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Research Article

Synthesis of C-14 and C-13, H-2-labeled IKK inhibitor: [^{14}C] and [$^{13}C_4$,D_3]-*N*-(6-chloro-7-methoxy-9H-pyrido[3,4-b]indol-8-yl)-2-methyl-3-pyridinecarboxamide

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Summary

[^{14}C]-*N*-(6-Chloro-7-methoxy-9H-pyrido [3,4-b]indol-8-yl)-2-methyl-3-pyridinecarboxamide (5B), an IKK inhibitor, was synthesized from [^{14}C]-barium carbonate in two steps in an overall radiochemical yield of 41%. The intermediate, [carboxyl-^{14}C]-2-methylnicotinic acid, was prepared by the lithiation and carbonation of 3-bromo-2-methylpyridine. [$^{13}C_4$,D_3]-*N*-(6-chloro-7-methoxy-9H-pyrido [3,4-b]indol-8-yl)-2-methyl-3-pyridinecarboxamide (5C) was synthesized from [1,2,3,4-$^{13}C_4$]-ethyl acetoacetate and [D_4]-methanol in six steps in an overall yield of 2%. [$^{13}C_4$]-2-methylnicotic acid, was prepared by condensation of [$^{13}C_4$]-ethyl 3-aminocrotonate and acrolein, followed by hydrolysis with lithium hydroxide.

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Introduction

Inhibitors of IkB kinase (IKK) are useful for treating IKK-2-mediated diseases, such as inflammatory diseases and cancer. The biological activity of the recently discovered IKK inhibitor, *N*-(6-chloro-7-methoxy-9H-pyrido

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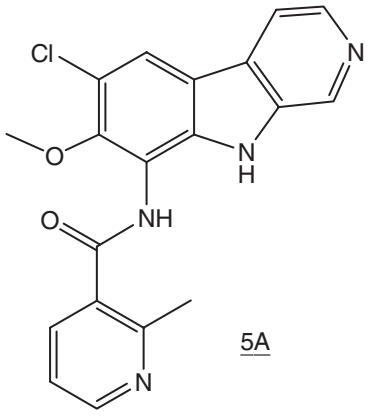

5A

Figure 1.

[3,4-b]indol-8-yl)-2-methyl-3-pyridinecarboxamide (5A),[1–4] led to detailed investigations on its disposition characteristics. The radiolabeled version was required to assist such investigations, especially in metabolite profiling and whole body autoradiography studies in experimental animals. The stable isotope labeled version was also required as an internal standard for mass spectrometry-based bioanalytical assays.

The amide structure of *N*-(6-chloro-7-methoxy-9H-pyrido [3,4-b]indol-8-yl)-2-methyl-3-pyridinecarboxamide (5A, Figure 1) provides two potential sites for radiolabeling, either on the beta-carboline portion or the nicotinic acid portion of the molecule. Preclinical metabolism results indicated that radiolabeling either portion of the molecule would be adequate for the intended ADME studies in animals. Therefore, labeling the 2-methylnicotinic acid for both stable isotope and radiolabeled versions was chosen.

Results and discussion

Although there are numerous methods in the literature for labeling nicotinic acid, there are no published reports for the 2-methyl analog. To prepare the [carboxyl-^{14}C]-2-methylnicotinic acid (4B), we first attempted to make 3-cyano-2-methylpyridine (3) by three commonly used cyanation protocols (Scheme 1).[5–7] The starting material for this cyanation, 3-bromo-2-methylpyridine, was prepared by bromination of 2-picoline (1). Both 3-bromo-2-methylpyridine (2A) and 5-bromo-2-methylpyridine (2B) resulted from the bromination of 2-picoline (1).[8] Because of structural similarities of these two isomers, we utilized chromatography and crystallization techniques to obtain the pure required isomer. The residue from the bromination workup was purified chromatographically to remove the unreacted starting material and leave a few pure fractions. The remaining impure fractions were crystallized to

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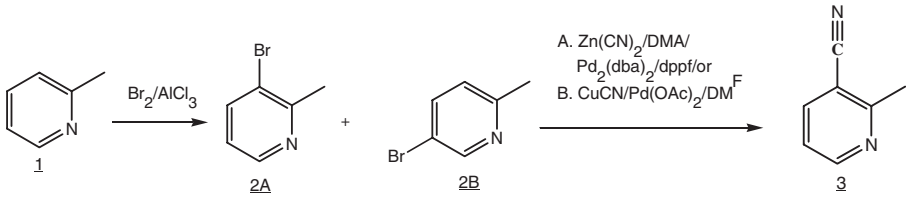
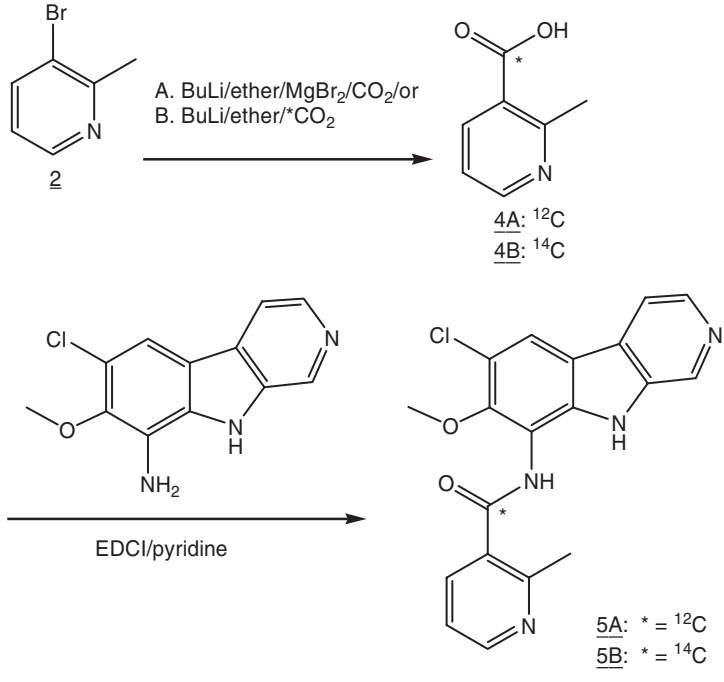


Scheme 1.



Scheme 2.

remove most of the 5-bromo-2-methylpyridine and further purified chromatographically to obtain 3-bromo-2-methylpyridine (2A).

Cyanation of 3-bromo-2-methylpyridine (2) with KCN and finely ground CuI in DMF at 150°C under nitrogen for 70 h returned only starting material. Cyanation of 3-bromo-2-methylpyridine (2) with $Zn(CN)_2$, $Pd_2(dba)_3$, dppf, and Zn in DMA at 150°C for 5 h indicated a 40% conversion to the product. No further improvement in yield could be obtained by prolonged heating. Similar cyanation of 3-bromo-2-methylpyridine (2) with copper (I) cyanide and palladium acetate in DMF at 150°C for 22 h indicated a 50% conversion. With further heating at 150°C for 48 h, neither the starting material nor the product could be identified. Because of poor cyanation results, we attempted to prepare [carboxyl-^{14}C]-2-methylnicotinic acid (4B) by carbonation reactions of metallated 2-methylpyridine (Scheme 2).[9,10]

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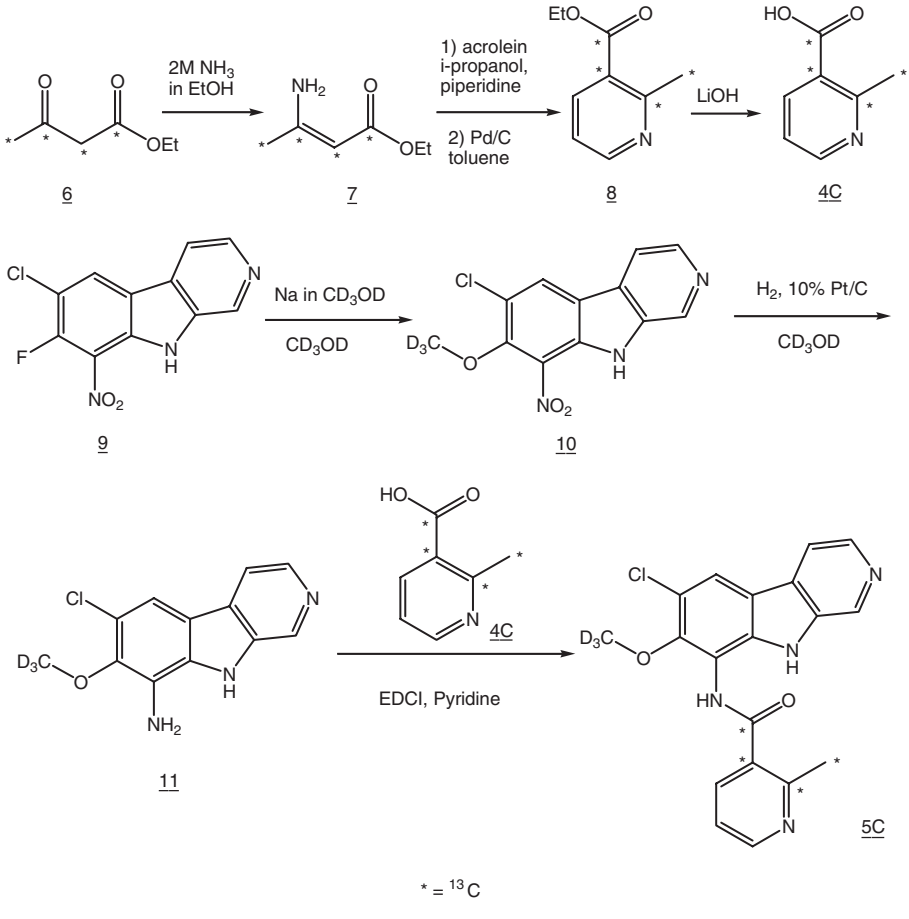
Carbonation via preparation of Grignard reagent did not provide the desired acid. Lithiation of 3-bromo-2-methylpyridine by *n*-BuLi followed by treatment with $MgBr_2$ and carbon dioxide provided the acid in a yield of 29%.[10] But lithiation of 3-bromo-2-methylpyridine followed by carbon dioxide provided the acid in yields ranging from 61 to 77%. Adaptation of this procedure provided the C-14-labeled acid. [^{14}C]-*N*-(6-chloro-7-methoxy-9H-pyrido [3,4-b]indol-8-yl)-2-methyl-3-pyridinecarboxamide (5B) was prepared by EDCI-mediated coupling of [^{14}C]-2-methylnicotinic acid with 8-amino-6-chloro-7-methoxy-9H-β-carboline in pyridine (52%).[2–4]

To prepare [$^{13}C_4$]-2-methylnicotinic acid (4C),[11,12] commercially available [1,2,3,4-$^{13}C_4$]-ethyl acetoacetate (6) was used as the starting material (Scheme 3). Treatment of 6 with ammonia in ethanol provided imine 7. Condensation of this imine with acrolein followed by oxidation of the resulting compound yielded [$^{13}C_4$]-ethyl 2-methylnicotinate (8). Hydrolysis of 8 with lithium hydroxide provided the labeled acid.



Scheme 3.

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Because 5A contains a chlorine atom, a labeled version that is 5 amu higher than the unlabeled version is required to ensure complete separation of labeled and unlabeled molecular ion clusters during mass spectrometric assays of 5A. To increase the molecular weight, labeling of the beta-carboline portion of the molecule, or utilization of the labeled acrolein was required. Labeling the methoxy protons with deuterium was chosen. Due to poor yields obtained by demethylation of 5A and subsequent remethylation of resulting phenol with methyliodide, utilization of the nucleophilic substitution of fluorine of 9 with sodium deuterio-methoxide was chosen. No loss of chlorine was observed during this sodium deuterio-methoxide reaction. The selective replacement of the fluoro group is due to its location, that is, ortho to the nitro group (a strong electron-withdrawing group). This approach is also patterned after the PCT publication of the synthetic route for preparation of 5A.[1] Reduction of 10 followed by coupling of the amine with [$^{13}C_4$]-2-methylnicotinic acid provided [$^{13}C_4$,D_3]-*N*-(6-chloro-7-methoxy-9H-pyrido [3,4-b]indol-8-yl)-2-methyl-3-pyridinecarboxamide (5C).[2–4] Yield during nitro reduction was lower due to slight loss of chlorine.

Conclusion

In summary, a practical method for the preparation of carbon-14 and carbon-13 labeled 2-methylnicotinic acids was developed. Lithiation and carbonation of 3-bromo-2-methylpyridine provided a convenient method of preparing [carboxyl-^{14}C]-2-methylnicotinic acid in a satisfactory yield. The coupling of [1,2,3,4-^{13}C]-ethyl 3-aminocrotonate and acrolein provided a practical method of preparing [$^{13}C_4$]-2-methylnicotinic acid. Labeled 2-methylnicotinic acid is a useful precursor for preparation of labeled pharmaceutical or agrochemical agents that are either esters or amides of 2-methylnicotinic acid.[13,14] [^{14}C]-*N*-(6-chloro-7-methoxy-9H-pyrido [3,4-b]indol-8-yl)-2-methyl-3-pyridinecarboxamide was synthesized in two steps in an overall radiochemical yield of 41%. [$^{13}C_4$,D_3]-*N*-(6-chloro-7-methoxy-9H-pyrido [3,4-b]indol-8-yl)-2-methyl-3-pyridinecarboxamide was prepared in six steps in an overall yield of 2%.

Experimental

General

All commercial reagents were used as supplied unless otherwise noted. [^{14}C]-Barium carbonate was purchased from American Radiolabeled Chemicals, USA. [1,2,3,4-$^{13}C_4$]-Ethyl acetoacetate and [D_4]-methanol were purchased from Cambridge Isotope Laboratories, USA. Radioactivity was quantified by liquid scintillation counting using a Beckman LS6500 counter. Purities were determined by HPLC (Agilent 1100) on Waters Symmetry C18 column (5μ, 4.6 × 250 mm) eluted at 1 ml/min with A (0.1% formic acid in 99% water and

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1% CH_3CN) and B (0.1% formic acid in 5% water and 95% CH_3CN). Elution was 0% B for 5 min, 0–100% B over 15 min, and 100% B holding for 5 min. UV detection was at 254 nm. Radioactive detection was with a Packard 500 TR flow detector using an Ultima Flo M scintillant at 3 ml/min. NMR spectra were recorded on either a Varian 600 or a 300 MHz spectrometer. LC-MS analyses were performed on a Micromass Platform LCZ and a ThermoFinnigan LCQ mass spectrometer. Column chromatography was performed on silica gel (230–400 mesh) supplied by SiliCycle, Canada.

*3-Bromo-2-methylpyridine (**2A**) and 5-Bromo-2-methylpyridine (**2B**)*[8]

2-Picoline (14.8 ml, 0.15 mol) was added under nitrogen to magnetically stirred solid aluminum chloride (60 g, 0.45 mol). This slurry was heated under nitrogen to 100°C, and bromine (11.53 ml, 0.225 mol) was added dropwise. The heating was continued at 100°C for 19 h. The reaction mixture was poured into 600 ml of ice water containing 23 ml of concentrated HCl. Additional concentrated HCl was added until acidic. $NaHSO_3$ was added until a colorless solution was obtained. The solution was washed with methylene chloride (3×100 ml). 50% NaOH aqueous solution was added to the aqueous phase in an ice bath until alkaline, and extracted with ether (4×80 ml). The ether solution was washed with 10% NaCl solution and dried with $MgSO_4$. The solvent was removed and the residue was chromatographed on silica gel using a mixture of ether–petroleum ether (1:15) as an eluant. The fractions containing two isomers were crystallized from pentane to remove most of the 5-bromo-2-methylpyridine. The mother liquor was chromatographed on silica gel using the same eluant. The respective pure fractions of the two isomers were combined and dried with $MgSO_4$ to provide 4.2 g yield (98% pure) of 3-bromo-2-methylpyridine (2A) and 5.3 g yield (97% pure) of 5-bromo-2-methylpyridine (2B). LC–MS/MS (3-bromo-2-methylpyridine): m/z 172 (M + 1), 174 (M + 1), 93. LC–MS/MS (5-bromo-2-methylpyridine): m/z 172 (M + 1), 174 (M + 1), 93. ^{1}H-NMR (3-bromo-2-methylpyridine, $(CD_3)_2SO$): δ 8.46 (1H, d), 8.00 (1H. d), 7.18 (1H, quartet), 2.70 (CH_3, s). ^{1}H-NMR (5-bromo-2-methylpyridine, $(CD_3)_2SO$): δ 8.55 (1H, s), 7.91 (1H, d), 7.25 (1H, d), 2.45 (CH_3, s).

*2-Methylnicotinic acid (**4A**)*[9,10]

Experimental 1: To a stirred suspension of Mg powder (29.2 mg, 50 mesh, 1.2 mmol) and $HgCl_2$ (5 mg) in anhydrous ether (5 ml) was added a solution of dry 3-bromo-2-methylpyridine (172 mg, 1.0 mmol) in anhydrous ether (2 ml). The mixture was stirred under nitrogen at room temperature for 3 h, then refluxed for 2 h. The resulting mixture was connected to a vacuum manifold, frozen with liquid nitrogen, evacuated, and reacted with dry CO_2 at −20°C for

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1 h. The reaction was quenched with water (10 ml). LC-MS/MS analysis showed no product.

Experimental 2: *n*-BuLi solution in hexanes (1.6 M, 0.47 ml, 0.75 mmol) was added dropwise to a solution of dry 3-bromo-2-methylpyridine (172 mg, 1.0 mmol) in anhydrous ether (6 ml) under nitrogen at −75°C. Stirring was continued for 0.5 h at −75°C, then a solution of $MgBr_2$ (0.75 mmol) in anhydrous THF (4 ml) was added dropwise to the reaction mixture. After stirring at −75°C for 0.5 h, the resulting mixture was connected to a vacuum manifold, frozen with liquid nitrogen, evacuated, and reacted with dry CO_2 (0.5 mmol) at −20°C for 1 h. The reaction was quenched with water (3 ml). To the reaction mixture 1 N NaOH was added until alkaline and separated. The aqueous phase was washed with ether (2 × 5 ml), 5 N HCl was added until acidic in an ice bath, and then concentrated. The residue was chromotographed on ion-exchange resin (DOWEX 500WX2–200) using water followed by 1 N NH_4OH as eluant to give 20.1 mg (29%) product. LC-MS/MS: *m*/*z* 138 (M + 1). ^{1}H-NMR ($(CD_3)_2SO$): δ 8.58 (1H, d), 8.15 (1H, d), 7.14 (1H, quartet), 2.70 (CH_3, s).

Experimental 3: *n*-BuLi solution in hexanes (1.6 M, 0.45 ml, 0.75 mmol) was added dropwise to a solution of dry 3-bromo-2-methylpyridine (172 mg, 1.0 mmol) in anhydrous ether (5 ml) under nitrogen at −50°C. After stirring was continued for 0.5 h at −50°C, the mixture was connected to a vacuum manifold, frozen with liquid nitrogen, evacuated, and reacted with dry CO_2 (0.5 mmol, generated from $BaCO_3$ and H_2SO_4) at −50°C for 1 h. The same procedures of purification were followed as above to give the product (42.4 mg, 61%). HPLC: 96% (UV at 254 nm). LC-MS/MS: *m*/*z* 138 (M + 1). ^{1}H-NMR ($(CD_3)_2SO$): δ 8.58 (1H, d), 8.15 (1H, d), 7.14 (1H, q), 2.70 (CH_3, s).

*[carboxyl-^{14}C]-2-Methylnicotinic acid (**4B**)*[9,10]

n-BuLi solution in hexanes (1.6 M, 1.8 ml, 2.88 mmol) was added dropwise to a solution of dry 3-bromo-2-methylpyridine (662 mg, 3.85 mmol) in anhydrous ether (20 ml) under nitrogen at −50°C. After stirring was continued for 0.5 h at −50°C, the mixture was connected to a vacuum manifold, frozen with liquid nitrogen, evacuated, and reacted with dry [^{14}C]-CO_2 (100 mCi, 1.923 mmol, 52 mCi/mmol, generated from [^{14}C]-$BaCO_3$ and H_2SO_4) at −50°C for 1 h. The reaction was quenched with water (10 ml). 1 N NaOH was added to the reaction mixture until alkaline and separated. The aqueous phase was washed with ether (2 × 20 ml), 5 N HCl was added until acidic in an ice bath, and then concentrated. The residue was chromotographed on ion-exchange resin (DOWEX 500WX2–200) using water followed by 1 N NH_4OH as eluant to give 205.8 mg (77 mCi, 52 mCi/mmol, 77%) product. HPLC: 96% (UV at 254 nm); radiochemical purity, 94%. LC-MS/MS: *m*/*z* 140 (M + 1), 138.

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^{1}H-NMR ($(CD_3)_2SO$): δ 13.20 (COOH, s), 8.58 (1H, d), 8.15 (1H, d), 7.14 (1H, quartet), 2.70 (CH_3, s).

*[^{14}C]-N-(6-chloro-7-methoxy-9H-pyrido [3,4-b]indol-8-yl)-2-methyl-3-pyridinecarboxamide (**5B**)*[1–4]

[carboxyl-^{14}C]-2-Methylnicotinic acid (101 mg, 0.73 mmol, 52 mCi/mmol), 6-chloro-7-methoxy-9H-β-carbolin-8-ylamine (164 mg, 0.66 mmol), and EDCI (381 mg, 1.99 mmol) were added to a dry flask. Pyridine (6 ml) was added via syringe under nitrogen. The resulting mixture was heated at 80°C under nitrogen overnight. Pyridine was removed by rotary evaporation and the residue was suspended in 3 ml of methanol. The methanolic solution was added to a stirring solution of saturated $NaHCO_3$ (30 ml). The resulting solid was collected by centrifuge and dissolved with CH_3OH/CH_2Cl_2 (1:3). The solution was dried with $MgSO_4$ and concentrated. The residue was chromatographed on silica gel using a mixture of CH_3OH/CH_2Cl_2 (7:93) as eluant to give 143.1 mg (20.2 mCi, 52mCi/mmol, 53%) of the title compound. HPLC: 99.6% (UV at 246 nm); radiochemical purity, 99.5%. LC-MS/MS: *m/z* 369 (M + 1), 367. ^{1}H-NMR ($(CD_3)_2SO$): δ 11.58 (1H, s), 10.35 (1H, s), 8.92 (1H, quartet), 8.60 (1H, s), 8.45 (1H, s), 8.35 (1H, s), 8.20 (1H, d), 8.15 (1H, s), 7.45 (1H, t), 3.85 (OCH_3, s), 2.70 (CH_3, s).

*[1,2,3,4-$^{13}C_4$]-Ethyl 3-aminocrotonate (**7**)*[11,12]

[1,2,3,4-$^{13}C_4$]-Ethyl acetoacetate (5, 4 g, 30 mmol) was transferred from ampoules to a round bottom flask. A solution of ammonia in ethanol (2 M, 45 ml) was added and the reaction was stirred at room temperature overnight. Completion of reaction was indicated by TLC (5/1 hexane/ethyl acetate) and the solvent was removed by rotary-evaporation to get the title compound, 3.92 g (98%). It was used directly in the next step.

*[$^{13}C_4$]-Ethyl 2-methylnicotinate (**8**)*[11,12]

To a stirred mixture of [1,2,3,4-$^{13}C_4$]-ethyl 3-aminocrotonate (7, 3.92 g, 29.46 mmol), isopropanol (7 ml) and piperidine (0.145 ml, 0.05 eq.) was added acrolein (2.75 ml, 1.4 eq.) over 20 min. The reaction mixture was heated to reflux for 3.5 h and then cooled to room temperature. A second portion of acrolein was added (2.75 ml), and the mixture was heated to reflux overnight. The solvent was removed under reduced pressure to give a black residue. The crude product was oxidized overnight by heating to reflux in toluene in the presence of Pd/C (10%). After cooling to room temperature, the black mixture was filtered through a Celite pad, the solution was then concentrated to a brown oil. The residue was redissolved in 50 ml chloroform and extracted with aqueous hydrochloric acid (2 N, 150 ml in two portions). The acidic aqueous

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solution was backwashed with fresh chloroform (3 × 100 ml). With cooling, the aqueous acid was basified with aqueous sodium hydroxide (2 N) until pH 14, and the ester was extracted into chloroform (3 × 200 ml). The organic extracts were dried over sodium sulfate, the solvent removed at reduced pressure, and the brown oil was purified by column chromatography (20% ethyl acetate in hexane) to provide the title compound as a light yellow oil, 505 mg (10%). No attempts were made to improve the yield because of the urgent requirement of the labeled compound for bioanalytical assays. ^{1}H-NMR ($CDCl_3$): δ 8.60 (1H, m), 8.18 (1H, m), 7.20 (1H, m), 4.37 (2H, m), 3.05 (1.5 H, quartet), 2.62 (1.5H, quartet), 1.40 (3H, t). ^{13}C-NMR ($CDCl_3$): δ 166 (1C, d), 160 (1C, t), 126 (1C, quartet), 25 (1C, d).

*[$^{13}C_4$]-2-Methylnicotinic acid (**4C**)*[11,12]

To a stirred solution of [$^{13}C_4$]-ethyl 2-methylnicotinate (8, 505 mg, 2.98 mmol) in 3 ml of methanol/tetrahydrofuran (1:1) mixture was added lithium hydroxide hydrate (188 mg, 1.5 eq.) solution in water (2 ml). The reaction mixture was heated at 50°C overnight. After cooling, the organic solvent was removed by rotary evaporation and the residue was suspended in water. Careful addition of strong acidic ion exchange residue (DOWEX 50WX4-400) until pH 4 liberated the free acid. The mixture was filtered and the filtrate was concentrated to yield a light brown solid (280 mg, 62%). ^{1}H-NMR (D_2O): δ 8.60 (2H, m), 8.18 (1H, m), 3.14 (1.5 H, quartet), 2.70 (1.5H, quartet). ^{13}C-NMR (D_2O): δ 173 (1C, d), 152 (1C, t), 138 (1C, t), 19 (1C, d). MS: m/z 152 (M + 1).

*[D_3]-6-Chloro-7-methoxy-8-nitro-9H-β-carboline (**10**)*[1,2]

A fresh solution of sodium in [D_4]-methanol (5 ml of 25% solution by weight) was added over 5 min to a vigorously stirred suspension of 6-chloro-7-fluoro-8-nitro-9H-β-carboline (9, 650 mg, 2.45 mmol) in 25 ml [D_4]-methanol cooled in an ice bath. The reaction mixture turned crimson red and it was completed after 1 h by LC-MS/MS analysis. The solvent was removed under reduced pressure to give a residue, to which 50 ml deuterium oxide was added to give a suspension of brown solid. Filtration of the suspension gave the desired product (500 mg, 73%). ^{1}H-NMR ($(CD_3)_2SO$): δ 11.28 (1H, s), 8.92 (1H, s), 8.30 (1H, d), 8.02 (1H, d), 7.60 (1H, s). MS: m/z 281 (M + 1).

*[D_3]-6-Chloro-7-methoxy -9H-β-carbolin-8-ylamine (**11**)*[1,2,3]

[D_3]-6-Chloro-7-methoxy-8-nitro-9H-β-carboline (10, 500 mg, 1.78 mmol) was suspended in 50 ml [D_4]-methanol and 100 mg of Pt/C (10%) was added. The flask was fitted with a balloon of hydrogen and the reaction mixture was stirred overnight at ambient temperature. Upon filtration through a pad of

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celite and the evaporation of methanol, a brown solid was obtained. This residue was purified by column chromatography (7% methanol in dichloromethane) to give the title compound (295 mg, 66%). ^{1}H-NMR ($(CD_3)_2SO$): δ 11.28 (1H, s), 8.92 (1H, s), 8.30 (1H, d), 8.02 (1H, d), 7.60 (1H, s), 5.43 (2H, s). MS: *m*/*z* 251 (M + 1).

*[$^{13}C_4D_3$]-N-(6-chloro-7-methoxy-9H-pyrido[3,4-b]indol-8-yl)-2-methyl-3-pyridinecarboxamide (**5C**)*[1–4]

[D_3]-6-Chloro-7-methoxy-9H-β-carbolin-8-ylamine (11, 258 mg, 1.032 mmol), [$^{13}C_4$]-2-methyl nicotinic acid (4C, 262 mg, 1.858 mmol, 1.8 eq.), and EDCI (80 mg, 2 eq.) were suspended in pyridine (4 ml). The resulting mixture was heated at 80°C overnight. Pyridine was then removed by rotary evaporation and the residue was purified by column chromatography (7% methanol in methylene chloride) to give an off-white solid (278 mg, 72%, 99% pure). ^{1}H-NMR ($(CD_3)_2SO$): δ 11.60 (1H, s), 10.35 (1H, s), 8.14–8.93 (6H, m), 7.45 (1H, m), 3.34 (1.3H, s), 2.93 (1.7H, s). ^{13}C-NMR (DMSO): δ 168 (1C, d), 156 (1C, t), 132 (1C, t), 24 (1C, d). MS: *m*/*z* 374 (M + 1).

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