Journal of Labelled Compounds and Radiopharmaceuticals
J Label Compd Radiopharm 2007; **50**: 65–71.
Published online in Wiley InterScience
(www.interscience.wiley.com). DOI: 10.1002/jlcr.1148

JLCR

Research Article

Synthesis of the anxiolytic agent (^{14}C) 6-hydroxy-buspirone for use in a human ADME study

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Received 26 July 2006; Revised 28 September 2006; Accepted 3 October 2006

Abstract: A reliable synthesis of ^{14}C labeled 6-hydroxy-buspirone is described. The molecule belongs to a unique class of compounds with the potential for anxiolytic activity. A radiolabeled analog was prepared to support the development of 6-hydroxy-buspirone. Specifically, a labeled variant was designed to meet the requirements of a human adsorption-distribution-metabolism-elimination (ADME) study. Multiple ^{14}C labels were needed to fully track the potential metabolic transformation of the molecule. Labeled 6-hydroxy-buspirone was prepared by oxidation of separately labeled versions of [^{14}C]buspirone. The final product was isolated in reasonable yield with a radiochemical purity of 99.8%. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: buspirone; 6-hydroxy-buspirone; carbon-14 synthesis

Introduction

Buspirone is marketed by Bristol-Myers Squibb as a novel antianxiety compound.[1–3] The compound is a member of the N-(4-heteroaryl-1-piperazinyl)alkylimide structural class (Figure 1) of psychotropic agents. Its affinity as a serotonin $5HT_{1a}$ receptor agonist is believed to be at least partially responsible for this anxiolytic activity.[4,5] Buspirone's clinical efficacy is similar to diazepam, but it is does not suffer from the usual side effects of benzodiazepines, namely sedation and addiction.[6]

Detailed studies of the metabolic profile of buspirone have been reported using radiolabeled tracer and LC-MS techniques.[7–10] Of the several identifiable metabolites of buspirone, 6-hydroxy-buspirone, and 1-pyrimidinylpiperizine (1-PP) are significant human metabolites.[8] The 6-hydroxy metabolite is biologically active, and has potential as a treatment for anxiety and depression.[11]

As part of the development of 6-hydroxy-buspirone, a detailed understanding of its pharmacokinetic and metabolic profiles was needed. To aid in this analysis, a radiolabeled analog was designed and synthesized. The criteria for labeling 6-hydroxy-buspirone centered around the anticipated human metabolism of the molecule. In particular, metabolic scission was a primary consideration for the labeling strategy. The phase I human biotransformation of Buspirone involves N-dealkylation at the piperazinyl nitrogen linking the 1-PP and imide moieties.[8] Apart from oxidative and phase 2 modifications to the molecule, this disconnection was anticipated for 6-hydroxy-buspirone and served to define the parameters for radiochemical labeling. To quantify and identify metabolites from each part of the molecule, 1-PP and 8-azaspiro[4.5]decane-7,9-dione, separate radiochemical tags on each piece were needed.

Results and discussion

Labeling the 1-PP, and 8-azaspiro[4.5]decane-7,9-dione pharmacophores of 6-hydroxy-buspirone presented some challenge. The availability of suitable precursors, and synthetic feasibility were key issues.

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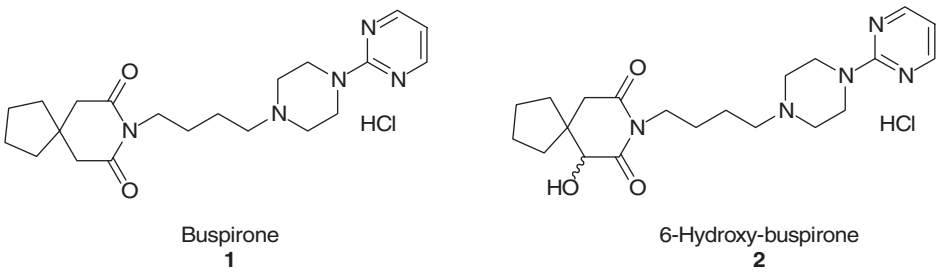
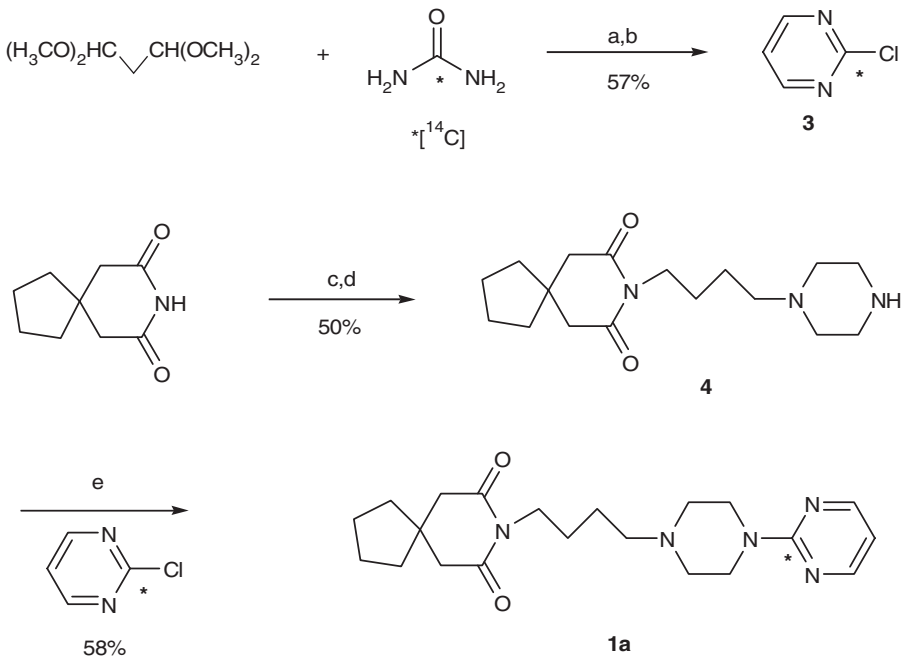


Figure 1



Scheme 1 [2-^{14}C]primidinyl buspirone: (a) EtOH, HCl, heat; (b) $POCl_3$, heat; (c) 1,4-dibromobutane, K_2CO_3, CH_3CN, heat; (d) piperazine, K_2CO_3, heat; (e) EtOH, Et_3N, heat.

From the onset of the project, it was understood that buspirone could be hydroxylated at the 6 position of the imide ring giving a racemic mixture of alcohols.[11] Chromatographic separation of the (*R*,*S*) isomer mixture has also been reported.[12] Since more than one labeled analog of 6-hydroxy-buspirone was required, it seemed reasonable to prepare separately labeled versions of buspirone and combine them into a single batch prior to hydroxylation. In this manner difficulties encountered in one synthetic sequence would not directly impact the other. In addition, we have observed a radiochemical stability advantage by limiting the number of labels incorporated on a per molecule basis.

The synthesis of buspirone and several related cyclic imides was reported by Wu and co-workers.[13] This chemistry relied on the reaction between an N-(ω-haloalkyl)imide and a functionalized piperazine, or alternatively, the condensation of an anhydride with 1-(ω-aminoalkyl)piperazine. A labeled synthesis existed for the preparation of the [^{14}C]1-PP variant of buspirone from the reaction of 2-Chloro-[2-^{14}C]pyrimidine with 8-(4-piperazinylbutyl)-azaspiro[4.5]decane-7,9-dione.[16,17] Labeling the azaspiro portion of the molecule and the subsequent hydroxylation to prepare the [^{14}C]6-hydroxy-buspirone product had not been reported.

Labeling the 8-azaspiro[4.5]decane-7,9-dione moiety required construction of the core via an accessible labeled precursor. Cipollina and co-workers reported on the synthesis Tiospirone.[18] They describe the preparation of 8-azaspiro[4.5]dec-2-ene-7,9-dione in six steps from 3-cyclopentene-1,1-dicarboxylate. We adapted this approach for the preparation of the labeled azaspiro-dione using K^{14}CN as the radiolabel source.

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DOI: 10.1002.jlcr

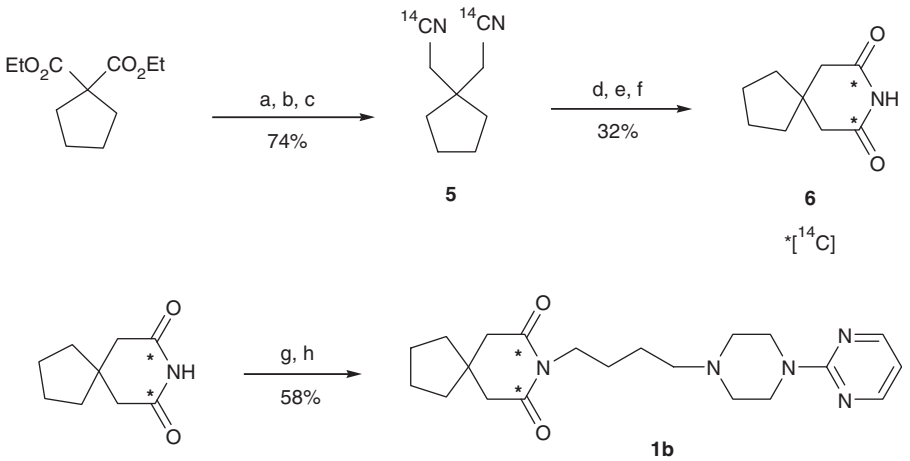
Synthesis of the [^{14}C]1-PP analog, **1a** (Scheme 1), was accomplished in five steps from commercially available [^{14}C]urea.[16] The isotopic activity of the labeled urea (53 mCi/mmol) was diluted to accommodate scale, and it was reacted with malonaldehyde bis(dimethyl acetal) to give [^{14}C]2-hydroxy pyrimidine. The pyrimidine product was further reacted with $POCl_3$, and the resulting radiolabeled chloride coupled with 8-(4-piperazinylbutyl)-azaspiro[4.5]decane-7, 9-dione affording **1a** in an overall radiochemical yield of 17%.

The synthesis of **1b** is illustrated in Scheme 2. Commercially available cyclopentane-1,1-dicarboxylic acid diethyl ester was reduced with $LiAlH_4$. The diol product was tosylated and reacted with K^{14}CN to give the key intermediate **5**. The ^{14}C labeled nitrile was hydrolyzed, and the resulting acid was dehydrated in the presence of acetic anhydride to give the cyclic anhydride. Imide **6** was prepared by conversion of the anhydride with ammonia hydroxide at an elevated temperature. Alkylation with 1,4-dibromobutane furnished the penultimate bromide which was coupled with 1-(2-pyrimidyl)piperizine HCl giving **1b** in 15% overall yield from K^{14}CN.

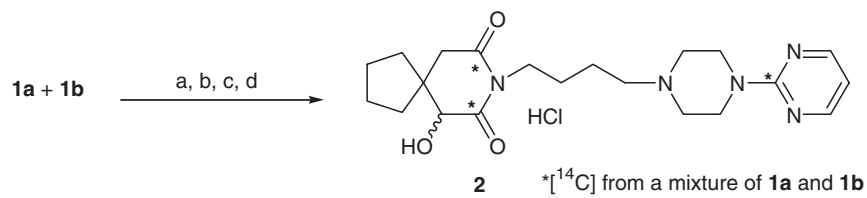
6-Hydroxy-buspirone, **2**, was prepared by the hydroxylation of a combined batch of **1a** and **1b** (Scheme 3). Although buspirone could be hydroxylated with Davis reagent,[19] yields were substantially improved using an alternate sequence. Radiolabeled buspirone was treated with NaHMDS at −78°C. Complete formation of the anion was achieved by briefly warming the reaction to −50°C The resulting anion was reacted with O_2 in the presence of $P(OEt)_3$ giving a racemic mixture of alcohols. The chemical purity of the crude product was improved by HPLC chromatography. Final purification of **2** was achieved by formation of its HCl salt, then crystallization. The radiochemical yield for the transformation of labeled buspirone to **2** was 39%. The radiochemical purity of the final product was 99.8%.

Experimental procedure

Reactions were run under an inert atmosphere of argon and stirred magnetically unless otherwise noted. Solvent removal under vacuum was accomplished using a Buchi R-124 rotary evaporator. Column chromatography was performed using a Biotage® flash chromatography system. Proton NMR spectra were



Scheme 2 8-Azaspiro[4.5]decane-[^{14}C-7,9]-dione buspirone: (a) 1.0 M $LiAlH_4$, diethylether; (b) TsCl, $CHCl_3$, pyridine; (c) K^{14}CN, DMSO, heat; (d) 40% KOH, ethylene glycol, heat; (e) Ac_2O, heat; (f) NH_4OH, heat; (g) 1,4-dibromobutane, CH_3CN, K_2CO_3, $DMAP_{(cat)}$; (h) K_2CO_3, Kl, CH_3CN, 1-(2-pyrimidyl)piperizine HCl.



Scheme 3 [^{14}C]6-Hydroxy-buspirone: (a) NaHMDS, THF, −78°C to −50°C; (b) $P(OEt)_3$, O_2, −78°C; (c) HPLC purification; (d) 6N, HCl, water/isopropyl alchohol, 60°C to r.t.

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DOI: 10.1002.jlcr

recorded on a 500 MHz Varian Unity/Inova or a 300 MHz Bruker Avance spectrometers. Radiochemical purities were determined by HPLC (Rainin Model SD-200, Varian PDA-2 detector and Beta-Ram detector (IN/US Systems Inc.) and TLC (Merck 60 F_{254} silica gel coated plates) using radiochemical detection (QC-Scan, Bioscan Model B-QC). Specific activity was determined by gravimetric analysis using liquid scintillation counting (Wallac Model 1409). Mass spectra were recorded on a Finnigan LCQ system. Reactions were monitored by HPLC, TLC and NMR. Radiolabeled products were compared with authentic standards when possible. All reagents and solvents were ACS grade or better.

HPLC: HPLC method described below was used for in process, and final product analyses. Co-injections with authentic samples where used when possible. All HPLC purities are measurements of radiochemical purity. Retention times were determined by UV detection of the desired component. Additional methods are described in the experimental section below.

HPLC in process method:

Column: YMC-Pack Pro 5.0 μm (4.6 × 150 mm)

Mobile Phase A: 90% Water/10% CH_3CN with 0.075% TFA

Mobile Phase B: 80% Water/20% CH_3CN with 0.075% TFA

Program: Isocratic (100% A) 0–10 min, Gradient (100% B) 10–20 min, Isocratic (100% B) 20–30 min, Gradient (100% A) 30–35 min, Isocratic (100% A) 35–45 min; Flow rate: 1 ml/min, Injection size: 20 μl.

Detection: UV at 220 nm and radiochemical.

2-chloro-(2-^{14}c)pyrimidine (3)

A commercial sample of [^{14}C]urea (113 mg, 1.88 mmol, 53 mCi/mmol) was dissolved in 5 ml of ethanol. Additional urea (508 mg, 8.5 mmol) was added giving 621 mg (10.3 mmol, 9.7 mCi/mmol) of [^{14}C]urea in absolute ethanol. Malonaldehyde bis(dimethyl acetal) (1.72 g, 10.5 mmol) and concd HCl (1.6 ml) were added to the stirred solution at r.t. Following the addition of all reactants, the reaction was heated to 85°C in an oil bath for 65 min. After cooling, the solvent was removed, and the residue was triturated with 6 ml of isopropyl alcohol at 0°C for 1 h. The resulting 2-hydroxy-[2-^{14}C]pyrimidine hydrochloride was collected by filtration, and dried under vacuum to a constant weight (1.1 g, 8.3 mmol). This material was transferred to a round-bottomed flask and used directly in the next step without purification. Phosphorous oxychloride (29.6 g, 193 mmol) was added, and the stirred reaction solution was heated to reflux (125°C) for 5 h. Excess $POCl_3$ was removed by distillation, and the residue was dissolved in chloroform (8 ml), then passed through a pad of celite. The celite was rinsed with additional chloroform (8 ml) giving a yellow colored filtrate. This filtrate was slowly added to a flask containing 5 g of crushed iced with stirring. The pH was adjusted to 8 with 40% NaOH, and the organic phase separated. Additional caustic was added as necessary to maintain a pH of 8. The aqueous phase was extracted further with chloroform (10 ml), and the organic phases combined, dried over Na_2SO_4, filtered, and concentrated to give **3** as a yellow solid (678 mg, 5.9 mmol, 57%).

8-(4-piperazinylbutyl)-azaspiro(4.5)decane-7,9-dione (4)

A round-bottomed flask fitted with a mechanical stirrer and reflux condenser was charged with 3,3-tetramethylene glutarimide (10 g, 60 mmol), 1,4-dibromobutane (13.1 g, 60 mmol), and potassium carbonate (24.9 g, 180 mmol). Anhydrous acetonitrile (150 ml) was added, and the reaction heated to reflux in an oil bath for 18 h. The reaction mixture was filtered while hot, and the resulting cake rinsed with additional CH_3CN (50 ml). The filtrate was concentrated to a clear oil (17.8 g, 58.8 mmol, 98%). Analytical characterization of the oil was consistent with 8-(4-bromobutyl)-8-azaspiro[4.5]decane-7,9-dione).[16] Without purification, anhydrous piperazine (25.3 g, 294 mmol), potassium carbonate (16.3 g, 118 mmol), and acetonitrile (130 ml) were added to the reaction flask. The mixture was mechanically stirred while heating at reflux for 22 h. The mixture was hot filtered through a bed of celite and the cake rinsed with hot methanol. At this point, the filtrate was concentrated and the residue dissolved in CH_2Cl_2 (200 ml). The organic solution was extracted with two portions of 2 N HCl (30 ml). The aqueous extracts were pooled and cooled in an ice bath, then the pH was adjusted to 9 with 40% NaOH. The resulting mixture was extracted with CH_2Cl_2 (2 × 50 ml), and the organic phases combined, washed with saturated brine (50 ml), and dried over Na_2SO_4. The organic solution was filtered, and concentrated to give **4** as a pale yellow oil (11.5 g, 30 mmol, 51%). The oil was shown to be consistent with the literature characterization of **4**.[16]

8-(4-(4-((2-^{14}c)primidinyl)-1-piperazinyl)butyl)-8-azaspiro(4.5)decane-7,9-dione (1a)

Radiolabeled chloride **3** (0.678 g, 5.92 mmol), piperazinyl dione **4** (2.66 g, 8.22 mmol), and triethylamine (1.23 g, 12.2 mmol) were combined together in a stainless steel Parr bomb. The contents were dissolved in absolute ethanol (40 ml), and the sealed bomb was heated at 125°C for 24 h. The reaction was cooled to

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DOI: 10.1002.jlcr

r.t., and the contents transferred to a flask allowing for solvent removal *in vacuo*. The crude solid was chromatographed on silica gel (96% $CHCl_3$/4% CH_3OH) giving a purified oil weighing 2.8 g. 1.0 N HCl (15 ml) was added to the oil, and the resulting mixture was extracted with CH_2Cl_2 (2×25 ml) leaving behind a clear aqueous solution. This solution was cooled in an ice bath, and made slightly basic by the addition of 5 N NaOH. The liberated free base was isolated by extraction with CH_2Cl_2 (2×25 ml). The organic extracts were combined, dried over Na_2SO_4, filtered, and the solvent removed to give an oil (1.63 g) which slowly solidified. The product was recrystallized from hot isopropyl alcohol (11 ml) affording **1a** as a white solid (1.32 g, 3.43 mmol, 42% from **3**). The radiochemical purity (HPLC) of **1a** was 99.7% and the specific activity was 10.4 mCi/mmol. ^{1}H NMR was consistent with the literature description of labeled buspirone.[16]

3,3-tetramethyleneglutaryl (1,5-^{14}c)dinitrile (5)

Cyclopentane-1,1-dicarboxylic acid diethyl ester (5 g, 23.4 mmol) was added dropwise to a cooled (0°C) solution of 1.0 M lithium aluminum hydride in ether (50 ml). Following this addition, the reaction flask was heated to 60°C for 3 h. The flask was then cooled to r.t. Water (2 ml), 15% w/w NaOH (2 ml), and water (5 ml) were added slowly in this order. The mixture was stirred for 16 h, followed by filtration. The ethereal solution was concentrated yielding 2.86 g (2.2 mmol, 94%) of cyclopentane-1,1-dimethanol. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.6 (s, 4 H), 2.4 (s, 2 H), 1.61 (m, 4 H), 1.42 (m, 4 H). The diol was used directly in the next step without purification. Cyclopentane-1,1-dimethanol (2.25 g, 17.3 mmol) was dissolved in $CHCl_3$ (50 ml). Pyridine (5.5 g, 69.2 mmol) was added slowly, then the reaction was cooled in an ice-bath and toluenesulfonyl chloride (9.9 g, 51.9 mmol) was added. The reaction was slowly warmed to r.t., and stirred for 16 h. Ether (200 ml), and 1N HCl (50 ml) were added to the flask. The phases were partitioned, and the organic phase was dried over Na_2SO_4, filtered, and concentrated to a solid. The crude product was recrystallized from hot methanol giving 6.6 g (15 mmol, 87% from diol) of the ditosylate (HPLC purity 98%, R_t 24.7 min). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.96 (d, $J = 8.3$ Hz, 4 H), 7.58 (d, $J = 8.3$ Hz, 4 H), 4.0 (s, 4 H), 2.68 (s, 6 H), 1.76 (m, 4 H), 1.61 (m, 4 H). Synthesis of the dinitrile, **5**, was completed by adding the following to a round-bottomed flask; K^{14}CN (55 mCi/mmol, 243 mg, 3.63 mmol), KCN (1.35 g, 20.8 mmol), ditosylate (5.37 g, 12.24 mmol), and DMSO (25 ml). The mixture was heated to 125°C for 16 h, then cooled slowly to r.t. The contents of the flask were transferred to a separatory funnel and partitioned between ether (50 ml) and water (50 ml). The organic phase was retained and washed with brine (25 ml). The ether phase was dried, filtered, and evaporated to a crude solid. The dinitrile was purified by column chromatography (70% hexanes/30% EtOAc) giving **5** as a white solid (HPLC purity 99%, R_t 21.9 min) weighing 1.48 g (10 mmol, 82% from ditosylate). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.48 (s, 4 H), 1.72 (m, 4 H), 1.66 (m, 4 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 117.2, 43.4, 36.9, 27.6, 24.4.

8-azaspiro(4.5)decane-(7,9-^{14}c)dione (6)

A 100 ml flask was charged with **5** (1.48 g, 10 mmol), ethylene glycol (10 ml), and KOH (5 ml, 40% w/w in water). The reaction was heated at 110°C for 16 h. The reaction was cooled to r.t., and then poured into a flask containing 6 N HCl (100 ml) with cooling at 0°C. 3-Cyclopentane-[1,1-^{14}C]diacetic acid (1.83 g, 9.7 mmol, 17.1 mCi/mmol) was isolated by extraction with ether (3×35 ml) and used in the next step without purification. The labeled acid was dissolved in acetic anhydride (10 g, 98 mmol), and heated to 150°C. After 18 h, the reaction was cooled to 65°C to facilitate solvent removal under a stream of N_2. The crude product was chromatographed (70% EtOAc/30% hexanes) on silica gel, and 1.3 g (7.6 mmol) of 3,3-tetramethyleneglutaric anhydride was isolated as a white solid (HPLC purity 97%, R_t 18.9 min) with a molar specific activity of 17 mCi/mmol. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.51 (s, 4 H), 1.6 (m, 4H), 1.4 (m, 4 H). The anhydride (1.1 g, 6.52 mmol) was dissolved in 30% $NH_4OH_{(aq)}$ (8.5 ml). The flask was then equipped with a cold-finger condenser (−78°C), and heated to 100°C for 3 h. Most of the water was then removed by rotary evaporation after cooling to r.t. *p*-Xylene (40 ml) was added to the residue along with 250 μl of 30% $NH_4OH_{(aq)}$. The flask was re-fitted with a Dean-Stark apparatus, and was heated to 175°C for 18 h. Under these conditions a 50% conversion of the glutaryl anhydride to Imide **6** was achieved. The crude product mixture was chromatographed (80% hexanes/20% EtOAc). Imide **6** (489 mg, 2.93 mmol), (HPLC purity 98%, R_t 16.7 min) and unreacted anhydride (420 mg, 2.5 mmol) were isolated as solids. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.03 (br s, 1H), 2.55 (s, 4 H), 1.75 (m, 4 H), 1.57 (m, 4 H).

8-(4-(4-(2-pyrimidyl)-1-piperazinyl)butyl)-8-azaspiro (4.5)decane-(7,9-^{14}c)dione (1b)

A flask was charged with **6** (480 mg, 2.84 mmol), 1,4-dibromobutane (766 mg, 3.55 mmol), DMAP (7 mg, 2 mol%), K_2CO_3 (1.18 g, 8.52 mmol), $CH_3CN_{(anh)}$

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DOI: 10.1002.jlcr

(10 ml). The flask was fitted with a reflux condenser and heated to 95°C. After 16 h, the flask was cooled to r.t., and the reaction was filtered to remove salts. The filtrate was concentrated and the residue was chromatographed (75% hexanes/25% EtOAc) to give 8-(4-bromobutyl)-8-azaspiro[4.5]-decane-[7,9-^{14}C]dione (598 mg, 1.98 mmol, 17 mCi/mmol), (TLC 75% hexanes/25% EtOAc, R_f 0.6). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.8 (t, $J = 7.2$ Hz, 2 H), 3.42 (t, $J = 6.7$ Hz, 2 H), 2.6 (s, 4 H), 1.86 (m, 2 H), 1.7 (m, 6 H), 1.5 (m, 4 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.2, 44.9, 39.5, 38.5, 37.6, 33, 30.1, 26.7, 24.2. The labeled bromide was combined with KI (16.4 mg, 5 mol%), 1-(2-pyrimidyl)piperazine (326 mg, 1.98 mmol), K_2CO_3 (821 mg, 5.94 mmol), and dissolved in $CH_3CN_{(anh)}$ (10 ml). The reaction mixture was heated to 95°C for 16 h, and filtered while hot. The filtrate was concentrated to an oil and chromatographed (95% CH_2Cl_2/5% CH_3OH) giving 637 mg (1.65 mmol, 17.1 mCi/mmol) of **1b** as a white solid (HPLC purity 96%, R_t 16.8 min). ^{1}H NMR consistent with structure.[16]

6-hydroxy-8-(4-(4-((2-^{14}c)pyrimidyl)-1-piperazinyl)-butyl)-8-azaspiro(4.5)decane-(7,9-^{14}c)dione Hydrochloride (2)

A mixture of **1a** (0.649 g, 1.68 mmol, 16.85 mCi, 25.95 μCi/mmol), **1b** (0.380 g, 0.99 mmol, 16.85 mCi, 44.36 μCi/mmol) and THF (27 ml) were added to a 100-ml three-neck flask. The solution was stirred at room temperature under argon for several minutes to ensure proper mixing and then cooled to −78°C. The water content (measured by KF) for a model reaction mixture containing unlabeled **1** was measured and found to be 119 ppm (0.17 mmol of water). Freshly titrated sodium bis(trimethylsilyl)amide (0.59 M in THF, 4.81 ml, 2.84 mmol, 1 eq.) was added dropwise to the solution of **1a** and **1b**. The internal temperature of the solution was kept below –70°C during the addition. The yellow solution was warmed to −50°C and stirred for 1 h. The mixture was cooled back to −78°C and triethyl phosphite (1.83 ml, 1.77 g, 10.7 mmol, 4 eq.) was added. At this time the solution was sparged with anhydrous oxygen, and the addition rate was adjusted to maintain an internal temperature below −73°C. After 2 h of sparging the reaction was quenched with HCl (1 N, 5.34 mL, 5.34 mmol, 2 eq.), and warmed to room temperature. The reaction mixture was diluted with water (25 ml) and the pH was adjusted to 7.0. The aqueous solution was extracted with CH_2Cl_2 (4 × 20 ml). The organic layers were combined and washed with 25% aqueous NaCl (25 ml). The organic solution was concentrated under reduced pressure, then dried under vacuum to give 1.61 g of a yellow solid. The solid was dissolved in 80:20 MeOH/water (40 ml), and the desired product was isolated by semi-preparative HPLC (2 ml injections on a YMC basic column s-5 μm 20 × 250 mm. Solvent: A=60:40 water/MeOH with 10 mM NH_4OAc; B=80:20 MeOH/water with 10 mM NH_4OAc. Conditions: 100% A 0–3 min, 100–0% A 3–23 min, 0% A 23–33 min, 0–100% A 33–34 min, 100% A 34–35 min.Flow: 9 ml/min. Wavelength: 220 nm). The fractions containing **2** were combined and partially concentrated. The aqueous solution that remained was diluted with water (50 ml) and extracted with CH_2Cl_2 (4 × 40 ml). The organic layers were combined and concentrated under reduced pressure. The white solid that remained was dried by dissolving in *tert*-butylmethyl ether (2 × 30 ml) and concentrated under reduced pressure. The white solid was vacuum dried (5 h) to give 0.520 g (49% yield) of **2** (free base) with a radiochemical purity of 99.43% (R_t = 19.54 min, HPLC conditions: YMC basic column s-5 μm 4.6 × 250 mm. Solvent: A=60:40 water/MeOH with 10 mM NH_4OAc; B=80:20 MeOH/water with 10 mM NH_4OAc. Conditions: 100% A 0–3 min, 100–0% A 3–23 min, 0% A 23–26 min, 0–100% A 26–27 min, 100% A 27–35 min. Flow: 1 ml/min, β-ram detector).

A suspension of **2** (free base, 0.520 g, 1.30 mmol) and isopropyl alcohol (4.4 ml) were heated to 60°C in a 25-ml flask. Water (0.11 ml) was added and the suspension was stirred until it became homogenous. The clear solution was treated with HCl (6.00 N, 1.30 mmol, 217 μl, 1 eq.) and stirred at 60°C for 15 min. The mixture was cooled to room temperature over 30 min, and then allowed to stand at room temperature for 2 h. The precipitate that formed was collected by vacuum filtration and then rinsed with isopropyl alcohol (5 ml). The off-white solid was vacuum dried for 20 h to give 0.472 g (83% yield) of **2** (HCl salt) (HPLC purity 99.87%, R_t 19.40 min, HPLC conditions: same as above for the free base **2**). The specific activity was measured as 29.37 μCi/mg (12.85 μmCi/mmol, 13.87 mCi). The radiochemical activity of the sample was diluted to the desired value by dissolution with unlabeled **2** in *tert*-butylmethyl ether. The suspension was concentrated under reduced pressure to give a white solid. The white solid was vacuum dried for 3 days to give 1.41 g of **2** (HPLC purity 99.9%, R_t 19.6 min) and a specific activity of 9.68 μCi/mg (4.23 mCi/mmol, 13.20 mCi). The white solid was shown to be the desired product by comparison of its NMR spectra with an authentic sample: ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.52 (br s, 1 H), 8.43 (d, $J = 4.7$ Hz, 2 H), 6.75 (t, $J = 4.7$ Hz, 1 H), 5.94 (brs, 1 H), 4.74–4.61 (m, 2 H), 4.11 (brs, 1 H), 3.68–3.41 (m, 4 H), 3.18–2.86 (m, 5 H), 2.65 (d, $J = 4.7$ Hz, 2 H), 1.78–1.11 (m, 13 H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.09, 171.35, 160.65, 158.13, 111.24, 73.42, 55.00, 50.29,

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DOI: 10.1002.jlcr

44.05, 42.59, 40.18, 38.26, 34.84, 31.01, 25.04, 25.00, 24.65, 20.42.

Conclusion

[^{14}C] Labeled 6-hydroxy-buspirone was prepared from separately labeled analogs of [^{14}C]buspirone. The yields for [^{14}C]buspirone, **1a** and **1b** were 17 and 15%, respectively. [^{14}C]6-Hydroxy-buspirone was prepared with a radiochemical purity of 99.8% (39% from labeled **1**), and utilized in a human ADME study. The present work adds to the body of literature describing the synthesis of buspirone and related compounds. Practical syntheses of radiolabeled 8-azaspiro[4.5]decane-7,9-dione and 6-hydroxy-buspirone were demonstrated.

Acknowledgements

The authors want to thank the BMS Pharmaceutical Research Institute and members of the BMS Radiochemical Synthesis Group for helpful discussions.

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