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NEW [^{11}C]PHOSGENE BASED SYNTHESIS OF [^{11}C]PYRIMIDINES FOR POSITRON EMISSION TOMOGRAPHY

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Abstract – Thymine, 5-FU, and uracil were successfully synthesized through a procedure involving a cyclocondensation of triphosgene with newly developed α-substituted β-aminoacrylamides intermediates (**1a**, X= Me; **1b**, X= F; **1c**, X= H). The radioligands [2-^{11}C]thymine and [2-^{11}C]5-fluorouracil were synthesized in high radiochemical yields in 16-17 minutes from the end of bombardment by applying the cyclocondensation method with [^{11}C]$COCl_2$.

INTRODUCTION

Thymidine phosphorylase (TP; (EC 2.4.2.4)) is an important enzyme which catalyses reversible deoxyribosylation of thymine (Thy) to thymidine (thymidine + phosphate ⇌ thymine + 2-deoxy-α-D-ribose 1-phosphate). 5-Substituted uracils (5-XUra) including uracil (Ura) can also be substrates for TP. TP is also known to activate 5-fluorouracil (5-FU) to 5-fluoro-2'deoxyribonucleoside, which acts as TP inhibitor.[1] Furthermore, it is reported that TP is associated with angiogenesis as a growth factor, and its expression is strongly associated with the growth of tumors.[2] Thus, TP is an attractive target for imaging and therapy,[3] and many pyrimidine-based radiopharmaceuticals including thymine have been developed for clinical diagnosis in the field of single photon computed tomography (SPECT) or positron emission tomography (PET).[4] In 1991, Vander Borght *et al.* synthesized

This paper is dedicated to Professor Emeritus Keiichiro Fukumoto on the occasion of his 75th birthday.

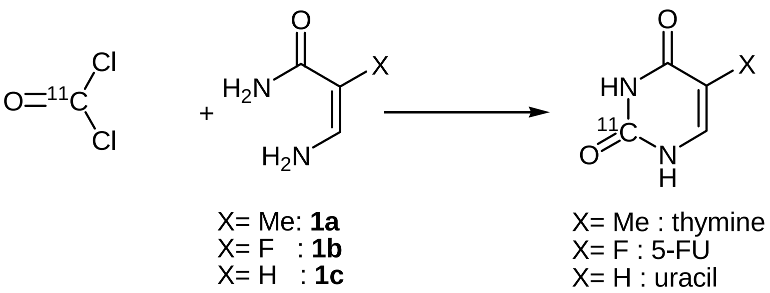
[2-^{11}C]thymine *via* cyclocondensation of diethyl β-methyl malate with [^{11}C]urea.[5] Although other investigators have attempted to improve this method or develop methodologies involving condensation of a malate intermediate with the labeling agent [^{11}C]urea derived from phosgene,[6] cyanide[7] or carbon dioxide as the key ring closure reactions,[8] those condensation reactions were carried out under conditions as drastic as those employed for ^{14}C labeled thymine synthesis.[9] The complexity of the currently available synthetic routes along with the extended length of preparation time has limited the extensive applicability of ^{11}C-labeled nucleosides in PET studies.

5-FU, which was originally synthesized in 1957[10,11] as one of a new class of antitumor fluoroprymidines, has been also an attractive target as a possible PET ligand. 5-FU-mediated inhibition of thymidylate synthetase [12] was subsequently shown to be one of the major mechanisms responsible for the antitumor activity of these compounds. Today, almost 50 years later, 5-FU remains front-line therapy, alone or in combination with other drugs or radiation, for gastric,[13,14] colorectal,[15,16] and other cancers including advanced pancreatic cancer.[17] Development of a diagnostic PET tracer based on 5-FU would be very important to assess or predict more successful outcomes in selecting drugs for cancer chemotherapy. Indeed, it has been demonstrated, for example, that tumor uptake of fluorine-18 labeled 5-FU (5-[^{18}F]FU) serves a positive prognostic role in selection of patients for 5-FU therapy (Strauss 5-[^{18}F]FU test).[18,19] Underutilization of the 'Strauss 5-[^{18}F]FU test' may be due, in part, to the proposed need for complex kinetic modeling rather than simple tumor uptake,[20] and/ or to the electrophilic F-18 radiosynthetic method developed in the early 1970's.[21] The latter method remains the sole method of 5-[^{18}F]FU radiosynthesis today, and is thus not popular in units using the ^{18}O(p, n)^{18}F nuclear reaction on $H_2{}^{18}O$ to produce aqueous radiofluoride for routine clinical radiofluorinations.

Thus, there are still clinical needs to develop efficient PET tracers directed towards TP for evaluating the grade of malignancy,[22] the proliferative activity of tumor cells,[23] and the outcome of cancer chemotherapy.

Meanwhile, we have recently developed a highly efficient synthesis of [^{11}C]$COCl_2$ with high specific activity.[24] This method has been successfully applied to producing [^{11}C]CGP-12177, a PET tracer for β-adrenoreceptors which is now supplied for clinical use.[25] Application of this [^{11}C]$COCl_2$ to the synthesis of [^{11}C]pyrimidines would provide a potential procedure for tumor targeting novel PET tracers.

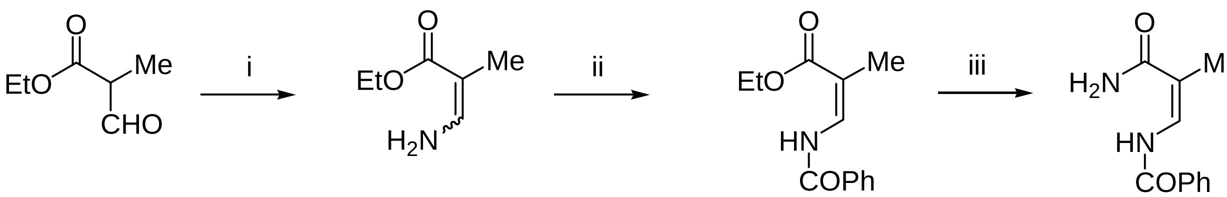
These contexts prompted us to develop a facile and efficient synthesis of [^{11}C]$COCl_2$-based [^{11}C]5-XUra (X = Me, F, H) by developing a synthetic route to the common precursors, β-aminoacrylamide derivatives (**1a**-**c**) with substituents such as Me (**a**), F (**b**), H (**c**) at the α-position, that is to be subjected to the cyclization with [^{11}C]$COCl_2$ to form versatile [2-^{11}C]pyrimidine derivatives in the last step (Scheme 1). We report herein a novel and facile synthesis of [2-^{11}C]thymine and [2-^{11}C]5-fluorouracil through direct condensation of [^{11}C]$COCl_2$ with the key intermediates β-aminoacrylamides (**1a**-**b**).



Scheme 1. Synthetic route of [2-^{11}C]pyrimidine derivatives.

RESULTS AND DISCUSSION

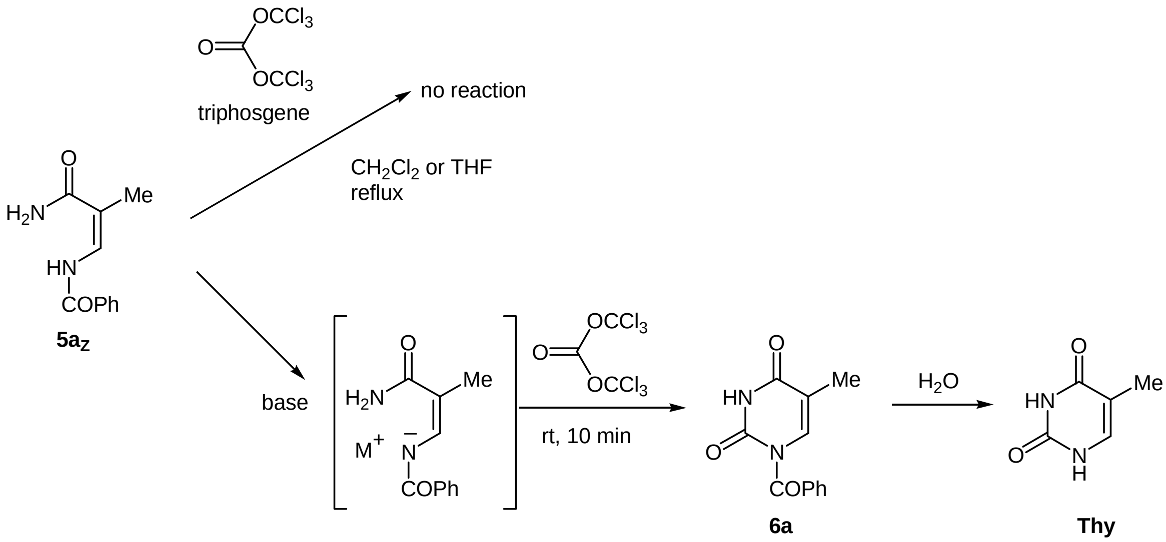
Synthesis of thymine (Thy). The key intermediate, β-aminomethacrylamide (**1a**), was readily synthesized from ethyl α-formylpropionate (**2a**).[26] Treatment of **2a** with ammonia afforded diastereomixture of β-aminomethacrylate (**3a$_Z$** and **3a$_E$**). The *Z* -isomer **3a$_Z$** was benzoylated with benzoyl chloride to give *N*-benzoylacrylate **4a$_Z$** with the desired stereochemistry (*Z*-form). Treatment of the resulting **4a$_Z$** with ammonia exclusively afforded the desired intermediate (*Z*)-β-(*N*-benzoylamino)-methacrylamide (**5a$_Z$**) with the stereochemistry maintained in the desired *Z*-form (Scheme 2). Hydrolysis of the benzoylated compound (**5a$_Z$**) failed to give β-aminomethacrylamide (**1a**). Therefore, **5a$_Z$** was used as a key intermediate for the subsequent ring closure with triphosgene, to Thy. For the synthesis of non-radio-labeled (cold) Thy, triphosgene was used as a safe and stable replacement for phosgene.



Reagents and Conditions: i) NH_3 in MeOH, reflux, 2 h,
ii) PhCOCl, pyridine, $CHCl_3$, 0°C, 2 h, then rt, overnight, iii) NH_3 in MeOH,rt, 1 week

Scheme 2. Synthesis of β-(*N*-benzoylamino) methacrylamide (**5a$_Z$**)

The key intermediate **5a$_Z$** was converted to the alkali metal salts (**5a$_Z$-Na** or **5a$_Z$-K**) with a base. Addition of triphosgene to the resulting salt under various conditions gave rise to the formation of benzoylthymine (**6a**), which was readily hydrolyzed with NH_3, or by passing through a short column of silica gel, to give Thy (Scheme 3).[27]



Scheme 3. Synthesis of Thy

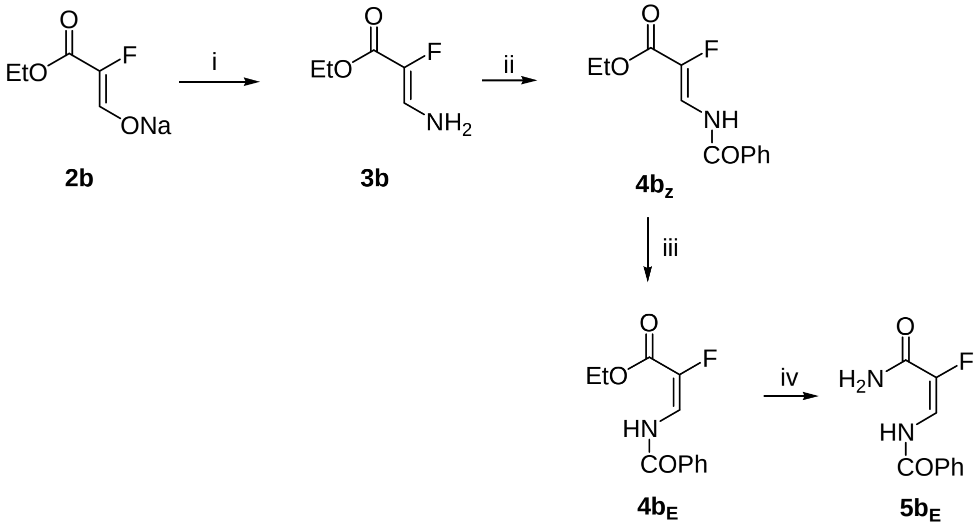
As summarized in Table 1, the best result was obtained when the reaction was performed with the sodium salt (**5a$_Z$-Na**) in DMF.

Table 1. Yields (%) of Thy

Solvent	Base	Mol. eq.	Yield (%)
THF or CH_2Cl_2	--	--	ND
DMF	NaH	5	99
DME	NaH	5	60
DME	*t*-BuOK	2	48
DME	*t*-BuOK	1	56

ND: not detected.

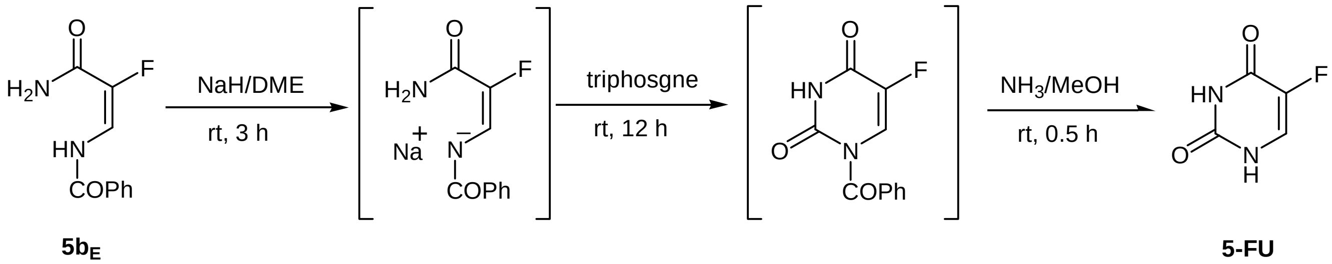
Synthesis of 5-fluorouracil (5-FU). The key intermediate *β*-(*N*-benzoylamino)-α-fluoroacrylamide (**5b$_E$**) was synthesized according to the procedure for **5a$_Z$**. Sodium ethyl 2-fluoro-3-hydoxyacrylate (**2b**), derived from ethyl formate and ethyl fluoroacetate in the presence of sodium methoxide[28] was treated with ammonia and ammonium chloride in methanol to give β-aminoacrylate (**3b**).[29] Benzoylation of the resulting **3b** with benzoyl chloride in pyridine afforded (*Z*)-ethyl β-benzoylamino-α-fluoroacrylate (**4b$_Z$**), wherein the ethoxycarbonyl group and the benzoylamino group occupy the undesired *trans*-stereochemistry on the ethylene moiety for the subsequent cyclization with phosgene. In order to effect geometric isomerization of the *Z*-isomer **4b$_Z$** into the *E*-isomer **4b$_E$**, UV-irradiation of **4b$_Z$** with a high-pressure mercury lamp afforded the equilibrium mixture of **4b$_Z$** and **4b$_E$** in the ratio of 1 : 9. The desired *E*-isomer **4b$_E$** was further treated with ammonia to furnish the key intermediate **5b$_E$** with the desired stereochemistry (*E*-form) in quantitative yield (Scheme 4).



Reagents and Conditions: i) NH_4Cl-MeOH, NH_3, rt, 1 week,
ii) PhCOCl, pyridine, $CHCl_3$, 0°C, 2 hr, then rt, overnight, iii) $h\nu$ (high pressure-Hg-lamp), 4 h
iv) NH_3 in MeOH,rt, 1 week

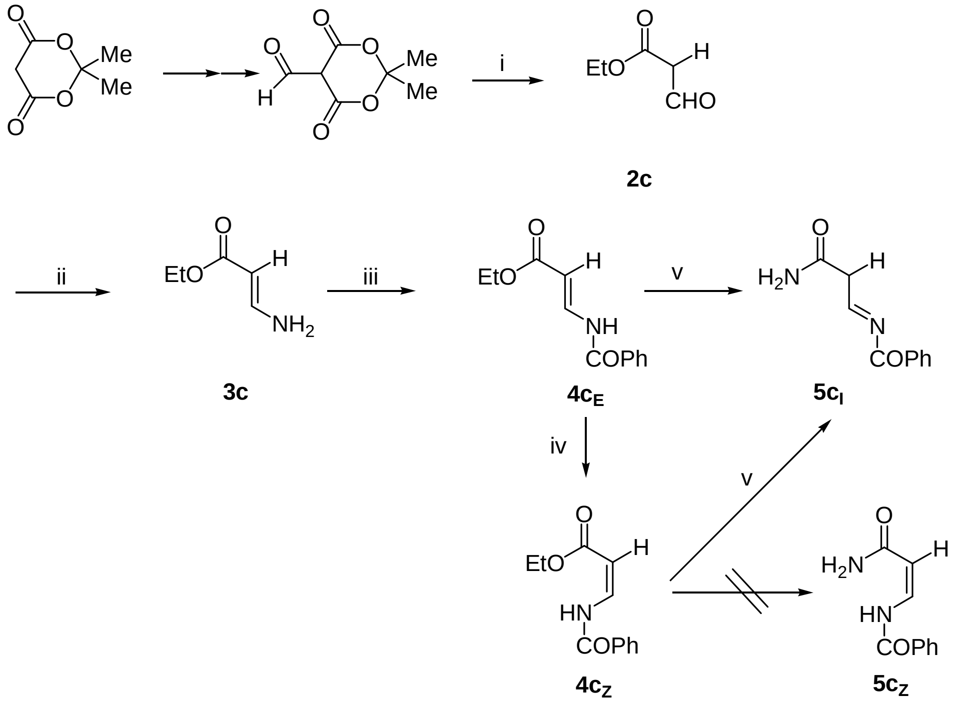
Scheme 4. Synthesis of β-(*N*-benzoylamino)methacrylamide (**5b**$_E$)

The sodium salt of the key intermediate **5b**$_E$ was subjected to cyclocondensation with triphosgene at room temperature, followed by hydrolysis with ammonia in methanol, resulting in the formation of 5-FU in high yield (75%, after purification on HPLC) (Scheme 5).



Scheme 5. Synthesis of 5-FU

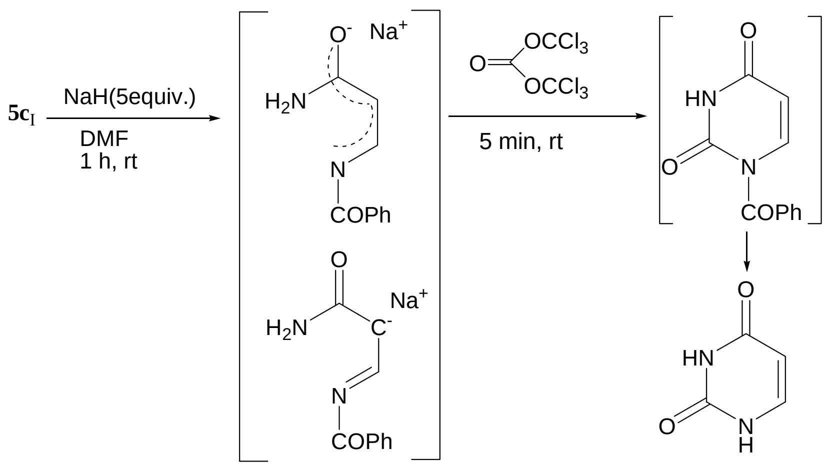
Synthesis of uracil (Ura). Ethyl formylacetate (**2c**), prepared from Meldrum's acid (2,2-dimethyl-1,3-dioxane-4,6-dione) and ethyl formate was treated with ammonia to afford β-aminoacrylate (**3c**), albeit in an undesired (*E*)-stereochemistry, which was benzoylated with benzoyl chloride to give benzoyl aminoacrylate **4c**$_E$. Treatment of the resulting **4c**$_E$ with ammonia afforded benzoyliminopropanamide, (**5c**$_I$), instead of giving either the desired *Z*-β-(*N*-benzoylamino)acrylamide (**5c**$_Z$) or *E*-β-(*N*-benzoylamino)acrylamide (**5c**$_E$). In order to obtain a key intermediate **5c**$_Z$ in the desired *Z*-stereochemisitry, E-β-(*N*-benzoylamino)acrylate (**4c**$_E$) was irradiated with a 500 W high-pressure mercury lamp to give the stereoisomer **4c**$_Z$. Treatment of the resulting ester (**4c**$_Z$) with ammonia, however,



Reagents and Conditions: i) EtOH, dry benzene, reflux, 90 min, ii) NH_4Cl-MeOH, NH_3, rt, 1 week, iii) PhCOCl, pyridine, $CHCl_3$, 0°C, 2 h, then rt, overnight, iv) $h\nu$ (high pressure-Hg-lamp), 4 h, v) NH_3 in MeOH, rt, 1 week

Scheme 6. Synthesis of **5c$_I$**

failed to give the desired **5c$_Z$**, but afforded the imino tautomer (**5c$_I$**), which is identical with that obtained from **4c$_E$** *via* ammonolysis (Scheme 6). The resulting imine (**5c$_I$**) is the tautomeric isomer of either **5c$_E$** or **5c$_Z$**, and hence the sodium or potassium salts, if formed sufficiently, can be inter-convertible through the tautomerism. Therefore, we decided to use **5c$_I$** for the subsequent cyclocondensation with triphosgene.



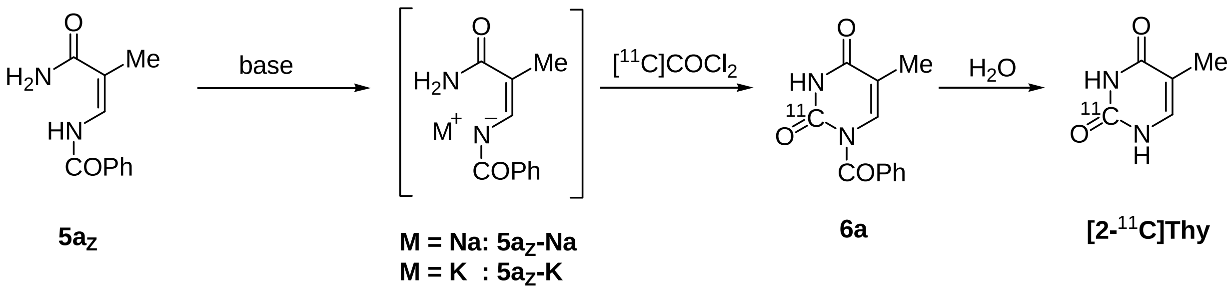
Ura

Scheme 7. Synthesis of Ura

Thus, imine **5c$_I$** was converted to its sodium salts (**5c$_I$-Na**) with sodium hydride, and resulting **5c$_I$-Na** was treated with triphosgene at room temperature for 5 min, to furnish the desired Ura, albeit in poor yield (2.4%) (Scheme 7). The formation of Ura was confirmed by comparison of the spectroscopic data and chromatographic behaviors with those of the authentic sample.

[^{11}C] $COCl_2$ based synthesis of [^{11}C]pyrimidines as potential tumor targeting PET tracers. As described above, pyrimidine rings were formed by the cycloaddition of the developed *N*-benzoyl aminoacrylamide intermediates and triphosgene in short reaction times, suggesting that the present reaction would provide a versatile method for the synthesis of pyrimidine based PET tracers. Then, we carried out the synthesis of [2-^{11}C]Thy and [2-^{11}C]5-FU, which could serve clinically as useful PET tracers for the evaluation of the cell proliferation ([2-^{11}C]Thy) and for the assessment and prediction of outcomes of 5-FU in chemotherapeutic treatment ([2-^{11}C]5-FU).

Synthesis of Synthesis of [2-^{11}C]Thy. ^{11}C-labeled thymine was readily prepared in a similar manner as described above, using the same automated synthesis system as used for [^{11}C]CGP-12177 production.[25] The direct ring closure reaction of [^{11}C]$COCl_2$ with non-activated precursor (**5a$_Z$**) only restored the starting material **5a$_Z$**. Therefore the precursor (**5a$_Z$**) was treated with a base to convert it to the alkali metal salt (**5a$_Z$-Na 5a$_Z$-K**), into which [^{11}C] $COCl_2$ gas was introduced to give [2-^{11}C]Thy (Scheme 8).



5a$_Z$ **6a** **[2-^{11}C]Thy**

Scheme 8. Synthesis of [2-^{11}C]Thy

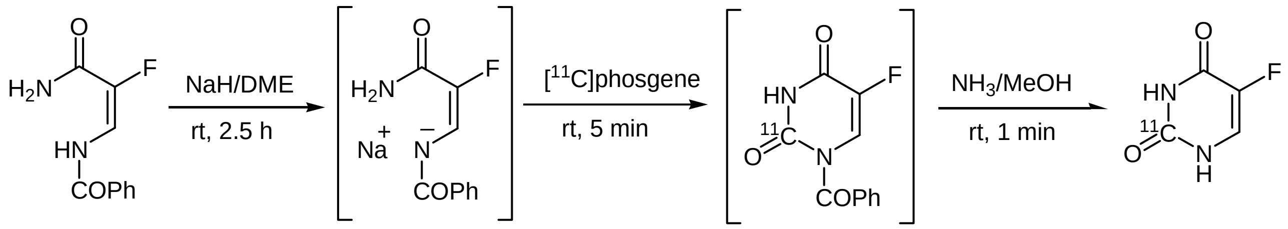
Table 2. Yields of [2-^{11}C]Thy

Solvent	Base	Mol. Eq.	Yield (MBq, EOS)
DME	--	-	ND
DMF	NaH	5	ND
toluene	NaH	5	41 (n=2)
DME	NaH	2	68 ± 65 (n=3)
DME	*t*-BuOK	2	137 (n=2)
DME	*t*-BuOK	1	362 ± 53 (n=3)

Bombardment was carried out with a 10 μA beam of 18 MeV protons for 10 min.
EOS: end of synthesis. ND: not detected.

The yield of [2-^{11}C]Thy under these conditions was 362 ± 53 MBq at EOS (n=3) (Table 2). The radiochemical yield of [2-^{11}C]thymine was ca. 24% from [^{11}C]$COCl_2$.[30] [2-^{11}C]Thymine produced was identical with authentic thymine by comparison of their chromatographic behavior on HPLC. The formation of [2-^{11}C]thymine was further confirmed by the enzymatic conversion to [2-^{11}C]thymidine.[6, 31] Thus, the present cyclocondensation of an appropriate activated *N*-benzoyl aminoacrylamide intermediate with highly reactive radiolabeling reagent [^{11}C]$COCl_2$ is a viable method for supplying [2-^{11}C]Thy and [2-^{11}C]thymidine for clinical PET tracer studies.

Synthesis of [2-^{11}C]5-FU. The key intermediate **5b$_E$**, activated as the sodium salt, was subjected to cyclocondensation with [^{11}C]$COCl_2$[29] on the same automated synthesis system used for the production of *S*-[^{11}C]CGP-12177.[25] The total synthesis took 17 minutes from the end of bombardment (EOB) to isolation of [2-^{11}C]5-FU. The yield of [2-^{11}C]5-FU was 380 MBq at EOS (Scheme 9), for a radiochemical yield of ca. 25%.



5bE

5-FU

Scheme 9. Synthesis of [2-^{11}C]5-FU

In all previous reports, labeling of the 2-position of thymine was accomplished by condensation of [^{11}C]urea and malate at 130 °C in fuming sulfuric acid. Recently, Steel et al reported an improved method for the preparation of [2-^{11}C]Thy via a multi-step process using [^{11}C]urea derived from [^{11}C]$COCl_2$. This radiosynthesis of [2-^{11}C]thymine took approximately 30 min from EOB.[6] On the other hand, our strategy involving the cyclocondensation with [^{11}C]$COCl_2$ for the direct production of [2-^{11}C]thymine is operationally simple, and offers fewer reaction steps at lower temperature. The total synthesis described herein takes 16 minutes from EOB to isolation of [2-^{11}C]Thy, thus significantly shortening reaction time, which is a crucial consideration for the preparation of short half-life radiopharmaceuticals. The success in the synthesis lies in the synthesis of the key precursor **5a$_Z$** bearing proper stereochemistry, and on the application of the highly reactive species, [^{11}C]$COCl_2$, for cyclocondensation in the final step.

Synthesis of [2-^{11}C]5-FU proceeded in the same way, resulting in the comparable radiochemical yields with those of [2-^{11}C]thymine using [^{11}C]$COCl_2$.[29] Importantly, the radiochemical yields are adequate for *in vivo* studies of [2-^{11}C]5-FU uptake in patients, and would appear sufficient for analysis of 1-h time-activity curves[32] using the catenary, three-compartment, five-parameter model developed for

5-[^{18}F]FU *in vivo*.[20]

We have provided a substantially more useful method for the synthesis of [2-^{11}C]Thy and [2-^{11}C]5-FU. Because of fewer reaction steps, mild reaction conditions, and reliability of product yield, the present methodology should find wide application in the preparation of many ^{11}C labeled radiopharmaceuticals.

EXPERIMENTAL

Materials and Analyses

Triphosgene was purchased from Aldrich Chemical Co. Ltd. (St. Louis, MO). All solvents were reagent grade and distilled using the appropriate methods. Column chromatography was performed using silica gel 60N (100-210 μm) and Aluminiumoxid 90 active neutral (70-230) Mesh ASTM, 0.063-0.200 mm, purchased from Merck. Silica gel HPLC was conducted on a Shim-Pack PREP-Sil (H) (250 mm x 20 mm *i.d.*, Silca gel) using a LC-6A (Shimadzu, Kyoto, Japan) apparatus with monitoring at 254 nm. All melting points are uncorrected. NMR spectra were measured with a JEOL JNM-EA500 (500 MHz) spectrometer, and ^{1}H-NMR chemical shift are given on the δ (ppm) scale based on those of the signals of solvents. MS spectra and high-resolution MS (HRMS) spectra were recorded with JEOL JMS-FABmate (EI). The elemental analyses (C, H, N) were within ± 0.4% of the theoretical values for C, H and N. UV-Irradiation was carried out externally with a 500 W high-pressure mercury (h.p. Hg) lamp (Eiko-sha, Osaka) in a degassed Pyrex tube (> 300 nm) on a merry-go-round apparatus.

Synthesis of Thy

Ethyl α-formylpropionate (2a). A solution of ethyl formate (10.4 mL, 130 mmol) and ethyl propionate (7.5 mL, 65 mmol) in dry Et_2O (80 mL) were added to a suspension of NaH (ca. 1.7 g, ca. 70 mmol) at 0 °C. The reaction mixture was stirred at ambient temperature for 60 h, and then neutralized with aqueous hydrochloric acid. The reaction mixture was extracted three times with CH_2Cl_2. After drying over anhydrous Na_2SO_4, the solvent was removed under atmospheric pressure to give **2a** (3.1 g, 37%) as oil.

Ethyl β-aminomethacrylate ($\mathbf{3a_Z}$, $\mathbf{3a_E}$). To an ethereal solution (5 mL) of **2a** (3.1g, 24 mmol) was added 7M methanolic ammonia (9 mL, 63 mmol). After heating under reflux for 2 h, the solvent was removed under reduced pressure, to give a mixture of $\mathbf{3a_Z}$ and $\mathbf{3a_E}$ (3.0 g, 98%) as oil. The Z-isomer $\mathbf{3a_Z}$ gradually isomerized itself into $\mathbf{3a_E}$, to afford a mixture of $\mathbf{3a_Z}$ and $\mathbf{3a_E}$ (1 : 1) when kept at ambient temperature for 21 h. Thus, the isolation of $\mathbf{3a_E}$ was not achieved.

(*Z*)-Ethyl β-aminomethacrylate ($\mathbf{3a_Z}$): H-NMR ($CDCl_3$) δ: 1.25 (3H, t, *J* =6.9 Hz), 1.68 (3H, s), 4.03 (2H, br s), 4.14 (2H, q, *J* =7.1 Hz), 7.43 (1H, t, *J* =10.3 Hz). EI-MS *m/z*: 129 $[M]^+$. EI-HRMS *m/z*: 129.0795 (Calcd for $C_6H_{11}NO_2$: 129.0790).

(*E*)-Ethyl β-aminomethacrylate (**3a$_E$**): ^{1}H-NMR ($CDCl_3$) δ: 1.25 (3H, t, *J* =6.9 Hz), 1.70 (3H, s), 4.03 (2H, br s), 4.13 (2H, q, *J* =6.9 Hz), 6.64 (1H, t, *J* =11.2 Hz).

Ethyl β-(*N*-benzoylamino)methacrylate (4a$_Z$, 4a$_E$). A mixture of **3a$_Z$** and **3a$_E$** (260 mg, 2 mmol) dissolved in $CHCl_3$ (10 mL) was added to a solution of pyridine (320 μL, 0.4 mmol) and benzoyl chloride (235 μL, 2 mmol) in $CHCl_3$ (20 mL) at 0 °C and kept overnight at rt. After removal of the solvent, 10% hydrochloric acid was added to the residual oil and extracted with Et_2O. After drying over anhydrous Na_2SO_4, the ethereal layer was subjected to silica-gel column chromatography with 10% AcOEt-hexane, to afford **4a$_Z$** (240 mg, 35%).

(*Z*)-Ethyl β-(*N*-benzoylamino)methacrylate (**4a$_Z$**): Colorless crystals, mp 61-62 °C, recrystalized from AcOEt. ^{1}H-NMR ($CDCl_3$) δ: 1.34 (3H, t, *J* =6.9 Hz), 1.90 (3H, d, *J* =1.2), 4.26 (2H, q, *J* =7.0 Hz), 7.48 (2H, t, *J* =7.4 Hz), 7.55 (1H, t, *J* =7.5 Hz), 7.62 (1H, dd, *J* =1.1, 10.9 Hz), 7.93 (2H, d, *J* =6.9 Hz), 11.4 (1H, d, *J* =9.8 Hz). EI-MS *m/z*: 233 $[M]^+$. EI-HRMS *m/z*: 233.1053 (Calcd for $C_{13}H_{15}NO_3$: 233.1052).

(*E*)-Ethyl β-(*N*-benzoylamino)methacrylate **(4a$_E$)**: ^{1}H-NMR ($CDCl_3$) δ: 1.32 (3H, t, *J* =7.5 Hz), 2.00 (3H, s), 4.24 (2H, q, *J* =7.2 Hz), 7.47 (1H, m), 7.50 (2H, t, *J* =7.5 Hz), 7.64 (1H, t, *J* =7.5 Hz), 8.13 (2H, d, *J* =8.0 Hz), 8.45 (1H, d, *J* =1.2 Hz).

(*Z*)-β-(*N*-Benzoylamino)methacrylamide (5a$_Z$). A solution of **4a$_Z$** in MeOH was added to an excess of liquid ammonia and allowed to stand at rt for a week. The reaction mixture was subjected to column chromatography over alumina with 50% AcOEt-hexane, to give **5a$_Z$** as colorless crystals. The ^{1}H-NMR measurement showed the reaction proceeded quantitatively. Colorless crystals, mp 182-184 °C (recrystallized from 50% AcOEt-Hexane). ^{1}H-NMR ($CDCl_3$) δ: 1.95 (3H, d, *J* =1.2 Hz), 5.40-5.80 (2H, br d, NH_2), 7.46 (2H, t, *J* =7.2 Hz), 7.54 (1H, t, *J* =7.5 Hz), 7.54 (1H, d, *J* =7.5 Hz), 7.93 (2H, d, *J* =6.9 Hz), 12.3 (1H, br s). EI-LRMS *m/z*: 204 $[M]^+$. EI-HRMS *m/z*: 204.0895 (Calcd for $C_{11}H_{12}N_2O_2$: 204.0899). *Anal.* Calcd for $C_{11}H_{12}N_2O_2$: C, 64.69; H, 5.92; N, 13.72. Found: C, 64.50; H, 6.05; N, 13.60.

Non-radio-labeled (cold) Thy. To a solution of **5a$_Z$-Na** (100.4 mg, 0.5 mmol) freshly prepared from NaH in DMF (8 mL), triphosgene (25 mg, 0.08 mmol) dissolved in THF (2.5 mL) was added, and stirred for 5 min. Then MeOH was added to the reaction mixture and the solvent was removed under reduced pressure. The residue was dissolved in water and extracted with $CHCl_3$. Aqueous NaOH (10%) was added to the chloroform layer. After neutralization with 10% hydrochlolic acid, the aqueous solution was submitted to reverse-phase HPLC with 6% aq. EtOH (3 mL/min), to give **Thy** quantitatively (32.4 mg).

Synthesis of 5-FU

***N*-[(1*E*)-2-carbamoyl-2-fluorovinyl]benzamide (5b$_E$)**. A solution of ethyl formate (2.2 mL, 27.5 mmol) and ethyl fluoroacetate (2 mL) in dry Et_2O (80 mL) was added to a solution of sodium ethoxide

(1.4 g, 20 mmol) in benzene (25 mL) at 0 °C. The reaction mixture was stirred overnight at ambient temperature. The solvent was evaporated under reduced pressure to give crude **2b** as oil. Crude **2b** (0.64 mmol, 100 mg) was dissolved in NH_4Cl saturated MeOH and 2 M NH_3-MeOH and stirred for 3 days at rt. After removal of the solvent, the residue was passed through a short column of alumina by using Et_2O as the eluent to give **3b** (69 mg, 0.6 mmol). To a solution of **3b** thus obtained in Et_2O, benzoyl chloride (70 μL, 0.6 mmol) and dry pyridine (48.5 μL, 0.6 mmol) were added at -10 °C. The reaction mixture was allowed to stand overnight at 0 °C, and subjected to HPLC with 10% AcOEt in hexane, to afford **4b$_Z$-ethyl ester** (0.06 mmol, 14.8 mg) and **4b$_Z$-methyl ester** (0.08 mmol, 18.4 mg), respectively.

Ethyl (2*Z*)-2-fluoro-3-(phenylcarbonylamino)prop-2-enoate **(4b$_Z$-ethyl ester)**: Colorless crystals, mp 101-102 °C (recrystallized from 20% AcOEt in hexane). ^{1}H-NMR (C_6D_6) δ: 0.83 (3H, t, *J* =7.45 Hz, CH_3), 3.88 (2H, q, *J* =7.45 Hz, CH_2), 6.86 (2H, aromatic), 6.99 (1H, aromatic), 7.35 (2H, aromatic), 7.62 (1H, br s, NH), 8.03 (1H, dd, *J*=11.45 Hz, $J_{H\text{-}F}$ =25.75 Hz, CH). EI-LRMS *m/z*: 237 $[M]^+$. EI-HRMS *m/z*: 237.0799 (Calcd for $C_{12}H_{12}NO_3$: 237.0801). Anal. Calcd for $C_{12}H_{12}NO_3$: C, 60.76; H, 5.10; N, 5.90. Found: C, 60.80; H, 5.10; N, 5.89.

Methyl (2*Z*)-2-fluoro-3-(phenylcarbonylamino)prop-2-enoate **(4b$_Z$-methyl ester)**: Colorless crystals, mp 142-143 °C (recrystallized from 20% AcOEt in hexane). ^{1}H-NMR (C_6D_6) δ: 3.27 (3H, s, CH_3), 6.84 (2H, aromatic), 6.97 (1H, aromatic), 7.26 (2H, aromatic), 7.31 (1H, br s, NH), 7.97(1H, dd, *J*=11.45 Hz, $J_{H\text{-}F}$ =25.75 Hz, CH). EI-LRMS *m/z*: 223 $[M]^+$. EI-HRMS *m/z*: 223.0647 (Calcd for $C_{11}H_{10}NO_3$: 223.0644). Anal. Calcd for $C_{11}H_{10}NO_3$: C, 59.19; H, 4.52; N, 6.28. Found: C, 59.44; H, 4.59; N, 6.16.

Photochemical isomerization of 4b$_Z$ ester. A solution of **4b$_Z$-ethyl ester** (50 mg) in MeCN (10 mL) was irradiated externally in a Pyrex tube at rt for 4 h. The reaction mixture was concentrated *in vacuo*, and the residue was submitted to HPLC with 30% AcOEt in hexane to give the *E*-form **4b$_E$-ethyl ester** and unchanged **4b$_Z$-ethyl ester** in 10% and 90% yields, respectively. Similar results were obtained from **4b$_Z$-methyl ester**.

Ethyl (2*E*)-2-fluoro-3-(phenylcarbonylamino)-2-propenoate **(4b$_E$-ethyl ester):** Colorless crystals, mp 91-92 °C (recrystallized from 20% AcOEt-hexane). ^{1}H-NMR (C_6D_6) δ: 0.83 (3H, t, *J* =7.45 Hz, CH_3), 3.84 (2H, q, *J* =7.45 Hz, CH_2), 6.89 (2H, aromatic), 6.95 (1H, aromatic), 7.79 (2H, aromatic), 7.86 (1H, dd, $J_{H\text{-}F}$, $J_{H\text{-}H}$ =11.45 Hz, CH), 10.2 (1H, br s, D_2O exchangeable, NH). EI-LRMS *m/z*: 237 $[M]^+$. EI-HRMS *m/z*: 237.0792 (Calcd for $C_{12}H_{12}NO_3$: 237.0801). Anal. Calcd for $C_{12}H_{12}NO_3$: C, 60.76; H, 5.10; N, 5.90. Found: C, 60.56; H, 5.23; N, 5.87.

Methyl (2*E*)-2-fluoro-3-(phenylcarbonylamino)prop-2-enoate **(4b$_E$-methyl ester):** Colorless crystals, mp 80-81 °C (recrystallized from 20 % AcOEt-hexane). ^{1}H-NMR (C_6D_6) δ: 3.19 (3H, s, CH_3), 6.89 (2H, aromatic), 6.94 (1H, aromatic) 7.79 (2H, aromatic), 7.84 (1H, dd, $J_{H\text{-}F}$, $J_{H\text{-}H}$ =11.45 Hz, CH), 10.16 (1H, br s, D_2O exchangeable, NH). EI-LRMS *m/z*: 223 $[M]^+$. EI-HRMS *m/z*: 223.0642 (Calcd for $C_{11}H_{10}NO_3$: 223.0644). Anal. Calcd for $C_{11}H_{10}NO_3$: C, 59.19; H, 4.52; N, 6.28. Found: C, 59.29; H, 4.54; N, 6.25.

***N*-[(1*E*)-2-carbamoyl-2-fluorovinyl]benzamide** (**5b$_E$**). A solution of **4b$_E$** (10 mg, 0.037 mmol) and excess liq. ammonia in MeOH (1 mL) was allowed to stand in a high-pressure reaction vessel for 4 days at rt. After evaporation of the solvent *in vacuo*, the reaction mixture gave *N*-[(1*E*)-2-carbamoyl-2-fluorovinyl]benzamide (**5b$_E$**) in quantitative yield. **5b$_E$**: Colorless crystals, mp 160-161 °C (recrystallized from 20% AcOEt-hexane). ^{1}H-NMR ($CDCl_3$) δ: 5.68, 6.10 (1H, br s, D_2O exchangeable, NH_2), 7.49 (2H, aromatic), 7.57 (1H, aromatic), 7.79(1H, dd, J_{H-F}, J_{H-H} =10.85 Hz, CH), 7.91 (2H, aromatic), 10.8 (1H, br s, D_2O exchangeable, NH). EI-LRMS *m/z*: 208 $[M]^+$. EI-HRMS *m/z*: 208.0639 (Calcd for $C_{10}H_9N_2O_2$: 208.0648). *Anal.* Calcd for $C_{10}H_9N_2O_2$: C, 57.69; H, 4.36; N, 13.46. Found: C, 57.65; H, 4.52; N, 13.34.

Non-radio-labeled (cold) 5-FU. A solution of the sodium salts of **5b$_E$**, prepared from **5b$_E$** (20.0 mg, 0.1 mmol) and NaH, and triphosgene (33 mg, 0.1 mmol, 3 eq) in DME (5mL) was stirred at rt for 16 h. After addition of 2 M NH_3-MeOH (2 mL), the reaction mixture was condenced under reduced pressure. The residue was submitted to reverse-phase HPLC with 3% MeOH in water (1 mL/min) to give 5-FU in 75 % yield.

Synthesis of uracil (Ura)

Ethyl *N*-benzoylaminoacrylate (4c$_E$). Ethyl formylacetate (**2c**) was prepared by refluxing a mixture of Meldrum's acid (480 mg, 2.5 mmol) and EtOH (1.2 equv. molar) in benzene for 90 min, according to the reported procedure.[33] A solution of crude **2c** (107 mg, 0.92 mmol, 37%), thus prepared, and liquid ammonia in MeOH (2.0 mL) was allowed to stand for a week at rt, to give **3c**. To a solution of crude **3c** (69 mg, 0.6 mmol) and pyridine (48.5 μL, 0.6 mmol) in Et_2O (2 mL), benzoyl chloride (70 μL, 0.6 mmol) dissolved in $CHCl_3$ (20 mL) was added dropwise at -10 °C, and the reaction mixture was kept overnight at -10 °C. After removal of the solvent, 10% hydrochloric acid was added to the residual oil and extracted with ether. After drying over anhydrous Na_2SO_4, the ethereal layer was subjected to silica-gel column chromatography with 10% AcOEt-hexane as an eluent, to afford ethyl *N*-benzoylaminoacrylate (**4c$_E$**) (14.8 mg, 0.068 mmol, 0.3%). ^{1}H-NMR ($CDCl_3$) δ: 1.27 (3H, t, *J* =6.9 Hz), 4.17 (2H, q, *J* =6.9 Hz), 5.64 (1H, d, *J* =14.3 Hz), 7.45 (2H, t, *J* =7.5 Hz), 7.55 (1H, t, *J* =7.5 Hz), 7.85 (1H, d, *J* = 7.5 Hz), 8.22 (1H, dd, *J* =11.5, 14.3 Hz), 8.67 (1H, d, *J* =11.5 Hz, -NH). EI-MS *m/z*: 219 $[M]^+$.

Photochemical isomerization of *E*-form 4c$_E$-ethyl ester to *Z*-form. A solution of *E*-form **4c$_E$** (50 mg) in MeCN (10 mL) in a Pyrex tube was irradiated externally at rt for 4 h. The reaction mixture was concentrated *in vacuo,* and submitted to HPLC with 30% AcOEt in hexane, to give **4c$_Z$** in the desired-*Z*-form in 30% yield, together with unchanged **4c$_E$** in 70% yield.

Ethyl N-benzoylaminoacrylate (**4c$_Z$**): ^{1}H-NMR ($CDCl_3$) δ: 1.27 (3H, t, *J* =6.9 Hz), 4.17 (2H, q, *J* =6.9 Hz), 5.64 (1H, d, *J* =14.3 Hz), 7.45 (2H, t, *J* =7.5 Hz), 7.55 (1H, t, *J* =7.5 Hz), 7.85 (1H, d, *J* = 7.5 Hz), 8.22 (1H, dd, *J* =11.5, 14.3 Hz), 8.67 (1H, d, *J* =11.5 Hz, -NH). EI-MS *m/z*: 219 $[M]^+$.

***N*-[(1*E*)-2-carbamoylvinyl]benzamide 5c$_I$.** A solution of **4c$_Z$** (10 mg, 0.046 mmol) and excess liq. ammonia in MeOH (2.0 mL) was kept at rt for 4 days. After evaporation of the solvent *in vacuo*, the residue was submitted to column chromatography on alumina with AcOEt – hexane (1 : 1) to give **5c$_I$** in the yield of 90% (7.5 mg, 0.040 mmol) as colorless crystals.

Similarly **4c$_E$** afforded **5c$_I$** in 90 % yield. ^{1}H-NMR (CD_3OD) δ: 2.66 (2H, d, *J* =5.7 Hz), 5.15 (1H, bs or t, *J* =5.7 Hz), 7.45 (2H, t, *J* =7.5 Hz), 7.52 (1H, t, *J* =7.5 Hz), 7.80 (1H, d, *J* = 7.5 Hz),. EI-MS *m/z*: 190 $[M]^+$.

Non-radio-labeled (cold) Ura. A solution of **5c$_I$** (19.0 mg, 0.1 mmol) and NaH (21.6 mg, 60% oil, 0.5 mmol) in DME was kept at rt for 4 h. Triphosgene (33 mg, 0.1 mmol, 3 eq) was added to the solution, which was allowed to stand overnight at rt. Then, 7 M ammonia in MeOH (2 mL) was added to the solution, and the reaction mixture was kept for 2 min at rt. Removal of the solvent under reduced pressure gave Ura in 2.4% (0.3 mg, 0.0024 mmol) yield.

Synthesis of 2-[^{11}C]Thymine

According to the procedure adopted for non-radio-labeled (cold) Thy, [^{11}C]phosgene was bubbled with helium carrier into a solution of **5az-Na** (0.2 mg) in DME (500 μL) for 1 min at 30 °C . After removal of the solvent by evaporation, the residue consisting of [2-^{11}C]*N*-benzoylthymine (**6a**) was treated with 1.5 M methanolic ammonia for 1 min at rt, and subjected to reverse-phase HPLC (column; *μ*-Bondapak C_{18}, 25 cm × 0.39 cm i.d., solvent; 3% EtOH–saline, flow rate; 0.5 mL/min at 40 °C), equipped with a UV monitor (detected at 254 nm) and a γ counter. The radioactive peak at 11 min was identified as the desired thymine. Radiochemical purity of [2-^{11}C]thymine was estimated to be 99% by HPLC.

Synthesis of [2-^{11}C]5-FU

According to the procedure for non-radio-labeled (cold) 5-FU, [^{11}C]phosgene was infused with helium into a solution of **5b$_E$-Na** (0.2 mg) in DME (500 μL) for 1 min at 30 °C. After removal of the solvent by evaporation, the residue consisting of [2-^{11}C]*N*-benzoyl-5-FU was treated with 1.5 M methanolic ammonia for 1 min at rt, and subjected to reverse-phase HPLC (column; Inertsil ODS-3, 250 mm x 4.6 mm i.d., solvent; 3% EtOH - Saline, flow rate; 0.5 mL/min at 40 °C), equipped with a UV monitor (detected at 270 nm) and a γ counter. The radioactive peak at 9.3 min was identified as authentic cold 5-FU.

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