THE SYNTHESIS OF 1,3,4-THIADIAZOL-2-YLCYANAMIDE SODIUM, A POTENTIALLY USEFUL ANTI-INFLUENZA AGENT AND ITS [5-^{14}C] AND [UL-$^{13}C_3$] ISOTOPOMERS

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SUMMARY

As an aid to the study of the metabolism and disposition of LY217896 in laboratory animals and humans, ^{14}C-labeled material was prepared utilizing formic-[^{14}C] acid as the starting material. Uniformly labeled LY217896-[^{13}C] was also synthesized for use in metabolite identification. Formic acid, cyanogen bromide, and potassium cyanide which were >99% isotopically enriched with ^{13}C were utilized; once the appropriate starting materials were synthesized, the synthesis of the ^{13}C and ^{14}C-labeled materials were parallel.

Keywords: anti-viral, LY217896 Na, C-14, UL-C-13

INTRODUCTION

The anti-viral activity of 1,3,4-thiadiazol-2-ylcyanamide (**1**, LY217896) has been discussed.[2] While not active against poliovirus and rhinovirus, the *in vitro* spectrum of LY217896 includes activity against influenza, herpes, vaccinia, parainfluenza, and several strains of the paramyxovirus family. LY217896 has also been shown to be active in a mouse influenza model.[3] Particularly impressive is the fact that unlike the commercially available anti-influenza agents, LY217896 is active against both A and B strains of influenza both *in vitro* and *in vivo*.[4] Because of the unique activity of LY217896, a clinical evaluation has been launched. In support of pre-clinical as well as clinical drug metabolism and disposition studies, preparation of ^{14}C-labeled material was undertaken. In order to facilitate the identification of metabolites, material uniformly labeled with ^{13}C was also synthesized. The preparation of these labeled isotopomers of LY217896 is the subject of this report.

DISCUSSION

Preliminary accounts of the synthesis of LY217896 (**1**) have been published.[5,6] Thiosemicarbazide (**2a**) was formylated by reaction with formic-[^{14}C] acid in the

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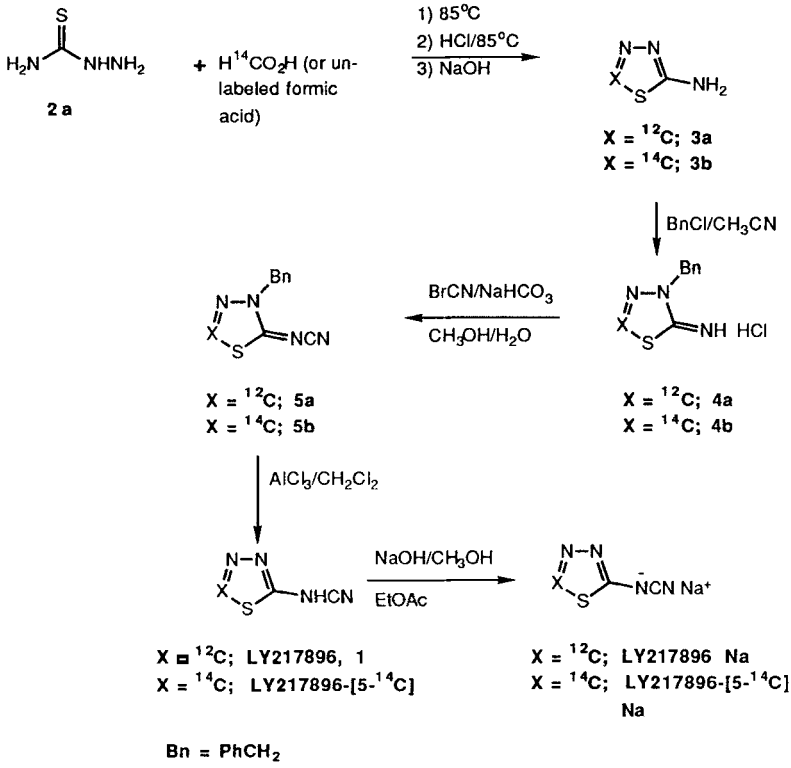
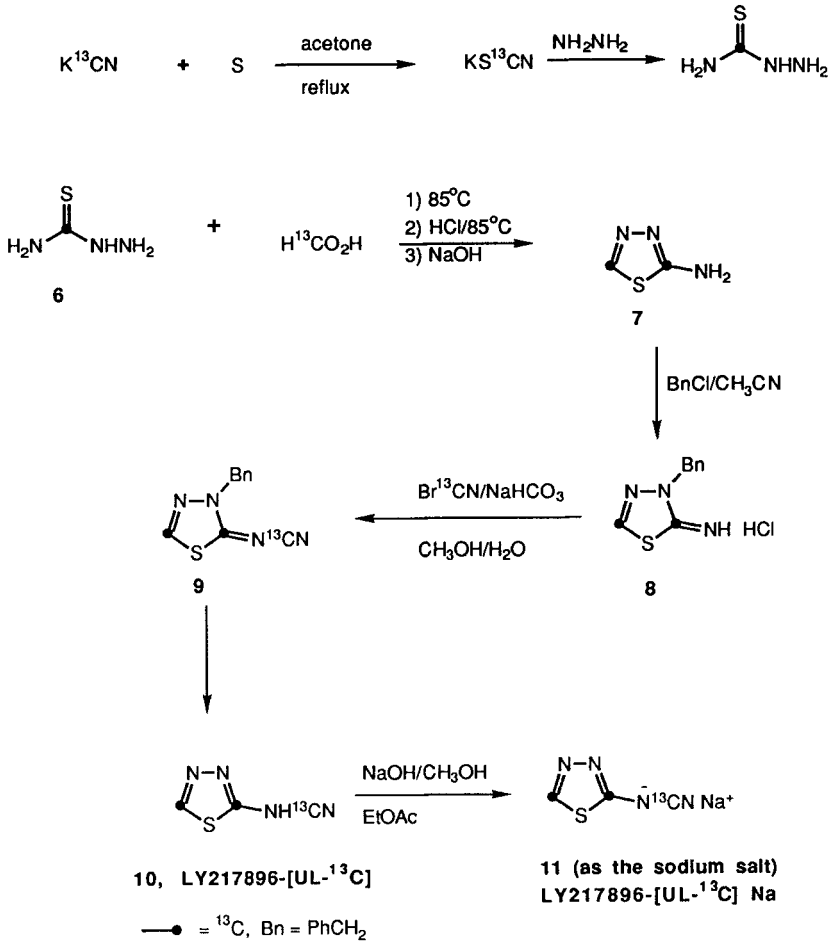
absence of solvent at 80-90°C for 0.5 hr (**Scheme 1**). The crystalline product was not isolated, but dissolved in 12N hydrochloric acid and heated for an additional 1 hr at 85°C to yield after work-up 2-amino-[1,3,4]-thiadiazole(**3a,b**) in 60% yield for the two steps. Reaction of **3** with benzyl chloride in acetonitrile, yielded the 3-benzylated product **4a,b**. Victor *et al* had earlier shown by unambiguous synthesis of both regioisomers, that benzylation of **3a** occurs

SCHEME 1



primarily in the three position.[7] Reaction of **4a,b** with cyanogen bromide in methanol, followed by deprotection of the resulting cyanamide **5a,b** with aluminum chloride yielded **LY217896** (or its 5-^{14}C-isotopomer). Reaction of **LY217896** (or its 5-^{14}C-isotopomer) with 5N sodium hydroxide, followed by crystallization from methanol/ethyl acetate provided **LY217896 Na** (or its 5-^{14}C-isotopomer). **LY217896-[5-^{14}C] Na** was identical to its unlabeled counterpart when examined by TLC (EtOAc/CH_3OH/NH_4OH, 70:30:1 and EtOAc/CH_2Cl_2/CH_3OH/NH_4OH, 20:20:10:1) and HPLC (Zorbax CN, hexanes/CH_3OH/CH_2Cl_2, 79:11:10 at 1 mL/min). The radiochemical purity was ≥99.2% in all systems examined. The specific activity was 34.75 μCi/mg (5.14 mCi/mmol)

SCHEME 2



Potassium cyanide-[^{13}C] was reacted with sulphur in refluxing acetone to yield potassium thiocyanate-[^{13}C] **(Scheme 2)**.[8] Utilizing a procedure described by Clark and Roth for the synthesis of thiosemicarbazide-[^{35}S][9], the crude potassium thiocyanate-[^{13}C] was reacted with hydrazine in 2-methoxyethanol to yield thiosemicarbazide-[^{13}C] (**6**). Further reaction as described above using ^{13}C-labeled formic acid and cyanogen bromide yielded LY217896-[UL-^{13}C] Na (**11**). LY217896-[UL-^{13}C] Na (**11**) co-eluted with authentic **LY217896 Na** on TLC; however, the ^{13}C-isotopomer eluted slightly ahead (R_T = 13.69 min) of its unlabeled counterpart (R_T = 14.60 min) by HPLC. Such isotopic fractionation has previously been reported.[10]

EXPERIMENTAL

Formic -[^{14}C] acid was purchased from DuPont NEN. Formic-[^{13}C] acid and potassium cyanide-[^{13}C] were purchased from Aldrich Chemical Co.; cyanogen bromide was purchased from Merck and Co/Isotopes. NMR spectra were

obtained on a General Electric QE-300 nuclear magnetic resonance spectrometer at 300 (^{1}H) and 75 (^{13}C) MHz . Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane. Direct chemical ionization mass spectra (DCI-MS) and electron impact mass spectra (EI-MS) were recorded on a Nermag R30-10 triple stage quadrapole mass spectrometer. High resolution FAB mass spectra were recorded on a VG Analytical VG-ZAB 3F mass spectrometer.[11] Microanalytical, IR, and UV data were provided by the Physical Chemistry Research Department of the Lilly Research Laboratories.

Flash chromatography was performed as described by Still *et al.*, using E.M. Science silica gel 60 (230-400 mesh).[12] Unless otherwise noted, the organic extracts were dried over anhydrous sodium sulfate.

Radiochemical purity (RCP) was assessed by autoradiography employing E. Merck silica gel F-254 TLC plates and Kodak BB-5 x-ray film. The radioactive lane was divided, suspended in methanol, and after sonication, the mixture was diluted with Aquassure™ scintillation cocktail (DuPont NEN) and counted. As a further check of the radiochemical purity, the sample was subjected to radio-HPLC; 30 sec samples of the eluent were collected, diluted with Aquassure™ and counted.

2-Amino-1,3,4-thiadiazole-[5-^{14}C] (3b): A mixture of thiosemicarbazide (**2**) (3.64 g, 40 mmol), formic acid (1.357 g, 1.112 mL, 29.5 mmol), and formic acid-[^{14}C] (200 mCi, 19.0 mCi/mmol, 10.5 mmol) was heated at 80-90°C for 0.5 hr. The reaction mixture was then dissolved in 33.3 mL of conc. hydrochloric acid; heating was continued at 85°C for 1 hr, then the reaction was allowed to stand overnight at room temperature. The mixture was concentrated *in vacuo* and the residue was redissolved in water. The resulting solution was made basic by the portionwise addition of sodium hydroxide (1N), whereupon a solid crystallized. The mixture was chilled in an ice bath for 1 hr and the solid was collected by filtration, washed with water, and dried to yield **3b** (2.40467 g, 59.5%).

This material co-migrated with authentic 2-amino-1,3,4-thiadiazole (**3a**) on TLC (methylene chloride/ethyl acetate/ methanol/ammonium hydroxide 20:20:10:1).

3-Phenylmethyl)-1,3,4-thiadiazol-2(3H)-imine Monohydrochloride (4a): A 2-propanol (IPA) (35 mL) suspension of 2-amino-1,3,4-thiadiazole (**3a**) (2.4 g, 23.76 mmol) was heated at reflux and after dissolution treated dropwise with benzyl chloride (3.0 mL, 26.25 mmol). The resulting solution was stirred at reflux for 2 hr and then allowed to cool to room temperature. A white precipatate formed which was collected by filtration, washed with fresh IPA, and dried to yield crude **4a** (1.787 g).

This material was mixed with material from another run (1.869 g) and was recrystallized from water (15 mL) to yield **4a** (2.484 g): ^{1}H-NMR (DMSO/d_6) δ 5.61 (s, 2H, CH_2), 7.39 (m, 5H, aromatic), and 9.95 ppm (s, 1H, H-5); ^{13}C-NMR (DMSO/d_6) δ 52.89, 128.08, 128.28, 128.68, 132.89, 145.29, and 166.72;

UV(EtOH) λ (ε_M) 242 (5010) and 204 (11853); EIMS M^+ 192.
Analysis calc'd for $C_9H_{10}ClN_3S$: C, 47.47; H, 4.43; N, 18.45; S, 14.08; and Cl, 15.57. Found: C, 47.61; H, 4.37; N, 18.61; S, 14.30; and Cl, 15.77.

3-Phenylmethyl)-1,3,4-thiadiazol-2(3H)-imine-[5-^{14}C] Monohydrochloride (4b): 2-Amino-1,3,4-thiadiazole-[5-^{14}C] (**3b**) (2.40467 g, 23.8 mmol) was suspended in acetonitrile, stirred at room temperature, and treated dropwise with benzyl chloride (3.308 g, 3.0 mL, 26.25 mmol). The mixture was stirred at reflux overnight and then allowed to cool to room temperature. The resulting white solid was collected by filtration, washed with water, and dried. Recrystallization from water (13 mL) yielded **4b** (1.57581 g, 29.2%).

TLC (EtOAc/CH_2Cl_2/CH_3OH/NH_4OH, 20:20:10:1) showed that **4b** was a single component which co-eluted with **4a**.

3-(Phenylmethyl)-1,3,4-thiadiazol-2(3H)-ylidenecyanamide, (5a): A mixture of **4a** (925 g, 4.06 mol) in 5.2 L of 1:1 methanol/water was stirred at room temperature and sodium bicarbonate (809 g, 9.63 mol) was added portionwise. A solution of cyanogen bromide (505 g, 4.76 mol) in methanol (1.37 L) was added dropwise over 10-15 min. The resulting mixture was heated at 50-60°C for 2 hr and then cooled to 35-40°C. Water (5.2 L) was added and the reaction mixture was cooled to 10°C and stirring was continued for 2 hr. The resulting solid was collected by filtration, washed with water, and dried to yield **5a** (870 g, 98.9%): ^{1}H-NMR (DMSO/d_6) δ 5.26 (s, 2H, CH_2), 7.35 (m, 5H, aromatic), and 8.89 ppm (s, 1H, H-5); ^{13}C-NMR (DMSO/d_6) δ 52.09, 117.25, 127.96, 128.10, 134.61, 142.59, and 171.16; UV(EtOH) λ (ε_M) 277 (10449), 236 (5596) and 203 (11993); FT-IR 2181.77 cm^{-1}(CN), EIMS M^+ 217.

Analysis calc'd for $C_{10}H_8N_4S$: C, 55.54; H, 3.73; N, 25.91; and S, 14.82. Found: C, 55.64; H, 3.80; N, 25.89; and S, 14.94.

3-(Phenylmethyl)-1,3,4-thiadiazol-2(3H)-ylidenecyanamide-[5-^{14}C], (5b): A suspension of **4b** (1.57581 g, 6.94 mmol) in 1:1 methanol/ water (13 mL) was treated portionwise with sodium bicarbonate (1.407 g, 16.75 mmol). The resulting mixture was stirred at room temperature and treated dropwise with cyanogen bromide (0.881 g, 8.31 mmol) in methanol (1.78 mL). The reaction was heated at 60-70°C for 1 hr, allowed to cool to 40°C, and then treated dropwise with water (6 mL). Stirring at 40°C was continued for an additional 1 hr whereupon the reaction mixture was allowed to cool to room temperature. After cooling to 4°C, the solid was collected by filtration. TLC (silica gel, heptane/ethyl acetate 1:1) showed the presence of two lower R_f impurities; the

material was purified by flash chromatography on silica gel, eluting with 10 mL fractions of 1:1 heptane/EtOAc. Fractions 28-55 were combined, concentrated *in vacuo*, and crystallized from 2-propanol to yield **5b** as a white solid (0.79641 g, 53.1%).

TLC (heptane/EtOAc 1:1) showed a single component which co-migrated with **5a**.

1,3,4-Thiadiazol-2-ylcyanamide, Sodium (LY217896 Na): A mixture of aluminum chloride (1.45 kg, 10.9 mol) and methylene chloride (3.6 L) was chilled with stirring at 5-10°C. A methylene chloride solution (8.7 L) of **5a** (650 g, 3.0 mol) was added dropwise over 1 hr, keeping the temperature below 25°C. After stirring for 24 hr, tetrahydrofuran (1.85 L) was added dropwise. A polymeric side product was removed by filtration (the solid material was washed with 2 x 1.85 L of methylene chloride) and the filtrates were concentrated to half of the original volume. The remaining solution was added to water (3.6 L) with stirring (temperature >25°C), whereupon crude **LY217896** precipitated. This material was collected by filtration, washed with water, and dried (234 g, 62%). This material was slurried in water (1.2 L) and treated with 50% NaOH to pH 7.0-8.0; charcoal was added and the mixture was stirred for 3 hr. The mixture was filtered and the filtrate was acidified to pH 2.0 with 12N HCl. The resulting solid was collected and dried (215 g).

The solid was mixed with methanol (1.0 L), made basic with 50% NaOH, and charcoal treated. The mixture was filtered and the solid was washed with methanol (2 x 200 mL). The combined filtrates were concentrated by one half in vacuo (a solid formed), and diluted with EtOAc (1.0 L). The resulting mixture was concentrated to remove additional methanol and more EtOAC was added (2.1 L). The resulting slurry was chilled to 5-10°C and after 2 hr, filtered to yield **LY217896 Na** (244.2 g, 55%) as a white solid: ^{1}H-NMR (DMSO/d_6) δ 8.47 (s, 1H, H-5); ^{13}C-NMR (DMSO/d_6) δ 123.37 (CN), 141.67(C-5), and 175.41(C-2); UV(EtOH) λ (e_M) 283 (9753), 229 (3943) and 203 (5239); FT-IR 2167 and 2181 cm^{-1}.

Analysis calc'd for C_3HN_4NaS: C, 24.53; H, 0.63; N, 37.82; and S, 21.65. Found: C, 24.61; H, 0.70; N, 37.84; and S, 21.54.

1,3,4-Thiadiazol-2-ylcyanamide-[5-^{14}C], Sodium (LY217896-[5-^{14}C] Na): Methylene chloride (7.5 mL) was chilled to -5° to 0°C. Aluminum chloride (1.77 g, 13.3 mmol) was added, followed by the dropwise addition of **5b** (0.79641 g, 3.69 mmol) in methylene chloride (7.5 mL). The mixture was allowed to warm to room temperature and stirring was continued overnight. The mixture was re-chilled to -5° to 0°C and treated dropwise with tetrahydrofuran

(15 mL) and stirred for 1 hr, followed by the dropwise addition of water (38 mL). Stirring was continued (1 hr), while the reaction mixture warmed to room temperature. The mixture was filtered to remove a yellow polymeric material. The filter cake was washed with methylene chloride (2 x 25 mL); the combined filtrates were extracted with water (5 mL) and made basic with 1N sodium hydroxide (4.5 mL, pH = 8-9). The aqueous layer was washed with methylene chloride and then acidified with 1N hydrochloric acid (pH = 2). A precipitate slowly formed; the mixture was allowed to stand at room temperature for 1 hr and then was chilled to 4°C. The solid was collected by filtration, washed with water, and dried *in vacuo* to yield **LY217896-[5-^{14}C]** (0.21378 g, 46%).

A methanolic suspension (2 mL) of **LY217896-[5-^{14}C]** (0.21378 g, 1.698 mmol) was treated with 5N sodium hydroxide (0.339 mL). Charcoal was added, the mixture was stirred for ten minutes and then the mixture was filtered through a plug of Hi-Flo super-cel. The filter cake was washed with methanol (2 x 5 mL) and the combined filtrates were concentrated *in vacuo*. The residue was re-dissolved in ethanol (10 mL) and re-concentrated. The residue was suspended in ethanol (5 mL), chilled to 4°C, and diluted with ethyl acetate (5 mL). After 2 hr at 4°C, the solid was collected by filtration, washed with ethyl acetate, and dried *in vacuo* to yield **LY217896-[5-^{14}C] Na** (0.13520 g, 24.8%).

LY217896-[5-^{14}C] Na was identical to **LY217896 Na** when examined by TLC (EtOAc/CH_3OH/NH_4OH, 70:30:1 and EtOAc/CH_2Cl_2/ CH_3OH/NH_4OH, 20: 20:10:1) and HPLC (Zorbax CN, hexanes/ CH_3OH/CH_2Cl_2, 79:11:10 at 1 mL/ min). The radiochemical purity was ≥99.2% in all systems examined. The specific activity was 34.75 μCi/mg (5.14 mCi/mmol)

Thiosemicarbazide-[^{13}C], (6): An acetone (200 mL) solution of potassium cyanide-[^{13}C] (5 g, 75.63 mmol) and sulphur (2.42, 75.63 mg-a) was stirred at reflux for 4 hr. The mixture was allowed to cool to room temperature and concentrated *in vacuo*, yielding potassium thiocyanate-[^{13}C] as an off-white solid.

The solid was redissolved in water (25 mL), mixed with a 15.4 mL aliquot of a hydrazine stock solution (30 g of hydrazine hydrate was mixed with 66.7 g of hydrazine sulfate and diluted to 100 mL with water), and stirred for 0.5 hr at room temperature. The resulting mixture was diluted with 2-methoxyethanol (260 mL) and distilled at 150-160°C (external temperature) until the reaction temperature reached 130°C; refluxing at 130°C was then continued for 2 hr. The mixture was allowed to cool to room temperature and then chilled at -10°C overnight. The white solid was collected by filtration, washed with water, and dried *in vacuo* to yield **6** (4.011 g, 57%): ^{1}H-NMR (DMSO/d_6) δ 4.47 (s, 2H, N-NH_2), 7.17 and 7.53 (bs, 2H, C-NH_2), and 8.61 (d, 1H, J_{13C-H} = 8.32 Hz, C-NH);

^{13}C-NMR (DMSO/d_6) δ 181.54. This material co-eluted with authentic thiosemicarbazide on TLC (CH_2Cl_2/EtOAc/CH_3OH/NH_4OH, 20:20:10:1).

HR-EIMS calc'd for $^{13}CH_5N_3S$: 92.1201. Found: 92.02379.

2-Amino-1,3,4-thiadiazole-[3,5-$^{13}C_2$] (7): A mixture of **6** (3.91 g, 42.6 mmol) and formic-[13C] acid (4.0 g, 85.2 mmol) was heated at 85°C for 0.5 hr and then worked up as described above to yield **7** (4.56g, > 100%): ^{1}H-NMR (DMSO/d_6) δ 7.17 (s, 2H, NH_2), 8.55 (dd, 1H, J_{13C-H} = 214.5, 3.05 Hz, 5-H); ^{13}C-NMR (DMSO/d_6) δ 142.96 (C-5) and 168.65 (C-2). TLC (CH_2Cl_2/EtOAc/CH_3OH/NH_4OH, 20:20:10:1) showed a single component co-eluting with authentic 2-amino-1,3,4-thiadiazole.

HR-FABMS calc'd for $^{13}C_2H_4N_3S$ + H: 104.0193. Found: 104.0195.

3-Phenylmethyl)-1,3,4-thiadiazol-2(3H)-imine-[3,5-$^{13}C_2$] Monohydrochloride (8): An acetonitrile solution (30 mL) of 7 (2.619 g, 25.4 mmol) was treated with benzyl chloride as described above to yield **8** (2.917 g, 50%) as a light tan solid. Re-crystallization from water yielded purified **8** (1.254 g, 21.7%) as a white solid: ^{1}H-NMR (DMSO/d_6) δ 5.49 (d, 2H, J = 2.55 Hz, CH_2), 7.41 (m, 5H, aromatic), 8.90 (dd, 1H, J_{13C-H} = 225.6, 2.50 Hz, 5-H), and 10.42 (bs, 2H, NH HCl); ^{13}C-NMR (DMSO/d_6) δ 145.66 (C-5) and 167.29 (C-2) The peaks arising from the benzyl were absent due to the limited sample and number of scans. TLC (CH_2Cl_2/EtOAc/CH_3OH/NH_4OH, 20:20:10:1) showed a major spot co-eluting with authentic **4a** along with a minor contaminant of lower R_f (unreacted starting material **7**).

HR-FABMS calc'd for $^{13}C_2{}^{12}C_7H_{10}N_3S$ + H: 194.0663. Found: 194.0674.

3-(Phenylmethyl)-1,3,4-thiadiazol-2(3H)-ylidenecyanamide-[3,5,cyano-$^{13}C_3$], (9): A suspension of **8** (1.20 g, 5.26 mmol) in 1:1 methanol/ water (10 mL) was treated portionwise with sodium bicarbonate (1.067 g, 12.7 mmol). The resulting mixture was stirred at room temperature and treated dropwise with cyanogen bromide-[^{13}C] (0.674 g, 6.3 mmol) in methanol (1.35 mL). The reaction was heated at 60-70°C for one hour, allowed to cool to 40°C, and then treated dropwise with water (4.6 mL). Stirring at 40°C was continued for an additional 1 hr whereupon the reaction mixture was allowed to cool to room temperature. After cooling to 4°C, the solid was collected by filtration. TLC (silica gel, heptane/ethyl acetate 1:1) showed the presence of two lower R_f impurities; the material was purified by flash chromatography on silica gel, eluting in 10 mL fractions of 1:1 pentane/ethyl

acetate. Fractions 17-29 were combined, concentrated *in vacuo*, and crystallized from 2-propanol to yield **9** as a white solid (0.73857 g, 60.7%).

TLC (heptane/ethyl acetate 1:1) showed a single component which co-migrated with **5a**.

1,3,4-Thiadiazol-2-ylcyanamide-[UL-$^{13}C_3$], Sodium (LY217896-[UL-$^{13}C_3$] Na): Methylene chloride (7 mL) was chilled to -5° to 0°C. Aluminum chloride (1.62 g, 12.14 mmol) was added, followed by the dropwise addition of **9** (0.7386 g, 3.37 mmol) in methylene chloride (7 mL). The mixture was allowed to warm to room temperature and stirring was continued overnight. The reaction mixture was treated as described above to yield LY217896-[UL-$^{13}C_3$] (0.1643 g). This material was dissolved in methanol (3 mL) and treated with 5N NaOH (0.253 mL, 1.265 mmol) and concentrated *in vacuo*. The mixture was re-dissolved in methanol, treated with charcoal, and filtered. The solid was washed with methanol (2 x 5 mL) and filtered. The combined filtrates were combined and concentrated *in vacuo*. The residue was re-dissolved in ethanol (5 mL), chilled, and treated dropwise with EtOAc (5 mL). The resulting solid was collected by filtration to yield LY217896-[UL-$^{13}C_3$] Na (0.136 g, 26.7%): ^{1}H-NMR (DMSO/d_6) δ 8.40 (dd, 1H, $J_{^{13}C\text{-}H}$ = 211.7, 2.55 Hz, 5-H); ^{13}C-NMR (DMSO/d_6) δ 123.87 (CN), 141.97 (C-5), and 175.72 ppm (C-2). TLC (EtOAc/CH_3OH/NH_4OH, 70:30:1 and EtOAc/CH_2Cl_2/CH_3OH/NH_4OH, 20:20:10:1) showed a single component which co-migrated with LY217896 Na. On HPLC (Zorbax CN, hexanes/ CH_3OH/CH_2Cl_2, 79:11:10 at 1 mL/min) the ^{13}C-isotopomer eluted slightly ahead (R_T = 13.69 min) of its unlabeled counterpart (R_T = 14.60 min). Such isotopic fractionation has previously been reported.[10]

HR-FABMS calc'd for $^{13}C_3HN_4SNa$ + Na: 173.9811. Found: 173.9818.

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