Supporting Information

Target analysis of α-alkylidene-γ-butyrolactones in uropathogenic *E. coli*

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1) Materials

All chemicals were of reagent grade or better and used without further purification. Chemicals and solvents were purchased from Sigma Aldrich or Acros Organics. For all reactions, only commercially available solvents of purissimum grade, dried over molecular sieve and stored under argon atmosphere were used. Solvents for chromatography and workup purposes were generally of reagent grade and purified before use by distillation. In all reactions, temperatures were measured externally. All experiments were carried out under argon.

Column chromatography was performed on Merck silica gel (Acros Organics 0.035–0.070 mm, mesh 60 Å).

^{1}H- and ^{13}C-NMR spectra were recorded on a *Bruker Avance I 360* (360 MHz), a *Bruker Avance I* (500 MHz) or a *Bruker Avance III 500* (500 MHz) NMR-System and referenced to the residual proton and carbon signal of the deuterated solvent, respectively.

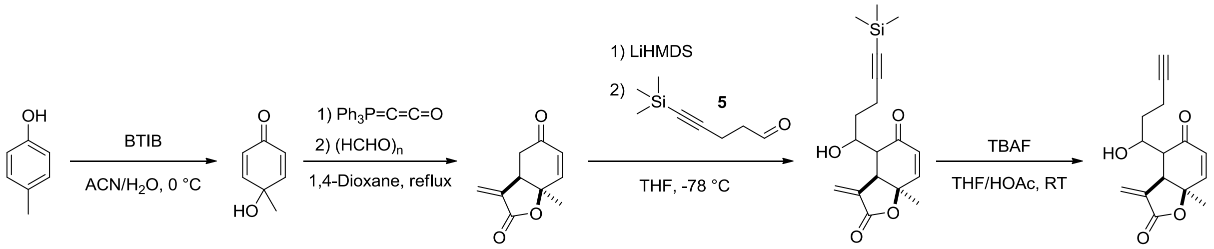
HR-ESI-MS, HR-LC-ESI-MS, HR-APCI-MS and HR-LC-APCI-MS mass spectra were recorded with a *Thermo Finnigan LTQ FT Ultra* coupled with a *Dionex UltiMate 3000* HPLC system. ESI-MS and LC-ESI-MS mass spectra were recorded with a *Thermo Finnigan LCQ ultrafleet* coupled with a *Dionex UltiMate 3000* HPLC system.

HPLC analysis was accomplished with a *Waters 2695 separations module*, an *X-Bridge™ C18 3.5 µm OBD™* column (4.6 x 100 mm) and a *Waters 2996 PDA detector*.

HPLC separation was accomplished with a *Waters 2545 quaternary gradient module*, an *X-Bridge™ Prep C18 10 µm OBD™* (50 x 250 mm), an *X-Bridge™ Prep C18 5 µm OBD™* (30 x 150 mm) or an *YMC Triart C18 5 µm* column (10 x 250 mm), a *Waters 2998 PDA detector* and a *Waters Fraction Collector III*.

2) Synthesis of the γ-lactone probe library

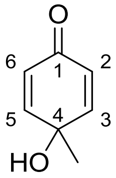
2.1) Synthesis of 7



1 **2** (48 %) **3** (71 %) **6** (46 %) **7** (53 %)

Compounds **3**, **6** and **7** were isolated as racemic mixtures.

2.1.1) 4-hydroxy-4-methylcyclohexa-2,5-dienone (**2**)



[Bis(trifluoroacetoxy)iodo] benzene (BTIB) (4.73 g, 11.0 mmol, 1.10 eq.) was added to a stirred solution of *p*-cresol (**1**) (1.08 g, 10.0 mmol, 1.00 eq.) in acetonitrile/water (3:1, 40 mL) at 0 °C and stirring was continued until TLC analysis (diethyl ether) showed completion of the reaction after 5-15 min. The brown reaction mixture was quenched by addition of water (40 mL) and the resulting mixture was extracted with dichloromethane (4 x 50 mL). The combined extracts were dried (Na_2SO_4) and evaporated under reduced pressure. This crude product was purified by column chromatography (diethyl ether/hexane 3:1) followed by recrystallization (hexane/ethyl acetate) to give **2** (518 mg, 4.82 mmol, 48 %) as a brown solid.

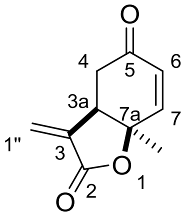
R_f = 0.20 (diethyl ether/hexane, 3:1).

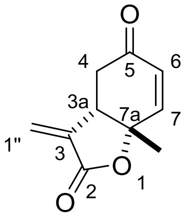
^{1}H-NMR (500 MHz, $CDCl_3$) $\delta = 6.90$ (d, $^3J_{2,3} = {^3J_{5,6}} = 9.9$ Hz, 2 H, 2-H, 6-H), 6.14 (d, $^3J_{2,3} = {^3J_{5,6}} = 9.9$ Hz, 2 H, 3-H, 5-H), 2.19 (br s, 1 H, OH), 1.50 (s, 3 H, Me).

^{13}C-NMR (90 MHz, $CDCl_3$) δ = 185.4, 152.1, 127.2, 67.2, 26.7.

Data is consistent with that reported in the literature.[1]

2.1.2) (3a*R**,7a*R**)-7a-Methyl-3-methylidene-3a,7a-dihydro-3*H*,4*H*-benzofuran-2,5-dione (**3**)





Triphenylphosphoranylideneketene (Bestmann's ylide) (317 mg, 1.05 mmol, 1.05 equiv.), handled under an atmosphere of argon, was added in one portion to a stirred solution of 4-hydroxy-4-methylcyclohexa-2,5-dienone (**2**) (124 mg, 1.00 mmol, 1.00 equiv.) in 1,4-dioxane (20 mL) at r.t. under argon and the resulting solution was heated to reflux for 15 h. The septum was removed and paraformaldehyde (300 mg, 10.0 mmol, 10.0 equiv.) was added in one portion, rapidly replacing the septum once the addition was complete. The mixture was then heated to reflux for 30 min before being cooled and the solvent was removed in vacuo. The residue was purified by flash column chromatography on SiO_2 eluting with EtOAc/hexane (2:1) to afford compound **3** (127 mg, 0.713 mmol, 71 %) as a colorless solid.

R_f = 0.66 (EtOAc/hexane, 2:1).

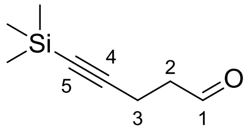
^{1}H-NMR (500 MHz, $CDCl_3$) δ = 6.60 (d, $^3J_{6,7}$ = 10.3 Hz, 1 H, 7-H), 6.31 (d, $^4J_{1''a,3a}$ = 3.4 Hz, 1 H, $1''_a$-H), 5.99 (d, $^3J_{6,7}$ = 10.3 Hz, 1 H, 6-H), 5.59 (d, $^4J_{1''b,3a}$ = 3.0 Hz, 1 H, $1''_b$-H), 3.35-3.39 (m, 1 H, 3a-H), 2.85 (d, $^3J_{3a,4}$ = 4.2 Hz, 2 H, 4-H), 1.75 (s, 3 H, CH_3).

^{13}C-NMR (90 MHz, $CDCl_3$) δ = 194.3, 168.1, 146.7, 137.5, 128.8, 122.4, 80.0, 45.1, 36.0, 23.9.

MS-ESI (m/z): $C_{10}H_{11}O_3$ $[M+H]^+$, calc.: 179.1, found: 179.0.

Data is consistent with that reported in the literature.[2]

2.1.3) 5-(Trimethylsilyl)pent-4-ynal (**5**)



5-(Trimethylsilyl)-4-pentyn-1-ol (**4**) (16.5 mmol, 2.58 g, 3.00 mL, 1.00 eq.) was added slowly to a suspension of Dess-Martin periodinane (7.21 g, 17.0 mmol, 1.03 eq.) in dichloromethane (80 mL). The mixture was stirred for 3 h and the solvent was removed in vacuo (500 mbar). The residue was purified by flash column chromatography on SiO_2 eluting with hexane/diethyl ether (6:1) to afford **5** (1.95 g, 12.7 mmol, 77 %) as colorless oil.

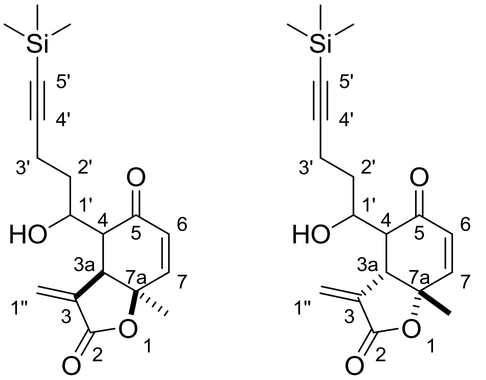
R_f = 0.40 (hexane/diethyl ether, 6:1).

^{1}H-NMR (500 MHz, $CDCl_3$) δ = 9.82 (s, 1 H, 1-H), 2.69 (t, $^3J_{2,3}$ = 7.2 Hz, 2 H, 2-H), 2.57 (t, $^3J_{2,3}$ = 7.2 Hz, 2 H, 3-H), 0.16 (s, 9 H, TMS).

^{13}C-NMR (90 MHz, $CDCl_3$) δ = 200.4, 104.7, 85.8, 42.5, 13.1, 0.0.

Data is consistent with that reported in the literature.[3]

2.1.4) (3a*R**7a*R**)-4-(1-hydroxy-5-(trimethylsilyl)pent-4-yn-1-yl)-7a-methyl-3-methylid-ene-3a,7a-dihydro-3*H*,4*H*-benzofuran-2,5-dione (**6**)



To **3** (40.0 mg, 0.224 mmol, 1.00 eq.) in THF (10 mL) was added LiHMDS (1 M in THF, 0.224 mL, 0.224 mmol, 1.00 eq.) at -78 °C and the mixture was stirred for 1 h. **5** (45.0 mg, 0.291 mmol, 1.30 eq.) was added and the mixture was stirred for further 4 h. The reaction mixture was quenched with saturated NH_4Cl_{aq} (10 mL) and extracted with EtOAc (3 x 20 mL). The organic phase was dried with $MgSO_4$ and the solvents were removed in vacuo. The residue was purified by flash column chromatography on SiO_2 eluting with hexane/EtOAc (2:1) to afford **6** (34.5 mg, 0.104 mmol, 46 %) as colorless oil.

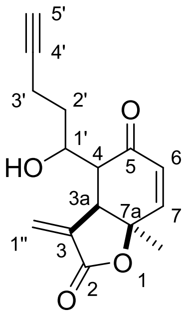
R_f = 0.29 (hexane/EtOAc, 2:1).

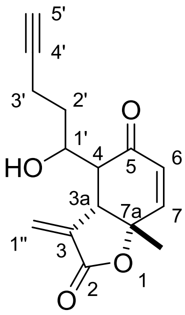
^{1}H-NMR (500 MHz, CDCl3) δ = 6.66 (d, $^3J_{6,7}$ = 10.4 Hz, 0.86 H, 7-H), 6.55 (d, $^3J_{6,7}$ = 10.4 Hz, 0.14 H, 7-H), 6.33-6.36 (m, 1 H, 1''$_a$-H), 6.14 (d, $^4J_{1''b,3a}$ = 3.0 Hz, 0.14 H, 1''$_b$-H), 6.00 (d, $^3J_{6,7}$ = 10.4 Hz, 0.86 H, 6-H), 5.98 (d, $^3J_{6,7}$ = 10.4 Hz, 0.14 H, 6-H), 5.60 (d, $^4J_{1''b,3a}$ = 3.0 Hz, 0.86 H, 1''$_b$H), 4.50-4.56 (m, 0.14 H, 1'-H), 4.10-4.17 (m, 0.86 H, 1'-H), 3.76-3.78 (m, 0.14 H, 3a-H), 3.75-3.73 (m, 0.86 H, 3a-H), 2.86-2.90 (m, 0.14 H, 4-H), 2.80-2.85 (m, 0.86 H, 4-H), 2.48-2.56 (m, 1 H, 3'$_a$-H), 2.37-2.45 (m, 1 H, 3'$_b$-H), 1.93-2.00 (m, 0.14 H, 2'-H), 1.82-1.89 (m, 0.86 H, 2'-H), 1.83 (s, 2.58 H, CH_3), 1.66-1.80 (m, 1 H, 2'-H), 1.77 (s, 0.42 H, CH_3), 0.18 (s, 1.26 H, TMS), 0.17 (s, 7.74 H, TMS).

^{13}C-NMR (90 MHz, $CDCl_3$) δ = 196.8, 168.1, 146.7, 138.34, 129.1, 128.60, 125.6, 122.8, 106.0, 87.2, 79.9, 70.3, 67.6, 54.5, 51.0, 47.5, 44.6, 34.7, 33.3, 26.88, 25.9, 22.3, 17.0, 0.05, 0.0, -0.03.

HRMS-ESI (m/z): $C_{18}H_{25}O_4Si$ $[M+H]^+$, calc.: 333.15219, found: 333.15166, δ = 1.6 ppm.

2.1.5) (3a*R**7a*R**)-4-(1-hydroxypent-4-yn-1-yl)-7a-methyl-3-methylidene-3a,7a-dihydro-3*H*,4*H*-benzofu-ran-2,5-dione (**7**)





To **6** (20.0 mg, 60.0 µmol, 1.00 eq.) in THF (5 mL) was added acetic acid (1 mL) and TBAF (200 µmol, 3.33 eq.) at r.t. and the reaction was stirred until TLC analysis showed completion of the reaction. Saturated NH_4Cl_{aq} (20 mL) was added and the reaction mixture was extracted with EtOAc. The organic phase was washed with saturated $NaCl_{aq}$, dried with $MgSO_4$ and the solvents were removed in vacuo. The residue was purified by flash column chromatography on SiO_2 eluting with hexane/EtOAc (2:1) to afford **7** (8.20 mg, 31.8 µmol, 53 %) as colorless oil.

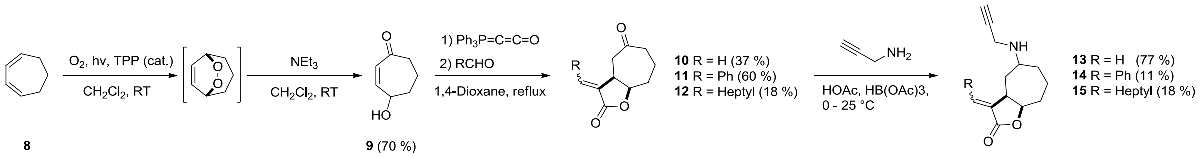
R_f = 0.34 (hexane/EtOAc, 2:1).

^{1}H-NMR (500 MHz, $CDCl_3$) δ = 6.69 (dd, $^3J_{6,7}$ = 10.3 Hz, $^4J_{3a,7}$ = 0.8 Hz, 1 H, 7-H), 6.41 (d, $^4J_{1''a,3a}$ = 2.6 Hz, 1 H, 1''$_a$-H), 6.08 (d, $^3J_{6,7}$ = 10.3 Hz, 1 H, 6-H), 5.74 (d, $^4J_{1''b\text{-}3a}$ = 2.3 Hz, 1 H, 1''$_b$-H), 4.05 (dt, J = 9.3, 4.0 Hz, 1 H, 1'-H), 3.42 (dtd, $^3J_{3a,4}$ = 5.8 Hz, $^4J_{1'',3a}$ = 2.4 Hz, $^4J_{3a,7}$ = 0.8 Hz, 1 H, 3a-H), 2.65 (dd, $^3J_{3a,4}$ = 6.1 Hz, $^3J_{4,1'}$ = 4.3 Hz, 1 H, 4-H), 2.41-2.46 (m, 2 H, 3'-H), 2.02-2.08 (m, 1 H, 2'$_a$-H), 2.00 (t, $^4J_{3',5'}$ = 2.7 Hz, 1 H, 5'-H), 1.84-1.92 (m, 1 H, 2'$_b$-H), 1.73 (s, 3 H, CH_3).

^{13}C-NMR (90 MHz, $CDCl_3$) δ = 197.8, 167.9, 145.5, 137.9, 129.6, 124.5, 83.2, 79.2, 69.8, 69.6, 52.1, 47.2, 34.0, 26.9, 15.0.

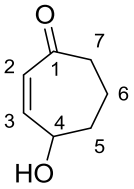
HRMS-ESI (m/z): $C_{15}H_{17}O_4$ $[M+H]^+$, calc.: 261.11214, found: 261.11210, δ = 0.2 ppm.

2.2) Synthesis of B7a, B7s, B7Ph and B7Hp



All compounds were isolated as racemic mixtures.

2.2.1) 4-hydroxycyclohept-2-enone (9)



A solution containing 1,3-cycloheptadiene (**8**) (942 mg, 10.0 mmol, 1.09 mL, 1.00 eq.) and tetraphenylporphyrin (6.0 mg, 9.8 µmol) in dry CH_2Cl_2 (30 mL) was irradiated with a tungsten halogen lamp at 4 °C while oxygen was continuously bubbled through it. The reaction was monitored by TLC analysis and after completion (3 h) the solvent was evaporated in vacuo. The residue was dissolved in CH_2Cl_2 (10 mL) and NEt_3 (2.80 mL, 20.0 mmol, 2.00 eq.) was added over 20 min at 0 °C. The mixture was stirred 1 h at 0 °C, 12 h at r.t. and HCl_{aq} (2 M, 15 mL) was added. The reaction mixture was extracted with CH_2Cl_2 (2 × 15 mL, the organic phase was dried over $MgSO_4$ and the solvent was removed in vacuo. The residue was purified by flash column chromatography on SiO_2 eluting with hexane/EtOAc (1:1) to afford **9** (873 mg, 6.92 mmol, 70 %) as colorless oil.

R_f = 0.47 (hexane/EtOAc, 1:1).

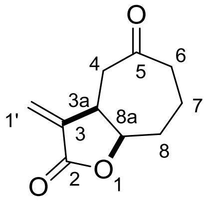
^{1}H-NMR (500 MHz, $CDCl_3$) δ = 6.59 (ddd, *J* = 12.6, 3.1, 1.2 Hz, 1 H, 3-H), 5.97 (ddd, *J* = 12.6, 2.2, 0.7 Hz, 1 H, 2-H), 4.56-4.63 (m, 1 H, 4-H), 2.54-2.67 (m, 2 H, 7-H), 2.18-2.28 (m, 1 H, 5_a-H), 2.09-2.18 (s, 1 H, OH), 1.81-1.91 (m, 3 H, 5_b-H, 6-H).

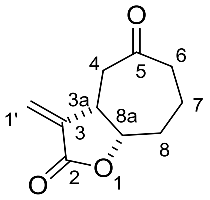
^{13}C-NMR (90 MHz, $CDCl_3$) δ = 203.1, 149.0, 129.9, 70.5, 43.1, 35.2, 18.3.

HRMS-ESI (m/z): $C_7H_{11}O_2$ $[M+H]^+$, calc.: 127.07536, found: 127.07527, δ = 0.7 ppm.

Data is consistent with that reported in the literature. [4]

2.2.2) (3a*R**8a*R**)-3-methylenehexahydro-2*H*-cyclohepta[*b*]furan-2,5(3*H*)-dione (**10**)





Triphenylphosphoranylideneketene (720 mg, 2.39 mmol, 1.05 eq.) was added to a stirred solution of **9** (288 mg, 2.29 mmol, 1.00 eq.) in 1,4-dioxane (45 mL) at r.t. and the reaction was heated to reflux for 15 h. Paraformaldehyde (684 mg, 22.8 mmol, 10.0 eq.) was added and the reaction mixture heated to reflux for additional 1.5 h. After the reaction cooled to r.t. the solvent was removed in vacuo and the residue was purified by flash column chromatography on SiO_2 eluting with hexane/EtOAc (1:1 → 1:2). The resulting product was further purified by HPLC yielding **10** (152 mg, 0.844 mmol, 37 %) as colorless solid.

R_f = 0.67 (hexane/EtOAc, 1:2).

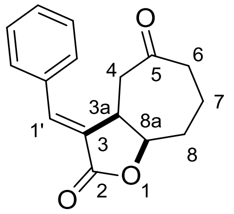
^{1}H-NMR (500 MHz, $CDCl_3$) δ = 6.37 (d, *J* = 2.7 Hz, 1 H, 1’$_a$-H), 5.71 (d, *J* = 2.4 Hz, 1 H, 1’$_b$-H), 4.73 (ddd, *J* = 9.5, 7.9, 3.8 Hz, 1 H, 8a-H), 3.39 (dddd, *J* = 14.4, 7.0, 4.6, 2.5 Hz, 1 H, 3a-H), 2.85 (dd, *J* = 13.0, 11.5 Hz, 1 H, 4$_a$-H), 2.59 (dd, *J* = 13.0, 4.3 Hz, 1 H, 4$_b$-H), 2.53 (t, *J* = 6.8 Hz, 2 H, 6-H), 2.23 (dddd, *J* = 14.4, 8.3, 3.8, 2.1 Hz, 1 H, 8$_a$-H), 1.95-2.10 (m, 2 H, 7$_a$-H, 8$_b$-H), 1.57-1.68 (m, 1 H, 7$_b$-H).

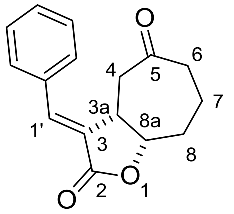
^{13}C-NMR (90 MHz, $CDCl_3$) δ = 208.8, 169.0, 138.0, 123.7, 79.7, 44.4, 43.8, 38.5, 29.9, 18.0.

HRMS-ESI (m/z): $C_{10}H_{13}O_3$ $[M+H]^+$, calc.:181.08592, found: 181.08608, δ = 0.9 ppm.

Data is consistent with that reported in the literature. [2]

2.2.3) (3a$R*$8a$R*$$E$)-3-benzylidenehexahydro-2*H*-cyclohepta[*b*]furan-2,5(3*H*)-dione (**11**) [2]





Triphenylphosphoranylideneketene (500 mg, 1.65 mmol, 1.05 eq.) was added to a stirred solution of **9** (199 mg, 1.58 mmol, 1.00 eq.) in 1,4-dioxane (40 mL) at r.t. and the reaction was heated to reflux for 15 h. Benzaldehyde (838 mg, 7.90 mmol, 5.00 eq.) was added and the reaction mixture heated to reflux for additional 48 h. After the reaction cooled to r.t. the solvent was removed in vacuo and the residue was purified by flash column chromatography on SiO_2 eluting with hexane/EtOAc (2:1 → 1:2). The resulting product was further purified by HPLC yielding **11** (243 mg, 0.948 mmol, 60 %) as colorless solid. E/Z > 95:5 based on comparison of chemical shift and coupling constant of 1'-H with related compounds.[5]

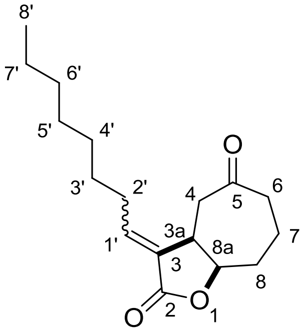
R_f = 0.34 (hexane/EtOAc, 1:2).

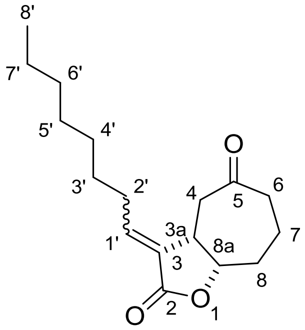
^{1}**H-NMR** (500 MHz, $CDCl_3$) δ = 7.57 (d, *J* = 7.4 Hz, 2 H, *o*-Ar-H) 7.55 (d, *J* = 1.9 Hz, 1 H, 1'-H), 7.48 (t, *J* = 7.4 Hz, 2 H, *m*-Ar-H), 7.43 (t, *J* = 7.3 Hz, 1 H, *p*-Ar-H), 4.69 (ddd, *J* = 8.9, 6.5, 4.7 Hz, 1 H, 8a-H), 3.72 (dddd, *J* = 11.9, 6.4, 3.0, 1.9 Hz, 1 H, 3a-H), 2.80 (t, 12.8 Hz, 1 H, 4_a-H), 2.51-2.64 (m, 3 H, 4_b-H, 6-H), 2.28 (dddd, *J* = 15.2, 8.8, 4.7, 2.0 Hz, 1 H, 8_a-H), 2.20 (dddd, *J* = 15.0, 10.6, 8.8, 1.9 Hz, 1 H, 8_b-H), 2.05-2.15 (m, 1 H, 7_a-H), 1.59-1.69 (m, 1 H, 7_b-H).

^{13}**C-NMR** (90 MHz, $CDCl_3$) δ = 209.4, 171.2, 137.9, 133.3, 130.5, 129.4, 128.6, 79.3, 77.4, 44.7, 40.3, 39.1, 29.4, 17.3.

HRMS-ESI (m/z): $C_{16}H_{17}O_3$ $[M+H]^+$, calc.: 257.11722, found: 257.11687, δ = 1.4 ppm.

2.2.4) (3a_R_*8a_R_*)-3-octylidenehexahydro-2_H_-cyclohepta[_b_]furan-2,5(3_H_)-dione (**12**) [2]





Triphenylphosphoranylideneketene (500 mg, 1.65 mmol, 1.05 eq.) was added to a stirred solution of **9** (199 mg, 1.58 mmol, 1.00 eq.) in 1,4-dioxane (40 mL) at r.t. and the reaction was heated to reflux for 15 h. Octanal (1.01 g, 7.90 mmol, 5.00 eq.) was added and the reaction mixture heated to reflux for additional 48 h. After the reaction cooled to r.t. the solvent was removed in vacuo and the residue was purified by flash column chromatography on SiO_2 eluting with hexane/EtOAc (10:1 → 1:2). The resulting product was further purified by HPLC yielding **12** (57.0 mg, 0.205 mmol, 13 %) as colorless solid. E/Z = 1:5 based on comparison of chemical shift and coupling constant of 1’-H with related compounds. [5]

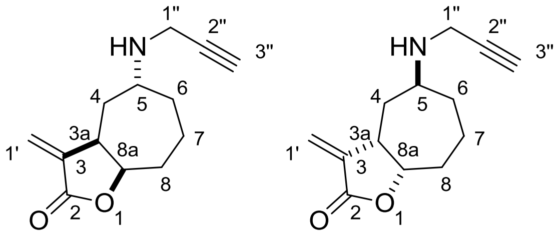
R_f = 0.42 (hexane/EtOAc, 1:2).

^{1}H-NMR (500 MHz, $CDCl_3$) δ = 6.77 (td, _J_ = 7.7, 2.0 Hz, 0.8 H, 1’-H(_Z_)), 6.25 (td, _J_ = 7.7, 2.0 Hz, 0.2 H, 1’-H(_E_)), 4.63 (ddd, _J_ = 8.4, 7.0, 4.6 Hz, 1 H, 8a-H(_E_+_Z_)), 3.31 (ddt, _J_ = 12.1, 5.7, 2.5 Hz, 0.8 H, 3a-H(_Z_)), 3.21-3.28 (m, 0.2 H, 3a-H(_E_)), 2.85 (t, _J_ = 12.4 Hz, 0.2 H, 4_a-H(_E_)) 2.80 (t, _J_ = 13.0 Hz, 0.8 H, 4_a-H(_Z_)), 2.69-2.76 (m, 0.4 H, 2’-H(_E_)), 2.48-2.61 (m, 2 H, 6-H(_E_+_Z_)), 2.43 (dd, _J_ = 12.9, 4.1 Hz, 0.2 H, 4_b-H(_E_)), 2.35 (dd, _J_ = 13.3, 3.6 Hz, 0.8 H, 4_b-H(Z)), 2.21-2.32 (m, 1.6 H, 2’-H(_Z_)), 2.10-2.21 (m, 2 H, 8-H(_E_+_Z_)), 1.99-2.10 (m, 1 H, 7_a-H(_E_+_Z_)), 1.57-1.68 (m, 1 H, 7_b-H(_E_+_Z_)), 1.40-1.54 (m, 2 H, 3’-H(_E_+_Z_)), 1.22-1.38 (m, 8 H, 4’-H(_E_+_Z_), 5’-H(_E_+_Z_), H6’-H(_E_+_Z_), 7’-H(_E_+_Z_)), 0.87-0.92 (m, 3 H, 8’-H(_E_+_Z_)).

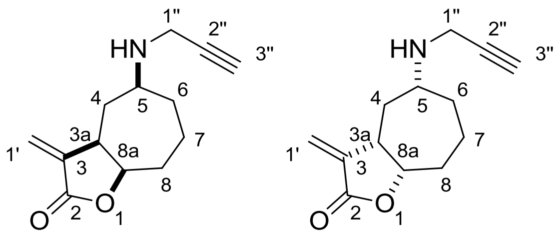
^{13}C-NMR (90 MHz, $CDCl_3$) δ = 209.5, 209.4, 169.9, 168.8, 146.5, 142.9, 130.0, 128.0, 79.1, 78.9, 44.7, 44.5, 44.2, 42.8, 40.2, 37.7, 31.8, 31.7, 29.8, 29.5, 29.3, 29.2, 29.2, 29.1, 29.0, 28.5, 27.7, 22.6, 22.6, 17.6, 17.0, 14.1, 14.1.

HRMS-ESI (m/z): $C_{17}H_{27}O_3$ $[M+H]^+$, calc.: 279.19547, found: 279.19510, δ = 1.3 ppm.

2.2.5) (3a_R_*5_R_*8a_R_*)-3-methylene-5-(prop-2-yn-1-ylamino)-octahydro-2_H_-cyclohepta[_b_]fu-ran-2-one (**13a**) and (3a*_R_5_S_*8a_R_*)-3-methylene-5-(prop-2-yn-1-ylamino)-octahydro-2_H_-cyclohepta[_b_]fu-ran-2-one (**13b**)[6]



13a



13b

To a solution of **10** (18.0 mg, 0.100 mmol, 1.00 eq.) in THF (10 mL) were added propargylamine (6.06 mg, 7.05 µL, 0.110 mmol, 1.10 eq.) and acetic acid (6.01 mg, 5.72 µL, 0.100 mmol, 1.00 eq.) at r.t. and the reaction was stirred for 15 min. Sodium triacetoxyborohydride (31.8 mg, 0.150 mmol, 1.50 eq.) was added and the reaction mixture was stirred at r.t. for further 36 h. Saturated $NaHCO_3$ (15 mL) and H_2O (5 mL) were added and the mixture was extracted with EtOAc (3 × 20 mL). The organic phase was dried over $MgSO_4$, the solvents were removed in vacuo and the residue was purified by flash column chromatography on SiO_2 eluting with EtOAc. The product was separated by HPLC yielding **13a** (11.5 mg, 52.4 µmol, 49 %) and **13b** (6.50 mg, 29.6 µmol, 28 %) as colorless solids.

Diastereomer 13a (for determination of relative stereochemistry see 2D-NMR spectrum)

R_f = 0.36 (hexane/EtOAc, 1:2).

^{1}H-NMR (500 MHz, $CDCl_3$) δ = 6.31 (d, J = 3.2 Hz, 1 H, 1’$_a$-H), 5.58 (d, J = 2.8 Hz, 1 H, 1’$_b$-H), 4.79 (ddd, J = 10.0, 8.7, 3.5 Hz, 1 H, 8a-H), 3.38-3.52 (m, 3 H, 3a-H, 1’’-H), 3.18-3.23 (m, 1 H, 5-H), 2.24 (t, J = 2.4 Hz, 1 H, 3’’-H), 2.00-2.07 (m, 2 H, 4$_a$-H, 8$_a$-H), 1.87 (ddd, J = 14.9, 10.1, 1.4 Hz, 1 H, 4$_b$-H), 1.71-1.81 (m, 1 H, 8$_b$-H), 1.55-1.70 (m, 4 H, 6-H, 7-H), 1.34 (br s, 1 H, NH).

^{13}C-NMR (90 MHz, $CDCl_3$) δ = 170.3, 140.2, 121.8, 81.7, 77.3, 71.7, 52.2, 37.44, 35.7, 35.7, 34.6, 31.5, 18.8.

HRMS-ESI (m/z): $C_{13}H_{18}NO_2$ $[M+H]^+$ calc.: 220.13321 found: 220.13360, δ = 1.8 ppm.

Diastereomer 13b (for determination of relative stereochemistry see 2D-NMR spectrum)

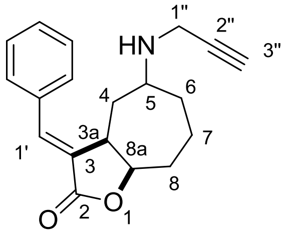
R_f = 0.25 (hexane/EtOAc, 1:2).

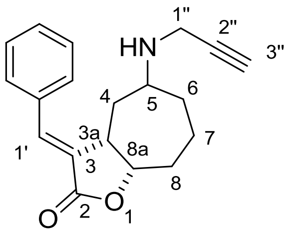
^{1}H-NMR (500 MHz, $CDCl_3$) δ = 6.31 (d, J = 3.0 Hz, 1 H, 1’$_a$-H), 5.62 (d, J = 2.6 Hz, 1 H, 1’$_b$-H), 4.64 (ddd, J = 12.2, 8.4, 4.1 Hz, 1 H, 8a-H), 3.51 (d, J = 2.5 Hz, 2 H, 1’’-H), 3.25-3.33 (m, 1 H, 3a-H), 2.82 (ddd, J = 10.0, 9.0, 2.6 Hz, 1 H, 5-H), 2.25 (t, J = 2.4 Hz, 1 H, 3’’-H), 2.14-2.21 (m, 1 H, 8$_a$-H), 1.98-2.02 (m, 3 H, 4$_a$-H, 6$_a$-H, 7$_a$-H), 1.58-1.73 (m, 2 H, 4$_b$-H, 8$_b$-H), 1.50 (br s, 1 H, NH), 1.22-1.41 (m, 2 H, 6$_b$-H, 7$_b$-H).

^{13}C-NMR (90 MHz, $CDCl_3$) δ = 169.9, 140.0, 122.5, 81.7, 77.2, 71.7, 56.8, 40.2, 37.5, 36.8, 35.4, 30.8, 21.5.

HRMS-ESI (m/z): $C_{13}H_{18}NO_2$ $[M+H]^+$ calc.: 220.13321 found: 220.13360, δ = 1.8 ppm.

2.2.6) (3a*R**8a*R***E*)-3-benzylidene-5-(prop-2-yn-1-ylamino)octahydro-2*H*-cyclohepta-[*b*]furan-2-one (**14**)[6]





To a solution of **11** (81.0 mg, 0.316 mmol, 1.00 eq.) in THF (40 mL) were added propargylamine (19.1 mg, 22.3 µL, 0.348 mmol, 1.10 eq.) and acetic acid (19.0 mg, 18.1 µL, 0.316 mmol, 1.00 eq.) at r.t. and the reaction was stirred for 15 min. Sodium triacetoxyborohydride (100 mg, 0.474 mmol, 1.50 eq.) was added and the reaction mixture was stirred at r.t. for further 36 h. Saturated $NaHCO_3$ (40 mL) and H_2O (20 mL) were added and the mixture was extracted with EtOAc (3 × 40 mL). The organic phase was dried over $MgSO_4$, the solvents were removed in vacuo and the residue was purified by flash column chromatography on SiO_2 eluting with hexane/EtOAc (2:1 → 0:1). The resulting product was further purified by HPLC yielding **14** (12.5 mg, 33.9 µmol, 11 %) as colorless solid.

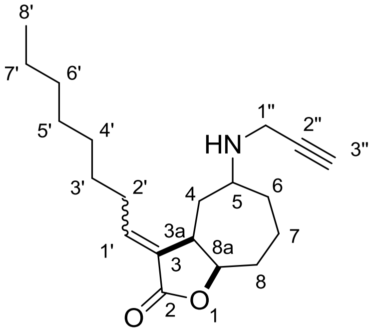
R_f = 0.14 (EtOAc).

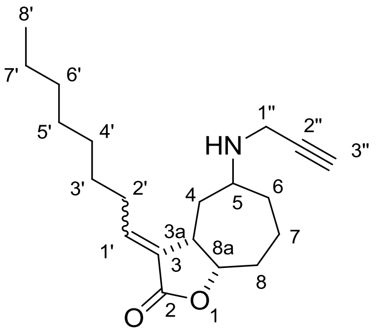
^{1}H-NMR (500 MHz, $CDCl_3$) δ = 7.59-7.65 (m, 2 H, Ar-H), 7.49-7.54 (m, 2 H, Ar-H), 7.43-7.49 (m, 2 H, Ar-H, 1'-H), 4.93 (ddd, *J* = 8.7, 6.7, 2.2 Hz, 0.15 H, 8a-H, Isomer 2), 4.67 (ddd, *J* = 11.2, 7.1, 6.2 Hz, 0.85 H, 8a-H, Isomer 1), 4.17 (ddt, *J* = 8.8, 6.2, 3.3 Hz, 0.15 H, 3a-H, Isomer 2), 3.84 (ddt, *J* = 9.7, 7.0, 2.5 Hz, 0.85 H, 3a-H, Isomer 1), 3.72 (ddd, *J* = 29.7, 17.0, 2.6 Hz, 1.7 H, 1''-H, Isomer 1), 3.48-3.56 (m, 0.85 H, 5-H, Isomer 1), 3.20 (ddd, *J* = 103.2, 16.8, 2.6 Hz, 0.3 H, 1''-H, Isomer 2), 2.94-3.00 (m, 0.15 H, 5-H, Isomer 2), 2.88 (t, *J* = 2.5 Hz, 0.85 H, 3''-H, Isomer 1), 2.71 (t, *J* = 2.6 Hz, 0.15 H, 3''-H, Isomer 2), 2.36 (ddd, *J* = 14.4, 8.7, 6.1 Hz, 1 H, 8_a-H), 2.19-2.29 (m, 1 H, 6_a-H), 1.86-2.02 (m, 3 H, 4-H, 7_a-H), 1.69 (m, 1 H, 8_b-H), 1.56-1.65 (m, 1 H, 6_b-H), 1.33-1.43 (m, 1 H, 7_b-H).

^{13}C-NMR (90 MHz, $CDCl_3$) δ = 170.8, 160.6, 137.4, 136.3, 133.64, 133.59, 130.4, 130.1, 130.1, 130.0, 129.3, 129.1, 79.6, 79.0, 77.4, 74.1, 57.8, 55.3, 39.0, 36.7, 33.8, 33.7, 32.9, 32.8, 31.7, 29.8, 29.5, 29.4, 29.0, 28.6, 22.4, 18.3, 17.5, 13.4.

HRMS-ESI (m/z): $C_{19}H_{22}NO_2$ $[M+H]^+$, calc.: 296.16451, found: 296.16427, 0.8 ppm.

2.2.7) (3a*R**8a*R**)--3-octylidene-5-(prop-2-yn-1-ylamino)octahydro-2*H*-cyclohepta[*b*]-furan-2-one (**15**)[6]





To a solution of **12** (57.0 mg, 0.205 mmol, 1.00 eq.) in THF (20 mL) were added propargylamine (12.4.1 mg, 14.5 µL, 0.226 mmol, 1.10 eq.) and acetic acid (12.3 mg, 11.7 µL, 0.205 mmol, 1.00 eq.) at r.t. and the reaction was stirred for 15 min. Sodium triacetoxyboro-hydride (65.3 mg, 0.308 mmol, 1.50 eq.) was added and the reaction mixture was stirred at r.t. for further 36 h. Saturated NaHCO$_3$ (20 mL) and H$_2$O (10 mL) were added and the mixture was extracted with EtOAc (3 × 30 mL). The organic phase was dried over MgSO$_4$, the solvents were removed in vacuo and the residue was purified by flash column chromatography on SiO$_2$ eluting with hexane/EtOAc (1:1 → 0:1). The resulting product was further purified by HPLC yielding **15** (12.0 mg, 37.8 µmol, 18 %) as colorless solid.

R_f = 0.23 (EtOAc).

^{1}H-NMR (500 MHz, CDCl$_3$) δ = 6.90 (ddd, *J* = 9.5, 5.6, 2.4 Hz, 0.30 H, 1'-H), 6.80 (ddd, *J* = 8.6, 6.4, 2.0 Hz, 0.47 H, 1'-H), 6.35 (dt, *J* = 8.0, 7.9, 2.3 Hz, 0.12 H, 1'-H), 6.25 (dt, *J* = 7.9, 7.7, 2.0 Hz, 0.09 H, 1'-H), 4.81-4.87 (m, 0.43 H, 8a-H), 4.54-3.63 (m, 0.57 H, 8a-H), 3.71-3.92 (m, 2 H, 1''-H), 3.16-3.56 (m, 2 H), 2.78-2.94 (m, 0.22 H), 2.60-2.74 (m, 0.22 H), 2.51-2.58 (m, 1 H, 3''-H), 2.40-2.48 (m, 0.50 H), 2.19-2.40 (m, 3 H), 2.08-2.19 (m, 1 H), 1.97-2.06 (m, 1.5 H), 1.82-1.93 (m, 0.41 H), 1.59-1.82 (m, 2.29 H), 1.40-1.59 (m, 2 H), 1.22-1.39 (m), 0.87-0.94 (m, 3 H).

^{13}C-NMR (90 MHz, CDCl$_3$) δ = 170.4, 170.1, 169.3, 168.9, 162.9, 162.7, 162.4, 162.1, 147.8, 147.1, 144.8, 143.7, 130.4, 128.8, 128.5, 126.8, 117.4, 115.1, 79.5, 79.5, 78.8, 78.4, 78.4, 77.99, 77.96, 77.94, 72.7, 72.6, 72.5, 72.3, 57.9, 57.5, 54.2, 54.0, 41.4, 39.1, 38.6, 36.8, 34.7, 34.0, 33.9, 33.8, 33.6, 33.6, 33.2, 32.7, 32.4, 32.2, 31.9, 31.9, 31.8, 31.6, 30.5, 30.4, 30.3, 29.6, 29.5, 29.5, 29.4, 29.4, 29.3, 29.3, 29.2, 29.22, 29.21, 29.1, 29.1, 28.6, 27.9, 27.8, 22.8, 22.6, 20.2, 18.9, 17.6, 17.5, 14.2, 14.2.

HRMS-ESI (m/z): C$_{20}$H$_{32}$NO$_2$ [M+H]$^+$, calc.: 318.24276, found: 318.24255, δ = 0.6 ppm.

3) Primer for recombinant expression

katG, *E. coli* CFT 073

Forward primer:

5’-GGGGACAAGTTTGTACAAAAAAGCAGGCTTTATGAGCACGTCAGACGAT

Reverse primer:

5’-GGGGACCACTTTGTACAAGAAAGCTGGGTGTTACAGCAGGTCGAAACG

thiI, *E. coli* CFT 073

Forward primer:

5’-GGGGACAAGTTTGTACAAAAAAGCAGGCTTTATGAAGTTTATCATTAAATTGTTCCCG

Reverse primer:

5’-GGGGACCACTTTGTACAAGAAAGCTGGGTGTTACGGGCGATACACCTTCA

ahpC, *E. coli* CFT 073

Forward primer:

5’-GGGGACAAGTTTGTACAAAAAAGCAGGCTTTATGTCCTTGATTAACACCAA

Reverse primer:

5’-GGGGACCACTTTGTACAAGAAAGCTGGGTGTTAGATTTTACCAACCAGGT

c2450, *E. coli* CFT 073

Forward primer:

5’-GGGGACAAGTTTGTACAAAAAAGCAGGCTTTATGGCTGTTCCATCATCAAAA

Reverse primer:

5’-GGGGACCACTTTGTACAAGAAAGCTGGGTGCTATTCTGCAAGACATTTCTG

c2450, *E. coli* CFT 073 (C-strep)

Forward primer:

5’-GGGGACAAGTTTGTACAAAAAAGCAGGCTTTGAAGGAGATAGAACCATGGCTGTTCCATCATCAAA

Reverse primer:

5’- GGGGACCACTTTGTACAAGAAAGCTGGGTGCTATTTTTCGAACTGCGGGTGGCTCCATTCTGCAAGACATTTCTGCA

4) Supporting tables and figures

Table S1. Proteins identified by mass spectrometry in *E. coli* K12, CFT073 and UTI89.

Protein	Protein ID	Score	Coverage [%]	Unique peptides	Peptides	PSM
Catalase-peroxidase (katG)	P13029 Q8FBA9 Q1R3X0	108.43 282.53 251.28	32.02 48.21 50.96	19 28 30	19 28 30	33 87 84
tRNA sulfurtransferase (thiI)	P77718 Q8FKB7 Q1RFB7	15.35 5.55 8.64	9.75 4.98 5.60	4 2 2	4 2 2	5 2 3
Alkyl hydroperoxide reductase subunit C (ahpC)	P0AE08 P0AE09 Q1REV6	9.52 23.27 21.48	21.39 24.06 31.55	3 4 5	3 4 5	3 8 8
Only in pathogenic *E. coli*	-	-	-	-	-	-
c2450 (identical to C2206)	Q8FGD0	267.26	55.29	10	10	97
C2206 (identical to c2450)	Q1RAE0	122.74	52.35	9	9	43

This list of proteins shows Protein ID, Score, Sequence coverage in percent, number of unique peptides, number of peptides, number of PSM's and number of replicates in the *E. coli* strains K12, CFT73 and UTI89.

Table S2. Labeled cysteines in *E. coli* ahpC and c2450.

Cysteine	**Sequence of peptide**	**XCorr**	**Probability**	**Charge**	**MH+ [Da]**	**ΔM [ppm]**
ahpC: C166	AAQYVASHPGEVC*PAK	2.27	27.46	3	1846.917	0.95
ahpC: C166	AAQYVASHPGEVC*PAK	2.30	19.68	3	1846.899	9.06
c2450: C167	C*LAEWSHPQFEK	3.08	25.30	3	1693,804	0,18
c2450: C167	C*LAEWSHPQFEK	2.87	33.35	2	1693,809	3,10

* **13a** labeled cysteine

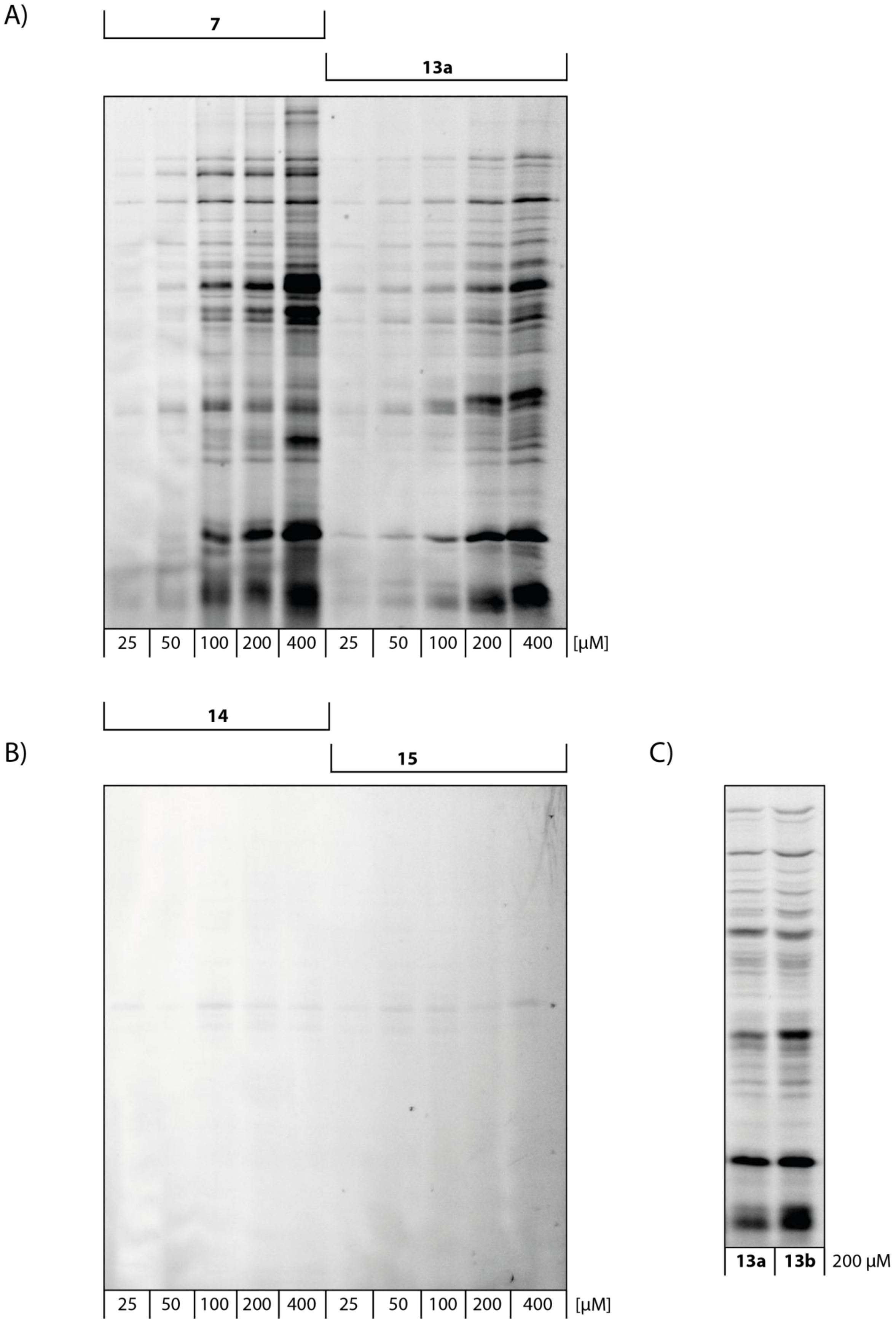
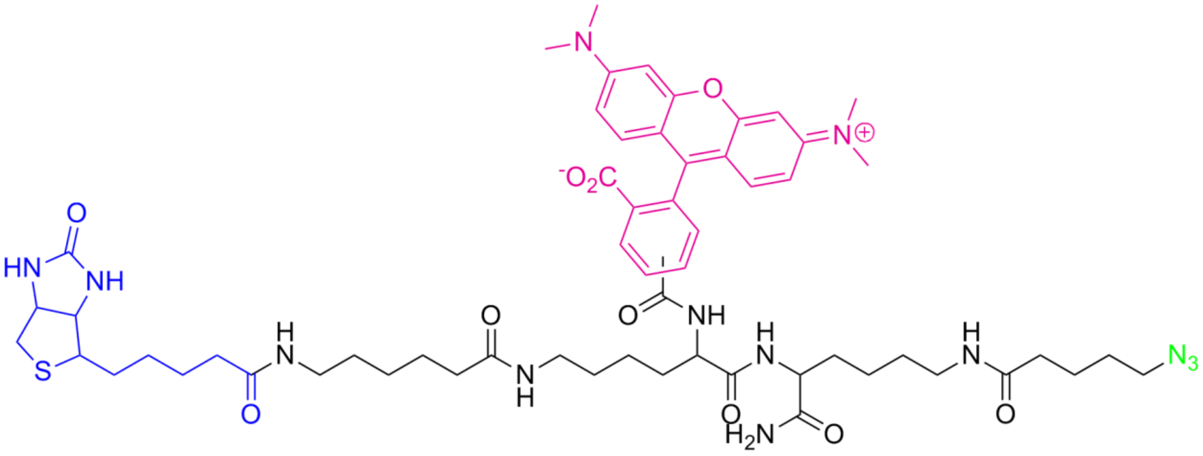
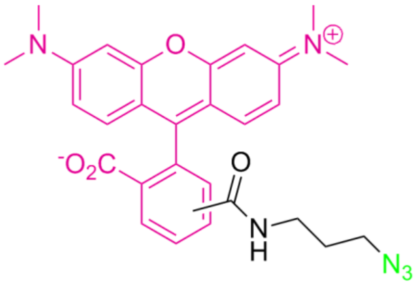


Figure S1. *In situ* labeling of *E. coli* K12 to determine the optimal labeling concentration of the corresponding probe.



biotin-rhodamine-azide

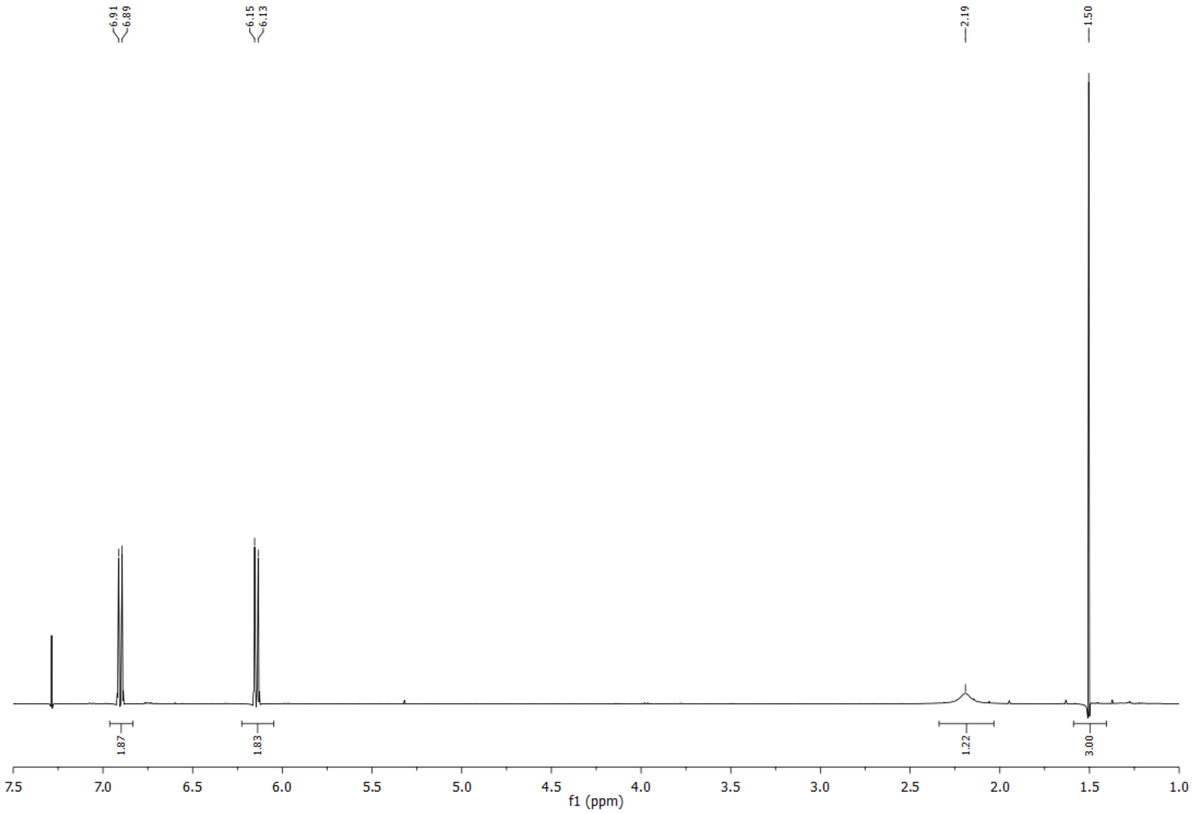


rhodamine-azide

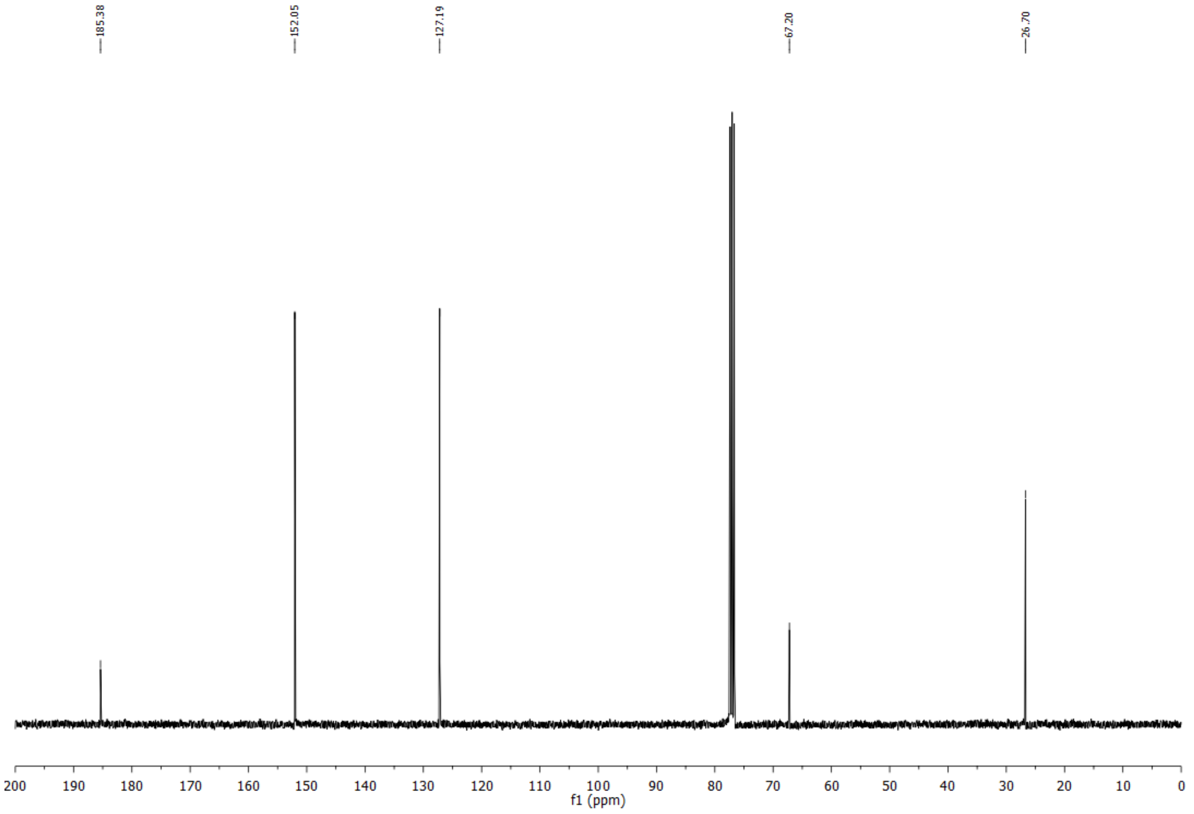
Figure S2. Structure of rhodamine-azide and biotin-rhodamine-azide (trifunctional linker).[7]

5) NMR-Spectra

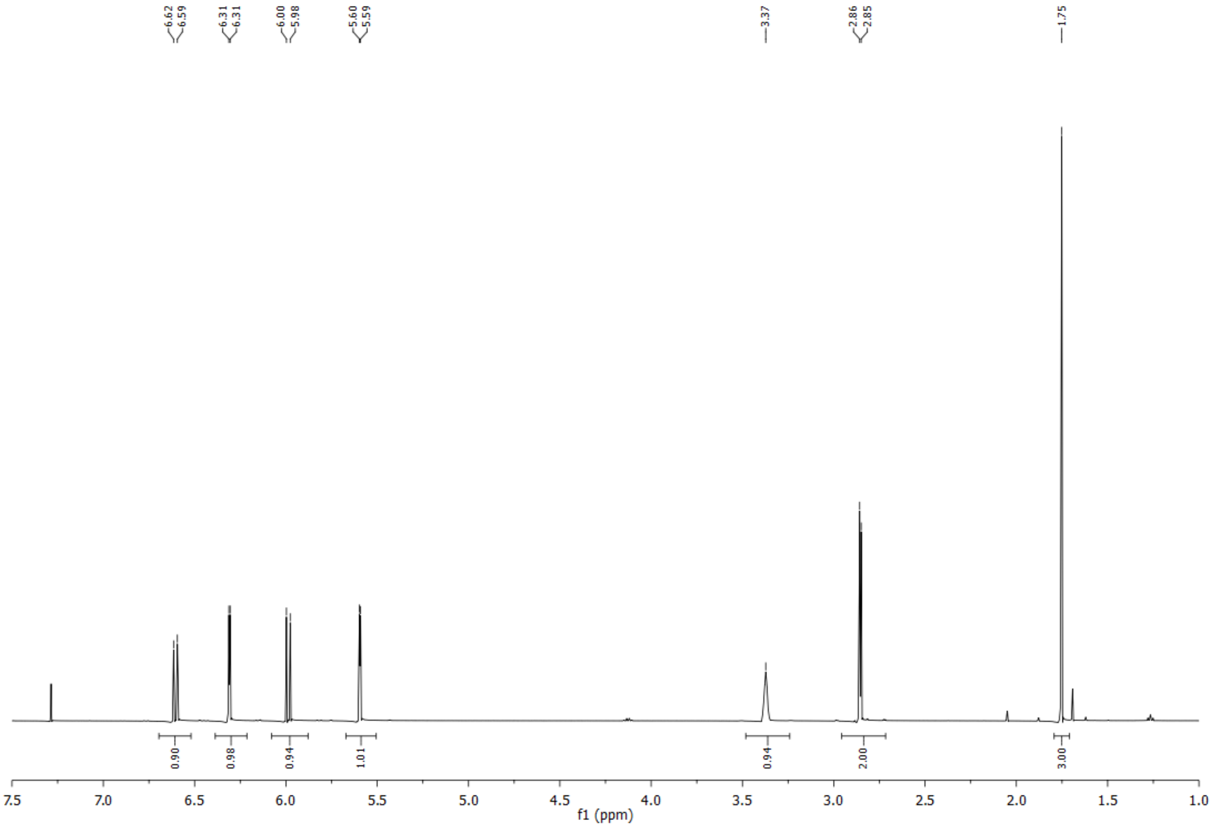
^{1}H-NMR (500 MHz, $CDCl_3$) of **2**



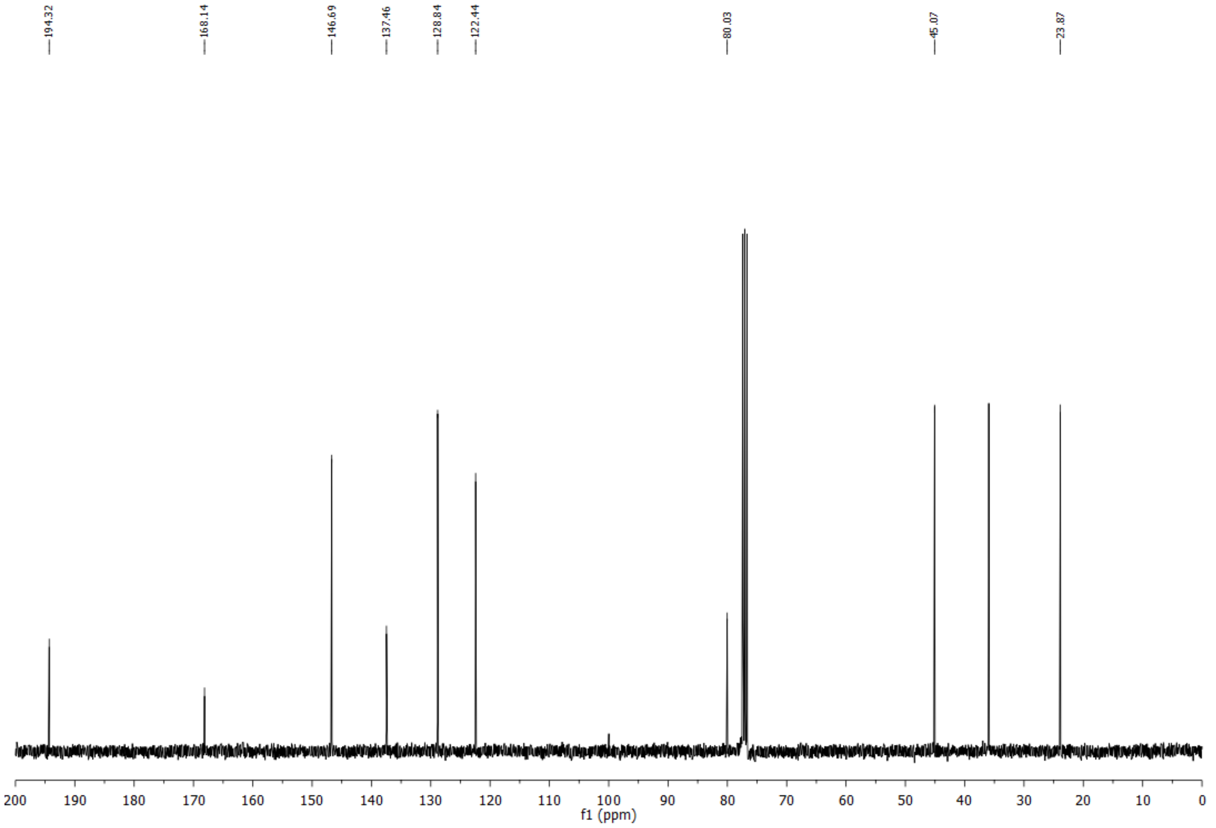
^{13}C-NMR (90 MHz, $CDCl_3$) of **2**



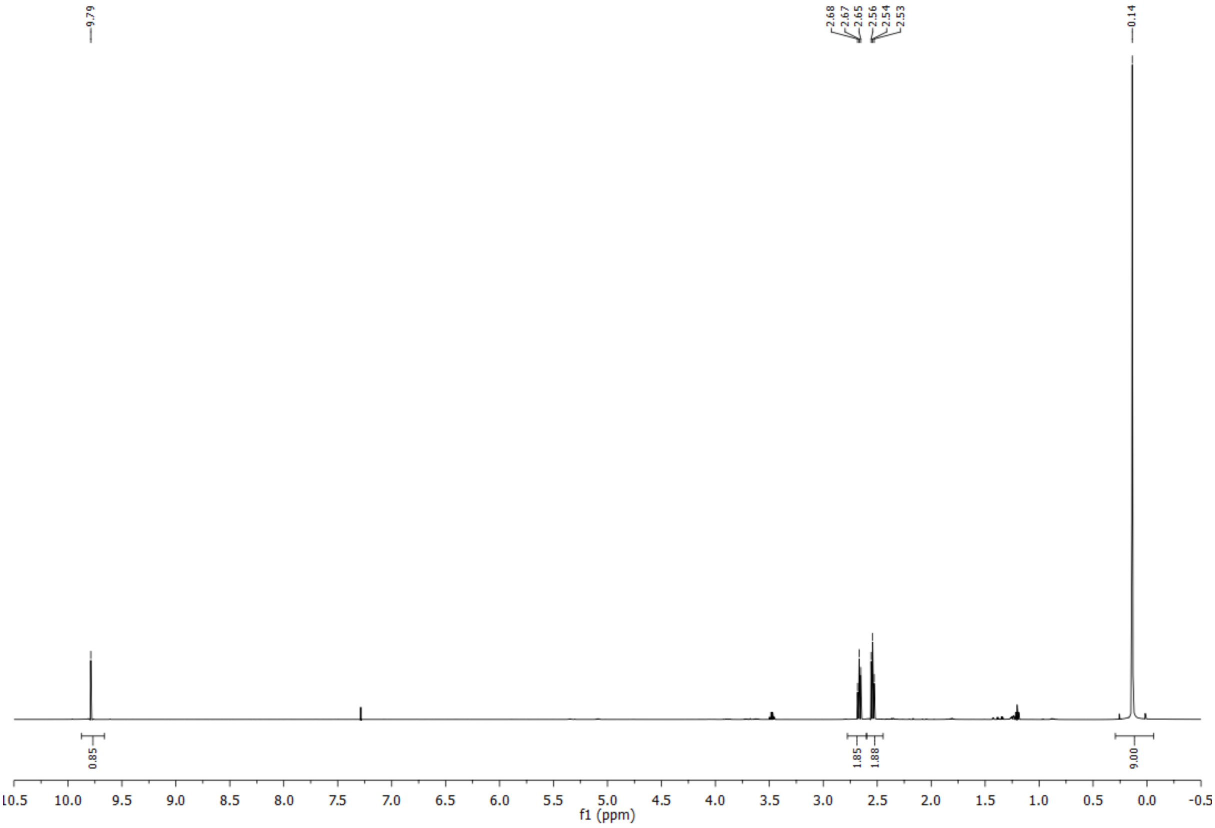
^{1}H-NMR (500 MHz, $CDCl_3$) of **3**



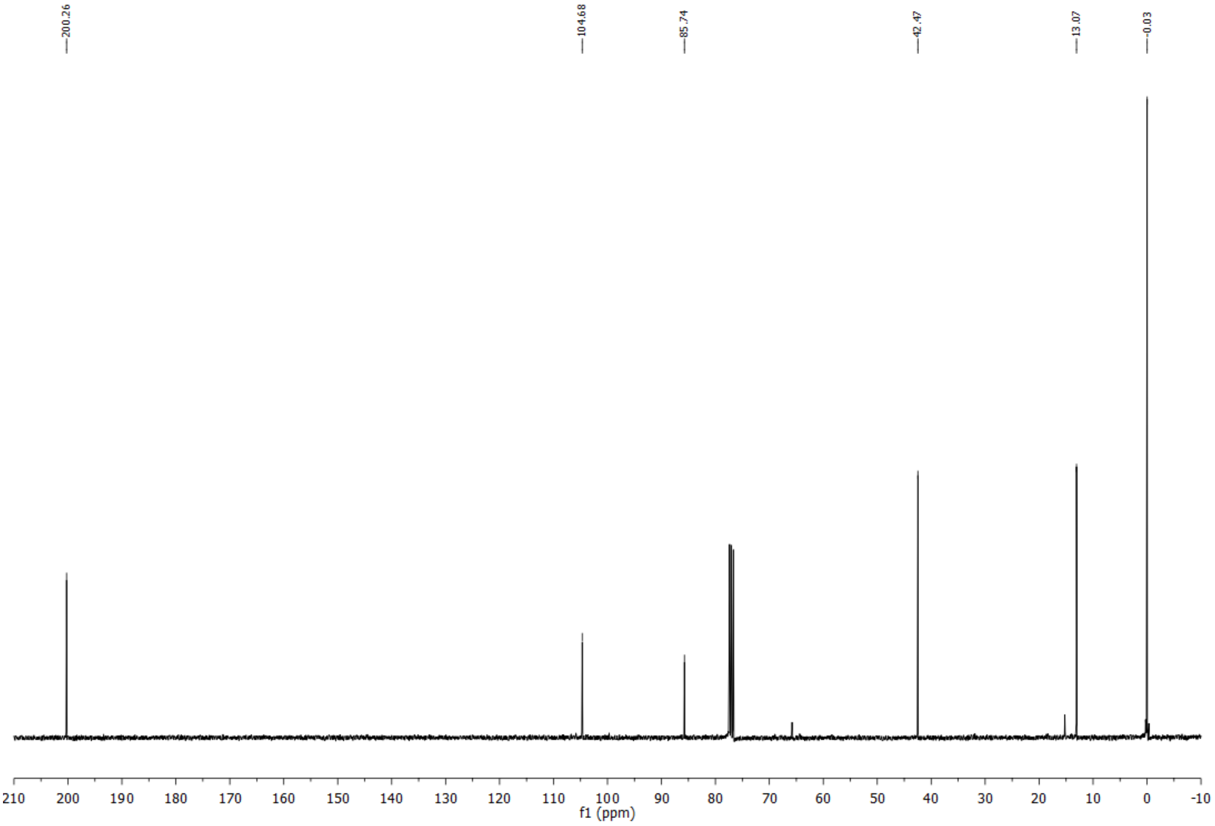
^{13}C-NMR (90 MHz, $CDCl_3$) of **3**



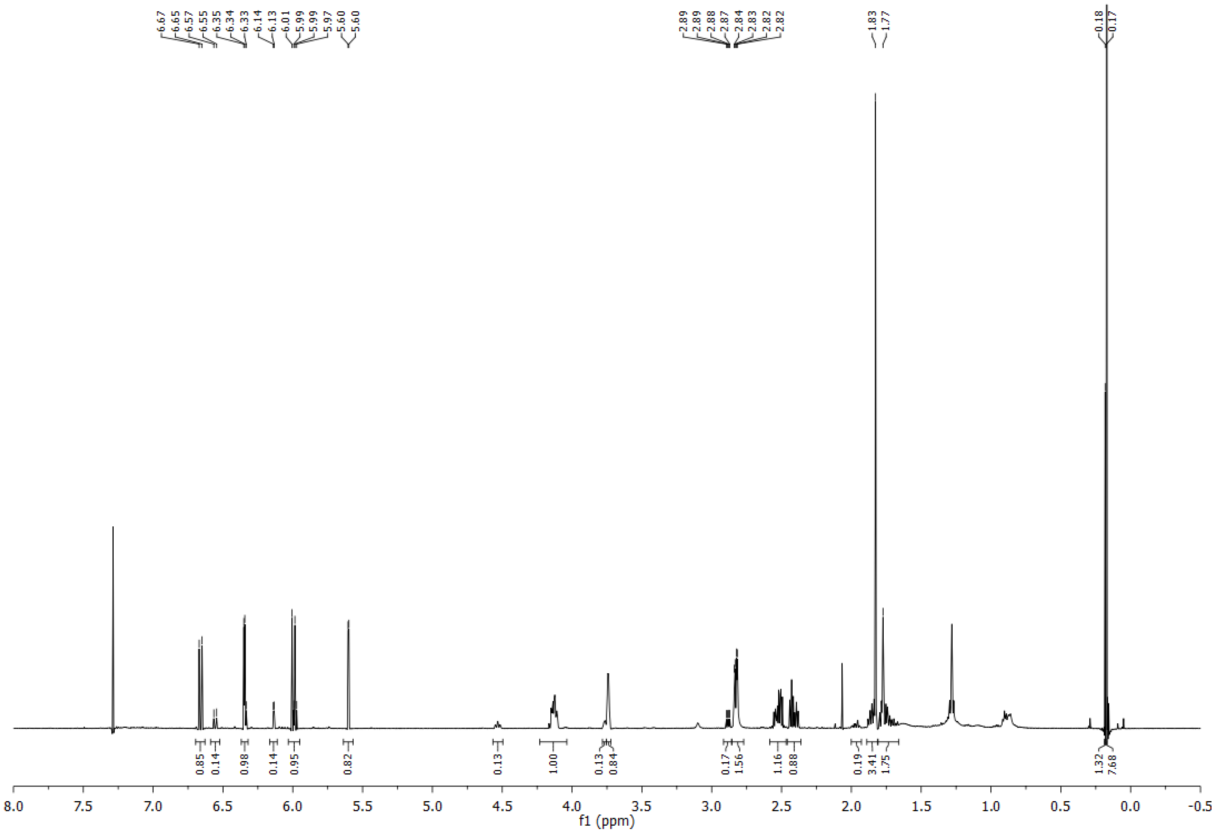
^{1}H-NMR (500 MHz, CDCl3) of **5**



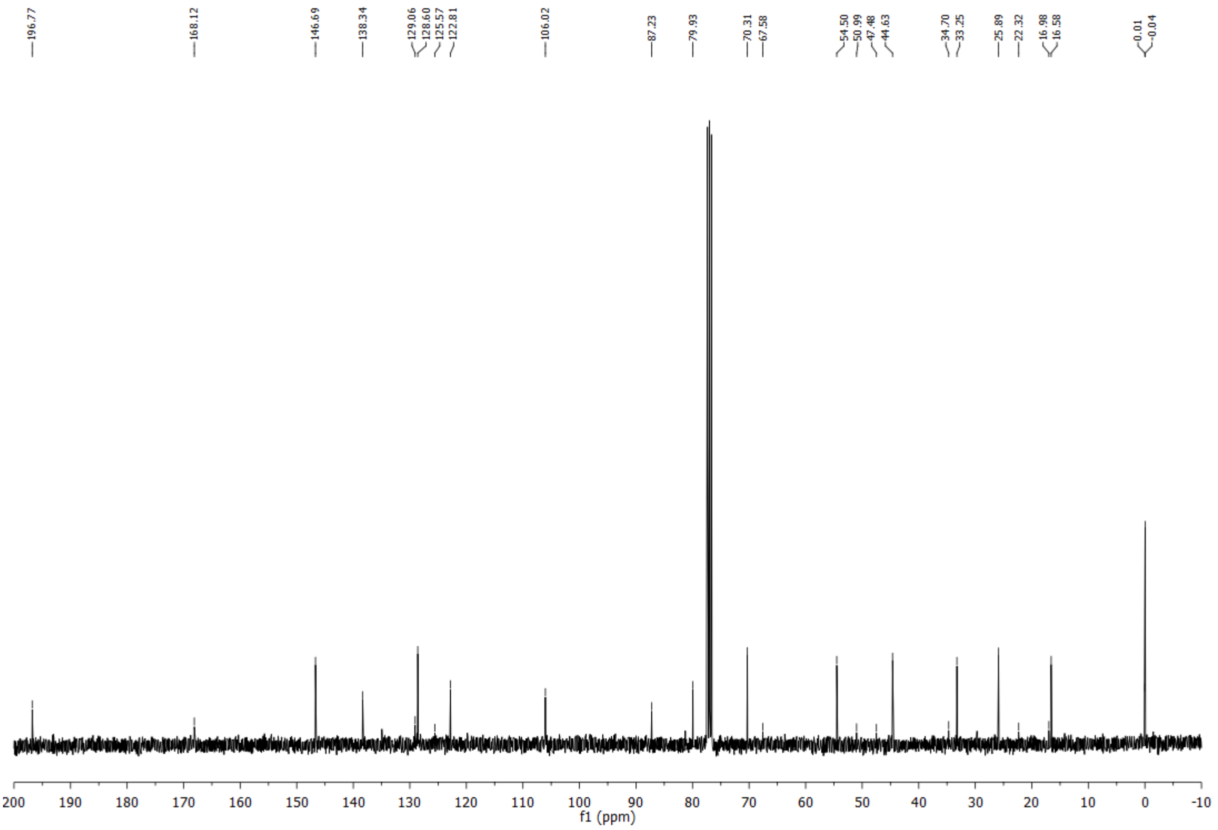
^{13}C-NMR (90 MHz, $CDCl_3$) of **5**



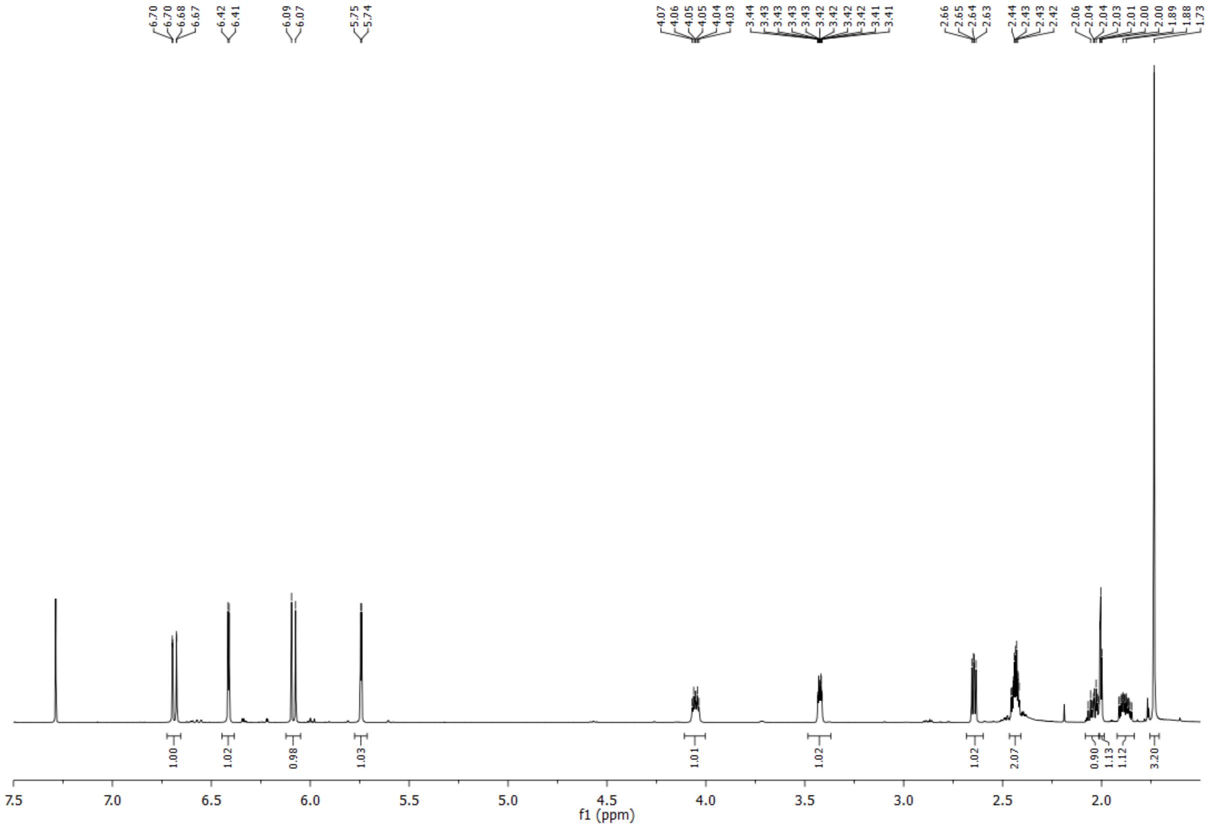
^{1}H-NMR (500 MHz, CDCl$_3$) of **6**



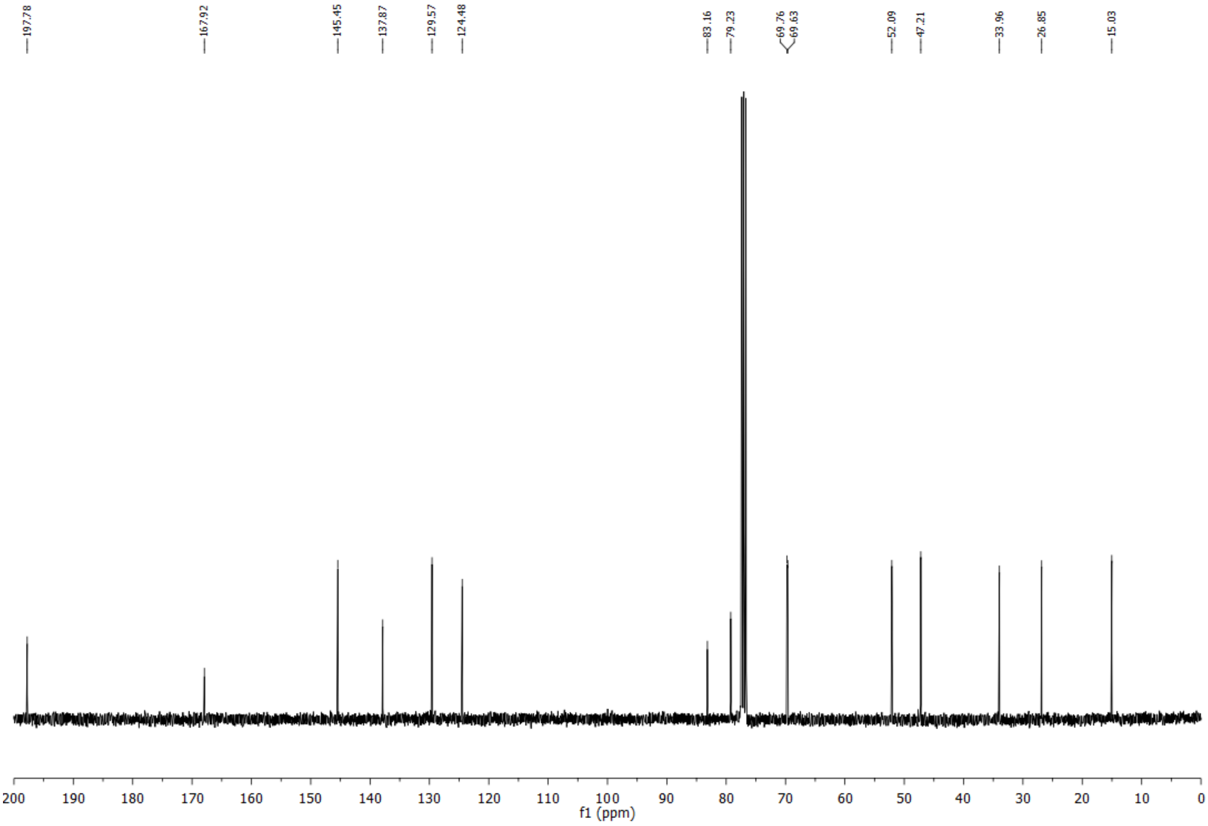
^{13}C-NMR (90 MHz, CDCl$_3$) of **6**



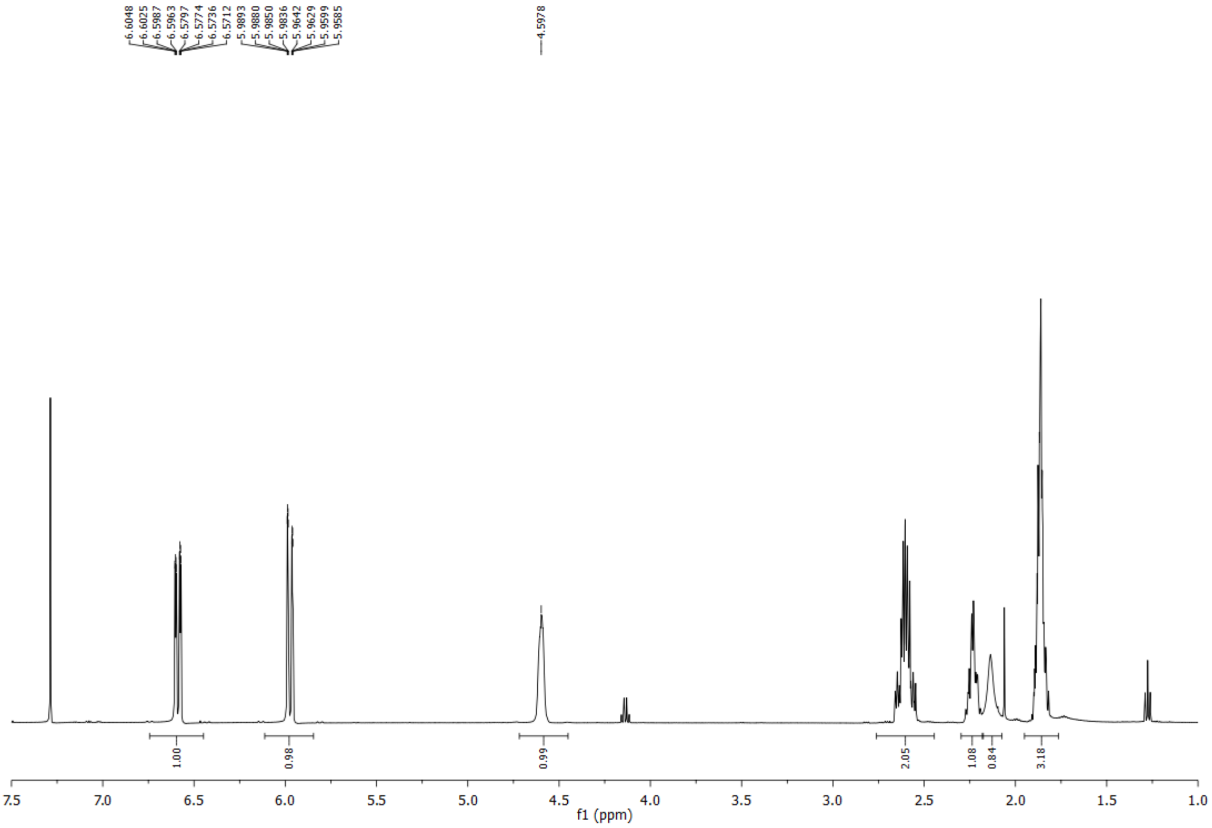
^{1}H-NMR (500 MHz, CDCl$_3$) of **7**



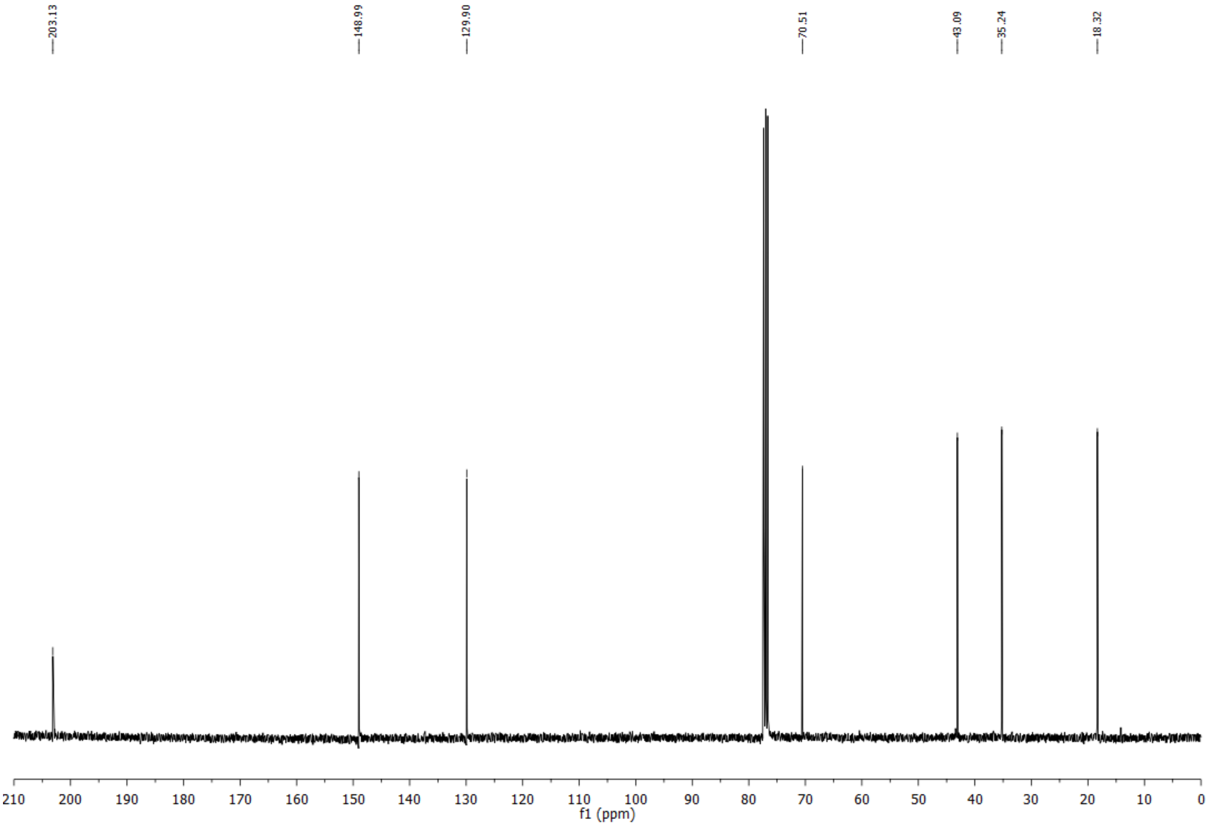
^{13}C-NMR (90 MHz, CDCl$_3$) of **7**



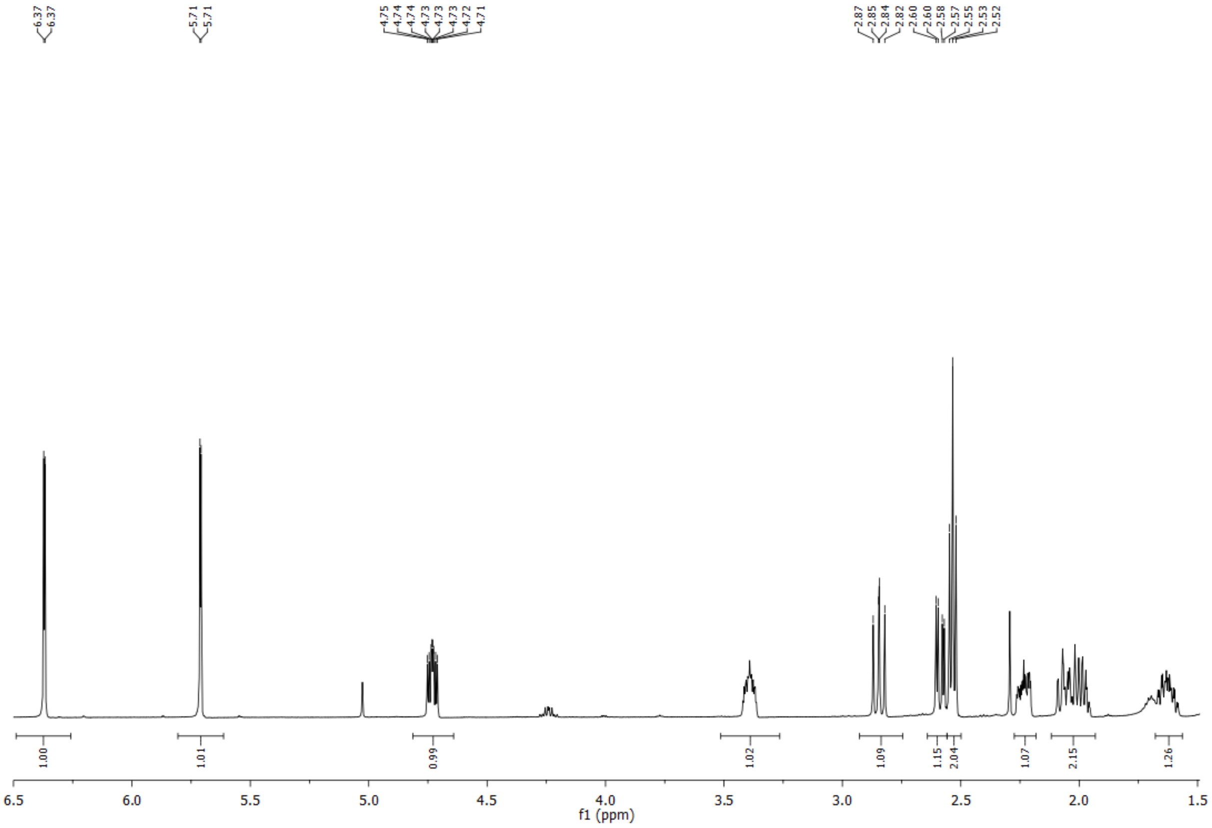
^{1}H-NMR (500 MHz, CDCl$_3$) of **9**



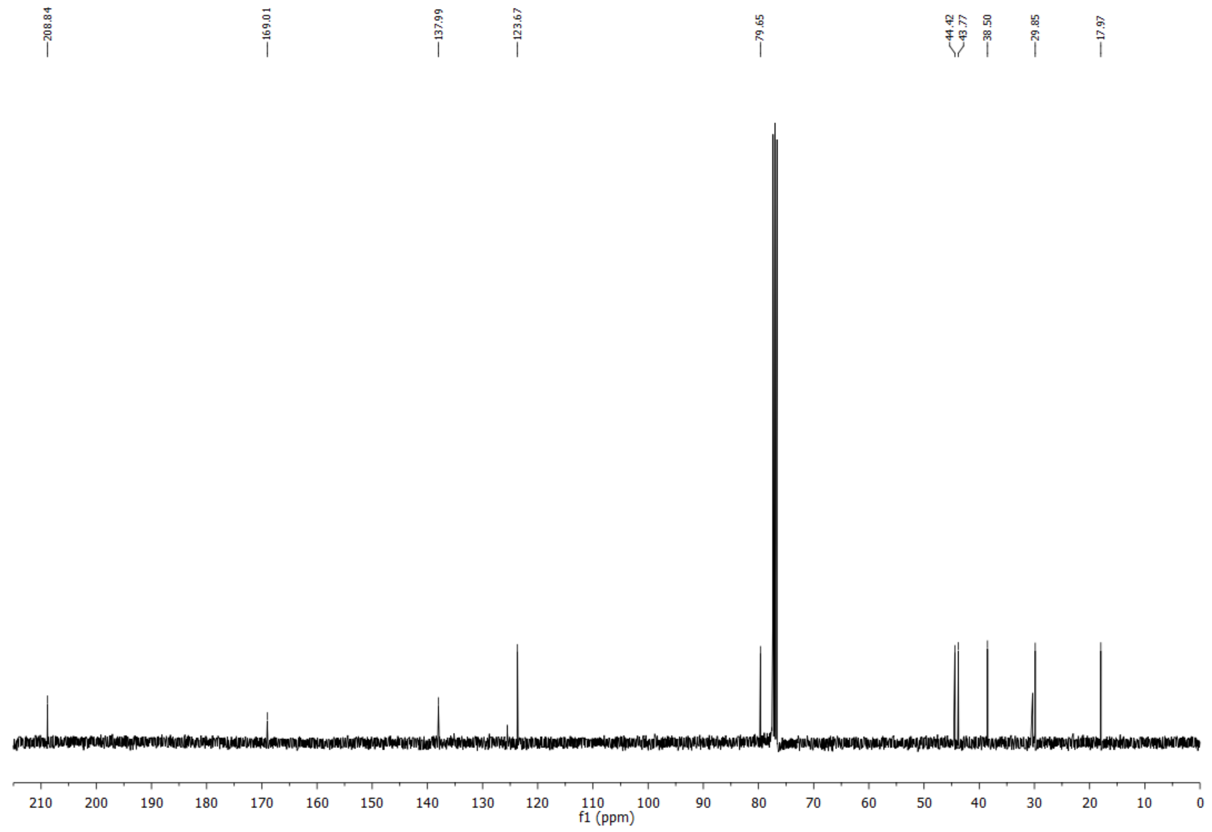
^{13}C-NMR (90 MHz, CDCl$_3$) of **9**



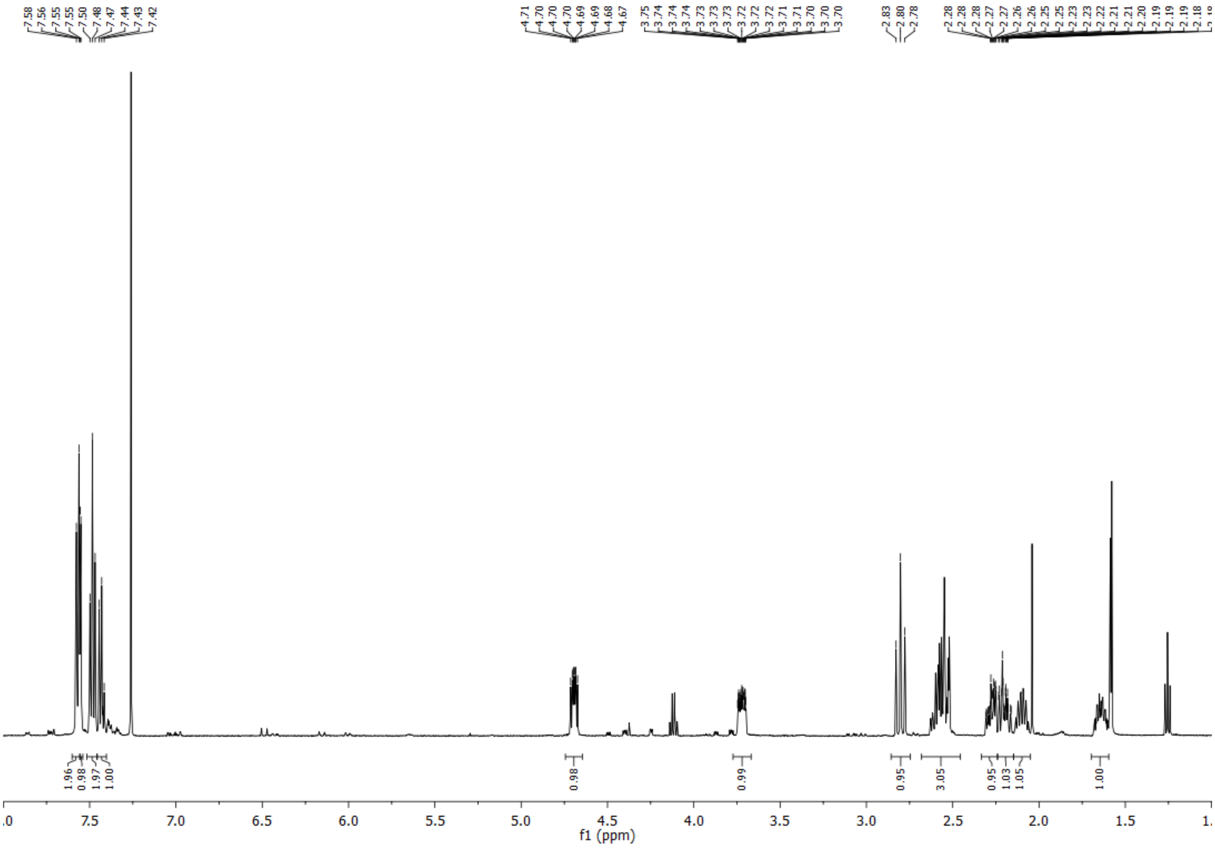
^{1}H-NMR (500 MHz, $CDCl_3$) of **10**



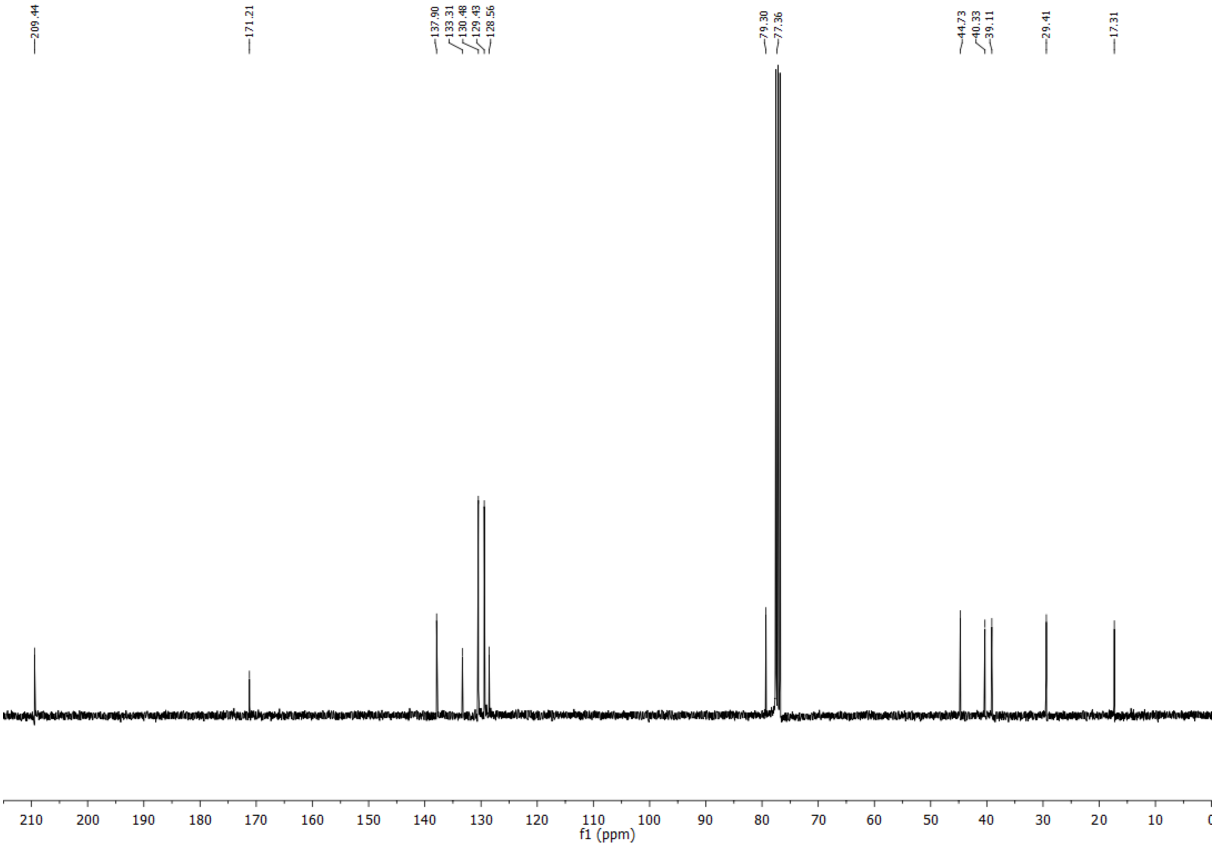
^{13}C-NMR (90 MHz, $CDCl_3$) of **10**



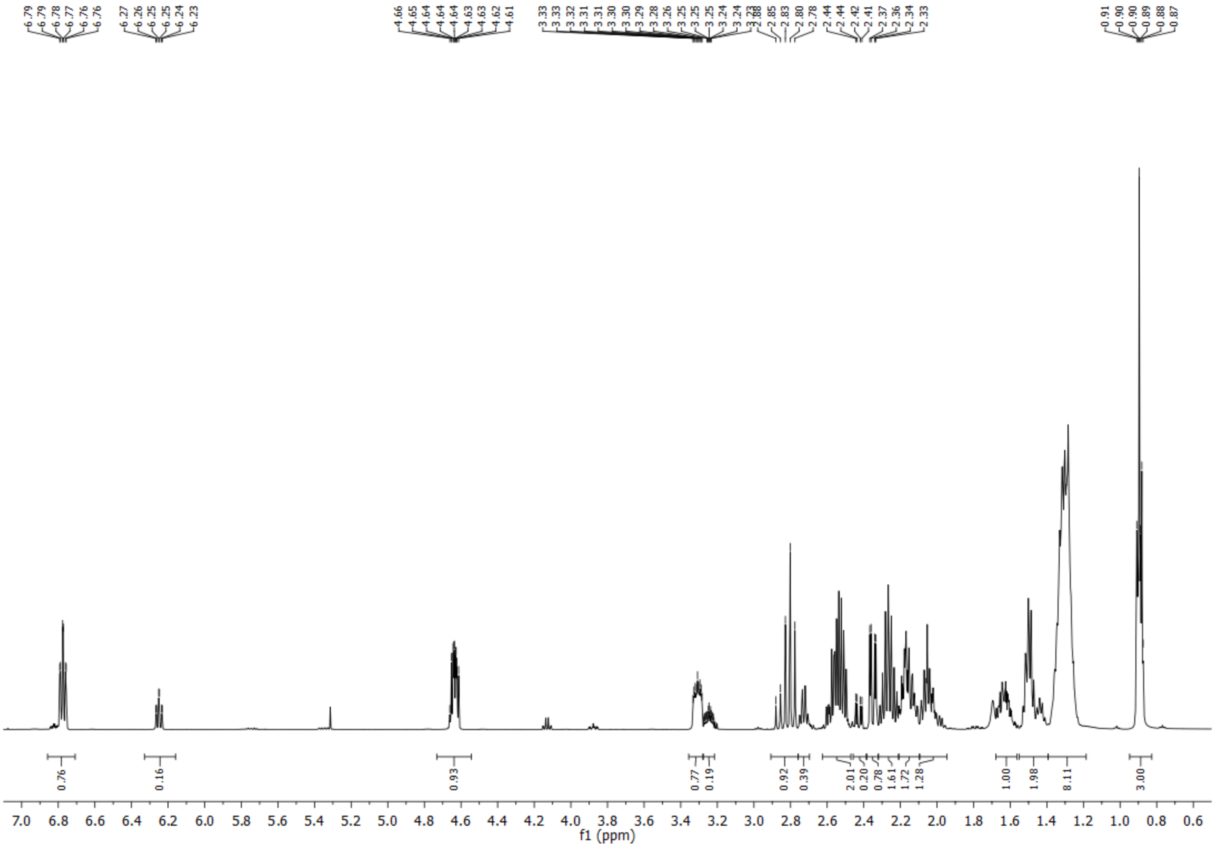
^{1}H-NMR (500 MHz, CDCl$_3$) of **11**



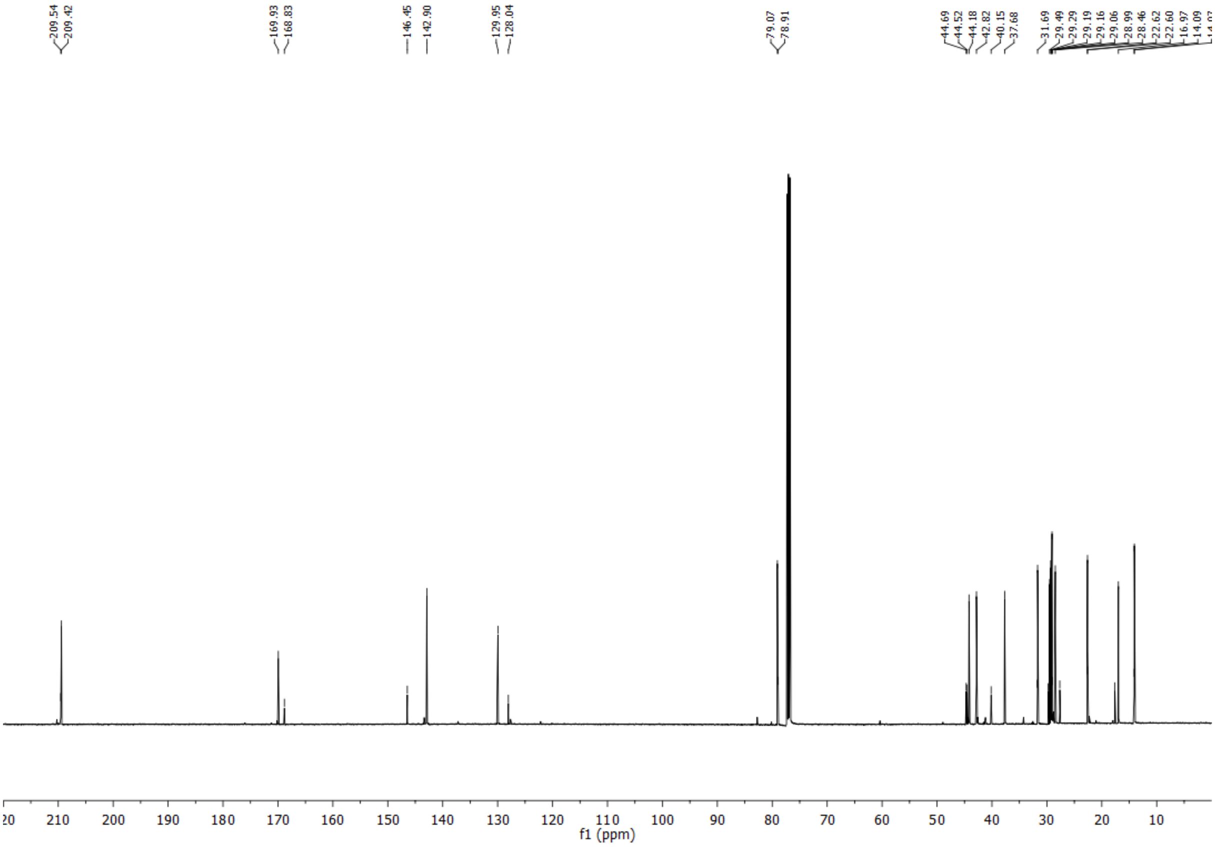
^{13}C-NMR (90 MHz, CDCl$_3$) of **11**



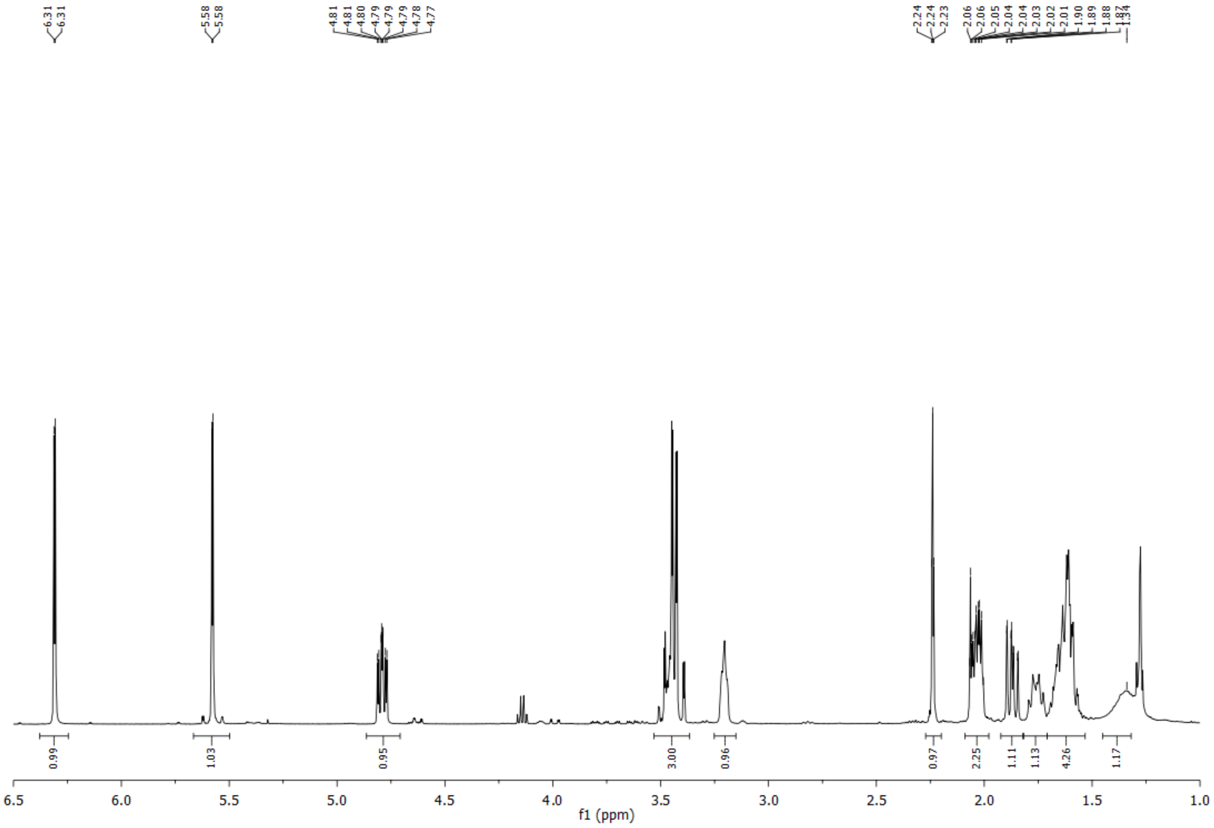
^{1}H-NMR (500 MHz, $CDCl_3$) of **12**



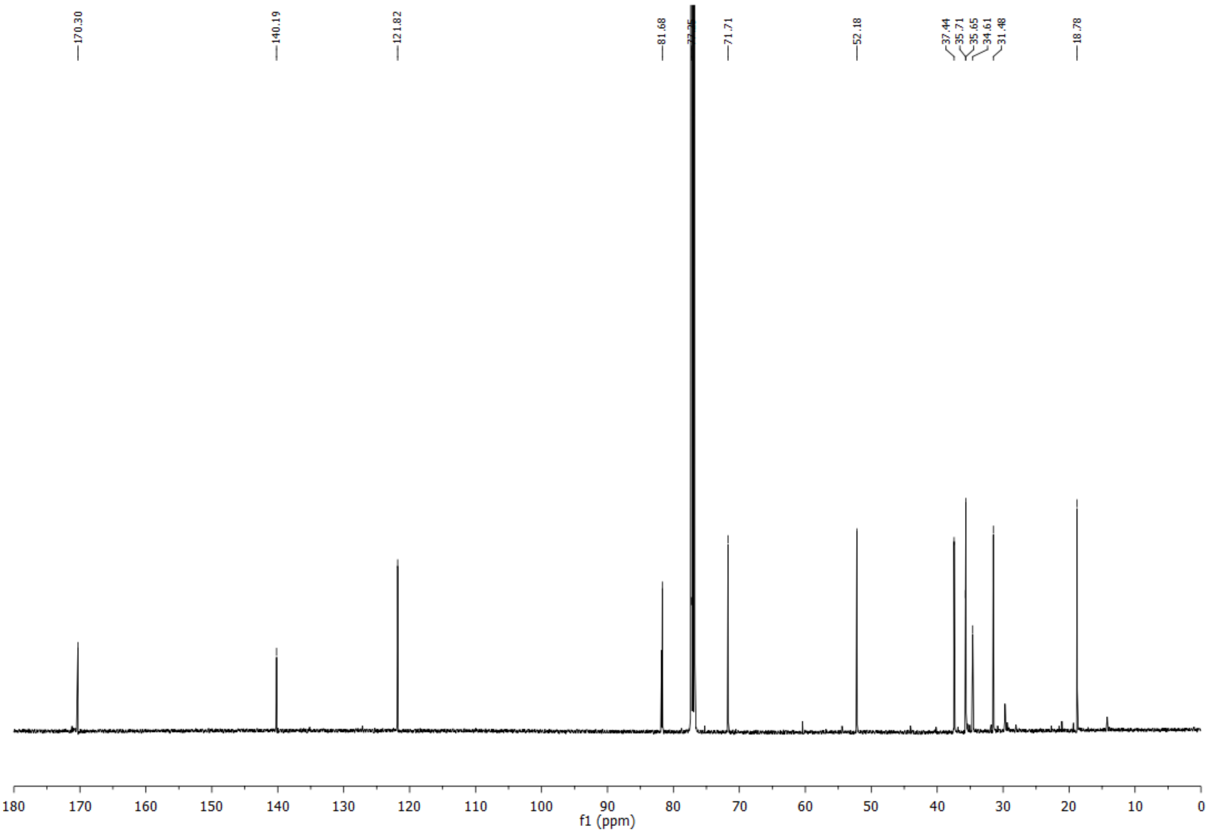
^{13}C-NMR (90 MHz, $CDCl_3$) of **12**



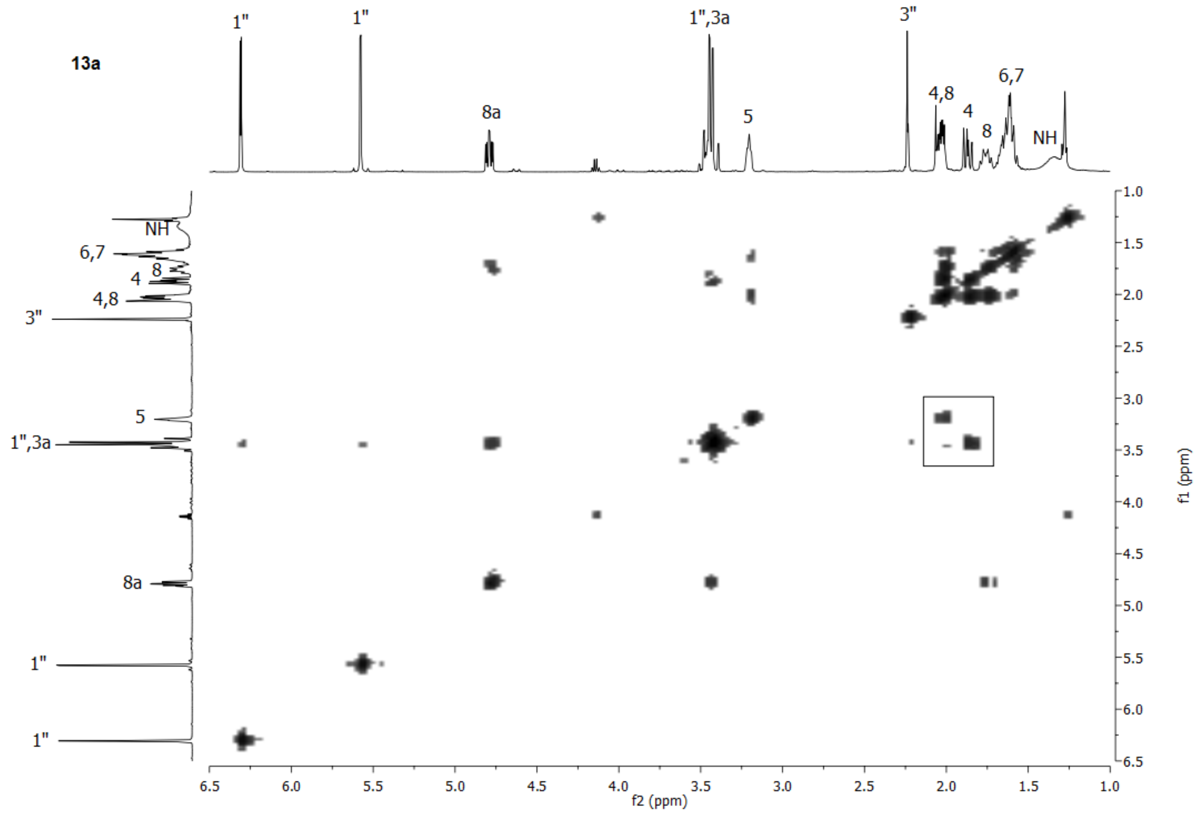
^{1}H-NMR (500 MHz, CDCl$_3$) of **13a**

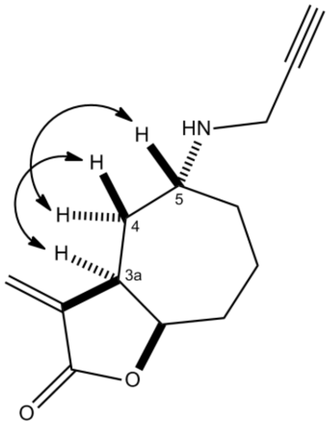


^{13}C-NMR (90 MHz, CDCl$_3$) of **13a**



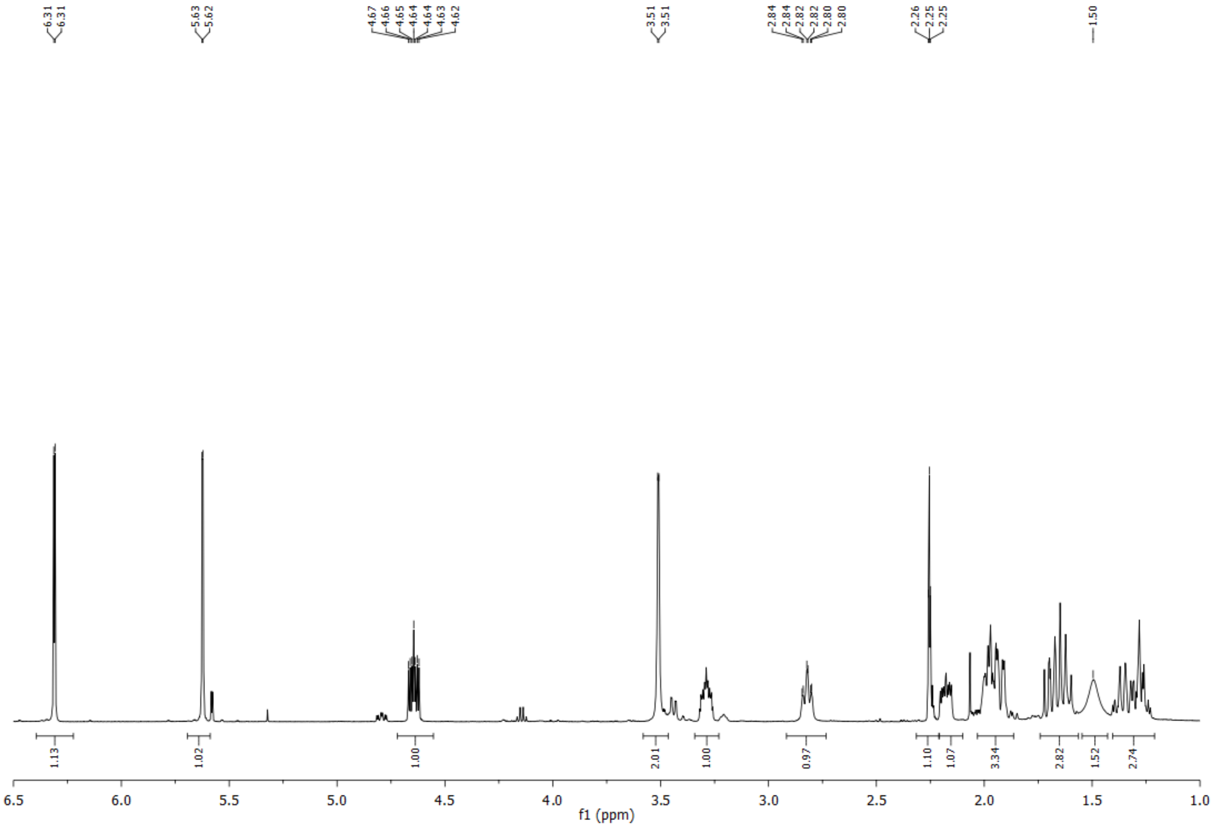
^{1}H,^{1}H-COSY-NMR (500 MHz, CDCl$_3$) of **13a**



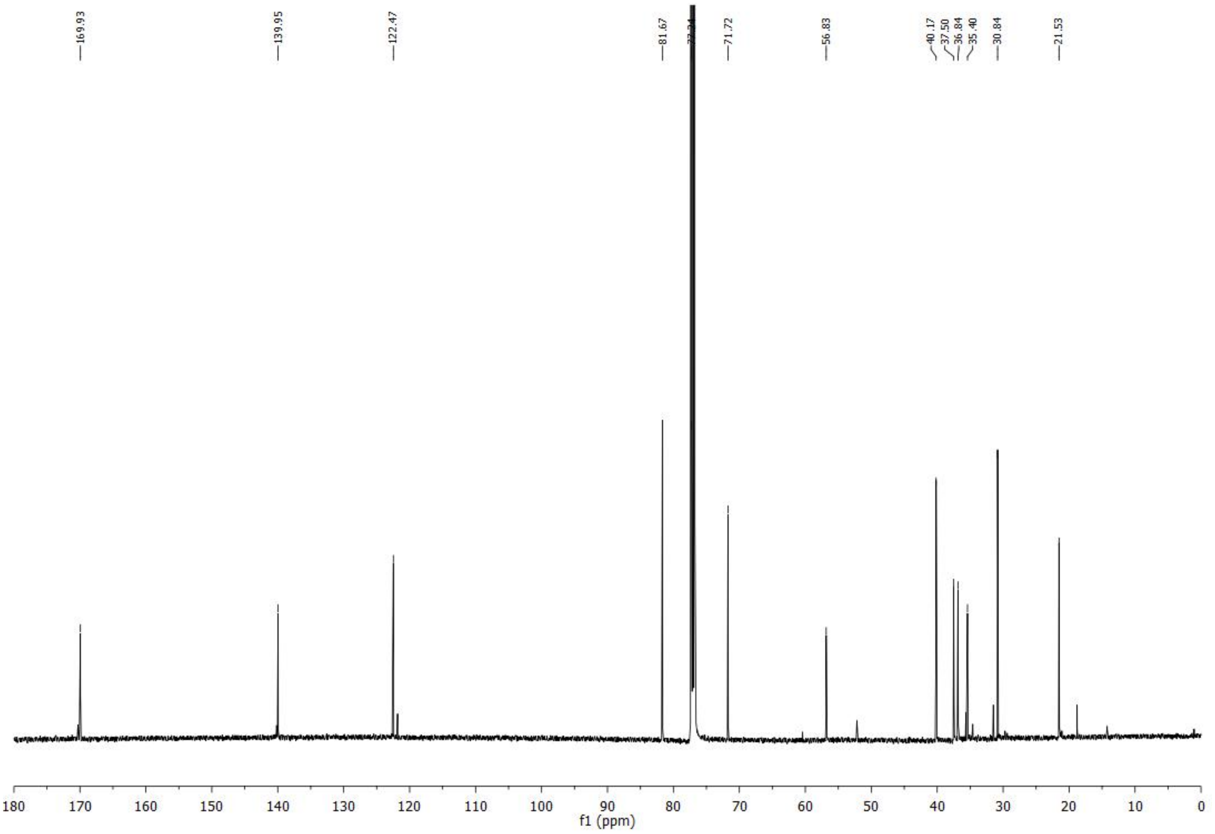


3a-H couples to 4-H$_a$, 5-H to 4-H$_b$

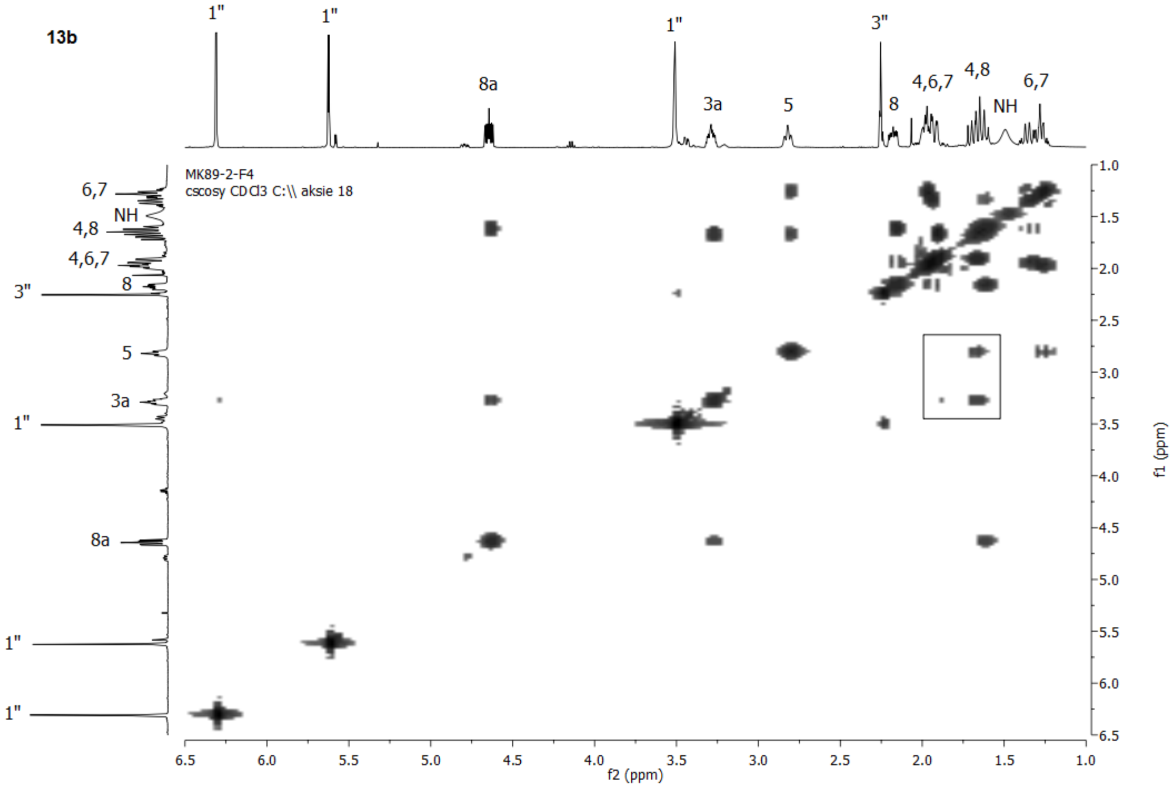
^{1}H-NMR (500 MHz, $CDCl_3$) of **13b**

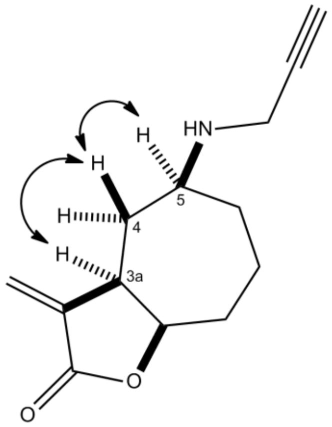


^{13}C-NMR (90 MHz, $CDCl_3$) of **13b**



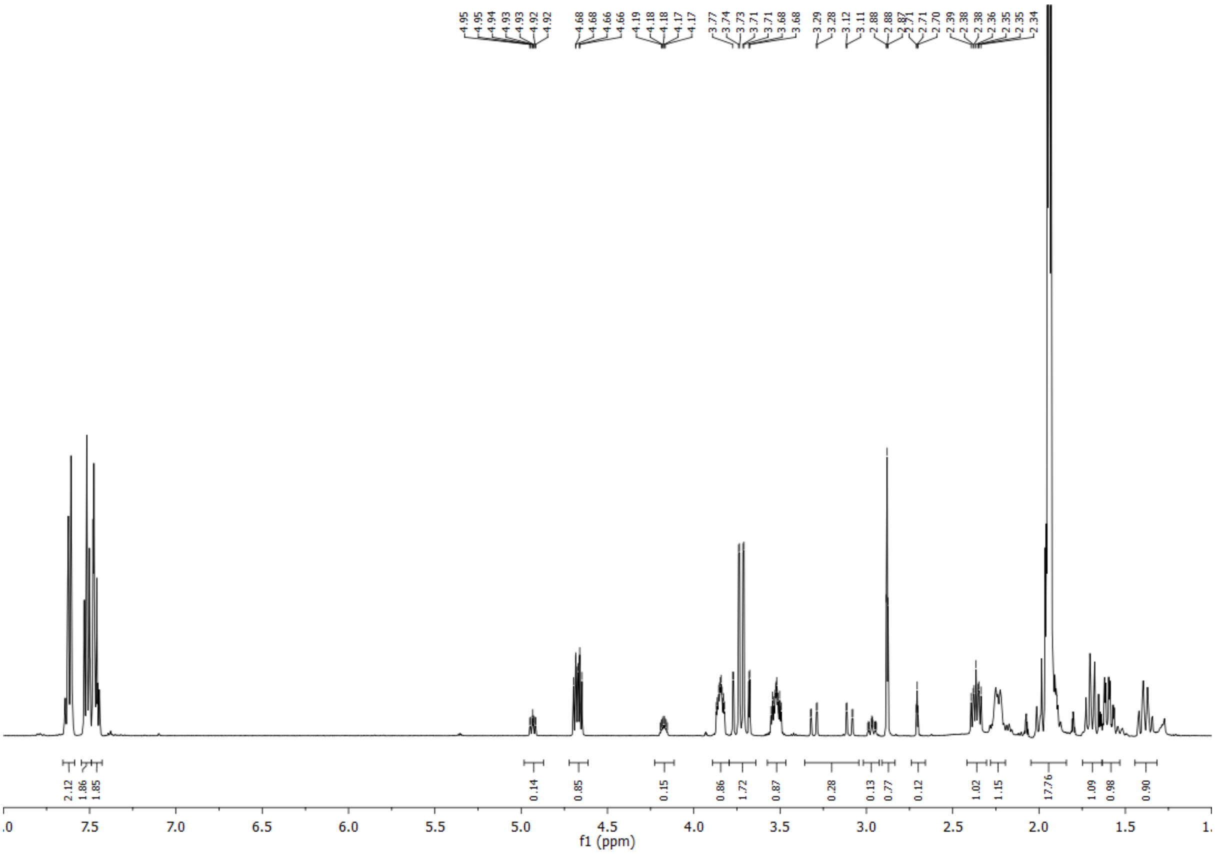
^{1}H,^{1}H-COSY-NMR (500 MHz, CDCl$_3$) of **13b**



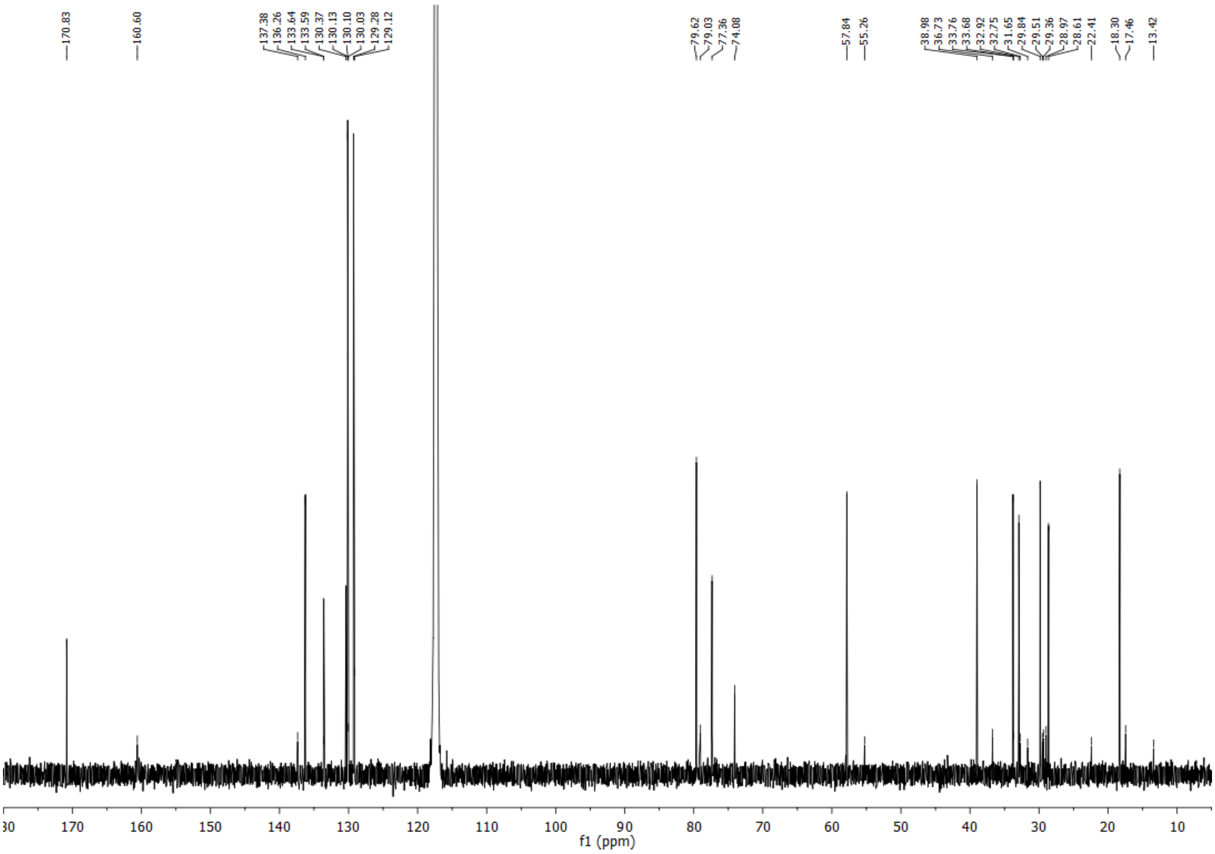


3a-H and 5-H couple to 4-H$_a$

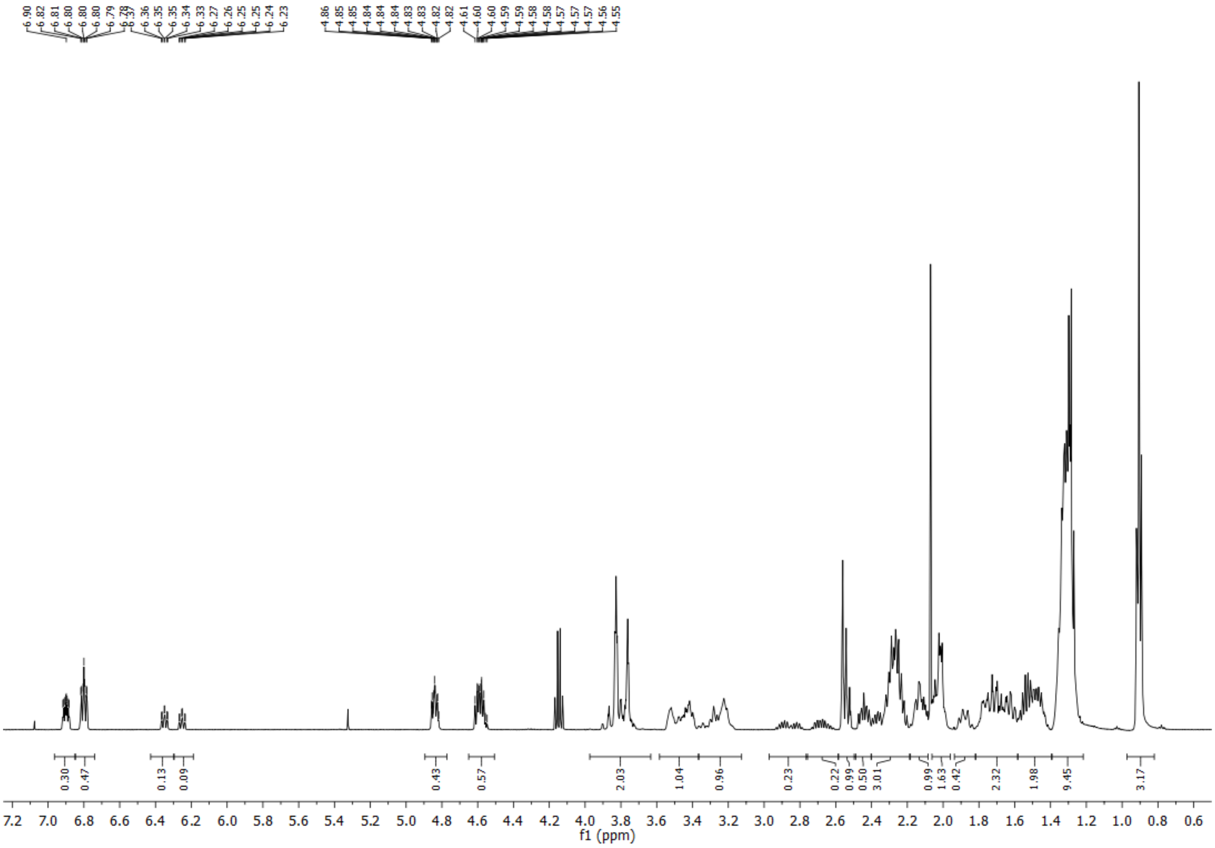
^{1}H-NMR (500 MHz, CDCl$_3$) of **14**



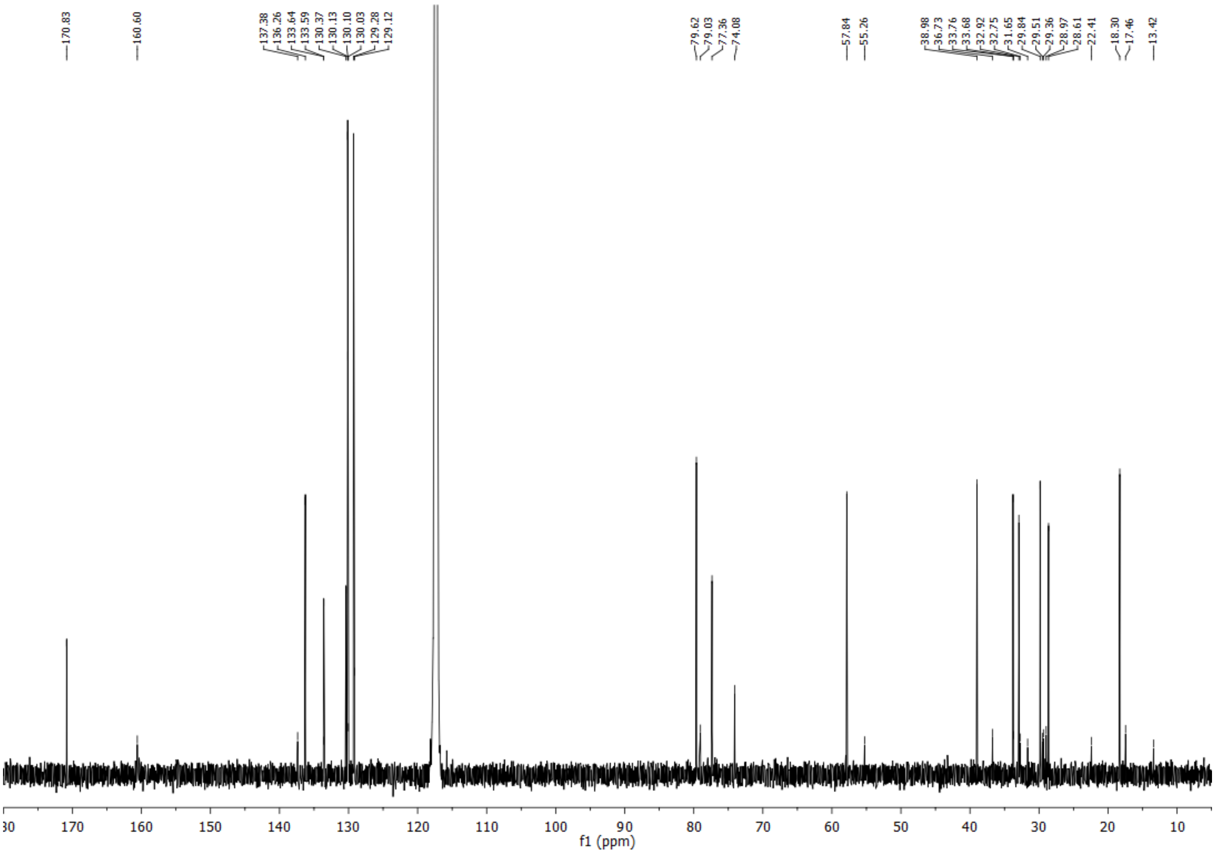
^{13}C-NMR (90 MHz, CDCl$_3$) of **14**



^{1}H-NMR (500 MHz, CDCl$_3$) of **15**



^{13}C-NMR (90 MHz, CDCl$_3$) of **15**



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