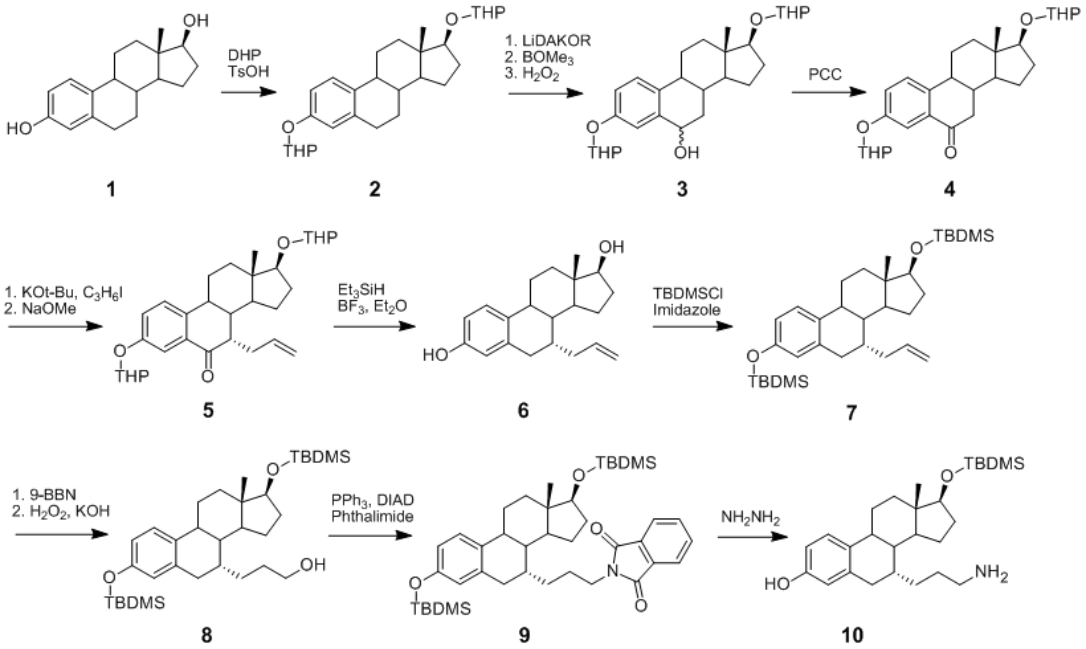
Supplementary material (ESI) for Molecular BioSystems

Supporting information



Scheme S.1. Synthesis of the C-7 derivatized estradiol

Experimental:

2: Estradiol (8.00 g, 29.4 mmol) was dissolved in CH_2Cl_2 (100 ml) and p-toluene sulfonic acid (TsOH) (56 mg, 0.29 mmol) was added followed by Dihydropyran (DHP) (13.3 ml, 146.8 mmol) and reaction was stirred at room temperature for 2 hours. After 2 hours water was added and product was extracted using ether. Column chromatography was performed to isolate THP protected products as clear oil (12 g, 94%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.79, 0.81 (2s, 3H), 2.83-2.87 (m, 2H), 3.47-3.52 (m, 1H), 3.57-3.61 (m, 1H), 3.72 (t, *J* = 8.5 Hz, 1H), 3.89-3.97 (m, 2H), 4.64 - 4.65 (m, 1H), 5.38 - 5.39 (m, 1H), 6.78 (d, J = 2.5 Hz, 1H), 6.84 (dd, J = 8.5, 2.5 Hz, 1H), 7.22 (d, J = 8 Hz, 1H)

3: To a -78 °C solution of 2.5 M *n*-BuLi in hexanes (30 mL, 75.0 mmol) in THF (50 mL) was added diisopropylamine (10.5 mL, 75.0 mmol), followed by 1 M KO*t*-Bu in THF (75.0 mL, 75.0 mmol) (yellow color change with addition of KO*t*-Bu). After 5 min, a solution of 2 (4.18 g, 9.49 mmol) in THF (20 mL) was added resulting in a dark red color reaction mixture which was stirred for 1.5 hours at -78 °C under N_2. The dry ice/acetone bath was replaced with an ice bath and trimethylborate ($BOMe_3$) (20 mL, 171.0 mmol) was slowly added. The reaction was stirred for an additional 2 hours at 0° C (reaction became turbid upon adition of trimethylborate). 35% H_2O_2 (25 mL) was then added and the reaction was stirred for 1 hour at room temperature after which time reaction was cooled to 0 °C, and 10% $Na_2S_2O_3$ (100 mL) was added. Product was extracted using EtOAc, dried over Na_2SO_4 and solvents were

removed. The crude mixture was dissolved in CH_2Cl_2 and flash chromatography was don (3:1, Hex:EtOAc) to afford the 6-OH compound as a pale yellow foam (2.86 g, 66%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.79, 0.81 (2s, 3H), 3.45-3.49 (m, 1H), 3.56 - 3.59 (m, 1H), 3.68 – 3.73 (m, 1H), 3.87-3.93 (m, 2H), 4.63 - 4.67 (m, 1H), 4.78 – 4.81 (m, 1H), 5.40 - 5.43 (m, 1H), 6.92 (2dd, *J* = 8.5, 2.5 Hz, 1H), 7.17, 7.18 (2d, *J* = 8 Hz, 1H), 7.26 (d, *J* = 2.5 Hz, 1H).

4: The 6-OH compound (2.86 g, 6.26 mmol) was dissolved in CH_2Cl_2 (30 mL) and cooled to 0 °C, PCC (2.7 g, 12.5 mmol) was then added in portions within 15 min. After 15 min at 0 °C, the mixture was warmed to room temperature and stirred for 2 hours. The reaction was diluted with ether (50 mL) and then filtered through Florisil to remove the chromium salts. The solvent was evaporated and the residue was dissolved in CH_2Cl_2 and purified via flash chromatography (3:1, Hex: EtOAc) to yield 4 as a white foam (2.28 g, 80%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.81, 0.82 (2s, 3H), 2.73 (dd, J = 16.5, 3.5 Hz, 1H), 3.47 – 3.52 (m, 1H), 3.58 – 3.62 (m, 1H), 3.73, 3.75 (2t, J = 8.5 Hz, 1H), 3.86-3.94 (m, 2H), 4.63 - 4.69 (m, 1H), 5.46 – 5.48 (m, 1H), 7.21 – 7.26 (2dd, *J* = 8.5, 3 Hz, 1H), 7.33, 7.34 (2d, *J* = 8.5 Hz, 1H), 7.71, 7.72 (2d, *J* = 3 Hz, 1H).

5: Ketone (1.91 g, 4.20 mmol) was dissolved in dry THF and cooled to 0° C, then 1M KO*t*-Bu (4.6 ml, 4.60 mmol) was added and reaction was stirred under N_2 at 0 °C for 30 min and then cooled to -78°C. Allyl iodide (383 μL, 4.60 mmol) was then added dropwise to the solution and after 10 min the reaction was quenched with water and warmed to room temperature. The solvents were removed, redissolved in ether, and then passed through a plug of silica. After solvent was evaporated, the residue was dissolved in MeOH (25 mL), and several small pieces of sodium were added. The mixture was stirred for 2 hours at room temperature, and then quenched with water, the MeOH was evaporated, and the product was extracted from water with ether. The solvents were evaporated and the residue was dissolved in CH_2Cl_2 and purified by flash chromatography (5:1 Hex:EtOAc) to give (5) as a white foam (272 mg, 13%), recovered (4) 896 mg, corrected yield 25%. This process was repeated two more times with recovered starting material to provide a total (575 mg, overall uncorrected 28%, corrected 36%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.80, 0.82 (2s, 3H), 3.47 – 3.52 (m, 1H), 3.60 – 3.62 (m, 1H), 3.74, 3.77 (2t, *J* = 8.5 Hz, 1H), 3.87-3.94 (m, 2H), 4.63 - 4.69 (m, 1H), 4.92 – 5.00 (m, 1H), 5.45 – 5.48 (m, 1H), 5.74 – 5.82 (m, 1H), 7.22 (2dd, *J* = 8.5, 2.5 Hz, 1H), 7.32, 7.34 (2d, *J* = 8.5 Hz, 1H), 7.69 (d, *J* = 2 Hz, 1H).

6: Triethylsilane (Et_3SiH) (4.37 ml) was added to a solution of (5) (205 mg, 0.41 mmol) in CH_2Cl_2 and the mixture was cooled to 0 °C. $BF_3.Et_2O$ (15 ml) was added drop-wise and the mixture was warmed to room temp and stirred overnight (greenish-yellow color change). Reaction was then carefully hydrolyzed with 10% K_2CO_3 (72 ml) and filtered through a buccner funnel. The filtrate was extracted with CH_2Cl_2, dried over Na_2SO_4 and flash chromatography performed (1:1, Hex:EtOAc) to yield a white solid (116 mg, 90%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ

0.79 (s, 3H), 2.72 (d, *J* = 16 Hz, 1H), 2.83 (dd, *J* = 17, 5.5 Hz, 1H), 3.77 (t, *J* = 8.5 Hz, 1H), 4.91 - 5.00 (m, 1H), 5.74 – 5.82 (m, 1H), 6.54 (d, *J* = 3 Hz, 1H), 6.64 (dd, *J* = 8.5, 2.5 Hz, 1H), 7.15 (d, *J* = 8.5 Hz, 1H).

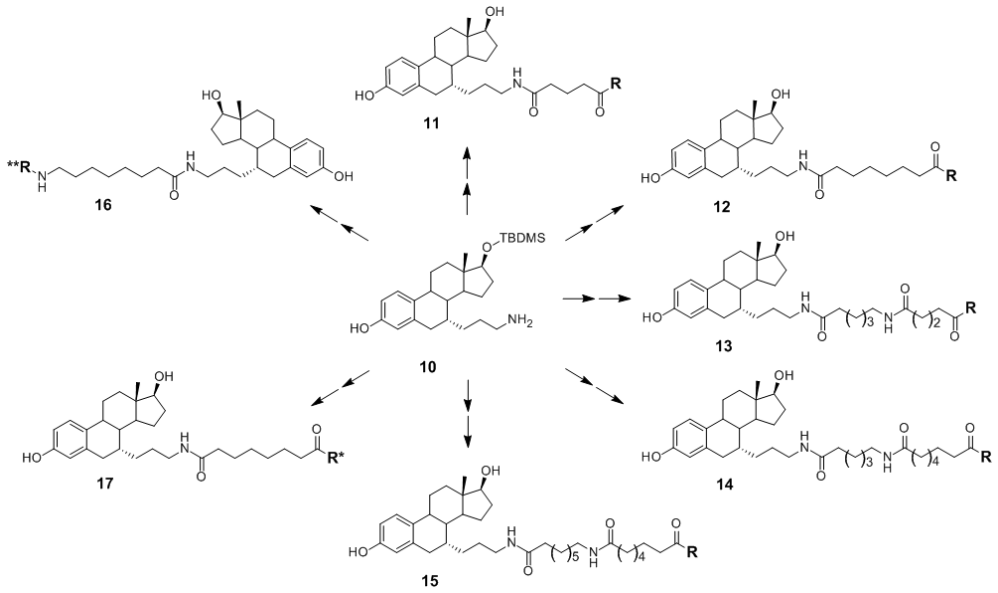
7: Imidazole (303 mg, 4.45 mmol) in CH_2Cl_2 and DMF (10 ml and 2 mL) was cooled to 0 °C for about ten mins, and then TBSCl (335 mg, 2.23 mmol) was added. The reaction was warmed to room temperature and a solution of (6) (116 mg, 0.37 mmol in 3 ml DMF) was added to the mixture. The reaction was stirred overnight at room temeprature. CH_2Cl_2 was removed via roto-vap and DMF via high vacuum, 0.1% K_2CO_3 (30 mL) was added and mixture extracted with CH_2Cl_2. The organic fractions were dried over Na_2SO_4 and passed through a short column (3:1 Hex:EtOAc) to yield (7) as a yellow oil (170 mg, 85%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.03 (s, 3H), 0.04 (s, 3H), 0.18 (s, 6 H), 0.75 (s, 3H), 0.89 (s, 9H), 0.97 (s, 9H), 2.70 (d, *J* = 16 Hz, 1H), 2.83 (dd, *J* = 17, 5.5 Hz, 1H), 3.65 (t, *J* = 8.5 Hz, 1H), 4.88 – 4.98 (m, 1H), 5.74 – 5.82 (m, 1H), 6.52 (d, *J* = 2.5 Hz, 1H), 6.61 (dd, *J* = 8.5, 2.5 Hz, 1H), 7.11 (d, *J* = 8.5 Hz, 1H).

8: A 0.5 M solution of 9-BBN in THF (3.14 mL, 1.57 mmol) was added to a solution of steroid (7) (170 mg, 0.31 mmol) in THF (10 mL). After stirring overnight at room temperature the reaction was cooled to 0 °C and quenched with 3 M KOH (2 mL), followed by 35% H_2O_2 (2 mL) after 5 min. The reaction was stirred for an addidional 3 h and saturated NaHCO3 was added. The mixture was extracted with CH_2Cl_2. The organic fractions were dried over Na_2SO_4 and evaporated in vacuo, and the residue was purified by flash chromatography (5:1 Hex:EtOAc) to provide (8) as a viscous oil (116 mg, 66%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.02 (s, 3H), 0.03 (s, 3H), 0.19 (s, 6 H), 0.74 (s, 3H), 0.89 (s, 9H), 0.97 (s, 9H), 2.68 (d, *J* = 16 Hz, 1H), 2.88 (dd, *J* = 16.7, 5.5 Hz, 1H), 3.58 – 3.60 (m, 2H), 3.65 (t, *J* = 8.5 Hz, 1H), 6.53 (d, *J* = 2.5 Hz, 1H), 6.61 (dd, *J* = 8.5, 2.5 Hz, 1H), 7.11 (d, *J* = 8.5 Hz, 1H).

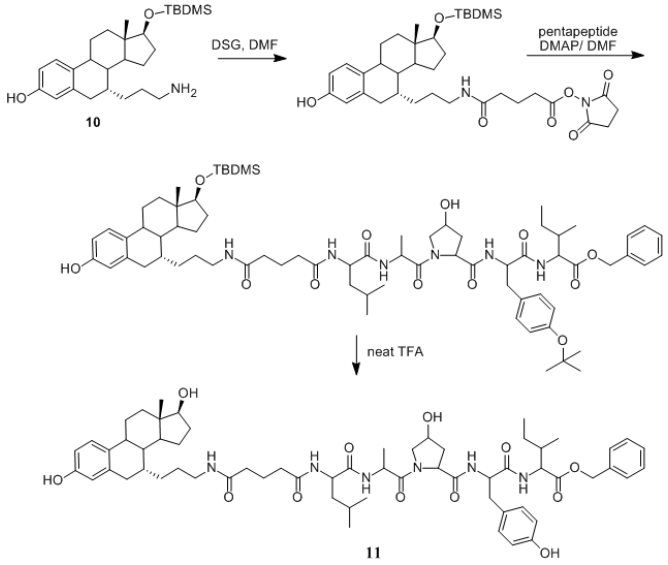
9: To a cooled solution (0 °C) of PPh3 (97 mg, 0.415 mmol) in THF (5 mL) was added DIAD (dropwise) (77 µl, 0.415 mmol). A white precipitate of the ylide was observed, and the reaction was stirred for 40 min at 0 °C. A solution of (8) (116 mg, 0.207 mmol) and phthalimide (58 mg, 0.415 mmol) in THF (2 mL) was then added to the ylide. The reaction was stirred for 1 h at 0 °C and then at room temperature overnight. The solvent was evaporated in vacuo, and the residue was purified via flash chromatography (5:1 Hex:EtOAc) to give (9) as a white foam (118 mg, 83%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.03 (s, 3H), 0.04 (s, 3H), 0.18 (s, 6 H), 0.74 (s, 3H), 0.89 (s, 9H), 0.99 (s, 9H), 2.67 (d, *J* = 16.5 Hz, 1H), 2.87 (dd, *J* = 17, 5 Hz, 1H), 3.55 – 3.68 (m, 3H), 6.52 (d, J = 2.5 Hz, 1H), 6.60 (dd, *J* = 8.5, 2.5 Hz, 1H), 7.11 (d, *J* = 8.5 Hz, 1H), 7.69 – 7.72 (m, 2H), 7.81 – 7.83 (m, 2H).

10: Anhydrous hydrazine (800 μl) was added to a solution of (9) (118 mg, 0.171 mmol) in DME (1.6 mL) and EtOH (1.6 mL). The mixture was refluxed for 2 h, during which time slightly brown precipitate formed on the sides and the solution turned slightly green. The reaction was cooled, and 5% NaOH (1.6 mL) was added, dissolving the precipitate. After 30 min, water was added, and the solution was extracted with CH_2Cl_2, dried over Na_2SO_4. The

solvent was evaporated in vacuo, and the residue was purified via flash chromatography (CH_2Cl_2: MeOH, 95:5) to give 10 as a white foam solid (56.7 mg, 76%). The TBDMS group at the 3-OH position is lost during this reaction. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.02 (s, 3H), 0.03 (s, 3H), 0.74 (s, 3H), 0.89 (s, 9H), 2.68 (d, *J* = 16.5 Hz, 1H), 2.88 (dd, *J* = 17, 5 Hz, 1H), 3.65 (t, J = 8.5 Hz, 1H), 6.50 (d, *J* = 2.5 Hz, 1H), 6.59 (dd, *J* = 8.5, 2.5 Hz, 1H), 7.11 (d, *J* = 8.5 Hz, 1H)



Scheme S.2. Synthesis of PROTACs based on estradiol C-7 derivative



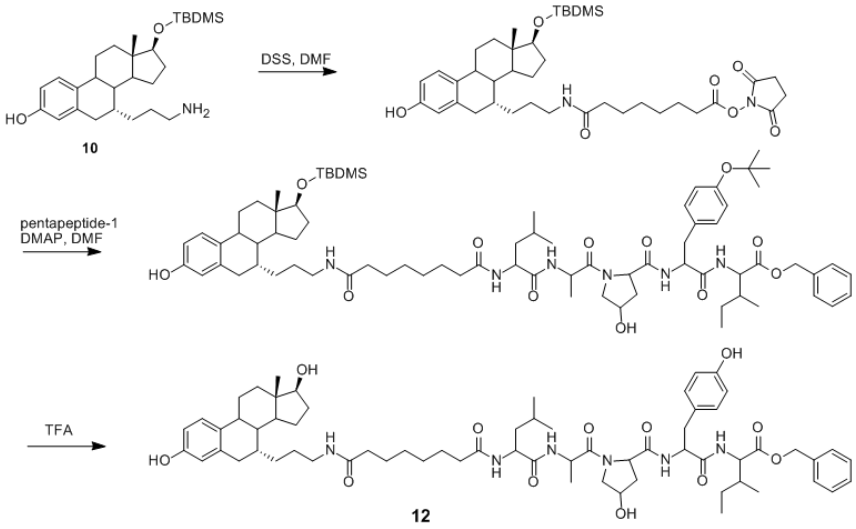
Scheme S.3. Synthesis of compound **11**

Disuccinimidyl gluterate DSG (54 mg, 0.166 mmol) was added to free NH_2, **10** (37 mg, 0.083 mmol) and dissolved in DMF (2 ml) mixture was stirred at room temp overnight. DMF was removed via high vacuum and column was

performed CH_2Cl_2: MeOH 95:5) to give a white solid (25 mg, 46%). For this reaction two products were isolated having similar mobility on TLC. The upper product which was later identified by NMR to be the correct product was reactive while the other isomer was unreactive in subsequent reactions. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.03 (s, 3H), 0.04 (s, 3H), 0.73 (s, 3H), 0.89 (s, 9H), 2.52 (t, *J* = 7 Hz, 2H), 2.63 (d, *J* = 16.5 Hz, 1H), 2.83 (s, 4H), 3.18 – 3.26 (m, 2H), 3.65 (t, *J* = 8.5 Hz, 1H), 5.92 (t, *J* = 5.5 Hz, 1H), 6.50 (d, *J* = 2.5 Hz, 1H), 6.62 (dd, *J* = 8.5, 2.5 Hz, 1H), 7.11 (d, *J* = 8.5 Hz, 1H).

Pentapeptide-1 (24 mg, 0.009 mmol) was added to the resulting compound (34 mg, 0.009 mmol) and dissolved in DMF (1.5 ml). The reaction was stirred at room temp overnight. Solvent was removed *in vacuo* and column chromatography was performed (95:5 CH_2Cl_2: MeOH) to yield a white solid (34 mg, 70%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.03 (s, 3H), 0.04 (s, 3H), 0.74 (s, 3H), 0.77 – 0.85 (m, 6H), 0.87 – 0.94 (m, 6H), 0.89 (s, 9H), 1.30 (s, 9H), 2.63 (d, *J* = 16 Hz, 1H), 2.77 (s, 1H), 2.83 (dd, *J* = 17, 4.5 Hz, 1H), 2.93 – 3.10 (m, 3H), 3.57 (m, 1H), 3.64 (t, *J* = 8.5 Hz, 1H), 3.75 (d, *J* = 11 Hz, 1H), 4.36 – 4.67 (m, 6H), 5.11 (s, 2H), 6.53 (d, *J* = 2.5 Hz, 1H), 6.62 (dd, *J* = 8.5, 2.5 Hz, 1H), 6.74 (t, *J* = 5.5 Hz, 1H), 6.84 (d, *J* = 8 Hz, 2H), 7.04 (d, *J* = 8.5 Hz, 2H), 7.09 (d, *J* = 8.5 Hz, 1H), 7.16 (d, *J* = 7.5 Hz, 1H), 7.28 – 7.33 (m, 4H), 7.55 (d, *J* = 7.5 Hz, 1H), 7.59 (d, *J* = 7 Hz, 1H).

11: t-Butyl from Tyr-OH and TBDMS protecting group on O-17 position was simultaneously removed from 12 (34 mg, 0.027 mmol) using neat TFA (2 ml) and stirring at room temperature until starting material disappeared ~30 min. After removal of TFA via high vacuum column chromatography was performed (CH_2Cl_2: MeOH 95:5) initially, then MeOH to isolate **11** as a white solid (13.8 mg, 47%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.75 (s, 3H), 0.82 – 0.96 (m, 12H), 2.63 (d, *J* = 17 Hz, 1H), 3.19 (m, 1H), 3.49 (m, 1H), 3.57 (m, 1H), 3.68 (t, *J* = 8.5 Hz, 1H), 4.32 – 4.38 (m, 2H), 4.49 – 4.55 (m, 4H), 5.12 (s, 2H), 6.53 (d, *J* = 2.5 Hz, 1H), 6.62 (dd, *J* = 8.5, 2.5 Hz, 1H), 6.72 (d, *J* = 8.5 Hz, 2H), 7.00 (d, *J* = 8.5 Hz, 2H), 7.08 (d, *J* = 8.5 Hz, 1H), 7.28 (d, *J* = 8 Hz, 1H), 7.31 – 7.34 (m, 4H), 7.51 (d, *J* = 7 Hz, 1H), 7.71 (2d, *J* = 7 Hz, 2H). MS (MALDI, DHB) *m/z* 1130 ($M+Na^+$, calcd for $C_{62}H_{86}N_6O_{12}$ requires 1129.63).



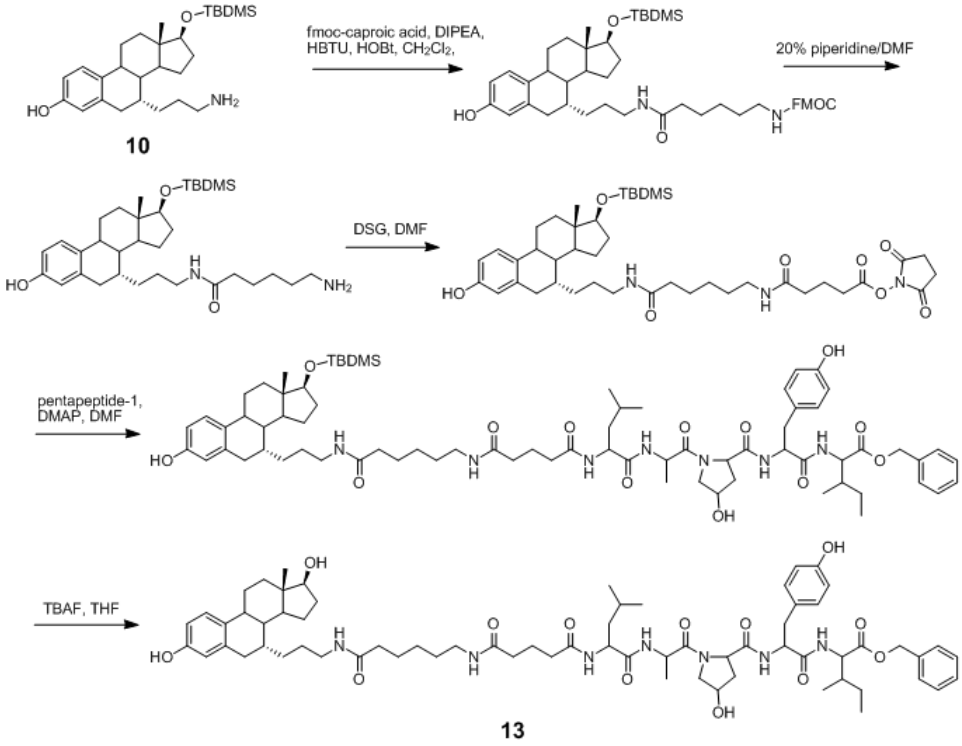
Scheme S.4. Synthesis of compound **12**

Disuccinimidyl suberate DSS (12 mg, 0.033 mmol) was added to free NH_2, **10** (14.6 mg, 0.033 mmol) and dissolved in DMF (1ml) mixture was stirred at room temp overnight. DMF was removed via high vacuum and column was performed (95:5 CH_2Cl_2: MeOH) to give a white solid (5.7 mg, 25%). For this reaction two products were isolated having similar mobility on TLC. The upper product which was later identified by NMR to be the correct product was reactive while the other isomer was unreactive in subsequent reactions. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.03 (s, 3H), 0.04 (s, 3H), 0.73 (s, 3H), 0.89 (s, 9H), 2.12 (t, *J* = 7.5 Hz, 2H), 2.58 (t, *J* = 7.5 Hz, 2H), 2.65 (d, *J* = 16.5 Hz, 1H), 2.82 – 2.87 (m, 5H), 3.08 – 3.12 (m, 1H), 3.24 – 3.29 (m, 1H), 3.65 (t, *J* = 8.5 Hz, 1H), 5.76 (t, *J* = 5.5 Hz, 1H), 6.53 (d, *J* = 2.5 Hz, 1H), 6.61 (s, 1H), 6.64 (dd, *J* = 8.5, 2.5 Hz, 1H), 7.11 (d, *J* = 8.5 Hz, 1H).

Pentapeptide-1 (6 mg, 0.008 mmol) was added to the resulting compound (5.7 mg, 0.008 mmol) and dissolved in DMF (1.5 ml). The reaction was stirred at room temp overnight. Solvent was removed in vacuo and column chromatography was performed (95:5 CH_2Cl_2: MeOH) to yield a white solid (6.6 mg, 66%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.03 (s, 3H), 0.04 (s, 3H), 0.74 (s, 3H), 0.79 – 0.85 (m, 6H), 0.87 – 0.95 (m, 6H), 0.89 (s, 9H), 1.13 – 1.33 (m, 13H), 1.31 (s, 9H), 2.63 (d, *J* = 16.5 Hz, 1H), 2.83 (dd, *J* = 16.5, 4.5 Hz, 1H), 2.97 – 3.10 (m, 3H), 3.23 – 3.27 (m, 1H), 3.56 (dd, *J* = 11.1, 3.9 Hz, 1H), 3.64 (t, *J* = 8.5 Hz, 1H), 3.75 (d, *J* = 11 Hz, 1H), 4.47 – 4.62 (m, 6H), 5.11 (2s, 2H), 6.53 (d, *J* = 2.5 Hz, 1H), 6.62 (dd, *J* = 8.5, 2.5 Hz, 1H), 6.66 (d, *J* = 8 Hz, 1H), 6.84 (d, *J* = 8.5 Hz, 2H), 7.05 (d, *J* = 8.5 Hz, 2H), 7.08 (d, *J* = 8.5 Hz, 1H), 7.30 – 7.37 (m, 4H), 7.51 (d, *J* = 7 Hz, 1H).

12: The resulting white solid (6.6 mg, 0.005 mmol) was dissolved in THF (0.5 ml) and TBAF in THF (3 drops) was added to remove TBDMS protecting group from O-17 position. Mixture was stirred at room temperature until starting material disappeared (5 days). Column chromatography was performed (CH_2Cl_2: MeOH 95:5) initially, then flushed with MeOH to isolate **12** as a white solid (2.5 mg, 42%). In subsequent reactions the OH on tyrosine

was protected and TFA was used to remove both protecting groups (TBDMS and t-Bu). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.74 (s, 3H), 0.79 – 0.85 (m, 6H), 0.87 – 0.95 (m, 6H), 2.63 (d, *J* = 16.5 Hz, 1H), 2.83 (dd, *J* = 16.5, 4.5 Hz, 1H), 2.97 – 3.10 (m, 3H), 3.23 – 3.27 (m, 1H), 3.53 (dd, *J* = 11.1, 3.9 Hz, 1H), 3.64 (t, *J* = 8.5 Hz, 1H), 3.75 (d, *J* = 11 Hz, 1H), 4.36 – 4.54 (m, 6H), 5.14 (s , 2H), 6.53 (d, *J* = 2.5 Hz, 1H), 6.62 (dd, *J* = 8.5, 2.5 Hz, 1H), 6.74 (d, *J* = 8 Hz, 1H), 7.02 (d, *J* = 8.5 Hz, 2H), 7.12 (d, *J* = 8.5 Hz, 1H), 7.25 (d, *J* = 8 Hz, 1H), 7.38 (s, 4H), 7.48 (d, *J* = 7.5 Hz, 1H), 7.54 (d, *J* = 7 Hz, 1H). MS (MALDI, DHB) *m/z* 1172 ($M+Na^+$, calcd for $C_{65}H_{92}N_6O_{12}$ requires 1171.68).



Scheme S.5. Synthesis of compound **13**

Fmoc-6-aminohexanoic acid (6-Ahx-OH/caproic acid) (13.4 mg, 0.038 mmol) was added to free NH_2, **10**, (17 mg, 0.038 mmol) dissolved in CH_2Cl_2. HBTU (21.6 mg, 0.057 mmol) and HoBt (8.73 mg, 0.057 mmol) was added to the mixture followed by DIPEA (2 drops) and mixture was stirred at room temp until all free NH_2 was coupled (15 min-1.5 hr). Column chromatography was performed (CH_2Cl_2: MeOH 99:1) yielding a white foamy solid (27 mg, 91%). A small amount of di-coupled product was isolated as well (5%). In some coupling reactions EDC was used as a coupling agent instead of HBTU providing cleaner reaction (only mono-coupled) product, but lower yields. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.02 (s, 3H), 0.03 (s, 3H), 0.73 (s, 3H), 0.89 (s, 9H), 2.62 (d, *J* = 16.5 Hz, 1H), 2.85 (dd, *J* = 16.5, 4.7 Hz 1H), 3.04 – 3.10 (m, 1H), 3.14 (q, *J* = 6.5 Hz, 2H), 3.29 – 3.34 (m, 1H), 3.64 (t, *J* = 8.3 Hz, 1H), 4.21 (t, *J* = 7 Hz, 1H), 4.41 (d, *J* = 7 Hz, 2H), 4.97 (t, *J* = 6 Hz, 1H), 5.47 (t, *J* = 6 Hz, 1H), 6.53 (d, *J* = 2.5 Hz, 1H), 6.62 (dd, *J* = 8.5,

2.5 Hz, 1H), 7.10 (d, *J* = 8.5 Hz, 1H), 7.30 – 7.33 (td, *J* = 7.5, 1.2 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.59 (d, *J* = 7.5 Hz, 2H), 7.76 (d, *J* = 7.5 Hz, 2H).

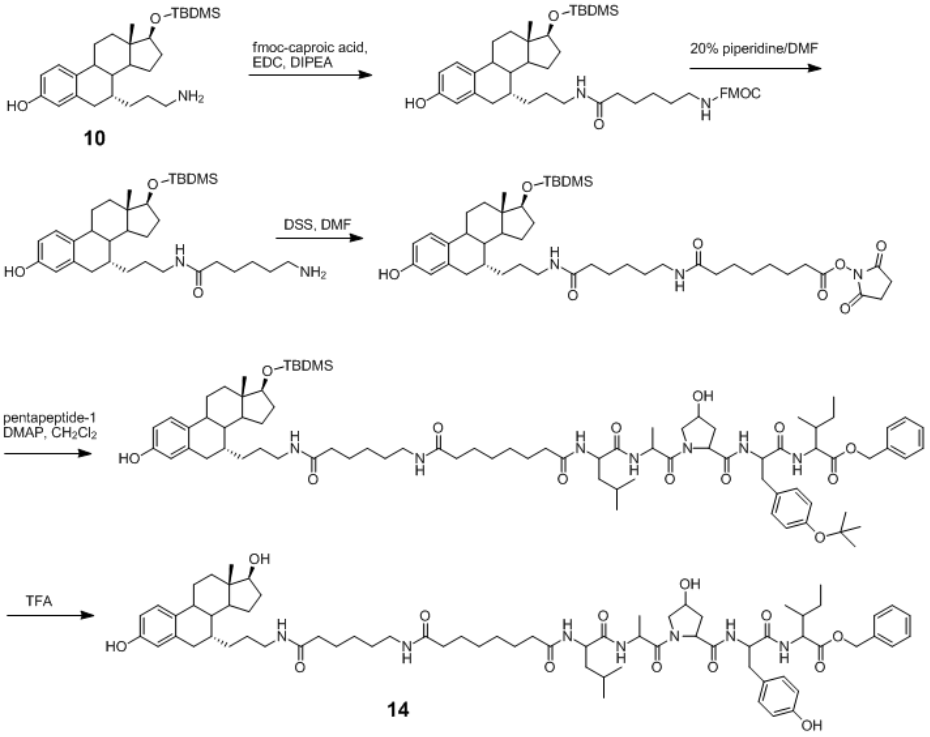
Fmoc from the resulting compound was deprotected using 20% piperidine in DMF following removal of solvents in vacuo. Flash column chromatography was performed using (CH_2Cl_2: MeOH 95:5) initially to remove by products and finally flushing with MeOH to isolate a white solid (14 mg, 73%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.03 (s, 3H), 0.04 (s, 3H), 0.74 (s, 3H), 0.89 (s, 9H), 2.60 (d, *J* = 16.5 Hz, 1H), 2.87 (dd, *J* = 16.5, 4.7 Hz 1H), 3.36 – 3.48 (m, 4H), 3.65 (t, *J* = 8 Hz, 1H), 5.50 (t, *J* = 5.4 Hz, 1H), 6.49 (d, *J* = 2.5 Hz, 1H), 6.62 (dd, *J* = 8.5, 2.5 Hz, 1H), 7.11 (d, *J* = 8.5 Hz, 1H).

Disuccinimidyl gluterate DSG (16.4 mg, 0.050 mmol) was added to the resulting compound (14 mg, 0.025 mmol) and dissolved in DMF (1.5 ml) the mixture was stirred at room temp 15 min. DMF was removed via high vacuum and column was performed 95:5 CH_2Cl_2: MeOH 95:5) to remove DSG and then (95:5 CH_2Cl_2: MeOH) to give a white solid (7 mg, 37%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.03 (s, 3H), 0.04 (s, 3H), 0.74 (s, 3H), 0.89 (s, 9H), 2.60 (d, *J* = 16.5 Hz, 1H), 2.31 (t, *J* = 7 Hz, 2H), 2.67 (t, *J* = 7 Hz, 2H), 2.87 (s, 4H), 3.00 – 3.05 (m, 1H), 3.18 – 3.24 (m, 2H), 3.31 – 3.35 (m, 1H), 3.65 (t, *J* = 8 Hz, 1H), 5.69 (t, *J* = 5.5 Hz, 1H), 6.39 (t, *J* = 5.5 Hz, 1H), 6.56 (d, *J* = 2.5 Hz, 1H), 6.64 (dd, *J* = 8.5, 2.5 Hz, 1H), 7.11 (d, *J* = 8.5 Hz, 1H).

Pentapeptide-1 (10 mg, 0.013 mmol) was added to the resulting compound (7 mg, 0.009 mmol) with catalytic amount of DMAP and dissolved in DMF (1.5 ml). The reaction was stirred at room temp overnight. Solvent was removed in vacuo and column chromatography was performed (95:5 CH_2Cl_2: MeOH) to yield a white solid (9 mg, 74%). ^{1}H NMR (DMSO, 500 MHz): δ 0.03 (s, 3H), 0.04 (s, 3H), 0.69 (s, 3H), 0.79 - 0.86 (m, 12H), 0.87 (s, 9H), 1.25 (s, 9H), 2.58 (d, *J* = 16.5 Hz, 1H), 3.66 (t, *J* = 8 Hz, 1H), 4.22 – 4.33 (m, 4H), 4.42 (t, *J* = 6.7 Hz, 1H), 4.47 – 4.51 (m, 1H), 5.11 (s, 2H), 6.42 (d, *J* = 2.5 Hz, 1H), 6.51 (dd, *J* = 8.5, 2.5 Hz, 1H), 6.82 (d, J = 8.5 Hz, 2H), 7.05 (d, *J* = 8.5 Hz, 1H), 7.11 (d, *J* = 8.5 Hz, 2H), 7.36 – 7.37 (m, 4H), 7.71 (t, *J* = 5.5 Hz, 1H), 7.74 (t, *J* = 5.5 Hz, 1H), 7.82 (d, *J* = 8 Hz, 1H), 7.92 (d, J = 8 Hz, 1H), 8.03 (t, *J* = 7 Hz, 1H).

13: The resulting compound (9 mg, 0.005 mmol) was dissolved in THF (0.5 ml) and TBAF in THF (0.5 ml) was added to remove TBDMS protecting group from O-17 position. Mixture was stirred at room temperature until starting material disappeared (48 hrs). Column chromatography was performed (CH_2Cl_2: MeOH 95:5) initially, then flushed with MeOH to isolate KC3 as a white solid (2.8 mg, 34%). In subsequent reactions the OH on tyrosine was protected and TFA was used to remove both protecting groups (TBDMS and t-Bu). ^{1}H NMR (DMSO, 500 MHz): δ 0.65 (s, 3H), 0.79 - 0.86 (m, 13H), 2.56 (d, *J* = 16.5 Hz, 1H), 2.75 (dd, *J* = 16.5, 4.5 Hz, 1H), 3.50 – 3.54 (m, 2H), 4.20 – 4.24 (m, 2H), 4.28 – 4.33 (m, 2H), 4.40 – 4.43 (m, 2H), 4.50 – 4.51 (m, 1H), 5.10 (s, 2H), 6.41 (d, *J* = 2.5 Hz, 1H), 6.49 (dd, *J* = 8.5, 2.5 Hz, 1H), 6.61 (d, *J* = 8.5 Hz, 2H), 6.98 (d, *J* = 8 Hz, 2H), 7.04 (d, *J* = 8.5 Hz, 1H), 7.36 (s, 4H), 7.71

(t, *J* = 5.5 Hz, 1H), 7.74 (t, J = 5.5 Hz, 1H), 7.77 (d, *J* = 8.5 Hz, 1H), 7.91 (d, J = 8 Hz, 1H), 8.00 (d, J = 8 Hz, 1H), 8.02 (d, *J* = 7 Hz, 1H). MS (MALDI, CHCA) *m/z* 1243 ($M+Na^+$, calcd for $C_{68}H_{97}N_7O_{13}$ requires 1242.71)



Scheme S.6. Synthesis of compound **14**

Fmoc-6-aminohexanoic acid (6-Ahx-OH/caproic acid) (37 mg, 0.104 mmol) was added to free NH_2, **10**, (46 mg, 0.104 mmol) dissolved in CH_2Cl_2. EDC (28 mg, 0.156 mmol) was added to the mixture followed by DIPEA (2 drops) and mixture was stirred at room temp until all free NH_2 was coupled (15 min-1.5 hr). Column chromatography was performed (CH_2Cl_2: MeOH 99:1) yielding a white foamy solid (61 mg, 75%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.02 (s, 3H), 0.03 (s, 3H), 0.73 (s, 3H), 0.89 (s, 9H), 2.62 (d, *J* = 16.5 Hz, 1H), 2.85 (dd, J = 16.5, 4.7 Hz 1H), 3.04 – 3.10 (m, 1H), 3.14 (q, J = 6.5 Hz, 2H), 3.29 – 3.34 (m, 1H), 3.64 (t, J = 8.3 Hz, 1H), 4.21 (t, J = 7 Hz, 1H), 4.41 (d, J = 7 Hz, 2H), 4.97 (t, J = 6 Hz, 1H), 5.47 (t, J = 6 Hz, 1H), 6.53 (d, J = 2.5 Hz, 1H), 6.62 (dd, J = 8.5, 2.5 Hz, 1H), 7.10 (d, *J* = 8.5 Hz, 1H), 7.30 – 7.33 (td, J = 7.5, 1.2 Hz, 2H), 7.40 (t, J = 7.5 Hz, 2H), 7.59 (d, J = 7.5 Hz, 2H), 7.76 (d, J = 7.5 Hz, 2H).

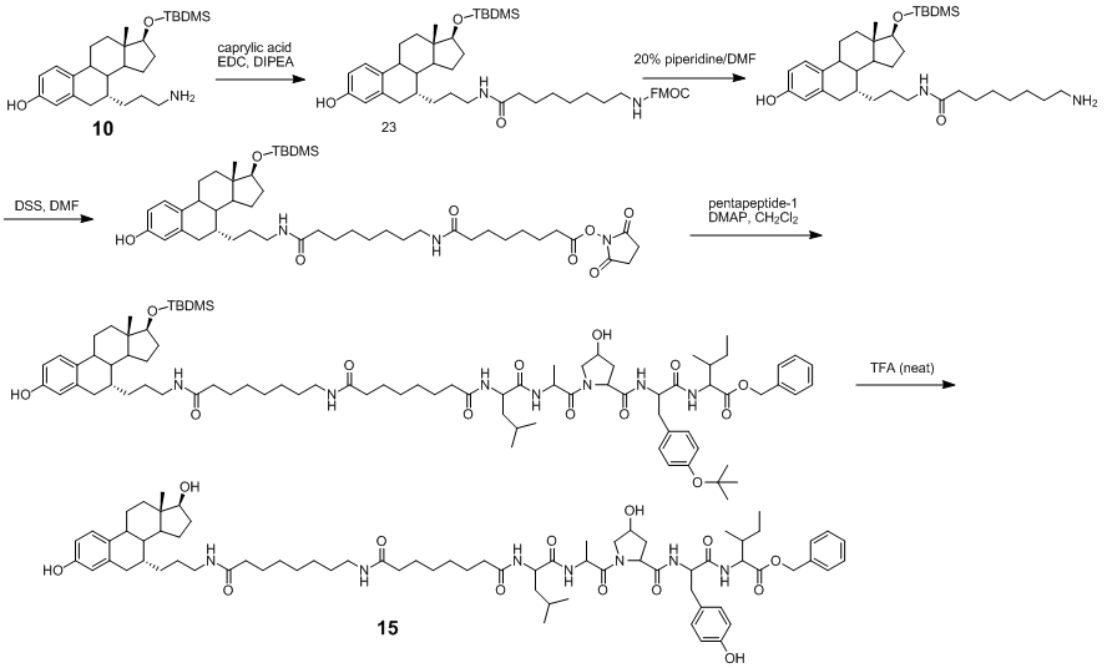
Fmoc from the white foamy compound was deprotected using 20% piperidine in DMF. Following removal of solvents in vacuo flash column chromatography was performed (short column) (CH_2Cl_2: MeOH 95:5) initially to remove by-products and finally flushing with MeOH to isolate white solid (39.5 mg, 91%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.03 (s, 3H), 0.04 (s, 3H), 0.74 (s, 3H), 0.89 (s, 9H), 2.60 (d, *J* = 16.5 Hz, 1H), 2.87 (dd, J = 16.5, 4.7 Hz 1H),

3.36 – 3.48 (m, 4H), 3.65 (t, J = 8 Hz, 1H), 5.50 (t, J = 5.4 Hz, 1H), 6.49 (d, J = 2.5 Hz, 1H), 6.62 (dd, J = 8.5, 2.5 Hz, 1H), 7.11 (d, *J* = 8.5 Hz, 1H).

DSS (52 mg, 0.142 mmol) was added to the resulting white solid (39.5 mg, 0.071 mmol) and dissolved in DMF (1.5 ml) the mixture was stirred at room temp overnight. DMF was removed via high vacuum and column was performed (99:1 CH_2Cl_2: MeOH) to give a white solid (17.6 mg, 31%). Coupling confirmed by negative Kaiser test.

Pentapeptide-1 (16 mg, 0.022 mmol) was added to the resulting white solid (17.6 mg, 0.022 mmol) with catalytic amount of DMAP and dissolved in CH_2Cl_2 (1.5 ml). The reaction was stirred at room temp overnight. Solvent was removed under reduced pressure and column chromatography was performed (95:5 CH_2Cl_2: MeOH) to yield a white solid (19 mg, 61%). ^{1}H NMR (500 MHz): δ 0.03 (s, 3H), 0.04 (s, 3H), 0.74 (s, 3H), 0.80 - 0.95 (m, 12H), 0.89 (s, 9H), 1.31 (s, 9H), 2.63 (d, J = 16.8 Hz, 1H), 2.85 (dd, J = 16.8, 4.2, 1H), 3.06 (d, J = 7.2 Hz, 3H), 3.13 – 3.27 (m, 4H), 3.55 (dd, J = 11.1, 3.6 Hz, 1H), 3.65 (t, J = 8 Hz, 1H), 4.40 – 4.43 (m, 2H), 4.48 – 4.56 (m, 4H), 4.47 – 4.51 (m, 1H), 5.12, (s, 2H), 6.35 (t, J = 5.7 Hz, 1H), 6.53 (d, *J* = 2.4 Hz, 1H), 6.63 (dd, J = 8.4, 2.4 Hz, 2H), 6.68 (t, J = 5.8 Hz, 1H), 6.86 (d, J = 8.4 Hz, 2H), 6.92 (d, J = 5 Hz, 1H), 6.95 (d, J = 5.7 Hz, 1H), 7.09 (d, J = 8.4 Hz, 1H), 7.10 (d, J = 8.4 Hz, 1H), 7.34 (s, 4H).

14: TBDMS and t-Bu protecting groups were removed simultaneously by adding neat TFA (1ml) to the product from the previous reaction (19 mg, 0.013 mmol) and stirring at room temperature until all starting material was gone (30 mim). TFA was removed in vacuo and column chromatography was performed (CH_2Cl_2: MeOH 95:5) to isolate **14** as a white solid (3.6 mg, 22%). 4 mg of product lacking the t-Bu group was also isolated, therefore corrected yield 27%. MS (MALDI, SA) *m/z* 1262.8 (M+H, calcd for $C_{71}H_{103}N_7O_{13}$ requires 1261.76).



Scheme S.7. Synthesis of compound **15**

Fmoc-caprylic acid (18 mg, 0.047 mmol) was added to free NH_2, **10**, (21 mg, 0.047 mmol) dissolved in CH_2Cl_2. EDC (13.5 mg, 0.070 mmol) was added to the mixture followed by DIPEA (2 drops) and mixture was stirred at room temp until all free NH_2 was coupled (15 min-1.5 hr). Column chromatography was performed (CH_2Cl_2: MeOH 99:1) yielding a white foamy solid (32 mg, 84%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.03 (s, 3H), 0.04 (s, 3H), 0.73 (s, 3H), 0.89 (s, 9H), 2.60 (d, *J* = 17 Hz, 1H), 2.85 (dd, J = 16.5, 4.7 Hz 1H), 3.04 – 3.10 (m, 1H), 3.12 (q, J = 7.4 Hz, 2H), 3.64 (t, J = 8.3 Hz, 1H), 4.17 (t, J = 7 Hz, 1H), 4.34 (d, J = 7 Hz, 2H), 5.00 (t, J = 6 Hz, 1H), 5.82 (t, J = 6 Hz, 1H), 6.51 (d, J = 2.5 Hz, 1H), 6.62 (dd, J = 8.5, 2.5 Hz, 1H), 7.06 (d, *J* = 8.5 Hz, 1H), 7.30 – 7.31 (td, J = 7.5, 1.0 Hz, 2H), 7.39 (t, J = 7.5 Hz, 2H), 7.56 (d, J = 7.5 Hz, 2H), 7.72 (d, J = 7.5 Hz, 2H).

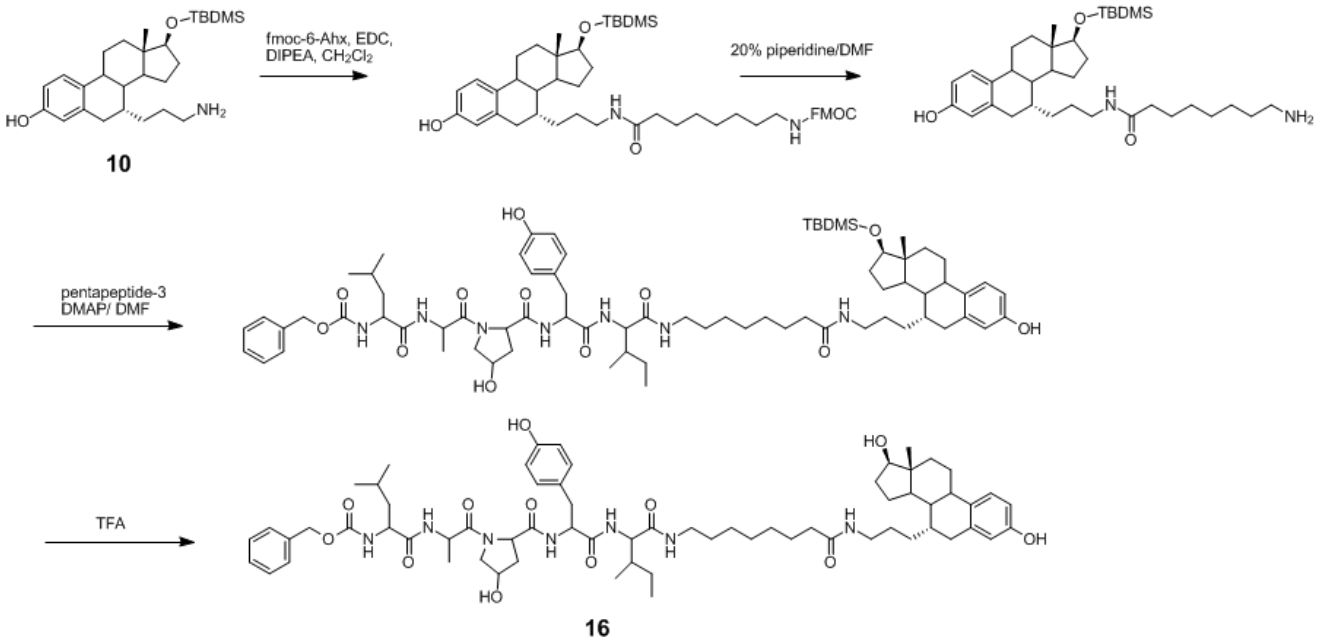
Fmoc from the above product (32 mg, 0.040 mmol) was removed using 20% piperidine in DMF. Following removal of solvents *in vacuo* flash column chromatography was performed (short column) (CH_2Cl_2: MeOH 95:5) initially to remove by-products and finally flushing with MeOH to isolate a white solid (13 mg, 56%). Deprotection confirmed by Kaiser test.

DSS (42 mg, 0.111 mmol) was added to the resulting white solid (13 mg, 0.022 mmol) and dissolved in DMF (1.5 ml) the mixture was stirred at room temp overnight. DMF was removed via high vacuum and column was performed (99:1 CH_2Cl_2: MeOH) to give a white solid (5.8 mg, 31%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.03 (s, 3H), 0.04 (s, 3H), 0.73 (s, 3H), 0.89 (s, 9H), 2.60 (t, *J* = 7.2 Hz, 1H), 2.84 (s, 2H), 3.21 – 3.28 (m, 2H), 3.64 (t, J = 8.3 Hz, 1H),

5.61(t, J = 5.7 Hz, 1H), 5.87 (t, J = 5.7 Hz, 1H), 6.55 (d, J = 2.5 Hz, 1H), 6.66 (dd, J = 8.5, 2.5 Hz, 1H), 7.11 (d, *J* = 8.5 Hz, 1H).

Pentapeptide-1 (5.2 mg, 0.007 mmol) was added to the resulting white solid (5.8 mg, 0.007 mmol) with catalytic amount of DMAP and dissolved in CH_2Cl_2 (1.5 ml). The reaction was stirred at room temp overnight. Solvent was removed under reduced pressure and column chromatography was performed (95:5 CH_2Cl_2: MeOH) to yield a white solid (4.8 mg, 48%). ^{1}H NMR (500 MHz): δ 0.03 (s, 3H), 0.04 (s, 3H), 0.74 (s, 3H), 0.80 - 0.95 (m, 12H), 0.89 (s, 9H), 1.31 (s, 9H), 2.63 (d, J = 16.8 Hz, 1H), 2.85 (dd, J = 16.8, 4.2, 1H), 3.06 (d, J = 7.2 Hz, 3H), 3.13 – 3.27 (m, 4H), 3.55 (dd, J = 11.1, 3.6 Hz, 1H), 3.65 (t, J = 8 Hz, 1H), 4.40 – 4.43 (m, 2H), 4.48 – 4.56 (m, 4H), 4.47 – 4.51 (m, 1H), 5.13 (s, 2H), 6.35 (t, J = 5.7 Hz, 1H), 6.53 (d, *J* = 2.4 Hz, 1H), 6.63 (dd, J = 8.4, 2.4 Hz, 2H), 6.68 (t, J = 5.8 Hz, 1H), 6.86 (d, J = 8.4 Hz, 2H), 6.92 (d, J = 5 Hz, 1H), 6.95 (d, J = 5.7 Hz, 1H), 7.09 (d, J = 8.4 Hz, 1H), 7.10 (d, J = 8.4 Hz, 1H), 7.34 (s, 4H).

15: TBDMS and t-Bu protecting groups were removed simultaneously by adding neat TFA (1ml) to the resulting white solid (4.8 mg, 0.003 mmol) and stirring at room temperature until all starting material was gone (30 mim). TFA was removed in vacuo and column chromatography was performed (CH_2Cl_2: MeOH 95:5) to isolate **15** as a white solid (0.8 mg, 19%). MS (MALDI, SA) *m/z* 1312 ($M+Na^+$, calcd for $C_{73}H_{107}N_7O_{13}$ requires 1312.79)



Scheme S.8. Synthesis of compound **16**

Fmoc-caprylic acid (29.2 mg, 0.077 mmol) was added to free NH_2, **10**, (34 mg, 0.077 mmol) dissolved in CH_2Cl_2. EDC (22 mg, 0.115 mmol) was added to the mixture followed by DIPEA (2 drops) and mixture was stirred at room temp until all free NH_2 was coupled (15 min-1.5 hr). Column chromatography was performed (CH_2Cl_2: MeOH 99:1) yielding a white foamy solid (53 mg, 62%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.03 (s, 3H), 0.04 (s, 3H), 0.73 (s, 3H), 0.89 (s, 9H), 2.62 (d, *J* = 17 Hz, 1H), 2.85 (dd, J = 16.5, 4.7 Hz 1H), 3.06 – 3.30 (m, 4H), 3.64 (t, J = 8.3 Hz, 1H), 4.20 (t, J = 7 Hz, 1H), 4.40 (d, J = 7 Hz, 2H), 4.91 (t, J = 5.5 Hz, 1H), 5.54 (t, J = 5.5 Hz, 1H), 6.53 (d, J = 2.5 Hz, 1H), 6.64 (dd, J = 8.5, 2.5 Hz, 1H), 7.10 (d, *J* = 8.5 Hz, 1H), 7.32 (m, 2H), 7.39 (t, J = 7.5 Hz, 2H), 7.58 (d, J = 7.5 Hz, 2H), 7.75 (d, J = 7.5 Hz, 2H).

Fmoc from the white solid product (53 mg, 0.065 mmol) was deprotected using 20% piperidine in DMF. Following removal of solvents in vacuo flash column chromatography was performed (short column) (CH_2Cl_2: MeOH 95:5) initially to remove by-products and finally flushing with MeOH to isolate a white solid (30 mg, 79%).

Pentapeptide-3 (17 mg, 0.023 mmol) was added to the white solid (13.6 mg, 0.023 mmol) and dissolved in DMF (1.5 ml). The reaction was stirred at room temp overnight. Solvent was removed in vacuo and column chromatography was performed (4:1 CH_2Cl_2: MeOH) to yield a white solid (17 mg, 56 %). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.03 (s, 3H), 0.04 (s, 3H), 0.73 (s, 3H), 0.89 (s, 9H), 2.62 (d, *J* = 17 Hz, 1H), 2.85 (dd, J = 16.5, 4.7 Hz 1H), 3.00 – 3.26 (m, 5H), 3.64 (t, J = 8.3 Hz, 1H), 4.20 – 4.25 (m, 2H), 4.22 – 4.56 (m, 3H), 5.10 (s, 2H), 6.53 (d, J = 2.5 Hz, 1H), 6.63 (dd, J = 8.5, 2.5 Hz, 1H), 6.74 (t, *J* = 8 Hz, 2H), 6.94 (d, J = 8 Hz, 1H), 7.00 (d, J = 8 Hz, 2H), 7.11 (d, J = 8 Hz, 1H), 7.22 (d, J = 8 Hz, 1H), 7.31(m, 2H), 7.89 (d, J = 6 Hz, 1H).

16: TBDMS protecting group was removed by adding neat TFA (1ml) to 29 (9.8 mg, 0.007 mmol) and stirring at room temperature for 30 mim. TFA was removed in vacuo and column chromatography was performed (CH_2Cl_2: MeOH 95:5) to isolate **16** as a white solid (6 mg, 67%). ^{1}H NMR ($CDCl_3$, 300 MHz): 0.77 (s, 3H), 2.64 (d, *J* = 17 Hz, 1H), 2.85 (dd, J = 16.5, 4.7 Hz 1H), 3.04 – 3.20 (m, 4H), 3.67 (t, J = 8.3 Hz, 1H), 4.20 – 4.23 (m, 2H), 4.37 – 4.56 (m, 3H), 5.10 (s, 2H), 5.90 (d, J = 8 Hz, 1H), 5.96 (d, J = 8.5 Hz, 1H), 6.16 (d, J = 6 Hz, 1H), 6.53 (d, *J* = 2.5 Hz, 1H), 6.64 (dd, J = 8.5, 2.5 Hz, 1H), 6.73 (t, J = 8.5 Hz, 2H), 6.92 (d, J = 8.5 Hz, 1H), 6.98 (d, J = 8.5 Hz, 1H), 7.12 (d, J = 8.5 Hz, 2H), 7.27 – 7.32 (m, 2H), 7.56 (d, J = 6 Hz, 1H), 7.79 (d, J = 7 Hz, 1H). MS (MALDI, CHCA) *m/z* 1200.7 ($M+Na^+$, calcd for $C_{66}H_{95}N_7O_{12}$ requires 1200.7)

Western blot quantification

The intensities of the bands on western blot films were quantified using volumetric densitometry (Quantity One, Bio-Rad). The estrogen receptor-alpha values were normalized to β-actin and DMSO was

arbitrarily assigned a value of 100% for comparison purposes. The results were graphed in GraphPad Prism (San Diego, USA) with means and standard deviations calculated from at least two independent experiments.

Fig 3

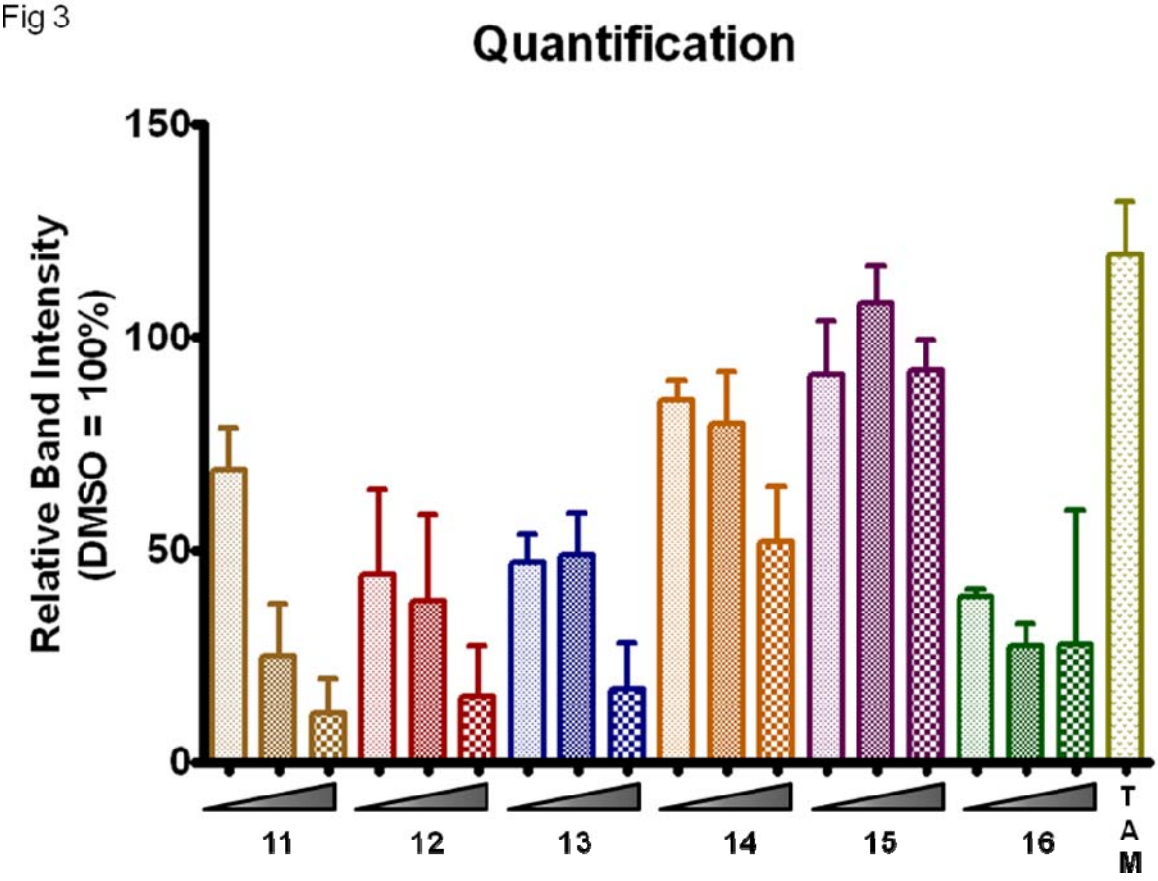
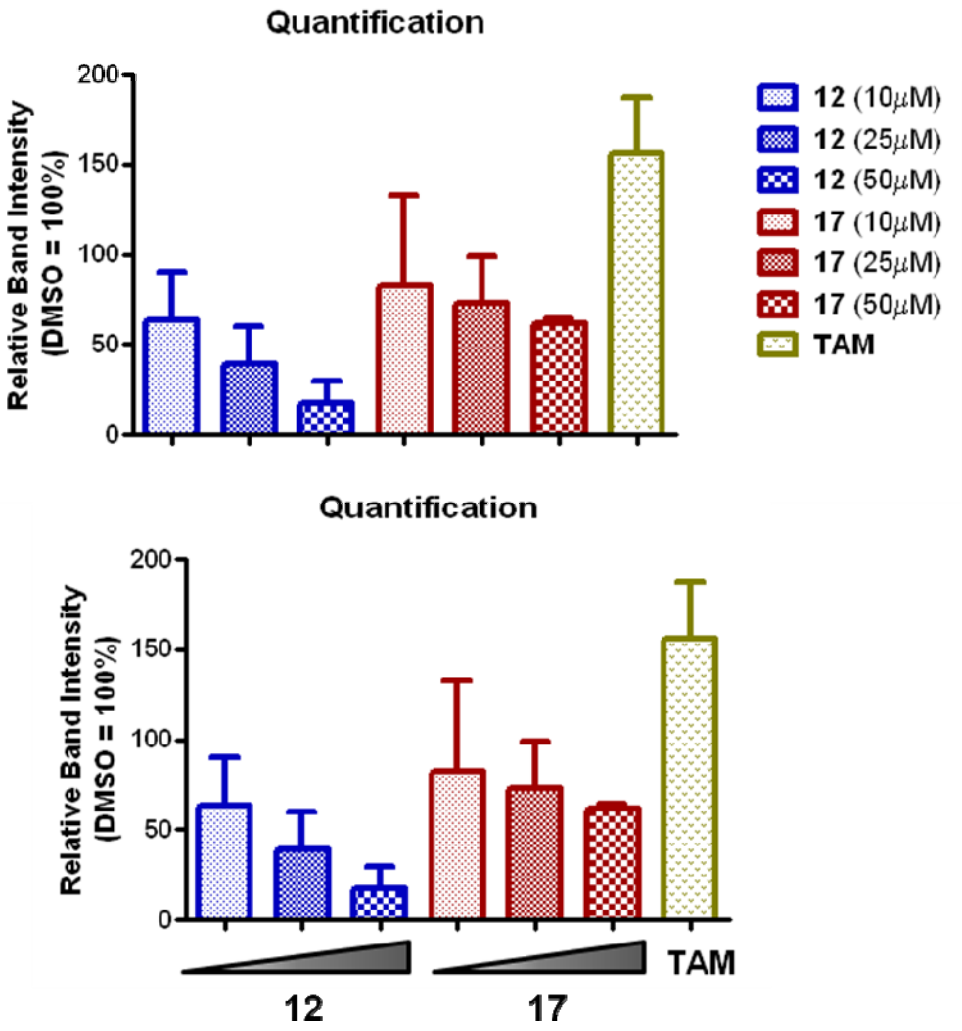


Fig 4



Supplementary material (ESI) for Molecular BioSystems