Synthesis and Discovery of Estra-1,3,5(10),6,8-pentaene-2,16α-diol

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Supporting Information

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1. Materials and Methods

A. Reagents and Solvents

All reagents and starting materials were purchased from commercial sources and used as received, unless otherwise indicated. Anhydrous dichloromethane (CH_2Cl_2), dimethylformamide (DMF), tetrahydrofuran (THF) and toluene (PhMe) were obtained by passing HPLC grade solvents through a column of activated alumina using a Glass Contour Solvent Purification System by Pure Process Technology, LLC. Anhydrous methanol (MeOH) was purchased in a Sure-Seal™ bottle from Sigma-Aldrich. For flash column chromatography, HPLC grade solvents were used without further purification. Solutions of *n*-BuLi and *t*-BuLi were purchased from Sigma-Aldrich and titrated against *N*-benzylbenzamide in accordance with the procedure reported by Chong.[1]

B. Reaction Set-Up and Purification

All reactions were conducted in flame-dried glassware under an atmosphere of dry nitrogen unless otherwise indicated. Reaction mixtures were magnetically stirred and their progress was monitored by thin layer chromatography (TLC) on EMD TLC silica gel 60 F_{254} glass-backed plates. Compounds were visualized by exposure of TLC plates to UV-light (254 nm), and staining with *p*-anisaldehyde.

Purification of crude isolates was achieved by flash column chromatography on a Biotage® Isolera One™ Automated Liquid Chromatography System using Biotage® SNAP Ultra HP-Sphere 10-25 g or Biotage® KP-Sil 10 g silica gel cartridges, or performed using a forced flow of the indicated solvent system on Sorbent Technologies™ silica gel 60 Å (40-63 μm particle size). Concentration of reaction product solutions and chromatography fractions was accomplished by rotary evaporation at 30–35 ºC under the appropriate pressure, followed by concentration at room temperature on a vacuum pump (approx. 0–1 mbar). Yields refer to chromatographically purified and spectroscopically pure compounds, unless otherwise indicated.

C. Characterization Data for New Compounds

i. Nuclear Magnetic Resonance Spectroscopy

^{1}H-NMR data were recorded on a Bruker Avance III 500 MHz NMR spectrometer (TBI probe) and a Bruker Avance III 600 MHz spectrometer (BBFO probe). ^{1}H chemical shifts are reported in parts per million (ppm, δ scale) downfield from tetramethylsilane and are referenced to the residual protium in $CDCl_3$ (7.26 ppm), CD_3OD (3.31ppm), $(CD_3)_2SO$ (2.50 ppm) and $(CD_3)_2CO$ (2.05 ppm). NMR coupling constants are measured in Hertz (Hz), and

[1] Burchat, A. F.; Chong, J. M.; Nielsen, N. *J. Organomet. Chem.* **1997**, *542*, 281-283.

splitting patterns are indicated as follows: br, broad; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. ^{13}C {1H decoupled} NMR data were recorded at 150 MHz on a Bruker Avance III 600 MHz spectrometer (BBFO probe). ^{13}C chemical shifts are reported in parts per million (ppm, δ scale) and are referenced to the central line of the carbon resonances of the solvents: $CDCl_3$ (77.16 ppm), CD_3OD (49.86 ppm), $(CD_3)_2SO$ (39.52 ppm) and $(CD_3)_2CO$ (29.92 ppm).

Structural assignments for new compounds were supported by two-dimensional NMR experiments (COSY, HSQC, and NOESY) recorded on a Bruker Avance III 600 MHz spectrometer (BBFO probe).

ii. Infrared Spectroscopy

Infrared spectra were collected on a JASCO FT/IR-4100 Fourier Transform Infrared Spectrometer.

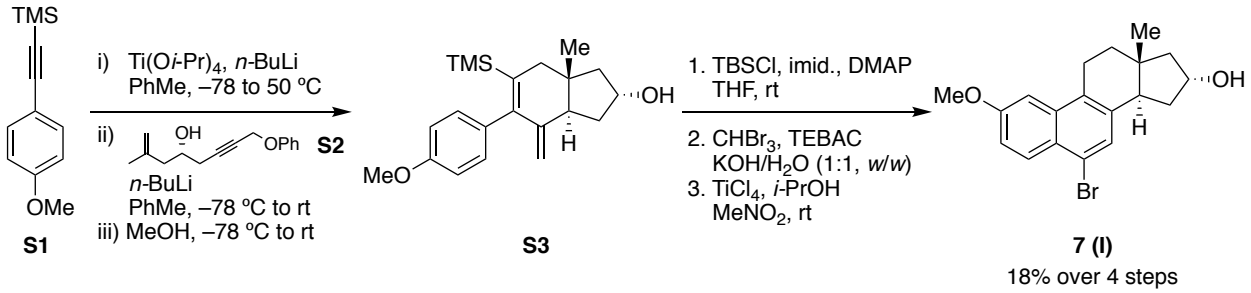
iii. Accurate Mass Determination

HRMS (ESI-TOF) analyses were performed at the Mass Spectrometry Laboratory of the University of Illinois at Urbana-Champaign.

iv. Optical Rotation

Optical rotations (α) were obtained on a JASCO-P-2000 polarimeter equipped with a tungsten-halogen lamp (WI) and interface filter set to 589 nm, using a sample cell with a pathlength of 100 nm. Specific rotations are reported as: $[\alpha]_{589}^{T\,(^\circ C)}$ (c, solvent) and are based on the equation $[\alpha]_{589}^{T\,(^\circ C)} = (100 \bullet \alpha)/(l \bullet c)$, where the concentration (c) is reported as g/100 ml and the pathlength (l) in decimeters.

2. Experimental Procedures



Synthesis of steroid 7 (I): This compound was prepared according to a procedure reported by Micalizio *et al*.[2] To a flask containing alkyne **S1** (48 g, 234.47 mmol) in anhydrous toluene (1.6 L) under nitrogen atmosphere was added Ti(O*i*-Pr)$_4$ (70 mL, 234.47 mmol). The flask was cooled to –78 ºC and *n*-BuLi (2.6 M in hexanes, 200 mL, 494.99 mmol) was added dropwise. After the addition of *n*-BuLi was complete, the flask was warmed to room temperature and then heated to 50 ºC for one hour. After the indicated time, the flask was re-cooled to room temperature, and then to –78 ºC in an acetone–CO_2 bath.

Meanwhile, *n*-BuLi (2.6 M in hexanes, 35 mL, 86.84 mmol) was added at –78 ºC to a separate flask containing a solution of enyne **S2**[2] (20 g, 86.84 mmol) in anhydrous toluene (250 mL). The lithium-alkoxide was transferred, via a cannula, to the Ti-alkyne complex at –78 ºC, and the resulting mixture was stirred, with gradual warming to room temperature, overnight (approximately 24 h). The next morning, the contents of the reaction flask were transferred dropwise, via a cannula, into anhydrous MeOH (1.6 L) at –78 ºC under an atmosphere of nitrogen. The resulting mixture was concentrated *in vacuo* and a saturated aqueous solution of $NaHCO_3$ was carefully added to the residue. The aqueous layer and organic phases were separated. The aqueous layer was extracted with ethyl acetate (× 3). The combined organic layers were dried over anhydrous $MgSO_4$, and the resulting mixture was vacuum filtered through a coarse fritted glass funnel. The filtrate was concentrated *in vacuo* to afford the tentatively assigned hydrindane **S3** (14.21 g, 50%) as a yellow oil.

To a solution of hydrindane **S3** (14.21 g, 41.48 mmol) in anhydrous THF (600 mL) were added TBSCl (13 g, 91.08 mmol), imidazole (6 g, 91.08 mmol) and DMAP (0.3 g, 2.33 mmol). The reaction mixture was stirred at room temperature under nitrogen atmosphere overnight (approximately 15 h). The next day, the reaction was quenched by the addition of a saturated aqueous solution of $NaHCO_3$. The two phases were separated and the aqueous layer was extracted with ethyl acetate (× 3). The combined organic layers were dried over anhydrous $MgSO_4$, and filtered through a coarse fritted glass funnel. The filtrate was concentrated *in vacuo* to afford the silylated product (16 g), as a yellow oil, which was used in the subsequent step without further purification.

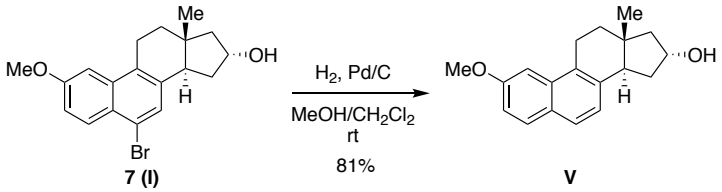
[2] Kim, W. S.; Du, K.; Eastman, A.; Hughes R. P.; Micalizio, G. C. *Nat. Chem.* **2017**, *10*, 70-77.

To a solution of the above product (16 g, 35.02 mmol) in CH_3Br (75 mL) were added benzyltriethylammonium chloride (TEBAC) (2 g, 8.78 mmol) and a premixed solution of KOH (20 g, 356 mmol) in DI water (20 mL). The reaction mixture was heated to 45 ºC and reacted overnight (approximately 16 h). The following morning, the reaction was quenched by the addition of DI water. The two phases were separated, and the aqueous layer was extracted with ethyl acetate (× 3). The combined organic extracts were washed with brine, and dried over anhydrous $MgSO_4$, and the drying agent was removed by filtration through a coarse fritted glass funnel. The filtrate was concentrated *in vacuo* to afford the crude product (20 g), which was used in the next step without further purification.

To a solution of the crude product from the previous step (20 g, 31 mmol) in $MeNO_2$ (620 mL) was added $TiCl_4$ (8.5 mL, 77.5 mmol) and *i*-PrOH (23.5 mL, 310 mmol). After the reaction mixture was stirred at room temperature for an hour, a second portion of $TiCl_4$ (8.5 mL, 77.5 mmol) and *i*-PrOH (23.5 mL, 310 mmol) were added. The reaction mixture was stirred at room temperature for an hour and then quenched by the addition of a saturated aqueous solution of $NaHCO_3$. The aqueous and organic phases were separated, and the aqueous phase was extracted with CH_2Cl_2 (× 3). The combined organic layers were dried over anhydrous $MgSO_4$, and filtered through a coarse fritted glass funnel and then concentrated *in vacuo* to afford the crude product, which was purified by flash column chromatography on silica gel with 90:10 to 70:30 hexanes-ethyl acetate gradient elution to afford steroid **7 (I)** (5.76 g, 18% over 4 steps) as a yellow solid.

Analytical data for 7 (I):

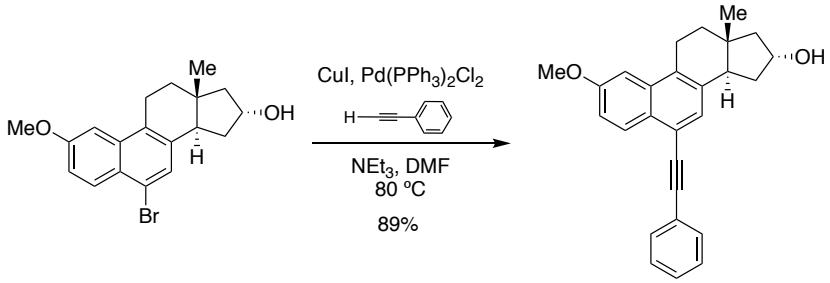
TLC (SiO_2) R_f = 0.26 (hexanes:ethyl acetate-60:40); $[\alpha]_{589}^{19.8}$ = +33.9 (*c* 0.0055, $CHCl_3$); **^{1}H NMR** (600 NMR, $CDCl_3$) δ 8.12 (d, *J* = 9.1 Hz, 1H), 7.34 (s, 1H), 7.24 – 7.08 (m, 2H), 4.76 – 4.64 (m, 1H), 3.93 (s, 3H), 3.24 – 3.11 (m, 2H), 3.07 (m, 1H), 2.30 (dd, *J* = 12.5, 7.3 Hz, 1H), 2.24 – 2.15 (m, 2H), 2.15 – 2.07 (m, 1H), 1.89 (td, *J* = 11.8, 7.8 Hz, 1H), 1.74 (br s, 1H), 1.42 (dd, *J* = 12.5, 5.7 Hz, 1H), 0.65 (s, 3H); **^{13}C NMR** (150 MHz, $CDCl_3$) δ 158.1, 137.9, 134.5, 129.4, 129.4, 126.6, 125.8, 120.4, 118.1, 102.3, 72.1, 55.3, 50.5, 47.8, 41.4, 37.2, 35.83, 24.7, 17.8; **IR** (thin film, cm^{-1}) 3360, 2998, 2937, 2854, 2831, 1713, 1623, 1590, 1509, 1448, 1430, 1416, 1376, 1359, 1347, 1315, 1158, 1118, 1088, 1060; **HRMS** (ESI-TOF) *m/z*: [M+H] Calculated for $C_{19}H_{22}O_2Br$ 361.0803; Found 361.0786.



Synthesis of steroid V: To a stirred solution of steroid **7** (**I**) (30 mg, 0.083 mmol) in 0.6 mL MeOH and 0.15 mL CH_2Cl_2 under nitrogen atmosphere was added 10% Pd/C (8.8 mg, 0.0083 mmol). The nitrogen atmosphere in the flask was exchanged for hydrogen gas, and the reaction was stirred under a slightly positive pressure of hydrogen for approximately 10 h. The mixture was filtered through a cotton-plugged pipet and rinsed forward with CH_2Cl_2. The filtrate was concentrated *in vacuo* to afford the crude product, which was purified by flash column chromatography on silica gel with 60:40 hexanes-ethyl acetate to afford steroid **V** (19 mg, 81%) as a white solid.

Analytical data for V:

TLC (SiO_2) R_f = 0.16 (hexanes:ethyl acetate-75:25); $[\alpha]_{589}^{19.8}$ = +40.7 (*c* 0.0089, $CHCl_3$); **^{1}H NMR** (600 NMR, $CDCl_3$) δ 7.72 (d, *J* = 8.9 Hz, 1H), 7.60 (d, *J* = 8.3 Hz, 1H), 7.19 (d, *J* = 2.5 Hz, 1H), 7.15 – 7.05 (m, 2H), 4.76 – 4.65 (m, 1H), 3.94 (s, 3H), 3.28 – 3.19 (m, 2H), 3.19 – 3.10 (m, 1H), 2.32 (dd, *J* = 12.4, 7.3 Hz, 1H), 2.27 – 2.17 (m, 2H), 2.18 – 2.09 (m, 1H), 1.93 (td, *J* = 11.8, 7.8 Hz, 1H), 1.48 – 1.39 (m, 1H), 0.67 (s, 3H), O-H was not identified; **^{13}C NMR** (150 MHz, $CDCl_3$) δ 157.7, 136.9, 133.0, 130.2, 129.3, 127.5, 125.8, 122.7, 116.9, 101.9, 72.3, 55.3, 50.8, 48.0, 41.5, 37.3, 36.0, 24.6, 17.8; **IR** (thin film, cm^{-1}) 3343, 2998, 2937, 2858, 1623, 1596, 1518, 1512, 1456, ; **HRMS** (ESI-TOF) *m/z*: [M+H] Calculated for $C_{19}H_{23}O_2$ 283.1698; Found 283.1688.



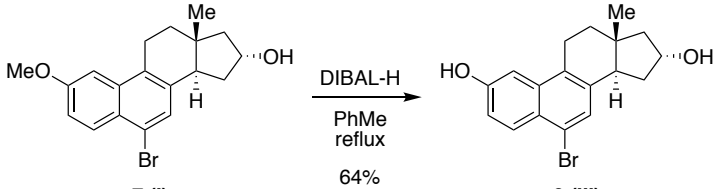
7 (I) **10 (XII)**

Synthesis of steroid 10 (XII): Anhydrous triethylamine (15 μL, 0.1107 mmol), anhydrous DMF (2 mL) and phenylacetylene (12 μL, 0.1107 mmol) were sequentially added to a Schlenk tube containing **7** (**I**) (20 mg, 0.0553 mmol), $Pd(PPh_3)_2Cl_2$ (1.94 mg, 0.0027 mmol) and CuI (1.05 mg, 0.00553 mmol) under nitrogen atmosphere at room temperature. The reaction mixture was then heated to 80 ºC and stirred overnight (approximately 15 h). The following morning, the volatile components of the reaction mixture were removed by rotary evaporation under reduced pressure. The remaining materials were partitioned between diethyl ether and water. The phases were separated, and the organic layer was washed several times with water to ensure complete removal of DMF. The organic phase was then concentrated *in vacuo* to afford the crude product,

which was purified by flash column chromatography on silica gel with 60:40 hexanes-ethyl acetate to afford steroid **10** (**XII**) (19 mg, 89%) as a light yellow solid.

Analytical data for 10 (XII):

TLC (SiO_2) R_f = 0.26 (hexanes:ethyl acetate-60:40); $[\alpha]_{589}^{19.8}$ = +30.3 (*c* 0.385, $CHCl_3$); **^{1}H NMR** (600 NMR, $CDCl_3$) δ 8.35 (d, *J* = 9.1 Hz, 1H), 7.64 (d, *J* = 6.8 Hz, 2H), 7.46 – 7.33 (m, 4H), 7.21 (d, *J* = 8.4 Hz, 2H), 4.72 (p, *J* = 5.8 Hz, 1H), 3.95 (s, 3H), 3.20 (m, 3H), 2.32 (dd, *J* = 12.4, 7.3 Hz, 1H), 2.28 – 2.20 (m, 2H), 2.15 (dd, *J* = 12.5, 7.6 Hz, 1H), 1.94 (td, *J* = 11.6, 8.3 Hz, 1H), 1.60 (s, 1H), 1.45 (dd, *J* = 12.4, 5.7 Hz, 1H), 0.67 (s, 3H); **^{13}C NMR** (150 MHz, $CDCl_3$) δ 158.0, 136.7, 133.1, 131.6, 130.9, 128.5, 128.4, 128.2, 127.2, 127.1, 123.6, 118.5, 117.6, 102.3, 93.4, 88.2, 72.3, 55.3, 50.6, 47.8, 41.4, 37.3, 35.9, 24.9, 17.8; **IR** (thin film, cm^{-1}) 3423, 2955, 2924, 2857, 1556, 1540, 1519, 1506, 1487, 1336, 1267, 1227, 1059, 1047, 1029; **HRMS** (ESI-TOF) *m/z*: [M+H] Calculated for $C_{27}H_{27}O_2$ 383.2011; Found 383.1995.



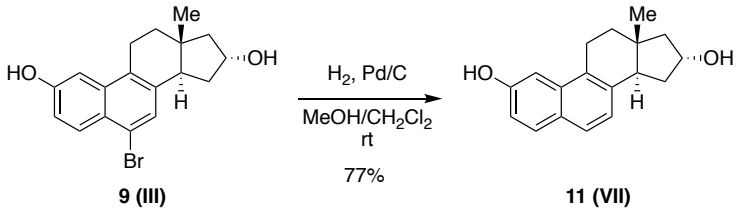
7 (I) **9 (III)**

Synthesis of steroid 9 (III): DIBAL-H (1.0 M in hexanes, 11.2 mL, 11.2 mmol) was added to a solution of **7** (**I**) (100 mg, 0.373 mmol) in anhydrous toluene (12 mL) at room temperature. The reaction mixture was then heated to reflux under nitrogen and stirred overnight (approximately 15 h). The next morning, the reaction mixture was cooled to room temperature, and quenched by sequential addition of 20 mL of CH_2Cl_2 and then 20 mL of a saturated aqueous solution of Rochelle's salt. The biphasic system was stirred vigorously for about 30 mins. The phases were separated and the aqueous layer was extracted with CH_2Cl_2 (× 3). The combined organic layers were then washed with brine, then dried over anhydrous $MgSO_4$, filtered through a coarse fritted glass funnel, and concentrated *in vacuo* to afford the crude product, which was purified by flash column chromatography on silica gel with 70:30 to 50:50 hexanes-ethyl acetate gradient elution to afford **9** (**III**) (121 mg, 64%) as a white solid.

Analytical data for 9 (III):

TLC (SiO_2) R_f = 0.21 (hexanes:ethyl acetate-60:40); $[\alpha]_{589}^{22.1}$ = +31.8 (*c* 0.605, CH_3OH); **^{1}H NMR** (600 NMR, CD_3OD) δ 7.93 (d, *J* = 9.1 Hz, 1H), 7.16 (s, 1H), 7.13 (d, *J* = 2.3 Hz, 1H), 7.00 (dd, *J* = 9.1, 2.3 Hz, 1H), 4.50 (m, 1H), 3.06 – 2.97 (m, 2H), 3.05 – 2.97 (m, 1H), 2.14 (dd, *J* = 12.4, 7.3 Hz, 1H), 2.08 – 1.96 (m, 3H), 1.76 (td, *J* = 11.7, 8.0 Hz, 1H), 1.30 (dd, *J* = 12.3, 5.9 Hz, 1H), 0.54 (s, 3H); **^{13}C NMR** (150 MHz, CD_3OD) δ 155.9, 137.6, 134.9, 128.8, 128.8, 125.4, 124.9, 119.9, 117.7, 105.0, 71.1, 49.8, 48.0, 47.9, 47.8, 47.6, 47.5, 47.4, 47.3, 47.2, 40.9, 36.3,

35.6, 24.1, 16.5; **IR** (thin film, cm^{-1}) 3398, 2927, 2859, 1585, 1565, 1552, 1528, 1511, 1481, 1426; **HRMS** (ESI-TOF) *m/z*: [M+H] Calculated for $C_{18}H_{20}O_2Br$ 347.0647; Found 347.0657.

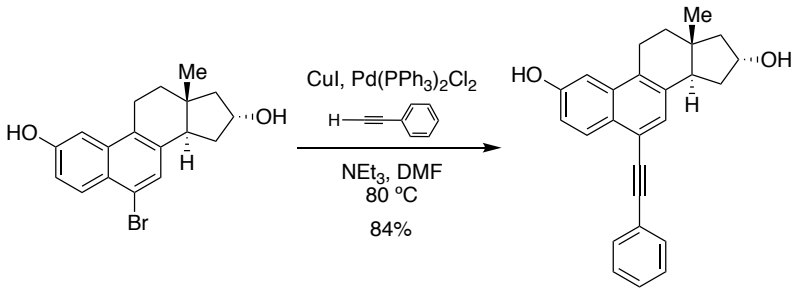


9 (III) **11 (VII)**

Synthesis of steroid 11 (VII): To a stirring mixture of steroid **9** (**III**) (30 mg, 0.086 mmol) in 0.6 mL MeOH and 0.15 mL CH_2Cl_2 under nitrogen atmosphere was added 10% Pd/C (8.8 mg, 0.0086 mmol) at room temperature. The nitrogen atmosphere in the flask was exchanged for hydrogen gas, and the reaction was stirred under a slightly positive pressure of hydrogen for approximately 10 h. The mixture was filtered through a cotton-plugged pipet and rinsed forward with CH_2Cl_2. The filtrate was concentrated *in vacuo* to afford the crude product, which was purified by flash column chromatography on silica gel with 70:30 to 60:40 hexanes-ethyl acetate gradient elution to afford steroid **11** (**VII**) (19 mg, 77%) as a white solid.

Analytical data for 11 (VII):

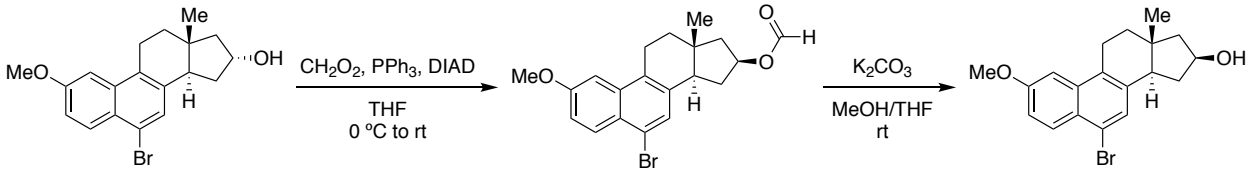
TLC (SiO_2) R_f = 0.20 (hexanes:ethyl acetate-60:40); $[\alpha]_{589}^{19.7}$ = +51.3 (*c* 0.890, CH_3OH); **^{1}H NMR** (600 NMR, CD_3OD) δ 7.55 (d, *J* = 8.8 Hz, 1H), 7.43 (d, *J* = 8.3 Hz, 1H), 7.11 (d, *J* = 2.2 Hz, 1H), 6.90 (dd, *J* = 8.6, 2.8 Hz, 2H), 4.62 – 4.54 (m, 1H), 3.10 – 3.01 (m, 2H), 2.98 (dt, *J* = 17.9, 9.0 Hz, 1H), 2.14 (dd, *J* = 12.3, 7.3 Hz, 1H), 2.12 – 2.03 (m, 2H), 2.01 (dd, *J* = 12.7, 7.1 Hz, 1H), 1.79 (td, *J* = 11.7, 8.1 Hz, 1H), 1.31 (dt, *J* = 11.2, 5.6 Hz, 1H), 0.55 (s, 3H); **^{13}C NMR** (150 MHz, CD_3OD) δ 155.0, 136.4, 133.4, 129.7, 128.2, 127.1, 125.5, 121.5, 116.3, 104.5, 71.2, 49.9, 48.0, 47.9, 47.8, 47.7, 47.6, 47.6, 47.5, 47.3, 47.2, 40.9, 36.5, 35.9, 24.1, 16.5; **IR** (thin film, cm^{-1}) 3421, 2948, 2937, 2921, 2983, 2854, 1623, 1516, 1436, 1375, 1364, 1335, 835; **HRMS** (ESI-TOF) *m/z*: [M+H] Calculated for $C_{18}H_{21}O_2$ 269.1542; Found 269.1534.



9 (III) **XV**

Synthesis of steroid XV: Anhydrous trimethylamine (16 μL, 0.1152 mmol), anhydrous DMF (2 mL) and phenylacetylene (12 μL, 0.1152 mmol) were sequentially added to a Schlenk tube containing **9** (**III**) (20 mg, 0.058 mmol), $Pd(PPh_3)_2Cl_2$ (2.03 mg, 0.0029 mmol) and CuI (1.1 mg, 0.0058 mmol) under nitrogen atmosphere at room temperature. The reaction mixture was then heated to 80 ºC and reacted overnight (approximately 15 h). The following morning, the volatile components of the reaction mixture were removed by rotary evaporation under reduced pressure. The remaining materials were partitioned between diethyl ether and water. The phases were separated, and the organic layer was washed several times with water to ensure complete removal of DMF. The organic phase was then concentrated *in vacuo* to afford the crude product, which was purified by flash column chromatography on silica gel with 60:40 hexanes-ethyl acetate to afford steroid **XV** (18 mg, 84%) as a yellow solid.

Analytical data for XV:
TLC (SiO_2) R_f = 0.20 (hexanes:ethyl acetate-60:40); $[\alpha]_{589}^{19.8}$ = +26.2 (*c* 0.105, $CHCl_3$); **^{1}H NMR** (600 NMR, $CDCl_3$) δ 8.34 (d, *J* = 8.9 Hz, 1H), 7.73 – 7.53 (m, 2H), 7.49 – 7.31 (m, 4H), 7.28 (d, *J* = 2.5 Hz, 1H), 7.14 (dd, *J* = 8.9, 2.5 Hz, 1H), 5.15 (br s, 1H), 4.78 – 4.67 (m, 1H), 3.20 (dd, *J* = 18.0, 7.9 Hz, 2H), 3.12 (m, 1H), 2.32 (dd, *J* = 12.4, 7.3 Hz, 1H), 2.28 – 2.17 (m, 2H), 2.13 (dd, *J* = 12.7, 7.5 Hz, 1H), 1.92 (td, *J* = 11.7, 7.7 Hz, 1H), 1.44 (dd, *J* = 12.5, 5.7 Hz, 1H), 0.66 (s, 3H); **^{13}C NMR** (150 MHz, $CDCl_3$) δ 153.9, 136.8, 131.6, 130.7, 128.9, 128.4, 128.2, 127.3, 127.2, 123.5, 118.6, 116.9, 105.9, 93.5, 88.1, 77.1, 76.9, 72.3, 50.6, 47.8, 41.4, 37.2, 35.9, 29.7, 24.7, 17.8; **IR** (thin film, cm^{-1}) 3421, 3237, 2973, 2916, 2852, 1717, 1698, 1682, 1658, 1647, 1635, 1619, 1558, 1223; **HRMS** (ESI-TOF) *m/z*: [M+H] Calculated for $C_{26}H_{25}O_2$ 369.1855; Found 369.1837.



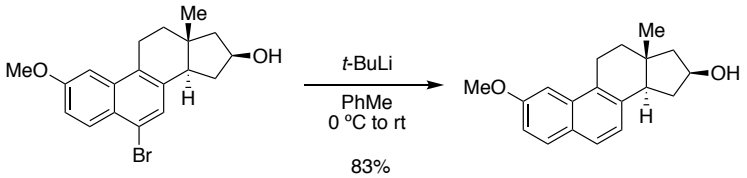
Synthesis of steroid 14 (II): Formic acid (0.167 mL, 4.43 mmol), PPh_3 (1.162 g, 4.43 mmol) and DIAD (0.872 mL, 4.43 mmol) were added to a solution of steroid **7** (**I**) (800 mg, 2.21 mmol) in THF (6 mL) at 0 ºC. The reaction mixture was stirred at 0 ºC for 10 mins, and then warmed to room temperature. After one hour, the reaction mixture was concentrated *in vacuo* and then purified by flash column chromatography on silica gel with 90:10 to 70:30 hexanes-ethyl acetate gradient elution to afford the intermediate **S4** as a yellow oil.

To a solution of the intermediate **S4** (720 mg, 1.85 mmol) in THF (2 mL) and MeOH (3 mL) was added K_2CO_3 (513 mg, 3.71 mmol) at room temperature. The reaction mixture was stirred for 2

h, and the solids were removed by filtering through a cotton pipet. The filtrate was concentrated *in vacuo* to afford the crude product, which was purified by flash column chromatography on silica gel with 80:20 to 60:40 hexanes-ethyl acetate gradient elution to afford steroid **14** (**II**) (673 mg, 84% over 2 steps) as a white solid.

Analytical data for 14 (II):

TLC (SiO_2) R_f = 0.32 (hexanes:ethyl acetate-60:40); $[\alpha]_{589}^{19.7}$ = +37.1 (*c* 0.560 , $CHCl_3$); **^{1}H NMR** (600 NMR, $CDCl_3$) δ 8.16 – 8.10 (m, 1H), 7.36 (s, 1H), 7.21 – 7.16 (m, 2H), 4.72 – 4.64 (m, 1H), 3.94 (s, 3H), 3.21 – 3.05 (m, 2H), 2.81 – 2.69 (m, 2H), 2.13 (ddd, *J* = 12.7, 6.6, 2.4 Hz, 1H), 1.88 – 1.71 (m, 4H), 0.91 (s, 3H); **^{13}C NMR** (150 MHz, $CDCl_3$) δ 158.2, 137.6, 134.6, 129.5, 129.5, 126.5, 125.8, 120.5, 118.1, 102.3, 72.4, 55.4, 49.9, 49.4, 40.0, 37.0, 35.9, 24.6, 18.1; **IR** (thin film, cm^{-1}) 3410, 2946, 2920, 2858, 2848, 1623, 1464, 1451, 1428, 1373, 1343, 1272, 1227, 1032; **HRMS** (ESI-TOF) *m/z*: [M+H] Calculated for $C_{19}H_{22}O_2Br$ 361.0803; Found 361.0818.



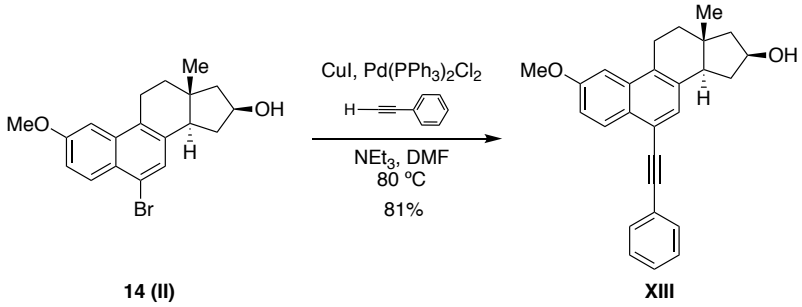
14 (II) **VI**

Synthesis of steroid VI: *t*-BuLi (1.58 M in toluene, 87 µL, 0.138 mmol) was added dropwise to a stirring mixture of steroid **14** (**II**) (17 mg, 0.055 mmol) inanhydrous toluene (2 mL) at 0 ºC, and then reacted 0 ºC for one hour. After this time, the cooling bath was removed and stirring was continued at room temperature for an additional hour. The reaction was quenched by careful addition of water. The aqueous and organic phases were separated, and the aqueous layer was extracted with ethyl acetate (× 3). The combined organic layers were dried over anhydrous Na_2SO_4, filtered through a coarse fritted glass funnel and then concentrated *in vacuo* to afford the crude product, which was purified by flash column chromatography on silica gel with 80:20 to 60:40 hexanes-ethyl acetate gradient elution to afford **VI** (13 mg, 83%) as a white solid.

Analytical data for VI:

TLC (SiO_2) R_f = 0.33 (hexanes:ethyl acetate-60:40); $[\alpha]_{589}^{21.9}$ = +65.4 (*c* 0.178, $CHCl_3$) In progress; **^{1}H NMR** (600 NMR, $CDCl_3$) δ 7.72 (d, *J* = 8.8 Hz, 1H), 7.61 (d, *J* = 8.3 Hz, 1H), 7.20 (d, *J* = 2.5 Hz, 1H), 7.15 – 7.03 (m, 2H), 4.75 – 4.63 (m, 1H), 3.94 (s, 3H), 3.26 – 3.11 (m, 2H), 2.89 – 2.74 (m, 2H), 2.14 (m, 1H), 1.92 – 1.82 (m, 1H), 1.83 – 1.70 (m, 3H), 0.96 – 0.88 (m, 3H); **^{13}C NMR** (150 MHz, $CDCl_3$) δ 157.7, 136.5, 133.1, 130.1, 129.3, 127.5, 125.8, 122.6, 116.9, 101.9, 72.6, 55.3, 50.1, 49.6, 40.0, 37.1, 37.0, 36.2, 24.5, 18.1; **IR** (thin film, cm^{-1}) 3295, 3050, 3002, 2955, 2941, 2917, 2892, 2871, 2838, 2824, 1621, 1430, 1415, 1335, 1307, 1153,

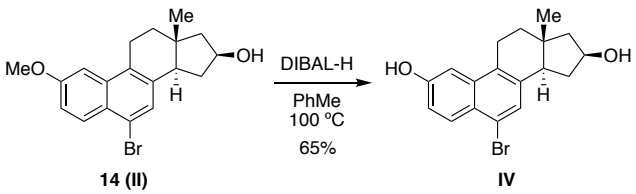
1134, 1029, 835, 754, 720, 701; **HRMS** (ESI-TOF) *m/z*: [M+H] Calculated for $C_{19}H_{23}O_2$ 283.1698; Found 283.1688.



Synthesis of steroid XIII: Anhydrous triethylamine (15 μL, 0.1107 mmol), anhydrous DMF (2 mL) and phenylacetylene (12 μL, 0.1107 mmol) were sequentially added to a Schlenk tube containing **14** (**II**) (20 mg, 0.0553 mmol), $Pd(PPh_3)_2Cl_2$ (1.94 mg, 0.0027 mmol) and CuI (1.05 mg, 0.00553 mmol) under nitrogen atmosphere at room temperature. The reaction mixture was then heated to 80 ºC and reacted overnight (approximately 15 h). The following morning, the volatile components of the reaction mixture were removed by rotary evaporation under reduced pressure. The remaining materials were partitioned between diethyl ether and water. The phases were separated, and the organic layer was washed several times with water to ensure complete removal of DMF. The organic phase was then concentrated *in vacuo* to afford the crude product, which was purified by flash column chromatography on silica gel with 70:30 to 60:40 hexanes-ethyl acetate to afford steroid **XIII** (15.3 mg, 81%) as a yellow solid.

Analytical data for XIII:

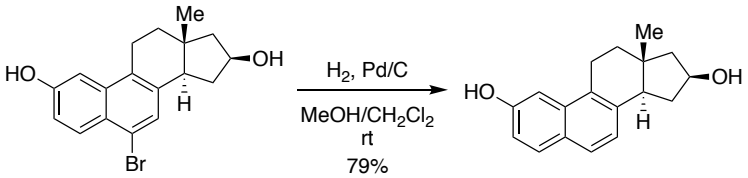
TLC (SiO_2) R_f = 0.30 (hexanes:ethyl acetate-60:40); $[\alpha]_{589}^{19.8}$ = +15.6 (*c* 0.295, CHCl3); **^{1}H NMR** (600 NMR, $CDCl_3$) δ 8.35 (d, *J* = 9.2 Hz, 1H), 7.64 (dd, *J* = 8.1, 1.4 Hz, 2H), 7.50 – 7.27 (m, 4H), 7.21 (d, *J* = 8.6 Hz, 2H), 4.69 (t, *J* = 7.6 Hz, 1H), 3.95 (s, 3H), 3.22 (m, 2H), 2.91 – 2.71 (m, 2H), 2.15 (ddd, *J* = 12.6, 6.6, 2.0 Hz, 1H), 1.95 – 1.81 (m, 2H), 1.81 – 1.69 (m, 2H), 1.65 br (s, 1H), 0.92 (s, 3H); **^{13}C NMR** (150 MHz, $CDCl_3$) δ 158.0, 136.3, 133.1, 131.6, 130.9, 128.5, 128.4, 128.2, 127.2, 127.1, 123.6, 118.5, 117.6, 102.3, 93.4, 88.2, 72.6, 55.3, 50.0, 49.4, 39.9, 37.1, 36.1, 24.8, 18.1; **IR** (thin film, cm^{-1}) 3477, 3059, 2979, 2952, 2935, 2904, 2853, 1770, 1759, 1747, 1718, 1685, 1663, 1636, 1618, 1581, 1455, 1429, 1418, 1373, 1337, 1301, 1268, 1048, 1027, 821, 762, 690; **HRMS** (ESI-TOF) *m/z*: [M+H] Calculated for $C_{27}H_{27}O_2$ 383.2011; Found 383.2023.



Synthesis of steroid IV: DIBAL-H (1.0 M in toluene, 2.2 mL, 2.21 mmol) was added to a solution of steroid **14** (**II**) (80 mg, 0.221 mmol) in anhydrous toluene (2.2 mL) at room temperature. The reaction mixture was then heated to reflux and reacted overnight (approximately 15 h). The next morning, the reaction mixture was cooled to room temperature, and quenched by the careful addition of ~5 mL of a saturated aqueous solution of Rochelle's salt. The biphasic system was stirred vigorously for about 30 mins. The phases were separated and the aqueous layer was extracted with ethyl acetate (× 3). The combined organic layers were dried over anhydrous Na_2SO_4, filtered through a coarse fritted glass funnel, and concentrated *in vacuo* to afford the crude product, which was purified by flash column chromatography on silica gel with 70:30 to 50:50 hexanes-ethyl acetate gradient elution to afford **IV** (50 mg, 65%) as a white solid.

Analytical data for IV:

TLC (SiO_2) R_f = 0.22 (hexanes:ethyl acetate-60:40); $[\alpha]_{589}^{22.2}$ = –887.1 (*c* 0.105, $CD_3OD/CDCl_3$); **^{1}H NMR** (600 NMR, $(CD_3)_2SO$) δ 10.00 (s, 1H), 7.95 (d, *J* = 9.0 Hz, 1H), 7.23 (s, 1H), 7.19 (d, *J* = 2.2 Hz, 1H), 7.15 (dd, *J* = 9.1, 2.3 Hz, 1H), 4.75 (s, 1H), 4.41 (t, *J* = 7.2 Hz, 1H), 3.06 – 2.87 (m, 2H), 2.68 – 2.54 (m, 2H), 2.03 – 1.91 (m, 1H), 1.64 (m, 2H), 1.56 (d, *J* = 12.7 Hz, 1H), 1.51 (m, 1H), 0.77 (s, 3H); **^{13}C NMR** (150 MHz, $(CD_3)_2SO$) δ 156.7, 138.1, 135.2, 129.3, 129.2, 125.8, 124.5, 120.0, 119.1, 105.8, 70.5, 49.9, 48.8, 36.9, 35.9, 24.4, 18.2; **IR** (thin film, cm^{-1}) 3388, 2953, 2926, 2858, 1728, 1698, 1659, 1623, 1587, 1465, 1430, 1369, 1161, 1132, 1024, 984, 888, 860, 833, 814. **TLC-MS** (ESI-TOF) *m/z*: [M+H] Calculated for $C_{18}H_{20}BrO_2$ 347.0647; Found 347.1 and 349.2.

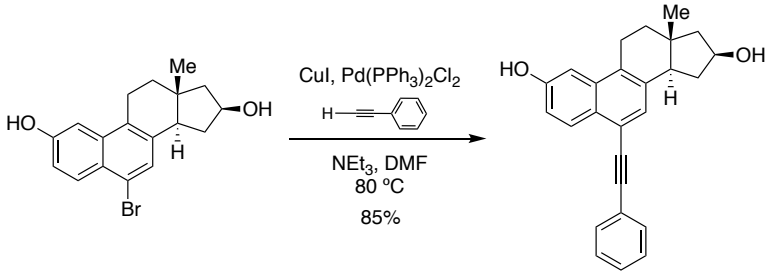


IV **VIII**

Synthesis of steroid VIII: To a stirring mixture of steroid **IV** (20 mg, 0.0576 mmol) in 1 mL MeOH and 0.25 mL CH_2Cl_2 under nitrogen atmosphere was added 10% Pd/C (6.1 mg, 0.0058 mmol) at room temperature. The nitrogen atmosphere in the flask was exchanged for hydrogen gas, and the reaction was stirred under a slightly positive pressure of hydrogen for approximately 10 h. The mixture was filtered through a cotton-plugged pipet and rinsed forward with CH_2Cl_2. The filtrate was concentrated *in vacuo* to afford the crude product, which was purified by flash column chromatography on silica gel with 65:35 hexanes-ethyl acetate to afford steroid **VIII** (12.2 mg, 79%) as a white solid.

Analytical data for VIII:

TLC (SiO_2) R_f = 0.23 (hexanes:ethyl acetate-60:40); $[\alpha]_{589}^{19.7}$ = +36.4 (*c* 0.310, CD_3OD); **^{1}H NMR** (600 NMR, CD_3OD) δ 7.56 (d, *J* = 8.8 Hz, 1H), 7.45 (d, *J* = 8.3 Hz, 1H), 7.12 (d, *J* = 2.2 Hz, 1H), 6.99 – 6.82 (m, 2H), 4.61 – 4.54 (m, 1H), 3.19 – 3.02 (m, 2H), 2.74 – 2.57 (m, 2H), 2.00 (ddd, *J* = 12.5, 6.3, 2.4 Hz, 1H), 1.72 (dd, *J* = 13.2, 8.6 Hz, 1H), 1.69 – 1.58 (m, 3H), 0.78 (s, 3H); **^{13}C NMR** (150 MHz, CD_3OD) δ 155.0, 136.1, 133.5, 129.7, 128.2, 127.1, 125.5, 121.4, 116.3, 104.4, 71.5, 49.1, 49.0, 39.6, 36.3, 36.1, 23.9, 16.97; **IR** (thin film, cm^{-1}) 3404, 3040, 2950, 2915, 2870, 2855, 1656, 1638, 1623, 1434, 1367, 1329, 1135, 1027, 835; **HRMS** (ESI-TOF) *m/z*: [M+H] Calculated for $C_{18}H_{21}O_2$ 269.1542; Found 269.1553.

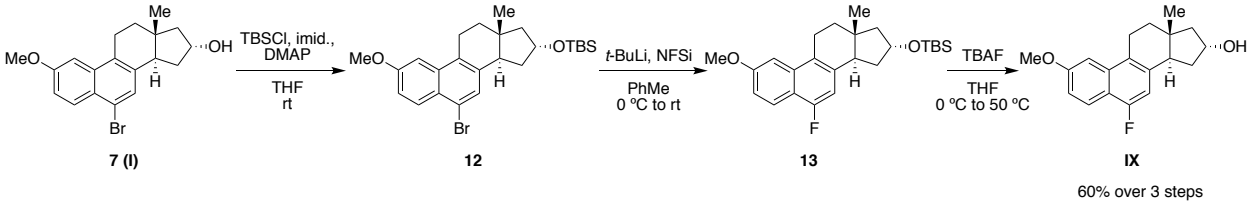


IV **XIV**

Synthesis of steroid XIV: Anhydrous triethylamine (16 μL, 0.1152 mmol), anhydrous DMF (2 mL) and phenylacetylene (12 μL, 0.1152 mmol) were sequentially added to a Schlenk tube containing **IV** (20 mg, 0.058 mmol), $Pd(PPh_3)_2Cl_2$ (2.03 mg, 0.0029 mmol) and CuI (1.1 mg, 0.0058 mmol) under nitrogen atmosphere at room temperature. The reaction mixture was then heated to 80 ºC and reacted overnight (approximately 15 h). The following morning, the volatile components of the reaction mixture were removed by rotary evaporation under reduced pressure. The remaining materials were partitioned between diethyl ether and water. The phases were separated, and the organic layer was washed several times with water to ensure complete removal of DMF. The organic phase was then concentrated *in vacuo* to afford the crude product, which was purified by flash column chromatography on silica gel with 65:35 hexanes-ethyl acetate to afford steroid **XIV** (18.3 mg, 85 %) as a yellow solid.

Analytical data for XIV:

TLC (SiO_2) R_f = 0.24 (hexanes:ethyl acetate-60:40); $[\alpha]_{589}^{22.1}$ = +7.4 (*c* 0.145, $(CH_3)_3CO$); **^{1}H NMR** (600 NMR, $(CD_3)_2CO$) δ 8.74 (s, 1H), 8.32 (d, *J* = 9.0 Hz, 1H), 7.77 – 7.60 (m, 2H), 7.53 – 7.37 (m, 3H), 7.37 – 7.30 (m, 2H), 7.21 (dd, *J* = 9.0, 2.4 Hz, 1H), 4.69 – 4.55 (m, 1H), 3.23 – 3.06 (m, 2H), 2.82 – 2.72 (m, 3H), 2.14 – 2.07 (m, 1H), 1.84 – 1.72 (m, 3H), 0.91 (s, 3H); **^{13}C NMR** (150 MHz, $(CD_3)_2CO$) δ 156.9, 137.6, 134.7, 132.3, 131.7, 129.6, 129.3, 129.2, 127.4, 127.4, 124.5, 119.2, 118.7, 106.5, 94.0, 89.1, 72.1, 50.8, 49.9, 40.7, 37.9, 36.9, 25.3, 18.3; **IR** (thin film, cm^{-1}) 3415, 3226, 1669, 1641, 1623; **HRMS** (ESI-TOF) *m/z*: [M+H] Calculated for $C_{26}H_{25}O_2$ 369.1855; Found 369.1837.



Synthesis of steroid IX:[3] TBSCl (417 mg, 2.768 mmol), imidazole (188 mg, 2.768 mmol) and DMAP (8.45 mg, 5 mol%) were added to a stirring solution of **7 (I)** (500 mg, 1.384 mmol) in anhydrous THF (20 mL) at room temperature under nitrogen atmostphere. The reaction mixture was stirred overnight (approximately 15 h). The following morning, the contents of the reaction flask was quenched with water. The aqueous and organic phases were separated, and the aqueous layer was extracted with ethyl acetate (× 3). The combined layers were dried over anhydrous Na_2SO_4, filtered through a coarse fritted glass funnel and then concentrated *in vacuo* to afford the tentatively assigned **12** (509 mg, 77%) as a yellow oil.

To a stirring solution of **12** (500 mg, 1.051 mmol) in anhydrous toluene (20 mL) at 0 ºC was added *t*-BuLi (1.58 M in toluene, 1.66 mL, 2.63 mmol). After the reaction mixture was stirred for about an hour at 0 ºC, NFSi (663 mg, 2.102 mmol) in toluene (20 mL) was added to the flask. The ice bath was removed after the addition of NFSi, and the resulting mixture was stirred for 2 h at room temperature. The reaction mixture was quenched by the addition of a saturated aqueous solution of NH_4Cl. The aqueous and organic phases were separated, and the aqueous layer was extracted with ethyl acetate (× 3). The combined organic layers were dried over anhydrous Na_2SO_4, filtered through a coarse fritted glass funnel and then concentrated *in vacuo* to afford the tentatively assigned **13** (360 mg, 83%) as a yellow oil.

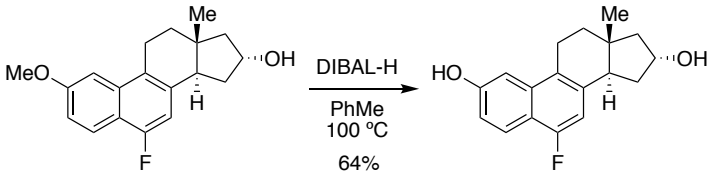
To a solution of **13** (360 mg, 0.8682 mmol) in anhydrous THF (5 mL) was added TBAF (1.1 mL, 1.041 mmol) at 0 ºC. The mixture was stirred for an hour at 0 ºC, then heated at 50 ºC for 2 h. The reaction was quenched with the addition of a saturated aqueous solution of NH_4Cl. The aqueous and organic phases were separated and the aqueous layer was extracted with diethyl ether (× 3). The combined organic layers were dried over anhydrous Na_2SO_4, filtered through a coarse fritted glass funnel and concentrated *in vacuo* to afford the crude product, which was purified by flash column chromatography on silica gel with 70:30 to 50:50 hexanes-ethyl acetate gradient elution to afford **IX** (245 mg, 60% over 3 steps) as a white solid.

Analytical data for IX:

TLC (SiO_2) R_f= 0.28 (hexanes:ethyl acetate-65:35); $[\alpha]_{589}^{22.4}$ = +60.7 (*c* 0.138, $CHCl_3$); **^{1}H NMR** (600 NMR, $CDCl_3$) δ 7.99 (d, *J* = 8.9 Hz, 1H), 7.18 – 7.09 (m, 2H), 6.74 (d, *J* = 11.1 Hz, 1H), 4.78 – 4.63 (m, 1H), 3.93 (s, 3H), 3.26 – 3.02 (m, 3H), 2.31 (dd, *J* = 12.5, 7.3 Hz, 1H), 2.20 – 2.09 (m, 3H), 1.91 (td, *J* = 11.8, 7.8 Hz, 1H), 1.47 – 1.40 (m, 1H), 0.66 (s, 3H); **^{13}C NMR** (150 MHz, $CDCl_3$) δ 158.4, 157.0 (d =, *J* = 249.47 Hz) 137.5 (d, *J* = 7.74 Hz), 130.1, 124.88 (d, *J* =

[3] Zajc, B. *J. Org. Chem.* **1999**, *64*, 1902-1907.

4.48 Hz), 122.7 (d, J = 5.55 Hz), 117.4 (d, J = 16.54 Hz), 117.2, 106.3 (d, J = 19.09 Hz), 102.0, 72.2, 55.4, 50.8, 48.1, 37.4, 36.0, 24.5, 17.9; **IR** (thin film, cm^{-1}) 3576, 3555, 3533, 3516, 3336, 2998, 2933, 2857, 1658, 1631, 1612, 1580, 1570, 1562, 1454, 1429, 1418, 1388, 1376, 1366, 1345, 1327, 1272, 1175, 1138, 1121, 1097, 1045, 1027; **HRMS** (ESI-TOF) *m/z*: [M+H] Calculated for $C_{19}H_{22}O_2F$ 301.1604; Found 301.1615.

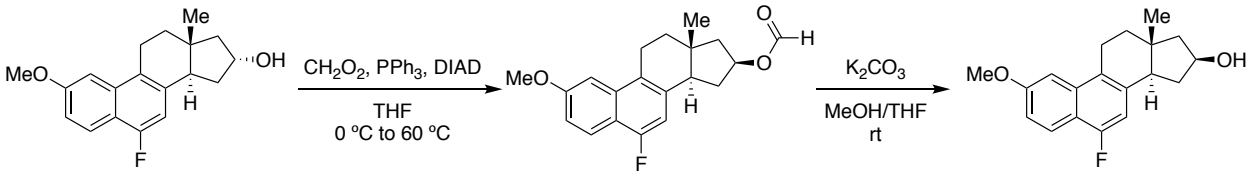


IX **X**

Synthesis of steroid X: DIBAL-H (1.0 M in toluene, 0.998 mL, 0.998 mmol) was added to a solution of **IX** (30 mg, 0.0998 mmol) in anhydrous toluene (1 mL) at room temperature. The reaction mixture was refluxed at 100 ºC overnight (approximately 15 h). The reaction mixture was then heated to reflux and reacted overnight (approximately 15 h). The next morning, the reaction mixture was cooled to room temperature, and quenched by the careful addition of ~2 mL of a saturated aqueous solution of Rochelle's salt. The biphasic system was stirred vigorously for about 30 mins. The phases were separated and the aqueous layer was extracted with ethyl acetate (× 3). The combined organic layers were dried over anhydrous Na_2SO_4, filtered through a coarse fritted glass funnel, and concentrated *in vacuo* to afford the crude product, which was purified by flash column chromatography on silica gel with 70:30 to 50:50 hexanes-ethyl acetate gradient elution to afford **X** (18 mg, 64%) as a yellow oil.

Analytical data for X:

TLC (SiO_2) R_f = 0.21 (hexanes:ethyl acetate-60:40); $[\alpha]_{589}^{22.7}$ = +62.7 (*c* 0.125, CD_3OD); **^{1}H NMR** (600 NMR, CD_3OD) δ 7.81 (d, J = 9.0 Hz, 1H), 7.12 (s, 1H), 6.98 (dd, J = 9.0, 2.3 Hz, 1H), 6.60 (d, J = 11.4 Hz, 1H), 4.58 – 4.46 (m, 1H), 3.14 – 2.98 (m, 2H), 3.00 – 2.88 (m, 1H), 2.16 (dd, J = 12.4, 7.3 Hz, 1H), 2.12 – 1.98 (m, 3H), 1.81 (tt, J = 11.9, 6.4 Hz, 1H), 1.33 (dd, J = 12.5, 5.9 Hz, 1H), 0.58 (s, 3H); **^{13}C NMR** (150 MHz, CD_3OD) δ 159.5 (d, J = 248.69 Hz), 159.0, 127.8, 126.4 (d, J = 4.48 Hz), 124.1 (d, J = 5.53 Hz), 118.9, 118.8 (d, J = 16.40 Hz), 118.5, 107.1, 106.9 (d, J = 21.32 Hz), 73.3, 52.1, 49.9, 38.6, 37.9, 26.0, 18.7; **IR** (thin film, cm^{-1}) 3531, 3508, 3424, 2978, 2954, 2940, 2878, 2857, 1647, 1640, 1628, 1619, 1582, 1573, 1448, 1439, 1411, 1402, 1389, 1369, 1331, 1277, 1219, 1118, 1097, 1041; **HRMS** (ESI-TOF) *m/z*: [M+H] Calculated for $C_{18}H_{20}O_2F$ 287.1447; Found 287.1447.



Synthesis of steroid XI: Formic acid (29 μL, 0.779 mmol), PPh_3 (204 mg, 0.779 mmol) and DIAD (0.15 mL, 0.779 mmol) were added to a solution of steroid **IX** (117 mg, 0.3895 mmol) in THF (4 mL) at 0 ºC. The reaction mixture was heated to 60 ºC and reacted for 3 h. The resulting solution was concentrated *in vacuo* to afford the crude intermediate, which was purified by flash column chromatography on silica gel with 95:5 to 90:10 hexanes-ethyl acetate gradient elution to afford the intermediate **S5** as a yellow oil.

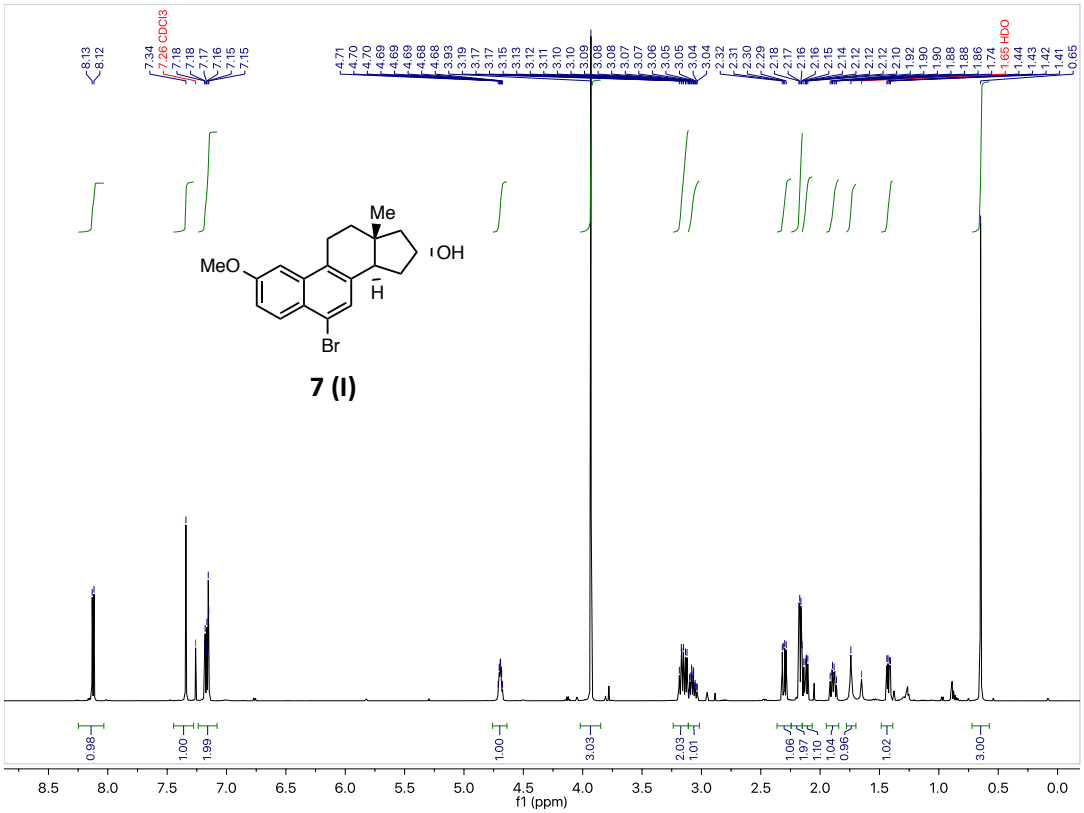
To a solution of the intermediate **S5** (100 mg, 0.305 mmol) in THF (2 mL) and MeOH (3 mL) was added K_2CO_3 (84 mg, 0.609 mmol) at room temperature. The reaction mixture was stirred for 2 h and the solids were removed by filtering through a cotton pipet. The filtrate was concentrated *in vacuo* to afford the crude product, which was purified by flash column chromatography on silica gel with 80:20 to 60:40 hexanes-ethyl acetate gradient elution to afford steroid **XI** (53.5 mg, 46% over 2 steps) as a white solid.

Analytical data for XI:

TLC (SiO_2) R_f = 0.25 (hexanes:ethyl acetate-60:40); $[\alpha]_{589}^{22.4}$ = +82.3 (*c* 0.256, $CHCl_3$) In progress; **^{1}H NMR** (600 NMR, $CDCl_3$) δ 8.00 (d, *J* = 8.9 Hz, 1H), 7.24 – 7.10 (m, 2H), 6.75 (d, *J* = 11.1 Hz, 1H), 4.76 – 4.59 (m, 1H), 3.94 (s, 3H), 3.30 – 3.01 (m, 2H), 2.88 – 2.66 (m, 2H), 2.19 – 2.09 (m, 1H), 1.85 (dd, *J* = 13.6, 8.0 Hz, 1H), 1.81 – 1.72 (m, 3H), 0.91 (s, 3H); **^{13}C NMR** (150 MHz, $CDCl_3$) δ 158.4, 157.6 (d, *J* = 248.93 Hz), 137.1 (d, *J* = 7.8 Hz), 130.1, 124.9 (d, *J* = 4.45 Hz), 122.7 (d, *J* = 5.67 Hz), 117.4 (d, 15.99 Hz), 117.2, 106.2 (d, *J* = 19.26 Hz), 102.0, 72.5, 55.4, 50.1, 49.7, 37.2, 36.2, 22.1, 18.2; **IR** (thin film, cm^{-1}) 3418, 2971, 2956, 2938, 2917, 2889, 2873, 2856, 1773, 1706, 1657, 1630, 1610, 1572, 1428, 1388, 1375, 1272, 1260, 1176, 1161, 1149, 1136, 1103, 1082, 1069; **HRMS** (ESI-TOF) *m/z*: [M+H] Calculated for $C_{19}H_{22}O_2F$ 301.1604; Found 301.1606.

3. NMR Spectra

Figure S1: ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) of **7 (I)**



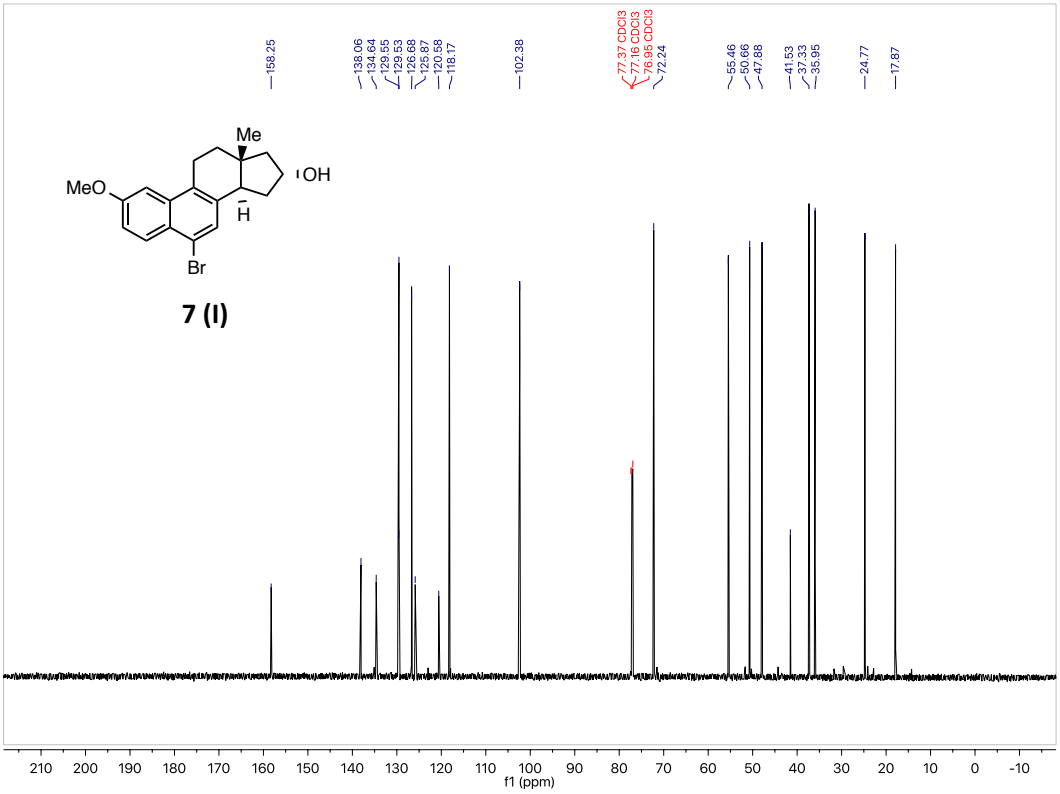
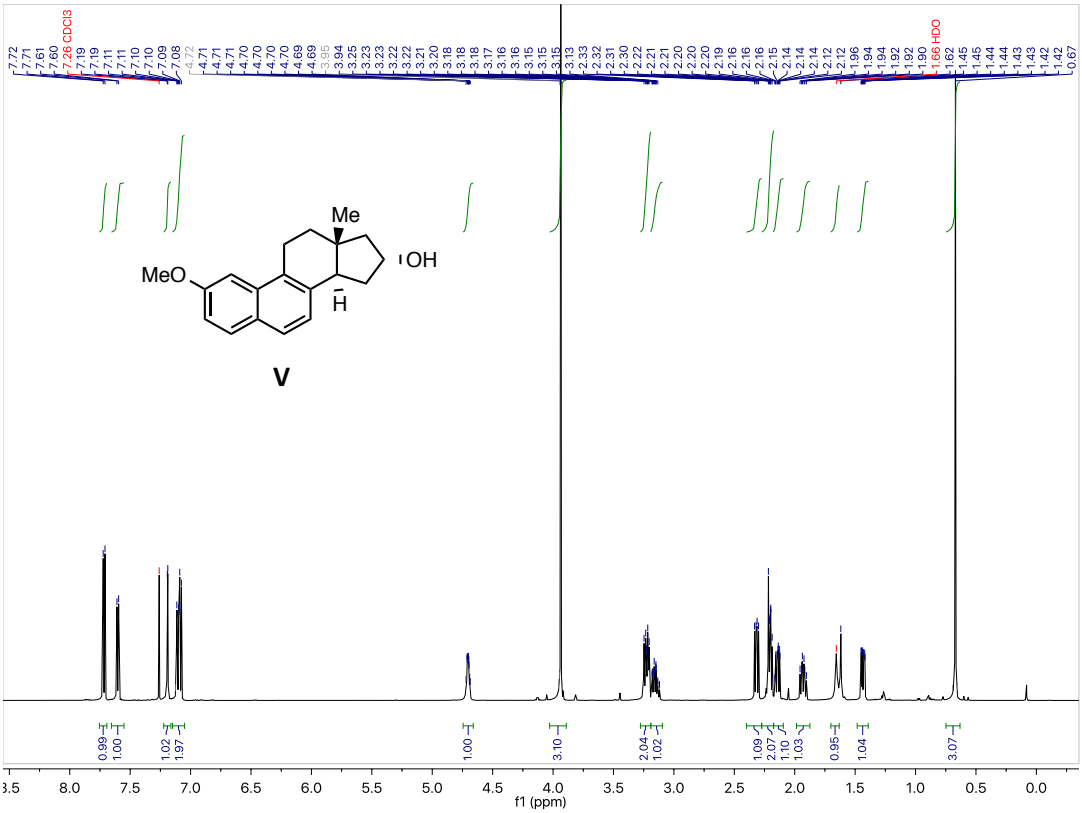


Figure S2: ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) of **V**



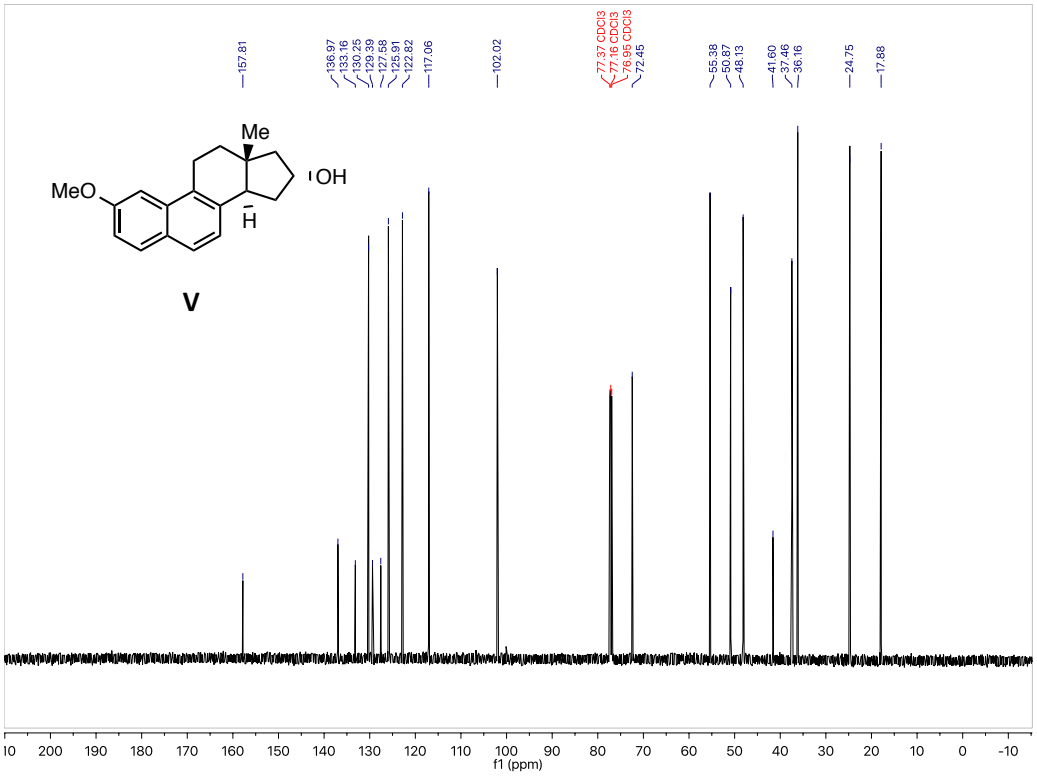
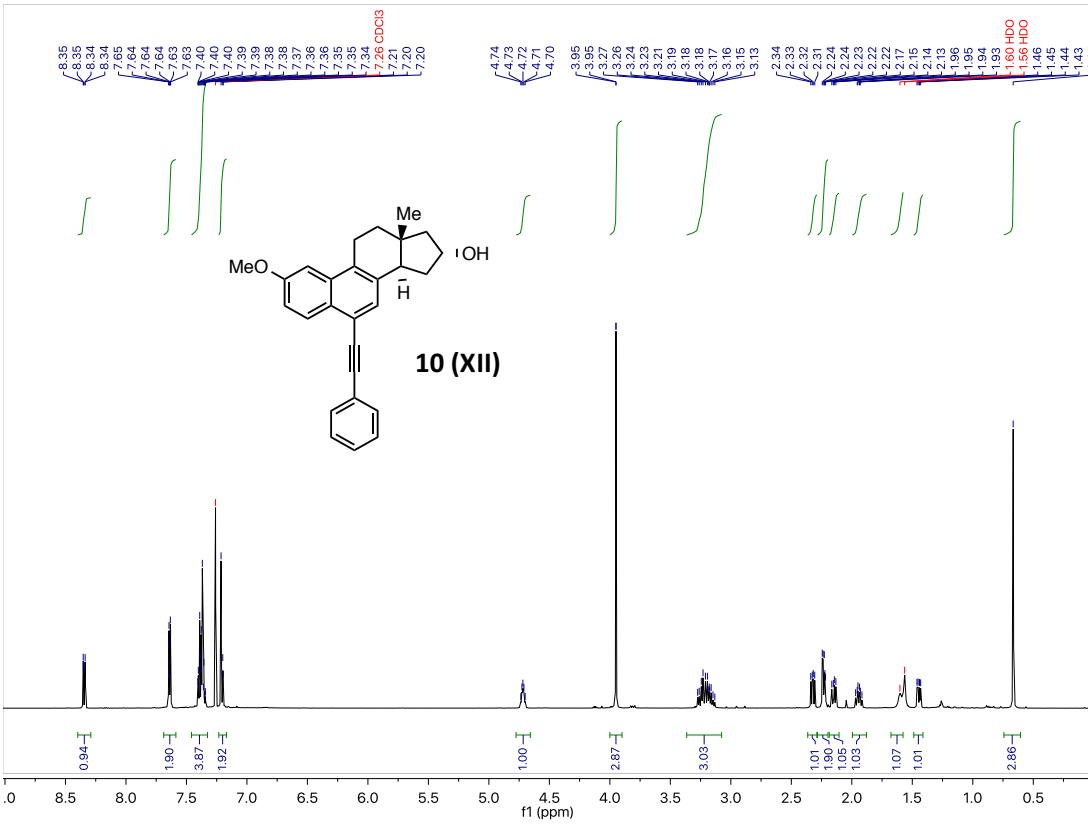


Figure S3: ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) of **10 (XII)**



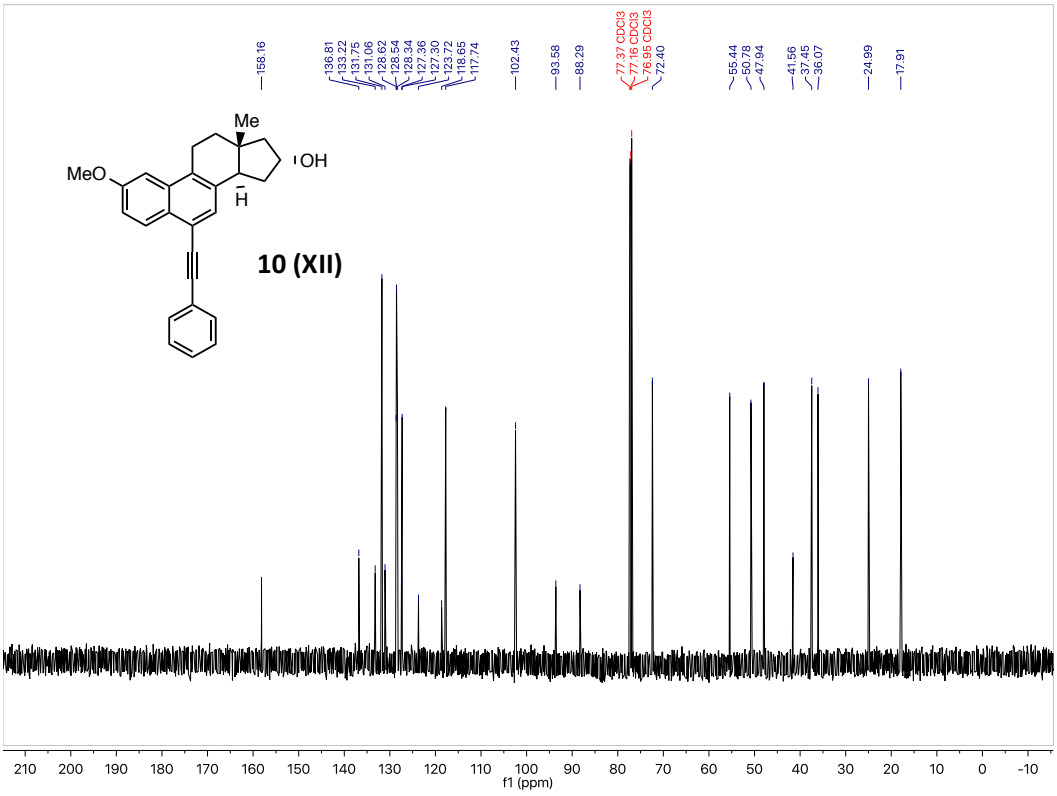
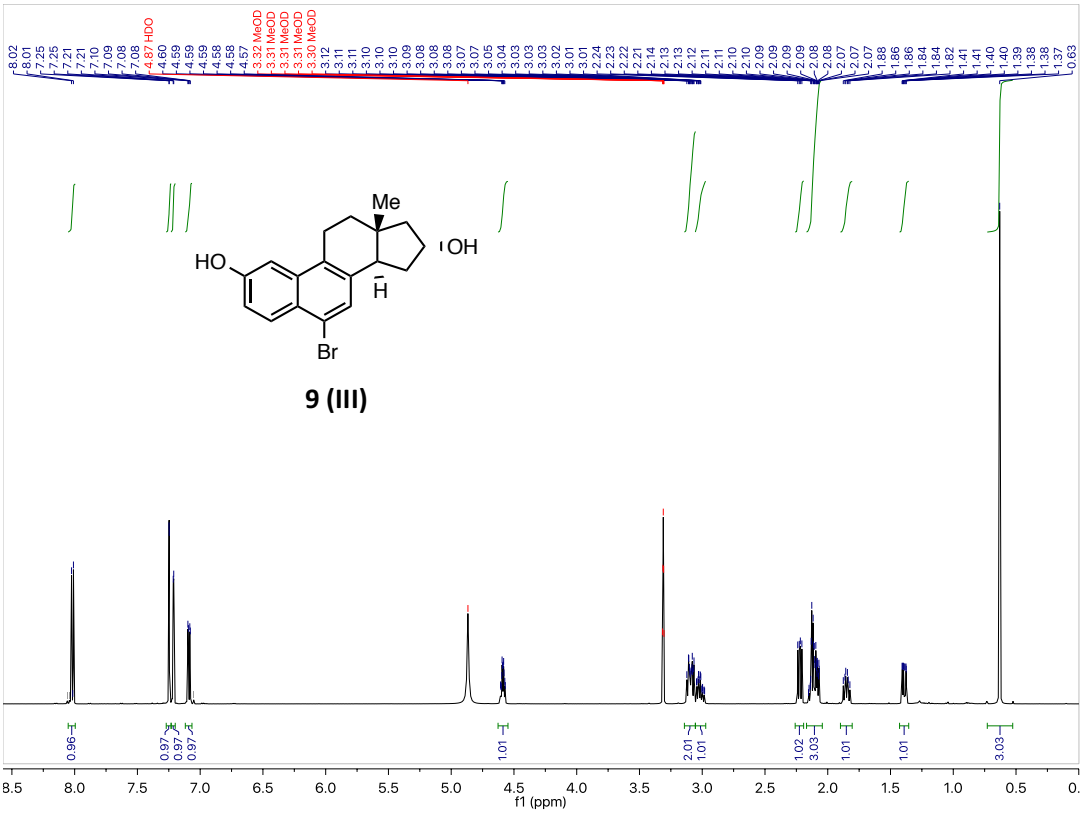


Figure S4: ^{1}H NMR (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD) of **9 (III)**



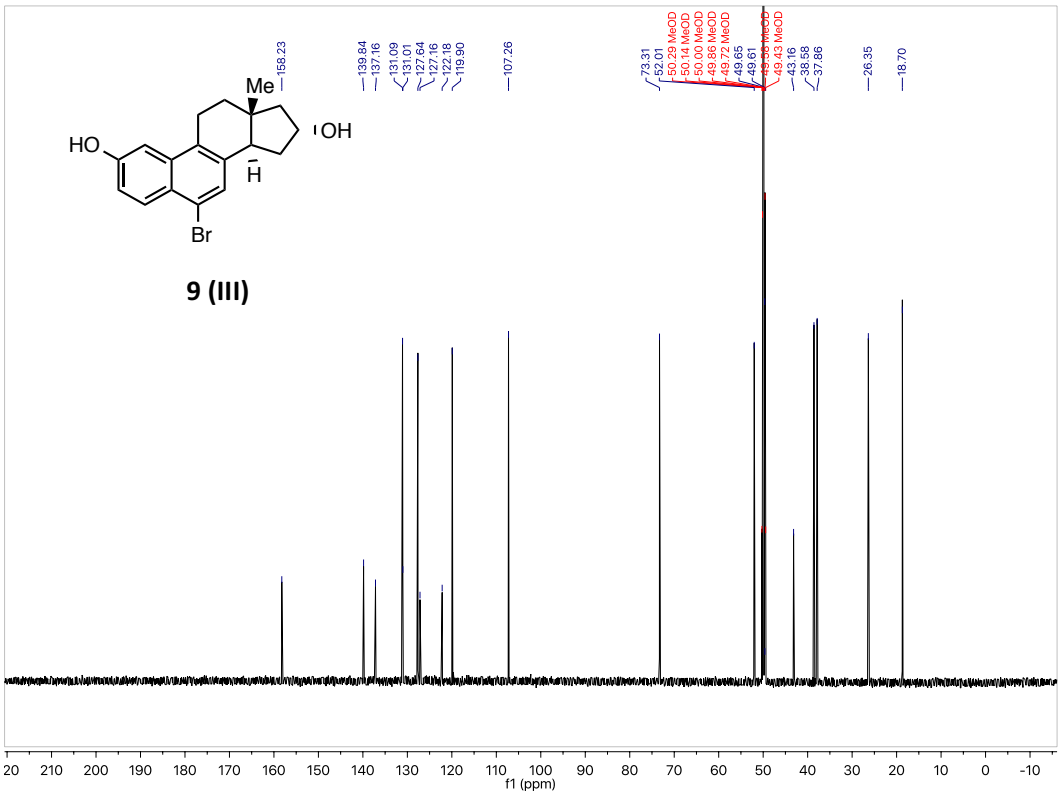
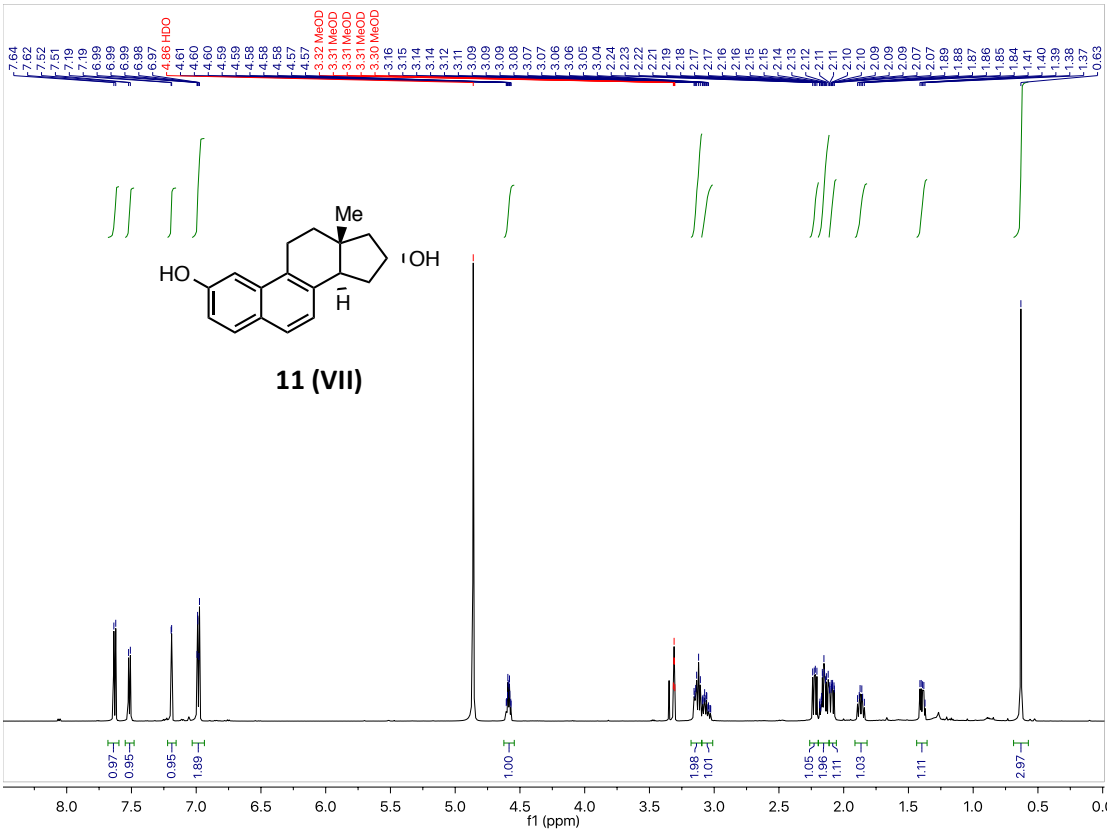


Figure S5: ^{1}H NMR (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD) of **11 (VII)**



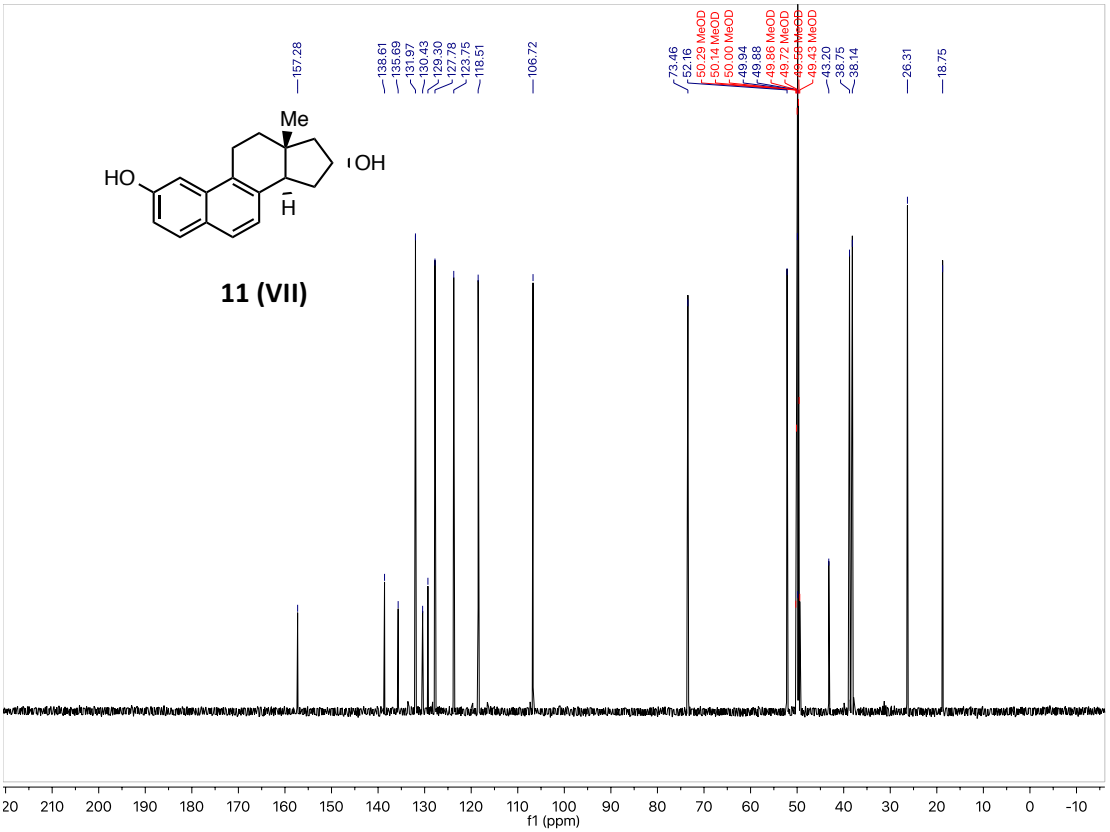
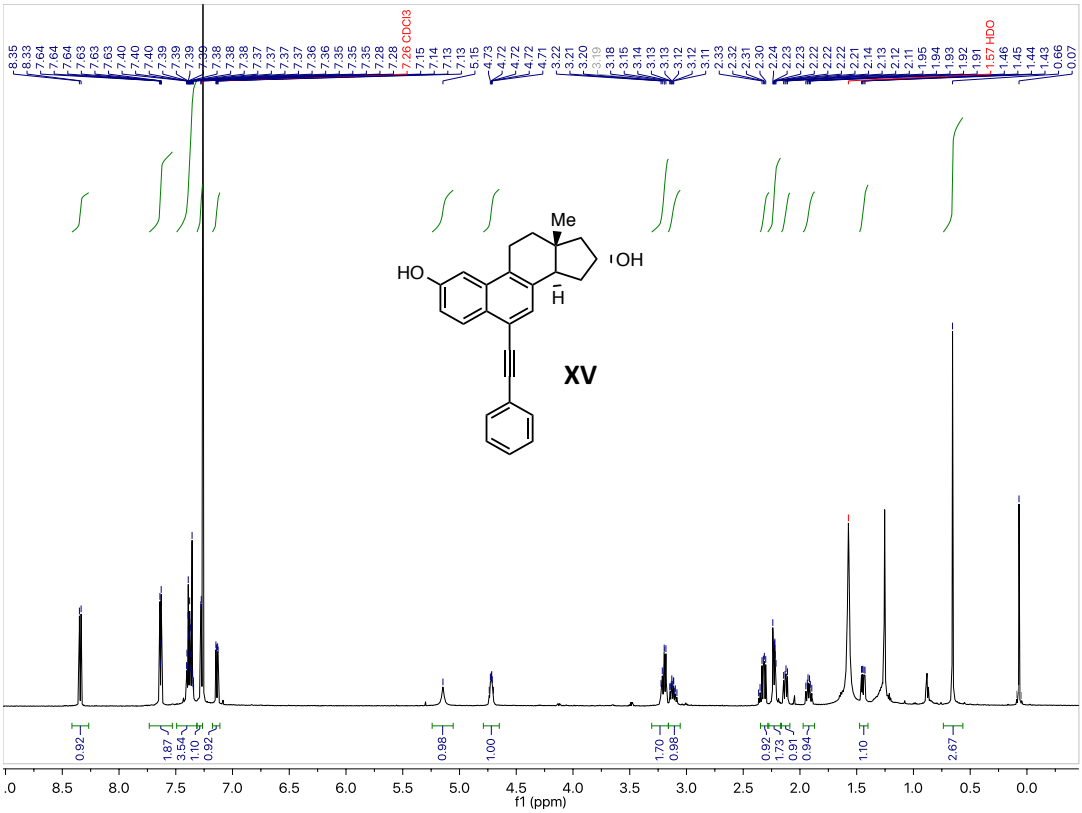


Figure S6: ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) of **XV**



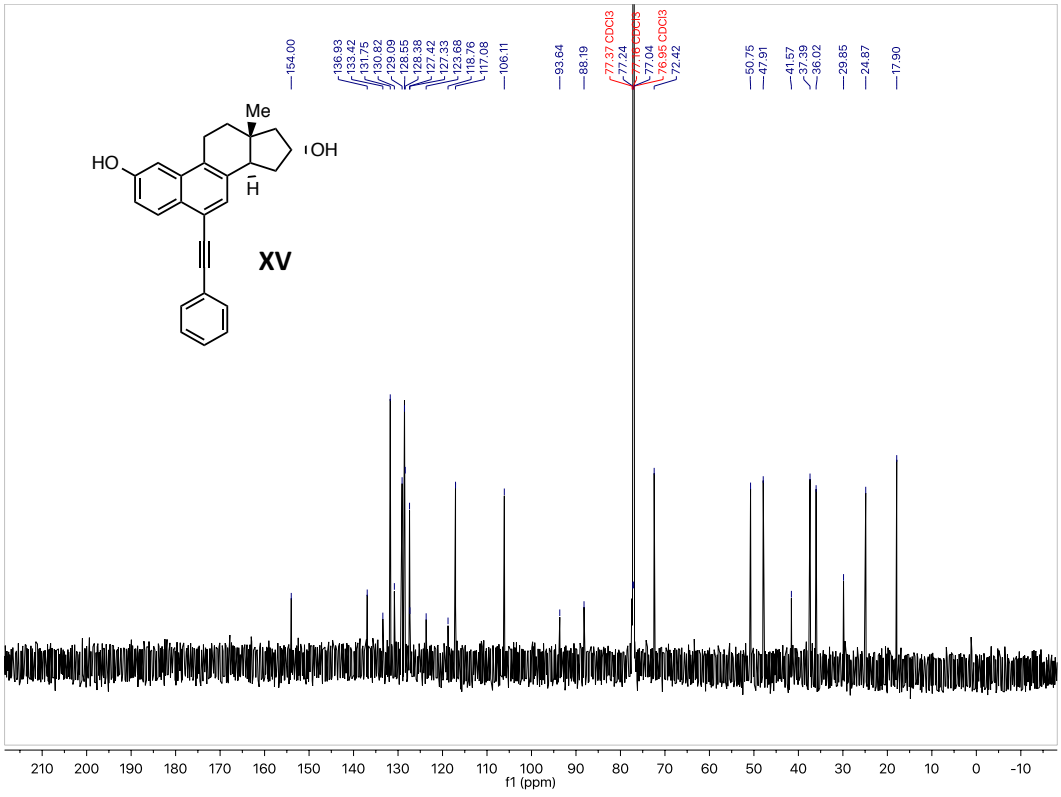
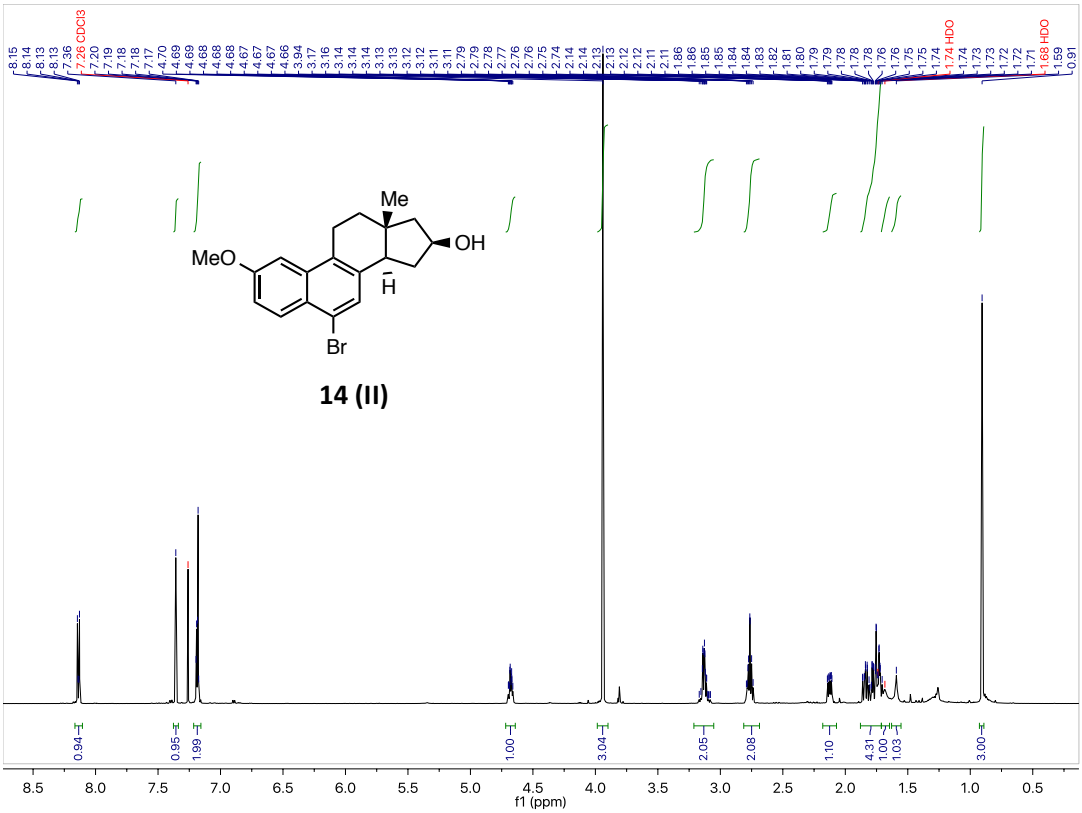


Figure S7: ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) of **14 (II)**



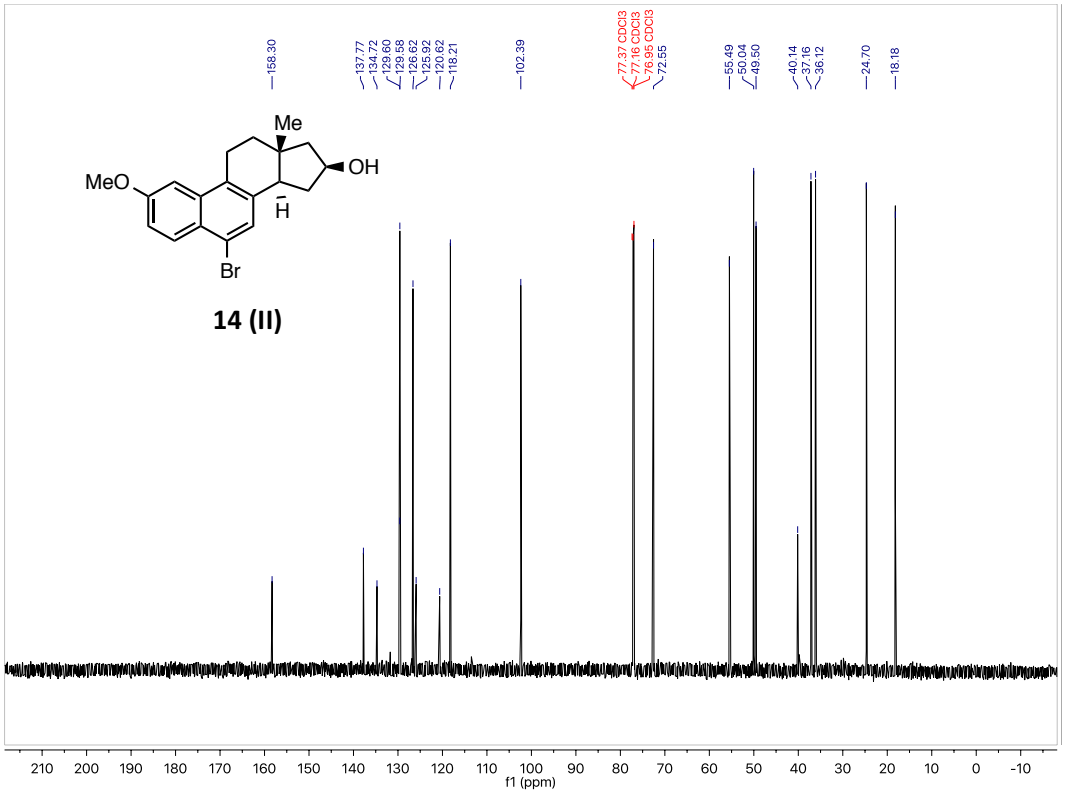
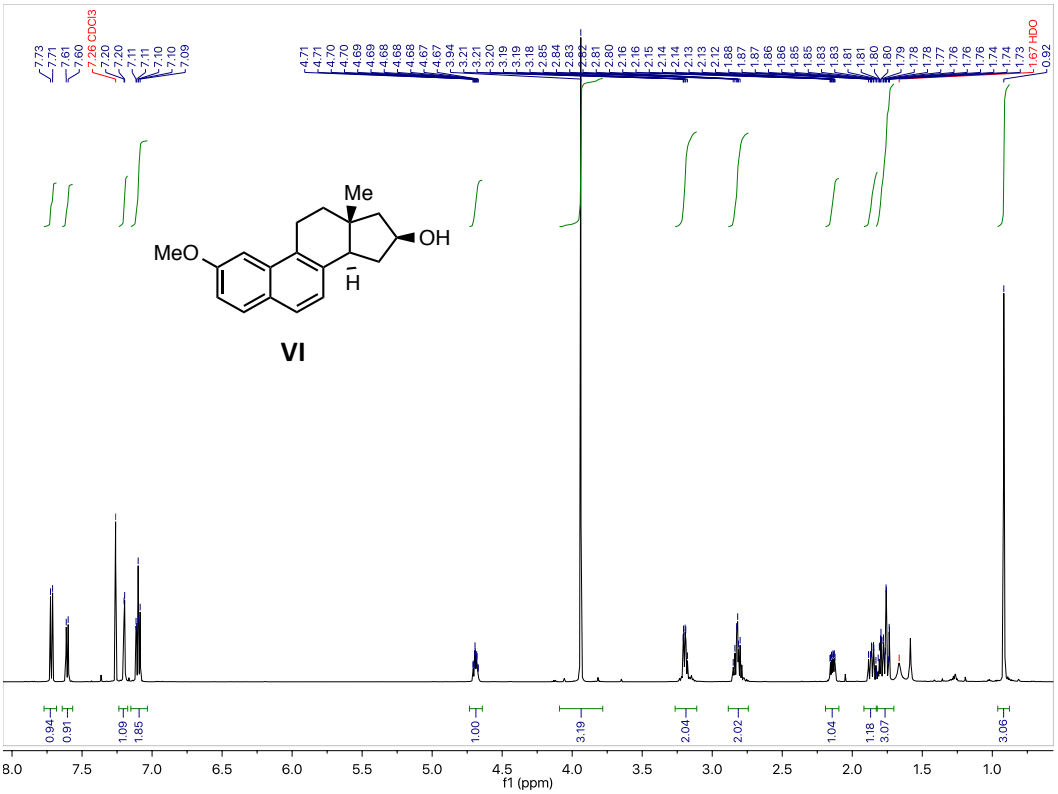


Figure S8: ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) of **VI**



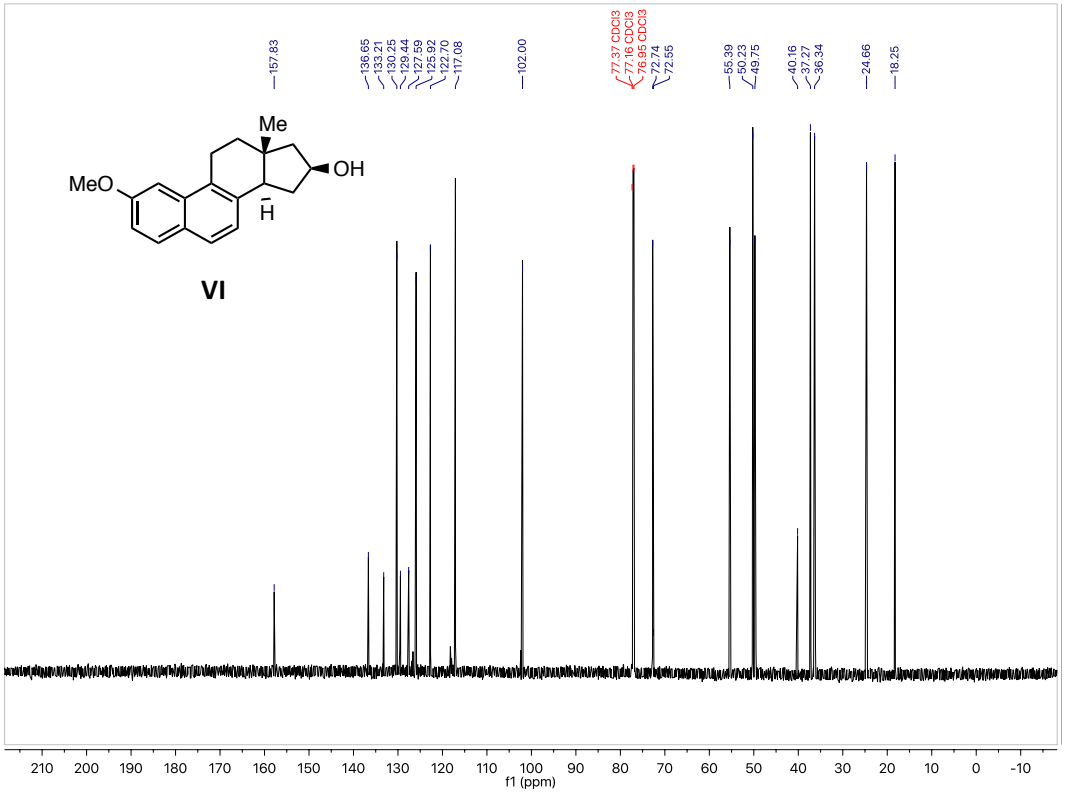


Figure S9: ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) of **XIII**

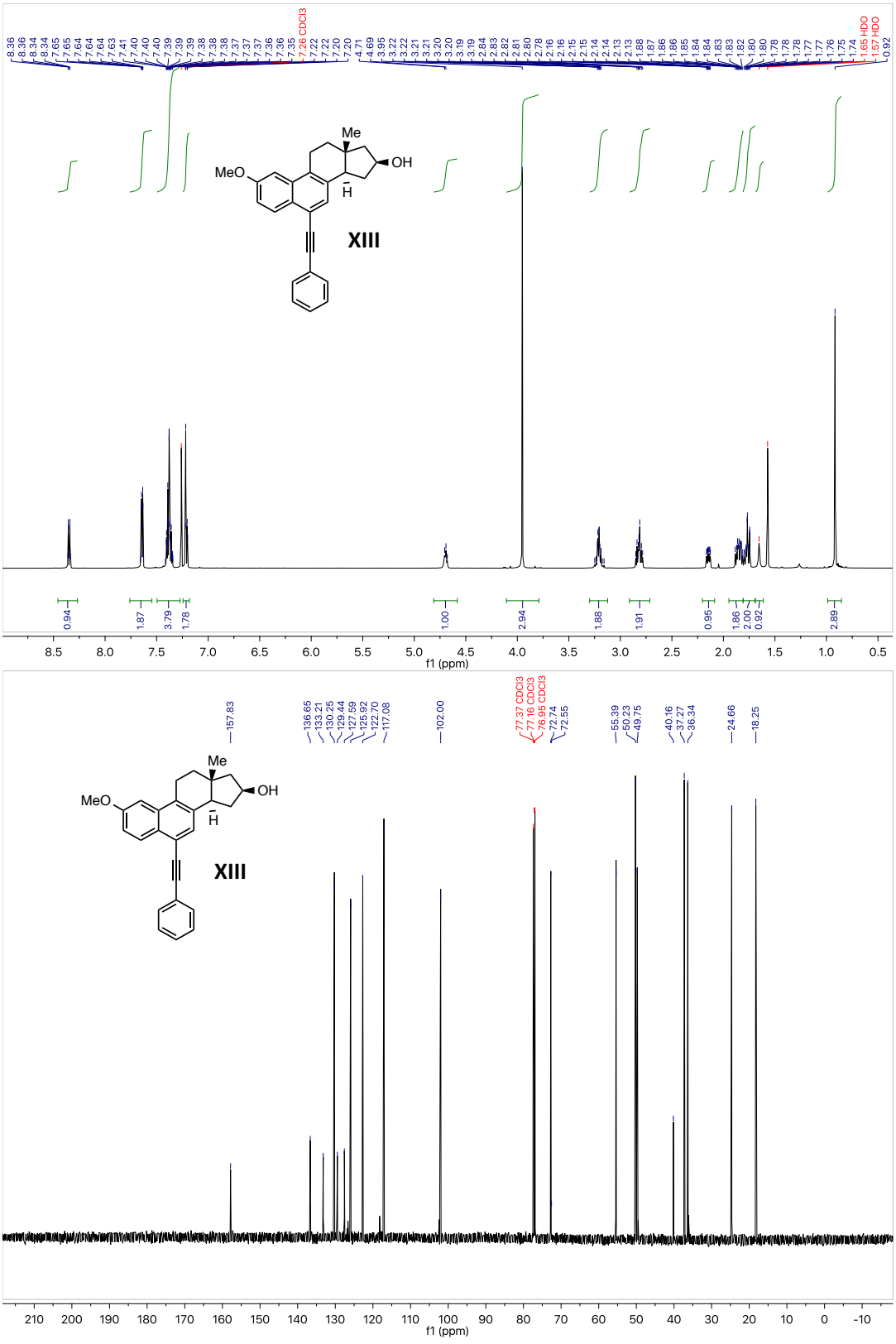
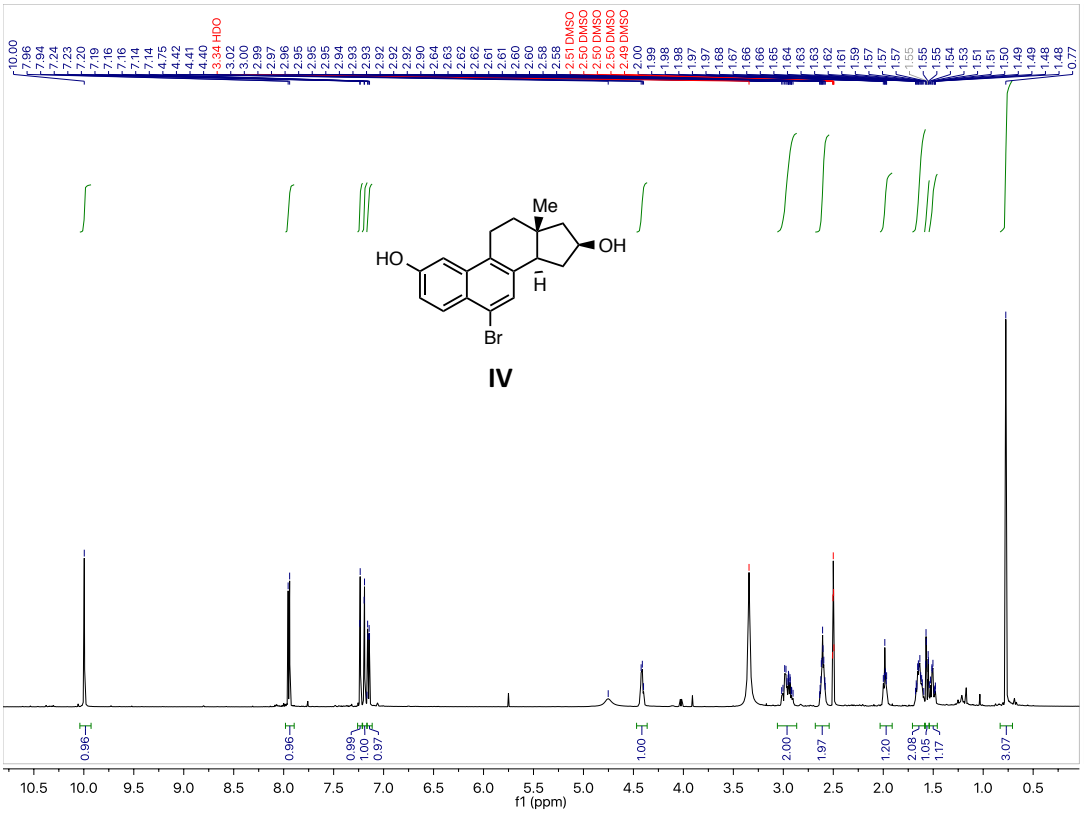


Figure S10: ^{1}H NMR (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD) of **IV**



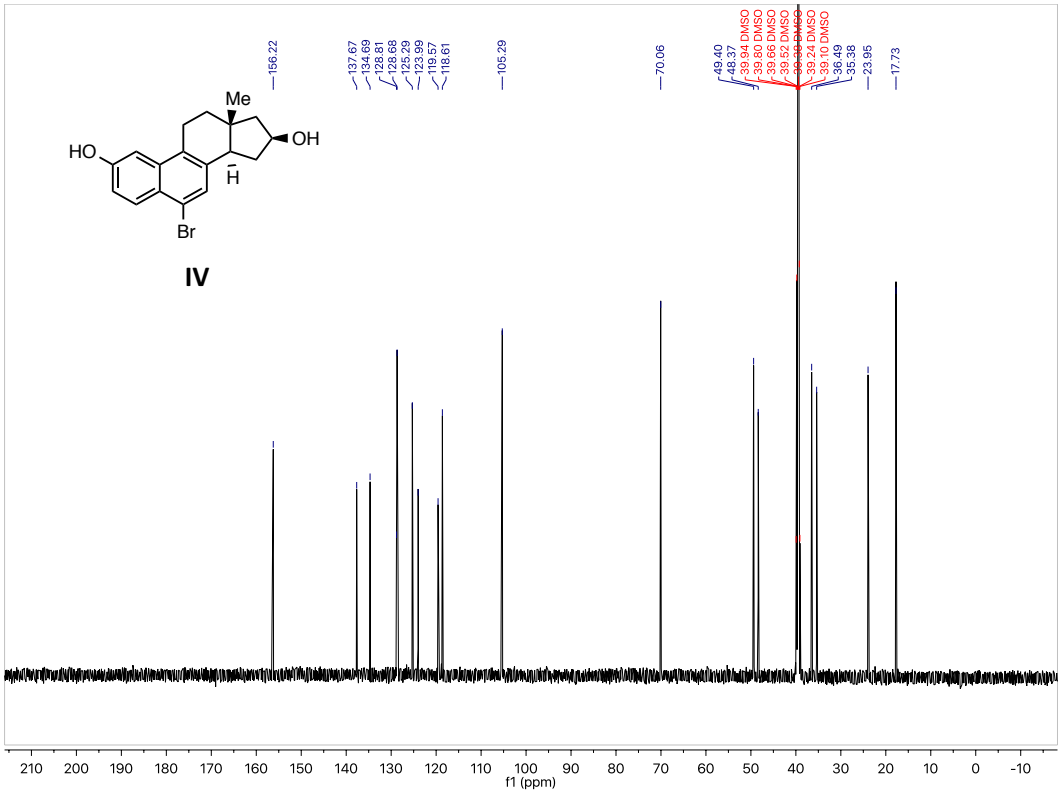


Figure S11: ^{1}H NMR (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD) of **VIII**

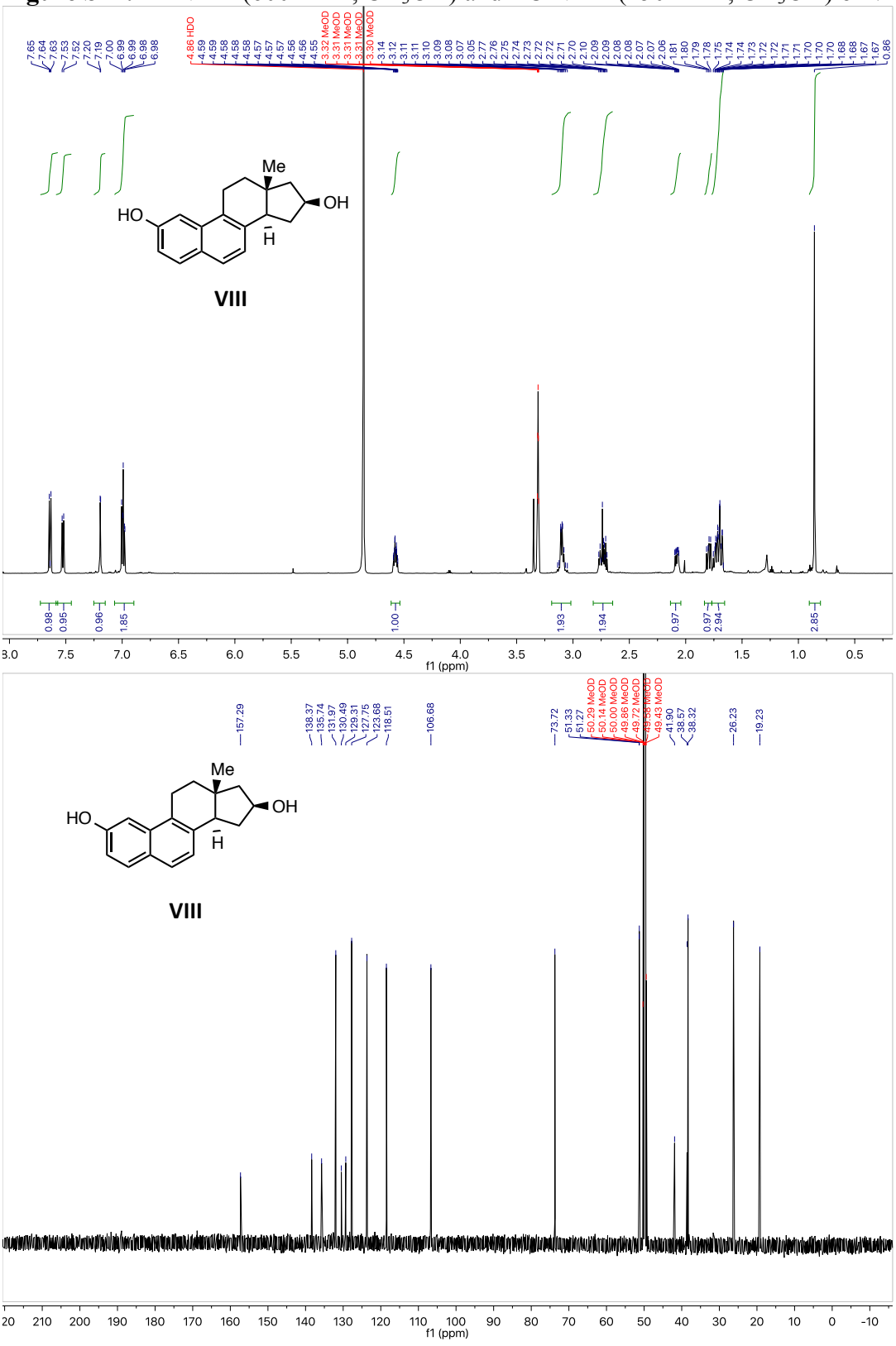
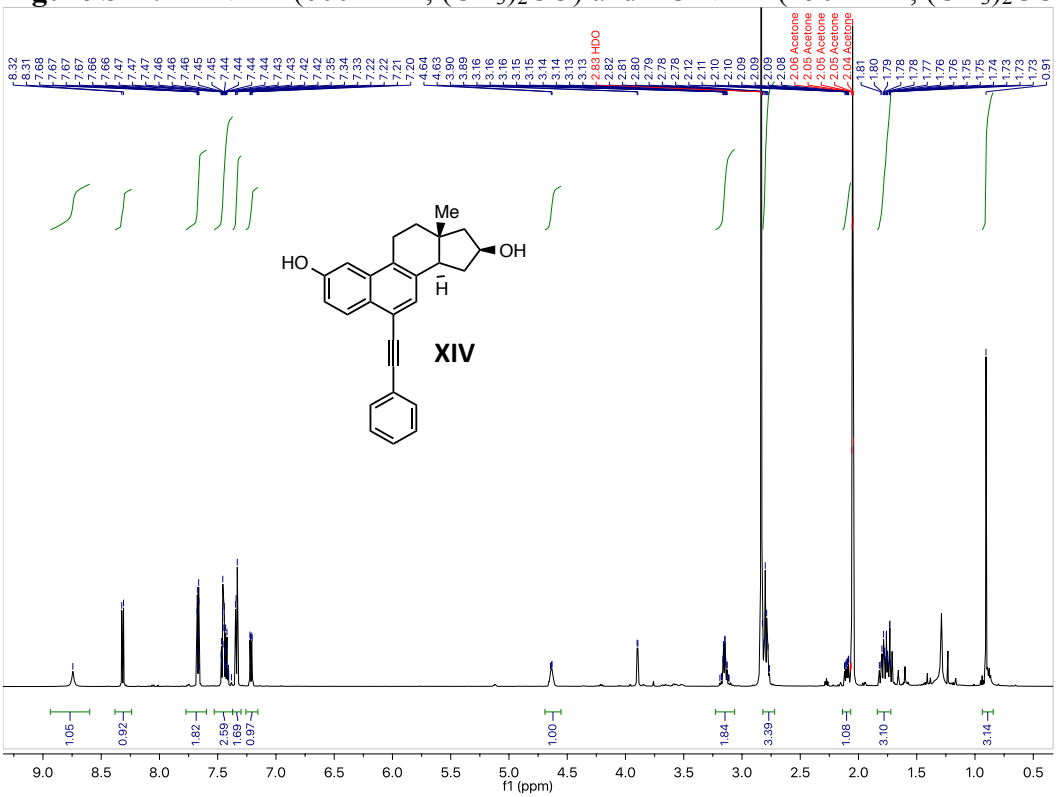


Figure S12: ^{1}H NMR (600 MHz, $(CD_3)_2CO$) and ^{13}C NMR (150 MHz, $(CD_3)_2CO$) of **XIV**



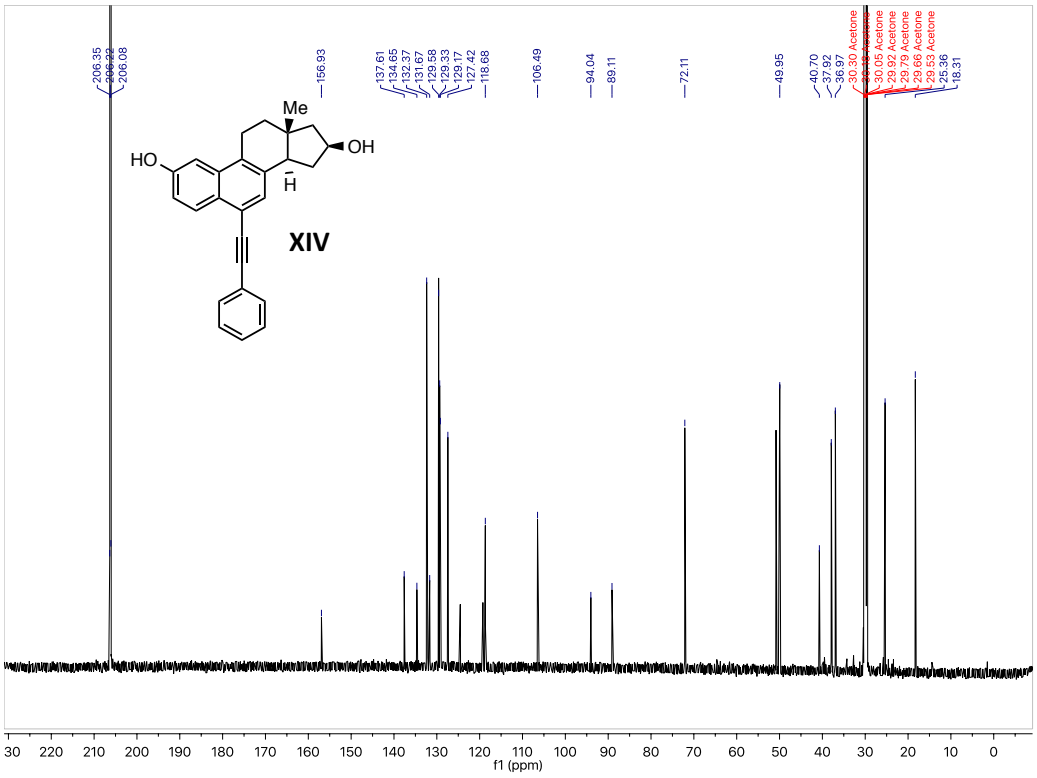


Figure S13: ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) of **IX**

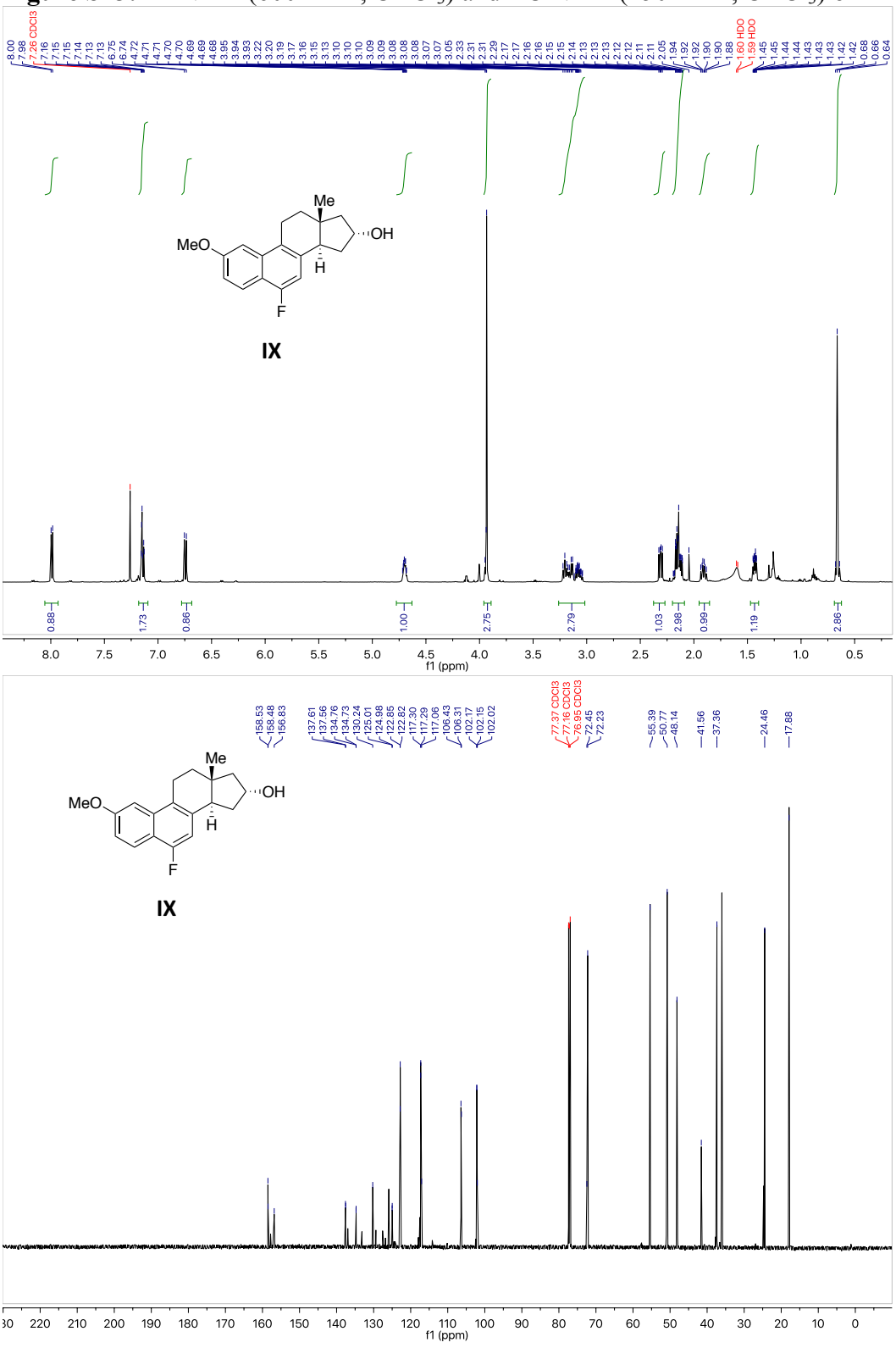


Figure S14: ^{1}H NMR (600 MHz, CD_3OD) and ^{13}C NMR (150 MHz, CD_3OD) of **X**

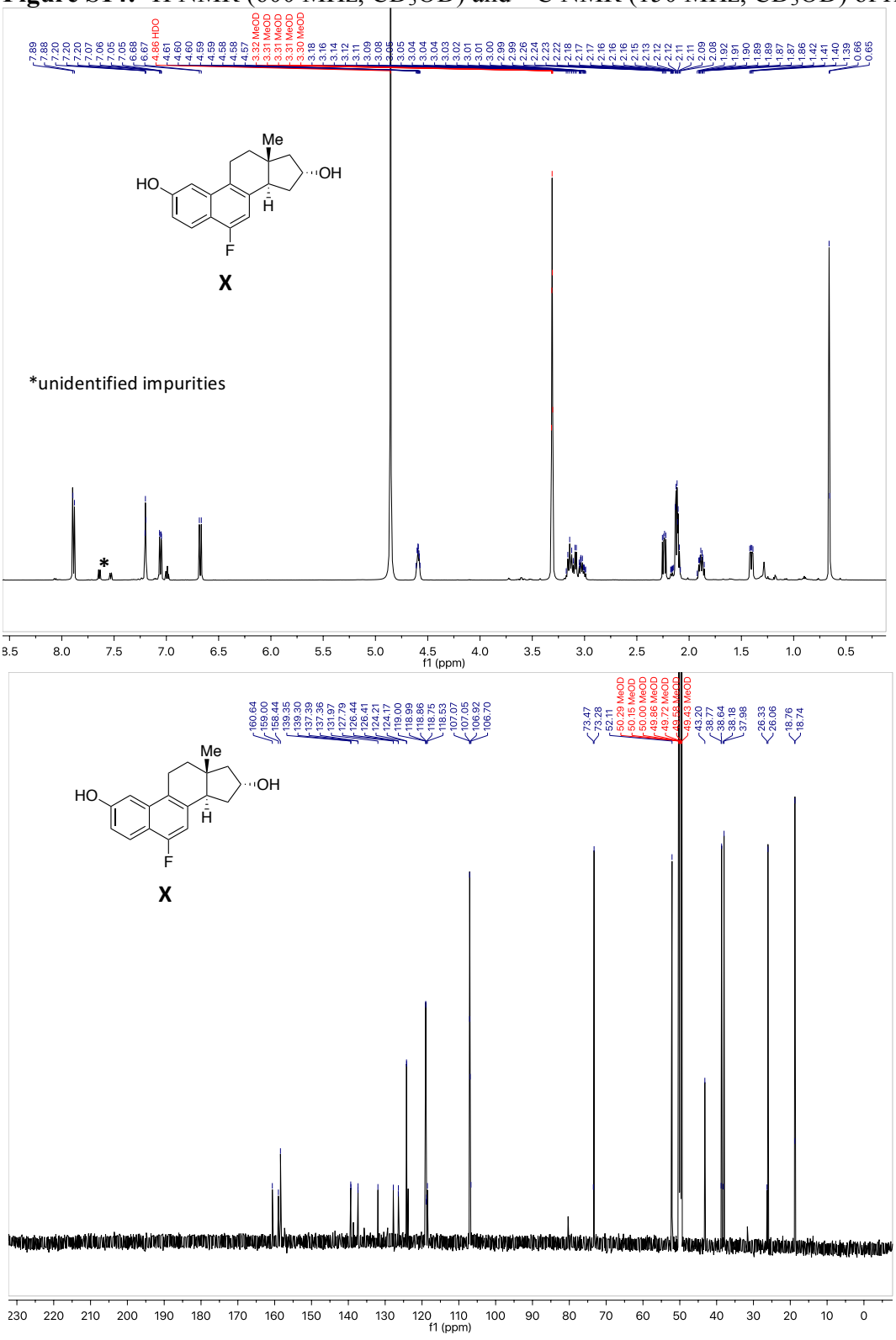
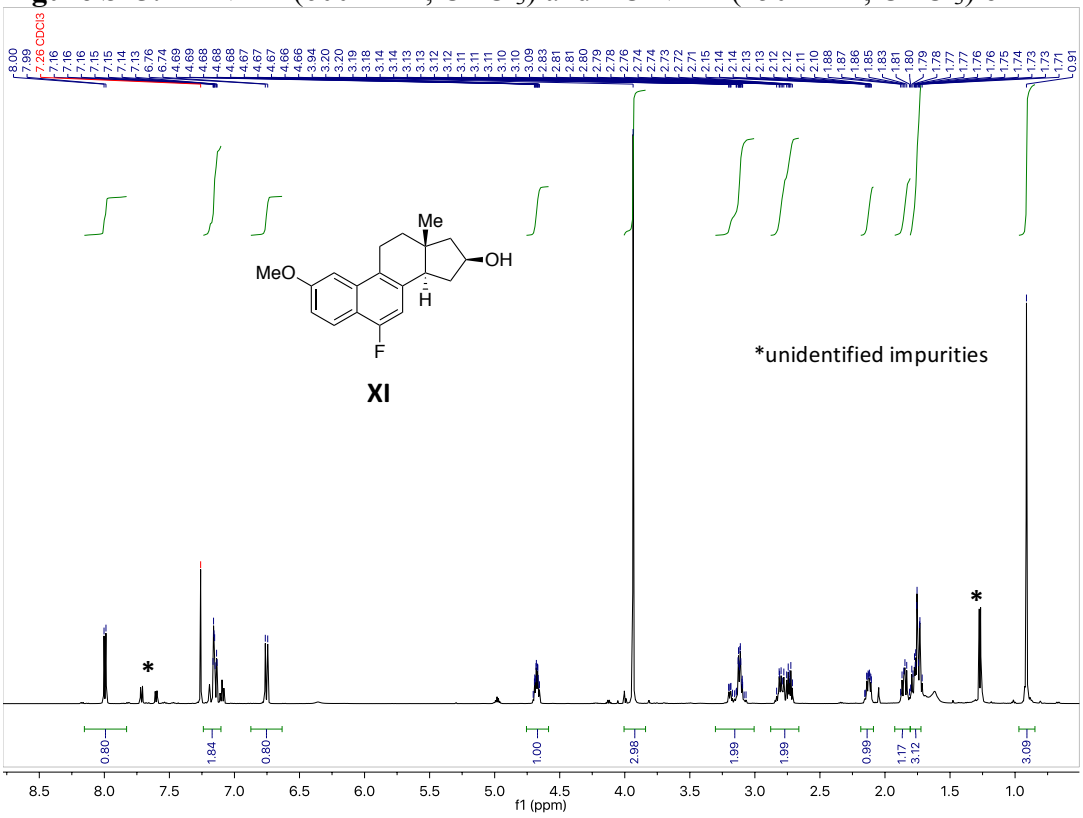
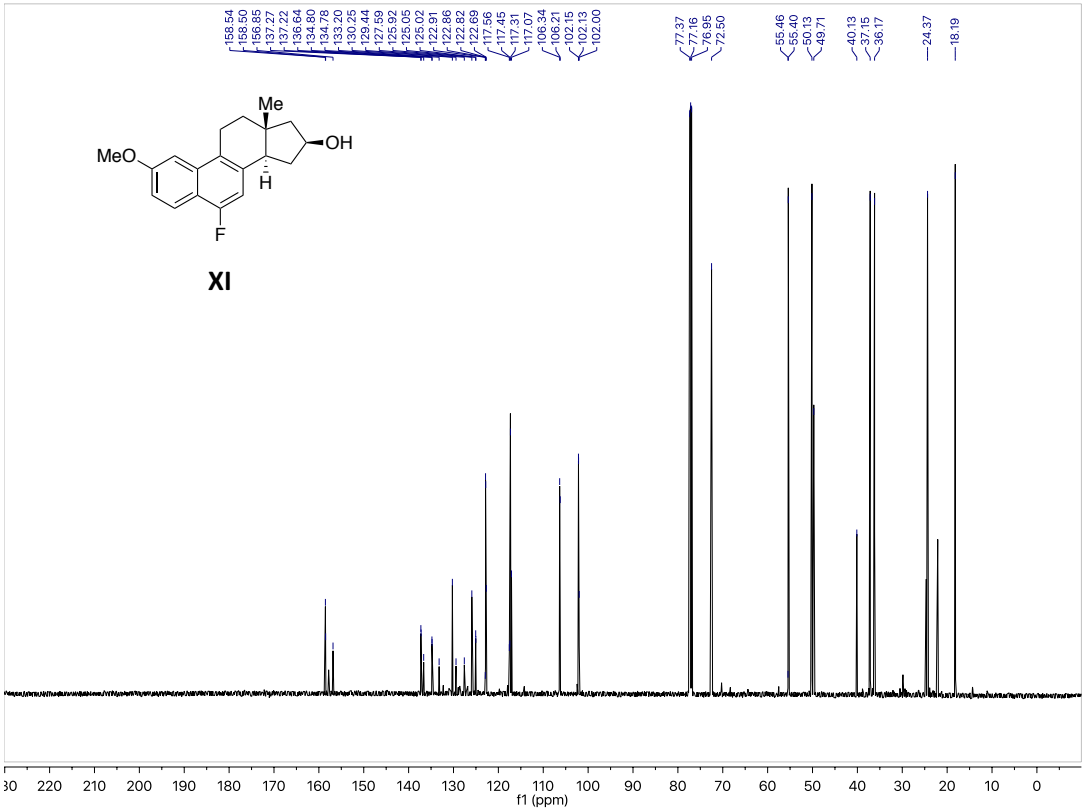


Figure S15: ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) of **XI**





4. Biological Evaluations

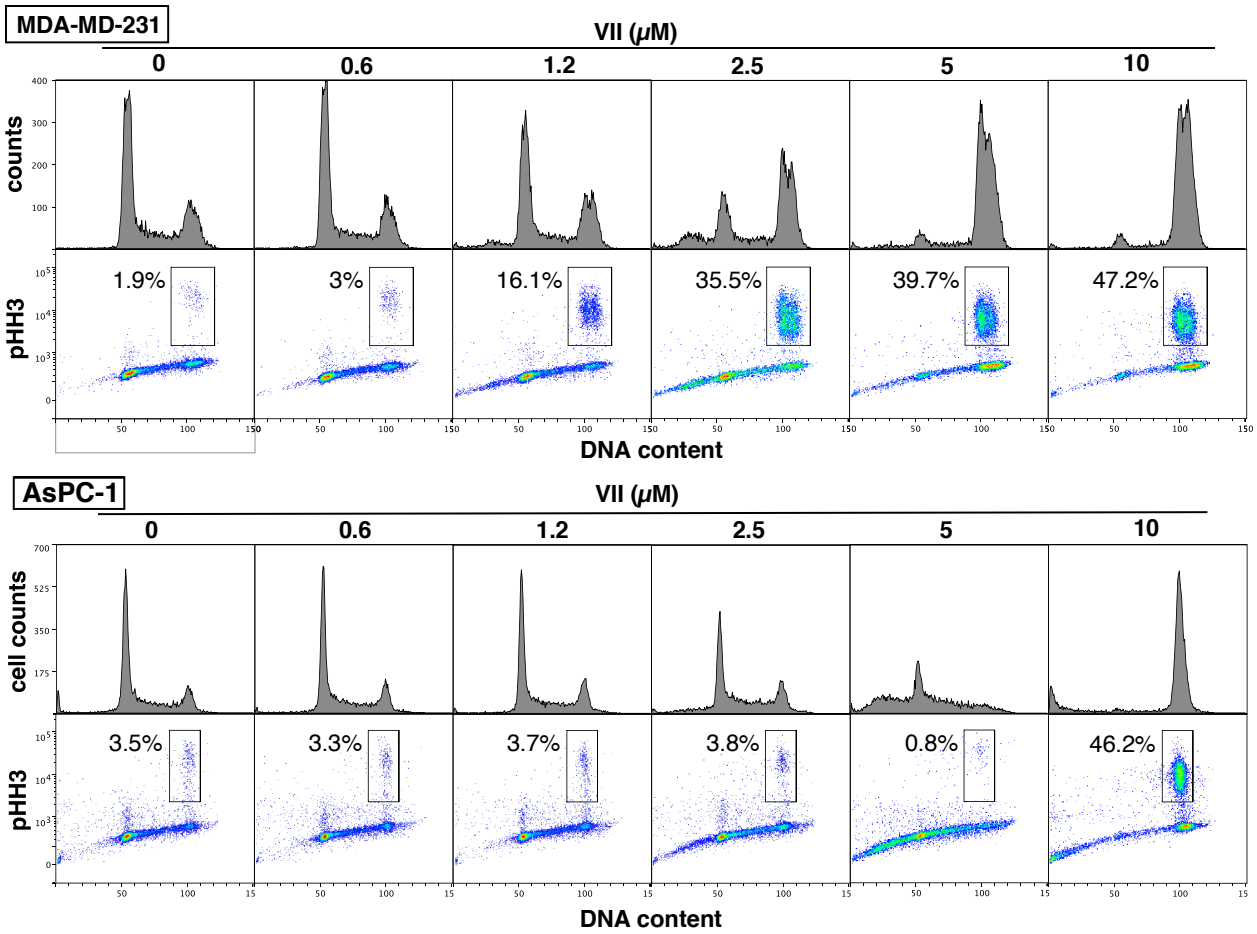


Figure S16: VII induces mitotic arrest in cells. MDA-MB-231 and AsPC-1 cells were incubated with VII for 24 h, then fixed in 70% ethanol, stained for DNA content (propidium iodide) and for phospho-histone H3 – a marker of cells in mitosis (antibody detecting pHH3, conjugated to a fluorescent tag), and analyzed by flow cytometry. The cells that accumulated with G2/M DNA content stained positive for pHH3 demonstrating that they are arrested in mitosis. The % values reflect the cells with pHH3. At concentrations that begin to cause arrest, some cells exhibit less than G1 DNA content suggesting they are undergoing apoptosis (2.5 μM for MDA-MB-231 and 5 μM for AsPC-1). This response is fairly typical of partial microtubule disruption, whereas complete microtubule disruption fully arrests cells in G2/M.

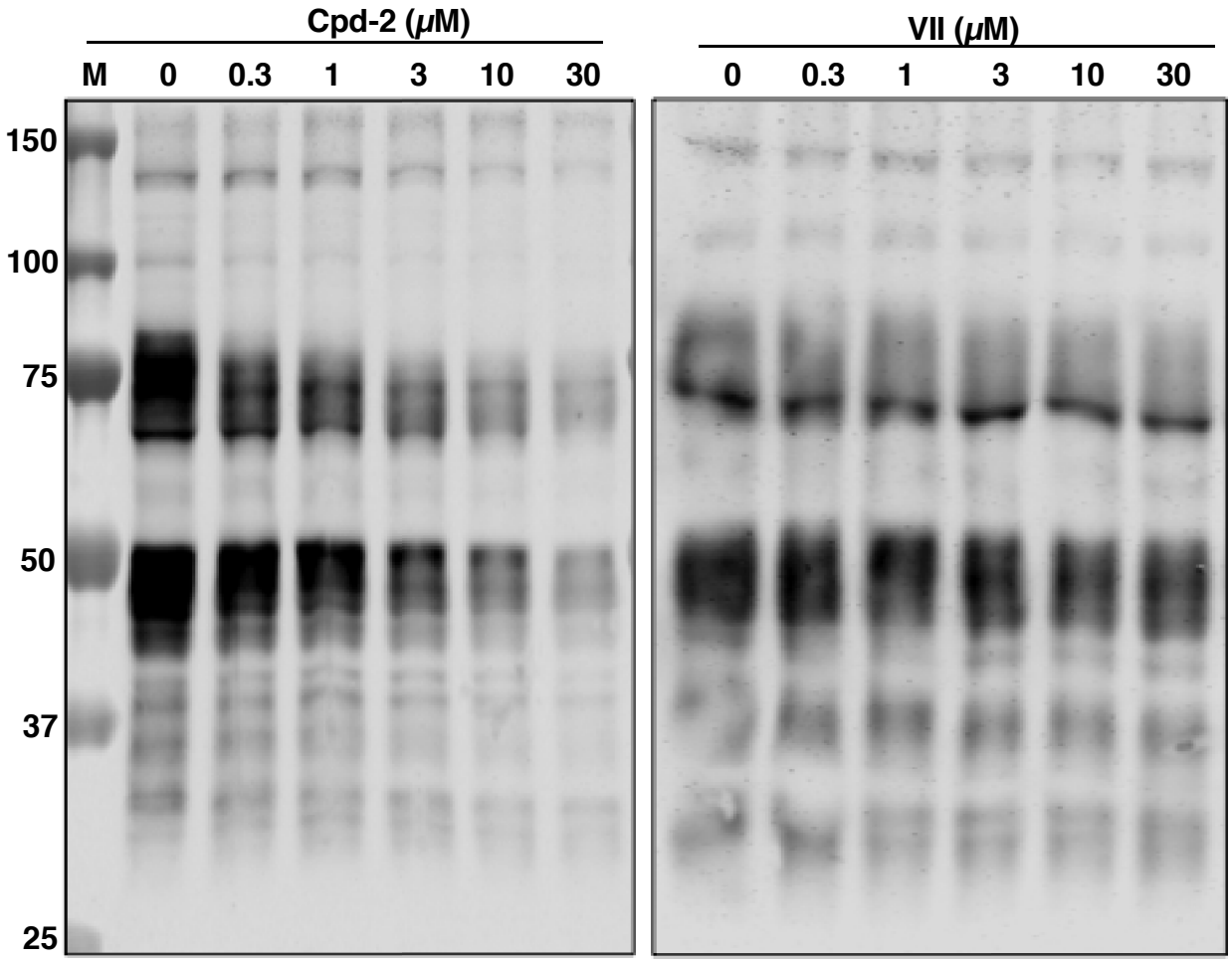


Figure S17: Compound **VII** does not inhibit CLK1/2/4 in cells. CLK1/2/4 are involved in mRNA splicing and phosphorylate serine-arginine (SR) proteins. The phosphorylated-SR proteins are detected on a western blot using an antibody to this phosphorylated peptide in the SR proteins. MDA-MB-468 cells were incubated with Cpd-2 for 3 h as a positive control demonstrating the reported inhibition of pSR (ref 20). A parallel incubation with **VII** had no significant impact on the levels of pSR proteins up to at least 30 μM. M=molecular weight markers from 25 – 150 kDa.

Estrogen Receptor Analyses

Assay Methods

▪ *Reporter Cells.* Reporter Cells used in the various ER assays express the ER native receptors. The reporter gene, firefly luciferase, is functionally linked to upstream receptor- specific genetic response elements (GRE), as summarized in **Tables S1 and S2**.

▪ *Compound Handling.* The compounds were stored as directed by the Study Sponsor. ▪ *Setup of ER Agonist Assays.* The ERs assays were performed as depicted in **Figure S18**.

Step 1: A suspension of Reporter Cells was prepared in Cell Recovery Medium (CRM; containing 10% charcoal stripped FBS).

For *agonist* assays, 100 of the Reporter Cell suspension was dispensed into wells of a white 96-well assay plate.

Step 2: Immediately prior to assay setup, Master stocks were diluted appropriately using compound screening medium (CSM; containing 10% charcoal stripped FBS) to generate '2x-concentration' treatment media. DMSO concentration across treatment groups was normalized to final concentration of 0.1% in the assay wells. 100 µl of each treatment medium was dispensed into duplicate assay wells pre-dispensed with Reporter Cells. Assay plates were incubated at 37°C for 24 hr.

Step 3: Following the incubation period, for *agonist* assays, treatment media were discarded and 100 µl/well of Luciferase Detection Reagent was added. RLUs were quantified from each assay well to determine NR *agonist* activity.

Table S1: Reporter Cells used in the various assays express the ER native receptors. The reporter gene, firefly luciferase, is functionally linked to upstream receptor- specific genetic response elements (GRE). The cell line background of respective Reporter Cells is indicated, as are the reference compounds used to confirm the performance of the respective assays in this study

Receptor (gene symbol)	**Receptor form ··· Reporter Vector**	**Host Cell Line**	**INDIGO Reference Agonist**
Nuclear Receptor Assays			
ERβ (NR3A2)	Native Receptor ··· ER GRE-Luciferase	CHO	17β-Estradiol

Table S2: provides a concise Summary of ERβ Agonist Assay Results. *Agonist* data values ≥ 2-fold activation (highlighted with green) are deemed to be significant and may warrant further consideration (Note: Compound **JA-1-58** in the table is compound **VII** in this manuscript)

Compound	nM	ERβ	
		Fold activation	EC50, nM
17β-Estradiol	0.800	8.4	0.0163
DMSO	0.10%	1.0	—
JA-1-58	0.004	1.0	20.9
	0.015	1.3	
	0.061	1.2	
	0.244	1.0	
	0.977	1.2	
	3.91	1.7	
	16	4.9	
	63	10	
	250	10	
	1,000	12	

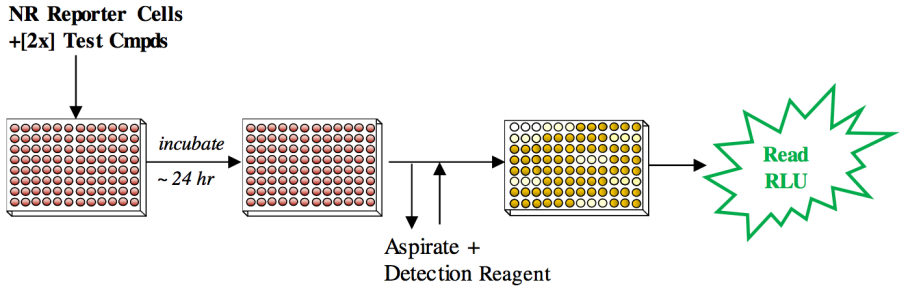


Figure S18: Agonist Assays

Assay Validation

Reference compounds were utilized to confirm the performance of the specific lot of Reporter Cells treated with the Sponsor's test compounds. Reference Compound and Test Compound assays were performed at the same time and, hence, were exposed to the same assay reagents and environmental conditions. Refer to individual data sets for the identities of specific reference agonist and their respective treatment concentration ranges. Reference groups always include a 'Vehicle' control to determine background activity in the assay and to calculate values of fold-activation of receptor activities.

Data Reduction

Microsoft Excel was used to manage and archive assay data, as well as to calculate average RLU values +/- Standard Deviation (SD), Fold-activation, Percent Coefficients of Variation (%CV),

and Z' values.

- **Percent Coefficient of Variation** (%CV): 100*(SD/Ave. RLU)
- ***Fold*-Activation** in ***Agonist*** assays: [Ave $RLU^{TestCmpd}$ / Ave $RLU^{Vehicle}$]
- **Z' for Reference *Agonists***: 1 - [(3*[$SD^{Vehicle}$ + SD^{RefMax}]) / (RLU^{RefMax} - $RLU^{Vehicle}$)]

Graphical Data Methods

Dose-response curve (DRC) analyses of reference compounds were performed *via* non-linear curve-fitting of Fold-Activation *vs.* Log[Cmpd] for agonist assay using GraphPad Prism software.

Reports regarding outsourced biological evaluations uploaded as separate files:

1. **DiscoverX "BioMAP Diversity PLUS Data Report"**
2. **DiscoverX "KINOME*scan*" profiling service**
3. **DiscoverX "Kd report"**
4. **Indigo Biosciences estrogen receptor analyses.**

Note: Compound **JA-1-58** in these reports is compound **VII** in this manuscript