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Research Article

Synthesis of several isotopically labeled pyrrolo[1,3-d]pyrimidine analogs

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Summary

Four members of a novel class of pyrrolo[2,3-d]pyrimidines that show potential for the treatment of asthma and neurodegenerative disorders, have been prepared with radioisotope labels and in one case with multiple stable isotope labels for ADME studies as part of the drug development process. The syntheses utilize an isotopically labeled 2,4,6-trisubstituted pyrimidine as a common building block, readily prepared from isotopically labeled urea. Cyclizations of the pyrimidine with bromo-ketones generate the ring fused pyrrolo[2,3-d]-pyrimidines with elegant efficiency as demonstrated by the preparation of structures **4**, **5**, **8** and **12**. Copyright © 2001 John Wiley & Sons, Ltd.

Key Words: radioactive; stable isotopes; pyrrolo[2,3-d]pyrimidines

Introduction

In 1990, Jacobsen *et al*. reported that the novel pyrrolo[2,3-d]pyrimidine, **4** was a potent antioxidant and inhibitor of lipid peroxidation.[1] Since then, several hundred analogs have been synthesized at the Pharmacia Corporation. A number of the analogs have exhibited potential as orally active antioxidants for the treatment of asthma and

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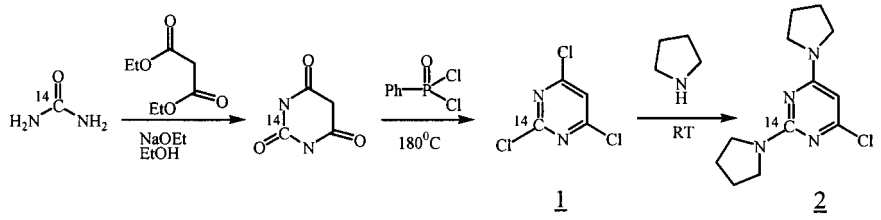
neurodegenerative disorders.[2] This paper describes the synthesis of four of these analogs labeled with carbon-14 at the C-2 position of the pyrimidine ring, and in one case, labeled with multiple stable isotopic labels, for preclinical absorption, distribution, metabolism, and excretion (ADME) studies, as part of the drug development process.

Discussion and results

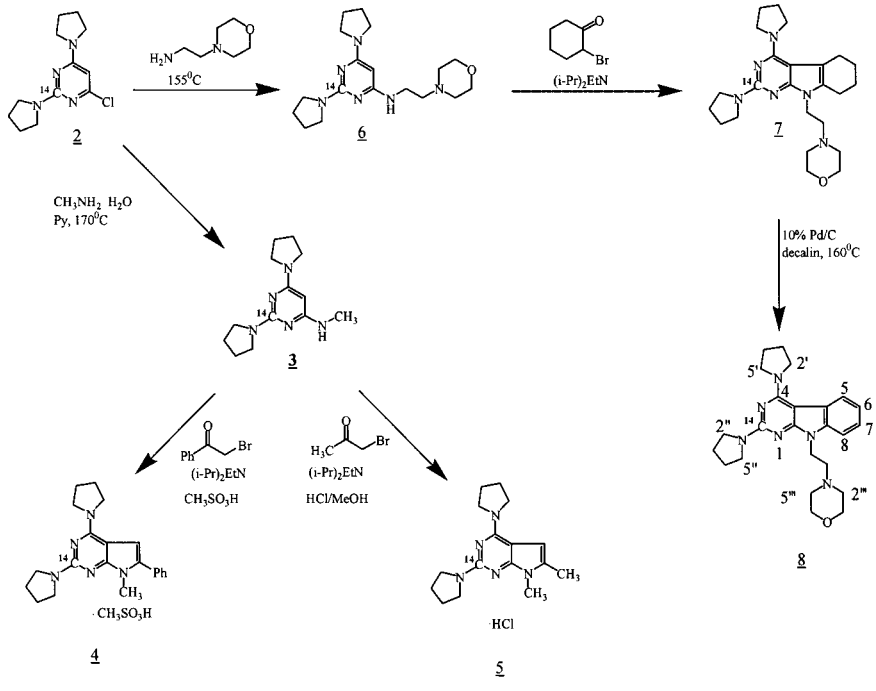
The preparation of **2**, as the penultimate intermediate for the synthesis of the first three pyrrolopyrimidines, **4**, **5**, and **8** is illustrated in Scheme 1. Following the chemistry presented in an earlier publication,[3] carbon-14 labeled urea (obtained from American Radiolabeled Chemicals) was converted to [^{14}C]barbituric acid and chlorodehydroxylated with phenylphosphonic dichloride to give 2,4,6-trichloro-[2-^{14}C]pyrimidine. Treatment with an excess of pyrrolidine at low temperature gave the dipyrrolidine-substituted intermediate **2**, in excellent yield. Due to the antioxidant nature of this class of compounds, and hence their sensitivity toward molecular oxygen especially when in solution, all solvents and solutions used were purged with nitrogen prior to use.

As shown in Scheme 2, a 25 mCi portion of **2** was treated with methylamine in a sealed pressure tube at 170°C for 26 h to give N-methyl-2,6-di-1-pyrrolidinyl-4-[2-^{14}C]pyrimidinamine, **3** in 87% radiochemical yield (RCY). This intermediate was used in a novel cyclization/dehydration reaction for the preparation of **4** and **5**.[2] In the presence of the non-nucleophilic base, diisopropylethylamine, **3** underwent cyclization and dehydration with 2-bromoacetophenone to give 7.8 mCi of product. The mesylate salt was prepared by treating with one equivalent of methanesulfonic acid to give a 74% RCY of **4** having a specific activity of 22.2 μCi/mg (9.85 mCi/mmol) and radiochemical purity (RCP) of at least 98% by both TLC and HPLC. In a similar manner, the cyclization/dehydration reaction of **3** and 2-bromoacetone gave 8.2 mCi of **5** in 71%



1 **2**

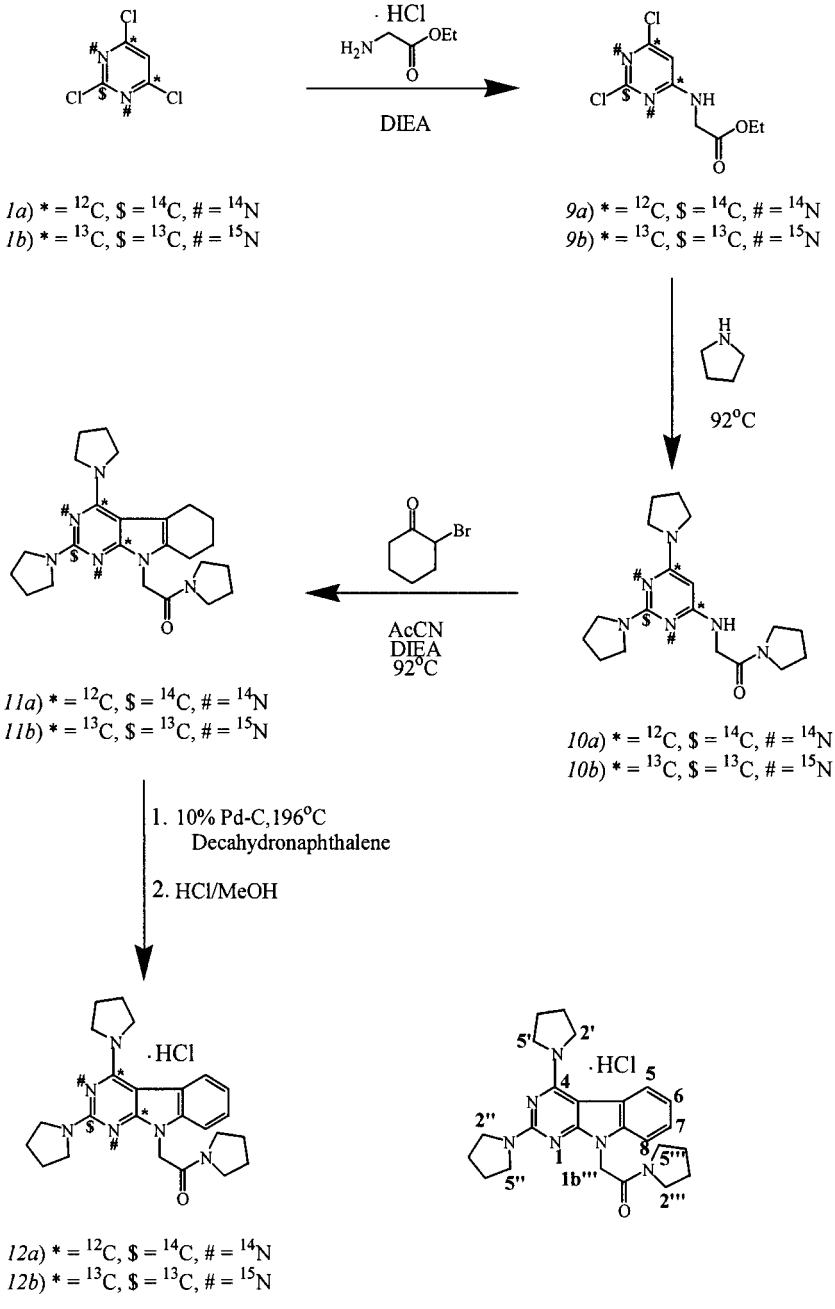
Scheme 1.



Scheme 2.

RCY, having a specific activity of 30.8 μCi/mg (9.9 mCi/mmol) after salt formation with dry hydrogen chloride in methanol. The RCP as determined by HPLC was 97% and by TLC was 98%.

A number of amines could be used to prepare 2,4,6-trisubstitued pyrimidines as illustrated by the preparation of **8**. The chloropyrimidine **2** was warmed in a pressure tube with aminoethylmorpholine (neat) for 24 h at 155°C. Reaction of the crude displacement product **6** with freshly prepared 2-bromocyclohexanone in the presence of base, gave 15.3 mCi of **7** in 50% RCY. Dehydrogenation of **7** with 10% palladium on carbon as the catalyst in anhydrous decahydronaphthalene at 160°C. This method was superior to the use of quinoline dehydrogenation reagents (chloranil and DDQ). After 4.5 h, only a small amount of starting material remained, no other side products were produced. Filtration to remove the catalyst, followed by acid/base workup to remove the decahydronaphthalene and purification by column chromatography gave, 4.9 mCi of **8**, in 82% RCY, with a specific activity of 23.9 μCi/mg (10 mCi/mmol), and a RCP of 99% by both HPLC and TLC.



1a) * = ^{12}C, $ = ^{14}C, # = ^{14}N
1b) * = ^{13}C, $ = ^{13}C, # = ^{15}N

9a) * = ^{12}C, $ = ^{14}C, # = ^{14}N
9b) * = ^{13}C, $ = ^{13}C, # = ^{15}N

10a) * = ^{12}C, $ = ^{14}C, # = ^{14}N
10b) * = ^{13}C, $ = ^{13}C, # = ^{15}N

11a) * = ^{12}C, $ = ^{14}C, # = ^{14}N
11b) * = ^{13}C, $ = ^{13}C, # = ^{15}N

12a) * = ^{12}C, $ = ^{14}C, # = ^{14}N
12b) * = ^{13}C, $ = ^{13}C, # = ^{15}N

Scheme 3.

A different approach was needed to prepare isotopically labeled **12** as shown in Scheme 3. Direct nucleophilic displacement of chloride from **2** with glycine pyrrolidine amide was unsuccessful, possibly due to the poor solubility of the amide in solvents like N,N-dimethylformamide.

A sample of 221 mCi of **1a** was warmed with glycine ethylester hydrochloride in the presence of diisopropylethylamine, to give a mixture of products from either the displacement of the chloride at the C-2 or C-4 of the pyrimidine ring in a rough statistical ratio of 1:2 (v/v). Subsequent column chromatography using 25:75 v/v ethyl acetate : hexanes resulted in the isolation of 164 mCi of **9a**, in 55% yield. Reaction of **9a** with excess pyrrolidine at reflux gave **10a** in excellent yield. Cyclization of **10a** with freshly prepared 2-bromocyclohexanone in the presence of base gave **11a**, in 89% RCY. Dehydrogenation of **11a** using 10% palladium on carbon in anhydrous decahydronaphthalene at 196°C gave 31 mCi (10.3% overall RCY) of **12a**, having a specific activity of 112 μCi/mg (51 mCi/mmol) after salt formation with dry hydrogen chloride in methanol. The RCP as determined by HPLC was 99.8% and by TLC was 98.7%.

A stable isotopomer of **12** was prepared using a parallel route shown in Scheme 3, by substituting [^{13}C, $^{15}N_2$]urea, and diethyl [1,3-$^{13}C_2$]-malonate as the unlabeled reagent precursors. Isolation of 379 mg (0.84 mmol) of **12b** was obtained with an isotopic enrichment of 99% for ^{13}C and >98% for ^{15}N (nominally).

Materials and methods

A. Thin layer chromatography (TLC)

TLC analyses were performed on 2.5 × 10 cm glass plates precoated with a 250 μm layer of silica gel GF (Analtech, Newark, DE). The developed zones were visualized by UV (254 nm) light. TLC radiochromatograms were obtained with a Bioscan System 200 Imaging Scanner. Integrated peak ratios were used to determine radiochemical purity (RCP).

B. High performance liquid chromatography (HPLC)

HPLC analyses were carried out with a Spectra Physics 8700 solvent delivery system. Chromatography for **4** and **5** was performed using a Supelcosil LC-18 DB, 5 μ, 4.6 mm I.D. × 250 mm column eluted with

85:15 v/v methanol:buffer (buffer = 1000 ml water, 5 ml triethylamine, and 1.4 ml formic acid), pumped isocratically at 1.5 ml/min. Chromatography for compounds **8** and **12** was performed using a Zorbax Rx-C8, 5 μ, analytical column (4.6 mm I.D. $\times$ 250 mm), eluted with 60:40 v/v CH_3CN:buffer (buffer = 1000 ml water, 5 ml triethylamine, 5 ml formic acid), pumped isocratically at 1.0 ml/min. All solvents were HPLC grade. UV detection of the column effluent was performed with a LDC/Milton Roy Spectromonitor-D variable wavelength detector set at 254 nm. Peak integration used the Radiomatic Flo-One Beta A280. Integrated peak ratios at the stated wavelength were used to determine the chemical purity. Radiochemical purity was determined from integrated radiochromatograms using the Radiomatic Flo-One Beta A280 radioactivity detector. A 2:1 (v/v) predetection mixing of Ultima Flo-M® (Packard Corp) liquid scintillation cocktail and column effluent was used for the measurement.

C. Radioactivity measurement

Radioactivity determinations were performed with a Pharmacia Wallac Model 1410 liquid scintillation spectrometer using the external standard method. Ultima Gold® (Packard Corp) was used as the liquid scintillation cocktail. Specific activity determinations were done by weighing out the compound of interest ($\sim$0.2 mg), dissolving it in methanol, and counting a known volume of the solution in 10 ml of the scintillation cocktail.

D. Nuclear magnetic resonance (NMR) spectroscopy

Structural determination was confirmed by proton NMR (^{1}H-NMR) using a Bruker AM 300 or a Bruker ARX 400 spectrometer at 300 or 400 MHz. The carbon-13 NMR (^{13}C-NMR) was performed on the same instruments at 75.5 and 100 MHz.

Experimental

*N-Methyl-2,6-di-1-pyrrolidinyl-4-[2-^{14}C]pyrimidinamine, **3***

A mixture of 391.8 mg (1.55 mmol) of unlabeled **2** and 240.6 mg (0.95 mmol, 25 mCi) of carbon-14 labeled **2** was placed in a 15 ml pressure tube equipped with a Teflon magnetic stirring bar, and a Teflon

stopper with a Viton® O-ring seal. The solids were dissolved in 2 ml of pyridine and to the solution, 8 ml of 40% methylamine/water was added quickly under nitrogen atmosphere. A fine precipitate formed. The pressure tube was stoppered and immersed in an oil bath at 140°C, which dissolved the fine solids. The oil bath temperature was increased to 170°C and the reaction mixture was stirred for 26 h. The tube was cooled in an ice bath, carefully opened under nitrogen atmosphere, and the reaction mixture was mixed with 5 ml of water and 2 ml of ethyl acetate. The mixture was briefly concentrated under reduced pressure. The aqueous mixture was further diluted with 5 ml of water, and cooled in an ice bath for 30 min. The resulting solids were collected by filtration, washed with water, and dried under high vacuum at room temperature to afford 537 mg of **3**, in 86% RCY, having a specific activity of 40.2 μCi/mg (9.94 mCi/mmol), and RCP of 94% by TLC (97:3 v/v, methylene chloride:4 M ammonia in methanol).

7-Methyl-6-phenyl-2,4-di-1-pyrrolidinyl-7H-pyrrolo[2,3-d,2-^{14}C]pyrimidine monomethanesulfonate, ***4***

A solution of 248 mg (1.0 mmol, 10 mCi) of the freshly prepared **3** in 6 ml of acetonitrile was mixed with 1.43 mmol of diisopropylethylamine and 231 mg (1.16 mmol) of 2-bromoacetophenone. The resulting solution was stirred at room temperature overnight under nitrogen atmosphere. Copious solids had precipitated. The mixture was then refluxed for 1 h and cooled under nitrogen. The resulting solids were filtered, washed with cold acetonitrile and dried under high vacuum at room temperature to afford 269 mg of product. The freshly prepared free base, 265 mg (0.76 mmol), was suspended in 2 ml of methanol at room temperature, and mixed with 1 ml of methanesulfonic acid solution (prepared from 1.83 g of methanesulfonic acid, brought to 25 ml with methanol). The suspension cleared, and after 10 min, 4 ml of ethyl acetate was added, and the solution was concentrated under reduced pressure. The residue was triturated with 4 ml of ethyl acetate and 8 ml of ether. The solids were filtered, washed with cold ether, and dried under high vacuum at room temperature to afford 331 mg of **4**, in 75% yield, having a specific activity of 22.2 μCi/mg (9.85 mCi/mmol), and a RCP of 99% by HPLC and 98% by TLC (95:5 v/v methylene chloride:acetone, sample prepared in 4 M ammonia in methanol).

6,7-Dimethyl-2,4-di-1-pyrrolidinyl-7H-pyrrolo[2,3-d,2-^{14}C]pyrimidine hydrochloride, **5**

To a stirred solution of 286 mg (1.15 mmol, 11.5 mCi) of **3**, in 6 ml of acetonitrile (solvent was purged with nitrogen prior to use) under nitrogen atmosphere at 0°C 260 µl (1.5 mmol) of diisopropylethylamine and 145 µl (1.73 mmol) of 2-bromoacetone were added. The reaction mixture was stirred overnight and allowed to reach room temperature as the ice bath melted. Copious solids resulted. The mixture was refluxed for 1 h, dissolving all solids. After cooling to room temperature, solids precipitated. Acetonitrile was removed under reduced pressure to about $\frac{1}{2}$ volume, and the resulting solids were filtered, washed with 2 ml of cold acetonitrile, and dried under high vacuum at room temperature to afford 247 mg of product. The material was mixed with 2 ml of methanol and 300 µl of 9.4 M hydrogen chloride in methanol. The resulting clear solution was briefly concentrated under reduced pressure to an oil, then mixed with 2 ml of ether. After stirring for 10 min at room temperature, solids precipitated. An additional 8 ml of ether was added, and the solids were filtered, washed with 4 ml of ether and dried under high vacuum at room temperature to afford 266 mg of **5**, in 71% RCY, having a specific activity of 30.8 µCi/mg (9.9 mCi/mmol), with RCP of 97% by HPLC and 98% by TLC (95:5 v/v methylene chloride : acetone, the sample was prepared in 4 M ammonia in methanol).

N-[2,(4-Morpholinyl)ethyl]2,6-di-1-pyrrolidinyl-4-[2-^{14}C]pyrimidinamine, **6**

A mixture of 101.5 mg (0.40 mmol) of unlabeled **2** and 289.3 mg (1.14 mmol, 26.35 mCi/mmol, 30 mCi) of carbon-14 labeled **2** was placed in a 15 ml pressure tube equipped with a Teflon magnetic stirring bar, and a Teflon stopper with a Viton® O-ring seal. Two millilitres of aminoethylmorpholine was added, the tube was purged with argon and tightly capped. The solution was placed in an oil bath at 155°C for 24 h. The pressure tube was cooled in an ice bath and carefully opened under nitrogen atmosphere. The bright yellow solution was partitioned with 10 ml of water and 20 ml of 1:1 v/v ethyl acetate:hexanes. The aqueous phase was extracted with an additional 20 ml of 1:1 v/v ethyl acetate:hexanes. The combined organic extracts were washed with 10 ml of water, 10 ml of saturated brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to afford 594 mg (28.8 mCi) of **6**, RCP of 93% by HPLC and 87% TLC (97:3 v/v

methylene chloride:4 M ammonia in methanol). This material was used without further purification in the next step.

2-Bromocyclohexanone

2-Bromocyclohexanone was prepared in a similar manner to a literature procedure for 1-bromo-3-methyl-2-butanone.[4] Freshly distilled cyclohexanone, 24.5 g (250 mmol) was mixed with 150 ml of methanol and cooled to −10°C. Bromine, 40 g (250 mmol) was added quickly to the vigorously stirred solution. There was no noticeable exotherm. After 30 min, the temperature was raised to 0°C. Stirring was continued for 8 h during which the reaction mixture came to room temperature. The colorless solution was quenched with 75 ml of water and stirred overnight at room temperature. An additional 75 ml of water was added and the two-phase mixture was concentrated under reduced pressure to remove the methanol. The remaining mixture was extracted with 2 × 100 ml portions of 1:1 v/v ethyl acetate:hexanes. The combined extracts were washed with 70 ml of water, 30 ml of 1.2 M sodium bicarbonate, 75 ml of saturated brine, and dried over anhydrous sodium sulfate. The dry extract was concentrated under reduced pressure and the residue was distilled under vacuum (B.P. 55–59°C/0.9 mm) to afford 34.5 g (78%) of a colorless liquid. A small sample was stored at room temperature under nitrogen, but within 4 days became a thick black tar. The bromoketone when stored in the frozen state at −10°C under argon, remain colorless for several weeks.

6,7,8,9-Tetrahydro-9-[2-(4-morpholinyl)ethyl]-2,4-di-1-pyrrolidinyl-5-H-[2-^{14}C]pyrimido[4,5-b]indole, 7

The freshly prepared **6** (theory: 1.54 mmol) was transferred to a 15 ml pressure tube with 4 ml of methylene chloride. The reaction mixture was concentrated under a stream of nitrogen, then under high vacuum to remove traces of solvent. The remaining yellow glass-like material was mixed with 700 µl (6.0 mmol) of 2-bromocyclohexanone, 1.1 ml (6.0 mmol) of diisopropylethylamine, and 1.0 ml of acetonitrile. The pressure tube was flushed with argon, tightly capped, and placed in an oil bath at 95°C for 34 h. Solids appeared as the reaction mixture cooled to room temperature. The mixture was partitioned with 10 ml of water and 20 ml of methylene chloride. The layers were separated and the aqueous layer was extracted with 20 ml of methylene chloride. The

combined organic extracts were washed with 10 ml of water, 10 ml of saturated brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The dark yellow residue was dissolved in methylene chloride and eluted through a 1 g Whatman Si solid phase extraction cartridge with 95:5 v/v methylene chloride:methanol to remove some polar impurities. The eluate was concentrated under reduced pressure. The residue was mixed with 4 ml of acetonitrile which on stirring and cooling gave a solid. The solid was filtered, washed with 3 ml of cold acetonitrile, and dried under high vacuum at room temperature to afford 363 mg (16.6 mCi) of **7**. The mother liquor (10.2 mCi) was isotopically diluted with 445 mg of unlabeled **7**, crystallized from acetonitrile to afford 408 mg of low specific activity material. The two crystalline lots of **7** were combined, dissolved in 6 ml of methylene chloride, concentrated under reduced pressure to an oil, and quickly mixed with 6 ml of acetonitrile at room temperature. The mixture was cooled in an ice bath for 10 min, then the solids were filtered, washed with 3 ml of cold acetonitrile, and dried under high vacuum at room temperature to afford 668 mg of **7**, having a specific activity of 22.9 μCi/mg (9.72 mCi/mmol). The RCP as determined was 99% by HPLC and 97% by TLC (95:5 v/v methylene chloride:methanol).

9-[2-(4-Morpholinyl)ethyl]2,4-di-1-pyrrolidinyl-9-H-[2-^{14}C]pyrimido[4,5-b]indole, ***8***

A mixture of 262 mg (0.617 mmol, 6.0 mCi) of **7**, 50 mg of 10% palladium on carbon catalyst, and 4.5 ml of decahydronaphthalene were stirred in an oil bath at 160°C for 4.25 h. A long 22 gauge stainless steel needle was used to sweep nitrogen through the mixture to remove the hydrogen gas. The reaction mixture was cooled to room temperature and filtered through a bed of Celite® and the bed washed with 2 ml of methylene chloride and 2 ml of hexanes. The filtrate and washings were partitioned with 10 ml of 0.5 N hydrochloric acid and 20 ml of hexanes. The aqueous phase was extracted again with 20 ml of hexanes to aid removal of the decahydronaphthalene. The aqueous phase was basified with 5 ml of 1.2 M sodium bicarbonate solution, and extracted with 2×20 ml portions of methylene chloride. The combined organic extracts were washed with 15 ml of saturated brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The resulting oil was purified by chromatography on 35 g of silica gel 60 (70-230 Mesh) packed in and eluted with 70:30 v/v methylene

chloride:acetonitrile. Fractions of **8** were collected, pooled, and concentrated under reduced pressure. The resulting oil was mixed with 1 ml of absolute ethanol at room temperature. Crystals formed quickly and were further precipitated by dropwise addition of 7 ml of water. The solids were filtered, washed with 5 ml of water, and dried under high vacuum at room temperature to afford 205 mg of **8**, in 81.7% yield, having a specific activity of 23.9 μCi/mg (10.0 mCi/mmol) and a RCP of 99% by HPLC and TLC. ^{1}H-NMR ($CDCl_3$) δ:1.95–1.97 (m, 8H, H-3′, 4′, 3″, 4″), 2.58 (bs, 4H, H-2‴, 5‴), 2.74 (t, 2H, $^{3}J = 6.9$ Hz, H-la‴), 3.60–3.62 (m, 8H, H-2″, 5″, 3‴, 4‴), 3.91 (bs, 4H, H-2′, 5′), 4.39 (t, 2H, $^{3}J = 6.9$ Hz, H-lb‴), 7.08 (dd, lH, $^{3}J = 8.0$, $^{3}J = 8.0$ Hz, H-6), 7.18 (dd, lH, $^{3}J = 7.8$, $^{3}J = 7.8$ Hz, H-7), 7.28 (d, lH, $^{3}J = 7.9$ Hz, H-8), 7.89 (d, lH, $^{3}J = 7.9$ Hz, H-5). ^{13}C-NMR ($CDCl_3$) δ:26.02 (C-3′, 4′, 3″, 4″), 38.75 (C-lb‴), 46.87 (C-2″, 5″), 49.97 (C-2′, 5′), 54.26 (C-2‴, 5‴), 57.04 (C-la‴), 67.36 (C-3‴, 4‴), 89.97 (C-4a), 108.58 (C-8), 119.98 (C-6), 120.88 (C-5), 121.85 (C-7), 122.45 (C-4b), 137.41 (C-8a), 158.4, 158.8 (C-9a, 4), 159.7 (C-2).

[(2,4-Dichloro-4-[2-^{14}C] pyrimidinyl)amino]acetic acid, ethyl ester, ***9a****, and stable isotope analog,* ***9b***

A room temperature solution of 802 mg (4.37 mmol, 221 mCi) of **1a** in 6 ml of dry tetrahydrofuran (THF) was treated with 628 mg (4.75 mmol) of glycine ethyl ester hydrochloride. To this mixture under nitrogen atmosphere, with stirring, 1.6 ml (9 mmol) of diisopropylethylamine (DIEA) was added dropwise over 10 min at room temperature. After the addition, the suspension was stirred at room temperature for 30 min, then refluxed for 5 h. After cooling to room temperature, the reaction mixture was concentrated under reduced pressure. The residue was partitioned between 20 ml of saturated sodium bicarbonate and 25 ml of methylene chloride. The layers were separated and the aqueous layer was extracted with 2×25 ml portions of methylene chloride. The combined methylene chloride extracts were dried over anhydrous sodium sulfate and concentrated under reduced pressure to afford a crude mixture containing the regioisomers. The crude mixture was purified by column chromatography on 90 g of silica gel (70-230 Mesh) packed in and eluted with 25:75 (v/v) ethyl acetate:hexanes. Fractions of **9** were pooled and concentrated under reduced pressure to afford 546 mg, 164 mCi (2.19 mmol, 74% RCY) of **9a**, RCP radiochemically pure by TLC (25:75 (v/v) ethyl acetate:hexanes). In a similar manner,

stable isotope labeled **9b** was prepared from 2,4,6-trichloro-[1,3-$^{15}N_2$, 2,4,6-$^{13}C_3$]pyrimidine to give 624 mg (2.49 mmol) of **9b**, representing a 54% yield, a single zone by TLC (25:75 v/v ethyl acetate:hexanes). ^{1}H-NMR ($CDCl_3$) δ:1.52 (t, 3H, $^3J = 7.14$ Hz, CH_3CH_2-), 4.19 (bs, 2H, $NHCH_2-$), 4.28 (q, 2H, $^3J = 7.12$ Hz, OCH_2), 5.7 (bs, lH, NH), 6.38 (bs, lH, H-5). ^{13}C-NMR ($CDCl_3$) δ : 14.50 (CH_3CH_2-), 43.42 (OCH_2), 62.43 (N-CH_2), 159.25 (C-6, enriched), 160.72 (C-4, enriched), 163.8 (C-2, enriched).

*[(2,6-Di-1-pyrrolidinyl-4[2-^{14}C]-pyrimidinyl)amino]acetyl pyrrolidine, **10a**, and stable isotope analog, **10b***

The solid **9a** (546 mg, 2.19 mmol) was treated with 10 ml of pyrrolidine at 0°C. The reaction mixture was placed under nitrogen atmosphere and stirred while warming to room temperature, and then heated at reflux for 5.5 h. After cooling to room temperature, the mixture was concentrated under reduced pressure, followed by high vacuum at room temperature to remove residual traces of pyrrolidine. The resulting residue was partitioned between 20 ml of saturated sodium bicarbonate and 25 ml of methylene chloride. The layers were separated, and the aqueous layer extracted with 2×25 ml portions of methylene chloride. The combined methylene chloride extracts were dried over anhydrous sodium sulfate and concentrated under reduced pressure to afford 103 mCi (63% RCY) of **10a**, RCP 99.8% by HPLC and RCP 95.9% by TLC (95:5 (v/v) methylene chloride:4 M ammonia in methanol). In a similar manner, stable isotope labeled **10b** was prepared from **9b** to give 857 mg (2.49 mmol) of **10b**, representing a 100% yield, a single zone by TLC (95:2 v/v methylene chloride:4 M ammonia in methanol). ^{1}H-NMR ($CDCl_3$) δ : 2.01−2.85 (m, 12H, H-3′, 3″, 3‴, 4′, 4″, 4‴), 3.35–3.69 (m, 12H, H-2′, 2″, 2‴, 5′, 5″, 5‴), 4.00 (bs, 2H, H-lb‴), 4.91 (bs, lH, H-5). ^{13}C-NMR ($CDCl_3$) δ : 24.45, 25.60, 25.70, 26.51 (C-3′, 3″, 3‴, 4′, 4″, 4‴), 46.40, 46.54, 46.84, 46.92, 47.07 (C-2′, 2″, 2‴, 5′, 5″, 5‴), 157.80 (C-6, enriched), 159.03 (C-4, enriched), 161.27 (C-2, enriched).

*(5,6,7,8-Tetrahydro-2,4-di-1-pyrrolidinyl-9H[2-^{14}C]-pyrimido[4,5-b]indoyl-9-yl)-acetic acid, pyrrolidine amide, **11a**, and stable isotope analog, **11b***

The **10a** from above was transferred to a 15 ml pressure tube (Ace glass No. 8648-04) with 5 ml of methylene chloride. The solution was

concentrated under a stream of nitrogen, then under high vacuum to remove traces of solvent. The remaining yellow solid was mixed with 1.4 g (7.81 mmol) of 2-bromocyclohexanone, 1.4 ml (7.81 mmol) of diisopropylethylamine and 4 ml of acetonitrile (purged in nitrogen). The pressure tube was flushed with nitrogen, tightly closed with a Teflon lined screw cap, and placed in an oil bath at 92°C for 34 h. All solids went into solution after 4 h of heating. After cooling to room temperature, the reaction mixture was partitioned between 25 ml of water and 25 ml of methylene chloride. The layers were separated and the aqueous layer was extracted with 2×25 ml portions of methylene chloride. The combined methylene chloride extracts were washed with 25 ml of water, 25 ml of brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure at room temperature. The resulting residue was triturated with 50 ml of hexane to induce solidification. The solids were filtered, washed with cold hexane and dried under high vacuum at room temperature to afford 845 mg (1.99 mmol, 89% RCY) of **11a**, RCP 87.4% by HPLC and 73.7% by TLC (95:5 (v/v) methylene chloride:4 M ammonia in methanol). In a similar manner, 988 mg (2.33 mmol, 94% yield) of **11b** was prepared, 94.6% pure by HPLC at 254 nm.

*1-[(2,4-D-1-pyrrolidinyl-9H-[2-^{14}C]-pyrimido-[4,5-b]indol-9-yl)-acetyl]pyrrolidine monohydrochloride, **12a**, and stable isotope analog **12b***

A mixture of 845 mg (1.99 mmol) of **11a** and 400 mg of 10% palladium on carbon catalyst and 10 ml of anhydrous decahydronaphthalene under nitrogen atmosphere was stirred in an oil bath at 196°C for 6 h. The reaction mixture was cooled to room temperature and filtered through a bed of Celite®, and the bed was washed with 10 ml of methylene chloride. The combined filtrate and wash were concentrated under reduced pressure. The residue was partitioned between 10 ml of 1 N HCl and 50 ml of hexanes. The layers were separated and the aqueous layer was extracted with 25 ml of hexanes to aid the removal of the decahydronaphthalene. The aqueous phase was basified with saturated sodium bicarbonate and extracted with 3×25 ml of methylene chloride. The combined methylene chloride extracts were washed with 25 ml of brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. TLC analysis of the residue showed the reaction to be incomplete. A mixture of the residue, 400 mg of fresh 10% palladium on carbon and 10 ml of decahydronaphthalene under

nitrogen atmosphere were stirred in an oil bath at 196°C for 6 h. The reaction mixture was worked up the same as above to remove the decahydronaphthalene to afford the crude free base. The crude mixture was purified by column chromatography on 90 g of silica gel (70-230 Mesh) packed in and eluted with 85:15 (v/v) methylene chloride:acetonitrile. Fractions of **12a** were pooled and concentrated under reduced pressure to afford 277 mg, 32.1 mCi (0.66 mmol) of **12a**, RCP 99.1% by HPLC. The purified free base was dissolved in 0.5 ml of a 1.2 M HCl in methanol solution. While cooling the methanolic solution, ether was added dropwise with stirring to a volume of 10 ml to precipitate the salt. After cooling at −18°C for 2 h, the white solids were filtered, washed with cold ether and dried under high vacuum at room temperature to afford 277 mg (0.61 mmol, 31 mCi, 14% overall yield) of **12a**, sp. act. 112 μCi/mg (51 mCi/mmol), RCP 99.8% by HPLC and RCP 98.7% by TLC. In a similar manner, 379 mg (0.84 mmol, 28% overall yield) of **12b**, 99% pure by HPLC at 254 nm. ^{1}H-NMR ($CDCl_3$) δ : 1.8−1.9, 2.0–2.1 (m, 4H, H-3‴, 4‴), 2.03–2.09 (m, 8H, H-3′, 3″, 4′, 4″) 3.48 (t, 2H, H-2‴, 5‴), 3.87 (t, 2H, H-2‴, 5‴), 4.00 (m, 8H, H-2′, 5′, 2″, 5″), 6.08 (s, 2H, H-lb‴), 7.22–7.33 (m, 3H, H-5, 6, 7), 7.89 (d, lH, H-8).

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