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Antitumor activity of phaeosphaeride A modified with nitrogen heterocyclic groups

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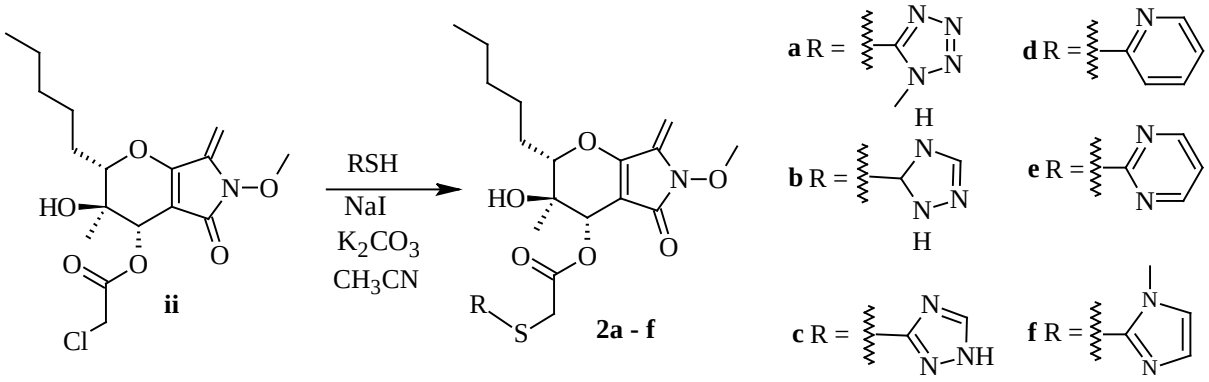
I. General Remarks

^{1}H NMR spectra were acquired on a Bruker AVANCE III 400 MHz NMR spectrometer in DMSO-d_6. The same solvent was used as an internal standard. *J*-values are given in Hz. Multiplicities are given as: s (singlet), d (doublet), t (triplet) and m (multiplet). 2-D COSY, HSQC, HMBC and ROESY experiments were carried out to aid assignment and establish the relative stereochemistry where appropriate. Optical rotations were acquired on an Optical Activity Polaar 3005 Polarimeter using a 2.5 cm cell with a Na 589 nm filter and the concentration of samples was denoted as *c*. Mass spectra data were acquired on a Thermo Scientific TSQ Quantum Access Max Mass spectrometer. High-resolution mass spectra (HRMS) were acquired on a LTQ Orbitrap Velos spectrometer. FTIR spectra were acquired on a Shimadzu IR Affinity-1 spectrometer. Organic solvents used were dried by standard methods when necessary. Commercially available reagents were used without further purification. All reactions were monitored by TLC with EMD/Merck KGaA silica gel coated plates, with visualization by UV light and by charring with 0.1% ninhydrine in EtOH. Column chromatography was performed using Merck 60 Å 70-230 mesh silica gel. Absorbance data obtained during cytotoxicity assessment was Acquired on a Multiscan Ascent microplate

photometer when using the MTT assay, and on an Epoch microplate spectrophotometer when using the SRB assay.

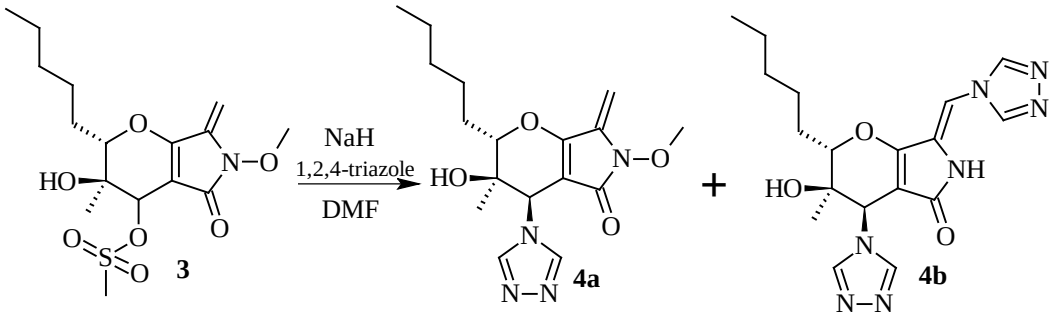
The synthesis of compounds **1** and **3** was reported previously[7,10].

II. General procedure for the synthesis of 2a-f



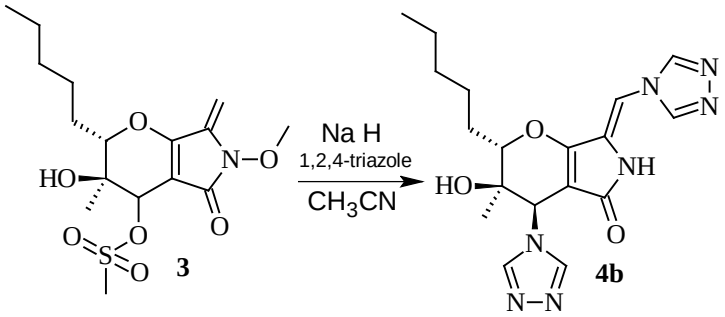
Potassium carbonate (0.11 mmol, 1.5 equiv) and NaI (0.007 mmol, 0.1 equiv) were added to a solution of the corresponding thiol (0.07 mmol, 1 equiv) in acetonitrile (20 mL). The reaction mixture was heated at 55°C for 10-15 min, and O-(chloroacetyl)phaeosphaeride (0.1 mmol, 1.4 equiv) was added. Thereafter, the mixture was heated at 55°C for 2 h. Dichloromethane (10 mL) was added to the red-brown solution, and the resulting salts were filtered off. The solvent was removed under vacuum, and the residue was purified by column chromatography on SiO_2 (DCM−methanol, 100:1).

III. Synthesis of 4a and 4b



Sodium hydride (60% dispersion, 0.789 mmol) was added to a solution of 1,2,4-triazole (0.412 mmol) in dry DMF. The reaction mixture was stirred at 25°C for 30 minutes. MsPPA **3** (0.343 mmol) was added, and the reaction mixture was heated at 50°C for 24 hours. The solution was cooled to 0°C, and the insoluble material was removed by filtration. The filtrate was diluted with DCM and washed with water, brine, dried over $MgSO_4$, filtered and evaporated to dryness. The residue was purified by column chromatography on SiO_2 (DCM−methanol, 100:1).

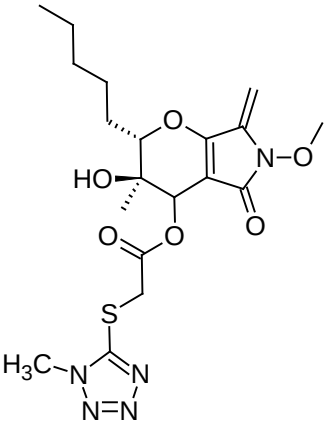
IV. Synthesis of 4b



1,2,4-Triazole (0.107 mol) and $CsCO_3$ dissolved in dry MeCN. The reaction mixture was stirred at 25°C for 30 minutes. MsPPA **3** (0.0533 mol) was added, and the reaction mixture was stirred at room temperature for 24 hours. The solution was cooled to 0°C, and the insoluble material was removed by filtration. The filtrate was diluted with DCM and washed with water, brine, dried over $MgSO_4$, filtered and evaporated to dryness. The residue was purified by column chromatography on SiO_2 (DCM−methanol, 100:1).

V. Characterization and ^{1}H NMR spectral data

(2*S*,3*S*)-3-Hydroxy-6-methoxy-3-methyl-7-methylidene-5-oxo-2-pentyl-2,3,4,5,6,7-hexahydropyrano[2,3-*c*]pyrrol-4-yl [(1-methyl-1*H*-tetrazol-5-yl)thio]acetate 2a



White powder, 12% yield. R_f 0.65 (20:1 CH_2Cl_2-MeOH). $[\alpha]_{20}^{D}$ = -34.84 (c 2.03, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.73 (s, 1H), 5.06 (d, 1.4 Hz, 1H), 5.00 (d, 1.4 Hz, 1H), 4.20 (d, *J* = 16.3 Hz, 1H), 4.12 (m, 1H), 4.08 (d, *J* = 16.4 Hz, 1H), 3.96 (s, 3H), 3.87 (s, 3H), 3.82 (br s, 1H), 1.88-1.51 (m, 3H), 1.44-1.26 (m, 5H), 1.22 (s, 3H), 0.88 (t, *J* = 6.5 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.79 (s), 164.68 (s), 158.09 (s), 153.19 (s), 136.21 (s), 100.85 (s), 92.68 (s), 86.33 (s), 70.73 (s), 64.66 (s, *J* = 5.4 Hz), 35.00 (s), 33.81 (s, *J* = 4.0 Hz), 31.53 (s), 27.36 (s), 26.08 (s), 22.59 (s), 17.03 (s), 14.09 (s). IR (KBr) 3391, 2956, 2933, 2860, 1720, 1671, 1637, 1450, 1265, 1156, 993, 737 cm^{-1}. HRMS $[M + H]^+$ calcd for $C_{19}H_{27}N_5O_6S$ 454.17548, found 454.17493.

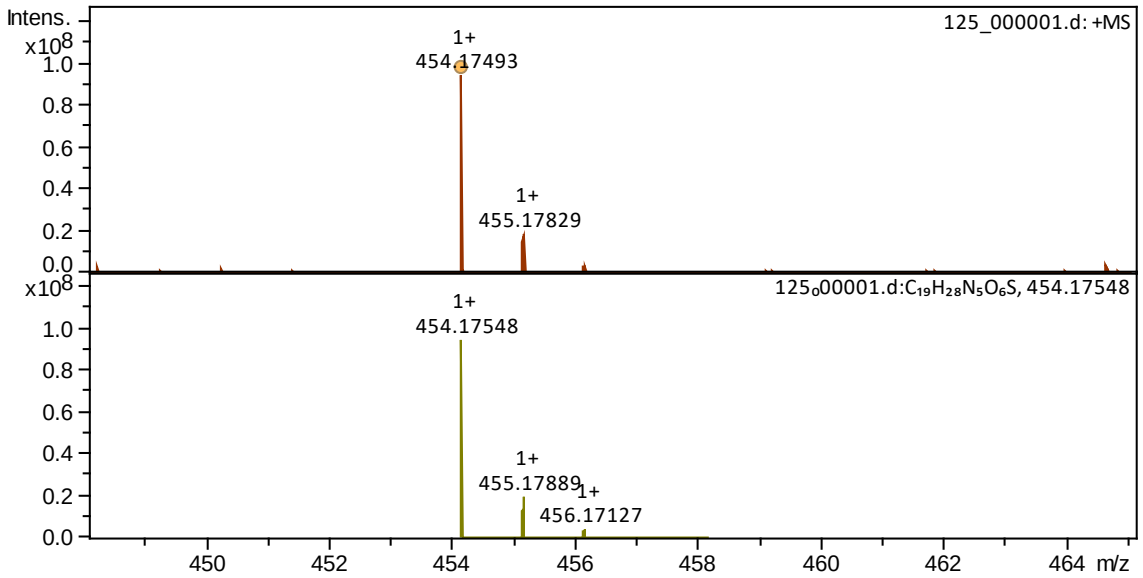


Figure S1. HRMS of (2*S*,3*S*)-3-hydroxy-6-methoxy-3-methyl-7-methylidene-5-oxo-2-pentyl-2,3,4,5,6,7-hexahydropyrano[2,3-*c*]pyrrol-4-yl [(1-methyl-1*H*-tetrazol-5yl)thio]acetate **2a**

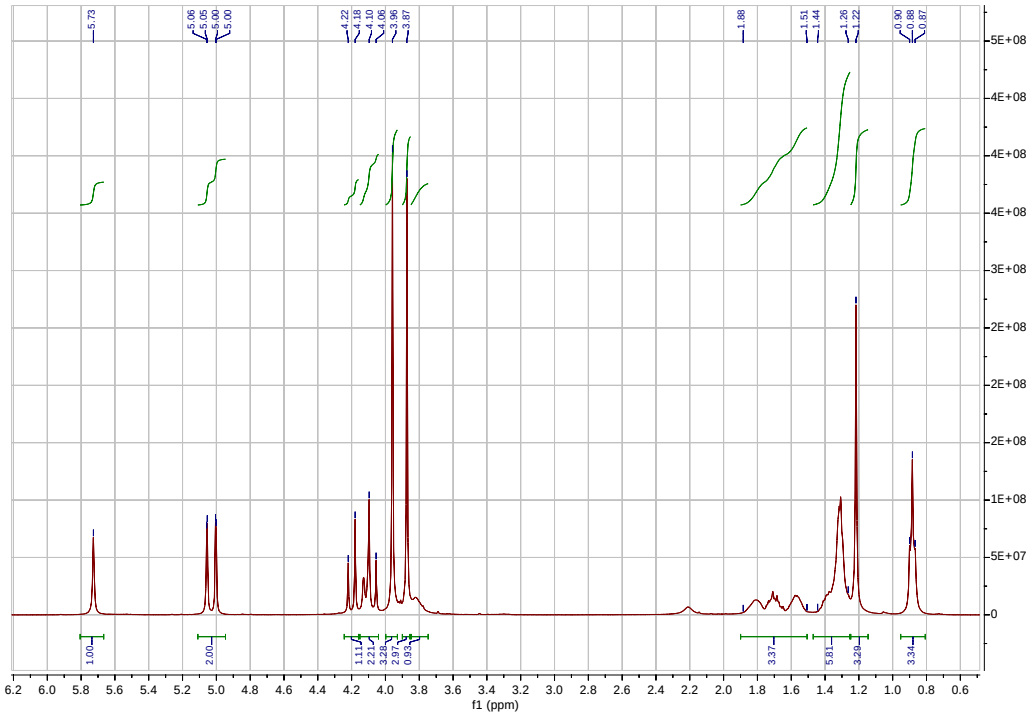


Figure S2. [1]H NMR of (2*S*,3*S*)-3-hydroxy-6-methoxy-3-methyl-7-methylidene-5-oxo-2-pentyl-2,3,4,5,6,7-hexahydropyrano[2,3-*c*]pyrrol-4-yl [(1-methyl-1*H*-tetrazol-5yl)thio]acetate **2a**

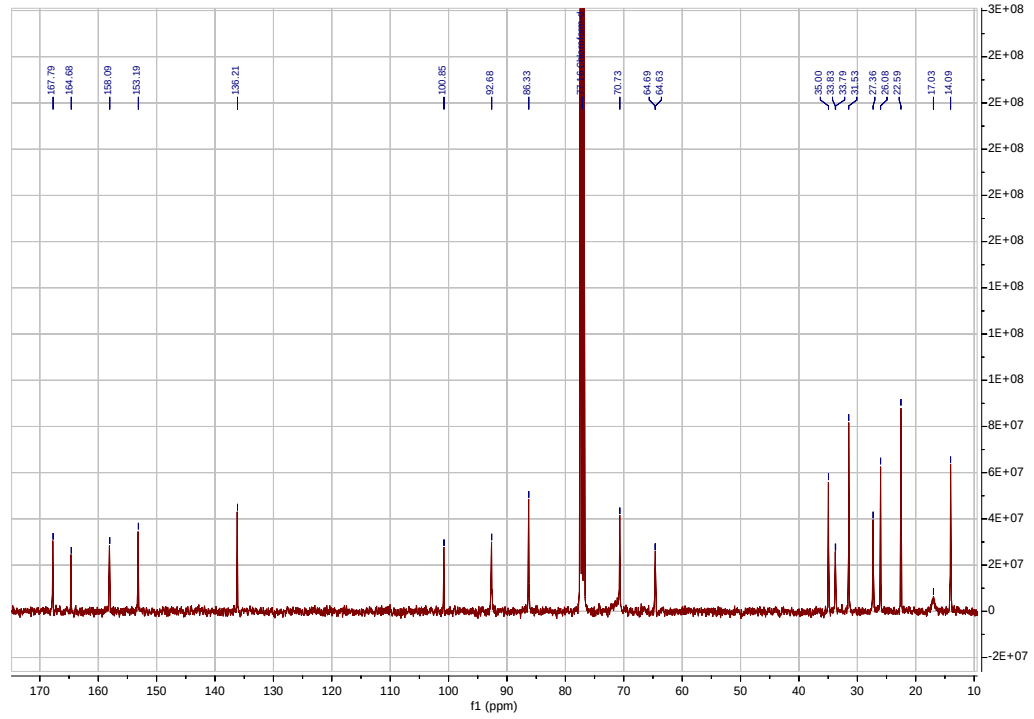


Figure S3. [13]C NMR of (2*S*,3*S*)-3-hydroxy-6-methoxy-3-methyl-7-methylidene-5-oxo-2-pentyl-2,3,4,5,6,7-hexahydropyrano[2,3-*c*]pyrrol-4-yl [(1-methyl-1*H*-tetrazol-5yl)thio]acetate **2a**

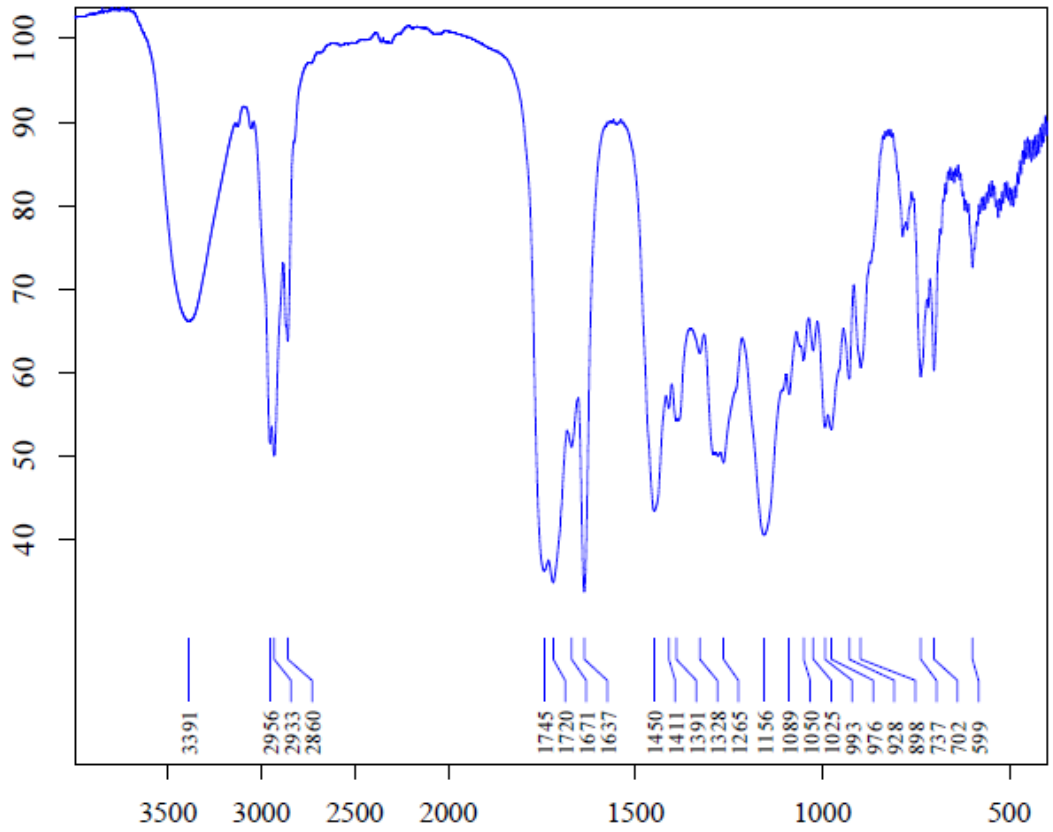
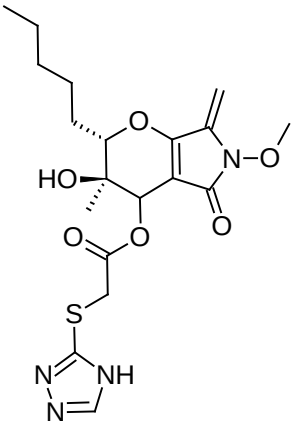


Figure S4. IR spectrum of (2*S*,3*S*)-3-hydroxy-6-methoxy-3-methyl-7-methylidene-5-oxo-2-pentyl-2,3,4,5,6,7-hexahydropyrano[2,3-*c*]pyrrol-4-yl [(1-methyl-1*H*-tetrazol-5yl)thio]acetate **2a**

(2*S*,3*S*)-3-Hydroxy-6-methoxy-3-methyl-7-methylidene-5-oxo-2-pentyl-2,3,4,5,6,7-hexahydropyrano[2,3-c]pyrrol-4-yl (4*H*-1,2,4-triazol-3-ylthio)acetate 2b



Orange oil, 12% yield. R_f 0.61 (20:1 CH_2Cl_2-MeOH). $[\alpha]_{20}^{D}$ = -58.31 (c 2.03, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (s, 1H), 5.76 (s, 1H), 5.11 (s, 1H), 5.07 (s, 1H), 4.14-4.11 (m, 1H), 3.92 (s, 3H), 3.92-3.78 (m, 3H), 1.90 – 1.55 (m, 3H), 1.48-1.29 (m, 5H), 1.22 (s, 3H), 0.91 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.52 (s), 164.93 (s), 158.29 (s), 154.16 (s), 148.25 (s), 136.10 (s), 101.10 (s), 93.26 (s), 86.36 (s), 71.04 (s), 64.84 (s), 53.57 (s), 35.52 (s), 31.62 (s), 27.44 (s), 26.11 (s), 22.66 (s), 16.84 (s), 14.16 (s). IR (KBr) 3404, 2956, 2932, 2860, 1734, 1636, 1451, 1379, 1277, 1145, 977, 720 cm^{-1}. HRMS $[M + H]^+$ calcd for $C_{19}H_{26}N_4O_6S$ 439.16558, found 439.16420.

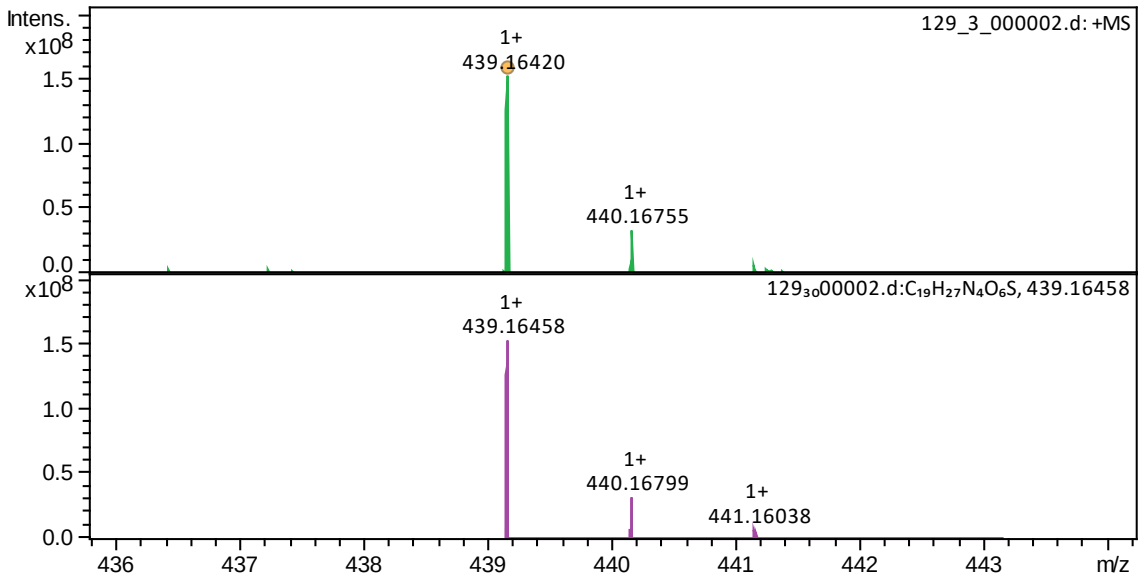


Figure S5. HRMS of (2*S*,3*S*)-3-hydroxy-6-methoxy-3-methyl-7-methylidene-5-oxo-2-pentyl-2,3,4,5,6,7-hexahydropyrano[2,3-*c*]pyrrol-4-yl (4*H*-1,2,4-triazol-3-ylthio)acetate **2b**

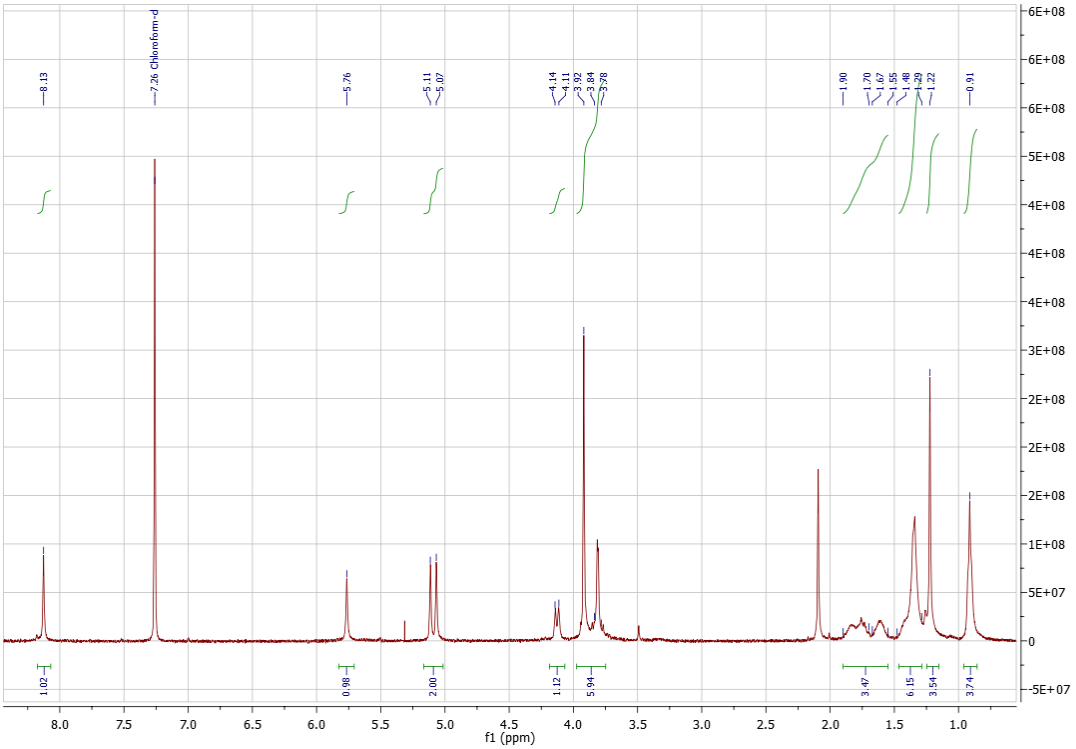


Figure S6. [1]H NMR of (2*S*,3*S*)-3-hydroxy-6-methoxy-3-methyl-7-methylidene-5-oxo-2-pentyl-2,3,4,5,6,7-hexahydropyrano[2,3-*c*]pyrrol-4-yl (4*H*-1,2,4-triazol-3-ylthio)acetate **2b**

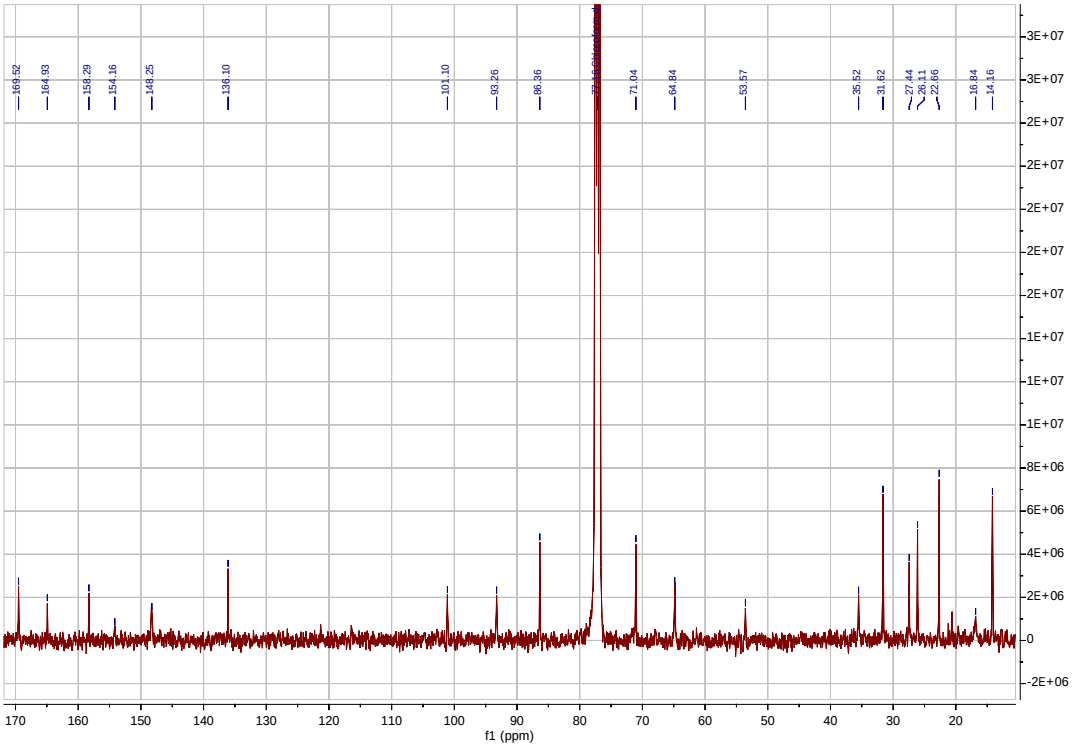


Figure S7. [13]C NMR of (2*S*,3*S*)-3-hydroxy-6-methoxy-3-methyl-7-methylidene-5-oxo-2-pentyl-2,3,4,5,6,7-hexahydropyrano[2,3-*c*]pyrrol-4-yl (4*H*-1,2,4-triazol-3-ylthio)acetate **2b**

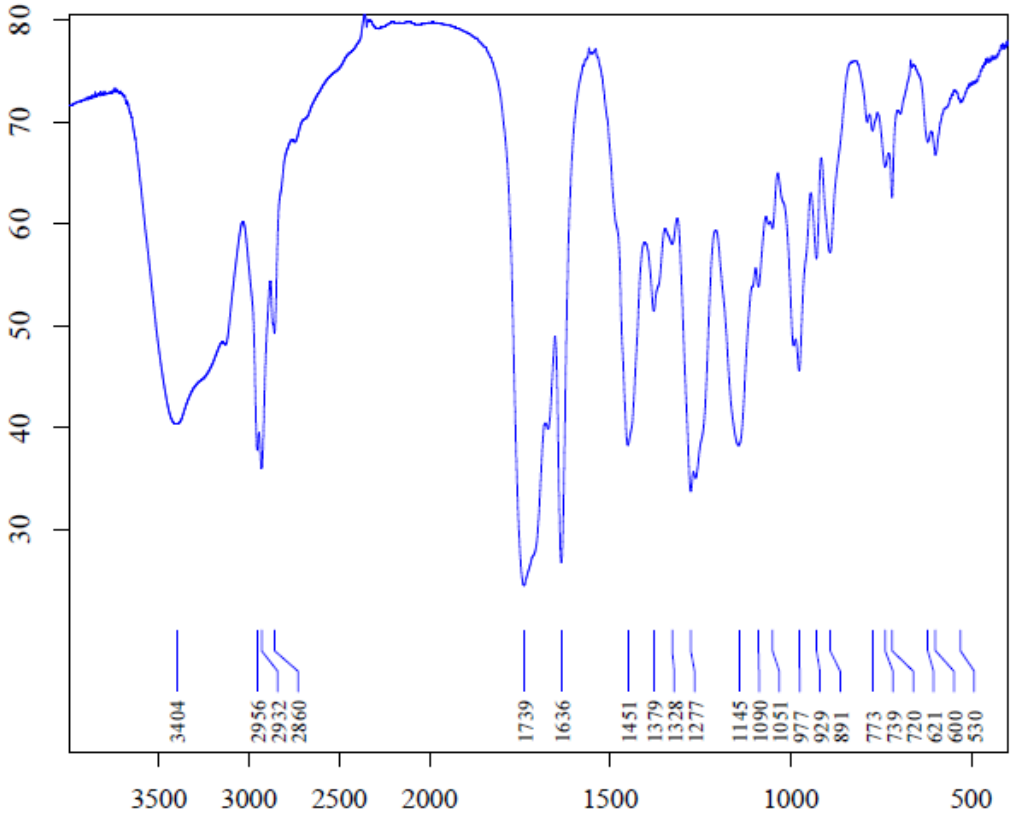
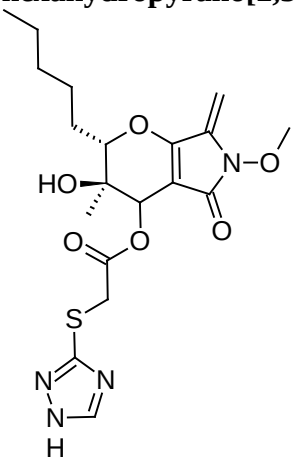


Figure S8. IR spectrum of (2*S*,3*S*)-3-hydroxy-6-methoxy-3-methyl-7-methylidene-5-oxo-2-pentyl-2,3,4,5,6,7-hexahydropyrano[2,3-*c*]pyrrol-4-yl (4*H*-1,2,4-triazol-3-ylthio)acetate **2b**

(2*S*,3*S*)-3-Hydroxy-6-methoxy-3-methyl-7-methylidene-5-oxo-2-pentyl-2,3,4,5,6,7-hexahydropyrano[2,3-*c*]pyrrol-4-yl (1*H*-1,2,4-triazol-3-ylthio)acetate 2c



Colorless oil, 15% yield. R_f 0.62 (20:1 CH_2Cl_2-MeOH). $[\alpha]_{20}^{D}$= -57.29 (c 2.03, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (s, 1H), 6.6 (br s, 1H), 5.75 (s, 1H), 5.10 (s, 1H), 5.04 (s, 1H), 4.14 (m, 1H), 3.88 (s, 3H), 3.84 (s, 2H), 1.86-1.68 (m, 2H), 1.65-1.51 (m, 1H), 1.45-1.26 (m, 5H), 1.20 (s, 3H), 0.90 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.57 (s), 165.18 (s), 158.26 (s), 155.37 (s), 147.25 (s), 136.14 (s), 101.02 (s), 93.26 (s), 86.46 (s), 70.92 (s), 64.76 (s, *J* = 4.9 Hz), 35.15 (s), 31.60 (s), 27.50 (s), 26.16 (s), 22.66 (s), 14.14 (s).). IR (KBr) 3296, 2925, 2854, 1721, 1665, 1637, 1453, 1262, 1143, 994, 799 cm^{-1}. HRMS $[M + H]^+$ calcd for $C_{19}H_{26}N_4O_6S$ 439.16458, found 439.16407.

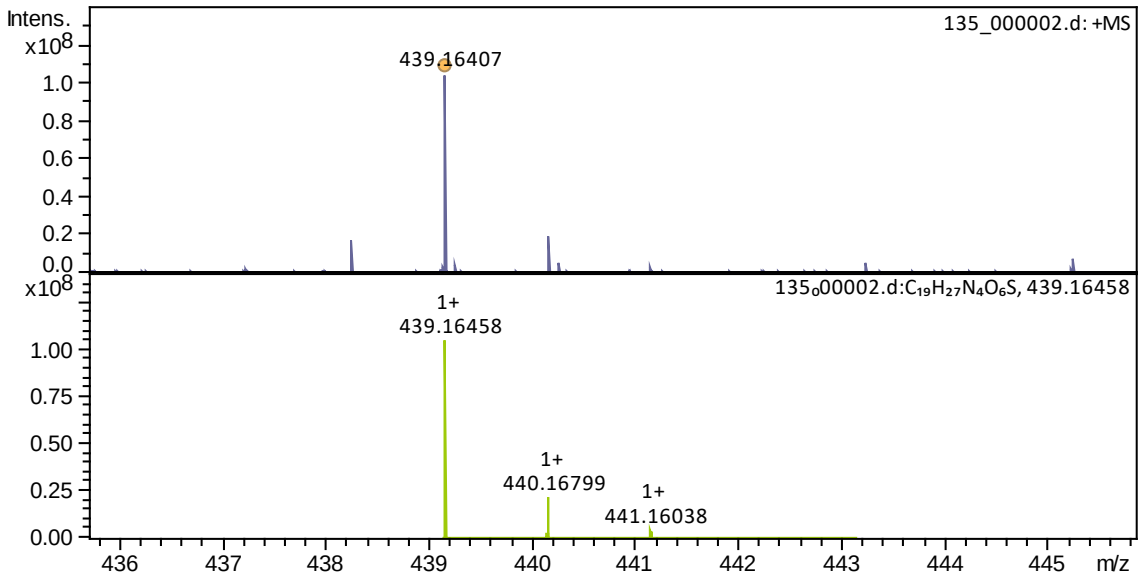


Figure S9. HRMS of (2*S*,3*S*)-3-hydroxy-6-methoxy-3-methyl-7-methylidene-5-oxo-2-pentyl-2,3,4,5,6,7-hexahydropyrano[2,3-*c*]pyrrol-4-yl (1*H*-1,2,4-triazol-3-ylthio)acetate **2c**

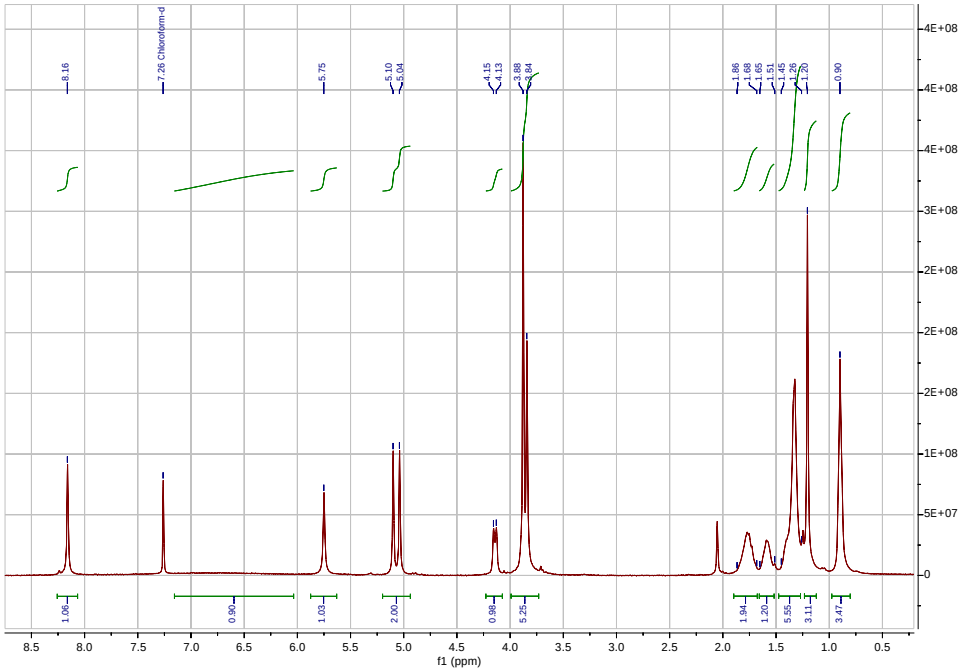


Figure S10. ^{1}H NMR of (2*S*,3*S*)-3-hydroxy-6-methoxy-3-methyl-7-methylidene-5-oxo-2-pentyl-2,3,4,5,6,7-hexahydropyrano[2,3-*c*]pyrrol-4-yl (1*H*-1,2,4-triazol-3-ylthio)acetate **2c**

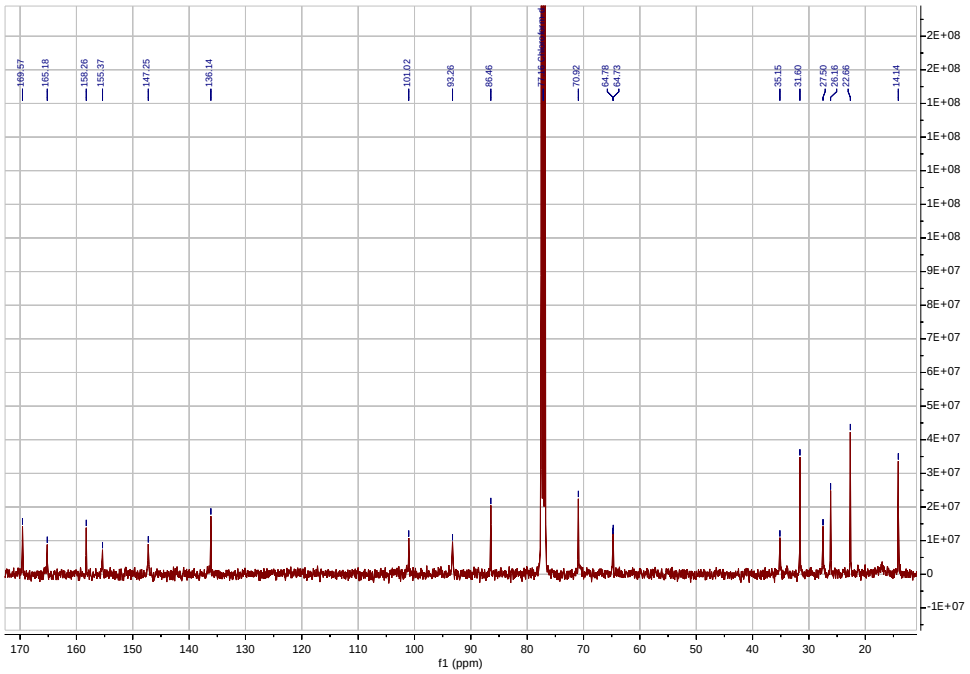


Figure S11. ^{13}C NMR of (2*S*,3*S*)-3-hydroxy-6-methoxy-3-methyl-7-methylidene-5-oxo-2-pentyl-2,3,4,5,6,7-hexahydropyrano[2,3-*c*]pyrrol-4-yl (1*H*-1,2,4-triazol-3-ylthio)acetate **2c**

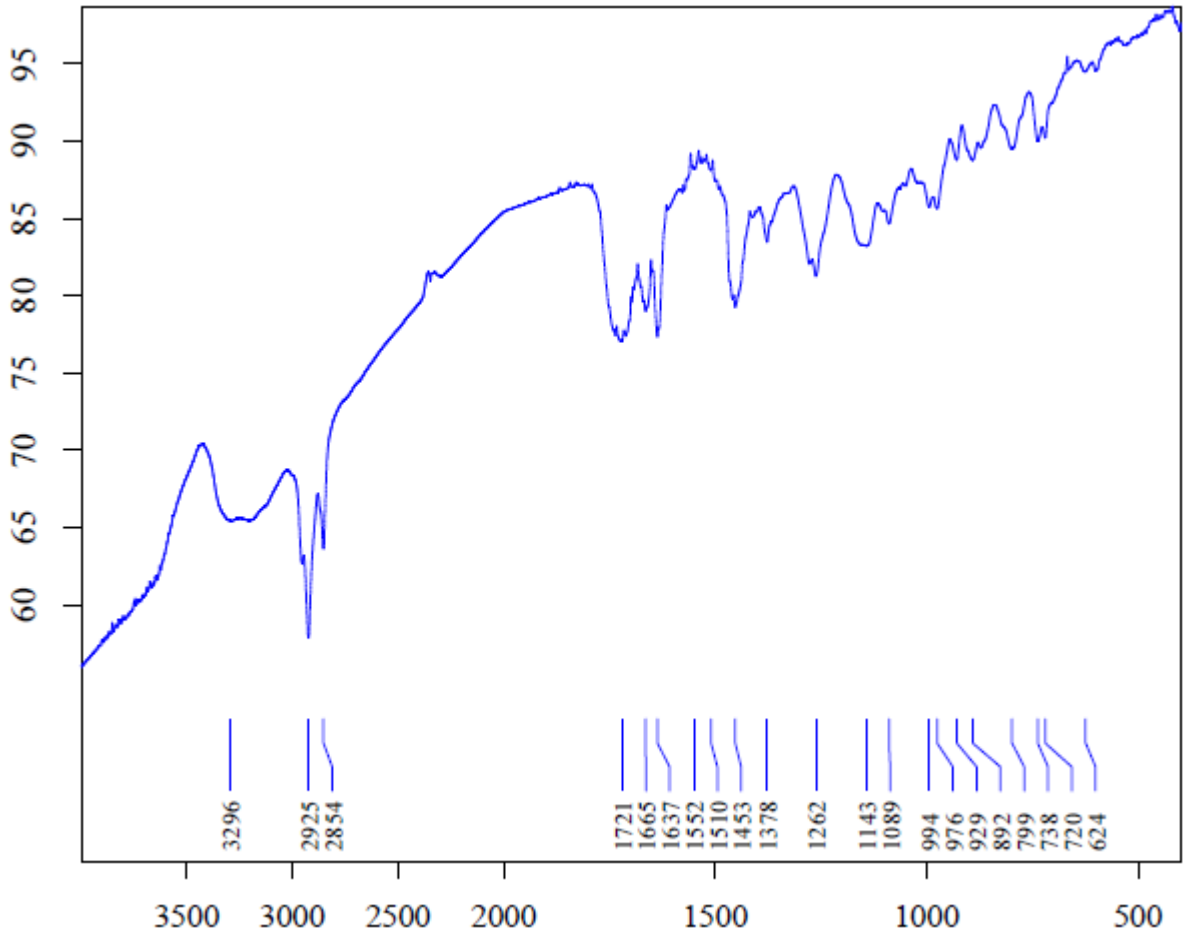
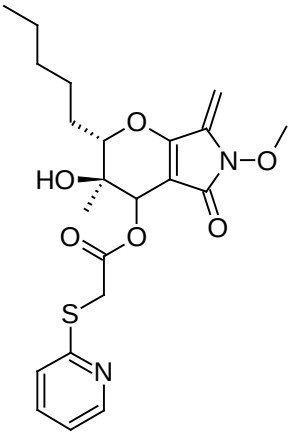


Figure S12. IR spectrum of (2*S*,3*S*)-3-hydroxy-6-methoxy-3-methyl-7-methylidene-5-oxo-2-pentyl-2,3,4,5,6,7-hexahydropyrano[2,3-*c*]pyrrol-4-yl (1*H*-1,2,4-triazol-3-ylthio)acetate **2c**

(2*S*,3*S*)-3-Hydroxy-6-methoxy-3-methyl-7-methylidene-5-oxo-2-pentyl-2,3,4,5,6,7-hexahydropyrano[2,3-*c*]pyrrol-4-yl (pyridin-2-ylthio)acetate 2d



Yellow oil, 12% yield. R_f 0.63 (20:1 CH_2Cl_2-MeOH). $[\alpha]_{20}^{D}$= -71.96 (c 2.03, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39-8.37 (m, 1H), 7.53-7.48 (m, 1H), 7.24 (m, 1H), 7.03-6.98 (m, 1H), 5.06 (d, *J* = 1.6 Hz, 1H), 5.02 (d, *J* = 1.6 Hz, 1H), 4.08-4.04 (m, 1H), 4.06 (d, *J* = 16.3 Hz, 2H), 3.96 (s, 3H), 1.78-1.70 (m, 2H), 1.43-1.29 (m, 6H), 1.20 (s, 3H), 0.90 (t, *J* = 6.3 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.63 (s), 164.66 (s), 158.13 (s), 157.02 (s), 149.58 (s), 136.36 (s, *J* = 11.2 Hz), 122.29 (s), 120.12 (s), 101.09 (s), 92.25 (s), 86.33 (s), 71.28 (s), 64.68 (s, *J* = 4.3 Hz), 32.75 (s), 31.57 (s), 29.82 (s), 27.51 (s), 26.20 (s), 22.62 (s), 17.50 (s), 14.14 (s). IR (KBr) 3294, 2931, 2859, 1727, 1667, 1638, 1579, 1454, 1416, 1262, 1123, 1023,893 cm^{-1}. HRMS $[M + H]^+$ calcd for $C_{22}H_{28}N_2O_6S$ 449.17408, found 449.17412.

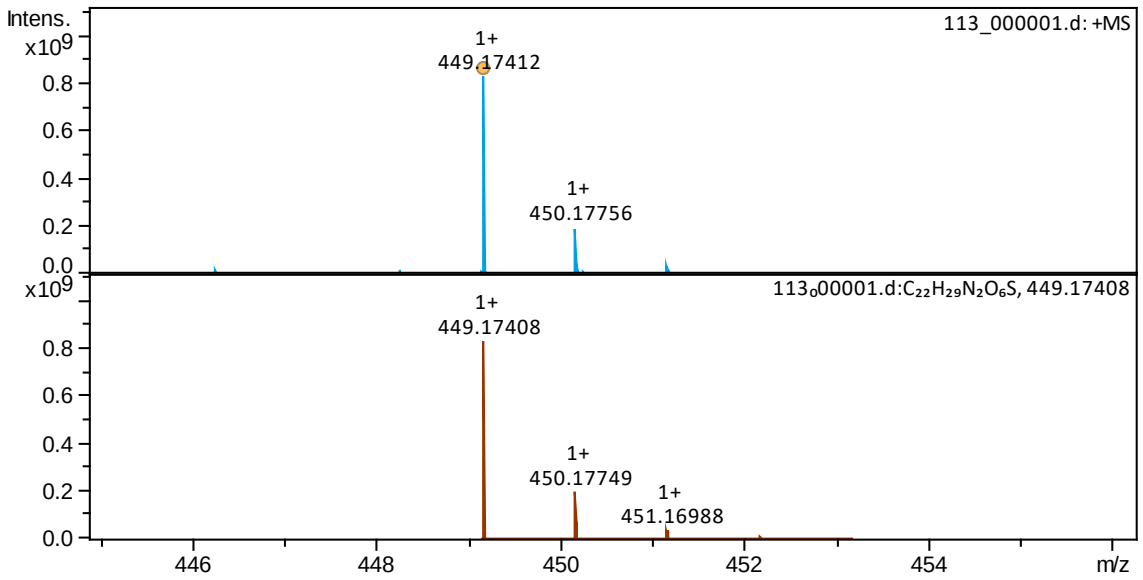


Figure S13. HRMS of (2*S*,3*S*)-3-hydroxy-6-methoxy-3-methyl-7-methylidene-5-oxo-2-pentyl-2,3,4,5,6,7-hexahydropyrano[2,3-*c*]pyrrol-4-yl (pyridin-2-ylthio)acetate **2d**

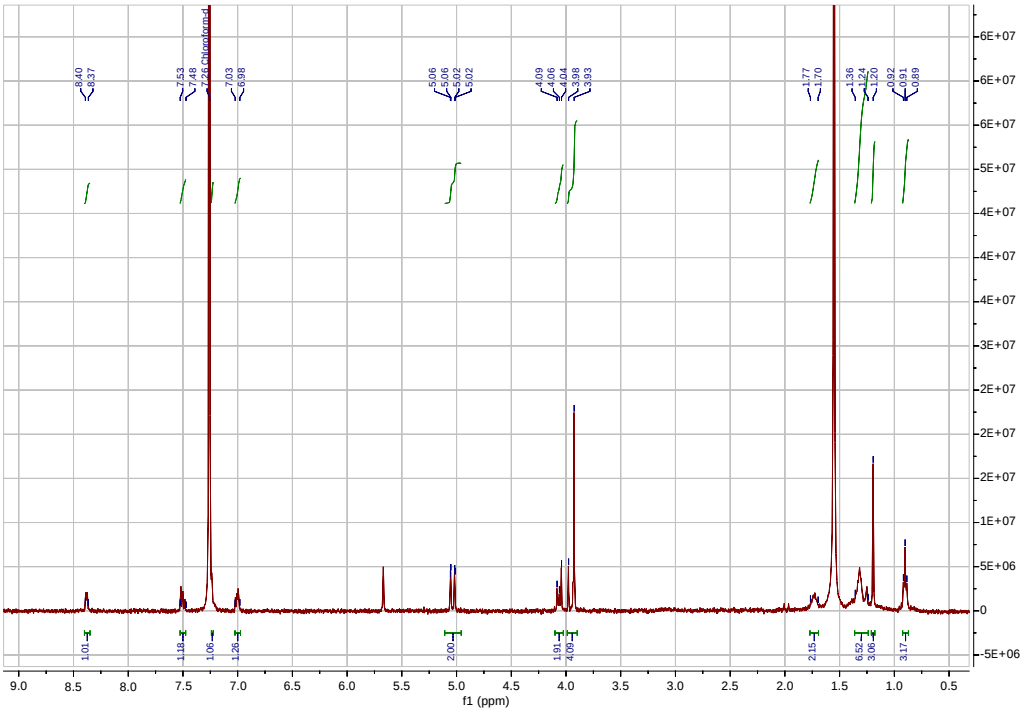


Figure S14. [1]H NMR of (2*S*,3*S*)-3-hydroxy-6-methoxy-3-methyl-7-methylidene-5-oxo-2-pentyl-2,3,4,5,6,7-hexahydropyrano[2,3-*c*]pyrrol-4-yl (pyridin-2-ylthio)acetate **2d**

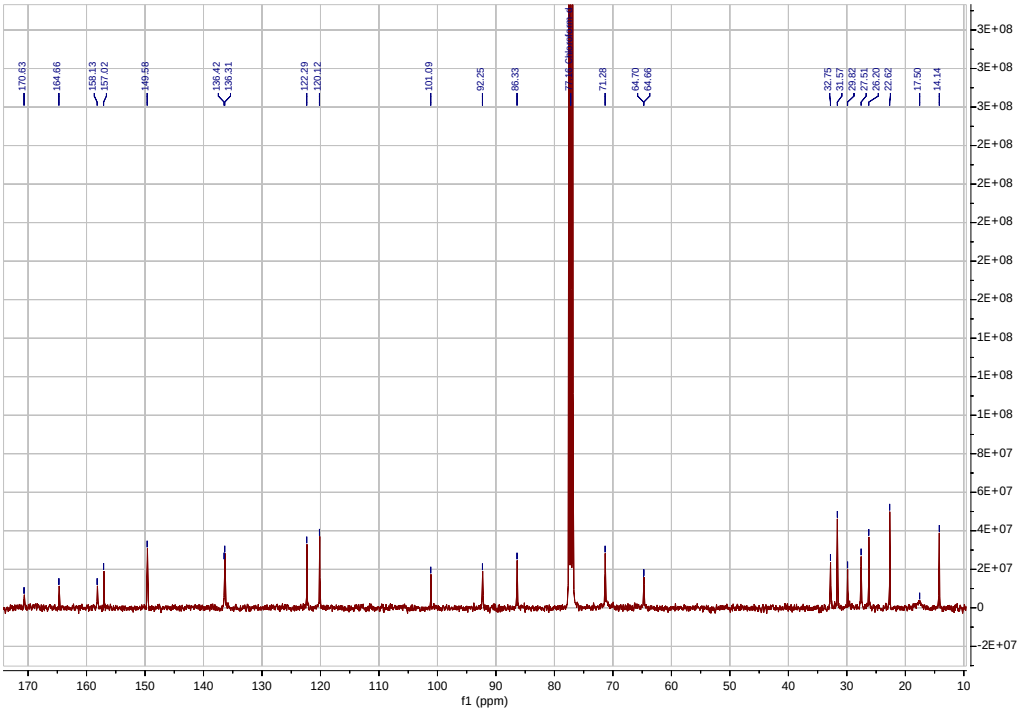


Figure S15. [13]C NMR of (2*S*,3*S*)-3-hydroxy-6-methoxy-3-methyl-7-methylidene-5-oxo-2-pentyl-2,3,4,5,6,7-hexahydropyrano[2,3-*c*]pyrrol-4-yl (pyridin-2-ylthio)acetate **2d**

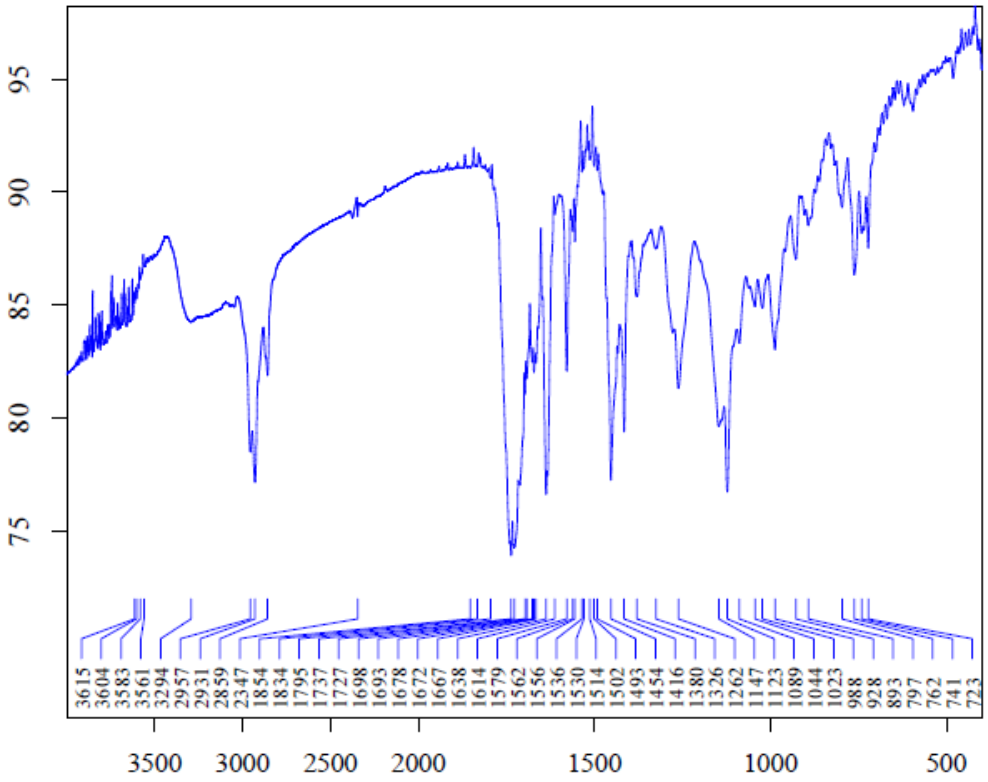
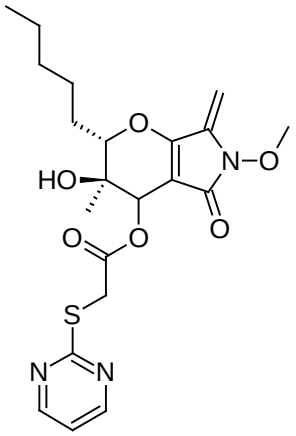


Figure S16. IR spectrum of (2*S*,3*S*)-3-hydroxy-6-methoxy-3-methyl-7-methylidene-5-oxo-2-pentyl-2,3,4,5,6,7-hexahydropyrano[2,3-*c*]pyrrol-4-yl (pyridin-2-ylthio)acetate **2d**

(2*S*,3*S*)-3-Hydroxy-6-methoxy-3-methyl-7-methylidene-5-oxo-2-pentyl-2,3,4,5,6,7-hexahydropyrano[2,3-*c*]pyrrol-4-yl (pyrimidin-2-ylthio)acetate 2e



Yellow oil, 18% yield. R_f 0.61 (20:1 CH_2Cl_2-MeOH). $[\alpha]_{20}^{D}$ = -53.97 (c 2.03, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (d, *J* = 4.8 Hz, 2H), 6.98 (t, *J* = 4.8 Hz, 1H), 5.64 (s, 1H), 5.05 (s, 1H), 5.00 (s, 1H), 4.10-4.07 (m, 1H), 4.02 – 3.94 (m, 2H), 3.89 (s, 3H), 3.35 (s, 1H), 1.82-1.60 (m, 2H), 1.60-1.45 (m, 1H), 1.45-1.23 (m, 5H), 1.19 (s, 3H), 0.88 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.75 (s), 170.05 (s), 164.70 (s), 158.08 (s), 157.51 (s), 136.37 (s), 117.06 (s), 100.88 (s), 92.37 (s), 86.36 (s), 71.24 (s), 64.65 (s, *J* = 5.0 Hz), 33.66 (s), 31.53 (s), 27.58 (s), 26.21 (s), 22.60 (s), 17.92 (s), 14.11 (s). IR (KBr) 3362, 2956, 2932, 2860, 1724, 1670, 1636, 1551, 1450, 1384 1262, 1140, 981, 799 cm^{-1}. HRMS [M + H]+ calcd for $C_{21}H_{27}N_3O_6S$ 450.16933, found 450.16881.

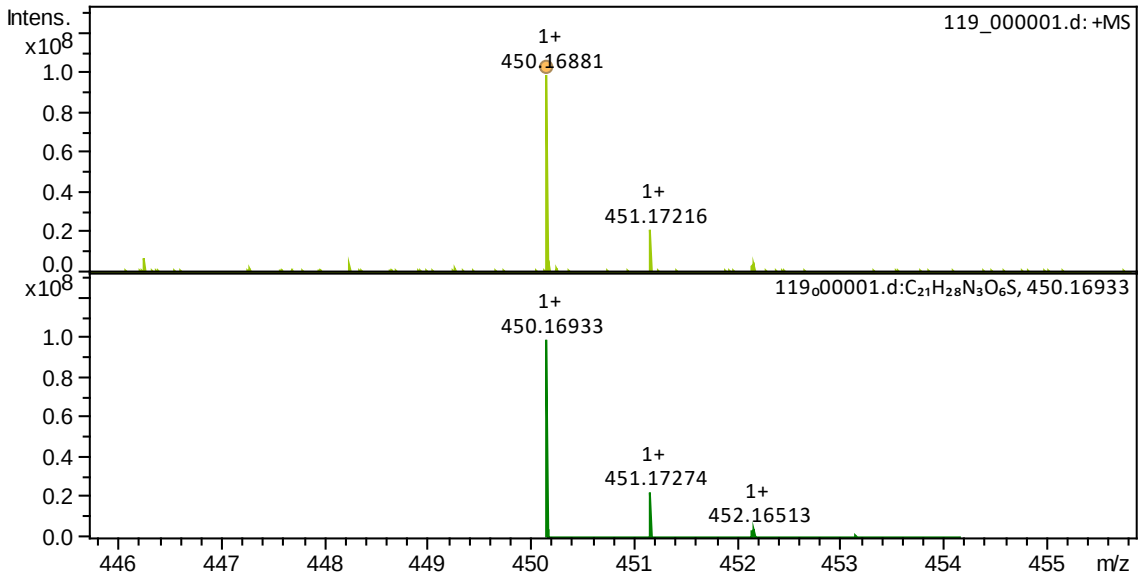


Figure S17. HRMS of (2*S*,3*S*)-3-hydroxy-6-methoxy-3-methyl-7-methylidene-5-oxo-2-pentyl-2,3,4,5,6,7-hexahydropyrano[2,3-*c*]pyrrol-4-yl (pyrimidin-2-ylthio)acetate **2e**

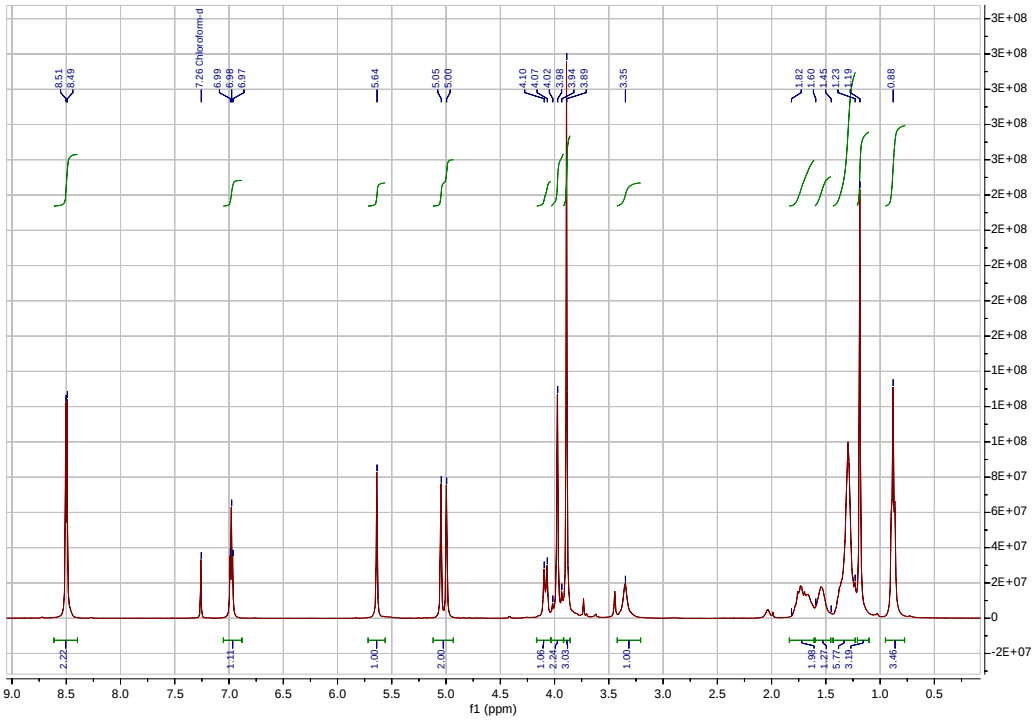


Figure S18. ^{1}H NMR spectrum of (2*S*,3*S*)-3-hydroxy-6-methoxy-3-methyl-7-methylidene-5-oxo-2-pentyl-2,3,4,5,6,7-hexahydropyrano[2,3-*c*]pyrrol-4-yl (pyrimidin-2-ylthio)acetate **2e**

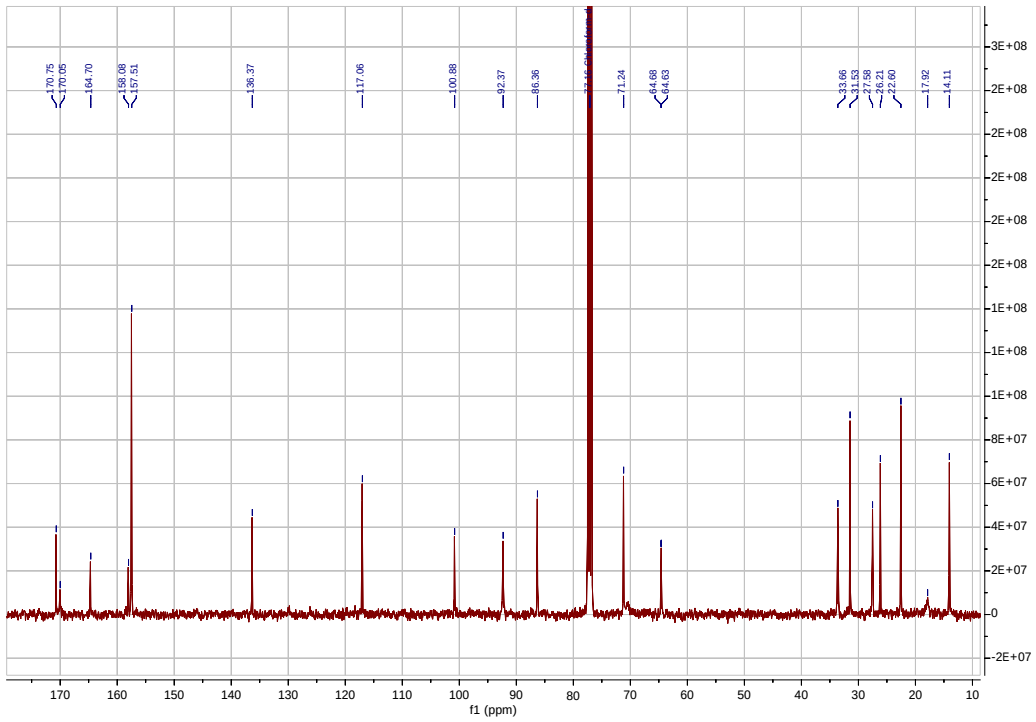


Figure S19. ^{13}C NMR spectrum of (2*S*,3*S*)-3-hydroxy-6-methoxy-3-methyl-7-methylidene-5-oxo-2-pentyl-2,3,4,5,6,7-hexahydropyrano[2,3-*c*]pyrrol-4-yl (pyrimidin-2-ylthio)acetate **2e**

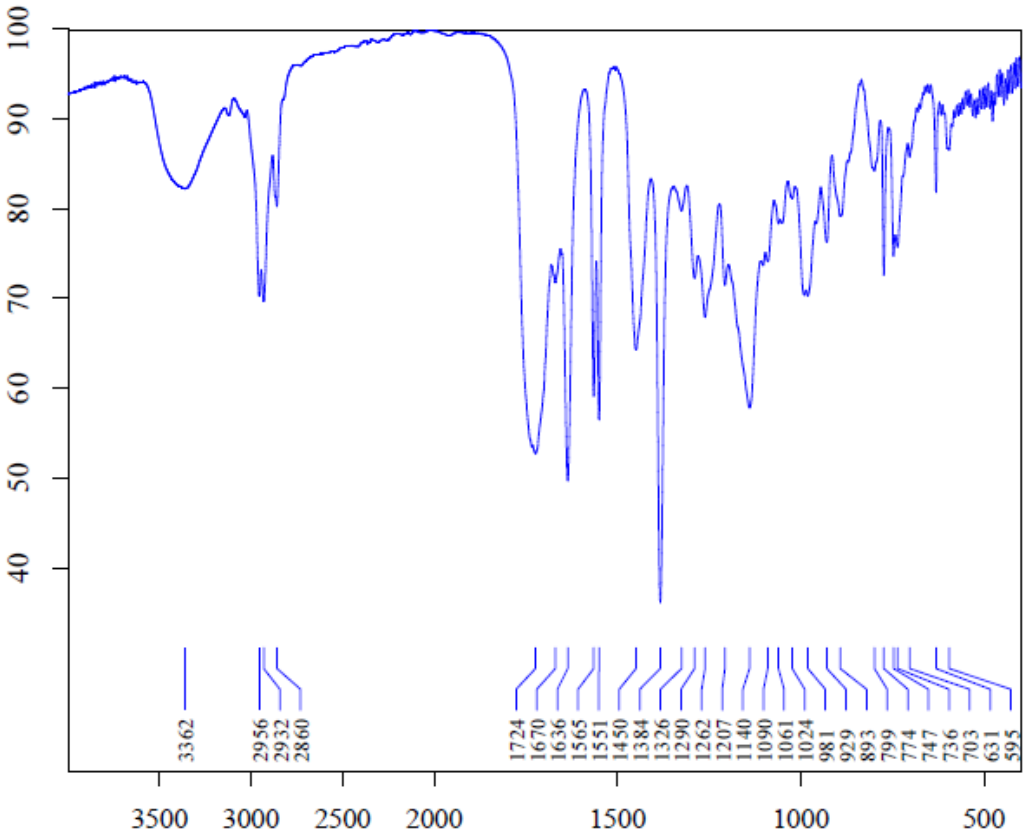
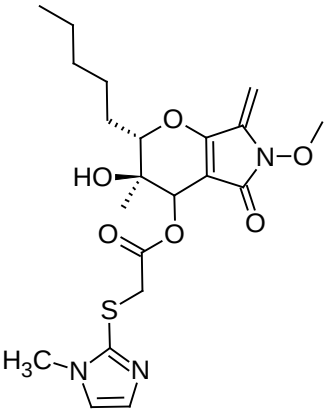


Figure S20. IR spectrum of (2*S*,3*S*)-3-hydroxy-6-methoxy-3-methyl-7-methylidene-5-oxo-2-pentyl-2,3,4,5,6,7-hexahydropyrano[2,3-*c*]pyrrol-4-yl (pyrimidin-2-ylthio)acetate **2e**

(2*S*,3*S*)-3-Hydroxy-6-methoxy-3-methyl-7-methylidene-5-oxo-2-pentyl-2,3,4,5,6,7-hexahydropyrano[2,3-*c*]pyrrol-4-yl [(1-methyl-1*H*-imidazol-2-yl)thio]acetate 2f



Orange oil, 8% yield. R_f 0.62 (20:1 CH_2Cl_2-MeOH). $[\alpha]_{20}^{D}$ = -203.09 (c 2.03, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.03 (s, *1*H), 6.97 (s, 1H), 6.76 (br. S, 1H), 5.84 (s, 1H), 5.03 (s, 1H), 4.99 (s, 1H), 4.11 (m, 1H), 3.88 (s, 3H), 3.73 (s, 3H), 3.61 (s, 2H), 2.06-2.00 (m, 1H), 1.75-1.59 (m, 2H), 1.46-1.35 (m, 5H), 1.30 (s, 3H), 0.91 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) 168.41 (s), 164.66 (s), 158.30 (s), 138.35 (s), 136.33 (s), 129.70 (s), 123.44 (s), 101.84 (s), 92.09 (s), 86.36 (s), 72.31 (s), 70.36 (s), 64.59 (s, *J* = 4.9 Hz), 37.11 (s, *J* = 5.2 Hz), 33.93 (s, *J* = 5.9 Hz), 31.73 (s), 27.27 (s), 26.03 (s), 22.66 (s), 15.61 (s), 14.15 (s). IR (KBr) 3303, 2956, 2932, 2860, 1739, 1672, 1637, 1456, 1280, 1133, 994, 928 cm^{-1}. HRMS $[M + H]^+$ calcd for $C_{21}H_{29}N_3O_6S$ 452.19498, found 452.19440.

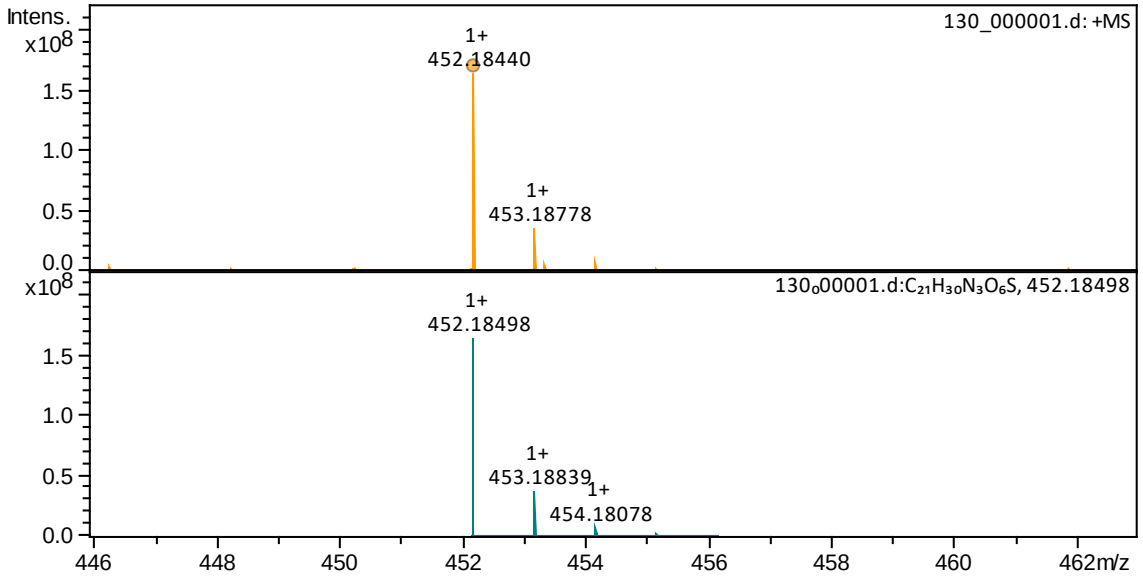


Figure S21. HRMS of (2*S*,3*S*)-3-hydroxy-6-methoxy-3-methyl-7-methylidene-5-oxo-2-pentyl-2,3,4,5,6,7-hexahydropyrano[2,3-*c*]pyrrol-4-yl [(1-methyl-1*H*-imidazol-2-yl)thio]acetate **2f**

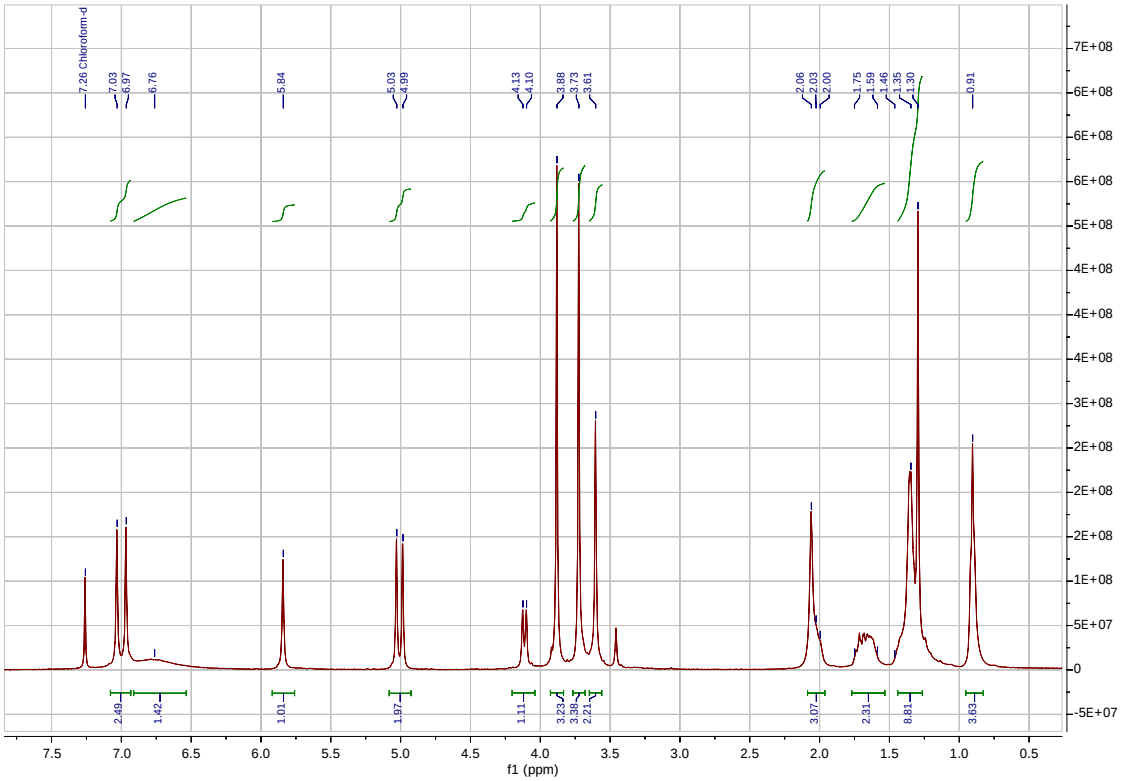


Figure S22. ^{1}H NMR of (2*S*,3*S*)-3-hydroxy-6-methoxy-3-methyl-7-methylidene-5-oxo-2-pentyl-2,3,4,5,6,7-hexahydropyrano[2,3-*c*]pyrrol-4-yl [(1-methyl-1*H*-imidazol-2-yl)thio]acetate **2f**

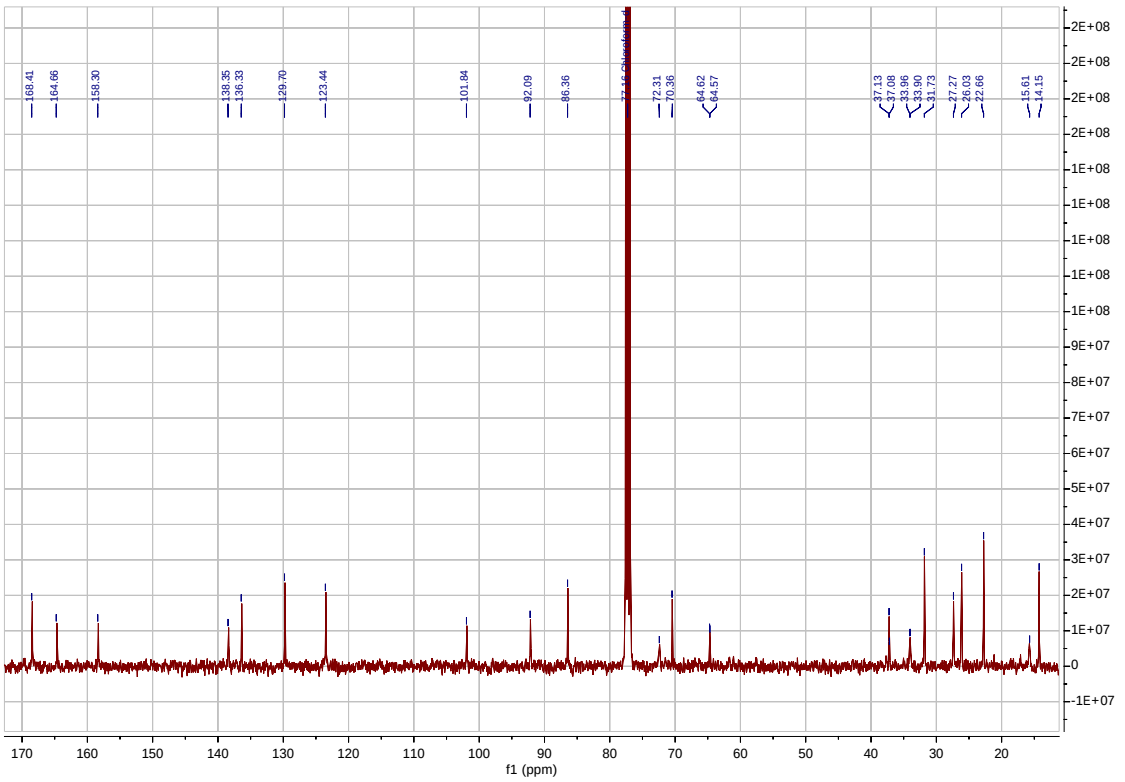


Figure S23. ^{13}C NMR of (2*S*,3*S*)-3-hydroxy-6-methoxy-3-methyl-7-methylidene-5-oxo-2-pentyl-2,3,4,5,6,7-hexahydropyrano[2,3-*c*]pyrrol-4-yl [(1-methyl-1*H*-imidazol-2-yl)thio]acetate **2f**

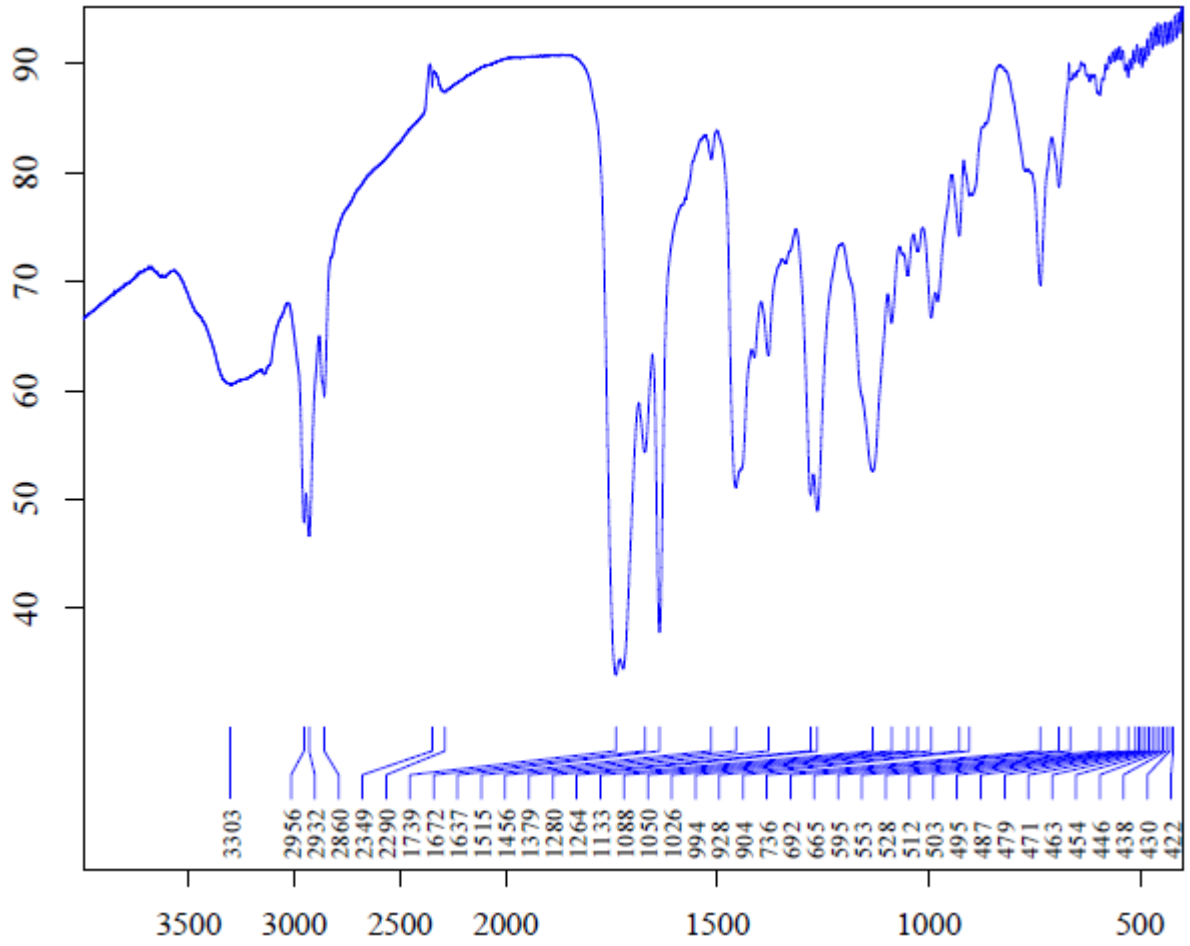
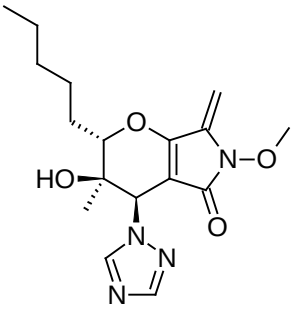


Figure S24. IR spectrum of (2*S*,3*S*)-3-hydroxy-6-methoxy-3-methyl-7-methylidene-5-oxo-2-pentyl-2,3,4,5,6,7-hexahydropyrano[2,3-*c*]pyrrol-4-yl [(1-methyl-1*H*-imidazol-2-yl)thio]acetate **2f**

(2*S*,3*R*,4*R*)-3-Hydroxy-6-methoxy-3-methyl-7-methylidene-2-pentyl-4-(1*H*-1,2,4-triazol-1-yl)-3,4,6,7-tetrahydropyrano[2,3-*c*]pyrrol-5(2*H*)-one 4a



White powder, 7.5% yield. R_f 0.45 (20:1 CH_2Cl_2-MeOH). $[\alpha]_{20}^{D}$ = -135.08 (c 2.03, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.23 (s, 1H), 7.94 (s, 1H), 5.19 (d, *J* = 1.8 Hz, 1H), 5.12 (d, *J* = 1.8 Hz, 1H), 4.93 (s, 1H), 4.25 (m, 1H), 3.92 (s, 3H), 3.89 (s, 1H), 1.95-1.81 (m, 1H), 1.74-1.58 (m, 2H), 1.48-1.30 (m, 5H), 1.28 (s, 3H), 0.90 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.75 (s), 159.70 (s), 147.04 (s), 144.31 (s), 135.97 (s), 97.80 (s), 94.52 (s), 70.39 (s), 64.93 (s, *J* = 6.5 Hz), 58.28 (s), 31.68 (s), 29.84 (s), 27.81 (s), 25.99 (s), 22.60 (s), 20.53 (s), 14.11 (s). IR (KBr) 3425, 2958, 2930, 2860, 1686, 1642, 1454, 1206, 1144, 978, 913 cm^{-1}. HRMS $[M + H]^+$ calcd for $C_{17}H_{24}N_4O_4$ 349.18407, found 349.18779.

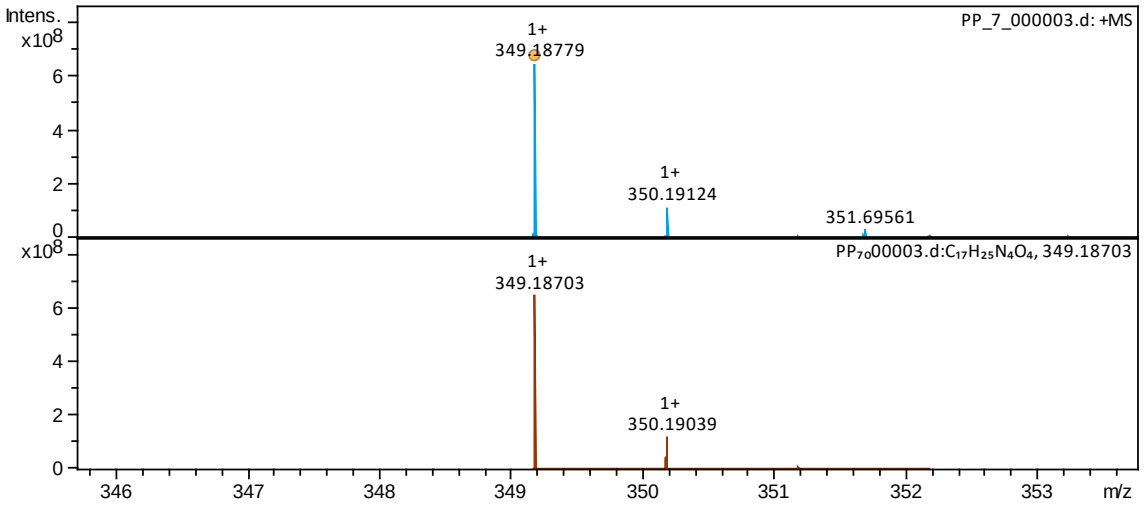


Figure S25. HRMS of (2*S*,3*R*,4*R*)-3-hydroxy-6-methoxy-3-methyl-7-methylidene-2-pentyl-4-(1*H*-1,2,4-triazol-1-yl)-3,4,6,7-tetrahydropyrano[2,3-*c*]pyrrol-5(2*H*)-one **4a**

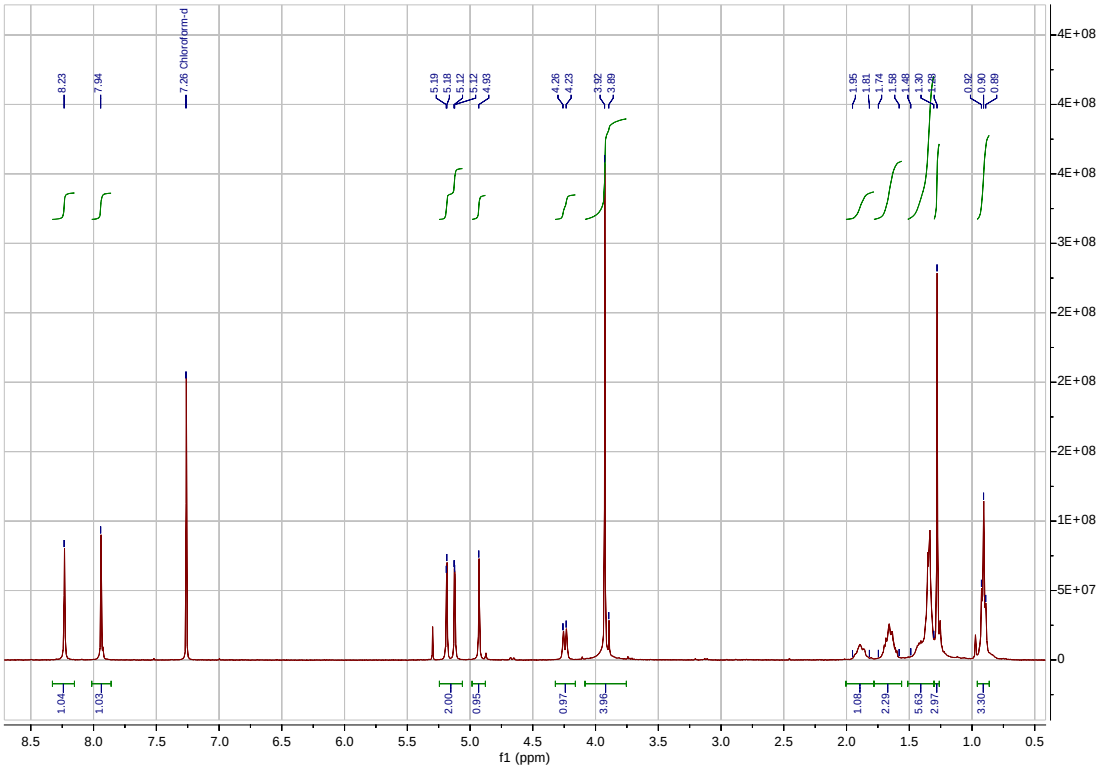


Figure S26. ^{1}H NMR of (2*S*,3*R*,4*R*)-3-hydroxy-6-methoxy-3-methyl-7-methylidene-2-pentyl-4-(1*H*-1,2,4-triazol-1-yl)-3,4,6,7-tetrahydropyrano[2,3-*c*]pyrrol-5(2*H*)-one **4a**

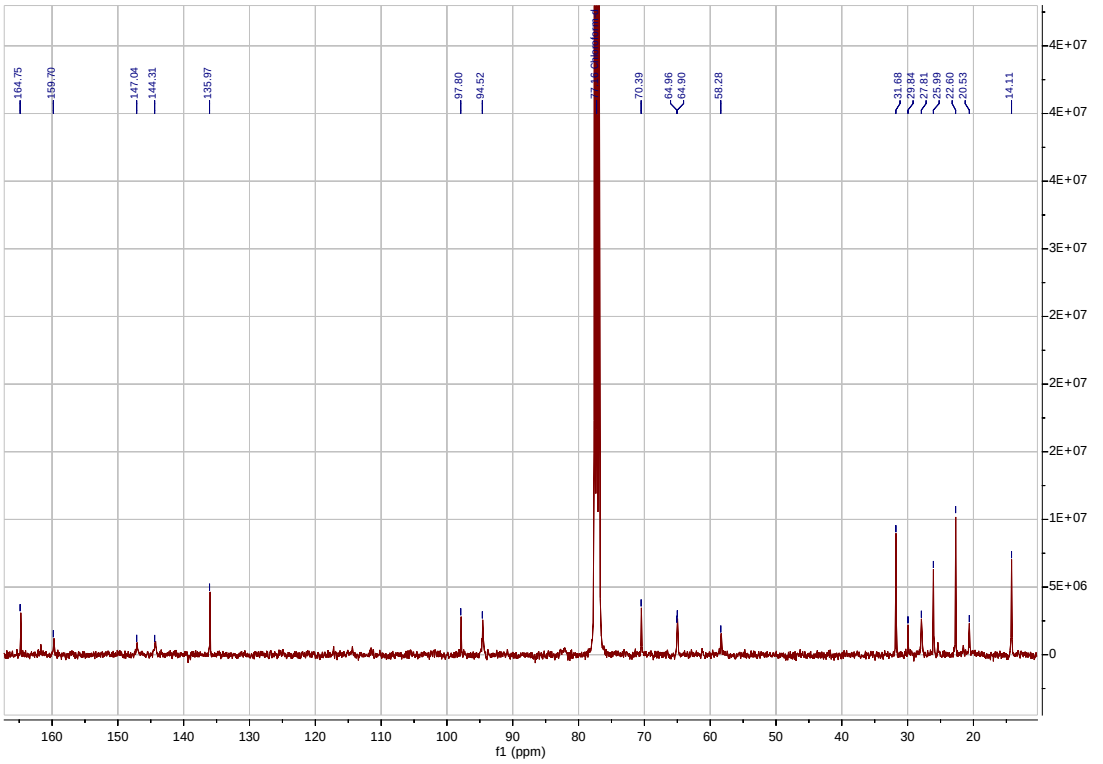


Figure S27. ^{13}C NMR of (2*S*,3*R*,4*R*)-3-hydroxy-6-methoxy-3-methyl-7-methylidene-2-pentyl-4-(1*H*-1,2,4-triazol-1-yl)-3,4,6,7-tetrahydropyrano[2,3-*c*]pyrrol-5(2*H*)-one **4a**

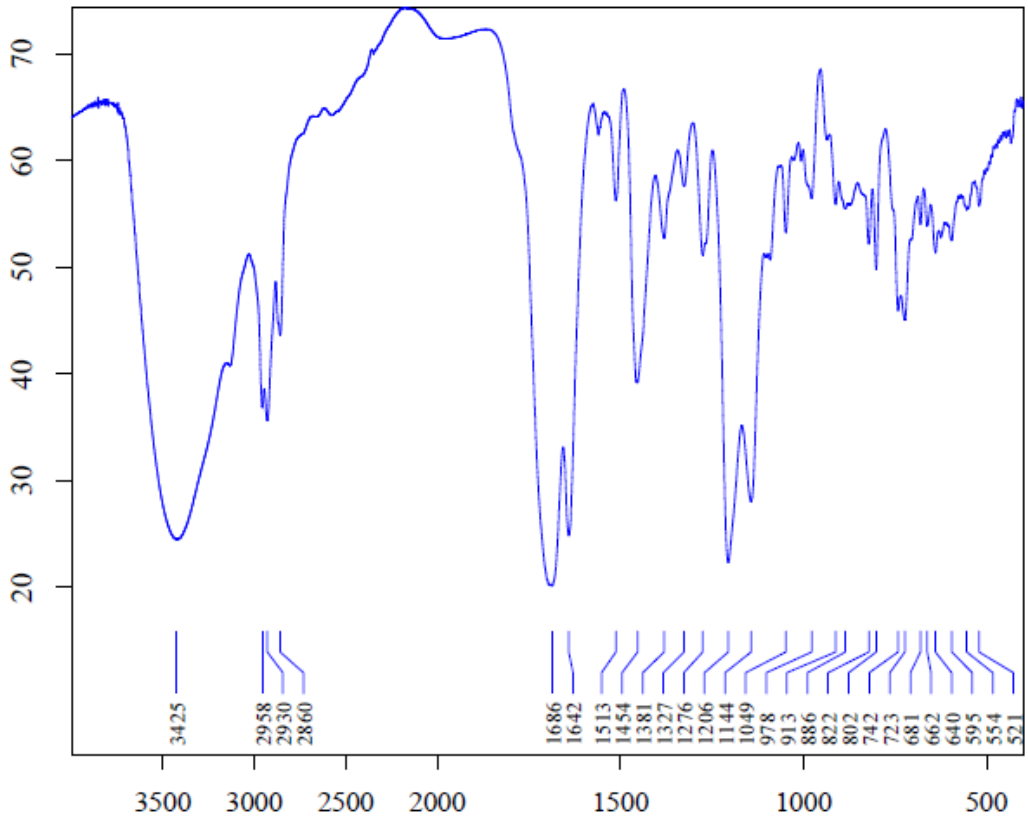
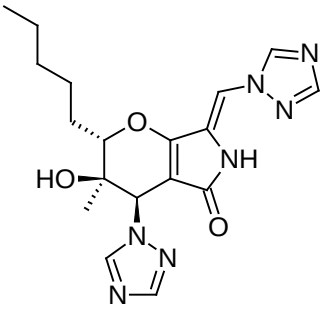


Figure S28. IR spectrum of (2*S*,3*R*,4*R*)-3-hydroxy-6-methoxy-3-methyl-7-methylidene-2-pentyl-4-(1*H*-1,2,4-triazol-1-yl)-3,4,6,7-tetrahydropyrano[2,3-*c*]pyrrol-5(2*H*)-one **4a**

(2*S*,3*R*,4*R*,7*Z*)-3-Hydroxy-3-methyl-2-pentyl-4-(1*H*-1,2,4-triazol-1-yl)-7-(1*H*-1,2,4-triazol-1-ylmethylidene)-3,4,6,7-tetrahydropyrano[2,3-*c*]pyrrol-5(2*H*)-one 4b



Colorless oil, 10,1% yield. R_f 0.3 (20:1 CH_2Cl_2-MeOH). $[\alpha]_{20}^{D}$ = -84.81 (c 2.03, CH_2Cl_2). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H), 8.33 (s, 1H), 8.23 (s, 1H), 8.10 (s, 1H), 7.87 (s, 1H), 6.90 (s, 1H), 4.99 (s, 1H), 4.49-4.32 (br s, 1H), 4.35 (s, 1H), 4.32 (s, 1H), 1.94-1.87 (m, 1H), 1.73-1.61 (m, 2H), 1.49-1.34 (m, 5H), 1.30 (s, 3H), 0.91 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.08 (s), 162.77 (s), 153.31 (s, *J* = 7.9 Hz), 151.76 (s, *J* = 9.1 Hz), 145.13 (s, *J* = 6.3 Hz), 144.10 (s, *J* = 5.3 Hz), 122.79 (s), 101.66 (s, *J* = 4.5 Hz), 101.11 (s), 82.69 (s), 70.81 (s), 57.16 (s, *J* = 11.0 Hz), 31.73 (s), 27.96 (s), 26.18 (s), 22.70 (s), 21.65 (s, *J* = 199.0 Hz), 14.15 (s). IR (KBr) 3409, 3123, 2956, 2929, 2859, 1699, 1652, 1509, 1463, 1394, 1276, 1135, 994, 813 cm^{-1}. HRMS $[M + H]^+$ calcd for $C_{18}H_{24}N_7O_3$ 386.19351, found 386.19330.

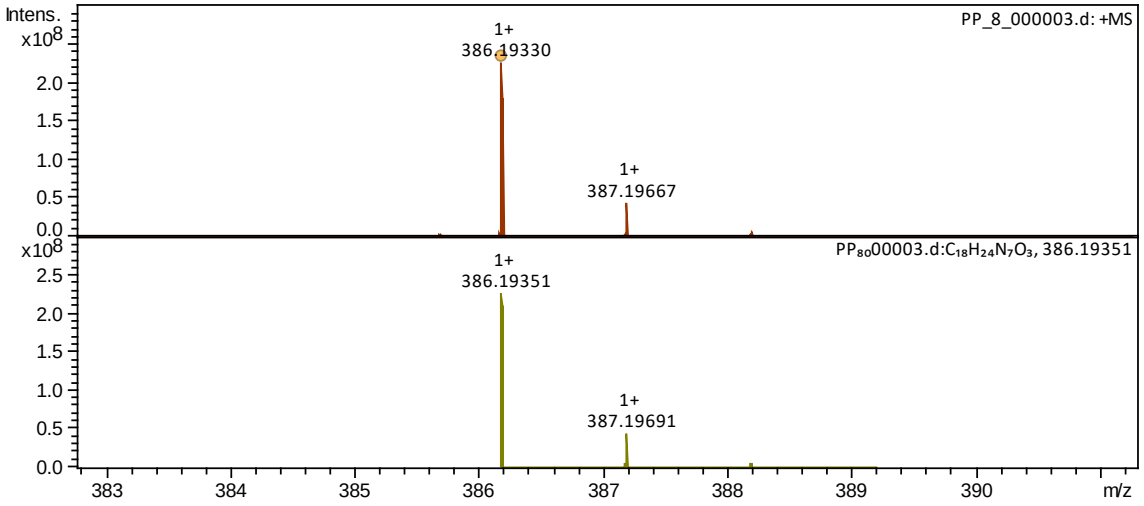


Figure S29. HRMS of (2*S*,3*R*,4*R*,7*Z*)-3-hydroxy-3-methyl-2-pentyl-4-(1*H*-1,2,4-triazol-1-yl)-7-(1*H*-1,2,4-triazol-1-ylmethylidene)-3,4,6,7-tetrahydropyrano[2,3-*c*]pyrrol-5(2*H*)-one **4b**

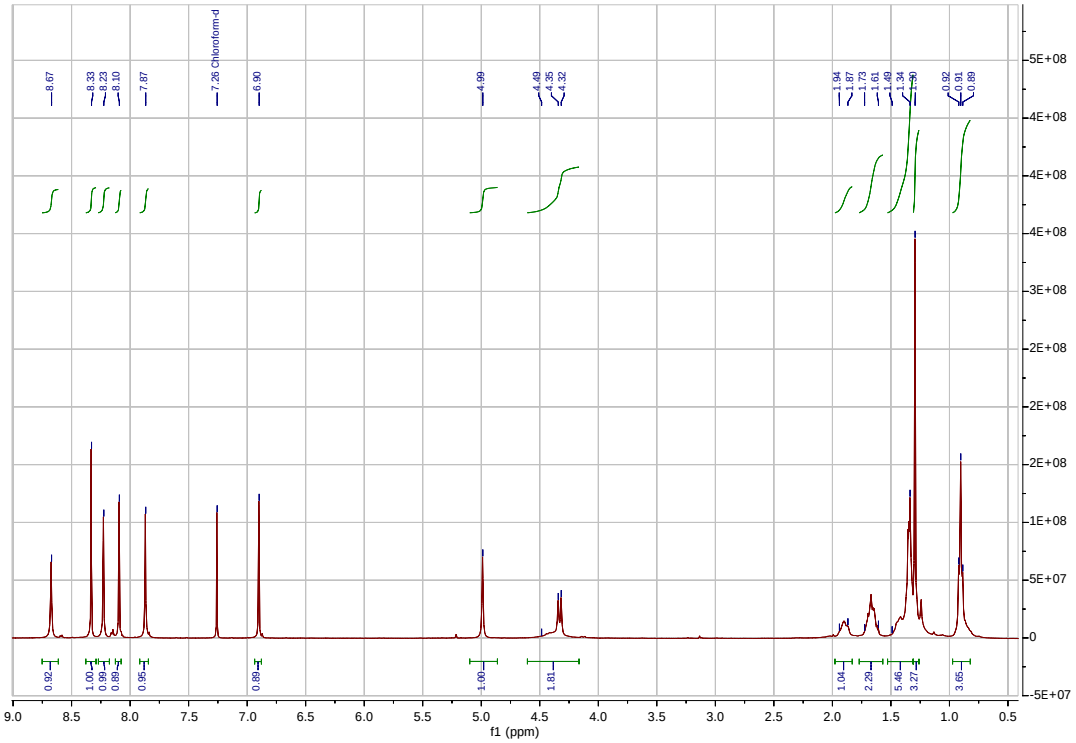


Figure S30. ^{1}H NMR of (2*S*,3*R*,4*R*,7*Z*)-3-hydroxy-3-methyl-2-pentyl-4-(1*H*-1,2,4-triazol-1-yl)-7-(1*H*-1,2,4-triazol-1-ylmethylidene)-3,4,6,7-tetrahydropyrano[2,3-*c*]pyrrol-5(2*H*)-one **4b**

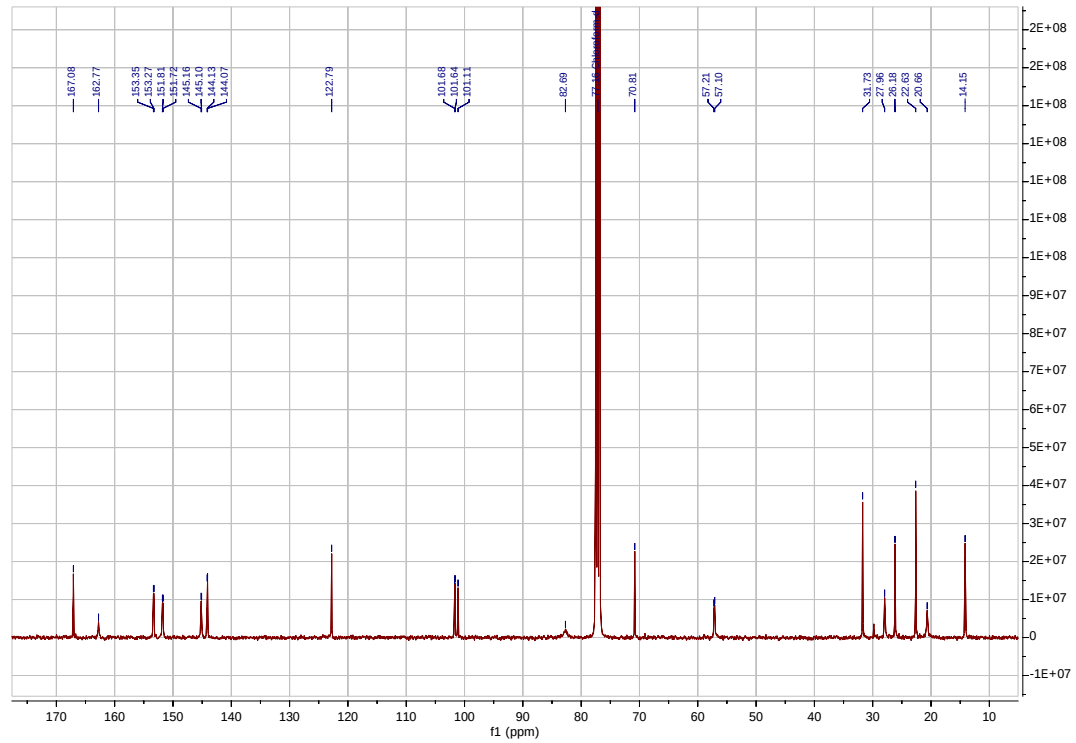


Figure S31. ^{13}C NMR of (2*S*,3*R*,4*R*,7*Z*)-3-hydroxy-3-methyl-2-pentyl-4-(1*H*-1,2,4-triazol-1-yl)-7-(1*H*-1,2,4-triazol-1-ylmethylidene)-3,4,6,7-tetrahydropyrano[2,3-*c*]pyrrol-5(2*H*)-one **4b**

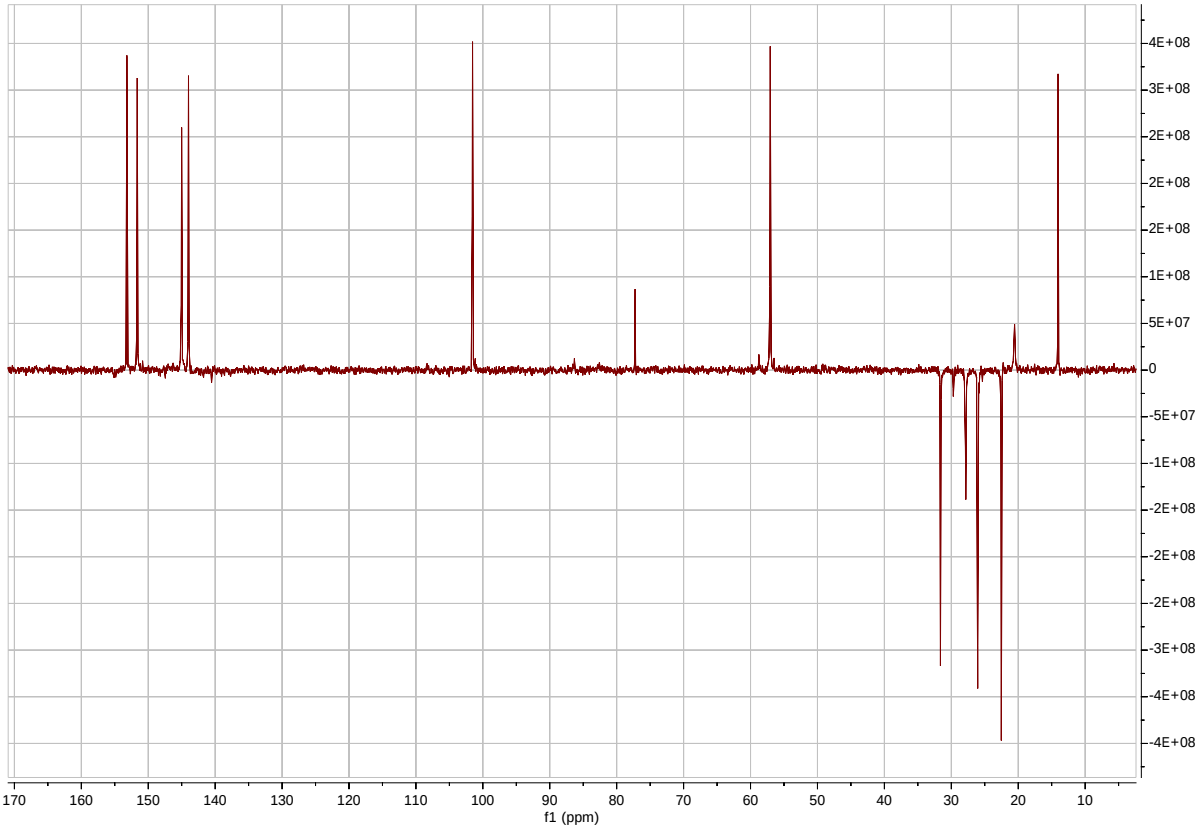


Figure S32. DEPT of (2*S*,3*R*,4*R*,7*Z*)-3-hydroxy-3-methyl-2-pentyl-4-(1*H*-1,2,4-triazol-1-yl)-7-(1*H*-1,2,4-triazol-1-ylmethylidene)-3,4,6,7-tetrahydropyrano[2,3-*c*]pyrrol-5(2*H*)-one **4b**

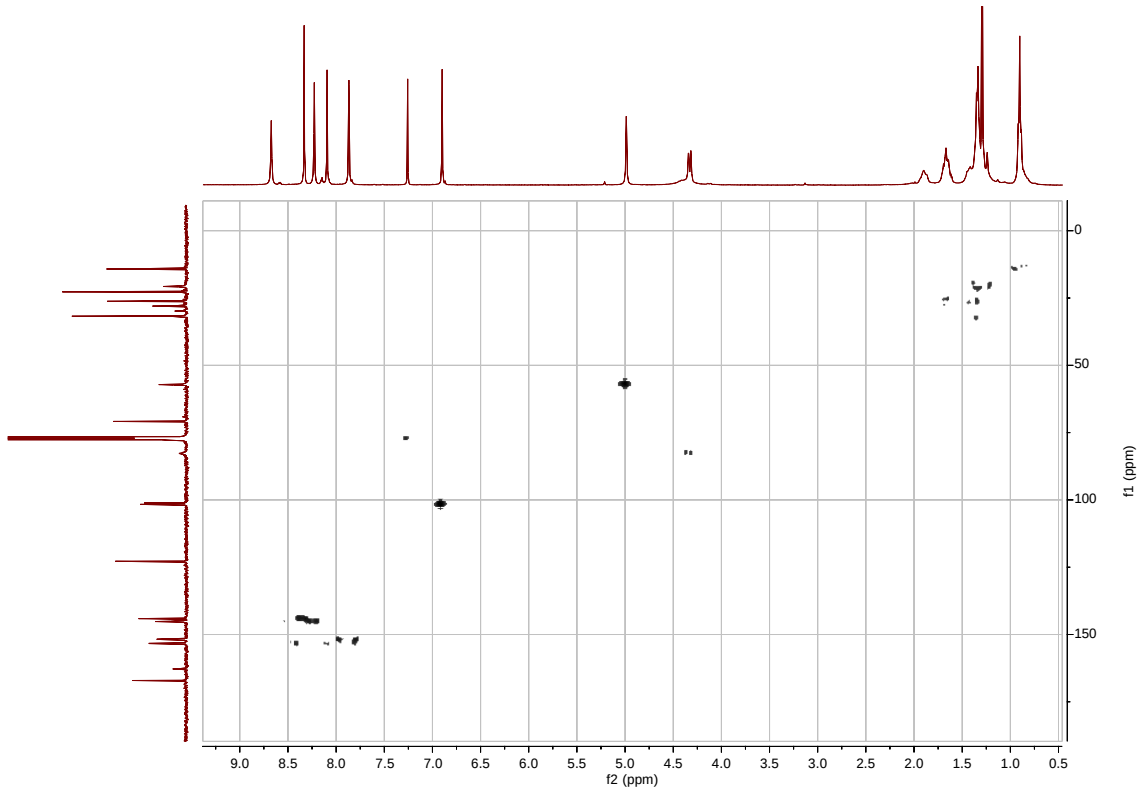


Figure S33. HMQC of (2*S*,3*R*,4*R*,7*Z*)-3-hydroxy-3-methyl-2-pentyl-4-(1*H*-1,2,4-triazol-1-yl)-7-(1*H*-1,2,4-triazol-1-ylmethylidene)-3,4,6,7-tetrahydropyrano[2,3-*c*]pyrrol-5(2*H*)-one **4b**

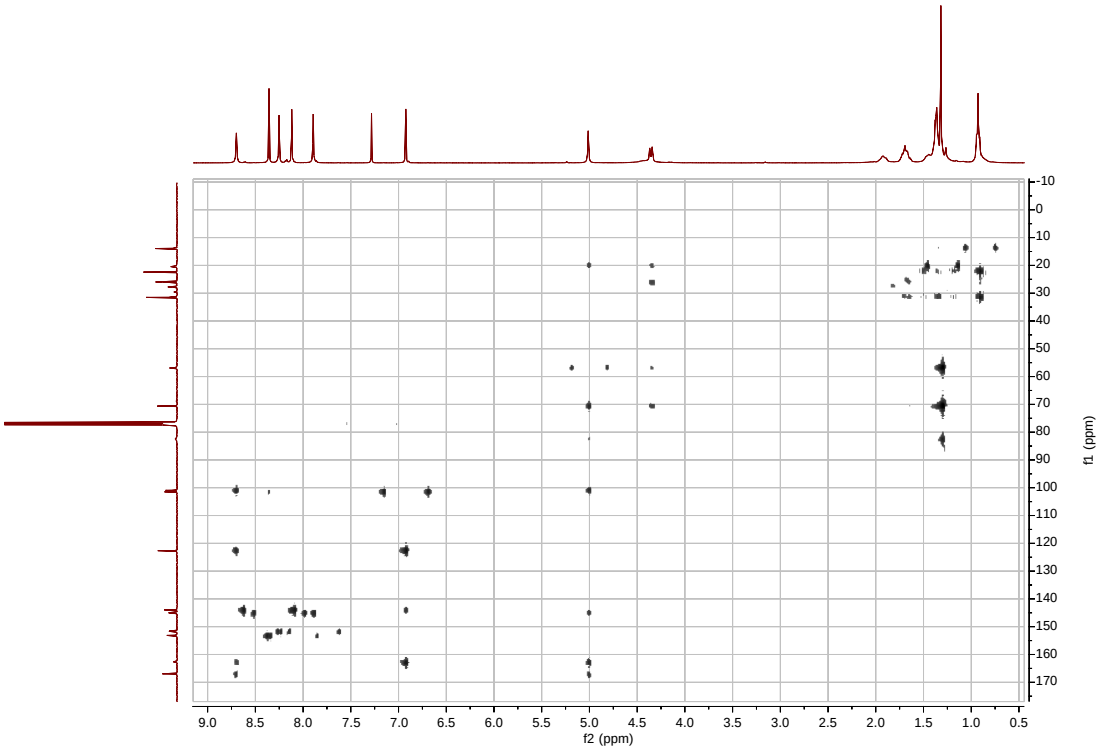


Figure S34. HMBC of (2*S*,3*R*,4*R*,7*Z*)-3-hydroxy-3-methyl-2-pentyl-4-(1*H*-1,2,4-triazol-1-yl)-7-(1*H*-1,2,4-triazol-1-ylmethylidene)-3,4,6,7-tetrahydropyrano[2,3-*c*]pyrrol-5(2*H*)-one **4b**

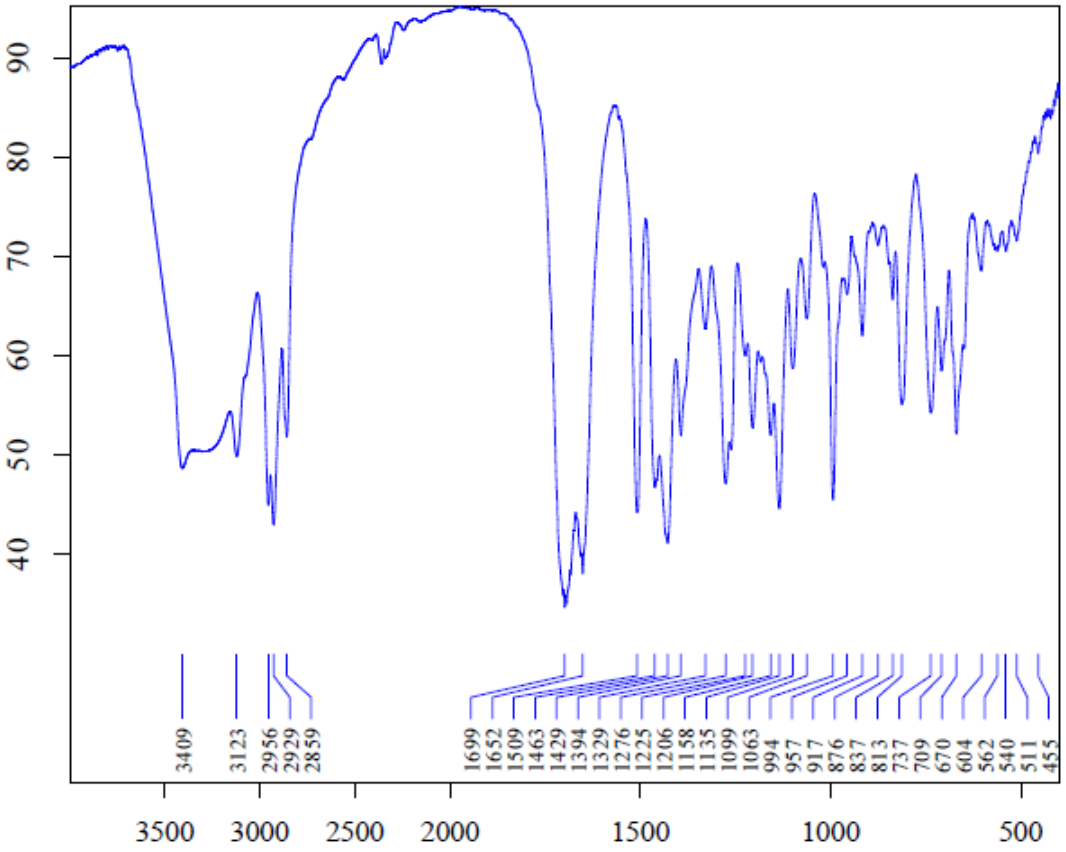
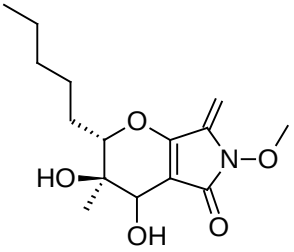


Figure S35. IR of (2*S*,3*R*,4*R*,7*Z*)-3-hydroxy-3-methyl-2-pentyl-4-(1*H*-1,2,4-triazol-1-yl)-7-(1*H*-1,2,4-triazol-1-ylmethylidene)-3,4,6,7-tetrahydropyrano[2,3-*c*]pyrrol-5(2*H*)-one **4b**

PPA



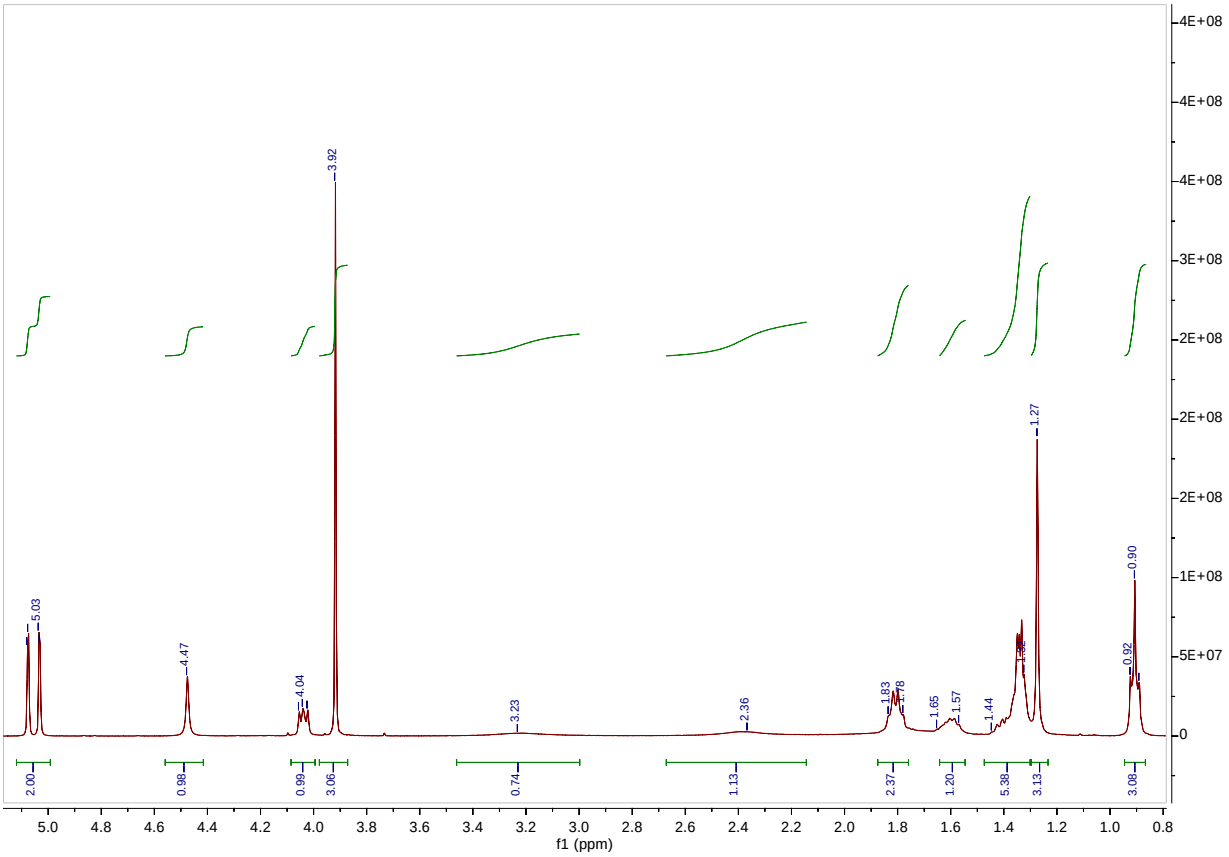


Figure S36. ^{1}H NMR spectrum of **PPA**

VII. Biological Studies

Materials and methods

Cell culture

MCF-7 (breast cancer), PC-3 (prostate adenocarcinoma), HCT-116 (colorectal cancer cell), A549 (lung cancer), K562 (chronic myelogenous leukemia), THP-1 (acute monocytic leukemia), and RPMI8226 (multiple myeloma), Jurkat (acute T-cell leukemia) was purchased from the Russian Academy of Sciences Cells Bank (Institute of Cytology of the Russian Academy of Sciences, Saint Petersburg, Russian Federation). Multiple myeloma cell line NCI-H929 was purchased from ATCC (USA). The MCF-7, A549 and were cultured in DMEM medium. Other cell lines were cultured in RPMI1640 medium, (PanEco, Russia) supplemented with 10% fetal bovine serum (GE Healthcare LifeSciences, São Paulo, Brazil), and gentamicin at a concentration of 40 g/mL and cultured at 37 C in a humidified atmosphere containing 5% CO_2. All experiments were performed with cells at passages 3 to 7 in the logarithmic phase of growth.

Cell treatment with the compounds and control drug

Cells were seeded into 96-well plates of 5×10^3 for adhesive cultures and 20×10^3 for suspension cultures per well in 90 L and 135 L of culture medium, respectively. The test substances were dissolved in DMSO to a concentration of 1×10^{-2} M. For subsequent dilution of the substances, a serum-free culture medium was used as the diluent. Final concentration of DMSO in wells was no more than 1%. It was found that this concentration of DMSO didn't affect the cells. Substances were added to the cells in 3–4 replicates after 24 h for adhesive cultures and immediately for suspension cultures. 10–15 L of serum-free medium was added to the control wells with non-exposed cells. Cells were cultured for 72 h at 37°C in an atmosphere of 5% CO_2.

Cytotoxicity evaluation by the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)) assay

The MTT test is a colorimetric method used to determine the number of living cells after various influence. The MTT reagent 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide is metabolized by mitochondrial dehydrogenases of living cells to formazan. Further, formazan crystals are dissolved in DMSO, and the percentage of cell survival is calculated from the level of optical density of the solution.

The MTT reagent (USA) dissolved in phosphate buffer saline (PBS) was added to the wells at a concentration of 5 mg/ml in a volume of 20 μl per well. After 2-3 hours the medium with the reagent was removed and the precipitate of the crystals of the formazan was dissolved in 60 μl of DMSO. The optical density is determined using a Multiscan FC spectrophotometer (Thermo Scientific, USA) at a wavelength of 540 nm.

Statistical Analysis

In a program, written in Microsoft Excel in the laboratory of tumor cell genetics "Program for the calculation of IC50 (half-minimal inhibitory concentration of a substance) chemotherapeutic substances", a plot was plotted for the level of optical density (percentage of control) versus the concentration of the substance and IC_{50} concentration was calculated. Results were expressed as the mean ± S.D.