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Synthesis of [^{2}H, ^{13}C] and [^{14}C] labeled vasopressin V_{1a}/V_2 receptor antagonist RWJ-676070 and its stable labeled *N*-des-benzoyl metabolite

Yong Gong,[a]* David C. Hoerr,[b] Larry E. Weaner,[b] and Ronghui Lin[a]

Stable isotope-labeled ([$^{13}C_2$, D_3]) and carbon 14-labeled vasopressin receptor antagonist RWJ-676070, a spirobenzazepine amide, were prepared in separate syntheses for use in drug metabolism studies. The stable isotopically labeled sample was prepared starting from [$^{13}C_2$, D_6]dimethyl sulfate and [^{13}C]copper (I) cyanide in nine steps with a 14% overall isotopic yield. The ^{14}C label was introduced in five steps starting from [^{14}C]potassium cyanide to provide material having a specific activity of 58 mCi/mmol (2.15 GBq/mmol). Selective hydrolysis of the metabolically labile amide bond in [$^{13}C_2$, D_3]RWJ-676070 gave the corresponding labeled metabolite in one step.

Keywords: isotope label; vasopressin receptor antagonist; spirobenzazepine; metabolite

Introduction

Vasopressin V_{1a}/V_2 receptor antagonists are promising in treating certain cardiovascular-related syndromes.[1] A series of nonpeptide-substituted spirobenzazepines was discovered to be potent and orally active vasopressin antagonists.[2–5] One of the representative compounds, RWJ-676070, was selected for clinical studies.[6] To support drug development efforts, both stable-labeled and radio-labeled RWJ-676070 were required. The stable-labeled compound was designed and synthesized as an internal standard for LC-MS-MS quantitative analysis.[7] Following completion of the synthesis, a request for next-day delivery of a labeled major metabolite of RWJ-676070 was received to support an on-going study. The metabolite, formed by *in vivo* cleavage of an amide bond with subsequent loss of the chlorofluorobenzoyl moiety, was facilely prepared to meet the request by direct hydrolysis of [$^{13}C_2$, D_3]RWJ-676070 to provide stable-labeled metabolite **12**. The carbon-14-labeled sample was prepared using chemistry similar to that used for preparation of the stable-labeled compound, but with the label in a known, metabolically stable position.

Results and discussion

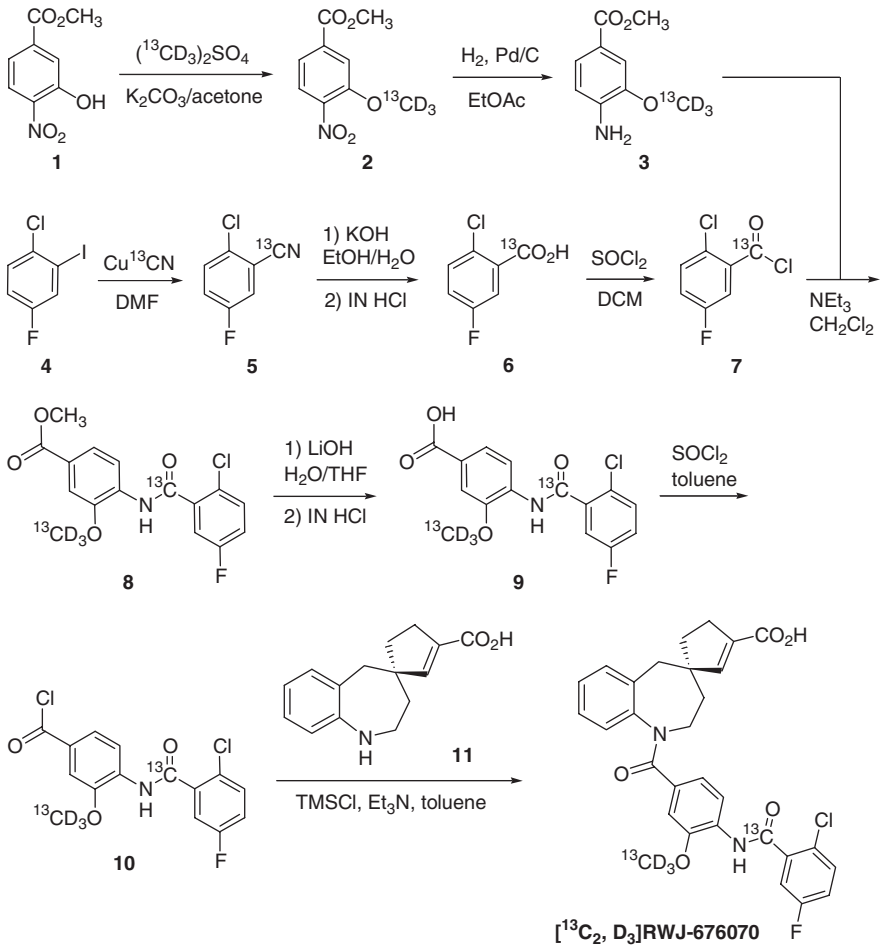
The synthesis of stable-labeled [$^{13}C_2$, D_3]RWJ-676070 is outlined in Scheme 1. The five stable labels were incorporated into two different functional groups, $-O^{13}CD_3$ and $-^{13}CN$, in intermediates **2** and **5**, respectively. The methoxy-$^{13}CD_3$ group was introduced through *O*-methylation of methyl 3-hydroxy-4-nitrobenzoate (**1**) with [$^{13}C_2$, D_6]dimethyl sulfate and the resulting [^{13}C, D_3]-labeled nitro compound **2** was hydrogenated to provide aniline intermediate **3**. Both steps proceeded in nearly quantitative chemical yields. Cyanation of 1-chloro-4-fluoro-2-iodobenzene (**4**) with [^{13}C]copper (I) cyanide introduced the fifth label to provide [^{13}C]nitrile **5**, which was hydrolyzed to the corresponding acid **6** followed by conversion to acid chloride **7**. Coupling of **7** with aniline **3** generated amido ester **8** with the required five isotopic labels [$^{13}C_2$, D_3] in 22% isotopic yield for six steps. Amido acid **9** from hydrolysis of ester **8** was converted to acid chloride **10** and coupled with spirobenzazepine **11**. The resulting [$^{13}C_2$, D_3]RWJ-676070 was obtained in 14% overall isotopic yield after high-performance liquid chromatography (HPLC) purification.

For the synthesis of [^{13}C, D_3]*N*-des-benzoyl metabolite **12**, a multi-step synthetic route similar to, but shorter than, the above procedure to [$^{13}C_2$, D_3]RWJ-676070 was obviously the normal choice. However, the urgent request for next-day delivery of a small amount of material prompted us to look for an alternative synthesis. The desired-labeled metabolite was prepared in one step by selective hydrolysis of the metabolically labile amide bond in [$^{13}C_2$, D_3]RWJ-676070. The reaction was completed at an elevated temperature (up to 150°C) with a reduced reaction time (10 min) using 1 N sodium hydroxide. The [$^{13}C_2$, D_3]RWJ-676070 was cleanly hydrolyzed to the target [^{13}C, D_3]aniline metabolite **12**, along with [^{13}C]2-chloro-5-fluorobenzoic acid (**6**) as the co-product (Scheme 2). The resulting [^{13}C, D_3]metabolite was purified by HPLC.

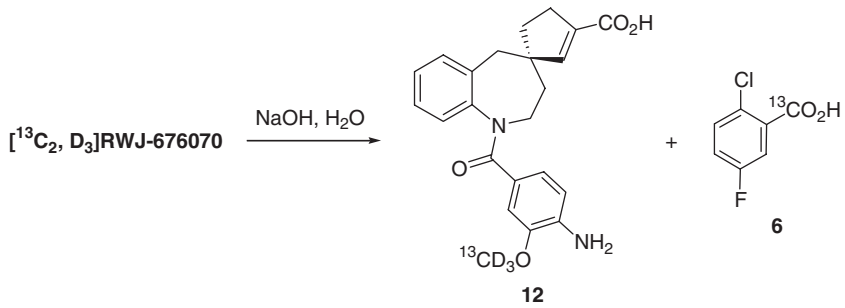
[a]*Global Preclinical Development, Johnson & Johnson Pharmaceutical Research & Development, Raritan, NJ 08869, USA*

[b]*Global Preclinical Development, Johnson & Johnson Pharmaceutical Research & Development, Spring House, PA 19477, USA*

**Correspondence to: Yong Gong, Global Preclinical Development, Johnson & Johnson Pharmaceutical Research & Development, Raritan, NJ 08869, USA. E-mail: ygong@prdus.jnj.com*



Scheme 1. Synthesis of [$^{13}C_2$, D_3]RWJ-676070



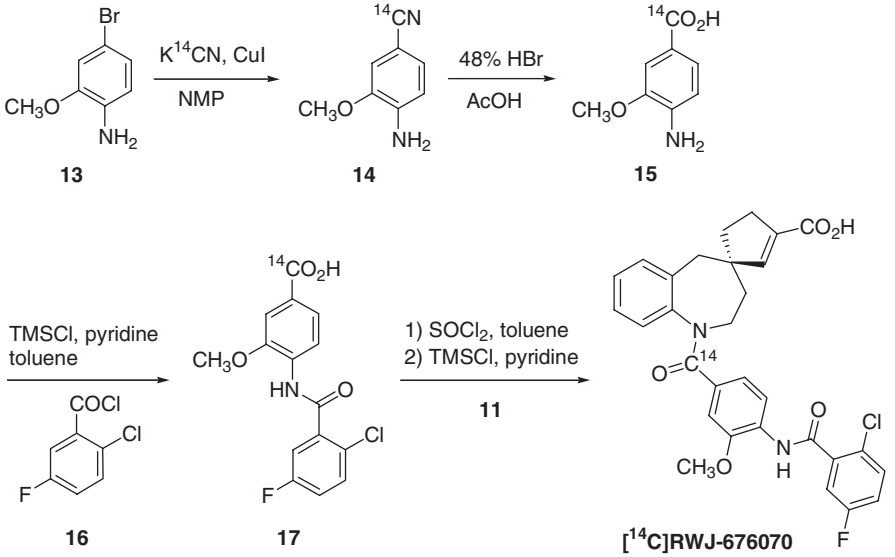
Scheme 2. Synthesis of [^{13}C, D_3]N-des-benzoyl metabolite (12)

The synthesis of [^{14}C]RWJ-676070 is outlined in Scheme 3. Cyanation of 4-bromo-2-methoxyphenylamine (**13**) with K^{14}CN (400 mCi) and CuI yielded nitrile **14** in 65% yield. Hydrolysis of **14** under acidic conditions provided aminobenzoic acid **15**, which was purified by HPLC, treated with chlorotrimethylsilane and acylated with 2-chloro-5-fluorobenzoyl chloride **16** to provide amido acid **17**. The latter was converted to the acid chloride by treatment with thionyl chloride and coupled with spirobenzazepine **11**. The resulting [^{14}C]RWJ-676070 (61.4 mCi, 15.3% radiochemical yield) was obtained with a specific activity of 58 mCi/mmol after HPLC purification.

Experimental

General

[$^{13}C_2$, D_6]Dimethyl sulfate (99.81 at% D, 99.57 at% ^{13}C) and [^{13}C]copper (I) cyanide (99 at% ^{13}C) were purchased from Isotec (Division of Sigma-Aldrich, St. Louis, MO). [^{14}C]Potassium cyanide was obtained from PerkinElmer (Boston, MA, 54.8 mCi/mmol). Methyl 3-hydroxy-4-nitrobenzate (**1**) was obtained from Lancaster Synthesis (Windham, NH) and 1-chloro-4-fluoro-2-iodobenzene (**4**) from Oakwood Product Inc (West Columbia, SC). 4-Bromo-2-methoxy-phenylamine (**13**) was purchased from



Scheme 3. Synthesis of [^{14}C]RWJ-676070

AstaTech (Bristol, PA). Other reagents and solvents were obtained from Sigma-Aldrich, VWR International and other suppliers, and used as received.

^{1}H and ^{13}C NMR spectra were acquired on a Bruker 300-Avance (300 MHz) spectrometer with TMS as an internal standard. Chemical shifts are expressed in parts per million (ppm, δ scale). LC-MS was performed on an Agilent 1100 series LC/MSD with an Agilent Zorbax SB C18 column (3 μm, 2.1 × 50 mm), gradient 10–100% CH_3CN–H_2O, 0.05% TFA or 0.05% NH_4OAc over 3.5 min, held at 100% CH_3CN for 2.5 min, flow rate 0.5 mL/min, detection at 214 and 254 nm, mass scan range 120–1500 amu. Flash chromatography was performed using a Teledyne Isco CombiFlash Companion system and a RediSep® silica gel column. Reverse-phase preparative HPLC purifications were performed using a Gilson system equipped with a Phenomenex Gemini C18 column (5 μm, 21.2 × 250 mm, 110 Å) eluted at 20 mL/min with UV detection at 214 and 254 nm, with a 20-min gradient from 10 to 90% CH_3CN in H_2O, 0.05% TFA. Analytical HPLC was performed with an Agilent Zorbax SB C8 column (5 μm, 4.6 × 250 mm) eluted with solvent B: CH_3CN–TFA (0.1%) and solvent A: H_2O–TFA (0.1%) starting from 10% B to 50% B at 20 min then increased to 90% B at 30 min. The flow rate was 1 mL/min, column temperature was 35°C and UV detection was at 254 nm. The sample was injected in a mixture of CH_3CN–H_2O–TFA (1:1:0.1%). HPLC with radioactive monitoring of the effluent was performed with an Agilent Zorbax Eclipse XDB C18 column (1.8 μm, 4.6 × 100 mm), eluted with a gradient from 10% solvent D (10 mM NH_4OAc in CH_3CN, 15:85, v/v) and 90% solvent C (10 mM NH_4OAc in CH_3CN, 95:5, v/v) to 100% solvent D at 8 min then held at this composition for 4 min before returning to 90% solvent C and 10% solvent D for 1 min. Run time was 13 min, the flow rate was 1 mL/min, column temperature was 55°C and UV detection was at 275 nm. Sample was injected in 5 μL of a CH_3CN–H_2O (2:1, v/v) solvent mixture for analysis with the UV detector. The final ^{14}C-labeled sample was analyzed with a Waters Xterra RP18 column (3.5 μm, 4.6 × 150 mm) using a 35-min solvent gradient from 10% B to 100% B, where solvent A contained 5% CH_3CN in 10 mM aqueous NH_4OAc buffer and solvent B contained 85% CH_3CN in 10 mM aqueous NH_4OAc buffer. The flow rate was 1 mL/min, column temperature was 55°C, and UV detection was at 275 nm. The sample was injected in 10 μL of a solvent mixture of CH_3CN–H_2O (70:30, v/v). Radioactivity in the HPLC effluent stream was measured by mixing the HPLC flow stream with EcoScint scintillation cocktail at 2 mL/min in a radioactive flow detector equipped with a 0.5 mL flow cell. The sample size for the radioactive measurements was 1 μL.

[Methoxy-^{13}C, D_3]methyl 3-methoxy-4-nitrobenzoate (2)

Methyl 3-hydroxy-4-nitrobenzoate (**1**, 0.98 g, 5.0 mmol) was stirred with [$^{13}C_2$, D_6]dimethyl sulfate (1.0 g, 7.5 mmol) in acetone (20 mL) in the presence of K_2CO_3 (1.38 g, 10 mmol) at ambient temperature for 17 h. The mixture was concentrated to dryness. The crude solid was partitioned between H_2O (12 mL) and EtOAc (12 mL). The organic layer was separated and the aqueous layer was extracted with EtOAc (4 mL). The combined EtOAc extracts were washed with H_2O (4 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure at 37°C to give **2** as a yellow solid (1.06 g, 99% chemical yield and 33% isotopic yield). ^{1}H NMR ($CDCl_3$) δ 7.85 (d, 1H, J = 8.3 Hz), 7.76 (d, 1H, J = 1.5 Hz), 7.70 (dd, 1H, J = 8.3, 1.5 Hz), 3.97 (s, 3H). MS m/z 216.0 $(MH)^+$.

[Methoxy-^{13}C, D_3]methyl 4-amino-3-methoxybenzoate (3)

Nitrobenzoate **2** (1.06 g, 4.9 mmol) was hydrogenated in EtOAc (10 mL) in the presence of 10% Pd/C (56 mg) at ambient temperature for 16 h. The Pd/C catalyst was then filtered and washed with EtOAc (3 × 2 mL). Removal of the solvent under reduced pressure at 37°C gave **3** as an off-white crystalline solid (0.90 g, 99%). ^{1}H NMR ($CDCl_3$) δ 7.55 (dd, 1H, J = 8.1, 1.8 Hz), 7.45 (d, 1H, J = 1.7 Hz), 6.67 (d, 1H, J = 8.2 Hz), 4.23 (br, 2H), 3.87 (s, 3H). MS m/z 186.1 $(MH)^+$.

[Nitrile-^{13}C]2-chloro-5-fluorobenzonitrile (5)

A mixture of 1-chloro-4-fluoro-2-iodobenzene (**4**, 2.30 g, 9.0 mmol) and $Cu^{13}CN$ (0.72 g, 8.0 mmol) in dimethyl formamide (DMF) (6 mL) was heated at 120°C oil bath for 24 h, then at 140°C for 4 h. The reaction mixture was cooled to ambient

temperature and filtered. The filter cake was washed with CH_2Cl_2 (2 × 2 mL). The filtrate was diluted with CH_2Cl_2 (10 mL), washed with H_2O (6 × 10 mL), and concentrated under reduced pressure at ambient temperature to give **5** as an off-white crystalline solid (1.12 g, 89%) after air drying (Caution: sublimes). ^{1}H NMR ($CDCl_3$) δ 7.48–7.57 (m, 1H), 7.37–7.43 (m, 1H), 7.25–7.32 (m, 1H).

[Carboxyl-^{13}C]2-chloro-5-fluorobenzoic acid (6)

To a suspension of benzonitrile **5** (1.12 g, 7.15 mmol) in EtOH (6 mL) was added KOH (1.22 g, 21.8 mmol) in H_2O (6 mL). The mixture was heated in a sealed tube at 140°C in an oil bath for 100 min and then concentrated under reduced pressure at 37°C. The resulting slurry was acidified with 1 N HCl (22 mL) and filtered. The filter cake was washed with H_2O (3 × 10 mL) and dried *in vacuo* at ambient temperature to give **6** as an off-white solid (1.09 g, 87%). ^{1}H NMR (DMSO-d_6) δ 13.71 (s, 1H), 7.58–7.66 (m, 2H), 7.41–7.47 (m, 1H). MS *m/z* 176.1 $(MH)^+$.

[Carbonyl-^{13}C]2-chloro-5-fluorobenzoyl chloride (7)

To a suspension of benzoic acid **6** (0.392 g, 2.23 mmol) in CH_2Cl_2 (8 mL) was added $SOCl_2$ (1 mL, 14 mmol). The mixture was heated to 60°C and refluxed for 4 h. The resulting solution was concentrated under reduced pressure at 37°C to give crude **7**, which was dissolved in CH_2Cl_2 (3 mL) and used directly in the next step.

[Methoxy-^{13}C, D_3, amido-^{13}C]methyl 4-(2-chloro-5-fluorobenzoylamino)-3-methoxybenzoate (8)

The solution of benzoyl chloride **7** (≤2.23 mmol) in CH_2Cl_2 prepared in the previous step was added slowly to a solution of aminobenzoate **3** (0.414 g, 2.23 mmol) in CH_2Cl_2 (7 mL) containing NEt_3 (0.93 mL, 6.7 mmol) while cooling in an ice bath. The mixture was stirred at ambient temperature for 2 h and then quenched with 1 N HCl (10 mL). The organic layer was separated, washed with saturated $NaHCO_3$ (10 mL) and concentrated under reduced pressure at 37°C. The crude product was purified by flash chromatography on silica gel, eluted with 0–30% EtOAc in heptane, to yield **8** as a white solid (0.663 g, 86% in two steps). ^{1}H NMR ($CDCl_3$) δ 8.85 (s, 1H), 8.61 (d, 1H, *J* = 8.4 Hz), 7.75 (d, 1H, *J* = 8.4 Hz), 7.52–7.60 (m, 2H), 7.43–7.48 (m, 1H), 7.12–7.19 (m, 1H), 3.93 (s, 3H). MS *m/z* 343.0 $(MH)^+$.

[Methoxy-^{13}C, D_3, amido-^{13}C]4-(2-chloro-5-fluorobenzoylamino)-3-methoxybenzoic acid (9)

To a solution of methyl benzoate **8** (0.40 g, 1.17 mmol) in THF (4 mL) was added 1.0 N LiOH (3.5 mL, 3.5 mmol). The mixture was stirred at ambient temperature for 5 h and the solvent was removed under reduced pressure at 37°C. The remaining mixture was acidified with 1 N HCl (4 mL) and filtered. The filter cake was washed with H_2O (3 × 2 mL) and dried *in vacuo* for 18 h at ambient temperature to give product **9** as a white solid (0.38 g, 100%). ^{1}H NMR (DMSO-d_6) δ 12.96 (s, 1H), 10.03 (s, 1H), 8.22 (d, 1H, *J* = 8.4 Hz), 7.50–7.62 (m, 4H), 7.36–7.42 (m, 1H). MS *m/z* 329.1 $(MH)^+$.

[Methoxy-^{13}C, D_3, amido-^{13}C]4-(2-chloro-5-fluorobenzoylamino)-3-methoxybenzoyl chloride (10)

To a suspension of benzoic acid **9** (0.38 g, 1.1 mmol) in toluene (4 mL) was added $SOCl_2$ (0.15 mL, 2.0 mmol) and DMF (2 drops). The mixture was heated at 97°C in an oil bath for 100 min and then concentrated under reduced pressure at 37°C to give **10** as a white solid, which was dissolved in CH_2Cl_2 (4 mL) and used directly in the next step reaction.

[Methoxy-^{13}C, D_3, amido-^{13}C]RWJ-676070

To an ice–water bath cooled solution of compound **11** (0.523 g, 1.1 mmol, as a camphorsulfonic acid salt) in toluene (4 mL) containing NEt_3 (0.83 mL, 6.0 mmol) was added chlorotrimethylsilane (0.46 mL, 3.6 mmol) dropwise and stirred at 0–4°C for 1 h. To the resulting slurry was added benzoyl chloride **10** in CH_2Cl_2 prepared above, followed by a CH_2Cl_2 rinse (2 mL) in a dropwise manner. The cooling bath was removed and the slurry was stirred at ambient temperature for an additional 17 h. The reaction was quenched with 1 N HCl (4 mL) and stirred for 20 min. The crude mixture was concentrated under reduced pressure at 37°C and the remaining crude product was dissolved in CH_3CN (6.5 mL) and purified by preparative HPLC as described above. The combined product fractions were concentrated and the product was obtained as a white solid (0.37 g, 61% in two steps). ^{1}H NMR (DMSO-d_6) δ 12.34 (br, 1H), 9.78 (s, 1H), 7.84 (d, 1H, *J* = 8.8 Hz), 7.56 (m, 1H), 7.45 (m, 1H), 7.10–7.38 (m, 3H), 7.02 (m, 1H), 6.68–6.81 (m, 3.6 H), 6.24 (s, 0.4 H), 4.70 (m, 1H), 3.38 (m, 1H, partial overlap with H_2O in DMSO), 2.50–3.02 (m, 4H, partial overlap with DMSO), 1.95 (m, 2H), 1.58 (m, 2H). ^{13}C NMR (DMSO-d_6) δ 163.7, 54.8. MS *m/z* 554.1 $(MH)^+$.

[Methoxy-^{13}C, D_3]metabolite 12

A mixture of [methoxy-^{13}C, D_3, amido-^{13}C]RWJ-676070 (10 mg, 18 μmol) and 1.0 N NaOH (0.30 mL) in a sealed tube was immersed in an oil bath heated at 130°C and the temperature was then increased to 150°C. The mixture was stirred at 150°C for 10 min, cooled to ambient temperature and acidified with 1 N HCl (0.30 mL). The resulting white slurry was concentrated to dryness, treated with MeOH (1.4 mL) and filtered. The filtrate was purified by preparative HPLC as described above. The product fraction was concentrated to give [methoxy-^{13}C, D_3] metabolite **12** as an off-white solid (7 mg as TFA salt, 76%). ^{1}H NMR (CD_3CN) δ 6.98–7.26 (m, 3H), 6.61–6.72 (m, 3.6 H), 6.46 (d, 1H, *J* = 8.0 Hz), 6.33 (br, 0.4 H), 4.76 (m, 1H), 3.35 (m, 1H), 2.59–3.04 (m, 4H), 1.58–1.96 (m, 4H, partial overlap with CD_3CN). MS *m/z* 397.1 $(MH)^+$.

[Nitrile-^{14}C]4-cyano-2-methoxyphenylamine (14)

A mixture of 4-bromo-2-methoxyphenylamine (**13**, 1.475 g, 7.3 mmol), K^{14}CN (490 mg, 7.3 mmol, 400 mCi, 54.8 mCi/mmol) and CuI (685 mg, 3.6 mmol) in 1-methyl-2-pyrrolidinone (10 mL) was heated at 220°C for 7 h. The reaction was cooled to ambient temperature and EtOAc (2 mL) and NaCN (2 g) in H_2O (6 mL) were added. This mixture was stirred for 20 min and extracted with EtOAc. The organic extracts were combined, washed with H_2O (4 × 10 mL) and dried over anhydrous $MgSO_4$. Filtration and removal of the solvent on a rotary evaporator gave an oil (**14**, 262 mCi, 54.8 mCi/mmol, 65.5% radiochemical yield), which was used directly in the next reaction step.

[Carboxyl-^{14}C]4-amino-3-methoxybenzoic acid (15)

To the crude nitrile **14** (4.85 mmol) obtained from above was added glacial AcOH (2.9 mL) and 48% HBr (4.85 mL). This mixture

was stirred and heated at 130°C for 7 h. The reaction was cooled to ice-bath temperature and EtOAc and 50% NaOH were added to pH 6. The solvent was removed to dryness and the product was extracted with a 95:5 (v/v) mixture of EtOAc–CH_3OH. Filtration and removal of the solvent *in vacuo* gave a dark brown solid. This was dissolved in CH_3CN–H_2O (3:2, v/v, 18 mL) and injected in 2 mL portions onto a 2.14 × 25 cm Dynamax C18 preparative HPLC column (Varian). The compound was eluted using a linear gradient solvent mixture from 10 to 22% CH_3CN–H_2O containing 0.05% TFA over 30 min. The solvent flow rate was 10 mL/min, column temperature was at ambient temperature and UV detection was at a wavelength of 254 nm. The product eluted from the column between 16 and 22 min and was collected and combined. The solvent was removed on a rotary evaporator at 30°C. Product **15** was dried *in vacuo* to an off-white solid (as the TFA salt, 639 mg, 123.7 mCi, 54.8 mCi/mmol, 47% yield) that was 98% radiochemically pure.

[Carboxyl-^{14}C]4-(2-chloro-5-fluorobenzoylamino)-3-methoxybenzoic acid (17)

Under a nitrogen gas atmosphere was placed aminobenzoic acid TFA salt **15** (639 mg, 2.25 mmol), toluene (5.6 mL) and dry pyridine (0.80 mL 9.89 mmol). This mixture was cooled in an ice bath and chlorotrimethylsilane (0.70 mL, 5.52 mmol) was slowly added over 10 min. The resulting mixture was stirred for 30 min at 0°C and a solution of 2-chloro-5-fluorobenzoyl chloride (**16**, 463 mg, 2.4 mmol) in toluene (1.65 mL) was slowly added and the reaction was stirred at 0°C for 3 h. The reaction was quenched by the addition of a mixture of concentrated HCl (0.4 mL), H_2O (1.5 mL), and EtOH (1.9 mL). After stirring for 15 min, the mixture was heated to 80°C for 30 min then cooled to room temperature. The resulting suspension was filtered and the filter cake was washed with H_2O and toluene. Product **17** was obtained as a white solid (593 mg, 1.82 mmol, 99.9 mCi) after drying *in vacuo*.

[Amido-^{14}C]RWJ-676070

To a solution of benzoic acid **17** (593 mg, 1.82 mmol) in toluene (3.9 mL) under a nitrogen gas atmosphere was added $SOCl_2$ (0.4 mL) and a catalytic amount of DMF (5 μL). This mixture was heated at 95°C for 1 h then at 100°C for an additional hour. Toluene and excess $SOCl_2$ were removed by vacuum distillation and the acid chloride residue was dissolved in CH_2Cl_2 (6 mL). Into a second reaction vessel was placed compound **11** (0.856 g, 1.80 mmol, as a camphorsulfonic acid salt), pyridine (0.832 mL, 10.9 mmol) and toluene (2.8 mL) under a nitrogen gas atmosphere. Chlorotrimethylsilane (0.716 mL, 5.65 mmol) was added at 0°C and the mixture was stirred for 1 h. At this temperature was added the above solution of the acid chloride to the reaction mixture and this was stirred for 5 h at ambient temperature. The reaction was quenched by the addition of 18% HCl (1.3 mL) and the solvent was removed on a rotary evaporator. The remaining solid was dissolved in EtOAc (30 mL) and this solution was washed with H_2O. The aqueous phase was back extracted with EtOAc (4 × 10 mL) and the organic extracts were washed with H_2O and dried over $MgSO_4$. Filtration and removal of the solvent gave a yellow solid (862 mg) that was dissolved in a mixture of CH_3CN–H_2O (10:2.2, v/v) and purified by preparative HPLC on a Supelcosil LC-ABZ+PLUS column (5 μm, 2.1 × 25 cm, Supelco). The product was eluted from the column using a 40-min linear gradient from 20 to 85% CH_3CN in H_2O with 0.05% TFA present at a flow rate of 10 mL/min. After a 10-min hold at the end of the gradient at 85% CH_3CN in H_2O–TFA, the column was re-equilibrated to the initial conditions. The product eluted from the column with a 34-min retention time. The center portion of the fractions containing product were collected and the solvent was immediately removed on a rotary evaporator at ambient temperature. The product having a radiochemical purity of 99.6% was recovered as a white solid (583 mg, 61.4 mCi, 58 mCi/mmol), dissolved in EtOH–H_2O (90:10, v/v) and stored at −20°C.

Conclusion

An efficient synthesis of stable-labeled [methoxy-^{13}C, D_3, amido-^{13}C]RWJ-676070 is described. The nine-step procedure achieved five isotopic labels in a 14% overall isotopic yield. A novel one-step route to the [^{13}C, D_3]-labeled *N*-des-benzoyl metabolite by selective hydrolysis of the metabolically labile amide bond in [methoxy-^{13}C, D_3, amido-^{13}C]RWJ-676070 was revealed. This successful approach enabled us to meet a next-day delivery challenge. Finally, a carbon-14 label was introduced in the metabolically stable carboxyl group of RWJ-676070 via a five-step sequence that provided material having a specific activity of 58 mCi/mmol.

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