Hirsch, D., Murelli, R.P. *et al.*

Synthesis and Biological Assessment of 3,7-Dihydroxytropolones

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Electronic Supplementary Information

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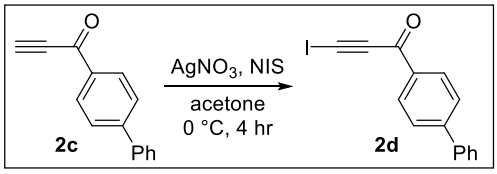
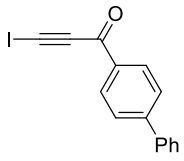
I. General Information

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 the reaction. ^1H NMR shifts are measured using the solvent residual peak as the internal standard ($CHCl_3$ δ 7.26, CD_3OD δ 3.31, $(CD_3)_2SO$ δ 2.50), 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), and integration. ^{13}C NMR shifts are measured using the solvent residual peak as the internal standard ($CDCl_3$ δ 77.2, CD_3OD δ 49.0, $(CD_3)_2SO$ δ 39.5), and reported as chemical shifts. Infrared (IR) spectral bands are characterized as broad (br), strong (s), medium (m), and weak (w). Microwave reactions were performed via the Biotage Initiator 2.5. Purification via normal phase column chromatography was performed on the Biotage Isolera Prime, with Biotage SNAP 10 g or 25 g cartridges, in a solvent system of ethyl acetate and hexanes. Reverse phase chromatography was performed on the Biotage Isolera Prime with Biotage SNAP C18 12 g cartridges, in a solvent system of water and acetonitrile with a 0.05% trifluoroacetic acid additive. Column gradients are measured in terms of column volumes (CV). Mass spectra were recorded on a spectrometer by the electrospray ionization (ESI) technique with a time-of-flight (TOF) mass analyzer.

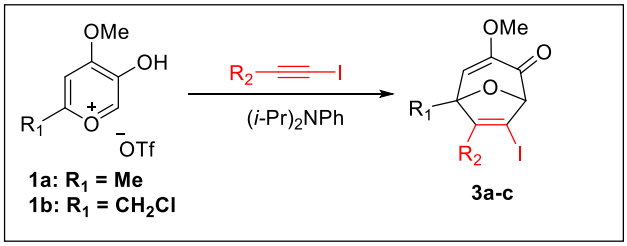
II. Synthesis and characterization of 1-([1,1'-biphenyl]-4-yl)-3-iodoprop-2-yn-1-one (2d)



Procedure: To a solution of 1-([1,1'-biphenyl]-4-yl)prop-2-yn-1-one **2c**[1] (228.0 mg, 1.105 mmol) in acetone (2.22 mL) was added N-iodosuccinimide (298.0 mg, 1.326 mmol). After stirring for 5 minutes, silver nitrate (9.3 mg, 0.055 mmol) was added slowly at 0 °C. The reaction was allowed to stir at 0 °C for 4 hours in the dark before being quenched by 10 mL of water. The reaction mixture was added to a separatory funnel containing 20 mL of CH_2Cl_2. The organic layer was isolated and the aqueous layer was extracted with CH_2Cl_2 (3 x 10 mL). Combined organics were extracted with 20 mL of water, 20 mL of saturated sodium bicarbonate solution, and 20 mL of aqueous sodium chloride. Combined organics were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting oil was purified by chromatography (Biotage Isolera Prime, 10 g silica gel column, solvent gradient: 1% EtOAc in hexanes (3 CV); 1-10% EtOAc in hexanes (15 CV)). Product fractions were concentrated to yield **2d** as a light yellow solid (211.5 mg, 57% yield). Melting point (mp) = 177-180°C. Rf= 0.10 in 5% EtOAc in hexanes. **IR (thin film, KBr)** 3054 (w), 3026 (w), 2916 (w), 2850 (w), 2149 (s), 1617 (s), 1597 (s), 1554 (w), 1472 (w), 1265 (w), 1048 (m), 856 (m), 777 (m), 740 (s), 691 (m) cm^{-1}. **^{1}H NMR (200 MHz, $CDCl_3$)** δ 8.23 – 8.17 (m, 2H), 7.74 – 7.69 (m, 2H), 7.66 – 7.61 (m, 2H), 7.51 – 7.39 (m, 3H). **^{13}C NMR (100 MHz, $CDCl_3$)** δ 176.1 (s), 147.3 (s), 139.8 (s), 135.1 (s), 130.6 (s), 129.2 (s), 128.7 (s), 127.5 (s), 127.5 (s), 94.2 (s), 19.1 (s). **HRMS (ESI+)** *m/z* calc'd for $C_{15}H_{10}IO^+$: 332.9771. Found: 332.9775.



III. Synthesis and characterization of iodo-8-oxabicyclo[3.2.1]octenes (3a-c)



[1] T. Schubert, W. Hummel, M.-R. Kula, M. Müller, *Eur. J. Org. Chem.*, **22**, 2001, 4181-4187.

General Procedure A. To a solution of salt **1**[2] and alkyne (10 equiv) in CH_2Cl_2 (0.5 M) was added N,N-diisopropylaniline (1.2 equiv). The reaction mixture was subjected to microwave irradiation at 120 °C for 20 minutes. The reaction mixture was then loaded directly onto column for chromatography and purified.

General Procedure B. To a solution of dimer **7b**[2] in CH_2Cl_2 (0.5 M) was added alkyne (20 equiv). The reaction was subjected to microwave irradiation at 100 °C for one hour. The reaction mixture was then loaded directly onto column for chromatography and purified.

Methyl 7-iodo-3-methoxy-5-methyl-2-oxo-8-oxabicyclo[3.2.1]octa-3,6-diene-6-carboxylate (3a).

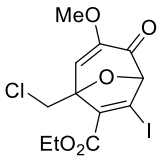
Procedure A: To a solution of salt **1a** (185.0 mg, 0.6373 mmol) and methyl 3-iodopropiolate[3] (1.3365 g, 6.3734 mmol) in CH_2Cl_2 (2 mL) was added N,N-diisopropylaniline (148.8 μL, 0.7649 mmol). After microwave irradiation at 120°C for 20 min, the reaction mixture was purified by chromatography (Biotage Isolera Prime, 25 g silica gel column, solvent gradient: 5% EtOAc in hexanes (3 CV); 5-10% EtOAc in hexanes (8 CV); 10-15% EtOAc in hexanes (10 CV); 15-20% EtOAc in hexanes (10 CV); 20-25% EtOAc in hexanes (10 CV); 25-35% EtOAc in hexanes (8 CV)). Product fractions were concentrated to yield **3a** as a yellow solid (126.2 mg, 57% yield). Melting point (mp) = 132-135 °C. Rf = 0.25 in 25% EtOAc in hexanes. **IR (thin film, KBr)** 2953 (w), 2840 (w), 1711 (s), 1610 (m), 1436 (w), 1379 (w), 1311 (m), 1205 (m), 1121 (w), 860 (w), 693 (w) cm^{-1}. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 6.06 (s, 1H), 5.03 (s, 1H), 3.84 (s, 3H), 3.55 (s, 3H), 1.75 (s, 3H). **^{13}C NMR (100 MHz, $CDCl_3$)** δ 186.9 (s), 163.0 (s), 151.0 (s), 145.1 (s), 119.2 (s), 103.4 (s), 93.8 (s), 88.0 (s), 54.9 (s), 52.3 (s), 21.8 (s). **HRMS (ESI+)** *m/z* calc'd for $C_{11}H_{12}IO_5^+$: 350.9724. Found: 350.9752.

Ethyl 7-iodo-3-methoxy-5-methyl-2-oxo-8-oxabicyclo[3.2.1]octa-3,6-diene-6-carboxylate (3b).

Procedure A: To a solution of salt **1a** (12.47 mg, 0.0430 mmol) and ethyl 3-iodopropiolate[3] (0.0963 g, 0.430 mmol) in CH_2Cl_2 (0.2 mL) was added N,N-diisopropylaniline (10.03 μL, 0.0516 mmol). After microwave irradiation at 120 °C for 20 min, the reaction mixture was purified by chromatography (Biotage Isolera Prime, 10 g silica gel column, solvent gradient: 5% EtOAc in hexanes (3 CV); 5-10% EtOAc in hexanes (8 CV); 10-15% EtOAc in hexanes (10 CV); 15-20% EtOAc in hexanes (10 CV); 20-25% EtOAc in hexanes (10 CV); 25-35% EtOAc in hexanes (8 CV)). Product fractions were concentrated to yield **3b** as a yellow oil (15.3 mg, 98% yield). Rf= 0.29 in 25% EtOAc in hexanes. **IR (thin film, KBr)** 2981 (w), 2936 (w), 1710 (s), 1609 (m), 1448 (w), 1324 (w), 1307 (w), 1259 (m), 1122 (w), 869 (w), 693 (m) cm^{-1}. **^{1}H NMR (200 MHz, $CDCl_3$)** δ 6.02 (s, 1H), 4.96 (s, 1H), 4.24 (q, *J* = 11.1 Hz, 2H), 3.50 (s, 3H), 1.70 (s, 3H), 1.32 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR (100 MHz, $CDCl_3$)** δ 187.0 (s), 162.6 (s), 150.9 (s), 145.1 (s), 119.3 (s), 103.0 (s), 93.7 (s), 88.0 (s), 61.7 (s), 55.0 (s), 21.7 (s), 14.3 (s). **HRMS (ESI+)** *m/z* calc'd for $C_{12}H_{14}IO_5^+$: 364.9880. Found: 364.9886.

[2] C. Meck, N. Mohd and R.P. Murelli, *Org. Lett.*, 2012, **14**, 5988-5991.
[3] B.M. Kuijpers, Triazole-linked glycosyl amino acids and peptides: synthesis, scope, and applications. Ph.D. Dissertation, Radboud University Nijmegen, Netherlands, 2008.

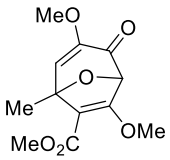
Ethyl 5-(chloromethyl)-7-iodo-3-methoxy-2-oxo-8-oxabicyclo[3.2.1]octa-3,6-diene-6-carboxylate (3c).



Procedure B: To a solution of dimer **7b** (369.4 mg, 1.06 mmol) in CH_2Cl_2 (2.12 mL) was added ethyl 3-iodopropiolate[3] (4.86 g, 21.7 mmol). After microwave irradiation at 100 °C for one hour, the reaction mixture was purified by chromatography (Biotage Isolera Prime, 50 g silica gel column, solvent gradient: 5% EtOAc in hexanes (3 CV); 5-10% EtOAc in hexanes (10 CV); 10-20% EtOAc in hexanes (10 CV); 20-35% EtOAc in hexanes (8 CV)). Product fractions were concentrated to yield **3c** as a yellow oil (596.0 mg, 71% yield). Rf = 0.32 in 25% EtOAc in hexanes. **IR (thin film, KBr)** 2980 (w), 2936 (w), 2839 (w), 1713 (s), 1613 (s), 1453 (w), 1369 (w), 1311 (m), 1270 (m), 1130 (w), 834 (w), 639 (m) cm^{-1}. **^{1}H NMR (200 MHz, $CDCl_3$)** δ 5.99 (s, 1H), 5.13 (s, 1H), 4.44 – 4.22 (m, 2H), 4.26 – 4.00 (dd, 2H), 3.59 (s, 3H), 1.38 (t, *J* = 7.1 Hz, 2H). **^{13}C NMR (100 MHz, $CDCl_3$)** δ 186.1 (s), 162.1 (s), 148.0 (s), 146.1 (s), 114.6 (s), 103.6 (s), 93.8 (s), 89.9 (s), 62.1 (s), 55.2 (s), 44.7 (s), 14.3 (s). **HRMS (ESI+)** *m/z* calc'd for $C_{12}H_{12}ClINaO_5^+$: 420.9310. Found: 420.9314.

IV. Synthesis and characterization of methoxy-8-oxabicyclo[3.2.1]octenes (4a-d)

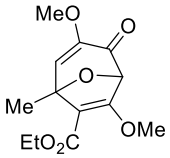
Methyl 3,7-dimethoxy-5-methyl-2-oxo-8-oxabicyclo[3.2.1]octa-3,6-diene-6-carboxylate (4a).



Procedure: To a solution of bicycle **3a** (8.6 mg, 0.025 mmol) in methanol (1.23 mL) was added 4-dimethylaminopryidine (3.0 mg, 0.025 mmol). After microwave irradiation at 120°C for 20 min, the reaction mixture was concentrated under reduced pressure, taken up in CH_2Cl_2 and purified by chromatography (Biotage Isolera Prime, 10 g silica gel column, solvent gradient: 5% EtOAc in hexanes (3 CV); 5-35% EtOAc in hexanes (40 CV)). Product fractions were concentrated to yield **4a** as a clear oil (5.8 mg, 94% yield). Melting point (mp) = 138-141 °C. Rf= 0.19 in 25% EtOAc in hexanes. **IR (thin film, KBr)** 2953 (w), 1696 (s), 1635 (s), 1606 (s), 1449 (w), 1371 (m), 1209 (m), 1112 (s), 834 (w), 723 (m) cm^{-1}. **^{1}H NMR (200 MHz, $CDCl_3$)** δ 6.20 (s, 1H), 5.05 (s, 1H), 3.96 (s, 3H), 3.76 (s, 3H), 3.56 (s, 3H), 1.75 (s, 3H). **^{13}C NMR (100 MHz, $CDCl_3$)** δ 188.8 (s), 168.7 (s), 163.8 (s), 145.1 (s), 121.8 (s), 114.2 (s), 86.0 (s), 83.4 (s), 60.7 (s), 54.9 (s), 51.7 (s), 23.0 (s). **HRMS (ESI+)** *m/z* calc'd for $C_{12}H_{15}O_6^+$: 255.0863. Found: 255.0873.

Ethyl 3,7-dimethoxy-5-methyl-2-oxo-8-oxabicyclo[3.2.1]octa-3,6-diene-6-carboxylate (4b).

Procedure: To a solution of bicycle **3b** (155.1 mg, 0.4259 mmol) in methanol (12 mL) was added 4-dimethylaminopryidine (139.5 mg, 1.142 mmol). After microwave irradiation at 120 °C for 20 min, the reaction mixture was purified by chromatography (Biotage Isolera Prime, 10 g silica gel column, solvent gradient: 5% EtOAc in hexanes (3 CV); 5-35% EtOAc in hexanes (35 CV)). Product fractions were concentrated to yield **4b** as a clear oil (73.85 mg, 64% yield). Melting point (mp) = 84-86 °C. Rf = 0.48 in 50% EtOAc in hexanes. **IR (thin film, KBr)** 2937 (w), 1709 (s), 1692 (s), 1635 (m), 1604 (m), 1451 (w), 1352 (w), 1241 (w), 1132 (w), 1112 (w), 986 (m), 723 (w) cm^{-1}. **^{1}H NMR (200 MHz, $CDCl_3$)** δ 6.20 (s, 1H), 5.04 (s, 1H), 4.23 (q, *J* = 7.1 Hz, 2H), 3.97 (s, 1H), 3.58 (s, 1H), 1.75 (s, 3H), 1.31 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR**



(100 MHz, $CDCl_3$) δ 188.7 (s), 168.2 (s), 163.2 (s), 144.9 (s), 121.7 (s), 114.2 (s), 85.8 (s), 83.3 (s), 60.5 (s), 60.4 (s), 54.7 (s), 22.9 (s), 14.3 (s). **HRMS (ESI+)** *m/z* calc'd for $C_{13}H_{17}O_6^+$: 269.1020. Found: 269.1019.

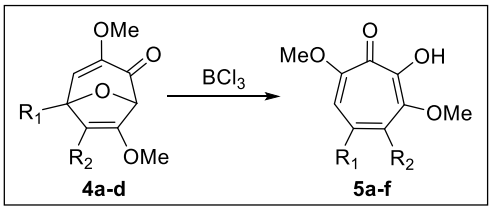
Ethyl 5-(chloromethyl)-3,7-dimethoxy-2-oxo-8-oxabicyclo[3.2.1]octa-3,6-diene-6-carboxylate (4c).

Procedure: To a solution of bicycle **3c** (348.9 mg, 0.8753 mmol) in methanol (43 mL) was added 4-dimethylaminopryidine (106.9 mg, 0.8753 mmol) in a sealed tube. After heating at 150 °C for 20 min in a silicon oil bath, the reaction mixture was purified by chromatography (Biotage Isolera Prime, 25 g silica gel column, solvent gradient: 5% EtOAc in hexanes (3 CV); 5-35% EtOAc in hexanes (30 CV)). Product fractions were concentrated to yield **4c** as a clear oil (161.1 mg, 61% yield). R_f = 0.24 in 25% EtOAc in hexanes. **IR (thin film, KBr)** 2980 (w), 1714 (s), 1690 (m), 1637 (m), 1607 (m), 1464 (w), 1380 (w), 1224 (m), 1133 (m), 1072 (w), 983 (w), 725 (w) cm^{-1}. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 6.13 (s, 1H), 5.11 (s, 1H), 4.21 (qd, *J* = 7.1, 2.8 Hz, 2H), 4.14 – 4.05 (m, 2H), 3.98 (s, 3H), 3.57 (s, 3H), 1.28 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR (100 MHz, $CDCl_3$)** δ 188.0 (s), 168.7 (s), 162.9 (s), 146.2 (s), 117.3 (s), 111.8 (s), 87.9 (s), 83.6 (s), 61.0 (s), 60.9 (s), 55.2 (s), 46.3 (s), 14.5 (s). **HRMS (ESI+)** *m/z* calc'd for $C_{13}H_{16}ClO_6^+$: 303.0630. Found: 303.0634.

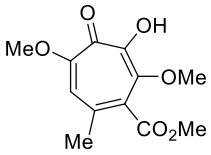
6-([1,1'-biphenyl]-4-carbonyl)-3,7-dimethoxy-5-methyl-8-oxabicyclo[3.2.1]octa-3,6-dien-2-one (4d).

Procedure: To a solution of dimer **7a** (85.0 mg, 0.303 mmol) in CH_2Cl_2 (606 μL) was added **2d** (201.0 mg, 0.607 mmol). After microwave irradiation at 120 °C for 30 minutes, the reaction mixture was concentrated under reduced pressure to yield a brown oil 6-([1,1'-biphenyl]-4-carbonyl)-7-iodo-3-methoxy-5-methyl-8-oxabicyclo[3.2.1]octa-3,6-dien-2-one (315.6 mg). To a solution of this crude iodobicycle (315.6 mg) in methanol (5.01 mL) was added 4-dimethylaminopryidine (74 mg, 0.606 mmol). After microwave irradiation at 70 °C for 15 min, the reaction mixture was concentrated under reduced pressure, taken up in CH_2Cl_2 and purified by chromatography (Biotage Isolera Prime, 10 g silica gel column, solvent gradient: 5% EtOAc in hexanes (3 CV); 5-35% EtOAc in hexanes (40 CV)). Product fractions were concentrated to yield **4d** as a clear oil (154.68 mg, 68% yield over 2 steps). Melting point (mp) = 138-141 °C. Rf= 0.19 in 25% EtOAc in hexanes. **IR (thin film, KBr)** 3060 (w), 3031 (w), 2979 (w), 2855 (w), 2839 (w), 1709 (s), 1616 (s), 1601 (s), 1511 (m), 1449 (m), 1370 (m), 1345 (m), 1299 (m), 1132 (m), 1109 (m), 933 (m), 755 (m) cm^{-1}. **^{1}H NMR (200 MHz, $CDCl_3$)** δ 7.89 (d, *J* = 8.2 Hz, 2H), 7.70 - 7.62 (m, 4H) 7.47 (t, *J* = 7.6 Hz, 2H), 7.42 – 7.36 (m, 1H), 6.42 (s, 1H), 5.21 (s, 1H), 3.77 (s, 3H), 3.62 (s, 3H), 1.73 (s, 3H). **^{13}C NMR (100 MHz, $CDCl_3$)** δ 189.8 (s), 188.5 (s), 165.7 (s), 145.7 (s), 145.0 (s), 140.0 (s), 137.0 (s), 129.8 (s), 129.0 (s), 128.2 (s), 127.3 (s), 126.9 (s), 122.5 (s), 122.3 (s), 87.4 (s), 83.4 (s), 60.3 (s), 54.8 (s), 22.2 (s). **HRMS (ESI+)** *m/z* calc'd for $C_{23}H_{21}O_5^+$: 377.1384. Found: 377.1380.

V. Synthesis and characterization of 3,7-dimethoxytropolones via BCl_3



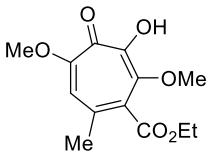
Methyl 6-hydroxy-4,7-dimethoxy-2-methyl-5-oxocyclohepta-1,3,6-triene-1-carboxylate (5a).



Procedure: To a solution of bicyclic compound **4a** (6.0 mg, 0.0236 mmol) in CH_2Cl_2 (1.69 mL) was added a 1M solution of BCl_3 in CH_2Cl_2 (165.2 µL, 0.1652 mmol). The reaction was allowed to stir at room temperature for 10 minutes before being quenched to pH 7 with pH 7 phosphate buffer. The organic layer was isolated and the aqueous layer was extracted with CH_2Cl_2 (5 x 10 mL). Combined organics were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to yield **5a** as a red solid (5.8 mg, 97% yield). Melting point (mp) = 93-97 °C. **IR (thin film, KBr)** 3192 (br), 2950 (w), 1734 (m), 1554 (w), 1459 (w), 1329 (s), 1268 (s), 1217 (s), 1138 (m), 1078 (w), 923 (w), 796 (w). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 6.89 (s, 1H), 3.98 (s, 3H), 3.95 (s, 3H), 3.93 (s, 3H), 2.37 (s, 3H). **^{13}C NMR (100 MHz, $CDCl_3$)** δ 168.6 (s), 167.7 (s), 158.3 (s), 155.2 (s), 150.1 (s), 134.0 (s), 131.1 (s), 119.5 (s), 61.4 (s), 56.8 (s), 52.8 (s), 24.9 (s). **HRMS (ESI+)** *m/z* calc'd for $C_{12}H_{14}NaO_6^+$: 277.0683. Found: 277.0689.

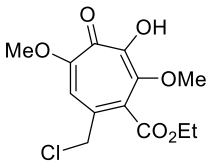
Ethyl 6-hydroxy-4,7-dimethoxy-2-methyl-5-oxocyclohepta-1,3,6-triene-1-carboxylate (5b).

Procedure: To a solution of bicyclic compound **4b** (20.9 mg, 0.0780 mmol) in CH_2Cl_2 (5.6 mL) was added a 1M solution of BCl_3 in CH_2Cl_2 (546 µL, 0.546 mmol). The reaction was allowed to stir at room temperature for 10 minutes before being quenched to pH 7 with pH 7 phosphate buffer. The organic layer was isolated and the aqueous layer was extracted with CH_2Cl_2 (5 x 10 mL). Combined organics were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to yield **5b** as a yellow oil (16.9 mg, 81% yield). **IR (thin film, KBr)** 3734 (w), 2940 (w), 1731 (s), 1553 (s), 1454 (w), 1328 (m), 1267 (m), 1217 (s), 1138 (s), 1017 (w), 901 (w), 669 (s). **^{1}H NMR (200 MHz, $CDCl_3$)** δ 6.89 (s, 1H), 4.41 (q, *J* = 7.1 Hz, 2H), 3.98 (s, 3H), 3.96 (s, 3H), 2.39 (s, 3H), 1.39 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR (100 MHz, $CDCl_3$)** δ 168.3 (s), 167.0 (s), 158.0 (s), 155.1 (s), 149.9 (s), 134.1 (s), 130.9 (s), 119.3 (s), 61.8 (s), 61.2 (s), 56.6 (s), 24.6 (s), 14.2 (s). **HRMS (ESI+)** *m/z* calc'd for $C_{13}H_{17}O_6^+$: 269.1020. Found: 269.1016.



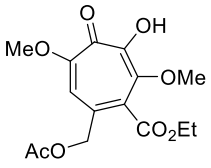
Ethyl 2-(chloromethyl)-6-hydroxy-4,7-dimethoxy-5-oxocyclohepta-1,3,6-triene-1-carboxylate (5c).

Procedure: To a solution of 1M BCl_3 in CH_2Cl_2 (80.6 μL, 0.0806 mmol) was added a solution of bicyclic compound



4c (6.1 mg, 0.0202 mmol) in CH_2Cl_2 (2.88 mL) at 0 °C. After stirring for 6 minutes, the reaction was slowly added to 6 mL of pH 5 phosphate buffer in a separatory funnel. After shaking, the pH of the aqueous layer was further adjusted to pH 4 via the gradual addition of an additional 12 mL of pH 5 phosphate buffer. The organic layer was isolated and the aqueous layer was extracted with CH_2Cl_2 (5 x 5 mL). Combined organics were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to yield **5c** as a brown oil (4.7 mg, 77% yield) which was immediately taken on to the next step. **IR (thin film, KBr)** 2924 (w), 2851 (w), 1731 (s), 1558 (m), 1464 (w), 1335 (m), 1268 (s), 1218 (m), 1135 (w), 1047 (w), 941 (w), 669 (w). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.07 (s, 1H), 4.48 (s, 2H), 4.45 (q, *J* = 7.2 Hz, 2H), 4.04 (s, 3H), 3.98 (s, 3H), 1.42 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR (100 MHz, $CDCl_3$)** δ 169.4 (s), 166.0 (s), 158.7 (s), 155.8 (s), 149.0 (s), 134.7 (s), 129.1 (s), 117.6 (s), 62.4 (s), 61.3 (s), 56.8 (s), 47.2 (s), 14.2 (s). **HRMS (ESI+)** *m/z* calc'd for $C_{13}H_{15}ClNaO_6^+$: 325.0449. Found: 325.0443.

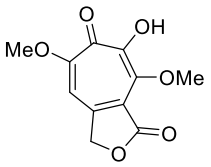
Ethyl 2-(acetoxymethyl)-6-hydroxy-4,7-dimethoxy-5-oxocyclohepta-1,3,6-triene-1-carboxylate (5d).



Procedure: To a solution of dimethoxytropolone **5c** (16.8 mg, 0.055 mmol) in acetic acid (5.55 mL) was added sodium acetate (91.05 mg, 1.11 mmol). The reaction was allowed to stir at room temperature for 15 hours before being quenched to pH 3 with pH 7 phosphate buffer. The organic layer was isolated and the aqueous layer was extracted with CH_2Cl_2 (5 x 10 mL). Combined organics were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to yield **5d** as a brown/yellow oil (14.7 mg, 93% yield). **IR (thin film, KBr)** 3734 (w), 2950 (br), 1737 (s), 1558 (w), 1462 (w), 1366 (w), 1333 (w), 1221 (s), 1139 (w), 1073 (w), 669 (w). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.14 (s, 1H), 5.03 (s, 2H), 4.43 (q, *J* = 7.1 Hz, 2H), 4.02 (s, 3H), 3.98 (s, 3H), 2.12 (s, 3H), 1.40 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR (100 MHz, $CDCl_3$)** δ 170.6 (s), 168.8 (s), 166.2 (s), 158.4 (s), 156.8 (s), 149.8 (s), 135.2 (s), 128.2 (s), 118.0 (s), 66.9 (s), 62.4 (s), 61.5 (s), 57.0 (s), 21.0 (s), 14.3 (s). **HRMS (ESI+)** *m/z* calc'd for $C_{15}H_{18}NaO_8^+$: 349.0894. Found: 349.0898.

7-Hydroxy-5,8-dimethoxy-1H-cyclohepta[c]furan-1,6(3H)-dione (5e).

Procedure A: A solution of dimethoxytropolone **5d** (12.9 mg, 0.034 mmol) in 2N aqueous NaOH (4.78 mL) was

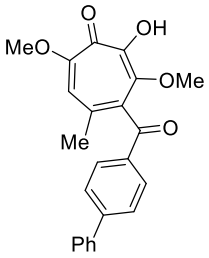


allowed to stir at room temperature for 3 hours before being diluted with 10 mL of CH_2Cl_2 and quenched to pH 3 with pH 3 phosphate buffer. The organic layer was isolated and the aqueous layer was extracted with CH_2Cl_2 (5 x 10 mL). Combined organics were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to yield **5e** as a yellow solid (9.1 mg, 81% yield).

Procedure B: Dimethoxytropolone **5c** (4.6 mg, 0.015 mmol) was subjected to reverse phase column chromatography conditions (Biotage Isolera Prime, SNAP 12g C18 silica gel column, solvent gradient: 5% acetonitrile in water (3 CV); 5-100% acetonitrile in water (35 CV); acetonitrile and water each contained 0.05% TFA). Product fractions were

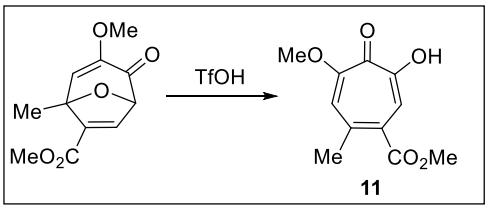
concentrated to yield **5e** as a yellow solid (2.9 mg, 81% yield). Melting point (mp) = 180-184 °C. Rf= 0.36 in 10% methanol in dichloromethane. **IR (thin film, KBr)** 3734 (w), 3217 (br), 2945 (w), 1760 (s), 1573 (s), 1457 (w), 1338 (m), 1284 (m), 1122 (w), 1051 (s), 868 (w), 668 (s). **^{1}H NMR (400 MHz, $CDCl_3$)** δ 6.83 (s, 1H), 5.17 (s, 2H), 4.11 (s, 3H), 4.09 (s, 3H). **^{13}C NMR (100 MHz, $CDCl_3$)** δ 171.7 (s), 168.5 (s), 162.4 (s), 154.7 (s), 149.6 (s), 144.6 (s), 119.2 (s), 106.6 (s), 69.8 (s), 62.1 (s), 57.4 (s). **HRMS (ESI+)** *m/z* calc'd for $C_{11}H_{11}O_6^+$: 239.0550. Found: 239.0558.

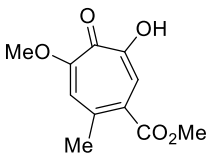
4-([1,1'-biphenyl]-4-carbonyl)-2-hydroxy-3,7-dimethoxy-5-methylcyclohepta-2,4,6-trien-1-one (5f).



Procedure: To a solution of 1M BCl_3 in CH_2Cl_2 (531.0 µL, 0.531 mmol) was added a solution of bicyclic compound **4d** (50.0 mg, 0.133 mmol) in CH_2Cl_2 (19.0 mL) at 0 °C. After stirring for 6 minutes at 0 °C, the reaction mixture was quenched to pH 7 with pH 7 phosphate buffer. The organic layer was isolated and the aqueous layer was extracted with CH_2Cl_2 (5 x 10 mL). Combined organics were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to yield a crude mixture of brown oil. This crude mixture was purified by reverse phase chromatography (Biotage Isolera Prime, 12 g C18 gel column, solvent gradient: 40% acetonitrile in water (3 CV); 40-55% acetonitrile in water (20 CV)). Product fractions were concentrated to yield **5f** as a clear, yellow oil (32.2 mg, 64% yield). Melting point (mp) = 150-153 °C. **IR (thin film, KBr)** 2940 (w), 2851 (w), 1674 (m), 1602 (m), 1312 (w), 1264 (w), 1092 (w) cm^{-1}. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.90 (d, *J* = 8.4 Hz, 2H), 7.69 (d, *J* = 8.6 Hz, 2H), 7.64-7.60 (m, 2H), 7.51-7.38 (m, 3H), 6.98 (s, 1H), 4.04 (s, 3H), 3.79 (s, 3H), 2.28 (s, 3H). **^{13}C NMR (100 MHz, $CDCl_3$)** δ 194.5 (s), 168.2 (s), 158.0 (s), 155.6 (s), 150.9 (s), 146.9 (s), 139.8 (s), 138.3 (s), 135.1 (s), 131.4 (s), 129.8 (s), 129.1 (s), 128.6 (s) 127.8 (s) 127.5 (s), 120.3 (s), 61.1 (s), 56.8 (s), 24.6 (s). HRMS (ESI+) *m/z* calc'd for $C_{23}H_{21}O_5^+$: 377.1384. Found: 377.1378.

VI. Synthesis and characterization of α-methoxytropolone via TfOH



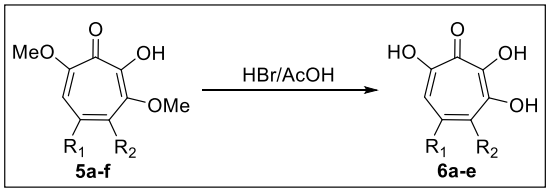


Methyl 6-hydroxy-4-methoxy-2-methyl-5-oxocyclohepta-1,3,6-triene-1-carboxylate (11). To a solution of methyl 3-methoxy-5-methyl-2-oxo-8-oxabicyclo[3.2.1]octa-3,6-diene-6-carboxylate[4] (484 mg, 2.16 mmol) in CH_2Cl_2 (22 mL) was added triflic acid (763 µL, 8.65 mmol). The reaction was allowed to stir for 30 min at rt before quenching with pH 7 phosphate

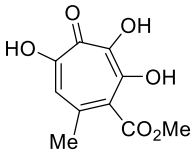
[4] M.P. D'Erasmo, T. Masaoka, J.A. Wilson, E.M. Hunte, Jr., J.A. Beutler, S.F.J. Le Grice and R.P. Murelli, *Med. Chem. Commun.*, 2016, **9**, 1789-1792.

buffer. The reaction mixture was then extracted with CH_2Cl_2 (3 x 20 mL), and the combined organics were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to yield **9** as a brown solid (377 mg, 78% yield). Product can be further purified via crystallization in MeOH to yield a yellow solid (233 mg, 48% yield). Melting point (mp) = 168-171 °C. **IR (thin film, KBr)** 3251 (br), 2918 (w), 2848 (w), 1717 (s), 1554 (m), 1483 (w), 1457 (m), 1335 (s), 1297 (m), 1220 (s), 1139 (w), 1053 (s), 907 (w), 788 (m) cm^{-1}. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.56 (s, 1H), 6.99 (s, 1H), 3.99 (s, 3H), 3.89 (s, 3H), 2.53 (s, 3H). **^{13}C NMR (100 MHz, $CDCl_3$)** δ 171.0, 169.1, 159.6, 158.3, 137.4, 131.5, 121.5, 117.4, 56.6, 52.9, 26.0. **HRMS (ESI+)** *m/z* calc'd for $C_{11}H_{13}O_5^+$: 225.0757. Found: 225.0753.

VII. Synthesis and characterization of 3,7-dihydroxytropolones via HBr/AcOH

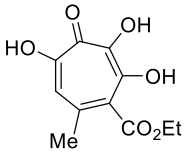


Methyl 4,6,7-trihydroxy-2-methyl-5-oxocyclohepta-1,3,6-triene-1-carboxylate (6a).



Procedure: To dimethoxytropolone **5a** (3.3 mg, 0.0130 mmol) was added 145 µL of 33% HBr/AcOH. The reaction was heated to reflux at 120 °C for 35 minutes before being quenched to pH 4 with pH 7 phosphate buffer. The organic layer was isolated and the aqueous later was extracted with CH_2Cl_2 (5 x 10 mL). Combined organics were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to yield **6a** as a red/brown solid (1.6 mg, 55%). Melting point (mp) = 131-135 °C. **IR (thin film, KBr)** 3198 (br), 2925 (w), 1733 (s), 1586 (w), 1525 (m), 1431 (s), 1325 (s), 1201 (s), 1057 (w), 878 (w), 796 (w). **^{1}H NMR (400 MHz, CD_3OD)** δ 7.00 (s, 1H), 3.91 (s, 3H), 2.32 (s, 3H). **^{13}C NMR (100 MHz, $CDCl_3$)** δ 167.2 (s), 157.3 (s), 154.5 (s), 153.4 (s), 152.3 (s), 136.8 (s), 125.0 (s), 120.8 (s), 52.1 (s), 24.7 (s). **HRMS (ESI+)** *m/z* calc'd for $C_{10}H_{11}O_6^+$: 227.0550. Found: 227.0556.

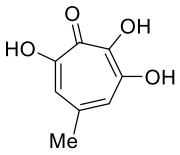
Ethyl 4,6,7-trihydroxy-2-methyl-5-oxocyclohepta-1,3,6-triene-1-carboxylate (6b).



Procedure: To dimethoxytropolone **5b** (15.5 mg, 0.0578 mmol) was added 682 µL of 33% HBr/AcOH in a sealed 0.5-2.0 mL sealed microwave vessel. The reaction was heated to reflux at 120 °C for 45 minutes in a silicon oil bath before being quenched to pH 4 with pH 7 phosphate buffer. The organic layer was isolated and the aqueous later was extracted with CH_2Cl_2 (5 x 10 mL). Combined organics were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to yield **6b** as a red/brown oil (12.0 mg, 87%). **IR (thin film, KBr)** 3210 (br), 2929 (w), 1730 (s), 1581 (w), 1445 (w), 1326 (w), 1193 (s), 1056 (m), 1012 (w), 860 (w), 779 (w) cm^{-1}. **^{1}H NMR (400 MHz, CD_3OD)** δ 7.02 (s, 1H), 4.39 (q, *J* = 6.8 Hz,

2H), 2.33 (s, 3H), 1.37 (t, *J* = 6.8 Hz, 3H). **^{13}C NMR (100 MHz, CD_3OD)** δ 169.3 (s), 158.7 (s), 157.5 (s), 157.4 (s), 153.2 (s), 137.0 (s), 126.9 (s), 121.0 (s), 62.8 (s), 24.4 (s), 14.4 (s). **HRMS (ESI+)** *m/z* calc'd for $C_{11}H_{12}NaO_6^+$: 263.0526. Found: 263.0528.

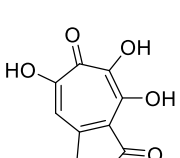
2,3,7-Trihydroxy-5-methylcyclohepta-2,4,6-trien-1-one (6c).



Procedure: To dimethoxytropolone **5a** (6.5 mg, 0.0256 mmol) was added 4.6 µL (10 equiv.) of water and 284 µL of 33% HBr/AcOH in a sealed 0.5-2.0 mL sealed microwave vessel. The reaction was heated to reflux at 120 °C for 35 minutes. The membrane of the sealed vessel was punctured with an 18G needle to release gas buildup approximately 10 minutes after subjecting reaction to heat. Upon completion, the reaction was quenched to pH 4 with pH 7 phosphate buffer. The organic layer was isolated and the aqueous later was extracted with CH_2Cl_2 (5 x 10 mL). Combined organics were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to yield **6c** as a red/brown oil (1.8 mg, 42%). **IR (thin film, KBr)** 3509 (br), 3218 (br), 2918 (m), 1591 (w), 1517 (m), 1434 (s), 1394 (w), 1339 (w), 1199 (s), 1092 (m), 1068 (w), 668 (m) cm^{-1}. **^{1}H NMR (400 MHz, CD_3OD)** δ 7.01 (s, 2H), 2.40 (s, 3H). **^{13}C NMR (100 MHz, CD_3OD)** δ 158.2 (s), 156.6 (s), 141.2 (s), 120.3 (s), 27.0 (s). **HRMS (ESI+)** *m/z* calc'd for $C_8H_9O_4^+$: 169.0495. Found: 169.0492.

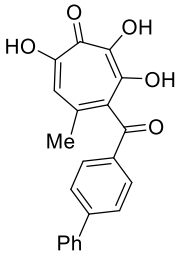
5,7,8-Trihydroxy-1H-cyclohepta[c]furan-1,6(3H)-dione (6d).

Procedure: To dimethoxytropolone **5e** (10.0 mg, 0.042 mmol) was added 453 µL of 33% HBr/AcOH in a sealed 0.5-2.0 mL sealed microwave vessel. The reaction was heated to reflux in a silicon oil bath at 120 °C for 30 minutes before being quenched to pH 1.5 with pH 5 phosphate buffer. The organic layer was isolated and the aqueous layer was extracted with CH_2Cl_2 (3 x 2 mL). Combined organics were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to yield **6d** as a yellow solid (5.0 mg, 57%). **IR (thin film, KBr)** 3502 (br), 3215 (br), 2962 (w), 2918 (s), 2849 (m), 1747 (m), 1622 (w), 1517 (w), 1260 (s), 1096 (m), 1022 (s), 799 (s) cm^{-1}. **^{1}H NMR (400 MHz, DMSO-d_6)** δ 6.83 (s, 1H), 5.17 (s, 2H). **^{13}C NMR (150 MHz, DMSO-d_6)** δ 171.0 (s), 164.7 (s), 159.9 (s), 154.3 (s), 150.9 (s), 149.1 (s), 109.8 (s), 105.1 (s), 69.7 (s). **HRMS (ESI+)** *m/z* calc'd for $C_9H_7O_6^+$: 211.0237. Found: 211.0231.



4-([1,1'-biphenyl]-4-carbonyl)-2,3,7-trihydroxy-5-methylcyclohepta-2,4,6-trien-1-one (6e).

Procedure: To dimethoxytropolone **5f** (10.6 mg, 0.0282 mmol) was added 500 µL of 33% HBr/AcOH. The reaction was heated to reflux at 120 °C for 50 minutes before being quenched to pH 4 with pH 7 phosphate buffer. During addition of pH 7 phosphate buffer to the solution, solid crashed out and the solution was filtered over cotton. The solid was taken up in CH_2Cl_2 (10 mL). This solution was dried over Na_2SO_4, filtered, and concentrated under reduced pressure to yield **6e** as a light brown solid (6.9 mg, 70%). Melting point (mp) = 183-186 °C. **IR (thin film, KBr)** 3447 (br), 2925 (w), 2937 (m), 2906 (w), 2856 (m), 1602 (m), 11404 (m), 753 (w), 721 (s) cm^{-1}. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.92 (d, *J* = 8.3 Hz, 2H), 7.69 (d, *J* = 8.4, 2H), 7.65 – 7.59 (m, 2H) 7.50 – 7.39(m, 3H), 7.21 (s, 1H), 2.30 (s, 3H). **^{13}C NMR (100 MHz, $CDCl_3$)** δ 194.2 (s), 157.6 (s), 154.4 (s), 153.5 (s), 152.2 (s), 147.1 (s), 139.7 (s), 137.4 (s), 134.5

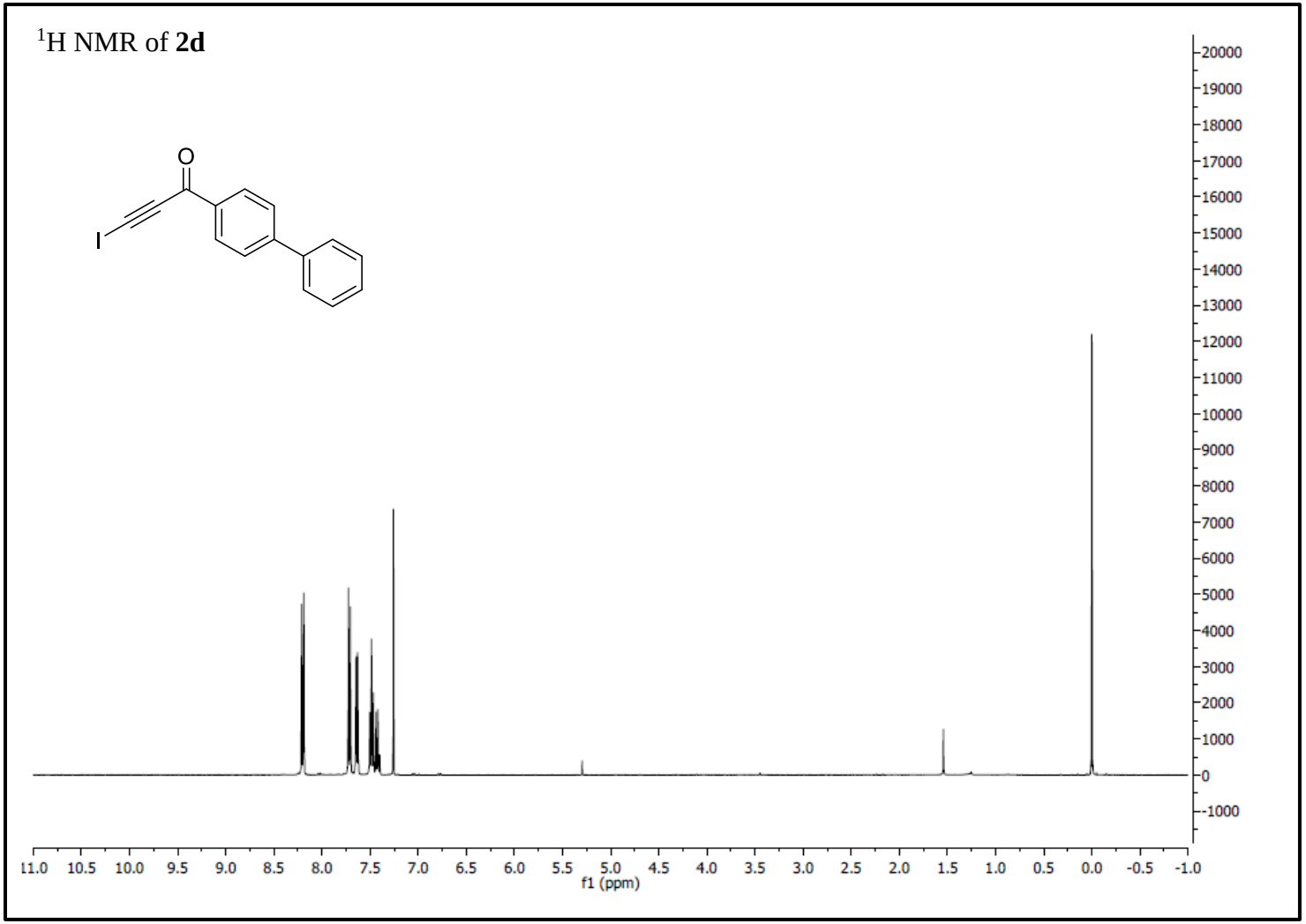


(s), 129.8 (s), 129.5 (s), 129.0 (s), 128.5 (s), 127.7 (s), 127.4 (s), 121.5 (s), 24.7 (s). **HRMS (ESI+)** *m/z* calc'd for $C_{21}H_{15}O_4^+$: 331.0965. Found: 331.0961.

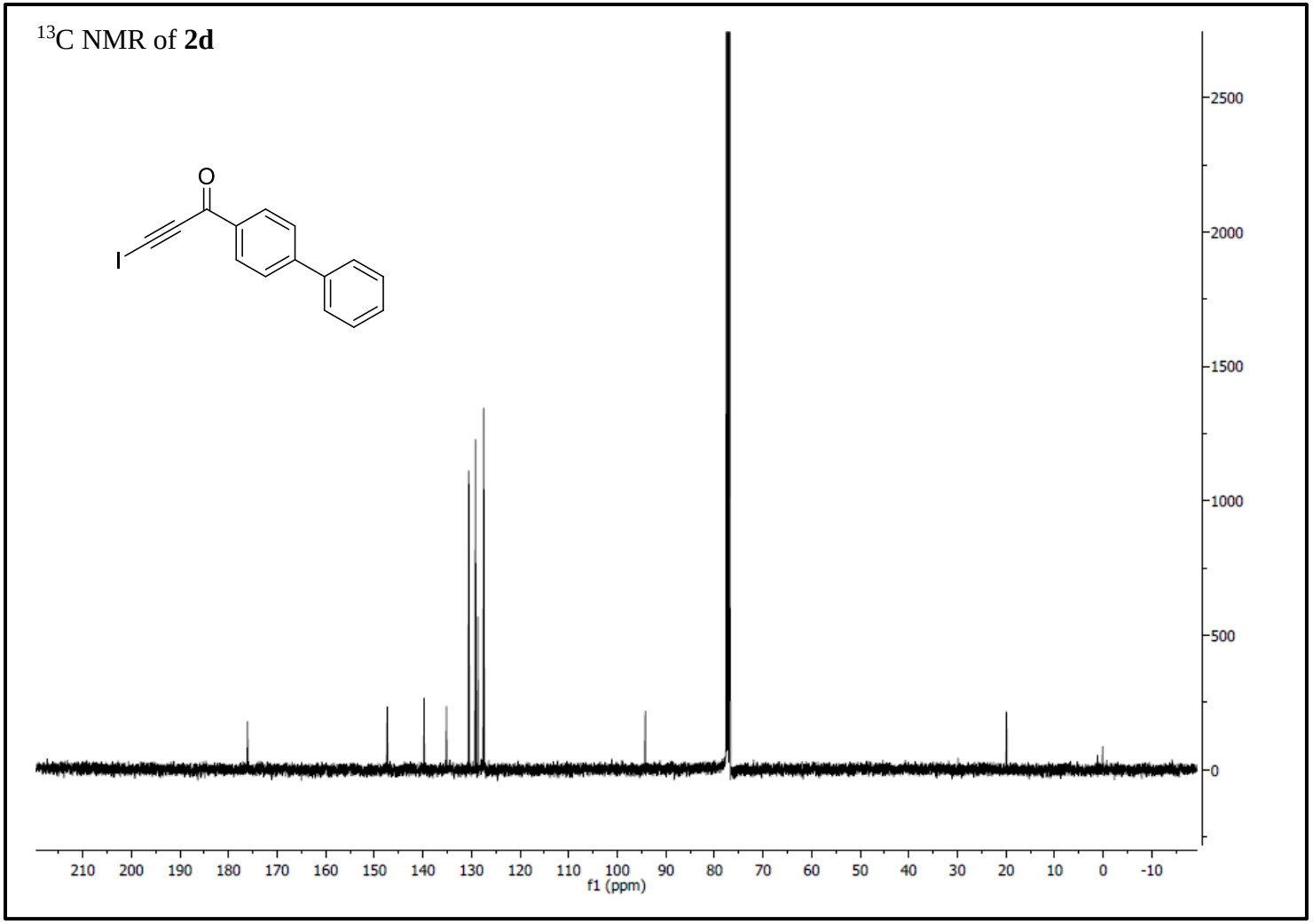
VIII. NMR spectra of new compounds

1-([1,1'-biphenyl]-4-yl)-3-iodoprop-2-yn-1-one **(2d)**

^{1}H NMR of **2d**

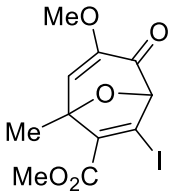


^{13}C NMR of **2d**

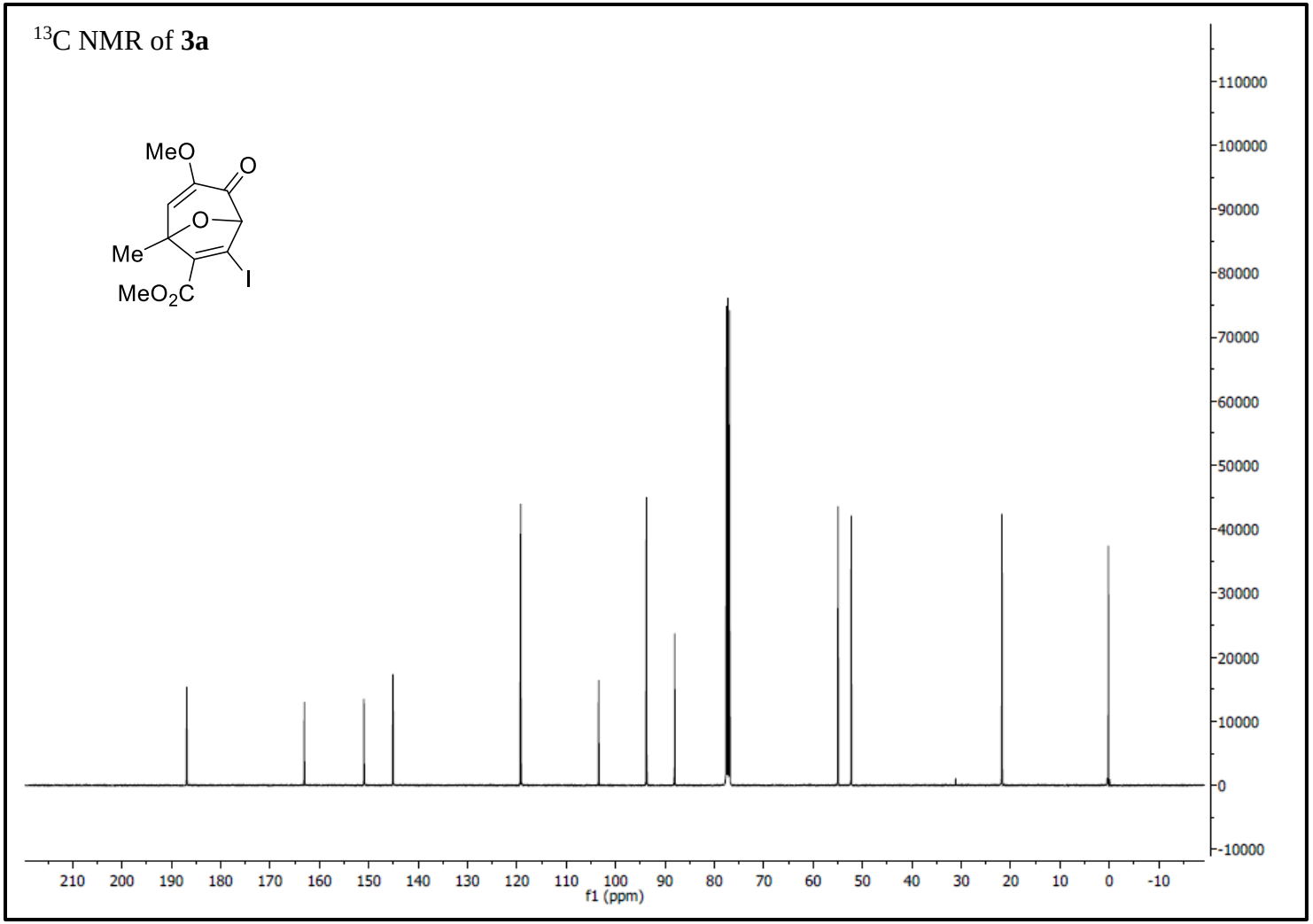

O
I
2500
2000
1500
1000
500
0
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

Methyl 7-iodo-3-methoxy-5-methyl-2-oxo-8-oxabicyclo[3.2.1]octa-3,6-diene-6-carboxylate **(3a)**

^{1}H NMR of **3a**



^{13}C NMR of **3a**


MeO
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O
Me
I
MeO2C
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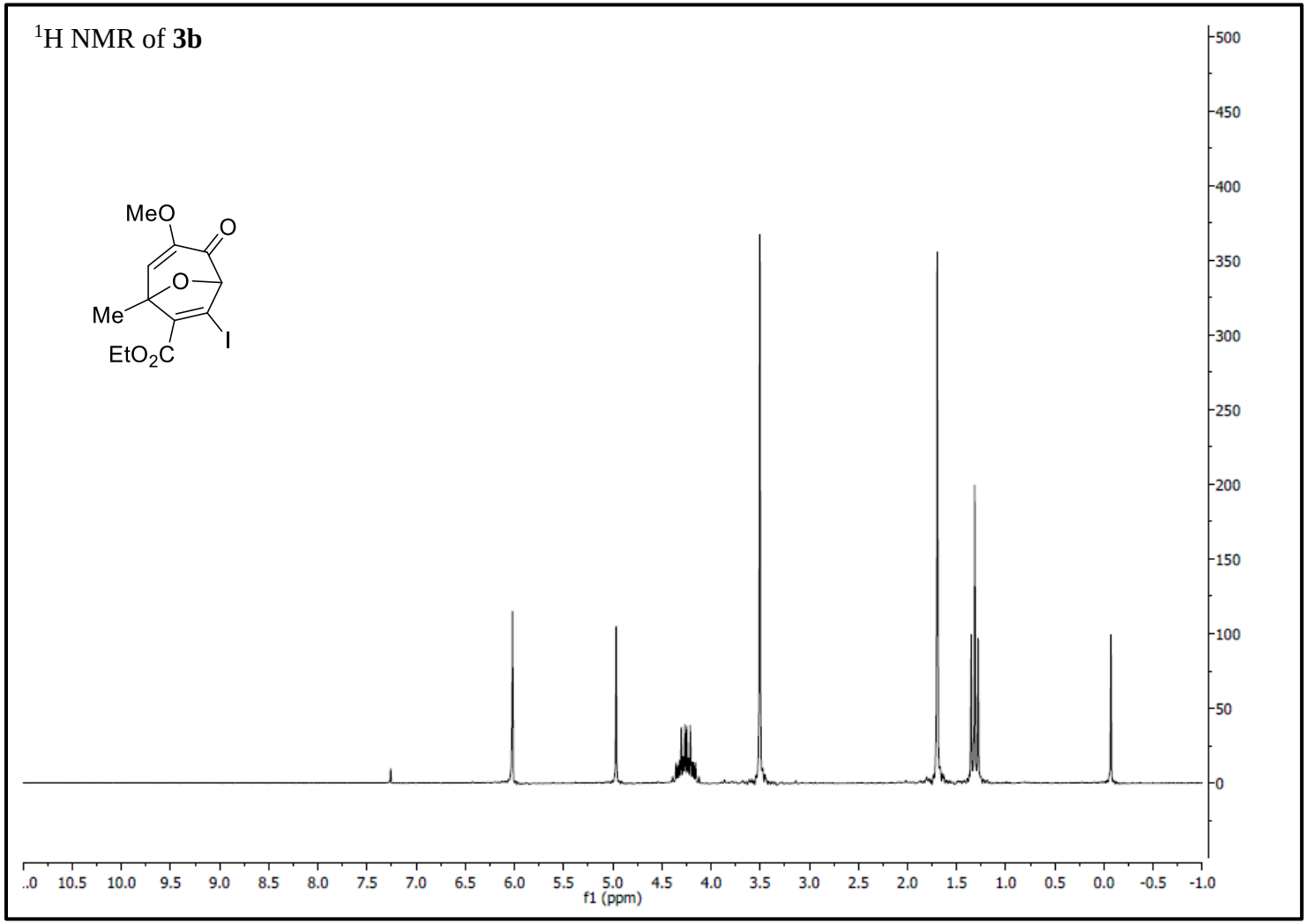
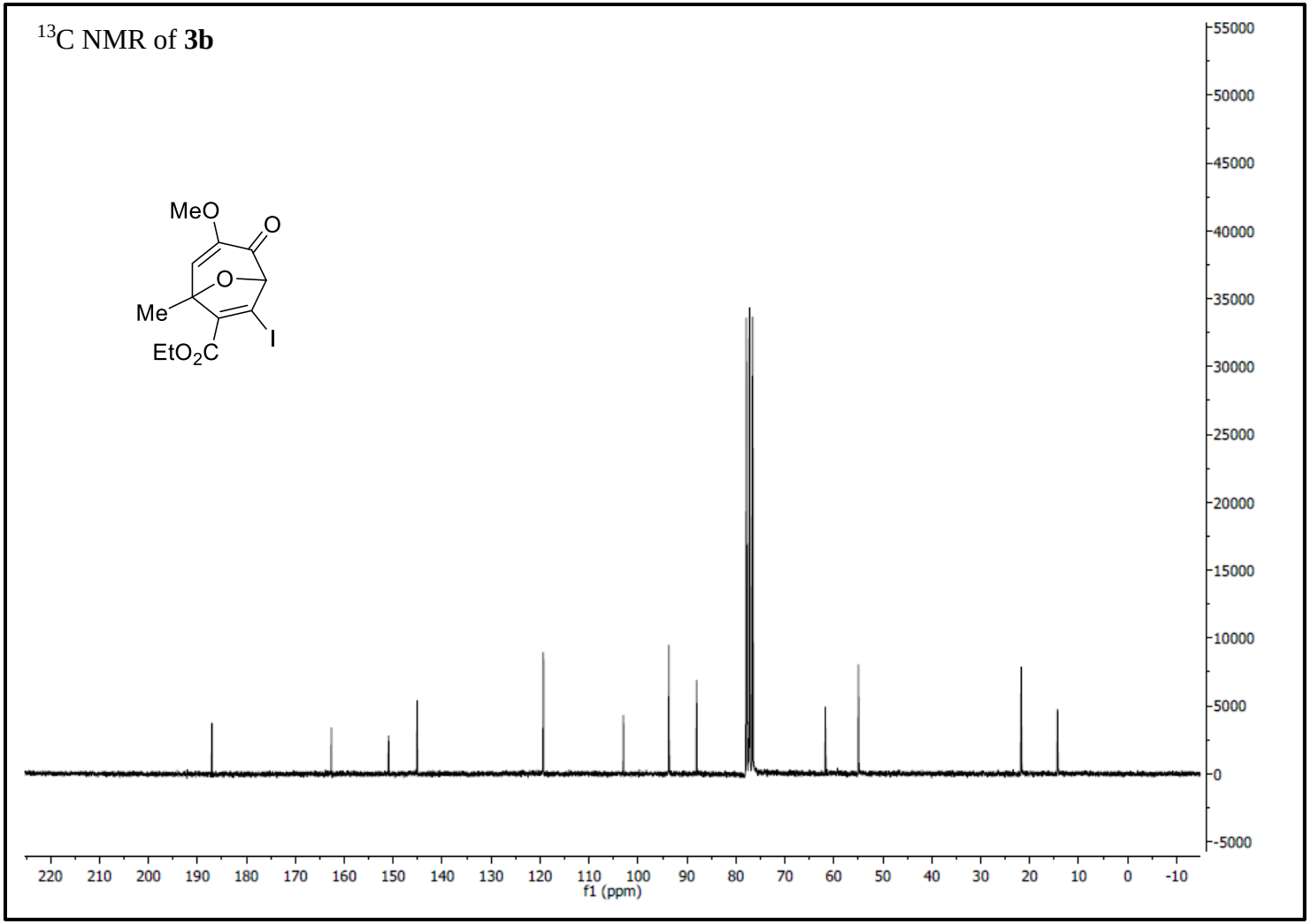
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
-10000
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

Ethyl 7-iodo-3-methoxy-5-methyl-2-oxo-8-oxabicyclo[3.2.1]octa-3,6-diene-6-carboxylate **(3b)**



^{13}C NMR of **3b**


MeO
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O
Me
I
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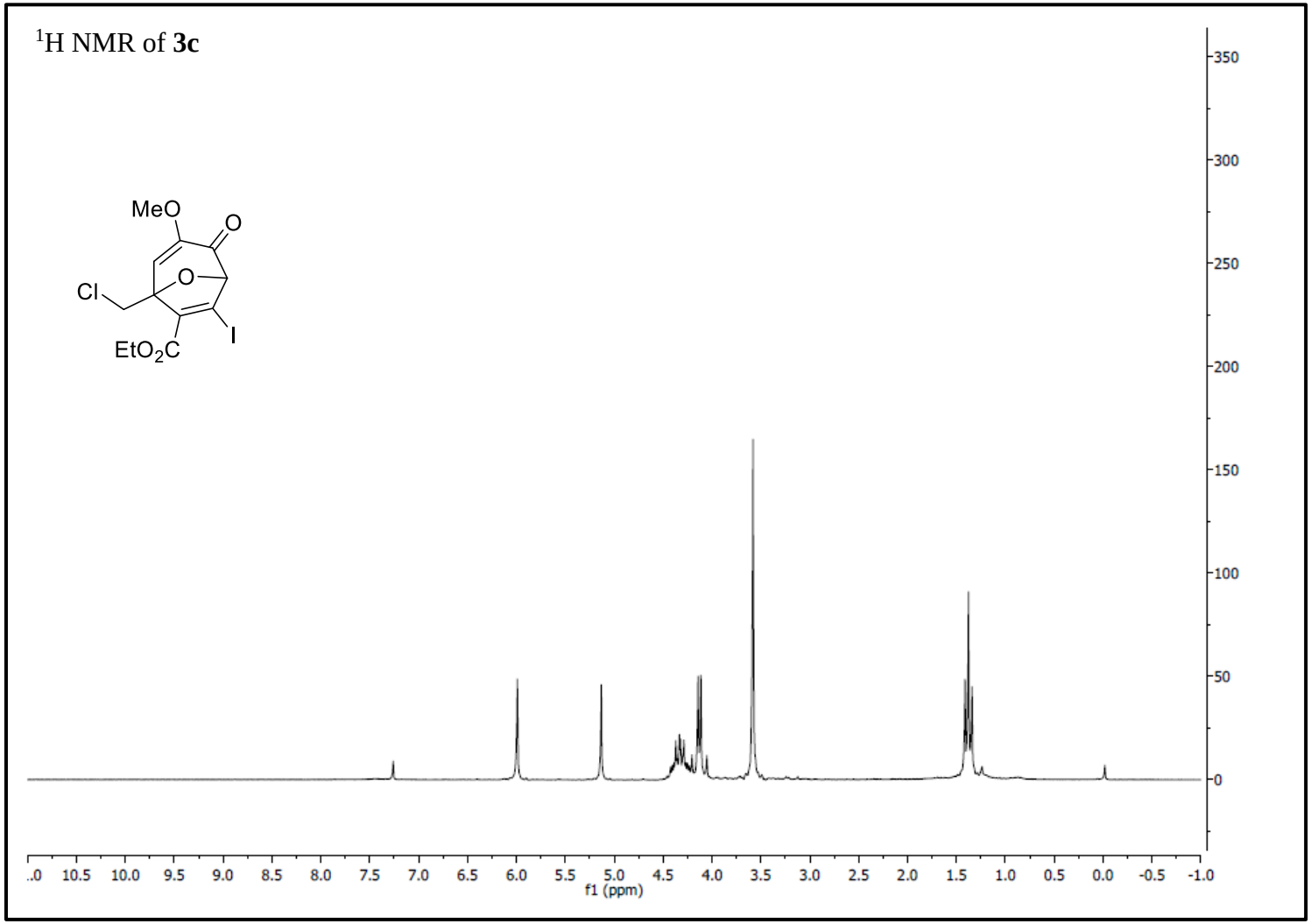
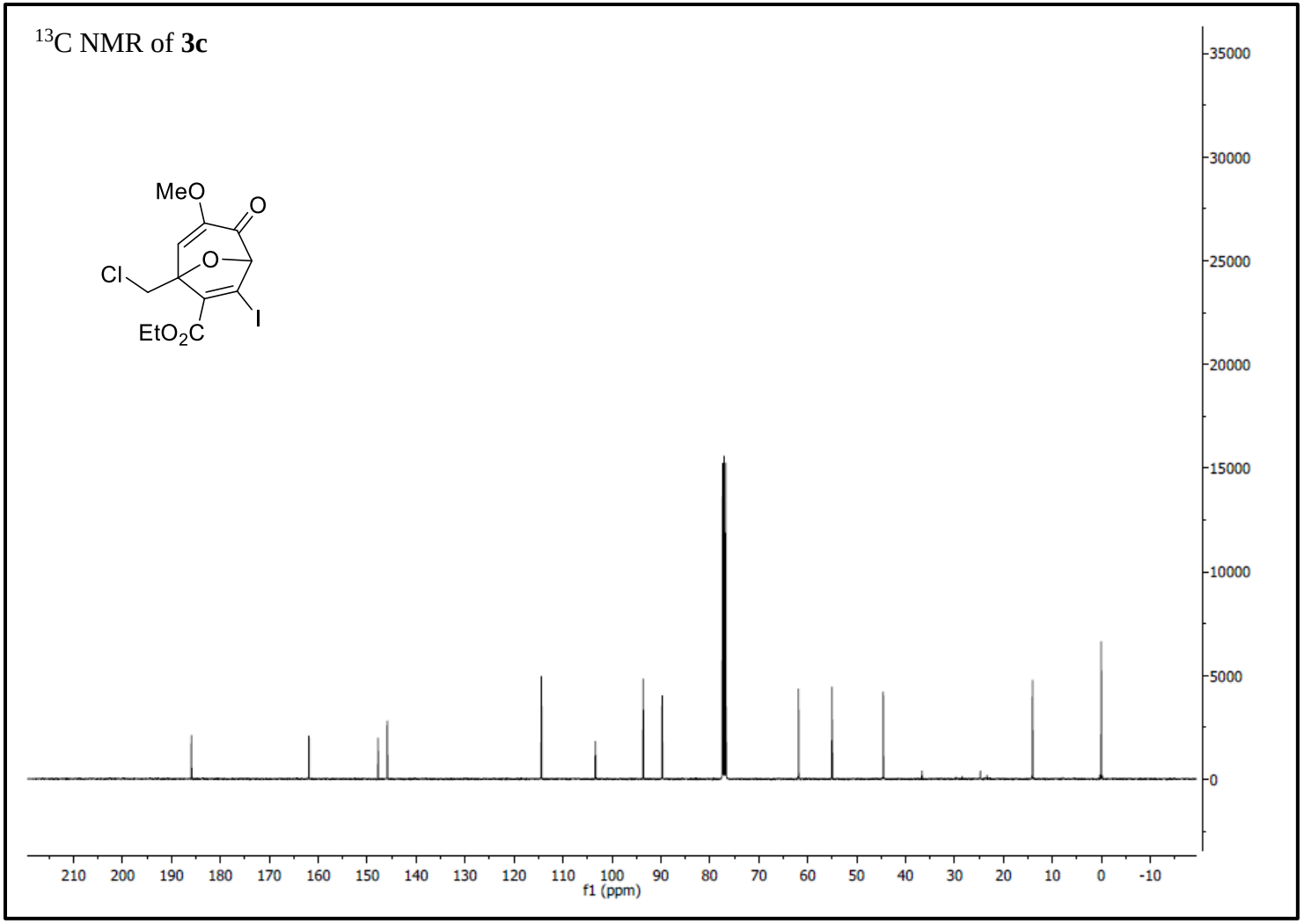
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
-5000
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

Ethyl 5-(chloromethyl)-7-iodo-3-methoxy-2-oxo-8-oxabicyclo[3.2.1]octa-3,6-diene-6-carboxylate **(3c)**

^{1}H NMR of **3c**



^{13}C NMR of **3c**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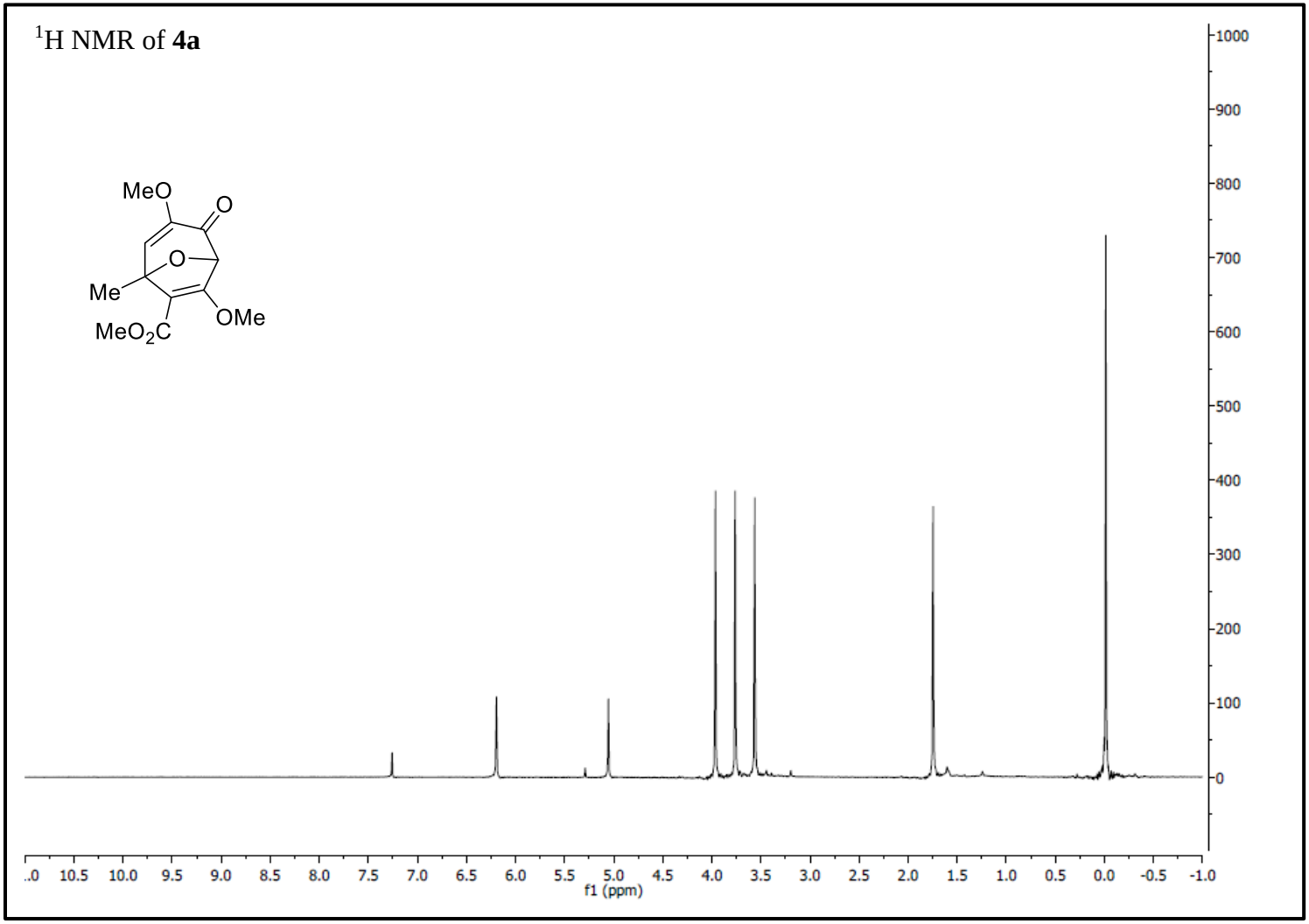
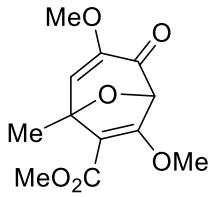
O
Cl
I
EtO2C
35000
30000
25000
20000
15000
10000
5000
0
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

Methyl 3,7-dimethoxy-5-methyl-2-oxo-8-oxabicyclo[3.2.1]octa-3,6-diene-6-carboxylate **(4a)**

^{1}H NMR of **4a**



^{13}C NMR of **4a**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O
Me
OMe
MeO2C

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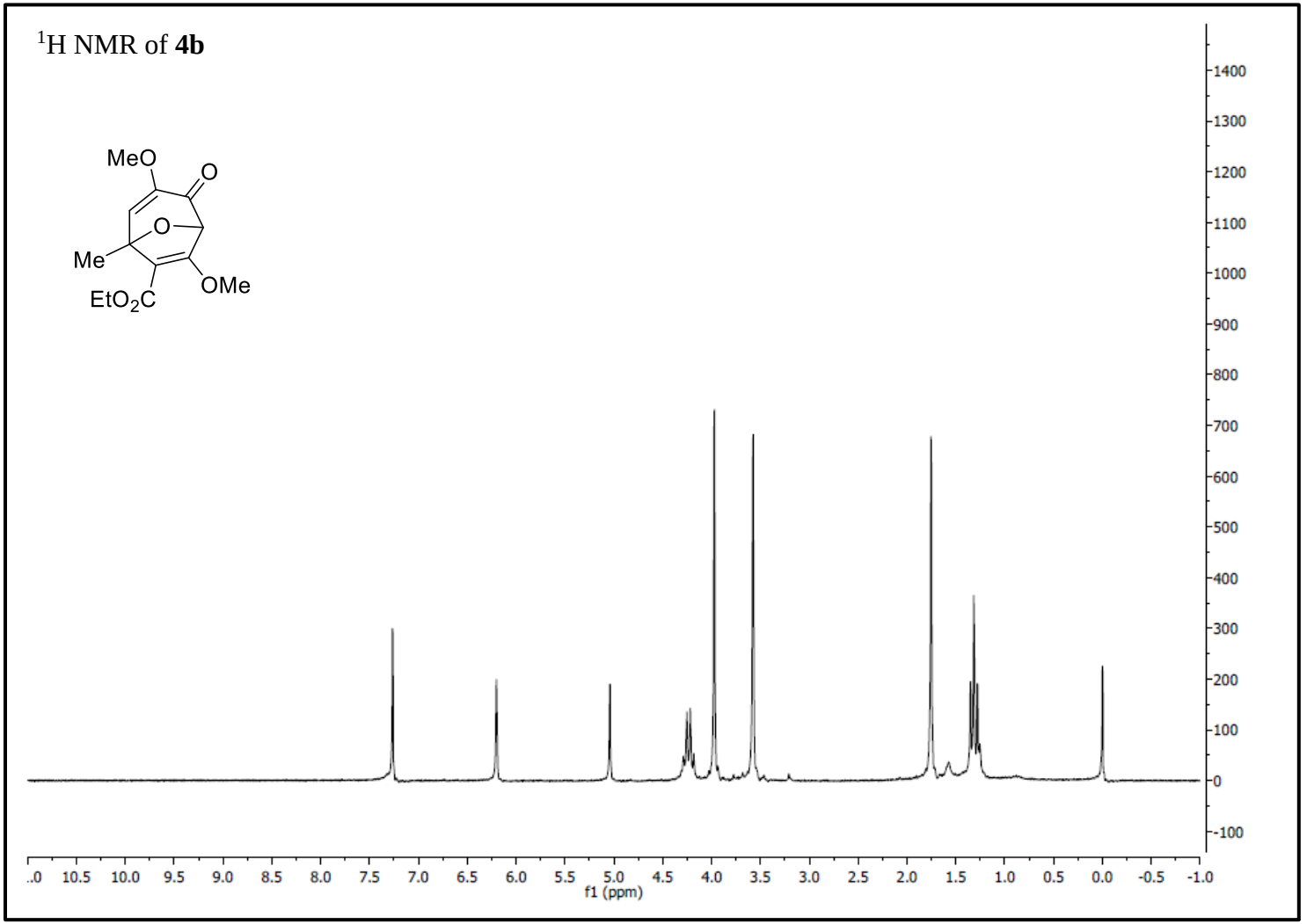
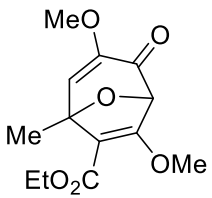
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

Ethyl 3,7-dimethoxy-5-methyl-2-oxo-8-oxabicyclo[3.2.1]octa-3,6-diene-6-carboxylate **(4b)**

^{1}H NMR of **4b**



^{13}C NMR of **4b**


MeO
O
O
Me
OMe
EtO2C

24000
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21000
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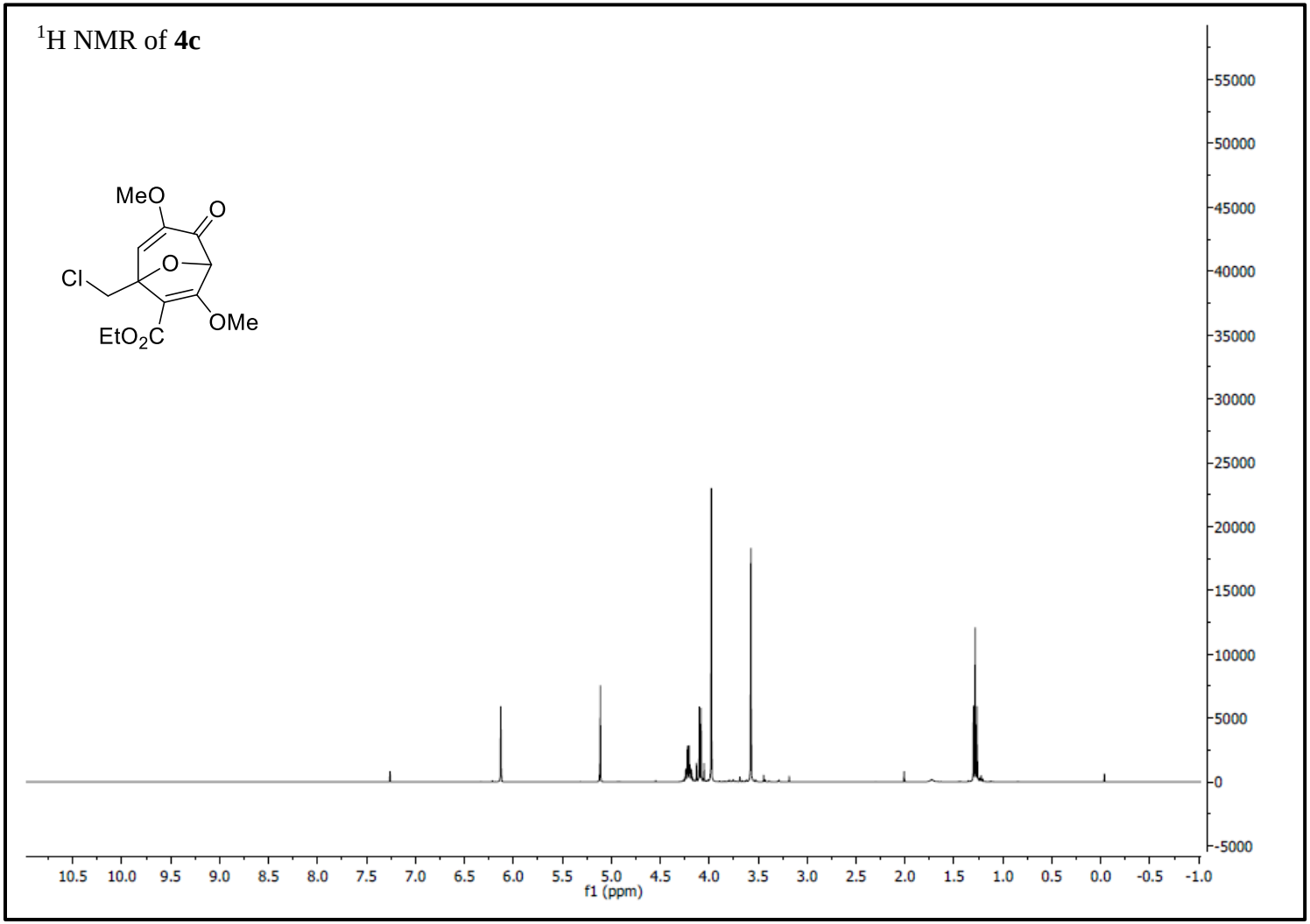
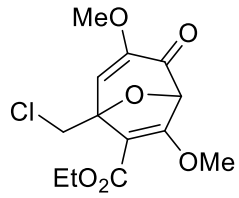
18000
17000
16000
15000
14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
-2000
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

Ethyl 5-(chloromethyl)-3,7-dimethoxy-2-oxo-8-oxabicyclo[3.2.1]octa-3,6-diene-6-carboxylate **(4c)**

^{1}H NMR of **4c**



^{13}C NMR of **4c**


MeO
O
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Cl
OMe
EtO2C

16000
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14000
13000
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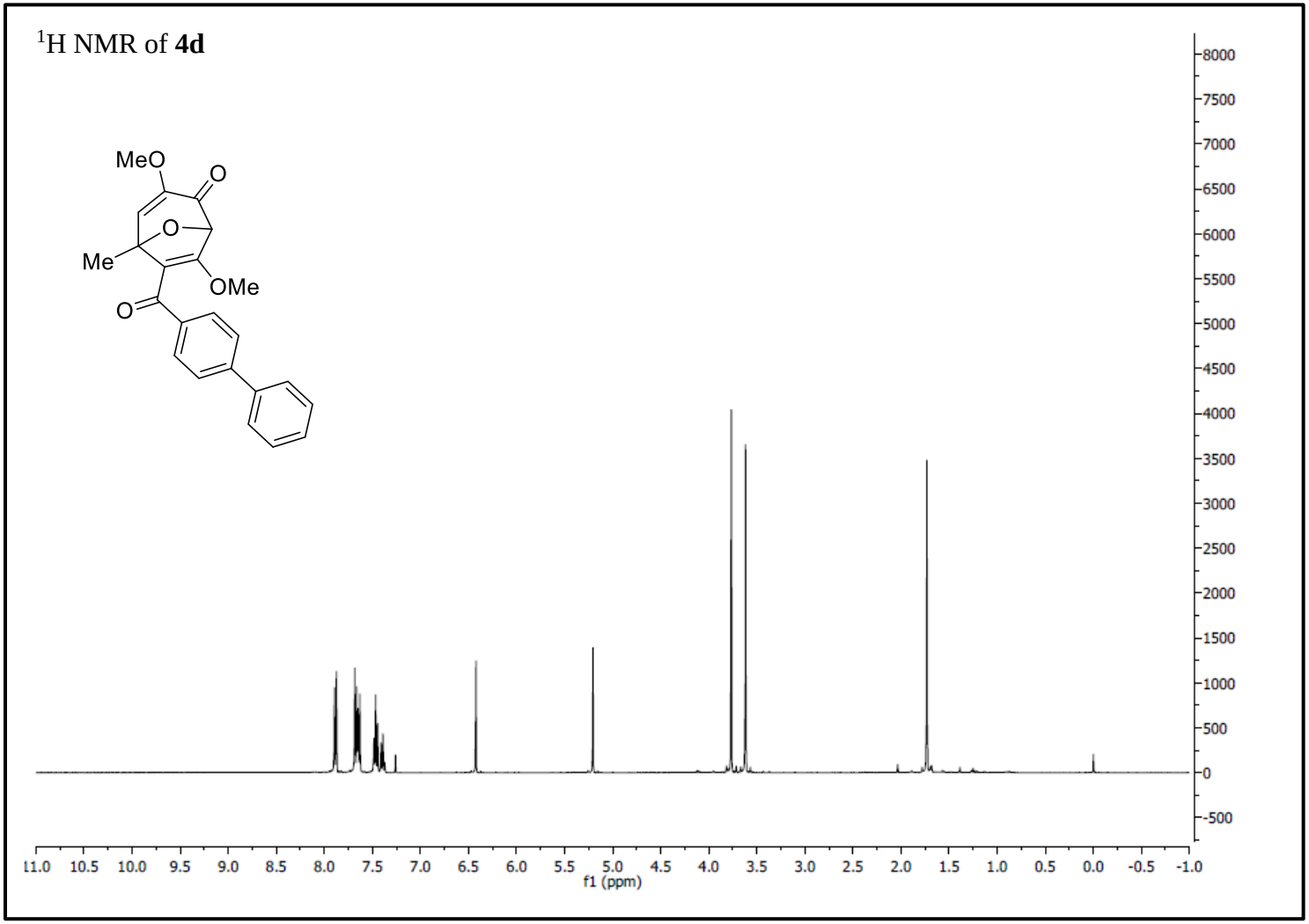
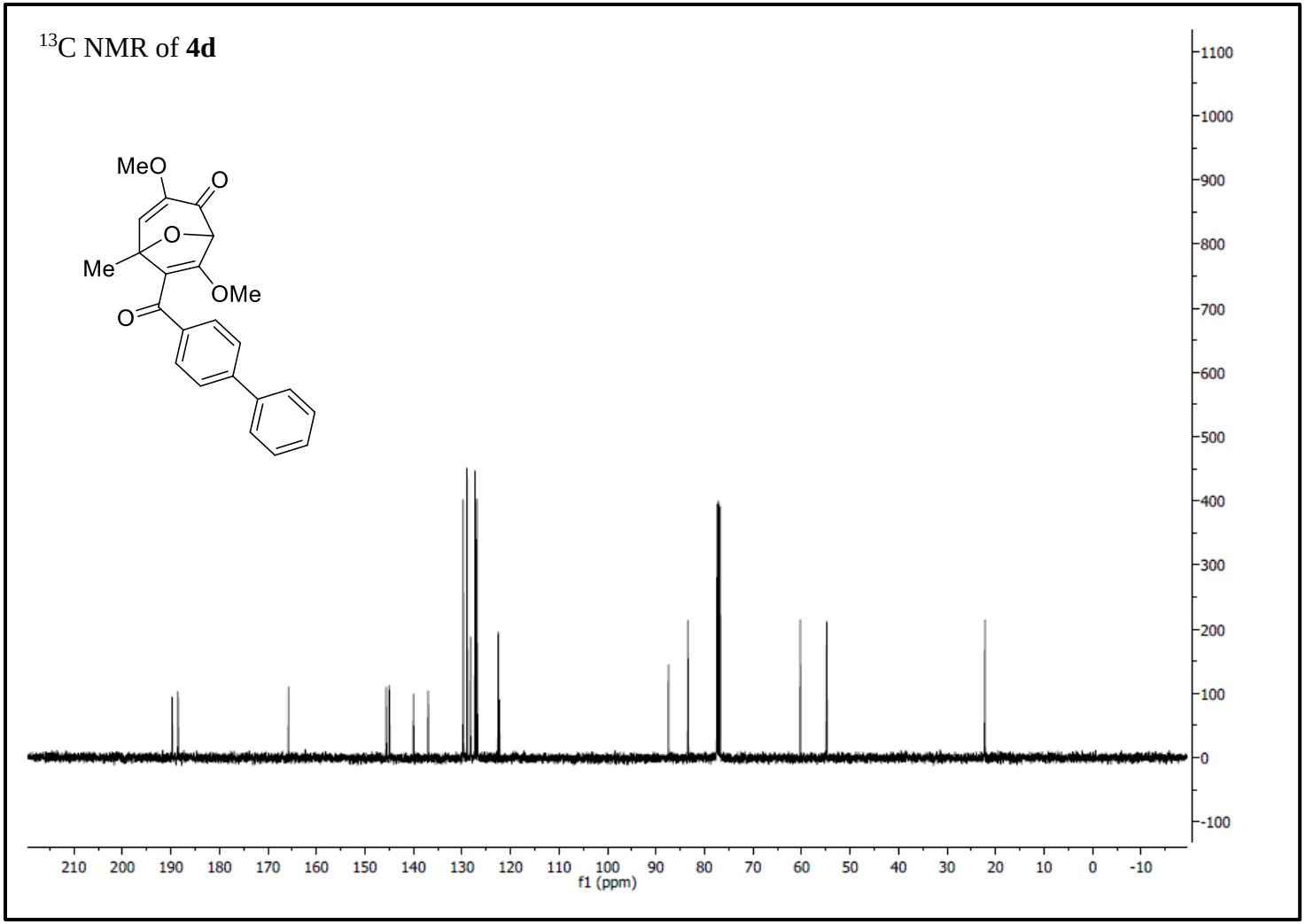
7000
6000
5000
4000
3000
2000
1000
0
-1000
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

6-([1,1'-biphenyl]-4-carbonyl)-3,7-dimethoxy-5-methyl-8-oxabicyclo[3.2.1]octa-3,6-dien-2-one **(4d)**

^{1}H NMR of **4d**



^{13}C NMR of **4d**


MeO
O
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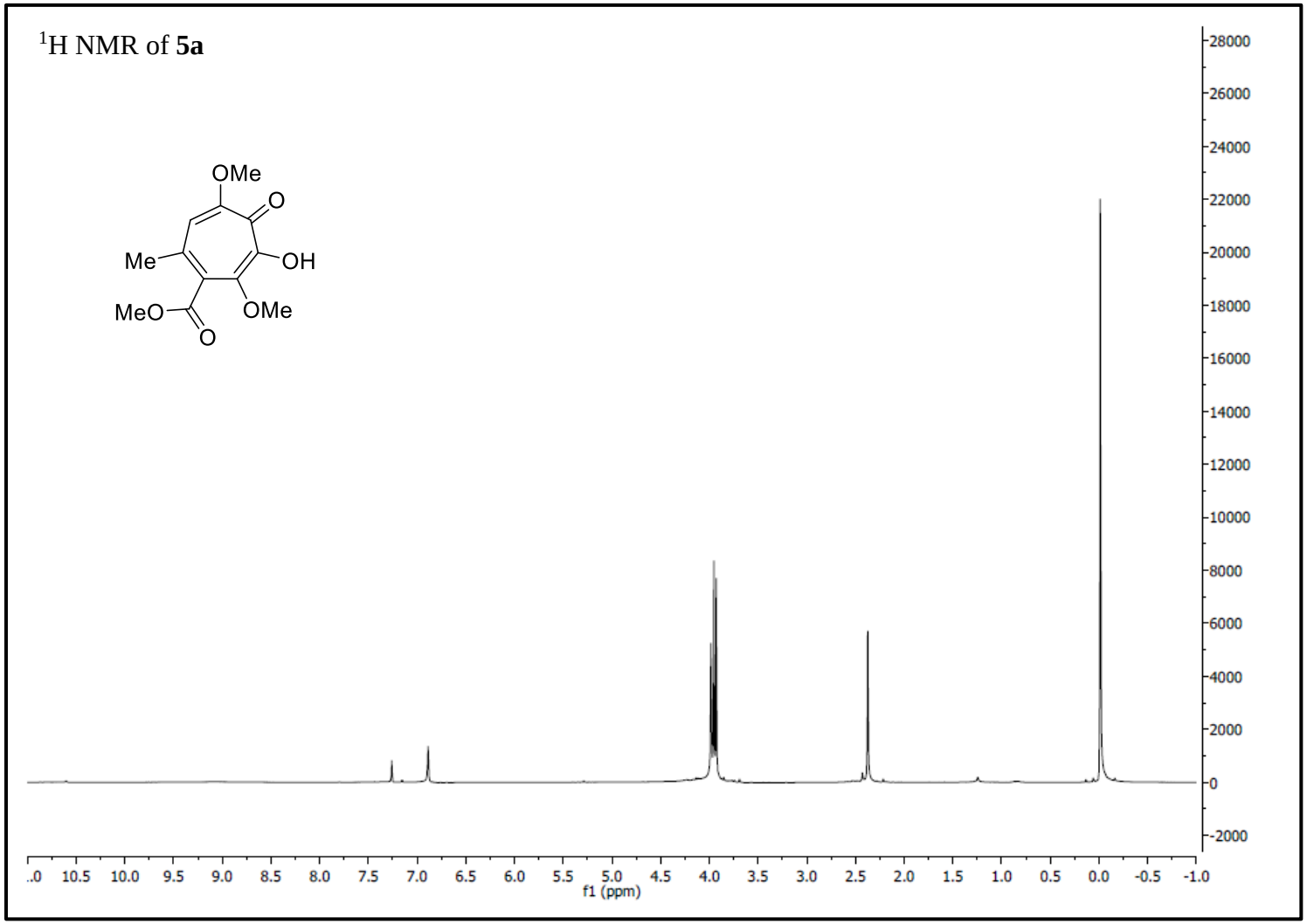
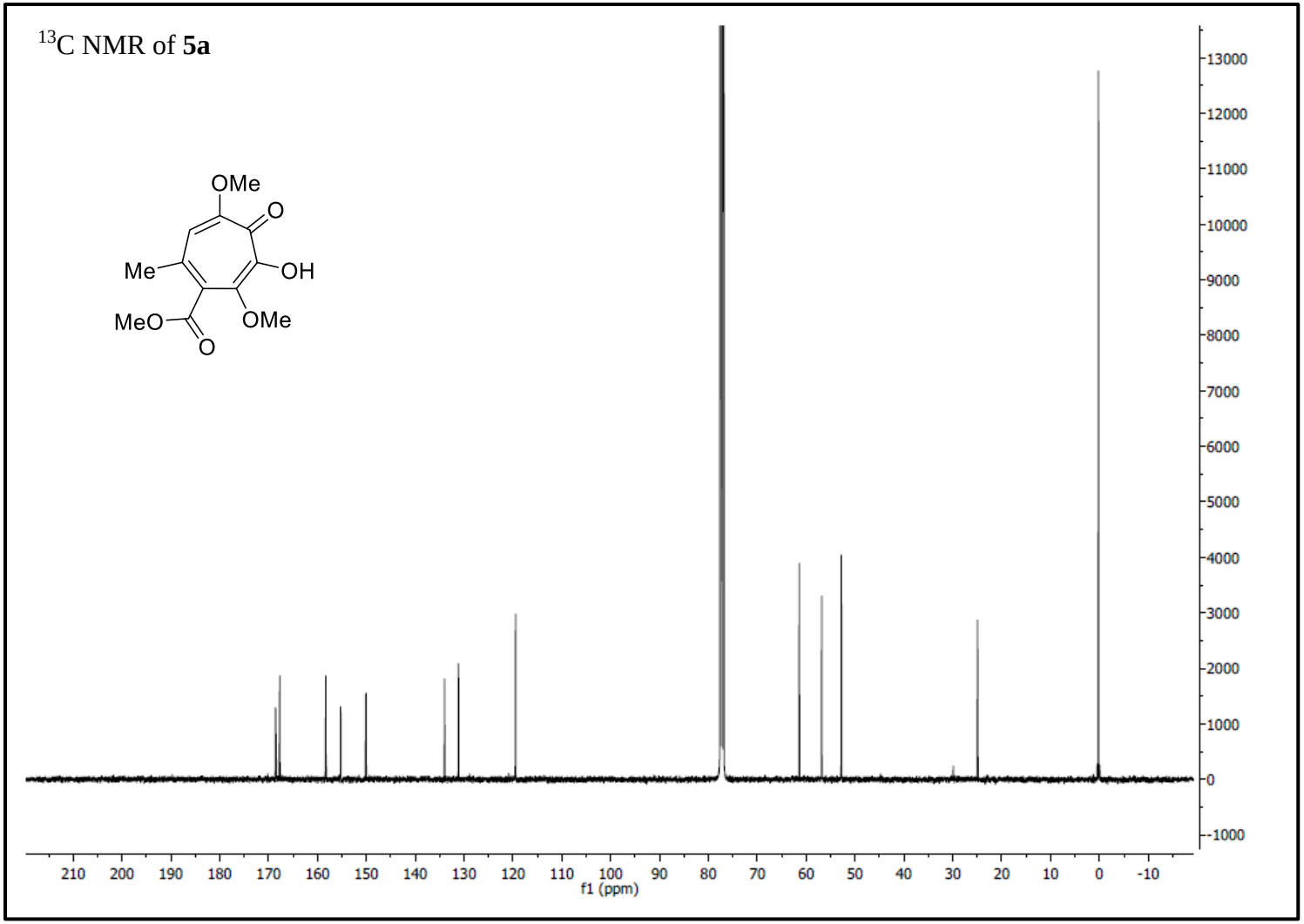
Me
OMe
O
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

Methyl 6-hydroxy-4,7-dimethoxy-2-methyl-5-oxocyclohepta-1,3,6-triene-1-carboxylate **(5a)**



^{13}C NMR of **5a**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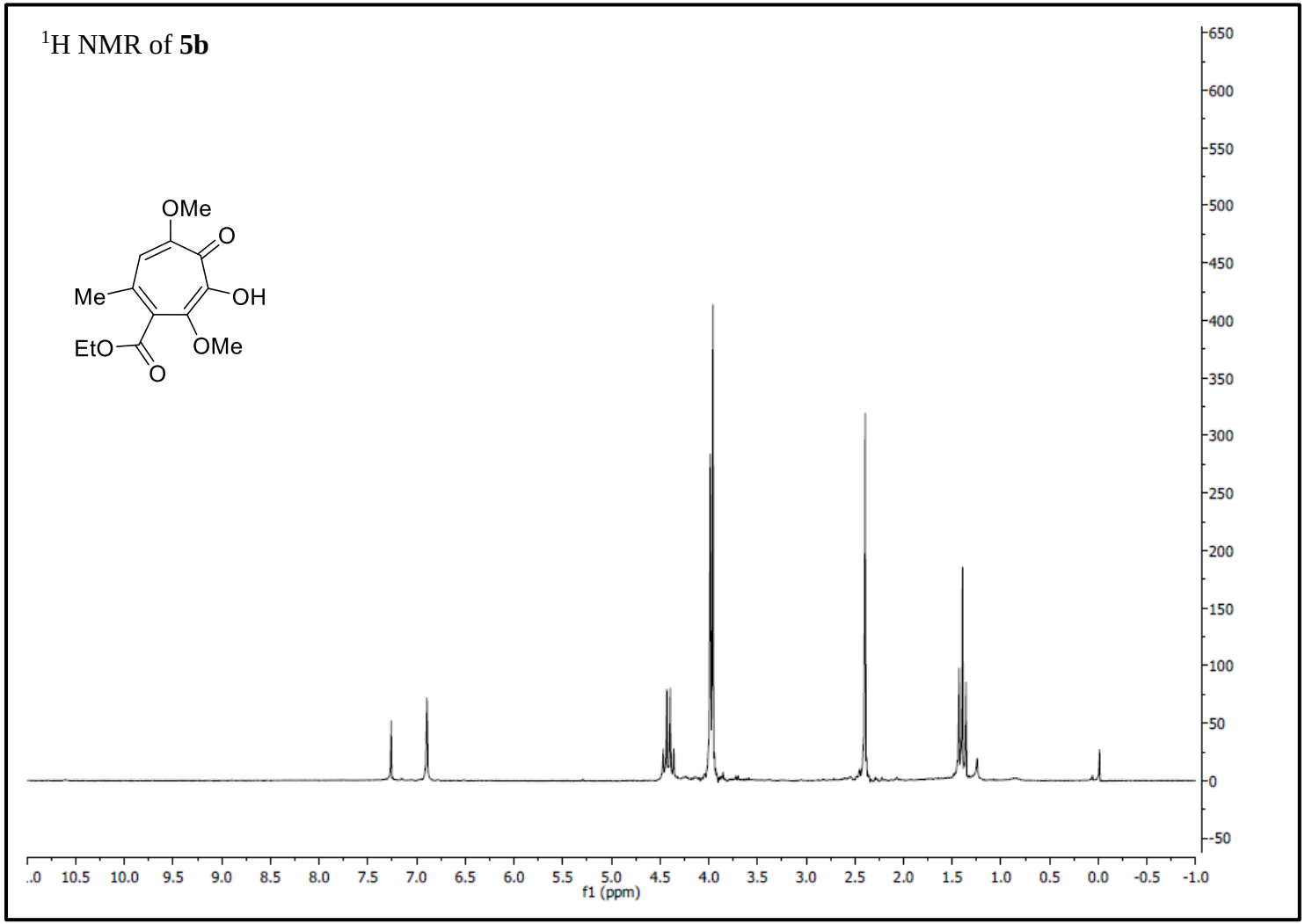
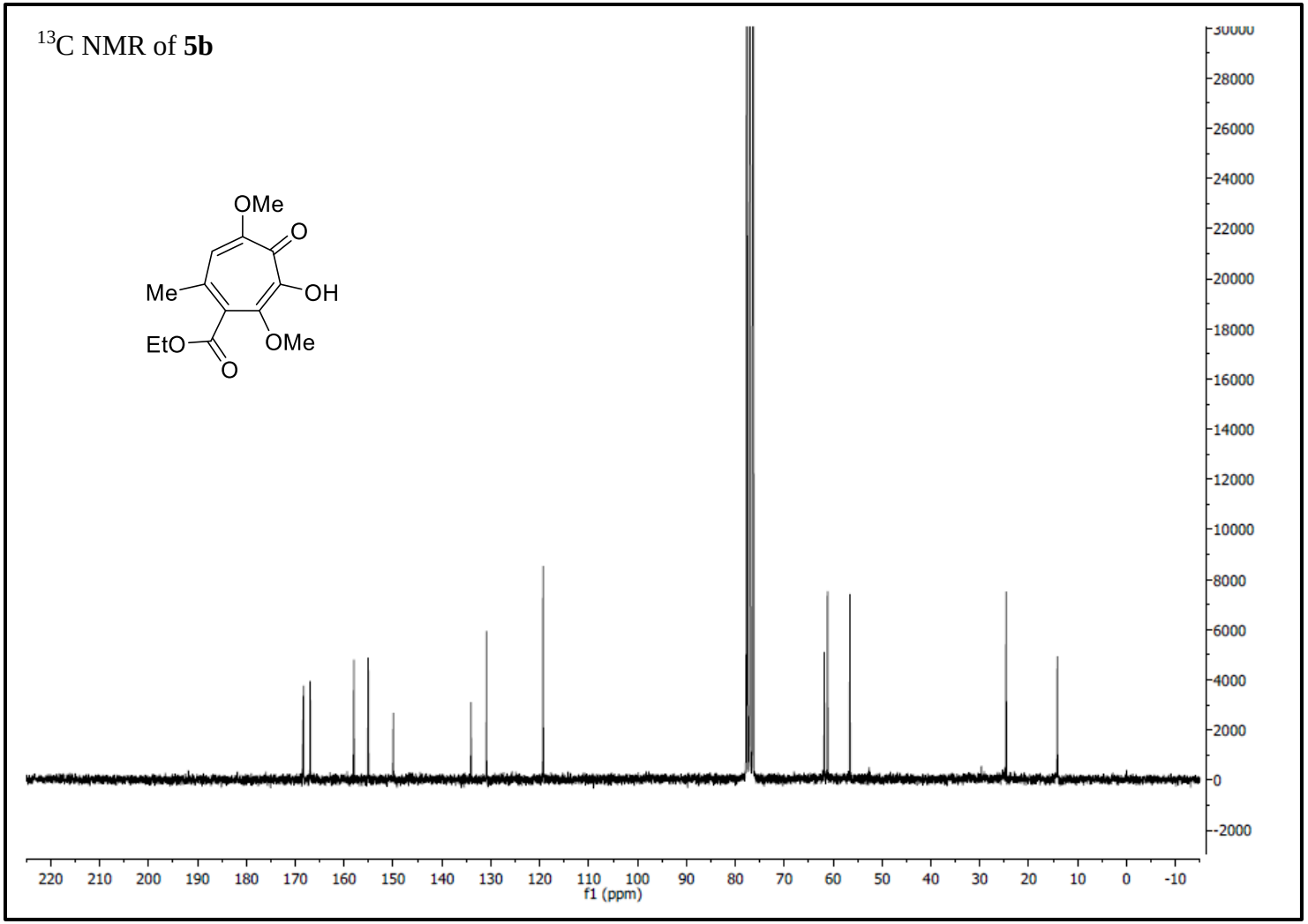
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

Ethyl 6-hydroxy-4,7-dimethoxy-2-methyl-5-oxocyclohepta-1,3,6-triene-1-carboxylate **(5b)**



^{13}C NMR of **5b**


OMe
O
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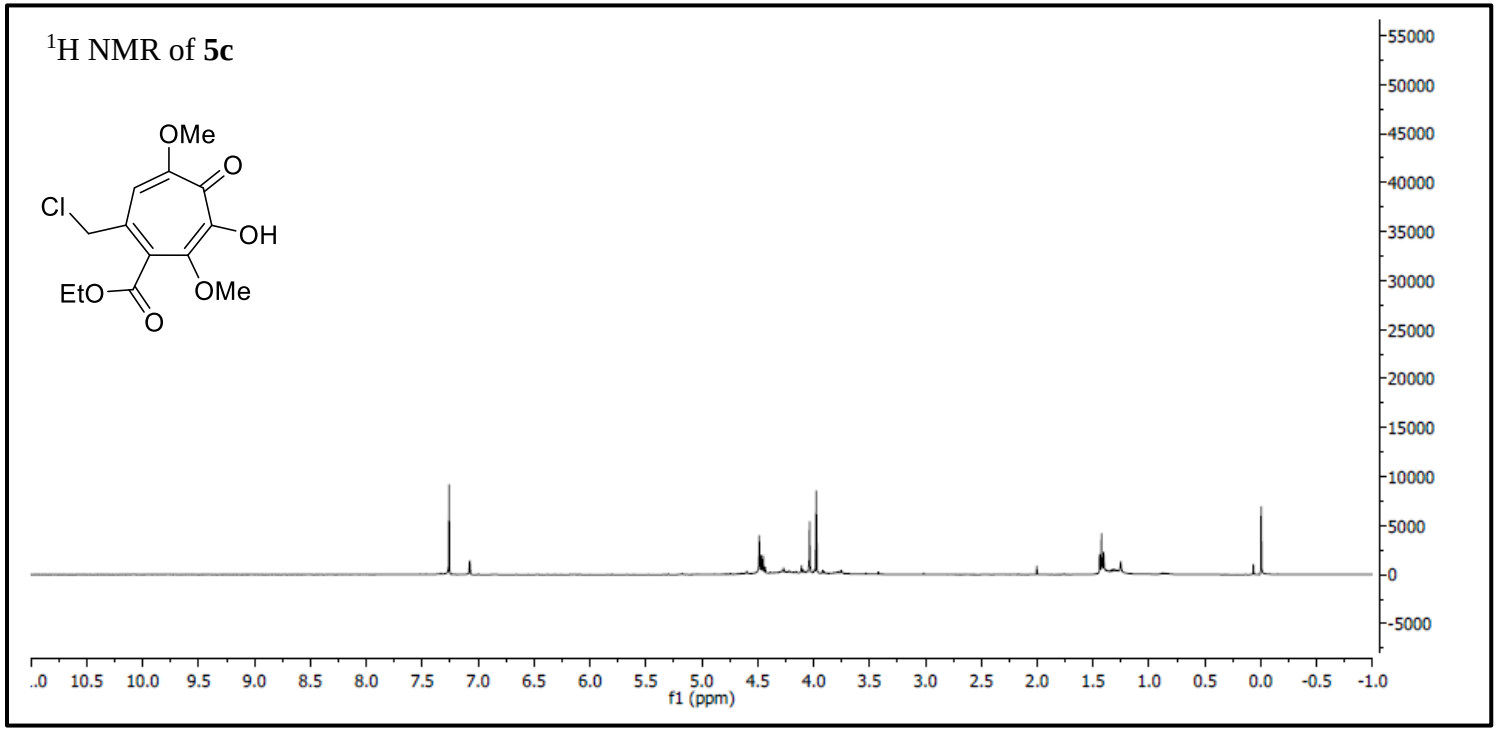
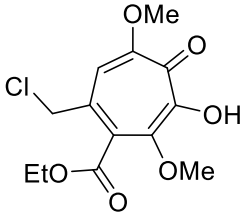
OH
EtO
OMe
O
28000
26000
24000
22000
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
-2000
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

Ethyl 2-(chloromethyl)-6-hydroxy-4,7-dimethoxy-5-oxocyclohepta-1,3,6-triene-1-carboxylate **(5c)**

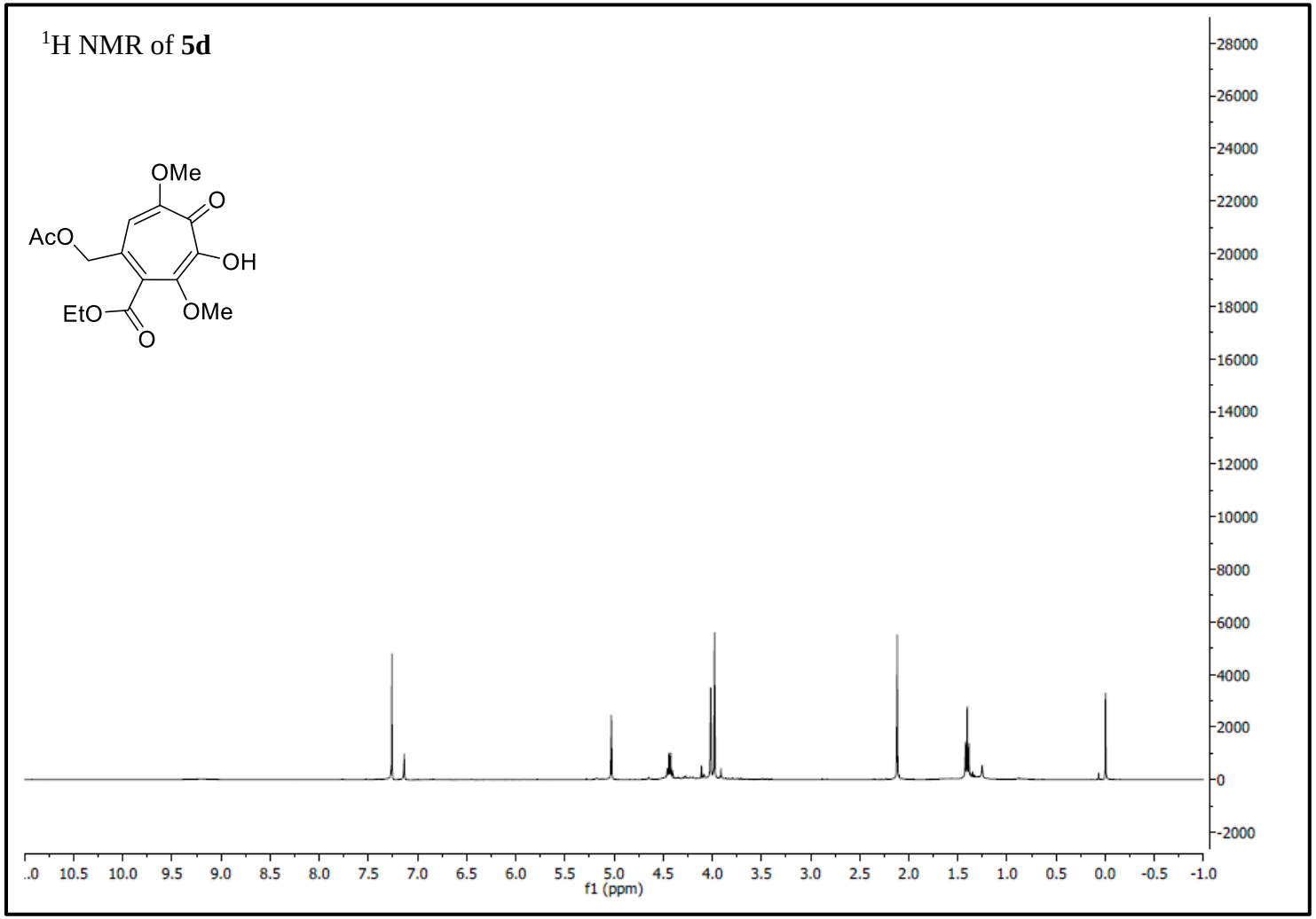


^{13}C NMR of **5c**

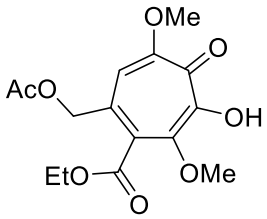

OMe
O
Cl
OH
EtO
OMe
O

10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

Ethyl 2-(acetoxymethyl)-6-hydroxy-4,7-dimethoxy-5-oxocyclohepta-1,3,6-triene-1-carboxylate **(5d)**

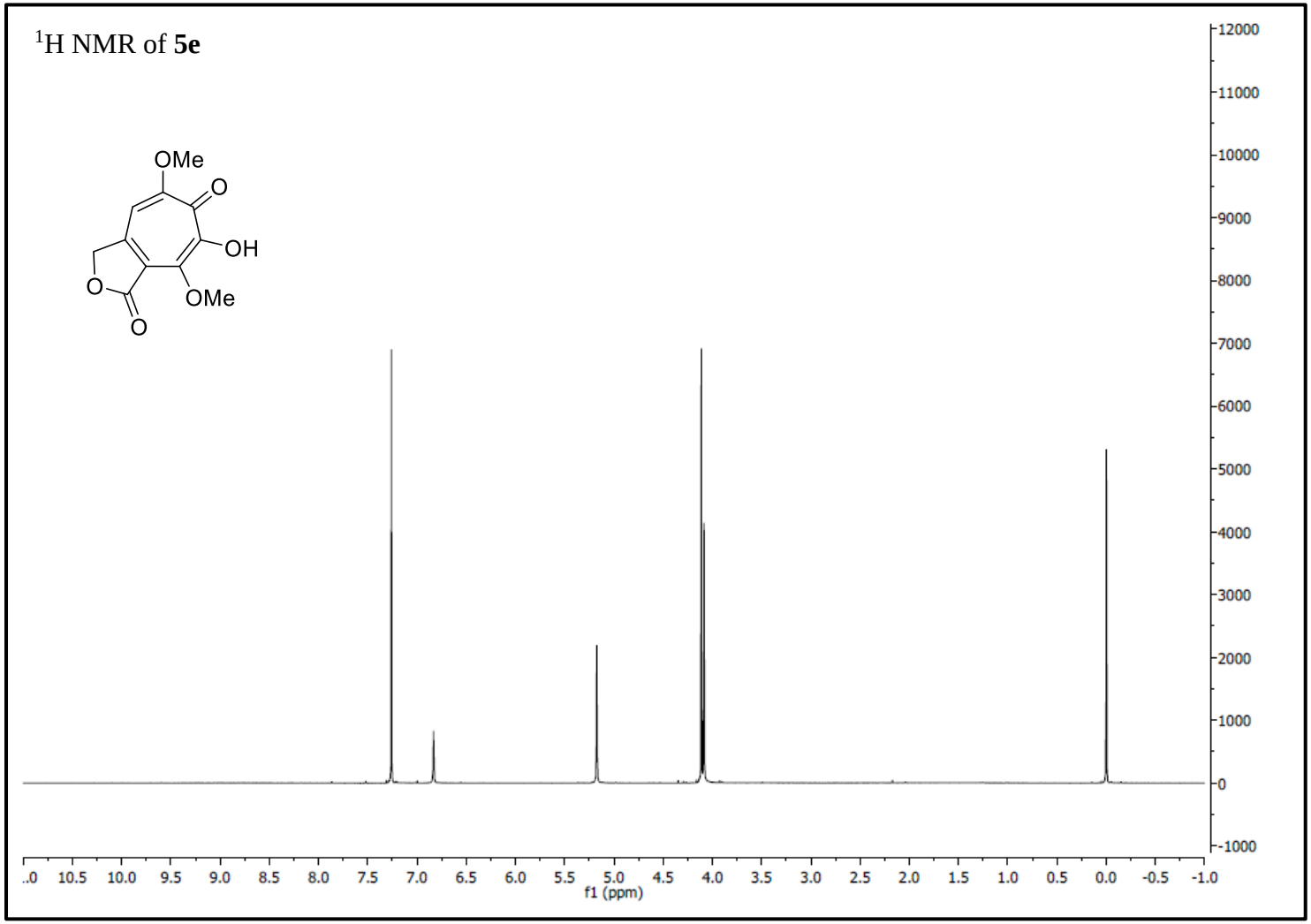


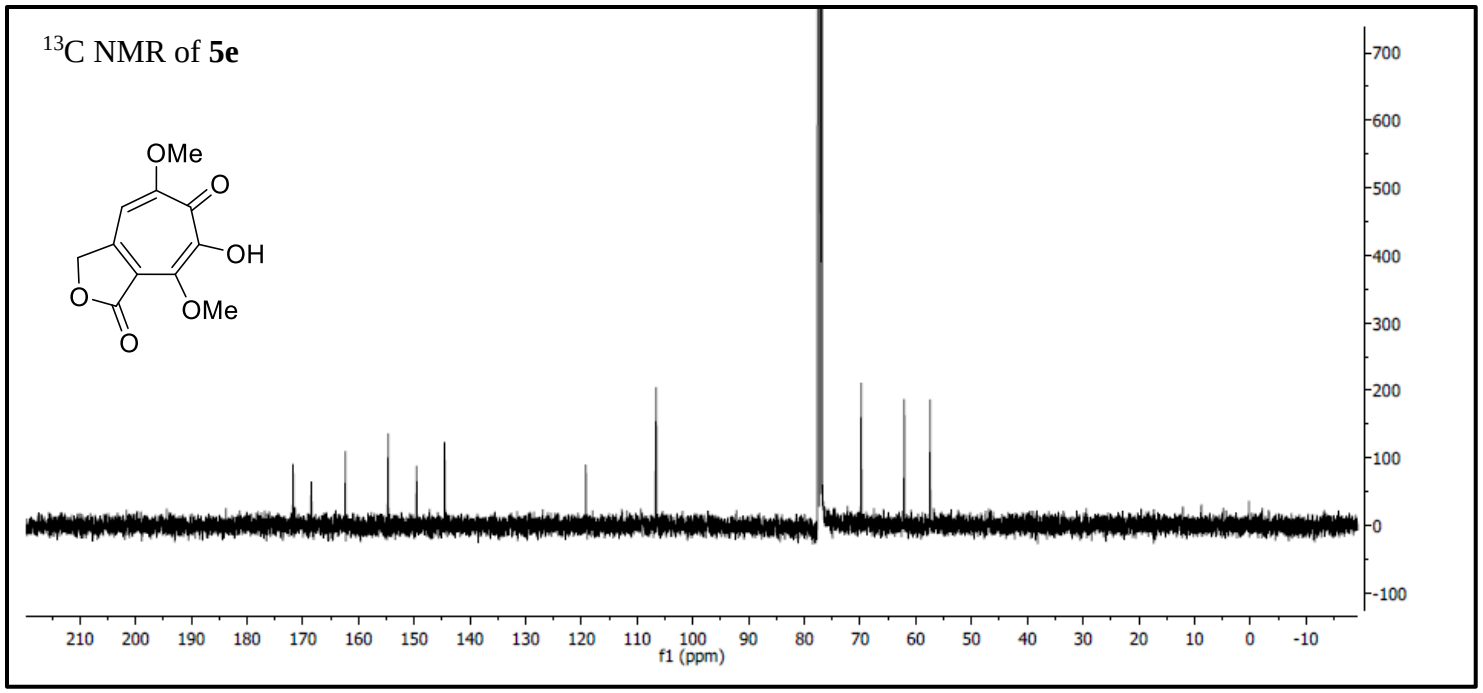
^{13}C NMR of **5d**


OMe
O
AcO
OH
EtO
OMe
O

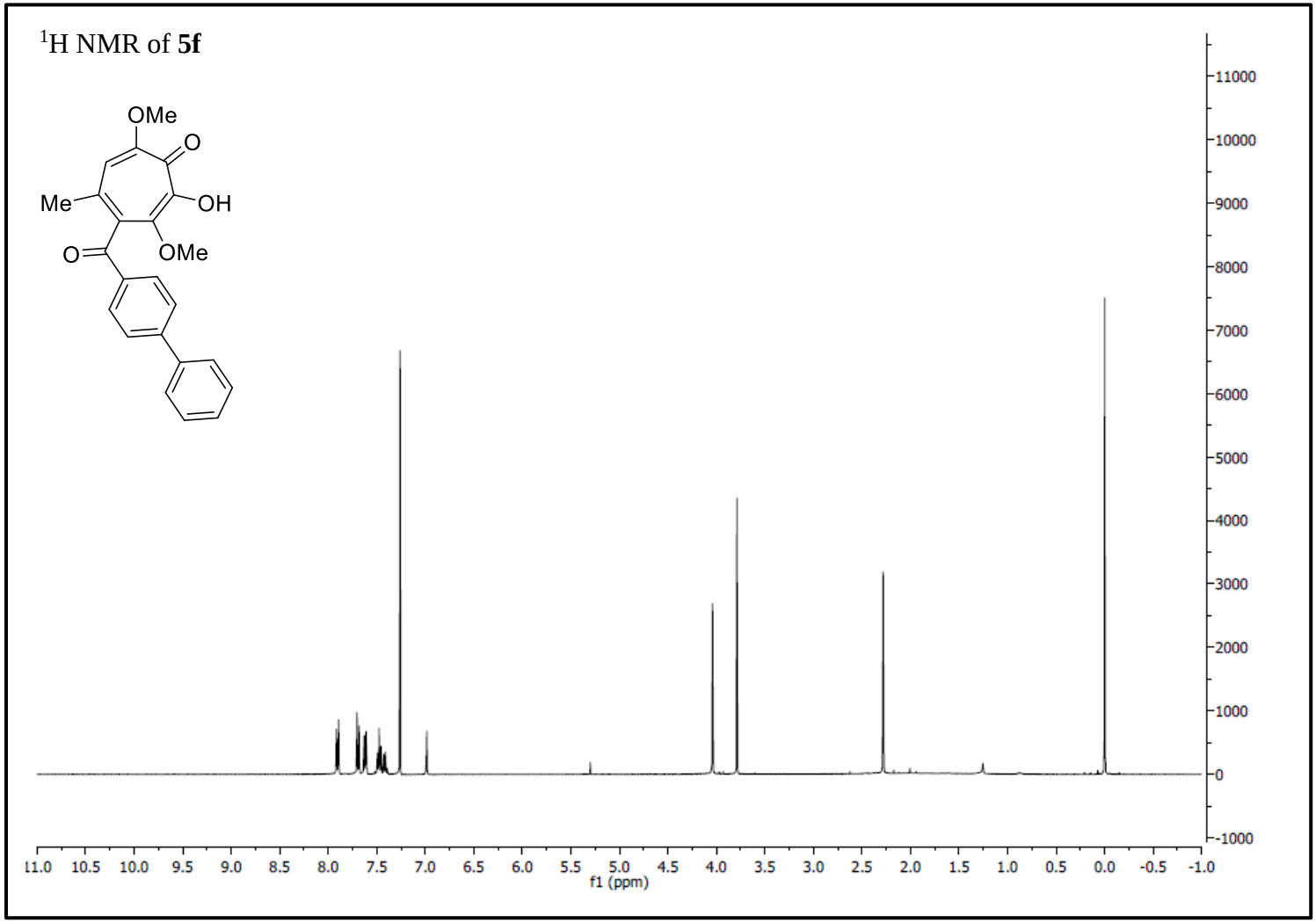
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

7-Hydroxy-5,8-dimethoxy-1H-cyclohepta[c]furan-1,6(3H)-dione **(5e)**

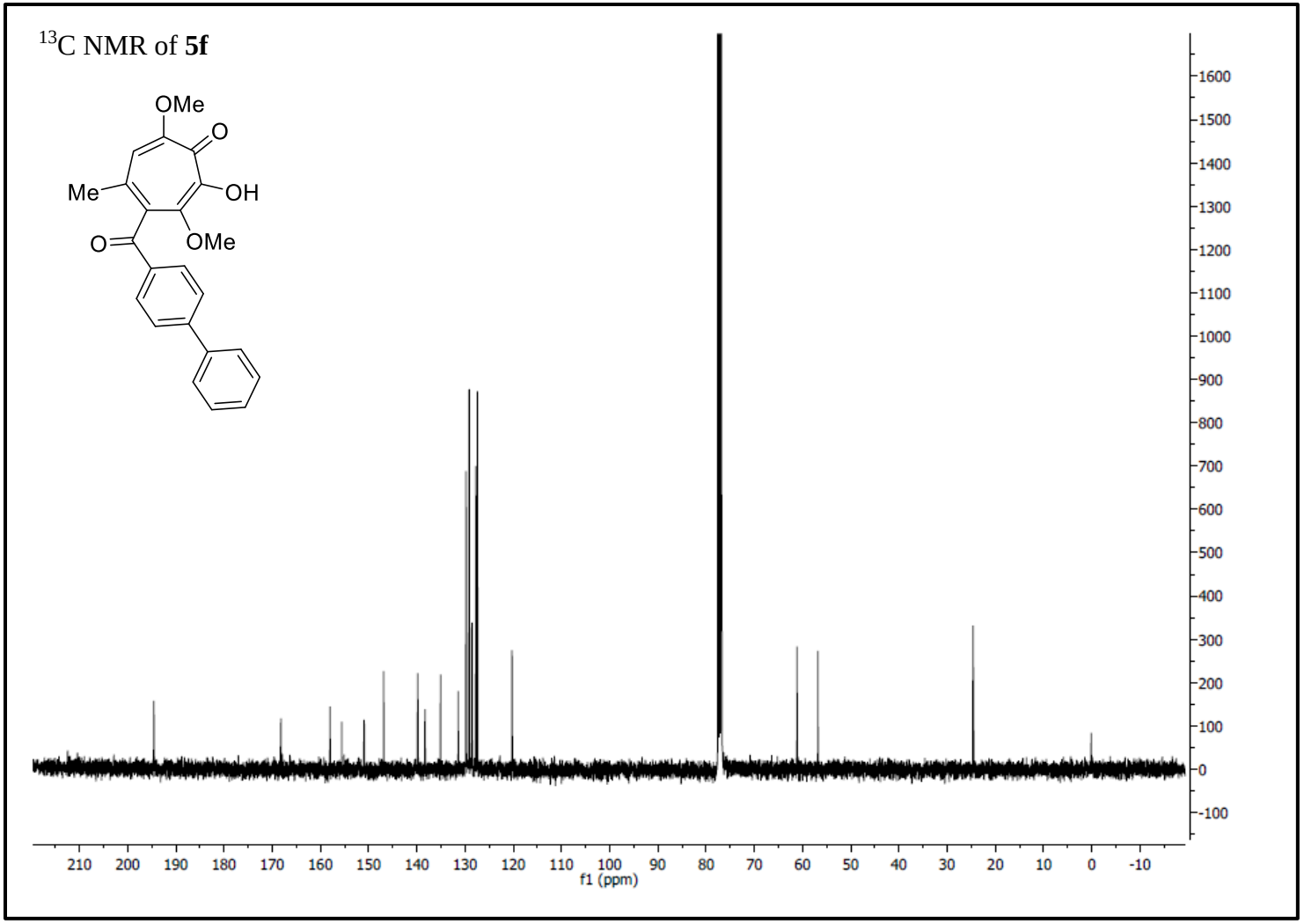



^{13}C NMR of **5e**
OMe
O
OH
O
OMe
O
700
600
500
400
300
200
100
0
-100
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

4-([1,1'-biphenyl]-4-carbonyl)-2-hydroxy-3,7-dimethoxy-5-methylcyclohepta-2,4,6-trien-1-one **(5f)**



^{13}C NMR of **5f**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O
Me
OH
O
OMe
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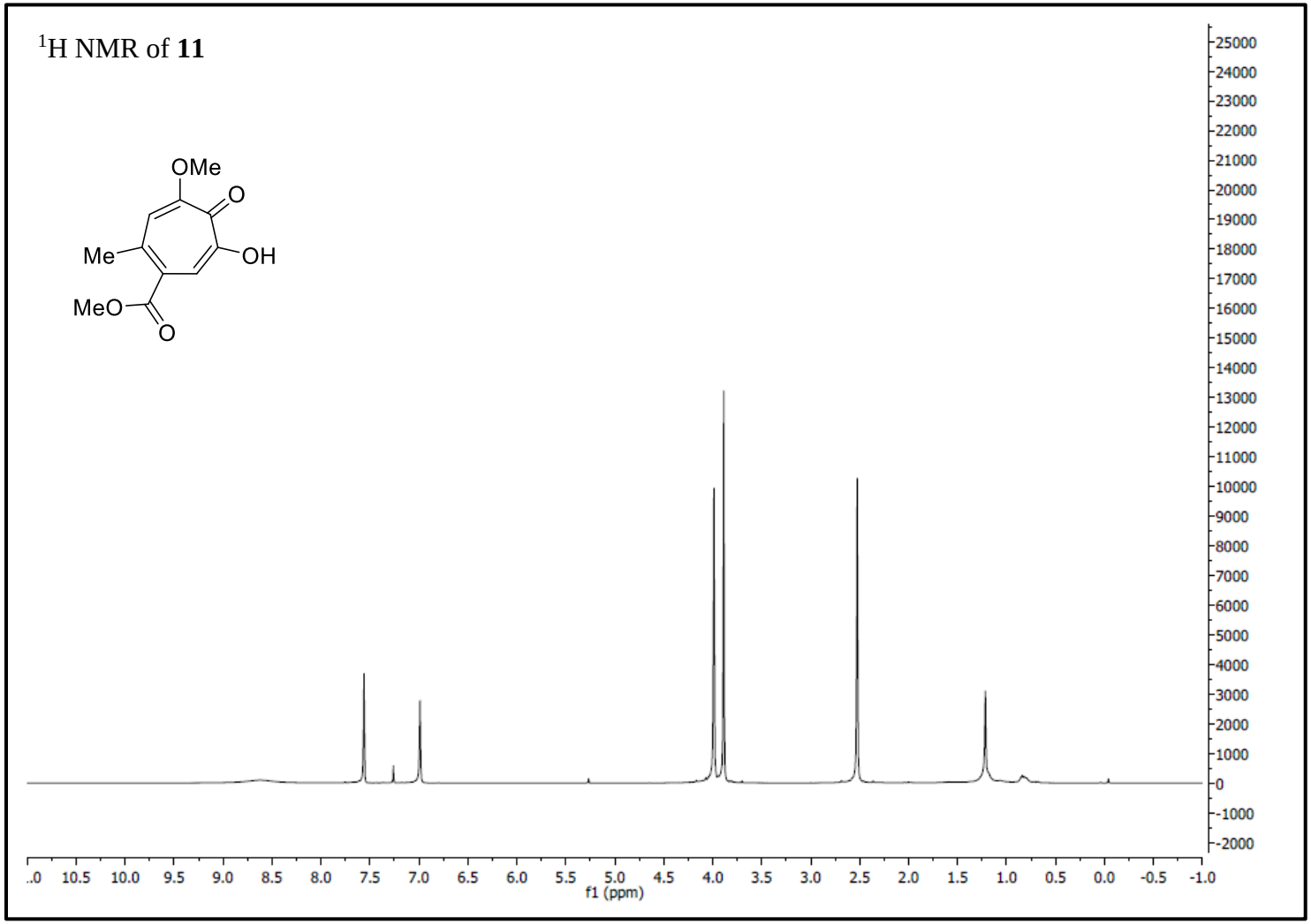
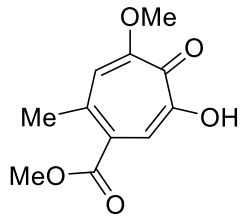
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

Methyl 6-hydroxy-4-methoxy-2-methyl-5-oxocyclohepta-1,3,6-triene-1-carboxylate **(11)**

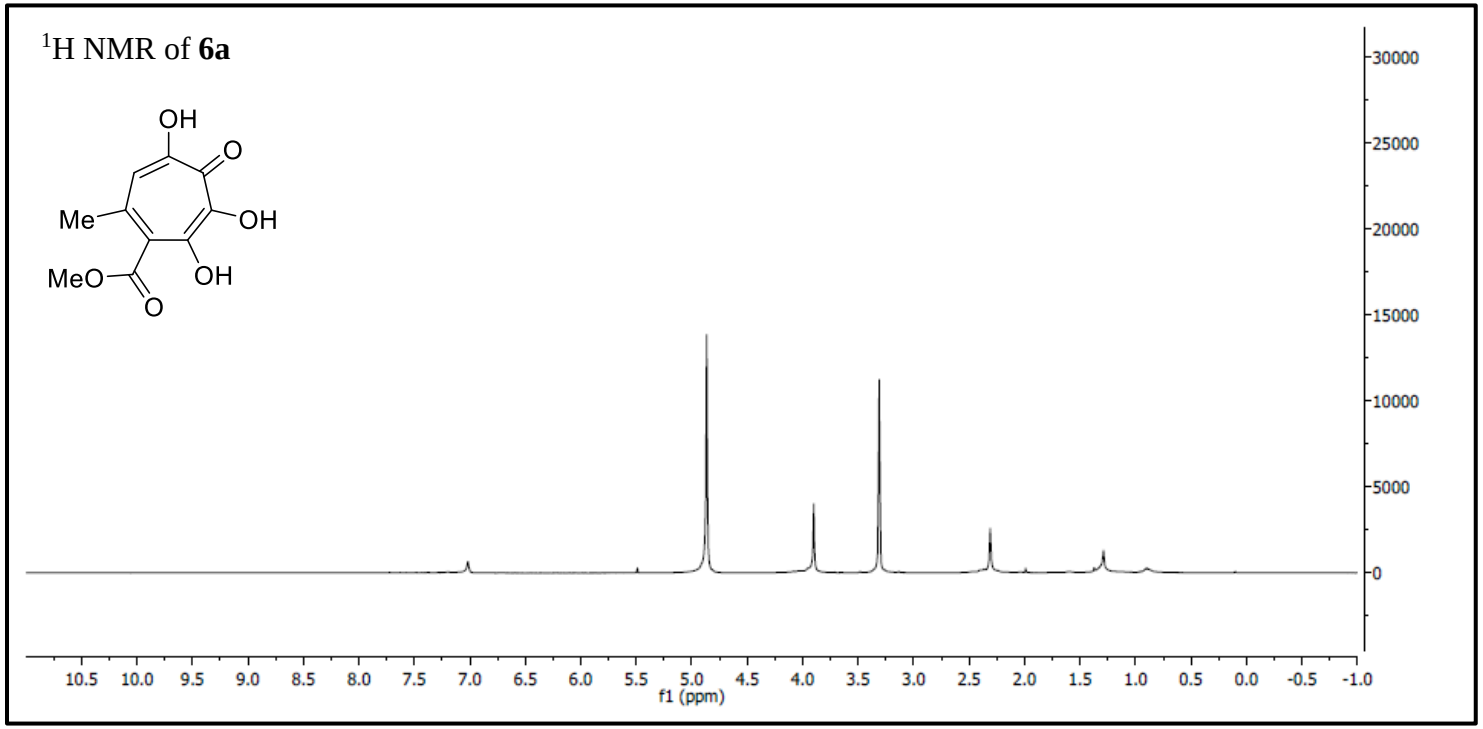


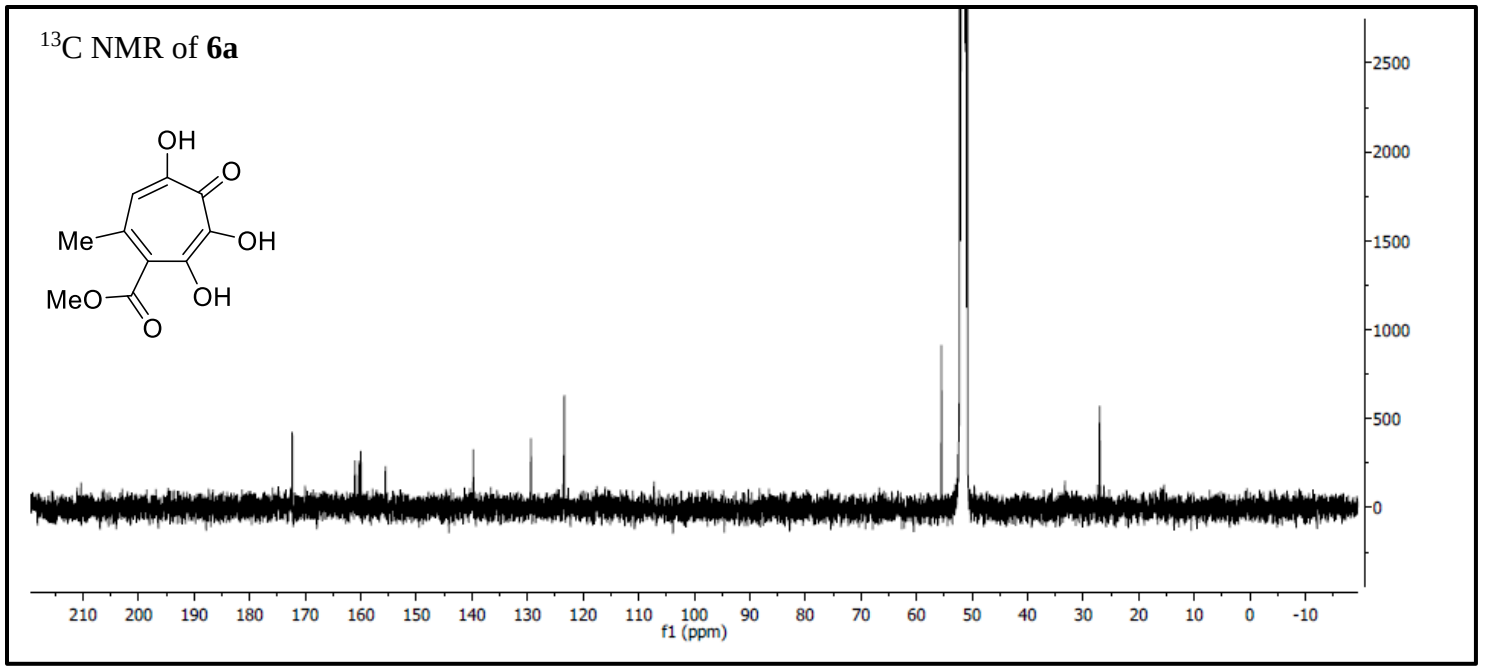
^{13}C NMR of **11**


OMe
O
Me
OH
MeO
O

3500
3000
2500
2000
1500
1000
500
0
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

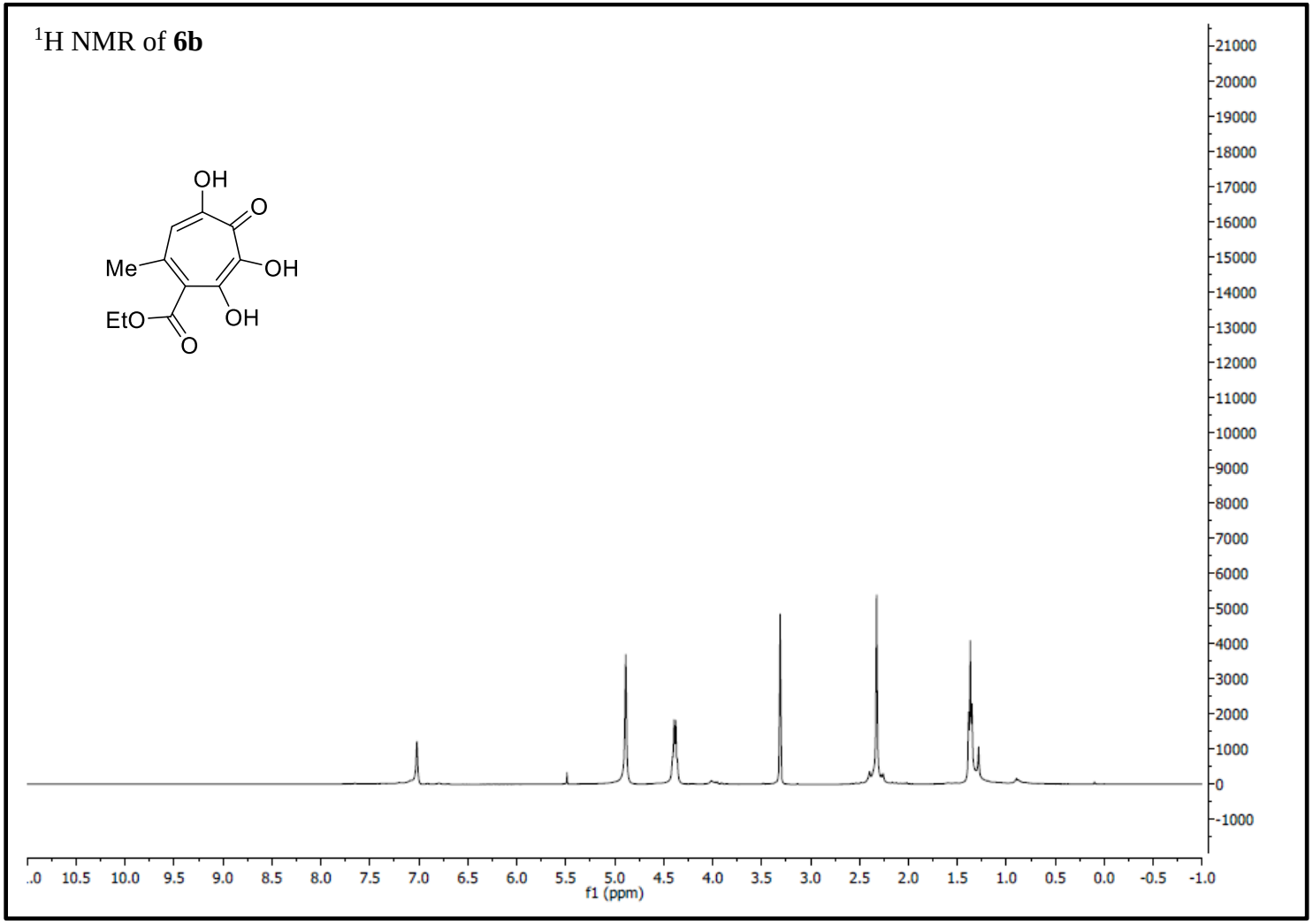
Methyl 4,6,7-trihydroxy-2-methyl-5-oxocyclohepta-1,3,6-triene-1-carboxylate **(6a)**



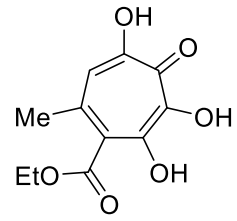

^{13}C NMR of **6a**
OH
O
Me
OH
MeO
OH
O
2500
2000
1500
1000
500
0
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

2,3,7-Trihydroxy-5-methylcyclohepta-2,4,6-trien-1-one **(6b)**

^{1}H NMR of **6b**

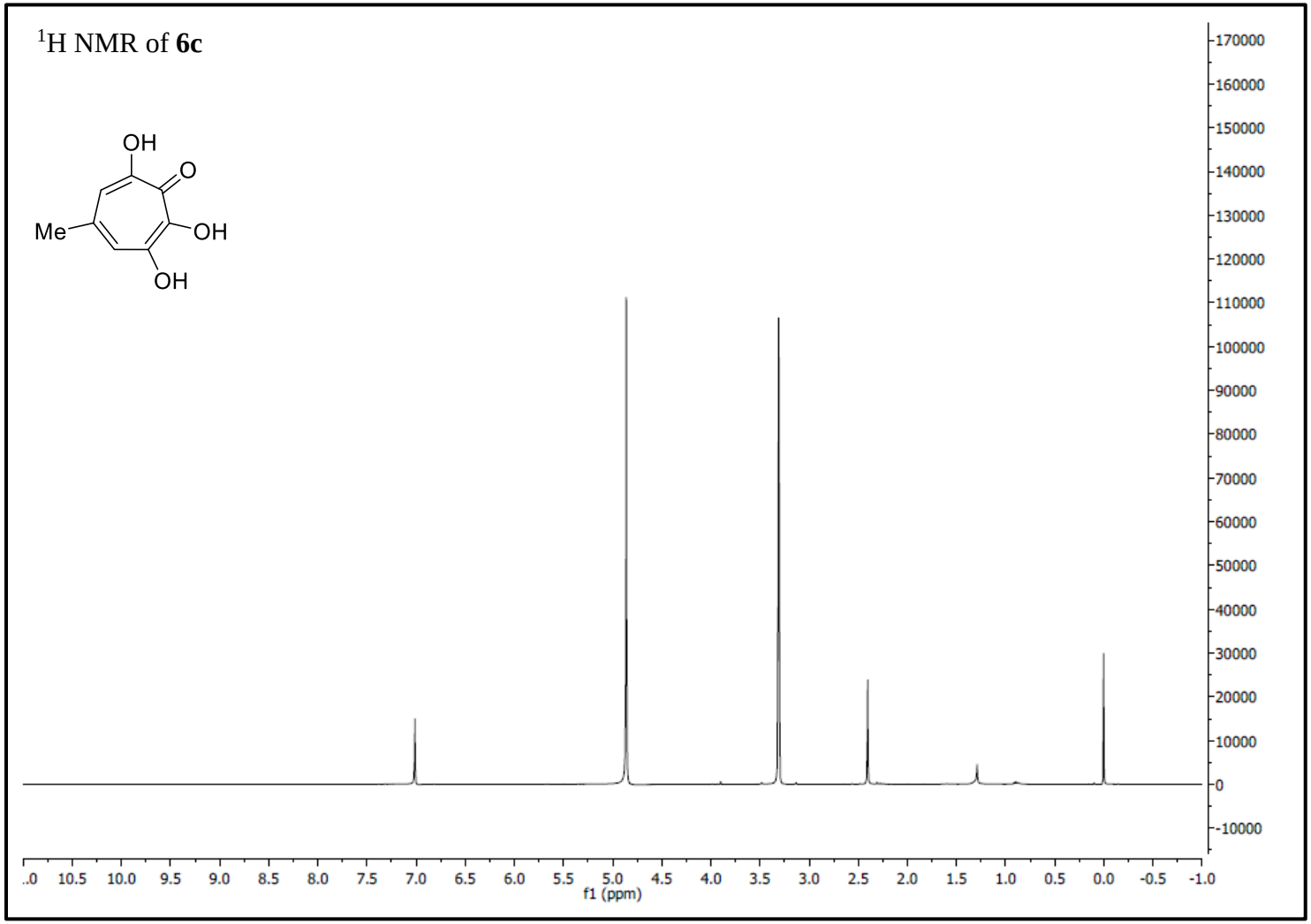


^{13}C NMR of **6b**

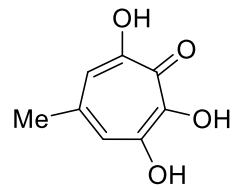

OH
O
Me
OH
EtO
OH
O

11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
20 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

Ethyl 4,6,7-trihydroxy-2-methyl-5-oxocyclohepta-1,3,6-triene-1-carboxylate **(6c)**



^{13}C NMR of **6c**


OH
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Me
OH
OH

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26000
24000
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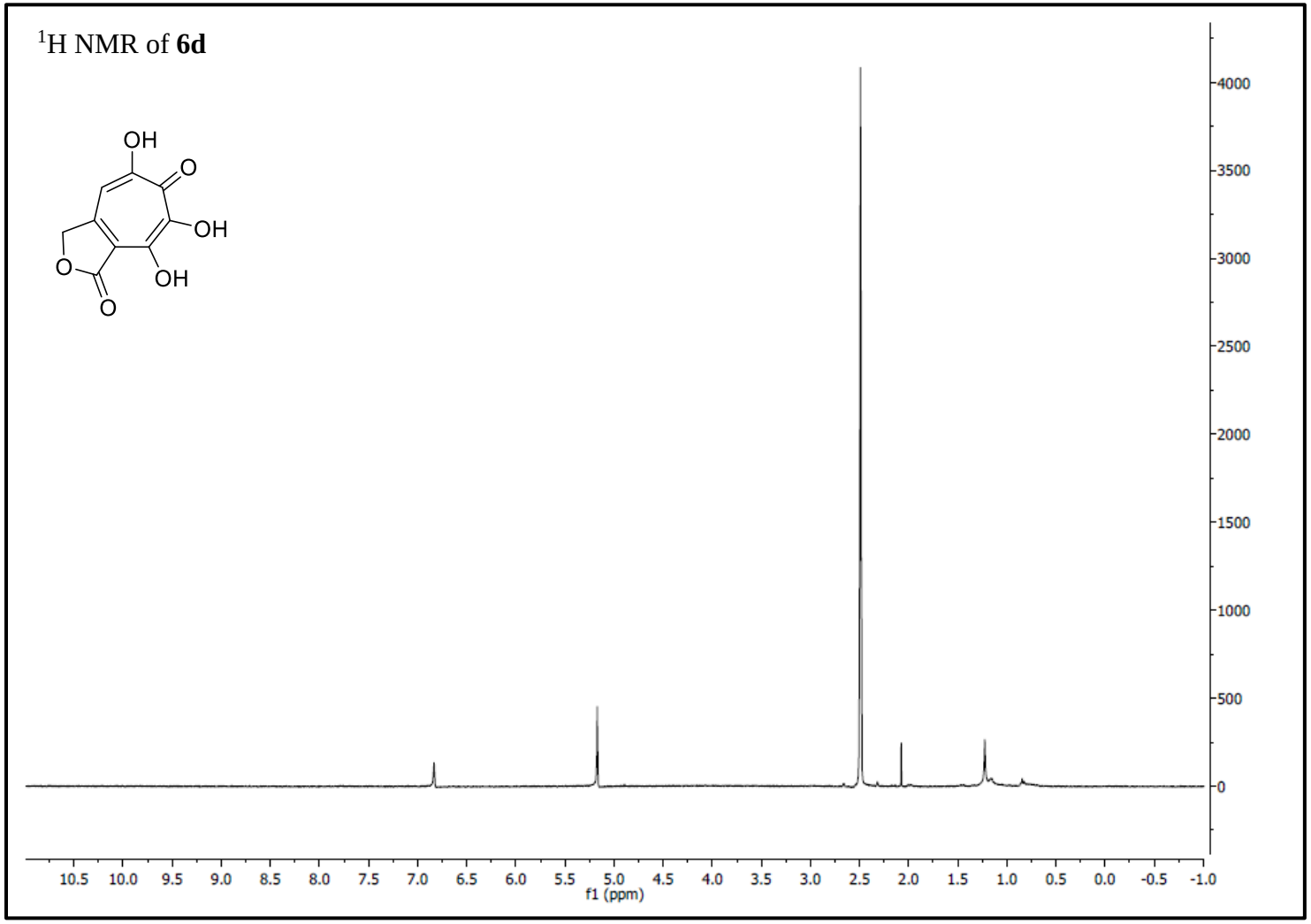
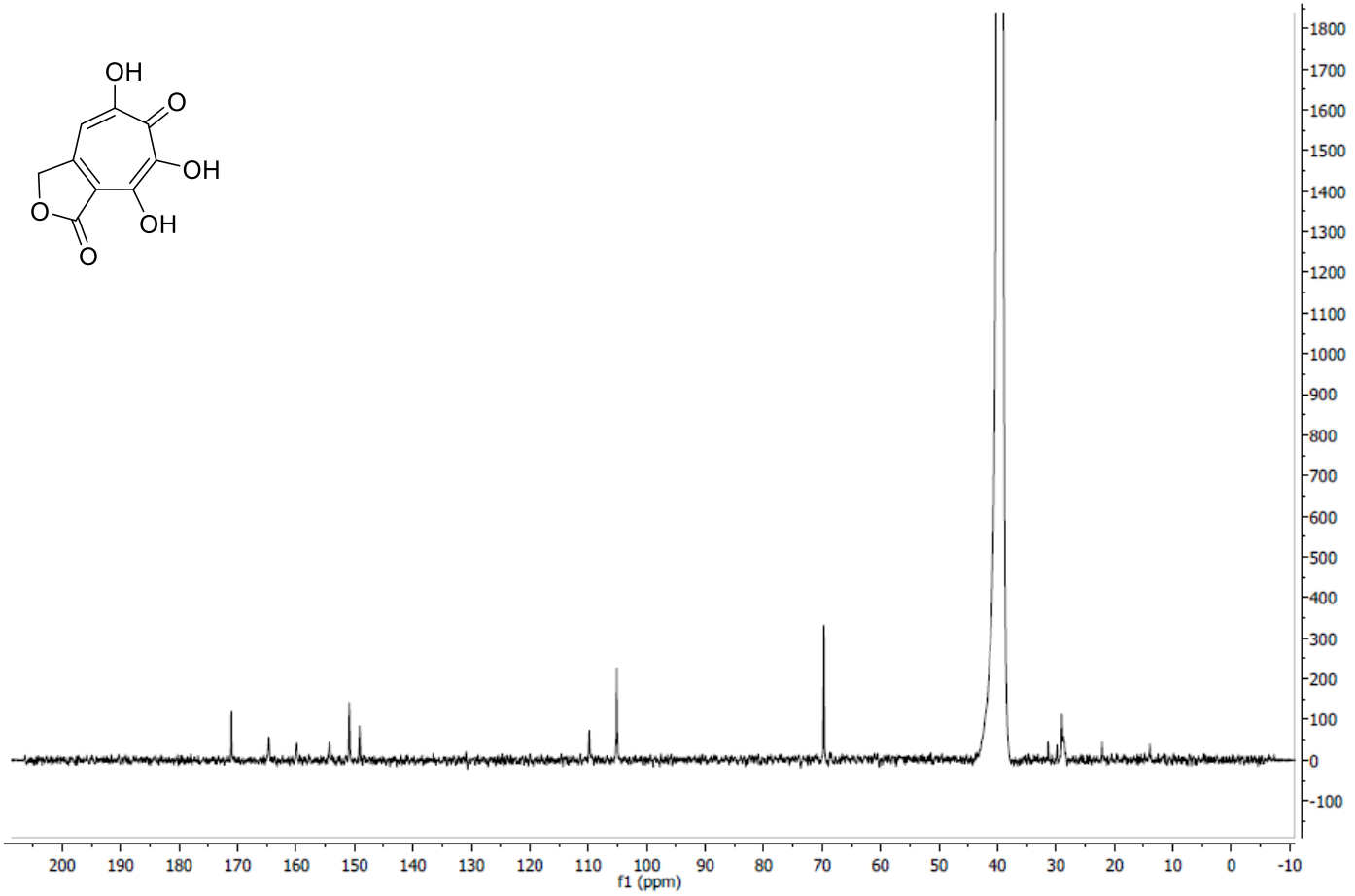
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
-2000
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

5,7,8-Trihydroxy-1H-cyclohepta[c]furan-1,6(3H)-dione **(6d)**



^{13}C NMR of **6d**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O
OH
O
OH
O
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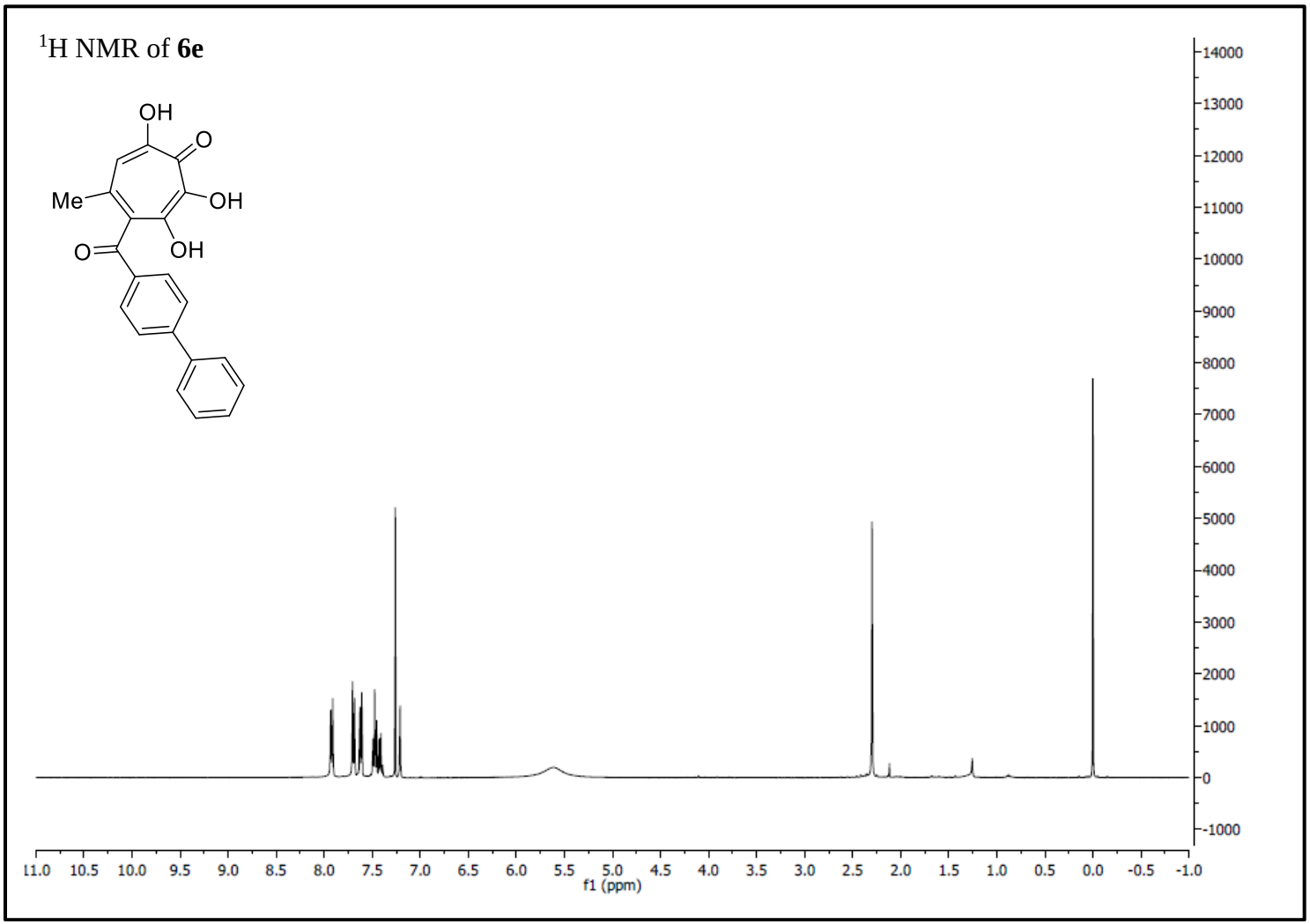
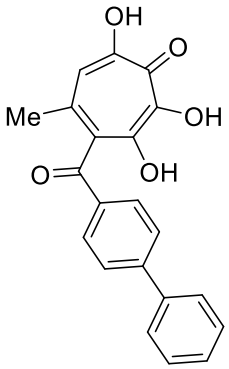
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
-100
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

4-([1,1'-biphenyl]-4-carbonyl)-2,3,7-trihydroxy-5-methylcyclohepta-2,4,6-trien-1-one **(6e)**

^{1}H NMR of **6e**



^{13}C NMR of **6e**


OH
O
Me
OH
O
OH

12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

IX. Biological Studies

1. HIV-Associated Assays

1a. RNase H Inhibitor Analysis. IC_{50} values were determined as previously reported[5] using an 18-nucleotide 3'-fluorescein-labeled RNA annealed to a complementary 18-nucleotide 5'-dabsyl-labeled DNA. The reaction was initiated by adding 10 μL of 20 ng/μL HIV-1 RT to 90 μL of mixture containing 50 mM Tris·HCl, pH 8.0, 60 mM KCl, 10 mM $MgCl_2$, 1% DMSO, 250 nM substrate, and increasing concentrations of α-hydroxytropolones. Plates were incubated at 37°C in a Infinite® M1000 PRO plate reader (Tecan, Switzerland) for 10 minutes, and fluorescence (λ_{ex} = 485 nm; λ_{em} = 520 nm) was measured at 1-minute intervals such that linear initial rates could be measured in the presence (v_i) and absence (v_o) of inhibitor. Percent inhibition was calculated as $100(v_o - v_i)/v_o$, and plotted against log[I]. IC_{50} values were calculated using Prism6 (GraphPad Software). All assays were performed in triplicate.

1b. Differential Scanning Fluorimetry (ThermoFluor). Thermal stability assays were performed according to Nettleship et al.[6] 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 64 ng/μL HIV-1 RT, 20 mM HEPES, pH 7.5, 10 mM $MgCl_2$, 100 mM NaCl, and a 1:1000 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 triplicate.

1c. HIV-1 Cytopathicity Assay. This assay was conducted as previously reported.[7] 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}).

2. Hepatitis B-Associated Assays

2a. HBV Replication Assay. This assay was conducted analogously to previous studies in the Tavis lab, using a HepDES19 cell system.[8] Cells were seeded in 12-well plates and incubated in Dulbecco's modified Eagle's medium (DMEM)/F12, 10% fetal bovine serum (FBS), and 1% penicillin and streptomycin (P/S). The test compounds (20 or 5 μM) was applied to duplicate wells 48 h later in medium containing a final DMSO concentration of 1%. Cells were harvested three days after compound addition, and nonencapsidated nucleic acids were digested with micrococcal

[5] 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*, 1249-56.
[6] Nettleship, J. E.; Brown, J.; Groves, M. R.; Geerlof, A. *Methods Mol. Biol.*, **2008** , *426*, 299-318.
[7] Weislow, O. S.; Kiser, R.; Fine, D. L.; Bader, J.; Shoemaker, R. H.; Boyd, M. R. J. *Natl. Cancer Inst.* **1989**, *81*, 577–86.
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nuclease (New England BioLabs). HBV DNA was purified from capsids using a QIAamp cador pathogen minikit (Qiagen) with proteinase K incubation overnight at 37°C. TaqMan PCR was performed for 40 cycles at an annealing temperature of 60°C. The primers and probe (IDT Inc.) for the plus-polarity strand were 5'CATGAACAAGAGATGATTAGGCAGAG3', 5'GGAGGCTGTAGGCATAAATTGG3', and 5'/56-FAM/CTGCGCACC/ZEN/AGCACCATGCA/3IABkFQ. The primers and probe for the minus-polarity strand were 5'GCAGATGAGAAGGCACAGA3', 5'CTTCTCCGTCTGCCGTT3', and 5'/56-FAM/AGTCCGCGT/ZEN/AAAGAGAGGTGCG/3IABkFQ.

2b. MTS Cytotoxicity Assay. Cytotoxicity measurements were conducted as described previously.[8] HepDES19 cells ($1.0x10^4$ cells per well) were seeded in 96-well plates and incubated in DMEM/F12 with 10% FBS plus 1% P/S. The compounds were diluted in the medium to the indicated concentrations plus 1% DMSO and added to the cells 24 h after plating, with each concentration tested in triplicate. MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] and phenazine ethosulfate (PES) (Promega). (Promega) were added to 0.33 mg/ml, the cultures were incubated for 60 min, and the absorbance was read at 490 nm. The 50% cytotoxic concentration (CC_{50}) values were calculated with GraphPad Prism using the four-parameter variable-response log(inhibitor)-versus-response algorithm with the bottom value set to zero.

3. Herpes Simplex Virus-Associated Assays

3a. HSV replication inhibition assays. HSV replication assays were conducted as described previously.[9] Test compounds were diluted in PBS containing 2% newborn calf serum and 2 mM L–glutamine and were added to confluent cell monolayers in 24-well plates. Equivalent dilutions of DMSO were used as vehicle controls. HSV-1 and HSV-2 were diluted in supplemented PBS medium and added so that the final compound concentrations were 5 or 1 μM and the HSV MOI was 0.1. The cells were incubated at 37°C for 1 h, the virus-containing inoculum was removed, the wells were washed once in PBS, and the compound (5 or 1 μM) in supplemented DMEM was added. Cells were incubated at 37°C for an additional 23 h, and the plates were then inspected by phase-contrast microscopy for cytopathic effect (CPE) or toxicity. Cells in wells showing less CPE than in DMSO-treated control wells were harvested by scraping, along with DMSO control wells and cells from additional wells with significant CPE for comparison. Samples were frozen at -80°C, thawed, and sonicated, and virus titers were then determined by a plaque assay on Vero cells. Each experiment was repeated at least once. The 50% effective concentrations (EC_{50}s) were determined for compounds **6a** and **6b** as described above except that serial dilutions of the compounds were employed. Values were calculated with GraphPad Prism using the three-parameter log(inhibitor)-versus-response algorithm with the bottom value set to zero.

3b. Vero cytotoxicity assays. Cytotoxicity experiments were carried out as described previously.[9] Vero cells ($1x10^4$ cells per well) were seeded into 96-well plates and incubated in DMEM/10% FBS plus PS. Test compounds were

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diluted in medium to the indicated concentrations with 1% DMSO and added to the cells 24 h after plating, with each concentration being tested in triplicate. Twenty-four hours after compound addition, [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS)] and phenazine ethosulfate (PES) (Promega) were added to 0.33 mg/ml, the cultures were incubated for 60 min, and the absorbance was read at 490 nm.MT. The 50% cytotoxic concentration (CC_{50}) values were calculated with GraphPad Prism using the four-parameter variable-response log(inhibitor)-versus-response algorithm with the bottom value set to zero.

3c. HSV-1 pUL15C Inhibition Assay. pUL15C inhibition experiments were carried out using a fluorescence quenching assay as described previously.[10] Substrate for the fluorescence quenching assay was generated using a dual-labeled 42-nt DNA oligonucleotide, Q670-42D-BHQ2plus:
5′ Q670-TATGTATTTAGGATTGGGATTATACCCAATCCTAAATACATA-BHQ2plus 3′, which forms duplex structure with a short intervening stem-loop. Denaturation and re-annealing was performed by heating a mixture containing 10 mM Q670-42D-BHQ2plus, 10 mM Tris/HCl (pH 7.6), and 25 mM NaCl to 80°C and slow cooling to 4°C. To a 96-well plate was added 1 μL of each inhibitor (in DMSO), followed by 89 μL of reaction buffer containing the substrate. Hydrolysis was initiated by adding 10 μL of 100 ng/μL HSV-1 pUL15C. Final assay conditions were 20 mM Tris-HCl, pH 7.0, 10 mM NaCl, 1 mM $MnCl_2$, 1% DMSO, 10 ng/μL pUL15C, 250 nM substrate, and increasing concentrations of inhibitor. Wells containing only DMSO were used as control. Plates were incubated at 37 ˚C in a Infinite® M1000 PRO plate reader (Tecan, Switzerland) for 10 min, and fluorescence (λ_{ex} = 646 nm; λ_{em} = 670 nm) was measured at 1 min intervals such that linear initial rates could be measured in the presence (v_i) and absence (v_o) of inhibitor. Percent inhibition was calculated as $100(v_o–v_i)/v_o$, and plotted against log[I]. IC_{50} values were calculated using Prism6. All assays were performed in triplicate.

X. Homology Docking Studies

1. Generation of pUL1C homology model.

The metal cations bound structural model of pUL1C employed in the molecular docking calculations was obtained by structural alignment of the metal-binding DEDD motif residues D509, E581, D706, and D707 of apo pUL15C crystal structure (PDB ID 4IOX)[11] with the DEDD motif of the crystal of HIV RT RNase H bound to divalent manganese cations and β-thujaplicinol (PDB ID 3K2P).[12] The resulting structure of holo pUL15C was refined by energy minimization and simulated annealing using the Impact molecular modeling program.[13]

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2. Molecular docking of αHT 12 and 3,7-dHT 6e.

The receptor grid was generated using the default parameters available in the Schrodinger Suite 2016-3 with one special adjustment where metal constraints were applied to allow metal-ligand interaction at the binding site. Glide docking[14] was performed with the ligands αHT **12** and 3,7-dHT **6e**. Several binding poses were obtained for the molecule **6e**, out of which the best binding pose is shown in Figure 4.

This conformation reveals favorable pi-cation interaction between the quaternary ammonium cation of Lys 640 and the phenyl ring pi system of the biphenyl group of the ligand. The biphenyl group is thought to be further stabilized by accommodating within a hydrophobic groove formed between Lys 640 and Asn 583 with possible hydrophobic interaction with the Leu 636 present inside the groove.

Further stabilization could also be achieved through the interactions of the biaryl side chain of 3,7-dHT **6e** with Asn 583 through NH-pi interactions. These interactions are never observed during the extensive molecular docking and molecular dynamics calculations of the αHT **12**, lacking an additional metal-coordinating oxygen, which cannot adopt an orientation compatible with the formation of these favorable products.

[14] Schrodinger Release 2016-3: Glide, Schrodinger, LLC, New York, NY, 2016.