Research Article

Journal of Labelled Compounds and Radiopharmaceuticals

Received 16 June 2011, Revised 1 September 2011, Accepted 12 September 2011 Published online 18 October 2011 in Wiley Online Library

(wileyonlinelibrary.com) DOI: 10.1002/jlcr.1940

A novel synthesis of [2-^{14}C]2,5-dichloropyrimidine

Scott B. Tran,[a]* Brad D. Maxwell,[a] Hong Wu,[b] and Samuel J. Bonacorsi, Jr.[a]

[2-^{14}C]2,5-dichloropyrimidine is a useful reagent for labeling biologically active compounds for use in hepatocyte transport studies, protein covalent binding, and metabolic profiling. This paper describes a novel five-step synthesis of [2-^{14}C]2,5-dichloropyrimidine from readily available [^{14}C]urea by way of a boronic acid intermediate. A total of 4.34 mCi of [2-^{14}C]2,5-dichloropyrimidine was obtained with a specific activity of 226.0 μCi/mg (33.7 mCi/mmol). The radiochemical purity was 95.8%, and the overall radiochemical yield was 22% based on 20 mCi of [^{14}C]urea starting material.

Keywords: pyrimidine; 2,5-dichloropyrimidine; ^{14}C; isotope labeling; boronic acid

Introduction

Pyrimidine and its substituted analogs are present throughout nature in various forms and are the building blocks of numerous biologically active compounds from antibiotics to vitamins and liposaccharides.[1,2] Pyrimidines are bases in RNA and DNA, in which the most abundant are cytosine, thymine, or uracil. Substituted pyrimidines are also common components of many drugs such as buspirone (antianxiety agent), dasatinib (tyrosine kinases inhibitor), bosentan (antihypertensitive agent), and sildenafil (vasodilator agent).[3] For the the drug discovery and development activities within Bristol-Myers Squibb to be supported, it was necessary to prepare [2-^{14}C]2,5-dichloropyrimidine, 5. We herein described a novel five-step synthesis of [2-^{14}C]2,5-dichloropyrimidine, 5.[4] This sequence improves the existing routes to 5 by being more robust and higher yielding.

Experimental

General: Radioactivity was measured with a PerkinElmer Liquid Scintillation Analyzer, Tri-Carb Model 2900TR (PerkinElmer Life Sciences, Inc., Boston, MA, USA). Mass spectra were obtained with a Finnigan LXQ mass spectrometer (Thermo Electron Corp., Marietta, OH, USA). Proton NMR spectra were recorded on a Bruker Advance Ultrashield 300 MHz. UV and radiochemical purities were determined by HPLC (Agilent Technologies 1100 Series HPLC System and IN/US System β-Ram radiometric flow detector with a 0.5-ml flow cell). Analytical HPLC method: Phenomenex C18 Luna (Phenomenex Inc., Torrance, CA, USA), 5 μm, 4.6 × 150 mm, detected at 220 nm. Mobile phase A: 0.05% trifluoroacetic acid (TFA) in water, mobile phase B: 0.05% TFA in acetonitrile. Gradient: 0 min 5% B, 6 min 5% B, 11 min 95% B, 13 min 95% B, 15 min 5% B. Flowrate = 1.0 ml/min. Semi-preparative HPLC was performed with Varian Model 218 PrepStar Pumps with 25-ml heads equipped with a Varian ProStar Model 320 UV-Vis Detector (Varian Medical Systems, Inc., Palo Alto, CA, USA) and Rheodyne Model 7725i injector. Semi-preparative HPLC Conditions: Phenomenex Luna C18, 21.2 × 250 mm. UV detection: 220 nm, Flowrate: 15 ml/min. Mobil phase A: 0.1% TFA in water, Mobile phase B: 0.1% TFA in acetonitrile. Gradient: 0 min 15% B, 11 min 95% B, 13 min 95% B, 15 min 15% B. TLC was performed on 60 F_{254} silica gel plates (Merck) with UV and/or iodine detection. The distillation was performed with a micro distillation apparatus from Sigma-Aldrich (catalog number: Z129607). Flash chromatography was conducted using Teledyne Isco RediSep Rf (San Diego, CA, USA) packed columns using an AnaLogix BSR pump. Radiolabeled products were compared with authentic standards when possible. All reagents and solvents were ACS grade or better. The specific activity of [2-^{14}C]2,5-dichloropyrimidine was determined gravimetrically.

5-Bromopyrimidin-2[^{14}C]-ol hydrochloride, 2

To a 10-ml reaction flask was added pyrimidin-2[^{14}C]-ol hydrochloride prepared according to the procedure of Bonacorsi *et al.*[5], 1 (93 mg, 0.70 mmol) and concentrated HCl (1.5 ml). To this solution was slowly added a solution of bromine (64.9 μl, 1.26 mmol) in 0.3 ml concentrated HCl. A precipitate that formed after the bromine solution was completely added. The reaction was stirred at room temperature. Additional bromine, 7 μl and 9 μl, was added after 1 h and 18 h, respectively, to drive the reaction to completion. The solution was concentrated to near dryness by rotovap. The wet crude product was partially azeotroped with acetonitrile (2 × 2 ml) and rinsed with ether (3 × 1 ml) then partially dried under vacuum to produce a light yellow solid as the HCl salt (181.7 mg, >quantitative crude yield due to the product still containing solvent). HPLC, product retention time = 3.80 min, radiochemical purity = 92.3%. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.51 (s 2H). MS ESI$^+$ $[M+H]^+$ = 175 (57%), 177 (100%), 179 (44%).

[a]*Department of Chemical Synthesis, Radiochemistry Group, Bristol-Myers Squibb, Pharmaceutical Research and Development, P.O. Box 4000, Princeton, NJ 08543, USA*

[b]*Discovery Chemistry, Bristol-Myers Squibb, Pharmaceutical Research and Development, P.O. Box 4000, Princeton, NJ 08543, USA*

**Correspondence to: Scott B. Tran, Department of Chemical Synthesis, Radiochemistry Group, Bristol-Myers Squibb, Pharmaceutical Research and Development, P.O. Box 4000, Princeton, NJ 08543, USA.*
E-mail: scott.tran@bms.com

[2-^{14}C]5-Bromo-2-chloropyrimidine, 3

To a 10-ml dry reaction flask was added crude 5-bromopyrimidin-2[^{14}C]-ol hydrochloride, 2 (181.0 mg) from the previous step, and neat phosphoryl trichloride (801 μl, 8.59 mmol). To the reaction was added N,N-dimethylaniline (109 μl, 0.86 mmol) slowly at room temperature resulting in a clear brown solution. The reaction was heated to 120°C for 1 h. TLC analysis (25%:75% EtOAc : hexane, product 2 $R_f = 0.40$, product 3 $R_f = 0.85$) showed the reaction to be complete. HPLC analysis showed the disappearance of product 2 and the formation of product 3 (retention time = 8.45 min). Phosphoryl trichloride was quickly, but carefully distilled at 120°C with a microdistillation apparatus. Some of the product sublimed on the upper walls of the flask. The flask was cooled to 0°C in an ice water bath, and diethyl ether (3 ml) was added followed by saturated $NaHCO_3$ (6 ml) and NaOH (20 mg). The aqueous layer was extracted with diethyl ether (4 × 3 ml). The combined organic extracts were washed with brine, dried over Na_2SO_4, and filtered. The majority of the ether was removed by distillation to give a brown oil (148 mg, >100% crude yield, 95.2% radiochemically pure). The oil was used in the next reaction without further purification. ^{1}H-NMR (300 MHz, DMSO-d_6) δ ppm 8.90 (s, 2H).

[2-^{14}C]2-Chloropyrimidin-5-ylboronic acid, 4

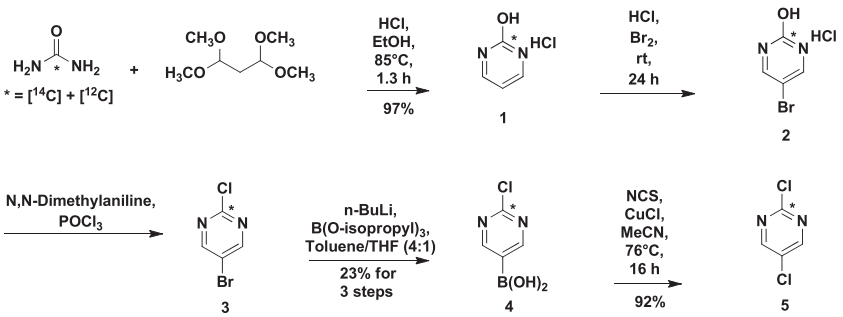
To an oven-dried 5-ml vial with a stir bar under nitrogen was added THF (240 μl), toluene (960 μl), 5-bromo-2[^{14}C]-chloropyrimidine, 3 (148 mg) from the previous step, and triisopropyl borate (197 μl, 0.842 mmol). The clear solution was cooled to −78°C to form a thick white suspension. *n*-Butyllithium (478 μl, 0.77 mmol) was added dropwise over 20 min. The reaction was vigorously stirred at −78°C for 1.5 h. The reaction was quenched at −78°C with 2 N HCl (1.2 ml) and then warmed slowly to room temperature resulting in two layers. HPLC analysis indicated that both layers contained product with the majority of the product being present in the aqueous layer. The aqueous layer was collected and evaporated to dryness by azeotroping with acetonitrile (3 × 10 ml) then further dried under vacuum. The crude product was dissolved in 1:1 water : acetonitrile (1.0 ml) and was purified by preparative HPLC. The pure fractions (retention time = 9.17 min) were pooled, and the solvents were removed by rotovap. The product was dried under vacuum to give a white solid as the TFA salt (44 mg, 23% yield after 3 steps). HPLC analysis showed that the product (retention time = 5.33 min) was 95.0% radiochemically pure. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.98 (s, 2H), 8.75 (br s, 2H).

[2-^{14}C]2,5-Dichloropyrimidine, 5

To a 5-ml flask equipped with a condenser under nitrogen was added [2-^{14}C]2-chloropyrimidin-5-ylboronic acid TFA salt, 4 (39 mg, 0.14 mmol) and acetonitrile (750 μl) to produce a suspension. To the suspension was added N-chlorosuccinimide (32.9 mg, 0.25 mmol) and copper (I) chloride (24.4 mg, 0.25 mmol). The mixture was heated to 76°C. After 16 h, HPLC analysis indicated complete formation of the product (retention time = 8.32 min) and loss of boronic acid starting material. The flask was cooled to 0°C in an ice water bath, diethyl ether (3 ml) was added, and pH of the solution was adjusted to 9 by slowly adding saturated $NaHCO_3$ (6 ml). After separation of the layers, the aqueous layer was extracted with diethyl ether (3 × 3 ml). The combined organic extracts were washed with brine, dried over Na_2SO_4, and filtered. The solvent was removed by distillation to afford an off white solid (19 mg, 0.13 mmol, 92% yield). The product radiochemical purity was 95.8%, total activity obtained = 4.34 mCi, and specific activity = 226.0 μCi/mg or 33.7 mCi/mmol. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.95 (s, 2H). MS (+H_2O, −HCl) ESI$^+$ $[M+H]^+$ = 131 (98%), 133 (100%), 135 (25%).

Results and discussion

2,5-dichloropyrimidine is chemically labile, volatile, and has a low melting point (57.0–57.5°C). It sublimes slowly upon standing at room temperature. At room temperature under vacuum at 50-μm mercury, approximately 45% of 2,5-dichloropyrimidine is lost after 1 h, and 85% is lost after 2 h. In unlabeled pilot reactions, we initially chose to follow the work reported by Tsantrizos[6] because of its convenience and quick access to the desired product, 2,5-dichloropyrimidine, after only two steps from pyrimidin-2-ol. However, on smaller scale, chlorination of pyrimidin-2-ol (95 mg, 0.70 mmol) gave low yields, produced multiple products, and required preparative HPLC to purify 5-chloropyrimidin-2-ol. Because of the low recovery and difficult purification and isolation, we developed a new synthetic pathway that produced the desired product in reasonable yield requiring less challenging purification conditions (Scheme 1). The synthesis of [2-^{14}C]2,5-dichloropyrimidine, 5 was completed in five steps from commercially available [^{14}C]urea. The specific activity of [^{14}C]urea (58 mCi/mmol) was reduced with unlabeled urea to approximately 30 mCi/mmol to accommodate the scale of the synthesis. It was reacted with 1,1,3,3-tetramethoxypropane in a heterocyclic condensation reaction to give pyrimidin[2-^{14}C]2-ol hydrochloride, 1 in 97% yield following the procedure of Bonacorsi.[5] Conversion of 1 to 5-bromopyrimidin[2-^{14}C]2-ol hydrochloride, 2 required 2.25 equivalents of bromine in concentrated HCl to ensure



Scheme 1. Preparation of [2-^{14}C]2,5-dichloropyrimidine, 5.

complete conversion. After solvent removal by rotovap, the residue was partially azeotroped with acetonitrile and purified by trituration with diethyl ether to afford the wet product greater than quantitative yield. This and other intermediates were only partially dried to avoid the loss of product under high vacuum. Crude compound 2 was subsequently used in the chlorination reaction with neat phosphoryl trichloride in the presence of N,N-dimethylaniline. The reaction was completed after 1 h at 120 °C. In the absence of N,N-dimethylaniline, extended reaction times (>36 h) and product decomposition were observed. Unreacted phosphoryl trichloride was carefully removed by distillation at 120 °C at ambient pressure. This step was performed quickly to avoid chemical decomposition at elevated temperatures. Although the product could have been purified by sublimation, distillation was preferred to remove residual phosphoryl trichloride and to avoid loss of product. This was followed by a basic work-up to afford crude chlorinated product 3 that was determined to be 95.2% radiochemically pure. This was sufficiently pure for use directly in the subsequent reaction with *n*-BuLi and triisopropyl borate to form [2-^{14}C]2-chloropyrimidin-5-ylboronic acid TFA salt, 4 in 23% yield after three steps. On larger scale unlabeled pilot boronation reactions, yields as high as 97% were obtained for this step. The lower yield obtained during the radiochemical synthesis was due to residual water being present in the previous crude product, which inhibited the Li/Br exchange and boronation. The final step involved the mild conversion of 4 to [2-^{14}C]2,5-dichloropyrimidine, 5 with stoichiometric amounts of N-chlorosuccinimide and copper chloride in 92% yield. This represents the first-reported heteroaromatic extension of the copper (I) mediated aromatic boronic acid to chloride conversion reported by Wu and Hynes.[7] HPLC analysis of the reaction progress indicated the desired product to be 95.8% radiochemically pure. Because of its chemical instability and volatility, it is recommended that [2-^{14}C]2, 5-dichloropyrimidine be used promptly upon its preparation. In our case, it was pure enough to be used directly in the next reaction without additional purification after aqueous work-up.

Acknowledgements

We gratefully acknowledge Huiping Zhang and Xiaoping Hou of Bristol-Myers Squibb Discovery Chemical Synthesis and colleagues at the Biocon Bristol-Myers Squibb Research and Development Center for providing us with unlabeled 2,5-dichloro-pyrimidine. We also thank John Hynes of Bristol-Myers Squibb Discovery Chemistry for providing us with the chlorination methodology used in the last step of the synthesis.

References

[1] The University of Michigan website, Chemistry on the Pyrimidine Ring. http://deepblue.lib.umich.edu/bitstream/2027.42/62242/1/riderl_2.pdf.
[2] a) D. J. Brown, R. F. Evans, W. B. Cowden, M. D. Fenn. *Chemistry of Heterocyclic Compounds: The Pyrimidines*, Volume 52. Print ISBN: 9780471506560. Online ISBN: 9780470187395. DOI: 10.1002/9780470187395. Copyright © 1994 by John Wiley & Sons, Inc.; b) C. Pierra, J. L. Imbach, E. De Clercq, J. Balzarini, A. Van Aerschot, P. Herdewijn, A. Faraj, A. G. Loi, J. P. Sommadossi, G. Gosselin, *Antiviral Res.* **2000**, *45*, 169–183.
[3] The DrugBank website. http://www.drugbank.ca/drugs?type=small molecule.
[4] This work was presented as a poster at the International Isotope Society 25th Northeast US Chapter Symposium held October 21–22, 2010 in Morristown, NJ.
[5] S. J. Bonacorsi, Jr, R. C. Burrell, G. M. Luke, J. S. Depue, J. K. Rinehart, B. Balasubramanian, L. J. Christopher, R. A. Iyer, *J. Label. Compd. Radiopharm.* **2007**, *50*, 65–71.
[6] Y. S. Tsantrizos, PCT WO 2007019674.
[7] a) H. Wu, J. Hynes, *Org. Lett.* **2010**, *12*, 1192–1195; b) C. Savarin, J. Srogl, L. S. Liebeskind, *Org. Lett.* **2002**, *4*, 4309–4312.

