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Rapid and efficient synthesis of stable isotope labeled [$^{13}C_4$, D_4]-5-(hydroxymethyl)thiazole: versatile building block for biologically interesting compounds

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5-(Hydroxymethyl)thiazole is a versatile building block for many biologically active compounds. A rapid and efficient four-step synthesis of its stable isotope labeled counterpart with four ^{13}C and four deuterium atoms in 32% total yield is reported. Condensation of [$^{13}C_2$]-chloro acetic acid with [^{13}C]-thiourea gave [$^{13}C_3$]-2,4-thiazolidinedione. Reaction of [$^{13}C_3$]-2,4-thiazolidinedione with phosphorus oxybromide and [^{13}C, D]-DMF ($Me_2N^{13}CDO$) produced [$^{13}C_4$, D]-2,4-dibromothiazole-5-carboxaldehyde. The resultant aldehyde was then reduced by sodium borodeuteride to [$^{13}C_4$, D_2]-(2,4-dibromothiazol-5-yl)-methanol. Catalytic deuteration of [$^{13}C_4$, D_2]-(2,4-dibromo-thiazol-5-yl)-methanol by palladium black with deuterium gas at 1 atm pressure and room temperature produced completely de-brominated [$^{13}C_4$, D_4]-5-(hydroxymethyl)thiazole. De-bromination of the 2,4-dibromothiazole by the catalysis of palladium black provides a simple and convenient synthetic method for the stable isotope labeled and potentially radioactive isotope labeled thiazole compounds.

Keywords: stable isotope label; synthesis; 5-(hydroxymethyl)thiazole; catalytic de-bromination; palladium black

Introduction

5-(Hydroxymethyl)thiazole (**1**, Figure 1) is a versatile and important building block for many biologically active compounds. For example, it was built into anti-viral protease inhibitors,[1–4] anti-diabetes agents,[5] and anti-cancer agents.[6] In particular, thiazol-5-ylmethyl carbamate commonly resides in many anti-viral agents, such as anti-HIV drug Ritonavir (**2**).[1] To support our drug development efforts stable isotope-labeled 5-(hydroxymethyl)thiazole with at least six isotope atoms is required.

Stable isotope-labeled compounds are powerful tools as internal standards for liquid chromatography–mass spectrometry (LC–MS–MS) quantitative analysis.[7] In order to better evaluate biological behavior of drug candidates, accurate analysis is required to establish their exposure in both animal and human subjects. LC–MS has been widely used to quantify the low levels of drug compounds found in biological samples such as blood, plasma, serum, urine, or tissues. The nature of the internal standard is an important aspect of any quantitative analytical procedure. For LC–MS analysis, the optimum internal standard is a pure, stable, isotopically labeled counterpart of the drug compound, with a sufficiently large mass difference to nullify the effect of naturally abundant heavy isotopes in the drug molecule. This mass difference depends upon the molecular weight and elements of the analyte. The stable isotopes should be incorporated at non-exchangeable positions. The stable isotope-labeled compounds should be chemically pure, free or less than 0.1% of unlabeled compound, and usually possess a difference of at least 3 amu's compared with the unlabeled parent molecules.

Several methods for the synthesis of 5-(hydroxymethyl)thiazole from small molecules have been reported in literature; for example, multiple-step synthesis from chloro-acetic acid ethyl ester, ethyl formate, and thioformate,[8] from 1,3-dichloropropenes and sodium thiocyanate,[9] from 3-oxo-propionic acid ethyl ester and thioformate,[10] as well as from 2,4-thiazolidinedione and DMF.[11] However, none of these methods were suitable to be directly used for the stable isotope-labeled synthesis due to various reasons such as not commercially available starting materials, poor yields, or drastic reaction conditions. Herein, we report a rapid and efficient synthesis of the stable isotope-labeled 5-(hydroxymethyl)thiazole (**3**).

Results and discussion

The synthesis of stable-labeled [$^{13}C_4$, D_4]-5-(hydroxymethyl)thiazole starting from [$^{13}C_2$]-chloro acetic acid (**4**) and [^{13}C]-thiourea was outlined in Scheme 1.

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First at all, commercially available [$^{13}C_2$]-chloro acetic acid (**4**) was condensed with [^{13}C]-thiourea upon heating to give compound **5** in 92% yield.

Next, reaction of compound **5** with excess of phosphorus oxybromide and *N*,*N*-dimethyl(formyl-^{13}C, D)amide produced compound **6** in 43% isolated yield. Slight H/D exchange on the formyl group was observed in a trial reaction. To avoid the H/D exchange exchangeable proton was exchanged to deuterium with deuterated methanol prior to its reaction with phosphorus oxybromide and *N*,*N*-dimethyl(formyl-^{13}C, D)amide. In addition, worthy of mention is that some 2,4-dibromo-thiazole-5-carboxaldehyde was converted to its corresponding dimethyl acetal when methanol-containing solvent system was used at the isolation and purification process. Thus, the crude product was purified by chromatography with gradient 0–5% ethyl acetate/heptane on silica gel.

Then, the resultant aldehyde was reduced readily by sodium borodeuteride in deuterated methanol to the corresponding alcohol **7** in 81% yield.

Catalytic hydrogenation of (2,4-dibromo-thiazol-5-yl)-methanol utilizing 10% palladium on charcoal, in a Parr hydrogenation reactor at 4 atm pressure, has been reported by Kerdesky and Seif.[11,12] For the catalytic reduction, deuteration at near atmosphere pressure is preferred for the small-scale reaction and convenience. An exploratory model on hydrogenation of (2,4-dibromo-thiazol-5-yl)-methanol was first investigated. It was found that, at 1 atm pressure, 10% palladium on charcoal catalyzed hydrogenation of 2,4-dibromo-thiazol-5-yl-methanol in the presence of sodium acetate in methanol, produced only the mono-de-brominated product, (4-bromo-thiazol-5-yl)-methanol, but none of the desired 5-(hydroxymethyl)thiazole. Under the same conditions, the catalytic hydrogenation of (2,4-dibromo-thiazol-5-yl)-methanol with Adam's catalyst (platinum oxide) also produced only quantitative (4-bromo-thiazol-5-yl)-methanol. It is known that the 4-bromo substituent on the thiazole is much less reactive than the 2-bromo substituent.[11,12] Remarkably, when much more reactive palladium black was used as catalyst for the hydrogenation (2,4-dibromo-thiazol-5-yl)-methanol was converted to completely de-brominated 5-(hydroxymethyl)thiazole at 1 atm pressure. Similarly, under the same conditions, (4-bromo-thiazol-5-yl)-methanol was completely de-brominated to 5-(hydroxymethyl)thiazole quantitatively. Thus, the catalytic hydro-de-bromination of the 2,4-dibromo thiazole could be achieved stepwise, first at 2-position, then at 4-position. In addition, the catalytic reaction in the absence of the base (sodium acetate) failed to produce desired product due to formation of un-identified by-products. Replacement of sodium acetate with other bases such as triethyl amine produced comparable yield. Finally, toward the desired [$^{13}C_4$, D_4]-5-(hydroxymethyl)thiazole (**3**), catalytic deuteration of [$^{13}C_4$, D_2]-(2,4-dibromo-thiazol-5-yl)-methanol (**7**), at 1 atm pressure and room temperature, by palladium black with deuterium gas in the presence of sodium acetate in deuterated methanol produced targeted compound **3** in near quantitative yield.

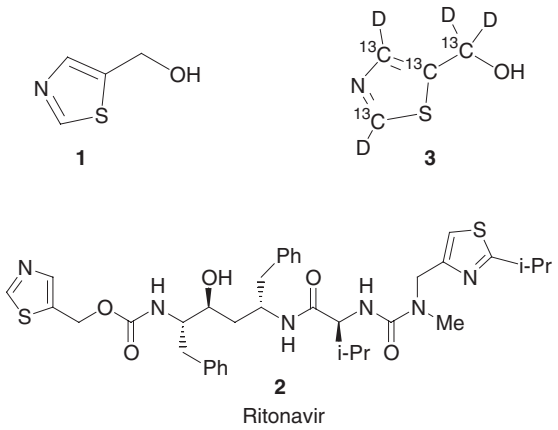
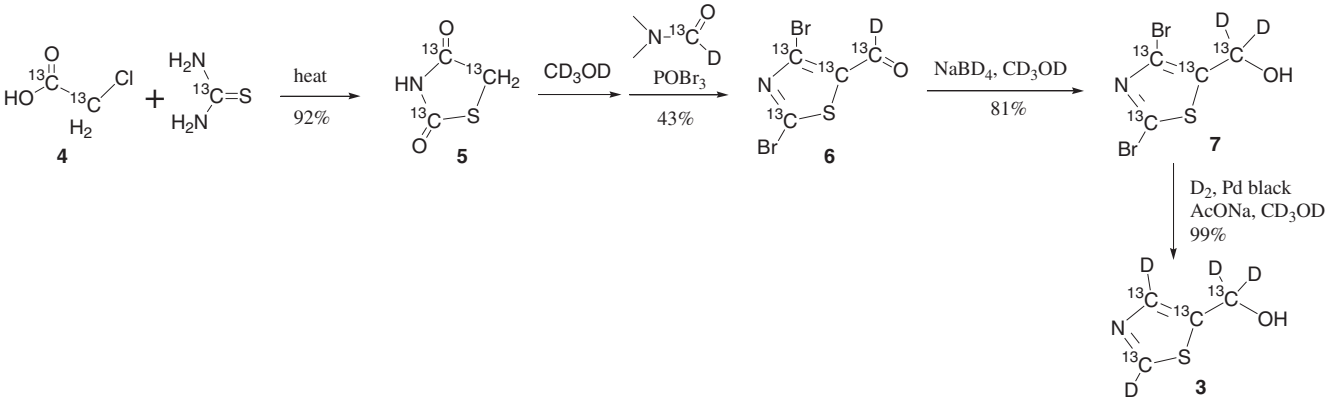


Figure 1. 5-(Hydroxymethyl)thiazole (**1**), Ritonavir (**2**), and the stable isotope labeled 5-(hydroxymethyl) thiazole (**3**).

Experimental

[$^{13}C_2$]-chloro acetic acid, [^{13}C]-thiourea, and *N*,*N*-dimethyl(formyl-^{13}C,D)amide were purchased from Isotec (a division of Sigma-Aldrich, St. Louis, MO). Phosphorus oxybromide, sodium acetate, and palladium black were purchased from Sigma-Aldrich and were used as received. Other reagents and solvents were obtained from VWR International.

^{1}H NMR spectra were acquired on a Bruker 300-Avance spectrometer operated at 300.13 MHz with TMS as an internal standard. ^{13}C NMR spectra were acquired at 75.47 MHz in deuterated chloroform unless stated otherwise. Chemical shifts are expressed in parts per million (ppm, δ scale). LC–MS analysis was performed on an Agilent 1100 series LC/MSD with an Agilent Zorbax® SB C18 column (3 μm, 2.1 × 50 mm), gradient 10–100% CH_3CN-H_2O, 0.05% TFA or 0.05% NH_4OAc over 3.5 min,



Scheme 1. Synthesis of stable isotope labeled 5-(hydroxymethyl)thiazole (**3**).

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hold at 100% CH_3CN for 2.5 min, flow rate 0.5 mL/min, detection at 214 and 254 nm, mass scan range 120–1500 amu. Flash chromatography was performed using a Teledyne Isco Combi-Flash Companion system and a RediSep® silica gel column.

[$^{13}C_3$]-2,4-thiazolidinedione (5)

This was prepared by modifying a reported literature procedure.[13]

To a solution of [^{13}C]-thiourea (1.611 g, 21.16 mmol) in water (2 mL) in a flask under nitrogen was added dropwise a solution of [$^{13}C_2$]-chloro acetic acid (**4**, 2.0 g, 21.16 mmol) in water (2 mL). The reaction mixture was stirred at room temperature for 30 min, then at 105°C overnight. The mixture was then cooled to room temperature, and a white solid was collected by suction–filtration, rinsed with water, and dried at 45°C under vacuum to give 2.178 g of the desired product. The filtrate was concentrated to dryness and re-dissolved in methanol and water, loaded with silica gel, evaporated to dryness, and subjected to column chromatography separation with 0–5% MeOH/CH_2Cl_2 to give a second batch of the desired compound as white solid (1.268 g). The two batches of desired compound were combined to give 3.446 g (92% yield). ^{1}H NMR (DMSO-d_6) δ 12.0 (s, br, 1H), 4.40 and 3.90 (each m, 2H). ^{13}C NMR (DMSO-d_6) δ 174.1–172.9 (m, ^{13}C-2, and ^{13}C-4), 36.2 (d, $^{13}CH_2$, J = 45.3 Hz). MS m/z 121 $(MH)^+$.

[$^{13}C_4$, D]-2,4-dibromo-thiazole-5-carboxaldehyde (6)

[$^{13}C_3$]-2,4-thiazolidinedione (**5**, 1.268 g, 10.57 mmol) was first dissolved in deuterated methanol (5 mL). The resultant solution was stirred for 5 min at room temperature and then evaporated to dryness under vacuum. This process was repeated one more time. The resultant dry residue was then thoroughly mixed with phosphorus oxybromide (15.15 g, 52.83 mmol, 5 eq) under nitrogen. To the well-mixed reaction mixture at 0°C under nitrogen was added *N*,*N*-dimethyl(formyl-^{13}C, D)amide (0.942 ml, 889 mg, 10.84 mmol, 1.12 eq). The reaction mixture was stirred at room temperature for 1 h, then at 70–80°C for another hour, and then at 105°C for 4 h. The mixture was then cooled to 0°C; and quenched with 100 ml of ice water; and then extracted with CH_2Cl_2 (total ~600 mL). The extracts were combined, washed with saturated $NaHCO_3$, dried over Na_2SO_4, filtered, and concentrated. The residue was then loaded with silica gel and evaporated to dryness. The mixture loaded on silica gel was purified by column chromatography with gradient 0–5% EtOAc/heptane on silica gel to give the desired compound as a pale yellow solid (1.32 g, 43%). ^{13}C NMR ($CDCl_3$) δ 182.1–180.6 (m, ^{13}C(O)D), 145.2–145.1 (m, ^{13}C-2), 137.4–135.5 (m, ^{13}C-5), 133.2–132.2 (m, ^{13}C-4). MS m/z 274 $(MH)^+$.

[$^{13}C_4$, D_2]-(2,4-dibromo-thiazol-5-yl)-methanol (7)

To a solution of [$^{13}C_4$, D]-2,4-dibromo-thiazole-5-carboxaldehyde (**6**, 3.315 g, 12.77 mmol) in dry CH_2Cl_2 (4 mL) was added anhydrous deuterated methanol (60 mL). To the resultant solution at 0°C was added sodium borodeuteride (507 mg, 12.11 mmol). The reaction mixture was stirred at 0°C for 2 h, then at room temperature for another 2 h. The mixture was then loaded with silica gel, and evaporated to dryness. This mixture was further purified by column chromatography separation on silica gel with gradient 0–5% MeOH/CH_2Cl_2 to give the desired product as yellowish solid (2.875 g, 81% yield). ^{1}H NMR ($CDCl_3$) δ 2.2 (s, br, 1H). ^{13}C NMR ($CDCl_3$) δ 138.5–136.7 (m, ^{13}C-5), 135.8 (s, ^{13}C-2), 121.6 (d, ^{13}C-4, J = 75.5 Hz), 58.0–56.7 (m, $^{13}CD_2OH$). MS m/z 279 $(MH)^+$.

[$^{13}C_4$, D_4]-5-(hydroxymethyl)thiazole (3)

Into a flask under nitrogen with a mixture of [$^{13}C_4$, D_2]-(2,4-dibromo-thiazol-5-yl)-methanol (**7**, 2.875 g, 10.34 mmol) and sodium acetate (1.790 g, 21.72 mmol, 2.1 eq) was added palladium black (1.437 g) and anhydrous deuterated methanol (83 mL). Deuterium gas was slowly bubbled into the suspension under stirring for 5 h, and then the reaction flask under vigorous agitation was connected to a balloon filled with deuterium overnight. The reaction was followed by TLC analysis with 5% MeOH/CH_2Cl_2. The palladium black was filtered off and rinsed with deuterated methanol. The filtrate was concentrated; and re-charged with CH_2Cl_2 (50 mL) and sonicated. The remaining white solid (presumably sodium bromide) was filtered and rinsed with CH_2Cl_2 (50 mL). The filtrate was again concentrated to give the crude desired product as oil (1.50 g). Distillation of the crude product under vacuum at 138–140°C/10 mmHg (lit.[12] 140–142°C/10 mmHg) gave the pure product as pale yellowish oil (1.27 g, 99%). The product [$^{13}C_4$, D_4]-5-(hydroxymethyl)thiazole was confirmed by ^{1}H and ^{13}C NMR in $CDCl_3$ and CD_3OD in comparison with authentic non-labeled compound, 5-(hydroxymethyl)thiazole (**1**), and by LCMS analysis, and HPLC analysis. For compound **3**, ^{1}H NMR ($CDCl_3$) δ 2.2 (s, br, 1H). ^{13}C NMR ($CDCl_3$) δ 153.5 (t, ^{13}C-2, J = 30.9 Hz), 141.4–137.7 (m, ^{13}C-5 and ^{13}C-4), 57.4–55.8 (m, $^{13}CD_2OH$). ^{13}C NMR (CD_3OD) δ 155.6 (t, ^{13}C-2, J = 32.4 Hz), 141.5–140.7 (m, ^{13}C-5 and ^{13}C-4), 57.6–56.0 (m, $^{13}CD_2OD$). MS m/z 124.2 (MH^+, 100% relative intensity), 123.2 (M-1)H^+, 13% relative intensity. For non-labeled compound **1**, ^{13}C NMR (CD_3OD) 155.5 (s, C-2), 141.6 (s, C-5), 141.1 (s, C-4), 57.3 (s, CH_2OD).

Conclusion

A rapid and efficient four-step synthesis for stable isotope labeled [$^{13}C_4$, D_4]-5-(hydroxymethyl)thiazole (**3**) with four ^{13}C and four deuterium atoms in 32% total yield is reported. [$^{13}C_2$]-chloro acetic acid (**4**) was condensed with [^{13}C]-thiourea to give [$^{13}C_3$]-2,4-thiazolidinedione (**5**). Reaction of compound **5** with excess phosphorus oxybromide and [^{13}CD]-DMF ($Me_2N^{13}CDO$) produced [$^{13}C_4$, D]-2,4-dibromo-thiazole-5-carboxaldehyde (**6**). Facile reduction of the resultant aldehyde **6** by sodium borodeuteride provided [$^{13}C_4$, D_2]-(2,4-dibromo-thiazol-5-yl)-methanol (**7**). Remarkably, catalytic deuteration of compound **7** by palladium black with deuterium gas at 1 atm pressure produced completely de-brominated [$^{13}C_4$, D_4]-5-(hydroxymethyl)thiazole (**3**). The stable isotope labeled 5-(hydroxymethyl)thiazole (**3**) may be utilized as a valuable building block in synthesis of isotope labeled biologically interesting compounds and pharmaceutical compounds. Catalytic hydrogenation of the 2,4-dibromothiazole with palladium black at 1 atm pressure and room temperature provides simplicity and convenience for the stable isotope labeling synthesis and potential for radioactive isotope labeling synthesis of thiazole compounds.

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