Supporting Information

Progress Toward the Asymmetric de Novo Synthesis of Lanostanes: A Counter Biomimetic Cucurbitane-to-Lanostane Type Transformation

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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 tetrahydrofuran (THF), dichloromethane (DCM) 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. For flash column chromatography, HPLC grade solvents were used without further purification.

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 initial exposure of TLC plates to UV-light (254 nm), followed by 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® Sfar Silica HC D 5–10 g cartridges. 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). NMR coupling constants are measured in Hertz (Hz), and 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 solvent: $CDCl_3$ (77.16 ppm).

Structural assignments for new compounds were supported by two-dimensional NMR experiments (COSY, HSQC, HMBC 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.

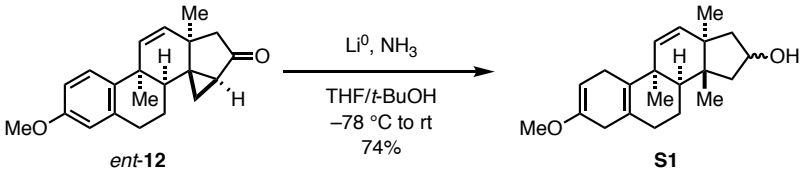
iii. Accurate Mass Determination

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

iv. Optical Rotation

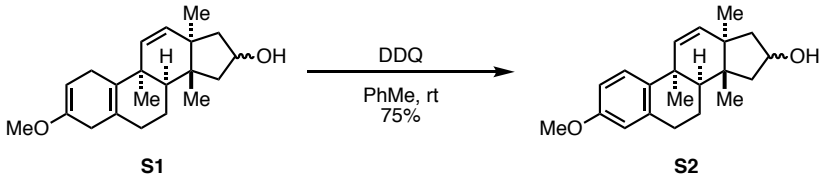
Optical rotations (α) were obtained on a JASCO-P-2000 polarimeter equipped with tungsten-halogen lamp (WI) and interface filter set to 589 nm, using a sample cell with a pathlength of 100 nm. Specific rotations are reported as: $[\alpha]_{589}^{T\,(^{\circ}C)}$ (*c*, solvent) and are based on the equation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

3. Experimental Procedures



Synthesis of enol ether S1: A three-necked, 250-mL round bottom flask was equipped with a Dewar condenser. Ammonia gas was condensed (approx. 50 mL) into the round bottom flask at –78 °C, and then the flask was placed under nitrogen atmosphere. Lithium metal (0.31g, 44.94 mmol, 66 equiv) was added in one portion. The resulting dark blue solution was stirred for 15 minutes at –78 °C. Then, *ent*-**12**[1] (0.210 g, 0.681 mmol, 1 equiv) was added in a 1:1 solution of THF/*t*-BuOH (25 mL total, prepared from 12.5 mL THF and 12.5 mL *t*-BuOH). The dark blue solution was stirred for 1 hour at –78 °C, then was allowed to warm to room temperature and was stirred for an additional 1 hour, maintaining the –78 °C Dewar condenser above the reaction flask the entire time. The solution was then returned to –78 °C and ~20 mL of a saturated aqueous solution of NH_4Cl was added, slowly. When the entire solution had changed in appearance from dark blue to clear, it was allowed to warm to room temperature. The ammonia was allowed to evaporate under a stream of nitrogen gas. The solution was then diluted with DCM (~50 mL) and DI water (~50 mL). The organic layer was separated, and the aqueous layer was extracted with DCM (5 x 50 mL). The combined organic layers were dried over Na_2SO_4, filtered through Celite, and concentrated *in vacuo*. The crude product was purified by flash column chromatography on a Biotage® Sfar Silica HC D 10 g cartridge with 93:7 to 40:60 hexanes–ethyl acetate gradient elution to afford the tentatively assigned diastereomeric mixture of enol ether **S1** (0.159 g, 74%) as a clear oil.

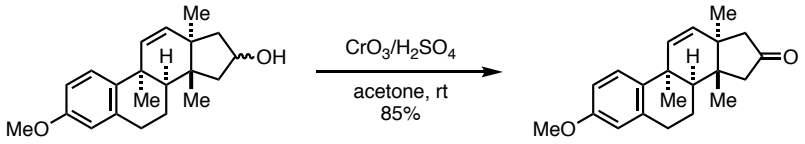
Analytical data for diastereomeric mixture of enol ether S1: ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (151 MHz, $CDCl_3$) are reported in Section 5, "NMR Spectra."
TLC (SiO_2) R_F = 0.4 (30% EtOAc/Hexanes).
IR (Thin film, cm^{-1}) 3790, 3662, 2954, 2927, 1218.
HRMS (ESI-TOF) m/z: [M+H]+ Calculated for $C_{21}H_{31}O_2$ 315.2324; found 315.2313.



Synthesis of alcohol S2: To a 25-mL round bottom flask equipped with stir bar was added enol ether **S1** (79.0 mg, 0.251 mmol, 1 equiv) in PhMe (5 mL). To this solution was added 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (0.120 g, 0.528 mmol, 2.1 equiv) in one portion. The resulting dark red solution was allowed to stir at room temperature for 3.5 hours, monitored by

TLC. Upon consumption of starting material as determined by TLC analysis, the solution was concentrated under reduced pressure to give a dark red residue which was purified by flash column chromatography on a Biotage® Sfar Silica HC D 5 g cartridge with 93:7 to 40:60 hexanes–ethyl acetate gradient elution to afford the tentatively assigned diastereomeric mixture of alcohol **S2** (58.9 mg, 75%) as a white foam.

Analytical data for diastereomeric mixture of S2: ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (151 MHz, $CDCl_3$) are reported in Section 5, "NMR Spectra."
TLC (SiO_2) R_F = 0.4 (40% EtOAc/Hexanes).
IR (Thin film, cm^{-1}) 3371, 2947, 1605, 1255.
HRMS (ESI-TOF) m/z: [M+H]+ Calculated for $C_{21}H_{29}O_2$ 313.2168; found 313.2154.

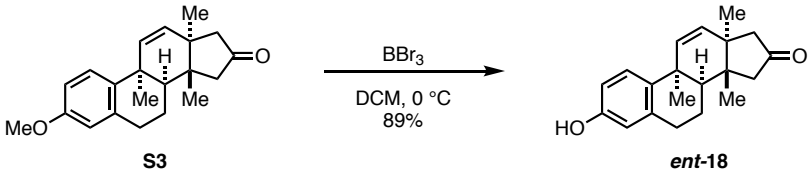


S2 **S3**

Synthesis of ketone S3: To prepare the Jones reagent[2] (CrO_3/H_2SO_4, ~3.8 M in H_2O): In a 10-mL round bottom flask with stir bar, chromium (IV) oxide (0.167 g) was dissolved in DI H_2O (0.31 mL) at room temperature. To this solution was added concentrated sulfuric acid (0.15 mL), slowly. An additional 0.13 mL DI H_2O was added to dissolve insoluble salts, resulting in a red solution.
In a separate 50-mL round bottom flask, a solution of alcohol **S2** (48.0 mg, 0.156 mmol, 1 equiv) in acetone (16 mL) was allowed to stir at room temperature and the freshly prepared Jones reagent (81 μL, 0.311 mmol, 2 equiv) was added dropwise. The opaque green solution was allowed to stir 30 minutes at room temperature. Upon consumption of starting material as determined by TLC analysis, *i*PrOH (1.2 mL) was added to quench remaining oxidant. The solvent was removed *in vacuo*. The resulting green residue was diluted with H_2O (25 mL) and EtOAc (25 mL), and the organic layer was separated. The aqueous layer was extracted with EtOAc (3 x 25 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give ketone **S3** (41.0 mg, 85%) as an orange residue, which was used without further purification.

Analytical data for ketone S3:
TLC (SiO_2) R_F = 0.8 (30% EtOAc/Hexanes).
Specific Rotation $[\alpha]_{589}^{20.4} = +172.2$ (*c* 0.25, $CHCl_3$).
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.28 (d, *J* = 8.6 Hz, 1H), 6.72 (dd, *J* = 8.6, 2.5 Hz, 1H), 6.67 (d, *J* = 2.1 Hz, 1H), 6.17 (d, *J* = 10.0 Hz, 1H), 5.96 (d, *J* = 9.9 Hz, 1H), 3.78 (s, 3H), 2.86 (ddd, *J* = 16.0, 10.7, 5.5 Hz, 1H), 2.74 (dt, *J* = 15.5, 5.3 Hz, 1H), 2.63 (t, *J* = 7.8 Hz, 1H), 2.51 (d, *J* = 17.8 Hz, 1H), 2.29 (d, *J* = 17.4 Hz, 1H), 2.16 – 2.06 (m, 1H), 1.98 (dd, *J* = 17.5, 6.6 Hz, 2H), 1.58 – 1.49 (m, 1H), 1.32 (s, 3H), 1.21 (s, 3H), 0.69 (s, 3H).

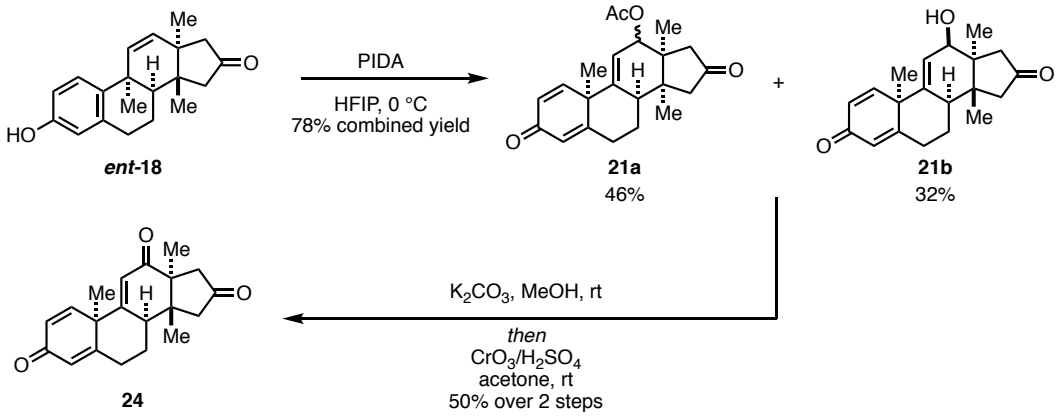
^{13}C NMR (151 MHz, $CDCl_3$) δ 218.86, 157.22, 139.39, 137.08, 134.85, 130.47, 125.31, 114.11, 111.81, 55.24, 48.74, 48.35, 45.35, 44.79, 43.01, 39.46, 33.46, 28.69, 27.04, 24.02, 19.74.
IR (Thin film, cm^{-1}) 2954, 1741, 1609.
HRMS (ESI-TOF) m/z: [M+H]+ Calculated for $C_{21}H_{27}O_2$ 311.2011, found 311.2003.



S3 ***ent*-18**

Synthesis of *ent*-phenol 18: Ketone **S3** (34 mg, 0.112 mmol, 1 equiv) was dissolved in DCM (11 mL) and the solution was cooled to 0 °C. Boron tribromide (33 µL, 0.335 mmol, 3 equiv) was added dropwise. The solution was allowed to stir 2.5 hours at 0 °C, progress monitored by TLC. Upon consumption of the starting material as determined by TLC analysis, MeOH (10 mL) was added to quench the Lewis acid. The solution was poured over a cold saturated aqueous $NaHCO_3$ solution (25 mL). The resulting mixture was transferred to a separatory funnel and diluted with DCM (25 mL). The organic layer was separated and the aqueous layer was extracted with DCM (3 x 25 mL). Combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was purified by flash column chromatography on a Biotage® Sfar Silica HC D 5 g cartridge with 93:7 to 40:60 hexanes–ethyl acetate gradient elution to afford *ent*-phenol **18** (29 mg, 89%) as a clear oil.

Analytical data for *ent*-phenol 18:
TLC (SiO_2) R_F = 0.5 (30% EtOAc/Hexanes).
Specific Rotation $[\alpha]_{589}^{20.5} = +72.2$ (*c* 0.25, $CHCl_3$).
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.21 (d, *J* = 8.4 Hz, 1H), 6.65 (dd, *J* = 8.4, 2.6 Hz, 1H), 6.61 (d, *J* = 2.2 Hz, 1H), 6.15 (d, *J* = 10.0 Hz, 1H), 5.95 (d, *J* = 10.0 Hz, 1H), 5.19 (s, 1H), 2.82 (ddd, *J* = 16.0, 10.9, 5.6 Hz, 1H), 2.69 (dt, *J* = 15.5, 5.3 Hz, 1H), 2.62 (t, *J* = 7.9 Hz, 1H), 2.51 (d, *J* = 17.9 Hz, 1H), 2.29 (d, *J* = 17.4 Hz, 1H), 2.09 (td, *J* = 13.7, 5.5 Hz, 1H), 1.99 (dd, *J* = 17.5, 12.3 Hz, 2H), 1.50 (tt, *J* = 13.2, 6.5 Hz, 1H), 1.31 (s, 3H), 1.20 (s, 3H), 0.66 (s, 3H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 219.46, 153.25, 139.69, 137.14, 134.86, 130.44, 125.51, 115.53, 113.39, 48.77, 48.38, 45.37, 44.80, 42.98, 39.48, 33.44, 28.50, 27.05, 23.97, 19.72.
IR (Thin film, cm^{-1}) 3376, 2954, 1730.
HRMS (ESI-TOF) m/z: [M+H]+ Calculated for $C_{20}H_{25}O_2$ 297.1849; found 297.1855.
Analysis of relative stereochemistry: HSQC, COSY and HMBC experiments were used to assign all ^{1}H NMR signals for *ent*-phenol **18**, and 1D NOESY experiments shown in Section 5: NMR Spectra revealed the shown correlations, allowing relative stereochemistry to be assigned.



Synthesis of *ent*-lanostane-based dienones 21a and 21b: *Ent*-**18** (20 mg, 0.068 mmol, 1 equiv) was dissolved in HFIP (2 mL) and the clear solution was cooled to 0 °C. PIDA (25 mg, 0.769 mmol, 1.1 equiv) was added in one portion and the resulting yellow solution was allowed to stir 60 seconds. Then, a saturated aqueous solution of $NaHCO_3$ (2 mL) was added. HFIP was removed *in vacuo* and the remaining slurry was diluted with EtOAc (2 mL). The organic layer was separated and the aqueous layer was extracted with EtOAc (3 x 5 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by pipet column silica gel chromatography from 100:0 to 70:30 hexanes–ethyl acetate gradient elution to afford both the diastereomeric mixture of *ent*-lanostane-based **21a** (11 mg, 46%) and **21b** (7 mg, 32%) in a 78% combined yield, both products as clear oils.

Analytical data for *ent*-lanostane-based dienone 21a: ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (151 MHz, $CDCl_3$) are reported in Section 4, "NMR Spectra."
TLC (SiO_2) R_F = 0.3 (50% EtOAc/Hexanes).
IR (Thin film, cm^{-1}) 2961, 1739, 1661, 1631, 1240.
HRMS (ESI-TOF) m/z: [M+H]+ Calculated for $C_{22}H_{27}O_4$ 355.1909; found 355.1905.

Analytical data for *ent*-lanostane-based dienone 21b:
TLC (SiO_2) R_F = 0.1 (50% EtOAc/Hexanes)
Specific Rotation $[\alpha]_{589}^{20.6} = +36.9$ (*c* 0.05 $CHCl_3$).
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.29 (d, *J* = 10.2 Hz, 1H), 6.31 (d, *J* = 11.7 Hz, 1H), 6.12 (s, 1H), 5.93 (d, *J* = 5.4 Hz, 1H), 3.98 (app. s, 1H), 2.94 (d, *J* = 18.2 Hz, 1H), 2.71 – 2.62 (m, 2 H), 2.53 – 2.44 (m, 1H), 2.36 (d, *J* = 17.7 Hz, 1H), 2.05 (d, *J* = 17.2 Hz, 1H), 1.91 (d, *J* = 18.2 Hz, 1H), 1.89 – 1.82 (m, 1H), 1.70 – 1.56 (m, 1H), 1.46 (s, 3H), 1.02 (s, 3H), 1.00 (s, 3H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 217.58, 186.07, 165.38, 152.86, 145.90, 127.80, 124.51, 122.25, 71.74, 49.94, 45.72, 45.12, 44.84, 42.18, 40.77, 32.32, 30.20, 28.92, 22.61, 20.60.
IR (Thin film, cm^{-1}) 3418, 1735, 1658, 1616.
HRMS (ESI-TOF) m/z: [M+H]+ Calculated for $C_{20}H_{25}O_3$ 313.1804; found 313.1800.

Analysis of relative stereochemistry: HSQC, COSY and HMBC experiments were used to assign all ^{1}H NMR signals for allylic alcohol **21b**, and 1D NOESY experiments revealed the

shown correlations, allowing relative stereochemistry to be assigned. See Section 5: NMR Spectra.

Synthesis of *ent*-lanostane-based trienone 24: The combined products *ent*-lanostane-based dienone **21a** and **21b** (0.050 mmol total, 1 equiv) were dissolved in a 1:1 solution of MeOH/THF (1.6 mL total, 0.8 mL each) at room temperature. Potassium carbonate (21 mg, 0.155 mmol, 5 equiv) was added in one portion and the mixture was allowed to stir at room temperature, progress monitored by TLC (5 hours). Upon consumption of starting material as judged by TLC analysis, to the solution was added a saturated aqueous NH_4Cl solution (0.5 mL). The solution was diluted with EtOAc (2 mL) and the organic layer was separated. The remaining aqueous solution was extracted with EtOAc (3 x 5 mL). The combined organic layers were allowed to dry over $MgSO_4$, filtered, and concentrated *in vacuo*.

The crude residue was transferred to a vial and diluted in acetone (0.5 mL) at room temperature. In a separate flask, to prepare the Jones reagent[2] (CrO_3/H_2SO_4, ~3.8 M in H_2O): In a 5-mL round bottom flask with stir bar, chromium (IV) oxide (56 mg) was dissolved in DI H_2O (100 μL) at room temperature. To this solution was added concentrated sulfuric acid (50 μL), slowly. An additional 40 μL DI H_2O was added to dissolve insoluble salts, resulting in a red solution. To the vial containing the solution of the crude residue of the allylic alcohol in acetone was added the freshly prepared Jones reagent (26 μL, 0.100 mmol, 2 equiv). Reaction progress was monitored by TLC (15 minutes). Upon consumption of starting material, remaining oxidant was quenched by the addition of *i*PrOH (0.5 mL). The solvent was removed *in vacuo* and the red residue was diluted with EtOAc (10 mL) and water (10 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (5 x 10 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude residue was purified by pipet column silica gel chromatography with 100:0 to 60:40 hexanes–ethyl acetate gradient elution to afford the *ent*-lanostane-based trienone **24** (8.4 mg, 50% over 2 steps) as a clear oil.

Analytical data for *ent*-lanostane-based trienone 24:
TLC (SiO_2) R_F = 0.2 (50% EtOAc/Hexanes).
Specific Rotation $[\alpha]_{589}^{20.8} = +190.4$ (*c* 0.05, $CHCl_3$).
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.20 (d, *J* = 10.2 Hz, 1H), 6.36 (dd, *J* = 10.2, 1.9 Hz, 1H), 6.19 (d, *J* = 1.7 Hz, 1H), 6.04 (d, *J* = 2.5 Hz, 1H), 3.10 (ddd, *J* = 12.9, 5.4, 2.6 Hz, 1H), 2.84 (d, *J* = 18.9 Hz, 1H), 2.78 (tdd, *J* = 13.7, 4.6, 1.6 Hz, 1H), 2.59 (dt, *J* = 13.5, 3.5 Hz, 1H), 2.51 (d, *J* = 17.3 Hz, 1H), 2.20 (d, *J* = 17.4 Hz, 1H), 2.10 – 2.02 (m, 1H), 2.00 – 1.94 (m, 1H), 1.72 (qd, *J* = 13.2, 4.1 Hz, 1H), 1.60 (s, 3H), 1.30 (s, 3H), 0.89 (d, *J* = 1.5 Hz, 3H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 214.45, 200.60, 185.38, 162.85, 162.11, 150.75, 128.55, 125.62, 123.11, 53.45, 48.37, 45.92, 44.62, 43.81, 42.04, 31.86, 29.78, 29.09, 21.37, 18.54.
IR (Thin film, cm^{-1}) 3052, 2986, 1741, 1686, 1668.
HRMS (ESI-TOF) m/z: [M+H]+ Calculated for $C_{21}H_{29}O_2$ 311.1647; found 311.1647.
Analysis of relative stereochemistry: HSQC, COSY and HMBC experiments were used to assign all ^{1}H NMR signals for *ent*-lanostane-based trienone **24.** See Section 5: NMR Spectra.

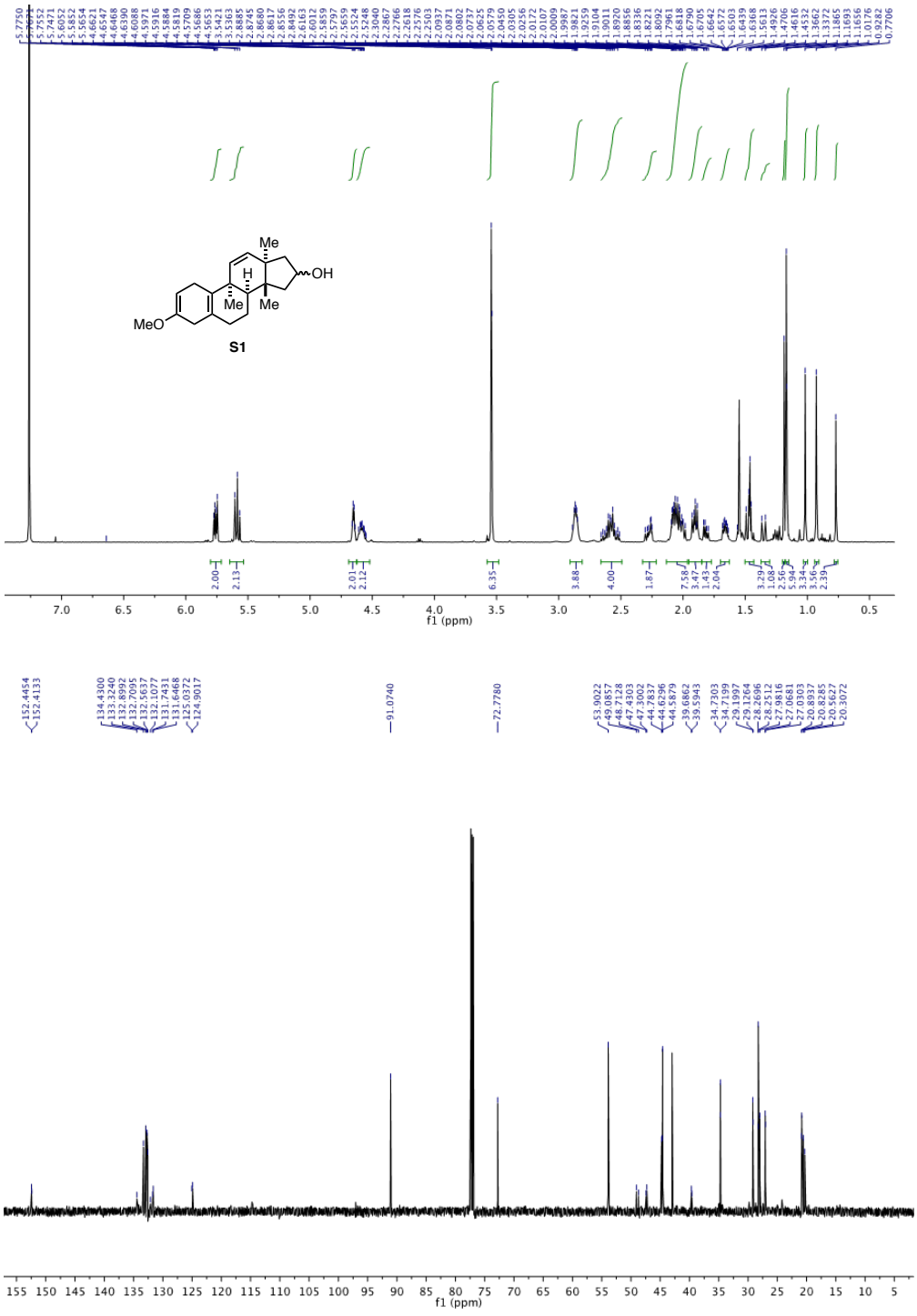
4. References

(1) Preparation of cyclopropyl ketone **12**: Bucknam, A. R.; Micalizio, G. C. Asymmetric De Novo Synthesis of a Cucurbitane Triterpenoid: Total Synthesis of Octanorcucurbitacin B. *J. Am. Chem. Soc.* **2022**, *144*, 8493–8497.

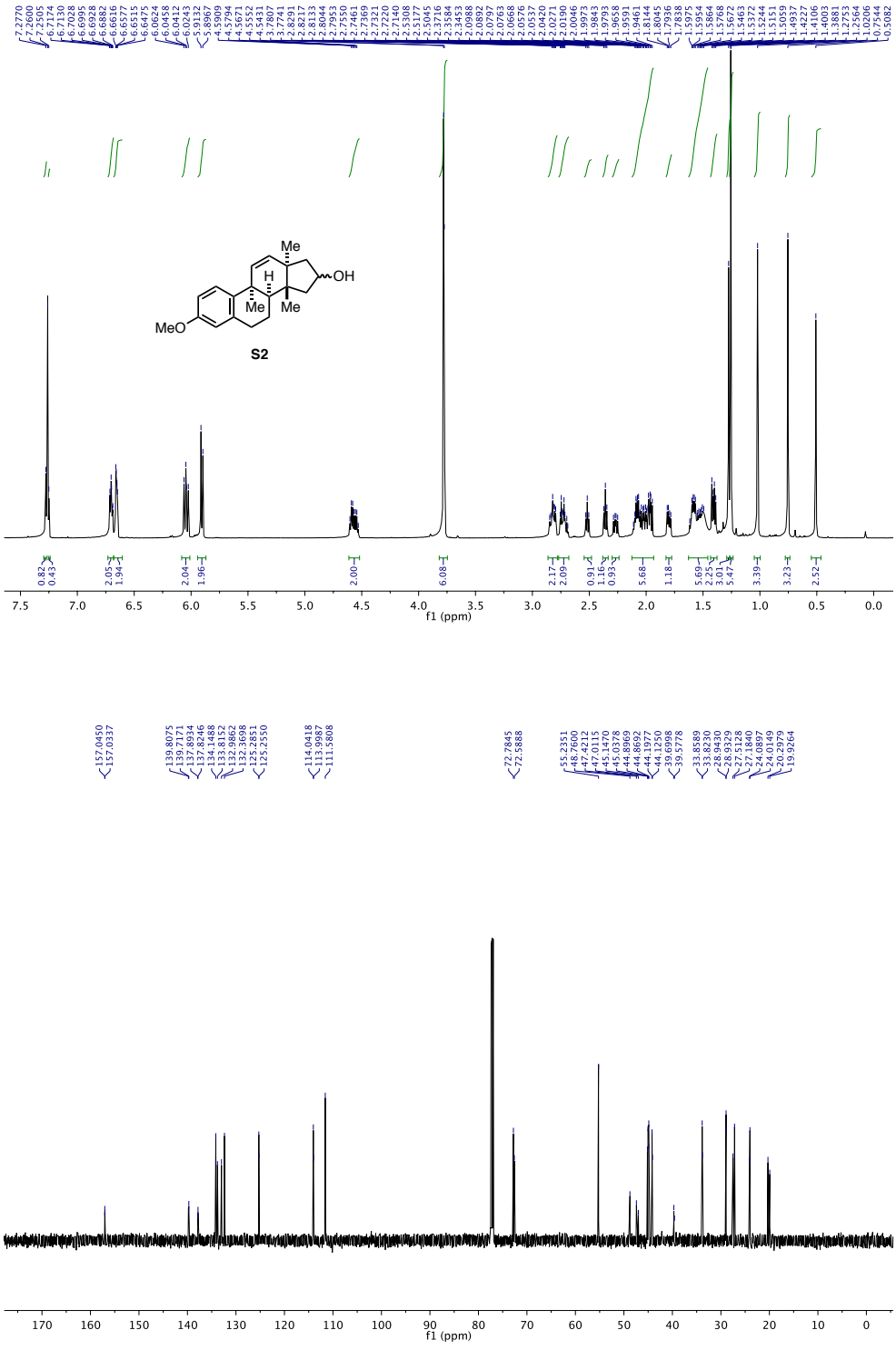
(2) Jones reagent preparation: Cyclooctanone. *Org. Synth.* **1965**, *45*, 28.

5. NMR Spectra

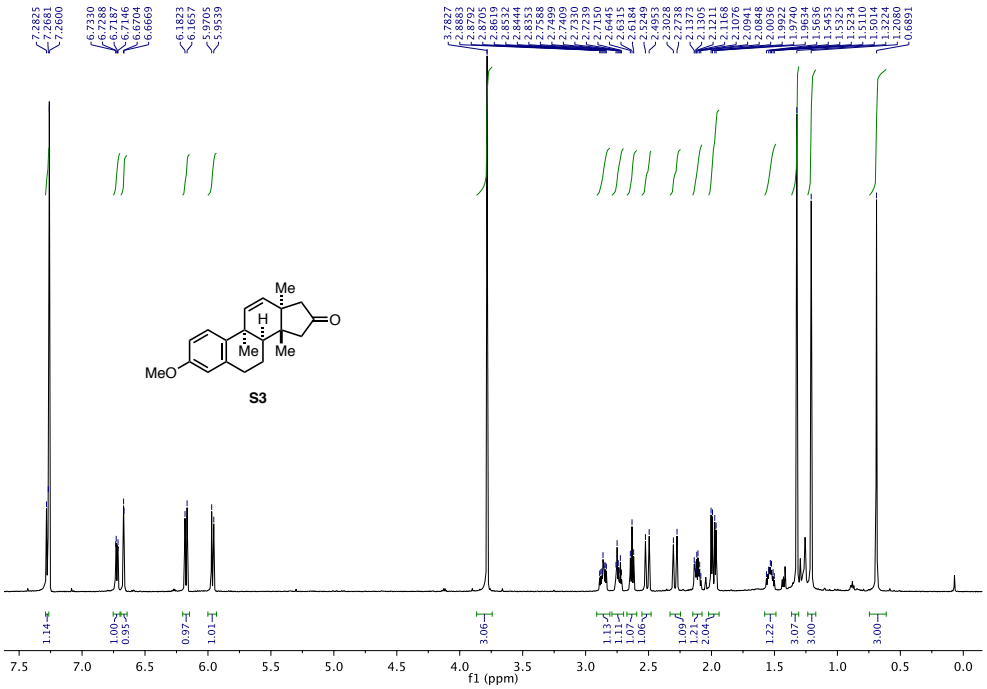
^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) of diastereomeric mixture **S1**

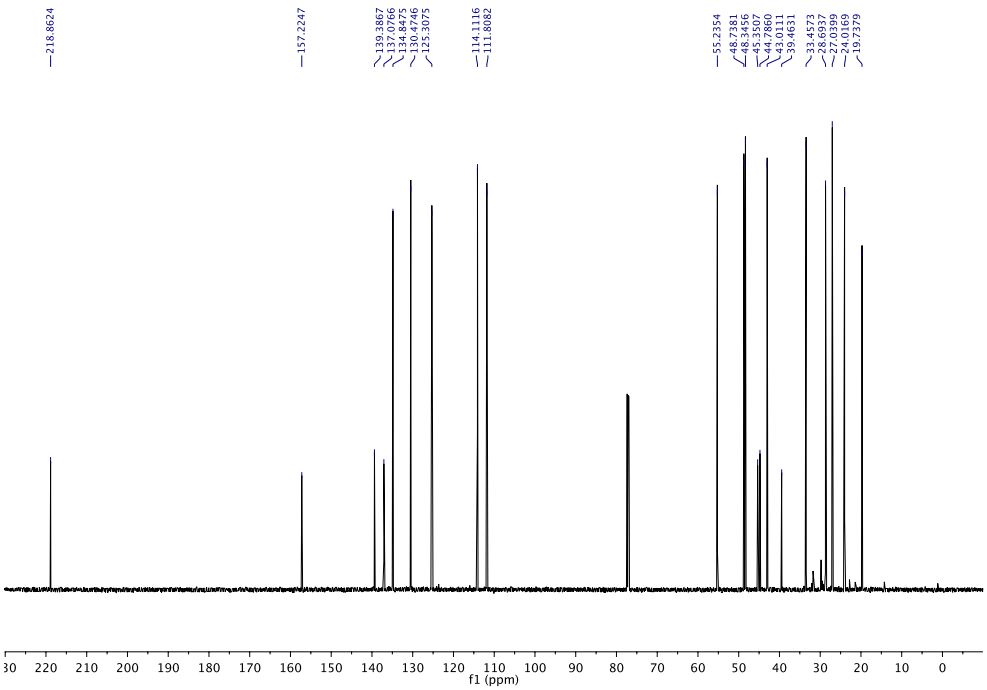


^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) of diastereomeric mixture **S2**

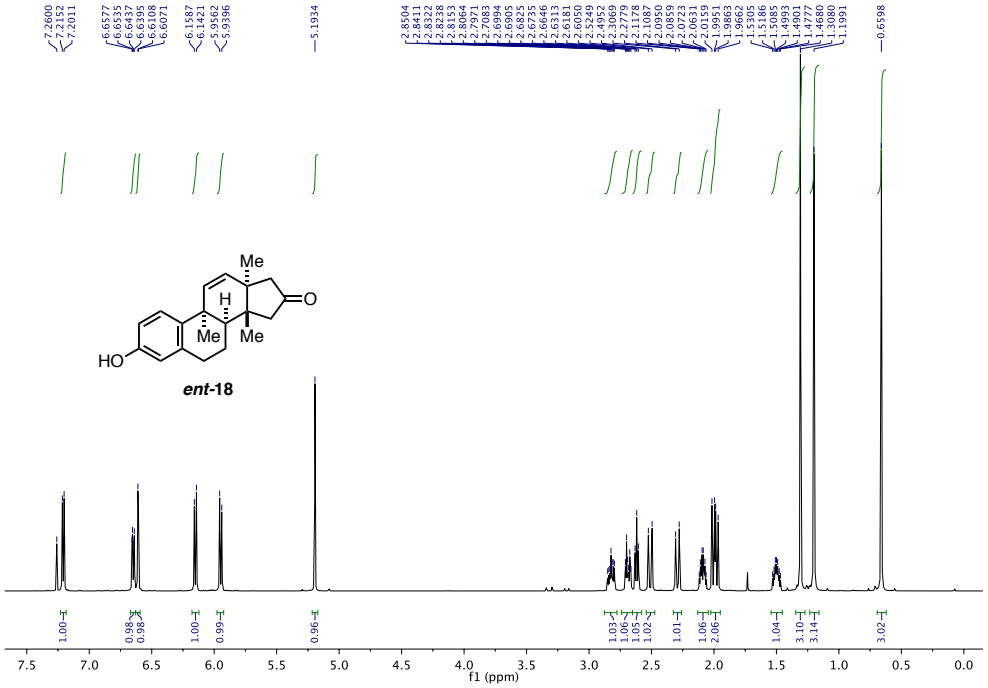


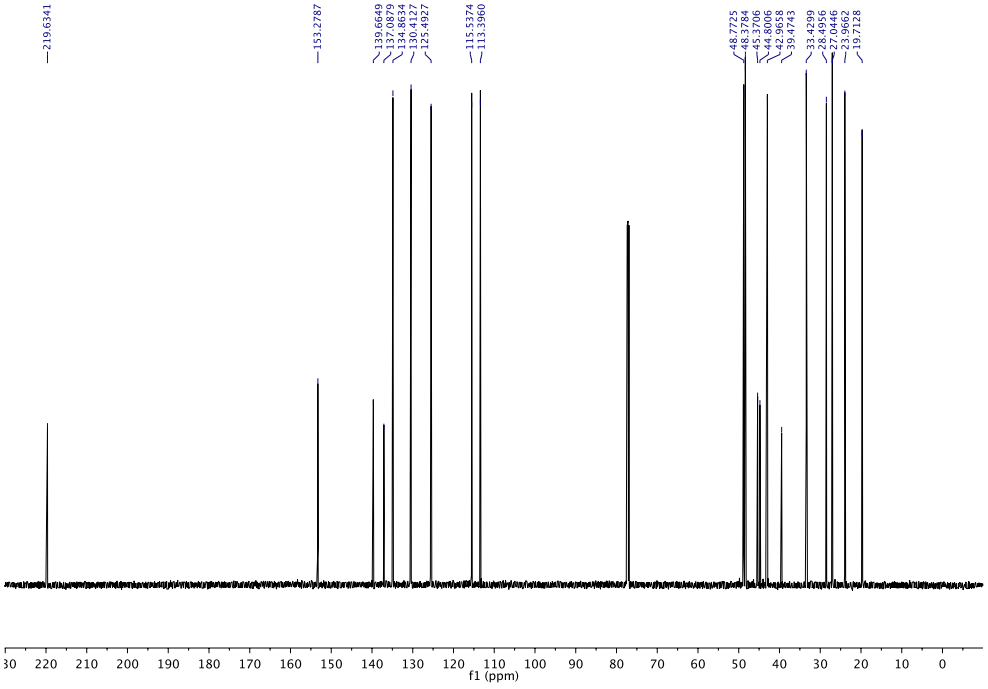
^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) of ketone **S3**



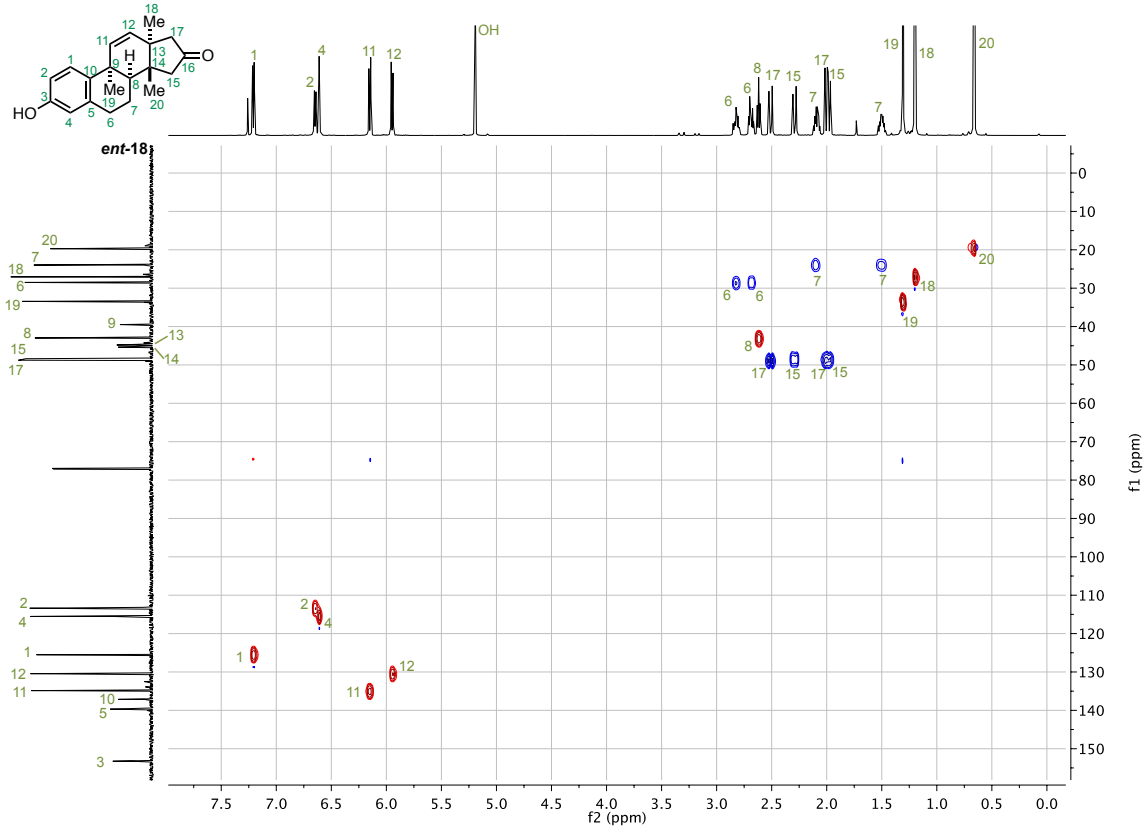


^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) of *ent*-phenol **18**

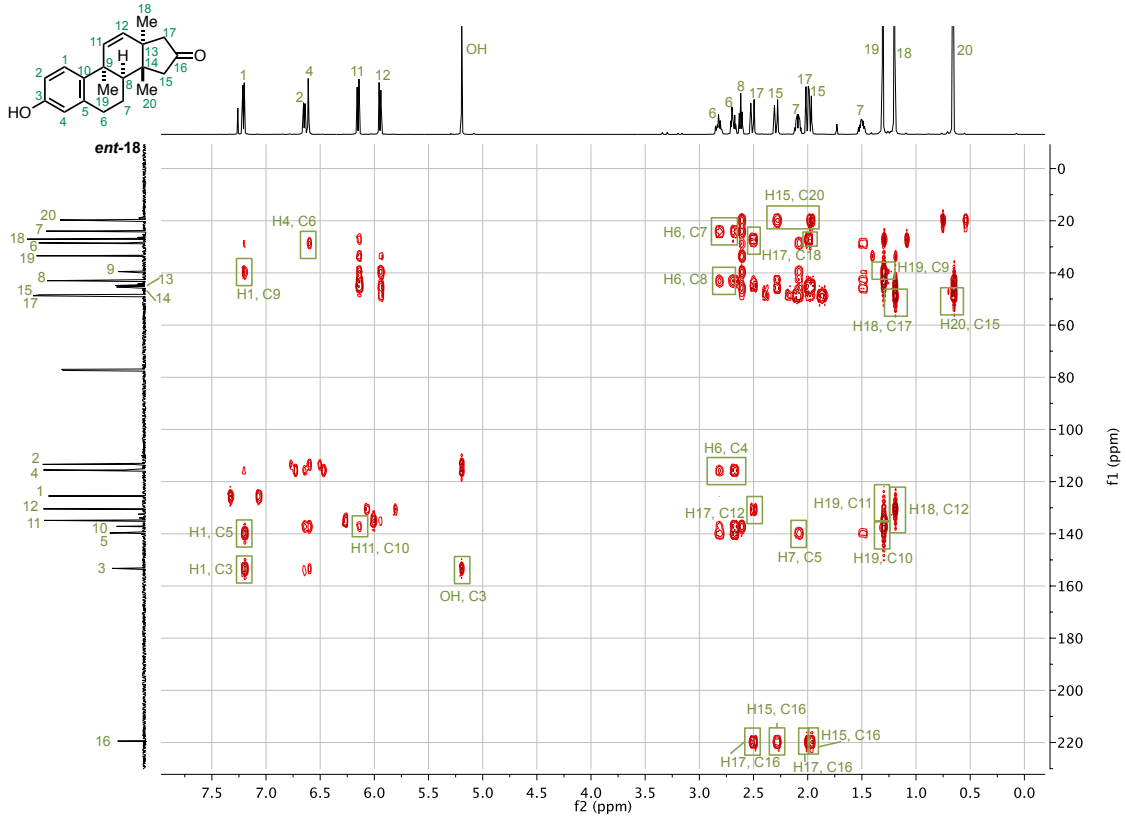




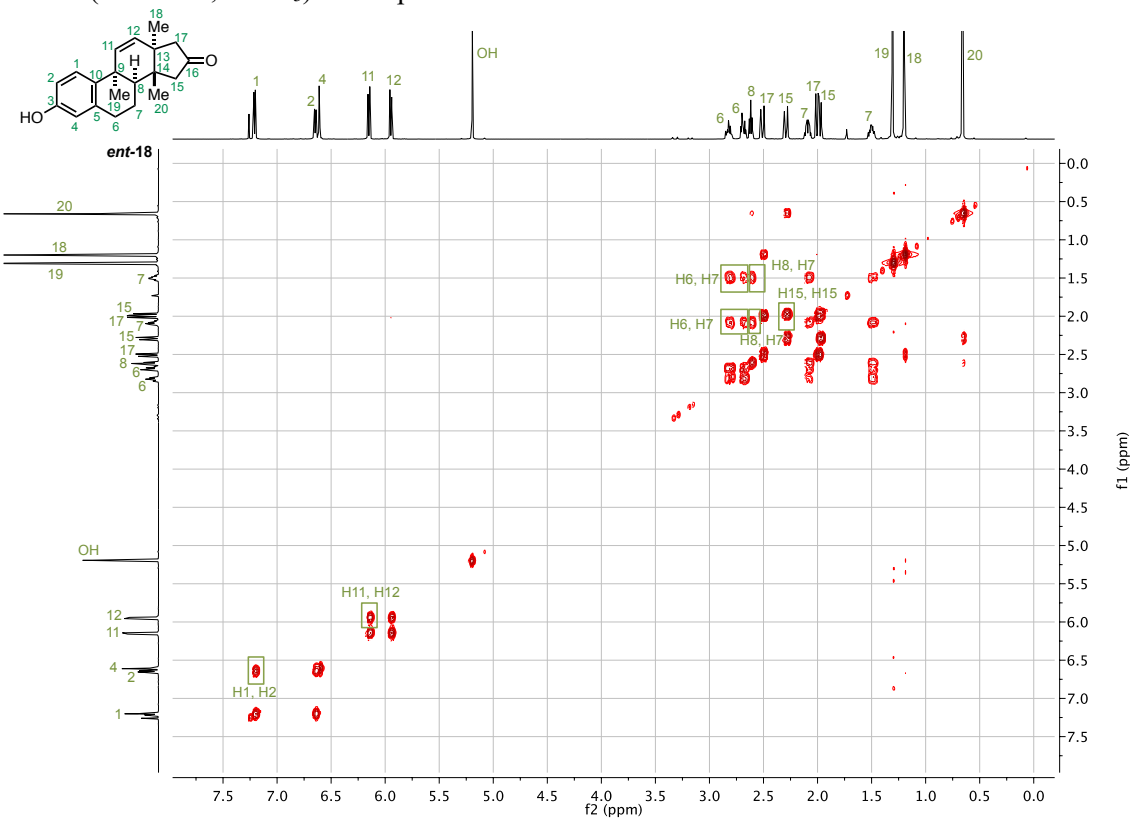
HSQC (600 MHz and 150 MHz, $CDCl_3$) of *ent*-phenol **18**



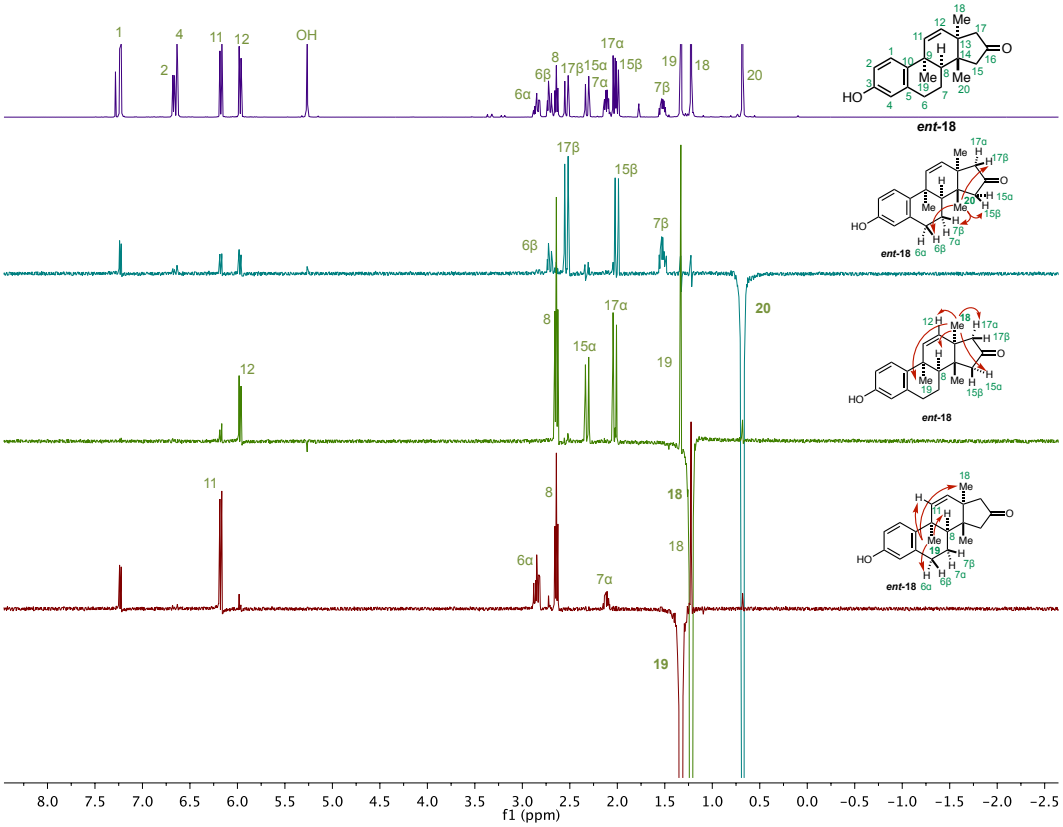
HMBC (600 MHz and 150 MHz, $CDCl_3$) of *ent*-phenol **18**



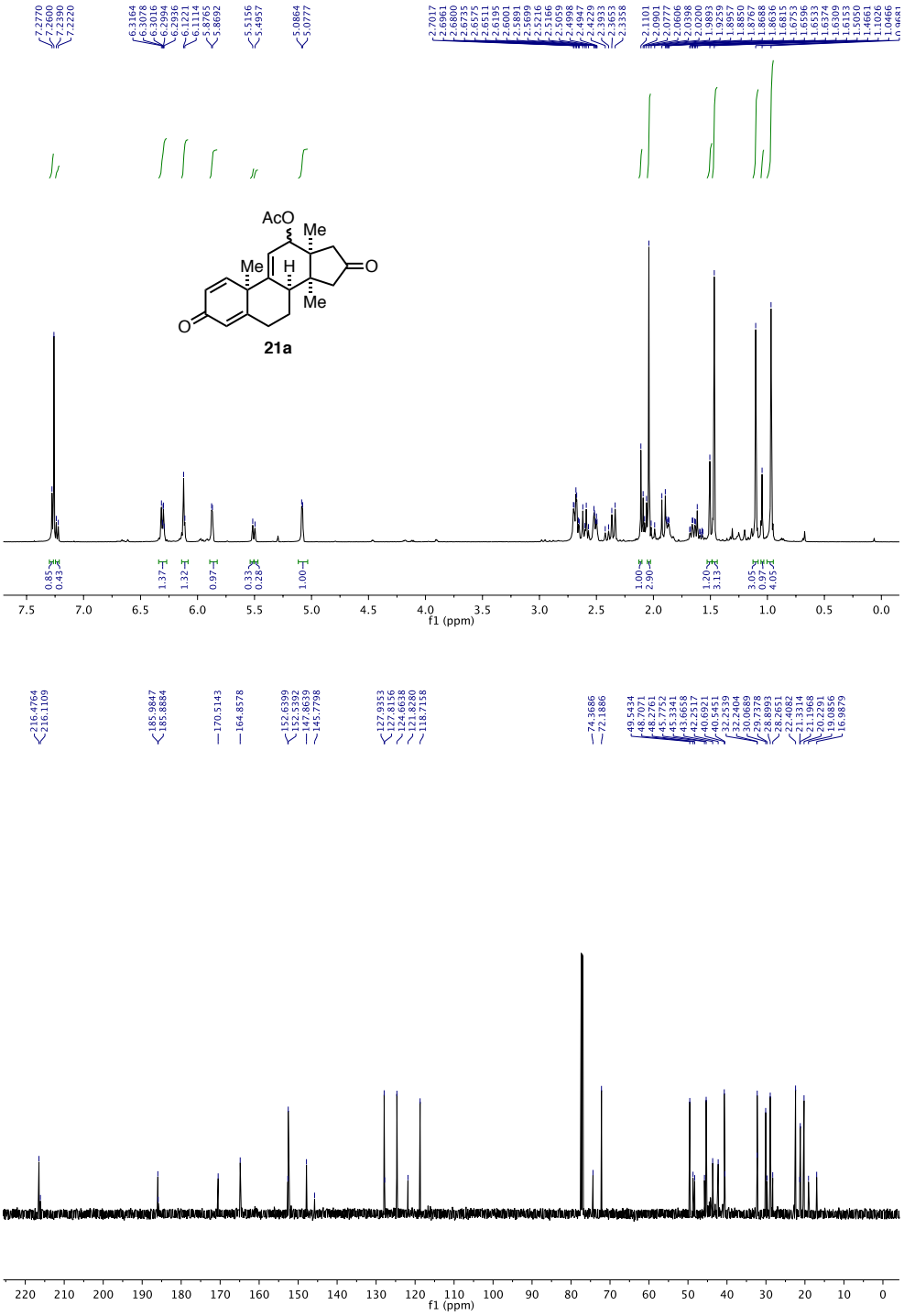
COSY (600 MHz, $CDCl_3$) of *ent*-phenol **18**



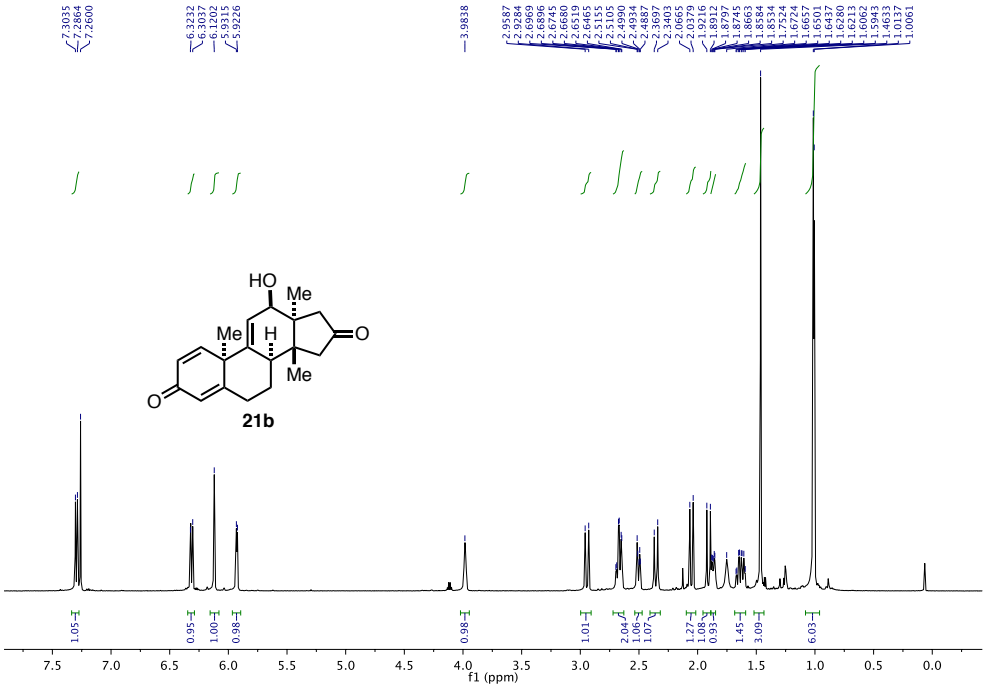
Selected 1D nOe interactions (500 MHz, $CDCl_3$) for *ent*-phenol **18**

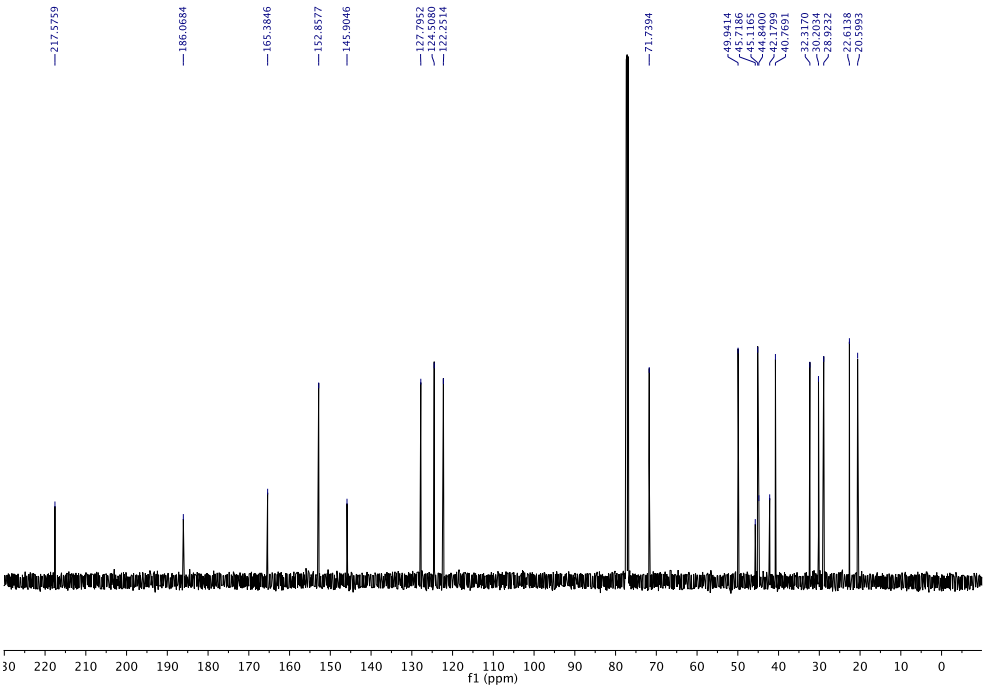


^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) of diastereomeric mixture of *ent*-lanostane-based dienone **21a**

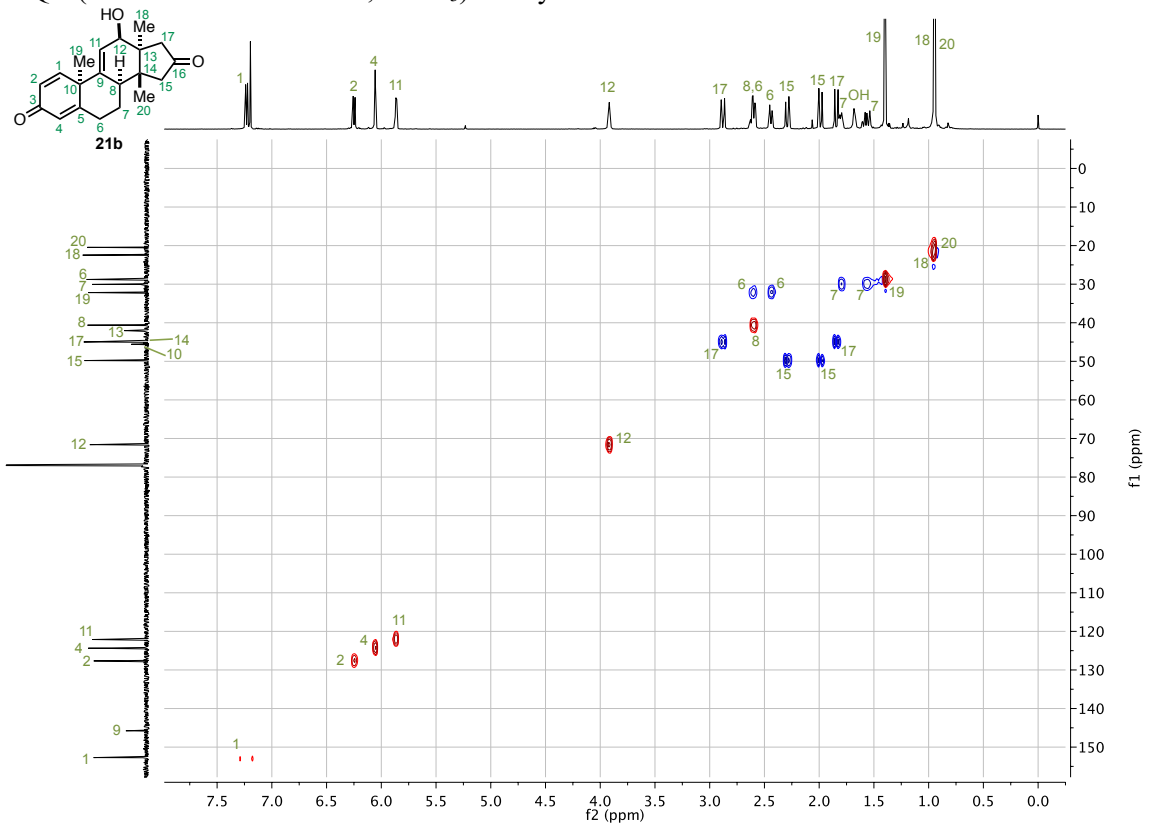


^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) of *ent*-lanostane-based allylic alcohol **21b**

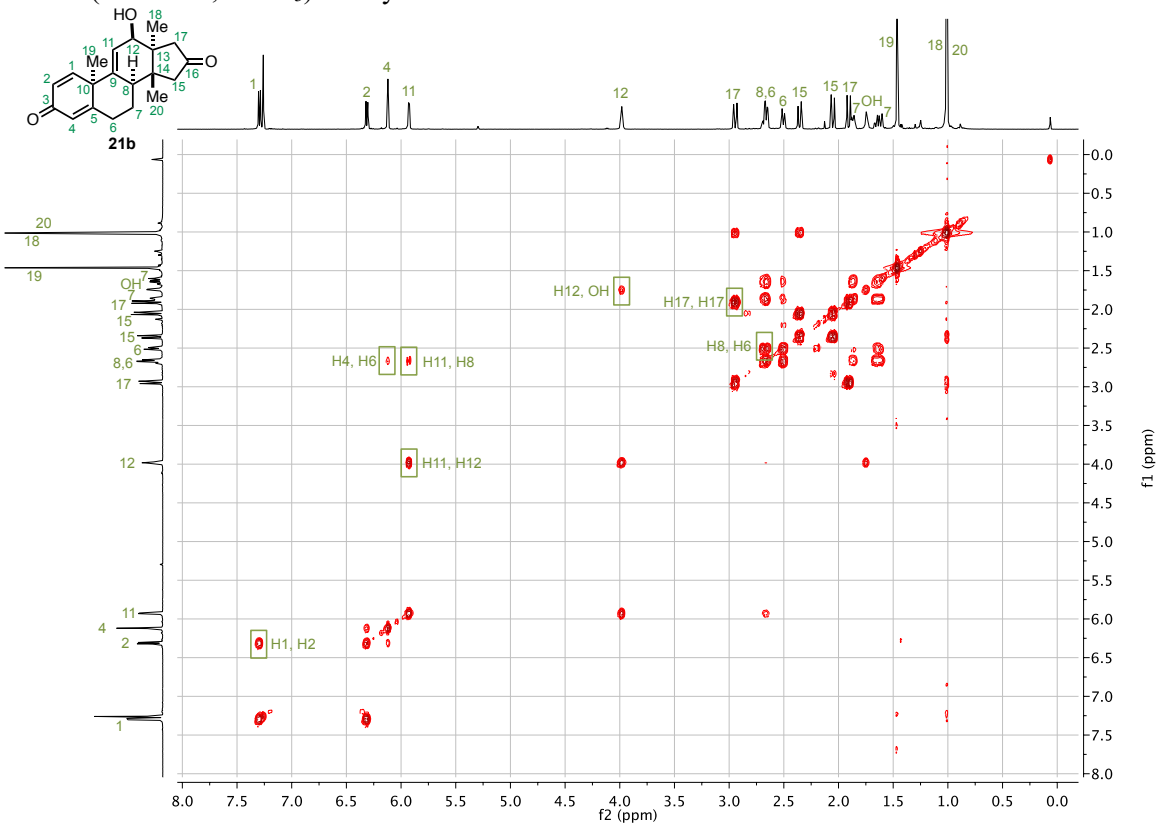




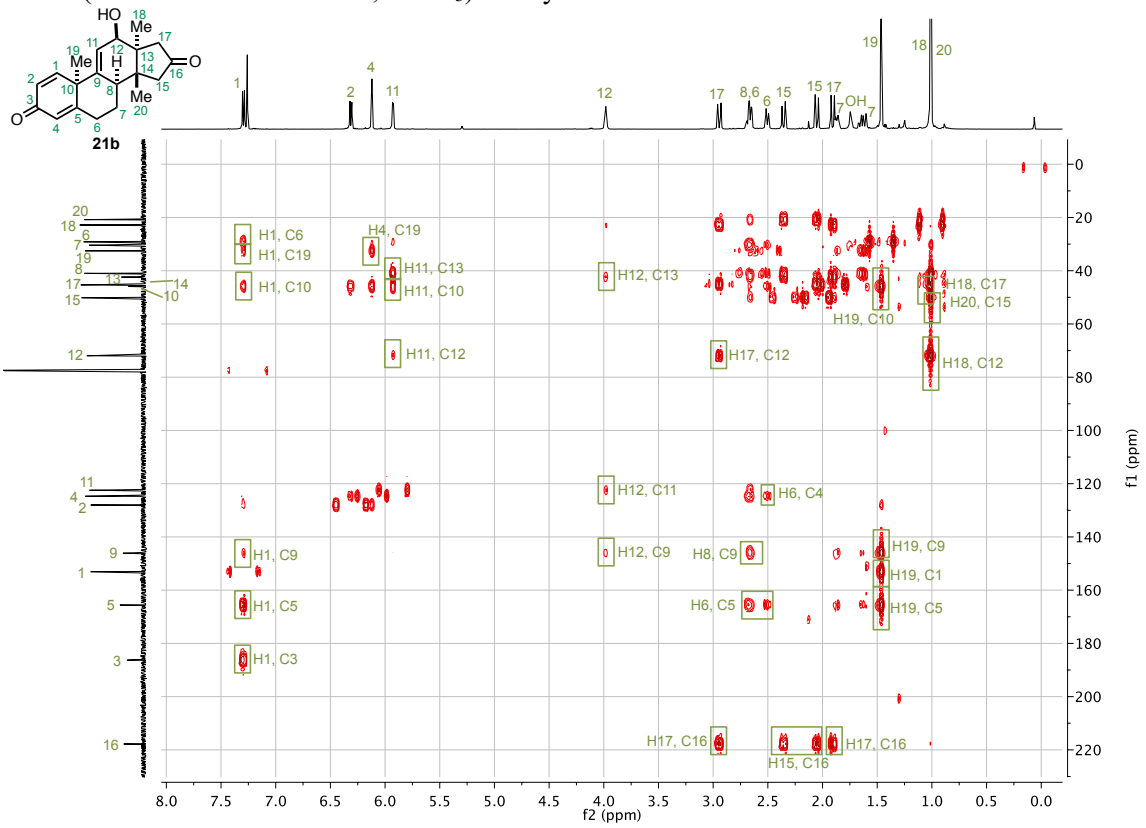
HSQC (600 MHz and 150 MHz, $CDCl_3$) of allylic alcohol **21b**



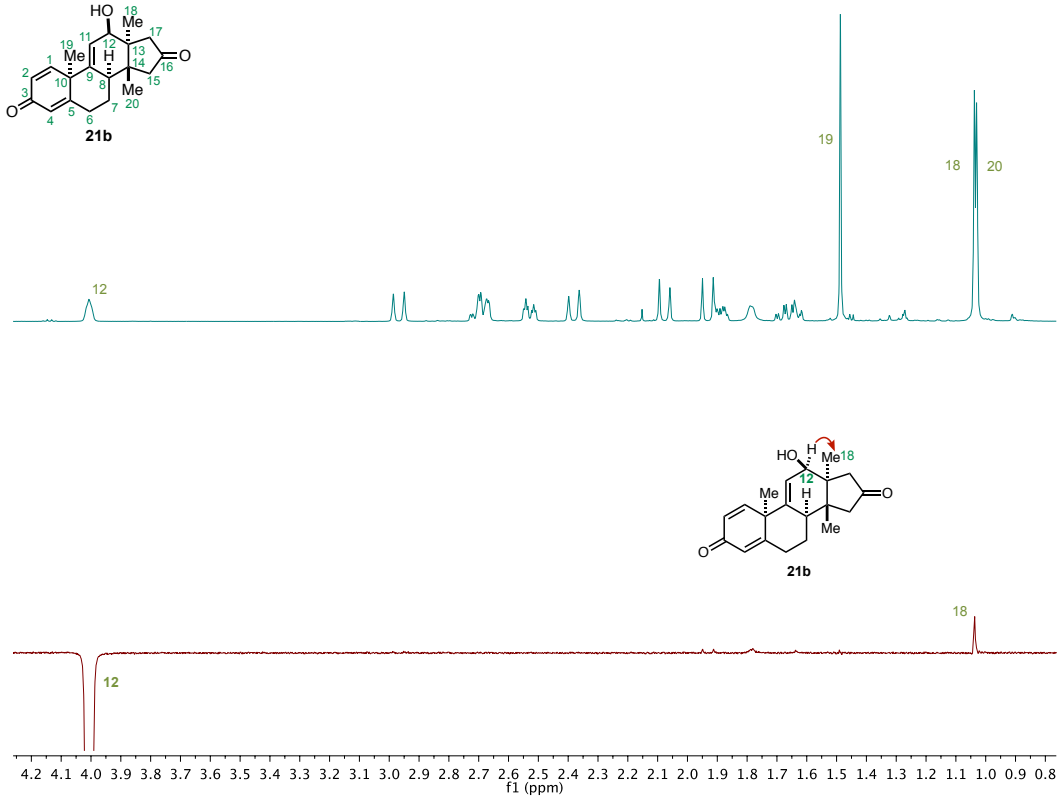
COSY (600 MHz, $CDCl_3$) of allylic alcohol **21b**



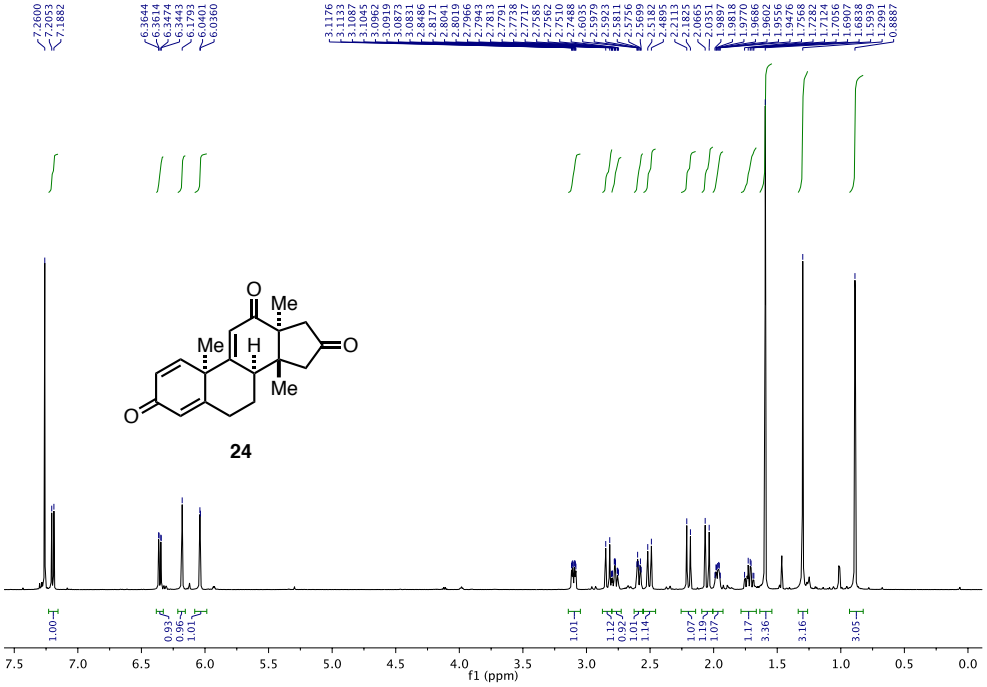
HMBC (600 MHz and 150 MHz, $CDCl_3$) of allylic alcohol **21b**

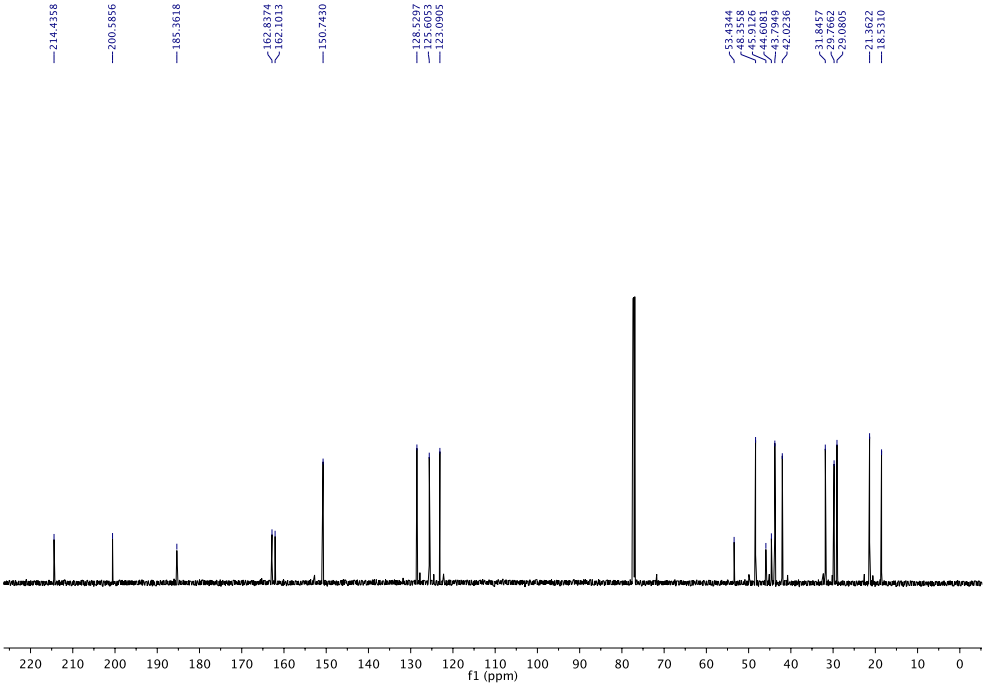


Selected 1D nOe interaction (500 mHz, $CDCl_3$) for allylic alcohol **21b**

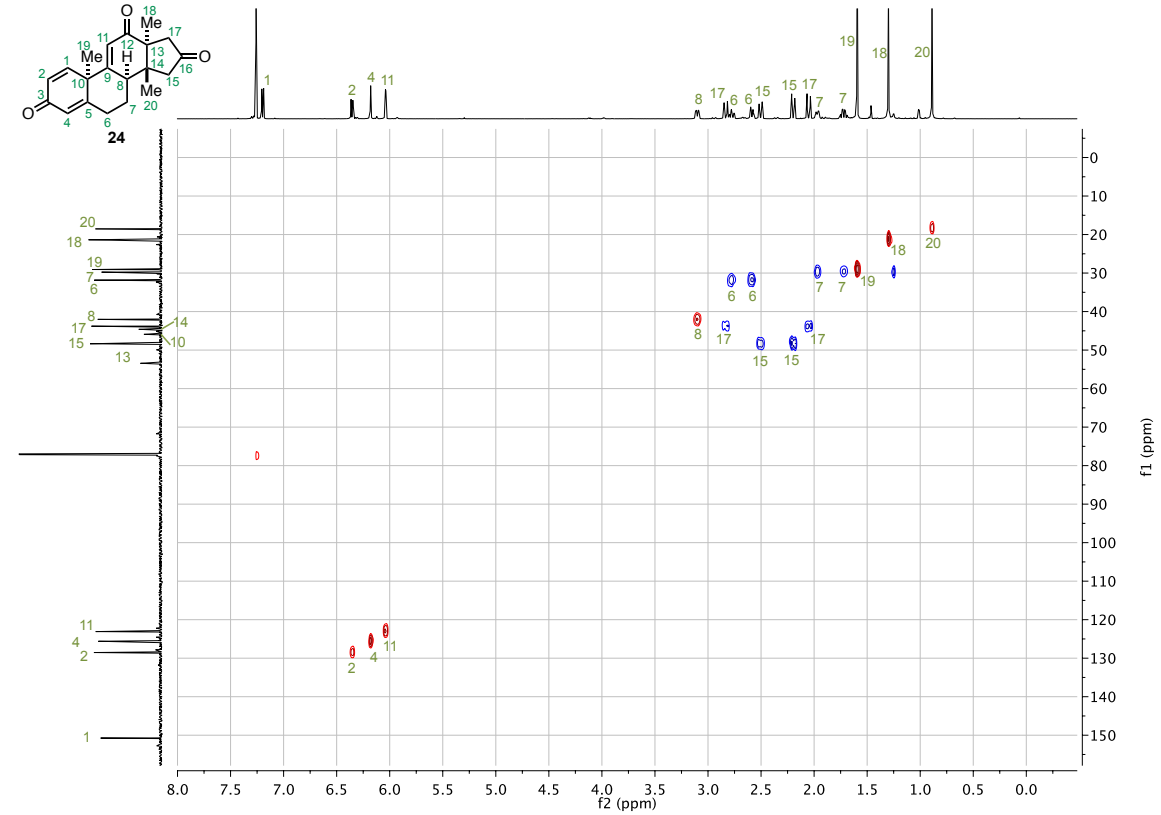


^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) of *ent*-lanostane-based trienone **24**

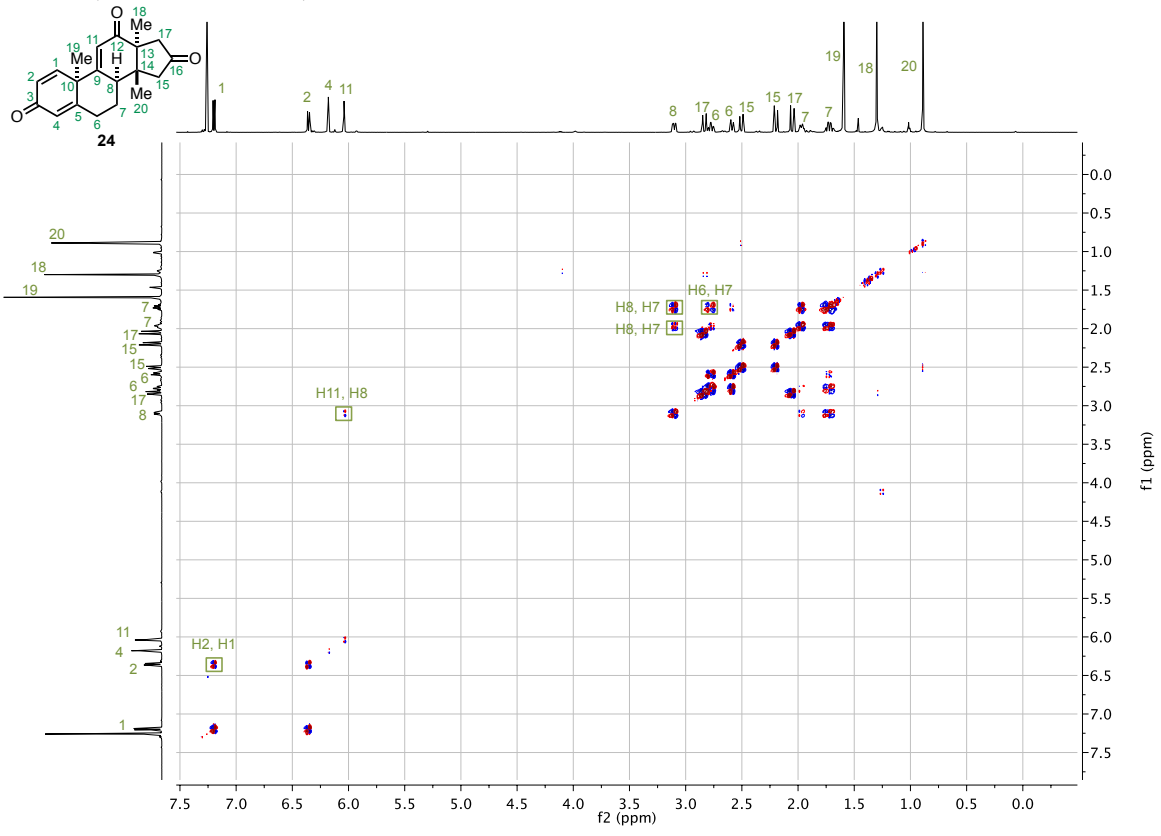




HSQC (600 MHz and 150 MHz, $CDCl_3$) of trienone **24**



COSY (600 MHz, $CDCl_3$) of trienone **24**



HMBC (600 MHz and 150 MHz, $CDCl_3$) of trienone **24**