A new route towards fimbrolide analogues: importance of the exomethylene motif in LuxR dependent quorum sensing inhibition

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Supplementary Information

I. SYNTHESIS

I.1. General

Solvents were distilled and dried before use: dichloromethane from calcium hydride, tetrahydrofuran from sodium benzophenone ketyl. Organic solutions were dried over anhydrous sodium sulfate. The reactions were performed under a constant flow of nitrogen. The reactions were monitored by t.l.c. on Silica Gel 60 F254 (Merck) and detection was carried out by UV light (254 nm) and/or charring with a 5 % phosphomolybdic acid solution in ethanol containing 10 % of H_2SO_4, or a 1 % potassium permanganate solution in water. Silica gel (Kieselgel 60, 70-230 mesh ASTM, Merck) was employed for column chromatography. Melting points were determined on a Kofler block apparatus.

The ^{1}H NMR (300 MHz or 400 MHz) and ^{13}C NMR (75 MHz or 100 MHz) spectra were recorded with a Brucker ALS300, DRX300, and DRX400 spectrometers. Chemical shifts are given in ppm. Coupling constants are expressed in Hertz and splitting pattern abbreviations are: s, singulet; d, doublet; t, triplet; q, quartet; m, multiplet; M, massif, p, pseudo. Multiplicity (^{13}C NMR) was determined by DEPT sequences. High resolution mass spectra were obtained by electro spray technique, positive mode with a ThermoFinnigan MAT 95 XL spectrometer. **α-phenylthio-γ-butyrolactone (4)** obtained following the procedures reported in literature.[1]

I.2. General procedure A for aldolisation reaction of lactone 4

To a stirred solution of lithium diisopropylamide (LDA, 1.5 eq), prepared from diisopropylamine (2 eq.) and 1.6 M of n-BuLi in hexane (1.5 eq.), in anhydrous THF at -78 °C, was slowly added a solution of lactone **4** (1 eq) in THF. The temperature was raised to -50 °C and the solution was stirred for 1 h. Then, a solution of aldehyde (1 eq.) in THF was added dropwise. The crude mixture was left at -50 °C for 2 h, at 0 °C for 30 mn and it was poured over saturated ammonium chloride solution. The ether extracts were dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by column chromatography through silica gel.

I.2.1. 3-butyl-3-(phenylthio)dihydrofuran-2(*3H*)-one (5a)

Diisopropylamine (3.6 mL, 25.7 mmol) was added to anhydrous THF (20 mL) under nitrogen. After cooling the solution to 0 °C, n-butyllithium (12 mL of a 1.6 M solution in hexane, 19.2 mmol) was added and the reaction mixture was stirred for 30 min. The solution was then colled to -78 °C and subsequently treated with the lactone **4** (2.5 g, 12.8 mmol), dissolved in dry THF (4 mL). After 30 min, a solution of iodobutane (1.5 mL, 12.8 mmol) dissolved in 1 eq. (1.4 mL) of hexamethylphosphoramide (HMPA) was added. When addition was complete, stirring was maintained at -78 °C for 4 h. The reaction was then allowed to warm to room temperature and stirred overnight. The mixture was then quenched by addition of saturated solution of ammonium chloride**.** The phases were separated and the aqueous layer was extracted with 3 portions (60 mL) of ether. The combined organic extract was washed with water, and saturated NaCl solution (50 mL portions of each). The organic solution was dried over anhydrous Na_2SO_4, and concentrated in vacuum to yield the desired crude products as slightly yellow oils. This crude product was purified by flash chromatography through silica gel using pentane-ether (8/2) as eluent to provide **5a** (2.6 g, 76 %) as mixture of enantiomers. Orange oil; Rf (SiO_2 ; P/Et_2O : 8/2) = 0.23. ^{1}H NMR (300MHz, $CDCl_3$) : δ 0.89 (t, *J* = 7.2Hz, 3H); 1.20 – 1.70 (m, 6H); 2.24 (m, 1H); 2.45 (m, 1H) ; 4.22 (m, 2H); 7.33-7.40 (m, 3H); 7.53 (m, 2H). ^{13}C NMR (75MHz, $CDCl_3$) : δ 14.0; 22.8; 26.7; 34.3; 34.6; 54.1; 65.1; 129.1; 129.5 (2C); 130.1 (2C); 137.2; 176.2. HRMS (CI+): Calculated for $C_{14}H_{18}O_2SNa^+$, 273.0920; founded MNa^+ = 273.0919.

I.2.2. 3-hexyl-3-(phenylthio)dihydrofuran-2(*3H*)-one (5b)

Diisopropylamine (3.6 mL, 25.7 mmol) was added to anhydrous THF (20 mL) under nitrogen. After cooling the solution to 0 °C, n-butyllithium (12 mL of a 1.6 M solution in hexane, 19.2 mmol) was added and the reaction mixture was stirred for 30 min. The solution was then colled to -78 °C and subsequently treated with the lactone **4** (2.5 g, 12.8 mmol), dissolved in dry THF (4 mL). After 30 min, a solution of iodohexane (1.9 mL, 12.8 mmol) dissolved in 1 eq. (1.4 mL) of hexamethylphosphoramide (HMPA) was added. When addition was complete, stirring was maintained at -78 °C for 4 h. The reaction was then allowed to warm to room temperature and stirred overnight. The mixture was then quenched by addition of saturated solution of ammonium chloride**.** The phases were separated and the aqueous layer was extracted with 3 portions (60 mL) of ether. The combined organic extract was washed with water, and saturated NaCl solution (50 mL portions of each). The organic solution was dried

over anhydrous Na_2SO_4, and concentrated in vacuum to yield the desired crude products as slightly yellow oils. This crude product was purified by flash chromatography through silica gel using pentane-ether (80:20) as eluent to provide **5b** (2.7 g, 64 %) as mixture of enantiomers. Orange oil; Rf (SiO_2; P/Et_2O : 80/20) = 0.29 and 0.47. ^{1}H NMR (300MHz, $CDCl_3$) : δ 0.81 (t, *J* = 7.2Hz, 3H); 1.22 (m, 8H); 1.50-1.78 (m, 2H); 2.19 (m, 1H); 2.35 (m, 1H) ; 4.17 (m, 2H); 7.30-7.36 (m, 3H); 7.40-7.50 (m, 2H). ^{13}C NMR (75MHz, $CDCl_3$) : δ 14.1; 22.6; 24.5; 29.3; 31.7; 34.3; 34.8; 54.1; 65.1; 129.1 (2C); 129.4; 130.1; 137.2 (2C); 176.2. HRMS (CI+): Calculated for $C_{16}H_{22}O_2SNa^+$, 301.1233; founded MNa^+ = 301.1232.

I.2.3. 3-(1-hydroxybutyl)-3-(phenylthio)dihydrofuran-2(*3H*)-one (5c)

According to general procedure A, freshly distilled butanal (926 µL, 10.3 mmol), LDA (1.5 eq), and lactone **4** (2 g, 10.3 mmol) gave the products **5c** (2.2 g, 80 %) as mixture of 3 diastereoisomers (by TLC) after purification by flash chromatography on silica gel. Eluent: dichloromethane-ether (97/3). Characterization of one diastereoisomer was done: yellow solid; mp = 97- 98 °C; Rf (SiO_2; CH_2Cl_2/Et_2O: 97/3) = 0.31. ^{1}H NMR (300MHz, $CDCl_3$) : δ 0.89 (t, *J* = 7.2Hz, 3H); 1.24 (m, 2H); 1.55 (m, 2H); 1.88 (ddd, *J* = 7.5; 6.6; 1.7Hz, 1H); 2.7 (m, 1H); 3.23 (s, 1H, OH); 3.78 (dd, *J* = 9; 2.8 Hz, 1H); 4.2 (m, 2H); 7.1-7.34 (m, 3H); 7.44 (m, 2H). ^{13}C NMR (75MHz, $CDCl_3$) : δ 14.0; 19.4; 29.0; 32.3; 58.1; 65.7; 70.5; 128.4; 129.1 (2C); 130.3 (2C); 137.2; 175.8. HRMS (CI+): Calculated for $C_{14}H_{18}O_3SNa^+$, 289.0869; founded MNa^+: 289.0870.

I.2.4. 3-(1-hydroxyhexyl)-3-(phenylthio)dihydrofuran-2(*3H*)-one (5d)

According to general procedure A, hexanal (614 µl, 5.12 mmol), LDA and lactone **4** (1 g, 5.12 mmol) gave the products **5d** (836 mg, 55 %) were obtained as mixture of diastereoisomers as yellow oil after purification by flash chromatography on silica gel. Eluent: pentane-ether (6/4). Characterization of one diastereoisomer was done: yellow oil; Rf (SiO_2; P/Et_2O: 60/40) = 0.29. ^{1}H NMR (300MHz, $CDCl_3$) : δ 0.87 (t, *J* = 7.0Hz, 3H); 1.29 (m, 6H); 1.57-1.64 (m, 2H); 2.14 (ddd, *J* = 7.5; 6.6; 1.7Hz, 1H); 2.55 (m, 1H); 3.19 [s(OH) , 1H]; 3.78 (d, *J* = 10.0Hz, 1H); 4.13 (m, 1H); 4.25 (p td, *J* = 8.9; 1.5Hz, 1H); 7.33-7.44 (m, 3H); 7.58 (m, 2H). ^{13}C NMR (75MHz, $CDCl_3$) : δ 14.3; 22.8 (2C); 26.4; 31.4; 32; 59.5; 65.8; 73.0; 129.0; 129.4 (2C); 130.6 (2C); 137.6; 175.7. HRMS (ESI+): Calculated for $C_{16}H_{22}O_3S\ Na^+$, 317.1187; founded MNa^+, 317.1186.

I.2.5. 3-(1-hydroxyoctyl)-3-(phenylthio)dihydrofuran-2(*3H*)-one (5e, 5e')

According to general procedure A, octanal (1.6 mL, 10.24 mmol), LDA and lactone **4** (2 g, 10.24 mmol), gave the diastereoisomers **5e, 5e'** [2.29 g (3/7), 69 %] in elution order after purification by repeated column chromatography through silica gel: Eluent 1 pentane-ether (6/4), Eluent 2 pure dichloromethane. **5e:** white solid; mp: 42-44°C; Rf (SiO_2; CH_2Cl_2) = 0.52. ^{1}H NMR of **5e** (300MHz, $CDCl_3$) : δ 0.87 (t, *J* = 7.0Hz, 3H); 1.26-1.37 (m, 10H); 1.64 (m, 2H); 2.14 (ddd, *J* = 14.1; 6.6; 1.7Hz, 1H); 2.55 (m, 1H); 3.18 (s, 1H, OH) ; 3.78 (d, *J* = 10.0Hz, 1H); 4.13 (m, 1H); 4.27 (p td, *J* = 9; 1.7Hz, 1H); 7.35-7.44 (m, 3H); 7.58 (m, 2H). ^{13}C NMR of **5e** (75MHz, $CDCl_3$) : δ 14.2; 22.7 (2C); 26.7; 29.3; 29.6; 31.3; 31.9; 59.3; 65.7; 72.8; 128.6; 129.3 (2C); 130.5 (2C); 137.5; 175.4. **5e':** white solid; mp: 54-56°C; Rf (SiO_2 ; CH_2Cl_2) = 0.44. ^{1}H NMR of **5e'** (300MHz, $CDCl_3$) : δ 0.90 (t, *J* = 7.0Hz, 3H); 1.32 (m, 10H); 1.57-1.65 (m, 1H); 2.00 (m, 1H); 2.15 (m, 1H); 2.74-2.85 (m, 1H); 3.61 (s, 1H, OH); 3.83 (m, 1H); 4.29 (m, 2H); 7.34-7.38 (m, 3H); 7.53 (m, 2H). ^{13}C NMR of **5e'** (75MHz, $CDCl_3$): δ 14.2; 22.8; 26.4; 29.0; 29.4; 29.6; 30.2; 32.0; 58.2; 65.7; 70.9; 129.2 (3C); 130.3 (2C); 137.3; 175,8. HRMS (ESI+): Calculated for $C_{18}H_{26}O_3S\ Na^+$, 345.1500; founded MNa^+, 345.1500.

I.3. General procedure B for oxydation-elimination reaction of lactones 5

To a stirred solution of lactones **5** in dichloromethane at 0 °C, was slowly added a solution of *m*-chloroperbenzoic acid (1.5 eq) in dichloromethane during 5 min. The solution was kept for 20 mn at 0 °C. The precipitate was filtered off and dichloromethane was added. The resulting organic phase was washed successively with aqueous 10 % sodium bisulfite, saturated $NaHCO_3$ solution, water, dried over anhydrous sodium sulphate and the solvent removed under reduced pressure to afford crude sulphoxides. This material was dissolved in toluene and was heated at reflux during 90 min. The solvent was removed under reduced pressure and the crude product was purified by column chromatography through silica gel.

I.3.1. 3-butylfuran-2(*5H*)-one (6a)[1]

According to general procedure B, lactones **5a** (1.2g, 4.7 mmol) and *m*-CPBA (1g, 6.3 mmol) gave furanone **6a** (530 mg, 81 %) after purification by flash chromatography on silica gel. Eluent: pentane-ether (60/40). Aspect: yellow oil; Rf (SiO_2; P/Et_2O: 60/40) = 0.15. ^{1}H NMR

(300MHz, $CDCl_3$): δ 0.93 (t, *J* = 7.3 Hz, 3H); 1.30-1.80 (m, 4H); 2.29 (m, 2H); 4.83 (dd, *J* = 1.8; 2 Hz); 7.09 (ps, 1H). ^{13}C NMR (75MHz, $CDCl_3$): δ 13.8; 22.3; 25.0; 29.5; 70.2; 134.4; 144.2; 174.6. HRMS (IC+): Calculated for $C_8H_{12}O_2$ Na^+ = 163.0730; founded MNa^+ = 163.0730.

I.3.2. 3-hexylfuran-2(*5H*)-one (6b)

According to general procedure B, lactone **5d** (900 mg, 3.2 mmol) and *m*-CPBA (615 mg, 3.6 mmol) gave furanone **8d** (468 mg, 86 %) after purification by flash chromatography on silica gel. Eluent: pentane-ether (50/50). Aspect: yellow oil; Rf (SiO_2; P/Et_2O: 50/50) = 0.56. ^1H NMR (300MHz, $CDCl_3$): δ 0.87 (t, *J* = 7.5 Hz, 3H); 1.29 (m, 6H); 1.54 (m, 2H); 2.27 (m, 2H); 4.75 (d, *J* = 2 Hz, 2H); 7.09 (ps, 1H). ^{13}C NMR (75MHz, $CDCl_3$): δ 14.1; 22.6; 25.4; 27.4; 28.9; 31.6; 70.2; 134.6; 144.1; 174.6. HRMS (IC+): Calculated for $C_{10}H_{16}O_2$ Na^+ = 191.1043; founded MNa^+ = 191.1043.

I.3.3. 3-(1-hydroxybutyl)furan-2(*5H*)-one (6c)

According to general procedure B, lactones **5c** (3g, 4,2 mmol) and *m*-CPBA (943 mg, 5.46 mmol) gave furanone **6c** (1.35 g, 78 %) after purification by flash chromatography on silica gel. Eluent: dichloromethane-ether (85/15). Aspect: yellow oil; Rf (SiO_2; CH_2Cl_2/Et_2O: 85/15) = 0.15. ^1H NMR (300MHz, $CDCl_3$): δ 0.96 (t, *J* = 7.3 Hz, 3H); 1.30-1.80 (m, 4H); 2.50 (s, 1H, OH); 4.53 (m, 1H); 4.83 (t, *J* = 1.7Hz, 2H); 7.30 (ps, 1H). ^{13}C NMR (75MHz, $CDCl_3$): δ 13.8; 18.5; 37.5; 66.7; 70.6; 136.7; 145.4; 173.5. HRMS (IC+): Calculated for $C_8H_{12}O_3$ Na^+ = 179.0679; founded MNa^+ = 179.0680.

I.3.4. 3-(1-hydroxyhexyl)furan-2(*5H*)-one (6d)

According to general procedure B, lactones **5d** (283 mg, 96 mmol) and *m*-CPBA (166 mg, 96 mmol) gave furanone **6d** (148 mg, 84 %) after purification by flash chromatography on silica. Eluent: dichloromethane-ether (85/15). Aspect: uncolored oil; Rf (SiO_2; CH_2Cl_2/Et_2O: 85/15) = 0.28. ^1H NMR (300MHz, $CDCl_3$) : δ 0.89 (pt, 3H); 1.31-1.48 (m, 6H); 1.70-1.78 (m, 2H); 2.50 (s(OH), 1H, OH); 4.21 (m, 1H); 4.84 (t, *J* = 1.7Hz, 2H); 7.30 (td, *J* = 3.2Hz; 1.7Hz, 1H). ^{13}C NMR (75MHz, $CDCl_3$) : δ 14.1; 22.6; 25.0; 31.6; 35.5; 67.2; 70.6; 136.7; 144.9; 173.4. HRMS (IC+): Calculated for $C_{10}H_{16}O_3$ H^+, 185.1178; founded MH^+ = 185.1180.

I.3.5. 3-(1-hydroxyoctyl)furan-2(*5H*)-one (6e)

According to general procedure B, lactones **5e**, **5e'** (1.1 g, 3.4 mmol) and *m*-CPBA (1 g, 5.1 mmol) gave furanone **6e** (516 mg, 71 %) after purification by flash chromatography on silica gel using dichloromethane-ether (85/15). Uncolored oil Rf (SiO_2; CH_2Cl_2-Et_2O: 85/15)= 0.33. ^{1}H NMR (300MHz, $CDCl_3$): δ 0.84 (t, *J* = 6.6Hz, 3H); 1.24-1.41 (m, 10H); 1.66-1.74 (m, 2H); 2.84 (s(OH), 1H); 4.47 (m, 1H); 4.80 (t, *J* = 1.5Hz, 2H); 7.31 (td, *J* = 3.0; 1.5Hz, 1H). ^{13}C NMR (75MHz, $CDCl_3$): δ 14.3; 22.9; 25.5; 29.5; 29.6; 32.0; 35.7; 67.3; 70.8; 136.9; 145.2; 173.6. HRMS (ESI+): Calculated for $C_{12}H_{20}O_3Na^+$ = 235.1310; founded MNa^+ = 235.1311.

I.4. General procedure C for methylenation reaction of furanone 6

Diisopropylamine (2 eq.) was added to anhydrous THF under nitrogen. After cooling the solution to 0 °C, n-butyllithium (1.5 eq. of a 1.6 M solution in hexane) was added and the reaction mixture was stirred for 15 min. The solution was then cooled to -78 °C and subsequently treated with furanones **6** (1eq.), dissolved in dry THF. Stirring was continued for one hour followed by addition of Eshenmoser's salt (7 eq.). The reaction mixture was stirred for one hour then allowed to warm to room temperature and stirred for 2h. The reaction mixture was poured into saturated ammonium chloride solution and extracted with ethyl acetate. The organic phase extracted was washed with water and brine and dried over anhydrous sodium sulphate, filtered and the solvent removed under reduced pressure. The crude product was purified by column chromatography through silica gel. Elimination of the amine occurs spontaneously on the silica gel.

I.4.1. 3-butyl-5-methylenefuran-2(*5H*)-one (8a)

According to general procedure C, furanone **6a** (1.2 g, 8.5 mmol) and Eshenmoser's salt (6.46 g, 34.9 mmol) gave methylene-furanone **8a** (435 mg, 34 %) after purification by flash chromatography on silica gel. Eluent: dichloromethane-ether (90:10). Aspect: yellow oil; Rf (SiO_2; CH_2Cl_2/Et_2O: 90/10) = 0.63. ^{1}H NMR (300MHz, $CDCl_3$): δ 0.93 (t, *J* = 7.8 Hz, 3H); 1.31-1.40 (m, 2H); 1.42-1.70 (m, 2H); 2.37 (m, 2H); 4.76 (d, *J* = 2 Hz, 1H); 5.10 (d, *J* = 2 Hz, 1H); 7.02 (s, 1H). ^{13}C NMR (75MHz, $CDCl_3$) : δ 13.7; 22.3; 24.9; 29.5; 95.5; 136.4; 136.5; 154.0; 170.6. HRMS (IC+): Calculated for $C_9H_{12}O_2$ H^+ = 153.0916; founded MH^+ = 153.0916.

I.4.2. 3-hexyl-5-methylenefuran-2(*5H*)-one (8b)[2]

According to general procedure C, furanone **6b** (1.1 g, 6.5 mmol) and Eshenmoser's salt (8.4 g, 45.7 mmol) gave methylene-furanone **8b** (680 mg, 58 %) after purification by flash chromatography on silica gel. Eluent: dichloromethane-pentane (90:10). Aspect: yellow oil; Rf (SiO_2; CH_2Cl_2/P: 90/10) = 0.8. ^{1}H NMR (300MHz, $CDCl_3$): δ 0.89 (t, *J* = 7.8 Hz, 3H); 1.20-1.45 (m, 6H); 1.50-1.60 (m, 2H); 2.37 (t, *J* = 9 Hz, 2H); 4.76 (d, *J* = 2 Hz, 1H); 5.10 (d, *J* = 2 Hz, 1H); 7.01 (s, 1H). HRMS (IC+): Calculated for $C_{11}H_{16}O_2$ Na^+ = 203.1043; founded MNa^+ = 203.1041.

I.4.3. 3-(1-hydroxybutyl)-5-methylenefuran-2(*5H*)-one (8c)[2]

According to general procedure C, furanone **6c** (300 mg, 1.92 mmol) and Eshenmoser's salt (2.48 g, 13.44 mmol) gave methylene-furanone **8c** (174 mg, 54 %) after purification by flash chromatography on silica gel. Eluent: dichloromethane-ether (90:10). Aspect: yellow oil; Rf (SiO_2; CH_2Cl_2/Et_2O: 90/10) = 0.38. ^{1}H NMR (300MHz, $CDCl_3$): δ 0.96 (t, *J* = 7.3 Hz, 3H); 1.38-1.80 (m, 4H); 2.33 (s, 1H, OH); 4.60 (m, 1H); 4.89 (d, *J* = 2.5Hz, 1H); 5.20 (d, *J* = 2.5Hz, 1H); 7.20 (d, *J* = 1.2Hz, 1H). ^{13}C NMR (75MHz, $CDCl_3$) : δ 14.7; 18.4; 37.7; 66.4; 97.6; 136.7; 138.6; 153.6; 169.4. HRMS (IC+): Calculated for $C_9H_{12}O_3$ H^+ = 169.0865; founded MH^+ = 169.0864.

I.4.4. 3-(1-hydroxyhexyl)-5-methylenefuran-2(*5H*)-one (8d) [3]

According to general procedure C, furanone **6d** (400 mg, 2.17 mmol) and Eshenmoser's salt (2.81 mg, 15.2 mmol) gave methylene-furanone **8d** (268 mg, 63 %) after purification by flash chromatography on silica gel. Eluent: pentane- ethyl acetate (60:40). Aspect: yellow oil; Rf (SiO_2; P/AcOEt: 60/40) = 0.51. ^{1}H NMR (300MHz, $CDCl_3$): δ 0.89 (pt, 3H); 1.30-1.42 (m, 6H); 1.69-1.77 (m, 2H); 2.30 (s, 1H, OH); 4.59 (m, 1H); 4.89 (d, *J* = 2.5Hz, 1H); 5.20 (d, *J* = 2.6Hz, 1H); 7.20 (m, 1H). ^{13}C NMR (75MHz, $CDCl_3$) : δ 14.3; 22.7; 25.2; 31.8; 36.0; 67.3; 97.9; 136.6; 138.6; 154.0; 169.5.

I.4.5. 3-(1-hydroxyoctyl)-5-methylenefuran-2(*5H*)-one (8e)[2]

According to general procedure C, furanone **6e** (475 mg, 2.56 mmol) and Eshenmoser's salt (2.9 g, 15.7 mmol) gave methylene-furanone **8e** (208 mg, 42 %) after purification by flash chromatography on silica gel. Eluent: pentane- ethyl acetate (60:40). Aspect: yellow oil; Rf (SiO_2; P/AcOEt: 60/40) = 0.74. ^{1}H NMR (300MHz, $CDCl_3$): δ 0.87 (pt, 3H); 1.27-142 (m, 10H); 1.71-1.80 (m, 2H); 2.28 (s, 1H, OH); 4.56-4.62 (m, 1H); 4.89 (d, *J* = 2.6Hz, 1H); 5.20

(dd, *J* = 2.5; 0.4Hz, 1H); 7.20 (m, 1H). ^{13}C NMR (75MHz, $CDCl_3$): δ 14.2; 22.7; 25.3; 29.3; 29.4; 31.9; 35.8; 67.1; 97.7; 136.4; 138.4; 153.8; 169.3. HRMS (IC+): Calculated for $C_{13}H_{20}O_3$ H^+ = 225.1491; founded MH^+ = 225.1492.

I.5. General procedure D for bromation reaction of methylene-furanone 8 [4]

A solution of Br_2 (1 eq.) in CCl_4 was added to the solution of methylene-furanone **8** (1 eq.) in CCl_4 at 0 °C. The mixture was stirred at room temperature for 30 mn and then the solvent was removed in vacuum. The obtained residue was dissolved in $CHCl_3$ and Et_3N (1 eq.), one drop of DBU was added at 0 °C. The mixture was further stirred at room temperature for 1 to 3 h. The solvent was removed in vacuum and the obtained crude product was purified by column chromatography through silica gel.

I.5.1. (Z)-5-(bromomethylene)-3-butylfuran-2(*5H*)-one (10a)

According to general procedure D, furanone **8a** (60 mg, 0. 39mmol) and Br_2 (63 mg, 0.39 mmol) gave bromofuranone **10a** (51 mg, 56 %) after purification by flash chromatography on silica gel. Eluent: pentane-ether (80:20). Aspect: yellow oil; Rf (SiO_2; P/ether : 80/20) = 0.27. ^{1}H NMR (300MHz, $CDCl_3$): δ 0.93 (t, *J* = 7.3Hz, 3H); 1.30-1.40 (m, 2H); 1.53-1.80 (m, 2H); 2.34 (m, 2H); 5.93 (s, 1H); 7.01 (d, *J* = 1.3 Hz, 1H). ^{13}C NMR (75MHz, $CDCl_3$): δ 13.8; 22.3; 25.2; 29.5; 89.6; 135.0; 136.0; 151.5; 169.3. HRMS (IC+): Calculated for $C_9H_{11}BrO_2$ H^+ = 231.0021; founded MH^+ = 231.0020.

I.5.2. (Z)-5-(bromomethylene)-3-hexylfuran-2(*5H*)-one (10b)[5]

According to general procedure D, furanone **8b** (75 mg, 0.41 mmol) and Br_2 (66 mg, 0.41 mmol) gave bromofuranone **10a** (40 mg, 37 %) after purification by flash chromatography on silica gel. Eluent: pentane-ether (80:20). Aspect: yellow oil; Rf (SiO_2; P/ether : 80/20) = 0.49. ^{1}H NMR (300MHz, $CDCl_3$): δ 0.89 (t, *J* = 7.3Hz, 3H); 1.30-1.40 (m, 6H); 1.53-1.80 (m, 2H); 2.34 (m, 2H); 5.93 (s, 1H); 7.01 (d, *J* = 1.3 Hz, 1H). ^{13}C NMR (75MHz, $CDCl_3$): δ 14.2; 22.6; 25.5; 27.4; 28.9; 31.5; 89.6; 135.0; 136.2; 151.5; 169.3. HRMS (IC+): Calculated for $C_{11}H_{15}BrO_2$ Na^+ = 281.0148; founded MNa^+ = 281.0148.

I.5.3. (Z)-5-(bromomethylene)-3-(1-hydroxybutyl)furan-2(*5H*)-one (10c)

(E)-5-(bromomethylene)-3-(1-hydroxybutyl)furan-2(*5H*)-one (10c)

According to general procedure D, furanone **8c** (65 mg, 0.38 mmol) and Br_2 (62 mg, 0.39 mmol) gave bromofuranone **10c-Z** and **10c-E** (45 mg and 6 mg, 48 %) after purification and separation by flash chromatography on silica gel. Eluent: dichloromethane-ether (95:5). Aspect **10c-Z**: yellow oil; Rf (SiO_2; CH_2Cl_2/ether : 95/5) = 0.42. ^{1}H NMR **10c-Z** (300MHz, $CDCl_3$) : δ 0.96 (t, *J* = 7.3Hz, 3H); 1.30-1.55 (m, 2H); 1.58-1.80 (m, 2H); 2.45 (s, 1H, OH); 4.57 (m, 1H); 6.06 (s, 1H); 7.22 (d, *J* = 1.3 Hz, 1H). ^{13}C NMR **10c-Z** (75MHz, $CDCl_3$): δ 13.9; 18.5; 37.7; 66.9; 91.8; 135.1; 137.8; 151.3; 167.9. Aspect **10c-E**: yellow oil ; Rf (SiO_2; CH_2Cl_2/ether : 95/5) = 0.6 ^{1}H NMR **10c-E** (300MHz, $CDCl_3$) : δ 0.97 (t, *J* = 7.3 Hz, 3H); 1.4-1.55 (m, 2H); 1.58-1.80 (m, 2H); 2.25 (1H, OH); 4.61 (m, 1H); 6.46 (s, 1H); 7.56 (s, 1H). HRMS (IC+): Calculated for $C_9H_{11}BrO_3$ H^+ = 246.9970; founded MH^+ = 246.9972.

I.5.4. (Z)-5-(bromomethylene)-3-(1-hydroxyhexyl)furan-2(*5H*)-one (10d)

According to general procedure D, furanone **8d** (130 mg, 0.66 mmol) and Br_2 (149 mg, 0.66 mmol) gave bromofuranone **10d** (56 mg, 31 %) after purification by flash chromatography on silica gel. Eluent: pentane-ethyl acetate (80:20). Aspect: yellow oil; Rf (SiO_2; P/AcOEt : 80/20)= 0.30. ^{1}H NMR (300MHz, $CDCl_3$): δ 0.87 (t, *J* = 7.3 Hz, 3H); 1.25-1.41 (m, 6H); 1.67-1.80 (m, 2H); 2.29 (s(OH), 1H); 4.56 (m, 1H); 6.06 (s, 1H); 7.21 (pd, 1H). ^{13}C NMR (75MHz, $CDCl_3$): δ 14.1; 22.7; 24.9; 31.6; 35.7; 67.2; 91.8; 135.1; 137.8; 151.3; 167.9. HRMS (IC+): Calculated for $C_{11}H_{15}BrO_3$ H^+ = 275.0283; founded MH^+ = 275.0283.

I.5.5. (Z)-5-(bromomethylene)-3-(1-hydroxyoctyl)furan-2(*5H*)-one (10e)

According to general procedure D, furanone **8e** (86 mg, 0.38 mmol) and Br_2 (61 mg, 0.38 mmol) gave bromofuranone **10e** (57 mg, 49 %) after purification by flash chromatography on silica gel. Eluent: pentane-ethyl acetate (80:20). Aspect: yellow oil; Rf (SiO_2; P/AcOEt : 80/20) = 0.32. ^{1}H NMR (300MHz, $CDCl_3$): δ 0.87 (t, *J* = 7.3Hz, 3H); 1.24-1.44 (m, 10H); 1.66-1.80 (m, 2H); 2.42 (s (OH), 1H); 4.55 (m, 1H); 6.06 (s, 1H); 7.22 (pd, 1H). ^{13}C NMR (75MHz, $CDCl_3$) : δ 14.2; 22.7; 25.3; 29.3; 29.4; 31.9; 35.7; 67.2; 91.8; 135.1; 137.8; 151.3; 167.9. HRMS (ESI+): Calculated for $C_{13}H_{19}BrO_3$ Na^+ =325.0415; founded MNa^+: 325.0415.

II. Biological evaluations

The recombinant *Escherichia coli* strain NM522 containing the sensor plasmid pSB401 was used. In pSB401, the *LuxR* and the *LuxI* promoter from *V. fischeri* have been coupled to the entire *Lux* structural operon (*LuxCDABE*) from *Photorhabdus luminescens*. Bacterial cultures were grown in exponential phase in Luria broth, in the presence of tetracycline (20 μg mL^{-1}), at 30 °C.

II.1. Agonistic activity

The inducing activity of the various acyl-HSL analogues was monitored using the *E. coli* biosensor strain. Acyl-HSL activity was measured in a microtitre plate format, with bioluminescence quantified using a Luminoskan luminometer. Concentrations of analogues, ranging from 0.1 to 200 μM, were made up to 0.1 mL volumes with growth medium. The amount of light produced by the bacteria was detected after 4–5 h. The amount of light measured was expressed in relative light units (RLU).

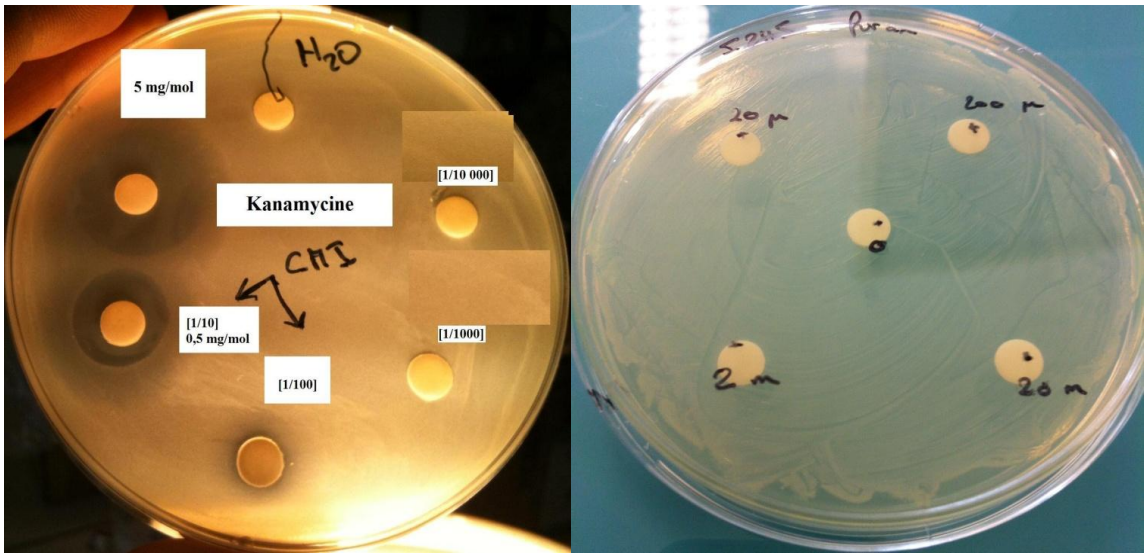
II.2. Antagonistic activity

The influence of acyl-HSL analogues on the induction of bioluminescence by 3-oxo-C_6-HSL was determined at concentrations ranging from 1 to 200 μM as described above, except that 3-oxo-C_6-HSL was included at a final concentration of 200 nM together with the analogue. 3-Oxo-C_6-HSL at 200 nM induced a 124 ± 8 RLU after 4-5 h, when the ratio of induced to background light was at its maximum.

II.3. Antibacterial activity

Furanone **10a**, the strongest inhibitor of this series of compounds, was tested using the disk diffusion assay, a common assay for antibiotic susceptibility standardized according to the Clinical and Laboratory Standards Institute guidelines.[6] A control disk diffusion assay was conducted using the relevant kanamycin antibiotic. The compound **10a** was tested at concentrations 20 μM, 200 μM, 2 mM and 20 mM. After overnight incubation at 37 °C, the inhibition zones of each compound were measured. No inhibition zone appears for the

furanone **10a** in contrast with the witness compound, where these zones are very clear for this antibiotic.

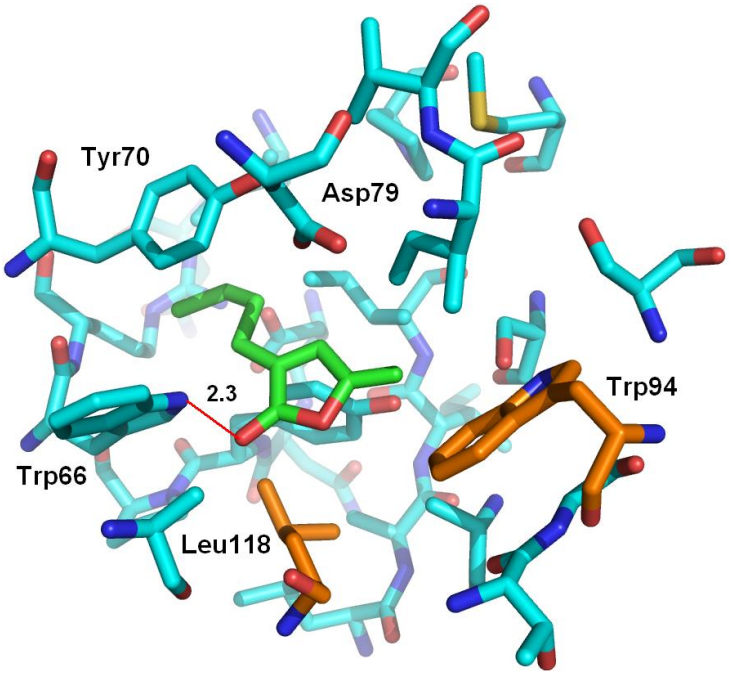


Kanamycin antibiotic ***Furanone 10a***

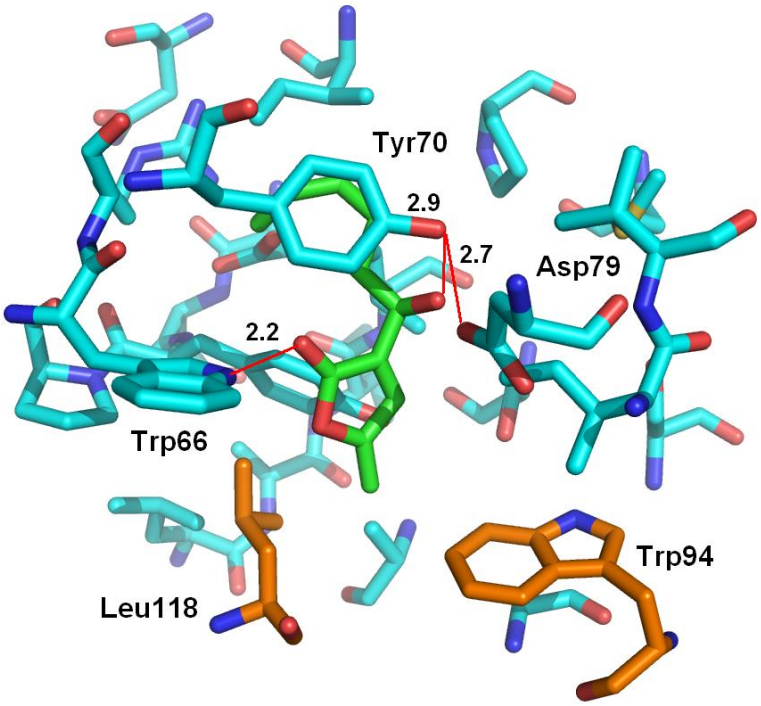
III. Molecular modelling.

All calculations were performed with ArgusLab[7] as software on a Dell OPTIPLEX GX620 PC. Docking experiments were performed with the docking module of ArgusLab. The protein model of LuxR[8] was created using SWISS-MODEL[9] with ClustalW.[10]

Docking studies were performed using the ligand binding site of LuxR created as previously described,[8] subsequently to the docking of 3-oxo-C_6-HSL.[11] Docking experiments were performed with the following parameters: Docking box centred on 3-oxo-C_6-HSL: X = Y = Z = 15 Å, ligand option: flexible; calculation type: Dock; Docking engine: GADock (Genetic Algorithm).[12] Genetic algorithm dock engine settings: default advanced parameters; hydrogen bonds were assigned within a distance of 3 Å.



8a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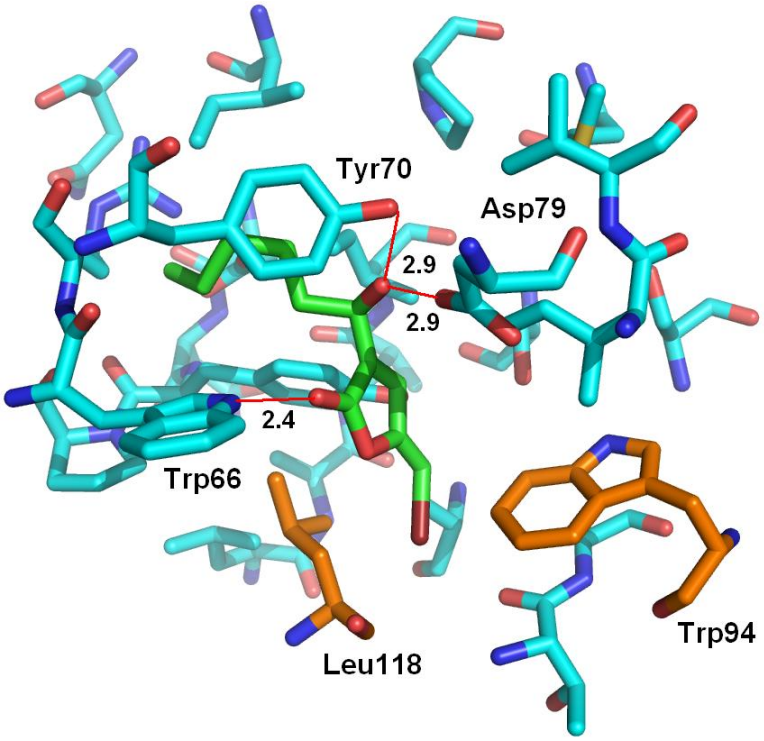
2.7
Asp79
2.2
Trp66
Trp94
Leu118

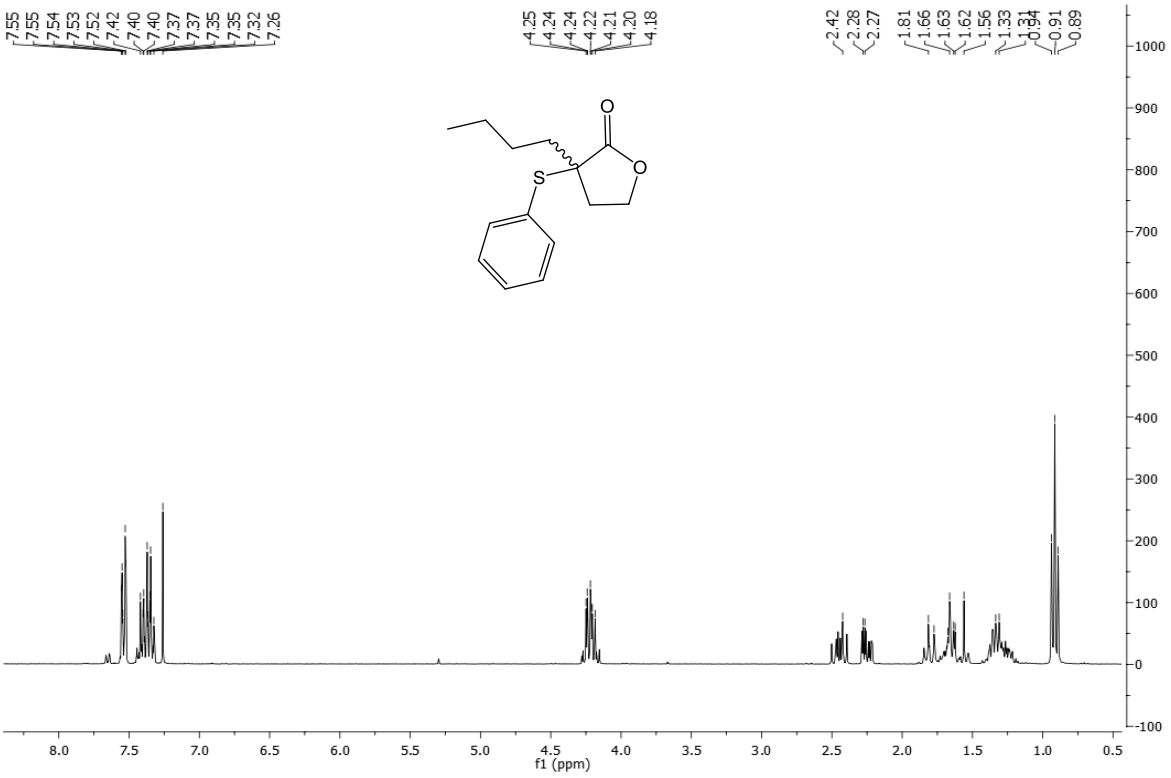
8d

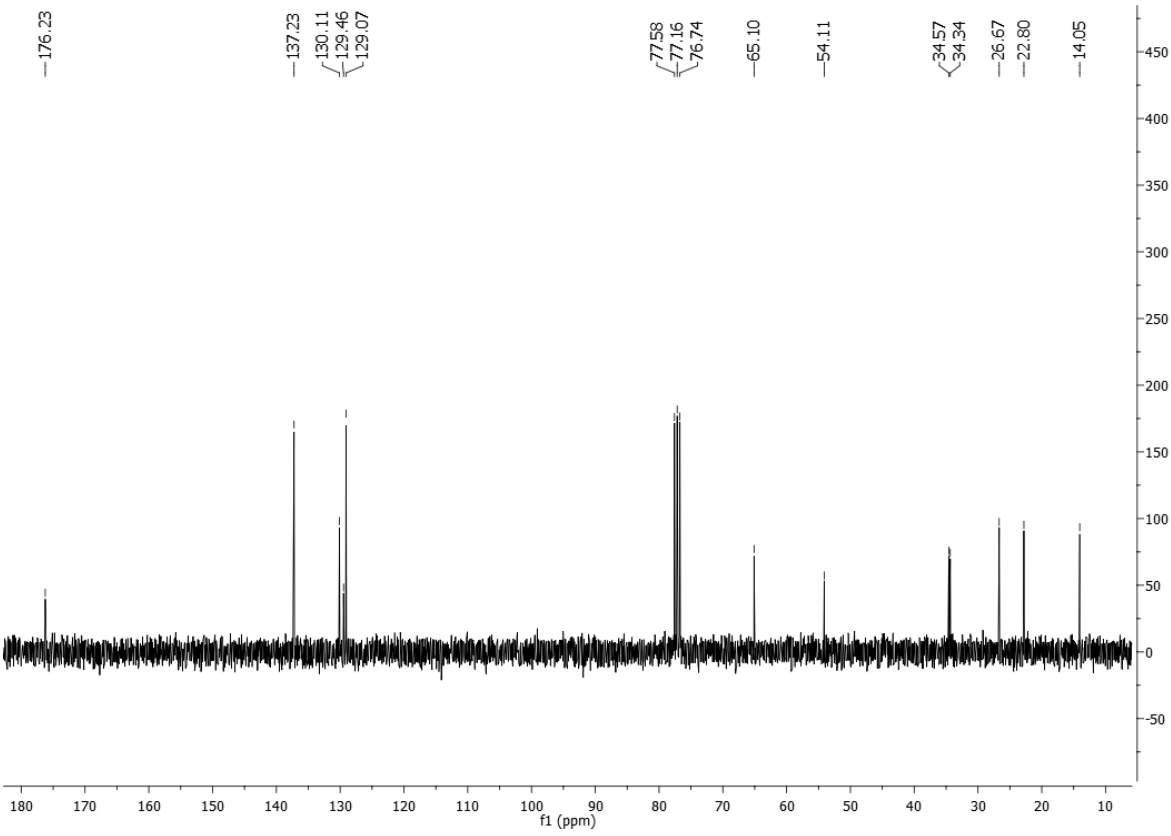

Tyr70
Asp79
2.9
2.9
2.4
Trp66
Trp94
Leu118

10d

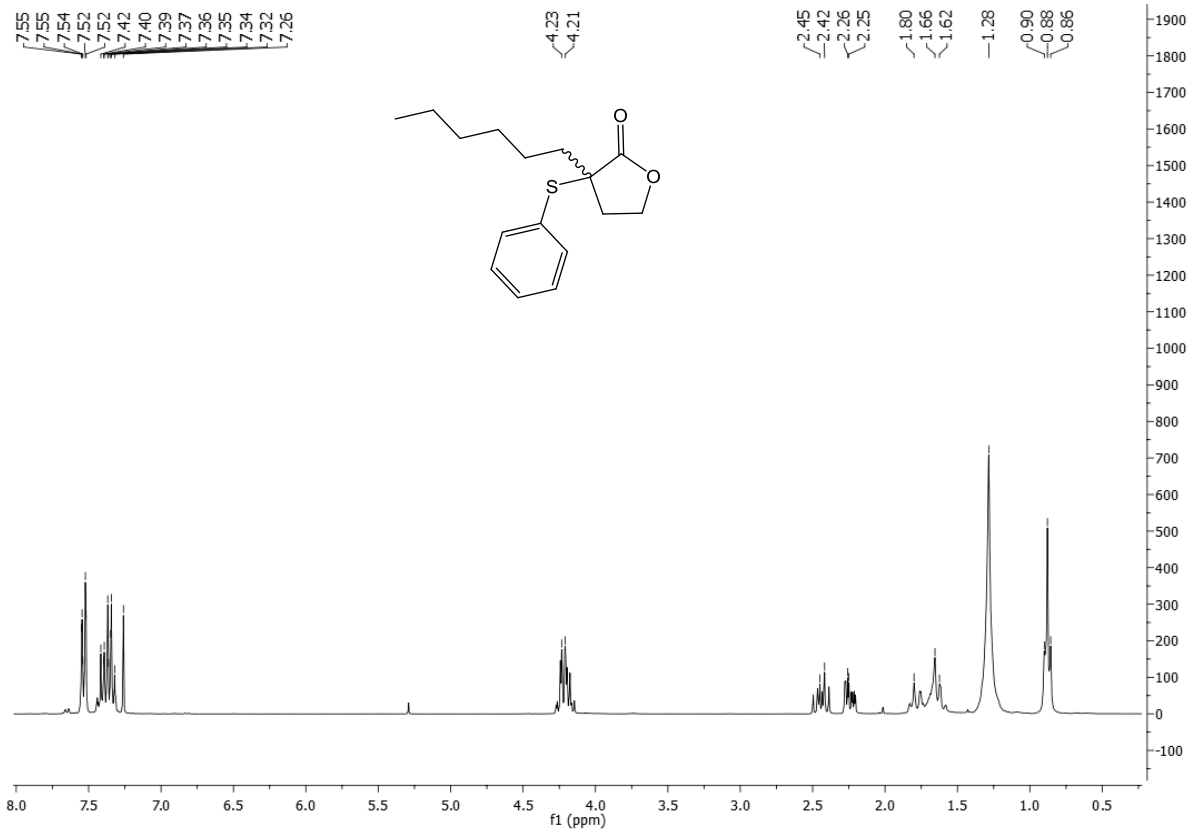
IV. ^{1}H and ^{13}C NMR spectrum

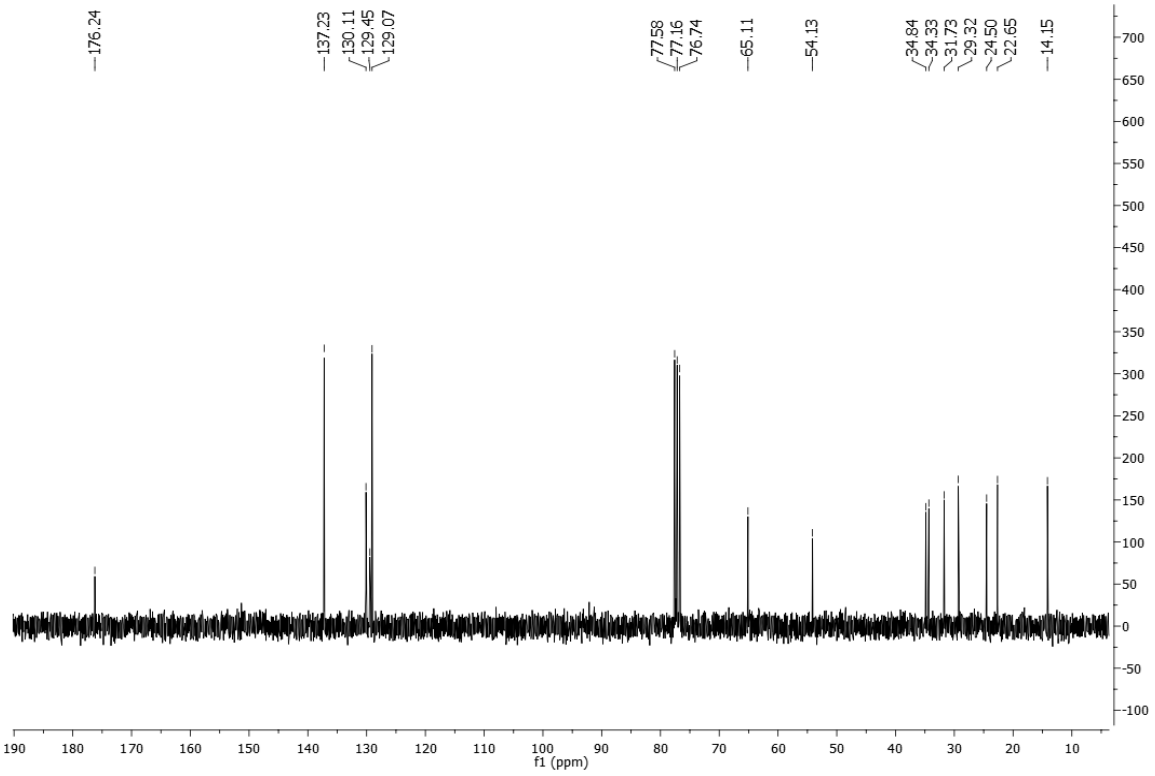
IV.1.1. 3-butyl-3-(phenylthio)dihydrofuran-2(*3H*)-one (5a)



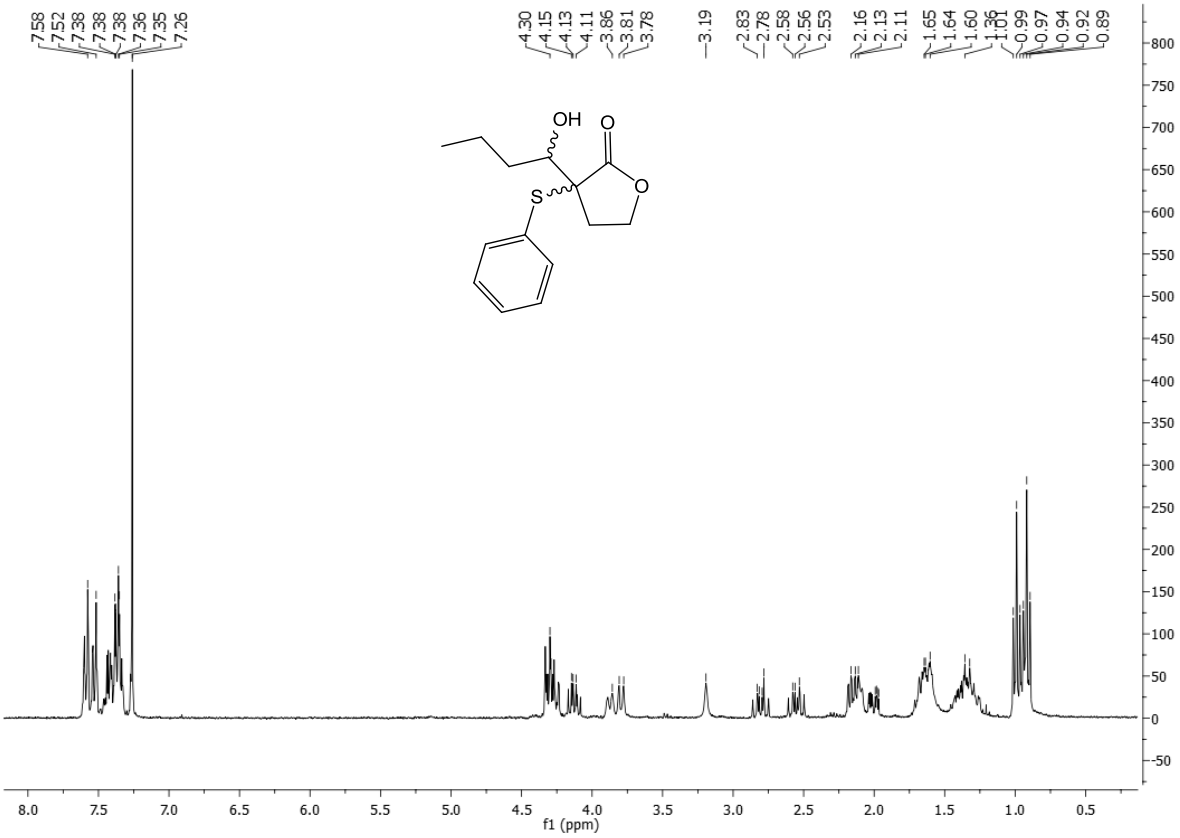


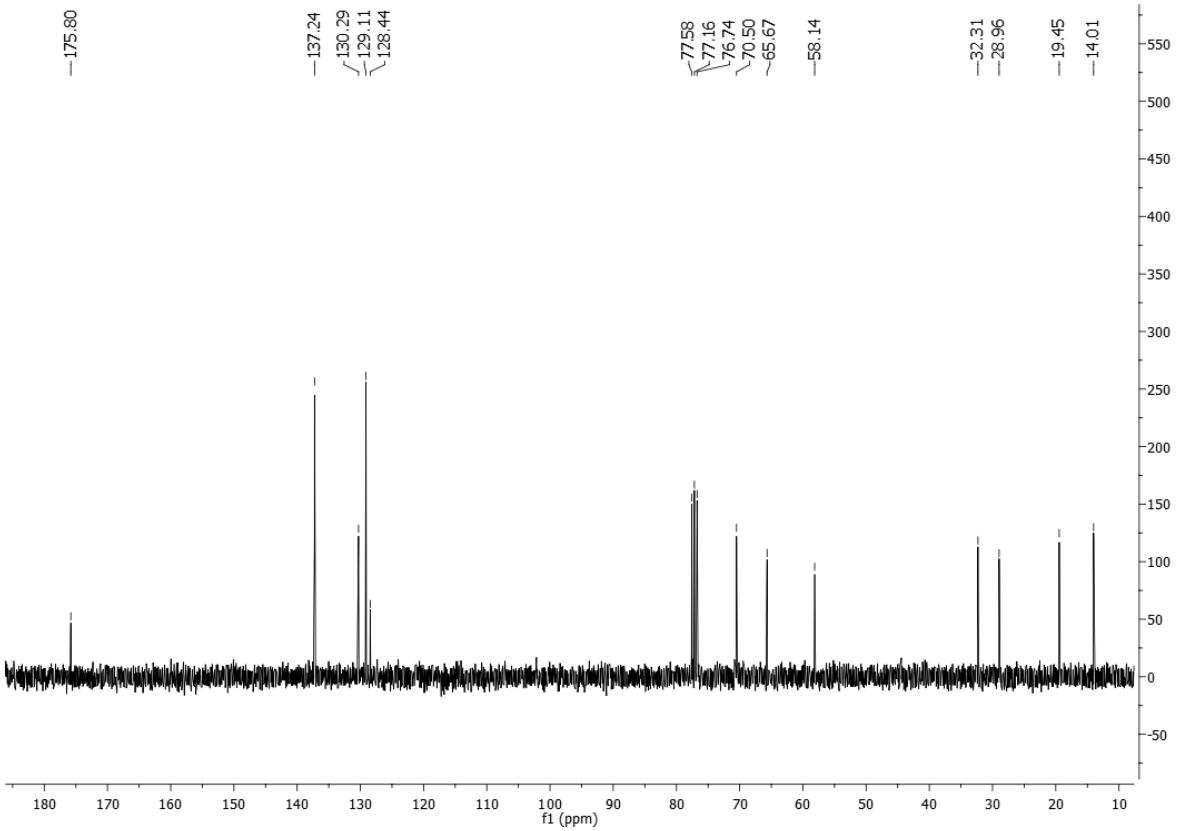
IV.1.2. 3-hexyl-3-(phenylthio)dihydrofuran-2(*3H*)-one (5b)



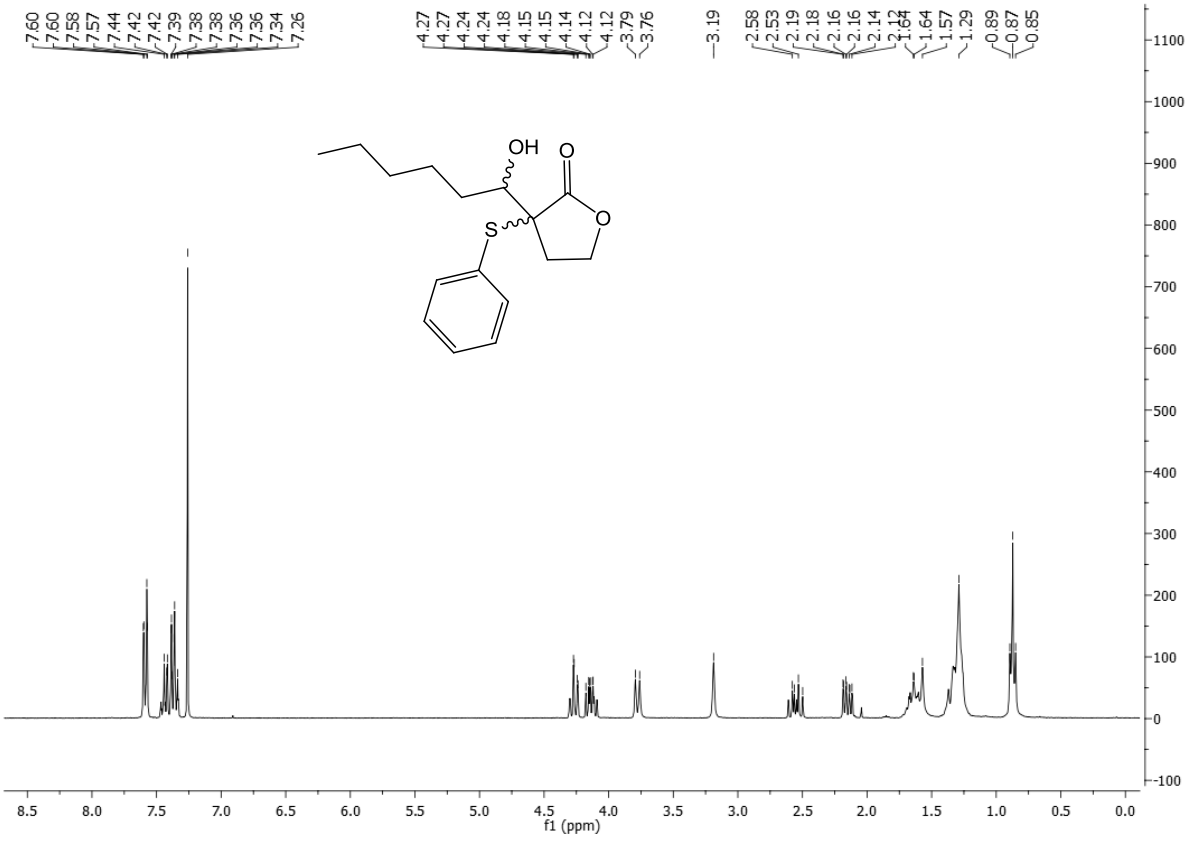


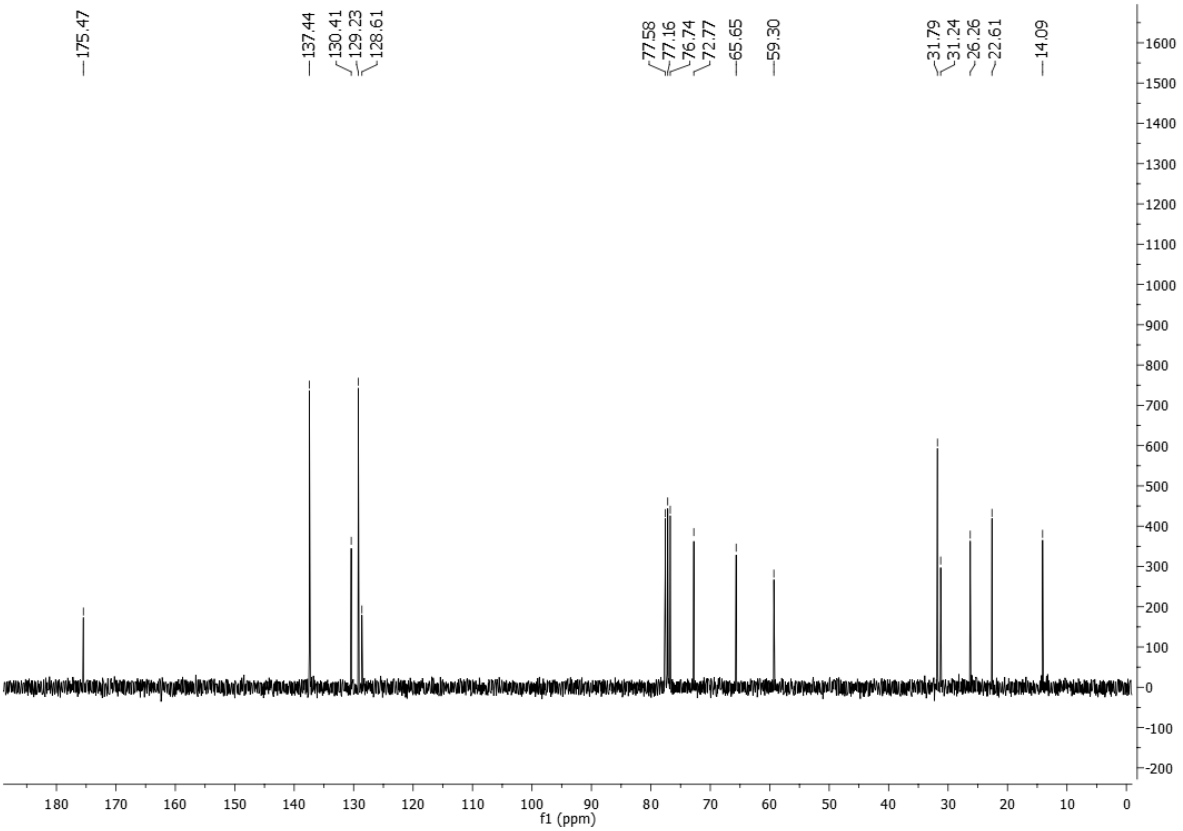
IV.1.3. 3-(1-hydroxybutyl)-3-(phenylthio)dihydrofuran-2(*3H*)-one (5c)



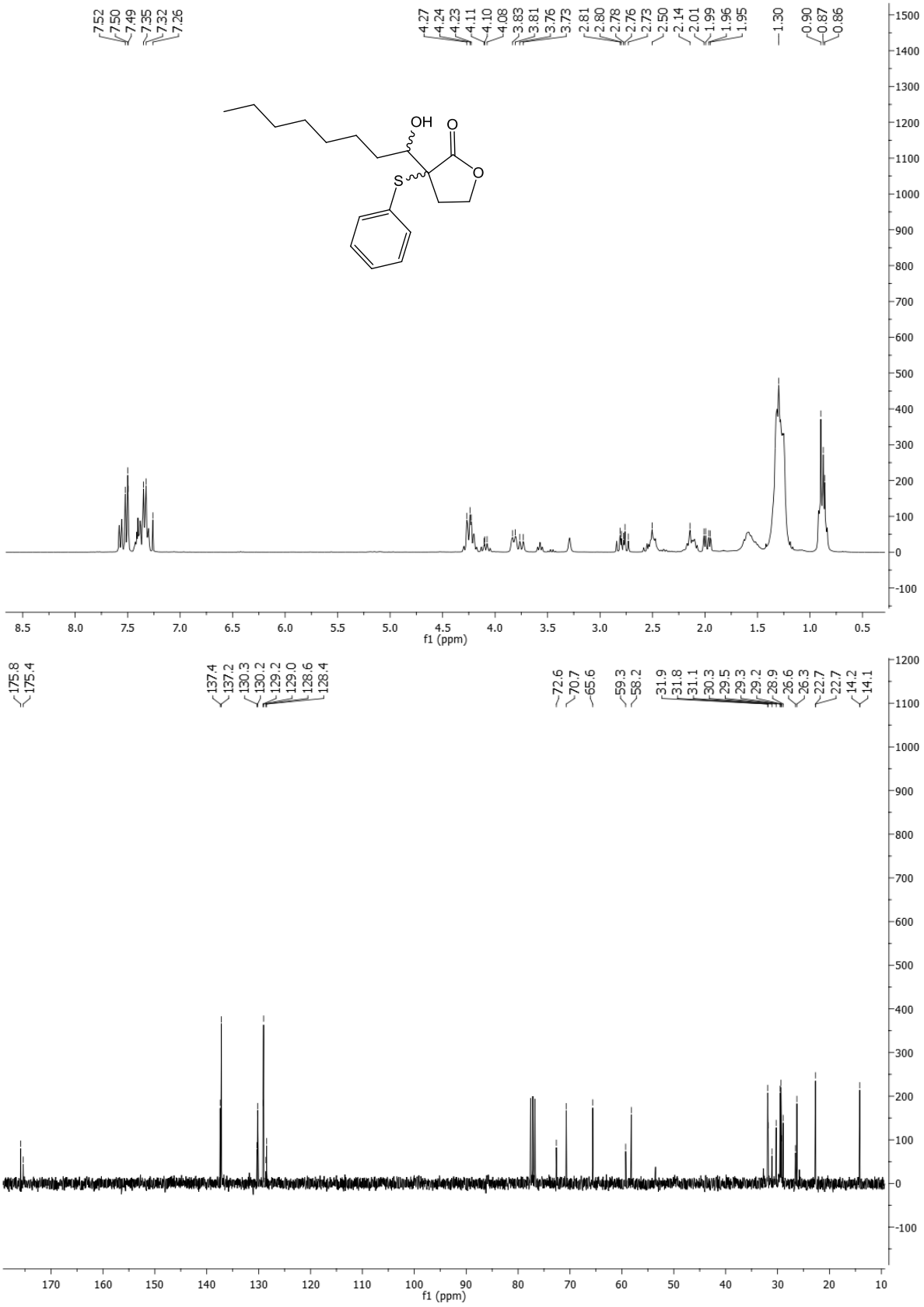


IV.1.4. 3-(1-hydroxyhexyl)-3-(phenylthio)dihydrofuran-2(*3H*)-one (5d)

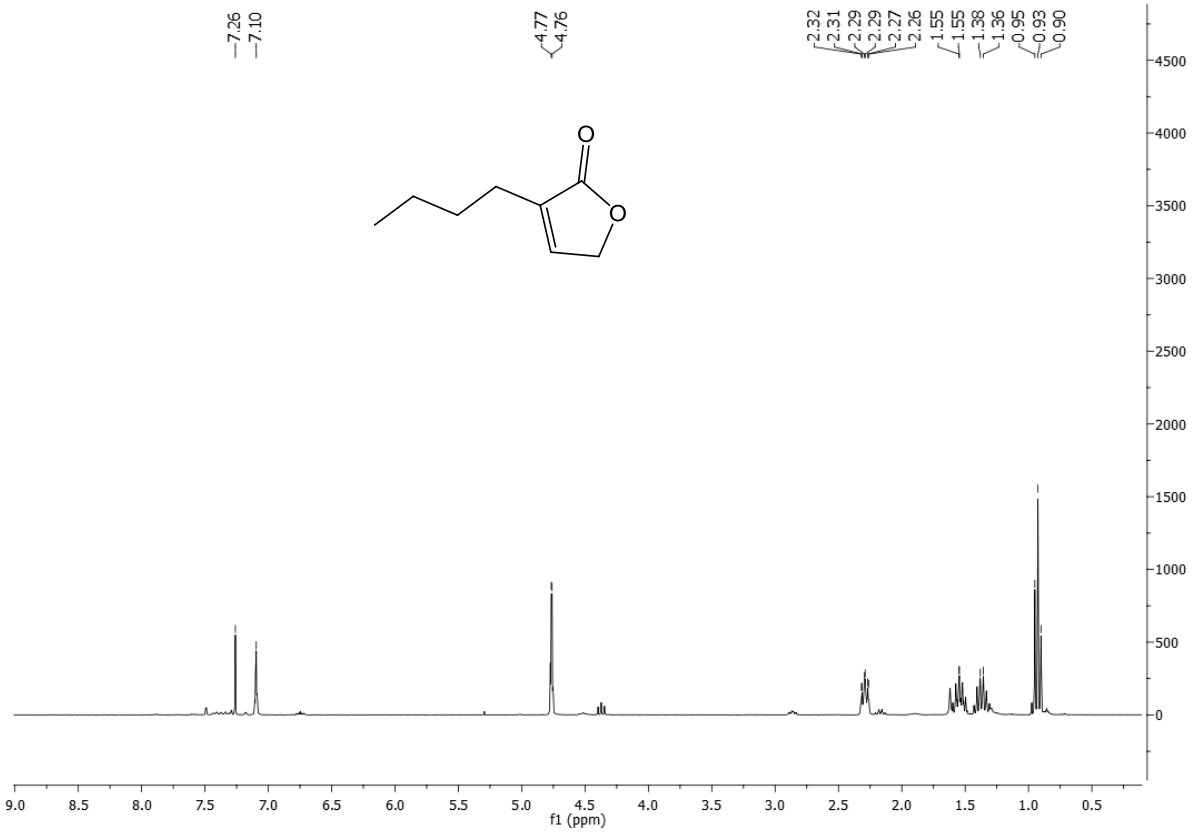


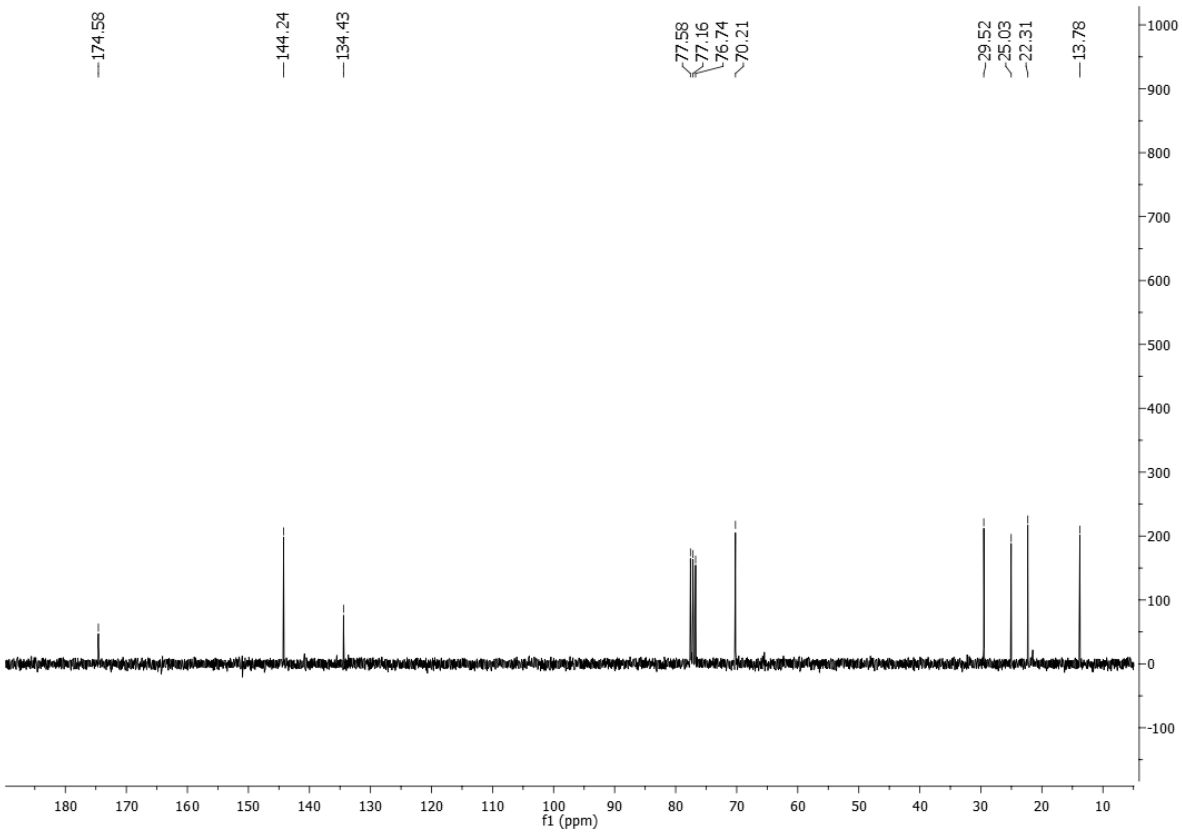


IV.1.5. 3-(1-hydroxyoctyl)-3-(phenylthio)dihydrofuran-2(*3H*)-one (5e, 5e')

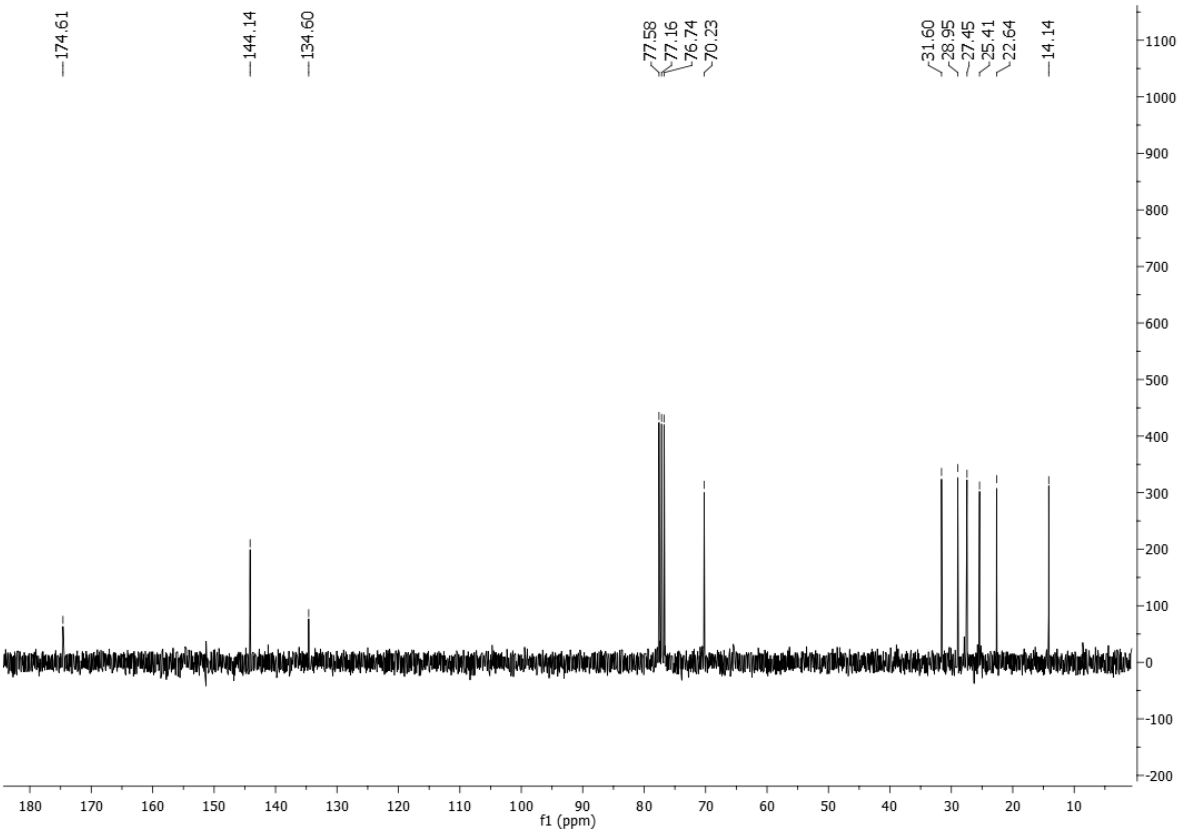


IV.1.6. 3-butylfuran-2(*5H*)-one (6a)

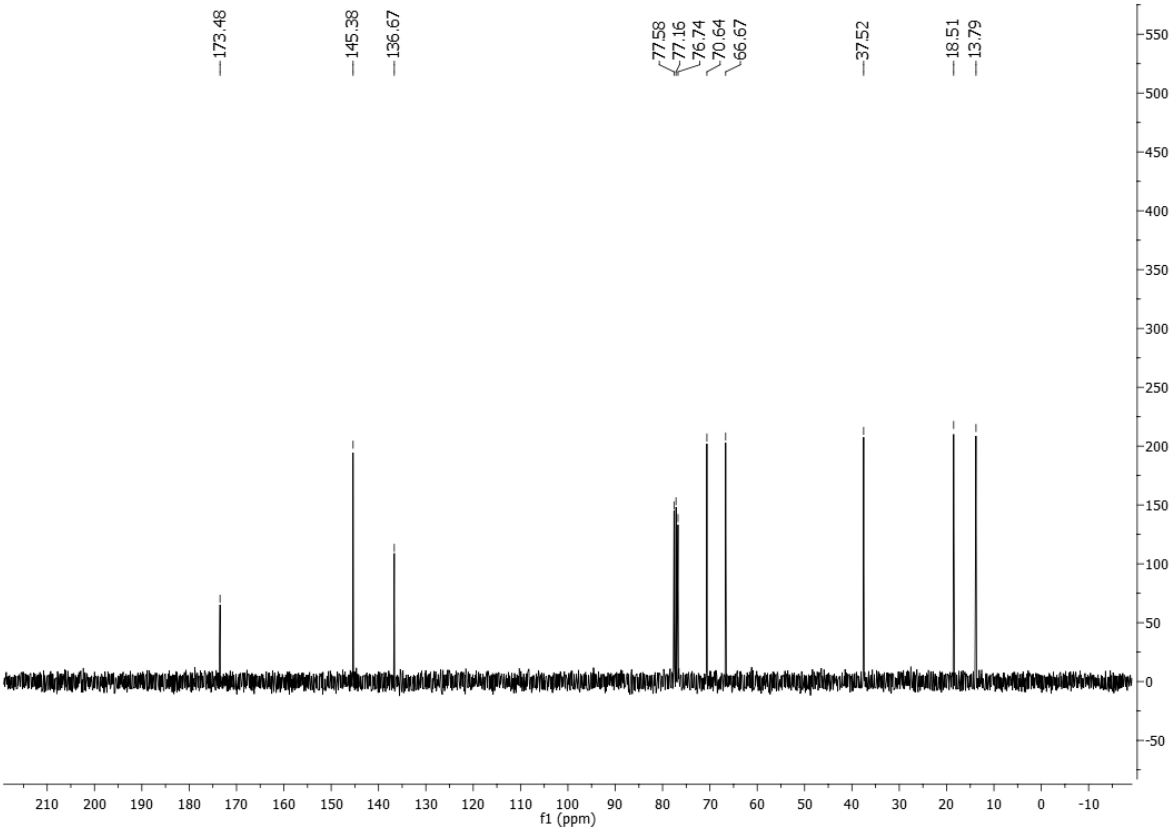




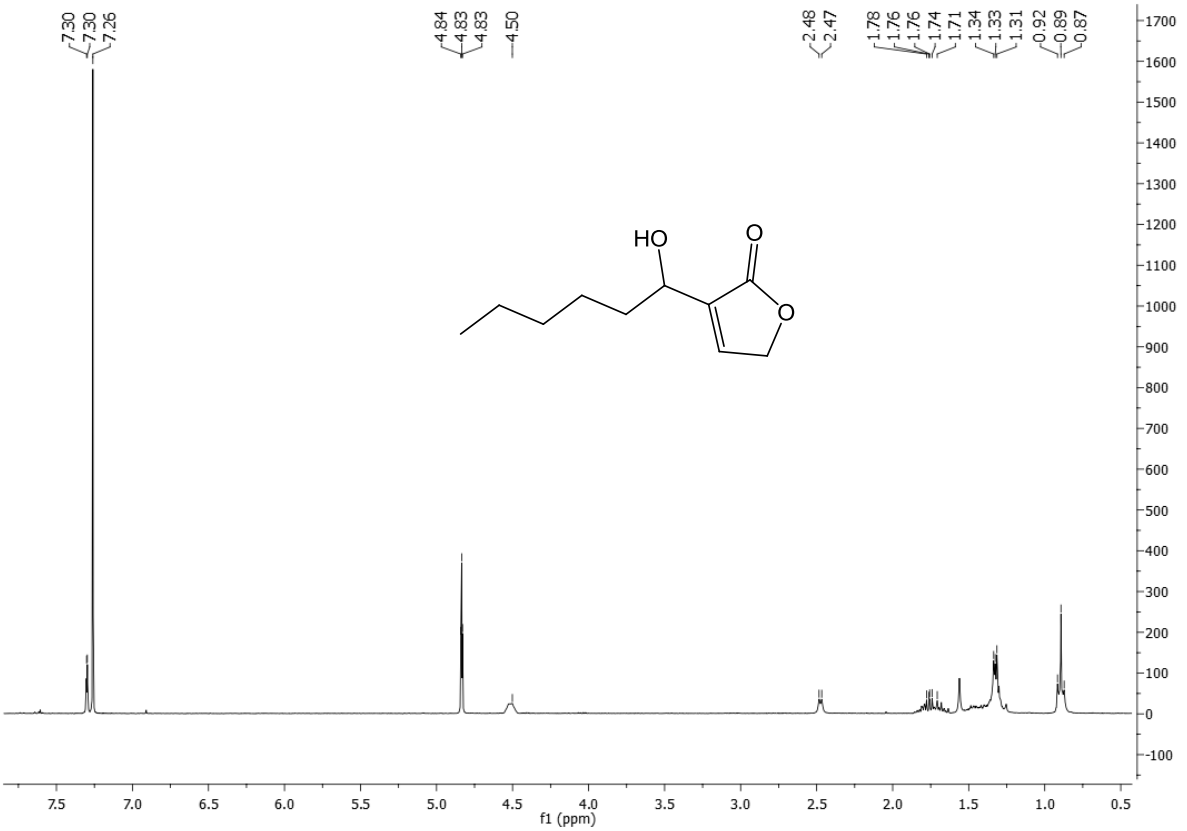
IV.1.7. 3-hexylfuran-2(*5H*)-one (6b)

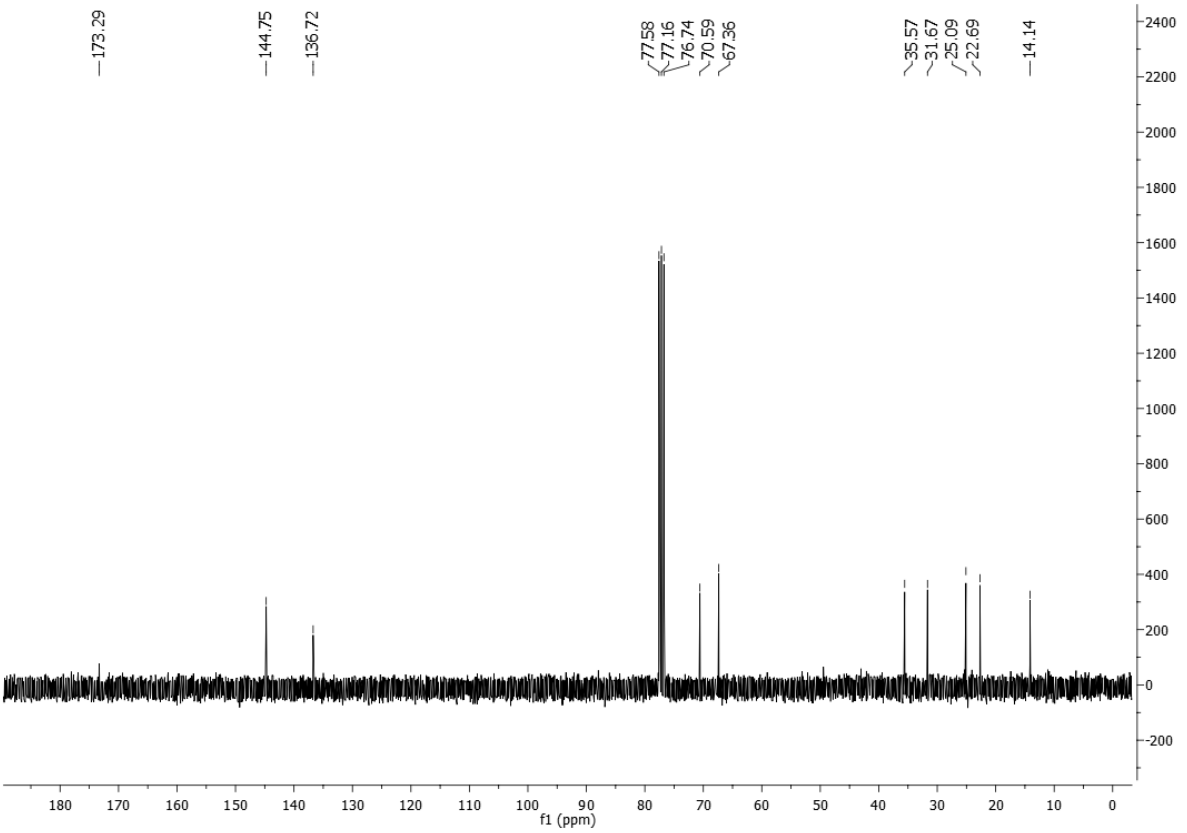


IV.1.8. 3-(1-hydroxybutyl)furan-2(*5H*)-one (6c)

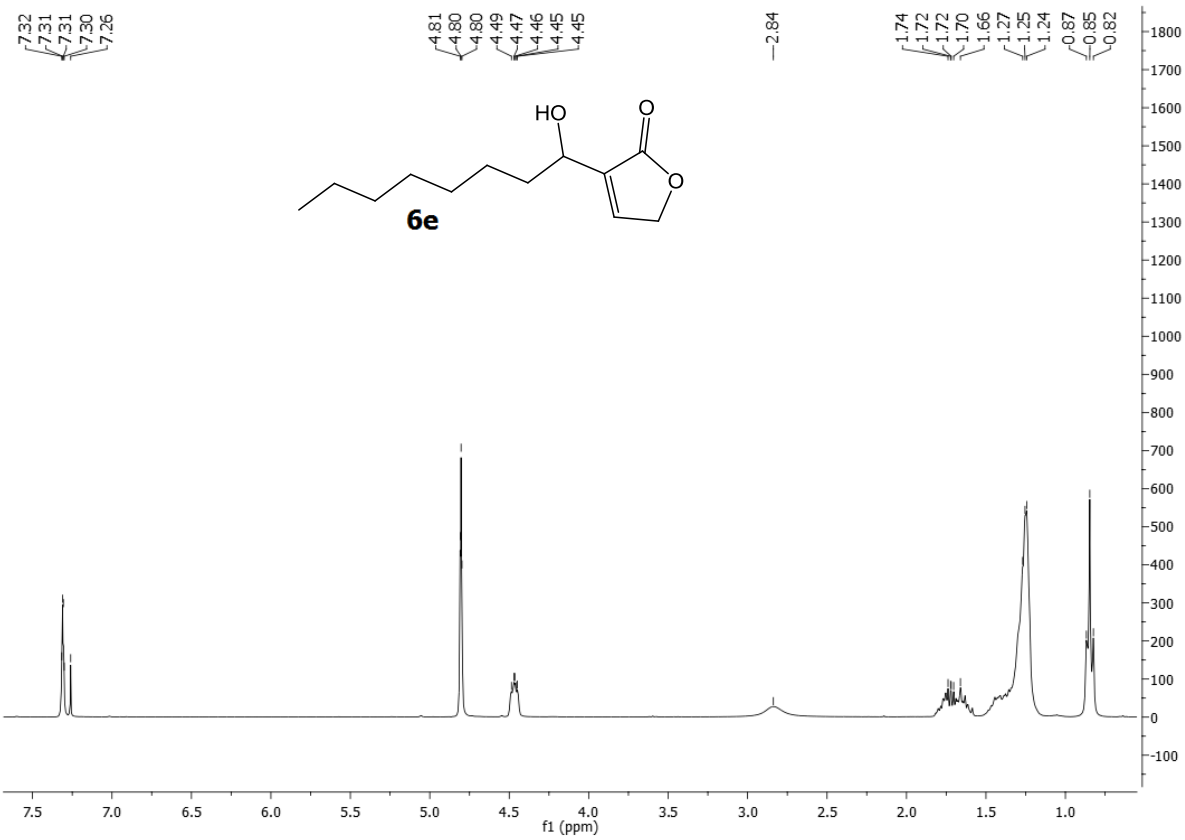


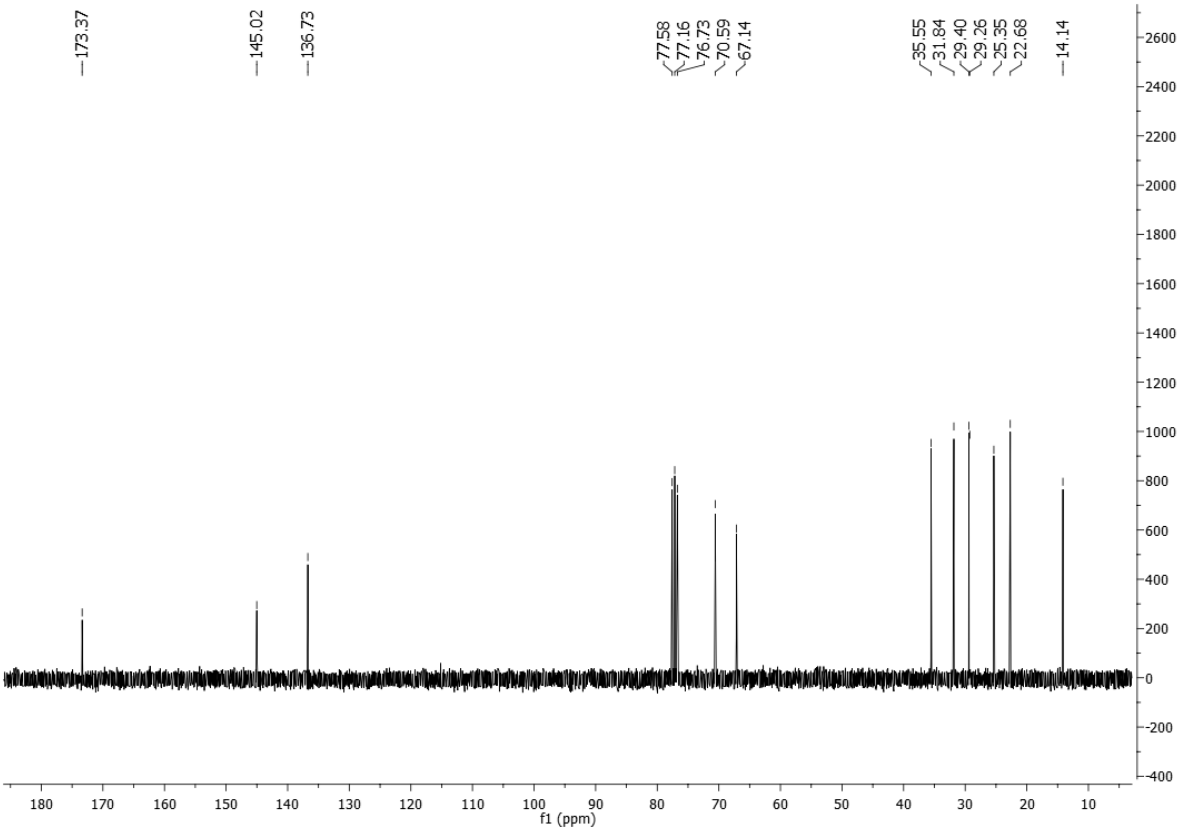
IV.1.9. 3-(1-hydroxyhexyl)furan-2(*5H*)-one (6d)



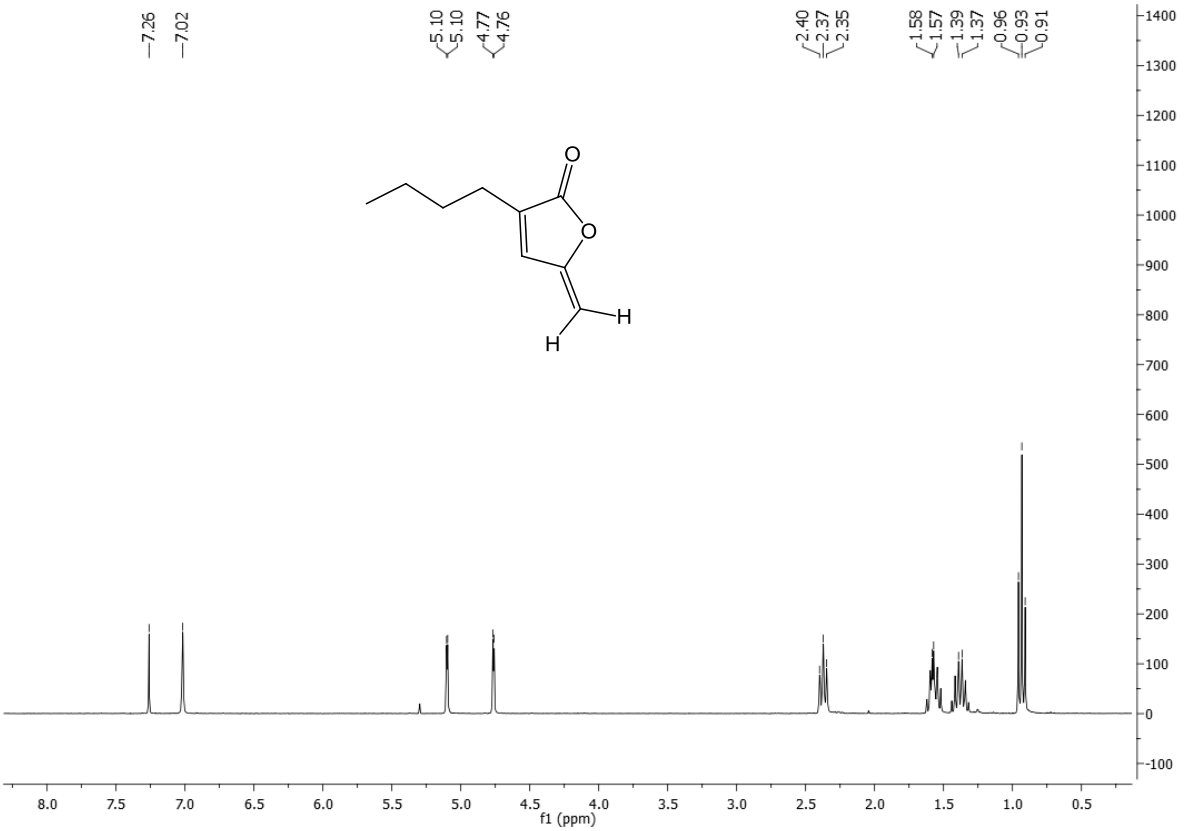


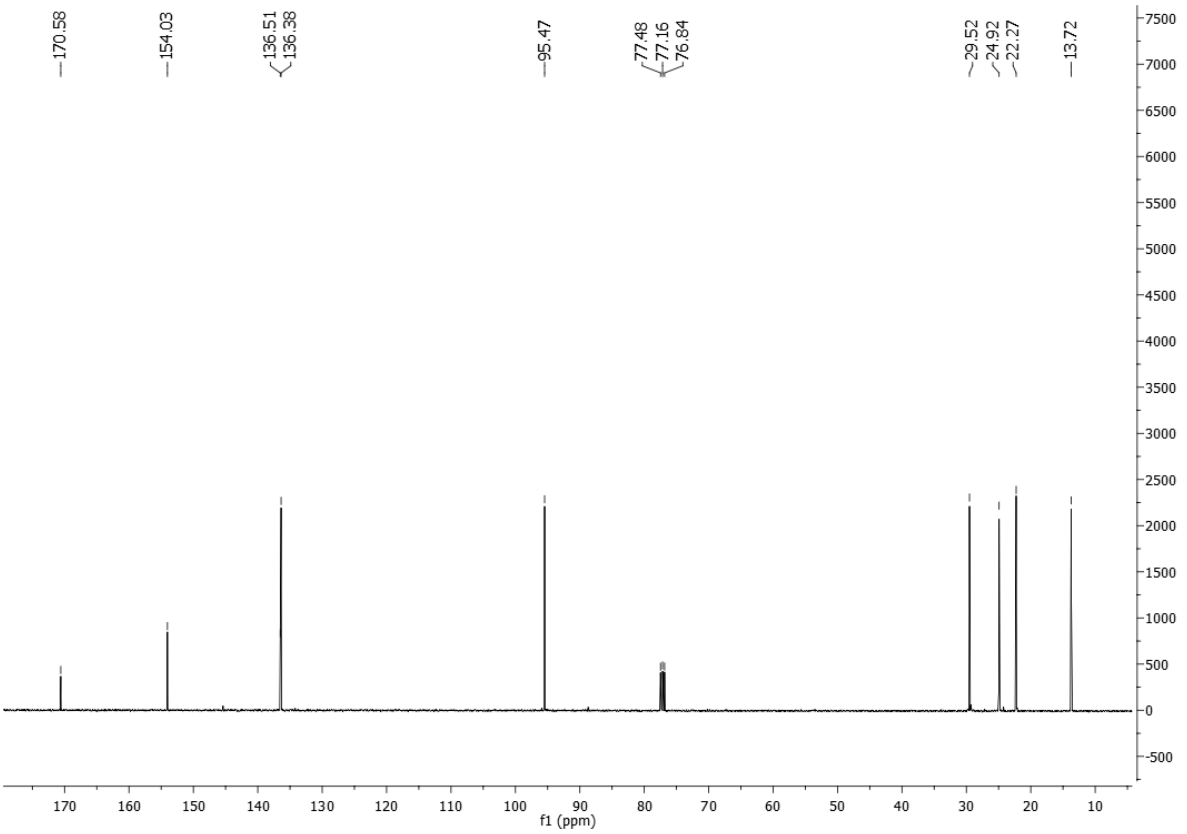
IV.1.10. 3-(1-hydroxyoctyl)furan-2(*5H*)-one (6e)

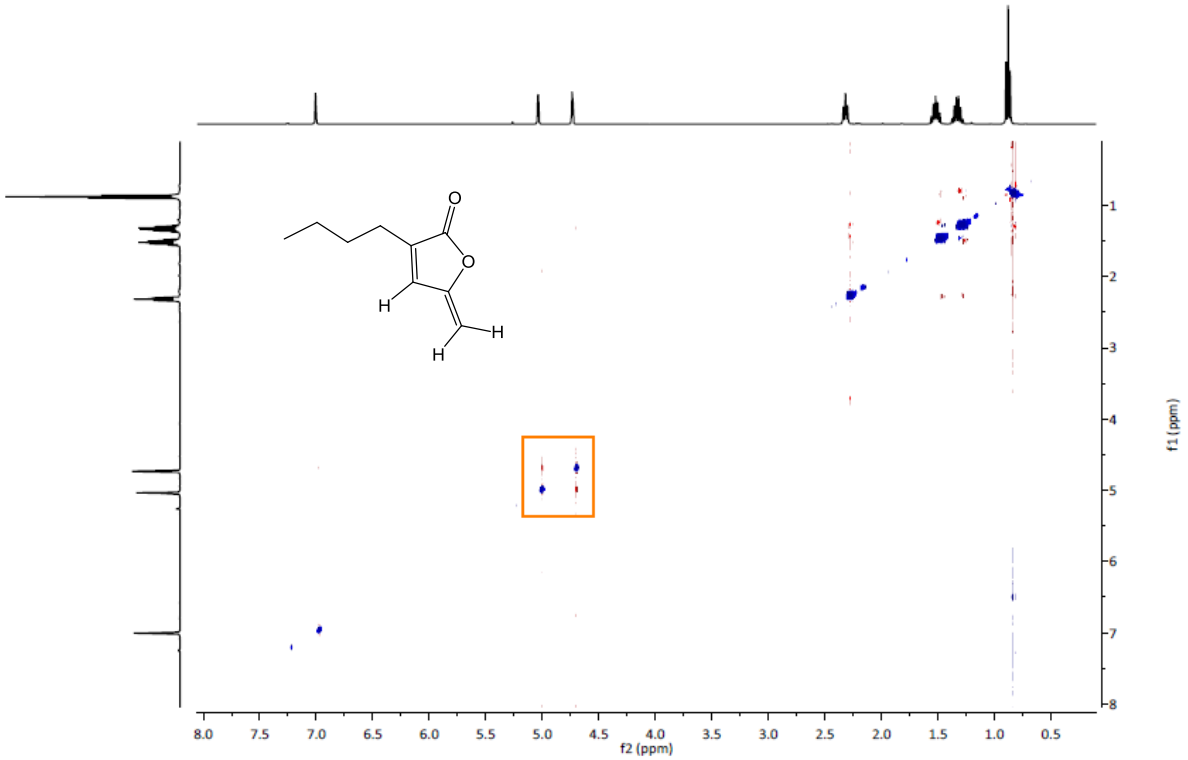




IV.1.11. 3-butyl-5-methylenefuran-2(*5H*)-one (8a)







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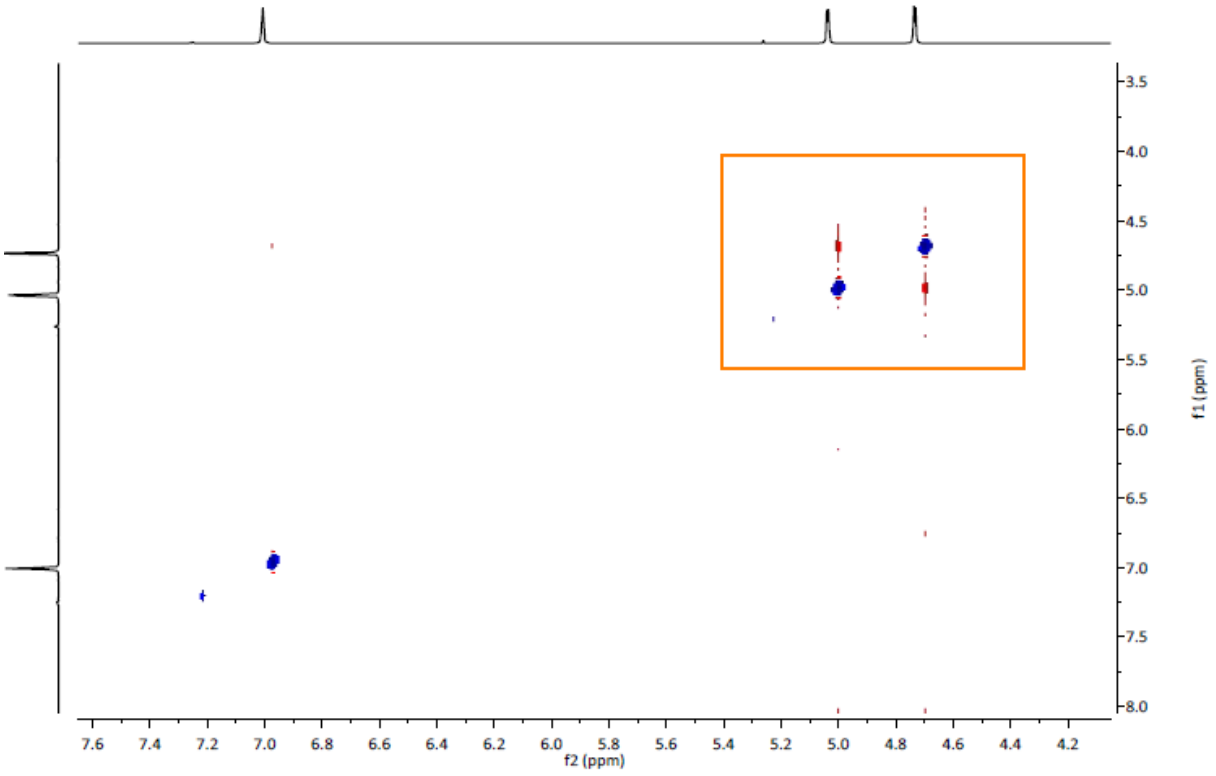
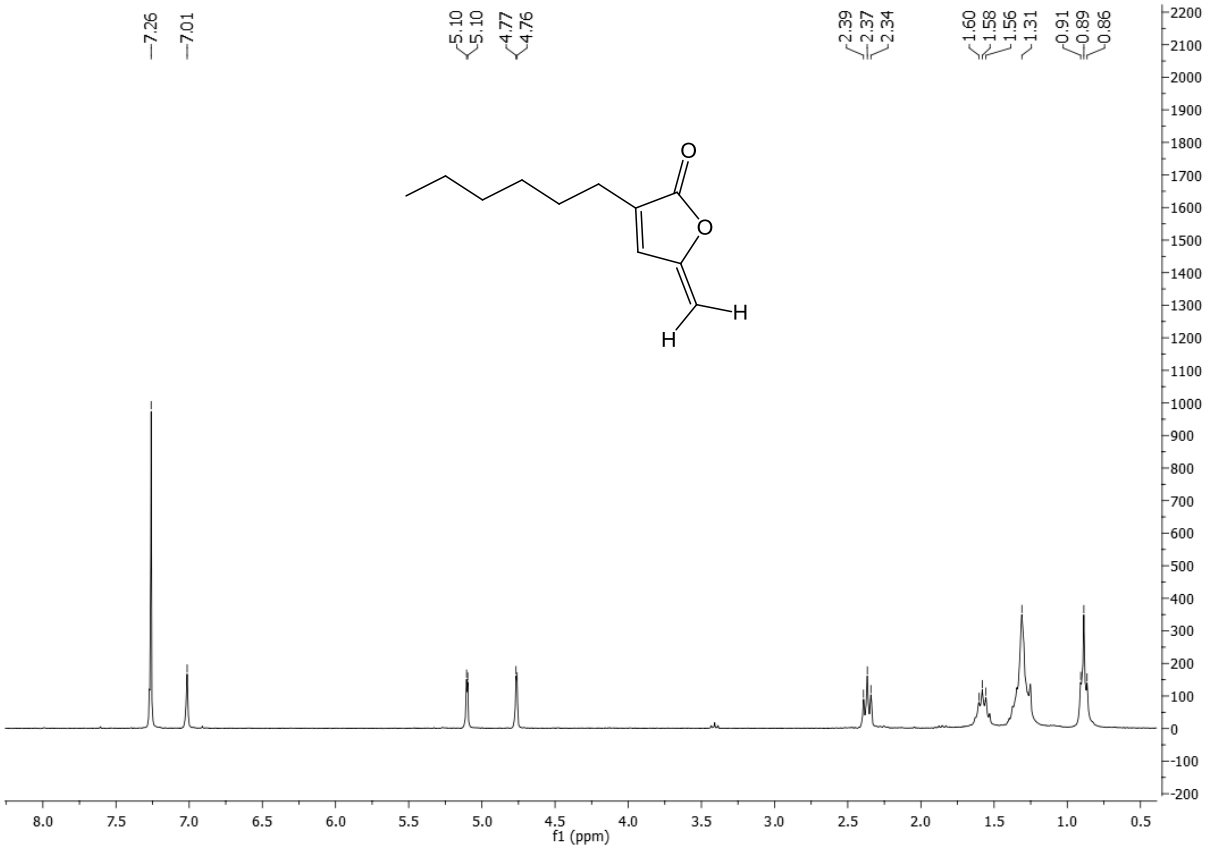
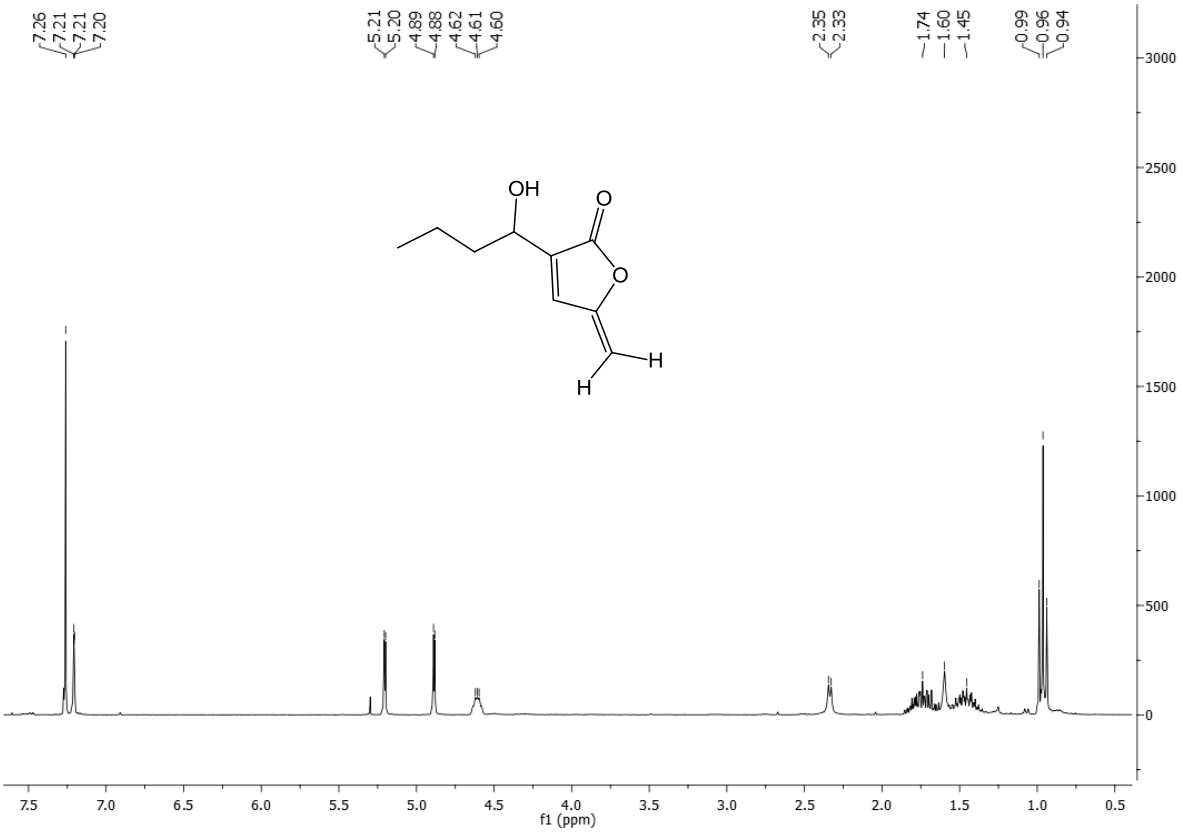


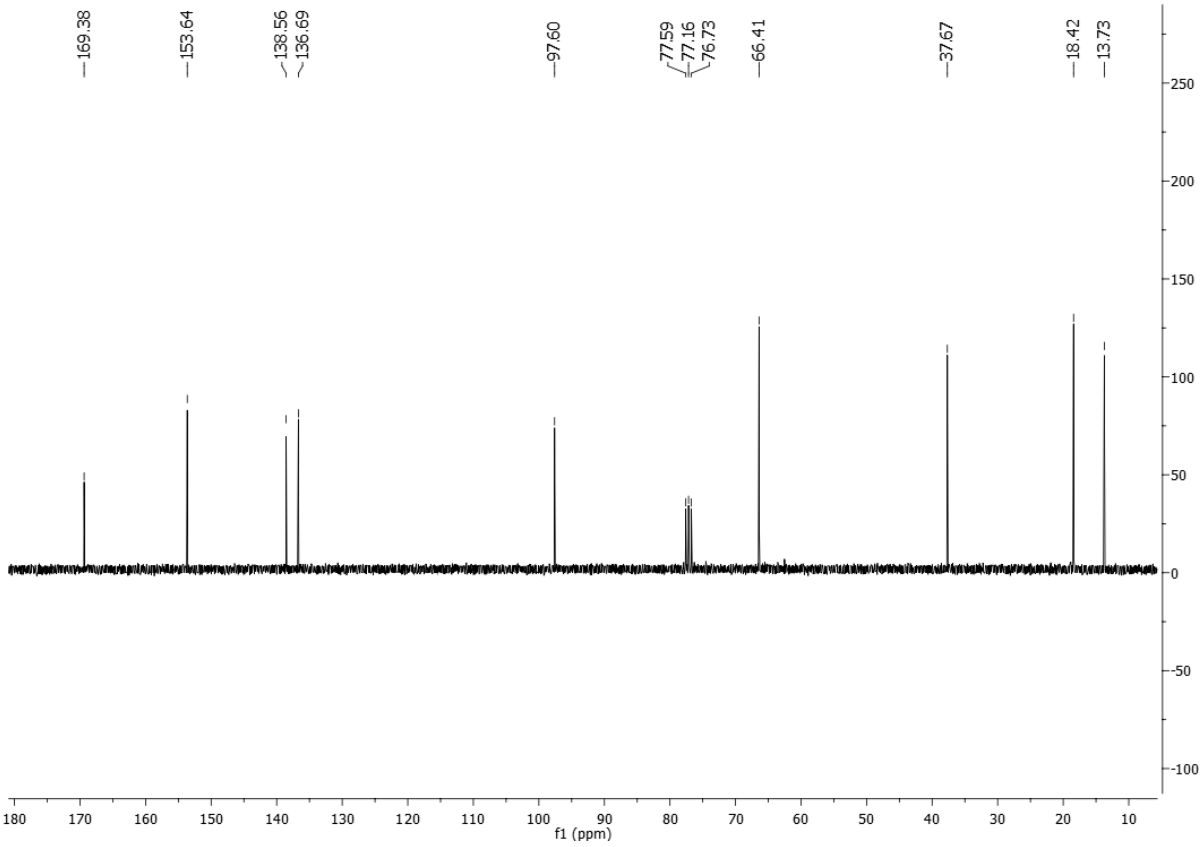
NOESY (Zoom)

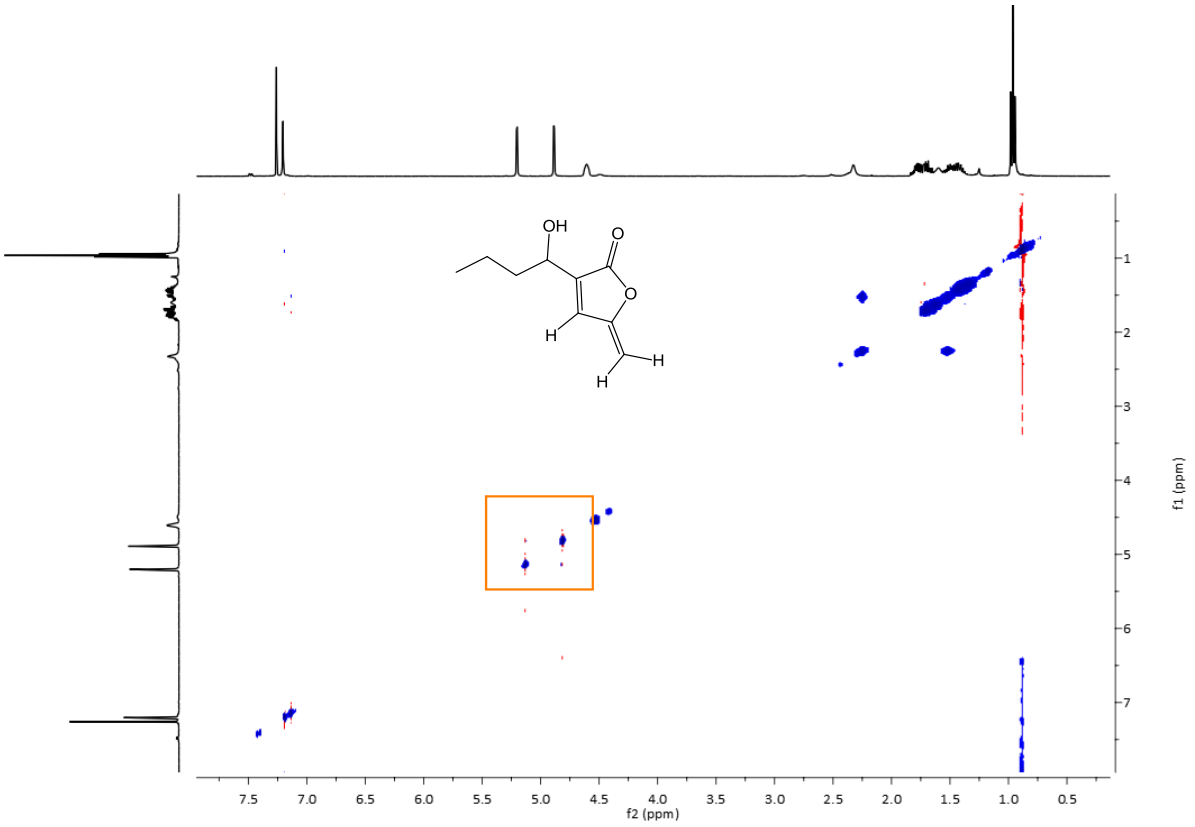
IV.1.12. 3-hexyl-5-methylenefuran-2(*5H*)-one (8b)



IV.1.13. 3-(1-hydroxybutyl)-5-methylenefuran-2(*5H*)-one (8c)







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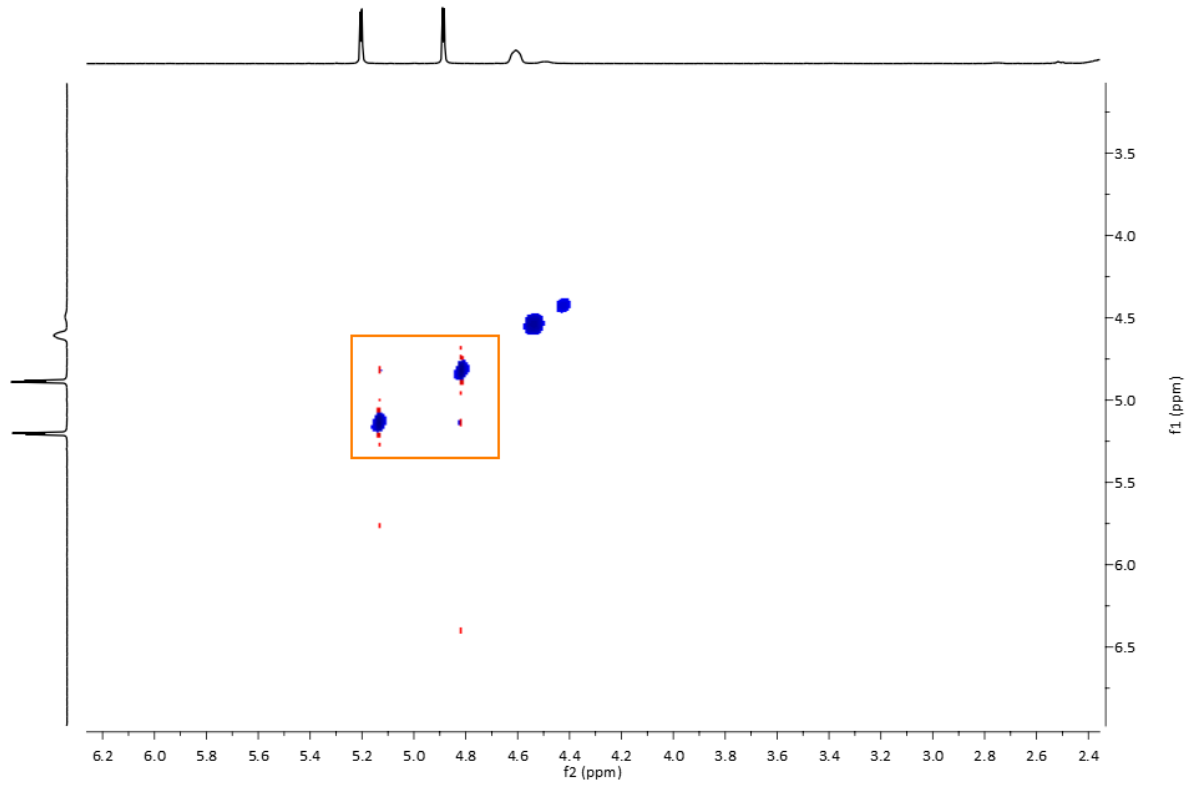
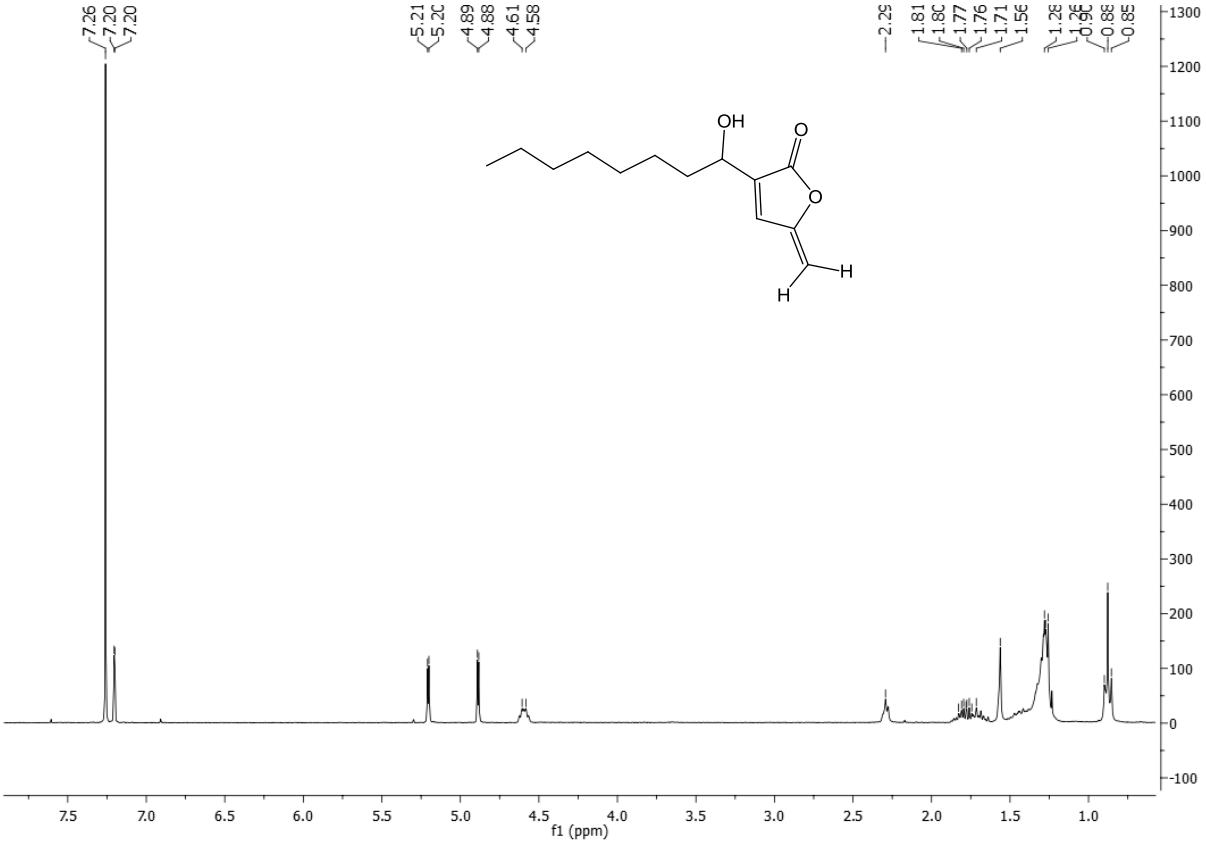
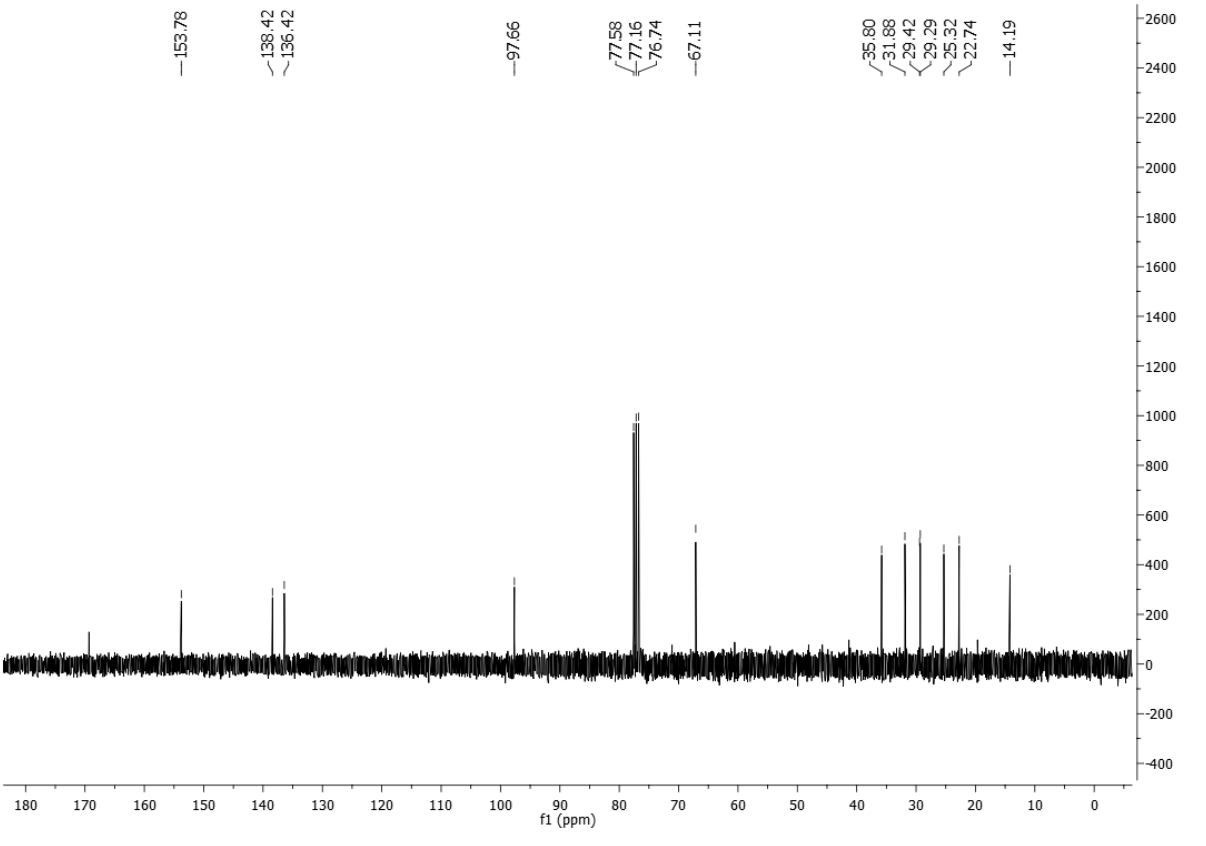


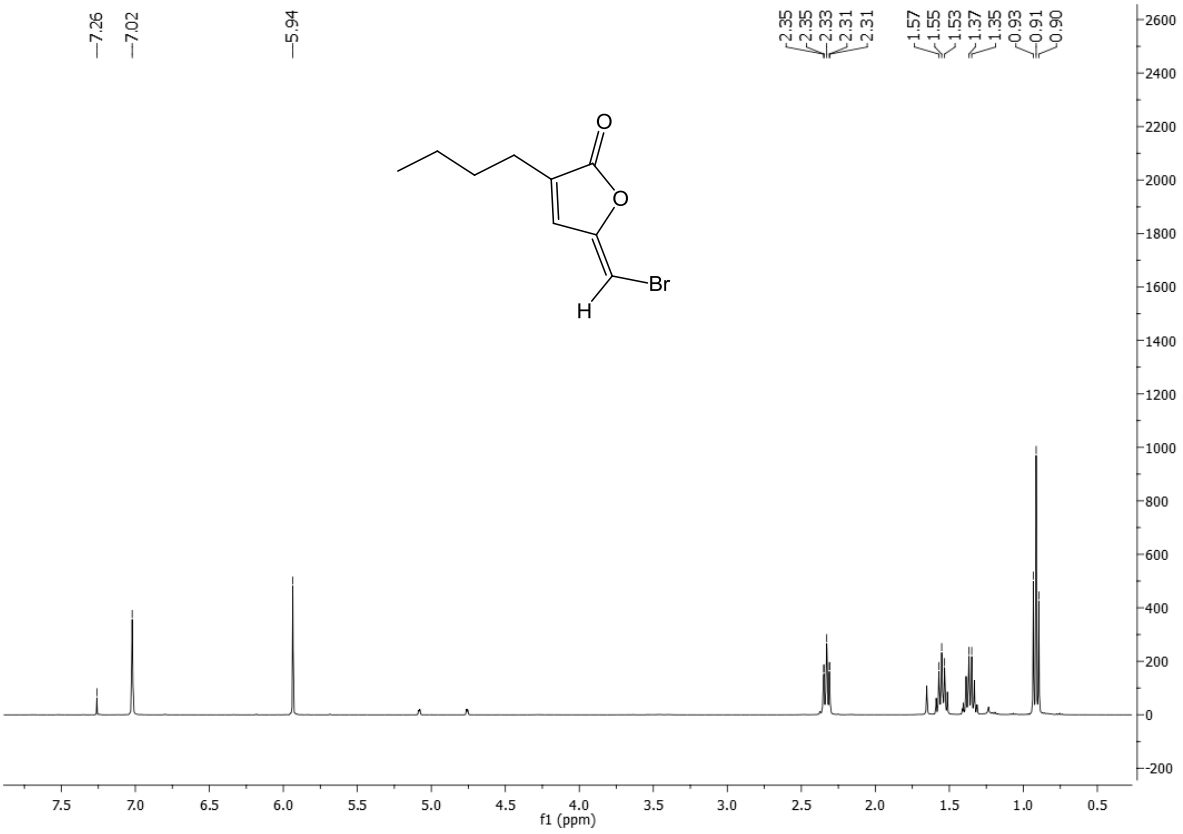
NOESY (Zoom)

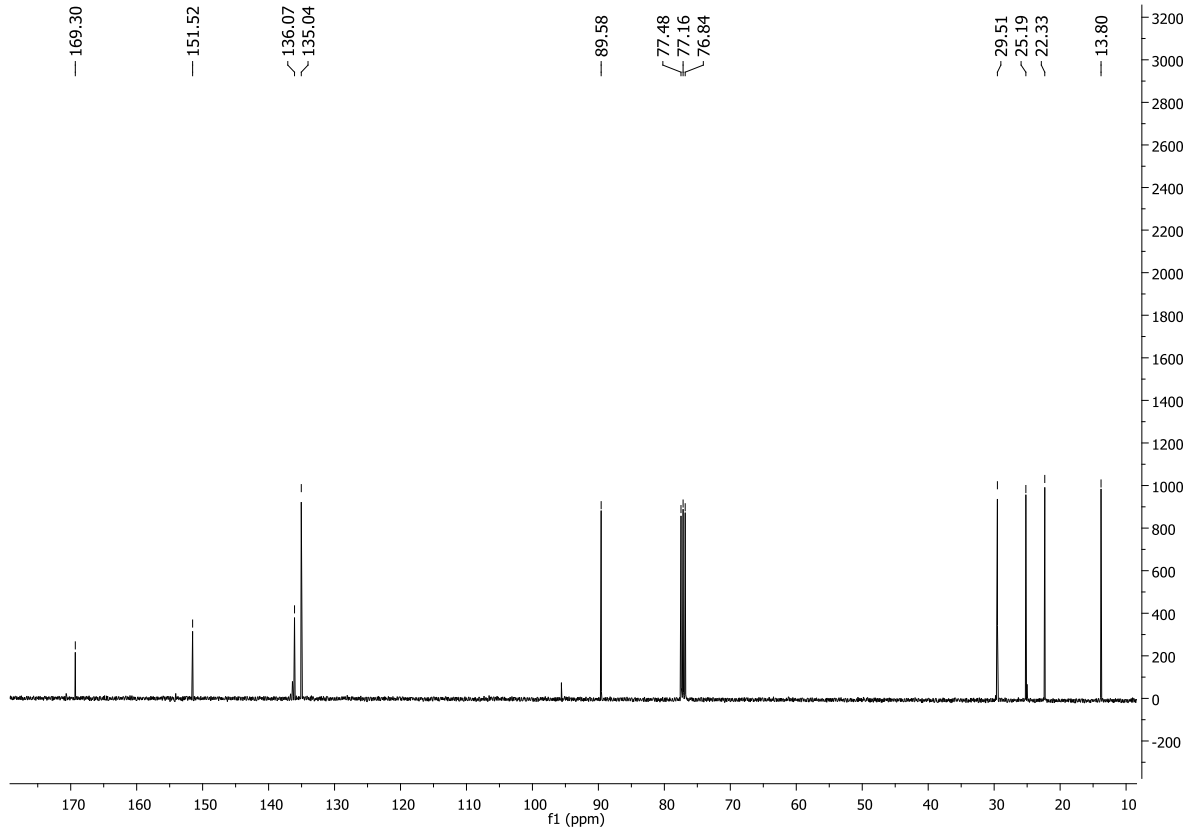
IV.1.14. 3-(1-hydroxyoctyl)-5-methylenefuran-2(*5H*)-one (8e)

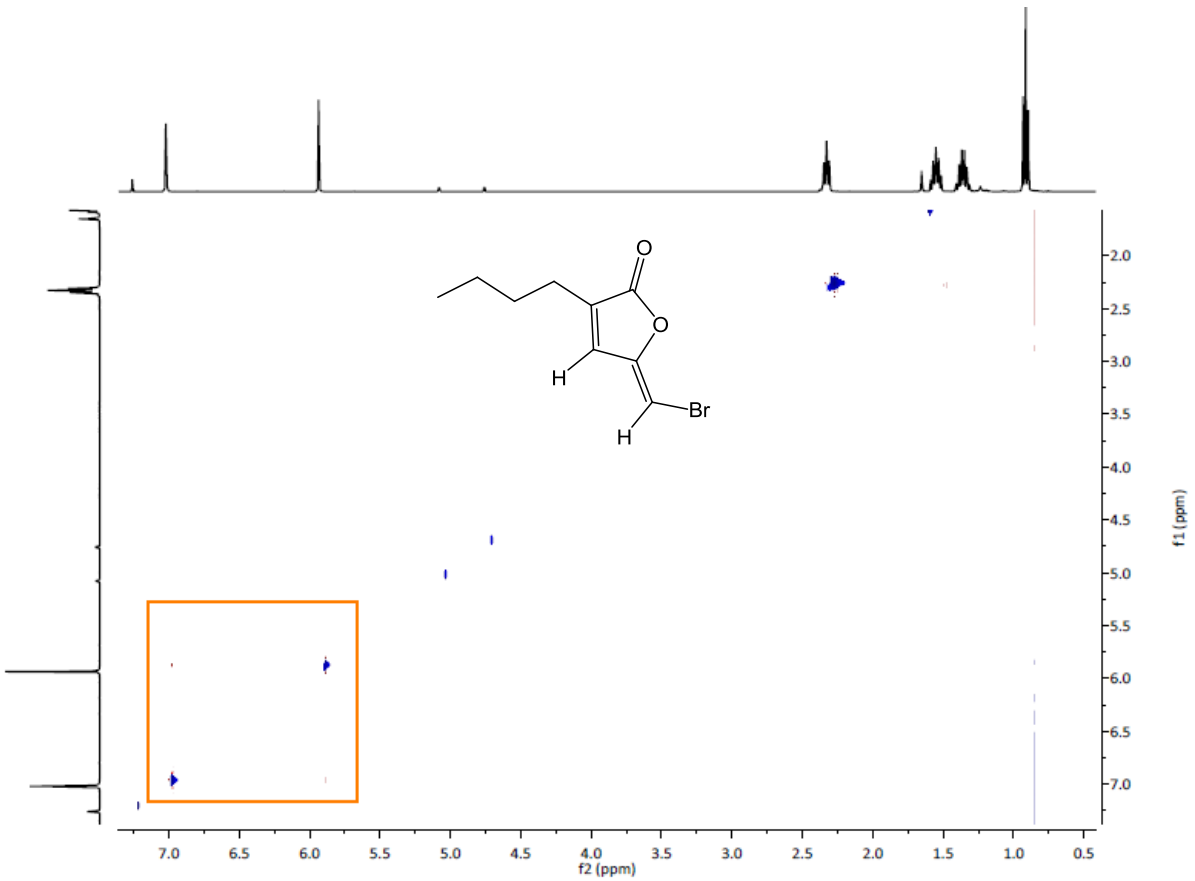




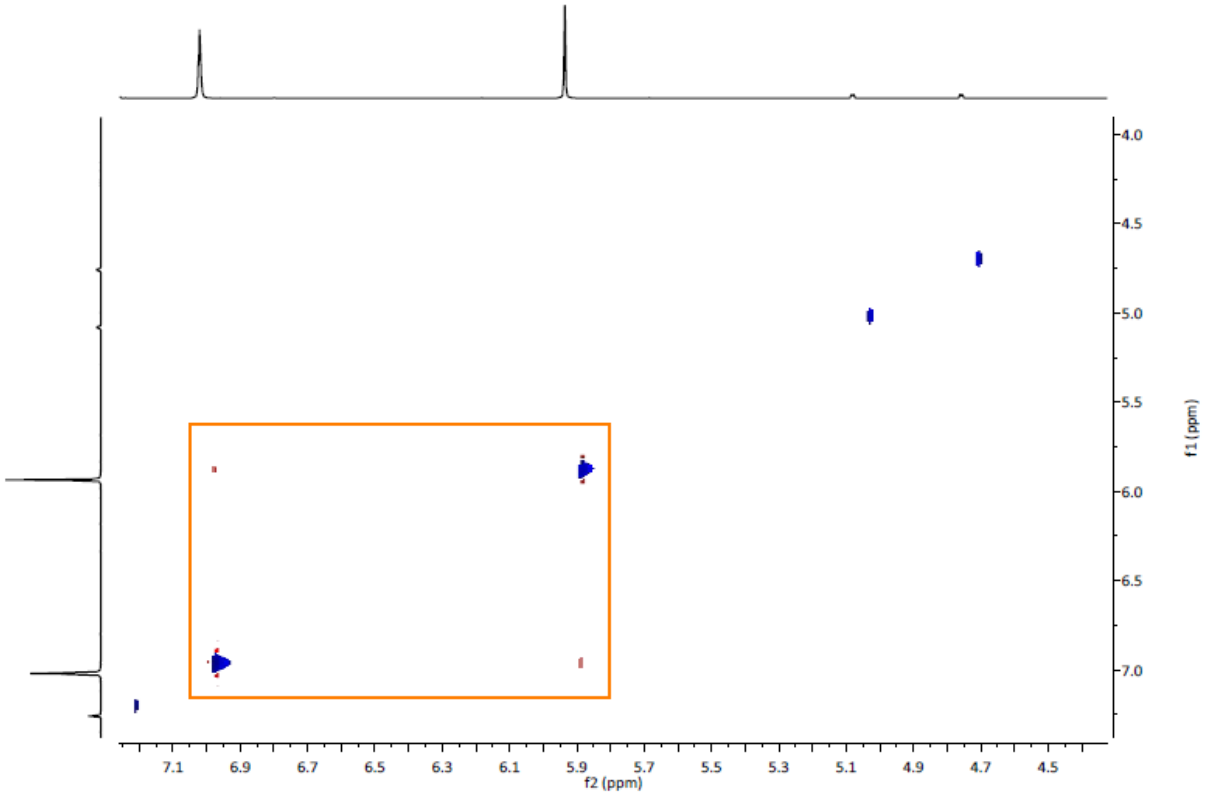
IV.1.15. (Z)-5-(bromomethylene)-3-butylfuran-2(5*H*)-one (10a)




169.30
151.52
136.07
135.04
89.58
77.48
77.16
76.84
29.51
25.19
22.33
13.80
f1 (ppm)

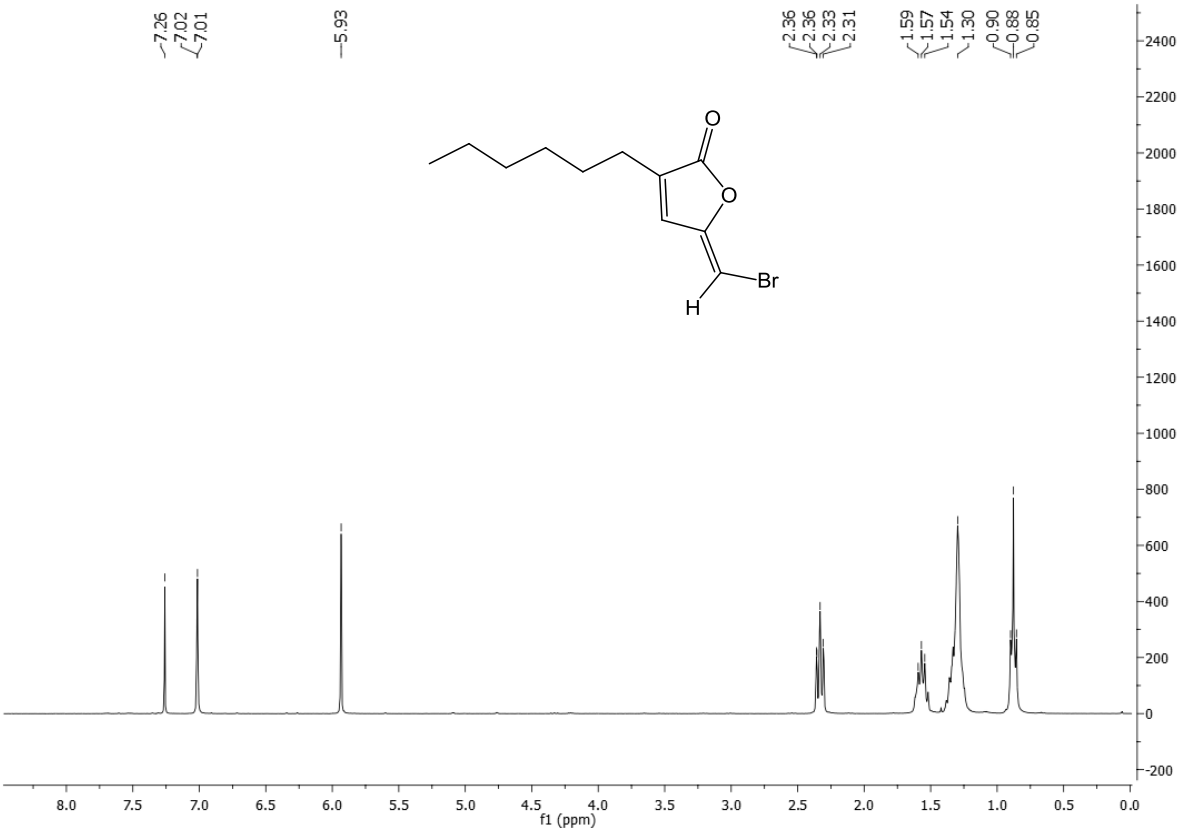

O
O
H
Br
H
f2 (ppm)
f1 (ppm)

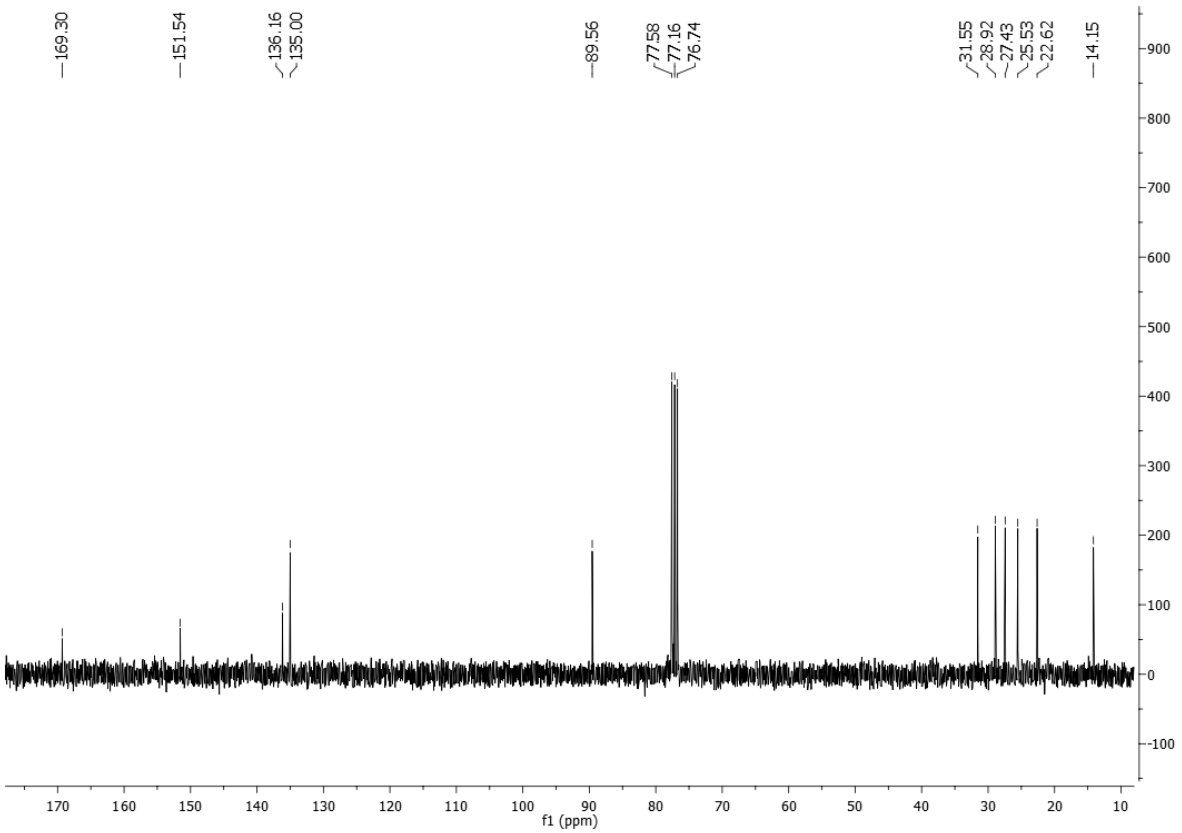
NOESY


4.0
4.5
5.0
5.5
6.0
6.5
7.0
f1 (ppm)
7.1
6.9
6.7
6.5
6.3
6.1
5.9
5.7
5.5
5.3
5.1
4.9
4.7
4.5
f2 (ppm)

NOESY (Zoom)

IV.1.16. (Z)-5-(bromomethylene)-3-hexylfuran-2(5*H*)-one (10b)

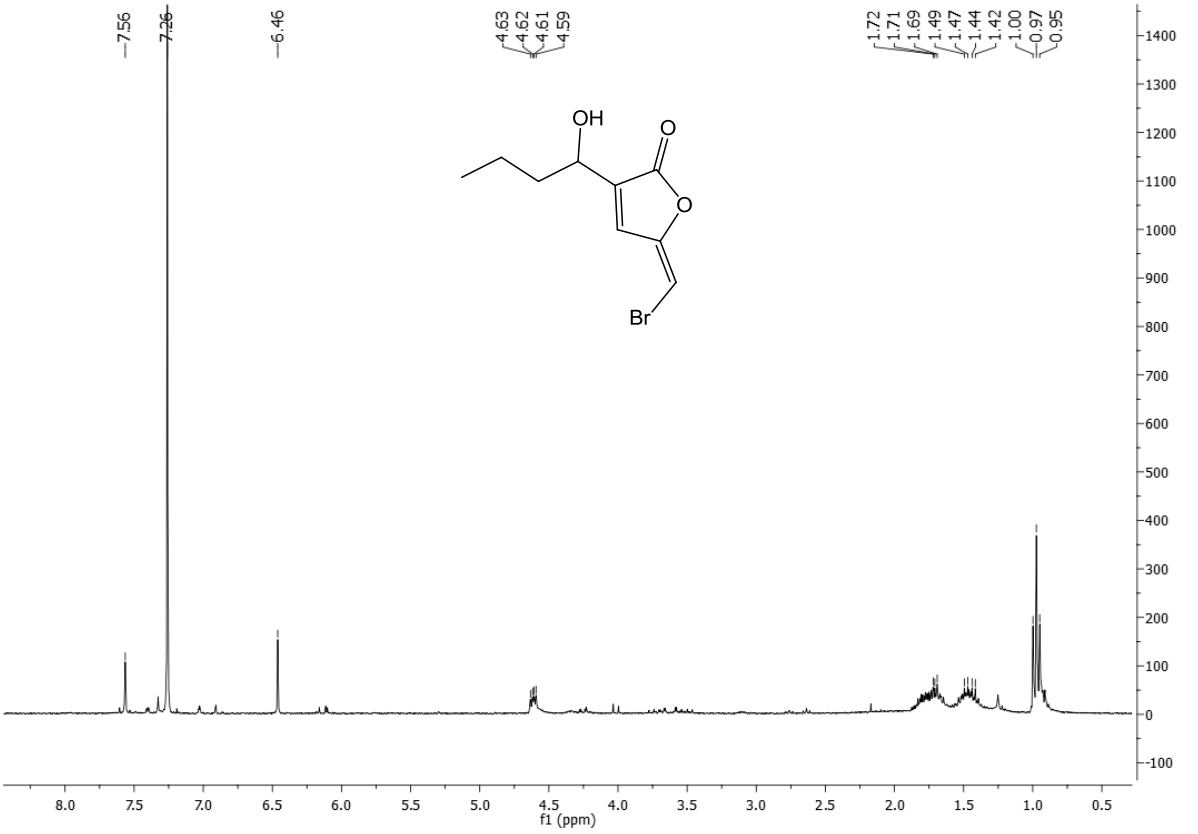


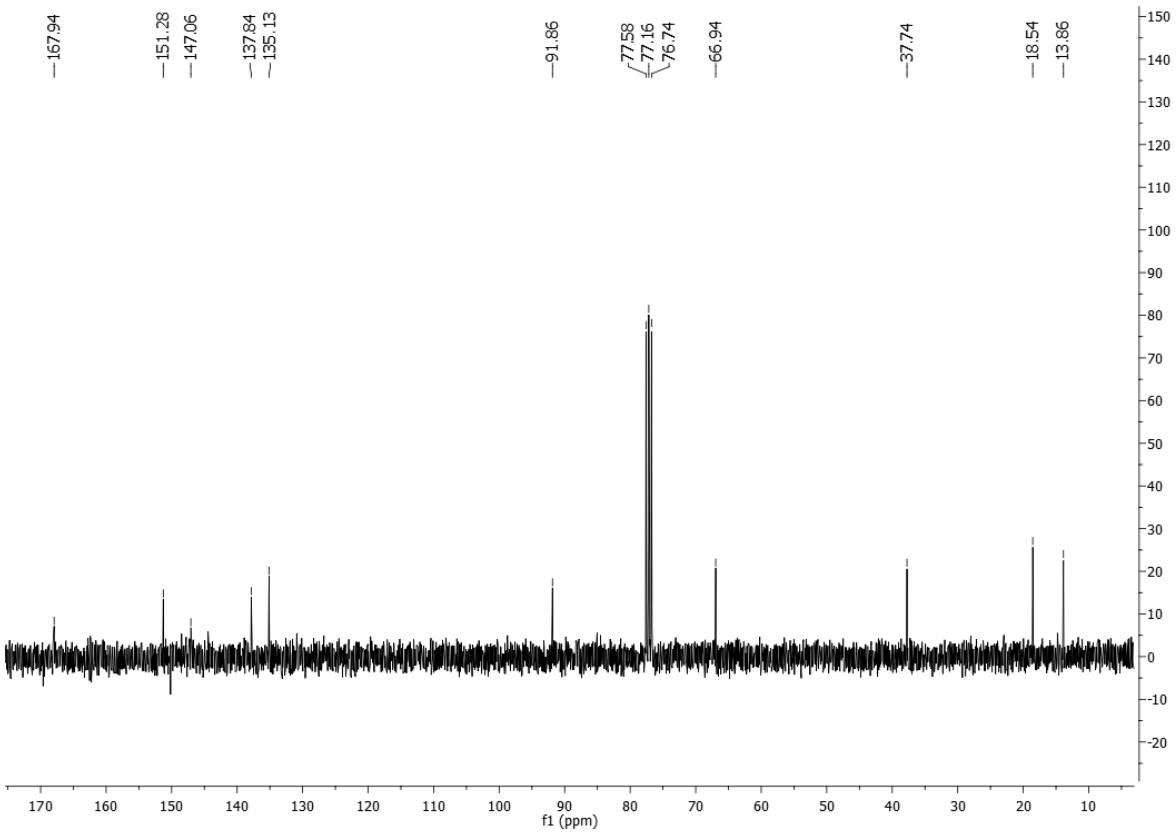


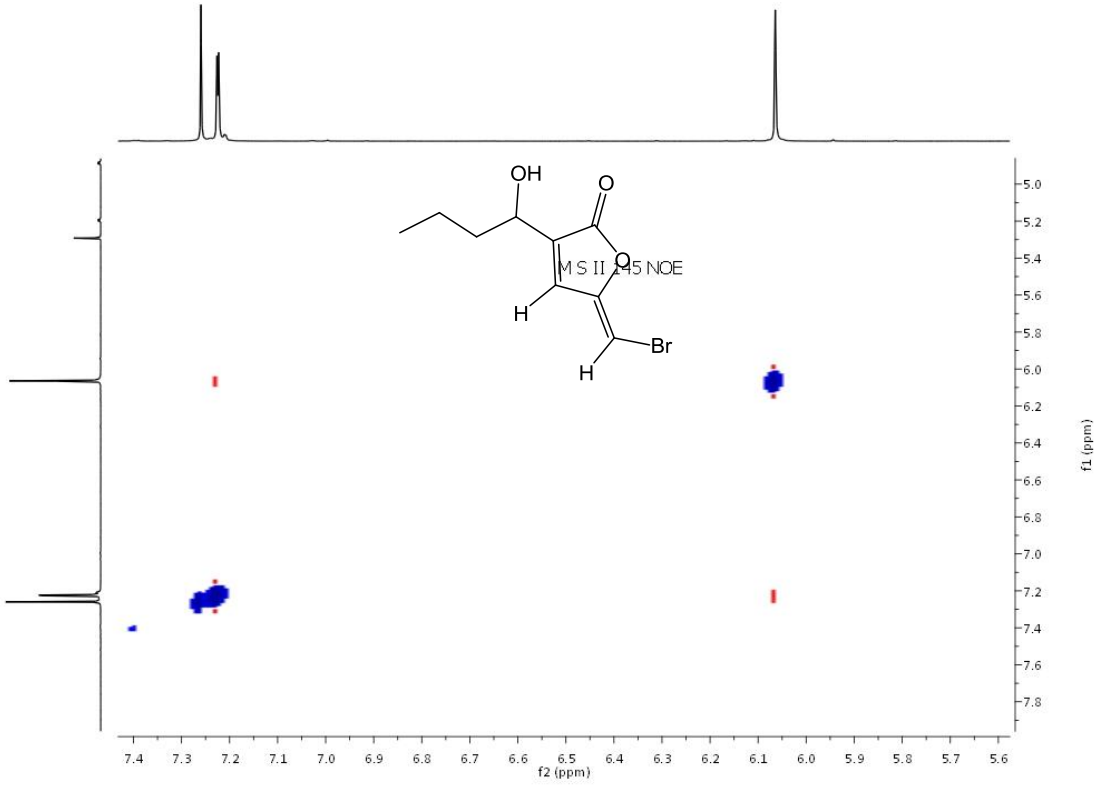
IV.1.17. (Z)-5-(bromomethylene)-3-(1-hydroxybutyl)furan-2(*5H*)-one (10c)

(E)-5-(bromomethylene)-3-(1-hydroxybutyl)furan-2(*5H*)-one (10c)


7.26
7.22
7.21
6.06
4.60
4.59
4.58
4.57
2.17
1.74
1.70
1.67
1.48
1.45
0.99
0.96
0.94
OH
O
O
Br
f1 (ppm)

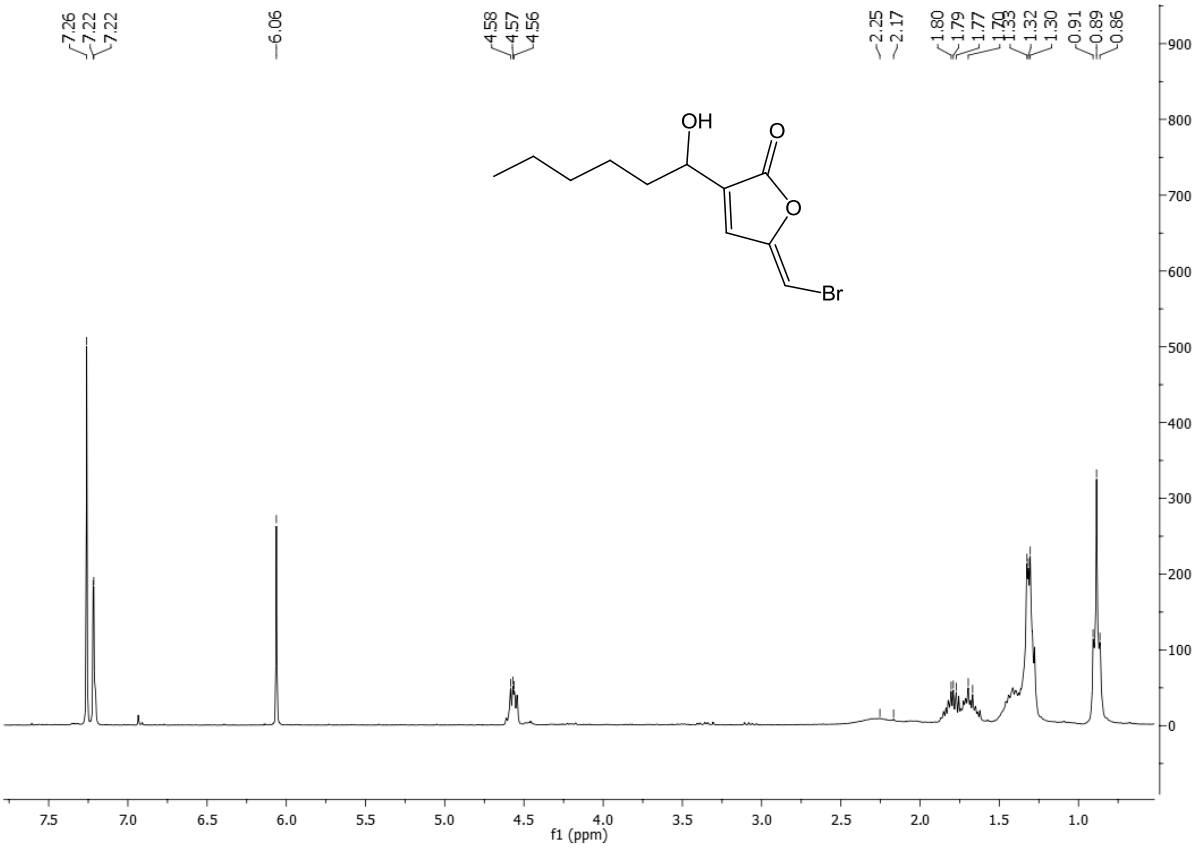

7.56
7.26
6.46
4.63
4.62
4.61
4.59
1.72
1.71
1.69
1.49
1.47
1.44
1.42
1.00
0.97
0.95
OH
O
O
Br
f1 (ppm)

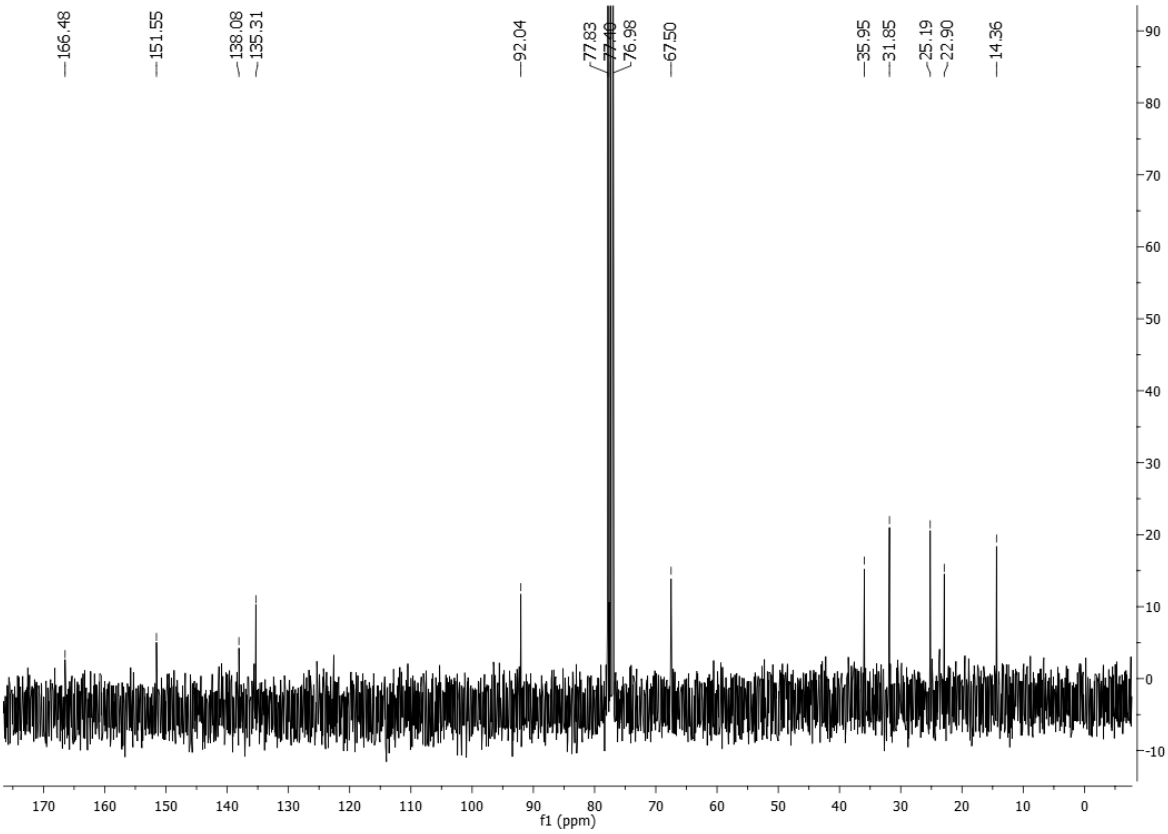

167.94
151.28
147.06
137.84
135.13
91.86
77.58
77.16
76.74
66.94
37.74
18.54
13.86
f1 (ppm)


OH
O
O
H
Br
H
M S II 245 NOE
f2 (ppm)
f1 (ppm)

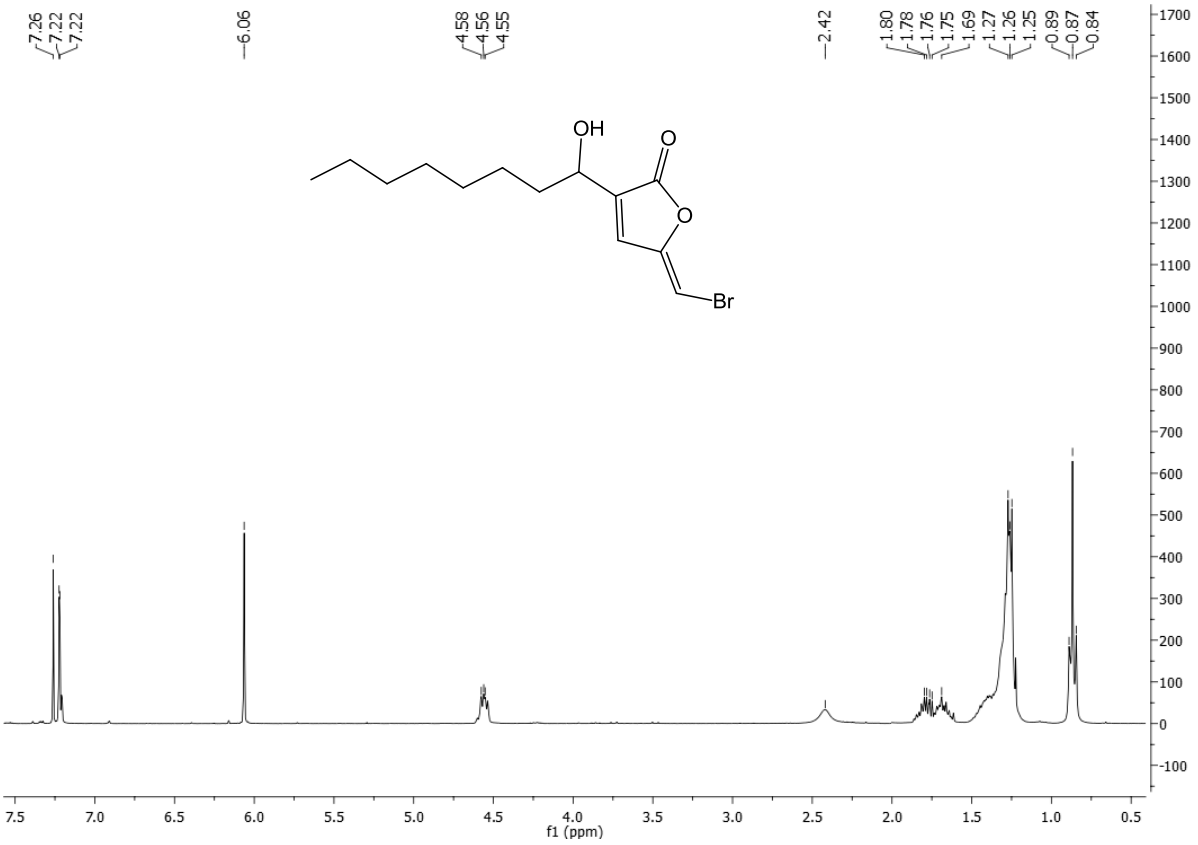
NOESY (Zoom)

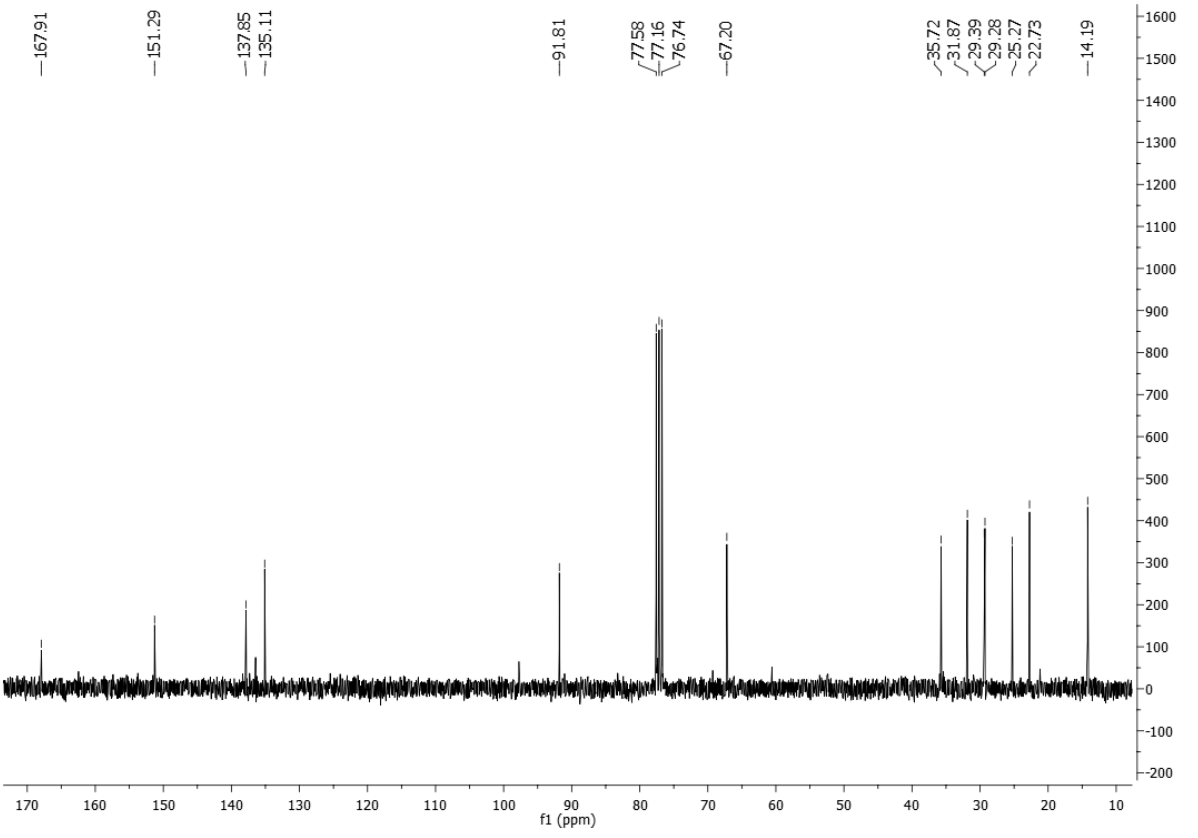
IV.1.18. (Z)-5-(bromomethylene)-3-(1-hydroxyhexyl)furan-2(*5H*)-one (10d)





IV.1.19. (Z)-5-(bromomethylene)-3-(1-hydroxyoctyl)furan-2(5*H*)-one (10e)





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