Supplemental Information to Accompany

Synthesis of a Stable and Orally Bioavailable Englerin Analogue

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General Information: All reactions were performed in single-neck oven- or flame-dried round-bottom flasks fitted with rubber septa under a positive pressure of argon, unless otherwise noted. Air- and moisture-sensitive liquids were transferred via syringe or stainless steel cannula. Organic solutions were concentrated by rotary evaporation below 35 °C at 10 Torr (diaphragm vacuum pump) unless otherwise noted. Analytical thin-layer chromatography (TLC) was performed using glass plates pre-coated with silica gel (0.25 mm, 60-Å pore size, 230-400 mesh, Sorbent Technologies) impregnated with a fluorescent indicator (254 nm). TLC plates were visualized by exposure to ultraviolet light (UV), then were stained by submersion in aqueous acidic dinitrophenylhydrazine solution (DNP), Ceric Ammonium Molybdate (CAM), or aqueous basic potassium permanganate solution ($KMnO_4$), followed by brief heating on a hot plate (215 °C, 10–30 s). Flash chromatography was performed as described by Still et al.[1], employing silica gel (60-Å pore size, 40–63 μm, standard grade, Sorbent Technologies).

Materials: Commercial reagents and solvents were used as received with the following exceptions. Triethylamine, dichloromethane, ethyl ether, dimethylsulfoxide, tetrahydrofuran, hexane, toluene, and benzene were purified by the method of Pangborn, et. al[2] 2-Chloropropanoate, 3-methyl-2-butanone, hexamethyldisilazane, and *N*,*N*-diisopropylamine were distilled from calcium hydride under an atmosphere of argon at 760 Torr. Hexamethylphosphoramide (HMPA) and *N*,*N*-dimethylformamide (DMF) were distilled from calcium hydride under reduced pressure (0.1 Torr) and stored under argon. 1,2-Diiodoethane was recrystallized from ethyl ether and stored under an atmosphere of argon. Lithium chloride was flame dried under vacuum (0.1 Torr, 10 min), cooled under an atmosphere of argon, and the dried solid was stored at 150 °C (drying oven, 760 Torr); the dried solid was also flame dried under vacuum (0.1 Torr, 10 min) immediately prior to use. The molarity of solutions of *n*-butyllithium was determined by titration against diphenylacetic acid as an indicator (average of three determinations).[3] Where noted, solvents were deoxygenated before use by bubbling with argon for 20 minutes.

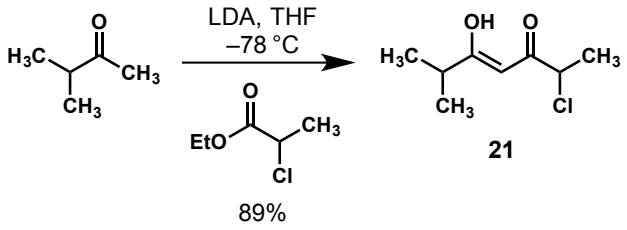
Instrumentation: Proton nuclear magnetic resonance (^{1}H NMR) spectra, carbon nuclear magnetic resonance (^{13}C NMR), and fluorine nuclear magnetic resonance (^{19}F NMR) spectra were recorded on Varian Mercury 300 MHz/75 MHz, Varian INOVA 500 MHz/125 MHz, Bruker CryoPlatform 400 MHz/100/376 MHz, or Bruker SMART 600 MHz/151 MHz NMR spectrometers

[1] Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978,** *43,* 2923–2925.
[2] Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J. *Organometallics* **1996**, *15*, 1518–1520.
[3] Kofron, W. G.; Baclawski, L. M. *J. Org. Chem.* **1976**, *41*, 1879–1880.

at 23 °C. Proton chemical shifts are expressed in parts per million (ppm, δ scale) downfield from tetramethylsilane and are referenced to residual protium in the NMR solvent ($CHCl_3$: δ 7.26, CD_2HOD: δ 3.31, CD_3SOCD_2H: δ 2.50). Carbon chemical shifts are expressed in parts per million (ppm, δ scale) downfield from tetramethylsilane and are referenced to the carbon resonance of the NMR solvent ($CDCl_3$: δ 77.00, CD_3OD: δ 49.00, CD_3SOCD_3: δ 39.52). Data are represented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, app = apparent), integration, and coupling constant (*J*) in Hertz (Hz). Infrared (IR) spectra were obtained using a Perkin Elmer 1600 FT-IR spectrophotometer referenced to a polystyrene standard and data are represented as frequency of absorption (cm^{-1}). Optical rotations were determined using a JASCO-DIP-370 polarimeter equipped with a sodium lamp source (589 nm). Reported readings are the average of three determinations for each sample. High-resolution mass spectra were obtained using an Agilent 1100 quaternary LC system coupled to an Agilent 6210 LC/MSD-TOF fitted with an ESI or an APCI source, or Thermo Q-Exactive Orbitrap using electrospray ionization (ESI) or a Waters GCT Premier spectrometer using chemical ionization (CI).

Experimental Procedures:

The following procedure for the preparation of the known 3-furanone **21**[4] was adapted from a literature report for the synthesis of 3-silyloxyfurans.[5]

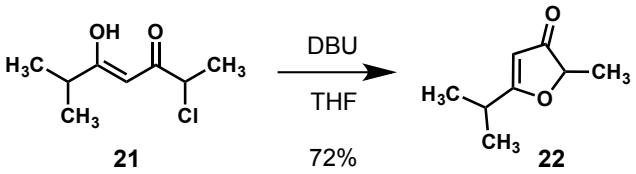


A solution of *n*-butyllithium (2.50 M, 14.4 mL, 36.0 mmol, 1.20 equiv) was added to a stirred solution of *N*,*N*-diisopropylamine (5.34 mL, 38.0 mmol, 1.26 equiv) in tetrahydrofuran (250 mL) at –78 °C. The resultant solution was warmed briefly to 0 °C, then was cooled to –78 °C whereupon a solution of 3-methyl-2-butanone (3.20 mL, 30.0 mmol) in tetrahydrofuran (15 mL) was added dropwise. The resultant mixture was stirred at –78 °C for 30 min, whereupon ethyl 2-chloropropanoate (4.20 mL, 33.0 mmol, 1.10 equiv) was added. The resultant mixture was

[4] Mukerji, S. K.; Sharma, K. K.; Torssell, K. B. G. *Tetrahedron* **1983**, *39*, 2231–2235.
[5] Winkler, J. D.; Oh, K.; Asselin, S. M. *Org. Lett.* **2005**, *7*, 387–389.

allowed to warm to 23 °C and stirred at that temperature for 8 h. The resultant yellow solution was cooled to 0 °C and was quenched with saturated aqueous ammonium chloride solution (30 mL). The layers were separated and the aqueous layer was extracted with dichloromethane (3 × 30 mL). The combined organic layers were dried over anhydrous sodium sulfate and the dried solution was concentrated. Purification of the residue by flash column chromatography (4% ethyl acetate-hexanes), afforded **21** (4.60 g, 89%) as a colorless oil.

Chlorodiketone **21**: TLC: 4% ethyl acetate–hexanes, R_f = 0.50 (UV, $KMnO_4$). ^{1}H NMR: (300 MHz, $CDCl_3$), δ: 5.84 (s, 1H), 4.38 (q, *J* = 6.9 Hz, 1H), 2.58–2.49 (m, 1H), 1.68 (d, *J* = 7.2 Hz, 3H), 1.18 (d, *J* = 7.0 Hz, 6H). ^{13}C NMR: (75 MHz, $CDCl_3$), δ: 198.6, 192.1, 94.8, 56.6, 36.7, 21.9, 19.5. FTIR (NaCl, thin film), cm-1: 2974, 1604. HRMS: ESI $[M + H]^+$ Calcd. for $C_8H_{14}ClO_2$: 177.0677. Found: 177.0675.

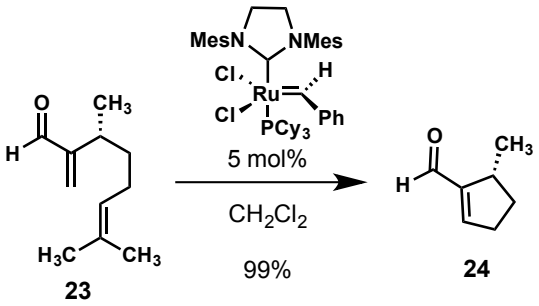


21 **22**

1,8-Diazabicyclo[5.4.0]undec-7-ene (6.00 mL, 40.0 mmol, 1.40 equiv) was added dropwise to a stirred solution of **21** (3.50 g, 28.5 mmol, 1 equiv) in tetrahydrofuran (100 mL) at 23 °C. A pale yellow precipitate formed immediately. The resultant suspension was stirred at 23 °C for 12 h, then was partitioned between water (100 mL) and ethyl acetate (100 mL). The layers were separated and the aqueous phase was extracted with ethyl acetate (6 × 50 mL). The combined organic layers were dried over anhydrous sodium sulfate and the dried solution was concentrated. Purification of the residue flash column chromatography (20% ethyl acetate–hexanes) gave **22** (2.80 g, 72%) as a pale yellow liquid.

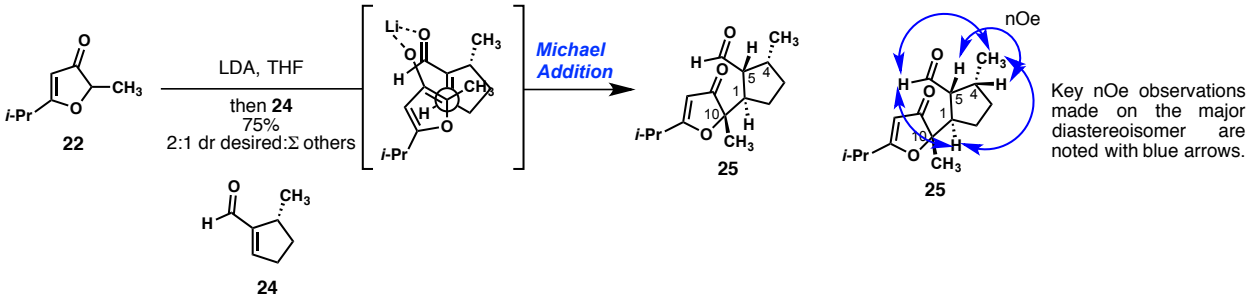
3-furanone **22**: TLC: 20% ethyl acetate–hexanes, R_f = 0.35 (UV, $KMnO_4$). ^{1}H NMR (300 MHz, $CDCl_3$), δ: 5.38 (s, 1H), 4.49 (q, *J* = 6.9 Hz, 1H), 2.76–2.66 (m, 1H), 1.43 (d, *J* = 7.0 Hz, 3H), 1.24 (s, 3H), 1.22 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$), δ: 206.1, 198.7, 100.7, 82.6, 30.5, 19.7, 19.7, 16.6. FTIR (NaCl, thin film), cm^{-1}: 2975, 1742, 1707, 1586. HRMS: ESI $[M + H]^+$ Calcd for $C_8H_{13}O_2$: 141.0910. Found: 141.0911.

The following literature procedure for the synthesis of **24** is provided for reader convenience:[6]



The second generation Grubbs catalyst (933 mg, 1.10 mmol, 0.05 equiv) was added in portions to a solution of **23**[7] (3.60 g, 22.0 mmol) in dichloromethane (200 mL) heated at reflux. The resultant brown solution was heated at reflux for 48 h, then was cooled and concentrated. Purification of the residue by flash column chromatography (4% ethyl acetate–hexanes) gave **24** (2.40 g, 99%) as a pale yellow oil.

Aldehyde **24**: TLC: 4% ethyl acetate–hexanes, R_f = 0.30 (UV, $KMnO_4$). $[\alpha]_D^{23}$ = –6.2° (c = 0.58, CH_2Cl_2). ^{1}H NMR (300 MHz, $CDCl_3$), δ: 9.76 (s, 1H), 6.80 (m, 1H), 3.06–2.98 (m, 1H), 2.68–2.55 (m, 1H), 2.27–2.14 (m, 1H), 1.63–1.53 (m, 2H), 1.13 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$), δ: 190.0, 153.2, 151.8, 36.8, 32.6, 32.1, 19.4. FTIR (NaCl, thin film), cm^{-1}: 2957, 1716, 1683, 1458. HRMS: ESI $[M - H]^+$ Calcd for C_7H_9O: 111.0804. Found: 111.0810. Assay of enantiomeric excess: Chiral HPLC analysis (Regis (*S*, *S*)-Whelk-O #1 25 cm x 4.6 mm, 1 mL/min flow rate, 5% isopropanol–hexanes, t_R (major) = 7.30 min, t_R (minor) = 6.92 min) 79% ee, average of three determinations.

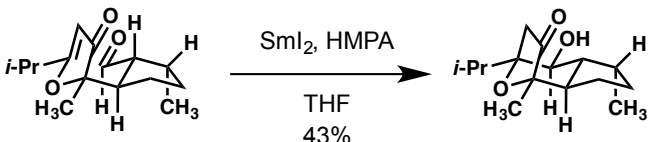


A solution of *n*-butyllithium (2.50 M, 2.20 mL, 5.50 mmol, 1.28 equiv) was added to a stirred solution of *N*,*N*-diisopropylamine (0.85 mL, 6.00 mmol, 1.40 equiv) in tetrahydrofuran (50 mL) at

[6] Chavez, D. E.; Jacobsen, E. N. *Org. Lett.* **2003,** *5* (14), 2563–2565.
[7] Takano, S.; Inomata, K.; Samizu, K.; Tomita, S.; Yanase, M.; Suzuki, M.; Iwabuchi, Y.; Sugihara, T.; Ogasawara, K. *Chem. Lett.* **1989**, 1283–1284.

–78 °C. The resultant solution was warmed briefly to 0 °C, then was cooled to –78 °C whereupon a solution of the 3-furanone **22** (600 mg, 4.30 mmol, 1 equiv) in tetrahydrofuran (5 mL) was added dropwise. The resultant mixture was stirred at –78 °C for 30 min, whereupon a solution of **24** (550 mg, 5.00 mmol, 1.16 equiv) in tetrahydrofuran (5 mL) was added. The reaction mixture was stirred at –78 °C for 30 min, then was warmed to 23 °C and stirred at that temperature for 1 h. The reaction mixture was then cooled to 0 °C whereupon saturated aqueous ammonium chloride solution (30 mL) was added carefully. The layers were separated and the aqueous layer was extracted with dichloromethane (3 × 30 mL). The combined organic layers were dried over anhydrous sodium sulfate and the dried solution was concentrated. Purification of the residue by flash column chromatography (20% ethyl acetate–hexanes) afforded the Michael adduct **25** (807 mg, 75%, 2:1 dr desired:Σ others) as a yellow oil.

Michael adduct **25**: TLC: 20% ethyl acetate–hexanes, R_f = 0.35 (UV, DNP). $[\alpha]_D^{23}$ = +36.1° (c = 0.38, $CHCl_3$). Major isomer: ^{1}H NMR (300 MHz, $CDCl_3$), δ: 9.62 (d, *J* = 3.2 Hz, 1H), 5.32 (s, 1H), 2.90 (dd, J_1 = 8.8 Hz, J_2 = 15.6 Hz, 1H), 2.69–2.62 (m, 2H), 2.38–2.27 (m, 1H), 1.99–1.90 (m, 1H), 1.83–1.73 (m, 2H), 1.59–1.48 (m, 1H), 1.29 (s, 3H), 1.19 (d, *J* = 7.0 Hz, 6H), 0.98 (d, *J* = 7.0 Hz, 3H). Major isomer: ^{13}C NMR: (75 MHz, $CDCl_3$), δ: 206.9, 204.4, 197.7, 101.2, 91.2, 54.5, 43.9, 39.0, 34.6, 30.5, 26.7, 21.0, 19.8, 19.5, 16.0. FTIR (NaCl, thin film), cm^{-1}: 2964, 1717, 1588. HRMS: ESI $[M + H]^+$ Calcd for $C_{15}H_{23}O_3$: 251.1642. Found: 251.1649.



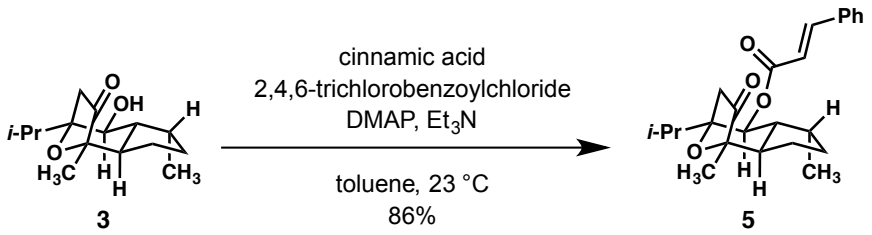
25 **3**

A solution of samarium(II) iodide in tetrahydrofuran [0.1 M, 8.64 mL, 864 μmol, 4.00 equiv, freshly prepared from samarium powder (195 mg, 1.30 mmol, 6.00 equiv) and 1,2-diiodoethane (244 mg, 864 μmol, 4.00 equiv)][8] was added dropwise to a solution of the Michael adduct **25** (54.0 mg, 216 μmol, 1 equiv) and HMPA (700 μL, 4.00 mmol, 18.5 equiv) in deoxygenated tetrahydrofuran (10 mL). The resultant deep purple mixture was stirred at 23 °C for 3 h, then was cooled to 0 °C and quenched by the addition aqueous hydrochloric acid solution (1 N, 10 mL). The layers were separated and the aqueous layer was extracted with dichloromethane (3

[8] Reisman, S. E.; Ready, J. M.; Weiss, M. M.; Hasuoka, A.; Hirata, M.; Tamaki, K.; Ovaska, T. V.; Smith, C. J.; Wood, J. L., *J. Am. Chem. Soc.* **2008,** *130*, 2087–2100.

× 10 mL). The combined organic layers were washed with saturated aqueous sodium bicarbonate solution (10 mL), dried over anhydrous sodium sulfate, and the dried solution was concentrated. Purification of the residue by flash column chromatography (10% ethyl acetate–hexanes) gave the ketoalcohol **3** (16.0 mg, 43%) as a pale yellow oil.

Ketoalcohol **3**: TLC: 20% ethyl acetate–hexanes, R_f = 0.30 (CAM). $[\alpha]_D^{23}$ = –47.4° (c = 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$), δ: 3.90 (dd, J_1 = 10.3 Hz, J_2 = 4.3 Hz, 1H), 2.46 (d, J = 18.5 Hz, 1H), 2.31 (d, J = 18.5 Hz, 1H), 2.33–2.24 (m, 1H), 2.10 (sept, J = 7.0 Hz, 1H), 1.98–1.94 (m, 1H), 1.67-1.58 (m, 2H), 1.39 (d, J = 4.3 Hz, 1H), 1.22 (s, 3H), 1.18–1.12 (m, 2H), 1.09 (d, J = 7.0 Hz, 3H), 1.08 (d, J = 7.0 Hz, 3H), 0.901 (d, J = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$), δ: 215.7, 83.5, 82.8, 70.4, 49.3, 46.4, 41.5, 32.3, 31.0, 30.4, 24.2, 17.9, 17.5, 17.0, 16.7. FTIR (NaCl, thin film), cm^{-1}: 3470, 2958, 1749. HRMS: ESI $[M + H]^+$ Calcd for $C_{15}H_{25}O_3$: 253.1798. Found: 253.1792.

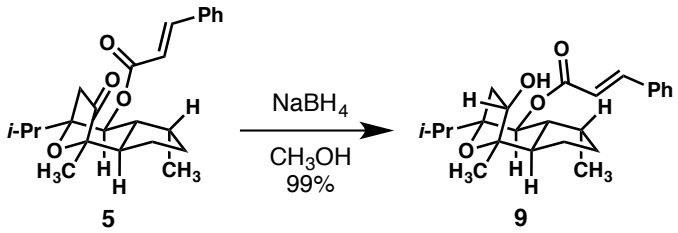


3 **5**

Cinnamic acid (18.0 mg, 120 μmol, 2.00 equiv), triethylamine (25.0 μL, 180 μmol, 3.00 equiv), 2,4,6-trichlorobenzoyl chloride (25.0 μL, 155 μmol, 2.58 equiv) and 4-dimethylaminopyridine (1.0 mg, 8.2 μmol, 0.14 equiv) were added sequentially to a solution of **3** (16.0 mg, 60.0 μmol, 1 equiv) in toluene (2 mL). The resultant mixture was stirred at 23 °C for 2 d, then was quenched with aqueous hydrochloric acid solution (1 N, 5 mL). The layers were separated and the aqueous layer was extracted with dichloromethane (3 × 5 mL). The combined organic layers were washed with saturated aqueous sodium bicarbonate (10 mL), then was dried over anhydrous sodium sulfate and the dried solution was concentrated. Purification of the residue by flash column chromatography (4% ethyl acetate-hexanes) afforded **5** (20.0 mg, 86%) as a colorless oil.

Ketoester **5**: TLC: 4% ethyl acetate–hexanes, R_f = 0.30 (UV, CAM). $[\alpha]_D^{23}$ = –52.2° (c = 0.32, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$), δ: 7.67 (d, J = 16.1 Hz, 1H), 7.54–7.50 (m, 2H), 7.49–7.37 (m, 3H), 6.39 (d, J = 16.1 Hz, 1H), 5.37 (d, J = 10.6 Hz, 1H), 2.52 (ab, 1H), 2.14–2.06 (m, 1H), 2.01–1.92 (m, 1H), 1.91–1.75 (m, 2H), 1.68–1.47 (m, 2H), 1.256 (s, 3H), 1.23–1.18 (m, 2H), 1.03 (t, J = 7.4 Hz, 6H), 0.94 (d, J = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$), δ: 215.5, 165.7,

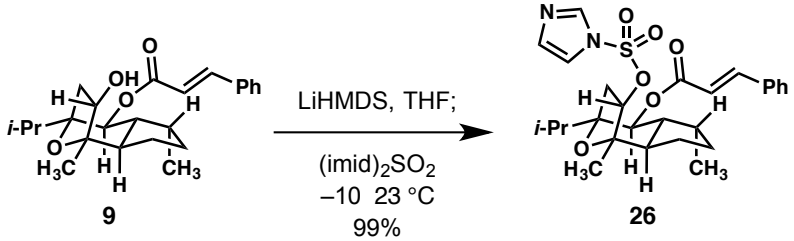
145.7, 134.4, 130.7, 129.2, 128.4, 117.9, 83.7, 82.6, 71.0, 48.6, 46.3, 42.9, 33.3, 31.3, 30.9, 23.5, 18.2, 17.7, 17.1, 17.0. FTIR (NaCl, thin film), cm^{-1}: 2960, 1754, 1713, 1636. HRMS: ESI $[M + H]^+$ Calcd. for $C_{24}H_{31}O_4$: 383.2217. Found: 383.2213.



5 9

Sodium borohydride (2.50 mg, 65.0 μmol, 1.00 equiv) was added to a solution of **5** (25 mg, 65 μmol, 1 equiv) in methanol (2 mL) at 0 °C. The resultant mixture was stirred at 0 °C for 30 min, and excess borohydride was quenched by the addition of saturated aqueous ammonium chloride solution (5 mL). The resultant mixture was extracted with dichloromethane (3 × 5 mL), the combined organic layers were dried over anhydrous sodium sulfate, and the dried solution was concentrated. Purification of the residue by flash column chromatography (20% ethyl acetate–hexanes) afforded **9** (25 mg, quantitative) as a colorless oil.

Ketoalcohol **9**: TLC: 20% ethyl acetate–hexanes, R_f = 0.40 (UV, CAM). $[\alpha]_D^{23}$ = –32.6° (c = 0.30, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$), δ: 7.66 (d, *J* = 16.2 Hz, 1H), 7.54–7.50 (m, 2H), 7.39–7.36 (m, 3H), 6.40 (d, *J* = 16.2 Hz, 1H), 5.22 (d, *J* = 10.3 Hz, 1H), 4.19 (dd, J_1 = 10.7 Hz, J_2 = 4.8 Hz, 1H), 2.39–2.29 (m, 2H), 2.16–2.04 (m, 2H), 1.98–1.67 (m, 6H), 1.32 (s, 3H), 1.29–1.21 (m, 1H), 0.98–0.93 (m, 9H). ^{13}C NMR (75 MHz, $CDCl_3$), δ: 166.1, 145.1, 134.6, 130.5, 129.1, 128.3, 118.6, 84.8, 81.6, 81.2, 72.7, 49.4, 46.4, 39.6, 33.1, 31.7, 31.4, 24.6, 23.5, 17.9, 17.3, 17.1. FTIR (NaCl, thin film), cm^{-1}: 2923, 1710, 1636. HRMS: ESI $[M + H]^+$ Calcd. for $C_{24}H_{33}O_4$: 385.2373. Found: 385.2389.

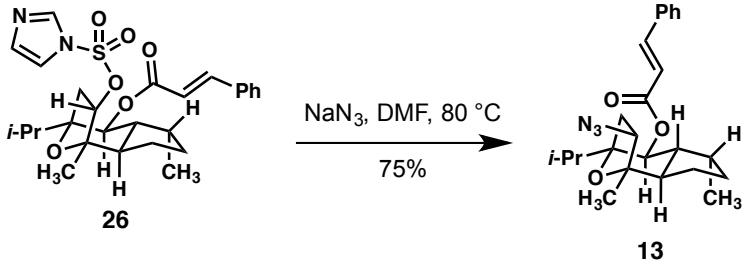


9 26

A solution of *n*-butyllithium (2.50 M, 72.8 μL, 182 μmol, 7.00 equiv) was added to a stirred solution of hexamethyldisilazane (42 μL, 200 μmol, 7.70 equiv) in tetrahydrofuran (2 mL) at 0 °C. The resultant solution was warmed briefly to 23 °C, then was cooled to 0 °C whereupon a

solution of **9** (10 mg, 26 μmol, 1 equiv) in tetrahydrofuran (1 mL) was added. The resultant mixture was stirred at 0 °C for 30 min, then was cooled to –10 °C whereupon *N*,*N'*-sulfuryldiimidazole (40 mg, 200 μmol, 7.70 equiv) was added. The reaction mixture was warmed to 23 °C, stirred at that temperature for 12 h. Excess *N*,*N'*-sulfuryldiimidazole was quenched by the addition of methanol (0.2 mL), and the resultant mixture was concentrated. The residue was partitioned between saturated aqueous sodium bicarbonate solution (5 mL) and dichloromethane (5 mL). The layers were separated and the aqueous phase was extracted with dichloromethane (3 × 5 mL). The combined organic layers were dried over anhydrous sodium sulfate and the dried solution was concentrated. Purification of the residue by flash column chromatography (20% ethyl acetate-hexanes) gave **26** (13 mg, quantitative) as a colorless oil.

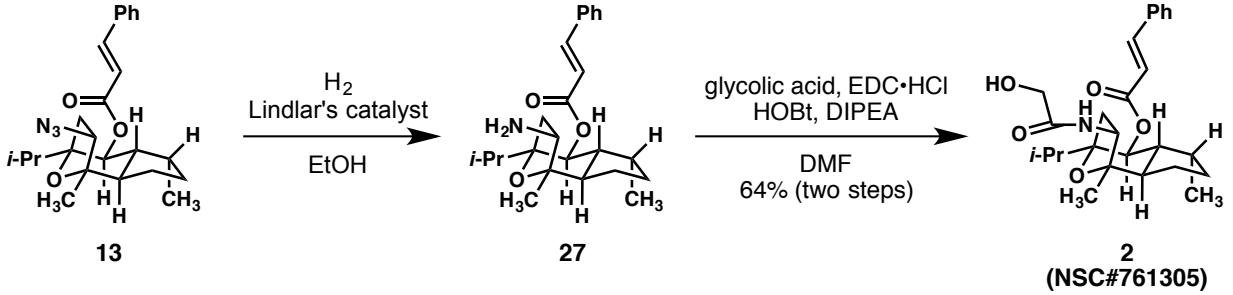
Imidazole **26**: TLC: 20% ethyl acetate–hexanes, R_f = 0.35 (UV, CAM). $[\alpha]_D^{23}$ = –14.0° (c = 0.71, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$), δ: 8.03 (s, 1H), 7.65 (d, *J* = 16.1 Hz, 1H), 7.56–7.53 (m, 2H), 7.41–7.38 (m, 4H), 7.24 (s, 1H), 6.38 (d, *J* = 16.4 Hz, 1H), 5.20 (d, *J* = 10.1 Hz, 1H), 4.59 (dd, J_1 = 10.7 Hz, J_2 = 4.8 Hz, 1H), 2.31–2.22 (m, 1H), 2.18–2.13 (m, 2H), 2.06–1.99 (m, 2H), 1.92–1.88 (m, 1H), 1.83–1.67 (m, 3H), 1.23–1.21 (m, 1H), 1.19 (s, 3H), 0.95–0.89 (m, 9H). ^{13}C NMR (75 MHz, $CDCl_3$), δ: 165.8, 145.7, 137.4, 134.4, 131.8, 130.7, 129.2, 128.4, 118.2, 117.9, 90.7, 85.4, 81.3, 71.3, 49.0, 46.5, 35.9, 32.8, 31.4, 31.3, 24.1, 22.7, 17.7, 17.0, 17.0. FTIR (NaCl, thin film), cm^{-1}: 2960, 1713, 1636. HRMS: ESI $[M + H]^+$ Calcd. for $C_{27}H_{35}N_2O_6S$: 515.2210. Found: 515.2215.



26 **13**

Sodium azide (25.0 mg, 380 μmol, 20.0 equiv) was added in one portion to a stirred solution of **26** (10.0 mg, 19.0 μmol, 1 equiv) in *N*,*N*-dimethylformamide (2 mL). The resultant mixture was heated at 80 °C for 2 d. The reaction mixture was cooled, diluted with dichloromethane (10 mL) and washed with water (10 mL). The aqueous layer was extracted with dichloromethane (3 × 5 mL), the combined organic layers were dried over anhydrous sodium sulfate, and the dried solution was concentrated. Purification of the residue by flash column chromatography (2% ethyl acetate-hexanes) afforded **13** (6.0 mg, 75%) as a colorless oil.

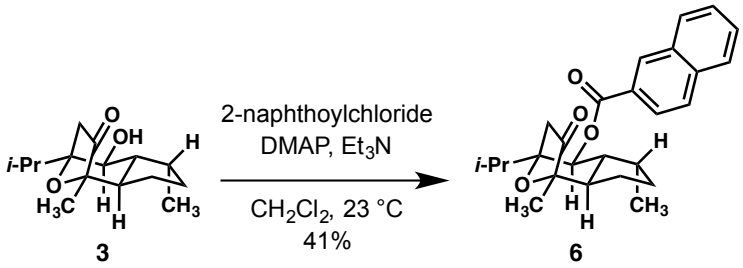
13: TLC: 4% ethyl acetate–hexanes, R_f = 0.50 (UV, $KMnO_4$). $[\alpha]_D^{23}$ = –37.7° (c = 0.5, $CHCl_3$). ^{1}H NMR: (500 MHz, $CDCl_3$), δ: 7.67 (d, *J* = 16.0 Hz, 1H), 7.54-7.52 (m, 2H), 7.40-7.38 (m, 3H), 6.50 (d, *J* =16.0 Hz, 1H), 5.13 (d, *J* = 10.3 Hz, 1H), 3.64 (dd, *J* = 8.4, 3.4, 1H), 2.60 (dd, *J* = 14.4, 8.5 Hz, 1H), 2.15-2.10 (m, 1H), 1.95-1.89 (m, 3H), 1.81-1.70 (m, 2H), 1.50-1.44 (m, 1H), 1.33 (s, 3H), 1.30-1.24 (m, 2H), 1.03 (d, *J* = 6.8 Hz, 3H), 0.98 (d, *J* = 7.0 Hz, 3H), 0.94 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR: (125 MHz, $CDCl_3$), δ: 165.6, 145.2, 134.2, 130.4, 128.9, 128.1, 117.9, 85.6, 85.4, 71.3, 63.3, 48.1, 46.9, 38.7, 32.9, 31.1, 30.9, 24.7, 20.2, 18.2, 17.5, 17.0. FTIR (NaCl, thin film), cm-1: 2930, 2092, 1711, 1640. HRMS: ESI $[M + H]^+$ Calcd. for $C_{24}H_{32}N_3O_3$: 410.2438. Found: 410.2443.



Lindlar's catalyst (5 mg) was added to a stirred solution of **13** (LZW-II-5-1, 9.0 mg, 22 μmol, 1 equiv) in ethanol (2 mL). The resultant mixture was bubbled with hydrogen (H_2, balloon) for 5 minutes and then stirred under a hydrogen atmosphere at room temperature for 30 min. The reaction mixture was then filtered through a pad of Celite, and the filtrate was concentrated. Purification of the residue by flash column chromatography (2% triethylamine-ethyl acetate) afforded **27** as a colorless oil that was advanced without further characterization.

Glycolic acid (26.0 mg, 350 μmol, 16.0 equiv), *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (67.0 mg, 350 μmol, 16.0 equiv) and 1-hydroxybenzotriazole (47.0 mg, 350 μmol, 16.0 equiv) were added sequentially to a stirred solution of **27** in *N*,*N*-dimethylformamide (4 mL) at 23 °C. The reaction mixture was cooled to 0 °C whereupon *N*,*N*-diisopropylethylamine (152 μL, 880 μmol, 40 equiv) was added. The resultant mixture was stirred at 0 °C for 1 h, then allowed to warm to 23 °C and stirred for an additional 24 h. The reaction mixture was diluted with water (10 mL), and the aqueous layer was extracted with dichloromethane (3 × 5 mL). The combined organic layers were dried over anhydrous sodium sulfate and the dried solution was concentrated. Purification of the residue by flash column chromatography (67% ethyl acetate–hexanes) afforded **2** (6.0 mg, 64% two steps) as a colorless oil.

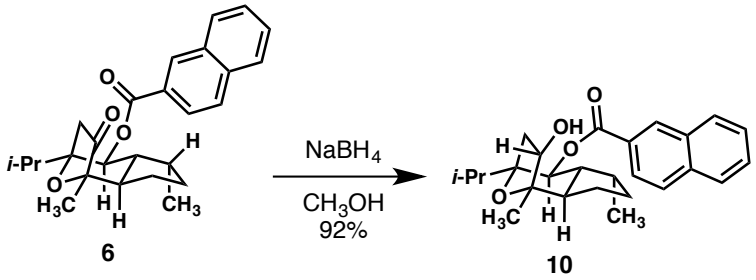
2 (NCI – **NSC#761305**): TLC: 67% ethyl acetate–hexanes, R_f = 0.50 (UV, CAM). $[\alpha]_D^{23}$ = –37.7° (c = 0.4, MeOH). ^{1}H NMR: (500 MHz, MeOH-d_4), δ: 7.69 (d, J = 15.9 Hz, 1H), 7.62-7.60 (m, 2H), 7.41-7.39 (m, 3H), 6.50 (d, J = 16.0 Hz, 1H), 5.13 (d, J = 9.6 Hz, 1H), 4.43 (dd, J_1 = 9.1 Hz, J_2 = 4.2 Hz, 1H), 4.00 (d, J = 4.3 Hz, 2H), 2.71 (dd, J_1 = 14.1 Hz, J_2 = 9.1 Hz, 1H), 2.15-2.11 (m, 1H), 2.04-1.96 (m, 1H), 1.92-1.87 (m, 1H), 1.80-1.76 (m, 2H), 1.72-1.66 (m, 3H), 1.41-1.36 (m, 1H), 1.30-1.27 (m, 2H), 1.14 (s, 3H), 1.04 (d, J = 6.8 Hz, 3H), 0.96 (d, J = 7.1 Hz, 3H), 0.93 (d, J = 7.2 Hz, 3H). ^{13}C NMR: (125 MHz, MeOH-d_4), δ: 174.1, 167.4, 146.8, 135.7, 131.7, 130.0, 129.3, 118.9, 86.4, 86.2, 72.7, 62.4, 52.0, 49.6, 47.9, 40.9, 34.7, 32.4, 32.0, 25.4, 20.1, 18.7, 17.8, 17.3. FTIR (NaCl, thin film), cm-1: 3399, 2961, 1709, 1665, 1532. HRMS: ESI [M + H]$^+$ Calcd. for $C_{26}H_{36}NO_5$: 442.2588. Found: 442.2567.



2-Naphthoyl chloride (59 mg, 0.31 mmol, 3.0 equiv) and 4-(dimethylamino)pyridine (38 mg, 0.31 mmol, 3.0 equiv) were added sequentially to a solution of **3** (26 mg, 0.10 mmol, 1.0 equiv) in 2:1 dichloromethane:triethylamine (30 mL). The reaction mixture was stirred at 23 °C for 2 d, then excess acid chloride was quenched by the addition of 1N aqueous hydrochloric acid solution (20 mL). The layers were separated and the aqueous layer was extracted with dichloromethane (3 × 20 mL). The combined organic extracts were washed with saturated aqueous sodium bicarbonate solution (20 mL), dried over anhydrous sodium sulfate, and the dried solution was concentrated. The resulting residue was purified by flash column chromatography (5% ethyl acetate–hexanes) to afford **6** as a pale yellow oil (17 mg, 41%).

Ketoester **6**: TLC: 20% ethyl acetate–hexanes, R_f = 0.68 (UV, CAM). ^{1}H NMR (500 MHz, $CDCl_3$), δ: 8.55 (s, 1H), 8.00 (d, J = 8.5 Hz, 1H), 7.95 (d, J = 8.0 Hz, 1H), 7.89 (d, J = 9.0 Hz, 2H), 7.63–7.55 (m, 2H), 5.57 (d, J = 10.5 Hz, 2H), 2.65 (ab, 1H), 2.15–2.11 (m, 1H), 2.05–1.99 (m, 1H), 1.93–1.85 (m, 2H), 1.73–1.67 (m, 2H), 1.30 (s, 3H), 1.27–1.22 (m, 2H), 1.07 (d, J = 7.0 Hz, 3H), 1.03 (d, J = 7.0 Hz, 3H), 0.99 (d, J = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$), δ: 215.3, 165.2, 135.6, 132.4, 131.2, 129.3, 128.5, 128.3, 127.8, 127.0, 126.8, 125.1, 83.6, 82.5, 71.3, 48.4, 46.2, 42.9, 33.1, 31.1, 30.7, 29.7, 23.3, 17.9, 17.6, 16.9. FTIR (NaCl, thin film), cm^{-1}:

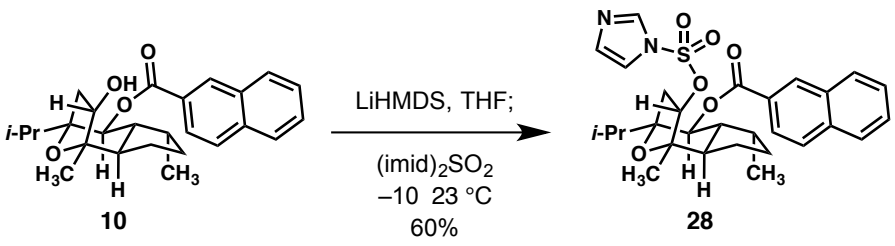
2925, 1715, 1631, 1276, 1196, 778. HRMS: APCI [M + H]$^+$ Calcd. for $C_{26}H_{32}O_4$: 407.2222. Found: 407.2226.



6 **10**

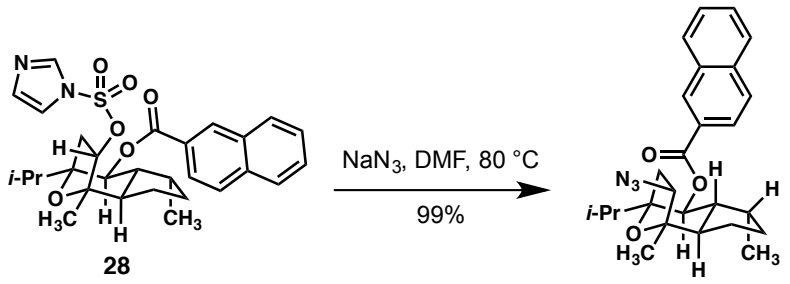
Sodium borohydride (15 mg, 0.40 mmol, 3.0 equiv) was added to a solution of **6** (54 mg, 0.13 mmol, 1.0 equiv) in methanol (25 mL) at 0 °C. The resultant mixture was stirred at 0 °C for 1 h, and then the excess borohydride was quenched by the addition of saturated aqueous ammonium chloride solution (25 mL). The mixture was extracted with dichloromethane (3 × 20 mL), the combined organic layers were dried over anhydrous sodium sulfate, and the dried solution was concentrated. Purification of the residue by flash column chromatography (20% ethyl acetate–hexanes) afforded **10** (50 mg, 92%) as a colorless oil.

Hydroxy ester **10**: TLC: 20% ethyl acetate–hexanes, R_f = 0.36 (UV, CAM). ^{1}H NMR (500 MHz, $CDCl_3$), δ: 8.58 (s, 1H), 8.05 (d, *J* = 9.0 Hz, 1H), 7.95 (d, *J* = 8.0 Hz, 1H), 7.88 (d, *J* = 8.5 Hz, 2H), 7.61–7.53 (m, 2H), 5.42 (d, *J* = 10.5 Hz, 2H), 4.25 (dd, J_1 = 11.0 Hz, J_2 = 5.0 Hz, 1H), 2.54–2.48 (m, 1H), 2.43 (dd, J_1 = 13.5 Hz, J_2 = 11.0 Hz, 1H), 2.25 (dd, J_1 = 13.5 Hz, J_2 = 5.0 Hz, 1H), 2.19–2.15 (m, 2H), 2.05–1.83 (m, 6H), 1.36 (s, 3H), 1.32–1.20 (m, 2H), 1.00 (d, *J* = 7.0 Hz, 3H), 0.98 (d, *J* = 7.0 Hz, 3H), 0.95 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$), δ: 165.4, 135.5, 132.5, 131.1, 129.3, 128.2, 128.1, 127.7, 127.6, 126.6, 125.3, 84.7, 81.5, 81.1, 73.0, 49.2, 46.3, 39.5, 33.0, 31.5, 31.1, 29.7, 24.4, 23.3, 17.8, 17.0. FTIR (NaCl, thin film), cm^{-1}: 2924, 1714, 1631, 1276, 1196, 778. HRMS: APCI [M + H]$^+$ Calcd. for $C_{26}H_{34}O_4$: 409.2379. Found: 409.2364.



A solution of *n*-butyllithium (2.50 M in hexanes, 340 μL, 0.86 mmol, 7.00 equiv) was added to a stirred solution of hexamethyldisilazane (230 μL, 0.94 mmol, 7.70 equiv) in tetrahydrofuran (15 mL) at 0 °C. The resultant solution was warmed briefly to 23 °C, then was cooled to 0 °C whereupon a solution of **10** (50 mg, 0.12 mmol, 1 equiv) in tetrahydrofuran (5 mL) was added. The resultant mixture was stirred at 0 °C for 30 min, then was cooled to –10 °C whereupon *N*,*N'*-sulfuryldiimidazole (218 mg, 1.10 mmol, 9.00 equiv) was added. The reaction mixture was warmed to 23 °C and stirred at that temperature for 12 h. Excess *N*,*N'*-sulfuryldiimidazole was quenched by the addition of methanol (5 mL), and the resultant mixture was concentrated. The residue was partitioned between saturated aqueous sodium bicarbonate solution (20 mL) and dichloromethane (20 mL). The layers were separated and the aqueous phase was extracted with dichloromethane (3 × 25 mL). The combined organic layers were dried over anhydrous sodium sulfate and the dried solution was concentrated. Purification of the residue by flash column chromatography (20% ethyl acetate-hexanes) gave **28** (39 mg, 60%) as a colorless oil.

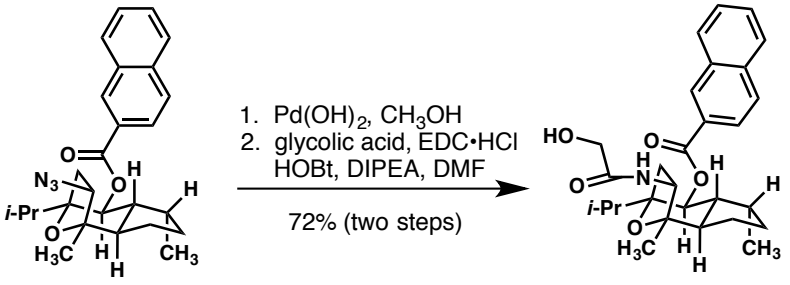
Imidazole **28**: TLC: 20% ethyl acetate–hexanes, R_f = 0.33 (UV, CAM). ^{1}H NMR (500 MHz, $CDCl_3$), δ: 8.53 (s, 1H), 8.07 (s, 1H), 8.02 (d, *J* = 8.0 Hz, 1H), 7.98 (d, *J* = 8.5 Hz, 1H), 7.90 (d, *J* = 8.5 Hz, 2H), 7.63–7.58 (m, 2H), 7.42 (s, 1H), 7.28 (s, 1H), 5.38 (d, *J* = 9.5 Hz, 2H), 4.64 (dd, J_1 = 11.0 Hz, J_2 = 5.0 Hz, 1H), 2.37 (dd, J_1 = 14.5 Hz, J_2 = 11.0 Hz, 1H), 2.27–2.22 (m, 1H), 2.19–2.14 (m, 2H), 2.02–1.90 (m, 2H), 1.83–1.77 (m, 1H), 1.74–1.60 (m, 2H), 1.28–1.24 (m, 1H), 1.22 (s, 3H), 0.98 (d, *J* = 7.0 Hz, 3H), 0.94 (d, *J* = 7.0 Hz, 3H), 0.92 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$), δ: 165.2, 135.6, 132.5, 131.2, 129.4, 128.5, 128.3, 127.8, 127.0, 126.8, 125.2, 90.5, 85.0, 81.2, 71.6, 48.9, 46.4, 35.7, 32.7, 31.2, 31.1, 29.7, 23.9, 22.5, 17.5, 16.8. FTIR (NaCl, thin film), cm^{-1}: 2920, 1715, 1632, 1198, 778. HRMS: ESI $[M + Na]^+$ Calcd. for $C_{29}H_{34}N_2NaO_6S$: 561.2035. Found: 561.2025.



28 14

Sodium azide (94.0 mg, 1.44 mmol, 20.0 equiv) was added to a solution of **28** (39.0 mg, 72.4 μmol, 1.0 equiv) in *N*,*N*-dimethylformamide (5 mL). The reaction mixture was heated at 80 °C for 18 h, and then quenched by the addition of 10% aqueous lithium chloride solution (20 mL). The mixture was extracted with dichloromethane (3 × 25 mL), the combined organic layers were dried over anhydrous sodium sulfate, and the dried solution was concentrated. Purification of the residue by flash column chromatography (5% ethyl acetate–hexanes) afforded the azide **14** (31 mg, 99%) as a colorless oil.

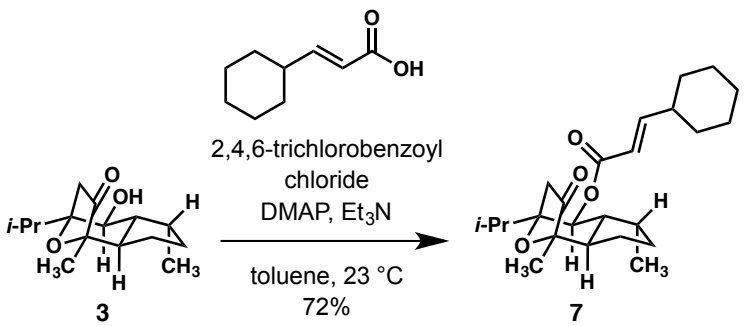
Azide **14**: TLC: 20% ethyl acetate–hexanes, R_f = 0.66 (UV, CAM). ^{1}H NMR (500 MHz, $CDCl_3$), δ: 8.56 (s, 1H), 8.02 (d, *J* = 8.5 Hz, 1H), 7.96 (d, *J* = 8.0 Hz, 1H), 7.89 (d, *J* = 8.5 Hz, 2H), 7.63–7.55 (m, 2H), 5.32 (d, *J* = 10.0 Hz, 2H), 3.72 (dd, J_1 = 8.5 Hz, J_2 = 3.5 Hz, 1H), 2.77 (dd, J_1 = 14.0 Hz, J_2 = 8.5 Hz, 1H), 2.17–2.13 (m, 1H), 2.05–2.02 (m, 1H), 1.99–1.92 (m, 2H), 1.88–1.75 (m, 3H), 1.63–1.60 (m, 2H), 1.37 (s, 3H), 1.05 (d, *J* = 7.0 Hz, 3H), 1.00 (d, *J* = 7.0 Hz, 3H), 0.90 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$), δ: 165.2, 135.6, 132.5, 131.1, 129.3, 128.3, 128.2, 127.8, 127.3, 126.8, 125.2, 85.7, 85.5, 71.9, 63.4, 48.2, 47.0, 38.9, 33.0, 31.1, 30.9, 29.7, 24.8, 20.2, 18.3, 17.5, 17.0. FTIR (NaCl, thin film), cm^{-1}: 2957, 2927, 2094, 1720, 1276, 1195. HRMS: APCI $[M + H]^+$ Calcd. for $C_{26}H_{32}N_3O_3$: 434.2444. Found: 434.2459.



14 17 (NSC#764081)

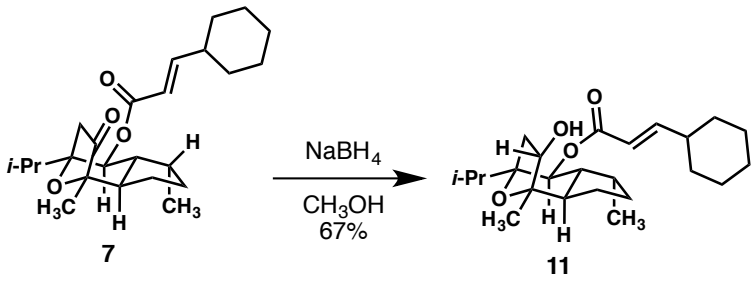
Palladium hydroxide on carbon (Pearlman's catalyst, 1.4 mg, 20 wt. % loading) was added to a solution of **14** (7.0 mg, 16 μmol, 1.0 equiv) in methanol (5 mL). The reaction mixture was bubbled with hydrogen (H_2, balloon) for 10 min, then was stirred under a hydrogen atmosphere (balloon) for 5 h. The reaction flask was purged with argon, the reaction mixture was filtered through a pad of Celite, and the pad was washed with methanol. The combined organic filtrates were concentrated and the resultant oily residue was dissolved in *N*,*N*-dimethylformamide (10 mL). Glycolic acid (12.0 mg, 0.161 mmol, 10.0 equiv), *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (31.0 mg, 0.161 mmol, 10.0 equiv), and 1-hydroxybenzotriazole (22.0 mg, 0.161 mmol, 10.0 equiv) were added sequentially to the stirred solution at 23 °C. The reaction mixture was cooled to 0 °C whereupon *N*,*N*-diisopropylethylamine (84 μL, 0.48 mmol, 30 equiv) was added. The resultant mixture was stirred at 0 °C for 1 h, then allowed to warm to 23 °C and stirred for an additional 18 h. The reaction mixture was quenched with 10% aqueous lithium chloride solution (20 mL), then extracted with dichloromethane (3 × 25 mL). The combined organic layers were dried over anhydrous sodium sulfate and the dried solution was concentrated. Purification of the residue by flash column chromatography (50% ethyl acetate–hexanes) afforded the glycolamide **17** (5.4 mg, 72% over two steps) as a colorless oil.

Glycolamide **17**: TLC: 50% ethyl acetate–hexanes, R_f = 0.20 (UV, CAM). ^{1}H NMR (500 MHz, CD_3OD), δ: 8.56 (s, 1H), 8.02 (d, *J* = 8.7 Hz, 1H), 7.96 (d, *J* = 7.5 Hz, 1H), 7.88 (d, *J* = 8.4 Hz, 2H), 7.63–7.53 (m, 2H), 6.52 (d, *J* = 9.6 Hz, 2H), 4.58–4.50 (m, 1H), 4.17 (s, 3H), 3.51 (bs, 1H), 2.90 (dd, J_1 = 14.4 Hz, J_2 = 9.0 Hz, 1H), 2.33–2.28 (m, 1H), 2.20–2.13 (m, 1H), 1.99–1.65 (m, 7H), 1.38–1.31 (m, 2H), 1.22 (s, 3H), 1.04 (d, *J* = 6.6 Hz, 3H), 0.98 (d, *J* = 6.9 Hz, 3H), 0.97 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (125 MHz, CD_3OD), δ: 174.1, 166.9, 137.1, 134.0, 132.2, 130.4, 129.7, 129.5, 128.9, 128.6, 128.0, 126.1, 86.5, 86.3, 73.3, 62.4, 52.2, 49.7, 48.0, 41.2, 34.9, 32.5, 32.1, 25.5, 20.1, 18.8, 17.8, 17.3. FTIR (NaCl, thin film), cm^{-1}: 2954, 2926, 1714, 1659, 1276, 1196, 1096, 968. HRMS: APCI $[M + H]^+$ Calcd. for $C_{28}H_{36}NO_5$: 466.2588. Found: 466.2607.



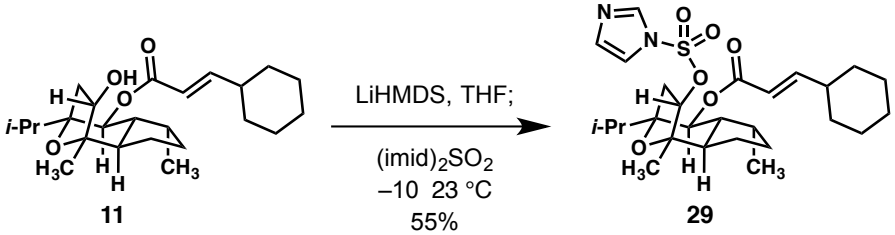
(*E*)-3-cyclohexylacrylic acid (45 mg, 0.29 mmol, 2.0 equiv), triethylamine (61 µL, 0.44 mmol, 3.0 equiv), 2,4,6-trichlorobenzoyl chloride (57 µL, 0.37 mmol, 2.5 equiv), and 4-(dimethylamino)pyridine (3.6 mg, 29 µmol, 0.2 equiv) were added sequentially to a solution of **3** (37 mg, 0.15 mmol, 1.0 equiv) in toluene (30 mL). The reaction mixture was stirred at 23 °C for 2 d, then excess acid chloride was quenched with 1N aqueous hydrochloric acid solution (20 mL). The layers were separated and the aqueous layer was extracted with dichloromethane (3 × 20 mL). The combined organic extracts were washed with saturated aqueous sodium bicarbonate solution (20 mL), dried over anhydrous sodium sulfate, and the dried solution was concentrated. The resulting residue was purified by flash column chromatography (5% ethyl acetate–hexanes) gave **7** as a pale yellow oil (41 mg, 72%).

Ketoester **7**: TLC: 20% ethyl acetate–hexanes, R_f = 0.69 (CAM). ^{1}H NMR (500 MHz, $CDCl_3$), δ: 6.90 (dd, J_1 = 15.5 Hz, J_2 = 7.0 Hz, 1H), 5.70 (d, J = 15.5 Hz, 1H), 5.29 (d, J = 10.5 Hz, 1H), 2.47 (ab, 1H), 2.14–2.06 (m, 2H), 1.95–1.86 (m, 2H), 1.78–1.75 (m, 4H), 1.69–1.64 (m, 2H), 1.52–1.49 (m, 1H), 1.43 (s, 3H), 1.33–1.28 (m, 2H), 1.21–1.12 (m, 4H), 1.02 (d, J = 7.0 Hz, 3H), 0.99 (d, J = 7.0 Hz, 3H), 0.92 (d, J = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$), δ: 215.4, 165.6, 155.4, 118.4, 83.5, 82.4, 70.3, 48.3, 46.0, 42.7, 40.5, 33.0, 31.6, 31.0, 30.7, 30.3, 29.7, 25.9, 25.7, 23.2, 17.9, 17.5, 16.9, 16.8. FTIR (NaCl, thin film), cm^{-1}: 2927, 2853, 1722, 1651. HRMS: APCI $[M + H]^+$ Calcd. for $C_{24}H_{37}O_4$: 389.2692. Found: 389.2661.



Sodium borohydride (30 mg, 0.78 mmol, 4.0 equiv) was added to a solution of **7** (76 mg, 0.20 mmol, 1.0 equiv) in methanol (40 mL) at 0 °C. The resultant mixture was stirred at 0 °C for 30 min, and then the excess sodium borohydride was quenched by the addition of saturated aqueous ammonium chloride solution (25 mL). The mixture was extracted with dichloromethane (3 × 20 mL), the combined organic layers were dried over anhydrous sodium sulfate, and the dried solution was concentrated. Purification of the residue by flash column chromatography (20% ethyl acetate–hexanes) afforded **11** (41 mg, 67%) as a colorless oil.

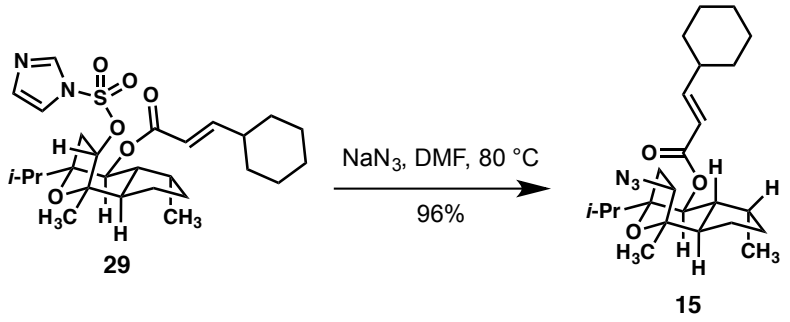
Hydroxyester **11**: TLC: 20% ethyl acetate–hexanes, R_f = 0.30 (CAM); ^{1}H NMR (600 MHz, $CDCl_3$), δ: 6.89 (dd, J_1 = 16.0, J_2 = 6.5 Hz, 1H), 5.71 (d, J = 16.0 Hz, 1H), 5.12 (d, J = 10.5 Hz, 1H), 4.17 – 4.12(m, 1H), 2.30 – 2.25 (m, 2H), 2.13 – 2.08 (m, 2H), 2.04 – 1.99 (m, 1H), 1.79 – 1.71 (m, 6H), 1.69 – 1.64 (m, 2H), 1.29 (s, 3H), 1.26 – 1.24 (m, 4H), 1.16 – 1.09 (m, 5H), 0.94 (d, J = 6.5 Hz, 3H), 0.91 (d, J = 7.0 Hz, 3H), 0.90 (d, J = 7.0 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$), δ: 166.1, 154.7, 119.1, 84.7, 72.2, 54.5, 49.2, 46.3, 40.5, 39.5, 33.0, 32.3, 31.8, 31.8, 31.6, 31.3, 26.1, 25.9, 24.4, 23.4, 17.8, 17.1, 17.0. FTIR (NaCl, thin film), cm^{-1}: 3310, 2925, 2853, 1549, 1375. HRMS: APCI $[M + H]^+$ Calcd. for $C_{24}H_{39}O_4$: 391.2848. Found: 391.2845.



A solution of *n*-butyllithium (2.50 M in hexanes, 0.33 mL, 0.82 mmol, 7.00 equiv) was added to a stirred solution of hexamethyldisilazane (0.19 mL, 0.91 mmol, 7.7 equiv) in tetrahydrofuran (10 mL) at 0 °C. The resultant solution was warmed briefly to 23 °C, then was cooled to 0 °C whereupon a solution of **11** (46 mg, 0.12 mmol, 1.0 equiv) in tetrahydrofuran (5 mL) was added. The resultant mixture was stirred at 0 °C for 30 min, then was cooled to –10 °C whereupon *N*,*N*'-sulfuryldiimidazole (210 mg, 1.06 mmol, 9.00 equiv) was added. The reaction mixture was warmed to 23 °C and stirred at that temperature for 12 h. Excess *N*,*N*'-sulfuryldiimidazole was quenched by the addition of methanol (5 mL), and the resultant mixture was concentrated. The residue was partitioned between saturated aqueous bicarbonate solution (20 mL) and dichloromethane (20 mL). The layers were separated and the aqueous phase was extracted with dichloromethane (3 × 25 mL). The combined organic layers were dried over anhydrous sodium sulfate and the dried solution was concentrated. Purification of the residue by flash column chromatography (20% ethyl acetate–hexanes) gave **29** (34 mg, 55%) as a colorless oil.

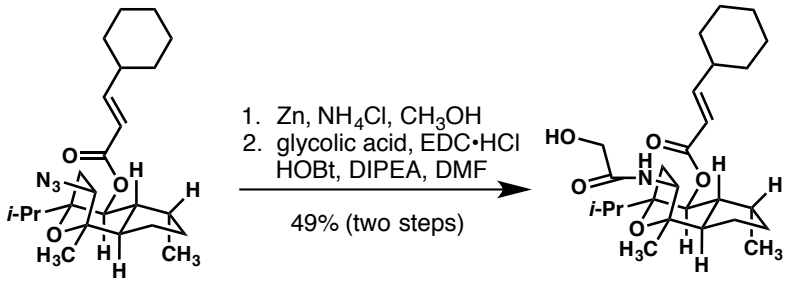
Imidazole **29**: TLC: 20% ethyl acetate–hexanes, R_f = 0.32 (UV, CAM). ^{1}H NMR (500 MHz, $CDCl_3$), δ: 8.02 (s, 1H), 7.37 (s, 1H), 7.23 (s, 1H), 6.89 (dd, J_1 = 16.0 Hz, J_2 = 6.5 Hz, 1H), 5.79 (d, J = 16.0 Hz, 1H), 5.11 (d, J = 10.0 Hz, 1H), 4.56 (dd, J_1 = 11.0 Hz, J_2 = 5.0 Hz, 1H), 2.27–2.20 (m, 1H), 2.15–2.05 (m, 4H), 2.01–1.95 (m, 2H), 1.87–1.83 (m, 1H), 1.78–1.75 (m, 6H), 1.68–1.65 (m, 3H), 1.60–1.54 (m, 3H), 1.17 (s, 3H), 0.91–0.87 (m, 12H). ^{13}C NMR (125 MHz, $CDCl_3$), δ: 165.6, 155.3, 131.6, 118.4, 90.5, 85.2, 81.0, 70.6, 48.7, 46.2, 40.5, 35.6, 32.5, 31.9,

31.6, 31.1, 30.3, 29.7, 25.9, 25.7, 23.9, 22.4, 17.4, 16.7. FTIR (NaCl, thin film), cm^{-1}: 2924, 2852, 1720, 1648, 1423. HRMS: ESI $[M + Na]^+$ Calcd. for $C_{27}H_{40}N_2NaO_6S$: 543.2505. Found: 543.2476.



Sodium azide (85 mg, 1.3 mmol, 20 equiv) was added in one portion to a solution of **29** (34 mg, 65 μmol, 1.0 equiv) in *N*,*N*-dimethylformamide (5 mL). The reaction mixture was heated at 80 °C for 18 h, and then quenched with 10% aqueous lithium chloride solution (20 mL). The mixture was extracted with dichloromethane (3 × 20 mL), the combined organic layers were dried over anhydrous sodium sulfate, and the dried solution was concentrated. Purification of the residue by flash column chromatography (5% ethyl acetate–hexanes) afforded the azide **15** (26 mg, 96%) as a colorless oil.

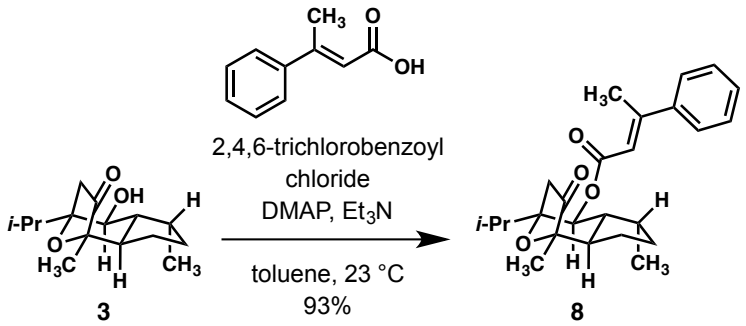
Azide **15**: TLC: 20% ethyl acetate–hexanes, R_f = 0.74 (CAM). ^{1}H NMR (500 MHz, $CDCl_3$), δ: 6.89 (dd, J_1 = 16.0 Hz, J_2 = 6.5 Hz, 1H), 5.71 (d, J = 15.5 Hz, 1H), 5.04 (d, J = 10.5 Hz, 1H), 3.60 (dd, J_1 = 8.0 Hz, J_2 = 3.0 Hz, 1H), 2.53 (dd, J_1 = 14.5 Hz, J_2 = 8.5 Hz, 1H), 2.14–2.09 (m, 3H), 1.91–1.84 (m, 4H), 1.79–1.72 (m, 6H), 1.70–1.66 (m, 2H), 1.60–1.54 (m, 3H), 1.43–1.40 (m, 1H), 1.31 (s, 3H), 1.00 (d, J = 7.0 Hz, 3H), 0.94 (d, J = 7.0 Hz, 3H), 0.91 (d, J = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$), δ: 165.7, 155.0, 118.6, 85.6, 85.4, 70.9, 63.3, 48.0, 46.9, 40.4, 38.6, 32.8, 31.7, 31.1, 30.9, 25.9, 25.7, 24.7, 20.2, 18.2, 17.5, 16.9. FTIR (NaCl, thin film), cm^{-1}: 2928, 2853, 2094, 1722, 1713. HRMS APCI $[M + H]^+$ Calcd. for $C_{24}H_{38}N_3O_3$: 416.2908. Found: 416.2912.



Ammonium chloride (5.0 mg, 96 μmol, 10 equiv) and zinc (dust, 6.0 mg, 96 μmol, 10 equiv) were added sequentially to a solution of **15** (4.0 mg, 9.6 μmol, 1.0 equiv) in methanol (3 mL). The reaction mixture was stirred vigorously at room temperature for 2 h, then was concentrated. The resulting residue was triturated with diethyl ether (3 × 10 mL) and the combined organic fractions were filtered through a pad of silica gel to afford the amine (3.5 mg, 93%), which was advanced without further purification. Glycolic acid (8.0 mg, 0.10 mmol, 10 equiv), *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (20.0 mg, 0.103 mmol, 10.0 equiv), and 1-hydroxybenzotriazole (14 mg, 0.10 mmol, 10 equiv) were added sequentially to a stirred solution of the amine intermediate in *N*,*N*-dimethylformamide (10 mL) at 23 °C. The reaction mixture was cooled to 0 °C whereupon *N*,*N*-diisopropylamine (54 μL, 0.31 mmol, 30.0 equiv) was added. The reaction mixture was stirred at 0 °C for 1 h, then allowed to warm to 23 °C and stirred for an additional 18 h. The reaction mixture was quenched with 10% aqueous lithium chloride solution (20 mL), then was extracted with dichloromethane (3 × 25 mL). The combined organic layers were dried over anhydrous sodium sulfate and the dried solution was concentrated. Purification of the residue by flash column chromatography (50% ethyl acetate–hexanes) afforded the glycolamide **18** (2.1 mg, 49% over two steps) as a colorless oil.

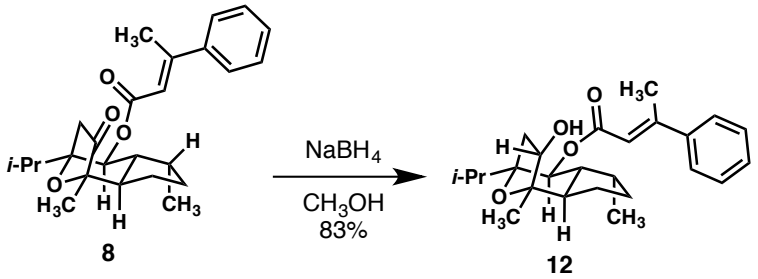
Glycolamide **18** (**NSC#768544**): TLC: 50% ethyl acetate–hexanes, R_f = 0.35 (CAM). ^{1}H NMR (600 MHz, $CDCl_3$) δ: 6.89 (dd, J_1 = 15.8, J_2 = 6.7 Hz, 1H), 6.45 (d, J = 9.6 Hz, 1H), 5.71 (d, J = 15.8 Hz, 1H), 5.05 (d, J = 10.3 Hz, 1H), 4.48 - 4.38 (m, 1H), 4.13 (s, 2H), 2.65 (dd, J_1 = 14.3, J_2 =9.0 Hz, 1H), 2.15 – 2.05 (m, 2H), 2.00 - 1.98 (m, 1H), 1.86 – 1.79 (m, 1H), 1.79 -1.73 (m, 4H), 1.72 – 1.64 (m, 2H), 1.64 – 1.57 (m, 2H), 1.49 (dd, J_1 14.4, J_2 =4.0 Hz, 1H), 1.35 – 1.20 (m, 6H), 1.16 (s, 3H), 1.16 – 1.11 (m, 2H), 1.00 (d, J = 6.8 Hz, 3H), 0.93 (d, J = 7.0 Hz, 3H), 0.91 (d, J = 7.1 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$), δ: 170.3, 165.9, 155.1, 118.9, 85.3, 84.8, 71.2, 62.3, 50.9, 48.3, 46.8, 40.9, 40.6, 33.4, 31.8, 31.3, 31.1, 26.1, 25.9, 24.6, 20.1, 18.4, 17.6, 17.1. FTIR

(NaCl, thin film), cm^{-1}: 3358, 2926, 2855, 1719, 1653. HRMS: APCI $[M + H]^+$ Calcd. for $C_{26}H_{42}NO_5$: 448.3063. Found: 448.3051.



(*E*)-3-phenylbut-2-enoic acid (129 mg, 0.793 mmol, 2.0 equiv), triethylamine (0.17 mL, 1.2 mmol, 3.0 equiv), 2,4,6-trichlorobenzoyl chloride (0.15 mL, 0.99 mmol, 2.5 equiv), and 4-dimethylaminopyridine (10 mg, 79 μmol, 0.2 equiv) were added sequentially to a solution of **3** (0.100 mg, 0.396 mmol, 1.00 equiv) in toluene (50 mL). The reaction mixture was stirred at 23 °C for 2 d, then excess acid chloride was quenched by the addition of 1N aqueous hydrochloric acid solution (20 mL). The layers were separated and the aqueous layer was extracted with dichloromethane (3 × 20 mL). The combined organic layers were washed with saturated aqueous sodium bicarbonate solution (20 mL), dried over anhydrous sodium sulfate, and the dried solution was concentrated. The resulting residue was purified by flash column chromatography (5% ethyl acetate–hexanes) to afford **8** (146 mg, 93%) as a pale yellow oil.

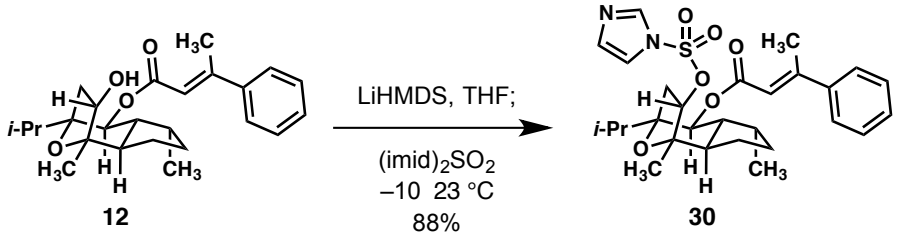
Ketoester **8**: TLC: 20% ethyl acetate–hexanes, R_f = 0.57 (UV, CAM). ^{1}H NMR (500 MHz, $CDCl_3$), δ: 7.48–7.47 (m, 2H), 7.38–7.37 (m, 3H), 6.06 (s, 1H), 5.34 (d, *J* = 11.0 Hz, 1H), 2.58 (s, 3H), 2.49 (ab, 1H), 2.15–2.11 (m, 1H), 2.00–1.93 (m, 1H), 1.91–1.78 (m, 2H), 1.68–1.63 (m, 1H), 1.55–1.49 (m, 1H), 1.26 (s, 3H), 1.22–1.19 (m, 2H), 1.05 (d, *J* = 7.0 Hz, 3H), 1.03 (d, *J* = 7.0 Hz, 3H), 0.96 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$), δ: 215.3, 165.2, 156.6, 142.0, 129.2, 128.5, 126.3, 116.7, 83.5, 82.4, 70.0, 48.4, 46.1, 42.7, 33.0, 31.1, 30.7, 23.3, 18.1, 17.9, 17.5, 16.9. FTIR (NaCl, thin film), cm^{-1}: 2955, 2925, 1756, 1717, 1153. HRMS: APCI $[M + H]^+$ Calcd. for $C_{25}H_{33}O_4$: 397.2373. Found: 397.2393.



8 12

Sodium borohydride (5.2 mg, 0.14 mmol, 3.0 equiv) was added to a solution of **8** (18 mg, 45 μmol, 1.0 equiv) in methanol (25 mL) at 0 °C. The resultant mixture was stirred at 0 °C for 30 min, and then the excess sodium borohydride was quenched by the addition of saturated aqueous ammonium chloride solution (25 mL). The mixture was extracted with dichloromethane (3 × 20 mL), the combined organic layers were dried over anhydrous sodium sulfate, and the dried solution was concentrated. Purification of the residue by flash column chromatography (20% ethyl acetate–hexanes) afforded **12** (15 mg, 83%) as a colorless oil.

Hydroxyester **12**: TLC: 20% ethyl acetate–hexanes, R_f = 0.30 (UV, CAM). ^{1}H NMR (500 MHz, $CDCl_3$), δ: 7.49–7.47 (m, 2H), 7.39–7.35 (m, 3H), 6.08 (s, 1H), 5.19 (d, J = 10.0 Hz, 1H), 4.17 (dd, J_1 = 11.0 Hz, J_2 = 5.0 Hz, 1H), 2.58 (s, 3H), 2.36–2.30 (m, 2H), 2.19–2.15 (m, 1H), 2.03 (dd, J_1 = 14.0 Hz, J_2 = 5.0 Hz, 1H), 1.99–1.93 (m, 1H), 1.85–1.78 (m, 3H), 1.69–1.62 (m, 1H), 1.32 (s, 3H), 1.22–1.19 (m, 1H), 0.98–0.95 (m, 3H). ^{13}C NMR (125 MHz, $CDCl_3$), δ: 215.3, 165.2, 156.6, 142.0, 129.2, 128.5, 126.3, 116.7, 83.5, 82.4, 70.0, 48.4, 46.1, 42.7, 33.0, 31.1, 30.7, 23.3, 18.1, 17.9, 17.5, 16.9. FTIR (NaCl, thin film), cm^{-1}: 3447, 2964, 2926, 1714, 1626, 1165. HRMS: APCI $[M + H]^+$ Calcd. for $C_{25}H_{35}O_4$: 399.2535. Found: 399.2519.

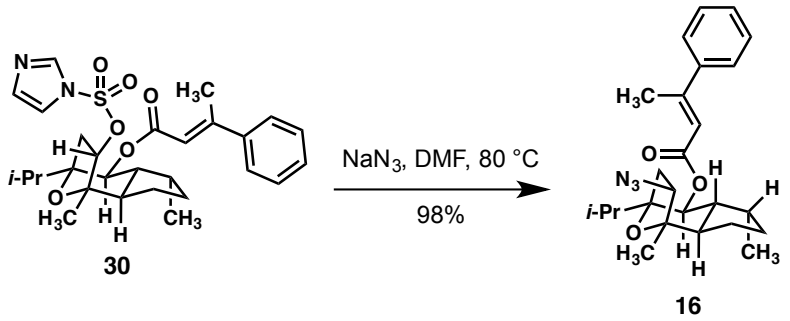


12 30

A solution of *n*-butyllithium (2.50 M in hexanes, 84.0 μL, 0.21 mmol, 7.00 equiv) was added to a stirred solution of hexamethyldisilazane (49 μL, 0.23 mmol, 7.7 equiv) in tetrahydrofuran (5 mL) at 0 °C. The reaction mixture was briefly warmed to 23 °C, then was cooled to 0 °C whereupon a solution of **12** (12 mg, 30 μmol, 1.0 equiv) in tetrahydrofuran (2 mL) was added. The resultant mixture was stirred at 0 °C for 30 min, then was cooled to –10 °C whereupon *N*,*N*'-

sulfuryldiimidazole (54 mg, 0.27 mmol, 9.0 equiv) was added. The reaction mixture was warmed to 23 °C and stirred at that temperature for 12 h. Excess *N*,*N*'-sulfuryldiimidazole was quenched by the addition of methanol (5 mL), and the resultant mixture was concentrated. The residue was partitioned between saturated aqueous bicarbonate solution (20 mL) and dichloromethane (20 mL). The layers were separated and the aqueous phase was extracted with dichloromethane (3 × 25 mL). The combined organic layers were dried over anhydrous sodium sulfate and the dried solution was concentrated. The residue was purified by flash column chromatography (20% ethyl acetate–hexanes) afforded **30** (14 mg, 88%) as a colorless oil.

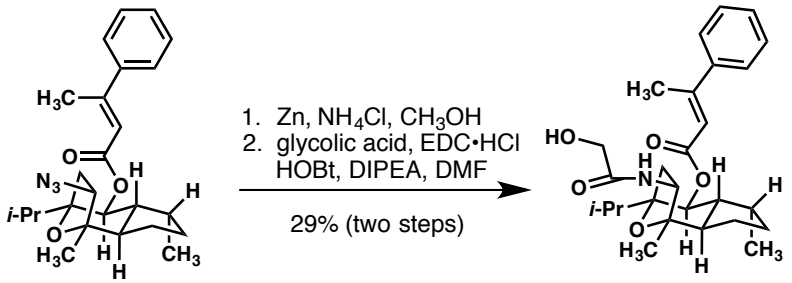
Imidazole **30**: TLC: 20% ethyl acetate–hexanes, R_f = 0.33 (UV, CAM). ^{1}H NMR (500 MHz, $CDCl_3$), δ: 8.01 (s, 1H), 7.49–7.47 (m, 3H), 7.39–7.35 (m, 4H), 7.22 (s, 1H), 6.06 (s, 1H), 5.16 (d, J = 10.5 Hz, 1H), 4.57 (dd, J_1 = 11.0 Hz, J_2 = 4.5 Hz, 1H), 2.57 (s, 3H), 2.27–2.16 (m, 2H), 2.12–2.08 (dd, J_1 = 14.5 Hz, J_2 = 4.5 Hz, 1H), 2.04–1.94 (m, 2H), 1.89–1.83 (m, 1H), 1.76–1.67 (m, 3H), 1.23–1.22 (m, 1H), 1.19 (s, 3H), 0.95 (d, J = 7.0 Hz, 3H), 0.93 (d, J = 6.5 Hz, 3H), 0.92 (d, J = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$), δ: 165.2, 156.5, 142.0, 137.1, 131.6, 129.2, 128.5, 126.3, 117.9, 116.8, 90.5, 85.3, 81.1, 70.4, 48.8, 46.4, 35.7, 32.5, 31.1, 29.7, 24.0, 22.5, 18.1, 17.4, 16.8. FTIR (NaCl, thin film), cm^{-1}: 2925, 2854, 1718, 1627, 1423, 1203, 1158. HRMS: ESI $[M + H]^+$ Calcd. for $C_{28}H_{37}N_2O_6S$: 529.2367. Found: 529.2346.



Sodium azide (44.0 mg, 0.530 mmol, 20.0 equiv) was added in one portion to a solution of **30** (14 mg, 27 μmol, 1.0 equiv) in *N*,*N*-dimethylformamide (5 mL). The reaction mixture was heated at 80 °C for 2 d, and then quenched by the addition of 10% aqueous lithium chloride solution (20 mL). The mixture was extracted with dichloromethane (3 × 25 mL), the combined organic layers were dried over anhydrous sodium sulfate, and the dried solution was concentrated.

Purification of the residue by flash column chromatography (5% ethyl acetate–hexanes) afforded the azide **16** (11 mg, 98%) as a colorless oil.

Azide **16**: TLC: 20% ethyl acetate–hexanes, R_f = 0.79 (UV, CAM). ^{1}H NMR (500 MHz, $CDCl_3$), δ: 7.49–7.45 (m, 2H), 7.38–7.37 (m, 3H), 6.06 (s, 1H), 5.09 (d, J = 10.5 Hz, 1H), 3.61 (dd, J_1 = 8.5 Hz, J_2 = 3.5 Hz, 1H), 2.58 (s, 3H), 2.54 (dd, J_1 = 14.5 Hz, J_2 = 8.5 Hz, 2H), 2.16–2.12 (m, 1H), 2.07–1.99 (m, 2H), 1.92–1.89 (m, 3H), 1.81–1.68 (m, 2H), 1.47–1.41 (m, 1H), 1.33 (s, 3H), 1.03 (d, J = 7.0 Hz, 3H), 0.98 (d, J = 7.0 Hz, 3H), 0.95 (d, J = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$), δ: 165.3, 156.1, 142.2, 129.1, 128.5, 126.3, 117.1, 85.6, 85.5, 70.7, 63.4, 48.2, 47.1, 38.6, 32.9, 31.2, 29.7, 24.8, 20.2, 18.2, 17.5, 17.0. FTIR (NaCl, thin film), cm^{-1}: 2927, 2855, 2094, 1717, 1626, 1270, 1162. HRMS: APCI [M + H]$^+$ Calcd. for $C_{25}H_{34}N_3O_3$: 424.2600. Found: 424.2613.

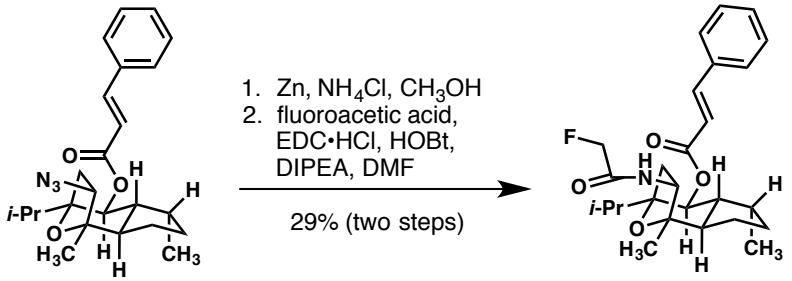


16 **19 (NSC#778312)**

Ammonium chloride (16 mg, 0.31 mmol, 10 equiv) and zinc (dust, 20 mg, 0.31 mmol, 10 equiv) were added sequentially to a solution of **16** (13 mg, 31 μmol, 1.0 equiv) in methanol (3 mL). The reaction mixture was stirred vigorously at room temperature for 2 h, then was concentrated. The resulting residue was triturated with diethyl ether (3 × 10 mL) and the combined organic fractions were filtered through a pad of silica gel to afford the amine (8.0 mg, 66%), which was advanced without further purification. Glycolic acid (23 mg, 0.31 mmol, 10 equiv), *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (59 mg, 0.31 mmol, 10.0 equiv), and 1-hydroxybenzotriazole (41 mg, 0.31 mmol, 10 equiv) were added sequentially to a stirred solution of the amine intermediate in *N*,*N*-dimethylformamide (15 mL) at 23 °C. The reaction mixture was cooled to 0 °C whereupon *N*,*N*-diisopropylamine (0.16 mL, 0.92 mmol, 30.0 equiv) was added. The reaction mixture was stirred at 0 °C for 1 h, then allowed to warm to 23 °C and stirred for an additional 18 h. The reaction mixture was quenched with 10% aqueous lithium chloride solution (20 mL), then extracted with dichloromethane (3 × 25 mL). The combined organic layers were dried over anhydrous sodium sulfate and the dried solution was

concentrated. Purification of the residue by flash column chromatography (50% ethyl acetate–hexanes) afforded the glycolamide **19** (4.0 mg, 29% over two steps) as a colorless oil.

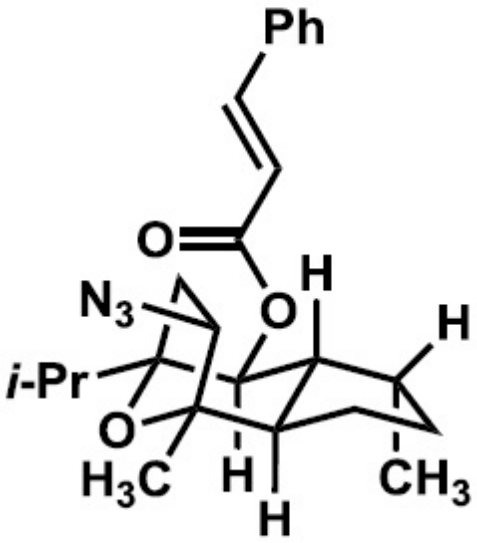
Glycolamide **19** (**NSC#778312**): TLC: 50% ethyl acetate–hexanes, R_f = 0.35 (UV, CAM). ^{1}H NMR: (500 MHz, $CDCl_3$), δ 6.89 (dd, J_1 = 15.5 Hz, J_2 = 7.0 Hz, 1H), 6.42 (d, J = 10.0 Hz, 1H), 5.71 (d, J = 15.5 Hz, 1H), 5.03 (d, J = 10.5 Hz, 1H), 4.46–4.41 (m, 1H), 4.14 (s, 2H), 2.65 (dd, J_1 = 14.5 Hz, J_2 = 9.0 Hz, 1H), 2.13–2.10 (m, 2H), 2.04–2.00 (m, 4H), 2.04–1.93 (m, 6H), 1.84–1.79 (m, 2H), 1.77–1.75 (m, 5H), 1.16 (s, 3H), 1.14–1.09 (m, 1H), 1.00 (d, J = 7.0 Hz, 3H), 0.93 (d, J = 7.0 Hz, 3H), 0.91 (d, J = 7.0 Hz, 3H); ^{13}C NMR: (125 MHz, $CDCl_3$), δ 170.1, 166.3, 155.0, 118.7, 85.1, 84.7, 71.0, 62.1, 50.8, 48.1, 46.7, 40.7, 40.4, 33.2, 31.9, 31.7, 30.9, 29.7, 25.9, 25.7, 24.5, 22.7, 20.0, 18.2, 17.5, 16.9. FTIR (NaCl, thin film), cm^{-1}: 3397, 2965, 2925, 2854, 1717, 1653. HRMS: APCI $[M + H]^+$ Calcd. for $C_{27}H_{38}NO_5$: 456.2750. Found: 456.2763.



Ammonium chloride (26 mg, 0.49 mmol, 10 equiv) and zinc (dust, 32 mg, 0.49 mmol, 10 equiv) were added sequentially to a solution of **13** (20 mg, 49 μmol, 1.0 equiv) in methanol (3 mL). The reaction mixture was stirred vigorously at room temperature for 2 h, then was concentrated. The resulting residue was triturated with diethyl ether (3 × 10 mL) and the combined organic fractions were filtered through a pad of silica gel to afford the amine, which was advanced without further purification. Fluoroacetic acid (38 mg, 0.49 mmol, 10 equiv), *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (94 mg, 0.49 mmol, 10.0 equiv), and 1-hydroxybenzotriazole (66 mg, 0.49 mmol, 10 equiv) were added sequentially to a stirred solution of the amine intermediate in *N*,*N*-dimethylformamide (15 mL) at 23 °C. The reaction mixture was cooled to 0 °C whereupon *N*,*N*-diisopropylamine (0.26 mL, 1.47 mmol, 30.0 equiv) was added. The reaction mixture was stirred at 0 °C for 1 h, then allowed to warm to 23 °C and stirred for an additional 18 h. The reaction mixture was quenched with 10% aqueous lithium chloride solution (20 mL), then extracted with dichloromethane (3 × 25 mL). The combined organic layers were dried over anhydrous sodium sulfate and the dried solution was

concentrated. Purification of the residue by flash column chromatography (50% ethyl acetate–hexanes) afforded the glycolamide **20** (6.3 mg, 29% over two steps) as a colorless oil.

Glycolamide **20** (**NSC#777412**): TLC: 20% ethyl acetate–hexanes, R_f = 0.16 (UV, CAM). ^{1}H NMR: (500 MHz, $CDCl_3$), δ: 7.66 (d, *J* = 16.0 Hz, 1H), 7.54-7.52 (m, 2H), 7.40-7.38 (m, 3H), 6.40 (d, *J* = 16.0 Hz, 1H), 6.30-6.28 (m, 1H), 5.15 (d, *J* = 10.5 Hz, 1H), 4.87 (s, 1H), 4.78 (s, 1H), 4.51-4.46 (m, 1H), 2.73 (dd, J_1 = 14.5 Hz, J_2 = 9.0 Hz, 1H), 2.17-2.13 (m, 1H), 1.97-1.93 (m, 1H), 1.90-1.78 (m, 2H), 1.75-1.63 (m, 2H), 1.38-1.29 (m, 2H), 1.27-1.23 (m, 5H), 1.19 (s, 3H), 1.03 (d, *J* = 6.5 Hz, 3H), 0.97 (d, *J* = 7.0 Hz, 3H), 0.94 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR: (101 MHz, $CDCl_3$), δ: 166.8 (d, *J* = 17.0 Hz), 165.8, 145.4, 134.4, 130.6, 129.0, 128.3, 118.1, 85.3, 84.8, 80.3 (d, *J* = 186.2 Hz), 71.5, 50.8, 48.3, 46.9, 40.9, 33.4, 31.3, 31.1, 29.5, 24.7, 22.9, 20.1, 18.4, 17.6, 17.1. ^{19}F NMR: (376 MHz, $CDCl_3$), δ: –182.4 (d, *J* = 145.0 Hz). FTIR (NaCl, thin film), cm^{-1}: 2955, 2924, 1710, 1637, 1169. HRMS: APCI $[M + H]^+$ Calcd. for $C_{26}H_{35}FNO_4$: 444.2550. Found: 444.2570.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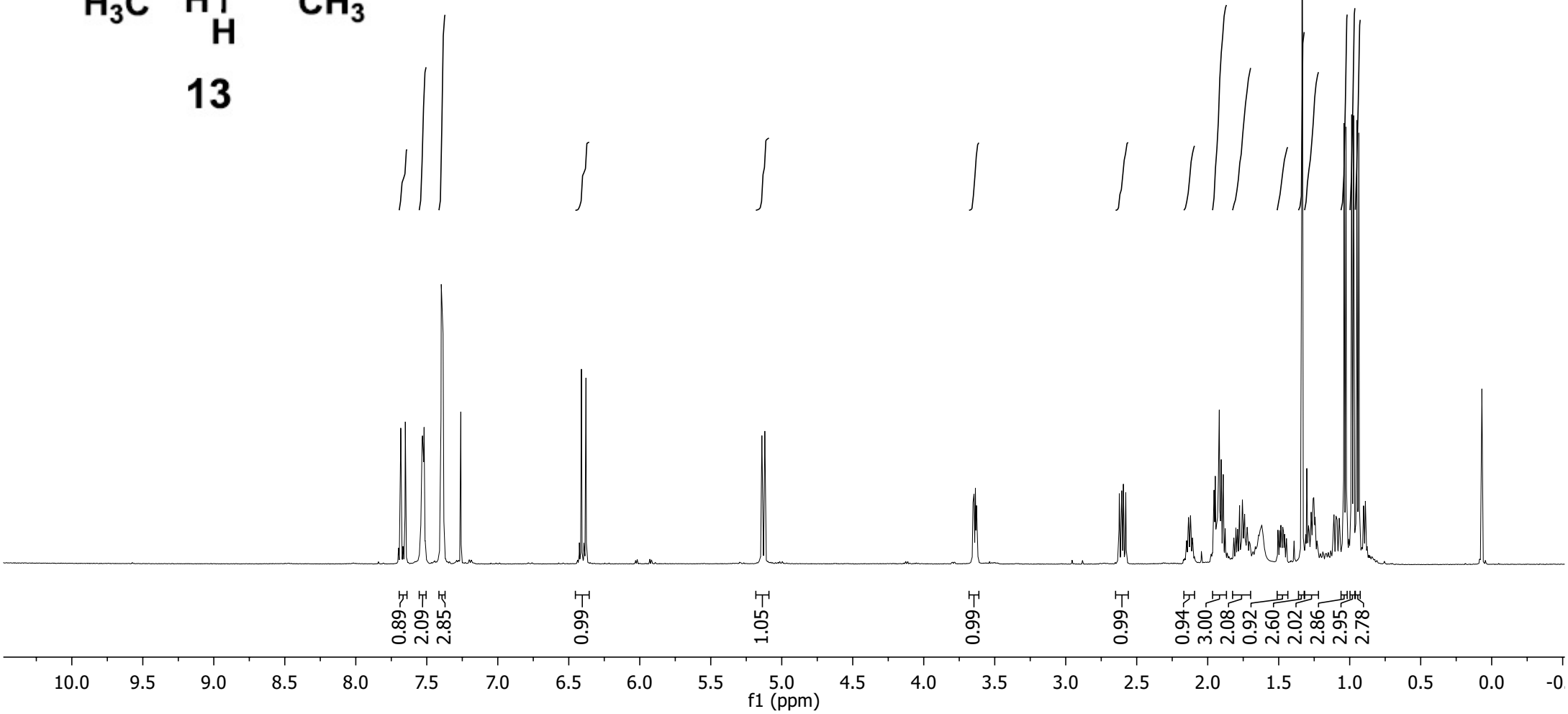
H
N3
H
i-Pr
O
H3C
H
H
CH3

13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8.5
8.0
7.5
7.0
6.5
6.0
5.5
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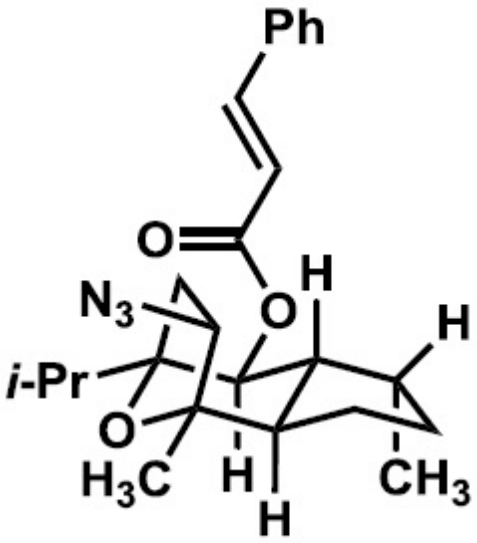
f1 (ppm)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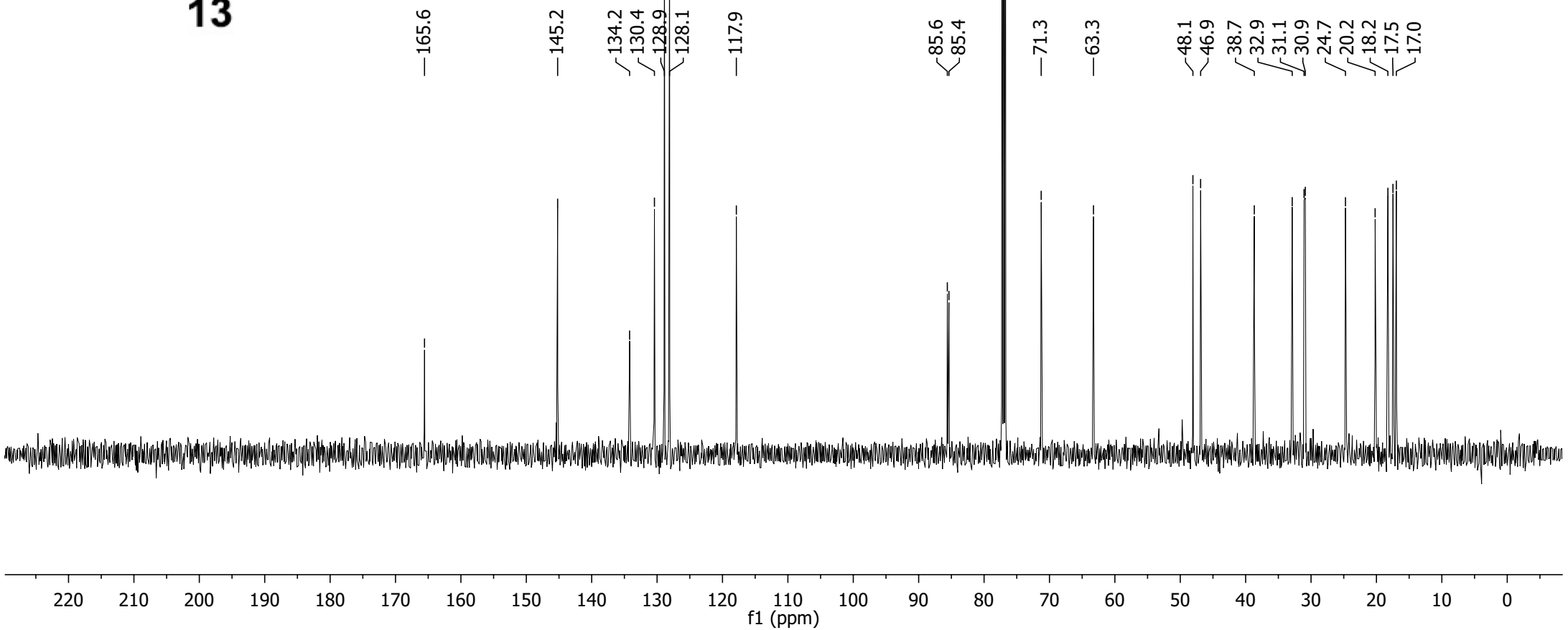
N3
H
i-Pr
O
H3C
H
H
CH3

13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128.1
117.9
85.6
85.4
71.3
63.3
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46.9
38.7
32.9
31.1
30.9
24.7
20.2
18.2
17.5
17.0
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210
200
190
180
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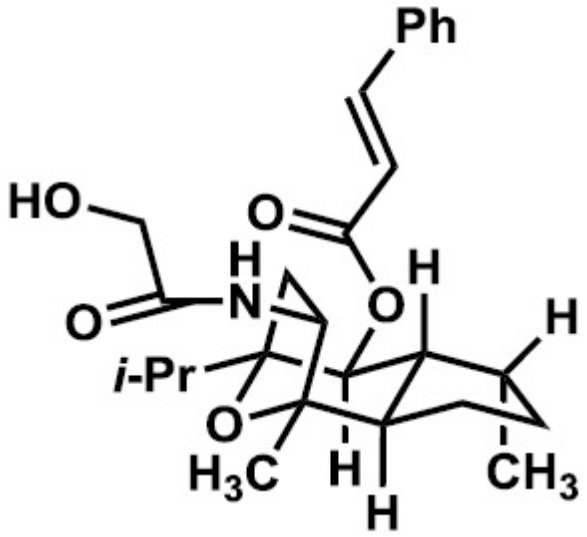
f1 (ppm)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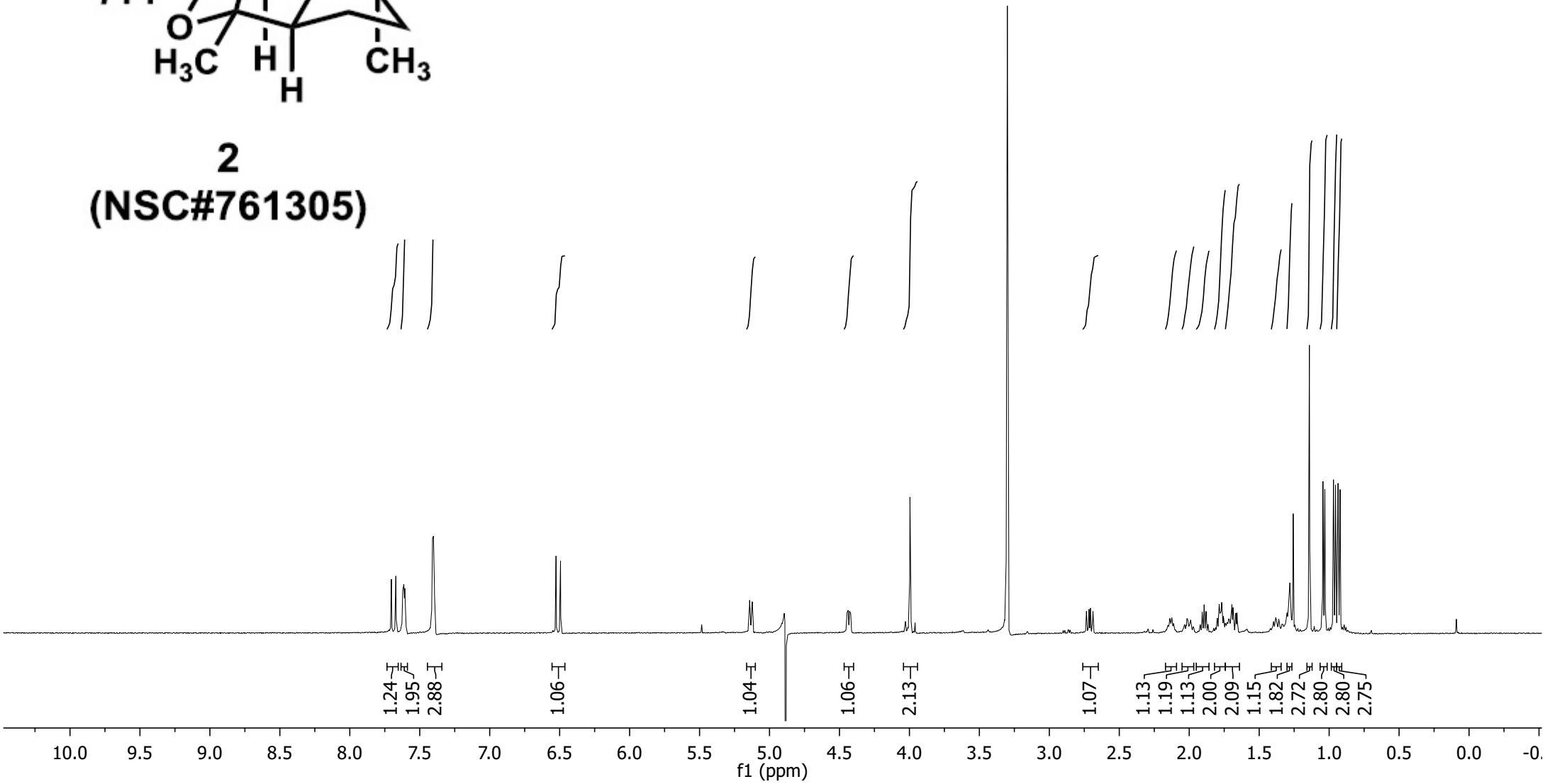
O
H
O
H
i-Pr
O
H3C
H
CH3
H

2

(NSC#761305)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1.19
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2.09
1.15
1.82
2.72
2.80
2.80
2.75
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9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
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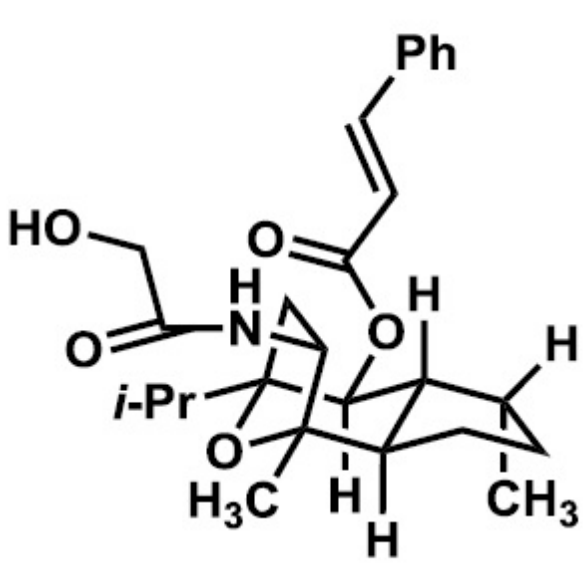
f1 (ppm)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H
N
O
O
H
H
i-Pr
O
H3C
H
CH3
H

2

(NSC#761305)

174.1
167.3
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135.7
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129.3
118.8
86.4
86.2
72.7
62.4
52.0
49.6
47.9
40.9
34.7
32.4
32.0
25.4
20.1
18.7
17.8
17.3
210
200
190
180
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90
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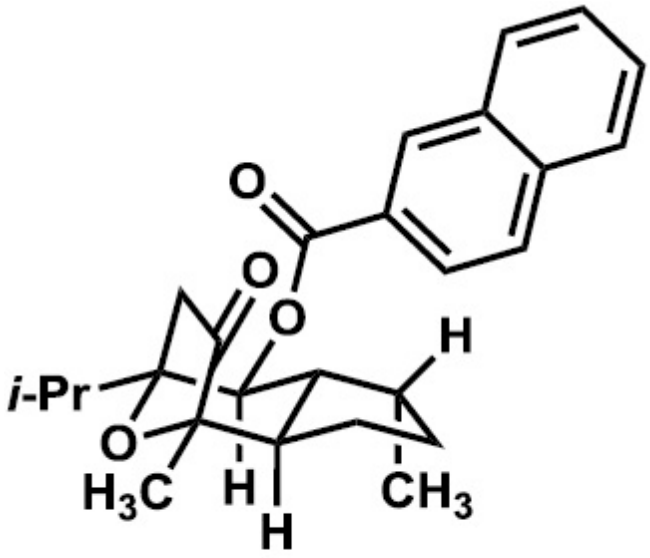
f1 (ppm)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i-Pr
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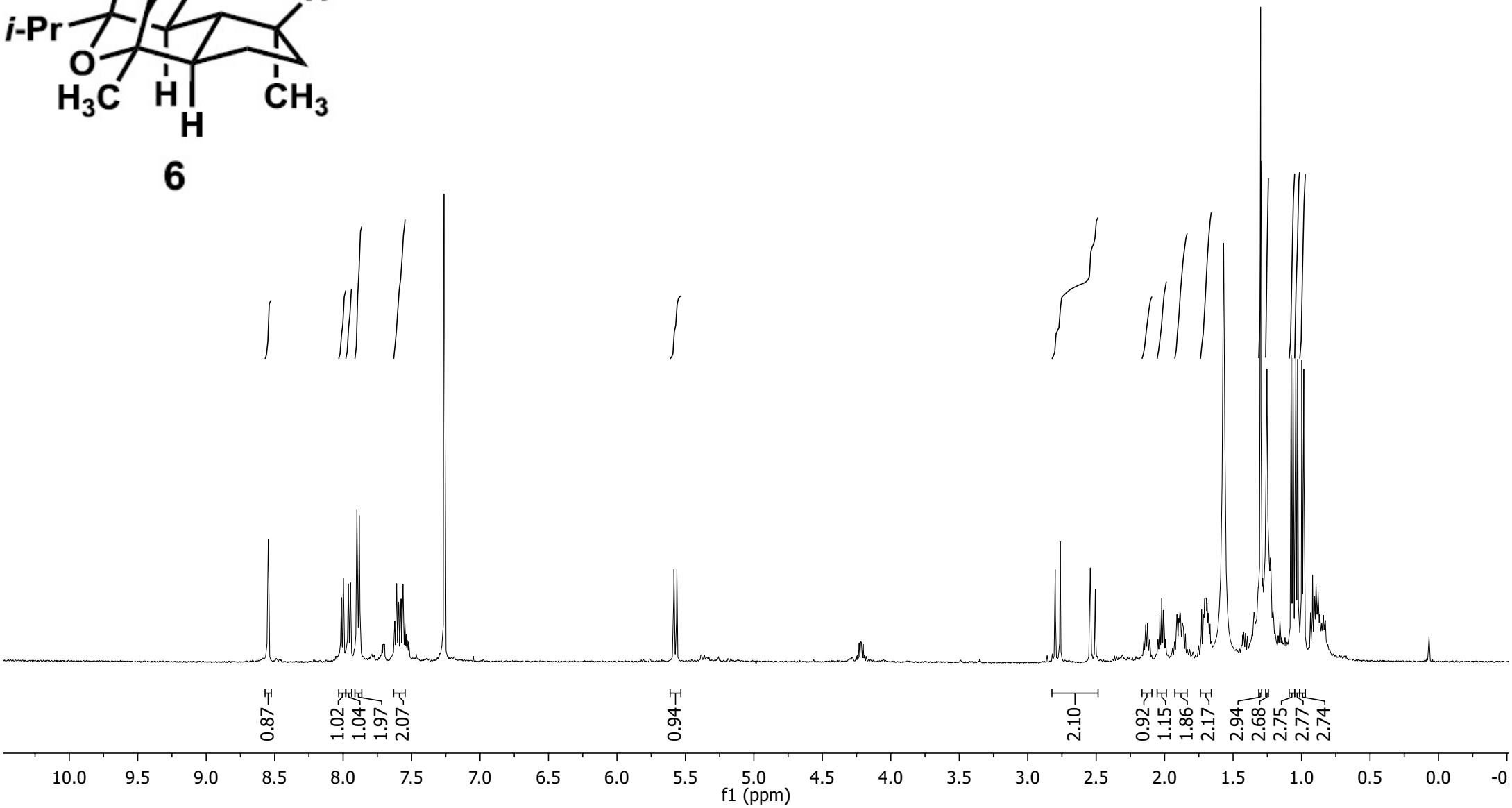
H
H
CH3

6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2.77
2.74
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9.0
8.5
8.0
7.5
7.0
6.5
6.0
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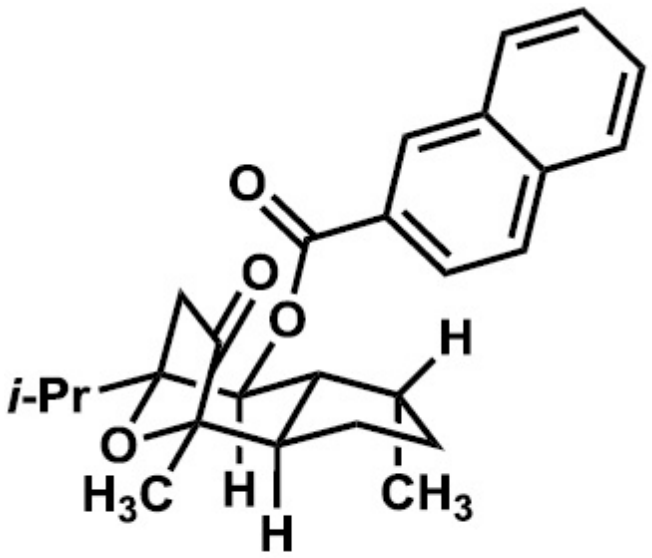
f1 (ppm)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i-Pr
O
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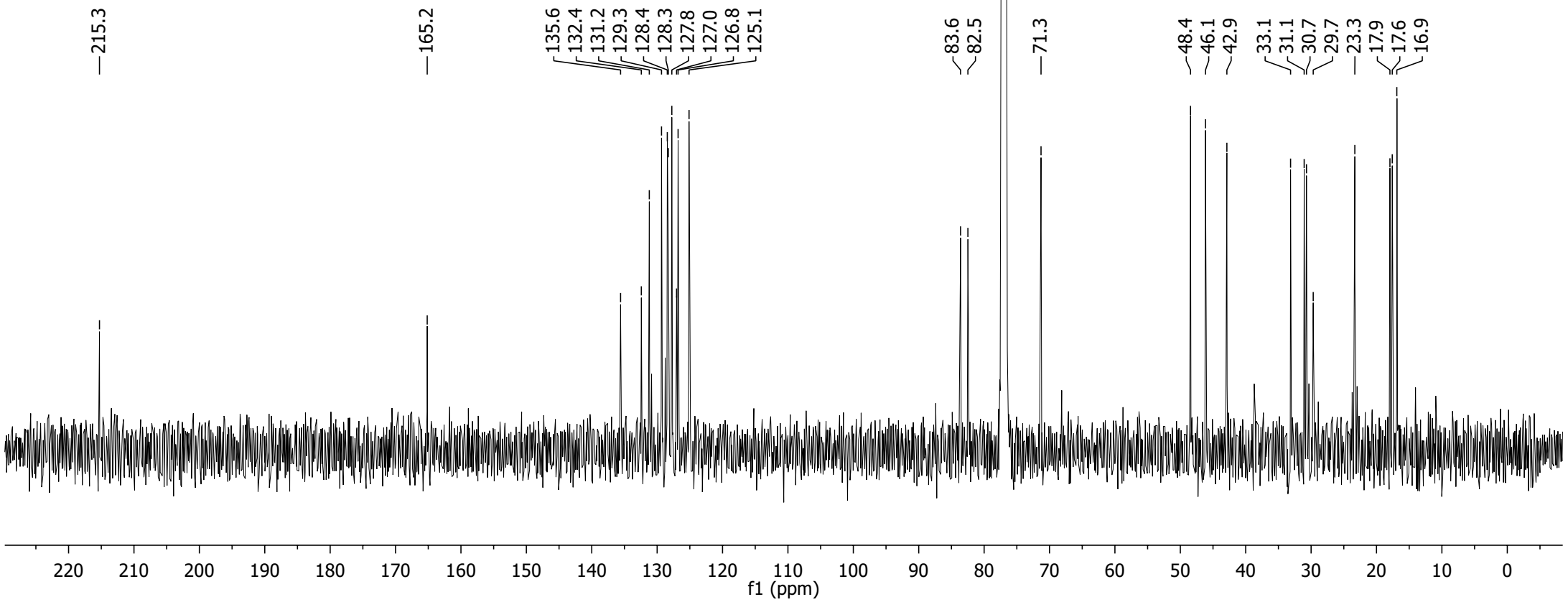
H
H
CH3

6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132.4
131.2
129.3
128.4
128.3
127.8
127.0
126.8
125.1
83.6
82.5
71.3
48.4
46.1
42.9
33.1
31.1
30.7
29.7
23.3
17.9
17.6
16.9
220
210
200
190
180
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130
120
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50
40
30
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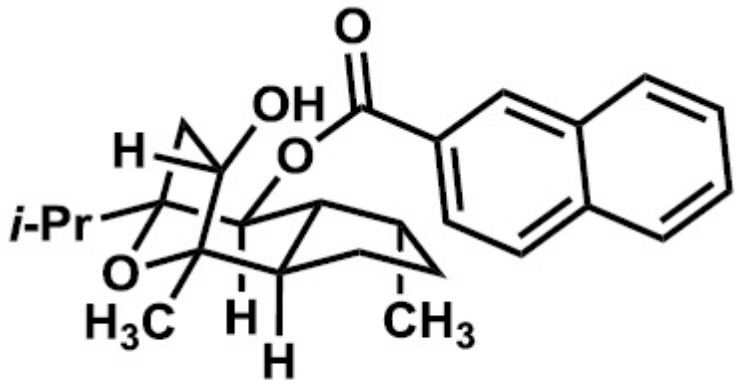
f1 (ppm)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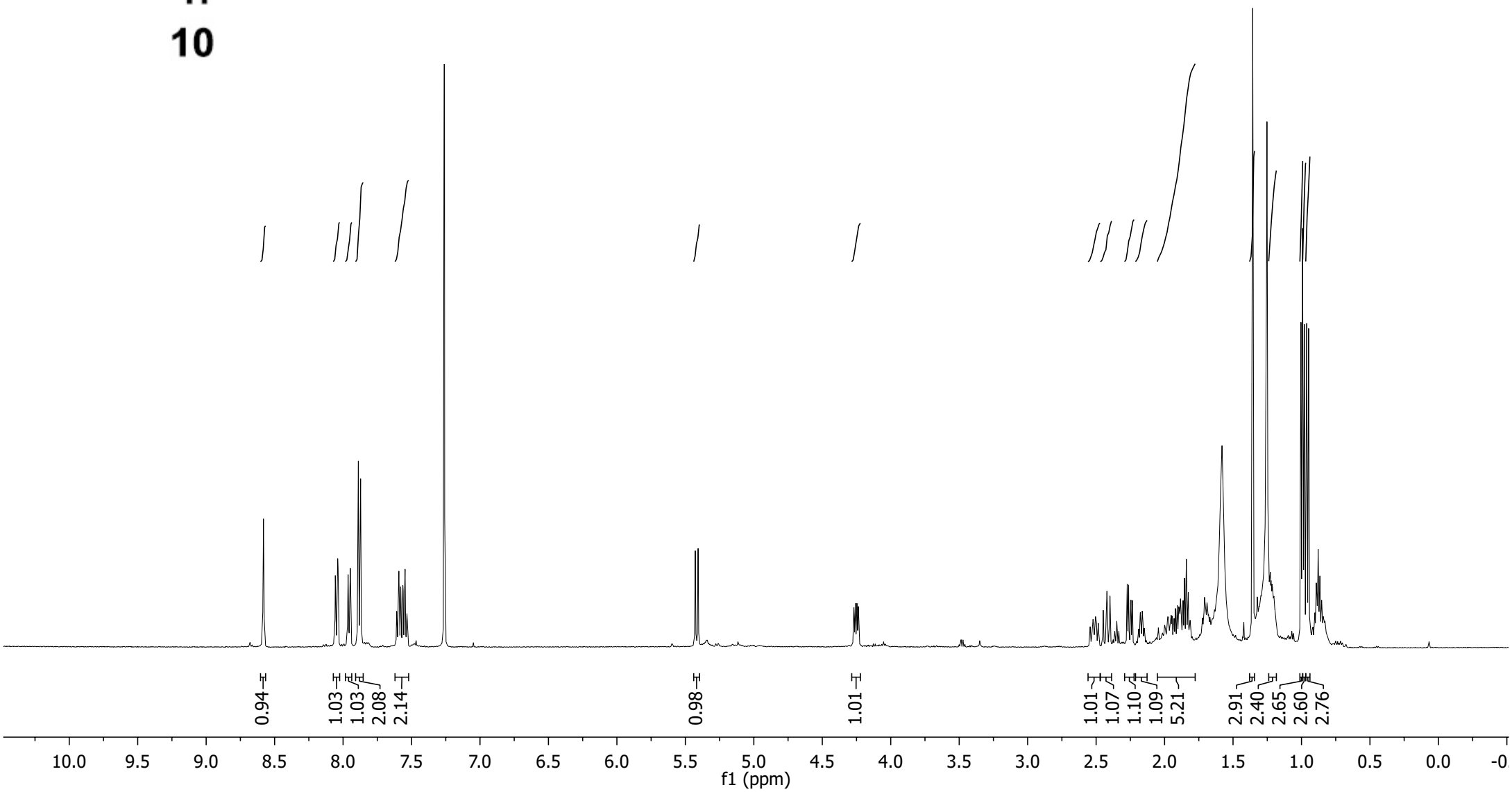
O
i-Pr
O
H3C
H
CH3
H

10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1.01
1.07
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1.09
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2.91
2.40
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2.60
2.76
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9.0
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8.0
7.5
7.0
6.5
6.0
5.5
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3.0
2.5
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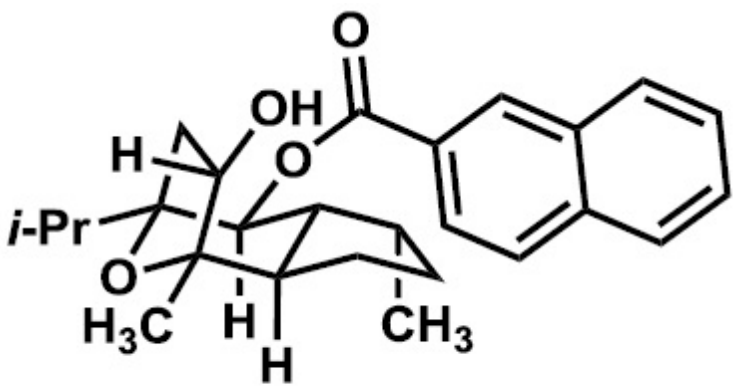
0.0
f1 (ppm)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O
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H
CH3
H

10

165.43
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129.32
128.21
128.11
127.72
127.65
126.63
125.33
84.64
81.44
81.06
72.99
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31.14
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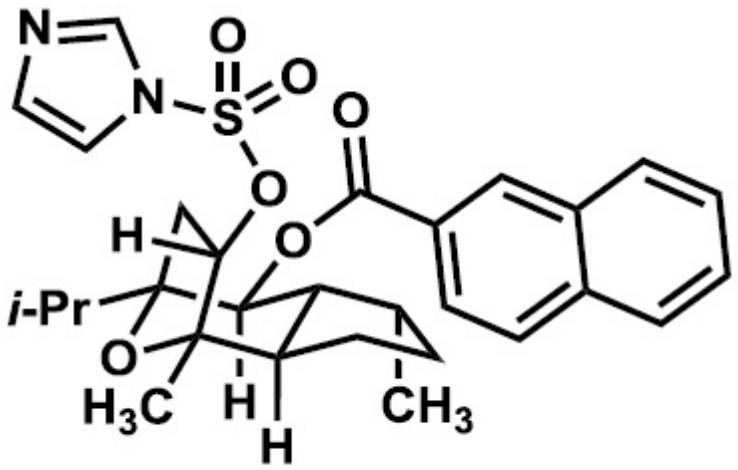
24.40
23.32
17.80
17.03
f1 (ppm)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N
S
O
O
O
H
O
i-Pr
O
H3C
H
CH3
H

28

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1.00
1.95
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1.07
1.06
1.23
1.02
1.14
1.39
2.03
2.09
1.19
2.75
0.81
2.60
2.34
2.34
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9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
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3.5
3.0
2.5
2.0
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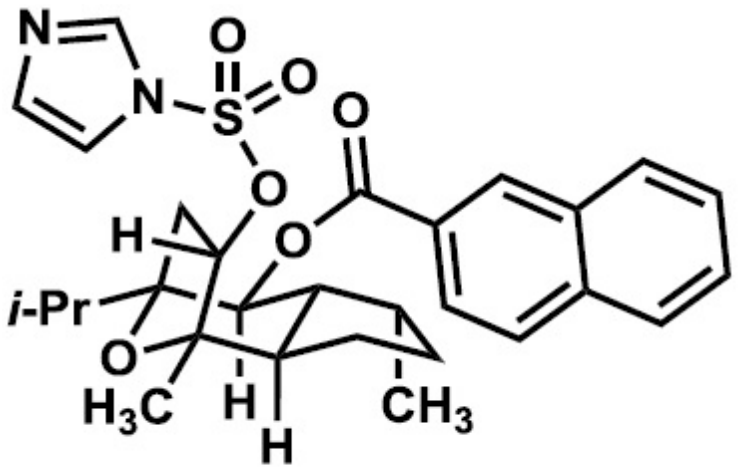
1.0
0.5
0.0
f1 (ppm)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O
N
S
O
O
O
H
O
i-Pr
O
H3C
H
CH3
H

28

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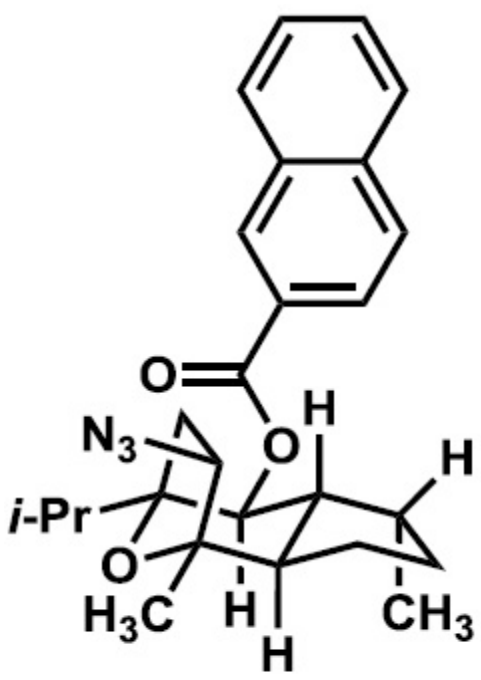
135.6
132.5
131.2
129.4
128.4
128.3
127.8
127.0
126.8
125.2
90.5
85.3
81.2
71.6
48.8
46.4
35.7
32.7
31.2
31.1
29.7
23.9
22.5
17.5
16.8
f1 (ppm)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H
N3
O
H
i-Pr
O
H3C
H
H
CH3

14

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1.05
0.99
0.99
1.06
1.70
2.66
2.54
2.97
3.29
2.94
2.86
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
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2.5
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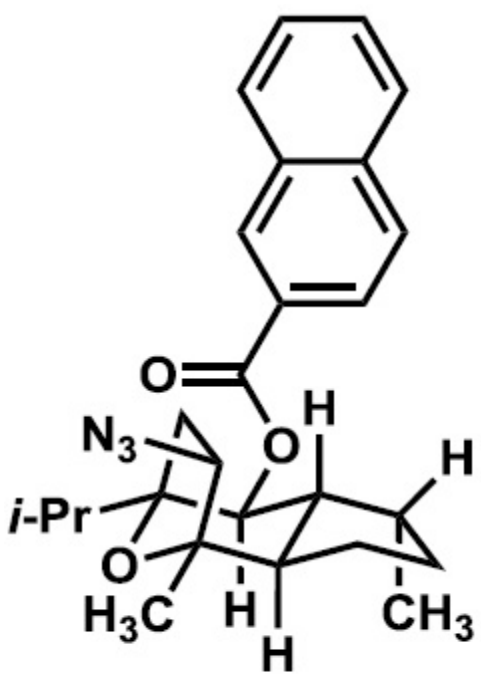
f1 (ppm)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H
N3
O
H
i-Pr
O
H3C
H
H
CH3

14

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135.6
132.5
131.1
129.3
128.4
128.2
127.8
126.7
125.2
85.7
85.5
71.9
63.4
48.2
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29.7
24.8
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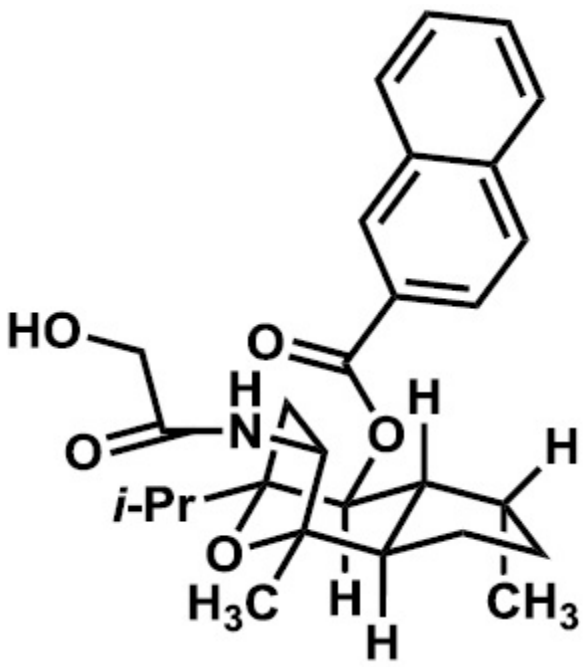
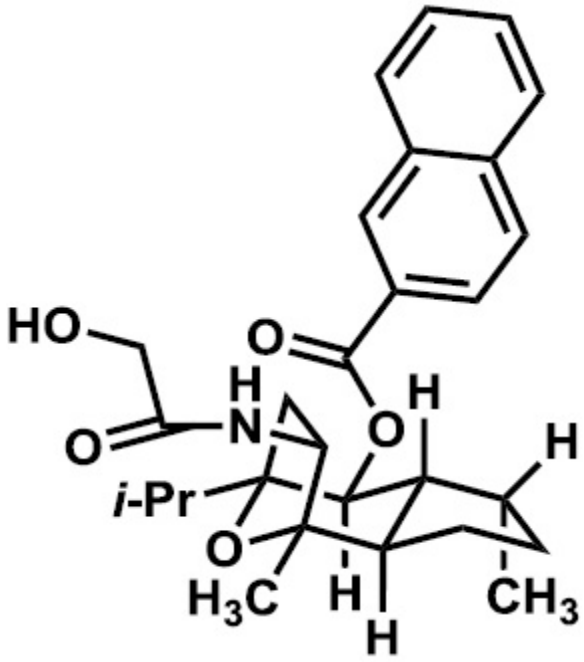
17.5
17.0
f1 (ppm)



17
(NSC#764081)



17

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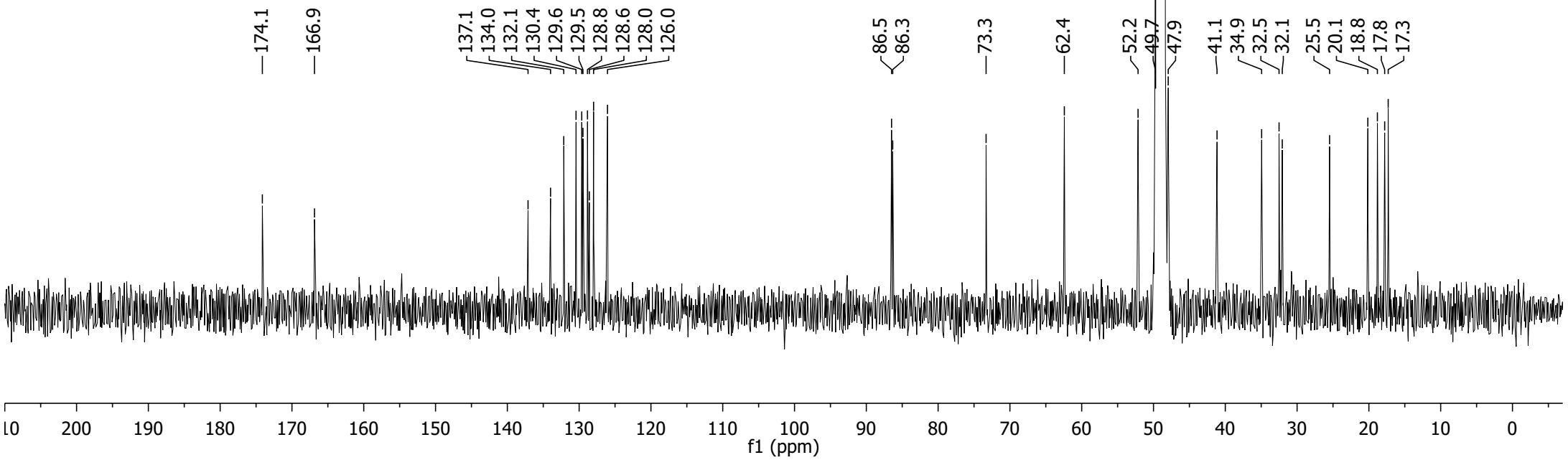

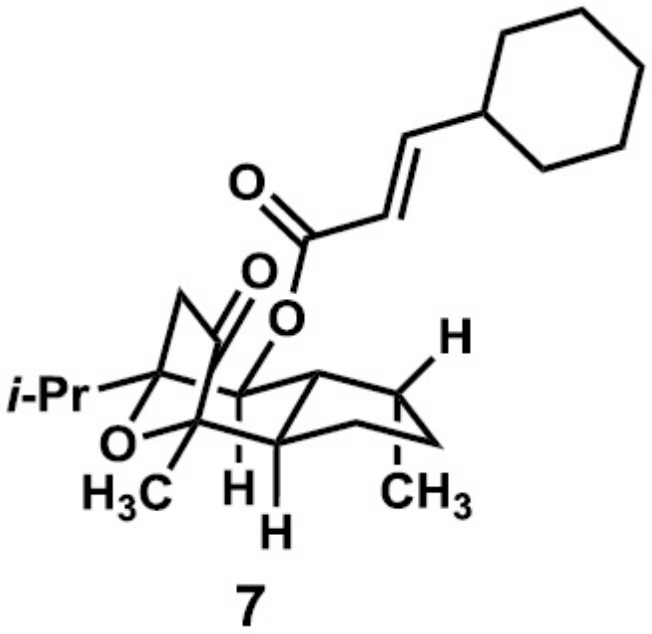
O
O
O
H
i-Pr
O
H3C
H
H
CH3

7

1.08
1.04
1.01
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2.19
1.96
4.75
2.09
1.03
2.40
3.37
4.25
2.74
3.12
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9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
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3.0
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2.0
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1.0
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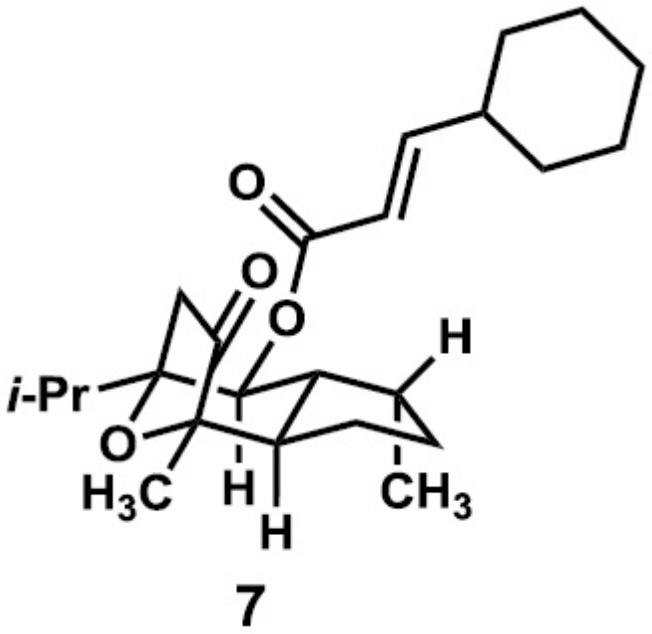
f1 (ppm)


O
O
O
H
i-Pr
O
H3C
H
H
CH3

7

215.4
165.6
155.4
118.4
83.5
82.4
70.3
48.3
46.0
42.7
40.5
33.0
31.6
31.0
30.7
30.3
29.7
25.9
25.7
23.2
17.9
17.5
16.9
220
210
200
190
180
170
160
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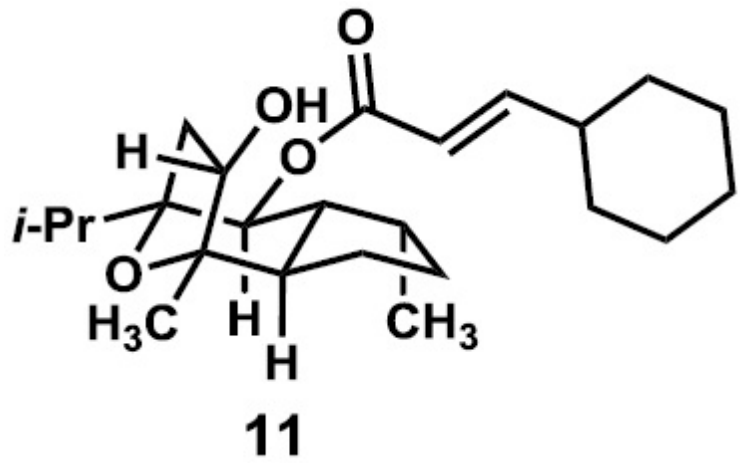
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


O
OH
H
O
i-Pr
O
H3C
H
CH3
H

11

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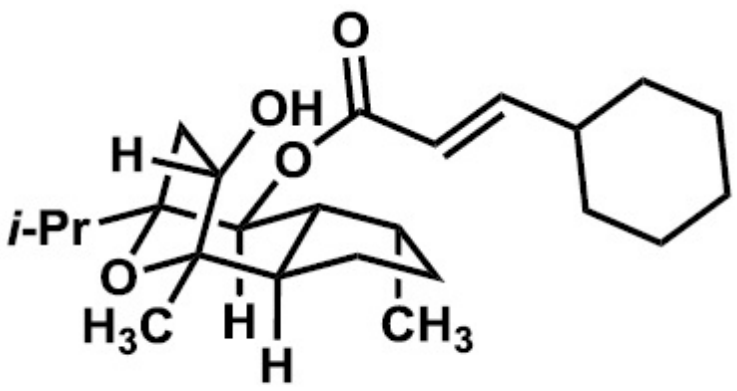
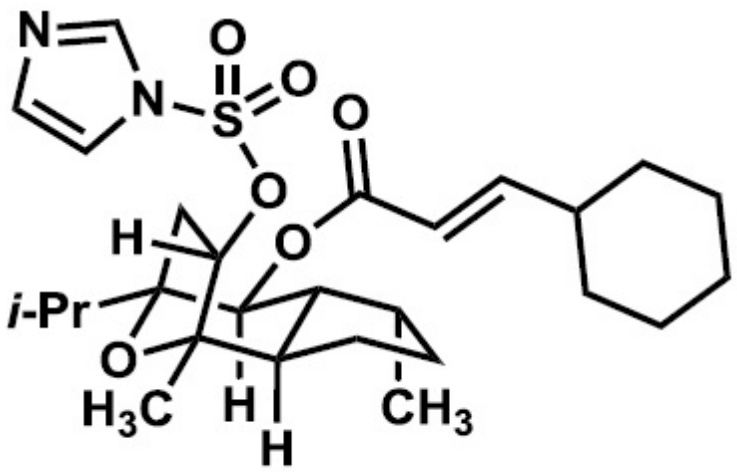
1.01
1.04
1.15
1.79
1.71
1.16
6.31
2.64
3.03
4.35
2.54
3.47
3.36
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


O
OH
H
O
i-Pr
O
H3C
H
CH3
H

11

165.9
154.5
118.9
84.5
81.0
72.0
54.3
49.0
46.2
40.4
39.3
32.8
32.2
31.7
31.4
31.1
25.9
25.7
24.3
23.3
20.3
17.7
16.9
f1 (ppm)


N
O
N
S
O
O
O
H
O
i-Pr
O
H3C
H
CH3
H

29

0.95
1.02
1.02
1.03
1.10
1.15
1.04
1.27
3.71
2.14
1.37
4.77
3.92
3.56
11.96
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
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1.0
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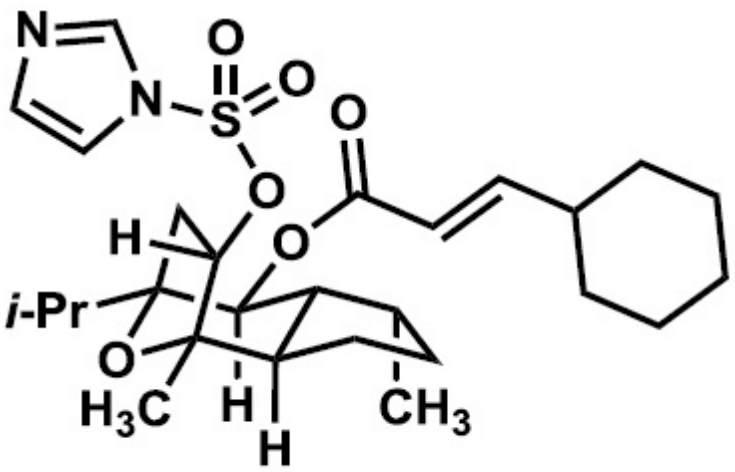
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O
N
S
O
O
O
H
O
i-Pr
O
H3C
H
CH3
H

29

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155.3
131.6
118.4
90.5
85.2
81.0
70.6
48.7
46.2
40.5
35.6
32.5
31.9
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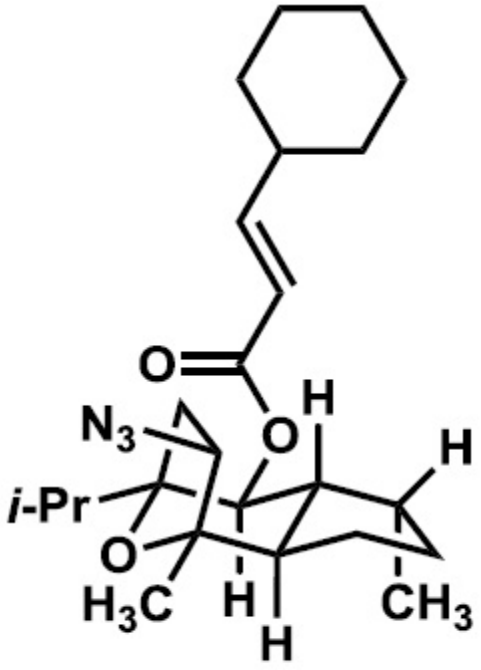
f1 (ppm)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H
H
CH3

15

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1.13
2.83
4.00
6.16
1.94
2.65
0.66
3.15
3.50
3.57
2.99
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
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3.5
3.0
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2.0
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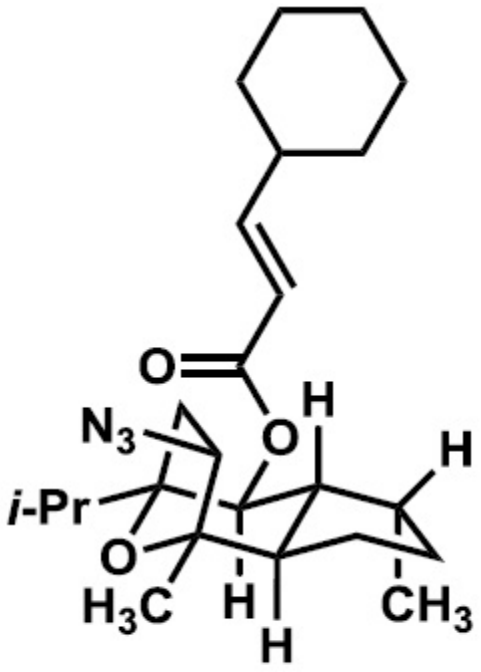
1.0
0.5
0.0
f1 (ppm)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H
N3
O
H
i-Pr
O
H3C
H
H
CH3

15

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155.0
118.6
85.6
85.4
70.9
63.3
48.0
46.9
40.4
38.6
32.8
31.7
31.1
30.9
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200
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180
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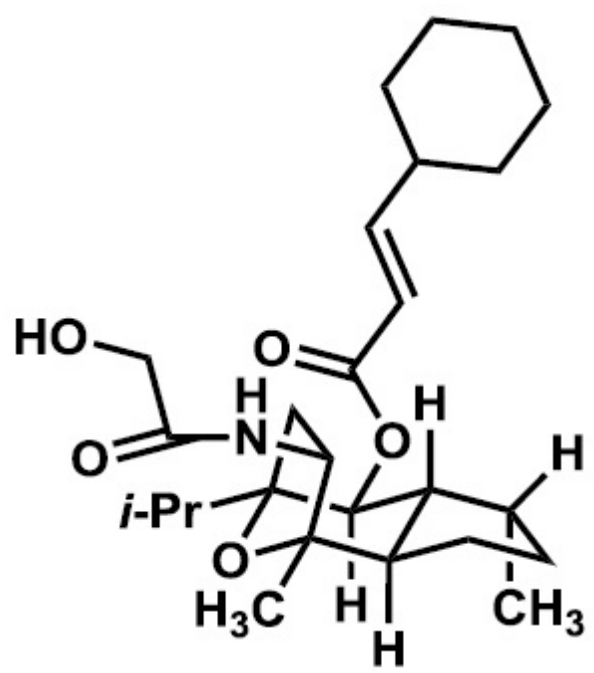

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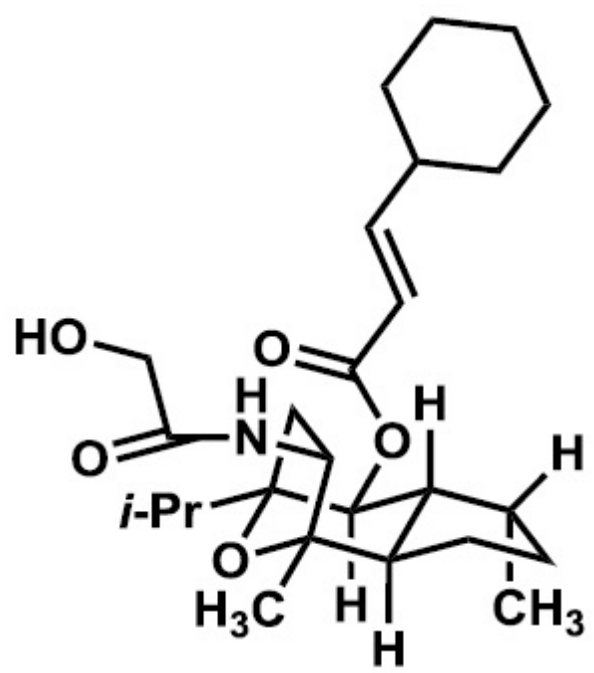
(NSC#768544)



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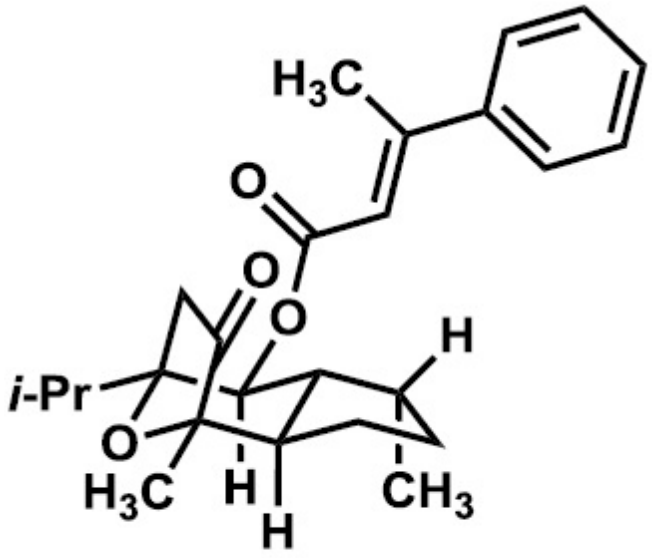
(NSC#768544)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O
O
O
i-Pr
O
H3C
H
H
H
CH3

8

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3.20
1.87
0.91
0.98
2.01
1.15
1.25
3.81
2.81
2.60
2.88
2.70
10.0
9.5
9.0
8.5
8.0
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6.5
6.0
5.5
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2.5
2.0
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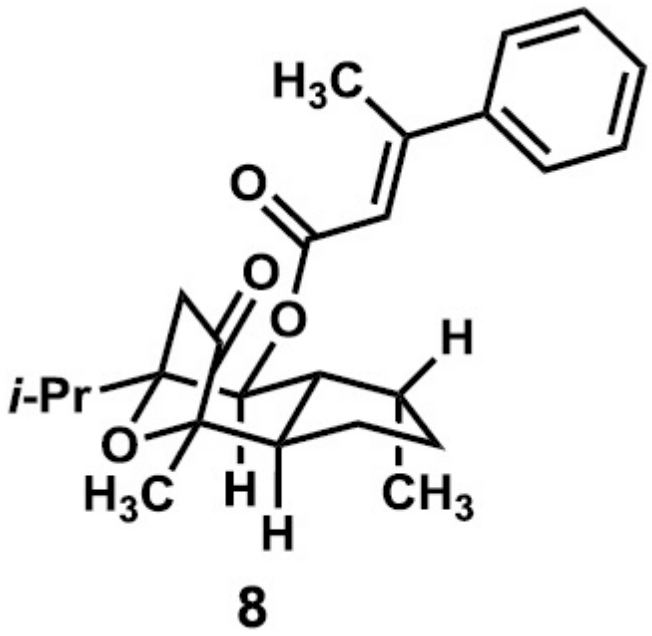
f1 (ppm)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O
O
H
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O
H3C
H
H
CH3

8

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128.5
126.3
116.7
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70.0
48.4
46.1
42.6
33.0
31.1
30.7
23.3
18.1
17.9
17.5
16.9
16.9
220
210
200
190
180
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90
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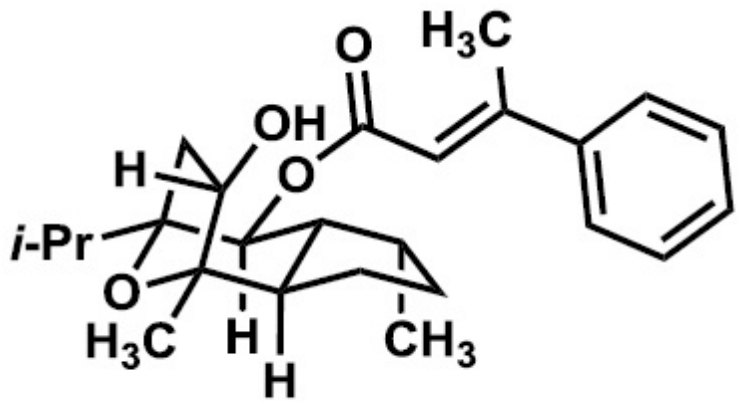
f1 (ppm)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i-Pr
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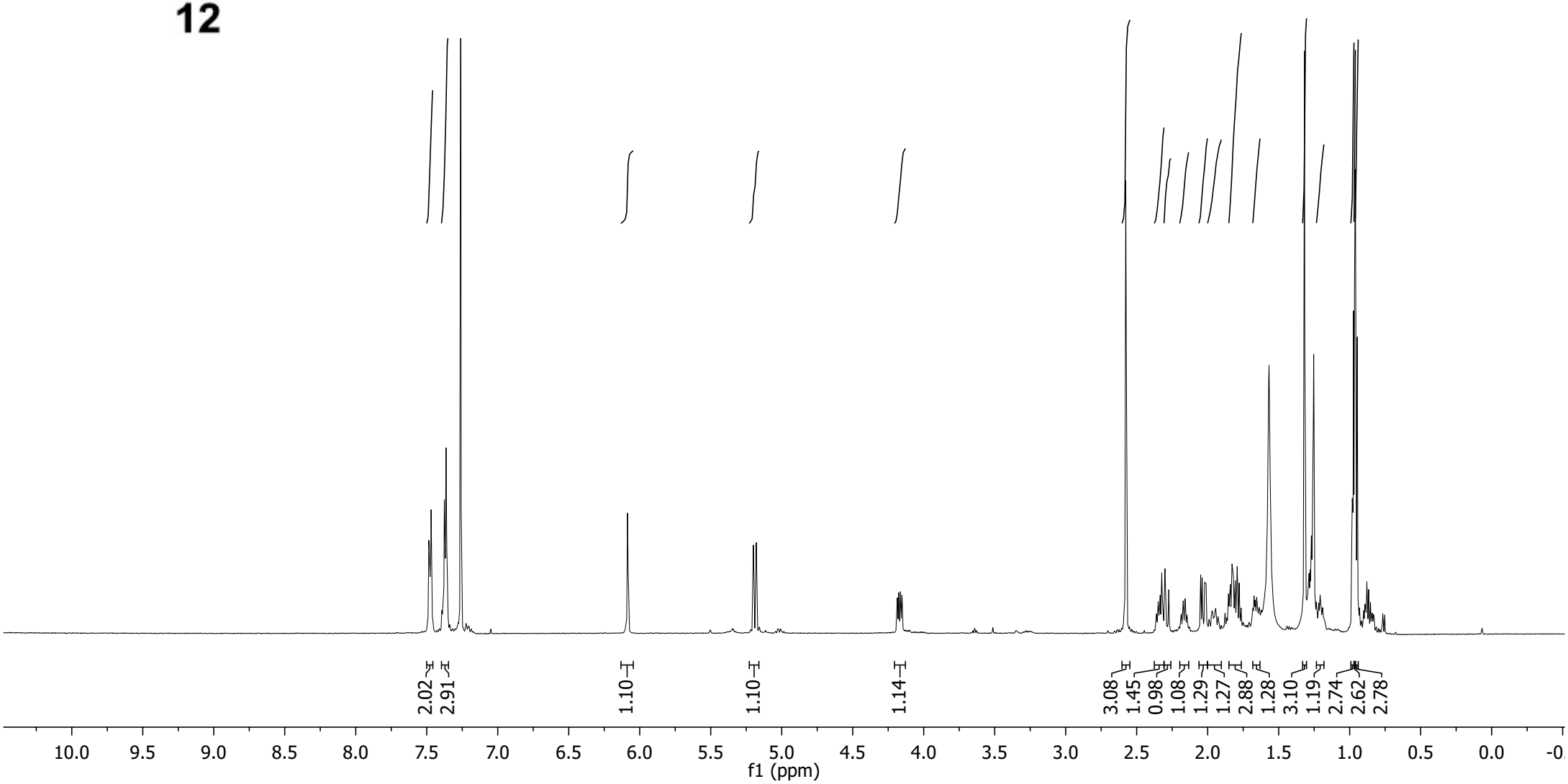
H3C
H
CH3
H

12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3.10
1.19
2.74
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7.0
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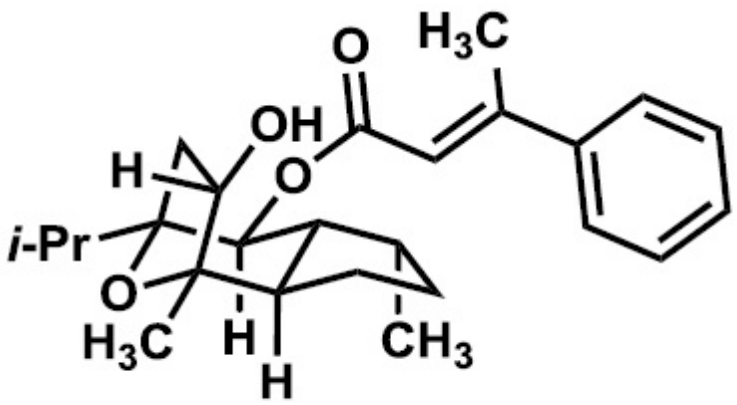
f1 (ppm)


O
H3C
OH
H
O
i-Pr
O
H3C
H
CH3
H

12

165.7
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142.4
129.1
128.6
126.5
117.6
84.8
81.5
81.2
71.9
49.3
46.5
39.6
33.0
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31.3
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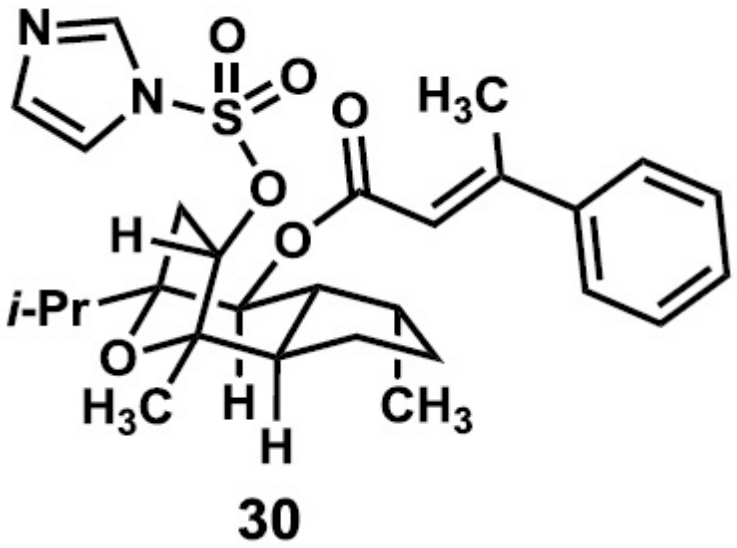
24.5
23.5
18.2
17.9
17.2
17.2
f1 (ppm)


N
O
N
S
O
O
H3C
O
H
O
i-Pr
O
H3C
H
CH3
H

30

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2.27
3.28
1.00
0.96
1.01
1.01
3.13
2.27
1.10
2.36
1.15
2.81
0.95
3.04
2.88
2.99
2.90
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9.5
9.0
8.5
8.0
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7.0
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6.0
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3.5
3.0
2.5
2.0
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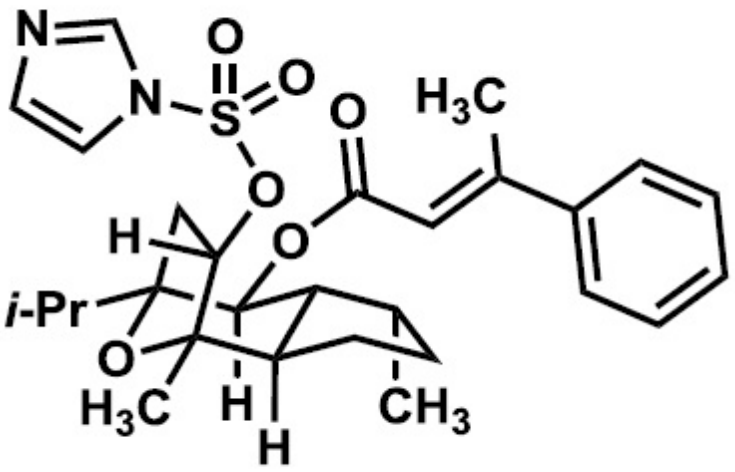
1.0
0.5
0.0
f1 (ppm)


N
O
N
S
O
O
H3C
O
O
H
i-Pr
O
H3C
H
CH3
H

30

165.2
156.4
142.0
137.0
131.6
129.1
128.5
126.3
117.9
116.8
90.5
85.3
81.0
70.4
48.8
46.4
35.7
32.5
31.2
31.1
29.7
23.9
22.5
18.1
17.4
16.8
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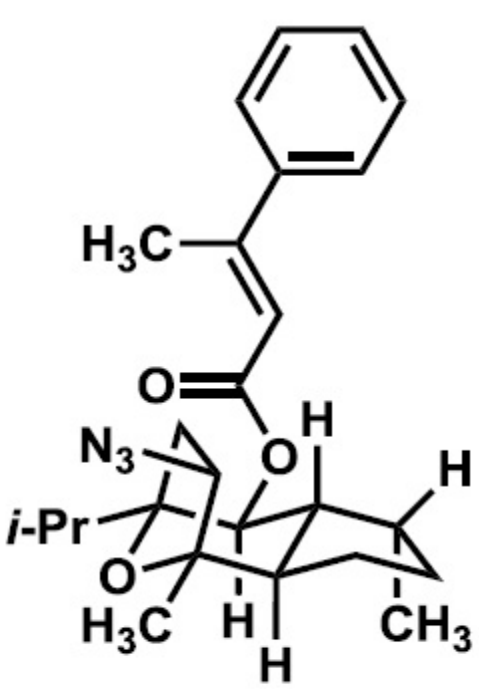
H3C
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H
N3
O
H
i-Pr
O
H3C
H
H
CH3

16

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1.12
0.91
2.84
0.91
1.06
1.62
2.13
2.31
1.76
3.69
2.87
2.99
3.07
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9.0
8.5
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6.0
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2.5
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1.0
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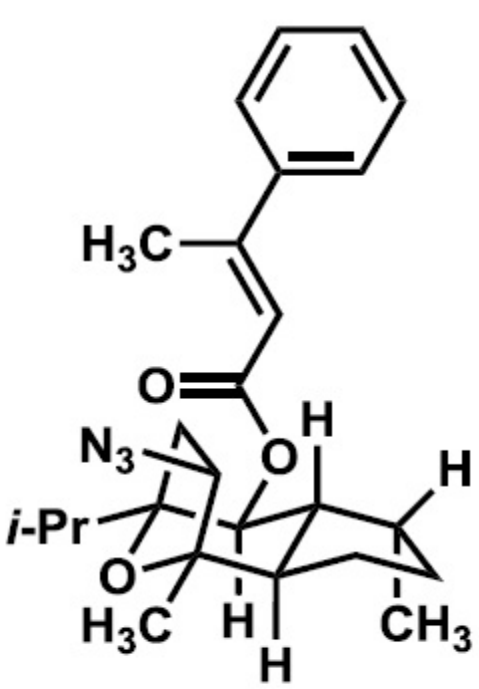
f1 (ppm)


H3C
O
O
H
N3
O
H
i-Pr
O
H3C
H
H
CH3

16

165.3
156.1
142.2
129.1
128.5
126.3
117.1
85.6
85.5
70.7
63.4
48.2
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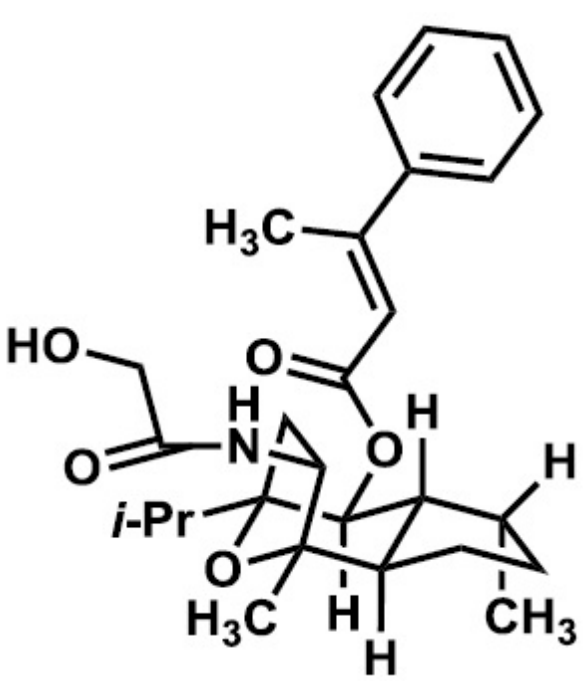
32.9
31.2
29.7
24.8
20.2
18.2
17.5
17.0
f1 (ppm)



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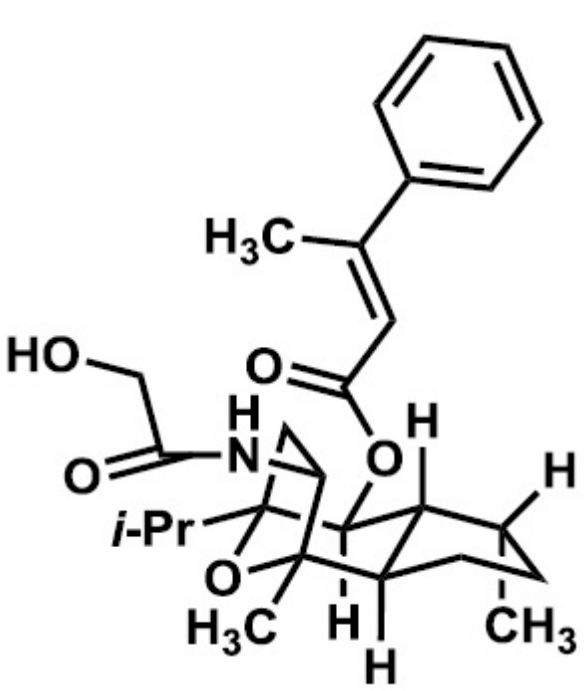

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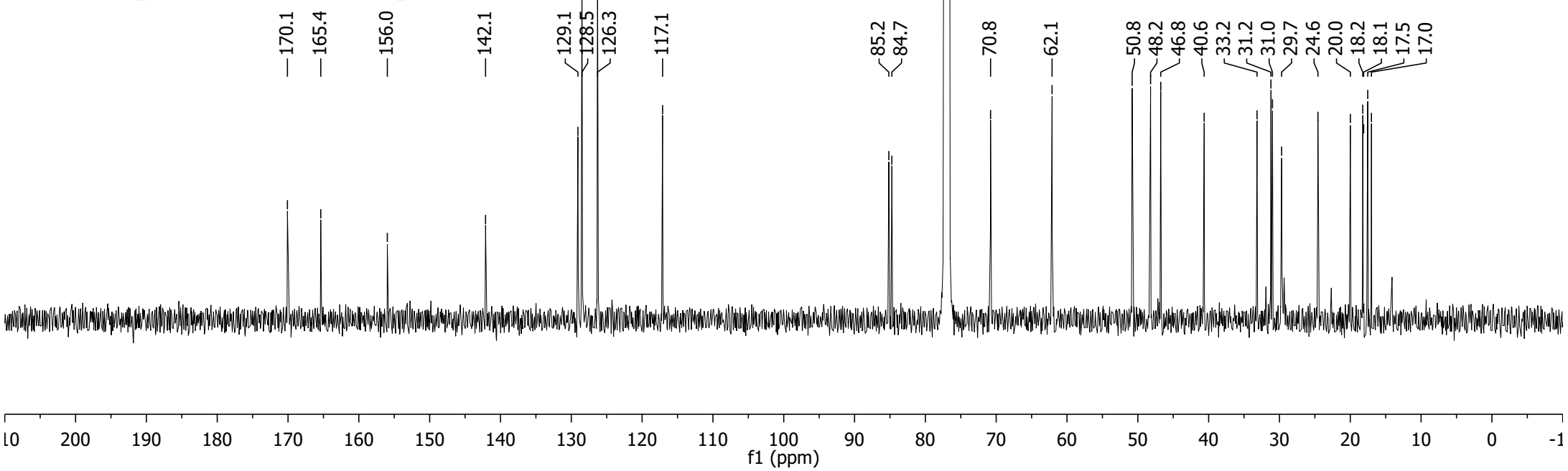

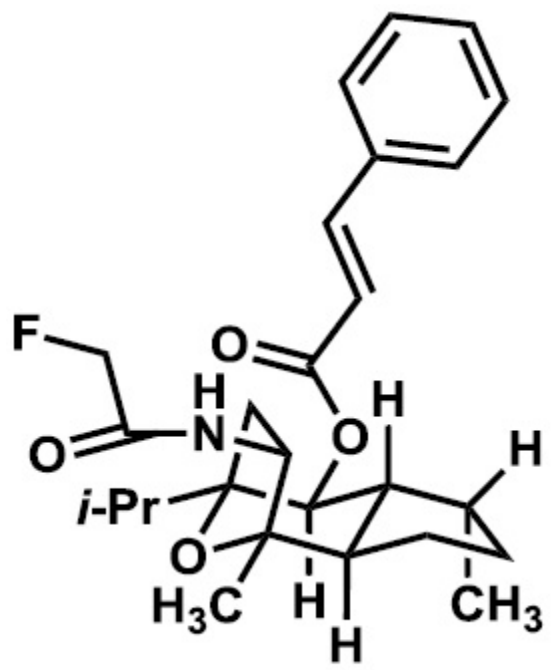

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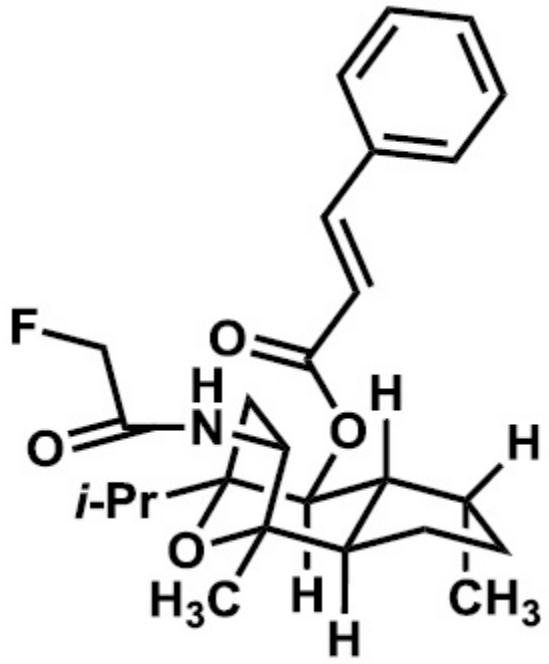

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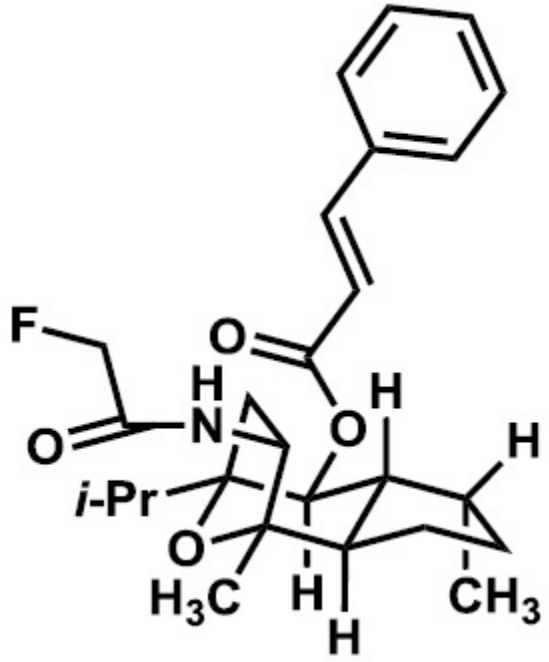


20
(NSC#777412)

Formulations

Parenteral Formulation

Vehicle Ingredients were ethanol, propylene glycol (PG), PEG 400, hydroxypropyl β-eyclodextrin (HPBCD, 8% w/v in H_2O). All vehicle ingredients were purchased from Sigma-Aldrich. A vial containing the dry drug compound (10 mg) was allowed to come to room temperature before proceeding to formulation. 150 µl of ethanol was added to the drug vial and mixed thoroughly using a vortex mixer. Next 400 µl of PG was added and mixed vigorously using a vortex mixer. Then, 450 µl of PEG 400 was added and mixed thoroughly. Last, 450 µl of HPBCD (8%) was added and mixed thoroughly. A clear solution with a concentration of 5.7 mg/ml was obtained at the final step.

To prepare dilutions of the reconstituted drug, a solution containing 8% HPBCD in water was used. To prepare the dilution vehicle, 8 g of HPBCD powder was weighed out and added to 100 mL of water. The concentrated formulation was added to the diluent in a dropwise fashion with stirring and made to 20 mL, for a working concentration of 0.5 mg/mL. The solution was then sterile filtered using an 0.2 µ Acrodisc (Pall) filter into a 20 mL sterile serum vial.

The 5.7 mg/ml solution of reconstituted drug was stable for over one week at 4°C. Drug dilutions prepared from the 5.7 mg/ml solution of reconstituted drug were also found to be stable for one week.

Oral Formulation

The dry compound was dissolved directly in Labrasol™ at a concentration of 20 mg/mL. The preparation was administered by gavage.

HPLC-MS/MS Assay for Englerins

This method was developed for mouse or rat plasma. The calibration range was 5-500 ng/mL. Lorazepam was used as an internal standard. UPLC was performed using a Waters Acquity

binary pump. Mobile Phase A1: 0.5% formic acid (aq), Mobile Phase B1: 0.5% formic acid in Acetonitrile, Mobile Phase A2: (90/10) water/CAN, Mobile Phase B2: CAN, Strong Needle Wash: 0.1% formic acid in CAN, Weak Needle Wash/Seal Wash: (80/20) water/CAN. Solvent programming started at 70% A for 0.40 min, with a linear gradient to 20% A at 0.55 min, isocratic until 1.50 min, and return to initial conditions at 1.70 min. Total run time was 2.0 minutes. Retention times were Aza-Englerin (**2**) 1.22 min, internal standard 0.97 min, flow rate 0.5 ml/min, injection volume 10 µL. The column was a Waters Acquity UPLC® BEH C18 1.7 um, 2.1x50mm column + guard column, column compartment temperature was 30°C.

Mass Spectrometry Conditions:

Ion Spray Voltage 5500 V, Source Temp 400°C, GS1/GS2: 60/50, Declustering Potential (DP) 35, Entrance Potential (EP) 8, Collision Energy (CE) 25,
Collision Exit Potential (CXP) 14, Dwell Times 200 msec, MRM 1 foraza-englerin (**2**) *m/z* 442.2 → 219.1 (CE=21), MRM 2 for lorazepam (IS) *m/z* 321.0 → 275.0 (CE=15).

Stock solutions

Aza-englerin master stock was prepared by adding 1.0 mg of **2** film to 1 mL of ACN to make a 1 mg/mL master stock and stored at –80°C. Lorazepam (IS) master stock was prepared by adding 2 mL of ethanol to a 1 mg vial make a 0.5 mg/mL solution, which was stored at –80°C.

Internal Standard Working Solutions

40 uL of 0.5 mg/mL lorazepam (LZP) master stock was mixed with 960 uL of ACN to make a 20 µg/mL solution. 50 uL of 20 µg/mL LZP was added to 100 mL of ACN to make 10 ng/mL LZP in 0.1% Formic acid /ACN.

Sample Preparation

Plasma samples were defrosted, vortexed for 15 sec, then kept on ice. To 100 uL of the thawed experimental sample, was added 100 uL of IS solution in a microcentrifuge tube, then 1 mL CAN, was added, the tube was capped and vortexed for 5 sec. The tubes were then centrifuged for 5 minutes at 13,000 rpm in a mini centrifuge @ 4 ºC. Approximately 850 uL of supernatent was transferred into a 96-well collection plate, which was then dried under desiccated air in a TurboVap 96 (40 C plate temp; <50 Fh). The sample was reconstituted with 100 uL of (50/50) ACN/H_2O added directly into the plate, which was vortexed thoroughly while covered with parafilm. The plate was inserted directly into Waters ACQUITY UPLC autosampler tray.

The original ultra-high performance liquid chromatography with tandem mass spectrometric detection (uHPLC-MS/MS) assay for englerin A (**1**) was modified in order to measure the N-substituted analogue (**2**). First, **1** has a molecular weight of 442 and gave a protonated molecular cation detected in the mass spectrometer at *m/z* 443.2. This intact cation was then fragmented and the predominant product cation was observed at *m/z* 201.2. In order to maximize this signal in the mass spectrometer, several instrument parameters were tuned for optimal signal performance, including the ion spray voltage (5500 V), TurboIon® source temperature (400 °C), declustering potential (70 V), entrance potential (10 V), collision energy (25 V), and the collision cell exit potential (12 V). Additionally, **1** was chromatographically separated from matrix contaminants remaining in the organic solvent extract (with acetonitrile) through the use of a bridged-ethylene hybrid bead-containing uHPLC column with an octadecyl carbon moiety (C18; 2.1x50mm, 1.7um particle size). The mobile phase consisted of (70/30, v/v) 0.5% formic acid(aq)/0.5% formic acid in acetonitrile that was ramped up to (20/80, v/v) over the course of 30 seconds and held there for an additional minute to solvate and elute the injected **1** that partitioned into the C18 stationary phase. The eluted **1** then entered the mass spectrometer at 1.40 min post injection.

To accommodate measurement of the aza analogue (**2**), the mass spectrometer was tuned for optimal signal performance on the molecular cation observed at *m/z* 442.2, and the subsequent predominant product cation observed at *m/z* 219.1. Several tuning parameters changed as a result of the different ions being meausured: declustering potential (35 V), entrance potential (13 V), collision energy (19 V), and the collision cell exit potential (10 V). The chromatographic scheme for the **2** was identical to EA, however the aza-analogue eluted from the column and entered the mass spectrometer at 1.22 min.

Oral Bioavailability Studies

Mice were dosed at 5 mg/kg, 10 mg/kg, 50 mg/kg, and 100 mg/kg compound **2** in Labrasol vehicle via oral gavage. 30 minutes after dosing, plasma was assayed for the concentration of compound **2** using LC-MS. The results are depicted graphically in Figure S1. As is apparent from the results shown in Figure S1, compound **2** was found in plasma concentrations up to approximately 700 ng/mL after dosing at 100 mg/kg.

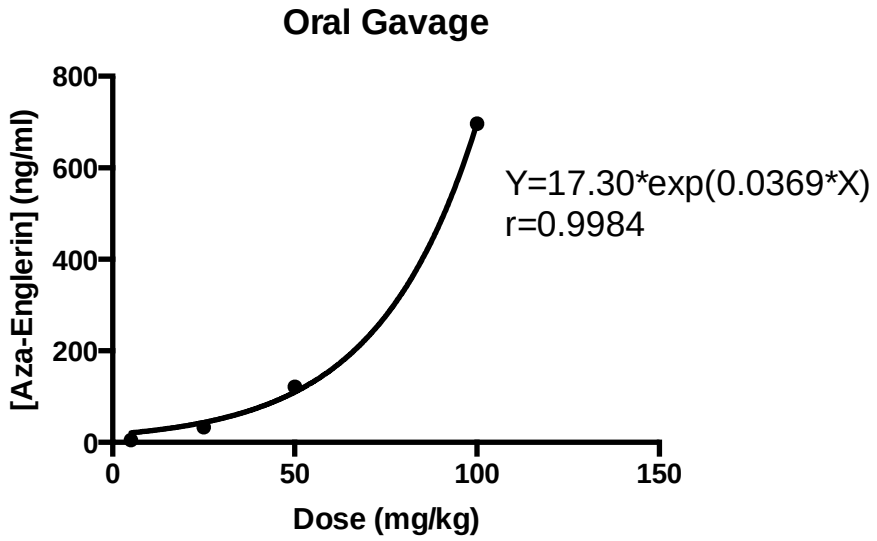


Figure S1. Nonlinear increase in plasma concentrations of **2** 30 min after dosing.

In a second experiment, ten mice were administered 20 mg/kg of compound **2** in Labrasol vehicle via oral gavage. Two mice at a time were sacrificed at the 5 min, 15 min, 1 h, 4 h, and 8 h. Plasma concentrations of compound **2** were measured by LC-MS/MS, and the results set forth in Table S1 and depicted graphically in Figure 1.

Table S1.

Animal	Time (hr)	Concentration of **2** (ng/mL)	Mean
1	0.083	6.87	10.04
2	0.083	13.20	
3	0.25	22.76	40.73
4	0.25	58.70	
5	1.0	87.16	73.20
6	1.0	59.24	
7	4.0	31.12	58.97
8	4.0	86.81	
9	8.0	87.28	67.75
10	8.0	48.21	

The AUC_{LAST} was calculated as 524.4 hr*ng/mL (SE=97.3 hr*ng/mL). As is apparent from the results set forth in Table S1 and the graphical results depicted in Figure 1, compound **2** is rapidly absorbed and the plasma concentrations of compound **2** are sustained for greater than 8 h. A nonlinear, more-than-dose proportional increase in plasma concentrations was observed. It appears that **2** is rapidly absorbed and plasma concentrations sustained for a longer than expected period. The elimination period likely dominates after 8 h and thus beyond the observed period from this experiment. Further pharmacokinetic experiments are needed that measure plasma concentrations out to 24-36 h post dose.

NCI 60 Testing

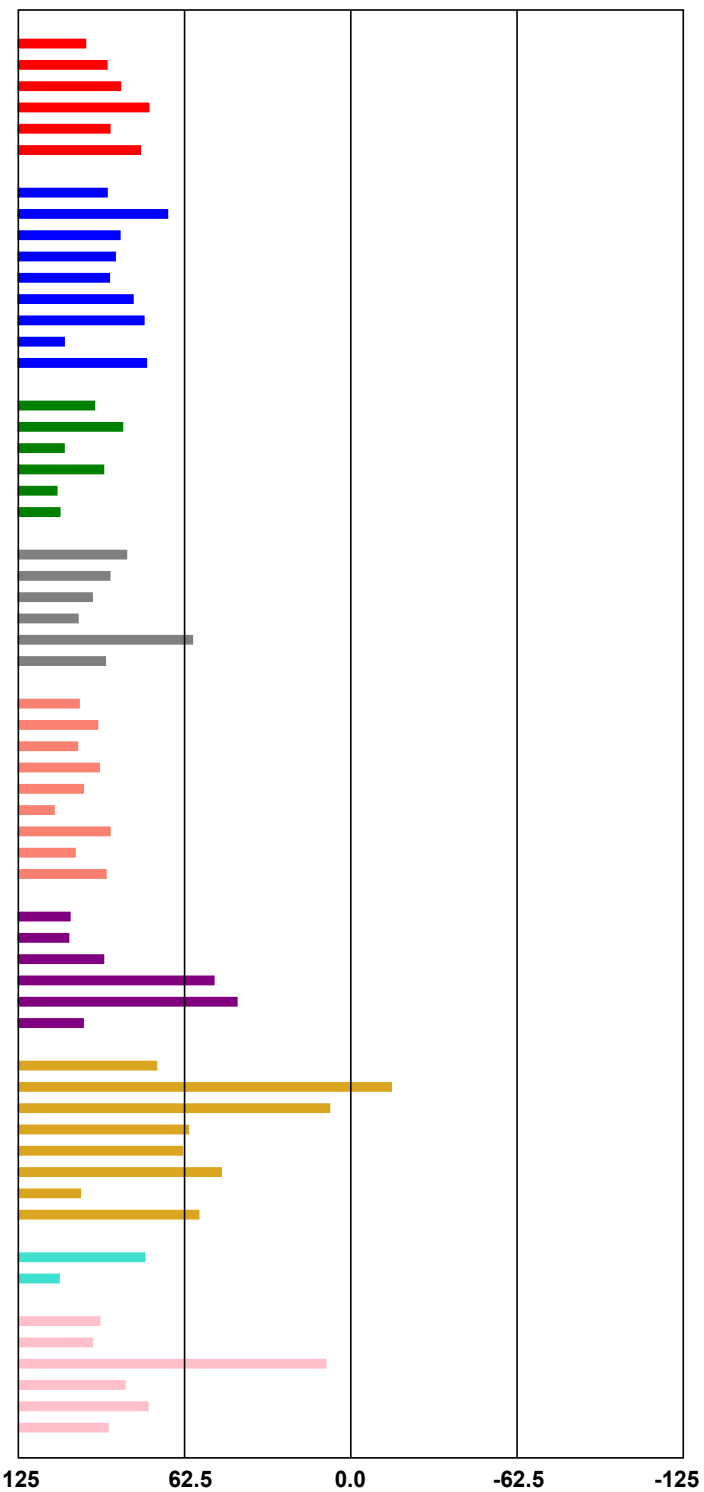
Samples were tested in the standard National Cancer Institute 60-cell line protocol. The drug exposure was for two days, with an SRB endpoint. First, compounds were tested against all 60 cell lines at a single final concentration of 10 μM. Results are shown in Figures S2-S6. They were then separately tested in five 10-fold dilutions, starting with a high concentration of 100 μM. The results are depicted as dose-response curves are found in Figures S7 for compound **2**, Figure S8 for compound **17**, Figure S9 for compound **18**, and Figure S10 for compound **19**.

DTP Figure S2a. Compound 2
One Dose Bar Graph

NSC: D-761305 / 1	Conc: 1.00E-5 Molar	Test Date: Aug 22, 2011
Experiment ID: 1108OS12		

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	99.78
HL-60(TB)	91.72
K-562	86.62
MOLT-4	75.99
RPMI-8226	90.61
SR	79.14
Non-Small Cell Lung Cancer	
A549/ATCC	91.65
EKVX	68.95
HOP-62	86.84
HOP-92	88.61
NCI-H226	90.81
NCI-H23	81.92
NCI-H322M	77.82
NCI-H460	107.78
NCI-H522	76.88
Colon Cancer	
COLO 205	96.41
HCT-116	85.85
HCT-15	107.84
HT29	93.00
KM12	110.57
SW-620	109.43
CNS Cancer	
SF-268	84.37
SF-295	90.63
SF-539	97.25
SNB-19	102.60
SNB-75	59.57
U251	92.32
Melanoma	
LOX IMVI	102.17
MALME-3M	95.23
M14	102.78
MDA-MB-435	94.63
SK-MEL-2	100.60
SK-MEL-28	111.58
SK-MEL-5	90.57
UACC-257	103.72
UACC-62	92.07
Ovarian Cancer	
IGROV1	105.63
OVCAR-3	106.12
OVCAR-5	92.99
OVCAR-8	51.51
NCI/ADR-RES	42.85
SK-OV-3	100.64
Renal Cancer	
786-0	73.08
A498	-15.20
ACHN	8.01
CAKI-1	61.10
RXF 393	63.32
SN12C	48.75
TK-10	101.69
UO-31	57.21
Prostate Cancer	
PC-3	77.46
DU-145	109.70
Breast Cancer	
MCF7	94.41
MDA-MB-231/ATCC	97.20
HS 578T	9.45
BT-549	84.94
T-47D	76.26
MDA-MB-468	91.31

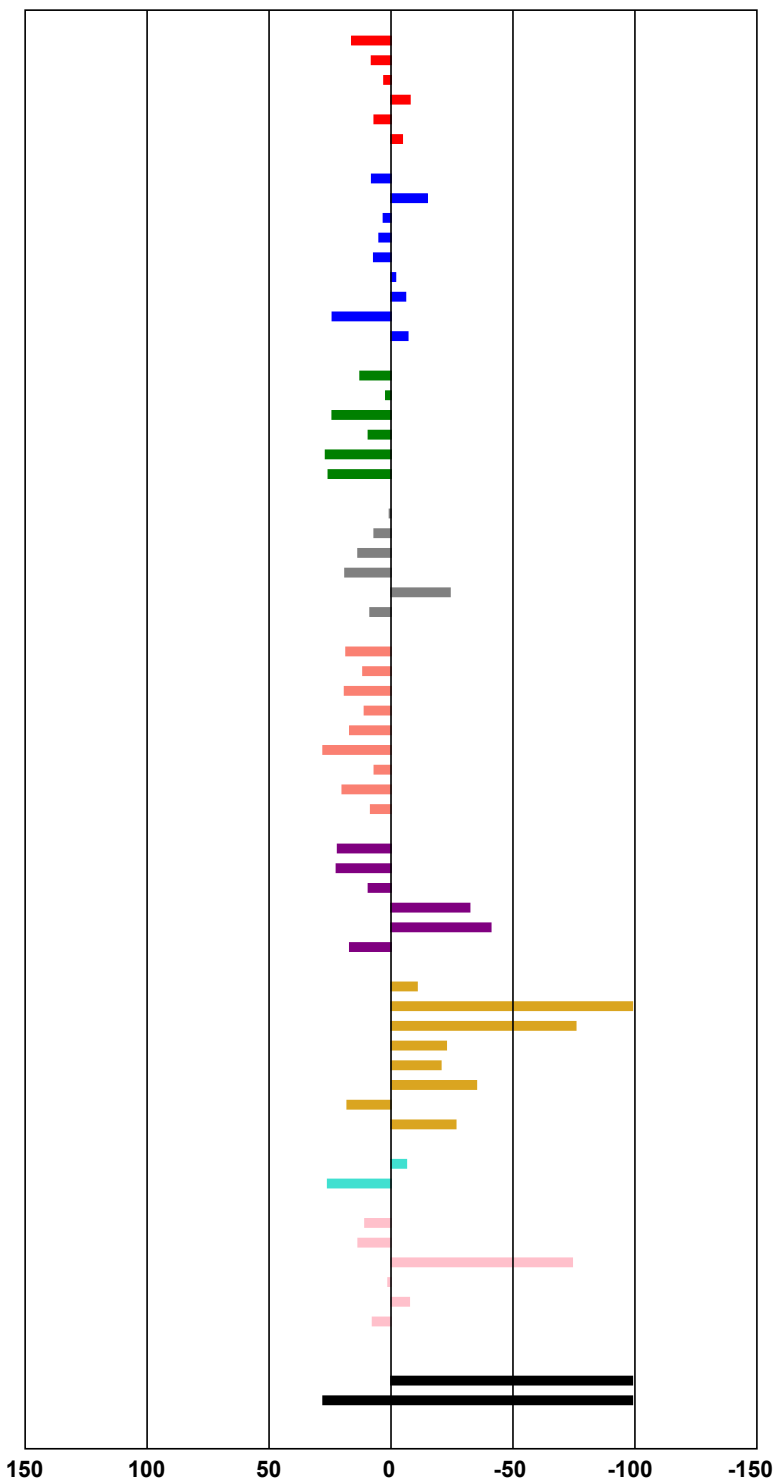
Bar Graph



DTP Figure S2b. Compound 2 One Dose Mean Graph	NSC: D-761305 / 1	Conc: 1.00E-5 Molar	Test Date: Aug 22, 2011
	Experiment ID: 1108OS12		Compound 2

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	99.78
HL-60(TB)	91.72
K-562	86.62
MOLT-4	75.99
RPMI-8226	90.61
SR	79.14
Non-Small Cell Lung Cancer	
A549/ATCC	91.65
EKVX	68.95
HOP-62	86.84
HOP-92	88.61
NCI-H226	90.81
NCI-H23	81.92
NCI-H322M	77.82
NCI-H460	107.78
NCI-H522	76.88
Colon Cancer	
COLO 205	96.41
HCT-116	85.85
HCT-15	107.84
HT29	93.00
KM12	110.57
SW-620	109.43
CNS Cancer	
SF-268	84.37
SF-295	90.63
SF-539	97.25
SNB-19	102.60
SNB-75	59.57
U251	92.32
Melanoma	
LOX IMVI	102.17
MALME-3M	95.23
M14	102.78
MDA-MB-435	94.63
SK-MEL-2	100.60
SK-MEL-28	111.58
SK-MEL-5	90.57
UACC-257	103.72
UACC-62	92.07
Ovarian Cancer	
IGROV1	105.63
OVCAR-3	106.12
OVCAR-5	92.99
OVCAR-8	51.51
NCI/ADR-RES	42.85
SK-OV-3	100.64
Renal Cancer	
786-0	73.08
A498	-15.20
ACHN	8.01
CAKI-1	61.10
RXF 393	63.32
SN12C	48.75
TK-10	101.69
UO-31	57.21
Prostate Cancer	
PC-3	77.46
DU-145	109.70
Breast Cancer	
MCF7	94.41
MDA-MB-231/ATCC	97.20
HS 578T	9.45
BT-549	84.94
T-47D	76.26
MDA-MB-468	91.31
Mean	83.74
Delta	98.94
Range	126.78

Mean Growth Percent - Growth Percent



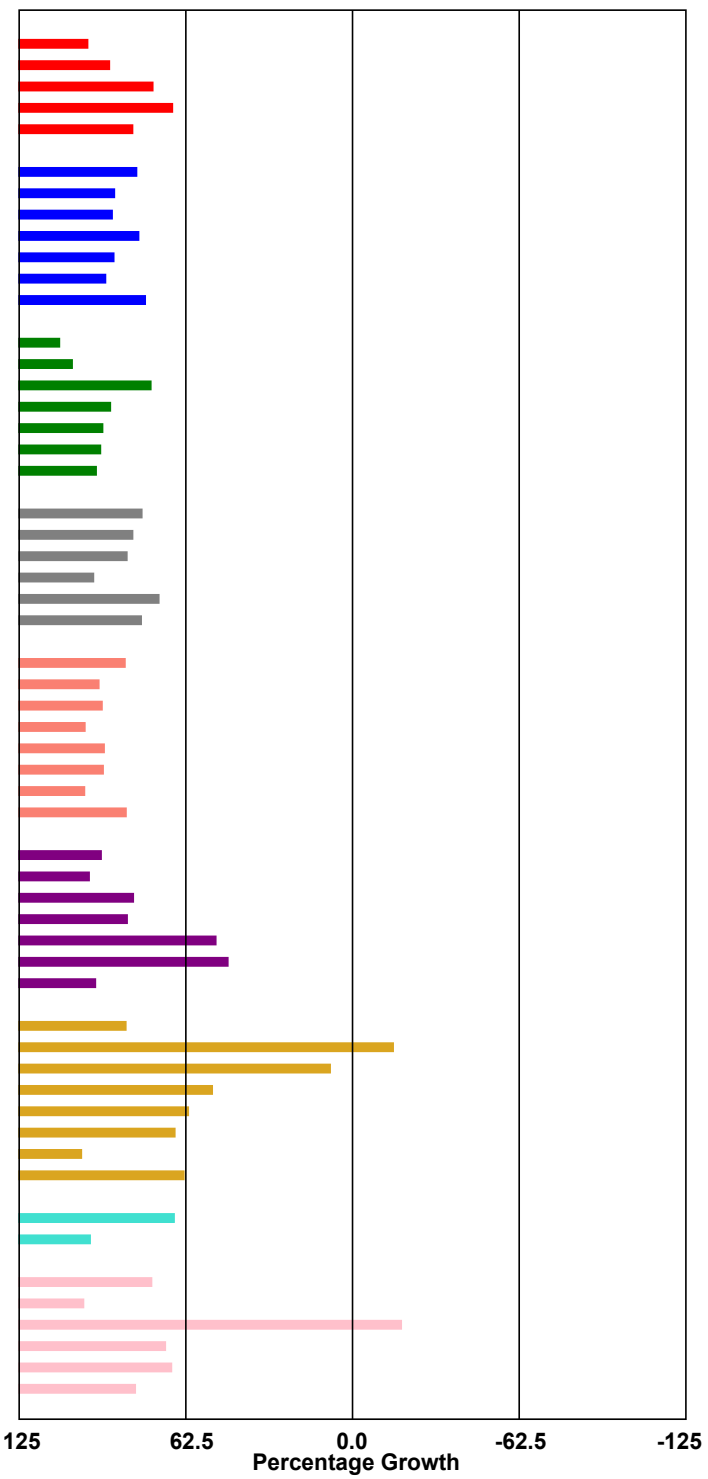
DTP Figure S3a. Compound 17

One Dose Bar Graph

NSC: D-764081 / 1	Conc: 1.00E-5 Molar	Test Date: Mar 19, 2012
Experiment ID: 1203OS38		Compound 17

Panel/Cell Line	Growth Percent
Leukemia	
HL-60(TB)	99.35
K-562	91.19
MOLT-4	74.92
RPMI-8226	67.56
SR	82.49
Non-Small Cell Lung Cancer	
A549/ATCC	81.00
HOP-62	89.31
NCI-H226	90.16
NCI-H23	80.23
NCI-H322M	89.55
NCI-H460	92.64
NCI-H522	77.74
Colon Cancer	
COLO 205	109.92
HCC-2998	105.15
HCT-116	75.65
HCT-15	90.82
HT29	93.73
KM12	94.54
SW-620	96.15
CNS Cancer	
SF-268	79.03
SF-295	82.48
SF-539	84.63
SNB-19	97.16
SNB-75	72.66
U251	79.26
Melanoma	
MALME-3M	85.34
M14	95.15
MDA-MB-435	93.95
SK-MEL-2	100.37
SK-MEL-28	93.16
SK-MEL-5	93.53
UACC-257	100.53
UACC-62	84.95
Ovarian Cancer	
IGROV1	94.29
OVCAR-3	98.78
OVCAR-4	82.22
OVCAR-5	84.53
OVCAR-8	51.29
NCI/ADR-RES	46.77
SK-OV-3	96.43
Renal Cancer	
786-0	85.02
A498	-15.23
ACHN	8.40
CAKI-1	52.62
RXF 393	61.60
SN12C	66.65
TK-10	101.69
UO-31	63.27
Prostate Cancer	
PC-3	66.92
DU-145	98.40
Breast Cancer	
MCF7	75.37
MDA-MB-231/ATCC	100.88
HS 578T	-18.26
BT-549	70.21
T-47D	67.91
MDA-MB-468	81.48

Bar Graph

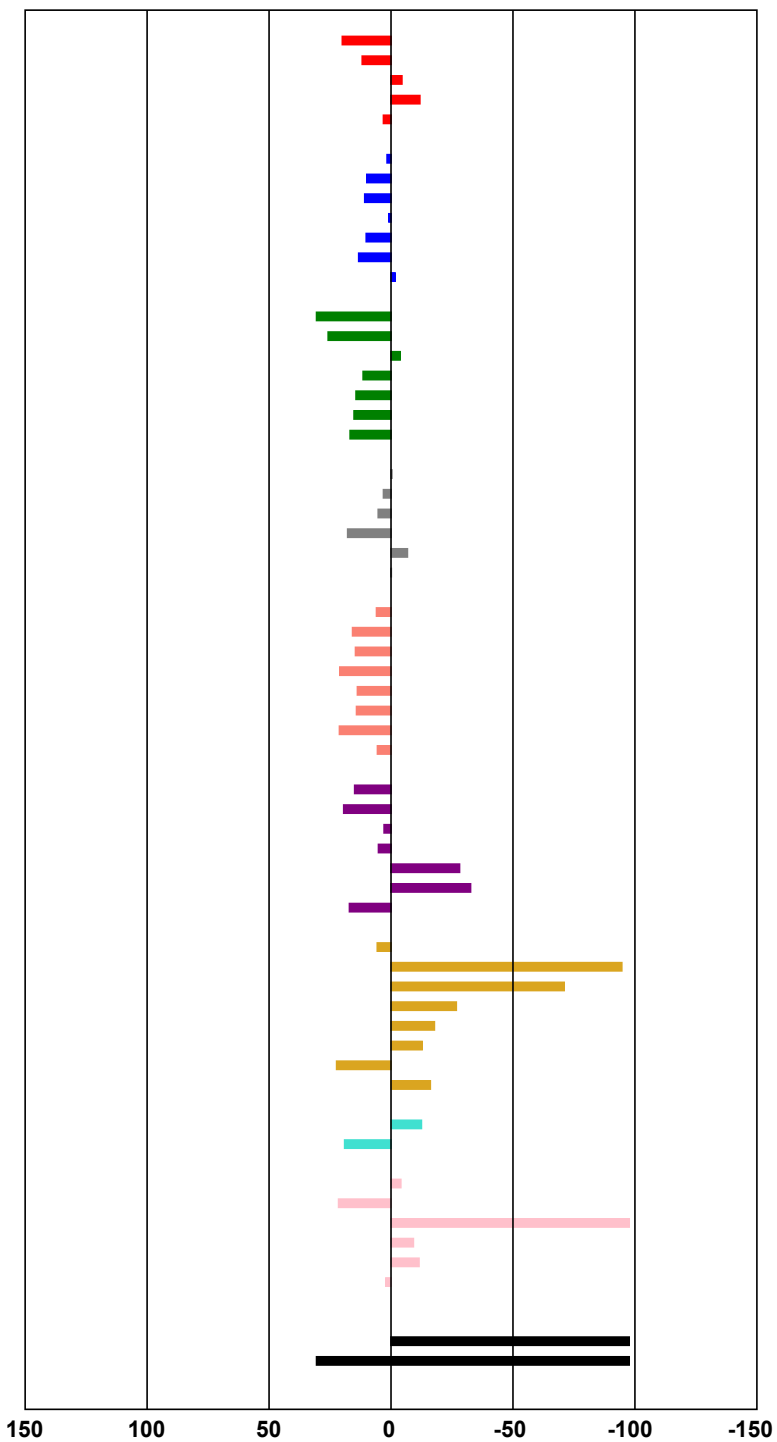


DTP Figure S3b. Compound 17
One Dose Mean Graph

NSC: D-764081 / 1	Conc: 1.00E-5 Molar	Test Date: Mar 19, 2012
Experiment ID: 1203OS38		

Panel/Cell Line	Growth Percent
Leukemia	
HL-60(TB)	99.35
K-562	91.19
MOLT-4	74.92
RPMI-8226	67.56
SR	82.49
Non-Small Cell Lung Cancer	
A549/ATCC	81.00
HOP-62	89.31
NCI-H226	90.16
NCI-H23	80.23
NCI-H322M	89.55
NCI-H460	92.64
NCI-H522	77.74
Colon Cancer	
COLO 205	109.92
HCC-2998	105.15
HCT-116	75.65
HCT-15	90.82
HT29	93.73
KM12	94.54
SW-620	96.15
CNS Cancer	
SF-268	79.03
SF-295	82.48
SF-539	84.63
SNB-19	97.16
SNB-75	72.66
U251	79.26
Melanoma	
MALME-3M	85.34
M14	95.15
MDA-MB-435	93.95
SK-MEL-2	100.37
SK-MEL-28	93.16
SK-MEL-5	93.53
UACC-257	100.53
UACC-62	84.95
Ovarian Cancer	
IGROV1	94.29
OVCAR-3	98.78
OVCAR-4	82.22
OVCAR-5	84.53
OVCAR-8	51.29
NCI/ADR-RES	46.77
SK-OV-3	96.43
Renal Cancer	
786-0	85.02
A498	-15.23
ACHN	8.40
CAKI-1	52.62
RXF 393	61.60
SN12C	66.65
TK-10	101.69
UO-31	63.27
Prostate Cancer	
PC-3	66.92
DU-145	98.40
Breast Cancer	
MCF7	75.37
MDA-MB-231/ATCC	100.88
HS 578T	-18.26
BT-549	70.21
T-47D	67.91
MDA-MB-468	81.48
Mean	79.38
Delta	97.64
Range	128.18

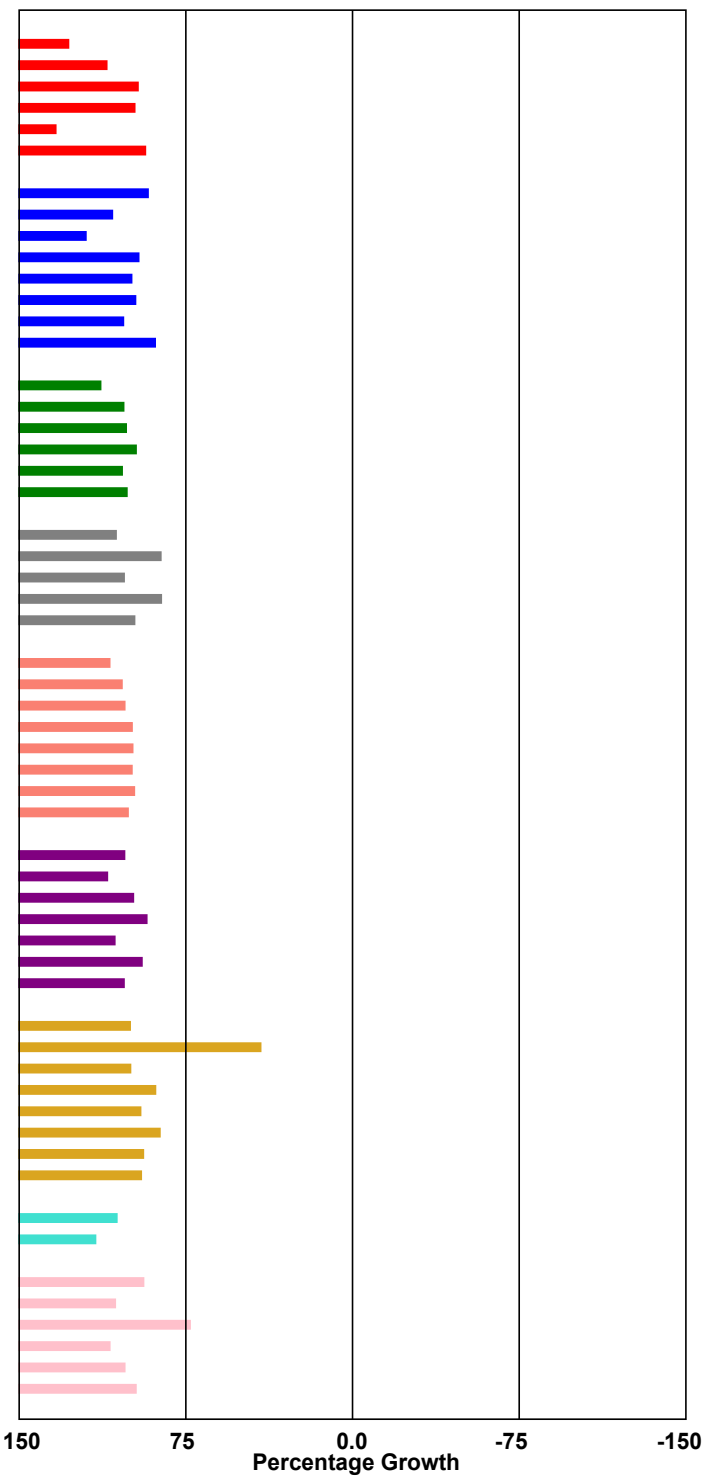
Mean Growth Percent - Growth Percent



DTP Figure S4a. Compound 18 One Dose Bar Graph	NSC: D-768544 / 1	Conc: 1.00E-5 Molar	Test Date: Nov 26, 2012
	Experiment ID: 1211OS83		

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	127.81
HL-60(TB)	110.60
K-562	96.54
MOLT-4	98.01
RPMI-8226	133.54
SR	93.21
Non-Small Cell Lung Cancer	
A549/ATCC	92.01
HOP-62	108.07
HOP-92	119.97
NCI-H226	96.22
NCI-H23	99.40
NCI-H322M	97.65
NCI-H460	103.10
NCI-H522	88.80
Colon Cancer	
COLO 205	113.35
HCT-116	102.99
HCT-15	101.85
HT29	97.40
KM12	103.67
SW-620	101.53
CNS Cancer	
SF-268	106.40
SF-295	86.26
SNB-19	102.78
SNB-75	86.06
U251	98.07
Melanoma	
MALME-3M	109.27
M14	103.75
MDA-MB-435	102.50
SK-MEL-2	99.21
SK-MEL-28	98.93
SK-MEL-5	99.27
UACC-257	98.18
UACC-62	101.01
Ovarian Cancer	
IGROV1	102.59
OVCAR-3	110.32
OVCAR-4	98.62
OVCAR-5	92.59
OVCAR-8	106.97
NCI/ADR-RES	94.76
SK-OV-3	102.83
Renal Cancer	
786-0	100.06
A498	41.34
ACHN	99.90
CAKI-1	88.67
RXF 393	95.36
SN12C	86.67
TK-10	94.12
UO-31	95.07
Prostate Cancer	
PC-3	106.05
DU-145	115.64
Breast Cancer	
MCF7	94.03
MDA-MB-231/ATCC	106.76
HS 578T	73.09
BT-549	109.23
T-47D	102.50
MDA-MB-468	97.46

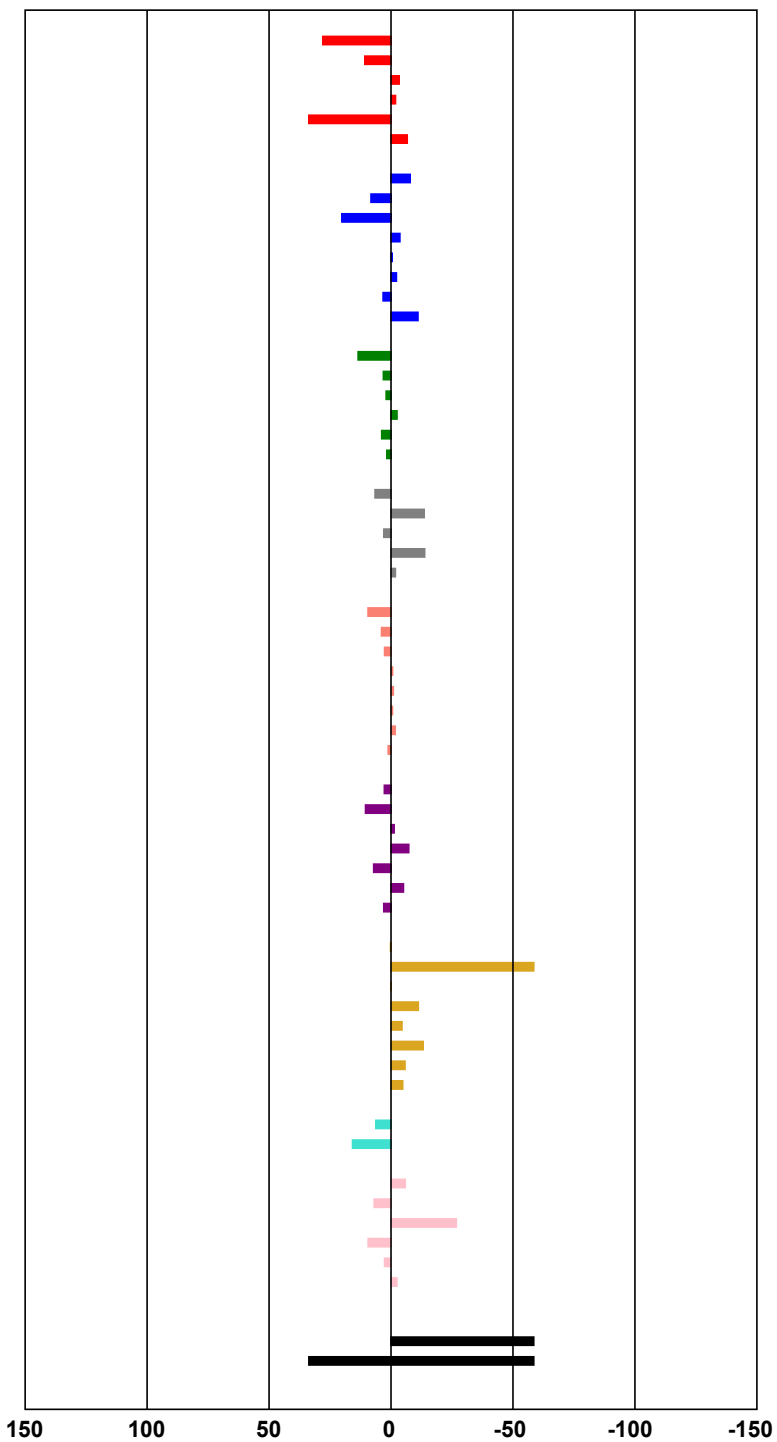
Bar Graph



DTP Figure S4b. Compound 18
One Dose Mean Graph

NSC: D-768544 / 1	Conc: 1.00E-5 Molar	Test Date: Nov 26, 2012
Experiment ID: 1211OS83		

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	127.81
HL-60(TB)	110.60
K-562	96.54
MOLT-4	98.01
RPMI-8226	133.54
SR	93.21
Non-Small Cell Lung Cancer	
A549/ATCC	92.01
HOP-62	108.07
HOP-92	119.97
NCI-H226	96.22
NCI-H23	99.40
NCI-H322M	97.65
NCI-H460	103.10
NCI-H522	88.80
Colon Cancer	
COLO 205	113.35
HCT-116	102.99
HCT-15	101.85
HT29	97.40
KM12	103.67
SW-620	101.53
CNS Cancer	
SF-268	106.40
SF-295	86.26
SNB-19	102.78
SNB-75	86.06
U251	98.07
Melanoma	
MALME-3M	109.27
M14	103.75
MDA-MB-435	102.50
SK-MEL-2	99.21
SK-MEL-28	98.93
SK-MEL-5	99.27
UACC-257	98.18
UACC-62	101.01
Ovarian Cancer	
IGROV1	102.59
OVCAR-3	110.32
OVCAR-4	98.62
OVCAR-5	92.59
OVCAR-8	106.97
NCI/ADR-RES	94.76
SK-OV-3	102.83
Renal Cancer	
786-0	100.06
A498	41.34
ACHN	99.90
CAKI-1	88.67
RXF 393	95.36
SN12C	86.67
TK-10	94.12
UO-31	95.07
Prostate Cancer	
PC-3	106.05
DU-145	115.64
Breast Cancer	
MCF7	94.03
MDA-MB-231/ATCC	106.76
HS 578T	73.09
BT-549	109.23
T-47D	102.50
MDA-MB-468	97.46
Mean	99.86
Delta	58.52
Range	92.20



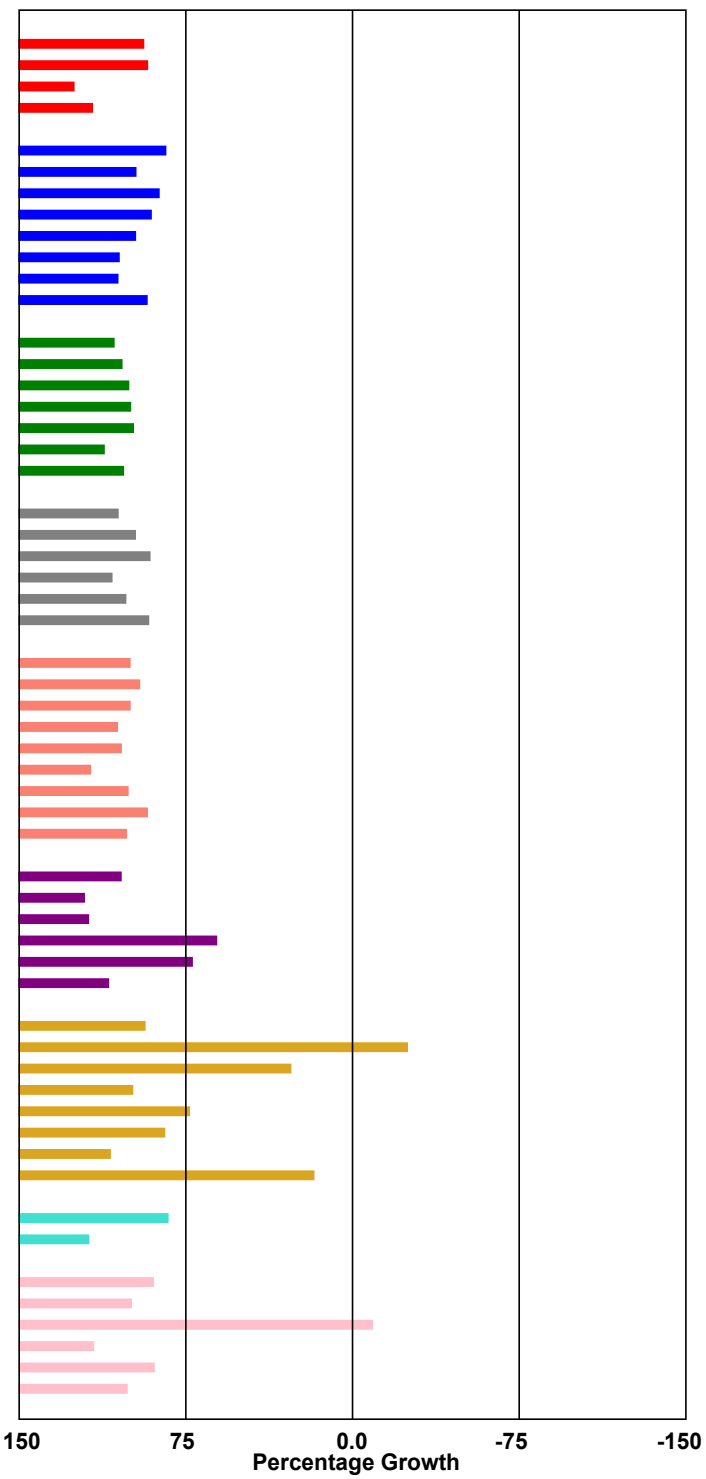
DTP Figure S5a. Compound 19

One Dose Bar Graph

NSC: D-778312 / 1	Conc: 1.00E-5 Molar	Test Date: Dec 02, 2013
Experiment ID: 1312OS99		

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	94.07
K-562	92.32
MOLT-4	125.43
SR	117.13
Non-Small Cell Lung Cancer	
A549/ATCC	84.13
HOP-62	97.57
HOP-92	87.13
NCI-H226	90.67
NCI-H23	97.74
NCI-H322M	105.10
NCI-H460	105.67
NCI-H522	92.53
Colon Cancer	
COLO 205	107.40
HCC-2998	103.84
HCT-116	100.80
HCT-15	99.96
HT29	98.69
KM12	111.83
SW-620	103.14
CNS Cancer	
SF-268	105.59
SF-295	97.79
SF-539	91.23
SNB-19	108.35
SNB-75	102.12
U251	91.84
Melanoma	
LOX IMVI	100.20
MALME-3M	95.90
M14	100.16
MDA-MB-435	105.86
SK-MEL-2	104.13
SK-MEL-28	117.95
SK-MEL-5	101.11
UACC-257	92.39
UACC-62	101.83
Ovarian Cancer	
IGROV1	104.22
OVCAR-3	120.76
OVCAR-5	118.88
OVCAR-8	61.25
NCI/ADR-RES	72.19
SK-OV-3	109.87
Renal Cancer	
786-0	93.47
A498	-24.59
ACHN	27.88
CAKI-1	99.02
RXF 393	73.51
SN12C	84.62
TK-10	109.09
UO-31	17.49
Prostate Cancer	
PC-3	83.12
DU-145	118.84
Breast Cancer	
MCF7	89.73
MDA-MB-231/ATCC	99.61
HS 578T	-8.92
BT-549	116.58
T-47D	89.34
MDA-MB-468	101.49

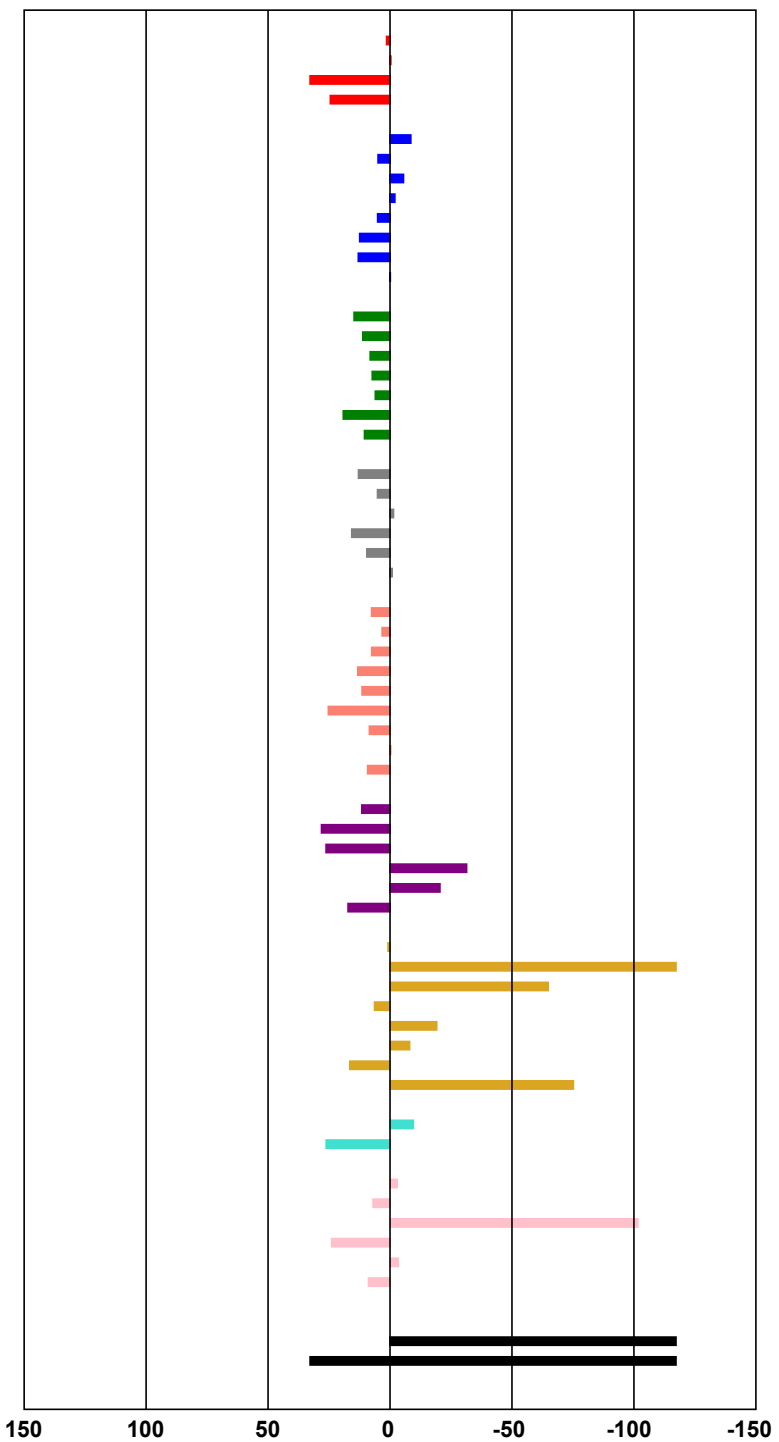
Bar Graph



DTP Figure S5b. Compound 19 One Dose Mean Graph	NSC: D-778312 / 1	Conc: 1.00E-5 Molar	Test Date: Dec 02, 2013
	Experiment ID: 1312OS99		

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	94.07
K-562	92.32
MOLT-4	125.43
SR	117.13
Non-Small Cell Lung Cancer	
A549/ATCC	84.13
HOP-62	97.57
HOP-92	87.13
NCI-H226	90.67
NCI-H23	97.74
NCI-H322M	105.10
NCI-H460	105.67
NCI-H522	92.53
Colon Cancer	
COLO 205	107.40
HCC-2998	103.84
HCT-116	100.80
HCT-15	99.96
HT29	98.69
KM12	111.83
SW-620	103.14
CNS Cancer	
SF-268	105.59
SF-295	97.79
SF-539	91.23
SNB-19	108.35
SNB-75	102.12
U251	91.84
Melanoma	
LOX IMVI	100.20
MALME-3M	95.90
M14	100.16
MDA-MB-435	105.86
SK-MEL-2	104.13
SK-MEL-28	117.95
SK-MEL-5	101.11
UACC-257	92.39
UACC-62	101.83
Ovarian Cancer	
IGROV1	104.22
OVCAR-3	120.76
OVCAR-5	118.88
OVCAR-8	61.25
NCI/ADR-RES	72.19
SK-OV-3	109.87
Renal Cancer	
786-0	93.47
A498	-24.59
ACHN	27.88
CAKI-1	99.02
RXF 393	73.51
SN12C	84.62
TK-10	109.09
UO-31	17.49
Prostate Cancer	
PC-3	83.12
DU-145	118.84
Breast Cancer	
MCF7	89.73
MDA-MB-231/ATCC	99.61
HS 578T	-8.92
BT-549	116.58
T-47D	89.34
MDA-MB-468	101.49
Mean	92.66
Delta	117.25
Range	150.02

Mean Growth Percent - Growth Percent

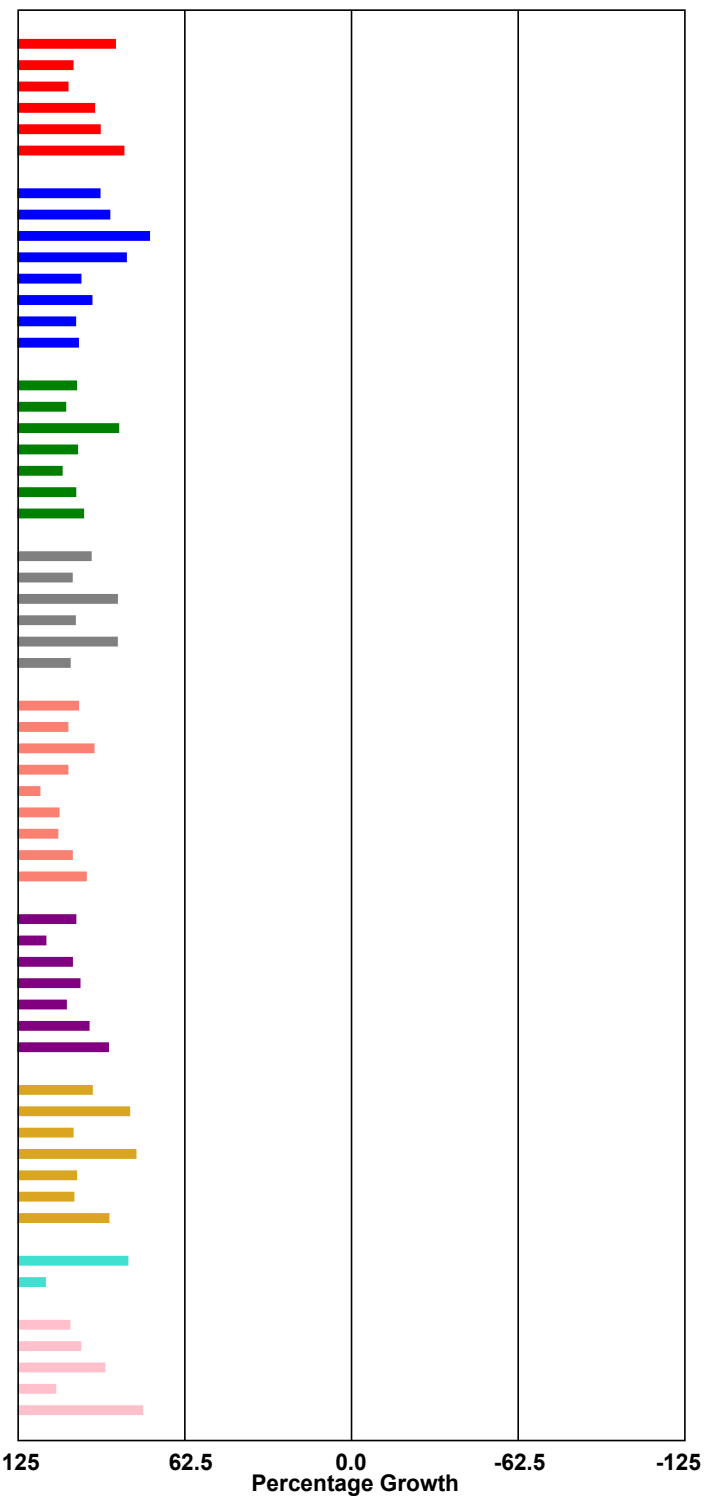


DTP Figure S6a. Compound 20
One Dose Bar Graph

NSC: D-777412 / 1	Conc: 1.00E-5 Molar	Test Date: Sep 30, 2013
Experiment ID: 1309OS80		

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	88.63
HL-60(TB)	104.52
K-562	106.42
MOLT-4	96.46
RPMI-8226	94.34
SR	85.46
Non-Small Cell Lung Cancer	
A549/ATCC	94.42
HOP-62	90.75
HOP-92	75.85
NCI-H226	84.54
NCI-H23	101.57
NCI-H322M	97.44
NCI-H460	103.56
NCI-H522	102.51
Colon Cancer	
COLO 205	103.21
HCC-2998	107.28
HCT-116	87.42
HCT-15	102.83
HT29	108.62
KM12	103.52
SW-620	100.57
CNS Cancer	
SF-268	97.74
SF-295	104.84
SF-539	87.89
SNB-19	103.67
SNB-75	87.93
U251	105.60
Melanoma	
LOX IMVI	102.48
MALME-3M	106.46
M14	96.67
MDA-MB-435	106.48
SK-MEL-2	116.95
SK-MEL-28	109.79
SK-MEL-5	110.24
UACC-257	104.80
UACC-62	99.56
Ovarian Cancer	
IGROV1	103.48
OVCAR-3	114.72
OVCAR-4	104.77
OVCAR-5	101.93
OVCAR-8	107.04
NCI/ADR-RES	98.53
SK-OV-3	91.26
Renal Cancer	
786-0	97.31
A498	83.31
ACHN	104.52
CAKI-1	80.94
SN12C	103.21
TK-10	104.20
UO-31	91.08
Prostate Cancer	
PC-3	83.98
DU-145	114.90
Breast Cancer	
MCF7	105.67
MDA-MB-231/ATCC	101.66
HS 578T	92.63
BT-549	111.03
T-47D	78.42

Bar Graph

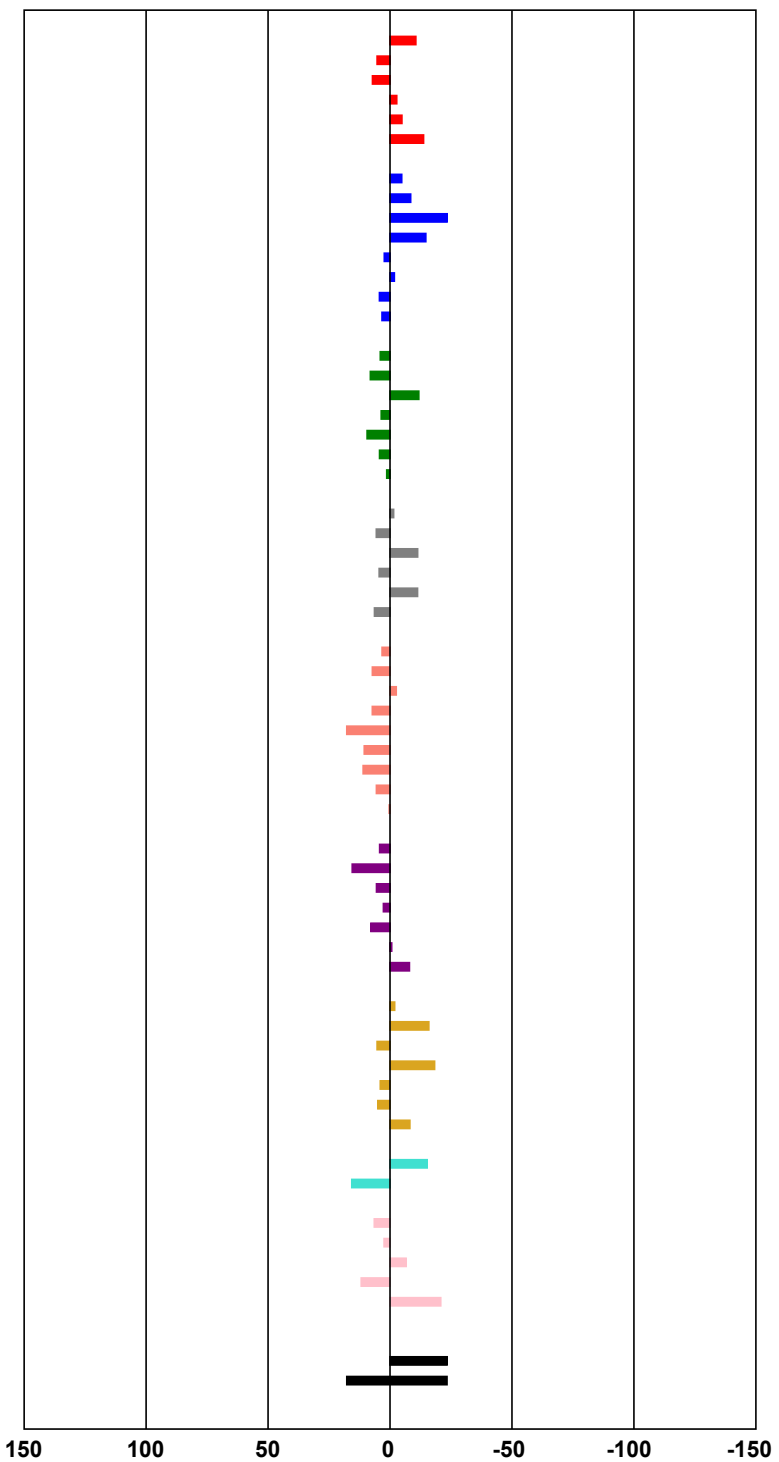


DTP Figure S6b. Compound 20
One Dose Mean Graph

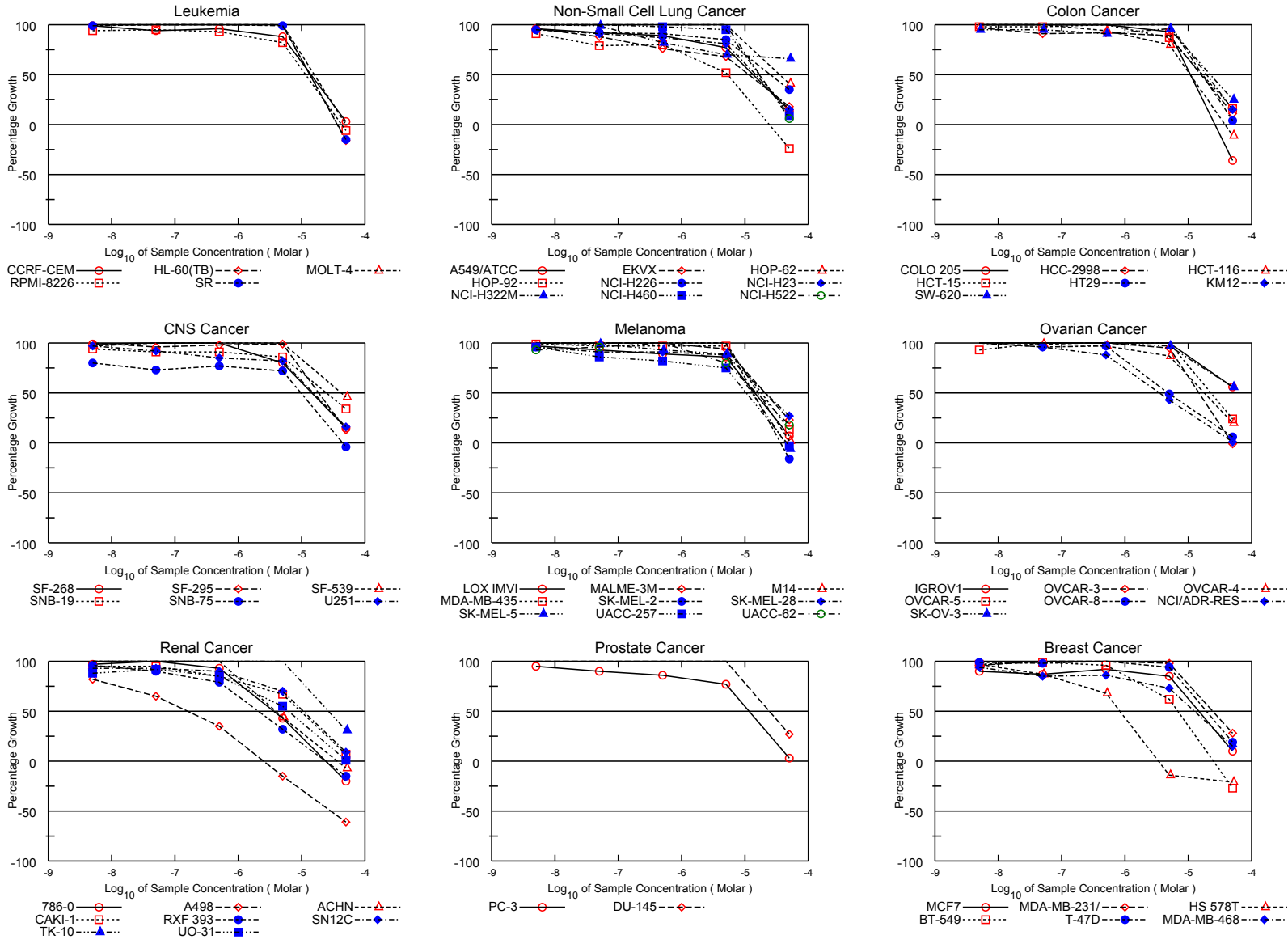
NSC: D-777412 / 1	Conc: 1.00E-5 Molar	Test Date: Sep 30, 2013
Experiment ID: 1309OS80		

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	88.63
HL-60(TB)	104.52
K-562	106.42
MOLT-4	96.46
RPMI-8226	94.34
SR	85.46
Non-Small Cell Lung Cancer	
A549/ATCC	94.42
HOP-62	90.75
HOP-92	75.85
NCI-H226	84.54
NCI-H23	101.57
NCI-H322M	97.44
NCI-H460	103.56
NCI-H522	102.51
Colon Cancer	
COLO 205	103.21
HCC-2998	107.28
HCT-116	87.42
HCT-15	102.83
HT29	108.62
KM12	103.52
SW-620	100.57
CNS Cancer	
SF-268	97.74
SF-295	104.84
SF-539	87.89
SNB-19	103.67
SNB-75	87.93
U251	105.60
Melanoma	
LOX IMVI	102.48
MALME-3M	106.46
M14	96.67
MDA-MB-435	106.48
SK-MEL-2	116.95
SK-MEL-28	109.79
SK-MEL-5	110.24
UACC-257	104.80
UACC-62	99.56
Ovarian Cancer	
IGROV1	103.48
OVCAR-3	114.72
OVCAR-4	104.77
OVCAR-5	101.93
OVCAR-8	107.04
NCI/ADR-RES	98.53
SK-OV-3	91.26
Renal Cancer	
786-0	97.31
A498	83.31
ACHN	104.52
CAKI-1	80.94
SN12C	103.21
TK-10	104.20
UO-31	91.08
Prostate Cancer	
PC-3	83.98
DU-145	114.90
Breast Cancer	
MCF7	105.67
MDA-MB-231/ATCC	101.66
HS 578T	92.63
BT-549	111.03
T-47D	78.42
Mean	99.22
Delta	23.37
Range	41.10

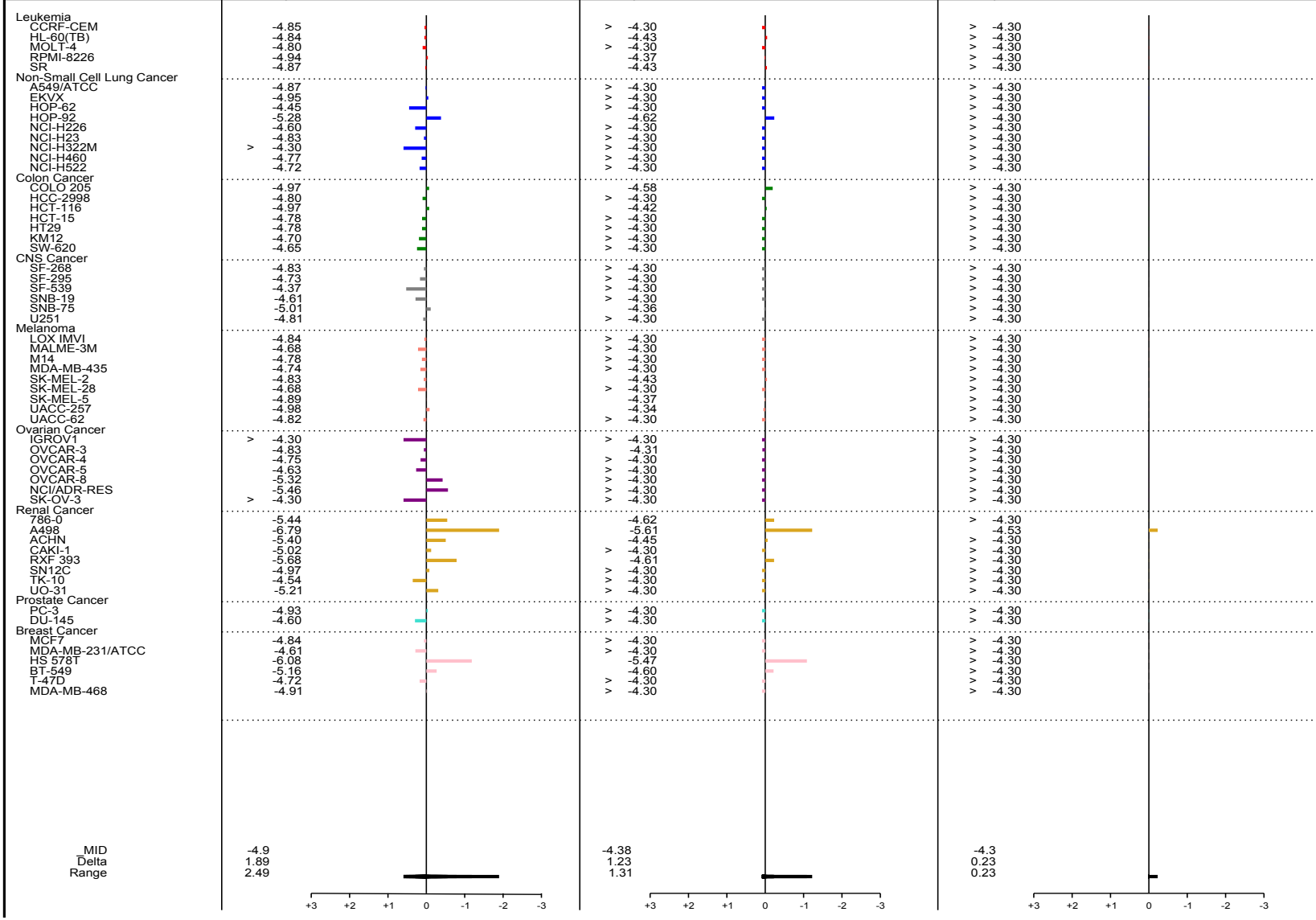
Mean Growth Percent - Growth Percent



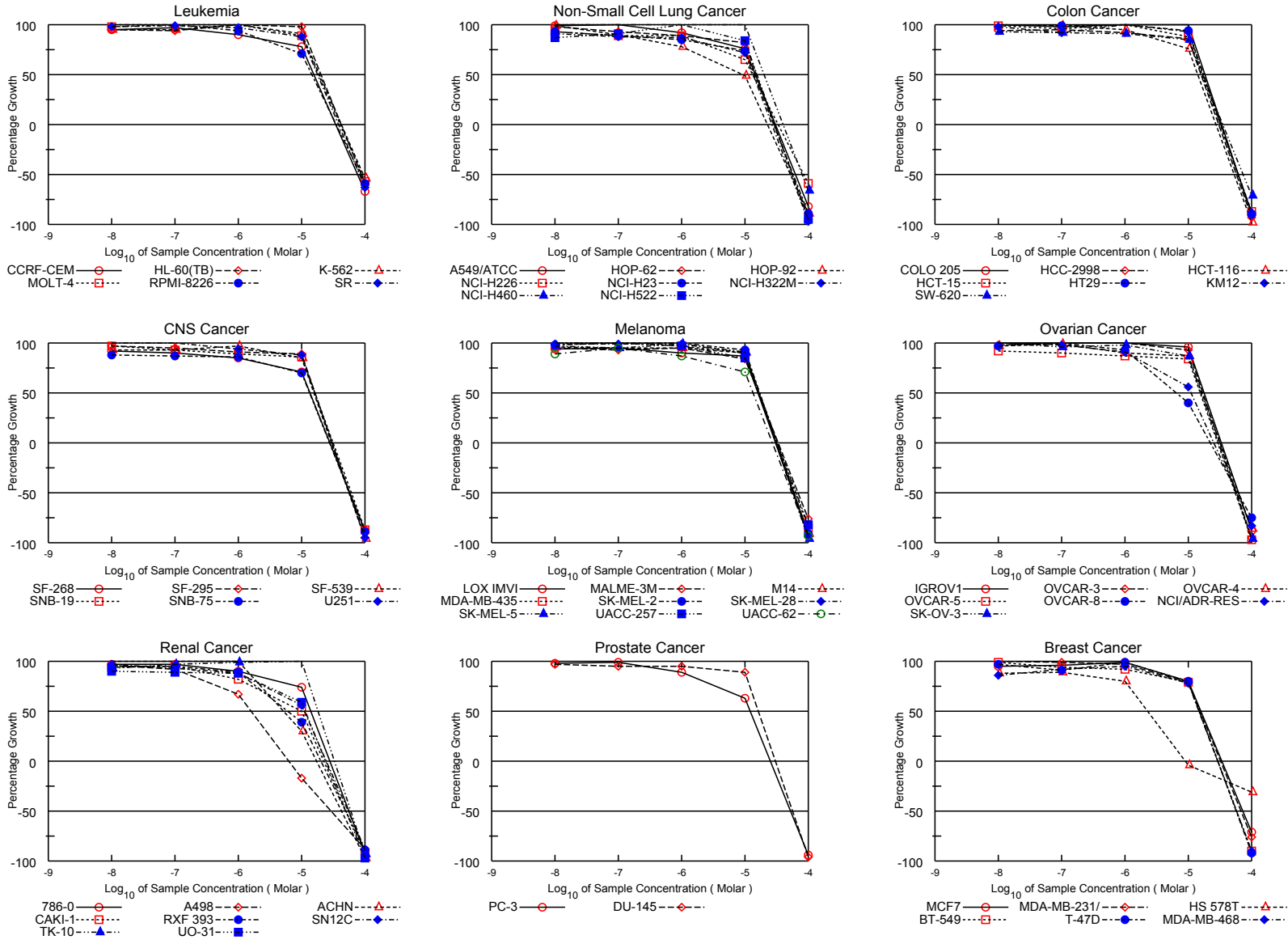
DTP, NCI	Figure S7a. Compound 2 Dose Response Curves	NSC: D - 761305 / 1	SSPL: 0GZS	EXP. ID: 1109NS27
			Test Date: September 26, 2011	



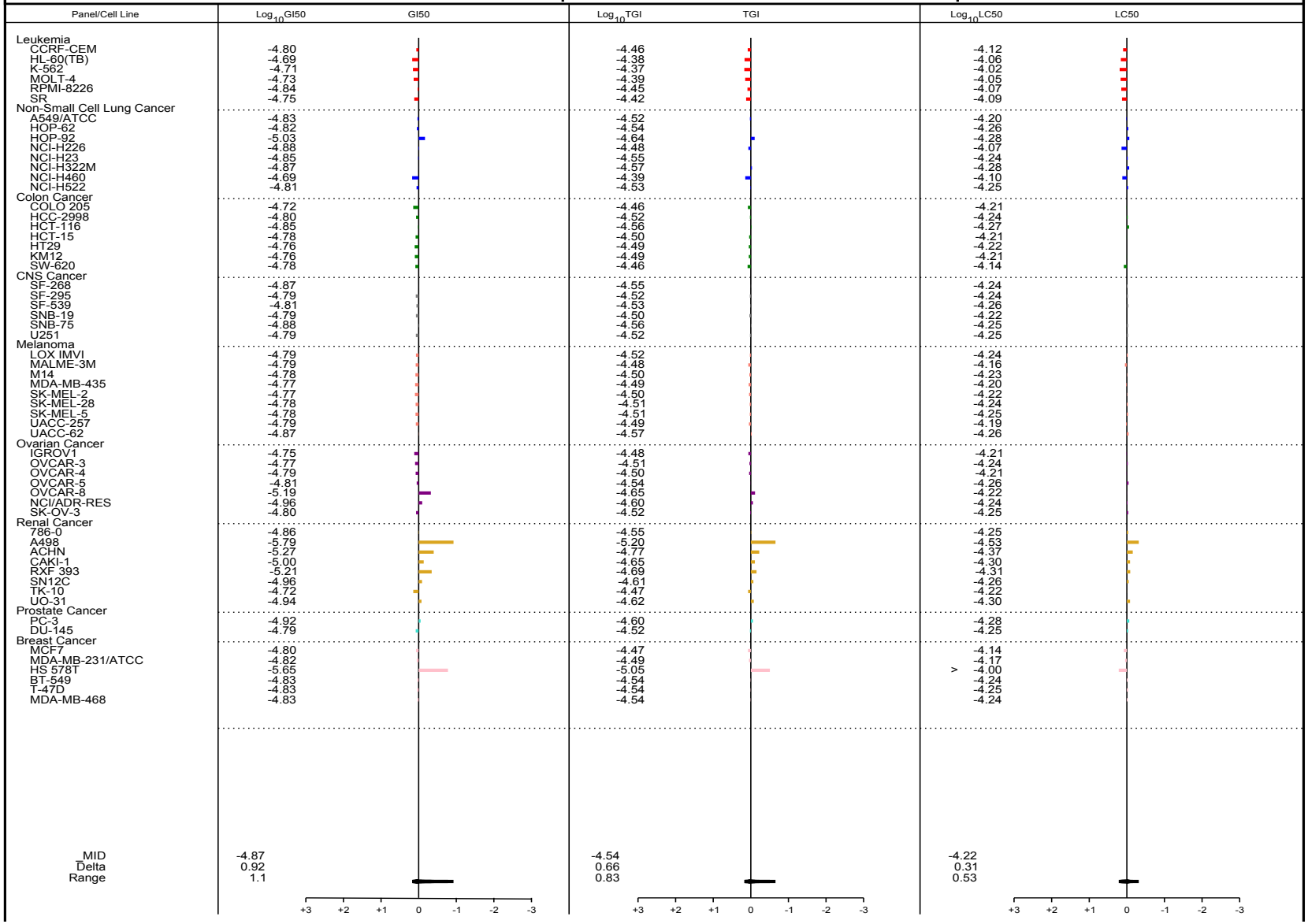
DTP, NCI	Figure S7b. Compound 2 Mean Graphs	NSC : D - 761305/1	Units :Molar	SSPL :0GZS	EXP. ID :1109NS27
				Test Date :September 26, 2011	



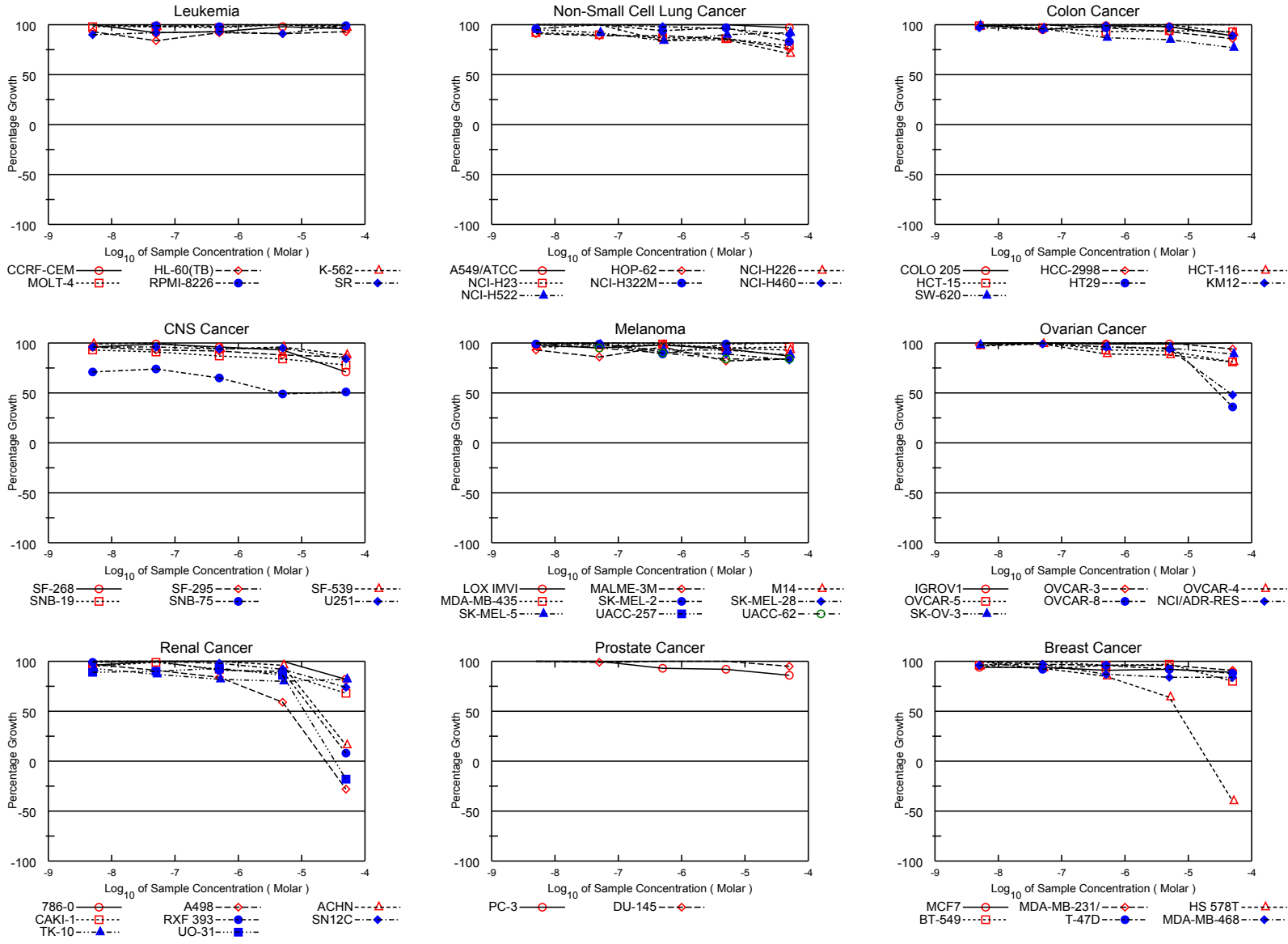
DTP, NCI	Figure S8a. Compound 17 Dose Response Curves	NSC: D - 764081 / 1	SSPL: 0GZS	EXP. ID: 1204NS58
			Test Date: April 30, 2012	



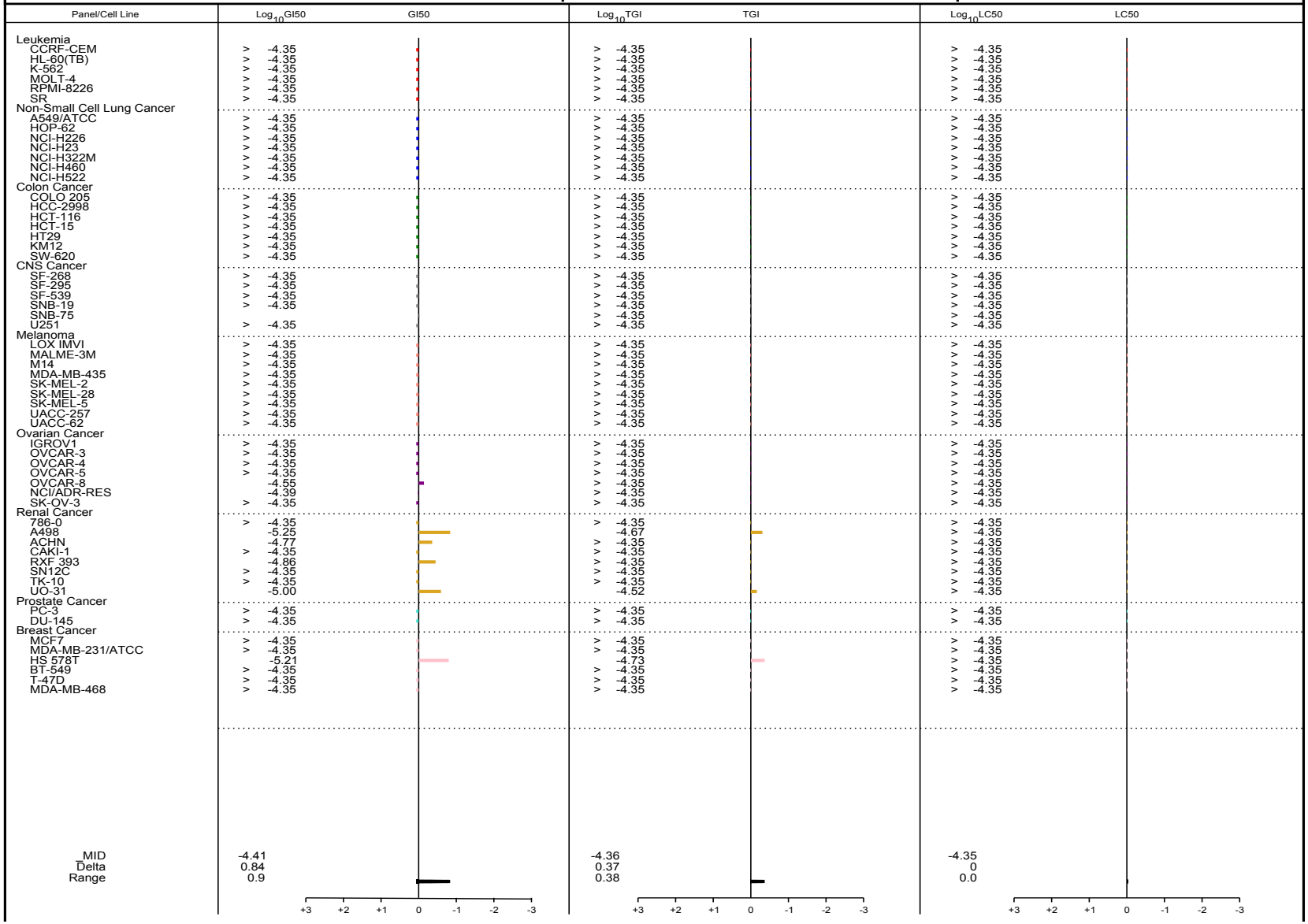
DTP, NCI	Figure S8b. Compound 17 Mean Graphs	NSC : D - 764081/1	Units :Molar	SSPL :0GZS	EXP. ID :1204NS58
				Test Date :April 30, 2012	



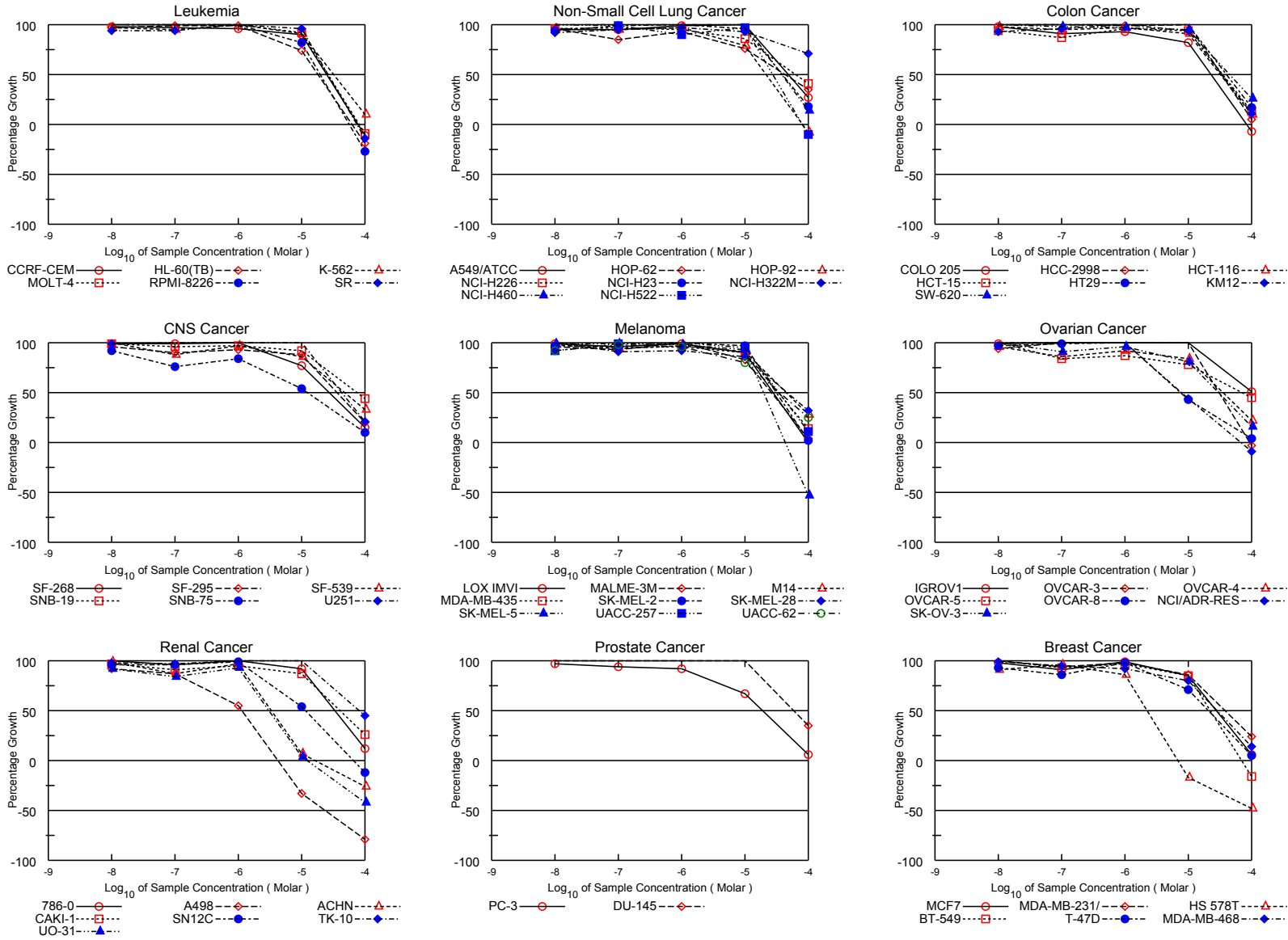
DTP, NCI	Figure S9a. Compound 18 Dose Response Curves	NSC: D - 768544 / 1	SSPL: 0GZS	EXP. ID: 1305NS81
			Test Date: May 13, 2013	



DTP, NCI	Figure S9b. Compound 18 Mean Graphs	NSC : D - 768544/1	Units :Molar	SSPL :0GZS	EXP. ID :1305NS81
				Test Date :May 13, 2013	



DTP, NCI	Figure S10a. Compound 19 Dose Response Curves	NSC: D - 778312 / 1	SSPL: 0GZS	EXP. ID: 1402NS22
			Test Date: February 03, 2014	



DTP, NCI	Figure S10b. Compound 19 Mean Graphs	NSC : D - 778312/1	Units :Molar	SSPL :0GZS	EXP. ID :1402NS22
				Test Date :February 03, 2014