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Synthesis of [^{14}C] labeled 2-methoxypyrimidine-5-carboxylic acid

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A versatile method for ^{14}C labeling of 2-methoxypyrimidine-5-carboxylic acid at the 2-position has been developed after encountering difficulties with traditional approaches to label the carboxyl function. The method developed can also be used for ^{14}C labeling other positions of the pyrimidine ring system.

Keywords: [^{14}C] 2-methoxyprimidine-5-carboxylic acid; [^{14}C] *O*-methylisourea hydrochloride

Introduction

The syntheses of 2-methoxypyrimidine-5-carboxylic acids with a ^{14}C label at the 2-position of the pyrimidine ring **2**, or at 4-, 5- and 6-positions of **3** involve multi-step process compared to the simple introduction of ^{14}C on the carboxyl function of **1** (Figure 1). This report discusses our initial attempts to synthesize carboxyl labeled 2-methoxypyrimidine-5-carboxylic acid and our eventual success in preparing ring labeled title compound.

Results and discussion

Labelling of carboxyl function of **1** was attempted by employing known methods such as metal–halogen exchange/Grignard reactions[1–2] and treatment with [^{14}C] CO_2. None of these methods resulted in the synthesis of desired ^{14}C labeled carboxylic acid derivative. Thus, when 5-bromo-2-methoxypyrimidine was reacted with *n*-BuLi followed by [^{14}C] CO_2 at −78 to −20°C, the reaction gave an unidentified product along with unreacted starting material.

Aromatic nitriles are well-known precursors for the corresponding carboxylic acids.[3] Aryl nitriles have been prepared by cyanation of iodides or bromides using CuCN or KCN in absence of solvent or in solvents like pyridine, quinoline, DMF, NMP at elevated temperature.[4–11] Under Von Braun conditions the reaction of 5-bromo-2-methoxypyrimidine with [^{14}C] CuCN in DMF at 145°C for 48 h did not yield cyano derivative.

Palladium catalyzed cyanations of aryl bromides promoted by organotin compounds have been successfully used for the preparation of a number of aryl cyanides including 3-cyanopyridine and 3-cyanoquinolines.[12] However, reaction of 5-bromo-2-methoxypyrimidine with [^{14}C] KCN under these conditions resulted only in the recovery of starting material.

The synthesis of 2-methoxypyrimidine-5-carboxylic acid **2** with a label at 2-position of pyrimidine ring is an alternative choice. A number of synthetic methods are reported in the literature for pyrimidine 5-carboxylic acid derivatives.[13–16] However, a few direct approaches leading to pyrimidine ring that lacks substitution at 4-position have been reported.[17–19]

Our approach to compound **2** is as depicted in Scheme 1. [^{14}C] barium cyanamide (**4**) was converted to [^{14}C] cyanamide (**5**) by the treatment with aqueous H_2SO_4 in 75% yield. Reaction of [^{14}C] cyanamide (**5**) with anhydrous CH_3OH in the presence of dry HCl gas for 3 days according to the literature method[20] gave [^{14}C] *O*-methylisourea hydrochloride (**6**).

In parallel, the sodium salt of 3,3-dimethoxy-2-methoxycarbonylpropen-1-ol (**7**) was synthesized by the condensation of methyl formate with methyl 3,3-dimethoxypropionate in the presence of NaH as described in the literature.[19] The sodium salt **7** was reacted with [^{14}C] *O*-methylisourea hydrochloride (**6**) in DMF at 100°C to give [2-^{14}C] methyl 2-methoxypyrimidine-5-carboxylate (**8**) in 70% yield, identified by ^{1}H NMR. The hydrolysis of ester **8** was carried out by heating with 2 N NaOH in aqueous dioxane.[21] [2-^{14}C] 2-methoxypyrimidine-5-carboxylic acid (**2**) was isolated after acidification of the mixture with 2 N HCl in 97% yield and radiochemical purity of 98.41% by HPLC. Specific activity was determined to be 56.4 mCi/mmol. The overall yield of [2-^{14}C] 2-methoxypyrimidine-5-carboxylic acid was 13.7% from **4**.

This methodology led to a successful labeling in the 2-position. This approach may also be applied to other pyrimidine carboxylic acid derivatives **3** with ^{14}C labeling at the 4-, 5- and/or 6-position by using appropriately labeled methyl formate or methyl 3,3-dimethoxypropionate. Labeling can also be extended to the carboxyl moiety as well using this scheme starting from **7** labeled at the ester carbon.

Experimental

All reagents and solvents were purchased from Sigma-Aldrich Chemical Company. [^{14}C] Barium cyanamide was obtained from

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I.U.T. GmbH, Germany. ^{1}H NMR spectra were recorded on a Bruker 300 MHz spectrometer. Internal TMS was used as a reference standard. The final product was identified by HPLC comparison with commercially available material on a Zorbax SB C-18 column using (A) 0.1% TFA in H_2O (B) CH_3CN, 0–50% B linear gradient in 25 min, holding 50% B for 5 min, 1 ml/min, UV 254 nm.

[^{14}C] cyanamide (5)

To a suspension of [^{14}C] barium cyanamide (**4**) (1200 mCi, specific activity 57.6 mCi/mmol, 20.8 mmol) in H_2O (14 ml) cooled to 0–5°C, was added conc. H_2SO_4 (1.05 ml, 20.8 mmol) drop wise during about 15 min. The resulting white suspension was stirred at 0–5°C for 1 h and centrifuged. The supernatant liquid was decanted and the residue stirred with H_2O (10 ml) and centrifuged. The process was repeated and the combined supernatant was extracted with EtOAc (10 × 30 ml). The organic extract was dried over Na_2SO_4, filtered and the solvent was removed in vacuum to yield a colorless solid (904 mCi, 75.3%). The product co-chromatographed with standard by radio-TLC on avicel plate using *n*-BuOH: NH_4OH: H_2O (12:3:5) as solvent system.

[^{14}C] *O*-methylisourea hydrochloride (6)

Anhydrous HCl gas was bubbled through a solution of **5** (335 mCi, 5.82 mmol) in anhydrous CH_3OH (5.0 ml) for 15 min. The reaction flask containing colorless solid was sealed and set aside at ambient temperature for 3 days. The solvent was removed under reduced pressure and the solid obtained was dried in a vacuum desiccator over P_2O_5 and KOH overnight. The [^{14}C] *O*-methylisourea hydrochloride (**6**) obtained (0.46 g, 242 mCi, 72.2%) was used in the next reaction without further purification. ^{1}H NMR (DMSO-d_6): δ 8.7 (bs, NH_2), 3.96 (s, 3H, CH_3).

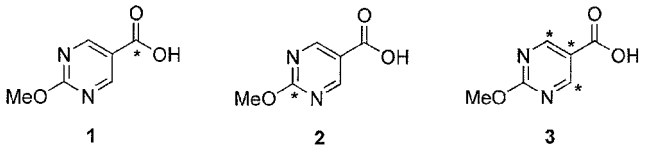

Figure 1. 2-Methoxyprimidine-5-carboxylic acids with [^{14}C] label at different positions.

[2-^{14}C] methyl 2-methoxypyrimidine-5-carboxylate (8)

A mixture of **6** (242 mCi, 4.20 mmol) and sodium 3,3-dimethoxy-2-carbomethoxyprop-1-en-1-oxide (**7**)[19] (0.96 g, 4.83 mmol) in DMF (8.0 ml) was heated to 110°C and maintained for 2 h. The reaction mixture was cooled to ambient temperature and H_2O (30.0 ml) was added. The solid separated was extracted with CH_2Cl_2 (3 × 30.0 ml). The combined organic extracts was dried over $MgSO_4$ and filtered. The filtrate (214 mCi) was concentrated under vacuum to yield a yellow solid. Radio-TLC analysis on silica gel plate [hexane: EtOAc (1:1)] indicated about 93% of the product co-eluting with the standard. The crude material was purified by a silica gel flash chromatography using hexane: EtOAc (9:1) as the eluent. Homogenous fractions were pooled and the solvent was removed under vacuum to yield [2-^{14}C] methyl 2-methoxypyrimidine-5-carboxylate (**8**) as a colorless solid (170 mCi, 70.3%). ^{1}H NMR ($CDCl_3$): δ 9.08 (s, 2H, H-4 and 6), 4.09 (s, 3H, CH_3) and 3.9 (s, 3H, CH_3)

[2-^{14}C] 2-methoxypyrimidine-5-carboxylic acid (2)

To a solution of **8** (170 mCi, 2.95 mmol) in a mixture of dioxane: H_2O (1:1, 34.0 ml) was added 2 N NaOH (1.77 ml, 3.54 mmol) and stirred at ambient temperature overnight. The clear solution was concentrated under reduced pressure to ~1 ml and diluted with H_2O (10.0 ml). Resulting turbid aqueous solution was washed with $CHCl_3$ (10.0 ml) and acidified with 2 N HCl to pH 2. Colorless solid was filtered, washed sequentially with H_2O (2 × 2.0 ml), H_2O: CH_3CN (1:1, 2 × 2.0 ml) and dried to a constant weight. The product obtained was identified as [2-^{14}C] 2-methoxypyrimidine-5-carboxylic acid (**2**) (0.45 g, 96.84%) with a radiochemical purity of 98.4% by HPLC. ^{1}H NMR (DMSO-d_6): δ 9.03 (s, 2H, H- at 4 and 6), 4.00 (s, 3H, CH_3), specific activity: 56.4 mCi/mmol determined by weight assay.

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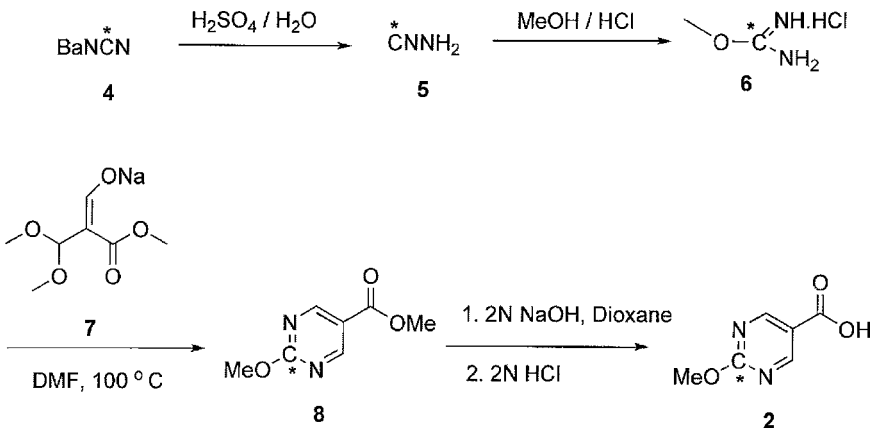

Scheme 1. Synthesis of [2-^{14}C] 2-methoxypyrimidine-5-carboxylic acid.

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