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SYNTHESIS OF [^{3}H]CI-980, ETHYL[5-AMINO-1,2-DIHYDRO-2(*S*)-METHYL-3(3-[^{3}H]PHENYL)PYRIDO[3,4-*b*]PYRAZIN-7-YL]CARBAMATE ISETHIONATE SALT, A TUBULIN-BINDING, ANTIMITOTIC, BROAD-SPECTRUM ANTITUMOR AGENT

Helen T. Lee and Peter W. K. Woo
Parke-Davis Pharmaceutical Research,
Division of Warner-Lambert Company,
Ann Arbor, Michigan 48105, U.S.A.

SUMMARY

[^{3}H]CI-980 (**10b**) was synthesized in an eight-step sequence with an overall yield of 2.4%. Reaction of *m*-bromophenyl lithium (**2**) with *N*-ethoxycarbonyl-L-alanine gave the chiral ketone **3**. Reduction of **3** with sodium borohydride followed by alkaline *N*-deprotection and condensation with ethyl 6-amino-4-chloro-5-nitro-2-pyridine carbamate (**6**) gave **7**. Chromium trioxide oxidation of **7** followed by reductive cyclization with iron-acetic acid gave the key bromo intermediate **9**. Palladium catalyzed ^{3}H-hydrogenolysis of **9** gave the free base form of [^{3}H]CI-980 (**10a**), which was converted to the isethionate salt (**10b**) before use.

Key Words: [^{3}H]CI-980, antitumor agent, catalytic ^{3}H-hydrogenolysis

INTRODUCTION

As indicated in the previous communication, the biologically more active 2(*S*) enantiomeric component of ethyl[5-amino-1,2-dihydro-2-methyl-3-phenylpyrido[3,4-*b*]pyrazin-7-yl]carbamate (*R*,*S*-**10**), a broad spectrum antitumor agent,[1] is being developed clinically as CI-980. During the process, the labeled forms of CI-980 were needed for pharmacokinetics and drug metabolism studies. This paper presents the synthesis of [^{3}H]CI-980.

RESULTS AND DISCUSSION

[^{3}H]CI-980 was synthesized in eight steps (Scheme I). Lithiation of **1** with *n*-butyl lithium at -110°C, followed by reaction with *N*-ethoxycarbonyl-L-alanine[2]

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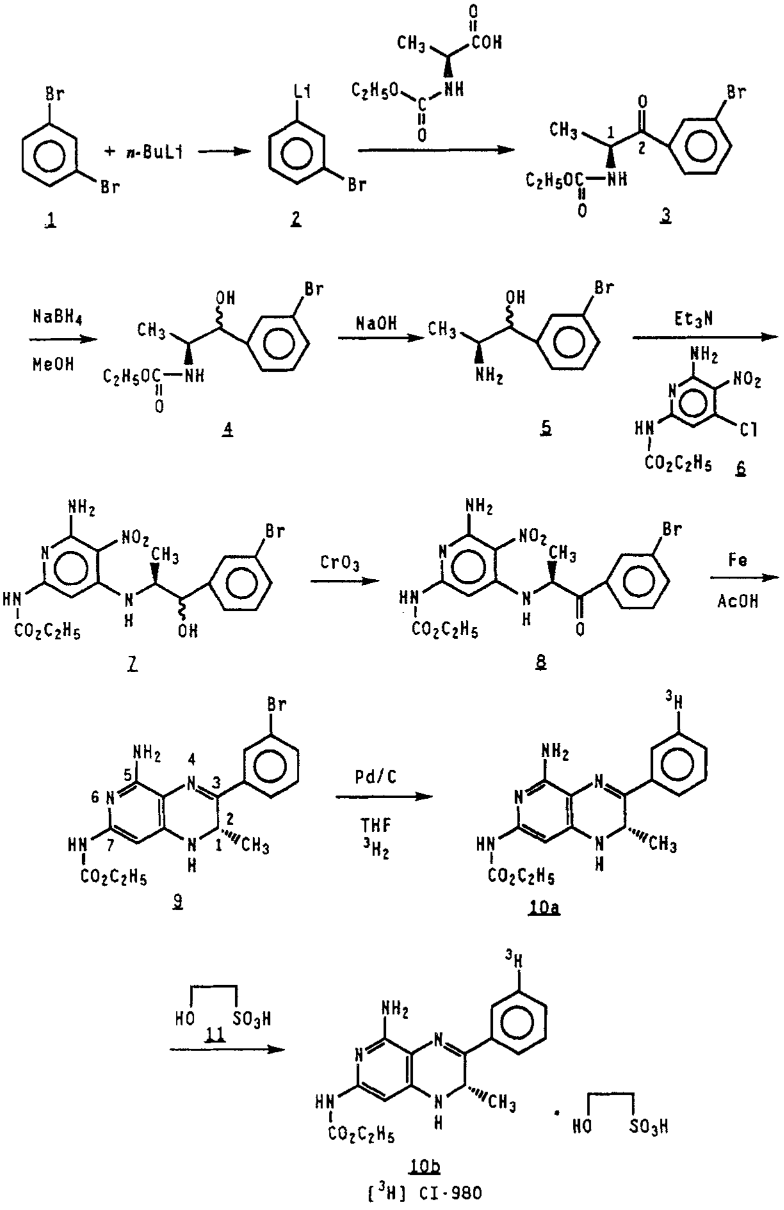
Scheme I. Synthesis of [^{3}H]CI-980



gave, after chromatography, 21% of pure **3**. Reduction of **3** with sodium borohydride in methanol at room temperature, followed by alkaline hydrolysis with potassium hydroxide in aqueous methanol (2 h, reflux), gave a crude product which was purified by trituration with ethanol; pure **5** (77%) was isolated in the mother liquor. Condensation of **5** with **6** in the presence of triethylamine in ethanol (24 h, reflux) gave, after chromatography, 30% of **7**. Oxidation of **7** with chromium trioxide in pyridine-dichloromethane (2 h, room temperature) gave 66% of **8**. Reductive cyclization of **8** with iron powder in acetic acid-ethanol (3.5 h, reflux) gave 75% of **9**.

Replacement of the bromine in **9** was readily accomplished in 1 h with either hydrogen or deuterium, using Pd/C as catalyst and THF as solvent. Tritiation was then carried out under similar conditions but in the presence of 15 equivalents of unlabeled CI-980 free base as carrier, as no product could be isolated in its absence. The product, after purification by silica gel chromatography, was characterized and stored as the free base **10a**. The free base was converted to [^{3}H]CI-981 (**10b**), the isethionate salt, for pharmacokinetic and metabolic studies immediately before use, as the latter was found to be unstable.

EXPERIMENTAL

Tritiation was performed by Amersham Co. Radioactivity was determined with a Packard Tri-Carb 4530 liquid scintillation counter, using Beckman Ready-Gel as the counting medium. TLC plates, E. Merck silica gel 60 F-254, were scanned on a Berthold LB2832 automatic TLC linear analyzer. Column chromatography was performed using E. Merck silica gel, 230-400 mesh. HPLC was performed using a Spectra Physics SP8700 solvent delivery system, Kratos Spectroflow 773 variable wavelength UV detector, and Radiomatic Beta Flow 1 radioactivity flow detector. Unless otherwise specified, Alltech Econosil Columns, C18 4.7 mm x 20 cm were used. MS data were obtained by electron impact at 70 eV using a V G Analytical 7070E/HF mass spectrometer.

Ethyl [2-(3-Bromophenyl)-1(*S*)-methyl-2-oxoethyl]carbamate (3).

n-Butyl lithium (30 mL, 75 mmol, 2.5 M) was added dropwise to an ether solution (125 mL) of *m*-dibromobenzene (17.7 g, 75 mmol) at -110°C and stirred at this temperature for 0.5 h. *N*-(Ethoxycarbonyl)-L-alanine[2] (0.4 g, 24.8 mmol) in ether (50 mL) was then added slowly. This reaction mixture was allowed to warm up from -110°C to -10°C. 1 M H_3PO_4 (100 mL) was added all at once, and the temperature was kept below 5°C. The residue from evaporation of the ether layer was purified by chromatography over silica gel (3:2, $CHCl_3$:hexane) to give product 3 (1.6 g, 21%). ^1H NMR ($CDCl_3$): δ 1.05-1.5 (m, 6H, CH_3), 4.05 (q, 2H, CH_2), 5.0-5.36 (m, 1H, CH), 5.5 (b, 1H, NH), 7.25-8.0 (m, 4H, Ar). Elemental analysis: calc. for $C_{12}H_{14}N_1O_3Br_1$: C 48.02; H 4.70; N 4.67. Found: C 48.13; H 4.82; N 4.83.

α-(1-Aminoethyl)-3-bromobenzenemethanol (**5**).

Sodium borohydride (361 mg, 9.54 mmol) was added in two portions over 0.5 h to a stirred solution of **3** (1.9 g, 6.33 mmol) in CH_3OH (25 mL) at room temperature. After 0.5 h, acetic acid (2 mL) was added and the reaction was evaporated to dryness. The residue was distributed between CH_2Cl_2 and saturated $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2 (2 x 20 mL), and the combined CH_2Cl_2 portions were evaporated to an oil. Aqueous MeOH (25 mL, 3:1, MeOH:H_2O) containing 0.9 g of KOH was added and the solution was refluxed for 2 h, cooled, and evaporated to dryness. An insoluble impurity was removed by recrystallization from EtOH. Evaporation of the mother liquor gave pure **5**, 1.12 g (77%).

Ethyl [6-Amino-4-[[2-(3-bromophenyl)-2-hydroxy-1-methylethyl]amino]-5-nitro-2-pyridinyl]carbamate (**7**).

A mixture of **6** (1.017 g, 4.45 mmol), **5** (1.12 g, 4.87 mmol), and Et_3N (0.65 mL) in EtOH (17 mL) was heated under reflux for 24 h, cooled, and evaporated to dryness. The residue was purified by silica gel column chromatography (1% EtOH in CH_2Cl_2) to give 670 mg of **7** (30%). ^1H NMR ($CDCl_3$): δ 1.18 (d, 3H, CH_3), 1.3 (t, 3H, CH_3), 3.8-4.0 (m, 1H, CH), 4.25 (q, 2H, CH_2), 5.05 (m, 1H, CH), 6.95-7.65 (m, 5H, Ar).

Ethyl [6-Amino-4-[[2-(3-bromophenyl)-1-methyl-2-oxoethyl]amino]-5-nitro-2-pyridinyl]carbamate (8).

A solution of 7 (640 mg, 1.41 mmol) in CH_2Cl_2 (25 mL) was added to a stirred solution of pyridine (1.77 mL in 25 mL CH_2Cl_2), followed by CrO_3 (1.078 g, 10.78 mmol). After stirring at room temperature for 2 h, the mixture was filtered through celite. The filtrate was evaporated, and the product (8) was obtained after purified by silica gel column chromatography (3% EtOH in $CHCl_3$) as yellow powder (420 mg, 66%). ^1H NMR ($CDCl_3$): δ 1.3 (t, 3H, CH_3), 1.6 (d, 3H, CH_3) 4.22 (q, 2H, CH_2), 5.16 (t, 1H, CH), 6.8 (s, H, NH), 7.1-7.98 (m, 4H, Ar), 8.05 (s, 1H, Ar). Elemental analysis: calc. for $C_{17}H_{18}N_5O_5Br \cdot 0.15CHCH_3$: C 43.81; H 3.89; N 14.90. Found: C 43.75; H 3.60; N 15.14. MS: *m/z* 452, 454 [$(M+H)^{\cdot+}$; ^{79}Br, ^{81}Br].

Ethyl [5-Amino-3-(3-bromophenyl)-1,2-dihydro-2(*S*)-methylpyrido[3,4-*b*]pyrazin-7-yl]carbamate (9).

Iron powder (39.6 mg, 0.71 nmol) was added to a solution of 8 (80 mg, 0.18 mmol) in 6.2 mL of glacial acetic acid and 6.2 mL of abs EtOH. The mixture was heated under reflux for 3.5 h. After addition of H_2O (1 mL) and neutralization with solid Na_2CO_3, the mixture was extracted with CH_2Cl_2 (3 x 20 mL), and the combined extracts were dried over $MgSO_4$ and concentrated under vacuum. Pure 9 was isolated by column chromatography on silica gel using Et_3N:EtOH:CH_2Cl_2 (3:3:94) as eluent. The yellow powder (9) weighed 70 mg (98%). MS: *m/z* 404, 406 [$(M+H)^{\cdot+}$; ^{79}Br, ^{81}Br].

[^3H]CI-980 (10b)

The ^2H analog of 10a was first prepared in order to optimize the reaction conditions and isolation procedures. Compound 9 (10 mg, 0.025 mmol) was subjected to ^2H-hydrogenolysis in the presence of 5% Pd/C (10 mg) in 4 mL THF under deuterium for 1 h at 1 atm and room temperature. The mixture was filtered and washed with methanol, and the filtrate was neutralized with K_2CO_3 (0.1 g) and evaporated to dryness. The pure ^{2}H analog (6 mg, 75%) was isolated by column chromatography using 2% EtOH in $CHCl_3$ as eluant. MS: m/z 327 [$(M+H)^{\cdot+}$; 326 for the reference ^{1}H analog).

Using similar procedure as for the ^{2}H analog above but in the presence of unlabeled CI-980 free base as carrier, ^{3}H-hydrogenolysis of **9** was carried out (8 mg of **9**, 100 mg of unlabeled **10a**, 20 mg Pd/C, 10 mL THF, 5 Ci of tritium gas), and the crude product was isolated as the free base (5 mL methanol rinse, 100 mg K_2CO_3). The crude product was coevaporated three times with ethanol to remove tritium gas, purified by chromatography, and further diluted with unlabeled **10a** to give the tritiated free base product (210 mg; 113 mCi 19.5% of theoretical). The purity of **10a**, according to TLC, was 98.5% (2:98 EtOH:$CHCl_3$; R_f 0.22), and according to HPLC (55% 0.05 M triethylamine adjust to pH 3.1 with formic acid, 45% acetonitrile, 1 mL/min, retention time 5.1 min), was 98.5% radiochemical and 100% chemical (270 nm). ^{1}H NMR ($CDCl_3$): δ 1.1-1.35 (m, 6H, CH_3), 4.1-4.35 (q, 2H, CH_2), 4.45 (b, 1H, NH), 4.8-4.9 (m, 1H, CH), 5.0 (b, 2H, NH_2), 7.35-7.05 (s, 1H, Ar), 7.75 (s, 1H, Ar), 7.9-8.05 (m, 2H, Ar). The specific activity was 175 mCi/mmol. Compound **10a** was treated with 1 eq of isethionic acid to give the salt **10b** immediately before pharmacokinetic and metabolic studies.

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