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SYNTHESIS OF ^{11}C-LABELED URACIL DERIVATIVE FOR A PET TRACER TARGETING THYMIDINE PHOSPHORYLASE

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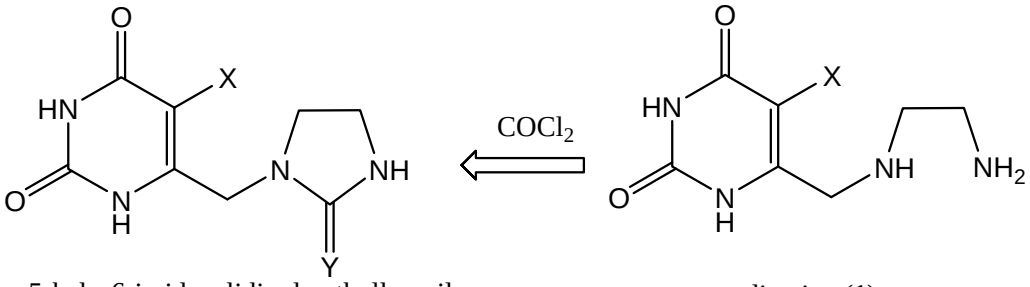
Abstract – The expression of thymidine phosphorylase (TP) is closely associated with angiogenesis in tumors. For developing a TP-expression-based positron emission tomography (PET) radiotracer for diagnosis and prognosis of cancer chemotherapy, we synthesized a novel ^{11}C-labeled oxoimidazolidinylmethyluracil ([^{11}C]-**2a**), which was designed on the basis of one of the most potent inhibitors, 5-bromo-6-[(2-iminoimidazolidinyl)-methyl]uracil hydrobromide (5BIMU), through a ring closure reaction of [^{11}C]phosgene with a developed diamine precursor (**1a**). After purification by HPLC, the total synthesis was accomplished in just 23 min after bombardment, and the yield was 1653 MBq at the end of synthesis (EOS).

Positron emission tomography (PET) is a powerful non-invasive imaging modality for staging, restaging, therapeutic response monitoring, and prognostication in patients with various cancers, and much attention has been paid on the development of useful PET tracers based on the changes in biological processes in cancers. Angiogenesis plays an important role in the growth and spread of cancer. Platelet-derived endothelial cell growth factor (PD-ECGF), which is one of various angiogenesis factors, is overexpressed in many solid tumors such as ovarian and breast tumors.[1] Interestingly, PD-ECGF is identical to thymidine phosphorylase (TP), which catalyzes the reversible phosphorolysis of thymidine to thymine and 2-deoxyribose 1-phosphate.[2]

In this regard, although several TP substrates have been synthesized and examined, no substance has been proved effective for non-invasive imaging of TP activity to date. Therefore, our study is focused on a new

approach of using potential PET tracers for probing tumor-selective, enzyme-utilizing radiolabeled TP inhibitors.

Because of short-lived positron-emitting radionuclides (ca. ^{11}C: 20.4 min, ^{13}N: 10.0 min, ^{15}O: 2.0 min) and the radioactive level, a very rapid and simple labeling process with an efficient organic reaction is essential for PET radiotracer synthesis. In recent years, various TP inhibitors have been designed and synthesized by estimating binding modes and energetics of the ligands in the active site residues of TP using computational methods.[3] These include the 5- and 6-substituted uracil analogues (e.g., 6-amino-5-chlorouracil)[4] and 6-methylene-bridged bicyclic uracils having either a pyrrolidine group or an imidazole group, which best match the active site of TP (Scheme 1).[5]



5-halo-6-imidazolidinylmethylluracil
Y = NH, O

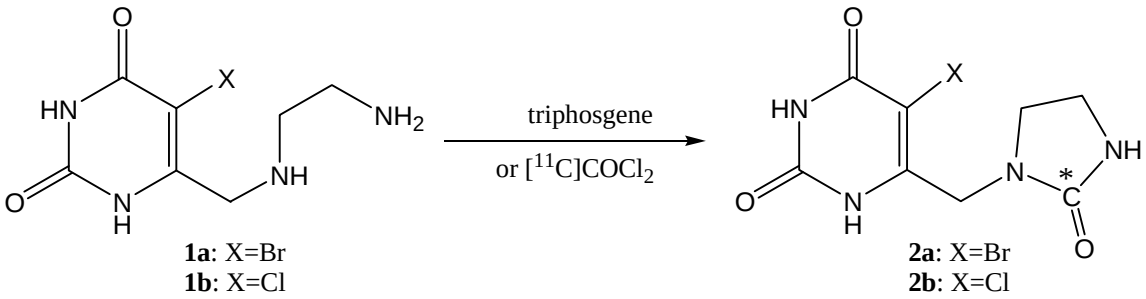
diamine (**1**)

Scheme 1

We recently developed a method for efficient synthesis of [^{11}C] $COCl_2$,[6] which is a highly potent and versatile agent for introducing a ^{11}C carbonyl group, and it was successfully applied to the synthesis of [^{11}C] CGP 12177,[7] a radioligand for β-adrenoreceptors, [2-^{11}C] thymidine,[8] a PET tracer for the assessment of cell proliferation, and [2-^{11}C] 5-FU[9] as an efficient agent for predicting the outcomes of 5-FU in cancer chemotherapy. Based on this, we planned to synthesize novel PET radiotracers: ^{11}C-labeled 6- methylene-bridged uracil derivatives of TP inhibitory activity. In this paper, we describe the synthesis of a novel TP target, ^{11}C-labeled oxoimidazolidinylmethyluracil, as a potential imaging agent for PET.

Our strategy for the synthesis of imidazolidinylmethyluracil with TP inhibitory activity is described in Scheme 1. Compound **1** was prepared by treating 5-X-6-chloromethyluracil (X = Br, Cl) with excess ethylenediamine in water.[10] Diamine **1** was treated with triphosgene in *t*-butanol in the presence of two equivalent moles of potassium *t*-butoxide (*t*-BuOK) at room temperature for 5 min, resulting in the formation of **2** (**2a**[11]: 82%, **2b**[12]: 46%): For the synthesis of cold compounds **2**, triphosgene was used for cyclocondensation as a safe and stable replacement for phosgene (Scheme 2). The ring closure process requires a base such as *t*-BuOK to avoid precipitation of diamine hydrochloride formed by the consumption of triphosgene.

The TP inhibitory effects of 6-methylene-bridged uracils **2** were assessed by the TP-catalyzed



1a: X=Br
1b: X=Cl

2a: X=Br
2b: X=Cl

Scheme 2

formation of thymine, according to the previous procedure.[13] The standard TP inhibitor 6-amino-5-chlorouracil (6A5CU) and the most potent inhibitor 5-bromo-6-[(2-imino-imidazolidinyl)methyl]uracil hydrobromide (5BIMU) were the reference compounds (Table 1).

Table 1. Inhibitory effect of uracil derivatives on *Escherichia coli* TP

	Compounds			
	2a	**2b**	6A5CU	5BIMU
IC_{50} (μM)	4.50	17.5	0.85	3.69×10^{-3}

The IC_{50} values of **2a** and **2b** were 4.50 and 17.5 μM for *Escherichia coli* TP, respectively. The inhibitory potency of these compounds was lower compared with that of 5BIMU, but **2a** showed comparable potency to that of 6A5CU as a standard inhibitor.

The precursor **1a** was subjected to cyclocondensation in the presence of *t*-BuOK with $[^{11}C]COCl_2$, prepared by our reported method,[6,7] to afford ^{11}C-labeled **2a** in a yield of 1653 ± 172 MBq at EOS with a specific activity of 818 ± 40 GBq/μmol (n = 3).[14] These results were obtained when the reactions were conducted using 1.0 mg of **1a** in the presence of three equivalent molar of *t*-BuOK in *t*-BuOH.[15]

The radio-HPLC trace is shown in Figure 1. After HPLC purification, the chemical purity of [^{11}C]-**2a** was >99%, as assessed by peak area evaluation of UV chromatograms. The radiochemical purity was >99%. The total synthesis was accomplished in just 23 min after bombardment.

In conclusion, a positron-emitting, nuclear-labeled TP inhibitor, ^{11}C-labeled oxoimidazolidinyl-methyluracil **2a**, was synthesized for the first time. The tracer exhibited moderate TP inhibitory potency. The corresponding diamine precursor was labeled with C-11 using $[^{11}C]\ COCl_2$ to produce a satisfactory yield and acceptable specific activity. [^{11}C]-**2a** was subsequently prepared as a formulation suitable for *in vivo* studies. The new radiotracer can be used as a PET radiopharmaceutical for imaging angiogenic enzyme TP expression due to its TP inhibitory potency.

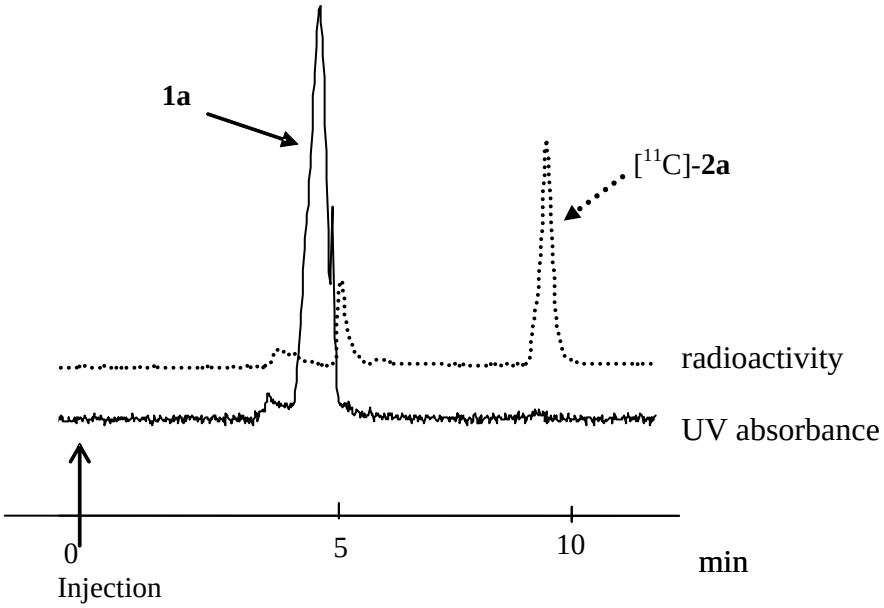


Figure 1. Preparative HPLC-chromatogram for the radiosynthesis of [^{11}C]-**2a**[15]

ACKNOWLEDGMENTS

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11. 5-Bromo-6-[2-(oxoimidazolidinyl)methyl]uracil (**2a**): mp 243-245 °C. ^{1}H-NMR (CD_3OD-d_4) δ 3.47-3.49 (m, 4H), 4.56 (s, 2H). FAB-MS *m/z*: 289, 291 $(M+H)^+$. Anal. Calcd for $C_8H_9BrN_4O_3$: C, 33.24; H, 3.14; N, 19.38. Found: C, 33.33; H, 3.09; N, 19.39.
12. 5-Chloro-6-[2-(oxoimidazolidinyl)methyl]uracil (**2b**): mp 233-235 °C. ^{1}H-NMR (CD_3OD-d_4) δ: 3.43–3.48 (m, 4H), 4.56 (s, 2H). FAB-MS *m/z*: 245, 247 $(M+H)^+$. HRFAB-MS: Calcd for $C_8H_{10}{}^{35}ClN_4O_3$: 245.0041. Found: 245.0044. Calcd for $C_8H_{10}{}^{37}ClN_4O_3$: 247.0412. Found: 247.0421.
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14. [^{11}C]$COCl_2$ was synthesized from [^{11}C]methane *via* [^{11}C]CCl_4. [^{11}C]methane was produced using an ultracompact cyclotron by the ^{14}N (p, α) ^{11}C nuclear reaction on nitrogen containing hydrogen (5%) in an aluminum target. Bombardment was performed using a 20 μA beam of 18 MeV protons for 20 min.
15. [^{11}C]$COCl_2$ was bubbled with helium flow into a reaction vial containing a solution of **1a** (1 mg) in *t*-BuOH (0.5 mL) in the presence of *t*-BuOK at 30 °C for 1min. The reaction mixture was subjected to reverse-phase HPLC equipped with UV monitor and radio detector: Megapak CIL C18, 25 cm × 1.0 cm i.d., 10% EtOH in water containing phosphoric acid (pH 2.32), flow rate 5.0 mL/min.