Received: 6 July 2016 | Revised: 19 August 2016 | Accepted: 5 September 2016

DOI 10.1002/jlcr.3463

WILEY Journal of Labelled Compounds and Radiopharmaceuticals

RESEARCH ARTICLE

Buscopan labeled with carbon-14 and deuterium

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Hyosine butyl bromide, the active ingredient in Buscopan, is an anticholinergic and antimuscarinic drug used to treat pain and discomfort caused by abdominal cramps. A straightforward synthesis of carbon-14– and deuterium-labeled Buscopan was developed using scopolamine, *n*-butyl-1-^{14}C bromide, and *n*-butyl-$^{2}H_9$ bromide, respectively. In a second carbon-14 synthesis, the radioactive carbon was incorporated in the tropic acid moiety to follow its metabolism. Herein, we describe the detailed preparations of carbon-14– and deuterium-labeled Buscopan.

KEYWORDS

Buscopan, carbon-14, deuterium, hyoscine butyl bromide, radiosynthesis, tropic acid

1 | INTRODUCTION

Tropane alkaloids are a class of natural compounds that act as anticholinergic and antimuscarinic agents. They are widely used in preoperative procedures because of their ability to decrease saliva and gastrointestinal tract secretions, and as antispasmodic like in the treatment of bladder spasm because they decrease motility of smooth muscles. They are used as stimulators of the respiratory system, in the treatment of colic, cystitis, and pancreatic peptic ulcer, as agents that induce dilation of the eye pupil (mydriatic). Tropane alkaloids are also used as antidotes to organophosphorus compounds.[1] Hyoscine, on the other hand, has a more central effect. It is used as a sedative, a hypnotic, and an antiemetic.[1] Butylscopolamine, also known as scopolamine butyl bromide, butylhyoscine, hyoscine butyl bromide or by its IUPAC name ([7(*S*)-(1α,2β,4β,5α,7β)]-9-butyl-7-(3-hydroxy-1-oxo-2-phenyl-propoxy)-9-methyl-3-oxa-9-azoniatrycylco [3.3.2.0^{2,4}] nonane bromide), the active ingredient in Buscopan, is an alkaloid extracted from the leaves of the *Duboisia* genus.[2] It is an anticholinergic and antimuscarinic agent. What makes hyoscine butylbromide so effective against the discomfort and pain of abdominal cramps is its extremely targeted effect (http://www.buscopan.com). Unlike other antispasmodics, Buscopan does not cross the blood-brain barrier and thus is unlikely to cause drowsiness. The butyl group introduces a permanent positive charge on the molecule and reduces its permeability across biological membranes.[3] Buscopan has been known for a long time for its antispasmodic and gastric antisecretory activities and is structurally similar to Spiriva, which is used in the treatment of chronic obstructive pulmonary diseases (Figure 1). Butylscopolamine is registered in more than 40 countries worldwide and has been clinically used for more than 50 years. It is on the list of the most important medications needed in a basic health system (see the World Health Organization Model List of Essential Medicines; http://www.who.int/medicines/publications/essentialmedicines/en/index.html). Buscopan is prescribed to treat pain and discomfort caused by abdominal cramps, menstrual cramps, or other spasmodic activities in the digestive systems and is an antiemetic.[4] It is also effective in managing some of the symptoms of irritable bowel syndrome.[1,5] Like many compounds containing quaternary nitrogen atoms, Buscopan can potentially block nicotinic acetylcholine receptors[6] and may be effective in suppressing the side effects of opiates.[7] Stable and radioactive isotope–labeled Buscopan was essential to the development of this drug and its advancement in clinical trials. Herein, we report the detailed synthesis of deuterium- and carbon-14–labeled Buscopan.

2 | RESULTS AND DISCUSSION

To prepare stable isotope– and carbon-14–labeled Buscopan, we used a straightforward synthesis using scopolamine and labeled *n*-butyl bromide[8] (Scheme 1).

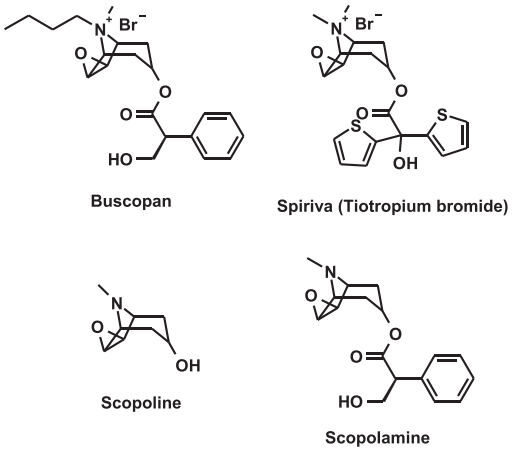
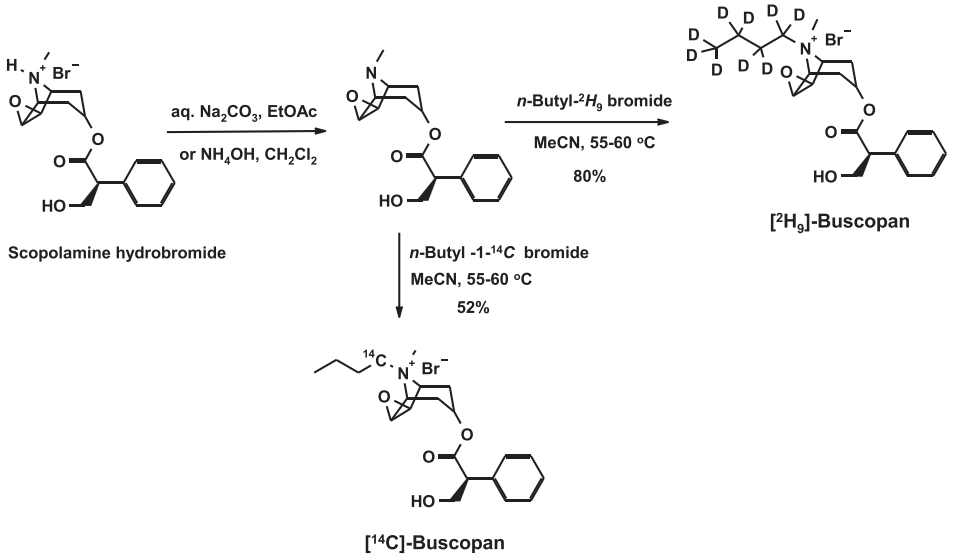


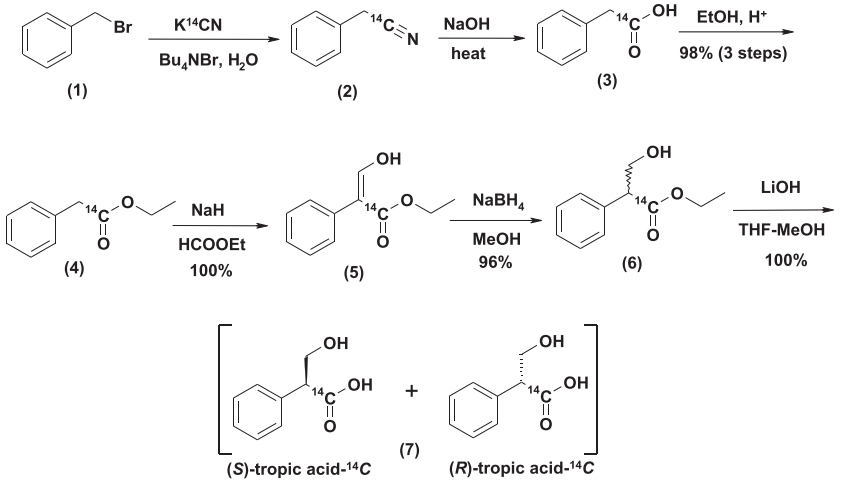
FIGURE 1 Chemical structures of Buscopan, Spiriva, scopoline, and scopolamine

Scopolamine, also known as hyoscine, is an ester of *l*-tropic acid and scopoline base, which is commercially available as a hydrogen bromide salt. Both *n*-butyl-$^{2}H_{9}$-bromide and *n*-butyl-1-^{14}C-bromide are also available commercially. Thus, to prepare the deuterium-labeled Buscopan, the scopolamine free base was first obtained from treatment of scopolamine hydrobromide salt with a base like sodium carbonate or concentrated ammonia and then reacted with excess *n*-butyl-$^{2}H_{9}$-bromide in acetonitrile at 60°C. Deuterium-labeled Buscopan was isolated in 80% yield after crystallization from anhydrous ethyl ether with more than 98% isotopic enrichment. Labeled Buscopan using deuterated *n*-butyl bromide has been reported by others as well.[9] Analogously, carbon-14–labeled Buscopan was obtained in 52% yield with a specific activity of 32 mCi/mmol.

Buscopan is prone to hydrolysis especially in aqueous media, and there was an obvious need to prepare Buscopan with the radioactive carbon in the tropic acid moiety to follow its metabolism in several animal species. Carbon-14–labeled tropic acid was reported before, although little or no details on the synthesis were given.[10,11] The synthesis of unlabeled racemic and chiral tropic acid chemically or biosynthetically was also reported by several groups.[12–20] Our preparation of



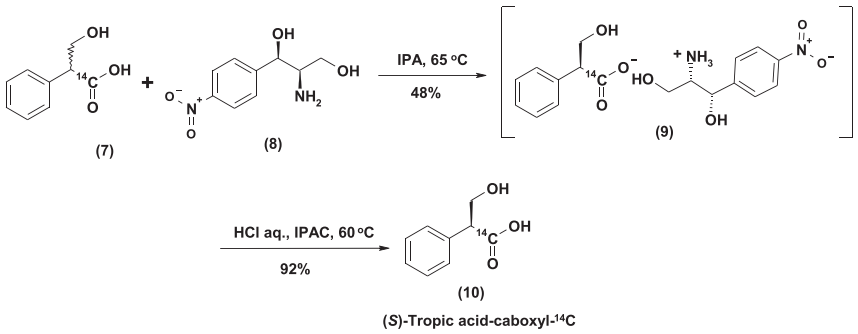
SCHEME 1 Synthesis of deuterium- and carbon-14–labeled Buscopan



SCHEME 2 Synthesis of *dl*-tropic acid-[carboxyl-^{14}C]

carbon-14–labeled racemic tropic acid provides access to both enantiomers of Buscopan, and a viable method of separating the enantiomers of tropic acid is developed. The synthesis of racemic tropic acid (Scheme 2) started from benzyl bromide, which was converted to the nitrile (**2**) in water in the presence of potassium cyanide-^{14}C and tetrabutylammonium bromide.[21,22] Hydrolysis of the nitrile group to (**3**) and esterification in ethanol afforded phenylacetic acid ethyl ester (**4**) in excellent yield. The phenyl ethyl acetate (**4**) was then reacted with ethyl formate and sodium hydride to furnish 3-hydroxy-2-phenyl-acrylic acid ethyl ester (**5**).[23,24] Reduction of the double bond was accomplished using sodium borohydride in methanol. Finally, ester hydrolysis gave *dl*-tropic acid-[carboxyl-^{14}C] (**7**).

With carbon-14–labeled racemic tropic acid in hand, a crystallization process was developed to separate the (*S*)-enantiomer or *l*-tropic acid along with a chiral high-performance liquid chromatography (HPLC) method. It is worth mentioning that enzymatic chiral resolution of tropic acid has been reported.[25–27] Different solvent systems



SCHEME 3 Separation of (*S*)-tropic acid-carboxyl-^{14}C

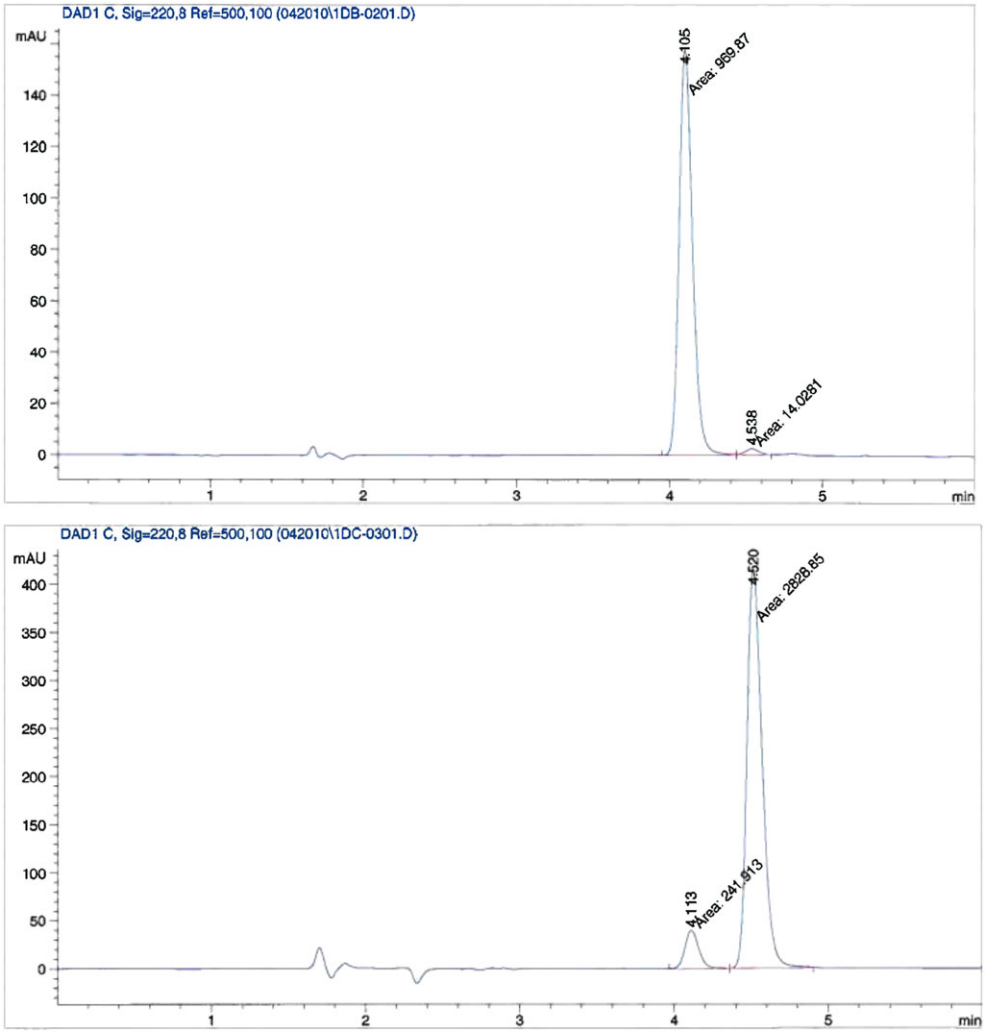
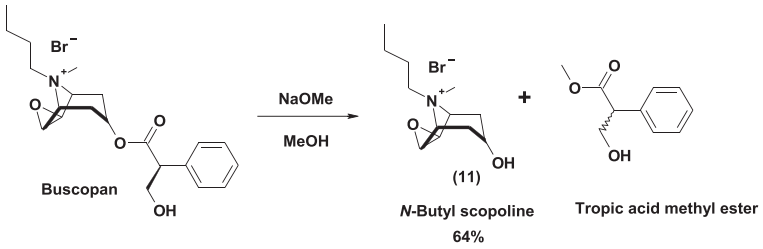


FIGURE 2 (Top) Chiral high-performance liquid chromatography of *l*-tropic acid and (bottom) *d*-tropic acid isolated from the mother liquor



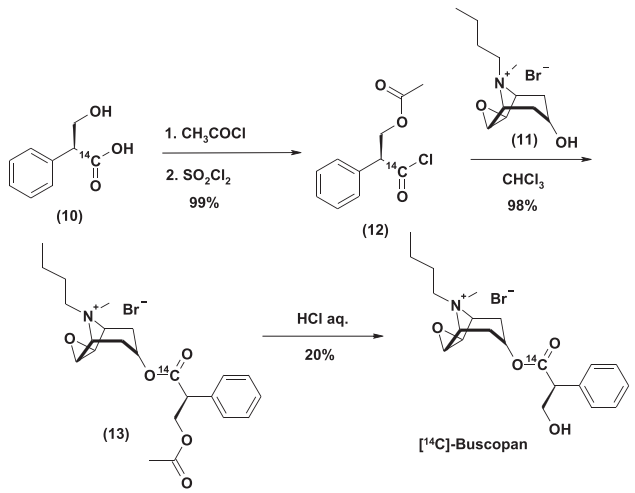
SCHEME 4 Hydrolysis of Buscopan to scopoline and tropic acid

were used to evaluate the chiral separation of racemic tropic acid using D-(−)-*threo*-2-amino-1-(4-nitrophenyl)-1,3-propanediol (**8**) as a resolving reagent.[28] In our hands, the best separation of the (*S*)-isomer from the mixture was achieved in either water or isopropanol solvent systems. Isopropanol provided better enantiopurity (approximately 97% enantiomeric excess [ee] after isopropanol wash) and with very good recovery. The (*S*)-tropic acid was then liberated from the salt (**9**) by treatment with concentrated HCl in isopropyl acetate (IPAC) in 92% yield (Scheme 3).

Chiral HPLC analysis indicated that the (*S*)-tropic acid was isolated in 97.5% ee. The mother liquor contained mainly the (*R*)-tropic acid contaminated with about 7% of (*S*)-tropic acid (Figure 2).

Only the ester bond formation between (*S*)-tropic acid and *N*-butyl scopoline (**11**) was then needed for the final assembly of carbon-14–labeled Buscopan. Chemical synthesis of the scopoline base has been reported by several groups,[29–31] but we opted for a direct 1-step synthesis of scopoline from Buscopan itself by a direct hydrolysis using sodium methoxide in methanol (Scheme 4). The tropic acid methyl ester is washed with organic solvents, and *N*-butyl scopoline bromide salt (**11**) is easily isolated with high chemical purity as a solid by a simple filtration.

(*S*)-Tropic acid was first protected as the acetate, and the carboxylic acid was converted to acyl chloride in 1 pot using acetyl chloride and then thionyl chloride, respectively[32] (Scheme 5). Reaction with *N*-butyl scopoline bromide gave the desired protected Buscopan (**13**). Removal of the acetyl protecting group led to competing ester hydrolysis of Buscopan. Nevertheless, about 8 mCi of [^{14}C]-Buscopan was obtained with a radiochemical purity of 98.5% and with more than 97% ee (Figure 3) and a specific activity of 42.4 mCi/mmol. We found that the produced tropic acid can be recycled to make carbon-14 Buscopan with little loss of optical purity.



SCHEME 5 Synthesis of [^{14}C]-Buscopan using *l*-tropic acid-[carboxyl-^{14}C]

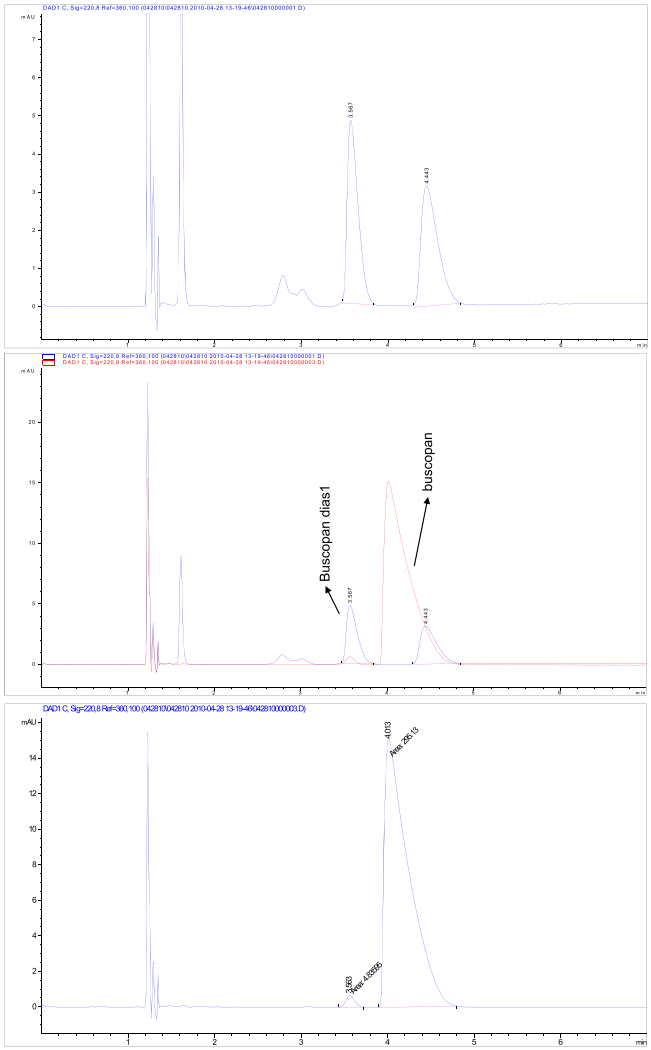


FIGURE 3 Chiral high-performance liquid chromatography of racemic Buscopan (top), [^{14}C]-Buscopan (bottom), and overlap of both chromatograms (middle)

3 | CONCLUSION

Buscopan is a drug registered in more than 40 countries worldwide and has been clinically in use for more than 50 years. It is on the list of the most important medications needed in a basic health system. The synthesis of stable isotope– and carbon-14–labeled Buscopan was accomplished using commercially available scopolamine, *n*-butyl-$^{2}H_9$-bromide and *n*-butyl-1-^{14}C-bromide, respectively. In a second synthesis of carbon-14–labeled Buscopan, carbon-14–labeled racemic tropic acid was prepared in 6 radiochemical steps in quantitative yield, and the (*S*)-tropic acid was efficiently separated from the (*R*)-enantiomer using (1*R*,2*R*)-(−)-2-amino-1-(4-nitrophenyl)-1,3-propanediol as a resolving reagent. The scopine moiety of Buscopan was obtained from the treatment of Buscopan itself with sodium methoxide. Finally, reaction of *N*-butylscopoline with the protected (*S*)-tropic acid followed by deprotection gave carbon-14 Buscopan.

4 | EXPERIMENTAL PROCEDURES

4.1 | Materials and methods

Nuclear magnetic resonance (NMR) spectra of radioactive compounds were recorded on a Bruker 500 MHz spectrometer using double encapsulated NMR tubes in deuterated dimethyl sulfoxide. ^{1}H NMR and ^{13}C NMR spectra of nonradioactive compounds were acquired using Bruker 400 MHz. Liquid scintillation counting was accomplished using a Beckman LS6500TA Liquid Scintillation Counter and UltimaGold cocktail (PerkinElmer, Boston, Massachusetts). HPLC analysis was performed on an Agilent 1200 instrument. HPLC conditions: method A; column: LiChrosorb 10RP8 (4 × 250 mm, 10 μm), mobile phase methanol (680 mL), water (0.001 N HCl, 2 g dodecyl hydrogen sulfate sodium salt in 370 mL), flow rate 2 mL/min. Method B; column: Chiralpak IA (4.6 × 250 mm), run time 6 minutes, mobile phase isocratic THF/heptane (30/70, 0.1% trifluoroacetic acid [TFA]), flow rate 1.8 mL/min, injection volume 5 μL, detection 220 nm. Method C; column: Kromasil 3-CelluCoat Rp (4.6 × 150 mm, 3 μm), temperature 40°C, detector 220 nm, flow rate 1.5 mL/min, injection volume 2 μL, run time 7.0 minutes, sample diluent: MeOH, mobile phase: A: 1% $HClO_4$ in water; B: MeCN, isocratic: 75/25 (V/V) A/B. Liquid chromatography-mass spectrometry (LCMS) was performed using a fast medium polar method: run time 2.0 minutes, gradient 95% water (0.1% TFA) and 5% MeCN (0.1% TFA) to 5% water in 1.7 minutes, hold to 2 minutes at 5% water, flow 2.5 mL/min, column: Agilent Zorbax C18 SB (4.6 mm × 30 mm, 3.5 μm). The data were acquired on a Waters Acquity Ultra Performance LC (Milford, Massachusetts). The radiochemical purity was measured using a radio-HPLC detector β-Ram model 3 (LabLogic Systems, Inc, Brandon, Florida) connected to Agilent HPLC instrument using IN-FLOW 2:1 liquid scintillation (LabLogic Systems, Inc, Brandon, Florida). Potassium cyanide-^{14}C was purchased from ViTrax (Placentia, California) *n*-Butyl-1-^{14}C bromide was purchased from Farbwerke Hoechst, Germany). *n*-Butyl-$^{2}H_9$-bromide (99.35 at.% ^{2}H) was purchased from Isotec (Miamisburg, Ohio). Scopolamine hydrobromide trihydrate, (−)-*N*-butylscopolamine bromide (Buscopan), and the rest of the reagents were purchased from Sigma-Aldrich Company (Milwaukee, Wisconsin).

5 | SYNTHESIS

5.1 | [$^{2}H_9$]-Buscopan

Scopolamine hydrobromide trihydrate (877 mg, 2.0 mmol) was added in 1 portion to a solution of Na_2CO_3 (255 mg, 2.4 mmol) in water (5 mL) at 0°C. The resulting colorless solution was stirred at room temperature for 15 minutes and then extracted with ethyl acetate (15 mL × 3). The combined extracts were dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give 0.6 g of foam. This material was dissolved in anhydrous acetonitrile (4 mL), and *n*-butyl-$^{2}H_9$ (1.75 g, 12 mmol) was added. The flask was sealed and heated to 60°C for 11 days. The solution was cooled to room temperature and concentrated under reduced pressure to give a white solid. Anhydrous ether was added (10 mL), and the resultant mixture was heated to reflux for 30 minutes. After cooling to room temperature, the solid was collected by filtration to afford 717 mg of product in 80% yield. Thin-layer chromatography (TLC) in 10% MeOH/CH_2Cl_2 using a plate that was treated with 6% w/w of NaBr in MeOH and stained with the Dragendorff reagent showed only 1 orange spot, R_f = 0.1. ^{1}H NMR (DMSO-d_6) δ: 7.22 to 7.40 (m, 5H), 5.25 (t, J = 6.2 Hz, 1H), 5.01 (t, J = 4.1 Hz, 1H), 4.21 (m, 2H), 3.91 (m, 2H), 3.80 (m, 2H), 3.11 (s, 2H), 2.99 (s, 3H), 2.51 to 2.70 (m, 2H), 1.90 (d, J = 16.1 Hz, 1H), 1.62 (d, J = 16.1 Hz, 1H). ^{13}C NMR (DMSO-d_6) δ: 170.87, 136.05, 128.52, 128.19, 128.09, 127.42, 62.89, 62.83, 62.68, 62.14, 54.09, 53.68, 53.50, 40.23, 28.09, 23.4 (m), 19.2 (m), 14.1 (m). LCMS R_t = 0.89 minutes (369.39, 100%). LC/ESI/MS: 368.77, 369.40 (100%), 370.31, 371.34, 372.34. LC/MS/MS: 221.26, 203.19 (100%), 157.08, 138.05.

5.2 | Synthesis of [^{14}C]-Buscopan

5.2.1 | Using *n*-butyl-1-^{14}C bromide

Scopolamine hydrobromide (1.76 g, 4.0 mmol) was dissolved in water (3 mL), and the base was precipitated by the addition of concentrated ammonia (3 mL, 7M in MeOH) and extracted with methylene chloride (10 mL × 3). The combined extracts were dried over Na_2SO_4, filtered, and concentrated to give 1.2 g of scopolamine base. This base was dissolved in

acetonitrile (10 mL) and cooled in liquid nitrogen (−192°C) under vacuum. *n*-Butyl-1-^{14}C bromide (370 mg, 2.7 mmol, 84 mCi, 3.12 GBq) was distilled from an ampoule with a breakable neck into the ampoule containing the scopolamine solution, and the latter ampoule was sealed. The ampoule was stored at 55°C for 21 days. It was then opened, chloroform (3 mL) was added, and the solution was chromatographed on a silica gel column (2-cm diameter, 20 g of silica gel, 0.05-0.2 mm) and eluted with up to 50% methanol in chloroform to remove unreacted scopolamine. The fractions containing the desired product were combined and concentrated to afford 625 mg of white crystals corresponding to 52% yield (based on *n*-butyl-^{14}C bromide). A total of 45 mCi of material with a specific activity of 32 mCi/mmol or 72 μCi/mg was obtained. TLC, solvent system *n*-butanol/formic acid/water 75:15:10 shows 1 spot coeluted with unlabeled Buscopan. Melting point 139°C to 140°C. HPLC method A: R_t = 6.41 minutes (99% chemical purity, with about 0.1% scopolamine) and radiochemical purity of 99%.

5.2.2 | Using (*S*)-tropic acid-carboxyl-^{14}C

5.2.2.1 | Benzonitrile-^{14}C (2)

Potassium cyanide-^{14}C (200 mCi, 45 mCi/mmol) and tetrabutyl ammonium bromide (60 mg) were added in a 20-mL screw cap vial. Methylene chloride (10 mL) and water (2.5 mL) were added followed by benzyl bromide (613 μL, 5.0 mmol), and the mixture was stirred vigorously for 48 hours. Stirring was stopped and the organic phase was syringed out. The aqueous layer was extracted with CH_2Cl_2 (6 mL × 2). The combined extracts were concentrated *in vacuo*, and the residue was purified by Combiflash using a 40-g disposable silica gel cartridge and up to 1% EtOAc:CH_2Cl_2. The tubes containing the product (TLC: 10% EtOAc:hexanes) were combined and concentrated *in vacuo* to give 596 mg of colorless oil in quantitative yield (200 mCi).

5.2.2.2 | Phenyl acetic acid-1-^{14}C (3)

A mixture of (**2**) (596 mg, 5.0 mmol) and NaOH (4.0 N, 4 mL) and water (6.0 mL) was heated to 105°C for 12 hours. After cooling in an ice bath, concentrated HCl (6 N, 3 mL) was added to give a white solid, which was filtered and washed with cold water. The aqueous layer was extracted with CH_2Cl_2 (6 mL × 3) and combined with the solid. The mixture was concentrated *in vacuo* to dryness to give 690 mg of the product as a white solid in excellent yield (198 mCi).

5.2.2.3 | Ethylphenyl acetate-1-^{14}C (4)

A solution of the crude (**3**) (650 mg, 4.7 mmol) in absolute ethanol (35 mL) was heated to reflux for 14 hours with 0.2 mL of concentrated H_2SO_4. After cooling to room temperature, the solution was concentrated *in vacuo*, and the residue was dissolved in CH_2Cl_2 (20 mL) and stirred. A saturated solution of $NaHCO_3$ was added slowly until no gas evolution was observed (35 mL). The organic layer was removed, and the aqueous layer was extracted with CH_2Cl_2 (40 mL × 2). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated to afford 0.83 g of yellow oil in quantitative yield (196 mCi).

5.2.2.4 | 3-Hydroxy-2-phenyl-acrylic acid ethyl ester-1-^{14}C (5)

To a solution of (**4**) (771 mg, 4.64 mmol) in ethyl formate (10 mL, 147 mmol) was added sodium hydride (0.8 g, 60% oil dispersion, 20.0 mmol) portion wise in 20 minutes at room temperature under nitrogen. An exotherm reaction and gas evolution were observed with each addition of NaH. The mixture was stirred at room temperature overnight, then poured into a solution of 1.0 N aq. HCl (40 mL), and extracted with ether (60 mL × 2). The combined ether extracts were dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give 1.42 g of colorless oil. Flash chromatography purification using a 40-g disposable silica gel column and up to 20% EtOAc in Hexanes gave 727 mg (157 mCi) of a colorless oil in 80% yield.

5.2.2.5 | 3-Hydroxy-2-phenyl-propionic acid ethyl ester-1-^{14}C (6)

A solution of the enol (**5**) (727 mg, 3.74 mmol) in anhydrous methanol (80 mL) was stirred in an ice bath. Sodium borohydride (183 mg, 4.8 mmol) was added in small portions in a 20-minute period. Gas evolution was observed with each addition. The resulting solution was stirred for 6 hours at 0°C and then concentrated *in vacuo*. The residue was treated with water (20 mL) and extracted with ether (40 mL × 3). The combined ether extracts were dried over Na_2SO_4, filtered, and concentrated *in vacuo* to give 732 mg (156 mCi) of colorless oil in 99% yield.

5.2.2.6 | 3-Hydroxy-2-phenyl-propionic acid-1-^{14}C (*dl*-tropic acid-carboxyl-^{14}C) (7)

To a solution of (**6**) (732 mg, 3.73 mmol) in THF:MeOH (1:1, 12 mL) was added a solution of LiOH. H_2O (198.5 mg, 5.25 mmol) in water (3 mL) was added at ambient temperature. The mixture was stirred for 14 hours and then concentrated *in vacuo*. The residue was treated with aqueous 1 N HCl (8 mL) and extracted with EtOAc (20 mL × 3). The combined EtOAc extracts were filtered through a phase separator column and concentrated *in vacuo* to give 670 mg of viscous oil. Flash chromatography purification using a 40-g disposable silica gel column and eluting with up to 30% MeOH/CH_2Cl_2 gave 441 mg (105 mCi) of a white solid in 67% yield.

5.2.2.7 | (1*R*,2*R*)-2-(λ^4-Azanyl)-1-(4-nitrophenyl)propane-1,3-diol, (*S*)-3-hydroxy-2-phenylpropanoate-2-^{14}C salt (9)

A mixture of racemic tropic acid (**7**) (440 mg, 2.65 mmol) and (**8**) (275 mg, 1.28 mmol) in isopropanol was heated to 65°C and stirred for 1 hour. The mixture was then cooled to room temperature in 4 hours, filtered, and washed with isopropanol (2.2 mL × 2). The white solid was dried on the

filter funnel for 2 hours and then transferred to a flask to give 400 mg (50 mCi) of a white solid in 48% yield.

5.2.2.8 | (*S*)-3-Hydroxy-2-phenyl-propionic-1-^{14}C acid (*l*-tropic acid-carboxyl-^{14}C) (10)

To a mixture of the salt (**9**) (400 mg, 1.05 mmol) in IPAC (6 g) was added concentrated aqueous HCl (112 µL, 12 N). The mixture was heated to 60°C and stirred for 30 minutes and then cooled to room temperature for more than 2 hours. The mixture was filtered and washed twice with IPAC (2.5 mL × 2), and the IPAC solution was concentrated *in vacuo* to give 163 mg of a white solid in 92% yield. Total activity = 45.7 mCi and specific activity = 45.7 mCi/mmol. HPLC method B: R_t = 4.1 minutes, 97.2% ee, 98.6% radiochemical purity.

5.3 | Hydrolysis of Buscopan

5.3.1 | (2*R*,4*S*)-9-Butyl-7-hydroxy-9-methyl-3-oxa-9-azatricyclo [3.3.1.0^{2,4}]nonan-9-ium bromide, *N*-butyl scopoline (11)

To a suspension of Buscopan (6.61 g, 15 mmol) in methanol (22 mL) was added a solution of sodium methoxide (1.3 mL, 0.65 mmol, 0.5M in MeOH), and the mixture was heated to 40°C. The resulting colorless solution was stirred at this temperature for 3 hours. LCMS showed only 40% conversion. A solution of sodium methoxide (0.43 mL, 0.5M in MeOH) was added, and stirring was continued for another 5 hours. HPLC showed about 60% conversion at 254 nm. A solution of acetic acid (0.1 mL in 1.0 mL of isopropyl alcohol) was added and stirred for 10 minutes. Then, IPAC (77 mL) was added to give a suspension that was stirred at 40°C for 30 minutes. The reaction was cooled to room temperature and stirred overnight. Stirring was stopped, and the mixture was left to settle. The mixture was filtered, and the solid was washed with *i*-PrOAc (80 mL) and dried *in vacuo* to give 2.81 g of a white solid in 64% yield. LCMS: R_t = 0.21 minutes, M^+–Br = 212.6 (100%). ^{1}H NMR (DMSO-d_6) δ: 5.07 (t, J = 4.1 Hz, 1H), 4.15 (m, 2H), 3.98 (m, 1H), 3.55 (m, 2H), 3.28 (s, 2H), 2.97 (s, 3H), 2.51 (m, 2H), 2.46 (t, J = 6.1 Hz, 1H), 1.79 (d, J = 16.1 Hz, 1H), 1.65 (m, 2H), 1.26 (m, 2H), 0.91 (t, J = 8.1 Hz, 3H).

5.3.2 | (*S*)-3-Chloro-3-oxo-2-phenylpropyl-3-^{14}C acetate (12)

A mixture of (*S*)-tropic acid (**10**) (163 mg, 0.97 mmol) and acetyl chloride (5 mL, 69.6 mmol) was heated at 58°C for 2 hours. The resulting colorless oil was concentrated to remove excess acetyl chloride to give 243 mg of a residue. To this residue, thionyl chloride was added (5 mL, 58.82 mmol), and the solution was heated at 60°C for 2.5 hours. The solution was concentrated *in vacuo*, and the residue was diluted with cyclohexane (5 mL) and concentrated. This procedure was repeated 3 times to evaporate excess thionyl chloride. The yellowish residue (220 mg, 99% yield) was used as is in the next step.

5.3.3 | (2*R*,4*S*)-7-((((*S*)-3-Acetoxy-2-phenylpropanoyl-1-^{14}C) oxy)-9-butyl-9-methyl-3-oxa-9-azatricyclo[3.3.1.0^{2,4}]nonan-9-ium bromide (13)

A solution of (**12**) (220 mg, 0.97 mmol) in anhydrous chloroform (6 mL) was treated with *N*-butylscopoline bromide (**11**) (200 mg, 0.7 mmol) and heated to 40°C for 5 hours. HPLC of the mixture showed the desired product. The reaction solution was cooled to room temperature and concentrated *in vacuo* to give 460 mg of a residue, which was used as is in the next step.

5.3.4 | [^{14}C]-Buscopan

A solution of the acetate derivative (**13**) (450 mg, 0.93 mmol) was treated with water (2 mL) and concentrated HCl (0.5 mL, 6 mmol). The mixture was stirred overnight. HPLC analysis showed that the starting material was converted to both the tropic acid and Buscopan. The reaction was extracted with ether, and the aqueous layer was frozen in liquid nitrogen and then lyophilized to give 310 mg of viscous oil. This residue was dried under reduced pressure and then dissolved in acetonitrile (0.2 mL), and anhydrous ether (3 mL) was added while heating. The cloudy mixture was seeded with few crystals of unlabeled Buscopan and left to crystallize at 0°C for 2 hours. The solution was pipetted out and concentrated *in vacuo* to give 112 mg (30 mCi) of crude (*S*)-tropic acid (76% yield). The solid, carbon-14 Buscopan, was dried to give 252 mg of a white solid in 20% yield. Total activity = 7.83 mCi. HPLC method C: R_t = 4.44 minutes, 98.5% radiochemical purity, and 97.1% ee with a specific activity of 42.4 mCi/mmol (117.7 µCi/mg); R_t of (*R*)-Buscopan 3.57 minutes. ^{1}H NMR (DMSO-d_6) δ: 7.36 (m, 5H), 5.09 (t, J = 4.1 Hz, 1H), 5.03 (t, J = 6.1 Hz, 1H), 4.18 (m, 2H), 3.91 to 4.01 (m, 2H), 3.75 to 3.82 (m, 2H), 3.71 (m, 1H), 3.51 to 3.58 (m, 2H), 3.04 (s, 3H), 2.55 to 2.71 (m, 2H), 1.91 (d, J = 16.1 Hz, 1H), 1.77 (d, J = 16.1 Hz, 1H), 1.65 (m, 2H), 1.24 (m, 2H), 0.90 (t, J = 8.1 Hz, 3H).

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How to cite this article: Latli, B., Stiasni, M., Hrapchak, M., Li, Z., Grinberg, N., Lee, H., Busacca, C. A., and Senanayake, C. H. (2016) Buscopan labeled with carbon-14 and deuterium. *J Label Compd Radiopharm*, doi: 10.1002/jlcr.3463