SYNTHESIS OF ^{13}C- AND ^{14}C-LABELED 1192U90, AN *ORTHO*-AMINO BENZAMIDE WITH A PRECLINICAL ATYPICAL ANTIPSYCHOTIC PROFILE

Mark H. Norman,*† and Stephen D. Gabriel‡

Division of Medicinal Chemistry† and Chemical Development Division‡,
Glaxo Wellcome Inc., Research Triangle Park, North Carolina 27709

SUMMARY

Three isotopic forms of potential antipsychotic agent 1192U90 (2-amino-N-(4-(4-(1,2-benzisothiazol-3-yl)-1-piperazinyl)butyl)benzamide) were synthesized: one containing ^{13}C-isotopes and two containing ^{14}C-isotopes. The compound in which the *ortho*-amino benzamide ring is completely ^{13}C-labeled was prepared in a four-step sequence starting from [$^{13}C_6$]aniline. The ^{14}C-labeled compounds were prepared by methods analogous to those previously described for the unlabeled material. The key step involved the condensation of 3-(4-(4-aminobutyl)-1-piperazinyl)-1,2-benzisothiazole with isatoic anhydride. The first ^{14}C-labeled compound (**3**) was prepared from ^{14}C-labeled 3-(4-(4-aminobutyl)-1-piperazinyl)-1,2-benzisothiazole, while the second compound (**4**) derived its isotopic label from [^{14}C]isatoic anhydride. Compound **3** had a specific activity of 26.55 mCi/mmol, a radiochemical purity of 99.3%, and a radiochemical yield of 3.4% based on **9**. Compound **4** had a specific activity of 22.67 mCi/mmol and a radiochemical purity of 99.2%.

Keywords: [$^{13}C_6$]1192U90, [^{14}C]1192U90, antipsychotic, dopamine, serotonin, antagonist.

INTRODUCTION

We recently reported the synthesis and biological evaluation of a series of substituted benzamides possessing mixed dopamine and serotonin antagonist activity.[1,2] In addition to being potent dopamine D_2 and serotonin 5-HT_2 antagonists, these derivatives demonstrate *in vivo* activities that suggest they would be useful in the treatment of schizophrenia and would have a low propensity to induce extrapyramidal side effects. From these investigations, 1192U90 (2-amino-N-(4-(4-(1,2-benzisothiazol-3-yl)-1-piperazinyl)butyl)benzamide hydrochloride; (**1**) was selected for further evaluation and is currently in phase I clinical trials as a potential atypical antipsychotic agent. To support the clinical development of this agent, three labeled forms of 1192U90 (**1**) were synthesized. [$^{13}C_6$]1192U90 (**2**) was prepared as an internal standard for analytical LC-MS-MS determinations, and two ^{14}C-labeled forms of 1192U90 (**3** and **4**) were prepared for use in pharmacokinetics and drug metabolism studies.

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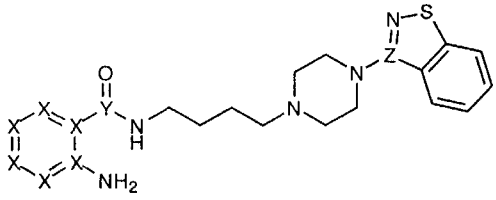
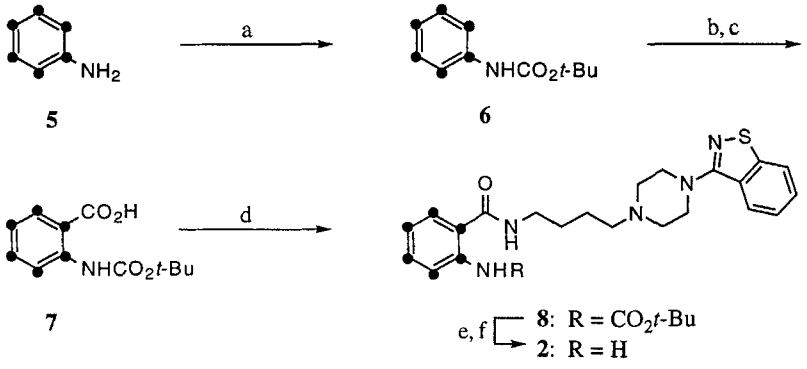


1: X = ^{12}C; Y = ^{12}C; Z = ^{12}C.
2: X = ^{13}C; Y = ^{12}C; Z = ^{12}C.
3: X = ^{12}C; Y = ^{12}C; Z = ^{14}C.
4: X = ^{12}C; Y = ^{14}C; Z = ^{12}C.

RESULTS AND DISCUSSION

To develop a suitable analytical method using mass spectrometry, it is desirable to have an isotopically labeled form of the drug substance that has a mass significantly different from the parent drug. Therefore, we chose to prepare [$^{13}C_6$]1192U90 (**2**), which was 6 amu heavier than **1**. The synthesis of **2** is outlined in Scheme I. Treatment of [$^{13}C_6$]aniline (**5**) with di-*tert*-butyl dicarbonate in refluxing tetrahydrofuran provided carbamate **6** in 94% yield. The BOC-protected anthranilic acid **7** was prepared according to a modification of the procedure described by Muchowski *et al.*[3] *Ortho*-metalation of carbamate **6** was accomplished by treatment with 2.4 equivalents of *tert*-butyllithium at -10 to -20 °C. The resulting dianion was quenched by bubbling carbon dioxide through the solution to provide acid **7**. We found that it was critical to closely monitor and maintain the temperature of the lithiation reaction at -10 to -20 °C.

Scheme I



● = ^{13}C. (a) $O(CO_2t\text{-}Bu)_2$, THF, reflux. (b) *t*-BuLi, THF, -78 °C to -10 °C. (c) CO_2 (gas), -78 °C. (d) DCC, HOBT, DMF, 3-(4-(4-aminobutyl)-1-piperazinyl)-1,2-benzisothiazole.[4] (e) CF_3CO_2H, $CHCl_3$, anisole, 25 °C. (f) HCl, ether.

Incomplete *ortho*-metalation resulted at lower temperatures, while decomposition occurred at higher temperatures, resulting in decreased yields. We also obtained better results when the dianion of **6** was quenched with gaseous rather than solid carbon dioxide. Amide **8** was prepared from a dicyclohexylcarbodiimide coupling of protected anthranilic acid **7** with 3-(4-(4-aminobutyl)-1-piperazinyl)-1,2-benzisothiazole (**10**).[4] Hydrolysis of carbamate **8** with trifluoroacetic acid in chloroform provided [$^{13}C_6$]1192U90 (**2**) in good yield. This material was treated with ethereal hydrogen chloride and isolated as its hydrochloride salt. Spectral and physical data for compound **2** were consistent with [$^{13}C_6$]1192U90, wherein all of the carbons in the benzamide ring were ^{13}C-labeled (>99 atom %). To confirm the structural assignment of **2**, we conducted a series of NMR experiments and compared the resulting spectra to the ^1H NMR spectrum of unlabeled 1192U90. These results are illustrated in Figure 1. The aromatic benzamide protons of **2** showed complex splitting patterns due to heteronuclear ^1H-^{13}C coupling (Figure 1B). However, when this coupling was eliminated, the signals for the four benzamide protons coincided with the corresponding signals of the unlabeled material (Figures 1C and 1D vs Figure 1A). Investigations are currently underway to develop an analytical LC-MS-MS method capable of measuring plasma levels of 1192U90 employing [$^{13}C_6$]1192U90 (**2**) as an internal standard.

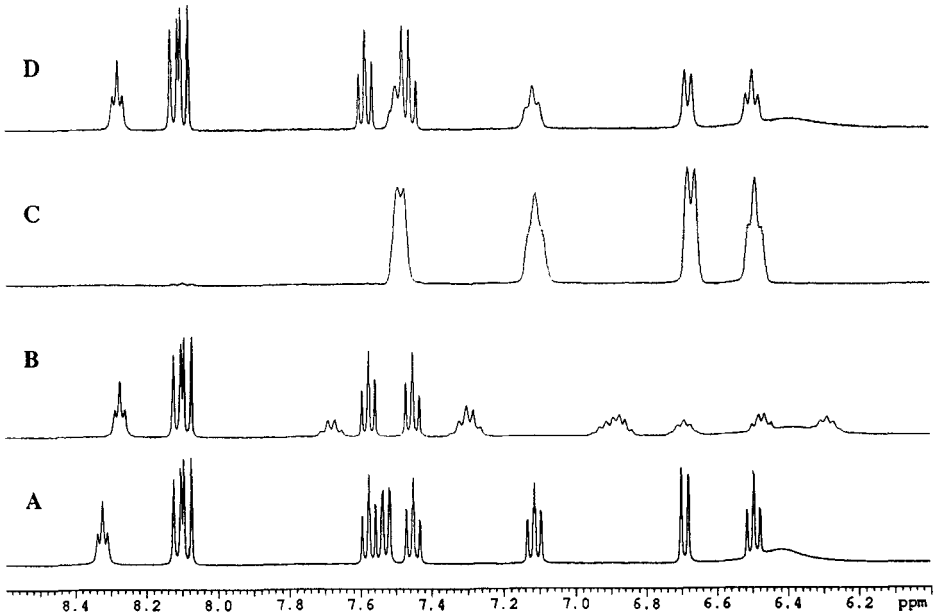
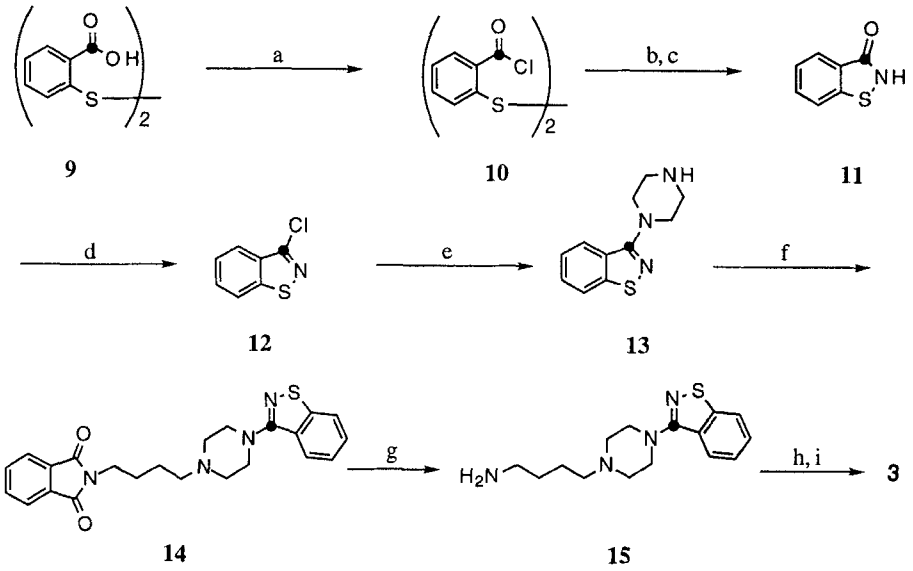


Figure 1. ^1H NMR spectra (6.1-8.4 ppm region) of 1192U90 taken in DMSO-d_6 at 400 MHz.
(a) Unlabeled 1192U90 (**1**). (b) [$^{13}C_6$]1192U90 (**2**). (c) First block of the HMQC spectrum (^{13}C-decoupled) of **2** showing only ^{13}C-labeled positions. (d) ^{13}C-decoupled ^1H NMR of **2**.

The second isotopic form of 1192U90 that we prepared contained a ^{14}C-label at the "imine"-like carbon in the piperazine benzisothiazole ring (compound **3**, Scheme II). Intermediate **13** was prepared according to a modification of the procedure described by Yevich and coworkers for the corresponding unlabeled compound.[5] Commercially available 2,2'-dithiosalicylic acid, labeled with ^{14}C at the carboxylate carbon, was treated with oxalyl chloride and dimethylformamide in tetrahydrofuran to give 2,2'-dithiobisbenzoyl chloride (**10**). This acid chloride was converted to 1,2-benzisothiazole-3(2*H*)-one (**11**) by cleaving the disulfide bond with chlorine gas in dichloromethane and reacting the resulting intermediate dichloride with ammonium hydroxide.

Scheme II



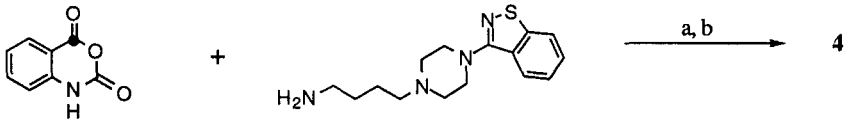
● = ^{14}C. (a) Oxalyl Chloride, THF, DMF, 25 °C. (b) Cl_2, CH_2Cl_2, 25 °C. (c) NH_4OH.
(d) PCl_5, 106 °C. (e) Piperazine, 170 °C. (f) N-(4-Bromobutyl)phthalimide, CH_3CN, Et_3N, 82 °C.
(g) $H_2NNH_2 \cdot H_2O$, CH_3OH, 65 °C. (h) Isatoic anhydride, EtOH, 25 °C. (i) 3.6*M* HCl/EtOAc, EtOAc.

Treatment of benzisothiazolone **11** with phosphorous pentachloride provided 3-chloro-1,2-benzisothiazole (**12**) in good yield. Piperazine benzisothiazole **13** was obtained by heating chloride **12** with six equivalents of piperazine at 170 °C. The secondary piperazine nitrogen of **13** was alkylated with N-(4-bromobutyl)phthalimide to give the corresponding phthalimide **14**. Primary amine **15** was easily obtained by the deprotection of phthalimide **14** with hydrazine

hydrate in refluxing methanol. Condensation of **15** with isatoic anhydride followed by treatment with 3.6*M* HCl in ethyl acetate provided the desired ^{14}C-labeled 1192U90 **3** as its hydrochloride salt.

The synthesis of the final ^{14}C-labeled form of 1192U90, wherein the ^{14}C-label is at the amide carbonyl carbon, is shown in Scheme III. This material was prepared by a method analogous to that used in the final step of the synthesis of compound **3**. Treatment of [^{14}C]isatoic anhydride (**16**) with 3-(4-(4-aminobutyl)-1-piperazinyl)-1,2-benzisothiazole (**17**)[4] in absolute ethanol provided [^{14}C]1192U90 (**4**) in good yield. ^{14}C-Labeled compounds **3** and **4** are currently being employed in studies investigating the pharmacokinetics and drug metabolism of 1192U90.

Scheme III



16 **17**

● = ^{14}C. (a) EtOH, 25 °C. (b) 3.6*M* HCl/EtOAc, EtOAc.

EXPERIMENTAL

General. Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. Anhydrous solvents such as dimethylformamide (DMF), tetrahydrofuran (THF), and dichloromethane were obtained from Aldrich Chemical Co. in Sure/Seal bottles. Triethylamine was distilled from CaH_2 prior to use. All reactions involving air- or moisture-sensitive compounds were performed under an argon or nitrogen atmosphere. Flash chromatography was performed using EM Science silica gel 60 (230–400-mesh ASTM). Thin-layer chromatography (TLC) was performed with Analtech silica gel GF TLC plates (250 μm) or EM Science silica gel 60 F254 TLC plates. HPLC analyses were carried out with a Waters 510 solvent delivery system and a Shimadzu RF530 fluorescence detector in series with a Beckman 171 flow through radioactivity detector using Flo-Scint III as the liquid scintillation cocktail. HPLC radiochemical purity was determined using a Zorbax C-18 (5 μm, 4.6 x 150 mm) analytical column with a mobile phase of CH_3CN/0.25*M* formic acid (77:23) containing

0.25% triethylamine. TLC radiochromatograms were obtained with a Bioscan System 200 Imaging Scanner. ^{1}H NMR spectra were determined with superconducting FT NMR spectrometers operating at 300 and 400 MHz. ^{13}C NMR were measured at 75.43 MHz. Chemical shifts are expressed in ppm downfield from internal tetramethylsilane. Significant ^{1}H and ^{13}C NMR data are reported in the following order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), number of protons, and coupling constants in Hz. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. Melting points were determined with a Thomas Hoover capillary melting point apparatus and are uncorrected.

***tert*-Butyl N-phenylcarbamate (6).** Anhydrous THF (15 mL), [$^{13}C_6$]aniline (1.47 g, 14.8 mmol, Isotec Inc., min. 99 atom %) and di-*tert*-butyl dicarbonate (3.56 g, 16.3 mmol, 1.1 eq) were placed in an oven-dried 100-mL round-bottomed flask. The colorless reaction mixture was placed under N_2 and heated at reflux for 3 h. The solvent was removed with a rotary evaporator and the resulting white solids were dissolved in EtOAc. The organic solution was washed with 1*N* HCl (2 x 15 mL) and with saturated aqueous $NaHCO_3$ (1 x 15 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated to give 3.26 g of a white solid. This crude material was recrystallized from hexanes and dried in a vacuum oven at 55 °C to give 2.65 g of carbamate **6** as white needles. A second crop of 0.12 g was obtained to give a total of 2.77 g (94%). MP: 135-136 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.52 (s, 9), 6.45 (br s, 1), 7.00 (dm, 1, J = 144.0), 7.32 (dm, 4 , J = 165.0). ^{13}C NMR ($CDCl_3$, 75.43 MHz): δ 28.24 (s, 1), 80.38 (s, 1), 118.32 (tm, 1, J = 59.9), 122.81 (tdm, 1, J = 55.6, 9.6), 128.85 (tm, 1, J = 57.7), 138.17 (dt, 1, J = 63.7, 9.9), 152.61 (s, 1). MS (CI CH_4 Dep 50 mA/sec): 200 (M+1), 144 (base). IR (CCl_4): 3449, 1739, 1159 cm^{-1}. Anal. calcd for $^{13}C_6C_5H_{15}NO_2$: C, 66.33; H, 7.59; N, 7.03. Found: C, 66.26; H, 7.60; N, 7.01.

2-((*tert*-Butoxycarbonyl)amino)benzoic acid (7). *tert*-Butyl N-phenylcarbamate (**6**) (2.77 g, 13.9 mmol) and anhydrous THF (40 mL) were placed in an oven-dried 100-mL round-bottomed flask. The colorless solution was placed under N_2 and cooled to -78 °C in a dry ice/acetone bath. *tert*-Butyllithium (19.4 mL, 33.0 mmol, 2.4 eq of a 1.7*M* solution in pentane) was added dropwise via a syringe over a 10-min period. The solution turned a bright-yellow color upon this addition, but the color quickly dissipated upon stirring. After one equivalent of *tert*-butyllithium was added, the yellow color persisted. The light-yellow solution was allowed to stir at -78 °C for 15 min and allowed to warm to -10 to -20 °C. The reaction temperature was maintained between -10 to -20 °C for 2 h. The solution became a dark-orange color. The

reaction mixture was cooled to -78 °C and allowed to stir for 0.5 h. CO_2 (g) was bubbled through a gas dispersion tube into the anionic solution. As the CO_2 was introduced, the dark-orange color lightened to give a pale-yellow solution. The reaction mixture was allowed to warm to room temperature and was extracted with saturated aqueous $NaHCO_3$. The organic phase was dried over $MgSO_4$, filtered, and concentrated with a rotary evaporator to give 1.5 g of a light-yellow solid (recovered starting material). The basic aqueous phase was acidified by the addition of 1*N* HCl and extracted with Et_2O (5 x 50 mL). The Et_2O extracts were dried over $MgSO_4$, filtered, and concentrated with a rotary evaporator to give 2.39 g of an orange solid. The crude material was dissolved in a minimum amount of hot Et_2O. Hexane was added until the solution became cloudy and the solution was cooled in a freezer. The resulting solids were filtered, washed with cold hexanes, and dried in a vacuum oven to give 1.05 g of **7** as a yellow powder. An additional 0.47 g of material was obtained as a second crop to give a total of 1.52 g (45%; 62% based on recovered starting material). MP: 152-153 °C. ^1H NMR ($CDCl_3$, 400 MHz): δ 1.55 (s, 9), 7.03 (d quintet, 1, J = 163.9, 7.6), 7.56 (d quintet, 1, J = 159.9, 8.0), 8.08 (dq, 1, J = 144.0, 7.8), 8.49 (dq, 1, J = 147.7, 7.9), 10.02 (br s, 1). ^{13}C NMR ($CDCl_3$, 75.43 MHz): δ 28.20 (s, 1), 80.75 (s, 1), 112.98 (dddd, 1, J = 67.5, 59.7, 8.1, 1.5), 118.77 (dddd, 1, J = 60.4, 56.4, 8.1, 2.5), 121.14 (tdm, 1, J = 55.4, 8.1), 131.76 (dddd, 1, J = 59.2, 56.9, 8.1, 1.5), 135.47 (dddd, 1, J = 57.7, 54.4, 8.1, 1.0), 142.78 (ddd, 1, J = 65.5, 62.0, 8.1), 152.62 (dd, 1, J = 3.5, 2.5), 172.48 (dm, J = 68.5). MS (CI, CH_4, Dep 50 mA/sec): 200 (M+1), 144 (base). IR (CCl_4/$CDCl_3$): 3532, 3337, 3000 (br), 1738, 1708, 1669 cm^{-1}. Anal. calcd for $^{13}C_6C_6H_{15}NO_4$: C, 59.26; H, 6.21; N, 5.76. Found: C, 59.46; H, 6.32; N, 5.86.

***tert*-Butyl N-(2-(((4-(4-(1,2-benzisothiazol-3-yl)-1-piperazinyl)butyl)amino)carbonyl) phenyl)carbamate Hydrochloride (8).** Anhydrous DMF (35 mL), 2-((*tert*-butoxycarbonyl) amino)benzoic acid (**7**) (1.70 g, 7.00 mmol), 3-(4-(4-aminobutyl)-1-piperazinyl)-1,2-benzisothiazole (**17**)[4] (2.13 g, 7.34 mmol, 1.05 eq), and 1-hydroxybenzotriazole hydrate (1.09 g, 8.04 mmol, 1.15 eq) were placed in a round-bottomed flask. The reaction mixture was cooled in an ice-water bath and a solution of dicyclohexylcarbodiimide (1.66 g, 8.04 mmol, 1.15 eq) in DMF (10 mL) was added via a pipet. The cold bath was removed and the solution was allowed to stir under N_2 at room temperature for 19 h. A white solid precipitated from the solution as the reaction proceeded. The solvent was removed with a rotary evaporator under high vacuum. The resulting orange oil was taken up in CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$. The organic layer was dried over $MgSO_4$, filtered, and concentrated with a rotary evaporator to give

an orange oil containing white solids. This material was dissolved in EtOAc, filtered, and concentrated to give 4.72 g of a dark orange oil. This material was purified by flash chromatography eluting with EtOAc/hexanes (3:1) followed by EtOAc/hexanes (4:1) to give 3.02 g (84%) of the free base as a viscous pale-orange oil. To obtain an analytical sample, a portion of this material was converted to its hydrochloride salt. The free base (0.77 g) was dissolved in CH_2Cl_2 and 1*N* ethereal HCl (1.5 mL, 1.0 eq) was added. The resulting salt was dissolved in hot EtOH and the solution was filtered hot. Ether was added to the filtrate until the solution was cloudy. The solids that formed upon cooling were filtered, washed with cold Et_2O, and dried in a vacuum oven at 70 °C to give 0.56 g of **8** as a pale-yellow glass. MP: 115-120 °C (effervesced and decomposed). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.44 (s, 9), 1.59 (m, 2), 1.80 (m, 2), 3.20 (m, 2), 3.28 (m, 4), 3.21 (m, 4), 4.05 (br d, 2, J = 13.4), 7.05 (br dt, 1, J = 163.0, 6.4), 7.45 (tm, 1, J = 7.5), 7.52 (br dt, 1, J = 160.0, 7.5), 7.58 (tm, 1, J = 7.6), 7.78 (dm, 1, J = 159.0), 8.10 (t, 2, J = 8.5), 8.19 (br dq, 1, J = 164.9, 7.3), 8.89 (br t, 1, J = 5.4), 10.75 (s, 1), 11.13 (br s, 1). ^{13}C NMR (DMSO-d_6, 75.43 MHz): δ 20.97 (s, 1), 26.35 (s, 1), 28.30 (s, 1), 38.78 (s, 1), 46.72 (s, 1), 50.80 (s, 1), 55.46 (s, 1), 80.03 (s, 1), 118.72 (td, 1, J = 58.9, 6.0), 119.52 (td, 1, J = 62.7, 7.6), 121.60 (td, 1, J = 55.1, 8.6), 124.34 (s, 1), 124.95 (s, 1), 127.31 (s, 1), 128.61 (td, 1, J = 57.2, 7.1), 132.39 (td, 1, J = 55.6, 7.6), 139.97 (td, 1, J = 63.5, 8.6), 149.85 (tm, 1, J = 61.2), 152.45 (s, 1), 162.56 (s, 1), 168.68 (d, 1, J = 64.5). APCI MS: 516.4 (M+1, base). IR (CCl_4/$CDCl_3$): 3280 (br), 2400 (br), 1720, 1633, 1158, 1393, 1368 cm^{-1}. Anal. calcd for $^{13}C_6C_{21}H_{36}N_5O_3SCl$• HCl•0.5 H_2O: C, 57.80; H, 6.65; N, 12.48; S, 5.71; Cl, 6.32; H_2O, 1.61. Found: C, 58.05; H, 6.62; N, 12.51; S, 5.79; Cl, 6.38; H_2O, 1.34.

2-Amino-N-(4-(4-(1,2-benzisothiazol-3-yl)-1-piperazinyl)butyl)-[1,2,3,4,5,6-$^{13}C_6$] benzamide Hydrochloride (2). Carbamate **8** (2.20 g, 4.27 mmol) was dissolved in $CHCl_3$ (30 mL) and the resulting yellow-orange solution was placed under N_2. Trifluoroacetic acid (30 mL) and anisole (3.0 mL) were added to the carbamate solution via syringe. The reaction mixture was allowed to stir at room temperature for 0.5 h. The solution was concentrated with a rotary evaporator and the resulting residue was dissolved in EtOAc and washed with saturated aqueous K_2CO_3. The organic phase was dried over $MgSO_4$, filtered, and concentrated with a rotary evaporator to give 7.5 g of an orange oil containing sticky white solids. This material was dissolved in EtOAc and washed with saturated aqueous K_2CO_3. The EtOAc solution was dried over $MgSO_4$, filtered, and concentrated to give 2.29 g of a clear viscous orange oil. This material was purified by flash chromatography on silica gel eluting with CH_2Cl_2/CH_3OH (95:5)

to give 1.40 g of an off-white glass. This material was dissolved in CH_2Cl_2 and 1*N* ethereal HCl (3.37 mL, 1.0 eq) was added. The resulting salt was heated with 95% EtOH. As the solution was heated, distilled H_2O was slowly added until the solids were dissolved. The solution was filtered hot and allowed to cool. The solids that formed upon cooling were filtered, washed with cold EtOH and Et_2O, and dried in a vacuum oven at 70 °C for 6 h to give 1.03 g (67%) of **2** as tan crystals. MP: 225-227 °C. ^1H NMR (DMSO-d_6, 400 MHz): δ 1.55 (quintet, 2, J = 7.4), 1.78 (m, 2), 3.17 (t, 2, J = 8.0), 3.26 (m, 4), 3.48 (t, 2, J = 12.4), 3.56 (d, 2, J = 12.4), 4.05 (d, 2, J = 13.2), 6.37 (br s, 2), 6.49 (d quintet, 1, J = 162.0, 7.1), 6.67 (dq, 1, J = 157.6, 7.0), 7.10 (dm, 1, J = 156.2), 7.49 (dq, 1, J = 155.5, 7.9), 7.45 (ddd, 1, J = 8.1, 7.0, 0.9), 7.57 (ddd, 1, J = 8.1, 7.0, 0.9), 8.08 (d, 1, J = 8.2), 8.11 (d, 1, J = 8.2), 8.27 (t, 1, J = 5.5), 10.92 (br s, 1). ^{13}C NMR (DMSO-d_6, 75.43 MHz): δ 21.80 (s, 1), 27.50 (s, 1), 39.19 (s, 1), 47.51 (s, 1), 51.59 (s, 1), 56.34 (s, 1), 115.84 (tdd, 1, J = 56.5, 18.1, 8.1), 117.42 (td, 1, J = 59.4, 7.5), 122.31 (s, 1), 125.15 (s, 1), 125.76 (s, 1), 128.12 (s, 1), 129.23 (td, 2, J = 58.2, 7.5), 132.67 (ddd, 1, J = 60.4. 54.0, 7.0), 150.63 (ddd, 1, J = 63.0, 59.4, 8.1), 153.27 (s, 1), 163.37 (s, 1), 170.04 (d, 1, J = 64.5). APCI MS: 416 (M+1, base). IR (KBr): 3389, 3006, 1640, 1548, 744 cm^{-1}. Anal. calcd for $^{13}C_6C_{16}H_{27}N_5OS \cdot HCl$: C, 58.46; H, 6.24; N, 15.50; S, 7.09; Cl, 7.84. Found: C, 58.56; H, 6.28; N, 15.49; S, 7.15; Cl, 7.90.

2,2'-Dithio-[7,7'-$^{14}C_2$]bisbenzoyl chloride (10). To a solution of 2,2'-dithio-[7,7'-$^{14}C_2$]salicylic acid (**9**) (0.865 g, 2.81 mmol, Wizard Laboratories, 120 mCi, 42.66 mCi/mmol) and THF (6.4 mL) was added oxalyl chloride (0.820 g, 6.46 mmol, 2.3 eq) in THF (2.0 mL) and one drop of DMF. Following a vigorous outgassing, the solution was stirred under argon at room temperature for 1.5 h. The volatiles were removed *in vacuo* and the resulting yellow solid was used in the subsequent reaction without further purification.

1,2-Benzisothiazole-3-(2*H*)-one-[carbonyl-^{14}C] (11). Chlorine gas was bubbled through a stirred suspension of acid chloride **10** in CH_2Cl_2 (7.7 mL) for 0.25 h. Bubbling was discontinued and the resulting yellow solution was stirred at room temperature under argon for 1 h. The solution was added dropwise with vigorous stirring to concentrated NH_4OH (3.5 mL) cooled in an ice-water bath. The reaction mixture was allowed to warm to room temperature and stirred for 1 h. The slurry was filtered and the solid collected, washed with water (125 mL), and dried at 40 °C under vacuum to give 0.332 g of **11** as a tan solid. The aqueous phase of the filtrate was separated and concentrated *in vacuo*. The resulting residue was purified by flash chromatography eluting with CH_2Cl_2/CH_3OH (9:1) to give an additional 0.050 g of **11** as a

second crop for a total yield of 0.382 g (45% based on **9**). This material co-eluted with authentic unlabeled **11** by TLC (CH_2Cl_2/CH_3OH, 9:1, R_f 0.54).

3-Chloro-1,2,[3-^{14}C]benzisothiazole (12). Phosphorus pentachloride (1.57 g, 7.54 mmol, 3.0 eq) was added to benzisothiazolone **11** (0.382 g, 2.51 mmol) and the mixture was heated at 106 °C with stirring under argon for 4 h. The reaction mixture was cooled in an ice-water bath for 5 min, quenched with water (16 mL), and extracted with CH_2Cl_2 (2 x 40 mL). The combined CH_2Cl_2 extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated to a yellow powder. This material was purified by flash chromatography eluting with $CHCl_3$ to yield **12** (0.182 g, 43%) as a yellow oil. This material co-eluted with authentic unlabeled **12** by TLC ($CHCl_3$, R_f 0.88).

3-(1-Piperazinyl)-1,2,[3-^{14}C]benzisothiazole (13). Piperazine (0.553 g, 6.42 mmol, 6.0 eq) was added to chloride **12** (0.182 g, 1.07 mmol) and the resulting mixture was heated at 170 °C with stirring under argon for 2 h. The melt was allowed to cool to room temperature, whereupon the mixture solidified upon cooling. The resulting solid was partitioned between CH_2Cl_2 (20 mL) and 1*M* NaOH (20 mL). The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (20 mL). The combined CH_2Cl_2 extracts were washed with 1*M* NaOH (20 mL) and brine, dried over Na_2SO_4, filtered, and concentrated to give **13** as a brown semi-solid, which was used without further purification. Analysis of this material by TLC indicated that the major spot co-eluted with authentic unlabeled **13** ($EtOH/NH_4OH$, 9:1, R_f 0.66).

2-(4-(4-(1,2,[3-^{14}C]Benzisothiazole-3-yl)-1-piperazinyl)butyl)phthalimide (14). To a solution of compound **13** in CH_3CN (2 mL) was added N-(4-bromobutyl)phthalimide (0.302 g, 1.07 mmol) and triethylamine (0.141 g, 1.39 mmol, 1.3 eq) in CH_3CN (3.0 mL). The mixture was heated at reflux with stirring under argon for 4 h then stirred at room temperature for 17 h. The mixture was concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 and purified by flash chromatography eluting with hexanes/EtOAc (1:1). The fractions were combined and evaporated to give 0.145 g of **14** (32% based on **12**) as a yellow solid. This material co-eluted with authentic unlabeled **14** by TLC (hexanes/EtOAc, 1:1, R_f 0.19).

3-(4-(4-Aminobutyl)-1-piperazinyl)-1,2,[3-^{14}C]benzisothiazole (15). To a solution of **14** (0.145 g, 0.34 mmol) in CH_3OH (3.0 mL) was added hydrazine hydrate (0.026 g, 0.52 mmol, 1.5 eq). The mixture was heated at reflux with stirring under argon for 5 h then stirred at room temperature for 108 h. The mixture was quenched with 2*M* HCl (1.7 mL) and stirred for 20 min.

The suspension was filtered over celite and the filter cake was washed with CH_3OH (50 mL). The filtrate was concentrated *in vacuo* and the resulting solid was partitioned between 1*M* NaOH (4.3 mL) and CH_2Cl_2 (20 mL). The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (15 mL). The combined CH_2Cl_2 extracts were dried over Na_2SO_4, filtered, and concentrated to give **15** as a yellow oil, which was used without further purification.

2-Amino-N-(4-(4-(1,2,[3-^{14}C]benzisothiazol-3-yl)-1-piperazinyl)butyl)benzamide Hydrochloride (3). Isatoic anhydride (0.056 g, 0.34 mmol) and anhydrous EtOH (1.4 mL) were added to a solution of primary amine **15** in anhydrous EtOH (0.6 mL). The resulting suspension was stirred at room temperature under argon for 19 h. The EtOH was removed *in vacuo* and the residue was purified by flash chromatography eluting with CH_2Cl_2/CH_3OH (95:5). The fractions were combined and evaporated. The residue was purified further by flash chromatography eluting with CH_2Cl_2/CH_3OH (95:5) to give the free base of **3** (0.112 g) as a clear oil. The oil was dissolved in EtOAc (1.0 mL) and 3.6*M* HCl in EtOAc (0.076 mL, 0.27 mmol) was added with stirring. The volatiles were removed *in vacuo* and the residue was crystallized from 2-propanol and dried under vacuum at room temperature for 16 h to give **3** (0.086 g, 56% based **14**) as an off-white solid: specific activity 26.55 mCi/mmol (overall radiochemical yield of 3.4% based on **9**). This material co-eluted (after neutralization with 1*M* NaOH) with authentic **1** by TLC (CH_2Cl_2/CH_3OH, 95:5, R_f 0.55). Radiochemical purity by TLC using the same TLC system was 99.8%. Radiochemical purity by HPLC was 99.3%.

2-Amino-N-(4-(4-(1,2-benzisothiazol-3-yl)-1-piperazinyl)butyl)benzamide-[carbonyl-^{14}C] Hydrochloride (4). 3-(4-(4-Aminobutyl)-1-piperazinyl)-1,2-benzisothiazole (**17**)[4] (0.592 g, 2.04 mmol), isatoic anhydride-[carbonyl-^{14}C] (**16**) (0.335 g, 2.05 mmol, Wizard Laboratories, 40 mCi, 19.56 mCi/mmol), and anhydrous EtOH (8.0 mL) were combined and stirred at room temperature under argon for 23 h. The solvent was removed *in vacuo* to give a brown oil. The crude material was purified by flash chromatography eluting with CH_2Cl_2/CH_3OH (95:5). The fractions were combined and concentrated to give the free base of **4** (0.708 g) as a light brown oil. Radiochemical purity of the free base was found to be 98.4% by TLC. The free base was dissolved in EtOAc (6.0 mL), and 3.6*M* HCl in EtOAc (0.48 mL, 1.73 mmol, 1.0 eq) was added with stirring. The volatiles were removed *in vacuo*, and the residue was crystallized from 2-propanol (10.6 mL) and dried under vacuum at room temperature for 3 h to give **4** (0.513 g, 56%) as an off-white solid: specific activity 22.67 mCi/mmol. This material co-eluted (after neutralization with 1*M* NaOH) with authentic **1** by TLC (CH_2Cl_2/CH_3OH, 95:5,

R_f 0.55). Radiochemical purity by TLC using the same TLC system was 99.0%. Radiochemical purity by HPLC was 99.2%.

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