Supplemental Data

SYNTHESIS OF CORTISOL DERIVATIVE

Melting point determinations were made using a Reichert Thermopan instrument. Proton and carbon nuclear magnetic resonance (NMR) spectra were acquired using both a 300 MHz Bruker AC300 FT-NMR and a 400 MHz Bruker Avance DRX400 FT-NMR. Chemical shifts (δ) are reported in parts per million (ppm) and coupling constants (J) are reported in Hz. Electrospray mass spectra were acquired using a VG Platform II Electrospray Mass Spectrometer (ES-MS) instrument.

4,5-Epoxy-11β,17,21-trihydroxypregna-3,20-dione (**1**). Adapted from published methods.[30-32] Cortisol (362.5 mg, 1.0 mmol) was partially dissolved in methanol (13 mL) and ethanol (5 mL) and chilled to 0 ^{o}C. Sodium hydroxide solution (10%w/v in distilled water, 1 mL) was added followed by 30%w/v hydrogen peroxide solution (400 μL). The reaction was kept stirring at 0 ^{o}C on ice for 3 h. The reaction mixture was then raised to room temperature; any remaining solid was filtered off. The filtrate pH was carefully adjusted to 7.0 using acetic acid and the resulting solution dried *in vacuo* to yield a clear, colourless oil. This sample was then constituted in distilled water (30 mL) and extracted with 3 x 30 mL of ethyl acetate. The organic phase was then washed with 1 x 30 mL of distilled water and dried over sodium sulfate. The supernatant was then passed through a bed of calcined alumina (~10 g) and the solvent removed *in vacuo* to yield a clear, colourless oil. The product was then column purified using 1:1 ethyl acetate: *n*-hexane to yield **1** (86.6 mg, 23%) as a colourless oil. R_f = 0.36 (1:1 ethyl acetate: *n*-hexane). Melting point (mp): 157-160 ^{o}C β epimer. 166-169 ^{o}C α epimer. Literature mp: β 147-148 ^{o}C, α 167-168 ^{o}C.

HPLC: 1mL/min. 60% methanol, 100% purity, R_t = 4.60 and 4.85 min. for the two epimers, λ_{max} = 204 nm. IR (KBr disc): 1450, 1701, 1724, 2369, 2928, 3449cm^{-1}. ^{1}H NMR (δ): 1.14 (3H, s, 18-CH_3), 1.36 (3H, s, 19-CH_3), 3.03 and 3.06 (1H, s, 4-H, β and α respectively), 4.30, 4.40 (1H each, d, *J* = 3.7Hz, 21-H). ^{13}C NMR (δ): 15.9 (18-CH_3), 20.0, 21.1, 22.2, 25.8, 28.3, 28.6, 29.0, 29.4, 30.4 (19-CH_3), 32.9, 35.2, 35.3, 40.6, 52.2, 62.8, 62.9, 68.0, 68.6, 206.5, 218.9. ESMS (-40 V, MeOH): m/z 363.2 $[M+H_2O-H]^-$.

4-(2'-Carboxyethylthio)-11β,17α,21-trihydroxypregn-4-ene-3,20-dione (**2**). Adapted from a published method.[33] **1** (586.8 mg, 1.559 mmol) was dissolved in ethanol (dried over molecular sieves, 5 mL). A solution of potassium hydroxide (25%w/v in distilled water, 730 μL) was stirred whilst 3-mercaptopropionic acid (224 μL) was added. The stirring solution then had the epoxide solution added dropwise and was immediately placed under nitrogen and stirred at room temperature for four hours. Distilled water (30 mL) was added. The aqueous phase was then extracted with diethyl ether (3 x 30 mL) before adjusting the pH of the aqueous phase to 1.5 with 1M HCl. The aqueous phase was then extracted with 3 x 30 mL of ethyl acetate. The organic phase was then dried over sodium sulfate and the liquor decanted and solvent removed *in vacuo*. The sample was then column separated using chloroform, 15:1 chloroform: methanol and methanol eluent. The sample was then dried to yield **2** (479.9 mg, 66%) as a clear, colourless oil. R_f = 0.42 (5:1 chloroform: methanol). Mp: 132-136 ^{o}C. Lit. mp: 177-178 ^{o}C. HPLC: 1 mL/min. 60%v/v methanol. R_t = 1.95min. % Purity = 100%. IR (neat): 1108, 1657, 2360, 2920. ^{1}H NMR: δ 0.89 (3H, s, 18-CH_3), 1.21 (1H, t, *J* = 7.0Hz), 1.47 (3H, s, 19-CH_3), 2.47 (2H, t, *J* = 7.0Hz, C**H$_2$**-COOH), 2.84 (2H, t, *J* = 7.1Hz, S-C**H$_2$**), 3.66 (1H, q, *J* = 7.0Hz), 4.28 (1H, d, *J* =

19.4Hz, 21-H), 4.66 (1H, d, *J* = 19.4Hz, 21-H). ^{13}C NMR: δ, 21.4, 22.1, 26.0, 26.2 (S-CH_2), 33.1 (19-CH_3), 35.4, 38.1, 38.4, 39.5, 46.3, 51.7, 53.3, 54.1, 56.1, 60.2, 71.1, 72.1, 93.2, 130.5, 179.6 (carboxylic acid), 182.9 (17-C), 200.8 (20-carbonyl), 216.9 (3-carbonyl). ES-MS (40 V, MeOH): m/z 466.1 $[M+H]^+$, 488.0 $[M+Na]^+$.

2,5-Dioxo-1-pyrrolidinyl-3-(11β,17α,21-trihydroxypregn-4-ene-3,20-dione-4-yl)thiopropanoate (**3**). **2** (479.9 mg, 1.029 mmol) was dissolved in dry dimethylformamide (DMF) (4 mL, dried over molecular sieves) and dicyclohexylcarbodimide (275.9 mg, 1.337 mmol, in 1 mL dry DMF) was added dropwise to the stirring steroid solution. This was followed by *N*-hydroxysuccinimide (153.9 mg, 1.337 mmol, in 1 mL dry DMF) also added dropwise. The reaction was stirred overnight at room temperature in the dark. The white solid formed was then filtered off and washed with dry DMF and the filtrate solvent removed *in vacuo*. The sample was then column purified using chloroform, 15:1 chloroform: methanol, 10:1 chloroform: methanol to yield **3** (486.9 mg, 84%) as a pale yellow semi-solid. R_f = 0.69 (5:1 chloroform: methanol). Mp: 139-142 ^oC. HPLC: 30% methanol, R_t = 1.86 min, % Purity = 90%. IR (KBr disc): 1078, 1655, 1736, 2928 cm^{-1}. ^{1}H NMR: δ 0.90 (3H, s, 18-CH_3), 1.50 (19-CH_3), 2.64 (2H, t, *J* = 6.8Hz), 2.83 (2H, t, *J* = 6.5Hz), 2.88 (4H, d, *J* = 1.2 Hz, NHS protons), 4.29 (1H, s, broad, 21-H). ^{13}C NMR: δ 16.9 (18-CH_3), 21.8, 23.8, 25.1, 25.8 (S-CH_2), 28.1, 30.6, 31.9, 33.1 (19-CH_3), 33.7, 34.0, 34.4, 39.4, 42.3, 47.7, 48.7, 52.0, 56.4, 68.0, 89.6, 125.6, 158.4, 167.7, 171.0, 179.6 (17-C), 196.4 (20-carbonyl), 206.8 (3-carbonyl). ES-MS (40 V, MeOH): m/z 695.7 $[M+DMF + 2H_2O + Na]^+$.

N-(t-Butoxycarbonyl)-4,7,10-trioxa-1,13-tridecanediamine (**4**). Synthesized according to previous method.[23]

N-(13-(t-butoxycarbonylamino)4,7,10-trioxatridecanyl)-3-(11β,17α,21-trihydroxypregn-4-ene-3,20-dione-4-yl)thiopropanimide (**5**)**. 3** (486.9 mg, 0.864 mmol) was dissolved in dry DMF (3.5 mL, dried over molecular sieves). To the stirring steroid solution, was added **4** (416.0 mg, 1.296 mmol, in 1.25 mL of dry chloroform (dried over molecular sieves)) dropwise, with an additional 2 x 250 μL of dry chloroform used to wash. The stirring solution had dry triethylamine added (750 μL, dried over molecular sieves). The reaction was then stirred at room temperature in the dark for 60 h. After 12 h, another 1 mL of dry DMF was added to aid solubility. The reaction was then stopped and solvent removed and sample dried *in vacuo* before column purification using chloroform, 15:1 chloroform: methanol and 10:1 chloroform: methanol as eluents, yielding **5** (413.6 mg, 62%) as a semi-solid. R_f = 0.32 (10:1 chloroform: methanol). Mp: 32-33 ^{o}C. HPLC: Purity: 99%. Methanol mobile phase, 1 mL/min. R_t = 1.92 min, λ_{max} = 206 nm. IR (KBr disc) 1707, 2930, 3437 cm^{-1}. ^{1}H NMR: δ 0.90 (3H, s, 18-CH_3), 1.43 (9H, s, Boc methyls), 1.50 (3H, s, 19-CH_3), 1.71-1.78 (6H, m, 4H from O-CH_2-C**H$_2$**-CH_2-NH, 2H from steroid fine structure), 2.60 (2H, m, C**H$_2$**-COOH), 2.82 (2H, m, C**H$_2$**-S), 3.11 (2H, t, *J* = 6.6Hz, CH_2-CO-NH-C**H$_2$**), 3.26 (2H, m, C**H$_2$**-NH-CO), 3.50-3.70 (14H, m, 12H from O-C**H$_2$**, 2H from steroid fine structure). ^{13}C NMR: δ 16.8 (18-CH_3), 21.5, 22.0, 25.6, 27.7, 27.9, 28.1, 28.3 and 28.6 (O-CH_2-**C**H_2-CH_2-NH), 29.5 (S-CH_2), 29.8 (CH_2), 30.3, 33.8 (19-CH_3), 34.5, 35.0, 37.9 (C), 42.4 (CH_2), 47.9, 48.1, 48.4, 48.6, 52.2, 52.4, 56.7, 69.0, 69.1, 69.8, 70.1 and 70.3 and 70.6 (**C**H_2-O), 79.0, 89.6, 126.1, 126.4,

157.3 (Boc terminal amide), 172.7 (steroid terminal amide), 178.9, 196.5 (3-carbonyl), 206.0 (20-carbonyl). ES-MS: (40 V, MeOH) m/z 385.4 $[M+2H]^{2+}$.

(13-Amino-4,7,10-trioxatridecanyl)-3-(11β,17α,21-trihydroxypregn-4-ene-3,20-dione-4-yl)thiopropanimide. (**6**). **5** (104.3 mg) was dissolved in formic acid (4 mL) and stirred for 4 h at room temperature in the dark. The solvent was removed *in vacuo* to yield an orange oil. $R_f = 0.13$ (10:1 methanol: Acetic Acid). ES-MS: (40 V, MeOH + formic acid) m/z 669.6 $[M+H]^+$, 718.1 $[M+H_2O+MeOH+H]^+$.

Abbreviations:

NMR, nuclear magnetic resonance; ppm, parts per million; J, coupling constant; ES-MS, electrospray mass-spectrometry; mp, melting point; HPLC, high performance liquid chromatography.

Discussion

A synthetic scheme is given in Figure S-1.

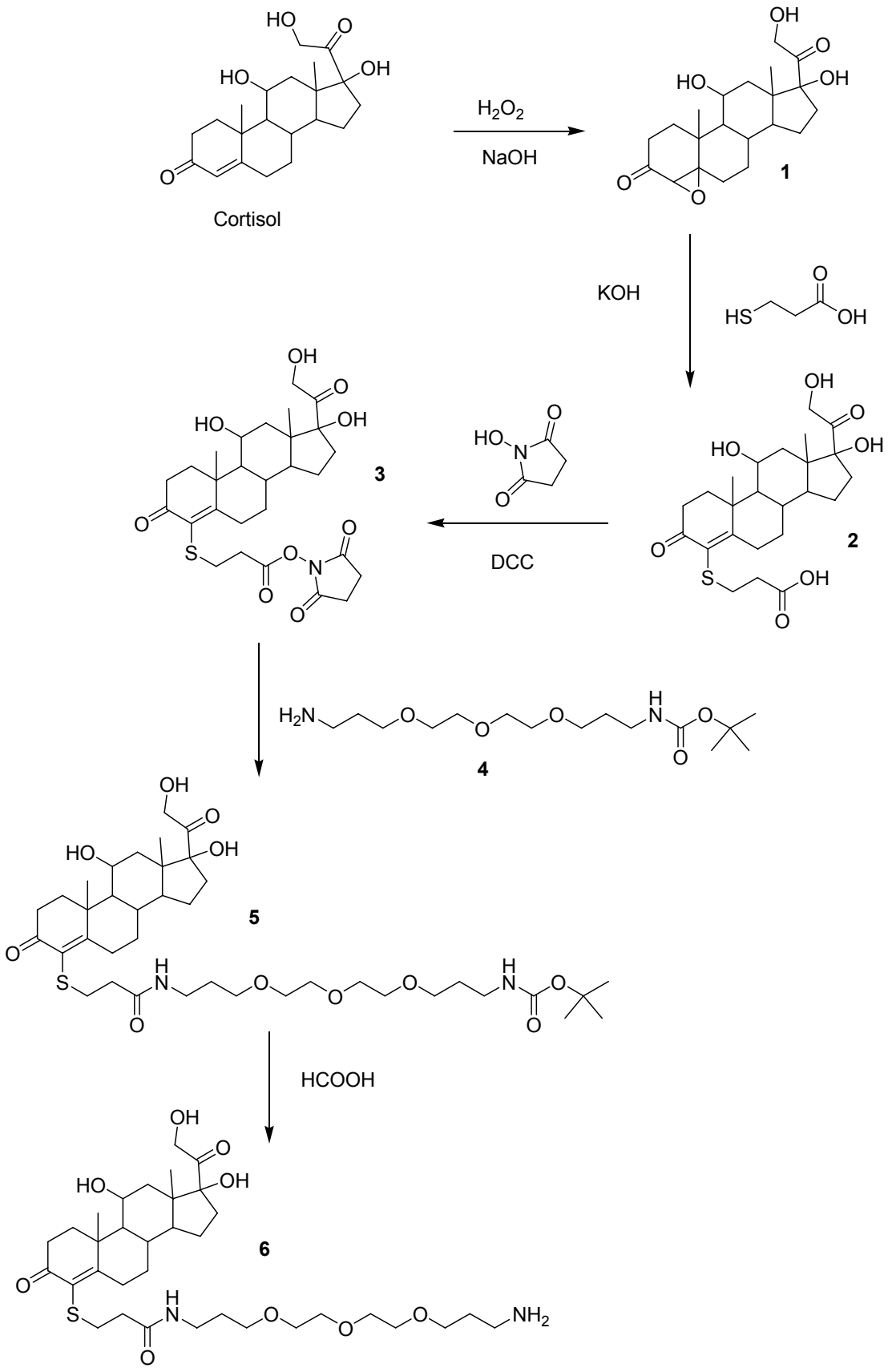

Figure S-1. Synthesis of the cortisol-linker derivative for immobilization on the SPR biosensor surface