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SYNTHESIS OF RADIOACTIVE AND STABLE ISOTOPE LABELED TIRILAZAD MESYLATE

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SUMMARY

Tirilazad mesylate, 21-[4-(2,6-di-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl]-16α-methyl-pregna-1,4,9(11)-triene-3,20-dione monomethanesulfonate, is a potent lipid peroxidation inhibitor capable of suppressing progression of tissue damage caused by trauma or ischemia. Several isotopically labeled versions of the compound have been synthesized for conducting *in vitro* and *in vivo* metabolic transformations of this experimental drug. These include labeling with carbon-14 at the 16α-methyl group of the steroid portion of the molecule, or at the C-2 position of the pyrimidine ring; also with deuterium at the steroid 16α-methyl group, and/or with carbon-13 at C-2, C-4, and C-6, and with nitrogen-15 at N-1 and N-3 of the pyrimidine ring.

Key Words: Tirilazad mesylate, multiple labels, radioactive, stable isotopes

INTRODUCTION

Lipid peroxidation is recognized as the deleterious process responsible for irreversible damage to nerve tissues following trauma or ischemic injury to the brain and spinal cord. A group of 21-aminosteroids[1] has been found to suppress such damage *in vitro*[2] by inhibiting iron-dependent lipid peroxidation. They have also exhibited *in vivo* activity[3] in animal models of trauma and ischemia to the central nervous system. One of these 21-aminosteroids, tirilazad mesylate, or 21-[4-(2,6-di-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl]-16α-methylpregna-1,4,9(11)-triene-3,20-dione monomethanesulfonate (*1*) is under clinical investigation for treating brain and spinal cord injury, stroke, subarachnoid hemorrhage, and possibly other degenerative neurological diseases such as Parkinson's disease. To study disposition and biotransformation of this compound in test animals andhuman subjects, we synthesized a variety of radioactive and stable isotope labeled forms of tirilazad mesylate, ***1a - 1e***.

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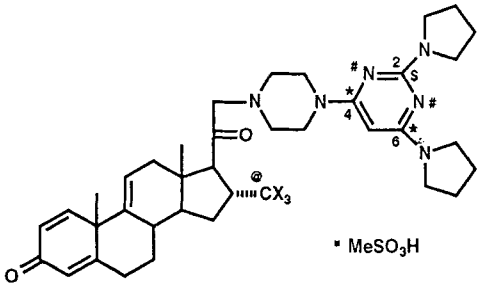


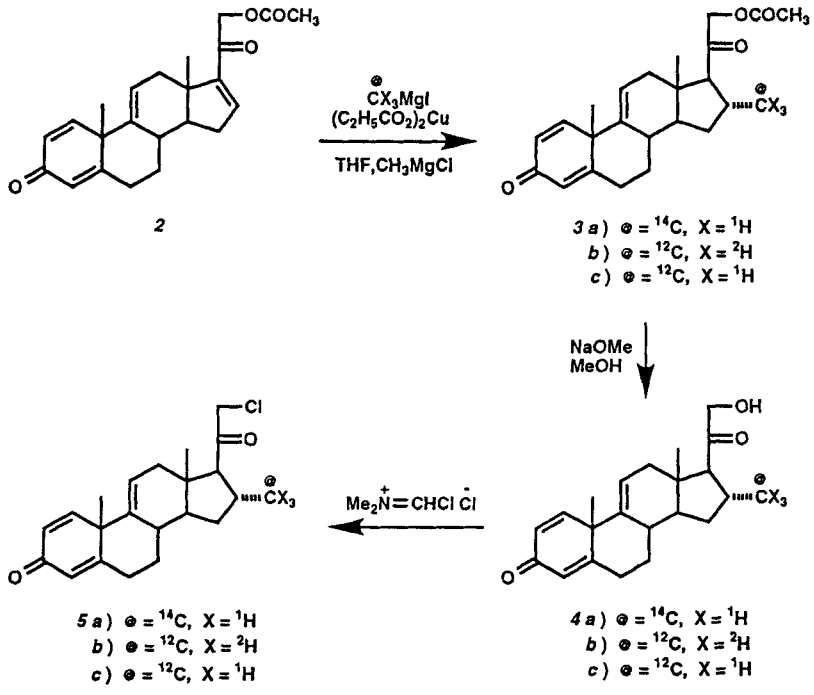
1a) @ = ^{12}C, * = ^{12}C, $ = ^{14}C, # = ^{14}N, X = ^{1}H
b) @ = ^{12}C, * = ^{13}C, $ = ^{13}C, # = ^{15}N, X = ^{1}H
c) @ = ^{12}C, * = ^{12}C, $ = ^{12}C, # = ^{14}N, X = ^{2}H
d) @ = ^{12}C, * = ^{13}C, $ = ^{13}C, # = ^{15}N, X = ^{2}H
e) @ = ^{14}C, * = ^{12}C, $ = ^{12}C, # = ^{14}N, X = ^{1}H
f) @ = ^{12}C, * = ^{12}C, $ = ^{12}C, # = ^{14}N, X = ^{1}H

DISCUSSION AND RESULTS

Tirilazad mesylate consists of two distinct structural sections, a steroid and a polyamine. In order to facilitate tracking of all drug-related materials, it appeared appropriate to radiolabel both segments. Therefore, carbon-14 was incorporated into the 16α-methyl group in the steroid portion of the molecule (***1e***), and at the C-2 position of the pyrimidine ring (***1a***). In addition, stable isotope-labeled drug was needed for conducting pharmacokinetic and metabolism studies using mass spectrometric methods to detect and quantify drug-related materials. To overcome the effects of carbon-13 natural abundance during mass spectrometric measurements, we synthesized labeled versions of tirilazad mesylate with masses of 3 (***1c***), 5 (***1b***), and 8 (***1d***) units above normal, by utilizing deuterium, carbon-13, and nitrogen-15 isotopes in appropriate combinations.

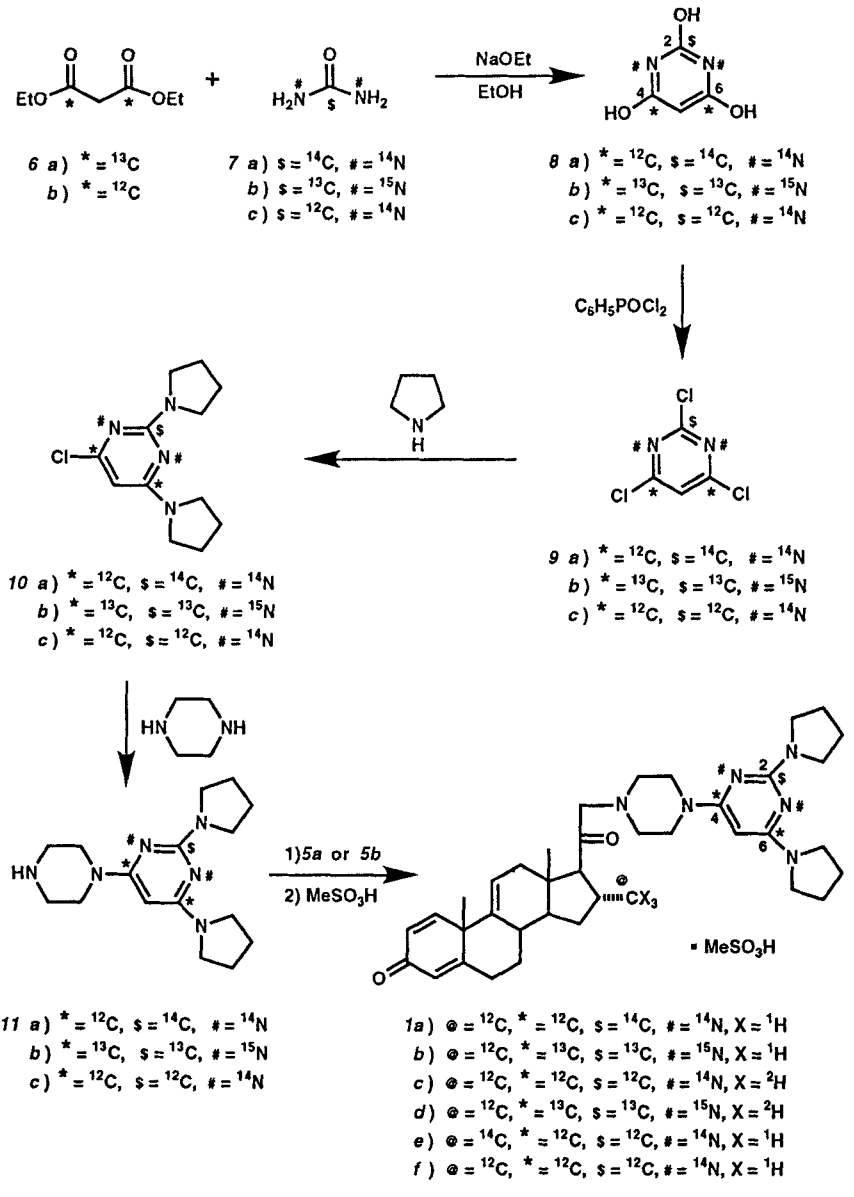
The synthesis of carbon-14 and deuterium labeled steroid precursors, shown in Scheme 1, is based on known methods[4]. The conjugate Grignard addition to the Δ^{16}-20-keto steroid ***2*** can normally be carried in high yield of the 16α-methyl steroid ***3c*** with methyl magnesium chloride in large excess. However, for the preparation of carbon-14 labeled ***3a*** and deuterium labeled ***3b***, the gaseous methyl chloride for preparing the Grignard reagent is not available in labeled forms, and use of labeled materials in large excess would not be appropriate in any case. Instead, the conjugate addition was carried out with appropriately labeled methyl magnesium iodide in 10% excess (relative to ***2***) in the presence of cupric propionate. This afforded the desired carbon-14 and deuterium labeled products ***3a*** and ***3b*** in modest yields (35-45%). Careful base hydrolysis of the 21-acetate in ***3c*** with exclusion of air (presence of air caused cleavage of the C_{20}-C_{21} bond to give the C-20 acid) afforded the C-21 alcohol ***4c***. Treatment of ***4c*** with Vilsmeier reagent gave the 21-chloro steroid ***5c***[5]. Analogous procedures were used to convert ***3a*** and ***3b*** to the correspondingly labeled ***5a*** and ***5b***.

SCHEME 1



The synthesis of 2,6-di(1-pyrrolidinyl)-4-[1-piperazinyl]pyrimidine (***11***) is outlined in Scheme 2. Dickey and Gray[6] reported the preparation of barbituric acid (***8c***) from diethyl malonate (***6b***) and urea (***7c***). Use of carbon-14 labeled urea (***7a***) afforded [2-^{14}C]barbituric acid (***8a***). Similarly, [2,4,6-^{13}C-1,3-^{15}N]barbituric acid ***8b*** was obtained by condensing diethyl[1,3-^{13}C]malonate (***6a***) and [$^{13}C^{15}N_2$]urea (***7b***). Treatment of barbituric acid with phenylphosphonic dichloride according to the procedure of Robinson[7] produced 2,4,6-trichloropyrimidine (***9c***). The same reaction on labeled barbituric acids ***8a*** and ***8b*** led to the correspondingly labeled ***9a*** and ***9b***. All three chlorines of 2,4,6-trichloropyrimidine readily underwent nucleophilic displacement by amines, albeit at different rates, with the chlorine at C-2 being the most reactive. By carrying out the displacements first with pyrrolidine at room temperature, followed by reaction with piperazine at elevated temperatures, one obtained ***11c***.[1] The same processes were used to convert labeled ***9a*** and ***9b*** to ***11a*** and ***11b***. Finally, the displacement of chlorine at C-21 of the steroid ***5c*** by the polyamine ***11c*** produced unlabeled tirilazad, isolated as the monomethanesulfonate salt ***1f***. The various labeled versions of tirilazad mesylate were obtained by mixing and matching appropriately labeled and/or unlabeled steroid ***5*** and polyamine ***11***, as indicated in the experimental procedures described below.

SCHEME 2



6 a) * = ^{13}C
b) * = ^{12}C

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

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

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

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

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

1a) @ = ^{12}C, * = ^{12}C, $ = ^{14}C, # = ^{14}N, X = ^{1}H
b) @ = ^{12}C, * = ^{13}C, $ = ^{13}C, # = ^{15}N, X = ^{1}H
c) @ = ^{12}C, * = ^{13}C, $ = ^{12}C, # = ^{14}N, X = ^{2}H
d) @ = ^{12}C, * = ^{13}C, $ = ^{13}C, # = ^{15}N, X = ^{2}H
e) @ = ^{14}C, * = ^{12}C, $ = ^{12}C, # = ^{14}N, X = ^{1}H
f) @ = ^{12}C, * = ^{12}C, $ = ^{12}C, # = ^{14}N, X = ^{1}H

EXPERIMENTAL SECTION

Radioactivity determinations were carried out with a Pharmacia Wallac 1410 Liquid Scintillation counter using the external standard method with Ultima Gold as the scintillation cocktail. Thin layer chromatographic (TLC) analyses were done on 2.5 x 10 cm glass plates precoated with a 250

μm layer of silica gel GF (Analtech). Developed zones were visualized under UV light. Radioactive zones were detected with a Bioscan System 200 Imaging Scanner. High performance liquid chromatographic (HPLC) analyses were carried out with a Spectra Physics Model 8700 Solvent Delivery System. The eluate was analyzed with an LDC/Milton Roy SpectroMonitor D variable wavelength UV detector set at 254 nm, and where appropriate, also a Radiomatic Model Flo-One Beta A280 or an IN/US β-RAM radioactivity detector.

16α-[^{14}C]Methyl-21-hydroxy-pregna-1,4,9(11)-triene-3,20-dione (***4a***)

All glassware items used in the Grignard reaction were flame-dried and flushed with dry nitrogen gas immediately prior to use. Magnesium turnings (86 mg. 3.5 mmol) which had been treated with 1 N HCl, washed with water, ethanol and acetone in that order, and flame-dried were covered with 3 ml of anhydrous ether in a 15 ml round bottom flask. [^{14}C]Methyl iodide, nominally 150 mCi at 59 mCi/mmol, dissolved in 3 ml of anhydrous ether was added and the mixture was stirred at room temperature under N_2. The mixture became cloudy and warm, then returned to ambient temperature during 30 min of stirring. This Grignard reagent was set aside while a solution of 1.10 g of ***2*** (3.0 mmol) in 40 ml of freshly distilled tetrahydrofuran (THF) was cooled to -10°C (ice-brine bath). To this solution was added with stirring a mixture containing 0.5 ml of 1.9 M cupric propionate in THF, 0.1 ml of 1.96 M methyl magnesium chloride in THF, and 2 ml of anhydrous THF. To this stirred mixture was added dropwise from a syringe, over 15 min under N_2, the ether solution of [^{14}C]methyl magnesium iodide. The resulting mixture containing copious amounts of precipitates was stirred at -9° to -7°C for 40 min, and treated with a mixture of 2 ml of methanol and 1 ml of 6N HCl. The mixture was diluted with 100 ml of ethyl acetate, washed with 3 x 80 ml of water, 80 ml of brine, and dried over anhydrous sodium sulfate. After the solvents were removed, the residue was dissolved in 20 ml of methanol and stirred with 0.5 ml of 25% sodium methoxide in methanol for 30 min. The mixture was partitioned with 50 ml of water, 25 ml of brine, and 75 ml of methylene chloride. The aqueous phase was extracted with 2 x 50 ml of methylene chloride. The combined organic phases were washed with 80 ml of brine and dried over sodium sulfate. Removal of solvent afforded 1.1 g of foamy residue which was chromatographed on a column of 60 g of silica gel packed in and eluted with 50 ml of 3:7 v/v ethyl acetate:hexane. The eluent was then changed to 1:1 v/v ethyl acetate:hexane. After a forerun of 150 ml, the eluate was collected in 10 ml fractions at 2.5 ml/min. Fractions containing ***4a*** were pooled and concentrated to give a partially crystalline

residue, which was triturated with 3 ml of warm ether, mixed with 3 ml of hexane and a few more ml of 1:1 ether:hexane. The mixture was filtered to give 214 mg of ***4a***, sp. act. 154 μCi/mg. From the mother liquor there was obtained, after isotopic dilution, 2.8 mCi of ***4a*** of sp. act. 16.1 μCi/mg. Both radioactive samples of ***4a*** co-chromatographed with an authentic sample of unlabeled ***4c*** by TLC and HPLC.

16α-[$^2H_3$]Methyl-21-hydroxy-pregna-1,4,9(11)-triene-3,20-dione(***4b***)

Similarly, from 25 g of ***2*** (68.2 mmol), 25 g of [$^2H_3$]methyl iodide (75 mmol), 1.82 g of magnesium turnings (75 mmol), and 1.8 ml of 1.9M cupric propionate (3.42 mmol), there was obtained 13.61 g of ***4b*** (40% yield), crystallized from EtOAc to give 7.51 g of fine white needles, mp. 157-160°C, single component by TLC identical to a standard sample of ***4c***; ^{1}H-NMR*(CD_3OD; TMS): δ 0.64(s, 3H), 1.13(dq, 1H,J_d=4.14Hz, J_q=13.47Hz), 1.35(s, 3H), 1.39-1.50(m, 2H), 1.60-1.74(m, 1H), 1.99-2.13(m, 4H), 2.2-2.4(m, 1H), 2.39(br d, 1H, J=13.6Hz), 2.60(dt, 1H, J_t=13.51Hz, J_d=8.86Hz), 2.73(br t, 1H, J=9.16Hz), 3.24(br s, 1H), 4.22(d, 2H, J=4.99Hz), 5.05-5.1(m, 1H), 6.01(s, 1H), 6.31(dd, 1H, J=2.1Hz, J=10.18Hz), 7.21(d, 1H, J=10.22Hz); ^{13}C-NMR*(CD_3OD; TMS): ppm 13.56, 26.36, 31.48, 31.89, 34.23, 34.47, 36.13, 40.17, 44.56, 45.72, 51.75, 67.69, 69.69, 119.47, 123.69, 127.17, 143.31, 154.27, 166.34, 186.09, 209.61; Infrared: υ_{max}(mull)3452, 2208, 2119, 2066, 1709, 1697, 1666, 1626, 1612, 1600, 1450, 1405, 1320, 1270, 1241, 1237, 1123, 1080, 1057, 888 cm^{-1}, peaks at 2208, 2119, and 2066 cm^{-1} consistent for C-D stretch of a CD_3 group; Mass Spectrum (EI, 70eV):343(M^+), 328, 315, 312, 284, 225.

21-Chloro-16α-[^{14}C]methyl-pregna-1,4,9(11)-triene-3,20-dione (***5a***)

A solution of 380 mg of ***4a*** (1.1 mmol, 21 mCi, 91.4% radiochemically pure (RCP)) in 3 ml of toluene and 0.6 μl of dimethylformamide (DMF) was added dropwise with stirring under N_2 to a mixture of 151 mg of Vilsmeier reagent (1.2 mmol, Aldrich) in 3 ml of toluene and 250 μl of pyridine. The mixture was stirred at room temperature for 5 h, quenched with 10 ml of water, and extracted with 3 x 25 ml of EtOAc. The combined extracts were washed with brine, dried over anhydrous Na_2SO_4,

*The ^{1}H-NMR spectrum of ***4b*** showed no signal at δ 0.92 (d, 3H, J=6.6Hz) for C-16 protons found in the ^{1}H-NMR spectrum of unlabeled ***4c***.

*The signal at 21.78 ppm for C-16 of unlabeled ***4c*** is missing in the ^{13}C-NMR spectrum of ^{2}H-labeled ***4b***, because ^{13}C-^{2}H coupling divides the signal into 7 lines, making it obscure and buried in the background.

filtered, and concentrated at reduced pressure to give ***5a***, 92% RCP by HPLC, 97% RCP by TLC (3:7 v/v EtOAc:hexane). This crude material was used without further purification to prepare [Me-^{14}C]tirilazad mesylate (***1e***).

21-Chloro-16α-[$^{2}H_3$]methylpregna-1,4,9(11)-triene-3,20-dione(***5b***)

A solution of 8.8 g of 21-hydroxy-16α-[$^{2}H_3$]methylpregna-1,4,9(11)-triene-3,20-dione (***4b***, 26 mmol) in 60 ml of toluene and 7 ml of DMF was added dropwise with stirring under N_2 over 15 min to a mixture of 3.9 g of Vilsmeier reagent (30 mmol, Aldrich), 15 ml of toluene, and 4.2 ml of pyridine. The mixture was then stirred at room temperature for 5 h, quenched with 20 ml of water, and extracted with 3 x 60 ml of EtOAc. The combined extracts were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated at reduced pressure to give 11.4 g of crude ***5b***. The crude product was chromatographed on a column of 200 g of silica gel packed in and eluted with 3:7 v/v EtOAc:hexane. The eluate was collected in 20 ml fractions at 5 ml/min. The fractions containing pure ***5b***, as monitored by TLC (3:7 v/v EtOAc:hexane), were pooled and concentrated to give 3.95 g of ***5b***, 95.7% pure by HPLC. A second batch of ***5b*** obtained from the column was found to be less pure (83.2% by HPLC), and was rechromatographed on silica gel (200 g) with 3:7 v/v EtOAc:hexane to give 3.0 g of ***5b***, 94.3% pure by HPLC. The combined yield was 74.7%.

[2-^{14}C]Barbituric Acid (***8a***)

Sodium metal was cut into small cubes in N_2-purged n-hexane under a nitrogen atmosphere (glove box). A piece of the metal, 257 mg (11 mmol), was added to a dry 50 ml round bottom flask containing 7 ml of absolute ethanol. The mixture was stirred under N_2 until the metal dissolved. To the freshly prepared solution of sodium ethoxide in ethanol was added 629 mg of [^{14}C]urea (sp. act. 26.1 mCi/mmol, 10.17 mmol, supplied by DuPont NEN) along with 3 ml of absolute ethanol, followed by 1.65 g of diethyl malonate (***6b***, 10.3 mmol) in 1 ml of absolute ethanol. The homogeneous mixture was refluxed with stirring under N_2 overnight (15 h) with precipitation of white solids. The mixture was cooled to room temperature, and 6 ml of water was added with stirring, followed by 4 ml of 6N HCl, which dissolved the solids present. Formation of new white precipitates followed quickly. The mixture was concentrated at 20 torr and 35°C to remove ethanol and water. The residue was dried under high vacuum at room temperature for 3 h. The resulting white, somewhat soft, solids were used without further purification for preparing ***9a***.

[2,4,6-$^{13}C_3$-1,3-$^{15}N_2$]Barbituric Acid (*8b*)

A solution of sodium ethoxide in ethanol was prepared by stirring chunks of freshly cut (under hexane) sodium metal, weighing a total of 1.265 g (55 mmol), in 35 ml of absolute ethanol in a nitrogen filled glove box. After the metal dissolved (~ 10 min), 3.153 g of [$^{13}C^{15}N_2$]urea (*7b*, 50 mmol, 99% isotopic enrichment) was added to the solution with stirring, followed by 8.109 g of diethyl [1,3-$^{13}C_2$]malonate (*6a*, 50 mmol, 99% isotopic enrichment). The addition was completed with aid of 1 ml of absolute ethanol. The homogeneous mixture was refluxed with stirring under N_2 for 20 h with precipitation of white solids. After cooling to room temperature, the mixture was mixed with 25 ml of water and acidified with 12 ml of 6N HCl. The clear solution was concentrated at reduced pressure to remove ethanol and water. The residue was dried under high vacuum to give crude *8b* which was used without further purification to prepare *9b*.

2,4,6-Trichloro-[2-^{14}C]pyrimidine (*9a*)

The crude [2-^{14}C]barbituric acid (*8a*) from above was heated with stirring in a nitrogen atmosphere under a condenser at 180°C (oil bath) with 7.1 ml of freshly distilled phenylphosphonic dichloride (9.749 g, 50 mmol, freshly distilled at 110-111°C and 6-7 torr). Evolution of HCl gas was accompanied by darkening of the mixture to an orange-red color. After 3.75 h, the dark viscous mixture was added dropwise with stirring to 50 ml of water to decompose the excess chlorinating agent. The mixture containing white precipates was filtered and the solids were washed with 10 ml of water followed by 70 ml of hexane. The filtrate was partitioned and the aqueous layer was extracted with 20 ml of hexane. The combined hexane extracts were washed with 20 ml of saturated $NaHCO_3$ solution, followed by 20 ml of brine, and dried over anhydrous Na_2SO_4. The filtered hexane solution was concentrated at 20°C and 20 torr to give 1.386 g of *9a* as an oil, 204 mCi, 75.6% radiochemical yield (RCY) from [^{14}C]urea, 97% RCP by HPLC. This material was used without further purification to prepare *10a*.

2,4,6-Trichloro-[2,4,6-$^{13}C_3$-1,3-$^{15}N_2$]pyrimidine (*9b*)

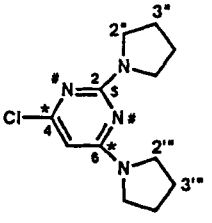
Similarly, from the crude *8b* and 48.75 g of freshly distilled phenylphosphonic dichloride, there was obtained 6.85 g of *9b* as a yellow oil, 72% overall yield from the labeled urea. TLC analysis (10% v/v Et_2O in pentane) showed presence of only traces of impurity. This material was used "as is" to prepare *10b*; ^{1}H-NMR (300 MHz, in $CDCl_3$ with TMS, δ) 7.39 (t, 1H, $^2J_{C\text{-}H}$ = 1 Hz, proton at C-5) .

4-Chloro-2,6-dipyrrolidinyl-[2-^{14}C]pyrimidine (***10a***)

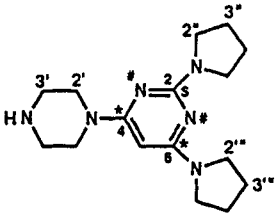
A solution of 3.20 g of pyrrolidine (45 mmol, supplied by Aldrich, redistilled at 86°C) in 5 ml of freshly distilled dry tetrahydrofuran (THF) was added dropwise with stirring at -12°C to -5°C (ice/ethanol bath) over 50 min to a solution of the crude ***9a*** from above in 10 ml of THF. Stirring at -5°C was continued for 1 h and the mixture was allowed to come to room temperature overnight. The mixture was treated with 5 ml of 5% $NaHCO_3$ solution to dissolve solids, and concentrated at reduced pressure to remove THF. The aqueous residue was partitioned with 25 ml each of methylene chloride and half-saturated Na_2CO_3 solution. The aqueous phase was extracted with 25 ml of methylene chloride. The combined extracts were washed with 25 ml of brine, dried over anhydrous Na_2SO_4, filtered, and the filtrate concentrated at 35°C and 20 torr to give 2.07 g of crude ***10a***. The crude was chromatographed on a column of 60 g of silica gel packed in and eluted with 15% v/v Et_2O in pentane. The eluate was collected in 12 ml fractions at 3 ml/min. The fractions containing pure desired product, as monitored by TLC (15% v/v Et_2O in pentane), were pooled and concentrated at reduced pressure to give 1.64 g (83.5% RCY from ***9a***) of pure ***10a*** after drying under vacuum, sp.act. 103.9 μCi/mg with >99% RCP by TLC.

4-Chloro-2,6-dipyrrolidinyl-[2,4,6-$^{13}C_3$-1,3-$^{15}N_2$]pyrimidine (***10b***)

The same procedure as the one used to prepare ***10a*** was followed. From 6.756 g of ***9b*** (36.4 mmol) and 15.36 g of redistilled pyrrolidine (216 mmol), there was obtained 8.07 g (86.0% yield) of ***10b***, a single component by TLC (15% v/v Et_2O) in pentane) with the same Rf as a standard sample of ***10c***; ^{1}H-NMR (see structure ***12***) (300 MHz, in $CDCl_3$,δ) 1.91 (m, 8H, protons at C-3″ and C-3‴ of pyrrolidine ring), 3.53 (m, 8H, protons at C-2″ and C-2‴ of pyrrolidine ring), 5.63 (dd, 1H, $^2J_{C\text{-}H}$ = 3.42 Hz, 1.68 Hz, H at C-5); ^{13}C-NMR (75 MHz, in $CDCl_3$, ppm from TMS) 25.3 (C-3″, C-3‴), 46.03, 46.35 (C-2″, C-2‴), 90.4 (C-5, unenriched) 158.7, 159.6 (C-2 and C-6, enriched), 161.1 (C-4, enriched); expansion of C-5 signal showed multiple coupling: $^1J_{C\text{-}C}$ = 84.36 Hz, 61.33 Hz by C-4 and C-6, $^3J_{C\text{-}C}$ =



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



13) * = ^{13}C, $ = ^{13}C, # = ^{15}N

8.0 Hz by C-2, $^{2}J_{C\text{-}N}$ = 2.47 Hz, 1.50 Hz by N-1 and N-3; ^{15}N-NMR (30.4 MHz, in $CDCl_3$, NH_4Cl external standard, ppm) 179.6 (t, $^{1}J_{N\text{-}C}$ = 5.7 Hz, N-3), 188.5 (dd, $^{1}J_{N\text{-}C}$ = 6.6, 2.6 Hz, N-1).

2,6-Dipyrrolidinyl-4-(1-piperazinyl)-[2-^{14}C]pyrimidine (***11a***)

A mixture of 390 mg of ***10a*** (1.54 mmol, 40.5 mCi), 663 mg of piperazine (7.7 mmol, Aldrich), and 2 ml of pyridine was refluxed with stirring under N_2 in a 170°C oil bath for 18 h. The mixture containing some precipitates was concentrated at 35°C and 20 torr. The solid residue* was partitioned with 15 ml of CH_2Cl_2 and 10 ml of 0.5N NaOH solution. The aqueous layer was extracted with 10 ml of CH_2Cl_2. The combined extracts were washed with 10 ml of brine, dried over Na_2SO_4, filtered, and concentrated at reduced pressure to give 469 mg of crude ***11a***. The crude material was chromatographed on a column of 30 g of silica gel packed in and eluted with 95:5 v/v CH_2Cl_2:4M NH_3 in methanol. The eluate was collected in 9 ml fractions at 3-4 ml/min. The fractions containing pure ***11a***, as monitored by TLC (95:5 v/v CH_2Cl_2:4M NH_3 in methanol), were pooled and concentrated at 25°C and 20 torr to give 424 mg of pure ***7a***, sp.act. 87.1 μCi/mg* with >99% RCP by TLC, 91% RCY.

2,6-Dipyrrolidinyl-4-(1-piperazinyl)-[2,4,6-$^{13}C_3$-1,3-$^{15}N_2$]pyrimidine (***11b***)

The same procedure as the one described above for preparing ***11a*** was used. From 7.951 g of ***10b*** (30.85 mmol), 13.286 g of piperazine (154 mmol, Aldrich), and 26 ml of pyridine, there was obtained, after purification by chromatography on a column of 200 g of silica gel, 8.70 g of pure ***11b*** (91.7% yield, single component by TLC (95:5 v/v CH_2Cl_2:4M NH_3 in methanol, and 3:7 v/v EtOAc : hexane) with the same Rf as a standard sample of ***11c***; ^{1}H-NMR (see Structure ***13)*** (300 MHz, $CDCl_3$ with TMS δ) 1.90 (m, 8H, protons at C-3″ and C-3‴ of pyrrolidine rings), 2.91 (m, 4H, protons at C-3′ of piperazine ring), 3.42 (m, 4H, protons at C-2′ of piperazine ring), 3.50 (m, 8H, protons at C-2″ and C-2‴ of pyrrolidine rings), 4.84 (s, 1H, proton at C-5 of pyrimidine ring); ^{13}C-NMR(75MHz,$CDCl_3$

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* The entire workup was carried out in a N_2 filled glove box because the product is highly sensitive to air oxidation. Degasing solvents and working under N_2 were done also during the preparation of tirilazad mesylate (***1***). These precautions consistently led to products of high purity, and are highly recommended when dealing with the polyamines, even during chromatographic purifications. Silica gel should be flushed with N_2 before being packed into a column.

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* Compound ***11*** has a tendency to adsorb onto glass surfaces. Caution should be exercised with working with this material in solution of very low concentrations.

with TMS, ppm) 25.20 (d, $^3J_{C\text{-}C}$ = 2.6 Hz), 25.46 (d, $^3J_{C\text{-}C}$ = 2.7 Hz) (C-3″, C-3‴; 45.54 (d, $^2J_{C\text{-}C}$ = 2.8 Hz), 45.84 (d, $^2J_{C\text{-}C}$ = 2.1 Hz), 45.89, 46.04 (d, $^2J_{C\text{-}C}$ = 1.7 Hz) (C-2″, C-2‴, C-2′, C-3′) 72.1 (m, unenriched C-5 of pyrimidine ring coupled with enriched ^{13}C at 2,4,6-positions and enriched ^{15}N at 1- and 3- positions of the pyrimidine ring); 160.1 (m, C-2, enriched, coupled with C-4, C-6, N-1, N-3, J = 5.3, 5.3, <1, <1 Hz); 162.3 (m, C-4 and C-6, enriched, coupled with N-1 and N-3, $^1J_{C\text{-}N}$ = 6.6, 6.3 Hz); 164.2 (m, C-6, enriched, coupled with N-1, N-3, C-2 or C-4, J=7.6, 3.0, 1.0 Hz); ^{15}N-NMR (30.4 MHz, in $CDCl_3$, NH_4Cl external standard, ppm with negative NOE) 165.8 (t, N-1 or N-3 enriched, coupled with ^{13}C, $^1J_{N\text{-}C}$ = 6.2 Hz), 168.7 (t, N-1 or N-3, enriched, coupled with ^{13}C, $^1J_{N\text{-}C}$ = 6.2 Hz).

[Pym-^{14}C]Tirilazad Mesylate (***1a***)

A mixture of 424 mg of ***11a*** (1.39 mmol, 36.9 mCi), 753 mg of (***5c***), 80 mg of NaI, 0.5 ml of 47% K_2CO_3 solution, and 6 ml of acetone was stirred under N_2 at room temperature for 5 h. The reaction was treated with 10 ml of water, and the mixture was extracted with 2 x 25 ml of CH_2Cl_2. The combined extracts were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated at reduced pressure to give 35.8 mCi of crude ***1a***. The crude was chromatographed on a column of 80 g of silica gel packed in and eluted with 70:30 v/v EtOAc:hexane. The eluate was collected in 15 ml fractions at 4 ml/min. Fractions containing pure ***1a*** as detected by TLC were pooled and concentrated at reduced pressure to afford 26.05 mCi of the free base of ***1a*** as a white solid, >99% RCP by HPLC (Supelcosil LC-18 5μ column, 85:15 v/v AcCN:Buffer (1000:5:1.35 v/v H_2O:Et_3N:HCO_2H))and TLC (70:30 v/v EtOAc:hexane). The free base was dissolved in 10 ml of freshly distilled dry THF, heated to boiling, and treated with 2.5 ml of 0.4M $MeSO_3H$* in dry THF. Two ml of hexane was added to the solution and the mixture was cooled with stirring to induce crystallization. Another 10 ml of hexane was added and the mixture was kept in the refrigerator overnight. The resulting crystals were filtered, washed with hexane, and dried to give 835 mg of ***1a*** (1.15 mmol), sp. act. 33.1 μCi/mg, 74.9% RCY, with >99% RCP by HPLC and TLC.

[Pym-$^{13}C_3{}^{15}N_2$]Tirilazad Mesylate (***1b***)

The procedure described above for the preparation of ***1a*** was followed. From 2.0 g of ***11b*** (6.5 mmol), 2.41 g of unlabeled 21-chloro steroid ***5c*** (6.7 mmol), 250 mg of NaI, and 1.5 ml of 47% K_2CO_3 in 25 ml of acetone, there was obtained, after purification of the crude product free base by

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* Slightly less than 1 equivalent of $MeSO_3H$ was used to ensure the product would contain only the mono mesylate salt.

column chromatography on 200 g of silica gel and conversion to the mesylate salt, 4.31 g of ***1b***, 91.4% yield, >99% pure by HPLC, single component identical to a standard sample of tirilazad mesylate by TLC, NMR and mass spectra consistent with proposed structure.

[Me-$^{2}H_{3}$]Tirilazad Mesylate (***1c***)

The procedure for preparing ***1a*** and ***1b*** was followed. From 2.0 g of ***5b*** (5.5 mmol), 1.66 g of ***11c*** (5.5 mmol), 210 mg of NaI, and 2 ml of 47% K_2CO_3 solution in 25 ml of acetone, there was obtained, after chromatographic purification on silica gel and conversion of the pure free base to the mesylate salt, 3.17 g of ***1c***, 80% yield, 98.7% pure by HPLC, single component identical to a standard sample of tirilazad mesylate by TLC, NMR and mass spectra consistent with proposed structure.

[Me-$^{2}H_{3}$-Pym-$^{13}C_{3}{}^{15}N_{2}$]Tirilazad Mesylate (***1d***)

Reaction of 2.3 g of ***5b*** (6.3 mmol) with 1.9 g of ***11b*** (6.3 mmol) under the same conditions as labeled tirilazad mesylate ***1d***. There was obtained 3.58 g of the product, 89.3% yield, >99% pure by described above for the preparation of ***1a***, ***1b***, and ***1c*** gave rise to the M+8 variety of stable isotope HPLC; single component identical to a standard sample of tirilazad mesylate by TLC; NMR and mass spectra consistent with proposed structure.

[Me-^{14}C]Tirilazad Mesylate (***1e***)

The crude ***5a*** from above, nominally 1.1 mmol, 18.8 mCi was treated with 450 mg of unlabeled ***11c*** (1.5 mmol) in 5 ml of acetone in the presence of 40 mg of NaI and 0.5 ml of 47% K_2CO_3, using the same procedure as described above for the preparation of ***1a***, to give 14.5 mCi of crude free base of ***1e***. Purification by column chromatography yielded 8.1 mCi of free base, which proved to be only 96% pure radiochemically. This material was further purified by preparative HPLC on a 22.5 mm ID x 250 mm Phenomenex Ultra Carb ODS-30 column, using a mobile phase of 700:300:5:3.5 v/v CH_3CN:H_2O:Et_3N:HCO_2H, to give pure tirilazad free base of >99% RCP. Conversion to the mesylate salt with $MeSO_3H$ afforded 192 mg of ***1e***, sp. act. 19.1 μCi/mg, 99% RCP by HPLC and TLC. There was obtained another 77 mg of ***1e*** from the mother liquor as the second crop. The low yield in this preparation resulted at least in part from the impurities in the starting labeled ***5a***, which necessitated additional purifications of tirilazad free base to obtain ***1e*** with high RCP.

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