Supporting Information

Synthesis of Fusidic Acid Derivatives Yields a Potent Antibiotic with an Improved Resistance Profile

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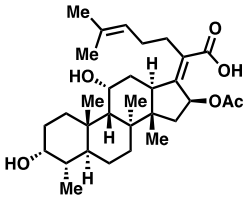
Table S1. Structures of **FA** and compounds **16**, **FA-CP**, **26**, and **27**. MICs of **FA** and **16**, **FA-CP**, **26**, **27** in *S. aureus* 29213 without human serum and fold change increase in MIC of **FA**, **16**, **FA-CP**, **26**, **27** in *S. aureus* 29213 with 50% human serum. MICs in μg/mL, (n=3).



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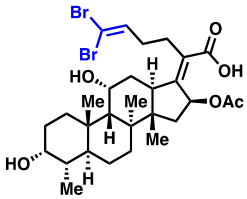

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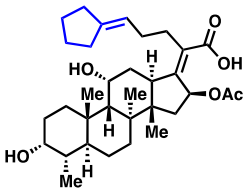

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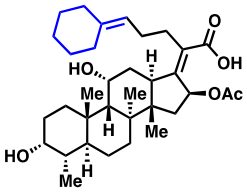

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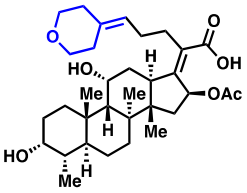

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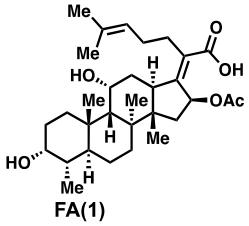
	MIC (μg/mL) Without Human Serum					Fold Change Increase in MIC with 50% Human Serum				
WT Strain	**FA**	**16**	**FA-CP**	**26**	**27**	**FA**	**16**	**FA-CP**	**26**	**27**
***S. aureus* 29123**	**0.125**	**0.125**	**0.125**	**0.125**	**0.125**	**16X**	**32X**	**8X**	**16X**	**32X**

Table S2. Structures of **FA** and compounds **16**, **FA-CP**, **26**, and **27**. Metabolic stability of **FA**, **16**, **FA-CP**, **26**, and **27** after incubation for 3h at 37 °C in mouse liver microsomes. Percent remaining was quantified using LC-MS/MS. Error = standard error of the mean of 3 biological replicates.



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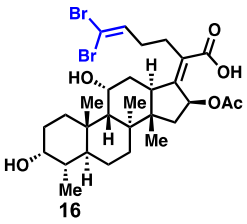

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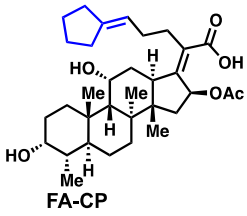

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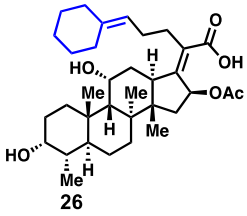

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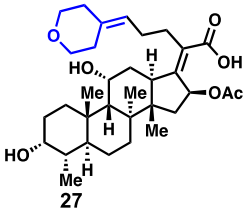

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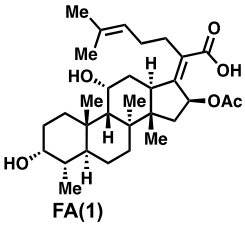
Compound	Metabolic Stability in Mouse Liver Microsomes
FA(1)	82 ± 0.8 %
16	88 ± 2 %
FA-CP	78 ± 4 %
26	61 ± 1 %
27	86 ± 5 %

Table S3. Structures of **FA** and compounds **16**, **FA-CP**, **26**, and **27**. IC_{50} values (μM) in human foreskin fibroblasts (HFF-1) of **FA**, **16**, **FA-CP**, **26**, and **27**. Cells were incubated for 72 h before viability was assessed by the Alamar Blue Assay. Error is SEM, n=2.



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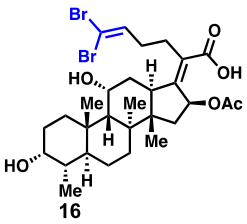

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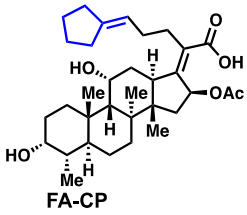
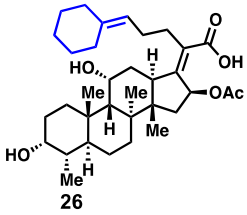


FA-CP



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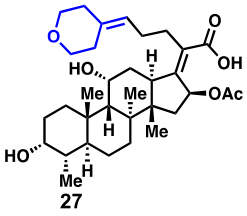

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Compound	Mammalian Toxicity in HFF-1
FA(1)	146 ± 11 μM
16	75 ± 3 μM
FA-CP	112 ± 2 μM
26	53 ± 4 μM
27	409 ± 16 μM

Table S4. List of antibiotics and associated reference numbers with clinically relevant levels of resistance in *S. aureus* and *E. faecium* clinical isolates assessed in Table S5 and Table S6.

Antibiotic Reference Number	Antibiotic
1	Cefoxitin
2	Ampicillin
3	Clindamycin
4	High-level Gentamicin
5	Doxycycline
6	Erythromycin
7	Gentamicin
8	Levofloxacin
9	High-level Streptomycin
10	Mupirocin
11	Oxacillin
12	Penicillin
13	Rifampin
14	Quinupristin/dalfopristin
15	Tetracycline
16	Trimethoprim/sulfamethoxazole
17	Vancomycin
18	Teicoplanin
19	Ciprofloxacin

Table S5. Antimicrobial assessment of **FA**, vancomycin, and **FA-CP** in a panel of multidrug-resistant clinical isolates of *S. aureus*. This data is depicted in Figure 5A. Full list of antibiotic resistance is provided. MICs determined using CLSI guidelines and are listed in μg/mL. All experiments were performed in biological triplicate.

Strain	Drug Resistance	Vancomycin MIC	FA MIC	FA-CP MIC
S. aureus AR0561[a]	1, 8, 10-12, 15	1	0.125	0.125
S. aureus AR0562[a]	1, 3, 6, 8, 11-12, 15	2	0.125	0.125
S. aureus AR0563[a]	1, 6-7, 10-12	1	0.125	0.125
S. aureus AR0564[a]	1, 3, 6-8, 10-12	2	0.125	0.125
S. aureus AR0565[a]	1, 3, 5-8, 11-12, 15-16	2	0.0625	0.0625
S. aureus AR0566[a]	1, 7-8, 11-12	0.5	0.125	0.125
S. aureus AR0567[a]	1, 3, 6-8, 10-12, 17	1	0.125	0.125
S. aureus AR0568[a]	3, 6, 8, 12	0.5	0.125	0.125
S. aureus AR0570[a]	1, 3, 6-8, 10-12, 15	1	0.125	0.125
S. aureus NRS3[b]	7, 11-12, 19	4	0.0625	0.0625
S. aureus NRS4[b]	11-12, 19	4	0.0625	0.0625
S. aureus NRS17[b]	11-12, 19	8	0.0625	0.0625
S. aureus NRS18[b]	11-12, 19	4	0.125	0.125
S. aureus NRS21[b]	7, 11-12, 15, 16, 19	4	0.0625	0.0625
S. aureus NRS22[b]	3, 6-7, 11-12, 16, 19	4	0.0625	0.0625
S. aureus NRS23[b]	11-12, 16, 19	4	0.125	0.125
S. aureus NRS24[b]	7, 11-12, 19	4	0.0625	0.0625
S. aureus NRS26[b]	7, 11-12, 16, 19	2	0.0625	0.0625
S. aureus NRS27[b]	7, 11-12, 16, 19	4	0.125	0.125
S. aureus NRS29[b]	7, 11-12, 16, 19	2	0.125	0.125
S. aureus NRS51[b]	11-12, 19	4	0.0625	0.0625
S. aureus NRS68[b]	3, 6, 11-12, 19	4	0.125	0.125
S. aureus NRS73[b]	7, 11-12, 16, 18 19	4	0.125	0.125
S. aureus NRS74[b]	3, 6-7, 11-12, 19	8	0.125	0.125
S. aureus NRS76[b]	6, 11-12, 19	4	0.125	0.125
S. aureus NRS77[b]	No noted resistance	1	0.0625	0.0625
S. aureus NRS382[b]	3, 6, 11-12, 19	2	0.0625	0.0625
S. aureus NRS383[b]	3, 6-7, 11-12, 19	0.5	0.125	0.125
S. aureus NRS384[b]	6, 11-12	1	0.125	0.125

[a]Obtained from the Centers for Disease Control and Prevention (CDC)
[b]Obtained from the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA)

Table S6. Antimicrobial assessment of **FA**, vancomycin, and **FA-CP** in a panel of multidrug-resistant clinical isolates of *E. faecium*. This data is depicted in Figure 5B. Full list of antibiotic resistance is provided. MICs determined using CLSI guidelines and are listed in μg/mL. All experiments were performed in biological triplicate.

Strain	Drug Resistance	Vancomycin MIC	FA MIC	FA-CP MIC
E. faecium AR0572[a]	2, 5, 8, 12, 13, 17-18	512	4	4
E. faecium AR0574[a]	2, 5, 8-9, 12, 13	0.5	2	2
E. faecium AR0575[a]	2, 8, 12, 13, 17-18	512	2	2
E. faecium AR0576[a]	2, 5, 8, 12-13	1	4	4
E. faecium AR0578[a]	2, 4-5, 8, 12, 13	0.5	2	2
E. faecium AR0579[a]	2, 4-5, 9, 12, 13-14	0.5	1	1

[a]Obtained from the Centers for Disease Control and Prevention (CDC)

7. Experimental Information for Biological Data

Bacterial Strains

S. aureus ATCC 29213, *E. faecium* ATCC 19434, and *S. aureus* ATCC BAA-1721 were obtained from the American Type Culture Collection (ATCC). Resistant strains of *E. faecium* were obtained from the Centers for Disease Control and Prevention (CDC). Resistant strains of *S. aureus* were obtained from the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA) and the Centers for Disease Control and Prevention (CDC).

Antimicrobial Susceptibility Tests

Susceptibility testing was performed in biological triplicate, using the micro-dilution broth method as outlined by the Clinical and Laboratory Standards Institute. Bacteria were cultured with cation-adjusted Muller-Hinton broth (Sigma-Aldrich, Cat# 90922) in round-bottom 96-well plates (Corning, Cat# 3788). Human serum (Ultrafiltrate, unspecified gender, 30K Dalton membrane filtered) was purchased from BioIVT (Hicksville, NY).

Cell Culture

HFF-1 cells were obtained from ATCC. HFF-1 cells were grown in DMEM with 15% fetal bovine serum (Gemini Benchmark, Cat# 100-106), 100 µg/mL penicillin, and 100 µg/mL streptomycin. All cells were cultured at 37 ºC in a 5% CO_2 environment. Media was prepared by the University of Illinois School of Chemical Sciences Cell Media Facility.

Cell Viability

Cells were harvested, seeded in a 96 well-plate and allowed to adhere. Cells were treated with investigational compounds in DMSO (1% final concentration). Cells were incubated for 72 h before viability was assessed by the Alamar Blue Assay. Raptinal (20 μM) was used as a dead control.

Mouse Liver Microsome Stability Assay

A mixture of PBS (pH 7.4), NADPH regenerating system solution A (Corning Life Sciences), and NADPH regenerating system solution B (Corning Life Sciences) was incubated at 37ºC in a shaking incubator for 5 min. Next, compound was added in DMSO (final concentration 50 µM, 0.5% DMSO) before ice-cold mouse liver microsomes (Thermo Fisher, male CD-1 mice, pooled) were added (final protein concentration of 1 mg/mL). An aliquot was immediately removed, quenched with an equal volume of 100 µM internal standard and centrifuged at 13,000 rcf for 3 min. The reactions were incubated at 37ºC in a shaking incubator for 3 h. A second aliquot was removed and quenched. Samples were analyzed with the 5500 QTRAP LC-MS/MS system (Sciex, Framingham, MA) in the Metabolomics Laboratory of Roy J. Carver Biotechnology Center, University of Illinois at Urbana-Champaign. Software Analyst 1.6.2 was used for data acquisition and analysis. The 1200 series HPLC system (Agilent Technologies, Santa Clara, CA) includes a degasser, an autosampler, and a binary pump. The LC separation was performed on an Agilent Sb-Aq column (4.6 x 50mm, 5µm) with mobile phase A (0.1% formic acid in water) and mobile phase B (0.1% formic acid in acetonitrile). The flow rate was 0.3 mL/min. The linear gradient was as follows: 0-3min, 100% A; 10-16min, 5% A; 16.5-22min, 100% A. The autosampler was set at 10°C. The injection volume was 1 µL. Mass spectra were acquired under both positive (ion spray voltage was +5500 V) and negative (ion spray voltage was -4500 V) electrospray ionization (ESI). The source temperature was 450 °C. The curtain gas, ion source gas 1, and ion source gas 2 were 33, 65, and 60 psi, respectively. Multiple reaction monitoring (MRM) was used for quantitation.

Selection of Resistant Mutants

Resistant mutants were selected via the large inoculum method. Briefly, *S. aureus* ATCC 29213 ($1x10^9$ CFU) were plated on 100 mm plates of LB agar containing 4, 2, 1, 0.5, and 0.25 µg/mL. Colonies were

visible after incubation at 37 ºC for 24 h. Resistant colonies were confirmed by streaking on selective media with the same concentration of fusidic acid and compounds **16**, FA-CP **(25)**, **26**, **27**.

Sequencing of *fusA*

FusA was amplified by colony PCR. Colonies were picked and diluted in 100 µL sterile H_2O. PCR reactions are setup by combining MiFi Mix (Bioline, London, UK), 20 µM primer mix [fusA-F2, fusA_seq1, Forward EF-G2, and Reverse EF-G] (*S. aureus* ATCC 29213), template DNA, and H_2O. Reactions were performed on C1000 Thermal Cycler (Bio-Rad, Hercules, CA) with the following conditions: 5 minutes denaturation at 95 ºC, followed by 30 cycles of 20 seconds at 95 ºC, 20 seconds at 50 ºC, and either 1 minute (*fusA1*) or 1.5 minutes (*fusA2*) at 72 ºC. A 10 µL portion of the PCR reaction mixture was analyzed by agarose gel to confirm the product. PCR reactions were purified using GeneJET PCR Purification Kit (Thermo Scientific). PCR amplicons were submitted to the Core DNA Sequencing Facility at the University of Illinois at Urbana-Champaign for Sanger sequencing with the following primers to sequence the *fusA* [fusA2, fusA_seq1, Forward EF-G2, Forward EF-G3, Forward EF-G4].

Primers for sequencing of *fusA* gene in *S. aureus*:

Portion of *fusA* Gene	Elongation Factor G Domain (D)	Oligonucleotide	Sequence	Reference
fusA1	D1	*fusA*-F2	5' –CTC GTA ATA TCG GTA TCA TG– 3'	This study
fusA1	D1	*fusA*_seq1	5' –TAA GGG TCA GTC ATA ACT TT– 3'	Reference 1
fusA1	D1	Forward EF-G2	5' –TGA TCG TTT ACA AGC TAA CGC– 3'	Reference 1
fusA2	D1 and D5	Forward EF-G2	5' –TGA TCG TTT ACA AGC TAA CGC– 3'	Reference 1
fusA2	D1 and D5	Reverse EF-G	5' –AGA AAT TAT TTA TAG CGA TGC– 3'	Reference 1
fusA2	D1 and D5	Forward EF-G3	5' –ATT CTT CCG TGT GTA CTC AGG– 3'	This study
fusA2	D1 and D5	Forward EF-G4	5' –TGG TCA ATA CGG TGA TGT TCA– 3'	Reference 1

Mouse MTD of Sodium Fusidate and FA-CP (25)

The protocol was approved by the IACUC at the University of Illinois at Urbana-Champaign (Protocol Number: 16144 and 19181). In these studies, 10- to 12- week-old female C57BL/6 mice purchased from Charles River were used. The maximum tolerated dose (MTD) of single compound was determined first. Sodium fusidate and FA-CP (25) were formulated in 5 % DMSO, 10 % Tween 20, 85 % PBS. Sodium fusidate and FA-CP (25) were given by IP injection. All the mice were monitored for signs of toxicity for 2 weeks. For multiple dose, the compound was given by daily IP for 4 consecutive days, and mice were monitored for signs of toxicity for 1 month. MTD was the highest dosage with acceptable toxicity (e.g. <20 % weight loss). Sodium fusidate and FA-CP (25) were well tolerated as a single dose of 50 mg/kg. Further analysis showed that sodium fusidate and FA-CP (25) were well tolerated with daily dosing of 50 mg/kg for 4 consecutive days. The MTD of FA-CP (25) was used to inform the dosing schedule used in subsequent efficacy studies.

Pharmacokinetic Assessment of Sodium Fusidate and FA-CP (25)

The protocol was approved by the IACUC at the University of Illinois at Urbana-Champaign (Protocol Number: 16144, 19181). In these studies, 10- to 12- week-old female C57BL/6 mice purchased from Charles River were used. The compounds were formulated in 5 % DMSO, 10 % Tween 20, and 85 % PBS. Mice were treated with sodium fusidate or FA-CP (25) (50 mg/kg) via intraperitoneal injection with three mice per time point (15, 30, 45, 60, 120, and 240 min). At specific time points, mice were sacrificed, blood was collected and centrifuged, and the serum was frozen at -80 ºC until analysis. The proteins in a 10 µL aliquot of serum were precipitated by the addition of 50 µL acetonitrile with the addition of 10 µL of 1.6 ug/mL internal standard (sodium fusidate was the internal standard when measuring FA-CP (25), and FA-CP (25) was the internal standard when measuring sodium fusidate). The sample was then vortexed and centrifuged to remove the proteins. Supernatants were analyzed with the QTRAP 5500 LC/MS/MS system (Sciex) in the Metabolomics Laboratory of the Roy J. Carver Biotechnology Center, University of Illinois at Urbana-Champaign. Software Analyst 1.6.2 was used for data acquisition and analysis. The 1200 Series HPLC System (Agilent Technologies) includes a degasser, an autosampler and a binary pump. The liquid chromatography separation was performed on an Agilent Zorbax SB-Aq column (4.6 mm x 50 mm; 5 µm) with mobile phase A (0.1 % formic acid in water) and mobile phase B (0.1 % formic acid in acetonitrile). The flow rate was 0.3 mL/min. The linear gradient was as follows: 0-1 min: 95 % A; 8-13 min: 0% A; 8.1-18.5 min: 95% A. The autosampler was set at 10 ºC. The injection volume was 5 µL. Mass spectra were acquired under negative electrospray ionization with a voltage of -4,500 V. The source temperature was 450 ºC. The curtain gas, ion source gas 1, and ion source gas 2 were 32, 60 and 60 psi, respectively. Multiple reaction monitoring was used for quantitation: fusidic acid: m/z 515.3 --> m/z 393.3, FA-CP (25): m/z 541.4 --> m/z 437.2. The limit of quantitation of (S/N =10) was 1 nM. Pharmacokinetic parameters were calculated with a one-compartment model using a nonlinear regression program (Phoenix WinNonlin Version 8.1; Certara USA).

Neutropenic Thigh Infection Burden Study with Sodium Fusidate and FA-CP (25)

Mouse studies were carried out in strict accordance with the recommendations in the guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Animal protocol was approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Illinois at Urbana-Champaign (protocol #17271). Briefly, seven-week-old male CD1 mice (cohorts of 8) were rendered neutropenic by intraperitoneal injection of cyclophosphamide (150 mg/kg on Day-4 to Day-2 and 100 mg/kg Day-1). On Day-1, mice were anesthetized with a combination of xylazine/ketamine, and furs on the right hind thigh were removed by clipping with a pair of scissors followed by application of depilating gel (Veet Aloe Vera Legs & Body Hair Remover Gel Cream). After 24 hours, mice were anesthetized with isoflurane, and infected with *S. aureus* ATCC 29213 or the *S. aureus* FA-resistant strain 32X-B at concentration of ~ 1 x 10^6 CFUs (in 50 µl) by injection into the thigh muscle (bicep femoris) with a 25G 5/6” needle. Infected mice were intraperitoneally treated with vehicle (85% PBS, 10% Tween, 5% DMSO), 50 mg/kg of sodium fusidate, or FA-CP (25) at 1, 2, 3 hours post-infection (hpi) individually in 100 µl volume. Infected animals were monitored for myositis and lameness until euthanasia. At indicated times (24-hpi for the 50 mg/kg cohorts for the *S. aureus* 29213 infection model, and 8-hpi for the 50 mg/kg cohorts for the *S. aureus* (FA-32X-B) infection model), mice were euthanized with CO_2 asphyxiation from a compressed gas source followed by cervical dislocation. Infected thigh muscle tissues were harvested and homogenized with a Omni Soft Tissue Tip™ Homogenizer (OMNI International) in 2 ml of sterile PBS. Bacterial burden in the tissue homogenates were determined by serial dilution plating onto tryptic soy agar.

8. Materials and Methods for Synthesis of Derivatives

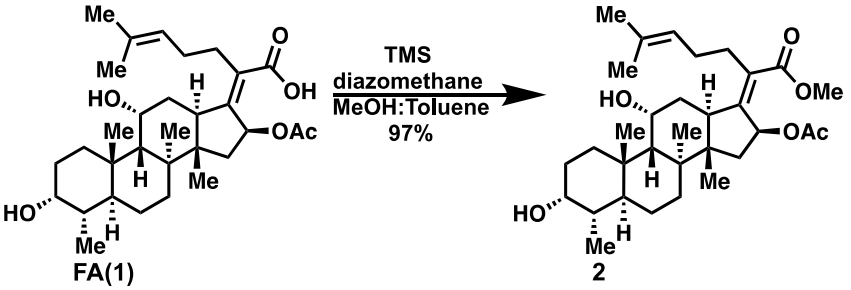
Fusidic acid was purchased from J&K Scientific, Ltd. and Hebei Shengmei Medical Technology Co. Ltd. Sodium fusidate was purchased from Alfa Aesar and AvaChem Scientific. Other chemical reagents were purchased from commercial sources and used without further purification. Anhydrous solvents used during these studies were dried after being passed through columns with activated alumina under nitrogen using a PureSolv MD-5 (Inert previously Innovative Technology, Inc.) solvent purification system. Flash chromatography was performed using silica gel (230–400 mesh).

Various NMR experiments were conducted in the NMR facilities at UIUC. ^{1}H NMR and ^{13}C NMR experiments were recorded on Varian Unity spectrometers at 500 MHz and 125 Hz, respectively and/or a Bruker Avance III HD 500 MHz NMR system equipped with a CryoProbe. Spectra were obtained in the following solvents (reference peaks also included for ^{1}H and ^{13}C NMRs): $CDCl_3$ (^{1}H NMR: 7.26 ppm; ^{13}C NMR: 77.20 ppm), and CD_3OD (^{1}H NMR: 3.31 ppm; ^{13}C NMR: 49.10 ppm). NMR experiments were performed at room temperature unless otherwise indicated. Chemical shift values are reported in parts per million (ppm) for all ^{1}H NMR and ^{13}C NMR spectra. ^{1}H NMR multiplicities are reported as: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, pentet = pent., sextet, = sex, heptet = hept. High–resolution mass spectra were obtained using Waters Q-TOF Ultima ESI and Agilent 6230 ESI TOF LC/MS spectrometers.

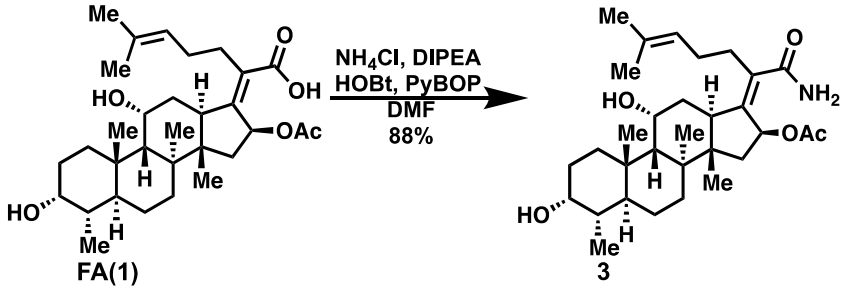
Final compounds for biological assays were purified using a Teledyne ISCO ACCQPrep HP125 Preparative HPLC (Column: Teledyne ISCO RediSep Prep C18 - 20 mm x 250 mm, 100 Å, 5-micron).

Purity of final compounds assessed for biological activity were purified to ≥ 95% as assessed by an Agilent 6230 LC/MS TOF (Column: Agilent ZORBAX Eclipse Plus C18 Rapid Resolution HT 2.1 x 100mm, 1.8-micron) and/or and Agilent Technologies 1290 Infinity II UHPLC equipped with a Phenomenex Kinetex column (2.1 mm ID x 50mm, 1.7-micron particle size, 100 Å pore size).

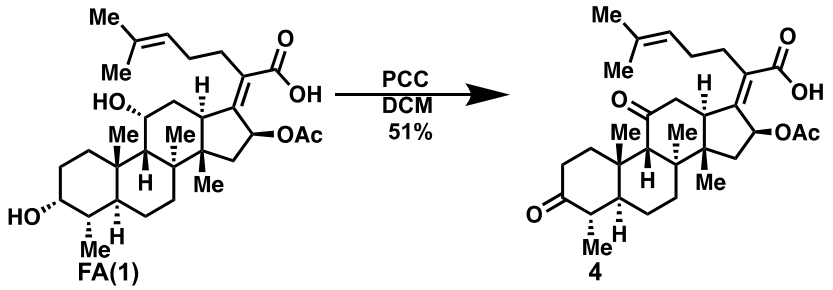
9. Preparation and Characterization of Core Fusidic Acid Analogues



Experimental procedure and spectra for compound **2** have been previously reported.[1]



Experimental procedure and spectra for compound **3** have been previously reported.[2]



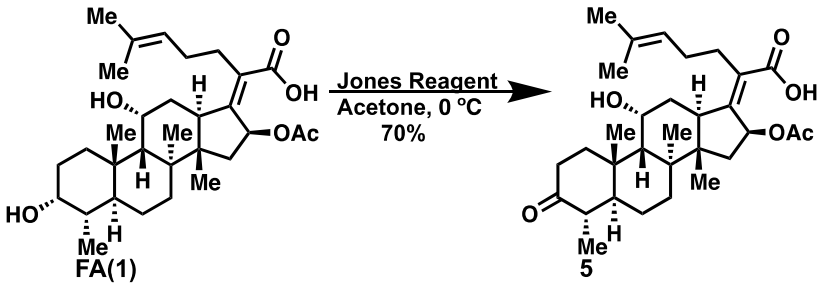
(*Z*)-2-((4*S*,5*S*,8*S*,9*S*,10*S*,13*R*,14*S*,16*S*)-16-hydroxy-4,8,10,14-tetramethyl-3,11-dioxohexadecahydro-17*H*-cyclopenta[*a*]phenanthren-17-ylidene)-6-methylhept-5-enoic acid (4)

Procedure: In a 500 mL RB flask, **FA** (3.00 g, 5.80 mmol, 1.0 eq.) was dissolved in DCM (200 mL). PCC (3.12 g, 14.5 mmol, 2.5 eq.) was added to the reaction. The reaction was then stirred at room temperature for 2 hrs. The solution was then concentrated, and the reaction was filtered through celite using EtOAc as the solvent. The eluent was then collected and concentrated. The resulting crude product was then purified by silica gel column chromatography (100% EtOAc) followed by preparatory HPLC (C18 20x250mm, 10–100% ACN in H_2O) to afford **4** (1.53 g, 51%) as a white solid.

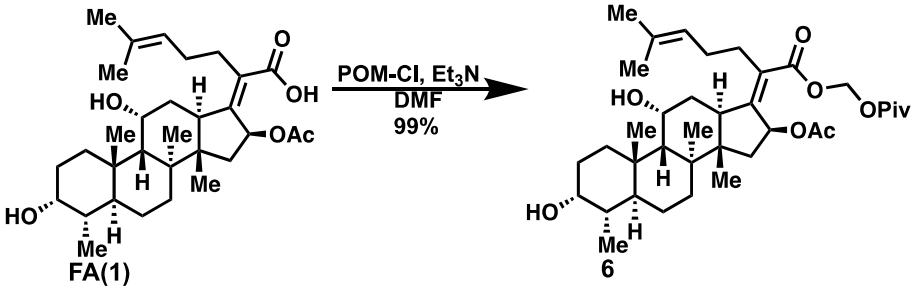
^{1}H NMR (500 MHz, Chloroform-*d*) δ 5.92 (d, *J* = 8.3 Hz, 1H), 5.10 – 5.05 (m, 1H), 2.96 – 2.86 (m, 2H), 2.77 – 2.65 (m, 2H), 2.60 (s, 1H), 2.54 – 2.47 (m, 1H), 2.44 – 2.31 (m, 3H), 2.26 – 2.08 (m, 3H), 2.08 – 1.99 (m, 3H), 1.98 (s, 3H), 1.84 – 1.77 (m, 1H), 1.66 (s, 3H), 1.58 (s, 3H), 1.58 – 1.55 (m, 1H), 1.45 (d, *J* = 14.3 Hz, 1H), 1.25 – 1.20 (m, 1H), 1.19 (s, 3H), 1.18 – 1.16 (m, 1H), 1.15 (s, 3H), 1.07 – 1.03 (m, 6H). (43 non-exchangeable protons)

^{13}C NMR (125 MHz, Chloroform-*d*) δ 216.02, 209.69, 174.27, 170.44, 148.46, 133.38, 130.96, 122.60, 74.32, 58.42, 48.83, 47.21, 46.36, 44.95, 44.53, 40.90, 38.22, 36.85, 36.74, 33.45, 32.55, 28.93, 28.16, 25.86, 24.11, 22.65, 21.35, 20.69, 17.91, 17.19, 14.25.

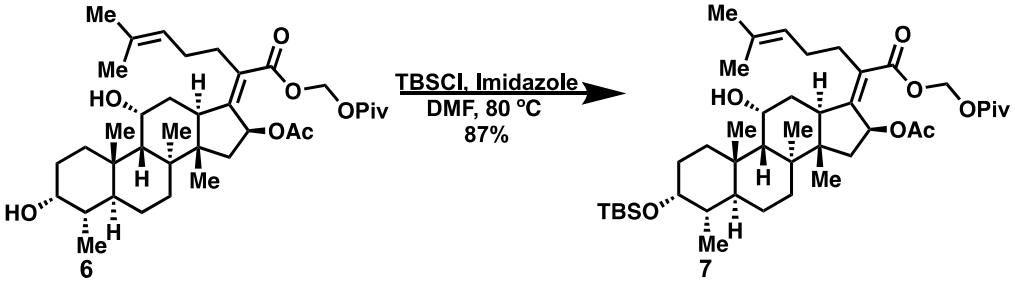
HRMS(ESI): m/z calc. for $C_{31}H_{44}O_6$ $[M]^+$: 513.3216, found: 513.3228.



Experimental procedure and spectra for compound **5** have been previously reported.[3]



Experimental procedure and spectra for compound **6** have been previously reported.[4, 5]



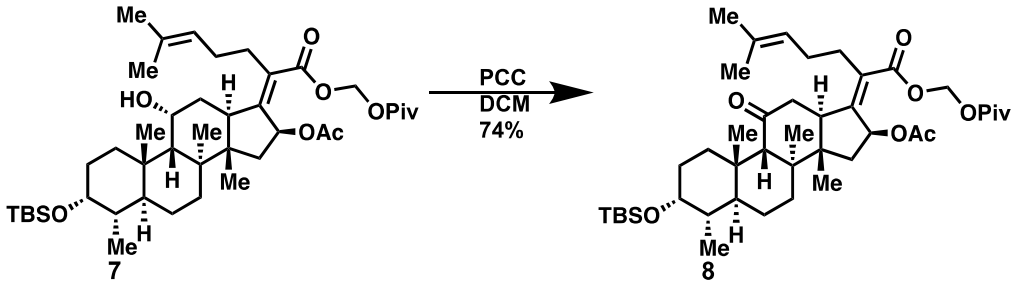
(pivaloyloxy)methyl(*Z*)-2-((3*R*,4*S*,5*S*,8*S*,9*S*,10*S*,11*R*,13*R*,14*S*,16*S*)-16-acetoxy-3-((*tert*-butyldimethylsilyl)oxy)-11-hydroxy-4,8,10,14-tetramethylhexadecahydro-17*H* cyclopenta[*a*]phenanthren-17-ylidene)-6-methylhept-5-enoate (7)

Procedure: In a 25 mL round bottom flask, **6** (2.08 g, 3.29 mmol, 1.0 eq.) was dissolved in DMF (10 mL). Then imidazole (526 mg, 7.72 mmol, 2.3 eq.) and TBSCl (1.01 g, 7.72 mmol, 2.3 eq.) were added, respectively. The reaction was then heated to 80 °C for 6 hrs. EtOAc and brine were then added, and the layers were separated. The aqueous layer was extracted three times with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. Silica gel column chromatography (5:1 Hex/EtOAc) afforded **7** as a white solid (2.14 g, 87%).

^{1}H NMR (500 MHz, Chloroform-*d*) δ 5.85 (d, *J* = 8.3 Hz, 1H), 5.78 (d, *J* = 5.4 Hz, 1H), 5.69 (d, *J* = 5.4 Hz, 1H), 5.09 – 5.04 (m, 1H), 4.34 – 4.28 (m, 1H), 3.70 – 3.66 (m, 1H), 3.07 – 3.00 (m, 1H), 2.50 – 2.36 (m, 2H), 2.34 – 2.28 (m, 1H), 2.24 – 2.07 (m, 4H), 1.96 (s, 3H), 1.83 – 1.71 (m, 3H), 1.66 (s, 3H), 1.58 (s, 3H), 1.54 – 1.46 (m, 3H), 1.45 – 1.38 (m, 2H), 1.34 (s, 3H), 1.29 (d, *J* = 14.2 Hz, 1H), 1.24 (s, 1H), 1.19 (s, 9H), 1.12 – 1.01 (m, 2H), 0.94 (s, 3H), 0.89 (s, 12H), 0.80 (d, *J* = 6.7 Hz, 3H), 0.01 (d, *J* = 6.4 Hz, 6H). (71 non-exchangeable protons)

^{13}C NMR (125 MHz, Chloroform-*d*) δ 177.18, 170.39, 168.25, 151.33, 132.77, 129.42, 123.10, 79.90, 74.45, 72.06, 68.52, 49.19, 49.00, 44.53, 39.61, 39.15, 38.90, 37.34, 36.67, 36.40, 35.43, 33.44, 30.99, 30.72, 28.92, 28.38, 27.04(3C), 26.06(3C), 25.87, 24.46, 22.63, 20.98, 20.53, 18.30, 18.27, 17.94, 16.92, -4.19, -4.72.

HRMS(ESI): m/z calc. for $C_{43}H_{72}O_8NaSi$ $[M+Na]^+$: 767.4894, found: 767.4921



7 8

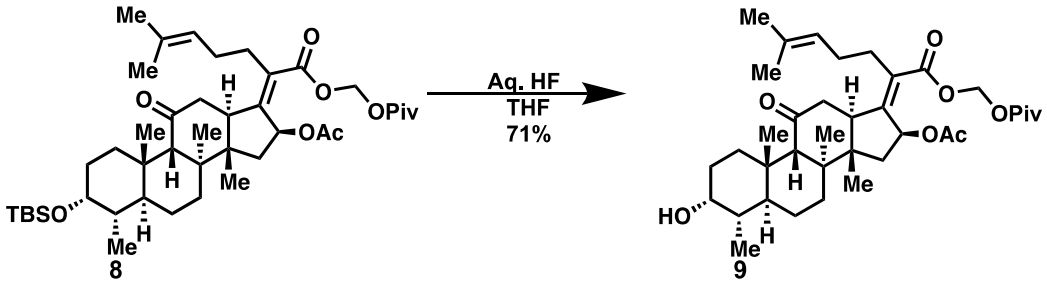
(pivaloyloxy)methyl (*Z*)-2-((3*R*,4*S*,5*S*,8*S*,9*S*,10*S*,13*R*,14*S*,16*S*)-16-acetoxy-3-((*tert*-butyldimethylsilyl)oxy)-4,8,10,14-tetramethyl-11-oxohexadecahydro-17*H*-cyclopenta[*a*]phenanthren-17-ylidene)-6-methylhept-5-enoate (8)

Procedure: In a 65 mL RB flask, **7** (1.71 g, 2.37 mmol, 1.0 eq.) was dissolved in DCM (34.0 mL). PCC (1.48 g, 6.87 mmol, 3.0 eq.) was added to the reaction. The reaction was then stirred at room temperature for 2 hrs. The reaction was the filtered and washed with EtOAc. The eluent was concentrated. The resulting crude product was then purified by silica gel column chromatography (3:1 Hex/EtOAc) affording **8** as a white solid (1.26 g, 74%).

^{1}H NMR (500 MHz, Chloroform-*d*) δ 5.87 (d, *J* = 8.3 Hz, 1H), 5.77 (d, *J* = 5.4 Hz, 1H), 5.70 (d, *J* = 5.4 Hz, 1H), 5.05 – 5.01 (m, 1H), 3.70 – 3.67 (m, 1H), 2.88 (dd, *J* = 13.1, 4.8 Hz, 1H), 2.84 – 2.79 (m, 1H), 2.67 – 2.60 (m, 1H), 2.50 (s, 1H), 2.40 – 2.28 (m, 2H), 2.23 – 2.18 (m, 1H), 2.17 – 2.08 (m, 2H), 2.07 – 2.00 (m, 1H), 1.98 (s, 3H), 1.97 – 1.83 (m, 2H), 1.76 – 1.69 (m, 1H), 1.64 (s, 3H), 1.60 – 1.58 (m, 1H), 1.56 (s, 3H), 1.55 – 1.52 (m, 1H), 1.49 – 1.42 (m, 1H), 1.39 (d, 1H), 1.24 (s, 1H), 1.19 (s, 9H), 1.15 (s, 3H), 1.13 (s, 3H), 0.98 (s, 3H), 0.97 – 0.90 (m, 1H), 0.88 (s, 9H), 0.86 – 0.84 (m, 1H), 0.78 (d, *J* = 6.7 Hz, 3H), 0.00 (d, *J* = 9.6 Hz, 6H).

^{13}C NMR (125 MHz, Chloroform-*d*) δ 210.33, 177.12, 170.15, 167.72, 148.48, 133.19, 130.41, 122.63, 79.89, 74.29, 72.35, 58.84, 48.94, 47.40, 44.81, 41.00, 38.89, 38.46, 38.33, 37.94, 35.26, 33.54, 31.02, 28.95, 28.17, 27.95, 27.01(3C), 26.03(3C), 25.82, 23.26, 21.07, 20.90, 20.21, 18.25, 17.90, 17.23, 17.03, -4.24, -4.74.

HRMS(ESI): m/z calc. for $C_{43}H_{70}O_8NaSi$ $[M+Na]^+$: 765.4738, found: 765.4743.



8 9

(pivaloyloxy)methyl (*Z*)-2-((3*R*,4*S*,5*S*,8*S*,9*S*,10*S*,13*R*,14*S*,16*S*)-16-acetoxy-3-hydroxy-4,8,10,14-tetramethyl-11-oxohexadecahydro-17*H*-cyclopenta[*a*]phenanthren-17-ylidene)-6-methylhept-5-enoate (9)

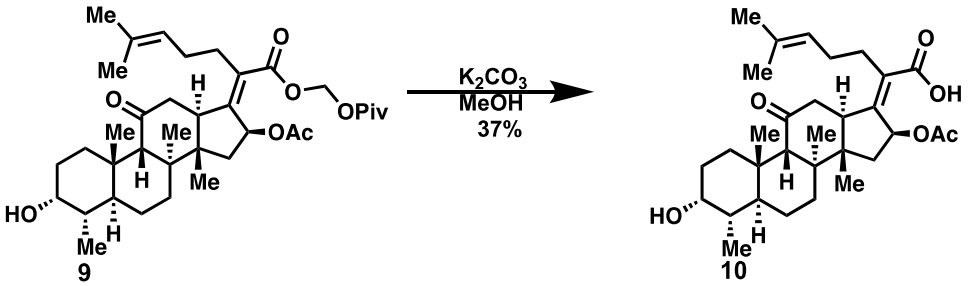
Procedure: In a 25 mL polystyrene bottle, **8** (161.8 mg, 0.217 mmol, 1.0 eq.) was dissolved in THF (10.0 mL). Aqueous HF (48% by weight) (1.50 mL) was added to the reaction. The reaction was then stirred at room temperature for 14 hrs. An additional (1.50 mL) of aqueous HF were added, and the

reaction was stirred for an additional 8 hrs. The reaction was then quenched by the addition of 10 % NaOH. The solution was then transferred to a polystyrene bottle where the reaction was extracted three times using DCM. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. Silica gel column chromatography (3:1 Hex/EtOAc) afforded **9** as a white solid (97.2 mg, 71%).

^{1}H NMR (500 MHz, Chloroform-*d*) δ 5.85 (d, *J* = 8.3 Hz, 1H), 5.76 (d, *J* = 5.4 Hz, 1H), 5.68 (d, *J* = 5.4 Hz, 1H), 5.05 – 5.00 (m, 1H), 3.75 – 3.72 (m, 1H), 2.91 – 2.85 (m, 1H), 2.83 – 2.78 (m, 1H), 2.68 – 2.60 (m, 1H), 2.55 (s, 1H), 2.38 – 2.27 (m, 2H), 2.22 – 2.10 (m, 2H), 2.07 – 1.98 (m, 2H), 1.97 (s, 3H), 1.96 – 1.81 (m, 4H), 1.68 – 1.65 (m, 1H), 1.63 (s, 3H), 1.55 (s, 3H), 1.53 – 1.46 (m, 2H), 1.38 (d, 1H), 1.23 – 1.19 (m, 1H), 1.18 (s, 9H), 1.14 (s, 3H), 1.13 (s, 3H), 1.05 – 0.99 (m, 1H), 0.99 (s, 3H), 0.88 (d, *J* = 6.9 Hz, 3H). (55 non-exchangeable protons)

^{13}C NMR (125 MHz, Chloroform-*d*) δ 210.21, 177.09, 170.18, 167.69, 148.21, 133.18, 130.36, 122.61, 79.88, 74.26, 71.50, 58.68, 48.87, 47.66, 44.90, 41.19, 38.86, 38.48, 38.30, 37.85, 34.81, 32.73, 30.32, 28.89, 28.26, 27.92, 26.98(3C), 25.79, 23.24, 21.15, 20.86, 20.44, 17.87, 17.19, 16.16.

HRMS(ESI): m/z calc. for $C_{37}H_{56}O_8Na$ $[M+Na]^+$: 651.3873, found: 651.3878.



(*Z*)-2-((3*R*,4*S*,5*S*,8*S*,9*S*,10*S*,13*R*,14*S*,16*S*)-16-acetoxy-3-hydroxy-4,8,10,14-tetramethyl-11-oxohexadecahydro-17*H*-cyclopenta[*a*]phenanthren-17-ylidene)-6-methylhept-5-enoic acid (10)

Procedure: In a 5 mL vial, **9** (97.2 mg, 0.15 mmol, 1.0 eq.) was dissolved in MeOH (1.22 mL). K_2CO_3 (42.7 mg, 0.30 mmol, 2.0 eq.) was added to the reaction. The reaction was then stirred at room temperature for 1 hr. The reaction was then filtered and concentrated. The resulting crude product was then purified by preparatory HPLC (C18 20x250mm, 10–100% ACN in H_2O) to afford **10** (29.3 mg, 37%) as a white solid.

^{1}H NMR (500 MHz, Methanol-d_4) δ 5.80 (d, *J* = 8.3 Hz, 1H), 5.19 – 5.14 (m, 1H), 3.72 – 3.68 (m, 1H), 2.91 – 2.85 (m, 1H), 2.76 – 2.70 (m, 3H), 2.36 – 2.26 (m, 2H), 2.20 – 2.03 (m, 5H), 2.03 (s, 3H), 2.02 – 1.97 (m, 2H), 1.91 – 1.83 (m, 1H), 1.67 (s, 3H), 1.66 – 1.63 (m, 1H), 1.62 (s, 3H), 1.57 – 1.50 (m, 1H), 1.32 (d, *J* = 13.3 Hz, 1H), 1.26 – 1.22 (m, 1H), 1.22 (s, 3H), 1.21 – 1.19 (m, 1H), 1.18 (s, 3H), 1.11 – 1.03 (m, 1H), 1.03 (s, 3H), 0.89 (d, *J* = 6.8 Hz, 3H). (44 non-exchangeable protons)

^{13}C NMR (125 MHz, Methanol-d_4) δ 213.77, 178.91, 173.30, 140.61, 135.90, 132.70, 125.24, 75.88, 72.36, 59.98, 50.17, 47.66, 46.46, 42.75, 39.79, 39.49, 38.97, 36.32, 34.02, 31.25, 31.07, 29.75, 28.79, 25.98, 23.50, 21.66, 21.49, 21.17, 17.95, 17.42, 16.66.

HRMS(ESI): m/z calc. for $C_{31}H_{46}O_6Na$ $[M+Na]^+$: 537.3192, found: 537.3192.

Preparation and Characterization of Fusidic Acid Side Chain Analogues

6 11

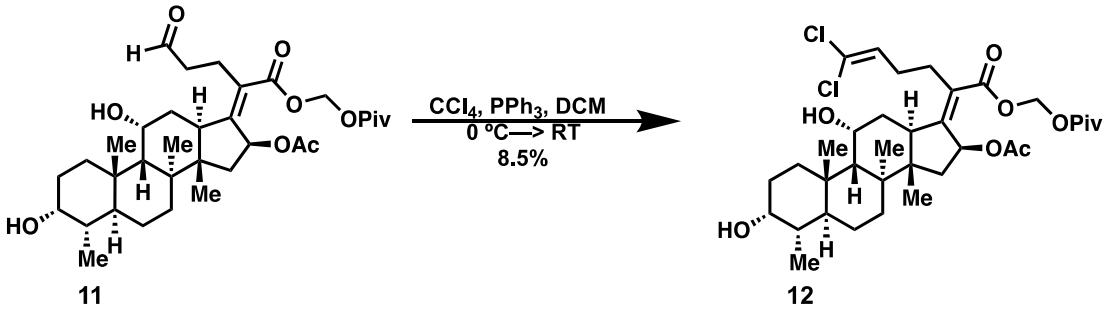
(pivaloyloxy)methyl (*Z*)-2-((3*R*,4*S*,5*S*,8*S*,9*S*,10*S*,11*R*,13*R*,14*S*,16*S*)-16-acetoxy-3,11-dihydroxy-4,8,10,14-tetramethylhexadecahydro-17*H*-cyclopenta[*a*]phenanthren-17-ylidene)-5-oxopentanoate (11)

Procedure: In a 65 mL round bottom flask, **6** (980 mg, 1.55 mmol, 1.0 eq.) was dissolved in acetone (9.25 mL) and distilled H_2O (0.80 mL). Then NMO (281 mg, 2.39 mmol, 1.5 eq.) and OsO_4 (0.15 mL, 0.028 mmol, 0.02 eq.) in a 0.2 M solution in MeCN were added, respectively. The solution was then stirred at room temperature under a N_2 atmosphere for 1 hr. The reaction was then quenched by the addition of 10 mL of saturated sodium thiosulfate. The reaction was stirred for an additional 45 minutes. The reaction was then concentrated *in vacuo* to remove the acetone. EtOAc and brine were then added, and the layers were separated. The aqueous layer was extracted five times with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. Silica gel column chromatography (100% EtOAc) afforded the crude dihydroxylation intermediate that was used without further purification.
In a 50 mL round bottom flask, the dihydroxylation intermediate (1.02 g, 1.53 mmol, 1.0 eq.) was dissolved in MeCN (10.0 mL) and distilled H_2O (10.0 mL). Then $NaIO_4$ (673 mg, 3.14 mmol, 2.0 eq.) was added. The solution was then stirred at room temperature for 6 hrs. The reaction was then cooled to 0 °C and quenched by the addition of 10 mL of saturated sodium sulfite. The reaction was stirred for an additional 45 minutes. EtOAc and brine were then added, and the layers were separated. The aqueous layer was extracted three times with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. Silica gel column chromatography (100% EtOAc) afforded **11** as a white solid (895 mg, 95%).

^{1}H NMR (500 MHz, Chloroform-*d*) δ 9.73 (s, 1H), 5.85 (d, *J* = 8.3 Hz, 1H), 5.77 (d, *J* = 5.4 Hz, 1H), 5.69 (d, *J* = 5.4 Hz, 1H), 4.35 – 4.31 (m, 1H), 3.74 – 3.70 (m, 1H), 3.10 – 3.04 (m, 1H), 2.75 – 2.65 (m, 3H), 2.55 – 2.45 (m, 1H), 2.31 – 2.23 (m, 1H), 2.19 – 2.08 (m, 4H), 1.96 (s, 3H), 1.85 – 1.78 (m, 2H), 1.74 – 1.66 (m, 2H), 1.56 – 1.51 (m, 2H), 1.51 – 1.43 (m, 1H), 1.35 (s, 3H), 1.29 (d, *J* = 13.6 Hz, 1H), 1.20 (s, 9H), 1.15 – 1.05 (m, 2H), 0.96 (s, 3H), 0.91 – 0.88 (m, 6H). (50 non-exchangeable protons)

^{13}C NMR (125 MHz, Chloroform-*d*) δ 201.23, 177.25, 170.29, 167.55, 153.29, 127.35, 80.06, 74.40, 71.58, 68.20, 49.49, 49.01, 44.74, 43.96, 39.66, 39.05, 38.93, 36.95, 36.75, 35.84, 35.69, 31.93, 30.14, 29.99, 27.03(3C), 23.90, 23.43, 21.47, 21.09, 20.94, 18.05, 16.14.

HRMS(ESI): m/z calc. for $C_{34}H_{52}O_9Na$ $[M+Na]^+$: 627.3509, found: 627.3497.



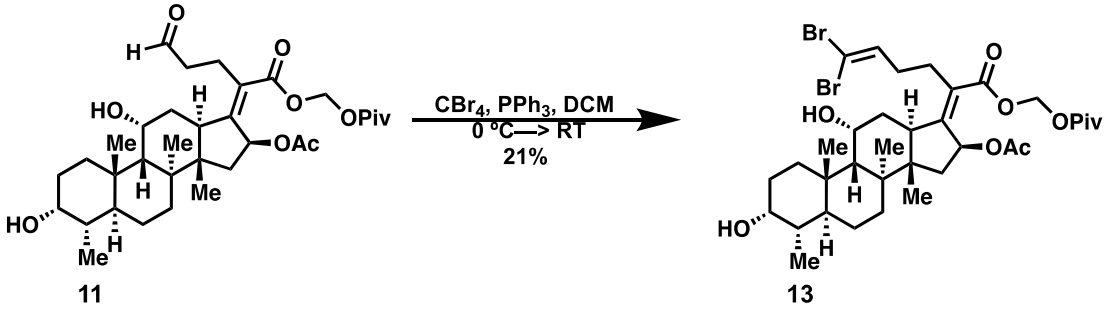
(pivaloyloxy)methyl (*Z*)-2-((3*R*,4*S*,5*S*,8*S*,9*S*,10*S*,11*R*,13*R*,14*S*,16*S*)-16-acetoxy-3,11-dihydroxy-4,8,10,14-tetramethylhexadecahydro-17*H*-cyclopenta[*a*]phenanthren-17-ylidene)-6,6-dichlorohex-5-enoate (12)

Procedure: In a 10 mL round bottom flask, CCl_4 (0.18 mL, 1.85 mmol, 2.0 eq.) was dissolved in DCM (3.00 mL). The solution was then cooled to 0 °C. This was followed by the addition of PPh_3 (967 mg, 3.68 mmol, 4.0 eq.). The reaction was then allowed to stirred at 0 °C for 20 minutes. Compound **11** (556 mg, 0.92 mmol, 1.0 eq.) was then added and the reaction was stirred at 0 °C for 3 hrs. The reaction was then stirred at room temperature for 12 hrs. EtOAc and brine were then added, and the layers were separated. The aqueous layer was extracted three times with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. Silica gel column chromatography (1:1 Hex/EtOAc) afforded **12** as a white solid (52.6 mg, 8.5%).

^{1}H NMR (500 MHz, Chloroform-*d*) δ 5.87 (d, *J* = 8.3 Hz, 1H), 5.84 (t, *J* = 7.4 Hz, 1H), 5.79 (d, *J* = 5.4 Hz, 1H), 5.69 (d, *J* = 5.4 Hz, 1H), 4.36 – 4.33 (m, 1H), 3.76 – 3.72 (m, 1H), 3.11 – 3.05 (m, 1H), 2.55 – 2.51 (m, 2H), 2.36 – 2.08 (m, 7H), 1.97 (s, 3H), 1.89 – 1.80 (m, 2H), 1.77 – 1.71 (m, 2H), 1.63 – 1.57 (m, 3H), 1.53 – 1.47 (m, 1H), 1.37 (s, 3H), 1.30 (d, *J* = 13.4 Hz, 1H), 1.21 (s, 9H), 1.14 – 1.09 (m, 1H), 0.97 (s, 3H), 0.92 – 0.90 (m, 6H). (50 non-exchangeable protons)

^{13}C NMR (125 MHz, Chloroform-*d*) δ 177.21, 170.34, 167.70, 153.25, 128.50, 127.83, 121.15, 80.05, 74.41, 71.54, 68.28, 49.37, 49.03, 44.74, 39.64, 39.09, 38.94, 37.18, 36.43, 36.21, 35.88, 32.45, 30.38, 30.10, 29.82, 27.24, 27.06(3C), 24.28, 23.03, 20.97, 20.93, 18.10, 16.12.

HRMS(ESI): m/z calc. for $C_{35}H_{52}O_8NaCl_2$ $[M+Na]^+$: 693.2937, found: 693.2946.



(pivaloyloxy)methyl (*Z*)-2-((3*R*,4*S*,5*S*,8*S*,9*S*,10*S*,11*R*,13*R*,14*S*,16*S*)-16-acetoxy-3,11-dihydroxy-4,8,10,14-tetramethylhexadecahydro-17*H*-cyclopenta[*a*]phenanthren-17-ylidene)-6,6-dibromohex-5-enoate (13)

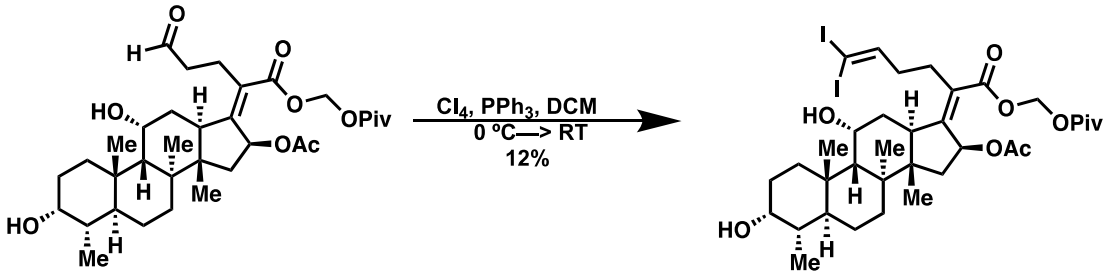
Procedure: In a 65 mL round bottom flask, CBr_4 (1.56 g, 4.70 mmol, 3.0 eq.) was dissolved in DCM (38.0 mL). The solution was then cooled to 0 °C. This was followed by the addition of PPh_3 (1.65 g, 6.29 mmol, 4.0 eq.). The reaction was then allowed to stir at 0 °C for 20 minutes. Compound **11** (951

mg, 1.57 mmol, 1.0 eq.) was then added and the reaction was stirred at 0 °C for 3 hrs. EtOAc and brine were then added, and the layers were separated. The aqueous layer was extracted three times with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. Silica gel column chromatography (1:1 Hex/EtOAc) afforded **13** as a white solid (249 mg, 21%).

^{1}H NMR (500 MHz, Chloroform-*d*) δ 6.38 (t, *J* = 7.4 Hz, 1H), 5.87 (d, *J* = 8.3 Hz, 1H), 5.79 (d, *J* = 5.4 Hz, 1H), 5.69 (d, *J* = 5.4 Hz, 1H), 4.37 – 4.33 (m, 1H), 3.76 – 3.72 (m, 1H), 3.11 – 3.05 (m, 1H), 2.59 – 2.50 (m, 2H), 2.31 – 2.07 (m, 6H), 1.97 (s, 3H), 1.89 – 1.80 (m, 2H), 1.77 – 1.69 (m, 2H), 1.62 – 1.54 (m, 4H), 1.52 – 1.47 (m, 1H), 1.37 (s, 3H), 1.30 (d, *J* = 14.3 Hz, 1H), 1.21 (s, 9H), 1.15 – 1.10 (m, 1H), 0.97 (s, 3H), 0.92 – 0.89 (m, 6H). (50 non-exchangeable protons)

^{13}C NMR (125 MHz, Chloroform-*d*) δ 177.21, 170.33, 167.65, 153.38, 137.27, 127.71, 90.04, 80.09, 74.43, 71.54, 68.27, 49.39, 49.05, 44.78, 39.65, 39.10, 38.95, 37.17, 36.46, 36.21, 36.00, 33.11, 32.43, 30.37, 30.11, 27.08(3C), 26.85, 24.26, 23.05, 20.97, 20.95, 18.15, 16.12.

HRMS(ESI): m/z calc. for $C_{35}H_{52}O_8NaBr_2$ $[M+Na]^+$: 781.1927, found: 781.1938.



11 14

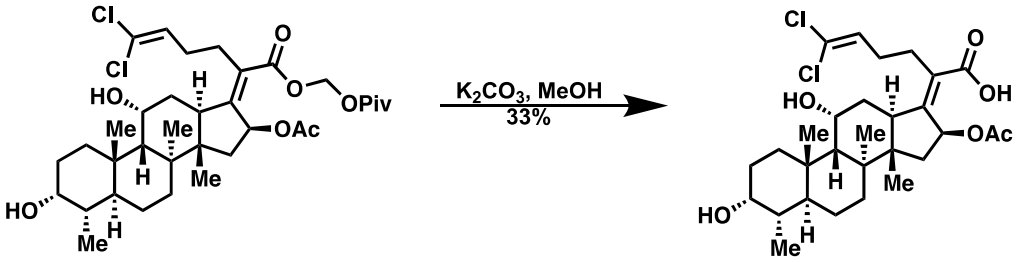
(pivaloyloxy)methyl (*Z*)-2-((3*R*,4*S*,5*S*,8*S*,9*S*,10*S*,11*R*,13*R*,14*S*,16*S*)-16-acetoxy-3,11-dihydroxy-4,8,10,14-tetramethylhexadecahydro-17*H*-cyclopenta[*a*]phenanthren-17-ylidene)-6,6-diiodohex-5-enoate (14)

Procedure: In a 10 mL round bottom flask, CI_4 (1.26 g, 2.42 mmol, 2.0 eq.) was dissolved in DCM (22.0 mL). This was followed by the addition of PPh_3 (1.27 g, 4.84 mmol, 4.0 eq.). The reaction was then stirred at room temperature for 30 minutes. Compound **11** (732 mg, 1.21 mmol, 1.0 eq.) was then added and the reaction was stirred at room temperature for 2 hrs. EtOAc and brine were then added, and the layers were separated. The aqueous layer was extracted three times with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. Silica gel column chromatography (1:1 Hex/EtOAc) afforded **14** as a white solid (127mg, 12%).

^{1}H NMR (500 MHz, Chloroform-*d*) δ 6.92 (t, *J* = 7.2 Hz, 1H), 5.86 (d, *J* = 8.3 Hz, 1H), 5.78 (d, *J* = 5.4 Hz, 1H), 5.68 (d, *J* = 5.4 Hz, 1H), 4.36 – 4.32 (m, 1H), 3.73 – 3.68 (m, 1H), 3.10 – 3.04 (m, 1H), 2.58 – 2.46 (m, 2H), 2.29 – 2.23 (m, 1H), 2.21 – 2.03 (m, 4H), 1.99 – 1.97 (m, 1H), 1.96 (s, 3H), 1.86 – 1.78 (m, 2H), 1.73 – 1.67 (m, 2H), 1.62 – 1.51 (m, 3H), 1.50 – 1.45 (m, 1H), 1.35 (s, 3H), 1.28 (d, *J* = 14.3 Hz, 1H), 1.19 (s, 9H), 1.13 – 1.06 (m, 2H), 0.95 (s, 3H), 0.90 – 0.87 (m, 6H). (50 non-exchangeable protons)

^{13}C NMR (125 MHz, Chloroform-*d*) δ 177.16, 170.30, 167.57, 153.49, 151.60, 127.44, 80.08, 74.39, 71.52, 68.13, 49.42, 48.97, 44.79, 39.60, 39.58, 39.01, 38.90, 36.93, 36.65, 36.10, 35.82, 31.98, 30.15, 30.00, 27.08(3C), 26.34, 23.92, 23.36, 21.04, 20.94, 18.03, 16.14, 13.10.

HRMS(ESI): m/z calc. for $C_{35}H_{52}O_8NaI_2$ $[M+Na]^+$: 877.1649, found: 877.1650.



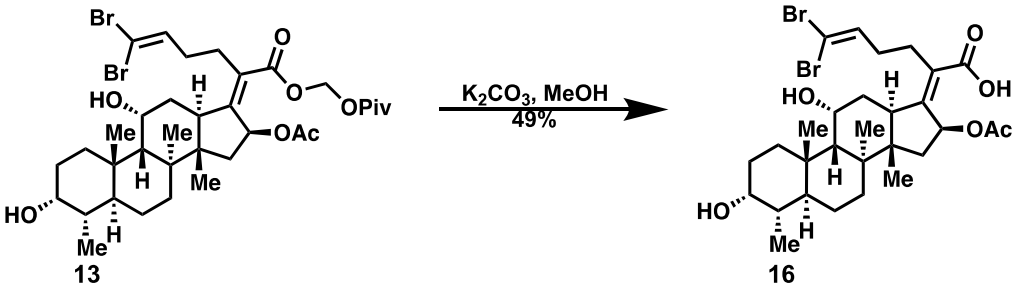
(*Z*)-2-((3*R*,4*S*,5*S*,8*S*,9*S*,10*S*,11*R*,13*R*,14*S*,16*S*)-16-acetoxy-3,11-dihydroxy-4,8,10,14-tetramethylhexadecahydro-17*H*-cyclopenta[*a*]phenanthren-17-ylidene)-6,6-dichlorohex-5-enoic acid (15)

Procedure: In a 5 mL vial, **12** (82.4 mg, 0.12 mmol, 1.0 eq.) was dissolved in MeOH (1.39 mL). K_2CO_3 (33.9 mg, 0.24 mmol, 2.0 eq.) was added to the reaction. The reaction was then stirred at room temperature for 1 hr. The reaction was then filtered and concentrated. The resulting crude product was then purified by preparatory HPLC (C18 20x250mm, 10–100% ACN in H_2O) to afford **15** (22.7 mg, 33%) as a white solid.

^{1}H NMR (500 MHz, Methanol-*d*$_4$) δ 6.03 (t, *J* = 7.4 Hz, 1H), 5.79 (d, *J* = 8.3 Hz, 1H), 4.32 – 4.27 (m, 1H), 3.67 – 3.64 (m, 1H), 3.06 – 2.99 (m, 1H), 2.66 – 2.59 (m, 1H), 2.47 – 2.40 (m, 1H), 2.36 – 2.23 (m, 4H), 2.20 – 2.08 (m, 2H), 2.00 (s, 3H), 1.87 – 1.64 (m, 5H), 1.61 (s, 1H), 1.57 – 1.44 (m, 2H), 1.39 (s, 3H), 1.20 (d, *J* = 14.0 Hz, 1H), 1.16 – 1.10 (m, 2H), 0.99 (s, 3H), 0.95 (s, 3H), 0.89 (d, *J* = 6.8 Hz, 3H). (39 non-exchangeable protons)

^{13}C NMR (125 MHz, Methanol-*d*$_4$) δ 178.94, 173.42, 139.76, 138.09, 131.33, 120.68, 75.94, 72.60, 68.86, 50.78, 50.15, 43.87, 40.77, 40.27, 38.32, 37.89, 37.57, 36.91, 33.00, 31.11, 31.03, 30.68, 29.04, 23.88, 23.87, 22.53, 21.17, 17.96, 16.59.

HRMS(ESI): m/z calc. for $C_{29}H_{42}O_6NaCl_2$ $[M+Na]^+$: 579.2256, found: 579.2245.



(*Z*)-2-((3*R*,4*S*,5*S*,8*S*,9*S*,10*S*,11*R*,13*R*,14*S*,16*S*)-16-acetoxy-3,11-dihydroxy-4,8,10,14-tetramethylhexadecahydro-17*H*-cyclopenta[*a*]phenanthren-17-ylidene)-6,6-dibromohex-5-enoic acid (16)

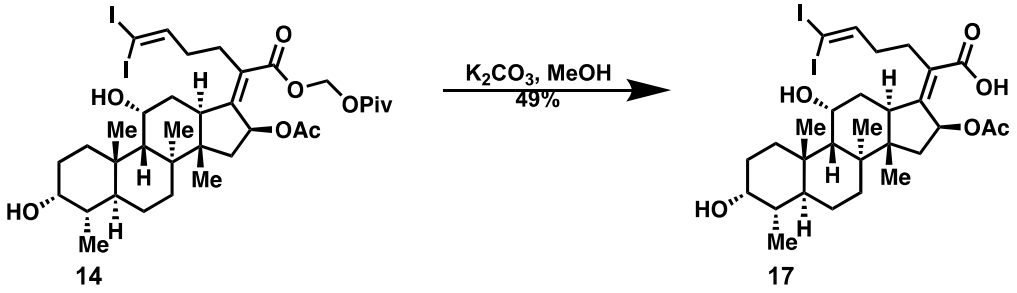
Procedure: In a 5 mL vial, **13** (48.4 mg, 0.06 mmol, 1.0 eq.) was dissolved in MeOH (0.90 mL). K_2CO_3 (17.5 mg, 0.12 mmol, 2.0 eq.) was added to the reaction. The reaction was then stirred at room temperature for 1 hr. The reaction was then filtered and concentrated. The resulting crude product was then purified by preparatory HPLC (C18 20x250mm, 10–100% ACN in H_2O) to afford **16** (20.1 mg, 49%) as a white solid.

^{1}H NMR (500 MHz, Methanol-*d*$_4$) δ 6.55 (t, *J* = 7.4 Hz, 1H), 5.78 (d, *J* = 8.3 Hz, 1H), 4.32 – 4.28 (m, 1H), 3.67 – 3.63 (m, 1H), 3.05 – 2.99 (m, 1H), 2.66 – 2.58 (m, 1H), 2.49 – 2.41 (m, 1H), 2.32 – 2.22 (m, 4H), 2.20 – 2.08 (m, 2H), 2.00 (s, 3H), 1.85 – 1.63 (m, 5H), 1.61 (s, 1H), 1.56 – 1.45 (m, 2H), 1.39 (s,

3H), 1.20 (d, *J* = 14.0 Hz, 1H), 1.17 – 1.09 (m, 2H), 1.00 (s, 3H), 0.95 (s, 3H), 0.89 (d, *J* = 6.7 Hz, 3H). (39 non-exchangeable protons)

^{13}C NMR (125 MHz, Methanol-*d*$_4$) δ 178.96, 173.42, 140.05, 139.60, 138.12, 89.28, 75.99, 72.62, 68.89, 50.80, 50.15, 43.84, 40.78, 40.29, 38.32, 37.90, 37.63, 36.93, 34.06, 33.01, 31.12, 31.04, 28.74, 23.87(2C), 22.53, 21.17, 17.98, 16.58.

HRMS(ESI): m/z calc. for $C_{29}H_{42}O_6NaBr_2$ $[M+Na]^+$: 667.1246, found: 667.1255.



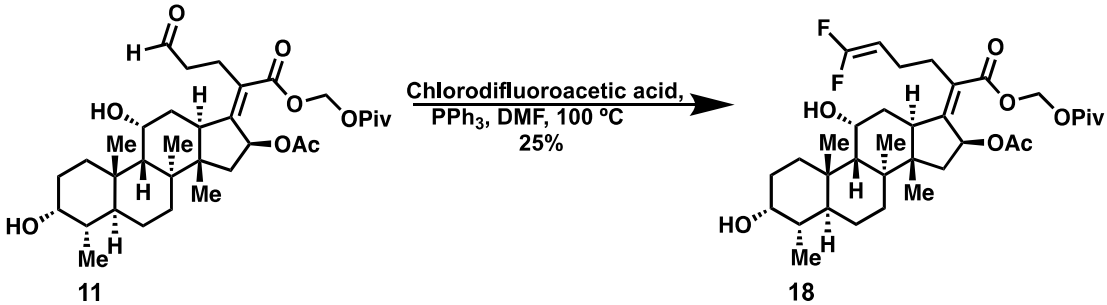
(*Z*)-2-((3*R*,4*S*,5*S*,8*S*,9*S*,10*S*,11*R*,13*R*,14*S*,16*S*)-16-acetoxy-3,11-dihydroxy-4,8,10,14-tetramethylhexadecahydro-17*H*-cyclopenta[*a*]phenanthren-17-ylidene)-6,6-diiodohex-5-enoic acid (17)

Procedure: In a 5 mL vial, **14** (127 mg, 0.15 mmol, 1.0 eq.) was dissolved in MeOH (1.70 mL). K_2CO_3 (41.3 mg, 0.30 mmol, 2.0 eq.) was added to the reaction. The reaction was then stirred at room temperature for 1 hr. The reaction was then filtered and concentrated. The resulting crude product was then purified by preparatory HPLC (C18 20x250mm, 10–100% ACN in H_2O) to afford **17** (54.0 mg, 49%) as a white solid.

^{1}H NMR (500 MHz, Methanol-*d*$_4$) δ 7.05 (t, *J* = 7.2 Hz, 1H), 5.79 (d, *J* = 8.3 Hz, 1H), 4.34 – 4.29 (m, 1H), 3.68 – 3.64 (m, 1H), 3.05 – 3.00 (m, 1H), 2.64 – 2.57 (m, 1H), 2.48 – 2.41 (m, 1H), 2.34 – 2.23 (m, 2H), 2.19 – 2.07 (m, 4H), 2.00 (s, 3H), 1.88 – 1.80 (m, 2H), 1.79 – 1.63 (m, 3H), 1.61 (s, 1H), 1.55 – 1.47 (m, 2H), 1.39 (s, 3H), 1.19 (d, *J* = 14.0 Hz, 1H), 1.16 – 1.10 (m, 2H), 1.00 (s, 3H), 0.96 (s, 3H), 0.89 (d, *J* = 6.8 Hz, 3H). (39 non-exchangeable protons)

^{13}C NMR (125 MHz, Methanol-*d*$_4$) δ 178.91, 173.42, 154.06, 139.66, 138.02, 75.90, 72.57, 68.85, 50.76, 50.16, 43.86, 40.74, 40.59, 40.26, 38.30, 37.88, 37.76, 36.90, 33.00, 31.11, 31.01, 28.36, 23.91, 23.86, 22.52, 21.18, 18.02, 16.59, 12.96.

HRMS(ESI): m/z calc. for $C_{29}H_{42}O_6NaI_2$ $[M+Na]^+$: 763.0969, found: 763.0955.



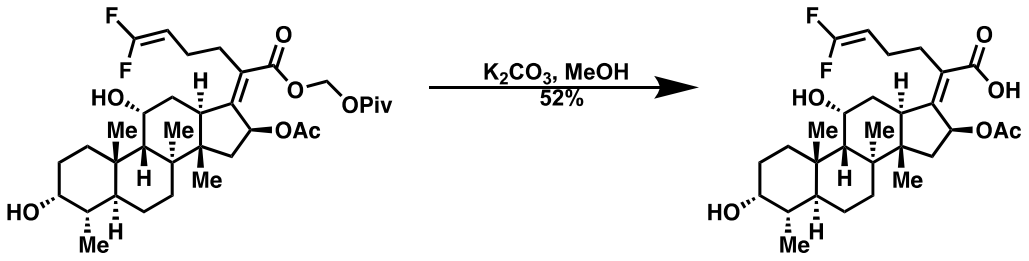
(pivaloyloxy)methyl (*Z*)-2-((3*R*,4*S*,5*S*,8*S*,9*S*,10*S*,11*R*,13*R*,14*S*,16*S*)-16-acetoxy-3,11-dihydroxy-4,8,10,14-tetramethylhexadecahydro-17*H*-cyclopenta[*a*]phenanthren-17-ylidene)-6,6-difluorohex-5-enoate (18)

Procedure: In a 5 mL vial, **11** (104 mg, 0.17 mmol, 2.0 eq.) was dissolved in DMF (1.00 mL). This was followed by the addition of PPh_3 (90.5 mg, 0.34 mmol, 2.0 eq.) and chlorodifluoroacetic acid (52.6 mg, 0.34 mmol, 2.0 eq.). The reaction was then heated to 100 °C for 4 hrs. EtOAc and brine were then added, and the layers were separated. The aqueous layer was extracted three times with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. Silica gel column chromatography (1:1 Hex/EtOAc) afforded **18** as a white solid (27.8 mg, 25%).

^{1}H NMR (500 MHz, MeOD) δ 5.85 (d, *J* = 8.3 Hz, 1H), 5.77 (d, *J* = 5.7 Hz, 1H), 5.72 (d, *J* = 5.7 Hz, 1H), 4.33 – 4.31 (m, 1H), 4.31 – 4.23 (m, 1H), 3.68 – 3.65 (m, 1H), 3.14 – 3.09 (m, 1H), 2.63 – 2.56 (m, 1H), 2.52 – 2.45 (m, 1H), 2.31 – 2.24 (m, 2H), 2.21 – 2.12 (m, 3H), 2.10 – 2.02 (m, 1H), 1.96 (s, 3H), 1.91 – 1.80 (m, 2H), 1.77 – 1.62 (m, 3H), 1.61 – 1.58 (m, 1H), 1.56 – 1.51 (m, 1H), 1.50 – 1.46 (m, 1H), 1.40 (s, 3H), 1.27 (d, *J* = 14.3 Hz, 1H), 1.21 (s, 9H), 1.18 – 1.10 (m, 2H), 1.00 (s, 3H), 0.94 (s, 3H), 0.90 (d, *J* = 6.8 Hz, 3H). (50 non-exchangeable protons)

^{13}C NMR (125 MHz, MeOD) δ 178.39, 172.13, 169.30, 157.96 (dd, *J* = 284.8, 282.7 Hz, 1C), 154.32, 129.41, 81.15, 78.18 (dd, *J* = 22.5, 20.5, Hz 1C), 75.71, 72.50, 68.54, 50.68, 50.14, 45.85, 40.79, 40.07, 39.86, 38.26, 37.90, 37.35, 36.89, 32.95, 31.10, 31.04, 29.24, 27.33(3C), 24.00, 23.87, 23.65 (d, *J* = 4.3 Hz, 1C), 22.41, 20.95, 18.25, 16.57.

HRMS(ESI): m/z calc. for $C_{35}H_{52}O_8NaF_2$ $[M+Na]^+$: 661.3528, found: 661.3506.



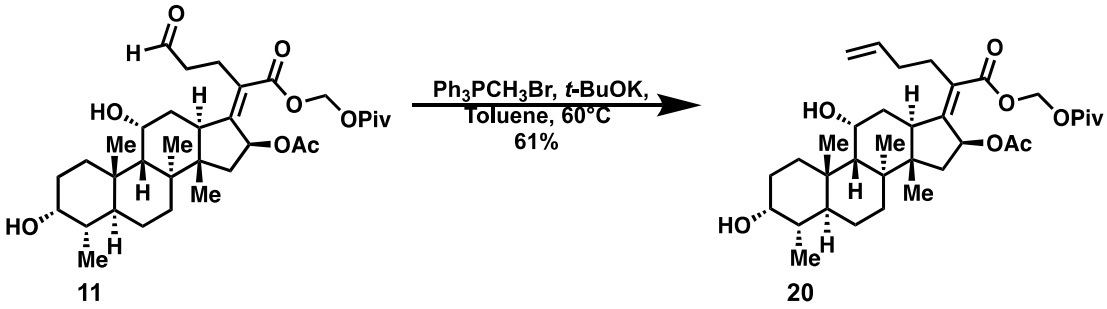
(*Z*)-2-((3*R*,4*S*,5*S*,8*S*,9*S*,10*S*,11*R*,13*R*,14*S*,16*S*)-16-acetoxy-3,11-dihydroxy-4,8,10,14-tetramethylhexadecahydro-17*H*-cyclopenta[*a*]phenanthren-17-ylidene)-6,6-difluorohex-5-enoic acid (19)

Procedure: In a 5 mL vial, **18** (89.6 mg, 0.14 mmol, 1.0 eq.) was dissolved in MeOH (1.60 mL). K_2CO_3 (38.7 mg, 0.28 mmol, 2.0 eq.) was added to the reaction. The reaction was then stirred at room temperature for 1 hr. The reaction was then filtered and concentrated. The resulting crude product was then purified by preparatory HPLC (C18 20x250mm, 10–100% ACN in H_2O) to afford **19** (38.0 mg, 52%) as a white solid.

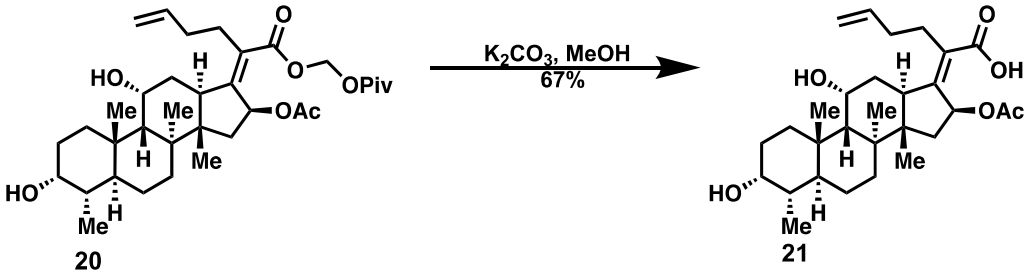
^{1}H NMR (500 MHz, Methanol-*d*$_4$) δ 5.78 (d, *J* = 8.3 Hz, 1H), 4.38 – 4.31 (m, 1H), 4.30 – 4.28 (m, 1H), 3.67 – 3.63 (m, 1H), 3.04 – 2.99 (m, 1H), 2.64 – 2.57 (m, 1H), 2.44 – 2.36 (m, 1H), 2.30 – 2.23 (m, 2H), 2.20 – 2.08 (m, 4H), 2.00 (s, 3H), 1.88 – 1.79 (m, 2H), 1.79 – 1.62 (m, 3H), 1.62 – 1.60 (m, 1H), 1.55 – 1.50 (m, 1H), 1.50 – 1.45 (m, 1H), 1.38 (s, 3H), 1.19 (d, *J* = 14.0 Hz, 1H), 1.16 – 1.08 (m, 2H), 0.99 (s, 3H), 0.94 (s, 3H), 0.89 (d, *J* = 6.8 Hz, 3H). (39 non-exchangeable protons)

^{13}C NMR (125 MHz, Methanol-*d*$_4$) δ 179.22, 173.44, 159.67 (dd, *J* = 283.6, 281.7 Hz, 1C), 139.34, 138.54, 79.11 (dd, *J* = 21.6, 20.7 Hz, 1C), 75.97, 72.62, 68.89, 50.80, 50.12, 43.80, 40.77, 40.29, 38.33, 37.89, 37.48, 36.91, 32.99, 31.11, 31.03, 30.52, 23.89, 23.86, 23.25 (d, *J* = 4.1 Hz 1C), 22.54, 21.18, 17.94, 16.58.

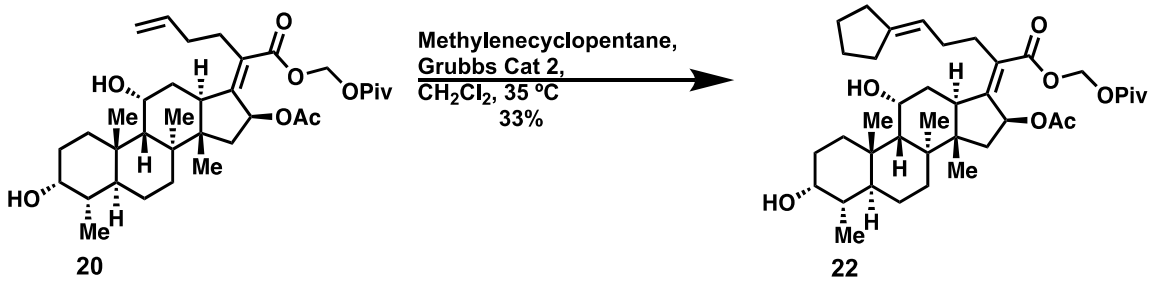
HRMS(ESI): m/z calc. for $C_{29}H_{42}O_6NaF_2$ $[M]^-$: 523.2871, found: 523.2872.



Experimental procedure and spectra for compound **20** have been previously reported.[6]



Experimental procedure and spectra for compound **21** have been previously reported.[6]



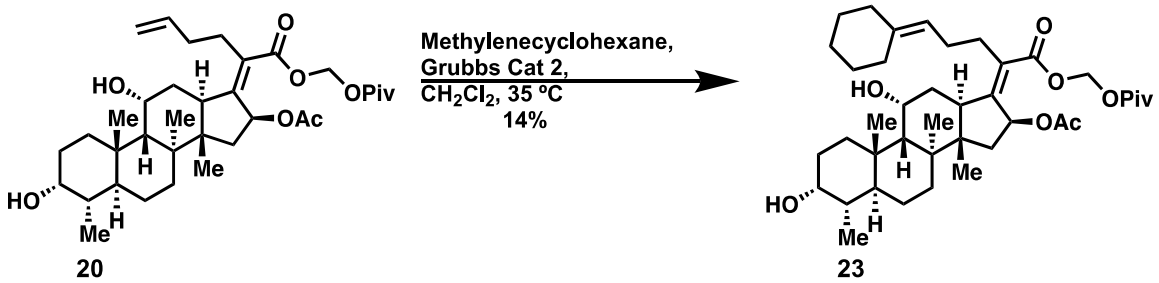
(pivaloyloxy)methyl (*Z*)-2-((3*R*,4*S*,5*S*,8*S*,9*S*,10*S*,11*R*,13*R*,14*S*,16*S*)-16-acetoxy-3,11-dihydroxy-4,8,10,14-tetramethylhexadecahydro-17*H*-cyclopenta[*a*]phenanthren-17-ylidene)-5-cyclopentylidenepentanoate (22)

Procedure: In a 5 mL vial, **20** (158 mg, 0.26 mmol, 1.0 eq.) and Grubbs Cat 2 (13.8 mg, 0.015 mmol, 0.06 eq.) were added. The vial was then degassed and backfilled with nitrogen three times. DCM (1.60 mL) and methylenecyclopentane (0.11 mL, 1.04 mmol, 4.0 eq.) were then added, respectively. The reaction was then heated to 35 °C for 24 hours. EtOAc and brine were then added, and the layers were separated. The aqueous layer was extracted three times with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. Silica gel column chromatography (3:1 Hex/EtOAc to 1:1 Hex/EtOAc) afforded **22** as a white solid (57.5 mg, 33%).

^{1}H NMR (500 MHz, Chloroform-*d*) 5.83 (d, *J* = 8.3 Hz, 1H), 5.77 (d, *J* = 5.4, 1.3 Hz, 1H), 5.69 (d, *J* = 5.4, 1.3 Hz, 1H), 5.21 – 5.14 (m, 1H), 4.34 – 4.29 (m, 1H), 3.73 – 3.70 (m, 1H), 3.06 – 3.00 (m, 1H), 2.49 – 2.40 (m, 2H), 2.33 – 2.27 (m, 1H), 2.19 – 2.09 (m, 7H), 2.03 – 1.97 (m, 1H), 1.96 (s, 3H), 1.84 – 1.72 (m, 5H), 1.64 – 1.52 (m, 7H), 1.51 – 1.46 (m, 1H), 1.35 (s, 3H), 1.28 (d, *J* = 14.3 Hz, 1H), 1.19 (s, 9H), 1.14 – 1.05 (m, 2H), 0.96 (s, 3H), 0.91 – 0.88 (m, 6H). (58 non-exchangeable protons)

^{13}C NMR (125 MHz, Chloroform-*d*) δ 177.17, 170.40, 168.30, 151.02, 144.58, 129.43, 118.50, 79.92, 74.45, 71.52, 68.34, 49.42, 48.89, 44.46, 39.61, 39.12, 38.89, 37.08, 36.52, 36.09, 35.73, 33.73, 32.29, 30.30, 30.07, 29.92, 28.83, 28.72, 27.03(3C), 26.50(2C), 24.10, 23.12, 20.99, 20.95, 18.00, 16.10.

HRMS(ESI): m/z calc. for $C_{39}H_{60}O_8Na$ $[M+Na]^+$: 679.4186, found: 679.4182.



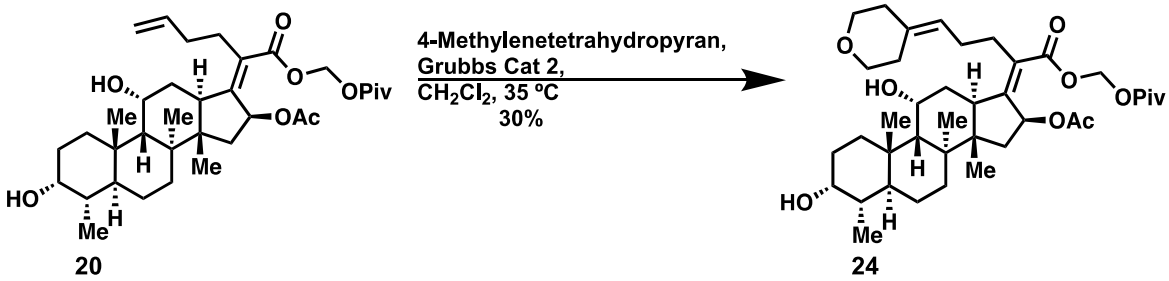
(pivaloyloxy)methyl (*Z*)-2-((3*R*,4*S*,5*S*,8*S*,9*S*,10*S*,11*R*,13*R*,14*S*,16*S*)-16-acetoxy-3,11-dihydroxy-4,8,10,14-tetramethylhexadecahydro-17*H*-cyclopenta[*a*]phenanthren-17-ylidene)-5-cyclohexylidenepentanoate (23)

Procedure: In a 5 mL vial, **20** (140 mg, 0.23 mmol, 1.0 eq.) and Grubbs Cat 2 (11.9 mg, 0.013 mmol, 0.06 eq) were added. The vial was then degassed and backfilled with nitrogen three times. DCM (1.42 mL) and methylenecyclohexane (0.11 mL, 0.93 mmol, 4.0 eq.) were then added, respectively. The reaction was then heated to 35 °C for 24 hours. EtOAc and brine were then added, and the layers were separated. The aqueous layer was extracted three times with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. Silica gel column chromatography (3:1 Hex/EtOAc to 1:1 Hex/EtOAc) afforded **23** as a white solid (22.0 mg, 14%).

^{1}H NMR (500 MHz, Chloroform-*d*) δ 5.85 (d, *J* = 8.3 Hz, 1H), 5.79 (d, *J* = 5.4, 1.3 Hz, 1H), 5.69 (d, *J* = 5.4, 1.3 Hz, 1H), 5.06 – 5.00 (m, 1H), 4.36 – 4.31 (m, 1H), 3.76 – 3.71 (m, 1H), 3.07 – 3.00 (m, 1H), 2.50 – 2.38 (m, 2H), 2.35 – 2.29 (m, 1H), 2.20 – 2.02 (m, 8H), 1.97 (s, 3H), 1.89 – 1.79 (m, 2H), 1.78 – 1.70 (m, 2H), 1.60 – 1.47 (m, 11H), 1.36 (s, 3H), 1.29 (d, *J* = 14.3 Hz, 1H), 1.20 (s, 9H), 1.16 – 1.04 (m, 2H), 0.97 (s, 3H), 0.93 – 0.88 (m, 6H). (60 non-exchangeable protons)

^{13}C NMR (125 MHz, Chloroform-*d*) δ 177.19, 170.42, 168.32, 151.05, 140.95, 129.45, 119.68, 79.93, 74.48, 71.53, 68.45, 49.38, 48.94, 44.49, 39.63, 39.16, 38.93, 37.33, 37.22, 36.39, 36.33, 35.79, 32.57, 30.43, 30.14, 29.23, 28.91, 28.71, 28.03, 27.47, 27.06(4C), 24.32, 22.93, 20.99, 20.93, 18.11, 16.11.

HRMS(ESI): m/z calc. for $C_{40}H_{62}O_8Na$ $[M+Na]^+$: 693.4342, found: 693.4344.



(pivaloyloxy)methyl (*Z*)-2-((3*R*,4*S*,5*S*,8*S*,9*S*,10*S*,11*R*,13*R*,14*S*,16*S*)-16-acetoxy-3,11-dihydroxy-4,8,10,14-tetramethylhexadecahydro-17*H*-cyclopenta[*a*]phenanthren-17-ylidene)-5-(tetrahydro-4*H*-pyran-4-ylidene)pentanoate (24)

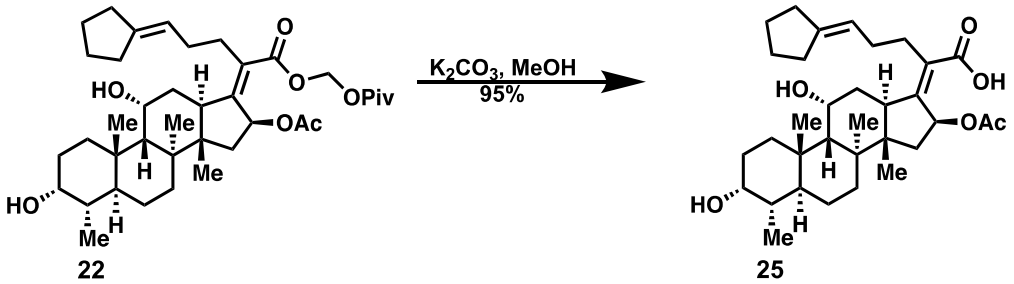
Procedure: In a 5 mL vial, **20** (220 mg, 0.36 mmol, 1.0 eq.) and Grubbs Cat 2 (18.5 mg, 0.021 mmol, 0.06 eq) were added. The vial was then degassed and backfilled with nitrogen three times. DCM (2.20 mL) and 4-methylenetetrahydropyran (0.16 mL, 1.45 mmol, 4.0 eq.) were then added, respectively. The reaction was then heated to 35 °C for 24 hours. EtOAc and brine were then added, and the layers were separated. The aqueous layer was extracted three times with EtOAc. The combined organic layers

were dried over $MgSO_4$, filtered, and concentrated. Silica gel column chromatography (1:1 Hex/EtOAc to 100% EtOAc) afforded **24** as a white solid (73.7 mg, 30%).

^{1}H NMR (500 MHz, Chloroform-*d*) δ 5.83 (d, *J* = 8.3 Hz, 1H), 5.76 (d, *J* = 5.4 Hz, 1H), 5.67 (d, *J* = 5.4 Hz, 1H), 5.17 – 5.11 (m, 1H), 4.33 – 4.29 (m, 1H), 3.73 – 3.70 (m, 1H), 3.67 – 3.55 (m, 4H), 3.05 – 3.00 (m, 1H), 2.48 – 2.41 (m, 2H), 2.33 – 2.26 (m, 1H), 2.22 – 2.08 (m, 8H), 1.95 (s, 3H), 1.86 – 1.70 (m, 5H), 1.60 – 1.52 (m, 3H), 1.50 – 1.45 (m, 1H), 1.34 (s, 3H), 1.27 (d, *J* = 14.3 Hz, 1H), 1.18 (s, 9H), 1.14 – 1.06 (m, 2H), 0.95 (s, 3H), 0.90 – 0.87 (m, 6H). (58 non-exchangeable protons)

^{13}C NMR (125 MHz, Chloroform-*d*) δ 177.16, 170.37, 168.23, 151.45, 135.64, 129.12, 121.66, 79.92, 74.42, 71.48, 69.69, 68.97, 68.38, 49.39, 48.94, 44.56, 39.62, 39.13, 38.91, 37.20, 37.06, 36.36, 36.27, 35.67, 32.53, 30.44, 30.10, 29.89, 29.00, 27.23, 27.05(3C), 24.33, 22.95, 20.97, 20.90, 18.11, 16.11.

HRMS(ESI): m/z calc. for $C_{39}H_{60}O_9Na$ $[M+Na]^+$: 695.4135, found: 695.4134.



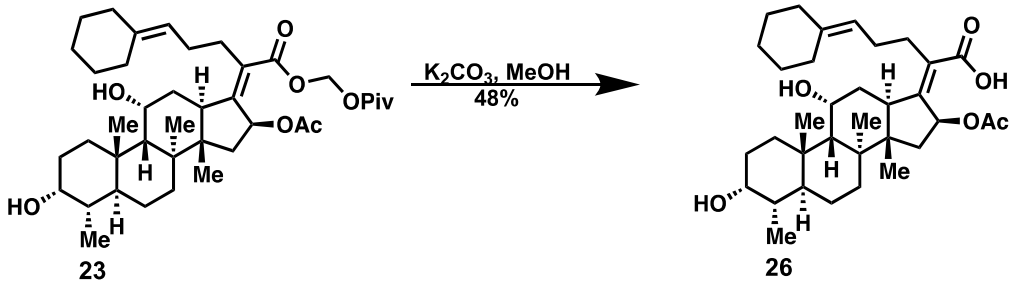
(*Z*)-2-((3*R*,4*S*,5*S*,8*S*,9*S*,10*S*,11*R*,13*R*,14*S*,16*S*)-16-acetoxy-3,11-dihydroxy-4,8,10,14-tetramethylhexadecahydro-17*H*-cyclopenta[*a*]phenanthren-17-ylidene)-5-cyclopentylidenepentanoic acid (25)

Procedure: In a 5 mL vial, **22** (139 mg, 0.21 mmol, 1.0 eq.) was dissolved in MeOH (2.42 mL). K_2CO_3 (58.7 mg, 0.42 mmol, 2.0 eq.) was added to the reaction. The reaction was then stirred at room temperature for 1 hr. The reaction was then filtered and concentrated. The resulting crude product was then purified by preparatory HPLC (C18 20x250mm, 10–100% ACN in H_2O) to afford **25** (110 mg, 95%) as a white solid.

^{1}H NMR (500 MHz, Methanol-d_4) δ 5.77 (d, *J* = 8.3 Hz, 1H), 5.30 – 5.23 (m, 1H), 4.31 – 4.28 (m, 1H), 3.67 – 3.63 (m, 1H), 3.03 – 2.97 (m, 1H), 2.59 – 2.52 (m, 1H), 2.37 – 2.25 (m, 3H), 2.24 – 2.17 (m, 4H), 2.17 – 2.07 (m, 4H), 2.00 (s, 3H), 1.87 – 1.69 (m, 4H), 1.68 – 1.56 (m, 6H), 1.56 – 1.46 (m, 2H), 1.38 (s, 3H), 1.19 (d, *J* = 14.3 Hz, 1H), 1.16 – 1.09 (m, 2H), 0.99 (s, 3H), 0.95 (s, 3H), 0.89 (d, *J* = 6.8 Hz, 3H). (47 non-exchangeable protons)

^{13}C NMR (125 MHz, Methanol-d_4) δ 179.70, 173.54, 144.12, 139.81, 137.90, 121.06, 76.08, 72.62, 68.96, 50.83, 50.06, 43.75, 40.77, 40.36, 38.33, 37.89, 37.51, 36.92, 34.63, 33.01, 31.11, 31.04, 30.90, 30.71, 29.58, 27.53, 27.49, 23.89, 23.86, 22.55, 21.23, 17.96, 16.58.

HRMS(ESI): m/z calc. for $C_{33}H_{50}O_6Na$ $[M+Na]^+$: 565.3505, found: 565.3503.



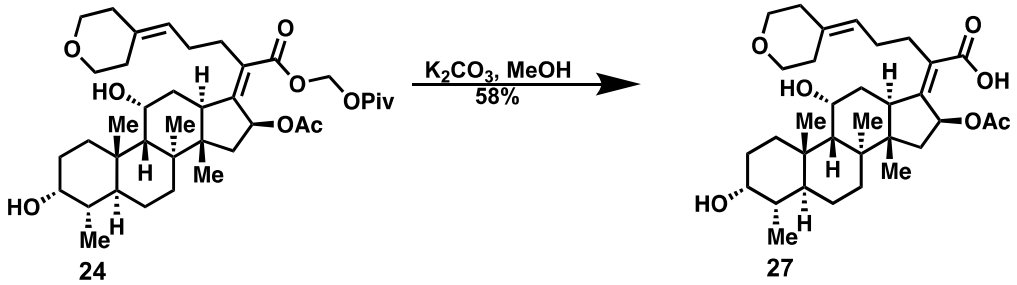
(*Z*)-2-((3*R*,4*S*,5*S*,8*S*,9*S*,10*S*,11*R*,13*R*,14*S*,16*S*)-16-acetoxy-3,11-dihydroxy-4,8,10,14-tetramethylhexadecahydro-17*H*-cyclopenta[*a*]phenanthren-17-ylidene)-5-cyclohexylidenepentanoic acid (26)

Procedure: In a 5 mL vial, **23** (75.5 mg, 0.11 mmol, 1.0 eq.) was dissolved in MeOH (1.28 mL). K_2CO_3 (31.1 mg, 0.22 mmol, 2.0 eq.) was added to the reaction. The reaction was then stirred at room temperature for 1 hr. The reaction was then filtered and concentrated. The resulting crude product was then purified by preparatory HPLC (C18 20x250mm, 10–100% ACN in H_2O) to afford **26** (29.9 mg, 48%) as a white solid.

^{1}H NMR (500 MHz, Methanol-d_4) δ 5.76 (d, *J* = 8.3 Hz, 1H), 5.13 – 5.08 (m, 1H), 4.31 – 4.28 (m, 1H), 3.66 – 3.64 (m, 1H), 3.03 – 2.97 (m, 1H), 2.54 – 2.48 (m, 1H), 2.33 – 2.22 (m, 3H), 2.19 – 2.09 (m, 6H), 2.08 – 2.04 (m, 2H), 2.00 (s, 3H), 1.85 – 1.67 (m, 4H), 1.65 – 1.59 (m, 2H), 1.57 – 1.47 (m, 8H), 1.38 (s, 3H), 1.19 (d, *J* = 14.0 Hz, 1H), 1.16 – 1.09 (m, 2H), 0.99 (s, 3H), 0.95 (s, 3H), 0.89 (d, *J* = 6.8 Hz, 3H). (49 non-exchangeable protons)

^{13}C NMR (125 MHz, Methanol-d_4) δ 179.67, 173.50, 140.74, 139.47, 138.11, 122.24, 76.06, 72.63, 68.96, 50.80, 50.05, 43.73, 40.75, 40.32, 38.34, 38.32, 37.86, 37.45, 36.86, 32.98, 31.55, 31.09, 31.01, 29.94, 29.83, 29.13, 28.38, 28.19, 23.92, 23.86, 22.56, 21.21, 17.93, 16.61.

HRMS(ESI): m/z calc. for $C_{34}H_{52}O_6Na$ $[M+Na]^+$: 579.3662, found: 579.3660.



(*Z*)-2-((3*R*,4*S*,5*S*,8*S*,9*S*,10*S*,11*R*,13*R*,14*S*,16*S*)-16-acetoxy-3,11-dihydroxy-4,8,10,14-tetramethylhexadecahydro-17*H*-cyclopenta[*a*]phenanthren-17-ylidene)-5-(tetrahydro-4*H*-pyran-4-ylidene)pentanoic acid (27)

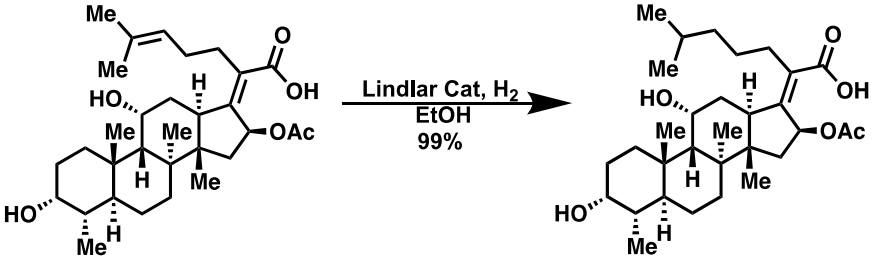
Procedure: In a 5 mL vial, **24** (75.1 mg, 0.11 mmol, 1.0 eq.) was dissolved in MeOH (1.27 mL). K_2CO_3 (30.8 mg, 0.22 mmol, 2.0 eq.) was added to the reaction. The reaction was then stirred at room temperature for 1 hr. The reaction was then filtered and concentrated. The resulting crude product was then purified by preparatory HPLC (C18 20x250mm, 10–100% ACN in H_2O) to afford **27** (36.0 mg, 58%) as a white solid.

^{1}H NMR (500 MHz, Methanol-d_4) δ 5.78 (d, *J* = 8.3 Hz, 1H), 5.30 – 5.24 (m, 1H), 4.30 – 4.28 (m, 1H), 3.67 – 3.61 (m, 5H), 3.03 – 2.97 (m, 1H), 2.58 – 2.50 (m, 1H), 2.36 – 2.26 (m, 5H), 2.20 – 2.07 (m, 6H),

2.00 (s, 3H), 1.87 – 1.65 (m, 4H), 1.65 – 1.60 (m, 2H), 1.55 – 1.51 (m, 1H), 1.50 – 1.46 (m, 1H), 1.38 (s, 3H), 1.19 (d, *J* = 14.3 Hz, 1H), 1.16 – 1.10 (m, 2H), 0.99 (s, 3H), 0.95 (s, 3H), 0.89 (d, *J* = 6.8 Hz, 3H). (47 non-exchangeable protons)

^{13}C NMR (125 MHz, Methanol-*d*$_4$) δ 179.54, 173.51, 139.42, 138.28, 135.42, 124.42, 76.05, 72.60, 70.84, 70.10, 68.94, 50.83, 50.09, 43.79, 40.78, 40.33, 38.31, 38.03, 37.89, 37.51, 36.92, 33.01, 31.27, 31.11, 31.05, 30.82, 28.09, 23.88(2C), 22.53, 21.22, 17.97, 16.59.

HRMS(ESI): m/z calc. for $C_{33}H_{50}O_7Na$ $[M+Na]^+$: 581.3454, found: 581.3452.



(*Z*)-2-((3*R*,4*S*,5*S*,8*S*,9*S*,10*S*,11*R*,13*R*,14*S*,16*S*)-16-acetoxy-3,11-dihydroxy-4,8,10,14-tetramethylhexadecahydro-17*H*-cyclopenta[*a*]phenanthren-17-ylidene)-6-methylheptanoic acid (28)

Procedure: In a 25 mL RB Flask, **FA** (200 mg, 0.387 mmol, 1.0 eq.) was dissolved in EtOH (10.0 mL). Then the solution was degassed with nitrogen. Next, 5 % Pd with $CaCO_3$ (179 mg, 0.29 mmol, 0.09 eq.) was added, and the reaction was degassed again with nitrogen. The reaction was stirred under a hydrogen atmosphere for 2 hours. The reaction was then filtered. The resulting crude product was then purified by preparatory HPLC (C18 20x250mm, 10–100% ACN in H_2O) to afford **28** (198 mg, 99%) as a white solid.

^{1}H NMR (500 MHz, Chloroform-*d*) δ 5.89 (d, *J* = 8.3 Hz, 1H), 4.38 – 4.32 (m, 1H), 3.79 – 3.71 (m, 1H), 3.07 – 3.01 (m, 1H), 2.46 – 2.33 (m, 2H), 2.30 – 2.25 (m, 1H), 2.20 – 2.08 (m, 3H), 1.97 (s, 3H), 1.90 – 1.81 (m, 2H), 1.78 – 1.71 (m, 2H), 1.62 – 1.55 (m, 3H), 1.53 – 1.43 (m, 3H), 1.37 (s, 3H), 1.35 – 1.31 (m, 1H), 1.29 (d, *J* = 14.3 Hz, 1H), 1.20 – 1.05 (m, 4H), 0.97 (s, 3H), 0.93 – 0.90 (m, 6H), 0.86 (d, *J* = 6.6 Hz, 6H). (47 non-exchangeable protons)

^{13}C NMR (125 MHz, Chloroform-*d*) δ 174.56, 171.03, 149.45, 130.79, 74.52, 71.64, 68.50, 49.43, 48.91, 44.23, 39.64, 39.16, 38.94, 37.22, 36.43, 36.33, 35.64, 32.52, 30.43, 30.07, 29.08, 28.01, 27.90, 24.29, 23.00, 22.80, 22.76, 20.98, 20.84, 18.04, 16.10.

HRMS(ESI): m/z calc. for $C_{31}H_{50}O_6$ $[M+Na]^+$: 541.3505, found: 541.3514.

10. References

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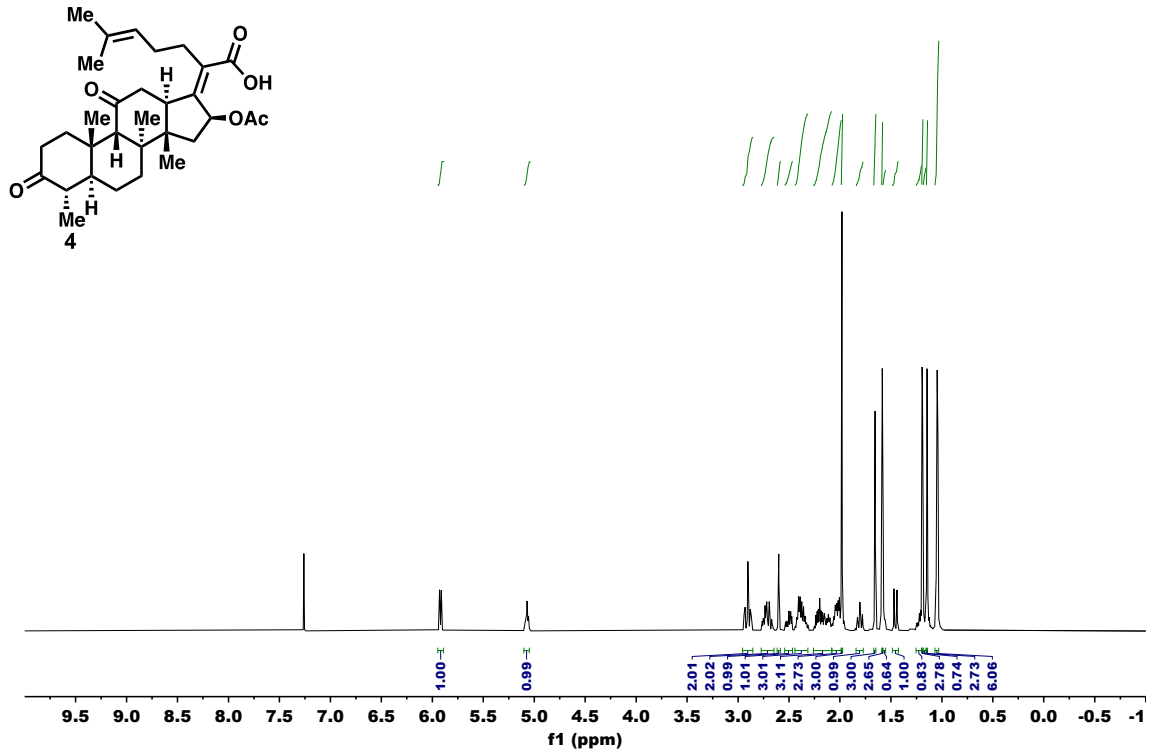
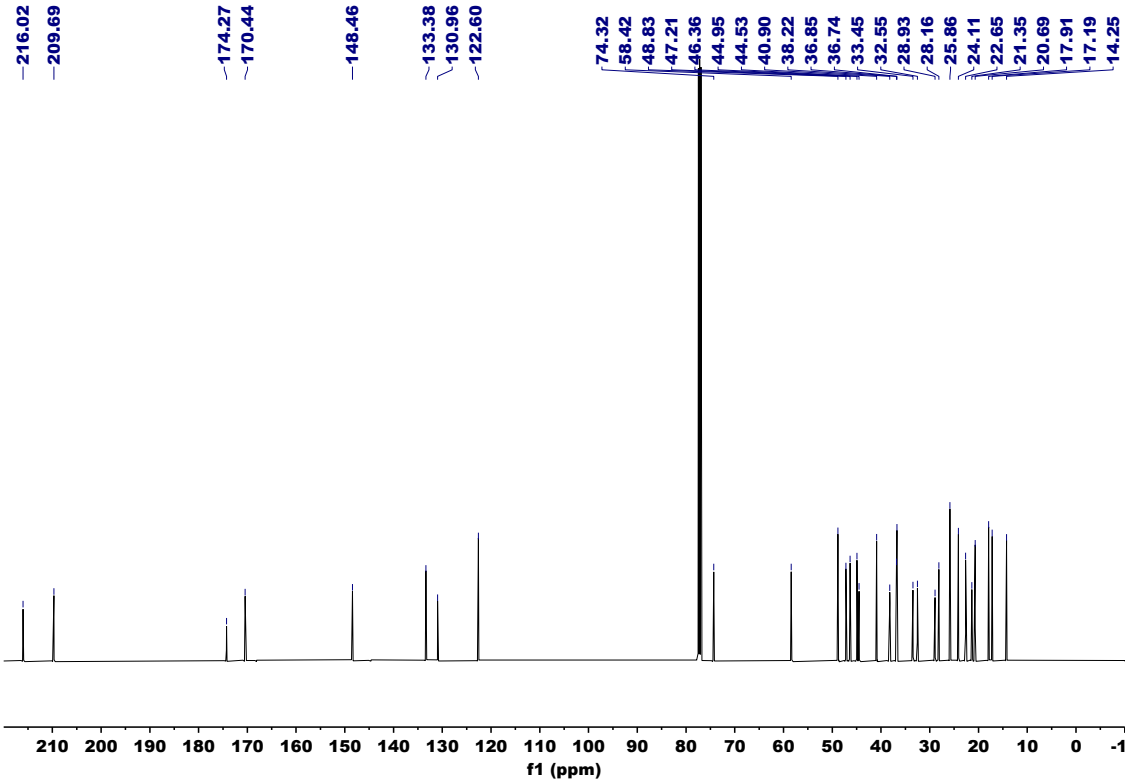
11. ^{1}H and ^{13}C NMR Spectra



^{1}H NMR



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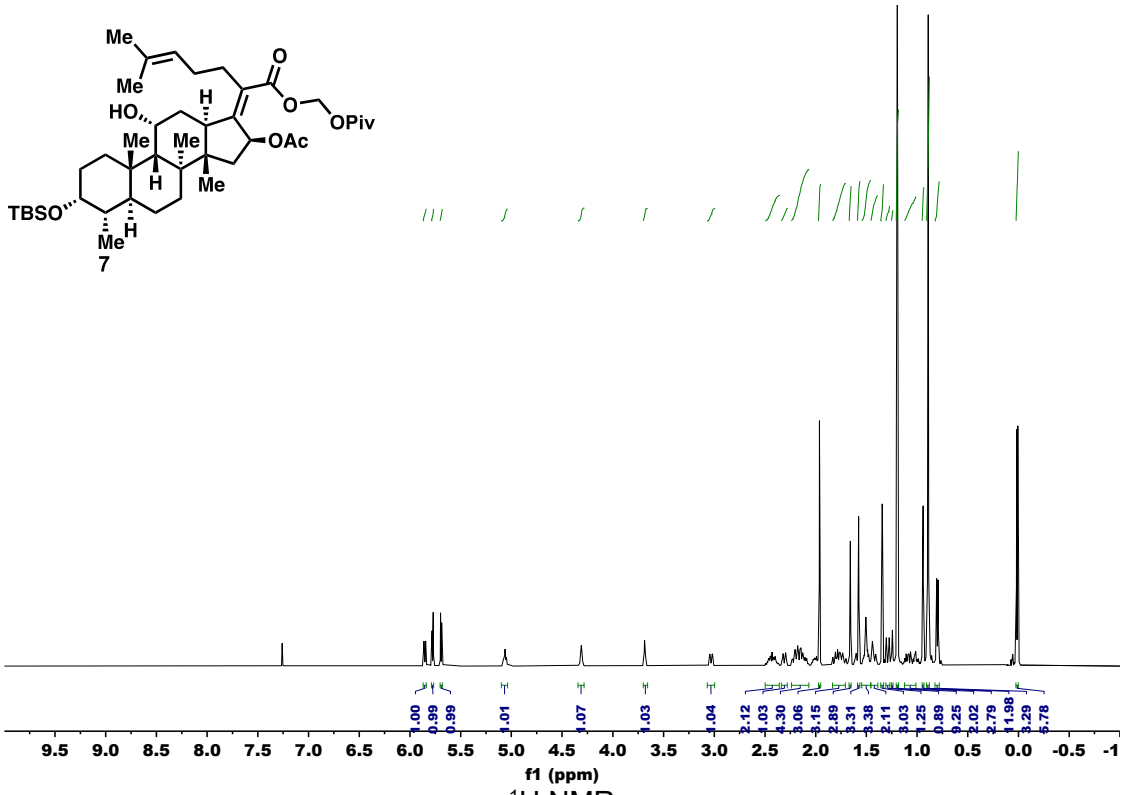

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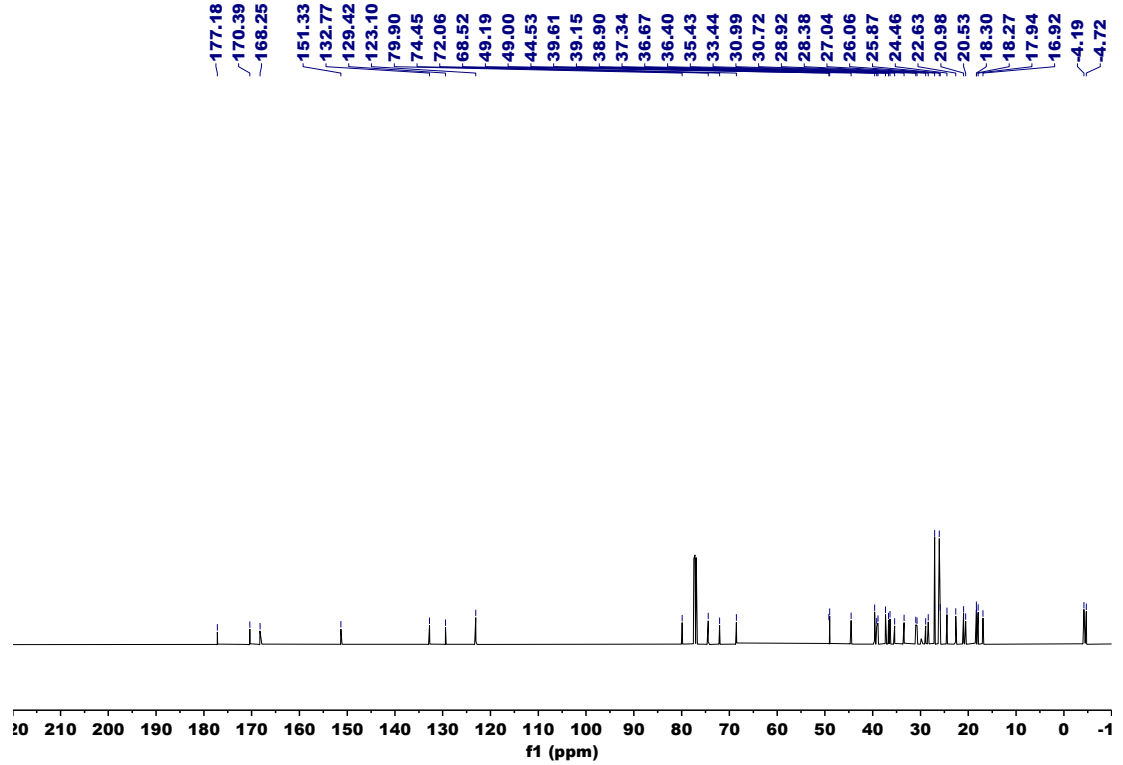

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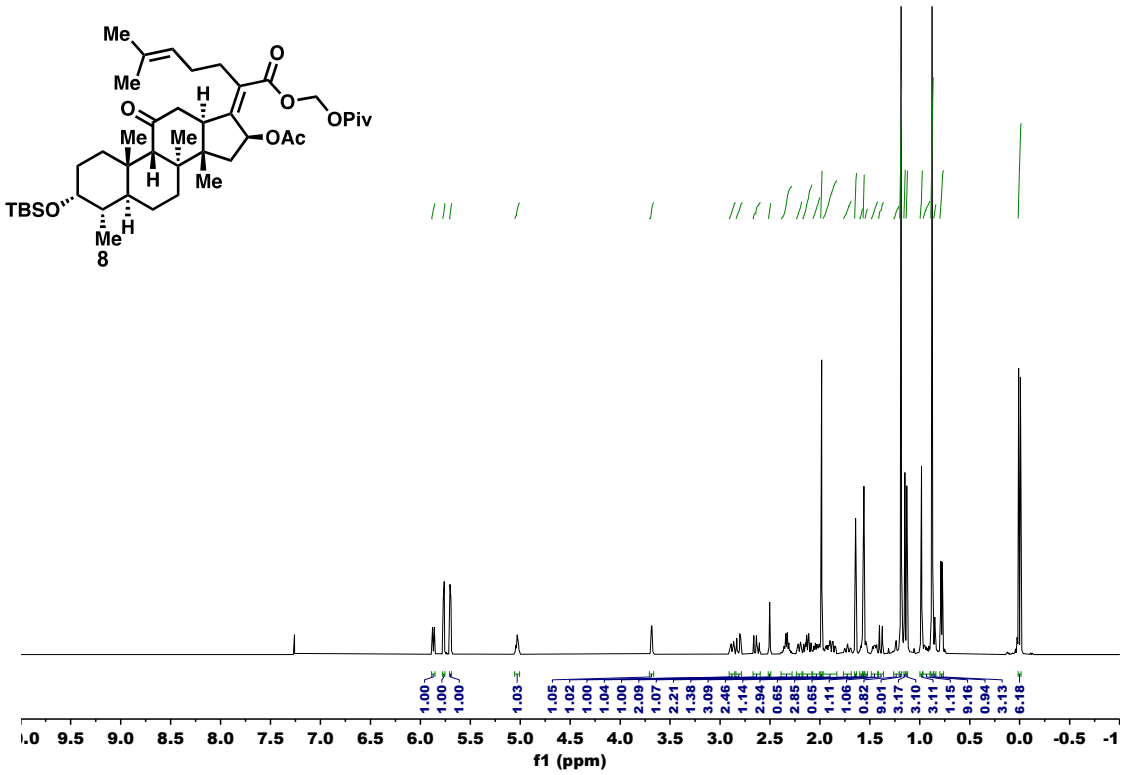

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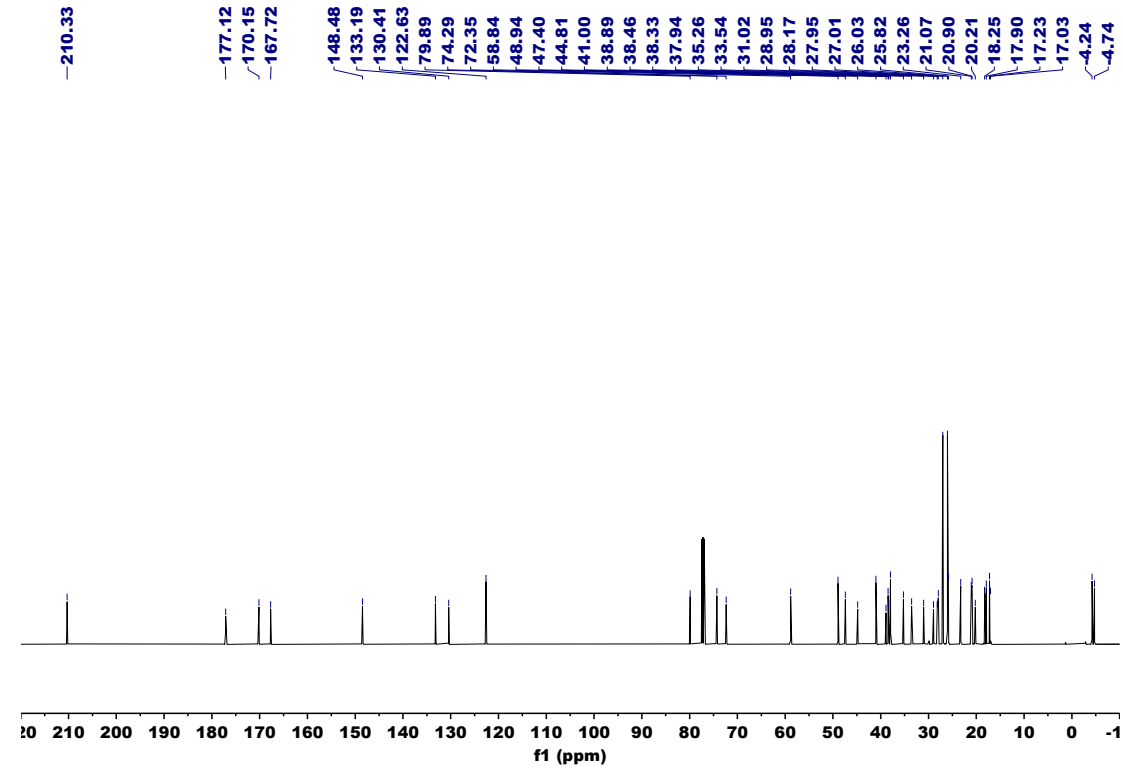

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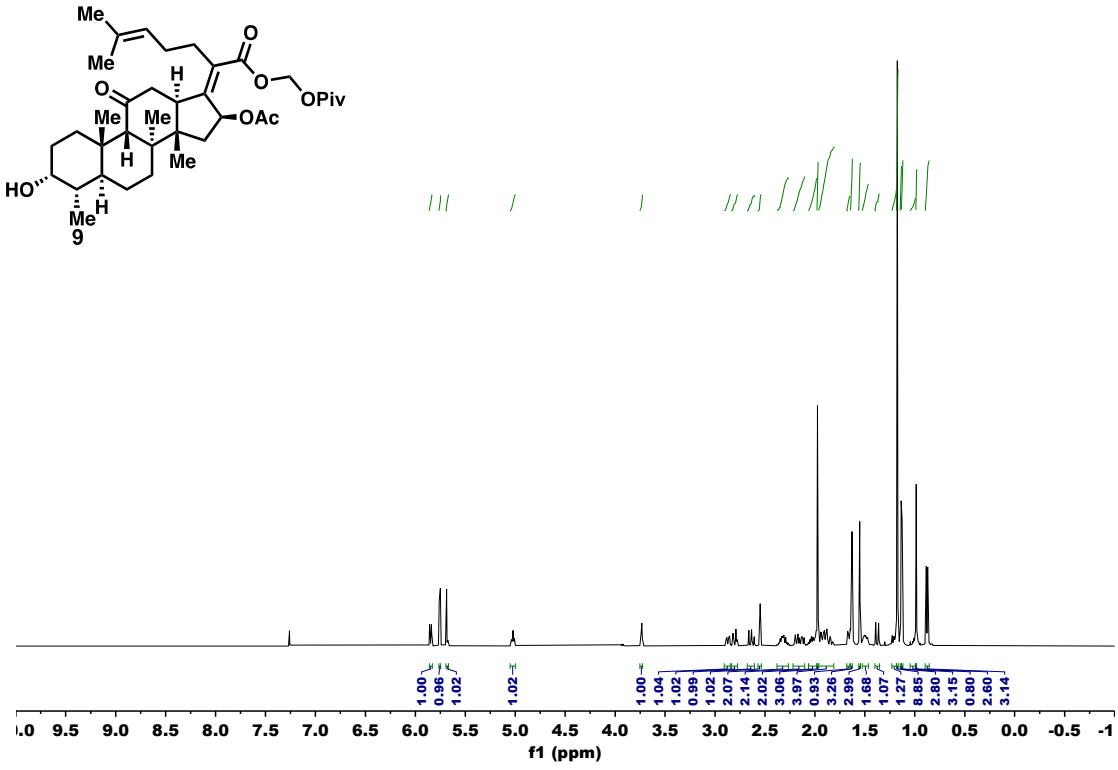

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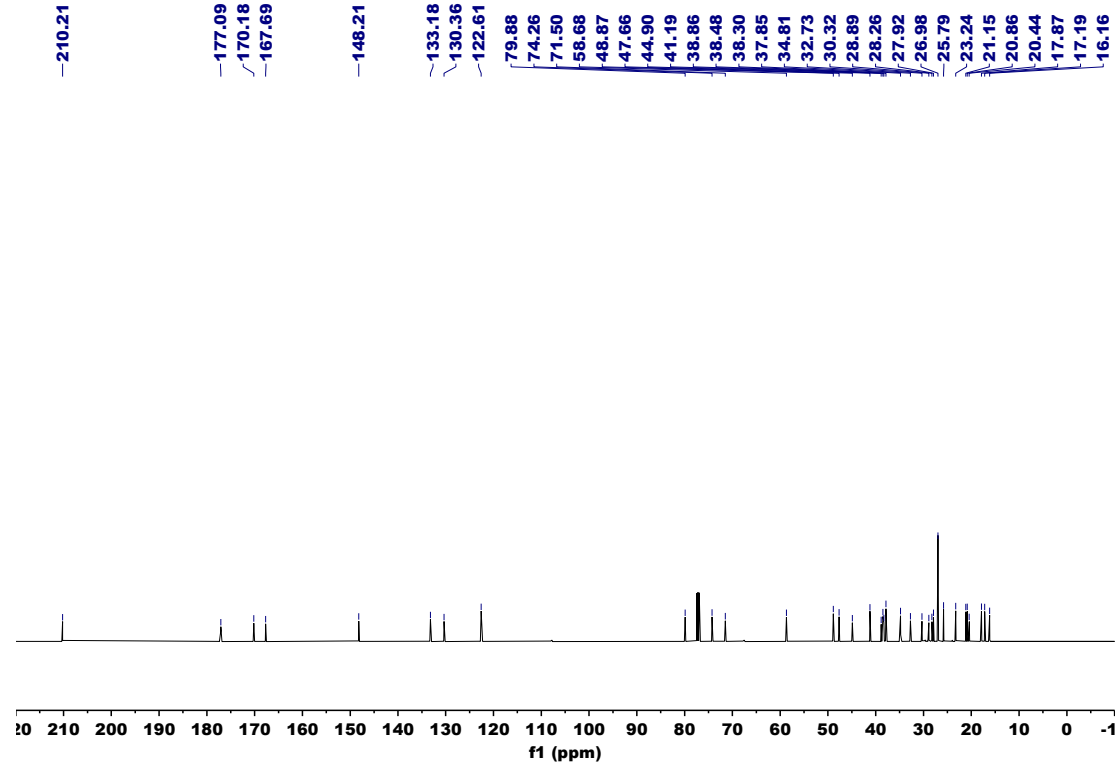

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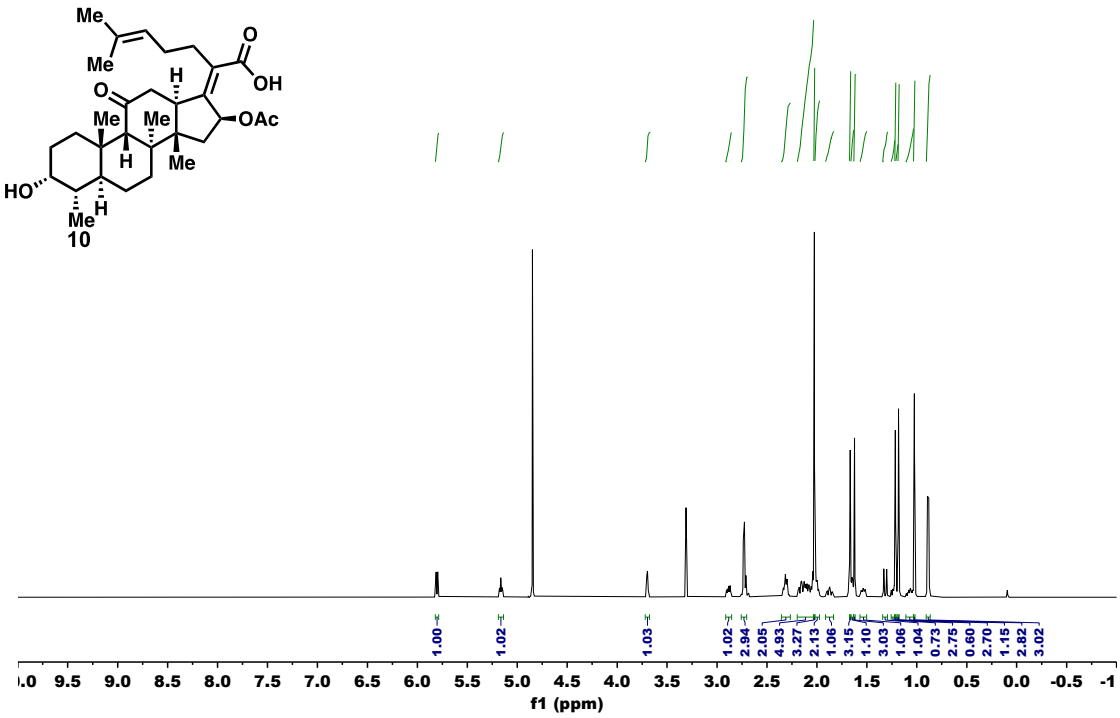

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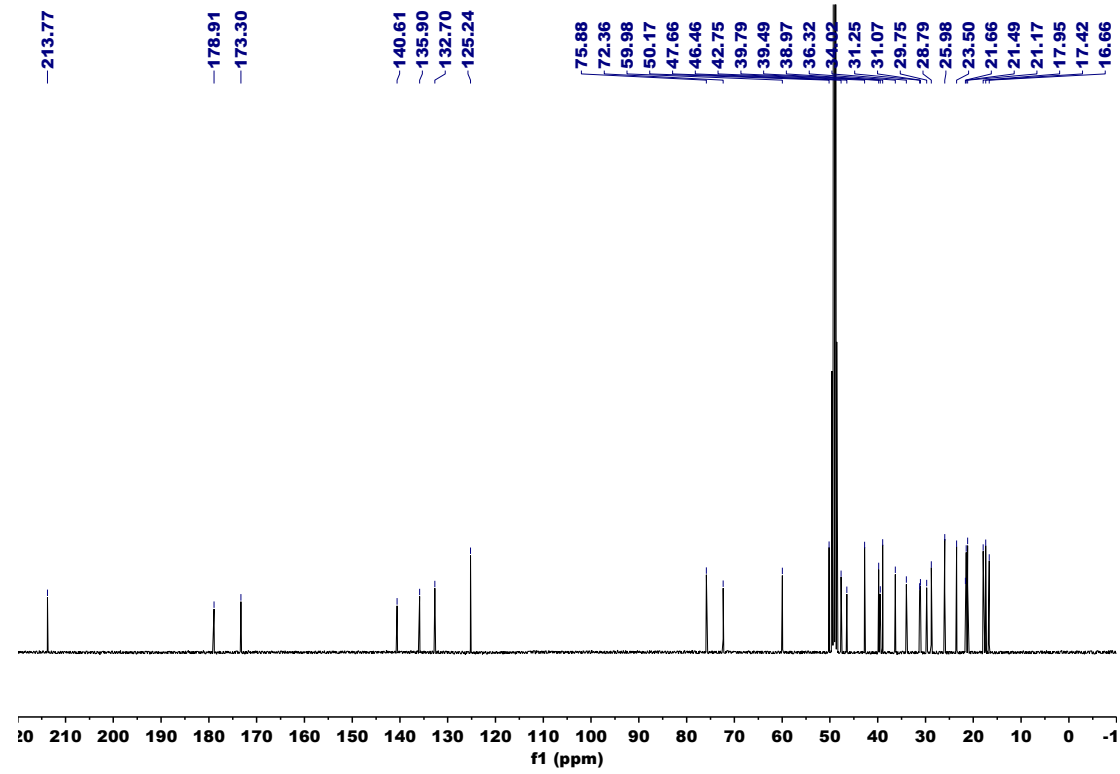

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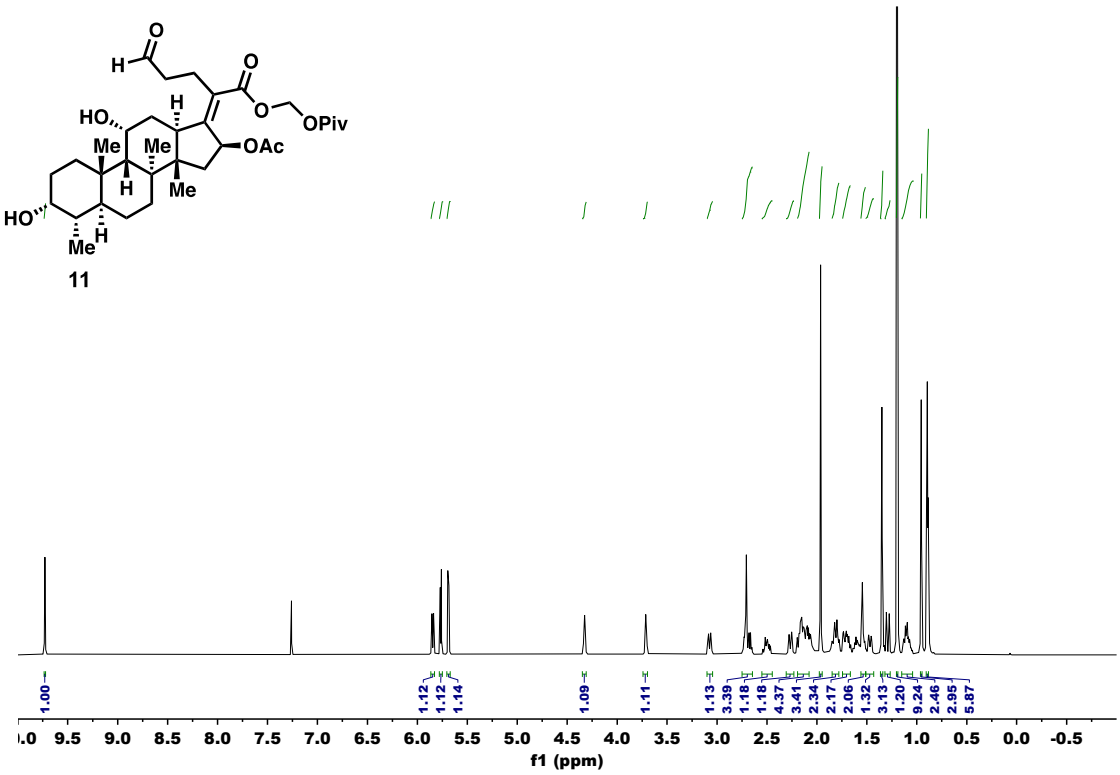

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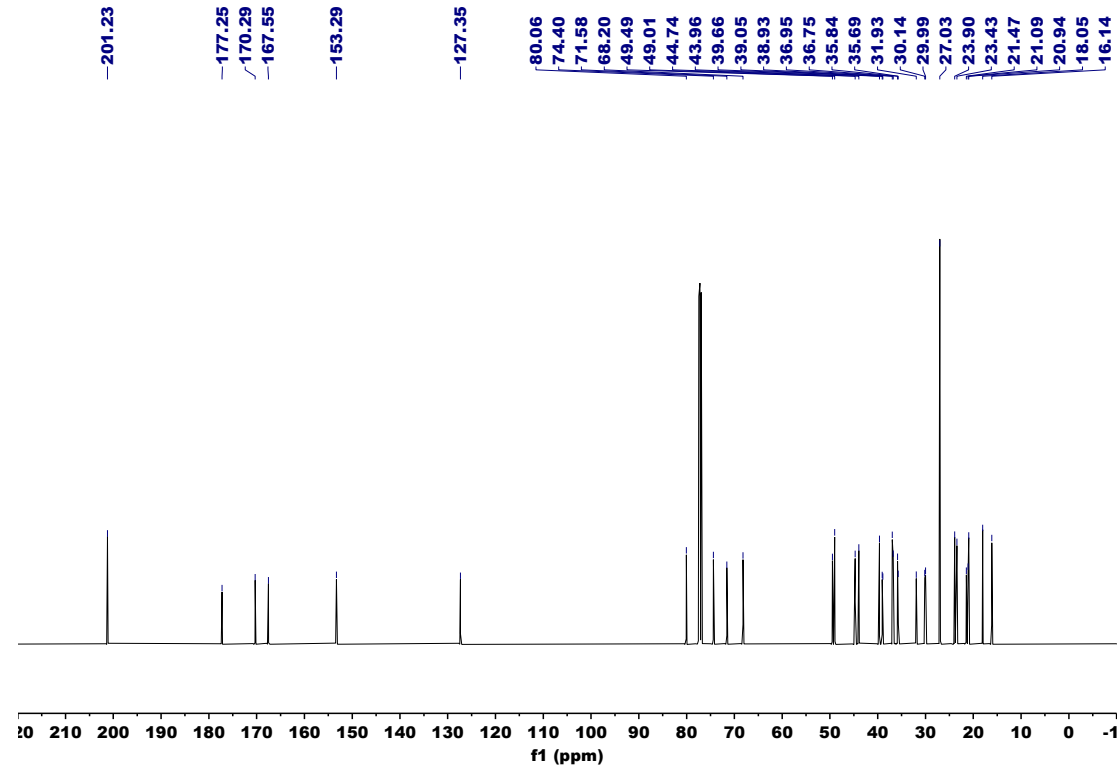

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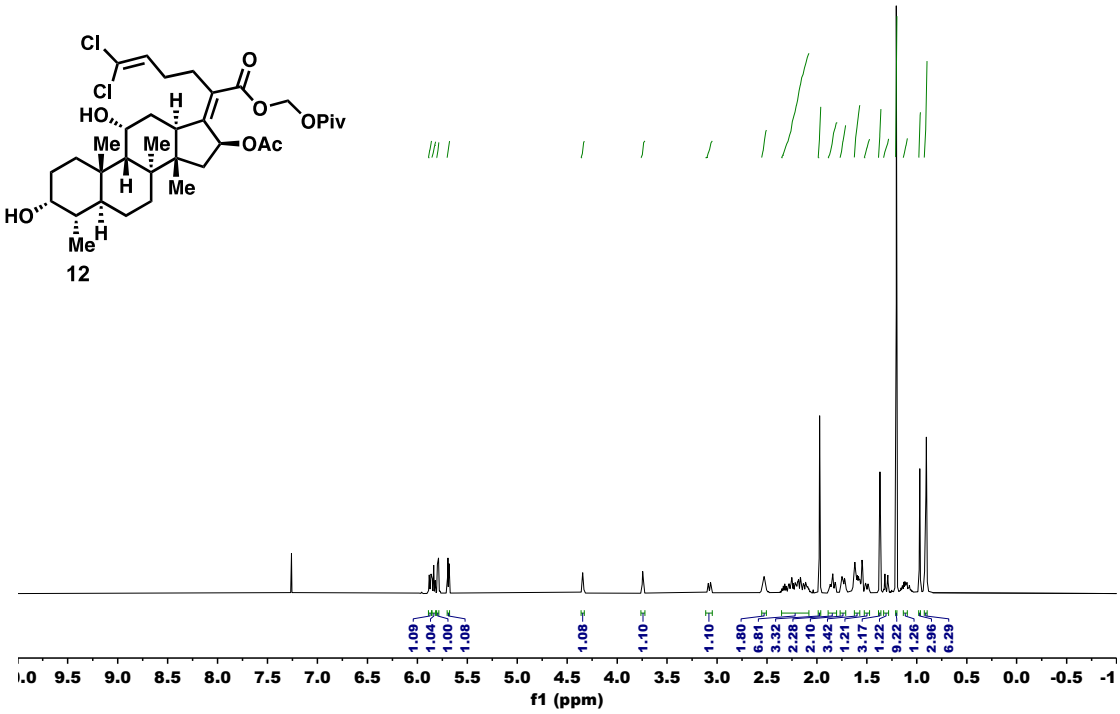

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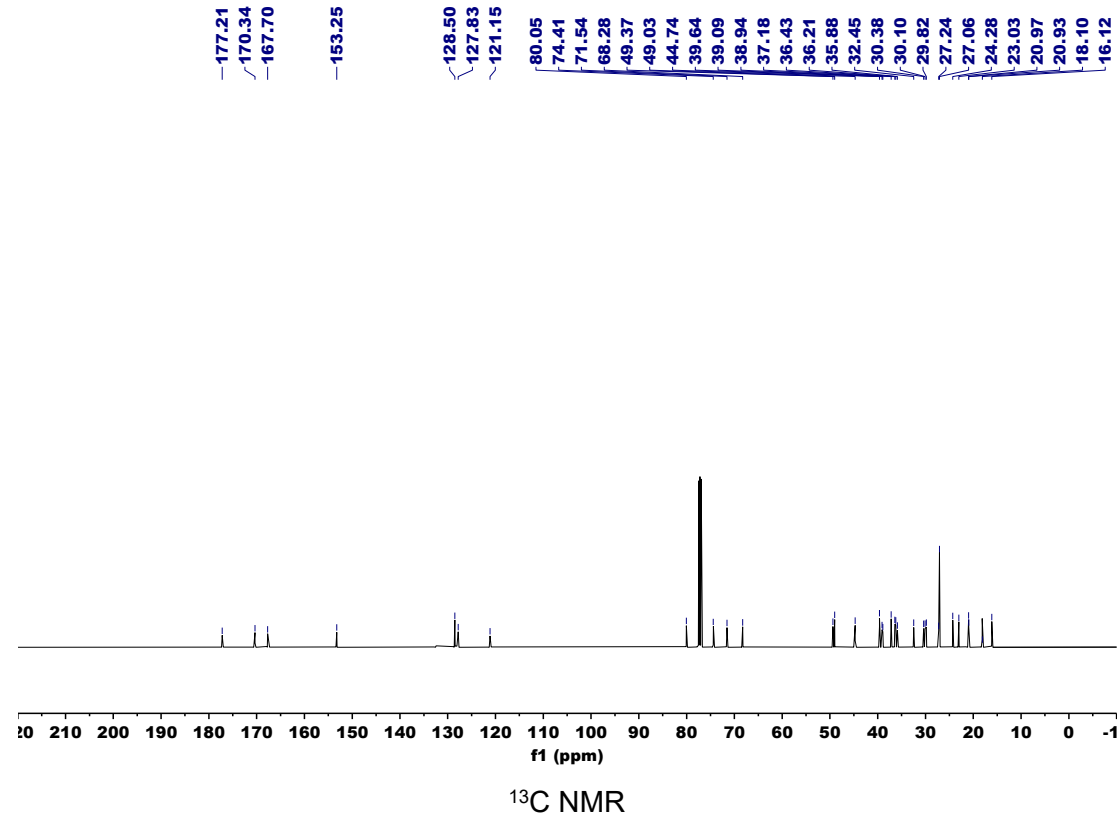

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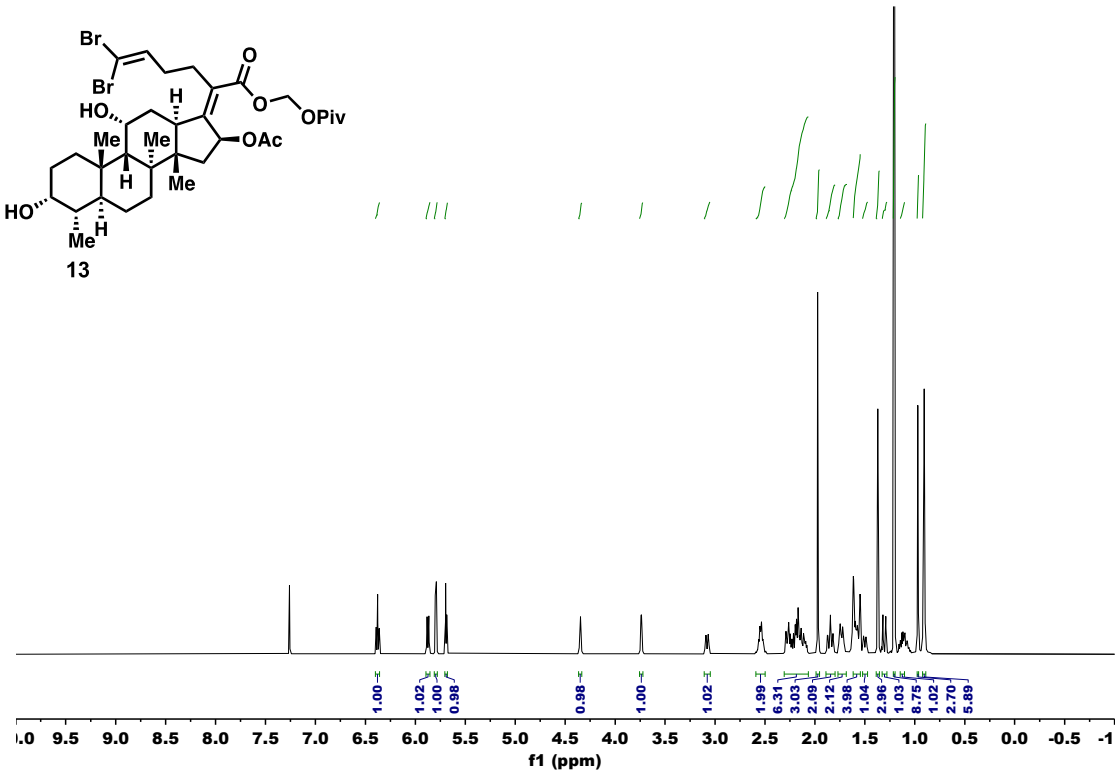

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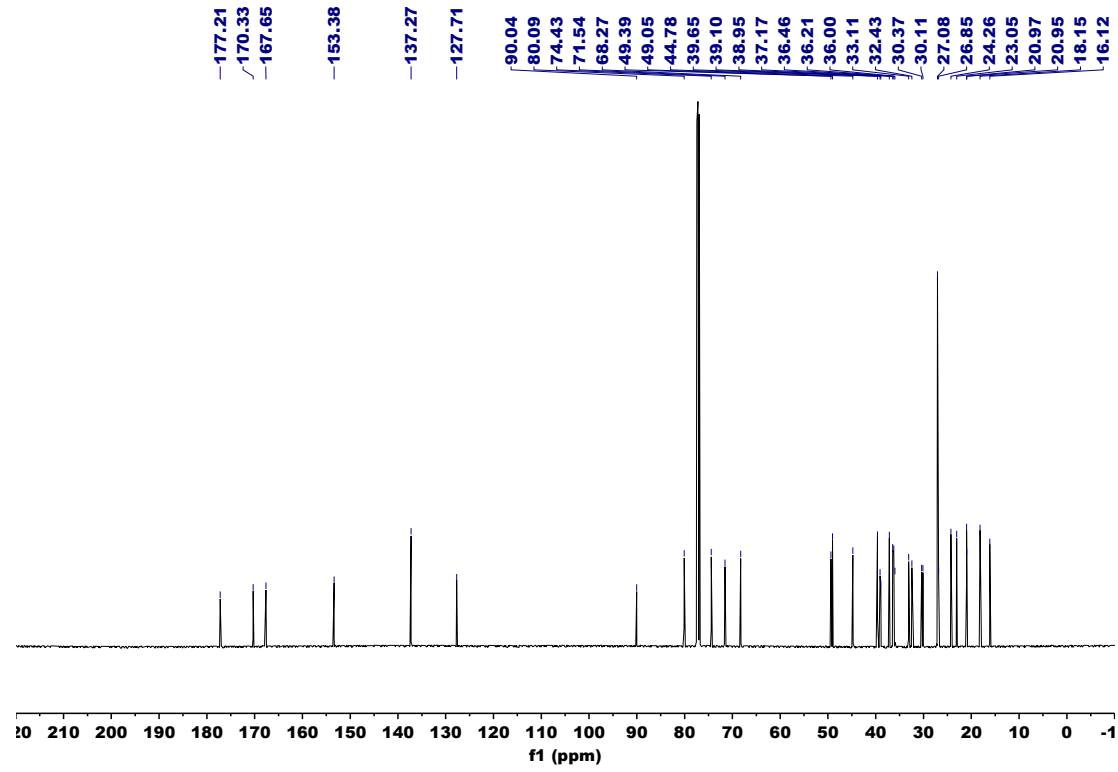

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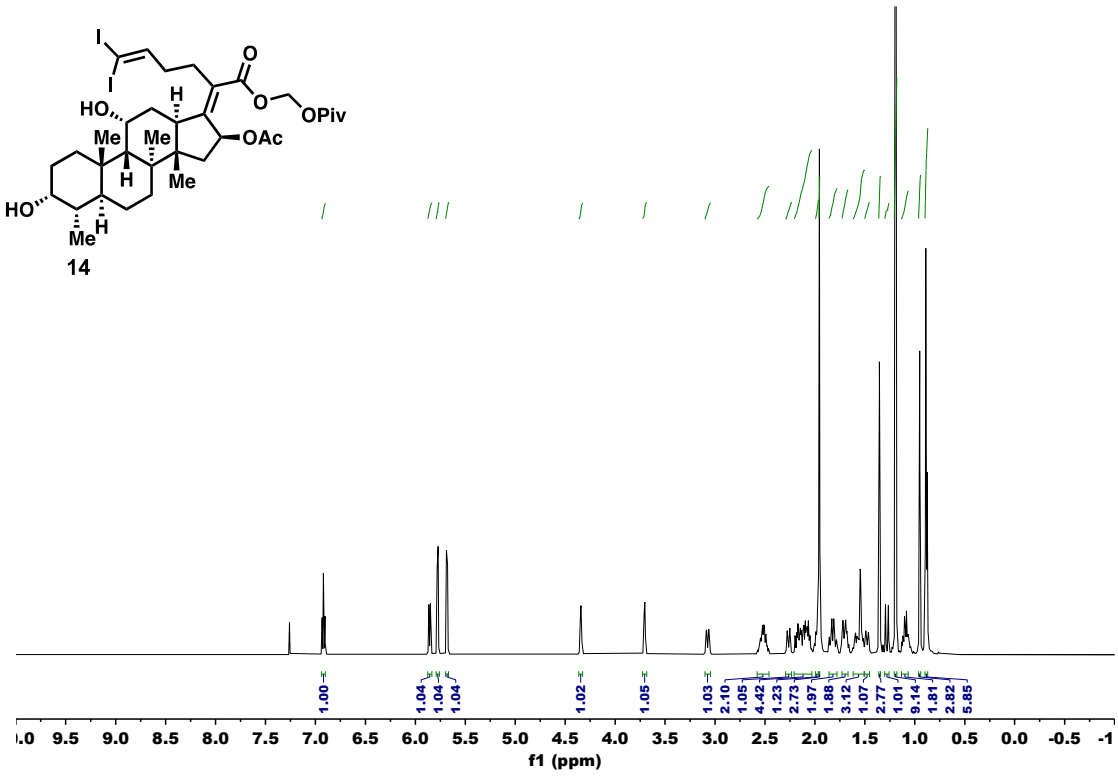
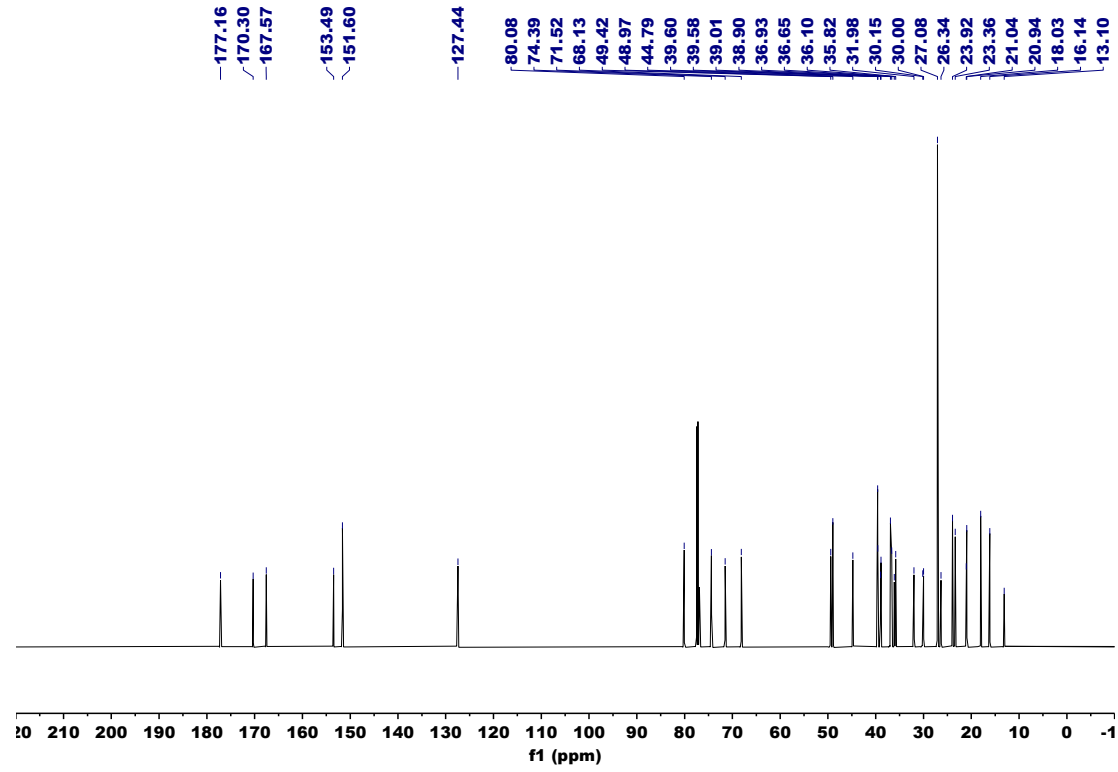
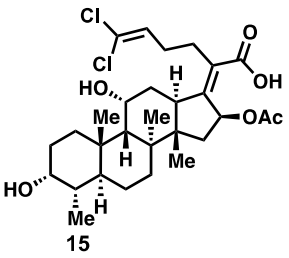


^{1}H NMR



^{13}C NMR



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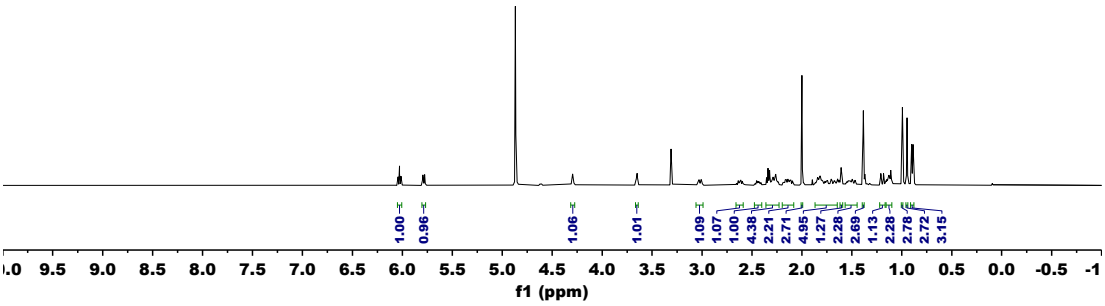

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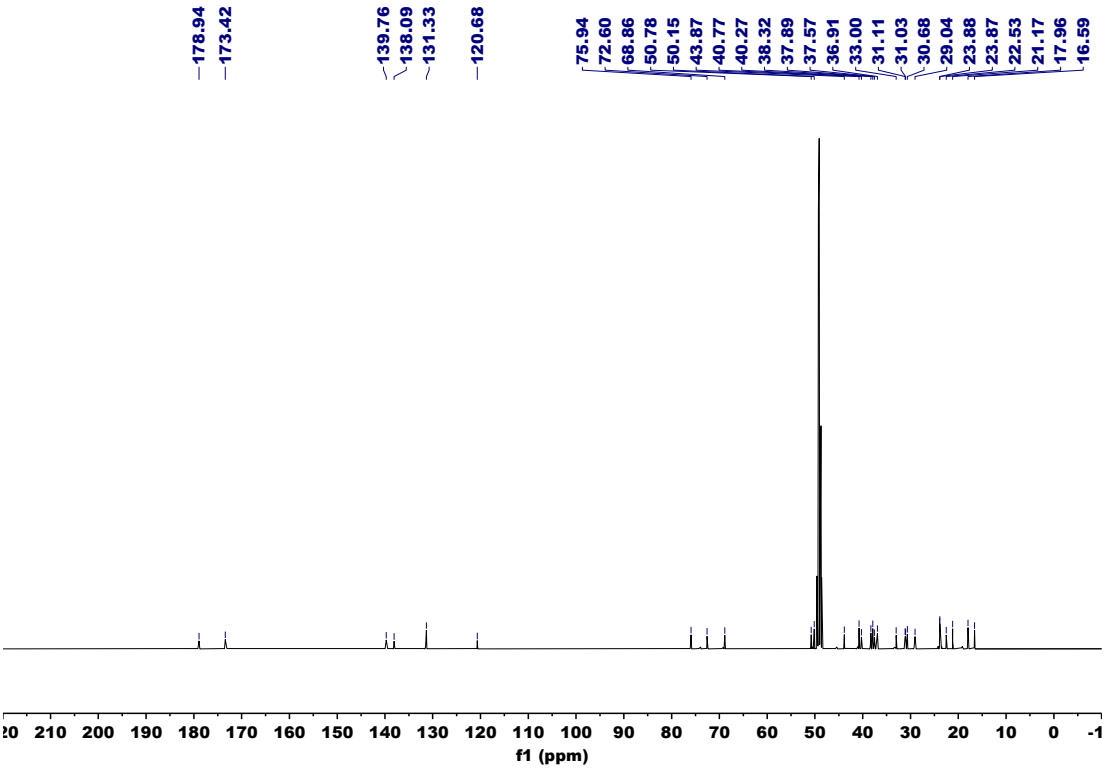

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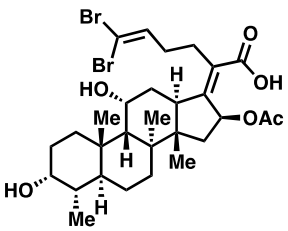

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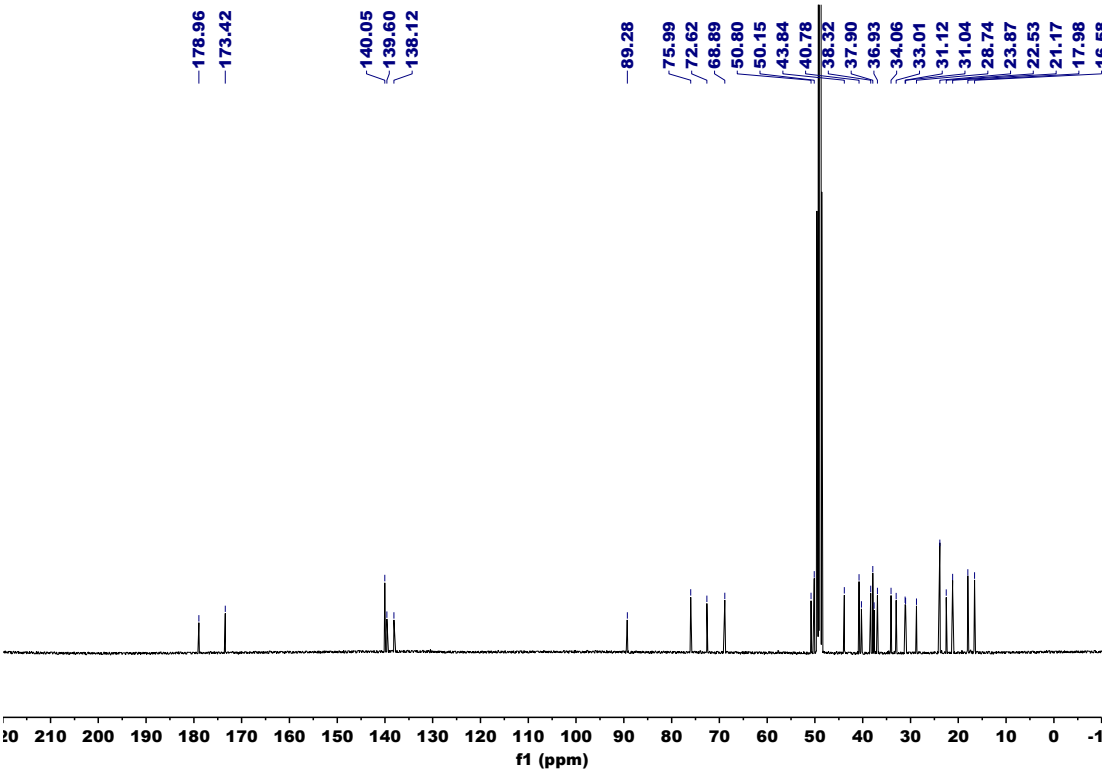

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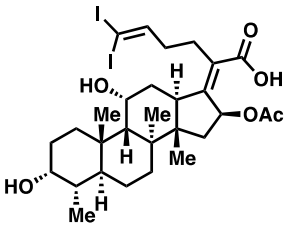

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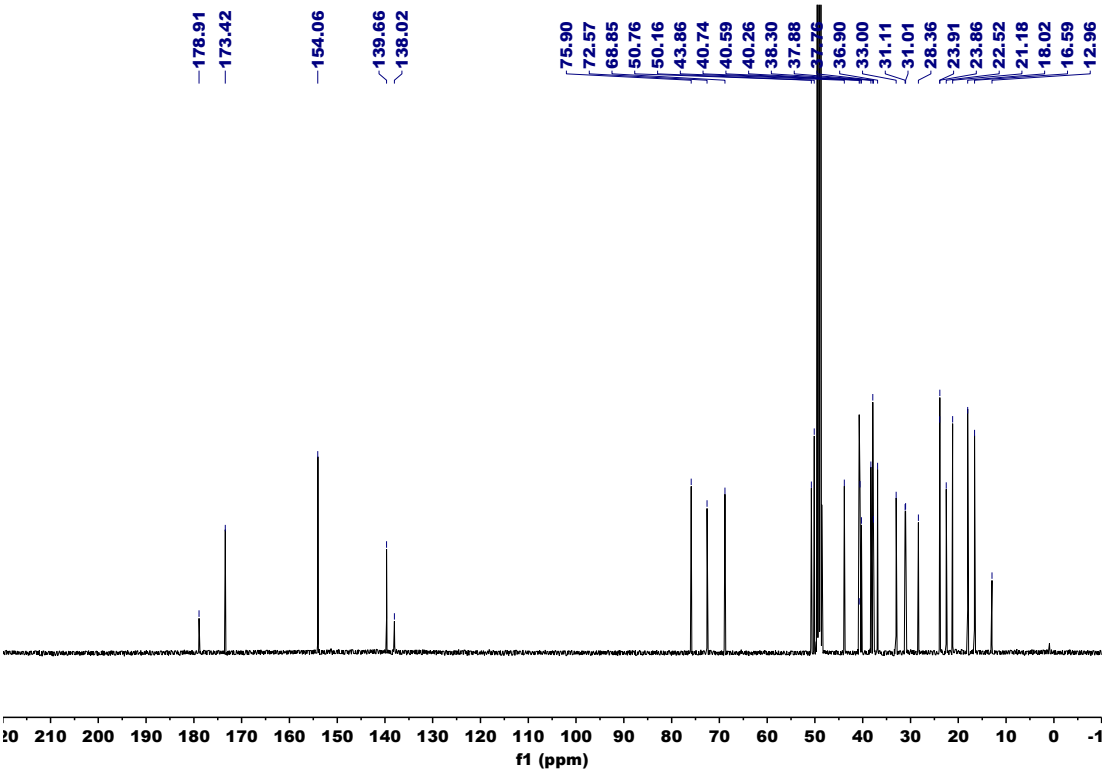

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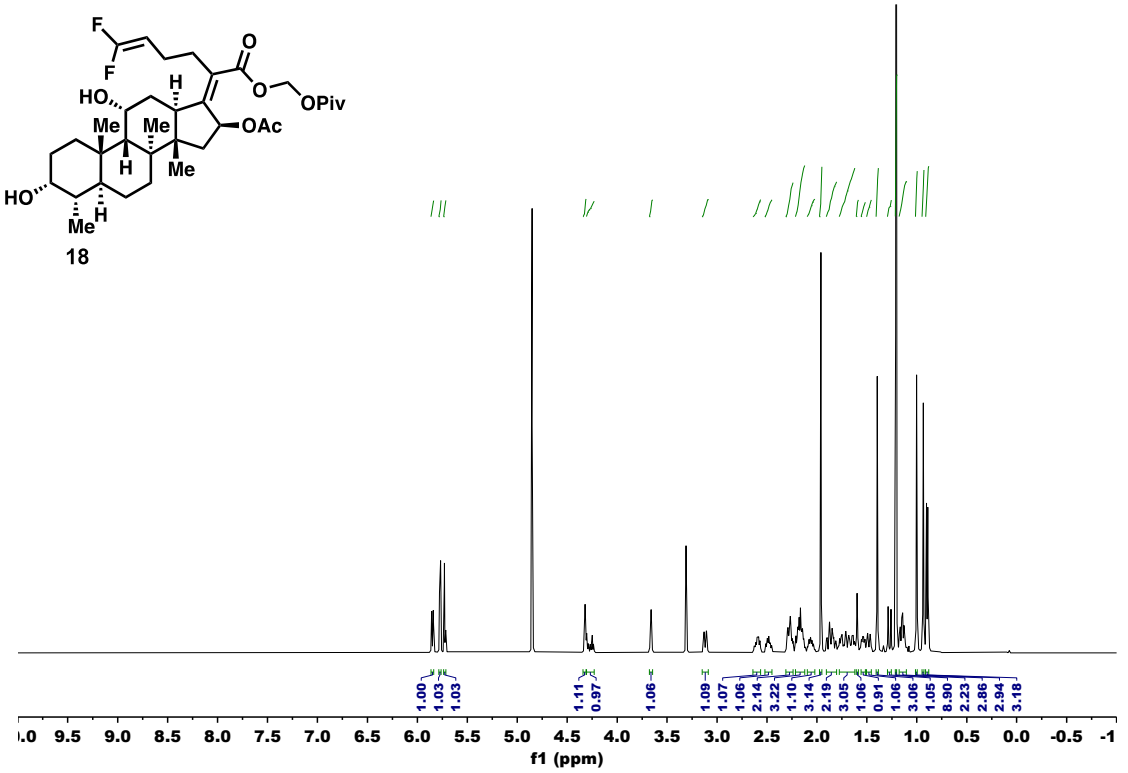

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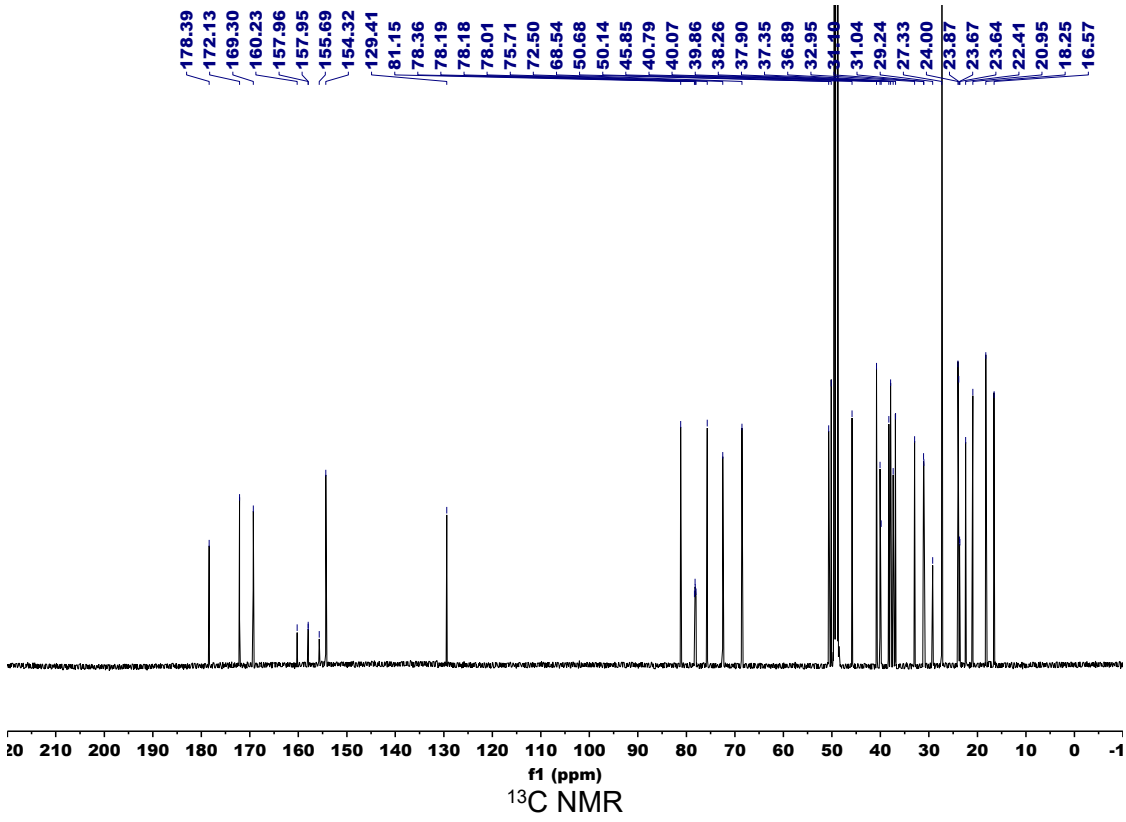

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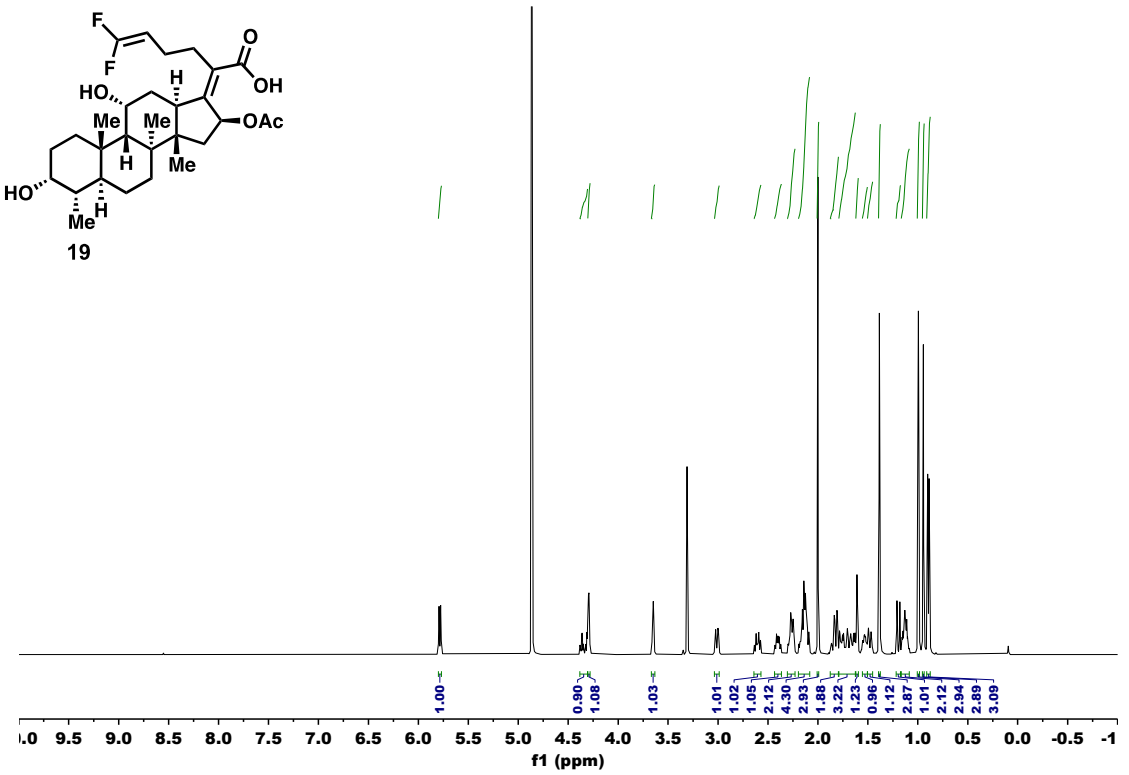

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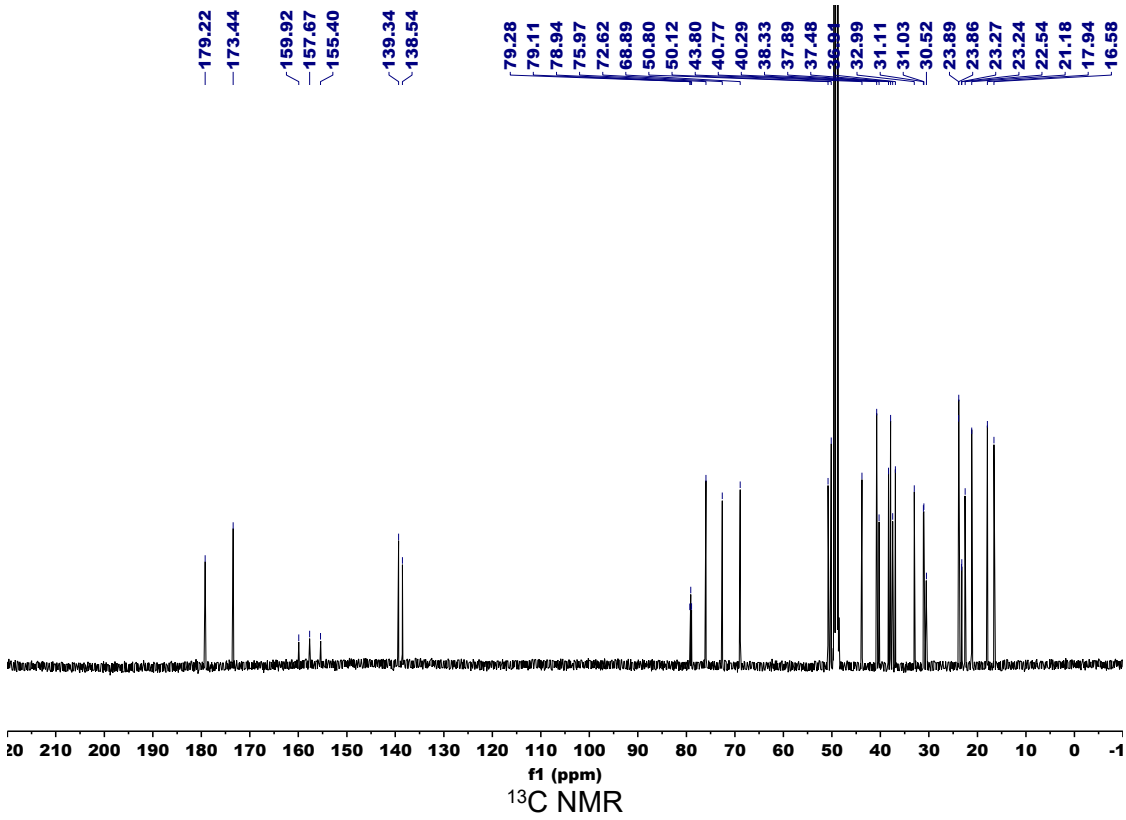

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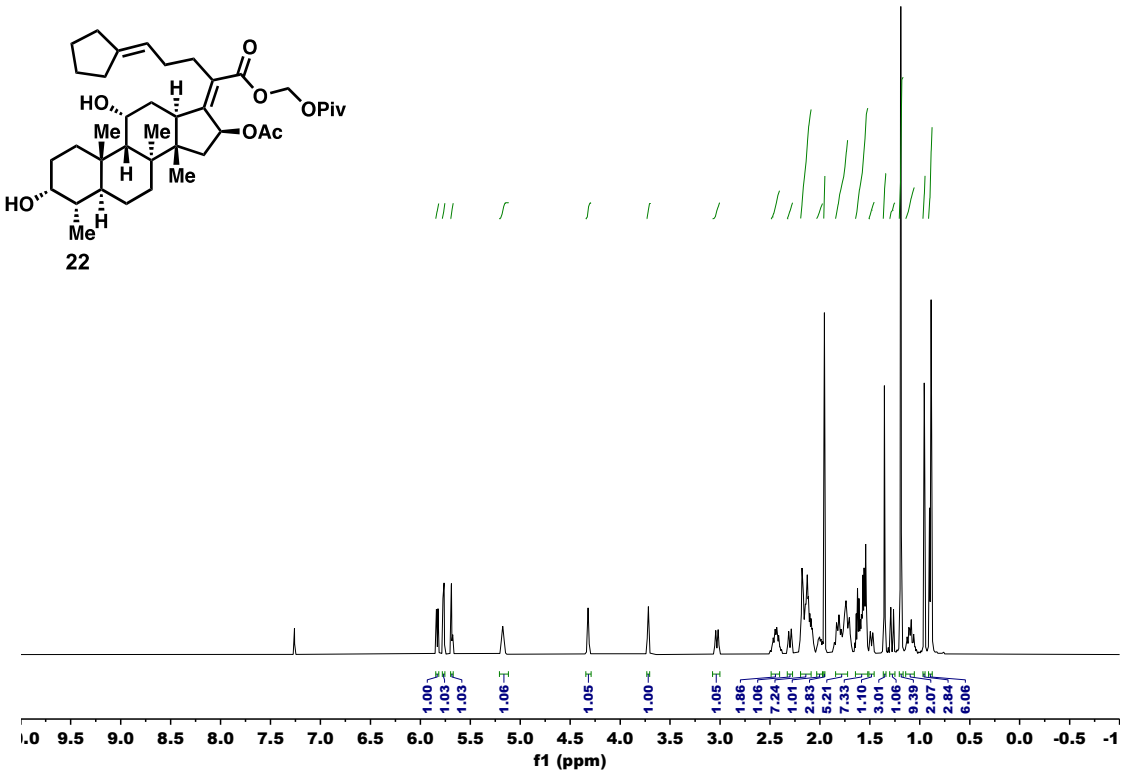

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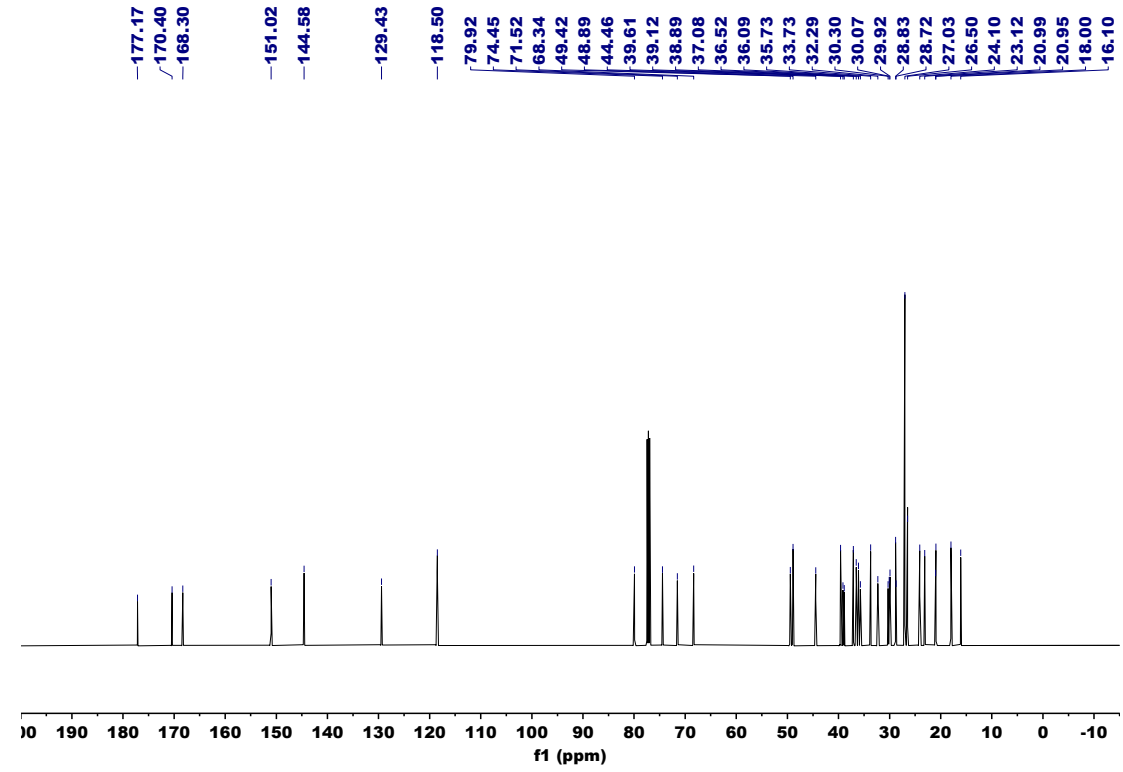

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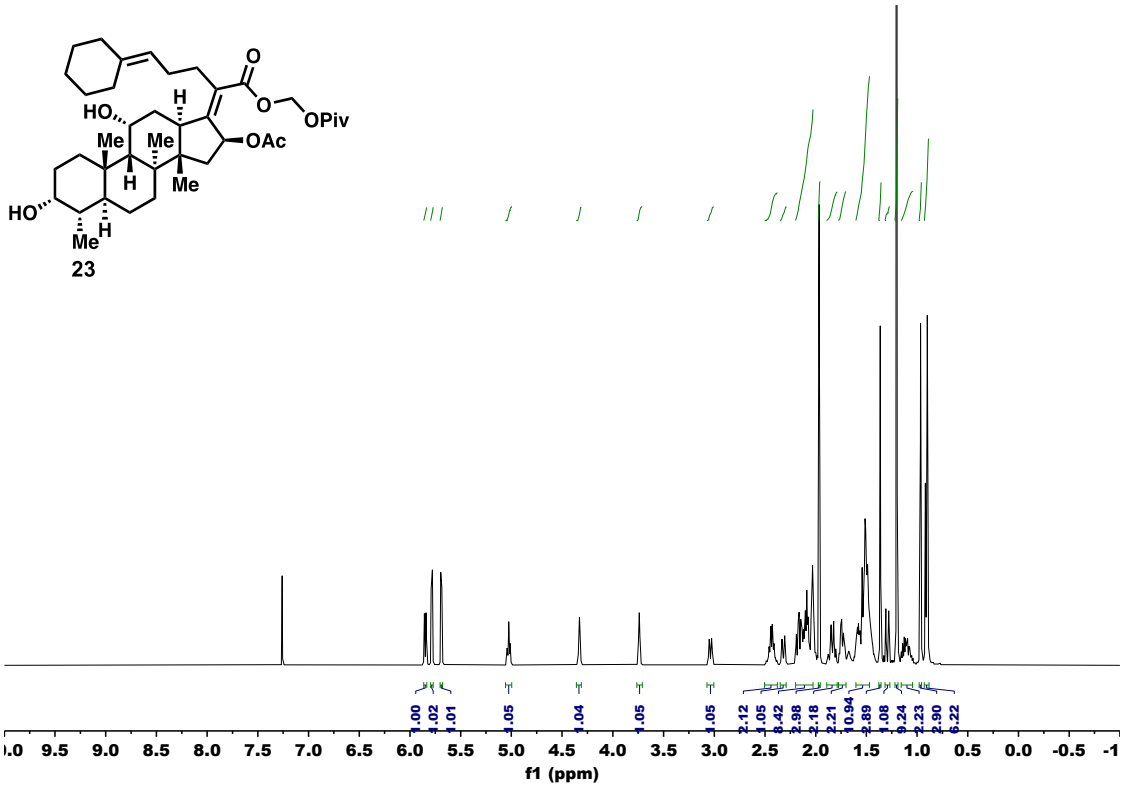

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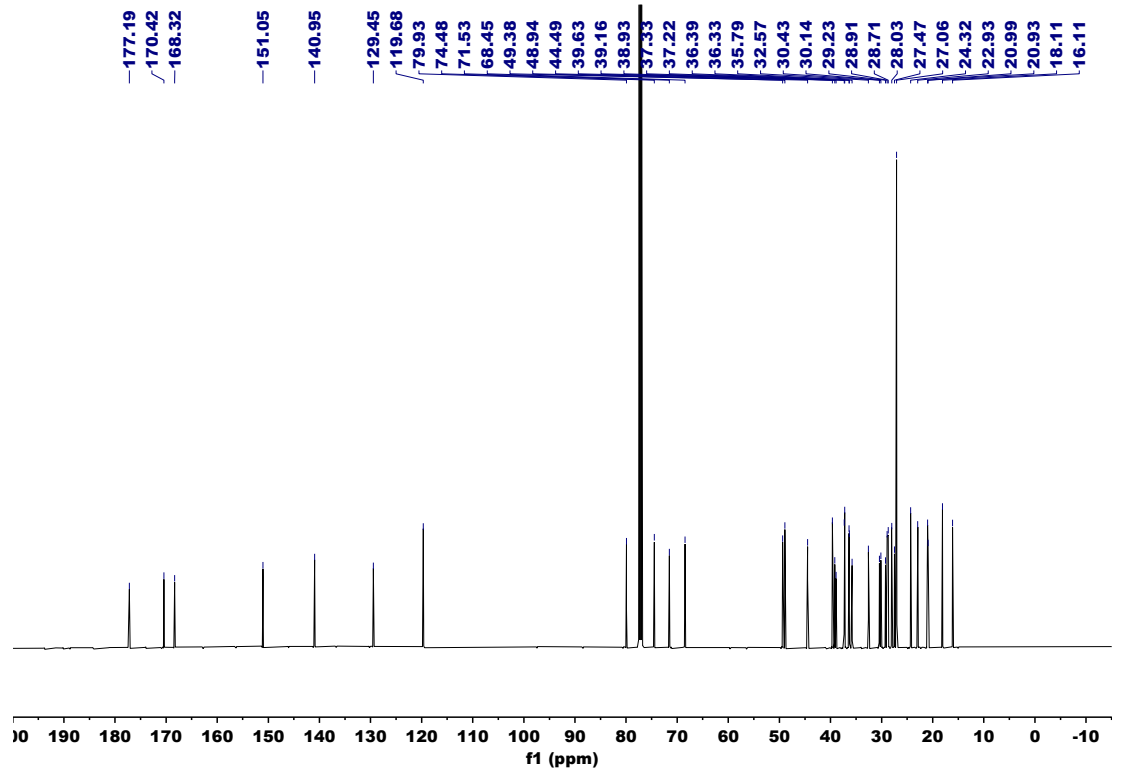

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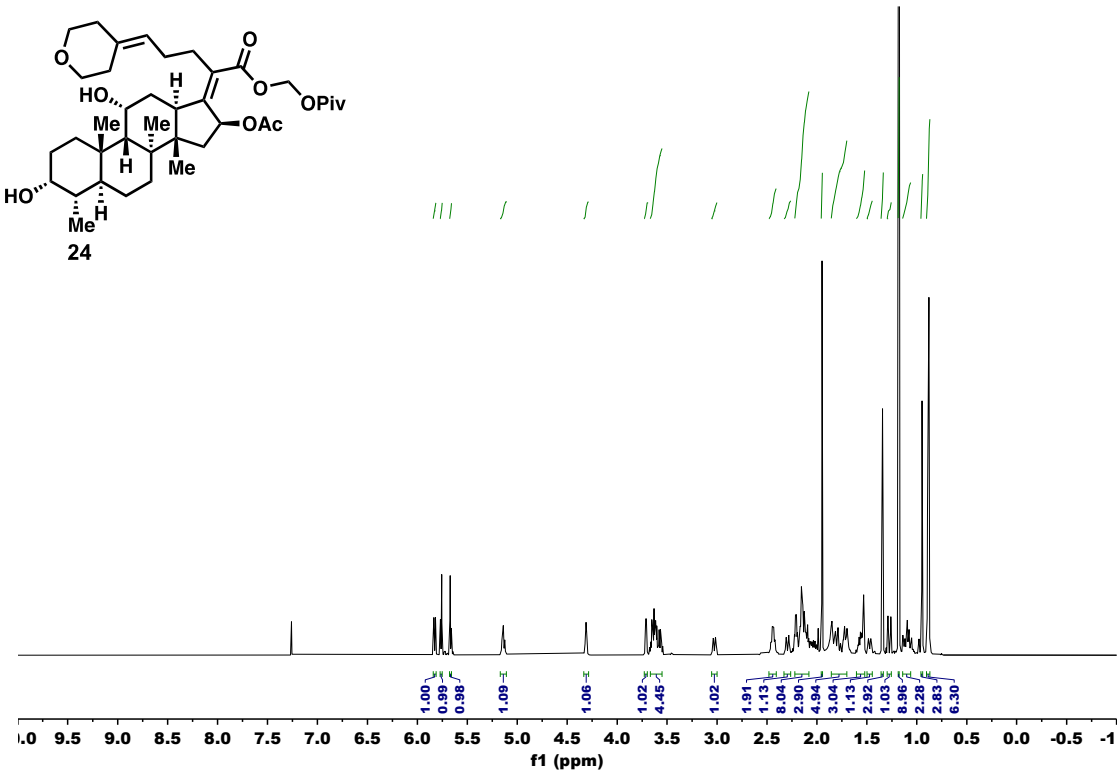

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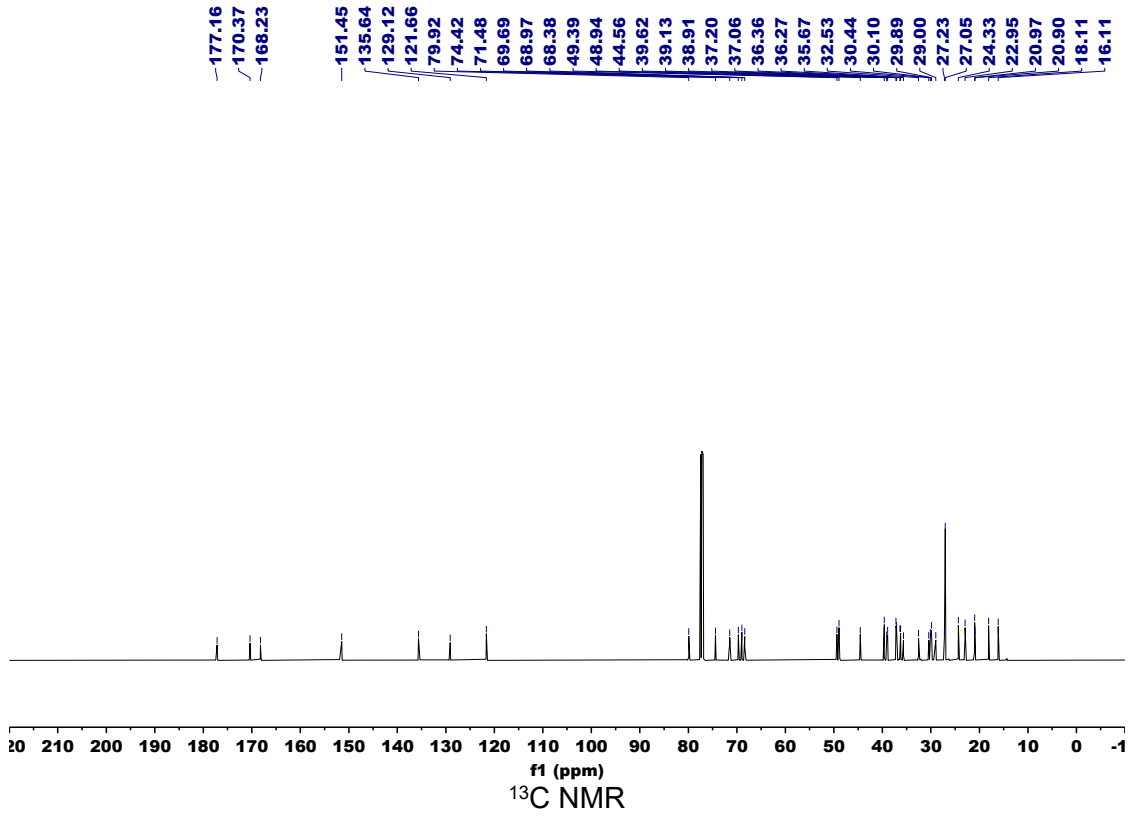

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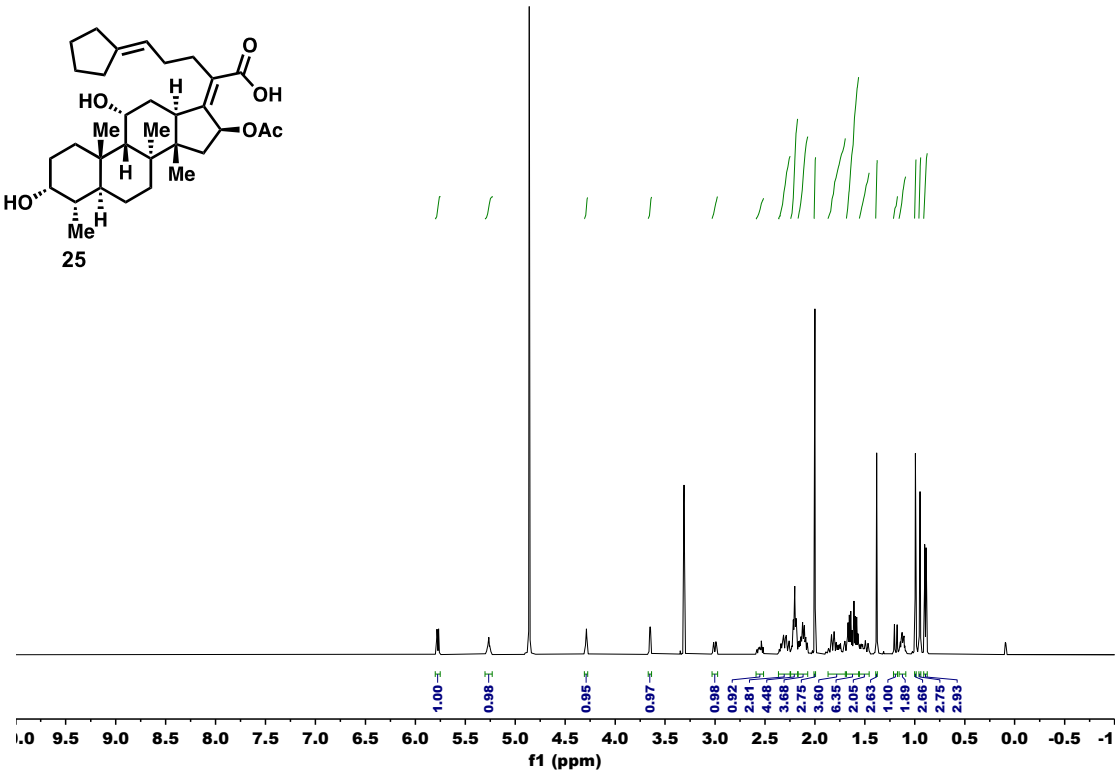

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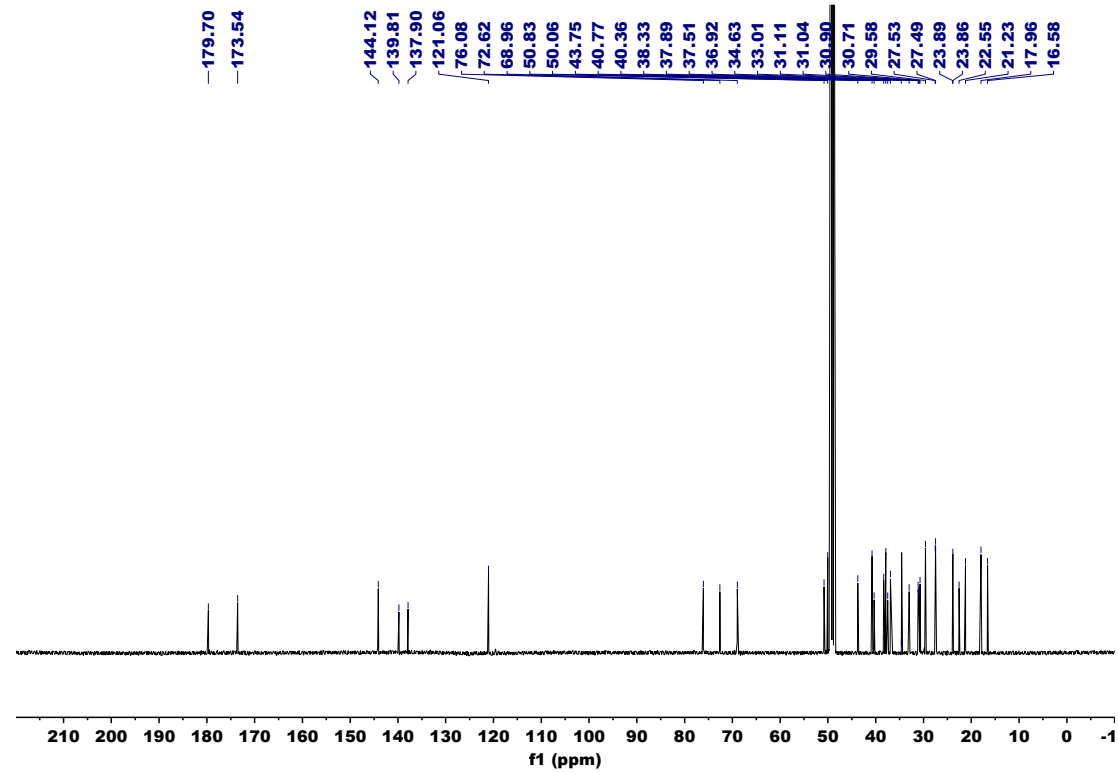

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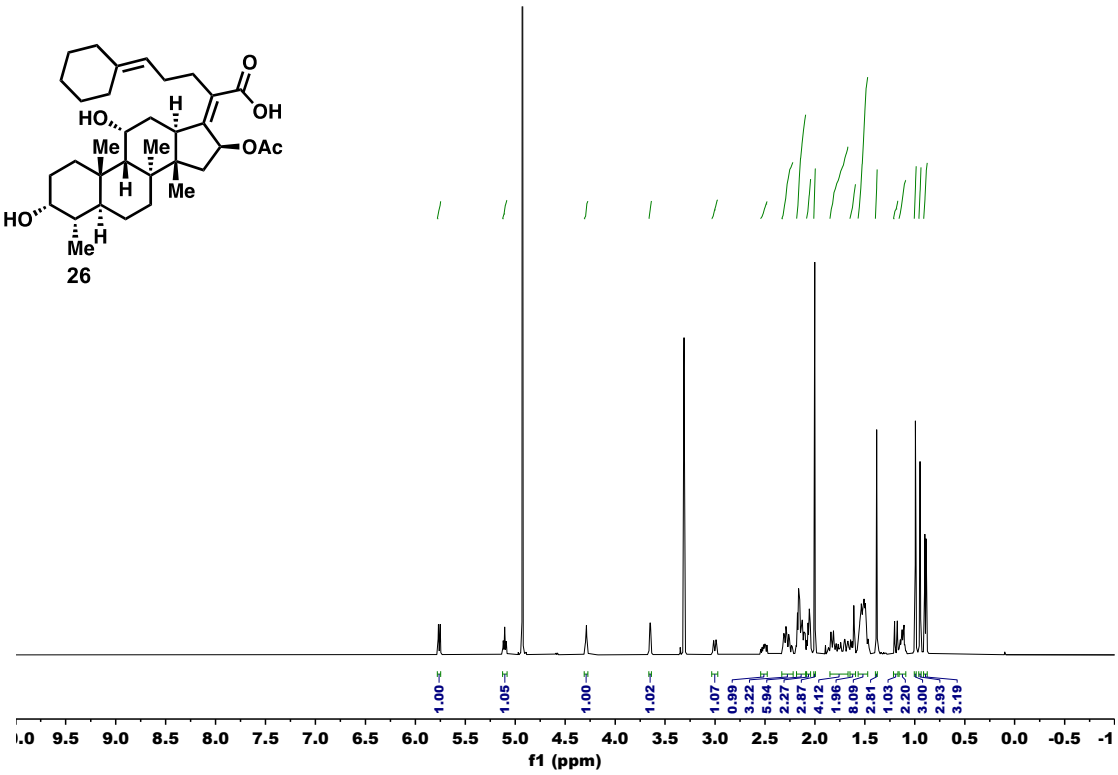

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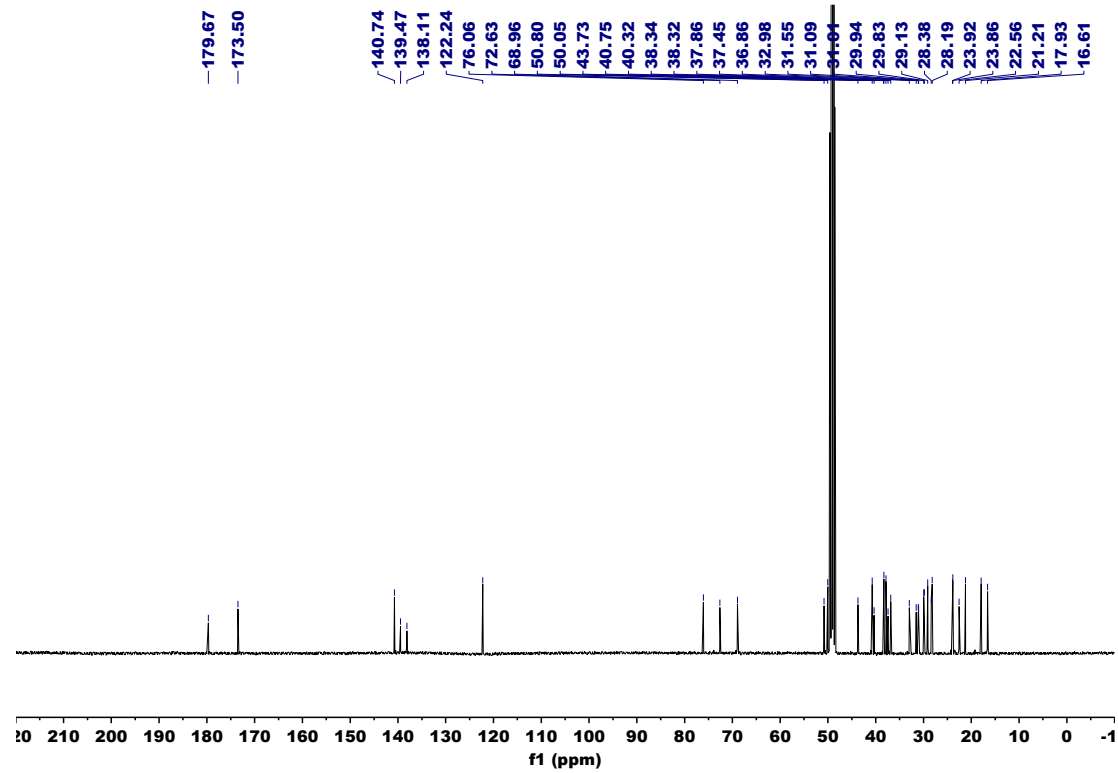

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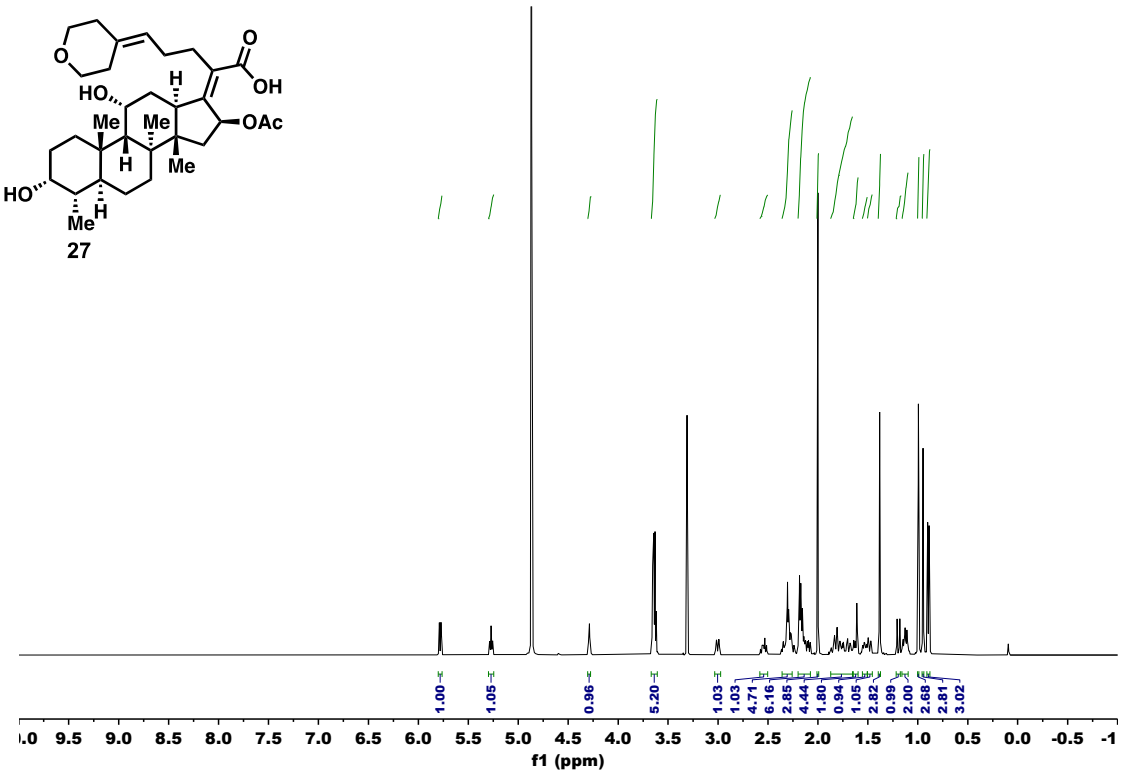

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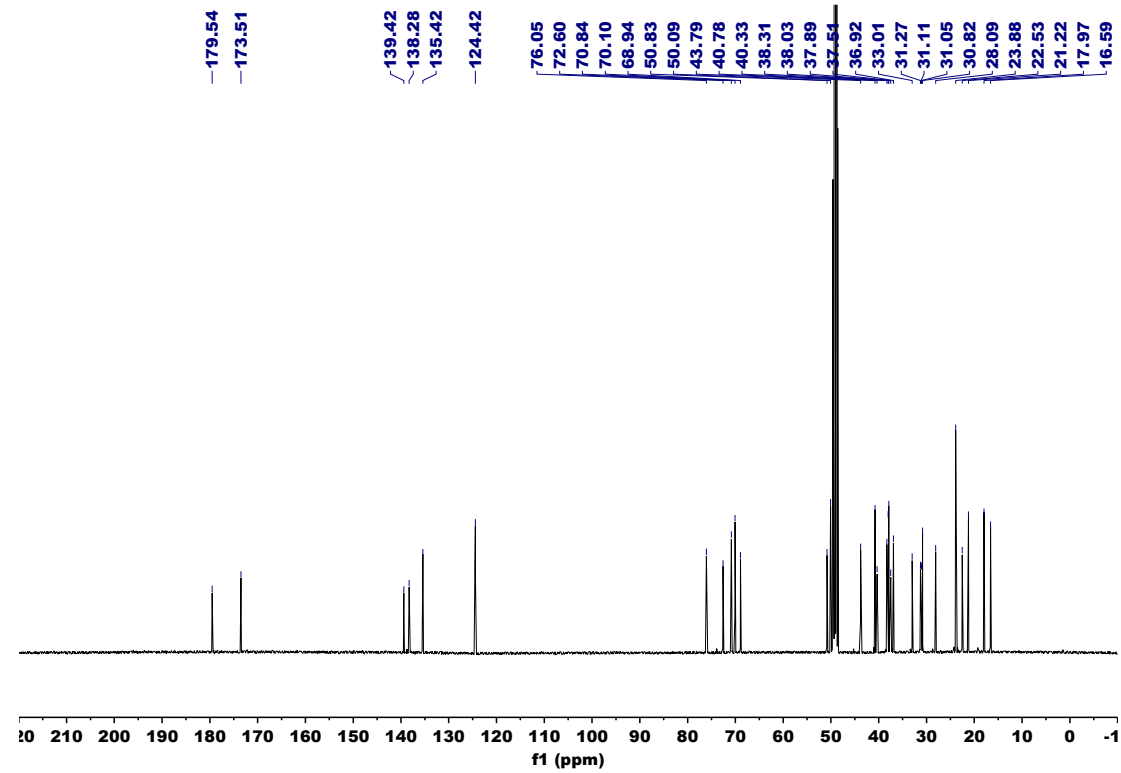

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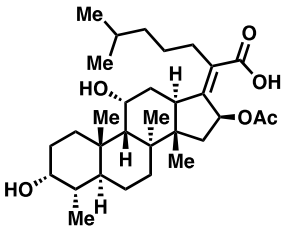

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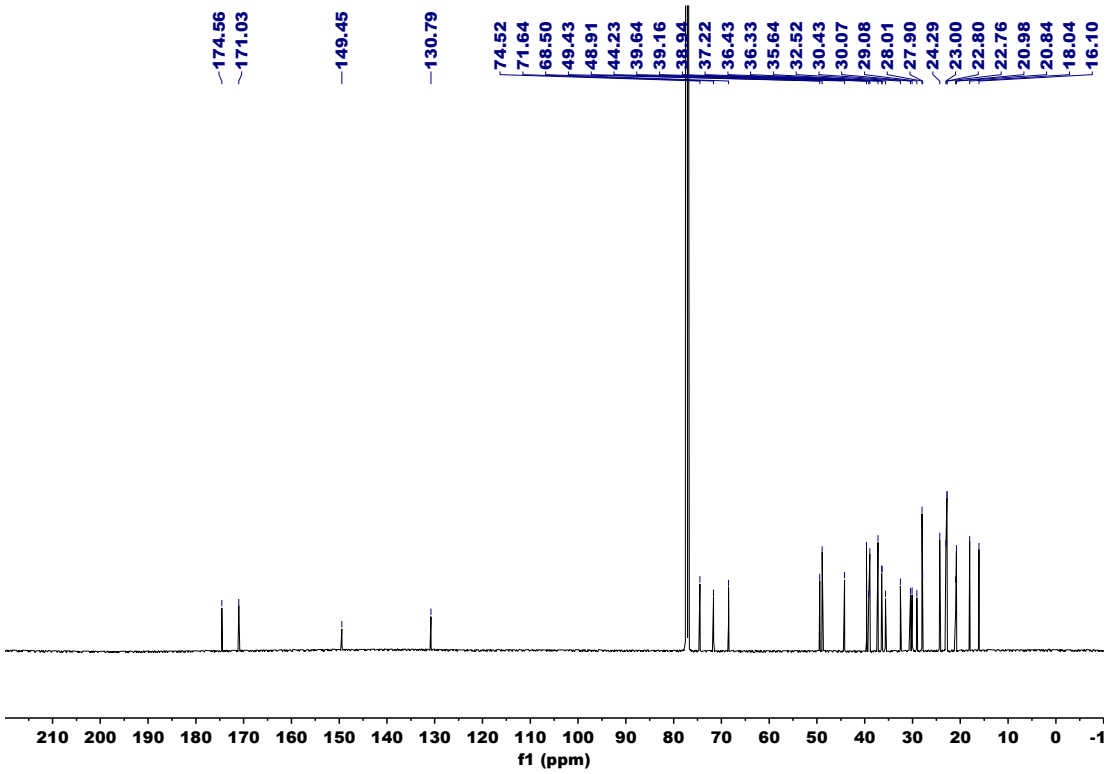
^{1}H NMR



^{13}C NMR