Development of selective steroid inhibitors for the glucose-6-phosphate dehydrogenase from *Trypanosoma cruzi*

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SUPPORTING INFORMATION

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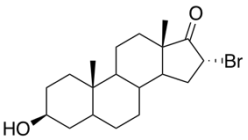
1. CHEMISTRY – experimental procedures

Reagents and solvents were commercial grade and were used as supplied, except when specified in the experimental procedure. For reactions requiring anhydrous conditions, flame dried glassware and nitrogen atmosphere were used. THF was distilled from calcium hydride and redistilled from sodium/benzophenone and dichloromethane, toluene, diisopropylamine and triethylamine were distilled from calcium hydride immediately prior to use. Anhydrous pyridine was distilled from and stored over potassium hydroxide. Reactions were monitored by thin-layer chromatography (TLC) on Silica gel 60 F254 aluminium sheets and exposed to UV radiation, followed by treatment with adequate stains and heating. Chromatographic separations were carried out on Merck 60 silica gel (230–400 mesh). ^{1}H NMR and ^{13}C NMR data were recorded on a Bruker Avance (250 MHz for ^{1}H and 62.5 MHz for ^{13}C NMR), or on an Agilent DD2 (500 MHz for ^{1}H and 125 MHz for ^{13}C NMR) spectrometer using as internal standard TMS, or the residual non deuterated solvent $CDCl_3$. Chemical shifts (δ) were expressed in ppm and multiplicities were reported as singlet (s), broad singlet (bs), doublet (d), double doublet (dd), triplet (t), apparent triplet (at), double triplet (dt), quartet (q), apparent quartet (aq), quintuplet (quint), multiplet (m). Coupling constants (*J*) are expressed in Hertz (Hz). Melting points (mp) were recorded on a PF 1500 FARMA apparatus with a heating rate of 5 °C min^{-1} and are uncorrected. High-resolution electrospray ionization mass spectrometry (HRMS-ESI) was performed on a BRUKER Impact II mass spectrometer in positive mode.

Note 1: In the ^{1}H NMR spectra, only peaks above 2.0 ppm, to the exception of C-18 and C-19 methyl groups and other unequivocal signals were recorded in the present analytical data.^{13}C NMR spectra are fully characterized. Both ^{1}H NMR and ^{13}C NMR spectra are included.

Note 2: Purity of compounds was verified by LC-HRMS, generally yielding >90% purity, to the exception of compounds **14**, **27**, **31** (>85%), which did ionize poorly, however, for which NMR spectra indicated high purity. ^{1}H and ^{13}C NMR spectra are included as ANNEX.

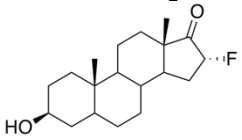
16α-Bromoepiandrosterone (3, BrEA)



A mixture of EA **2** (2.0 g, 6.89 mmol) and copper(II) bromide (3.85 g, 17.22 mmol) in MeOH (70 mL; 0.1M) was stirred at 65 ºC for 18 h. The solvent was then evaporated, and the dark residue purified on silica gel eluting with hexane/ethyl acetate (1:1) to give a compound that proved impure by ^{1}H NMR. Recrystallization from acetone/hexane yielded the pure *title compound* (1.69 g, 67%) as a white powder; m.p. 159-161 ºC (*lit.*[1] 155-156.5 ºC; δ_H (250 MHz; $CDCl_3$) 4.52 (1 H, d, *J* 6.0), 3.60 (1 H, s), 2.26-2.07 (2 H, m), 0.89 (3 H, s), 0.82 (3 H, s); δ_C (62.5 MHz; $CDCl_3$) 213.5, 71.0, 54.2, 47.9, 47.8, 46.3, 44.7, 37.9, 36.8, 35.6, 34.3, 34.0, 32.3, 31.3, 30.7 28.2, 20.4, 14.2, 12.1; HRMS (ESI) calcd for $C_{19}H_{33}BrNO_2^+$ $[M+NH_4]^+$: 386.1689, found: 386.1687.

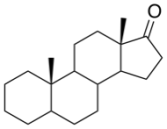
[1] Glazier, E. R. *J. Org. Chem.* **1962**, *27* (8), 2937–2938.

16α-Fluoroepiandrosterone (4, FEA)



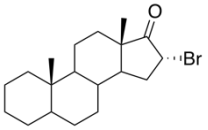
A mixture of EA **2** (100 mg, 0.34 mmol), Selectfluor® (146 mg, 0.41 mmol) and concentrated sulfuric acid (2 µL, 0.03 mmol) in MeOH (1.7 mL; 0.2M) was stirred at reflux over 18 h. The solvent was removed and the residue purified on silica gel eluting with hexane/ethyl acetate (1:1) to the desired *title compound* (76 mg, 75%) as a white powder; m.p. 171-173 ºC (*lit.*[2] 160-162 ºC); δ_H (250 MHz; $CDCl_3$) 5.06 (1 H, dd, $^1J_{HF}$ 50.7, ^2J_H 7.3), 3.64-3.52 (1 H, m), 0.89 (3 H, s), 0.81 (3 H, s); δ_C (62.5 MHz; $CDCl_3$) 213.2 (d, $^2J_{CF}$ 12.4), 90.1 (d, $^1J_{CF}$ 186.5), 71.0, 54.2, 48.3, 47.7, 44.7, 37.9, 36.8, 35.6, 34.8, 31.3, 31.2, 30.5, 29.75 (d, $^2J_{CF}$ 20.7), 28.2, 20.0, 14.0, 12.2; HRMS (ESI) calcd for $C_{19}H_{33}FNO_2^+$ $[M+NH_4]^+$: 326.2490, found: 326.2496. The coupling constants for C16-H (5.06 ppm) are in agreement with Stavber's report, thus confirming the expected stereoselectivity of the α-fluorination, although it seems that about 4% of β-product was also formed, according to our NMR spectra (see annex).[2]

Androstan-17-one (5, dEA)



To a solution of triphenylphosphine (677 mg, 2.58 mmol), and imidazole (176 mg, 2.58 mmol) in anhydrous CH_2Cl_2 (8 mL) was added iodine (655 mg, 2.58 mmol), followed by a solution of EA **2** (500 mg, 1.72 mmol) in anhydrous CH_2Cl_2 (3.5 mL; 0.15M total) at room temperature under inert atmosphere. After stirring for 72 h, the solvent was removed and the residue purified on silica gel eluting with hexane/ethyl acetate (0:1) to remove the excess reagents and by-products (δ_H (250 MHz; $CDCl_3$) 4.93 (1 H, m), 2.49-2.38 (1 H, m), 2.14-2.00 (1 H, m), 0.85 (3 H, s), 0.81 (3 H, s); δ_C (62.5 MHz; $CDCl_3$) 221.1, 53.9, 51.4, 47.7, 42.0, 38.6, 37.6, 36.6, 35.8, 34.9, 34.3, 32.6, 31.5, 30.6, 27.4, 21.7, 20.0, 13.8, 13.3. The resulting solid was taken up in acetic acid (21.5 mL; 0.08M) and zinc powder (1.35 g, 20.66 mmol) was added. The suspension was heated at 55 ºC under vigorous stirring for 1 h. After cooling, the mixture was diluted with CH_2Cl_2 (60 mL), filtered through a pad of Celite® and the cake washed with CH_2Cl_2 (60 mL). The filtrate was washed with a 0.1M NaOH solution (2 × 50 mL), water (60 mL), brine (60 mL), dried over $MgSO_4$, filtered and evaporated. The resulting solid was recrystallized from MeOH to yield the desired *title compound* (308 mg, 65%) as colourless crystals; m.p. 118-120 ºC (*lit.*[3] 123-123.5 ºC); δ_H (250 MHz; $CDCl_3$) 2.47-2.36 (1 H, m), 2.11-1.96 (1 H, m), 0.84 (3 H, s), 0.79 (3 H, s); δ_C (62.5 MHz; $CDCl_3$) 221.4, 54.8, 51.5, 47.8, 47.0, 38.6, 36.4, 35.8, 35.0, 31.6, 30.9, 28.9, 28.7, 26.7, 22.1, 21.7, 20.0, 13.8, 12.2; HRMS (ESI) calcd for $C_{19}H_{34}NO^+$ $[M+NH_4]^+$: 292.2635, found: 292.2631.

16α-Bromoandrostan-17-one (6, dBrEA)

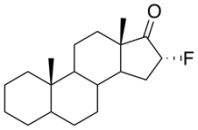


A mixture of dEA **5** (100 mg, 0.36 mmol) and $CuBr_2$ (203 mg, 0.91 mmol) in MeOH (3.6 mL; 0.1M) was stirred at reflux over 18 h. The solvent was removed and the dark residue purified on silica gel

[2] Stavber, S.; Jereb, M.; Zupan, M. *Synthesis* **2002**, *2002* (17), 2609–2615.
[3] Schwartz, A. G.; Lewbart, M. L.; Pashko, L. L. *Cancer Res* **1988**, *48* (17), 4817–4822.

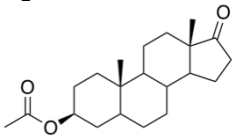
eluting with hexane/ethyl acetate (9:1) to give the desired *title compound* (74 mg, 69%) as a white powder; mp 142-145 ºC (*lit.*[4] 197 ºC); δ_H (250 MHz; $CDCl_3$) 4.52 (1 H, d, *J* 6.7), 2.26-2.07 (2 H, m), 0.89 (3 H, s), 0.80 (3 H, s); δ_C (62.5 MHz; $CDCl_3$) 213.6, 54.6, 48.0, 47.8, 46.9, 46.5, 38.5, 36.4, 34.3, 34.1, 32.4, 30.8 28.9, 28.6, 26.7, 22.1, 19.9, 14.2, 12.1; HRMS (ESI) calcd for $C_{19}H_{33}{}^{79}BrNO^+$ $[M+NH_4]^+$: 370.1740, found: 370.1739.

16α-Fluoroandrostan-17-one (7, dFEA)



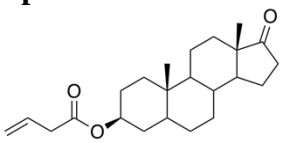
A mixture of dEA **5** (100 mg, 0.36 mmol), Selectfluor® (155 mg, 0.44 mmol) and concentrated sulfuric acid (2 µL, 0.04 mmol) in MeOH (1.8 mL; 0.2M) was stirred at reflux over 18 h. The solvent was removed and the residue purified on silica gel eluting with hexane/ethyl acetate (92:8) to the desired *title compound* (79 mg, 74%) as a white wax; δ_H (250 MHz; $CDCl_3$) 5.06 (1 H, dd, $^1J_{HF}$ 50.7, ^2J_H 7.3), 0.90 (3 H, s), 0.80 (3 H, s); δ_C (62.5 MHz; $CDCl_3$) 213.4 (d, $^2J_{CF}$ 12.5), 90.2 (d, $^1J_{CF}$ 186.5), 54.6, 48.5, 47.8, 46.9, 38.5, 36.4, 34.9, 31.3, 30.6, 29.75 (d, $^2J_{CF}$ 20.7), 28.9, 28.6, 26.6, 22.0, 19.9, 14.2, 12.1; HRMS (ESI) calcd for $C_{19}H_{33}FNO^+$ $[M+NH_4]^+$:310.2541, found: 310.2543. The coupling constants for C16-H (5.06 ppm) are in agreement with Stavber's report, thus confirming the expected stereoselectivity of the α-fluorination, although it seems that about 6% of β-product was also formed, according to our NMR spectra (see annex).[2]

Epiandrosterone acetate (8)



To a solution of EA **2** (200 mg, 0.69 mmol) in anhydrous CH_2Cl_2 (3.4 mL; 0.2M) at 0 °C was added Et_3N (144 µL, 1.03 mmol), followed by acetyl chloride (59 µL, 0.83 mmol) under a nitrogen atmosphere. After 30 min, the reaction mixture was directly purified on silica gel eluting with hexane/ethyl acetate (9:1) to yield the *title compound* (219 mg, 96%) as colourless crystals; m.p. 112-115 ºC (*lit.*[5] 104-105 ºC); δ_H (250 MHz; $CDCl_3$) 4.70-4.61 (1 H, m), 2.46-2.35 (1 H, m), 0.82 (6 H, s); δ_C (62.5 MHz; $CDCl_3$) 221.1, 170.6, 73.4, 54.2, 51.3, 47.7, 44.6, 36.6, 35.7, 35.6, 34.9, 33.9, 31.4, 30.7, 28.2, 27.3, 21.7, 21.3, 20.4 13.7, 12.1; HRMS (ESI) calcd for $C_{21}H_{36}NO_3^+$ $[M+NH_4]^+$: 350.2690, found: 350.2697.

Epiandrosterone but-3-enoate (9)

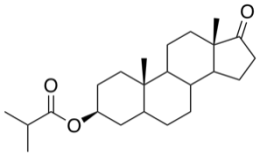


To a solution of EA **2** (50 mg, 0.17 mmol) in anhydrous CH_2Cl_2 (0.9 mL; 0.2M) at 0 °C was added Et_3N (36 µL, 0.26 mmol), followed by crotonyl chloride (20 µL, 0.21 mmol) under a nitrogen atmosphere. After 30 min, the reaction mixture was directly purified on silica gel eluting with hexane/ethyl acetate (4:1) to yield the *title compound* (38 mg, 58%) as a white wax; δ_H (250 MHz;

[4] Schwartz, A. G.; ML Lewbart US Patent 4,898,694 (1990).
[5] Chambers, R. D.; Nakano, T.; Parsons, M.; Sandford, G.; Batsanov, A. S.; Howard, J. A. K. *Journal of Fluorine Chemistry* **2008**, *129* (9), 811–816.

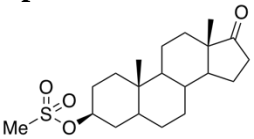
$CDCl_3$) 5.90 (1 H, ddt, *J* 16.8, 9.8, 6.9), 5.13 (2 H, ad), 4.75-4.63 (1 H, m), 3.03 (2 H, d, *J* 6.9), 2.47-2.36 (1 H, m), 0.83 (6 H, s); δ_C (62.5 MHz; $CDCl_3$) 221.1, 171.0, 130.5, 118.2, 73.7, 54.2, 51.3, 47.7, 44.5, 39.4, 36.6, 35.8, 35.6, 34.9, 33.8, 31.4, 30.7, 28.2, 27.3, 21.7, 20.4, 13.7, 12.1; HRMS (ESI) calcd for $C_{23}H_{38}NO_3^+$ $[M+NH_4]^+$:376.2846, found: 376.2858.

Epiandrosterone isobutyrate (10)



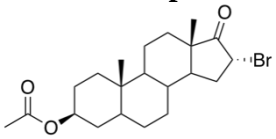
To a solution of EA **2** (110 mg, 0.38 mmol) in anhydrous CH_2Cl_2 (4.0 mL; 0.1M) at 0 °C was added isobutyryl chloride (100 µL, 0.83 mmol), followed by Et_3N (140 µL, 0.98 mmol) under a nitrogen atmosphere. After 30 min, the reaction mixture was directly purified on silica gel eluting with hexane/ethyl acetate (8:2) to yield the *title compound* (105 mg, 77%) as a white powder; m.p. 160-164 ºC (*lit.*[6] 158-160 ºC); δ_H (500 MHz; $CDCl_3$) 4.71 – 4.65 (1 H, m), 2.53 – 2.40 (2 H, m), 2.10 – 2.03 (1 H, m), 1.96 – 1.90 (1 H, m), 1.15 (3 H, s), 1.14 (3 H, s); δ_C (125 MHz; $CDCl_3$) 221.2, 176.7, 73.0, 54.3, 51.4, 47.8, 44.6, 36.7, 35.8, 35.7, 35.0, 34.2, 33.9, 31.5, 30.8, 28.3, 27.4, 21.8, 20.5, 19.0, 18.9, 13.8, 12.2; HRMS (ESI) calcd for $C_{23}H_{40}NO_3^+$ $[M+NH_4]^+$: 378.3003, found: 378.3006.

Epiandrosterone methanesulfonate (11)



To a solution of EA **2** (35 mg, 0.12 mmol) in anhydrous CH_2Cl_2 (0.6 mL; 0.2M) at 0 °C was added Et_3N (25 µL, 0.18 mmol), followed by methanesulfonyl chloride (11 µL, 0.14 mmol) under a nitrogen atmosphere. After 30 min, the reaction mixture was directly purified on silica gel eluting with hexane/ethyl acetate (7:3) to yield the *title compound* (43 mg, 99%) as colourless crystals; m.p. 146-148 ºC (*lit.*[7] 155 ºC); δ_H (250 MHz; $CDCl_3$) 4.65-4.52 (1 H, m), 2.97 (3 H, s), 2.47-2.36 (1 H, m), 0.83 (6 H, s); δ_C (62.5 MHz; $CDCl_3$) 220.9, 81.7, 54.1, 51.2, 47.6, 44.7, 38.7, 36.6, 35.7, 35.3, 35.0, 34.9, 31.4, 30.6, 28.5, 28.1, 21.6, 20.4 13.7, 12.1; HRMS (ESI) calcd for $C_{20}H_{36}NO_4S^+$ $[M+NH_4]^+$: 386.2360, found: 386.2366.

16α-Bromoepiandrosterone acetate (12)

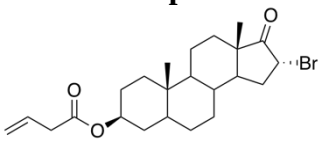


To a solution of BrEA **3** (100 mg, 0.27 mmol) in anhydrous CH_2Cl_2 (1.4 mL; 0.2M) at 0 °C was added Et_3N (57 µL, 0.41 mmol), followed by acetyl chloride (23 µL, 0.33 mmol) under a nitrogen atmosphere. After 30 min, the reaction mixture was directly purified on silica gel eluting with hexane/ethyl acetate (9:1) to yield the *title compound* (83 mg, 74%) as colourless crystals; m.p. 159-162 ºC (*lit.*[8] 166-167 ºC); δ_H (250 MHz; $CDCl_3$) 4.71-4.60 (1 H, m), 4.51 (2 H, d, *J* 5.9), 2.22-2.08 (2 H, m), 0.87 (3 H, s),

[6] Harding, J. R.; King, C. D.; Perrie, J. A.; Sinnott, D.; Stachulski, A. V. *Org. Biomol. Chem.* **2005**, *3* (8), 1501–1507.
[7] Kamijo, S.; Matsumura, S.; Inoue, M. *Org. Lett.* **2010**, *12* (18), 4195–4197.
[8] Glazier, E. R. *J. Org. Chem.* **1962**, *27* (8), 2937–2938.

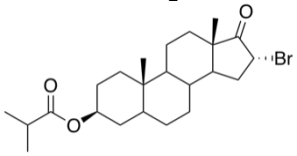
0.83 (3 H, s); δ_C (62.5 MHz; $CDCl_3$) 213.3, 170.5, 73.3, 54.0, 47.8, 47.7, 46.3, 44.5, 36.5, 35.6, 34.2, 34.0, 33.8, 32.2, 30.6, 28.0, 27.3, 21.4, 20.3 14.1, 12.1; HRMS (ESI) calcd for $C_{21}H_{35}{}^{79}BrNO_3{}^+$ $[M+NH_4]^+$: 428.1795, found: 428.1799.

16α-Bromoepiandrosterone but-3-enoate (13)



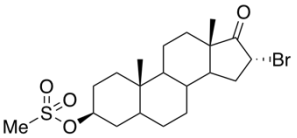
To a solution of BrEA **3** (50 mg, 0.14 mmol) in anhydrous CH_2Cl_2 (0.7 mL; 0.2M) at 0 °C was added Et_3N (38 µL, 0.27 mmol), followed by crotonyl chloride (20 µL, 0.20 mmol) under a nitrogen atmosphere. After 30 min, the reaction mixture was directly purified on silica gel eluting with hexane/ethyl acetate (4:1) to yield the *title compound* (34 mg, 57%) as an amorphous solid; δ_H (250 MHz; $CDCl_3$) 5.90 (1 H, ddt, *J* 17.4, 9.9, 6.9), 5.13 (2 H, ad), 4.73-4.63 (1 H, m), 4.51 (2 H, d, *J* 5.9), 3.03 (2 H, d, *J* 6.9), 2.18-2.11 (2 H, m), 0.87 (3 H, s), 0.83 (3 H, s); δ_C (62.5 MHz; $CDCl_3$) 213.4, 171.0, 130.5, 118.2, 73.6, 54.0, 47.8, 47.7, 46.3, 44.5, 39.5, 36.5, 35.6, 34.2, 34.0, 33.8, 32.2, 30.6, 28.0, 27.3, 20.3 14.1, 12.1; HRMS (ESI) calcd for $C_{23}H_{37}{}^{79}BrNO_3{}^+$ $[M+NH_4]^+$: 454.1951, found: 454.1947.

16α-Bromoepiandrosterone isobutyrate (14)



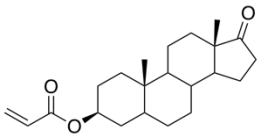
To a solution of BrEA **3** (50 mg, 0.14 mmol) in anhydrous CH_2Cl_2 (0.7 mL; 0.2M) at 0 °C was added isobutyryl chloride (17 µL, 0.16 mmol), followed by Et_3N (28 µL, 0.20 mmol) under a nitrogen atmosphere. After 30 min, the reaction mixture was directly purified on silica gel eluting with hexane/ethyl acetate (85:15) to yield the *title compound* (44 mg, 72%) as an amorphous solid; δ_H (500 MHz; $CDCl_3$) 4.72-4.65 (1 H, m), 4.53 (1 H, d, *J* 7.0), 2.53-2.45 (1 H, m), 2.24-2.11 (1 H, m), 0.90 (3 H, s), 0.86 (3 H, s); δ_C (125 MHz; $CDCl_3$) 213.4, 176.7, 72.9, 54.1, 47.9, 47.8, 46.3, 44.5, 36.6, 35.6, 34.3, 34.15, 34.06, 33.8, 32.3, 30.6, 28.1, 27.3, 20.3, 19.00, 18.97, 14.2, 12.2; HRMS (ESI) calcd for $C_{23}H_{39}{}^{79}BrNO_3{}^+$ $[M+NH_4]^+$: 456.2108, found: 456.2115.

16α-Bromoepiandrosterone methanesulfonate (15)



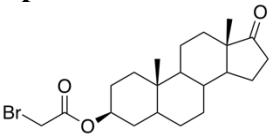
To a solution of BrEA (**3**) (50 mg, 0.14 mmol) in anhydrous CH_2Cl_2 (0.7 mL; 0.2M) at 0 °C was added methanesulfonyl chloride (23 µL, 0.30 mmol), followed by Et_3N (45 µL, 0.33 mmol) under a nitrogen atmosphere. After 30 min, the reaction mixture was directly purified on silica gel eluting with hexane/ethyl acetate (7:3) to yield the *title compound* (55 mg, 90%) as an amorphous solid; δ_H (250 MHz; $CDCl_3$) 4.67-4.50 (2 H, m + d, *J* 6.2), 2.99 (3 H, s), 2.25-2.10 (2 H, m), 0.88 (3 H, s), 0.84 (3 H, s); δ_C (62.5 MHz; $CDCl_3$) 213.2, 81.6, 53.9, 47.74, 47.68, 46.2, 44.6, 38.7, 36.6, 35.3, 34.9, 34.1, 34.0, 32.2, 30.5, 28.5, 27.9, 20.3 14.1, 12.0; HRMS (ESI) calcd for $C_{20}H_{35}{}^{79}BrNO_4S^+$ $[M+NH_4]^+$: 464.1465, found: 464.1462.

Epiandrosterone acrylate (16)



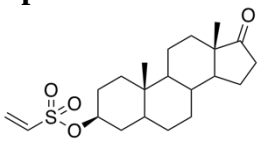
To a solution of EA **2** (50 mg, 0.17 mmol) in anhydrous CH_2Cl_2 (0.9 mL; 0.2M) at room temperature was added Et_3N (36 µL, 0.26 mmol), followed by acryloyl chloride (17 µL, 0.21 mmol) under a nitrogen atmosphere. After 30 min, the reaction mixture was directly purified on silica gel eluting with hexane/ethyl acetate (7:3) to yield the *title compound* (36 mg, 61%) as an amorphous solid; δ_H (250 MHz; $CDCl_3$) 6.36 (1 H, dd, *J* 17.2, 1.6), 6.07 (1 H, dd, *J* 17.2, 10.3), 5.77 (1 H, dd, *J* 10.3, 1.6), 4.82-4.69 (1 H, m), 2.47-2.36 (1 H, m), 0.84 (6 H, s); δ_C (62.5 MHz; $CDCl_3$) 221.1, 165.7, 130.2, 129.0, 73.6, 54.2, 51.3, 47.7, 44.6, 36.6, 35.8, 35.6, 35.0, 33.9, 31.5, 30.7, 28.2, 27.3, 21.7, 20.4, 13.7, 12.2 HRMS (ESI) calcd for $C_{22}H_{36}NO_3^+$ $[M+NH_4]^+$: 362.2690, found: 362.2692.

Epiandrosterone α-bromoacetate (17)



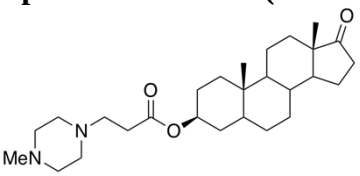
To a solution of EA **2** (100 mg, 0.34 mmol) in anhydrous CH_2Cl_2 (1.8 mL; 0.2M) at room temperature was added Et_3N (180 µL, 1.29 mmol), followed by bromoacetyl bromide (90 µL, 1.03 mmol) under a nitrogen atmosphere. After 2.5 h, the reaction mixture was directly purified on silica gel eluting with hexane/ethyl acetate (4:1) to yield the *title compound* (127 mg, 70%) as an amorphous solid; δ_H (250 MHz; $CDCl_3$) 4.80-4.67 (1 H, m), 3.77 (2 H, s), 2.47-2.36 (1 H, m), 0.84 (6 H, s); δ_C (62.5 MHz; $CDCl_3$) 221.1, 166.7, 75.6, 54.2, 51.3, 47.7, 44.6, 36.6, 35.8, 35.6, 35.0, 33.5, 31.5, 30.7, 28.2, 27.1, 26.3, 21.7, 20.4, 13.8, 12.2; HRMS (ESI) calcd for $C_{21}H_{35}{}^{79}BrNO_3^+$ $[M+NH_4]^+$: 428.1795, found: 428.1793.

Epiandrosterone vinylsulfonate (18)



To a solution of EA **2** (200 mg, 0.69 mmol) in anhydrous CH_2Cl_2 (3.4 mL; 0.2M) at 0 ºC was added 2-chloroethylsulfonyl chloride (86 µL, 0.83 mmol), followed by Et_3N (230 µL, 1.65 mmol) under a nitrogen atmosphere. After 30 min, the reaction mixture was concentrated and directly purified on silica gel eluting with hexane/ethyl acetate (7:3) to yield the *title compound* (160 mg, 61%) as an amorphous solid; δ_H (250 MHz; $CDCl_3$) 6.54 (1 H, dd, *J* 16.6, 9.7), 6.36 (1 H, d, *J* 16.6), 6.04 (1 H, d, *J* 9.7), 4.51-4.38 (1 H, m), 2.47-2.36 (1 H, m), 0.835 (3 H, s), 0.830 (3 H, s); δ_C (62.5 MHz; $CDCl_3$) 220.9, 133.8, 128.8, 82.6, 54.1, 51.2, 47.7, 44.7, 36.7, 35.7, 35.3, 34.9, 34.9, 31.4, 30.6, 28.4, 28.1, 21.7, 20.4 13.7, 12.1; HRMS (ESI) calcd for $C_{21}H_{36}NO_4S^+$ $[M+NH_4]^+$: 398.22360, found: 398.2367.

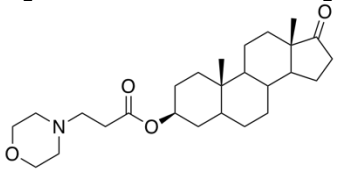
Epiandrosterone 3-(4-methylpiperazin-1-yl)propanoate (19)



To a solution of **16** (24 mg, 0.07 mmol) in anhydrous CH_2Cl_2 (0.28 mL; 0.25M) at room temperature was added *N*-methylpiperazine (15 µL, 0.14 mmol) under a nitrogen atmosphere. After 16 h, the reaction mixture was directly purified on silica gel eluting with chloroform/methanol (6-8%) to yield

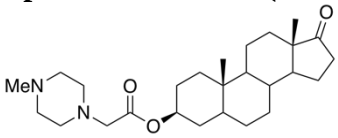
the *title compound* (30 mg, 97%) as a yellow oil; δ_H (500 MHz; $CDCl_3$) 4.75-4.68 (1 H, m), 2.69 (2 H, t, *J* 7.4), 2.60-2.33 (10 H, m + t, *J* 7.5), 2.28 (3 H, s), 2.10-2.03 91 H, m), 0.86 (3 H, s), 0.85 (3 H, s); δ_C (125 MHz; $CDCl_3$) 221.2, 172.0, 73.5, 55.0, 54.3, 53.5, 52.8, 51.3, 47.7, 46.0, 44.6, 36.7, 35.8, 35.6, 35.0, 33.9, 32.6, 31.5, 30.8, 28.2, 27.4, 21.7, 20.4, 13.8, 12.2; HRMS (ESI) calcd for $C_{27}H_{45}N_2O_3^+$ $[M+H]^+$: 445.3425, found: 445.3409.

Epiandrosterone 3-morpholinopropanoate (20)



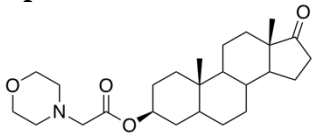
To a solution of **16** (29 mg, 0.08 mmol) in anhydrous CH_2Cl_2 (0.34 mL; 0.25M) at room temperature was added morpholine (15 µL, 0.17 mmol) under a nitrogen atmosphere. After 16 h, the reaction mixture was directly purified on silica gel eluting with chloroform/methanol (0.5%) to yield the *title compound* (26 mg, 72%) as a yellow oil; δ_H (500 MHz; $CDCl_3$) 4.76-4.69 (1 H, m), 3.69 (4 H, at, *J* 4.7), 2.67 (2 H, t, *J* 7.3), 2.47-2.41 (7 H, m), 2.11-2.03 (1 H, m), 0.86 (3 H, s), 0.85 (3 H, s); δ_C (125 MHz; $CDCl_3$) 221.2, 171.9, 73.5, 66.9, 54.3, 54.0, 53.3, 51.3, 47.8, 44.6, 36.7, 35.8, 35.6, 35.0, 33.9, 32.5, 31.5, 30.8, 28.3, 27.4, 21.7, 20.4, 13.8, 12.2; HRMS (ESI) calcd for $C_{26}H_{42}NO_4^+$ $[M+H]^+$: 432.3108, found: 432.3107.

Epiandrosterone 2-(4-methylpiperazin-1-yl)acetate (21)



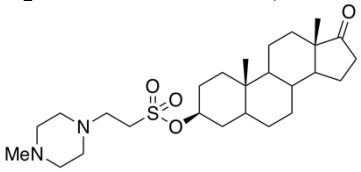
To a solution of **17** (53 mg, 0.13 mmol) in CH_2Cl_2 (0.7 mL; 0.2M) was added 1-methylpiperazine (36 µL, 0.32 mmol) under a nitrogen atmosphere. After 16 h, the reaction mixture was directly purified on on silica gel eluting with $CHCl_3$/MeOH (95:5) to yield the *title compound* (48 mg, 87%) as a yellowish wax; δ_H (500 MHz; $CDCl_3$) δ 4.79-4.72 (1H, m), 3.18 (2H, s), 2.75-2.37 (9H, m), 2.30 (3H, s), 2.12-2.00 (2H, m), 0.86 (3H,s), 0.85 (3H, s); δ_C (125 MHz; $CDCl_3$) 221.3, 169.8, 73.9, 59.7, 54.8, 54.3, 52.9, 51.4, 47.8, 46.0, 44.6, 36.7, 35.8, 35.6, 35.0, 33.9, 31.5, 30.8, 28.3, 27.4, 21.8, 20.5, 13.8, 12.2; HRMS (ESI) calcd for $C_{26}H_{43}N_2O_3^+$ $[M+H]^+$: 431.3268, found: 431.3279.

Epiandrosterone 2-morpholinoacetate (22)



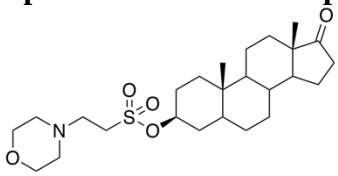
To a solution of bromoacetate **17** (50 mg, 0.12 mmol) in anhydrous CH_2Cl_2 (0.4 mL; 0.3M) at 0 ºC was added morpholine (21 µL, 0.24 mmol) under a nitrogen atmosphere. After 16 h, the reaction mixture was directly purified on silica gel eluting with chloroform/methanol (0.5%) to yield the *title compound* (50 mg, 99%) as an amorphous solid; δ_H (500 MHz; $CDCl_3$) 4.79-4.73 (1 H, m), 3.75 (4 H, t, *J* 4.6), 3.18 (2 H, s), 2.58 (4 H, t, *J* 4.6), 2.46-2.41 (1 H, m), 2.10-2.03 (1 H, m), 0.86 (3 H, s), 0.85 (3 H, s); δ_C (125 MHz; $CDCl_3$) 222.1, 169.6, 74.0, 66.8, 59.9, 54.3, 53.2, 51.3, 47.7, 44.8, 36.7, 35.8, 35.6, 35.0, 33.9, 31.5, 30.8, 28.2, 27.4, 21.7, 20.4, 13.8, 12.2; HRMS (ESI) calcd for $C_{25}H_{40}NO_4^+$ $[M+H]^+$: 418.2952, found: 418.2948.

Epiandrosterone 2-(4-methylpiperazin-1-yl)ethane-1-sulfonate (23)



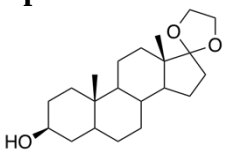
To a solution of vinylsulfonate **18** (22 mg, 0.06 mmol) in anhydrous CH_2Cl_2 (0.23 mL; 0.2M) at room temperature was added *N*-methylpiperazine (13 µL, 0.12 mmol) under a nitrogen atmosphere. After 16 h, the reaction mixture was directly purified on silica gel eluting with $CHCl_3$/MeOH (95:5) to yield the *title compound* (26 mg, 93%) as an amorphous solid; δ_H (500 MHz; $CDCl_3$) 4.65-4.59 (1 H, m), 3.26 (2 H, t, *J* 7.8), 2.88 (2 H, t, *J* 7.8), 2.53 (4 H, bs), 2.47-2.41 (4 H, m), 2.28 (3 H, s), 2.11-2.03 (1 H, m), 0.86 (6 H, s); δ_C (125 MHz; $CDCl_3$) 221.0, 81.7, 54.9, 54.1, 52.8, 51.8, 51.3, 49.0, 47.7, 45.9, 44.8, 36.7, 35.8, 35.4, 35.2, 34.9, 31.4, 30.7, 28.7, 28.1, 21.7, 20.4, 13.8, 12.1; HRMS (ESI) calcd for $C_{26}H_{45}N_2O_4S^+$ $[M+H]^+$: 481.3095, found: 481.3089.

Epiandrosterone 2-morpholinoethanesulfonate (24)



To a solution of vinyl sulfonate **18** (50 mg, 0.13 mmol) in anhydrous CH_2Cl_2 (0.7 mL; 0.2M) at room temperature was added morpholine (23 µL, 0.26 mmol) under a nitrogen atmosphere. After 16 h, the reaction mixture was directly purified on silica gel eluting with $CHCl_3$/MeOH (98:2) to yield the *title compound* (60 mg, 98%) as an amorphous solid; δ_H (500 MHz; $CDCl_3$) 4.66-4.59 (1 H, m), 3.70 (4 H, t, *J* 4.6), 3.26 (2 H, at, *J* 7.3), 2.85 (2 H, at, *J* 7.6), 2.48 (4 H, t, *J* 4.6), 2.46-2.40 (1 H, m), 2.10-2.02 (1 H, m), 0.85 (6 H, s); δ_C (125 MHz; $CDCl_3$) 221.0, 81.7, 66.7, 54.1, 53.3, 52.2, 51.2, 48.9, 47.7, 44.8, 36.7, 35.8, 35.4, 35.2, 34.9, 31.4, 30.7, 28.7, 28.1, 21.7, 20.4 13.7, 12.1; HRMS calcd for $C_{25}H_{42}NO_5S^+$ $[M+H]^+$: 468.2778, found: 468.2781.

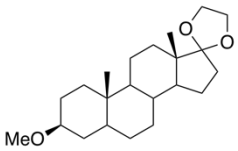
Epiandrosterone 17-ketal (25)



A mixture of EA **2** (3.0 g, 10.3 mmol), ethylene glycol (11.55 mL, 206.6 mmol), trimethyl orthoformate (11.30 mL, 103.3 mmol) and *p*-toluenesulfonic acid (422 mg, 2.2 mmol) in CH_2Cl_2 (52 mL; 0.2M) was stirred for 16 h at room temperature under a nitrogen atmosphere. The reaction mixture was washed with a 1M NaOH solution (2 × 50 mL), brine (50 mL), dried over $MgSO_4$, filtered and evaporated. The residue was purified on silica gel eluting with hexane/ethyl acetate (3:2) to yield the *title compound* (2.90 g, 84%) as colourless crystals; m.p. 140-143 ºC (*lit.*[9] 152-154 ºC); δ_H (500 MHz; $CDCl_3$) 3.93-3.83 (4 H, m), 3.62-3.56 (1 H, m), 0.84 (3 H, s), 0.81 (3 H, s); δ_C (125 MHz; $CDCl_3$) 119.5, 71.2, 65.1, 64.5, 54.1, 50.3, 45.9, 44.8, 38.2, 37.0, 35.7, 35.5, 34.2, 31.5, 31.3, 30.7, 28.6, 22.6, 20.6, 14.4, 12.3; HRMS calcd for $C_{21}H_{35}O_3^+$ $[M+H]^+$: 335.2581, found: 335.2577.

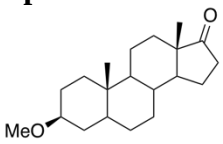
Epiandrosterone 17-ketal methyl ether (26)

[9] Hitchin, J. R.; Hamilton, N. M.; Jordan, A. M.; Lyons, A. J.; Ogilvie, D. J. *Tetrahedron Letters* **2012**, *53* (23), 2868–2872.



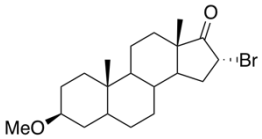
To a solution of ketal **25** (106 mg, 0.318 mmol) in dry THF (4.8 mL; 65 mM), was added sodium hydride (60% dispersion in mineral oil; 127 mg, 3.18 mmol), and the mixture was stirred under an argon atmosphere at reflux for 1 h. Methyl iodide (0.2 mL, 2.54 mmol) was added via syringe and the reaction mixture was further stirred at reflux overnight. Once cooled to room temperature, water (5 mL) was added and the mixture extracted with ethyl acetate (3 x 5 mL). The organic phase was washed with brine, dried over $MgSO_4$, and evaporated. The residue was purified on silica gel eluting with hexane/ethyl acetate (85:15) to yield the *title compound* (85 mg, 77%) as an amorphous solid; m.p. 120-122 ºC; δ_H (500 MHz; $CDCl_3$) 3.98-3.78 (m, 4 H); 3.34 (s, 3 H); 3.17-3.08 (m, 1 H); 2.01-1.92 (m, 1 H); 0.83 (s, 3 H); 0.80 (s, 3 H); δ_C (125 MHz; $CDCl_3$) 119.5, 79.8, 65.2, 64.5, 55.5, 54.2, 50.4, 46.0, 44.8, 37.0, 35.8, 35.7, 34.3, 34.2, 31.4, 30.7, 28.7, 27.9, 22.7, 20.6, 14.4, 12.3; HRMS calcd for $C_{22}H_{37}O_3^+$ $[M+H]^+$: 349.2737, found: 349.2743.

Epiandrosterone methyl ether (27)



To a solution of ketal **27** (85 mg; 0.24 mmol) in 1,4-dioxane (3.0 mL; 80 mM) was added a 5% aqueous H_2SO_4 (0.4 mL; 0.36 mmol) solution. The resulting mixture was stirred at room temperature for 5 h. The solution was then neutralized by addition of a saturated $NaHCO_3$ solution and extracted into ethyl acetate (3 x 5 mL). The organic phase was washed with water (10 mL) and brine (10 mL), dried over $MgSO_4$, and evaporated. The residue was purified on silica gel eluting with hexane/ethyl acetate (85:15) to yield the *title compound* (58 mg, 77%) as a white solid; m.p. 110-112 ºC (*lit.*[10] 112-114 ºC); δ_H (500 MHz; $CDCl_3$) 3.34 (s, 3 H), 3.17-3.09 (m, 1 H), 2.43 (1 H, dd, *J* 19.4, 8.9), 2.11-2.01 (m, 1 H), 0.86 (s, 3 H), 0.82 (s, 3 H), δ_C (125 MHz; $CDCl_3$) 221.3, 79.7, 55.6, 54.5, 51.5, 47.8, 44.8, 36.9, 36.0, 35.8, 35.1, 34.3, 31.6, 30.9, 28.6, 27.8, 21.8, 20.5, 13.8, 12.3; HRMS calcd for $C_{20}H_{33}O_2^+$ $[M+H]^+$: 305.2475, found: 305.2481.

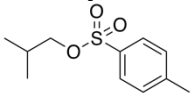
16α-Bromoepiandrosterone methyl ether (28)



A solution of **28** (49 mg, 0.16 mmol) and copper(II) bromide (90 mg, 0.4 mmol) in MeOH (1.7 mL; 0.1M) was heated at 65 ºC for 16 h. The solvent was then evaporated, and the residue was purified on silica gel eluting with hexane/ethyl acetate (95:5, 90:10, 85:15) to yield the *title compound* (44 mg, 71%) as a white solid; m.p. 160-162 ºC (*lit.*[11] 180-181 ºC); δ_H (500 MHz; $CDCl_3$) 4.53 (1 H, d, *J* 7.0); 3.35 (s, 3 H); 3.18-3.07 (m, 1 H); 2.27-2.10 (m, 2 H); 0.90 (s, 3 H) 0.82 (s, 3 H); δ_C (125 MHz; $CDCl_3$) 213.7, 79.8, 55.8, 54.5, 48.2, 48.0, 46.6, 44.9, 37.0, 36.2, 34.5, 34.4, 34.3, 32.6, 31.0, 28.6, 28.0, 20.6, 14,4, 12.4; HRMS calcd for $C_{20}H_{35}{}^{79}BrNO_2^+$ $[M+NH_4]^+$: 400.1846, found: 400.1848.

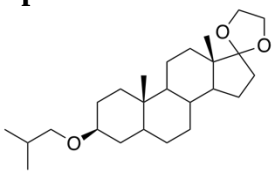
[10] Hora, J. *Collection of Czechoslovak Chemical Communications* **1966**, *31* (7), 2737–2744.
[11] Fajkoš, J. *Collection of Czechoslovak Chemical Communications* **1955**, *20* (2), 312–335.

Isobutyl-4-toluenesulfonate (29)



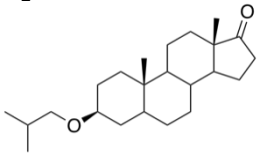
To a solution of iBuOH (500 mg, 6.77 mmol) in anhydrous CH_2Cl_2 (7.0 mL; 0.2M) at 0 °C was added Et_3N (1.0 mL, 6.77 mmol) and 4-(dimethylamino)pyridine (83.0 mg, 0.67 mmol), followed by *p*-toluenesulfonyl chloride (260 mg, 1.35 mmol) under a nitrogen atmosphere. The mixture was stirred at 25 ºC for 12 h. The reaction was quenched upon addition of a 0.1 M aqueous solution of HCl (2.4 mL). The CH_2Cl_2 layer was separated, diluted with diethyl ether, washed with 0.1 M aq solution of HCl (5 mL) and water (5 mL), dried over $MgSO_4$, and evaporated. The residue was purified on silica gel eluting with hexane/ethyl acetate (90:10) to yield the *title compound* (267 mg, 86%) as a yellowish oil; δ_H (500 MHz; $CDCl_3$) 7.79 (2 H, d, *J* = 8.4 Hz), 7.34 (2 H, d, *J* = 8.4 Hz), 3.79 (2 H, d, *J* = 6.5 Hz), 2.45 (3 H, s), 1.94 (1H, sept, *J* = 6.7 Hz), 0.90 (3H s), 0.88 (3H s). ; δ_C (125 MHz; $CDCl_3$) 144.6, 133.2, 129.8, 127.9, 76.3, 28.0, 21.6, 18.6; HRMS (ESI) calcd for $C_{11}H_{20}NO_3S^+$ $[M+NH_4]^+$: 246.1158, found: 246.1157.

Epiandrosterone 17-ketal isobutyl ether (30)



To a solution of ketal **25** (102 mg, 0.31 mmol) in dry DMF (2.0 mL; 0.15M), was added sodium hydride (60% dispersion in mineral oil; 122 mg, 3.05 mmol), and the mixture was stirred under an argon atmosphere at reflux for 1 h. The reaction mixture was then cooled to room temperature, upon which time sulfonate **29** (0.6 mL, 3.05 mmol) and NaI (5 mg, 0.03 mmol) were added, and heating at reflux was resumed for 21 h. Once cooled to room temperature, the reaction mixture was diluted with water (5 mL) and extracted with ethyl acetate (3 x 5 mL). The organic phase was washed with brine (10 mL), dried over $MgSO_4$, and evaporated. The residue was purified on silica gel eluting with hexane/ethyl acetate (90:10) to yield the *title compound* (116 mg, 60%) as a white wax; δ_H (500 MHz; $CDCl_3$) 3.96-3.81 (m, 4 H), 3.23-3.13 (m, 3 H), 2.01-1.92 (m, 1 H), 0.89 (s, 3 H), 0.88 (s, 3 H), 0.83 (s, 3 H); 0.80 (s, 3 H), δ_C (125 MHz; $CDCl_3$) 119.5, 78.6, 75.1, 65.2, 64.5, 54.3, 50.4, 46.0, 44.9, 37.1, 35.9, 35.8, 34.9, 34.2, 31.4, 30.8, 28.8, 28.7, 28.4, 22.7, 20.6, 19.5, 19.4, 14.4, 12.3; HRMS calcd for $C_{25}H_{43}O_3^+$ $[M+H]^+$: 391.3207, found: 391.3206.

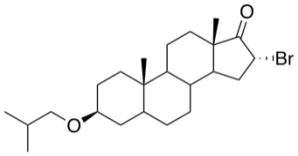
Epiandrosterone isobutyl ether (31)



To a solution of ketal **30** (106 mg, 0.27 mmol) in a (2:1) mixture of THF and acetone (2.7 mL; 0.1M) was added an aqueous HCl solution (2M; 1.9 mL), and the resulting mixture was stirred at room temperature for 2 h. The solution was then neutralized by addition of a saturated $NaHCO_3$ solution and extracted with ethyl acetate (3 x 5 mL). The organic phase was washed with water (10 mL) and brine (10 mL), dried over $MgSO_4$, and evaporated. The residue was purified on silica gel eluting with hexane/ethyl acetate (100%, 90:10, 80:20) to yield the *title compound* (84 mg, 88%) as a colourless wax; δ_H (500 MHz; $CDCl_3$) 3.26-3.10 (m, 3 H), 2.43 (1 H, dd, *J* 19.2, 8.8), 2.12-2.00 (m, 1 H), 0.90 (s,

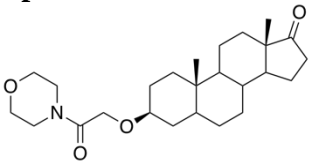
3 H), 0.89 (s, 3 H), 0.86 (s, 3 H), 0.83 (s, 3 H); δ_C (125 MHz; $CDCl_3$) 221.4, 78.5, 75.2, 54.6, 51.5, 47.8, 45.0, 37.0, 36.0, 35.9, 35.1, 34.9, 31.6, 31.0, 28.8, 28.5, 28.3, 21.8, 20.8, 19.5, 19.4, 13.8, 12.3; HRMS calcd for $C_{23}H_{39}O_2^+$ $[M+H]^+$: 347.2945, found: 347.2944.

16α-Bromoepiandrosterone isobutyl ether (32)



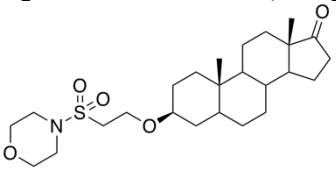
A solution of ketone **31** (63 mg, 0.18 mmol) and copper(II) bromide (122 mg, 0.55 mmol) in MeOH (2.0 mL; 0.1M) was heated at 65 ºC for 13 h. The solvent was then evaporated and the residue purified on silica gel eluting with hexane/ethyl acetate (95:5, 90:10, 85:15) to yield the *title compound* (58 mg, 75%) as a colourless wax; δ_H (500 MHz; $CDCl_3$) 4.53 (1 H, d, *J* 7.1), 3.23-3.13 (m, 3 H), 2.26-2.09 (m, 2 H), 0.92-0.86 (m, 9 H), 0.82 (s, 3 H); δ_C (125 MHz; $CDCl_3$) 213.6, 78.4, 75.2, 54.3, 48.0, 47.8, 46.4, 44.9, 36.9, 36.0, 34.8, 34.3, 34.1, 32.4, 30.8, 28.8, 28.4, 28.2, 20.4, 19.5, 19.4, 14.2, 12.3; HRMS calcd for $C_{23}H_{41}{}^{79}BrNO_2^+$ $[M+NH_4]^+$: 442.2315, found: 442.2314.

Epiandrosterone 2-morpholino-2-oxoethoxy ether (33)



To a solution of ketal **37** (100 mg, 0.22 mmol) in a (2:1) mixture of THF and acetone (2.2 mL; 0.1M) at room temperature was added an aqueous HCl solution (2M; 1.55 mL). After 3 h, LCMS analysis showed complete conversion of the starting material. The reaction mixture was diluted with water (5 mL) and extracted with chloroform (2 × 5 mL). The combined organic layers were dried over MgSO4, filtered and evaporated to dryness. The resulting solid was recrystallized from a mixture of hexane and CH_2Cl_2 (9:1) to yield the *title compound* (70 mg, 78%) as an amorphous solid; δ_H (500 MHz; $CDCl_3$) 4.17 (2 H, s), 3.69-3.67 (4 H, m), 3.62-3.58 (4 H, m), 3.36-3.30 (1 H, m), 2.46-2.41 (1 H, m), 2.10-2.02 (1 H, m), 0.86 (3 H, s), 0.83 (3 H, s); δ_C (125 MHz; $CDCl_3$) 221.3, 168.5, 79.0, 67.9, 66.83, 66.78, 54.4, 51.4, 47.7, 45.8, 44.7, 42.1, 36.8, 35.84, 35.81, 35.0, 34.4, 31.5, 30.8, 28.4, 28.0, 21.7, 20.4 13.8, 12.2; HRMS calcd for $C_{25}H_{40}NO_4^+$ $[M+H]^+$: 418.2952, found: 418.2948.

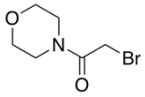
Epiandrosterone 2-(morpholinosulfonyl)ethyl ether (34)



To a solution of ketal **38** (117 mg; 0.23 mmol) in a (2:1) mixture of THF and acetone (2.3 mL; 0.1M) was added an aqueous HCl solution (2M; 1.6 mL), and the resulting mixture was stirred at room temperature for 4 h. The solution was then neutralized by addition of a saturated $NaHCO_3$ solution and extracted with ethyl acetate (3 x 5 mL). The organic phase was washed with water (10 mL) and brine (10 mL), dried over $MgSO_4$, and evaporated. The residue was purified on silica gel eluting with hexane/ethyl acetate (70:30) to yield the *title compound* (89 mg, 83%) as a white wax; δ_H (500 MHz; $CDCl_3$) δ 3.88-3.79 (m, 2 H), 3.75 (4 H, at, *J* 4.8), 3.33-3.25 (m, 5 H), 3.20 (2 H, t, *J* 6.1), 2.44 (1 H, dd, *J* 19.3, 8.7), 2.12-2.00 (m, 1 H), 0.86 (s, 3 H), 0.82 (s, 3 H); δ_C (125 MHz; $CDCl_3$) 221.2, 79.2, 66.7,

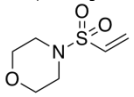
61.3, 54.4, 51.4, 50.2, 47.8, 45.6, 44.7, 36.8, 35.9, 35.8, 35.0, 34.6, 31.6, 30.9, 28.5, 28.1, 21.8, 20.5, 13.8, 12.3; HRMS calcd for $C_{25}H_{42}NO_5S^+$ $[M+H]^+$: 468.2778, found: 468.2777.

2-Bromo-1-morpholinoethan-1-one (35)



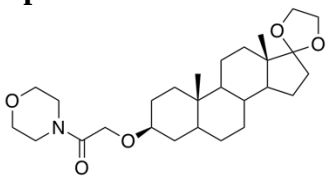
To a solution morpholine (440 mg; 5.05 mmol) and triethylamine (0.70 mL; 5.05 mmol) in anhydrous CH_2Cl_2 (10 mL; 0.5M) at 0 ºC was added a solution of bromoacetyl bromide (0.44 mL; 5.05 mmol) in anhydrous CH_2Cl_2 (10 mL; 0.5M) dropwise under an argon atmosphere. After 1 h at 0 ºC, TLC analysis indicated complete conversion of the starting material, so the reaction mixture was poured onto water (20 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The organic layer was filtered over a hydrophobic frit and evaporated to yield the *title compound* (780 mg, 74%) as a colourless oil, which was used without further purification. Analytical data were in accordance to the literature;[12] δ_H (500 MHz; $CDCl_3$) 3.86 (2 H, s), 3.74 (2 H, at, *J* 4.9), 3.70 (2 H, at, *J* 4.6), 3.64 (2 H, at, *J* 4.6), 3.52 (2 H, at, *J* 4.9); δ_C (125 MHz; $CDCl_3$) 165.6, 66.6, 66.4, 47.2, 42.5, 25.3.

4-(Vinylsulfonyl)morpholine (36)



To a solution morpholine (0.28 mL; 3.07 mmol) and triethylamine (1.50 mL; 10.74 mmol) in anhydrous CH_2Cl_2 (10 mL; 0.3M) at 0 ºC was added 2-chloroethanesulfonyl chloride (0.32 mL; 3.07 mmol) dropwise under an argon atmosphere. After 2 h at 0 ºC, TLC analysis indicated complete conversion of the starting material, so the reaction mixture was diluted with chloroform (10 mL) and washed with brine (2 × 10 mL), dried over $MgSO_4$, filtered and evaporated. The residue was purified on silica gel eluting with hexane/ethyl acetate (1:1) to yield the *title compound* (384 mg, 71%) as a colourless oil; δ_H (500 MHz; $CDCl_3$) 6.44 (1 H, dd, *J* 16.6, 10.0), 6.27 (1 H, d, *J* 16.7), 6.10 (1 H, d, *J* 9.9), 3.77 (1 H, t, *J* 4.8), 3.14 (1 H, t, *J* 4.8); δ_C (125 MHz; $CDCl_3$) 131.7, 129.5, 66.2, 45.6; HRMS calcd for $C_6H_{12}NO_3S^+$ $[M+H]^+$: 178.0532, found: 178.0530.

Epiandrosterone 17-ketal 2-morpholino-2-oxoethoxy ether (37)

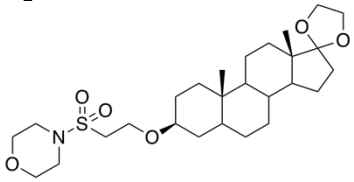


To a solution of ketal **25** (200 mg, 0.60 mmol) in anhydrous THF (1.70 mL; 0.35M) at 0 ºC was added a solution of n-butyllithium (1.86M; 0.32 mL; 0.60 mmol) dropwise under an argon atmosphere. After 1 h, a solution of acetyl bromide **35** (125 mg, 0.60 mmol) and TBAI (23 mg, 0.07mmol) in anhydrous THF (0.6 mL; 1M) was added dropwise. The reaction mixture was allowed to warm to room temperature, and stirred for 16 h, upon which time TLC analysis still showed the presence of the starting material. Nevertheless, the mixture was diluted with a (1:1) mixture of ethyl acetate and diethyl ether (10 mL) and washed with water (6 mL) and brine (6 mL). The organic layer was dried over MgSO4, filtered and evaporated, and the residue was purified on silica gel eluting with hexane/ethyl acetate (3:7) to yield the *title compound* (115 mg, 90% brsm) as an amorphous solid; δ_H (500 MHz; $CDCl_3$) 4.16 (2 H, s), 3.95-3.83 (4 H, m), 3.69-3.67 (4 H, m), 3.61-3.58 (4 H, m), 3.35-3.29 (1 H, m), 0.86 (3 H, s),

[12] Roiser, L.; Waser, M. *Org. Lett.* **2017**, *19* (9), 2338–2341.

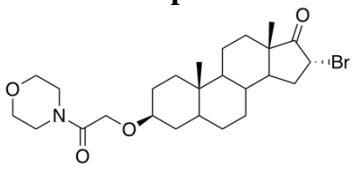
0.83 (3 H, s); δ_C (125 MHz; $CDCl_3$) 168.6, 119.4, 79.2, 67.9, 66.83, 66.78, 64.5, 54.1, 50.3, 45.9, 45.8, 44.7, 42.1, 36.9, 35.71, 35.69, 34.5, 34.1, 31.3, 30.7, 28.6, 28.0, 22.6, 20.6 14.4, 12.2; HRMS calcd for $C_{27}H_{44}NO_5^+$ $[M+H]^+$: 462.3214, found: 462.3226.

Epiandrosterone 17-ketal 2-(morpholinosulfonyl)ethyl ether (38)



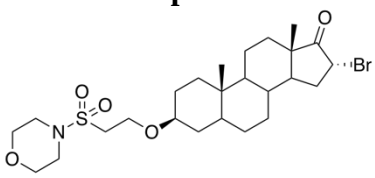
A mixture of ketal **25** (116 mg, 0.35 mmol), TBAB (11 mg, 0.03 mmol) and vinyl sulfone **36** (155 mg, 0.88 mmol) in toluene (3.5 mL; 0.1M), and aqueous NaOH (6M; 0.2 mL, 1.2 mmol) was stirred at room temperature for 42 h, upon which time the solvent was removed *in vacuo*. The residue was taken up in water (10 mL) and extracted with chloroform (3 x 10 mL). The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified on silica gel eluting with hexane/ethyl acetate (70:30) to yield the *title compound* (117 mg, 66%) as a white solid; m.p. 145-148 ºC; δ_H (500 MHz; $CDCl_3$) δ 3.96-3.87 (m, 2 H); 3.87-3.78 (m, 4 H); 3.75 (4 H, at, *J* 4.6); 3.33-3.24 (m, 5 H); 3.20 (2 h, t, *J* 6.1); 2.02-1.92 (m, 1 H); 0.83 (s, 3 H); 0.79 (s, 3 H); δ_C (125 MHz; $CDCl_3$) 119.5, 79.3, 66.7, 65.2, 64.5, 61.3, 54.2, 50.3, 50.2, 46.0, 45.6, 44.7, 36.9, 35.8, 35.7, 34.6, 34.2, 31.3, 30.7, 28.7, 28.2, 22.6, 20.6, 14.4, 12.3; HRMS calcd for $C_{27}H_{46}NO_6S^+$ $[M+H]^+$: 512.3040, found: 512.3038.

16α-Bromoepiandrosterone 2-morpholino-2-oxoethoxy ether (39)



A solution of ketone **33** (37 mg, 0.09 mmol) and copper(II) bromide (44 mg, 0.19 mmol) in MeOH (1.0 mL; 0.1M) was stirred at 65 ºC for 15 h. The solvent was then evaporated, and the residue purified on silica gel eluting with hexane/ethyl acetate (70:30) to yield the *title compound* (37 mg, 74%) as a white solid; m.p.149-152 ºC; δ_H (500 MHz; $CDCl_3$) 4.52 (1 H, d, *J* 6.9); 4.17 (s, 2 H); 3.70-3.66 (m, 4 H); 3.64-3.55 (m, 4 H); 3.37-3.31 (m, 1 H); 2.24-2.12 (m, 2 H); 0.89 (s, 3 H); 0.83 (s, 3 H); δ_C (125 MHz; $CDCl_3$) 213.4, 168.5, 79.0, 68.0, 66.9, 66.8, 54.2, 47.9, 47.8, 46.4, 45.9, 44.6, 42.2, 36.7, 35.9, 34.4, 34.3, 34.1, 32.3, 30.7, 28.3, 28.0, 20.4, 14.2, 12.2; HRMS calcd for $C_{25}H_{39}{}^{79}BrNO_4^+$ $[M+H]^+$: 496.2057, found: 496.2057.

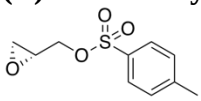
16α-Bromoepiandrosterone 2-(morpholinosulfonyl)ethyl ether (40)



A solution of ketone **34** (47 mg, 0.10 mmol) and copper(II) bromide (50 mg, 0.22 mmol) in MeOH (1.1 mL; 0.1M) was stirred at 65 ºC for 15 h. The solvent was then evaporated, and the residue purified on silica gel eluting with hexane/ethyl acetate (70:30) to yield the *title compound* (41 mg, 74%) as a white solid; m.p:158-161 ºC; δ_H (500 MHz; $CDCl_3$) 4.53 (1 H, d, *J* 6.9), 3.88-3.80 (m, 2 H), 3.75 (4 H, at, *J* 4.6), 3.33-3.25 (m, 5 H), 3.20 (2 H, t, *J* 6.2), 2.27-2.10 (m, 2 H), 0.90 (s, 3 H), 0.82 (s, 3 H); δ_C (125 MHz; $CDCl_3$) 213.4, 79.1, 66.7, 61.4, 54.2, 50.2, 47.9, 47.8, 46.4, 45.6, 44.7, 36.7, 35.9, 34.5, 34.3,

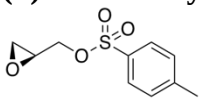
34.1, 32.3, 30.7, 28.3, 28.1, 20.4, 14.2, 12.2; HRMS calcd for $C_{25}H_{41}{}^{79}BrNO_5S^+$ $[M+H]^+$: 546.1883, found: 546.1883.

(*R*)-Oxiran-2-ylmethyl 4-methylbenzenesulfonate (41)



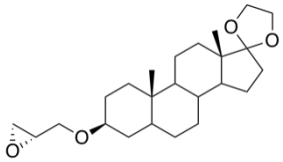
To a solution of (*S*)-(+)-glycidol (2.0 g, 27.0 mmol), *p*-toluenesulfonyl chloride (7.72 g, 40.5 mmol) and DMAP (330 mg, 2.70 mmol) in anhydrous CH_2Cl_2 (90 mL; 0.3M) at 0 ºC was added triethylamine (5.64 mL; 40.5 mmol) dropwise under an argon atmosphere. The reaction mixture was allowed to stir at 5 ºC for 3 h, upon which time TLC analysis showed complete conversion of the starting material. The reaction mixture was thus washed with a 2M HCl solution (2 × 50 mL), a saturated aqueous $NaHCO_3$ solution (50 mL), brine (50 mL), dried over $MgSO_4$, filtered and evaporated. The residue was purified on silica gel eluting with hexane/ethyl acetate (4:1 to 1:1) to yield the *title compound* (5.23 g, 85%) as a clear oil; δ_H (500 MHz; $CDCl_3$) 7.81 (1 H, d, *J* 8.3), 7.36 (1 H, d, *J* 8.5), 4.26 (1 H, dd, *J* 11.5, 3.5), 3.96 (1 H, dd, *J* 11.5, 6.1), 3.20-3.17 (1 H, m), 2.81 (1 H, at, *J* 4.5), 2.61 (1 H, dd, *J* 4.8, 2.5), 2.45 (3 H, s); δ_C (125 MHz; $CDCl_3$) 145.1, 132.7, 129.9, 127.9, 70.4, 48.8, 44.6, 21.6; HRMS calcd for $C_{10}H_{16}NO_4S^+$ $[M + Na]^+$: 246.0795, found: 246.0796.

(*S*)-Oxiran-2-ylmethyl 4-methylbenzenesulfonate (42)



To a solution of (*R*)-(+)-glycidol (2.0 g, 27.0 mmol), *p*-toluenesulfonyl chloride (5.15 g, 27.0 mmol) in anhydrous CH_2Cl_2 (39 mL; 0.7M) at 0 ºC was added triethylamine (4.89 mL; 35.0 mmol) dropwise under an argon atmosphere. The reaction mixture was allowed to stir at 5 ºC for 2 h, upon which time TLC analysis showed complete conversion of the starting material. The reaction mixture was filtered and washed with a 2M HCl solution (2 × 20 mL), a saturated aqueous $NaHCO_3$ solution (2 × 20 mL), brine (20 mL), dried over Na_2SO_4, filtered and evaporated, to yield the *title compound* (5.22 g, 85%) as a clear oil that solidified upon storage in a refrigerator; δ_H (500 MHz; $CDCl_3$) 7.81 (1 H, d, *J* 8.3), 7.36 (1 H, d, *J* 8.2), 4.26 (1 H, dd, *J* 11.4, 3.4), 3.95 (1 H, dd, *J* 11.4, 6.0), 3.20-3.17 (1 H, m), 2.82 (1 H, at, *J* 4.5), 2.60 (1 H, dd, *J* 4.7, 2.5), 2.45 (3 H, s); δ_C (125 MHz; $CDCl_3$) 145.1, 132.7, 129.9, 128.0, 70.4, 48.8, 44.6, 21.7; HRMS calcd for $C_{10}H_{16}NO_4S^+$ $[M + Na]^+$: 246.0795, found: 246.0795.

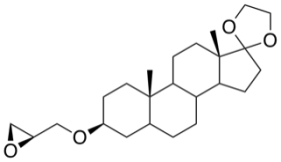
Epiandrosterone 17-ketal (*R*)-oxiran-2-yl)methyl ether (45)



To a solution of ketal **25** (200 mg, 0.60 mmol) in anhydrous DMF (4.0 mL; 0.7M) at 0 ºC was added sodium hydride (60% dispersion in mineral oil; 191 mg; 4.78 mmol) in one portion under an argon atmosphere. After the evolution of hydrogen gas had stopped (as observed through bubbler filled with oil after 10 min), a solution of tosylate **41** (273 mg, 1.20 mmol) in anhydrous DMF (2.0 mL; 0.3M) was added dropwise. The reaction mixture was allowed to warm to room temperature, and stirred for 21 h, upon which time TLC analysis still showed the presence of the starting material. Nevertheless, the mixture was diluted with diethyl ether (10 mL) and carefully quenched with water (excess NaH reacting). The mixture was washed with brine (3 × 10 mL) and the resulting organic layer was dried over MgSO4, filtered and evaporated. The residue was purified on silica gel eluting with hexane/ethyl

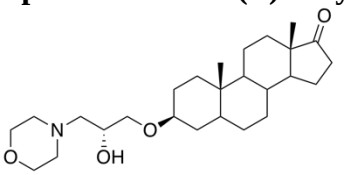
acetate (4:1 to 3:2) to yield the *title compound* (108 mg, 46%) as an amorphous solid; δ_H (500 MHz; $CDCl_3$) 3.95-3.82 (4 H, m), 3.70 (1 H, dd, *J* 11.3, 3.4), 3.47 (1 H, dd, *J* 11.4, 5.6), 3.32-3.26 (1 H, m), 3.14-3.11 (1 H, m), 2.79 (1 H, dd, *J* 4.9, 4.4), 2.61 (1 H, dd, *J* 5.0, 2.7), 0.83 (3 H, s), 0.80 (3 H, s); δ_C (125 MHz; $CDCl_3$) 119.5, 79.1, 68.7, 65.1, 64.5, 54.2, 51.2, 50.3, 45.9, 44.8, 44.7, 37.0, 35.8, 35.7, 34.8, 34.2, 31.3, 30.7, 28.6, 28.1, 22.6, 20.6 14.4, 12.2; HRMS calcd for $C_{24}H_{39}O_4^+$ $[M+H]^+$: 391.2843, found: 391.2850.

Epiandrosterone 17-ketal (*S*)-oxiran-2-yl)methyl ether (46)



To a solution of ketal **25** (200 mg, 0.60 mmol) in anhydrous DMF (4.0 mL; 0.7M) at 0 ºC was added sodium hydride (60% dispersion in mineral oil; 191 mg; 4.78 mmol) in one portion under an argon atmosphere. After the evolution of hydrogen gas had stopped (as observed through bubbler filled with oil after 10 min), a solution of tosylate **41** (546 mg, 2.39 mmol) in anhydrous DMF (2.0 mL; 0.3M) was added dropwise. The reaction mixture was allowed to warm to room temperature, and stirred for 21 h, upon which time TLC analysis still showed the presence of the starting material. Nevertheless, the mixture was diluted with diethyl ether (10 mL) and carefully quenched with water (excess NaH reacting). The mixture was washed with brine (3 × 10 mL) and the resulting organic layer was dried over MgSO4, filtered and evaporated. The residue was purified on silica gel eluting with hexane/ethyl acetate (4:1 to 3:2) to yield the *title compound* (97 mg, 41%) as an amorphous solid; δ_H (500 MHz; $CDCl_3$) 3.95-3.83 (4 H, m), 3.71 (1 H, dd, *J* 11.4, 3.3), 3.46 (1 H, dd, *J* 11.3, 5.6), 3.33-3.26 (1 H, m), 3.15-3.11 (1 H, m), 2.79 (1 H, at, *J* 4.6), 2.61 (1 H, dd, *J* 5.0, 2.7), 0.83 (3 H, s), 0.80 (3 H, s); δ_C (125 MHz; $CDCl_3$) 119.4, 79.1, 68.7, 65.1, 64.5, 54.2, 51.2, 50.3, 45.9, 44.8, 44.6, 37.0, 35.8, 35.7, 34.5, 34.2, 31.3, 30.7, 28.7, 28.2, 22.6, 20.6 14.4, 12.2; HRMS calcd for $C_{24}H_{39}O_4^+$ $[M+H]^+$: 391.2843, found: 391.2841.

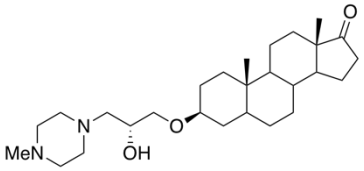
Epiandrosterone (*R*)-2-hydroxy-3-morpholinopropyl ether (47)



A solution of epoxide **45** (36 mg, 0.09 mmol) and morpholine (40 µL, 0.46 mmol) in MeOH (0.61 mL; 0.15M) was heated at 50 ºC for 1.5 h, upon which time complete conversion was observed by TLC analysis. The reaction mixture was diluted with chloroform (2 mL) and washed with water (5 × 2 mL) and brine (2 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated. The crude compound was taken up in a (2.5:1) mixture of THF and acetone (0.92 mL; 0.1M) and an aqueous HCl solution (2M; 0.66 mL) was then added. After stirring for 2 h at room temperature, the reaction mixture was diluted with ethyl acetate (3 mL), washed with saturated $NaHCO_3$ (3 × 3 mL) and brine (3 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated, and the resulting residue purified on silica gel eluting with chloroform/methanol (95:5) to yield the *title compound* (14 mg, 35%) as a white wax; δ_H (500 MHz; $CDCl_3$) 3.90-3.85 (1 H, m), 3.75-3.68 (4 H, m), 3.51 (1 H, dd, *J* 9.8, 4.3), 3.45 (1 H, dd, *J* 9.7, 5.8), 3.28-3.22 (1 H, m), 2.64-2.60 (2 H, m), 2.47-2.38 (5 H, m), 2.10-2.02 (1 H, m), 0.86 (3 H, s), 0.82 (3 H, s); δ_C (125 MHz; $CDCl_3$) 221.3, 79.1, 70.4, 67.0, 66.2, 61.4, 54.5, 53.8, 51.4, 47.8,

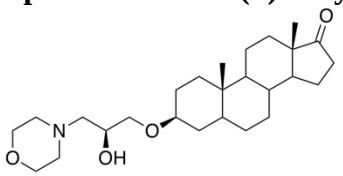
44.8, 36.9, 35.9, 35.8, 35.0, 34.6, 31.5, 30.9, 28.5, 28.1, 21.7, 20.5 13.8, 12.3; HRMS calcd for $C_{26}H_{44}NO_4^+$ $[M+H]^+$: 434.3265, found: 434.3257.

Epiandrosterone (*R*)-2-hydroxy-3-(4-methylpiperazin-1-yl)propyl ether (48)



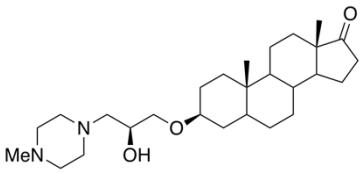
A solution of epoxide **45** (35 mg, 0.09 mmol) and *N*-methylpiperazine (50 µL, 0.45 mmol) in MeOH (0.60 mL; 0.15M) was heated at 50 ºC for 1.5 h, upon which time complete conversion was observed by TLC analysis. The reaction mixture was diluted with chloroform (3 mL) and washed with a (3:1) mixture of water and MeOH (5 × 2 mL) and brine (2 × 2 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated. The crude compound was taken up in a (2.5:1) mixture of THF and acetone (0.90 mL; 0.1M) and an aqueous HCl solution (2M; 0.64 mL) was then added. After stirring for 2 h at room temperature, the reaction mixture was diluted with ethyl acetate (3 mL), washed with saturated $NaHCO_3$ (3 × 3 mL) and brine (3 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated, and the resulting residue purified on silica gel eluting with chloroform/methanol (9:1) to yield the *title compound* (17 mg, 43%) as an amorphous solid; δ_H (500 MHz; $CDCl_3$) 3.87-3.83 (1 H, m), 3.50 (1 H, dd, *J* 9.8, 4.2), 3.44 (1 H, dd, *J* 9.8, 5.7), 3.28-3.22 (1 H, m), 2.67 (2 H, bs), 2.57-2.37 (8 H, m), 2.29 (3 H, s), 2.10-2.02 (1 H, m), 0.86 (3 H, s), 0.82 (3 H, s); δ_C (125 MHz; $CDCl_3$) 221.3, 79.1, 70.5, 66.3, 60.7, 55.2, 54.5, 51.4, 47.8, 46.0, 44.8, 36.9, 35.9, 35.8, 35.0, 34.7, 31.5, 30.9, 28.5, 28.1, 21.8, 20.5 13.8, 12.3; HRMS calcd for $C_{27}H_{47}N_2O_3^+$ $[M+H]^+$: 447.3581, found: 447.3588.

Epiandrosterone (*S*)-2-hydroxy-3-morpholinopropyl ether (49)



A solution of epoxide **46** (32 mg, 0.08 mmol) and morpholine (36 µL, 0.41 mmol) in MeOH (0.55 mL; 0.15M) was heated at 50 ºC for 1.5 h, upon which time complete conversion was observed by TLC analysis. The reaction mixture was diluted with chloroform (2 mL) and washed with water (5 × 2 mL) and brine (2 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated. The crude compound was taken up in a (2.5:1) mixture of THF and acetone (0.82 mL; 0.1M) and an aqueous HCl solution (2M; 0.59 mL) was then added. After stirring for 2 h at room temperature, the reaction mixture was diluted with ethyl acetate (3 mL), washed with saturated $NaHCO_3$ (3 × 3 mL) and brine (3 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated, and the resulting residue purified on silica gel eluting with chloroform/methanol (97:3) to yield the *title compound* (25 mg, 64%) as an amorphous solid; δ_H (500 MHz; $CDCl_3$) 3.89-3.84 (1 H, m), 3.75-3.68 (4 H, m), 3.52 (1 H, dd, *J* 9.8, 4.2), 3.44 (1 H, dd, *J* 9.7, 5.8), 3.28-3.22 (1 H, m), 2.64-2.60 (2 H, m), 2.47-2.38 (5 H, m), 2.10-2.02 (1 H, m), 0.86 (3 H, s), 0.82 (3 H, s); δ_C (125 MHz; $CDCl_3$) 221.3, 79.1, 70.4, 67.0, 66.2, 61.3, 54.5, 53.8, 51.4, 47.8, 44.8, 36.9, 35.9, 35.8, 35.0, 34.7, 31.5, 30.9, 28.5, 28.1, 21.7, 20.5 13.8, 12.2; HRMS calcd for $C_{26}H_{44}NO_4^+$ $[M+H]^+$: 434.3265, found: 434.3267.

Epiandrosterone (*S*)-2-hydroxy-3-(4-methylpiperazin-1-yl)propyl ether (50)



A solution of epoxide **46** (34 mg, 0.09 mmol) and *N*-methylpiperazine (48 µL, 0.44 mmol) in MeOH (0.58 mL; 0.15M) was heated at 50 ºC for 1.5 h, upon which time complete conversion was observed by TLC analysis. The reaction mixture was diluted with chloroform (3 mL) and washed with a (3:1) mixture of water and MeOH (5 × 2 mL) and brine (2 × 2 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated. The crude compound was taken up in a (2.5:1) mixture of THF and acetone (0.87 mL; 0.1M) and an aqueous HCl solution (2M; 0.62 mL) was then added. After stirring for 2 h at room temperature, the reaction mixture was diluted with ethyl acetate (3 mL), washed with saturated $NaHCO_3$ (3 × 3 mL) and brine (3 mL). The organic layer was dried over Na_2SO_4, filtered and evaporated, and the resulting residue purified on silica gel eluting with chloroform/methanol (9:1) to yield the *title compound* (28 mg, 72%) as a yellow wax; δ_H (500 MHz; $CDCl_3$) 3.87-3.83 (1 H, m), 3.51 (1 H, dd, *J* 9.8, 4.3), 3.44 (1 H, dd, *J* 9.8, 5.7), 3.28-3.22 (1 H, m), 2.67 (2 H, bs), 2.57-2.37 (8 H, m), 2.29 (3 H, s), 2.10-2.02 (1 H, m), 0.86 (3 H, s), 0.82 (3 H, s); δ_C (125 MHz; $CDCl_3$) 221.3, 79.1, 70.5, 66.3, 60.7, 55.1, 54.5, 51.4, 47.8, 46.0, 44.8, 36.9, 35.9, 35.8, 35.0, 34.7, 31.5, 30.9, 28.5, 28.1, 21.8, 20.5 13.8, 12.3; HRMS calcd for $C_{27}H_{47}N_2O_3^+$ $[M+H]^+$: 447.3581, found: 447.3581.

2. BIOLOGY – experimental procedures

2.1. Biochemical assays

Recombinant *Tc*G6PDH and *Hs*G6PDH purification and inhibition assay are described elsewhere.[13] Briefly, both proteins were expressed with his-tag in transformed *E. coli* and purified by chromatographic affinity in Ni-NTA (QIAGEN) columns. G6PDH-diaphorase coupled reaction were used for enzymatic inhibition assay based in resorufin fluorescence readout (ex. 545-20 nm, em. 600-40 nm) in ClarioStar plate reader (BMG LabTech). For IC50 determination, compounds were serially diluted in 11 points with a dilution factor of 0.4. The IC50 was calculated by nonlinear regression of the data using GraphPad Prism.

Table S1. Results of the enzymatic assays of target compounds against *Tc*G6PDH and *Hs*G6PDH.

Entry	IC50-TC (µM)	s.d.	IC50-HS (µM)	s.d.	pIC50_TC	pIC50_HS	S.I.[a]
1	30.40	0.80	5.81	0.28	4.5	5.2	0.2
2	7.14	0.36	1.57	0.04	5.1	5.8	0.2
3	0.11	0.01	0.25	0.01	7.0	6.6	2.2
4	0.66	0.04	0.25	0.00	6.2	6.6	0.4
5	24.23	1.78	0.63	0.04	4.6	6.2	0.0
6	0.98	0.07	0.56	0.02	6.0	6.3	0.6
7	0.59	0.03	0.08	0.00	6.2	7.1	0.1
8	5.01	1.04	47.27	1.96	5.3	4.3	9.4
9	7.88	0.44	n.d.	n.a.	5.1	< 4.0	> 10.1
10	10.27	3.84	n.d.	n.a.	5.0	< 4.0	> 7.8
11	8.75	0.64	15.33	0.64	5.1	4.8	1.8
12	0.15	0.01	24.03	8.49	6.8	4.6	**160.9**
13	0.38	0.03	45.47	6.58	6.4	4.3	**119.8**
14	0.20	0.03	n.d.	n.a.	6.7	< 4.0	**> 404**
15	0.76	0.04	4.57	0.14	6.1	5.3	6.0
19	12.60	0.20	n.d.	n.a.	4.9	< 4.0	> 6.3
20	13.17	0.42	77.00	2.67	4.9	4.1	5.8
21	12.90	0.47	n.d.	n.a.	4.9	< 4.0	> 6.2
22	16.30	1.27	n.d.	n.a.	4.8	< 4.0	> 4.9
23	4.53	0.17	33.80	7.93	5.3	4.5	7.5
24	4.34	0.30	13.67	0.16	5.4	4.9	3.1
27	4.30	0.80	21.23	1.42	5.4	4.7	4.9
28	0.74	0.04	36.20	4.20	6.1	4.4	**48.8**
31	53.97	6.18	19.57	11.89	4.3	4.7	0.4
32	1.68	0.19	n.d.	n.a.	5.8	< 4.0	**> 47.6**
33	47.80	2.53	n.d.	n.a.	4.3	< 4.0	> 1.7
34	27.17	1.78	25.00	0.60	4.6	4.6	0.9
39	2.31	0.14	22.33	0.42	5.6	4.7	9.7
40	1.35	0.17	6.38	3.41	5.9	5.2	4.7
47	50.27	3.29	n.d.	n.a.	4.3	< 4.0	> 1.6
48	41.77	2.24	n.d.	n.a.	4.4	< 4.0	> 1.9
49	58.57	3.42	46.17	0.78	4.2	4.3	0.8
50	48.83	2.09	77.47	1.22	4.3	4.1	1.6

[§]s.d. standard deviation; n.d., not detected; n.a., not applicable; S.I., selectivity index
[§§]All assays were performed in triplicate
[a]For compounds showing no activity (n.d.), the minimum S.I. value was calculated based on the highest concentration tested (80 µM).

[13] Mercaldi, G. F.; Ranzani, A. T.; Cordeiro, A. T. *J. Biomol. Screen.* **2014**, *19*, 1362–1371.

2.2. Intracellular *T. cruzi* image-based assay

2.2.1. Mammalian and T. cruzi cell lines

Mammalian cells LLC-MK2 (green monkey kidney epithelial cells) and H9c2 (rat cardiomyocytes) were cultivated in 75cm^2 T-flasks containing 15 mL of Dulbecco's Modified Eagle Medium (DMEM; Sigma-Aldrich) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Vitrocell), 100 U/ml penicillin (Gibco) and 100 µg/ml streptomycin (Gibco). *T. cruzi* (strain Y) trypomastigotes were obtained from supernatant of infected LLC-MK2 after 5 days of infection. LLC-MK2 infection was renewed weekly and maintained in DMEM medium supplemented with 2% FBS. Otherwise stated, cells were always incubated at 37°C, 5% CO_2 and >95% humidity.

2.2.2. Compound intermediary plate

Compounds were dissolved in anhydrous DMSO (Sigma-Aldrich) to a stock concentration of 10 mM. For single concentration assay compound stocks were diluted to 5 mM in anhydrous DMSO. For dose-response assays, compound stocks were diluted to 8 mM and six lower concentrations by applying a half-log factor serial dilution procedure. At this stage all samples were diluted in anhydrous DMSO. Intermediary plates were prepared diluting compounds in fresh DMEM medium in a proportion of 1:99. At this stage, DMSO concentration was 1%. Then, 30 µL of samples from intermediary plates were transferred to final assay plate pre-filled with 45 µL of DMEM medium and cells. DMSO concentration in assay plate was 0.4%.

2.2.3. Intracellular image-based assay

The current assay was adapted from previously published protocol.[14] Briefly, ($5x10^5$ cells) *T. cruzi* trypomastigotes collected from LLC-MK2 infected cells were used to infect ($1x10^6$ cells) H9c2 rat cardiomyocytes for 18h (overnight), in 75cm^2 T-flask. Then, cells were washed with phosphate buffered solution to remove extracellular parasites and resuspended in fresh DMEM medium to a concentration of $2.2x10^4$ cells per mL. Multidrop (Thermo Fisher Science) was used to dispense 45 µL of cellular suspension into 384 microplates ($1x10^3$ cells per well). Plates were incubated for addition 48h for *T. cruzi* differentiation into intracellular amastigotes. Next, 30 µL of compounds from intermediary plates were transferred to assay plates. At this stage DMSO concentration was reduced to 0.4%. After 72h incubation (treatment period), cells were fixed with 4% paraformaldehyde (PFA; Sigma-Aldrich) and stained with 4µg/mL Hoechst 33342 solution (Thermo Fisher Scientific) for 30 minutes at room temperature. Image acquisition was accomplished by the fluorescence microscope Operetta (PerkinElmer, Hamburg, DE) using a 20X long WD objective. Five images per well were acquired and processed in Columbus software (PerkinElmer, Hamburg, DE). Software default parameters were used to identify host cells nuclei, cytoplasm area and spot in cytoplasm. Infected cells were defined as cells with at least 3 spots in cytoplasm area. Infection ratio correspond to infected cells/total cells per well. Total cells, infected cells and infection ratio data were analyzed and plotted with GraphPad Prism software.

[14] Alonso-Padilla J, Cotillo I, Presa JL, Cantizani J, Peña I, Bardera AI, Martín JJ, Rodriguez A. *PLoS Negl Trop Dis.* **2015** Jan 23;9(1):e0003493. doi: 10.1371/journal.pntd.0003493. eCollection 2015 Jan.