Traceless Solid-Phase α-Hydroxytropolone Synthesis

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Supporting Information

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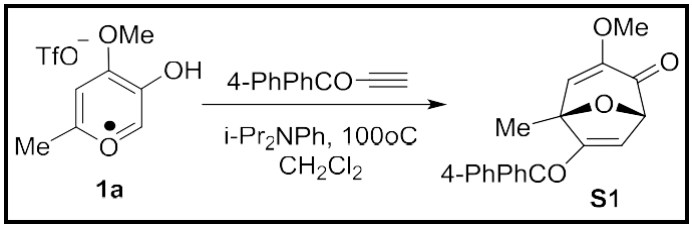
I. General Information

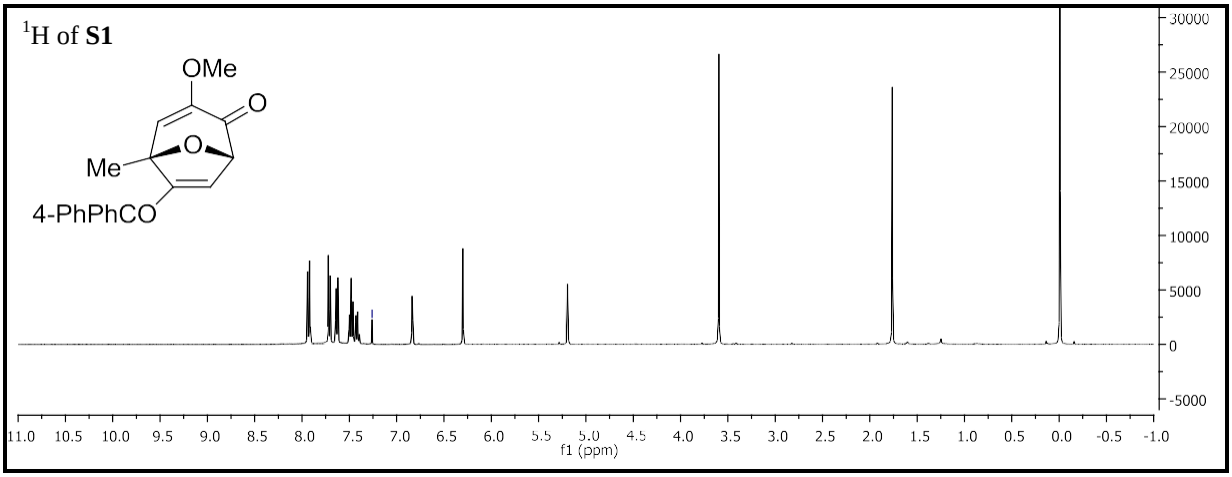
All starting materials and reagents were purchased from commercially available sources and used without further purification, with exception of CH_2Cl_2, which was purified on a solvent purification system prior to reactions.[1] ^1H NMR shifts are measured using the solvent residual peak as the internal standard ($CHCl_3$ δ 7.26), and reported as follows: chemical shift, multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, dd = doublet of doublet, q = quartet, m = multiplet), coupling constant (Hz), integration. ^{13}C NMR shifts are measured using the solvent residual peak as the internal standard ($CDCl_3$ δ 77.16), and reported as chemical shifts. Infrared (IR) spectral bands are characterized as broad (br), strong (s), medium (m), and weak (w). Mass spectra were recorded on a spectrometer by electrospray ionization (ESI) technique and time-of-flight (TOF) mass analyzer. Microwave reactions were performed via the Biotage Intiator (External IR Temperature Sensor). Where noted, reaction products were purified via silica gel chromatography using a Biotage® Isolera Prime, with Biotage® SNAP 10g cartridges, in a solvent system of ethyl acetate (EtOAc) in hexane.

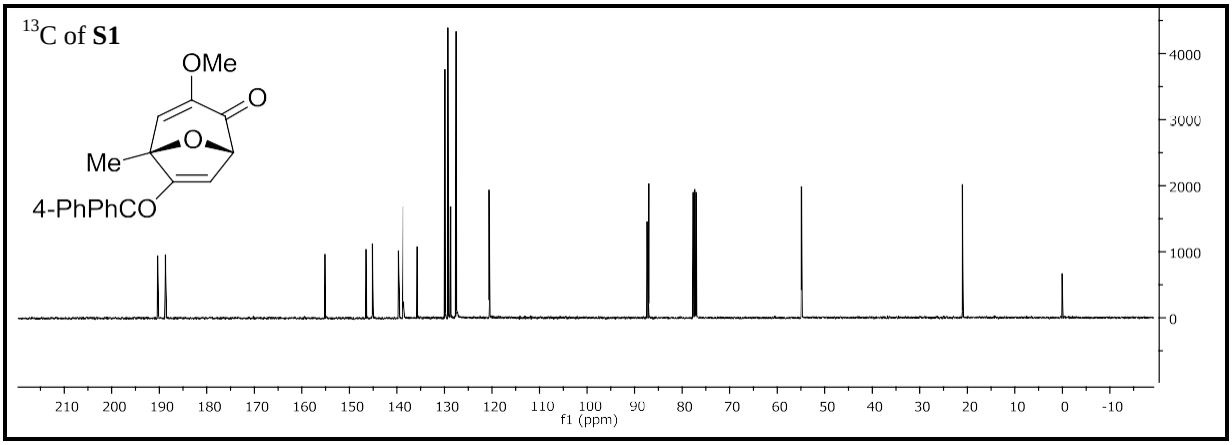
[1] Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J. *Organometallics*, **1996**, *15*, 1518-1520

II. Solution Phase Synthesis of 3-Methoxy-8-oxabicyclo[3.2.1]octenes

6-([1,1'-Biphenyl]-4-carbonyl)-3-methoxy-5-methyl-8-oxabicyclo[3.2.1]octa-3,6-dien-2-one (S1). To a suspension of triflate salt **1a** (100 mg, 0.345 mmol)[2] and 1-([1,1'-biphenyl]-4-yl)prop-2-yn-1-one (712 mg, 3.45 mmol, 10 eq)[3] in CH_2Cl_2 (5 mL) was added N,N-diisopropylaniline (81 µL, 0.414 mmol, 1.2 eq). After microwave irradiation at 100 °C for 1 hr, the reaction mixture was concentrated and purified by chromatography (Silica [10g], 0% EtOAc/hexane to 35% EtOAc/hexane gradient over 20 column volumes), yielding **S1** as an orange solid (92.8 mg, 77% yield). MP= 156-159 °C. R_f= 0.22 in 20% EtOAc in hexanes. **IR (thin film, KBr)** 3063 (w), 2979 (w), 2935 (w), 2837 (w), 1711 (s), 1641 (m), 1603 (s), 1449 (w), 1323 (m), 1127 (m), 1043 (w), 989 (w), 844 (m), 744 (s), 698 (m) cm^{-1}. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.93 (d, *J* = 8.3 Hz, 2H), 7.71 (d, *J* = 8.3 Hz, 2H), 7.65 – 7.61 (m, 2H), 7.51 – 7.38 (m, 3H), 6.83 (d, *J* = 2.4 Hz, 1H), 6.30 (s, 1H), 5.20 (d, *J* = 2.5 Hz, 1H), 3.60 (s, 3H), 1.77 (s, 3H). **^{13}C NMR (100 MHz, $CDCl_3$)** δ 190.37, 188.72, 155.32, 146.66, 145.31, 139.84, 138.77, 135.73, 129.91, 129.29, 128.71, 127.63, 127.54, 120.62, 87.37, 87.01, 54.98, 21.07. **HRMS (ESI+)** *m/z* calc'd for $C_{22}H_{19}O_4^+$: 347.1278. Found: 347.1280.

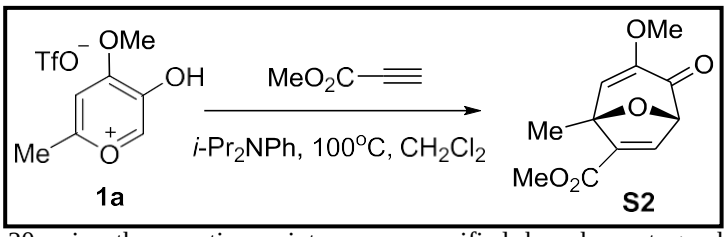


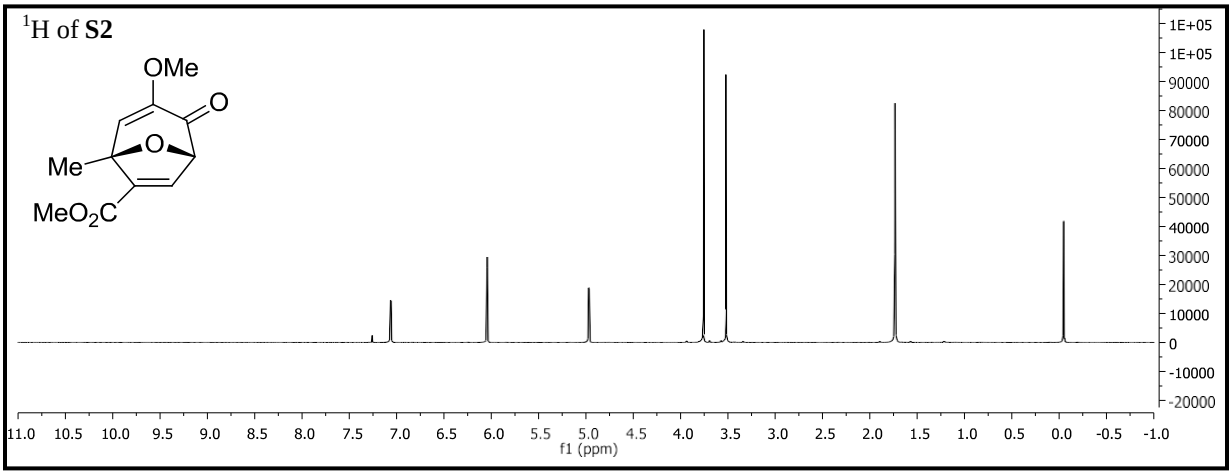


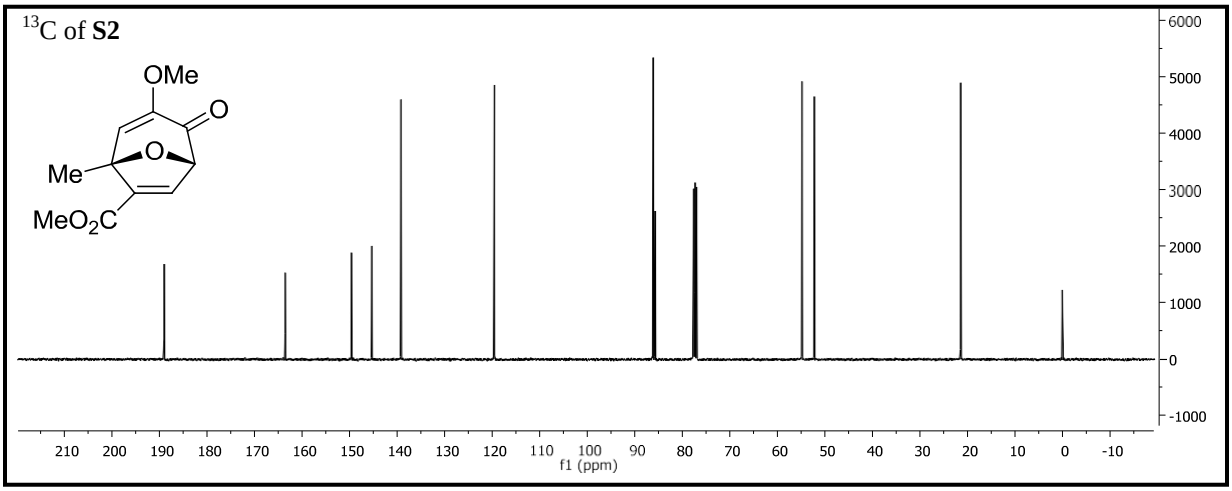


[2] Meck, C.; Mohd, N.; Murelli, R. P. *Org. Lett.* **2012**, *14*, 5988-5991.
[3] Schubert, T.; Hummel, W.; Kula, M.-R.; Müller, M. *Eur. J. Org. Chem.* **2001**, 4181-4187

Methyl 3-methoxy-5-methyl-2-oxo-8-oxabicyclo[3.2.1]octa-3,6-diene-6-carboxylate (S2). To a suspension of triflate salt **1a** (100 mg, 0.345 mmol) and methyl propiolate (307 µL, 3.45 mmol, 10 eq) in CH_2Cl2 (750 µL) was added N,N-diisopropylaniline (81 µL, 0.414 mmol, 1.2 eq). After microwave irradiation at 100 °C for 20 min, the reaction mixture was purified by chromatography (Silica [10 g], 0% EtOAc/hexane to 35% EtOAc/hexane gradient over 22 column volumes). Product fractions were concentrated to yield **S2** as a yellow oil (54.8 mg, 71% yield). R_f= 0.29 in 25% EtOAc in hexanes. **IR (thin film, KBr)** 3095 (w), 2954 (w), 2840 (w), 1712 (s), 1616 (m), 1604 (m), 1438 (w), 1323 (m), 1223 (m), 1127 (m), 1075 (m), 1035 (w), 873 (w), 859 (w), 752 (w) cm^{-1}. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.06 (d, *J* = 2.5 Hz, 1H), 6.05 (s, 1H), 4.98 (d, *J* = 2.5 Hz, 1H), 3.76 (s, 3H), 3.52 (s, 3H), 1.73 (s, 3H). **^{13}C NMR (100 MHz, $CDCl_3$)** δ 188.89, 163.49, 149.57, 145.27, 139.15, 119.53, 86.16, 85.75, 54.92, 52.32, 21.57. **HRMS (ESI+)** *m/z* calc'd for $C_{11}H_{13}O_5^+$: 225.0757. Found: 225.0757.

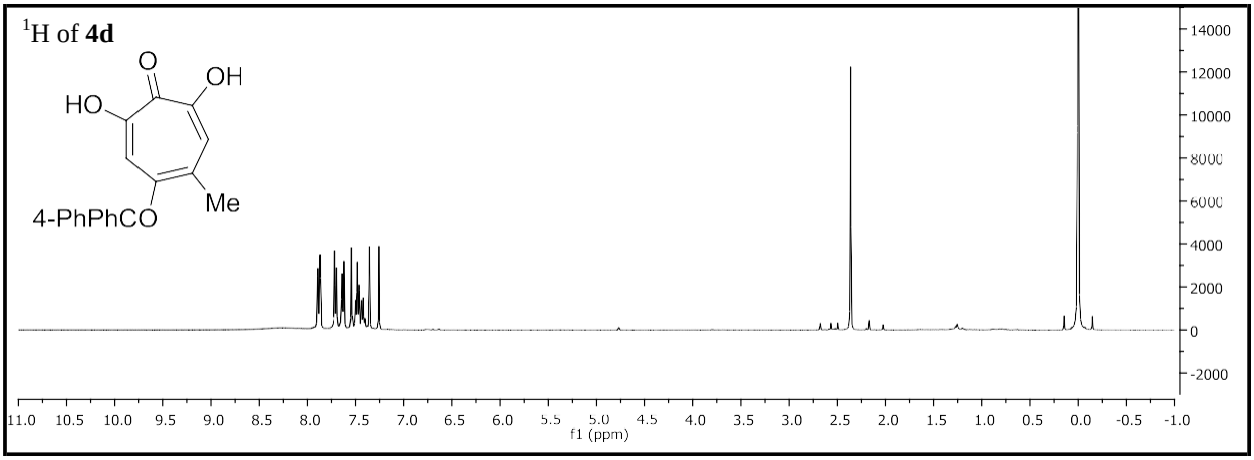


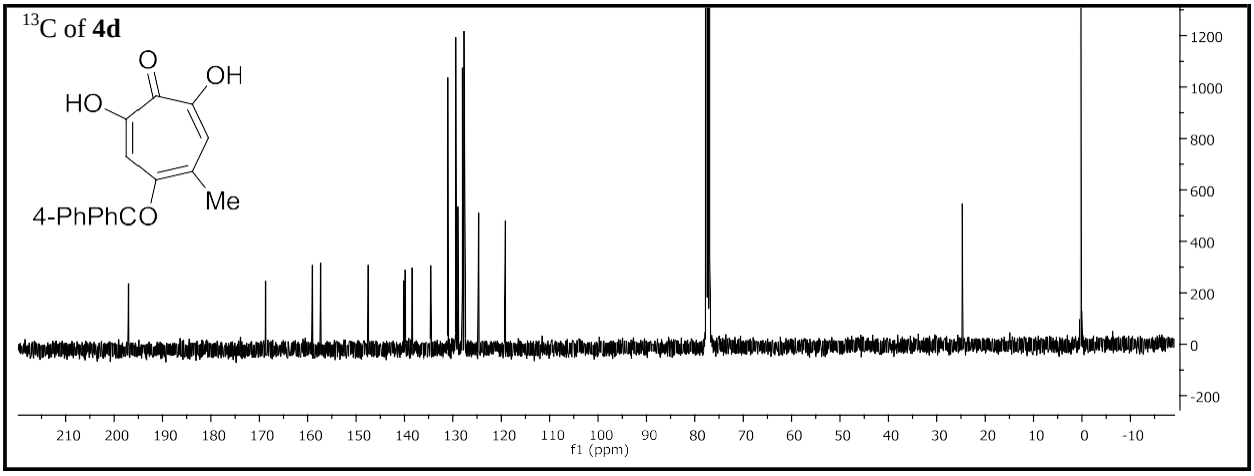




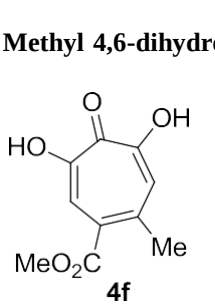
III. Solution Phase Synthesis of α-Hydroxytropolones

4-([1,1'-biphenyl]-4-carbonyl)-2,7-dihydroxy-5-methylcyclohepta-2,4,6-trien-1-one (4d). To a solution of bicycle **S1** (46.4 mg, 0.134 mmol) in CH_2Cl_2 (1.5 mL) was added trifluoromethanesulfonic acid (47.3 μL, 0.536 mmol, 4 eq). The reaction was allowed to stir for 30 minutes, at which time it was quenched with sodium acetate (110 mg, 1.34 mmol, 10 eq), stirred for 20 min, and concentrated under reduced pressure. The crude mixture was then dissolved in 25% HBr in acetic acid (2 mL), and heated to 90 ºC for 4 hr. The reaction was cooled to room temperature, quenched with pH 7 phosphate buffer (10 mL), and diluted with CH_2Cl_2 (5 mL). The organic layer was washed phosphate buffer (3 X 10 mL), dried over Na_2SO_4, filtered, and concentrated to yield **4d** as a brown oil (26.2 mg, 59% yield). **IR (thin film, KBr)** 3262 (br), 3060 (w), 2961 (w), 1669 (s), 1601 (s), 1534 (s), 1398 (m), 1284 (s), 1232 (s), 1191 (s), 1083 (s), 906 (m), 859 (m), 750 (s), 696 (m) cm^{-1}. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.88 (d, *J* = 8.2 Hz, 2H), 7.71 (d, *J* = 8.2 Hz, 2H), 7.63 (d, *J* = 7.4 Hz, 2H), 7.54 (s, 1H), 7.52-7.39 (m, 3H), 7.36 (s, 1H), 2.36 (s, 3H). **^{13}C NMR (100 MHz, $CDCl_3$)** δ 197.02, 168.70, 159.03, 157.33, 147.49, 140.15, 139.85, 138.42, 134.57, 131.05, 129.41, 128.96, 128.01, 127.70, 124.67, 119.18, 24.86. **HRMS (ESI+)** *m/z* calc'd for $C_{21}H_{17}O_4^+$: 333.1121. Found: 333.1124.

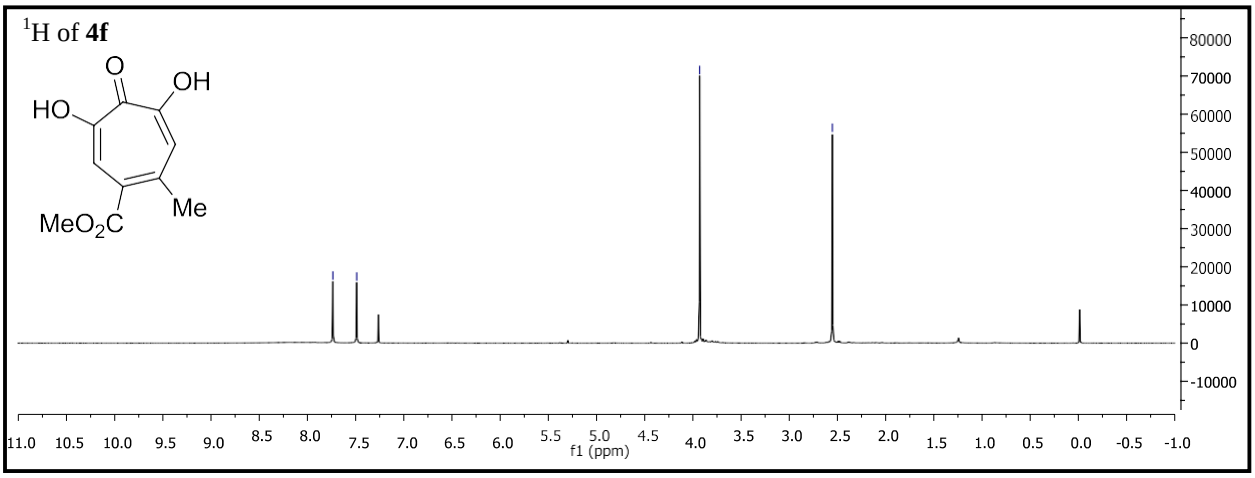


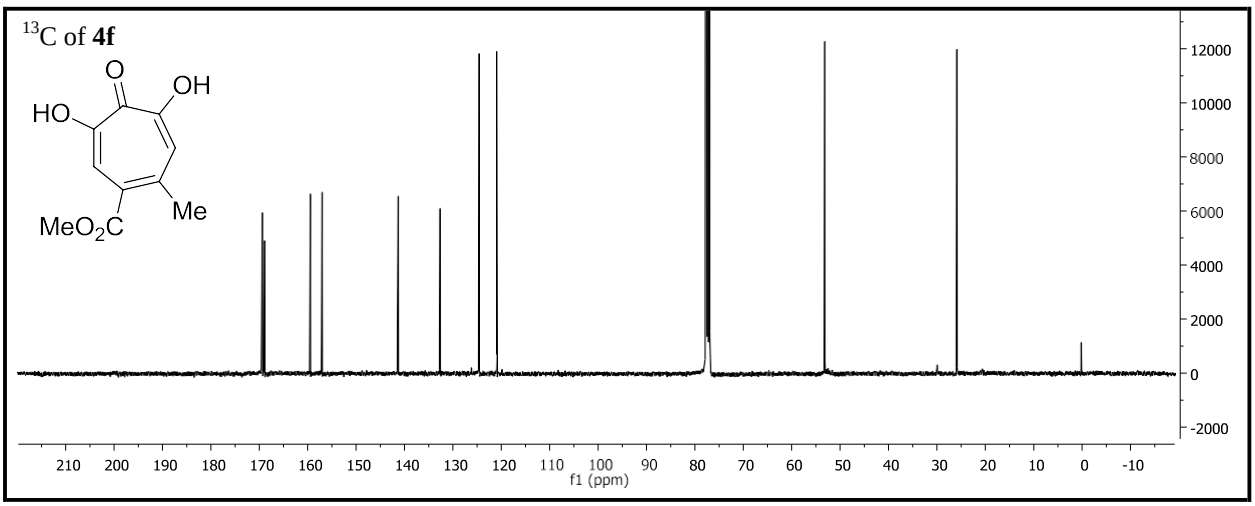


Methyl 4,6-dihydroxy-2-methyl-5-oxocyclohepta-1,3,6-trienecarboxylate (4f). To a solution of bicycle **S2** (54.8 mg, 0.244 mmol) in CH_2Cl_2 (1.5 mL) was added trifluoromethanesulfonic acid (86.3 µL, 0.978 mmol, 4 eq). The reaction was allowed to stir for 30 minutes, at which time it was quenched with phosphate buffer (pH 7, 10 mL), extracted with CH_2Cl_2 (3 X 5 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude mixture was then dissolved in 33% HBr in acetic acid (2 mL), and heated to reflux for 1.5 hr. The reaction was cooled to room temperature, quenched with pH 7 phosphate buffer (10 mL), and extracted with CH_2Cl_2 (3 X 5 mL), dried over Na_2SO_4, filtered, and concentrated to yield **4f** as a brown solid (30.0 mg, 58% yield). MP = 137-141 °C. **IR (thin film, KBr)** 3239 (br), 2963 (w), 1731 (s), 1544 (s), 1435 (m), 1400 (m), 1288 (s), 1214 (s), 1141 (m), 1100 (m), 1067 (w), 948 (w), 904 (w), 764 (w), 635 (w) cm^{-1}. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.73 (s, 1H), 7.48 (s, 1H), 3.93 (s, 3H), 2.56 (s, 3H). **^{13}C NMR (100 MHz, $CDCl_3$)** δ 169.30, 168.82, 159.40, 156.96, 141.27, 132.64, 124.60, 120.92, 53.22, 25.97. **HRMS (ESI+)** *m/z* calc'd for $C_{10}H_{11}O_5^+$: 211.0601. Found: 211.0602.

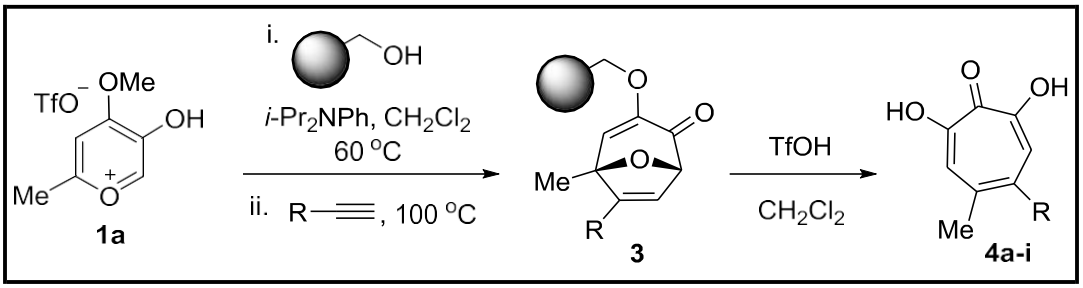


4f





IV. Solid Phase Synthesis of α-Hydroxytropolones



General Procedures for Liquid Alkynes (Procedure A): Polystyrene supported benzyl alcohol (3.5 mmol/g loading capacity, 1 eq) was added to a microwave reactor vial and allowed to swell for 30 min in CH_2Cl_2 before the addition of triflate salt **1a** (1.5 eq) and N,N-diisopropyl aniline (1.8 eq), and the reaction mixture was gently stirred at 60 ºC for 14 h. The beads were drained of CH_2Cl_2, submerged in alkyne, and heated to 100 ºC for various time points. After the reaction was cooled to room temperature, the beads were rinsed with CH_2Cl_2 (6 X 1 mL) and then resuspended in CH_2Cl_2. Trifluoromethanesulfonic acid (4 eq) was added to the reaction mixture, which was then allowed to stir at room temperature for 30 min. The reaction was diluted with CH_2Cl_2 (2 mL) and quenched with pH 7 phosphate buffer (10 mL). The aqueous layer was extracted with CH_2Cl_2 (3 X 5 mL), and combined organics were dried over Na_2SO_4, filtered, and concentrated to yield α-hydroxytropolones.

General Procedures for Solid Alkynes (Procedure B): Polystyrene supported benzyl alcohol (3.5 mmol/g loading capacity, 1 eq) was added to a microwave reactor vial and allowed to swell for 30 min in CH_2Cl_2 before the addition of triflate salt **1a** (1.5 eq) and N,N-diisopropyl aniline (1.8 eq), and the reaction mixture was gently stirred at 60 ºC for 14 h. Alkyne was added to the reactor, and the mixture was heated to 100 ºC for various time points. After the reaction was cooled to room temperature, the beads were rinsed with CH_2Cl_2 (6 X 1 mL) and then resuspended in CH_2Cl_2 (500 µL). Trifluoromethanesulfonic acid (4 eq) was added to the reaction mixture, which was then allowed to stir at room temperature for 30 min. The reaction was diluted with CH_2Cl_2 (2 mL) and quenched with pH 7 phosphate buffer (10 mL). The aqueous layer was extracted with CH_2Cl_2 (3 X 5 mL), and combined organics were dried over Na_2SO_4, filtered, and concentrated to yield α-hydroxytropolones.

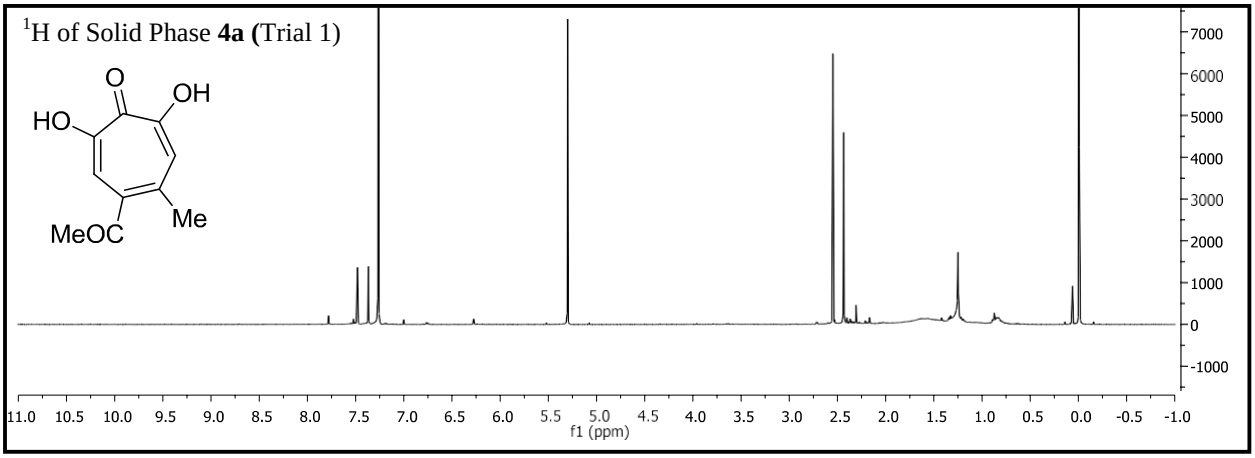
α-HT	Trial	Procedure	Solid Support (mg)	CH_2Cl_2 (µL) [a]	Triflate Salt (mg) [a]	Base (µL) [a]	Alkyne [a]	Time (h) [a]	Triflic Acid (µL) [b]	CH_2Cl_2 (µL) [b]	Total Mass (mg)	Overall Yield	Ratio (4:7:8)
R = COMe	1	A	33	500	50	41	500 µL	1.5	41	500	1.8	8%	9:1:0
	2	A	33	500	50	41	500 µL	2	41	500	1.6	7%	1:0:0
	3	A	66	720	100	81	700 µL	2	81	720	1.9	4%	9:1:0
R = COCy	1	A	33	500	50	41	500 µL	2	41	500	3	11%	15:1:4
R = COPh	1	B	33	500	50	41	105 mg	2	41	500	5.6	22%	1:0:1
	2	B	66	720	100	81	210 mg	2	81	720	3.2	6%	5:0:1
R = CO4-PhPh	1	B	33	500	50	41	240 mg	3	41	500	4.7	9%	1:0:1
R = CO_2Et	1	A	33	500	50	41	500 µL	1.5	41	500	1.7	7%	1:0:0
	2	A	33	500	50	41	500 µL	1.5	41	500	2.2	9%	1:0:0
	3	A	66	720	100	81	700 µL	1.5	81	720	1.8	4%	1:0:0
R = CO_2Me	1	A	33	500	50	41	500 µL	1.5	41	500	1.4	6%	1:0:0
	2	A	66	720	100	81	700 µL	1.5	81	720	3.1	6%	1:0:0
R= Ph	1	A	33	500	50	41	500 µL	4.5	41	500	1.8	7%	9:1:0
	2	A	33	500	50	41	500 µL	5.5	41	500	3.3	13%	20:1:0
	3	A	66	720	100	81	700 µL	5.5	81	720	2.8	6%	5:1:0
R = 4-CF_3Ph	1	A	33	500	50	41	500 µL	4.5	41	500	3.4	11%	4:1:0
	2	A	33	500	50	41	500 µL	5.5	41	500	2	6%	1:0:0
R = 1-Npth	1	A	33	500	50	41	500 µL	4.5	41	500	2.1	7%	5:1:0
	2	A	33	500	50	41	500 µL	5.5	41	500	1.7	6%	15:1:0
DMAD	1	A	33	500	50	41	500 µL	1	41	500	2.2	10%	0:0:1

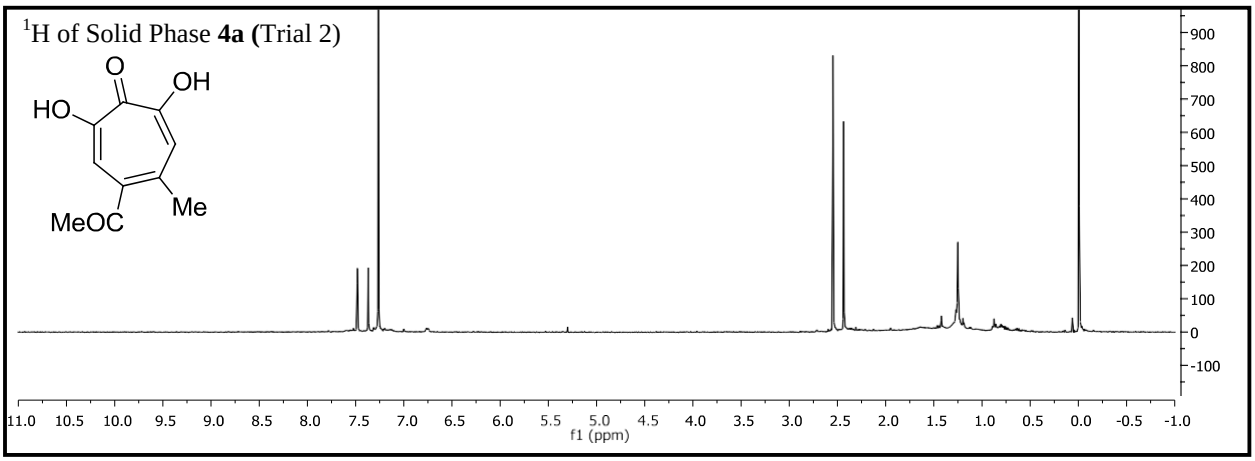
[a] Conditions used for oxidopyrylium cycloaddition.

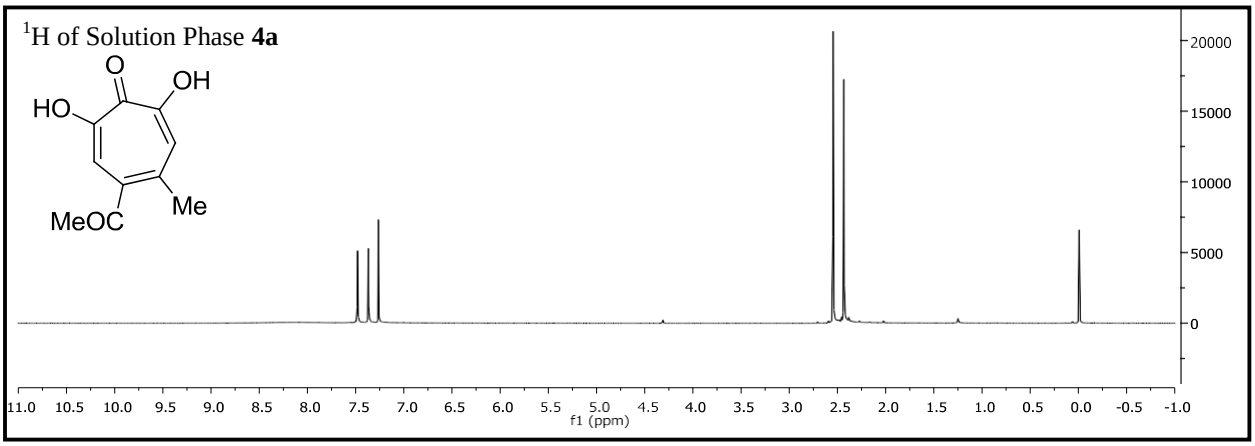
[b] Cleavage Conditions.

V. Solid and Solution Phase ^{1}H NMR Spectra

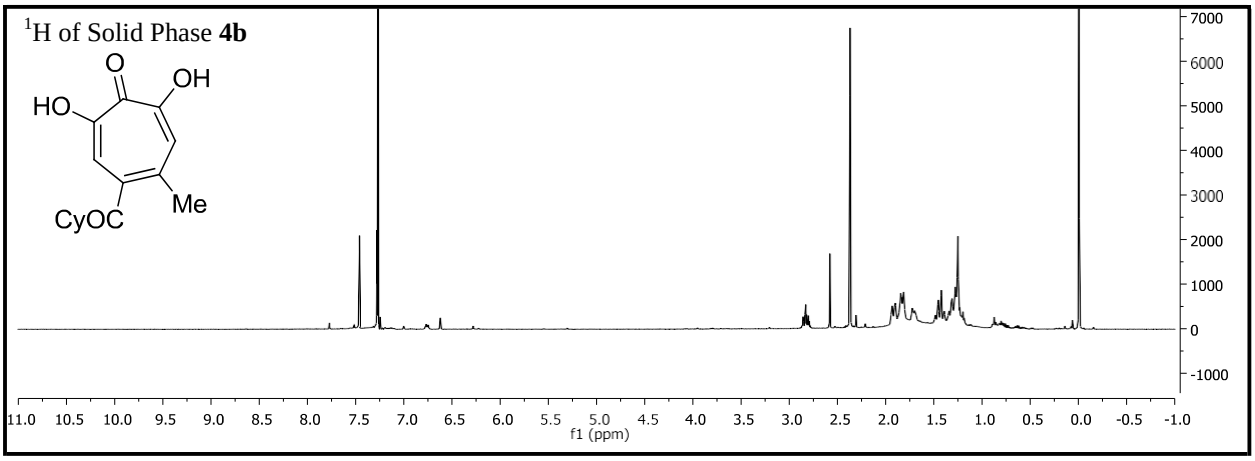
4-Acetyl-2,7-dihydroxy-5-methylcyclohepta-2,4,6-trienone (4a)

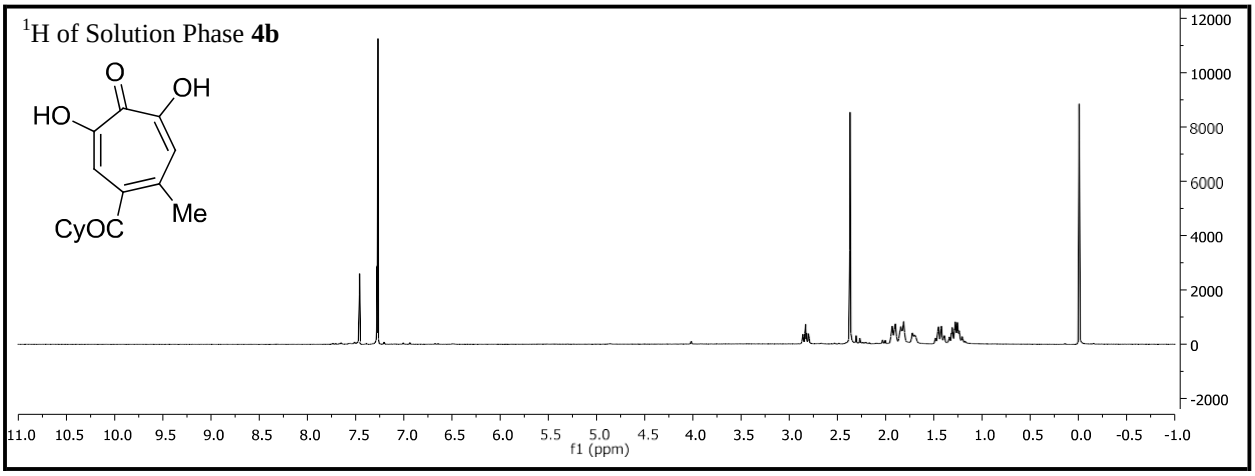




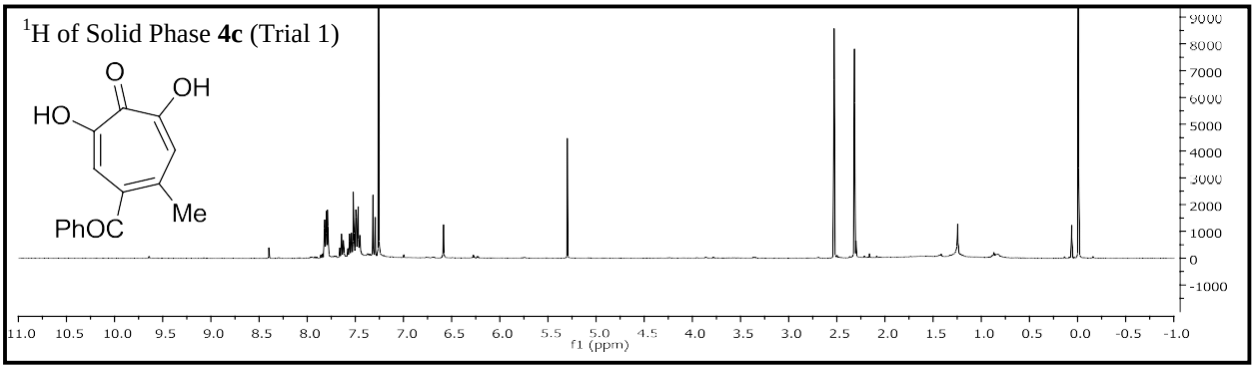


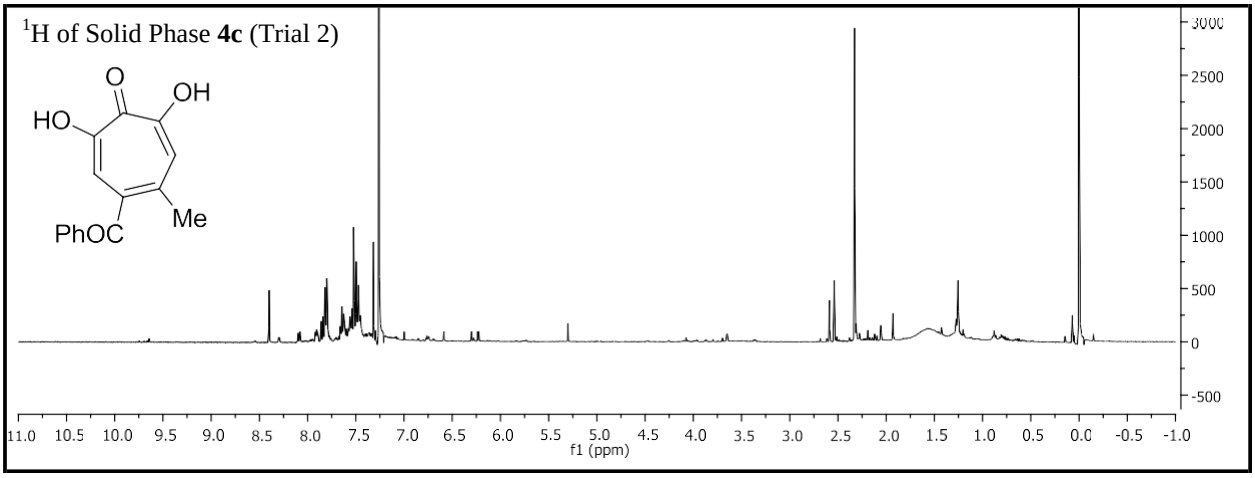
4-(Cyclohexanecarbonyl)-2,7-dihydroxy-5-methylcyclohepta-2,4,6-trienone (4b)

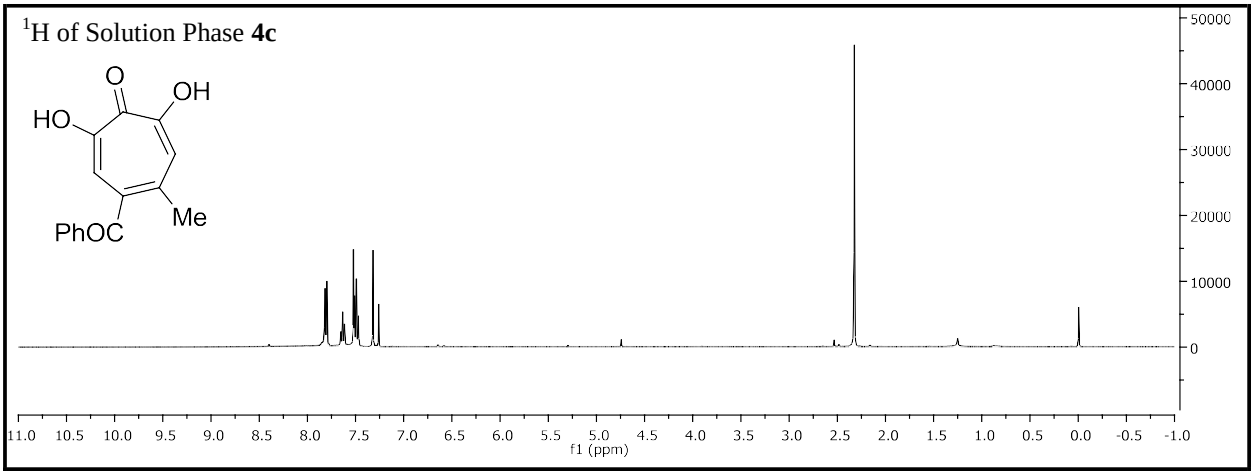




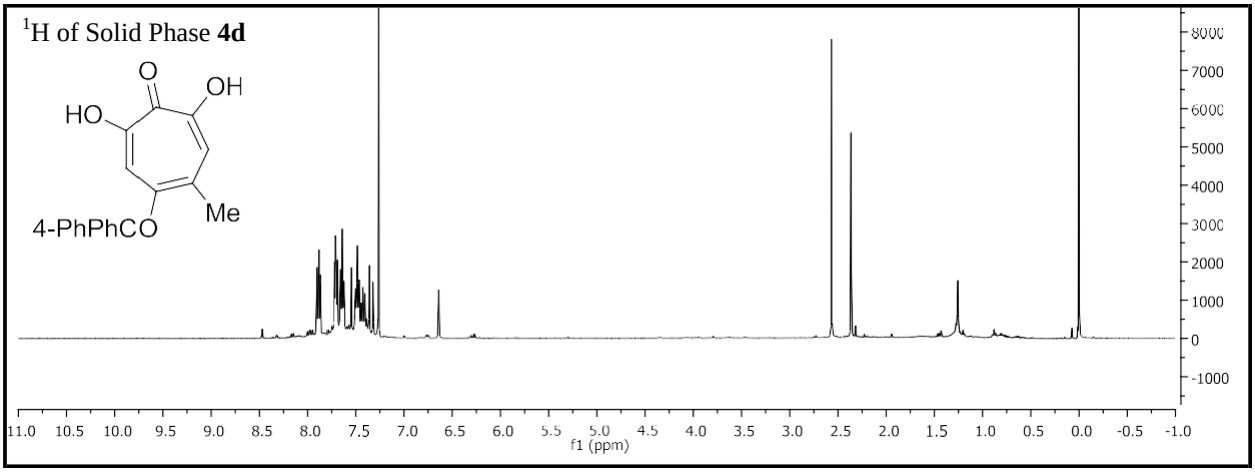
4-Benzoyl-2,7-dihydroxy-5-methylcyclohepta-2,4,6-trienone (4c)

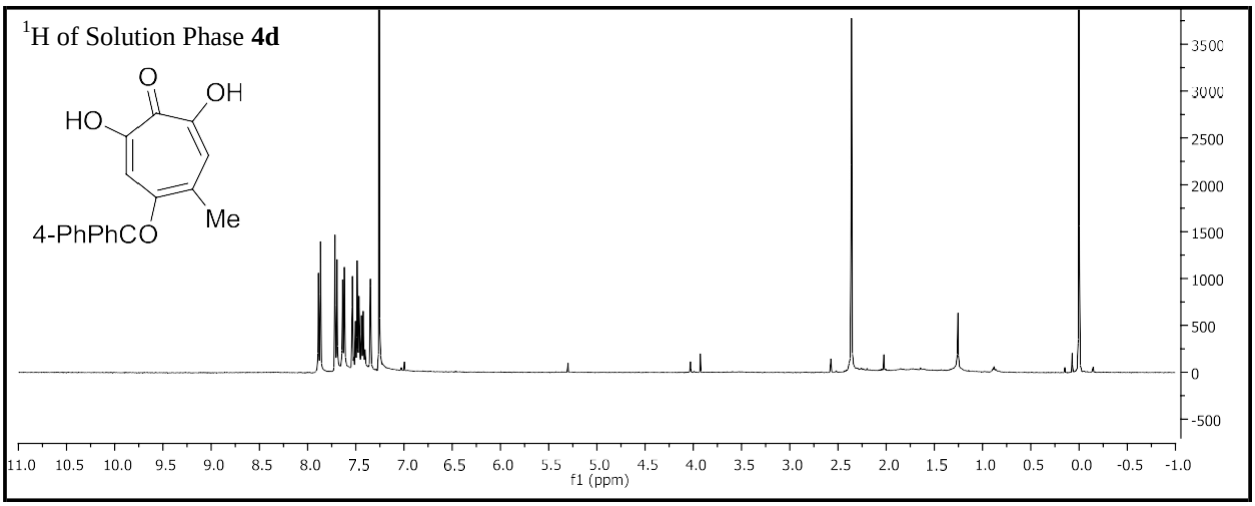




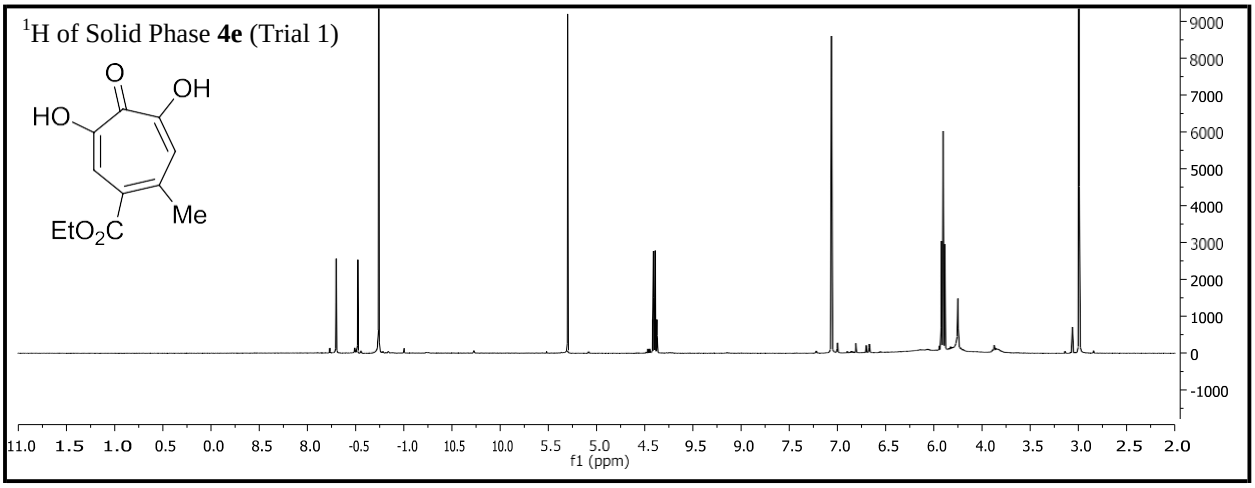


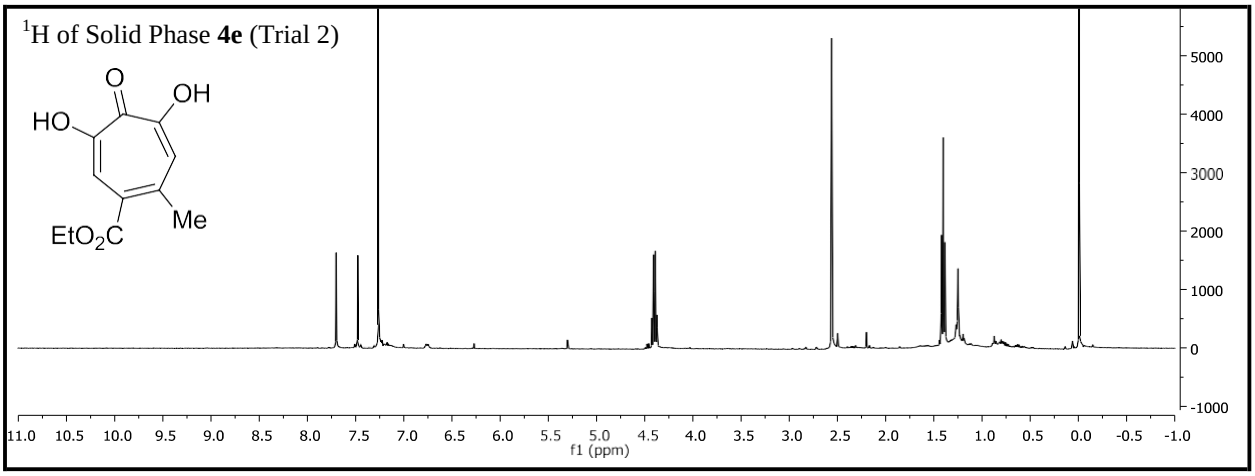
4-([1,1'-Biphenyl]-4-carbonyl)-2,7-dihydroxy-5-methylcyclohepta-2,4,6-trienone (4d)

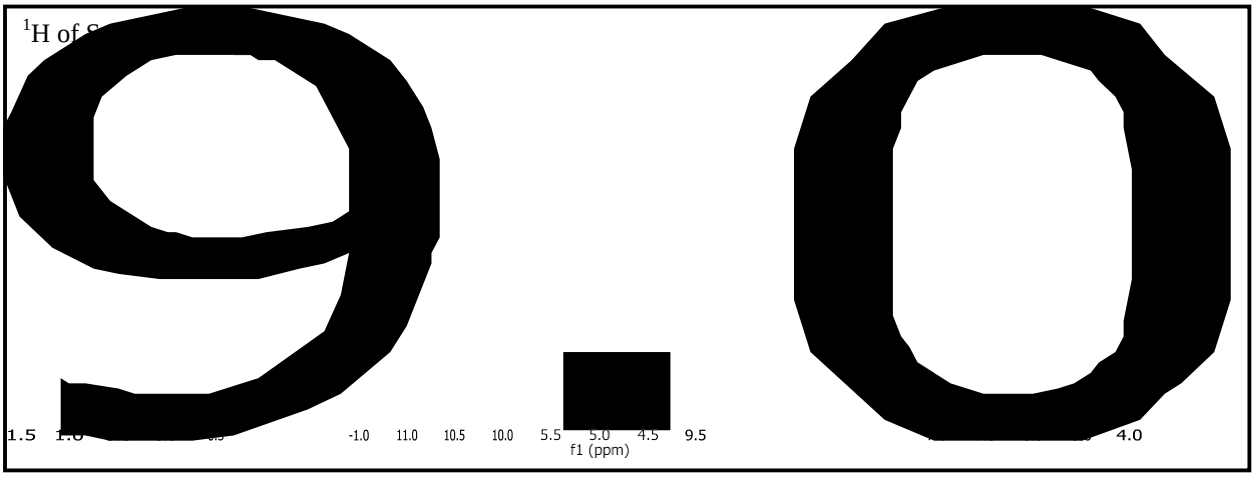


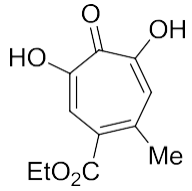


Ethyl 4,6-dihydroxy-2-methyl-5-oxocyclohepta-1,3,6-trienecarboxylate (4e)



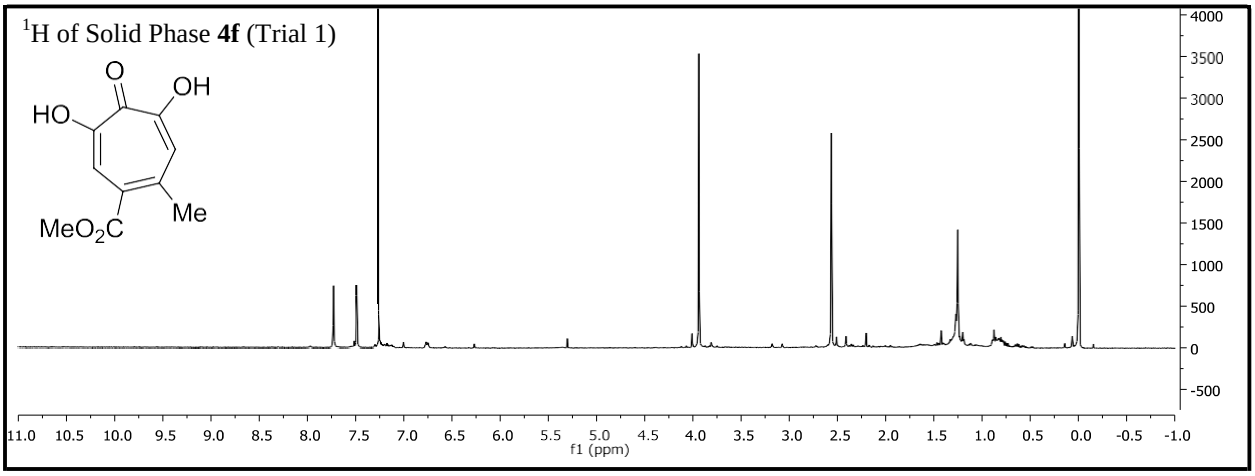




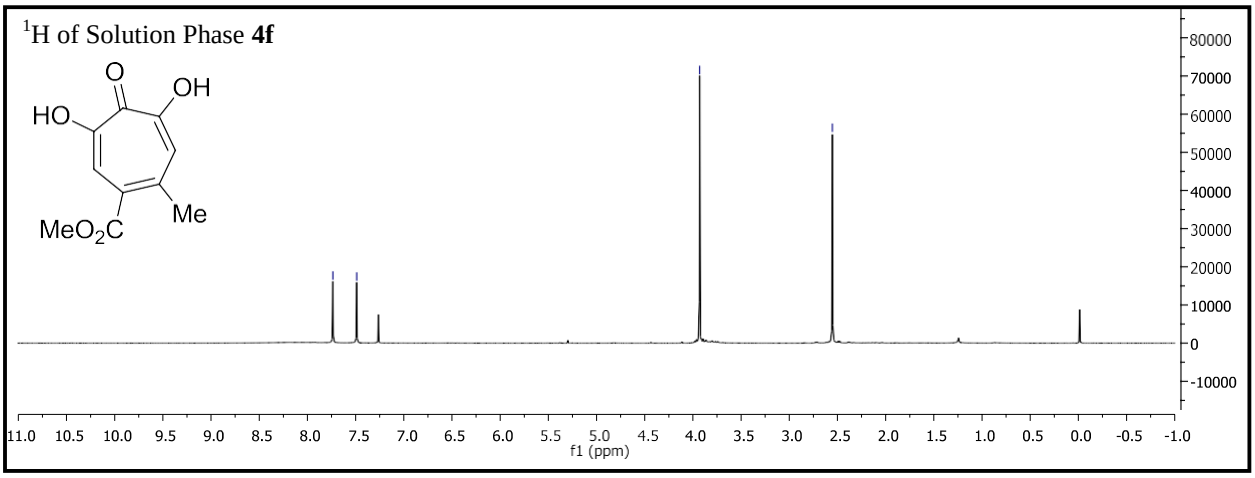


Methyl 4,6-dihydroxy-2-methyl-5-oxocyclohepta-1,3,6-trienecarboxylate (4f)

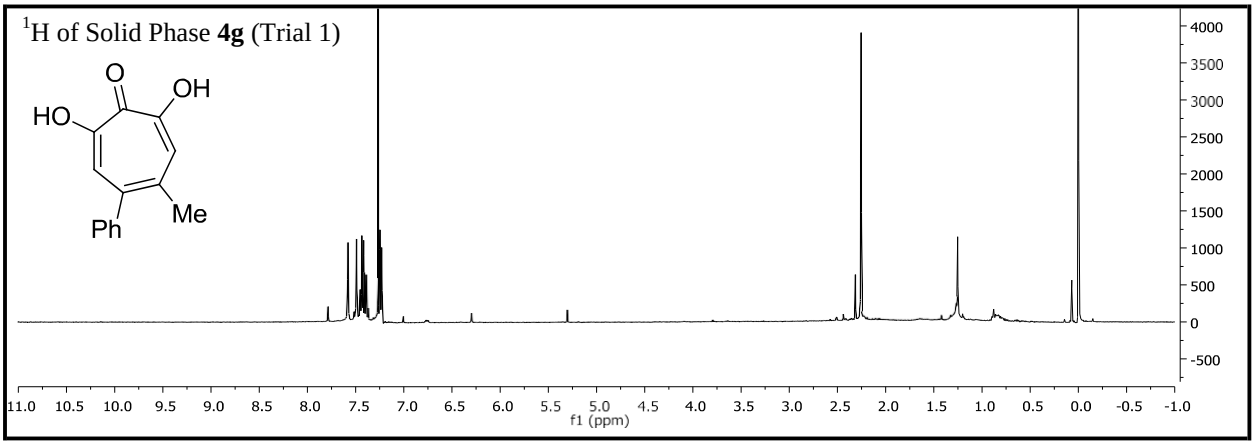
^{1}H of Solid Phase **4f** (Trial 1)

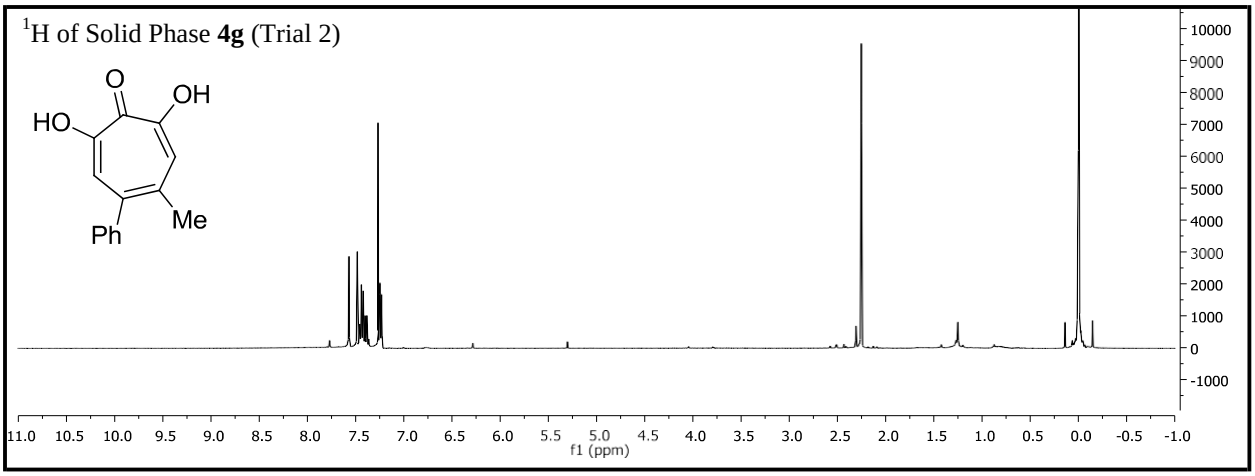


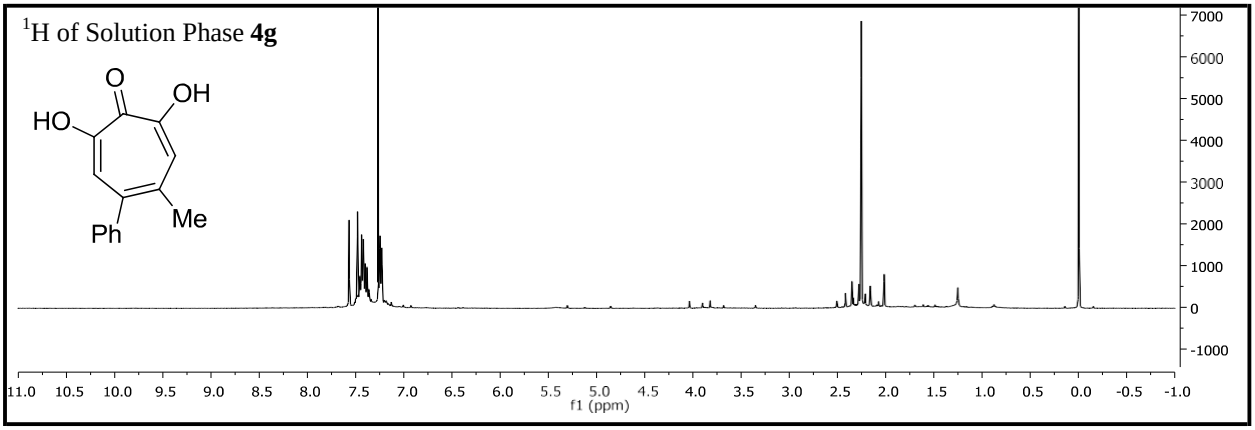
^{1}H of Solution Phase **4f**



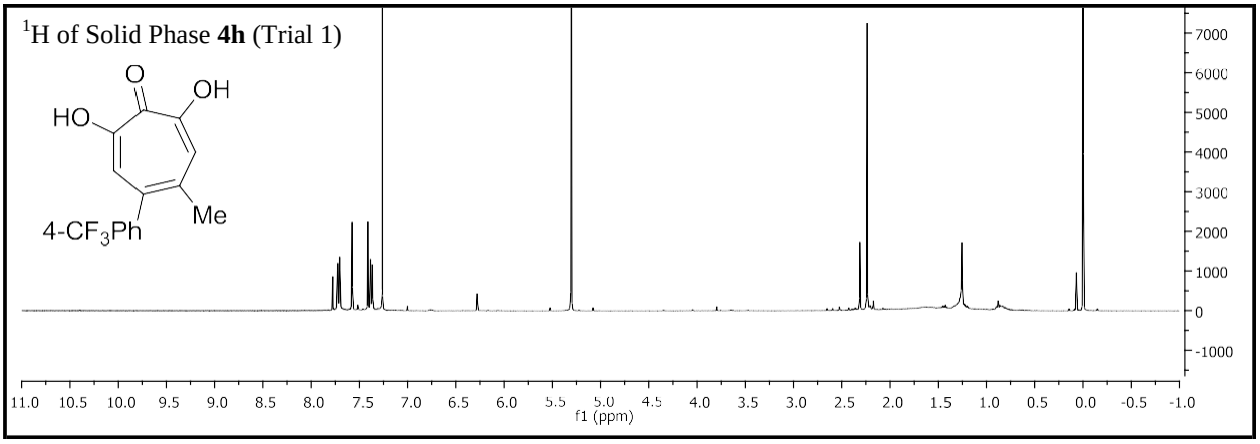
2,7-Dihydroxy-4-methyl-5-phenylcyclohepta-2,4,6-trienone (4g)

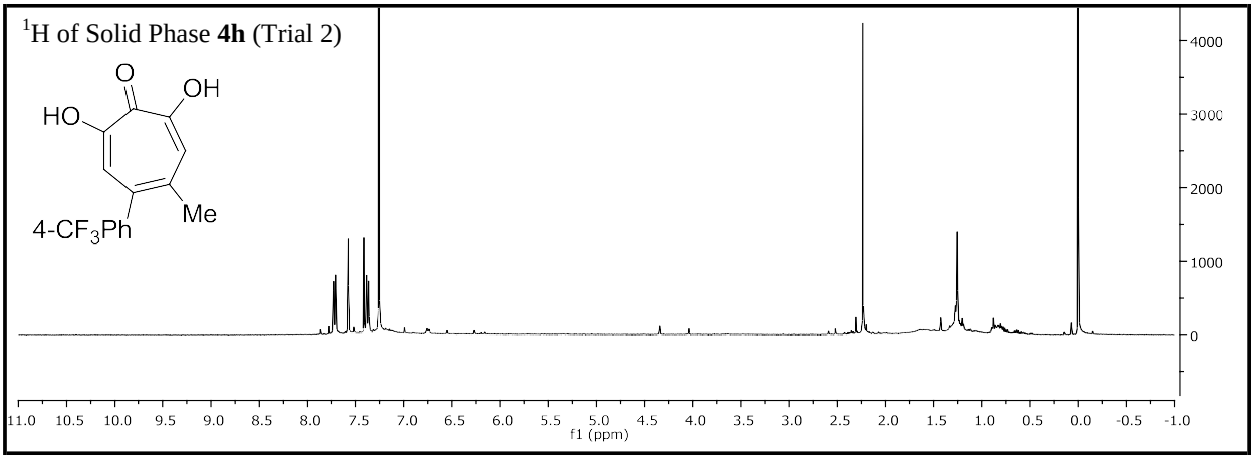


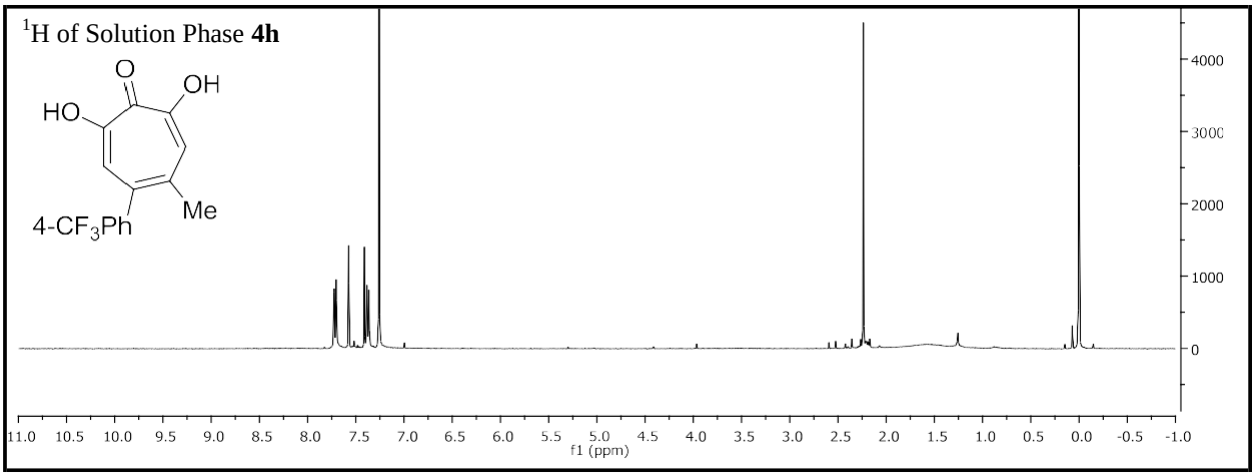




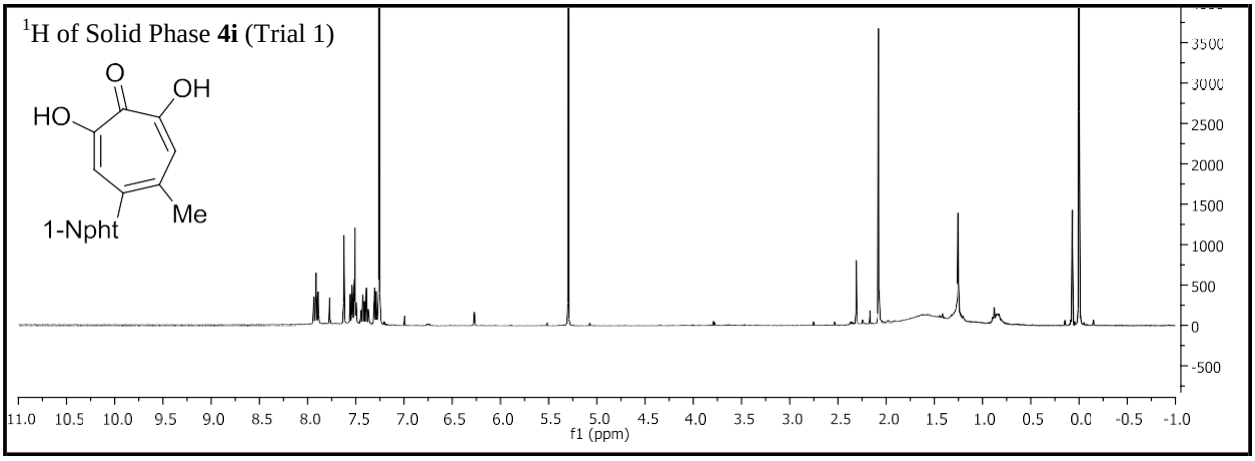
2,7-Dihydroxy-4-methyl-5-(4-(trifluoromethyl)phenyl)cycloheptа-2,4,6-trienone (4h)

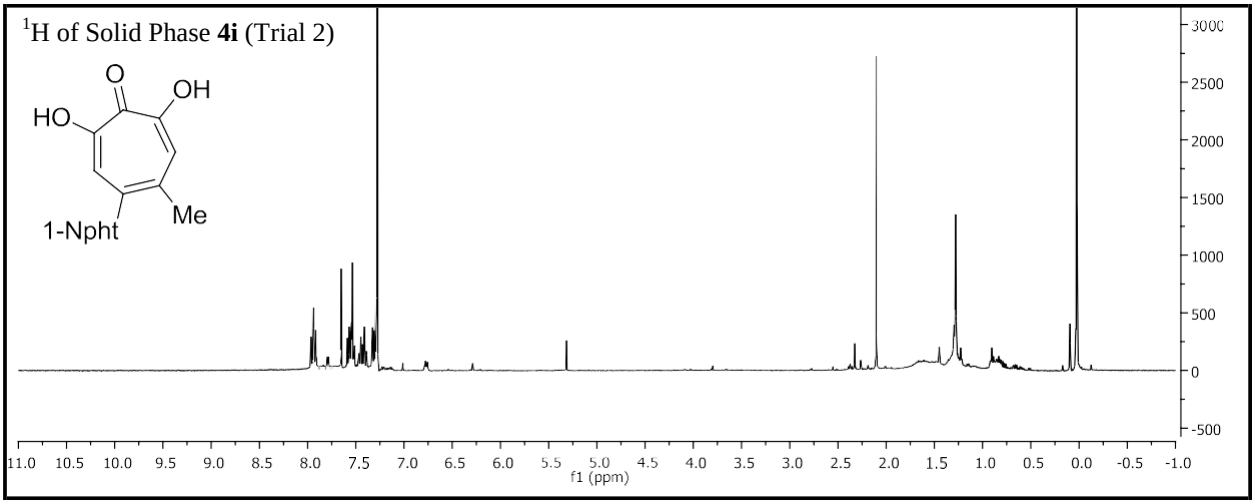


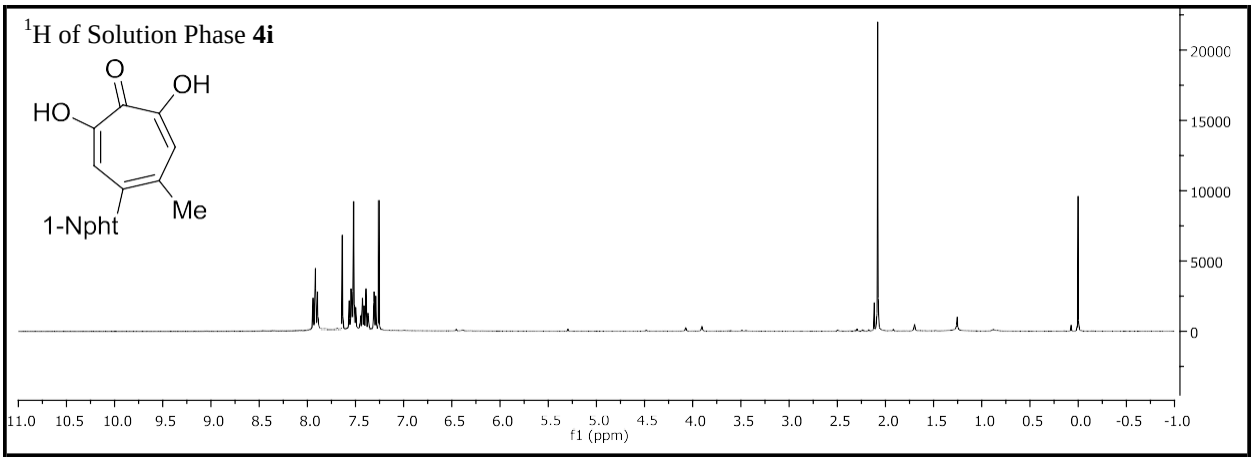




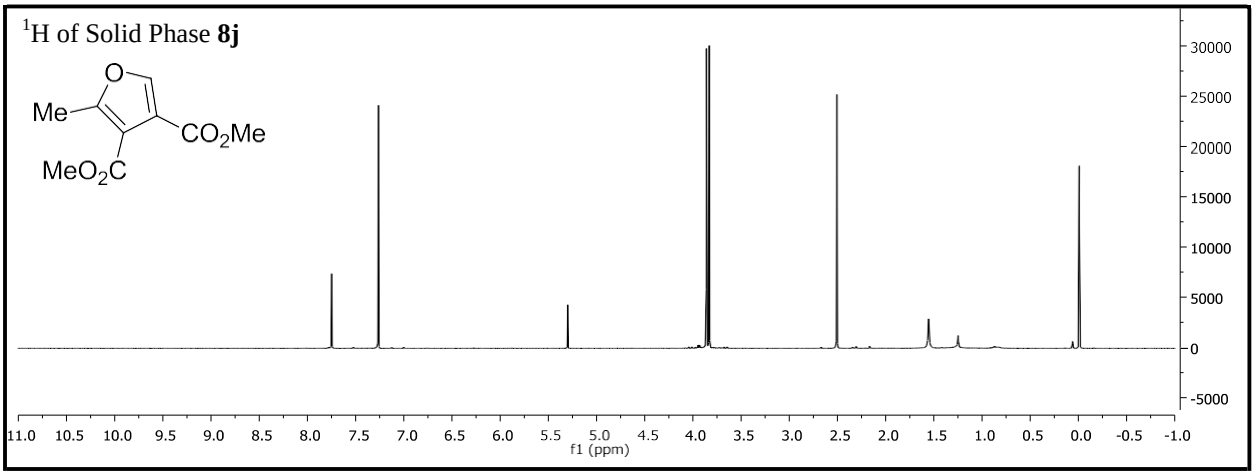
2,7-Dihydroxy-4-methyl-5-(naphthalen-1-yl)cyclohepta-2,4,6-trienone (4i)



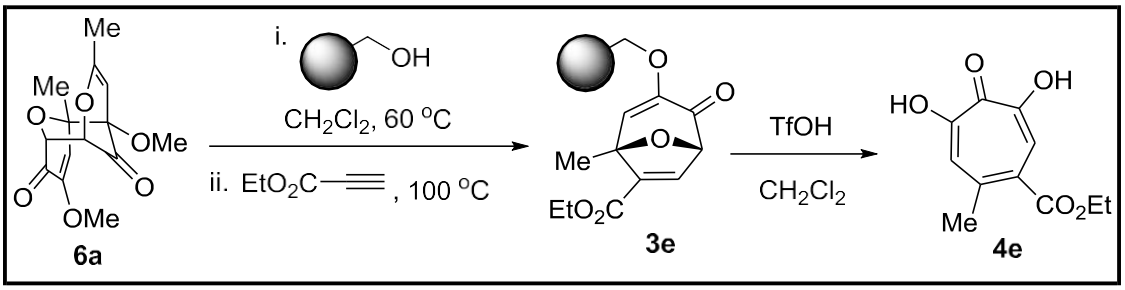




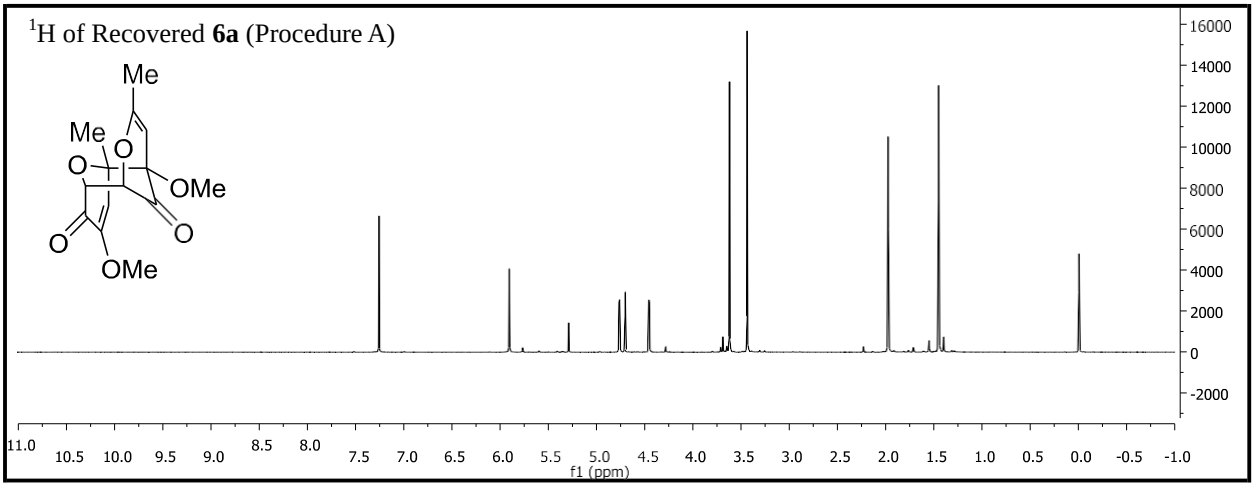
Dimethyl 2-methylfuran-3,4-dicarboxylate (8j)

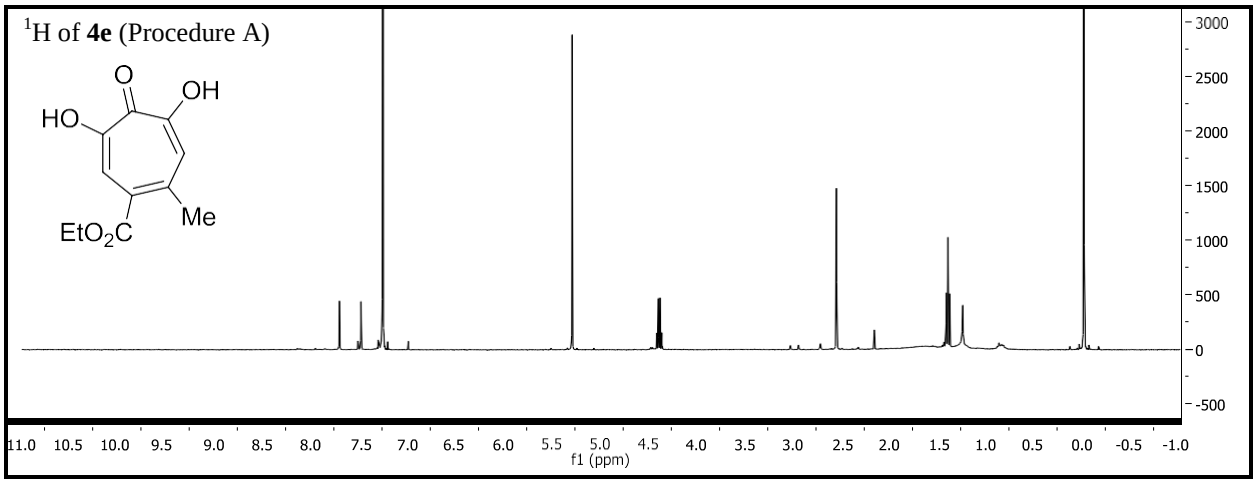


VI. Optimization of α-Hydroxytropolone Solid Phase Synthesis Using Oxidopyrylium Dimer

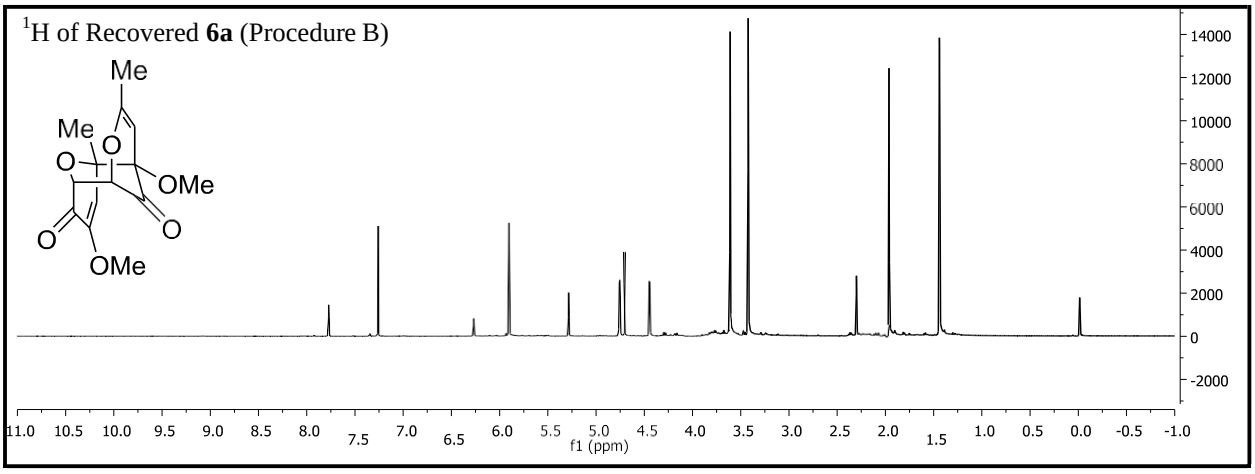


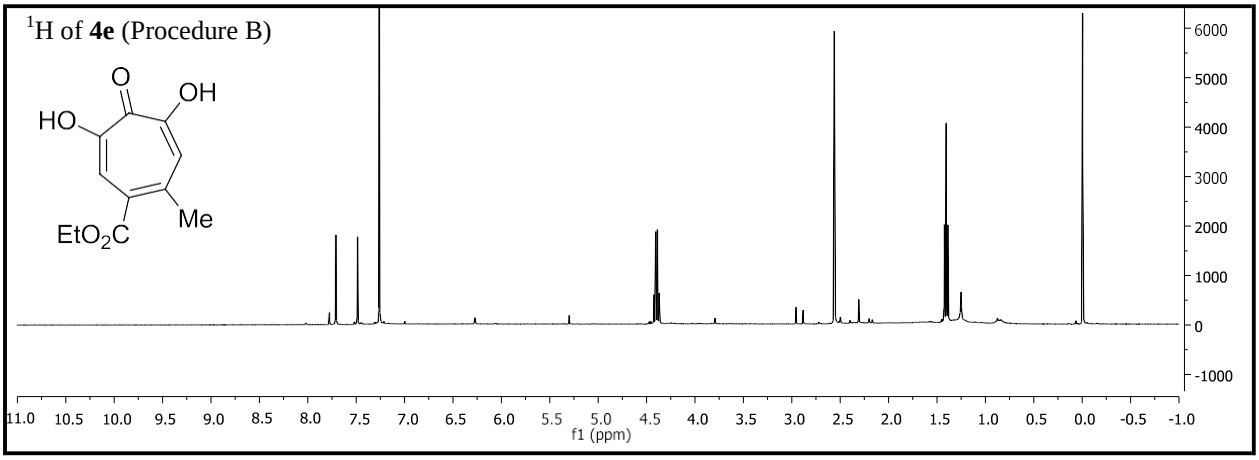
Procedure A: Polystyrene supported benzyl alcohol (3.5 mmol/g, 33 mg, 0.114 mmol, 1 eq) was added to a microwave reactor vial and allowed to swell for 30 min in CH_2Cl_2 before the addition of oxidopyrylium dimer **6a** (24 mg, 0.086 mmol, 0.74 eq), and the reaction mixture was gently stirred at 60 ºC for 14 h. The beads were rinsed with CH_2Cl_2 (6 X 1 mL), and the supernatant liquid was collected (16 mg of **6a**, 66% recovery). Ethyl propiolate (500 µL) was added to the reactor, and the mixture was heated to 100 ºC for 1.5 h. After the reaction was cooled to room temperature, the beads were rinsed with CH_2Cl_2 (6 X 1 mL) and the supernatant liquid was collected (3 mg, crude bicyclic intermediate). The resin was then resuspended in CH_2Cl_2 (500 µL) and trifluoromethanesulfonic acid (41 µL, 0.464 mmol, 4 eq) was added to the vessel. The mixture was stirred at room temperature for 30 min, diluted with CH_2Cl_2 (2 mL) and quenched with pH 7 phosphate buffer (10 mL). The aqueous layer was extracted with CH_2Cl_2 (3 X 5 mL), and combined organics were dried over Na_2SO_4, filtered, and concentrated to yield α-hydroxytropolone **4e** (1.5 mg, 6%).





Procedure B: Polystyrene supported benzyl alcohol (3.5 mmol/g, 33 mg, 0.114 mmol, 1 eq) was added to a microwave reactor vial and allowed to swell for 30 min in CH_2Cl_2 before the addition of oxidopyrylium dimer **6a** (120 mg, 0.428 mmol, 3.8 eq), and the reaction mixture was gently stirred at room temperature for 9 days. The beads were rinsed with CH_2Cl_2 (6 X 1 mL), and the supernatant liquid was collected (104 mg of **6a**, 87% recovery). Ethyl propiolate (500 µL) was added to the reactor, and the mixture was heated to 100 ºC for 1.5 h. After the reaction was cooled to room temperature, the beads were rinsed with CH_2Cl_2 (6 X 1 mL) and the supernatant liquid was collected (9 mg, crude bicyclic intermediate). The resin was then resuspended in CH_2Cl_2 (500 µL) and trifluoromethanesulfonic acid (41 µL, 0.464 mmol, 4 eq) was added to the vessel. The mixture was stirred at room temperature for 30 min, diluted with CH_2Cl_2 (2 mL) and quenched with pH 7 phosphate buffer (10 mL). The aqueous layer was extracted with CH_2Cl_2 (3 X 5 mL), and combined organics were dried over Na_2SO_4, filtered, and concentrated to yield α-hydroxytropolone **4e** (5 mg, 20%).




1H of 4e (Procedure B)
O
OH
HO
Me
EtO2C
6000
5000
4000
3000
2000
1000
0
-1000
11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)

VII. Biological Assays

RNase H Inhibitor Analysis. IC_{50} values were determined as previously reported[4] using an 18-nucleotide 3'-fluorescein-labeled RNA annealed to a complementary 18-nucleotide 5'-dabsyl-labeled DNA. Cleavage of the HIV-1 polypurine tract (PPT) primer was performed with a 29 nt Cy5-labeled RNA (5'-Cy5-*UUU UAA AAG AAA AGG GGG G*AC UGG AAG GG*-3', where *represents the PPT 3' terminus) hybridized to a 40 nt DNA (5'-ATT AGC CCT TCC AGT CCC CCC TTT TCT TTT AAA AAG TGG C-3'). The reaction was initiated by adding 1 μL of 100 mM $MgCl_2$ to 9 μL of mixture containing 4 ng enzyme, 200 nM substrate, 20 μM α-hydroxytropolones in 50 mM Tris, pH 8.0, 80 mM KCl, 2 mM DTT, and 10% DMSO at 37°C and quenched with 10 μL of a gel-loading buffer after 10 min. Hydrolysis products were fractionated by denaturing polyacrylamide gel electrophoresis and visualized by fluorescent imaging (Typhoon Trio+, GE Healthcare).

Differential Scanning Fluorimetry (ThermoFluor). Thermal stability assays were performed according to Nettleship et al.[5] To a LightCycler® 480 96-well plate (Roche) was added 1 μL of 1 mM each compound (in DMSO), followed by 49 μL of DSF buffer containing 1.6 μM HIV RT RNaseH, 20 mM HEPES, pH 7.5, 10 mM $MgCl_2$, 100 mM NaCl, and a 1:2000 dilution of Sypro® Orange dye (Invitrogen). The mixture was heated from 30 to 80°C in increments of 0.2 °C. Fluorescence intensity was measured using excitation/emission wavelengths of 483 nm and 568 nm, respectively. Changes in protein thermal stability (ΔT_m) upon compound binding were analyzed by using LightCycler® 480 Software. All assays were performed in duplicate.

HIV-1 Cytopathicity assay. This assay was conducted as previously reported.[4] Samples were dissolved in DMSO at 10 mM and diluted to a final high concentration of 50 μM in a 96-well assay plate, with 2-fold dilutions made to a low concentration of 0.78 μM. All samples were tested in duplicate. The HIV-1 virus strain RF was used to infect CEM-SS cells. Compound cytotoxicity was measured in the same assay plate using uninfected cells. Regression analysis was used to estimate the effective concentration (EC_{50}) as well as the cytotoxic concentration (CC_{50}).

[4] Budihas, S. R.; Gorshkova, I.; Gaidamakov, S.; Wamiru, A.; Bona, M. K.; Parniak, M. A.; Crouch, R. J.; McMahon, J. B.; Beutler, J. A.; Le Grice, S. F. J. *Nucleic Acids Res*. **2005**, *33*, 1249-1256.
[5] Nettleship, J. E.; Brown, J.; Groves, M. R.; Geerlof, A. *Methods Mol. Biol.*, **2008**, 426, 299-318.