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Steroids

Synthesis of N-desmethyl derivatives of 17α-acetoxy-11β-(4-N,N-dimethylaminophenyl)-19-norpregna-4,9-diene-3,20-dione and mifepristone[1]
Substrates for the synthesis of radioligands

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Abstract

The syntheses of N-desmethyl derivatives of CDB-2914 and the mono-N-desmethyl derivative of Mifepristone are described. We also describe the use of the mono-desmethyl derivatives as substrates for the synthesis of N-tritomethyl derivatives of CDB-2914 and Mifepristone with high specific activity (ca. 80 Ci/mmol), which serve as radioligands for radioimmunoassay.

Keywords: Steroids; Synthesis; N-desmethyl; CDB-2914; Mifepristone; Radioligands

Since its discovery in the early 1980s, Mifepristone **1** (RU-38 486) (Fig. 1) remains the most well-studied antiprogestin of clinical importance to date. Early metabolism studies [1] have demonstrated that the parent compound **1** is rapidly converted to mono-desmethyl derivative **2** (RU-42 633) and a non-demethylated propynyl alcohol metabolite **3** (RU-42 698). Further metabolism converts **2** to di-desmethyl metabolite **4** (RU-42 848). All three metabolites have been shown to retain powerful antiprogestational and antiglucocorticoid activity and thus, all contribute to the overall physiological action of Mifepristone.

In 1986, Cook et al. [2] reported the synthesis of 17α-acetoxy-11β-(4-N,N-dimethylaminophenyl)-19-norprenga-4,9-diene-3,20-dione **5** (CDB-2914). This compound was shown to be equipotent to Mifepristone with slightly lower antiglucocorticoid activity. For clinical trials, a large quantity of **5** was required, for which we developed a practical large scale synthesis [3,4]. We anticipated that the metabolism of **5** would be analogous to that of Mifepristone. Therefore, it was necessary to synthesize authentic reference samples of mono- and di-desmethyl CDB-2914 **6** and **7**, respectively. These samples helped to investigate their role as potential metabolites and gain insight into the pharmacokinetics of the parent compound.

The di-N-desmethyl derivative of CDB-2914 **7** was prepared through copper (I)-catalyzed conjugate addition of a suitably protected derivative of 4-bromoaniline to the well know 9(11)-en-5α,10α-epoxide. The mono-desmethyl derivative of CDB-2914 **6** was obtained in a single step from the parent compound using a novel oxidative N-demethylation reaction developed in our laboratories [5]. Similar oxidative N-demethylation of Mifepristone **1** gave the mono-N-desmethyl derivative (RU-42 633) **2**. The mono-desmethyl derivatives **6** and **2** were used for the synthesis of N-tritiomethyl derivatives of CDB-2914 **8** and RU-38 486 **9**.

1. Experimental

Melting points (mp) were determined on a Thomas-Hoover apparatus and are uncorrected. Nuclear magnetic resonance spectra were recorded on either a Varian EM-390 (90 MHz) or a General Electric GE-300 (300 MHz) spectrometers as deuteriochloroform solutions using tetrameth-

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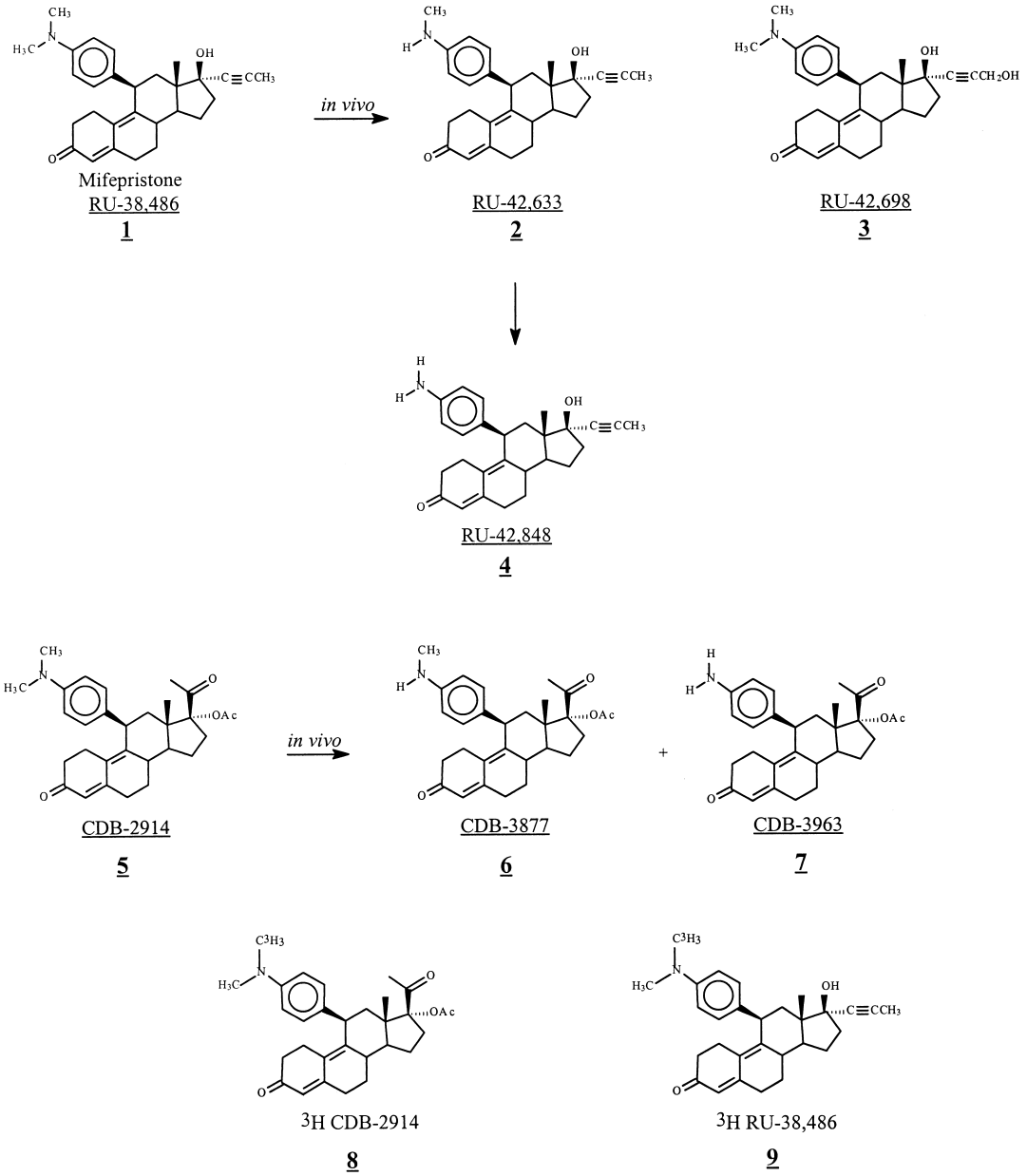

Fig. 1. Metabolites and radioligands.

ylsilane (TMS) as an internal standard ($\delta = 0.0$). Infrared (IR) spectra were recorded on a Perkin-Elmer model 1600 FTIR instrument equipped with a diffuse reflectance accessory using a KBr matrix. Mass spectral analyses(EI) were conducted by Dr Susan Weintraub of the University of Texas Health Science Center at San Antonio using a Finnigan-MAT model 4615. Combustion analyses were performed by Midwest Microlabs Ltd. (Indianapolis, IN, USA). Flash-column chromatography was performed on 32–63 μM silica gel obtained from Scientific Absorbents Inc. (Atlanta, GA, USA). Thin-layer chromatography (TLC) analyses were carried out on silica gel GF (Analtech) glass plates (2.5 $\times$ 10 cm with 250 μM and prescored).

Most chemicals and solvents were ACS reagent grade or better and were used without further purification. Anhydrous tetrahydrofuran (THF) was obtained via distillation of reagent grade material from the sodium benzophenone ketyl.

The reactions with ^{3}H methyl iodide on CDB-3877 and RU-42,633 were performed by American Radiolabeled Chemicals, Inc. (ARC) of St. Louis, MO, USA.

1.2. 4-Bromo-N,N-bis-(trimethylsilyl)aniline **12**

Compound **12** was prepared according to the procedure of Bock et al. [6] NMR (90 MHz) δ 0.1 (s, 18 H), 6.6 and 7.35 (d, J = 9 Hz, AA'BB' of aromatic protons) ppm. FTIR: ν_{max} 2953, 1479, 1251, 1218 cm^{-1}.

MS(m/e): $M^+ = 317$.

Analysis: For $C_{12}H_{22}BrNSi_2$ Calc: C 45.56; H 7.01; Br 25.25. Found: C 46.83; H 7.34: Br 22.50. Note: NMR and mass spectral analyses indicate the presence of a contaminant which was determined to be 4-trimethylsilyl-N,N-bis-(trimethylsilyl) aniline which is not removed during distillation.

2.2. 4-Bromo-N-methyl-N-trimethylsilylaniline **13**

Under nitrogen, a solution of N-methylaniline (22 ml, 203 mmol); methanol (0.8 ml, 19.8 mmol); and tetramethylammonium bromide (3.13 g, 20.3 mmol) in methylene chloride (200 ml) was cooled in an ice bath. A solution of bromine (11 ml, 213.5 mmol) in methylene chloride (200 ml) was added dropwise with stirring at such a rate to maintain the reaction temperature below 10°C (ca. 45 min for the addition). During the addition, the brown color disappears instantaneously. The end of the reaction is determined by the persistency of a slight brown coloration. At that point, triethylamine (30 ml, 215.7 mmol) was added and the solution allowed to warm to room temperature. The methylene chloride was removed under a stream of dry nitrogen with slight warming. The residue was subjected to vacuum distillation (bp 72–76°C @ 1 mm Hg) to afford 4-bromo-N-methylaniline **11** (26 g, 69%).

NMR (CCl_4, 90 MHz) δ 2.7 (s, NCH_3), 3.65 (br.s, NH), 6.85 (d of d, J = 75.6 Hz, J' = 9.0 Hz, 4 aromatic H) ppm.

Under nitrogen, a solution of 4-bromo-N-methylaniline **11** (15 g, 81.06 mmol) in dry THF (100 ml) was chilled to −78°C. Butyllithium (1.5 M/hexanes, 54 ml, 81 mmol) was added via a syringe and the mixture was allowed to warm to −30°C for about 5 min. The resulting slurry was cooled back to −78°C and chlorotrimethylsilane (11 ml, 86.7 mmol) was added. The mixture was stirred at −78°C for 30 min. and then allowed to warm to room temperature. The solvent was removed under a stream of dry nitrogen with occasional warming. The resulting liquid was filtered through a pad of Celite and the filtrate was concentrated in vacuo to give 20.3 g. Vacuum distillation (92–96°C @ 1 mm Hg) gave 16.2 g (77%) of **13**.

NMR(CCl_4, 90 MHz) δ 0.13 (s, 9 H, -Si$(CH_3)_3$), 2.7 (s, -NCH_3), 6.88 (d of d, J = 48.6 Hz, J' = 9 Hz, 4 H, aromatic) ppm.

2.3 3,20-bis-Ethylenedioxy-17α-hydroxy-5α,10α-epoxy-19-norpregn-9(11)-ene **15** [2]

Hexafluoroacetone trihydrate (1.42 g, 6.45 mmol), hydrogen peroxide (30 wt%, 0.8 ml, 7.76 mmol), and methylene chloride (10 ml) were combined in a 100 ml flask equipped with a magnetic stir bar and a polyethylene stopper. The system was flushed with nitrogen and the mixture was stirred in an ice bath for 30 min. A solution of 3,20-bisethylenedioxy-17α-hydroxy-19-norpregna-4,9-diene **14** (2.0 g, 4.97 mmol) in methylene chloride (10 ml) was then added dropwise over 10 min. The system was sealed and stirred overnight at 4°C. The mixture was diluted with methylene chloride (60 ml) and washed sequentially with 10% sodium sulfite solution, saturated sodium bicarbonate solution, and brine. The organic fractions were combined, dried over sodium sulfate, filtered, and concentrated in vacuo to give 1.6 g of a stable foam. NMR analysis indicated the crude material contained 62% of the desired α-epoxide **15**.

NMR (90 MHz) δ 0.77 (s, C-18 CH_3), 1.36 (s, C-21 CH_3), 3.75–4.3 (m, 8 H, ketal methylenes), 5.85 (br. m, 11-CH, β-epoxide), 6.04 (br. m, 11-CH, α-epoxide) ppm.

2.4. 3,20-bis-Ethylenedioxy-5α,17α-dihydroxy-11β-(4-aminophenyl)-19-norpregn-9-ene **16a**

Magnesium (410 mg, 16.7 mmol) was weighed into a 100-ml round bottom, three-neck flask equipped with a reflux condenser, addition funnel, a magnetic stir bar, and a rubber septum. A single crystal of iodine was added and the system was flushed with dry nitrogen. The entire system was flame dried under a positive pressure of nitrogen. After cooling, dry ether (20 ml) and a catalytic amount of 1,2-dibromoethane were added via a syringe. An ether (5.0 ml) solution of p-bromo-N,N-bis(trimethylsilyl)aniline **12** (5.3 g, 16.7 mmol) was prepared and 1.0 ml of this solution was added. The mixture was heated to reflux and the remaining bromo-aniline solution was added dropwise over 1 h. After refluxing for 5 h, the reaction mixture was allowed to cool to room temperature and stirred overnight. Dry THF (20 ml) was added followed by the addition of copper(I) chloride (166 mg, 1.67 mmol) and the mixture was stirred at room temperature for 30 min. After cooling to 0°C, a THF (10 ml) solution of epoxide **15** (1.0 g, 2.39 mmol) was added via a syringe and the mixture was stirred at 0°C for 15 min. The mixture was then allowed to warm to room temperature and stirred for 2 h. Saturated ammonium chloride solution (30 ml) was added slowly. Air was bubbled through the mixture for 30 min and then diluted with water. The aqueous mixture was extracted with methylene chloride (3x). The methylene chloride extracts were washed with water (2x) and brine. The combined extracts were dried over sodium sulfate, filtered, and evaporated in vacuo to give 5.5 g of a dark oil. The oil was loaded onto a column (Si) and eluted with 10% acetone/methylene chloride to afford 700 mg as a stable foam. Recrystallization of this material from methylene chloride/methanol (containing a trace of pyridine) gave 180 mg of **16a** as a high melting solid (no melt up to 310°C in a sealed tube).

NMR (90 MHz) δ 0.48 (s, 18-CH_3), 1.35 (s, 21-CH_3), 3.75–4.05 (br. m, 8 H, 3,20-diketal), 4.2 (br. d, 11β-H), 6.55 and 7.15 (d, J = 9 Hz, AA'BB', 4 H) ppm.

FTIR: ν_{max} 3553, 3357, 2942, 1621, 1512 cm^{-1}.

MS (m/e): $M^+ = 511$.

Analysis: for $C_{30}H_{41}NO_6$ Calc: C 70.42; H 8.08; N 2.74. Found: C 70.49; H 8.16; N 2.84.

2.5. 11β-(4-Aminophenyl)-17α-hydroxy-19-norpregna-4,9-diene-3,20-dione **17a**

A mixture of ethanol (50 ml) and 8.5% sulfuric acid (5 ml) was sparged with nitrogen for 30 min. Grignard product **16a** (2.6 g, 5.1 mmol) was added as a solid and the mixture was placed in an oil bath preheated to 95°C for 30 min. The mixture was cooled in an ice bath and quenched with the addition of saturated potassium carbonate (ca. pH = 10). The ethanol was removed in vacuo and the residue diluted with water. The resulting solid was filtered, washed with water (2x), and dried in vacuo. The material was purified via preparitive HPLC (Waters LC 2000 system, Prep Pak 500 silica cartridge, 3% isopropanol/methylene chloride with 0.1% triethylamine, 20 ml/min) to afford 1.23 g of **17a**, mp = 268–270°C (sealed tube).

NMR (300 MHz) δ 0.46 (s, 18-CH_3), 2.25 (s, 21-CH_3), 4.33 (d, 11α-H), 5.75 (s, C-4 H), 6.57 and 6.91 (d, J = 9 Hz, AA'BB' for aromatic H) ppm.

FTIR: ν_{max} 3367, 3257, 2932, 1703, 1643, 1583, 1512 cm^{-1}.

MS (m/e): M^+ = 405.

Analysis: for $C_{26}H_{31}NO_3$ Calc: C 77.01; H 7.70; N 3.43. Found: C 76.82; H 7.54; N 3.54.

2.6. 11β-(4-N-Trifluoroacetamidophenyl)-17α-acetoxy-19-norpregna-4,9-diene-3,20-dione **18a**

Trifluoroacetic anhydride (1.39 ml, 9.86 mmol) and acetic acid (0.56 ml, 9.86 mmol) were added to methylene chloride (10 ml) and stirred at room temperature for 30 min. The mixture was chilled to 0°C in an ice bath and treated with p-toluenesulfonic acid (86.3 mg, 0.45 mmol). The mixed anhydride was added to a pre-cooled (0°C) mixture of amino-alcohol **17a** (200 mg, 0.49 mmol) in methylene chloride (20 ml) and the mixture was stirred at 0°C for 2 h. The mixture was quenched with the cautious addition of saturated potassium carbonate (to pH = 10). After diluting with water, the mixture was extracted with methylene chloride (3x). The methylene chloride extracts were washed with water (2x), brine, combined, and dried over sodium sulfate. The mixture was filtered and the filtrate was concentrated in vacuo to afford 280 mg. The crude material was recrystallized from acetone/ether to yield 155 mg of a white solid, mp = 187–189°C (sealed tube).

NMR (300 MHz) δ 0.3 (s, 18-CH_3), 2.08 (s, 17-OAc), 2.15 (s, 21-CH_3), 4.45 (d, 11α-H), 5.8 (s, C-4 H), 7.06 and 7.7 (d, J = 9 Hz, AA'BB' of 4 aromatic H), 9.28 (s, -N*H*$COCF_3$) ppm.

FTIR: ν_{max} 3291, 2953, 1730, 1708, 1659, 1240, 1158 cm^{-1}.

MS (m/e): M^+ = 543.

Analysis: for $C_{30}H_{22}F_3NO_5$ Calc: C 66.29; H 5.93; N 2.58. Found: C 65.21; H 6.10; N 2.42.

2.7. 17α-Acetoxy-11β-(4-aminophenyl)-19-norpregna-4,9-diene-3,20-dione **7**

The acetamide-acetate **18a** (650 mg, 1.19 mmol) was suspended in 35% aqueous methanol (100 ml) and treated with potassium hydrogen carbonate (10 g). The mixture was stirred at room temperature for 18 h and monitored by TLC (10% acetone/methylene chloride). The solvent was removed in vacuo and the residue was taken up in water. The aqueous mixture was extracted with methylene chloride (3x). The organic extracts were washed with water (2x), brine, and combined. After filtering, the filtrate was evaporated in vacuo and the residue was purified via flash chromatography (10% acetone/methylene chloride) to afford 425 mg of **7** along with 60 mg of starting material **18a**. The desired product **7**, obtained above, was combined with similar materials from previous experiments and the combined materials (550 mg) were recrystallized from methylene chloride/ether two times to yield 440 mg, (no true melt at temperatures up to 310°C in a sealed tube. However, discoloration/decomposition began at 290°C).

NMR (300 MHz) δ 0.35 (s, 18-CH_3), 2.08 (s, 17-OAc), 2.12 (s, 21-CH_3), 4.40 (d, 11α-H), 5.78 (s, C-4 H), 6.5 and 7.05 (d, J = 9 Hz, AA'BB' of 4 aromatic H) ppm.

FTIR: ν_{max} 3466, 3378, 2964, 1730, 1708, 1665, 1599, 1512, 1261 cm^{-1}.

MS (m/e): M^+ = 447.

Analysis: for $C_{28}H_{33}NO_4$ Calc: C 75.14; H 7.43; N 3.13. Found: C 72.61; H 7.27; N 3.05.

2.8. 11β-(4-N-Methylaminophenyl)-17α-hydroxy-19-norpregna-4,9-diene-3,20-dione **17b**

Magnesium (0.8 g, 32.9 mmol) was weighed into a 100-ml round bottom 2-neck flask. The flask was fitted with a reflux condenser, magnetic stir bar, and a rubber septum. A small crystal of iodine was added and the system was flushed with nitrogen. The entire system was flame dried and then allowed to cool to room temperature under a positive pressure of nitrogen. THF was added, followed by the addition of dibromoethane (ca. 0.1 ml) the mixture was stirred at room temperature until there was evidence of reaction on the surface of the magnesium. A solution of 4-bromo-N-methyl-N-trimethylsilylaniline **13** (7.7 g, 29.8 mmol) in dry THF (10 ml) was added via a syringe. The mixture was stirred and heated to reflux for 4 h. The mixture was cooled to room temperature and solid copper(I) bromide-dimethyl sulfide complex (0.68 g, 3.3 mmol) was added and the mixture was stirred at room temperature for 30 min. The epoxide **6** (1.6 g, assume 4.97 mmol) as a THF (15 ml) solution was added and the mixture was stirred for 2 h. Saturated ammonium chloride solution (5.0 ml) was added and the mixture was stirred at room temperature while air was bubbled through the mixture. The mixture was diluted with water and extracted with methylene chloride. The methylene chloride extracts were washed with water

(2x) and brine. The combined methylene chloride extracts were dried over sodium sulfate, filtered, and evaporation of the solvent gave 11.2 g of a dark oil.

The crude material from above was diluted with ethanol (100 ml) and 10% sulfuric acid (20 ml) was added and the mixture was heated to reflux for 1 h. The solvent was removed in vacuo, diluted with water, and made basic with concentrated ammonium hydroxide. The mixture was extracted with methylene chloride. The methylene chloride extracts were washed with water (3x), brine, combined, and dried over sodium sulfate. After filtering, evaporation of the solvent gave 7.3 g of a black oil. The crude material was purified via flash chromatography (5% acetone/methylene chloride) to afford 0.4 g of **17b** as a green foam. The material was recrystallized from ethyl acetate to give 0.25 g (12%), mp = 81–83°C.

NMR (90 MHz) δ 0.43 (s, 18-CH_3), 2.23 (s, 21-CH_3), 2.78 (s, -NHCH_3), 4.40 (br. d, J = 7.5 Hz, 1 H, 11-CH), 5.78 (s, 1 H, 4-CH), 6.76 (d of d, J = 39 Hz, J' = 9 Hz, 4 H) ppm.

FTIR: ν_{max} 3537, 3408, 3294, 2952, 1730, 1704, 1657, and 1642 cm^{-1}.

2.9. 17α-Acetoxy-11β-(4-N-acetyl, N-methylaminophenyl)-19-norpregna-4,9-diene-3,20-dione **18b**

In dry glassware, trifluoroacetic anhydride (1.3 ml, 9.2 mmol) and glacial acetic acid (0.5 ml, 8.7 mmol) were added to dry methylene chloride (4.0 ml). The mixture was stirred at room temperature for 30 min. Solid p-toluenesulfonic acid monohydrate (0.082 g, 0.43 mmol) was added and the mixture was chilled to 0°C. A solution of the steroid **17b** (0.2 g, 0.43 mmol) in dry methylene chloride (1.0 ml) was added and the mixture was stirred at 0°C for 20 min. Saturated potassium carbonate solution (ca. 3.0 ml) was added dropwise until cessation of CO_2 evolution was observed. The mixture was diluted with water and extracted with methylene chloride. The organic extracts were washed with water (2x), brine, combined, dried (Na_2SO_4), filtered and concentrated in vacuo to give 0.22 g of an oil. NMR analysis indicated this material to be diacetate **18b**. Trituration of the residue with pentane gave 0.15g of a solid (69.3%).

NMR (90 MHz) δ 0.3 (s, 18-CH_3), 2.10, 2.13, and 2.23 (s, 3 H, 21-CH_3, -OAc, or NAc), 3.23 (s, NCH_3), 4.53 (br. d, J = 6.7 Hz, 1 H, 11-CH), 5.83 (s, 1 H, 4-CH), 7.21 (d of d, J = 13.5 Hz, J' = 9 Hz, 4 H) ppm.

FTIR: ν_{max} 2946, 1730, 1711, 1664, and 1604 cm^{-1}.

2.10. 17α-Acetoxy-11β-(4-N-methylaminophenyl)-19-norpregna-4,9-dien-3-one (CDB-3877) **6** [5]

Calcium oxide (8.03 g, 143.14 mmol) was added to a well stirred THF/methanol (100 ml) solution of CDB-2914 **5** (8.0 g, 16.84 mmol) and the mixture was chilled in an ice bath. A THF/methanol (20 ml) solution of iodine (21.37 g, 84.21 mmol) was added and the mixture was stirred at 0°C for 1 h. The mixture was filtered and the filter pad was washed with methanol. The filtrate was diluted with 10% sodium thiosulfate solution and the aqueous mixture was extracted with methylene chloride (3x). The methylene chloride extracts were washed with water and brine, combined, and dried over sodium sulfate. Evaporation of the solvent gave 9.72 g of a stable brown foam. The material was chromatographed (10% acetone/methylene chloride) to afford 3.88 g (50%) of CDB-3877 **6.** A sample was recrystallized from methylene chloride/hexanes to provide an analytical sample, mp = 239–240°C (dec.).

NMR (300 MHz) δ 0.37 (s, 18-CH_3), 2.10 (s, 17α-OAc), 2.17 (s, COCH_3), 2.87 (s, NHCH_3), 4.45 (d, J = 11 Hz, 11α-H), 5.83 (s, C-4 H), 6.57 and 7.02 (d, J = 9 Hz, AA'BB' of aromatic H) ppm.

FTIR (KBr, diffuse reflectance): 3417, 2891, 1729, 1708, 1660, 1604, 1581 cm^{-1}.

MS (m/z): 461 (M^+), 120, 107 (base).

Analysis: for $C_{29}H_{35}NO_4 \cdot 0.2\ CH_2Cl_2$ Calc: C 73.31; H 7.41; N 2.93. Found: C 73.13 H 7.60; N 3.08.

Note: Prolonged drying at elevated temperatures failed to remove traces of methylene chloride.

2.11. 17β-Hydroxy-17α-propynyl-(4-N-methylaminophenyl)-estra-4,9-dien-3-one (RU-42 633) **2** [6]

Similar treatment of RU-38 486 with iodine-calcium oxide in THF-methanol, as described above, gave RU-42 633 **2** in 82% yield as a stable foam.

NMR (300 MHz) δ 0.552 (s, 18-CH_3), 1.894 (s, $\equiv CCH_3$), 2.813 (s, -NCH_3), 4.340 (d, J = 6.6 Hz, 11α-H), 5.756 (s, C-4 H), 6.545 and 6.971 (d, AA'BB' of aromatic H) ppm.

FTIR (KBr, diffuse reflectance): 3380, 2945, 1656, 1614, 1518 cm^{-1}.

MS (m/e): 415 (M^+), 266, 120, 107 (base).

Analysis: for $C_{28}H_{33}NO_2 \cdot 0.3\ CH_2Cl_2$ Calc: C 77.06; H 7.68; N 3.18. Found: C 77.28; H 7.97; N 3.33.

Note: Prolonged drying at elevated temperatures failed to remove traces of methylene chloride.

2.12. 17α-Acetoxy-11β-(4-N-methyl, N-tritiomethyl-aminophenyl)-19-norpregna-4,9-dien-3-one **8**

A solution containing CDB-3877 **6** (2.0 mg, 4.8 nmol), DMF (0.5 ml), and ^3H methyl iodide (100 mCi, in 0.15 ml of THF; specific activity 80 Ci/mmol) was heated with stirring at 70°C for 90 h in a 1.0 ml sealed tube. After removal of unreacted methyl iodide ^3H under vacuum, the reaction mixture was first purified by prep TLC (silica gel) using hexane/acetone (3:1) as the solvent system. The band corresponding to N-tritiomethyl CDB-2914 was identified by exposing the TLC plate to X-ray film. The correct band was cut from the plate and eluted from the silica gel using ethanol to give 10 mCi. The product was further purified via

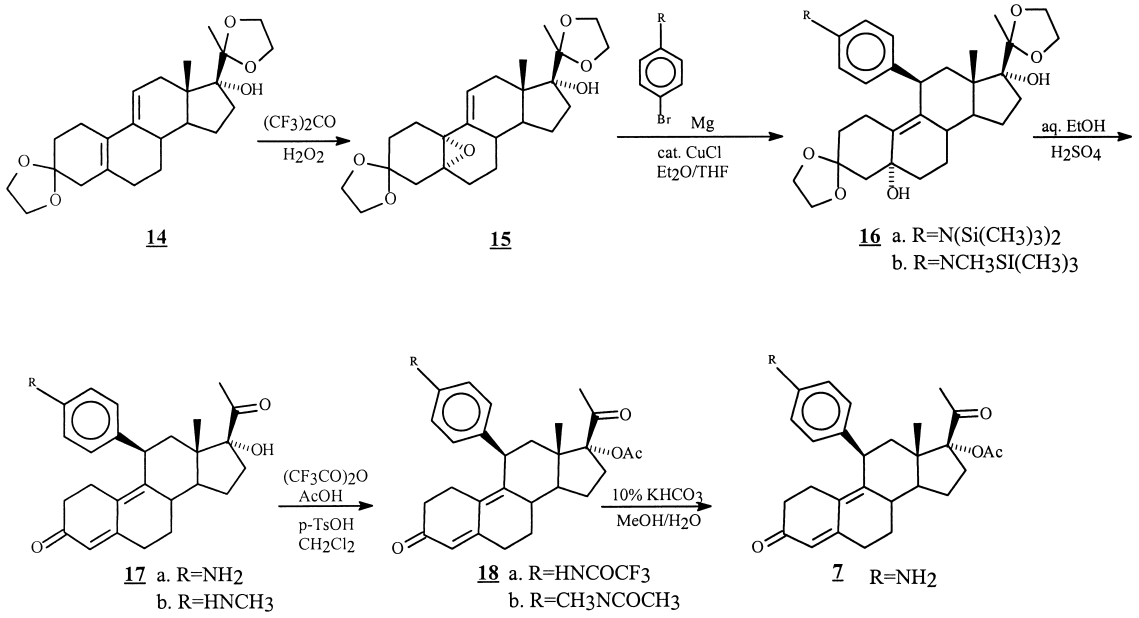

Fig. 2. Synthesis of di-N-desmethyl derivative.

prep HPLC (Zorbax XDB-C18, 3.5 micron 4.6 × 150 mm, acetonitrile/0.03% TEAA 1:1). Final yield of N-tritiomethyl CDB-2914 was 5.5 mCi, specific activity 80 Ci/mmol, radiochemical and chemical purity was 97%.

2.13. 17β-Hydroxy-17α-propynyl-11β-(4-N-methyl, N-tritiomethylaminophenyl)-estra-4,9- dien-3-one (^{3}H RU-38 486)

A solution containing RU-42 633 **2** (2.0 mg, 4.8 nmol), DMF (0.5 ml), and ^{3}H methyl iodide (100 mCi, in 0.15 ml of THF; specific activity 80 Ci/mmol) was heated with stirring at 70°C for 90 h in a 1.0 ml sealed tube. After removal of unreacted methyl iodide ^{3}H under vacuum, the reaction mixture was first purified by prep TLC (silica gel) using hexane/acetone (3:1) as the solvent system. The band corresponding to mifepristone (methyl ^{3}H) was identified by exposing the TLC plate to X-ray film. The correct band was cut from the plate and eluted from the silica gel using ethanol to give 10 mCi. The product was further purified via prep HPLC (Zorbax XDB-C18, 3.5 micron 4.6 × 150 mm, acetonitrile/0.03% TEAA 1:1). Final yield of tritiomethyl mifepristone was 5.5 mCi, specific activity 80 Ci/mmol, radiochemical and chemical purity 97%.

3. Results and discussion

The synthesis of the N-desmethyl derivatives (Fig. 2) was dependent upon the preparation of protected derivatives of 4-bromoaniline **10a** and 4-bromo-N-methylaniline **10b** (Fig. 3), which would allow for the successful formation of Grignard reagents. Attempts to form Grignard reagents directly from **10a** and **10b** were met with failure. 4-Bromo-*bis*-N,N- trimethylsilylaniline **12** was prepared using a stepwise silylation according to the procedure of Bock et al. [6]. Thus, reaction of **10a** with chlorotrimethylsilane, in triethylamine, gave 4-bromo-N-trimethylsilylaniline **11**. Subsequent reaction of **11** with butyllithium, followed by the addition of chlorotrimethylsilane afforded **12**. In a similar fashion, 4-bromo-N-methyl-N-trimethylsilylaniline **13** was obtained using the butyl lithium/chlorotrimethylsilane procedure.

The regioselective 5,10-epoxidation of 3,20-*bis*-ethylenedioxy-17α-hydroxy-19-nor-pregna-5(10),9(11)-diene **14** [7] was achieved using hexafluoroacetone hydroperoxide generated in situ from hexafluoroacetone and hydrogen peroxide following the general procedure of Teutsch et al. [8]. Fractional crystallization of the epoxide mixture gave the

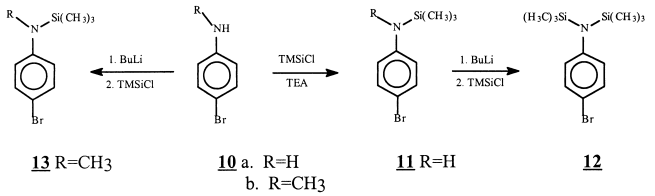

Fig. 3. Synthesis of protected 4-bromoanilines.

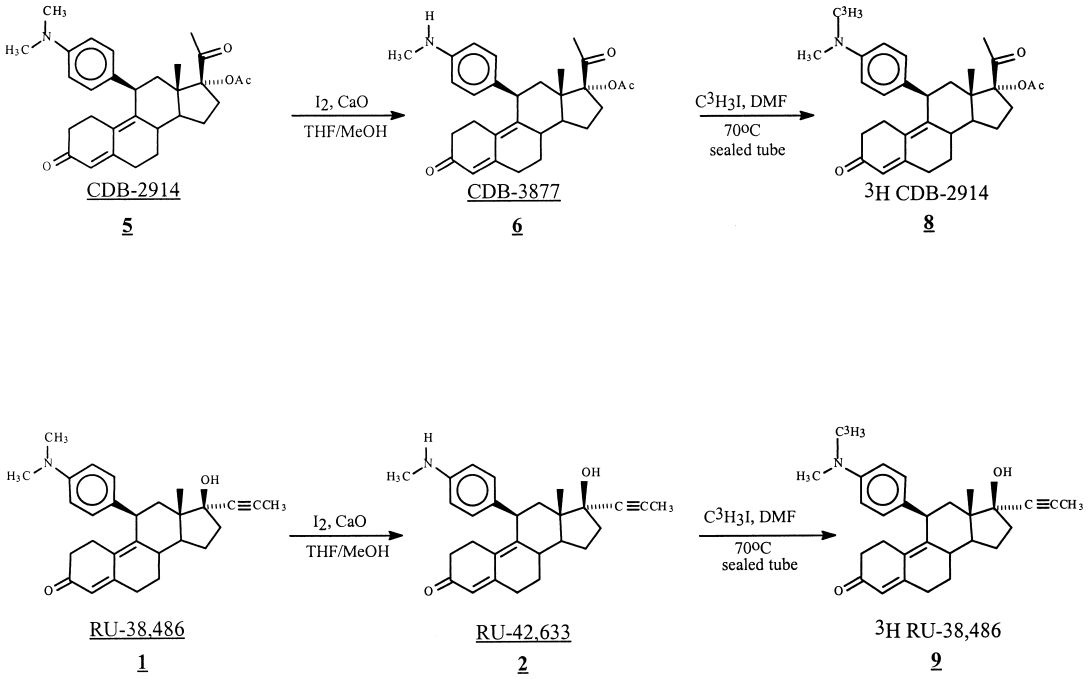

Fig. 4. Synthesis of mono-desmethyl and N-tritiomethyl derivatives.

pure 5α,10α-isomer **15** in 50% yield. Copper (I) catalyzed conjugate addition of the Grignard reagents, prepared from aryl bromides **12** and **13**, gave the Grignard adducts **16a** and **16b**. Acid hydrolysis of Grignard adducts **16a** and **16b** gave 4,9-dien-3,20-diones **17a** and **17b**. Acetylation of **17a** and **17b** with the mixed anhydride, generated from the reaction of trifluoroacetic anhydride and acetic acid, gave 17-acetate, N-trifluoroacetate **18a** and 17-acetate, N-acetate **18b**. Careful hydrolysis of **18a** using 10% potassium bicarbonate gave di-N-desmethyl derivative **7**. Attempts to selectively hydrolyze the N-acetate in **18b** were not successful.

Demethylation of CDB-2914 **5** was accomplished using iodine-calcium oxide in THF-methanol to afford N-desmethyl CDB-2914 **6** [5]. Similar treatment of RU-38 486 **1** and other 20-ketopreganes, with the 11β-N,N-dimethylaminophenyl group, consistently gave N-dealkylated products.

While we describe two methods to introduce the 4-methylaminophenyl group at C-11, only oxidative demethylation of **5** proved to be the only viable approach to obtain desmethyl derivative **6**. As was mentioned above, acetylation of **17b** gave 17-acetate-N-acetate **18b** and we were unable to selectively hydrolyze the N-acetate in **18b** to give **6**.

The ease with which the N-desmethyl derivatives of CDB-2914 and RU-38 486 can be obtained, made possible the facile synthesis of the N-tritiomethyl derivatives (Fig. 4). Thus, the reaction of the N-desmethyl derivatives **6** and **2** with ^{3}H methyl iodide (80 Ci/mmol) afforded the N-tritiomethyl derivatives **8** and **9** with high specific activity. The high specific activity of these materials makes them ideal as radioligands for use in radioimmunoassay (RIA) of the parent compounds. While the synthesis of N-tritiomethyl RU-38 486 **1** has been reported by Mais et al. [9], their synthesis of the substrate RU-42 633 **2** was achieved through a multi-step sequence, rendering the process more cumbersome.

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