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An improved synthesis of [2-^{14}C]2, 5-dichloropyrimidine

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A previously described, five-step synthesis of [2-^{14}C]2, 5-dichloropyrimidine was based on condensation of [^{14}C]urea with an acetal, followed by bromination, chlorination, boronic acid formation, and finally chlorination. This improved synthesis also started from readily available [^{14}C]urea, which was condensed with 2-chloromalonaldehyde, followed by chlorination with $POCl_3$ yielding [2-^{14}C]2, 5-dichloropyrimidine with a radiochemical purity of 99% in an overall radiochemical yield of 72%.

Keywords: pyrimidine; 2,5-dichloropyrimidine; urea; C-14; isotopic labeling

Introduction

Substituted pyrimidines are useful intermediates for many molecules of pharmaceutical interest such as buspirone (antianxiety), dasatinib (tyrosine kinases inhibitor), bosentan (antihypertensive), and sildenafil (vasodilator).[1] Labeling these compounds with carbon-14 can be readily achieved from carbon-14 labeled pyrimidines. Historically, labeled pyrimidines have been difficult to prepare in high yields. Previously, our group published a five-step synthesis of [2-^{14}C]2, 5-dichloropyrimidine from [^{14}C]urea by way of a boronic acid intermediate with an overall radiochemical yield of 22%.[2] To avoid volatile and difficult to handle intermediates in that sequence and shorten the number of steps, we investigated an alternative synthesis starting from the same [^{14}C]urea precursor. Here, we describe a new, more efficient two-step synthesis of [2-^{14}C]2, 5-dichloropyrimidine.

Experimental

General: All reagents and solvents used were of ACS grade or higher. [^{14}C] urea was purchased from Quotient Bioresearch, Cardiff, UK. Microwave reactions were conducted using a CEM Discovery system with magnetic stirring. Liquid chromatography–mass spectrometry (LC–MS) data were obtained on a Thermo LXQ 2.0 Mass Spectrometer System (Thermo Fisher Scientific Inc. 81 Wyman StreetWaltham, MA 02454) with electrospray ionization. ^{1}H NMR spectra were recorded on a Bruker AVANCE II 300 MHz Spectrometer with an Ultrashield™ Magnet (Bruker Scientific Instruments, Billerica, Massachusetts). Analytical HPLC analyses were performed on an Agilent 1100 HPLC system including solvent degasser, quaternary pump, autosampler, and diode array detector connected to an IN/US BetaRam flow detector with a 0.5-ml detector (LabLogic Systems, Inc., Brandon, FL) cell. Analytical HPLC method: Phenomenex LUNA C18 (Phenomenex Inc., Torrance, CA, USA), 5 μm, 4.6 × 150 mm, UV detection at 220, 320, and 280 nm, flow rate 1 ml/min. Mobile phase A: 0.1% trifluoroacetic acid in water, mobile phase B: 0.1% trifluoroacetic acid in acetonitrile. Gradient: 0 min 100% A, 3 min 100% A, 10 min 40% B, 15 min 95% B, 20 min 95% B, 25 min 100% A. Preparative HPLC was performed on a Varian HPLC system with two PrepStar 218 pumps and ProStar 320 variable UV detector. Preparative HPLC method: Phenomenex LUNA C18, 5 μm, 21.2 × 250 mm, UV detection at 323 nm, flow rate 20 ml/min. Mobile phase A: 0.1% trifluoroacetic acid in water, mobile phase B: 0.1% trifluoroacetic acid in acetonitrile. Gradient: 0 min 100% A, 3 min 100% A, 20 min 95% B, 25 min 100% A. Radioactivity was measured with a PerkinElmer TriCarb Model 2900Tr liquid scintillation counter (PerkinElmer Life Sciences Inc., Boston, MA, USA), using Ultima Gold (PerkinElmer Life Science) as liquid scintillation cocktail.

[2-^{14}C]5-Chloropyrimidin-2-ol, 6

[^{14}C]urea (21 mg, 0.34 mmol, 18 mCi, 56 mCi/mmol), unlabeled urea (15 mg, 0.25 mmol) (total 36 mg, 0.58 mmol), and 2-chloromalonaldehyde (89 mg, 0.84 mmol) were placed in a 7-ml microwave tube and acetic acid (1.6 ml) was added. The solution was subjected to microwave conditions: 130 °C, 40 min, PowerMax on, stirrer on. HPLC analysis showed approximately 80% conversion to the desired product. The crude mixture was added to water (10 ml) and injected onto the preparative HPLC system. Pooled HPLC fractions containing product were concentrated under reduced pressure to give [2-^{14}C]5-chloropyrimidin-2-ol **6** as a white solid (56 mg, 13 mCi, radiochemical yield 72%). ^{1}H NMR (300 MHz, DMSO-d6) δ 8.43 (s, 2H). MS ESI^+ $[M+H]^+ = 131, 133$.

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

[2-^{14}C]5-Chloropyrimidin-2-ol (**6**, 28 mg, 0.25 mmol, 6.5 mCi) and unlabeled 5-chloropyrimidin-2-ol (28 mg, 0.25 mmol) (total 56 mg, 0.49 mmol) were dissolved in MeOH (1 ml) and carefully transferred to a 7-ml microwave tube then evaporated under a gentle stream of nitrogen. $POCl_3$ (700 μl, 7.5 mmol) was added and the mixture subjected to microwave conditions: 130 °C, 20 min, PowerMax on, stirrer on. After cooling to room temperature, the reaction mixture was added dropwise to a 20-ml vial containing ice-cold water (5 ml) with stirring in an ice-bath. KOH pellets (~1 g) were added to adjust the pH to 9. The mixture was extracted with Et_2O (5 x 10 ml). Pooled organic extracts were dried over Na_2SO_4, filtered and concentrated under a steady stream of nitrogen for 2h to a volume of ~0.5 ml. The vial was capped and stored in the refrigerator overnight. The crude product solidified to form a light yellow solid, [2-^{14}C]2, 5-dichloropyrimidine, **5** (64 mg, 6.5 mCi, radiochemical yield: 100%, radiochemical purity: 99%). The product co-eluted with an authentic standard.

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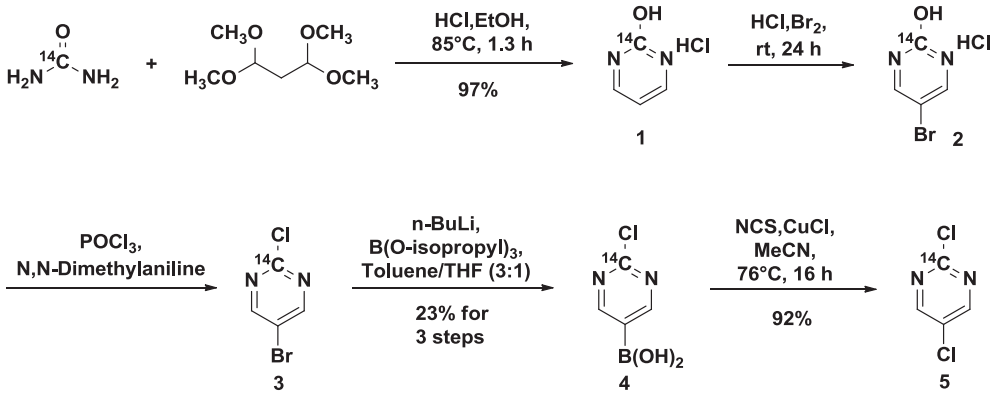
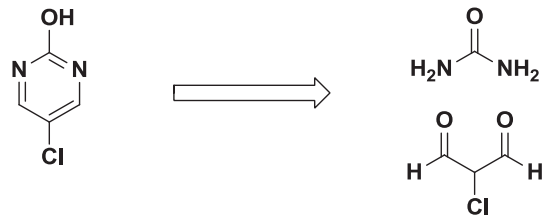


Scheme 1. Previously reported preparation of [2-^{14}C]2, 5-dichloropyrimidine **5** by our group.

Results and discussion

A five-step synthesis of [2-^{14}C]2,5-dichloropyrimidine was recently published by Tran *et al.*[2] In this synthesis, [^{14}C]urea was condensed with malonaldehyde bis(dimethyl acetal) to yield [2-^{14}C]pyrimidin-2-ol hydrochloride **1**, followed by bromination and chlorination to give [2-^{14}C]5-bromo-2-chloropyrimidine **3**. Subsequently, **3** was converted to [2-^{14}C]2-chloropyrimidin-5-ylboronic acid **4**. Finally, boronic acid **4** was reacted with *N*-chlorosuccinimide and copper chloride to give [2-^{14}C]2, 5-dichloropyrimidine in 22% overall radiochemical yield. The key intermediate [2-^{14}C]5-bromo-2-chloropyrimidine **3** was observed to be volatile and decomposed at elevated temperature.[2] (Scheme 1)



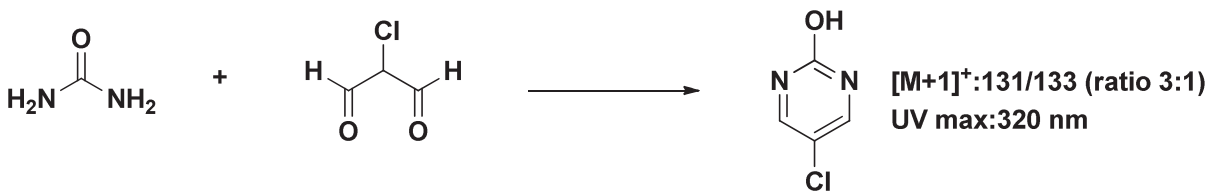
Scheme 2. Retrosynthetic analysis of 5-chloropyrimidin-2-ol.

To avoid this intermediate and develop a more efficient synthesis, we reviewed the synthetic strategy and decided to focus on the preparation of key intermediate 5-chloropyrimidin-2-ol. Retrosynthetic analysis indicated that direct condensation of urea with 2-chloromalonaldehyde could give the desired 5-chloropyrimidin-2-ol as shown in Scheme 2.

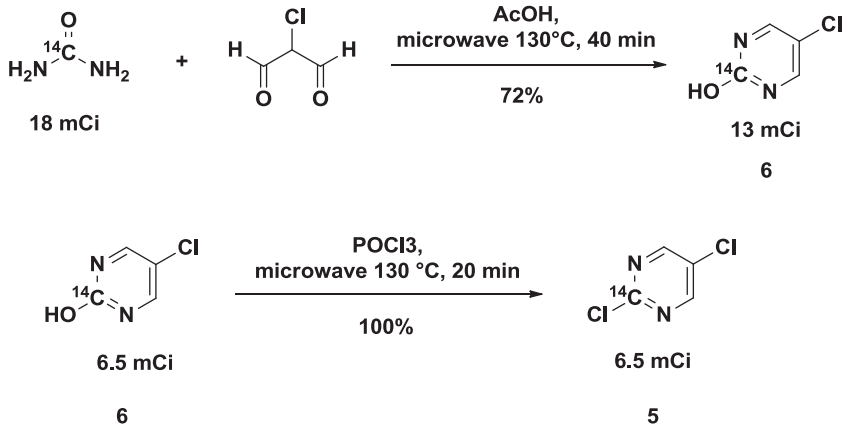
It was previously reported that 2-chloromalonaldehyde reacted with different benzamidine acetates in pyridine to form pyrimidine derivatives in good yields.[3] First, we tried the condensation reaction of urea with 2-chloromalonaldehyde in pyridine. A new product with a $[M+1]^+ = 149/151$ was observed with a UV max of 280 nm, the data suggests that this product is the result of condensation without the desired dehydration to form the pyrimidine system. (Table 1)

Modifying the reaction conditions with a Dean Stark trap at 120°C yielded the same product. We switched the solvent to acetic acid and a mixture of two products with $[M+1]^+ = 131/133$ and 149/151 were observed by LC–MS. The UV maximum of the new product was 320 nm, matching the product standard. The ^{1}H NMR spectrum also matched that of the standard. Unfortunately, at an elevated reaction temperature (120°C), extended reaction times did not improve conversion of the condensation product to the pyrimidine. Therefore, we switched to microwave radiation of the mixture in acetic acid to form the desired product as the

Table 1. Condensation of urea with 2-chloromalonaldehyde under different reaction conditions



Reaction conditions	Results
Pyridine, 90–105°C	$[M+H]^+$: 149/151 (ratio 3:1, 131/133 + water), UV max 280 nm
Pyridine, 120°C w/ dean stark trap	$[M+H]^+$: 149/151, UV max 280 nm
Acetic acid, 100°C	Mixture of products with $[M+H]^+$: 149/151, 131/133
Acetic acid, microwave 120°C, 20 min	Mostly desired product + hydrate ($[M+H]^+$: 149/151)
Conc. HCl, microwave 120°C, 20 min	Desired product, unidentified black particles
Conc. HCl/DMF (1:1), microwave 120°C, 20 min	No product
Acetic acid, microwave 130°C, 40 min	Complete conversion to form a clear solution



Scheme 3. Synthesis of [2-^{14}C]2, 5-dichloropyrimidine **5**.

major component of the reaction mixture but still some undehydrated, non-conjugated intermediate was observed. Concentrated HCl was also used in place of acetic acid, but black particles were formed because of the poor solubility of 2-chloromalonaldehyde in the aqueous medium. However, analysis of the aqueous phase showed complete conversion to the desired product. Next, a 1:1 mixture of concentrated HCl and Dimethylformamide (DMF) was used to address the solubility issue of 2-chloromalonaldehyde. Unfortunately, no desired product was observed. Eventually, we settled for the optimal conditions of acetic acid, microwave 130°C, 40 min, PowerMax on, stirrer on. LC–MS showed complete conversion to the desired product, 5-chloropyrimidin-2-ol.

As shown in Scheme 3, [^{14}C]urea reacted with 2-chloromalonaldehyde yielding 72% of [2-^{14}C]5-chloropyrimidin-2-ol **6** after preparative HPLC purification. The final chlorination was conducted in $POCl_3$ under microwave conditions at 130°C for 20 min with PowerMax on, giving the desired [2-^{14}C]2, 5-dichloropyrimidine **5** in quantitative yield. Because of the volatility of the 2, 5-dichloropyrimidine, a modified workup procedure was adapted. The reaction mixture was added dropwise to a small volume of water in an ice-bath. The reverse quench is important to ensure the safe consumption of excess $POCl_3$. KOH pellets were added directly to the quenched solution to adjust the pH to 9. Et_2O was then added to extract the product. After drying, most of the Et_2O was removed under a very gentle stream of nitrogen. The resulting solid product containing small amounts of Et_2O was used directly in the next reaction without further purification. In both reactions, carbon 14-labeled compounds were diluted with unlabeled compounds (urea and 5-chloropyrimidin-2-ol) to fit our specific activity requirement. Repeated run without any dilution with unlabeled compounds gave same result.

Conclusion

An efficient and improved two-step synthesis of [^{14}C]2, 5-dichloropyrimidine was developed. [2-^{14}C]2, 5-Dichloropyrimidine was prepared in 72% radiochemical yield. This chemistry should readily apply to the preparation of other 5-substituted pyrimidines, such as 5-bromo, 5-alkyl or 5-acetyl.

Acknowledgements

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Conflict of Interest

The authors did not report any conflict of interest.

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