SYNTHESIS OF 2-AMINO-3,5-DIHYDRO-7-(3-THIENYLMETHYL)-[6-^{14}C]-4H-PYRROLO[3,2-d]PYRIMIDIN-4-ONE MONOHYDROCHLORIDE ([^{14}C]CI-1000)

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SUMMARY

2-Amino-3,5-dihydro-7-(3-thienylmethyl)-[6-^{14}C]-4H-pyrrolo [3,2-d]pyrimidin-4-one monohydrochloride ([^{14}C]CI-1000), a potent purine nucleoside phosphorylase inhibitor, was made in six steps from potassium [^{14}C]cyanide. A key step was the reduction of a nitro and a nitrile group with sodium hypophosphite and RaNi followed by cyclization of the resulting intermediate to form a pyrrolo[3,2-d] pyrimidinone.

Key words: potassium [^{14}C]cyanide, methyl [^{14}C]cyanoacetate, CI-1000, PNP inhibitor, immunosuppressive agent

INTRODUCTION

2-Amino-3,5-dihydro-7-(3-thienylmethyl)-4H-pyrrolo[3,2-d]pyrimidin-4-one

monohydrochloride (CI-1000) is a purine nucleoside phosphorylase (PNP) inhibitor. The

compound shows a K_i of 0.067 µM in the human erythrocyte PNP enzyme assay and an IC$_{50}$ of

0.08 µM in the calf spleen PNP assay.[1] CI-1000 is under investigation as an immunosuppressive

agent. To study its pharmacokinetics and drug metabolism, a carbon-14 labeled material was

synthesized.
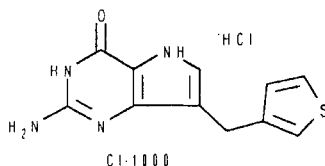
CI-1000

RESULTS AND DISCUSSION

Scheme 1 shows the synthesis of CI-1000 with carbon-14 in the 6 position of the pyrrolo[3,2-d]pyrimidine ring starting from potassium [^{14}C]cyanide. The intermediate 4 was made using a modification of the method reported by Anderson and Woodall.[2] The starting material for the reported reaction was methyl cyanoacetate. Carbon-14 introduced into methyl cyanoacetate provided an entry into the synthesis using the literature precedence. Potassium [^{14}C]cyanide and methyl bromoacetate in refluxing methanol gave 2. Base-catalyzed condensation of the resulting 2 with 3-thiophene carboxaldehyde gave the unsaturated cyanoester 3 in a 30% yield for both steps. Catalytic hydrogenation with 5% Pd/C reduced the double bond to give 4 in a 95% yield.

Intermediate 4 was converted to intermediate 7 as reported by Kostland and Sircar for the synthesis of CI-972.[3] Potassium carbonate was substituted for the reported use of sodium hydride in the reaction of the chloropyrimidine 5 and cyanoester 4 in DMSO. Hydrolysis and decarboxylation of 6 produced 7 in a 75 % yield for the 2 steps.

SCHEME 1
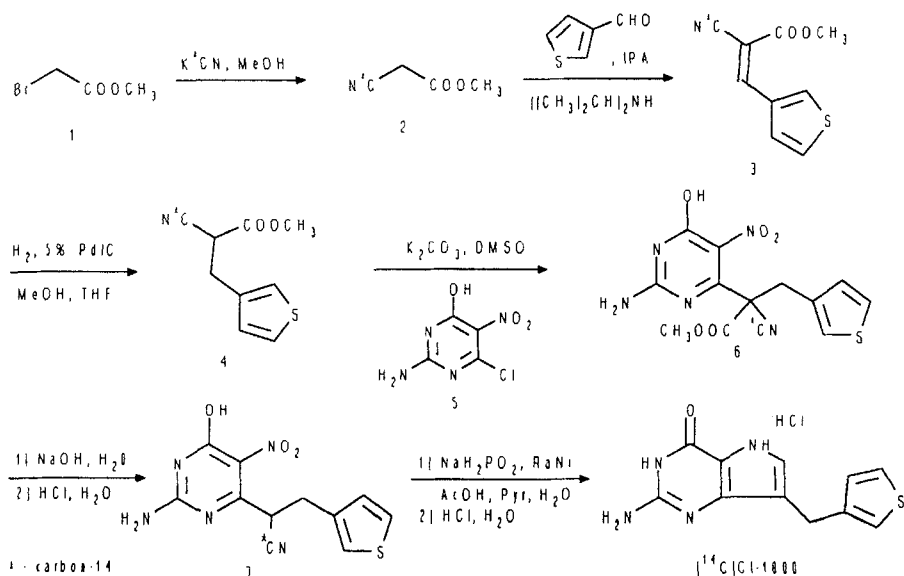
Scheme 2 shows how 7 was converted to CI-972,[3] and how CI-1000 was made by converting CI-972 to its diazonium salt and then reduction.[4] At this point investigation showed that it may be possible to go in one step to CI-1000. This would require the reduction of the

nitrile to the aldehyde and the reduction of the nitro group to the amine followed by

intramolecular cyclization. Catalytic hydrogenation showed success in forming CI-1000 but

CI-972 was found as an impurity in varying amounts in the product mixture.[5] There are several

other chemical means of nitrile reduction which could be useful in this transformation. Stephen

showed that stannous chloride and HCl under anhydrous conditions reduce nitriles to aldehydes.[6]

Such conditions may also reduce nitro groups and could give CI-1000. Treatment of 7 in Et$_2$O

with SnCl$_2$ and saturating the mixture with HCl gas gave exclusively CI-972. The reduction of

nitriles to aldehydes using Raney nickel and internally generated hydrogen was reported to be

successful. Blackberg and Staskun reported that Raney nickel and sodium hypophosphite in

water gave good yields.[7] They also reported the next year the use of aqueous formic acid as the

hydrogen source.[8] Van Es and Staskun showed in a later paper that the formic acid system also

reduced nitro groups to amines such as m-nitrobenzonitrile giving m-formamidobenzaldehyde.[9]

I explored both the system with Raney nickel, sodium hypophosphite in acetic acid, pyridine and

water; and the system with Raney nickel and aqueous formic acid. The reactions were followed

qualitatively by TLC. The first system gave predominately CI-1000 with some CI-972. The

formic acid method gave predominately CI-972 and another unidentified product. CI-1000 was

present only in a trace amount.

Scheme 2
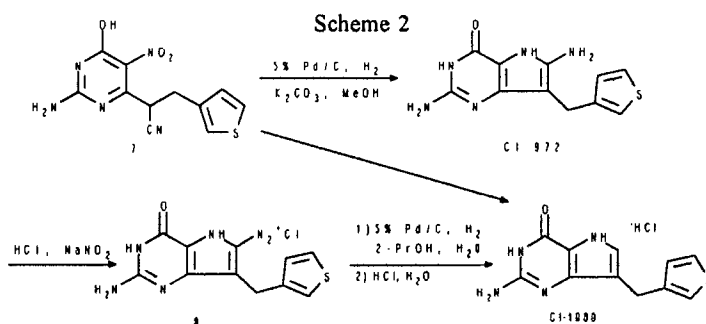
Although some of the high pressure catalytic hydrogenations were showing some promise

for giving good yields of CI-1000, the conditions with Raney nickle/sodium hypophosphite were

selected as the method for the radiolabeled synthesis. The conversion of 7 to [^{14}C]CI-1000 was

run in that system at 45-50°C for 18 hours. Radio-TLC showed the product distribution of the

isolated crude product to be CI-1000 45%, CI-972 25% and two unidentified polar impurities. Column chromatography gave [^{14}C]CI-1000 in a 27% yield. However, CI-972 was still present in 0.83%. Specifications required that the impurity be < 0.5 %. Further purification described in the experimental section was used to produce [^{14}C]CI-1000 that exceeded the required specifications. The final result was 6.52 mCi of [^{14}C]CI-1000 at a specific activity of 32.4 μCi/mg (9.17 mCi/mmole).

EXPERIMENTAL

Potassium [^{14}C]cyanide, at a specific activity of 58 mCi/mmol, was purchased from American Radiolabeled Chemicals, St. Louis, Missouri. Methyl bromoacetate and 3-thiophenecarboxaldehyde were purchased from Aldrich Chemical Company, Milwaukee, Wisconsin. 2-Amino-6-chloro-5-nitro-4(3H)-pyrimidine (5) was made by the method of Browne[10] and supplied by Parke-Davis Chemical Development, Holland, Michigan.

Liquid scintilation counting was performed with a Packard Tri-Carb 4530 liquid scintillation counter using Beckman Ready-solve MP liquid scintillation cocktail. Thin layer chromatography (TLC) was done with E. Merck silica gel (0.25 mm). The plates were anaylyzed for radiochemical purity (RCP) using a Berthold LB-2832 automatic TLC-linear analyzer. High performance liquid chromatography (HPLC) was performed using a Waters Associates model 600 solvent delivery system with a model 481 UV detector and a Radiomatic Flo-one/Beta model A250 radioactive flow detector. All compounds had identical R_f or t_R to that of authentic unlabeled standards. ^{1}H-NMR spectra were run on a Varian XL-200 (200 MHz) spectrometer. Chemical shifts were reported in δ units downfield from tetramethylsilane.

Methyl 2-[^{14}C]cyano-3-(3-thienyl)acrylate. Potassium [^{14}C]cyanide (336 mg, 5.17 mmol, 300 mCi) was suspended in MeOH (10 mL) and methyl bromoacetate(2.156 g, 14.1 mmol) was added. The mixture was heated at reflux for 45 min. Unlabeled potassium cyanide (673 mg, 10.3 mmol) was added and the reflux continued for 2 h. The cooled reaction mixture was filtered and the resulting solid was rinsed with MeOH. The solvent was evaporated and the resulting residue partitioned between water and dichloromethane. The organic layer was separated, dried

(MgSO₄) and evaporated *in vacuo* to give methyl [¹⁴C]cyanoacetate. This was dissolved in 2-propanol(5 mL) at 0° C and treated with 3-thiophenecarboxaldehyde (1.70 g, 15.2 mmol) in 2-propanol (5 mL) and diisopropylamine(0.3 mL). The reaction mixture was warmed to room temperature after 15 min. After an additional 15 min, the reaction was cooled and filtered to give a first crop of **3**. The filtrate was evaporated the the residue crystallized from 2-propanol. The combined crops gave 808 mg of **3** (30% yield from BrCH₂COOMe). TLC: R_f = 0.16, RCP = 98.9%, SiO₂, Hexane:EtOAc 9:1

Methyl 2-[¹⁴C]cyano-3-(3-thienyl)propionate (4). A mixture of **3** (808 mg, 4.19 mmol) and 5% Pd/C (300mg) in methanol (40 mL) and THF (10 mL) was shaken under H₂ (60 psi) for 15 h. The reaction mixture was filtered and evaporated to an oil. Crystallization from 2-propanol gave 776 mg of **4** (95% yield). TLC: R_f = 0.21, RCP = 97.4%, SiO₂ Hexane:EtOAc 4:1

Methyl 2-amino-α-[¹⁴C]cyano-6-hydroxy-5-nitro-α-(3-thienylmethyl)-4-pyrimidine acetate (6). A mixture of **4** (776 mg, 3.97 mmol), **5** (1.566 g, 4.00 mmol), potassium carbonate (1.1 g, 8.0 mmol) and DMSO (6.0 mL) was heated at 70 °C for 5.5 h. The DMSO was removed by vacuum distillation. The residue was mixed with ice and neutralized with 6 *M* HCl. The resulting solid **6** was isolated, rinsed with water, partially air dried and then used in the next step. TLC: R_f = 0.23, RCP = 100%, SiO₂ CHCl₃:MeOH:NH₄OH 30:5:1

(±)-2-Amino-6-hydroxy-5-nitro-α-(3-thienylmethyl)-4-pyrimidine [¹⁴C]acetonitrile (7). The cyanoester **6** was suspended in water (20 mL) at 0 °C and treated with 50% NaOH (1.6 g) over 5 min. The resulting red solution was stirred at room temperature for 3 h, then cooled and the pH was adjusted to four with 6 *M* HCl. The resulting solid was filtered, rinsed with water and dried *in vacuo* to give 868 mg (75% yield from **4**). TLC: R_f = 0.27, RCP = 96.4%, SiO₂ CHCl₃:MeOH:NH₄OH 30:5:1. Specific activity: 63.8 μCi/mg, 18.6 mCi/mmol.

2-Amino-3,5-dihydro-7-(3-thienylmethyl)-[6-¹⁴C]4H-pyrrolo[3,2-d]pyrimidin-4-one monohydrochloride ([¹⁴C]CI-1000). A mixture of **7** (867 mg, 2.98 mmol), unlabeled **7** (906 mg, 3.11 mmol), sodium hypophosphite (3.6 g) and Raney Ni (1.9 g, wet) in pyridine (24 mL), acetic acid (12 mL) and water (12 mL) was heated at 45-50 °C for 18 h. The mixture was cooled,

filtered (Celite) and the solid was rinsed with the solvent mixture. The solvent was removed by
vacuum distillation at 65 °C (0.25 torr). The dark residue was stirred with water and a yellow
solid was isolated. The crude solid was purified by flash chromotography on silica gel eluting
with $CHCl_3$:MeOH 7:1. Due to limited solubility of the product in the mobile phase, it co-eluted
with several other impurities. Impure fractions were chromatographed a second time. From the
chromatography were collected 455 mg of pure [14C]CI-1000 free base. The solid was slurried in
1 *M* HCl (15 mL) and heated with stirring for 20 min. After cooling, filtering and rinsing with
water, [14C]CI-1000 was isolated (465 mg, 27% yield). TLC: R_f = 0.33, RCP = 99.1%,
SiO_2 $CHCl_3$:MeOH:NH_4OH 30:5:1.

Purification of [14C]CI-1000. The above sample (containing 0.83% [14C]CI-972 by TLC) was
suspended in 1 *M* HCl (8 mL) with $NaNO_2$ (40 mg) for 4 h then filtered and dried to give
396 mg of [14C]CI-1000. HPLC analysis showed 1.3% of [14C]CI-972 present. The sample was
again treated with $NaNO_2$ in aqueous HCl, this time at 80 °C for 30 min. The isolated sample
was recrystallized from MeOH/H_2O 2:1 (charcoal) to give 238.2 mg of [14C]CI-1000. The RCP
was 98.2 % with new high R_f impurities. The solid was purified by flash chromatography (SiO_2
$CHCl_3$:MeOH 17:1) and then crystallized from $CHCl_3$:MeOH 4:1 to give 176 mg of
[14C]CI-1000. 1H-NMR showed less than one equivalent of HCl. The sample was triturated with
1 *M* HCl at reflux for one h. The solid was isolated and dried *in vacuo* to give 205 mg,
(6.58 mCi) of [14C]CI-1000. Specific Activity: 32.4 µCi/mg, 9.17 mCi/mmol; TLC: R_f = 0.35,
RCP = 99.9, SiO_2 $CHCl_3$:MeOH:NH_4OH 30:5:1. HPLC: t_R = 6.20 min, RCP = 100%,
Chemical Purity 99.3%. Alltech Econosil CN, 10 µ, 4.6 mm ID x 250 mm, 0.05 *M* Et_3N (pH = 3
with HCOOH):CH_3CN 95:5, flow rate = 2.0 mL/min UV @ 225 nm. 1H NMR (DMSO-d_6) δ
3.88 (s, 2H), 7.10 (d, J=3 Hz, 1H), 7.18 (s, 1H), 7.20 (s, 1H), 7.45, (d, J=3 Hz, 1H),
7.75, (s, 2H), 12.35, (s, 2H).

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