Mapping Two Neurosteroid Modulatory Sites in the Prototypic Pentameric Ligand Gated Ion Channel GLIC

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K-Selectride (1 M in THF, 0.15 mL, 0.15 mmol) was added to a stirred solution of (5'α)-spiro[3H-diazirine-3,6'-pregnane]-3',20'-dione1 (1, 52.9 mg, 0.15 mmol) in dry THF (15 mL) at −78 °C under N₂. Upon completion of addition, the cooling bath was removed and the reaction was stirred at room temperature for 6 h. The reaction was then cooled to 0 °C and MeOH (0.3 mL), 5 N NaOH (0.3 mL) and 30% H₂O₂ (0.3 mL) were added sequentially. The cooling bath was removed and the reaction was stirred overnight. The product was extracted into Et₂O and the organic layer was separated and washed with water, brine and dried over anhydrous Na₂SO₄. After removal of the solvent, the residue was purified by flash column chromatography on silica gel to give 5α-20-Azip (31 mg, 58%) as a white solid: mp 130–132 °C; ¹H NMR (300 MHz, CDCl₃) δ 3.96 (b, 1H), 2.54 (t, J = 9.0 Hz, 1H), 2.13 (s, 3H), 1.12 (s, 3H), 0.65 (s, 3H), 0.42 (dd, J = 13.5 Hz, 4.2 Hz, 1H); ¹³C NMR δ (75 MHz, CDCl₃) δ 209.6, 65.1, 63.5, 56.1, 53.5, 44.1, 39.4, 38.7, 38.0, 37.2, 33.7, 31.4, 30.7, 29.4, 27.9, 23.9, 22.6, 20.6, 13.3, 11.9; IR (film, cm⁻¹) 3427, 2928, 1704, 1354. Anal. Calcd for C₂₁H₃₂N₂O₂: C, 73.22%; H, 9.36%; N 8.13%. Found: C, 73.44%; H, 9.56%; N, 8.06%.
Synthesis of 5α-12-Azip

(3β,5α,12α)-3-(Acetyloxy)-12-hydroxy-pregnan-20-one (2). Steroid 1 (230 mg, 0.61 mmol) prepared according to the literature was dissolved in CH$_2$Cl$_2$ (1 mL) and EtOH (15 mL) was added. A small amount of steroid 1 precipitated upon EtOH addition and CH$_2$Cl$_2$ was added dropwise until the precipitate dissolved. NaBH$_4$ (67 mg) was added in portions and the reaction was stirred at room temperature for 10 min. Water (5 mL) was added and the pH was brought to ~ 6 with dilute HCl. The products were extracted into CH$_2$Cl$_2$ and the extract was washed with water and dried with anhydrous Na$_2$SO$_4$. The solvent was removed under reduced pressure and the residue was purified by flash column chromatograph (silica gel eluted with a gradient of 4:1 to 3:1 hexanes:EtOAc) to yield steroid 2 (67 mg, 29%). Also formed in this reaction were the more polar 12β-alcohol epimer (the major product) and the 12β,20-diol (very minor product). These two more polar products were isolated by flash column chromatography (silica gel eluted with 4:1 EtOAc:hexanes) when the reaction was repeated on a larger scale and these compounds were only partially
characterized. Steroid 2 had: $^1$H-NMR (300 MHz, CDCl$_3$) δ 4.68 (m, 1H), 3.99 (br s, 1H), 3.13 (t, $J =$ 9.1 Hz, 1H), 2.15 (s, 3H), 2.02 (s, 3H), 0.82 (s, 3H), 0.64 (s, 3H); $^{13}$C-NMR (75 MHz, CDCl$_3$) δ 210.5, 170.6, 73.5, 71.4, 55.6, 48.2, 48.0, 47.4, 44.6, 36.6, 35.5, 35.1, 33.9, 31.5, 30.7, 28.9, 28.4, 27.3, 23.7, 22.2, 21.4, 13.9, 12.0; IR (film, cm$^{-1}$) 3469, 2937, 2873, 1732, 1702, 1473, 1446, 1381, 1361, 1247.

(3β,5α,12α)-3,12-Bis(acetyloxy)-pregnan-20-one (3). Steroid 2 (0.52 g, 1.38 mmol) was dissolved in pyridine (5 mL) and Ac$_2$O (1.5 mL). DMAP (60 mg) was added and the reaction was stirred at room temperature for 20 min. The reaction was poured into aqueous NaHCO$_3$ and the product extracted into CH$_2$Cl$_2$. The extract was washed with water, dried with anhydrous Na$_2$SO$_4$ and the solvent removed under reduced pressure. The residue was purified by flash column chromatography (silica gel eluted with 1:6 EtOAc in hexanes) to yield steroid 3 (0.58 g, ca. 100%) which had: $^1$H-NMR (300 MHz, CDCl$_3$) δ 5.12 (br, s), 4.68 (m, 1H), 2.95 (t, $J =$ 8.8 Hz), 2.14 (s, 3H), 2.02 (s, 3H), 0.81, (s, 3H), 0.69 (s, 3H); $^{13}$C-NMR (75 MHz, CDCl$_3$) δ 208.7, 170.6, 170.3, 74.4, 73.4, 55.6, 49.4, 48.1, 46.7, 44.7, 36.7, 35.3, 35.1, 34.0, 31.6, 31.1, 28.4, 27.3, 25.8, 23.7, 22.3, 21.38, 21.31, 13.8, 12.0; IR (film, cm$^{-1}$) 2945, 2856, 1737, 1704, 1472, 1441, 1362, 1244.

(3β,5α,12α)-3,12-Bis(acetyloxy)-pregnan-20-one, cyclic-(1,2-ethanediyl acetal) (4). Steroid 3 (0.57 g, 1.36 mmol) was dissolved in toluene (60 mL) and the toluene was partially removed (~20 mL) by distillation using a Dean-Stark apparatus. Ethylene glycol (3 mL) and PPTS (0.19 g) were added and the reaction was refluxed for 5 h. The toluene was washed with water, dried with anhydrous Na$_2$SO$_4$ and removed under reduced pressure. The residue was purified by flash column chromatography (silica gel eluted with 6:1 hexane:EtOAc) to yield steroid 4 (0.63 g, ca. 100%) which had: $^1$H-NMR (300 MHz, CDCl$_3$) δ 5.08 (m, 1H), 4.67 (m, 1H), 3.88 (m, 4H), 2.29 (t, $J =$ 9.6 Hz, 1H), 2.06 (s, 3H), 2.01 (s, 3H), 1.20 (s, 3H), 0.82 (s, 3H), 0.81 (s, 3H); $^{13}$C-NMR (75 MHz, CDCl$_3$) δ 170.5, 170.4, 111.4, 75.3, 73.5, 64.8, 63.2, 49.8, 49.0, 48.3, 44.7, 44.5, 36.7, 35.0, 34.8, 34.0, 31.5, 28.4, 27.3, 25.5, 24.0, 23.0, 22.3, 21.4, 21.3, 13.4, 12.0; IR (film, cm$^{-1}$) 2947, 2874, 1735, 1472, 1447, 1371, 1247.

(3β,5α,12α)-3,12-Dihydroxypregnan-20-one, 20-cyclic-(1,2-ethanediyl acetal) (5). Diacetate 4 (0.63 g, 1.36 mmol) was hydrolyzed to diol 5 in the standard manner using a procedure similar to that described for the preparation of steroid 8. Steroid 5 (0.56 g, ca. 100%) had: $^1$H-NMR (300 MHz, CDCl$_3$) δ 3.95 (m, 4H), 3.58 (m, 1H), 2.22 (t, $J =$ 10.0 Hz, 1H), 0.788 (s, 3H), 0.785 (s, 3H); $^{13}$C-NMR (75 MHz, CDCl$_3$) δ 72.6, 71.1, 64.0, 63.7, 49.8, 48.1, 47.8, 46.1, 44.8, 38.2, 36.8, 35.1, 34.9, 31.5, 31.4, 28.6, 27.7, 23.6, 23.0, 22.8, 13.9, 12.1; IR (film, cm$^{-1}$) 3401, 2929.
(3α,5α,12α)-12-Hydroxy-3-(formyloxy)-pregnan-20-one, cyclic-(1,2-ethanediyl acetal) (6). Steroid 5 (0.56 g, 1.48 mmol) and Ph3P (0.78 g) were dissolved in THF (30 mL). HCO2H (136 mg) and DEAD solution (0.46 mL, 40 wgt. % in toluene) were added. After the reaction became slightly yellow, stirring at room temperature was continued for 1 h. The solvents were removed under reduced pressure and the residue was purified by flash column chromatography (silica gel eluted with a gradient of 7:1 to 5:1 hexanes:EtOAc containing 1% triethyl amine) to yield steroid 6 (0.47 g, 78%) which had: 1H-NMR (300 MHz, CDCl3) δ 8.04 (s,1H), 5.16 (br s, 1H), 3.95 (m, 4H), 2.23 (m, 2H), 1.26 (s,3H), 0.79 (s, 6H); 13C-NMR (75 MHz, CDCl3) δ 160.7, 112.2, 72.6, 70.2, 64.0, 63.8, 49.9, 48.2, 47.8, 46.1, 39.8, 35.4, 34.9, 32.9, 32.6, 31.4, 28.2, 27.3, 26.1, 23.6, 22.9, 22.8, 14.0, 11.2; IR (film, cm–1) 3468, 2931, 1720, 1446, 1371.

(3α,5α)-3-(Formyloxy)-pregnane-12,20-dione, 20-cyclic-(1,2-ethanediyl acetal) (7). Steroid 6 (0.45 g, 1.11 mmol) was dissolved in CH2Cl2 (100 mL) and solid NaOAc (0.30 g) and PCC (0.60 g) were added. The reaction was stirred at room temperature for 2 h. Diethyl ether was added and the organic phase was washed with water, dried over anhydrous Na2SO4 and removed under reduced pressure. The residue was purified by flash column chromatography (silica gel eluted with 7:1 hexanes:EtOAc) to give steroid 7 (0.40 g, 89%) which had: 1H-NMR (300 MHz, CDCl3) δ 8.05 (s, 1H), 5.17 (br s, 1H), 4.00 (m, 2H), 3.86 (m, 2H), 2.73 (t, J = 9.5 Hz, 1H), 2.55 (t, J = 12.8 Hz, 1 H), 2.18 (dd, J = 11.4 Hz, J = 5.0 Hz, 1H), 1.30 (s, 3H), 1.13, (s, 3H), 0.91 (s, 3H); 13C-NMR (75 MHz, CDCl3) δ 213.8, 160.5, 111.2, 69.7, 65.4, 62.8, 58.1, 56.8, 49.2, 39.7, 37.7, 36.5, 34.6, 32.6, 32.4, 31.1, 28.0, 25.8, 24.3, 23.8, 21.7, 12.9, 11.0; IR (film, cm–1) 2929, 1719, 1704, 1447, 1432, 1378.

(3α,5α)-3-Hydroxypregnane-12,20-dione, 20-cyclic-(1,2-ethanediyl acetal) (8). Steroid 7 (0.40 g, 0.99 mmol) was dissolved in MeOH (40 mL) and water (5 mL) and solid KHCO3 (0.20 g) were added. The reaction was refluxed for 15 min. The MeOH was removed under reduced pressure and the product was extracted into EtOAc. The extract was washed with water until the water phase had pH ~7 and the organic phase was dried over anhydrous Na2SO4 and removed under reduced pressure leaving steroid 8 (0.37 g, ca. 100%) as a white solid which was not further purified and had: 1H-NMR (300 MHz, CDCl3) δ 4.05 (overlapped br s, 1H), 3.98 (m, 2H), 3.86 (m, 2H), 2.73 (m, J = 9.5 Hz, 1H), 2.53 (t, J = 12.9 Hz), 2.19 (dd, J = 12.9 Hz, J = 4.8 Hz, 1H), 1.30 (s, 3H), 1.12 (s, 3H), 0.87 (s, 3H); 13C-NMR (75 MHz, CDCl3) δ 214.3, 111.3, 66.1, 65.5, 62.9, 58.2, 57.1, 56.9, 49.2, 38.9, 37.8, 36.9, 35.6, 34.7, 31.9, 31.3, 28.9, 28.3, 24.4, 23.8, 21.9, 13.0, 10.9; IR (film, cm–1) 3401, 3012, 2925, 2875, 1705, 1445, 1432.

(3α,5α)-3'-Hydroxy-spiro[3H-diazirine-3,12'-]pregnan-20'-one, cyclic-(1,2-ethanediyl acetal) (11). Steroid 8 (0.25 g, 0.66 mmol), cyclohexylamine (5 mL) and CF3CO2H (5 μL) were refluxed for 17 h. The
cyclohexylamine was removed under reduced pressure and crude enamine 9 was obtained as a light brown foam which was not purified or characterized.

The crude enamine 9 was dissolved in MeOH (25 mL), cooled to 0 °C and anhydrous NH₃ was bubbled into the MeOH solution until NH₃ saturation was achieved. The reaction was warmed to room temperature and stirred for 1 h. Hydroxylamine-O-sulfonic acid (0.48 g) was then added and stirring was continued for 20 h. The reaction was filtered to remove a solid and the MeOH was removed under vacuum to obtain diaziridine 10 as a light yellow solid which was used without purification or characterization.

The crude diaziridine 10 was dissolved in CH₂Cl₂ (25 mL) and the reaction was cooled to 0 °C. Triethyl amine (2.5 mL) was then added. I₂ (~ 0.7 g) dissolved in CH₂Cl₂ then was added dropwise until a yellow color persisted. The reaction was allowed to warm to room temperature and stirred for 1 h. The CH₂Cl₂ was washed with 5% aqueous Na₂S₂O₃, water, and dried over Na₂SO₄. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography (silica gel eluted with 4% to 5% EtOAc in CH₂Cl₂) to yield diazirine 11 (150 mg, 58%) which had: ¹H-NMR (300 MHz, CDCl₃) δ 4.02 (overlapped br s, 1H), 3.95 (m, 2H), 3.81 (t, J = 6.5 Hz, 2H), 1.96 (t, J = 13.8 Hz, 1H), 1.05 (s, 3H), 1.04, (s, 3H), 0.78 (s, 3H), 0.11 (dd, J = 4.7 Hz, J = 13.7 Hz, 1H); ¹³C-NMR (75 MHz, CDCl₃) δ 110.8, 66.1, 64.4, 62.6, 55.9, 51.9, 50.1, 44.7, 38.8, 35.7, 35.6, 34.7, 34.2, 31.8, 31.5, 28.8, 28.6, 28.3, 24.3, 23.9, 23.8, 12.6, 10.8; IR (film, cm⁻¹) 3401, 2924, 1705, 1566, 1445. Anal. Calcd for C₂₁H₃₂N₂O₂: C, 73.22; H, 9.36; N, 8.13; found: C, 72.98; H, 9.20; N, 8.26.

(3’α,5’α)-3’-Hydroxy-spiro[3H-diazirine-3,12’]-pregnan-20’-one (12, 5α-12-Azip). Steroid 11 (150 mg, 0.39 mmol) was dissolved in acetone (25 mL), PTSA (50 mg dissolved in 5 mL acetone) and water (1 mL) were added. The reaction was stirred at room temperature for 4 h and NaHCO₃ (25 mg) dissolved in water was added. The acetone was removed under reduced pressure and CH₂Cl₂ was added. The CH₂Cl₂ was washed with water, dried over anhydrous Na₂SO₄ and removed. The residue was purified by flash column chromatography (silica gel eluted with 5:1 hexanes:EtOAc) to yield diazirine 12 (135 mg, ca. 100%) which had: ¹H-NMR (300 MHz, CDCl₃) δ 4.04 (m, 1H), 1.88 (s, 3H), 1.00 (s, 3H), 0.78 (s, 3H), 0.24 (dd, J = 4.8 Hz, J = 14.4 Hz, 1H); ¹³C-NMR (75 MHz, CDCl₃) δ 66.1, 56.0, 55.9, 52.3, 46.5, 38.9, 35.6, 34.7, 34.4, 31.8, 31.5, 30.9, 28.8, 28.2, 28.0, 24.5, 24.2, 12.2, 10.9; IR (film, cm⁻¹) 3401, 2916, 2854, 1705, 1566, 1445. Anal. Calcd for C₂₁H₃₂N₂O₂: C, 73.22; H, 9.36; N, 8.13; found: C, 72.98; H, 9.20; N, 8.26.

Synthesis of 5α-15-Azip
(3α,5α)-3-(Acetyloxy)-17α-bromo-pregnan-20-one (2). A mixture of (3α,5α)-3-(acetyloxy)-pregnan-20-one (1, 1.20 g, 3.33 mmol, prepared by acetylation of (3α,5α)-3-hydroxypregnan-20-one using acetic anhydride, pyridine and DMAP), and NBS (1.0 g) in CCl₄ (40 mL) was illuminated and refluxed for 25 min. Then the mixture was chilled and the solid was removed by filtration. The filtrate was washed successively with 10% NaHCO₃, water and dried over anhydrous Na₂SO₄. Removal of solvent under reduced pressure gave a residue which was purified by flash column chromatography (silica gel eluted with hexanes/EtOAc, 10:1). Crystallization from MeOH gave steroid 2 (1.08 g, 75%) as white needles which had: mp 137–138 °C (from MeOH); 'H NMR (300 MHz, CDCl₃) δ 5.01 (s, 1H), 3.06 (m, 1H), 2.38 (s, 3H), 2.25 (m, 1H), 2.05 (s, 3H), 0.94 (s, 3H), 0.75 (s, 3H); '³C NMR (75 MHz, CDCl₃) δ 11.2, 14.0, 20.6, 21.4, 22.6, 25.9, 27.3, 28.0, 31.5, 32.6, 35.4, 35.6, 35.7, 35.8, 39.7, 47.0, 50.7, 53.1, 69.8, 73.3, 86.4, 170.4, 201.2; IR (film, cm⁻¹) 2940, 2868, 1736, 1702, 1362, 1240. Anal. Calcd. for C₂₃H₃₅BrO₃: C, 62.87; H, 8.03; found: C, 63.00; H, 8.12.
(3α,5α)-3-(Acetyloxy)-pregn-16-en-20-one (3). Steroid 2 (2.40 g, 5.47 mmol) in dry pyridine (55 mL) was refluxed for 6 h. The reaction mixture was poured into water, and the precipitated solid was extracted into diethyl ether. The ethereal solution was washed free of pyridine with water and 10% aqueous HCl, and then washed with 5% NaHCO₃, water and dried over anhydrous Na₂SO₄. Removal of solvent under reduced pressure gave a residue which was purified by flash column chromatography (silica gel eluted with hexanes/EtOAc, 6:1) to give steroid 3 (1.80 g, 91%) as white crystals which had: mp 162–163 °C (from hexanes); ¹H NMR (300 MHz, CDCl₃) δ 6.70 (dd, J = 1.8 Hz, J = 3.3 Hz, 1H), 5.01 (s, 1H), 2.26 (s, 3H), 2.05 (s, 3H), 0.88 (s, 3H), 0.83 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 11.3, 15.9, 20.6, 21.5, 26.0, 27.1, 28.2, 31.8, 32.1, 32.6, 32.8, 33.7, 34.7, 36.0, 40.2, 46.3, 54.6, 56.4, 70.1, 144.4, 155.5, 170.7, 196.8; IR (film, cm⁻¹) 2941, 2865, 1732, 1322, 1230 cm⁻¹. Anal. Calcd. for C₂₃H₃₄O₃: C, 77.05; H, 9.56; found: C, 76.94; H, 9.44.

(3α,5α)-3-(Acetyloxy)-20,20-[1,2-ethanediylbis(oxy)]-pregn-16-ene (4). Steroid 3 (2.90 g, 8.10 mmol), ethylene glycol (4.75 g) and PTSA (0.30 g) in toluene (100 mL) were refluxed overnight in a Dean-Stark apparatus under N₂. The reaction was cooled to room temperature and poured into water. The two phases were transferred to a separatory funnel and the product was extracted into EtOAc. The combined extracts were washed successively with saturated aqueous NaHCO₃, water, brine, and dried over anhydrous Na₂SO₄. The solvents were removed under reduced pressure and the residue was purified by flash column chromatography (silica gel eluted with hexanes/EtOAc, 8:1) to give steroid 4 (2.10 g, 64%) as white crystals which had: mp 95–96 °C (from hexanes); ¹H NMR (300 MHz, CDCl₃) δ 5.76 (d, J = 1.5 Hz, 1H), 5.01 (s, 1H), 3.92 (m, 4H), 2.05 (s, 3H), 1.50 (s, 3H), 0.91 (s, 3H), 0.82 (s, 3H); ¹³C NMR (300 MHz, CDCl₃) δ 11.3, 17.1, 20.7, 21.5, 25.9, 26.1, 28.3, 30.7, 31.7, 32.7, 32.9, 33.9, 35.5, 35.9, 40.2, 46.1, 54.5, 58.1, 64.2, 64.5, 70.1, 108.4, 126.8, 155.1, 170.7; IR (film, cm⁻¹) 2936, 2864, 1734, 1360. Anal. Calcd. for C₂₅H₃₈O₄: C, 74.59; H, 9.51; found: C, 74.63, H, 9.47.

(3α,5α)-3-(Acetyloxy)-pregn-16-ene-15,20-dione, 20-cyclic 1,2-ethanediyl acetal (5). A solution of CrO₃ (1.30 g) in CH₂Cl₂ (65 mL) was cooled to –15 °C and 3,5-dimethylpyrazole (9.52 g) was added in one portion. The resultant brown slurry was stirred for 30 min followed by addition of steroid 4 (1.96 g, 4.88 mmol) in CH₂Cl₂. The mixture was stirred at –15 °C for 4 h and a 1:1 mixture of hexanes and EtOH was added. The reaction mixture was filtered through a pad of silica gel. Solvent was removed under reduced pressure to give a residue which was purified by flash column chromatography (silica gel eluted with hexanes/EtOAc, 4:1) to give steroid 5 (1.12 g, 55%) as white crystals which had: mp 126–128 °C (from hexanes/EtOAc); ¹H NMR (300 MHz, CDCl₃) δ 5.91 (s, 1H), 5.01 (s, 1H), 3.92 (m, 4H), 2.70 (dd, J = 3.3 Hz, J = 12.9 Hz, 1H), 2.05 (s, 3H), 2.05 (s, 3H), 1.59 (s, 3H), 1.15 (s, 3H), 0.76 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ
(3α,5α)-3-(Acetyloxy)-pregnane-15,20-dione, 20-cyclic 1,2-ethanediyl acetal (6). Steroid 5 (1.02 g, 2.45 mmol) was dissolved in EtOH (16 mL) and hydrogenated (50 psi, 5% Pd/C, 110 mg) in a Paar hydrogenator at room temperature for 5 h. The reaction mixture was filtered through a pad of Celite 545® to remove catalyst and the solvent was removed under reduced pressure. The residue was passed through a short column (silica gel eluted with hexanes/EtOAc, 3:1) to give steroid 6 (1.00 g, 98%) as white crystals which had: mp 177–178 °C (from hexanes/EtOAc); 1H NMR (300 MHz, CDCl3) δ 5.00 (s, 1H), 3.95 (m, 4H), 2.68 (dd, J = 3.3 Hz, J =12.9 Hz, 1H), 2.05 (s, 3H), 1.35 (s, 3H), 0.83 (s, 3H), 0.80 (s, 3H); 13C NMR (75 MHz, CDCl3) δ 11.2, 13.8, 20.1, 21.4, 24.7, 26.0, 27.8, 30.4, 31.5, 32.6, 32.7, 35.8, 37.1, 39.4, 39.9, 41.5, 53.7, 53.8, 63.1, 65.4, 65.7, 69.8, 110.6, 170.5, 215.1; IR (film, cm–1) 2935, 2863, 1733, 1244. Anal. Calcd. for C25H38O5: C, 71.74; H 9.15; found: C, 71.87; H, 8.93.

(3'α,5'α)-20',20'-[1,2-Ethanediylbis(oxy)]-spiro[3H-diazirine-3,15']-pregnan-3'-ol, acetate (7). Anhydrous ammonia gas was bubbled into a stirred solution of steroid 6 (902 mg, 2.16 mmol) in MeOH (60 mL) at 0 °C until the MeOH was saturated with ammonia. The solution was further stirred at 0 °C for 2 h. Then a solution of hydroxylamine-O-sulphonic acid (1.01 g) in MeOH (ca. 95 mL) was added at 0 °C and the reaction was stirred at room temperature overnight. After filtration to remove precipitated (NH4)2SO4, the filtrate was mixed with EtOAc (ca. 35 mL) and triethylamine (ca. 3 mL). Iodine crystals were added in portions while stirring at 0 °C until a yellow color persisted. The mixture was diluted with EtOAc (ca. 35 mL) and the solution was washed successively with 10% aqueous Na2S2O3 (ca. 15 mL), water (ca. 15 mL), brine (ca. 15 mL), and dried over anhydrous Na2SO4. Removal of solvent under reduced pressure gave a residue which was purified by flash column chromatography (silica gel eluted with hexanes/EtOAc, 8:1) to give steroid 7 (41.3 mg, 4.5%), recovered steroid 6 (681 mg, 76%) and the 14β-epimer of steroid 6 (67 mg, 7.4%). Steroid 7 was obtained as white needles which had: mp 148–150 °C (from hexanes/EtOAc); 1H NMR (300 MHz, CDCl3) δ 4.97 (s, 1H), 3.95 (m, 4H), 2.04 (s, 3H), 1.30 (s, 3H), 1.17 (s, 3H), 0.74 (s, 3H); 13C NMR (75 MHz, CDCl3) δ 11.2, 14.3, 20.1, 21.5, 24.6, 26.0, 27.7, 30.1, 30.8, 31.3, 32.6, 32.7, 35.1, 35.8, 39.0, 39.8, 43.6, 54.1, 54.6, 55.8, 63.1, 65.3, 69.8, 110.9, 170.6; IR (film, cm–1) 2949, 1728, 1586, 1247.

(3’α,5’α)-20’,20’-[1,2-Ethanediylbis(oxy)]-spiro[3H-diazirine-3,15’]-pregnan-3’-ol (8). Steroid 7 (38.1 mg, 0.089 mmol), KOH (7.5 mg) and water (2 drops) in MeOH (10 mL) was stirred at room temperature
for 48 h. The reaction solution was poured into water (50 mL) and the product was extracted into EtOAc (3 X 20 mL). The combined extracts were washed successively with water (3 X 10 mL), brine (10 mL), and dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure and the residue was purified by flash column chromatography (silica gel eluted with hexanes/EtOAc, 2:1) to give steroid 8 (33.4 mg, 97%) as white crystals which had: mp 144–145 °C (from EtOAc); ¹H NMR (75 MHz, CDCl₃) δ 4.01 (s, 1H), 3.95 (m, 4H), 1.30 (s, 3H), 1.16 (s, 3H), 0.73 (s, 3H); ¹³C NMR (300 MHz, CDCl₃) δ 11.0, 14.4, 20.1, 24.6, 27.9, 28.9, 30.1, 30.8, 31.3, 32.0, 35.1, 35.7, 36.1, 38.9, 39.0, 43.6, 54.2, 54.5, 55.8, 63.2, 65.3, 66.3, 110.9; IR (film, cm⁻¹) 3304, 2928, 2852, 1582, 1450.

(3′α,5′α)-3′-Hydroxy-spiro[3H-diazirine-3,15′]-pregnan-20-one (9, 5[15-Azip]). Steroid 8 (35.8 mg, 0.093 mmol), PTSA (10 mg) and water (2 drops) in acetone (15 mL) was stirred at room temperature for 2 h. The reaction solution was poured into water (20 mL) and the product was extracted into EtOAc (3 X 15 mL) the combined extracts were washed successively with 10% aqueous NaHCO₃ (10 mL), water (10 mL), brine (10 mL) and dried over anhydrous Na₂SO₄. The solvent was removed under reduced pressure and the product was purified by flash column chromatography (silica gel eluted with hexanes/EtOAc/CH₂Cl₂, 2.5:1:0.5) to give steroid 9 (30.7 mg, 97%) as white crystals which had: mp 139–140 °C; ¹H NMR (300 MHz, CDCl₃) δ 4.02 (s, 1H), 2.73 (t, J = 9.3 Hz, 1H), 2.16 (s, 3H), 2.05 (m, 2H), 1.01 (s, 3H), 0.73 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 11.0, 14.8, 20.3, 27.8, 28.9, 30.6, 30.7, 30.8, 31.6, 32.1, 35.0, 35.6, 36.2, 38.5, 38.8, 45.1, 54.1, 54.5, 60.8, 66.2, 207.1; IR (film, cm⁻¹) 3310, 2928, 2850, 1704, 1450, 1360. Anal. Calcd. for C₂₁H₃₂N₂O₂: C, 73.22; H, 9.36; N, 8.13; found: C, 73.39; H, 9.24; N, 7.99.
Synthesis of KK200

1-Bromo-4-[(methyoxymethoxy)methyl]-benzene (2). Chloromethyl methyl ether (3.06 mL, 40.35 mmol) was added to a stirred, cold solution of 4-bromobenzyl alcohol (1, 5.0 g, 26.9 mmol) and Hunig’s base (14.05 mL, 80.7 mmol) in CH₂Cl₂ (30 mL) and the reaction was stirred at room temperature for 12 h. Aqueous NaHCO₃ (100 mL) was added and the product was extracted into CH₂Cl₂ (3 x 75 mL). The combined extracts were dried over anhydrous Na₂SO₄ and the solvents removed under reduced pressure to give an oil which was purified by flash column chromatography (silica gel eluted with 5-10% EtOAc in hexanes) to give compound 2 as a colorless liquid (6.0 g, 97%) which had: ¹H NMR (400 MHz, CDCl₃) δ 7.39, (d, J = 8.2 Hz, 2H), 7.16 (d, J = 8.2 Hz, 2H), 4.61 (s, 2H), 4.46 (s, 2H), 3.32 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 136.9, 131.5, 129.4, 121.5, 95.7, 68.4, 55.4.

2,2,2-Trifluoro-1-[4-[(methoxymethoxy)methyl]phenyl]-ethanone (3). Compound 2 (6.0 g, 26 mmol) dissolved in THF was added to a stirred hot suspension of Mg turnings (947 mg, 39
mmol) in THF (100 mL) and the mixture was heated to reflux for 15 minutes. Ethyl bromide (0.15 mL, 2 mmol) was added to the refluxing solution to activate the magnesium and the reaction was refluxed for another 90 min. The resulting THF solution of Grignard reagent was cooled to room temperature and transferred by cannula to a flask containing a cold solution of 1-trifluoroacetyl piperidine (18.1 g, 100 mmol) in THF (15 mL). The reaction was allowed to stir for another 13 h at room temperature. Saturated aqueous NH₄Cl was added and the product was extracted into EtOAc. The combined organic extracts were dried over anhydrous Na₂SO₄ and the solvents removed under reduced pressure to give a pale yellow oil. Flash column chromatography (silica gel eluted initially with hexanes and then with 2-8% EtOAc in hexanes) gave compound 3 as a liquid (4 g, 62%) which had: ¹H NMR (400 MHz, CDCl₃) δ 8.07 (d, J = 7.8 Hz, 2H), 7.54 (d, J = 7.8 Hz, 2H), 4.75 (s, 2H), 4.70 (s, 2H), 3.42 (s, 3H).

2,2,2-Trifluoro-1-[4-[(methoxymethoxy)methyl]phenyl]-ethanone, oxime (4). Compound 3 (3.8 g, 15 mmol), hydroxylamine hydrochloride (6.95 g, 100 mmol) and NaOAc (16.4 g, 200 mmol) were refluxed in MeOH (200 mL) for 48 h. The reaction was cooled and the MeOH was removed under reduced pressure. The residue was dissolved in water and extracted with CH₂Cl₂. The combined organic extracts were dried over anhydrous Na₂SO₄ and the solvents removed under reduced pressure to give compound 4 as an oil which was a mixture of E/Z oxime isomers (3.8 g, 96%) which had: ¹H NMR (400 MHz, CDCl₃) δ 9.28 & 9.05 (b s, 1H), 7.20–7.50 (m, 6H), 4.76 & 4.74 (s, 2H), 4.66 & 4.65 (s, 2H), 3.43 (s, 3H).

2,2,2-Trifluoro-1-[4-[(methoxymethoxy)methyl]phenyl]-ethanone, O-[(4-methylphenyl)sulfonyl]oxime (5). Tosyl chloride (3.29 g, 17.28 mmol) was added to a stirred, cold solution of compound 4 (3.8 g, 14.4 mmol) and triethyl amine (4.2 mL, 30 mmol) in CH₂Cl₂ and the reaction was stirred at 0 °C for 2 h. Aqueous NaHCO₃ was added and the product was extracted into CH₂Cl₂. The combined organic extracts were dried over anhydrous Na₂SO₄ and the solvents removed under reduced pressure to give compound 5 as a residue which was used without purification. Compound 5 had: ¹H NMR (400 MHz, CDCl₃) δ 7.90 (m, 2H), 7.30–7.55 (m, 4H), 4.75 & 4.72 (s, 2H), 4.65 & 4.64 (s, 2H), 3.43 & 3.42 (s, 3H), 2.49 & 2.47 (s, 3H).
3-[4-[(Methoxymethoxy)methyl]phenyl]-3-(trifluoromethyl)-3\(H\)-Diazirine (7). Freshly condensed anhydrous ammonia (20 mL) was added to a stirred, cold (-78 °C) solution of compound 5 (6.0 g, 14.4 mmol) in CH\(_2\)Cl\(_2\) (100 mL) and the mixture was slowly warmed to room temperature and stirred for 16 h. Water was added and the product was extracted into CH\(_2\)Cl\(_2\) (3 x 80 mL). The combined organic extracts were dried over anhydrous Na\(_2\)SO\(_4\) and the solvents removed under reduced pressure to give crude diaziridine 6 which was used without purification or characterization.

Crude diaziridine 6 was dissolved in MeOH (50 mL). Triethyl amine (10 mL) was added and a saturated solution of I\(_2\) in MeOH was added in portions until the I\(_2\) color persisted. Excess I\(_2\) was eliminated by adding a 5% aqueous Na\(_2\)S\(_2\)O\(_3\) (50 mL) and the reaction was diluted with water (100 mL). The product was extracted into CH\(_2\)Cl\(_2\) (75 mL x 3). The combined organic extracts were dried over anhydrous Na\(_2\)SO\(_4\) and the solvents removed under reduced pressure to give an oil which was purified by flash column chromatography (silica gel eluted with 2-10% EtOH in hexanes) to give the diazirine 7 (2.6 g, 70%) as a liquid which had: \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.40 (d, \(J = 8.2\) Hz, 2H), 7.19 (d, \(J = 8.2\) Hz), 4.71 (s, 2H), 4.61 (s, 2H), 3.41(s, 3H).

4-[3-(Trifluoromethyl)-3\(H\)-diazirin-3-yl]-benzenemethanol (8). Compound 7 (2.6 g, 10 mmol) dissolved in MeOH (10 mL) was added to 5-7 % dry HCl in MeOH (20 mL) and the reaction was stirred at room temperature for 13 h. Aqueous saturated NaHCO\(_3\) (100 mL) was carefully added and the product was extracted into CH\(_2\)Cl\(_2\) (3 x 75 mL). The combined organic extracts were dried over anhydrous Na\(_2\)SO\(_4\) and the solvents removed under reduced pressure to give an oil which was purified by flash column chromatography (silica gel eluted with 5-20% EtOAc in hexanes) to give compound 8 (2.1 g, 97%) which had: \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.39 (d, \(J = 7.8\) Hz, 2H), 7.20 (d, \(J = 7.8\) Hz, 2H), 4.71 (s, 2H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 142.4, 128.2, 126.9, 126.5, 122.1 (q, \(J = 275\) Hz), 63.9, 28.3 (q, \(J = 40.4\) Hz).

3-[4-(Iodomethyl)phenyl]-3-(trifluoromethyl)-3\(H\)-Diazirine (9). A mixture of imidazole (0.82 g, 12 mmol), triphenylphosphine (1.83 g, 7 mmol) and I\(_2\) (2.03 g, 8 mmol) in CH\(_2\)Cl\(_2\) (20 mL) was stirred at room temperature for 15 min. Compound 8 (864 mg, 4 mmol) dissolved in CH\(_2\)Cl\(_2\) (10 mL) was added and the reaction was stirred for 90 min at room temperature. Hexane (100 mL) was added, a precipitate was formed and stirring was continued for 10 min. The brown supernatant was removed from the precipitate, added to a silica gel column and purified by flash column chromatography to give compound 9 (587 mg, 55%) which had: \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.36 (d, \(J = 7.8\) Hz, 2H), 7.20 (d, \(J = 7.8\) Hz, 2H), 4.71 (s, 2H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 142.4, 128.2, 126.9, 126.5, 122.1 (q, \(J = 275\) Hz), 63.9, 28.3 (q, \(J = 40.4\) Hz).
chromatography (silica gel eluted with 2-10% EtOAc in hexanes). The solvents were removed under reduced pressure to give compound 9 (1.1 g, 85%) as a viscous liquid which on standing crystallized to give a pale yellow solid which had: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.40 (d, $J$ = 7.8 Hz, 2H), 7.12 (d, $J$ = 7.8 Hz, 2H), 4.43 (s, 2H). $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 141.1, 129.1 (2 x C), 128.7, 126.9 (2 x C), 122.0 (q, $J$ = 275 Hz), 28.3 (q, $J$ = 40.5 Hz).

(5α,17β)-17-[[4-[3-(Trifluoromethyl)-3H-diazirin-3-yl]phenyl]methoxy]-androstane-3-one, cyclic 1,2-ethanediyl acetal (11). (5α,17β)-17-Hydroxyandrostan-3-one, cyclic 1,2-ethanediyl acetal (10, 301 mg, 0.9 mmol) prepared by a literature procedure$^3$ and NaH (60% suspension in mineral oil, 0.8 g, 20 mmol) in DMF (10 mL) and THF (7 mL) were stirred at room temperature for 30 min. Compound 9 (652 mg, 2 mmol) was added and the reaction was stirred for 14 h. The reaction mixture was cooled, 2-propanol and then cold water were carefully added. After the NaH was consumed, ice and additional water (100 mL) were added. The product was extracted into EtOAc (4 x 40 mL). The combined organic extracts were washed with brine, dried over anhydrous Na$_2$SO$_4$ and the solvents removed under reduced pressure to give an oil which was purified by flash column chromatography (silica gel eluted with hexanes followed by 2-20% EtOAc in hexanes) to give steroid 11 as an oil (205 mg, 43%) which had: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.36 (d, $J$ = 8.2 Hz, 2H), 7.15 (d, $J$ = 8.2 Hz, 2H), 4.53 (s, 2H), 3.92 (s, 4H), 3.38 (t, $J$ = 8.2 Hz, 1H), 0.82 (s, 3H), 0.81 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 141.3, 127.8, 127.4 (2 x C), 126.3 (2 x C), 122.1 (q, $J$ = 275 Hz), 109.3, 88.7, 70.7, 64.1, 54.1, 51.1, 43.6, 43.1, 38.0, 37.9, 36.0, 35.5, 35.2, 31.5, 31.4, 31.1, 28.5, 28.4, 28.1, 27.8, 23.3, 22.6, 20.8, 14.1, 11.8, 11.4.

(5α,17β)-17-[[4-[3-(Trifluoromethyl)-3H-diazirin-3-yl]phenyl]methoxy]-androstane-3-one (12). Steroid 11 (197 mg, 0.37 mmol) and PTSA (50 mg) in acetone (25 mL) were stirred at room temperature of for 16 h. Aqueous NaHCO$_3$ was added and the acetone was removed under reduced pressure. Water was added and the product was extracted into EtOAc (3 x 50 mL). The combined organic extracts were washed with brine, dried over anhydrous Na$_2$SO$_4$ and the solvents removed under reduced pressure. The crude product was purified by flash column chromatography (silica gel eluted with 15-20% EtOAc in hexanes) to give compound 12 as an oil (163 mg, 90%) which had: $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.36 (d, $J$ = 8.2 Hz, 2H), 7.16 (d, $J$ = 8.2 Hz, 2H), 4.53 (s, 2H), 3.38 (t, $J$ = 8.2 Hz, 1H), 1.01 (s, 3H), 0.83 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 212.0, 141.2,
127.9, 127.4 (2 x C), 126.4 (2 x C), 122.1 (q, J = 275 Hz), 88.6, 70.8, 53.9, 51.0, 46.7, 44.7, 43.1, 38.5, 38.1, 37.8, 35.7, 35.2, 31.2, 28.8, 27.8, 23.4, 21.1, 11.8, 11.5.

(3α,5α,17β)-17-[[4-[3-(Trifluoromethyl)-3H-diazirin-3-yl]phenyl]methoxy]-androstane-3-ol (13). K-selectride (1 M in THF, 1 mL, 1 mmol) was added to a cold (–78 °C) solution of steroid 12 (160 mg, 0.33 mmol) in THF and the reaction was stirred at –78 °C for 1 h. The reaction was terminated by the addition of a few drops of water and the temperature was raised to 0 °C. A 1:1 mixture of 50% aqueous H2O2 (5 mL) and 4 N NaOH (5 mL) was added and stirring was continued at room temperature for 90 min. The reaction was diluted with water and the product extracted into EtOAc (3 x 50 mL). The combined organic extracts were washed with brined, dried over anhydrous Na2SO4 and the solvents removed under reduced pressure to give an oil. The crude product was purified by flash column chromatography (silica gel eluted with 20-40% ethyl acetate in hexanes) to give compound 13 as a white solid (140 mg, 87%): mp 121–123 °C; 1H NMR (400 MHz, CDCl3) δ 7.36 (d, J = 8.2 Hz, 2H), 7.16 (d, J = 8.2 Hz, 2H), 4.53 (s, 2H), 4.04 (s, 1H), 3.38 (t, J = 8.2 Hz, 1H), 0.81 (s, 3H), 0.78 (s, 3H); 13C NMR (100 MHz, CDCl3) δ 141.3, 127.9, 127.4 (2 x C), 126.4 (2 x C), 122.1 (q, J = 275 Hz), 88.7, 70.8, 66.5, 54.4, 51.2, 43.1, 39.1, 38.0, 36.1, 35.8, 35.3, 32.2, 31.5, 29.0, 28.6, 28.4, 28.1, 27.8, 23.3, 20.4, 11.8, 11.2; IR (film, cm−1) 3306, 2931, 2845, 1611, 1519, 1446, 1345, 1230. Anal. Calcd. for C28H37F3N2O2: C, 68.55; H, 7.60; N, 5.71. Found: C, 68.79; H, 7.77; N, 5.74.

References
