Synthesis of 17α-Cy3-estradiol

The synthesis procedure was depicted in Figure S1 and described in below. An amount of 2.4 g of t-Butyldimethylsilyl chloride (ALDRICH, St. Louis, MO, USA) and 2.8 g of imidazole (Fluka, Buchs, Switzerland) were added to a solution of the 2.0 g estrone (Fluka, Buchs, Switzerland) dissolved in 20 mL dry dimethylformamide (J.T. Baker, Phillipsburg, NJ, USA). The mixture was stirred at room temperature overnight and then diluted with ether. The organic phase was washed with brine and the organic solvents were evaporated to afford 2 (84%).

\[ ^1H\text{NMR} (\text{CDCl}_3): 7.12 (1H, d), 6.63 (1H, d), 6.57 (1H, s), 2.85 (2H, m), 1.25-2.54 (13H, m), 0.98 (9H, s), 0.91 (3H, s), 0.19 (6H, s). \]

To a 30 mL-THF solution containing 2.8 g of 3-butyn-1-ol tetrahydropyranyl (THP) (ALDRICH, St. Louis, MO, USA) ether were added with 6.9 mL of 2.5 M \( n-\text{butyllithium} (n-\text{BuLi}) \) (CHEMICALL, Japan) dissolved in \( n-\text{hexane} \) and then a 20-mL solution of 2 at -78 °C. The reaction mixture was slowly warmed to room temperature. After stirring for 15 hours, the reaction was quenched by the addition of water. The reaction mixture was then extracted with ethyl acetate, and the organic extracts were washed with water. The crude product was purified by column chromatography on silica gel (15% ethyl acetate in \( n-\text{hexane} \)) to afford 3. (60%)

\[ ^1H\text{NMR} (\text{CDCl}_3): 7.12 (1H, d), 6.63 (1H, d), 6.57 (1H, s), 4.66 (1H, m), 3.83 (2H, m), 3.53 (2H, m), 2.78 (2H, m), 2.55 (2H, m), 1.25-2.54 (13H, m), 0.98(9H, s), 0.91(3H, s), 0.19(6H, s). \]

The THP group of the E2 derivative of 3 was removed by treating 3 with 14.8 mg of catalytic 10-camphorsulfonic acid (CSA) (Fluka, Buchs, Switzerland) dissolved in 8 mL methanol for 10 min at room temperature. The crude product was purified by column chromatography on silica gel (20% ethyl acetate in \( n-\text{hexane} \)) to afford 4. (74%)

\[ ^1H\text{NMR} (\text{CDCl}_3): 7.12 (1H, d), 6.63 (1H, d), 6.57 (1H, s), 3.83 (2H, m), 2.78 (2H, m), 2.55 (2H, m), 1.25-2.54 (13H, m), 0.98(9H, s), 0.91(3H, s), 0.19(6H, s). \]

\( N-(\text{tert-butoxycarbonyl})-2\) nitrobenzenesulfonylamide (NsNHBOc) was first synthesized by the method described in ref. S1. To a mixture of 4, 151 mg of NsNHBOc and 131 mg of triphenylphosphine (TPP) (Fluka, Buchs, Switzerland) dissolved in 2 mL dry benzene was added with 200 \( \mu L \) of 2.2 M diethyl azodicarboxylate (TCL, Japan) dissolved in toluene, and the resulting mixture was stirred for 16 hours at room temperature. The reaction was then quenched by the addition of water, and the mixture was extracted with ethyl acetate. The organic extract was washed with water and the crude product was purified by column chromatography on silica gel (20% ethyl acetate in \( n-\text{hexane} \)) to afford 5. (67%)

\[ ^1H\text{NMR} (\text{CDCl}_3): 8.31 (1H, m), 7.75 (3H, m), 7.12 (1H, d), 6.63 (1H, d), 6.57 (1H, s), 3.93 (2H, t), 2.78 (2H, m), 2.70 (2H, t), 1.25-2.54 (22H, m), 0.98(9H, s), 0.91(3H, s), 0.19(6H, s). \]
A solution of 5 dissolved in 1 mL dry THF was treated with a 0.32 mL of 1M tetrabutylammonium fluoride (TBAF) (ALDRICH, St. Louis, MO, USA) in THF for 3 hours at room temperature. The solution was diluted with ethyl acetate and then thoroughly washed with water. The crude product was purified by column chromatography on silica gel (25% ethyl acetate in n-hexane) to afford 6, (77%) \( ^1H \text{NMR} (\text{CDCl}_3): 8.31 (1H, m), 7.75 (3H, m), 7.12 (1H, d), 6.63 (1H, d), 6.57 (1H, s), 3.93 (2H, t), 2.78 (2H, m), 2.70 (2H, t), 1.25-2.54 (22H, m), 0.91(3H, s) \)

The Ns group was removed by stirring 6 with 5µL of thiophenol (PhSH) (ALDRICH, St. Louis, MO, USA) and 40 mg of cesium carbonate (Cs₂CO₃) (ALDRICH, St. Louis, MO, USA) dissolved in 0.6 mL DMF for 1 hour at room temperature, and the resulting product was purified by thin-layer preparative chromatography on silica gel (50% ethyl acetate in n-hexane) to afford 7, (70%) \( ^1H \text{NMR} (\text{CDCl}_3): 7.12 (1H, d), 6.63 (1H, d), 6.57 (1H, s), 3.93 (2H, t), 2.78 (2H, m), 2.70 (2H, t), 1.25-2.54 (22H, m), 0.91(3H, s). \)

The Boc group was removed with 10µL of TFA dissolved in 0.2 mL dichloromethane for 3 hours reaction at room temperature. The reaction mixture was then evaporated to afford 8, (62%) \( ^1H \text{NMR} (\text{CD}_3\text{OD}): 7.12 (1H, d), 6.63 (1H, d), 6.57 (1H, s), 3.93 (2H, t), 2.78 (2H, m), 2.70 (2H, t), 1.25-2.54 (13H, m), 0.91(3H, s). \) MALDI-MS: \( m/z \) 340 (M+ H)+.

Finally, 17α-NH₂-estradiol derivative 8 was dissolved in methanol and then reacted with 0.1 mg of Cy3 (Amersham Life Science, US) dissolved in 20 µL of PBS buffer for 2 hours at room temperature to yield 17α-Cy3-estradiol (Cy3-E2). The crude product was purified by reversed-phase HPLC. MALDI-MS: \( m/z \) 952 (M+ H)+. The final concentration of Cy3-E2 was determined by the calibration curve constructed from the fluorescence measurements of free Cy3 dyes.

Figure S1

1. TBDMS-Cl, imidazole, DMF

2. n-BuLi, THF, -78°C to rt

3. TBDMS-Cl, imidazole, DMF

4. cat. CSA, MeOH

5. NsNHBOc, TPP, DEAD, benzene

6. Cs₂CO₃, PhSH, DMF

7. TFA, DCM

8. TBAF