A novel and efficient synthesis of [2-^{11}C]5-fluorouracil for prognosis of cancer chemotherapy

Koh-ichi Seki [1], Ken-ichi Nishijima [2], Yuji Kuge [3], Nagara Tamaki [3], Leonard I. Wiebe [4], and Kazue Ohkura [2]

[1] Central Institute of Radio Isotope Science, Hokkaido University, Sapporo, Japan; [2] Faculty of Pharmaceutical Sciences, Health Sciences University of Hokkaido, Hokkaido, Japan; [3] Graduate School of Medicine, Hokkaido University, Sapporo, Japan; [4] Faculty of Pharmacy & Pharmaceutical Sciences, University of Alberta, and Cross Cancer Institute, Edmonton, Alberta, Canada

In memory of Professor Antoine (Tony) A. Noujaim, whose outstanding contributions to radiopharmacy, diagnostic oncology and the immunotherapy of cancer will serve as an inspiration to us all.

Received January 2, 2007; Revision received January 15, 2007; Accepted January 15, 2007, Published June 14th 2007

ABSTRACT - Purpose: In order to facilitate the use of the PET-based 'Strauss test' for 5-FU sensitivity, a rapid and facile synthesis of [2-^{11}C]5-fluorouracil ([2-^{11}C]5-FU), based on [^{11}C]phosgene ([^{11}C]$COCl_2$), is reported. **Methods:** The key intermediate (*E*)-β-benzoylamino-α-fluoroacrylamide (**1**) and [^{11}C]phosgene was submitted to cyclo-condensation to give [2-^{11}C]5-fluorouracil. **Results:** [2-^{11}C]5-Fluorouracil was synthesized in 17 min with high (~25%) radiochemical yield. **Conclusion:** The present study provides a rapid, simple, and efficient synthesis of [2-^{11}C]5-FU, that would serve as a useful prognostic PET tracer for 5-FU chemotherapy.

INTRODUCTION

The original synthesis of 5-fluorouracil (5-FU), one of a new class of antitumor fluoroprymidines, was reported in 1957 (1,2). 5-FU-mediated inhibition of thymidylate synthetase (3) was subsequently shown to be one of the major mechanisms responsible for the antitumor activity of these compounds. Detailed studies have pointed to 5-FU interference with DNA and protein synthesis, as a result of conversion to the corresponding ribose nucleoside and substitution into RNA, as an equally important mechanism of toxicity (4). The first clinical studies of 5-FU were reported in 1958 (5). Radiolabeled 5-FU ([2-^{14}C]5-FU) (6) and [6-^{14}C]5-FU (7) have been used for biodistribution studies in animals and [2-^{14}C]5-FU biodistribution has been studied in cancer patients (8).

Today, almost 50 years later, 5-FU remains front-line therapy, alone or in combination with other drugs or radiation, for gastric (9,10), colorectal (11,12) and other cancers including advanced pancreatic cancer (13). In light of the limited success of cancer chemotherapy in general, including 5-FU, it is surprising that the medical community has not demanded, and relied upon, a rational basis for the prediction of more successful outcomes when selecting a chemotherapeutic drug. It has been demonstrated, for example, that tumor uptake of fluorine-18 labeled 5-FU ([^{18}F]5-FU) serves a positive prognostic role in selection of patients for 5-FU therapy (Strauss [^{18}F]5-FU test) (14, 15). Under-utilization of the 'Strauss [^{18}F]5-FU test' may be due, in part, to the proposed need for complex kinetic modeling rather than simple tumor uptake (16), and/or to the electrophilic F-18 radiosynthetic method developed in the early 1970's [17]. The latter method remains the sole method of 5-FU radiosynthesis today, and is thus not popular in units using the ^{18}O(p,n)^{18}F nuclear reaction to produce aqueous radiofluoride for routine clinical radiofluorinations. The ease of use of the latter approach has resulted in a largely 'nucleophilic' F-18 labeling world.

Since only F, N, O, H and C can be used to radiolabel 5-FU without altering its biochemistry, F-18 and C-11 offer the only opportunity for nuclear (positron emission tomographic; PET) imaging. Pyrimidine nucleosides have been labeled with C-11 at C-2 (18,19), and PET studies using C-11 nucleosides have been reported (20), but [2-^{11}C]5-FU is not among those reported. In order to facilitate the use of the PET-based 'Strauss test' for 5-FU sensitivity, a rapid, facile synthesis of [2-^{11}C]5-fluorouracil ([2-^{11}C]5-FU), based on [^{11}C]phosgene ([^{11}C]$COCl_2$) (21), has been developed and is now reported (Figure 1).

Corresponding author: Koh-ichi Seki, Ph. D, Central Institute of Radio Isotope Science, Hokkaido University, Kita-15, Nishi-7, Kita-ku, Sapporo Tel: +81-11-706-6090, E-mail: seki@ric.hokudai.ac.jp

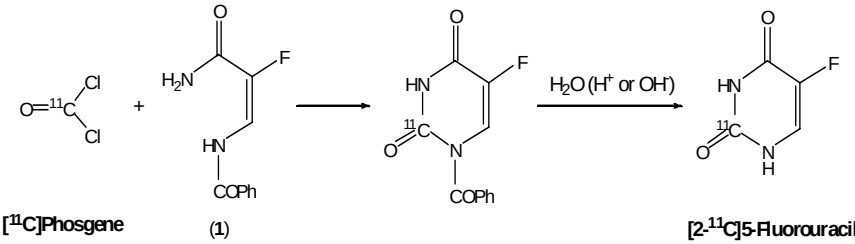
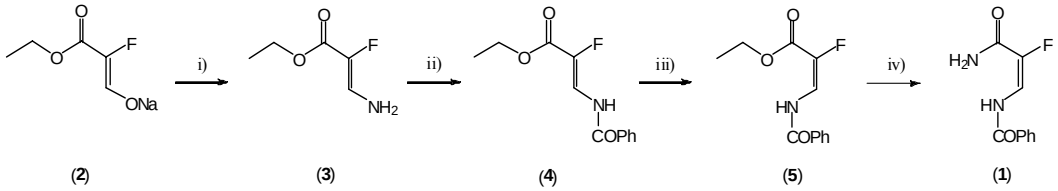


Figure 1. Synthetic route for [2-^{11}C]5-fluorouracil.



Reagents and Conditions :
i) NH_4Cl-CH_3OH, NH_3, 4 days, ii) $PhCOCl$, C_5H_5N, ether, overnight, iii) hn (h.p.-Hg-lamp), iv) NH_3, CH_3OH, 1 week

Figure 2: Synthesis of the 5-fluorouracil precursor (1).

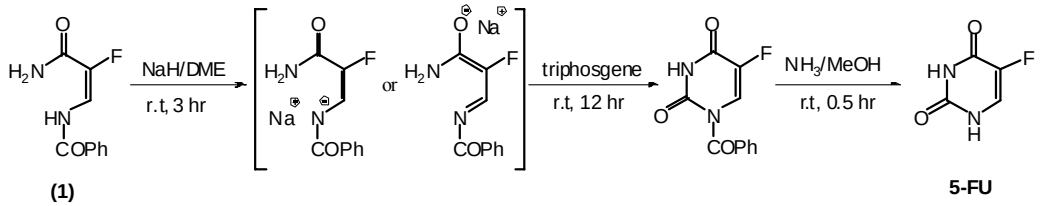


Figure 3. Synthesis of 5-fluorouracil (5-FU).

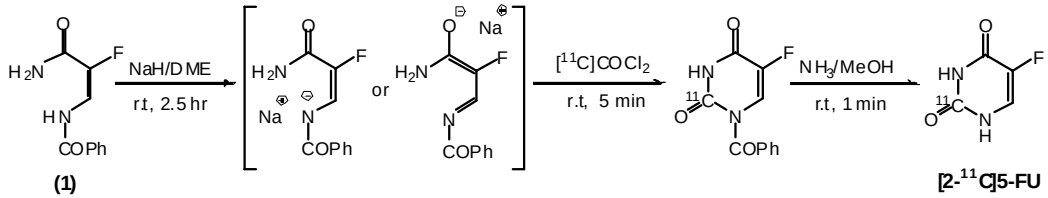


Figure 4. Synthesis of [2-^{11}C]5-FU.

MATERIALS AND METHODS

Chemicals

Triphosgene was purchased from Aldrich Chemical Co. Ltd. (St. Louis, MO). All solvents were reagent grade and distilled using the appropriate methods. [^{11}C]$COCl_2$ was synthesized from [^{11}C]CH_4 *via* [^{11}C]CCl_4 according to our previously reported method [21]. Bombardment was carried out with a 10 μA beam of 18 MeV protons for 10 min. The yield of [^{11}C]$COCl_2$ was estimated to be about 1500 MBq based on the yield of [^{11}C]diphenylurea produced with aniline under the same conditions.

RESULTS

Synthesis of (E)-β-benzoylamino-α-fluoroacrylic amide (1)

Our first goal was the synthesis of the key intermediate **1**, which would undergo cyclocondensation with [^{11}C]phosgene at the final step into [2-^{11}C]5-FU: Treatment of ethyl 2-fluoro-3-hydoxyacrylate sodium salt (**2**) [22] with ammonia (NH_3) and ammonium chloride in methanol gave rise to the formation of β-aminoacrylate (**3**) [23]. Benzoylation of the resulting **3** with benzoyl chloride in pyridine afforded (*Z*)-ethyl β-benzoyl-amino-α-fluoroacrylate (**4**), wherein the ethoxy-carbonyl group and the benzoylamino group occupy the *trans*-stereochemistry on the ethylene moiety. In order to effect geometric isomerization of the *Z*-isomer **4** into the *E*-isomer **5**, **4** was irradiated with a high-pressure mercury lamp, to afford the equilibrium mixture of **5** and **4** in the ratio of 1: 9. The *E*-isomer **5**, having a convenient stereochemistry for the ring closure with phosgene, was further treated with NH_3, resulting in the production of the key intermediate **1** in quantitative yield with the desired stereochemistry (*E*-form) (Figure 2).

Synthesis of cold 5-FU

The sodium salt of the key intermediate **1** was subjected to cyclocondensation with triphosgene at room temperature, followed by the hydrolysis with 2 M NH_3 in methanol, resulting in the formation of 5-FU in high yield (75%, after purification on HPLC) (Figure 3).

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

The key intermediate **1**, activated as the sodium salt, was subjected to cyclocondensation with [^{11}C]$COCl_2$ (23) on the same automated synthesis system used for the production of *S*-[^{11}C]CGP-12177 (24). The total synthesis took 17 minutes from the end of bombardment to isolation of [2-^{11}C]5-FU. The yield of [2-^{11}C]5-FU was 380 MBq at EOS (Figure 4). The radiochemical yield of [2-^{11}C]5-FU was approximately 25%. The radio-HPLC trace is shown in Figure 5.

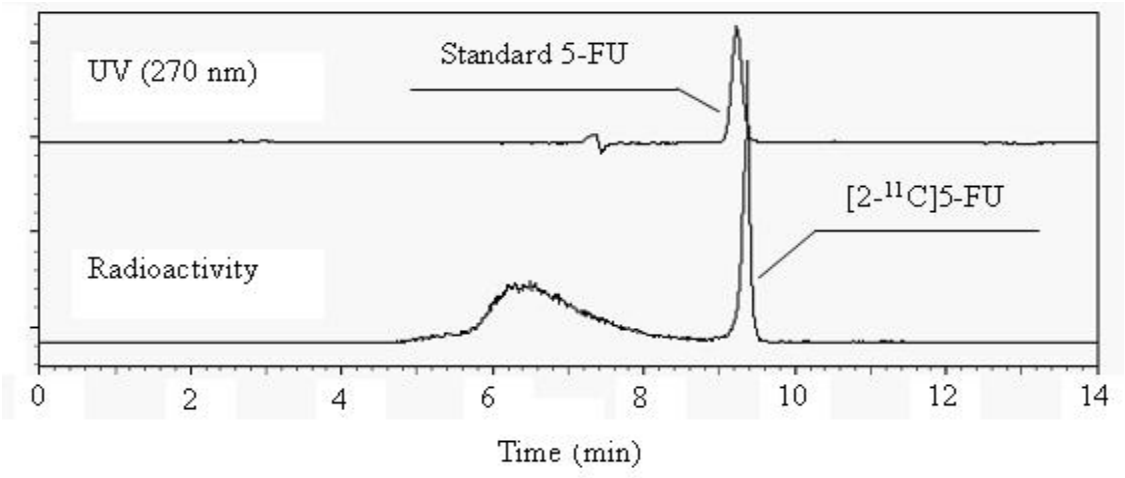


Figure 5. Chromatographic data for the analysis of [2-^{11} C]5-FU. [2-^{11}C]5-FU was analyzed by reverse-phase HPLC (Inertsil ODS-3, 250 mm x 4.6 mm *i.d.*) with elution of 3% ethanol in water at a flow rate of 0.5 mL/min. The chromatogram was monitored continuously based on radioactivity (RLC-700, Aloka, Tokyo, Japan) and absorbance at 270 nm (SPD-10 Avp, Shimadzu).

DISCUSSION

[2-^{11}C]5-FU was synthesized by the cyclocondensation of newly developed (*E*)-β-benzoylamino-α-fluoroacrylic amide (**1**) with [^{11}C]$COCl_2$. The success in the synthesis lies in the synthesis of the key intermediate **1** bearing proper stereochemistry, and on the application of highly reactive species [^{11}C]$COCl_2$ for cyclocondensation in the final step. These radiochemical yields (25%) compare favorably with yields (23%) reported previously for the synthesis of [2-^{11}C]thymine using [^{11}C]COCl2 (23). 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 (20)using the catenary, three-compartment, five-parameter model developed for 5-[^{18}F]FU in vivo (16).

CONCLUSION

The present study demonstrates the first efficient synthesis of [2-^{11}C]5-FU, which would be a useful PET-tracer for the assessment and prediction of outcomes of 5-FU in chemotherapeutic treatment.

ACKNOWLEDMENT

This work was in part supported by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

REFERNCES

[1] Heidelberger, C., Chaudhuri, N.K., Danneberg, P., Mooren, D., Griesbach, L., Duschinsky, R., Schnitzer, R.J., Pleven, E., Scheiner, J. Fluorinated pyrimidines, A new class of tumour-inhibitory compounds. Nature, 179: 663-666, 1957.

[2] Duschinsky, R., Pleven, E., Heidelberger, C. The synthesis of 5-fluoropyrimidines. J. Am. Chem. Soc., 79: 4559, 1957.

[3] Hartmann, K.U., Heidelberger C. Studies on fluorinated pyrimidines. XII. Inhibition of thymidylate synthetase. J. Biol. Chem., 236: 3006, 1961.

[4] Myers, C.E. The pharmacology of the fluoropyrimidines. Pharmacological Reviews, 33: 1-15, 1981.

[5] Curreri, A.R., Ansfield , F.J., McIver, F.A., Waisman, H.A., Heidelberger, C. Clinical studies with 5-fluorouracil. Cancer Res, 18: 478-484, 1958.

[6] Chaudhuri, N.K., Montag, B., Heidelberger, C. Studies on fluoropyrimidines. XVI. Metabolism of 5-fluorouracil-2-^{14}C and 5-fluoroorotic-2-^{14}C acid in vivo. Cancer Res., 18: 318-328, 1958.

[7] Mukherjee, K.L., Heidelberger, C. Studies on fluorinated pyrimidines. IX. The degradation of 5-fluorouracil-6-^{14}C. J. Biol. Chem., 235: 433-7, 1960.

[8] Mukherjee, K.L., Curreri, A.R., Jauid, M. Heidelberger, C. Tissue distribution of 5-fluorouracil-2-^{14}C and 5-fluoro-2'-deoxyuridine in cancer patients. Cancer Res., 23: 67, 1963.

[9] Tahara, M., Ohtsu, A., Boku, N., Nagashima, F., Muto, M., Sano, Y., Yoshida, M., Mera, K., Hironaka, S., Tajiri, H., Yoshida, S. Sequential methotrexate and 5-fluorouracil therapy for gastric cancer patients with peritoneal dissemination: a retrospective study. Gastric Cancer, 4: 212-218, 2001.

[10] Van Cutsem, E., Moiseyenko, V.M., Tjulandin, S., Majlis, A., Constenla, M., Boni, C., Rodigues, A., Fodor, M., Chao, Y., Voznyi, E., Risse, M.L., Ajani, J.A. Phase III study of docetaxel and cisplatin plus fluorouracil compared with cisplatin and fluorouracil as first-line therapy for advanced gastric cancer: a report of the V325 Study Group. J. Clin. Oncol., 24: 4991-4997, 2006.

[11] Patiyil, S., Alberts, S.R. Metastatic colorectal cancer: Therapeutic options. Curr. Treat. Options Oncol., 7: 389-398, 2006.

[12] Goyle, S., Maraveyas, A. Chemotherapy for colorectal cancer. Dig. Surg., 22: 401-414, 2005.

[13] Park, J.K., Ryu, J.K., Lee, J.K., Yoon, W.J., Lee, S.H., Kim, Y.T., Yoon, Y.B. Gemcitabine chemotherapy versus 5-fluorouracil-based concurrent chemoradio-therapy in locally advanced unresectable pancreatic cancer. Pancreas, 33: 397-402, 2006.

[14] Dimitrakopoulou-Strauss, A., Strauss, L.G., Schlag, P., Hohenberger, P., Irngartinger, G., Oberdoorfer, F., Doll, J., Van Kaick, G. Intravenous and intra-arterial oxygen-15-labeled water and fluorine-18-labeled fluorouracil in patients with liver metastases from colorectal carcinoma. J. Nucl. Med., 39: 465-473, 1998.

[15] Kissel, J., Brix, G., Bellemann, M.E., Strauss, L.G., Dimitrakopoulou-Strauss, A., Port, R., Haberkorn, U., Lorenz, W.J. Pharmacokinetic analysis of 5-[^{18}F]fluorouracil tissue concentrations measured with positron emission tomography in patients with liver metastases from colorectal adenocarcinoma. Cancer Res., 57: 3415-3423, 1997.

[16] Bading, J.R., Yoo, P.B., Fissekis, J.D., Alauddin, M.M., D'Argenio, D.Z., Conti, P.S. Kinetic modeling of 5-fluorouracil anabolism in colorectal adenocarcinoma: a positron emission tomography study in rats. Cancer Res., 63: 3667-3674, 2003.

[17] Fowler, J.S., Finn, R.D., Lambrecht, R.M., Wolf, A.P. The synthesis of ^{18}F-5-fluorouracil. VII. J. Nucl. Med., 14: 63-64, 1973.

[18] Sundoro-Wu, B.M., Schmall, B., Conti, P.S., Dahl, J.R., Drumm, P., Jacobsen, J.K. Selective alkylation of pyrimidyldianions: synthesis and purification of ^{11}C labeled thymidine for tumor visualization using positron emission tomography. Int. J. Appl. Radiat. Isot., 35: 705-708, 1984.

[19] Link, J.M., Grierson, J., Krohn, K. Alternatives in the synthesis of 2-[C-11]thymidine. J. Labelled Compd. Radiopharm., 37: 610-612, 1995.

[20] Mankoff, D.A., Shields, A.F., Graham, M.M., Link, J.M., Eary, J.F., Krohn, K.A. Kinetic analysis of 2-[carbon-11]thymidine PET imaging studies: compartmental model and mathematical analysis. J. Nucl. Med., 39: 1043-1055, 1998.

[21] Nishijima, K., Kuge, Y., Seki, K., Ohkura, K., Motoki, N., Nagatsu, K., Tanaka, A., Tsukamoto, E., Tamaki, N. A simplified and improved synthesis of [^{11}C]phosgene with iron and iron (III) oxide. Nucl. Med. Biol., 29: 345-350, 2002.

[22] Miyamoto, T., Egawa, H., Matsumoto, J. Pyridonecarboxylic acids as antibacterial agents. VIII. An alternative synthesis of enoxacin via fluoronicotinic acid derivatives. Chem. Pharm. Bull., 35: 2280-2285, 1987.

[23] Ohkura, K., Nishijima, K., Sanoki, K., Kuge, Y., Tamaki, N., Seki, K. A new convenient method for the synthesis of [2-^{11}C]thymine utilizing [2-^{11}C]phosgene. Tetrahedron Lett., 47: 5321-5626, 2006.

[24] Nishijima, K., Kuge, Y., Seki, K., Ohkura, K., Morita, K., Nakada, K., Tamaki, N. Preparation and pharmaceutical evaluation for clinical application of high specific activity S-(-)[^{11}C]CGP-12177, a radioligand for β-adrenoreceptors. Nucl. Med. Commun., 25: 845-849, 2004.