Identification of Vitamin D3-Based Hedgehog Pathway Inhibitors that Incorporate an Aromatic A-Ring Isostere.

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Supporting Information – Synthetic Procedures, Compound Characterization, Biological Assay Protocols, 1H and 13C NMR Spectra

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Table of Contents

General Information.

VD3 used for chemical synthesis was purchased from HBCChem, Inc. Hydroxy benzoic acids (HBA) and methyl ester phenols were purchased from either Sigma-Aldrich or Fisher Scientific. ACS or HPLC grade methanol, acetone, and tetrahydrofuran were purchased from Fisher Scientific. Anhydrous DCM (low water, <50 ppm water) was purchased from BrandNu Laboratories, Inc. (J.T. Baker solvent). NMR data was performed on a Bruker AVANCE 500 or 300 MHz spectrometer and analysis was done on MestReNova version 8.0.0. HRMS data was analyzed at the Mass Spectrometry Facility at the University of Connecticut, performed by Dr. You-Jun Fu. Infrared (IR) analysis was performed on a Shimadzu FTIR-8400S spectrophotometer with IR Solution software.

Experimental Procedures.

Preparation of benzyl protected methyl ester 5b. To a stirred solution of 3-phenolic methyl ester **5a** (1eq, 6.5 mmol) in acetone (30 mL), was added potassium carbonate (anhydrous, 6eq, 39 mmoL) followed by benzyl bromide (1.2 eq, 7.9 mmol). The reaction mixture was refluxed until methyl ester was consumed (~6 hr). The mixture was filtered over a celite pad, washed with EtOAC (30 mL), and concentrated. The crude residue was purified by column chromatography on silica gel (5% EtOAC in hexanes) to afford the benzyl-protected 3-phenolic methyl ester **5b** as a clear oil in high yield (85%).

Preparation of benzoic acid 5c. To a stirred solution of ester **5b** (3.7 mmol) in THF (30 mL) was added potassium hydroxide (aq., 20%; 20mL). The reaction mixture was stirred at room temperature for 12 hr at which time it was neutralized with portion-wise additions of 3N HCl and then the pH was brought to ~1 using 1N HCl. The aqueous mixture was extracted with EtOAc (3X100 mL). The combined organic fractions were dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude residue was purified by column chromatography on silica gel (30-50% EtOAC in hexanes) to afford pure **5c** as a white solid in good yield (70%).

General esterification coupling procedure. Grundmann's alcohol **4** (1 eq, 50 mg, 0.19 mmol), dicyclohexylcarbodiimide (3 eq, 117 mg, 0.57 mmol), and dimethylaminopyridine (3 eq, 70 mg, 0.57 mmol) were dissolved in anhydrous DCM (4 mL) in an oven-dried round bottom flask. The benzoic acid (**5c**, **6b**, **7b**, **8b**, benzoic acid, 3-chlorobenzoic acid, 3-methoxybenzoic acid, and 3-acetylbenzoic acid; 3 eq, 0.57 mmol) was added and the reaction mixture stirred overnight at RT. The crude mixtures were loaded directly on a silica column and purified with flash chromatography (4-60% EtOAc in Hexanes) to yield **9** – **20** in good yields (60-95%).

9

(1R,3aR,4S,7aR)-7a-methyl-1-((R)-6-methylheptan-2-yl)octahydro-1H-inden-4-yl 3-(benzyloxy)benzoate, (**9**). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68 (m, 2H), 7.46 (m, 2H), 7.40 (m, 2H), 7.35, (m, 1H), 7.18 (m, 1H), 5.41 (m, 1H), 5.12 (s, 2H), 2.08 (m, 1H), 2.00 (m, 1H), 1.83 (m, 2H), 1.54 (m, 5H), 1.44 (m, 2H), 1.37 (m, 2H), 1.25 (m, 2H), 1.15 (m, 4H), 1.04 (s, 3H), 0.95 (d, *J* = 6.4 Hz, 3H), 0.88 (dd, *J* = 6.6, 2.1 Hz, 6H). ^{13}C NMR (126 MHz $CDCl_3$) δ 166.2, 158.6, 136.5, 132.2, 129.3, 128.5, 128.0, 127.4, 122.2, 120.0, 114.9, 72.4, 70.0, 56.4, 51.6, 41.9, 39.9, 39.4, 35.9, 35.4, 30.5, 27.9, 27.0, 23.7, 22.8, 22.6, 22.5, 18.5, 18.0, 13.5. IR(film) *ν*max 3018, 2938, 2930, 2855, 2117, 1709, 1598, 1584, 1467, 1464, 1447, 1382, 1365, 1289, 1272,

1215, 1158, 1108, 1102, 1074, 1042, 1026, 946, 757, 696, 680, 668. DART-HRMS: a) *m/z* calcd. for $C_{32}H_{44}O_3$: 476.3290 $[M]^+$. Found: 476.3292. b) *m/z* calcd. for $C_{32}H_{43}O_3$: 475.3212 $[M-H]^+$. Found: 475.3216.

10

Benzyl ((1R,3aR,4S,7aR)-7a-methyl-1-((R)-6-methylheptan-2-yl)octahydro-1H-inden-4-yl) isophthalate, (**10**). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.76 (m, 1H), 8.26 (m, 2H), 7.54 (m, 1H), 7.46 (m, 2H), 7.40 (m, 2H), 7.35 (m, 1H), 5.42 (m, 1H), 5.38 (m, 2H), 2.07 (m, 1H), 2.01 (m, 1H), 1.81 (m, 2H), 1.54 (m, 5H), 1.44 (m, 1H), 1.37 (m, 3H), 1.24 (m, 6H), 1.13 (m, 2H), 0.99 (s, 3H), 0.94 (d, *J* = 6.4 Hz, 3H), 0.90 (d, *J* = 1.2 Hz, 3H), 0.88 (d, *J* = 1.2 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.5, 165.4, 135.6, 133.8, 133.6, 131.3, 130.7, 130.5, 128.5, 128.2, 72.7, 67.0, 56.4, 51.5, 41.8, 39.8, 39.4, 35.8, 35.3, 30.4, 29.6, 27.9, 27.0, 23.7, 22.7, 22.6, 22.5, 18.5, 17.9, 13.41. DART-HRMS: a) *m/z* calcd. for $C_{33}H_{44}O_4NH_4$: 522.3583 $[MNH_4]^+$. Found: 522.3581.

11

(1R,3aR,4S,7aR)-7a-methyl-1-((R)-6-methylheptan-2-yl)octahydro-1H-inden-4-yl 3-((tert-butoxycarbonyl)amino)benzoate, (**11**). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.91 (m, 1H), 7.84 (br s, 1H), 7.70 (m, 1H), 7.34 (m, 1H), 7.06 (s, 1H), 5.40 (m, 1H), 2.04 (m, 1H), 1.97 (m, 1H), 1.81 (m, 2H), 1.51 (s, 9H), 1.41 (m, 2H), 1.33 (m, 2H), 1.23 (m, 3H), 1.12 (m, 4H), 1.02 (s, 3H), 0.92 (d, *J* = 6.5 Hz, 3H), 0.87 (d, *J* = 1.9 Hz, 3H), 0.85 (d, *J* = 1.9 Hz, 3H). ^{13}C NMR (126 MHz $CDCl_3$) δ 166.2, 152.6, 138.7, 131.4, 128.9, 123.7, 122.8, 119.6, 80.4, 72.3, 56.3, 51.5, 41.8, 39.8, 39.3, 35.8, 35.3, 30.4, 29.6, 28.2, 27.9, 26.9, 23.6, 22.7, 22.5, 22.4, 18.5, 17.9, 13.4. DART-HRMS: *m/z* calcd. for $C_{30}H_{47}NO_4NH_4$: 503.3849 $[MNH_4]^+$. Found: 503.3868.

12

(1R,3aR,4S,7aR)-7a-methyl-1-((R)-6-methylheptan-2-yl)octahydro-1H-inden-4-yl 3-((tert-butoxycarbonyl)(methyl)amino)benzoate, (**12**). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.90 (m, 1H), 7.84 (m, 1H), 7.47 (m, 1H), 7.39 (m, 1H), 5.40 (m, 1H), 3.29 (s, 3H), 2.07 (m, 1H), 1.98 (m, 1H), 1.81 (m, 2H), 1.53 (m, 5H), 1.46 (s, 9H), 1.35 (m, 3H), 1.25 (m, 6H), 1.12 (m, 4H), 1.04 (s, 3H), 0.93 (m, *J* = 6.5 Hz, 3H), 0.86 (m, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.0, 154.4, 143.9, 131.4, 129.8, 128.4, 126.3, 126.1, 80.6, 72.4, 56.4, 51.6, 41.9, 39.9, 39.4, 37.0, 35.9, 35.4, 30.5, 29.6, 28.2, 27.9, 27.0, 23.7, 22.7, 22.6, 22.5, 18.6, 18.0, 13.5. DART-HRMS: a) *m/z* calcd. for $C_{31}H_{49}NO_4NH_4$: 517.4005 $[MNH_4]^+$. Found: 517.3971.

13

(1R,3aR,4S,7aR)-7a-methyl-1-((R)-6-methylheptan-2-yl)octahydro-1H-inden-4-yl benzoate, (**13**). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.06 (m, 2H), 7.55 (m, 1H), 7.45 (m, 2H), 5.41 (m, 1H), 2.08 (m, 1H), 2.00 (m, 1H), 1.84 (m, 2H), 1.54 (m, 5H), 1.36 (m, 3H), 1.25 (m, 3H), 1.14 (m, 4H), 1.05 (s, 3H), 0.94 (d, *J* = 6.5 Hz, 3H), 0.87 (dd, *J* = 6.5, 2.1 Hz, 6H). ^{13}C NMR (126 MHz $CDCl_3$) δ 166.5, 132.6, 130.9, 129.5, 128.3, 72.2, 56.4, 51.6, 41.9, 39.9, 39.4, 35.9, 35.4, 30.5, 28.0, 27.0, 23.7, 22.8, 22.6, 22.5, 18.6, 18.0, 13.5. IR(film) *ν*max 3017, 2953, 2933, 2925, 2868, 1706, 1457, 1451, 1314, 1284, 1273, 1265, 1215, 1117, 1095, 1026, 760, 712, 668. DART-HRMS: a) *m/z* calcd. for $C_{25}H_{38}O_2NH_4$: 388.3216 $[MNH_4]^+$. Found: 388.3199. b) *m/z* calcd. for $C_{25}H_{37}O_2$: 369.2794 $[M-H]^+$. Found: 369.2773.

14

(1R,3aR,4S,7aR)-7a-methyl-1-((R)-6-methylheptan-2-yl)octahydro-1H-inden-4-yl 3-chlorobenzoate (**14**). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.01 (m, 1H), 7.93 (m, 1H), 7.52 (m, 1H), 7.38 (m, 1H), 5.40 (m, 1H), 2.08 (m, 1H), 1.98 (m, 1H), 1.82 (m, 2H), 1.53 (m, 4H), 1.41 (m, 2H), 1.33 (m, 3H), 1.25 (m, 5H), 1.13 (m, 4H), 1.03 (s, 3H), 0.93 (d, *J* = 6.4 Hz, 3H), 0.87 (d, *J* = 2.0 Hz, 3H), 0.86 (d, *J* = 2.0 Hz, 3H). ^{13}C NMR (126 MHz $CDCl_3$) δ 165.2, 134.4, 132.6, 129.6, 127.6, 72.9, 56.4, 51.5, 41.9, 39.8, 39.4, 35.9, 35.4, 30.5, 28.0, 27.0, 23.7, 22.6, 22.5, 18.6, 18.0, 13.5. IR(film) *v*max 2949, 2921, 1721, 1463, 1455, 1428, 1381, 1292, 1252, 1159, 1127, 1121, 1060, 750. DART-HRMS: *m/z* calcd. for $C_{25}H_{36}ClO_2$: 403.2404 $[M-H]^+$. Found: 403.2347. *m/z* calcd. for $C_{25}H_{38}ClO_2NH_3$: 422.2826 $[MNH_4]^+$. Found: 422.2807.

15

(1R,3aR,4S,7aR)-7a-methyl-1-((R)-6-methylheptan-2-yl)octahydro-1H-inden-4-yl 3-methoxybenzoate, (**15**). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.65 (m, 1H), 7.59 (m, 1H), 7.35 (m, 1H), 7.10 (m, 1H), 5.40 (m, 1H), 3.85 (s, 3H), 2.07 (m, 1H), 1.99 (m, 1H), 1.82 (m, 2H), 1.52 (m, 5H), 1.35 (m, 3H), 1.24 (m, 2H), 1.12 (m, 3H), 1.05 (s, 3H), 0.93 (d, *J* = 6.5 Hz, 3H), 0.87 (d, *J* = 2.0 Hz, 3H), 0.86 (d, *J* = 2.1 Hz, 3H). ^{13}C NMR (126 MHz $CDCl_3$) δ 166.3, 159.5, 132.2, 129.3, 121.9, 119.2, 113.9, 72.3, 56.4, 55.3, 51.6, 41.9, 39.9, 39.4, 35.9, 35.4, 30.5, 27.9, 27.0, 23.7, 22.7, 22.6, 22.5, 18.5, 18.0, 13.5. IR(film) *v*max 2950, 2947, 2933, 2928, 2866, 1718, 1606, 1581, 1284, 1275, 1227, 1158, 1099, 1043, 755. DART-HRMS: *m/z* calcd. for $C_{26}H_{40}O_3$: 400.2977 $[M]^+$. Found: 400.2985. *m/z* calcd. for $C_{26}H_{41}O_3NH_3$: 418.3321 $[MNH_4]^+$. Found: 418.3341.

16

(1R,3aR,4S,7aR)-7a-methyl-1-((R)-6-methylheptan-2-yl)octahydro-1H-inden-4-yl 3-acetylbenzoate, (**16**). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.62 (m, 1H), 8.23 (m, 1H), 8.15 (m, 1H), 7.55 (m, 1H), 5.44 (m, 1H), 2.64 (s, 3H), 2.09 (m, 1H), 2.01 (m, 1H), 1.83 (m, 2H), 1.55 (m, 5H), 1.42 (m, 2H), 1.33 (m, 2H), 1.24 (m, 3H), 1.14 (m, 4H), 1.07 (s, 3H), 1.01 (m, 1H), 0.94 (d, *J* = 6.4 Hz, 3H), 0.87 (d, *J* = 2.0 Hz, 3H), 0.86 (d, *J* = 1.9 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 197.2, 165.6, 137.2, 133.8, 131.9, 131.4, 129.7, 128.8, 72.8, 56.4, 51.5, 41.9, 39.8, 39.4, 35.9, 35.4, 30.5, 28.0, 27.0, 26.6, 23.7, 22.7, 22.6, 22.5, 18.5, 18.0, 13.5. IR(film) *v*max 3440 (br), 3018, 2952, 2933, 2867, 2855, 1716, 1690, 1467, 1432, 1359, 1302, 1231, 1216, 1159, 1121, 1074, 937, 769, 684, 668, 596. DART-HRMS: a) *m/z* calcd. for $C_{27}H_{41}O_3NH_4$: 430.3321. $[MNH_4]^+$. Found: 430.3337.

17

(1R,3aR,4S,7aR)-7a-methyl-1-((R)-6-methylheptan-2-yl)octahydro-1H-inden-4-yl 3-hydroxybenzoate, (**17**). Protected ester **9** (0.1 mmol) was dissolved in MeOH:THF (2:1, 6 mL). $Pd(OH)_2$ (10 % on carbon, 10 mg) was then added. The flask was sealed with a rubber septum and charged with an atmosphere of hydrogen gas. The reaction was stirred overnight at RT. The reaction mixture was passed through a celite pad, rinsed with ethyl acetate (10 mL), and concentrated under reduced pressure. The crude residue was purified using flash chromatography over a packed silica column (10% EtOAc in hexanes) to yield analogue **17** in good yields (70%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.70 (m, 1H), 7.64 (m, 1H), 7.34 (m, 1H), 7.11 (m, 1H), 6.63 (br s, 1H), 5.43 (m, 1H), 2.08 (m, 1H), 2.01 (m, 1H), 1.84 (m, 2H), 1.56 (m, 5H), 1.37 (m, 3H), 1.26 (m, 3H), 1.15 (m, 4H), 1.04 (s, 3H), 0.95 (d, *J* = 6.5 Hz, 3H), 0.90 (dd, *J*

= 6.6, 1.9 Hz, 6H). ^{13}C NMR (126 MHz $CDCl_3$) δ 166.9, 156.1, 132.0, 129.7, 121.7, 120.2, 116.5, 72.2, 56.4, 51.5, 41.9, 39.8, 39.5, 35.9, 35.4, 30.5, 28.0, 27.0, 23.7, 22.8, 22.6, 22.5, 18.6, 18.0, 13.5. IR (film) *ν*max 3385 (br s), 3019, 2951, 2935, 2868, 1689, 1599, 1589, 1466, 1453, 1366, 1344, 1295, 1215, 1157, 1106, 1075, 1062, 982, 946, 886, 755, 680, 668. DART-HRMS: a) *m/z* calcd. for $C_{25}H_{37}O_3$: 385.2743 $[M-H]^+$. Found: 385.2745. b) *m/z* calcd. for $C_{25}H_{37}O_3NH_4$: 404.3165 $[MNH_4]^+$. Found: 404.3172.

18

3-((((1R,3aR,4S,7aR)-7a-methyl-1-((R)-6-methylheptan-2-yl)octahydro-1H-inden-4-yl)oxy)carbonyl)benzoic acid, (**18**). Protected ester **10** (0.1 mmol) was dissolved in ethyl acetate (6 mL). Pd/C (10 %, 10 mg) was then added. The flask was sealed with a rubber septum and charged with an atmosphere of hydrogen gas. The reaction was stirred overnight at RT. The reaction mixture was passed through a celite pad, rinsed with ethyl acetate (10 mL), and concentrated under reduced pressure. The crude residue was purified using flash chromatography over a packed silica column (30% EtOAc in hexanes) to yield final analogues in good yield (95%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.41 (br s, 1H), 8.81 (m, 1H), 8.30 (m, 2H), 7.58 (m, 1H), 5.46 (m, 1H), 2.09 (m, 1H), 2.02 (m, 1H), 1.85 (m, 2H), 1.55 (m, 5H), 1.43 (m, 2H), 1.35 (m, 2H), 1.25 (m, 6H), 1.14 (m, 3H), 1.08 (s, 3H), 0.94 (d, *J* = 6.5 Hz, 3H), 0.87 (d, *J* = 1.9 Hz, 3H), 0.86 (d, *J* = 1.9 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.2, 165.5, 134.5, 134.0, 131.5, 131.3, 129.8, 128.7, 72.9, 56.5, 51.6, 41.9, 39.8, 39.4, 35.9, 35.4, 30.5, 29.6, 27.9, 27.0, 23.7, 22.8, 22.6, 22.5, 18.5, 18.0, 13.5. IR(film) *ν*max 3019, 2992, 2950, 2926, 2907, 2889, 2869, 2853, 1712, 1699, 1608, 1466, 1446, 1415, 1383, 1303, 1288, 1257, 1235, 1215, 1141, 1096, 1074, 939, 762, 753, 731, 668. DART-HRMS: a) *m/z* calcd. for $C_{26}H_{38}O_4NH_4$: 432.3114. $[MNH_4]^+$. Found: 432.3140.

General procedure for Boc-deprotection.

Boc-protected ester **11** or **12** (0.1 mmol) was dissolved in DCM (2 mL) under Argon. Trifluoroacetic acid (neat, 1-2 mL) was then added. The reaction was stirred and monitored by TLC. When complete, sodium bicarbonate (aq., satd.) was added and the mixture was extracted with DCM (3X20mL). The combined organics were dried over sodium sulfate, filtered, and concentrated under reduced pressure. The crude residue was purified using flash chromatography over a packed silica column (30-50% EtOAc in hexanes) to yield analogues **19** and **20** in good yields (55-70%).

19

(1R,3aR,4S,7aR)-7a-methyl-1-((R)-6-methylheptan-2-yl)octahydro-1H-inden-4-yl 3-aminobenzoate, (**19**). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44 (m, 1H), 7.36 (m, 1H), 7.21 (m, 1H), 6.84 (m, 1H), 5.38 (m, 1H), 3.80 (br s, 2H), 2.06 (m, 1H), 1.97 (m, 1H), 1.81 (m, 2H), 1.51 (s, 5H), 1.41 (m, 2H), 1.34 (m, 2H), 1.23 (m, 2H), 1.12 (m, 4H), 1.03 (s, 3H), 0.93 (d, *J* = 6.5 Hz, 3H), 0.88 (d, *J* = 1.9 Hz, 3H), 0.86 (d, *J* = 1.9 Hz, 3H). ^{13}C NMR (126 MHz $CDCl_3$) δ 166.6, 146.4, 131.8, 129.1, 119.6, 119.1, 115.7, 72.1, 56.4, 51.5, 41.8, 39.9, 39.4, 35.8, 35.3, 30.5, 27.9, 27.0, 23.7, 22.7, 22.6, 22.5, 18.5, 18.0, 13.5. IR (film) *ν*max 3454 (br), 3407 (br), 3018, 2953, 2937, 2931, 2909, 2894, 2866, 2853, 2848, 1704, 1622, 1605, 1590, 1490, 1460, 1366, 1313, 1303, 1292, 1240, 1233, 1216, 1159, 1102, 946, 768, 752, 668. DART-HRMS: *m/z* calcd. for $C_{25}H_{39}NO_2$: 385.2981 $[M]^+$. Found: 385.2975.

20

(1R,3aR,4S,7aR)-7a-methyl-1-((R)-6-methylheptan-2-yl)octahydro-1H-inden-4-yl 3-(methylamino)benzoate, (**20**). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.85 (m, 2H), 7.44 (m, 1H), 7.37 (m, 2H), 5.41 (m, 1H), 2.07 (m, 1H), 1.98 (m, 1H), 1.81 (m, 2H), 1.53 (m, 5H), 1.37 (m, 4H), 1.25 (m, 6H), 1.13 (m, 4H), 1.02 (s, 3H), 0.93 (d, *J* = 6.5 Hz, 3H), 0.87 (d, *J* = 1.8 Hz, 3H), 0.86 (d, *J* = 1.8 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.5, 141.7, 132.8, 130.0, 126.2, 122.9, 119.4, 73.0, 56.4, 51.5, 41.9, 39.8, 39.4, 35.9, 35.3, 35.0, 30.5, 29.7, 28.0, 27.0, 23.7, 22.7, 22.6, 22.5, 18.5, 17.9, 13.4. IR(film) *v*max 3430 (br, s), 2952, 2922, 2847, 1703, 1606, 1456, 1275, 1242, 1235, 1158, 1109, 753. DART-HRMS: a) *m/z* calcd. for $C_{26}H_{42}NO_2$: 400.3215 $[MH]^+$. Found: 400.3245.

Biological Assay Protocols.

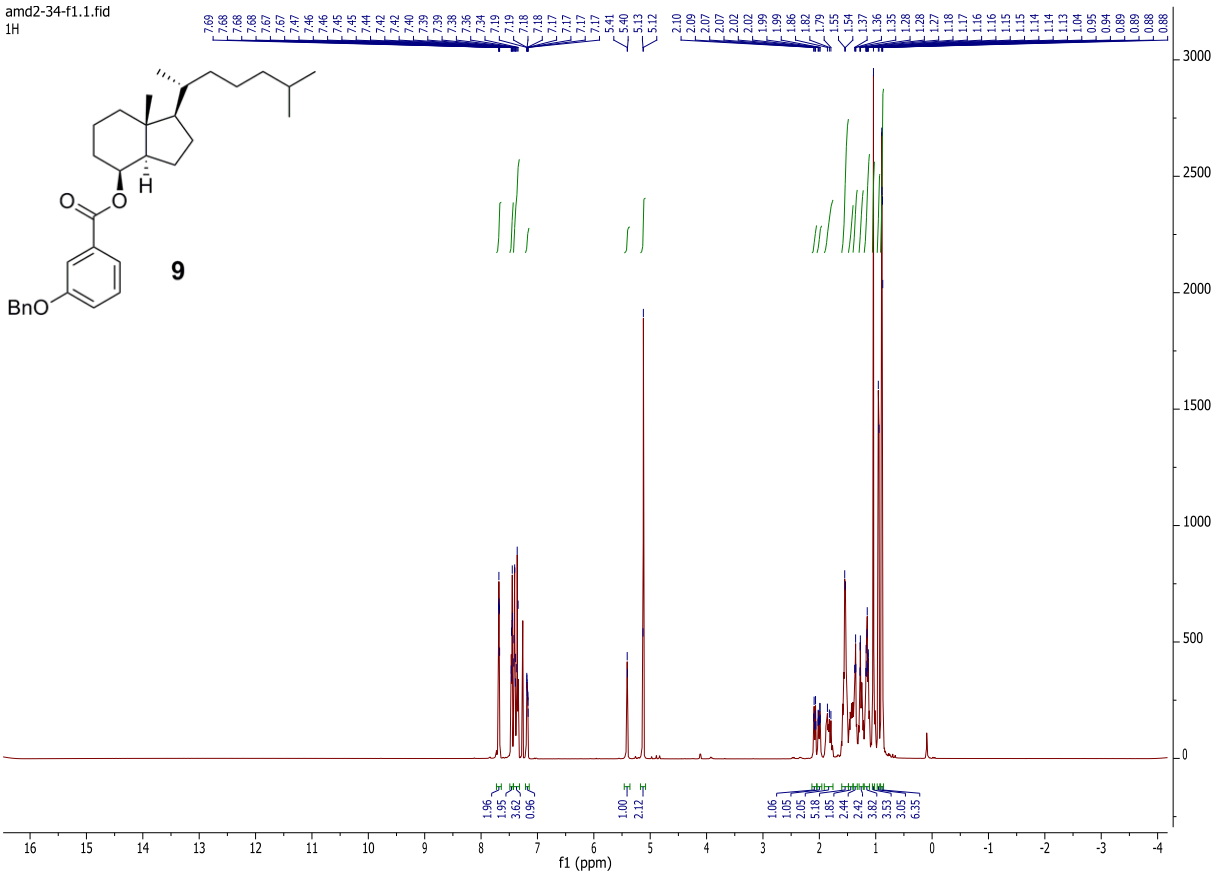
Cell Culture and Reagents. C3H10T1/2, M2-10B4, and DAOY cells were purchased from American Type Culture Collection (ATCC). ASZ001 cells were a generous gift of Dr. Ervin Epstein (Children's Hospital of Oakland Research Institute). Gibco by Life Technologies culture media was purchased from ABI. C3H10T1/2 cells were cultured in BME (Gibco) supplemented with 10% FBS (Atlanta Biologicals, Premium Select), 1% L-glutamine (Cellgro; 200 nM solution), and 0.5% penicillin/streptomycin (Cellgro; 10,000 I.U./mL penicillin, 10,000 µg/mL). M2-10B4 cells were cultured in RPMI Medium 1640 (Gibco) supplemented with 10% FBS and 1% penicillin/streptomycin. ASZ001 cells were cultured in 154CF media, supplemented with 2% FBS (chelexed, heat-inactivated), 1.0% penicillin/streptomycin, and a final concentration of 0.05mM $CaCl_2$. DAOY cells were cultured in DMEM supplemented with 10% FBS, 1% L-glutamine, and 1% penicillin/streptomycin. Cells were maintained using the media described above (denoted "growth" media). Media denoted as "low FBS" contained 0.5% FBS and the same percentage of other supplements as specified for growth media ("low FBS" media with this percentage FBS was used for C3H10T1/2, M2-10B4, and DAOY cell assays). Following plating and 24 hr growth period, no FBS supplemented media was used for ASZ001 cell assays. All cells were grown in Corning Cell Culture, canted neck T75 or T150 flasks (Fisher Scientific) in an Autoflow IR water-jacketed CO_2 incubator (37°C, 5% CO_2). Experiments with C3H10T1/2 and M2-10B4 cells were performed in BD Falcon sterile 60 mm dishes. 500,000 cells at ~80% confluence were plated in 5 mL media. Experiments with ASZ001 and DAOY cells were performed in BD Falcon 35 mm dishes or in 6-well, 35mm plates. For the ASZ001 line, 300,000 cells at ~80% confluence were plated in 2 mL media. For the DAOY line, 200,000 cells at ~80% confluence were plated in 2 mL media. DMSO was used as solvent to prepare all drug solutions and the final DMSO concentration did not exceed 0.3%. 20α-hydroxycholesterol and 22(*S*)-hydroxycholesterol (OHCs) were purchased from Sigma-Aldrich. VD3, for biological studies, was purchased from Sigma-Aldrich. Cyclopamine and GDC-0449 were purchased from LC Labs.

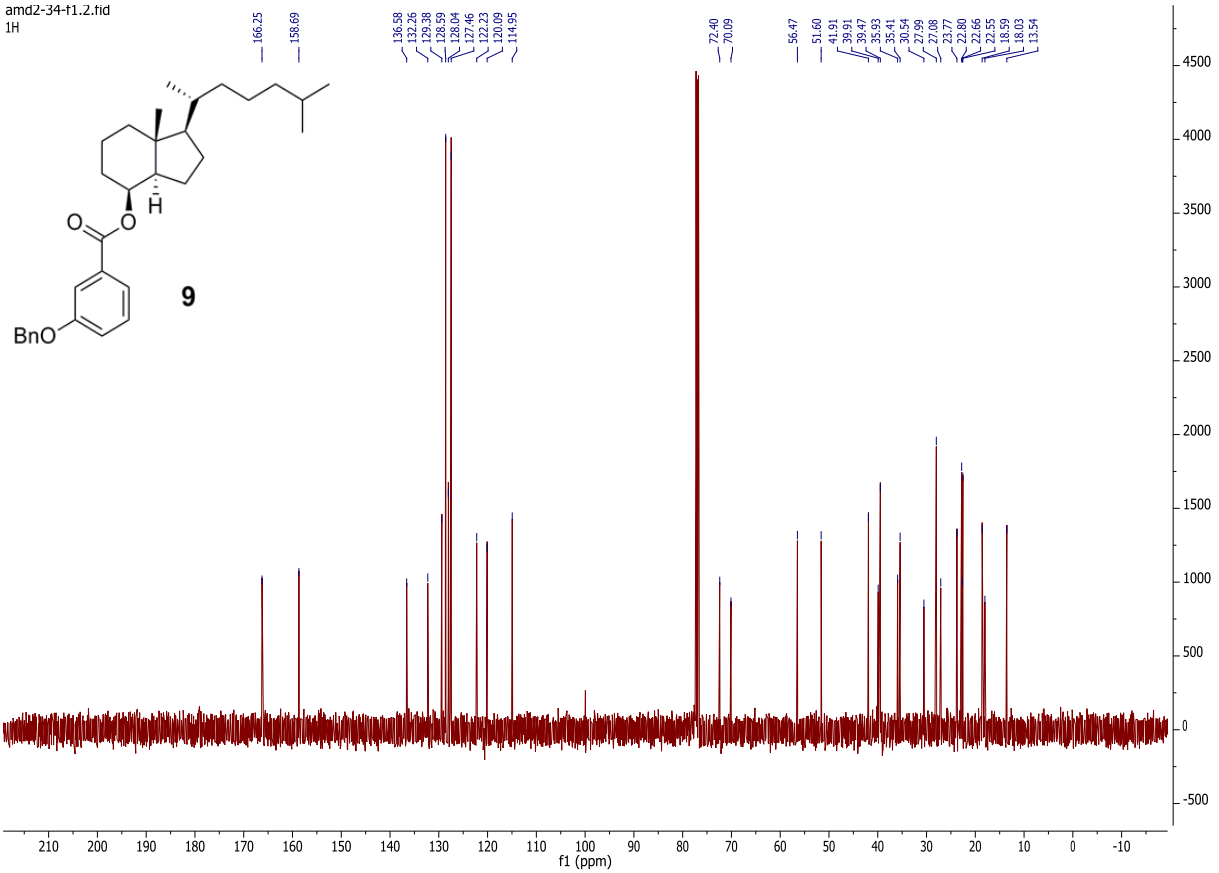
General protocol for analysis of Hh and VDR target gene regulation in mouse embryonic fibroblasts, C3H10T1/2 and M2-10B4. Cells were plated in growth media at ~80% confluence. Once cells reached confluence (approximately 24 hr), growth media was removed and replaced with low FBS media (5 mL). This was followed by addition of OHCs, OHC and analogue, or DMSO (vehicle control). Cells were incubated (37°C, 5% CO_2) for 24 hr period and RNA was isolated and evaluated as described below.

General protocol for analysis of Hh and VDR target gene regulation in murine-derived BCC (ASZ001) and human-derived DAOY cells. Cells were plated in growth media at ~80% confluence. After 24 hr, growth media was removed and replaced with low FBS (DAOYs) or no FBS (ASZ001) media (2 mL). Cells grew an additional 24 hrs. After this time, addition of DMSO (vehicle control) or analogues was performed. Cells were incubated (37°C, 5% CO_2) for 48 hr period and RNA was isolated and evaluated as described below.

General RT-PCR Protocol. Following treatment and incubation, total RNA was extracted with TRIZOL® Reagent (Invitrogen) following the manufacturer's instructions. RNA was quantified (absorbance at 260 ηM) and cDNA synthesis was performed utilizing the High Capacity cDNA Reverse Transcription Kit (ABI) per the manufacturer's instructions on a BioRad MyCycler. Quantitative RT-PCR was performed on an ABI 7500 system using the following Taqman Gene Expression Primer/Probe solutions (ABI): mouse ActB (Mm00607939_s1), mouse GLI1 (Mm00494645_m1); mouse PTCH1 (Mm00436026_m1); mouse CYP24A1 (Mm00487244_m1); human ActB (Hs99999903_m1); human GLI1 (Hs00171790_m1); human PTCH1 (Hs00181117_m1); human CYP24A1 (Hs00167999_m1). Relative gene expression levels were computed via the ΔΔ Ct method. ActinB mRNA expression levels of all samples were normalized to DMSO control ActinB mRNA levels (vehicle; set at 1.00). DMSO control Gli1 mRNA level were set to 1.0 and Gli1 mRNA levels for OHCs treated samples (upregulated) were set to 100% relative to DMSO control. The mRNA expression levels from drug treated samples were compared to OHC levels (Gli1, PTCH) and DMSO control (Cyp24a1). Data was analyzed using GraphPad Prism 5 and IC_{50} values represent mean ± SEM for at least two separate experiments performed in triplicate.

^{1}H and ^{13}C NMR spectrum.

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70.09
56.47
51.60
41.91
39.91
39.47
39.03
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18.03
13.54
O
O
H
9
BnO
4500
4000
3500
3000
2500
2000
1500
1000
500
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-500
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200
190
180
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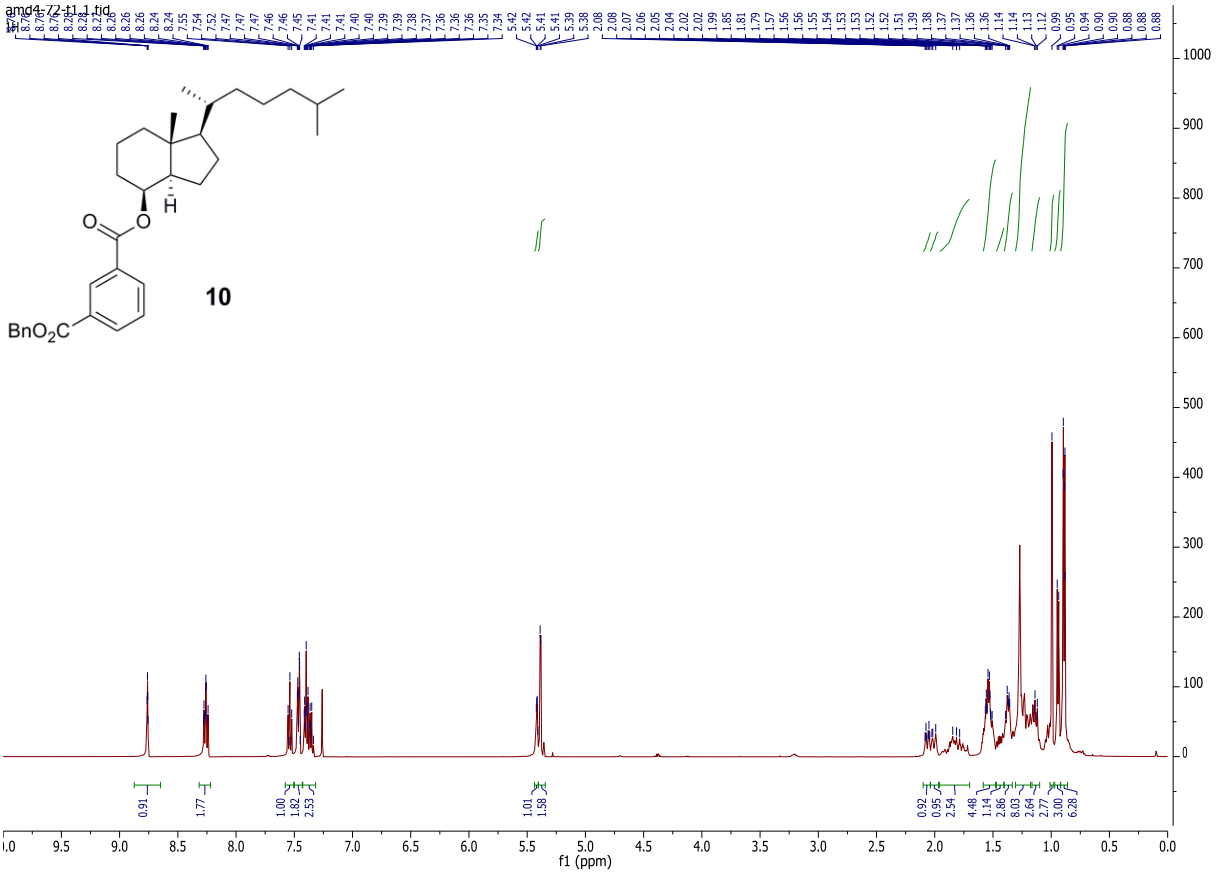
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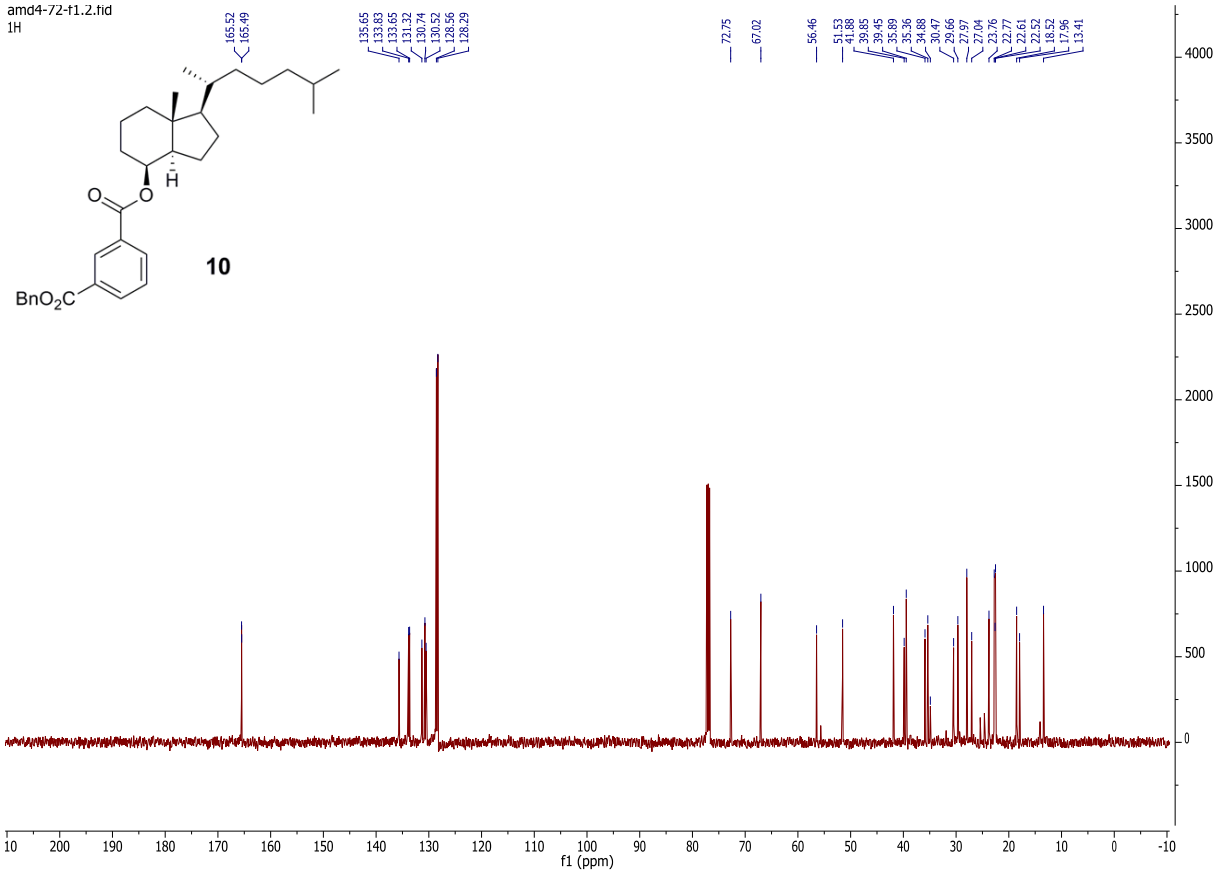
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51.53
41.88
39.85
39.45
39.26
35.89
35.36
34.88
30.47
29.66
27.97
27.04
23.76
22.77
22.61
22.52
18.52
17.96
13.41
O
O
H
10
BnO2C
4000
3500
3000
2500
2000
1500
1000
500
0
10
200
190
180
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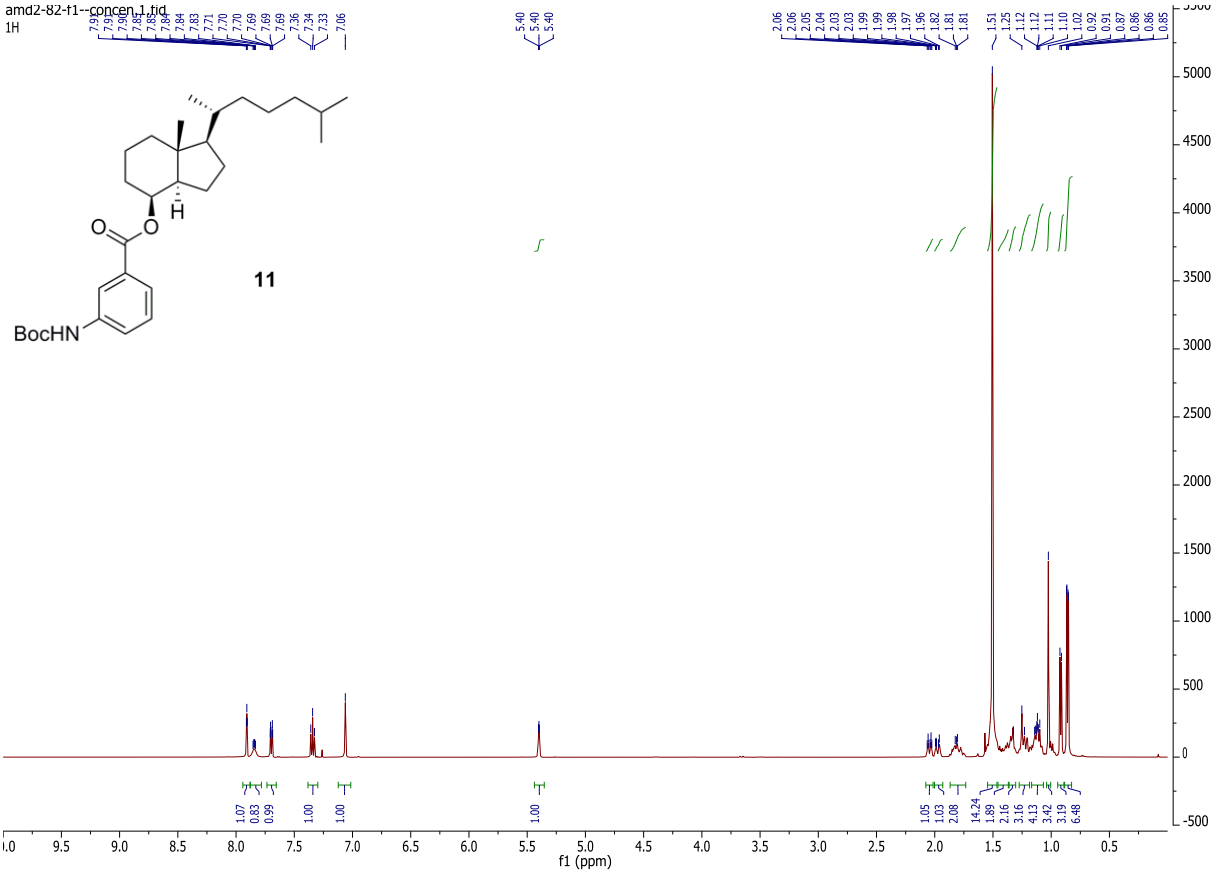
f1 (ppm)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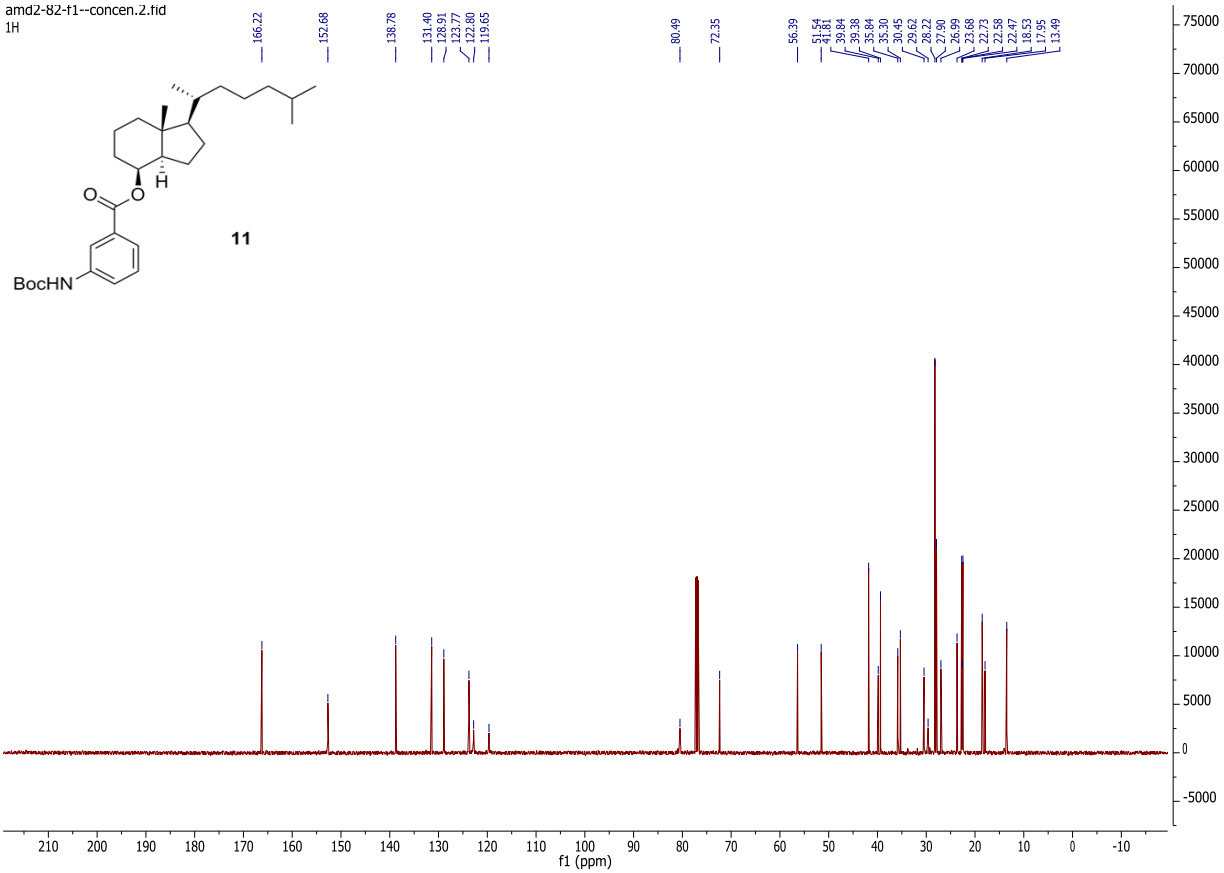
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39.38
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27.90
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H
11
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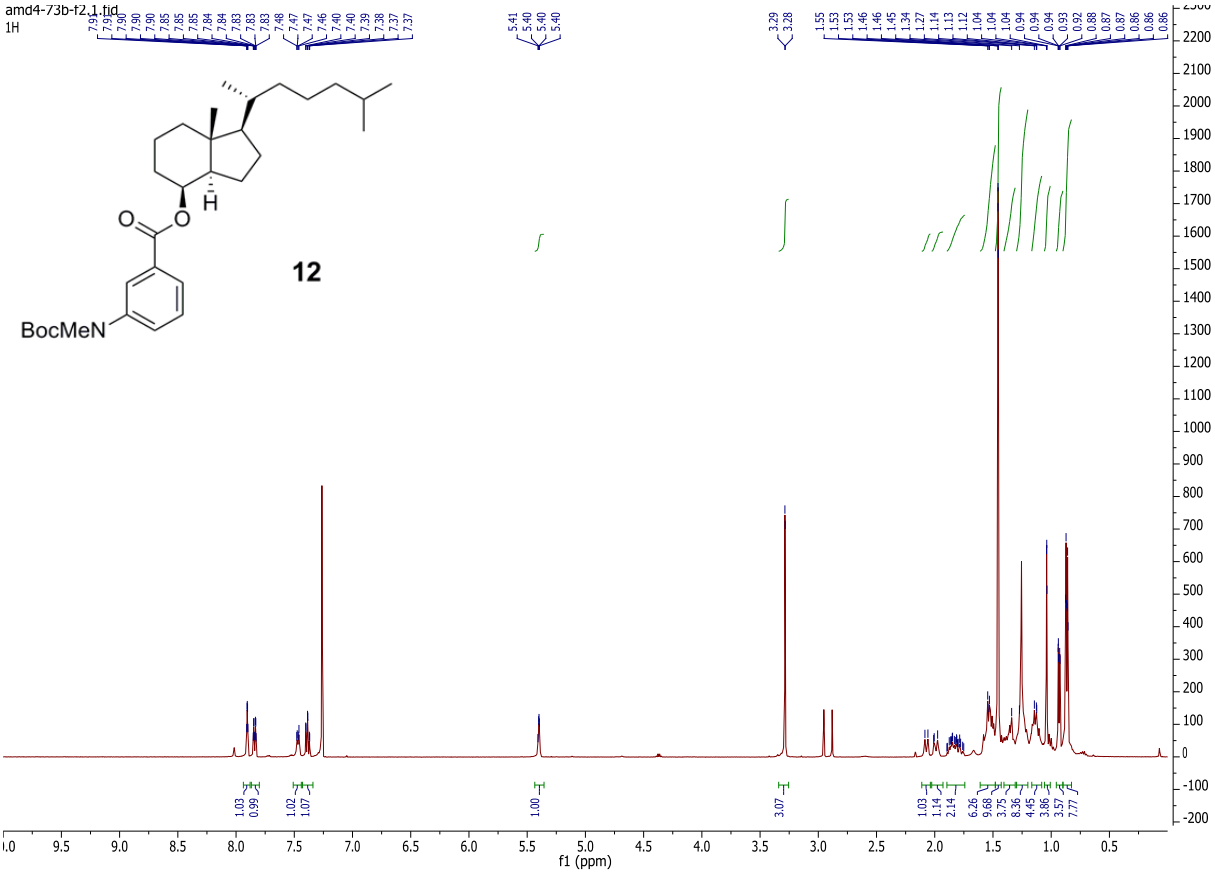
f1 (ppm)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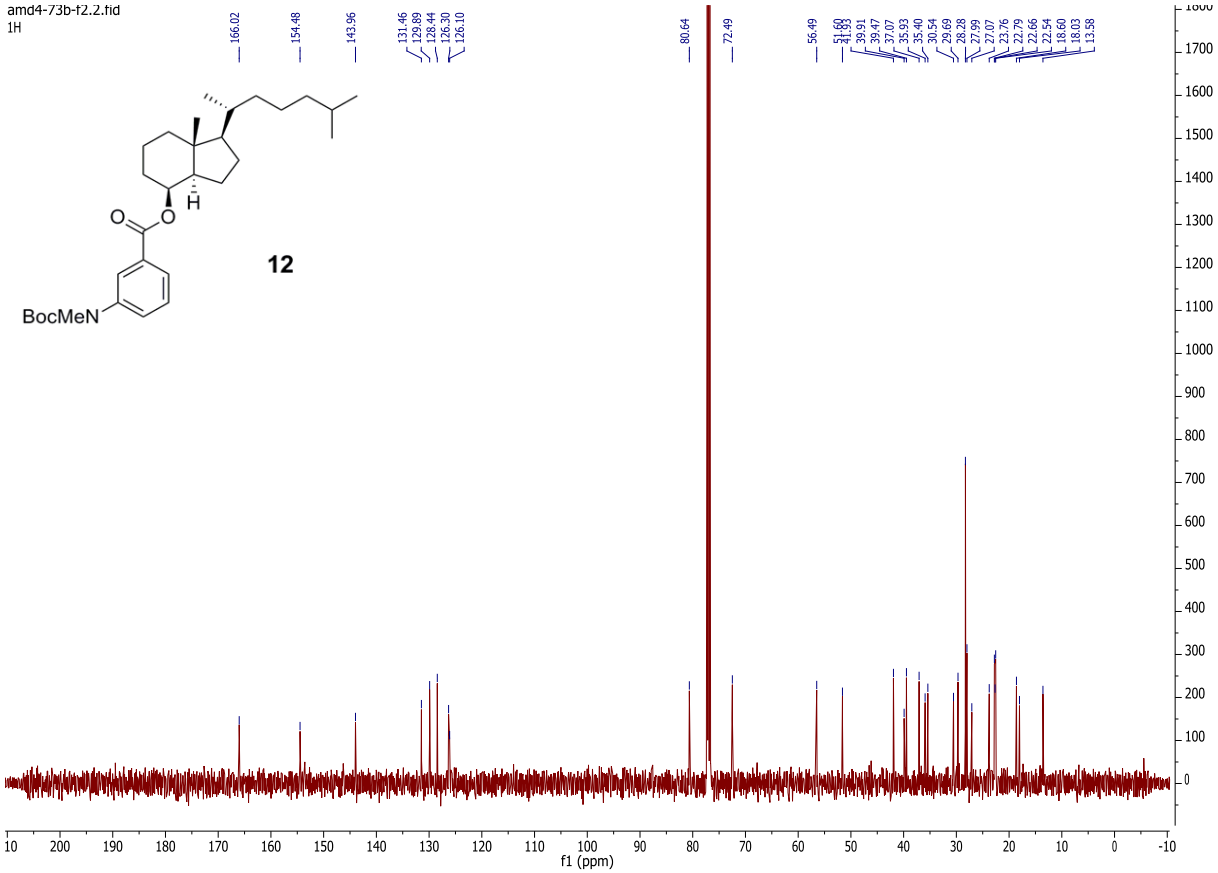
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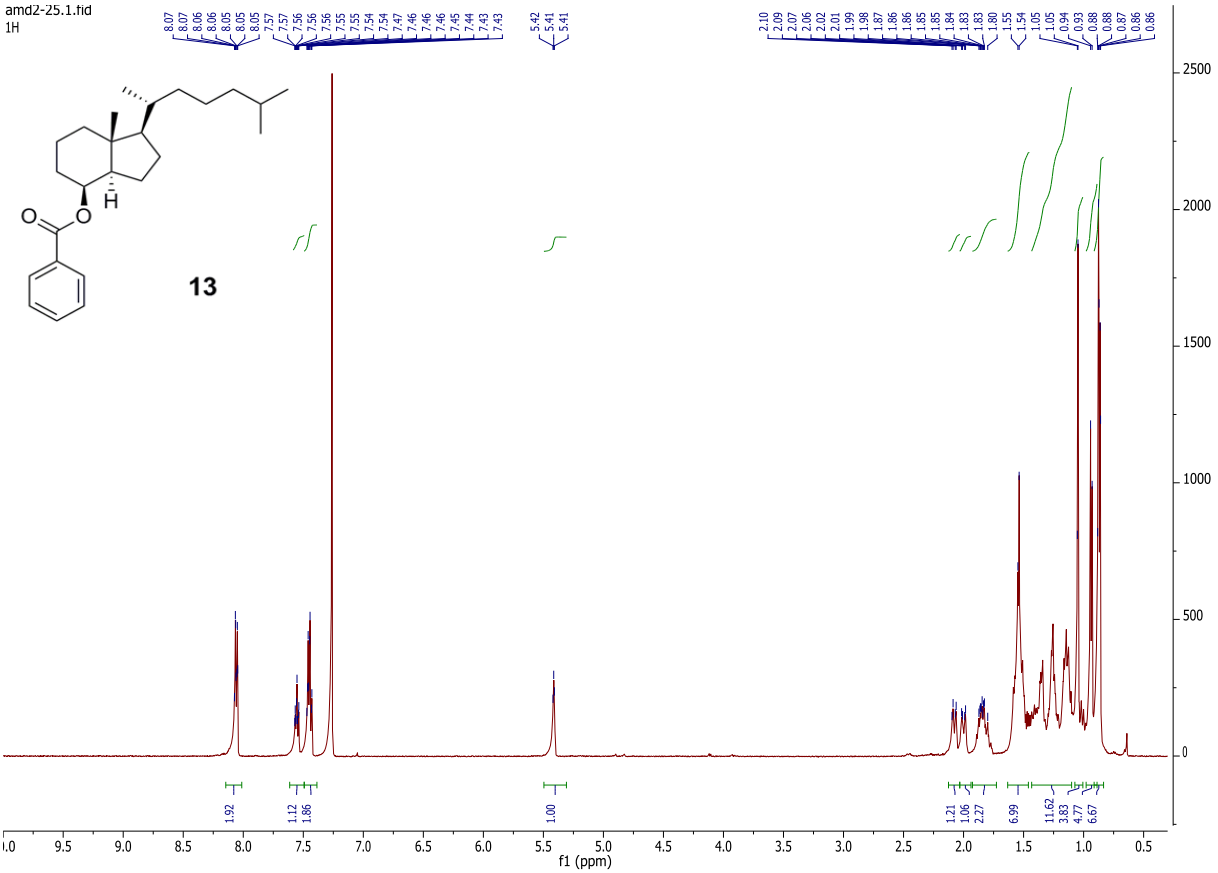
O
H
12
BocMeN
f1 (ppm)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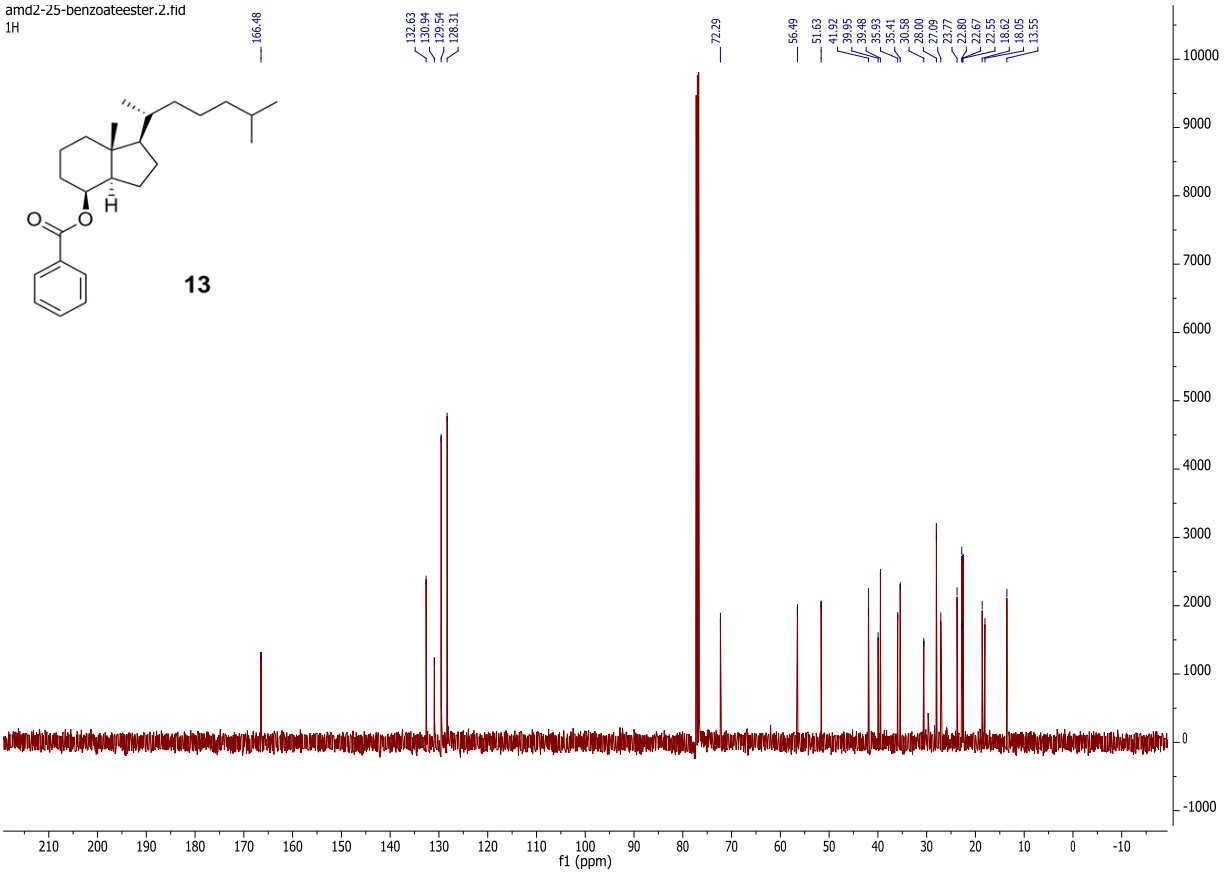
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13
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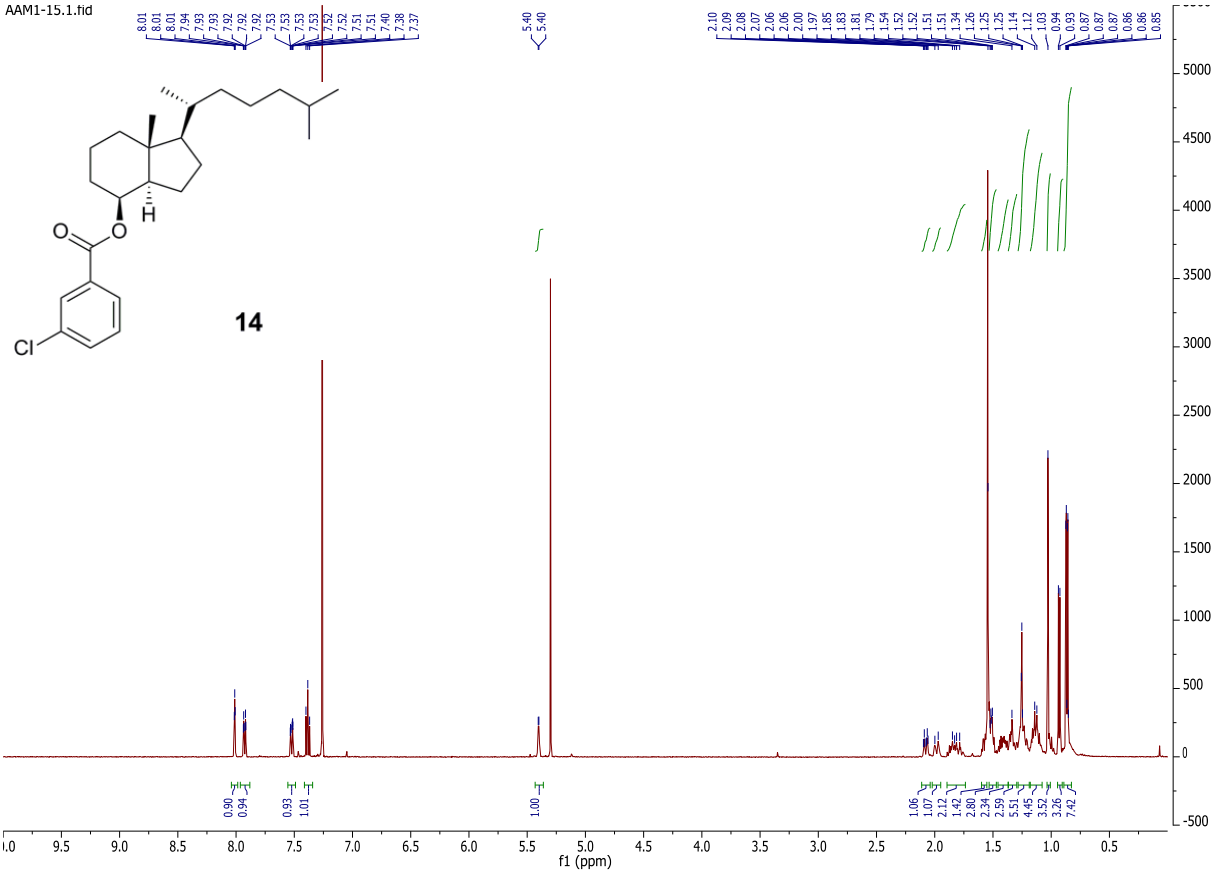
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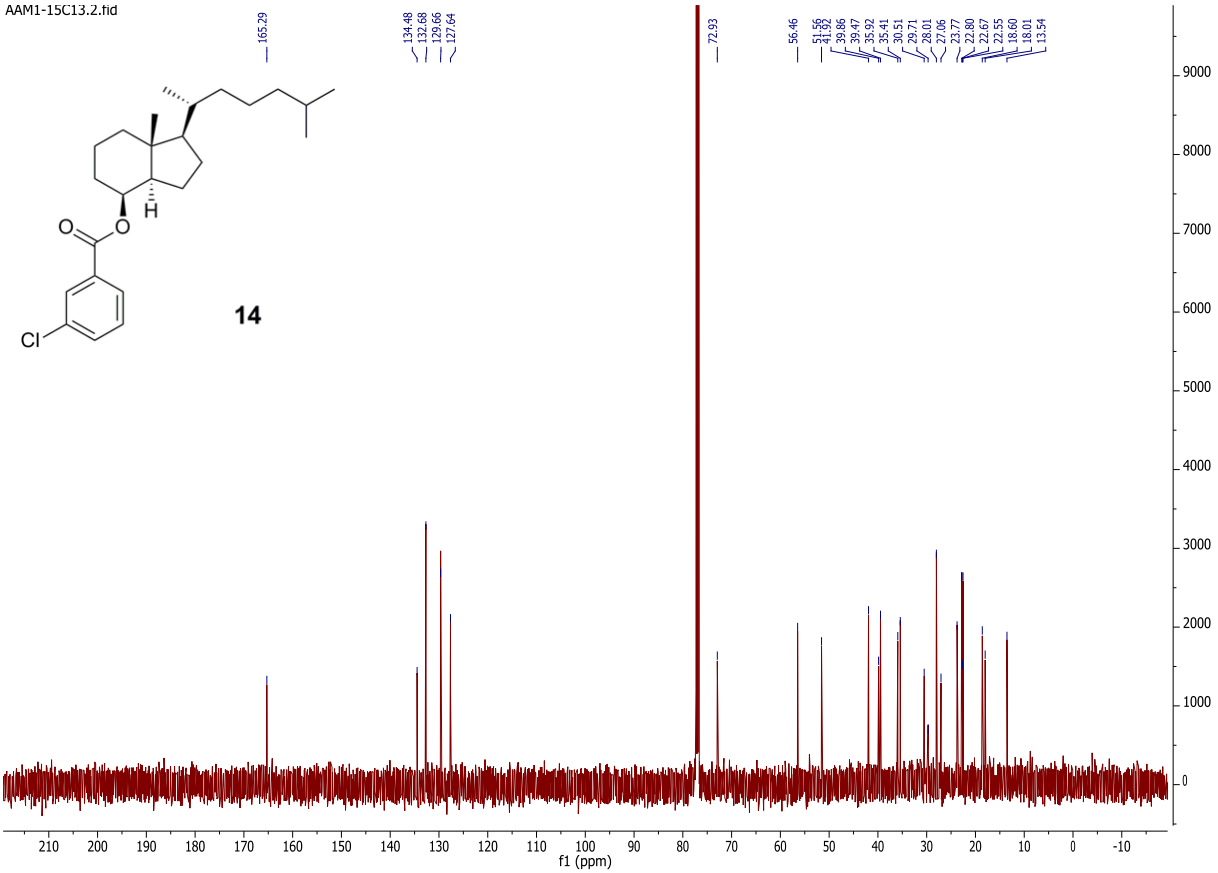
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Cl
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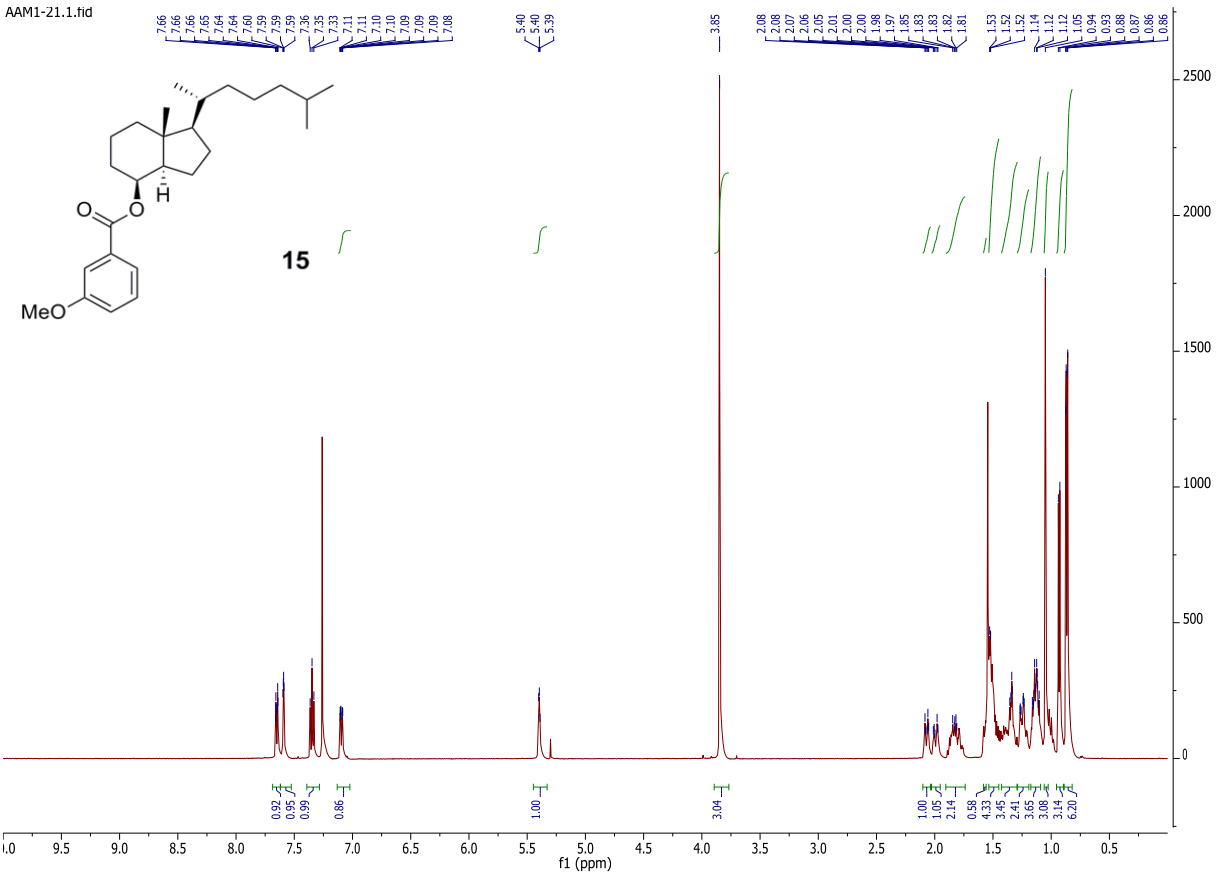
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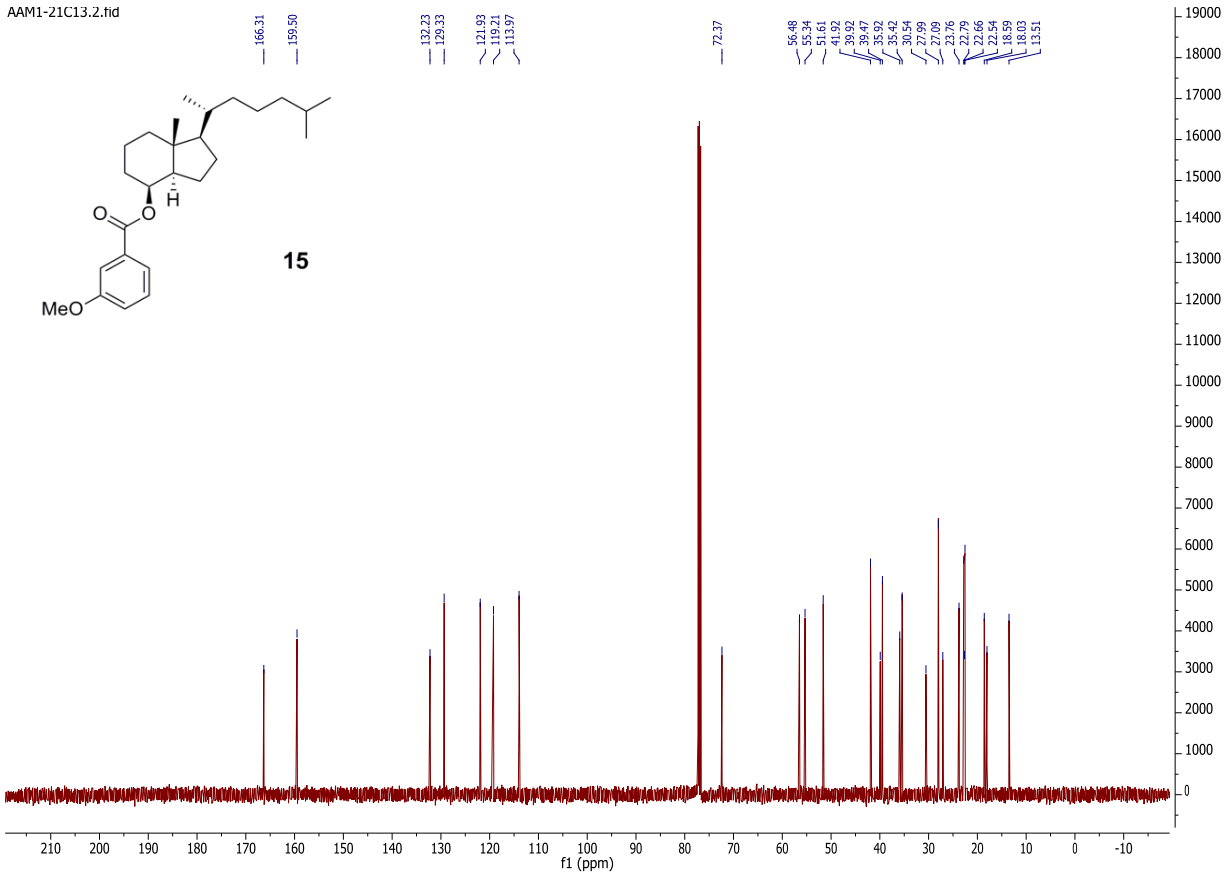
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15
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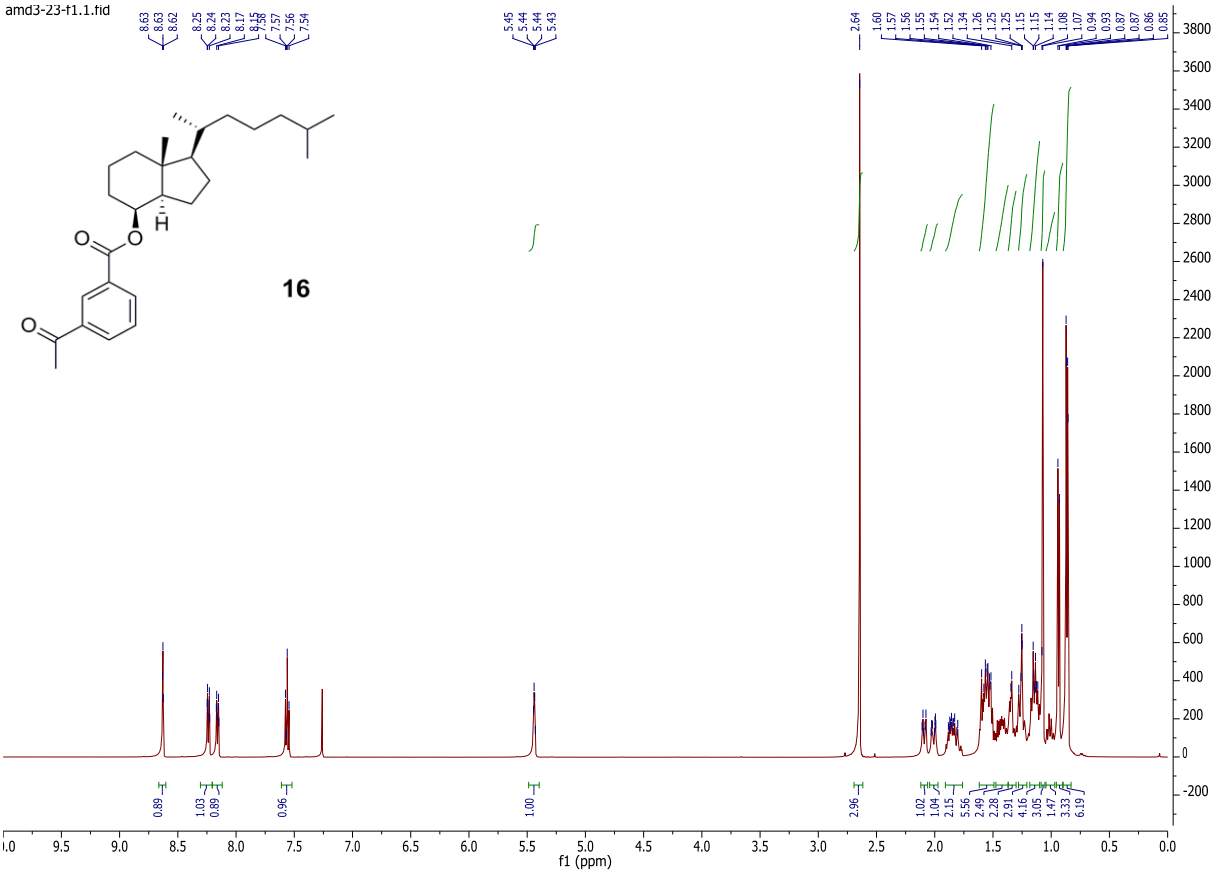
H
f1 (ppm)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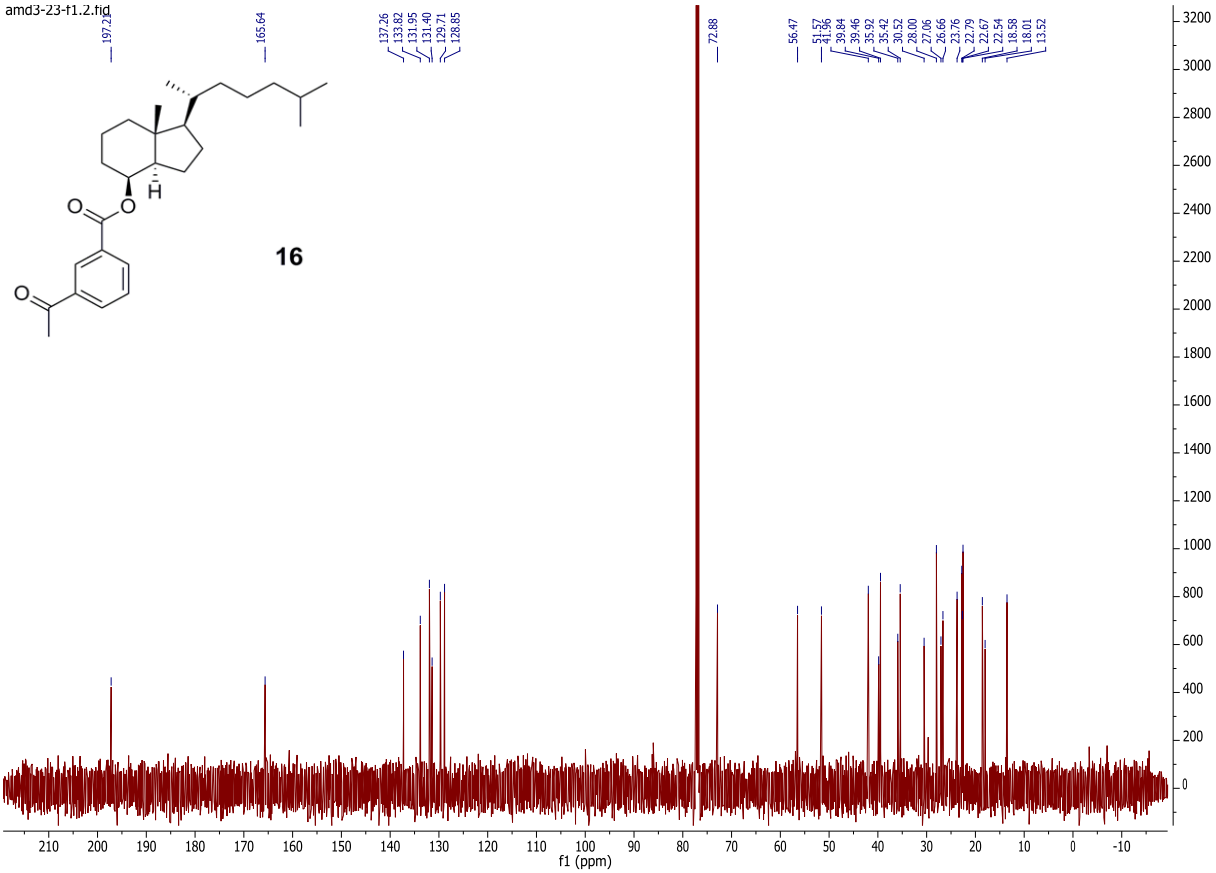
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16
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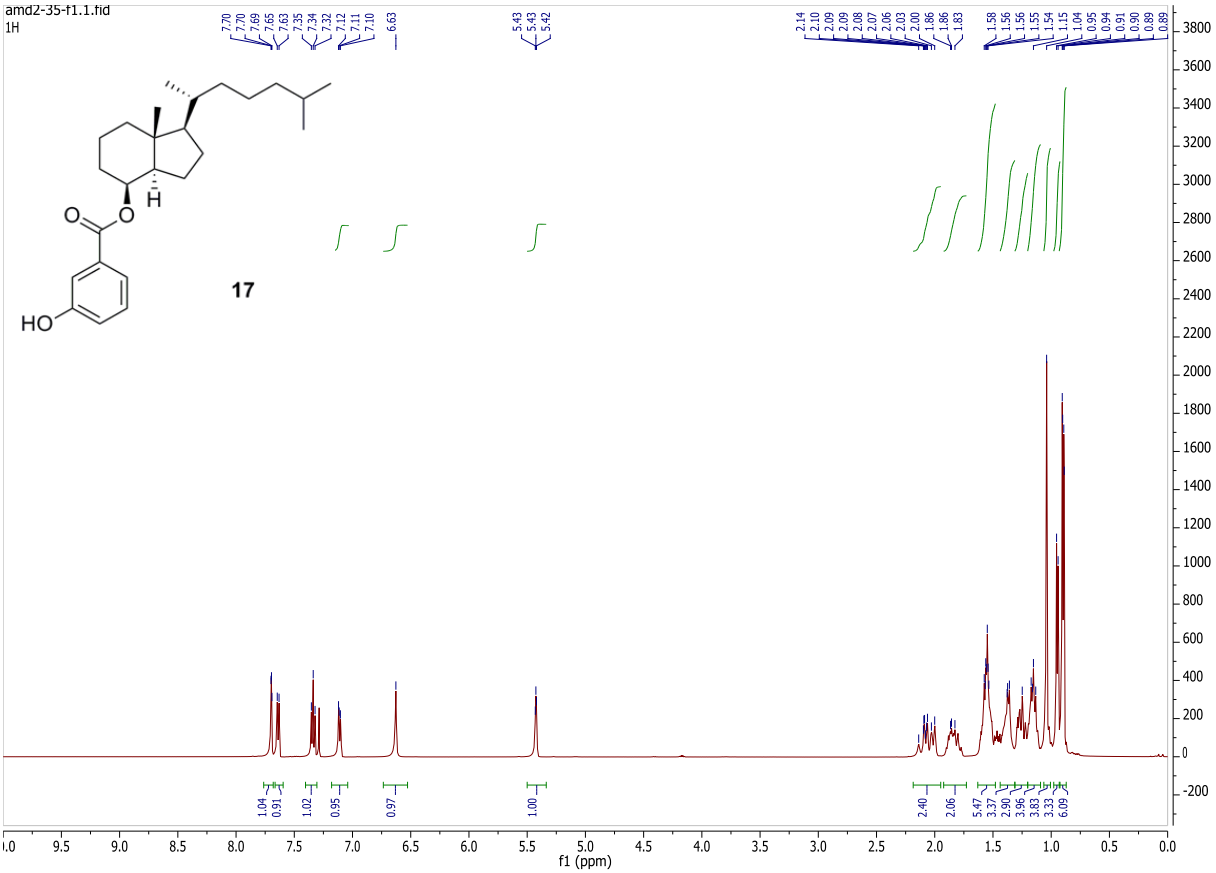
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O
H
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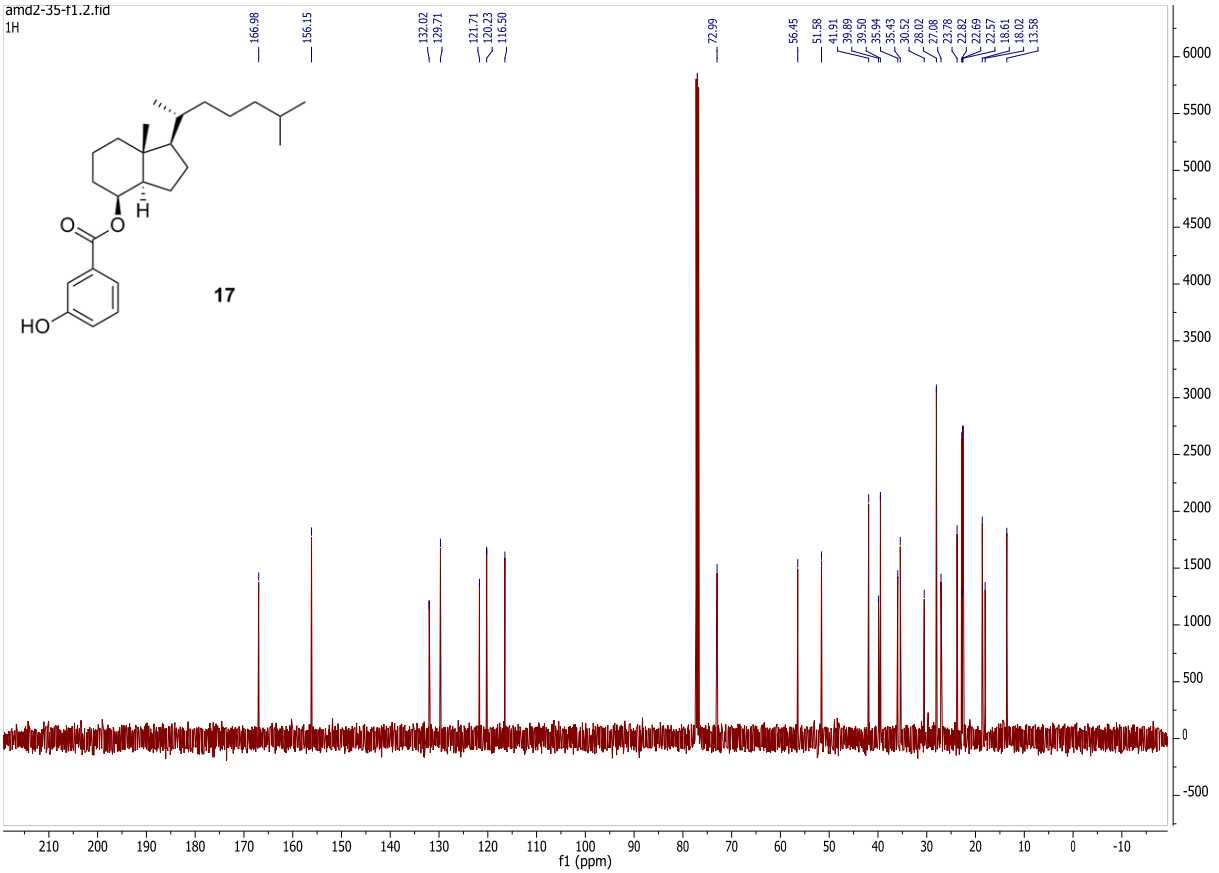
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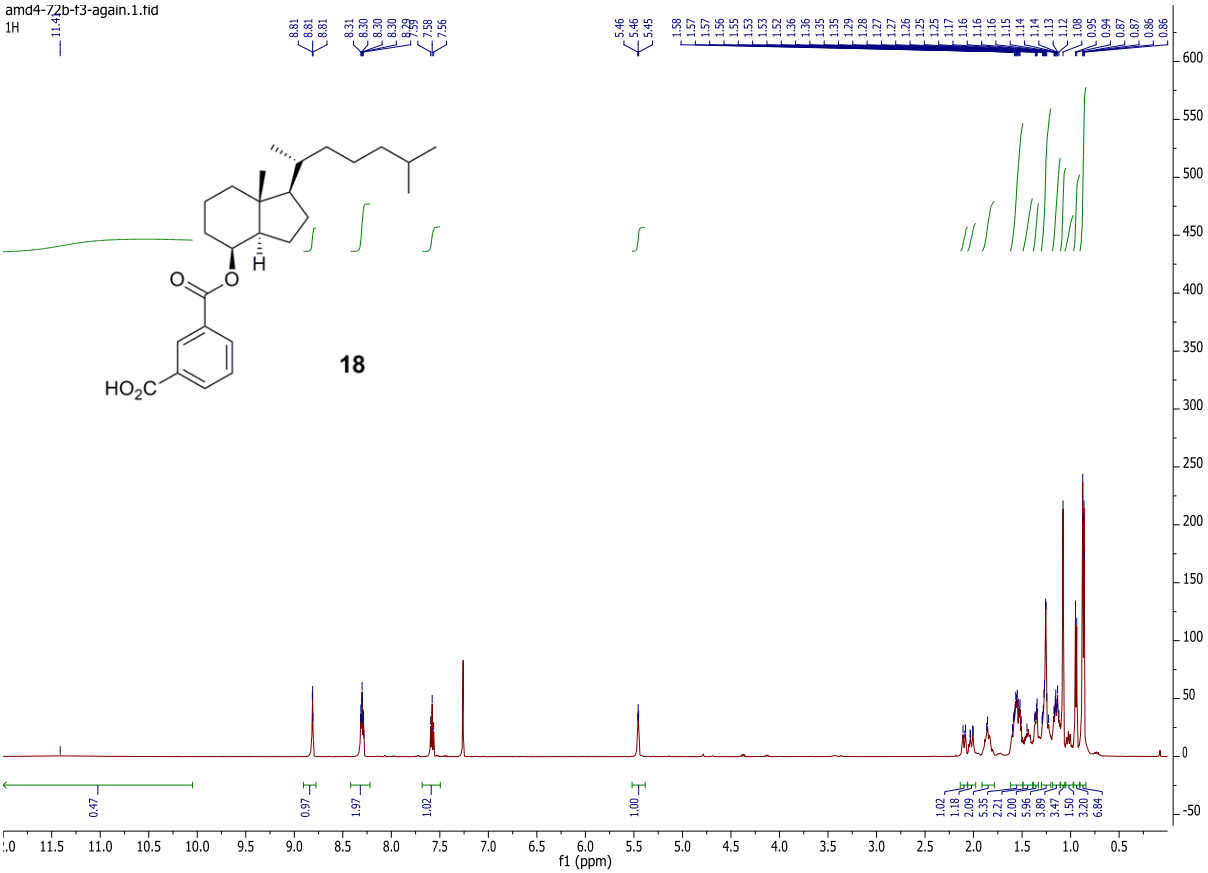
H
HO
17
f1 (ppm)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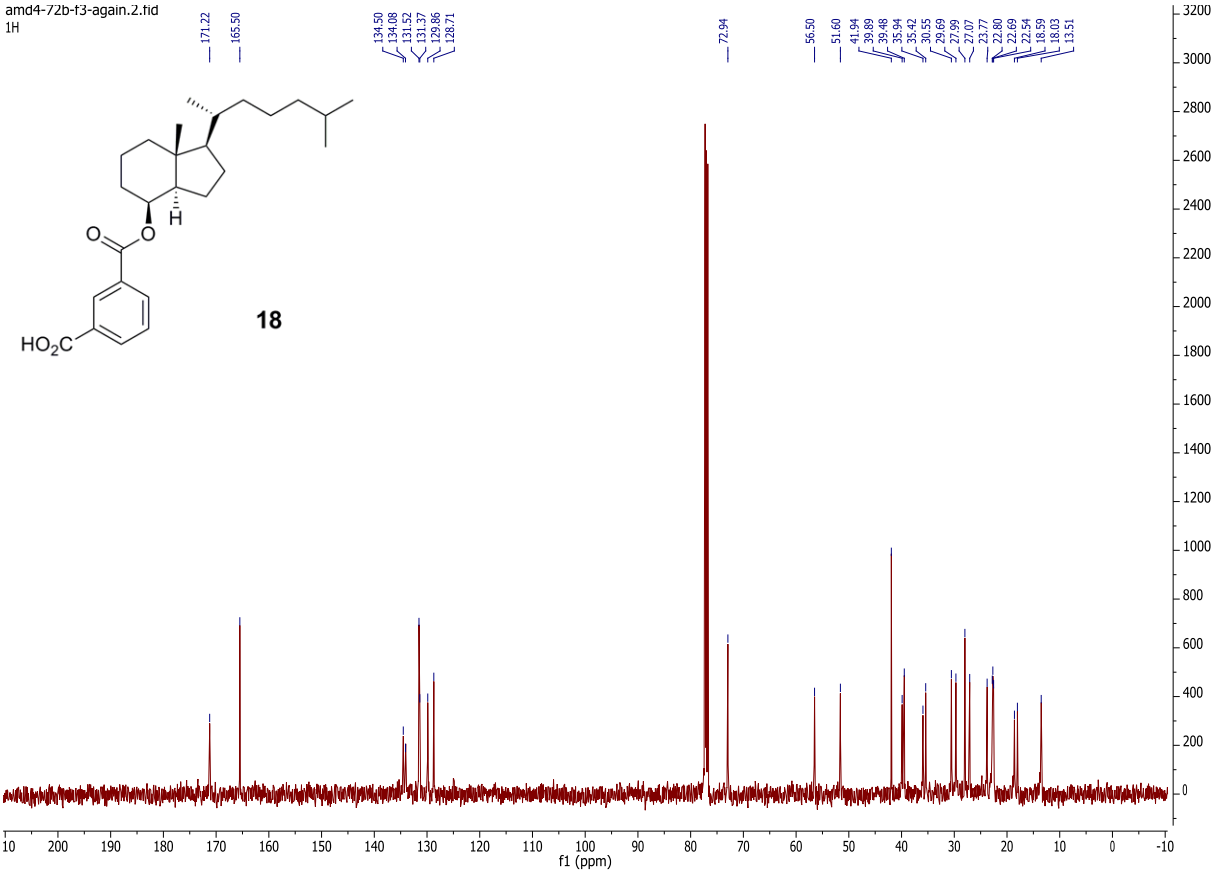
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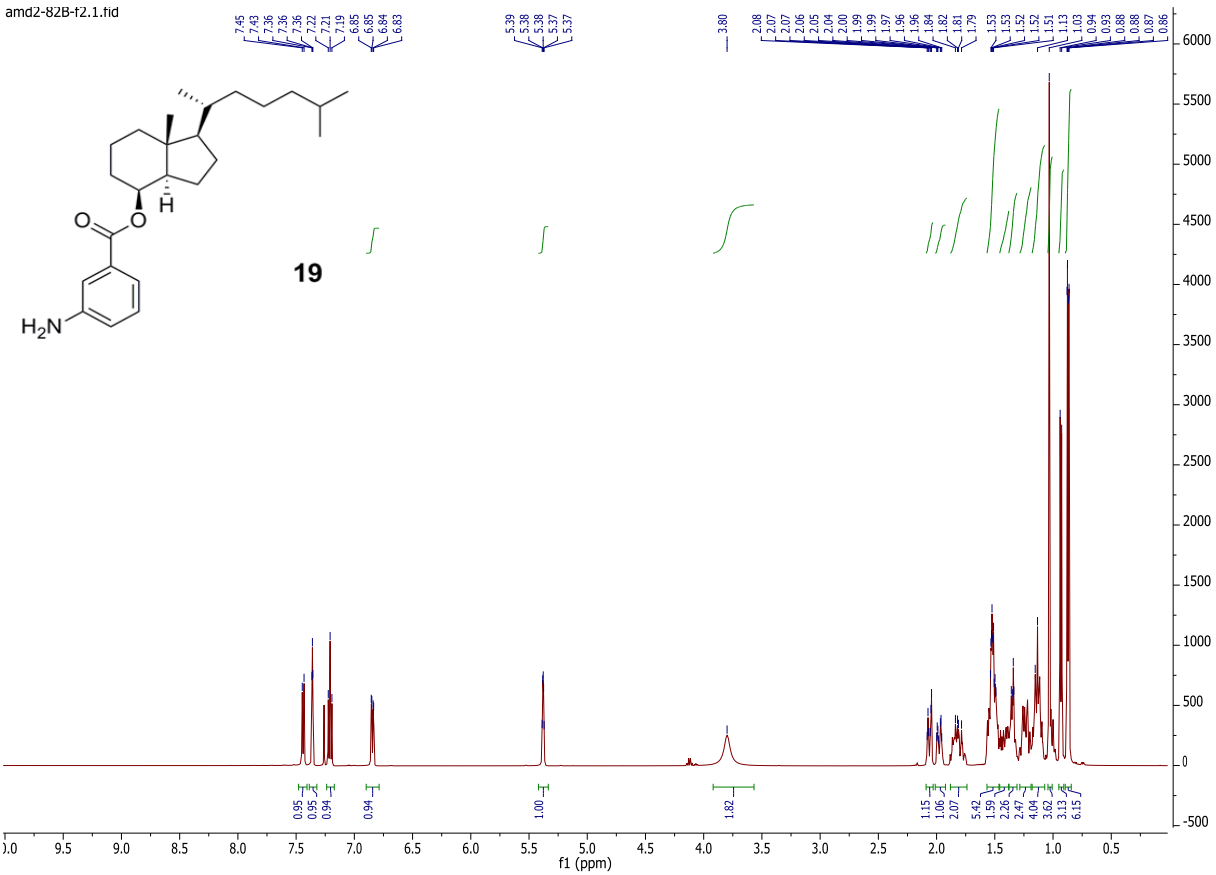
O
H
HO2C
18
f1 (ppm)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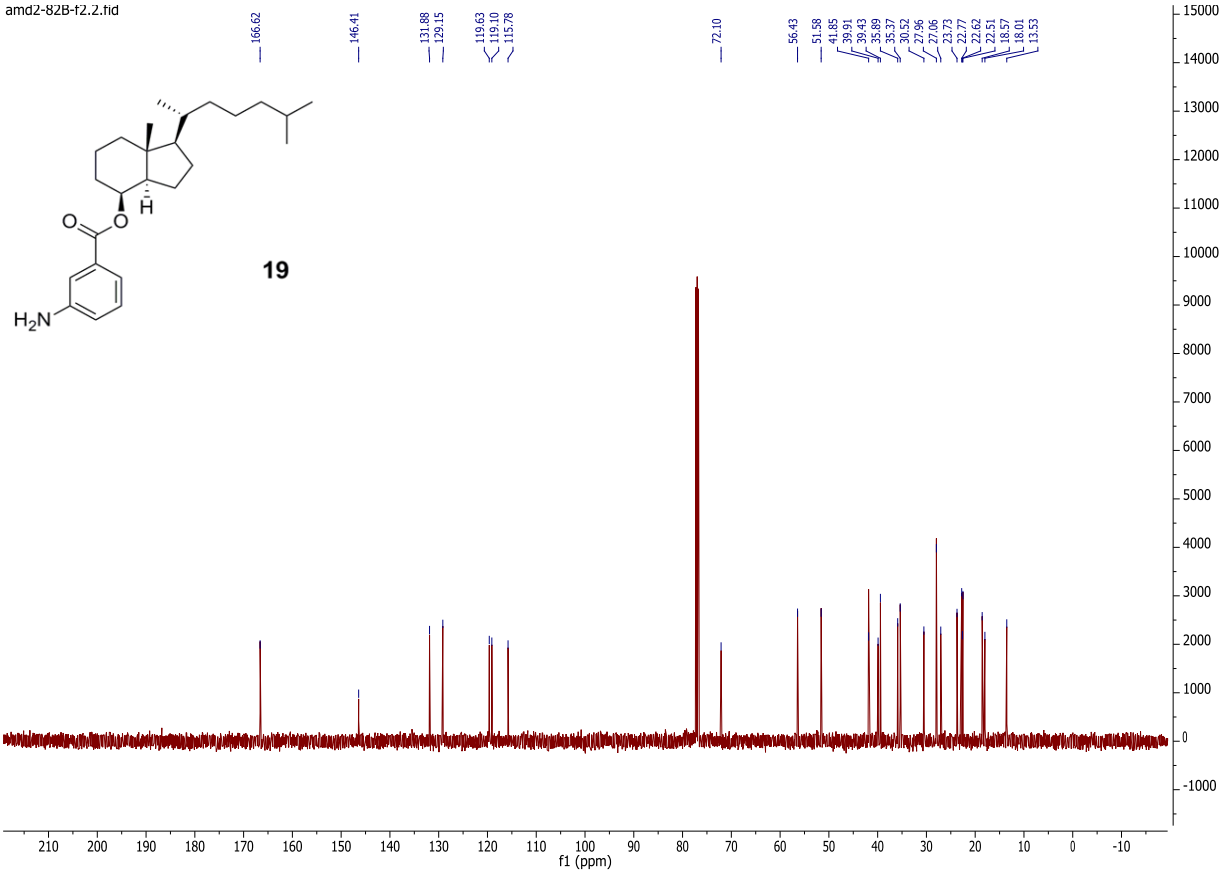
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22.51
18.57
18.01
13.53
19
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O
H
H2N
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14000
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
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200
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170
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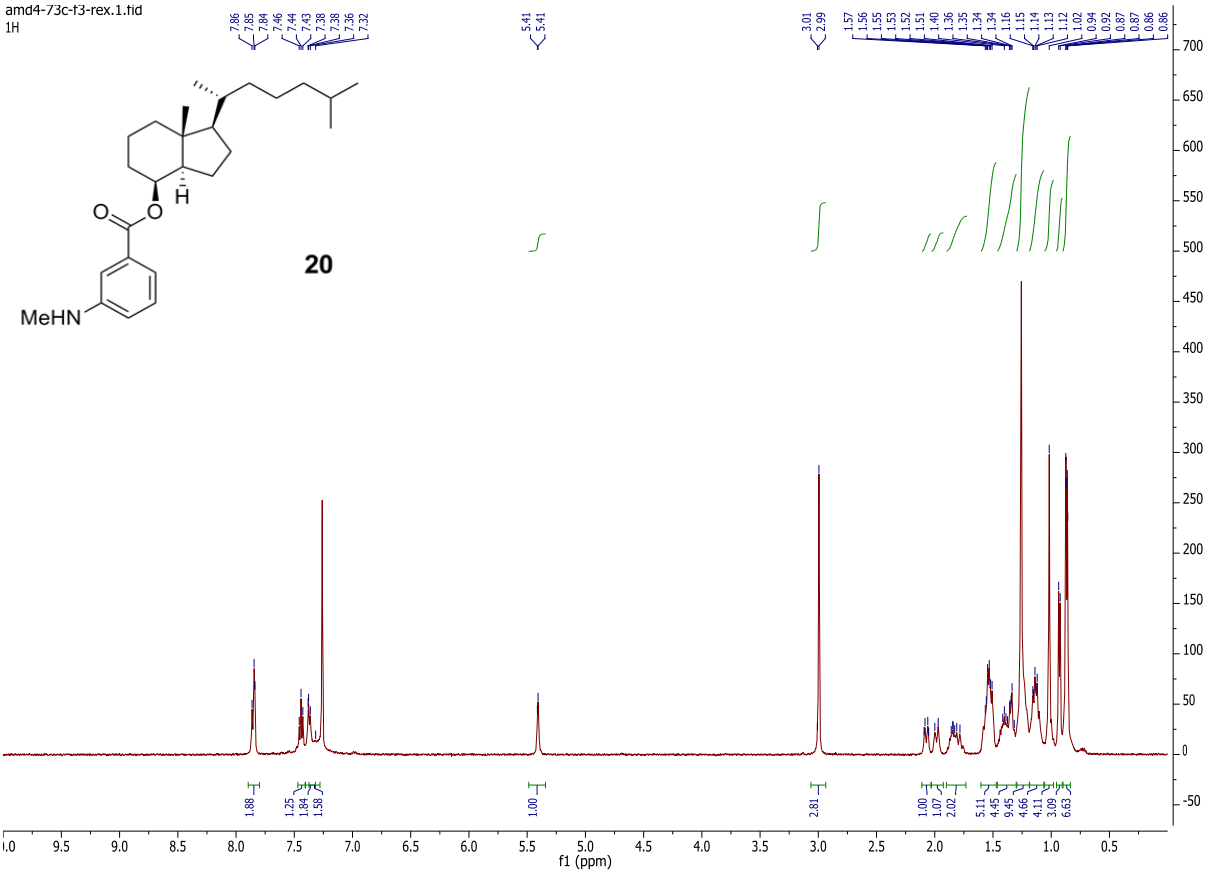
f1 (ppm)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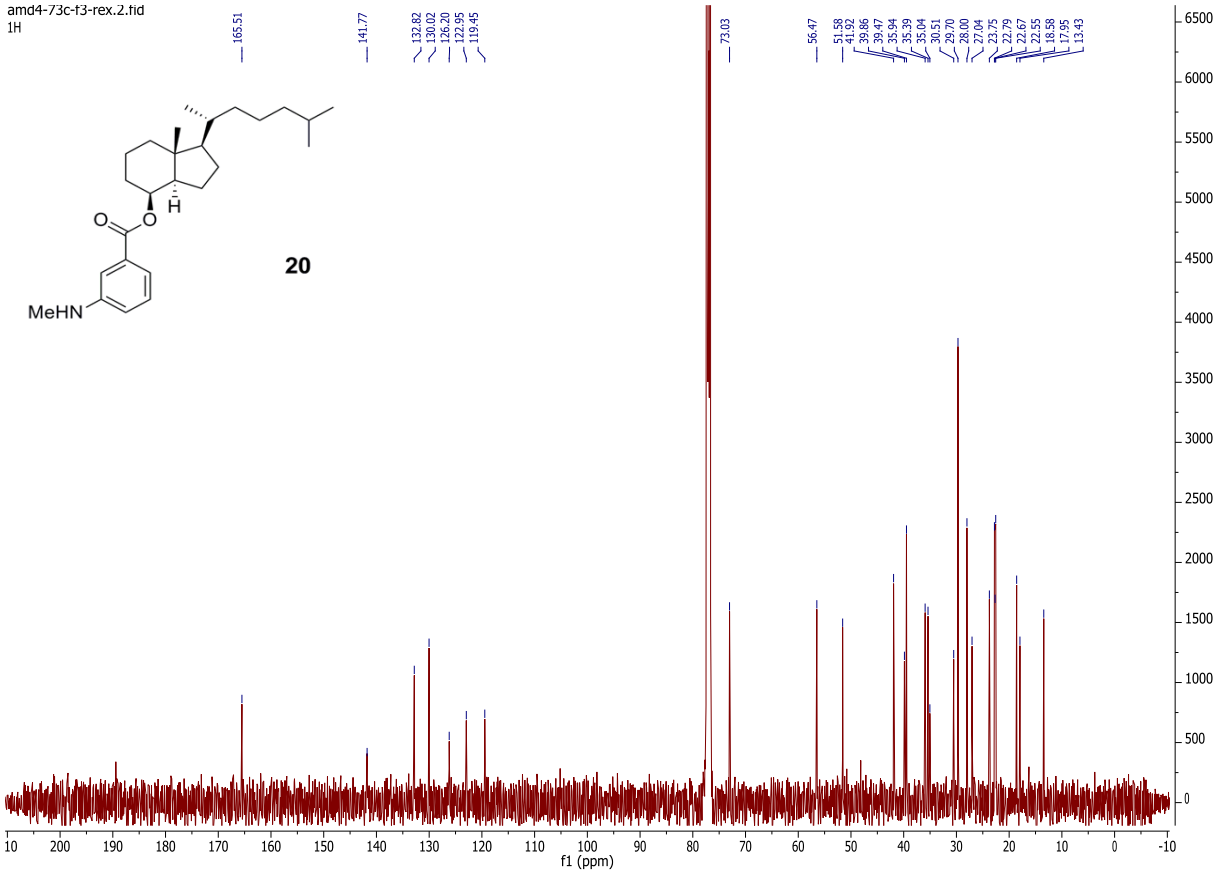
1H
20
MeHN
f1 (ppm)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23.75
22.79
22.67
22.55
18.58
17.95
13.43
O
O
H
MeHN
20
f1 (ppm)

Supplement Figure 1. Dose-dependent response for Cyp24A1 mRNA expression levels in ASZ001 and DAOY cell lines. Biological assay details described on supporting information pages S7-S8. Values shown for VD3 and analogues **17** and **19** from 10 to 0.5 µM.