Received: 24 March 2016 | Revised: 7 July 2016 | Accepted: 22 July 2016

DOI 10.1002/jlcr.3433

WILEY Journal of Labelled Compounds and Radiopharmaceuticals

SPECIAL ISSUE ARTICLE

The syntheses of isotopically labelled CB-1 antagonists for the treatment of obesity

Scott B. Tran[1]* | Brad D. Maxwell[1] | Richard Burrell[2] | Samuel J. Bonacorsi Jr.[1]

[1] Discovery Chemistry Platforms – Radiochemistry, Bristol-Myers Squibb, Inc, P.O. Box 4000, Princeton, NJ 08543, USA

[2] Discovery Chemistry Platforms – Radiochemistry, Bristol-Myers Squibb, Inc, P.O. Box 5100, Wallingford, CT 06492, USA

Correspondence
Scott Tran, Radiochemical Synthesis, Bristol-Myers Squibb, P.O. Box 4000, Princeton, NJ 08543, USA.
Email: scott.tran@bms.com

BMS-725519, BMS-811064, and BMS-812204 are potent and selective central cannabinoid receptor antagonists that have been investigated for the treatment of human obesity. To further understand their biotransformation profiles, radiolabelled and stable-labelled products were required. This paper describes the utility of [^{14}C]1,1-carbonyldiimidazole as a radiolabelling reagent for the syntheses of carbonyl-labelled [^{14}C]BMS-725519, [^{14}C]BMS-811064, and [^{14}C]BMS-812204. The syntheses of stable-labelled [$^{13}C_6$]BMS-725519 and [$^{13}CD_3{}^{13}CD_2$]BMS-812204 synthesized from of [$^{13}C_6$]4-chloroacetophenone and [$^{13}CD_3{}^{13}CD_2$]iodoethane, respectively, are also described.

KEYWORDS

carbon-13, CB-1 antagonists, deuterium, synthesis

1 | INTRODUCTION

Obesity is now the most common nutritional disorder in western industrialized countries.[1] The central cannabinoid (CB-1) receptors are believed to play a role in controlling food consumption that contributes to weight gain.[2,3] Understanding the connectivity between CB-1 and weight gain has important practical implications because blocking the CB-1 receptor was thought to offer a promising therapeutic approach to treating obesity.[4] From Bristol-Myers Squibb Discovery Chemistry efforts for the CB-1 program, BMS-725519,[5] BMS-811064, and BMS-812204[6,7] emerged as potent and selective CB-1 antagonists. They work by blocking endogenous cannabinoids binding to neuronal CB-1 receptors. To further the development of these compounds, radiolabelled and stable-labelled products of these drug candidates were required for use in metabolic profiling, protein covalent binding studies, hepatocyte cell transport studies, and LC-MS quantitation and qualification analyses. We envisioned the use of [^{14}C]1,1′-carbonyldiimidazole, ([^{14}C]CDI),[8,9] as a readily available labelled reagent to incorporate the carbon-14 label. The syntheses of stable-labelled [$^{13}C_6$]BMS-725519 and [$^{13}CD_3{}^{13}CD_2$]BMS-812204 were synthesized from [$^{13}C_6$]4-chloroacetophenone and [$^{13}CD_3{}^{13}CD_2$]iodoethane, respectively. We report herein details of the preparation and characterizations of the isotopically labelled molecules shown in Figure 1.

2 | RESULTS AND DISCUSSION

[^{14}C]CDI[8,9] offers great advantages in the syntheses of [^{14}C]carbonyl compounds because of its similar reactivity to phosgene without the associated stability and safety issues. It is highly recommended to use freshly prepared and dry [^{14}C]CDI to maximize yields of desired products. It is also important to check the purity of [^{14}C]CDI as it decomposes upon storage over time, even at −78°C. High-performance liquid chromatography (HPLC) analysis of [^{14}C]CDI after 5 years of storage showed two main radioactive peaks in which the larger peak was [^{14}C]CDI, at 83.1%. The radiochemical purity analysis result, however, was deceiving, because the chemical purity of the reagent as measured by proton nuclear magnetic resonance (^{1}H NMR) spectroscopy showed the material to be only 23% with the majority of the product being unlabelled chemical degradation products that did not interfere with the carbonylation reaction.

The synthesis of [^{14}C]BMS-725519 was achieved via a ring cyclization of 1.1 eq. of diluted isotopically labelled [^{14}C]CDI with 5-(4-chlorophenyl)-3-hydrazinyl-4-(pyridin-4-yl)pyridazine, **1**, to give cyclized intermediate **2** in a

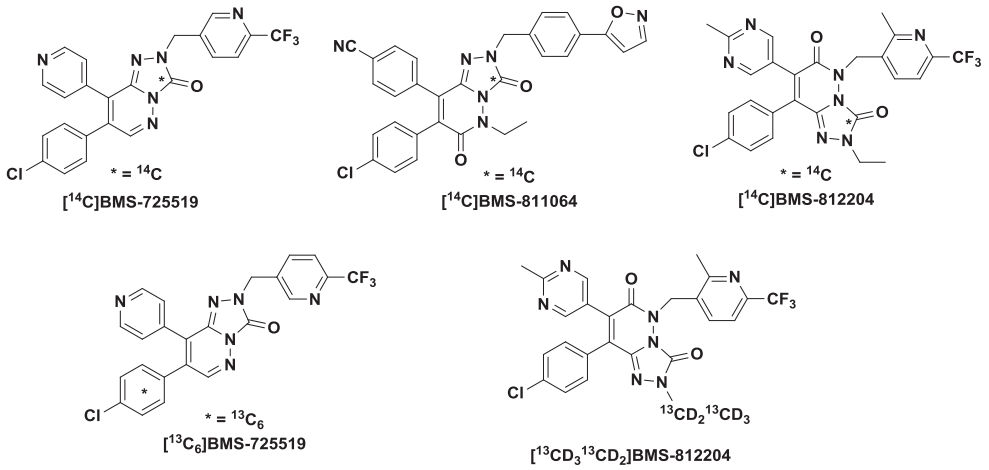


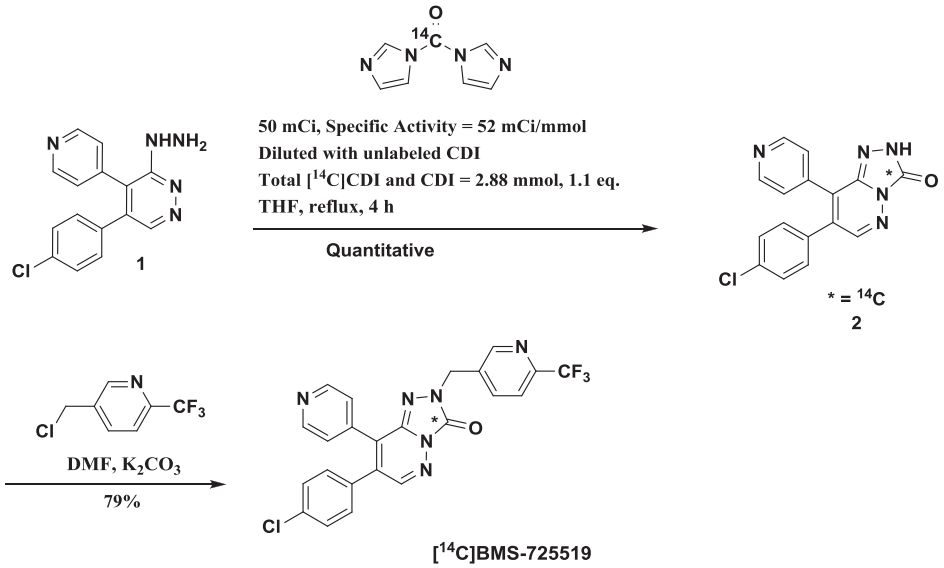
FIGURE 1 Isotopically labelled CB-1 receptor antagonists

quantitative yield. Product **2** was alkylated with 5-(chloromethyl)-2-(trifluoromethyl)pyridine to give the desired product [^{14}C]BMS-725519, in 79% overall yield at a specific activity of 19.1 mCi/mmol (39.6 μCi/mg) in 99.3% radiochemical purity and at 99.7% chemically purity as shown in Scheme 1.

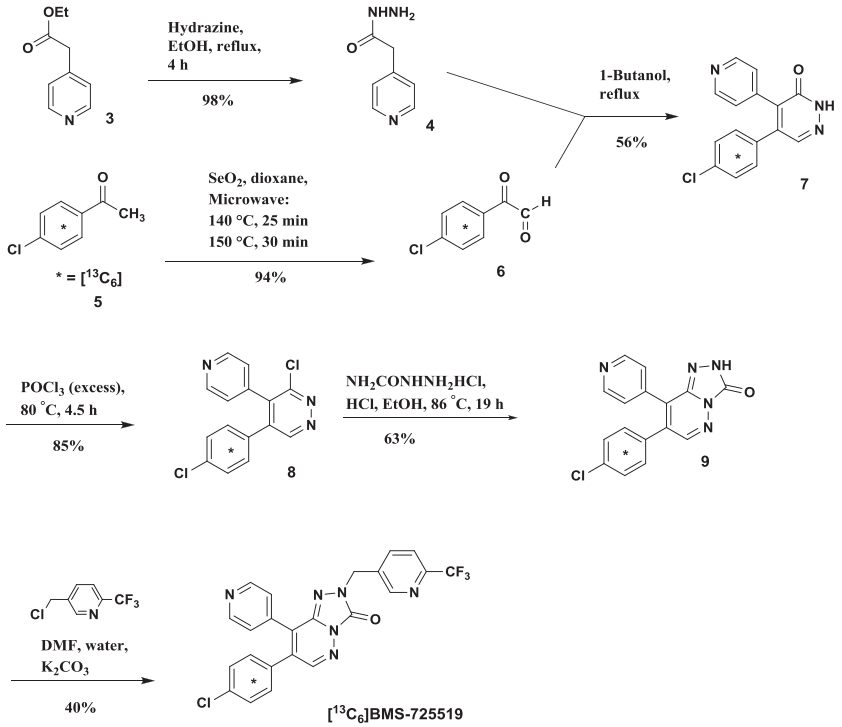
Stable-labelled [$^{13}C_6$]BMS-725519 was prepared by reacting **3** with hydrazine in ethanol to give product **4** as shown in Scheme 2. Oxidation of [$^{13}C_6$]4-chloroacetophenone, **5**, with selenium dioxide in 1,4-dioxane with heating by microwave gave compound **6** in 94% yield. Compounds **4** and **6** were cyclized to form [$^{13}C_6$]5-(4-chlorophenyl)-4-(pyridin-4-yl)pyridazin-3(2H)-one, **7** in 56% yield. Chlorination of **7** with phosphorus oxychloride followed by ring cyclization with hydrazinecarboxamide and alkylation with 5-(chloromethyl)-2-(trifluoromethyl) pyridine gave the desired [$^{13}C_6$]BMS-725519 in 21% overall yield and 98.9% chemical purity. The mass spectroscopic isotopic distribution of the product was 98% of the M + 6 labelled material, 2% of the M + 2 labelled product, and an undetectable level of the unlabelled product.

[^{14}C]BMS-811064 was formed by reacting hydrazine **10** with [^{14}C]CDI to form cyclic intermediate **11** in 80% yield. Alkylation of **11** with 5-(4-(bromomethyl)phenyl)isoxazole in DMF gave 16.4 mCi of the desired compound in an overall yield of 32.8% as shown in Scheme 3. [^{14}C]BMS-811064 was determined to be 99.7% chemically pure, 99.9% radiochemically pure, and had a specific activity of 24.1 mCi/mmol (42.8 μCi/mg).

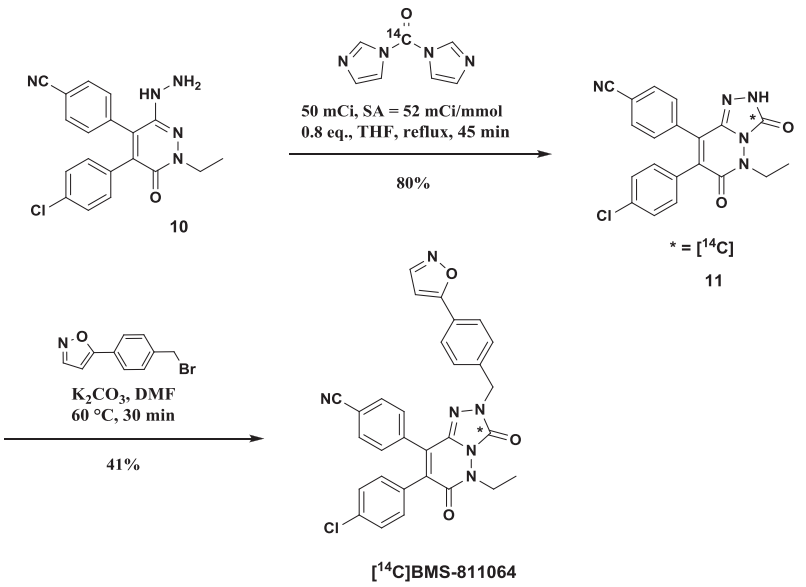
[^{14}C]BMS-812204 was synthesized in a similar way as [^{14}C]BMS-725519. Boc-protected **12** received from Bristol-Myers Squibb Discovery Chemistry was deprotected with 30% trifluroacetic acid in dichloromethane to give compound **13**. Compound **13** was not isolated, but was instead directly reacted with [^{14}C]CDI followed by ethyl alkylation with iodoethane to give compound **15** as a TFA salt after purification by preparative HPLC as shown in Scheme 4. The TFA salt was converted to its freebase form with 10% potassium carbonate to give 2.0 mCi of [^{14}C]



SCHEME 1 Synthesis of [^{14}C]BMS-725519



SCHEME 2 Synthesis of [$^{13}C_6$]BMS-725519



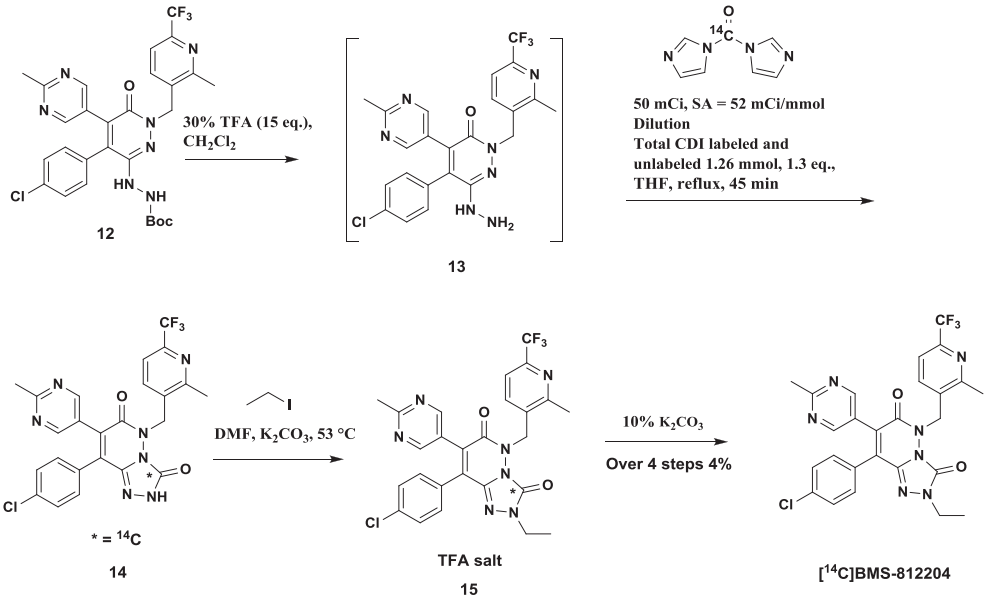
SCHEME 3 Synthesis of [^{14}C]BMS-811064

BMS-812204 with a radiochemical purity of 99.9%, a chemical purity of 98.7%, and with a specific activity of 34.1 mCi/mmol (59.1 μCi/mg).

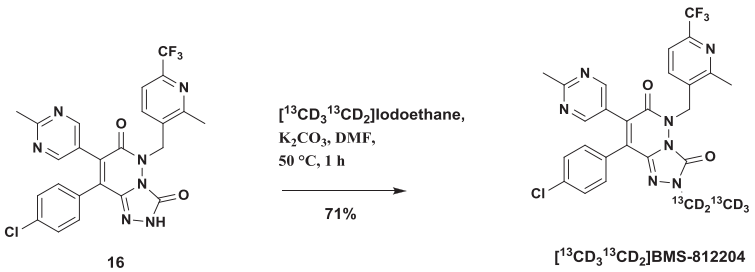
The synthesis of [$^{13}CD_3{}^{13}CD_2$]BMS-812204 was completed by alkylating the late-stage intermediate, compound **16,** with [$^{13}CD_3{}^{13}CD_2$]iodoethane as shown in Scheme 5. After aqueous work up and flash chromatography on silica gel, the desired product, [$^{13}CD_3{}^{13}CD_2$]BMS-812204, was isolated in 71% yield. The product was 99.0% chemically pure. The mass spectroscopic isotopic distribution showed that the product was >98% of the M + 7 labelled material and had an undetectable level of the unlabelled product.

3 | CONCLUSIONS

[^{14}C]1,1′Carbonyldiimidazole was used as the radiolabel reagent in the labelled carbonyl syntheses. This approach allowed for the rapid incorporation of the carbon-14–label late in the syntheses of three structurally diverse CB-1 candidates to be able to understand their Administration, Distribution,



SCHEME 4 Synthesis of [^{14}C]BMS-812204



SCHEME 5 Synthesis of [$^{13}CD_3{}^{13}CD_2$]BMS-812204

Metabolism, Excretion (ADME) properties in animals. They were also used for *in vitro* protein covalent binding and cell transporter studies. Stable-labelled [$^{13}C_6$]BMS-725519 and [$^{13}CD_2{}^{13}CD_3$]BMS-812204 were synthesized for use as mass spectrometry internal standards and showed undetectable levels of the unlabelled product in mass isotopic distributions.

4 | EXPERIMENTAL

4.1 | Materials and methods

Some of the experimental procedures were obtained from Bristol-Myers Squibb's Discovery Chemistry (Pennington, NJ) or Chemical Synthesis Departments (Princeton, NJ). These procedures were optimized for use with labelled reagents. Intermediates and authentic standards were obtained from either Bristol-Myers Squibb's Chemical Synthesis Department or Discovery Chemistry. Reactions were completed under an inert atmosphere of nitrogen or argon and stirred magnetically. [^{14}C]CDI (52 mCi/mmol) was obtained from ViTrax (Placentia, CA). [$^{13}C_6$]4-Chloroacetophenone and [$^{13}CD_3{}^{13}CD_2$]iodoethane were purchased from Isotec (Miamisburg, OH). Radioactivity was measured with either a Wallac (Waltham, MA) model 1409 or a PerkinElmer (Waltham, MA) Tri-Carb model 2900TR liquid scintillation counter. Microwave reactions were completed using a CEM Discover microwave system (Matthews, NC) with the power setting at 200 W. Mass spectra were obtained with a Finnigan TSQ or a Finnigan LCQ mass spectrometer (Thermo Fisher Scientific Inc. Waltham, MA). Proton nuclear magnetic resonance spectra were recorded on a Bruker (Billerica, MA) Advance Ultrashield 300 MHz or 400 MHz or a JEOL (Tokyo, Japan) EC+ 500 MHz spectrometer. For stable isotope-labelled products, only carbon-13–enriched positions are reported for carbon nuclear magnetic resonance (^{13}C NMR) characterization purposes. Chemical purities were determined by HPLC using either a Shimadzu (Columbia, MD) SCL-10A VP/UV-1 detector or a Varian (Santa Clara, CA) Prostar 330 PDA detector. Radiochemical purities were measured with a LabLogic (Brandon, FL) β-Ram radiometric flow detector with a 0.5 mL flow cell. Thin-layer chromatography (TLC) was performed on EMD (Darmstadt, Germany) 60 F_{254} silica gel plates. Flash chromatography was conducted on Biotage (Uppsala, Sweden) KP-Sil silica gel. Radiolabelled and stable isotope-labelled products were compared with authentic standards when possible. All reagents and solvents were American Chemical

Society grade or better. Specific activities were determined gravimetrically. The HPLC method described below was used for in process and final product analyses. Analytical HPLC method: YMC (Shimogyo-ku, Kyoto, Japan) ODS-AQ C18, 3 μm, 4.6 × 150 mm, ultraviolet detection at 230 nm. Mobile phase A: 0.1% TFA in water and mobile phase B: 0.1% TFA in acetonitrile. Gradient: 0 minute 5% B, 8 minutes 95% B, 10 minutes 95% B, 12 minutes 5% B, 15 minutes 5% B, and flow rate = 1.0 mL/minute. Preparative HPLC method: Phenomenex (Torrence, CA) Luna C18, 5 μm, 21.2 mm × 250 mm, ultraviolet detection at 254 nm. Mobile phase A: 0.1% TFA in water and mobile phase B: 0.1% TFA in acetonitrile. Gradient: 0 minute 40% B, 18 minutes 95% B, 22 minutes 95% B, 28 minutes 40% B, and flow rate = 12.0 mL/minute.

4.2 | Synthesis of [^{14}C]BMS-725519

4.2.1 | [^{14}C]7-(4-Chlorophenyl)-8-(pyridin-4-yl)-[1,2,4]triazolo[4,3-b]pyridazin-3(2H)-one, 2

[^{14}C]CDI (0.279 g, 0.96 mmol, 52 mCi/mmol, 50 mCi), carbonyldiimidazole (0.311 g, 1.92 mmol) (total carbonyldiimidazole labelled + unlabelled 2.88 mmol, 1.1 eq), compound **1** (0.78 g, 2.63 mmol), and THF (12 mL) were added to a dry flask. The yellow solution was heated to 65°C and stirred for 4 hours under argon. During the reaction, a yellow precipitate formed. The reaction was cooled to room temperature and the THF was removed under reduced pressure. To the yellow solid was added water (30 mL) and the suspension was transferred to a centrifuge tube. The suspension was centrifuged and the water was decanted. The water wash step was repeated. Analysis of the water washes by HPLC showed that they contained some of the desired product. The water washes were combined and extracted with dichloromethane (DCM) (4 × 20 mL). The DCM extracts were combined and concentrated to give a yellow solid. The yellow solid from the DCM extractions was combined with the yellow solid from the centrifugation and was vacuum dried for 16 hours to give 0.832 g of [^{14}C]7-(4-chlorophenyl)-8-(pyridin-4-yl)-[1,2,4]triazolo[4,3-b]pyridazin-3(2H)-one, **2** (50 mCi, quantitative yield). The product was analyzed by HPLC and found to be at 99.5% chemically pure with a retention time of 10.3 minutes and 99.2% radiochemically pure. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.26 to 7.31 (m, 2H), 7.31 to 7.35 (m, 2H), 7.41 to 7.47 (m, 2H), 8.42 (s, 1H), 8.55 to 8.63 (m, 2H), and 12.89 (br. s, 1H). The specific activity was determined to be 59.6 μCi/mg (19.3 mCi/mmol).

4.2.2 | [^{14}C]7-(4-Chlorophenyl)-8-(pyridin-4-yl)-2-((6-(trifluoromethyl)pyridin-3-yl)methyl)-[1,2,4]triazolo[4,3-b]pyridazin-3(2H)-one, [^{14}C]BMS-725519

[^{14}C]7-(4-Chlorophenyl)-8-(pyridin-4-yl)-[1,2,4]triazolo[4,3-b]pyridazin-3(2H)-one (**2**, 0.26 g, 0.80 mmol, 15.5 mCi), 5-(chloromethyl)-2-(trifluoromethyl)pyridine (0.19 g, 0.96 mmol), potassium carbonate (0.22 g, 1.6 mmol), and DMF (4 mL) were added to a dry flask. The yellow solution was heated to 65°C and stirred for 2.5 hours under argon. The reaction was cooled to room temperature, diluted with water (30 mL), and was extracted with EtOAc (4 × 20 mL). The pooled organic extracts were concentrated to give a dark brown oil. To the oil was added *tert*-butyl methyl ether (50 mL) and then it was concentrated. The crude product was vacuum dried for 16 hours to give a dark brown solid. The crude solid was purified by flash chromatography on silica gel and was eluted with EtOAc. Pure fractions analyzed by TLC (40% EtOAc/60% hexane, R_f = 0.17) were combined, concentrated, and vacuum dried for 18 hours to give 0.31 g of [^{14}C]BMS-725519 as a bright yellow solid (12.1 mCi, 79% radiochemical yield). The product was analyzed by HPLC to be 99.7% chemically pure with a retention time of 12.5 minutes and was 99.3% radiochemically pure. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 5.28 (s, 2H), 7.07 (*d*, *J* = 8.56 Hz, 2H), 7.20 to 7.26 (m, 2H), 7.33 (*d*, *J* = 8.56 Hz, 2H), 7.66 (*d*, *J* = 8.06 Hz, 1H), 7.94 (*d*, *J* = 8.06 Hz, 1H), 8.21 (s, 1H), 8.64 (br. s, 2H), and 8.78 (s, 1H). The specific activity was determined to be 39.6 μCi/mg (19.1 mCi/mmol).

4.3 | Synthesis of [$^{13}C_6$]BMS-725519

4.3.1 | 2-(Pyridin-4-yl)acetohydrazide, 4

To a 0.5 L flask was added ethyl 2-(pyridin-4-yl)acetate (**3**, 10 g, 60.5 mmol) and EtOH (104 mL) followed by the addition of hydrazine hydrate (9.16 g, 183 mmol, N_2H_4 65%). The solution was heated to 90°C for 4.5 hours. The progress of the reaction was monitored by TLC (5% MeOH/CH_2Cl_2, R_f (starting material) = 0.7, R_f (product) = 0.0). The reaction was cooled to room temperature and the solution was concentrated to dryness. The residue was further dried under high vacuum for 2 hours. The crude product was purified by trituration with hexane (0.4 L) and then collected by filtration to give **4**, 9.0 g of a white solid (98% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.26 (s, 1H), 8.46 to 8.45 (m, 2H), 7.25 to 7.24 (m, 2H), 4.23 (s, 2H), and 3.38 (s, 2H).

4.3.2 | [$^{13}C_6$]2-(4-Chlorophenyl)-2-oxoacetaldehyde, 6

[$^{13}C_6$-Phenyl]4-chloroacetophenone (**5**, 0.5 g, 3.11 mmol), SeO_2 (518.2 mg, 4.67 mmol), and dioxane (1.25 mL) were added to a microwave tube. The reaction was heated in a microwave oven at 140°C for 25 minutes and at 150°C for another 30 minutes while monitoring by HPLC (product retention time = 9.70 minutes). The mixture was extracted with EtOAc (3 × 20 mL). The combined EtOAc extracts were washed with water (20 mL), brine (10 mL), dried over $MgSO_4$, filtered, and concentrated to dryness to give 510 mg of a white product (94% crude yield). The crude product was used in the next reaction without further purification. ^{13}C NMR (126 MHz, DMSO-d_6) δ 140.94 to 137.30 (m), 132.40 to 130.23 (m), and 129.95 to 127.21 (m).

4.3.3 | [$^{13}C_6$]5-(4-Chlorophenyl)-4-(pyridin-4-yl)pyridazin-3(2H)-one, 7

[$^{13}C_6$]2-(4-Chlorophenyl)-2-oxoacetaldehyde (**6**, 455 mg, 2.6 mmol), 2-(pyridin-4-yl)acetohydrazide (**4**, 394 mg, 2.6 mmol), and 1-butanol (13.0 mL) were heated to 123°C for 2.5 hours. The reaction was monitored by TLC (5% MeOH/EtOAc, product R_f = 0.5) and by HPLC (product retention time = 6.30 minutes). The solution was evaporated to dryness and the residue dissolved in DCM (30 mL), washed with water (7 mL), brine (5 mL), dried over $MgSO_4$, filtered, and concentrated to dryness. The crude product was purified by silica gel flash chromatography eluting with 0% to 5% MeOH/DCM to afford 494 mg of a white solid (56% yield). HPLC analysis at 254 nm showed the product to be 85% pure. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.43 (s, 1H), 8.58 to 8.39 (m, 2H), 8.02 (*d*, *J* = 1.6 Hz, 1H), 7.56 (*d*, *J* = 3.3 Hz, 1H), 7.39 (*d*, *J* = 8.2 Hz, 1H), 7.23 (*d*, *J* = 4.9 Hz, 1H), 7.19 to 7.13 (m, 2H), and 7.11 to 7.03 (m, 1H).

4.3.4 | [$^{13}C_6$]3-Chloro-5-(4-chlorophenyl)-4-(pyridin-4-yl)pyridazine, 8

To a dry flask was added [$^{13}C_6$]5-(4-chlorophenyl)-4-(pyridin-4-yl)pyridazin-3(2H)-one, **7** (306 mg, 1.1 mmol), and $POCl_3$ (1.80 mL, 19.3 mmol). The mixture was heated to 80°C for 4.5 hours while monitoring by HPLC (product retention time = 6.58 minutes) and TLC (5% MeOH/EtOAc, product R_f = 0.7). The solution was evaporated to dryness. The residue was dissolved in EtOAc (30 mL), washed with saturated $NaHCO_3$ (20 mL), and 1 N NaOH (2.5 mL). The layers were separated and the organic layer was washed with brine (7 mL), dried over $MgSO_4$, filtered, and concentrated to dryness to give an 85% yield of crude product **8**. The crude product was used in the next step without purification.

4.3.5 | [$^{13}C_6$]7-(4-Chlorophenyl)-8-(pyridin-4-yl)-[1,2,4]triazolo[4,3-b]pyridazin-3(2H)-one, 9

To [$^{13}C_6$]3-chloro-5-(4-chlorophenyl)-4-(pyridin-4-yl)pyridazine (**8**, 297 mg, 0.96 mmol) and hydrazinecarboxamide HCl (183 mg, 1.64 mmol) was added 75% EtOH (6.8 mL) and concentrated HCl (33 μL). The mixture was heated to 86°C for 19 hours. The reaction was monitored by TLC (80% EtOAc/hexane, product R_f = 0.80) and by HPLC (product retention time = 5.97 minutes). The solution was evaporated to dryness. The residue was dissolved in EtOAc (60 mL), was washed with saturated $NaHCO_3$ (15 mL), 1 N NaOH (1.0 mL), brine (15 mL), dried over $MgSO_4$, filtered, and was concentrated to dryness. The crude product was purified by trituration with EtOAc to give 201 mg of a white solid (63% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.92 (s, 1H), 8.69 (*d*, *J* = 5.5 Hz, 2H), 8.44 (s, 1H), 7.62 (br. s, 1H), 7.53 to 7.42 (m, 3H), 7.28 (br. s, 1H), and 7.20 to 7.09 (m, 1H).

4.3.6 | [$^{13}C_6$]7-(4-Chlorophenyl)-8-(pyridin-4-yl)-2-((6-(trifluoromethyl)pyridin-3-yl)methyl)-[1,2,4]triazolo[4,3-b]pyridazin-3(2H)-one, [$^{13}C_6$]BMS-725519

In a similar way as [^{14}C]BMS-725519 was prepared, compound **9** (201 mg, 0.61 mmol) was reacted with 5-(chloromethyl)-2-(trifluoromethyl)pyridine (142.9 mg, 0.73 mmol) to obtain 100 mg of [$^{13}C_6$]BMS-725519 as a light yellow solid (40% yield). HPLC purity analysis at 254 nm showed the product to be 98.9% chemically pure with a retention time of 7.18 minutes. LC-MS $[M + H]^+$ = 489/491. Mass isotopic distribution, [M + 6] = 98%, [M + 2] = 2%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.73 (s, 1H), 8.63 to 8.57 (m, 2H), 8.50 (*d*, *J* = 1.6 Hz, 1H), 7.99 (*d*, *J* = 8.2 Hz, 1H), 7.90 (*d*, *J* = 8.2 Hz, 1H), 7.61 (br. s, 1H), 7.43 (br. s, 1H), 7.34 to 7.24 (m, 3H), 7.13 (*d*, *J* = 8.8 Hz, 1H), and 5.37 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 133.18 to 130.58 (m) and 128.98 to 128.03 (m).

4.4 | Synthesis of [^{14}C]BMS-811064

4.4.1 | [^{14}C]4-(7-(4-Chlorophenyl)-5-ethyl-3,6-dioxo-2,3,5,6-tetrahydro-[1,2,4]triazolo[4,3-b]pyridazin-8-yl)benzonitrile, 11

To 4-(5-(4-chlorophenyl)-1-ethyl-3-hydrazinyl-6-oxo-1,6-dihydropyridazin-4-yl)benzonitrile (**10**, 404 mg, 1.1 mmol) in THF (4.6 mL) was added [^{14}C]CDI (269 mg, 0.95 mmol, 50 mCi, 57% chemically pure, remainder imidazole and/or imidazole HCl, specific activity = 186 μCi/mg). The resulting suspension was heated at 65°C for 2 hours. The reaction was monitored by TLC (60% EtOAc/hexane, starting material R_f = 0.10, product R_f = 0.20) and by HPLC (starting material retention time = 7.20 minutes, product retention time = 9.97 minutes). The solution was evaporated to dryness. The residue was washed with water (8 mL) and extracted with DCM (3 × 12 mL). The combined organic extracts were washed with brine (8 mL), dried over $MgSO_4$, filtered, and concentrated to dryness. The crude product was purified by flash chromatography eluting with 0% to 5% MeOH/DCM to afford 285 mg of a light yellow solid (80% yield). The product was used in the next reaction without further purification.

4.4.2 | [^{14}C]4-(7-(4-Chlorophenyl)-5-ethyl-2-(4-(isoxazol-5-yl)benzyl)-3,6-dioxo-2,3,5,6-tetrahydro-[1,2,4]triazolo[4,3-b]pyridazin-8-yl)benzonitrile, [^{14}C]BMS-811064

To a dry flask was added compound (**11**, 140 mg, 0.36 mmol), 5-(4-(bromomethyl)phenyl)isoxazole (94 mg, 0.39 mmol), potassium carbonate (99 mg, 0.71 mmol), and DMF (1.2 mL). The yellow solution was heated to 65°C and stirred for 0.5 hour under nitrogen. The reaction was cooled to room temperature and diluted with water (10 mL). The aqueous solution was extracted with EtOAc (3 × 12 mL). The combined EtOAc extracts were concentrated to give a dark brown oil. The oil was vacuum dried for 16 hours to give a dark brown solid. The crude solid was purified by silica gel flash chromatography eluting with

5% to 55% EtOAc/hexane to give 233.4 mg of an off white solid. This product was then further purified by preparative HPLC to afford 205 mg of a white solid (16.4 mCi, 41% yield). The product was determined to be 99.7% chemically pure with a retention time of 14.3 minutes and 99.9% radiochemically pure. LC-MS $[M + H]^+$ = 551.33/553.25. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.66 (*dd*, *J* = 1.9, 0.9 Hz, 1H), 7.83 (*dd*, *J* = 18.5, 8.1 Hz, 4H), 7.47 to 7.38 (m, 4H), 7.32 (*d*, *J* = 8.5 Hz, 2H), 7.11 (*d*, *J* = 8.3 Hz, 2H), 7.06 to 7.00 (m, 1H), 5.14 (s, 2H), 4.60 (q, *J* = 6.9 Hz, 2H), and 1.34 (t, *J* = 6.8 Hz, 3H). The specific activity was determined to be 42.8 μCi/mg (24.1 mCi/mmol).

4.5 | Synthesis of [^{14}C]BMS-812204

4.5.1 | [^{14}C]8-(4-Chlorophenyl)-5-((2-methyl-6-(trifluoromethyl)pyridin-3-yl)methyl)-7-(2-methylpyrimidin-5-yl)-[1,2,4]triazolo[4,3-b]pyridazine-3,6(2H,5H)-dione, 14

To *tert*-butyl 2-(4-(4-chlorophenyl)-1-((2-methyl-6-(trifluoromethyl)pyridin-3-yl)methyl)-5-(2-methylpyrimidin-5-yl)-6-oxo-1,6-dihydropyridazin-3-yl)hydrazine-1-carboxylate (**12**, 0.52 g, 0.87 mmol) in DCM (3.0 mL) under nitrogen was added TFA slowly (1.0 mL, 13.6 mmol). The solution was stirred for 16 hours at room temperature. An aliquot of the reaction mixture was added to saturated sodium carbonate and was extracted with EtOAc. TLC analysis (70% EtOAc/hexane, product R_f = 0.0) of the EtOAc layer indicated complete conversion to the hydrazine intermediate. The reaction solution was evaporated under high vacuum to give a dry residue. To this residue under N_2 was added THF (2.8 mL) resulting in a light red, clear solution. To this was added carbonyldiimidazole (48.6 mg, 0.30 mmol) followed by [^{14}C]carbonyldiimidazole (269 mg, 0.95 mmol, 50 mCi, 57% chemically pure, remainder imidazole and/or imidazole HCl, specific activity = 186 μCi/mg). The mixture was heated to 60°C for 45 minutes. The solution was evaporated to dryness and the residue was taken up in water (8 mL) and extracted with DCM (3 × 12 mL). The combined organic extracts were washed with brine (8 mL), dried over $MgSO_4$, filtered, and concentrated to dryness. The crude product was purified by silica gel flash chromatography eluting with 10% to 75% EtOAc/hexane to afford 80.0 mg of the product as a light yellow foam. HPLC analysis at 254 nm showed the product to be 64% chemically pure and 99% radiochemically pure. The product was used in the next reaction without further purification.

4.5.2 | [^{14}C-]8-(4-Chlorophenyl)-2-ethyl-5-((2-methyl-6-(trifluoromethyl)pyridin-3-yl)methyl)-7-(2-methylpyrimidin-5-yl)-[1,2,4]triazolo[4,3-b]pyridazine-3,6(2H,5H)-dione.TFA, 15

In a 2-mL, V-shaped vial containing [^{14}C]8-(4-chlorophenyl)-5-((2-methyl-6-(trifluoromethyl)pyridin-3-yl) methyl)-7-(2-methylpyrimidin-5-yl)-[1,2,4]triazolo[4,3-b]pyridazine-3,6(2H,5H)-dione (**14**, 80 mg, 64% chemically pure, 0.10 mmol) was added DMF (0.25 mL), potassium carbonate (36.1 mg, 0.26 mmol), and iodoethane (15.8 μL, 0.19 mmol). The mixture was stirred at 53°C for 1.2 hours while monitoring by HPLC (product retention time = 10.97 minutes). The mixture was cooled to room temperature, water (3 mL) was added, and the solution was extracted with EtOAc (3 × 10 mL). The combined organic extracts were washed with brine (7 mL), dried over $MgSO_4$, filtered, and concentrated to dryness. The crude product (82 mg) was dissolved in 1.0 mL acetonitrile/water (9:1) and was purified by preparative HPLC to afford 39.2 mg of an off white solid as the TFA salt, **15**. The product was used in the next reaction without further purification.

4.5.3 | [^{14}C-]8-(4-Chlorophenyl)-2-ethyl-5-((2-methyl-6-(trifluoromethyl)pyridin-3-yl)methyl)-7-(2-methylpyrimidin-5-yl)-[1,2,4]triazolo[4,3-b]pyridazine-3,6(2H,5H)-dione, [^{14}C]BMS-812204

The TFA salt product, **15**, was dissolved in DCM (5 mL) and washed with 10% K_2CO_3 (3 mL). The aqueous wash was back extracted with DCM (2 × 6 mL). The combined organic extracts were washed with brine (2 mL), dried over $MgSO_4$, filtered, and concentrated to dryness. The product was dried under vacuum for 5 hours and then diluted with the unlabelled product (11 mg, 19.7 μmol) by dissolving in DCM (5 mL), removal of solvent under reduced pressure and vacuum drying to give 33.8 mg of a light yellow solid (2.0 mCi, 4% radiochemical yield over four steps). The product was analyzed by HPLC to be 98.7% chemically pure with a retention time of 10.97 minutes and 99.9% radiochemically pure. LC-MS $[M + H]^+$ = 558.33/560.33. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.44 (s, 2H), 7.94 (*d*, *J* = 8.1 Hz, 1H), 7.72 (*d*, *J* = 8.3 Hz, 1H), 7.50 (*d*, *J* = 8.5 Hz, 2H), 7.38 to 7.33 (m, 2H), 5.74 (s, 2H), 3.86 to 3.76 (m, 2H), 2.66 to 2.55 (m, 6H), and 1.23 to 1.03 (m, 3H). The specific activity was determined to be 59.1 μCi/mg (34.1 mCi/mmol).

4.5.4 | 8-(4-Chlorophenyl)-2-(ethyl-1,2-13C2-d5)-5-((2-methyl-6-(trifluoromethyl)pyridin-3-yl)methyl)-7-(2-methylpyrimidin-5-yl)-[1,2,4]triazolo[4,3-b]pyridazine-3,6(2H,5H)-dione, [$^{13}CD_3{}^{13}CD_2$]BMS-812204

In a 5-mL, V-shaped vial containing 8-(4-chlorophenyl)-5-((2-methyl-6-(trifluoromethyl)pyridin-3-yl)methyl)-7-(2-methylpyrimidin-5-yl)-[1,2,4]triazolo[4,3-b]pyridazine-3,6(2H,5H)-dione (**16**, 0.70 g, 1.3 mmol) was added DMF (2.5 mL) and potassium carbonate (0.37 g, 2.65 mmol) followed by the addition of [$^{13}CD_3{}^{13}CD_2$]iodoethane (0.16 mL, 2.0 mmol). The mixture was stirred at 50°C for 1 hour while monitoring by HPLC (product retention time = 10.97 minutes). The mixture was cooled to room temperature, water (15 mL) was added, and the solution was extracted with EtOAc (3 × 10 mL). The combined organic extracts were washed with brine (7 mL), dried over $MgSO_4$, filtered, and concentrated to dryness. The crude product was purified by silica gel flash chromatography eluting with 20%

EtOAc/hexane to 100% EtOAc to give 524 mg of [$^{13}CD_3{}^{13}CD_2$]BMS-812204 as a white solid (71% yield). HPLC analysis showed the product to be 99.0% chemically pure. LC-MS $[M + H]^+$ = 563.33/565.33. ^1H NMR (500 MHz, $CDCl_3$) δ 8.36 (s, 2H), 7.55 (*d*, *J* = 8.2 Hz, 1H), 7.43 (*d*, *J* = 8.2 Hz, 1H), 7.31 to 7.24 (m, 2H), 7.20 to 7.12 (m, 2H), 5.86 (s, 2H), and 2.65 (*d*, *J* = 14.8 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 40.15 (m) and 11.50 (m).

ACKNOWLEDGMENTS

We gratefully acknowledge the Bristol-Myers Squibb Scientists in the Departments of Discovery Chemistry and Discovery Synthesis for providing us with important intermediates, synthetic procedures, and helpful conversations. We thank Sharon Gong of Bristol-Myers Squibb Radiochemistry for providing the isotope ratio analyses by mass spectrometry. We thank Bruce Ellsworth, PhD, for providing valuable suggestions on the preparation of this manuscript.

REFERENCES

1. Ogden CL, Carroll MD, Curtin LR, McDowell MA, Tabak CJ, Flegal KM. Prevalence of overweight and obesity in the United States, 1999-2004. *JAMA*. 2006;295:1549–1555.

2. Antel J, Gregory PC, Nordheim U. CB_1 cannabinoid receptor antagonists for treatment of obesity and prevention of comorbid metabolic disorders. *J Med Chem.* 2006;49:4008–4016.

3. Pagotto U, Marsicano G, Cota D, Lutz B, Pasquali R. The emerging role of the endocannabinoid system in endocrine regulation and energy balance. *Endocr Rev.* 2006;27(1):73–100.

4. Ellsworth BA, Wang Y, Zhu Y, et al. Discovery of pyrazine carboxamide CB1 antagonists: The introduction of a hydroxyl group improves the pharmaceutical properties and in vivo efficacy of the series. *Bioorg Med Chem Lett*. 2007;17:3978–3982.

5. Yang Y, Miller KJ, Zhu Y, et al. Characterization of a novel and selective CB1 antagonist as a radioligand for receptor occupancy studies. *Bioorg Med Chem Lett*. 2011;21:6856–6860.

6. Ellsworth BA, Wu X, Sher P, et al. Discovery of BMS-812204, a cannabinoid receptor (CB1) antagonist, via reductions in log P in the series to balance volume of distribution and clearance (Abstract MEDI 232). Paper presented at: 238th American Chemical Society National Meeting; August 16-20, 2009; Washington, DC.

7. Ellsworth BA, Sher PM, Wu X, et al. Reductions in log P improved protein binding and clearance predictions enabling the prospective design of cannabinoid receptor (CB1) antagonists with desired pharmacokinetic properties. *J Med Chem*. 2013;56:9586–9600.

8. Walker DG, Leister WH, Weaner LE. Synthesis of 1,1'-carbonyldiimidazole-1-^{14}C and its use in preparing a methoxy(polyethylene)glycol semicarbazide linker. *J Label Compd Radiopharm*. 1995;36:661–669.

9. Staab HA, Wendel K. 1,1'-Carbonyldiimidazole. *Org Synth*. 1968;48:44–46.

How to cite this article: Tran, S. B., Maxwell, B. D., Burrell, R., and Bonacorsi, S. J., Jr. (2016) The syntheses of isotopically labelled CB-1 antagonists for the treatment of obesity. *J. Label Compd. Radiopharm*, doi: 10.1002/jlcr.3433