SYNTHESIS AND CHARACTERIZATION OF TRITIUM-LABELED RU486

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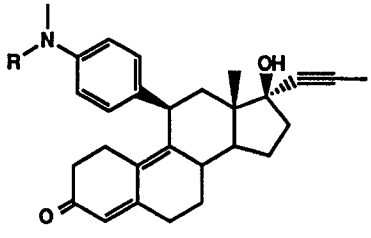
SUMMARY

[^{3}H]RU38486 (RU486) was synthesized from its penultimate precursor, desmethyl RU486, by substitution of the secondary amine with tritiated methyl iodide; specific activity 85 Ci/mmol. Ligand binding studies confirm that RU486 binds with high affinity to human progesterone receptor type A (PR-A) and progesterone receptor type B (PR-B).

Key Words: Progesterone receptor, tritiated RU486

INTRODUCTION

RU486 is a synthetic steroid possessing high affinity for both the glucocorticoid and progesterone receptors (1-2). The clinical usefulness of this compound has been established in contraception (3) and certain neoplastic diseases such as breast cancer(4). Although some studies on the mechanisms of action of RU486 have been conducted with this analog in its tritiated form (5-6), the availability of the molecule and its low specific



R = Me, RU486
R = H, Desmethyl RU486

activity have restricted further investigations (7). We set out, therefore, to synthesize [^{3}H]RU486 with a greater specific activity than previously described in order to undertake studies to understand more precisely the molecular basis for interactions of RU486 with its

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receptor(s), and to study receptor-ligand/DNA complex formation. Rather than introducing the tritium group(s) into the ring system as had been done previously, we introduced a tritritium methyl group as a final step into the penultimate precursor, desmethyl RU486. The synthetic route leading to desmethyl RU486 is shown in Figure 1.

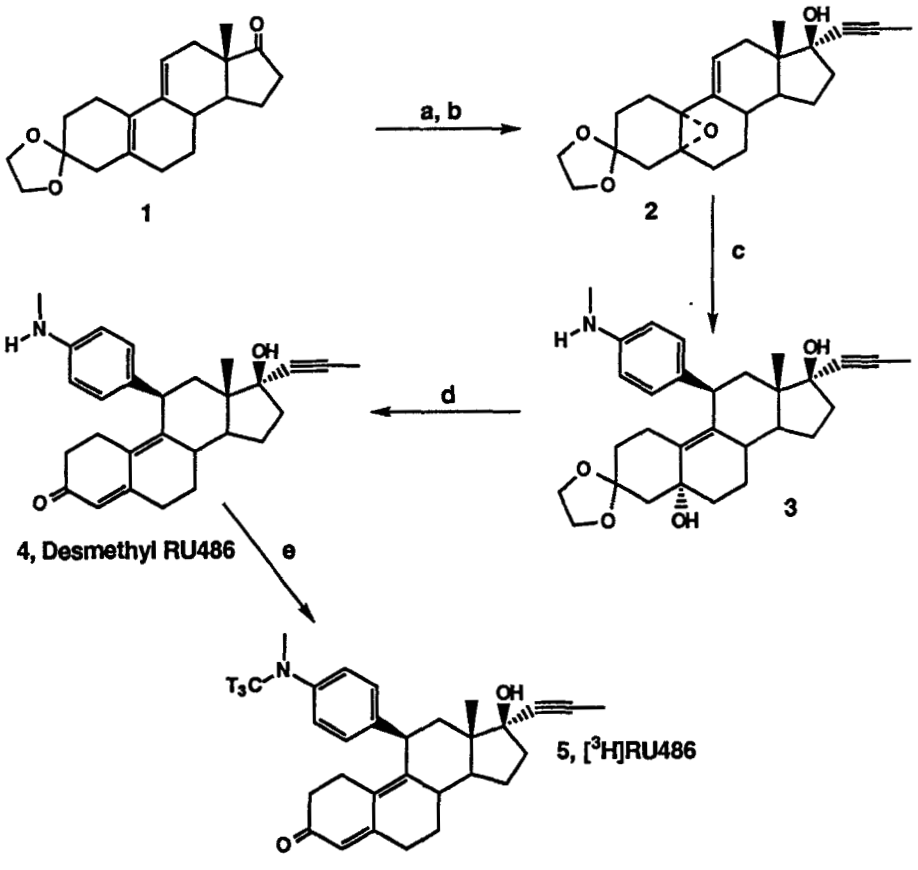

FIGURE 1. Synthetic route to [^{3}H]RU486. a, H_2O_2; b, Me—≡—Li; c, 4-(trimethylsilyl methylamino)phenylmagnesium bromide; d, PTS, EtOH; e, T_3MeI in DMF-toluene, 1:1, 72 hours at 70°C.

Ketal **1** was oxidized to the epoxide with hydrogen peroxide followed by reaction with the lithium salt of propyne to give β-hydroxy compound **2.** Condensation of **2** with the Grignard reagent of the trimethylsilyl protected aniline gave alcohol **3** upon work up of the reaction mixture. Deprotection of the 3-keto group and dehydration was accomplished with para-toluene sulfonic acid (PTS) in ethanol at 40°C to afford desmethyl RU486, **4.** The final step was carried out in toluene-DMF (1:1) using one equivalent of tritritio methyl iodide in a sealed

ampule at 70°C for 72 hours and gave a 40% yield of the desired [^{3}H]RU486 which co-migrated with authentic RU486 by HPLC and TLC. Labelled material was purified by reverse-phase HPLC and was cleanly seperated from the starting desmethyl RU486. As such, the specific activity of the compound resulted in that of the starting tritiated methyl iodide, 85 Ci/mmol.

In order to assess the potential of this ligand to bind to the human progesterone receptor, a saturation binding analysis was performed. The results are shown in Figure 2.

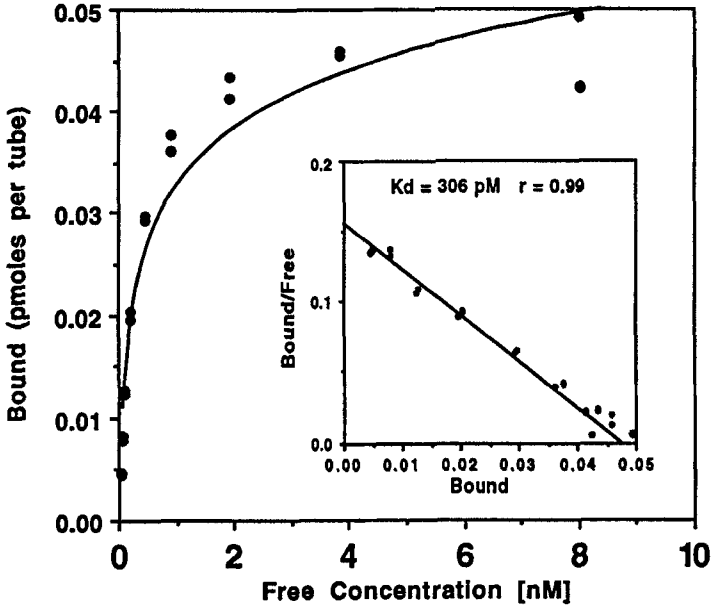

FIGURE 2. Saturation binding of RU486 to human PR-A. The inset shows the Scatchard replot of the saturation data.

Human progesterone receptor (A-form, PR-A) used in this study was derived from baculovirus infected Sf21 cells (8). It became saturated with [^{3}H]RU486 (a Scatchard replot of the saturation is shown in the inset of Figure 2) and provided a dissociation constant (K_d) of 309 pM. These results indicate that this ligand will be a useful tool to expand on earlier studies using [^{3}H]RU486 and the greater specific activity should provide increased sensitivity.

EXPERIMENTAL

Starting material **1** was obtained from Xianju Pharmaceutical Co., Ltd., People's Republic of China.

3,3-[1,2-ethylenedioxybis(oxy)]-17α-(1-propynyl)-estra-9(11)-en-5α,10α-epoxy-17β-ol **2**.

To a dried 100 ml three neck flask containing 5.0 g of **1** in 50 ml dichloromethane was added 5 ml of 30% hydrogen peroxide and 1.0 ml hexachloroacetone and the reaction continued for 72 hours. The organic layer was washed sequentially with 10% $Na_2S_2O_3$, saturated $NaHCO_3$ and brine, dried and concentrated under reduced pressure. The solid was recrystallized from EtOAc/isopropyl ether (1:3) to give 4.0 g (76.1% yield) of the epoxide, m.p. 158°C; IR 1090 cm^{-1}, 1740 cm^{-1} (17-keto).

Into a three neck dry flask containing 8.4 ml of anhydrous THF and 35 ml of butyllithium (1.6 M in hexane) was bubbled propyne to saturation at 0°C. After 30 minutes, a solution of the epoxide compound (3.5 g) in 10 ml dry THF was added over a 30-minute period. TLC analysis indicated the reaction was complete after one hour and the reaction mixture was poured into 30 ml water, the aqueous layer extracted with ethyl acetate and dried over Na_2SO_4. Removal of the solvent under reduced pressure following recrystallization from isopropyl ether afforded 3.5 g (89%) of **2**, m.p. 190°C; ^1H NMR (60 MHz, $CDCl_3$) (δ ppm) 0.85 (s, 3H, H-18), 1.85 (s, 3H, propyne), 3.9 (s, 4H, ketal), 6.05 (m, 1H, H-11).

11β-(4-methyaminophenyl)-[3,3-(1,2-ethylenedioxybisoxy)]-17α-(1-propynyl)-estra-9-en-5α,17β-diol **3**.

Compound **2** (3.5 g) was dissolved in 30 ml of dry THF in a three neck flask and cooled to 0°C. Copper(I) chloride was added followed by p-N-trimethylsilyl-N-methyl aminophenyl magnesium bromide in dry THF. After 6 hours, the reaction mixture was poured into 10 ml of saturated NH_4Cl, washed with the same solution followed by brine, drying and concentration under reduced pressure. Flash chromatography (silica gel, cyclohexane/acetone, 5:1) gave 3.8 g (78%) of the desired compound, m.p. 210°C; ^1H NMR (60 MHz, $CDCl_3$) (δ ppm) 0.5 (s, 3H, H-18), 1.9 (s, 3H, propyne), 2.95 (s, 3H, NCH3), 3.9 (s, 4H, ketal), 4.25 (m, 1H, H-11), 6.65, 7.05 (AA-BB, ArH).

N-Desmethyl RU486 **4**.

Compound **4** (2.8 g) and p-toluene sulfonic acid (1.4 g) were dissolved in 28 ml of 90% ethanol at 40°C for 4 hours and then poured into 300 ml of 1% NaOH. The crude N-desmethyl RU486 was filtered followed by flash chromatography (silica gel,

cyclohexane/acetone, 5:1) to give 1.9 g of a light yellow solid (60% yield), m.p. 112-114°C; ^{1}H NMR (60 MHz, $CDCl_3$) (δ ppm) 0.55 (s, 3H, H-18), 1.9 (s, 3H, propyne), 2.9 (s, 3H, NHCH3), 4.3 (broad s, 1H, H-11), 5.5 (s, 1H, H-4), 6.6,7.0 (AA'-BB', 4H, ArH).

N-tritritiomethyl RU486 5.

Into a dry, narrow glass tube was placed 1.0 μmole of 4,100 μl DMF and 100 μl of a toluene solution containing 20 mCi (Amersham) of tritiated methyl iodide (85 Ci/mmol). The glass tube was flame sealed and placed into a 70°C sand bath for 72 hours followed by opening the tube and evaporation of the solvent under a stream of nitrogen. The residue was dissolved into methanol and injected onto a semi-preparative reverse-phase column using methanol-0.1M NH_4Ac (7:3) as the mobile phase. Under these conditions, starting material eluted at 10 minutes and the desired product at 18 minutes to give a yield of 40% (8 mCi) and specific activity of 85 Ci/mmol.

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