Supporting Information:

Isolation and synthesis of a bacterially-produced inhibitor of rosette development in choanoflagellates

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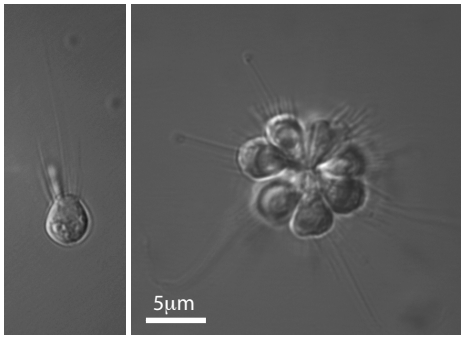


Choanoflagellates in their unicellular (left) and rosette (right) forms. Scale bar: 5 μm.

METHODS

Instrumentation:

All HPLC was performed on Agilent 1100 or 1200 series instruments. Specific columns used are specified in methods. LCMS was performed on an Agilent 1200 series HPLC with 6130 ESI mass spectrometer. High resolution mass spectrometry was performed on an Agilent 6530 QTOF LCMS (ESI) or a Waters Micromass (EI) 70-VSE (EI experiments conducted at the University of Illinois at Urbana-Champaign Mass Spectrometry Laboratory). Optical rotations were performed on a Jasco P-2000 polarimeter with a sodium lamp. NMRs were performed on the following instruments: Varian Inova 400 MHz, Varian Unity Inova 600 MHz, or a 500 MHz Oxford magnet with Varian Inova consul, equipped with a Varian HCN coldprobe.

Choanoflagellate husbandry

Salpingoeca rosetta strain SrEpac (Levin et al. 2013) was propagated in 5% Sea Water Complete media. 5% Sea Water Complete (SWC) media (250 mg/L peptone, 150 mg/L yeast extract, 150mL/L glycerol in artificial sea water) was made by diluting SWC to 5% (vol/vol) in artificial sea water.

SrEpac was passaged 1:10 into fresh medium once a day to stimulate rapid growth. For all bioassays, lipids were added to SrEpac shortly after passaging, at a density of approximately 10^4-10^5 cells/mL. Rosettes were quantified 22-25 hours post induction.

Activity profile of lipids

Lipid inducing/inhibitory activity was determined using a quantitative bioassay for rosette development. Lipid samples were resuspended in DMSO to a concentration of 2mg/mL. Lipids were first pre-mixed in 5% SWC to avoid precipitation of the sample, and then added to 100mL SrEpac, aliquoted into 96 well plates (Corning Costar), to yield the desired concentration.

To quantify rosette development, SrEpac was pipetted vigorously and fixed in 1% formaldehyde immediately before counting (Bright-Line hemacytometer, Hausser Scientific). To determine the fraction of cells in rosettes, single cells and cells within rosettes were scored until 1000 total cells had been counted. A group of four or more cells qualified as a rosette if the cells maintained an organized polarity (each cell oriented with the apical flagellum pointing outward) after vigorous physical perturbation. At least three biological replicates were performed for each assay.

Graphs were generated using GraphPad Prism 6 statistical software. Curves were fit to data using non-linear regression (curve fit, one site total).

Isolation of IOR-1 from *A. machipongonensis*

A. machipongonensis was grown in multi-liter scale in marine broth, shaking at 200 rpm at 30ºC for 3 days. Cells were pelleted by centrifugation and then extracted with 2:1 chloroform/methanol 2x and 1:1 chloroform/methanol 1x as previously described[1]. All phases were recombined and cell debris was removed by filtration. After drying, crude extract was fractionated by preparatory scale reverse phase HPLC using a Phenomenex Gemini NX C18 column (10μ, 110 A, 250 x 21.2 mm). Compounds were eluted at 10 ml/min in a gradient of solvents A (water + 0.1% NH_4OH) and B (MeOH + 0.1% NH_4OH) using the following method: 30% - 100% solvent B for 30 minutes, isocratic at 100% solvent B for 8 minutes, and ramp back down to 30% B over 2 minutes. Fractions containing IOR-1 eluted at around 80% B.

As this molecule is undetectable by UV, presence of IOR-1 was determined by LCMS on an Agilent 1200 series HPLC with 6130 series ESI mass spectrometer, injecting onto a Phenomenex Gemini NX-C18 column (110 A, 5 μm, 100 x 4.6 mm). Method for IOR-1detection: compounds eluted at 0.5 ml/min using a gradient of solvents A (water + 0.1% NH_4OH) and B (methanol + 0.1% NH_4OH), starting from 65% solvent B and increasing to 100% solvent B over 20 minutes.

To remove fatty acid impurities, fractions containing IOR-1 were resuspended in methanol and treated with TMS-diazomethane (Sigma Aldrich 362832), which was added until mixture turned yellow. Reaction was stirred vigorously at room temperature for ~15 minutes. Acetic acid was added dropwise to quench reaction. After drying *in vacuo*, entire mixture was purified by semi-prep HPLC using a Phenomenex Gemini NX-C18 (100A, 5μm, 250 x 10 mm) and the following method: With a flow rate of 2.4 ml/min, elute compounds with a gradient of 65 - 90% solvent B (MeOH + 0.1% NH4OH) over 20 minutes, 90-100% solvent B over 2 minutes, and isocratic at 100% B for 8 minutes. Compound was detected using an evaporative light scattering device (Agilent 1200 series ELSD).

To determine the concentration of IOR-1 in conditioned medium, 1 L *A. machipongonensis* was grown in marine broth for 3 days at 30 ºC, shaking at 200 rpm. Culture was spun down and sterile filtered to remove cells. 500 mls of conditioned medium was lyophilized and resuspended in 20 ml methanol. 50 μl of suspension was injected onto the LC/MS for comparison with a IOR-calibration curve. Calculated equation for calibration: y = 17415x-29972, revealed that based on integration of the MS spectrum, 19.9 ng of 351 were injected, which back-calculated to 536 ng/500 ml. We were thus able to determine the concentration of IOR-1 in *A. machipongonensis* conditioned medium was calculated to be 2.84 nM.

Synthesis of IOR-1A-1D

Undec-10-yn-1-yl toluene-p-sulfonate (**1**)

To a solution of undec-10-yn-1-ol (Alfa Aesar L11807, **1'**) (29.5 mmol, 5 g) in pyridine (11.8 mL) and dichloromethane (17.7 mL) at 0 ºC, 1.5 eq. toluene-p-sulfonyl chloride was added. Mixture was stirred overnight at 4º C. Mixture was then diluted with water and extracted with hexanes. Extract was washed with water, aq. HCl and brine, dried over sodium sulfate and concentrated under vacuum. Moved to next step without further purification[2]. MS (EI) observed: 345.1498, expected: 345.1500 ($C_{18}H_{26}O_3SNa$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d,

J = 8.3 Hz, 2H), 7.34 (d, J = 8.0 Hz, 2H), 4.02 (t, J = 6.5 Hz, 2H), 2.45 (s, 3H), 2.17 (td, J = 7.0, 2.7 Hz, 2H), 1.93 (t, J = 2.6 Hz, 1H), 1.67 – 1.59 (m, 2H), 1.56 – 1.46 (m, 3H), 1.30 – 1.16 (m, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 144.90, 133.54, 130.03, 128.09, 84.95, 70.95, 68.38, 29.48, 29.18, 29.11, 29.08, 28.91, 28.69, 25.57, 21.95, 21.86, 18.64.

14-methylpentadec-1-yne (**2**)

To a solution of **1** (15.5 mmol, 5 g) in THF (17.85 ml), 2.1 eq. i-pentylMgBr (31 mmol, 23.2 ml) was added dropwise at 0 ºC followed by immediate addition Li_2CuCl_4 (3.2 mL, 0.31 mmol). Mixture was allowed to return to room temperature overnight. Mixture was quenched with NH4Cl, extracted with hexanes and washed with water, sodium bicarbonate and brine.[2] Extract was dried over sodium sulfate and concentrated under vacuum yielding **2** (1.4 g, 42%) as a yellow oil. Product was purified by silica column using 100% hexanes yielding. Unable to obtain HRMS for this compound. Expected mass: 222.2348 ($C_{16}H_{30}$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.18 (td, J = 7.1, 2.7 Hz, 2H), 1.93 (d, J = 2.6 Hz, 1H), 1.56 – 1.48 (m, 3H), 1.41 – 1.36 (m, 2H), 1.27 (dd, J = 6.4, 3.2 Hz, 14H), 1.18 – 1.12 (m, 2H), 0.86 (d, J = 6.6 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 85.17, 68.36, 39.42, 30.30, 30.06, 30.01, 29.97, 29.87, 29.48, 29.13, 28.87, 28.33, 27.78, 23.05, 23.01, 18.77.

15-methylhexadec-2-yn-1-ol (**3**)

To a solution of **2** (2 g, 9 mmol) in THF (14 mL) at 4 ˚C, *n*-BuLi (20 mmol) was added. After 30 minutes, paraformaldehyde (10.8 mmol, 324 mg) was added in portions. Mixture was warmed to RT over 2 hours. Reaction quenched with 1:1 water/sat. NH_4Cl and extracted with hexanes.[3] Crude material was purified on silica gel using 100% hexanes followed by 3:2 hexanes/ethyl acetate to obtain pure **3** as a light yellow solid (1.4g, 66%). Expected mass 252.2453 ($C_{17}H_{32}O$). Unable to obtain HRMS for this compound. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.25 (t, *J* = 2.2 Hz, 2H), 2.20 (tt, *J* = 7.1, 2.2 Hz, 1H), 1.56 – 1.45 (m, 3H), 1.39 – 1.33 (m, 2H), 1.33 – 1.18 (m, 14H), 1.17 – 1.08 (m, 2H), 0.86 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 86.67, 78.23, 51.42, 39.04, 29.92, 29.69, 29.65, 29.62, 29.50, 29.13, 28.87, 28.59, 27.95, 27.40, 22.64, 18.72.

(*Z*)-15-methylhexadec-2-en-1-ol (**Z-4**)

To a solution of **3** (1 g, 4 mmol) in methanol (10 mL), Lindlar reagent was added (500 mg) and stirred for 30 minutes. Flask was charged with H_2 and stirred overnight at room temperature. Reaction mixture was filtered over celite and concentrated under vacuum yielding **Z-4** as a white solid (0.73 g, 74%). Crude extract was purified by HPLC (C18) using a gradient of 85-100% acetonitrile. MS (EI) observed: 254.2615, expected: 254.2610 ($C_{17}H_{34}O$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.64 – 5.50 (m, 2H), 4.19 (d, *J* = 6.1 Hz, 2H), 2.07 (q, *J* = 7.0 Hz, 2H), 1.58 – 1.45 (m, 1H), 1.34 – 1.20 (m, 18H), 1.17 – 1.11 (m, 2H), 0.86 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 133.29, 128.26, 58.61, 39.04, 29.92, 29.69, 29.65, 29.59, 29.58, 29.47, 29.21, 27.95, 27.42, 27.39, 22.64.

(*E*)-15-methylhexadec-2-en-1-ol (**E-4**)

To a solution of **3** (1 g, 4 mmol) in ether (20 mL), Red-Al was added at 0 ˚C and stirred for 2 hours, then stirred at RT overnight. Reaction quenched with sat. potassium sodium tartrate at 0 ºC and extracted with hexanes affording **E-4** as a white solid (0.68 g, 70%). Crude extract was

purified by HPLC (C18) using a gradient of 85-100% acetonitrile. MS (EI) observed: 254.2610, expected: 254.2610 ($C_{17}H_{34}O$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.74 – 5.58 (m, 2H), 4.08 (d, *J* = 5.5 Hz, 2H), 2.03 (q, *J* = 6.9 Hz, 2H), 1.51 (dp, *J* = 13.3, 6.6 Hz, 1H), 1.38 – 1.22 (m, 18H), 1.14 (q, *J* = 6.9 Hz, 2H), 0.86 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 133.55, 128.78, 63.80, 39.04, 32.19, 29.93, 29.69, 29.65, 29.59, 29.48, 29.17, 29.12, 27.95, 27.40, 22.63.

(2*R*,3*R*)-15-methylhexadecane-1,2,3-triol (**5A**)

A round bottom flask was charged with 10 ml water and 10 ml tert-butanol. 2.8 grams AD-mix (β) was added and stirred at room temperature until two phases appeared. Methanesulfonamide (180 mg, 1.9 mmol) was added and mixture cooled to 0 °C until salts start to precipitate out (~15 minutes)[4]. **E-4** (500 mg, 1.9 mmol) was added and stirred vigorously at 0 °C for 6-24 hours until production of 5A (0.44 g, 77%), a white solid. Reaction progress monitored by TLC (1:1 hexanes/ethyl acetate). MS (EI) observed: 270.2559 ($C_{17}H_{34}O_2$ – loss of water), expected: 288.2664 ($C_{17}H_{36}O_3$); ^{1}H NMR (400 MHz, CD_3OD) δ 3.63 (dd, *J* = 11.3, 4.6 Hz, 1H), 3.58 – 3.51 (m, 2H), 3.46 (dt, *J* = 6.2, 4.3 Hz, 1H), 1.51 – 1.43 (m, 3H), 1.26 (d, *J* = 11.3 Hz, 18H), 1.17 – 1.10 (m, 2H), 0.84 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (100 MHz, CD_3OD) δ 74.73, 72.32, 64.34, 49.00, 39.66, 33.84, 30.49, 30.28, 30.27, 30.23, 28.54, 27.98, 26.37, 22.89.

(2*S*,3*S*)-15-methylhexadecane-1,2,3-triol (**5B**)

Same protocol as above using α-mix. 5B was produced as a white solid (0.45 g, 82%). MS (EI) observed: 270.2559 ($C_{17}H_{34}O_2$), expected: 288.2664 ($C_{17}H_{36}O_3$); ^{1}H NMR (400 MHz, CD_3OD) δ 3.63 (dd, *J* = 11.1, 4.8 Hz, 1H), 3.60 – 3.51 (m, 2H), 3.47 (dt, *J* = 6.5, 4.4 Hz, 1H), 1.58 – 1.45 (m, 3H), 1.36 – 1.22 (m, 18H), 1.21 – 1.12 (m, 2H), 0.87 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (100 MHz, CD_3OD) δ 75.34, 72.58, 64.52, 40.10, 34.16, 30.90, 30.69, 30.67, 30.64, 28.99, 28.40, 26.86, 23.01.

(2*S*,3*R*)-15-methylhexadecane-1,2,3-triol (**5C**)

A round bottom flask was charged with 10 ml water and 10 ml tert-butanol. 2.8 grams AD-mix (β) was added and stirred at room temperature until two phases appeared. Methanesulfonamide (180 mg, 1.9 mmol) was added and mixture cooled to 0 °C until salts start to precipitate out (~15 minutes). **Z-4** (500 mg, 1.9 mmol) was added and stirred vigorously at 0 °C for 6 - 24 hours until production of 5C (0.49 g, 87%), a white solid. Reaction progress monitored by TLC (1:1 hexanes/ethyl acetate). MS (EI) observed: 270.2564 ($C_{17}H_{34}O_2$), expected: 288.2664 ($C_{17}H_{36}O_3$); ^{1}H NMR (400 MHz, CD_3OD) δ 3.72 (dd, *J* = 11.3, 3.7 Hz, 1H), 3.56 (dd, *J* = 11.3, 6.6 Hz, 1H), 3.49 (td, *J* = 6.3, 3.2 Hz, 1H), 3.42 (td, *J* = 6.6, 3.7 Hz, 1H), 1.71 – 1.63 (m, 1H), 1.58 – 1.47 (m, 2H), 1.40 – 1.24 (m, 18H), 1.21 – 1.15 (m, 2H), 0.88 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (100 MHz, CD_3OD) δ 76.35, 73.70, 64.77, 40.25, 34.14, 31.05, 30.86, 30.81, 30.79, 29.16, 28.54, 26.79, 23.08, 22.99.

(2*R*,3*S*)-15-methylhexadecane-1,2,3-triol (**5D**)

Same protocol as above using AD-mix (α). Reaction yielded **5D** (0.41, 73%), a white solid. MS (EI) observed: 270.2562 ($C_{17}H_{34}O_2$), expected: 288.2664 ($C_{17}H_{36}O_3$); ^{1}H NMR (400 MHz,

CD_3OD) δ 3.71 (dd, *J* = 11.3, 3.7 Hz, 1H), 3.55 (dd, *J* = 11.3, 6.5 Hz, 1H), 3.47 (td, *J* = 8.5, 7.7, 2.8 Hz, 1H), 3.42 (td, *J* = 6.5, 3.6 Hz, 1H), 1.69 – 1.60 (m, 1H), 1.56 – 1.46 (m, 2H), 1.29 (s, 18H), 1.20 – 1.13 (m, 2H), 0.87 (d, *J* = 6.5 Hz, 6H); ^{13}C NMR (100 MHz, CD_3OD) δ 76.28, 73.69, 64.77, 40.25, 34.13, 31.05, 30.86, 30.79, 29.16, 28.54, 26.79, 23.09, 22.99.

(2*R*,3*R*)-2,3-dihydroxy-15-methylhexadecyl 4-methylbenzenesulfonate (**6A**)

To a solution of **5A** (100 mg, 0.34 mmol) in pyridine (1.4 ml) and dichlororomethane (2 ml) stirring at O °C, toluene-*p*-sulfonyl chloride (1.5 eq, 0.52 mmol, 100 mg) was added. Reaction was stirred overnight at 4 °C. Mixture was then diluted with water and extracted with ethyl acetate. Extract was washed with water, 1M HCl and brine, dried over sodium sulfate and concentrated under vacuum. Crude extract was purified by cellulose column using 20% ethanol + 0.1% diethylamine and 80% hexanes + 0.1% diethylamine. Overall reaction and purification yielded enantiopure 6A (63 mg, 41%, ee 72%). HRMS – ESI (M+H) observed: 443.2821 ($C_{24}H_{43}O_5S^+$), (M+H) expected: 443.2831 ($C_{24}H_{43}O_5S^+$); ^{1}H NMR (400 MHz, CD_3OD) δ 7.80 (d, *J* = 8.4 Hz, 2H), 7.44 (d, *J* = 8.0 Hz, 2H), 4.10 (dd, *J* = 10.1, 4.4 Hz, 1H), 3.99 (dd, *J* = 10.1, 6.9 Hz, 1H), 3.61 (dt, *J* = 7.4, 4.0 Hz, 1H), 3.47 (dt, *J* = 8.2, 3.9 Hz, 1H), 2.46 (s, 3H), 1.59 – 1.47 (m, 1H), 1.44 – 1.37 (m, 3H), 1.34 – 1.23 (m, 17H), 1.21 – 1.14 (m, 2H), 0.88 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (100 MHz, CD_3OD) δ 146.46, 134.39, 131.13, 129.00, 72.90, 72.54, 72.12, 40.24, 33.83, 31.04, 30.81, 30.77, 30.72, 29.15, 28.54, 26.87, 23.09, 22.97, 21.65.

(2*S*,3*S*)-2,3-dihydroxy-15-methylhexadecyl 4-methylbenzenesulfonate (**6B**)

Followed same protocol as above using a solution of **5B** instead of 5A. Reaction + purification yielded enantiopure 6B (51 mg, 36%, ee 75%) HRMS – ESI (M+H) observed: 443.2827 ($C_{24}H_{43}O_5S^+$), (M+H) expected: 443.2831 ($C_{24}H_{43}O_5S^+$); ^{1}H NMR (400 MHz, CD_3OD) δ 7.80 (d, *J* = 8.3 Hz, 2H), 7.44 (d, *J* = 8.1 Hz, 2H), 4.10 (dd, *J* = 10.1, 4.3 Hz, 1H), 3.99 (dd, *J* = 10.1, 6.9 Hz, 1H), 3.61 (dt, *J* = 7.0, 4.0 Hz, 1H), 3.47 (dt, *J* = 8.1, 3.9 Hz, 1H), 2.46 (s, 3H), 1.57 – 1.46 (m, 1H), 1.45 – 1.35 (m, 3H), 1.29 (m, 17H), 1.22 – 1.13 (m, 2H), 0.88 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (100 MHz, CD_3OD) δ 146.48, 134.38, 131.12, 129.02, 72.90, 72.56, 72.13, 40.25, 33.83, 31.04, 30.80, 30.77, 30.71, 29.15, 28.53, 26.86, 23.07, 23.00, 21.64

(2*S*,3*R*)-2,3-dihydroxy-15-methylhexadecyl 4-methylbenzenesulfonate (**6C**)

Followed same protocol as above using a solution of **5C**. Reaction + purification yielded enantiopure 6C (36 mg, 26%, ee 68%) – a white solid. HRMS – ESI (M+H) observed: 443.2838 ($C_{24}H_{43}O_5S^+$), (M+H) expected: 443.2831 ($C_{24}H_{43}O_5S^+$); ^{1}H NMR (400 MHz, CD_3OD) δ 7.81 (d, *J* = 8.4 Hz, 2H), 7.44 (d, *J* = 8.0 Hz, 2H), 4.20 (dd, *J* = 10.1, 2.9 Hz, 1H), 3.99 (dd, *J* = 10.1, 6.9 Hz, 1H), 3.52 (td, *J* = 7.0, 2.9 Hz, 1H), 3.39 (dq, *J* = 8.5, 3.1 Hz, 1H), 2.46 (s, 3H), 1.66 – 1.57 (m, 1H), 1.56 – 1.46 (m, 2H), 1.35 – 1.22 (m, 18H), 1.21 – 1.14 (m, 2H), 0.88 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (100 MHz, CD_3OD) δ 146.43, 134.37, 131.02, 129.11, 73.74, 73.38, 72.66, 40.25, 34.30, 31.04, 30.81, 30.78, 30.74, 29.15, 28.53, 26.53, 23.03, 21.57.

(2*R*,3*S*)-2,3-dihydroxy-15-methylhexadecyl 4-methylbenzenesulfonate (**6D**)

Followed same protocol as above using a solution of **5D**. Reaction + purification yielded enantiopure 6C (52 mg, 38%, ee 78%) – a white solid. HRMS – ESI (M+H) observed: 443.2832 ($C_{24}H_{43}O_5S^+$), (M+H) expected: 443.2831 ($C_{24}H_{43}O_5S^+$); ^{1}H NMR (400 MHz, CD_3OD) δ 7.81 (d, *J* = 8.3 Hz, 2H), 7.44 (d, *J* = 8.2 Hz, 2H), 4.21 (dd, *J* = 10.2, 2.9 Hz, 1H), 3.99 (dd, *J* = 10.1, 6.8 Hz, 1H), 3.52 (td, *J* = 7.0, 2.9 Hz, 1H), 3.39 (dq, *J* = 8.3, 3.0 Hz, 1H), 2.46 (s, 3H), 1.65 – 1.57 (m, 1H), 1.55 – 1.44 (m, 2H), 1.36 – 1.23 (m, 18H), 1.22 – 1.13 (m, 2H), 0.88 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (100 MHz, CD_3OD) δ 146.43, 134.37, 131.02, 129.11, 73.74, 73.38, 72.66, 40.25, 34.30, 31.04, 30.81, 30.78, 30.74, 29.15, 28.53, 26.53, 23.04, 21.57.

(2*S*,3*R*)-2,3-dihydroxy-15-methylhexadecane-1-sulfonic acid (**IOR-1A**)

Sodium sulfite was dissolved in 1.5 ml water and heated to 62 °C. To this solution, **6A** (30 mg, 0.067 mmol) dissolved in ethanol (0.6 ml) was added. Reaction was stirred at 62 °C for 10 hours. Reaction was then extracted with ethyl acetate and washed with water and brine affording IOR-A – a white solid (3.3 mg, 14%). **IOR-1A** was purified by RP-HPLC (C18) using a gradient of Methanol/water + 01% ammonium hydroxide. Optical rotation: $[\alpha]_D^{21.8}$ +20 (*c* 0.25, MeOH); HRMS – ESI (M-H) observed: 351.2211 ($C_{17}H_{35}O_5S^-$), (M-H) expected: 351.2211 ($C_{17}H_{35}O_5S^-$); ^{1}H NMR (500 MHz, $CD_3OD/CDCl_3$) δ 4.04 (dt, *J* = 8.4, 3.3 Hz, 1H), 3.60 (dt, *J* = 7.9, 3.6 Hz, 1H), 3.09 (dd, *J* = 14.0, 3.5 Hz, 1H), 2.98 (dd, *J* = 14.0, 8.5 Hz, 1H), 1.61 – 1.49 (m, 3H), 1.42 – 1.28 (m, 18H), 1.24 – 1.17 (m, 2H), 0.91 (d, *J* = 6.5 Hz, 6H); ^{13}C NMR (126 MHz, $CD_3OD/CDCl_3$) δ 74.27, 71.36, 55.28, 40.19, 33.73, 31.00, 30.74, 29.10, 28.49, 27.10, 23.03.

(2*R*,3*S*)-2,3-dihydroxy-15-methylhexadecane-1-sulfonic acid (**IOR-1B**)

Same protocol as above using **6B** afforded IOR-1B (4.1 mg, 17%). Optical rotation: $[\alpha]_D^{22}$ -12 (*c* 0.5, MeOH) HRMS – ESI (M-H) observed: 351.2214 ($C_{17}H_{35}O_5S^-$), (M-H) expected: 351.2211 ($C_{17}H_{35}O_5S^-$); ^{1}H NMR (500 MHz, $CD_3OD/CDCl_3$) δ 4.05 (dt, *J* = 8.9, 3.3 Hz, 1H), 3.57 (dt, *J* = 7.8, 3.8 Hz, 1H), 3.07 (dd, *J* = 14.0, 3.2 Hz, 1H), 3.00 (dd, *J* = 14.0, 8.8 Hz, 1H), 1.58 – 1.47 (m, 3H), 1.37 – 1.23 (m, 18H), 1.20-1.15 (m, 2H), 0.88 (d, *J* = 6.5 Hz, 6H); ^{13}C NMR (126 MHz, $CD_3OD/CDCl_3$) δ 74.09, 71.09, 54.90, 39.90, 33.45, 30.72, 30.47, 28.79, 28.22, 26.80, 22.97.

(2*R,* 3*R*)-2,3-dihydroxy-15-methylhexadecane-1-sulfonic acid (**IOR-1C**)

Same protocol as above using **6C** yielded a white solid (4.5 mg, 19%). Optical rotation: $[\alpha]_D^{22}$ +10 (*c* 0.5, MeOH); HRMS – ESI (M-H) observed: 351.2215 ($C_{17}H_{35}O_5S^-$), (M-H) expected: 351.2211 ($C_{17}H_{35}O_5S^-$); ^{1}H NMR (500 MHz, $CD_3OD/CDCl_3$) δ 3.96 (ddd, *J* = 9.5, 5.5, 2.0 Hz, 1H), 3.54 (ddd, *J* = 8.8, 5.6, 3.2 Hz, 1H), 3.17 (dd, *J* = 14.0, 2.1 Hz, 1H), 2.89 (dd, *J* = 14.1, 9.6 Hz, 1H), 1.64 – 1.50 (m, 3H), 1.40 – 1.24 (m, 18H), 1.23 – 1.16 (m, 2H), 0.89 (d, *J* = 6.6 Hz, 6H) ; ^{13}C NMR (126 MHz, $CD_3OD/CDCl_3$) δ 74.61, 72.26, 54.08, 40.02, 33.54, 30.83, 30.57, 28.91, 28.33, 26.69, 23.00.

(2*S*, 3*S*)-2,3-dihydroxy-15-methylhexadecane-1-sulfonic acid (**IOR-1D**)

Same protocol as above using **6D** yielded a white solid (4.3 mg, 18%). Optical rotation: $[\alpha]_D^{22}$ -4 (*c* 0.5, MeOH); HRMS – ESI (M-H) observed: 351.2210 ($C_{17}H_{35}O_5S^-$), (M-H) expected: 351.2211 ($C_{17}H_{35}O_5S^-$); ^{1}H NMR (500 MHz, $CD_3OD/CDCl_3$) δ 3.95 (ddd, *J* = 9.8, 5.4, 2.1 Hz,

1H), 3.54 (ddd, *J* = 8.8, 5.3, 3.2 Hz, 1H), 3.14 (dd, *J* = 14.1, 2.0 Hz, 1H), 2.89 (dd, *J* = 14.1, 9.7 Hz, 1H), 1.61 – 1.49 (m, 3H), 1.36 – 1.23 (m, 18H), 1.18 – 1.12 (m, 2H), 0.86 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (126 MHz, $CD_3OD/CDCl_3$) δ 74.71, 72.36, 54.25, 40.10, 33.62, 30.91, 30.70, 29.00, 28.40, 26.76, 23.02.

References:

(1) Beemelmanns, C.; Woznica, A.; Alegado, R. A.; Cantley, A. M.; King, N.; Clardy, J. *J. Am. Chem. Soc.* **2014**, *136*, 10210–10213.

(2) Takikawa, H.; Nozawa, D.; Kayo, A.; Muto, S.-E.; Mori, K. *J. Chem. Soc., Perkin Trans. 1* **1999**, No. 17, 2467–2477.

(3) Romuald, C.; Cazals, G.; Enjalbal, C.; Coutrot, F. *Org. Lett.* **2013**, *15*, 184–187.

(4) Sharpless, K. B.; Amberg, W.; Bennani, Y. L.; Crispino, G. A.; Hartung, J.; Jeong, K. S.; Kwong, H. L.; Morikawa, K.; Wang, Z. M. *J. Org. Chem.* **1992**, *57*, 2768–2771.

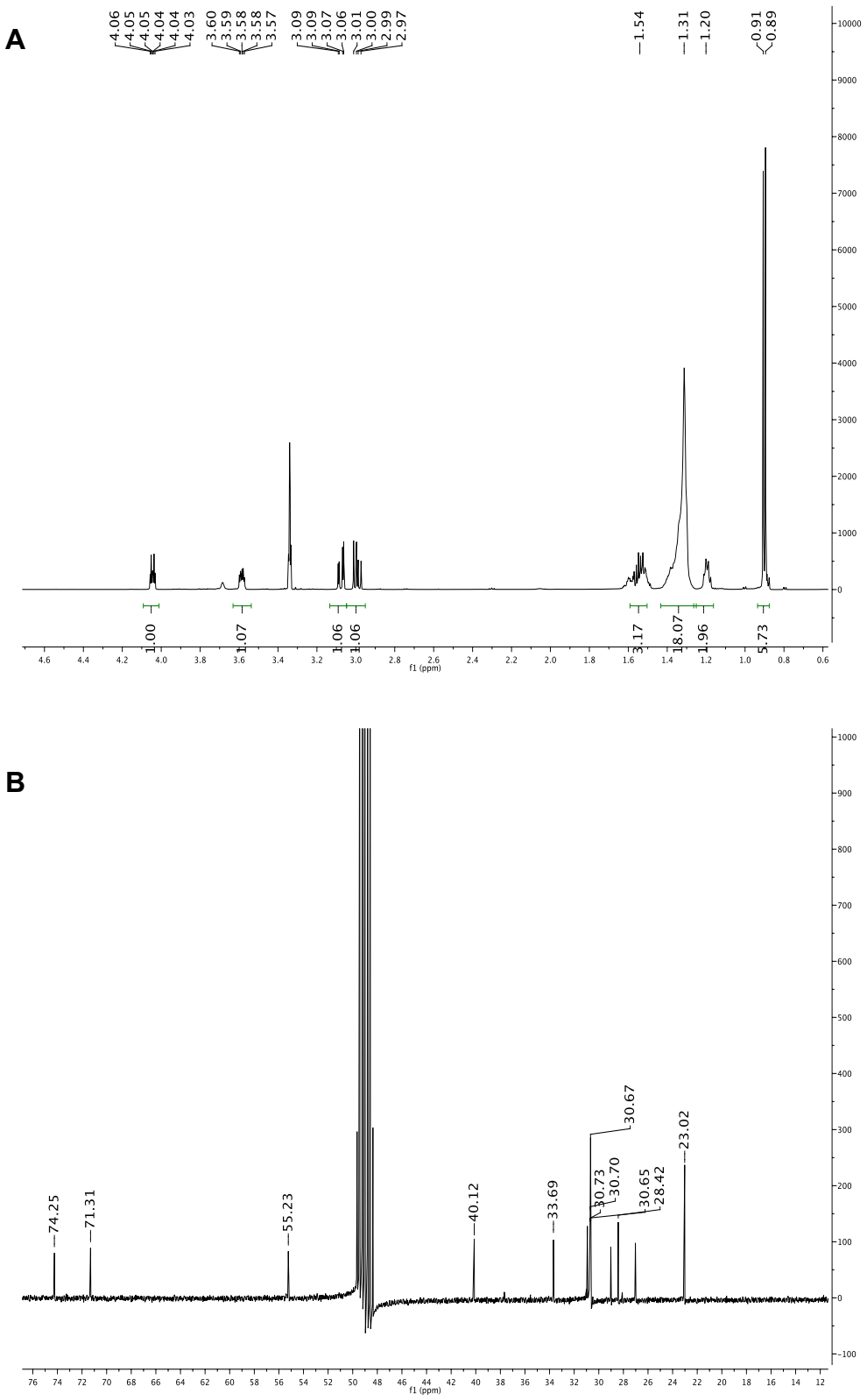
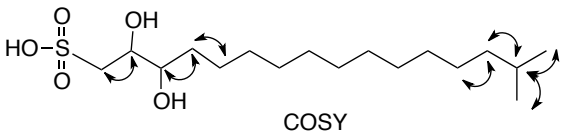


Figure S1: (A) ^{1}H spectrum of isolated IOR-1 (B) ^{13}C spectrum of isolated IOR-1

A



B

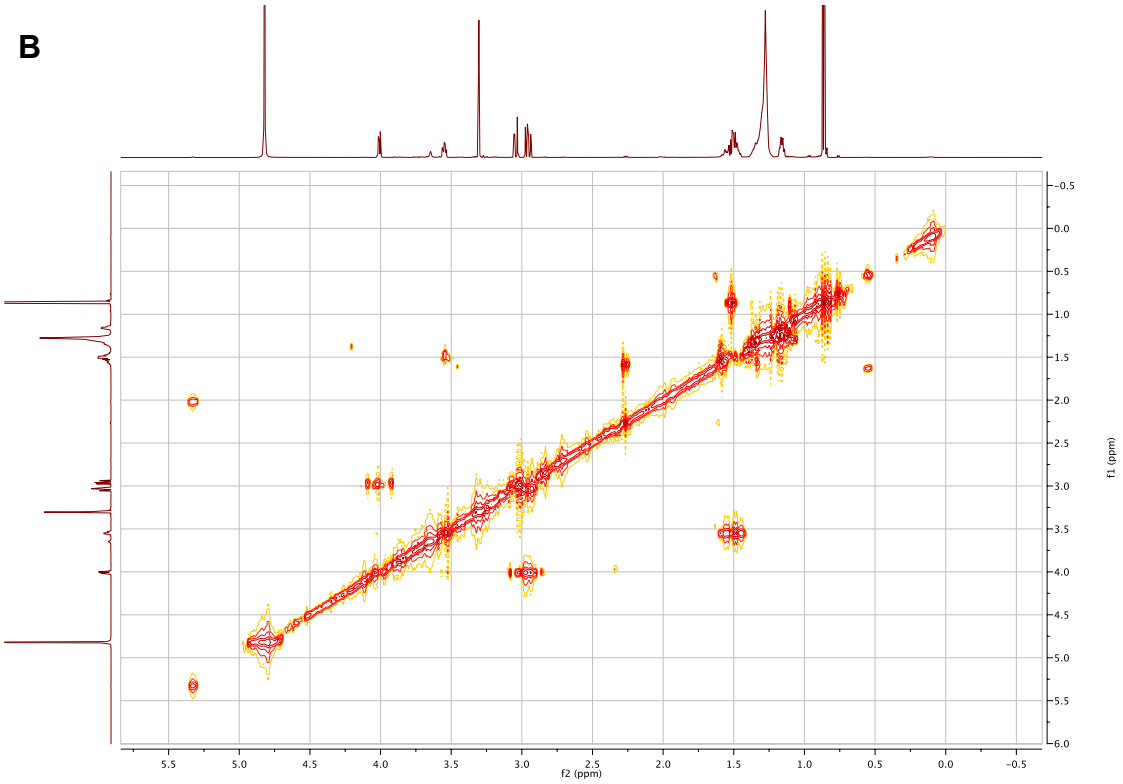


Figure S2: (A) key correlations observed in COSY spectrum of isolated IOR-1 (B) full COSY spectrum. Solvent: $CD_3OD/CDCl_3$, ^{13}C (151 MHz), ^{1}H (600 MHz)

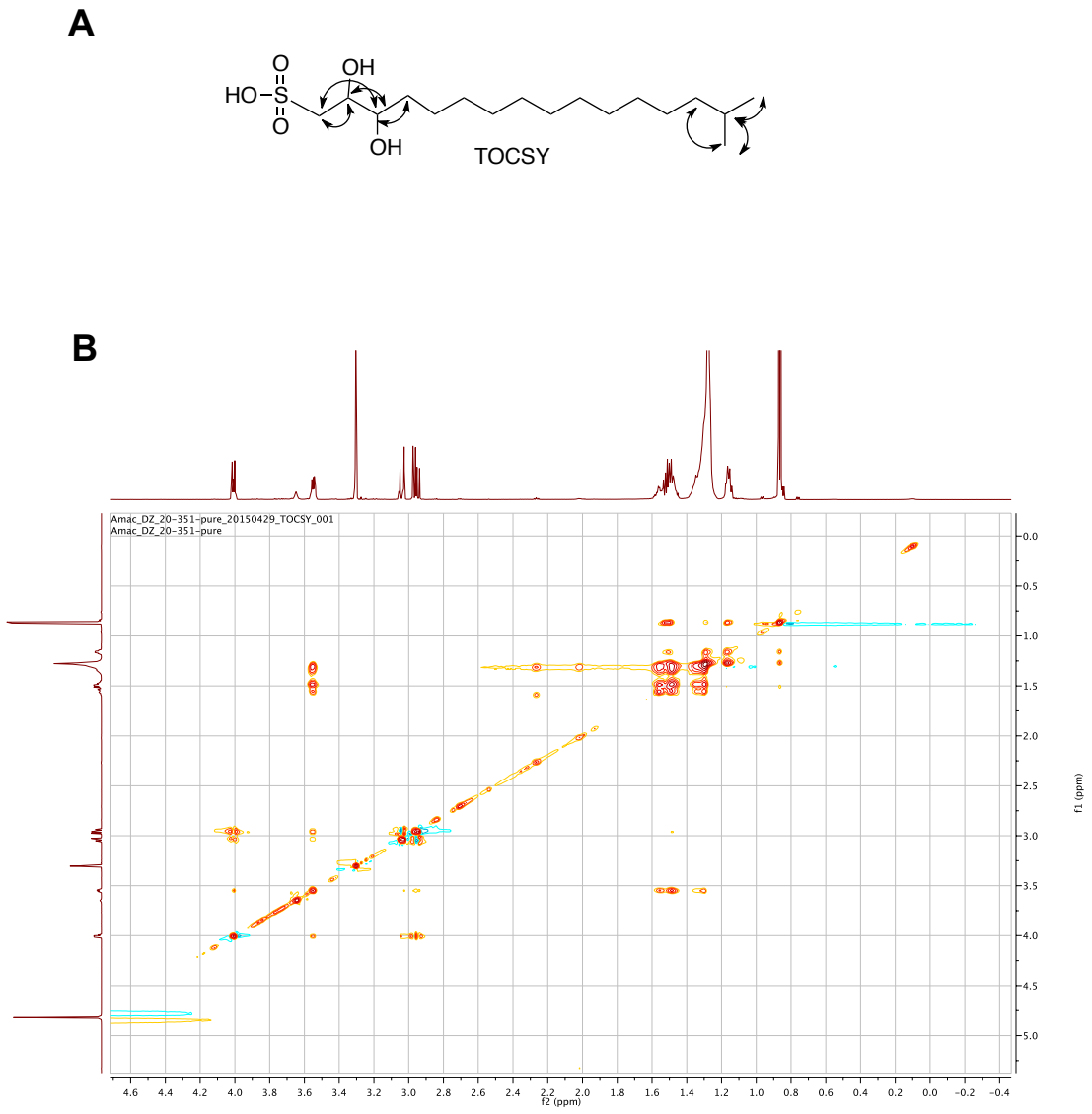


Figure S3: (A) key correlations observed in TOCSY spectrum of isolated IOR-1 (B) TOCSY full spectrum. Solvent: $CD_3OD/CDCl_3$, ^{13}C (151 MHz), ^{1}H (600 MHz)

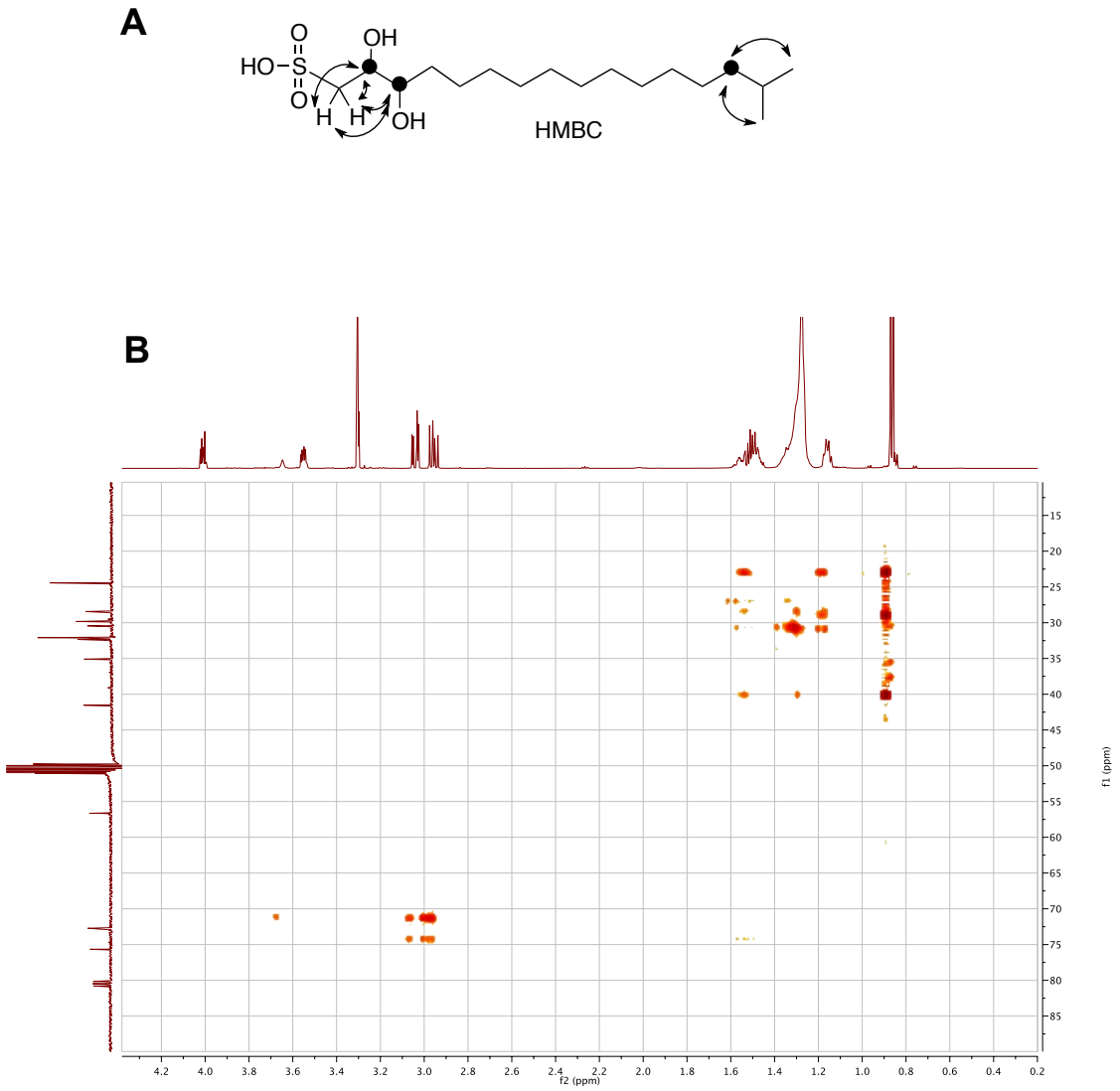


Figure S4: (A) key correlations observed in HMBC spectrum of isolated IOR-1 (B) HMBCAD full spectrum. Solvent: $CD_3OD/CDCl_3$, ^{13}C (151 MHz), ^{1}H (600 MHz)

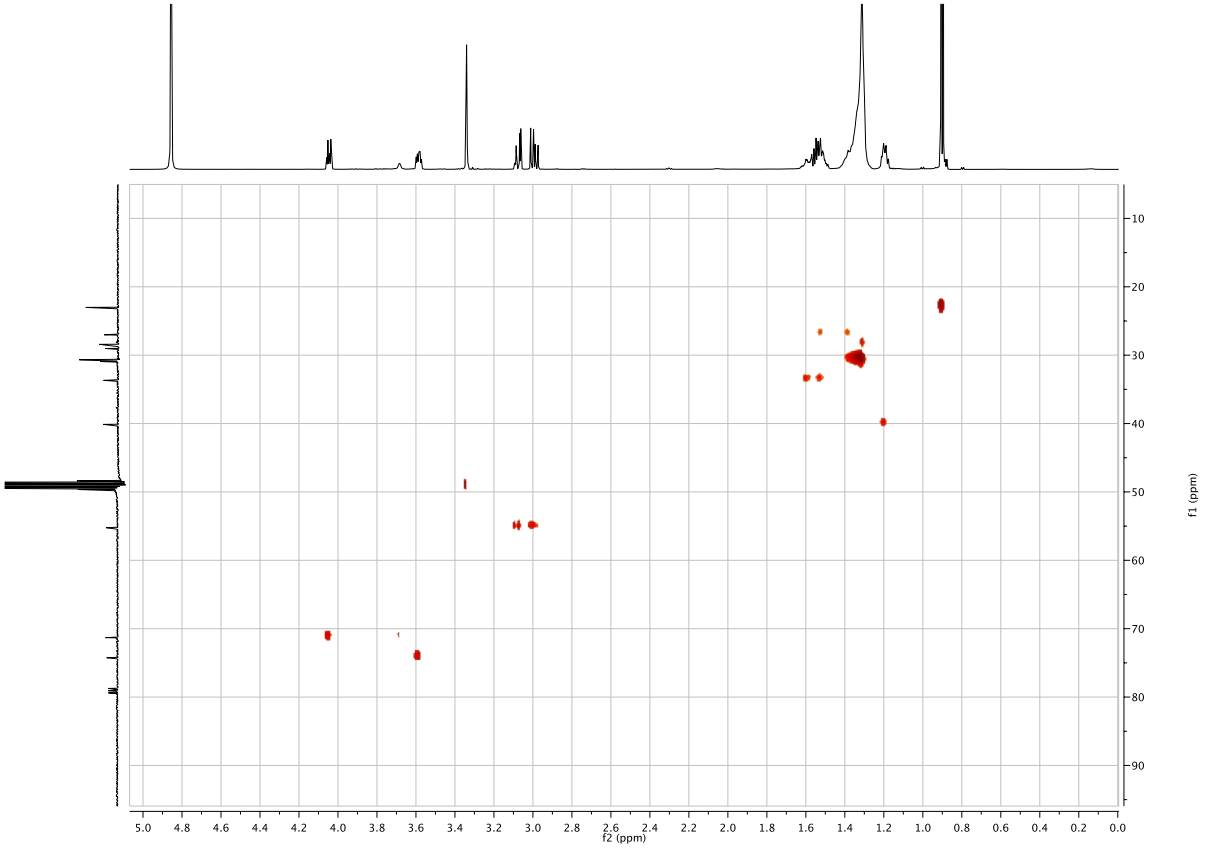


Figure S5: HSQCAD full spectrum of isolated IOR-1. Solvent: $CD_3OD/CDCl_3$, ^{13}C (151 MHz), ^{1}H (600 MHz)

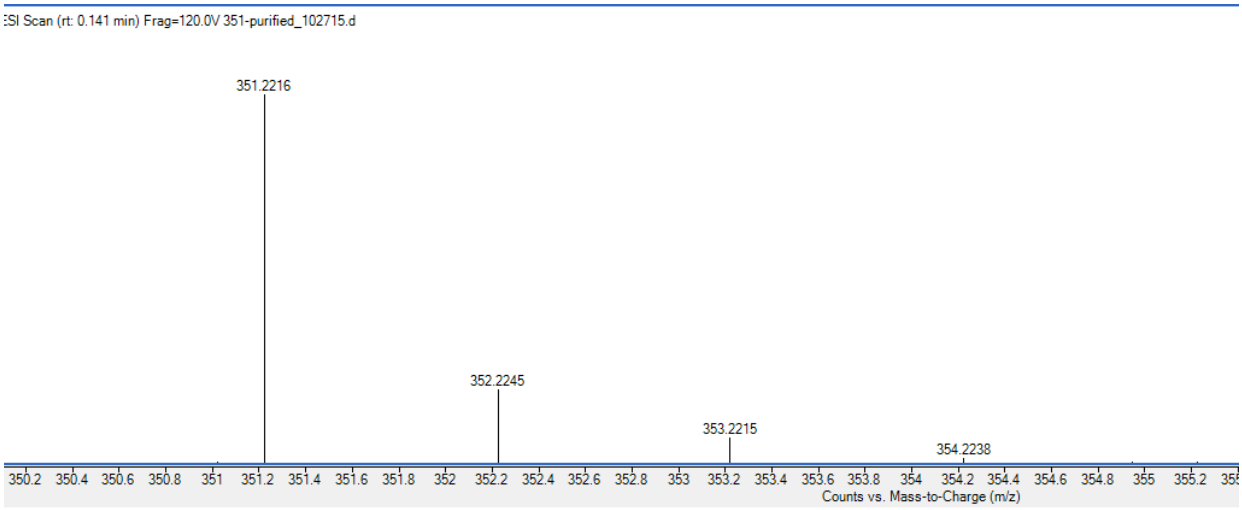


Figure S6: HRMS of isolated IOR-1

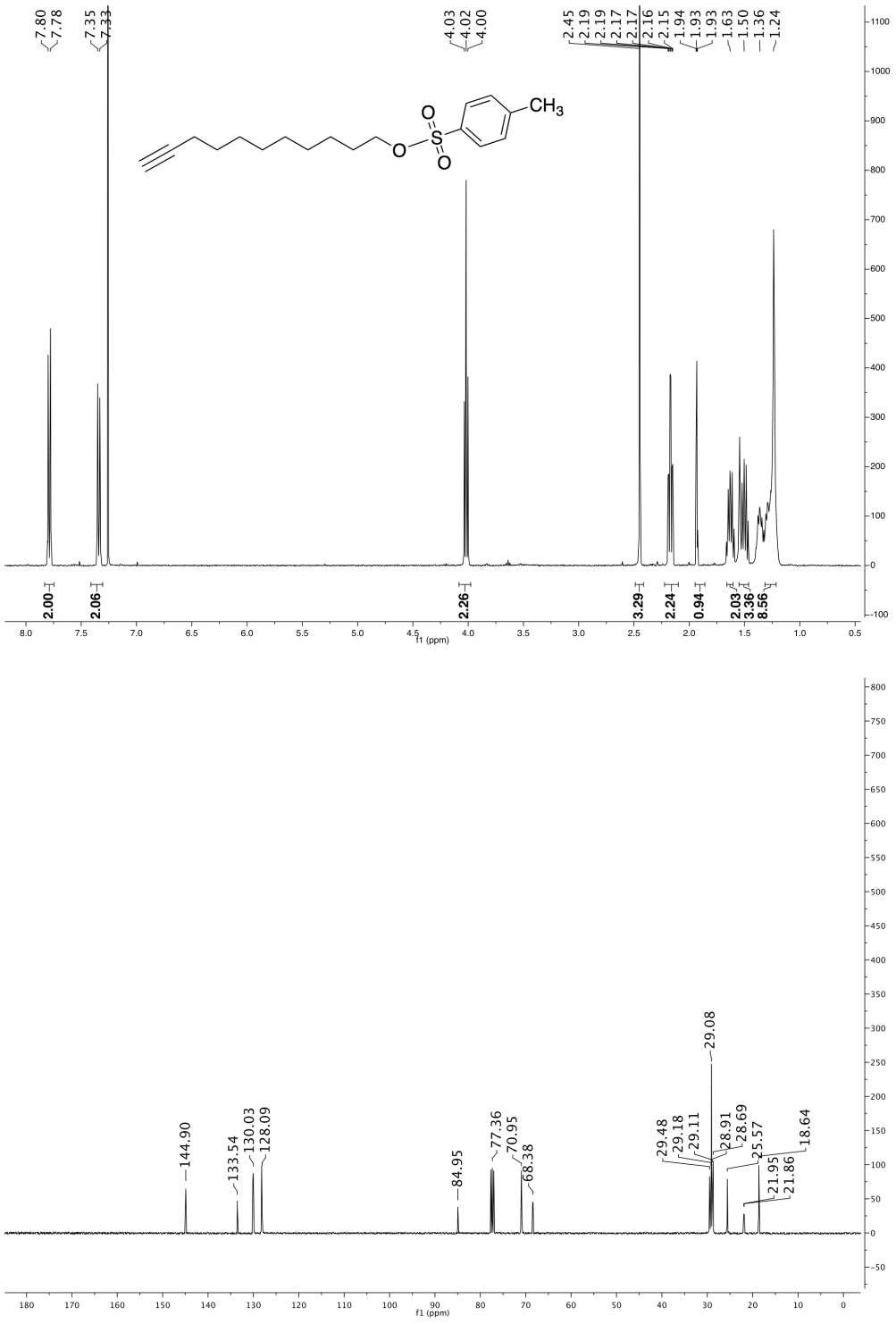


Figure S7: **1** ^{1}H NMR (top) and ^{13}C NMR (bottom). Solvent: $CDCl_3$

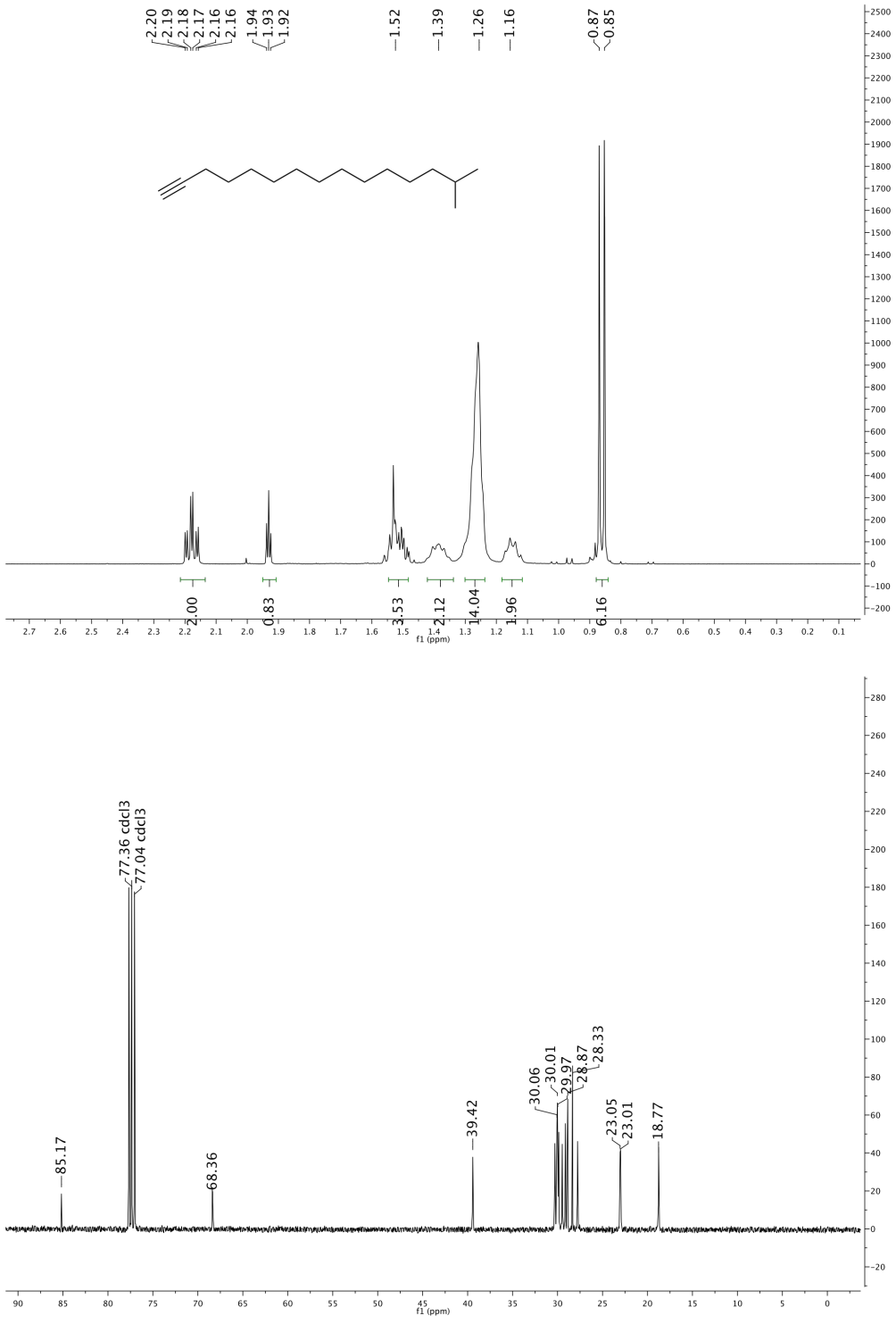


Figure S8: **2** ^{1}H NMR (top) and ^{13}C NMR (bottom). Solvent: $CDCl_3$

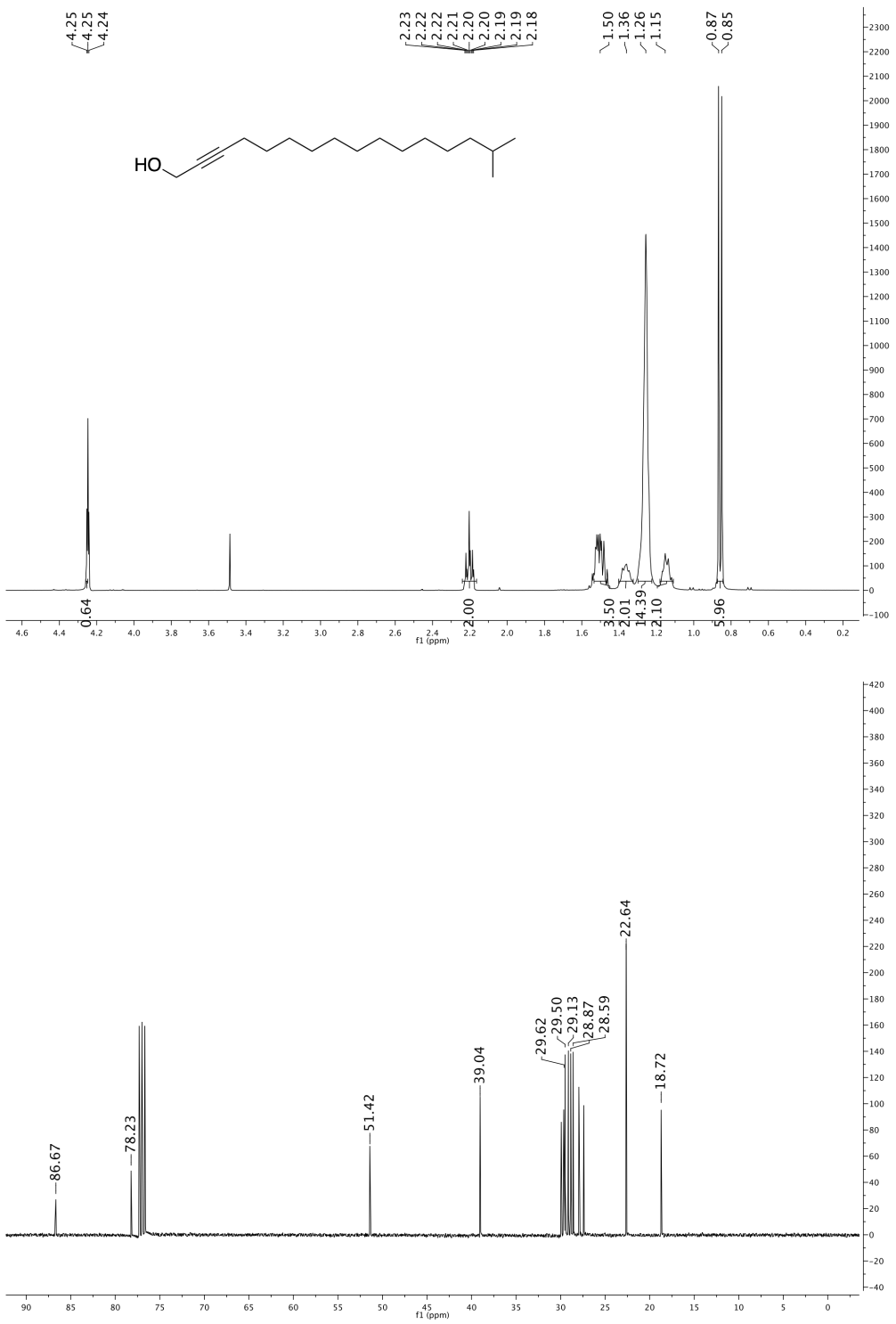


Figure S9: **3** ^{1}H NMR (top) and ^{13}C NMR (bottom). Solvent: $CDCl_3$

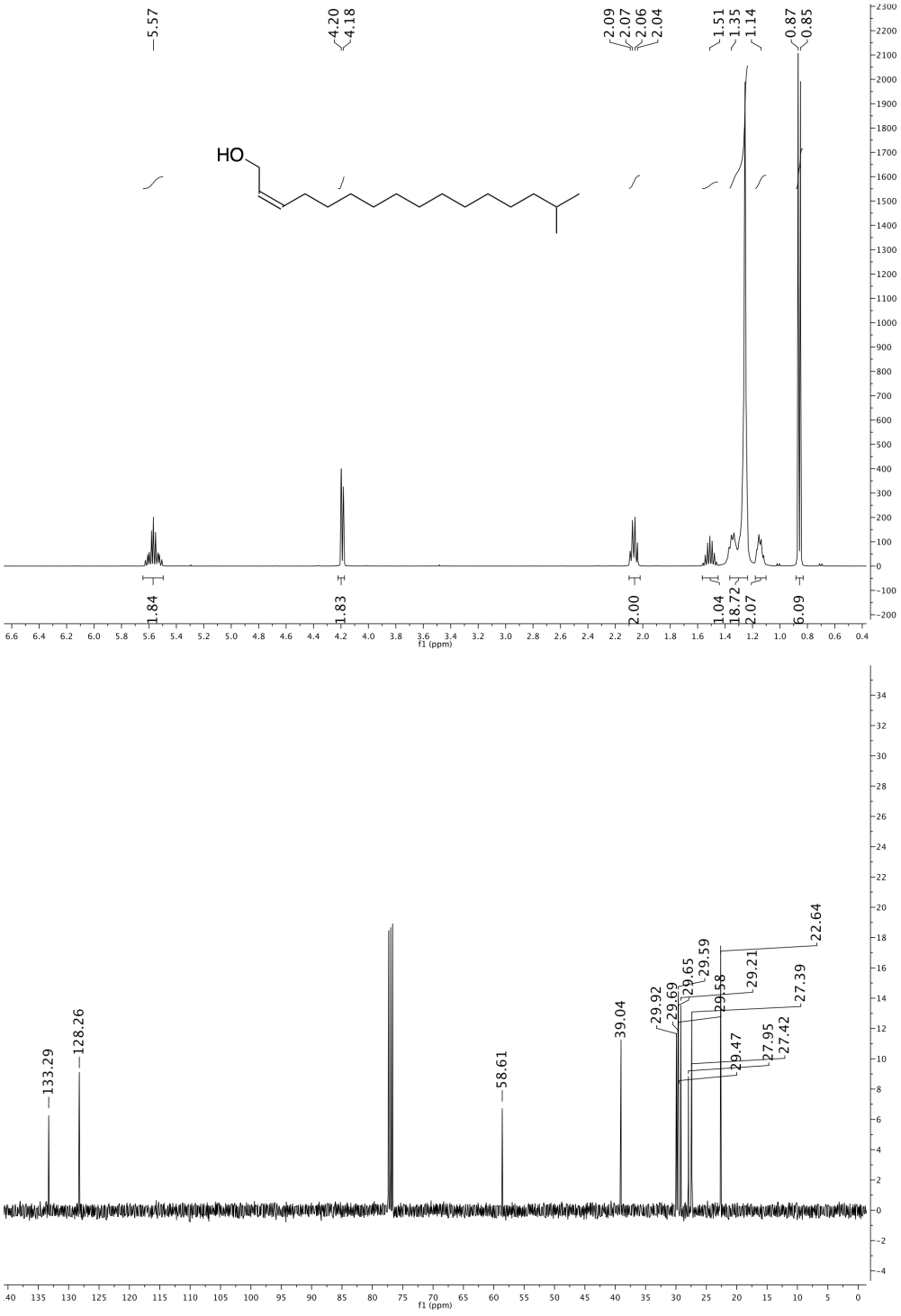


Figure S10: **Z-4** ^{1}H NMR (top) and ^{13}C NMR (bottom). Solvent: $CDCl_3$

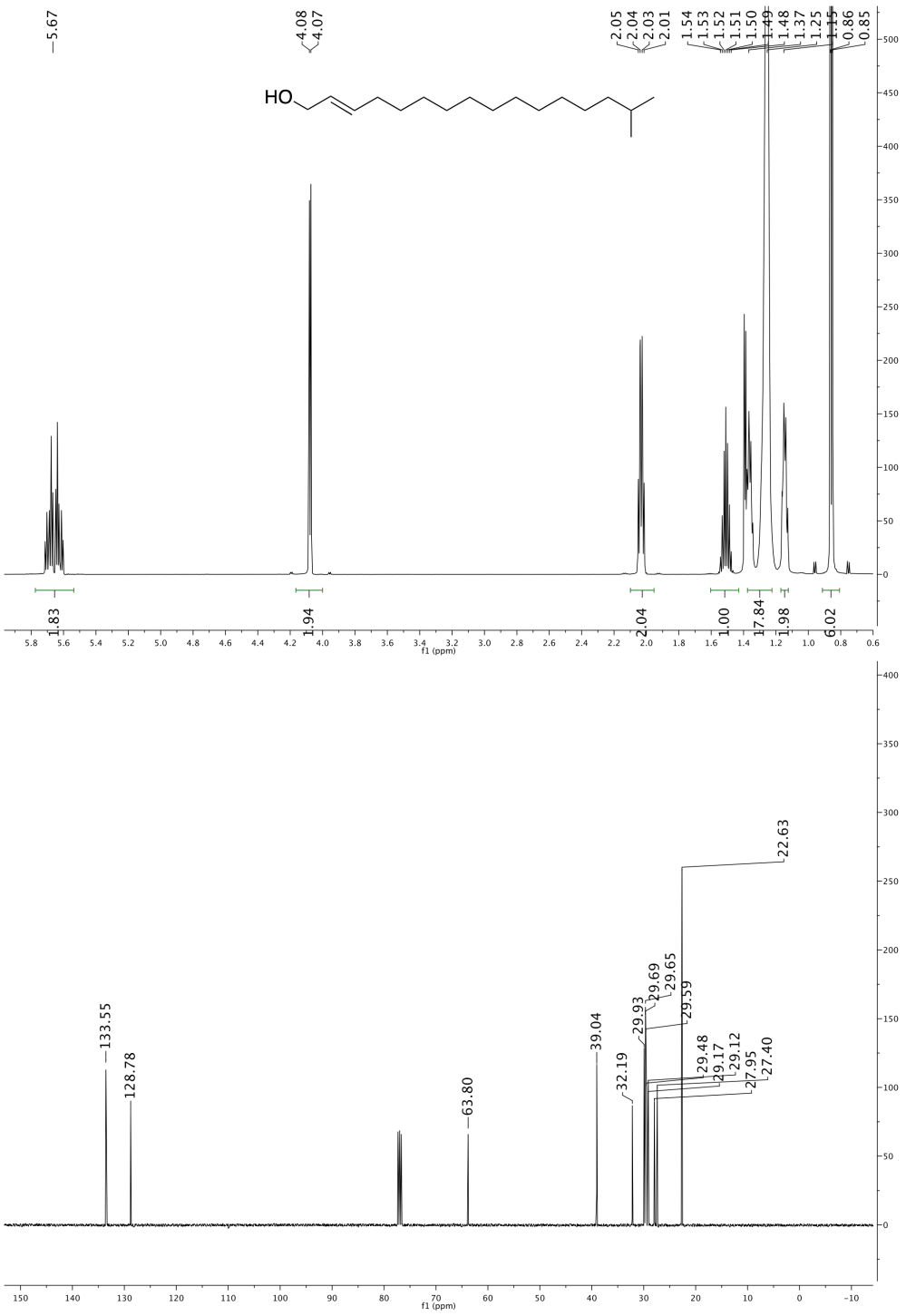


Figure S11: **E-4** ^{1}H NMR (top) and ^{13}C NMR (bottom). Solvent: $CDCl_3$

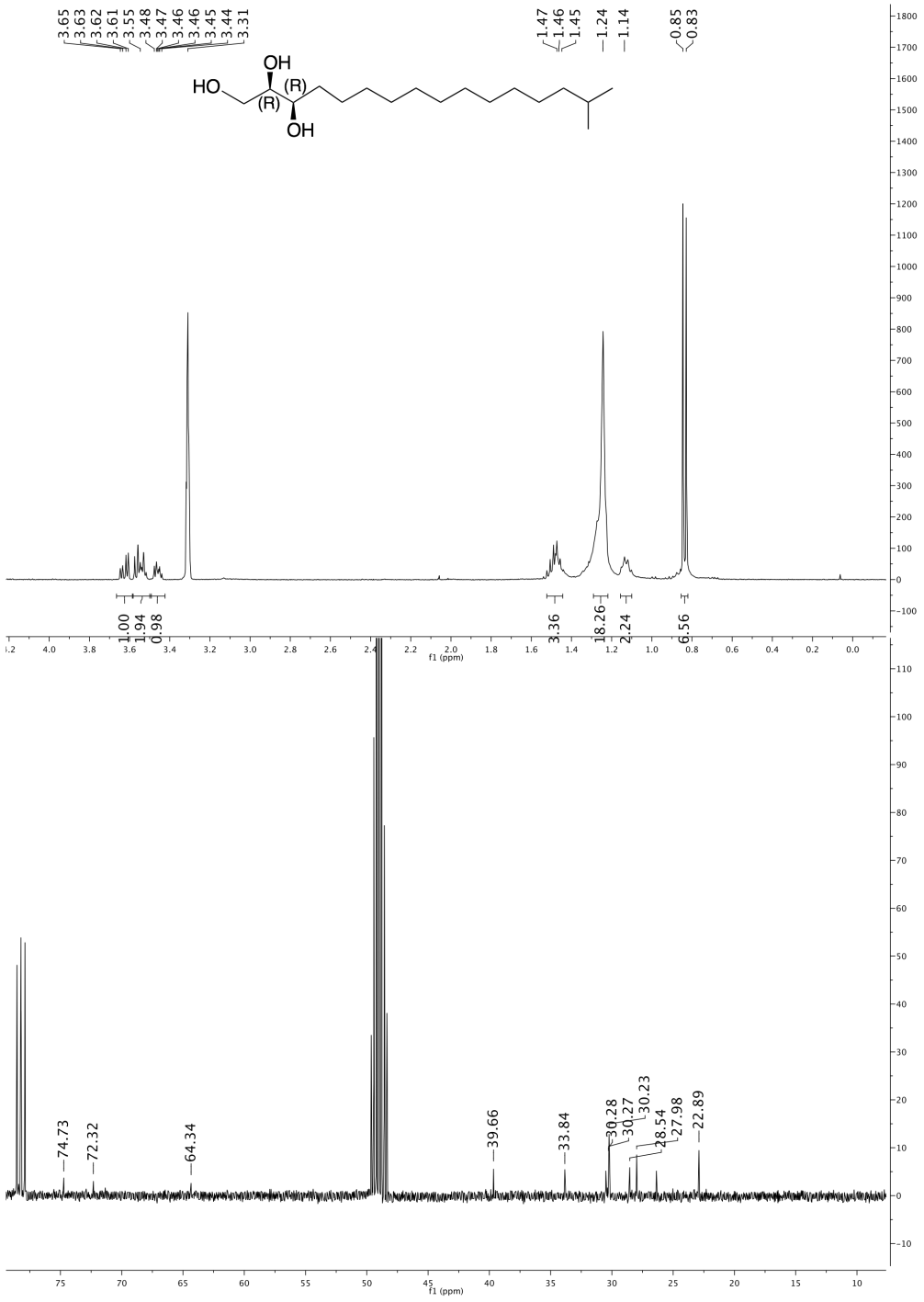


Figure S12: **5A** ^{1}H NMR (top) and ^{13}C NMR (bottom). Solvent: CD_3OD

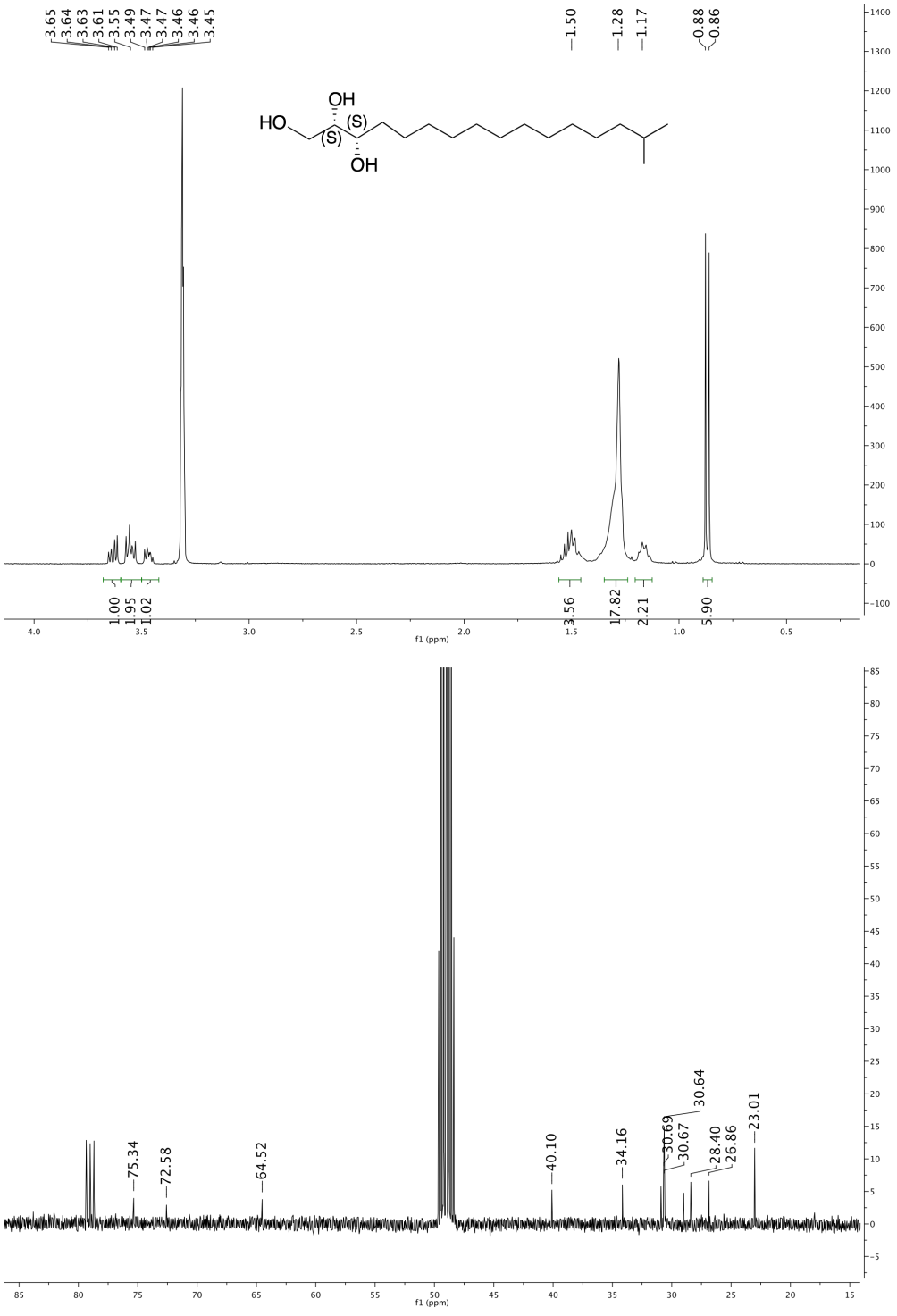


Figure S13: **5B** ^{1}H NMR (top) and ^{13}C NMR (bottom). Solvent: CD_3OD

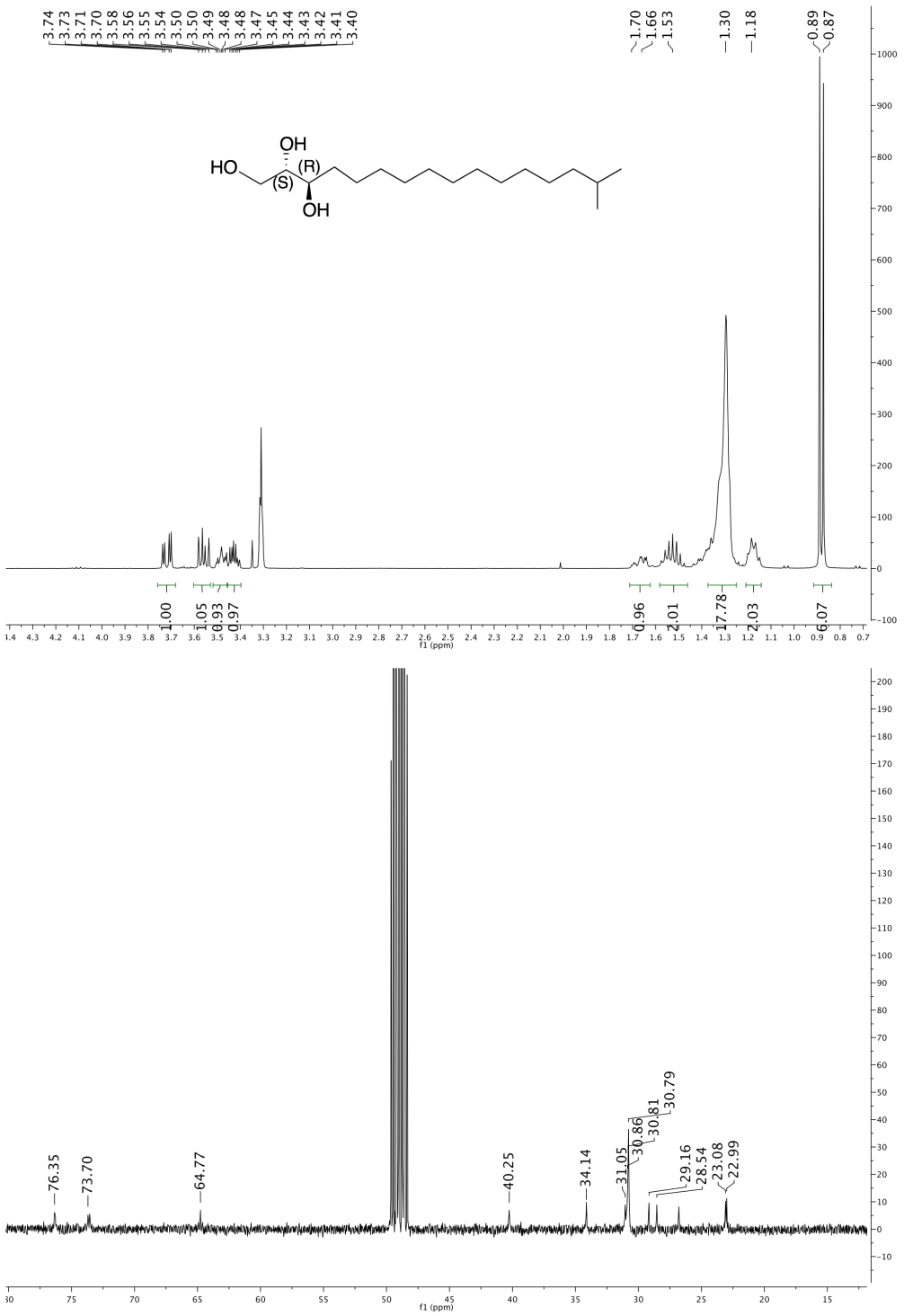


Figure S14: **5C** ^{1}H NMR (top) and ^{13}C NMR (bottom). Solvent: CD_3OD

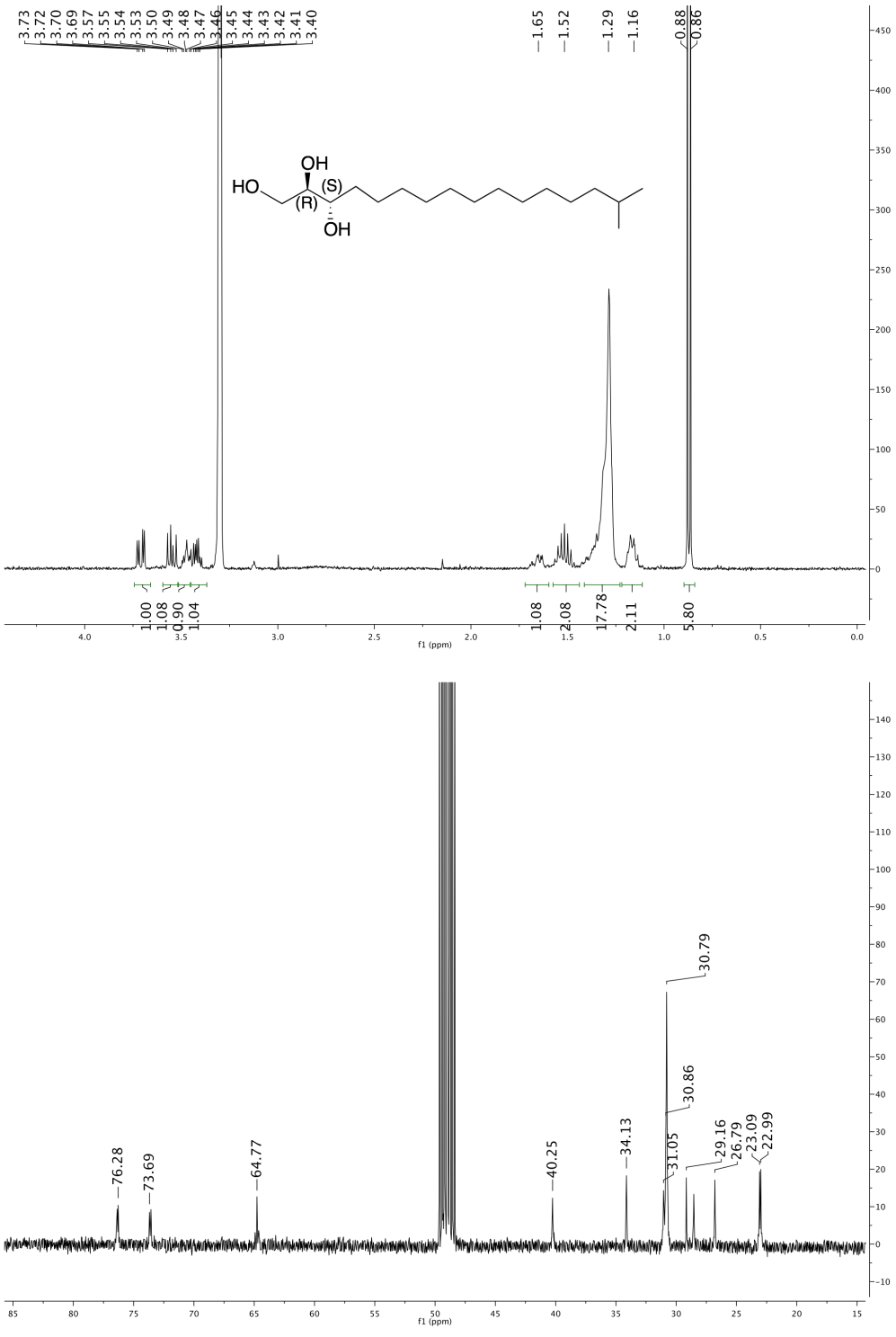


Figure S15: **5D** ^{1}H NMR (top) and ^{13}C NMR (bottom). Solvent: CD_3OD

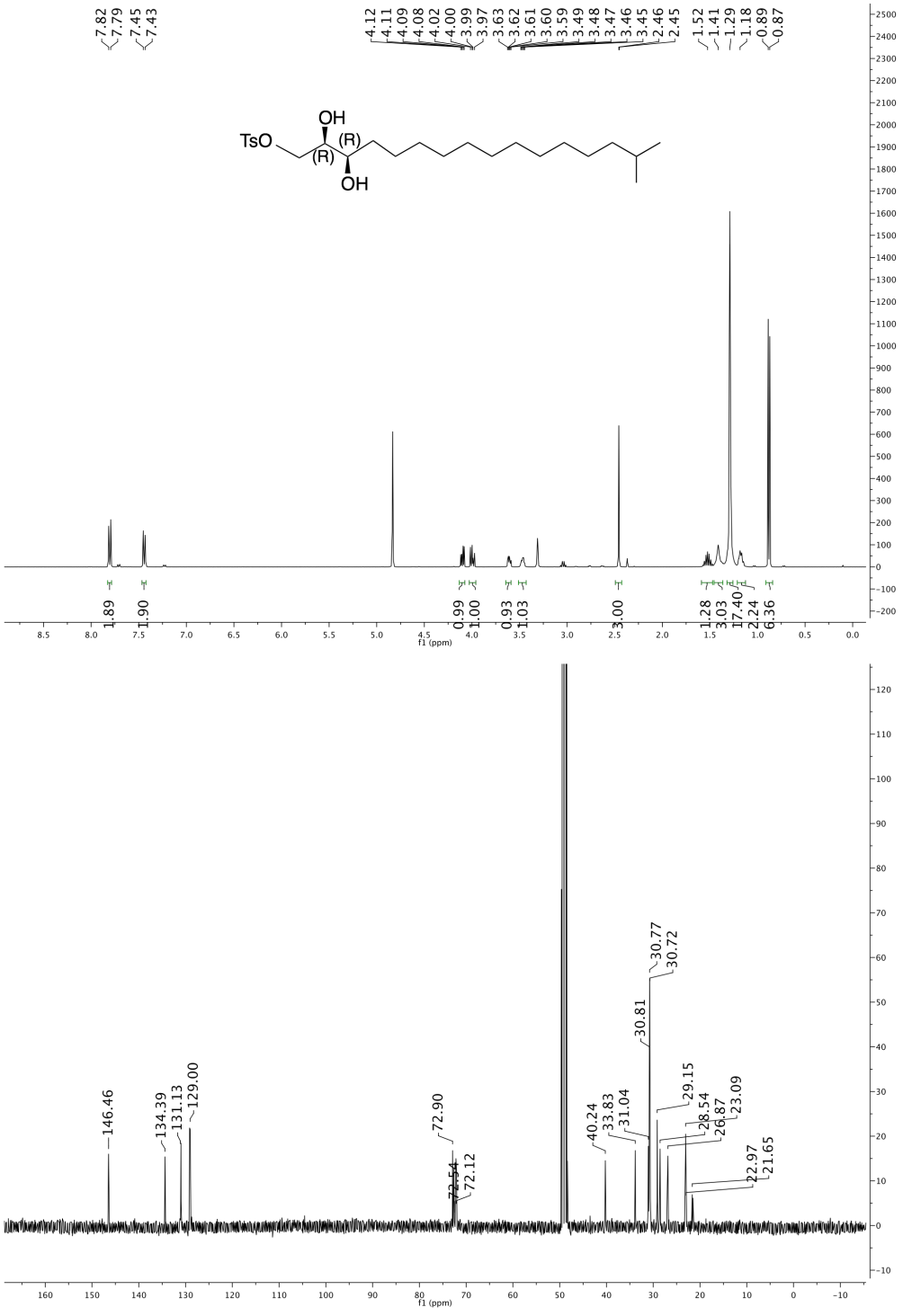


Figure S16: **6A** ^{1}H NMR (top) and ^{13}C NMR (bottom). Solvent: CD_3OD

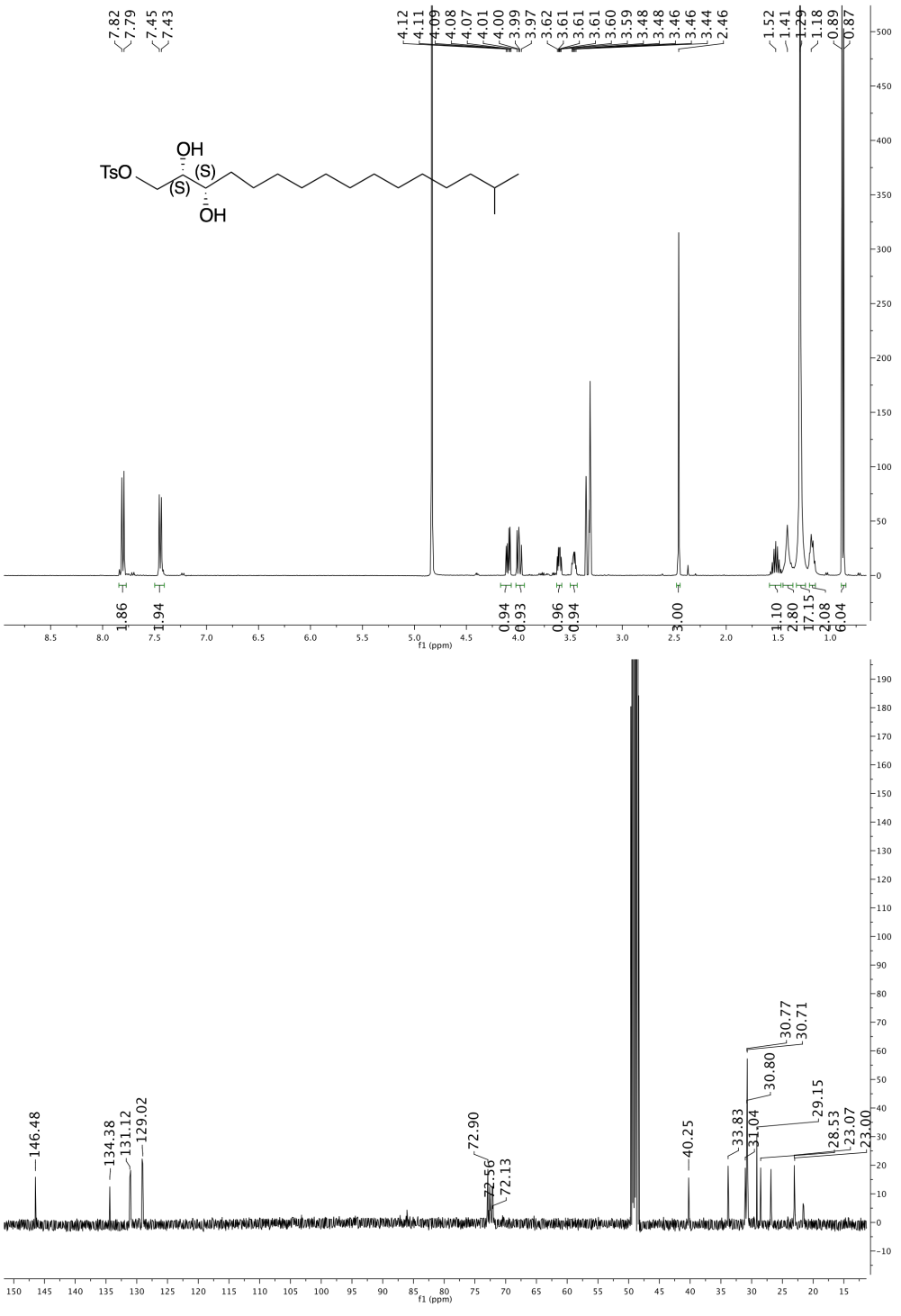


Figure S17: **6B** ^{1}H NMR (top) and ^{13}C NMR (bottom). Solvent: CD_3OD

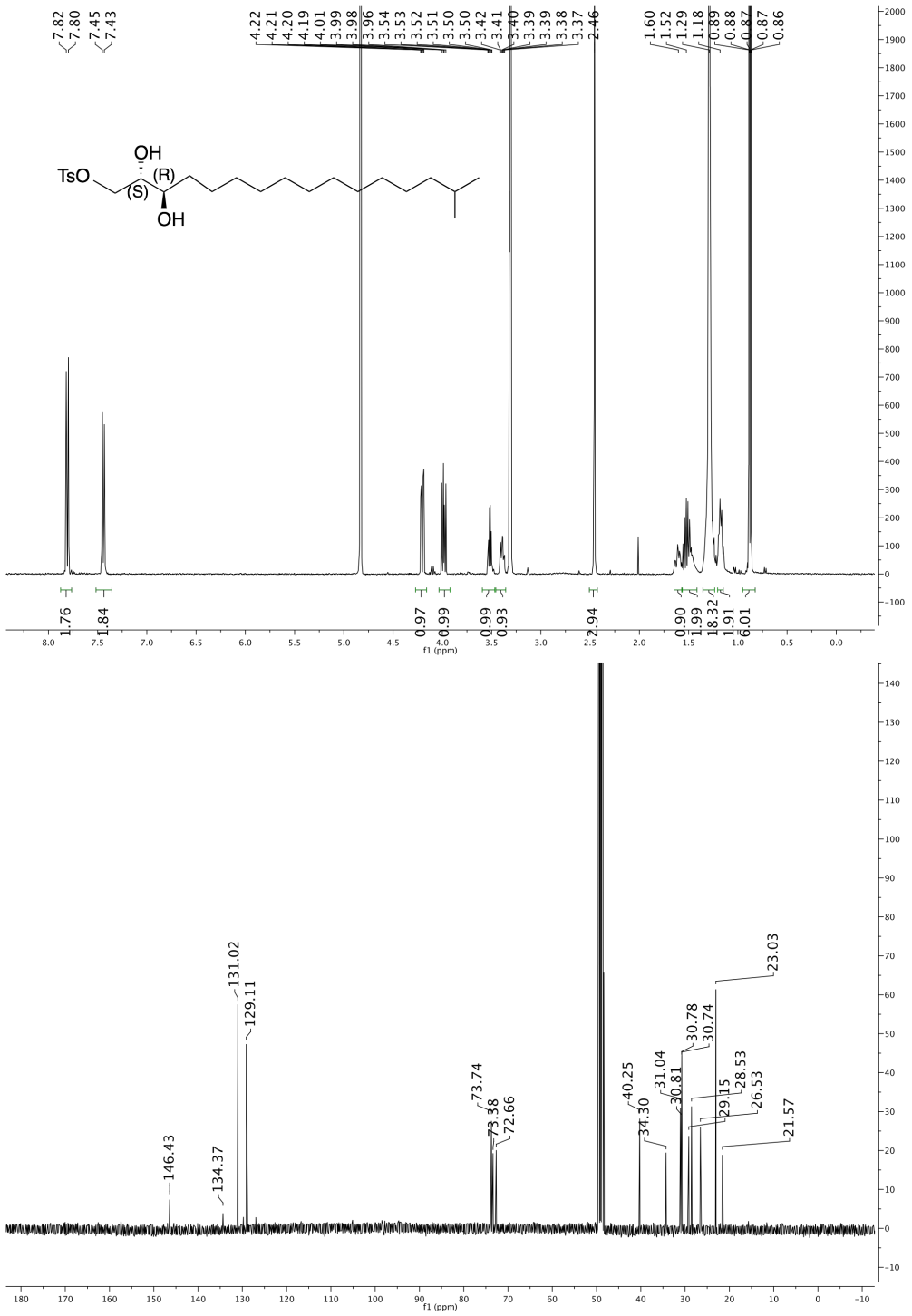


Figure S18: **6C** ^{1}H NMR (top) and ^{13}C NMR (bottom). Solvent: CD_3OD

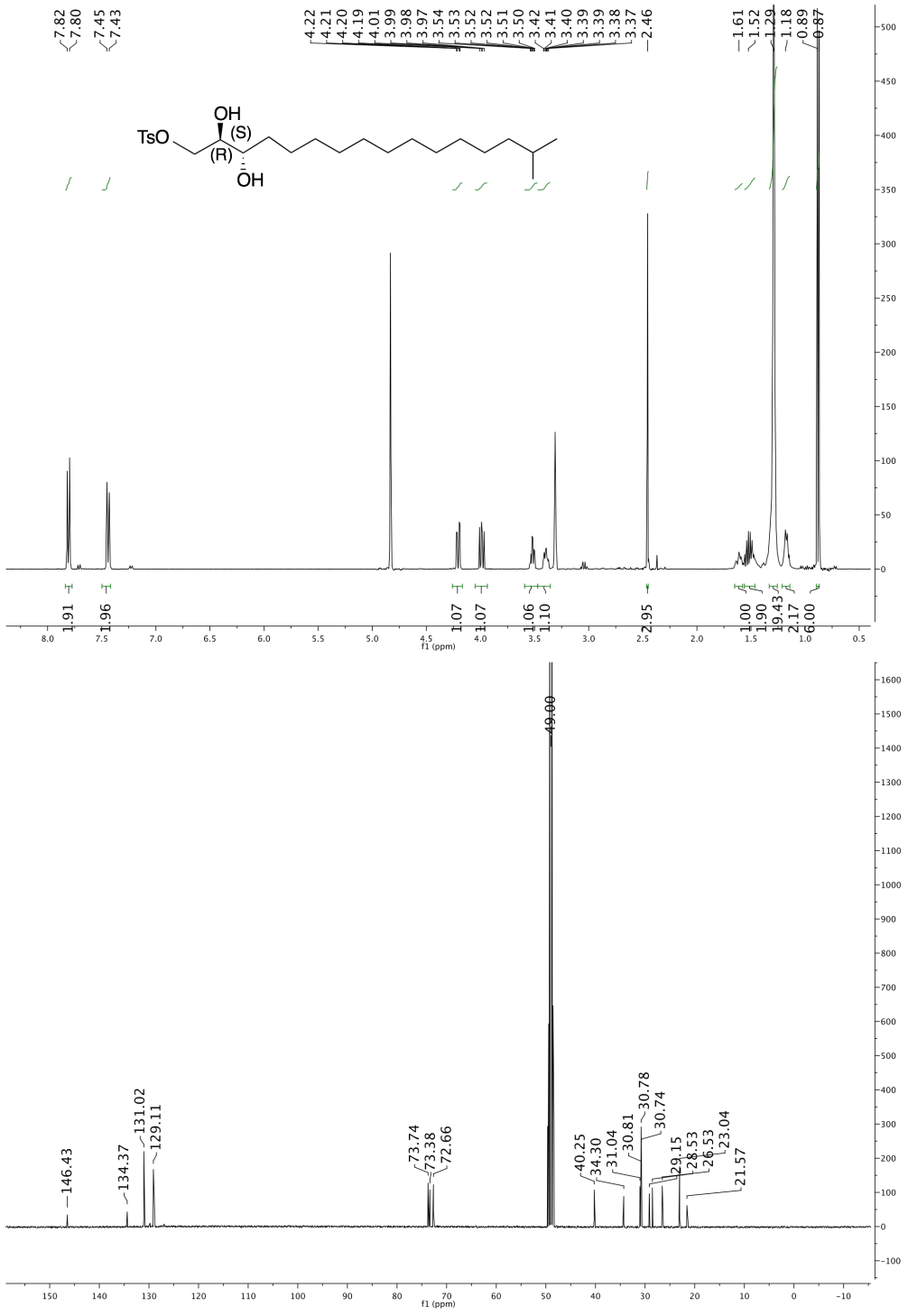


Figure S19: **6D** ^{1}H NMR (top) and ^{13}C NMR (bottom). Solvent: CD_3OD

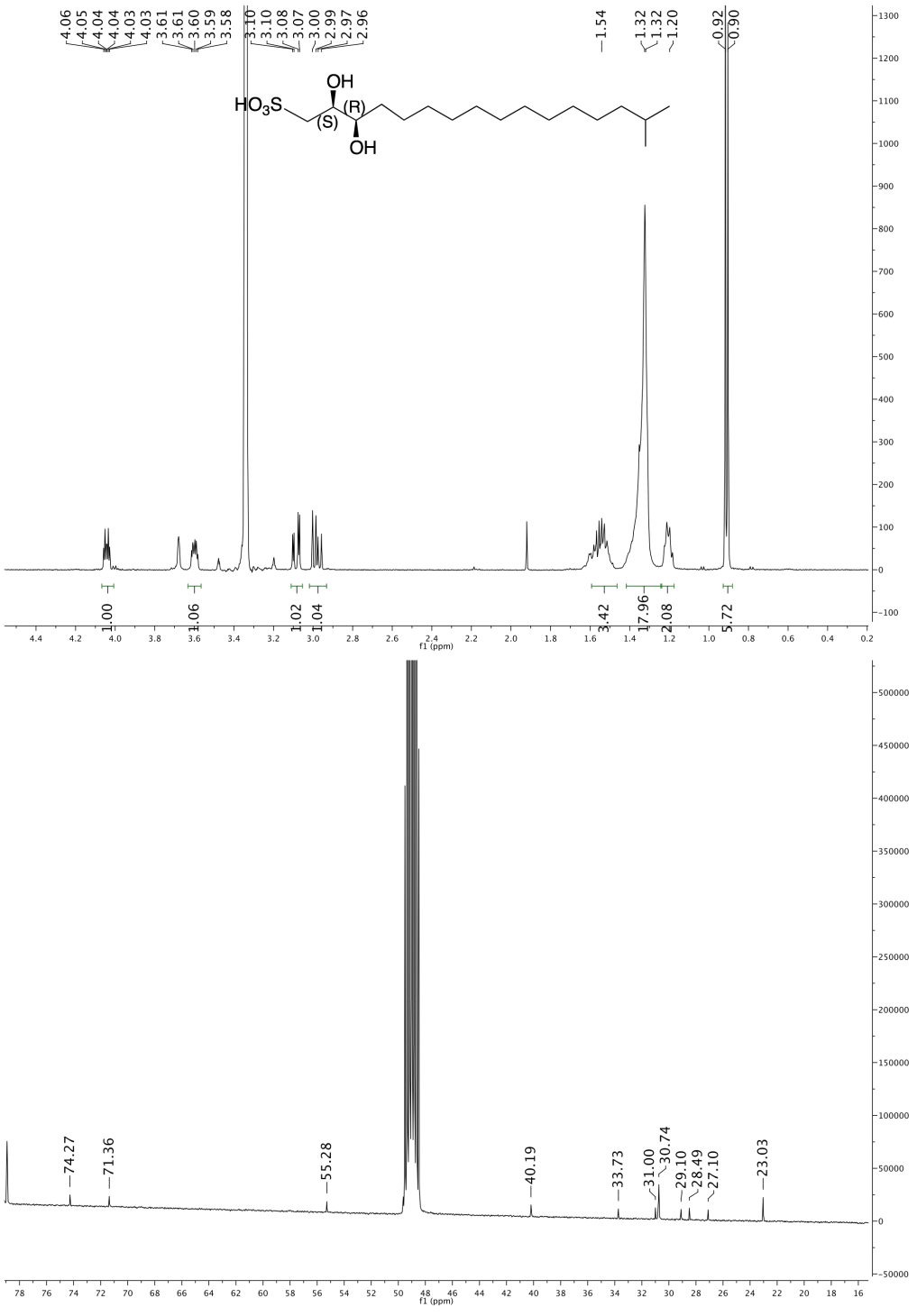


Figure S20: **IOR-1A** ^{1}H NMR (top) and ^{13}C NMR (bottom). Solvent: $CD_3OD/CDCl_3$

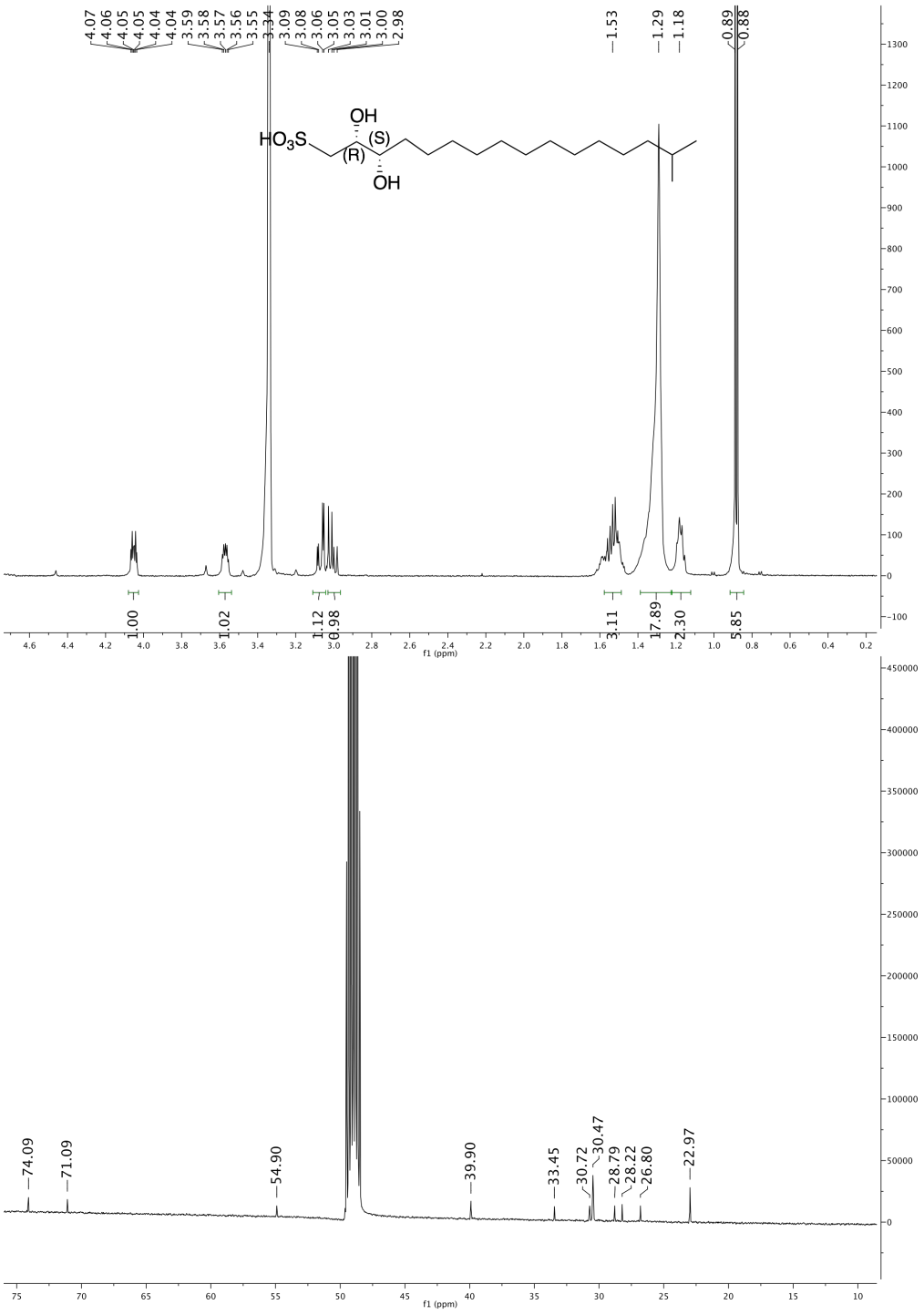


Figure S21: **IOR-1B** ^{1}H NMR (top) and ^{13}C NMR (bottom). Solvent: $CD_3OD/CDCl_3$

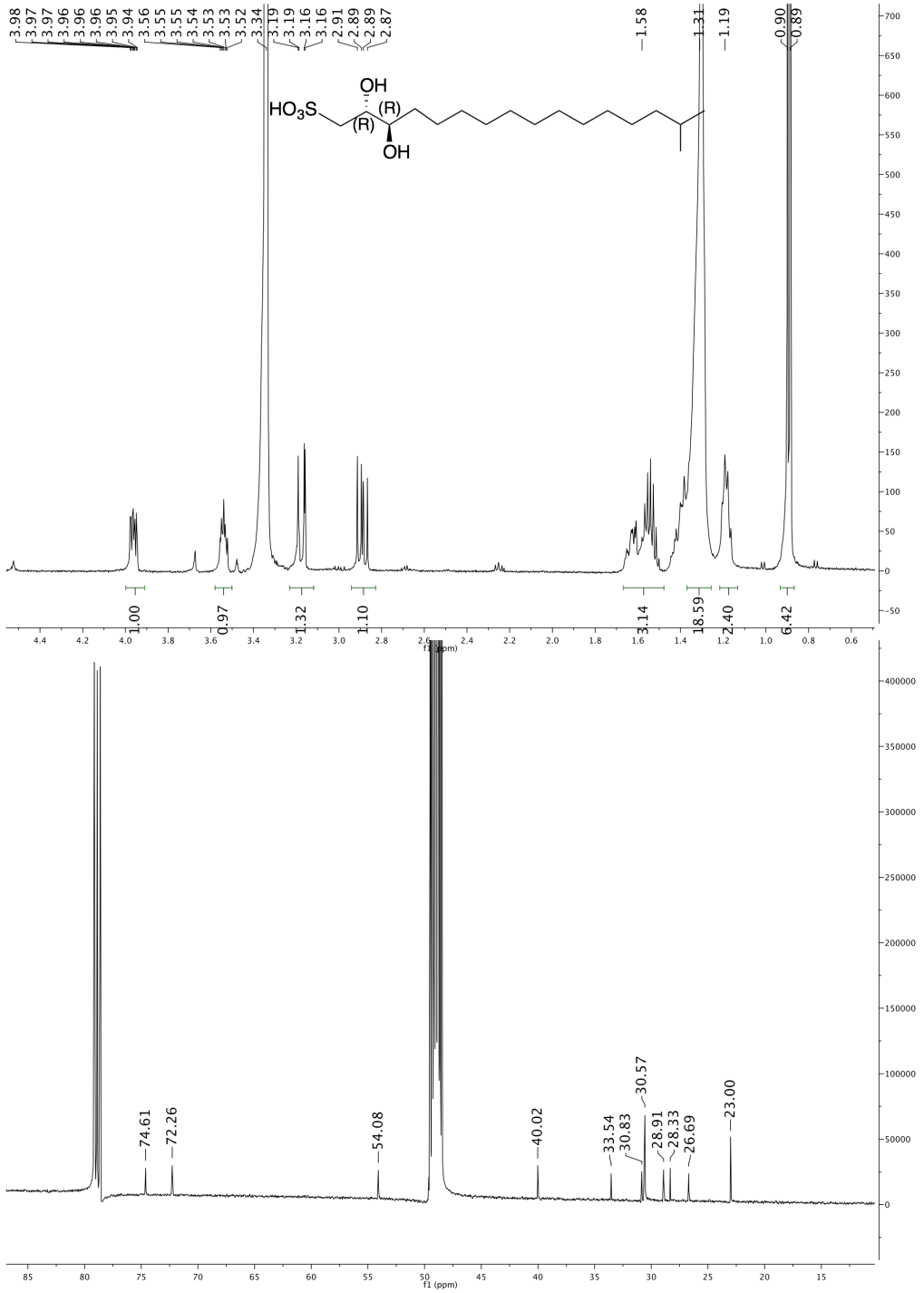


Figure S22: **IOR-1C** ^{1}H NMR (top) and ^{13}C NMR (bottom). Solvent: $CD_3OD/CDCl_3$

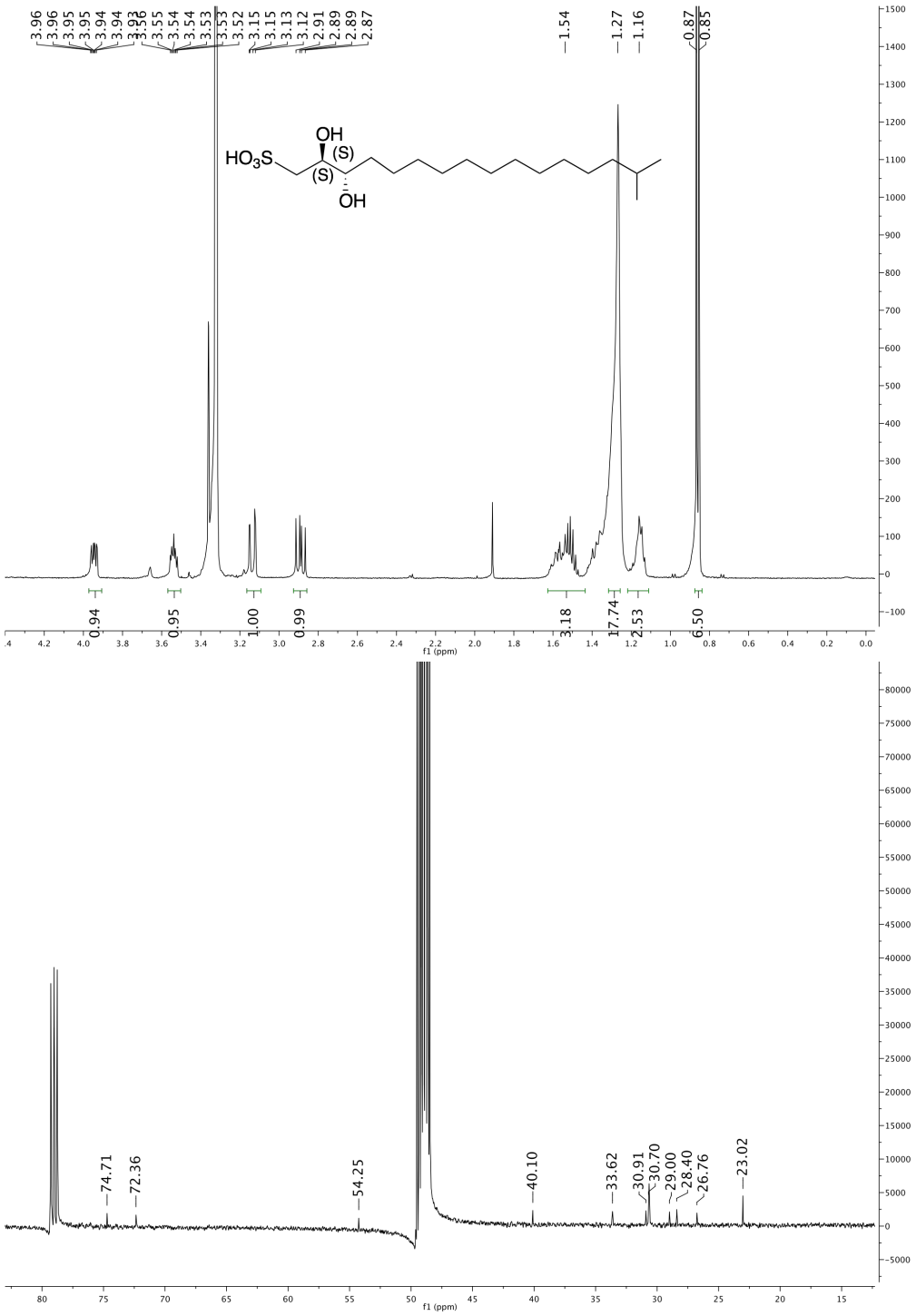
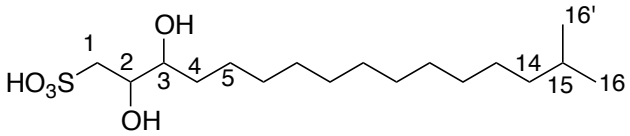


Figure S23: **IOR-1D** ^{1}H NMR (top) and ^{13}C NMR (bottom). Solvent: $CD_3OD/CDCl_3$



Position	Isolated IOR-1 δH, m, H (*J* in Hz)	^{13}C	IOR-1A δH, m, H (*J* in Hz)	^{13}C	IOR-1B δH, m, H (*J* in Hz)	^{13}C
1	3.08, dd, 1H (13.8, 3.1) 2.99, dd, 1H (13.9, 8.6)	55.23	3.09, dd, 1H (14.0, 3.5) 2.98, dd, 1H (14.0, 8.5)	55.28	3.07, dd, 1H (14.0, 3.2) 3.00, dd, 1H (14.0, 8.8)	54.90
2	4.04, dt, 1H (8.5, 3.2)	71.31	4.04, dt, 1H (8.4, 3.3)	71.36	4.05, dt, 1H (8.9, 3.3)	71.09
3	3.59, dt, 1H (7.6, 3.7)	74.25	3.60, dt, 1H (7.9, 3.6)	74.27	3.57, dt, 1H (7.8, 3.8)	74.09
4	1.58 – 1.50, m, 2H	33.69	1.61 – 1.49, m, 2H	33.73	1.58 – 1.47, m, 2H	33.45
5-13	1.37 – 1.27, m, 18H	28.4-30.9	1.42 – 1.28, m, 18H	28.49-31	1.37 – 1.23, m, 18H	28.22-33.45
14	1.22 – 1.17, m, 2H	40.12	1.24 – 1.17, m, 2H	40.19	1.20-1.15, m, 2H	39.90
15	1.58 – 1.50, m, 1H)	27.02	1.61 – 1.49, m, 1H	27.10	1.58 – 1.47, m, 1H	26.80
16, 16'	0.90, d, 6H (7.1)	23.02	0.91, d, 6H (6.5)	23.03	0.88, d, 6H (6.5)	22.97

Position	Isolated IOR-1 δH, m, H (*J* in Hz)	^{13}C	IOR-1C δH, m, H (*J* in Hz)	^{13}C	IOR-1D δH, m, H (*J* in Hz)	^{13}C
1	3.08, dd, 1H (13.8, 3.1) 2.99, dd, 1H (13.9, 8.6)	55.23	3.17, dd, 1H (14.0, 2.1) 2.89, dd, 1H (14.1, 9.6)	54.08	3.14, dd, 1H (14.1, 2.0) 2.89, dd, 1H (14.1, 9.7)	54.25
2	4.04, dt, 1H (8.5, 3.2)	71.31	3.96, ddd, 1H (9.5, 5.5, 2.0)	72.26	3.95, ddd, 1H (9.8, 5.4, 2.1)	72.36
3	3.59, dt, 1H (7.6, 3.7)	74.25	3.54, ddd, 1H (8.8, 5.6, 3.2)	74.61	3.54, ddd, 1H (8.8, 5.3, 3.2)	74.71
4	1.58 – 1.50, m, 2H	33.69	1.64 – 1.50, m, 2H	33.54	1.61 – 1.49, m, 2H	33.62
5-13	1.37 – 1.27, m, 18H	28.4-30.9	1.40 – 1.24, m, 18H	28.33-30.83	1.36 – 1.23, m, 18H	28.40-30.91
14	1.22 – 1.17, m, 2H	40.12	1.23 – 1.16, m, 2H	40.02	1.18 – 1.12, m, 2H	40.10
15	1.58 – 1.50, m, 1H)	27.02	1.64 – 1.50, m, 1H	26.69	1.61 – 1.49, m, 1H	26.76
16, 16'	0.90, d, 6H (7.1)	23.02	0.89, d, 6H (6.6)	23.00	0.86, d, 6H (6.6)	23.02

Figure S24: NMR shifts comparison between isolated IOR-1 and synthetic IOR-1A-D

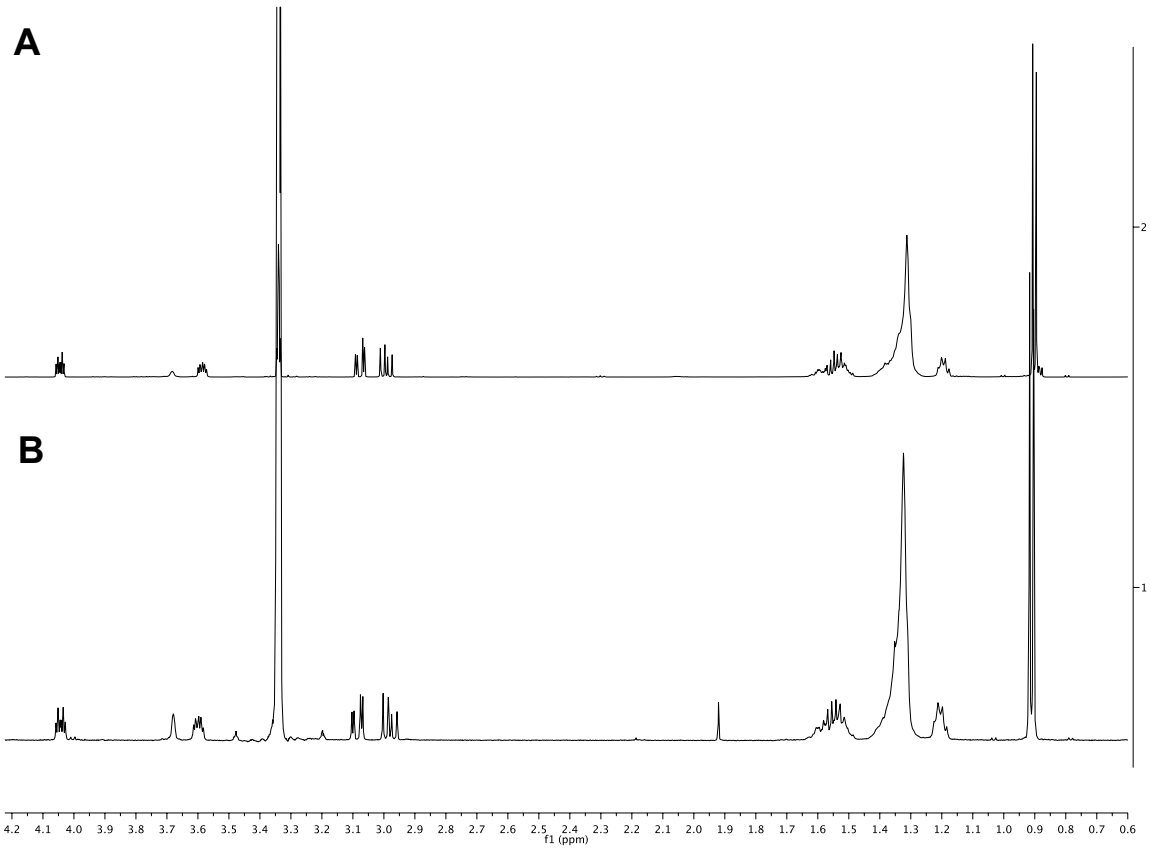


Figure S25: (A) 1H spectrum of isolated IOR-1. (B) 1H spectrum of synthetic IOR-1A

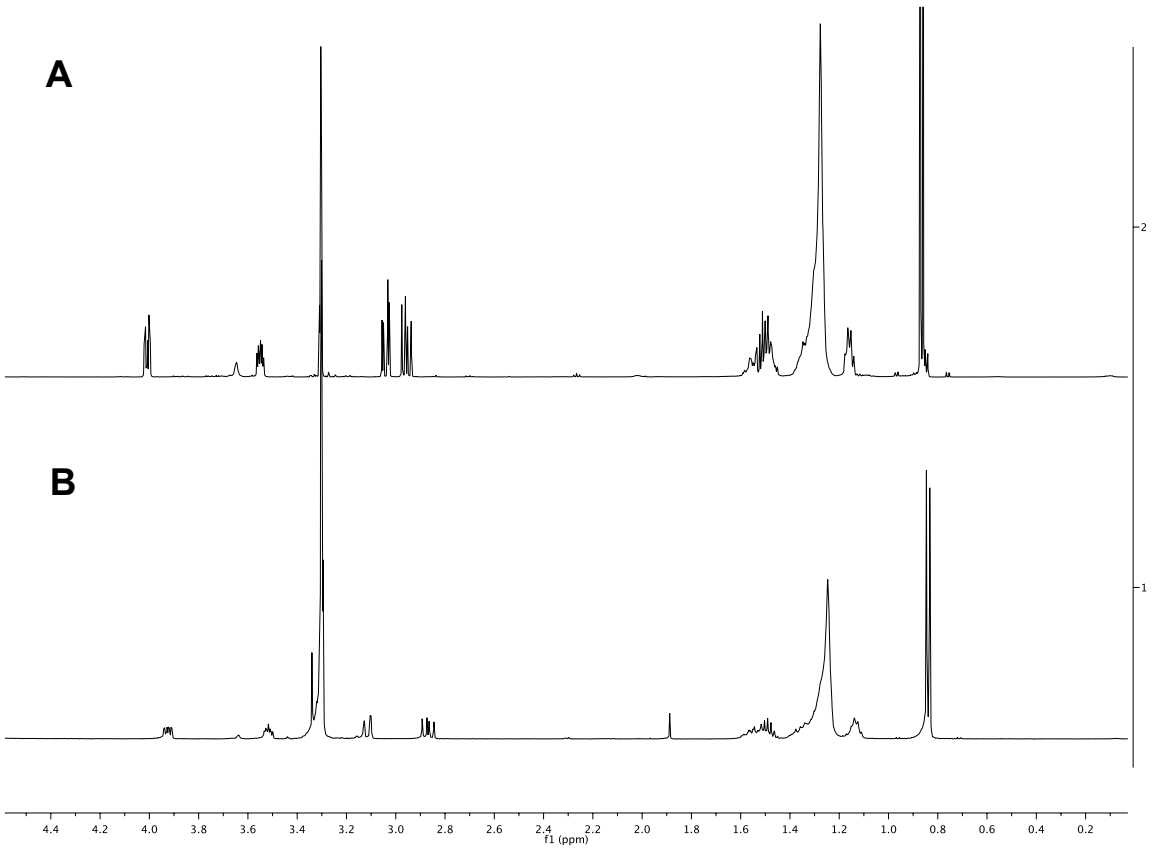


Figure S26: (A) 1H spectrum of isolated IOR-1. (B) 1H spectrum of synthetic IOR-1D

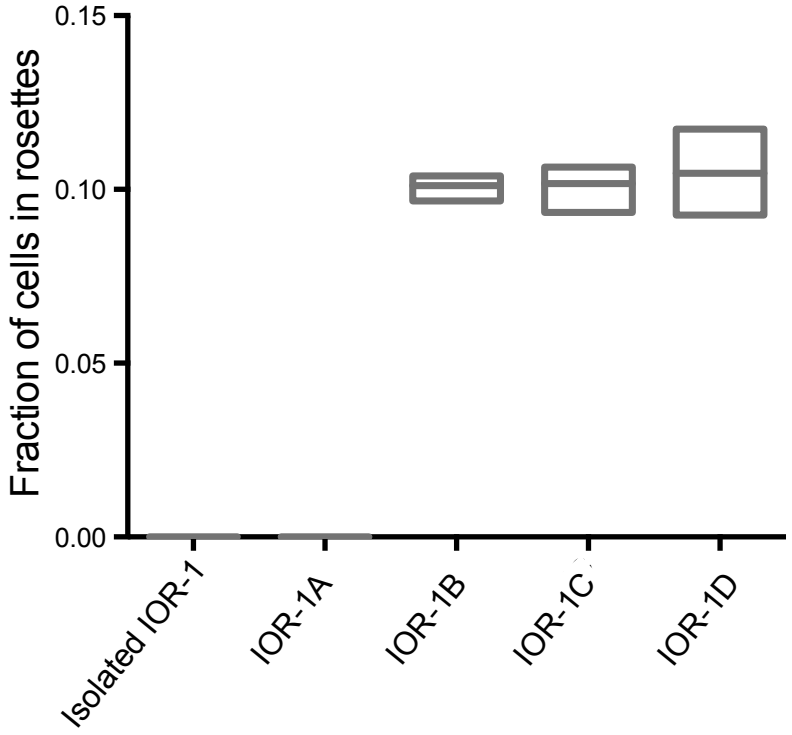
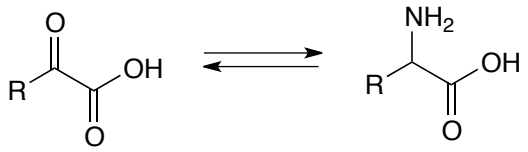


Figure S27. Activity of IOR synthetic stereoisomers. Rosettes were induced with 2 μM RIF-2 and treated with 2.5 nm IOR-1.

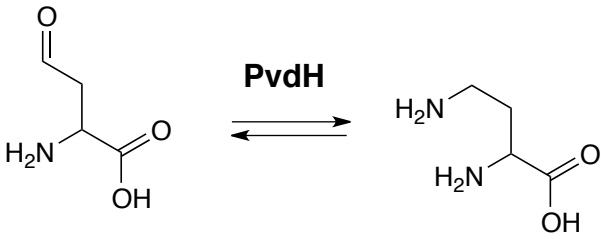
Compound name	Rosette inhibition
Safingol	No activity
D-erythro-sphinganine	No activity
Sphinganine-1-phosphate	No activity

Figure S28. Inhibition of rosettes with commercially available IOR-1 structural analogs. Rosettes induced with 2 μM RIF-2 and treated in 3-fold dilution between 0.1 ng/ml and 1 μg/ml.

A



B

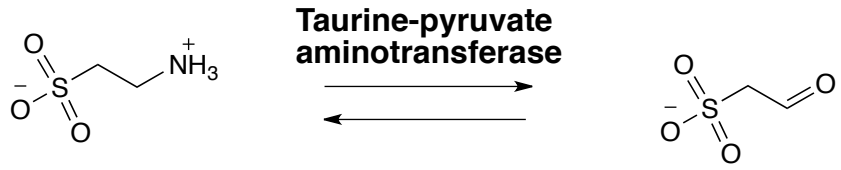


Pvdh [*Pseudomonas aeruginosa*]
Gbk ID: BAT65564.1
Length: 469 aa
Locus: BAT65564

RAST annotation	AA length	Identity (%)
Acetylornithine aminotransferase	397	31
Acetylornithine aminotransferase	393	30
Acetylornithine aminotransferase	376	28
Ornithine aminotransferase	411	26
Aminotransferase, class III	757	28
Glutamate-1-semialdehyde aminotransferase	430	28

Figure S29: (A) Generalized scheme of aminotransferase function. (B) Putative aminotransferases from *A. machipongonensis* genome (NCBI refseq: NZ_CM001023.1). Genome re-annotated using Rapid Annotation using Subsystem Technology (RAST). BLAST+ used for sequence comparison to previously characterized Pvdh[1] (pyoverdine biosynthesis). [1]

[1] Vandenande, C. S.; Vlasschaert, M.; Seah, S. Y. K. *J. Bacteriol.* **2004** 186 (17), 5596-5602



Taurine-pyruvate aminotransferase [*Bilophila wadsworthia*]

Gbk ID: AAG50296.1
Length: 456 aa
Locus: AAG50296

RAST annotation	AA length	Identity (%)
Acetylornithine aminotransferase	397	34
Acetylornithine aminotransferase	393	29
Ornithine aminotransferase	411	28
Aminotransferase, class III	757	29
Acetylornithine aminotransferase	376	29

Figure S30: Putative aminotransferases from *A. machipongonensis* genome (NCBI refseq: NZ_CM001023.1). Genome re-annotated using Rapid Annotation using Sybsystem Technology (RAST). BLAST+ used for sequence comparison to previously characterized taurine-pyruvate aminotransferase.[2]

[2] Laue, H.; Cook, A. M. *European Journal of Biochemistry* **2000**, 267 (23), 6841-6848