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Synthesis of three alpha 7 agonists in labeled form

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In support of a program to develop an alpha 7 agonist as a treatment for Alzheimer's disease, three drug candidates, 1, 2, and 3, were prepared in labeled forms. Compound 1 was prepared in C-14 labeled form by lithiation of [2,6-$^{14}C_2$] 2-chloropyridine and subsequent coupling with spirooxirane-2,3′-quinuclidine. When this same coupling was attempted using [3,4,5,6-$^{2}H_4$]2-chloropyridine, alcohol [$^{2}H_6$]-6 was the major product indicating that the primary isotope effect for the lithiation step was significant enough to shift the reaction pathway. Therefore, an alternate site of labeling was used to prepare [$^{2}H_4$]-1. [$^{13}C_5$]-2 was prepared in five steps from [$^{13}C_5$]2-furoic acid, but the C-14 labeled compound used [$^{14}C_2$]-1 as the starting material instead. [$^{14}C_2$]-3 was prepared in two steps from [carbonyl-^{14}C]nicotinic acid.

Keywords: deuterium isotope effect; alpha 7; [carbonyl-^{14}C]nicotinic acid; [$^{13}C_5$]2-furoic acid

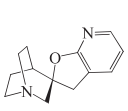
Introduction

Nicotinic receptors are a widely distributed family of ligand-gated ion channels. They are pentameric in structure, made up of various transmembrane subunits, denoted α, β, γ, δ, and ε. Assembly of these subunits in various combinations gives a diversity of receptors, with a range of electrical and binding properties.[1] In the brain, $\alpha4\beta2$ and $\alpha7$ are the two most prevalent neuronal nicotinic receptor stoichiometries.[2] Relative to $\alpha4\beta2$, the $\alpha7$ receptor possesses greater sensitivity to alpha-bungarotoxin (a nicotinic receptor antagonist),[3] lower sensitivities to acetylcholine and nicotine,[4] and higher calcium ion permeability. In spite of these differences, both $\alpha4\beta2$ and $\alpha7$ have been shown to be involved in attention, learning, and working memory.[5]

Nicotine itself is known to have positive effects on cognitive function in humans and nonhumans in various cognition models.[6,7] These effects are believed to be mediated, at least in part, by the $\alpha7$ receptor, as the $\alpha7$ subtype is highly expressed in regions of the brain that are implicated in cognitive processes, such as the hippocampus, thalamus, and cortex.[8] Nicotinic receptors are also involved in cognitive deficits seen in schizophrenia, as well as associated sensory gating deficits.[9]

Further highlighting a connection between $\alpha7$ and cognition, $\alpha7$ knockout mice show deficits in processes involving both attention and cognition.[10] Because of this apparent link, modulation of $\alpha7$ receptor function is thought to provide a means for the treatment of cognitive deficits, both in neurodegenerative and psychiatric disorders.[11]

Compound **1, 2**, and **3** were identified as orally available, high-affinity $\alpha7$ agonists, and so to further probe their biological activity, they were required in labeled form.



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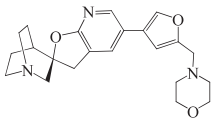

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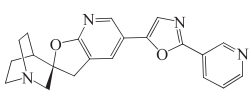

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Results and discussion

In support of a program to develop an alpha 7 agonist as a treatment for Alzheimer's disease, quinucleodine **1** was required in C-14 labeled form for initial DMPK studies.[12] The synthesis followed the medicinal chemistry route.[13] The first step consisted of a Johnson–Corey–Chaykovsky reaction[14,15] to give racemic epoxide **5** in 70% yield. Epoxide **5** was first isolated as an oil that failed to generate the desired product in the subsequent reaction, but azeotropic drying of **5** with $PhCH_3$ provided a waxy solid, which performed adequately in the next step. Coupling of epoxide **5** with [2,4-$^{14}C_2$]2-chloropyridine proceeded smoothly but in a modest crude yield of 46% after silica gel chromatography. Highly enantiomerically enriched **[$^{14}C_2$]-1** was obtained by crystallization with D-tartaric acid to give an overall yield of 12% (11% radiochemical yield) (Scheme 1).

Given this successful preparation, a similar route was envisioned for the preparation of a stable isotope-labeled isotopomer; the intent was to substitute [$^{2}H_4$]2-chloropyridine and [$^{2}H_9$]trimethylsulfoxonium iodide for [2,4-$^{14}C_2$]2-chloropyridine and unlabeled trimethylsulfoxonium iodide. As shown in Scheme 2, quinuclidone (**4**) was treated with [$^{2}H_9$]trimethylsulfoxonium iodide to afford **[$^{2}H_2$]-5** in 50% yield. The lower yield relative to the reaction of unlabeled material may be a result of

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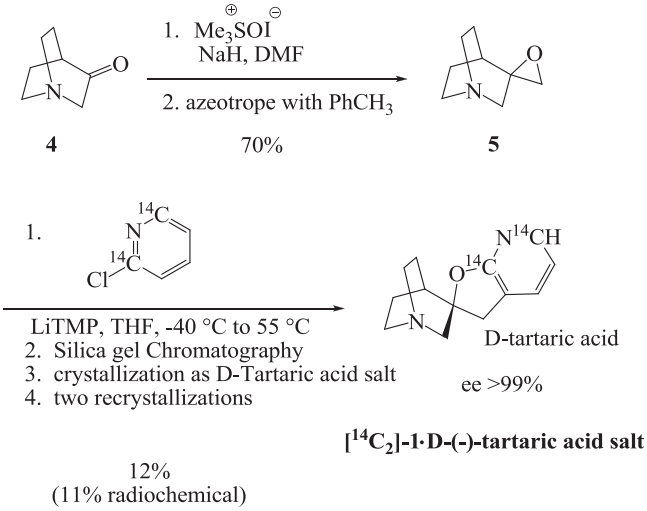
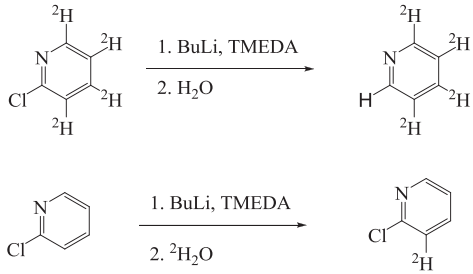


Scheme 1. Synthesis of $[^{14}C_2]$-1 · D-(−)-tartaric acid salt.

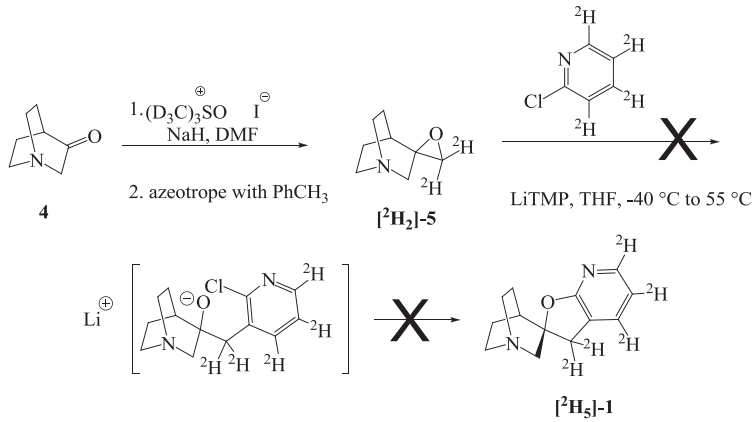
an isotope effect. $[^2H_4]$2-chloropyridine was then treated with lithium tetramethylpiperide followed by dropwise addition of the labeled epoxide. However, the expected spirocycle **$[^2H_5]$-1** was not produced. Instead, a more polar product was obtained that was identified as **$[^2H_6]$-6**. The apparent route to this product is shown in Scheme 3; we believe that treatment of $[^2H_4]$2-chloropyridine with lithium tetramethylpiperide gave halogen-metal exchange instead of deprotonation alpha to the chlorine which would afford quinuclidin-3-ol **$[^2H_5]$-6** in lieu of the desired spirocycle.

The deuterium isotope effect in the metalation reaction was tested by treating 2-chloropyridine and $[^2H_4]$2-chloropyridine under identical metalation conditions (Scheme 4). The labeled reaction was then neutralized with water, while the unlabeled reaction was neutralized with D_2O. Each reaction was monitored by TLC, and the resulting products were identified by GC/MS. Unlabeled 2-chloropyridine was recovered quantitatively and showed incorporation of a single deuterium. However, $[^2H_4]$ 2-chloropyridine showed $[^2H_4]$pyridine as the major product with the chlorine replaced by a proton. There was also a small amount of unreacted starting material recovered.

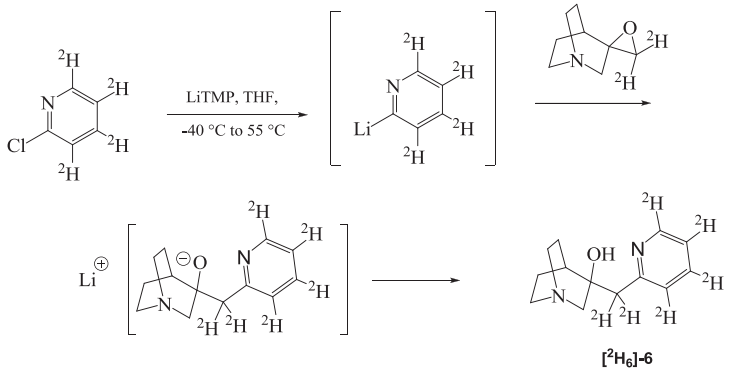
These results confirmed that the original route to the target compound was not viable for the synthesis of deuterated **1**. Clearly, an alternative route of synthesis was going to have to be developed, and this route would have to avoid, at the very least,



Scheme 4. Investigation of the route to **$[^2H_6]$-6**.



Scheme 2. Attempted preparation of stable isotope labeled **1**.

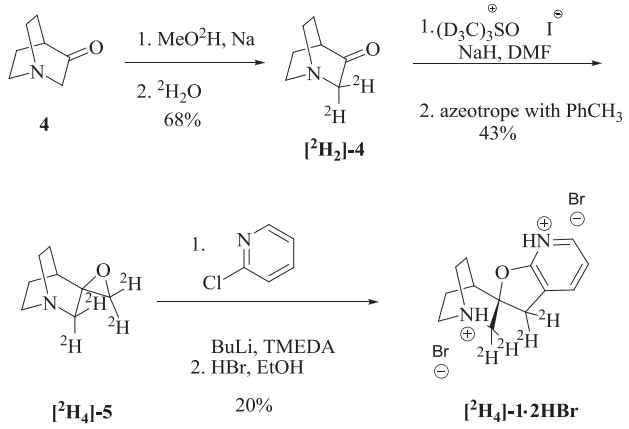


Scheme 3. Proposed synthetic route to **$[^2H_6]$-6**.

the use of a pyridine that had a deuterium ortho to the chlorine.

The successful route used for the synthesis of stable isotope labeled **1** is shown in Scheme 5. Quinuclidone (**4**) was labeled *via* deuterium exchange with CH_3OD and sodium followed by neutralization with D_2O. Deuterium incorporation was greater than 98% with less than 0.1% unlabeled starting material. This was treated as previously described with [$^2H_9$]trimethylsulfoxonium iodide to afford epoxide **[$^2H_4$]-5** in 43% yield. Spirocycle ring formation using unlabeled 2-chloropyridine successfully afforded the final compound in 20% yield.

A second alpha 7 agonist, quinucleodine **2**, was also required in stable isotope labeled form with a minimum mass increase of 4 AMU. The obvious site of labeling—the morpholine ring either with H-2 or C-13—was not pursued so that we could better prepare for our projected C-14 synthesis in which we planned to use [^{14}C]2-furoic acid as the C-14 precursor. We had previously

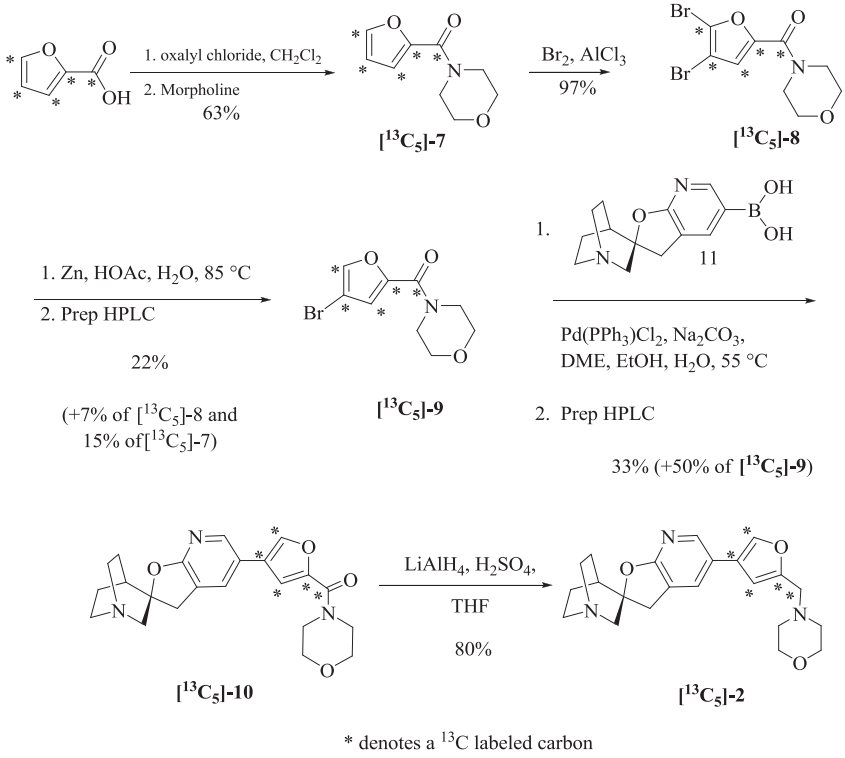


Scheme 5. Synthesis of **[$^2H_4$]-1 · 2HBr**.

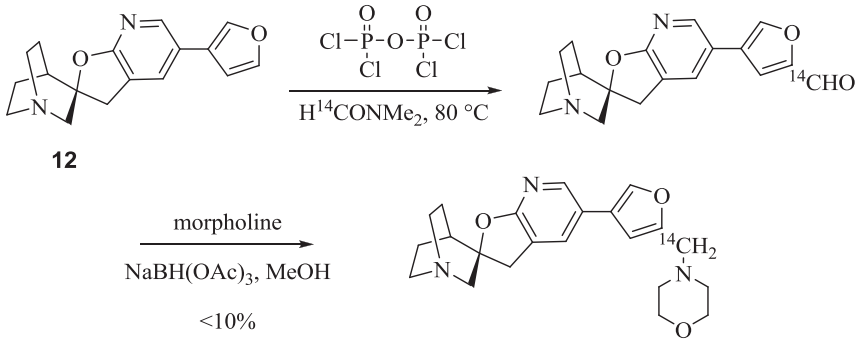
prepared [$^{13}C_5$]2-furoic acid[16] and thus started with a simple peptide coupling to give amide **[$^{13}C_5$]-7** (Scheme 6). The conversion of methyl 2-furoate to methyl 4-bromofuroate previously reported by Belen'kii and coworkers served as a good precedent for the next sequence of reactions.[17] Amide **[$^{13}C_5$]-7** was dibrominated with $AlCl_3$ in neat Br_2. The resulting dibromide **[$^{13}C_5$]-8** was debrominated to give a mixture of monobromide **[$^{13}C_5$]-9**, dibromide **[$^{13}C_5$]-8**, and des-bromide **[$^{13}C_5$]-7** in a 50:15:35 ratio. Monobromide **[$^{13}C_5$]-9** was isolated by preparative HPLC and the site of debromination confirmed by co-elution on HPLC and ^{1}H NMR analysis. The Suzuki coupling of **[$^{13}C_5$]-9** to give **[$^{13}C_5$]-10** proved to be very problematic. The yields of **[$^{13}C_5$]-10** varied dramatically from run-to-run, and the reaction gave many by-products; therefore, the reaction was run in several batches and the final compound purified by preparative HPLC to give an isolated yield of 33% yield. The final reduction proceeded smoothly to give agonist **[$^{13}C_5$]-2** in 80% yield.

While this route did deliver enough of the stable isotope-labeled compound for initial DMPK uses, it demonstrated that several of the steps were suboptimal for radiochemical use. The Suzuki coupling gave an unexpectedly poor and variable yield and attempts to improve the yield and make the chemistry more consistent were not successful. The mono-debromination was also not ideal although re-cycling of the dibromide and des-bromo compound partially compensated for this. Overall, we felt that this synthetic route was not ready for use with C-14, and yet, a rapid delivery of C-14 labeled **2** was required to support DMPK studies. Therefore, we looked into other options.

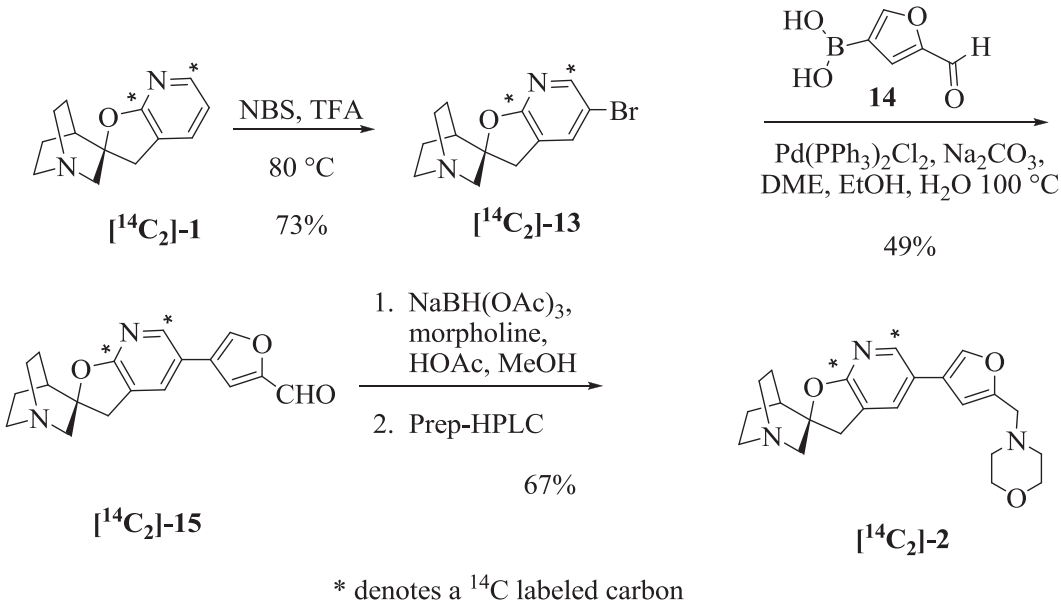
We first investigated the Vilsmeier–Haack reaction using C-14 DMF and furan **12** (Scheme 7). Various reaction conditions were probed and resulted in between 60% and 80% of the radioactivity in the organic extracts. However, this radioactivity consisted of a mixture of compounds with the same M + 1/Z ratio on LCMS as the target aldehyde. The two major radioactive HPLC peaks were



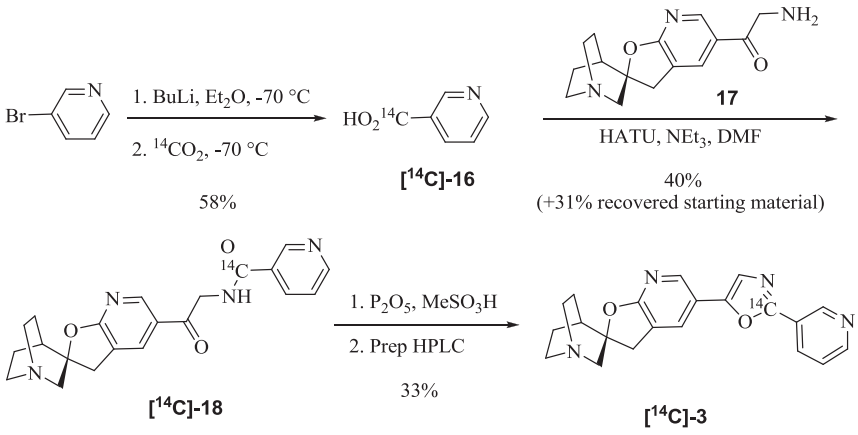
Scheme 6. Synthesis of **[$^{13}C_5$]-2**.



Scheme 7. Attempted synthesis of [^{14}C]-**2**.



Scheme 8. Synthesis of [$^{14}C_2$]-**2**.



Scheme 9. Synthesis of [^{14}C]-**3**.

broad and in approximately a 2:1 ratio. The same ratio was observed on different HPLC columns in both MeCN and MeOH and with different acid modifiers. The HPLC profile of authentic unlabeled aldehyde also gave a broad peak on HPLC that eluted with the minor peak from the Vilsmeier reaction. Reductive amination of the crude reaction mixture afforded quantitative recovery of radioactivity, but the desired compound represented less than 10% of the total radioactivity by HPLC. Therefore, this route was abandoned.

The need for C-14 labeled **2** was acute, so a secure option was pursued. A small amount of [$^{14}C_2$]-**1** was brominated using TFA and *N*-bromosuccinimide to give monobromide [$^{14}C_2$]-**13** in

good yield (Scheme 8). The bromide was reacted with boronic acid **14** in a Suzuki reaction to give aldehyde **[$^{14}C_2$]-15** in a quantitative recovery of radioactivity but only 49% radiochemical purity. The crude reaction mixture was converted to the final compound in three batches *via* reductive amination to give the target compound, **[$^{14}C_2$]-2**, in good purity after purification by HPLC.

A third alpha 7 agonist, **3**, was desired labeled with C-14 for evaluation by DMPK. [^{14}C]Nicotinic acid was prepared from 3-bromopyridine by lithiation with *n*-BuLi at −70°C and subsequent reaction with $^{14}CO_2$ to provide nicotinic acid **[^{14}C]-16** in a 59% yield (Scheme 9).[18,19] Coupling of **[^{14}C]-16** with amine **17** with HATU activation gave an incomplete reaction with many impurities. No effort was made to improve this step as the yield was deemed sufficient to deliver the requisite amount of material. Cyclization was achieved by heating amide **[^{14}C]-18** in Eaton's solution at 130°C overnight.[20] The crude yield of the reaction was good, but during purification by preparative HPLC, several products containing fractions had to be rejected because of impurities. The target oxazole **[^{14}C]-3** was isolated in 33% yield in high radiochemical purity and in 29% yield at a lower purity (81% radiochemical purity). The higher purity batch contained enough material to support the study, so the impure batch was stored for purification at a later date. The impurities were not characterized.

Conclusion

In summary, the synthesis of compounds **1** and **2** labeled with both C-14 and stable isotope labels and compound **3** with C-14 was successfully completed. During the preparation of a deuterated version of **1**, an unanticipated product was isolated which resulted from a change in the rate of deprotonation of 2-chloropyridine when the protons were substituted with deuterium. Therefore, an alternate route to deuterated **1** was developed.

Experimental

General: [2,6-$^{14}C_2$]2-chloropyridine was obtained from Quotient Bioresearch (Cardiff CF24 5JQ, UK). Ba$^{14}CO_3$ and [^{14}C]DMF were obtained from American Radiolabeled Chemicals, Inc. (St. Louis, MO, USA). [$^{13}C_5$]2-furoic acid was prepared according to the method of Dorff.[16] Quinuclidin-3-one, **11**, **12**, **14**, and **17** were provided by AstraZeneca CNS-Chemistry (Wilmington, DE, USA). All other reagents were obtained from Acros Organics (Pittsburgh, PA, USA), Sigma-Aldrich (St. Louis, MO, USA), Fisher (Waltham, MA, USA), and Strem (Newburyport, MA, USA) and were used without purification.

^{1}H NMR spectra were recorded on Bruker DPX 300, DRX 400, Avance 500, and DRX 600 spectrometers in $^2H_6$-DMSO, or $C^2H_3O^2H$ at 30°C and were referenced to the residual solvent peak. LC/MS analyses were performed on an HP MSD-1100 using a 4.6 × 100 mm Phenomenex Luna-C18(2) column, with a 10–100% gradient of MeOH-0.1% aq formic acid over 10 min and ESI.

High-performance liquid chromatography analyses were performed using an Agilent 1100 series HPLC system using the following conditions unless otherwise noted: 150 × 4.6 mm column, 20 min gradient elution with MeCN-0.1% TFA as mobile phase followed by a 5 min wash with 100% organic mobile phase and a flow rate of 1 mL/min using one of the following methods: method A: 100 × 4.6 mm Thermo Scientific beta basic C18, isocratic 5% aqueous MeCN with 0.026 M NH_4OAc over 30 min; method B: YMC Basic, 20–40% MeCN-0.2% diethylamine in water, 1.5 mL/mL; method C: Phenomenex Luna C-18(2), 5–100% MeOH-0.1% formic acid over 10 min followed by 3 min at 100%; method D: Phenomenex Luna C18(2), 2–20%; method E: Phenomenex Luna C18(2), 2–50%; method F: Phenomenex Curosil PFP, 2–20%; method G: Phenomenex Luna C-18(2), 10–20%; method H: Phenomenex RP-Polar, 13–17%.

GC analyses were performed on a Supelco SPB-S 30 M × 0.25 mm column with an injector temperature of 250°C, detector temperature of 280°C, and an over temperature of 70°C for 2 min then 10°C/min from 2 to 22 min.

Preparative HPLC purifications were performed on a 21.2 × 250 mm column using MeOH-0.1% TFA as the eluent and at a flow rate of 15 mL/min and were concluded with a 10 min wash with 100% organic unless otherwise noted using the following methods: method α: 30 × 100 mm Phenomenex Gemini NX C18 5 micron, 2–98% over 12 min EtOH in 0.5 M NH_4HCO_3 (pH 10); method β: Phenomenex Luna C18(2), 2–40% over 10 min then 40–70% 10–20 min; method γ: Phenomenex Luna C18, 2–20% over 15 min; 20–30% over 15–25 min; method δ: Phenomenex Luna C-18(2), 2–20% over 10 min then 20–35% over 36 min; method ε: Phenomenex Polar-RP, 5–25% MeCN-0.1% TFA over 40 min.

Silica gel purifications were performed on a Teledyne ISCO CombiFlash Companion using gradient elution. It is important to note that the quinucleodine ring is very nucleophilic and thus reacts with CH_2Cl_2 to give a quarternary amine adduct.

1′-azaspiro[oxirane-2,3′-bicyclo[2.2.2]octane] (5)

A slurry of 7.086 g (177.2 mmol) of a 60% w/w dispersion of NaH in mineral oil in 50 mL of petroleum ether was stirred for 20 min, and then the solvent was carefully removed. The residue was taken up in 20 mL of DMF, and 38.41 g (174.4 mmol) of trimethylsulphoxonium iodide was added to the slurry. The slurry was stirred for 5 h until gas evolution was complete. The reaction mixture was diluted with 20 mL of DMF, and 18.20 g (145.6 mmol) of 3-quinuclidinone in 20 mL of DMF was added. The mixture was stirred for 4.5 h and was then diluted with 350 mL of water. The resulting mixture was extracted four times with $CHCl_3$, and the combined organic extracts were washed three times with 100 mL of water and then dried ($MgSO_4$). The solution was filtered and then concentrated under reduced pressure to give an oil. The oil was diluted with $PhCH_3$ and then concentrated to azeotrope any remaining water. After drying under high vacuum, 14.11 g (70%) of **5** was obtained as a waxy solid. LC/MS (M + H): 140.1 (100%), 142.1 (10%). ^{1}H NMR (500 MHz, CDCl3): δ 3.09 (dd, J = 15, 2 Hz, 1H), 2.95 (m,1H), 2.85 (m,4H), 2.72 (m,2H), 1.97 (m,1H), 1.78 (m,2H), 1.56 (m,1H), 1.38 (m,1H). The purity of the compounds is estimated to be >95% based on the ^{1}H NMR.

(2R,4′S)-3H-1′-azaspiro[furo[2,3-b, 2,6-$^{14}C_2$]pyridine-2,3′-bicyclo[2.2.2]octane], D-(−)-tartaric acid salt, ([$^{14}C_2$]-1 · D-(−)-tartaric acid salt)

A solution of 1.6 mL (9.5 mmol) of 2,2,6,6-tetramethylpiperidine in 10 mL of THF was cooled to −60°C as 7.8 mL of a 2.5 M (19.5 mmol) solution of *n*-BuLi in hexanes was added over 10 min. The solution was stirred at −60°C for 1 h, and then a solution of 250 mCi (4.4 mmol) of [2,6-$^{14}C_2$]2-chloropyridine and 637 mg (5.61 mmol) of 2-chloropyridine in 4 mL of THF was added over 20 min. The solution was warmed to −40°C and was stirred for 40 min. Then, a solution of 1.32 g (9.51 mmol) of **5** in 10 mL of THF was added over 15 min at −40°C, and the reaction was warmed to −10°C and stirred for 5 h. It was then warmed to room temperature and stirred overnight and was then heated at 50°C for 6 h. The reaction mixture was then cooled to 0°C, and 10 mL of water and 10 mL of methyl t-butylether (MTBE) were added. The biphasic mixture was filtered through celite, and the celite washed twice with 40 mL of 1:1 MTBE:water. The pH of the aqueous layer was adjusted to 7 with 5 M HCl, and the layers were separated. The aqueous phase was washed with 10 mL of MTBE, basified to pH 10 with 47% NaOH, and then extracted three times with 20 mL of $CHCl_3$. The combined chloroform extracts were concentrated to dryness to give a brown oil, and the oil was purified by column chromatography on silica gel (85:15 $CHCl_3$:MeOH) to give 0.974 g of a brown solid after drying at reduced pressure. The solid was dissolved in 3 mL of refluxing EtOH, and 678 mg (4.52 mmol) of D-(−)-tartaric acid in 1 mL of water was slowly added. After 15 min, the solution was cooled to room temperature. A crystal of the D-(−)-tartaric acid salt of **1** was added, and the turbid mixture was

cooled to 0°C. The slurry was filtered, and the solid washed with 2 mL of cold EtOH. After drying under reduced pressure, 538 mg of **[^{14}C]-1** was obtained as the D-(−)-tartaric acid salt. HPLC showed a 96:4 ratio of R:S enantiomers (Daicel Chirocel OD 250 × 4.6 mm, 15% EtOH in isohexane). The solid was recrystallized twice from 2 mL of 1:1 EtOH:water to give 445 mg (12%, 11% radiochemical yield) of **[$^{14}C_2$]-1 · D-(−)-tartaric acid salt** which showed less than 0.5% of the S enantiomer to be present. The radiochemical purity of the sample was >99% (method A). The specific activity was determined to be 23 mCi/mmol by gravimetric analysis. LC/MS(M + H): 217 (100%), 221 (21.2%), 218 (14.6%), 219 (3.1%). ^{1}H NMR (D_6-DMSO, 500 MHz): δ 7.92 (d, *J* = 7.0 Hz, 1H), 7.60 (d, *J* = 7.0 Hz, 1H), 6.87 (t, *J* = 7.0 Hz, 1H), 4.05 (s, 2H), 3.45(d, *J* = 17 Hz, 1H), 3.34(d, *J* = 14 Hz, 1H), 3.24 (d, *J* = 14 Hz, 1H), 3.18 (d, *J* = 17 Hz, 1H), 3.03 (m, 2H), 2.95 (m, 2H), 2.07 (s, 2H), 1.79 (m, 2H), 1.61 (m, 1H).

[2,2-$^2H_2$]Quinuclidin-3-one, [$^2H_2$]-4

A solution of sodium methoxide in MeOD prepared from 150 mL of CH_3OD and 10.2 g of sodium metal[21] was stirred as 9.6 g (76.7 mmol) of quinuclidin-3-one was added and the resulting solution was heated to reflux under an argon atmosphere as 150 mL of D_2O was added. The reaction was heated at reflux for 2.5 h. The MeOH was removed under vacuum, and the water extracted with chloroform. The chloroform layer was dried over sodium sulfate, and after filtering, was concentrated to give 9.3 g of a pale, yellow, and waxy solid. This crude product was partitioned between ether and D_2O/dilute NaOD. The ether layer was removed, dried ($MgSO_4$), and filtered, and the resulting solution was concentrated under vacuum to give 6.6 g (68%) of **[$^2H_2$]-4** as a white solid.

[2′,2′,3,3-$^2H_4$]spirooxirane-2,3′-quinuclidine, [$^2H_4$]-5

A slurry of 2.16 g of NaH (60% dispersion in oil) in 45 mL of dry DMF was stirred as 11.1 g (48.4 mmol) of [$^2H_9$]trimethylsulfoxonium iodide[22] was added portionwise. After the addition was complete, the reaction mixture was stirred an additional 10 min. To this was added 6.6 g (53 mmol) of [2,2-$^2H_2$]quinuclidin-3-one. The reaction was stirred 30 min and was then poured into 150 mL of D_2O. The product was extracted with ether and concentrated under vacuum, and the residue was dried by azeotroping with toluene. This gave 2.4 g (43%) of a pale yellow oil.

[2′,2′,3,3-$^2H_4$]spiro[furo[2,3-b]pyridine-2,3′-quinuclidine HBr salt, [$^2H_4$]-1 · 2HBr

A solution of 1.30 mL (7.68 mmol) of tetramethylpiperidine in 9 mL of dry THF cooled to −60°C under argon was stirred as 3.08 mL of a 2.5 M solution (7.71 mmol) of *n*-butyl lithium in hexanes was added, and the reaction was stirred for 1 hour at −60°C. To this was added 0.668 mL (7.1 mmol) of 2-chloropyridine dropwise over 15 min. The reaction temperature was allowed to rise to −45°C over 45 min and then 1.0 g (6.98 mmol) of [2′,2′,3,3--$^2H_4$]spirooxirane-2,3′-quinuclidine in 4 mL of THF was added dropwise over 15 min. The reaction was warmed to −20°C during which it darkened. The temperature was maintained between −20°C and −15°C for 4 h. The reaction was neutralized by the addition of 10 mL of D_2O and 10 mL of MTBE. The mixture was vigorously stirred and was then filtered through celite. The celite was rinsed with 1:1 D_2O/MTBE, and the pH of the filtrate was adjusted to 7.0 by addition of DCl in D_2O. The layers were separated, and the aqueous layer was washed with 10 mL of MTBE. The aqueous layer was adjusted to pH 10 by the addition of NaOD in D_2O. This was extracted with chloroform, and the combined organic extracts were dried (Na_2SO_4). The solution was filtered and concentrated under vacuum to give 900 mg. The crude product was purified by flash chromatography (85:15 chloroform/MeOH on silica) to afford 363 mg of product as a reddish oil that crystallized upon standing. This was converted to its bis-HBr salt by treatment of an ethanol solution of the free base with an excess of 48% HBr in ethanol. This was stirred 2 h. Diethyl ether was added and the precipitate washed with ether. This gave 523 mg (20%) of **[$^2H_4$]-1 · 2HBr** as an off white solid. The purity by HPLC was 98.9% (method B), and the purity by GC was >99.5% (on the free base). LC/MS (M + H): 221 (100%), 222 (18.6%), 220 (4.8). (no peak for 217, 218, or 219 was observed. LC/MS detection limit was 0.1%) ^{1}H NMR (D_6-DMSO, 500 MHz): δ 7.96 (dd, *J* = 7.0, 2 Hz, 1H), 7.65 (dd, *J* = 7.5, 2 Hz, 1H), 6.92 (dd, *J* = 7.0, 7.5 Hz, 1H), 3.35 (m, 2H), 2.25 (s, 1H), 2.20 (m, 1H), 1.92 (m, 3H), 1.83 (m, 1H). ^{13}C NMR (D_6-DMSO, 150 MHz): δ 165.9, 146.4, 134.2, 118.8, 117.4, 83.0, 58.1 (m), 45.2, 44.8, 36.9 (m), 29.5, 18.5, 18.0.

[$^{13}C_4$]furan-2-yl(morpholino))[^{13}C]methanone, [$^{13}C_5$]-7

A solution of 106 mg (0.91 mmol) of [$^{13}C_5$]2-furoic acid[16] in 5 mL of CH_2Cl_2 was stirred as 2.3 mL (4.5 mmol) of oxalyl chloride and 5 μL of DMF were added. The solution was stirred for 90 min at room temperature, and then the solvent was evaporated. The residue was taken up in 3 mL of CH_2Cl_2, and 95 μL (1.1 mmol) of morpholine was added. After 30 min, the solution was diluted with 5 mL of CH_2Cl_2, and the resulting solution was washed twice with 10 mL of sat. $NaHCO_3$ and then with 5 mL of brine. The organic layer was dried ($MgSO_4$) and filtered, and then the filtrate was concentrated to dryness to give a 107 mg (63%) of **[$^{13}C_5$]-7** as a brown oil. LC/MS: 187 (100%), 186 (8.2%), 188 (6.2%). ^{1}H NMR (CD_3OD, 500 MHz): δ 7.67 (dm, *J* = 205 Hz, 1H), 7.09 (dm, *J* = 184 Hz, 1H), 7.03 (dm, *J* = 173 Hz, 1H), 6.62 (dm, *J* = 178 Hz, 1H), 3.75 (m, 8H).

(4,5-dibromo[$^{13}C_4$]furan-2-yl)(morpholino))[^{13}C]methanone, [$^{13}C_5$]-8

A reaction vessel was charged with 107 mg (0.57 mmol) of **[$^{13}C_5$]-7**, and 89 μL (1.7 mmol) of bromine was added. The resulting slurry was stirred to create a homogenous solution which hardened into a semi-solid after about 1 min. To the top of the hardened mixture was added 230 mg (1.72 mmol) of $AlCl_3$, and the reaction vessel was sealed. The mixture was heated for approximately 4 min with a heat gun during which the semi-solid melted and stirred freely. The reaction was cooled to room temperature, and 5 mL of water was carefully added. The aqueous solution was extracted five times with 4 mL of Et_2O, and the combined organic layers were dried ($MgSO_4$) and filtered. Concentration of the filtrate afforded 192 mg (97%) of **[$^{13}C_5$]-8** as a yellow oil. LC/MS: 345 (100%) 347 (50%), 343 (48%) ^{1}H NMR (CD_3OD, 500 MHz): δ 7.13 (dm, *J* = 184 Hz, 1H), 3.73 (m, 8H).

(5-bromo[$^{13}C_4$]furan-2-yl)(morpholino))[^{13}C]methanone, [$^{13}C_5$]-9

A suspension of 100 mg (0.29 mmol) of **[$^{13}C_5$]-8**, 57 mg (0.87 mmol) of Zn, 50 μL of AcOH, and 0.19 mL of water was stirred and heated at 85°C for 10 min. The reaction was cooled to room temperature, and 5 mL of $NaHCO_3$ was added. The slurry was then extracted four times with 3 mL of CH_2Cl_2, and the organic layer was dried ($MgSO_4$). The drying agent was removed by filtration, and the filtrate was concentrated to dryness to afford 50 mg of a pale yellow oil which contained 50% monobromide **[$^{13}C_5$]-9**, 15% dibromide **[$^{13}C_5$]-8**, and 35% desbromide **[$^{13}C_5$]-7** (method C). The compound was purified by preparative HPLC (method α) to give 17 mg (22%) of **[$^{13}C_5$]-9** as a white solid. LC/MS: 265 (100%), 267 (98%) 266 (24%), 264 (13%). ^{1}H NMR (CD_3OD, 500 MHz): δ 7.77 (dm, *J* = 212 Hz, 1H), 7.09 (dm, *J* = 184 Hz, 1H), 3.74 (m, 8H). ^{13}C NMR (CD_3OD, 125 MHz): δ 159.5 (d), 147.5 (dd), 143.8 (d), 118.4 (dd), 101.6 (dd).

(4-((2R,4′S)-3H-1′-azaspiro[[$^{13}C_4$]furo[2,3-b]pyridine-2,3′-bicyclo[2.2.2]octane]-5-yl)furan-2-yl)(morpholino)[^{13}C]-methanone, [$^{13}C_5$]-10

A stirred solution of 55 mg (0.21 mmol) of **11**, 28 mg (0.11 mmol) of **[$^{13}C_5$]-9**, 45 mg (0.42 mmol) of Na_2CO_3, 1 mL of dimethoxyethane, 0.4 mL of ethanol, and 0.3 mL of water was sparged with N_2 for 20 min after which 3.71 mg (5.3 μmol) of bis(triphenylphosphine)palladium(II) chloride was added. The solution was warmed to 55°C, and after 3 h, an additional 25 mg (0.1 mmol) of **11** was added. The solution was heated overnight. The reaction mixture was concentrated to dryness, and the

residue was taken up in 30 mL of $CHCl_3$:MeOH (1:1) and then filtered. The filtrate was concentrated to dryness, and the residue purified by preparative HPLC (method *β*). Product containing fractions were pooled, concentrated to half volume, and passed through an Oasis HLB SepPak. The SepPak was washed with water, sat $NaHCO_3$, and water, and the compound was eluted with MeOH. The resulting solution was concentrated to dryness to give 14 mg (33%) of **[$^{13}C_5$]-10**. A similar isolation procedure afforded 14 mg of starting bromide **[$^{13}C_5$]-9**.

(*R*)-5-(5-(morpholinomethyl)[$^{13}C_4$]furan-3-[^{13}C]-yl)-3H-1′-azaspiro[furo[2,3-b]pyridine-2,3′-bicyclo[2.2.2]octane], [$^{13}C_5$]-2

A solution of 23 mg (0.057 mmol) of **[$^{13}C_5$]-10** in 0.4 mL of THF was slowly added to a preformed solution of 0.24 mL of 1 M (0.24 mmol) $LiAlH_4$ in THF and 6.4 μL (0.12 mmol) of concentrated H_2SO_4 at room temperature. The reaction mixture was stirred for 20 min, and then 0.2 mL of water was added to the solution. The resulting slurry was filtered through a 0.45 μm syringe filter, and the filter was rinsed with 1 mL of water and 1 mL of THF. The sample was then purified by preparative HPLC (method γ). Pure fractions were pooled and concentrated to approximately half volume. The residue was then loaded onto a 50 mg Oasis HLB SepPak and the column was sequentially washed with 20 mL of water, 20 mL of saturated $NaHCO_3$ solution, and 40 mL of H_2O. The compound was eluted with 10 mL of MeOH, and the MeOH was evaporated to afford 19 mg of a yellow oil. The oil was diluted with 2 mL of ether, and the resulting slurry was sonicated for 2 min. The resulting white solid was collected by filtration to give 19 mg (80%) of [$^{13}C_5$]-**2** after drying under vacuum overnight. LC/MS: 387 (100%), 388 (21%), 386 (6.7%), 385 (0.1%). ^{1}H NMR (500 MHz, $CDCl_3$, ^{13}C decoupled): δ 8.11 (s, 1H), 7.58 (s, 1H), 7.50 (s, 1H), 6.46 (s, 1H), 3.75 (t, J = 4.6 Hz, 4H), 3.55 (m, 2H), 3.40 (t, J = 9 Hz, 2H), 2.8 (m, 6H), 2.52 (m, 4H), 2.26 (m, 1H), 2.01 (m, 1H), 1.68 (t, J = 7.4 Hz, 2H), 1.47 (m, 1H).

(2R,4′S)-5-bromo-3H-1′-azaspiro[furo[2,3-b, 2,6-$^{14}C_2$] pyridine-2,3′-bicyclo[2.2.2]octane], [$^{14}C_2$]-13

A solution of 73 mg (0.41 mmol) of *N*-bromosuccinimide in 1 mL of TFA was slowly added to 51 mg (0.13 mmol, 3.1 mCi, 23 mCi/mmol) of **[$^{14}C_2$]-1**, and the resulting solution was stirred and heated at 80°C for 2 h. The solvent was removed, and the residue partitioned between 5 mL of $CHCl_3$ and 3 mL of sat $NHCO_{3(aq)}$. The organic layer was removed, and the aqueous layer was extracted three times with 4 mL of $CHCl_3$. The combined organic layers were concentrated to dryness to give 2.4 mCi (73%) of **[$^{14}C_2$]-13**. HPLC analysis showed the radiochemical purity to be 94.3% (method d).

4-((2R,4′S)-3H-1′-azaspiro[furo[2,3-b, 2,6-$^{14}C_2$]pyridine-2,3′-bicyclo[2.2.2]octane]-5-yl)furan-2-carbaldehyde, [$^{14}C_2$]-15

A solution of 51 mg (0.17 mmol, 4.0 mCi, 23 mCi/mmol) of **[$^{14}C_2$]-13** in 8 mL of dimethoxyethane was stirred as a solution of 100 mg (0.24 mmol) of $NaCO_3$ in 4 mL of water and 2.8 mL of EtOH was added. N_2 was bubbled through the solution for 5 min, and then 126 mg (0.90 mmol) of 5-formylfuran-3-ylboronic acid and 19 mg (0.027 mmol) of dichlorobis (triphenylphosphine)palladium(II) were added. N_2 was bubbled through the solution for 10 additional minutes, and then the reaction mixture was stirred at room temperature for 20 min, at 60°C for 3 h, and at 100°C for 2 h. The reaction mixture was concentrated to dryness, and 10 mL of 50% MeOH in $CHCl_3$ was added. The solution was then concentrated to dryness. The residue was taken up in 10 mL of $PhCH_3$ to give 4.0 mCi (49% yield based on radiochemical purity) and then the solution concentrated to dryness. This was repeated three times. HPLC shows the purity of the product to be 49.9% (method e).

(R)-5-(4-(morpholinomethyl)furan-2-yl)-3H-1′-azaspiro[furo [2,3-b, 2,6-$^{14}C_2$]pyridine-2,3′-bicyclo[2.2.2]octane], [$^{14}C_2$]-2

A solution of 1.3 mCi (49% radiochemical purity, 0.023 mmol) of **[$^{14}C_2$]-15** in 6 mL of MeOH was stirred as 0.33 mL of morpholine was added. After 10 min, 0.25 mL (4.37 mmol) of acetic acid was added, and after a further 10 min, 613 mg (2.89 mmol) of sodium triacetoxyborohydride was added. The reaction was stirred for 1 h and was then concentrated to near dryness. The residue was taken up in 5 mL of MeOH and was purified by preparative HPLC (method δ) in five batches. The product containing fractions were combined and concentrated to approximately half volume. The solution was basified with NaOH to pH > 11 and was extracted three times with 40 mL of $CHCl_3$. The combined organic layers were concentrated to dryness to give 0.43 mCi (67%) of **[$^{14}C_2$]-2** as a white solid. HPLC analysis showed the compound to have a 99.5% radiochemical purity (methods d and f). The specific activity of the compound was determined to be 26.6 mCi/mmol by LC/MS. LC/MS: 382 (100%), 383 (30.7), 386 (26.1), 384 (7.3%), 387 (6.0%). ^{1}H NMR (500 MHz, D_3COD): δ 8.17 (s, 1H), 7.93 (s, 1H), 7.86 (s, 1H), 6.83 (s, 1H), 3.90 (br s., 2H), 3.78 (m, 4H), 3.62 (m, 3H), 3.45 (m, 2H), 3.39 (m, 3H), 2.80 (br s., 4H), 2.45 (t, J = 6.4 Hz, 1H), 2.37 (br s., 1H), 2.09 (m, 2H), 1.98 (dddd, J = 10.8, 10.6, 10.6, 1.7 Hz, 1H).

[carbonyl-^{14}C]nicotinic acid, [^{14}C]-16

A stirred solution of 342 mg (2.16 mmol) of 3-bromopyridine in 25 mL of diethyl ether was cooled to −70°C as 1.2 mL (1.92 mmol) of *n*-butyllithium was added over 10 min. The solution was stirred for 1 h at −70°C and was then cooled in a $N_{2(l)}$ bath and evacuated to 0.1 mm Hg. Approximately 56 mCi (1 mmol) of $^{14}CO_2$ (generated from 202 mg (1.01 mmol, 56 mCi/mmol) of $Ba^{14}CO_3$ and 20 mL of H_2SO_4) was transferred *via* gas transfer to the reaction flask,[23] and the solution was then warmed to −70°C and was stirred for 1 h. The solution was then warmed to room temperature, and 15 mL of water was added. The layers were separated, and the aqueous layer was concentrated to dryness to give 33 mCi (58%) of **[^{14}C]-16**. The compound was used directly in the next reaction.

[carboxyl-^{14}C]-N-(2-oxo-2-((2R,4′S)-3H-1′-azaspiro[furo[2,3-b]pyridine-2,3′-bicyclo[2.2.2]octane]-5-yl)ethyl) nicotinamide, [^{14}C]-18

A solution of 26.4 mCi (0.46 mmol) of **[^{14}C]-16** in 3 mL of DMF was stirred as 1.27 g (3.34 mmol) of HATU and 0.80 mL (5.7 mmol) of triethylamine were added. After 10 min, 324 mg (0.85 mmol) of **17** was added, and the reaction mixture was stirred for 3 h. The reaction mixture was then diluted with 50 mL of 1 M NaOH and 50 mL of $CHCl_3$, and the layers were separated. The organic layer was washed three times with 50 mL of 1 M NaOH and was then dried ($MgSO_4$). The drying agent was removed by filtration, and the resulting yellow solution was concentrated to dryness to give 16 mCi of **[^{14}C]-18** (40% yield corrected for purity) as a yellow oil. The oil was heated at 130°C for 1 h under vacuum with stirring to drive off residual solvents. HPLC analysis of the oil showed it to have a 65% radiochemical purity (method d). The aqueous layer consisted of 8 mCi of [^{14}C]nicotinic acid which was isolated by solid phase extraction for future use.

(2R,4′S)-5-(2-[^{14}C]-2-(pyridin-3-yl)oxazol-5-yl)-3H-1′-azaspiro [furo[2,3-b]pyridine-2,3′-bicyclo[2.2.2]octane], [^{14}C]-3

A solution of 10.2 mCi (0.18 mmol, 65% radiochemical purity) of **[^{14}C]-18** in 3 mL of Eaton's solution (7.7 wt% P_2O_5 in $MeSO_3H$)[20] was stirred at 130°C overnight. After cooling to room temperature, the solution was diluted with 50 mL of water and basified with 50% NaOH. The solution was extracted three times with 50 mL of $CHCl_3$ to give 10 mCi. The solution was concentrated to dryness and then purified batchwise by preparative HPLC (method ε). The pure product containing fractions were combined and concentrated to approximately 50% volume. They were diluted with

10 mL of sat $NaHCO_{3(aq)}$ and extracted three times with 40 mL of $CHCl_3$. The combined organic extracts were dried ($MgSO_4$) and filtered, and then the filtrate was concentrated to dryness to give 2.2 mCi (33%) of **[^{14}C]-3** as a white solid. HPLC analysis (methods G and H) showed the radiochemical purity to be 99.3%. The specific activity of the compound was determined to be 56 mCi/mmol by LC/MS. A second batch of material was isolated in the same manner from less pure column fractions to give 2.4 mCi at a radiochemical purity of 81.7%. LC/MS (M + 1): 363 (100%), 364 (23%), 361 (11%), 362 (3.1%), 365 (3.1%). ^{1}H NMR (500 MHz, MeOD): δ 9.25 (d, J = 1.5 Hz, 1H), 8.66 (dd, J = 4.9, 1.5 Hz, 1H), 8.48 (dt, J = 7.9, 1.8 Hz, 1H), 8.43 (d, J = 1.8 Hz, 1H), 8.04 (d, J = 1.8 Hz, 1H), 7.63 (s, 1H), 7.60 (dd, J = 7.3, 4.9 Hz, 1H), 3.60 (m, 1H), 3.24 (m, 2H), 3.09 (d, J = 15.0 Hz, 1H), 2.97 (m, 2H), 2.87 (m, 2H), 2.20 (m, 1H), 2.07 (br s., 1H), 1.80 (m, 2H), 1.62 (m, 1H). ^{13}C NMR (126 MHz, CD_3OD): δ 167.0, 150.4, 150.2, 146.5, 142.3, 133.9, 130.7, 124.2, 123.7, 122.8, 121.5, 118.1, 88.1, 61.9, 46.0, 45.4, 37.9, 31.2, 21.6, 20.7.

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Conflict of Interest

The authors did not report any conflict of interest.

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