Total synthesis and *in vivo* evaluation of 8-DeoxyPumiliotoxin 193H

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The total synthesis of both the double bond isomers of indolizine alkaloid 8-deoxypumiliotoxin 193H has been accomplished. Both the double bond isomers ***Z*-4** and ***E*-4** induced convulsions and inhibited neuro-muscular activity at a dose of 25 mg/kg after intraperitoneal injection in mice. The lethal dose of ***Z*-4** and ***E*-4** was 100 mg/kg, indicating that 8-deoxypumiliotoxin 193H is 10-times less toxic than the known pumiliotoxin (+)-251D.

Supporting Information

General experimental details and spectral data of all new compounds

General Experimental Details

Animals

Male CD-1 mice obtained from ENVIGO (Netherlands) weighing 23–25 g were housed under standard conditions (21–23 °C, 12 h light–dark cycle) with unlimited access to standard food (R-70 diet, Lactamin AB, Sweden) and water. All animal care and experimental procedures were performed in accordance with the guidelines of the EU Directive 2010/63/EU, local laws and policies and were approved by the Latvian Animal Protection Ethical Committee, Food and Veterinary Service, Riga, Latvia.

General central nervous system (CNS) tests

The effects of the compounds were evaluated before as a negative control and 30, 60, 120, 180 and 240 min after i.p. administration at doses of 10, 25, 50 and 100 mg/kg as described previously.[44] The animals were continuously observed for the first 30 minutes.

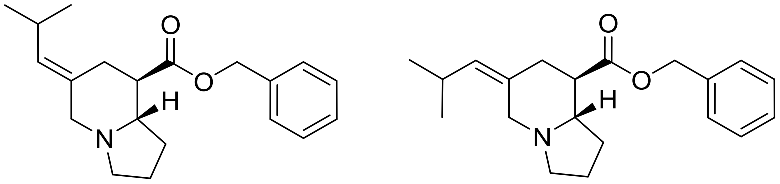
To measure the effects of the compounds on muscle strength and coordination, we employed the rota-rod, chimney and traction tests. One day before the rota-rod experiment, the animals were trained on the apparatus, and the animals that failed to remain on the rotating rod for at least 90 s were excluded from further testing. On the experimental day, the animals were placed on a rota-rod (16 rpm) and the number of animals falling off the rota-rod within the 180 s session was recorded. In the chimney test, the mice had to climb backwards up a Pyrex glass tube (30 cm length, 3 cm inner diameter). The mice successfully reaching the 20 cm mark within 30 s were selected for further testing. The effect of the drugs on muscle strength was also examined in the traction test. In this test, the forepaws of the mouse were placed on a horizontal firmly fixed stick. The untreated mice grasped the stick with both forepaws and, when allowed to hang free, placed at least one hind foot on the stick within 5 s. An inability to perform that movement was scored as a failure of traction. The rectal temperature of the animals was measured using a thermometer (Thermalert TH-5, USA).

Synthesis

All reactions were performed under an atmosphere of argon unless otherwise indicated. The reagents and starting materials were obtained from commercial sources and used as received. The solvents were purified and dried using standard procedures prior to use. Petroleum ether of boiling range 60–80 °C was used. Flash chromatography was carried out using Merck Kieselgel (230–400 mesh). NMR spectra were recorded on Varian Mercury (400 MHz) and Bruker (300 MHz) spectrometers. The chemical shift values are referenced against residual protons in the deuterated solvents, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad). Infrared spectra were recorded in the range 4000-600 cm^{-1} as a film. HRMS were obtained on a Micromass AutoSpec Ultima Magnetic sector mass spectrometer. Optical rotations were measured using a Perkin Elmer 141 polarimeter.

Experimental Procedures and Characterization Data

Figure S1. Benzyl (8*R*,8a*S*,*E*)-6-(2-methylpropylidene)octahydroindolizine-8-carboxylate and benzyl (8*R*,8a*S*,*Z*)-6-(2-methylpropylidene)octahydroindolizine-8-carboxylate ***E*-10** and ***Z*-10.**



Lactone **9**[1] (300 mg, 1.343 mmol) was placed in a MW tube and dried overnight over P_2O_5 in a vacuum drying chamber. The tube was tightly sealed and purged 3 times with argon. Dry DCM (6 mL) and 1.3 mL of freshly from sodium distilled Me_2NEt (884 mg, 12.091 mmol) was added and the mixture was then cooled to 0 °C and treated with Et_2BOTf (878 mg, 4.030 mmol). After 1 h, the reaction mixture was left to warm to ambient temperature and stirred for 1 h, then heated in MW at 50 °C for 1 h. After that, the mixture was diluted with water (~6 mL), 20 drops of 10% aqueous HCl were added and the biphasic mixture was stirred vigorously for 1 h followed by evaporation. Benzyl alcohol (435 mg, 4.030 mmol), EDC (772 mg, 4.030 mmol), HOBt*H_2O (411 mg, 2.686 mmol), Et_3N (1359 mg, 13.434 mmol) and dry DMF (6 mL) were added and the obtained slurry was stirred for 16 h. The reaction mixture was diluted with brine, and extracted twice with DCM. The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by preparative LC/MS. Fractions containing ***Z*-10** and ***E*-10** were concentrated *in vacuo*, the residue was dissolved in MeOH and treated with Dowex® 1X8100 basic resin and again concentrated *in vacuo*. The residue was purified by flash column chromatography (Pet:EtOAc 9:1 to 3:2)**.**

Benzyl (8*R*,8a*S*,*E*)-6-(2-methylpropylidene)octahydroindolizine-8-carboxylate ***E*-10** was isolated as a pale yellow oil (45 mg, 11%).

^{1}H NMR (400MHz; $CDCl_3$): δ 7.37 – 7.31 (m, 5H), 5.20 – 5.15 (m, 1H), 5.14 (d, *J* = 6.2 Hz, 2H), 3.33 (dd, *J* = 11.6, 1.4 Hz, 1H), 3.05 (td, *J* = 8.7, 2.3 Hz, 1H), 2.87 (ddd, *J* = 13.8, 4.0, 1.4 Hz, 1H), 2.67 (dt, *J* = 11.6, 1.4 Hz, 1H), 2.61 – 2.48 (m, 1H), 2.35 – 2.24 (m, 1H), 2.22 – 2.09 (m, 2H), 2.07 – 1.97 (m, 1H), 1.93 – 1.75 (m, 2H), 1.74 – 1.60 (m, 1H), 1.55 – 1.41 (m, 1H), 0.96 (d, *J* = 6.6 Hz, 3H), 0.91 (d, *J* = 6.7 Hz, 3H).

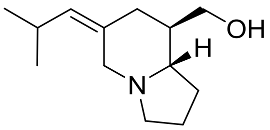
^{13}C NMR (100MHz, $CDCl_3$): δ 173.81, 136.05, 133.97, 129.68, 128.65, 128.31, 128.25, 66.36, 65.64, 60.39, 53.73, 48.08, 29.88, 29.02, 26.69, 23.42, 23.22, 21.08.

HRMS-ESI (m/z): [M+H] calculated for $C_{20}H_{28}NO_2$, 314.2120; found 314.2135.

$[\alpha]_D^{20} = +3.00^O$ (*c* 1, $CHCl_3$)

Benzyl (8*R*,8a*S*,*Z*)-6-(2-methylpropylidene)octahydroindolizine-8-carboxylate ***Z*-10**[1] was isolated as a pale yellow oil (160 mg, 38%). ^{1}H NMR (600MHz; CDCl$_3$): δ 7.39 – 7.30 (m, 5H), 5.12 (d, *J* = 3.2 Hz, 2H), 5.11 – 5.08 (m, 1H), 3.84 (d, *J* = 13.1 Hz, 1H), 3.06 (t, *J* = 8.9 Hz, 1H), 2.61 – 2.50 (m, 1H), 2.49 – 2.41 (m, 2H), 2.39 – 2.25 (m, 2H), 2.25 – 2.12 (m, 2H), 1.96 – 1.87 (m, 2H), 1.87 – 1.78 (m, 1H), 1.75 – 1.65 (m, 1H), 1.54 – 1.43 (m, 1H), 0.97 (d, *J* = 6.6 Hz, 3H), 0.90 (d, *J* = 6.6 Hz, 3H).

Figure S2. ((8*R*,8*aS*,*Z*)-6-(2-methylpropylidene)octahydroindolizin-8-yl)methanol ***Z*-11**



DIBAL-H (1.2 M in toluene, 2 mL, 340 mg, 2.393 mmol) was added to a stirred solution of ester ***Z*-10** (150 mg, 0.477 mmol) in dry DCM (2 mL) under an atmosphere of Ar at –78 °C, and the obtained solution was stirred for 5 h at –78 °C. The reaction mixture was quenched at the same temperature upon the addition of MeOH and then warmed to ambient temperature. The mixture was treated with a saturated aqueous solution of sodium potassium tartrate and stirred vigorously for 1 h, then extracted with DCM (2 times). The combined organic layers were dried over anhydrous Na$_2$SO$_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (DCM:MeOH:Et$_3$N 20:1:0.2). The title compound ***Z*-11** (100 mg, 80% Yield) was isolated as a colourless oil.

^{1}H NMR (400MHz; CDCl$_3$): δ 5.08 (d, *J* = 9.1 Hz, 1H), 3.85 (dd, *J* = 12.1, 1.5 Hz, 1H), 3.62 (dd, *J* = 10.8, 4.7 Hz, 1H), 3.47 (dd, *J* = 10.8, 6.6 Hz, 1H), 3.07 (td, *J* = 8.7, 2.3 Hz, 1H), 2.57 (ddt, *J* = 13.3, 9.0, 6.7 Hz, 1H), 2.39 (d, *J* = 12.0 Hz, 1H), 2.32 (ddd, *J* = 13.7, 4.1, 1.3 Hz, 1H), 2.14 (q, *J* = 9.0 Hz, 1H), 1.99 – 1.77 (m, 3H), 1.78 – 1.63 (m, 1H), 1.59 – 1.41 (m, 2H), 0.96 (d, *J* = 6.6 Hz, 3H), 0.89 (d, *J* = 6.8 Hz, 3H).

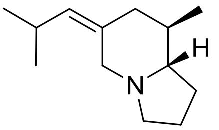
^{13}C NMR (100MHz, CDCl$_3$): δ 132.94, 130.95, 66.41, 65.40, 54.01, 52.77, 44.86, 37.41, 28.75, 26.66, 23.73, 23.39, 21.34.

HRMS-ESI (m/z): [M+H] 210.1858 calculated for $C_{13}H_{23}NO$, found 210.1859.

IR ($\nu_{max,}$ film): 3359, 2956, 2925, 2868, 2813, 1464, 1435, 1362, 1261, 1215, 1153, 1092, 1043, 970.

$[\alpha]_D^{20}$= +19.24^O (*c* 1, CHCl$_3$)

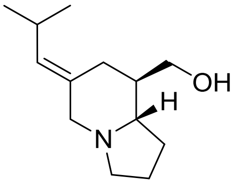
Figure S3. (8*R*,8a*S*,*Z*)-8-Methyl-6-(2-methylpropylidene)octahydroindolizine ***Z*-4**



TsCl (170 mg, 0.893 mmol) was added to a stirred solution of ***Z*-11** (170 mg, 0.812 mmol), DIPEA (157 mg, 0.200 mL, 1.218 mmol) and DMAP (10 mg, 0.081 mmol) in 3 mL of dry DCM under an Ar atmosphere. After stirring overnight, the reaction mixture was evaporated at rt and placed under Ar. Dry THF was added (3 ml) and the mixture was cooled in an ice bath, then Superhydride® (1.7 M in THF, 886 mg, 4.8 mL, 8.121 mmol) was added dropwise over 15 min. The cooling bath was removed and the mixture was stirred overnight at rt. The mixture was quenched with aqueous $NaHCO_3$ and extracted with Et_2O (2 times) followed by DCM (2 times). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (DCM:MeOH 20:1). The title compound ***Z*-4** (60 mg, 38% Yield over 2 steps) was isolated as a colourless oil.

^{1}H NMR 300 MHz, $CDCl_3$: 5.02 (dt, *J* = 9.1, 1.9 Hz, 1H), 3.85 (d, *J* = 12.1 Hz, 1H), 3.07 (td, *J* = 8.7, 2.3 Hz, 1H), 2.57 (dp, *J* = 8.9, 6.6 Hz, 1H), 2.36 (d, *J* = 12.1 Hz, 1H), 2.22 – 2.04 (m, 2H), 2.01 – 1.52 (m, 5H), 1.51 – 1.26 (m, 2H), 0.97 (d, *J* = 6.6 Hz, 3H), 0.89 (dd, *J* = 6.6, 2.2 Hz, 6H).

Figure S4. ((8*R*,8a*S*,*E*)-6-(2-methylpropylidene)octahydroindolizin-8-yl)methanol ***E*-11**



The title compound ***E*-11** was prepared in a similar manner as ***Z*-11** and was isolated as a colourless oil (35 mg, 81%).

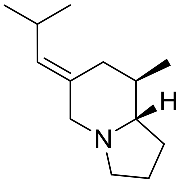
^{1}H NMR (400MHz; $CDCl_3$): δ 5.15 (dt, *J* = 9.0, 1.8 Hz, 1H), 3.67 (dd, *J* = 10.8, 4.6 Hz, 1H), 3.52 (dd, *J* = 10.8, 6.4 Hz, 1H), 3.34 (dd, *J* = 11.6, 1.4 Hz, 1H), 3.10 – 3.01 (m, 1H), 2.80 – 2.69 (m, 2H), 2.65 – 2.50 (m, 2H), 2.10 (q, *J* = 9.1 Hz, 1H), 2.02 – 1.89 (m, 1H), 1.90 – 1.75 (m, 1H), 1.74 – 1.65 (m, 1H), 1.66 – 1.54 (m, 1H), 1.56 – 1.41 (m, 1H), 1.16 (t, *J* = 7.2 Hz, 1H), 0.95 (d, *J* = 5.2 Hz, 3H), 0.92 (d, *J* = 6.7 Hz, 3H).

^{13}C NMR (100MHz, $CDCl_3$): δ 133.21, 131.13, 66.56, 65.55, 60.71, 53.88, 46.15, 44.62, 29.69, 28.88, 26.70, 23.49, 21.27.

HRMS-ESI (m/z): [M+H] 210.1858 calculated for $C_{13}H_{23}NO$, found 210.1873.

$[\alpha]_D^{20}$= -4.50^O (*c* 1, $CHCl_3$).

Figure S5. (8*R*,8a*S*,*E*)-8-methyl-6-(2-methylpropylidene)octahydroindolizine ***E*-4**



The title compound ***E*-4** was prepared in a similar manner as ***Z*-4** and was isolated as colourless oil (30 mg, 54%).

^{1}H NMR (400MHz; CDCl$_3$): δ 5.12 (d, *J* = 9.1 Hz, 1H), 3.34 (d, *J* = 11.2 Hz, 1H), 3.08 (td, *J* = 8.9, 2.6 Hz, 1H), 2.77 – 2.43 (m, 3H), 2.31 – 2.07 (m, 1H), 2.02 – 1.58 (m, 3H), 1.56 – 1.20 (m, 4H), 1.04 – 0.77 (m, 9H).

^{13}C NMR (100MHz, CDCl$_3$): δ 132.91, 131.10, 70.79, 60.24, 53.63, 36.48, 35.10, 28.58, 26.53, 23.33, 23.08, 20.78, 18.79.

HRMS-ESI (m/z): [M+H] 194.1909 calculated for $C_{13}H_{24}N$, found 194.1917.

IR ($\nu_{max,}$ film): 2955, 2929, 2869, 2778, 1461, 1375, 1313, 1258, 1156, 1128, 969, 891, 824.

$[\alpha]_D^{20}$= -3.60^{O} (*c* 1, CHCl$_3$)

[1] G. Smits, R. Zemribo, *Eur. J. Org. Chem.* **2015**, *2015*, 3152–3156.

Figure S6. ^{1}H NMR spectrum of **Z-10**, (400 MHz)

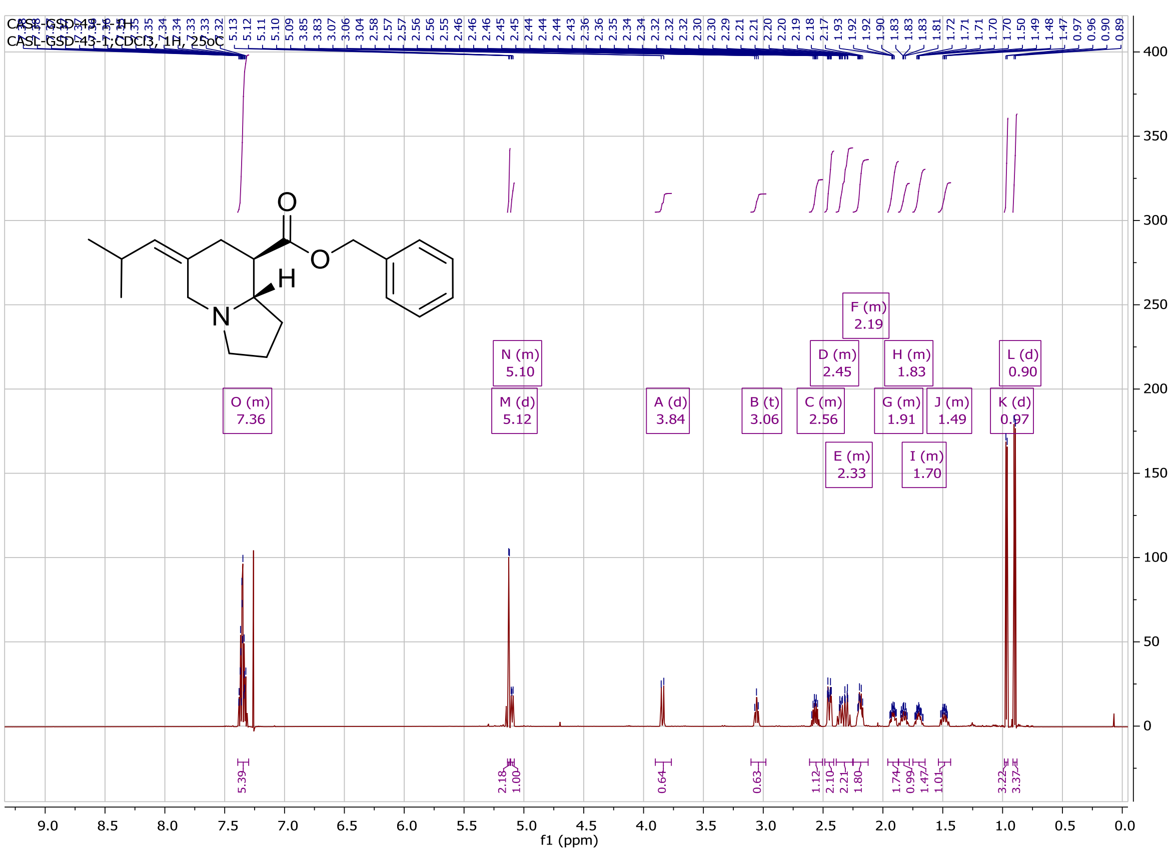


Figure S7. ^{1}H NMR spectrum of ***E*-10**, (400 MHz)

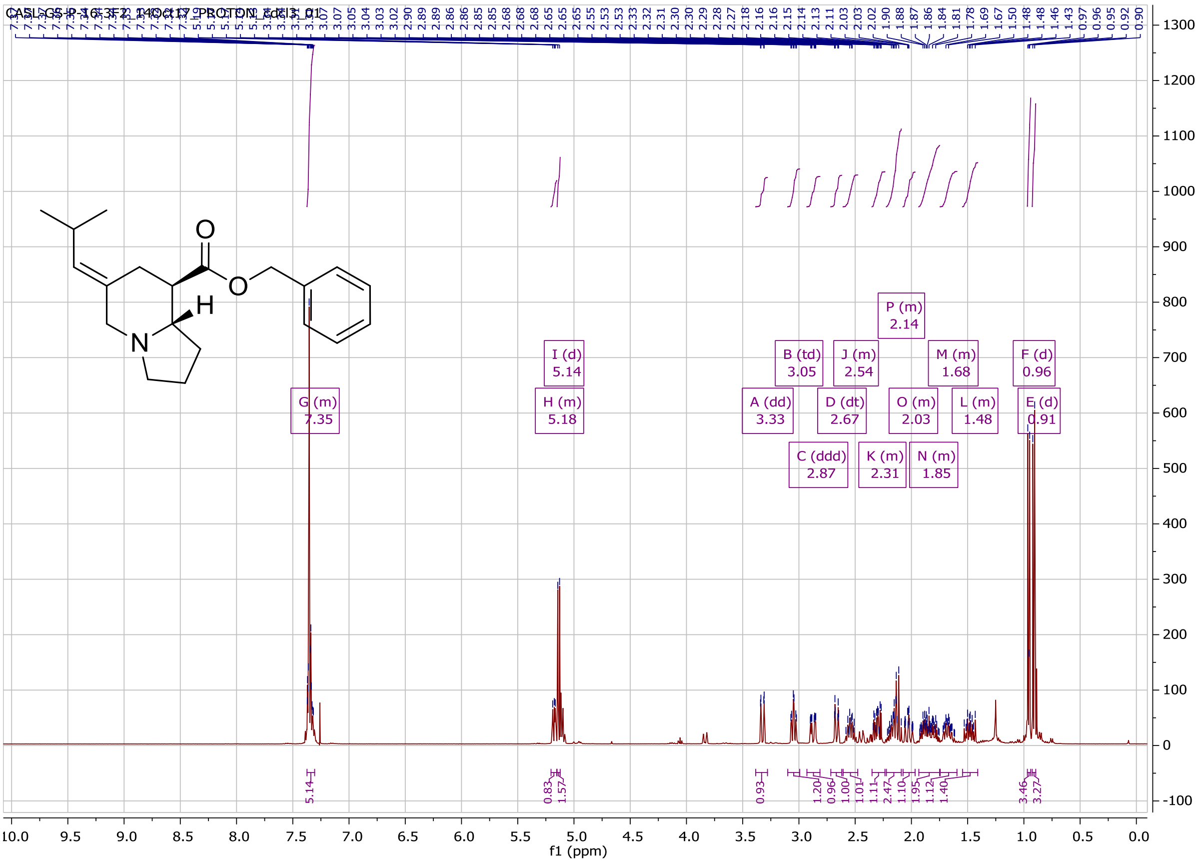


Figure S8. ^{13}C NMR spectrum of ***E*-10**, (100 MHz)

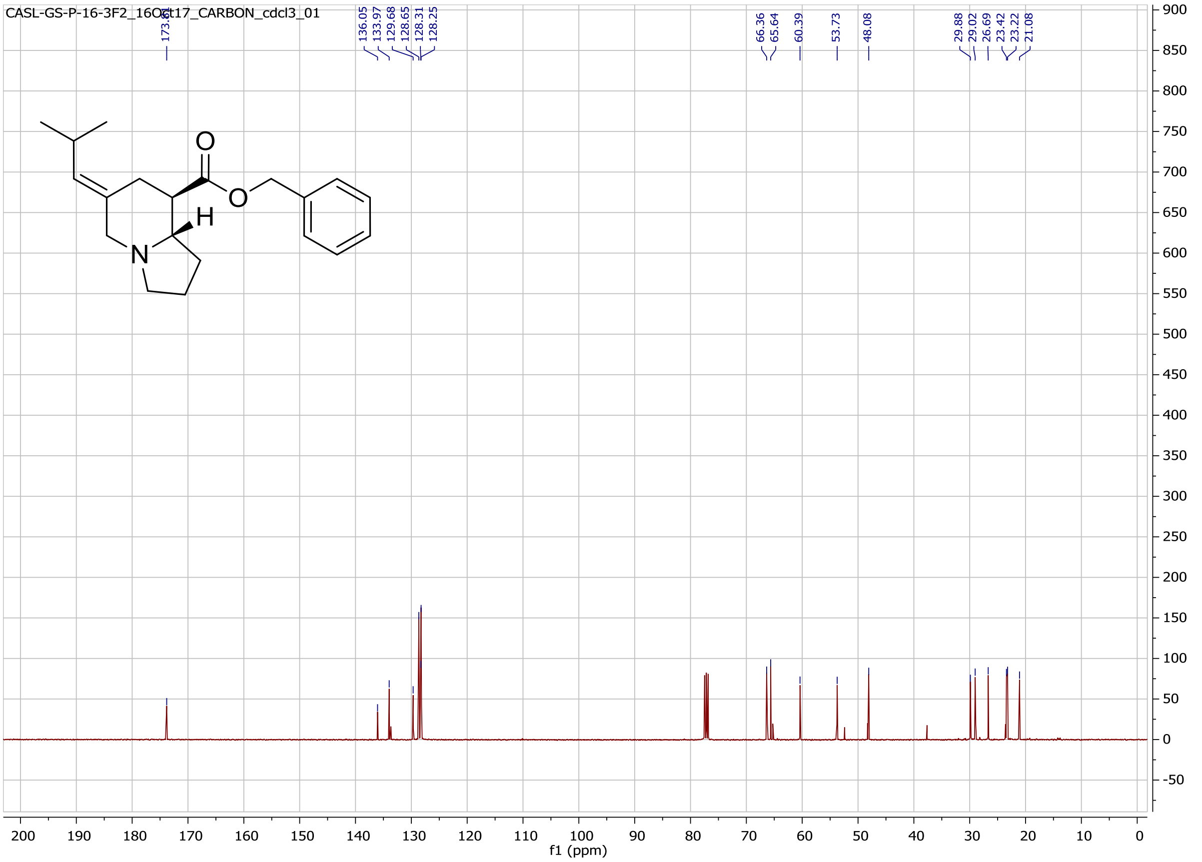


Figure S9. HSQC spectrum of ***E*-10**

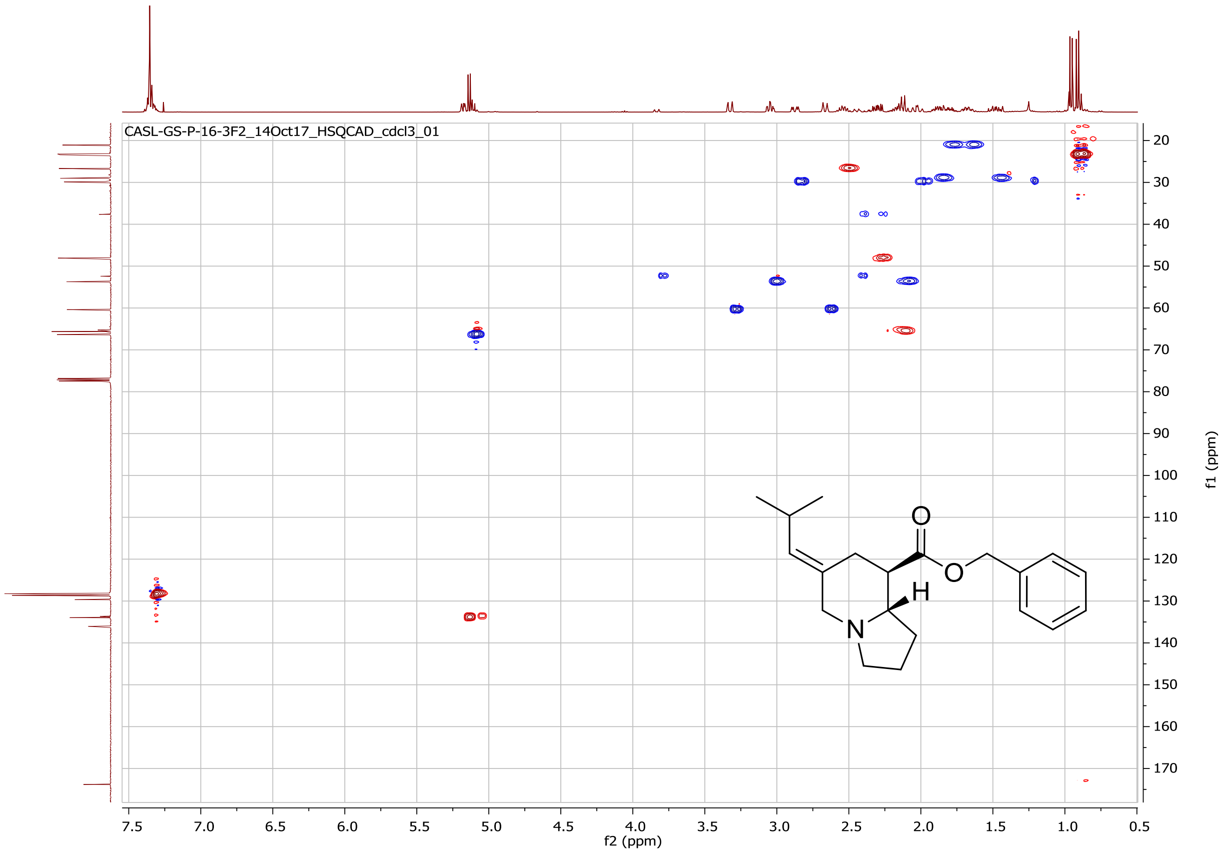


Figure S10. HMBC spectrum of ***E*-10**

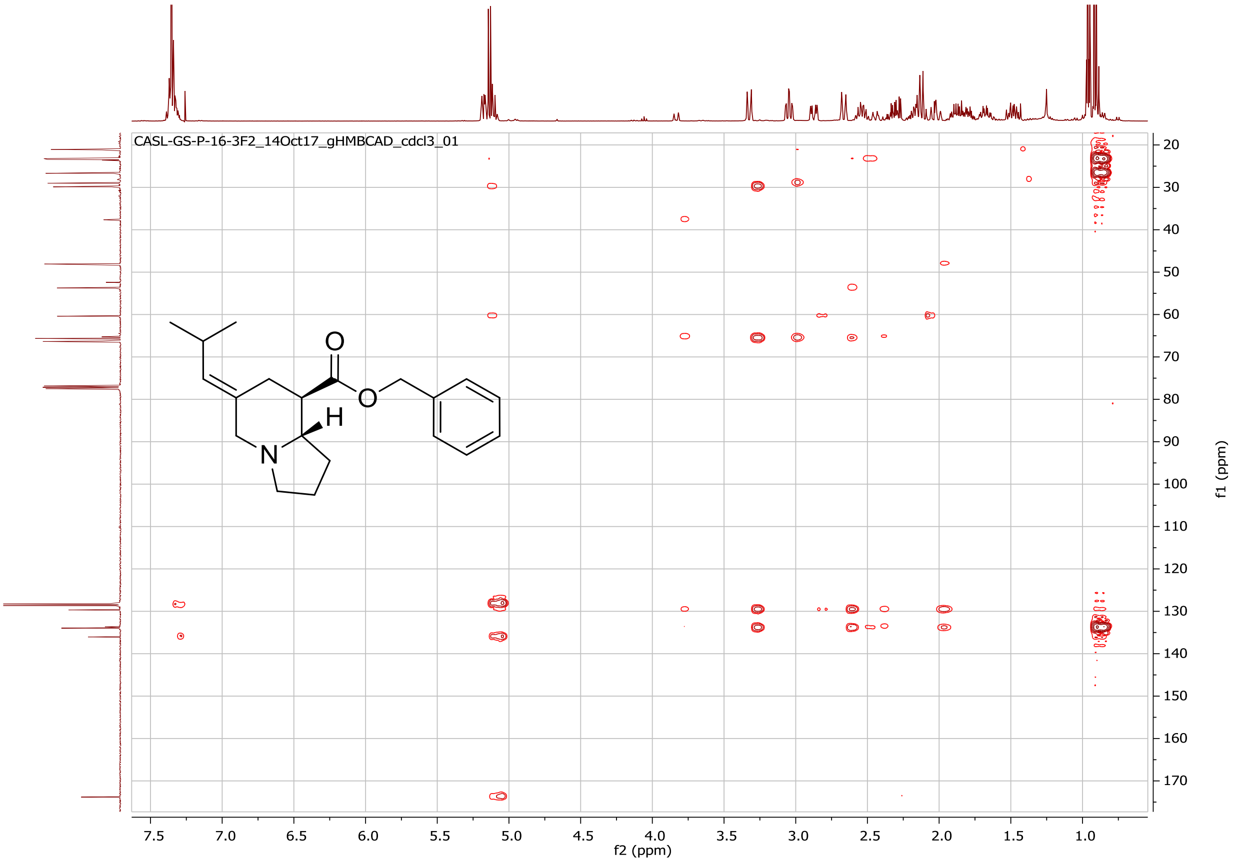


Figure S11. ^{1}H NMR spectrum of ***Z*-11**, (400 MHz)

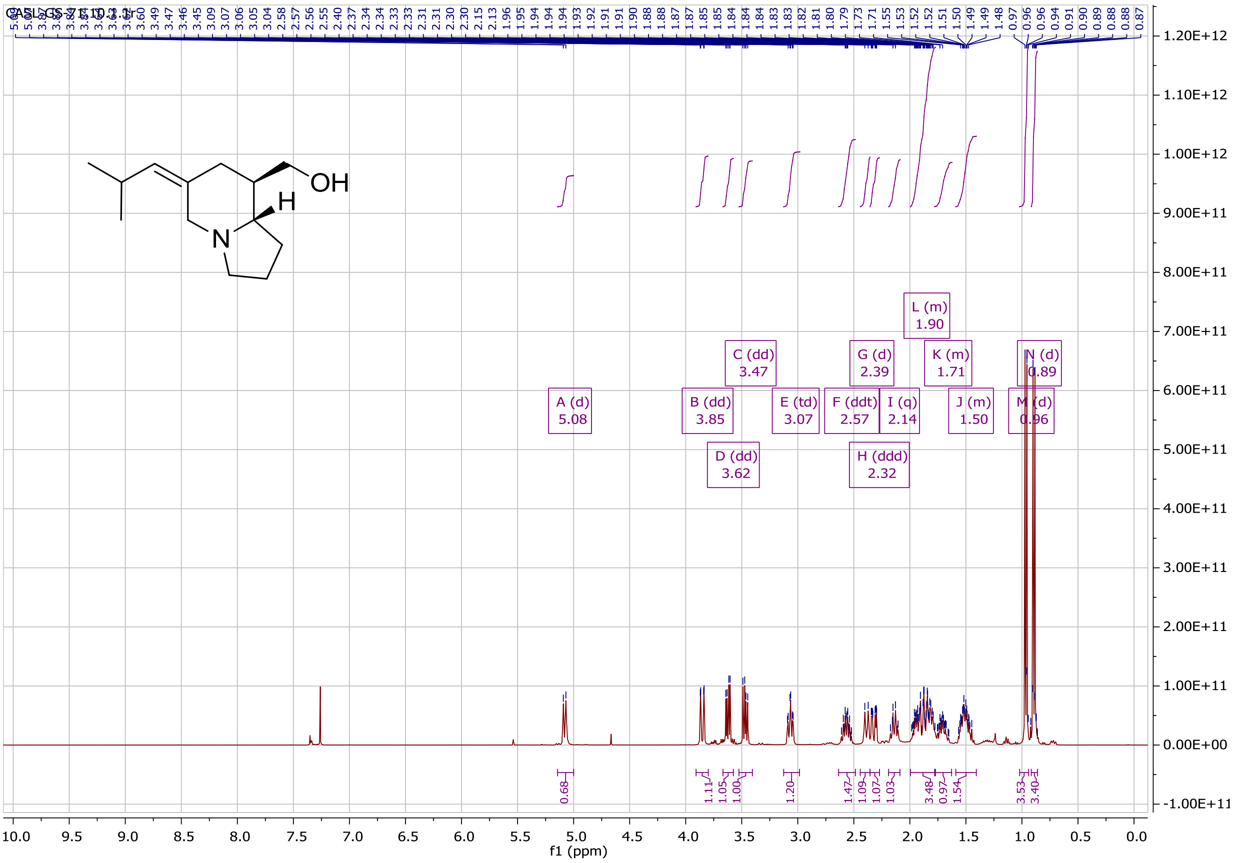


Figure S12. ^{13}C NMR spectrum of ***Z*-11**, (100 MHz)

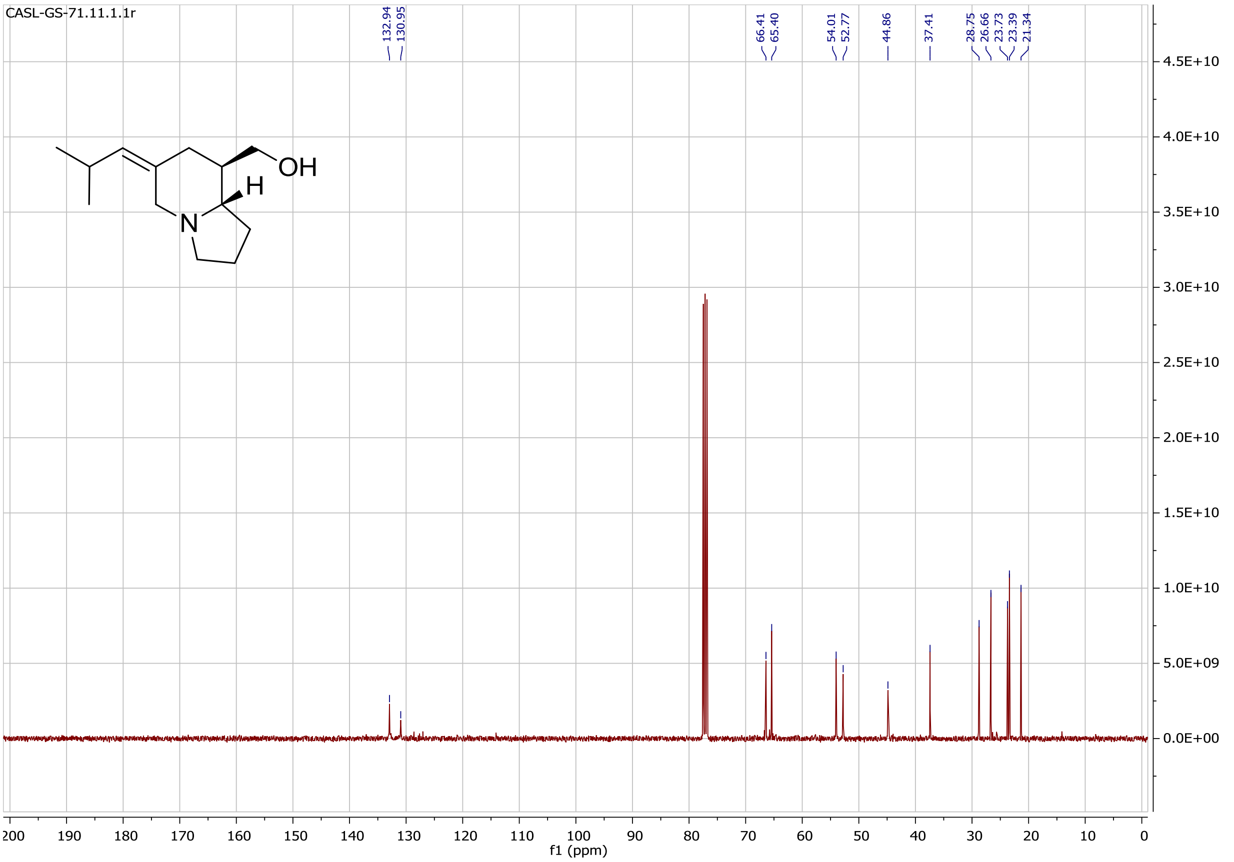


Figure S13. HSQC spectrum of ***Z*-11**

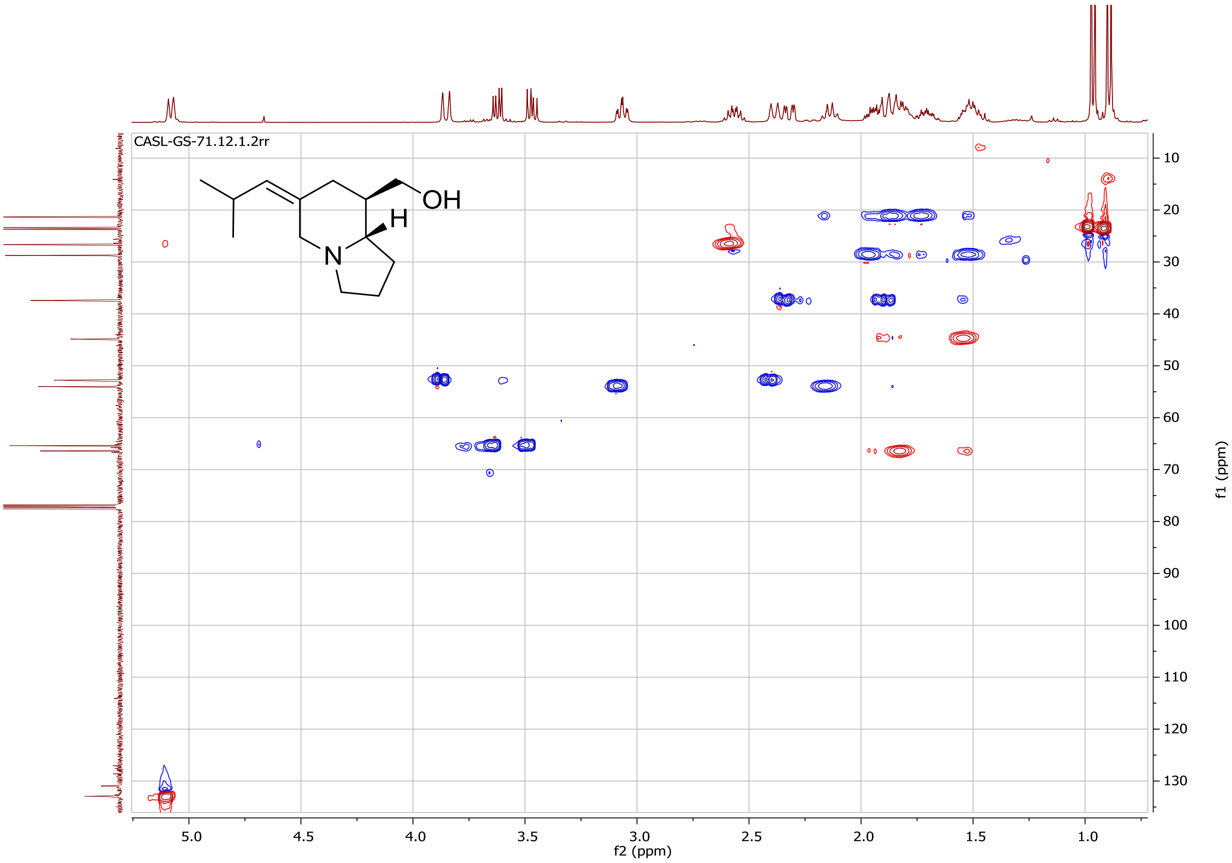


Figure S14. ^{1}H NMR spectrum of ***Z*-4**, (400 MHz)

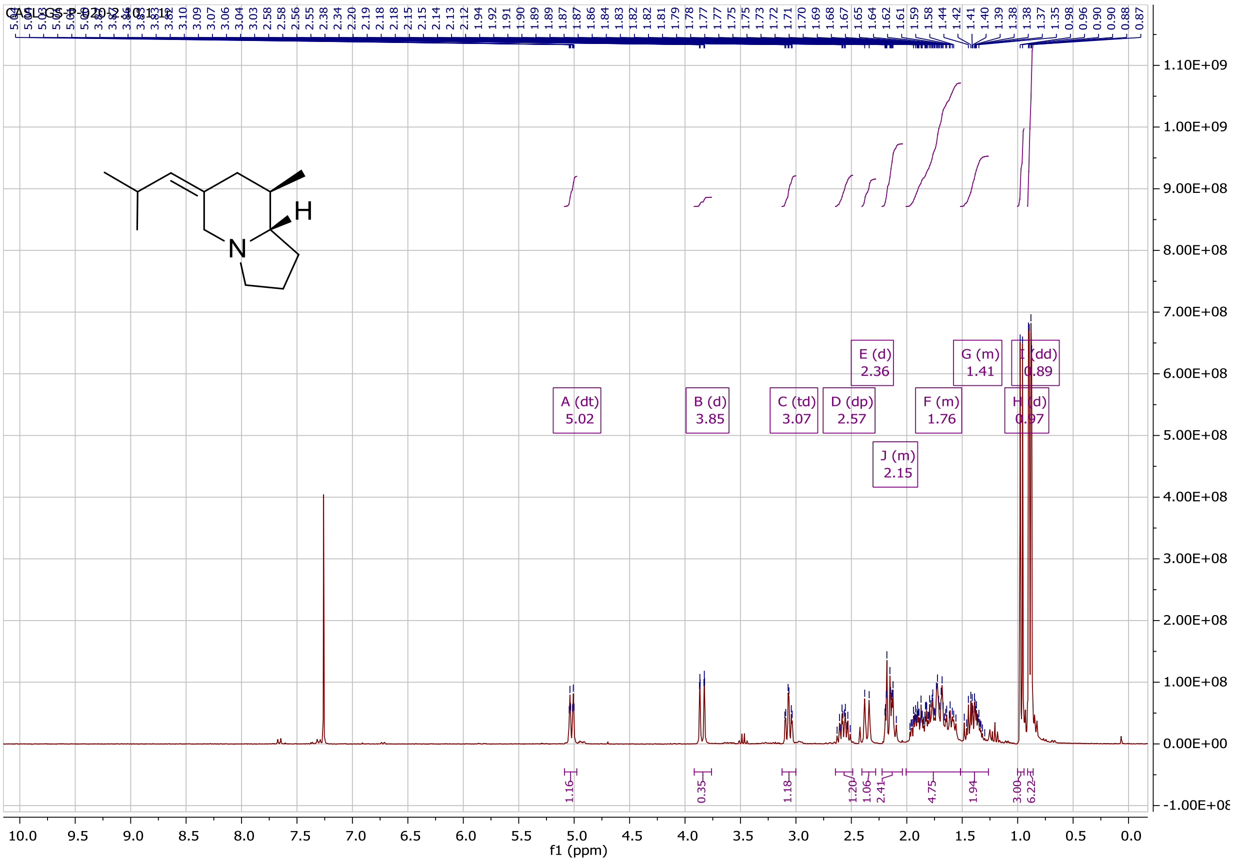


Figure S15. ^{1}H NMR spectrum of ***E*-11**, (400 MHz)

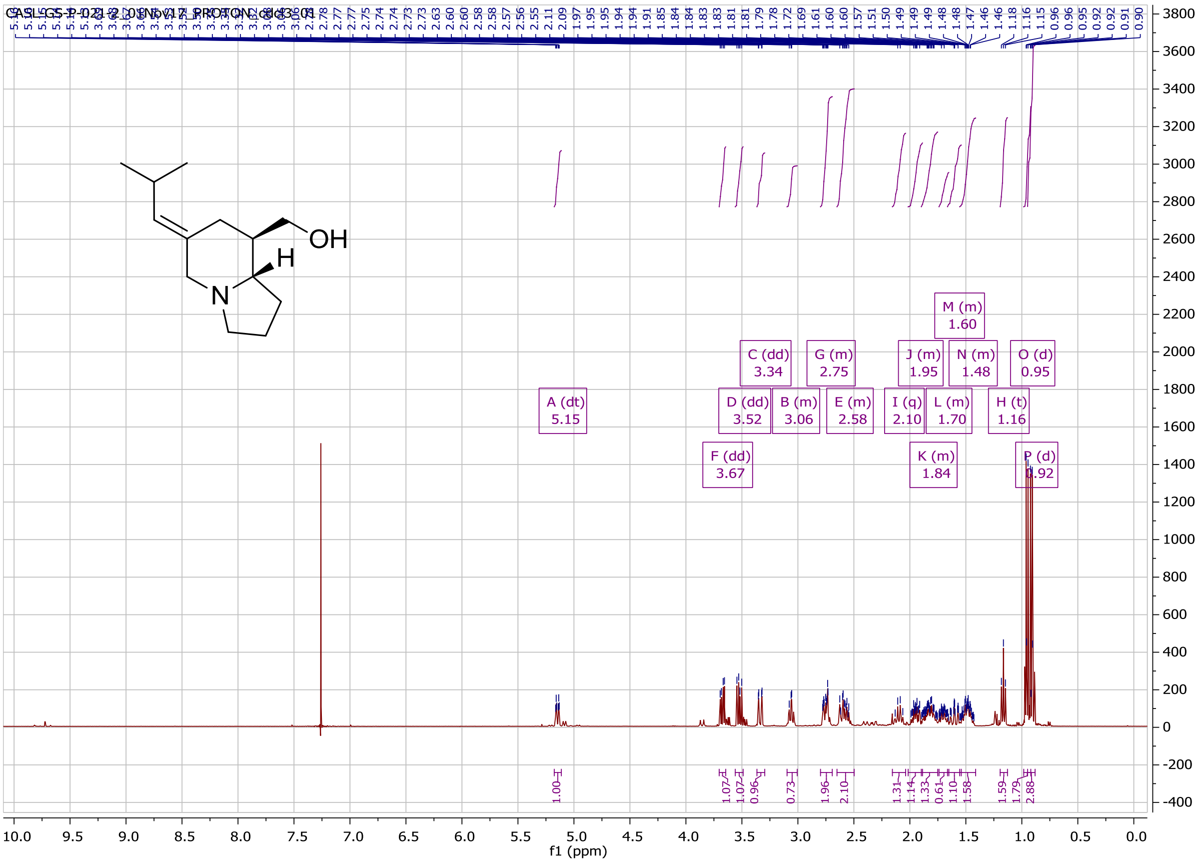


Figure S16. ^{13}C NMR spectrum of ***E*-11**, (100 MHz)

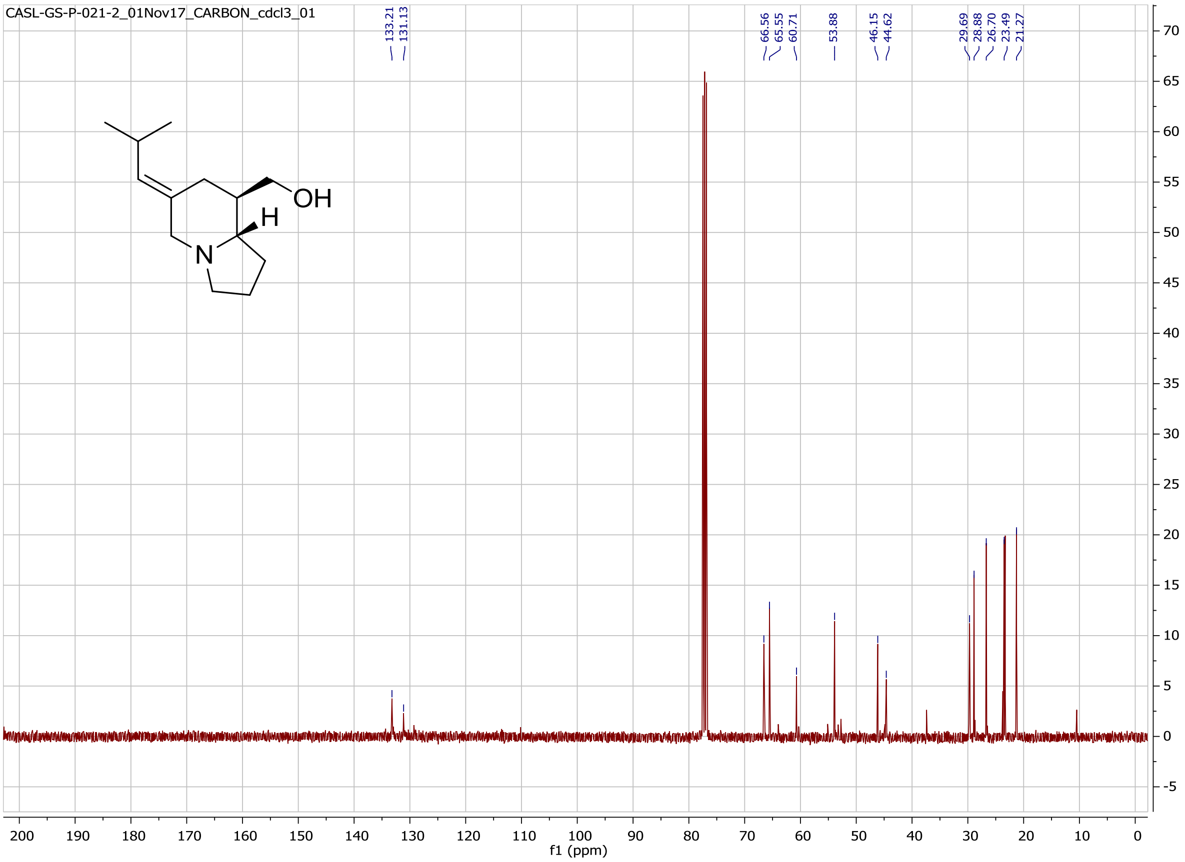


Figure S17. HSQC spectrum of ***E*-11**

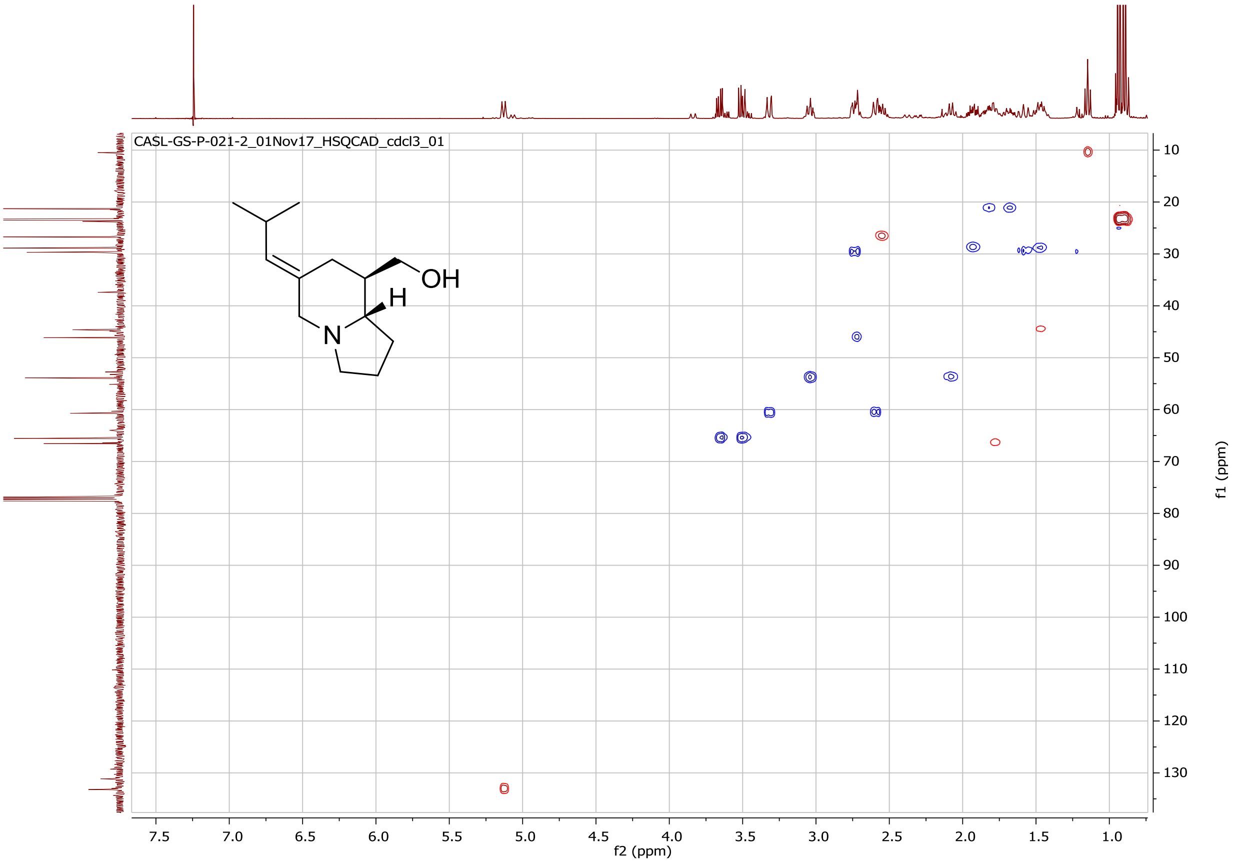


Figure S18. ^{1}H NMR spectrum of ***E*-4**, (400 MHz)

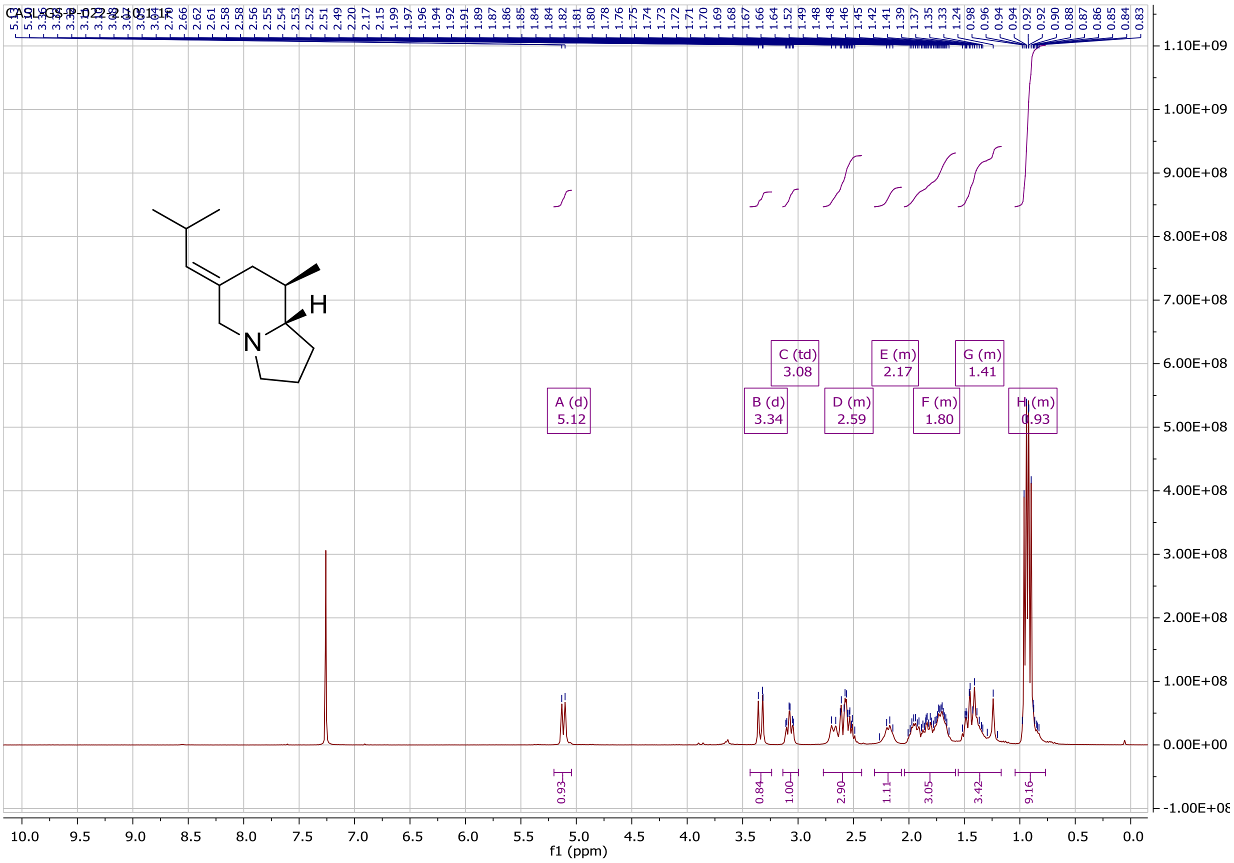


Figure S19. ^{13}C NMR spectrum of ***E*-4**, (100 MHz)

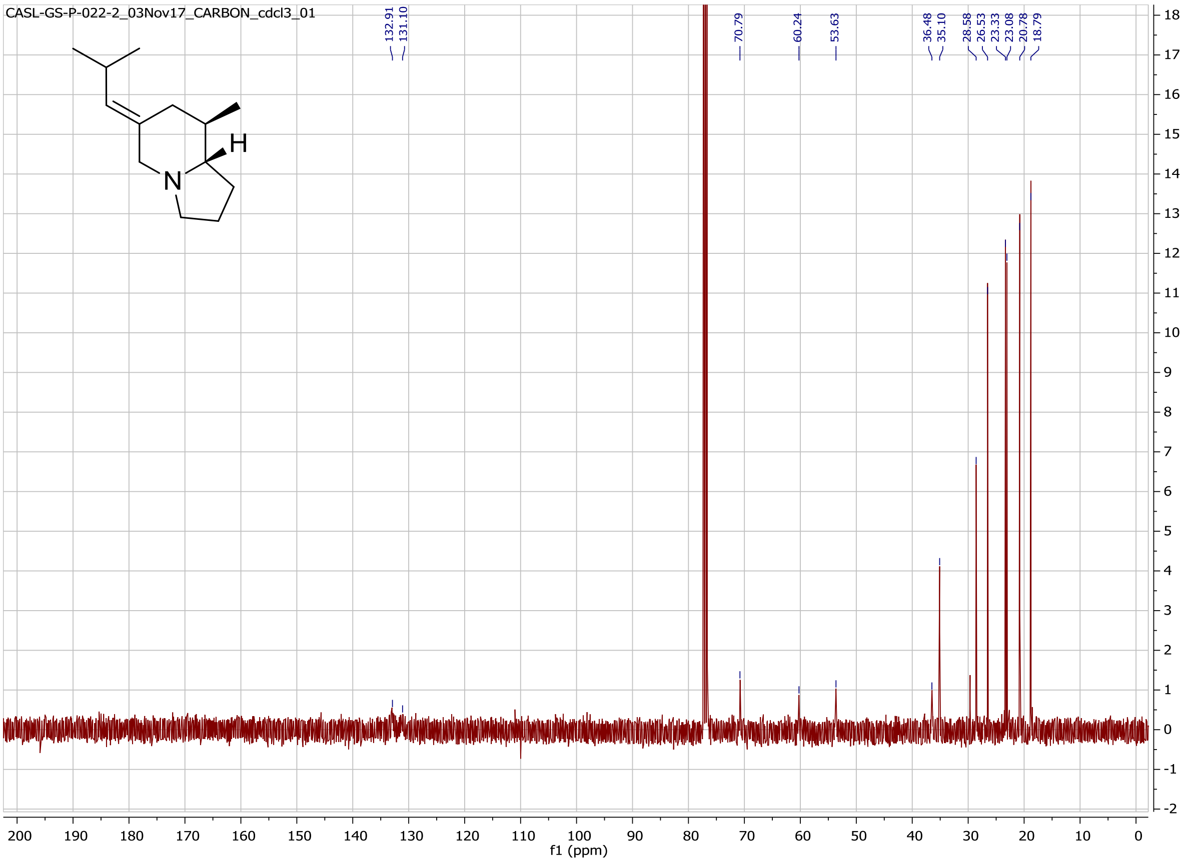


Figure S20. HSQC spectrum of ***E*-4**

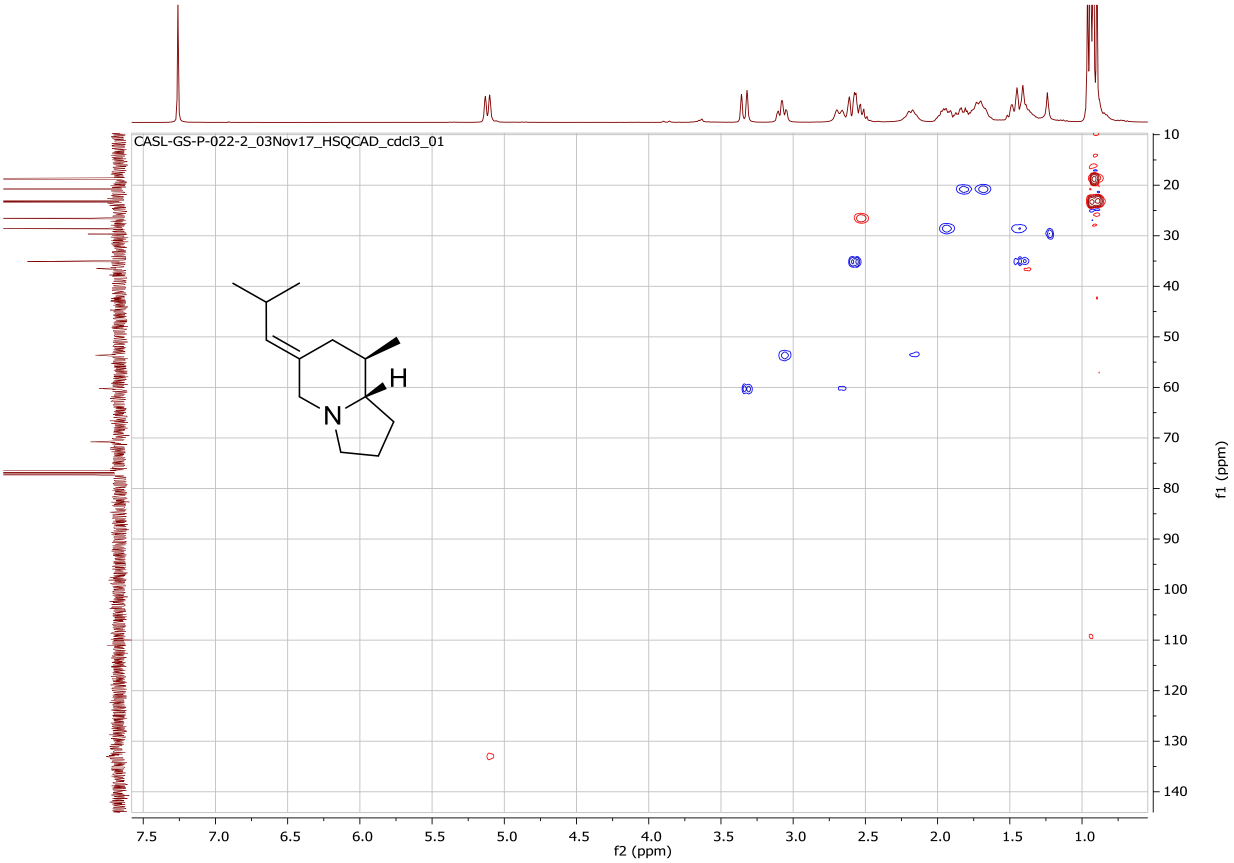


Figure S21. HMBC spectrum of ***E*-4**