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Carbon-14 labeling of K777•HCl, a therapeutic agent for Chagas disease†

Sung-Whi Rhee, Wallace W. Bradford, Jeremiah P. Malerich, and Mary J. Tanga*

The antitrypanosomal agent K777•HCl was labeled with carbon-14 to support absorption, distribution, metabolism, and excretion studies of this potential new drug for the treatment of Chagas disease. The radiolabeled compound was prepared in eight steps from [^{14}C(U)]-(L)-phenylalanine with a specific activity of 54.4 mCi/mmol and an overall radiochemical yield of 4.1%.

Keywords: K777; Chagas disease; Trypanosoma cruzi; vinyl sulfone

Introduction

Chagas disease (American trypanosomiasis) is a potentially life-threatening disease that affects 10 million people worldwide, primarily in Latin America.[1] The parasite *Trypanosoma cruzi*, which causes Chagas disease, is transmitted to animals and people through the fecal matter of the insect vector, triatomine or 'kissing bugs.' Left untreated, Chagas disease causes serious heart and digestive problems. The two frontline therapies for the treatment of Chagas disease, benznidazole and nifurtimox, are accompanied by significant toxicity.[2–4] K777 is a vinyl sulfone cysteine protease inhibitor, which irreversibly inhibits cruzain, a key protease required for the viability of *T. cruzi*. A novel chemical entity originally synthesized at Khepri Pharmaceuticals and shown to be active against *T. cruzi* at the University of California, San Francisco, K777 has been shown to be safe and effective in models of acute and chronic Chagas disease.[5,6] To support the preclinical development of the drug candidate, ^{14}C-K777•HCl was prepared for use in absorption, distribution, metabolism, and excretion studies.

Results and discussion

K777 can be disconnected retrosynthetically (Scheme 1) into *N*-methylpiperazine, phosgene (or a synthetic equivalent), (L)-phenylalanine, and chiral amine **1**, as was described previously by Somoza.[7] The choice of site for radiolabeling was twofold: (1) the phenylalanine unit is metabolically stable; and (2) phenylalanine uniformly labeled with carbon-14 is commercially available. A synthesis from [^{14}C(U)]-(L)-phenylalanine would allow us to prepare K777 following the established route and with high specific activity.

Synthetically, the radiosynthesis began with [^{14}C(U)]-(L)-phenylalanine and required the immediate protection of the carboxyl group (Scheme 2). To circumvent the protection and deprotection of the amino group during esterification, an *in situ* protection strategy was utilized.[8] [^{14}C(U)]-(L)-phenylalanine was treated with phosgene to generate oxazolidine dione **2**. Subsequent addition of benzyl alcohol and ethereal HCl provided H-[^{14}C(U)]-Phe-OBn as the HCl salt **3**. Reaction of **3** with phosgene gave the isocyanate **4**, which was reacted with *N*-methylpiperazine to produce urea **5**. Hydrogenolysis of the benzyl ester gave carboxylic acid **6**, setting the stage for coupling with amine **1**.[8] Treatment of **6** with isobutylchloroformate and *N*-methylmorpholine gave a mixed anhydride that was reacted with **1** as its hydrotosylate (HOTs) salt to give K777•HOTs. The tosylate counterion was exchanged by reaction with ethereal HCl to afford K777 as its hydrochloride salt. A single hot run produced 46.1 mg of [^{14}C$_9$]K777•HCl with total activity of 4.11 mCi and specific activity of 54.4 mCi/mmol. The overall radiochemical yield from [^{14}C(U)]-(L)-phenylalanine was 4.1%.

Experimental

General methods

All reactions were carried out under argon atmosphere by using commercial reagents of the highest available grade and without further purification. [^{14}C(U)]-(L)-phenylalanine was purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO). Unlabeled intermediates were analyzed by nuclear magnetic resonance (NMR) (Varian Mercury VX 300 MHz or Varian 400 MHz) and liquid chromatography-mass spectrometry (LCMS) (Thermo LCQ Fleet and Finnigan Surveyor System). Radioassays of labeled products were carried out in 10 mL of Scintisol cocktail (Isolab Inc.) with internal standards and counted using a

SRI International, Biosciences Division, 333-Ravenswood Ave, Menlo Park, CA 94025 USA

**Correspondence to: Mary J. Tanga, SRI International, Biosciences Division, 333-Ravenswood Ave, Menlo Park, CA 94025 USA.*
E-mail: mary.tanga@sri.com

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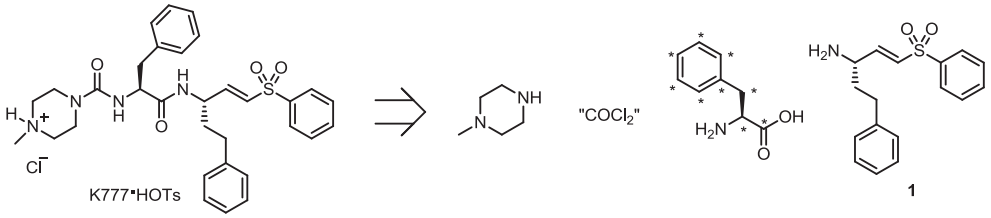
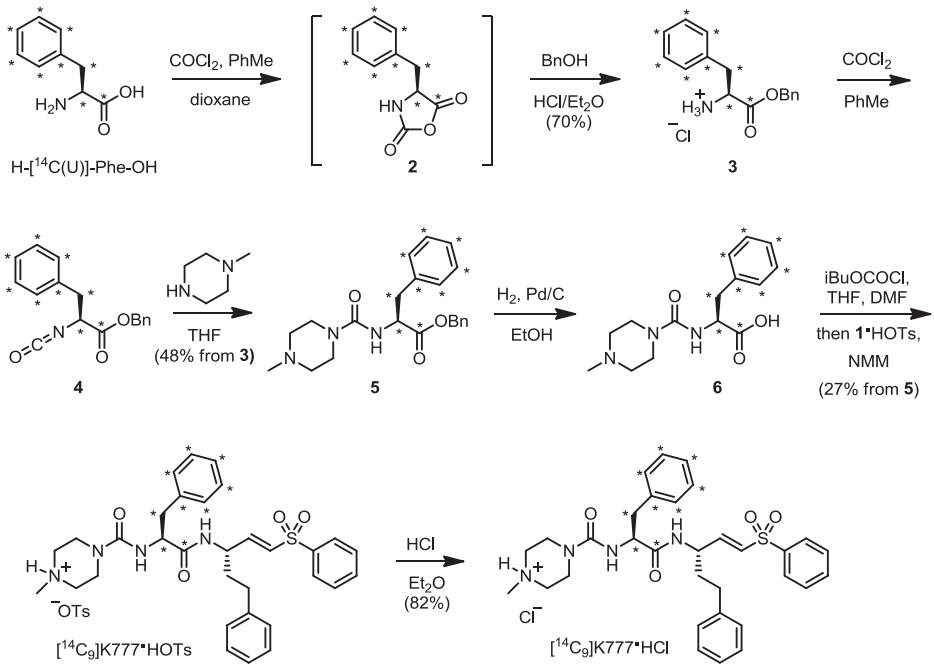


Scheme 1. Retrosynthetic analysis.



Scheme 2. Synthesis of [$^{14}C_9$]K777•HCl.

Beckman LS-6000 liquid scintillation system. Analyses by radio-HPLC were obtained with a Waters HPLC system (Waters Corporation, MA, USA; 600 Controller, 717 plus autosampler, and 996 photodiode array detector; column: Agilent Zorbax S-8 C_{18}, 4.6 × 150 mm) with detection by a Radiomatic 610 TR as a flow scintillation analyzer (PerkinElmer, Inc., MA).

[^{14}C(U)]-(L)-phenylalanine benzyl ester hydrochloride (3)

[^{14}C(U)]-(L)-phenylalanine (0.62 mmol, 103 mg, manufacturer specified specific activity: 150 mCi/mmol, total activity: 93.6 mCi) was dried under vacuum for 2 days. Unlabeled (L)-phenylalanine (0.49 mmol, 81.7 mg) and anhydrous 1,4-dioxane (8 mL) were added to give a solution of [^{14}C(U)]-(L)-phenylalanine with a calculated specific activity of 85.0 mCi/mmol. The solution was cooled in an ice bath, and a phosgene solution (17 mmol, 20% in toluene, 9 mL) was added. After stirring for 72 h at room temperature (RT), the mixture became clear and was concentrated under a stream of argon. The residue was redissolved in anhydrous 1,4–dioxane (9 mL) and cooled to 0°C. Benzyl alcohol (5.8 mmol, 0.6 mL) and ethereal HCl (9.0 mmol, 2 M in Et_2O, 4.5 mL) were added. The mixture was allowed to warm to RT. The reaction mixture became cloudy over 72 h, and the crude solid compound **3** was isolated by filtration. On cooling to 0°C, additional **3** precipitated. The combined solids were recrystallized from boiling water to give benzyl ester **3** as a white solid (206 mg, 70% yield, total activity 43.8 mCi, and specific activity of 61.0 mCi/mmol). HPLC (Zorbax 50% aqueous trifluoroacetic acid (0.1%)/50% MeCN isocratic, 0.5 mL/min flow rate, 210 nm): t_r 5.0 min. Data for cold sample are as follows: ^{1}H NMR (400 MHz, methanol-d_4) 3.19 (d, J = 6 Hz, 1H), 3.29 (m, 1H), 4.34 (t, J = 7 Hz, 1H), 5.21 (m, 2H), 7.12–7.20 (m, 2H), and 7.24–7.39 (m, 8H). ESI MS $(M+H)^+$ 257.

Benzyl (S)-2-isocyanato-3-[$^{14}C_6$]phenyl[$^{14}C_3$]propionate (4)

To a suspension of [^{14}C(U)]-(L)-phenylalanine, benzyl ester hydrochloride (**3**; 0.70 mmol, 206 mg, 43.8 mCi) in CH_2Cl_2 (15 mL) was added anhydrous pyridine (3.09 mmol, 0.25 mL). The mixture was cooled to −40°C and stirred vigorously while a phosgene solution (11.3 mmol, 20% in toluene, 6.0 mL) was added. The mixture was maintained at −40°C for an additional 5 h and then washed with ice-cold aqueous HCl (0.5 M, 10 mL). The aqueous phase was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phases were washed with ice-cold brine (20 mL), and the aqueous phase was back-extracted with CH_2Cl_2 (2 × 20 mL). The combined organic phases were dried over anhydrous $MgSO_4$ and filtered through a pad of $MgSO_4$/charcoal/Celite in a sintered glass funnel. The filtrate was concentrated to give crude **4** (150 mg), which was used without further purification.

Benzyl (S)-[4-methylpiperazin-1-carbonyl]amino-3-[$^{14}C_6$]phenyl[$^{14}C_3$] propionate (5)

A solution of compound **4** (0.47 mmol, 133 mg) in anhydrous THF (20 mL) was cooled to 0°C, and then a solution of *N*-methylpiperazine (0.65 mmol, 0.8 mL) in anhydrous THF (0.8 mL) was added. The mixture was stirred at RT for 2 h, and additional *N*-methylpiperazine (0.15 mmol, 0.2 mL) in THF (0.2 mL) was added. After an additional 2 h, the reaction mixture was concentrated on a rotary evaporator. The obtained residue was purified by column chromatography (silica gel, 2 × 13 cm, EtOAc/MeOH gradient). The piperazinyl urea **5** was isolated as viscous oil (131 mg, 48% yield from **3**). HPLC (Zorbax 5 mM aqueous NH_4OH/50% MeCN isocratic, 1.0 mL/min flow rate, 210 nm): t_r 5.1 min. Data for cold sample are as follows: ^{1}H NMR

(300 MHz, $CDCl_3$) δ 2.28 (s, 3H), 2.36 (m, 4H), 3.11 (d, J = 6 Hz, 1H), 3.25–3.41 (m, 4H), 4.77–4.94 (m, 2H), 5.09 (d, J = 13 Hz, 1H), 5.18 (d, J = 13 Hz, 1H), 6.97–7.03 (m, 2H), 7.16–7.24 (m, 3H), and 7.27–7.40 (m, 5H). ESI MS $(M+H)^+$ 382.

(S)-2-[4-methylpiperazine-1-carbonyl]amino-3-[$^{14}C_6$]phenyl[$^{14}C_3$] propionic acid (6)

To a solution of compound **5** (0.34 mmol, 131 mg) in absolute, EtOH (10 mL) was added Pd/C (40.6 mg, 30% Pd loading on activated carbon). The reaction mixture was degassed twice and exposed to hydrogen gas (1 atm) for 16 h. The remaining hydrogen gas was removed *in vacuo*, and the mixture was filtered through a pad of Celite, rinsed with additional ethanol (2 × 10 mL), and concentrated under vacuum. Crude **6** (100 mg, total activity: 20.5 mCi, specific activity: 59.8 mCi/mmol) was isolated as a pale brown solid, which was used without further purification. HPLC (Zorbax 5 mM aqueous NH_4OH/50% MeCN isocratic, 1.0 mL/min flow rate, 210 nm): t_r 3.1 min. Data for cold sample are as follows: ^{1}H NMR (DMSO-d_6) δ 2.15 (3H, s), 2.18 (4H, m), 2.84–2.94 (1H, dd, J = 15, 11 Hz), 2.95–3.04 (1H, dd, J = 15, 5 Hz), 3.14–3.30 (4H, m), 4.17 (1H, m), 6.65 (1H, d, J = 8 Hz), and 7.17–7.27 (5H, m). ESI MS $(M+H)^+$ 292.

4-Methyl-N-((S)-1-oxo-3-[$^{14}C_6$]phenyl-1-(((S,E)-5-phenyl-1-(phenylsulfonyl) pent-1-en-3-yl)amino) [$^{14}C_3$]propan-2-yl)piperazine-1-carboxamide hydrotosylate ([$^{14}C_9$]K777•HOTs)

A solution of compound **6** (0.34 mmol, 100 mg) in THF (12 mL) and dimethylformamide (3 mL) was cooled to 0°C. *N*-Methylmorpholine (0.41 mmol, 0.5 mL) was then added, followed by isobutyl chloroformate (0.43 mmol, 0.9 mL) in THF (0.9 mL). The reaction mixture was maintained at or below −20°C for 2 h. Vinyl sulfone **1**[9–11] (0.374 mmol, 177 mg) was added as a solid in one portion, followed by dropwise adding of a solution of *N*-methylmorpholine (0.41 mmol, 0.5 mL) in THF (0.5 mL). The temperature was rigorously kept between −15°C and −30°C to minimize epimerization of the phenylalanine residue. After stirring for 2 h, saturated aqueous $NaHCO_3$ (20 mL) was added. The mixture was extracted with ethyl acetate (3 × 20 mL), washed with brine (2 × 10 mL), dried over $MgSO_4$, and filtered. Concentration gave crude K777 free base (220 mg) as brown viscous oil. The oil was dissolved in acetonitrile (4 mL), and *p*-toluenesulfonic acid monohydrate (0.348 mmol, 66.2 mg) was added. The solution was allowed to stand for 16 h at RT. The resulting pale brown precipitate was washed with ether (20 mL) and air-dried. The precipitate was recrystallized twice from boiling acetonitrile to give [$^{14}C_9$]K777•HOTs (68 mg, 27% from **5**) as a white solid.

4-Methyl-N-((S)-1-oxo-3-[$^{14}C_6$]phenyl-1-(((S,E)-5-phenyl-1-(phenylsulfonyl) pent-1-en-3-yl)amino) [$^{14}C_3$]propan-2-yl)piperazine-1-carboxamide hydrochloride ([$^{14}C_9$]K777•HCl)

[$^{14}C_9$]K777•HOTs (68 mg) was dissolved in anhydrous CH_2Cl_2 (10 mL). The solution was cooled in an ice bath, and ethereal HCl (2 M, 10 mL) was added. The mixture was stirred and allowed to slowly warm to RT overnight. The mixture was concentrated, and the residue was washed with diethyl ether (25 mL) and hexanes (25 mL). The solid was isolated by centrifugation, and after removal of the supernatant, it was dried under high vacuum. [$^{14}C_9$]K777•HCl was obtained as a white solid (46.1 mg, 82% yield, total activity: 4.11 mCi, specific activity: 54.4 mCi/mmol, radiochemical purity of >94% determined by radio-HPLC). HPLC (Zorbax 50% aqueous trifluoroacetic acid (0.1%)/50% MeCN isocratic, 0.5 mL/min flow rate, 210 nm): t_r 6.3 min. Data for cold sample are as follows: ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.75 (1H, m), 1.88 (1H, m), 2.49–2.90 (4H, m), 2.68 (3H, d, J = 4 Hz), 3.05 (2H, m), 3.04–3.26 (2H, m), 3.30 (2H, d, J = 11 Hz), 3.97–4.15 (2H, m), 4.27 (1H, m), 4.43 (1H, m), 6.45 (1H, d, J = 15 Hz), 6.81 (1H, dd, J = 15, 4 Hz), 7.13–7.31 (10H, m), 7.61–7.74 (3H, m), 7.82 (2H, d, J = 8 Hz), 8.22 (1H, d, J = 8 Hz), 10.85 (1H, br s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 31.3, 34.7, 37.46, 40.8, 41.9, 48.7, 52.0, 56.4, 125.8, 126.3, 127.1, 128.0, 128.3, 128.4, 129.2, 129.6, 133.6, 138.2, 140.3, 141.2, 147.2, 156.7, and 172.0. ESI MS $(M+H)^+$ 575.

SUPPORTING INFORMATION

A radio-HPLC trace of [$^{14}C_9$]K777•HCl is provided.

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Conflict of Interest

The authors did not report any conflict of interest.

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