Pergamon

Bioorganic & Medicinal Chemistry 10 (2002) 929–934

BIOORGANIC & MEDICINAL CHEMISTRY

Novel Steroidal Vinyl Fluorides as Inhibitors of Steroid $C_{17(20)}$ Lyase

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Received 5 September 2001; accepted 25 September 2001

Abstract—20-Fluoro-17(20)-pregnenolone derivatives were designed as enol mimics of pregnenolone. All of the targeted, novel fluoroolefins were potent inhibitors of $C_{17(20)}$ lyase.

Introduction

Steroid $C_{17\alpha}$ hydroxylase/$C_{17(20)}$ lyase (EC1.14.99.9/EC 4.1.2.30), the enzyme responsible for converting progesterone and pregnenolone to androstenedione and dehydroepiandrosterone, respectively, is found in the testes, ovaries, adrenals, and placenta.[1] Two distinct steps comprise this conversion, for example, an initial 17α-hydroxylation and a subsequent cleavage of the $C_{17(20)}$ bond,[2,3] to give the C17-keto androgens. Inhibition of $C_{17(20)}$ lyase would, therefore, lead to the cessation of androgen production and also, indirectly, shut down estrogen production, since estrogens arise from androgens via the action of the enzyme aromatase. Mechanistic work on this intriguing enzyme has been performed by several investigators.[4–7] A mechanism proposed by Corina et al.[6] is shown in Scheme 1. A molecular model for this enzyme has been developed,[8–10] based on homology with $P450_{cam}$. This model is comprised of two orthogonal lobes, for example, a $C_{17\alpha}$ hydroxylase lobe and a $C_{17(20)}$ lyase lobe, which share a common heme at the connecting elbow. The human enzyme has been expressed and purified in bacteria.[11] The inhibition of lyase is a potential therapeutic approach for the treatment of prostate cancer and hormone-dependent breast cancer,[12–15] since androgens and oestrogens, respectively, are required for the growth of these tumors.

A variety of $C_{17(20)}$ lyase inhibitors of different mechanistic types and structural classes have been described.[16] These categories include steroidal mechanism-based inhibitors,[17–19] steroidal C_{17}-heterocyclic inhibitors,[20–26] nonsteroidal heterocyclic inhibitors,[27–38] and other inhibitors which do not precisely fall into these categories.[39–44] Fluoroolefins represented by structures **8** and **9** (Scheme 2) were proposed as (*Z*) and (*E*) enol mimics, respectively, of pregneolone. Vinyl halides have previously been designed to mimic an enolized ketone intermediate.[45] This report describes the synthesis of these and related vinyl fluorides and $C_{17(20)}$ lyase inhibition data which was generated for these de novo designed inhibitors.

Chemistry

Synthesis of the targeted vinyl fluorides are detailed in Schemes 3 and 4. Dehydroepiandrosterone (**6**) was silylated with *tert*-butyldimethylsilyl chloride to give the protected alcohol **10** in 88% yield. Treatment of **10** with triethyl 2-fluorophosphonoacetate gave a mixture of *E*- and *Z*-isomers **11**, which were inseparable by chromatography. Reduction of this mixture with diisobutylaluminum hydride gave a new mixture of primary alcohols which was separable, by virtue of the primary alcohols which rendered the geometric isomers more polar and allowed them to be differentiated on silica gel. The major material was *E*-isomer **12**, which was isolated in

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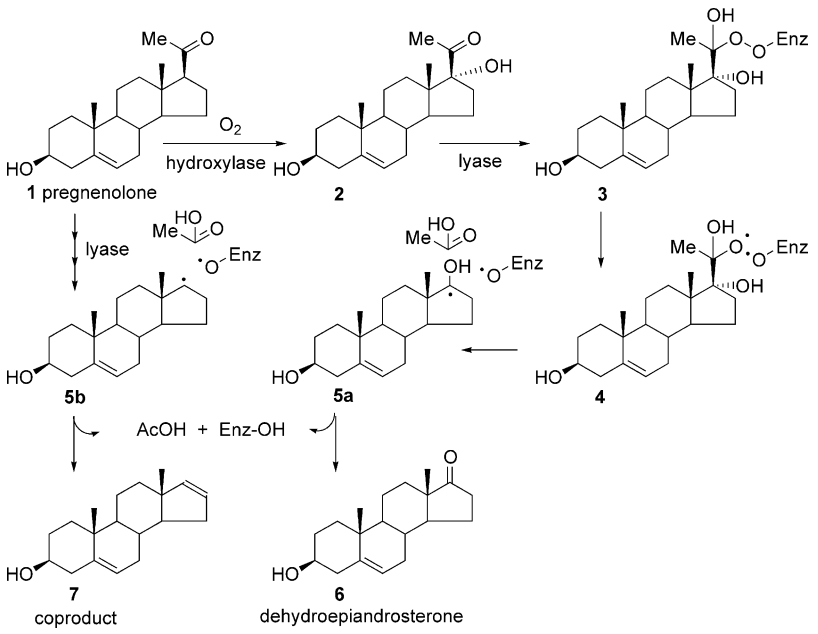
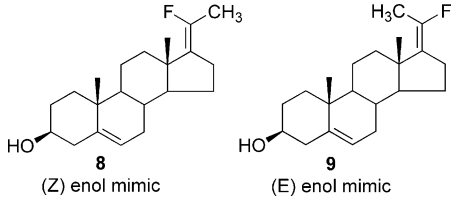
PII: S0968-0896(01)00354-6



Scheme 1. Proposed mechanism for $C_{17(20)}$ lyase.



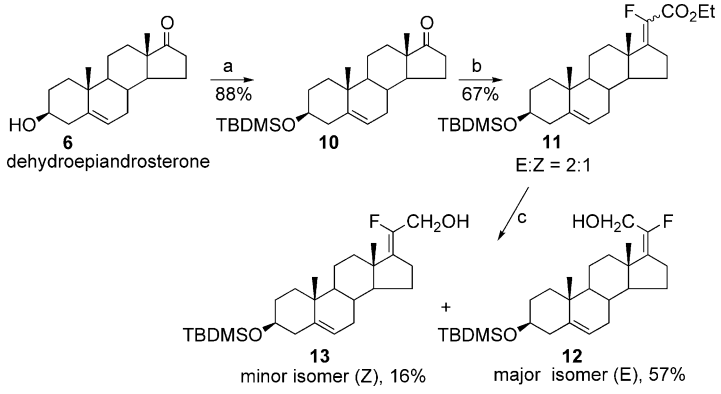
Scheme 2. Vinyl fluoride $C_{17(20)}$ pregnenolone enol mimics.

57% yield. The minor *Z*-isomer **13**, was isolated in 16% yield. Unequivocal assignment of these regioisomers was determined using high-resolution NMR techniques, for example, DQF-COSY and HMQC for resonance assignments, and NOESY and heteronuclear ^{1}H–^{19}F NOE difference experiments[46] for determination of fluoro olefin geometry.

The major isomer **12** from the Horner–Emmons vinyl homologation reaction was then manipulated as shown in Scheme 4 to produce two additional *E*-vinyl fluorides. Treatment of **12** with tetrabutylammonium fluoride removed the silyl protecting group to give diol **14** in 95% yield. Hydrogenolysis of the allylic alcohol gave fluoroolefin **15** in 76% yield; removal of the silyl protecting group, again with tetrabutylammonium fluoride, gave fluoroolefin **9** (*E*-isomer) in 94% yield. In similar fashion were prepared the fluoroolefins (*Z*-isomers) **16** and **8**, also shown in Scheme 4. Compounds **8**, **9**, **14**, and **16**, which were the designed enol mimics of pregnenolone, are novel compounds.

Enzymology

Percent inhibition values for fluoroolefins **14**, **9**, **16**, and **8** were evaluated against cynomolgous monkey testicular $C_{17(20)}$ lyase at given concentrations, with and without preincubation of enzyme with inhibitor, are presented in Table 1. Methods used for determination of the IC_{50} values in Table 1 are detailed in the Experimental.



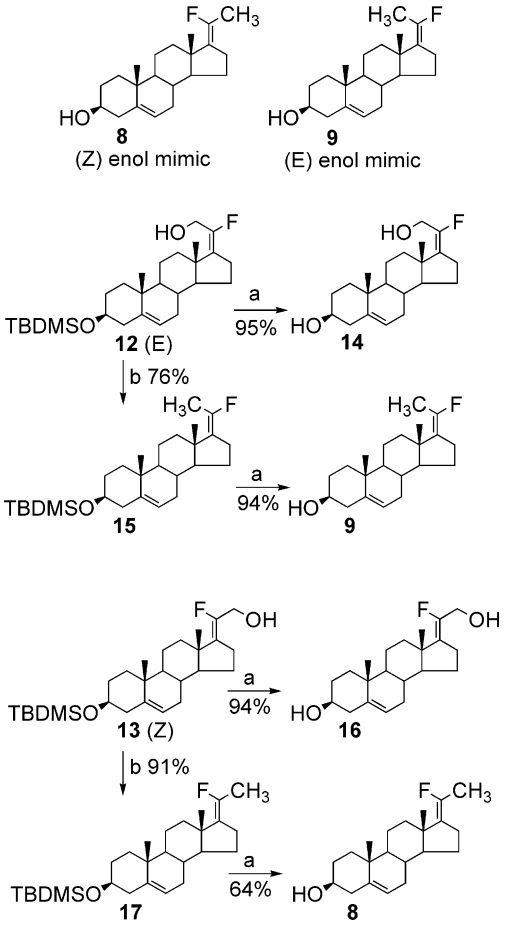
Scheme 3. Reagents: (a) TBDMS-Cl, DMAP, TEA, DMF; (b) $(EtO)_2P(O)CHFCO_2Et$, LHMDS, THF; (c) DIBAL-H, CH_2Cl_2.

Results and Discussion

From Table 1, it is evident that the 20-fluoro-17(20)-ene pregnenolones **14**, **9**, **16**, and **8** are potent inhibitors of cynomolgous monkey testicular $C_{17(20)}$ lyase. From a comparison of the % inhibition values for the compounds with and without preincubation, it appears that neither of the *E*-isomers (**14** and **9**) is time-dependent. Allyl alcohol **16** is the most potent inhibitor, and it is not clear whether this *Z*-isomer is displaying time-dependency from the data which was generated, since all values at both concentrations used are close to 100% inhibition. However, fluoroolefin **8**, also a *Z*-isomer but less potent than **16**, is clearly displaying time-dependence.

Conclusion

In summary, we have designed novel 20-fluoro-17(20)-ene pregnenolones which are potent inhibitors of cynomolgous monkey testicular $C_{17(20)}$ lyase. These fluoroolefins were designed as mimics of the enol tautomers of pregnenolone (**13**). Compound **8** was clearly time-dependent, and additional compounds in this set may also have a time-dependent component. Although it is interesting to speculate on possible reasons for this time-dependent behavior, the limited data which we have collected to date is insufficient to support a hypothesis. However, it is clear that this study supports the concept of designing enol mimics of ketone substrates as enzyme inhibitors.



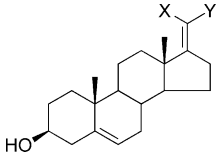
Scheme 4. Reagents: (a) TBAF, THF; (b) SO_3·pyridine complex, $LiAlH_4$, THF.

Experimental

General

Melting points were determined with a Thomas-Hoover capillary melting apparatus and are uncorrected. TLC analyses were performed with Merck DC-F254 silica gel plates, with visualization by alkaline permanganate and UV light. Flash chromatography was performed with Merck silica gel 60 (0.040–0.063 μm). NMR spectra were recorded on Unity 400 or Gemini-300 spectrometers in $CDCl_3$, unless otherwise stated. ^1H and ^{13}C NMR signals are reported in ppm from tetramethylsilane (s, d, t, m and br for singlet, doublet, triplet, multiplet and broad, respectively) and coupling constants are reported in hertz (Hz). Infrared spectra were recorded on a Perkin-Elmer Model 1800 or Mattson Galaxy 5020 FT-IR spectrophotometer. Mass spectral data were collected at 70 eV on a Finnigan MAT 4600, MAT TSQ-700 or VG Analytical Ltd ZAB2-SE mass spectrophometer and computerized for HRMS. Combustion analyses performed using a Perkin-Elmer Model 2400 elemental analyzer fell within ±0.4% of the calculated values. The organic extracts were dried over magnesium sulfate or sodium sulfate prior to evaporation in vacuum on a rotary evaporator. Anhydrous solvents and starting materials were purchased from Aldrich Chemical Company and used as obtained with the following exception: dehydroepiandrosterone was purchased from Proquina.

Table 1. Inhibition of cynomolgus monkey testicular $C_{17(20)}$ lyase with novel 20-fluoro-17,20-ene pregnenolone analogues



Compd	Isomer	X	Y	Concn (μM)	Preincubation (min)	Inhibition (%)
16	Z	F	CH_2OH	10	0	100
				1	0	96
				10	40	100
				1	40	94
14	E	CH_2OH	F	10	0	85
				1	0	63
				10	40	87
				1	40	61
8	Z	F	CH_3	10	0	78
				1	0	49
				10	40	94
				1	40	72
9	E	CH_3	F	10	0	88
				1	0	54
				10	40	94
				1	40	60

(3β)-3-[(1,1-Dimethylethyl)dimethylsilyl]oxy]androst-5-en-17-on-3-ol (10). To a stirred solution of dehydroepiandrosterone (**7**, 20.0 g, 69.3 mmol) in anhydrous DMF (350 mL) under nitrogen was added *t*-butyldimethylsilyl chloride (11.0 g, 72.8 mmol), 4-dimethylaminopyridine (0.42 g, 3.47 mmol) and triethylamine (10.6 mL, 76.3 mmol). The resultant suspension was stirred at room temperature for 3 days and then poured into rapidly stirred water (1.5 L). The resultant suspension was filtered and the white solid crystallized from aqueous acetone to give 10 (24.6 g, 88%) as a white crystalline solid: mp 146–148 °C. TLC R_f 0.78, ethyl acetate/hexane (1:1); ^{1}H NMR δ 5.38–5.32 (m, 1H, vinyl), 3.55–3.43 (m, 1H, OCH), 1.03 (s, 3H, Me), 0.89 [s, 9H, C(Me)$_3$], 0.88 (s, 3H, Me), 0.06 (s, 6H, 2×SiMe); MS (CI, CH_4) *m*/*z* (rel intensity) 403 (MH^+, 3), 401 (5), 387 (9), 345 (18), 271 (100). Anal. calcd for $C_{25}H_{42}SiO_2$: C, 74.57;H, 10.51. Found: C, 74.89; H, 10.84.

(3β)-[(1,1-Dimethylethyl)dimethylsilyl]oxy]-20-fluoropregna-5,17(20)-dien-21-oic acid, ethyl ester (13). To a stirred solution of triethyl 2-fluoro-2-phosphonoacetate (0.76 mL, 3.75 mmol) in THF (15 mL) under nitrogen was added lithium hexamethyldisilazane (3.50 mL of a 1.0 M solution in THF, 3.50 mM). After 1 h, a solution of **10** (1.01 g, 2.50 mL) in THF (5 mL) was added and the reaction mixture was heated to reflux. After 2.5 h, the reaction mixture was allowed to cool to room temperature and concentrated. The residue was partitioned between Et_2O (40 mL) and 0.4 M aqueous hydrochloric acid (40 mL). The layers were separated and the organic layer was washed with 0.5 M aqueous hydrochloric acid (20 mL), saturated aqueous sodium bicarbonate (20 mL), and brine (20 mL). Drying, filtration, and concentration gave crude 11. Flash chromatography (6×17 cm column) eluting with ethyl acetate/hexane (2:98) gave 11 (mixture of *E* and *Z* isomers, 0.83 g, 67%) as a white solid. TLC R_f 0.41 and 0.51, ethyl acetate/hexane (3:97); ^{19}F NMR δ −121.59 (s, *E* isomer) and −135.49 (s, *Z* isomer); MS (CI, CH_4) *m*/*z* (rel intensity) 491 (MH^+, 97), 475 (59), 445 (24), 433 (65), 359 (100), 339 (24). Anal. calcd for $C_{29}H_{47}FO_3Si$: C, 70.97: H, 9.65. Found: C, 71.32; H, 10.02.

(3β,17*E*)-3-[(1,1-Dimethylethyl)dimethylsilyl]oxy]-20-fluoropregna-5,17(20)-diene-3,21-diol (20) and (3β,17*Z*)-3-[(1,1-dimethylethyl)dimethylsilyl]oxy]-20-fluoropregna-5,17(20)-diene-3,21-diol (13). To a stirred solution of **11** (7.29 g, 14.9 mmol) in dichloromethane (135 mL) under nitrogen and cooled to −78 °C was slowly added diisobutylaluminum hydride (6.55 mL of a 1.0 M solution in dichloromethane, 65.5 mmol). After 1 h, the reaction was quenched with a solution of glacial acetic acid (3.8 mL) in dichloromethane (9 mL) and the reaction mixture was poured into dichloromethane (250 mL)/saturated aqueous potassium sodium tartrate (250 mL). The resultant emulsion was filtered through a Celite pad (3 cm), the filtrates transferred to a separatory funnel, and the layers separated. The organic layer was washed with saturated aqueous potassium sodium tartrate (130 mL), saturated aqueous sodium bicarbonate (250 mL), and brine (100 mL). Drying, filtration, and concentration gave crude product. Flash chromatography (two equal batches, 8×20 cm column) eluting with a gradient (10–15%) of ethyl acetate in hexane gave 12 (3.80 g, 57%) as a white solid: mp 144–147 °C. TLC R_f 0.37, ethyl acetate/ hexane (15:85); ^{1}H NMR (400 MHz) δ 5.34–5.29 (m, 1H, vinyl), 4.29 (ddd, 1H, *J* = 21.4, 13.4, 6.4 Hz, 0.5 CH_2O), 4.20 (ddd, 1H, *J* = 21.4, 13.4, 6.4 Hz, 0.5 CH_2O), 3.53–3.44 (m, 1H, OCH), 1.01 (s, 3H, 19-Me), 0.91 (d, 3H, *J* = 1.1 Hz, 18-Me), 0.89 (s, 9H, Si-*t*-Bu), 0.054 (s, 6H, 2×SiMe); ^{13}C NMR δ 150.8 (d, *J* = 241.3 Hz, 20-C), 141.7, 131.9 (d, *J* = 14.7 Hz, 17-C), 120.7, 72.6, 58.7 (d, *J* = 31.6 Hz, 21-C), 56.5, 50.1, 42.9, 42.6 (d, *J* = 5.6 Hz, 13-C), 37.4, 37.2, 36.7, 32.1, 31.8, 31.5, 31.4, 26.1 (d, *J* = 4.6 Hz, 16-C), 26.0, 23.9, 21.3, 19.4, 18.6 (d, *J* = 3.4 Hz, 18-Me), 18.2; ^{19}F NMR δ −114.32 (br t, *J* = 24 Hz); MS (CI, CH_4) *m*/*z* (rel intensity) 449 (MH^+, 3), 448 (5), 447 (19), 431(10), 429 (5), 391 (32), 299 (100), 297 (17). Anal. calcd for $C_{27}H_{45}FO_2Si$: C,72.27; H, 10.11. Found: C, 72.18; H, 10.28.

Also obtained was **13** (1.10 g, 16%) as a white solid: mp 174–176 °C. TLC R_f 0.29, ethyl acetate/hexane (15:85); ^{1}H NMR δ 5.36–5.31 (m, 1H, vinyl), 4.15 (dd, 2H, *J* = 21.2, 6.1 Hz, CH_2O), 3.56–3.44 (m, 1H, OCH), 1.03 (s, 3H, 19-Me), 0.94 (s, 3H, 18-Me), 0.91 (s, 9H, Si-*t*-Bu), 0.075 (s, 6H, 2×SiMe); ^{13}C NMR δ 149.9 (d, *J* = 245.0 Hz, 20-C), 141.8, 129.5 (d, *J* = 13.7 Hz, 17-C), 120.7, 72.6, 59.9 (d, *J* = 30.8 Hz, 21-C), 55.6, 50.1, 44.2, 42.8, 37.3, 36.7, 36.1 (d, *J* = 6.3 Hz, 16-C), 32.0, 31.8, 31.2, 25.9, 25.8 (d, *J* = 6.5 Hz, 15-C), 24.9, 21.0, 19.4, 18.3, 16.9, 16.88; ^{19}F NMR δ −128.10 (t, *J* = 21.1 Hz); MS (CI, CH_4) *m*/*z* (rel intensity) 449 (MH^+, 2), 448 (4), 447 (15), 431 (10), 429 (5), 391 (26), 317 (47), 299 (100) 297 (15). Anal. calcd for $C_{27}H_{45}FO_2Si$: C, 72.27; H, 10.11. Found: C, 72.06; H, 10.22.

(3β,17*E*)-20-Fluoropregna-5,17(20)-diene-3,21-diol (14). To compound **12** (307 mg, 0.68 mmol) under nitrogen was added tetrabutylammonium fluoride (3.0 mL of a 1.0 M solution in THF, 3.0 mmol) and the resultant solution was stirred at room temperature for 23 h. The reaction solution was added dropwise to vigorously stirred water (50 mL) and the resultant suspension was filtered and dried to give 14 (218 mg, 95%) as a white solid: mp 214–216 °C. TLC R_f 0.15, ethyl acetate/hexane (45:55). ^{1}H NMR (DMSO-d_6) δ 5.30–5.25 (m, 1H, vinyl), 4.92 (t, 1H, *J* = 5.5 Hz, OH), 4.60 (d, 1H, *J* = 4.8 Hz, OH), 4.15–3.89 (m, 2H, CH_2O), 3.35–3.19 (m OCH), 0.95 (s, 3H, 19-Me), 0.85 (d, 3H, *J* = 1.1 Hz, 18-Me); ^{19}F NMR (DMSO-d_6) δ −108.66 (dd, *J* = 28.0, 24.1 Hz), MS (CI, CH_4) *m*/*z* (rel intensity) 335 (MH^+, 4), 334 (9), 333 (18), 317 (100), 299 (93), 297 (28). Anal. calcd for $C_{21}H_{31}FO_2$: C, 75.41; H, 9.34. Found: C, 75.61; H, 9.50.

(3β,17*E*)-3-[(1,1-Dimethylethyl)dimethylsilyl]oxy]-20-fluoropregna-5,17(20)-dien-3-ol (15). To a stirred solution of **12** (1.35 g, 3.00 mmol) in THF (30 mL) under nitrogen and cooled in an ice water bath was added sulfur trioxide pyridine complex (0.84 g, 5.27 mmol). The resultant suspension was stirred at ice bath temperature for 3 h, and then stored in a refrigerator overnight. To the stirred suspension was carefully added lithium aluminum hydride (0.80 g, 21.1 mmol) in portions. The reaction was quenched by cautiously adding

0.6 mL of water, 0.6 mL of 1.0 N aq NaOH, and finally, another 0.6 mL of water. The resultant suspension was diluted with diethyl ether (80 mL) and stirred vigorously for several minutes. The suspension was filtered and the filtrate was concentrated to give crude **15**. Flash chromatography (5×14 cm column) eluting with ethyl acetate/hexane (5:95) gave 15 (0.99 g, 76%) as a white solid. A portion of **15** was crystallized from aqueous acetone to give a white crystalline solid: mp 128–130 °C. TLC R_f 0.52, ethyl acetate/hexane (2:98). ^{1}H NMR δ 5.35-5.31 (m, 1H, vinyl), 3.55–3.42 (m, 1H, OCH), 1.92 (dt, 3H, J = 18.9, 1.9 Hz, 21-Me) 1.01 (s, 3H, 19-Me) 0.89 (s, 9H, Si-*t*-Bu), 0.86 (d, 3H, J = 1.3 Hz, 18-Me), 0.06 (s, 6H, 2×SiMe); ^{19}F NMR δ −95.86 (q, J = 18.9 Hz); MS (CI,CH_4) m/z (rel intensity) 433 (MH^+, 9), 432 (10), 431 (38), 417 (43) 413 (70), 375 (55), 301 (100). Anal. calcd for $C_{27}H_{45}FOSi$: C, 74.94; H, 10.48. Found: C, 75.16; H, 10.46.

(3β,17*E*)-20-Fluoropregna-5,17(20)-dien-3-ol (9). In a fashion analogous to the preparation of **14** was prepared **9** (299 mg, 94%) as a white solid: mp 129–133 °C. TLC R_f 0.25, ethyl acetate/hexane (1:3). ^{1}H NMR δ 5.38–5.34 (m, 1H, vinyl), 3.60–3.47 (m, 1H, OCH), 1.92 (dt, 3H, J = 18.9, 1.9 Hz, 21-Me), 1.02 (s, 3H, 19-Me), 0.86 (d, 3H, J = 1.2 Hz, 18-Me); ^{19}F NMR δ −95.79 (q, J = 18.8 Hz); MS (CI, CH_4) m/z (rel intensity) 319 (MH^+, 9), 318 (17), 317 (33), 301 (100), 299 (65), 281 (9). Anal. calcd for $C_{21}H_{31}FO$:C, 79.20; H, 9.81. Found: C, 79.10, H, 9.81.

(3β,17*Z*)-20-Fluoropregna-5,17(20)-diene-3,21-diol (16). In a fashion analogous to the preparation of **14** was prepared **16** (299 mg, 94%) as a white solid: mp 198–203 °C. TLC R_f 0.19, ethyl acetate/hexane (45:55). ^{1}H NMR δ 5.29–5.25 (m, 1H, vinyl), 4.91 (t, 1H, J = 5.7 Hz, OH), 4.60 (d, 1H, J = 4.5 Hz, OH), 3.88 (dd, 1H, J = 23.1, 5.7 Hz, CH_2O), 3.34–3.19 (m, 1H, OCH), 0.95 (s, 3H, 19-Me), 0.86 (s, 3H, 18-Me); ^{19}F NMR δ −123.18 (t, J = 23.1 Hz); MS (CI, CH_4) m/z (rel intensity) 335 (MH^+, 4), 334 (6), 333 (15) 317 (100) 299 (46). Anal. calcd for $C_{21}H_{31}FO_2$: C, 75.41; H, 9.34. Found: C, 75.37; H, 9.43.

(3β,17*Z*)-3-[(1,1-Dimethylethyl)dimethylsilyl]oxy]-20-fluoropregna-5,17(20)-dien-3-ol (17). Compound **17** (0.59 g, 91%), a white solid, was prepared in an analogous fashion to compound **15**. Crystallization from acetone gave a white crystalline solid: mp 138–140 °C. TLC R_f 0.52, ethyl acetate/hexane (2:98); ^{1}H NMR δ 5.34–5.30 (m 1H, vinyl), 3.54–3.42 (m, 1H, OCH), 1.79 (dt, 3H, J = 17.2, 1.4 Hz, 21-Me), 1.01 (s, 3H, 19-Me), 0.89 (s, 12H, 18-Me and Si-*t*-Bu), 0.057 (2×SiMe); ^{19}F NMR δ −110.32 (q of q, J = 17.1, 2.0 Hz). MS (CI, CH_4) m/z (rel intensity) 433 (MH^+, 10), 432 (12), 431 (45), 417 (58), 413 (45), 375 (58), 301 (100). Anal. calcd for $C_{27}H_{45}FOSi$: C, 74.94; H, 10.48. Found: C, 75.20; H, 10.43.

(3β,17*Z*)-20-fluoropregna-5,17(20)-dien-3-ol (8). In a fashion analogous to the preparation of **14** was prepared **8** (204 mg, 64%) as a white crystalline solid, after crystallization from methanol: mp 153–155 °C. TLC R_f 0.27, ethyl acetate/hexane (1:3). ^{1}H NMR δ 5.37–5.33 (m, 1H, vinyl), 3.59–3.46 (m, 1H, OCH), 3.49 (s, 0.6H, MeOH solvate), 1.79 (dt, 3H, J = 17.2, 1.4 Hz, 21-Me), 1.02 (s, 3H, 19-Me), 0.89 (s, 3H, 18-Me); ^{19}F NMR δ −110.27 (q of q, J = 17.2, 2.0 Hz); MS (CI, CH_4) m/z (rel intensity) 319 (MH^+, 7), 318 (15), 317 (28), 301 (100), 299 (39), 281 (8). Anal. calcd for $C_{21}H_{31}FO$·0.2-MeOH: C, 78.38; H,9.87. Found: C, 78.40; H, 9.82.

Enzymes and assays

Compounds were tested for inhibition of cynomolgous monkey $C_{17(20)}$ lyase in vitro using microsomal preparations of the enzyme from testicular tissue. Testes were removed from anesthetized animals and flash frozen in liquid nitrogen. Microsomes were isolated as described previously.[47] The compound to be tested was dissolved in dimethyl sulfoxide and diluted in 0.05 M potassium phosphate buffer, pH 7.4, to give the desired concentrations of test compound; this contributes 0.1% v/v dimethyl sulfoxide to the final mix. Assays contained 0.05 M potassium phosphate, pH 7.4, an NADPH regenerating system (1 mM NADPH, 5 mM glucose-6-phosphate, 1 IU/mL glucose-6-phosphate dehydrogenase), test compound, substrate and microsomal protein in a total volume of 0.2 mL. Control assays contained all components, including dimethyl sulfoxide, but no test compound. All assays were performed in duplicate. The reaction was initiated by the addition of substrate, 7-^{3}H-17α-hydroxypregnenolone (11.2 mCi/mmol; 0.20 mCi per assay) plus unlabeled 17α-hydroxypregnenolone dissolved in dimethyl sulfoxide, contributing 2.5% v/v to the final assay mix, and phosphate buffer, yielding a final concentration of 0.05 mM 17α-hydroxypregnenolone (ca. equal to the K_m value) to the other assay components. The complete assay was incubated at 34 °C for 6 min. Each assay was terminated by addition of 5 mL of chloroform:methanol (2:1). Carrier steroids representing substrates and products (17α-hydroxypregnenolone, dehydroepiandrosterone, and androst-5-ene-3β,17β-diol) and 0.8 mL of distilled, deionized water were also added at this time. The steroids were extracted by the method of Moore and Wilson.[48] The organic phase containing the steroids was evaporated using nitrogen gas, the residues were dissolved in 18% tetrahydrofuran (v/v) in hexane, and the steroids were separated by HPLC on a Si60 (5 mm) column (250×4 mm) using a gradient of 18–22% tetrahydrofuran (v/v) in hexane. Radioactivity in the steroid peaks was measured using a Radiometric Model HS or Model A515 Flo-One detector.

The enzyme activity for each assay was calculated from the percent conversioon of substrate to products, and the results were expressed as percent inhibition of control.

References and Notes

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