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# Heck reaction and Stille coupling as the key steps in the synthesis of carbon-14-labeled gsk-3 inhibitor alsterpaullone

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**Glycogen synthase kinase 3 (GSK-3) is a serine/threonine protein kinase. Development of GSK3 inhibitor Alsterpaullone as a therapeutic agent for mood disorders, such as Alzheimer's disease and bipolar disorder, required the synthesis of a suitably labeled drug product for use in human metabolism and pharmacokinetic studies. Owing to the potential metabolic degradation of the molecule, a multi-labeled approach utilizing $^{14}C$ was adopted. The synthesis of [$^{14}C$]Alsterpaullone was accomplished in separate routes from methyl-[1, 2-$^{14}C$]-2-bromoacetate and [1, 2-$^{14}C$]-2-bromoethanol, respectively. Labeled versions were combined on the basis of molar radioactivity giving a final product with a radiochemical purity of 99.0% and a specific activity of 54.0 mCi/mmol.**

**Keywords:** carbon-14 labeling; glycogen synthase kinase; GSK-3 inhibitor; Alsterpaullone; Alzheimer's disease; Heck reaction; Stille coupling; indole synthesis

## Introduction

The novel indole derivative 9-nitro-7, 12-dihydroindolo[3, 2-d][1] benzazepin-6(5 H)-one is currently marketed as an unique GSK-3β inhibitor known commercially as Alsterpaullone.[1,2] This compound not only exhibits inhibition of GSK3β by preventing the Tau phosphorylation[3] at the sites that are typically phosphorylated by GSK3β, but also demonstrates remarkable *in vitro* antitumor activity. This dual activity affords Alsterpaullone as a novel and promising therapeutic agent for treatment of broad spectrum of disorders, especially mood disorders such as Alzheimer's disease.

Development of Alsterpaullone involved a detailed study of its pharmacology. A human clinical study was designed to measure the absorption, distribution, metabolism, elimination and toxicity (ADMET) of Alsterpaullone.[4] To measure these parameters, a radiotracer study utilizing $^{14}C$-labeled Alsterpaullone was devised. Conceivably, in addition to oxidation of the aromatic benzene and pyrrole rings, the molecule could also undergo oxidations of the lactam and incur further degradation. This hypothesis has been confirmed by one of our investigations in collaboration with our colleagues at the University of Illinois.[4b] According to a LC-MS/MS analysis of Alsterpaullone metabolites in comparison to synthesized intermediates, we were convinced that the majority *in vitro* metabolism of Alsterpaullone takes place on the seven-membered lactam, giving a mixture of metabolites include, but are not limited to α-hydroxyllactam, β,γ-epoxylactam and their corresponding conjugates.[4] Obviously, the complex nature of this metabolic situation limited the usefulness of mono-labeled Alsterpaullone for human ADME studies. In order to fully investigate the *in vivo* metabolism of Alsterpaullone and focus our attention on the transformation of the lactam ring, a multi-labeled version of [$^{14}C$] Alsterpaullone was adopted, thus assuring that metabolites from the lactam functionalization contain at least a $^{14}C$ label (Figure 1).

This article describes the synthesis of multi-labeled [$^{14}C$] Alsterpaullone for human clinical studies. The synthesis of Alsterpaullone has been previously reported.[5] However, this well-established 'cold' synthetic route can only be partially adopted, but not copied for the preparation of hot Alsterpaullone due to the unavailability of the corresponding [$^{14}C$]-labeled starting materials. Multi-step syntheses of separately labeled homologs of Alsterpaullone were completed and clinical supplies were prepared by combining labeled versions **1a** and **1b** based on equivalent amounts of radioactivity. The final product, multi-$^{14}C$-labeled Alsterpaullone, was prepared with a radiochemical purity of 99.0% with a specific activity of 54.0 mCi/mmol (Figure 2).

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## Experimental

Reactions were run under an inert atmosphere of argon and magnetically stirred at a constant rate. Solvent removal under vacuum was accomplished using a Buchi R-124 rotary evaporator. Column chromatography was performed using a Biotage® flash column chromatography system. Proton NMR spectra were recorded on a Varian Unity/Inova 300 MHz spectrometer. Radiochemical purity was determined by HPLC (Waters model 2695, PDA detector and Beta-Ram detector (IN/US Systems Inc.)) and TLC (Merck 60 $F_{254}$ silica gel coated plates) using radiochemical detection (QC-scan, Bioscan Model B-QC). Specific activity was determined by gravimetric analysis using liquid scintillation counting (Wallac Model 1409). Reactions were monitored by HPLC, TLC and NMR, and comparisons were made with authentic materials when available. All reagents were ACS grade or better, and radiolabeled precursors were supplied by Perkin Elmer Life Sciences.

HPLC method described below was used for in process and final product analyses as well as purification were described.

Column: YMC-Pack Pro 5.0 μm (4.6 × 150 mm). Mobile phase A: 80% water/20% $CH_3CN$ with 0.1% TFA. Mobile phase B: 20% water/80% $CH_3CN$ with 0.1% TFA. Program: Isocratic (100% A) 0–10 min, gradient (0–100% B) 10–30 min, isocratic (100% B), 30–35 min, gradient (0–100% A), 35–40 min. Flow rate: 1.0 mL/min, injection size: 10 μL.

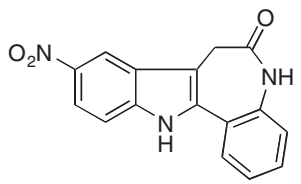



**Figure 1.** Alsterpaullone (**1**).

### 4-bromo-but-2-[1, 2-$^{14}$C]enoic acid methyl ester 3

A 125 mL round-bottom flask was charged with [1, 2-$^{14}$C] (methoxycarbonylmethyl) triphenylphosphonium bromide (2.3 g, 5.3 mmol, 305 mCi at 57.6 mCi/mmol) and anhydrous THF (100 mL). The flask was cooled to −78°C and LHMDS (1.0 M in hexane, 5.5 mL, 5.5 mol) was added dropwise. The resulting yellow solution was allowed to stir at the same temperature for 1 h before a solution of 2-bromoacetaldehyde (8.0 mmol) in anhydrous THF (10 mL) was added dropwise. The mixture was slowly warmed up to 0°C and stir at 0°C for another 1 h. Then the ice bath was allowed to thaw and the clear yellow solution was stirred at rt overnight. The next morning, iced water (50 mL) was added to the flask and the homogeneous mixture was transferred to a 250 mL separatory funnel and extracted with ethyl acetate (3 × 50 mL). The organic layers were combined and washed with saturated $NH_4Cl$ (3 × 30 mL), water (3 × 30 mL) and brine (3 × 30 mL) successively. The organic extracts were dried over $Na_2SO_4$, filtered and concentrated to give a crude product. The crude product was further purified by flash column chromatography followed by preparative TLC to afford the E isomer **3** as a colorless syrup (924 mg, 98%). TLC, $R_f = 0.32$ (25% ethyl acetate, hexanes). $^1$H NMR (300 MHz, $CDCl_3$) δ 2.51 (m, 2H), 3.33 (s, 3H), 5.82 (d, $J = 11.8$ Hz, 1H), 6.33 (d, $J = 12.1$ Hz, 1H).

### 4-(2-bromo-4-nitro-phenylamino)-but-2-[1, 2-$^{14}$C]enoic acid methyl ester 4

To a solution of 2-bromo-4-nitroaniline (1.74 g, 8.0 mmol) in anhydrous DMF (20 mL) at 0°C was added NaH (320 mg, 60% in mineral oil, 8.0 mmol). The off-white suspension was allowed to stir at the same temperature for about 45 min, until all the $H_2$ generation ceased. A solution of compound **3** (895 mg, 5.0 mmol) in anhydrous DMF (5.0 mL) was added dropwise and the mixture was allowed to warm up to 60°C and stir at this temperature overnight. The next morning, the reaction mixture

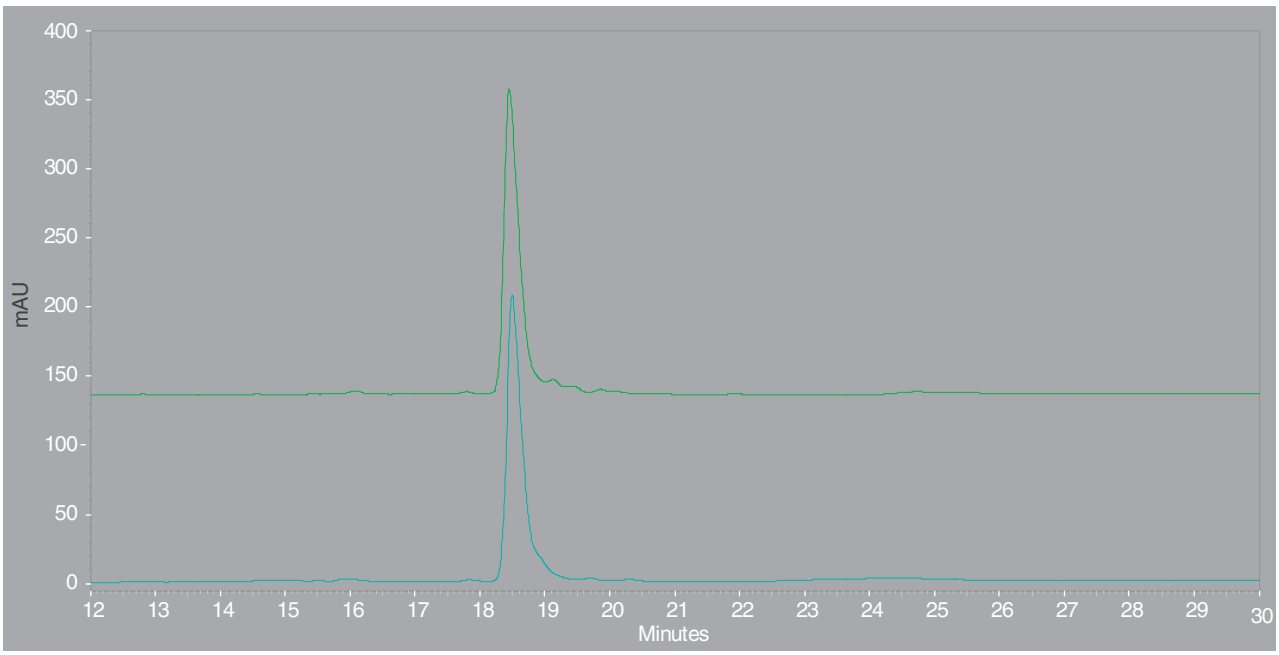



**Figure 2.** Radiochromatogram overlay of **1a** (bottom) and **1b** (top).

was cooled to rt and chipped ice (1 g) was added to the vigorously stirred mixture followed by water (1 mL). The reaction mixture was warmed up to rt and stirred further for 30 min before transferred to a 125 mL separatory funnel and partitioned. The aqueous phase was extracted with ethyl acetate (3 × 20 mL) and the organic layers were combined and washed successively with saturated $NH_4Cl$ (3 × 10 mL) and brine (3 × 10 mL). The organic extracts were dried over $Na_2SO_4$, filtered and concentrated to provide a brown solid as the crude product (2.84 g), which was further purified by flash column chromatography to provide compound **4** as a brown powder (1.45 g, 92%). $^1$H NMR (300 MHz, $CDCl_3$) δ 2.38 (d, $J$ = 2.3 Hz, 2H), 3.51 (s, 3H), 5.84 (d, $J$ = 12.1 Hz, 1H), 6.32 (d, $J$ = 12.2 Hz, 1H), 7.21 (d, $J$ = 2.1Hz, 1H), 7.34 (d, $J$ = 1.8 Hz, 1H), 7.88 (s, 1H). $^{13}$C NMR (75 MHz, $CDCl_3$) δ 202.04, 138.20, 131.60, 128.65, 128.54, 125.63, 124.39, 120.18, 113.15, 42.84, 27.13.

### 4-[(2-Bromo-4-nitro-phenyl)-*tert*-butoxycarbonyl-amino]-but-2-[1, 2-$^{14}$C]enoic acid methyl ester 5

To a solution of **4** (1.40 g, 4.4 mmol) in THF (20 mL) and saturated aqueous $NaHCO_3$ (5 mL) at 0°C was added $Boc_2O$ (1.25 g, 5.7 mmol). The solution was allowed to stir at rt overnight. The next morning, the solution was transferred to a 125 mL separatory funnel and partitioned. The mixture was extracted with ethyl acetate (3 × 20 mL) and all the organic layers were combined and washed successively with water (3 × 10 mL) and brine (3 × 10 mL). The organic extracts were dried over $Na_2SO_4$, filtered and concentrated to give a colorless residue, which after flash column chromatography purification, afforded compound **5** as an off-white solid (1.79 g, 98%). $^1$H NMR (300 MHz, $CDCl_3$) δ 0.88 (s, 9H), 2.41 (d, $J$ = 2.0 Hz, 2H), 3.50 (s, 3H), 5.82 (d, $J$ = 12.0 Hz, 1H), 6.33 (d, $J$ = 11.9 Hz, 1H), 7.18 (d, $J$ = 1.9 Hz, 1H), 7.33 (d, $J$ = 1.9 Hz, 1H), 7.85 (s, 1H). $^{13}$C NMR (75 MHz, $CDCl_3$) δ 201.18, 195.66, 136.78, 131.52, 127.48, 127.04, 126.60, 123.30, 117.10, 114.16, 45.84, 36.76, 25.28, 15.28.

### 3-[1, 2-$^{14}$C]Methoxycarbonylmethyl-6-nitro-4*H*-quinoline-1-carboxylic acid *tert*-butyl ester 6

A round bottom flask was charged with compound **5** (1.74 g, 4.2 mmol), $Pd(OAc)_2$ (112 mg), $Ph_3P$ (131 mg, 0.5 mmol), $NaHCO_3$ (706 mg, 8.4 mmol), LiCl (178 mg, 4.2 mmol) and anhydrous DMF (20 mL). The flask was heated to 130°C and the reaction was monitored at every 1 h interval. After all the starting material had been consumed, the flask was cooled to rt and the mixture was filtered through a small silica gel pad to remove all the precipitates. The silica gel pad was washed with EtOAc (3 × 10 mL) and the filtrates were combined and washed successively with water (3 × 10 mL) and brine (3 × 10 mL). The organic layers were dried over $Na_2SO_4$, filtered and concentrated to give a yellow residue (1.61 g) as the crude product. After a flash column chromatography, the desired compound was obtained as an off-white powder (1.11 g, 76%). $^1$H NMR (300 MHz, $CDCl_3$) δ 0.89 (s, 9H), 3.41 (m, 2H), 3.46 (s, 3H), 6.18 (s, 1H), 7.28 (d, $J$ = 1.9 Hz, 1H), 7.32 (d, $J$ = 2.1 Hz, 1H), 7.52 (s, 1H). $^{13}$C NMR (75 MHz, $CDCl_3$) δ 204.36, 197.28, 129.20, 129.15, 128.41, 128.00, 125.70, 123.30, 123.10, 119.16, 35.25, 33.26, 26.73, 17.26. HRMS: Cacd for $C_{17}H_{20}N_2O_6$ 348.1321, Found 348.1328.

### 3-[1, 2-$^{14}$C]Methoxycarbonylmethyl-6-nitro-2-trimethylstannanyl-4*H*-quinoline-1-carboxylic acid *tert*-butyl ester 7

To a −78°C solution of **6** (1.15 g, 3.3 mmol) in anhydrous THF (15 mL) was added freshly prepared LDA (1.2 M in THF, 2.8 mL, 3.3 mmol). The resulting yellow solution was stirred at −78°C for 30 min, then warmed up to 0°C and stirred for 1 h. The red solution was cooled to −78°C again and a solution of $Me_3SnCl$ (3.3 mmol) in THF was added dropwise with vigorous stirring. The mixture was kept at the same temperature for 30 min and allowed to slowly warm up to rt. A saturated aqueous $NH_4Cl$ solution (5.0 mL) was added and the mixture was partitioned in a 125 mL separatory funnel. Ethyl acetate (3 × 20 mL) was added for extraction and the organic layers were combined and washed successively with water (3 × 10 mL) and brine (3 × 10 mL). After filtration and rotatory evaporation, the organic phase was concentrated to give a yellow residue, which upon further flash column chromatography, delivered the desired compound **7** as a yellow syrup (1.11 g, 66%). $^1$H NMR (300 MHz, $CDCl_3$) δ 0.90 (s, 9H), 2.02 (s, 9H), 3.55 (m, 2H), 3.49 (s, 3H), 7.27 (d, $J$ = 2.0 Hz, 1H), 7.30 (d, $J$ = 2.1 Hz, 1H), 7.62 (s, 1H).

### 3-[1, 2-$^{14}$C]Methoxycarbonylmethyl-5-nitro-2-phenyl-indole-1-carboxylic acid *tert*-butyl ester 8

A Schlenk flask was charged with compound **7** (1.11 g, 2.17 mmol), bromobenzene (0.34 mL, 3.25 mmol), $Pd(PPh_3)_2Cl_2$ (40 mg, 0.05 mmol), Tri-*tert*-butylphosphine (20 μL, 0.11 mmol), CuI (21 mg, 0.11 mmol), CsF (660 mg, 4.34 mmol) and anhydrous toluene (20 mL). The suspension was heated to 120°C and vigorously stirred for 16 h. TLC monitoring proved that all the starting material **7** had been consumed. The brown mixture was filtered through a short silica gel column and the column was washed with EtOAc (3 × 5 mL). The light-colored filtrates were combined and rotatory evaporated to dryness. The crude product was further purified by flash column chromatography to give compound **8** as an off-white powder (846 mg, 95%). $^1$H NMR (300 MHz, $CDCl_3$) δ 0.87 (s, 9H), 3.36 (m, 2H), 3.46 (s, 3H), 7.27–7.33 (m, 6H, Ar-H), 7.39 (d, $J$ = 2.1 Hz, 1H), 7.68 (s, 1H). $^{13}$C NMR (75 MHz, $CDCl_3$) δ 205.15, 202.31, 133.20, 130.17, 129.60, 128.63, 128.28, 126.14, 125.85, 125.37, 125.02, 124.75, 123.86, 123.06, 122.58, 122.38, 35.35, 28.78, 27.26, 18.40.

### 8-Nitro-6*H*,11*H*-benzo[*a*][4, 5-$^{14}$C]carbazol-5-one 9

To a solution of LiOH (51 mg, 2.14 mmol) in $H_2O$ (1.0 mL) at 25°C was added a solution of **8** (800 mg, 1.95 mmol) in anhydrous THF (5 mL). To this solution was added $H_2O_2$ (30%, 5.0 mL). The solution was stirred at 25°C until the TLC monitoring showed that all the starting material **8** had been consumed. After cooling to 0°C, the solution was acidified with aqueous HCl (1.0 N) to pH 1–2. The mixture was extracted with EtOAc (3 × 10 mL) and all the extracts were combined and washed successively with water (3 × 10 mL), brine (3 × 10 mL) and dried over $Na_2SO_4$. The organic solvents were removed under reduced pressure to give a white powder (741 mg), which was dissolved in anhydrous pyridine (1.5 mL) and treated with cyanuric chloride (370 mg, 2.0 mmol) at 0°C. The mixture was allowed to warm up to rt and stirred for 3 h. After diluted with EtOAc (20 mL) and washed with water (3 × 5 mL) and brine (3 × 5 mL), all the solvents were removed under reduced pressure to deliver a colorless syrup (822 mg), which was used for next step reaction without further purification. The syrup was dissolved in anhydrous $CH_2Cl_2$ (10 mL) and cooled to −78°C. With vigorous stirring, $AlCl_3$ (267 mg, 2.0 mmol) was added in one portion. The solution was allowed to stir at this temperature for 1 h and slowly warmed up to rt and stirred for another 1 h. The mixture was again cooled to 0°C and aqueous HCl (4.0 N, 2.0 mL) was

added. The resulting clear solution was stirred at rt for 2 h and extracted with EtOAc (3 × 10 mL). The organic phase was washed successively with water (3 × 10 mL), brine (3 × 10 mL) and dried over $Na_2SO_4$. The solvents were removed under reduced pressure to give a crude product, which was purified by flash column chromatography to give the desired compound **9** as an off-white powder (331 mg, 61%). $^1$H NMR (300 MHz, $CDCl_3$) δ 3.12 (br, 1H, N-H), 3.52 (m, 2H), 7.24 (d, $J$ = 2.7 Hz, 1H), 7.28 (m, 2H, Ar-H), 7.31 (d, $J$ = 2.4 Hz, 1H), 7.35 (d, $J$ = 1.9 Hz, 1H), 7.37 (d, $J$ = 2.2 Hz, 1H), 7.56 (s, 1H). $^{13}$C NMR (75 MHz, $CDCl_3$) δ 215.28, 138.20, 136.60, 132.65, 131.56, 127.46, 127.00, 125.88, 125.67, 125.03, 123.38, 122.92, 122.10, 119.90, 118.25, 78.81. HRMS: Cacd for $C_{16}H_{10}N_2O_3$ 278.0691, Found 278.0688

#### 8-Nitro-6*H*, 11*H*-benzo[*a*][4, 5-$^{14}$C]carbazol-5-one oxime 10

To a solution of compound **9** (300 mg, 1.08 mmol) in absolute ethanol (10 mL) and anhydrous pyridine (2.5 mL) at 0°C was added $NH_2OH \cdot HCl$ (140 mg, 2.0 mmol) in one portion. The resulting yellow mixture was allowed to stir at rt overnight. TLC monitoring showed that all the starting material **9** had been consumed. The solution was diluted with EtOAc (20 mL) and partitioned in a 75 mL separatory funnel. After extraction with EtOAc ( × 10 mL), the organic layers were combined and washed successively with water (3 × 10 mL), brine (3 × 10 mL) and dried over $Na_2SO_4$. The solution was filtered, concentrated and purified by flash column chromatography to give a white powder as the desired product (215 mg, 68%). Compound **9** (89 mg) was recovered. $^1$H NMR (300 MHz, $CDCl_3$) δ 3.59 (m, 2H), 7.25–7.27 (m, 2H, Ar-H), 7.32–7.34 (m, 3H, Ar-H), 7.39 (d, $J$ = 2.1 Hz, 1H), 7.55 (s, 1H). $^{13}$C NMR (75 MHz, $CDCl_3$) δ 217.28, 137.55, 136.68, 134.60, 131.58, 127.66, 127.20, 125.99, 125.76 124.73, 123.78, 123.02, 122.10, 120.00, 118.95, 78.88.

#### 9-Nitro-5, 12-dihydro-7*H*-benzo[2, 3]azepino[4, 5-*b*]indol-[6, 7-$^{14}$C]-6-one 1a

To a solution of **10** (215 mg, 0.73 mmol) in toluene (8 mL) at 0°C was added PPA (750 mg). The mixture was heated to 80°C and stirred vigorously for 5 h. TLC monitoring clearly showed that starting compound **10** had been converted a new product. The resulting dark syrup was cooled to 0°C and treated with saturated aqueous $NaHCO_3$ (10 mL). The mixture was transferred to a 75 mL separatory funnel and partitioned. The aqueous phase was extracted with EtOAc (3 × 15 mL) and the organic layers were combined and washed successively with water (3 × 10 mL) and brine (3 × 10 mL). The combined organic phase was dried over $Na_2SO_4$, filtered and concentrated to afford a brown syrup, which was further subjected to a flash column chromatography purification, giving title compound **1a** as a light tan solid (165 mg, 77%) with a specific activity of 53.30 mCi/mmol and an HPLC radiochemical purity of 99.6%. $^1$H NMR (300 MHz, $CDCl_3$) δ 3.59 (m, 2H), 7.25–7.27 (m, 2H, Ar-H), 7.32–7.34 (m, 3H, Ar-H), 7.39 (d, $J$ = 2.1 Hz, 1H), 7.55 (s, 1H). $^{13}$C NMR (75 MHz, $CDCl_3$) δ 205.24, 133.50, 132.78, 132.03, 130.59, 126.89, 126.20, 124.88, 124.25, 123.18, 123.09, 122.89, 121.21, 120.05, 119.35, 85.56. HRMS: Cacd for $C_{16}H_{11}N_3O_3$ 293.0800, Found 293.0808.

#### 4-Bromo-but-2-[1, 2-$^{14}$C]enoic acid methyl ester 11

To a solution of 2-bromo[1, 2-$^{14}$C]ethanol (5.0 mmol, 280 mCi at 55 mCi/mmol) in $CH_2Cl_2$ (20 mL) at 0°C was added Dess–Martin reagent (2.54 g, 6.0 mmol) and the suspension was allowed to stir at the same temperature for 30 min before warmed up to rt. After 2 h, TLC and HPLC monitoring confirmed that all the starting material had been consumed. The reaction mixture was filtered through a celite pad and the filtration was collected and subject to a distillation under reduced pressure to remove most of the solvent. The concentrated residue which proved by HPLC to be > 90% pure and with a total activity of 230 mCi was used for next step reaction without further purification.

The chemical synthesis of compounds **11**–**18** and **1b**, 9-Nitro-5, 12-dihydro-7*H*-benzo[2,3]azepino[4, 5-*b*]indol-[7a, 12a-$^{14}$C]-6-one were performed according to the synthetic procedures of compounds **3**-**9** and **1a**, respectively. Compounds **11**–**18**, **1b** only differ from **3**–**9**, **1a** in the $^{14}$C labeling position, and the spectroscopic data of each compound were identical to its corresponding $^{14}$C-labeled region isomer.

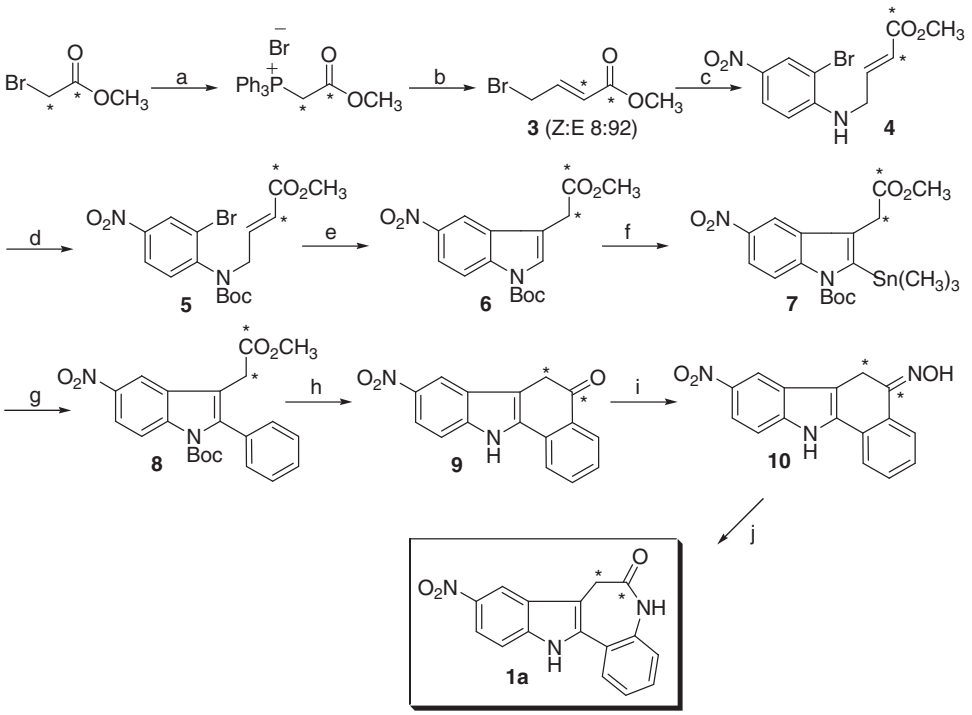
## Results and discussions

The synthesis of lactam ring-labeled [$^{14}$C] Alsterpaullone was accomplished in ten steps from methyl-[1, 2-$^{14}$C]-2-bromoacetate (Scheme 1). A Wittig reagent was obtained in a quantitative yield at the early stage of the synthesis, which was then treated at 0°C with 2-bromoacetaldehyde **2**, generated from a Dess–Martin oxidation of 2-bromoetahnol, to furnish compound **3** in 93% yield with a Z/E ration of 8:92 based on $^1$H NMR characterization. A conjugation of **3** with 2-bromo-4-nitroaniline was accomplished in the presence of NaH at 0°C using DMF as the solvent to deliver compound **4**. Following Boc protection of the free amine gave compound **5**, which was devised for the palladium-catalyzed indole synthesis. According to a well-established method reported by Mori and Ban,[6] the indole scaffold was built in an efficient and clean fashion.

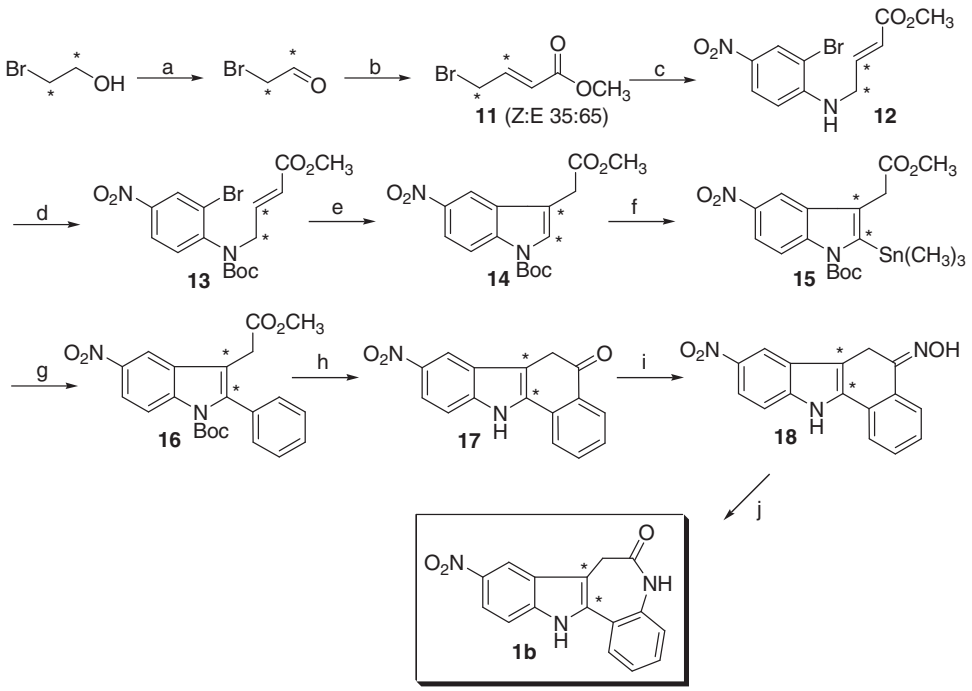
The obtained compound **6** was subject to an LDA-assisted deprotonation followed by a trimethyltin chloride treatment gave indolyl dimethyltin compound **7** as the key precursor for indole-phenyl conjugation and further lactam synthesis.

A Stille coupling between compound **7** and bromobenzene was carried out at 120°C in the presence of catalytic amount of $PdCl_2(PPh_3)_2$ to deliver compound **8** in an excellent 95% yield. The cyclization of **8** to ketone **9** was troublesome. In our first attempt of ring closure, a polyphosphoric acid (PPA)-mediated 'one-pot' reaction was employed.[7] However, instead of obtaining any desired compound, a black char was obtained, making further analysis and purification difficult. As an alternative, stepwise conversion of **8** to the corresponding carboxylic acid followed by a PPA-induced cyclization was investigated. As we have expected, compound **9** was isolated in 37%, which was unsatisfactory considering the lost of costly radiolabeled materials. Finally, after stepwise transformation of **8** to the corresponding acyl chloride, a conventional Friedel–Crafts[8] conversion delivered **9** in an acceptable 61% overall yield. The ketone **9** was successfully converted to an oxime **10** via an overnight treatment with hydroxylamine in basic condition. The final touch of the synthesis was accomplished by employing a PPA-mediated Beckman rearrangement,[7] which provided the [$^{14}$C] lactam **1a** in 77% yield as the only isomer. HPLC with on-line scintillation counter verified the radiochemical purity of **1a** as 99.5%. Further radioactivity assay determined the specific activity of **1a** as 53.7 mCi/mmol.

The pyrrole ring $^{14}$C-labeled **1b** was prepared in a similar fashion from commercially available [1, 2-$^{14}$C]-2-bromoethanol (Scheme 2). Following the methodology outlined in Scheme 1,




**Scheme 1.** Reagents and Conditions: (a) $PPh_3$, THF, 0°C to rt, 92%; (b) 2-bromoacetadehyde, LHMDS, THF/HMPA, −78–0°C, 98%; (c) NaH, DMF, 2-bromo-4-nitroaniline, rt to 60°C, 92%; (d) $Boc_2O$, $NaHCO_3$, aq THF, rt, 98%; (e) $Pd(OAc)_2$, $PPh_3$, $HaHCO_3$, LiCl, DMF, 130°C, 76%; (f) LDA (1.0 M in THF), $Me_3SnCl$, −78–0°C; (g) $PdCl_2(PPh_3)_2$, *t*-$Bu_3P$, CuI, CsF, DMF, 120°C, 16 h, 95%; (h) (1) LiOH, $H_2O_2$, THF, rt; (2) cyanuric chloride, pyridine, 0°C to rt; (3) $AlCl_3$, $CH_2Cl_2$, −78°C to rt, 2 h, 61%; (i) $NH_2OH \cdot HCl$, EtOH, Pyridine, rt, overnight, 99%; (j) PPA, toluene, 0–80°C, 5 h, 77%.




**Scheme 2.** Reagents and Conditions: (a) Dess–Martin reagent, $CH_2Cl_2$, 0°C to rt, 2 h; (b) methyl bromoacetate phosphonium salt, LHMDS, THF/HMPA, −78–0°C, 88%; (c) NaH, DMF, 2-bromo-4-nitroaniline, rt to 60°C, 92%; (d) $Boc_2O$, $NaHCO_3$, aq THF, rt, 94%; (e) $Pd(OAc)_2$, $PPh_3$, $HaHCO_3$, LiCl, DMF, 130°C, 66%; (f) LDA (1.0 M in THF), $Me_3SnCl$, −78°C to 0°C; (g) $PdCl_2(PPh_3)_2$, *t*-$Bu_3P$, CuI, CsF, DMF, 120°C, 16 h, 90%; (h) (1) LiOH, $H_2O_2$, THF, rt; (2) cyanuric chloride, pyridine, 0°C to rt; (3) $AlCl_3$, $CH_2Cl_2$, −78°C to rt, 2 h, 54%; (i) $NH_2OH \cdot HCl$, EtOH, Pyridine, rt, overnight, 98%; (j) PPA, toluene, 0°C to 80°C, 5 h, 84%.

the pyrrole ring-labeled lactam **1b** was obtained with 99.3% radiochemical purity and a specific activity of 54.4 mCi. The overall yield of **1b** from [1, 2-$^{14}$C]-2-bromoethanol was 26.7%. It is worth mentioning that the Wittig reaction was dramatically affected by the isotope effects incurred by dual $^{14}$C labeling. As a result, the reaction proceeded in a sluggish manner to provide

the Z/E isomers with a ratio of 35:65. The separation of the Z/E isomers required a flash column chromatography followed by a preparative TLC with highly non-polar eluent (5% ethyl acetate in hexanes). The PPA-induced Beckman rearrangement at the final stage of the synthesis was installed uneventfully, the phenyl migration giving the desired compound **1b** as the only isomer.

## Conclusions

Multiple-position $^{14}$C-labeled GSK-3 inhibitor Alsterpaullone was synthesized from readily available $^{14}$C-labeled building blocks. The key steps involved a Palladium-catalyzed Morri–Ban indole synthesis and a Stille coupling. The synthetic strategy reported herein provides a concise route to multi-$^{14}$C-labeled Alsterpaullone with excellent selectivity, good radiochemical purity and overall yields. With a focus on the understanding of *in vivo* transformation of the lactam ring, multiple $^{14}$C-labeling strategy will provide us an opportunity to screen and potentially identify every possible metabolite. Once the metabolites are mapped, further study will be carried out in our group for toxicology and drug safety evaluation. This multi-labeled Alsterpaullone will also be used as a probe for the investigation of Tau phosphorylation and GSK-3β inhibition for drug discover purpose. The research is currently on-going in our group and we will publish the results in due course.

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