Supplemental methods

1. Synthesis of ent-*VP1-001*

***ent*-Steroid 2**

ent-Testosterone (**1**) was prepared as described previously (Covey, D.F., *Polish J. Chem.,* 2006, **80**, 511-522; see also references therein). To a solution of *ent*-testosterone (**1**, 3.8 g, 13.2 mmol) in acetic anhydride (80 mL) was added NaI (7.92 g, 52 mmol) and trimethylsilyl chloride (5.8 mL, 52 mmol) at 0 °C under N_2. After addition, the reaction was allowed to warm to room temperature for 2 h. The reaction was added to Et_3N (40 mL) in diethyl ether (100 mL). The ether solution was washed with brine (50 mL x 4), aqueous $NaHCO_3$ (50 mL x 2) and dried over Na_2SO_4. After filtration, the solvent was removed under reduced pressure and the residue was purified by flash column chromatography (silica gel eluted with 25% EtOAc in hexanes) to give *ent*-steroid **2** (3.05 g, 70%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.33-5.32 (m, 1H), 4.60 (t, J = 8.3 Hz, 1H), 3.52-3.47 (m, 1H), 2.30-0.90 (m), 2.02 (s, 3H), 1.00 (s, 3H), 0.79 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.2, 140.9, 121.1, 82.7, 71.5, 51.0, 50.0, 42.3, 42.2, 37.2, 36.7, 36.5, 31.6, 31.5, 31.4, 27.4, 23.5, 21.1, 20.5, 19.3, 11.8.

***ent*-Steroid 3**

ent-Steroid **2** (3.05 g, 4.04 mmol) was dissolved in CH_2Cl_2 (50 mL) and cooled to 0 °C. (i-Pr)$_2$EtN (3.0 mL) and $ClCH_2OMe$ (1.35 ml, 18.0 mmol) were added and the reaction was stirred at room temperature for 16 h. The reaction was made basic by adding aqueous $NaHCO_3$ solution and the product was extracted into CH_2Cl_2. The combined

extracts were washed with brine, dried over Na_2SO_4, filtered and solvent removed to give a viscous liquid which was purified by flash column chromatography (silica gel eluted with 10% EtOAc in hexanes) to give *ent*-steroid **3** as a colorless liquid (2.65 g, 77%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.33-5.32 (m, 1H), 4.65 (s, 2H), 4.59 (t, *J* = 8.2 Hz, 1H), 3.39-3.35 (m, 1H), 3.34 (s, 3H), 2.35-0.89 (m), 2.01 (s, 3H), 0.99 (s, 3H), 0.78 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0, 140.7, 121.2, 94.6, 82.6, 76.7, 55.0, 50.9, 50.0, 42.3, 39.4, 37.1, 36.7, 31.6, 31.4, 28.8, 27.4, 23.5, 21.0, 20.4, 19.3, 11.8.

***ent*-Steroid 4**

To a solution of *ent*-steroid **3** (2.65 g, 7.05 mmol) in methanol (60 mL) was added K_2CO_3 (4.0 g) at room temperature. The mixture was refluxed for 16 h. Methanol was removed under reduced pressure and the residue was purified by flash column chromatography (silica gel eluted with 25% EtOAc in hexanes) to give *ent*-steroid **4** (2.31 g, 99%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.32-5.30 (m, 1H), 4.64 (s, 2H), 3.61 (t, *J* = 8.6 Hz, 1H), 3.40-3.34 (m, 1H), 3.33 (s, 3H), 2.31-0.87 (m), 0.95 (s, 3H), 0.72 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.7, 121.3, 94.5, 81.6, 76.7, 55.0, 51.2, 50.2, 42.6, 39.4, 37.2, 36.7, 36.5, 31.8, 31.4, 30.3, 28.8, 23.3, 20.5, 19.3, 10.9.

***ent*-Steroid 5**

To a solution of *ent*-steroid **4** (1.5 g, 4.54 mmol) in CH_2Cl_2 (60 mL) was added Dess–Martin periodinane (2.5 g, 6 mmol) at room temperature. After 1 h, water (50 mL) was

added, the product was extracted into CH_2Cl_2 (150 mL x 3) and the combined extracts were washed with brine (50 mL x 2). The organic layer was dried over Na_2SO_4, filtered, and the solvents removed. The residue was purified by flash column chromatography (silica gel eluted with 10% EtOAc in hexanes) to give *ent*-steroid **5** (1.5 g, 100%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.39-5.38 (m, 1H), 4.68 (s, 2H), 3.45-3.38 (m, 1H), 3.37 (s, 3H), 2.49-0.98 (m), 1.03 (s, 3H), 0.88 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 221.0, 140.9, 120.9, 94.7, 76.7, 55.1, 51.7, 50.2, 47.5, 39.5, 37.1, 36.8, 35.8, 31.4, 31.3, 30.8, 28.8, 21.8, 20.3, 19.3, 13.5.

***ent*-Steroid 6**

A solution of freshly prepared sodium ethoxide (sodium 0.4 g, 15 mmol dissolved in ethanol 15 mL) was added dropwise slowly to a solution of *ent*-steroid **5** (1.5 g, 4.54 mmol) and triethyl phosphonoacetate (3.44 g, 15 mmol) in anhydrous ethanol (25 mL) under N_2 while stirring at 35-40 °C. After addition, the reaction was refluxed for 16 h. After cooling to room temperature, the ethanol was removed and the residue was dissolved in ether which was washed with water, dried over Na_2SO_4 and filtered. Solvent was removed and the residue was purified by flash column chromatography (silica gel eluted with 10% EtOAc in hexanes) to give *ent*-steroid **6** (1.68 g, 87%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.52 (s, 1H), 5.35-5.34 (m, 1H), 4.66 (s, 2H), 4.15-4.09 (m, 2H), 3.43-3.33 (m, 1H), 3.35 (s, 3H), 2.84-2.79 (m, 2H), 2.36-0.93 (m), 1.01 (s, 3H), 0.82 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.1, 167.3, 140.7, 121.3, 108.6, 94.6,

76.7, 59.4, 55.1, 53.7, 50.2, 46.0, 39.5, 37.2, 36.8, 35.1, 31.6, 31.5, 30.4, 28.8, 24.4, 20.9, 19.3, 18.2, 14.3.

The reaction sequence reported below that converts *ent*-steroid **6** into *ent*-steroid **16** **(*ent*-VP1-001)** is based on that reported previously for the preparation of the natural stereoisomer of *ent*-steroid **16 (**Wicha, J.; Bal, K. *J. C. S. Perkin I*, **1978**, 1282-1288).

Unpurified *ent*-Steroid 7

To a solution of *ent*-steroid **6** (1.4 g, 3.48 mmol) in EtOAc (150 mL) was added PtO_2 (15 mg) at room temperature. Hydrogenation was carried out under 20 psi for 6 h. Solvent was removed and the residue was purified by flash column chromatography (silica gel eluted with 10% EtOAc in hexanes) to give unpurified *ent*-steroid **7** (1.4 g, 100%): ^{1}H NMR δ 4.63-4.60 (m, 1H), 4.08-4.03 (m, 2H), 3.48-3.32 (m, 1H), 3.31 (s, 3H), 2.34-0.57 (m), 0.76 (s, 3H), 0.54 (s, 3H); ^{13}C NMR δ 176.1, 140.7, 121.3, 94.4, 76.2, 60.0, 55.3, 55.0, 54.5, 46.9, 44.9, 42.1, 37.4, 37.0, 35.6, 35.5, 35.3, 35.2, 32.1, 28.7, 28.1, 24.4, 20.9, 14.2, 12.5.

Unpurified *ent*-steroid **7** contains minor amounts of the *ent*-steroid in which the Δ^5 double bond has been hydrogenated. This saturated *ent*-steroid could not be removed easily by chromatography on silica gel. To separate the two compounds chromatographically, *ent*-steroid **7** was converted first into *ent*-steroid **8** and then into

ent-steroid **9** which is easily purified. *ent*-Steroid **9** was then converted back via *ent*-steroid **8** into *ent*-steroid **7** and then subsequently into *ent*-steroid **10**.

Unpurified *ent*-Steroid 8

Acetyl chloride (2 mL) was slowly added to unpurified hydrogenation product *ent*-steroid **7** (1.4 g, 3.48 mmol) in ethanol (30 mL) at room temperature. After 2 h, water was added and the product was extracted into CH_2Cl_2 (100 mL x 2). The combined extracts were dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (silica gel eluted with 25% EtOAc in hexanes) to give unpurified *ent*-steroid **8** (1.2 g): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.35-5.34 (m, 1H), 4.13-4.07 (m, 2H), 3.55-3.47 (m, 1H), 2.38-0.81 (m), 1.10 (s, 3H), 0.61 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.9, 140.8, 121.5, 71.6, 60.1, 55.5, 50.3, 46.8, 42.2, 41.9, 37.3, 37.2, 36.5, 35.2, 31.9, 31.8, 31.6, 28.1, 24.5, 20.8, 19.4, 14.2, 12.4.

***ent*-Steroid 9**

To a solution of unpurified *ent*-steroid **8** (1.2 g, 3.33 mmol) in diethyl ether (100 mL) and acetic acid (5 mL) was slowly added Br_2 in HOAc (3 mL) until a brown color persisted. After 5 min, aqueous $Na_2S_2O_3$ was added and the reaction became colorless. EtOAc (100 mL) was added and the EtOAc solution was washed with aqueous $NaHCO_3$ (50 mL x 2), brine (50 mL) and dried over anhydrous Na_2SO_4. After filtration, the solvent

was removed under reduced pressure and the residue was purified by flash column chromatography (silica gel eluted with 20% EtOAc in hexanes) to give *ent*-steroid **9** (1.4 g, 81%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.82-4.81 (m, 1H), 4.44-4.37 (m, 1H), 4.12-4.06 (m, 2H), 2.72-1.08 (m), 1.43 (s, 3H), 0.62 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.8, 89.6, 68.9, 60.1, 56.0, 54.0, 47.6, 46.6, 45.6, 42.2, 42.0, 37.2, 37.0, 36.7, 35.2, 30.9, 30.1, 28.0, 24.2, 21.0, 20.3, 14.2, 12.7.

Purified *ent*-Steroid 8

Zinc dust (6.0 g) was added to a solution of *ent*-steroid **9** (1.4 g, 2.7 mmol) in HOAc (20 mL) and EtOAc (30 mL) at room temperature. After 16 h, the mixture was filtered through Celite and washed with EtOAc (200 mL). Solvent was removed under reduced pressure and the residue was purified by flash column chromatography (silica gel eluted with 25% EtOAc in hexanes) to give purified *ent*-steroid **8** (925 mg, 95%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.26-5.25 (m, 1H), 4.06-4.01 (m, 2H), 3.85 (s, br, 1H), 3.47-3.40 (m, 1H), 2.31-0.73 (m), 0.93 (s, 3H), 0.54 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.8, 140.7, 121.1, 71.2, 60.0, 55.4, 50.1, 46.6, 41.9, 41.7, 37.1, 37.0, 36.3, 35.0, 31.7, 31.7, 31.2, 27.9, 24.3, 20.6, 19.2, 14.0, 12.2.

Purified *ent*-Steroid 7

Purified *ent*-steroid **8** (925 mg, 2.57 mmol) was dissolved in CH_2Cl_2 (20 mL) and cooled to 0 °C. $(i\text{-Pr})_2$EtN (1.3 mL, 7.5 mmol) and $ClCH_2OMe$ (0.45 ml, 6.0 mmol) were added

and the reaction was stirred at room temperature for 16 h. The reaction mixture was made basic by adding aqueous saturated $NaHCO_3$ solution and the product extracted into CH_2Cl_2. The combined extracts were washed with brine, dried over anhydrous Na_2SO_4 and solvent removed to give a viscous liquid which was purified by flash column chromatography (silica gel eluted with 20% EtOAc in hexanes) to give purified *ent*-steroid **7** as a colorless liquid (1.02 g, 98%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.34-5.33 (m, 1H), 4.67 (s, 2H), 4.12 (q, *J* = 7.0 Hz, 2H), 3.42-3.36 (m, 1H), 3.35 (s, 3H), 2.37-0.80 (m), 1.00 (s, 3H), 0.60 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 173.8, 140.7, 121.5, 94.6, 76.8, 60.0, 55.5, 55.1, 50.3, 46.7, 41.9, 39.5, 37.2, 37.1, 36.7, 35.2, 31.9, 31.8, 28.9, 28.1, 24.5, 20.7, 19.3, 14.2, 12.3.

***ent*-Steroid 10**

To a solution of *ent*-steroid **7** (500 mg, 1.25 mmol) in THF (20 mL) was added LDA (1.25 mL, 2.0 M, 2.5 mmol) and HMPA (0.5 mL) at -78 °C. After 1 h, 2-(3-iodopropyl)-2-methyl-1,3-dioxolane (960 mg, 3.75 mmol) was added. After addition, the mixture was warmed to room temperature for 16 h. Aqueous NH_4Cl was added and the product was extracted into EtOAc (100 mL x 2), dried over Na_2SO_4 and filtered. Solvent was removed under reduced pressure and the residue was purified by flash column chromatography (silica gel eluted with 20% EtOAc in hexanes) to give *ent*-steroid **10** (594 mg, 90%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.34-5.33 (m, 1H), 4.67 (s, 2H), 4.14-4.08 (m, 2H), 3.94-3.86 (m, 4H), 3.42-3.38 (m, 1H), 3.36 (s, 3H), 2.32-0.90 (m), 0.99 (s, 3H), 0.70 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.1, 140.7, 121.5, 109.9, 94.7, 76.9, 64.6,

64.5, 59.7, 56.0, 55.1, 52.6, 50.1, 47.3, 41.9, 39.5, 38.9, 37.4, 37.2, 36.7, 32.2, 31.8, 31.7, 28.9, 27.0, 23.8, 23.7, 21.8, 20.8, 19.3, 14.2, 12.0.

***ent*-Steroid 11**

To a solution of *ent*-steroid **10** (594 mg, 1.12 mmol) in diethyl ether (20 mL) was added $LiAlH_4$ (2.0 M in THF, 4. 0 mL, 8.0 mmol) at room temperature. After 2 h, water (0.32 mL), 10 % aqueous NaOH (0.64 mL) and water (0.96 mL) were slowly added sequentially. After stirring for 30 min, the mixture was filtered through Celite and washed with CH_2Cl_2 (100 mL). Solvent was removed under reduced pressure and the residue was purified by flash column chromatography (silica gel eluted with 25% EtOAc in hexanes) to give *ent*-steroid **11** (530 mg, 97%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.31-5.30 (m, 1H), 4.63 (s, 2H), 3.92-3.85 (m, 4H), 3.70-3.33 (m, 3H), 3.31 (s, 3H), 2.32-0.76 (m), 0.96 (s, 3H), 0.65 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.5, 121.5, 110.0, 94.5, 77.3, 64.4, 62.3, 56.5, 55.0, 50.1, 50.0, 42.2, 41.9, 39.4, 39.3, 39.0, 37.1, 36.6, 31.7, 29.3, 28.8, 27.5, 24.0, 23.6, 20.9, 20.5, 19.2, 12.0.

***ent*-Steroid 12**

To a solution of *ent*-steroid **11** (530 mg, 1.08 mmol) in CH_2Cl_2 (15 mL) was added mesyl chloride (2 mmol, 0.15 mL) and Et_3N (0.42 mL, 3 mmol) at 0 °C. After 1 h, aqueous NH_4Cl was added and the product was extracted into CH_2Cl_2 (100 mL x 2). The combined extracts were dried over anhydrous Na_2SO_4, filtered and the solvents

removed under reduced pressure. The residue was purified by flash column chromatography (silica gel eluted with 10% EtOAc in hexanes) to give *ent*-steroid **12** (625 mg, 99%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.34-5.33 (m 1H), 4.67 (s, 2H), 4.37-4.08 (m, 3H), 3.95-3.88 (m, 4H), 3.43-3.38 (m, 1H), 3.35 (s, 3H), 2.99 (s, 3H), 2.35-0.79 (m, 30H), 0.99 (s, 3H), 0.69 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.7, 121.5, 109.8, 94.6, 76.8, 70.0, 64.5, 56.4, 55.1, 49.9, 42.1, 39.7, 39.5, 39.3, 39.0, 37.2, 37.1, 36.6, 31.8, 31.7, 29.4, 28.8, 27.4, 24.0, 23.7, 21.0, 20.1, 19.3, 12.2.

***ent*-Steroid 13**

To a solution of *ent*-steroid **12** (625 mg, 1.08 mmol) in diethyl ether (30 mL) was added $LiAlH_4$ (2.0 M in THF, 4. 0 mL, 8.0 mmol) at room temperature. After 2 h, water (0.32 mL), 10 % aqueous NaOH (0.64 mL) and water (0.96 mL) were slowly added sequentially. After stirring for 30 min, the mixture was filtered through Celite and washed with CH_2Cl_2 (100 mL). Solvent was removed under reduced pressure and the residue was purified by flash column chromatography (silica gel eluted with 10% EtOAc in hexanes) to give *ent*-steroid **13** (510 mg, 99%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.32-5.31 (m, 1H), 4.65 (s, 2H), 3.90-3.37 (m, 4H), 3.40-3.36 (m, 1H), 3.33 (s, 3H), 2.33-0.86 (m), 0.98 (s, 3H), 0.64 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.6, 121.6, 110.1, 94.6, 76.7, 64.5, 64.4, 56.6, 56.0, 55.0, 50.0, 42.2, 39.7, 39.6, 39.5, 37.1, 36.6, 36.0, 35.6, 31.8, 31.7, 28.8, 28.1, 24.2, 23.6, 20.9, 20.5, 19.3, 18.6, 11.7.

***ent*-Steroid 14**

To a solution of *ent*-steroid **13** (270 mg, 0.57 mmol) in acetone (30 mL) was added *p*-toluenesulfonic acid (100 mg) at room temperature. The reaction was stirred at room temperature for 2 h. Acetone was removed under reduced pressure and the residue was purified by flash column chromatography (silica gel eluted with 15% EtOAc in hexanes) to give *ent*-steroid **14** (235 mg, 96%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.29-5.27 (m, 1H), 4.62 (s, 2H), 3.37-3.33 (m, 1H), 3.37-3.33 (m, 1H), 3.30 (s, 3H), 2.35-0.83 (m), 2.07 (s, 3H), 0.95 (s, 3H), 0.62 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 208.9, 140.5, 121.5, 94.5, 76.8, 56.6, 55.7, 55.0, 50.0, 44.0, 42.2, 39.6, 39.4, 37.1, 36.5, 35.5, 35.3, 31.7, 31.7, 29.7, 28.8, 28.0, 24.1, 20.9, 20.2, 19.2, 18.4, 11.7.

***ent*-Steroid 15**

To a solution of *ent*-steroid **14** (235 mg, 0.55 mmol) in THF (20 mL) was added 6 N HCl (10 mL) at room temperature. After 2 h, the product was extracted into CH_2Cl_2 (100 mL x 2) and the combined extracts were washed with aqueous $NaHCO_3$ (50 ml x 2), dried over Na_2SO_4 and filtered. Solvent was removed under reduced pressure and the residue was purified by flash column chromatography (silica gel eluted with 25% EtOAc in hexanes) to give *ent*-steroid **15** (208 mg, 98%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.35-5.34 (m, 1H), 3.55-3.48 (m, 1H), 2.39-0.89 (m), 2.13 (s, 3H), 1.01 (s, 3H), 0.68 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 309.4, 140.8, 121.6, 71.7, 56.7, 55.8, 50.1, 44.2, 42.3, 42.2, 39.7, 37.2, 36.5, 35.6, 35.4, 31.9(2C), 31.6, 29.8, 28.2, 24.2, 21.0, 20.4, 19.4, 18.6, 11.8.

229

230 ***ent*-Steroid 16 (*ent*-VP1-001)**

231

232 To a solution of *ent*-steroid **15** (208 mg, 0.54 mmol) in benzene (6 mL) and diethyl ether
233 (10 mL) was added methyl lithium (1.6 M in diethyl ether, 2 mL, 3.2 mmol) at 0 °C. After
234 1 h, aqueous NH_4Cl was added and the product was extracted into EtOAc (100 mL x 2),
235 dried over Na_2SO_4 and filtered. Solvent was removed under reduced pressure and the
236 residue was purified by flash column chromatography (silica gel eluted with 25% EtOAc
237 in hexanes) to give *ent*-steroid **16 (*ent*-VP1-001)** (147 mg, 68%): mp 181-183 °C; $[\alpha]_D^{20}$
238 +38.3 (*c* = 0.06, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 5.36-5.35 (m, 1H), 3.56-3.50 (m,
239 1H), 2.31-0.93 (m), 0.95 (s, 3H), 0.69 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.8,
240 121.7, 71.8, 71.1, 56.7, 56.1, 50.1, 44.4, 42.3, 42.2, 39.8, 37.2, 36.5, 36.4, 35.7,
241 31.9(2C), 31.6, 29.3, 29.2, 28.2, 24.2, 21.1, 20.7, 19.4, 18.7, 11.8.

242

243

244