Supplementary Information for

Effects of Fluorine Substitution on Substrate Conversion by Cytochromes P450 17A1 and 21A2

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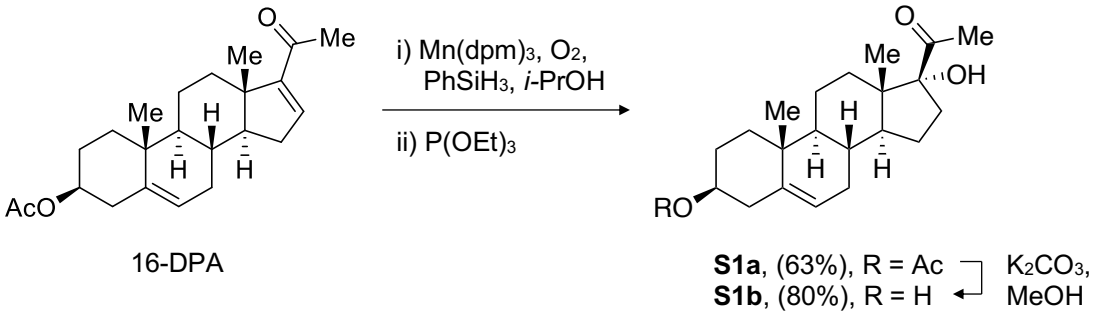
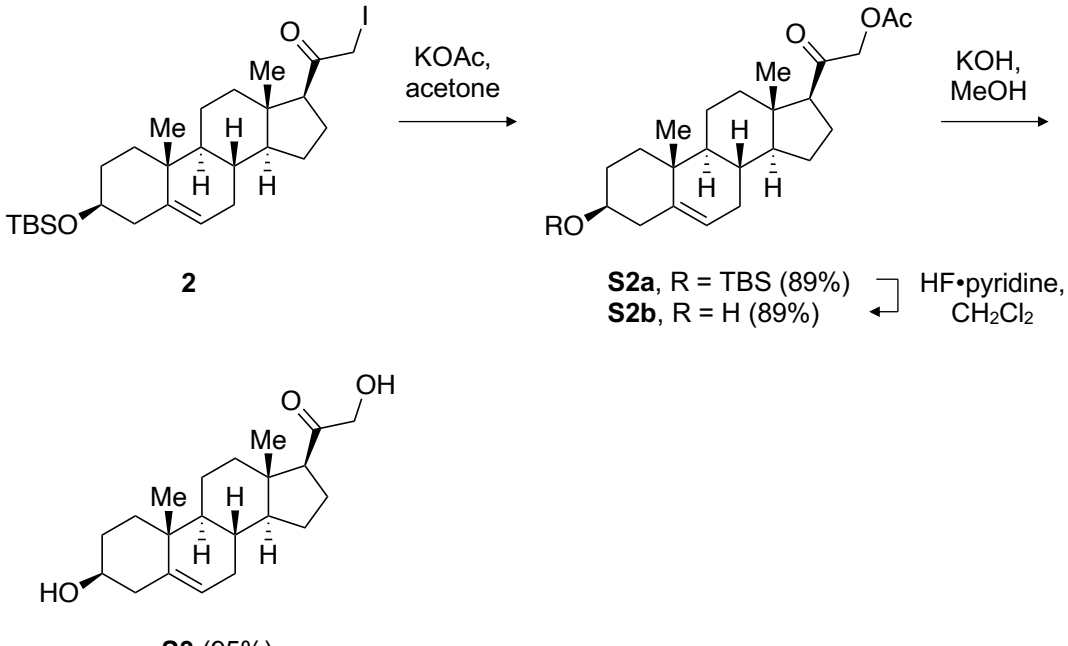
Scheme S1. Synthesis of 17α-Hydroxypregnenolone.



16-DPA

S1a, (63%), R = Ac
S1b, (80%), R = H
K_2CO_3, MeOH

Scheme S2. Synthesis of 21-Hydroxypregnenolone.



2

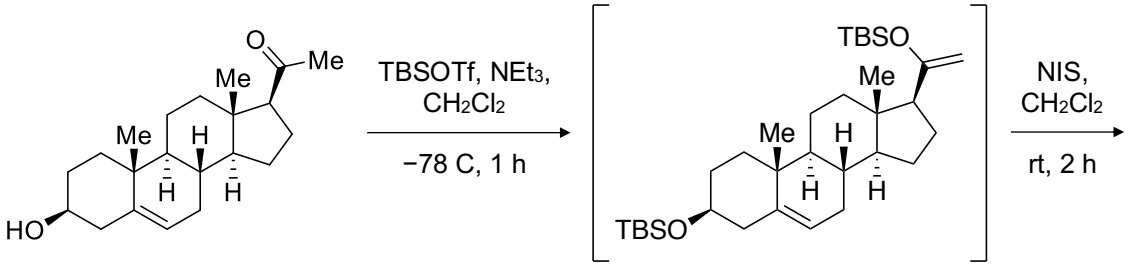
S2a, R = TBS (89%)
S2b, R = H (89%)
HF•pyridine, CH_2Cl_2

S3 (95%)

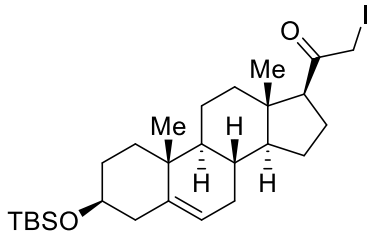
General Information. All chemicals and solvents were purchased from commercial sources (Alfa Aesar, Ark Pharm, Oakwood Chemical, or Sigma-Aldrich) and used as received. Unless stated otherwise, reactions were performed under ambient conditions and monitored by thin-layer chromatography using Analtech silica gel GHLF (250 microns) coated glass plates, which were visualized by either shortwave UV light or cerium ammonium molybdate stain. Normal phase column chromatography was carried out on a Teledyne Isco Combiflash purification system. Microwave reactions were performed using a Biotage Initiator Classic with an auto sampler. All nuclear magnetic resonance (NMR) spectra (^{1}H, ^{13}C, and ^{19}F) were recorded in deuterated solvents ($CDCl_3$ or DMSO-d_6) on a Varian 400 MR NMR spectrometer. Chemical shifts are reported in parts per million (ppm) and were adjusted using the residual undeuterated solvents ($CDCl_3$: 7.26 ppm for ^{1}H NMR, 77.2 ppm for ^{13}C NMR; DMSO-d_6: 2.50 ppm for ^{1}H NMR, 39.5 ppm for ^{13}C NMR) as an internal reference. Using the unified scale, ^{19}F NMR spectra were referenced with respect to the ^{1}H frequency of residual solvent or tetramethylsilane (TMS). Coupling constants are reported in Hertz (Hz) and peak multiplicities as either a singlet (s), doublet (d), triplet (t), multiplet (m), AB quartet (AB q), ABX, or complex. The purity of 17α-hydroxypregnenolone was determined by quantitative NMR using 1,3,5-trimethoxybenzene as the standard. The purity of 17α-hydroxypregnenolone was determined by quantitative NMR using 1,3,5-trimethoxybenzene as the standard. Infrared (IR) spectra were acquired on a Thermo Scientific Nicolet iS 5 FT-IR Spectrometer. Melting points were determined on an Optimelt MPA100 instrument and are uncorrected. Optical rotations were measured with a Rudolph AUTOPOL IV. High-resolution mass spectrometry (HRMS) data were collected on a Thermo Electron hybrid ion trap FT-ICR mass spectrometer equipped with a 7T ICR magnet (LTQ-FT) using either an electrospray ionization (ESI) or atmospheric-pressure chemical ionization (APCI) probe. Compound purity was measured using a Waters ACQUITY UPLC H-Class System coupled to the aforementioned mass spectrometer with an ESI or APCI probe. All samples were prepared in MeOH at a concentration of ca. 1 mg/mL, and 3 μL of each solution was introduced at a flow rate of 0.6 mL/min onto a Waters Acquity UPLC BEH C18 column (2.1 × 50 mm, 1.7 μm particle size) at 40 °C. The solvent gradient was as follows: H_2O with 0.1% formic acid ramped linearly over 9.8 min to 95% MeCN with 0.1% formic acid and held for 0.4 min. At 10.2 min, the gradient was switched back to H_2O with 0.1% formic acid and allowed to re-equilibrate for 1.1 min to prepare for the next sample. Purity was determined on the basis of peak integration (area under the curve) from the

total ion chromatogram, and HRMS data provided verification of chemical identity. All tested compounds had an LCMS purity of >95%.

Synthetic Procedures.



pregnenolone **1**

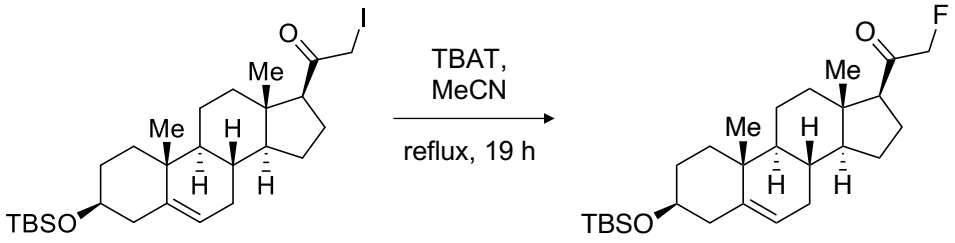


2 (87% over 2 steps)

3β-[*tert*-Butyl(dimethyl)silyl]oxy-21-iodopregn-5-en-20-one (2). A flame-dried 50 mL round-bottom flask under Ar was charged with pregnenolone (1.00 g, 3.16 mmol), NEt_3 (2.2 mL, 16 mmol, 5.1 equiv), and anhydrous CH_2Cl_2 (12 mL). The reaction mixture was cooled to −78 °C, and TBSOTf (2.2 mL, 9.6 mmol, 3.0 equiv) was added over 3 min. After stirring for 1 h, the reaction mixture was allowed to warm to rt, quenched with a solution of saturated $NaHCO_3$ in H_2O (10 mL), and the aqueous layer was extracted with CH_2Cl_2 (2 × 10 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in a 100 mL round-bottom flask.

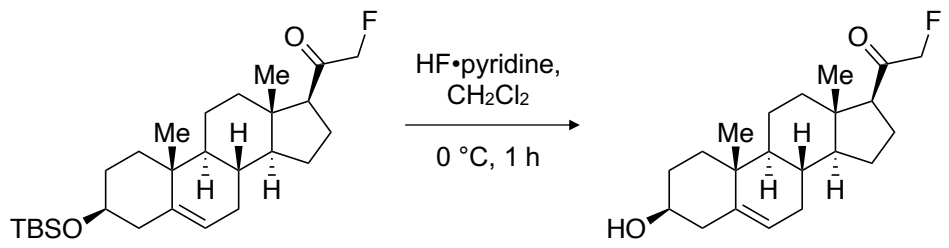
Crude **1** was dissolved in CH_2Cl_2 (20 mL), and NIS (0.856 g, 3.80 mmol, 1.2 equiv) was added in one portion. After stirring for 2 h, the reaction mixture was washed with a 10 wt% solution of $Na_2S_2O_3$ in H_2O (30 mL), and the aqueous layer was extracted with CH_2Cl_2 (2 × 10 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by column chromatography (40 g of silica gel, 0–5% EtOAc/hexanes) to afford **2** (1.54 g, 2.76 mmol, 87% yield) as a white, amorphous solid. R_f = 0.4 (5% EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.33–5.29 (m, 1H), 3.81 (AB q, $\Delta\nu_{AB}$ = 40.5 Hz, J_{AB} = 10.5 Hz, 2H), 3.53–3.43 (m, 1H), 2.93 (t, J = 8.8 Hz, 1 H), 2.31–2.13 (m,

3H), 2.04–1.90 (m, 2H), 1.84–1.68 (complex, 4H), 1.66–1.40 (complex, 6H), 1.35–1.22 (m, 1H), 1.21–1.12 (m, 1H), 1.10–0.93 (m, 2H), 1.00 (s, 3H), 0.89 (s, 9H), 0.65 (s, 3H), 0.06 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 203.5, 141.7, 120.9, 72.6, 60.2, 56.9, 50.1, 45.0, 42.9, 38.9, 37.5, 36.7, 32.2, 32.1, 31.9, 26.1 (3C), 24.8, 24.3, 21.2, 19.6, 18.4, 13.6, 8.6, −4.4 (2C); IR (film) 1695 cm^{-1}; mp 117–119 °C; $[\alpha]_D^{22}$ +57.3 (*c* 1.00, $CHCl_3$); HRMS (APCI) *m/z*: $[M + H]^+$ calcd for $C_{27}H_{46}IO_2Si$ 557.2306, found 557.2301.



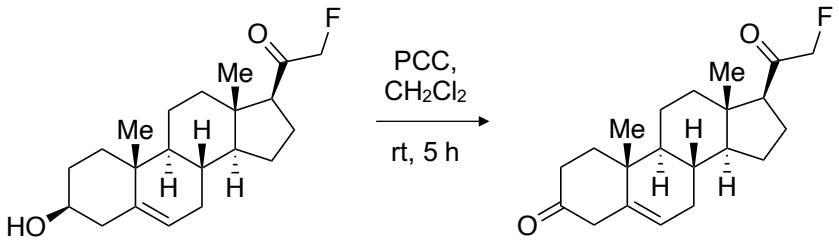
2 **3a** (74%)

3β-[*tert*-Butyl(dimethyl)silyl]oxy-21-fluoropregn-5-en-20-one (3a). A flame-dried 50 mL round-bottom flask under Ar was charged with **2** (0.699 g, 1.26 mmol), TBAT (1.02 g, 1.89 mmol, 1.5 equiv), and anhydrous MeCN (12 mL). After equipping a condenser, the reaction mixture was heated to reflux, stirred for 19 h, and allowed to cool to rt. Then, a 1.0 M solution of TBAF in THF (1.4 mL, 1.4 mmol, 1.1 equiv) was added in one portion, and the reaction mixture was stirred for 15 min before being concentrated. Note: This work-up was used to regenerate TBAT *in situ*, because the fluoro(triphenyl)silane produced in the reaction complicated purification. The crude product was purified by column chromatography (24 g of silica gel, 0–20% EtOAc/hexanes) to afford **3a** (0.418 g, 0.932 mmol, 74% yield) as a white, amorphous solid. R_f = 0.3 (25% EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.34–5.29 (m, 1H), 4.77 (AB of ABX, $\Delta\nu_{AB}$ = 29.8 Hz, J_{AB} = 16.1 Hz, J_{AX} = 48.1 Hz, J_{BX} = 47.6 Hz, 2H), 3.54–3.43 (m, 1H), 2.73 (td, J = 9.0 Hz, $^4J_{H17,F21}$ = 3.2 Hz, 1H), 2.32–2.13 (m, 3H), 2.06–1.96 (m, 1H), 1.92–1.39 (complex, 11H), 1.35–1.14 (m, 2H), 1.10–0.93 (m, 2H), 1.00 (s, 3H), 0.89 (s, 9H), 0.67 (s, 3H), 0.06 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.9 (d, J = 17 Hz), 141.7, 120.9, 85.6 (d, J = 186 Hz), 72.7, 58.1 (d, J = 2 Hz), 57.2, 50.1, 45.1, 42.9, 38.8 (d, J = 1 Hz), 37.5, 36.8, 32.2, 32.1, 32.0, 26.1 (3C), 24.8, 22.6 (d, J = 2 Hz), 21.2, 19.6, 18.4, 13.7, −4.4 (2C); ^{19}F NMR (376 MHz, $CDCl_3$) δ −225.4 (X of ABX, td, J_{AX} = J_{BX} = 48 Hz, $^4J_{H17,F21}$ = 3 Hz); IR (film) 1727 cm^{-1}; mp 158–161 °C; $[\alpha]_D^{22}$ +27.6 (*c* 1.00, $CHCl_3$); HRMS (APCI) *m/z*: $[M + H]^+$ calcd for $C_{27}H_{46}FO_2Si$ 449.3246, found 449.3242.



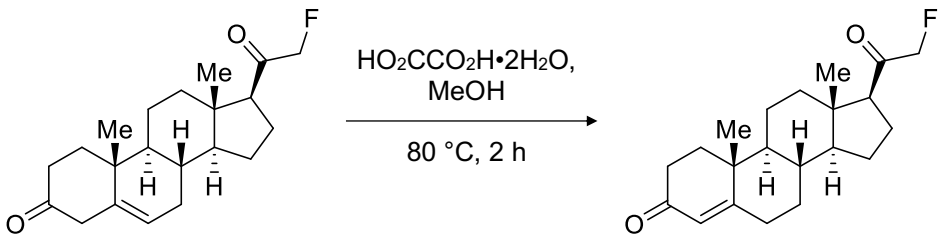
3a **3b** (86%)

21-Fluoro-3β-hydroxypregn-5-en-20-one (21-fluoropregnenolone, 3b). A 20 mL polypropylene vial was charged with **3a** (0.333 g, 0.743 mmol) and CH_2Cl_2 (15 mL). The reaction mixture was cooled to 0 °C, and Olah's reagent (0.39 mL, ca. 70 wt% HF in pyridine, ca. 20 equiv) was added over 3 min. After stirring at 0 °C for 1 h, the reaction mixture poured into an ice-cold solution of saturated $NaHCO_3$ in H_2O (10 mL), and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by column chromatography (24 g of silica gel, 0–50% EtOAc/hexanes) to afford **3b** (0.214 g, 0.641 mmol, 86% yield) as a white, amorphous solid. R_f = 0.5 (50% EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.37–5.33 (m, 1H), 4.77 (AB of ABX, $\Delta\nu_{AB}$ = 29.3 Hz, J_{AB} = 16.1 Hz, J_{AX} = 48.1 Hz, J_{BX} = 47.6 Hz, 2H), 3.58–3.47 (m, 1H), 2.73 (td, J = 9.1 Hz, $^4J_{H17,F21}$ = 3.3 Hz, 1H), 2.34–2.17 (m, 3H), 2.05–1.96 (m, 1H), 1.92–1.80 (m, 3H), 1.79–1.39 (complex, 9H), 1.36–1.15 (m, 2H), 1.14–1.04 (m, 1H), 1.03–0.94 (m, 1H), 1.01 (s, 3H), 0.67 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.7 (d, J = 17 Hz), 140.7, 121.3, 85.4 (d, J = 186 Hz), 71.7, 57.9 (d, J = 2 Hz), 57.0, 49.9, 44.9, 42.2, 38.6 (d, J = 1 Hz), 37.2, 36.5, 31.9, 31.7, 31.6, 24.7, 22.5 (d, J = 2 Hz), 21.0, 19.4, 13.6; ^{19}F NMR (376 MHz, $CDCl_3$) δ −225.4 (X of ABX, td, J_{AX} = J_{BX} = 48 Hz, $^4J_{H17,F21}$ = 3 Hz); IR (film) 3526, 1710 cm^{-1}; mp 183–185 °C, lit.[1] mp 184–185 °C (CH_2Cl_2–Et_2O); $[\alpha]_D^{21}$ +35.7 (*c* 1.00, $CHCl_3$), lit.[1] $[\alpha]_D^{26}$ +36.9 (*c* 1.03, $CHCl_3$); HRMS (APCI) *m/z*: $[M - OH]^+$ calcd for $C_{21}H_{30}FO$ 317.2275, found 317.2273; LCMS: t_R = 6.2 min.



3b **4** (56%)

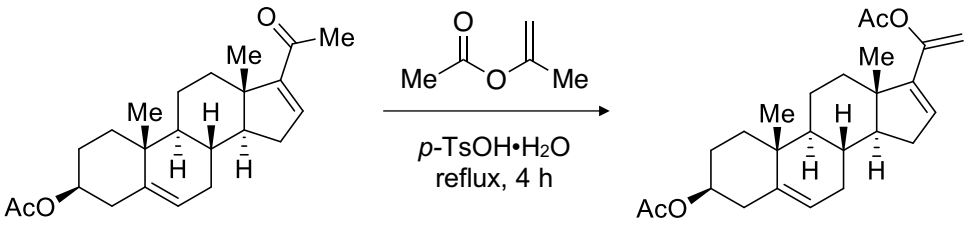
21-Fluoropregn-5-ene-3,20-dione (4). A 20 mL vial was charged with **3b** (0.162 g, 0.484 mmol), Celite (0.650 g), and CH_2Cl_2 (10.0 mL). The mixture was cooled to 0 °C, and PCC (0.115 g, 0.533 mmol, 1.1 equiv) was added in one portion. After warming to rt, the reaction mixture was stirred for 5 h and concentrated. The crude product adsorbed onto Celite was purified by column chromatography (12 g of silica gel, 0–25% EtOAc/hexanes) to afford **4** (90.4 mg, 0.272 mmol, 56% yield) as a white, amorphous solid. R_f = 0.4 (25% EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.35–5.31 (m, 1H), 4.76 (AB of ABX, $\Delta\nu_{AB}$ = 27.2 Hz, J_{AB} = 16.1 Hz, J_{AX} = 47.9 Hz, J_{BX} = 47.5 Hz, 2H), 3.31–3.22 (m, 1H), 2.81 (dd, J = 16.5, 2.3 Hz, 1H), 2.74 (td, J = 9.0 Hz, $^4J_{H17,F21}$ = 3.1 Hz, 1H), 2.47 (ddd, J = 15.2, 13.4, 5.8 Hz, 1H), 2.33–2.17 (m, 2H), 2.10–1.98 (m, 2H), 1.95–1.87 (m, 1H), 1.80–1.41 (complex, 8H), 1.36–1.19 (m, 2H), 1.17 (s, 3H),1.12–1.04 (m, 1H), 0.69 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 210.0, 206.8 (d, J = 17 Hz), 138.7, 122.6, 85.6 (d, J = 186 Hz), 58.0 (d, J = 2 Hz), 57.0, 49.1, 48.4, 45.0, 38.6 (d, J = 1 Hz), 37.7, 37.04, 36.99, 32.1, 31.8, 24.8, 22.6 (d, J = 2 Hz), 21.4, 19.3, 13.7; ^{19}F NMR (376 MHz, $CDCl_3$) δ −225.4 (X of ABX, td, J_{AX} = J_{BX} = 48 Hz, $^4J_{H17,F21}$ = 3 Hz); IR (neat) 1712 cm^{-1}; mp 156–159 °C (dec); $[\alpha]_D^{23}$ +79.4 (*c* 1.00, $CHCl_3$); HRMS (APCI) *m/z*: $[M + H]^+$ calcd for $C_{21}H_{30}FO_2$ 333.2224, found 333.2218.



4 **5** (85%)

21-Fluoropregn-4-ene-3,20-dione (21-fluoroprogesterone, 5). A 5 mL microwave vial was charged with **4** (90.4 mg, 0.272 mmol), $HO_2CCO_2H•2H_2O$ (0.171 g, 1.36 mmol, 5.0 equiv), and MeOH (5.5 mL). The vial was capped, heated to 80 °C, and stirred for 2 h. After cooling to rt, the reaction mixture was

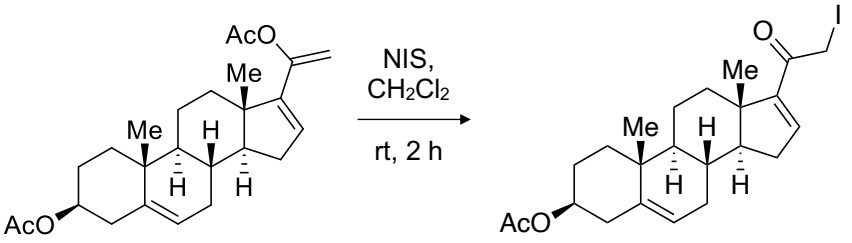
concentrated under a stream of N_2 and dissolved in Et_2O (5 mL). The organic layer was washed with a 2.0 M solution of NaOH in H_2O (2 mL) followed by H_2O (2 mL), dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by column chromatography (4 g of silica gel, 0–25% EtOAc/hexanes) to afford **5** (76.6 mg, 0.230 mmol, 85% yield) as a white, amorphous solid. R_f = 0.2 (25% EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.73 (s, 1H), 4.76 (AB of ABX, $\Delta\nu_{AB}$ = 27.3 Hz, J_{AB} = 16.1 Hz, J_{AX} = 47.9 Hz, J_{BX} = 47.5 Hz, 2H), 2.75 (J = 9.1 Hz, $^4J_{H17,F21}$ = 3.2 Hz, 1H), 2.48–2.17 (complex, 5H), 2.07–1.98 (m, 1H), 1.96–1.82 (m, 2H), 1.81–1.52 (complex, 5H), 1.51–1.19 (complex, 4H), 1.18 (s, 3H), 1.13–0.93 (m, 2H), 0.70 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.8 (d, J = 17 Hz), 199.5, 170.8, 124.1, 85.6 (d, J = 186 Hz), 57.9 (d, J = 2 Hz), 56.3, 53.7, 45.0, 38.7, 38.5 (d, J = 1 Hz), 35.9, 35.8, 34.1, 32.9, 32.0, 24.7, 22.6 (d, J = 2 Hz), 21.1, 17.5, 13.8; ^{19}F NMR (376 MHz, $CDCl_3$) δ −225.4 (X of ABX, td, J_{AX} = J_{BX} = 48 Hz, $^4J_{H17,F21}$ = 3 Hz); IR (film) 1727, 1668, 1615 cm^{-1}; mp 145–147 °C, lit.[1] mp 143–145 °C (Et_2O); $[\alpha]_D^{22}$ +202 (*c* 1.00, $CHCl_3$), lit.[1] $[\alpha]_D^{26}$ +206 (*c* 1.00, $CHCl_3$); HRMS (APCI) *m/z*: $[M + H]^+$ calcd for $C_{21}H_{30}FO_2$ 333.2224, found 333.2223; LCMS: t_R = 6.3 min.



16-DPA **6** (77%)

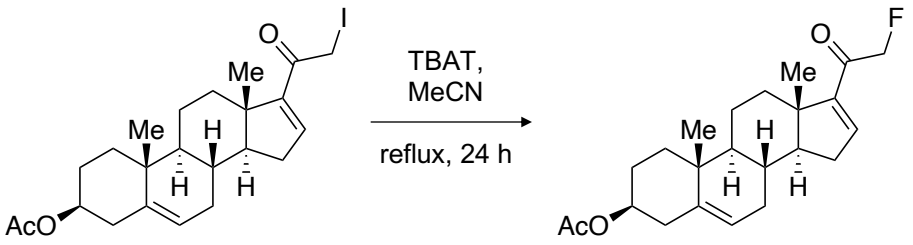
Pregna-5,16-20-triene-3β,20-yl diacetate (6). A 100 mL round-bottom flask was charged with 16-dehydropregnenolone acetate (4.00 g, 11.2 mmol), *p*-TsOH•H_2O (0.429 g, 2.26 mmol, 0.2 equiv), and isopropenyl acetate (56 mL, 510 mmol, 46 equiv). A 15 cm Vigreux column attached to a short path distillation apparatus was equipped, and the reaction mixture was heated to 130 °C. After an initial fraction (bp 50–60 °C) was removed, the temperature was raised to 150 °C, and most of the solvent (ca. 50 mL) was distilled over the course of 4 h. The reaction mixture was allowed to cool to rt, diluted with Et_2O (200 mL), and quenched with a saturated solution of $NaHCO_3$ in H_2O (3.6 mL). The organic layer was washed with H_2O (100 mL), dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by recrystallization from *i*-PrOH (slow cooling of hot solvent) to afford **6** (3.42 g, 8.58 mmol, 77% yield) as a light brown, crystalline solid. R_f = 0.6 (25% EtOAc/hexanes); ^{1}H NMR (400 MHZ, $CDCl_3$) δ 5.82 (dd, J =

3.5, 2.0 Hz, 1H), 5.41–5.37 (m, 1H), 5.06 (s, 1H), 4.78 (s, 1H), 4.65–4.55 (m, 1H), 2.39–2.26 (m, 2H), 2.21–2.13 (m, 1H), 2.17 (s, 3H), 2.12–2.07 (m, 1H), 2.03 (s, 3H), 2.02–1.96 (m, 3H), 1.95–1.81 (m, 3H), 1.75–1.45 (complex, 7H), 1.20–1.10 (m, 1H), 1.09–1.01 (m, 1H), 1.06 (s, 3H), 0.97 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.6, 169.2, 149.8, 148.3, 140.1, 129.9, 122.4, 102.3, 74.0, 57.1, 50.3, 46.2, 38.3, 37.0, 36.9, 35.4, 31.6, 31.1, 30.3, 27.9, 21.6, 21.1, 21.0, 19.4, 16.0; IR (film) 1760, 1732, 1636 cm^{-1}; mp 139–141 °C (*i*-PrOH); $[\alpha]_D^{23}$ −53.4 (*c* 1.00, $CHCl_3$), lit.[2] $[\alpha]_D^{25}$ −57.7 (*c* 1, $CHCl_3$); HRMS (APCI) *m/z*: $[M + H]^+$ calcd for $C_{25}H_{35}O_4$ 399.2530, found 399.2524.



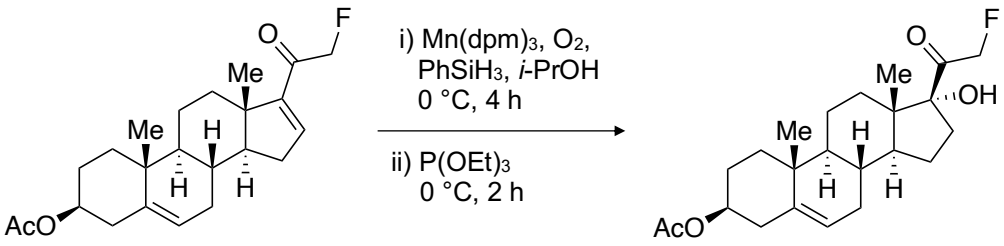
6 **7a** (68%)

21-Iodo-20-oxopregna-5,16-dien-3β-yl acetate (7a). A 250 mL round-bottom flask was charged with **6** (3.38 g, 8.48 mmol) and CH_2Cl_2 (85 mL). Then, NIS (2.10 g, 9.33 mmol, 1.1 equiv) was added in one portion, and the reaction mixture was a stirred for 2 h. A 10 wt% solution of $Na_2S_2O_3$ in H_2O (100 mL) was added, and the aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by column chromatography (120 g of silica gel, 0–25% EtOAc/hexanes) to afford **7a** (2.80 g, 5.80 mmol, 68% yield) as an off-white, amorphous solid. An analytical sample was prepared by recrystallization from EtOAc–hexanes (slow evaporation of solvent mixture). R_f = 0.3 (10% EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.79 (dd, *J* = 3.5, 1.9 Hz, 1H), 5.39–5.35 (m, 1H), 4.64–4.54 (m, 1H), 4.04 (AB q, $\Delta\nu_{AB}$ = 19.3 Hz, J_{AB} = 10.1 Hz, 2H), 2.40–2.25 (complex, 4H), 2.11 (ddd, *J* = 17.4, 12.1, 1.9 Hz, 1H), 2.05–1.96 (m, 1H), 2.02 (s, 3H), 1.89–1.81 (m, 2H), 1.75–1.32 (complex, 7H), 1.18–1.00 (m, 2H), 1.05 (s, 3H), 0.92 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 191.2, 170.6, 151.8, 145.7, 140.4, 122.0, 73.9, 56.2, 50.4, 46.5, 38.2, 37.0, 36.9, 34.4, 32.7, 31.6, 30.3, 27.8, 21.6, 20.7, 19.4, 15.6, 3.7; IR (film) 1722, 1658, 1585 cm^{-1}; mp 135–137 °C (EtOAc–hexanes, dec); $[\alpha]_D^{20}$ −55.6 (*c* 1.00, $CHCl_3$); HRMS (APCI) *m/z*: $[M + H]^+$ calcd for $C_{23}H_{32}IO_3$ 483.1391, found 483.1383.



7a **7b** (75%)

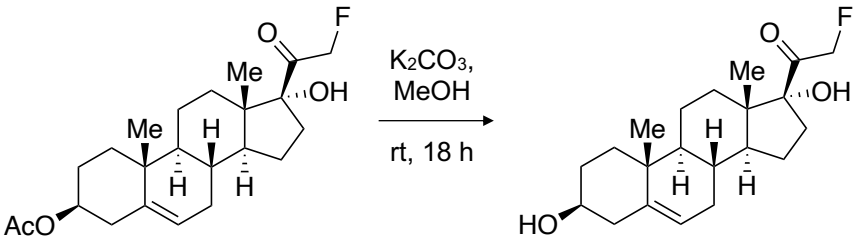
21-Fluoro-20-oxopregna-5,16-dien-3β-yl acetate (7b). A flame-dried 100 mL round-bottom flask under Ar was charged with **7a** (2.72 g, 5.64 mmol), TBAT (4.54 g, 8.41 mmol, 1.5 equiv), and anhydrous MeCN (56 mL). After equipping a condenser, the reaction mixture was heated to reflux, stirred for 24 h, allowed to cool to rt, and concentrated. The crude product was purified by column chromatography (80 g of silica gel, 0–5% EtOAc/hexanes) to afford **7b** (1.59 g, 4.24 mmol, 75% yield) as a white, amorphous solid. An analytical sample was prepared by recrystallization from MeOH–H_2O (slow cooling of hot solvent mixture). R_f = 0.6 (25% EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.80–6.75 (m, 1H), 5.41–5.36 (m, 1H), 5.09 (AB of ABX, $\Delta\nu_{AB}$ = 20.4 Hz, J_{AB} = 15.0 Hz, J_{AX} = J_{BX} = 47.3 Hz, 2H), 4.65–4.55 (m, 1H), 2.43–2.26 (complex, 4H), 2.09 (ddd, *J* = 17.4, 12.1, 1.9 Hz, 1H), 2.05–1.96 (m, 1H), 2.03 (s, 3H), 1.90–1.82 (m, 2H), 1.75–1.52 (complex, 5H), 1.48–1.32 (m, 2H), 1.19–1.01 (m, 2H), 1.06 (s, 3H), 0.96 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 192.2 (d, *J* = 16 Hz), 170.7, 151.6 (d, *J* = 2 Hz), 145.3 (d, *J* = 4 Hz), 140.4, 122.0, 83.4 (d, *J* = 183 Hz), 74.0, 55.9, 50.5, 47.0, 38.3, 37.0, 36.9, 34.5, 33.0, 31.7, 30.2, 27.9, 21.6, 20.7, 19.4, 16.0; ^{19}F NMR (376 MHz, $CDCl_3$) δ −228.9 (X of ABX, t, J_{AX} = J_{BX} = 47 Hz); IR (film) 1731, 1683, 1656, 1586 cm^{-1}; mp 191–192 °C (MeOH–H_2O); $[\alpha]_D^{22}$ −45.7 (*c* 1.00, $CHCl_3$); HRMS (APCI) *m/z*: $[M + H]^+$ calcd for $C_{23}H_{32}FO_3$ 375.2330, found 375.2324.



7b **8a** (66%)

21-Fluoro-17α-hydroxy-20-oxopregn-5-en-3β-yl acetate (8a). A 2 dram vial was charged with **7b** (0.149 g, 0.398 mmol), $Mn(dpm)_3$ (8.2 mg, 14 μmol, 0.04 equiv), and *i*-PrOH (4.0 mL). The reaction

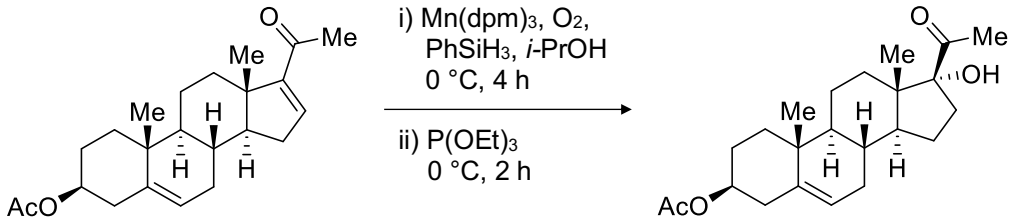
mixture was cooled to 0 °C and sparged with a balloon of O_2 for 15 min. Then, phenylsilane (100 μL, 0.810 mmol, 2.0 equiv) was added over 5 min, and the reaction mixture was stirred at 0 °C for 4 h under O_2. Afterward, the balloon was removed, and $P(OEt)_3$ (80 μL, 0.46 mmol, 1.2 equiv) was added. After stirring at 0 °C for 2 h, the reaction mixture was allowed to warm to rt, diluted with CH_2Cl_2 (10 mL), washed with H_2O (20 mL), and the aqueous layer was extracted with CH_2Cl_2 (2 × 10 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by column chromatography (12 g of silica gel, 0–20% EtOAc/hexanes) to afford **8a** (0.103 g, 0.262 mmol, 66% yield) as a white, amorphous solid. R_f = 0.2 (25% EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.35 (dd, *J* = 47.9, 17.0 Hz, 1H), 5.39–5.35 (m, 1H), 5.09 (dd, *J* = 47.7, 17.0 Hz, 1H), 4.65–4.54 (m, 1H), 2.72 (ddd, *J* = 14.8, 11.4, 3.0 Hz, 1H), 2.38–2.25 (m, 2H), 2.21–2.12 (m, 1H), 2.07–1.95 (m, 1H), 2.03 (s, 3H), 1.91–1.82 (m, 2H), 1.81–1.72 (m, 2H), 1.71–1.41 (complex, 8H), 1.40–1.28 (m, 1H), 1.20–1.09 (m, 1H), 1.06–0.95 (m, 1H), 1.02 (s, 3H), 0.71 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.2 (d, *J* = 13 Hz), 170.8, 139.8, 122.3, 90.3, 85.2 (d, *J* = 181 Hz), 74.0, 51.4, 49.7, 48.6, 38.2, 37.1, 36.7, 35.0, 32.1, 32.0, 30.6, 27.8, 24.0, 21.6, 20.7, 19.4, 15.0; ^{19}F NMR (376 MHz, $CDCl_3$) δ −231.1 (t, *J* = 48 Hz); IR (neat) 3568, 1723 cm^{-1}; mp 226–232 °C (dec); $[\alpha]^{22}_D$ −29.9 (*c* 1.00, $CHCl_3$); HRMS (APCI) *m/z*: $[M + H]^+$ calcd for $C_{23}H_{34}FO_4$ 393.2436, found 393.2433.



8a **8b** (89%)

21-Fluoro-3β,17α-dihydroxypregn-5-en-20-one (21-fluoro-17α-hydroxypregnenolone, 8b). A 2 dram vial was charged with **8a** (82.1 mg, 0.209 mmol), K_2CO_3 (0.289 g, 2.09 mmol, 10.0 equiv), and MeOH (7.0 mL). The reaction mixture was stirred for 18 h, and a saturated solution of NH_4Cl in H_2O (5 mL) was added. Then, the reaction mixture was concentrated under a stream of N_2, and the wet solid was suspended in H_2O (5 mL). The precipitate was isolated by vacuum filtration, washed with H_2O (3 × 1 mL) followed by Et_2O (2 × 1 mL), and dried to afford **8b** (64.7 mg, 0.185 mmol, 89% yield) as a white, amorphous solid. R_f = 0.5 (50% EtOAc/hexanes); ^{1}H NMR (400 MHZ, DMSO-d_6) δ 5.41 (dd, *J* = 47.7, 17.1 Hz, 1H),

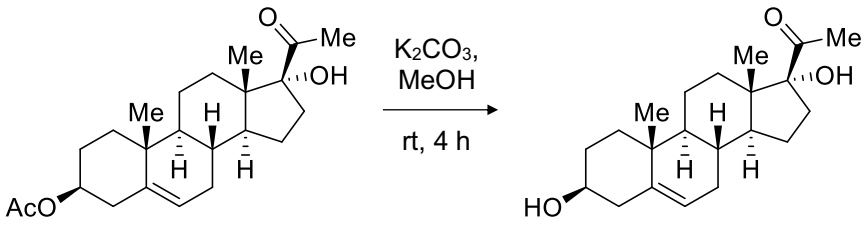
5.38 (s, 1H), 5.30–5.23 (m, 1H), 5.10 (dd, *J* = 47.8, 17.1 Hz, 1H), 4.59 (d, *J* = 4.6 Hz, 1H), 3.31–3.20 (m, 1H), 2.55–2.43 (m, 1H), 2.20–2.03 (m, 2H), 1.99–1.89 (m, 1H), 1.81–1.13 (complex, 13H), 1.04–0.82 (m, 2H), 0.94 (s, 3 H), 0.54 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 206.6 (d, *J* = 12 Hz), 141.2, 120.2, 88.6, 84.9 (d, *J* = 176 Hz), 69.9, 50.7, 49.4, 47.2, 42.2, 36.9, 36.1, 33.5, 31.6, 31.5, 31.4, 30.0, 23.4, 20.3, 19.1, 14.4; ^{19}F NMR (376 MHz, DMSO-d_6) δ −231.0 (t, *J* = 48 Hz); IR (neat) 3450, 3317, 1723 cm^{-1}; mp 237–243 °C (dec); $[\alpha]_D^{21}$ −34 (*c* 0.40, pyridine); HRMS (APCI) *m/z*: $[M - OH]^+$ calcd for $C_{21}H_{30}FO_2$ 333.2224, found 333.2223; LCMS: t_R = 5.4 min.



16-DPA **S1a** (63%)

17α-Hydroxy-20-oxopregn-5-en-3β-yl acetate (S1a). A 2 dram vial was charged with 16-dehydropregnenolone acetate (0.150 g, 0.422 mmol), Mn(dpm)$_3$ (7.7 mg, 13 μmol, 0.03 equiv), and *i*-PrOH (4.2 mL). The reaction mixture was cooled to 0 °C and sparged with a balloon of O_2 for 15 min. Then, $PhSiH_3$ (67 μL, 0.55 mmol, 1.3 equiv) was added over 5 min, and the reaction mixture was stirred at 0 °C for 3 h under O_2. Afterward, the balloon was removed, and P(OEt)$_3$ (79 μL, 0.46 mmol, 1.1 equiv) was added. After stirring at 0 °C for 3 h, the reaction mixture was allowed to warm to rt, diluted with CH_2Cl_2 (10 mL), washed with H_2O (20 mL), and the aqueous layer was extracted with CH_2Cl_2 (2 × 10 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by column chromatography (12 g of silica gel, 0–25% EtOAc/hexanes) to afford **S1a** (99.6 mg, 0.266 μmol, 63% yield) as a white, crystalline solid. An analytical sample was prepared by recrystallization from *i*-PrOH (slow cooling of hot solvent). R_f = 0.4 (25% EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.40–5.36 (m, 1H), 4.65–4.55 (m, 1H), 2.72 (s, 1H), 2.67 (ddd, *J* = 14.5, 11.7, 2.8 Hz, 1H), 2.38–2.28 (m, 2H), 2.27 (s, 3H), 2.05–1.96 (m, 1H), 2.03 (s, 3H), 1.90–1.77 (m, 3H), 1.76–1.27 (complex, 10H), 1.20–1.09 (m, 1H), 1.06–0.96 (m, 1H), 1.02 (s, 3H), 0.73 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 211.9, 170.7, 139.8, 122.5, 90.2, 74.0, 50.8, 49.7, 48.4, 38.2, 37.1, 36.7, 33.7, 32.0, 31.9, 30.2, 28.1, 27.9, 24.3, 21.6, 20.6, 19.5, 15.5;

IR (neat) 3381, 1729, 1688 cm^{-1}; mp 233–235 °C (*i*-PrOH); $[\alpha]_D^{22}$ −85.0 (*c* 1.00, $CHCl_3$), lit.[3] $[\alpha]_D^{20}$ −31 (*c* 1.0, $CHCl_3$); HRMS (APCI) *m/z*: $[M + H]^+$ calcd for $C_{23}H_{35}O_4$ 375.2530, found 375.2529.



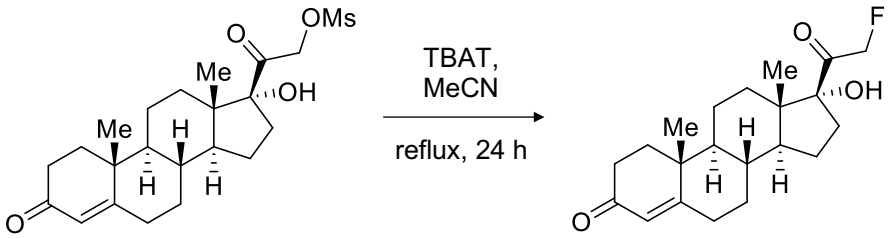
S1a **S1b** (80%)

3β,17α-Dihydroxypregn-5-en-20-one (17α-hydroxypregnenolone, S1b). A 2 dram vial was charged with **S1a** (39.9 mg, 0.107 mmol), K_2CO_3 (0.148 g, 1.07 mmol, 10.0 equiv), and MeOH (3.5 mL). The reaction mixture was stirred for 4 h, and a saturated solution of NH_4Cl in H_2O (5 mL) was added. Then, the reaction mixture was concentrated under a stream of N_2, and the wet solid was suspended in H_2O (2 mL). The precipitate was isolated by vacuum filtration, washed with H_2O (2 × 2 mL) followed by Et_2O (2 × 2 mL), and dried to afford **S1b** (28.3 mg, 85.1 μmol, 80% yield) as a white, amorphous solid. R_f = 0.5 (50% EtOAc/hexanes); ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.29–5.24 (m, 1H), 5.18 (s, 1H), 4.59 (d, *J* = 4.6 Hz, 1H), 3.31–3.21 (m, 1H), 2.59–2.51 (m, 1H), 2.18–2.03 (m, 2H), 2.09 (s, 3H), 1.99–1.88 (m, 1H), 1.87–1.49 (complex, 7H), 1.48–1.28 (complex, 5H), 1.19–1.06 (m, 1H), 1.04–0.82 (m, 2H), 0.93 (s, 3H), 0.49 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 210.4, 141.2, 120.3, 89.3, 70.0, 50.6, 49.4, 46.2, 42.2, 36.9, 36.1, 32.3, 31.6, 31.5, 31.4, 30.4, 26.8, 23.3, 20.3, 19.1, 14.4; IR (neat) 3389, 3330, 1681 cm^{-1}; mp 265–270 °C (dec); $[\alpha]_D^{22}$ −61 (*c* 0.40, pyridine); HRMS (APCI) *m/z*: $[M - OH]^+$ calcd for $C_{21}H_{31}O_2$ 315.2319, found 315.2317; LCMS: t_R = 5.4 min.

11-deoxycortisol **9** (91%)

17α-Hydroxy-3,20-dioxopregn-4-en-21-yl methanesulfonate (9). A flame-dried 20 mL vial under Ar was charged with 11-deoxycortisol (0.300 g, 0.867 mmol), NEt_3 (0.20 mL, 1.4 mmol, 1.6 equiv), and anhydrous THF (9.0 mL). The reaction mixture was cooled to 0 °C, and MsCl (0.11 mL, 1.4 mmol, 1.6 equiv)

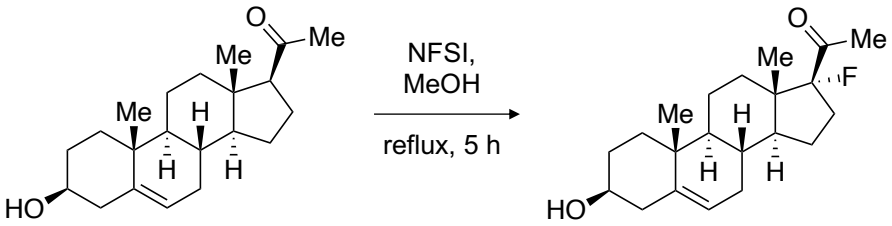
was added over 3 min. After stirring for 30 min, the reaction mixture was allowed to warm to rt, stirred for 1 h, poured into H_2O (15 mL), and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by column chromatography (24 g of silica gel, 0–50% EtOAc/hexanes) to afford **9** (0.336 g, 0.792 mmol, 91% yield) as a white, amorphous solid. An analytical sample was prepared by recrystallization from EtOAc–hexanes (slow evaporation of solvent mixture). R_f = 0.3 (50% EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.73 (s, 1H), 5.33 (d, *J* = 18.0 Hz, 1H), 5.04 (d, *J* = 18.0 Hz, 1H), 3.22 (s, 3H), 2.75 (ddd, *J* = 14.9, 11.5, 3.1 Hz, 1H), 2.48–2.24 (complex, 5H), 2.08–1.99 (m, 1H), 1.90–1.75 (m, 3H), 1.74–1.31 (complex, 8H), 1.18 (s, 3H), 1.15–1.02 (m, 1H), 0.96 (ddd, *J* = 12.4, 10.1, 4.1 Hz, 1H), 0.72 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 204.4, 199.8, 171.2, 124.1, 90.2, 72.6, 53.4, 50.7, 48.6, 39.5, 38.7, 35.85, 35.78, 35.3, 34.0, 32.9, 32.1, 30.4, 23.7, 20.8, 17.5, 14.9; IR (film) 3580, 3327, 3231, 1735, 1631 cm^{-1}; mp 166–168 °C (EtOAc–hexanes, dec); $[\alpha]_D^{22}$ +130 (*c* 0.50, $CHCl_3$); HRMS (APCI) *m/z*: $[M - SO_3CH_3]^+$ calcd for $C_{21}H_{29}O_3$ 329.2111, found 329.2111.



9 **10** (44%)

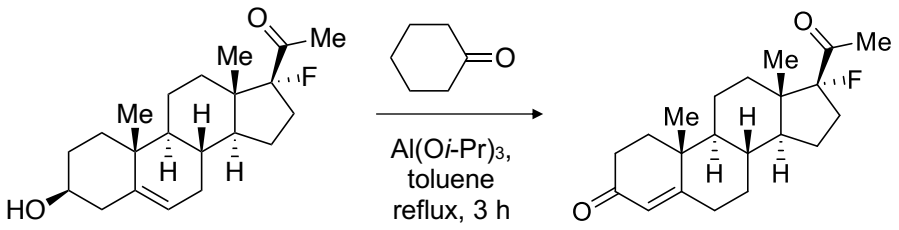
21-fluoro-17α-hydroxypregn-4-ene-3,20-dione (21-fluoro-17α-hydroxyprogesterone, 10). A flame-dried 10 mL round-bottom flask equipped under Ar was charged with **9** (0.101 g, 0.246 mmol), TBAT (0.186 g, 0.344 mmol, 1.4 equiv), and anhydrous MeCN (2.5 mL). After equipping a condenser, the reaction mixture was heated to reflux, stirred for 24 h, allowed to cool to rt, and concentrated. The crude product was purified by column chromatography (12 g of silica gel, 0–20% Et_2O/CH_2Cl_2) to afford **10** (37.8 mg, 0.108 mmol, 44% yield) as white, amorphous solids. R_f = 0.4 (20% Et_2O/CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.72 (s, 1H), 5.36 (dd, *J* = 47.9, 17.0 Hz, 1H), 5.09 (dd, *J* = 47.7, 17.1 Hz, 1H), 2.73 (ddd, *J* = 14.8, 11.4, 3.0 Hz, 1H), 2.49–2.23 (complex, 5H), 2.07–1.98 (m, 1H), 1.91–1.30 (complex, 11H), 1.18 (s, 3H), 1.15–1.02 (m, 1H), 1.00–0.91 (m, 1H), 0.73 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 206.2 (d, *J* = 13 Hz), 199.8, 171.2, 124.1, 90.0, 85.2 (d, *J* = 181 Hz), 53.4, 50.6, 48.6, 38.7, 35.9, 35.7, 35.0, 34.0, 32.9, 32.1, 30.5,

23.8, 20.7, 17.5, 15.1; ^{19}F NMR (376 MHz, $CDCl_3$) δ −231.2 (t, *J* = 48 Hz); IR (neat) 3395, 1728, 1664, 1613 cm^{-1}; mp 231–233 °C, lit.[4] mp 233–235 °C (acetone); $[\alpha]_D^{21}$ +130 (*c* 0.50, $CHCl_3$), lit.[4] $[\alpha]_D^{23}$ +130 (*c* 0.34, $CHCl_3$); HRMS (APCI) *m/z*: $[M + H]^+$ calcd for $C_{21}H_{30}FO_3$ 349.2173, found 349.2174; LCMS: t_R = 5.7 min.



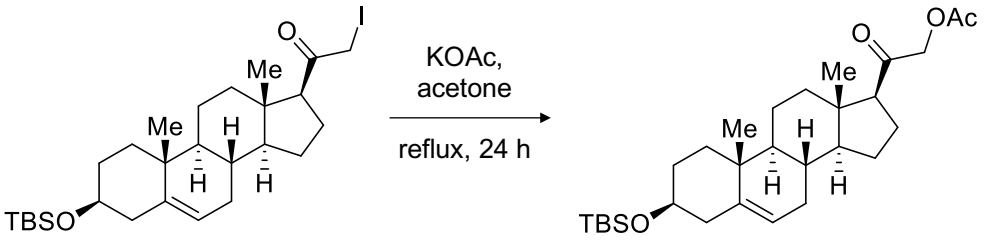
pregnenolone **11** (24%)

17α-Fluoro-3β-hydroxypregn-5-en-20-one (17α-fluoropregnenolone, 11). A flame-dried 100 mL round-bottom flask under Ar was charged with pregnenolone (4.01 g, 12.7 mmol), NFSI (5.00 g, 15.9 mmol, 1.3 equiv), and anhydrous MeOH (160 mL). After equipping a condenser, the reaction mixture was heated to reflux, stirred for 5 h, and allowed to cool to rt. Then, the reaction mixture was diluted with CH_2Cl_2 (240 mL) and washed with a 2.0 M solution of NaOH in H_2O (240 mL) followed by brine (240 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by column chromatography (160 g of silica gel, 0–25% EtOAc/hexanes) and then recrystallized from MeOH–H_2O (slow cooling of hot solvent mixture) to afford **11** (1.03 g, 3.08 mmol, 24% yield) as a white, crystalline solid. R_f = 0.3 (25% EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.38–5.32 (m, 1H), 3.57–3.48 (m, 1H), 2.66–2.50 (m, 1H), 2.36–2.17 (m, 2H), 2.22 (d, *J* = 5.3 Hz, 3H), 2.05–1.97 (m, 1H), 1.89–1.40 (complex, 13H), 1.36–1.23 (m, 1H), 1.14–1.05 (m, 1H), 1.04–0.96 (m, 1H), 1.01 (s, 3H), 0.66 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 207.8 (d, *J* = 34 Hz), 140.8, 121.4, 111.3 (d, *J* = 189 Hz), 71.8, 51.7, 49.7, 48.0 (d, *J* = 20 Hz), 42.4, 37.4, 36.6, 32.2 (d, *J* = 22 Hz), 32.0, 31.9, 31.7, 30.9 (d, *J* = 5 Hz), 27.8 (d, *J* = 1 Hz), 23.9 (d, *J* = 1 Hz), 20.7, 19.5, 14.2 (d, *J* = 5 Hz); ^{19}F NMR (376 MHz, $CDCl_3$) δ −156.0 (t, *J* = 34 Hz); IR (film) 3336, 1716 cm^{-1}; mp 163–165 °C (MeOH–H_2O); $[\alpha]_D^{24}$ +15.2 (*c* 1.00, $CHCl_3$); HRMS (APCI) *m/z*: $[M - OH]^+$ calcd for $C_{21}H_{30}FO$ 317.2275, found 317.2274; LCMS: t_R = 7.0 min.



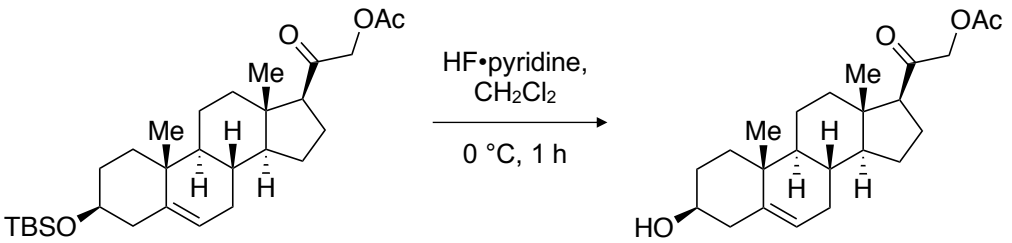
11 **12** (48%)

17α-Fluoropregn-4-ene-3,20-dione (17α-fluoroprogesterone, 12). A flame-dried 10 mL round-bottom flask under Ar was charged with **11** (0.115 g, 0.345 mmol), Al(O*i*-Pr)$_3$ (0.144 g, 0.705 mmol, 2.0 equiv), cyclohexanone (0.71 mL, 6.9 mmol, 20 equiv), and anhydrous toluene (2.6 mL). After equipping a condenser, the reaction mixture was heated to reflux, stirred for 3 h, allowed to cool to rt, and concentrated. Then, the residue was dissolved in CH_2Cl_2 (10 mL), washed with a 1.0 M solution of HCl in H_2O (20 mL), and the aqueous layer was extracted with CH_2Cl_2 (10 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography (24 g of silica gel, 0–50% EtOAc/hexanes) to afford **12** (54.5 mg, 0.164 mmol, 48% yield) as a white, amorphous solid. An analytical sample was prepared by recrystallization from MeOH–H_2O (slow cooling of hot solvent mixture). R_f = 0.3 (25% EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.73 (s, 1H), 2.67–2.49 (m, 1H), 2.47–2.25 (complex, 4H), 2.21 (d, *J* = 5.3 Hz, 3H), 2.02 (ddd, *J* = 13.4, 5.0, 3.2 Hz, 1H), 1.91–1.58 (complex, 8H), 1.56–1.28 (m, 3H), 1.18 (s, 3H), 1.17–1.06 (m, 1H), 1.00 (ddd, *J* = 12.2, 10.3, 4.2 Hz, 1H), 0.69 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 207.6 (d, *J* = 34 Hz), 199.4, 170.7, 124.2, 111.0 (d, *J* = 189 Hz), 53.3, 50.9, 48.0 (d, *J* = 20 Hz), 38.7, 35.8, 35.6, 34.1, 32.8, 32.1 (d, *J* = 22 Hz), 32.0, 30.7 (d, *J* = 5 Hz), 27.7 (d, *J* = 1 Hz), 23.7 (d, *J* = 1 Hz), 20.7, 17.5, 14.3 (d, *J* = 5 Hz); ^{19}F NMR (376 MHz, $CDCl_3$) δ −156.0 (t, *J* = 34 Hz); IR (film) 1716, 1673, 1618 cm^{-1}; mp 172–174 °C (MeOH–H_2O); $[\alpha]_D^{25}$ +186 (*c* 1.00, $CHCl_3$); HRMS (APCI) *m/z*: $[M + H]^+$ calcd for $C_{21}H_{30}FO_2$ 333.2224, found 333.2223; LCMS: t_R = 6.9 min.



2 **S2a** (89%)

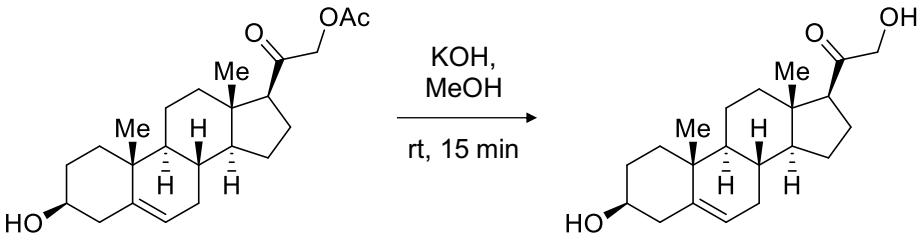
3β-[*tert*-Butyl(dimethyl)silyl]oxy-20-oxopregn-5-en-21-yl acetate (S2a). A 250 mL round-bottom flask was charged with **2** (1.27 g, 2.28 mmol), KOAc (0.717 g, 7.31 mmol, 3.2 equiv), and acetone (114 mL). After equipping a condenser, the reaction mixture was heated to reflux, stirred for 24 h, and allowed to cool to rt. Then, the reaction mixture was concentrated, dissolved in CH_2Cl_2 (30 mL), washed with H_2O (30 mL), and the aqueous layer was extracted with CH_2Cl_2 (2 × 30 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by column chromatography (40 g of silica gel, 0–10% EtOAc/hexanes) to afford **S2a** (0.987 g, 2.02 mmol, 89% yield) as a white, amorphous solid. R_f = 0.3 (10% EtOAc/hexanes); ^{1}H NMR (400 MHZ, $CDCl_3$) δ 5.33–5.29 (m, 1H), 4.62 (AB q, $\Delta\nu_{AB}$ = 67.4 Hz, J_{AB} = 16.8 Hz, 2H), 3.53–3.42 (m, 1H), 2.50 (t, J = 8.8 Hz, 1H), 2.33–2.13 (m, 3H), 2.16 (m, 3H), 2.07–1.94 (m, 2H), 1.81 (dt, J = 13.2, 3.5 Hz, 1H), 1.77–1.23 (complex, 10H), 1.20–0.93 (m, 3H), 0.99 (m, 3H), 0.88 (s, 9H), 0.66 (s, 3H), 0.05 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 203.9, 170.4, 141.7, 120.9, 72.7, 69.3, 59.5, 57.3, 50.2, 44.9, 42.9, 38.7, 37.5, 36.8, 32.2, 32.04, 31.95, 26.1 (3C), 24.7, 23.0, 21.2, 20.7, 19.6, 18.4, 13.2, −4.4 (2C); IR (neat) 1744, 1720 cm^{-1}; mp 159–160 °C; $[\alpha]_D^{22}$ +31.6 (*c* 1.00, $CHCl_3$); HRMS (APCI) *m/z*: $[M + H]^+$ calcd for $C_{29}H_{29}O_4Si$ 489.3395, found 489.3399.



S2a **S2b** (89%)

3β-Hydroxy-20-oxopregn-5-en-21-yl acetate (S2b). A 20 mL polypropylene vial was charged with **S2a** (0.398 g, 0.814 mmol) and CH_2Cl_2 (17 mL). The reaction mixture was cooled to 0 °C, and Olah's reagent (0.50 mL, ca. 70 wt% HF in pyridine, ca. 20 equiv) was added over 3 min. After stirring at 0 °C for

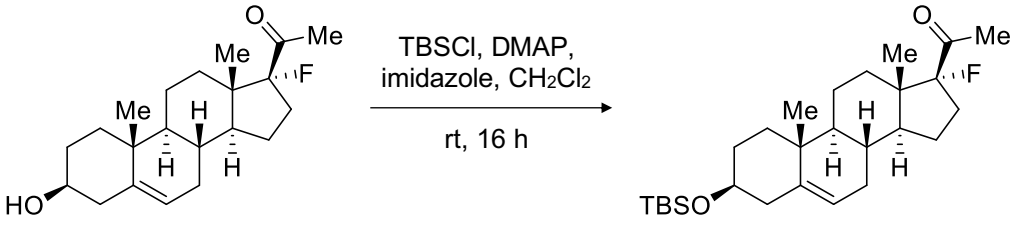
1 h, the reaction mixture poured into an ice-cold solution of saturated $NaHCO_3$ in H_2O (10 mL), and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by column chromatography (24 g of silica gel, 0–50% EtOAc/hexanes) to afford **S2b** (0.271 g, 0.724 mmol, 89% yield) as a white, amorphous solid. R_f = 0.5 (50% EtOAc/hexanes); ^{1}H NMR (400 MHZ, $CDCl_3$) δ 5.36–5.32 (m, 1H), 4.62 (AB q, $\Delta\nu_{AB}$ = 70.0 Hz, J_{AB} = 16.8 Hz, 2H), 3.57–3.46 (m, 1H), 2.50 (t, J = 8.8 Hz, 1H), 2.33–2.14 (m, 3H), 2.16 (s, 3H), 2.07–1f.96 (m, 2H), 1.89–1.80 (m, 2H), 1.77–1.23 (complex, 10H), 1.20–1.04 (m, 2H), 1.03–0.93 (m, 1H), 1.00 (s, 3H), 0.67 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.9, 170.4, 140.9, 121.4, 71.8, 69.3, 59.4, 57.2, 50.1, 44.8, 42.4, 38.7, 37.4, 36.7, 32.0, 31.9, 31.7, 24.7, 23.0, 21.2, 20.6, 19.5, 13.2; IR (neat) 3553, 1749, 1721 cm^{-1}; mp 184–185 °C, lit.[5] mp 184–185 °C (acetone); $[\alpha]_D^{22}$ +29.2 (*c* 1.00, $CHCl_3$); HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{23}H_{35}O_4$ 375.2530, found 375.2531.



S2b **S3** (95%)

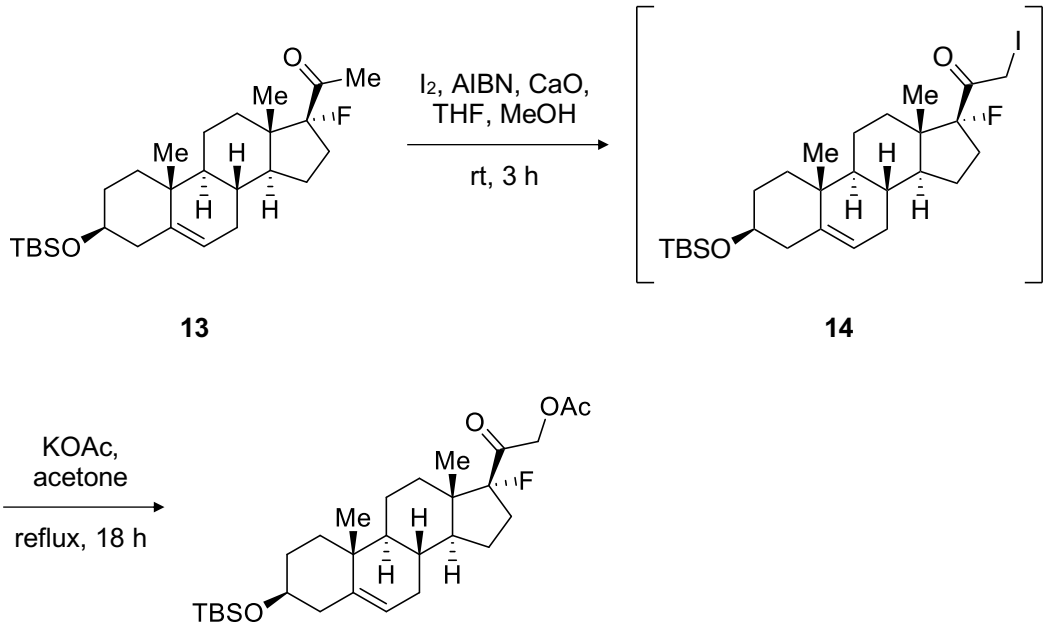
3β,21-Dihydroxypregn-5-en-20-one (21-hydroxypregnenolone, S3). A flame-dried 2 dram vial under Ar was charged with **S2b** (0.120 g, 0.322 mmol) and anhydrous MeOH (3.6 mL). The reaction mixture was sparged with Ar for 15 min, and a 2.6 M solution of KOH in MeOH (0.20 mL, 0.52 mmol, 1.6 equiv), which had been sparged with N_2 for 15 min, was added. After stirring for 15 min, the reaction mixture was neutralized with a 1.0 M solution of HCl in H_2O (2 mL), diluted with H_2O (8 mL), and the aqueous layer was extracted with CH_2Cl_2 (2 × 10 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography (12 g of silica gel, 0–50% EtOAc/hexanes) to afford **S3** (0.102 g, 0.306 mmol, 95% yield) as a white, amorphous solid. An analytical sample was prepared by recrystallization from acetone (slow evaporation of solvent). R_f = 0.4 (50% EtOAc/hexanes); ^{1}H NMR (400 MHZ, $CDCl_3$) δ 5.37–5.31 (m, 1H), 4.18 (AB of ABX, $\Delta\nu_{AB}$ = 21.9 Hz, J_{AB} = 18.9 Hz, J_{AX} = 4.8 Hz, J_{BX} = 4.3 Hz, 2H), 3.57–3.46 (m, 1H), 3.27 (X of ABX, t, J_{AX} = J_{BX} = 4.7 Hz, 1H), 2.45 (t, J = 9.0 Hz, 1H), 2.35–2.16 (m, 3H), 2.05–1.96 (m, 1H), 1.95–1.89 (m, 1H), 1.88–1.80 (m, 2H), 1.79–1.69

(m, 2H), 1.66–1.23 (complex, 8H),1.21–0.93 (m, 3H), 1.00 (s, 3H), 0.65 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 210.4, 140.9, 121.4, 71.8, 69.6, 59.4, 57.1, 50.1, 44.9, 42.3, 38.7, 37.4, 36.7, 32.0, 31.9, 31.7, 24.7, 23.1, 21.1, 19.5, 13.5; IR (neat) 3315, 1716, 1694 cm^{-1}; mp 175–178 °C (acetone), lit.[6] mp 168–170 °C (acetone); $[\alpha]_D^{23}$ +4.8 (*c* 1.00, $CHCl_3$), lit.[6] $[\alpha]_D^{20}$ +8.9 (*c* 1, $CHCl_3$); HRMS (ESI) *m/z*: $[M - OH]^+$ calcd for $C_{21}H_{31}O_2$ 315.2319, found 315.2322; LCMS: t_R = 4.3 min.



11 **13** (95%)

3β-[*tert*-Butyl(dimethyl)silyl]oxy-17α-fluoropregn-5-en-20-one (13). A flame-dried 25 mL round-bottom flask under Ar was charged with **11** (0.831 g, 2.48 mmol), TBSCl (0.679 g, 4.50 mmol, 1.8 equiv), DMAP (30.9 mg, 0.252 mmol, 0.1 equiv), imidazole (0.386 g, 5.67 mmol, 2.3 mmol), and anhydrous CH_2Cl_2 (14 mL). After stirring for 16 h, a saturated solution of NH_4Cl in H_2O (20 mL) was added, and the aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography (40 g of silica gel, 0–5 vol% EtOAc/hexanes) to afford **13** (1.06 g, 2.36 mmol, 95% yield) as a white, amorphous solid. R_f = 0.3 (5% EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.34–5.30 (m, 1H), 3.54–3.43 (m, 1H), 2.68–2.49 (m, 1H), 2.32–2.13 (m, 2H), 2.22 (d, *J* = 5.3 Hz, 3H), 2.06–1.97 (m, 1H), 1.90–1.39 (complex, 12H), 1.37–1.23 (m, 1H), 1.11–0.95 (m, 2H), 1.00 (s, 3H), 0.89 (s, 9H), 0.66 (s, 3H), 0.06 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 207.7 (d, *J* = 34 Hz), 141.6, 120.9, 111.4 (d, *J* = 189 Hz), 72.6, 51.8, 49.8, 48.0 (d, *J* = 20 Hz), 42.9, 37.5, 36.7, 32.19, 32.18 (d, *J* = 22.2 Hz), 32.02, 32.00, 30.9 (d, *J* = 5 Hz), 27.8 (d, *J* = 1 Hz), 26.1 (3C), 23.9, 20.7, 19.6, 18.4, 14.2 (d, *J* = 5 Hz), −4.4 (2C); ^{19}F NMR (376 MHz, $CDCl_3$) δ −156.0 (t, *J* = 35 Hz); IR (neat) 1717 cm^{-1}; mp 139–141 °C; $[\alpha]_D^{24}$ +20.0 (*c* 1.00, $CHCl_3$); HRMS (ESI) *m/z*: $[M + Na]^+$ calcd for $C_{27}H_{45}FO_2SiNa$ 471.3065, found 471.3064.



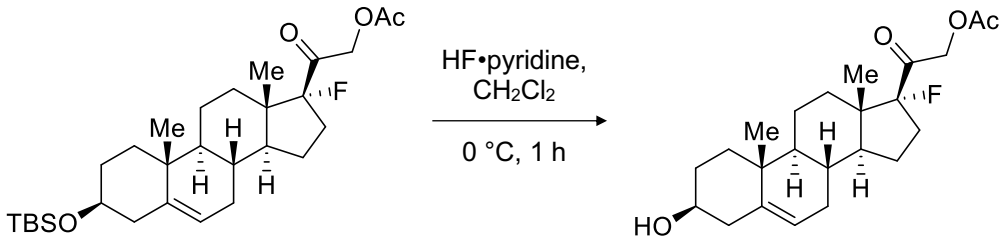
13 **14**

15 (61% over 2 steps)

3β-[*tert*-Butyl(dimethyl)silyl]oxy-17α-fluoro-20-oxopregn-5-en-21-yl acetate (15). A flame-dried 25 mL round-bottom flask under Ar was charged with **13** (0.700 g, 1.56 mmol), CaO (0.875 g, 15.6 mmol, 10.0 equiv), AIBN (77.6 mg, 0.473 mmol, 0.3 equiv), anhydrous MeOH (3.75 mL), and THF (3.8 mL). Then, a solution of I_2 (0.555 g, 2.19 mmol, 1.4 equiv) in anhydrous MeOH (1.7 mL) and THF (3.6 mL) was added over 5 min. After stirring for 3 h, the reaction mixture was filtered with CH_2Cl_2 (3 × 10 mL), the filtrate was washed with a 10 wt% solution of $Na_2S_2O_3$ in H_2O (30 mL), and the aqueous layer was extracted with CH_2Cl_2 (2 × 30 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated in a 250 mL round-bottom flask.

Crude **14** was dissolved in acetone (75 mL), and KOAc (0.499 g, 5.08 mmol, 3.3 equiv) was added in one portion. After equipping a condenser, the reaction mixture was heated to reflux, stirred for 16 h, allowed to cool to rt, and concentrated. The residue was dissolved in CH_2Cl_2 (30 mL), washed with H_2O (30 mL), and the aqueous layer was extracted with CH_2Cl_2 (2 × 30 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography (80 g of silica gel, 0–5% EtOAc/hexanes) to afford **15** (0.490 g, 0.967 mmol, 62% yield) as a white, amorphous solid. R_f = 0.2 (5% EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.35–5.28 (m, 1H), 4.91 (AB of ABX, $\Delta\nu_{AB}$ = 22.3 Hz, J_{AB} = 18.0 Hz, J_{AX} = 1.3 Hz, J_{BX} = 4.5 Hz, 2H), 3.54–3.42 (m, 1H), 2.66–2.44 (m,

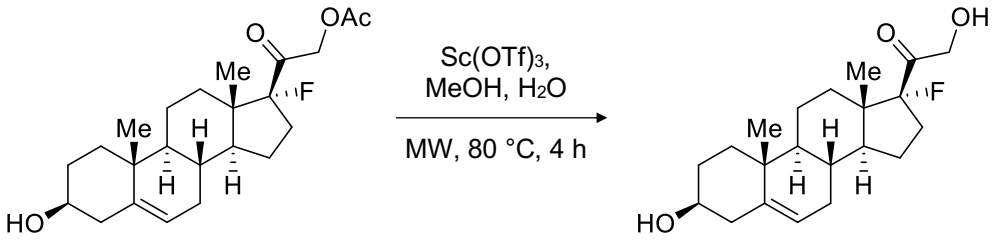
1H), 2.35–2.21 (m, 1H), 2.17 (s, 3H), 2.06–1.97 (m, 1H), 1.96–1.28 (complex, 14H), 1.12–0.95 (m, 2H), 1.00 (s, 3H), 0.89 (s, 9H), 0.70 (s, 3H), 0.06 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 202.1 (d, *J* = 33 Hz), 170.5, 141.7, 120.8, 111.8 (d, *J* = 185 Hz), 72.6, 68.0 (d, *J* = 8 Hz), 51.8, 49.7, 48.9 (d, *J* = 19 Hz), 42.9, 37.5, 36.7, 33.1 (d, *J* = 22 Hz), 32.2, 32.04, 32.01, 30.2 (d, *J* = 5 Hz), 26.1 (3C), 24.0, 20.7, 20.6, 19.6, 18.4, 13.7 (d, *J* = 5 Hz), −4.4 (2C); ^{19}F NMR (376 MHz, $CDCl_3$) δ −164.7 to −165.0 (X of ABX, m); IR (film) 1755, 1738 cm^{-1}; mp 127–130 °C; $[\alpha]_D^{24}$ +30.0 (*c* 1.00, $CHCl_3$); HRMS (ESI) *m/z*: $[M + Na]^+$ calcd for $C_{29}H_{47}FO_4SiNa$ 529.3120, found 529.3122.



15 **16a** (93%)

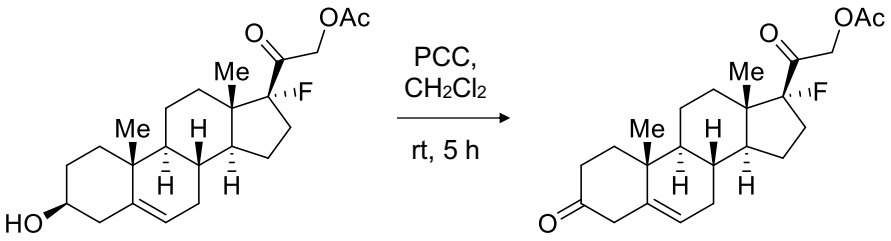
17α-Fluoro-3β-hydroxy-20-oxopregn-5-en-21-yl acetate (16a). A 20 mL polypropylene vial was charged with **15** (0.436 g, 0.861 mmol) and CH_2Cl_2 (17 mL). The reaction mixture was cooled to 0 °C, and Olah's reagent (0.45 mL, ca. 70 wt% HF in pyridine, ca. 20 equiv) was added over 3 min. After stirring at 0 °C for 1 h, the reaction mixture poured into an ice-cold solution of saturated $NaHCO_3$ in H_2O (30 mL), and the aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by column chromatography (40 g of silica gel, 0–50% EtOAc/hexanes) to afford **16a** (0.316 g, 0.805 mmol, 93% yield) as a white, amorphous solid. An analytical sample was prepared by recrystallization from acetone–H_2O (slow cooling of hot solvent mixture). R_f = 0.5 (50% EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.38–5.32 (m, 1H), 4.90 (AB of ABX, $\Delta\nu_{AB}$ = 27.2 Hz, J_{AB} = 17.9 Hz, J_{AX} = 1.5 Hz, J_{BX} = 4.5 Hz, 2H), 3.58–3.46 (m, 1H), 2.65–2.43 (m, 1H), 2.35–2.18 (m, 2H), 2.17 (s, 3H), 2.07–1.97 (m, 1H),1.96–1.43 (complex, 13H), 1.42–1.26 (m, 1H), 1.15–0.95 (m, 2H), 1.01 (s, 3H), 0.70 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 202.1 (d, *J* = 33 Hz), 170.5, 140.9, 121.3, 111.8 (d, *J* = 185 Hz), 71.8, 68.0 (d, *J* = 8 Hz), 51.7, 49.6, 48.9 (d, *J* = 19 Hz), 42.4, 37.4, 36.6, 33.1 (d, *J* = 22 Hz), 32.01, 31.96, 31.7, 30.2 (d, *J* = 5 Hz), 24.0, 20.7, 20.6, 19.5, 13.7 (d, *J* = 5 Hz); ^{19}F NMR (376 MHz, $CDCl_3$) δ −164.7 to −165.0 (X of ABX, m); IR (neat) 3580, 1749, 1734 cm^{-1}; mp 218–220 °C (acetone–

H_2O); $[\alpha]_D^{24}$ +35.0 (*c* 1.00, $CHCl_3$); HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{23}H_{34}FO_4$ 393.2436, found 393.2436.



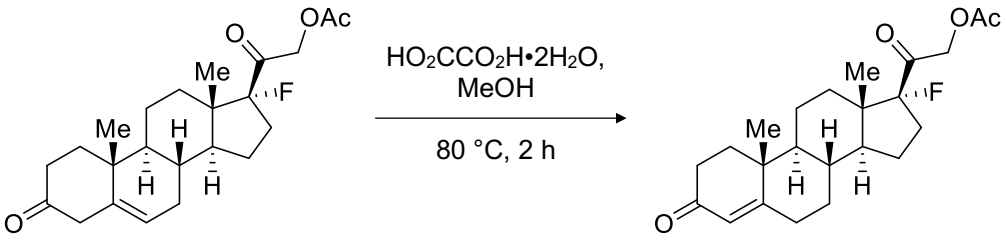
16a **16b** (36%)

17α-Fluoro-3β,21-dihydroxypregn-5-en-20-one (17α-fluoro-21-hydroxypregnenolone, 16b). A 5 mL microwave vial was charged with **16a** (0.100 g, 0.255 mmol), $Sc(OTf)_3$ (26.2 mg, 53.2 μmol, 0.2 equiv), MeOH (2.0 mL), and H_2O (0.50 mL). The reaction mixture was stirred at 80 °C for 4 h using a microwave reactor (time measured when the reaction mixture reached the programmed temperature after a ramp period of ca. 1 min). After cooling to rt, a saturated solution of $NaHCO_3$ in H_2O (10 mL) was added, and the aqueous layer was extracted with CH_2Cl_2 (2 × 10 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography (24 g of silica gel, 0–50% EtOAc/hexanes) to afford **16b** (32.0 mg, 91.3 μmol, 36% yield) as a white, amorphous solid. An analytical sample was prepared by recrystallization from Et_2O–hexanes (slow evaporation of solvent mixture). R_f = 0.4 (50% EtOAc/hexanes); ^1H NMR (400 MHz, $CDCl_3$) δ 5.38–5.32 (m, 1H), 4.60 (dt, *J* = 20.4, 4.4 Hz, 1H), 4.31 (ddd, *J* = 20.5, 5.2, 1.3 Hz, 1H), 3.58–3.47 (m, 1H), 3.01 (t, *J* = 5.0 Hz, 1H), 2.66–2.46 (m, 1H), 2.35–2.18 (m, 2H), 2.08–1.31 (complex, 15H), 1.15–0.95 (m, 2H), 1.01 (s, 3H), 0.71 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 209.3 (d, *J* = 34 Hz), 140.9, 121.3, 110.9 (d, *J* = 186 Hz), 71.7, 67.6 (d, *J* = 7 Hz), 51.7, 49.6, 49.0 (d, *J* = 19 Hz), 42.3, 37.3, 36.6, 33.5 (d, *J* = 22 Hz), 32.0, 31.9, 31.7, 30.4 (d, *J* = 5 Hz), 24.0, 20.6, 19.5, 14.1 (d, *J* = 5 Hz); ^{19}F NMR (376 MHz, $CDCl_3$) δ −166.1 to −166.3 (m); IR (neat) 3281, 1730 cm^{-1}; mp 173–176 °C (Et_2O–hexanes, dec); $[\alpha]_D^{24}$ +16.0 (*c* 1.00, $CHCl_3$); HRMS (ESI) *m/z*: $[M - OH]^+$ calcd for $C_{21}H_{30}FO_2$ 333.2224, found 333.2227; LCMS: t_R = 4.5 min.



16a **S4** (63%)

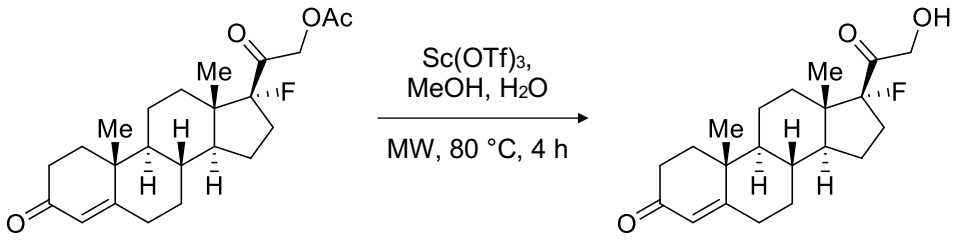
17α-Fluoro-3,20-dioxopregn-5-en-21-yl acetate (S4). A 20 mL vial was charged with **16a** (0.250 g, 0.637 mmol), Celite (1.00 g), and CH_2Cl_2 (13 mL). The reaction mixture was cooled to 0 °C, and PCC (0.156 g, 0.724 mmol, 1.1 equiv) was added in one portion. After warming to rt, the reaction mixture was stirred for 5 h and concentrated. The crude product adsorbed onto Celite was purified by column chromatography (24 g of silica gel, 0–25% EtOAc/hexanes) to afford **S4** (0.156 g, 0.399 mmol, 63% yield) as a white, amorphous solid. R_f = 0.4 (25% EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.37–5.32 (m, 1H), 4.90 (AB of ABX, $\Delta\nu_{AB}$ = 38.1 Hz, J_{AB} = 17.9 Hz, J_{AX} = 1.5 Hz, J_{BX} = 4.4 Hz, 2H), 3.32–3.23 (m, 1H), 2.83 (dd, *J* = 16.4, 2.2 Hz, 1H), 2.66–2.42 (m, 2H), 2.35–2.26 (m, 1H), 2.17 (s, 3H), 2.12–2.00 (m, 2H), 1.99–1.30 (complex, 11H), 1.19 (s, 3H), 1.15–1.06 (m, 1H), 0.73 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 210.0, 202.0 (d, *J* = 33 Hz), 170.5, 138.7, 122.5, 111.7 (d, *J* = 185 Hz), 68.0 (d, *J* = 8 Hz), 51.6, 48.9 (d, *J* = 19 Hz), 48.7, 48.4, 37.7, 37.04, 36.96, 33.1 (d, *J* = 22 Hz), 32.0, 31.8, 30.1 (d, *J* = 5 Hz), 23.9, 21.0, 20.6, 19.3, 13.7 (d, *J* = 5 Hz); ^{19}F NMR (376 MHz, $CDCl_3$) δ −164.8 to −165.1 (X of ABX, m); IR (neat) 1747, 1722 cm^{-1}; mp 154–157 °C (dec); $[\alpha]_D^{24}$ +73.8 (*c* 1.00, $CHCl_3$); HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{23}H_{32}FO_4$ 391.2279, found 391.2280.



S4 **17a** (90%)

17α-Fluoro-3,20-dioxopregn-4-en-21-yl acetate (17a). A 5 mL microwave vial was charged with **S4** (0.156 g, 0.399 mmol), $HO_2CCO_2H•2H_2O$ (0.253 g, 2.01 mmol, 5.0 equiv), and MeOH (4.0 mL). The vial was capped, heated to 80 °C, and stirred for 2 h. After cooling to rt, the reaction mixture was concentrated

under a stream of N_2 and dissolved in Et_2O (20 mL). The organic layer was washed with a saturated solution of $NaHCO_3$ in H_2O (10 mL) followed by H_2O (10 mL), dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by column chromatography (12 g of silica gel, 0–25% EtOAc/hexanes) to afford **17a** (0.140 g, 0.360 mmol, 90% yield) as a white, amorphous solid. R_f = 0.2 (25% EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.73 (s, 1H), 4.88 (AB of ABX, $\Delta\nu_{AB}$ = 39.5 Hz, J_{AB} = 17.9 Hz, J_{AX} = 1.2 Hz, J_{BX} = 4.4 Hz, 2H), 2.65–2.24 (complex, 5H), 2.17 (s, 3H), 2.06–1.99 (m, 1H), 1.97–1.58 (complex, 9H), 1.53–1.32 (m, 2H), 1.18 (s, 3H), 1.17–1.05 (m, 1H), 0.99 (ddd, *J* = 12.3, 9.9, 4.0 Hz, 1H), 0.73 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 201.9 (d, *J* = 33 Hz), 199.5, 170.6, 170.5, 124.2, 111.5 (d, *J* = 185 Hz), 68.0 (d, *J* = 8 Hz), 53.2, 50.9, 48.8 (d, *J* = 19 Hz), 38.7, 35.8, 35.6, 34.0, 33.0 (d, *J* = 22 Hz), 32.8, 32.0, 30.0 (d, *J* = 5 Hz), 23.8, 20.7, 20.6, 17.5, 13.7 (d, *J* = 5 Hz); ^{19}F NMR (376 MHz, $CDCl_3$) δ −164.8 to −165.1 (X of ABX, m); IR (neat) 1754, 1740, 1673, 1619 cm^{-1}; mp 167–169 °C; $[\alpha]_D^{24}$ +189 (*c* 1.00, $CHCl_3$); HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{23}H_{32}FO_4$ 391.2279, found 391.2278.



17a **17b** (57%)

17α-Fluoro-21-hydroxypregn-4-ene-3,20-dione (17α-fluoro-21-hydroxyprogesterone, 17b). A 5 mL microwave vial was charged with **17a** (0.100 g, 0.256 mmol), $Sc(OTf)_3$ (26.9 mg, 54.7 μmol, 0.2 equiv), MeOH (2.0 mL), and H_2O (0.52 mL). The reaction mixture was stirred at 80 °C for 4 h using a microwave reactor (time measured when the reaction mixture reached the programmed temperature after a ramp period of ca. 1 min). After cooling to rt, a saturated solution of $NaHCO_3$ in H_2O (10 mL) was added, and the aqueous layer was extracted with CH_2Cl_2 (2 × 10 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography (24 g of silica gel, 0–50% EtOAc/hexanes) to afford **17b** (50.7 mg, 0.145 mmol, 57% yield) as a white, amorphous solid. An analytical sample was prepared by recrystallization from EtOAc (slow evaporation of solvent). R_f = 0.4 (50% EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.74 (s, 1H), 4.60 (dt, *J* = 20.5, 4.6 Hz, 1H), 4.31 (ddd, *J* = 20.6, 5.4, 1.2 Hz, 1H), 2.99 (t, *J* = 4.9 Hz, 1H), 2.66–2.26 (complex, 5H), 2.07–1.98 (m, 1H), 1.93–

1.60 (complex, 8H), 1.50–1.35 (m, 3H), 1.21–1.06 (m, 1H), 1.19 (s, 3H), 1.03–0.65 (m, 1H), 0.75 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 209.1 (d, *J* = 35 Hz), 199.4, 170.4, 124.2, 110.6 (d, *J* = 186 Hz), 67.6 (d, *J* = 7 Hz), 53.2, 50.9, 49.0 (d, *J* = 19 Hz), 38.6, 35.8, 35.6, 34.0, 33.4 (d, *J* = 22 Hz), 32.8, 32.0, 30.3 (d, *J* = 5 Hz), 23.8, 20.6, 17.5, 14.2 (d, *J* = 5 Hz); ^{19}F NMR (376 MHz, $CDCl_3$) δ −166.1 to −166.3 (m); IR (neat) 3462, 1724, 1648, 1608 cm^{-1}; mp 176–178 °C (EtOAc, dec); $[\alpha]_D^{24}$ +189 (c 1.00, $CHCl_3$); HRMS (ESI) *m/z*: $[M + H]^+$ calcd for $C_{21}H_{30}FO_3$ 349.2173, found 349.2174; LCMS: t_R = 4.5 min.

Plasmid Constructs. Recombinantly expressed human CYP17A1, CYP21A2, human full-length NADPH cytochrome P450 reductase, and human full-length cytochrome b_5 enzymes were used in this study. The P450 genes were constructed synthetically with codon optimization for expression in *E. coli* and modified to omit the single, N-terminal transmembrane helix and add a C-terminal histidine tag for both CYP17A1Δ19H and CYP21A2dH as described.[7, 8] A synthetic, codon-optimized gene of human full-length NADPH-cytochrome P450 reductase in pET-29a(+) and human full-length cytochrome b_5 in pET-15b are as described.[9]

Enzyme Expression and Purification. Human cytochrome P450 enzyme CYP17A1 and CYP21A2 were expressed and purified as described.[8] The purity, quality, and quantity of P450 proteins were assessed by the UV–Vis spectrum, SDS–PAGE, and the reduced carbon monoxide difference spectrum. Concentrations of P450 enzymes for functional assays were determined from the reduced carbon monoxide difference spectra in the presence of 2 μM progesterone as a ligand. In spectral binding assays, the P450 concentration was determined by the Soret band in the absolute spectrum using an extinction coefficient of 100 mM^{-1}cm^{-1}. Human full-length NADPH-cytochrome P450 reductase (CPR) enzyme was expressed and purified as described.[9] Final reductase samples were evaluated on SDS–PAGE and by UV–Vis spectroscopy. CPR was quantitated by flavin absorbance of the fully oxidized protein at 454 nm with an extinction coefficient of 21.4 mM^{-1}cm^{-1}. Human full-length cytochrome b_5 (b_5) was expressed and purified as described[9] and quantified by the dithionite-reduced Soret band using a extinction coefficient of 185 mM^{-1}cm^{-1}.

Functional Assays. Endogenous substrates and their fluorinated analogs were evaluated for turnover by CYP17A1 and CYP21A2. The enzyme mixture used for the 17α-hydroxylase assays consisted of CYP17A1 (20 pmol) and CPR (80 pmol); b_5 (80 pmol) was also added for the 17,20-lyase assays.

Depending on the substrate, the enzyme mixture was varied for the 21-hydroxylase assays: 0.5 pmol of CYP21A2 and 2 pmol of CPR for progesterone, 17α-fluoroprogesterone (**12**), and 17α-hydroxyprogesterone; 5 pmol of CYP21A2 and 20 pmol of CPR for 21-fluoropregnenolone (**3b**) and 21-fluoroprogesterone (**5**); 20 pmol of CYP21A2 and 80 pmol of CPR for pregnenolone and 17α-fluoropregnenolone (**11**). Stocks of all substrates were prepared and serially diluted with DMSO. For the CYP17A1 functional assays, the following substate concentrations were used: 0.1–100 μM for pregnenolone; 0.4–100 μM for **3b**; 50 and 100 μM for **11**; 0.02–20 μM for 17α-hydroxypregnenolone (**S1b**) and 21-fluoro-17α-hydroxypregnenolone (**8b**); 0.8–100 μM for progesterone, **5**, and **12**; 0.2–50 μM for 17α-hydroxyprogesterone and 21-fluoro-17α-hydroxyprogesterone (**10**). For the CYP21A2 functional assays, the following substate concentrations were used: 0.1–100 μM for pregnenolone and **11**; 50 and 100 μM for **3b**; 0.01–10 μM for progesterone and **12**; 0.8–200 μM for **5**; 0.04–40 μM for 17α-hydroxyprogesterone. An equal volume (2.5 μL) of each serially diluted substrate was mixed with the reaction buffer (50 mM of Tris-HCl and 5 mM of $MgCl_2$ in H_2O, pH 7.4) to obtain the desired substrate molarity with constant final DMSO concentration (0.5%). Samples containing enzyme, substrate, and reaction buffer were incubated for 3 min in a 37 °C water bath, and reactions were initiated by adding a 1 mM solution of NADPH in H_2O, bring the total volume to 200 μL. After incubating at 37 °C for 10 min, reactions were sequentially stopped by adding a 10.0 M solution of HCl in H_2O (150 μL).

To quantify substrate and product concentrations by LC-MS/MS, the following deuterated internal standards (CDN Isotopes) were used: pregnenolone-d_4, 17α-hydroxypregnenolone-d_3, dehydroepiandrosteron-d_6, progesterone-d_9, 17α-hydroxyprogesterone-d_8, androstenedione-d_7, and 21-deoxycorticosterone-d_8. A 50 μM stock of each standard was prepared in MeCN, and solution were combined together to contain either (1) pregnenolone-d_4, 17α-hydroxypregnenolone-d_3, and dehydroepiandrosterone-d_6, or (2) progesterone-d_9, 17α-hydroxyprogesterone-d_8, and androstenedione-d_7 (replaced by 21-deoxycorticosterone-d_8 in the 21-hydroxylase assays). These two mixtures were diluted with HPLC-grade H_2O to obtain a final concentration of 33 nM (ca. 3 ng/mL) for each deuterated internal standard. In separate 1.5 mL vials, portions of the appropriate internal standard solution (200 μL) were mixed with aliquots (100 μL) taken from the functional assay samples. Each aqueous layer was then

extracted with MTBE (1 mL). After vortexing and allowing phase separation, a part of the upper organic layer (500 μL) was removed, placed into a new 1.5 mL vial, and dried using a SpeedVac.

If pregnenolone or one of its analogs was used as the substrate, samples were derivatized by adapting a previously reported procedure.[10] Specifically, a stock of picolinic acid (25 mg), 2-methyl-6-nitrobenzoic anhydride (20 mg), and DMAP (10 mg) in anhydrous MeCN (1 mL) was used for derivatization. An aliquot of this solution (100 μL) was added to each sample, which was then briefly vortexed. After sitting at rt for ca. 1 h, reactions were quenched with LCMS-grade H_2O (300 μL), dried on a SpeedVac, and reconstituted in 50% MeOH/H_2O (300 μL). Analyte concentrations were then measured with a Agilent 1260 binary pump HPLC coupled to a 6490 triple quadrupole mass spectrometer with an ESI probe. Separations were performed using a 2D-LC method with a ThermoScientific Hypersil GOLD C4 column (10 × 3 mm, 3 μm particle size) and a Phenomenex Kinetex biphenyl column (50 × 2.1 mm, 3 μm particle size) serving as the first and second dimensions, respectively. The mobile phase was comprised of 0.2 mM NH_4 in H_2O (solvent A) and 0.2 mM NH_4F in MeOH (solvent B). Each sample (1–5 μL) was injected onto the first dimension column at 30 °C, and the solvent gradient was as follows: 50% B held for 2.7 min (flow rate = 0.5 mL/min), 100% B held from 2.7–7.2 min (flow rate = 0.5 mL/min from 2.7–4.0 min, 0.1 mL/min from 4.0–5.2 min, and 0.5 mL/min from 5.2–7.2 min), and 50% B held from 7.2–9.0 min (flow rate = 0.5 mL/min). From 2.6–5.4 min, the valve was switched from waste to the second dimension (temperature = 45 °C), and analytes were resolved at a flow rate of 0.5 mL/min using the following solvent gradient: 60% B held for 5.4 min, 75% B ramped to 95% B from 5.4–9.0 min, 95% B held from 9.0–9.1 min, 100% B held from 9.1–10.2 min, and 60% B held from 10.2–10.3 min.

If progesterone or one of its analogs was used as the substrate, samples were immediately reconstituted in 50% MeOH/H_2O (300 μL) following extraction with MTBE. Using the same instrument and mobile phase from before, separations were performed using only the Phenomenex Kinetex biphenyl column (50 × 2.1 mm, 3 μm particle size). Each sample (1–5 μL) was introduced at a flow rate of 0.5 ml/min onto the column at 45 °C, and the solvent gradient was as follows: 40% B ramped to 83% B over 8.5 min, 100% B held from 8.5–9.5 min, and 40% B held from 9.5–11.5 min.

Analytes were detected on the mass spectrometer in multiple reaction monitoring mode and identified by their mass transitions (Table S1).

Table S1. Detection parameters for steroid analytes.

Analyte	Parent Ion	Transition (*m/z*)	Dwell (ms)	CE (V)	t_R (min)	Internal Standard
Internal Standards						
Pregnenolone-d_6	[M + C_6H_4NO]	426.2 → 303.2	30	9	8.4	N/A
17α-Hydroxypregnenolone-d_3	[M + C_6H_4NO]	441.2 → 318.2	30	9	7.1	N/A
Dehydroepiandrosterone-d_6	[M + C_6H_4NO]	400.2 → 277.2	30	9	7.7	N/A
Progesterone-d_9	[M + H]	324.1 → 100.1	20	28	8.1	N/A
17α-Hydroxyprogesterone-d_8	[M + H]	339.2 → 100.0	20	25	6.6	N/A
Androstendione-d_7	[M + H]	294.0 → 100.0	20	30	6.3	N/A
11-Deoxycorticosterone-d_8	[M + H]	339.2 → 113.0	20	25	6.9	N/A
Δ^5-Steroids						
Pregnenolone	[M + C_6H_4NO]	422.3 → 299.3	30	5	8.3	Pregnenolone-d_6
17α-Hydroxypregnenolone (**S1b**)	[M + C_6H_4NO]	438.2 → 315.1	30	5	7.1	17α-Hydroxypregnenolone-d_3
Dehydroepiandrosterone	[M + C_6H_4NO]	394.3 → 271.2	30	9	7.7	Dehydroepiandrosterone-d_6
21-Hydroxypregnenolone (**S3**)	[M + C_6H_4NO]	438.2 → 297.3	30	13	7.6	17α-Hydroxypregnenolone-d_3
21-Fluoropregnenolone (**3b**)	[M + C_6H_4NO]	440.2 → 317.3	20	9	8.1	Pregnenolone-d_6
21-Fluoro-17α-hydroxypregnenolone (**8b**)	[M + C_6H_4NO]	456.2 → 315.3	20	13	7.1	17α-Hydroxypregnenolone-d_3
17α-Fluoropregnenolone (**11**)	[M + C_6H_4NO]	440.2 → 317.3	20	7	8.2	Pregnenolone-d_6
17α-Fluoro-21-hydroxypregnenolone (**16b**)	[M + C_6H_4NO]	456.2 → 333.2	20	5	7.1	17α-Hydroxypregnenolone-d_3

Analyte	Parent Ion	Transition (*m/z*)	Dwell (ms)	CE (V)	t_R (min)	Internal Standard
Δ^4-Steroids						
Progesterone*	[M + H]	315.2 → 109.0	20	25	7.6 or 8.1	Progesterone-d_9
17α-Hydroxyprogesterone*	[M + H]	331.2 → 313.1	20	13	5.7 or 6.6	17α-Hydroxyprogesterone-d_8
Androstenedione	[M + H]	287.0 → 97.0	20	20	6.4	Androstendione-d_7
11-Deoxycorticosterone	[M + H]	331.2 → 97.0	20	25	6.9	11-Deoxycorticosterone-d_8
11-Deoxycortisol	[M + H]	347.2 → 109.0	20	25	7.5	11-Deoxycorticosterone-d_8
21-Fluoroprogesterone (**5**)	[M + H]	332.2 → 109.0	20	30	7.4	Progesterone-d_9
21-Fluoro-17α-hydroxyprogesterone (**10**)	[M + H]	349.2 → 109.1	20	34	5.7	17α-Hydroxyprogesterone-d_8
17α-Fluoroprogesterone (**12**)	[M + H]	333.2 → 97.0	20	26	8.2	Progesterone-d_9
17α-Fluoro-21-hydroxyprogesterone (**17b**)	[M + H]	349.2 → 109.1	20	34	6.8	11-Deoxycorticosterone-d_8

Note: Fragmentor and collision cell accelerator voltages were 380 and 4 V, respectively.

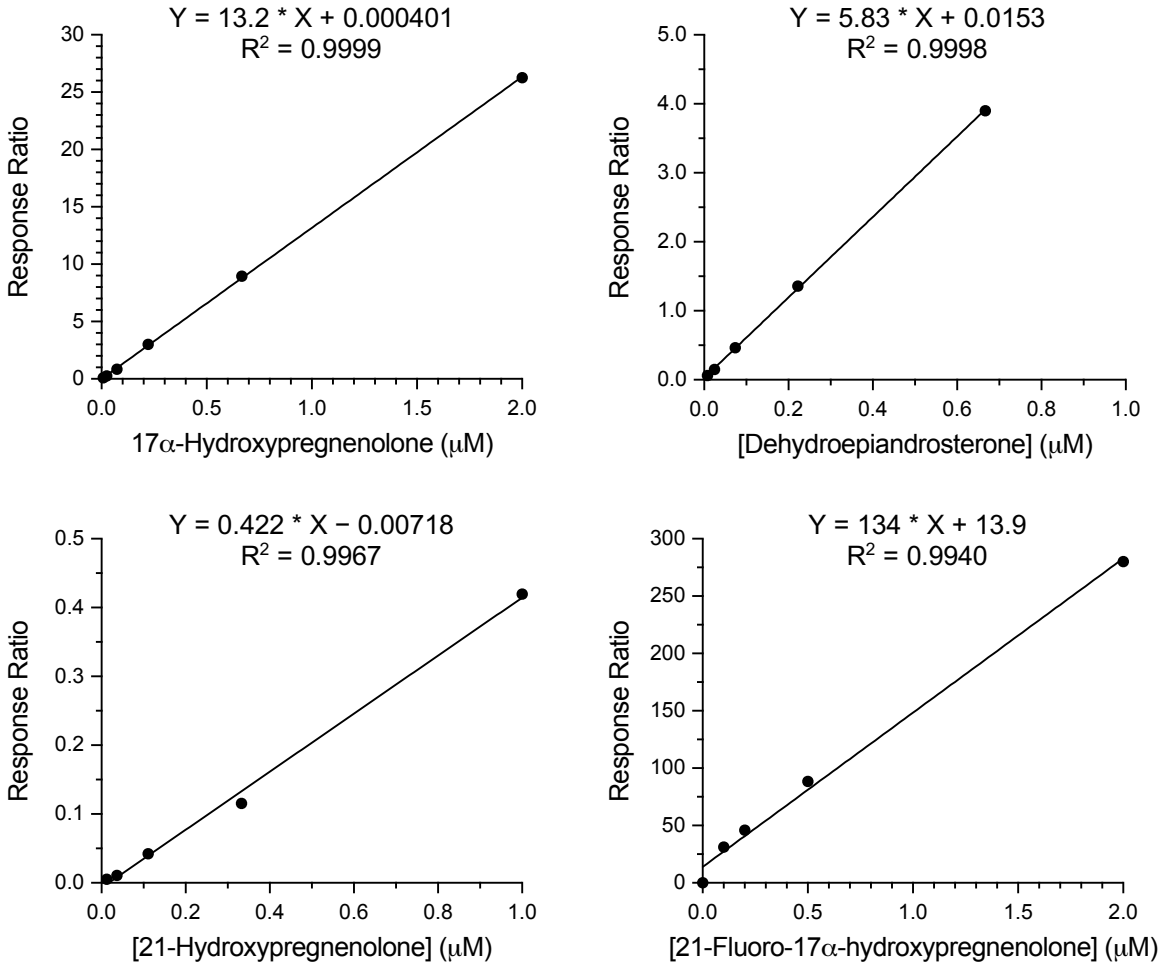
*Samples were run with a significant time gap between independent experiments. While the LC-MS/MS method did not change, differences in retention times were observed for a few products before and after this gap. However, the identity of each compound was validated by running the appropriate standards and monitoring the corresponding fragmentation patterns.

Using a standard curve, concentrations were then quantified based on the peak integration ratio (area under the curve) between the compound of interest and its internal standard (Figure S1). The peak areas for specific analytes were determined using the Agilent MassHunter quantitative analysis program. As shown in Table S2, limits of detection (LOD) assuming a 95% confidence level were calculated for the observed and predicted products using the following equation:

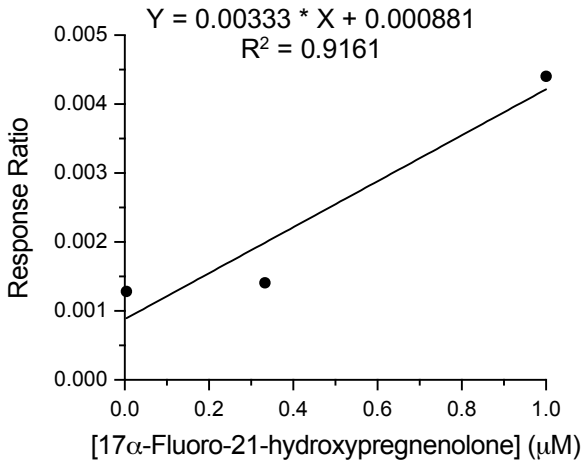
$$\text{LOD} = 3.3 * \frac{\sigma}{s}$$

where σ is the standard deviation (SD) of the response and s is the slope of the line.[11, 12]

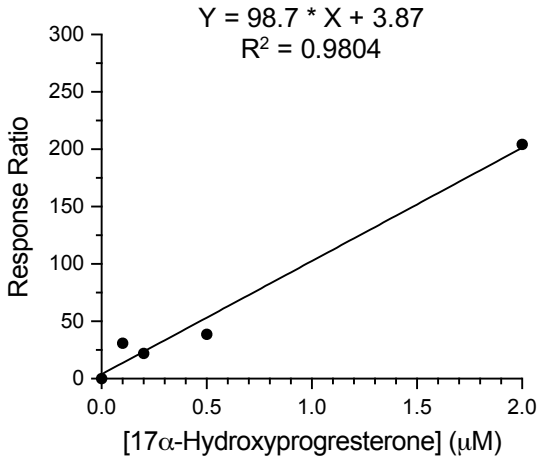
(a) Standard curves for Δ^5-steroids

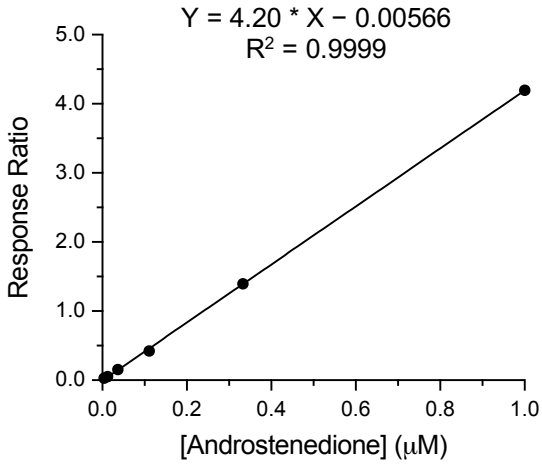


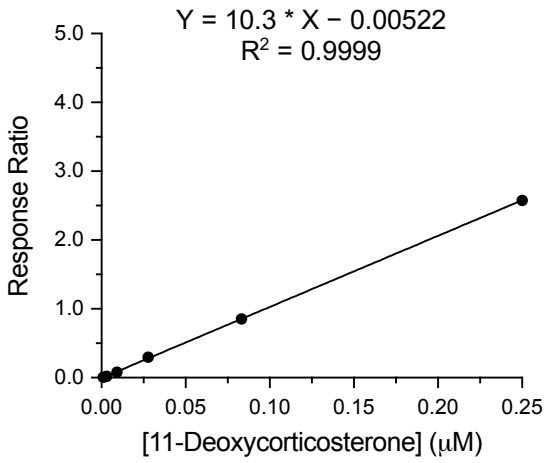
(a) Standard curves for Δ^5-steroids (Continued)

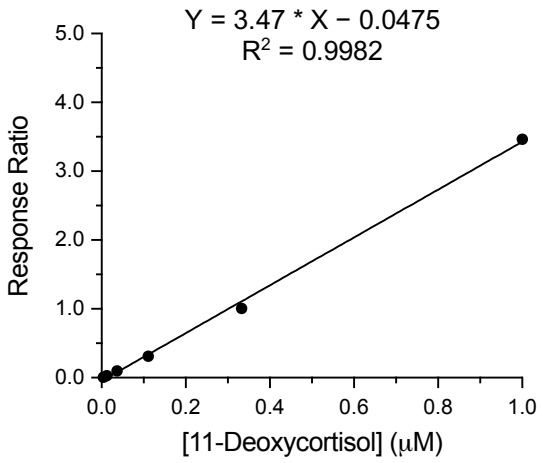


(b) Standard curves for Δ^4-steroids









(b) Standard curves for Δ^4-steroids (Continued)

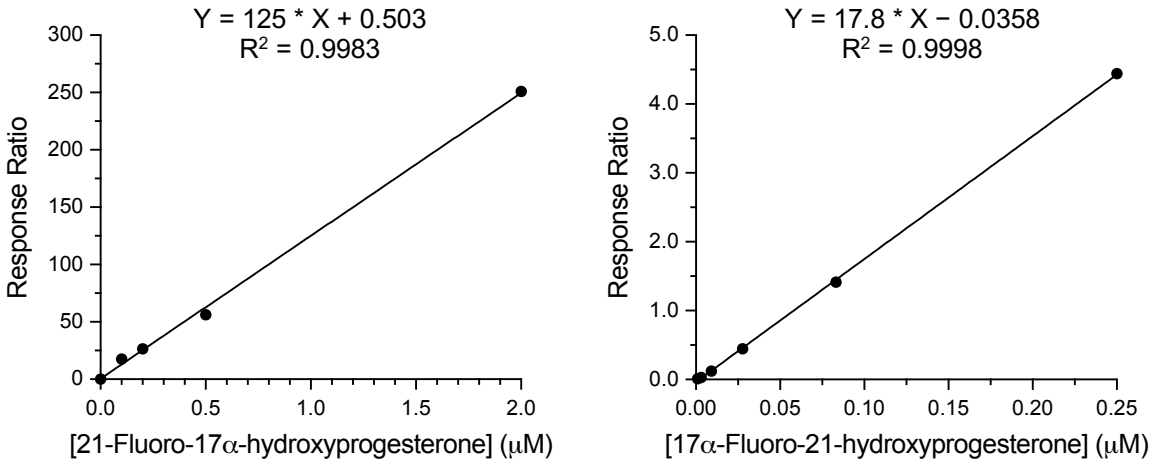


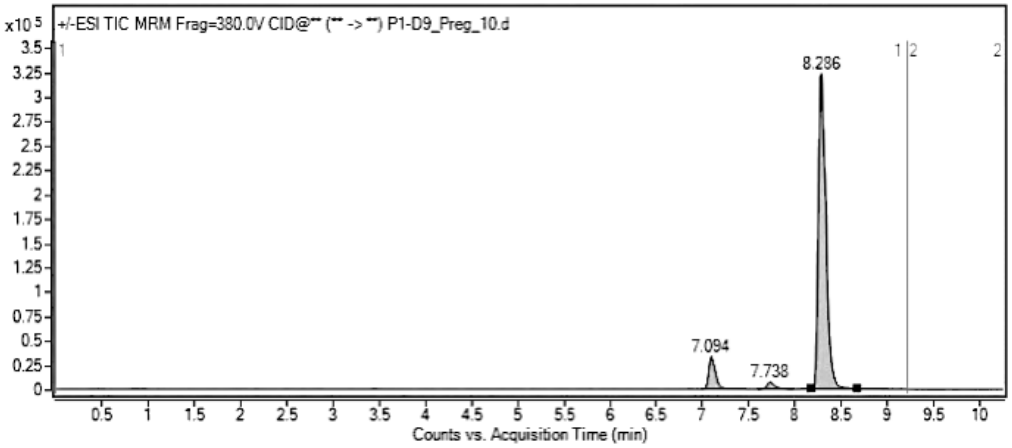
Figure S1. Standard curves for **(a)** Δ^5- and **(b)** Δ^4-steroids.

Table S2. Limits of detection for observed and predicted product analytes.

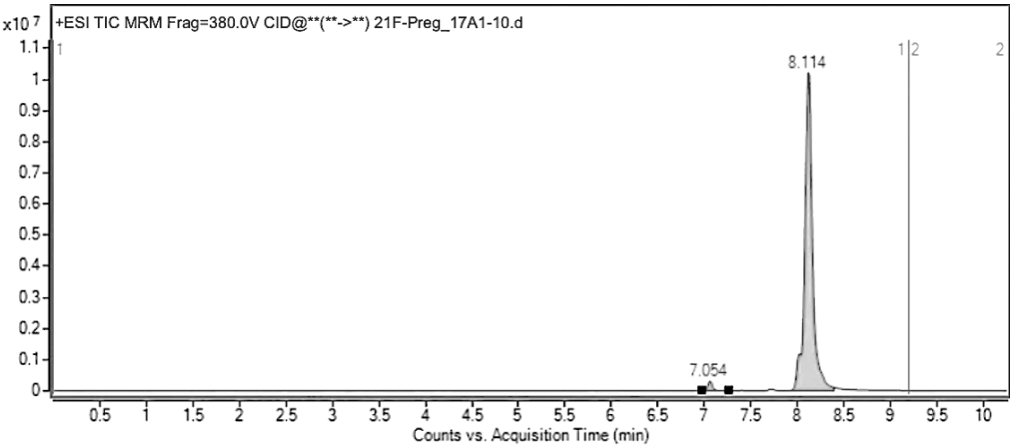
Analyte	SD of Response	Slope (uM^{-1})	LOD (μM)
Δ^5-Steroids			
17α-Hydroxypregnenolone (**S1b**)	0.0612	13.2	0.015
Dehydroepiandrosterone	0.0152	5.83	0.0087
21-Hydroxypregnenolone (**S3**)	0.00668	0.422	0.052
21-Fluoro-17α-hydroxypregnenolone (**8b**)	5.57	134	0.14
17α-Fluoro-21-hydroxypregnenolone (**16b**)	0.000614	0.00333	0.61
Δ^4-Steroids			
17α-Hydroxyprogesterone	7.47	98.7	0.25
Androstenedione	0.0110	4.20	0.0087
11-Deoxycorticosterone	0.0047	10.3	0.0015
11-Deoxycortisol	0.0319	3.47	0.030
21-Fluoro-17α-hydroxyprogesterone (**10**)	2.68	125	0.071
17α-Fluoro-21-hydroxyprogesterone (**17b**)	0.0129	17.8	0.0024

Representative chromatograms generated in the functional assays are presented in Figure S2.

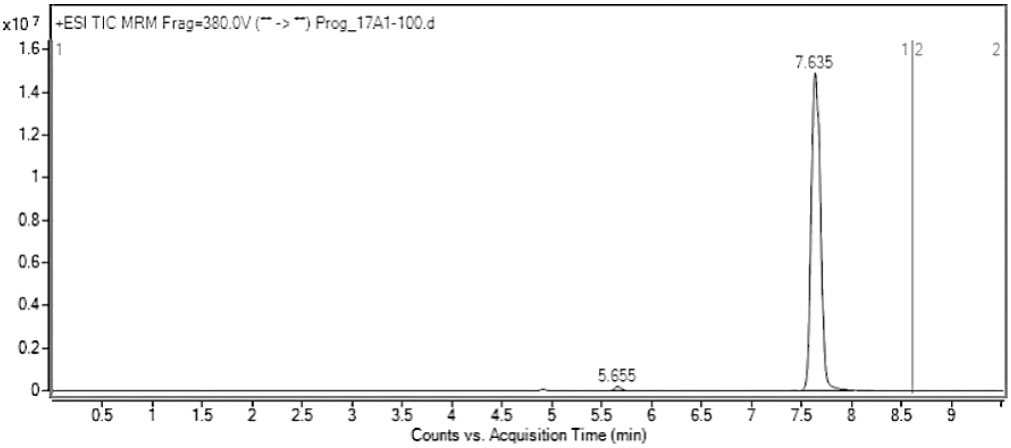
(a) 17α-Hydroxylase Activity of CYP17A1



Pregnenolone (8.3 min) to 17α-Hydroxypregnenolone (7.1 min)

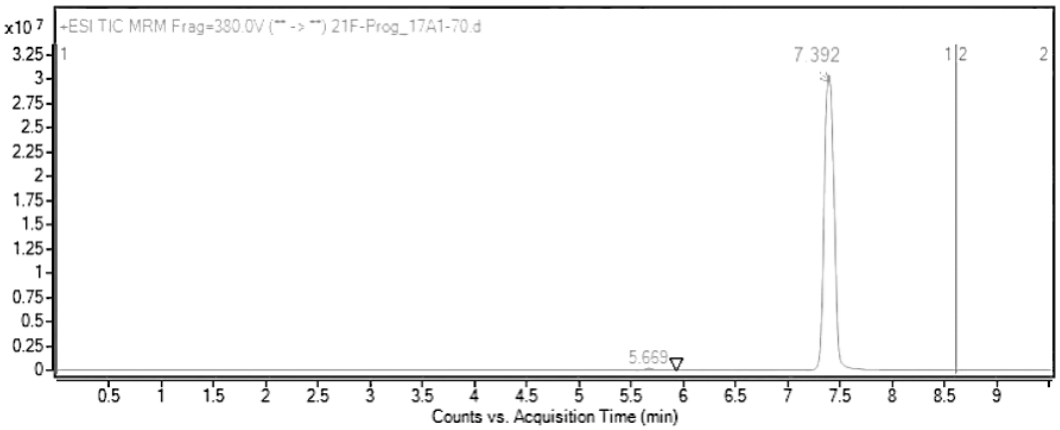


21-Fluoropregnenolone (8.1 min) to 21-Fluoro-17α-hydroxypregnenolone (7.1 min)



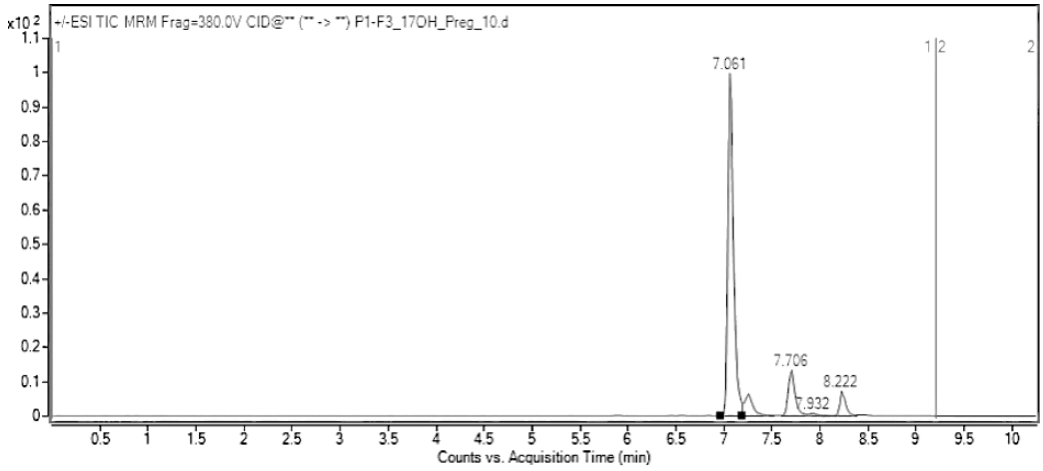
Progesterone (7.6 min) to 17α-Hydroxyprogesterone (5.7 min)

(a) 17α-Hydroxylase Activity of CYP17A1 (Continued)

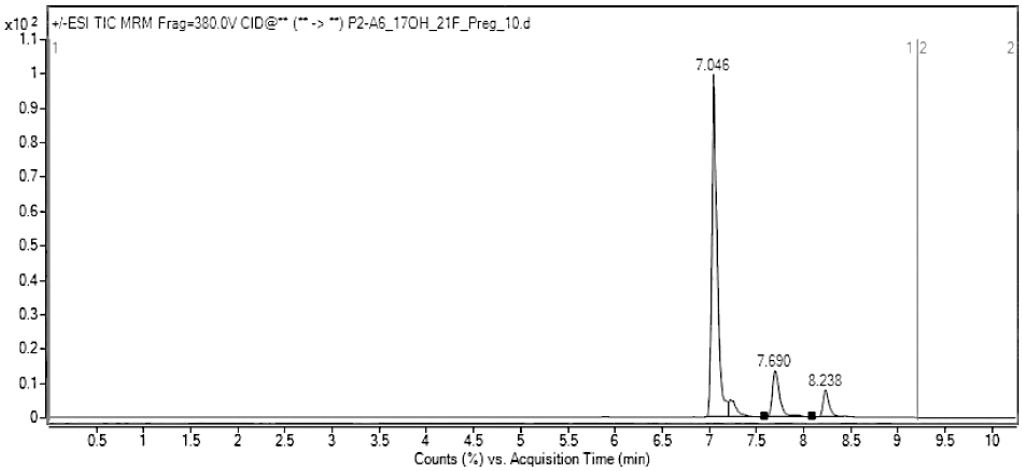


21-Fluoroprogesterone (7.4 min) to 21-Fluoro-17α-hydroxyprogesterone (5.7 min)

(b) 17,20-Lyase Activity of CYP17A1

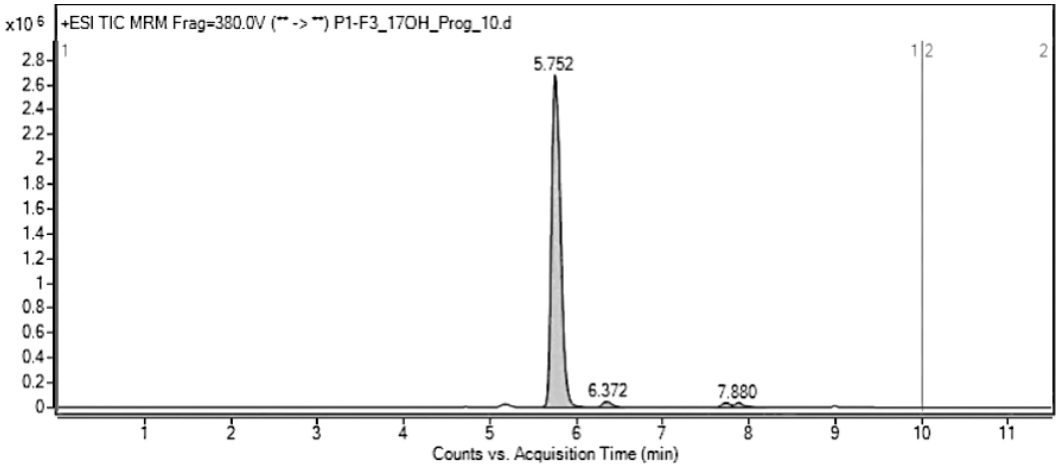


17α-Hydroxypregnenlone (7.1 min) to Dehydroepiandrosterone (7.7 min)

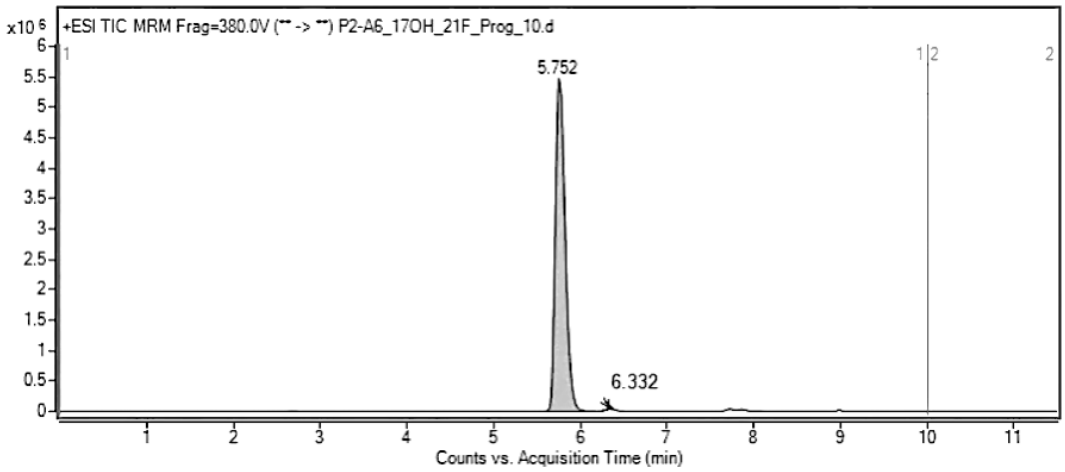


21-Fluoro-17α-hydroxypregnenolone (7.0 min) to Dehydroepiandrosterone (7.7 min)

(b) 17,20-Lyase Activity of CYP17A1 (Continued)

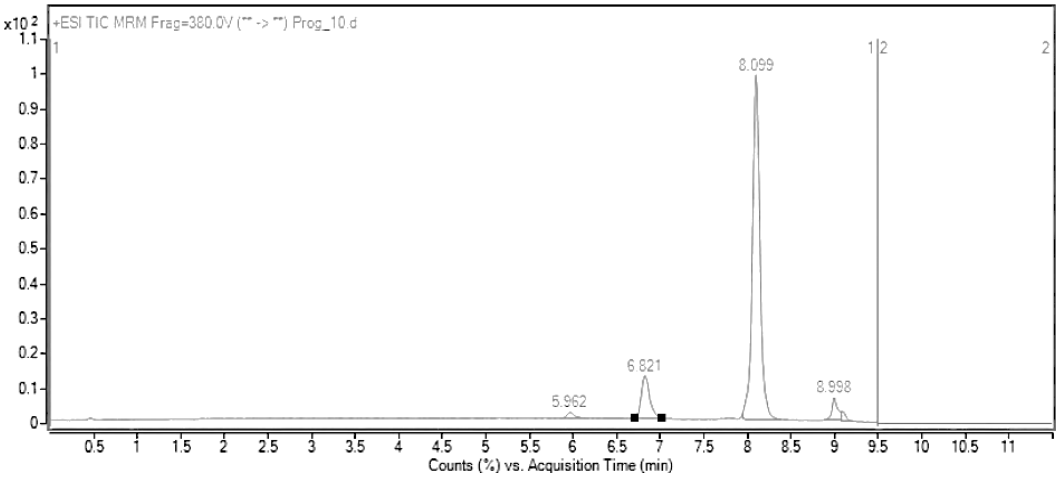


17α-Hydroxyprogesterone (5.8 min) to Androsterone (6.4 min)



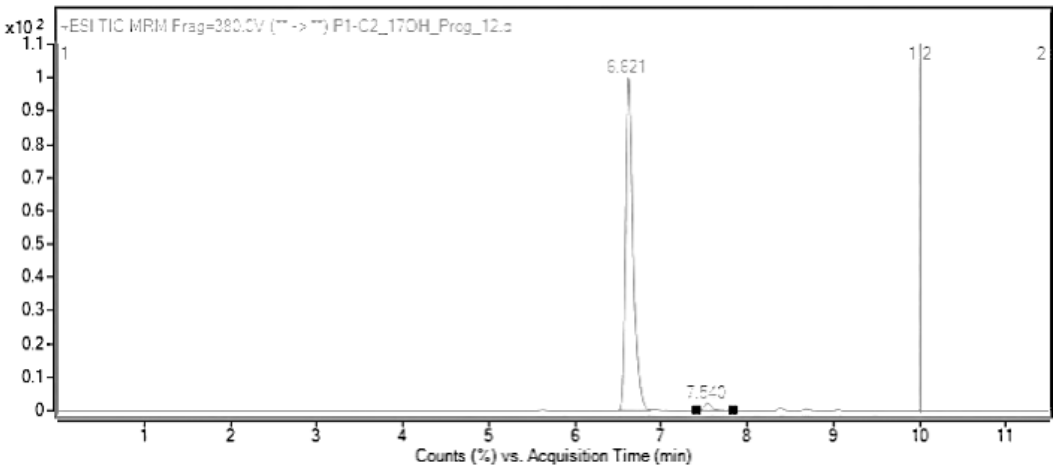
21-Fluoro-17α-hydroxyprogesterone (5.8 min) to Androsterone (6.3 min)

(c) 21-Hydroxylase Activity of CYP21A2

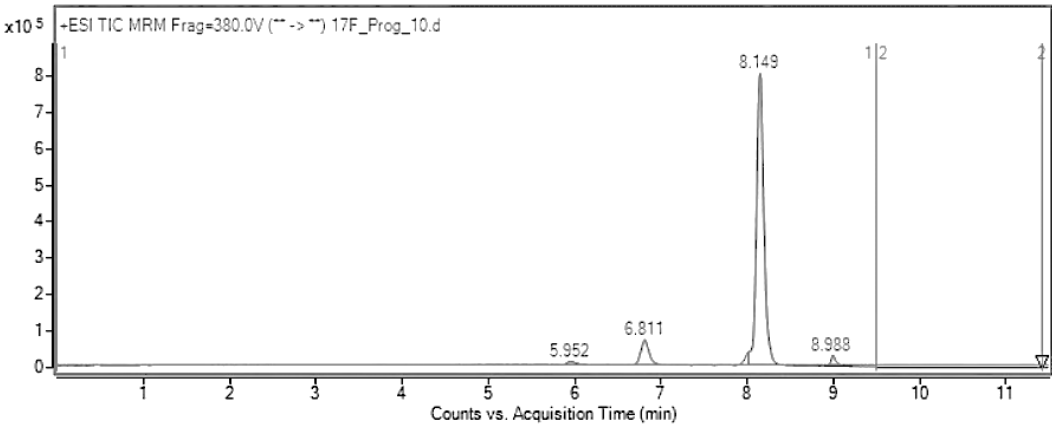


Progesterone (8.1 min) to 11-Deoxycortisone (6.8 min)

(c) 21-Hydroxylase Activity of CYP21A2 (Continued)



17α-Hydroxyprogesterone (6.6 min) to 11-Deoxycortisol (7.5 min)



17α-Fluoroprogesterone (8.1 min) to 17α-Fluoro-21-hydroxyprogesterone (6.8 min)

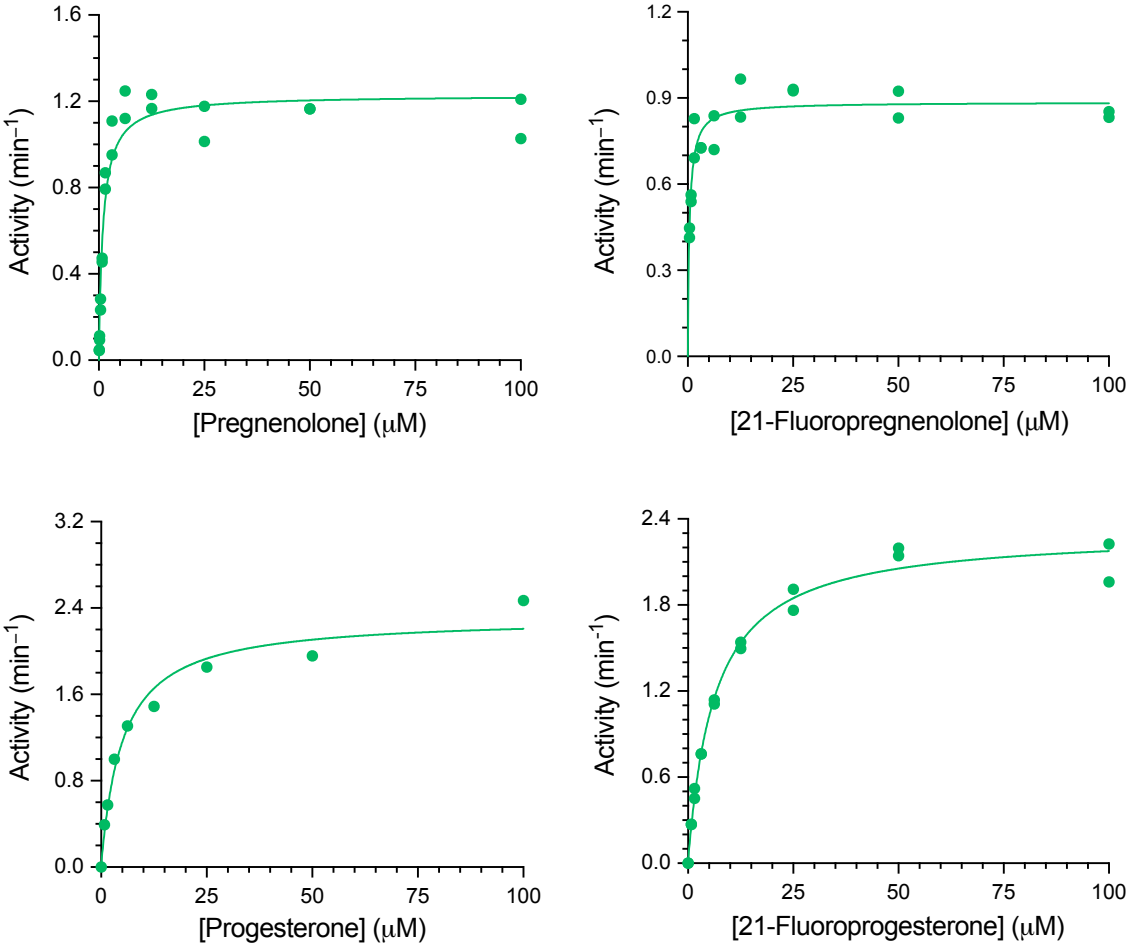
Figure S2. Representative chromatograms for the **(a)** 17α-hydroxylase and **(b)** 17,20-lyase activities of CYP17A1, as well as the **(c)** 21-hydroxylase activity of CYP21A2.

Product formed with each substrate concentration was determined and fitted with non-linear regression to the Michaelis-Menten equation in Prism (GraphPad Software, La Jolla, CA) to determine catalytic turnover parameters K_m and V_{max} (Figure S3). These parameters were determined from two independent replicates (n = 2).

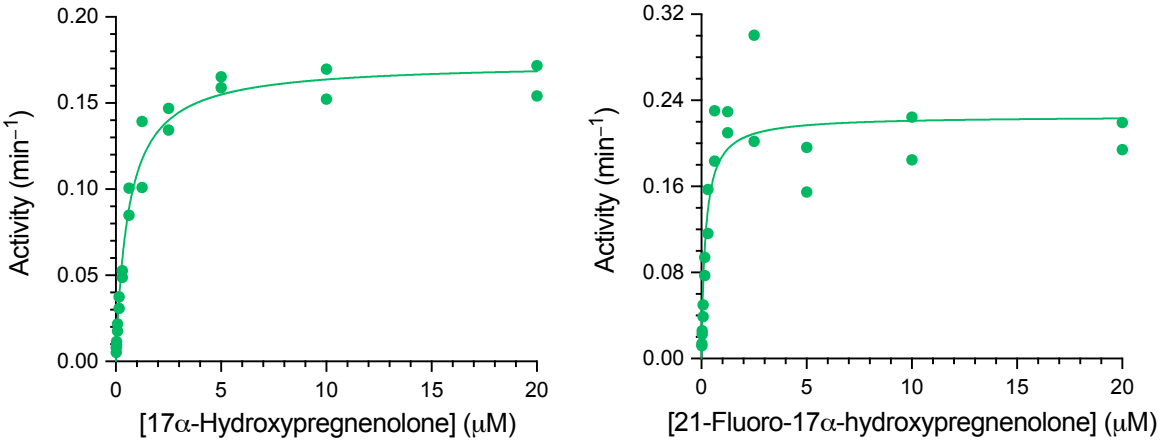
$$Y = \frac{V_{max} * X}{K_m + X}$$

Potential outliers were detected using the ROUT method with a value of Q = 1%. One point was eliminated from the 21-hydroxylase assay using 17α-hydroxyprogesterone as the substrate.

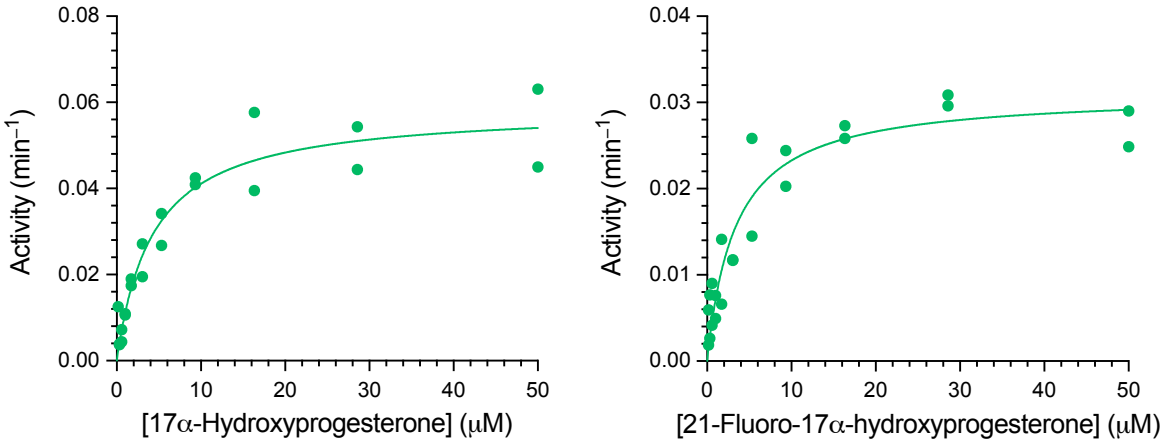
(a) 17α-Hydroxylase Activity of CYP17A1



(b) 17,20-Lyase Activity of CYP17A1



(b) 17,20-Lyase Activity of CYP17A1 (continued)



(c) 21-Hydroxylase Activity of CYP21A2

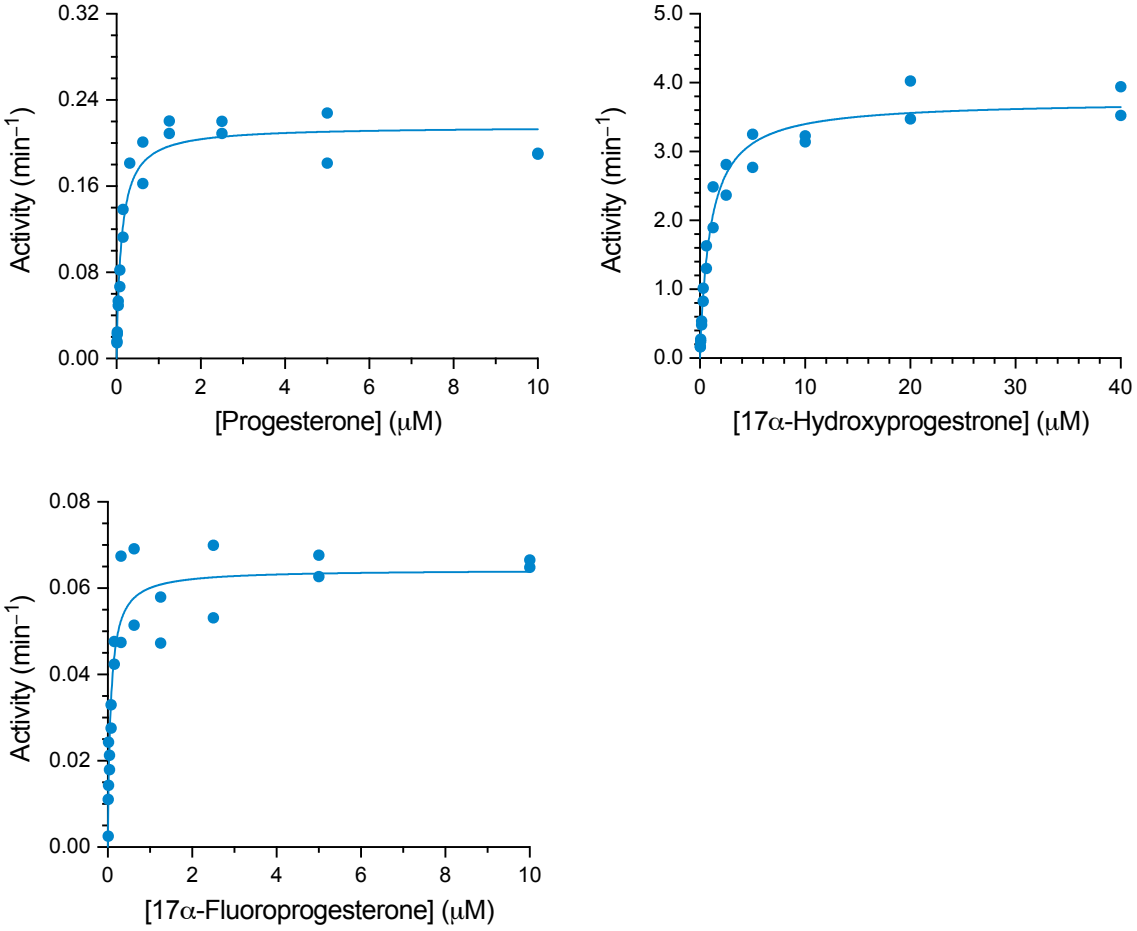


Figure S3. Enzyme kinetics determined for substrates of the **(a)** 17α-hydroxylase and **(b)** 17,20-lyase activities of CYP17A1, as well as the **(c)** 21-hydroxylase activity of CYP21A2.

Note: The kinetic parameters for CYP17A1 were determined without lipid, which was previously shown to not change k_{cat} and increase K_m compared to the full length enzyme.[7] Typically, there are no significant differences with or without lipid as measured across a number of different cytochrome P450 enzymes, so we did not repeated these experiments for CYP21A2. In addition, 16α-hydroxyprogesterone was generated as a minor product in the 17α-hydroxylase reaction at ca. 10% of the total progesterone metabolites, similar to the wild-type protein.[13]

Spectral Binding Assays. To characterize the binding mode and measure binding affinity, steroid ligands were titrated into CYP17A1 or CYP21A2 and changes to the heme absorbance were monitored using a method as described.[14] Specific modifications were as follows: CYP17A1 assays were performed using 5 cm path length cuvettes and a 0.1 μM solution of CYP17A1 in buffer (50 mM of K_3PO_4, 20% glycerol, and 500 mM of NaCl in H_2O, pH 7.4). CYP21A2 spectral binding assays were performed using 1 cm path length cuvettes and a 1 μM solution of CYP21A2 in buffer (100 mM of K_3PO_4 in H_2O, pH 7.4). Absorbance changes were either fit to a simple single-site binding equation for compounds with estimated K_d values greater than the protein concentration:

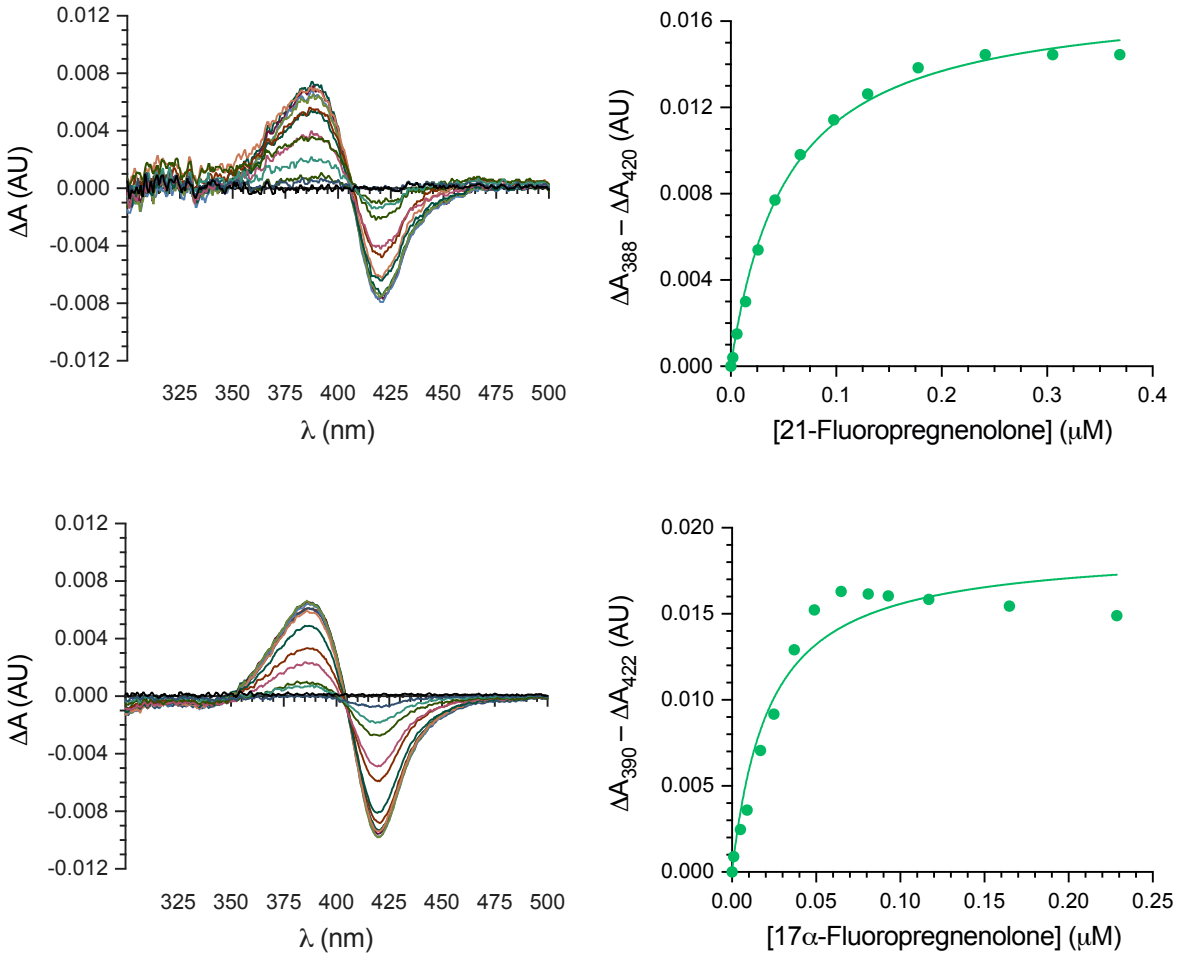
$$\Delta\Delta A = \frac{\Delta\Delta A_{max} * S}{K_d + S}$$

or the Morrison equation for compounds exhibiting K_d values equal or less than the protein concentration:

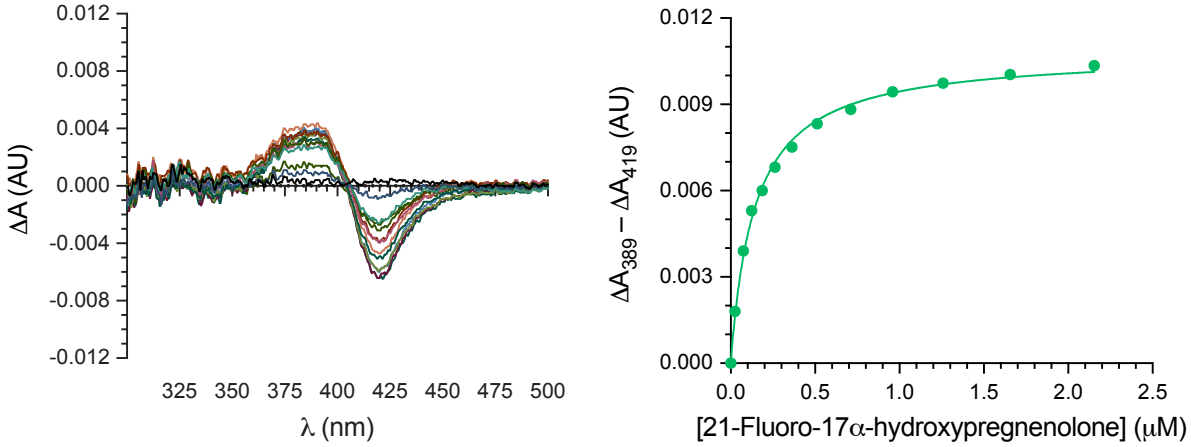
$$\Delta\Delta A = \Delta\Delta A_{max} \frac{(E + S + K_d) - \sqrt{(E + S + K_d)^2 - 4ES}}{2E}$$

where $\Delta\Delta A$ is the difference between the peak and trough in the absorbance spectrum, E is the protein concentration, and S is the ligand concentration. Both K_d and the maximum spectral change ($\Delta\Delta A_{max}$) were determined through nonlinear regression in Prism (GraphPad Software, La Jolla, CA) using the equations above (Figure S4).

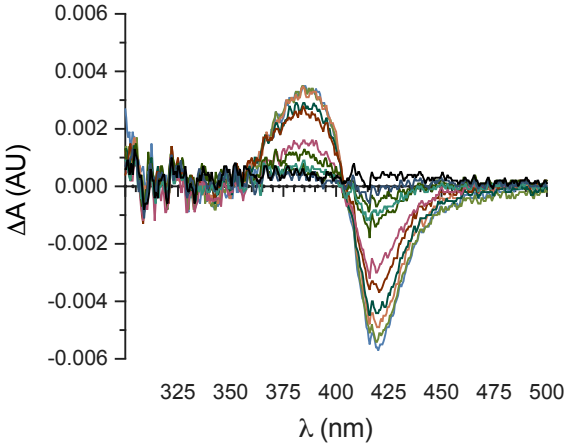
(a) Dissociation constants determined for CYP17A1

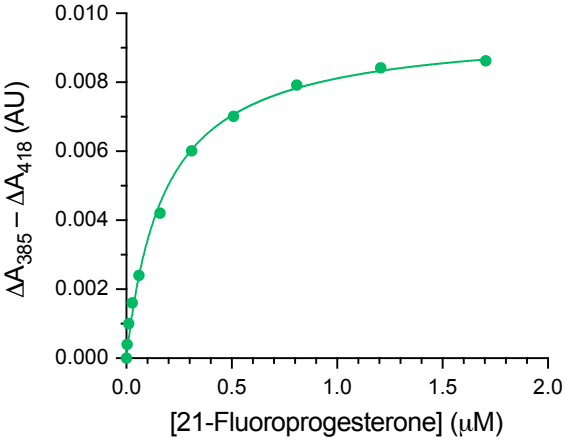


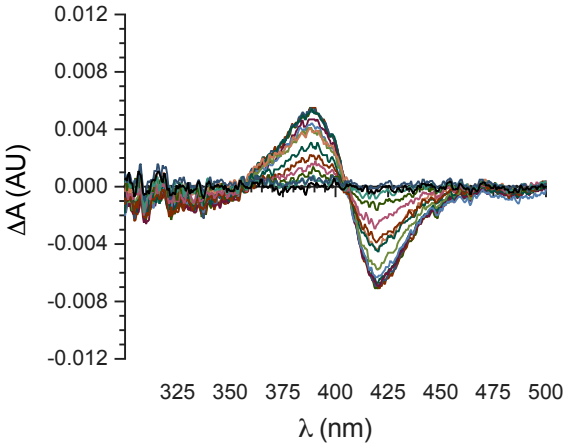
Note: The above ligand is a very tight binder, which precludes definition of a dissociation constant with similar accuracy to the other experiments.

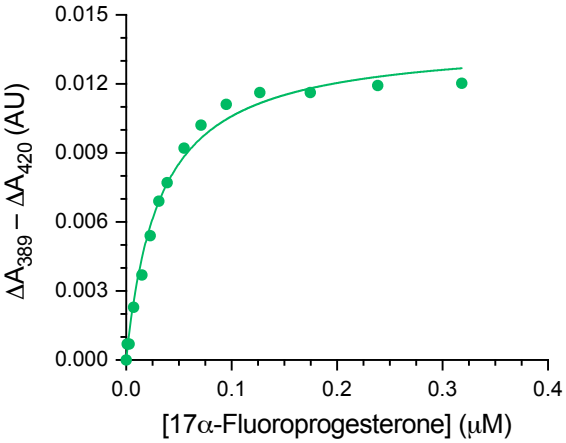


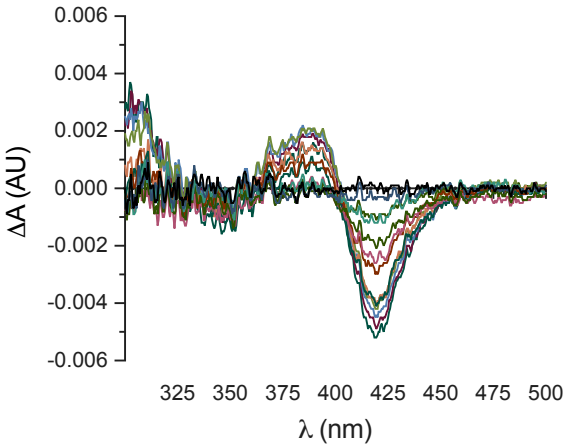
(a) Dissociation constants determined for CYP17A1 (Continued)

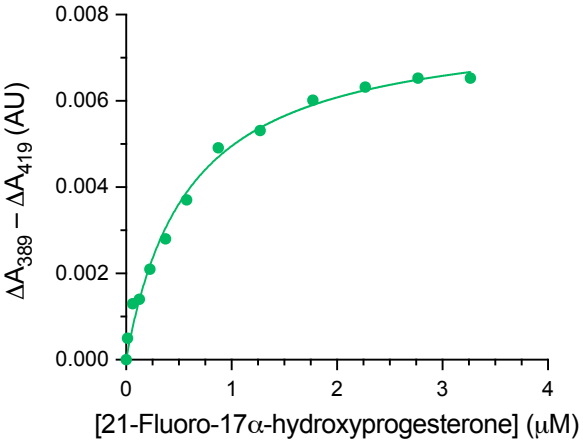




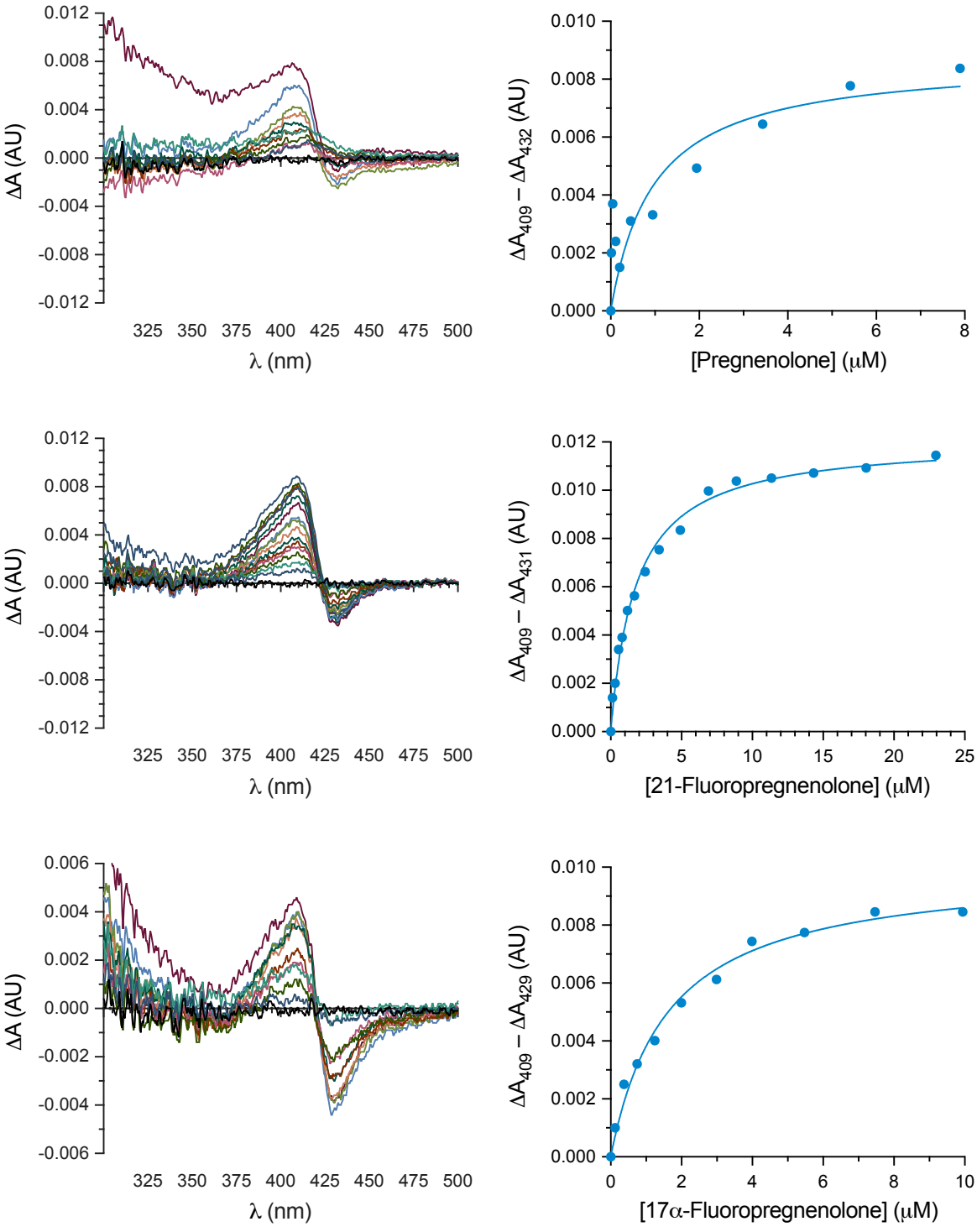




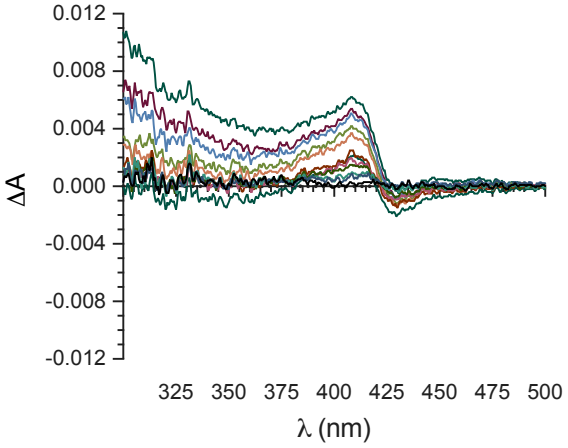


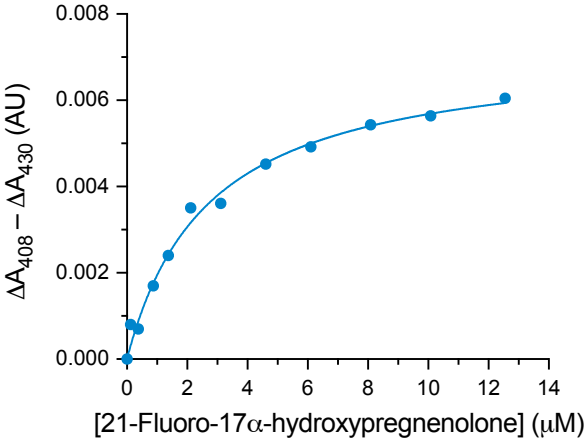


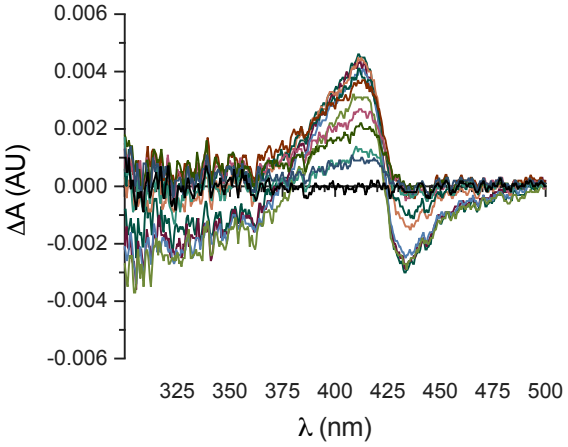
(b) Dissociation constants determined for CYP21A2

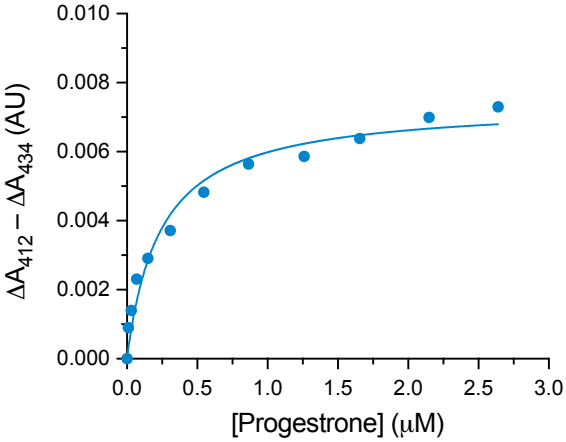


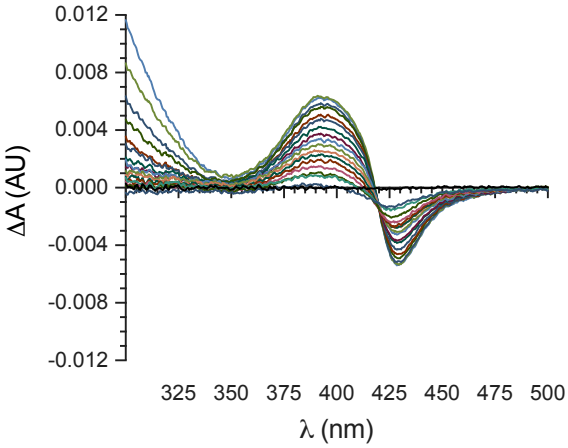
(b) Dissociation constants determined for CYP21A2 (Continued)

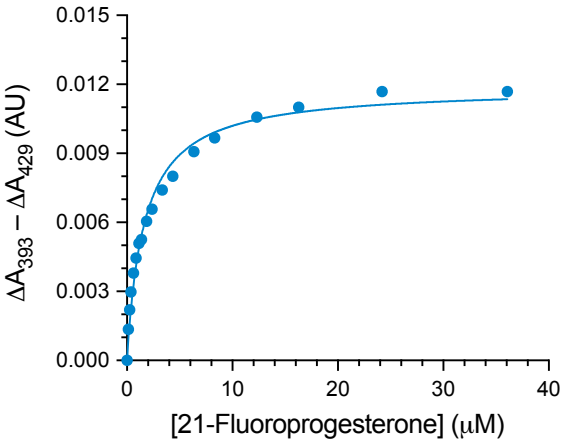












(b) Dissociation constants determined for CYP21A2 (Continued)

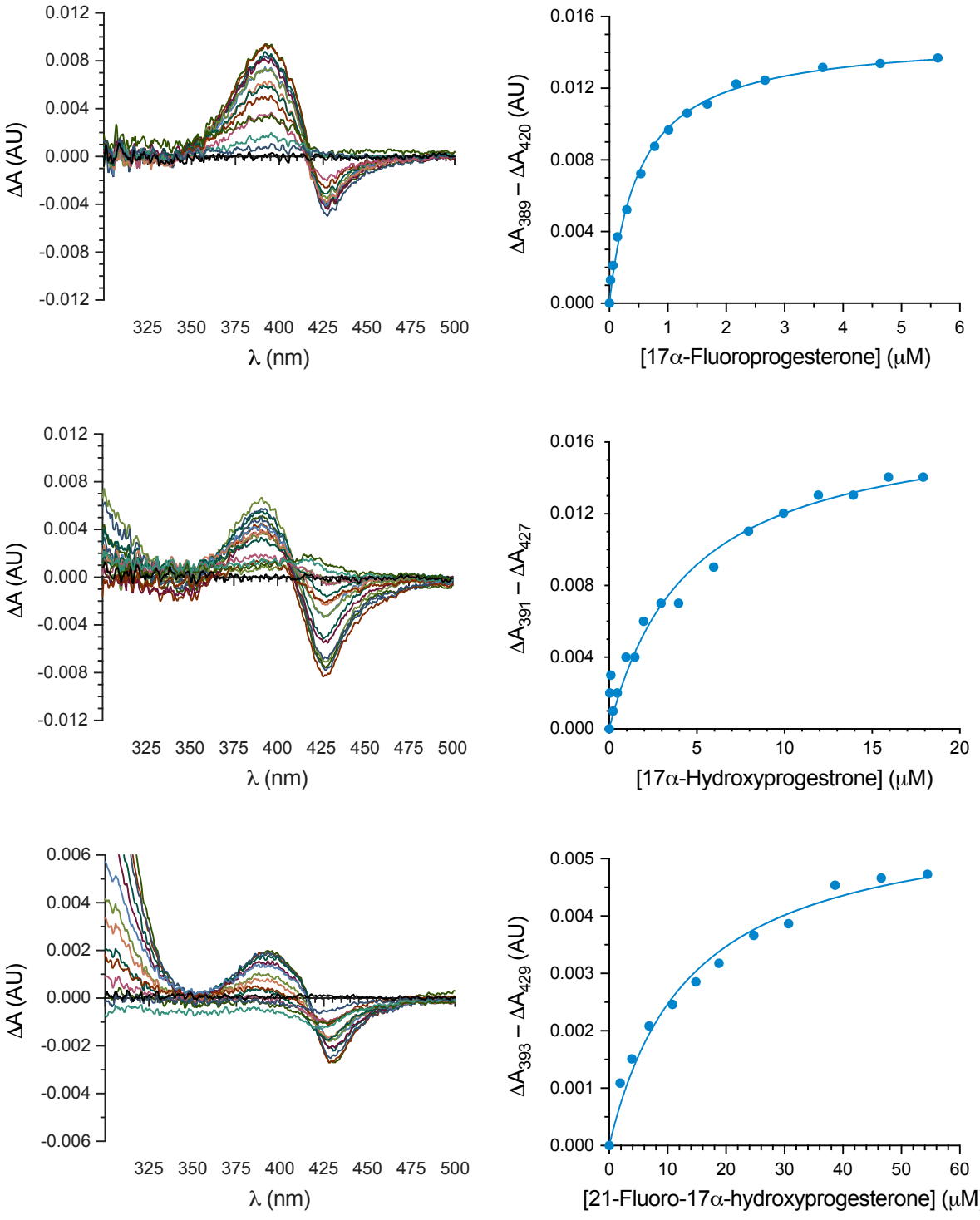
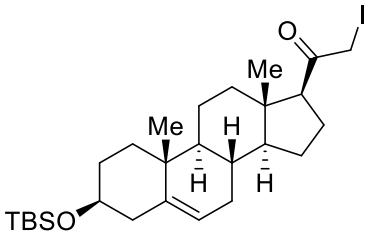


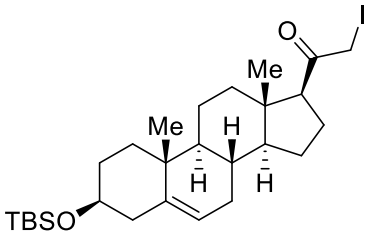
Figure S4. Spectral binding titrations to determine the dissociation constants for each ligand against **(a)** CYP17A1 or **(b)** CYP21A2.

^{1}H NMR (400 MHz, $CDCl_3$)



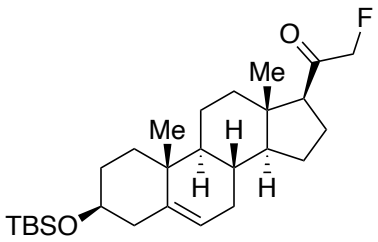
2

^{13}C NMR (101 MHz, $CDCl_3$)



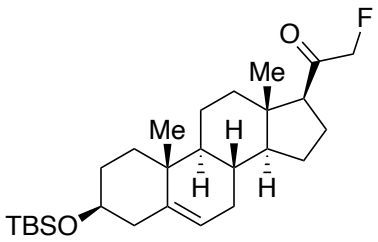
2

^{1}H NMR (400 MHz, $CDCl_3$)



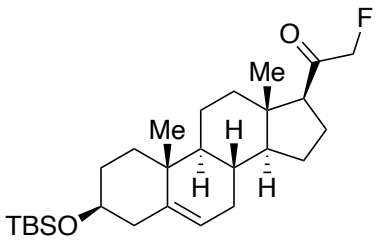
3a

^{13}C NMR (101 MHz, $CDCl_3$)



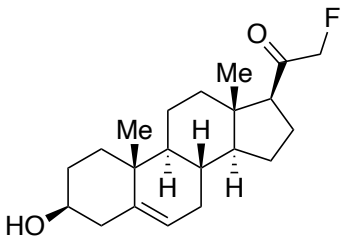
3a

^{19}F NMR (376 MHz, $CDCl_3$)



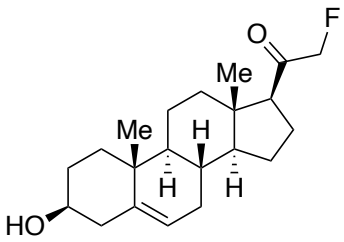
3a

^{1}H NMR (400 MHz, $CDCl_3$)



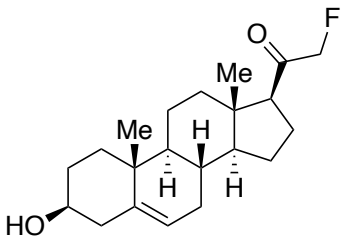
3b

^{13}C NMR (101 MHz, $CDCl_3$)



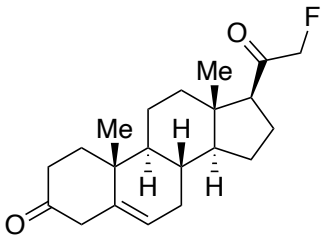
3b

^{19}F NMR (376 MHz, $CDCl_3$)



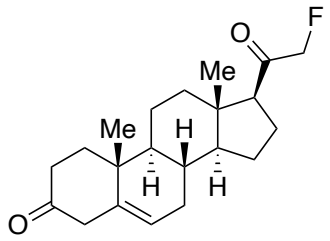
3b

^{1}H NMR (400 MHz, $CDCl_3$)



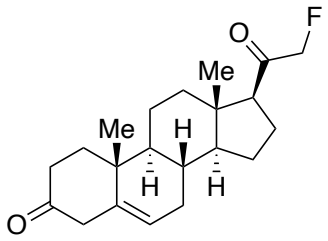
4

^{13}C NMR (101 MHz, $CDCl_3$)



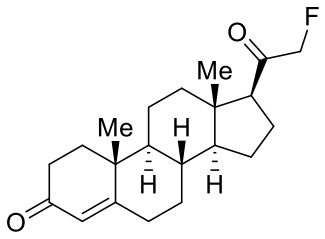
4

^{19}F NMR (376 MHz, $CDCl_3$)



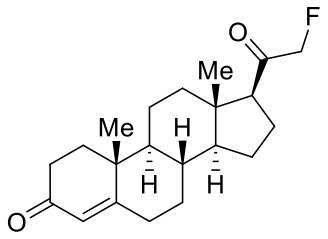
4

^{1}H NMR (400 MHz, $CDCl_3$)



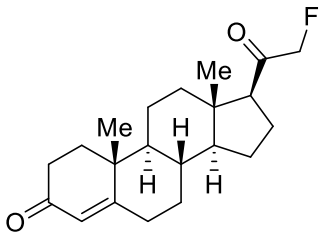
5

^{13}C NMR (101 MHz, $CDCl_3$)



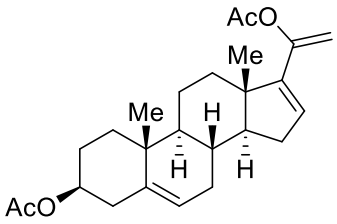
5

^{19}F NMR (376 MHz, $CDCl_3$)



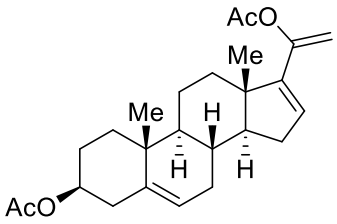
5

^{1}H NMR (400 MHz, $CDCl_3$)



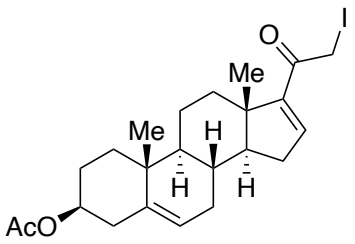
6

^{13}C NMR (101 MHz, $CDCl_3$)



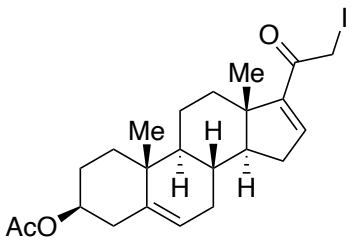
6

^{1}H NMR (400 MHz, $CDCl_3$)



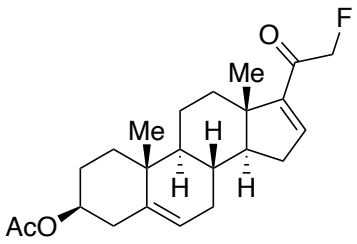
7a

^{13}C NMR (101 MHz, $CDCl_3$)



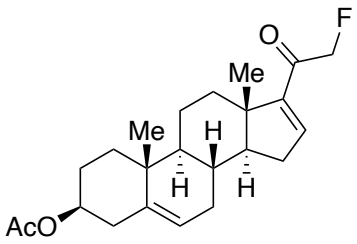
7a

^{1}H NMR (400 MHz, $CDCl_3$)



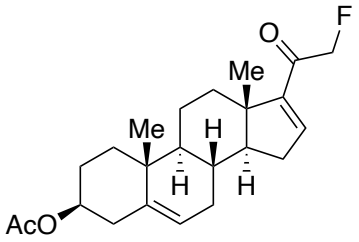
7b

^{13}C NMR (101 MHz, $CDCl_3$)



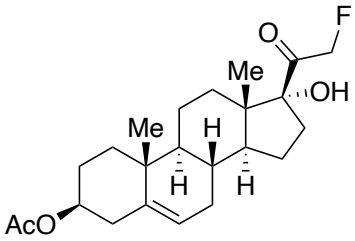
7b

^{19}F NMR (376 MHz, $CDCl_3$)



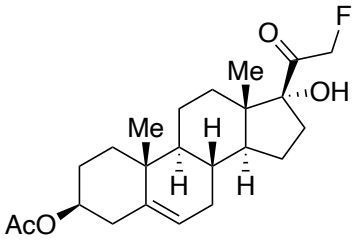
7b

^{1}H NMR (400 MHz, $CDCl_3$)



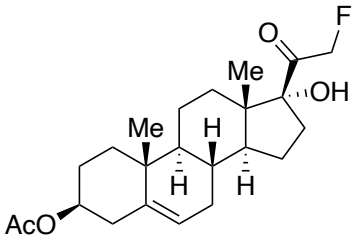
8a

^{13}C NMR (101 MHz, $CDCl_3$)



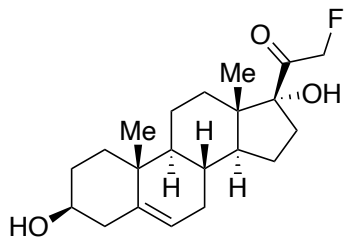
8a

^{19}F NMR (376 MHz, $CDCl_3$)



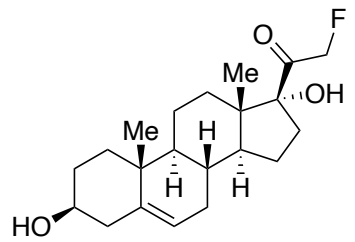
8a

^{1}H NMR (400 MHz, DMSO-d_6)



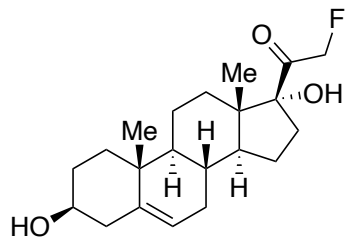
8b

^{13}C NMR (101 MHz, DMSO-d_6)



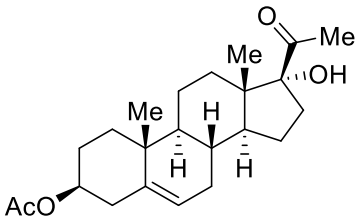
8b

^{19}F NMR (376 MHz, DMSO-d_6)



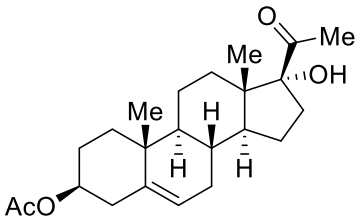
8b

^{1}H NMR (400 MHz, $CDCl_3$)



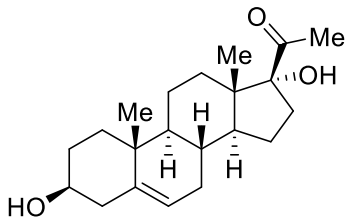
S1a

^{13}C NMR (101 MHz, $CDCl_3$)



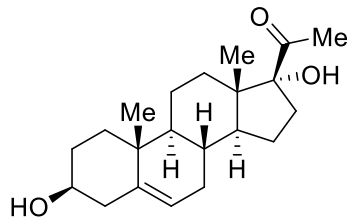
S1a

^{1}H NMR (400 MHz, DMSO-d_6)



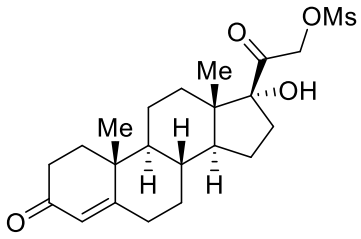
S1b

^{13}C NMR (101 MHz, DMSO-d_6)



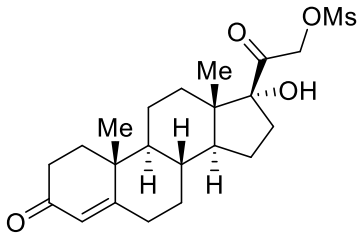
S1b

^{1}H NMR (400 MHz, $CDCl_3$)



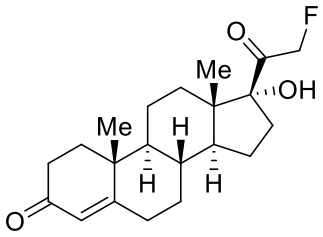
9

^{13}C NMR (101 MHz, $CDCl_3$)



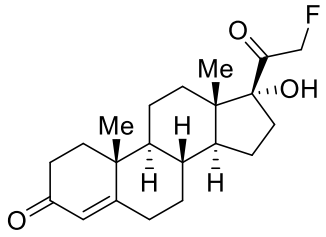
9

^{1}H NMR (400 MHz, $CDCl_3$)



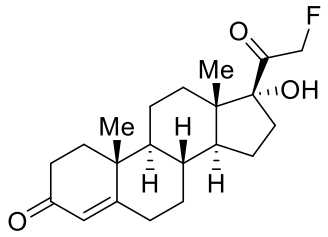
10

^{13}C NMR (101 MHz, $CDCl_3$)



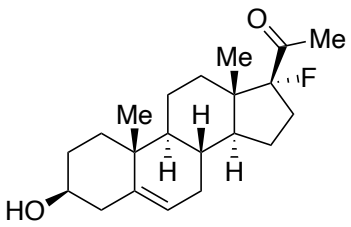
10

^{19}F NMR (376 MHz, $CDCl_3$)



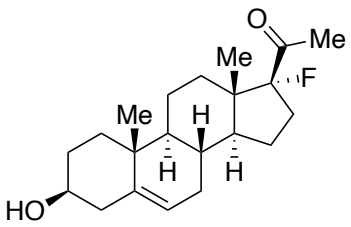
10

^{1}H NMR (400 MHz, $CDCl_3$)



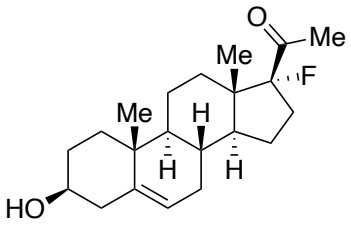
11

^{13}C NMR (101 MHz, $CDCl_3$)



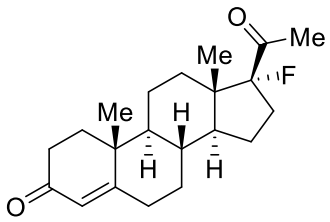
11

^{19}F NMR (376 MHz, $CDCl_3$)



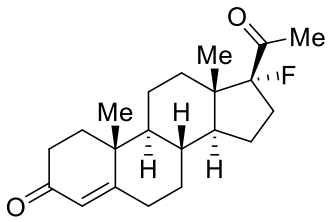
11

^{1}H NMR (400 MHz, $CDCl_3$)



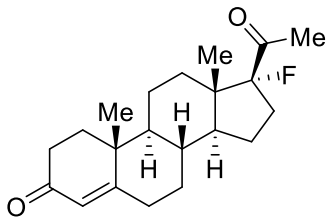
12

^{13}C NMR (101 MHz, $CDCl_3$)



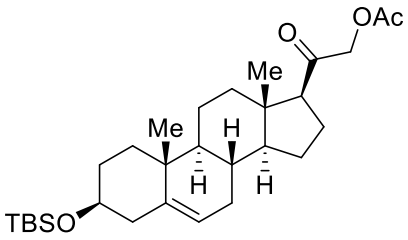
12

^{19}F NMR (376 MHz, $CDCl_3$)



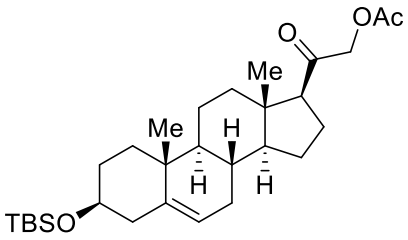
12

^{1}H NMR (400 MHz, $CDCl_3$)



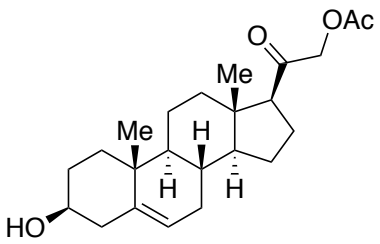
S2a

^{13}C NMR (101 MHz, $CDCl_3$)



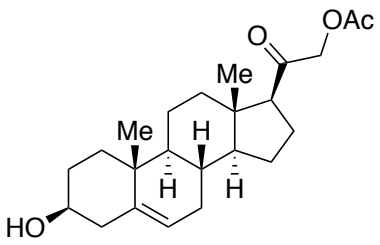
S2a

^{1}H NMR (400 MHz, $CDCl_3$)



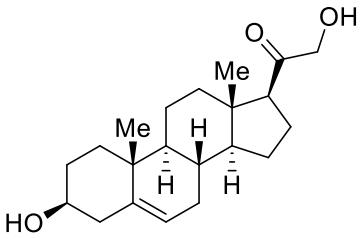
S2b

^{13}C NMR (101 MHz, $CDCl_3$)



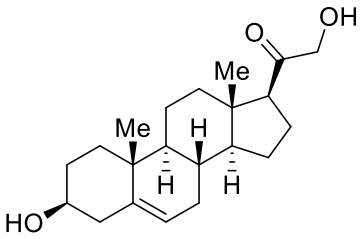
S2b

^{1}H NMR (400 MHz, $CDCl_3$)



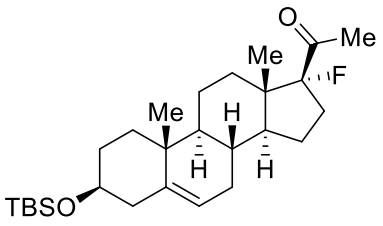
S3

^{13}C NMR (101 MHz, $CDCl_3$)



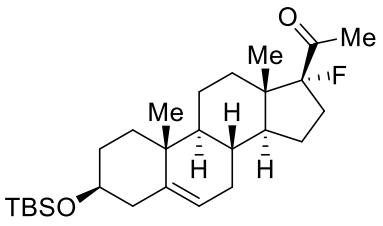
S3

^{1}H NMR (400 MHz, $CDCl_3$)



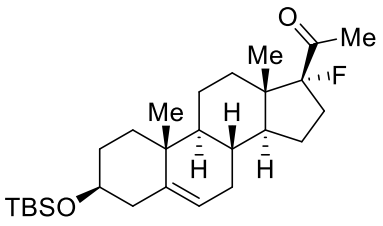
13

^{13}C NMR (101 MHz, $CDCl_3$)



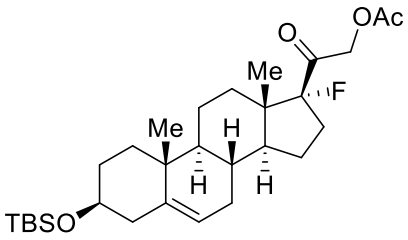
13

^{19}F NMR (376 MHz, $CDCl_3$)



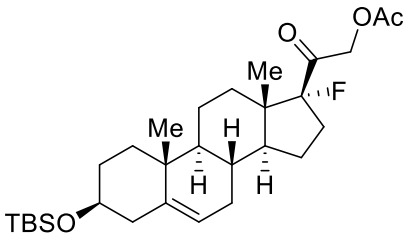
13

^{1}H NMR (400 MHz, $CDCl_3$)



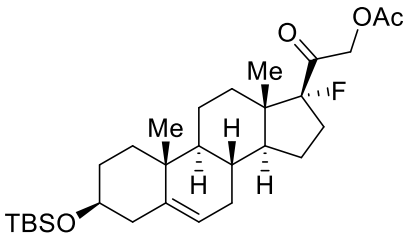
15

^{13}C NMR (101 MHz, $CDCl_3$)



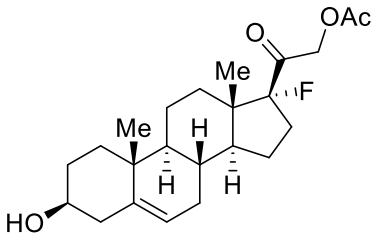
15

^{19}F NMR (376 MHz, $CDCl_3$)



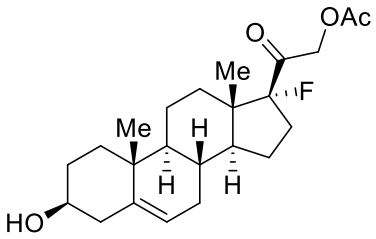
15

^{1}H NMR (400 MHz, $CDCl_3$)



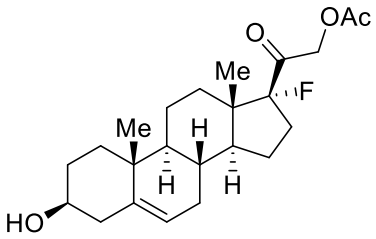
16a

^{13}C NMR (101 MHz, $CDCl_3$)



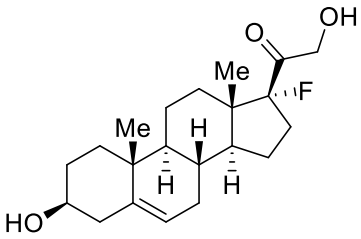
16a

^{19}F NMR (376 MHz, $CDCl_3$)



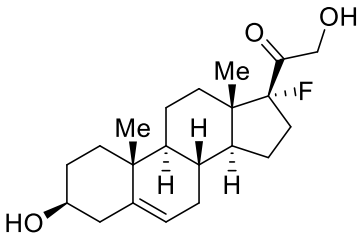
16a

^{1}H NMR (400 MHz, $CDCl_3$)



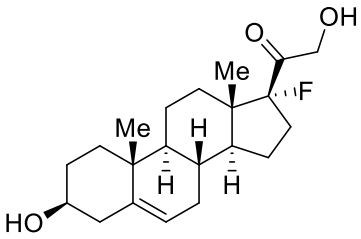
16b

^{13}C NMR (101 MHz, $CDCl_3$)



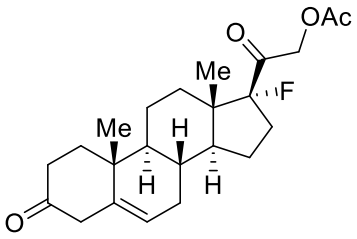
16b

^{19}F NMR (400 MHz, $CDCl_3$)



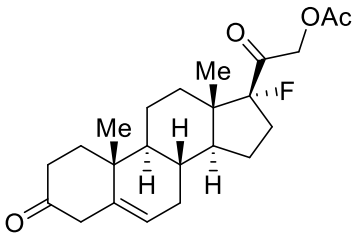
16b

^{1}H NMR (400 MHz, $CDCl_3$)



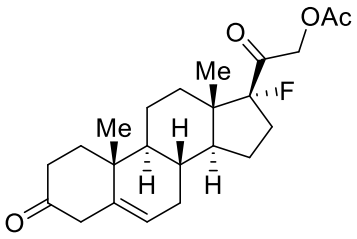
S4

^{13}C NMR (101 MHz, $CDCl_3$)



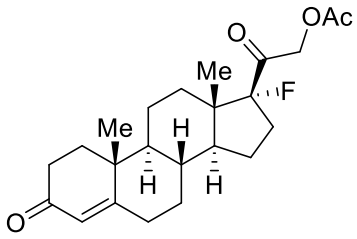
S4

^{19}F NMR (376 MHz, $CDCl_3$)



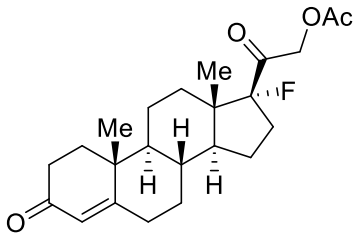
S4

^{1}H NMR (400 MHz, $CDCl_3$)



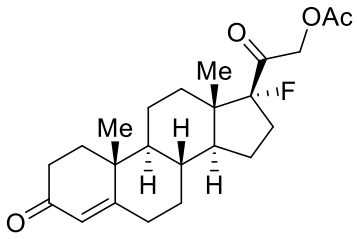
17a

^{13}C NMR (101 MHz, $CDCl_3$)



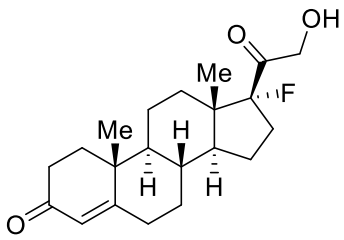
17a

^{19}F NMR (376 MHz, $CDCl_3$)



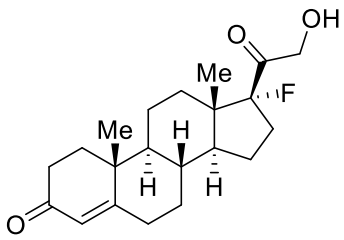
17a

^{1}H NMR (400 MHz, $CDCl_3$)



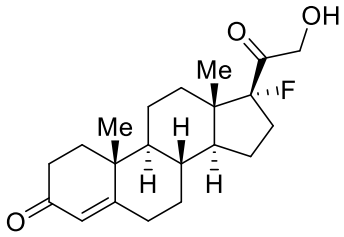
17b

^{13}C NMR (101 MHz, $CDCl_3$)



17b

^{19}F NMR (376 MHz, $CDCl_3$)



17b

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