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Synthesis and purification of [2-^{13}C]-5-fluorouracil

Huzaifa S. Rangwala,[a] John W. Giraldes,[a] and Vadim J. Gurvich[a,b]*

[2-^{13}C]-5-Fluoropyrimidine-2,4(*1*H,*3*H)-dione ([2-^{13}C]-5-fluorouracil or [2-^{13}C]-5-FU) is a potential diagnostic agent for measuring 5-FU-induced toxicity in cancer patients. It was prepared and purified with isotopic and chemical purity of > 99% on a multigram scale in a two-step synthesis from [^{13}C]-urea. Preparative separation of [2-^{13}C]-FU and [2-^{13}C]-uracil was carried out by automated medium pressure silica gel column chromatography. The method is applicable to a broader range of 5-FU isotopic analogs derived from labeled uracil.

Keywords: 5-FU; [2-^{13}C]-5-fluorouracil; carbon-13; stable isotope; 5-FU/uracil separation

Introduction

5-Fluoropyrimidine-2,4(*1*H,*3*H)-dione (5-fluorouracil or 5-FU) has been used as an effective anti-tumor agent for several decades.[1,2] The original strategy for its synthesis from pseudo-urea and α-fluoro-β-keto ester enolates was reported by Duschinsky over 50 years ago.[3–5] Hundreds of thousands of patients are treated with 5-FU every year of which a certain percent of patient population exhibit severe toxicity and even death. That, in part, can be attributed to the dihydropyrimidine dehydrogenase (DPD) deficiency, a result of decreased activity of DPD, that plays a key role in the uracil catabolic pathway (Scheme 1).[6–9] To assess this deficiency in cancer patients, a 5-FU that contains a nonradioactive isotope, such as carbon-13, in position 2, can be used as a diagnostic agent. As a result of catabolic activation in patients, it will produce a highly stable $^{13}CO_2$ that can be detected and quantitatively measured in a breath test.[10]

To enable this diagnostic test, a multigram quantity of a high-quality [2-^{13}C]-5-FU (**1**) is required. It can be prepared from [^{13}C]-urea (**2**) which is commercially available. The use of the labeled urea for introducing an isotope into position-2 of the uracil ring was first reported in 1952 by Mander and Brown[11] who reacted [^{14}C]-urea with malic acid in fuming sulfuric acid following the approach pioneered by Davidson and Baudusch.[12] The same year, Bennett reported a condensation of [^{14}C]-thiourea with diethoxypropionate to produce [2-^{14}C]-thiouracil that was subsequently converted to [2-^{14}C]-uracil.[13] A similar approach was reintroduced in 2001 by a Pfizer group that reported the use of [^{14}C]-thiourea for introduction of an isotope into position-2 followed by hydrolysis with chloroacetic acid to produce [2-^{14}C]-uracil.[14] The use of labeled [^{11}C]-phosgene was reported for the introduction of the C-11 moiety into the 2-position of 5-FU for use as an agent for the positron emission tomographic imaging-based diagnostic Strauss test.[15]

Most of the previously reported pathways either include direct fluorination of uracil with hydrogen fluoride HF,[16] fluorine,[17–19] trifluoromethyl hypofluorite CF_3OF,[16,20] acetyl hypofluorite,[21], selective F-Cl exchange,[22] or a lengthy multistep approach.[3,23] More recently, a thermal conversion of series of *O*-alkyl and *O*-acyl substituted 5-fluoro-6-hydroxy-5,6-dihydro-pyrimidinediones to 5-FU was reported.[24] All these strategies involve using dangerous reagents that also represent a challenge in scaling up. In this work, Selectfluor® was chosen as the effective and most safe agent for direct fluorination of uracil. The use of this reagent for the preparation of unlabeled 5-FU was reported in 1995 by Lal *et al*.[25]

Experimental

[^{13}C]-urea was obtained from Cambridge Isotope Laboratories, Andover, MA. All other reagents and supplies were purchased from Sigma Aldrich and Fisher Scientific. Nuclear magnetic resonance spectra were recorded on a 400 MHz Bruker Avance spectrophotometer. Chemical shifts are reported as δ values with reference to TMS. Fluorotrichloromethane was used as an internal reference for fluorine NMR. Purity and isotopic enrichment of the final product were determined using an Alliance 2695 HPLC System equipped with PDA detector and Shimadzu LCMS 2010 MS Detector. Chromatographic separation was performed on Teledyne ISCO Automated Chromatography system Combiflash® XL using Teledyne ISCO flash columns.

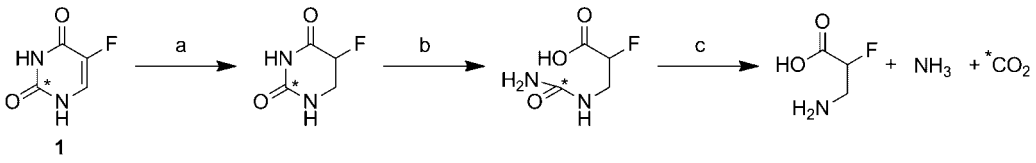
[2-^{13}C]-Pyrimidine-2,4(1*H*,3*H*)-dione (4)

Polyphosphoric acid (PPA; 405 g) was placed into a 1 L round bottom flask and heated at 110°C under stirring for 20 min. Then

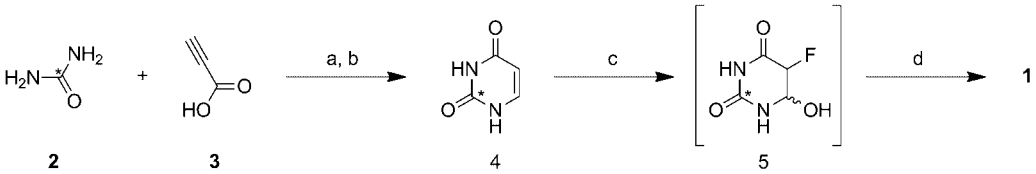
[a]*Institute for Therapeutics Discovery and Development, College of Pharmacy, University of Minnesota, Minneapolis, MN, USA*

[b]*Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, Minneapolis, MN, USA*

**Correspondence to: Vadim J. Gurvich, Institute for Therapeutics Discovery and Development, College of Pharmacy, University of Minnesota, 717 Delaware St. SE, Minneapolis, MN 55414, USA.*
E-mail: vadimg@umn.edu



Scheme 1. (a) Dihydropyrimidine dehydrogenase (PDP); (b) Dihydropyrimidinase (DHP); and (c) β-Ureidopropionase (BUP); * = ^{13}C.



Scheme 2. (a) PPA; (b) NaOH/H_2O; (c) Selectfluor®, $Ph_4B^-Na^+$; and (d) 220–230 °C; * = ^{13}C.

[^{13}C]-urea (**2**, 15 g, 246 mmol) was added. After the reaction mixture became completely homogenic, propiolic acid (**3**, 16.6 mL; 270 mmol) was slowly added in approx 4 mL portions. The heating bath temperature was lowered to 95°C and the reaction mixture stirred for 4 h. Water (600 mL) was slowly added followed by charcoal (3 g) and the mixture was stirred for approx 1 min. Charcoal was filtered out and the filtrate was placed into a refrigerator and left overnight. The precipitated solid was then collected by vacuum filtration and re-crystallized from boiling water (600 mL); charcoal (3 g) was added to the boiling solution and immediately filtered off. The solution was allowed to cool down, the precipitated solid was collected and dried *in vacuo* to produce 17.6 g (64% yield) of the desired product **4:** ^{1}H NMR (DMSO-d_6) δ 11.0 (s, 1H), 10.8 (s, 1H), 7.38 (m, 1H), 5.44 (dd, $J_1 = 7.56$, $J_2 = 1.85$, 1H). ^{13}C NMR (DMSO-d_6) δ 100, 142, 152 (high-intensity signal), 164. MS *m/z* 112 [M^+-1].

[2-^{13}C]-5-Fluoropyrimidine-2,4(1*H*,3*H*)-dione (1)

[2-^{13}C]-Pyrimidine-2,4(1*H*,3*H*)-dione **4** (28.4 g, 251 mmol) was placed into distilled water (850 mL). Selectfluor® (88.9 g, 251 mmol) was added to the suspension and the mixture was stirred at 105°C for 16 h. Sodium tetraphenylborate (192 g, 561 mmol) was added. The heat source was removed from the flask and the mixture was stirred on an ice bath for 30 min. The precipitated solid was then filtered out and washed with water. The filtrate and washing water were combined and concentrated under reduced pressure and the residue was dried overnight in a vacuum drying oven at 95°C. The residue was transferred into the sublimating apparatus base and flushed with nitrogen. The sublimating apparatus was heated in a silicon oil bath (bath temperature 195–205°C) under vacuum (0.5 mm Hg) while the temperature of the cold finger was maintained at the range of 10–50°C. The sublimation proceeded until the residue on the bottom of the apparatus was completely blackened. Then the sublimed material was removed, the sublimation apparatus was rinsed with water and acetone, and dried. The sublimation then proceeded with a silicon oil bath heated to a temperature of 220–230°C until the residue on the bottom of the apparatus was completely blackened. The sublimed material was removed from the apparatus and its composition was determined by ^{1}H NMR. If the content of the unreacted [2-^{13}C]-pyrimidine-2,4(1*H*,3*H*)-dione **4** was less than 5%, further purification by recrystallization from ethanol is possible. If it was greater than 5%, the material was dissolved in methanol, stirred with silica gel and the solvent was evaporated to dryness under reduced pressure. The residue was then loaded on the loading column of Teledyne ISCO Automated Chromatography system Combiflash® XL and purified on a silica gel column with eluent acetyl acetate–isopropanol using the following gradient: 0–5 min – 100% ethyl acetate, 5–15 min – 99.5% ethyl acetate, 15–30 min – 99% ethyl acetate, 35 min – 95% ethyl acetate. The fractions were analyzed by HPLC and those containing the pure product were combined and the solvents were removed under reduced pressure. After re-crystallization from ethanol, the material was dried *in vacuo* to produce 13.4 g (41% yield) of the desired product **1** with > 99% purity as determined by HPLC and > 99.9% isotopic enrichment as determined by LCMS: ^{1}H NMR (DMSO-d_6) δ 11.5 (bs, 1H), 10.7 (bs, 1H), 7.75 (m, 1H). ^{13}C NMR (DMSO-d_6) δ 158, 151 (high-intensity signal), 141, 139, 126. ^{19}F NMR (DMSO-d_6) δ 171.4. MS *m/z* 130 [M^+-1].

Results and discussion

The primary goal of this study was to develop a reliable and scalable methodology for the preparation of [2-^{13}C]-5-FU (**1**) on a multigram scale with the chemical and isotopic purity of > 99%. This approach is designed for the preparation of this material under FDA-regulated cGMP conditions that makes it suitable for use as an investigational new agent in clinical trials.

For the preparation of [2-^{13}C]-5-FU (**1**), a three-step chemical process represented in Scheme 2 has been chosen. [2-^{13}C]-Uracil (**4**) was prepared using a modified procedure of the Iida application[26] of the Harada and Suzuki condensation[27] of [^{13}C]-urea (**2**) with propiolic acid (**3**). The resulting [2-^{13}C]-uracil (**4**) was converted to [2-^{13}C]-5-FU (**1**) by direct fluorination with Selectfluor®.[25] Our initial experimental results following Lal's approach[25] revealed that the actual yield and purity of the final product were far lower than expected. It was unclear whether the reported 82% yield was referring to 5-FU or its immediate precursor, 5-fluoro-6-hydroxydihydropyrimidine-2,4(1*H*,3*H*)-dione, that is formed as a result of the direct fluorination of uracil with a reported yield of 82%. The purity of the final product was not reported.

We attempted to optimize the fluorination step by varying the amount of Selectfluor® from 0.9 to 1.5 eq of uracil. The conversion of uracil was only 75–80% at 0.9 eq and an

unidentified impurity was increasingly formed when we progressed from 1.1 to 1.5 eq of Selectfluor®. While the use of 1.0 eq did not result in full conversion of the starting material, the formation of the impurity was minimal. Our experiments revealed that an unidentified impurity formed at the fluorination step, was not being removed by sublimation. We also found this specific impurity to be difficult to remove by both chromatography and recrystallization. However, it was determined that the impurity is sublimed at a lower temperature range than the target material **1**. To resolve this problem, a fractionated sublimation process was developed. At the first step, the sublimation apparatus was heated to 195–205°C in which some [2-^{13}C]-5-FU (**1**) and the entire impurity were sublimed. The second step was carried out at a higher temperature range of 220–230°C that allowed the isolation of [2-^{13}C]-5-FU (**1**) contaminated only with 5–10% of [2-^{13}C]-uracil (**4**).

The composition of the fluorohydrin **5** products, as determined by NMR, was consistent with that described by Visser.[28] Our attempts to substitute the sublimation procedure with certain other types of hydrolysis and elimination previously described in literature,[29–31] as well as using microwave irradiation, did not result in sufficient conversion of the fluorohydrin **5** to the target compound **1** or any advantages for purification.

The sublimated material contained up to 10% of [2-^{13}C]-uracil (**4**) and our attempts to remove it by re-crystallizations were not successful. Chromatographic separation of 5-FU and uracil is considered a challenge that reportedly can only be achieved by preparative HPLC.[19,32–35] We applied the normal phase HPLC approach developed by Kažoka for detection of uracil in 5-FU[34,36] to a multigram preparative separation by Teledyne ISCO Automated Chromatography system Combiflash® XL. The separation was performed on a silica gel column using an acetyl acetate–isopropanol gradient. The chromatography step was followed by final re-crystallization from ethanol allowing us to achieve > 99% purity of [2-^{13}C]-5-FU (**1**); Figure 1. Crystallization from ethanol without a chromatography step allowed us to achieve a high level of purity but would not remove the residual [2-^{13}C]-uracil (**4**).

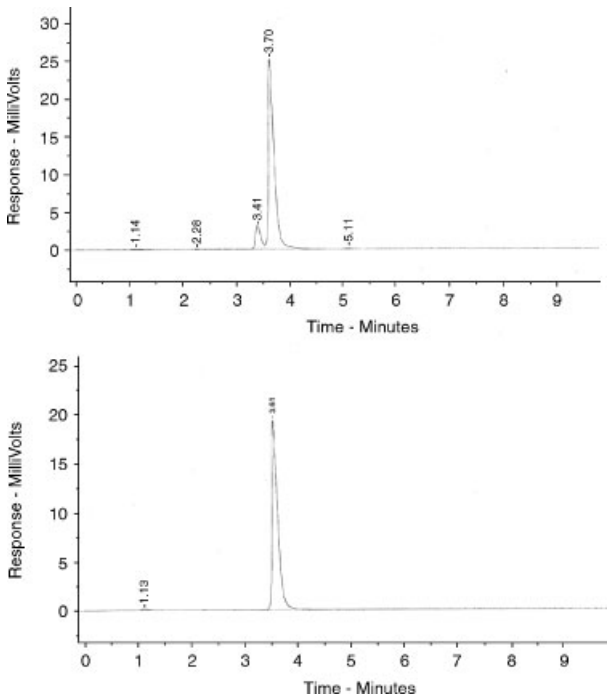


Figure 1. Chromatograms of analytical HPLC of (a) mixture of [2-^{13}C]-5-FU (**1**) and [2-^{13}C]-uracil (**4**) before chromatographic purification and (b) [2-^{13}C]-5-FU (**1**) after chromatographic purification.

Conclusion

[2-^{13}C]-5-FU (**1**) was successfully synthesized and purified by preparative chromatography on silica gel followed by recrystallization. The material was prepared on a multigram scale that can be further scaled up for obtaining larger quantities. The material is characterized by high chemical and isotopic purities and it is suitable for further clinical development as a diagnostic agent. This approach is applicable to any conversion of uracil to 5-FU on a larger scale.

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References

[1] G. C. Daher, B. E. Harris, R. B. Diasio, *Pharmacol. Ther.* **1990**, *48*, 189–222.
[2] J. L. Grem, *Invest. New Drugs* **2000**, *18*, 299–313.
[3] R. Duschinsky, E. Pleven, C. Heidelberger, *J. Am. Chem. Soc.* **1957**, *79*, 4559–4560.
[4] C. Heidelberger, R. Duschinsky, US 2802005, **1957**.
[5] C. Heidelberger, N. K. Chaudhuri, P. Danneberg, D. Mooren, L. Griesbach, R. Duschinsky, R. J. Schnitzer, E. Pleven, J. Scheiner, *Nature* **1957**, *179*, 663–666.
[6] A. Lee, H. Ezzeldin, J. Fourie, R. Diasio, *Clin. Adv. Hematol. Oncol.* **2004**, *2*, 527–532.
[7] H. R. Thomas, H. H. Ezzeldin, V. Guarcello, L. K. Mattison, B. L. Fridley, R. B. Diasio, *Pharmacogenetics and Genomics* **2007**, *17*, 973–987.
[8] H. R. Thomas, H. H. Ezzeldin, V. Guarcello, L. K. Mattison, B. L. Fridley, R. B. Diasio, *Pharmacogenetics and Genomics* **2008**, *18*, 25–35.
[9] H. H. Ezzeldin, R. B. Diasio, *J. Clin. Oncol.* **2008**, *26*, 2080–2082.
[10] L. K. Mattison, H. Ezzeldin, M. Carpenter, A. Modak, M. R. Johnson, R. B. Diasio, *Clin. Cancer Res.* **2004**, *10*, 2652–2658.
[11] H. G. Mandel, C. L. Brown, *J. Am. Chem. Soc.* **1952**, *74*, 2439–2440.
[12] D. Davidson, O. Baudisch, *J. Am. Chem. Soc.* **1926**, *48*, 2379–2383.
[13] L. L. Bennett, *J. Am. Chem. Soc.* **1952**, *74*, 2432–2433.
[14] K. S. Zandi, S. A. Miller, *Synth. Appl. Isot. Label. Compd. Proc. Int. Symp. 7th* **2001**, 268–271.
[15] K.-i. Seki, K.-i. Nishijima, Y. Kuge, N. Tamaki, L. I. Wiebe, K. Ohkura, *J. Pharm. Pharm. Sci.* **2007**, *10*, 212–216.
[16] R. H. Hesse, D. H. R. Barton, H. T. Toh, M. M. Pechet, *J. Org. Chem.* **1972**, *37*, 329–330.
[17] E. N. Vine, D. Young, W. H. Vine, W. Wolf, *Int. J. Appl. Radiat. Isot.* **1979**, *30*, 401–405.
[18] M. Diksic, P. Di Raddo, *Tetrahedron Lett.* **1984**, *25*, 4885–4888.
[19] F. Oberdorfer, E. Hofmann, W. Maier-Borst, *J. Label. Compd. Radiopharm.* **1989**, *27*, 137–145.
[20] O. Miyashita, K. Matsumura, H. Shimadzu, N. Hashimoto, *Chem. Pharm. Bull.* **1981**, *29*, 3181–3190.
[21] G. W. M. Visser, R. E. Herder, F. J. J. De Kanter, J. D. M. Herscheid, *J. Chem. Soc. Perkin Trans. 1: Org. Bio-Org. Chem.* (*1972–1999*) **1988**, 1203–1207.
[22] B. Baasner, E. Klauke, *J. Fluorine Chem.* **1989**, *45*, 417–430.
[23] G. Q. Shi, Q. Wang, M. Schlosser, *Tetrahedron* **1996**, *52*, 4403–4410.
[24] S. G. Semenov, B. N. Maximov, *Fluorine Notes* **2006**, *45*.
[25] G. S. Lal, W. Pastore, R. Pesaresi, *J. Org. Chem.* **1995**, *60*, 7340–7342.

[26] K. Iida, T. Chiyoda, R. Hirasawa, A. Iwata, M. Kajiwara, *J. Label. Compd. Radiopharm.* **1997**, *39*, 69–77.
[27] K. Harada, S. Suzuki, *Tetrahedron Lett.* **1976**, 2321–2322.
[28] G. W. M. Visser, S. Boele, B. W. Vonhalteren, G. Knops, J. D. M. Herscheid, G. A. Brinkman, A. Hoekstra, *J. Org. Chem.* **1986**, *51*, 1466–1471.
[29] H. A. Lozeron, M. P. Gordon, T. Gabriel, W. Tautz, R. Duschinsky, *Biochemistry* **1964**, *3*, 1844–1850.
[30] R. Duschinsky, T. Gabriel, W. Tautz, A. Nussbaum, M. Hoffer, E. Grunberg, J. H. Burchenal, J. J. Fox, *J. Med. Chem.* **1967**, *10*, 47–58.
[31] G. W. M. Visser, R. Wedzinga, R. P. Klok, J. D. M. Herscheid, *J. Chem. Soc. Perkin Trans. 2* **1994**, 231–236.
[32] A. A. Miller, J. A. Benvenuto, T. L. Loo, *J. Chromatogr. B*: *Biomed. Sci. Appl.* **1982**, *228*, 165–176.
[33] F. P. LaCreta, W. M. Williams, *J. Chromatogr. B*: *Biomed. Sci. Appl.* **1987**, *414*, 197–201.
[34] H. Kažoka, *Khimiko-Farmatsevticheskii Zh.* **1995**, *29*, 49–51.
[35] G. J. Peters, I. Kraal, E. Laurensse, A. Leyva, H. M. Pinedo, *J. Chromatogr.* **1984**, *307*, 464–468.
[36] H. Kažoka, *J. Chromatogr. A* **2003**, *994*, 221–225.

