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Carbon-14- and carbon-13-labeled phosphoric acid [2-4-(4-cyanophenyl)-thiazol-2-yl]-(2,4-difluorophenyl)-1-[1,2,4]triazol-4-yl-methylpropoxymethyl] *mono*ester dilysine salt, a prodrug of ravuconazole

I. Victor Ekhato* and J. Kent Rinehart

4-Bromobenzoic acid [carboxyl-^{14}C] and 4-(2-bromoacetyl) [Ar-$^{13}C_6$]benzonitrile were transformed into the title compounds containing [ring^{14}C-thiazol-4-yl] and [Ar-$^{13}C_6$-benzonitrile]. ^{14}C-Ravuconazole was prepared in 37% yield and Purity >99%. $^{13}C_6$-Ravuconazole was made in 56% overall yield and Purity of >98%. Each labeled compound was converted by additional reaction steps to the corresponding labeled prodrug.

Keywords: antifungal agents; 14α-demethylase; ravuconazole; prodrug; phosphoric acid dilysine salt

Introduction

Phosphoric acid [2-4-(4-cyanophenyl)-thiazol-2-yl]-(2,4-difluorophenyl)-1-[1,2,4]triazol-4-yl-methylpropoxymethyl] *mono*ester dilysine salt **1** (BMS-379224) in Figure 1 is a prodrug of ravuconazole.[1] Ravuconazole **2** (BMS-207147), licensed to BMS from Eisai Co. Ltd. (ER-30346), is an inhibitor of lanosterol 14α-demethylase activity, a P-450-dependent monooxygenase and a key enzyme in the biosynthesis of ergosterol.[2] Ergosterol is essential in fungi for cell viability and proliferation. Ravuconazole has been demonstrated by *in vivo* and animal model experiments to be more potent against aspergillosis, candidiasis and cryptococcosis than clinically useful amphotericin B, and the newer azole antifungals like fluconazole and intraconazole.[3] It is orally active, shows broad spectrum activity and has a generally favorable safety profile. BMS is further studying this agent to develop intravenous (iv) formulation for the treatment of systemic fungal infection. For neutropenic and immunocompromised patients who become seriously ill and are not able to tolerate oral administration, the parenteral administration of an antifungal agent is critical. In light of the superior antifungal activity, successful clinical development of an iv formulation of ravuconazole is highly desirable for this class of patients. A dilysine salt of the phosphoric acid prodrug is attractive since lysine and phosphate residues are readily cleared, unlike with cyclodextrins as carrier[4] and would not accumulate in the presence of renal insufficiency. We have made the radio-isotope and stable labeled versions of the prodrug **1** BMS 379224 to support further preclinical pharmacokinetics characterization. Our plan called for the radio-label to be placed in the thiazole ring instead of making [benzonitrile-^{14}CN] or {[U-^{14}C]benzonitrile} variant.[5] Studies in our laboratory showed a rare metabolic conversion of ravuconazole nitrile functionality into a thiocyanate.[6] The incorporation of carbon-14 in the thiazole moiety is intended to avert loss of the label by this rare metabolic route. After examining the synthetic literature on ravuconazole we decided that the thiazole unit could be constructed from readily accessible labeled reagents.[7] To make the stable labeled prodrug a more advanced intermediate bearing [Ar-$^{13}C_6$]benzonitrile was preferred since it would afford species with a sufficient molecular mass increase that overlap with other ions resulting by isotopic mass distribution is avoided. Once obtained, labeled ravuconazole could be taken through some additional steps to provide the radio and stable labeled prodrug targets. In the following section we wish to describe the synthesis of labeled versions of the dilysine salt of phosphoric acid prodrug of ravuconazole.

Experimental

All reactions were carried out under an atmosphere of argon unless otherwise specified. Solvents were commercial grade and used without purification or drying. Column chromatography was carried out on Merck Kiesegel 60 (230 μ) silica gel. Flash chromatographic separations were performed on a Biotage Flash System using pre-packed silica gel cartridges. TLC visualization reagents included (10% iodine plus 10% AcOH) in

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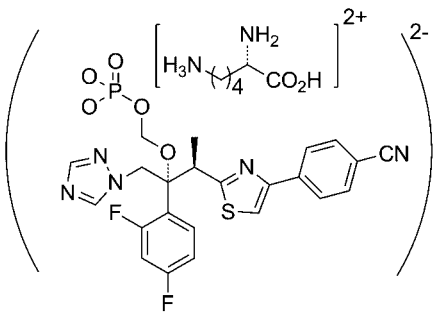
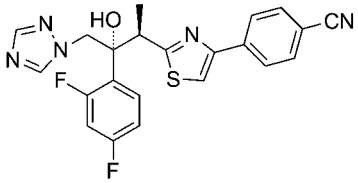


1 BMS-379224



2 BMS-207147

Figure 1. Prodrug and parent ravuconazole, targets of the labelling sequence.

40% aqueous KI, and cerium sulfate in 10% sulfuric acid. 1 H NMR spectra were recorded at 300, 400 or 500 MHz. Chemical shifts are given in ppm relative to tetramethylsilane (TMS). Agilent 1100 HPLC System including solvent degasser, pump, automated injector, and a variable UV detector connected to IN/US BetaRam model 4 Flow Detector with a 0.25 mL detector cell was used in the analyses of compounds. The HPLC column was Waters Xterra RP18, 3 μ, 4.6 × 150 mm, PN 18600004. Elution Solvent System A = Water +0.05% TFA and B = Acetonitrile+0.05% TFA and compound eluted under gradient condition with detection by UV at 254 nm and 280 nm. LC/MS analysis was performed on a Finnigan LXQ Mass Spectrometer System, LC/MS method: Generic-B20 (+p ESI full mass). Only the molecular ion and/or base peaks in MS are given. 4-Bromobenzoic acid [^{14}C-carboxyl] was purchased from ViTrax, Placentia, CA, USA and was diluted with 'cold' 4-bromobenzoic acid from Aldrich Chemical Company before it was used, and 4′-bromoaceto[Ar-$^{13}C_6$]phenone was custom synthesized by Isotec Inc., and supplied through Aldrich Chemicals.

1-(4-bromophenyl) [carbonyl −^{14} C]ethanone 4

4-Bromobenzoic acid [^{14}C-carboxyl] **3** (1.005 g, 5 mmol, 100 mCi) was stirred in anhydrous toluene (20 mL), oxalyl chloride (1.308 mL, 15 mmol) followed by catalytic DMF were added. After 1 h the reaction solution was concentrated under reduced pressure, and further co-evaporated with toluene to give a solid product. A solution of this material in CH_2Cl_2 (20 mL) was added at −5°C to *N,O*-dimethylhydroxylamine hydrochloride (634 mg, 6.3 mmol) in dichloromethane (30 mL) containing pyridine (1.158 mL, 14.31 mmol), and stirred for 1 h allowing it to warm to room temperature. The reaction mixture was diluted with chloroform, (30 mL), and washed with water, (30 mL), 1 N hydrochloric acid, (30 mL), brine (20 mL) and dried. Evaporation gave an amide (1.260 g, 5 mmol), and to a solution of this material in dry THF 20 mL at −5°C was added 3.0 M solution of methylmagnesium iodide (5 mL, 15 mmol). The reaction was stirred at room temperature overnight, quenched with diluted hydrochloric acid, 1.0 N, 20 mL and extracted with ether (3 × 30 mL). The ethereal extract was washed with 1 N HCl (2 × 20 mL), brine, (20 mL) and dried over anhydrous sodium sulfate. The solution was evaporated to yield **4** (1.0 g, ≈quant). ^{1}H-NMR (400 MHz, $CDCl_3$), δ 2.57 (s, 3H), 7.57 (d, *J* = 8.5 Hz, 2H), 7.79 (d, *J* = 8.5 Hz, 2H). ^{13}C-NMR (100.61 MHz, $CDCl_3$) 196.9, 135.8, 131.8, 129.8, 128.2 and 26.

4-Acetyl[carbonyl −^{14} C]benzonitrile 5

To tetrakis(triphenylphospine)palladium(0) (232.28 mg, 0.201 mmol) and zinc cyanide (353.72 mg, 3.01 mmol) in a 50 mL round bottom flask equipped with a reflux condenser and argon inlet was added 15 mL of anhydrous dimethylformamide. The reaction vessel was repeatedly evacuated and filled with argon to eliminate air. A solution of **4** (1.0 g, 5.02 mmol) in DMF (5 mL) was added to make a total reaction volume of 20 mL, and the degassing process was repeated. The mixture was stirred at 96°C overnight under argon atmosphere, cooled to room temperature and concentrated to a residue. The residue was diluted with toluene (30 mL), and the solution was washed with 3 N ammonium hydroxide solution (2 × 20 mL), brine (20 mL) and dried over $MgSO_4$. The solution was evaporated to provide **5** (680 mg, 93 mCi, 93%). ^{1}H-NMR (400 MHz, $CDCl_3$),δ 2.63 (s, 3 H), 7.76 (d, *J* = 8.5 Hz, 2H), 8.03 (d, *J* = 8.5 Hz, 2H). HPLC with Waters Xterra 4.6 × 150 mm column RP18 3.5 micron, elution solvent A = Water+0.05 TFA; B = acetonitrile+0.05% TFA, 0–12 min 10% B, 12–17 min 90%, 17–20 min 10% B, Flow rate 1 mL/min, at UV 254 and 270 nm gave retention time of 4.4 min.

4′-(2-Bromoacetyl) [carbony −^{14}C]-benzonitrile 6

Catalytic $AlCl_3$, 5 mg, was added to a solution of 4-acetylbenzonitrile [carbonyl-^{14}C] **5** (680 mg, 4.68 mmol) in ethyl acetate (20 mL) at 5°C. Bromine (239 μL, 4.68 mmol) in ethyl acetate was added under an atmosphere of argon, and the reaction was stirred for a further 30 min at room temperature. HPLC indicated 65:35 ratio of the desired compound to putative dibromo contaminant. After the reaction was quenched with 10% solution of sodium thiosulfate, the solution was washed with 20 mL of water, 20 mL of brine and dried on sodium sulfate. A solution of the crude product in dichloromethane was applied to a column of silica gel, and the unwanted dibromo compound was eluted with 10% ether in pet ether. Further elution of the column with 25% ether in pet ether gave **6** (516 mg, 49.2%). ^{1}H-NMR (400 MHz, $CDCl_3$), δ 4.42 (s, 3H), 7.99 (d, *J* = 8.5 Hz, 2H), 8.07 (d, *J* = 8.5 Hz, 2H). ^{13}C-NMR (100.61 MHz, $CDCl_3$) 190.0, 136.9, 132.6, 129.4, 117.6, 117.1 and 30. HPLC with Waters Xterra 4.6 × 150 mm column RP 18; elution solvent A = water+0.05% TFA, B = Acetonitrile+0.05 TFA, gradient condition, 0–12 min 30–90% B, 12–16 min, 90–30% B, Flow rate mL/min at UV detection wavelength of 254 and 270 nm to give retention time of 6.9 min.

4-{2-[2-(2,4-Difluoro-phenyl)-2-hydroxy-1-methyl-3-[1,2,4]triazol-4-yl-propyl]-}-[^{14}C]thiazol-4-yl-benzonitrile, 7 ^{14}C BMS-207147

A mixture of 4-(2-bromoacetyl)benzenenitrile [carbonyl-^{14}C]) **4** (516 mg, 2.30 mmol) and BMS-226630 (768.6 mg, 2.46 mmol) in anhydrous methanol (10 mL) was refluxed for 1.5 h under argon

atmosphere. After cooling to room temperature, the reaction mixture was diluted with 36 mL of dichloromethane, and 146 mg of $NaHCO_3$ followed by 20 mL of water. The aqueous portion was separated, and was further extracted with dichloromethane (2×15 mL). The combined organic extract was washed with 20 mL of water, 20 mL of brine, dried over $MgSO_4$ and evaporated to a residue. The residue was purified by column chromatography on silica gel. Elution of the column with 1–2% methanol in dichloromethane gave pure fractions that were combined, evaporated to a solid which crystalized from ethyl acetate-hexane to give **7** ^{14}C-BMS-207147, (634.6 mg, 63%, 36.83 mCi, specific activity 58.04 μCi/mg or 25 mCi/mmol). ^{1}H-NMR (400 MHz, $CDCl_3$), δ 8.01 (d, $J = 8.2$ Hz, 2H), 7.83 (s, 1H), 7.73 (d, $J = 8.2$ Hz, 1H), 7.68 (s, 1H), 7.63 (s, 1H), 7.50 (m, $J = 6.4$ Hz, $J = 6.4$ Hz, $J = 3.1$ Hz, 1H), 6.80 (m, 1H), 5.72 (s, 1H), 4.90 (d, $J = 14.4$ Hz, 1H), 4.25 (d, $J = 14.4$ Hz, 1H), 4.07 (q, $J = 7.3$ Hz, 1H), 1.22 (d, $J = 7.3$ Hz, 2H). Chemical purity > 99% and radiochemical purity > 99.8% at retention time 5.7 min as authentic BMS-207147-01.

Phosphoric acid mono-[2-4-(4-cyano-phenyl)-[^{14}C]-thiazol-2-yl]-1-(2,4-difluoro-phenyl)-1-[1,2,4]triazol-4-ylmethyl-propoxymethyl]ester dilysine 12, BMS-379224-04

^{14}C-BMS-207147 **7** (466 mg, 27.04 mCi, 1.05 mmol)) in dry THF (5 mL) was added slowly to a suspension of sodium hydride (60% dispersion, 130 mg, 3.2 mmol) in dry THF (6 mL). Next, a solution of iodine (134 mg, 0.529 mmol, 0.5 eq) in dry THF (3 mL) was added, and stirred at 17°C for 30 min. BMS-371000 (330 μL, 1.26 mmol) was added neat, and the mixture was stirred at room temperature overnight. After the reaction mixture was cooled in ice-bath, 4 mL of water was added followed by toluene, (15 mL), stirred briefly and allowed to settle. The aqueous phase was discarded and the organic portion was washed repeatedly with 5 mL portions of brine until it became a clear solution. The solution was concentrated under reduced pressure at < 30°C to give **8** as a creamy white solid. The material **8** was taken up in CH_2Cl_2 (8 mL), cooled in ice bath and trifluoroacetic acid 2.4 mL was added. After 10 min at room temperature, the solution was concentrated under reduced pressure, and further co-evaporated with toluene at < 20°C to afford the crude product. In a solution of minimum methanol, the compound was applied to a C_{18} silica gel column that had been equilibrated phosphate buffer pH 7.0. The column was eluted first with 10% methanol in water, and then by step gradient elution with 20, 40, 60% methanol in water. Fractions containing the pure desired compound were combined and concentrated by rotary evaporator to give a solid. To the material in warm (55°C) methanol (4 mL) was slowly added with stirring, a solution of lysine (2 eq) in water/MeOH, 2 mL (0.5/1.5). The reaction was cooled to −10°C and the solid which separated was collected by filtration, washed with 2% water in methanol and dried at 50°C under vacuum overnight to give **9** (395 mg, 43.8%, 6.7 mCi, 17 μCi/mg, 14.2 mCi/mmol based on Mol Wt 839.85). ^{1}H-NMR (400 MHz, DMSO-d_6), δ 9.12 (s, 1), 8.0 (d, 2H), 7.94 (s, 1H), 7.83 (s, 1H), 7.72 (d, 1H), 7.22 (q, 1H), 6.91 (m, 1H), 6.79 (m, 1H), 5.42 (tr, 1H), 5.35 (q, 1H), 5.24 (tr, 1H), 3.76 (q, 1H), 3.46 (m, 3H), 2.86 (tr, 6H), 1.83 (m, 3H), 1.75 (m, 3H), 1.63 (m, 6H), 1.49 (m, 8H). HPLC with Waters Xterra MS C8 4.6 × 150 mm column, gradient condition 0–12 min, 25–85% B, and 12–18 min, 85–25% B, where A = 0.1 M aq NH_4OAc/0.01 M NH_4OAc in MeOH (80:20) and B = 0.1 mmol aq NH_4OAc/0.01 M NH_4OAc in MeOH/MeCN (5:20:70) at a Flow rate of mL/min gave retention time 8.2 min, chemical purity > 98.0% and radiochemical purity > 99%.

4′-Acetyl [Ar −$^{13}C_6$]benzonitrile 11

$Zn(CN)_2$ (1.768 g, 15.06 mmol) and tetrakis(triphenylphospine)-palladium(0) (1.610 g, 1.34 mmol), 4-(2-bromoaceto[Ar-$^{13}C_6$])-phenone **10** (5.0 g, 25.11 mmol) under the reaction conditions described for 3 gave crystaline **11** (3.5 g, 95%). ^{1}H-NMR (400 MHz, $CDCl_3$), δ 8.04 (dm, $J_{C-H} = 164$ Hz, $J_{C-C} = 13.0$ Hz, 2H), 7.75 (dm, $J_{C-H} = 164$ Hz, $J_{C-C} = 13.0$ Hz, 2 H). ^{13}C-NMR (125.77 MHz, $CDCl_3$) 116.4 (td, $^1J_{C-C} = {}^1J_{C-C} = 60$ Hz, $^2J_{C-C} = 6.0$ Hz, CCN), 128.8 (td, $^1J_{C-C} = {}^1J_{C-C} = 65.8$ Hz, $^2J_{C-C} = 6.6$ Hz, 2CH), 132.7 (td, $^1J_{C-C} = {}^1J_{C-C} = 65$ Hz, $^2J_{C-C} = 6.6$ Hz, 2CH), 140.1 (td, $^1J_{C-C} = {}^1J_{C-C} = 58$ Hz, $^2J_{C-C} = 8.6$ Hz, CCO). HPLC (condition as in **5**) gave retention time 4.4 min.

4-(2-Bromoacetyl) [Ar −$^{13}C_6$]benzonitrile 12

Phenyltrimethylammonium tribromide (8.27 g, 22.0 mmol) was added portion-wise over 10 min to a stirred solution of **11** (3.20 g, 21.17 mmol) in 20 mL of dry THF. The pale solution was stirred over 20 min during which a heavy precipitate was formed. Ice-cold water (30 mL) was added and the solid was separated by filtration, washed with water and dried under high vacuum overnight. It was crystalized from hexane to give **12** (3.32 g, 68.1%). ^{1}H-NMR (400 MHz, $CDCl_3$), δ 8.10 (dm, $J_{C-H} = 158.9$ Hz, $J_{C-C} = 13.0$ Hz, 2H), 7.82 (dm, $J_{C-H} = 161.5$ Hz, $J_{C-C} = 13.0$ Hz, 2H). ^{13}C-NMR (125.77 MHz, $CDCl_3$) 117.10 (td, $^1J_{C-C} = {}^1J_{C-C} = 60$ Hz, $^2J_{C-C} = 9.6$ Hz, CCN), 128.8 (td, $^1J_{C-C} = {}^1J_{C-C} = 58.2$ Hz, $^2J_{C-C} = 8.3$ Hz, 2CH), 132.7 (td, $^1J_{C-C} = {}^1J_{C-C} = 56.2$ Hz, $^2J_{C-C} = 6.6$ Hz, 2CH), 140.1 (td, $^1J_{C-C} = {}^1J_{C-C} = 58.2$ Hz, $^2J_{C-C} = 9.5$ Hz, CCO). HPLC (condition as in **6**) retention time 6.9 min.

4-{2-[2-(2,4-Difluoro-phenyl)-2-hydroxy-1-methyl-3-[1,2,4] triazol-4-yl-propyl]-thiazol-4-yl}-[Ar −$^{13}C_6$]benzonitrile, 13 $^{13}C_6$ BMS-207147

Compound **12** (2.72 g, 11.8 mmol) and BMS-226630 (3.98 g, 14.8 mmol) as described for **5**, gave after crystalization from ethyl acetate-hexane, **13** (892 mg, 53%). ^{1}H-NMR (400 MHz, $CDCl_3$), δ 8.20 (m, 1H), 7.95 (m, 1H), 7.83 (s, 1H), 7.80 (m, 1H), 7.68 (s, 1H), 7.63 (d, 1H), 7.51 (m, 2H), 6.80 (m, 2H), 5.72 (s, 1H), 4.91 (d, 1H), 4.24 (d, 1H), 4.07 (q, 1H) and 1.22 (d, 3H). MS (EI) *m/z* 444.2 $[M+H]^+$, 375, 224. ^{13}C-NMR (125.77 MHz, $CDCl_3$) 111.6 (td, $^1J_{C-C} = {}^1J_{C-C} = 60.1$ Hz, $^2J_{C-C} = 10.5$ Hz, CCN), 126.7 (td, $^1J_{C-C} = {}^1J_{C-C} = 59.1$ Hz, $^2J_{C-C} = 8.5$ Hz, 2CH), 132.69 (td, $^1J_{C-C} = {}^1J_{C-C} = 60.1$ Hz, $^2J_{C-C} = 8.6$ Hz, 2CH), 137.8 (td, $^1J_{C-C} = {}^1J_{C-C} = 58.2$ Hz, $^2J_{C-C} = 10.5$ Hz, CC = C). HRMS (EI) $[M+H]^+$ 444.1390 ($^{12}C_{16}^{13}C_6H_{17}F_2N_5OS$ requires 444.1401). Isotopic distribution [M+6] 98 $^{13}C_6$-atom% for $^{12}C_{16}^{13}C_6H_{17}F_2N_5OS$; [M+5] 2.0 $^{13}C_5$ atom% for $^{12}C_{16}^{12}C^{13}C_5H_{17}F_2N_5OS$; [M+0] < 0.01 atom% for $C_{22}H_{17}F_2N_5OS$. HPLC with Xterra 4.6 × 150 mm column, gradient condition and detection at UV wavelength of 254 and 270 nm, flow rate of 1 mL/min gave same retention time 13.1 min as authentic BMS-207147-01 (070D3U-001-01A) and chemical purity of > 99.2%.

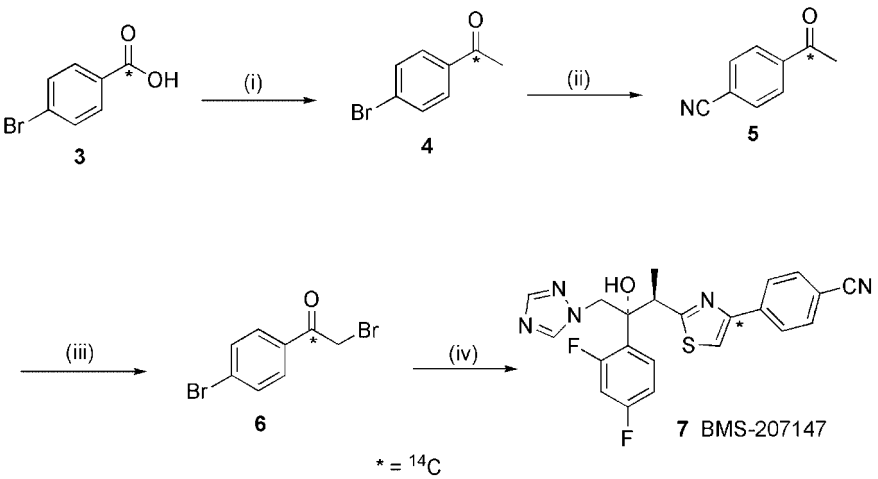
Phosphoric acid [2-4-(4-cyano-[Ar −$^{13}C_6$]phenyl)-thiazol-2-yl]-1-(2,4-difluoro-phenyl)-1-[1,2,4]triazol-4-ylmethyl-propoxymethyl] monoester dilysine salt 15 $^{13}C_6$ BMS-379224

From sodium hydride 60% dispersion in oil (137.15 mg, 3.42 mmol), **13** (500 mg, 1.14 mmol), iodine (145.04 mg, 0.571 mmol), BMS-371000 (353.87 mg, 354 uL, 1.36 mmol), lysine

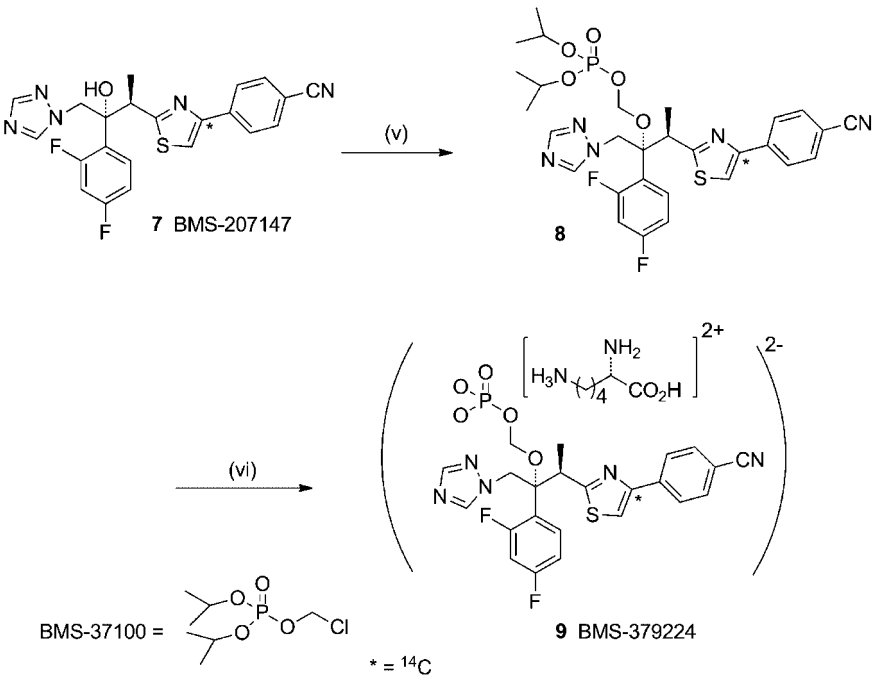
(340.15 mg, 2.28 mmol), as in experiment **9** gave compound **15** (650 mg, 67.8%). ^{1}H-NMR 400 MHz, DMSO-d_6), δ 9.12 (s, 1), 8.19 (m, 1H), 7.95 (s, 1H), 7.88 (m, 1H), 7.83 (s, 1H), 7.58 (q, 1H), 7.26 (q, 1H), 6.91 (m, 1H), 6.81 (m, 1H), 5.46 (tr, 1H), 5.35 (q, 2H), 5.28 (tr, 1H), 3.43 (tr, 3H), 2.85 (tr, 6H), 1.83 (m, 3H), 1.75 (m, 3H), 1.63 (m, 6H), 1.49 (m, 8H). H PLC with Waters Xterra MS C8 4.6 × 250 mm column, gradient 0–16 min, 25–85% B, and 16–18 min, 85–25% B, where A = 0.1 M aq NH_4OAc/0.01 M NH_4OAc in MeOH (80:20) and B = 0.1 mmol aq NH_4OAc/0.01 M NH_4OAc in MeOH/MeCN (5:20:70) at a flow rate of mL/min gave retention Time of 8.9 min and chemical purity > 98.7%.

Results and discussion

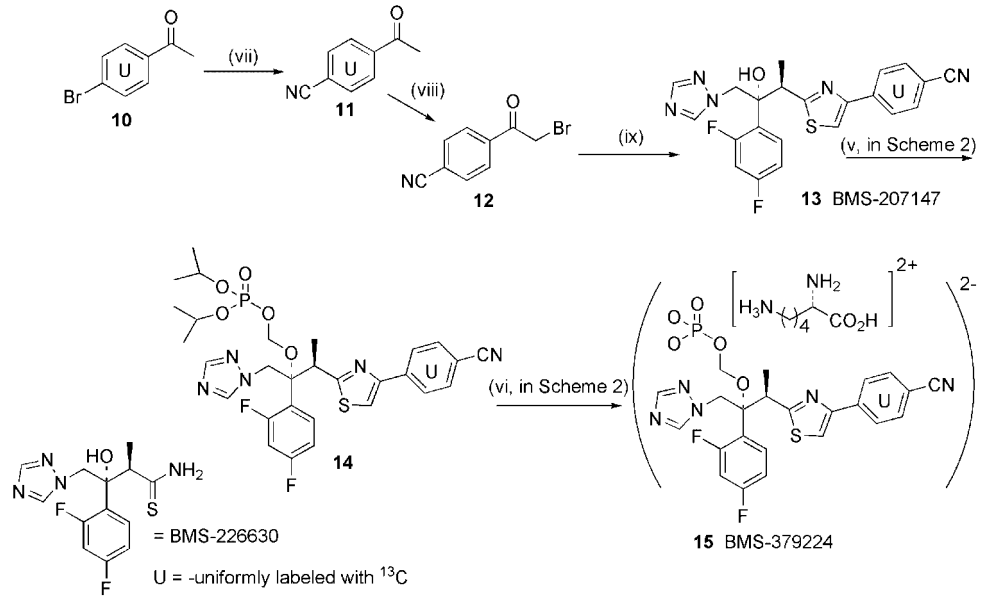
Scheme 1 describes the preparation of ^{14}C-ravuconazole, Scheme 2 illustrates its conversion to carbon-14-labeled prodrug, and Scheme 3 summarizes an analogous preparation of $^{13}C_6$-ravuconazole and the corresponding prodrug. 4-Bromobenzoic acid [^{14}C-carboxy] **3** is our starting reagent for the radiolabeled ravuconazole. We decided based on earlier work[7] to go through the sequence that includes the reaction of 4-(2-bromoacetyl[^{14}C-carbonyl])benzonitrile **6** with BMS-226630 to form 4-{2-[2,4-difluoro-phenyl)-2-hydroxy-1-methyl-3-[1,2,4] triazol-4-yl-propyl]-[^{14}C]thiazol-4-yl-benzonitrile **7**. The compound **6** was prepared from **3** by DMF-catalyzed exchange reaction with oxalyl chloride to make 4-bromo[^{14}C-carboxy]-benzoyl chloride. 4-Bromo-*N*-methoxy-*N*-methylbenzamide was generated from it according to the Weinreb's protocol,[8] and subsequently treated with methylmagnesium iodide to give 4-bromoaceto[carboxyl 2-^{14}C]phenone **4**. Palladium-catalyzed cyanation of **4** with $Zn(CN)_2$ at 95°C under argon atmosphere[9] overnight gave **5** in 86% yield from 4-bromobenzoic acid.



Scheme 1. (i) a. $(COCl)_2$, cat. DMF, Toluene, 23°, 1 h; b. Me(MeO)NH. HCl, pyr, $CHCl_3$ 0 to 23°C, 2 h; c. MeMgI, THF, −78°C, 18 h, quant; (ii) 4 mol % $Pd(PPh_3)_4$, $Zn(CN)_2$ DMF, 80°C, 18 h, 93 %; (iii) cat, $AlCl_3$, Br_2, EtOAc, 0.5 h, 23°C, 49%; (iv) BMS-226630, MeoH, reflux, 1.5 h, 63%.



Scheme 2. (v) NaH, I_2, THF, 17°C, 30 min, BMS-37100, 23°C, o.n; (vi) CH_2Cl_2, TFA, 10 min 23°C, C_{18} Column Isolation, 43.8% 2 steps.



Scheme 3. (vii) 4 mol % $Pd(PPh_3)_4$, $Zn(CN)_2$, 80°C, DMF, 95 %; (viii) ($C_6H_5N^+(CH_3)_3Br_3^-$), THF, 20 min 23°C, 89%, (ix) BMS-226630, MeoH, Reflux, 53 %; (v) and (viii) see Schemes 1 & 2.

We found the bromination of **5**, catalyzed by $AlCl_3$, to be problematic. An unexpectedly high amount (35%) of 4′-(2,2-dibromoacetyl)-benzonitrile was formed along with the desired 4-(2-bromoacetyl)-benzonitrile **6**. While a major weakness in the radio-labeling sequence, the impurity is easily removed by flash chromatography on silica gel column. Compound **6** was refluxed in methanol containing BMS-226630 to give **7** in 63% purified yield. Owing to the variable yield we observed during the method development we changed the method for transforming BMS-207147 into BMS-379224 to achieve enhanced and reproducible yield. Accordingly, the sodium salt of **7** was reacted with BMS-371000, in the presence of I_2 overnight, to make trialkyl phosphonate ester, **8**.[1] A non-extraction workup was adopted to isolate the *mono*ester in the subsequent de-protection step. After the solvent was evaporated the residue was subjected to a step gradient reverse phase chromatography. This procedure gave reproducible yields of the acid *mono*ester. Formation of the dilysine salt of the *mono*ester was completed by adding aqueous solution of lysine in methanol at 55°C to give **9** ^{14}C BMS-379224 in 64% yield.

The problematic bromination step encountered during the carbon-14 synthesis was solved in the stable label synthesis by substituting, as a superior brominating agent, phenyltrimethylammonium tribromide ($C_6H_5N^+(CH_3)_3Br_3^-$).[10] Beginning from 4-(2-bromoaceto-[Ar-$^{13}C_6$])phenone **10** the nitrile **11** was made in > 95% yield. This was followed by bromination with phenyltrimethylammonium tribromide to afford **12** in 89% yield. The putative dibromide was formed in about 10%, leading overall, to an improved yield of **13** $^{13}C_6$-BMS 207147. In a sequence of reactions similar to the radiosynthetic route, the compound **13** was treated with BMS-371000 to make the trialkylphosphate, deprotected with TFA to the phosphoric acid *mono*ester. It was isolated in the same manner and converted to the dilysine salt **15** $^{13}C_6$ BMS-379224 in 68% yield. Where possible these compounds were matched with authentic reference samples by HPLC and TLC techniques and gave analytical results consistent with the assigned structures.

Conclusion

In summary, we have successfully prepared a version of ravuconazole containing [^{14}C]-thiazole moiety in four steps from 4-bromo[carboxy-^{14}C]benzoic acid. By a similar sequence, a version of ravuconazole bearing [Ar-$^{13}C_6$]benzonitrile was made. These radio-isotope and stable labeled ravuconazole analogs were respectively transformed into the dilysine salt of the phosphoric acid *mono*ester prodrug. Our decision to incorporate carbon-14 in the thiazole ring system was based on considerations of metabolic stability and cost.

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