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11β-Substituted 13β-ethyl gonane derivatives exhibit reversal of antiprogestational activity

Pemmaraju N. Rao,* James W. Cessac,* Richard P. Blye,† and Hyun K. Kim†

** Department of Organic Chemistry, Southwest Foundation for Biomedical Research, San Antonio, Texas, USA; and † National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland, USA*

The syntheses of three 17α-acetoxy-13β-ethyl-11β-aryl-18,19-dinorpregna-4,9-diene-3,20 diones from levonorgestrel are described. Despite their close structural similarity to the antiprogesterone CDB-2914, one of the compounds exhibits agonistic progestational activity, and the other two compounds are totally inactive. (*Steroids* **63**:50–57, 1998) *© 1998 by Elsevier Science Inc.*

Keywords: synthesis; 13β-ethyl gonane derivatives; CDB-2914; progestin; antiprogestin

Introduction

The discovery of mifepristone (Figure 1) as an antiprogestational agent prompted spectacular progress in the basic understanding of progesterone and antiprogesterone action, as well as in the development of improved analogs[1] and compounds for other therapeutic uses.[2] Substitution at the 17-position by an α-acetoxy-β-acetyl group yielded a compound (CDB-2914, Figure 1) which exhibited antiprogestational and antiglucocorticoid activity similar to mifepristone.[3] Analogs with pure antiprogestational activity devoid of antiglucocorticoid activity would be of great interest for long-term use as contraceptive or therapeutic agents.

The increase in relative binding affinity (RBA) of levonorgestrel (Figure 1) for the progestin receptor (PR) over norethindrone[4] (Figure 1), along with a concomitant increase in progestational activity in rabbits,[5] led us to believe that a similar substitution of a 13β-ethyl group for the 18-methyl group of CDB-2914 could result in synthetic analogs with increased antiprogestational activity. A precedent for this type of modification in antiprogestins has been reported in the literature with regard to the synthesis and antiprogestational activity of a 13β-ethyl analog (RU43855, Figure 1)[6] of mifepristone. In order to extend this investigation into this type of structural modification, 13β-ethyl analogs of CDB-2914 with varying 11β-aryl substituents were synthesized and tested for biological activity.

Address reprint requests to Pemmaraju N. Rao, Department of Organic Chemistry, Southwest Foundation for Biomedical Research, P.O. Box 760549, San Antonio, Texas 78245-0549.
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Experimental

Chemistry

Melting points were determined on a Thomas–Hoover apparatus and are uncorrected. ^{1}H NMR were determined in deuterochloroform either at 300 MHz using a General Electric QE-300 spectrometer or at 90 MHz using a Varian EM-390 spectrometer. Infrared spectra were recorded on a Perkin–Elmer model 1600 FTIR instrument equipped with a diffuse reflectance accessory using a KBr matrix. Optical rotations were measured using a Rudolph Research Autopol II automatic polarimeter using a 1.0 dm cell. HPLC analysis was carried out on Waters Associates, Inc. equipment monitored by a Lambda max Model 481 LC Spectrophotometer. 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 mass spectrometer. Combustion analyses were performed by Midwest MicroLabs Ltd., Indianapolis, Indiana. "Flash column" chromatography was performed on 32–64 μM silica gel obtained from Scientific Adsorbents Inc., Atlanta, Georgia. TLC Analyses were carried out on silica gel GF (Analtech) glass plates (2.5 × 10 cm with 250 μM layer and prescored).

Most chemicals and solvents were analytical grade and used without further purification. 4-Bromoacetophenone ethylene ketal was prepared according to the procedure of Detty et al.[7] Levonorgestrel **1** was obtained from Wyeth–Ayerst Research, Princeton, New Jersey.

Ketalization of Levonorgestrel

A mixture of Levonorgestrel **1** (60 g, 192 mmol), ethylene glycol (60 mL, 1.07 mol), *p*-toluenesulfonic acid monohydrate (3.0 g, 15.8 mmol) in benzene (1.75 L), was heated to reflux under nitrogen in a flask equipped with a Dean–Stark trap for 16 h. The solvent was removed in vacuo and the residue taken up in methylene chloride. The organic fractions were washed with saturated

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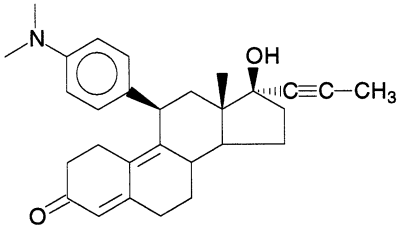

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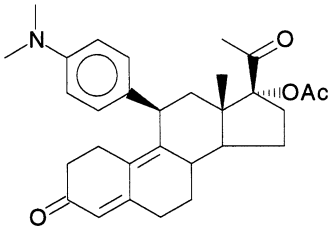

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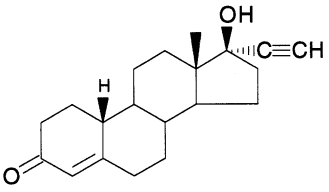

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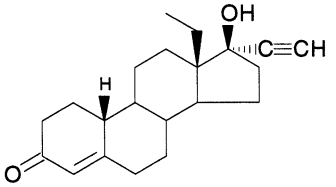
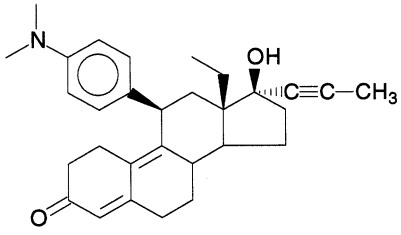


Norgestrel



RU43855

Figure 1.

sodium bicarbonate solution (1×), water (1×) and brine (1×), combined, dried (Na_2SO_4), filtered, and concentrated in vacuo to give the crude mixture of Δ^5- and $\Delta^{5,(10)}$-isomers of the 3-ketal **2** (79 g). Analysis by HPLC (Waters Associates NovaPak C_{18}; CH_3CN/H_2O 1:1; 1 mL/min; λ = 210 nm) indicated a 65:35 ratio of the $\Delta^{5,(10)}$-isomer to the Δ^5-isomer. ^{1}H NMR (90 MHz) δ 0.93 (m, 13β-CH_2CH_3), 2.57 (s, C≡CH), 3.97 (m, ethylenedioxy CH_2's), 5.47 (m, 6 C–H)ppm.

13β-Ethyl-17β-hydroxy-18,19-dinor-17α-pregn-5(10)-en-20-yn-3-one (4)

The ketal mixture **2** (79 g, assume 192 mmol) was dissolved in glacial acetic acid (840 mL) and the system was flushed with nitrogen. Water (360 mL) was added and the mixture was heated to 50°C for 1 h. Heating was discontinued and the mixture was stirred an additional 16 h. The reaction mixture was diluted to 6 L with water and the precipitate was collected by filtration and washed well with water. The solid was taken up in CH_2Cl_2, filtered through Na_2SO_4 and concentrated in vacuo to give 60.1 g solid residue. This material (**3**) was separated on a large Flash chromatography column (14 × 32 cm. bed of silica, 1.5% acetone in CH_2Cl_2 followed by 5% acetone in CH_2Cl_2) into levonorgestrel **1** (24 g, 40% recovery) and the $\Delta^{5,(10)}$-isomer **4** (36 g, 60% from **1**) both as white crystalline solids. A small amount of the $\Delta^{5,(10)}$-isomer **4** was crystallized from acetonitrile/water for characterization. m.p. = 169–171°C. ^{1}H NMR (300 MHz) δ 0.975 (t, J = 7.5 Hz, 13β-CH_2CH_3), 2.607 (s, C≡CH), 2.681 and 2.787 (each d, J_{AB} = 20.7 Hz, C4-H_2) ppm. IR (cm^{-1}) 3402, 3301, 2939, 1727, 1698. MS (*m/z*): M^+ = 312. Analysis calculated for $C_{12}H_{28}O_2 \cdot 1/3\ CH_3CN$: C, 79.79; H, 8.96. Found: C, 79.82; H, 8.86.

13β-Ethyl-17β-hydroxy-18,19-dinor-17α-pregna-4,9-dien-20-yn-3-one (5)

Under nitrogen, a solution of the $\Delta^{5,(10)}$ ketone **4** (36 g, 115.2 mmol) in dry pyridine (175 mL) was added to a solution of pyridinium bromide perbromide (45 g, 126.6 mmol) in dry pyridine (350 mL) preheated to 80°C. The reaction was stirred at 85–90°C for 1 h, cooled to room temperature and then poured into ice cold 2.5 *N* HCl solution (3 L). The crude product was collected by filtration, washed well with water, and taken up in ethyl acetate. The organic fractions were washed once with 1 *N* HCl, once with water, and once with brine, then combined, dried (Na_2SO_4), filtered and concentrated in vacuo. Crystallization of the residue from ether gave the pure diene **5** (21.1 g, 59%) in two crops. Concentration of the mother liquors followed by Flash chromatography (3% acetone in CH_2Cl_2) and crystallization from ether gave an additional 2.8 g of the pure diene **5** (total yield = 23.9 g, 66.8%). m.p. = 124–126°C [lit.[8] 130°C]. ^{1}H NMR (300 MHz) δ 1.067 (t, J = 7.4 Hz, 13β-CH_2CH_3), 2.605 (s, C≡CH), 5.685 (s, C4-H) ppm. IR (cm^{-1}) 3296, 2942, 1654, 1604. MS (*m/z*): M^+ = 310. Analysis calculated for $C_{21}H_{26}O_2$: C, 81.25; H, 8.44. Found: C, 81.28; H, 8.45.

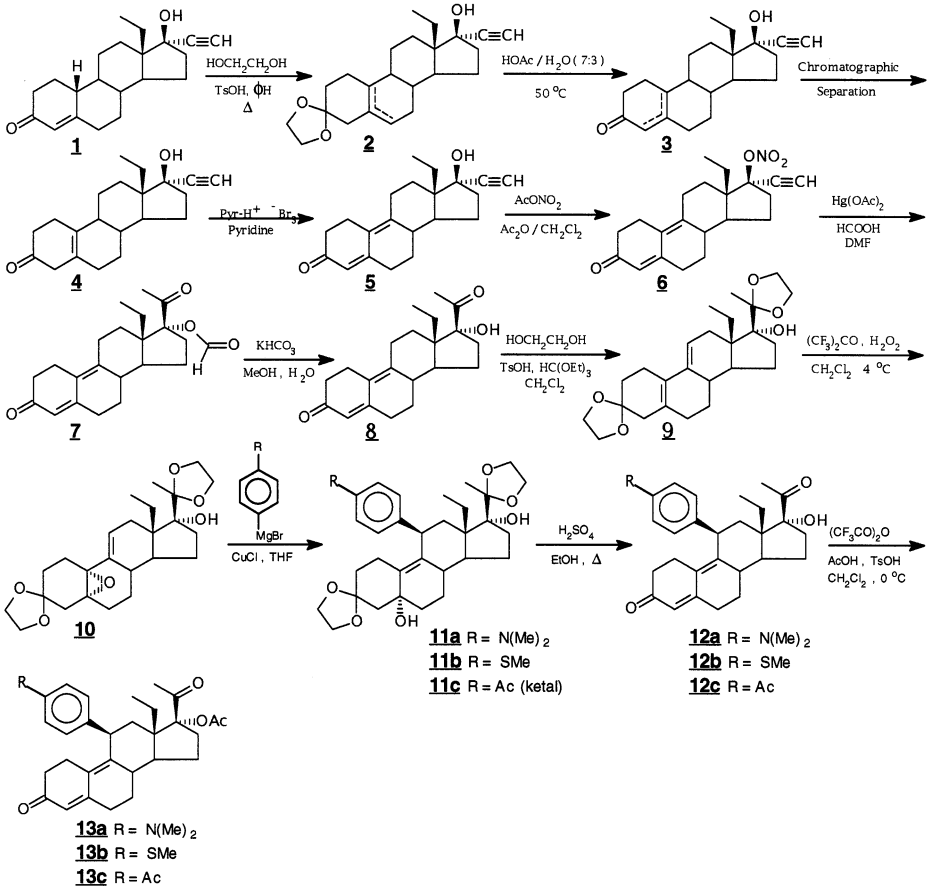


Figure 2. Synthesis of 17α-Acetoxy-13β-ethyl-11β-aryl-18,19-dinorpregna-3,20-diones.

*13β-Ethyl-17β-hydroxy-18,19-dinor-17α-pregna-4,9-dien-20-yn-3-one 17-nitrate ester (**6**)*

Under nitrogen, purified fuming nitric acid (5 mL, 113 mmol) was added dropwise to freshly distilled acetic anhydride (11 mL, 116.6 mmol) cooled to 0°C in an ice bath. The mixture was then stirred at 0°C for 10 min, room temperature for 30 min, then cooled again to 0°C. A portion (14 mL, ~99 mmol $AcONO_2$) of this solution was added to a solution of the dienone **5** (10 g, 32.2 mmol) in dry CH_2Cl_2 (30 mL) cooled to −15°C in an ice/solid NH_4Cl bath. This mixture was stirred at −15°C for 1 h then poured into a mixture of ice/saturated sodium bicarbonate solution (~400 mL). The mixture was stirred until the ice had melted and then extracted with CH_2Cl_2 (3×). The organic fractions were washed with saturated sodium bicarbonate solution (1×), water (1×) and brine (1×), then combined, filtered through Na_2SO_4 and concentrated in vacuo to give 11.7 g residue as a yellow-brown foam. This material was used directly in the subsequent reaction without further purification. A small amount of material from a previous preparation was purified by crystallization from ether for purposes of characterization. m.p. = 96–98°C. ^{1}H NMR (300 MHz) δ 1.058 (t, J = 7.4 Hz, 13β-CH_2CH_3), 2.741 (s, C≡CH), 5.701 (s, C-4H) ppm. IR (cm^{-1}) 3294, 3234, 2946, 2112, 1659, 1633, 1296. MS (*m/z*): M^+ = 355. Analysis calculated for $C_{21}H_{25}NO_4$: C, 70.96; H, 7.09; N, 3.94. Found: C, 70.73; H, 7.04; N, 3.88.

*17α-Formyloxy-13β-ethyl-18,19-dinorpregna-4,9-diene-3,20-dione (**7**)*

Under nitrogen, formic acid (99%, 80 mL) was added to dry DMF (20 mL) cooled in an ice bath. The ice bath was removed and the mixture stirred until warming to room temperature. Solid $Hg(OAc)_2$ (0.8 g, 2.5 mmol) was added and the mixture stirred at room temperature for ~10 min. This mixture was then added at once to a flask containing the crude nitrate product **6** (11.7 g, assume 32.2 mmol) and the reaction was stirred at room temperature and monitored by TLC (5% acetone/CH_2Cl_2) which indicated a complete reaction after 5.5 h. The reaction was then poured into ice water (~2 L) with stirring and the resulting precipitate was collected by filtration, washed with water and taken up in ethyl acetate. The organic fractions were washed with water (2×), brine (1×), combined, dried (Na_2SO_4), filtered and concentrated in vacuo. Purification of the residue by Flash chromatography (7% acetone/CH_2Cl_2) followed by crystallization from ether gave the formate ester **7** (3.3 g, 29% from **5**) as a light brown solid. m.p. = 156–158°C. ^{1}H NMR (300 MHz) δ 0.731 (t, J = 7.5 Hz, 13β CH_2CH_3), 2.197 (s, C21-H_3), 5.706 (s, C4-H), 8.034 (s, formate) ppm. IR (cm^{-1}) 2936, 1726, 1664, 1613. MS (m/z): M^+ = 356.

*17α-Hydroxy-13β-ethyl-18,19-dinorpregna-4,9-diene-3,20-dione (**8**)*

Under nitrogen, a 0.5 *M* solution of $KHCO_3$ (200 mL, 100 mmol) was added to a solution of the 17α-formate **7** (25 g, 70.1 mmol) in methanol (2.5 L). After stirring overnight at room temperature, TLC (5% acetone/CH_2Cl_2) indicated ~80% reaction. An additional 50 mL of the 0.5 *M* $KHCO_3$ solution was added and the mixture heated to reflux for 1 h. At the end of that time, TLC (10% acetone in CH_2Cl_2) indicated a complete reaction. The reaction mixture was cooled to room temperature and glacial acetic acid (3.2 mL, 55.7 mmol) was added. The methanol was removed in

vacuo and the suspended crude product in water was extracted with CH_2Cl_2 (3×). The organic fractions were washed with water (2×), dried (Na_2SO_4), filtered and concentrated in vacuo to give 23 g residue as an oil. Trituration of this material with ether gave 17 g **8** as a solid. Concentration of the mother liquors followed by Flash chromatography (10% acetone in CH_2Cl_2) and ether trituration gave an additional 3 g of **8**. Total amount of the 17α-hydroxy compound **8** obtained was 20 g (89%). m.p. = 208–211°C. ^{1}H NMR (300 MHz) δ 0.717 (t, J = 7.5 Hz, 13β-CH_2CH_3), 2.357 (s, C21-H_3), 5.681 (s, C4-H) ppm. IR (cm^{-1}) 3431, 2953, 1703, 1642, 1597. MS (*m/z*): M^+ = 328. Analysis calculated for $C_{21}H_{28}O_3$: C, 76.79; H, 8.59. Found: C, 76.83; H, 8.59.

*3,20-bis-Ethylenedioxy-13β-ethyl-17α-hydroxy-18,19-dinorpregna-4,9-diene (**9**)*

Under nitrogen, a mixture of the 17α-hydroxy compound **8** (18 g, 54.8 mmol), triethylorthoformate (23 mL, 138.1 mmol), ethylene glycol (16 mL, 286 mmol), and toluenesulfonic acid monohydrate (0.5 g, 2.6 mmol) in dry CH_2Cl_2 (300 mL) was stirred at room temperature overnight. The mixture was diluted with CH_2Cl_2 (~100 mL), washed with saturated sodium bicarbonate solution (1×), water (1×), and brine (1×). The organic fractions were combined, dried (Na_2SO_4), filtered and concentrated in vacuo to give 22 g residue as a brown solid. This material was crystallized from CH_2Cl_2/MeOH containing a trace of pyridine to give diketal **9** (19.2 g, 84%). m.p. = 110–113°C. ^{1}H NMR (300 MHz) δ 0.893 (t, J = 7.5 Hz, 13β-CH_2CH_3), 1.392 (s, C21-H_3), 3.986 (s over m, ethylenedioxy CH_2's), 5.581 (m, C11-H)ppm. IR (cm^{-1}) 3506, 2953, 2879. M/S (m/z): M^+ = 416. Analysis calculated for $C_{25}H_{26}O_5 \cdot 2/9\ CH_2Cl_2$: C, 69.57; H, 8.44. Found: C, 69.58; H, 8.30.

*3,20-bis-Ethylenedioxy-13β-ethyl-17α-hydroxy-5α,10α-epoxy-18,19-dinorpregn-9(11)-ene (**10**)*

Hydrogen peroxide (30%, 5.6 mL, 54.66 mmol) was added to a solution of hexafluoroacetone trihydrate (7.7 mL, 55.24 mmol) in CH_2Cl_2 (150 mL) cooled to 0°C in an ice bath. The mixture was stirred at 0°C for 30 min followed by the dropwise addition of diketal **9** (18 g, 43.2 mmol) in CH_2Cl_2 (150 mL) cooled to 0°C along with solid Na_2HPO_4 (8.6 g, 60.54 mmol). The mixture was stirred at 0°C for 3 h, then overnight at 4°C. The reaction was diluted with CH_2Cl_2 (~150 mL) and washed with 10% Na_2SO_3 solution (2×), and saturated sodium bicarbonate solution (1×). The organic fractions were combined, filtered through Na_2SO_4 and concentrated in vacuo to give 19 g residue as a foam. Analysis by TLC (2% acetone in CH_2Cl_2) indicated ~5–10% of starting material remained. Analysis by NMR indicated epoxide mixture to consist of a 65:35 mixture of the α- and β-isomers of the 5,10 epoxide. Trituration of this mixture with ether gave the pure solid 5α,10α-epoxide **10** (7.1 g, 38%). m.p. = 145–147°C. ^{1}H NMR (300 MHz) δ 0.911 (t, J = 7.5 Hz, 13β-CH_2CH_3), 1.377 (s, C21-H_3), 3.80–4.10 (m, ethylenedioxy CH_2's), 6.043 (m, C11-H) ppm. IR (cm^{-1}) 3506, 2953, 2879. MS (*m/z*): M^+ = 432. Analysis calculated for $C_{21}H_{36}O_6$: C, 69.42; H, 8.39. Found: C, 69.29; H, 8.49.

*3,20-bis-Ethylenedioxy-13β-ethyl-5α,17α-dihydroxy-11β-[4-(*N,N*-dimethylamino)phenyl]-18,19-dinorpregn-9-ene (**11a**)*

Under anhydrous conditions, magnesium (0.76 g, 31.3 mmol) was weighed into a 100 mL round bottom two-neck flask equipped with a reflux condenser, a magnetic stir bar and a rubber septum. A small crystal of I_2 was added and the system flushed with dry nitrogen. The system plus contents were flame dried under nitrogen. After cooling to room temperature, dry THF (25 mL) was added followed by dibromoethane (~0.1 mL) and the mixture was stirred at room temperature. After evidence of reaction was observed (bubbles), a solution of 4-bromo-*N*,*N*-dimethylaniline (5.72 g, 28.6 mmol) in dry THF (20 mL) was added via syringe. After stirring at room temperature for 5–10 min, the reaction was initiated and the reaction refluxed for about 20 min. The reaction mixture was stirred for an additional 1 h during which time it had cooled to room temperature. Solid copper (I) chloride (0.28 g, 2.8 mmol) was added with stirring followed 30 min later by a solution of epoxide **10** (2.2 g, 5.08 mmol) in dry THF (10 mL). The reaction was then stirred at room temperature for 1 h. Saturated ammonium chloride solution (~15 mL) was added dropwise to quench the reaction. In order to oxidize Cu(I) to Cu(II), air was drawn through the reaction mixture for 15 min via a 6 inch needle inserted through the rubber septum and a slight vacuum applied to the top of the reflux condenser. The reaction was then poured into water (~150 mL) and extracted with CH_2Cl_2 (3×). The organic fractions were washed with saturated NH_4Cl solution (2×), 2 *N* NH_4OH (2×), water (1×) and brine (1×), then combined, filtered through Na_2SO_4 and concentrated in vacuo to give 7 g blue-purple residue as an oil. Purification via Flash chromatography (5% acetone/CH_2Cl_2) followed by crystallization from ether gave **11a** (2.27 g, 78.8%). m.p. = 137–140°C (d). ^{1}H NMR (300 MHz) δ 0.317 (t, J = 7.5 Hz, 13β-CH_2CH_3), 1.370 (s, C21-H_3), 2.888 (s, NMe_2), 3.761–4.015 (m, ethylenedioxy CH_2's), 4.129–4.172 (m, C11α-H), 6.615 (d, J = 39.6 Hz, J_2 = 8.7 Hz, aromatic C3′-H and C5′-H), 7.044 (d, J = 8.7 Hz, aromatic C2′-H and C6′-H) ppm. IR (cm^{-1}) 3530, 3471, 2949, 2884. MS (*m/z*): M^+ = 553. Analysis calculated for $C_{33}H_{47}NO_6 \cdot 1/2\ H_2O$: C, 70.43; H, 8.60; N, 2.49. Found: C, 70.40; H, 8.48; N, 2.54.

*3,20-bis-Ethylenedioxy-13β-ethyl-5α,17α-dihydroxy-11β-[4-(methylthio)phenyl]-18,19-dinorpregn-9-ene (**11b**)*

Following the same procedure used in the preparation of **11a**, a solution of 4-bromothioanisole (9.78 g, 48.15 mmol) in dry THF (20 mL) was added to magnesium (1.17 g, 48.14 mmol), dibromoethane (~0.1 mL), and a small crystal of I_2 in dry THF (80 mL). After the reaction was initiated, the mixture was stirred at ambient temperature for 1 h. Solid copper (I) chloride (0.475 g, 4.8 mmol) was added with stirring, followed 30 min later by a solution of epoxide **10** (4.0 g, 9.6 mmol) in dry THF (20 mL). The reaction was then stirred at room temperature for 2 h. Analysis by TLC (10% acetone/CH_2Cl_2) of a small aliquot, quenched with saturated ammonium chloride and extracted with ethyl acetate, indicated a complete reaction at this time. Work up as previously described gave 9.7 g crude product as a reddish oil. Trituration of this material with pentane afforded compound **11b** (4.135 g, 77%) as a white solid. m.p. = 182–183°C. ^{1}H NMR (300 MHz) δ 0.280 (t, J = 7.5 Hz, 13β-CH_2CH_3), 1.356 (s, C21-H_3), 2.453 (s, SCH_3), 3.737–4.015 (m, ethylenedioxy CH_2's), 4.201 (d, J = 8.4 Hz, C11α-H), 7.122 (s, aromatic) ppm. IR (cm^{-1}) 3535, 3502, 2941. MS (*m/z*): M^+ = 556. Analysis calculated for $C_{32}H_{44}O_6S$: C, 69.03; H, 7.97; S, 5.76. Found: C, 68.99; H, 7.94; S, 5.68.

*3,20-bis-Ethylenedioxy-13β-ethyl-5α,17α-dihydroxy-11β-{4-[1,1-(ethylenedioxy)ethyl]phenyl}-18,19-dinorpregn-9-ene (**11c**)*

Following the same procedure used in the preparation of **11a**, a solution of 4-bromoacetophenone ethylene ketal[1] (8.5 g, 34.97 mmol) in dry THF (20 mL) was added to magnesium (0.85 g, 34.97 mmol), 1,1-dibromoethane and a small crystal of I_2 in dry

THF (40 mL). After the reaction had initiated, the mixture was stirred at ambient temperature for 2 h. Surprisingly, this Grignard reagent is only partially soluble in THF, giving a suspension of white solid upon complete formation. Solid copper (I) chloride (0.346 g, 3.49 mmol) was added followed 30 min later by a solution of epoxide **10** (3.0 g, 6.94 mmol) in dry THF (20 mL). The reaction was complete after 1 h at room temperature. Work up as previously described gave 9.5 g crude product mixture. Trituration of this material with ether afforded 2.5 g of the solid **11c**. Concentration of the filtrate in vacuo followed by Flash chromatography (Et_2O/CH_2Cl_2 1:1) gave an additional 0.7 g product. Total yield of **11c** was 3.2 g (77%). m.p. = 182–183°C. ^{1}H NMR (300 MHz) δ 0.151 (t, J = 7.5 Hz, 13β-CH_2CH_3), 1.353 (s, C21-H_3), 1.634 (s, CH_3 of 11β-substituent), 3.698–4.038 (m, ethylenedioxy CH_2's), 4.247 (d, J = 8.4 Hz, C11α-H), 7.175 (d, J = 8.4 Hz, aromatic C2′-H and C6′-H), 7.298 (d, J = 8.4 Hz, aromatic C3′-H and C5′-H) ppm. IR (cm^{-1}) 3501, 2945. MS (*m/z*): M^+ = 596. Analysis calculated for $C_{35}H_{48}O_8 \cdot 1/6\ H_2O$: C, 70.09; H, 8.12. Found: C, 70.19; H, 7.94.

*13β-Ethyl-11β-[4-(*N,N-*dimethylamino)phenyl]-17α-hydroxy-18,19-dinorpregna-4,9-diene-3,20-dione (**12a**)*

Nitrogen was bubbled through a mixture of ethanol (50 mL) and 8.5% sulfuric acid (5 mL) for 30 min to remove oxygen. The Grignard adduct **11a** (2.27 g, 4.1 mmol) was added as a solid and the mixture was refluxed for 1 h. After that time, TLC (10% acetone/CH_2Cl_2) indicated complete reaction. The mixture was cooled to room temperature, neutralized with saturated sodium carbonate solution, diluted with water (~100 mL) and extracted with CH_2Cl_2 (3×). The organic fractions were washed with saturated sodium bicarbonate solution (1×), water (1×) and brine (1×), then combined, filtered through sodium sulfate, and concentrated in vacuo to give 2 g residue as a foam. This material was purified by Flash chromatography (10% acetone/CH_2Cl_2) to give the 17α-ol **12a** (1.27 g, 69.2%) as a foam. ^{1}H NMR (90 MHz) δ–0.07 (t, J = 6.6 Hz, 13β-CH_2CH_3), 2.27 (s, C21-H_3), 2.89 (s, NMe_2), 4.35 (br. d, J = 7.2 Hz, C11α-H), 5.72 (s, C4-H), 6.60 (d, J = 9 Hz, aromatic C2′-H and C6′-H), 7.00 (d, J = 9 Hz, aromatic C3′-H and C5′-H) ppm. IR (cm^{-1}) 3469, 2944, 2883, 1706, 1658, 1649, 1612. MS (*m/z*): M^+ = 447.

*13β-Ethyl-11β-[4-(methylthio)phenyl]-17α-hydroxy-18,19-dinorpregna-4,9-diene-3,20-dione (**12b**)*

Following the same procedure used to prepare compound **12a**, Grignard adduct **11b** (3.79 g, 6.8 mmol) and 8.5% sulfuric acid (10 mL) were refluxed in ethanol (100 mL) for 45 min. After that time, TLC (5% acetone in CH_2Cl_2) indicated a complete reaction. The mixture was cooled to room temperature, diluted with water (~100 mL) and neutralized with concentrated NH_4OH solution. The reaction mixture was further diluted with water (~100 mL) and extracted with ether (3×). The organic fractions were washed with water (2×) and brine (1×), then combined, dried (Na_2SO_4), filtered and concentrated in vacuo to give 3.2 g residue as a yellow foam. This material was purified by Flash chromatography (5% acetone in CH_2Cl_2) followed by trituration with heptane to give the 17α-ol **12b** (1.95 g, 63.6%). m.p. = 115–120°C. ^{1}H NMR (90 MHz) δ–0.10 (t, J = 7.5 Hz, 13β-CH_2CH_3), 2.27 (s, C21-H_3), 2.43 (s, SMe), 4.37 (br. d, J = 7.5 Hz, C11α-H), 5.73 (s, C4-H), 7.13 (s, aromatic) ppm. IR (cm^{-1}) 3461, 2941, 1706, 1648, 1591. MS (*m/z*): M^+ = 450. Analysis calculated for $C_{28}H_{34}O_3S \cdot 1/4\ H_2O$: C, 73.89; H, 7.64; S, 7.04. Found: C, 73.94; H, 7.70; S, 6.88.

*13β-Ethyl-11β-(4-acetylphenyl)-17α-hydroxy-18,19-dinorpregna-4,9-diene-3,20-dione (**12c**)*

Following the same procedure used to prepare compound **12a**, Grignard adduct **11c** (3.2 g, 5.36 mmol) and 8.5% sulfuric acid (10 mL) were refluxed in ethanol (100 mL) for 1 h. After that time, TLC (10% acetone in CH_2Cl_2) indicated a complete reaction. The mixture was cooled to room temperature, diluted with water (~100 mL) and neutralized with concentrated NH_4OH solution. The reaction mixture was further diluted with water (~100 mL) and extracted with ether (3×). The organic fractions were washed with water (2×) and brine (1×), then combined, dried (Na_2SO_4), filtered and concentrated in vacuo to give 2.5 g residue as a foam. This material was purified by Flash chromatography (Et_2O/CH_2Cl_2 1:1) followed by trituration with ether to give the 17α-ol **12c** (2.06 g, 86%). m.p. = 151–154°C. ^{1}H NMR (300 MHz) δ–0.151 (t, J = 7.2 Hz, 13β-CH_2CH_3), 2.282 (s, C21-H_3), 2.562 (s, 4′-acetyl CH_3), 4.449 (d, J = 8.1 Hz, C11α-H), 5.755 (s, C4-H), 7.293 (d, J = 7.8 Hz, aromatic C2′-H and C6′-H), 7.853 (d, J = 7.8 Hz, aromatic C3′-H and C5′-H)ppm. IR (cm^{-1}) 3335, 2950, 1707, 1680, 1653, 1602. MS (*m/z*): M^+ = 446. Analysis calculated for $C_{29}H_{34}O_4 \cdot 2/3\ H_2O$: C, 75.95; H, 7.77. Found: C, 76.01; H, 7.78.

*17α-Acetoxy-13β-ethyl-11β-[4-(*N,N-*dimethylamino)phenyl]-18,19-dinorpregna-4,9-diene-3,20-dione (**13a**)*

Trifluoroacetic anhydride (8 mL, 56.6 mmol), glacial acetic acid (3.3 mL, 57 mmol) and dry CH_2Cl_2 (50 mL) were combined and stirred under nitrogen at room temperature for 30 min. Solid *p*-toluenesulfonic acid monohydrate (0.5 g, 2.6 mmol) was added and the mixture was cooled to 0°C in an ice bath. A solution of the 17α-alcohol **12a** (1.25 g, 2.8 mmol) in dry CH_2Cl_2 (10 mL) cooled to 0°C was added and the mixture stirred at 0°C for 1 h. At the end of that time, TLC (10% acetone in CH_2Cl_2) indicated complete reaction. The reaction was carefully quenched with saturated potassium carbonate solution (40 mL, ~200 mmol), diluted with water (100 mL) and extracted with CH_2Cl_2 (3×). The organic fractions were washed with water (2×) and brine (1×), then combined, filtered through Na_2SO_4 and concentrated in vacuo to give 1.5 g residue as a foam. Purification by Flash chromatography (5% acetone in CH_2Cl_2) followed by crystallization from CH_2Cl_2 gave compound **13a** (0.8 g, 57.6%). m.p. = 233–236°C. $[\alpha]_D^{26}$ = +210.73° (c = 1.03, $CHCl_3$). ^{1}H NMR (300 MHz) δ–0.057 (t, J = 7.2 Hz, 13β-CH_2CH_3), 2.116 (s, C21-H_3 or OAc), 2.128 (s, C21-H_3 or OAc), 2.894 (s, NMe_2), 4.367 (d, J = 8.7 Hz, C11α-H), 5.736 (s, C4-H), 6.608 (d, J = 8.7 Hz, aromatic C2′-H and C6′-H), 6.998 (d, J = 8.4 Hz, aromatic C3′-H and C4′-H)ppm. IR (cm^{-1}) 2943, 2878, 1730, 1707, 1654, 1610. MS (*m/z*): M^+ = 489. Analysis calculated for $C_{31}H_{39}NO_4 \cdot 1/4\ H_2O$: C, 75.35; H, 8.06; N, 2.83. Found: C, 75.22; H, 8.00; N, 2.84. Analysis by HPLC ($H_2O/CH_3CN/Et_3N$ 30:70:0.033; Waters Associates NovaPak C_{18}, 1 mL/min, λ = 260 nm) indicated the product to be >99% pure with a retention time of 7.8 min.

*17α-Acetoxy-13β-ethyl-11β-[4-(methylthio)phenyl]-18,19-dinorpregna-4,9-diene-3,20-dione (**13b**)*

Following the same procedure used to prepare compound **13a**, the 17α-alcohol **12b** (1.7 g, 3.77 mmol) in dry CH_2Cl_2 (15 mL) was reacted with the mixed anhydride prepared from trifluoroacetic anhydride (15 mL, 106.4 mmol), glacial acetic acid (6.1 mL, 106.7 mmol) and *p*-toluenesulfonic acid monohydrate (1.0 g, 5.25 mmol) in dry CH_2Cl_2 (75 mL) at 0°C. After stirring at 0°C for one hour, TLC (10% acetone in CH_2Cl_2) indicated a complete reaction. The

reaction was quenched at 0°C with concentrated NH_4OH solution (~22 mL, 326 mmol), diluted with water (100 mL) and extracted with CH_2Cl_2 (3×). The organic fractions were washed with water (2×) and brine (1×), then combined, filtered through Na_2SO_4 and concentrated in vacuo to give 2.05 g residue as a yellow solid. Purification via Flash chromatography (4% acetone in CH_2Cl_2) followed by crystallization from ethyl acetate gave the acetate **13b** (1.13 g, 61%). m.p. = 270–275°C (d). $[\alpha]_D^{26} = +213.9°$ (c = 1.01, $CHCl_3$). ^1H NMR (300 MHz) δ–0.103 (t, J = 7.2 Hz, 13β-CH_2CH_3), 2.118 (s, C21-H_3 or OAc), 2.131 (s, C21-H_3 or OAc), 2.444 (s, SMe), 4.406 (d, J = 8.1 Hz, C11α-H), 5.757 (s, C4-H), 7.087 and 7.142 (each d, J = 8.7 Hz, aromatic) ppm. IR (cm^{-1}) 2948, 1728, 1712, 1659, 1595. M/S (m/z): M^+ = 492. Analysis calculated for $C_{30}H_{36}O_4S \cdot 1/8\ H_2O$: C, 72.80; H, 7.38; S, 6.48. Found: C, 72.84; H, 7.41; S, 6.47. Analysis by HPLC (H_2O/CH_3CN 3:7, Phenomenex Prodigy 5 ODS-2; 1 mL/min; λ = 302 nm) indicated the product to be >99% pure with a retention time of 7.2 min.

*17α-Acetoxy-13β-ethyl-11β-(4-acetylphenyl)-18,19-dinorpregna-4,9-diene-3,20-dione (**13c**)*

Following the same procedure used to prepare compound **13a**, the 17α-alcohol **12c** (1.6 g, 3.58 mmol) in dry CH_2Cl_2 (10 mL) was reacted with the mixed anhydride prepared from trifluoroacetic anhydride (10 mL, 70.94 mmol), glacial acetic acid (4.1 mL, 71.7 mmol) and toluenesulfonic acid monohydrate (0.68 g, 3.6 mmol) in dry CH_2Cl_2 (50 mL) at 0°C. After stirring at 0°C for 45 min, TLC (10% acetone in CH_2Cl_2) indicated a complete reaction. The reaction was quenched at 0°C with concentrated NH_4OH solution (~15 mL, 222 mmol), diluted with water (100 mL) and extracted with CH_2Cl_2 (3×). The organic fractions were washed with water (2×) and brine (1×), then combined, filtered through Na_2SO_4 and concentrated in vacuo to give 1.6 g residue as a yellow solid. Crystallization of this material from CH_2Cl_2/Et_2O gave the acetate **13c** (1.2 g, 68.5%) as a white solid. m.p. = 268–270°C (d). $[\alpha]_D^{26} = +184.4°$ (c = 1.03, $CHCl_3$). ^1H NMR (300 MHz) δ–0.164 (t, J = 7.5 Hz, 13β-CH_2CH_3), 2.122 (s, C21-H_3 or OAc), 2.138 (s, C21-H_3 or OAc), 2.570 (s, 4′-acetyl), 4.500 (d, J = 8.7 Hz, C11α-H), 5.779 (s, 1 H, C4-H), 7.293 (d, J = 8.4 Hz, aromatic C2′-H and C6′-H), 7.863 (d, J = 8.4 Hz, aromatic C3′-H and C5′-H) ppm. IR (cm^{-1}) 2951, 1729, 1712, 1683, 1660, 1596. MS (*m/z*): M^+ = 488. Analysis calculated for $C_{31}H_{36}O_5$: C, 76.20; H, 7.43. Found: C, 76.14; H, 7.33. Analysis by HPLC (H_2O/CH_3CN 3:7, Phenomenex Prodigy 5 ODS-2; 1 mL/min; λ = 302 nm) indicated the product to be >99% pure with a retention time of 4.1 min.

Biological assays

Progestational assay.[9,10,11] Immature New Zealand White rabbits approximately 1 kg in body weight were maintained under standard conditions of housing and allowed access to food and water ad lib. The animals were randomized to groups of six rabbits each and received daily subcutaneous injections of 5.0 μg estradiol benzoate in sesame oil for six consecutive days. On the seventh day, animals received the standard or test material dissolved in sesame oil by subcutaneous or intraluminal injection. Subcutaneous injections were administered daily for five consecutive days. Intraluminal injections of test compounds dissolved in sesame oil were made into a 3–4 cm segment of one uterine horn and vehicle only in the other, following Ketamine–Xylazine, 1:0.2 by weight, (Ketasit Pitman Moore; Rompun Mobay Corp.) anesthesia. The segment was ligated to prevent migration of test material or vehicle. Animals were sacrificed 24 h after the last subcutaneous injection and 3 days after intraluminal administration and the uterine horns were excised, cleaned of fat and connective tissue, blotted on moist filter paper to express luminal fluid and weighed to the nearest 0.1 mg. Midsections of each uterine horn (central to the ligatures for intraluminal administration) were removed, fixed in Bouin's fluid, processed for sectioning at 5 microns and stained with hematoxylin and eosin. Slides were read in a blinded fashion and the degree of glandular proliferation was graded. Three dose levels of unknown and standard were usually tested. Means and standard error of the means were calculated and plotted on semilog paper. Curve fitting, potency ratios and statistics were undertaken using PROPHET.[11–14]

Antiprogestational assay. Antiprogestational activity was measured as the percent inhibition of the response to a standard stimulating dose (0.8 mg total dose) of progesterone when both substances were administered concomitantly using the protocol described for progestational activity. Where compounds were administered intraluminally, progesterone was injected subcutaneously for three days following estrogen priming. Treatment of data was similar to that described above except that they are reported in terms of percent inhibition.

Relative binding affinities for the progesterone and glucocorticoid receptors

Uteri and Thymus glands were obtained from estradiol-primed immature female rabbits of the New Zealand White strain. Tissues were excised and immediately placed in ice cold TEGDM buffer (10 mM Tris, pH 7.4; 1.5 mM EDTA; 10% glycerol vol/vol; 1 mM dithiothreitol [DTT]; and 20 mM sodium molybdate). The tissues were dissected free of connective tissue and fat, weighed, and minced finely. Minced tissues were homogenized in three volumes TEGDM/gm with four 10 s bursts of a VirTis Cyclone set at half maximum speed with a 30 s cooling period (in ice) between bursts. Homogenates were centrifuged at 109,663 ×g at 4°C for 1 h to yield the soluble cytosol fraction. Aliquots of cytosol were snap frozen and stored at −75°C. All binding assays were carried out at 2–4°C for 16–18 h. The following radioactive ligands were used: [1,2-^{3}H(*N*)]-progesterone (50.0 Ci/mmol) for the progesterone receptor (PR) and [6,7-^{3}H(*N*)]-dexamethasone (39.2 Ci/mmol) for the glucocorticoid receptor (GR). For the progesterone receptor RBA assays 0.02 mL uterine cytosol or TEGDM buffer, 0.05 mL of various concentrations of test compounds or progesterone, 0.13 mL TEGDM buffer and 0.05 mL [^{3}H]-progesterone were added to duplicate tubes. For the glucocorticoid receptor RBA assays, 0.1 mL thymus cytosol or TEGDM buffer, 0.05 mL of various concentrations of test compounds or dexamethasone, 0.05 mL TEGDM buffer and 0.05 mL [^{3}H]-dexamethasone were added to duplicate tubes. The concentrations of the test compounds, progesterone and dexamethasone ranged from 0.5 to 500 nM. Total binding was measured at radioactive ligand concentrations of 3.5 nM and nonspecific binding was measured in the presence of a 200-fold molar excess of unlabeled progesterone (PR) or dexamethasone (GR), respectively.

In all incubations bound and free ligand concentrations were separated using dextran-coated charcoal (DCC). A 0.1 mL aliquot of DCC (0.5% charcoal/0.05% Dextran T-70) was added to each tube. The tubes were vortexed and incubated on ice for 10 min. Five tenths mL TEG buffer (without DTT or molybdate) was then added to all tubes to improve supernatant recovery following centrifugation. The charcoal was pelleted by centrifugation at 2,100 ×g for 15 min at 4°C. The supernatants containing the [^{3}H]-steroid receptor complexes were decanted into vials containing 4 mL Optifluor (Packard Instrument Co.), vortexed, equilibrated in a liquid scintillation counter for 30 min and then counted for 2 min. This provided the quantity of receptor bound [^{3}H]-steroid at each competitor concentration.

Table 1 Relative Binding Affinities and Biological Potency in the Rabbit

Compound	Relative binding affinity		Progestational activity[c]	Antiprogestational activity[d]
	PR[a]	GR[b]		
1	263	8.5	100	—
CDB-2914	114	127	—	100
13a	117	175	Inactive	Inactive
13b	98	27	**12–21**	Inactive
13c	159	18	Inactive	Inactive

[a] Progesterone = 100%; immature estrogen-primed rabbit uterus.
[b] Dexamethasone = 100%; immature estrogen-primed rabbit thymus.
[c] Progestational activity = Clauberg: Oral administration to estrogen-primed immature rabbits; (−)-norgestrel (**1**) = 100 (assigned).
[d] Antiprogestational activity = Anti-Clauberg: Oral administration to estrogen-primed immature rabbits; CDB-2914 = 100 (assigned).

The EC_{50} (Effective Concentration) for each standard curve and each of the compound curves was determined by entering the counting data (receptor bound [^{3}H]-progesterone or [^{3}H]-dexamethasone) into a four parameter sigmoidal computer program (RiaSmart™ Immunoassay Data Reduction Program, Packard Instrument Co., Meridan, CT). The RBA for each test compound was calculated using the following equation:

$$\text{RBA} = \frac{EC_{50}\ \text{Standard}}{EC_{50}\ \text{Test Compound}} \times 100$$

where EC_{50} Standard = molar concentration of unlabeled progesterone or dexamethasone required to decrease bound [^{3}H]-progesterone (PR) or [^{3}H]-dexamethasone (GR) to 50% of the respective buffer control (100% bound ligand) and EC_{50} Test Compound = molar concentration of test compound required to decrease bound [^{3}H]-progesterone (PR) or [^{3}H]-dexamethasone (GR) to 50% of the respective buffer control (100% bound ligand).

Results and discussion

Chemistry

Ketalization of levonorgestrel **1** (Figure 2) gave a quantitative yield of the 3-ketal mixture **2**, indicated by NMR and HPLC to consist of a 65:35 ratio of the $\Delta^{5(10)}$ and the $\Delta^{5(6)}$ isomers. Mild acidic hydrolysis of mixture **2** followed by chromatographic separation of mixture **3** gave the $\Delta^{5(10)}$-ketone **4** in 60% yield along with a 40% recovery of levonorgestrel **1**. Following the procedure of Perelman et al,[16] bromination-dehydrobromination of compound **4** using pyridinium bromide perbromide in pyridine afforded the dienone **5** in 67% yield. Conversion of **5** to the 17β-nitrate ester **6** followed by the mercuric acetate catalyzed isomerization and hydrolysis procedure of Hofmeister et al[17] gave the formate ester **7** in 29% yield from compound **5**. All of the examples described by Hofmeister et al[17] in their patent consisted of Δ^4-3-keto, $\Delta^{1,4}$-3-keto, or $\Delta^{4,6}$-3-keto steroids and the overall yields ranged from 19−65%. In our experiments with the $\Delta^{4,9}$-3-keto **5**, the desired nitro derivative **6** was accompanied with other by-products, resulting in a lower yield. Mild basic hydrolysis of the formate ester **7** gave the 17α-hydroxy compound **8** in 89% yield. For the introduction of the 11β-aryl substituents, the general strategy developed by Teutsch et al[18] was adopted. Ketalization of diene-dione **8** proceeded with a double bond shift to form the 5(10), 9(11)-diene **9** in 84% yield. The regioselective 5,10-epoxidation of diene **9** was carried out using hexafluoroacetone hydroperoxide generated in situ from hexafluoroacetone trihydrate and hydrogen peroxide following the general procedure of Teutsch et al.[19] This technique was modified by the addition of solid Na_2HPO_4 as buffer and increasing the number of equivalents of hexafluoroacetone trihydrate to 1.5. Application of this method to diene **9** gave an epoxide mixture from which the pure 5α,10α-epoxide **10** could be isolated in 38% yield. The copper (I) catalyzed addition of the Grignard reagent prepared from the appropriate aryl bromide (4-bromodimethylaniline, 4-bromothioanisole, and 4-bromoacetophenone ketal[7]) gave the cor-

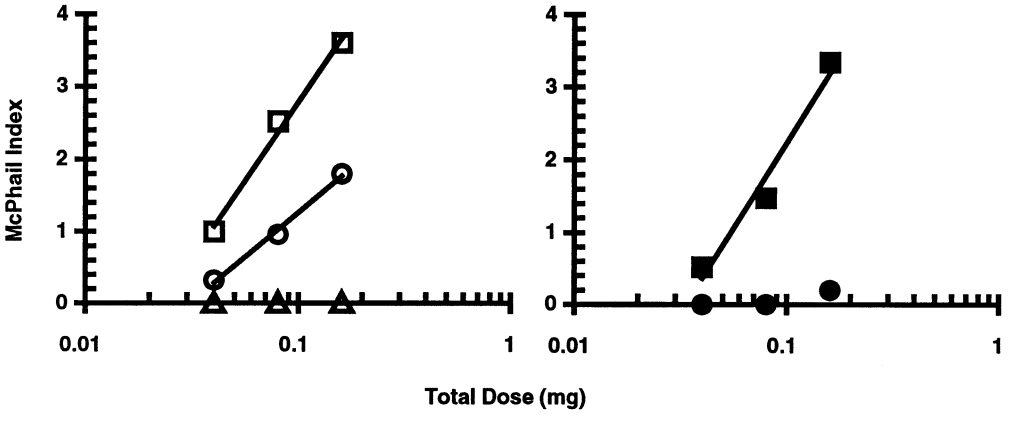


Figure 3. Oral progestational activity (Method of McPhail). Legend: ○ (compound *13b*); △ (compound *13c*); □ (compound *1*); ● (compound *13a*); ■ (compound *1*). Straight regression lines were computer fitted.

responding Grignard adducts **11a–c** in 77–79% yields. Acid catalyzed hydrolysis and dehydration of compounds **11a–c** gave the analogous 17α-hydroxy-diene-diones **12a–c** in yields ranging from 66 to 86%. Acetylation of compounds **12a–c** was carried out in 58–69% yields following the procedure of Carruthers and Garshasb[20] to give the final 17α-acetoxy products **13a–c**.

Biology

Results of the in vitro relative binding affinities for progesterone and glucocorticoid receptors and in vivo Clauberg and anti-Clauberg assays are shown in Table 1. Their relative binding affinities for the progesterone receptor increased with the exception of **13b**, and their relative binding affinities for the glucocorticoid receptor decreased with the exception of **13a**. Oral administration of CDB-2914 and levonorgestrel (**1**) were used as reference standards for the anti-Clauberg and the Clauberg assay respectively. Surprisingly, none of the 13β-ethyl analogs displayed antiprogestational activity in the rabbit. On the contrary, one 13β-ethyl analog (**13b**) was found to exhibit agonistic activity in the Clauberg assay. The 13β-ethyl-11β-(4-methylthiophenyl)-analog (**13b**) has 12 to 21% progestational activity relative to progesterone as shown in Table 1 and Figure 3. The analogs **13a** and **13c** were devoid of oral progestational activity (Method of McPhail, Figure 3). This is in contrast with the marked antiprogestational activity reported for most 11β-aryl-19-norpregna-4,9-diene series[3,6,21] and with the abortive activity of RU43855, which is the 13β-ethyl analog of mifepristone previously reported by Teutsch and Philibert.[6] The latter has approximately 30% of the abortive activity of mifepristone.[6] No explanation for these results can be given at the present time. However, the evidence presented here suggests that the methyl portion of the angular 13β-ethyl group appears to play a role in the reversal of antiprogestational activity of **13b** and the loss of activity of **13a** and **13c** in the rabbit. From the results shown in Table 1, it appears that agonistic and antagonistic activities are independent of relative binding affinity. A similar observation has been previously reported by Teutsch.[22] Earlier strategies[23] for the design of antiprogestins centered around mifepristone analogs targeted towards a high and specific binding to the progesterone receptor. This strategy has become of limited value.

Currently, computer-aided drug design involving molecular similarity methods[24] and CoMFA (Comparative Molecular Field Analysis) technique[25,26] using RBAs to progesterone and glucocorticoid receptors have already been applied and resulted in the synthesis of more potent antiprogestational agents than CDB-2914 with a decrease in antiglucocorticoid activity. The results of these studies will be reported in due course.

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