Supporting Information

Synthesis of "neoprofen", a rigidified analogue of ibuprofen, exemplifying synthetic methodology for enhancing the 3-D topology of pharmaceutical substances

*Ron R. Ramsubhag, Chelsea L. Massaro, Christina M. Dadich, Andrew J. Janeczek, Tung T. Hoang, Elizabeth A. Mazzio, Suresh Eyunni, Karam F. A. Soliman, and Gregory B. Dudley**

Department of Chemistry and Biochemistry, Florida State University, Tallahassee, Florida 32306-4390, USA

College of Pharmacy and Pharmaceutical Sciences, Florida Agricultural and Mechanical University, Tallahassee, Florida 32307, USA

C. Eugene Bennett Department of Chemistry, West Virginia University, Morgantown, West Virginia 26506, USA

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1. General information

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a 400 or 600 MHz spectrometer using $CDCl_3$ as the deuterated solvent. The chemical shifts (δ) are reported in parts per million (ppm) relative to the residual $CHCl_3$ peak (7.26 ppm for ^{1}H-NMR and 77.0 for ^{13}C-NMR). The coupling constants (*J*) are reported in Hertz (Hz). Mass spectra were recorded using electrospray ionization (ESI) and atmospheric-pressure chemical ionization (APCI). Yields refer to isolated material judged to be ≥ 95% pure by ^{1}H NMR spectroscopy following silica gel chromatography. All chemicals were used as received unless otherwise stated. The purifications were performed by flash chromatography using silica gel with 40-63 micron particle size.

Methods and Materials

Hanks Balanced Salt Solution, (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (HEPES), sulfanilamide, 96 well plates, general reagents and supplies, were all purchased from Sigma Scientific (Sigma -Aldrich, St Louis, MO) or VWR (Radnor, PA). COX Activity Kits were purchased from Abcam (Cambridge, MA) and Cayman Chemical (Ann Arbor, Michigan) .

Cell Culture

BV-2 microglia cells were provided by Elizabeta Blasi.[1] Cells were cultured in DMEM high glucose media [glucose 4500mg/L] containing 5% FBS, 4 mM L-glutamine, and penicillin/streptomycin (100 U /0.1 mg/ml). Culture conditions were maintained at 37°C in 5% CO_2/atmosphere and every 2–3 days, the media was replaced and cells sub-cultured. For experiments, plating media consisted of DMEM (minus phenol red) [glucose 4500mg/L], 2.5% FBS and penicillin/streptomycin (100 U /0.1 mg/ml). LPS O111:B4 was prepared in HBSS at 1 mg/ml and stored at –20°C. For experiments, LPS was added to the culture media at a working concentration of 1μg/ml.

Cell Viability

Cell viability was quantified using resazurin [7-Hydroxy-3H-phenoxazin-3-one 10-oxide] (Alamar Blue) indicator dye.[2] A working solution of resazurin was prepared in sterile HBSS minus phenol red (0.5 mg/ml), then added (15% v/v) to each sample. Samples were returned to the incubator for 2–4 hr, and reduction of the dye by viable cells (to resorufin, a fluorescent compound) was quantitatively analyzed using a Synergy HTX multi-mode reader (Bio-Tek, Winooski, VT) with settings at [550/580], [excitation/emission].

Cyclooxygenase 2 Activity

Quantification of PGF2 from arachadonic acid by human COX-2 was determined using enzyme activity combined with ELISA (Cayman Chemical , Ann Arbor MI) following the manufacturers guidelines. Samples were analyzed at 405 nm on a Synergy HTX multi-mode reader (Bio-Tek, Winooski, VT).

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1. Blasi E, Barluzzi R, et al. J Neuroimmunol. 1990;27(2–3):229–237.
2. Evans SM, Casartelli A, et al. Toxicol In Vitro. 2001;15(4–5):579–584.

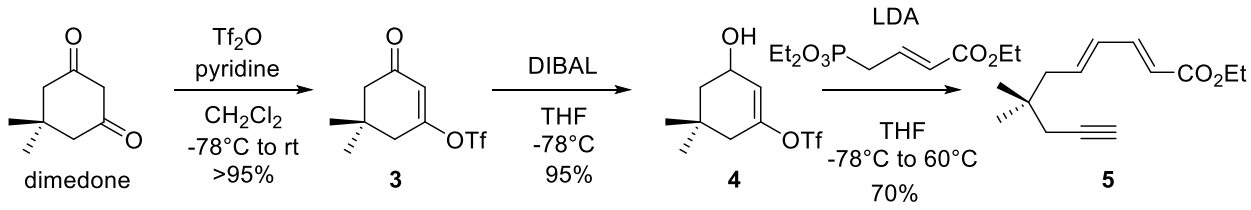
Nitrite (NO_2–)

Quantification of nitrite (NO_2−) was determined using the Greiss reagent.[3] Greiss reagent was prepared by mixing an equal volume of 1.0% sulfanilamide in 10% phosphoric acid and 0.1% N-(1-naphthyl)-ethylenediamine in deionized water, then added directly to the cell supernatant (experimental media consisting of DMEM - phenol red) and incubated at room temperature for 10 min. Controls and blanks were run simultaneously, and subtracted from the final value to eliminate interference. Samples were analyzed at 540 nm on a Synergy HTX multi-mode reader (Bio-Tek, Winooski, VT).

Data analysis

Statistical analysis was performed using Graph Pad Prism (version 3.0; Graph Pad Software Inc. San Diego, CA, USA) with significance of difference between the groups assessed using a one-way analysis of variance (ANOVA), followed by Tukey post hoc means comparison test, or Student's t test. IC_{50}s were determined by regression analysis using Origin Software (OriginLab, Northampton, MA)

2. Synthesis of ethyl (2E,4E)-7,7-dimethyldeca-2,4-dien-9-ynoate (5)



To a solution of dimedone (1.0 g, 7.1 mmol, 1 equiv) in dichloromethane (30 ml) was added pyridine (1.15 ml, 14.2 mmol, 2 equiv) under nitrogen. The resulting mixture was stirred for 10 minutes at -78°C before adding trifluoromethanesulfonic anhydride (1.44 ml, 8.6 mmol, 1.2 equiv) dropwise via syringe. The reaction mixture was stirred at -78°C for 20 minutes, warmed to 0°C for 20 minutes, and warmed further to room temperature for 30 minutes. The reaction was quenched using a 1M HCl solution (30 ml) when complete consumption of dimedone was observed by TLC and extracted 3 times with diethyl ether (3x10 ml). The combined organic layers were washed with aqueous Na_2CO_3 and water, dried over sodium sulfate, filtered and concentrated. The resulting oil was purified by flash column chromatography (0 to 5%

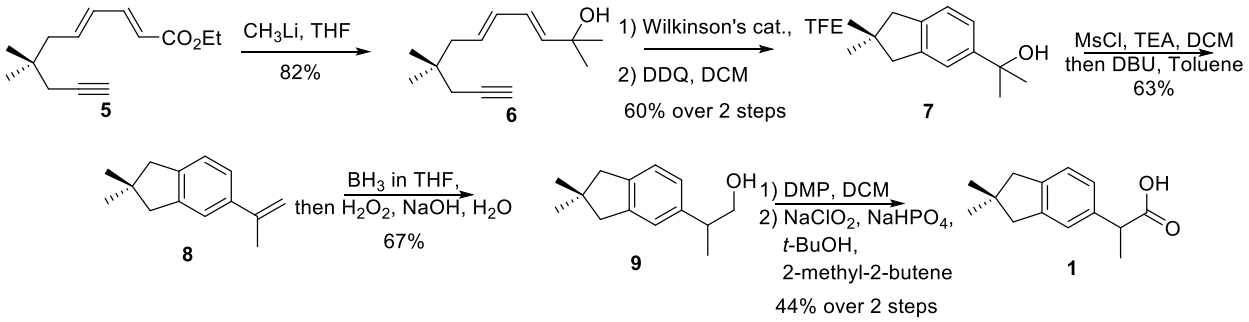
3. Ko SH, Ryu GR, et al.. Transplantation. 2008;85(3):323–330.

EtOAc/Hexanes) to yield 1.89g (>95%) of triflate **3** as a colorless oil. Characterization data were in agreement with the literature.[4]

To a stirred solution of triflate **3** (2.48 g, 9.1 mmol, 1 equiv) in 42 ml THF at -78°C was slowly added DIBAL-H (10.9 ml, 1.0 M solution in toluene, 1.2 equiv). The resulting mixture was stirred at -78°C for 10 minutes, warmed to 0°C for 10 minutes, and room temperature for 30 minutes. The reaction mixture was then diluted with ether and cooled to 0°C before quenching with 15% NaOH and water. The mixture was stirred for 15 minutes before added $MgSO_4$. Following the addition of $MgSO_4$, the mixture was stirred for an additional 15 minutes. Vacuum filtration and rotary evaporation gave the crude vinylogous hemiacetal triflate **4**. Purification by flash column chromatography (2% to 5% EtOAc/Hexane) gave 1.85g (>95%) of vinylogous hemiacetal triflate **4** as a colorless oil. Characterization data were in agreement with the literature.[4]

To a solution of diisopropylamine in THF (0.1 M) at -78°C was added dropwise *n*-BuLi (2.5 M solution in hexane). The mixture was then stirred at -78°C for 10 minutes, warmed to 0°C for 30 minutes, and then cooled back to -78°C prior to the successive addition of vinylogous hemiacetal triflate **4** (1.56 g, 5.63 mmol, 1 equiv) and phosphonate (1.56 g, 6.19 mmol, 1.1 equiv). The reaction mixture was stirred at -78°C for 10 minutes, warmed to 0°C for 10 minutes, and heated to 60°C in an oil bath for 2 hours. After 2 hours, the mixture was cooled to room temperature before quenching the reaction with half-saturated NH_4Cl. The mixture was extracted three times with diethyl ether and the combined organic layers washed with water, dried over $MgSO_4$, and concentrated. The resulting oil was purified by flash column chromatography (1% to 2.5% EtOAc/Hexane) to yield 0.868g (70%) of dienyne **5** as a light yellow oil.

3. Synthesis of neoprofen 1



4. Hoang, T. T.; Dudley, G. B. *Org. Lett.* **2013**, *15*, 4026-4029.

To a solution of THF (0.05 M) was added MeLi (1.7 ml, 1.6 M in diethylether, 5 equiv) under nitrogen at room temperature. The reaction mixture was brought down to -78°C. A solution of the dienyne ester **5** (0.12 g, 0.545 mmol, 1 equiv) in THF (1 ml) was added dropwise to the MeLi mixture -78°C. TLC revealed consumption of starting material after 1 hr. The reaction was then cooled to 0°C and then quenched with a saturated solution of NH_4Cl. The following solution was then extracted with EtOAc three times. The organic layers were then collected, dried with $MgSO_4$, and concentrated under reduced pressure to give an oil. The resulting oil was purified by flash column chromatography (5% to 10 EtOAc/Hexane) to yield 91 mg (82%) of alcohol **6** as a colorless oil.

To a solution of **6** (7 mg, 0.034 mmol, 1 equiv) in 2,2,2-trifluorethanol (1 ml) under nitrogen was added Wilkinson's cat. (4 mg, 0.004 mmol, 10 mol%). The reaction mixture was then heated to 55°C and left to stir overnight. TLC revealed the consumption of starting material. The reaction was then diluted with water and extracted with DCM three times. The organic layers were collected, dried with sodium sulfate, and concentrated under reduced pressure. No further purification was needed and the oil residue was taken on to the next step. The oil residue was then dissolved with DCM (1.5 ml). DDQ (0.015 g, 0.07 mmol, 2 equiv) was added to the reaction and left to stir at room temperature for 7 hours. The reaction was then concentrated to give a brown oil. The oil was purified by flash column chromatography (5% to 10% EtOAc/Hexane) to yield 4.2 mg (60%) of indane **7** as a colorless oil.

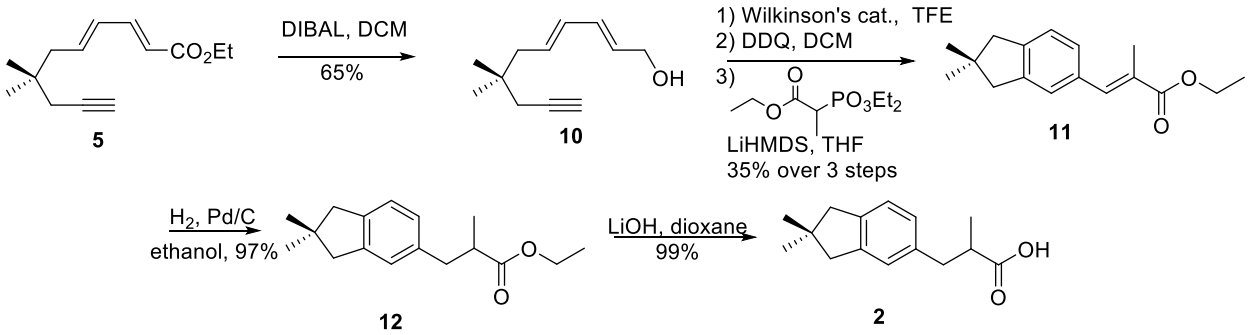
To a solution of tertiary alcohol **7** (0.03 g, 0.147 mmol, 1 equiv) in dry DCM (0.2 M) under nitrogen at 0°C was added TEA (0.061 ml, 0.441 mmol, 3 equiv). Methanesulfonyl chloride (0.034 ml, 0.441 mmol, 3 equiv) was then added to the stirring solution at 0°C over 5 min. The reaction continued stirring for an hour before quenching with aq. $NaHCO_3$ and extracted with $CHCl_3$ three times. The organic layers were collected, dried with sodium sulfate, and concentrated under reduced pressure. No further purification was needed and the oil residue was taken on to the next step. The oil residue was then dissolved with toluene (0.2 M) under nitrogen. DBU (0.066 ml, 0.441 mmol, 3 equiv) was then added dropwise to the reaction and heated to 50°C to stir overnight. TLC showed a new nonpolar spot as well as consumption of starting material. The reaction mixture was cooled to 0°C and quenched with a saturated solution of aq. $NaHCO_3$. The aqueous layer was extracted with diethyl ether three times. The organic layers were collected, washed with brine, dried with sodium sulfate, concentrated under reduced pressure to give an oil. The resulting oil was purified by flash column chromatography (100% Hexane) to yield 17 mg (63%) of alkene **8** as a colorless oil.

To a solution of the styrene derivative **8** (0.014g, 0.082 mmol, 1 equiv) in dry THF (0.68 M) under nitrogen at 0°C was added the borane-THF complex (0.091 ml, 1 M in THF, 1.1 equiv) dropwise. The reaction mixture was allowed to warm up to room temperature and stirred for an additional 5 hours. H_2O (0.012ml, 0.68 M), 15% aq. NaOH (0.06 ml, 1.36 M), and H_2O_2 (0.06 ml, 1.36 M) were added dropwise to the stirring solution and left to stir for an additional 4 hours. The reaction was then extracted with pentane three times and washed with brine. The organic

layers were collected, dried with $MgSO_4$, and concentrated under reduced presuure to give a colorless oil. The resulting oil was purified by flash column chromatography (5% to 10% EtOAc/Hexane) to yield 10.3 mg (67%) of primary alcohol **9** as a colorless oil.

Primary alcohol **9** (0.017g, 0.083 mmol, 1 equiv) was added to dry DCM (0.2 M) under nitrogen. $NaHCO_3$ (0.025g, 0.416 mmol, 5 equiv) was added to the reaction mixture and cooled to 0°C. Dess-Martin periodinane (0.053g, 0.125 mmol, 1.5 equiv) was then added to the cooled solution and left to stir for 8 hours at room temperature. The reaction was then quenched with a solution of aq. $NaHCO_3$ and extracted with diethyl ether three times. The organic layers were then collected and washed with a saturated solution of aq. Na_2SO_3. The organic layer was then collected, dried over sodium sulfate, and concentrated under reduced pressure to give an oil. A crude ^{1}H-NMR revealed the aldehyde intermediate. The oil was then dissolved in *t*-BuOH (0.151 ml), followed by the addition of 2-methylbut-2-ene (0.151 ml, 2 M in THF, 8.88 equiv). The reaction was cooled to 0°C. A mixture of $NaClO_2$ (9.5 mg, 0.105 mmol, 3.1 equiv) and NaH_2PO_4 (0.013g, 0.105 mmol, 3.1 equiv) in H_2O (0.151 ml) was slowly added dropwise to the cooled solution. The reaction was left to stir at room temperature for 6 hours. The reaction mixture was diluted with 1 M HCl solution and extracted with DCM three times. The organic layers were then collected and washed with a saturated solution of aq. Na_2SO_3. The organic layer was then collected, dried over sodium sulfate, and concentrated under reduced pressure to give an oil. The resulting oil was purified by flash column chromatography (5% to 20% EtOAc/Hexane) to yield 8 mg (44%) of carboxylic acid **1** as a colorless oil.

4. Synthesis of homo-neoprofen 2



To a solution of dienyne ester **5** (0.210g, 0.954 mmol, 1 equiv) in DCM (0.1 M) under nitrogen at -78°C was added DIBAL dropwise (2.38 ml, 1M in toluene, 2.5 equiv). The reaction was stirred at -78°C for 4 hours. The reaction was then diluted with ether and warmed to 0°C. Water (0.10 ml) was added dropwise to the cooled solution, followed by the addition of 15% aq NaOH (0.10 ml). Additional water (0.238 ml) was added to the mixture. The reaction was allowed to

warm up to room temperature and left to stir for 15 minutes. Anhydrous $MgSO_4$ was added to the mixture and left stirring for an added 15 minutes. The reaction mixture was filtered to remove salts and then concentrated under reduced pressure. The resulting crude oil was purified using flash column chromatography (5% to 10% EtOAc/Hexanes) to give 0.110g (65%) of primary alcohol **10** as a colorless oil.

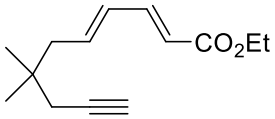
To a solution of **10** (0.01g, 0.056 mmol, 1 equiv) in 2,2,2-trifluorethanol (1 ml) under nitrogen was added Wilkinson's cat. (6 mg, 0.006 mmol, 10 mol%). The reaction mixture was then heated to 55°C and left to stir overnight. TLC revealed the consumption of starting material. The reaction was then diluted with water and extracted with DCM three times. The organic layers were collected, dried with sodium sulfate, and concentrated under reduced vacuum. No further purification was needed and the oil residue was taken on to the next step. The oil residue was then dissolved with DCM (1.5 ml). DDQ (0.057g, 0.252 mmol, 4.5 equiv) was added to the reaction and left to stir at room temperature for 7 hours. TLC showed a new nonpolar spot, tentatively identified as the indanecarboxaldehyde intermediate. The reaction mixture was then concentrated under reduced pressure, filtered through a silica plug (10% EtOAc/Hexanes), concentrated under reduced pressure, and carried forward without further purification.

To a stirred 1.0 M solution of LiHMDS in THF (0.073 mL, 1 M in THF, 1.3 equiv) at -78°C was slowly added triethyl 2-phosphonopriopionate (0.013 mL, 0.062 mmol, 1.1 equiv). The resulting mixture was stirred for 5 minutes before the dropwise addition of the presumed indanecarboxaldehyde intermediate from the previous step. The reaction mixture was stirred at -78°C for 10 minutes, warmed to 0°C for 10 minutes, and room temperature for 30 minutes. The reaction was quenched with half-saturated NH_4Cl and extracted three times with diethyl ether. The combined organic layers were washed with water, dried over $MgSO_4$, and concentrated. The resulting oil was purified by flash column chromatography (2.5% EtOAc/Hexane) to yield 4.5 mg (35% over the three steps) of α-β-unsaturated ester **11** as a colorless oil.

To a stirred solution of α-β-unsaturated ester **11** (53.7 mg, 0.208 mmol, 1 equiv) in ethanol (4 ml) was added palladium on activated carbon (19.9 mg). The reaction mixture was flushed and maintained under a hydrogen atmosphere and stirred overnight at 35°C. The solution was filtered over celite and extracted three times with ethyl acetate. The combined organic layers were washed with water, dried over Na_2SO_4, and concentrated. No further purification was required. The resulting oil gave 52 mg (97%) of the saturated ester **12** as a colorless oil.

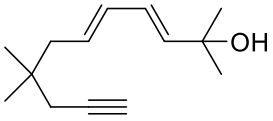
To a stirred solution of ester **12** (25.6 mg, 0.098 mmol, 1 equiv) in dioxane (0.77 ml) was added 0.5 M LiOH (0.39 ml) solution. The reaction mixture was stirred for 2 days at room temperature until complete consumption of the starting material **12** was observed by TLC. The reaction mixture was quenched with AcOH at 0°C. The resulting solution was extracted three times with ethyl acetate and the combined organic layers were washed with water, dried over Na_2SO_4, and concentrated. The resulting oil gave 22.5 mg (99%) of **2** as a colorless oil.

5. Characterization data of all compounds



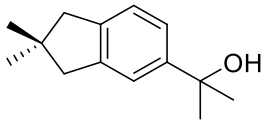
5

Mass = 0.86g; Yield = 70%
Light yellowish oil
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.30-7.22 (m, 1H, ($CDCl_3$ in this region)), 6.25-6.07 (m, 1H), 5.83-5.78 (m, 1H), 4.20 (q, *J*=7.12 Hz, 2H), 2.18 (d, *J*=7.36 Hz, 2H), 2.08-2.05 (m, 2H), 2.02-1.99 (m, 1H), 1.29 (t, *J*=7.12 Hz, 4H), 0.98 (s, 6H) ppm;
^{13}C NMR (600 MHz, $CDCl_3$): δ 167.2, 144.6, 140.5, 131.1, 119.8, 82.0, 70.3, 60.2, 44.4, 34.3, 31.5, 26.7, 14.3 ppm;
HRMS (ESI+) calcd for $C_{14}H_{20}O_2Na$ $(M+Na)^+$ 243.1361 found 243.1360



6

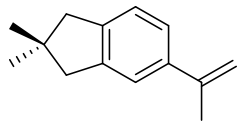
Mass = 0.091g; Yield = 82%
Colorless oil
^{1}H NMR (400 MHz, $CDCl_3$): δ 6.25-6.16 (m, 1H), 6.10-6.00 (m, 1H), 5.78-5.63 (m, 2H), 2.13-2.037 (m, 4H), 1.99 (t, *J*=2.61 Hz, 1H), 1.40 (broad s, 1H), 1.34 (s, 6H), 0.97 (s, 6H) ppm;
^{13}C NMR (600 MHz, $CDCl_3$): δ 139.3, 132.6, 130.7, 126.8, 82.5, 70.7, 70.0, 44.3, 34.2, 31.4, 29.8, 26.6 ppm;
HRMS (APCI) calcd for $C_{14}H_{23}O$ $(M+H)^+$ 207.17434 found 207.17484



7

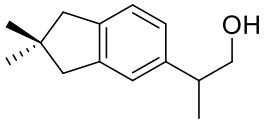
Mass = 4.2 mg; Yield = 60%
White solid
Melting point = 36°C

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.29 (s, 1H), 7.26-7.22 (m, 1H), 7.12 (d, *J*=7.53 Hz, 1H), 2.71 (d, *J*= 8.07 Hz, 4H), 1.59-1.53 (broad s, 7H), 1.14 (s, 6H) ppm;
^{13}C NMR (600 MHz, $CDCl_3$): δ 147.1, 143.6, 142.0, 124.3, 122.2, 120.8, 72.6, 47.9, 47.4, 40.2, 31.9, 28.9 ppm;
HRMS (ESI+) calcd for $C_{14}H_{20}O_2Na$ $(M+Na)^+$ 243.1361 found 243.1366



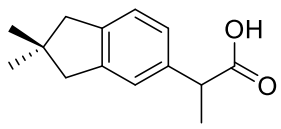
8

Mass = 17.2 mg; Yield = 63%
Colorless oil
^{1}H NMR (600 MHz, $CDCl_3$): δ 7.29 (s, 1H), 7.27-7.24 (m, 1H), 7.13 (d, *J*=7.69 Hz, 1H), 5.34-5.33 (broad s, 1H), 5.03 (t, *J*=1.50 Hz, 1H), 2.73 (d, *J*=7.49 Hz, 4H), 2.16 (s, 3H), 1.17 (s, 6H) ppm;
^{13}C NMR (600 MHz, $CDCl_3$): δ 143.7, 143.6, 143.0, 139.4, 124.4, 123.5, 121.9, 111.4, 47.8, 47.5, 40.3, 28.9, 22.1 ppm;
HRMS (APCI) calcd for $C_{14}H_{19}$ $(M+H)^+$ 187.14813 found 187.14820



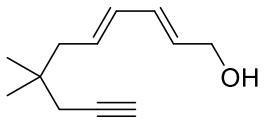
9

Mass = 10.3 mg; Yield = 67%
Colorless oil
^{1}H NMR (600 MHz, $CDCl_3$): δ 7.11 (d, *J*=7.04 Hz, 1H), 7.03 (s, 1H), 6.99 (d, *J*=8.50 Hz, 1H), 3.72-3.65 (m, 2H), 2.91 (sextet, *J*=7.20 Hz, 1H), 2.70 (d, *J*=8.00 Hz, 4H), 1.26 (d, *J*=7.20 Hz, (OH), 4H), 1.14 (s, 6H) ppm;
^{13}C NMR (600 MHz, $CDCl_3$): δ 144.1, 142.0, 141.3, 125.3, 124.8, 123.8, 68.9, 47.8, 47.4, 42.3, 40.2, 28.9, 17.8 ppm;
HRMS (ESI+) calcd for $C_{14}H_{20}Ona$ $(M+Na)^+$ 227.1412 found 227.1419



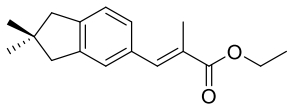
1

Mass = 8.0 mg; Yield = 44%
Colorless oil
^{1}H NMR (600 MHz, $CDCl_3$): δ 7.16-6.99 (m, 1H), 3.73-3.65 (m, 1H), 2.69 (d, *J*=9.11 Hz, 4H), 1.49 (d, *J*=7.16 Hz, 3H), 1.14 (s, 6H) ppm;
^{13}C NMR (600 MHz, $CDCl_3$): δ 180.4, 144.1, 142.8, 137.7, 125.4, 124.8, 123.9, 47.7, 47.4, 45.2, 40.2, 28.9, 18.3 ppm;
HRMS (ESI+) calcd for $C_{14}H_{18}O_2Na$ $(M+Na)^+$ 241.1204 found 241.1217



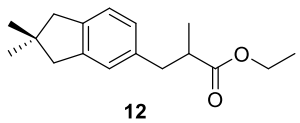
10

Mass = 104 mg; Yield = 65%
Colorless oil
^{1}H NMR (400 MHz, $CDCl_3$): δ 6.23 (dd, *J*= 15.16, 10.42 Hz, 1H), 6.07 (dd, *J* = 14.96, 10.42 Hz, 1H), 5.79-5.65 (m, 2H), 4.17 (t, *J*=5.40 Hz, 2H), 2.11-2.03 (m (CH_2, *J*=7.43 Hz) (CH_2, *J*=2.70 Hz), 4H), 1.99 (t, *J*=2.70 Hz, 1H), 1.32 (t, *J*=5.40 Hz, 1H), 0.95 (s, 6H) ppm;
^{13}C NMR (?, $CDCl_3$): δ 132.3, 131.8, 131.4, 130.0, 82.4, 70.0, 63.5, 44.2, 34.1, 31.4, 26.6 ppm;
HRMS (APCI) calcd for $C_{12}H_{19}O$ $(M+H)^+$ 179.14304 found 179.14322



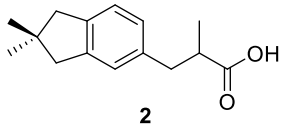
11

Mass = 4.6 mg; Yield = 35%
Colorless oil
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.67 (s, 1H), 7.24-7.15 (m, 3H), 4.26 (q, *J*=7.12 Hz, 2H), 2.76-2.71 (m, 4H), 2.15-2.11 (m, 3H), 1.34 (t, *J*=7.12, 3H), 1.16 (s, 6H) ppm;
^{13}C NMR (600 MHz, $CDCl_3$): δ 168.9, 144.3, 143.8, 139.3, 133.9, 128.0, 127.3, 126.0, 124.6, 60.8, 47.6, 40.3, 28.8, 14.4, 14.2 ppm;
HRMS (ESI+) calcd for $C_{17}H_{22}O_2Na$ $(M+Na)^+$ 281.1518 found 281.1514



12

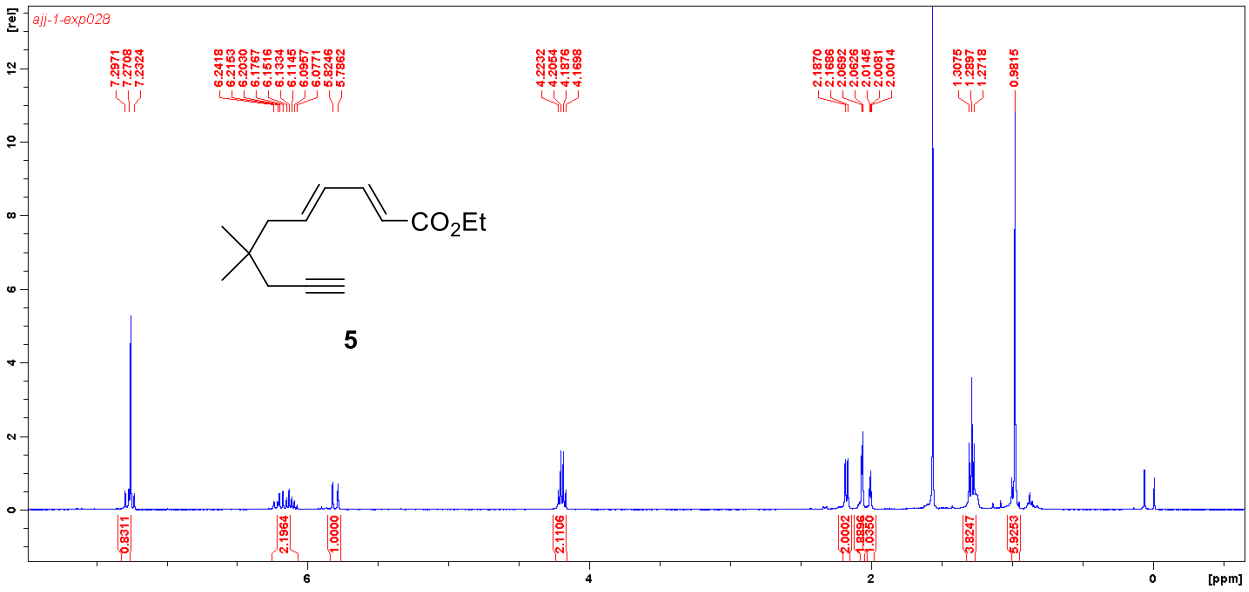
Mass = 52 mg; Yield = 97%
Colorless oil
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.05 (d, *J*=7.52 Hz, 1H), 6.96 (s, 1H), 6.91 (d, *J*=7.52, 1H), 4.09 (q, *J*=7.08, 2H), 2.99-2.95 (dd, *J*=13.1 Hz, 6.52 Hz, 1H), 2.74-2.56 (m, 6H), 1.22-1.11 (m, 12H (CH_3, t, *J*=7.12 Hz)) ppm;
^{13}C NMR (600 MHz, $CDCl_3$): δ 176.4, 143.7, 141.5, 137.2, 126.8, 125.4, 124.5, 60.2, 47.7, 47.4, 41.7, 40.2, 39.7, 28.8, 16.8, 14.2 ppm;
HRMS (ESI+) calcd for $C_{17}H_{24}O_2Na$ $(M+Na)^+$ 283.1674 found 283.1666

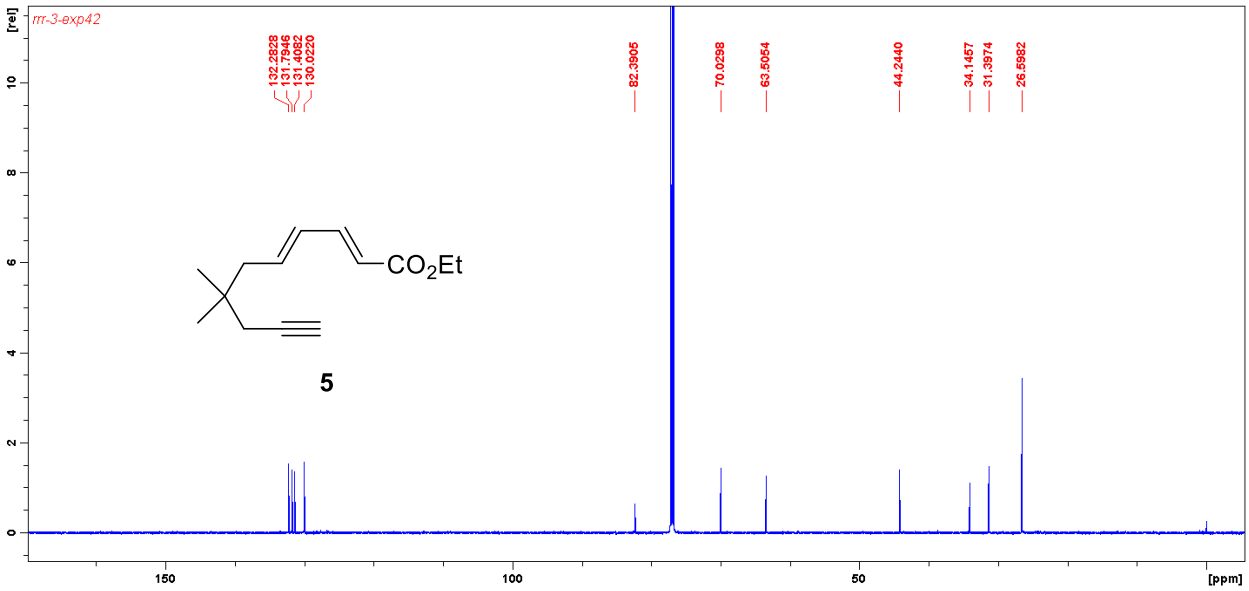


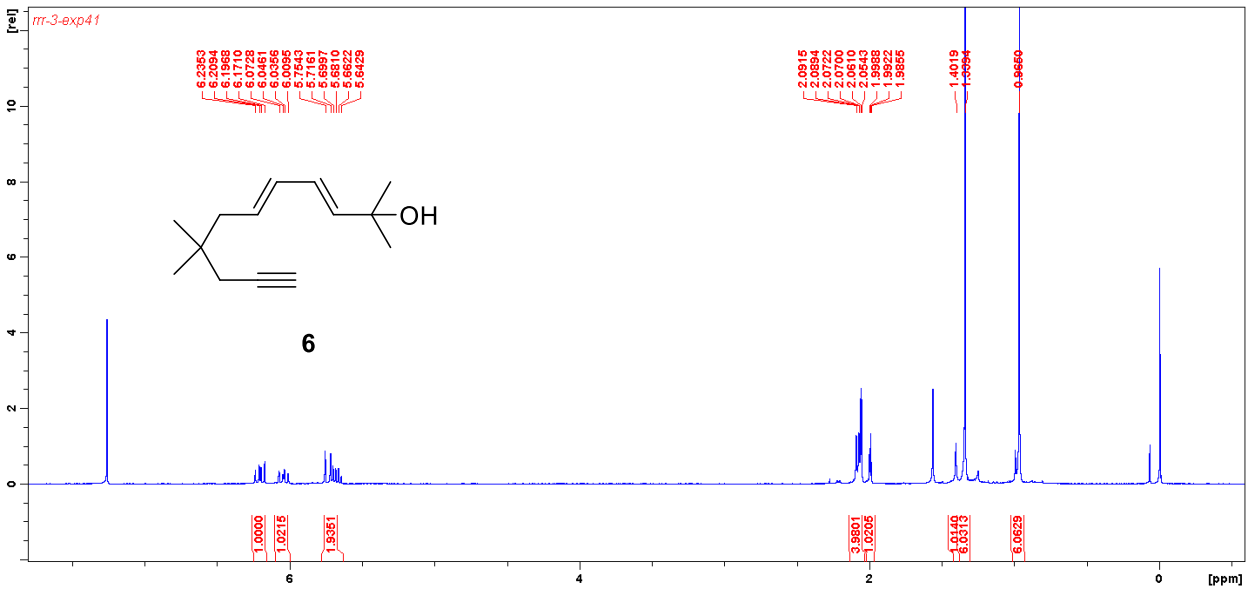
2

Mass = 22.5 mg; Yield = 99%
Colorless oil
^{1}H NMR (?, $CDCl_3$): δ 8.52 (br s, 1H), 7.06 (d, *J*=7.6 Hz, 1H), 6.98 (s, 1H), 6.93 (d, *J*=7.52, 1H), 3.06-3.03 (dd, *J*=13.2 Hz, 6.04 Hz, 1H), 2.79-2.55 (m, 6H), 1.19-1.12 (m, 9H) ppm;
^{13}C NMR (?, $CDCl_3$): δ 182.4, 143.8, 141.6, 136.9, 126.8, 125.4, 124.6, 47.7, 47.4, 41.6, 40.2, 39.3, 28.9, 16.5 ppm;
HRMS (ESI-) calcd for $C_{15}H_{19}O_2$ $(M-H^+)^-$ 231.1385 found 231.1405

6. Copies of ^{1}H-NMR and ^{13}C-NMR spectra of compounds



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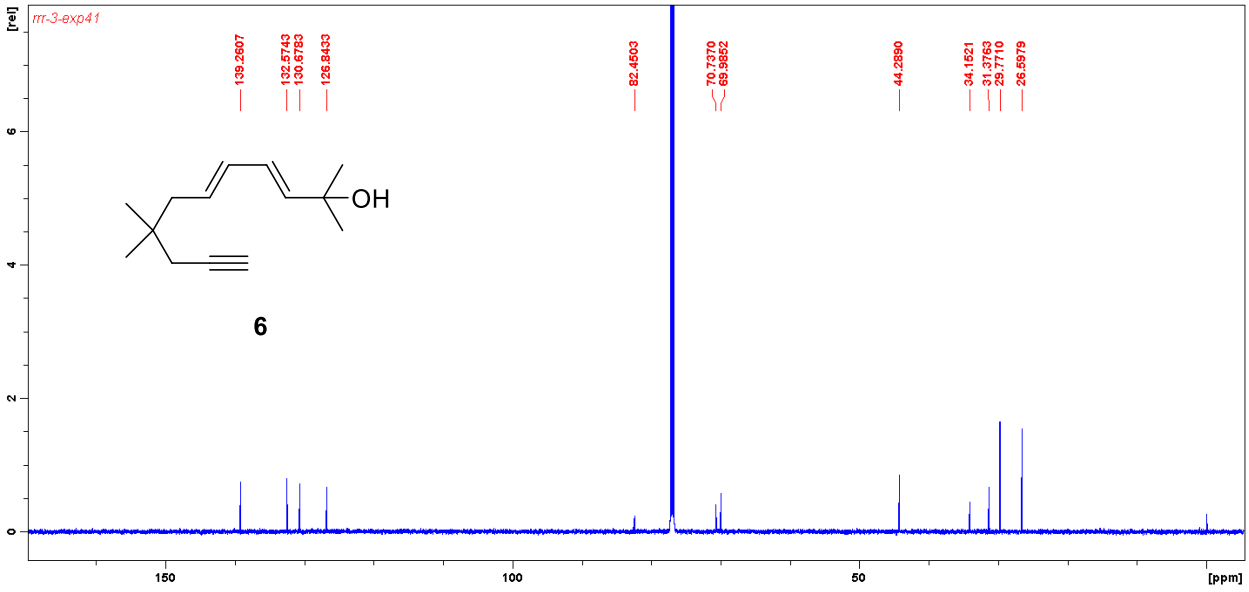
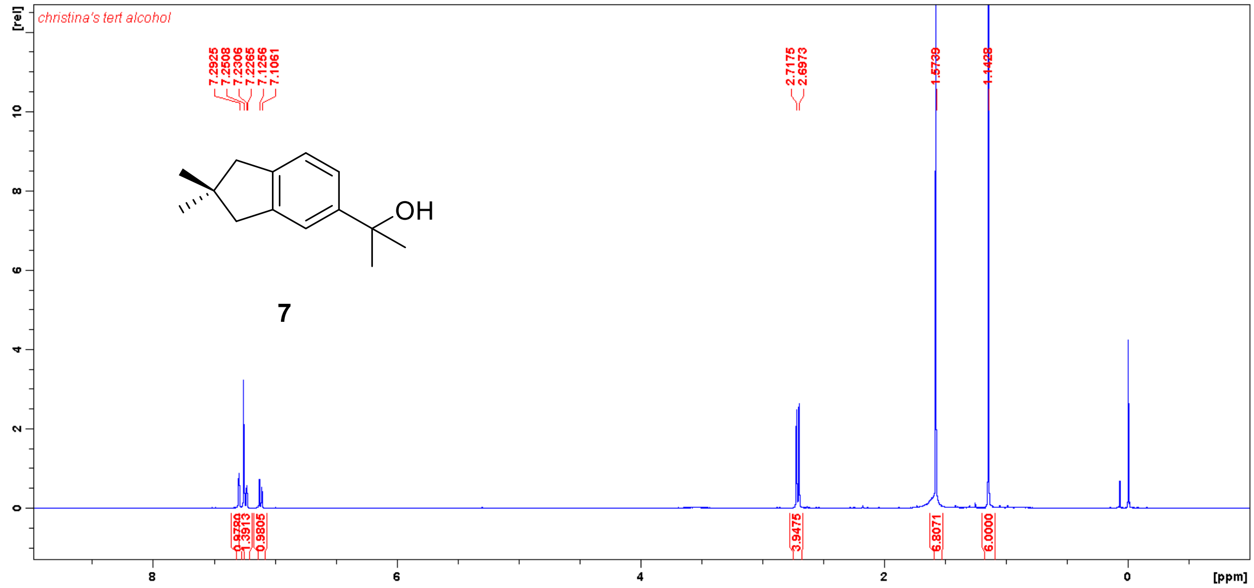
6.0094
5.1968
6.1710
6.0728
6.0461
6.0356
6.0095
5.7543
5.7141
5.6997
5.6810
5.6622
5.6429
2.0915
2.0694
2.0722
2.0700
2.0610
2.0543
1.9988
1.9922
1.9855
1.4019
1.0000
0.9660
OH
6
1.0000
1.0215
1.9351
3.9801
1.0206
1.0140
6.0313
6.0629
6
4
2
0
[ppm]


rrr-3-exp41
[rel]
139.2607
132.5743
130.6783
126.8433
82.4503
70.7370
69.9852
44.2890
34.1521
31.3763
29.7710
26.5979
OH
6
150
100
50
[ppm]


christina's tert alcohol
7.2925
7.2508
7.2306
7.2265
7.1256
7.1061
2.7175
2.6973
1.5739
1.1428
OH
7
3.9475
6.8071
6.0000
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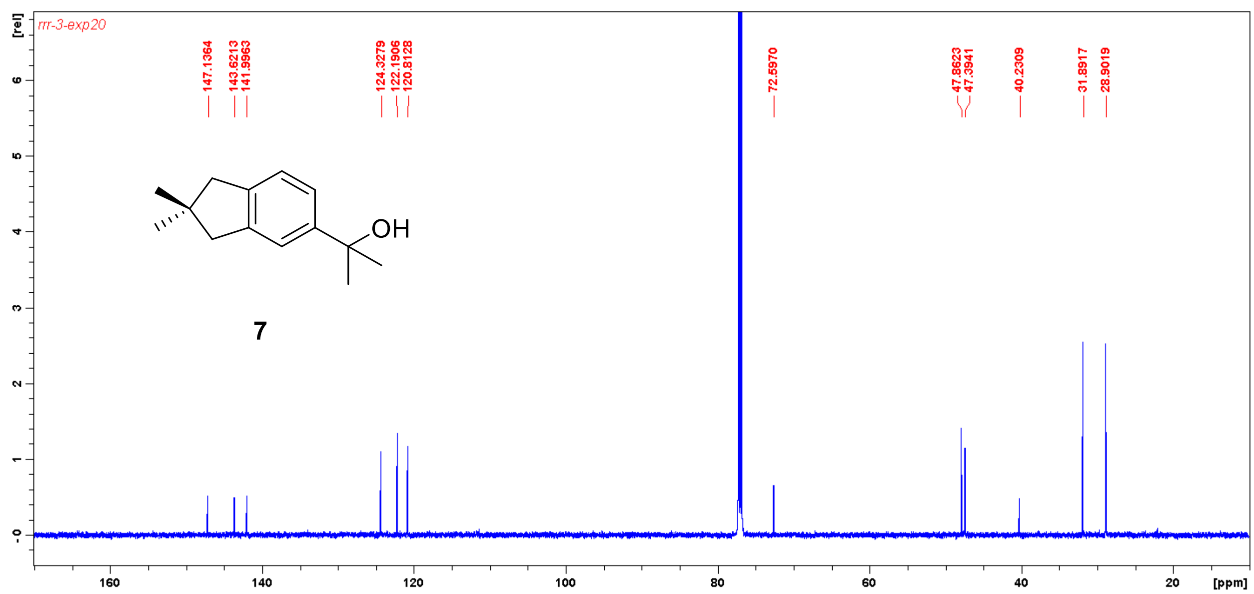
[ppm]


rrr-3-exp20
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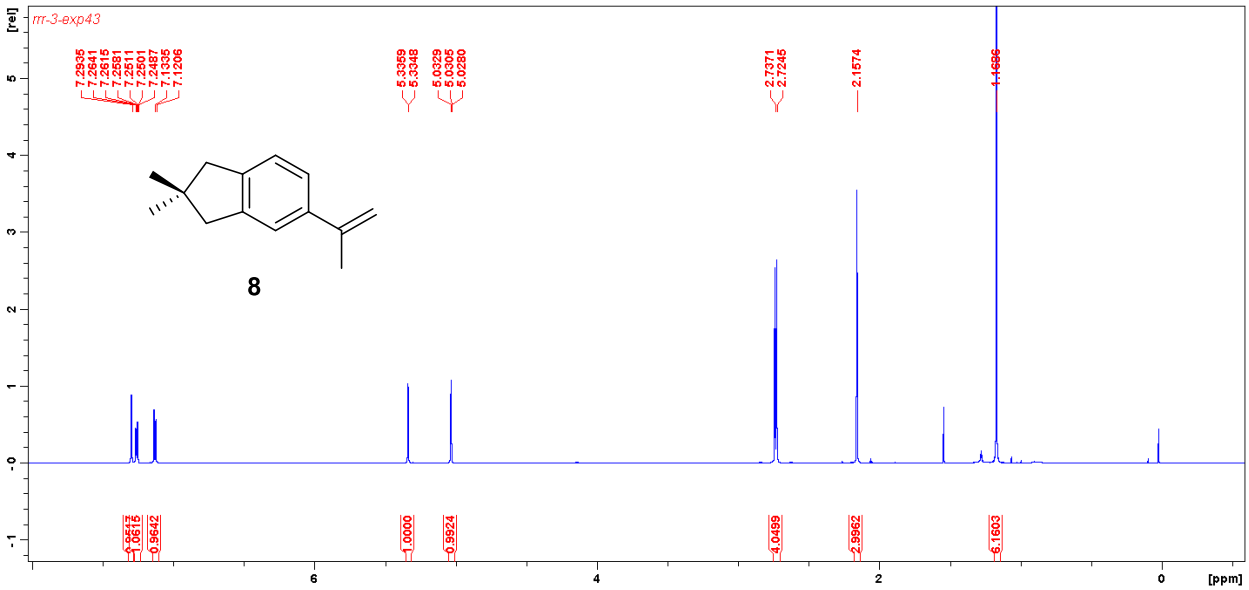
143.6213
141.9963
124.3279
122.1906
120.8128
72.5970
47.8623
47.3941
40.2309
31.8917
28.9019
OH
7
[rel]
[ppm]


rrr-3-exp43
[rel]
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7.2841
7.2615
7.2581
7.2511
7.2501
7.2487
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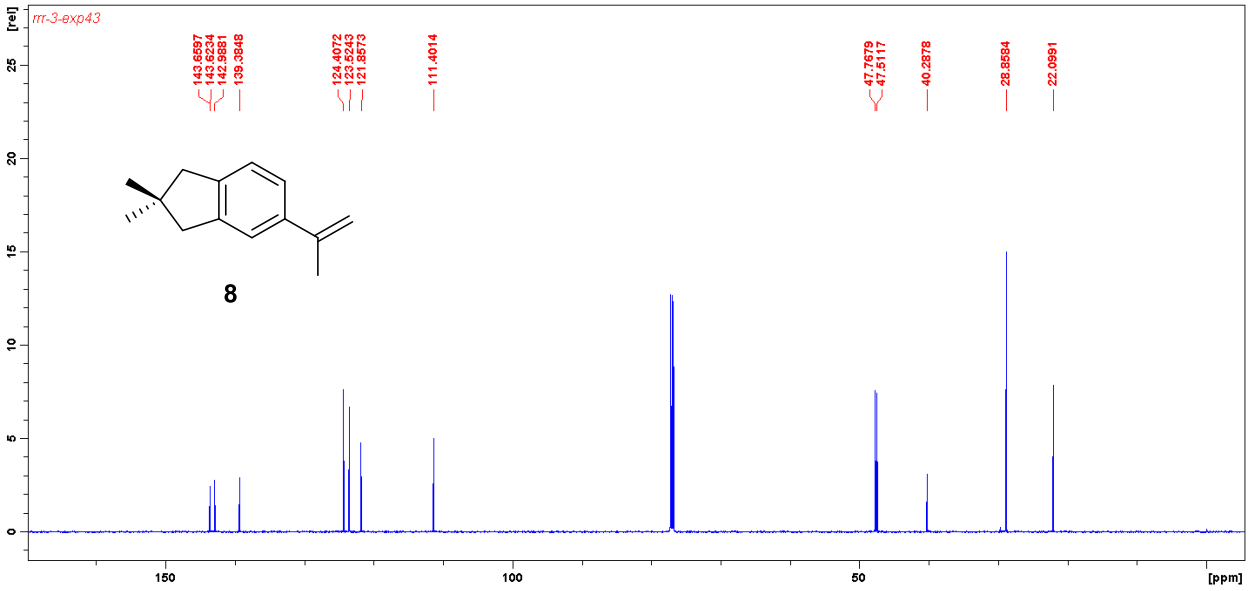
7.1206
5.3359
5.3348
5.0329
5.0305
5.0280
2.7371
2.7245
2.1574
1.1696
0.9643
1.0000
0.9924
4.0499
2.9962
6.1603
[ppm]
8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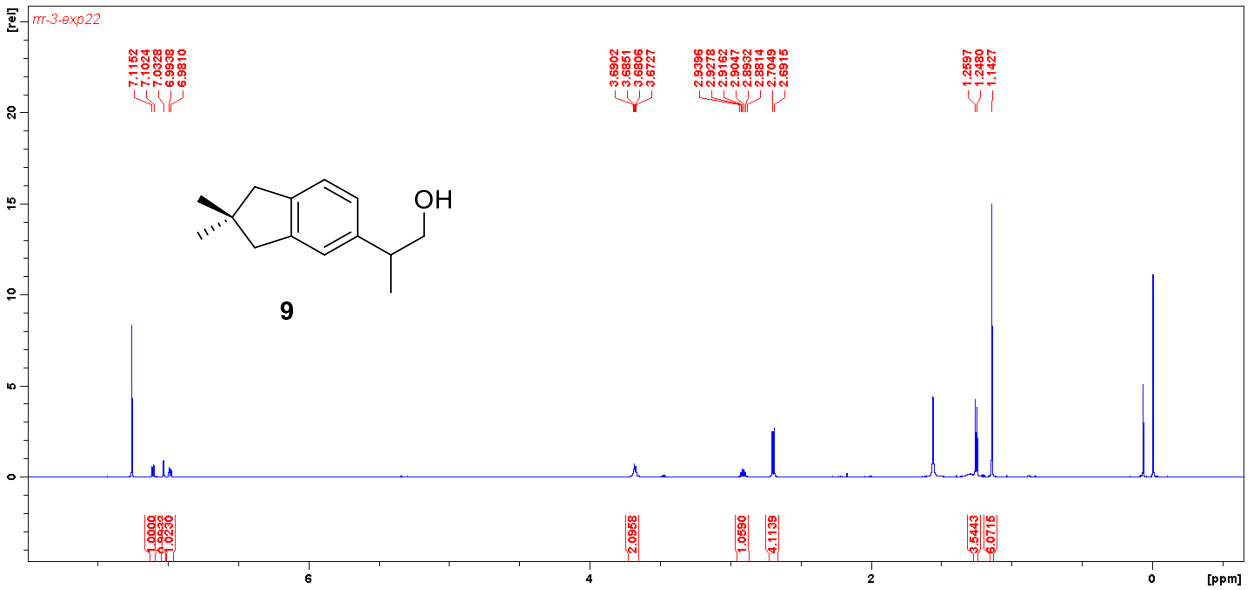
143.6234
142.9881
139.3848
124.4072
123.5243
121.8573
111.4014
47.7679
47.5117
40.2878
28.8584
22.0991
[ppm]
8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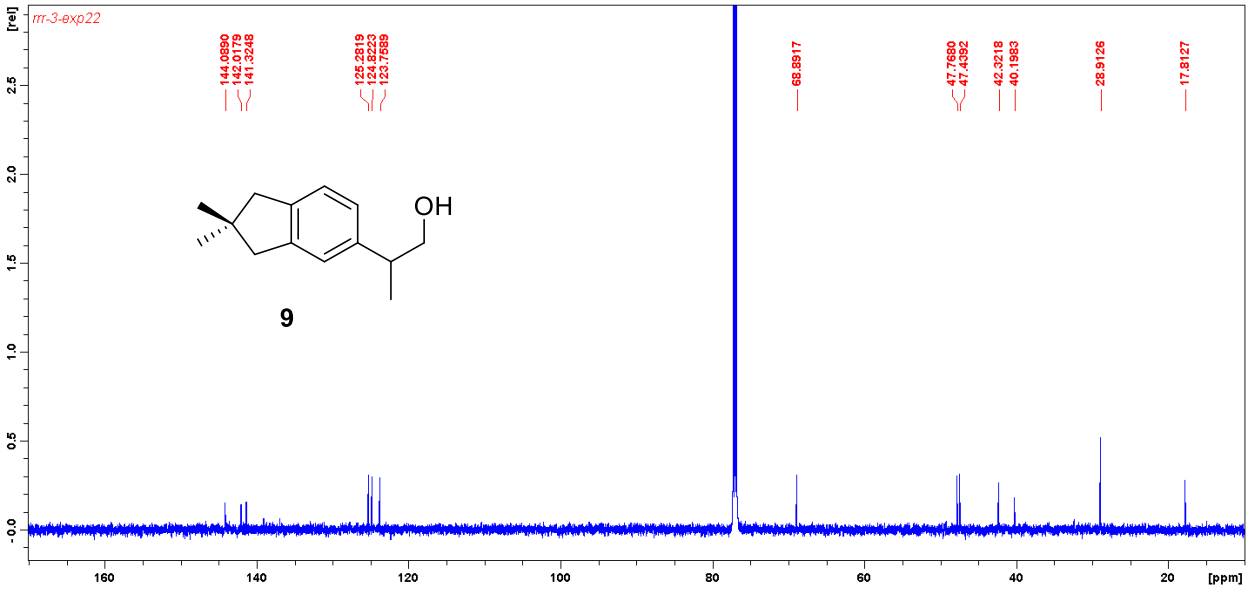
[rel]
7.1162
7.1024
7.0238
6.9938
6.9810
3.6902
3.6851
3.6806
3.6727
2.9396
2.9278
2.9162
2.9047
2.8932
2.8814
2.7049
2.6915
1.2597
1.2480
1.1427
OH
9
1.0000
1.0330
1.0230
2.0958
1.0590
4.1139
3.5443
6.0715
[ppm]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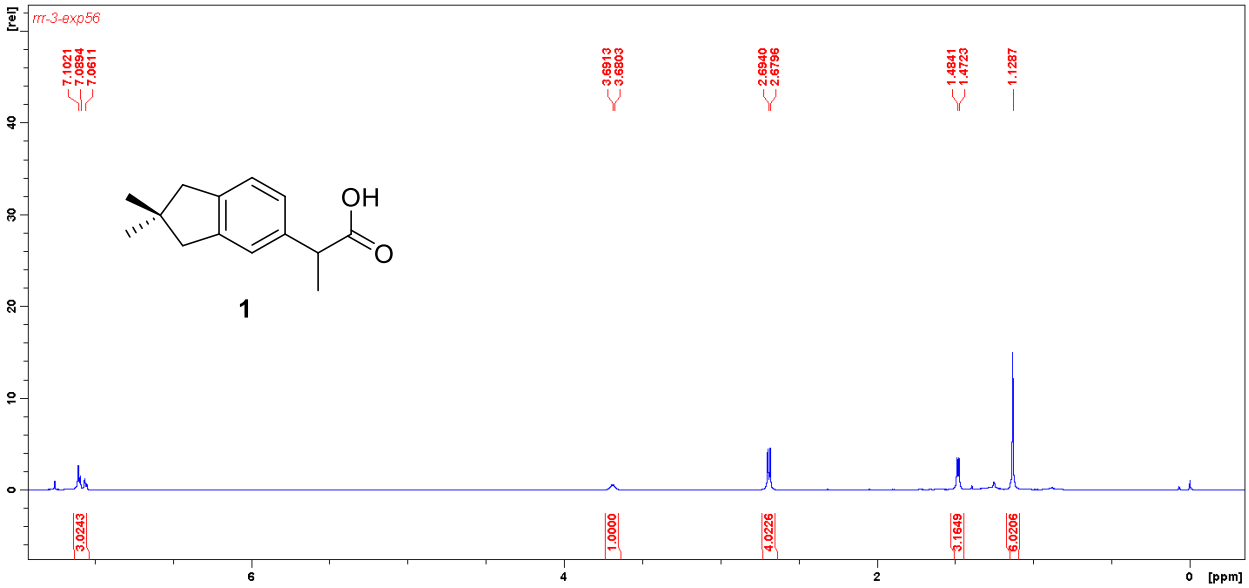
144.0890
142.0179
141.3248
125.2819
124.8223
123.7589
68.8917
47.7680
47.4392
42.3218
40.1983
28.9126
17.8127
OH
9
[ppm]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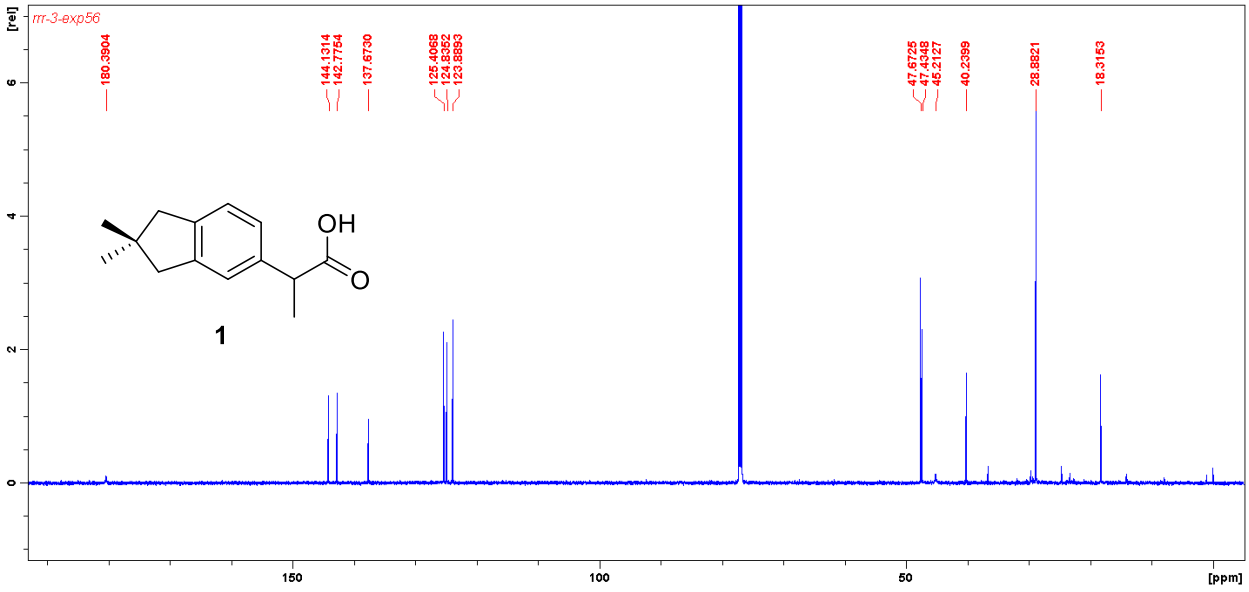
[rel]
7.1021
7.0894
7.0611
3.6913
3.6803
2.6940
2.6796
1.4841
1.4723
1.1287
OH
O
1
3.0243
1.0000
4.0226
3.1649
6.0206
6
4
2
0
[ppm]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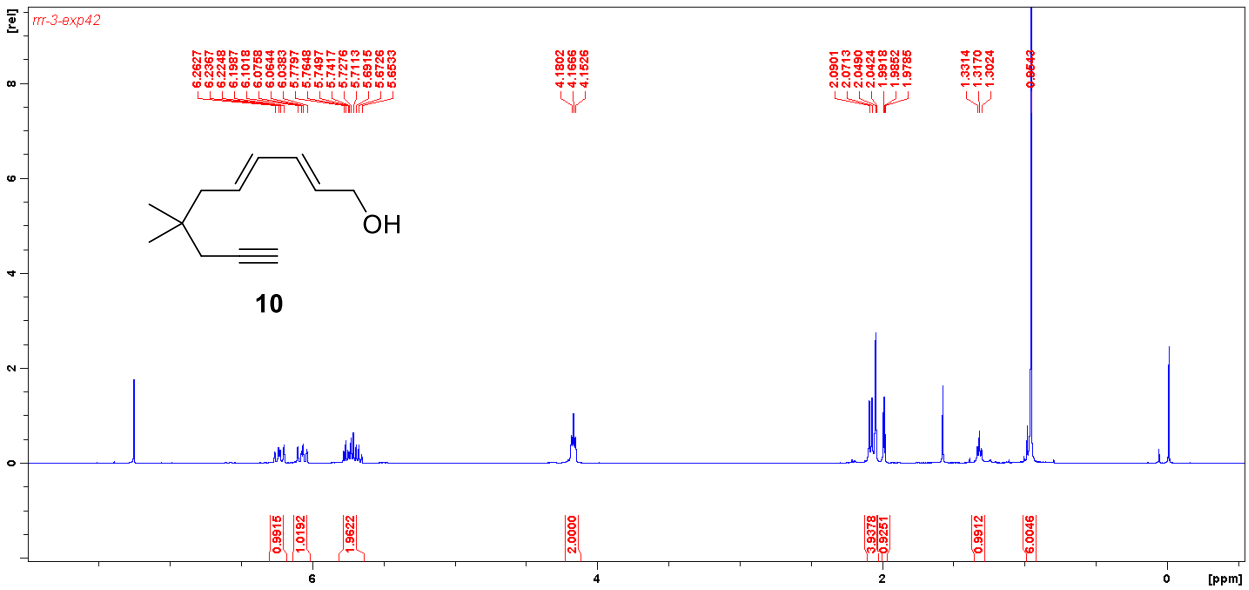
144.1314
142.7764
137.6730
125.4068
124.8352
123.8893
47.6725
47.4348
45.2127
40.2399
28.8821
18.3153
OH
O
1
150
100
50
[ppm]


rrr-3-exp42
[rel]
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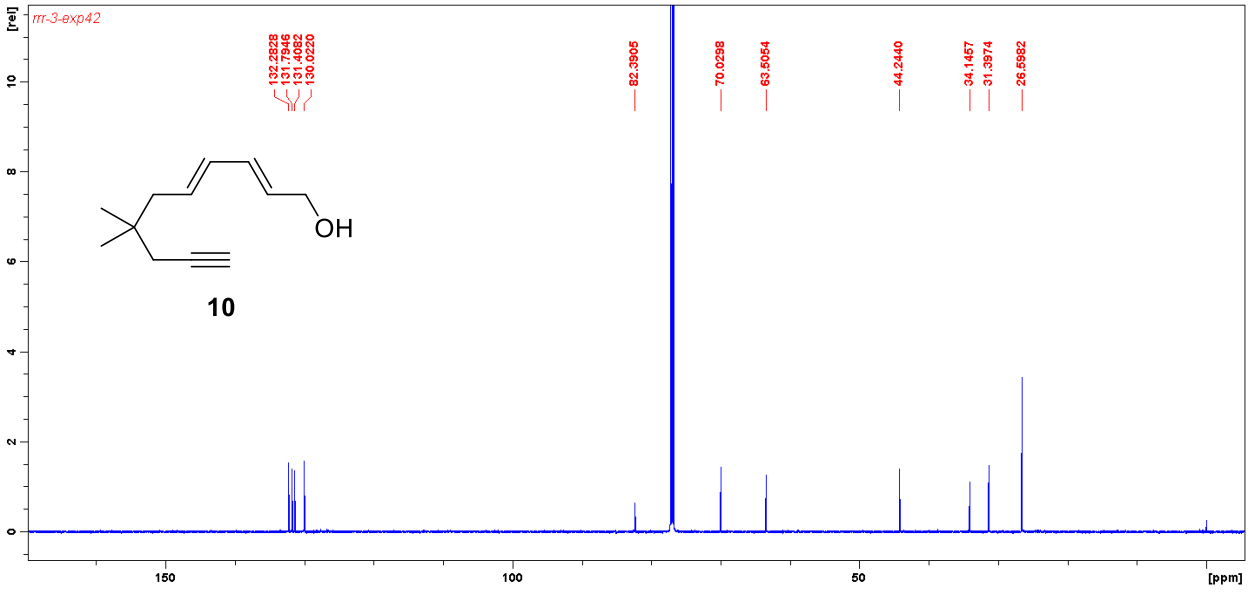
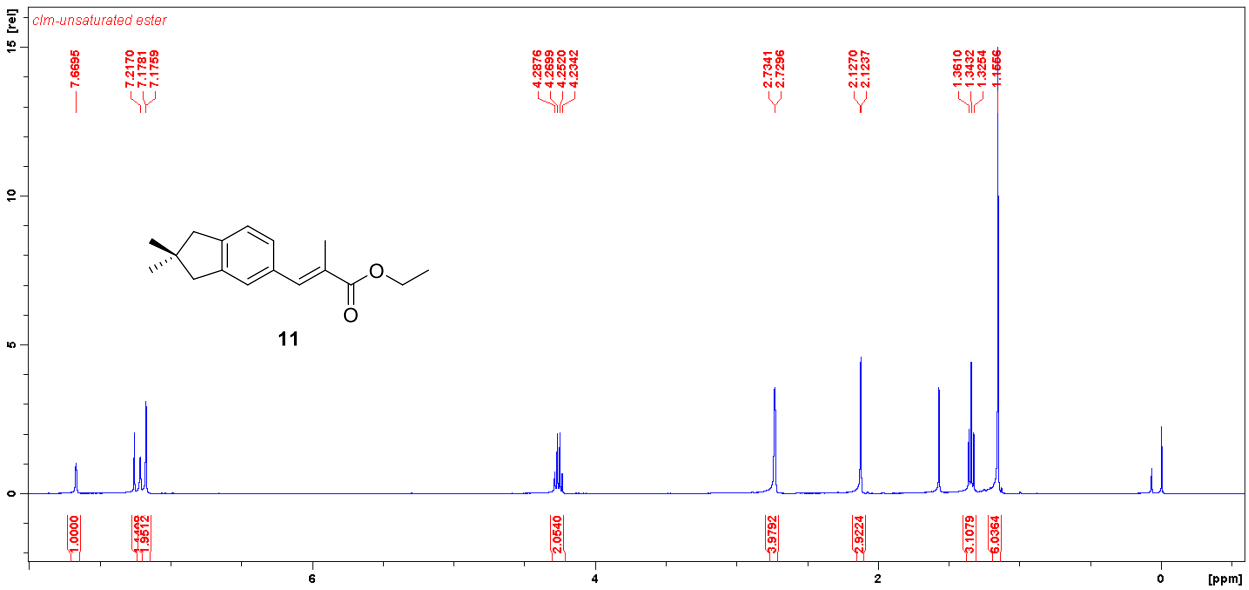
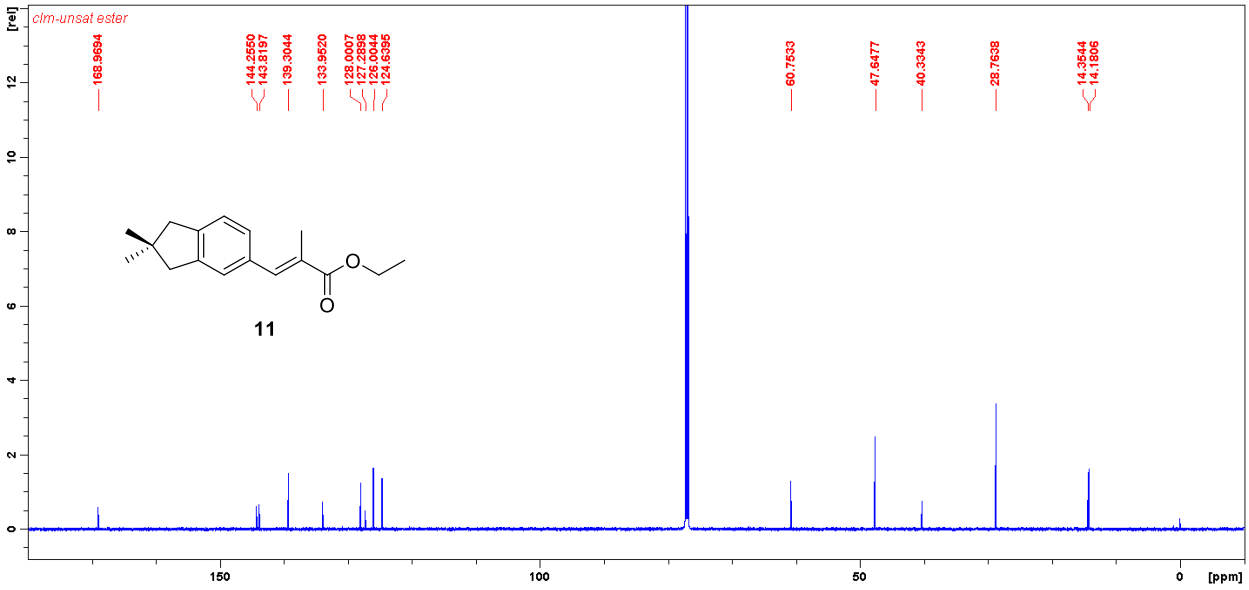
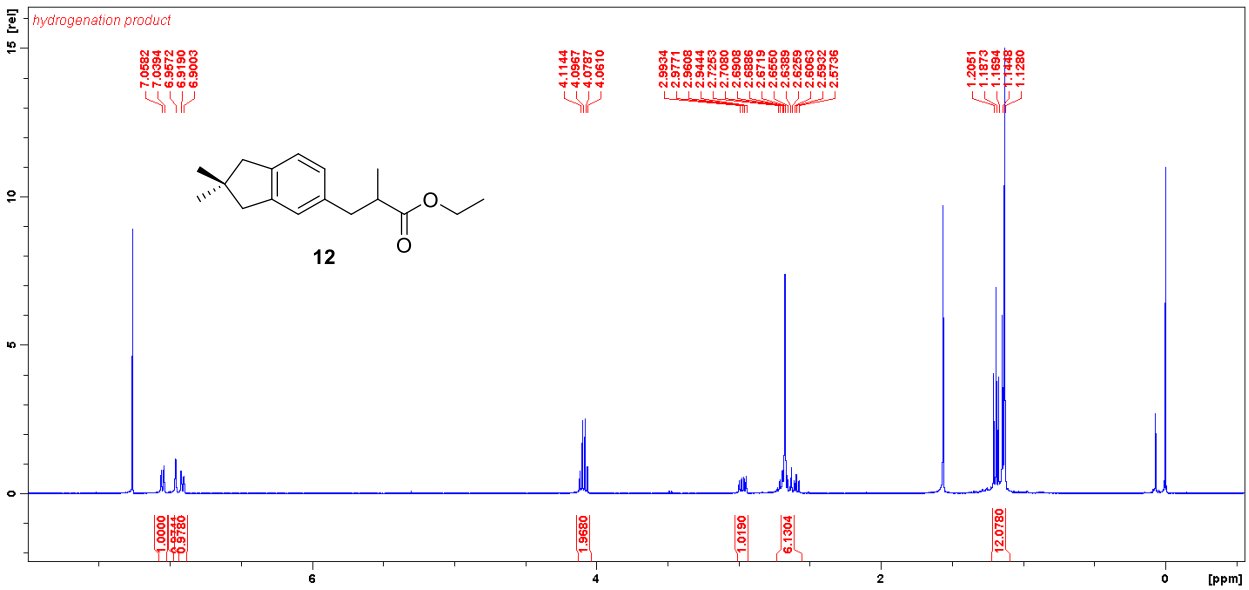
6.2367
6.2248
6.1987
6.1018
6.0788
6.0644
6.0383
5.7797
5.7648
5.7497
5.7417
5.7276
5.7113
5.6915
5.6726
5.6533
4.1802
4.1666
4.1526
2.0901
2.0713
2.0490
2.0424
1.9913
1.9852
1.9785
1.3314
1.3170
1.3024
0.8643
OH
10
0.9915
1.0192
1.9622
2.0000
3.9378
0.9251
0.9912
6.0046
6
4
2
0
[ppm]


rrr-3-exp42
[rel]
132.2828
131.7946
131.4092
130.0220
82.3905
70.0298
63.5054
44.2440
34.1457
31.3974
26.5982
OH
10
150
100
50
[ppm]


clm-unsaturated ester
7.6695
7.2170
7.1781
7.1759
4.2876
4.2658
4.2520
4.2342
2.7341
2.7296
2.1270
2.1237
1.3610
1.3432
1.3254
1.1656
11
1.0000
1.1462
1.9512
2.0540
3.9792
2.9224
3.1079
6.0364
[rel]
[ppm]


clm-unsat ester
168.9694
144.2550
143.8197
139.3044
133.9520
128.0007
127.2898
126.0044
124.6396
60.7533
47.6477
40.3343
29.7638
14.3544
14.1806
11
[rel]
[ppm]


hydrogenation product
7.0582
7.0384
6.9572
6.9190
6.9003
4.1144
4.0967
4.0787
4.0610
2.9934
2.9771
2.9608
2.9444
2.7253
2.7080
2.6908
2.6586
2.6419
2.6250
2.6089
2.6259
2.6063
2.5932
2.5736
1.2051
1.1873
1.1694
1.1448
1.1280
12
1.0000
0.9744
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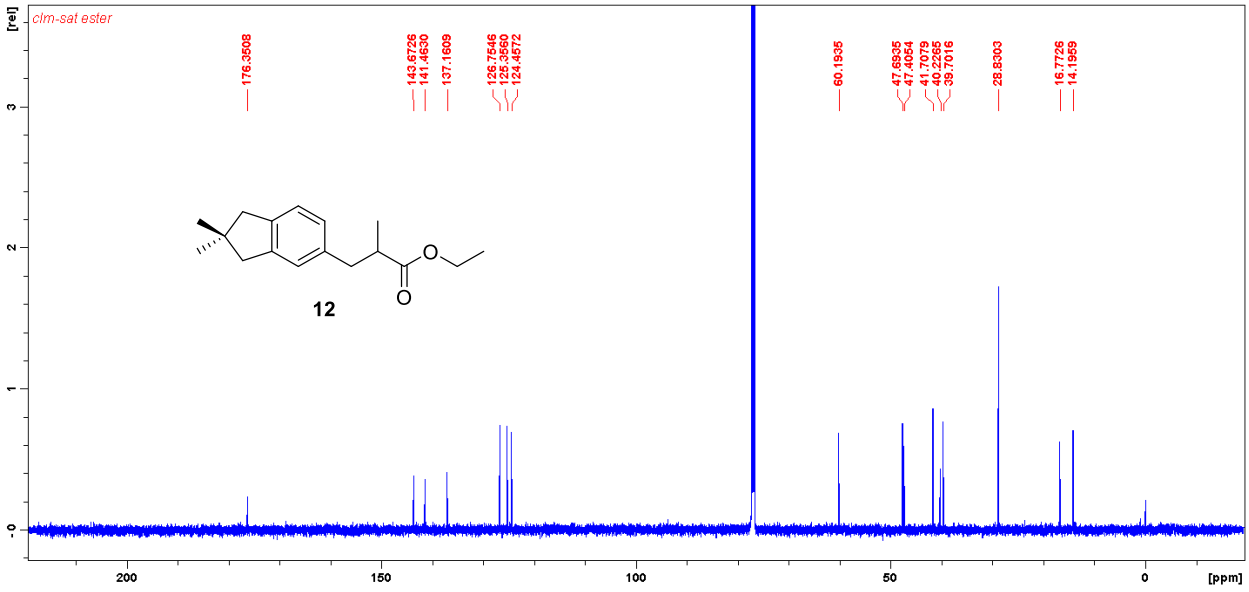
1.9680
1.0190
6.1304
12.0780
[rel]
[ppm]


clm-sat ester
176.3508
143.6726
141.4630
137.1609
126.7546
125.3560
124.4572
60.1935
47.6935
47.4054
41.7079
40.2265
39.7016
28.8303
16.7726
14.1959
12
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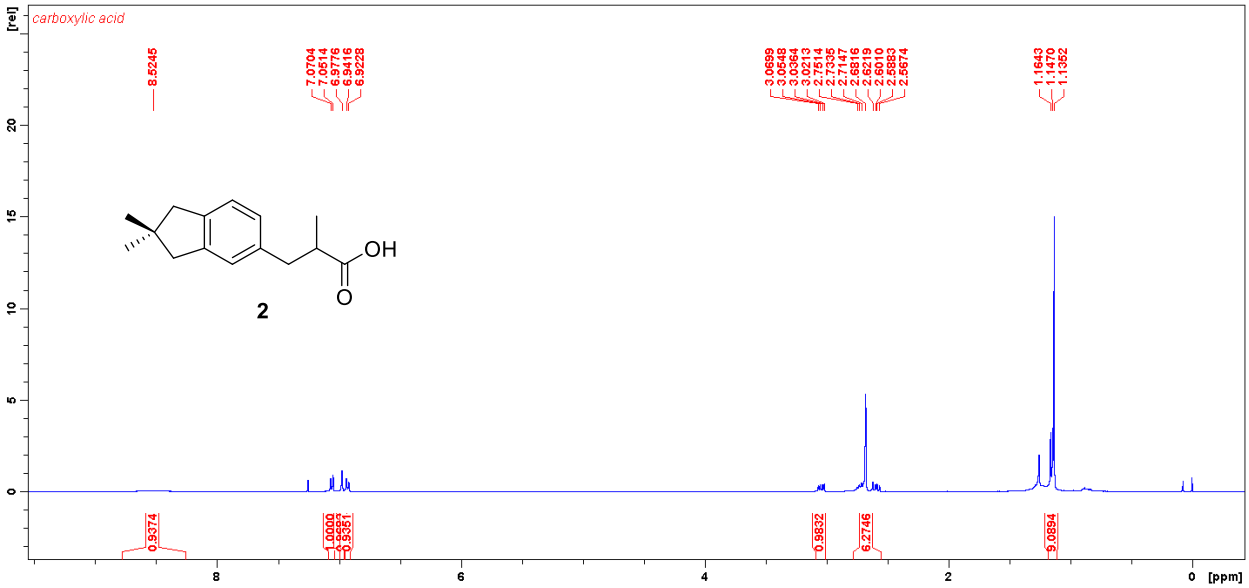
[ppm]


carboxylic acid
[rel]
8.5245
7.0704
7.0514
6.9776
6.9416
6.9228
3.0699
3.0548
3.0364
3.0213
2.7514
2.7347
2.7195
2.6816
2.6219
2.6010
2.5883
2.5674
1.1643
1.1470
1.1352
OH
O
2
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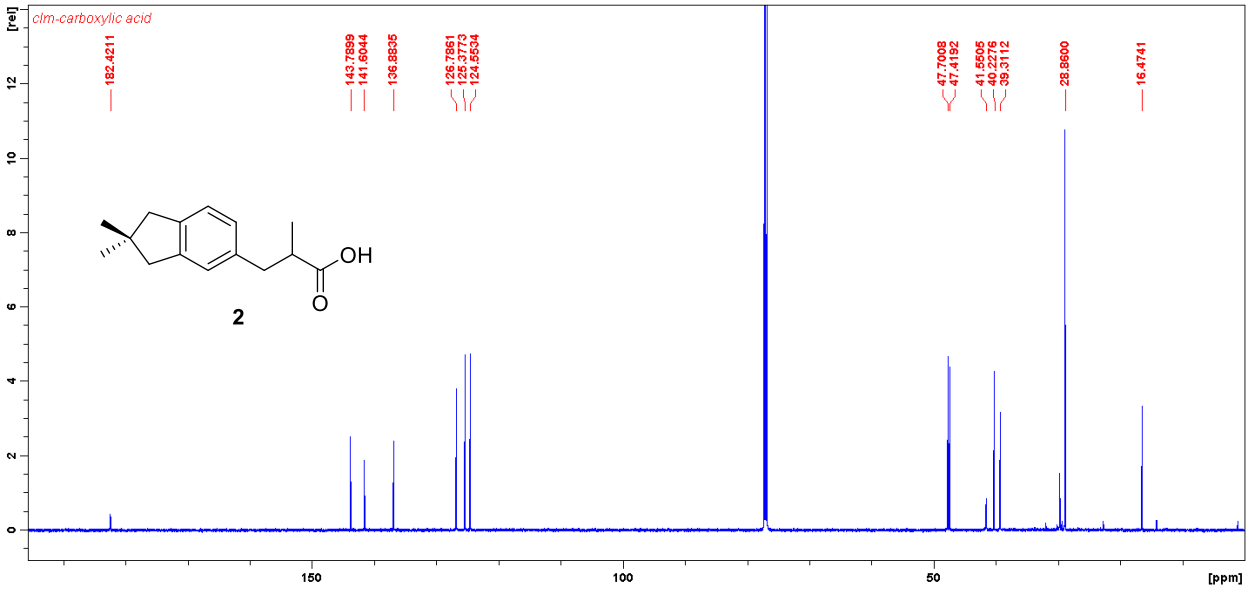
1.0000
0.9351
0.9832
6.2746
9.0694
[ppm]


clm-carboxylic acid
[rel]
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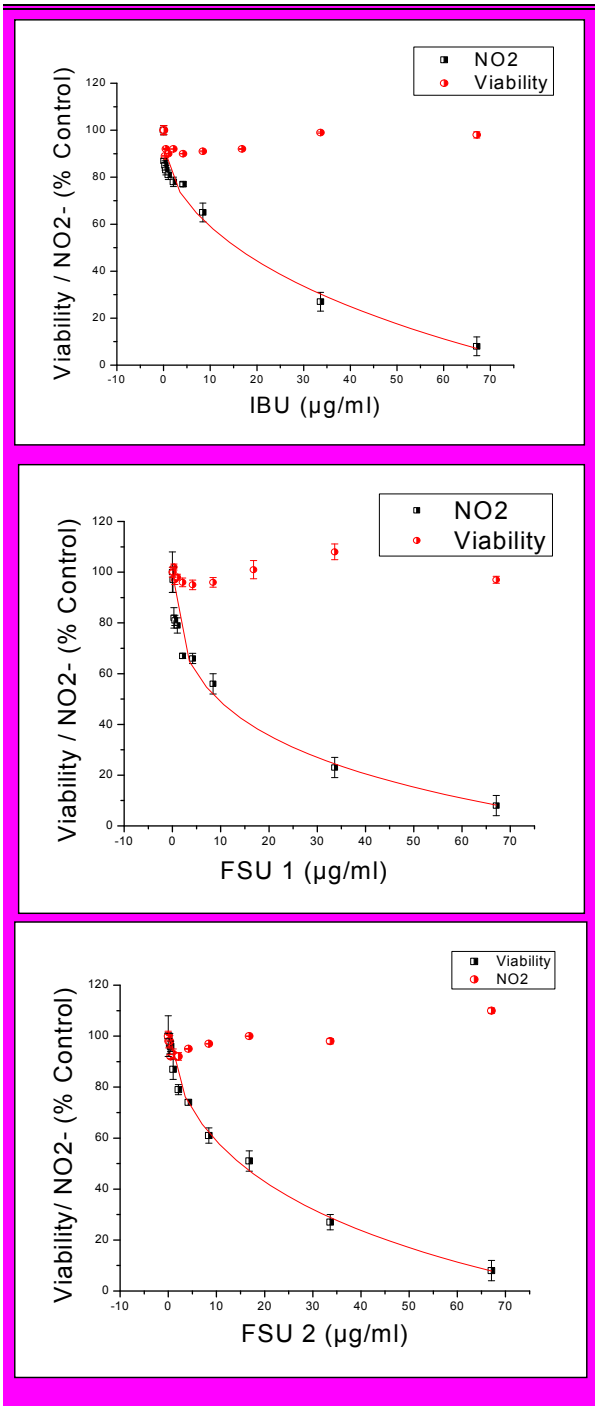
143.7899
141.6044
136.8835
126.7861
125.3773
124.5534
47.7008
47.4192
41.5505
40.2276
39.3112
28.8600
16.4741
OH
O
2
[ppm]

7. Nitric oxide – LPS activated BV2 Microglial Cells

Nitric Oxide - LPS activated BV2 Microglial Cells

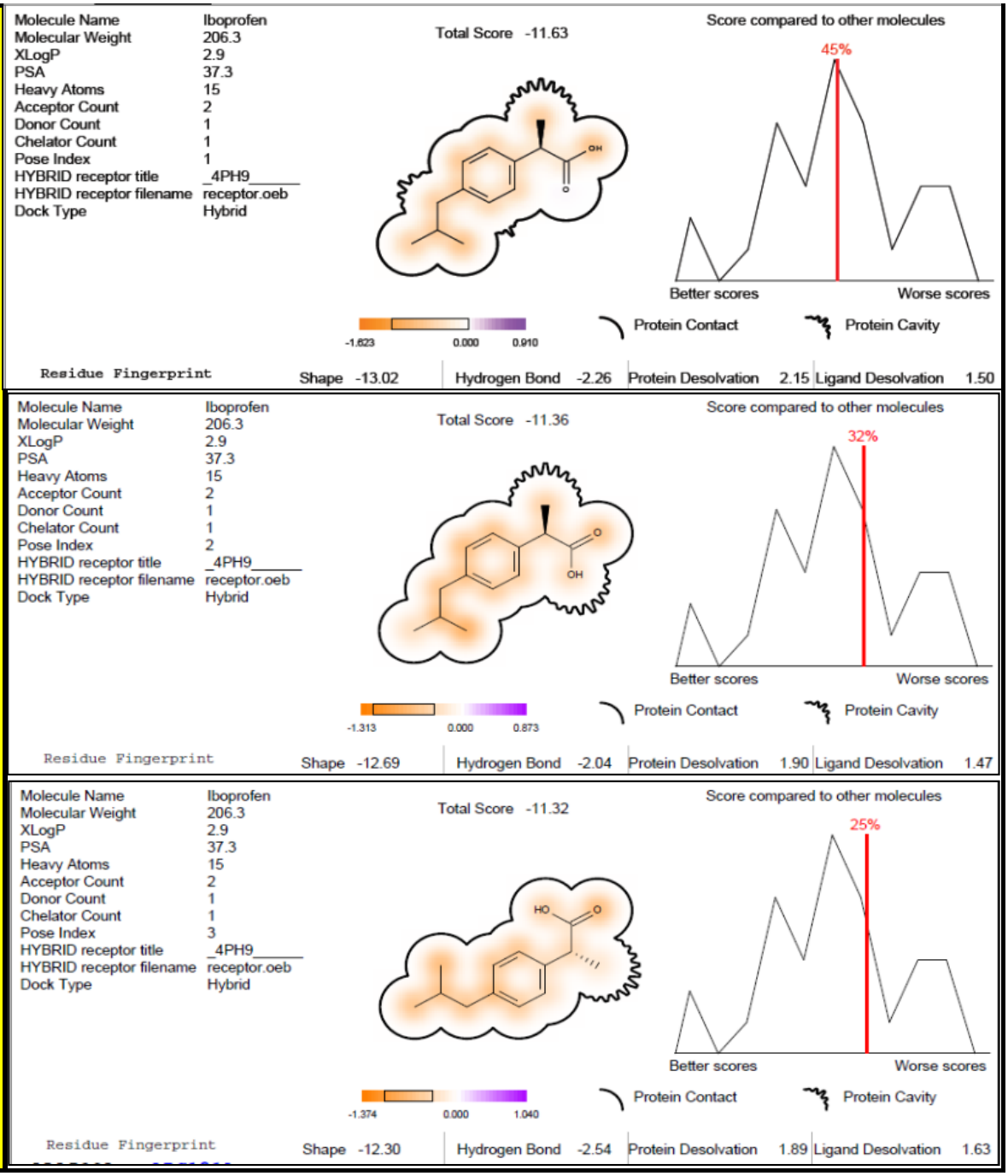


NO_2- inhibition and toxicity in BV-2 cells treated with LPS (1µg/ml). The data represent NO_2- and viability (% LPS Control), presented as the Mean ± S.E.M, n=4. Statistical difference from the Controls were determined by a one-way ANOVA, followed by a Tukey post – hoc test * P<.05 .

8. Molecular Docking of IBU vs IBU analogues on Murine Cyclooxygenase 2

Comparative Scoring : Molecular Docking of IBU vs IBU Analogues on Murine Cyclooxygenase 2.

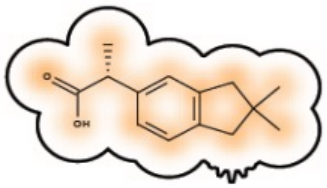
IBUPROFEN



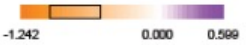
FSU 1

Molecule Name	FSU1
Molecular Weight	218.3
XLogP	2.7
PSA	37.3
Heavy Atoms	16
Acceptor Count	2
Donor Count	1
Chelator Count	1
Pose Index	1
HYBRID receptor title	_4PH9_____
HYBRID receptor filename	receptor.oeb
Dock Type	Hybrid

Total Score -12.28



Score compared to other molecules



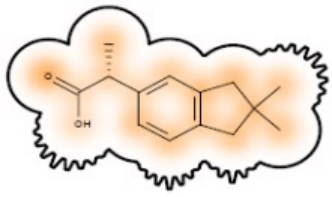
Protein Contact Protein Cavity

Residue Fingerprint

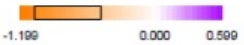
Shape -13.07 | Hydrogen Bond -2.34 | Protein Desolvation 2.02 | Ligand Desolvation 1.12

Molecule Name	FSU1
Molecular Weight	218.3
XLogP	2.7
PSA	37.3
Heavy Atoms	16
Acceptor Count	2
Donor Count	1
Chelator Count	1
Pose Index	2
HYBRID receptor title	_4PH9_____
HYBRID receptor filename	receptor.oeb
Dock Type	Hybrid

Total Score -12.19



Score compared to other molecules



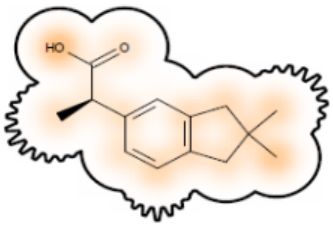
Protein Contact Protein Cavity

Residue Fingerprint

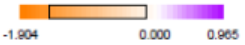
Shape -13.00 | Hydrogen Bond -2.29 | Protein Desolvation 1.98 | Ligand Desolvation 1.12

Molecule Name	FSU1
Molecular Weight	218.3
XLogP	2.7
PSA	37.3
Heavy Atoms	16
Acceptor Count	2
Donor Count	1
Chelator Count	1
Pose Index	3
HYBRID receptor title	_4PH9_____
HYBRID receptor filename	receptor.oeb
Dock Type	Hybrid

Total Score -12.10



Score compared to other molecules



Protein Contact Protein Cavity

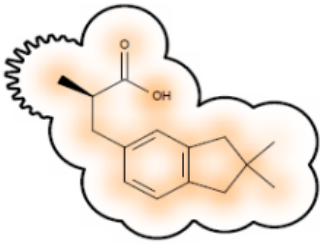
Residue Fingerprint

Shape -13.37 | Hydrogen Bond -2.48 | Protein Desolvation 2.24 | Ligand Desolvation 1.50

FSU 2

Molecule Name	FSU2
Molecular Weight	232.3
XLogP	2.8
PSA	37.3
Heavy Atoms	17
Acceptor Count	2
Donor Count	1
Chelator Count	1
Pose Index	1
HYBRID receptor title	_4PH9_____
HYBRID receptor filename	receptor.oeb
Dock Type	Hybrid

Total Score -13.05



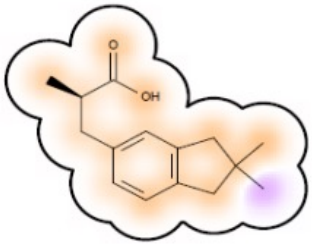
Score compared to other molecules

Protein Contact Protein Cavity

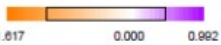
Residue Fingerprint Shape -14.05 Hydrogen Bond -2.63 Protein Desolvation 2.06 Ligand Desolvation 1.58

Molecule Name	FSU2
Molecular Weight	232.3
XLogP	2.8
PSA	37.3
Heavy Atoms	17
Acceptor Count	2
Donor Count	1
Chelator Count	1
Pose Index	2
HYBRID receptor title	_4PH9_____
HYBRID receptor filename	receptor.oeb
Dock Type	Hybrid

Total Score -13.00



Score compared to other molecules

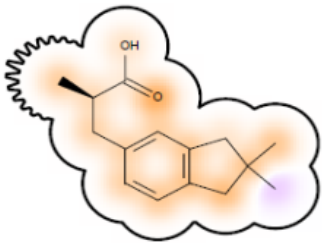


Protein Contact Protein Cavity

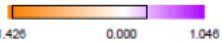
Residue Fingerprint Shape -14.22 Hydrogen Bond -2.58 Protein Desolvation 2.21 Ligand Desolvation 1.59

Molecule Name	FSU2
Molecular Weight	232.3
XLogP	2.8
PSA	37.3
Heavy Atoms	17
Acceptor Count	2
Donor Count	1
Chelator Count	1
Pose Index	3
HYBRID receptor title	_4PH9_____
HYBRID receptor filename	receptor.oeb
Dock Type	Hybrid

Total Score -12.49



Score compared to other molecules



Protein Contact Protein Cavity