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**Research Article**

# Synthesis of C-14-labeled novel IKK inhibitor: 2-[$^{14}$C]-*N*-(6-chloro-9H-pyrido [3,4-b]indol-8-yl)-3-pyridinecarboxamide

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## Summary

2-[$^{14}$C]-*N*-(6-Chloro-9H-pyrido [3,4-b]indol-8-yl)-3-pyridinecarboxamide (9A, also referred to as [$^{14}$C]-PS-1145) was synthesized from [$^{14}$C]-paraformaldehyde in five steps in an overall radiochemical yield of 15%. The key intermediate 1-[$^{14}$C]-6-chloro-1,2,3,4-tetrahydro-$\beta$-carboline was obtained by Pictet–Spengler cyclization of chlorotryptamine with [$^{14}$C]-paraformaldehyde. Similar reactions were conducted with tryptamine to address the generality of the methodology. Copyright © 2005 John Wiley & Sons, Ltd.

**Key Words:** 2-[$^{14}$C]-*N*-(6-chloro-9H-pyrido [3,4-b]indol-8-yl)-3-pyridine-carboxamide; [$^{14}$C]-paraformaldehyde

## Introduction

The transcription factor NF-kB mediates the expression of a number of pro-inflammatory cytokines, adhesion molecules, growth factors, and anti-apoptosis survival proteins. Inhibitors of IkB kinase (IKK) have been sought as specific regulators of NF-kB. These inhibitors are expected to be of clinical utility in the treatment of inflammatory or immune-related disorders or diseases. The remarkable biological activity of the recently discovered IKK inhibitor, *N*-(6-chloro-9H-pyrido [3,4-b]indol-8-yl)-3-pyridinecarboxamide (9)[1] led us to prepare a carbon-14(C-14)-labeled version. This radiolabeled version was required to assist in metabolite profiling and whole body autoradiography studies in experimental animals.

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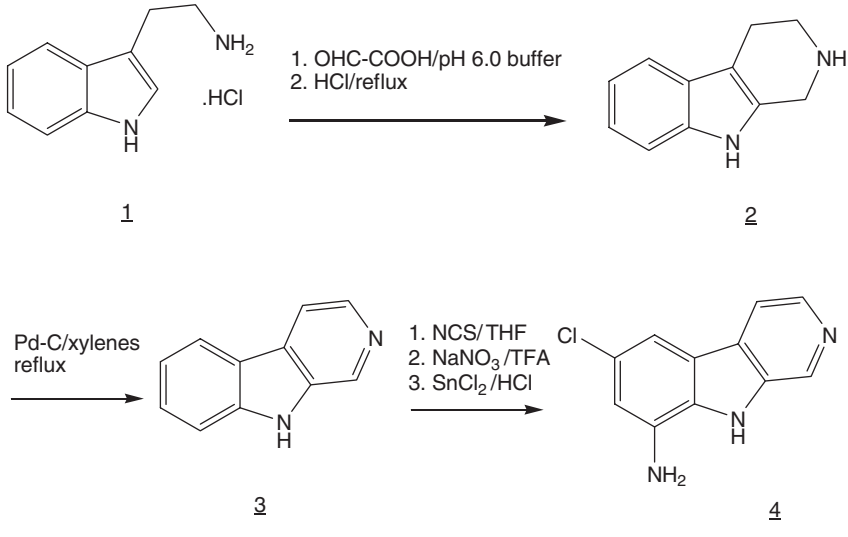
## Results and discussion

As delineated in Scheme 1, the published procedure for synthesis of 8-amino-6-chloro-$\beta$-carboline (*4*) utilized Pictet–Spengler cyclization of tryptamine with glyoxalic acid followed by chlorination, nitration and subsequent reduction of the nitro group.[1–3] Adaptation of this procedure for synthesis of PS-1145 labeled with C-14 in the beta carboline ring would have required [$^{14}$C]-glyoxalic acid. Along with the difficulty of obtaining or preparing [$^{14}$C]-glyoxalic acid[4,5] one has to contend with problems of regioselectivity during chlorination.

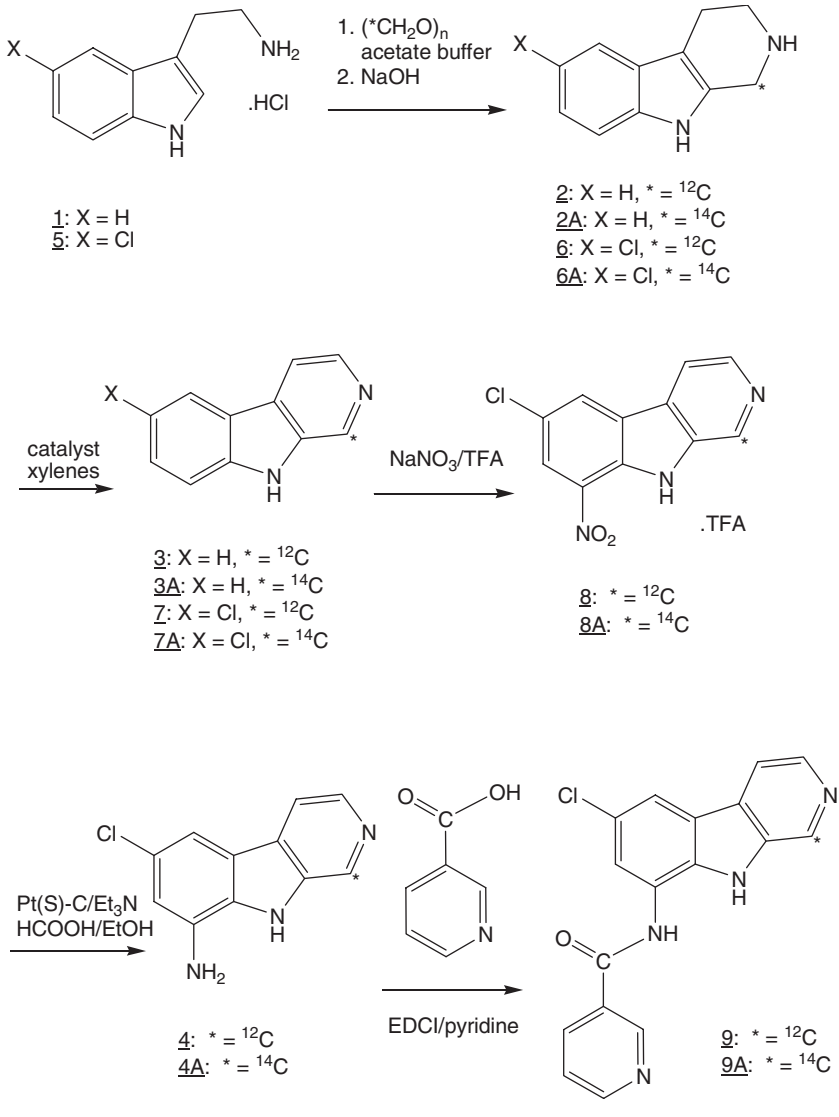
In an alternative approach, a similar carboline like 1-[$^{14}$C]-6-methoxy-1,2,3,4-tetrahydro-$\beta$-carboline has been synthesized from commercially available [$^{14}$C]-formaldehyde in a yield of 25%.[6] We believe that this low yield is because of instability and volatility of [$^{14}$C]-formaldehyde. Commercially available [$^{14}$C]-paraformaldehyde is more stable and can easily replace [$^{14}$C]-formaldehyde in such Pictect–Spengler cyclization reactions. In order to avoid poor regioselectivity during chlorination, we reasoned that Pictet–Spengler cyclization of 5-chlorotryptamine hydrochloride with [$^{14}$C]-paraformaldehyde would be a better alternative. Using this strategy, [$^{14}$C]-PS-1145 was synthesized from [$^{14}$C]-paraformaldehyde in five steps (Scheme 2).

In order to address generality of the methods, these reactions were also conducted with tryptamine. Cyclization reactions of 5-chlorotryptamine hydrochloride and tryptamine hydrochloride with [$^{14}$C]-paraformaldehyde in 3 M acetate buffer provided the desired $\beta$-carbolines in yields of 51 and 67%,




**Scheme 1.**




**Scheme 2.**

respectively. 1,2,3,4-Tetra-$\beta$-carbolines were oxidized to $\beta$-carbolines by refluxing with sulfur in xylenes[2,7] or Pd-C in xylenes.[1] Oxidation with Pd-C proceeded smoothly. Yields of oxidized products ranged between 60 and 65%. Nitration of 3A with sodium nitrate in TFA provided the nitro compound (73%), which was reduced with poisoned catalyst to provide the amine (94%).[1,8–10] Finally, [$^{14}$C]-PS-1145 (2-[$^{14}$C]-*N*-(6-chloro-9H-pyrido [3,4-b]indol-8-yl)-3-pyridinecarboxamide) (9A) was prepared by reacting 1-[$^{14}$C]-8-amino-6-chloro-$\beta$-carboline (4A), nicotinic acid, and EDCI (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride) in pyridine (73%).[1,11]

## Conclusion

Reaction of tryptamines with paraformaldehyde provides a convenient method of preparing 1,2,3,4-tetrahydro-$\beta$-carbolines in reasonable yields. C-14-labeled novel IKK inhibitor, 2-[$^{14}$C]-*N*-(6-chloro-9H-pyrido [3,4-b]indol-8-yl)-3-pyridine-carboxamide, was synthesized in five steps in an overall radiochemical yield of 15%.

## Experimental

### *General*

All commercial reagents were used as supplied unless otherwise stated. [$^{14}$C]-Paraformaldehyde was purchased from Amersham Biosciences. Radioactivity was quantified by liquid scintillation counting using Beckman LS6500 counter. HPLC analyses were performed on a Luna C18 (2) column (5 μ, 4.6 × 150 mm) with a flow rate of 1 ml/min. Solvent systems utilized consisted of A (0.1% formic acid in 99% water and 1% $CH_3CN$) and B (0.1% formic acid in 5% water and 95% $CH_3CN$) with a following gradient profile: 5% B for 1 min, 5–100% B over 12 min, and 100% B for 3 min. UV detection was at 254 (or 210) nm and radioactive detection was with a IN/US $\beta$-Ram Model 3 flow detector at a scintillant flow of 3 ml/min. NMR spectra were recorded on a Varian 600 (or 300) MHz spectrometer. LC–MS analyses were performed on a ThermoFinnigan LCQ mass spectrometer.

### *1-[$^{14}$C]-1,2,3,4-Tetrahydro-β-carboline* (2A)

To a stirred suspension of tryptamine hydrochloride (4.8 mmol), [$^{14}$C]-paraformaldehyde (10 mCi, 56 mCi/mmol) and paraformaldehyde (3.8 mmol, 0.114 g, specific activity of diluted paraformaldehyde, 2.2 mCi/mmol) in water (16 ml) was added 3 M acetate buffer (2.4 ml) under nitrogen. The mixture was heated under nitrogen at 105°C for 4 h. The reaction mixture was concentrated. The product was isolated by crystallization at 0°C. The dried solid was dissolved in methanol. This solution was basified with 1 N NaOH and evaporated to dryness. The solid was washed with cold water and dried to give 0.462 g (67%, 6.7 mCi, 2.2 mCi/mmol) product. HPLC: chemical purity 94% (UV area % at 210 nm); radiochemical purity, 94%. LC–MS/MS: $m/z$ 175 (M + 1), 173, 144.

### *1,2,3,4-Tetrahydro-β-carboline* (2)

Under similar reaction conditions, compound 2 was obtained in 71% yield (0.25 g) from paraformaldehyde (2 mmol). HPLC: 95% pure (UV at 210 nm). LC–MS/MS: $m/z$ 173 (M + 1), 144. $^1$H-NMR ($(CD_3)_2SO$): δ 7.33 (1H, d), 7.25 (1H, d), 6.97 (1H, t), 6.91 (1H, t), 3.85 (2H, s), 2.97 (2H, t), 2.60 (2H, t).

*1-[$^{14}$C]-6-Chloro-1,2,3,4-tetrahydro-β-carboline* (6A)

To a stirred suspension of 5-chlorotryptamine hydrochloride (3.77 mmol) and [$^{14}$C]-paraformaldehyde (175 mCi, 56 mCi/mmol), in water (12.5 ml) was added 3 M acetate buffer (1.88 ml) under nitrogen. The mixture was heated under nitrogen at 105°C for 4 h. The reaction mixture was cooled to room temperature. The product was isolated by crystallization at 0°C. The dried solid was dissolved in methanol. This solution was basified with 1 N NaOH and evaporated to dryness. The solid was washed with cold water and dried to give 91 mCi (55 mCi/mmol, 52%) product. HPLC: 99% (UV at 210 nm); radiochemical purity, 99%. LC–MS/MS: $m/z$ 209 (M + 1), 207, 178.

*6-Chloro-1,2,3,4-tetrahydro-β-carboline* (6)

The title compound was prepared similarly from paraformaldehyde (2 mmol) in 65% yield (0.27 g). HPLC: 99% (UV at 210 nm). LC–MS/MS: $m/z$ 207 (M + 1), 178. $^1$H-NMR ($(CD_3)_2SO$): δ 7.32 (1H, s), 7.24 (1H, d), 6.92 (1H, dd), 3.85 (2H, s), 2.95 (2H, t), 2.58 (2H, t).

*1-[$^{14}$C]-β-carboline* (3A)

1-[$^{14}$C]-1,2,3,4-Tetrahydro-β-carboline (2A, 6.7 mCi, 2.2 mCi/mmol) was suspended in xylenes (15 ml) in a 100 ml round-bottom flask equipped with a condenser that was open to the atmosphere. 5% Pd/C (0.24 g) was added. The mixture was heated in xylenes at 143°C with good stirring until LC–MS/MS indicated complete conversion (2 days). 5% Pd/C (0.12 g) was added twice a day to hasten the reaction. The reaction mixture was cooled to room temperature and methanol (150 ml) was added. The mixture was filtered through a pad of celite. The solvents were evaporated to give the desired (4.36 mCi, 2.2 mCi/mmol, 65%) product.[†] HPLC: 95% (UV at 210 nm); radiochemical purity, 96%. LC–MS/MS: $m/z$ 171 (M + 1), 169.

*β-Carboline* (3)

Oxidation of 1,2,3,4-tetrahydro-β-carboline (2, 0.5 mmol) using similar conditions provided 0.058 g (68%) of product. HPLC: 96% (UV at 210 nm). LC–MS/MS: $m/z$ 169 (M + 1). $^1$H-NMR ($(CD_3)_2SO$): δ 11.62 (NH, s), 8.90 (1H, s), 8.33 (1H, d), 8.24 (1H, d), 8.10 (1H, d), 7.58 (1H, t), 7.52 (1H, d), 7.24 (1H, t).

*1-[$^{14}$C]-6-Chloro-β-carboline* (7A)

1-[$^{14}$C]-6-Chloro-1,2,3,4-tetrohydro-β-carboline (6A, 29.8 mCi, 55 mCi/mmol) was suspended in xylenes (4.3 ml) in a 25 ml round-bottom flask equipped with

[†]The low recovery was probably due to adsorption of the product on the catalysts.

a condenser that was open to the atmosphere. 5% Pd/C (0.054 g) and $PtO_2$ (0.01 g) were added. The mixture was heated in xylenes at 143°C with good stirring until LC–MS/MS indicated complete conversion (3 days). Pd/C (0.027 g) was added three times a day to hasten the reaction. The same scale reaction was repeated twice (total activities, 61.2 mCi, 55 mCi/mmol). The reaction mixtures were cooled to room temperature and combined. Methanol (180 ml) was added. The mixture was filtered through a pad of celite. The solvents were evaporated to give 55 mCi (55 mCi/mmol, 60%) product (see footnote †). HPLC: 93% (UV at 210 nm); radiochemical purity, 96%. LC–MS/MS: $m/z$ 205 (M + 1), 203.

*6-Chloro-β-carboline* (7)

Following the similar conditions, oxidation of 6-chloro-1,2,3,4-tetrohydro-β-carboline (6, 1 mmol) provided 0.127 g (62%) product. HPLC: 97% (UV at 210 nm). LC–MS/MS: $m/z$ 203 (M + 1). $^1$H-NMR ($(CD_3)_2SO$): δ 8.96 (1H, s), 8.38 (1H, d), 8.35 (1H, d), 8.15 (1H, dd), 7.66 (1H, d), 7.54 (1H, dd).

*1-[$^{14}$C]-6-Chloro-8-nitro-β-carboline* (8A)

1-[$^{14}$C]-6-Chloro-β-carboline (7A, 55 mCi, 55 mCi/mmol) and $NaNO_3$ (2 mmol) were taken in a 25 ml round-bottom flask. Trifluoroacetic acid (3 ml) was added at 0°C under nitrogen. The mixture was stirred at room temperature for 18 h. The solvent was evaporated. The residue was washed with cold water and ether. The solid was dried to give the product (40 mCi, 55 mCi/mmol, 73%). HPLC: 98% (UV at 254 nm) and radiochemical purity, 99%. LC–MS/MS: $m/z$ 250 (M + 1), 248.

*6-Chloro-8-nitro-β-carboline* (8)

6-Chloro-β-carboline (7, 0.5 mmol) was nitrated similarly to provide 0.15 g (83%) product. HPLC: 98% (UV at 210 nm). LC–MS/MS: $m/z$ 248 (M + 1). $^1$H-NMR ($(CD_3)_2SO$): δ 13.18 (NH, s), 9.23 (1H, s), 9.16 (1H, d), 8.77 (1H, d), 8.72 (1H, d), 8.63 (1H, d).

*1-[$^{14}$C]-8-Amino-6-chloro-β-carboline* (4A)

1-[$^{14}$C]-6-Chloro-8-nitro-β-carboline (8A, 6.6 mCi, 55 mCi/mmol), 6-chloro-8-nitro-β-carboline (0.18 mmol, 0.065 g, specific activity of diluted carboline, 22 mCi/mmol), and 5% Pt(S)/C (0.0326 g) were taken in a 25 ml round-bottom flask. Ethanol (2.28 ml), triethylamine (1.5 mmol, 0.21 ml), and formic acid (1.2 mmol, 0.052 ml) were added at 0°C under nitrogen. The mixture was warmed to room temperature and heated at 78°C for 5 h. The reaction mixture was cooled, diluted with methanol (23 ml), and filtered through a pad of celite. The filtrate was basified with an aqueous solution of 1 M $Na_2CO_3$ to about pH

10. The solvents were evaporated. The residue was washed with water and dried to give 6.2 mCi (22 mCi/mmol, 94%) product. HPLC: 97% (UV at 254 nm), radiochemical purity, 98%. LC–MS/MS: $m/z$ 220 (M + 1), 218.

*8-Amino-6-chloro-β-carboline* (4)

Reduction of 6-chloro-8-nitro-β-carboline (8, 0.2 mmol) provided the desired (0.434 g, 100%) product. HPLC: 94% (UV at 254 nm). LC–MS/MS: $m/z$ 218 (M + 1). $^1$H-NMR ($(CD_3)_2SO$): δ 11.30 (NH, s), 8.94 (1H, s), 8.30 (1H, d), 8.03 (1H, d), 7.50 (1H, s), 6.76 (1H, s), 5.70 ($NH_2$, s).

*2-[$^{14}$C]-N-(6-Chloro-9H-pyrido [3,4-b]indol-8-yl)-3-pyridinecarboxamide (PS-1145)* (9A)

[$^{14}$C]-8-Amino-6-chloro-β-carboline (4A, 6.2 mCi, 22 mCi/mmol), nicotinic acid (0.39 mmol), and EDCI (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, 0.849 mmol) were taken in a 25 ml round-bottom flask. Pyridine (3 ml) was added under nitrogen. The mixture was stirred at room temperature for 5 h. The solvent was evaporated. To the residue was added methanol (0.2 ml) and saturated $NaHCO_3$ (3 ml). The mixture was stirred at room temperature for 0.5 h, then cooled in an ice bath. The solid product was isolated by centrifugation and dried. The residue was extracted with dichloromethane and methanol (1:1). The solution was concentrated and chromatographed on a silica gel column using a mixture of dichloromethane and methanol as eluants to provide the (4.6 mCi, 22 mCi/mmol, 74%) product. HPLC: 99.5% pure (UV at 254 nm) and radiochemical purity, 99.7%. LC–MS/MS: $m/z$ 325 (M + 1), 323. $^1$H-NMR ($(CD_3)_2SO$, 600 MHz): δ 11.58 (NH, s), 10.68 (NH, s), 9.25 (1H, s), 8.97 (1H, s), 8.85 (1H, d), 8.45 (1H, d), 8.38 (1H, d), 8.30 (1H, s), 8.18 (1H, d), 7.80 (1H, s), 7.64 (1H, dd).

*N-(6-Chloro-9H-pyrido [3,4-b]indol-8-yl)-3-pyridinecarboxamide (PS-1145)* (9)

Condensation of 8-amino-6-chloro-β-carboline (4, 0.3 mmol) similarly with nicotinic acid provided 0.0678 g (70%) product. HPLC: 99% (UV at 254 nm). LC–MS/MS: $m/z$ 323 (M + 1). $^1$H-NMR ($(CD_3)_2SO$): δ 11.58 (NH, s), 10.68 (NH, s), 9.25 (1H, s), 8.97 (1H, s), 8.85 (1H, d), 8.45 (1H, d), 8.38 (1H, d), 8.30 (1H, s), 8.18 (1H, d), 7.80 (1H, s), 7.64 (1H, dd).

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