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Synthesis, biological evaluations and molecular modelling of new analogs of the anti-cancer agent 2-methoxyestradiol: Potent inhibitors of angiogenesis

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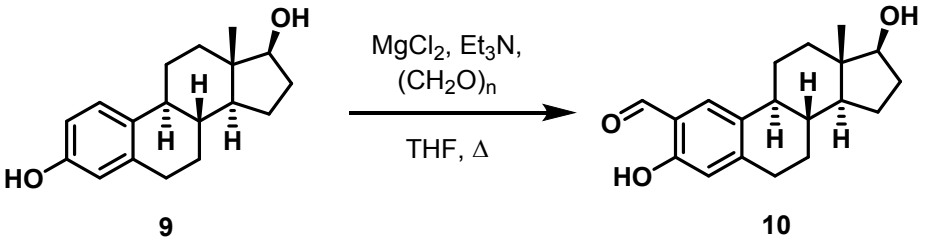
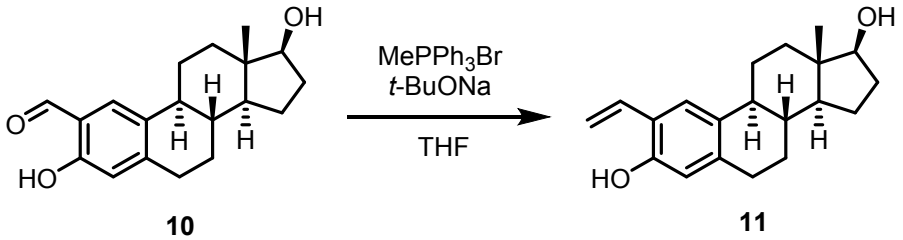
*2-Formylestradiol (**10**)*[1]



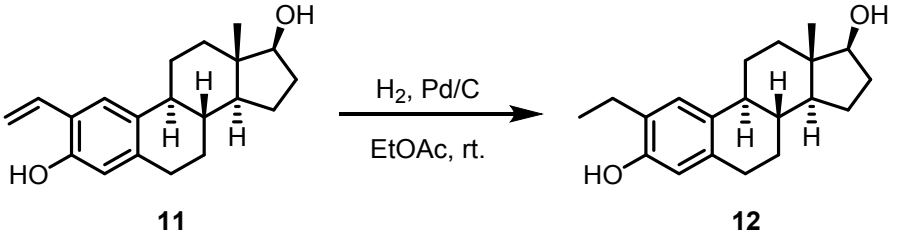
To a solution of estradiol (**9**) (2.72 g, 10 mmol) in dry THF (100 mL), anhydrous $MgCl_2$ (2.85 g, 30 mmol), Et_3N (4.2 mL, 30 mmol), and paraformaldehyde (1.2 g, 40 mmol) were added under an argon atmosphere. After refluxing the mixture for 4 hours, TLC (hexane/EtOAc, 8:2) revealed complete consumption of **9**, and the reaction mixture was cooled and quenched (1 M HCl, 50 mL). The aqueous phase was extracted with EtOAc (3x50 mL) and the combined organic phases were washed with brine and then dried ($MgSO_4$). The crude product was purified by flash chromatography (silica gel, 20% ethyl acetate in hexane, R_f = 0,43) to give the pure product in 81% yield 2.44 g, mp 231-233 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.77 (s, 1H), 9.81 (s, 1H), 7.42 (s, 1H), 6.70 (s, 1H), 3.74 (t, *J* = 8.4 Hz, 1H), 3.01 – 2.73 (m, 2H), 2.42 – 2.29 (m, 1H), 2.24 – 2.06 (m, 2H), 2.05 – 1.95 (m, 1H), 1.95 – 1.80 (m, 1H), 1.81 – 1.65 (m, 1H), 1.64 – 1.13 (m, 8H), 0.79 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 196.2, 159.4, 148.2, 132.9, 130.7, 119.1, 117.1, 81.9, 50.2, 43.6, 43.4, 38.6, 36.6, 30.7, 30.3, 26.9, 26.4, 23.3, 11.2.

*2-Allylestradiol (**11**)*



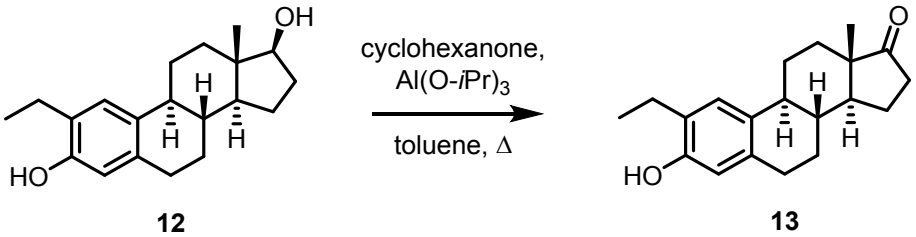
To a suspension of methyltriphenylphosphonium bromide (16.5 mmol, 5.9 g) in anhydrous THF (100 mL) at 0° C under argon *t*-BuONa (16.5 mmol, 1.6 g) was added. The suspension was stirred for 15-20 min at 0° C to give a yellow solution. 2-Formylestradiol **10** (5.5 mmol, 1.65 g) was added and the turbid mixture was stirred for 4 h at room temperature. A saturated ammonium chloride solution was added and the aqueous phase was extracted with DCM (3x20 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated. The compound was purified by flash chromatography (silica gel, 20% ethyl acetate in hexane, R_f = 0,43) to give the pure product in 84% yield 1.38 g, mp. 159-161 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31 (s, 1H), 6.91 (dd, *J* = 17.7, 11.2 Hz, 1H), 6.54 (s, 1H), 5.71 (dd, *J* = 17.7, 1.4 Hz, 1H), 5.32 (dd, *J* = 11.2, 1.4 Hz, 1H), 3.76 (t, *J* = 8.4 Hz, 1H), 2.93 – 2.71 (m, 2H), 2.48 – 2.29 (m, 1H), 2.27 – 2.09 (m, 2H), 2.03 – 1.93 (m, 1H), 1.94 – 1.83 (m, 1H), 1.79 – 1.66 (m, 1H), 1.63 – 1.10 (m, 9H), 0.81 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 150.9, 138.1, 133.0, 132.1, 124.4, 122.4, 115.9, 114.9, 82.1, 50.2, 44.0, 43.4, 39.0, 36.9, 30.8, 29.5, 27.3, 26.5, 23.3, 11.2.

*2-Ethylestradiol (**12**)*



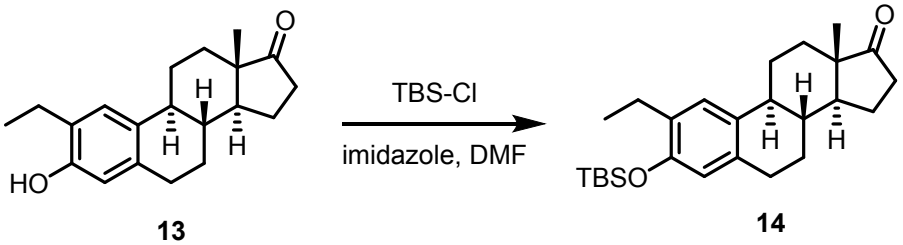
A solution of 2-allylestradiol **11** (7.3 mmol, 2.1 g) in EtOAc (70 mL) was added at room temperature to a stirred solution Pd-C (10% (w/w)) in EtOAc. The resulting mixture was stirred under H_2 gas under complete conversion of **11**. Upon completion, 20 mL dichloromethane was added, and the reaction mixture was filtered through celite and washed with dichloromethane (3 x 30 mL). The combined filtrates were concentrated *in vacuo*, and the residue purified by flash chromatography (silica gel, 20% ethyl acetate in hexane, R_f = 0.43) to give the pure **12** in 89% yield 1.95 g, mp. 165-166 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.06 (s, 1H), 6.50 (s, 1H), 4.82 (s, 1H), 3.75 (t, *J* = 8.5 Hz, 1H), 2.92 – 2.70 (m, 2H), 2.60 (q, *J* = 7.5 Hz, 2H), 2.41 – 2.27 (m, 1H), 2.22 – 2.00 (m, 2H), 2.00 – 1.92 (m, 1H), 1.92 – 1.80 (m, 1H), 1.80 – 1.62 (m, 1H), 1.58 – 1.27 (m, 7H), 1.23 (t, *J* = 7.6 Hz, 3H), 0.79 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 151.4, 135.6, 132.7, 127.4, 126.5, 115.3, 82.1, 50.2, 44.2, 43.4, 39.1, 36.9, 30.7, 29.4, 27.4, 26.6, 23.3, 23.2, 14.6, 11.2. HRMS calcd. for $C_{20}H_{28}O_2$ $[M]^{\bullet+}$ 300.2089. Found. 300.2086.

*2-Ethylestrone (**13**)*



2-Ethylestradiol **12** (2.41 g, 8.0 mmol) was placed in a flame dried 250 mL two-necked round-bottomed flask equipped with a 5 mL flame dried Dean-Stark trap and a reflux condenser. Toluene (80 mL) was added to dissolve the starting material. Aluminum-*iso*-propoxide (8.17 g, 40 mmol) and cyclohexanone (33.1 mL, 320 mmol) were added, and the reaction mixture was heated at reflux (145 °C) for 20 h. The reaction mixture was allowed to cool to room temperature. Then water (100 mL) and 1 M HCl (45 mL) were added. The organic material was extracted with ethyl acetate (3 x 150 mL). The aqueous emulsion was acidified with 1 M HCl (50 mL) and left until the emulsion separated. The aqueous layer was extracted once more with ethyl acetate (100 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated *in vacuo*. The residue was triturated with hexane to give **13**, as white crystals in 75% yield 1.79 g, mp. 201-204 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.06 (s, 1H), 6.52 (s, 1H), 4.73 (s, 1H), 3.03 – 2.81 (m, 2H), 2.60 (q, J = 7.5 Hz, 2H), 2.55 – 2.46 (m, 1H), 2.46 – 2.36 (m, 1H), 2.30 – 2.21 (m, 1H), 2.20 – 2.10 (m, 1H), 2.09 – 1.90 (m, 3H), 1.74 – 1.36 (m, 6H), 1.23 (t, J = 7.5 Hz, 3H), 0.92 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 221.4, 151.5, 135.3, 132.1, 127.5, 126.5, 115.4, 50.6, 48.2, 44.2, 38.6, 36.1, 31.8, 29.2, 26.7, 26.2, 23.2, 21.7, 14.5, 14.0. HRMS calcd. for $C_{20}H_{26}O_2$ $[M]^{\bullet+}$ 298.1933 Found. 298.1930.

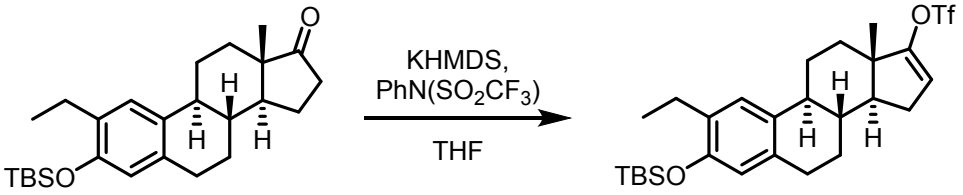
*3-tert-Butyldimethylsiloxy-2-ethylestrone (**14**)*



2-Ethylestrone **13** (1.51 g, 5.0 mmol) was placed in a 100 mL round bottomed flask and dissolved in dry DMF (25 mL). Imidazole (0.85 g, 12.5 mmol) and TBSCl (1.13 g, 7.5 mmol) were added. The reaction mixture was stirred at room temperature under argon atmosphere for 5 h. Saturated aqueous NaCl (20 mL) was added, and the mixture was extracted with ethyl acetate (3 x 20 mL). The combined organic extracts were washed with brine (10 mL), aqueous AcOH (10%, 10 mL) and aqueous $NaHCO_3$ (10 mL) before it was dried ($MgSO_4$) and evaporated *in vacuo*. The product was purified by flash chromatography (silica gel, 20% ethyl acetate in hexane, R_f = 0,43)

to give **14** as a colorless oil in 93% yield (1.93 g). $[\alpha]^{D}$ = - 109 (c = 0.1, MeOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.06 (s, 1H), 6.50 (s, 1H), 2.93 – 2.76 (m, 2H), 2.57 (q, *J* = 7.6 Hz, 2H), 2.54 – 2.46 (m, 1H), 2.43 (d, *J* = 11.9 Hz, 1H), 2.24 (dd, *J* = 13.6, 7.0 Hz, 1H), 2.11 (d, *J* = 8.6 Hz, 1H), 2.08 – 1.93 (m, 3H), 1.69 – 1.32 (m, 6H), 1.17 (t, *J* = 7.5 Hz, 3H), 1.02 (s, 9H), 0.92 (s, 3H), 0.23 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 221.1, 151.5, 134.7, 132.2, 132.1, 126.4, 118.4, 50.6, 48.2, 44.3, 38.6, 36.0, 31.8, 29.4, 26.8, 26.1, 25.9, 23.7, 21.8, 14.8, 14.0, -4.0, -4.0.

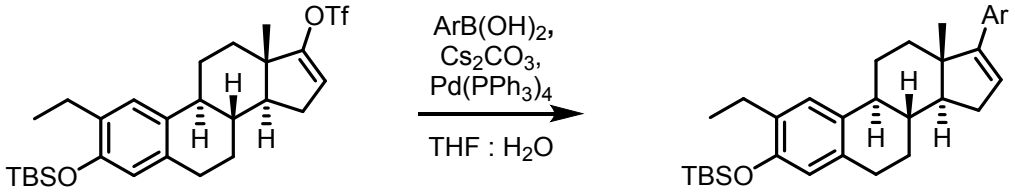
*(8R,9S,13S,14S)-3-((tert-butyldimethylsilyl)oxy)-2-ethyl-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-17-yl trifluoromethanesulfonate (**15**)*



14 **15**

TBS protected 2-ethylestrone **14** (861 mg, 2.5 mmol) and *N*-Phenyl-bis(trifluoromethanesulfonimide) (1.34 g, 3.75 mmol) were dissolved in dry THF (50 mL) and cooled to -78 °C. NaHMDS (12.5 mL 0.6 M in toluene, 7.5 mmol), was added dropwise at −78 °C and the resulting mixture was stirred at -78 °C for 3 h. Then the reaction was brought to room temperature and quenched by addition of saturated aqueous NH_4Cl (20 mL). The mixture was extracted with CH_2Cl_2 (2 x 15 mL); the combined organic extracts were washed with water and brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by flash chromatography (silica gel, 20% ethyl acetate in hexane, R_f = 0,64) to give 1,39 g of **15** as a colorless oil (81% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.03 (s, 1H), 6.50 (s, 1H), 5.62 (dd, *J* = 3.2, 1.6 Hz, 1H), 2.98 – 2.73 (m, 2H), 2.58 (q, *J* = 7.5 Hz, 2H), 2.49 – 2.24 (m, 3H), 2.17 – 2.02 (m, 1H), 1.96 – 1.86 (m, 2H), 1.86 – 1.73 (m, 1H), 1.68 – 1.54 (m, 3H), 1.49 – 1.36 (m, 1H), 1.18 (t, *J* = 7.5 Hz, 3H), 1.02 (s, 9H), 1.02 (s, 3H), 0.24 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.6, 151.5, 134.6, 132.3, 132.2, 126.0, 118.5, 114.6, 53.8, 45.3, 44.5, 36.9, 33.0, 29.2, 28.5, 27.0, 26.0, 25.9, 23.8, 18.4, 15.5, 14.8, -4.0, -4.0.

*General procedure for the Suzuki cross-coupling reaction and synthesis of (**16a-e**)*

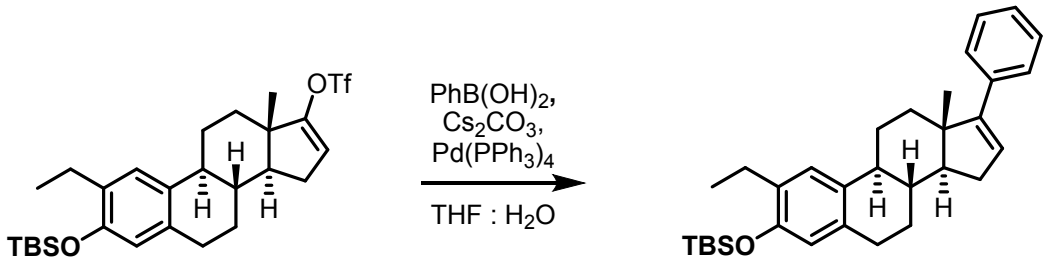


15 **16a-e**

The TBS protected steroid triflate **15** (0.5 mmol, 1 equiv.), cesium carbonate (2 equiv.) and the boronic acid (1.05 equiv.) was placed in a flame dried 50 mL round-bottomed flask under argon

atmosphere and dissolved in a 1:1 mixture of water and THF. $Pd(PPh_3)_4$ (5 mol%) was added and the reaction mixture was stirred at room temperature (18 – 22 h.). Upon completion the reaction mixture was poured into brine (15 mL) and extracted with ethyl acetate (4 x 5 mL). The combined organic extracts were dried ($MgSO_4$) and the solvent evaporated *in vacuo*. The residue was purified by flash chromatography (silica gel, 2 - 20% ethyl acetate in hexane) to give the pure products.

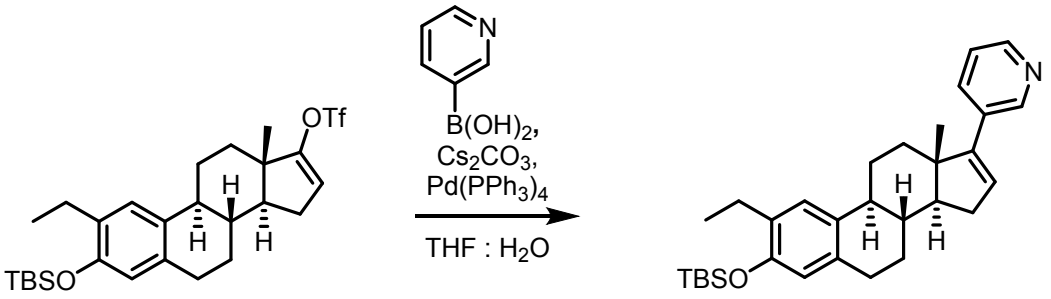
*tert-Butyl(((8S,9S,13S,14S)-2-ethyl-13-methyl-17-phenyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-3-yl)oxy)dimethylsilane (**16a**)*



15 **16a**

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41 (dd, *J* = 8.3, 1.3 Hz, 2H), 7.38 – 7.28 (m, 2H), 7.30 – 7.21 (m, 1H), 7.06 (s, 1H), 6.51 (s, 1H), 5.94 (dd, *J* = 3.2, 1.7 Hz, 1H), 2.84 (s, 2H), 2.58 (q, *J* = 7.5 Hz, 2H), 2.46 – 2.24 (m, 3H), 2.25 – 2.05 (m, 2H), 2.02 – 1.90 (m, 1H), 1.87 – 1.75 (m, 1H), 1.73 – 1.60 (m, 2H), 1.52 – 1.32 (m, 2H), 1.18 (t, *J* = 7.5 Hz, 3H), 1.07 (s, 3H), 1.02 (s, 9H), 0.28 – 0.19 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.2, 151.3, 137.5, 135.0, 133.0, 132.0, 128.3, 127.2, 126.9, 126.1, 118.4, 57.1, 47.8, 44.4, 37.6, 35.7, 31.5, 29.5, 28.0, 26.8, 25.9, 23.8, 18.4, 17.0, 14.9, -3.9, -4.0. Eluent 2% EtOAc in hexane R_f = 0.59, yield 173 mg, 73%, product colorless oil.

*3-((8S,9S,13S,14S)-3-((tert-butyldimethylsilyl)oxy)-2-ethyl-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-17-yl)pyridine (**16b**)*

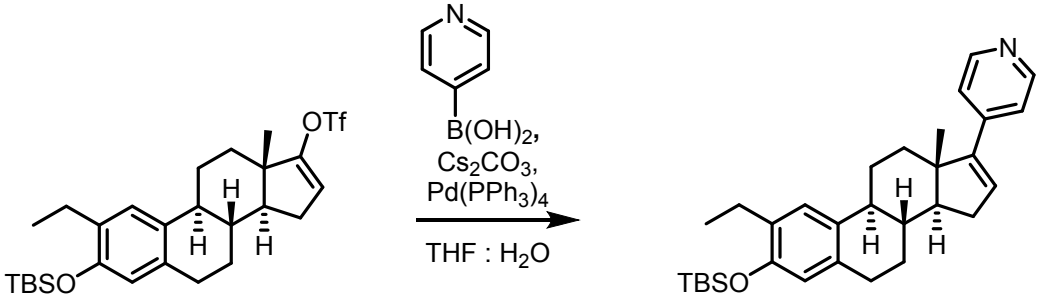


15 **16b**

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.54 (s, 1H), 8.35 (d, *J* = 4.7 Hz, 1H), 7.60 (d, *J* = 7.9 Hz, 1H), 7.14 (dd, *J* = 9.0, 6.3 Hz, 1H), 6.94 (s, 1H), 6.39 (s, 1H), 5.92 (s, 1H), 2.86 – 2.61 (m, 2H), 2.46 (q, *J* = 7.5 Hz, 2H), 2.35 – 2.12 (m, 3H), 2.09 – 1.98 (m, 2H), 1.89 – 1.79 (m, 1H), 1.76 – 1.64 (m, 1H), 1.62 – 1.46 (m, 3H), 1.42 – 1.27 (m, 1H), 1.06 (t, *J* = 7.5 Hz, 3H), 0.93 (s, 3H), 0.90 (s, 9H), 0.12 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 151.7, 151.3, 147.3, 134.7, 134.2, 133.3, 132.6,

131.9, 129.5, 125.9, 123.3, 123.3, 118.3, 56.8, 47.8, 44.2, 37.4, 35.4, 31.6, 29.3, 27.9, 26.5, 25.80, 23.7, 18.3, 16.8, 14.8, -4.1, -4.1. Eluent 20% EtOAc in hexane R_f = 0.26, yield 192 mg, 81%, product colorless oil.

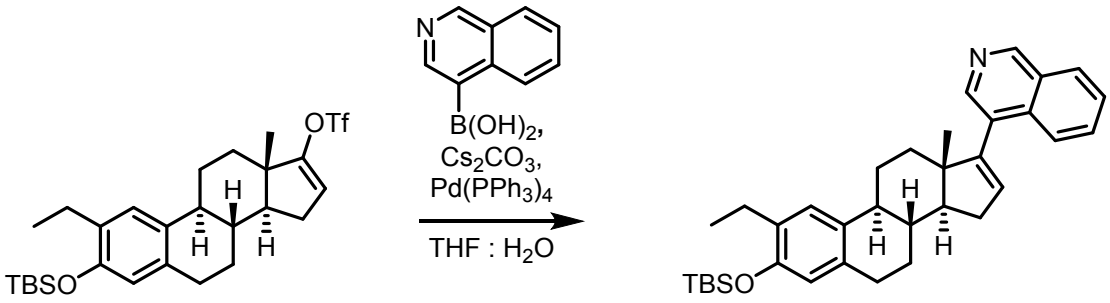
*4-((8S,9S,13S,14S)-3-((tert-butyldimethylsilyl)oxy)-2-ethyl-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-17-yl)pyridine (**16c**)*



15 **16c**

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.53 (d, *J* = 6.2 Hz, 2H), 7.43 – 7.28 (m, 2H), 7.06 (s, 1H), 6.52 (s, 1H), 6.22 (dd, *J* = 3.2, 1.8 Hz, 1H), 3.02 – 2.73 (m, 2H), 2.59 (q, *J* = 7.5 Hz, 2H), 2.47 – 2.11 (m, 5H), 2.00 – 1.90 (m, 1H), 1.86 – 1.76 (m, 1H), 1.68 (t, *J* = 10.7 Hz, 3H), 1.55 – 1.39 (m, 1H), 1.19 (t, *J* = 7.5 Hz, 3H), 1.09 (s, 3H), 1.03 (s, 9H), 0.25 (d, *J* = 1.4 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.7, 151.3, 149.4, 144.9, 134.7, 132.5, 132.0, 131.7, 125.9, 121.2, 118.3, 56.8, 47.6, 44.2, 37.3, 35.3, 31.6, 29.3, 27.8, 26.5, 25.8, 23.6, 18.2, 16.8, 14.7, -4.1, -4.1. Eluent 20% EtOAc in hexane R_f = 0.27, yield 185 mg, 78%, product colorless oil.

*4-((8S,9S,13S,14S)-3-((tert-butyldimethylsilyl)oxy)-2-ethyl-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-17-yl)isoquinoline (**16d**)*

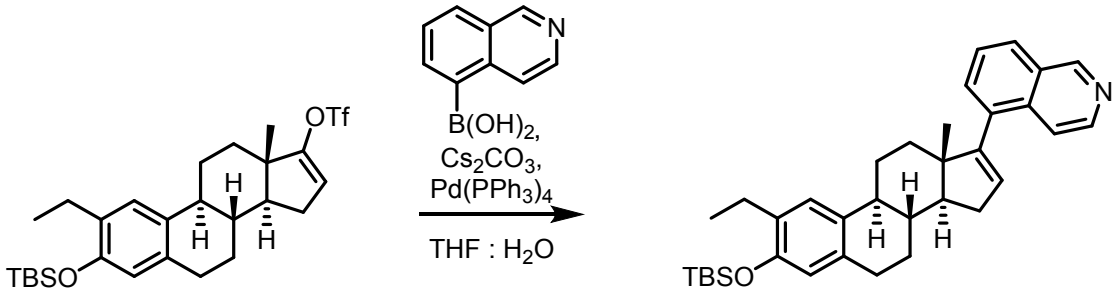


15 **16d**

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.05 (s, 1H), 8.22 (s, 1H), 8.00 (d, *J* = 8.5 Hz, 1H), 7.90 (d, *J* = 8.1 Hz, 1H), 7.63 (t, *J* = 7.6 Hz, 1H), 7.53 (t, *J* = 7.5 Hz, 1H), 6.93 (s, 1H), 6.42 (s, 1H), 5.81 (s, 1H), 2.92 – 2.64 (m, 2H), 2.47 (q, *J* = 7.4 Hz, 2H), 2.42 – 2.38 (m, 1H), 2.34 – 2.12 (m, 3H), 2.05 – 1.84 (m, 2H), 1.71 – 1.56 (m, 2H), 1.55 – 1.37 (m, 3H), 1.06 (t, *J* = 7.5 Hz, 3H), 0.91 (s, 12H), 0.14 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 151.7, 151.3, 147.3, 147.3, 135.7, 134.7, 134.2, 133.3, 132.6, 131.9, 129.5, 129.4, 126.4, 125.9, 123.3, 123.3, 118.3, 56.8, 47.8, 44.2, 37.4, 35.4,

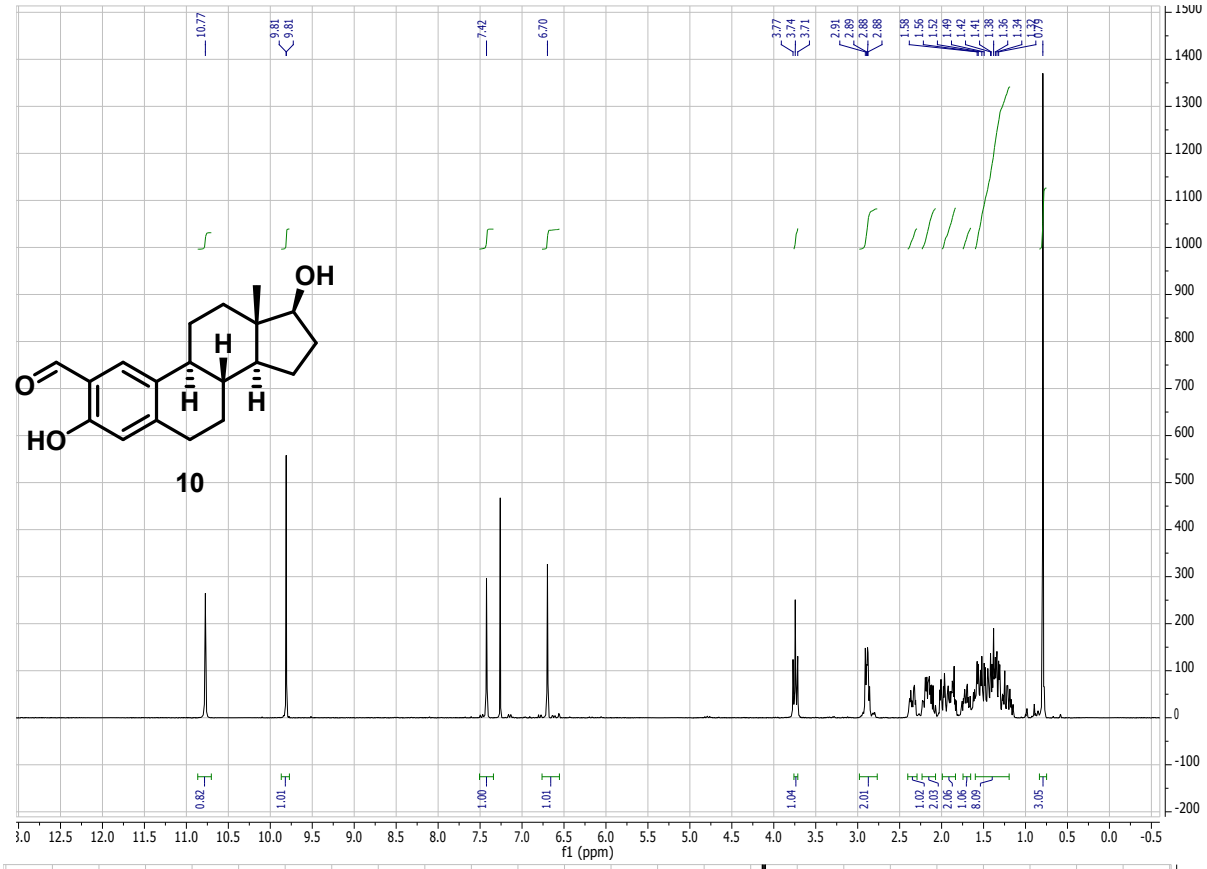
31.6, 29.3, 27.9, 26.5, 25.8, 23.7, 18.3, 16.8, 14.8, -4.1, -4.1. Eluent 20% EtOAc in hexane R_f = 0.34, yield 220 mg, 84%, product light yellow oil.

*5-((8S,9S,13S,14S)-3-((tert-butyldimethylsilyl)oxy)-2-ethyl-13-methyl-7,8,9,11,12,13,14,15-octahydro-6H-cyclopenta[a]phenanthren-17-yl)isoquinoline (**16e**)*



15 **16e**

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.25 (s, 1H), 8.51 (d, *J* = 6.0 Hz, 1H), 8.00 – 7.70 (m, 2H), 7.64 – 7.45 (m, 2H), 7.02 (s, 1H), 6.52 (s, 1H), 5.84 (dd, *J* = 3.0, 1.5 Hz, 1H), 2.89 (dd, *J* = 16.4, 9.2 Hz, 2H), 2.62 – 2.51 (m, 2H), 2.55 – 2.44 (m, 1H), 2.44 – 2.24 (m, 3H), 2.01 (tt, *J* = 11.4, 6.3 Hz, 2H), 1.72 (t, *J* = 12.3 Hz, 2H), 1.67 – 1.47 (m, 3H), 1.16 (t, *J* = 7.5 Hz, 3H), 1.02 (s, 12H), 0.24 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.7, 151.5, 151.4, 143.0, 135.6, 135.2, 134.9, 132.7, 132.0, 130.9, 129.7, 129.2, 126.7, 126.5, 126.1, 119.4, 118.4, 56.9, 50.1, 44.6, 37.9, 35.4, 32.2, 29.5, 28.2, 26.6, 25.9, 23.8, 18.4, 16.7, 14.9, -4.0, -4.0. Eluent 20% EtOAc in hexane R_f = 0.31, yield 196 mg (75%), product colorless oil.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9.81
9.81
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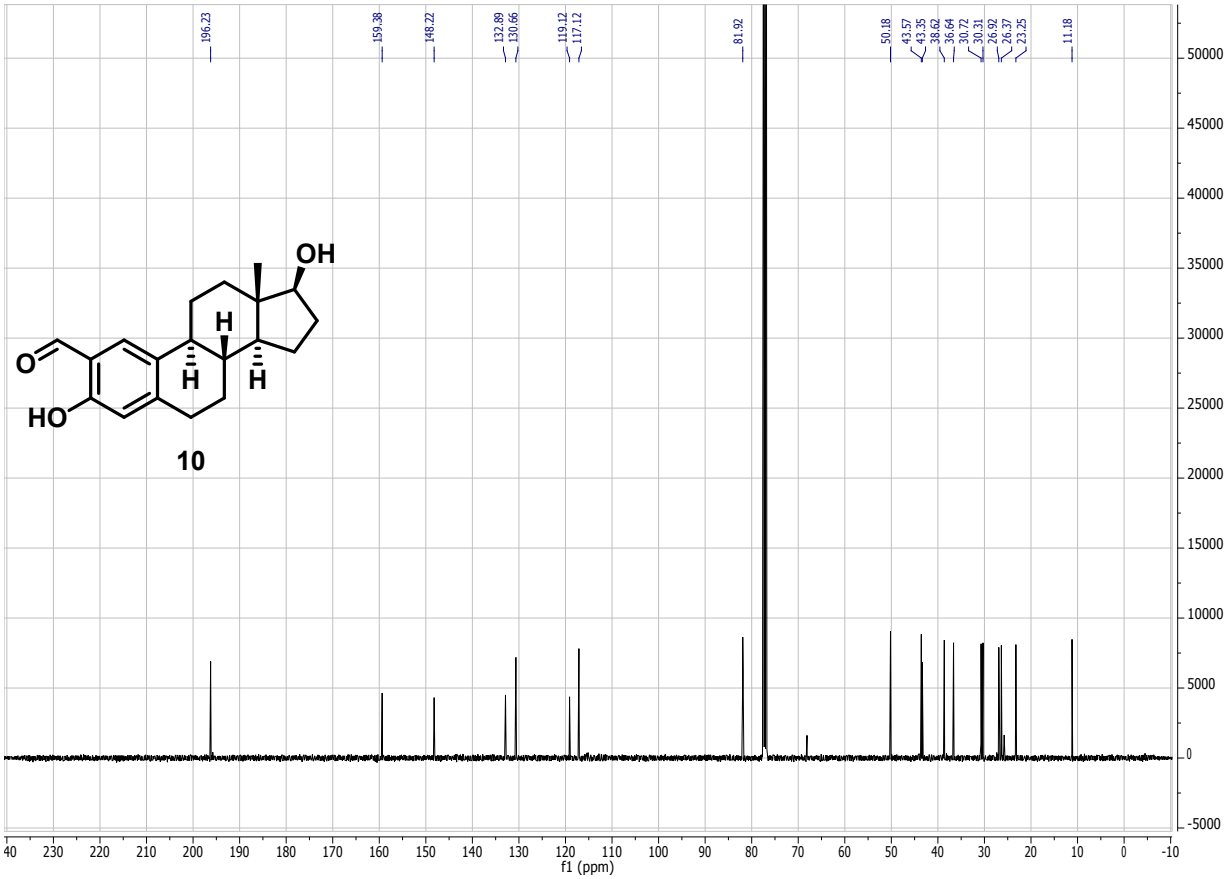
6.70
3.77
3.74
3.71
2.91
2.89
2.88
2.88
1.58
1.56
1.55
1.52
1.49
1.47
1.42
1.41
1.38
1.36
1.34
0.79
0.82
1.01
1.00
1.01
1.04
2.01
1.02
2.03
2.06
1.06
8.09
3.05
OH
H
O
H
H
HO
10
f1 (ppm)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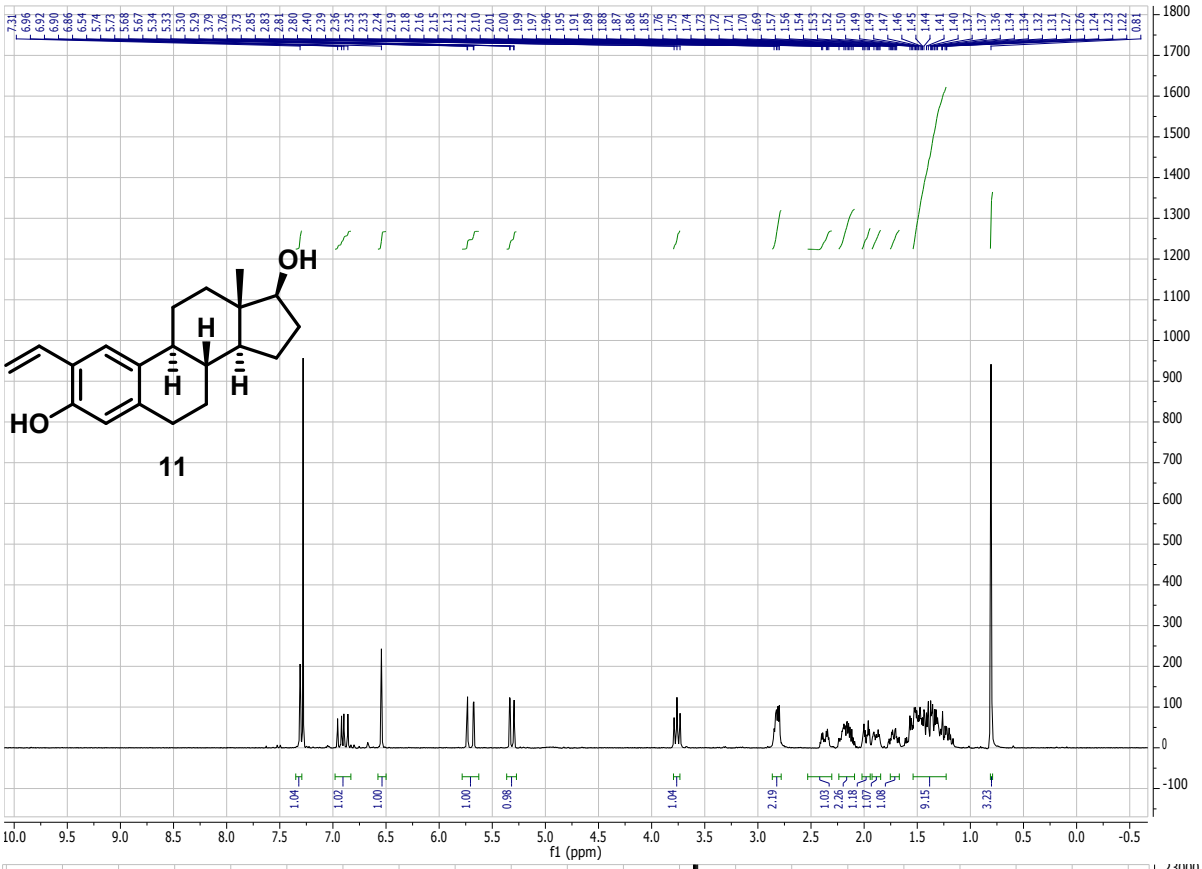
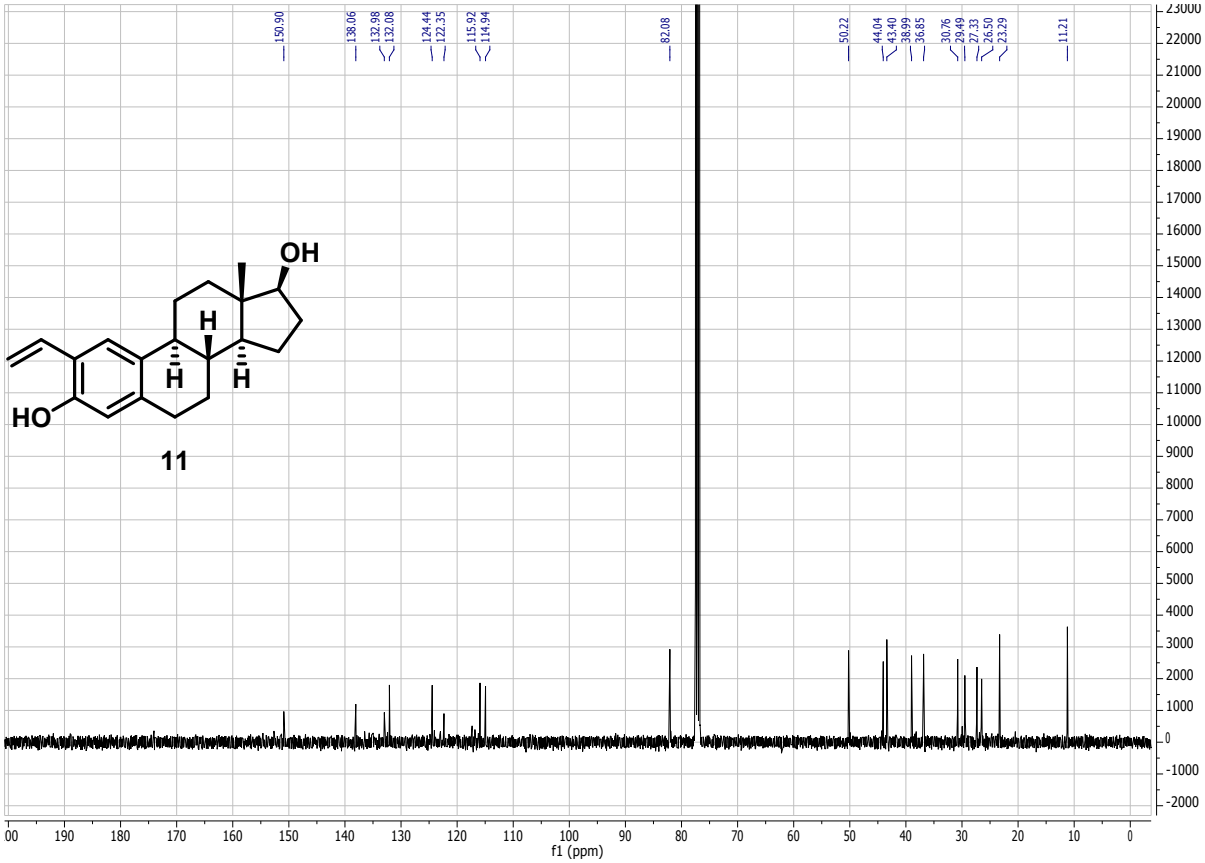
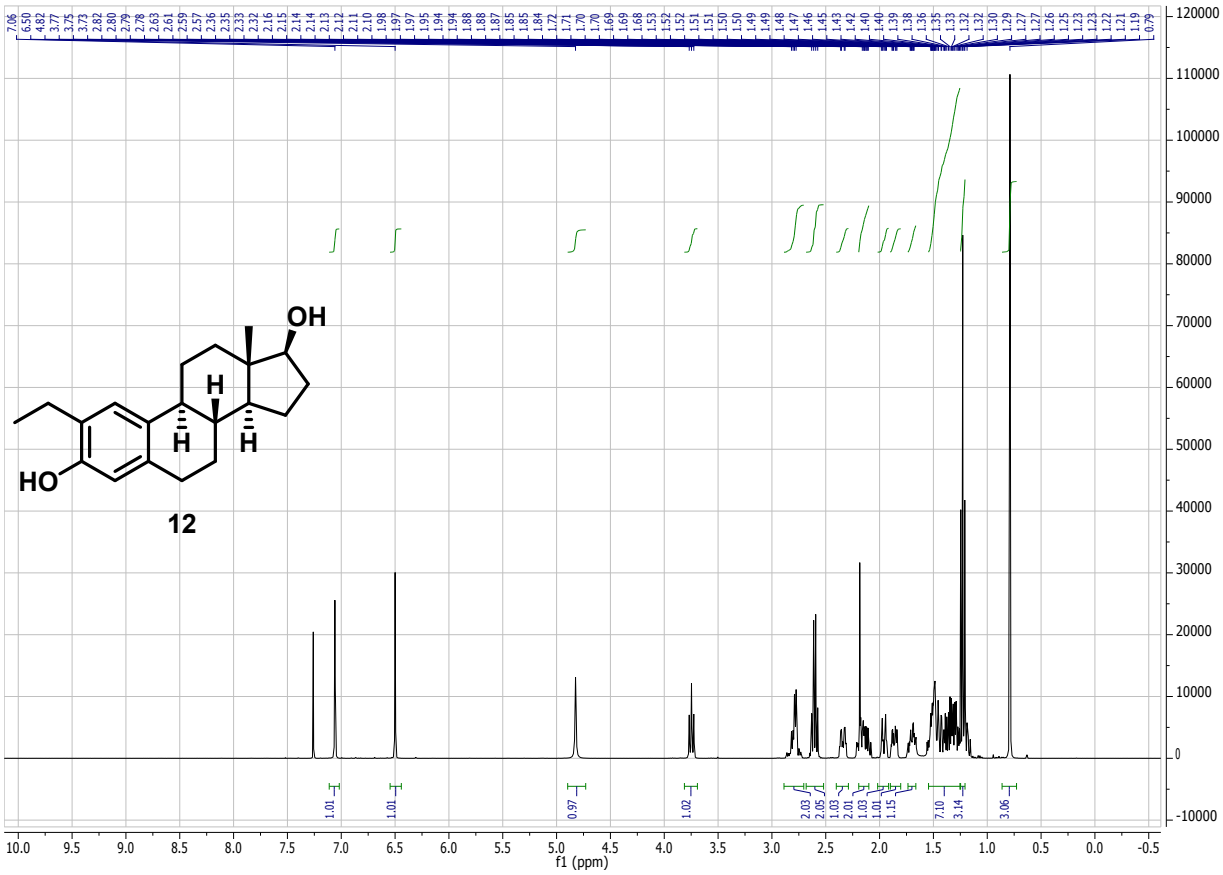
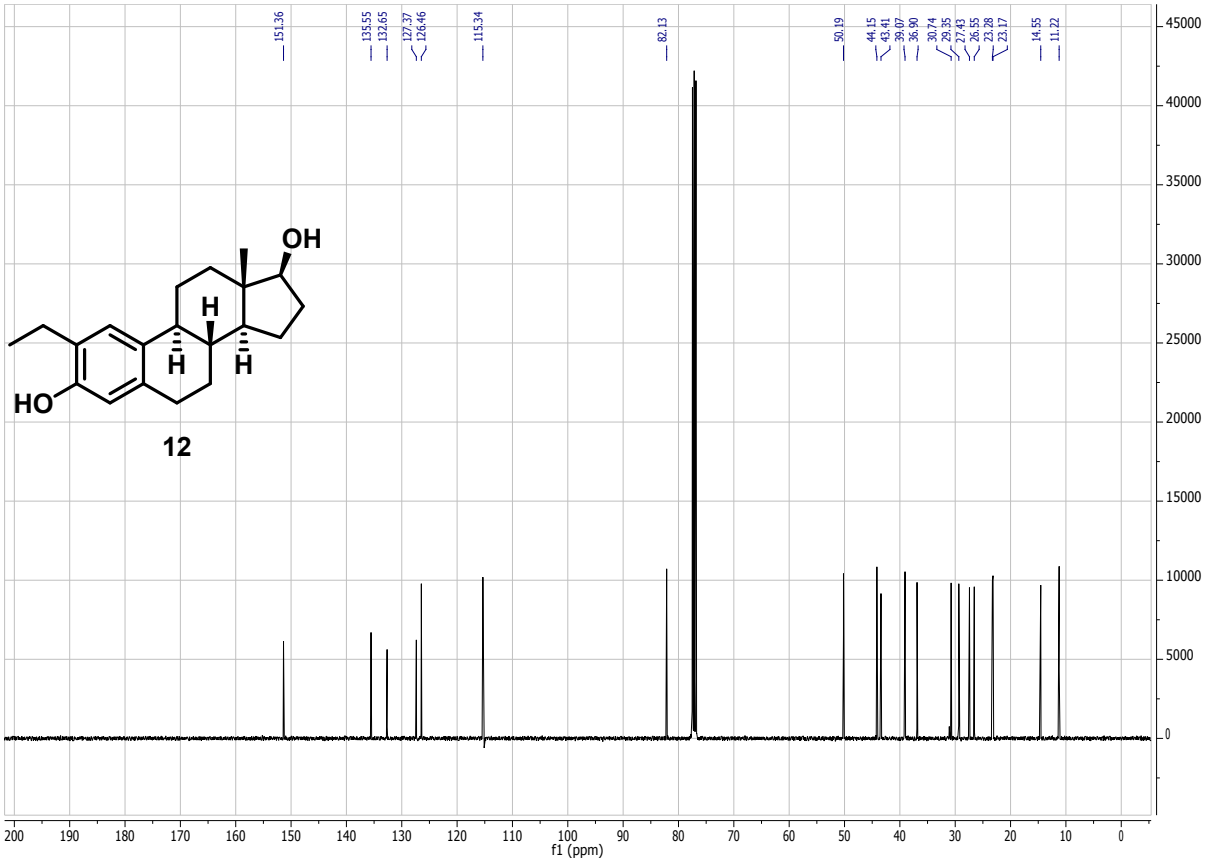
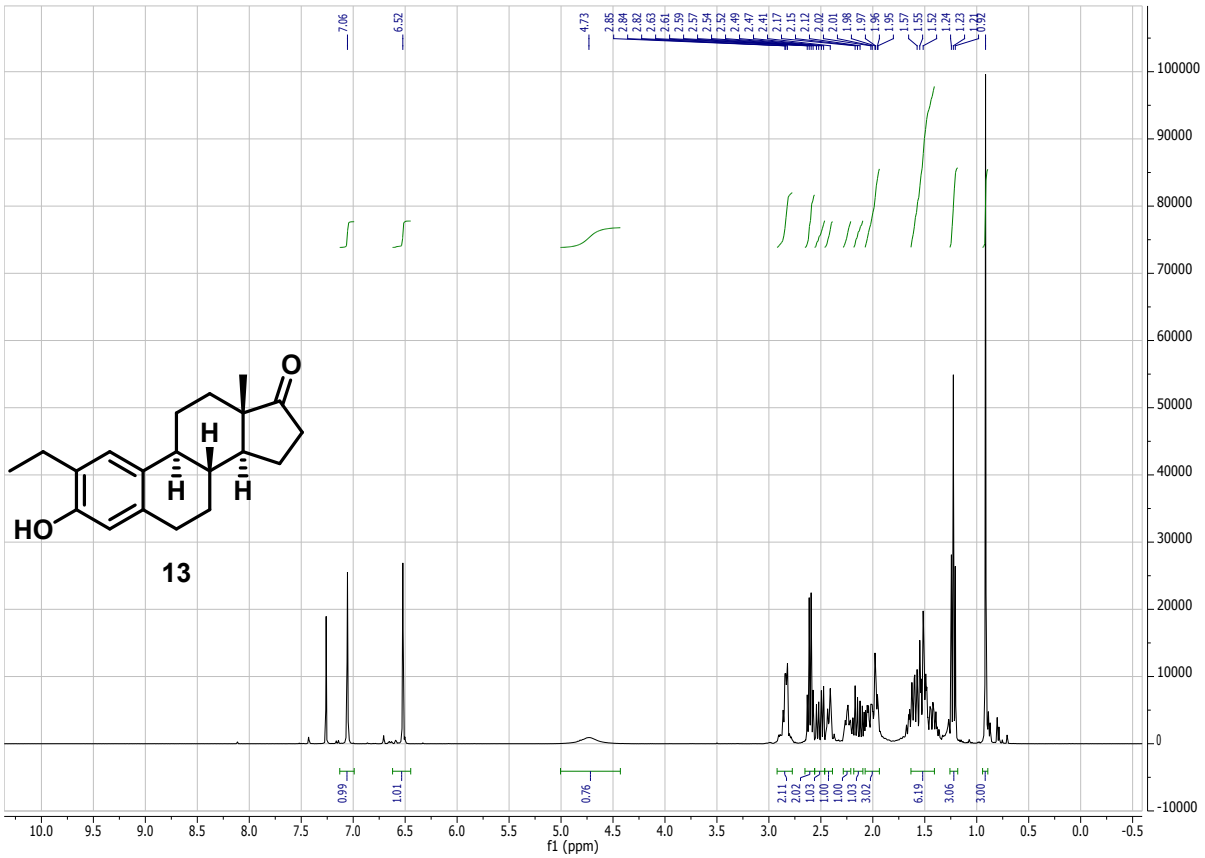
159.38
148.22
132.89
130.66
119.12
117.12
81.92
50.18
43.57
43.35
38.62
36.64
30.72
30.31
26.92
26.37
23.25
11.18
OH
H
O
H
H
HO
10
f1 (ppm)


OH
H
H
H
HO
11
f1 (ppm)


OH
H
H
H
HO
11
f1 (ppm)


OH
H
H
H
HO
12
f1 (ppm)


OH
H
H
H
HO
12
f1 (ppm)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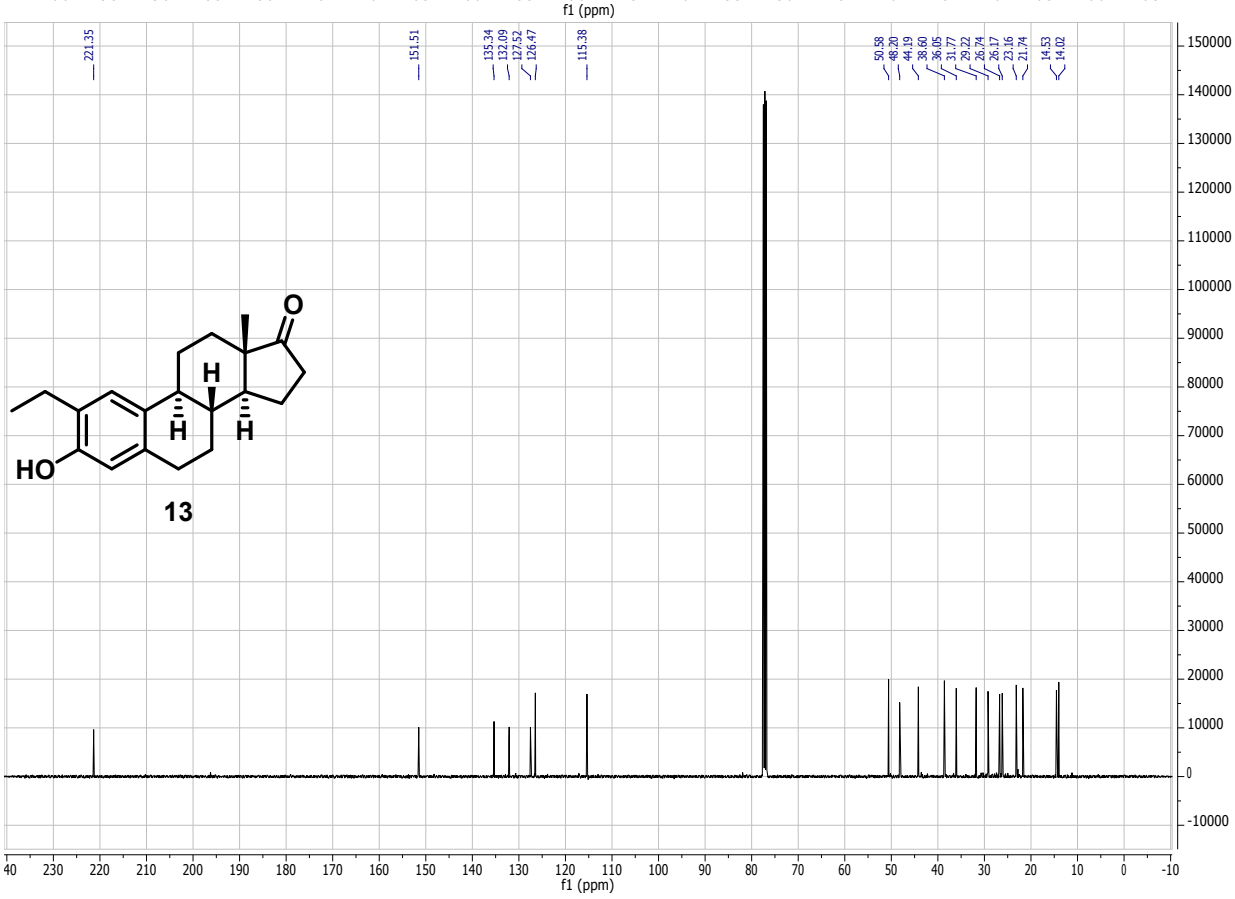
4.73
2.85
2.84
2.82
2.63
2.61
2.59
2.57
2.54
2.52
2.49
2.47
2.41
2.17
2.15
2.12
2.02
2.01
1.98
1.97
1.96
1.95
1.57
1.55
1.52
1.24
1.23
1.21
0.92
0.99
1.01
0.76
2.11
2.02
1.03
1.00
1.00
1.03
3.02
6.19
3.06
3.00
O
H
H
H
HO
13
f1 (ppm)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135.34
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127.52
126.47
115.38
50.58
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44.19
38.60
36.05
31.77
29.22
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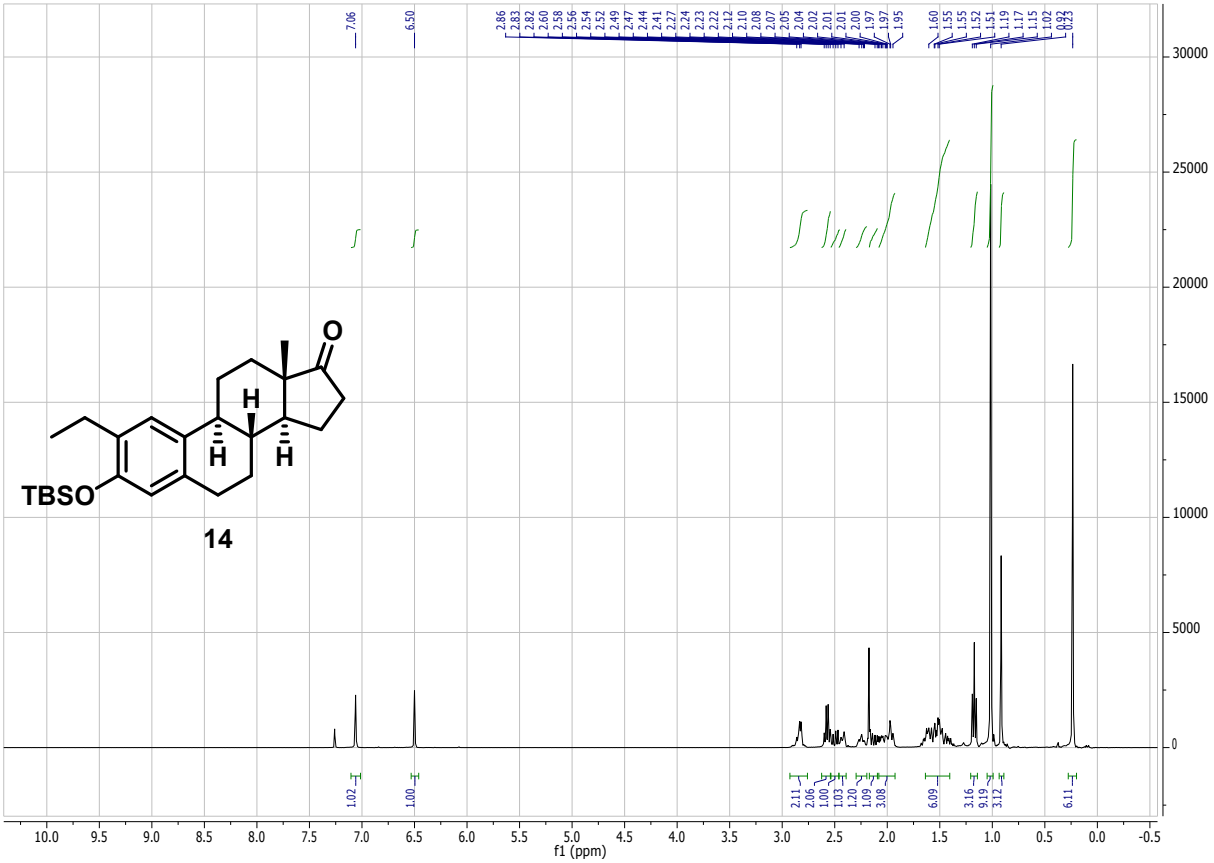
26.17
23.16
21.74
14.53
14.02
O
H
H
H
HO
13
f1 (ppm)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6.50
2.86
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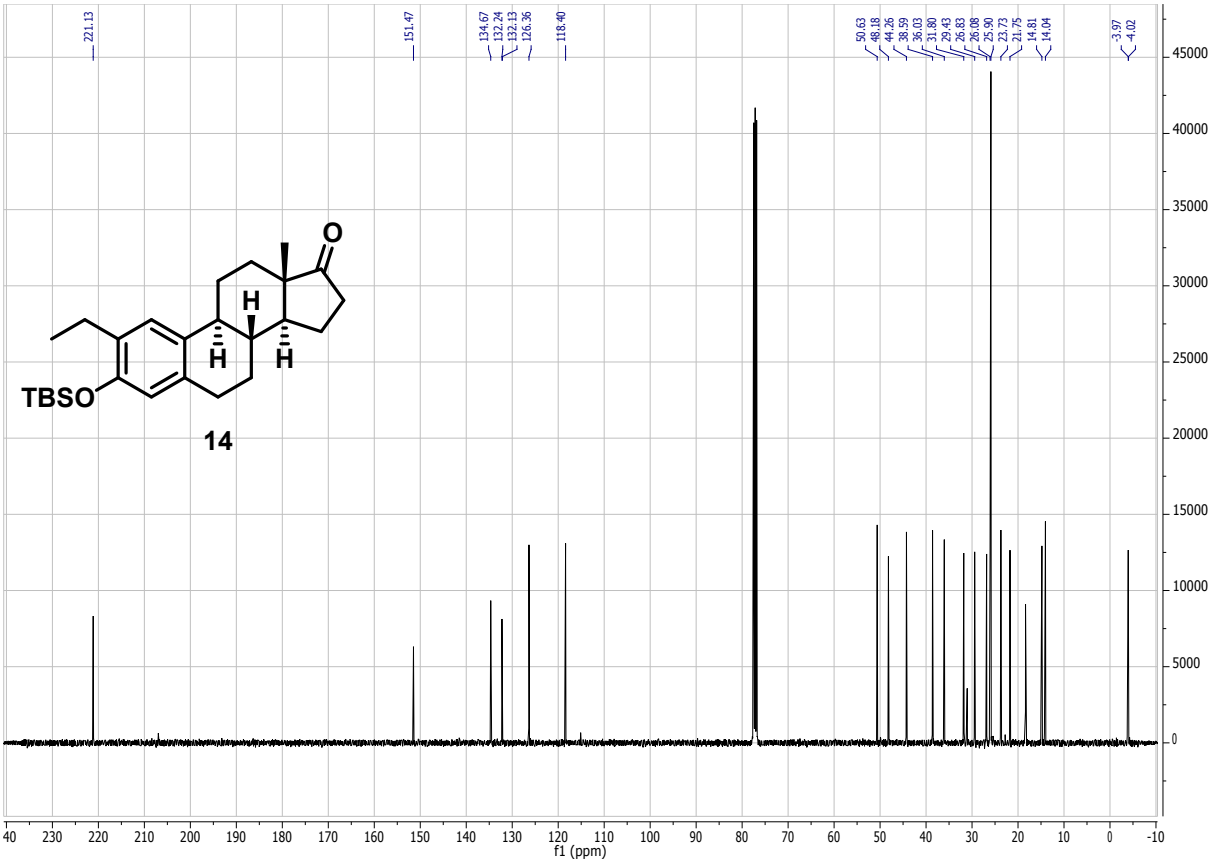
2.82
2.60
2.58
2.56
2.54
2.52
2.49
2.47
2.44
2.41
2.27
2.24
2.23
2.22
2.12
2.10
2.08
2.07
2.05
2.04
2.02
2.01
2.01
2.00
1.97
1.97
1.95
1.60
1.55
1.55
1.52
1.51
1.19
1.17
1.15
1.02
0.92
0.23
1.02
1.00
2.11
2.06
1.00
1.03
1.20
1.09
3.08
6.09
3.16
9.19
3.12
6.11
f1 (ppm)
O
H
H
H
TBSO
14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44.26
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29.43
26.83
26.08
25.90
23.73
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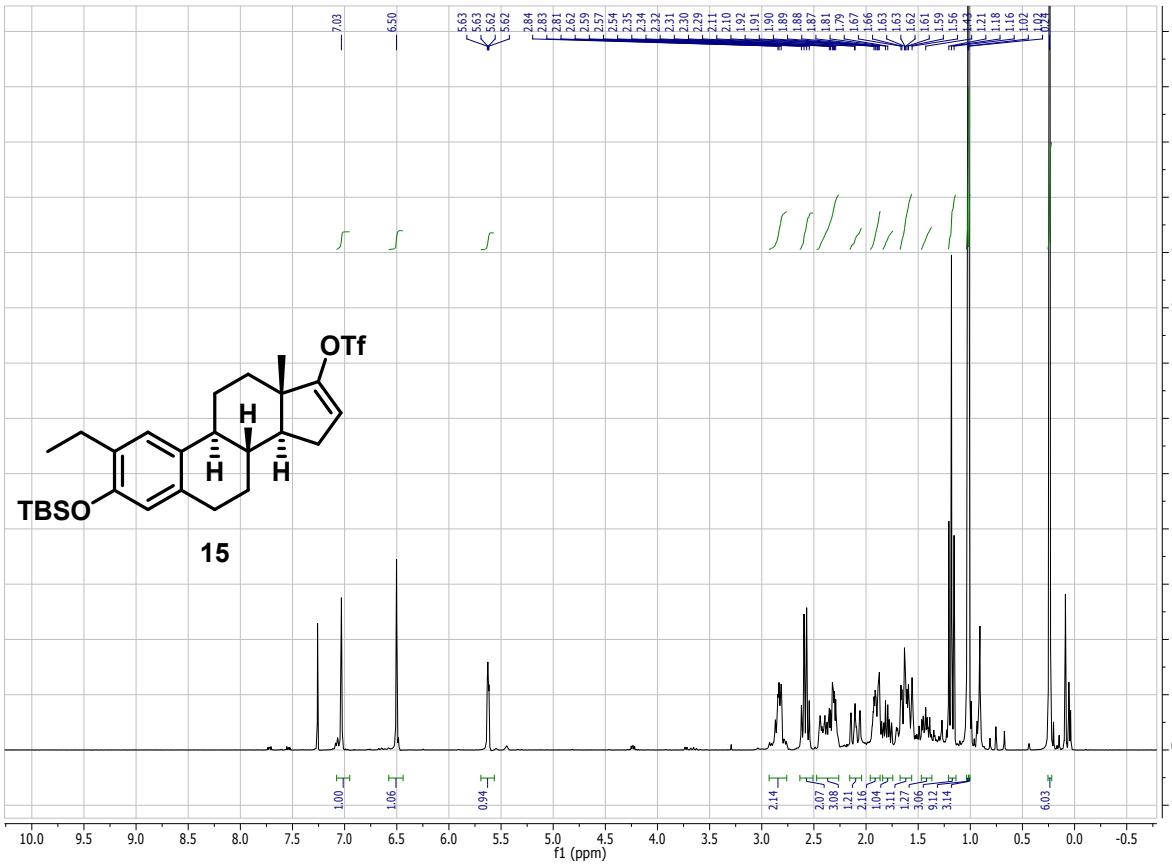
14.04
-3.97
-4.02
f1 (ppm)
O
H
H
H
TBSO
14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TBSO
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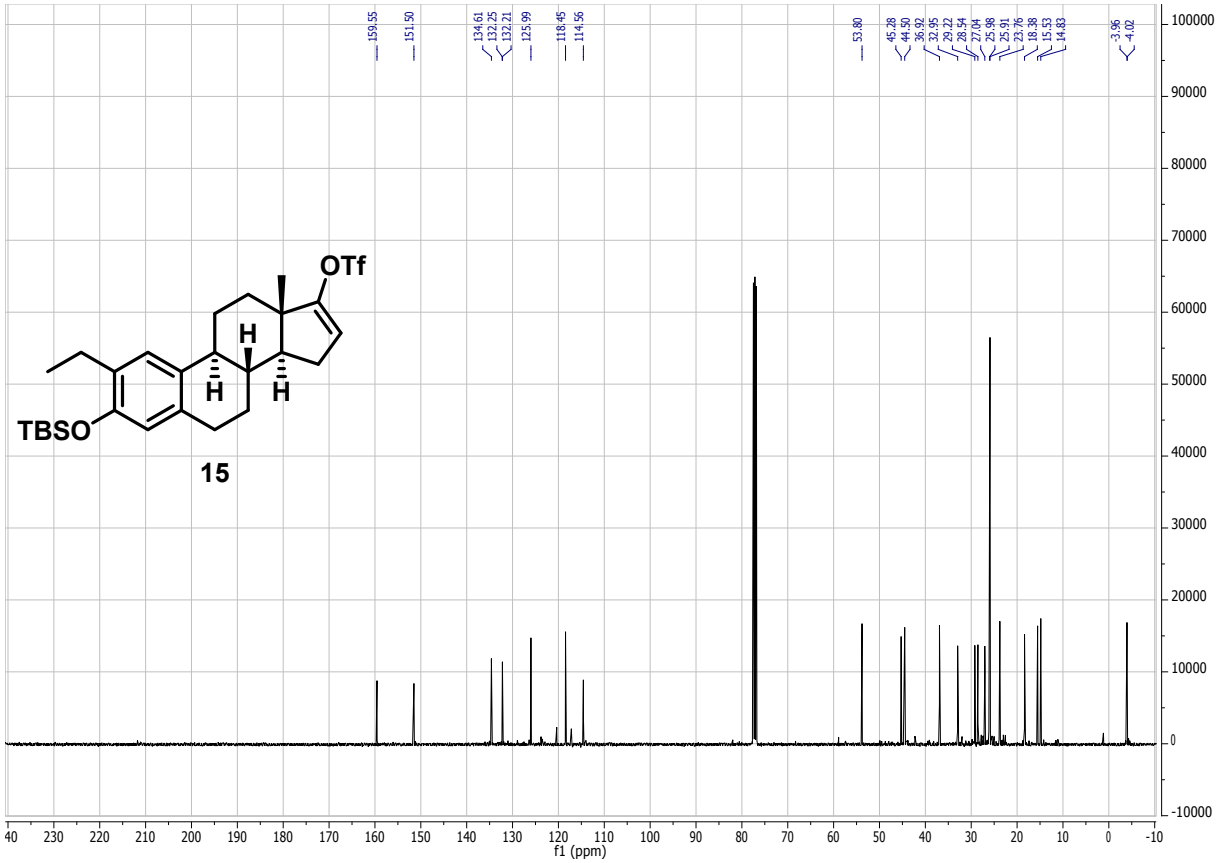
f1 (ppm)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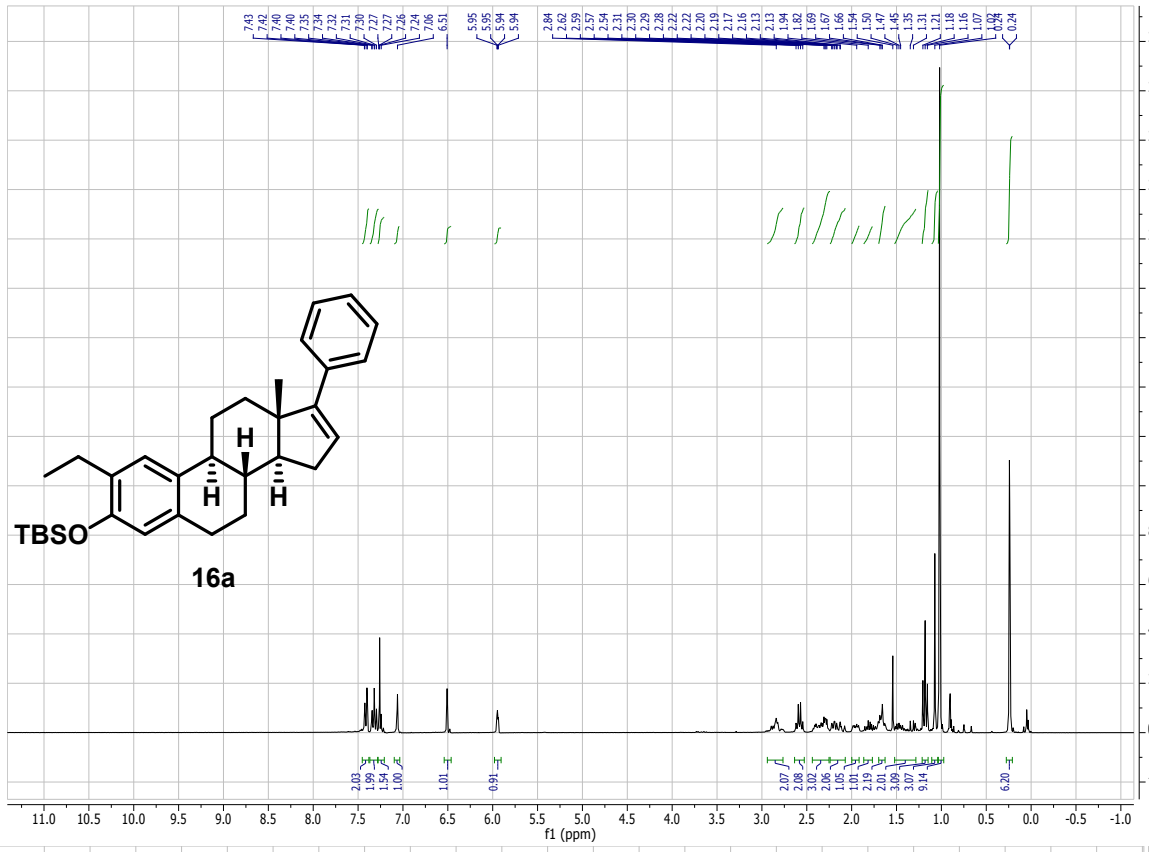
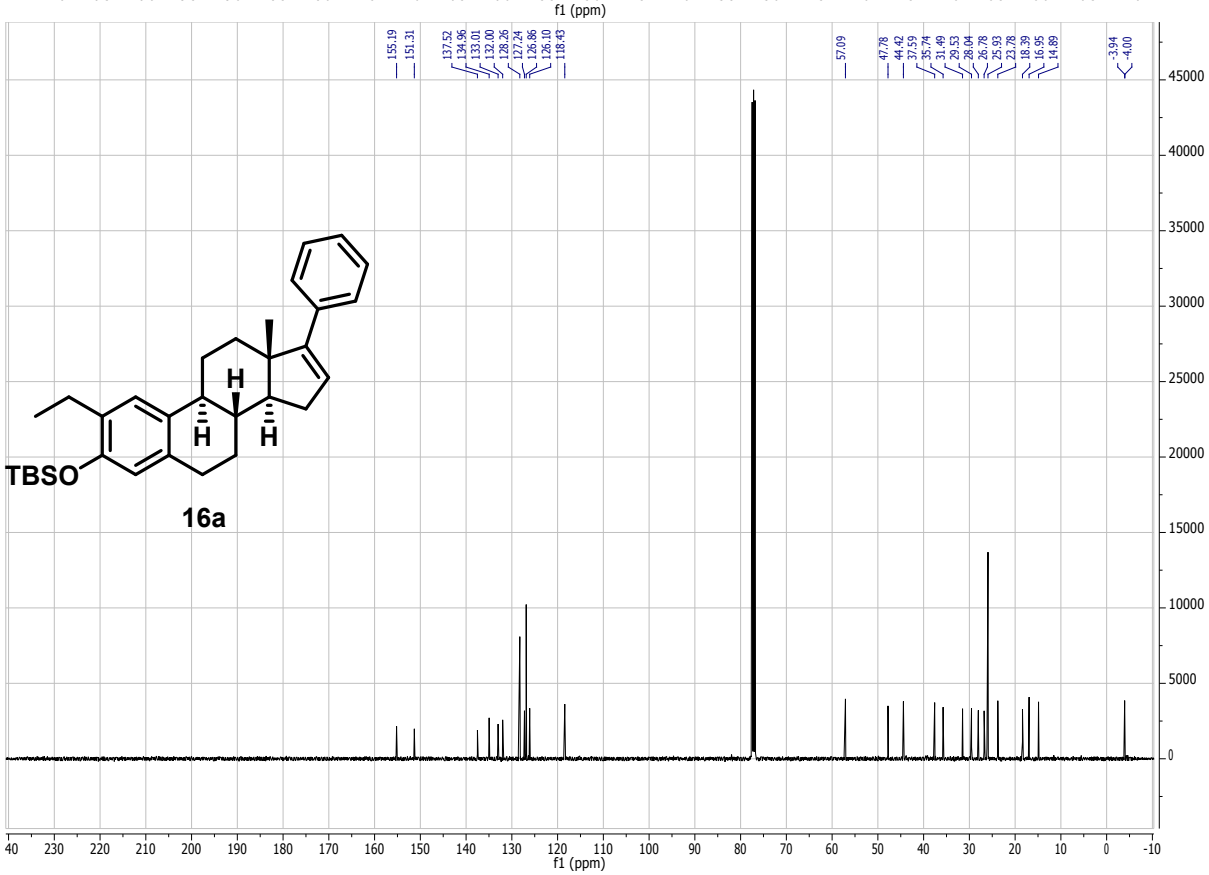
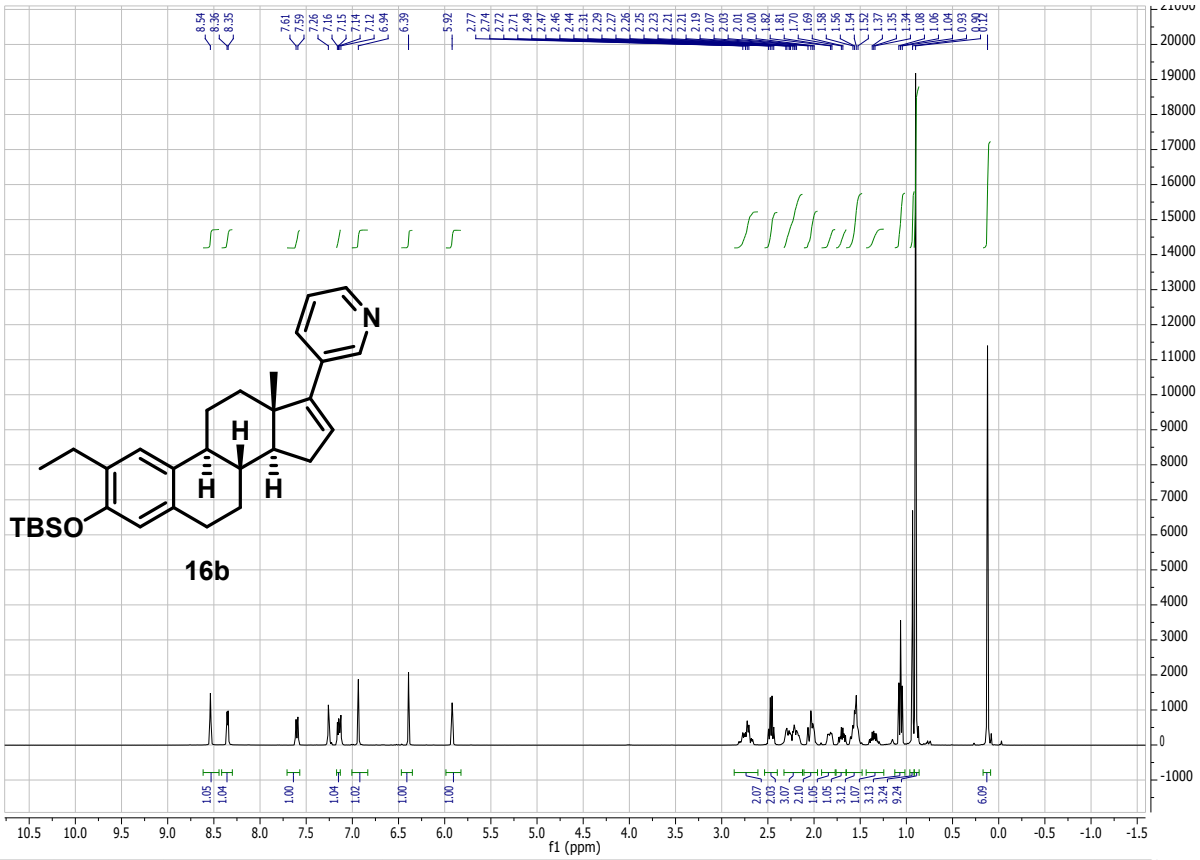
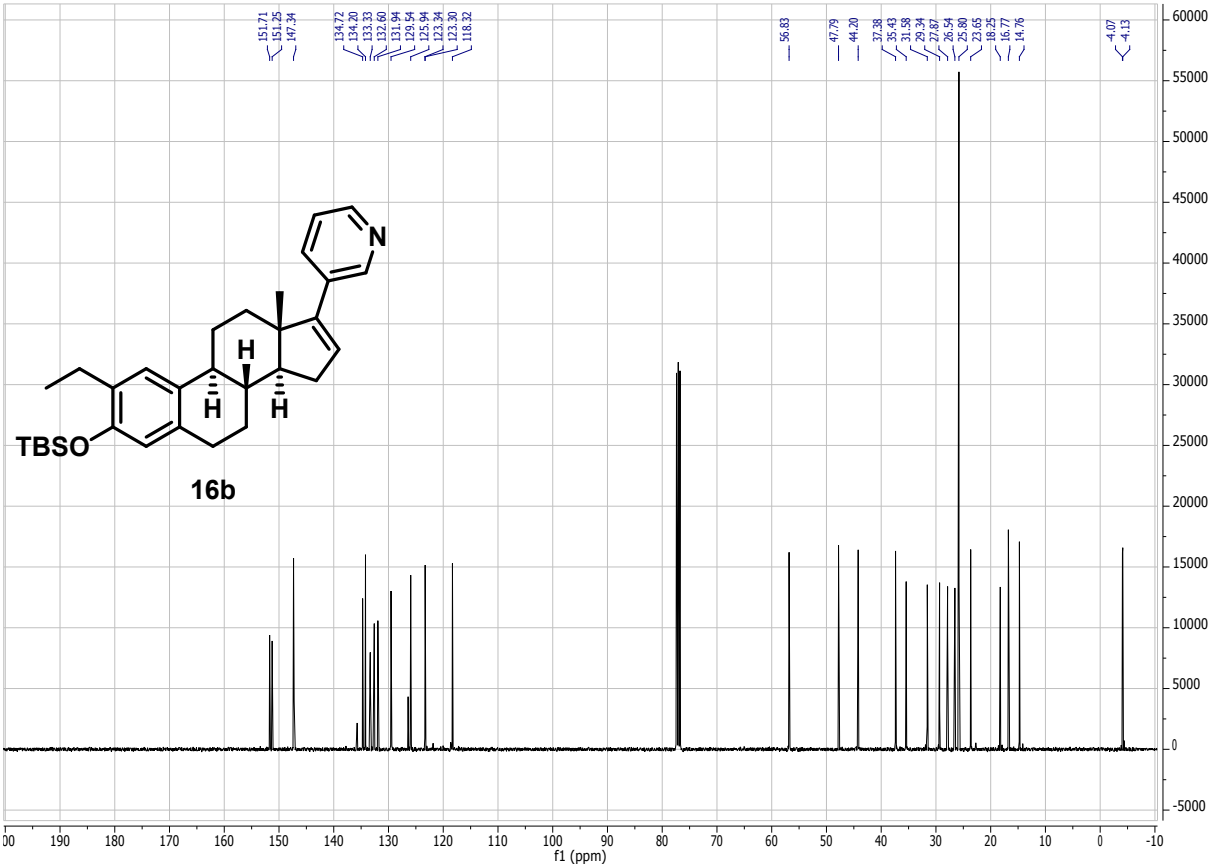
H
H
TBSO
15
f1 (ppm)


TBSO
H
H
H
16a
f1 (ppm)


TBSO
H
H
H
16a
f1 (ppm)


TBSO
16b
f1 (ppm)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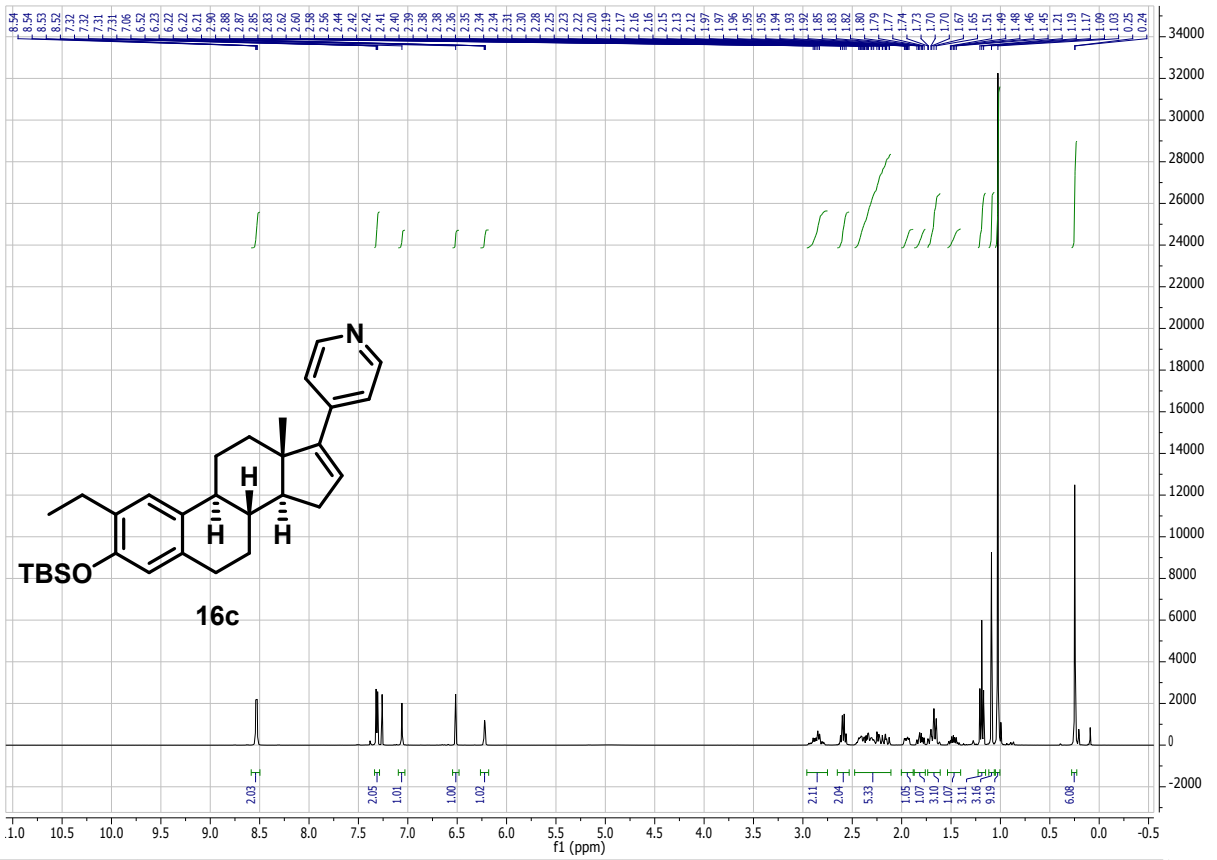
16b
f1 (ppm)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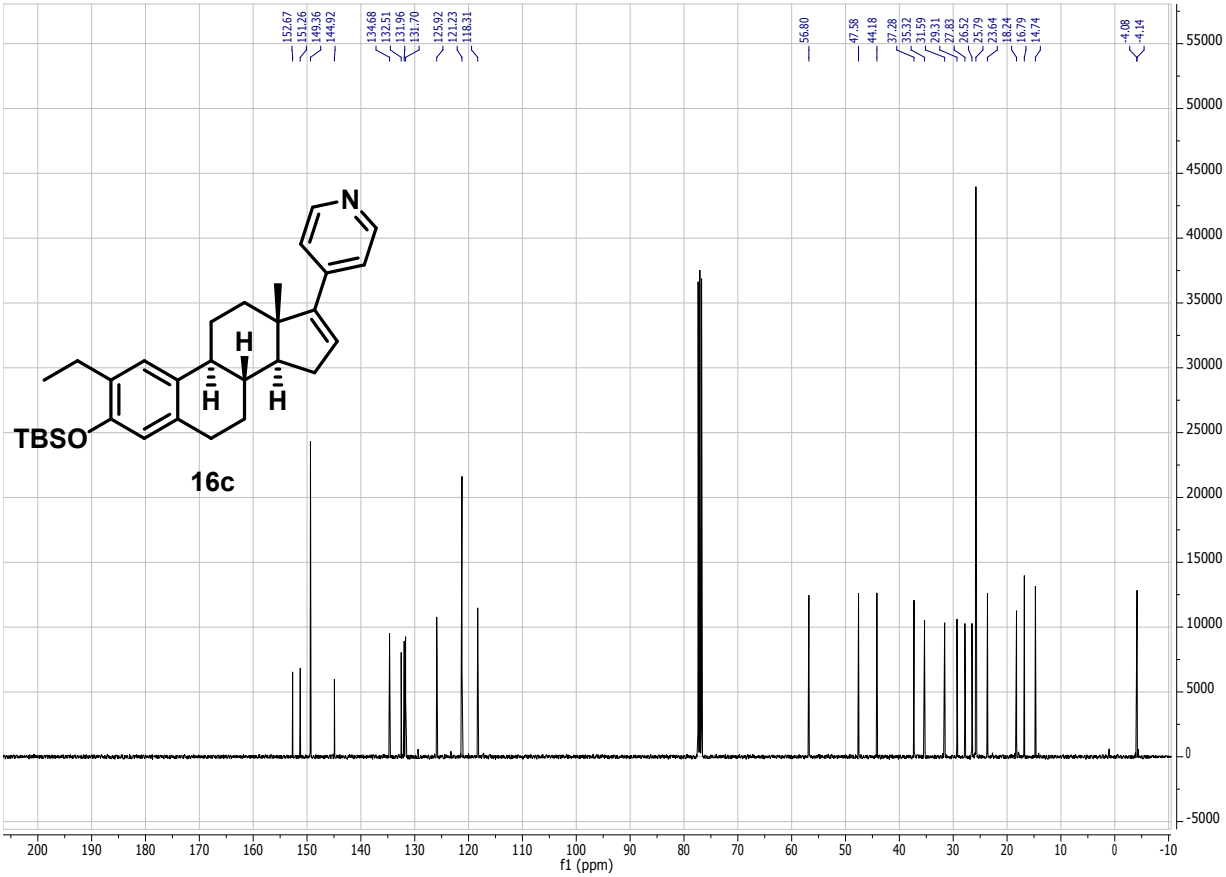
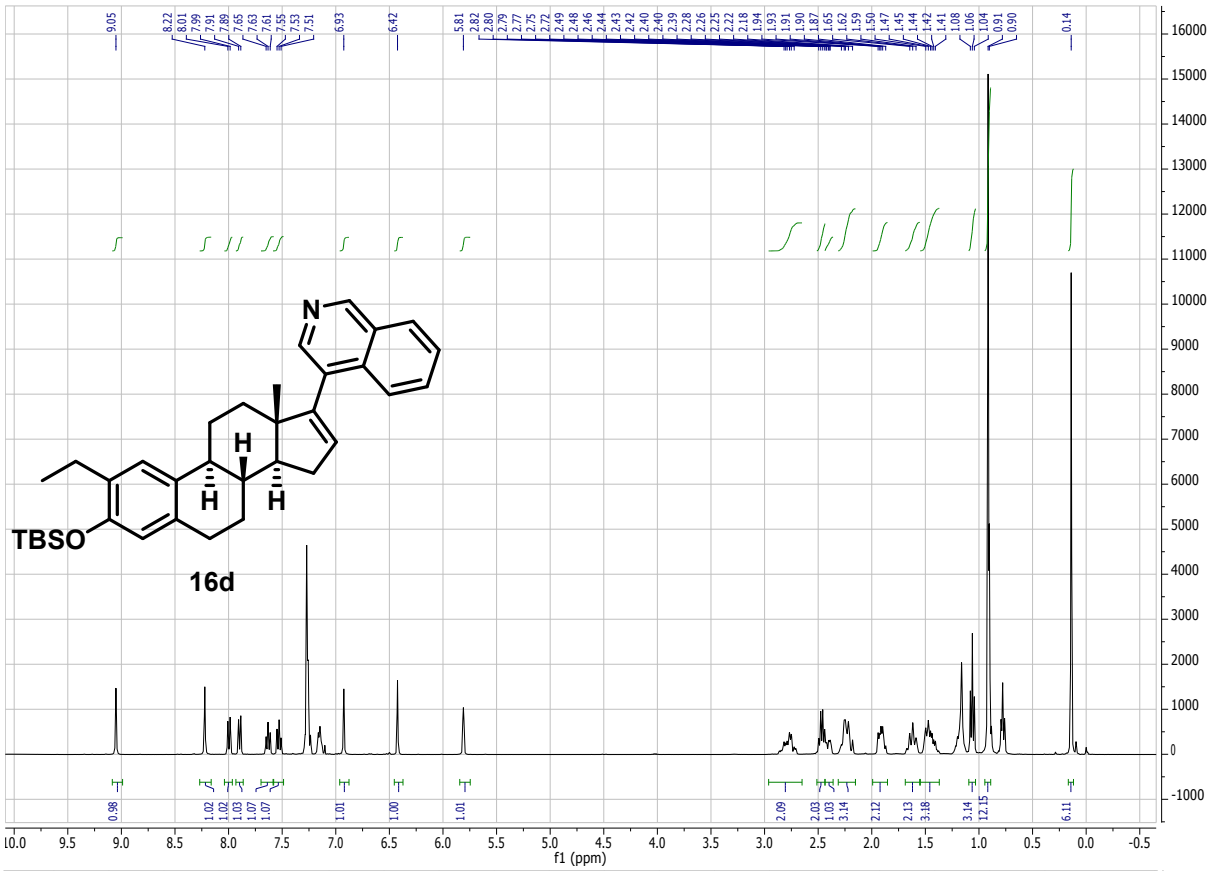
H
H
H
TBSO
16c
f1 (ppm)


N
H
H
H
TBSO
16c
f1 (ppm)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7.89
7.65
7.63
7.61
7.55
7.53
7.51
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6.42
5.81
2.82
2.80
2.79
2.77
2.75
2.72
2.49
2.48
2.46
2.44
2.43
2.42
2.40
2.40
2.39
2.28
2.26
2.25
2.22
2.18
2.14
1.94
1.93
1.91
1.90
1.87
1.65
1.62
1.59
1.50
1.47
1.45
1.44
1.42
1.41
1.08
1.06
1.04
0.91
0.90
0.14
N
H
H
H
TBSO
16d
0.98
1.02
1.02
1.03
1.07
1.07
1.01
1.00
1.01
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1.03
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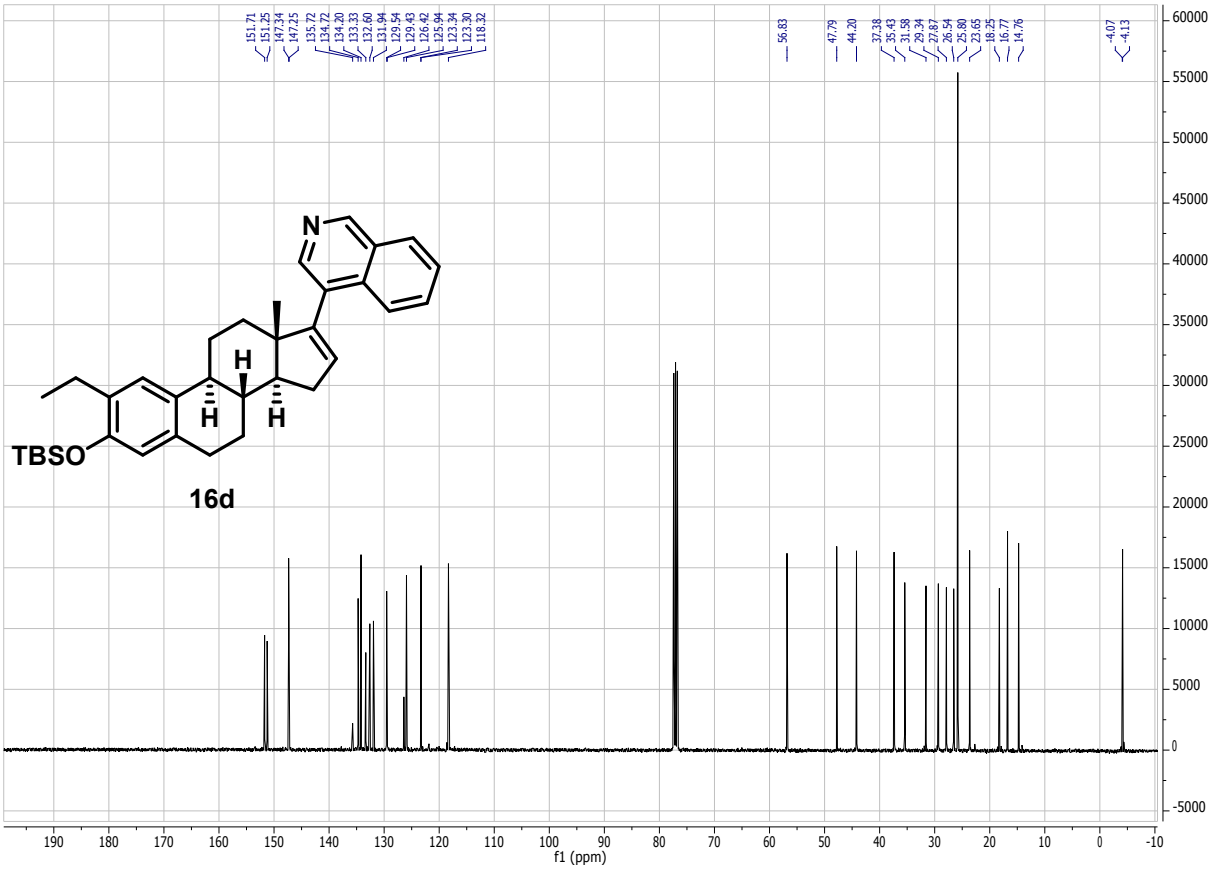
2.12
2.13
3.18
3.14
12.15
6.11
f1 (ppm)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147.25
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134.72
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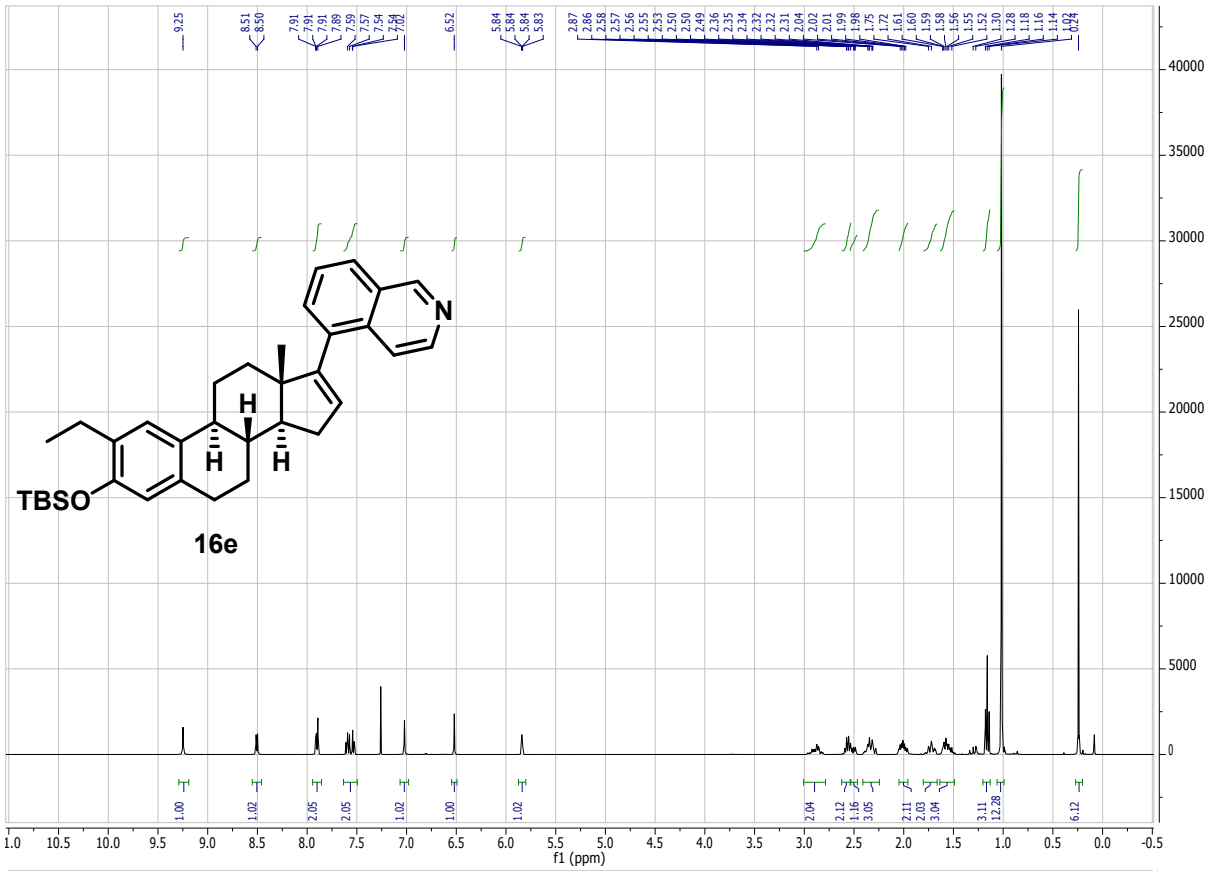
129.54
129.43
126.42
125.94
123.34
123.30
118.32
56.83
47.79
44.20
37.38
35.43
31.58
29.34
27.87
26.54
25.80
23.65
18.25
16.77
14.76
-4.07
-4.13
N
H
H
H
TBSO
16d
f1 (ppm)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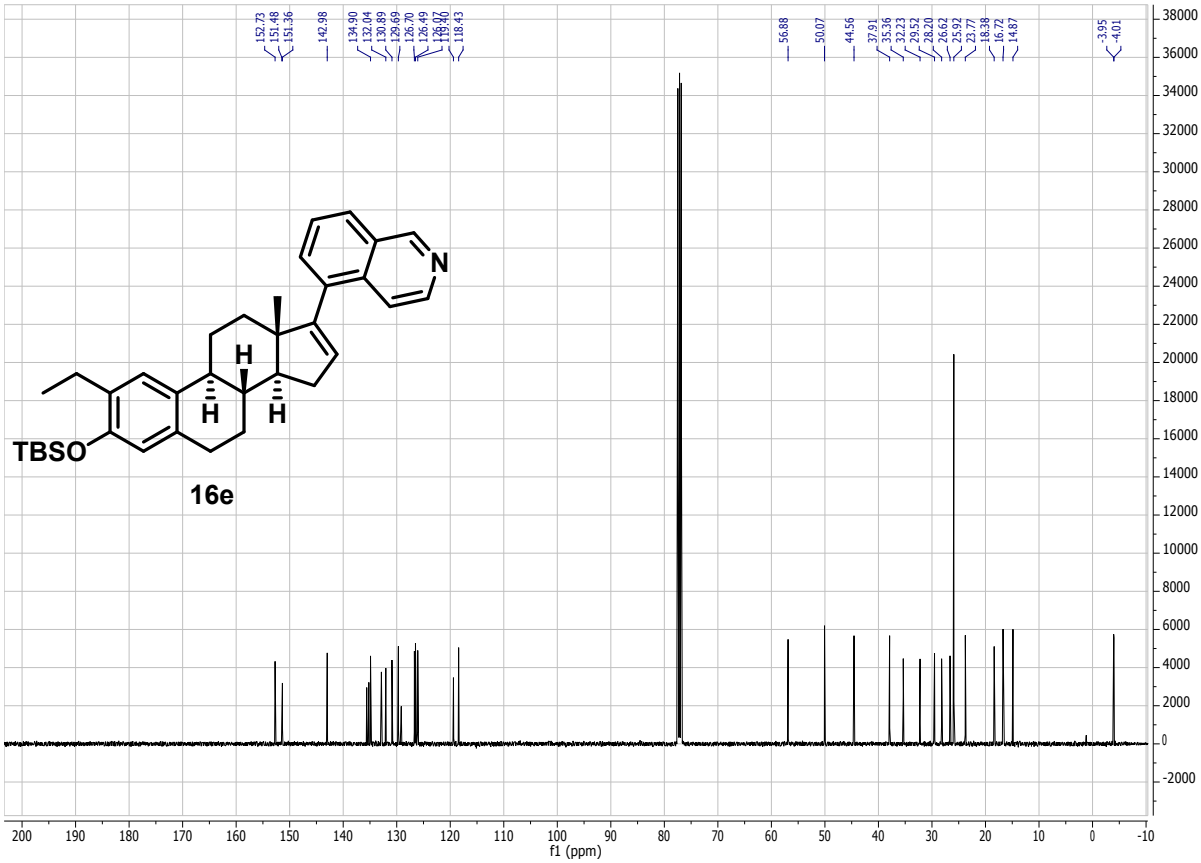
H
H
H
N
16e
f1 (ppm)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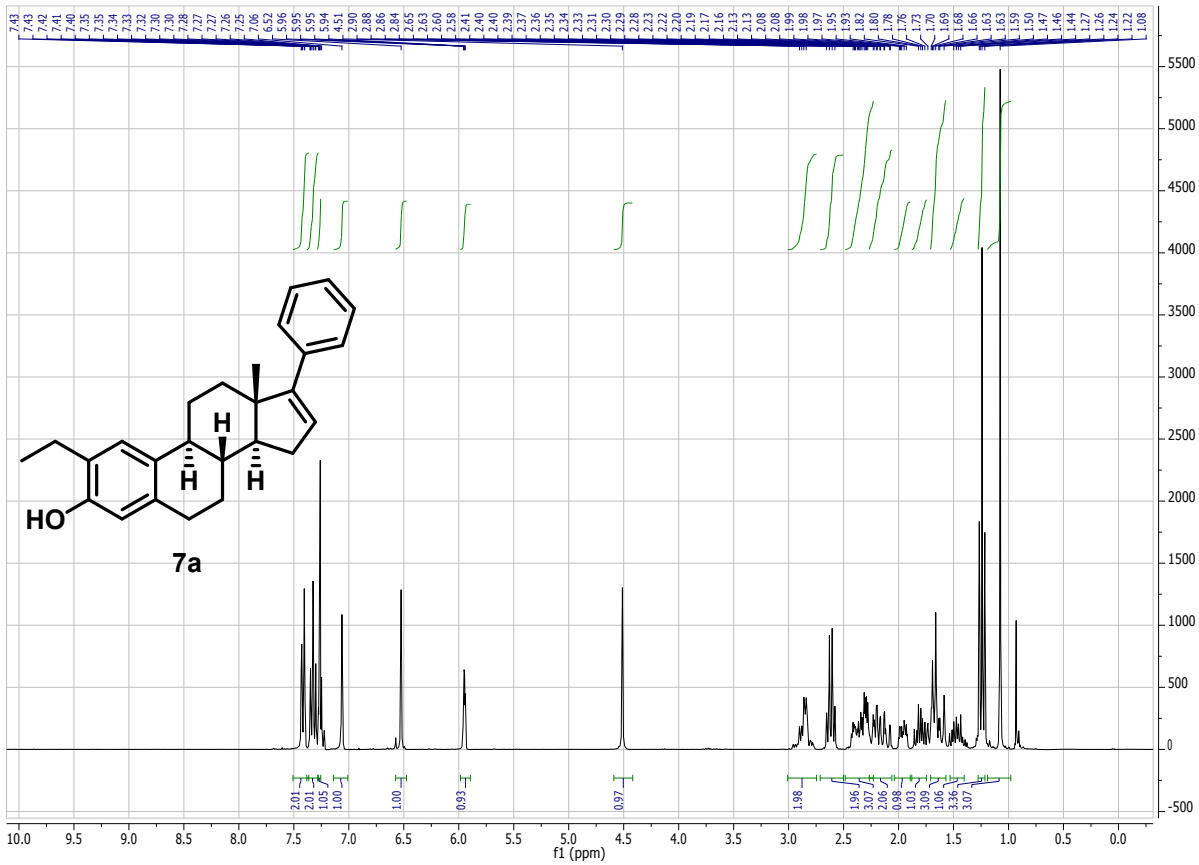
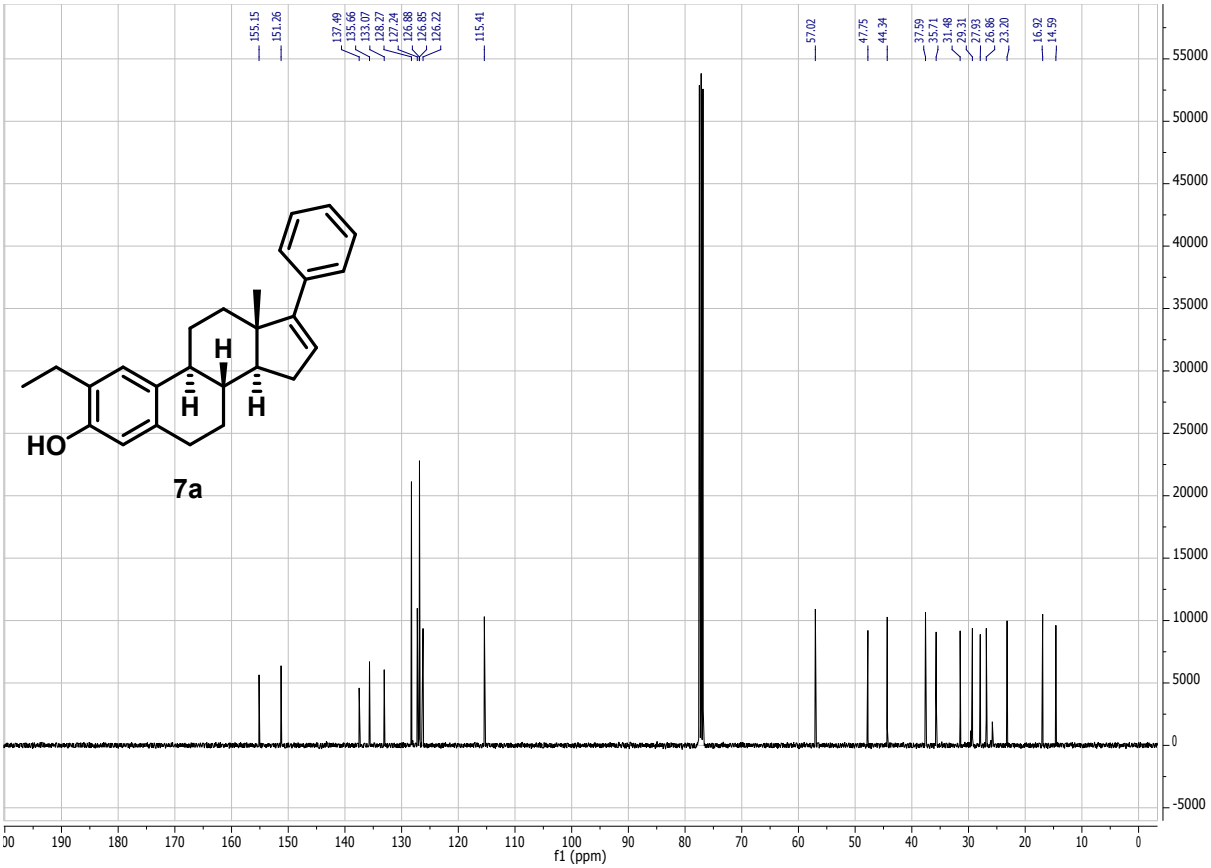

7a
HO
H
H
H
f1 (ppm)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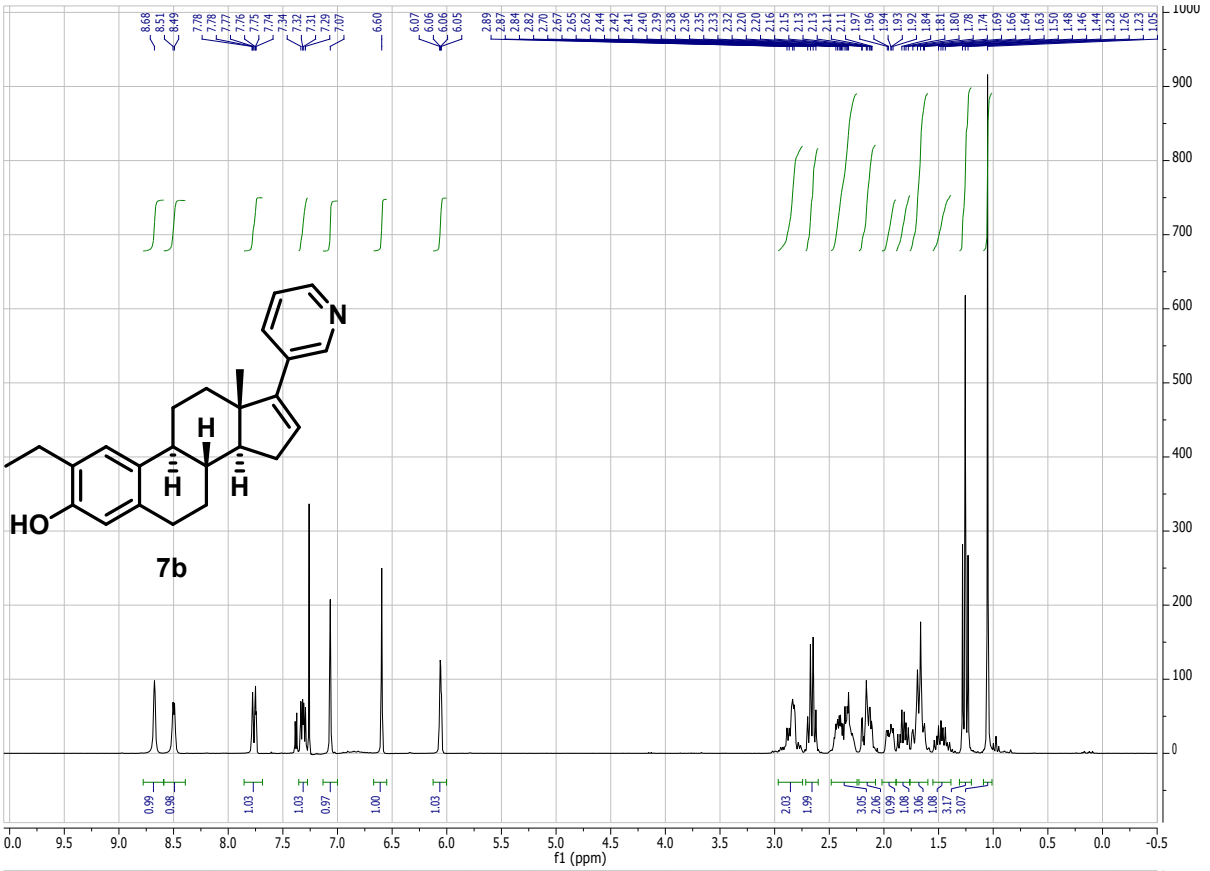
7b
HO
N
H
H
H
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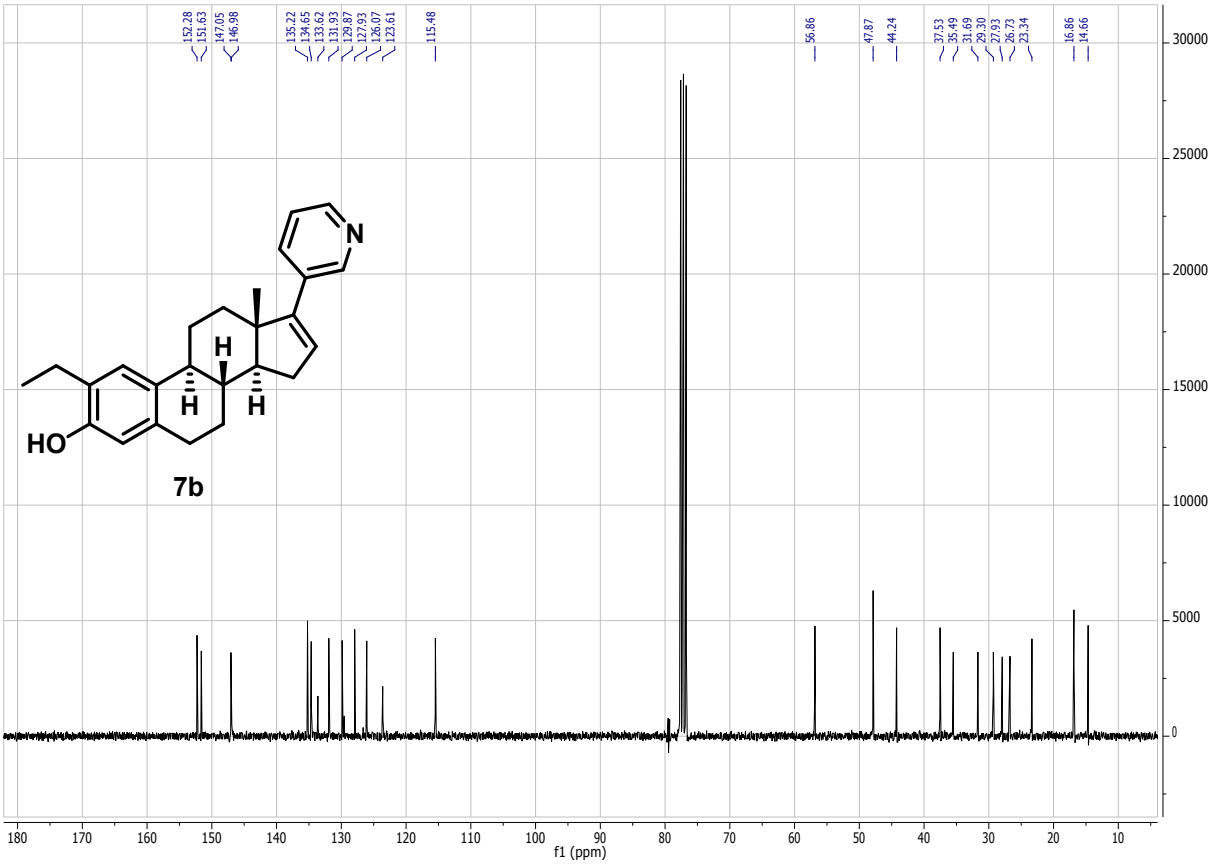
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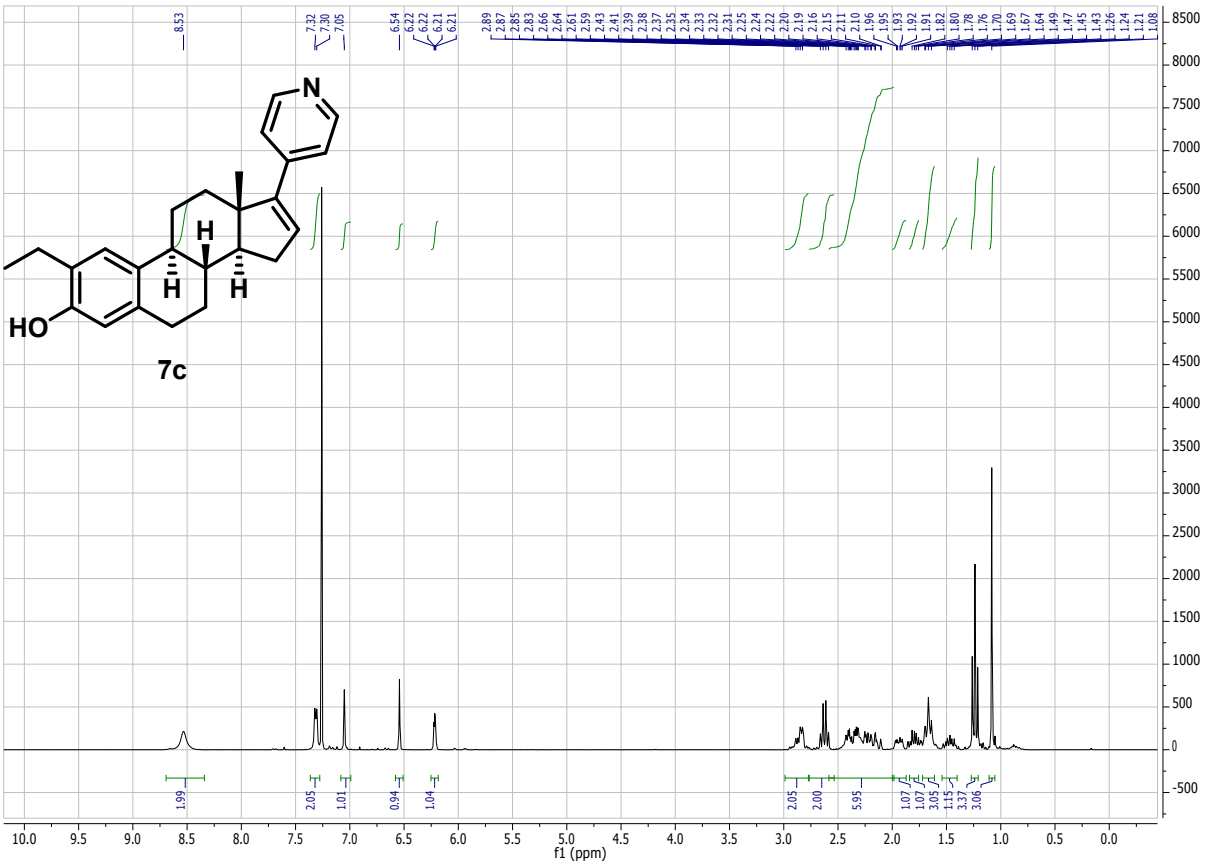
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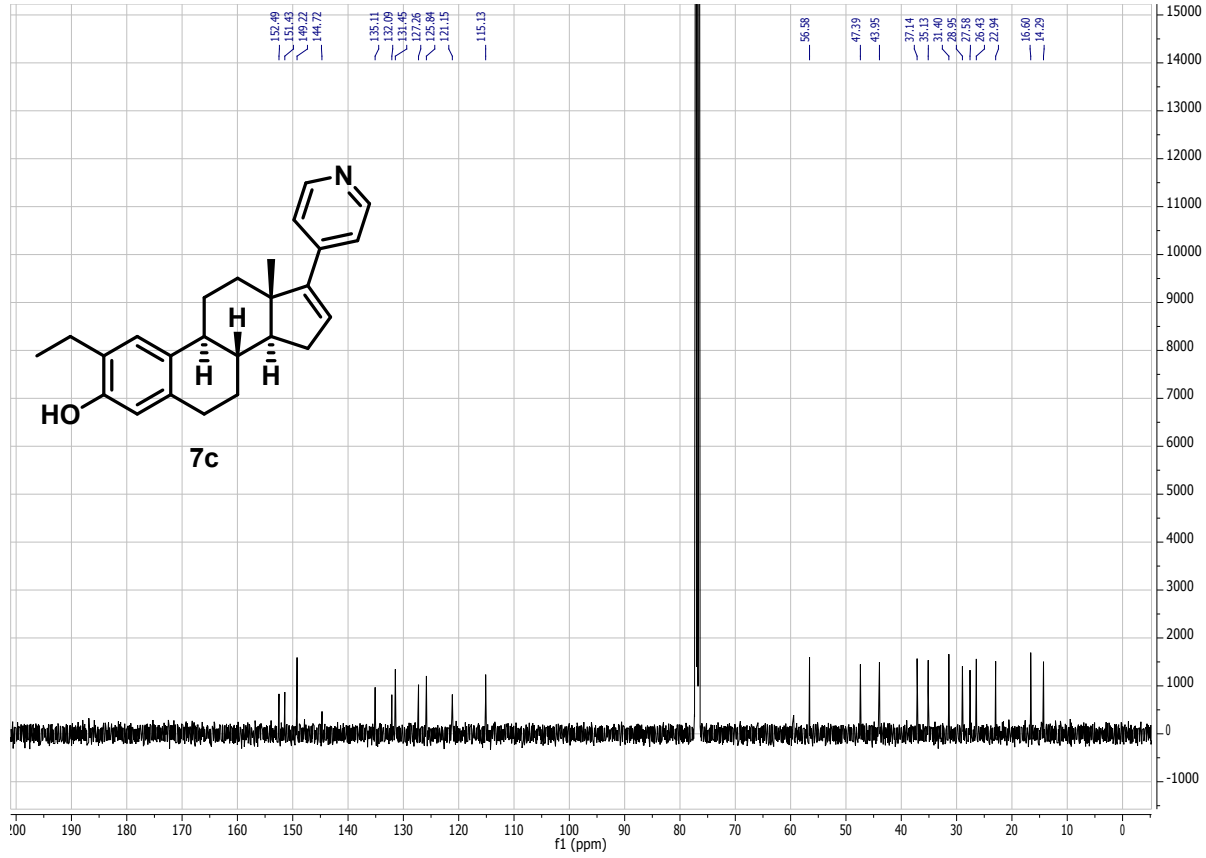
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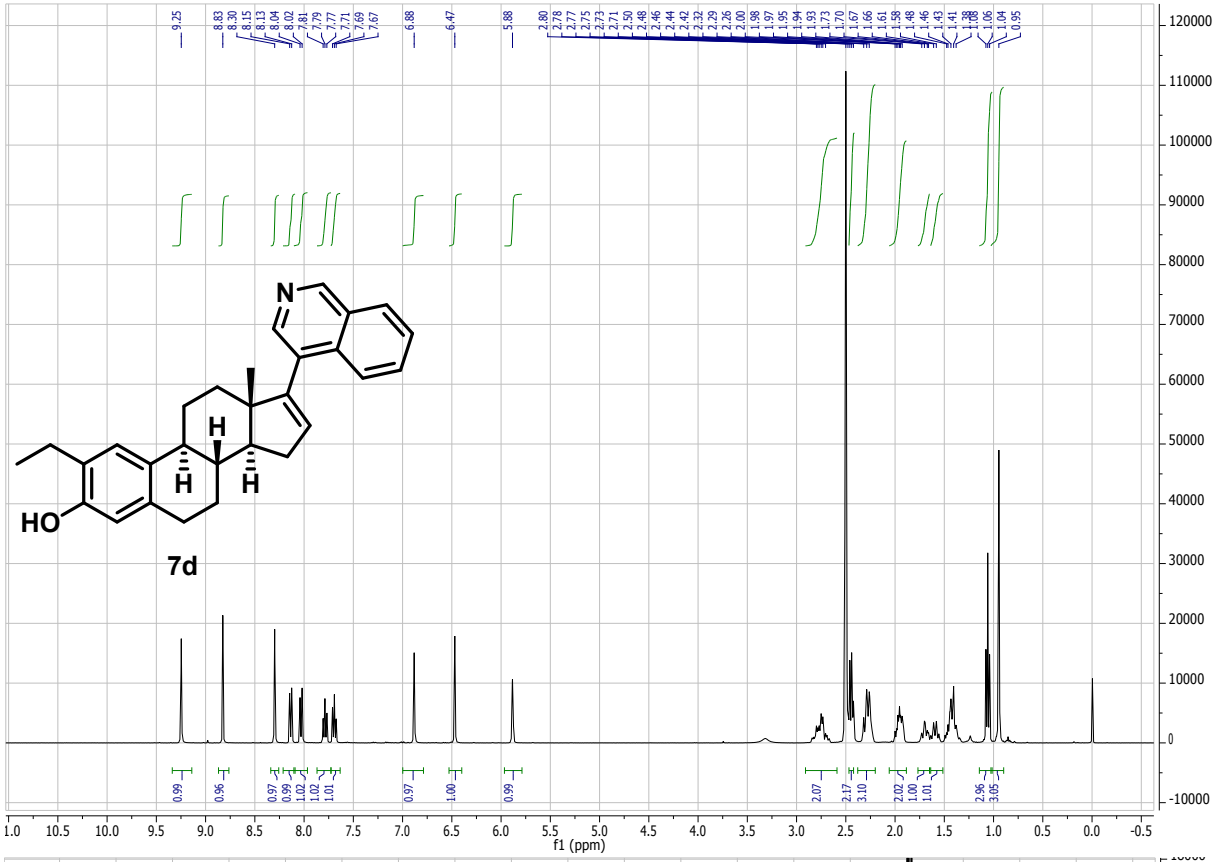
7d
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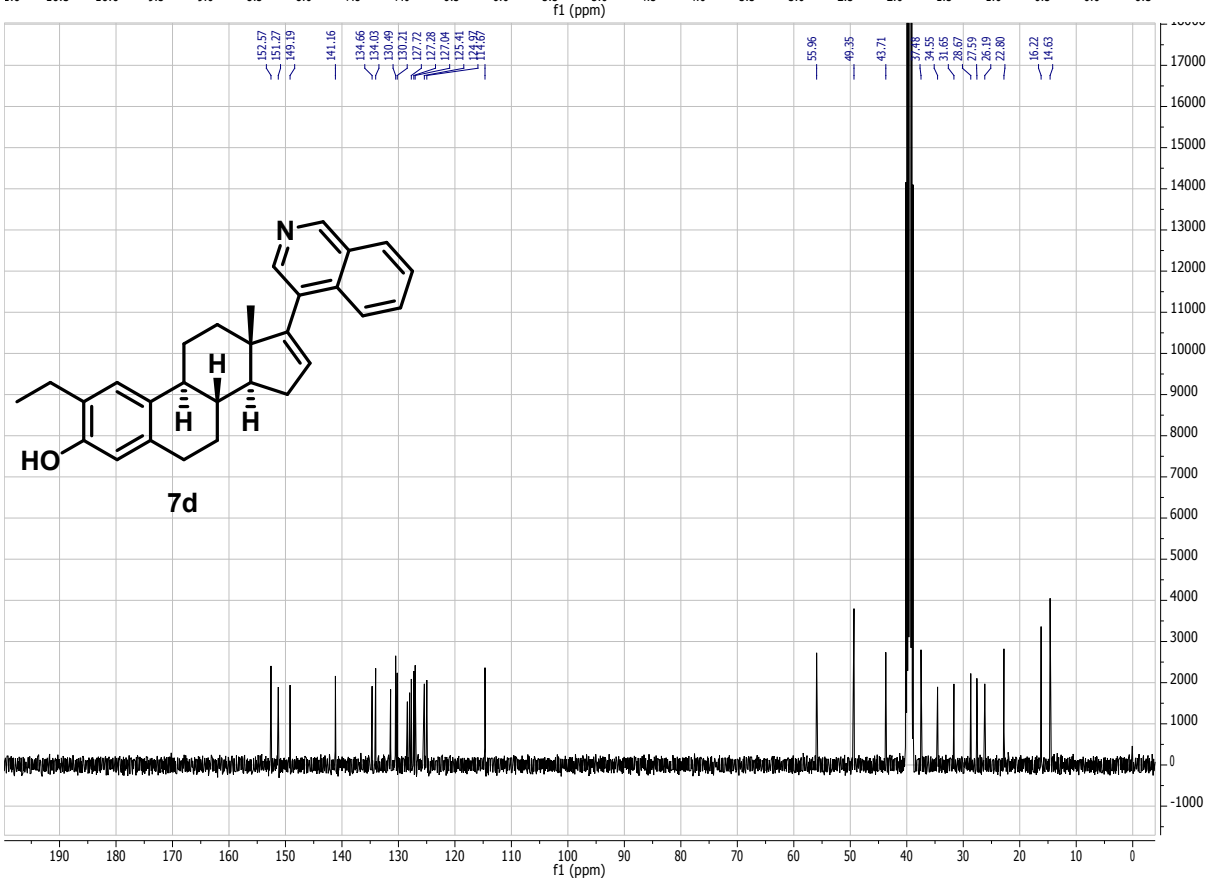
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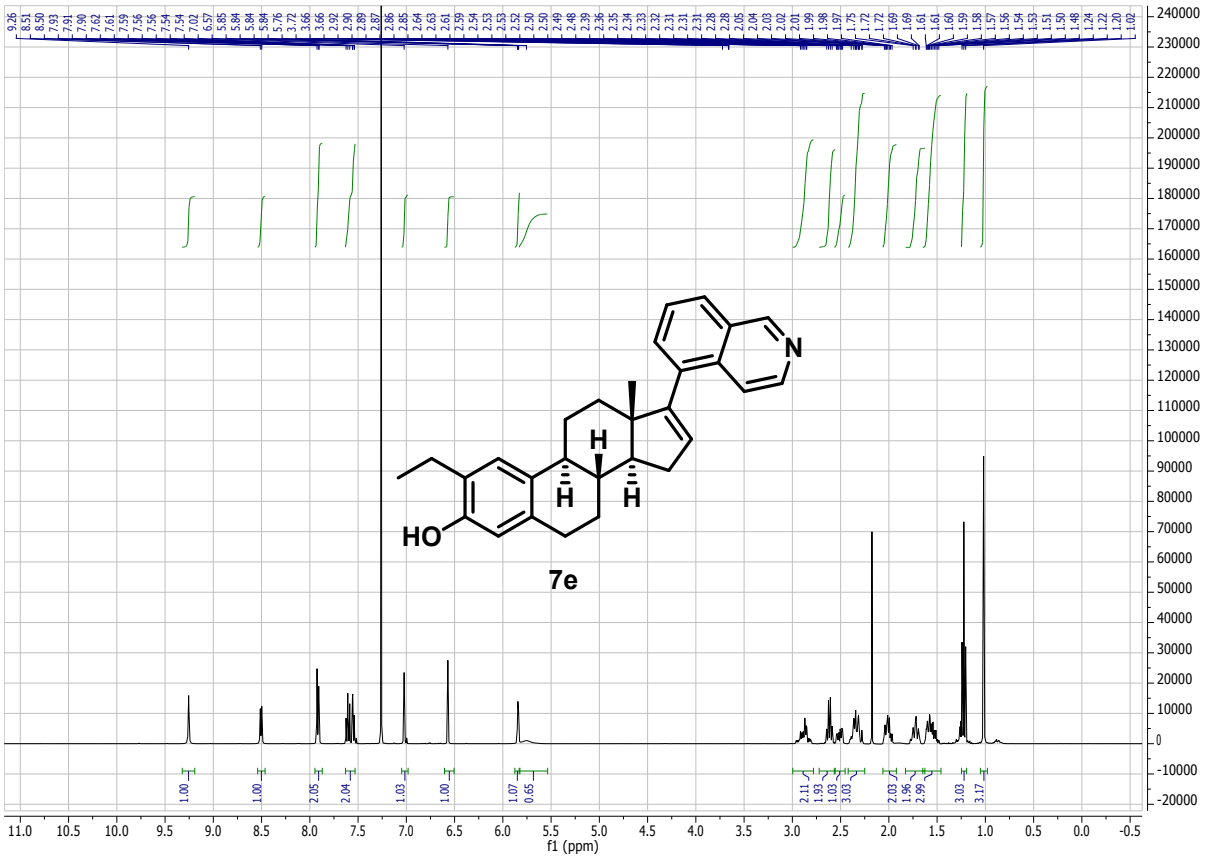
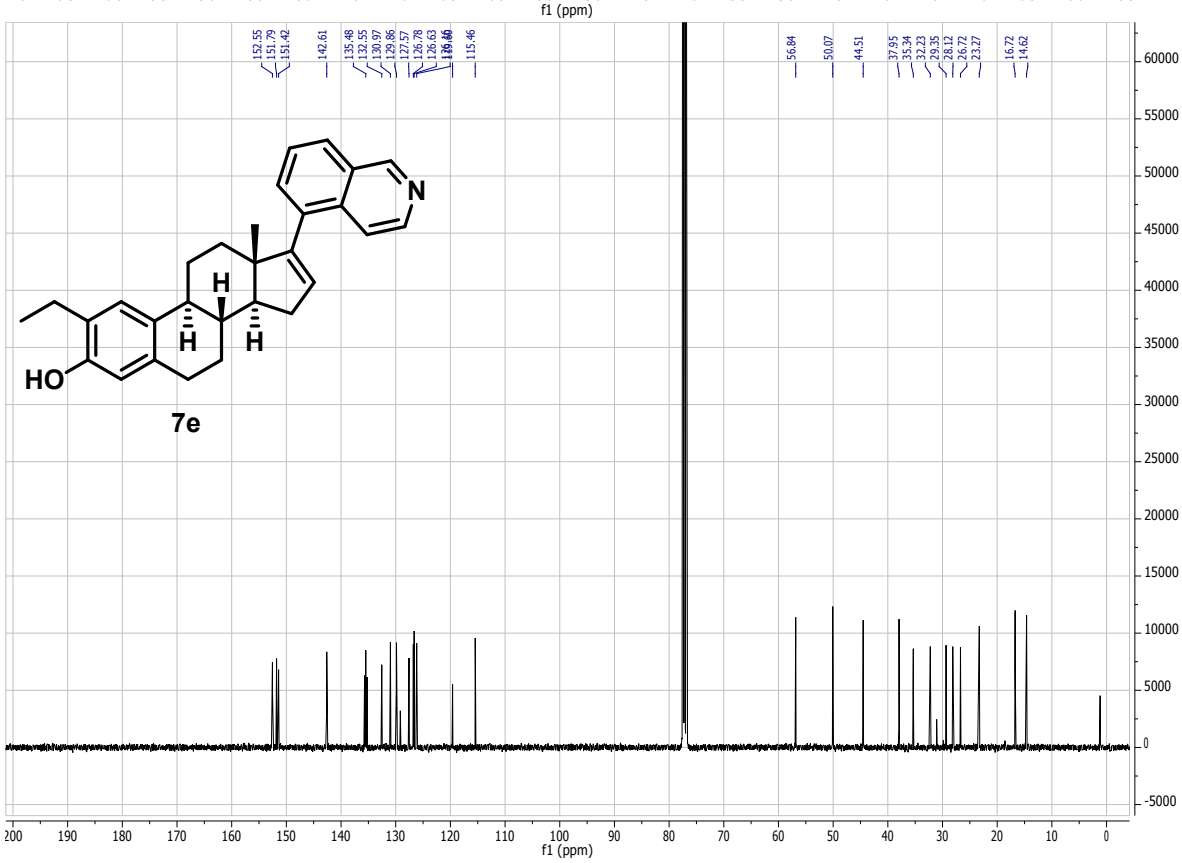
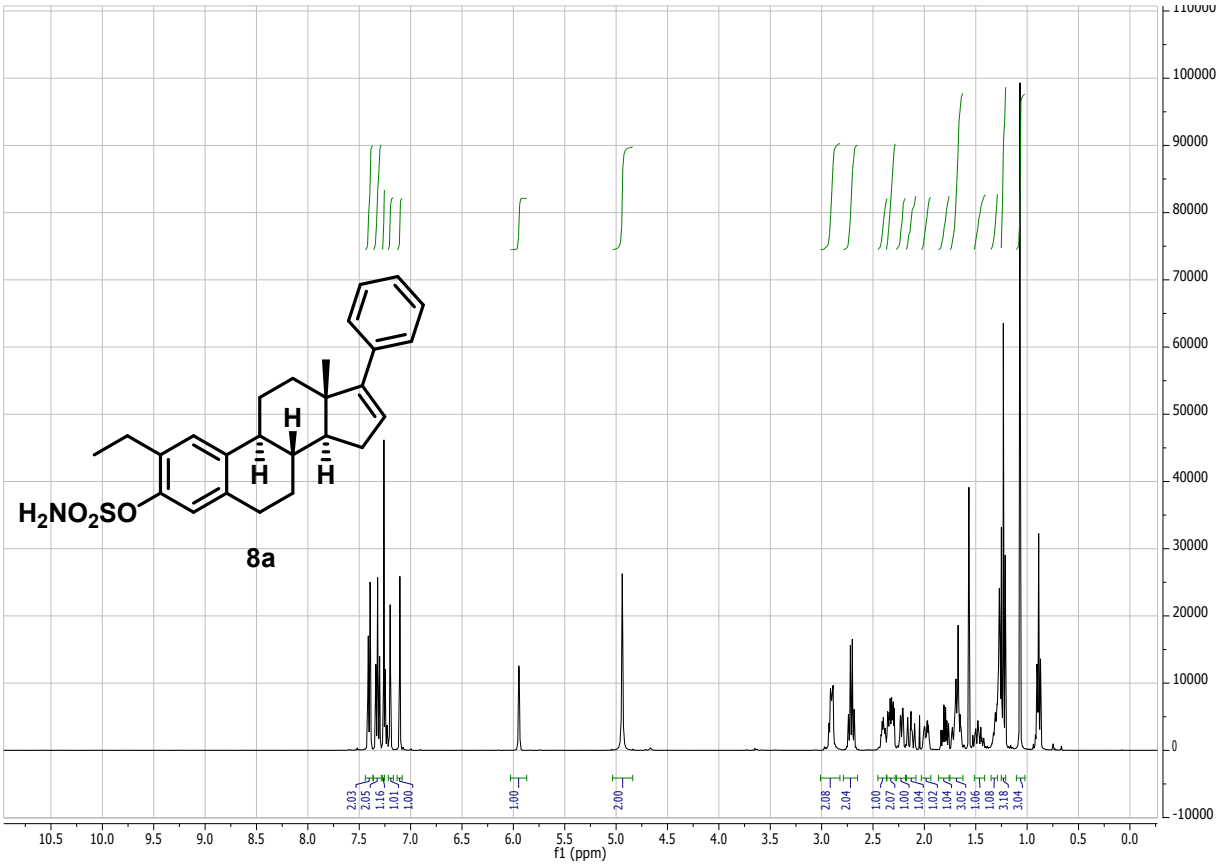
f1 (ppm)


N
H
H
H
HO
7e
f1 (ppm)


N
H
H
H
HO
7e
f1 (ppm)


H
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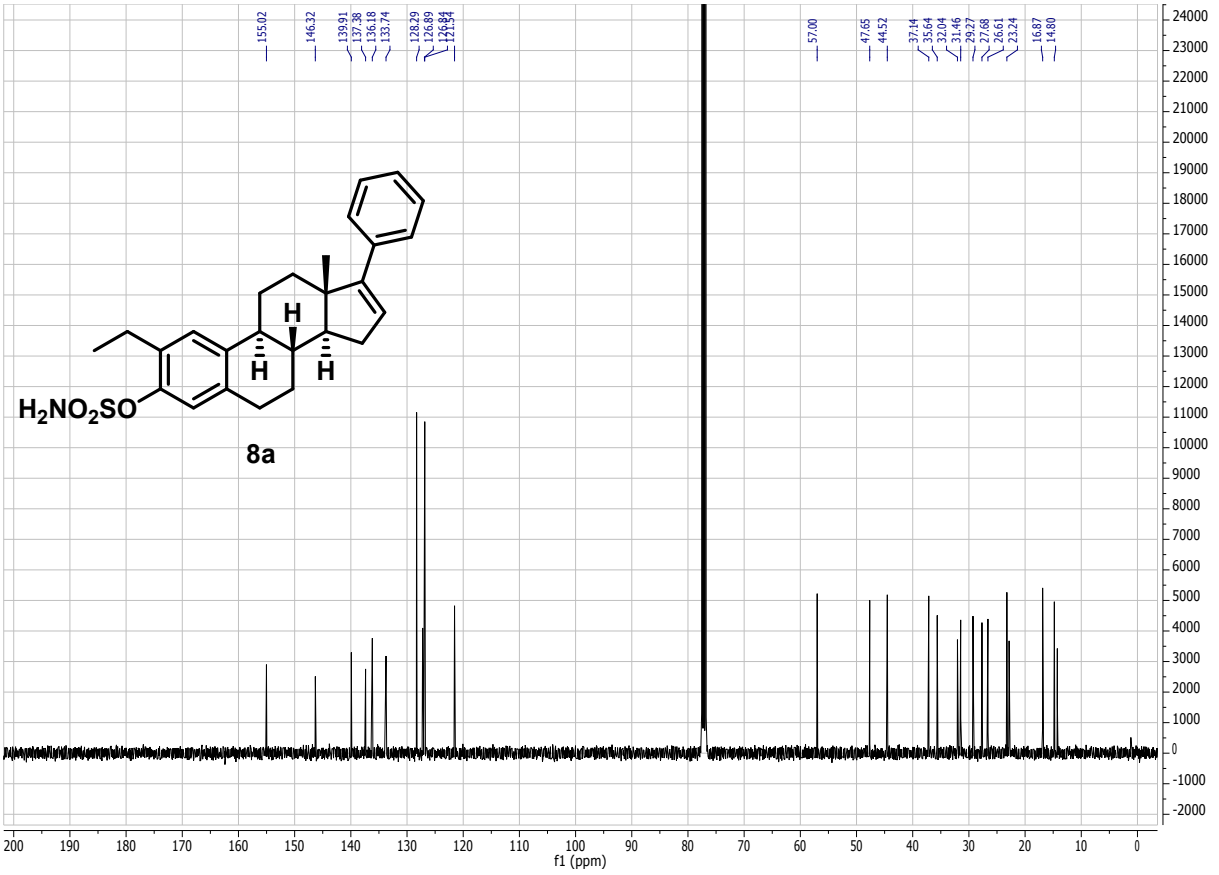
H
H_2NO_2SO
8a
f1 (ppm)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35.64
32.04
31.46
29.27
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23.24
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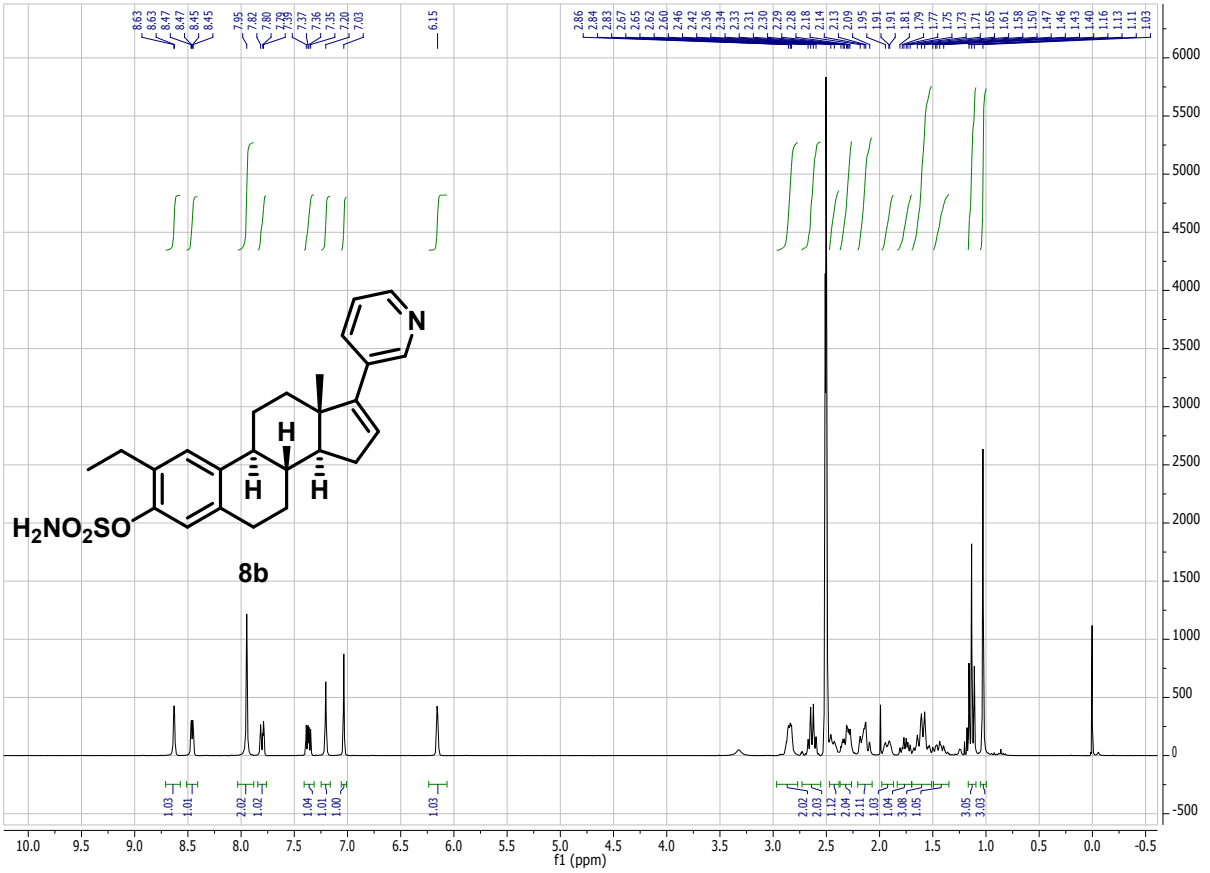
H
H
H
H_2NO_2SO
8a
f1 (ppm)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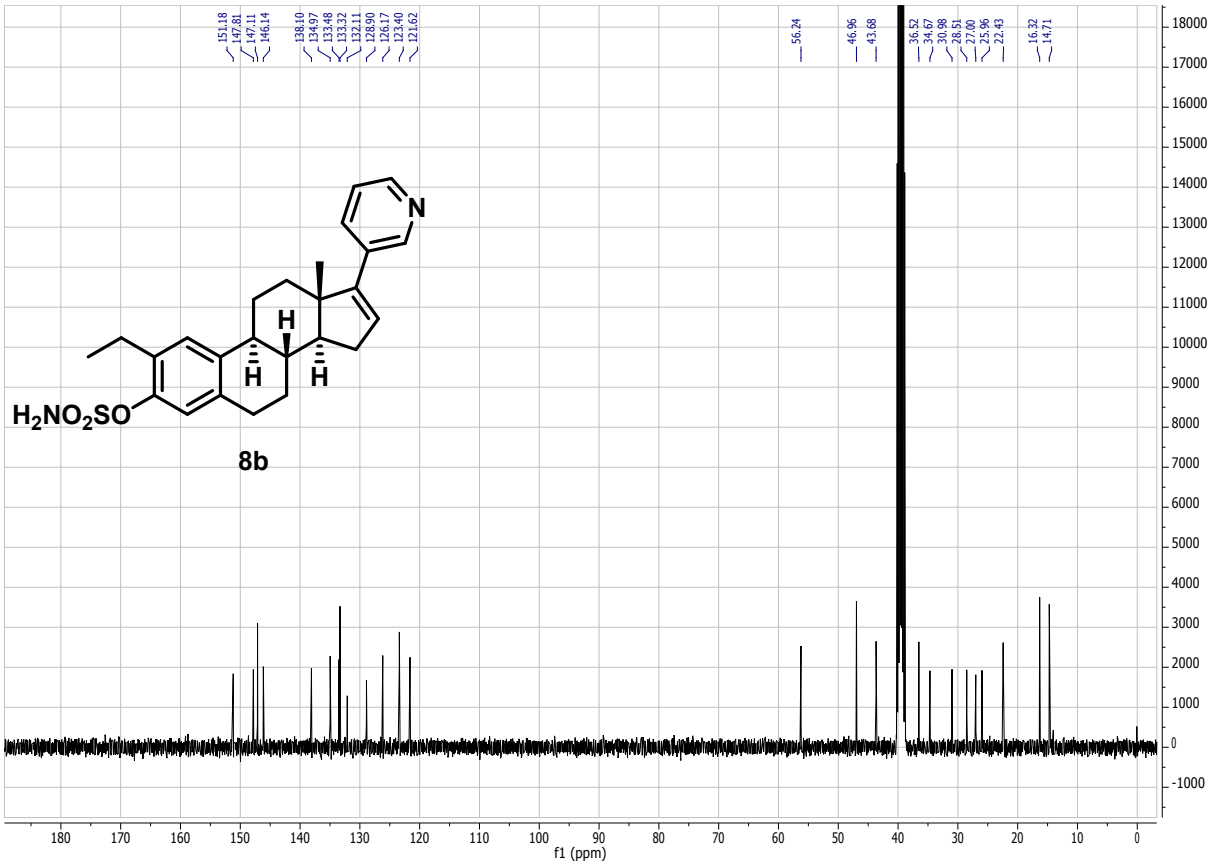
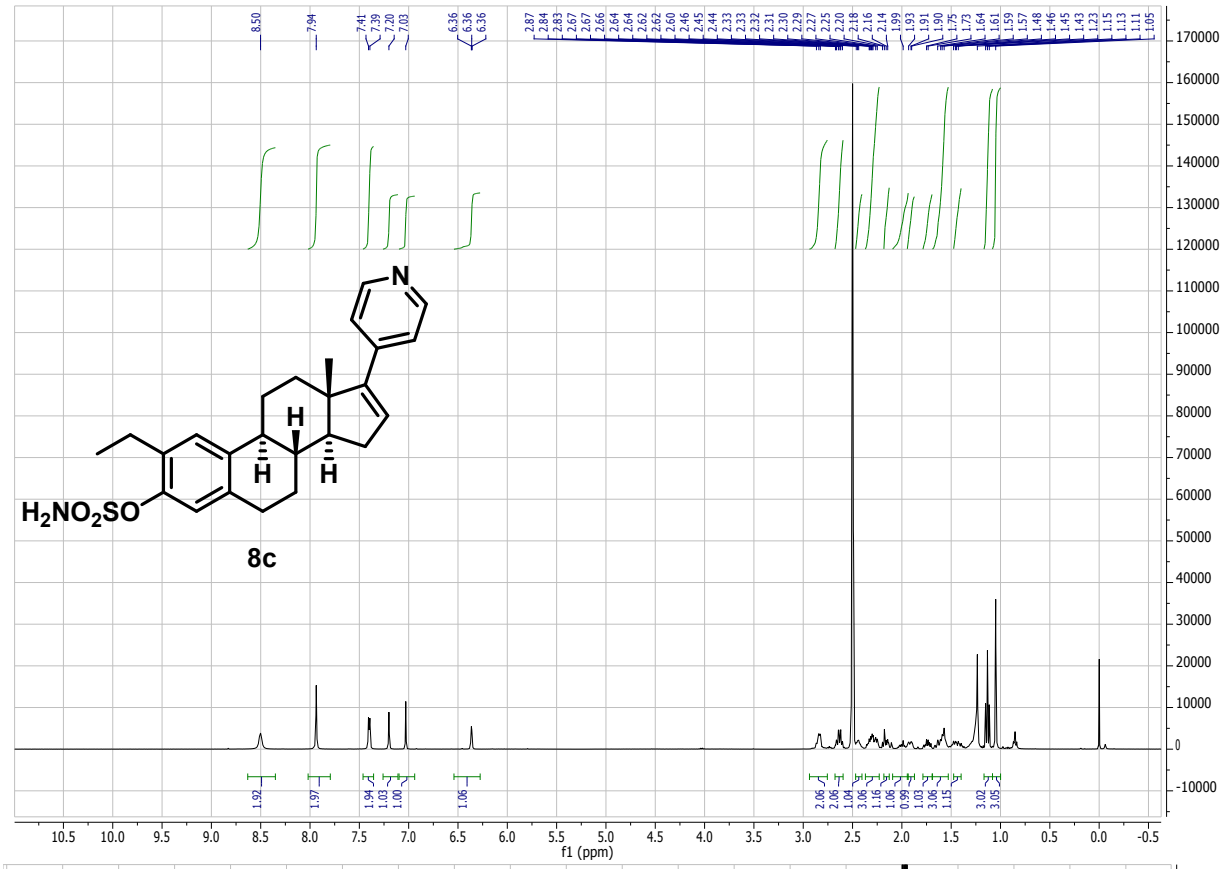
7.95 7.82 7.80 7.79 7.39 7.37 7.36 7.35 7.20 7.03
6.15
2.86 2.84 2.83 2.67 2.65 2.62 2.60 2.46 2.42 2.36 2.34 2.33 2.31 2.30 2.29 2.28 2.18 2.14 2.13 2.09 1.95 1.91 1.91 1.81 1.79 1.77 1.75 1.73 1.71 1.65 1.61 1.58 1.50 1.47 1.46 1.43 1.40 1.16 1.13 1.11 1.03
N
H
H
H
H_2NO_2SO
8b
1.03 1.01 2.02 1.02 1.04 1.01 1.00 1.03 2.02 2.03 1.12 2.04 2.11 1.03 1.04 3.08 1.05 3.05 3.03
f1 (ppm)


151.18 147.81 147.11 146.14 138.10 134.97 133.48 133.32 132.11 128.90 126.17 123.40 121.62
56.24 46.96 43.68 36.52 34.67 30.98 28.51 27.00 25.96 22.43 16.32 14.71
N
H
H
H
H_2NO_2SO
8b
f1 (ppm)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7.41
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7.20
7.03
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2.84
2.83
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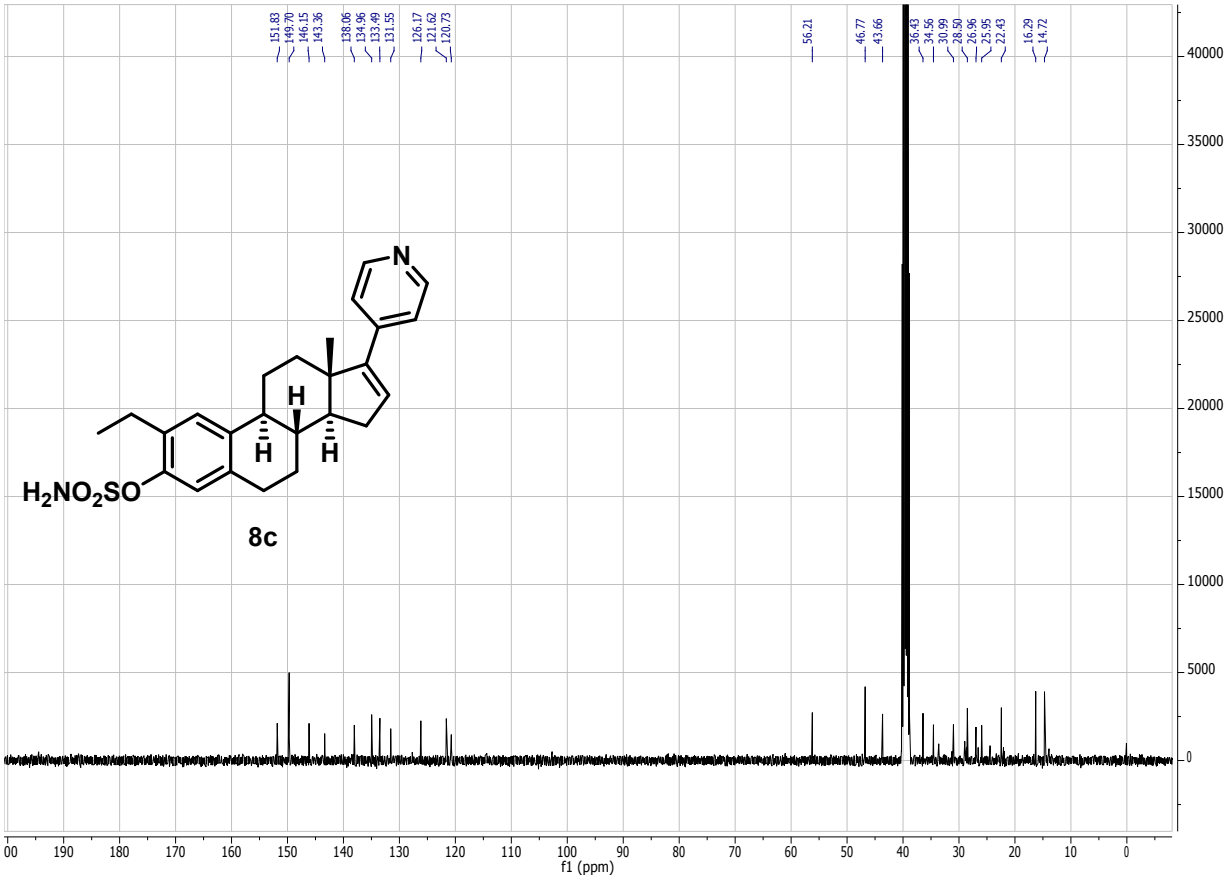
2.67
2.67
2.66
2.64
2.64
2.62
2.62
2.60
2.46
2.45
2.44
2.33
2.33
2.32
2.31
2.30
2.29
2.27
2.25
2.20
2.18
2.16
2.14
1.99
1.93
1.91
1.90
1.75
1.73
1.64
1.61
1.59
1.57
1.48
1.46
1.45
1.43
1.23
1.15
1.13
1.11
1.05
1.92
1.97
1.94
1.03
1.00
1.06
2.06
2.08
1.04
3.06
1.16
1.08
0.99
1.03
1.06
1.15
3.02
3.05
N
H
H
H
H_2NO_2SO
8c
f1 (ppm)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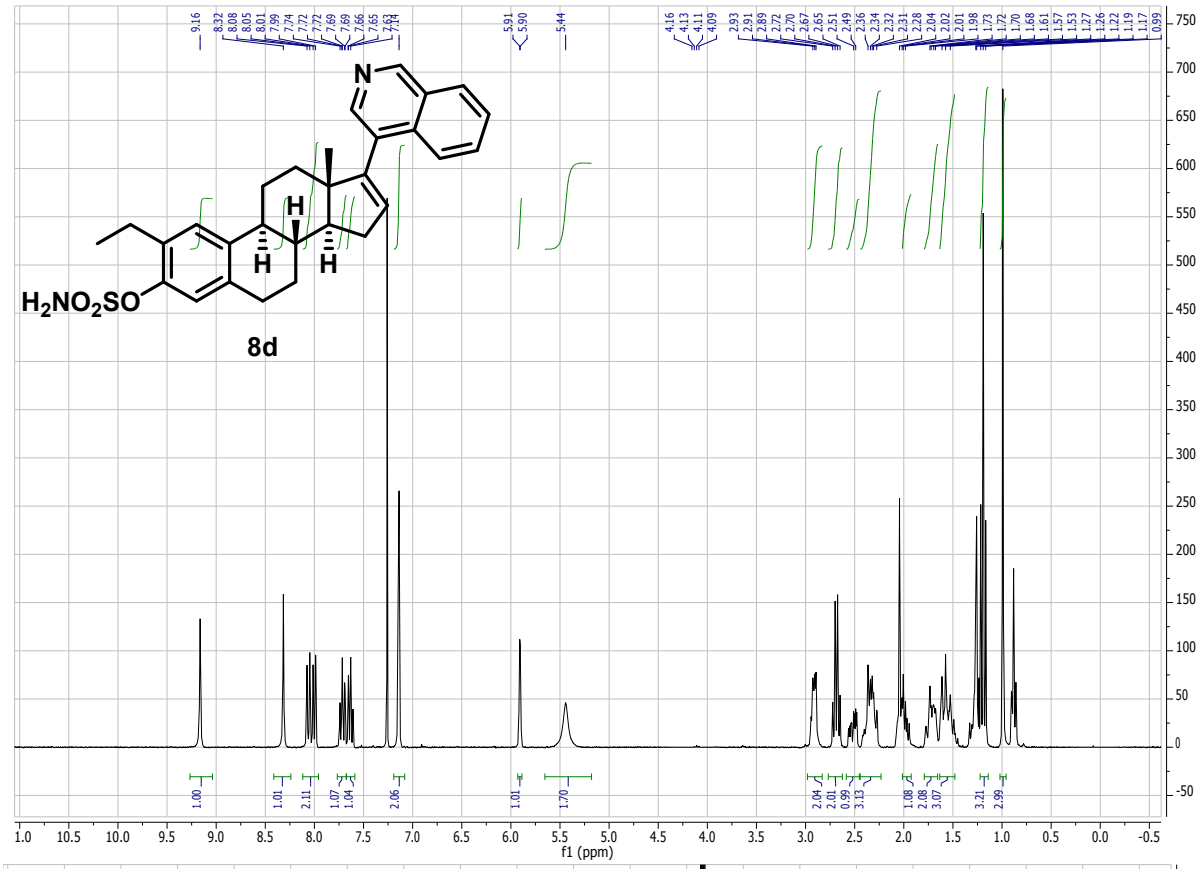
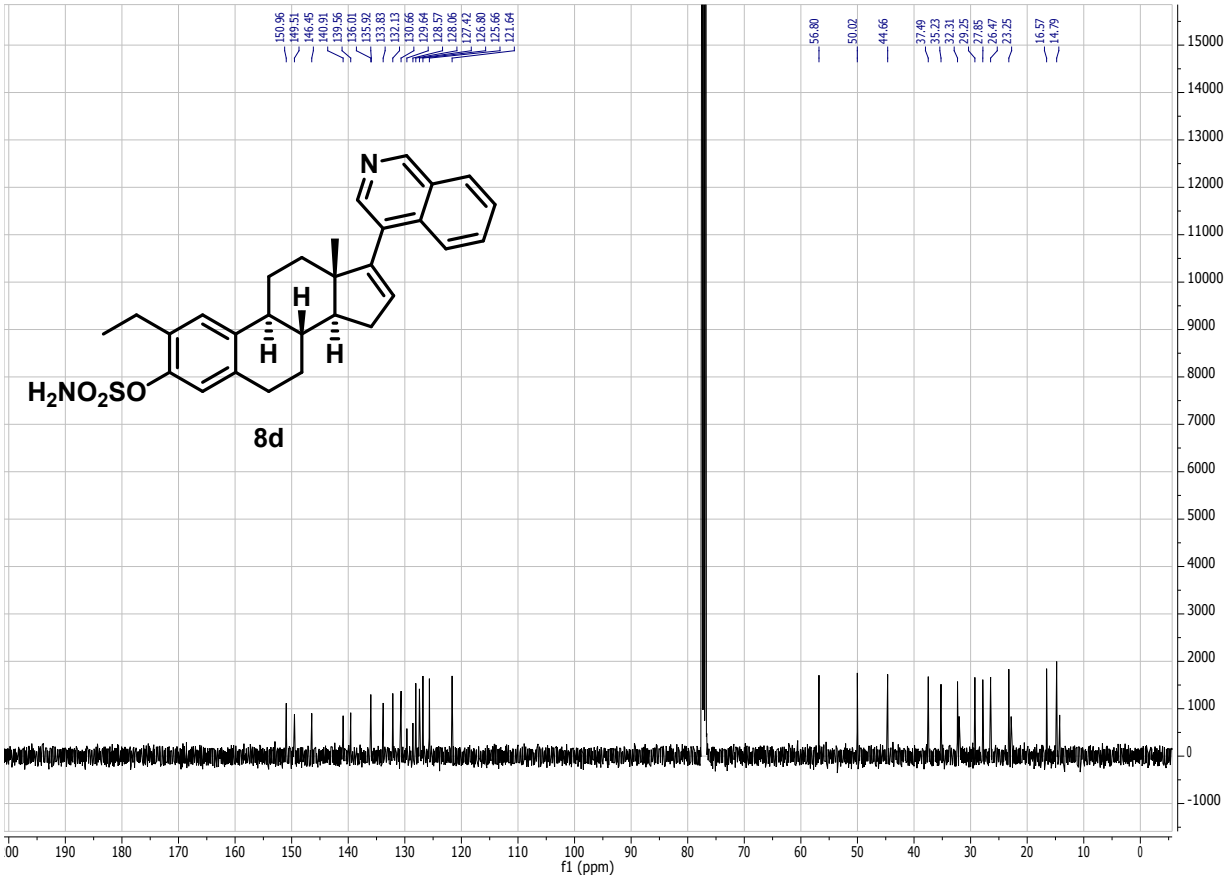
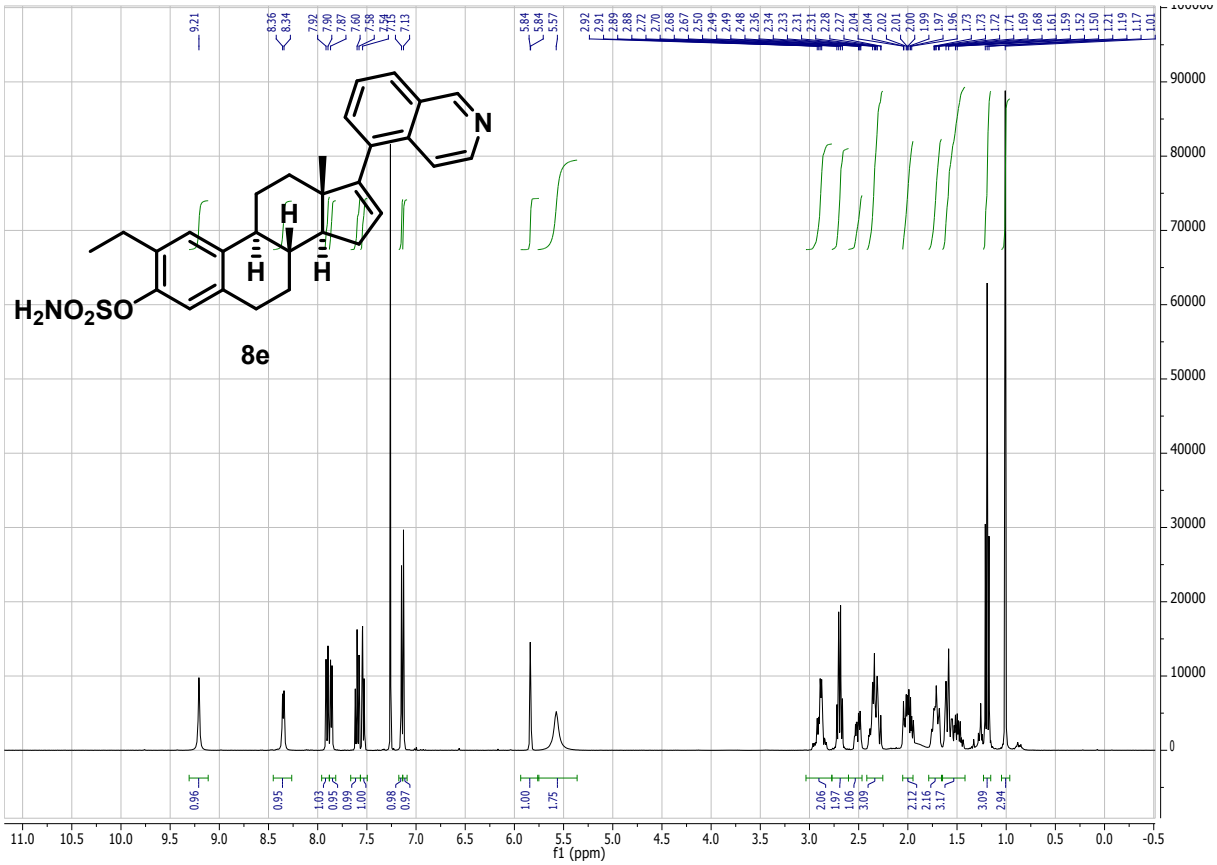
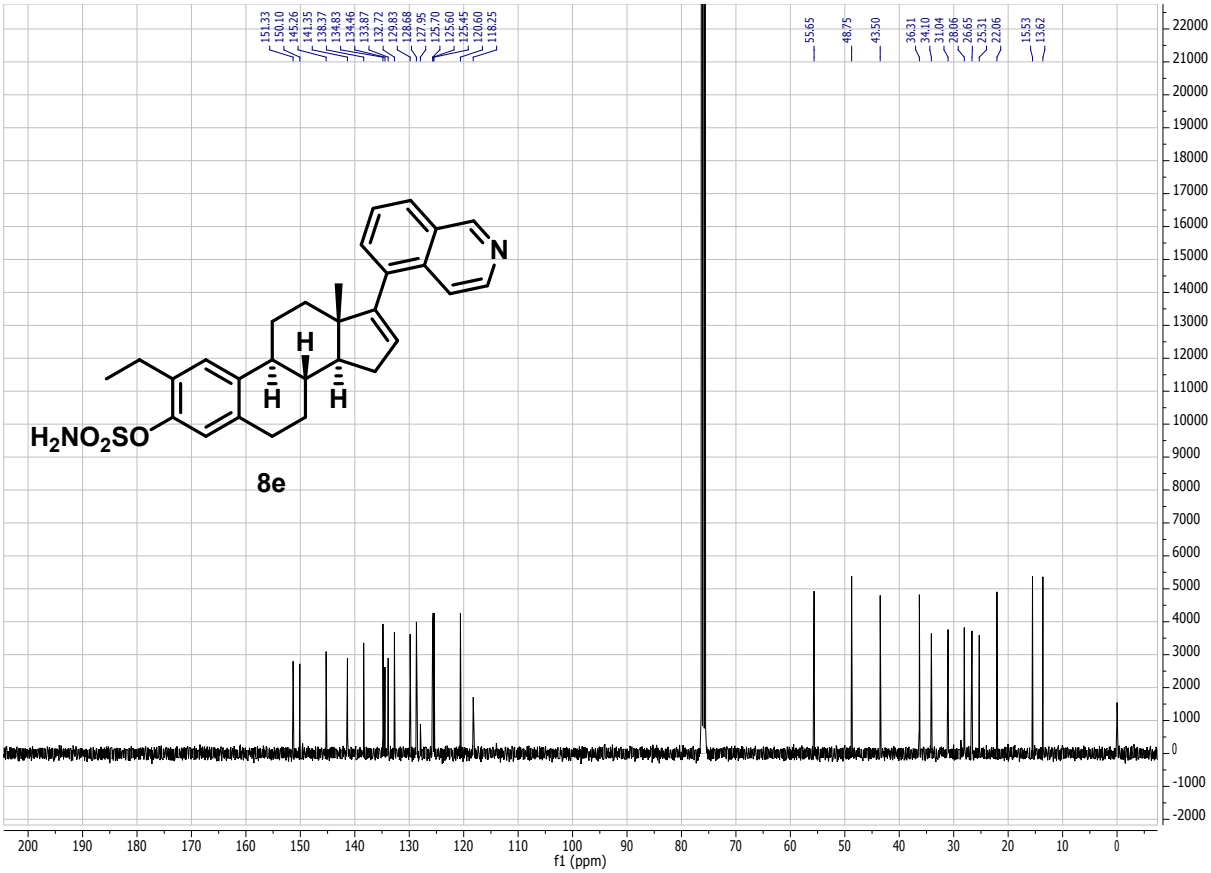
149.70
146.15
143.36
138.06
134.96
133.49
131.55
126.17
121.62
120.73
56.21
46.77
43.66
36.43
34.56
30.99
28.50
26.96
25.95
22.43
16.29
14.72
N
H
H
H
H_2NO_2SO
8c
f1 (ppm)


N
H
H
H
H_2NO_2SO
8d
f1 (ppm)


N
H
H
H
H_2NO_2SO
8d
f1 (ppm)


8e
f1 (ppm)


8e
f1 (ppm)

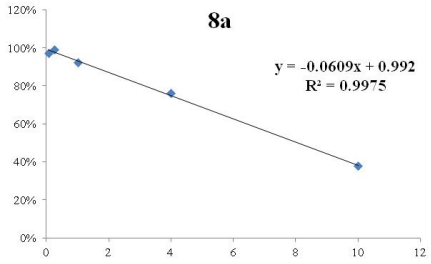
Determination of inhibition of tubulin polymerization data

The polymerization curve was made as OD of each sample (Y axis) vs time (X axis). The AUC (area under curve) between zero to 30 minutes was obtained to present the polymerization degree. The final concentration of each compound tested was 0.0625, 0.25, 1, 4 and 10 μM. The AUC (area under curve) between zero to 30 minutes was obtained to present the polymerization degree using Sigmaplot software. After AUC was obtained, the average AUC was calculated with the equation was shown as below to get the inhibition percentage. We set colchicine as 100% inhibition and paclitaxel as 0%.

Inhibition rate = Sample (paclitaxel)/Sample (colchicine - paclitaxel) x 100%

The IC_{50}–value was calculated after obtaining the curve equation of inhibition % (Y axis) and concentration (x axis) using Excel.

Compound **8a** is shown as an example:



Reference:

(1) Akselsen, O. W.; Hansen, T. V. *Tetrahedron* **2011**, *67*, 7738.