1–3] The biological principal of the imaging modality involves phosphorylation and, therefore, intracellularly retention of the radiolabeled nucleosides by the HSV1-TK. The current HSV1-TK substrate of nuclear imaging includes radioactive fluorine and iodine isotope-labeled nucleoside derivatives, such as 9-(4-[F-18]fluoro-3-hydroxymethylbutyl)guanine (FHBG)[4,5] and 5-[I-131/124]iodo-1-(2-fluoro-2-deoxy-β-D-arabinofuranosyl)uracil (FIAU).[6–8] However, use of radioactive fluorine- and iodine-labeled probes in routine procedures is hampered by either the limited supply of radioisotopes or by suboptimal imaging characteristics, such as iodine-131. A [Tc-99m]technetium-labeled TK substrate for nuclear gene imaging would address the concerns because of its ease of production and optimal imaging characteristics ($T_{1/2}$ = 6 h, 140 keV).

In this communication we report the synthesis and characterization of {2-[5-(2′-fluoro-2′-deoxyuridin-5-yl)-pent-4(*E*)-enyl] [2-(2-mercaptoethyl)aminoethyl]aminoethanethiolato(3)-*N,N′,S,S′*}oxo-[^{99m}Tc]technetium(V) (FTcAU). The fact that among the thymidine substrates of HSV1-TK the substitution on 5 position of thymine ring shows the most variability and flexibility[7,9–11] leads us to design our first Tc-99m-labeled probe using 5-substituted 2′-fluoro-2′-deoxyuridine as a template. Because of the requirement of cellular membrane penetration, the radiometal is labeled through a lipophilic, N2S2 metal chelator, *N,N′*-bis(2-mercaptoethyl)ethylenediamine. To characterize the chemical structure of the Tc-99m-labeled probe, a nonradioactive mimic compound, {2-[5-(2′-fluoro-2′-deoxyuridin-5-yl)pent-4(*E*)-enyl] [2-(2-mercaptoethyl)aminoethyl]aminoethanethiolato(3)-*N,N′,S,S′*}oxo-[^{188}Re] rhenium(V) (FReAU) is also synthesized for NMR and MS analysis and HPLC identification.

A convergent synthetic strategy is employed to obtain 5-{[2-(4-methoxybenzylsulfanyl)ethyl]{2-[2-(4-methoxybenzylsulfanyl)ethylamino]ethyl}amino} pent-4(*E*)-enyl-1-(3,5-diacetyl-2-fluoro-2-deoxy-1-β-D-ribofuranosyl)uracil **9**, the precursor of Tc-99m-labeled **12**, from two synthons, 5-(5-bromopent-1(*E*)-enyl)-1-(3,5-diacetyl-2-fluoro-2-deoxy-1-β-D-ribofuranosyl)uracil **5** and *N,N′*-bis-[2-(4-methoxybenzylsulfanyl)ethyl]ethylenediamine **8**.

Keywords: FTcAU; TK substrate; Nuclear imaging; Reporter probe.
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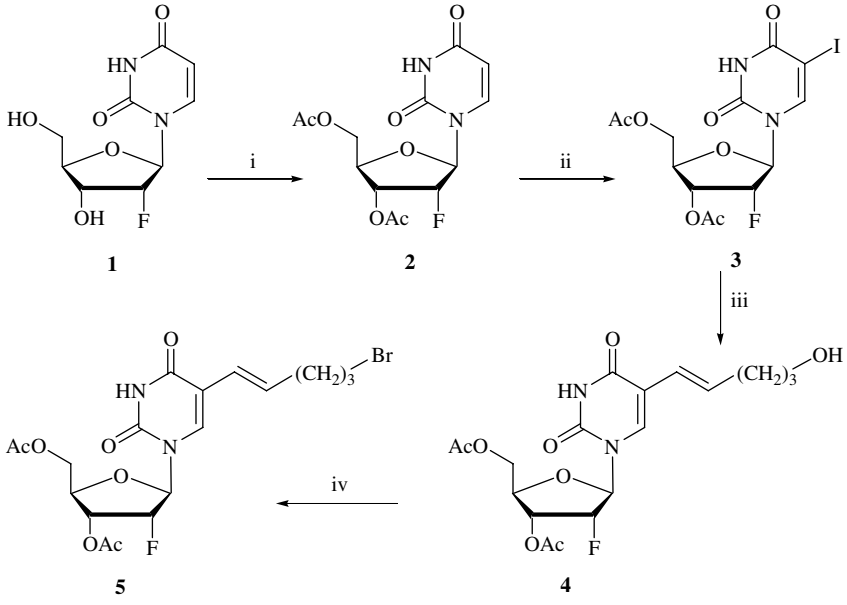
At first, the thymidine analog **4** is synthesized through a multiple-step reaction from 2′-fluoro-2′-deoxyuridine **1** (Scheme 1). The hydroxyl groups of **1** are protected by treating with acetic anhydride in pyridine to give **2**, followed by iodination to 5-iodo-3′,5′-diacetyl-2′-fluoro-2′-deoxyuridine **3** using iodonium chloride in methylene chloride with the iodination yield of 87%. Stille coupling reaction of **3** with 1-(tributylstannyl)-1(*E*)-penten-5-ol yields 5-(5-hydroxylpent-1(*E*)-enyl)-1-(3,5-diacetyl-2-fluoro-2-deoxy-1-β-D-ribofuranosyl)uracil **4** in 92% yield.[12] Treatment of **4** with NBS in the presence of triphenyl phosphorus gives the bromide **5** in 75% yield.[13]

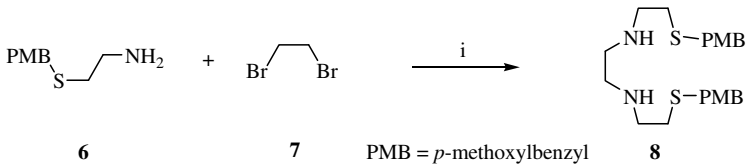
The metal chelating moiety, *N,N*′-bis-[2-(4-methoxybenzylsulfanyl)ethyl]ethylenediamine **8** is prepared from 2-(4-methoxybenzylsulfanyl)ethylamine **6**[14] and dibromoethylene **7** in yield of 41% (Scheme 2). Cesium hydroxide controls the *N*-alkylation reaction to obtain secondary amine as the major product.[15]

Coupling of thymidine analog **5** and chelator fragment **8** produces **9**.[16] The removal of acetyl protecting groups of **9** with potassium carbonate in aqueous methanol yields **10**[17] with a two-step yield of 76%. The thiol protecting groups, 4-methoxybenzyl, of **10** are removed with $Hg(OAc)_2$ in TFA to give trifluoroacetate salts of **11**. The crude air-sensitive compound **11** is conjugated with technetium immediately, without purification. Addition of [^{99m}Tc]pertechnetate in PBS into the aqueous methanol of the crude **11** in the presence of Sn-glucoheptonate in 80 °C water bath for 30 min and thereafter HPLC[18] purification yields the target compound, FTcAU **12** with a radiochemical yield of 42% (Scheme 3). To characterize the chemical structure of the FTcAU **12**, its analog of rhenium-188 conjugate, FReAU **13**, is synthesized with modifying a similar reaction condition [19] by adding tetrabutylammonium tetrachlorooxorhenate(V) into a solution of compound **11** in methanol and stirring for 12 h. The rhenium conjugate **13** is purified by flash chromatography and its chemical structure is characterized with ^{1}H NMR and high resolution ESI-MS.[20] The characterization of FTcAU is carried out using reverse phase HPLC by co-injection with FReAU.

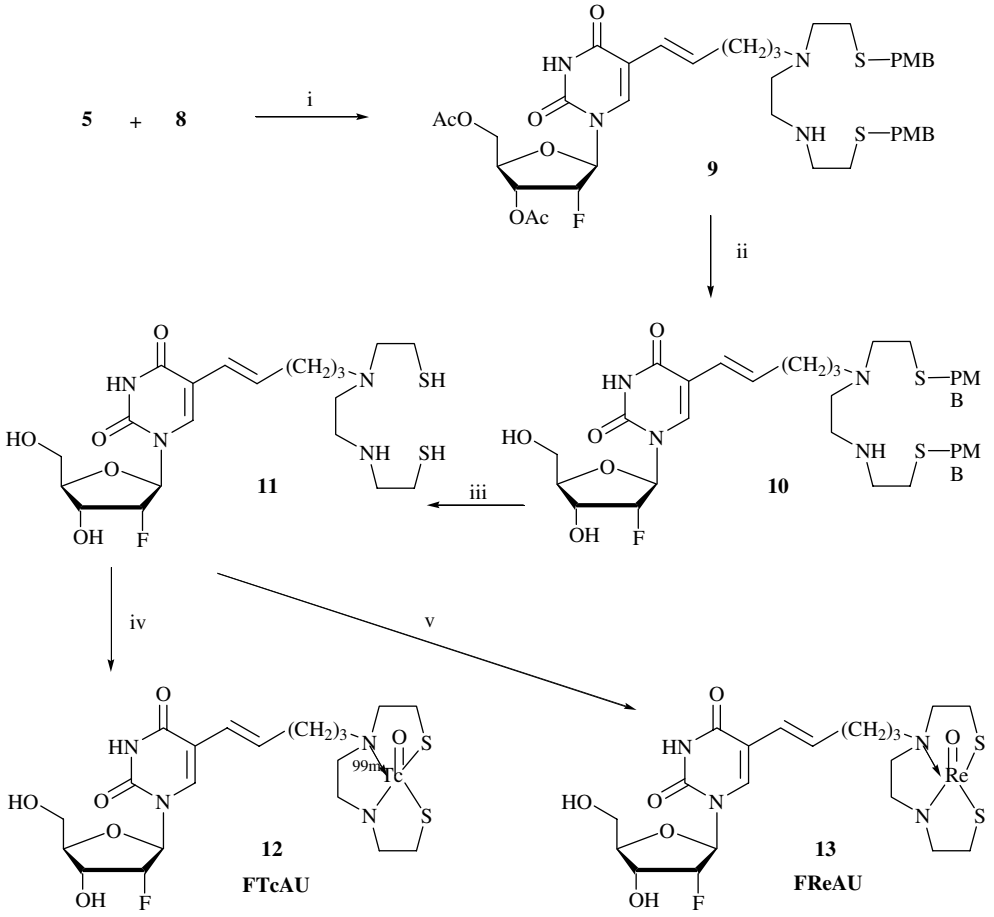
The present work demonstrates the synthesis of a neutral Tc-99m-labeled thymidine analog **12**, a potential HSV1-TK substrate. In vitro verification of the compound by cell uptake experiments is underway in our laboratory and the result will be published elsewhere.



Scheme 1. Reagents: (i) Ac_2O, Py; (ii) ICl, CH_2Cl_2; (iii) 1-(tributylstannyl)-1(*E*)-penten-5-ol, $(CH_3CN)_2PdCl_2$, DMF; (iv) NBS, PPh_3, DMF.



Scheme 2. Reagents: (i) CeOH, molecular sieves (4 Å), DMF.



Scheme 3. Reagents: (i) DIEA, CH_3CN; (ii) K_2CO_3; (iii) $Hg(OAc)_2$/TFA, H_2S; (iv) [^{99m}Tc]$NaTcO_4$, Sn-glucoheptonate; (v) $(Bu_4N)^+(ReOCl_4)^-$.

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12. Compound **4**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.72 (m, 2H), 2.13 (s, 3H), 2.17 (s, 3H), 2.25 (m, 2H), 3.68 (m, 2H), 4.34 (m, 1H), 4.44 (d, 2H), 5.17 (m, 1H), 5.40 (dq, 1H), 5.82 (d, 1H), 6.10 (d, 1H, J = 15.9 Hz), 6.47 (dt, 1H, J = 15.9 Hz), 7.31 (s, 1H). HRMS (ESI) $[M + H]^+$, obsd: 415.1514, calcd: 415.1517.
13. Compound **5**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.99 (m, 2H), 2.13 (s, 3H), 2.17 (s, 3H), 2.32 (m, 2H), 3.42 (m, 2H), 4.37 (m, 1H), 4.44 (d, 2H), 5.17 (m, 1H), 5.39 (dq, 1H), 5.82 (d, 1H), 6.10 (d, 1H, J = 16.2 Hz), 6.48 (dt, 1H, J = 16.2), 7.32 (s, 1H). HRMS (ESI) $[M + H]^+$, obsd: 477.0664, calcd: 477.0673.
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16. Compound **9**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.59 (m, 2H), 2.09 (s, 3H), 2.15 (s, 3H), 2.47 (m, 4H), 2.61 (s, 4H), 2.77 (m, 6H), 2.97 (t, 2H), 3.64 (s, 2H), 3.69 (s, 2H), 3.76 (s, 3H), 3.77 (s, 3H), 4.28–4.48 (m, 3H), 5.22 (m, 1H), 5.43 (dq, 1H), 5.81 (dd, 1H), 6.10 (d, 1H, J = 15.9 Hz), 6.44 (dt, 1H, J = 15.9 Hz), 6.82 (d, 4H), 7.22 (d, 4H), 7.33 (s, 1H). HRMS (ESI) $[M + H]^+$, obsd: 817.3322, calcd: 817.3316.
17. Compound **10**. ^{1}H NMR (300 MHz, THF-d_8): δ 1.57 (m, 2H), 2.13 (m, 2H), 2.42–2.73 (m, 12H), 2.88 (t, 2H), 3.63 (s, 2H), 3.67 (s, 2H), 3.75 (s, 3H), 3.76 (s, 3H), 3.94 (d, 2H), 4.35 (m, 1H), 4.91 (dq, 1H), 6.05 (d, 1H), 6.15 (d, 1H, J = 15.0 Hz), 6.44 (dt, 1H, J = 15.0 Hz), 6.83 (d, 4H), 7.23 (d, 4H), 8.36 (s, 1H). HRMS (ESI) $[M + H]^+$, obsd: 733.3272, calcd: 733.3105.
18. NOTE: HPLC purification: column: Econosil C18 10u, 250 × 10 mm; gradient: acetonitrile/PBS (pH 7.4) (35/65); flow rate: 3 mL/min.; retention time: 10.5 min.
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20. Compound **13**. ^{1}H NMR (300 MHz, THF-d_8): δ 1.42 (m, 2H), 2.17 (m, 2H), 2.62 (m, 2H), 2.77 (m, 2H), 3.04 (m, 2H), 3.29 (m, 6H), 3.43 (m, 2H), 3.95 (m, 1H), 4.03 (m, 2H), 4.30 (m, 1H), 5.22 (m, 1H), 4.93 (dq, 1H), 6.00 (dd, 1H), 6.15 (d, 1H, J = 15.9 Hz), 6.57 (dt, 1H, J = 15.9 Hz), 8.21 (s, 1H). HRMS (ESI) $[M + H]^+$, obsd: 693.1214, calcd: 693.1226.