Synthesis and Evaluation of *cis*-1-[4-(Hydroxymethyl)-2-cyclopenten-1-yl]-5-[^{124}I]iodouracil: A New Potential PET Imaging Agent for HSV1-tk Expression

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In our pursuit to find an appropriate reporter probe for herpes simplex virus type-1 thymidine kinase (HSV1-tk), a carbocyclic nucleoside analogue, *cis*-1-[4-(hydroxymethyl)-2-cyclopenten-1-yl]-5-[^{124}I]iodouracil, has been efficiently synthesized. A Pd(0)-catalyzed coupling reaction together with organotin and exchange reactions for radiolabeling gave more than 80% radiochemical yield with greater than 95% radiochemical purity and 1.15 GBq/μmol specific activity. Biological data reveal that the analogue is stable *in vitro*, less toxic than ganciclovir (GCV), and selective to HSV1-tk-expressed cells based on micro positron emission tomography (microPET) image analyses. Thus, this new carbocyclic nucleoside, referred to in this paper as carbocyclic 2′,3′-didehydro-2′,3′-dideoxy-5-iodouridine (carbocyclic d4IU) is a potential imaging probe for HSV1-tk.

Introduction

Gene therapy is a technique that is developing rapidly for the treatment of a number of different cancers. Among the various gene therapy approaches, the prodrug strategy is gaining popularity.[1] This strategy involves delivering a suicide gene (or reporter gene) to target cells and administering prodrugs. The suicide gene encodes novel nonmammalian enzymes, such as herpes simplex virus type-1 thymidine kinase (HSV1-tk), that can convert a relatively nontoxic prodrug into a highly toxic agent.[2] HSV1-tk is one of the most widely used effector gene systems for imaging gene expression. This is because it can be both a suicide gene and a reporter gene. It is a nonspecific nucleoside kinase, making it effective against a variety of tumor models both *in vitro* and *in vivo*.[3] Optimizing the therapeutic effect requires an adequate radiolabeled probe to image the reporter gene. Thus, these probes are currently of high interest in the radiopharmaceuticals and nuclear medicine fields. *In vivo* stability is of the utmost importance for an ideal imaging agent. Therefore, cleavage of the glycosidic bond by thymidine phosphorylase must be prevented. Various radiolabeled nucleoside analogues,[4−7] both pyrimidine and acycloguanosine derivatives, have been synthesized and evaluated for HSV1-tk imaging. Some nucleoside analogues have low uptake rates and low affinity for HSV1-tk.[3c] Others have high selectivity and uptake rates but show appreciable phosphorylation,[8] resulting in *in vivo* instability. To resolve the stability problem, continuous efforts to modify nucleoside analogues are in progress. These include modifying the sugar moiety, such as replacing the furanose O with S[9] or incorporating electron-withdrawing substituents (F and Br) at the 2′ position.[6,9,10] Recently, interest has grown in changing sugar moieties to carbocyclic nucleosides and their analogues. Replacing the furanose oxygen with carbon is of particular interest, because the resulting carbocyclic nucleosides possess greater metabolic stability. They are less susceptible to phosphorylase enzymes that cleave the glycosidic linkage in normal nucleosides.[11] In addition, carbocyclic nucleoside analogues show a wide field of application for antiviral chemotherapy.[12] This paper introduces the synthesis and preliminary evaluation of a new radiolabeled carbocyclic nucleoside analogue for use as a potential HSV1-tk imaging reporter probe.

Results and Discussion

Chemistry. In recent years, our group has been interested in synthesizing carbocyclic nucleosides and their analogues. We have prepared a triol **3**[13] (Scheme 1) that we utilize as our starting material. In this paper, we once again used this triol to synthesize a new carbocyclic nucleoside that is a potential positron emission tomography (PET) imaging agent for HSV1-tk expression. Conversion of **3** to the dicarbonate **5** was carried out using known procedures.[14] The coupling partner, 3-*N*-benzoyluracil (**2**), was prepared by protecting uracil with excess benzoyl chloride.[15] During the workup stage of the reaction, the benzoyl group at *N*-1 was readily deprotected to yield the desired product **2**. Compounds **5** and **2** were coupled using known palladium chemistry, initially introduced by Trost[16a] and Tsuji.[16b] The Pd(0) catalyst was prepared *in situ* from $Pd(OAc)_2$ in THF with triisopropyl phosphite and *n*-BuLi.[17] This in turn produced the π-allylpalladium complex of compound **5** which undergoes nucleophilic attack by the anion on the pyrimidine base **2**. The reaction proceeded *via* 1,4-addition because 1,2-addition was sterically hindered by a nonbonding interaction with the substituent in the cyclopentene ring. Hydrolysis of the coupling product **6** gave carbocyclic 2′,3′-didehydro-2′,3′-dideoxyuridine (**7**, carbocyclic d4U) in 90% yield. Iodination and radioiodination of **7** followed; however, this produced several side products. The side products are presumably due to the presence of multiple iodination sites, one in the pyrimidine base and another in the carbocyclic ring. This observation led us to modify our strategy.

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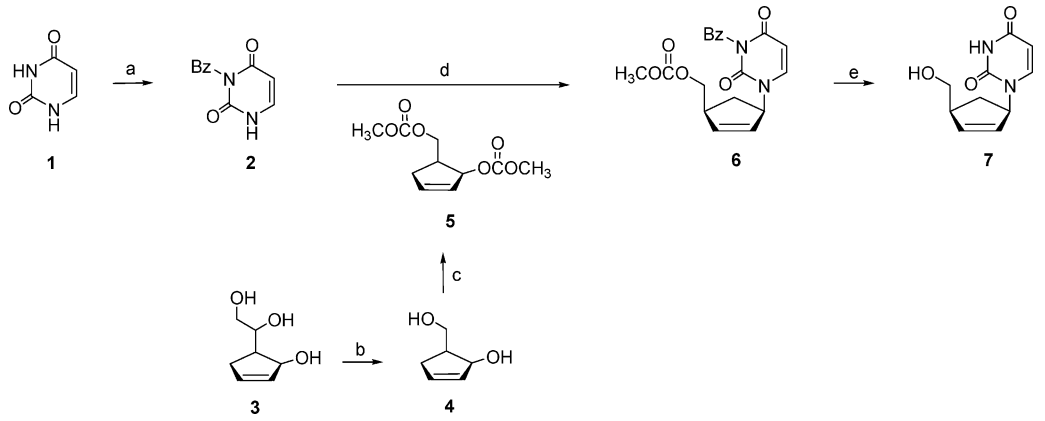
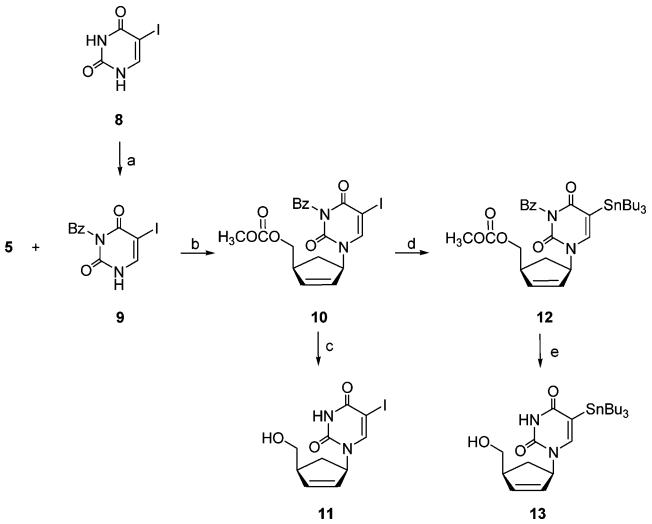
Scheme 1. Synthesis of Carbocyclic d4U[a]



[a] Reagents and conditions: (a) Benzoyl chloride, anhydrous CH_3CN, pyridine, 12 h, 0 °C, 65%; (b) (i) $NaIO_4$, diethyl ether, H_2O, 2 h, (ii) ethylene glycol, 1 h, (iii) $NaBH_4$, 2 h, 70%; (c) methyl chloroformate, DMAP (cat.), $CHCl_3$, pyridine, 0 °C, 1 h, 93%; (d) (i) $Pd(OAc)_2$, (*i*-PrO)$_3$P, THF, under argon, rt, (ii) *n*-BuLi, hexane, rt, (iii) **2** in DMSO, (iv) **5** in anhydrous THF, 12 h, 93%; (e) (i) 0.50 N aqueous K_2CO_3, rt, 1 d, (ii) 0.1 N HCl, 90%.

Scheme 2. Synthesis of Precursor **13**[a]

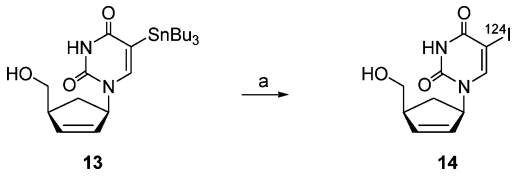


[a] Reagents and conditions: (a) Benzoyl chloride, anhydrous CH_3CN, pyridine, 12 h, 0 °C, 63%; (b) (i) $Pd(OAc)_2$, (*i*-PrO)$_3$P, THF, under argon, rt, (ii) *n*-BuLi, hexane, rt, (iii) **9** in DMSO, (iv) **5** in anhydrous THF, 12 h, 88%; (c) (i) 0.50 *N* aqueous K_2CO_3, rt, 12 h, (ii) dry ice, 70%; (d) (i) $Pd(PPh_3)_4$, 1,4-dioxane, (ii) bis(tributyltin), 100 °C, 7 h, 73%; (e) (i) 0.50 N aqueous K_2CO_3, rt, 12 h, (ii) dry ice, 74%.

A combination of organotin and exchange reaction has been report as a facile strategy to radiolabeled compounds.[18] Thus, we took advantage of this technique. To facilitate this transformation we coupled 3-*N*-benzoyl-5-iodouracil (**9**) (Scheme 2) to dicarbonate **5** in lieu of compound **2**. Compound **9** was synthesized from 5-iodouracil in the same manner as compound **2**. The coupling iodo product **10** was obtained using aforementioned procedures. Compound **10** was hydrolyzed to produce *cis*-1-[4-(hydroxymethyl)-2-cyclopenten-1-yl]-5-iodouracil (**11**), the reference compound. On the other hand, **10** was converted to a stannylated derivative **12** by the palladium-catalyzed condensation of bis(tributyltin), subsequent hydrolysis gave the desired stannylated nucleoside precursor **13**. All compounds prepared are racemic in nature.

The radioiodination of **13**, as shown in Scheme 3, was performed using a no-carrier-added iodine-124 produced *via* the ^{125}Te(p, 2n) ^{124}I nuclear reactions on enriched $^{125}TeO_2$. The labeled compound was separated, collected, and analyzed using reverse phase HPLC with UV and radioactivity detectors. The HPLC chromatographic retention time of **14** was 15.725 min with a radiochemical yield >80% and radiochemical purity

Scheme 3. Radioiodination of Precursor **13**[a]



[a] Reagents and conditions: (a) (i) Na^{124}I, 0.01 N NaOH, MeOH, (ii) 1.0 N HCl to pH 4.0, 30% H_2O_2, 10 min, (iii) $NaHSO_3$, 80%, radiochemical purity >99%, specific activity 1.15 GBq/μmol.

>99%. The specific activity was 1.15 GBq/μmol following purification. We confirmed that no side products were produced aside from the unreacted radioiodide based on radio-TLC. Moreover, HPLC analysis showed that radiolabeled and reference compounds have the same retention times. It should be noted that radioiodination was carried out using the unprotected stannyl precursor **13** despite the ease of handling the protected precursor **12**. Deprotection after radioiodination requires additional undesirable laboratory handling of radioactive materials, which may result in some loss of radioiodinated product and additional exposure to radioactivity. Finally, we proved that the unprotected stannyl precursor **13** exclusively gave the desired product *cis*-1-[4-(hydroxymethyl)-2-cyclopenten-1-yl]-5-[^{124}I]-iodouracil (**14**), which we will refer to as carbocyclic 2′,3′-didehydro-2′,3′-dideoxy-5-iodouridine (carbocyclic d4IU).

Biology. The newly synthesized carbocyclic d4IU was evaluated for biological characteristics. We hypothesized that using a cyclopentene ring in lieu of the sugar moiety would confer stability on the compound against glycosidic bond cleavage due to the absence of the anomeric effect. To examine the metabolic stability, we compared carbocyclic d4IU and 5-iododeoxyuridine (IDU) stability using the mouse liver microsome and mouse serum tests (Figures 1 and 2). In the mouse liver microsome stability test, the $t_{1/2}$ for IDU and carbocyclic d4IU was 1.24 h and 3.40 h, respectively (based on a 30 min incubation). This shows that carbocyclic d4IU is three times more stable than IDU. The mouse serum stability test showed that the $t_{1/2}$ for IDU and carbocyclic d4IU were 22.6 h and 248 h, respectively (based on a 6 h incubation), an eleven-fold difference in stability.

Furthermore, the cellular uptake of the carbocyclic d4IU was determined in two cell lines, the MCA hepatoma cell line (derived from the Morris hepatoma RH 7777 cell line) and the MCA-TK cell line (derived from HSV1-tk-expressing cells using a retroviral vector). The cellular uptake in these two cell lines

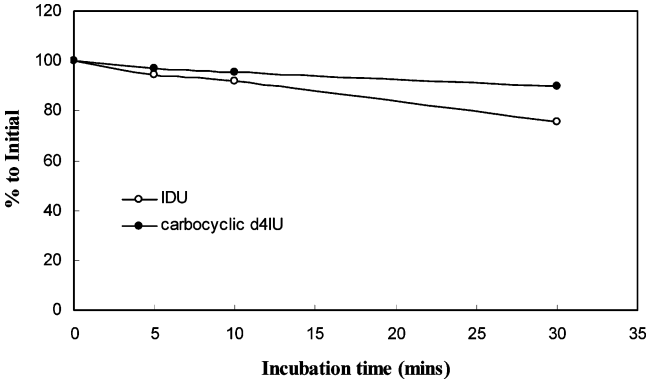


Figure 1. Mouse liver microsome stability.

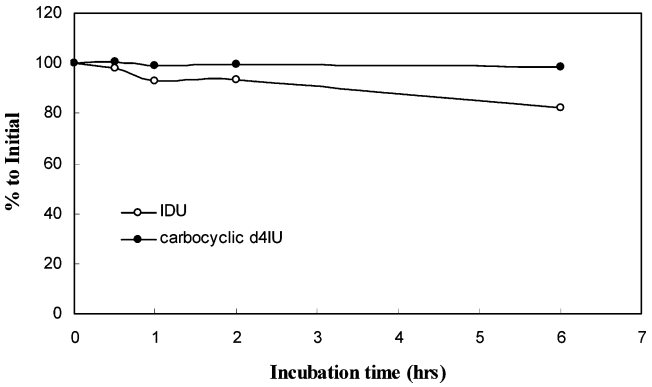


Figure 2. Mouse serum stability.

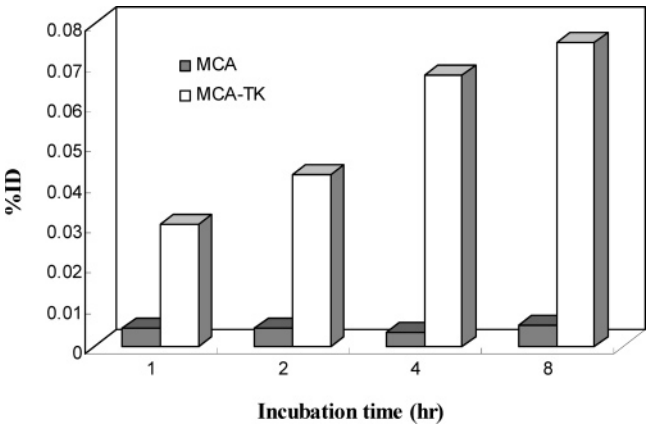


Figure 3. *In vitro* cellular uptake of carbocyclic [^{125}I]d4IU(0.6 μCi) in MCA-TK and MCA cell.

was measured using no-carrier-added carbocyclic [^{125}I]d4IU at 0.6 μCi. Figure 3 shows that the cellular uptake in MCA-TK cells was much higher than that in MCA cells, in a time-dependent manner. We checked the effect of the radioactivity dose on cellular uptake in term of %ID (injected dose) and confirmed that carbocyclic d4IU uptake increases proportionally (Table 1).

The *in vitro* cytotoxicity of carbocyclic d4IU was determined by measuring the cell survival at different concentrations (Figure 4). The IC_{50} was found to be 2.52 mM in MCA cells and 3.08 mM in MCA-TK cells. For comparison, these cytotoxicity data were matched up to ganciclovir (GCV) (Figure 5). Cytotoxicity test of GCV showed the IC_{50}s for MCA and MCA-TK cells to be 0.27 mM and 0.15 μM, respectively. Thus, carbocyclic d4IU is much less toxic than GCV (Figure 6).

MicroPET images (coronal and traverse) of normal mice using carbocyclic [^{124}I]d4IU are shown in Figure 7 to measure the *in vivo* effects. Tumors were grown with MCA cells: wild type cells on the left thigh and HSV1-tk-transduced cells on the right thigh. The microPET images revealed that the carbocyclic [^{124}I]-d4IU accumulated in the HSV1-tk-transduced tumor grafted mouse tissue with a maximum uptake at 3 h postinjection. There was more radioactivity in TK-expressing tumors than in wild type tumors. In the latter, accumulation did not differ significantly from body background levels. According to microPET images, carbocyclic d4IU uptake by MCA-TK decreases over time. These images suggest that even though carbocyclic d4IU is transported into cells and is phosphorylated, it is unlikely to be used as a DNA polymerase substrate and thus is eventually excreted from cell.

This data were corroborated by the radiation counts at the region of interest (Figure 8). The total radiation counts decreased with time while the MCA-TK/MCA ratio remained constant (Table 2). These data show that radioiodinated carbocyclic d4IU is selective to MCA-TK cells at any time and thus is a potential reporter probe for HSV1-tk monitoring.

In the microPET images, it is worth noting that the radioiodinated compound accumulates preferentially in the abdominal region. To verify the specific location, normal mice ($n = 4$) were injected intravenously with 100 μCi carbocyclic d4IU. After 5 h, the mice were sacrificed and organs were excised. The radioactivity of each organ was measured and expressed as a percentage of the injected dose per gram of tissue (% ID/g). The biodistribution data showed that accumulation in the abdominal region was restricted to the stomach (Figure 9). This intraluminal localization of radioactivity in the stomach was due to deiodination of carbocyclic [^{124}I]d4IU. The microPET images [^{124}I]d4IU could be improved by blocking with cold iodine. Furthermore, the lipophilicity of carbocyclic [^{124}I]d4IU was also checked and was found to be 0.29 (log *P*).

By and large, we have synthesized carbocyclic d4IU efficiently, and our biological data demonstrate that it is a potential agent for tumor imaging.

Experimental Section

^{1}H and ^{13}C NMR spectra were obtained using a Bruker 300 spectrometer (300 MHz). Infrared spectra were recorded on a Bio-Rad FTS 6000 FT-IR spectrometer. Uncorrected melting points were determined with a Gallenkamp melting point apparatus. HRMS were obtained on a JMS 700 spectrometer. Analytical thin layer chromatography (TLC) was conducted on E. Merck 60 F254 aluminum-backed silica gel plates (0.2 mm) with a fluorescent indicator. Radio-TLC was measured on a Bioscan AC-3000 scanner (Washington D.C.). High performance liquid chromatography (HPLC) was carried out on μBondapak C18 column (3.9 × 300 mm, Waters). Developed plates were visualized with UV light or a 2.0% phosphomolybdic acid staining solution. Flash column chromatography was performed using Merck silica gel 60 (230−400 mesh) under positive pressure according to the still procedure.[19] All reagents and solvents were reagent grade and purified by known procedures before use.[20]

***cis*-5-Hydroxymethylcyclopent-2-en-1-ol (4)**. The diol **4** was prepared from a triol **3** by a known procedure.[13] IR (thin film) 3423, 3058, 2930, 1615, 1441, 1350, 1155, 1019, 951, 891, 723 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.98 (m, 1H), 5.82 (m, 1H), 4.89 (m, 1H), 3.78 (m, 2H), 3.30 (br s, 1H), 3.06 (br s, 1H), 2.43 (m, 1H), 2.32 (m, 1H), 2.16 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 135.01, 132.30, 77.69, 62.69, 42.59, 33.65.

***cis*-Methyl 5-(methoxycarbonyloxymethyl)cyclopent-2-enyl-1-carbonate (5)**. Pyridine (10 mL) and DMAP (0.170 g, 1.38 mmol) were added to a diol **4** solution (2.00 g, 17.5 mmol) in anhydrous $CHCl_3$ (20 mL) at 0 °C. Methyl chloroformate (20.3 mL, 263.1 mmol) in pyridine (10 mL) was slowly added using a dropping funnel at 0 °C. After stirring for 1 h, the reaction mixture

Table 1. *In Vitro* Cellular Uptake of Carbocyclic [^{125}I]d4IU(0.5 μCi and 2 μCi) in MCA-TK and MCA Cells

		MCA		MCA-TK		
radiation dose, μCi	total uptake	uptake	%ID	uptake	%ID	%ID MCA-TK/MCA
0.50	361858.02	16.82	0.0046	804.17	0.2222	47.81
2.0	1887442.89	28.87	0.0015	3953.37	0.2095	136.94

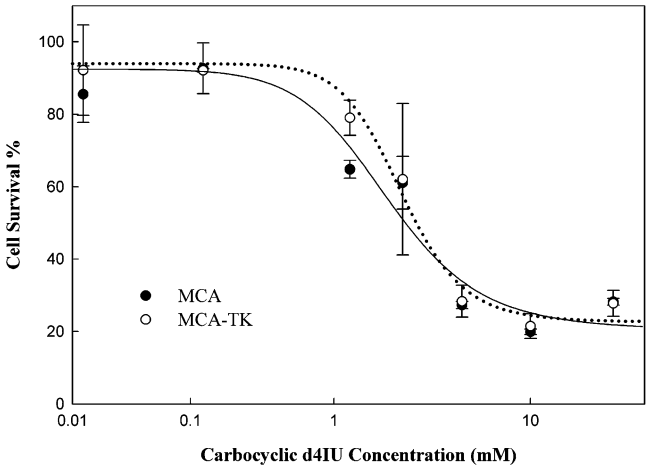


Figure 4. *In vitro* cytotoxicity of carbocyclic d4IU in MCA and MCA-TK cell.

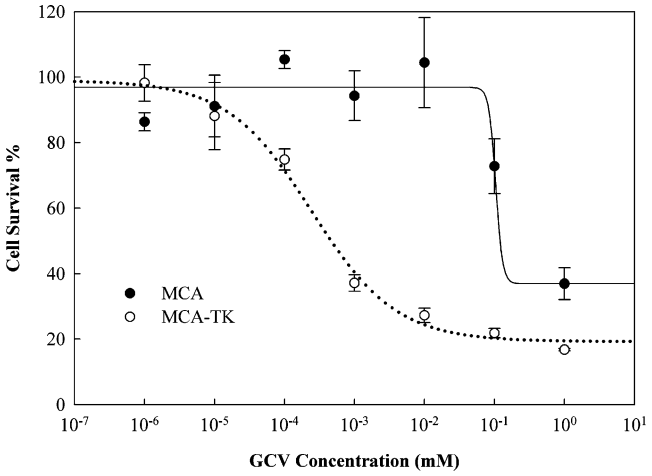


Figure 5. *In vitro* cytotoxicity of GCV in MCA and MCA-TK cell.

was diluted with $CHCl_3$ (30 mL) and washed with brine solution (30 mL × 3). The aqueous phase was extracted with $CHCl_3$ (30 mL × 3). The organic phase was collected, dried with anhydrous $MgSO_4$, and concentrated by rotary-evaporation. The residue was purified by silica gel column chromatography (ethyl acetate:hexane = 1:6, v/v) to give a colorless oil **5** (R_f = 0.26; 3.70 g, 93%). IR (thin film): 2958, 2916, 2855, 1742, 1442, 1252, 945, 788 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 6.14 (m, 1H), 5.91 (m, 1H), 5.62 (dd, J = 4.5, 1.8 Hz, 1H), 4.35 (m, 1H), 4.22 (m, 1H), 3.77 (s, 3H), 3.76 (s, 3H), 2.75 (dd, J = 14.57, 7.2 Hz, 1H), 2.50 (m, 1H), 2.30 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 155.52, 155.20, 137.67, 128.84, 82.01, 66.81, 54.84, 54.74, 39.92, 34.65.

3-*N*-Benzoyluracil (2).[15] Benzoyl chloride (4.2 mL, 35.7 mmol) was added to a uracil solution (2.00 g, 17.8 mmol) in anhydrous pyridine (30 mL) and anhydrous acetonitrile (75 mL) at 0 °C. After stirring for 12 h, cold water (50 mL) was added to the reaction mixture. The solvent was removed by rotary-evaporation, and the residue was diluted with methanol (50 mL). Silica gel (~10 g) was added to the solution, and the resulting suspension was dried under reduced pressure. A white solid **6** (R_f = 0.3; 2.37 g, 65%) was obtained from preloaded silica gel column chromatography ($CHCl_3$:MeOH = 30:1, v/v). ^{1}H NMR (DMSO-d_6): δ 11.60 (br s, 1H), 8.00−7.49 (m, 6H), 5.77 (dd, J = 19.5, 9.0 Hz, 1H).

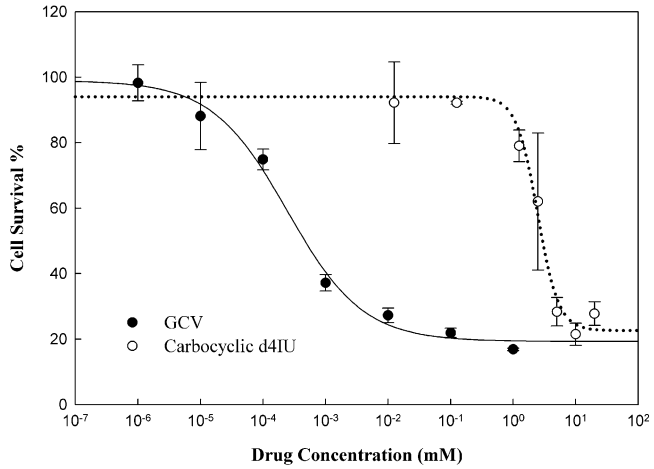


Figure 6. *In vitro* cytotoxicity comparison of carbocyclic d4IU and GCV in MCA-TK cell.

***cis*-3-*N*-Benzoyl-1-[4′-(methoxycarbonyloxymethyl)cyclopent-2′-enyl]uracil (6)**. Triisopropyl phosphite (0.086 mL, 0.35 mmol) was added to a solution of $Pd(OAc)_2$ (0.020 g, 0.087 mmol) at ambient temperature in anhydrous THF (4.0 mL) under argon. After stirring for 5 min, *n*-BuLi (1.6 N in hexane, 0.11 mL, 0.17 mmol) was added at ambient temperature. The resulting mixture was stirred for 5 min to obtain the tetrakis(triisopropyl phosphite)palladium-(0) catalyst. The *in situ*-prepared Pd(0) catalyst was added to the solution of **2** (0.21 g, 1.04 mmol) in anhydrous DMSO (7.0 mL) *via* cannula at ambient temperature. Next, a solution of **5** (0.20 g, 0.87 mmol) in anhydrous THF (3.0 mL) was added to the reaction mixture. After stirring for 12 h, the reaction mixture was diluted with $CHCl_3$ (15 mL) and washed with a brine solution (20 mL × 3). The aqueous phase was extracted with $CHCl_3$ (20 mL × 2). The organic phase was collected, dried with anhydrous $MgSO_4$, and concentrated by rotary-evaporation. The residue was purified by silica gel column chromatography (ethyl acetate:hexane = 2:3 to 1:1, v/v) to give a colorless syrup **6** (R_f = 0.25; 0.30 g, 93%). mp 114−117 °C; IR (KBr pellet) 1746, 1700, 1653, 1600, 1277 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.98−7.94 (m, 2H), 7.70−7.67 (m, 1H), 7.54−7.46 (m, 3H), 6.15 (m, 1H), 5.83 (d, J = 9.0 Hz, 1H), 5.77 (m, 2H), 4.34−4.15 (m, 2H), 3.82 (s, 3H), 3.14 (m, 1H), 2.83 (m, 1H), 1.54 (m, 1H); ^{13}C NMR ($CDCl_3$): δ 169.13, 162.17, 155.49, 149.87, 141.29, 138.46, 134.99, 131.50, 130.28, 130.01, 129.10, 101.94, 69.68, 61.63, 54.77, 44.12, 33.35; HRMS: calcd for $C_{19}H_{18}N_2O_6$ [(M + Na)$^+$, FAB] 393.1063 found 393.1057.

***cis*-1-[4′-(Hydroxymethyl)cyclopent-2′-enyl]uracil (7)**. Compound **6** (0.073 g, 0.197 mmol) was added to 0.50 N aqueous potassium carbonate (5.0 mL) and stirred at room temperature for 24 h. The reaction mixture was neutralized to pH 7−8 with dry ice. After removal of the solvent by rotary-evaporation, the residue was diluted with methanol (10 mL). Silica gel (~2.0 g) was added to the solution, and the resulting suspension was dried under reduced pressure. A white solid **7** (R_f = 0.23; 0.037 g, 90%) was obtained from preloaded silica gel column chromatography ($CHCl_3$:MeOH = 20:1 to 15:1, v/v). mp 165−167 °C; IR (KBr pellet) 3467, 3155, 1694, 1609, 1268, 1254 cm^{-1}; ^{1}H NMR (D_2O): δ 7.65 (d, J = 8.1 Hz, 1H), 6.21 (m, 1H), 5.83 (d, J = 7.8 Hz, 1H), 5.78 (m, 1H), 5.55 (m, 1H), 3.63 (m, 2H), 2.99 (m, 1H), 2.70 (m, 1H), 1.43 (m, 1H); ^{13}C NMR (D_2O): δ 163.80, 151.52, 142.24, 139.97, 130.00, 101.81, 63.03, 61.02, 47.85, 35.53; HRMS: calcd for $C_{10}H_{12}N_2O_3$ [(M + Na)$^+$, FAB] 231.0746 found 231.0744.

3-*N*-Benzoyl-5-iodouracil (9). Benzoyl chloride (2.35 mL, 20.17 mmol) was added to an iodouracil solution (2.00 g, 8.40 mmol) in

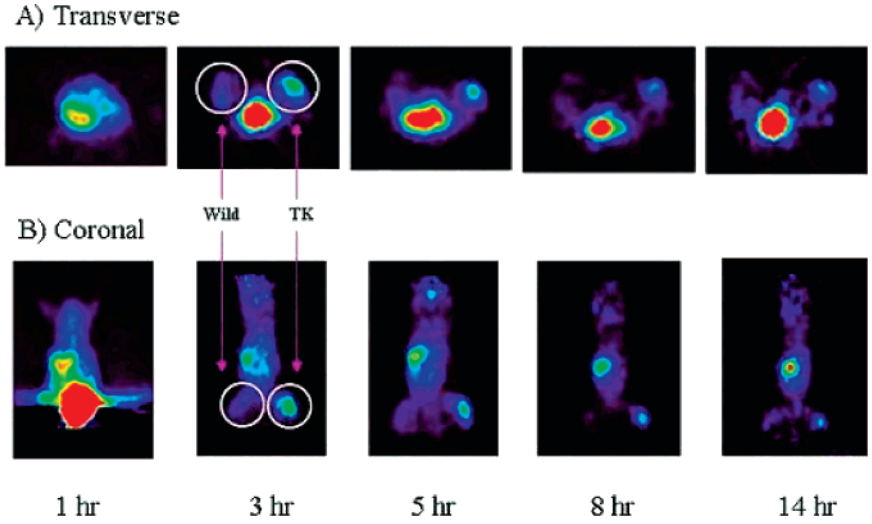


Figure 7. MicroPET Images of carbocyclic [^{124}I]d4IU at various times of MCA (left thigh) and MCA-TK (right thigh)-bearing nude mice.

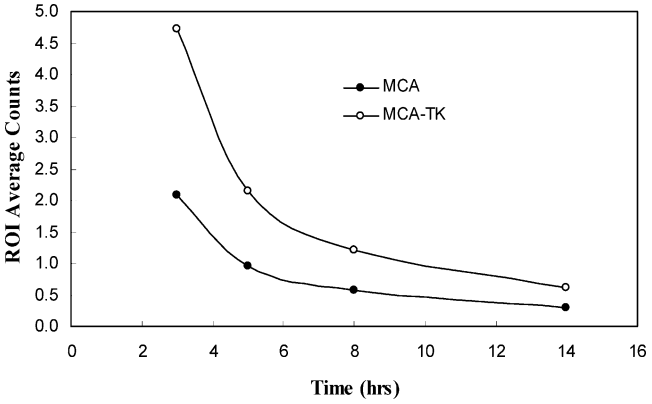


Figure 8. Radioactivity count of carbocyclic [^{124}I]d4IU in MCA and MCA-TK tumors from the microPET image.

Table 2. Radioactivity Count Ratio of Carbocyclic [^{124}I]d4IU in MCA and MCA-TK Tumors from the MicroPET Image

	average counts		
time (h)	MCA-TK(uCi/cc)	MCA(uCi/cc)	MCA-TK/MCA ratio
3	4.72	2.09	2.26
5	2.16	0.97	2.23
8	1.22	0.57	2.13
14	0.61	0.29	2.10

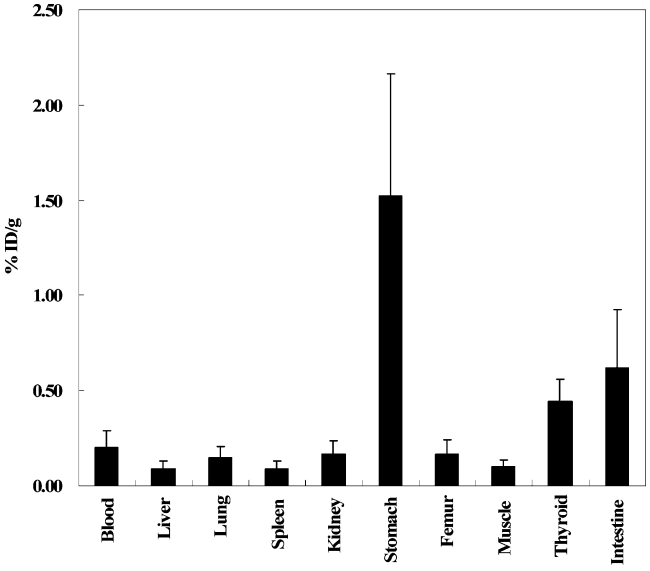


Figure 9. Biodistribution of carbocyclic [^{124}I]d4IU, 5 h after injection in normal mice ($n = 4$).

anhydrous pyridine (20 mL) and anhydrous acetonitrile (50 mL) at 0 °C. After stirring for 12 h, cold water (30 mL) was added to the reaction mixture. The solvent was removed by rotary-evaporation, and the residue was diluted with methanol (30 mL). Silica gel (~7 g) was added to this solution, and the resulting suspension was dried under reduced pressure. A white solid **9** ($R_f = 0.27$; 2.36 g, 63%) was obtained from preloaded silica gel column chromatography ($CHCl_3$:MeOH = 50:1 to 30:1, v/v). ^{1}H NMR (DMSO-d_6): δ 11.97 (br s, 1H), 8.16−8.15 (m, 1H), 8.07−7.98 (m, 2H), 7.81−7.76 (m, 1H), 7.69−7.53 (m, 2H).

***cis*-3-*N*-Benzoyl-1-[4′-(methoxycarbonyloxymethyl)-cyclopent-2′-enyl]-5-iodouracil (10).** Triisopropyl phosphite (0.11 mL, 0.46 mmol) was added to a solution of $Pd(OAc)_2$ (0.026 g, 0.14 mmol) in anhydrous THF (4.0 mL) at ambient temperature under argon. After stirring for 5 min, *n*-BuLi (1.6 N in hexane, 0.14 mL, 0.23 mmol) was added at ambient temperature. The resulting mixture was stirred for 5 min to obtain the tetrakis(triisopropyl phosphite)-palladium(0) catalyst. The *in situ*-prepared Pd(0) catalyst was added to a solution of 3-*N*-benzoyl-5-iodouracil **9** (0.59 g, 1.71 mmol) in anhydrous DMSO (8.0 mL) *via* cannula at ambient temperature. A solution of **5** (0.26 g, 1.14 mmol) in anhydrous THF (4.0 mL) was added to the reaction mixture. After stirring for 12 h, the reaction mixture was diluted with $CHCl_3$ (20 mL) and washed with brine solution (20 mL × 3). The aqueous phase was extracted with $CHCl_3$ (20 mL × 2). The organic phase was collected, dried with anhydrous $MgSO_4$, and concentrated by rotary-evaporation. The residue was purified by silica gel column chromatography (ethyl acetate:hexane = 1:2, v/v) to give a white solid **10** ($R_f = 0.24$; 0.50 g, 88%). mp 211−213 °C; IR (KBr pellet) 1741. 1691, 1656, 1600, 1274, 1254 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 7.95−7.92 (m, 2H), 7.84 (s, 1H), 7.70−7.65 (m, 1H), 7.54−7.49 (m, 1H), 6.19 (m, 1H), 5.78 (m, 1H), 5.71 (m, 1H), 4.63 (m, 1H), 4.39−4.14 (m, 2H), 3.85 (s, 3H), 3.14 (m, 1H), 2.85 (m, 1H), 1.57 (m, 1H); ^{13}C NMR ($CDCl_3$): δ 167.94, 159.00, 155.68, 149.75, 145.64, 139.46, 135.18, 131.13, ,130.67, 130.54, 129.93, 129.19, 68.96, 61.93, 55.00, 44.35, 33.48; HRMS: calcd for $C_{19}H_{17}IN_2O_6$ [$(M + Na)^+$, FAB] 519.0029 found 519.0026.

***cis*-1-[4′-(Hydroxymethyl)cyclopent-2′-enyl]-5-iodouracil (11).** Compound **10** (0.030 g, 0.060 mmol) was added to 0.50 N aqueous potassium carbonate (5.0 mL) and stirred at ambient temperature for 12 h. The reaction mixture was neutralized to pH 7−8 with 0.1 N aqueous HCl. After removal of the solvent by rotary-evaporation, the residue was diluted with methanol (10 mL). Silica gel (~1.0 g) was added to the solution, and the resulting suspension dried under the reduced pressure. A white solid **11** ($R_f = 0.22$; 0.014 g, 70%) was obtained from preloaded silica gel column chromatog-

raphy (CH_2Cl_2:MeOH = 25:1 to 20:1, v/v). mp 193−194 °C; IR (KBr pellet) 3466, 3156, 1693, 1609, 1268, 1254 cm^{-1}; ^1H NMR (CD_3OD): δ 8.05 (s, 1H), 6.18 (dd, J = 7.5, 2.4 Hz, 1H), 5.74 (dd, J = 7.2, 2.1 Hz, 1H), 5.60 (m, 1H), 3.73−3.53 (m, 2H), 2.94 (m, 1H), 2.66 (m, 1H), 1.53 (m, 1H); ^{13}C NMR (CD_3OD): δ 161.76, 151.40, 147.11, 140.01, 129.13, 127.22, 66.40, 63.26, 61.78, 47.60. 32.84; HRMS: calculated for $C_{10}H_{11}IN_2O_3$ [$(M + H)^+$, FAB] 334.9893 found 334.9894.

***cis*-3-*N*-Benzoyl-1-[4′-(methoxycarbonyloxymethyl)cyclopent-2′-enyl]-5-tributylstannyluracil (12)**. Bis(tributyltin) (0.13 mL, 0.26 mmol) and $Pd(PPh_3)_4$ (0.0070 g, 0.0060 mmol) were added to a solution of compound **10** (0.060 g, 0.12 mmol) in anhydrous 1,4-dioxane (5 mL) at ambient temperature. The reaction mixture was stirred at 100 °C for 7 h. Next, the reaction mixture was diluted with $CHCl_3$ (10 mL) and washed with brine solution (10 mL × 3). The aqueous phase was extracted with $CHCl_3$ (20 mL × 2). The organic phase was collected, dried with anhydrous $MgSO_4$, and concentrated by rotary-evaporation. The residue was purified by silica gel column chromatography (ethyl acetate:hexane = 1:6 to 1:4, v/v) to give a pale yellow oil **12** (R_f = 0.27; 2.73 g, 73%). IR (thin film) 1751, 1695, 1648, 1600, 1269, 1220 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.94−7.91 (m, 2H), 7.66−7.61 (m, 1H), 7.51−7.46 (m, 2H), 7.18 (s, 1H), 6.15 (dd, J = 7.5, 2.1 Hz, 1H), 5.80 (dd, J = 5.4, 2.1 Hz, 1H), 5.72 (m, 1H), 4.26−4.12 (m, 2H), 3.81 (s, 3H), 3.14 (m, 1H), 2.80 (m, 1H), 1.64−0.87 (m, 28H); ^{13}C NMR ($CDCl_3$): δ 169.75, 165.45, 155.73, 150.49, 144.17, 137.72, 134.74, 131.82, 130.75, 130.40, 128.99, 112.95, 69.68, 61.20, 54.84, 44.13, 34.00, 28.80, 27.16, 26.76, 13.52, 9.92.

***cis*-1-[4′-(Hydroxymethyl)-cyclopent-2′-enyl]-5-tributylstannyluracil (13)**. Compound **12** (0.036 g, 0.054 mmol) was added to 0.50 N aqueous potassium carbonate (5.0 mL). The mixture was stirred at ambient temperature for 12 h. The reaction mixture was neutralized to pH 7−8 with dry ice. After removal of the solvent by rotary-evaporation, the residue was diluted with methanol (10 mL). Silica gel (∼1.0 g) was added to the solution, and the resulting suspension was dried under reduced pressure. A white syrup **13** (R_f = 0.35; 0.020 g, 74%) was obtained from preloaded silica gel column chromatography (CH_2Cl_2:MeOH = 50:1 to 30:1, v/v). IR (thin film) 3402, 3183, 1695, 1653, 1612, 1254, 1220 cm^{-1}; ^1H NMR (CD_3OD): δ 7.49 (s, 1H), 6.18 (dd, J = 5.7, 1.8 Hz, 1H), 5.73 (dd, J = 5.7, 2.1 Hz, 1H), 5.65 (m, 1H), 3.59 (m, 2H), 2.94 (m, 1H), 2.65 (m, 1H), 1.57−0.89 (m, 28H); ^{13}C NMR (CD_3OD): δ 167.77, 152.09, 145.83, 139.09, 129.50, 111.54, 64.14, 61.07, 47.47, 33.17, 28.75, 26.87, 12.62, 9.21; HRMS: calculated for $C_{22}H_{38}N_2O_3Sn$ [$(M + Na)^+$, FAB] 521.1802 found 521.1801.

Production of Iodine-124 and Radioiodination. No-carrier-added iodine-124 was produced *via* the $^{125}Te(p, 2n)^{124}I$ nuclear reactions on enriched $^{125}TeO_2$ at the KIRAMS MC-50 cyclotron. Approximately 4.0 mg of the tributylstannylated compound **13** was dissolved in 5.0 mL of MeOH. A 10 mCi (370 MBq) amount of radioiodide ($Na^{124}I$) in 0.01 N aqueous NaOH was added to the precursor **13** solution (100 μL). The reaction mixture was acidified to pH 4.0 with 1.0 N aqueous HCl, and 30% H_2O_2 (50 μL) was added. After stirring for 10 min at ambient temperature, the reaction mixture was quenched with saturated $NaHSO_3$. Purification of the radioiodinated product **14** was performed by HPLC separation on a μBondapak C 18 column (3.9 mm × 300 mm). The product was eluted at a flow rate of 2.0 mL/min with 0.1% trifluoroacetic acid in water/EtOH (water:EtOH = 4:1, v/v). The retention time of the product was 15.725 min. The radiochemical purity of the product was determined by the HPLC system used for purification and radio-thin layer chromatography (radio-TLC). Radio-TLC was performed on a silica gel plate (Merck 60 F254 aluminum-backed silica gel plates), which was developed with acetonitrile/water (19:1, v/v) solvent (R_f = 0.8 for product and 0.08 for unreacted iodide). The total elapsed time was about 80 min, including radioiodination and HPLC purification. The overall radiochemical yield and purity were more than 80% (296 MBq) and 95%, respectively. The specific activity was 1.15 GBq/μmol following purification.

Mouse Serum Stability test. Mouse serum (Innovative Research Inc., # IMS-BCSER) was preincubated for 5 min at 37 °C. A 10 μL amount of 5.0 mM drug stock solution (in 100% DMSO) was added to 990 μL of mouse serum in duplicate and thoroughly mixed. A 200 μL amount of the mixture was dispensed into four glass tubes and incubated at 37 °C for 0, 30, 60, or 120 min. A 3.0 mL amount of ethyl acetate was added and mixed for 1 min to terminate the reaction. The mixture was centrifuged at 3000 rpm for 30 min at 4 °C. The supernatant was transferred to a new glass tube, and the solvent was evaporated for 40 min using a centrifugal concentrator. A 200 μL amount of the mobile phase was added to the concentrated mixture and mixed for 10 min. The mixture was centrifuged at 3000 rpm for 10 min at 4 °C again. The sample solution was transferred to a filter plate (Millipore, Solvinert plate, 0.45 μm) and filtered with centrifugation at 440*g* for 3 min at 4 °C. HPLC analysis followed (Instrument: Waters 2695 separations module, Waters 2487 UV detector; conditions: column: Hypersil GOLD, 2.1 × 150 mm, 5 μm, Thermo Electron Corporation; mobile phase: methanol:5.0 mM ammonium acetate = 10:90 (IDU) and methanol: 5.0 mM ammonium acetate = 20:80 (carbocyclic d4IU); Flow rate: 0.2 mL/min, λ = 260 nm; injection volume = 20 μL; column temperature: 40 °C).

Mouse Liver Microsome Stability Test. Mouse liver microsomes (Innovative Research Inc., no. IMS-BCSER) were preincubated for 3 min at 37 °C. The reaction was started with 25 μL of NADPH (10 mM) and 5 μL of 5.0 mM drug stock solution (in 100% DMSO). This was added to 990 μL of pooled mouse liver microsomes in duplicate and thoroughly mixed. A 200 μL amount of the mixture was dispensed into four glass tubes and incubated at 37 °C for 0, 30, 60, or 120 min, and 3.0 mL of ethyl acetate was added and mixed for 1 min to terminate the reaction. The mixture was centrifuged at 3000 rpm for 30 min at 4 °C. The supernatant was transferred to a new tube, and the solvent was evaporated for 40 min using centrifugal concentrator. A 250 μL amount of the mobile phase was added to the concentrated mixture and mixed for 10 min. The mixture was centrifuged at 3000 rpm for 10 min at 4 °C. The sample solution was transferred to a filter plate (Millipore, Solvinert plate, 0.45 μm) and filtered with centrifugation at 440*g* for 3 min at 4 °C. HPLC analysis followed (Instrument: Waters 2695 separations module, Waters 2487 UV detector; conditions: column: Hypersil GOLD, 2.1 × 150 mm, 5 μm, Thermo Electron Corporation; mobile phase: methanol:5.0 mM ammonium acetate = 10:90 (IDU) and methanol:5.0 mM ammonium acetate = 20:80 (carbocyclic d4IU); flow rate: 0.2 mL/min, λ = 260 nm; injection volume = 50 μL; column temperature: 40 °C).

***In Vitro* Cyctotoxicity Test.** Exponentially growing cells were trypsinized and collected in the respective culture medium. Cell concentrations were adjusted to 2.5 × 10^4 cells/mL in the corresponding medium. Cells (2 × 10^3/80 μL) were seeded into 96-well plates and incubated at 37 °C with 5.0% CO_2 and 95% air for a moment. Test compounds were dissolved in 20 μL growth medium at the desired concentration and added to each well. After 5 d incubation at 37 °C with 5.0% CO_2, MTS was added. Following an additional 1−2 h incubation, the absorbance at 492 nm was recorded using a 96-well plate reader (650 nm reference wavelength).

MicroPET Imaging. MCA and MCA-TK tumor cells (1 × 10^6 cells in 100 μL) were subcutaneously injected into the right and left thighs, respectively, of nude mice. After 3 weeks, the presence of a tumor mass was confirmed. Animals were injected with carbocyclic [^{124}I]d4IU (7.4 MBq) in the tail vein. Imaging was performed using a microPET R4 scanner (Concorde Microsystems, Inc.) at 1, 3, 5, 8, and 14 h after injection. Static scans were performed with 30-min acquisitions. Regional tumor radioactivity concentrations (kBq/cm^3) were estimated from the maximum pixels in regions of interest (drawn around the tumor on transverse and coronal slices of the reconstructed image sets). The radioactivity uptake ratio of carbocyclic [^{124}I]d4IU in MCA and MCA-TK tumors from ROI (region of interest) was calculated from the reconstructed images.

Biodistribution. Normal mice (n = 4) were injected intravenously with carbocyclic [^{125}I]d4IU (3.7 MBq). After 5 h, the mice

were sacrificed and each organ was excised and weighed. The radioactivity of each organ was measured with a gamma counter and expressed as a percentage of the injected dose per gram of tissue (% ID/g).

Lipophilicity Procedure. Carbocyclic [^{124}I]d4IU was dissolved in an octanol/H_2O mixture (1:1). The mixture was stirred for 5 min using a vortex machine. The octanol and water layers were separated. The activity (μCi/500μL) of each layer was measured using a dose calibrator. Measurements were performed 10 times, and their average produced the partition coefficient. Lipophilicity was reported as the log of the partition coefficient.

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