



Supporting Information

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Synthesis and Bioactivity of New Analogue of Bicyclic 1-Azafagomine

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Author Contributions

M.K. Supervision:Lead

A.H. Investigation:Equal

SUPPORTING INFORMATION

EXPERIMENTAL

General chemistry

Solvents were distilled under anhydrous conditions. All reagents were purchased and used without further purification. Glassware was dried prior to use. Compounds were purified by dry flash chromatography using silica 60, <0063 mm and water pump vacuum or by flash-chromatography using silica 60 Å 230–400 mesh as stationary phases. TLC plates (silica gel 60 F₂₅₄) were visualized either at a UV lamp or in iodine. Melting points are uncorrected. The specific rotation was determined by AUTOPOL III polarimeter.

Elemental analysis was performed on the analyzer Carlo Erba 1108.

NMR experiments: The NMR experiments have been performed on a BRUKER FT NMR spectrometer AVANCE 400 (Bruker, Karlsruhe, Germany) (400 MHz for ¹H and 100.6 MHz for ¹³C) with a BVT 3200 variable temperature unit in 5 mm sample tubes using Bruker Standard software (TopSpin 3.1). The ¹H and ¹³C chemical shifts were referenced to internal tetramethylsilane (TMS); the experimental parameters for ¹H are as follows: digital resolution = 0.23 Hz, SWH (spectral width in Hz) = 8224 Hz, TD (time domain) = 65 K, SI (Fourier transform size) = 32 K, 90 pulse-length = 10 μs, PL1 (power level for F1 channel) = 3 dB, ns (number of scans) = 1, ds (number of dummy scans) = 0, d1 (relaxation delay) = 1 s and for ¹³C as follows: digital resolution = 0.27 Hz, SWH = 25253 Hz, TD = 65 K, SI = 32 K, 90 pulse-length = 9 μs, PL1 = 2 dB, ns = 100, ds = 2, d1 = 3 s.

HSQC: pulse program = hsqcetgp, digital resolution = 1.97 Hz, SWH = 5342, TD = 4096, SI = 1024, 90 pulse-length = 20 μs, PL1 = 3 dB, ns = 4, ds = 16, d1 = 1.5 s.

HMBC: pulse program = hmbcgpplpndgf, digital resolution = 1.97 Hz, SWH = 6810, TD = 4096, SI = 2048, 90 pulse-length = 20 μs, PL1 = 3 dB, ns = 24, ds = 16, d1 = 1.5 s.

The NMR-grades DMSO-d₆, CDCl₃, CD₃OD, D₂O was used for the solutions of 1-7.

IR experiments: Infrared spectra were recorded on a Bomem MB 104. Samples were run as oils as thin films. The spectrum was taken in the range of 4000-400 cm⁻¹ at room temperature.

MS experiments: MS spectra were recorded on a Varian 500-MS LC Ion Trap Mass and VG Autopsc M spectrometer.

Synthesis of new analogue of bicyclic 1-azafagomine (1-7)

Synthesis of (E)-2,4-pentadienol (1)

2,4-Pentadienol **1** was obtained by repeating the literature procedure. In details, 250 ml of a necked bottom flask with a magnetic stir bar was placed in an ice bath, where 5.09 g of 2,4-pentadienic acid, which was dissolved in dried THF (77 ml) and 8.9 ml of NEt₃ were added. Then 5 ml of ethylchloroformate was dissolved in 13 ml of THF, placed in dropping funnel, connected with 250 ml three-bottom flask and left it to pour drop by drop for 30 min at -5°-0°C. After 30 min reaction was stopped, precipitate filtrated under vacuum and washed thoroughly with THF (3×50ml) (Solution A).

A 1-l three-necked round bottom flask was placed in an ice bath (7°C) where 515 ml of H₂O and 4.804 g of NaBH₄ were added and stirred. Solution A was then added dropwise over 30 min after which the solution was removed from the ice bath and allowed to stir at ambient temperature for 3.5 h. Thereafter, the solution was reinserted into an ice-water bath and 20 ml of 37% HCl was added dropwise using a dropping funnel under an N₂ atmosphere. The mixture obtained was extracted with diethyl ether (2 x 100 ml), and the organic layers were combined and extracted with 10% NaOH solution (50 ml), followed by extraction with H₂O (50 ml) and brine (50 ml), then dried over anhydrous MgSO₄ and concentrated to dryness under reduced pressure to give a colorless liquid in 31.4% yield, b.p. 58-62°C/11 mmHg (15 Pa). Yield 31.4%. The spectroscopic data corresponded to literature values [**46**], ν_{\max} (CHCl₃)/cm⁻¹ 3616, 3444, 2873, 2254, 1824, 1731, 1606, 1455, 1377, 1083, 994, 902; δ_{H} (400 MHz; CDCl₃) 6.25 (2H, m, CH₂=CH), 5.74 (1H, dt, J=14.7, 5.7, CH=CH₂OH), 5.13 (1H, d, J=16.4, CH), 5.01 (1H, d, J=9.6, CH), 4.07 (2H, m, CH₂OH), 3.12 (1H, br s, OH); δ_{C} (100 MHz; CDCl₃) 136.2 (CH), 132.5 (CH), 131.2 (CH), 116.9 (CH₂), 62.3 (CH₂). Anal. Calcd. for C₅H₈O: C, 71.42; H, 9.52. Found: C, 71.47; H, 9.48.

Synthesis of (S)-Diethyl-3-(hydroxymethyl)pyridazine-1,2(3H,6H)-dicarboxylate (2)

Compounds **2** was obtained according to the procedure. In general, in order to obtain compound **2**, two different solutions (A and B) were prepared.

Solution A: a solution of Me₂Zn 1.2 M in toluene (991 μ l; 1.19 mmol) was added to a solution of penta-2,4-dien-1-ol **1** (0.100 g; 1.19 mmol) in dry toluene (6 ml) at 0°C, and stirred for 5 min.

Solution B: a solution of MeMgBr 1.4 M in toluene/THF (849 μ L; 1.19 mmol) was added to a solution of (S)-BINOL (0.340 g; 1.19 mmol) in dry toluene (6 ml) at 0°C, and stirred for 5 min.

Thereafter, solution A was diluted in dry toluene (10 ml), added to solution B, stirred for 5 min and then refrigerated at -78°C. To this mixture, a solution of diethyl azodicarboxylate (543 μ L; 1.19 mmol) in dry toluene (10 ml) was added. The temperature was allowed to rise

gradually to room temperature and the reaction mixture was stirred for 18h. The reaction was quenched with a saturated solution of NaHCO₃ (1 ml), filtered through a pad of Celite®, which was washed with EtOAc (3 × 20 ml). The filtrates were combined and concentrated under reduced pressure giving a yellow oil. The crude oil was purified by “dry-flash” chromatography (silica, petroleum ether / diethyl ether). (*S*)-BINOL was recovered with petroleum ether (1): diethyl ether (1) (0.200 g; 69%) and product (-)-**2** with diethyl ether (0.225 g; 73%) as a yellow oil. $[\alpha]_D^{20}$ -23.4° (conc. 1.25% in CHCl₃). ν_{\max} (neat) 3483, 1707 cm⁻¹. δ_H (400 MHz, CDCl₃) 1.23-1.30 (12H, m, 4CH₃, A+B), 2.58 (1H, br s, OH), 3.35 (1H, dd, *J*=12.3, 9.5 Hz, H-3', A), 3.45 (1H, dd, *J*=12.0, 9.8 Hz, H-3', B), 3.56-3.69 (2H, m, 2H-3', A+B), 3.77 (1H, dd, *J*=13.5, 4.3 Hz, H-6, A), 3.91 (1H, br s, H-6, B), 4.11-4.26 (8H, m, 4CH₂, A+B), 4.30 (1H, tdd, *J*=6.0, 3.9, 2.2 Hz, H-6, B), 4.34-4.44 (1H, m, H-6, A), 4.72 (2H, br s, H-3, A+B), 5.66-5.88 (4H, m, H-4 + H-5, A+B) ppm. δ_C (100 MHz, CDCl₃) 14.3 (CH₃, A), 14.4 (CH₃, B), 42.2 (C-6, A), 43.6 (C-6, B), 55.9 (C-3, A), 56.9 (C-3, B), 61.9 (C-3', A+B), 62.6, 62.7, 62.8, 62.9 (CH₂, A+B), 123.4, 124.2, 124.6, 125.2 (C-4 or C-5, A+B), 154.9, 155.7, 156.2, 156.3 (C=O, A+B) ppm. HRMS (ESI): calcd for C₁₁H₁₈N₂NaO₅: 281.1108; found: 281.1109. Anal. Calcd. for C₁₁H₁₈N₂O₅: C, 51.16; H, 6.97; N, 10.85. Found: C, 51.21; H, 6.91; N, 10.81.

Synthesis (*S*)-(1,2,3,6-tetrahydropyridazin-3-yl)methanol (**3**)

To a stirred solution of cycloadduct **2** (0.119 g; 0.461 mmol) in THF (2 mL) was added 2ml of 2M NaOH. The mixture was left under reflux for 3h. After cooling down THF (2 ml) and a suspension of Amberlite (H⁺) in water was added to the reaction mixture. The mixture was swirled to promote contact and then immediately filtrated under vacuum. The solvent was evaporated. By adding acetone, water was co-evaporated in the rotary evaporator giving yellow oil (0.048 g; 91.0 %). $[\alpha]_D^{20}$ -20° (conc. 0.3% in EtOH). ν_{\max} (neat): 3422 (N-H), 1643 (C=C) cm⁻¹. δ_H (400 MHz, D₂O) 6.01 (1H, ddd, *J*=2.4, 5.6, 10.4 Hz H-5), 5.79 (1H, ddd, *J*=2.0, 4.4, 10.4 Hz, H-4), 3.65-3.57 (2H, m, H-3'), 3.54-3.48 (1H, m, H-3), 3.35 (1H, ddd, *J*=2.8, 5.2, 17.6 Hz H-6), 3.24 (1H, ddd, *J*=2.6, 3.2, 17.2 Hz, H-6) ppm. δ_C (100.6 MHz, D₂O) 127.4 (C-4 or C-5), 125.0 (C-5 or C-4), 62.5 (C-3'), 54.8 (C-3), 44.2 (C-6) ppm. HRMS (ESI): calcd for C₅H₁₀N₂O [M+H]⁺ 114.0793; found: [M+H]⁺ 115.0165. Anal. Calcd. for C₅H₁₀N₂O: C, 52.63; H, 8.77; N, 24.56. Found: C, 52.67; H, 8.74; N, 24.51.

Synthesis of *tert*-butyl (*S*)-3-(hydroxymethyl)-3,6-dihydropyridazine-1(2*H*)-carboxylate (*N*-Boc-1-azafagomine) (**4**)

To a stirred solution of compound **3** (0.044 g; 0.385 mmol) dissolved in ethanol (10 mL) was added solid NaOH (0.015 g; 0.385 mmol) and Boc₂O (0.015 g; 0.385 mmol). The reaction

mixture was left under magnetic stirring for 1 h at room temperature. Then the mixture was filtrated through a pad of celite and washed thoroughly with ethanol. The solution was concentrated to give a yellow solid. The yellow solid was dissolved in acetone, filtrated and the solution evaporated to give a yellow oil (0.077g; 93%) $[\alpha]_D^{20} + 67^\circ$ (conc. 0.78 % in DCM). $v_{\max}(\text{neat})$: 3269 (OH), 1694 (CO) cm^{-1} . δ_H (400 MHz, D_2O) 5.96 (1H, br d, $J=8.8$, H-5), 5.90 (1H, bd, $J=8.8$, H-4), 4.09 (1H, bd, $J=10.0$ Hz, H-6), 3.96 (1H, bd, $J=10.0$ Hz, H-6), 3.68-3.56 (3H, m, H-3, H-3'), 1.50 (9H, s, $\text{C}(\text{CH}_3)_3$) ppm. δ_C (100.6 MHz, D_2O), 156.8 (C=O), 125.1, 125.0 (C-4 and C-5), 82.7 (C), 61.8 (C-3'), 55.9 (C-3), 27.6 ($\text{C}(\text{CH}_3)_3$) ppm. HRMS (ESI): calcd for $\text{C}_{10}\text{H}_{18}\text{N}_2\text{O}_3$ $[\text{M}+\text{H}]^+$ 214.1317; found: $[\text{M}-\text{H}]^-$ 213.1591. Anal. Calcd. for $\text{C}_{10}\text{H}_{18}\text{N}_2\text{O}_3$: C, 56.07; H, 8.41; N, 13.08. Found: C, 56.11; H, 8.37; N, 13.05.

Synthesis of *tert*-butyl (S)-7-oxo-4a,5-dihydro-7H-oxazolo[3,4-b]pyridazine-1(2H)-carboxylate (5)

To a stirred solution of compound **4** (0.114 g; 0.532 mmol) in dry DCM (10 ml) was added DIPEA (10 equiv.; 0.93 mL; 0.532 mmol) and triphosgene (0.5 equiv.; 0.077 g; 0.532 mmol). The mixture was left stirring under nitrogen for 5 min at room temperature. The solvent was removed, and the residue was purified by column chromatography (EtOAc : Pet. ether, 1:1) to give yellow oil (0.077g; 93%). $[\alpha]_D^{20} +30^\circ$ (conc.0.4% DCM). $v_{\max}(\text{neat})$: 1717 (C=O) cm^{-1} . δ_H (400 MHz, DMSO) 5.89 (1H, dm, J 8.8) 5.86 (1H, (1H, ddd, $J=8.4, 4.8, 2.0$ Hz), 4.02 (2H, dd, $J=4.8, 2.4$ Hz), 3.71 (1H, br s), 3.55 (1H, dd, $J=11.2, 4.8$ Hz), 3.46 (1H, $J=11.2, 7.2$ Hz) ppm. δ_C (100.6 MHz, DMSO- d_6), 153.6 (CO), 152.6 (CO), 124.5-123.8 (C-H, Ar), 83.0, 81.6 (Cq), 60.4, 59.9 (CH_2O), 56.2, 55.6, 53.0 (CH), 43.8, 43.3 (CH_2N), 27.9, 27.8, 27.7, 27.4 (CH_3) ppm. HRMS (ESI): calcd for $\text{C}_{11}\text{H}_{16}\text{N}_2\text{O}_4$ $[\text{M}+\text{H}]^+$ 240.0793; found: $[\text{M}+\text{H}]^+$ 241.0165. Anal. Calcd. for $\text{C}_{11}\text{H}_{16}\text{N}_2\text{O}_4$: C, 55.00; H, 6.66; N, 11.66. Found: C, 55.05; H, 6.63; N, 11.69.

Synthesis of (3S,4R,4aR)-*tert*-butyl 3,4-dihydroxy-7-oxohexahydro-1H-oxazolo[3,4-b]pyridazine-1-carboxylate (6)

Compound **5** (0.076; 0.316 mmol) was dissolved in a mixture of acetone (1.2 ml) and water (0.11 ml). To this solution *N*-methylmorpholine *N*-oxide (1.3 equiv., 0.048 g; 0.316 mmol) followed by a solution of OsO_4 in water (4%; 34 μl ; 0.0053 mmol) were added. The reaction mixture was stirred for 1 day at room temperature. The solvents were evaporated until a residue, which was subsequently dissolved in ethyl acetate (20 ml) and washed with water (2 x 15 ml). The organic fractions were combined, dried over MgSO_4 , filtrated, and the solution concentrated in the rotary evaporator to give an orange oil (0.078g; 90%). $v_{\max}(\text{neat})$: 1693(C=O) cm^{-1} . NMR δ_H (400 MHz, CDCl_3), 1.5 (9H, s, 3 CH_3), 3.42 (1H, m), 3.6 (2H, s, OH), 3.81

(2H, d, $J=2.4$ Hz), 3.91 (2H, q, $J=3.6$ Hz), 4.34 (2H, t, $J=2$ Hz). δ_c (100.6 MHz, $CDCl_3$), 28.07 (3 CH_3), 29.6 (CH_2N), 36.5 (CHN), 65.1(CH_2O), 66.4 (CH-OH), 68.8 (CH-OH), 82.6 (C), 155.8 (CO), 162.6 (CO) ppm. HRMS (EI): m/z calcd for $C_{11}H_{18}N_2O_6$: 274.1165 $[M + H]^+$, found: $[M + H]^+$ 274.0658. Anal. Calcd. for $C_{11}H_{18}N_2O_6$: C, 48.17; H, 6.56; N, 10.21. Found: C, 48.21; H, 6.59; N, 10.17.

Synthesis of (3*S*,4*R*,4*aR*)-3,4-dihydroxytetrahydro-1*H*-oxazolo[3,4-*b*]pyridazin-7(2*H*)-one (7)

To a solution of compound **6** (0.099 g, 0.361 mmol) in acetonitrile (3 ml) was added H_2O (11 ml) and BiI_3 (0.199 g, 0.726 mmol). The mixture was refluxed for 16 h (water bath 110 °C). A column chromatography (DCM : EtOH, 7:3) was run giving a brown oil (0.069 g; 0.396 mmol, 55 %). ν_{max} (neat): 3404 (OH), 1745 (CO) cm^{-1} . (400 MHz, CD_3OD) δ_H 4.57 (1H, br s), 4.44 (1H, dd, $J=7.6, 8.8$ Hz, H-3'), 4.26 (1H, dd, $J=3.2, 8.8$ Hz, H-3'), 3.86 (1H, ddd, $J=3.6, 7.6, 10$ Hz, H-5), 3.83-3.81 (1H, m, H-3), 3.51 (1H, dd, $J=2.6, 9.8$ Hz, H-4), 2.97 (1H, dd, $J=2.8, 14.0$ Hz, H-6), 2.90 (1H, dd, $J=1.2, 13.2$ Hz, H-6) ppm. δ_c (100.6 MHz, CD_3OD) 159.2 (CO), 70.7 (C-4), 67.0 (C-3), 66.5 (CH_2O), 55.0 (C-5), 52.5 (CH_2 -6) ppm. HRMS (ESI): m/z calcd for $C_6H_{10}N_2NaO_4$: 197.0538 $[M + Na]^+$, found: $[M + Na]^+$ 197.0533. Anal. Calcd. for $C_6H_{10}N_2O_4$: C, 41.37; H, 5.74; N, 16.09. Found: C, 41.39; H, 5.71; N, 16.11.

Biological activity

Antibacterial activity of compounds **3-7** and pristine antibiotics (cefotaxime and ceftriaxone) were tested by diffusion method on *Staphylococcus aureus* and *Escherichia coli*, as described by Mayrhofer [47]. Pristine antibiotics (cefotaxime and ceftriaxone) were taken in amount 25 μg (indicator disks were purchased by Research-and-Development Center of Pharmacotherapy, 192236 St. Petersburg). The synthesized substances were also taken in the amount equal to 25 μg . *Escherichia coli* was cultivated on Endo's medium and *Staphylococcus aureus* on Baird-Parker agar. Microbiological tests were performed on Petri dishes. Due to the fact that this method provides only quality data, microdilution method was also performed, as it is written by Jorgensen and Lee [48]. By this method, the MICs of the prepared compounds **3-7** and usual antibiotics were identified and compared to each other. In order to perform the microdilution method, the stock solutions with different concentrations of the substances were prepared in distilled sterile water and were distributed in 96 multi-well plates. Each well was inoculated with 0.1 ml of microbial suspensions of 0.5 Mc Farland turbidity, prepared from 24h fresh culture. Sterility control wells (nutrient broth) and microbial growth controls (inoculated nutrient broth) were used. The plates were incubated for 24 h at 37°C.

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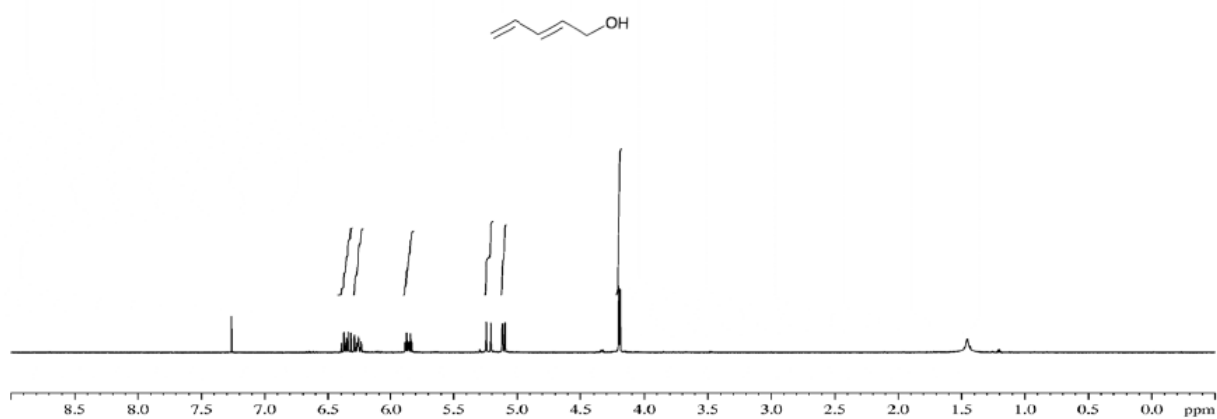


Figure S1. ¹H NMR (CDCl₃) spectrum of compound **1**

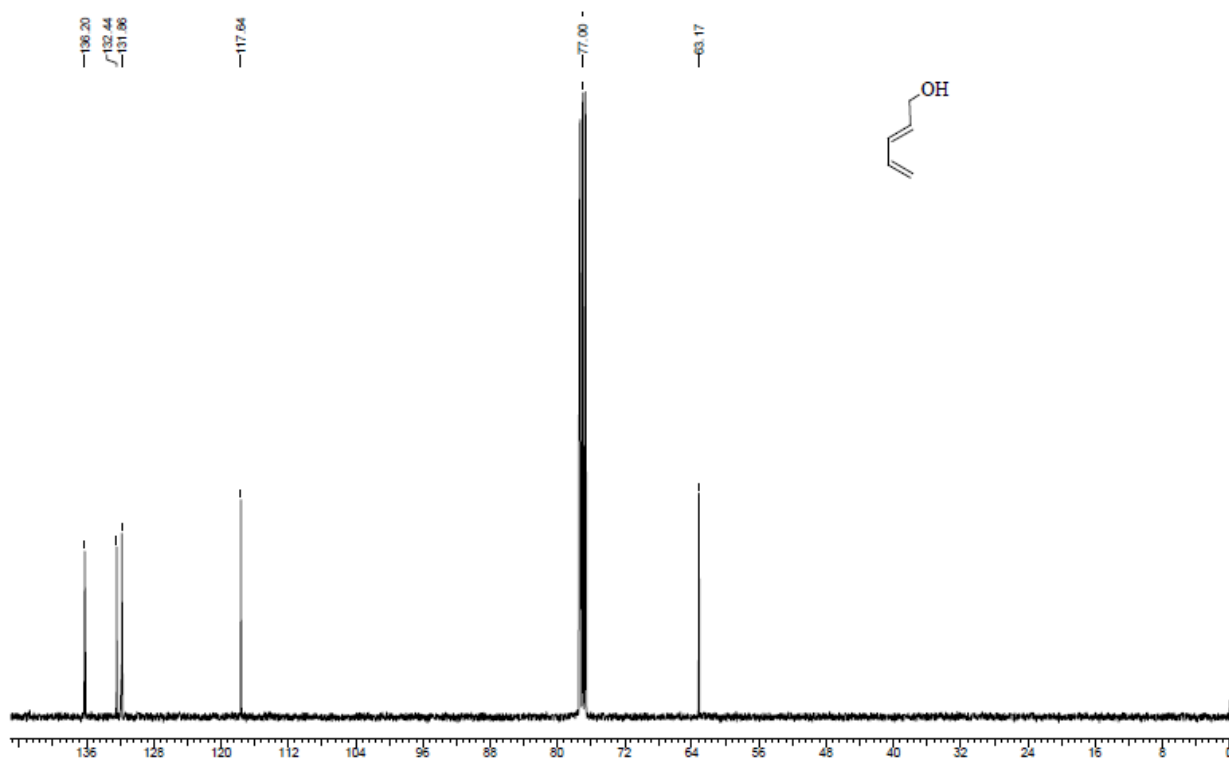


Figure S2. ¹³C NMR (CDCl₃) spectrum of compound 1

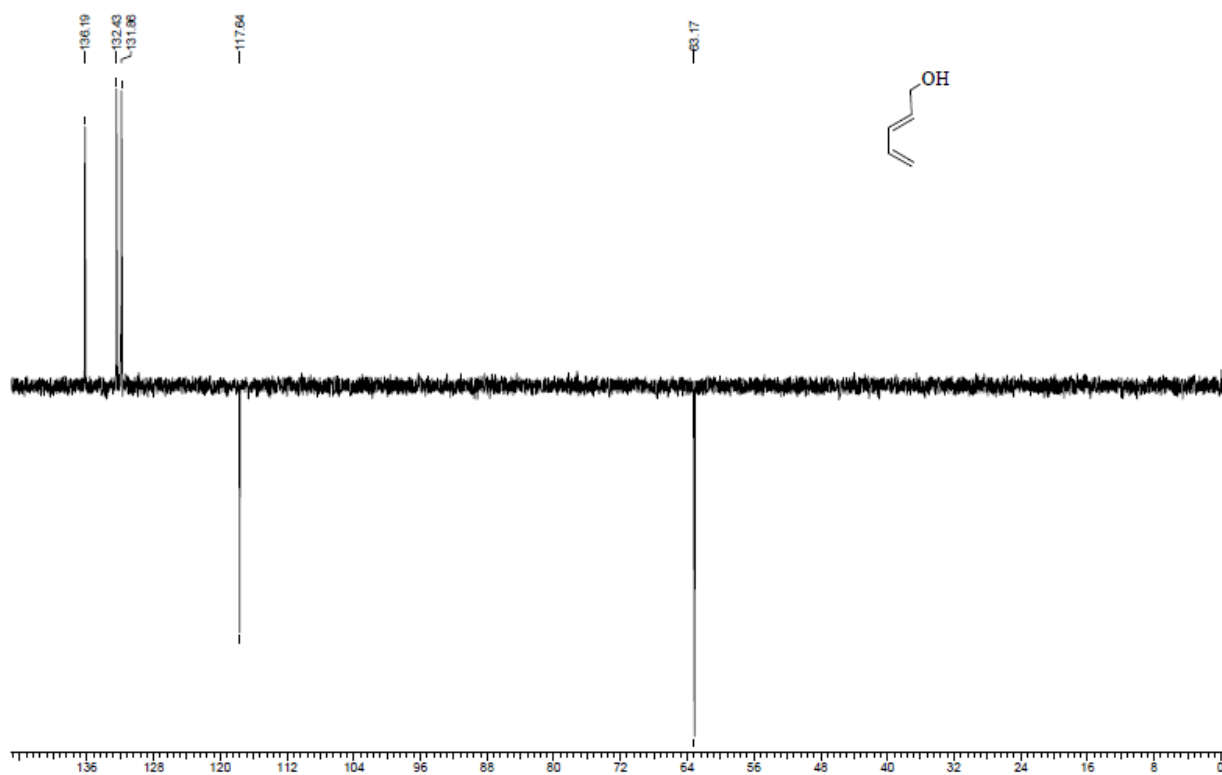


Figure S3. dept 135 NMR (CDCl₃) spectrum of compound 1

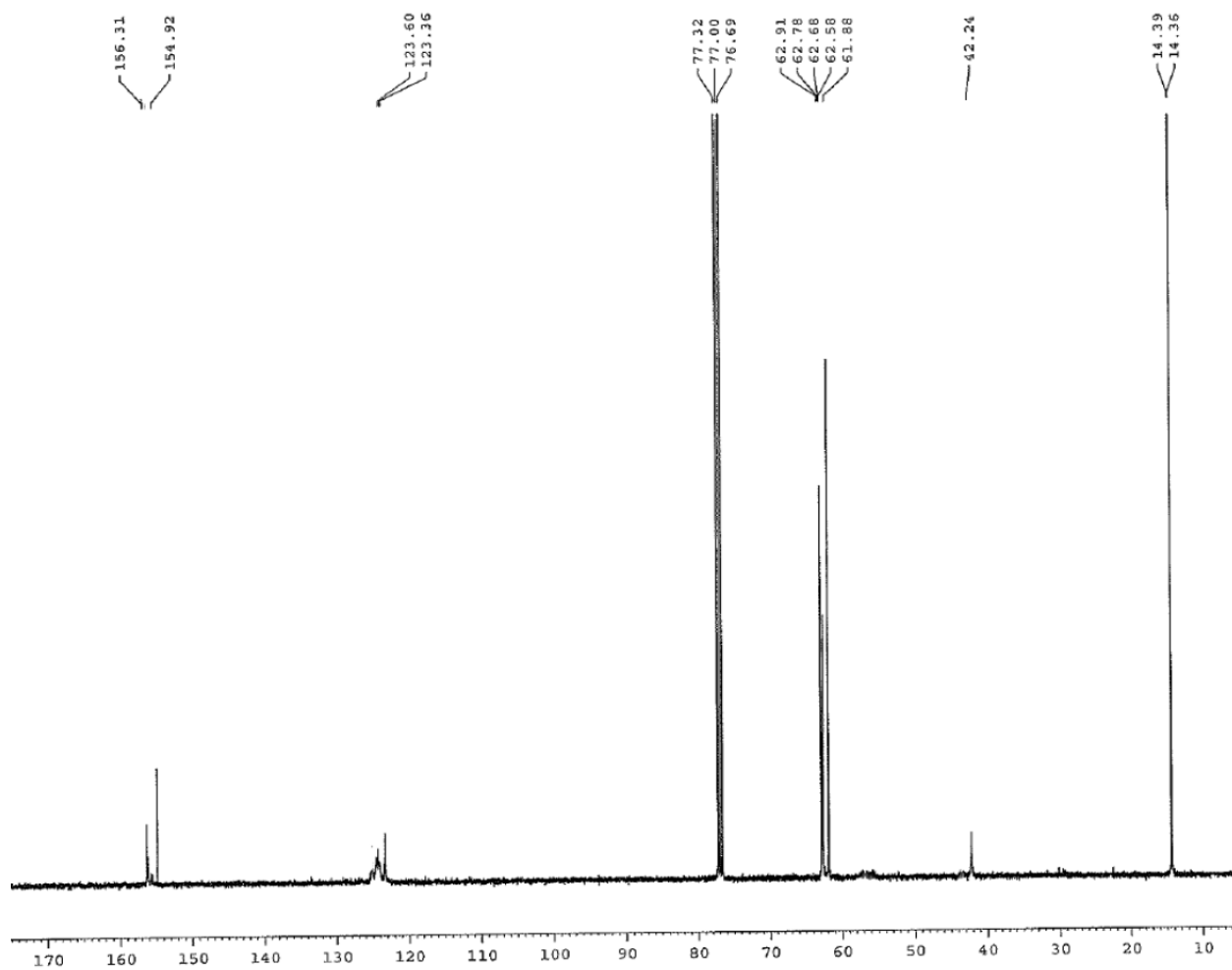


Figure S4. ^{13}C NMR (CDCl_3) spectrum of compound 2

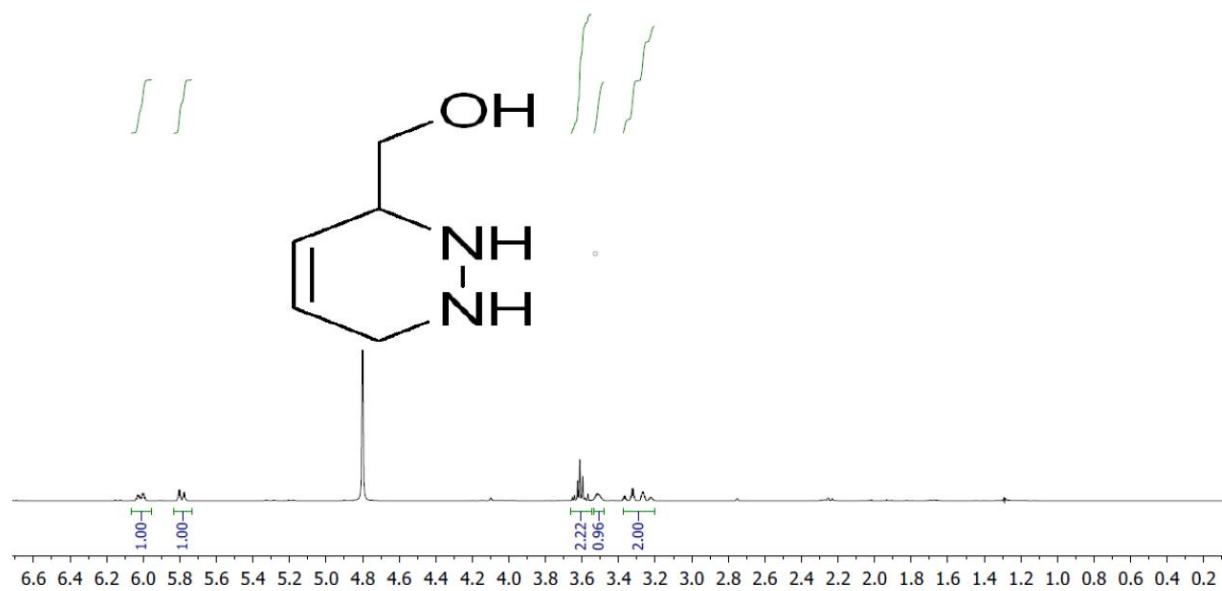


Figure S5. ^1H NMR (D_2O) spectrum of compound 3

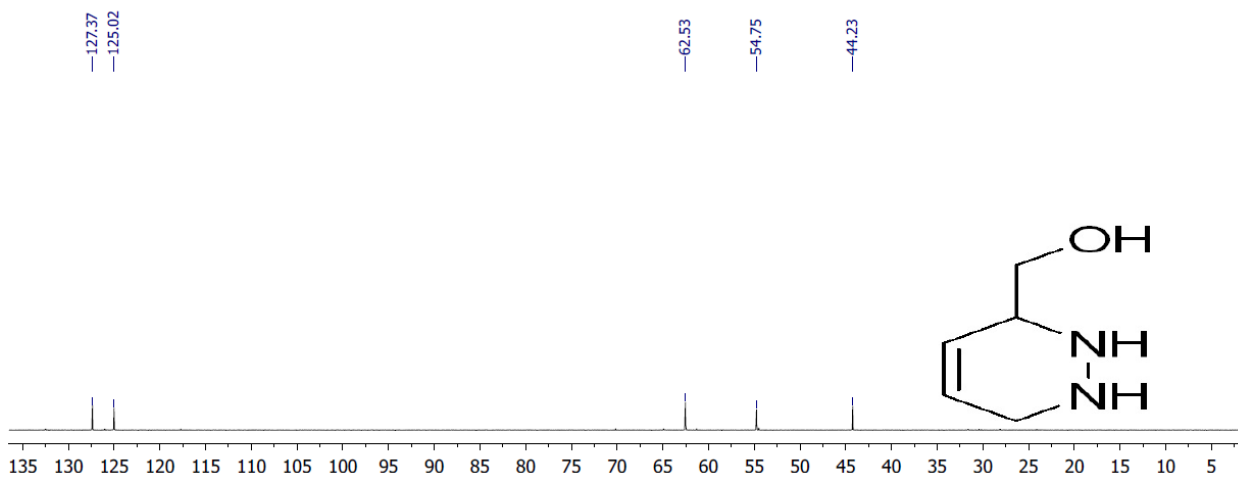


Figure S6. ^{13}C NMR (D₂O) spectrum of compound 3

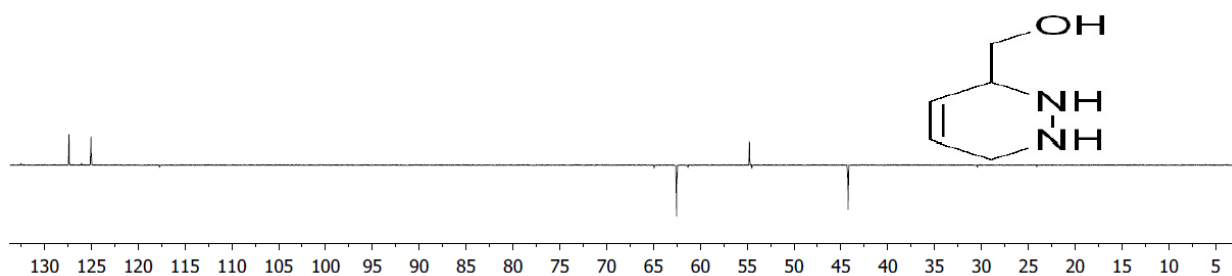


Figure S7. dept 135 NMR (D₂O) spectrum of compound 3

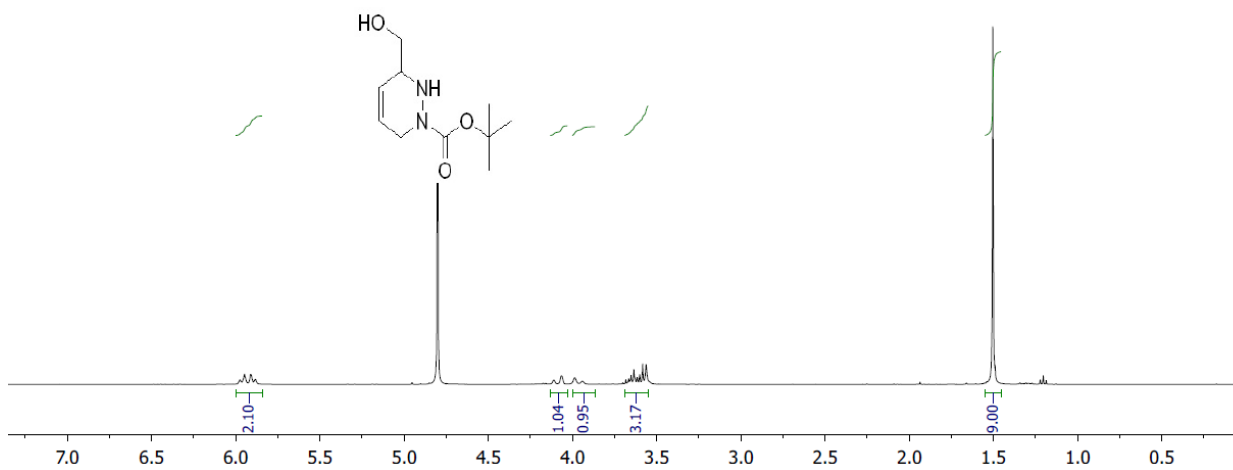


Figure S8. ^1H NMR (D₂O) spectrum of compound 4

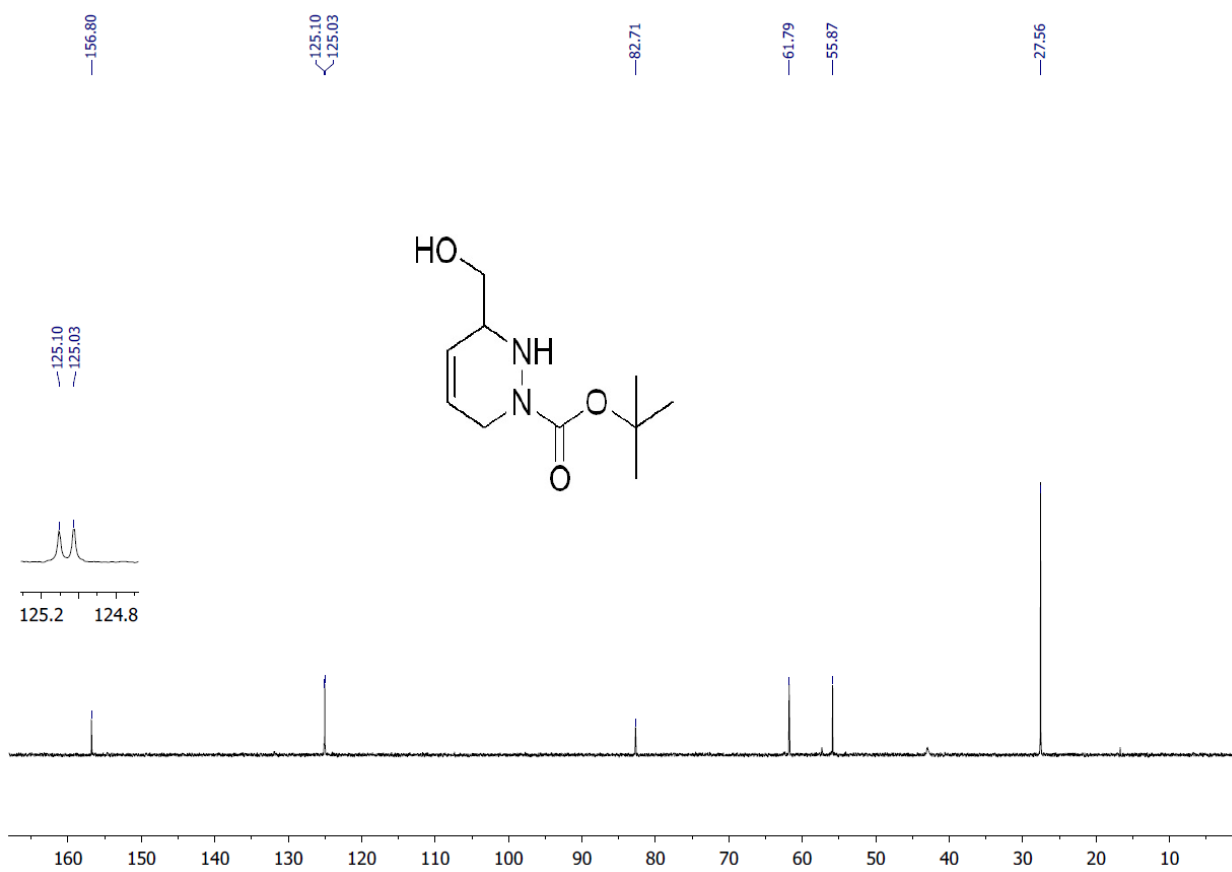


Figure S9. ^{13}C NMR (D₂O) spectrum of compound 4

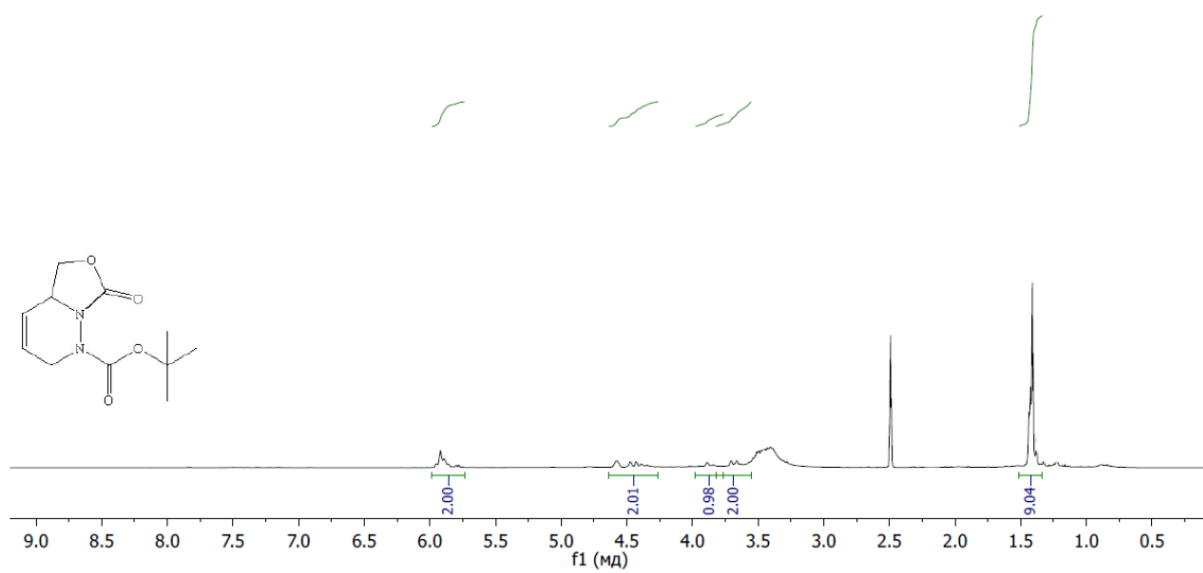


Figure S10. ^1H NMR (DMSO-d₆) spectrum of compound 5

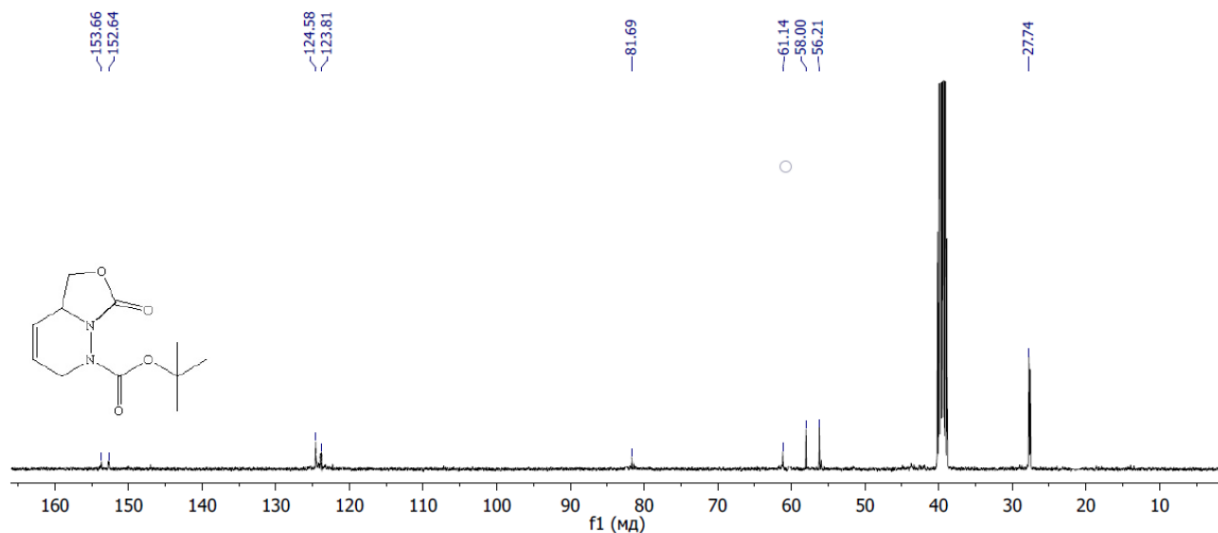


Figure S11. ^{13}C NMR (DMSO- d_6) spectrum of compound **5**

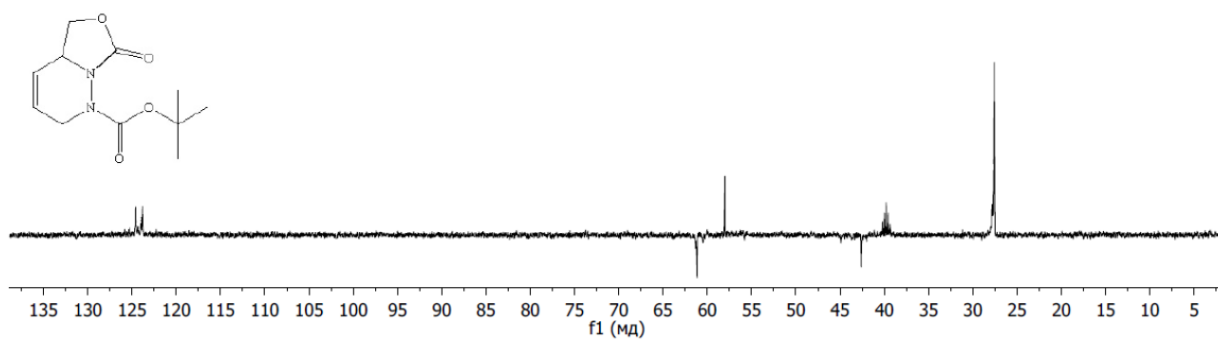


Figure S12. dept 135 NMR (DMSO- d_6) spectrum of compound **5**

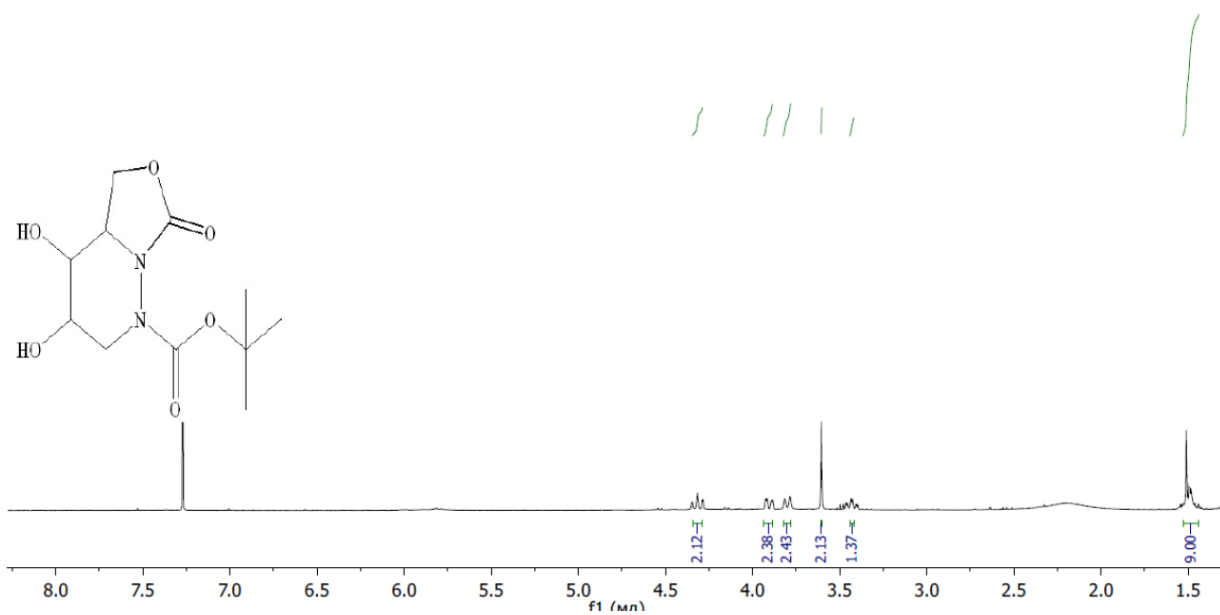


Figure S13. ^1H NMR (CDCl_3) spectrum of compound **6**

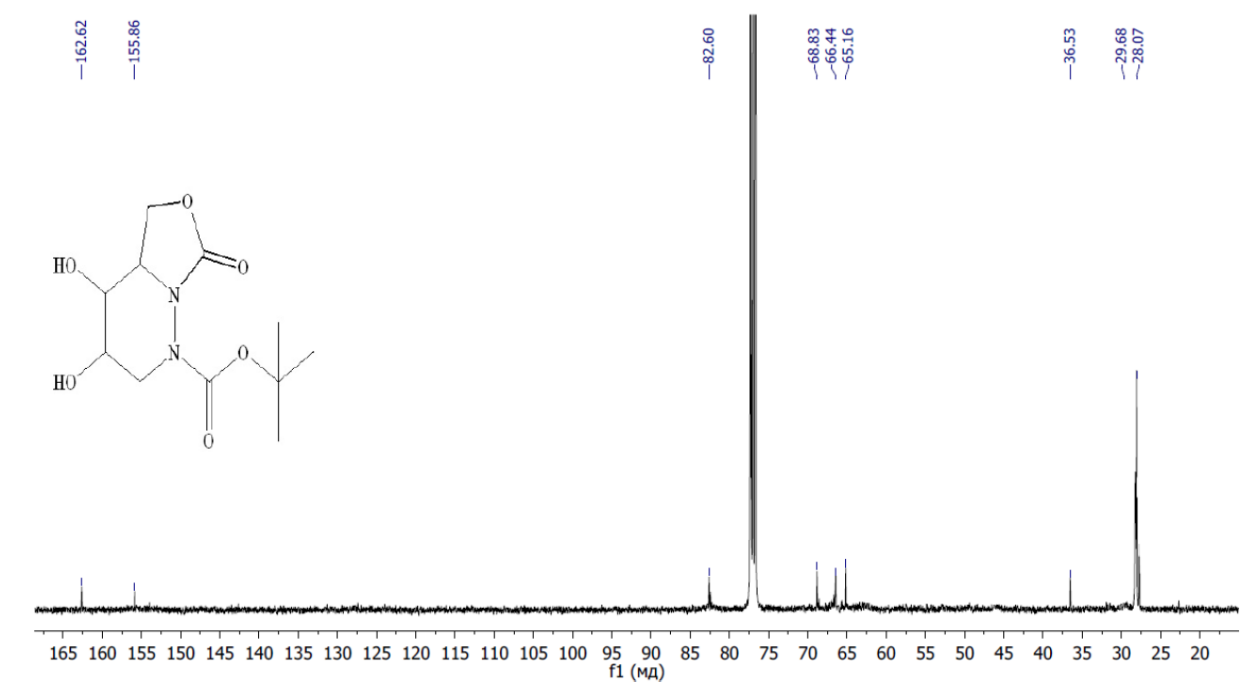


Figure S14. ^{13}C NMR (CDCl_3) spectrum of compound **6**

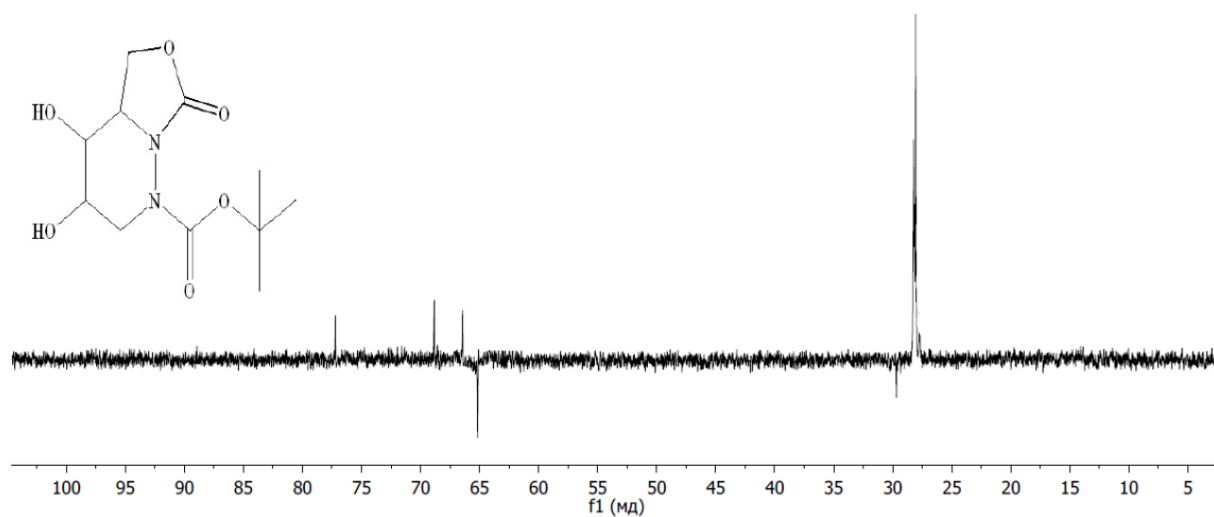
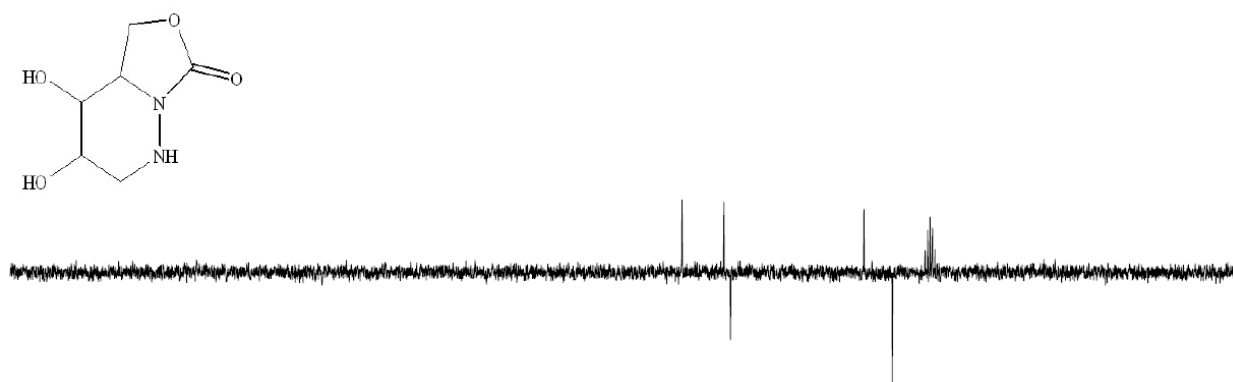
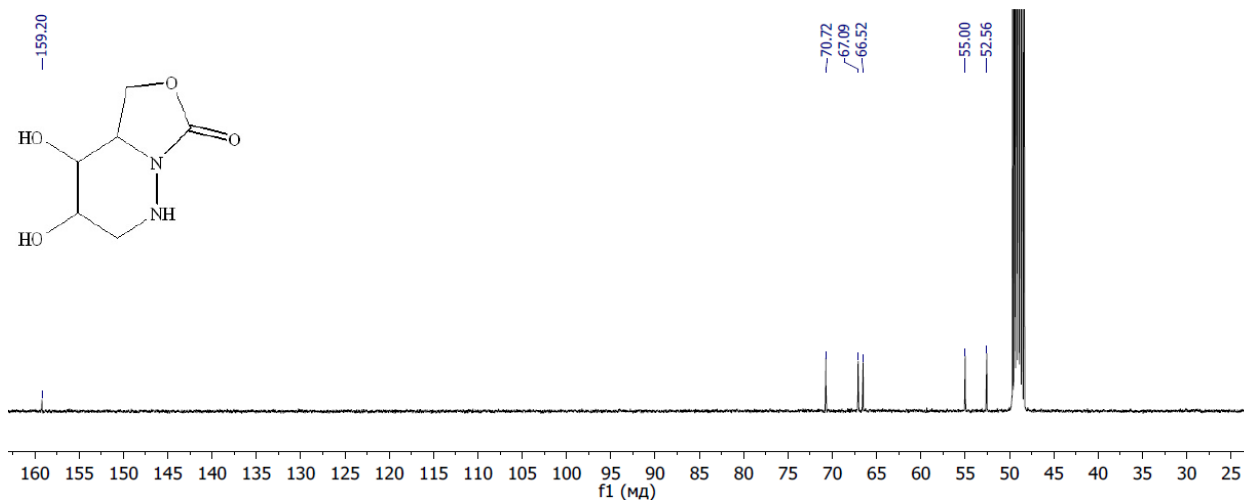
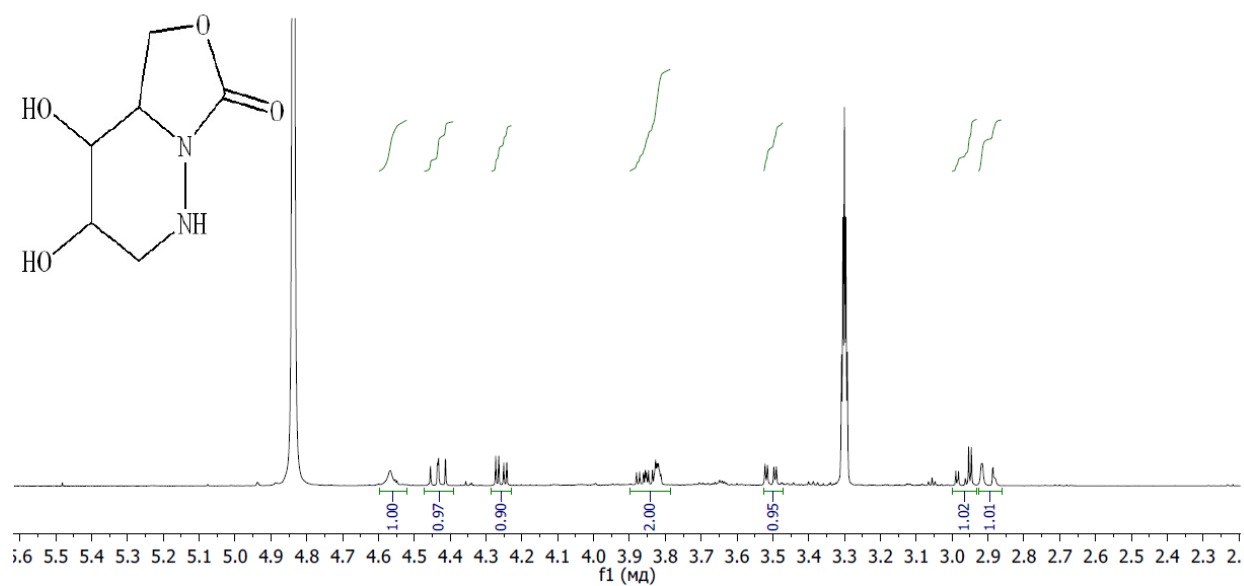


Figure S15. dept 135 NMR (CDCl_3) spectrum of compound **6**



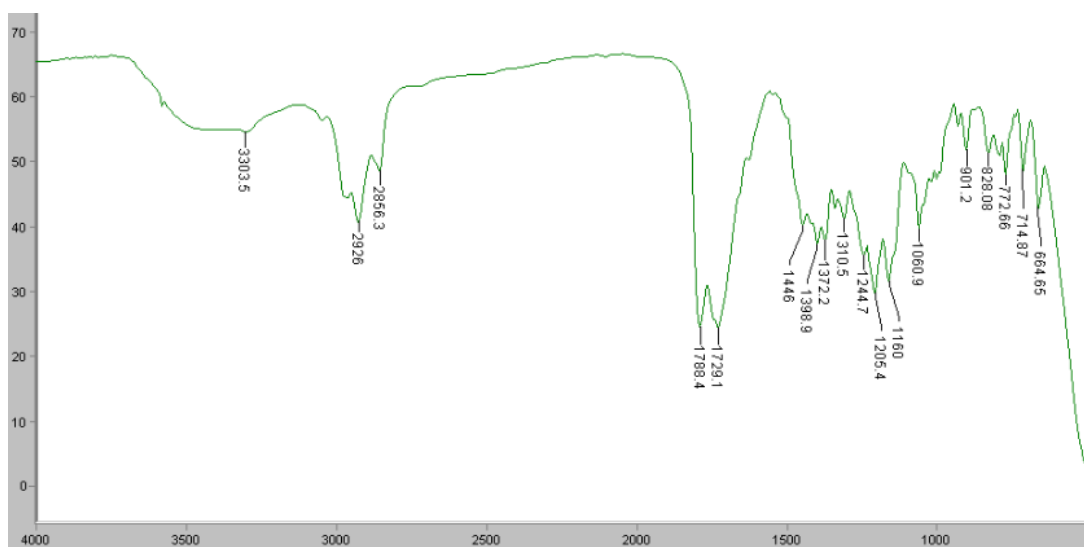


Figure S19. IR (ATR) spectrum of compound 2

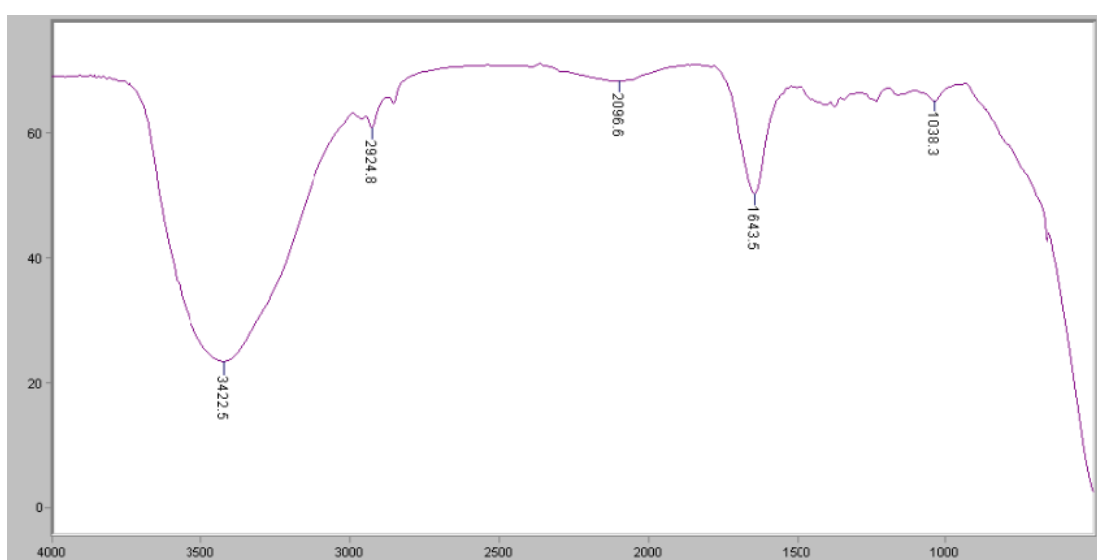


Figure S20. IR (ATR) spectrum of compound 3

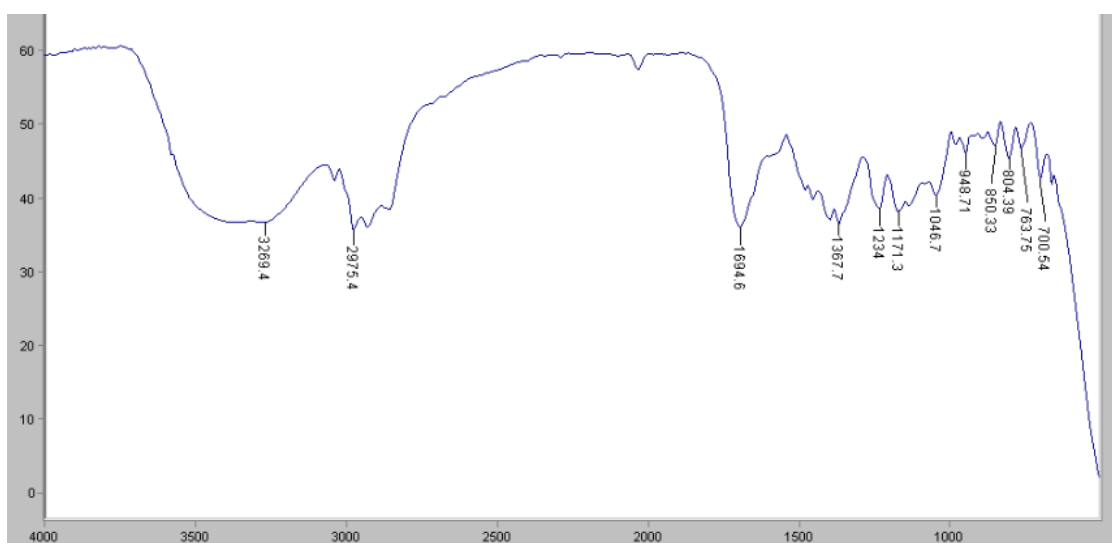


Figure S21. IR (ATR) spectrum of compound **4**

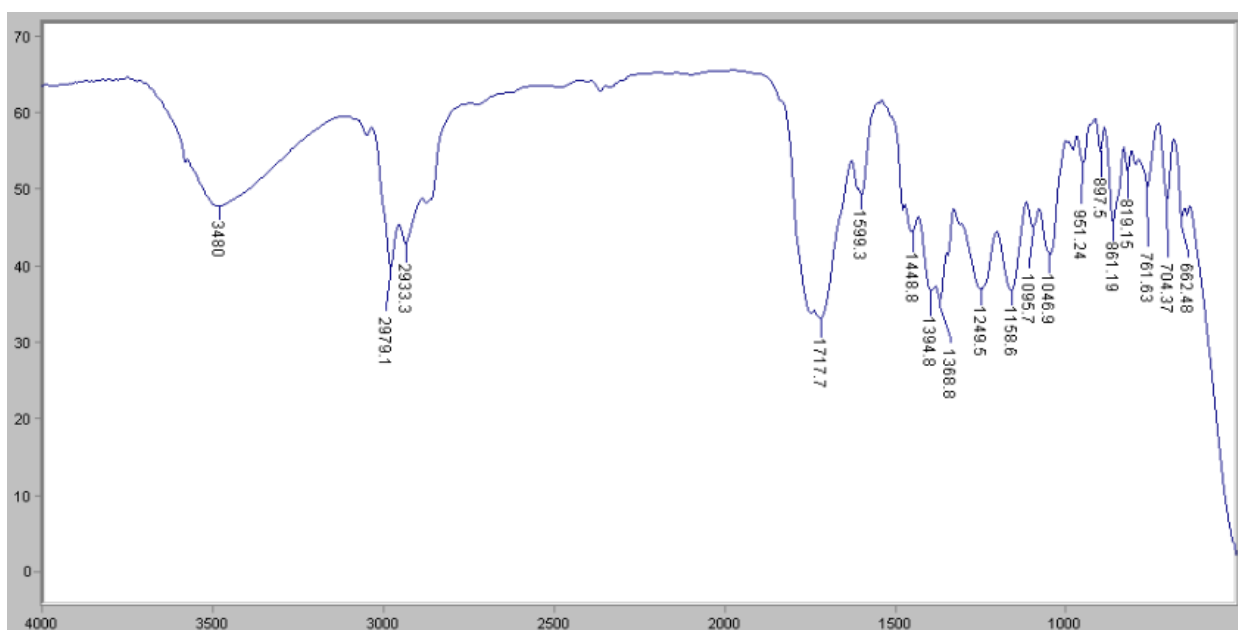


Figure S22. IR (ATR) spectrum of compound **5**

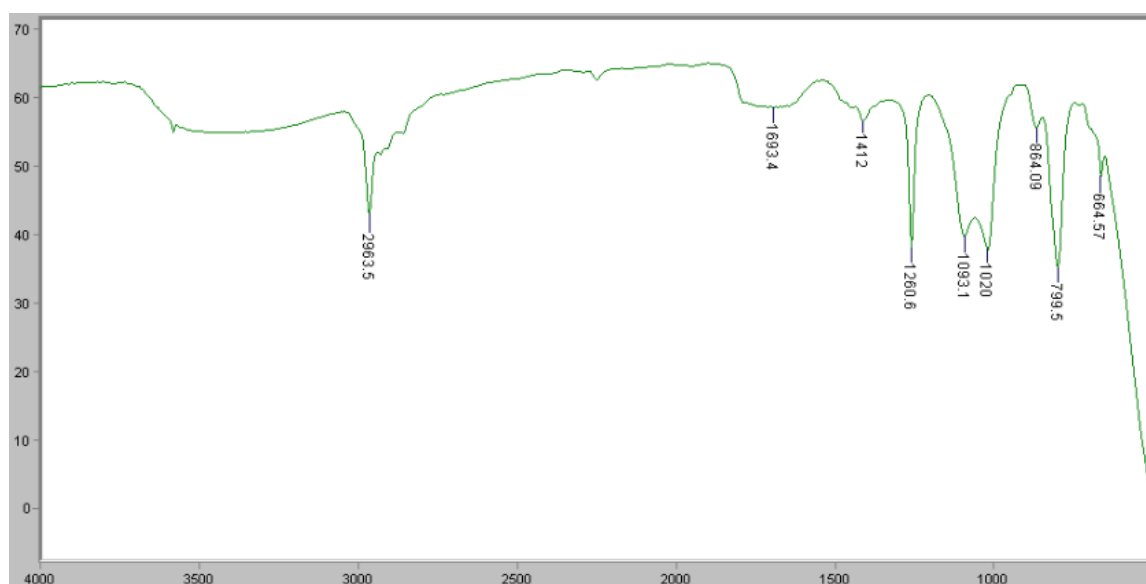


Figure S23. IR (ATR) spectrum of compound **6**

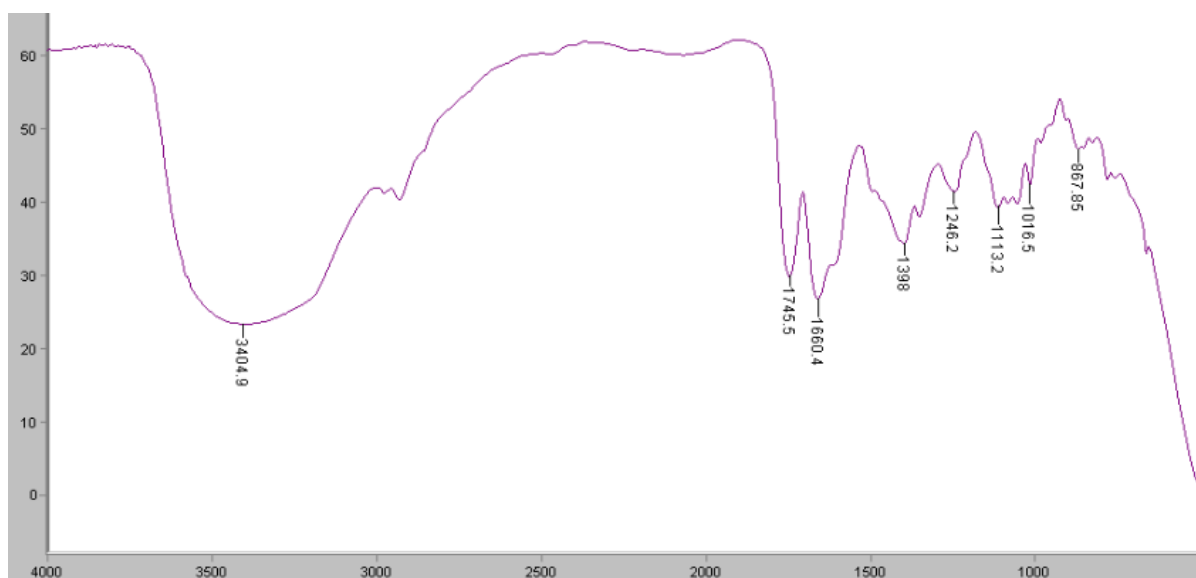


Figure S24. IR (ATR) spectrum of compound **7**

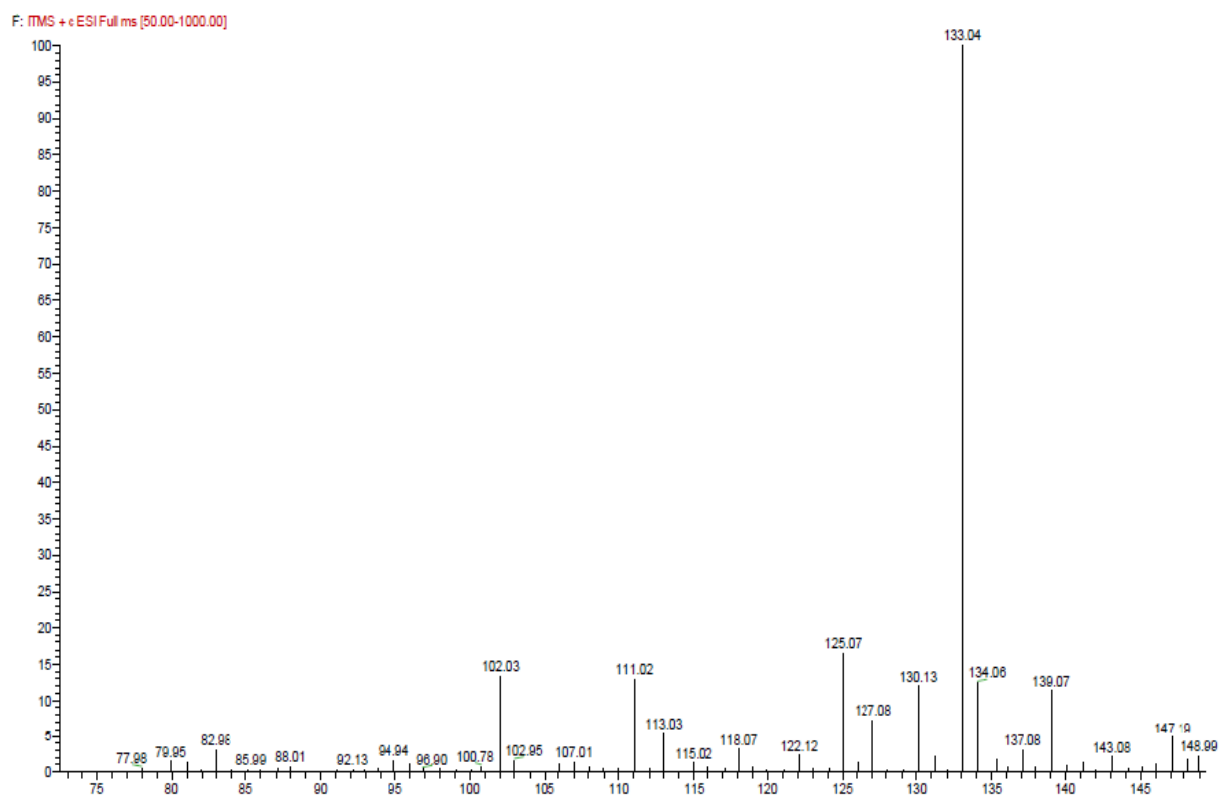


Figure S25. Mass spectra of compound **3**

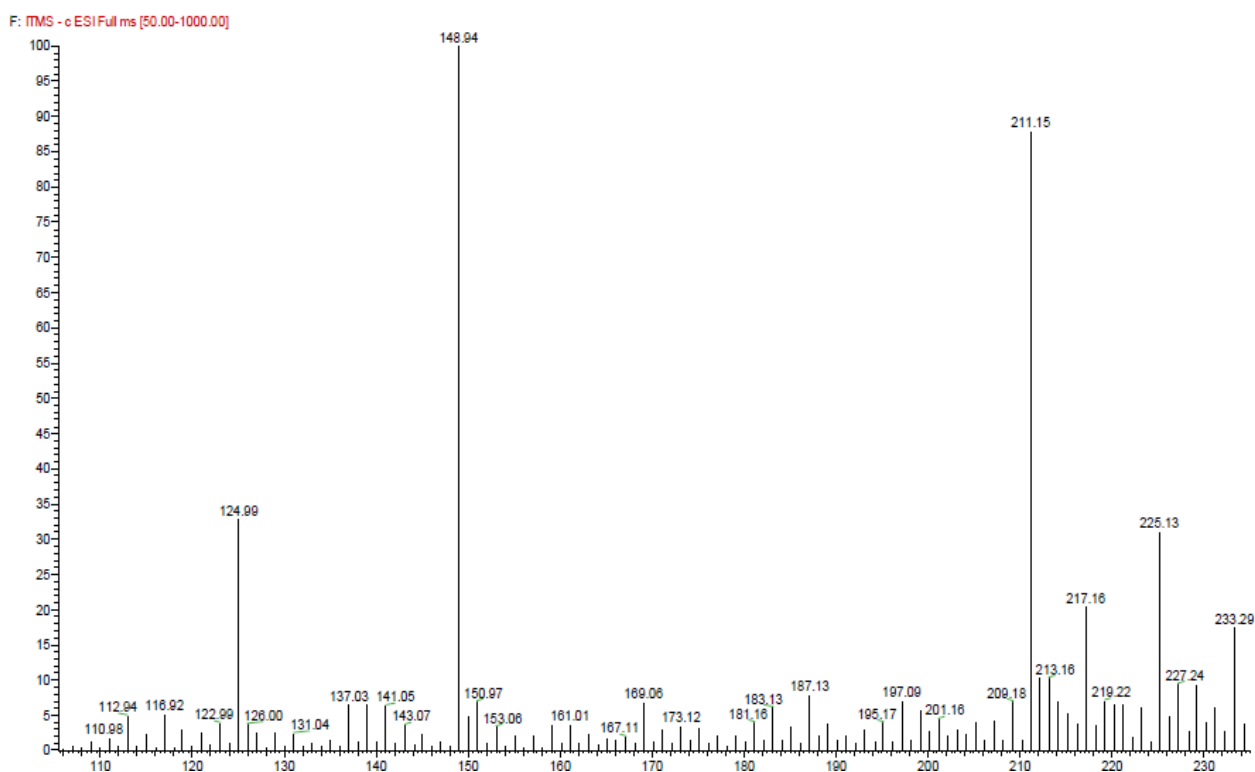


Figure S26. Mass spectra of compound **4**

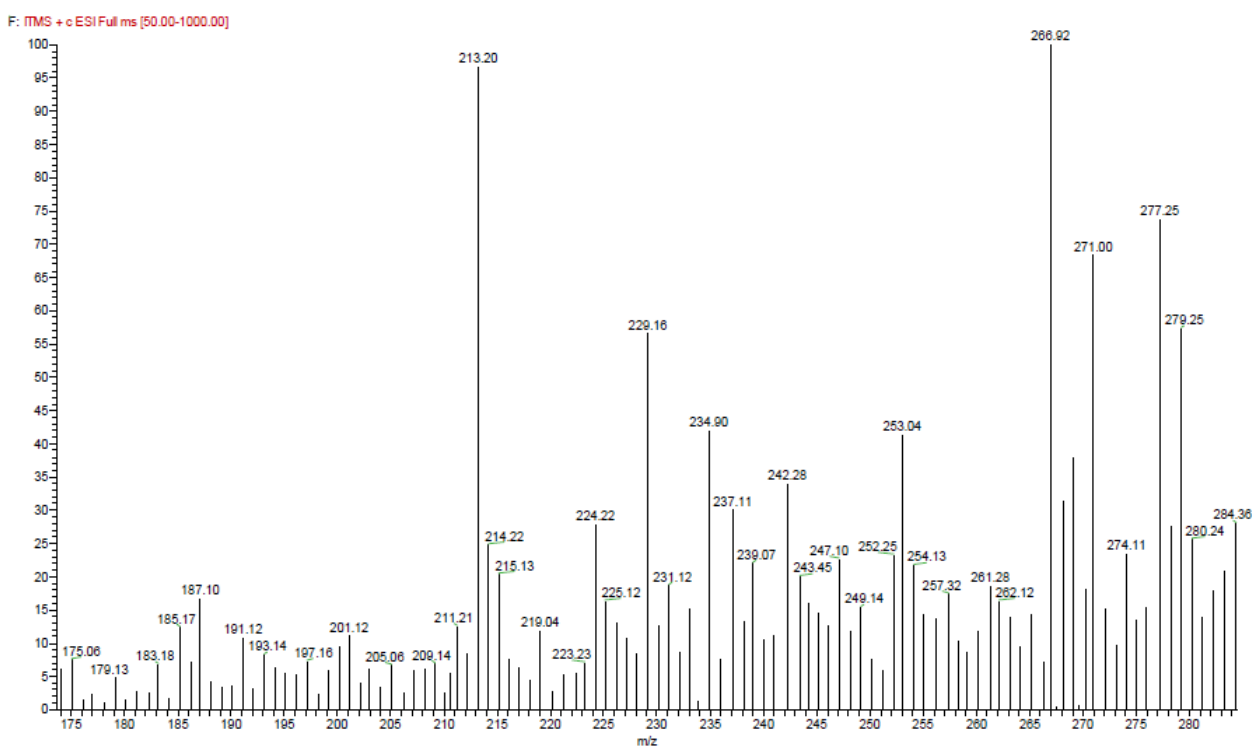
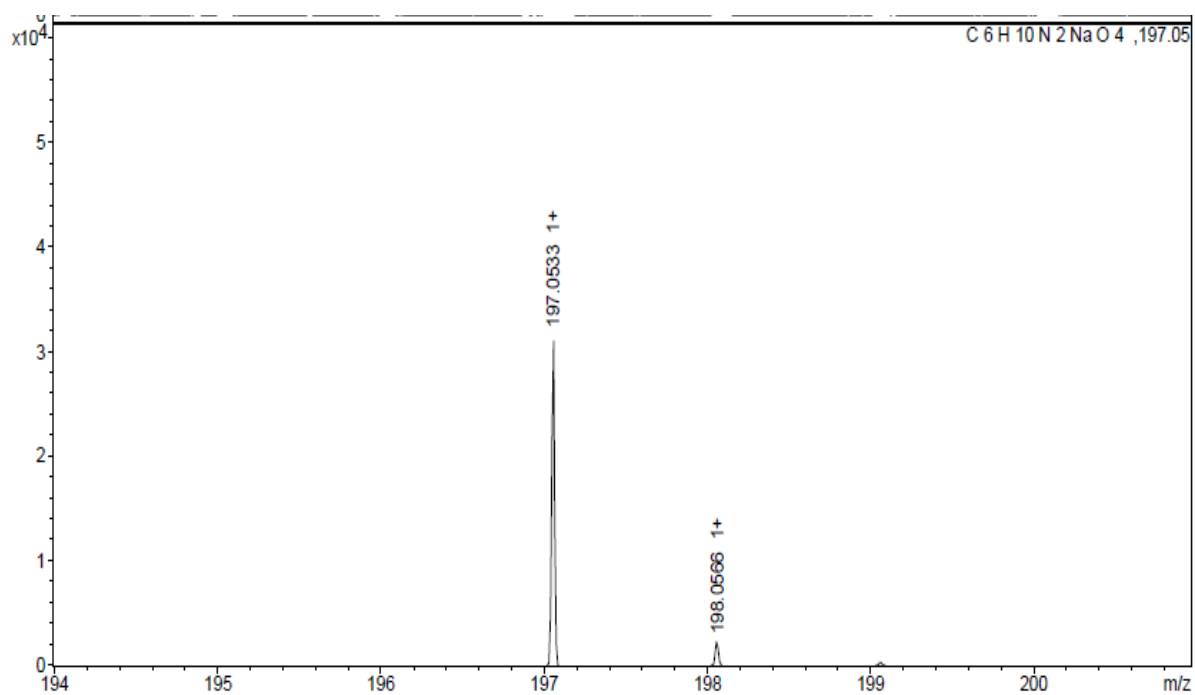


Figure S27. Mass spectra of compound **6**



Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e ⁻	Conf	N-Rule
197.0527	1	C ₆ H ₁₀ N ₂ NaO ₄	100.00	197.0533	0.6	3.1	10.5	2.5	even		ok

Figure S28. Mass spectra of compound 7