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Supporting Information

Extending the Salinilactone Family

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Abstract: Five new members of the salinilactone family, salinilactones D-H, are reported. These bicyclic lactones are produced by *Salinispora* bacteria and display extended or shortened alkyl side chains compared to the recently reported salinilactones A-C. They were identified by GC/MS, gas chromatographic retention index and comparison with synthetic samples. We further investigated the occurrence of salinilactones across six newly proposed *Salinispora* species to gain insight into how compound production varies among taxa. The growth inhibiting effect of this compound family on multiple biological systems including non-*Salinispora* actinomycetes were analyzed. Additionally, we found strong evidence for significant cytotoxicity of the title compounds.

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Part 1: Compound Overview

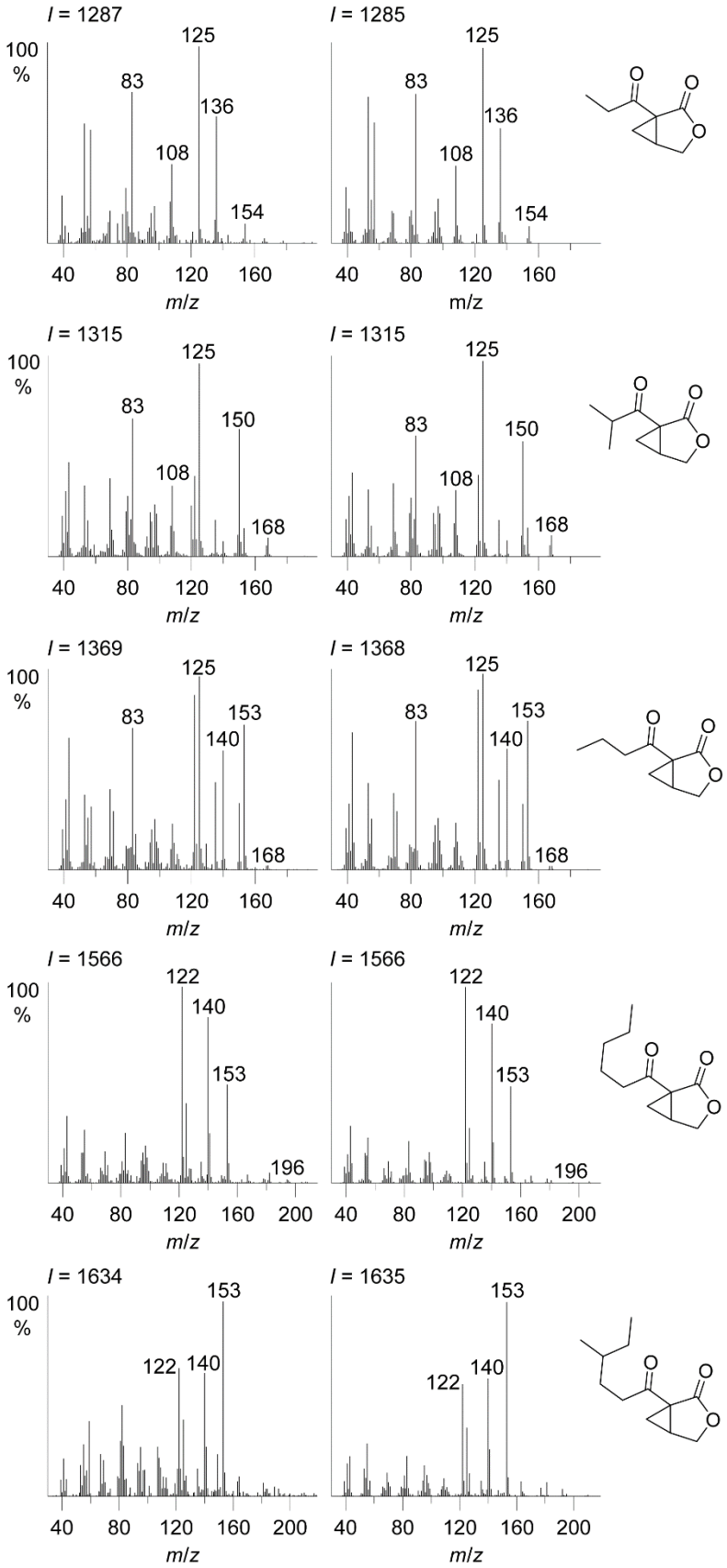

Figure S1. Comparison of mass spectra and gas chromatographic retention index *I* of the natural products (left) and the synthetic compounds (right).

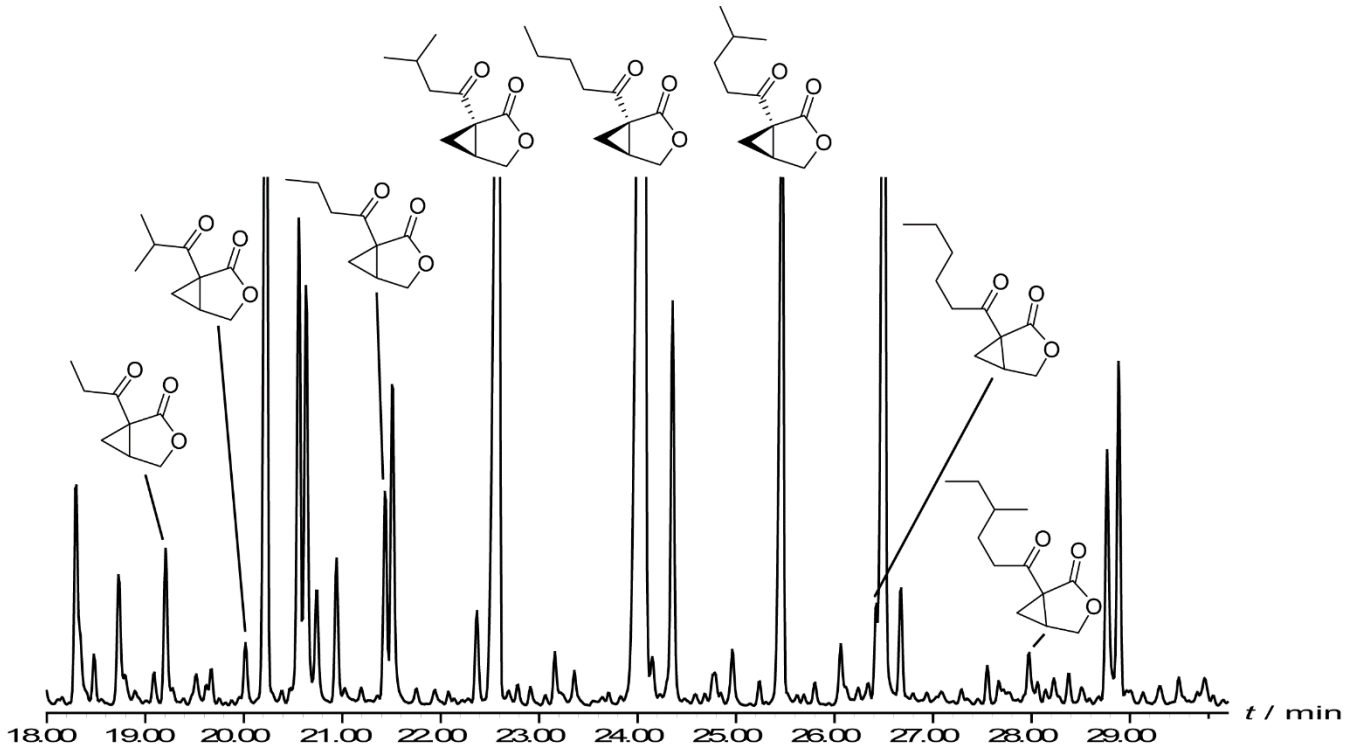

Figure S2. Section of the TIC-chromatogram of a 48 h CLSA extract of a liquid culture of *Salinispora arenicola* CNS-205 with salinilactone peaks tagged with the corresponding compound structures.

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Part 2: Bioassays / Species and Spt9 phylogenies

Table S1. Strains used in the study.

Strain	Species	NCBI Taxon ID/DSM No.
CNY-202	*S. "cortesiana"*	1305843
CNB-536	*S. tropica*	1136431
CNY-666	*S. "goodfellowii"*	1408341
CNT-854	*S. "oceanensis"*	1137265
CNS-237	*S. "mooreana"*	1288089
CNR-942	*S. "fenicalii"*	1169187
CNS-055	*S. "vitiensis*"	1169182
CNB-440	*S. tropica*	369723 / DSM 44818
CNS-205	*S. arenicola*	391037
CNR-114	*S. pacifica*	1137260 / DSM 45820
CNR-894	*S. pacifica*	1137261
	Streptomyces lavendulae	DSM 40069
	Streptomyces griseus	DSM 40236
	Micromonospora nigra	DSM 43818
	Micromonospora echinospora	DSM 43816
	Amycolatopsis mediterranei	DSM 43304
	Actinoplanes teichomyceticus	DSM 43866

Cultivation of bacteria

All cultures were cultivated at 28 °C on agar plates on their respective media. Liquid cultures were initiated with these plated cultures at 100 mL scale. Liquid cultures for CLSA-extraction had a volume of 250 mL. All liquid cultures were cultivated at 28 °C in a heated shaker.

Culture media

A1 medium (*Salinispora* bacteria):
1000 mL water, 4 g yeast extract, 2 g bactopeptone, 10 g soluble starch, 30 g instant ocean, (20 g agar).

Medium 65 (Actinomycetes)
1000 mL water, 4 g glucose, 4 g yeast extract, 10 g malt extract, (2 g calcium carbonate, 12 g agar).

GPHF medium (*M. nigra*)
1000 mL water, 10 g glucose, 5 g peptone from casein, 5 g yeast extract, 5 g beef extract, 0.74 g calcium chloride dihydrate, (15 g agar).

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Agar diffusion assays

Sterile cellulose discs were combined with appropriate amounts of methanolic solutions of salinilactone B. The soaked discs were than dried by the sterile air stream under a clean bench. The bacteria were spread on the agar plates and the loaded discs were placed on the agar.

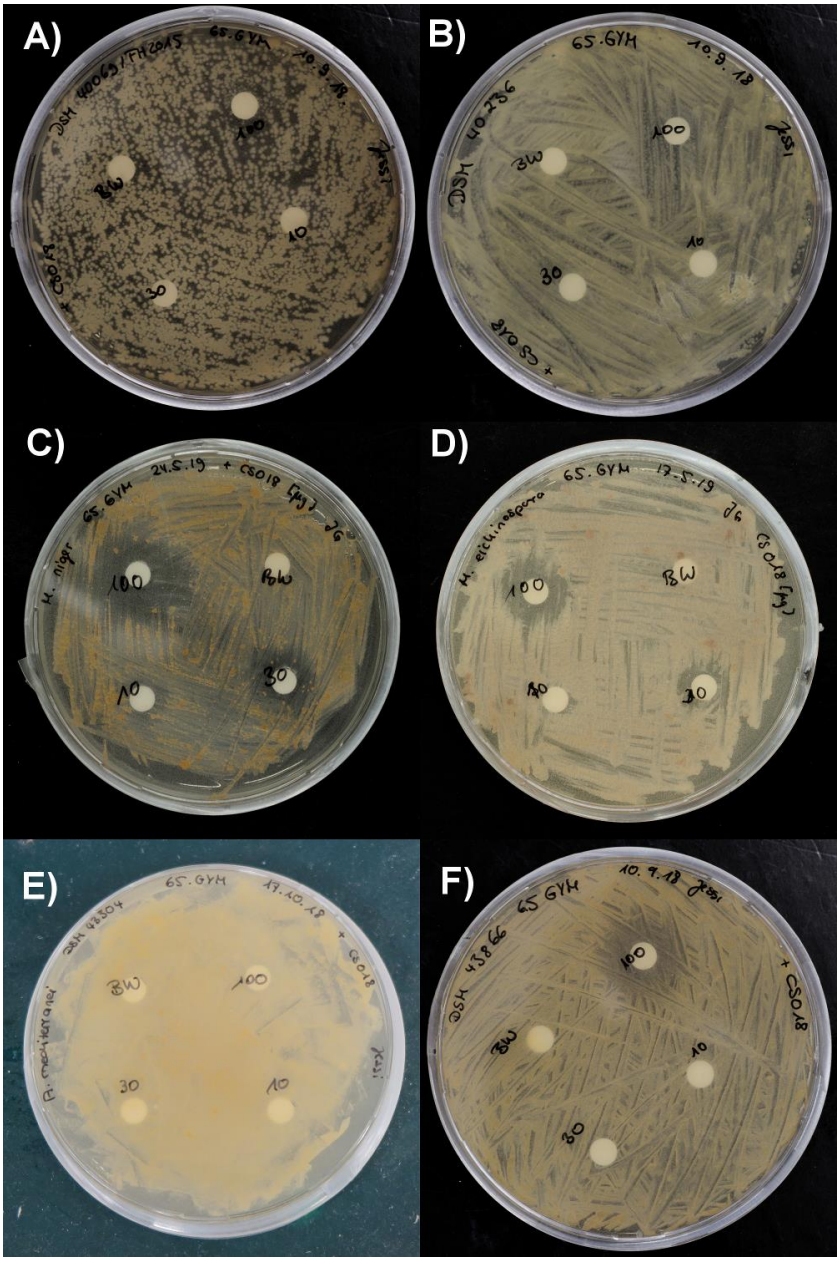

Figure S3. Agar diffusion tests of non-*Salinispora* actinomycetes using salinilactone B (**1b**) with loadings of 10 µg, 30 µg and 100 µg. A) *Streptomyces lavendulae* DSM40069; B) *Streptomyces griseus* DSM40236; C) *Micromonospora nigra* DSM43818; D) *Micromonospora echinospora* DSM43816; E) *Amycolatopsis mediterranei* DSM43304; F) *Actinoplanes teichomyceticus* DSM43866.

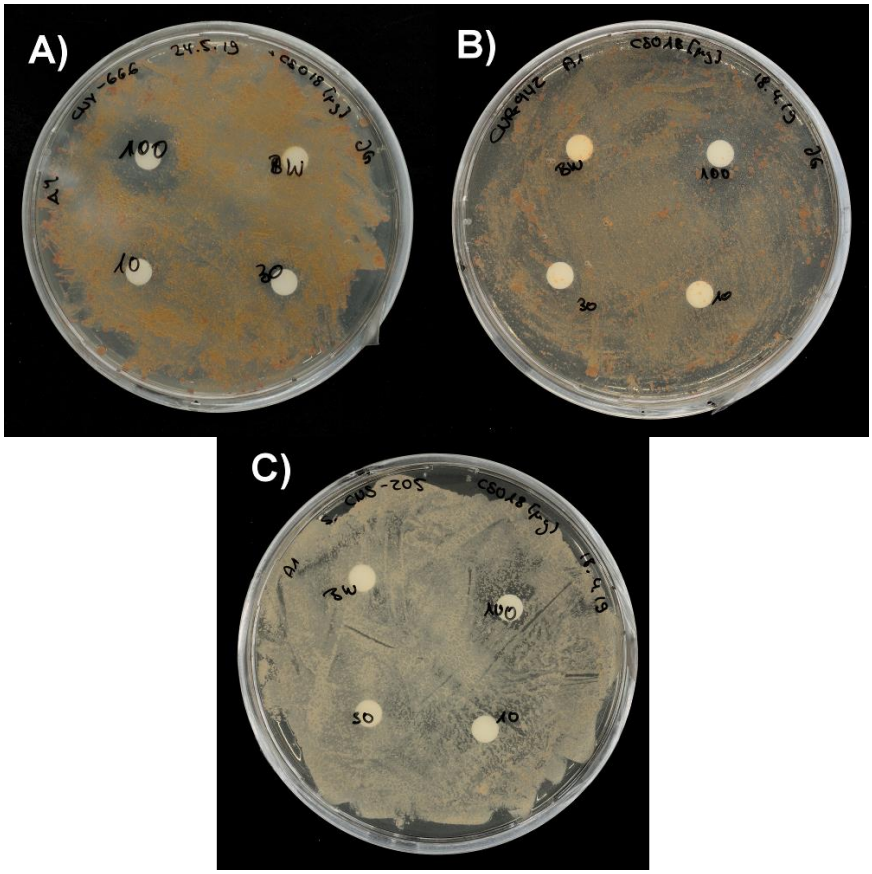

Figure S4. Agar diffusion tests of *Salinispora* bacteria using salinilactone B (**1b**) with loadings of 10 µg, 30 µg and 100 µg. A) *Salinispora "goodfellowii"* CNY-666; B) *Salinispora "fenicalii"* CNR-942; C) *Salinispora arenicola* CNS-205.

Artemia salina cytotoxic assay

Artemia salina eggs (Fisher scientific) hatched at 28°C in the dark in artificial seawater (40 g/L Instant Ocean, Marineland) that was oxygenated with an aquarium pump. After 48 hours, nauplii were separated from the eggs. The compounds were serially diluted in methanol, added in triplicate to 24 well plates, allowed to evaporate, and resuspended in 10 uL of DMSO prior to adding sea water for a final volume of 2.5 mL. 20 to 40 nauplii were added to each well including seawater and DMSO controls. After 24 hours of incubation at 28°C in the dark, dead nauplii were counted under a dissecting microscope. Odd swimming behavior was ignored and only organisms that showed no evidence of movement were considered dead. The experiment was repeated by triplicate using different batches of artificial sea water and eggs. LC_{50} values were calculated in R by Probit analysis.[S2]

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Table S2. Occurrence of salinilactones A-G[a] (**1a**-**1g**) in different *Salinispora* strains based on 24 h CLSA experiments of liquid and agar cultures. Compounds are ordered according to their retention index. (-): Not detected; (+) Compound peak lower than 10% height of highest peak in TIC; (++): Compound peak higher than 10% height of highest peak in TIC; (?): Traces of compound detected, identification uncertain.

Salinispora strain	Cultivation (time)	Optical density	Salinilactone D (**1d**)	Salinilactone E (**1e**)	Salinilactone F (**1f**)	Salinilactone A (**1a**)	Salinilactone B (**1b**)	Salinilactone C (**1c**)	Salinilactone G (**1g**)
S. "cortesiana" CNY-202	liquid (4 d)	2.00	-	-	-	?	?	-	-
	agar	-	-	-	-	+	+	-	-
S. tropica CNB-536	liquid (4 d)	2.00	-	-	-	-	?	-	-
	agar	-	-	-	+	?	?	-	-
S. "goodfellowii" CNY-666	liquid (8 d)	1.04	-	-	-	-	-	-	-
	agar	-	-	-	-	-	-	-	-
S. "oceanensis" CNT-854	liquid (12 d)	0.99	+	-	+	+	+	?	-
	agar	-	-	-	?	+	+	-	-
S. "mooreana" CNS-237	liquid (6 d)	1.31	-	-	-	+	+	-	-
	agar	-	-	-	?	+	?	+	-
S. "fenicalii" CNR-942	liquid (4 d)	1.90	-	-	-	-	-	-	-
	agar	-	-	-	-	-	-	-	-
S. "vitiensis" CNS-055	liquid (5 d)	2.00	-	-	-	-	-	-	-
	agar	-	+	-	?	+	+	-	-
S. tropica CNB-440	liquid (5 d)	1.60	-	?	+	+	+	+	+
	agar	-	-	?	+	+	+	?	-
S. arenicola CNS-205	liquid (6 d)	2.00	+	+	+	++	++	+	+
	agar	-	+	-	+	+	+	+	-
S. pacifica CNR-114	liquid (5 d)	1.72	+	?	+	++	+	+	+
	agar	-	+	+	++	++	+	+	?
S. pacifica CNR-894	liquid (3 d)	2.00	+	?	++	++	++	+	+
	agar	-	+	-	++	++	+	?	-
S. arenicola CNH-996A	agar	-	+	?	+	++	++	+	?

[a]Salinilactone H (**1h**) was not included because it was only detectable in very small amounts in long-time CLSA extracts (48 h) of *Salinispora arenicola* CNS-205.

Part 3: Experimental Procedures

Instrumentation

Each synthetic compound was analyzed by ^{1}H and ^{13}C NMR, IR, and GC/MS. NMR analyses were carried out on Bruker DPX-300, Bruker DRX 400, and Bruker AV-II 600 instruments using $CDCl_3$ as solvent with TMS as internal standard. IR spectra were recorded with a Bruker Tensor 27 (Diamond ATR) or a Dani Instruments DiscovIR IR detector coupled to an Agilent Technologies 7890B gas chromatograph. GC/MS analyses were performed using an HP5973 mass spectrometer coupled to a HP6890 gas chromatograph and an Agilent 5977A mass spectrometer coupled to an Agilent 7890B gas chromatograph. Agilent HP5-MS (GC/MS; AgilentTechnologies, 30 m × 0.25 mm i.d. × 0.25µm) and Agilent HP5 (GC/IR; 30 m × 0.25 mm i.d. × 0.25µm) columns with helium as carrier gas (GC/MS 1.2 ml/min, GC/IR 1.5 ml/min) were used for the analyses. A standard temperature program was used, starting at 50 °C for 5 min. Then the temperature was raised to 320 °C at a rate of 5°C/min.

General synthetic methods and materials

Reactions were carried out under a nitrogen atmosphere employing Schlenk techniques and rubber septa to seal the flasks. Liquids were transferred using syringes. Reactions were monitored by TLC on silica pre-coated polyester sheets (Polygram® SIL G/UV254, Macherey-Nagel), which were stained using a basic potassium permanganate solution with subsequent gentle heating. Flash column chromatography was performed on silica gel (Merck 40-63 µm). Starting materials were purchased from commercial suppliers and used without further purification. Solvents were purified employing standard techniques. Acetic acid was dried by distillation with acetic acid anhydride.

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Synthetic procedures

General procedure 1 for the preparation of terminal alkynes from alkyl bromides

Under a nitrogen atmosphere, lithium acetylide EDA-complex (1.50 eq.) and sodium iodide (0.05 eq.) were dissolved in dry DMSO (0.5 M in acetylide) and stirred for 5 minutes at room temperature. Then, a solution of the alkyl bromide (1.00 eq.) in dry DMSO (1 M) was added dropwise. The mixture was stirred for 5 h at room temperature and subsequently quenched with excess of water. The alkyne was distilled off together with water at a heating bath temperature of 140 °C. The pure alkyne was separated from the aqueous phase and dried over anhydrous sodium sulfate.[S3]

General procedure 2 for the preparation of 2-alkynoic acids from terminal alkynes

Under a nitrogen atmosphere, the alkyne (1.00 eq.) was dissolved in dry THF (0.75 M) and cooled to -78 °C. Then, *n*-butyllithium (1.6 M, 1.00 eq.) in hexane was added and the mixture was stirred at -78 °C for 45 minutes. Subsequently, the solution was warmed up to 0 °C and carbon dioxide was bubbled through it for 1 h. The reaction was quenched with water and the reaction mixture was extracted with a small amount of pentane. The pentane layer was separated and discarded while the aqueous layer was acidified with 2 M hydrochloric acid and extracted with ethyl acetate four times. The combined organic layers were washed with saturated sodium chloride solution and dried over anhydrous sodium sulfate. Evaporation of the solvent under reduced pressure yielded the product.[S4]

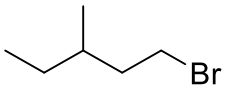
General procedure 3 for the preparation of allyl esters from carboxylic acids

A single neck flask was charged with the carboxylic acid (1.00 eq.), allyl alcohol (1.00 eq.) 4-dimethylaminopyridine (DMAP, 0.10 eq.) and dry DCM (DCM, 0.17 M in acid). The solution was cooled to 0 °C and *N*,*N*´-dicyclohexylcarbodiimide (DCC, 1.00 eq.) was added. The reaction mixture was allowed to warm up to room temperature and was stirred for 16 h. The resulting slurry was filtered and the solvent was removed from the filtrate under reduced pressure. The residue was purified by column chromatography over silica gel.[S5]

General procedure 4 for the Pd-catalyzed cyclization of allyl alk-2-ynoates

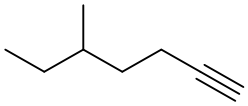
Acetic acid was dried and distilled under nitrogen before use. Under a nitrogen atmosphere, the allyl alkynoate (1.00 eq.) was dissolved in dry acetic acid (0.1 M) and combined with palladium-(II)-acetate (0.10 eq.) and (diacetoxyiodo)benzene (2.00 eq.). The mixture was heated to 80 °C and stirred for 3 h at this temperature. After quenching with water, the mixture was extracted with DCM three times. The combined organic phases were dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure. The crude product was purified by column chromatography over silica gel. In case of contamination with acetic acid, the product was dissolved in chloroform and washed with saturated sodium bicarbonate solution. The organic layer was separated, dried over sodium sulfate, and the solvent was evaporated at reduced pressure.[S6]

1-Bromo-3-methylpentane (2h)



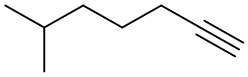
At 0 °C bromine (1.71 g, 10.8 mmol, 0.55 mL, 1.33 eq.) was slowly added to a solution of triphenylphosphine (2.82 g, 10.8 mmol, 1.33 eq.) in dry DCM (20 mL). After completion of the bromine addition, a solution of 3-methylpentan-1-ol (824 mg, 8.08 mmol, 1.00 mL, 1.00 eq.) in dry DCM was added. The reaction mixture was stirred for 3 h at 0 °C. Subsequently, the mixture was diluted with diethyl ether and washed with saturated aqueous $NaHSO_3$ solution. The phases were separated and the aqueous phase was extracted with diethyl ether (3 x 30 mL). The combined organic layers were dried over anhydrous sodium sulfate before the solvents were evaporated under reduced pressure to give the crude product. Stirring with pentane then precipitated the phosphine oxide formed during the reaction that was filtered off next. The residue was purified by column chromatography over silica gel (100% pentane) to yield the product as colorless oil (1.18 g, 7.13 mmol, 88%).[S7]
^{1}H NMR (300 MHz, $CDCl_3$) δ [ppm]: 0.86-0.91 (m, 6H), 1.19 (m, 1H), 1.36 (m, 1H), 1.55 (m, 1H), 1.68 (m, 1H), 1.89 (m, 1H), 3.36-3.51 (m, 2H).**^{13}C NMR (75 MHz, $CDCl_3$) δ [ppm]:**.11.1, 18.4, 29.0, 32.2, 33.2, 39.6. **MS (EI, 70eV): *m/z* (%):** 164 (M^+, 3), 85 (76), 69 (30), 57 (100), 55 (65), 41 (61). **IR (ATR diamond, ν_{max} [cm^{-1}]):** 568, 643, 1215, 1254, 1461, 2874, 2926, 2961.

5-Methylhept-1-yne (3h)



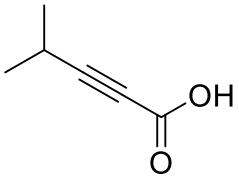
5-Methylhept-1-yne was prepared following general procedure 1. Amounts used: 1-bromo-3-methylpentane (**2h**) (800 mg, 4.85 mmol), lithium acetylide EDA-complex (669 mg, 7.27 mmol), sodium iodide (36.3 mg, 242 µmol), DMSO (21 mL). Yield: colorless liquid (340 mg, 3.09 mmol, 64%).
^{1}H NMR (300 MHz, $CDCl_3$) δ [ppm]: 0.83-0.92 (m, 6H), 1.07-1.24 (m, 1H), 1.27-1.41 (m, 2H), 1.41-1.64 (m, 2H), 1.92 (t, 1H, *J* = 2.7 Hz), 2.09-2.29 (m, 2H). **^{13}C NMR (75 MHz, $CDCl_3$) δ [ppm]:** 11.2, 16.2, 18.6, 29.0, 33.5, 35.2, 67.9, 84.9. **MS (EI, 70eV): *m/z* (%):** 109 ($M-H^+$, 3), 95 (92), 81 (100), 79 (57), 70 (50), 55 (58), 41 (82). **IR (ATR diamond, ν_{max} [cm^{-1}]):** 625, 1367, 1385, 1468, 2119, 2871, 2955, 3314.

6-Methylhept-1-yne (3i)



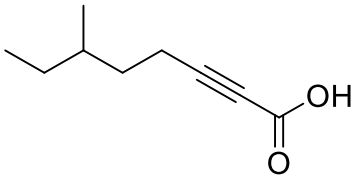
6-Methylhept-1-yne was prepared following general procedure 1. Amounts used: 1-bromo-4-methylpentane (624 mg, 3.78 mmol), lithium acetylide EDA-complex (522 mg, 5.67 mmol), sodium iodide (28.3 mg, 189 µmol), DMSO (16 mL). Yield: colorless liquid (173 mg, 1.57 mmol, 42%).
^{1}H NMR (600 MHz, $CDCl_3$) δ [ppm]: 0.89 (d, 6H, *J* = 6.6 Hz), 1.24-1.31 (2H,m), 1.49-1.60 (3H, m), 1.94 (t, 1H, *J* = 2.7 Hz), 2.17 (td, 2H, *J* = 7.3, 2.7 Hz). **^{13}C NMR (151 MHz, $CDCl_3$) δ [ppm]:** 18.4, 22.3, 26.2, 27.4, 37.8, 67.8, 84.6. **MS (EI, 70eV): *m/z* (%):** 110 (M^+, 1), 109 (2), 95 (100), 69 (100), 67 (86), 55 (67), 43 (82), 41 (85). **IR (ATR diamond, ν_{max} [cm^{-1}]):** 625, 1367, 1385, 1468, 2119, 2871, 2955, 3314.

4-Methylpent-2-ynoic acid (4e)



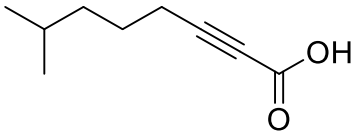
4-Methylpent-2-ynoic acid was prepared following general procedure 2. Amounts used: 3-methylbut-1-yne (333 mg, 4.89 mmol, 0.50 mL), *n*-butyllithium (1.6 M, 4.89 mmol, 3.06 mL), THF (6.0 mL). Yield: yellow liquid (492 mg, 4.39 mmol, 90%).
^{1}H NMR (300 MHz, $CDCl_3$) δ [ppm]: 1.25 (d, 6H, *J* = 7.0 Hz), 2.72 (spt, 1H, *J* = 7.0 Hz), 9.53 (s_{br}, 1H). **^{13}C NMR (75 MHz, $CDCl_3$) δ [ppm]:** 20.6, 21.6, 71.8, 97.2, 158.6. **IR (ATR diamond, ν_{max} [cm^{-1}]):** 721, 1224, 1679, 2228, 2977.

6-Methyloct-2-ynoic acid (4h)



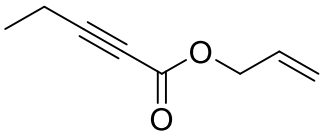
6-Methyloct-2-ynoic acid was prepared following general procedure 2. Amounts used: **3h** (300 mg, 2.72 mmol), *n*-butyllithium (1.6 M, 2.72 mmol, 1.70 mL), THF (4.0 mL). Yield: yellow liquid (386 mg, 2.50 mmol, 92%).
^{1}H NMR (300 MHz, $CDCl_3$) δ [ppm]: 0.81-0.97 (m, 6H), 1.08-1.25 (m, 1H), 1.28-1.55 (m, 3H), 1.55-1.72 (m, 1H), 2.29-2.44 (m, 2H), 10.7 (s_{br}, 1H). **^{13}C NMR (75 MHz, $CDCl_3$) δ [ppm]:** 11.1, 16.5, 18.5, 28.9, 33.6, 34.0, 72.5, 93.0, 158.6. **IR (ATR diamond, ν_{max} [cm^{-1}]):** 604, 754, 1275, 1682, 2236, 2876, 2928, 2961.

7-Methyloct-2-ynoic acid (4i)



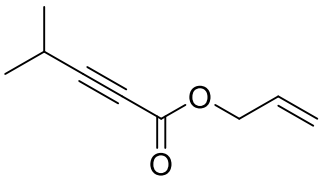
7-Methyloct-2-ynoic acid was prepared following general procedure 2. Amounts used: **3i** (160 mg, 1.45 mmol), *n*-butyllithium (1.6 M, 1.45 mmol, 0.91 mL), THF (1.8 mL). Yield: yellow liquid (213 mg, 1.38 mmol, 95%).
^{1}H NMR (300 MHz, $CDCl_3$) δ [ppm]: 0.89 (d, 6H, *J* = 6.6 Hz), 1.22-1.35 (m, 2H), 1.49-1.68 (m, 3H), 2.34 (t, 2H, *J* = 7.2 Hz), 10.6 (s_{br}, 1H). **^{13}C NMR (75 MHz, $CDCl_3$) δ [ppm]:** 19.0, 22.4, 25.3, 27.6, 38.0, 72.6, 92.7, 158.5. **IR (ATR diamond, ν_{max} [cm^{-1}]):** 756, 1276, 1682, 2236, 2954.

Allyl pent-2-ynoate (5d)



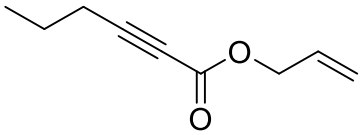
Allyl pent-2-ynoate was prepared following general procedure 3. Amounts used: Pent-2-ynoic acid (200 mg, 2.04 mmol), allyl alcohol (118 mg, 2.04 mmol, 0.14 mL), DCC (421 mg, 2.04 mmol), DMAP (24.9 mg, 204 µmol), DCM (12 mL). Column chromatography using a 4:1 mixture of pentane/DCM. Yield: colorless liquid (285 mg, 1.87 mmol, 92%).
^{1}H NMR (300 MHz, $CDCl_3$) δ [ppm]: 1.21 (t, 3H, *J* = 7.6 Hz), 2.35 (q, 2H, *J* = 7.6 Hz), 4.65 (ddd, 2H, *J* = 5.8, 1.5, 1.4 Hz), 5.28 (dq, 1H, *J* = 10.0, 1.4 Hz), 5.36 (dq, 1H, *J* = 17.1, 1.5 Hz), 5.93 (ddt, 1H, *J* = 17.1, 10.0, 5.8 Hz). **^{13}C NMR (75 MHz, $CDCl_3$) δ [ppm]:** 12.4, 12.5, 66.3, 72.3, 91.0, 119.2, 131.3, 153.5. **MS (EI, 70eV): *m/z* (%):** 137 ([M-1]$^+$, 3), 109 (4), 93 (14), 81 (100), 53 (39). **IR (solid, ν_{max} [cm^{-1}]):** 752, 1055, 1085, 1456, 1651, 1714, 2242, 2944, 2985, 3024, 3088.

Allyl 4-methylpent-2-ynoate (5e)



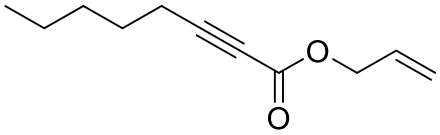
Allyl 4-methylpent-2-ynoate was prepared following general procedure 3. Amounts used: **4e** (471 mg, 4.20 mmol), allyl alcohol (244 mg, 4.20 mmol, 0.29 mL), DCC (866 mg, 4.20 mmol), DMAP (51.0 mg, 420 µmol), DCM (25 mL). Column chromatography using a 10:1 mixture of pentane/diethyl ether. Yield: colorless liquid (567 mg, 3.73 mmol, 89%).
^{1}H NMR (300 MHz, $CDCl_3$) δ [ppm]: 1.24 (d, 6H, *J* = 6.8 Hz), 2.70 (spt, 1H, *J* = 6.8 Hz), 5.28 (dq, 1H, *J* = 10.4, 1.2 Hz), 5.36 (dq, 1H, *J* = 17.2, 1.5 Hz), 5.93 (ddt, 1H, *J* = 17.2, 10.4, 5.9 Hz). **^{13}C NMR (75 MHz, $CDCl_3$) δ [ppm]:** 20.5, 21.7, 66.3, 72.1, 94.5, 119.2, 131.3, 153.6. **MS (EI, 70eV): *m/z* (%):** 152 (M$^+$, 2), 137 (12), 107 (14), 95 (100), 79 (20), 67 (75), 41 (56). **IR (solid, ν_{max} [cm^{-1}]):** 752, 1026, 1249, 1651, 1714, 2236, 2876, 3089.

Allyl hex-2-ynoate (5f)



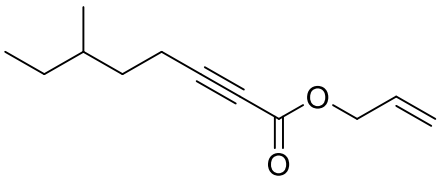
Allyl hex-2-ynoate was prepared following general procedure 3. Amounts used: Hex-2-ynoic acid (200 mg, 1.78 mmol), allyl alcohol (104 mg, 1.78 mmol, 0.12 mL), DCC (368 mg, 1.78 mmol), DMAP (21.8 mg, 178 µmol), DCM (10 mL). Column chromatography using a 4:1 mixture of pentane/DCM. Yield: colorless liquid (219 mg, 1.44 mmol, 81%).
^{1}H NMR (300 MHz, $CDCl_3$) δ [ppm]: 1.02 (t, 3H, *J* = 7.4 Hz), 1.62 (sxt, 2H, *J* = 7.3 Hz), 2.32 (t, 2H, *J* = 7.3 Hz), 4.65 (dt, 2H, *J* = 5.9, 1.3 Hz), 5.28 (dq, 1H, *J* = 10.4, 1.2 Hz), 5.36 (dq, 1H, *J* = 17.2, 1.5 Hz), 5.93 (ddt, 1H, *J* = 17.2, 10.4, 5.9 Hz). **^{13}C NMR (75 MHz, $CDCl_3$) δ [ppm]:** 13.4, 20.6, 21.0, 66.2, 73.0, 89.8, 119.1, 131.3, 153.5. **MS (EI, 70eV): *m/z* (%):** 152 (M^+, 1), 137 (4), 124 (8), 95 (100), 79 (27), 67 (19), 53 (20), 41 (28). **IR (solid, ν_{max} [cm^{-1}]):** 751, 1082, 1250, 1651, 1713, 2239, 2877, 1968, 3025, 3089.

Allyl oct-2-ynoate (5g)



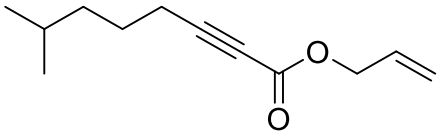
Allyl oct-2-ynoate was prepared following general procedure 3. Amounts used: Oct-2-ynoic acid (200 mg, 1.43 mmol), allyl alcohol (82.9 mg, 1.43 mmol), DCC (294 mg, 1.43 mmol), DMAP (17.4 mg, 143 µmol), DCM (10 mL). Column chromatography using a 4:1 mixture of pentane/DCM. Yield: colorless liquid (196 mg, 1.08 mmol, 76%).
^{1}H NMR (300 MHz, $CDCl_3$) δ [ppm]: 0.87-0.95 (m, 3H), 1.23-1.45 (m, 4H), 1.52-1.66 (m, 2H), 2.33 (t, 2H, *J* = 7.1 Hz), 4.65 (dt, 2H, *J* = 5.9, 1.4 Hz), 5.25-5.31 (m, 1H), 5.36 (dq, 1H, *J* = 17.2, 1.5 Hz), 5.93 (ddt, 1H, *J* = 17.2, 10.4, 5.9 Hz). **^{13}C NMR (75 MHz, $CDCl_3$) δ [ppm]:** 13.8, 18.6, 22.1, 27.2, 30.9, 66.2, 72.9, 90.0, 119.1, 131.3, 153.5. **MS (EI, 70eV): *m/z* (%):** 180 (M^+, 3), 137 (15), 123 (100), 79 (66), 67 (66), 55 (47), 41 (61). **IR (solid, ν_{max} [cm^{-1}]):** 752, 1077, 1251, 1710, 2241, 2863, 2873, 2957, 3024, 3087.

Allyl 6-methyloct-2-ynoate (5h)



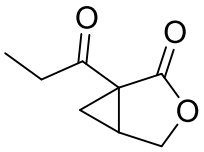
Acid **4h** (345 mg, 2.24 mmol, 1.00 eq.) and allyl alcohol (153 µL, 130 mg, 2.24 mmol, 1.00 eq.) were combined with DMAP (27.3 mg, 224 µmol, 0.10 eq.) and DCM (20 mL). Subsequently, *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC, 429 mg, 2.24 mmol, 1.00 eq.) was added and the reaction mixture was stirred at room temperature for 16 h. Then water (30 mL) was added to quench the reaction and the solution was washed with water (2 x 20 mL) and sat. aq. NaCl solution (30 mL). The organic phase was dried over anhydrous sodium sulfate, filtered, and the solvent was removed under reduced pressure. The residue was purified by column chromatography over silica gel (20:1 pentane/diethyl ether). Yield: Colorless oil (230 mg, 1.18 mmol, 53%).
^{1}H NMR (300 MHz, $CDCl_3$) δ [ppm]: 0.82-0.93 (m, 6H), 1.08-1.24 (m, 1H), 1.27-1.52 (m, 3H), 1.56-1.69 (m, 1H), 2.25-2.41 (m, 2H), 4.65 (dq, 2H, *J* = 5.9, 0.9 Hz), 5.27 (dq, 1H, *J* = 10.4, 1.2 Hz), 5.36 (dq, 1H, *J* = 17.2, 1.5 Hz), 5.93 (ddt, 1H, *J* = 17.2, 10.4, 5.8 Hz). **^{13}C NMR (75 MHz, $CDCl_3$) δ [ppm]:** 11.1, 16.4, 18.5, 28.9, 33.5, 34.1, 66.2, 72.8, 90.2, 119.1, 131.3, 153.5. **MS (EI, 70eV): *m/z* (%):** 194 (M^+, 3), 137 (56), 107 (41), 93 (37), 79 (83), 67 (71), 55 (47), 41 (100). **IR (solid, ν_{max} [cm^{-1}]):** 752, 1074, 1250, 1463, 1652, 1712, 2241, 2877, 2963, 3025, 3088.

Allyl 7-methyloct-2-ynoate (5i)



Allyl 7-methyloct-2-ynoate was prepared following general procedure 3. Amounts used: **4i** (203 mg, 1.31 mmol), allyl alcohol (76.3 mg, 1.31 mmol, 86.0 µL), DCC (271 mg, 1.31 mmol), DMAP (16.1 mg, 131 µmol), DCM (8.0 mL). Column chromatography using a 10:1 mixture of pentane/diethyl ether. Yield: colorless liquid (203 mg, 1.04 mmol, 79%), with slight impurities of DCC.
^{1}H NMR (300 MHz, $CDCl_3$) δ [ppm]: 0.89 (d, 6H, *J* = 6.6 Hz), 1.22-1.33 (m, 2H), 1.49-1.65 (m, 3H), 2.32 (t, 2H, *J* = 7.2 Hz), 4.63-4.67 (m, 2H), 5.28 (m, 1H), 5.36 (dq, 1H, *J* = 17.2, 1.5 Hz), 5.93 (ddt, 1H, *J* = 17.2, 10.4, 5.8 Hz). **^{13}C NMR (75 MHz, $CDCl_3$) δ [ppm]:** 18.9, 22.4, 25.5, 27.6, 38.1, 66.3, 72.9, 90.0, 119.1, 131.3, 153.5. **MS (EI, 70eV): *m/z* (%):** 194 (M^+, 1), 137 (18), 109 (25), 93 (27), 79 (56), 69 (100), 55 (28), 41 (78). **IR (solid, ν_{max} [cm^{-1}]):** 751, 1076, 1249, 1651, 1712, 2240, 2871, 2957, 3024, 3089.

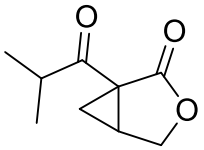
1-Propanoyl-3-oxabicyclo[3.1.0]hexan-2-one (1d)



1-Propanoyl-3-oxabicyclo[3.1.0]hexan-2-one was prepared following general procedure 4. Amounts used: **5d** (179 mg, 1.30 mmol), palladium-(II)-acetate (29.1 mg, 130 µmol), (diacetoxyiodo)benzene (836 mg, 2.59 mmol), dry acetic acid (11 mL). Column chromatography using a 1:1 mixture of pentane/diethyl ether. Yield: yellow liquid (113 mg, 730 µmol, 56%).

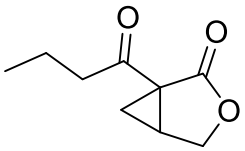
^{1}H NMR (500 MHz, $CDCl_3$) δ [ppm]: 1.10 (t, 3H, *J* = 7.2 Hz), 1.39 (dd, 1H, *J* = 5.5, 4.2 Hz), 2.06 (dd, 1H, *J* = 8.0, 4.2 Hz), 2.77 (m, 1H), 2.87 (dq, 1H, *J* = 19.0, 7.2 Hz), 3.20 (dq, 1H, *J* = 19.0, 7.2 Hz), 4.20 (d, 1H, *J* = 10.0 Hz), 4.34 (dd, 1H, *J* = 9.5, 4.7 Hz). **^{13}C NMR (126 MHz, $CDCl_3$) δ [ppm]:** 7.4, 23.9, 29.6, 35.1, 36.1, 67.2, 172.8, 203.3. **MS (EI, 70eV): *m/z* (%):** 154 (M^+, 9), 136 (62), 125 (100), 108 (40), 83 (75), 57 (59), 53 (72). **IR (solid, ν_{max} [cm^{-1}]):** 830, 994, 1091, 1303, 1386, 1461, 1699, 1766, 2912, 2941, 2979, 3097. ***I* (HP5-MS):** 1285.

1-(2-Methylpropanoyl)-3-oxabicyclo[3.1.0]hexan-2-one (1e)



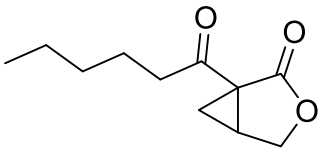
1-(2-Methylpropyl)-3-oxabicyclo[3.1.0]hexan-2-one was prepared following general procedure 4. Amounts used: **5e** (200 mg, 1.31 mmol), palladium-(II)-acetate (29.5 mg, 131 µmol), (diacetoxyiodo)benzene (844 mg, 2.62 mmol), dry acetic acid (11 mL). Column chromatography using at first 3:1 mixture of pentane/diethyl ether and 1:1 pentane/diethyl ether later. Yield: yellow liquid (141 mg, 838 µmol, 64%).
^{1}H NMR (500 MHz, $CDCl_3$) δ [ppm]: 1.11 (d, 3H, *J* = 6.7 Hz), 1.19 (d, 3H, *J* = 6.7 Hz), 1.39 (dd, 1H, *J* = 5.7, 4.1 Hz), 2.03 (dd, 1H, *J* = 8.1, 4.1 Hz), 2.71-2.77 (m, 1H), 3.67 (spt, 1H, *J* = 6.7 Hz), 4.21 (d, 1H, *J* = 9.5 Hz), 4.35 (dd, 1H, *J* = 9.5, 4.6 Hz). **^{13}C NMR (126 MHz, $CDCl_3$) δ [ppm]:** 17.9, 18.1, 23.6, 29.4, 35.2, 38.1, 67.1, 172.7, 206.5. **MS (EI, 70eV): *m/z* (%):** 168 (M^+, 10), 150 (50), 135 (19), 125 (100), 122 (45), 108 (37), 83 (69), 53 (72), 43 (72). **IR (solid, ν_{max} [cm^{-1}]):** 767, 1079, 1301, 1390, 1695, 1766, 2877, 2975. ***I* (HP5-MS):** 1315.

1-Butanoyl-3-oxabicyclo[3.1.0]hexan-2-one (1f)



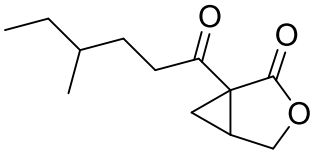
1-Butanoyl-3-oxabicyclo[3.1.0]hexan-2-one was prepared following general procedure 4. Amounts used: **5f** (100 mg, 657 µmol), palladium-(II)-acetate (35.0 mg, 156 µmol), (diacetoxyiodo)benzene (423 mg, 1.31 mmol), dry acetic acid (6.0 mL). Column chromatography using at first 1:1 mixture of pentane/DCM and 100% DCM later. Yield: yellow liquid (82.6 mg, 491 µmol, 75%).
^{1}H NMR (400 MHz, $CDCl_3$) δ [ppm]: 0.95 (t, 6H, *J* = 7.5 Hz), 1.39 (dd, 1H, *J* = 5.5, 4.3 Hz), 1.58-1.73 (m, 2H), 2.05 (dd, 1H, *J* = 7.9, 4.1 Hz), 2.77 (dddd, 1H, *J* = 8.0, 5.6, 4.8, 0.8 Hz), 2.85 (ddd, 1H, *J* = 17.8, 7.8, 6.8 Hz), 3.12 (ddd, 1H, *J* = 17.9, 7.8, 6.8 Hz), 4.20 (d, 1H, *J* = 9.3 Hz), 4.20 (d, 1H, *J* = 9.3 Hz), 4.34 (dd, 1H, *J* = 9.5, 4.8 Hz). **^{13}C NMR (100 MHz, $CDCl_3$) δ [ppm]:** 13.5, 16.8, 23.7, 29.6, 36.2, 43.4, 67.2, 172.8, 202.6. **MS (EI, 70eV): *m/z* (%):** 168 (M^+, 1), 153 (77), 140 (60), 135 (48), 125 (100), 122 (88), 83 (69), 53 (82), 43 (93). **IR (solid, ν_{max} [cm^{-1}]):** 768, 1085, 1392, 1697, 1768, 2965. ***I* (HP5-MS):** 1369.

1-Hexanoyl-3-oxabicyclo[3.1.0]hexan-2-one (1g)



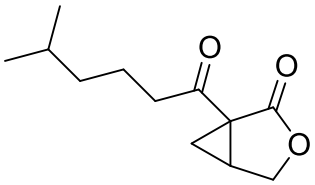
1-Hexanoyl-3-oxabicyclo[3.1.0]hexan-2-one was prepared following general procedure 4. Amounts used: **5g** (100 mg, 555 µmol), palladium-(II)-acetate (12.5 mg, 55.5 µmol), (diacetoxyiodo)benzene (357 mg, 1.11 mmol), dry acetic acid (5.0 mL). Column chromatography using at first 1:1 mixture of pentane/DCM and 100% DCM later. Yield: yellow liquid (93.1 mg, 474 µmol, 86%).
^{1}H NMR (400 MHz, $CDCl_3$) δ [ppm]: 0.86-0.93 (m, 3H), 1.27-1.36 (m, 4H), 1.39 (dd, 1H, *J* = 5.7, 4.1 Hz),1.55-1.68 (m, 2H), 2.05 (dd, 1H, *J* = 8.0, 4.0 Hz), 2.77 (dddd, 1H, *J* = 8.0, 5.6, 4.8, 0.8 Hz), 2.86 (ddd, 1H, *J* = 17.8, 8.0, 6.2 Hz), 3.12 (ddd, 1H, *J* = 17.8, 8.0, 6.2 Hz), 4.20 (d, 1H, *J* = 9.5 Hz), 4.34 (dd, 1H, *J* = 9.4, 4.9 Hz). **^{13}C NMR (100 MHz, $CDCl_3$) δ [ppm]:** 13.9, 22.4, 23.1, 23.8, 29.6, 31.2, 36.2, 41.6, 67.2, 172.8, 202.9. **MS (EI, 70eV): *m/z* (%):** 196 (M^+, 1), 167 (4), 153 (56), 140 (82), 125 (35), 122 (100), 83 (28), 53 (36). **IR (solid, ν_{max} [cm^{-1}]):** 767, 1086, 1391, 1696, 1769, 2873, 2957. ***I* (HP5-MS):** 1566.

1-(4-Methylhexanoyl)-3-oxabicyclo[3.1.0]hexan-2-one (1h)



1-(4-Methylhexanoyl)-3-oxabicyclo[3.1.0]hexan-2-one was prepared following general procedure 4. Amounts used: **5h** (100 mg, 515 µmol), palladium-(II)-acetate (11.6 mg, 51.5 µmol), (diacetoxyiodo)benzene (332 mg, 1.03 mmol), dry acetic acid (4.0 mL). Column chromatography using at first 4:1 mixture of pentane/diethyl ether and 1:1 pentane/diethyl ether later. Yield: yellow liquid (57.1 mg, 272 µmol, 53%), inseparable mixture of diastereomers.
^{1}H NMR (600 MHz, $CDCl_3$) δ [ppm]: 0.84-0.91 (m, 6H), 1.12-1.22 (m, 1H), 1.31-1.49 (m, 4H), 1.57-1.70 (m, 1H), 2.06 (ddd, 1H, *J* = 7.9, 4.2, 3.6 Hz), 2.73-2.79 (m, 1H), 2.82-2.93 (m, 1H), 3.07-3.19 (m, 1H), 4.20 (d, 1H, *J* = 9.4 Hz), 4.34 (dd, 1H, *J* = 9.4, 4.8 Hz). **^{13}C NMR (151 MHz, $CDCl_3$) δ [ppm]:** 11.3/11.3, 18.9/19.0, 23.8/23.8, 29.1/29.2, 29.6/29.7, 29.9, 33.9/33.9, 36.1/36.2, 39.5/39.5, 67.2, 172.8/172.8, 203.1/203.1. **MS (EI, 70eV): *m/z* (%):** 210 (M^+, 1), 181 (9), 153 (100), 140 (62), 125 (43), 122 (59), 83 (29), 55 (40). **IR (solid, ν_{max} [cm^{-1}]):** 767, 1087, 1390, 1463, 1697, 1770, 2875, 2929, 2962. ***I* (HP5-MS):** 1635.

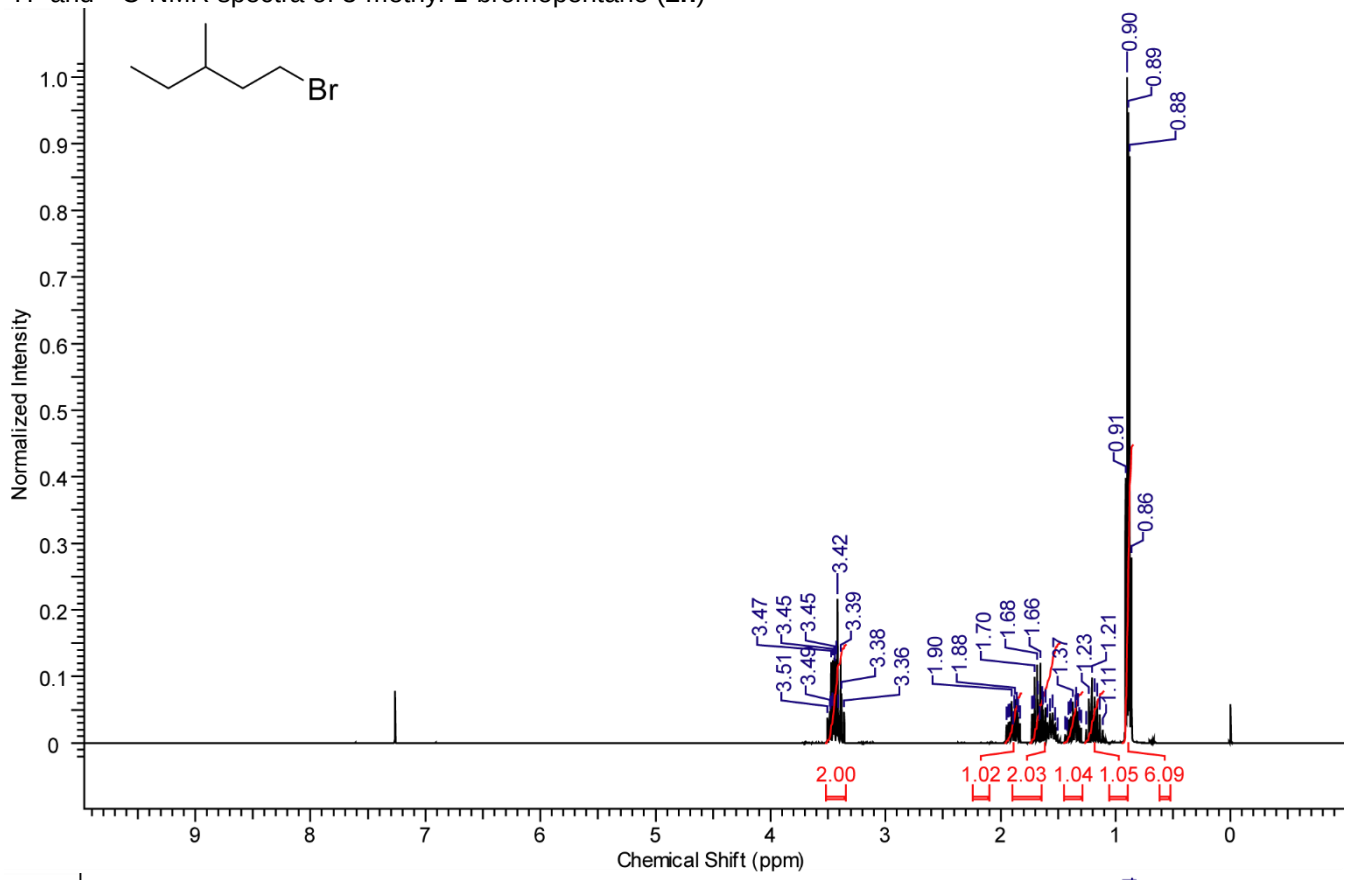
1-(5-Methylhexanoyl)-3-oxabicyclo[3.1.0]hexan-2-one (1i)

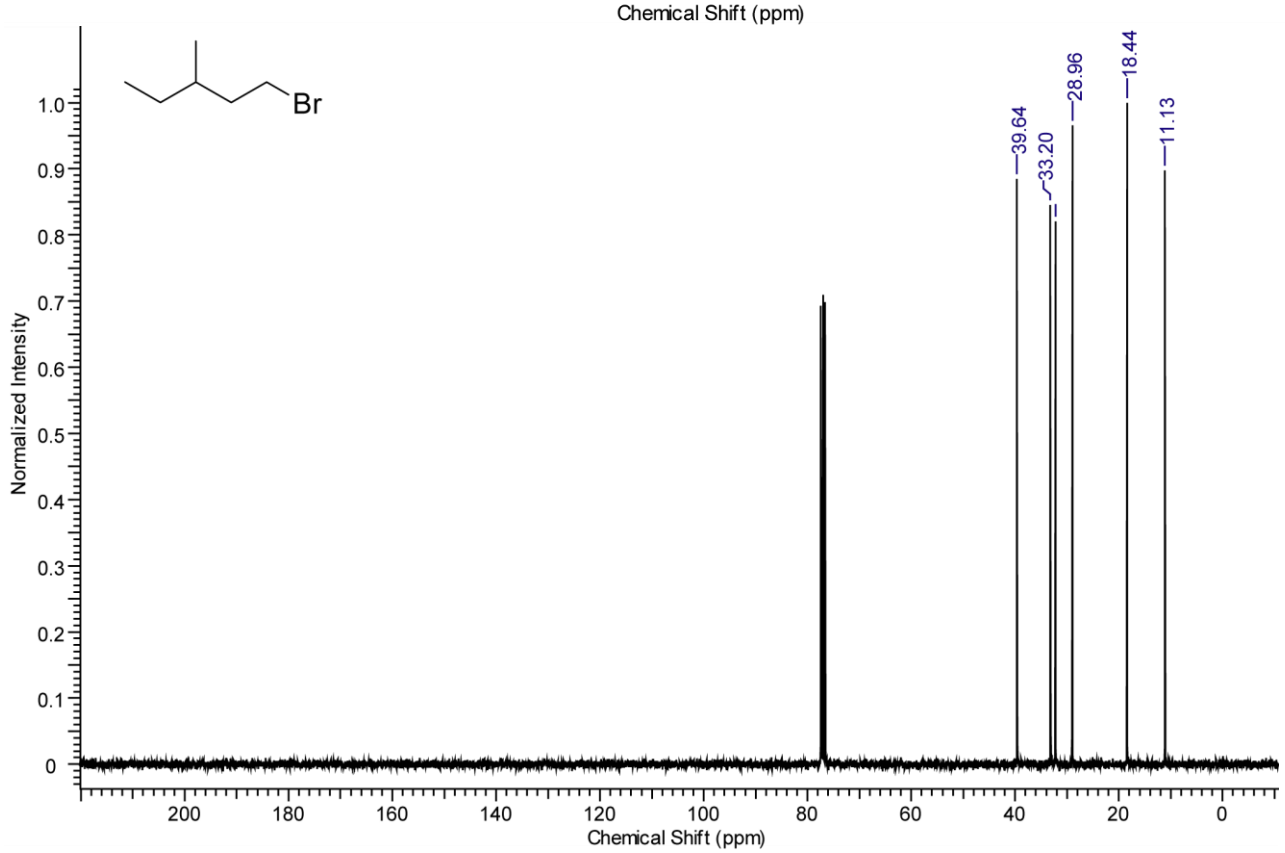


1-(5-Methylhexanoyl)-3-oxabicyclo[3.1.0]hexan-2-one was prepared following general procedure 4. Amounts used: **5i** (100 mg, 510 µmol), palladium-(II)-acetate (11.2 mg, 51.0 µmol), (diacetoxyiodo)benzene (332 mg, 1.03 mmol), dry acetic acid (4.4 mL). Column chromatography using at first 3:1 mixture of pentane/diethyl ether and 1:1 pentane/diethyl ether later. Yield: yellow liquid (53.5 mg, 254 µmol, 49%).
^{1}H NMR (400 MHz, $CDCl_3$) δ [ppm]: 0.88 (d, 6H, *J* = 6.5 Hz), 1.20 (dt, 2H, *J* = 8.7, 7.1 Hz), 1.39 (dd, 1H, *J* = 5.7, 4.1 Hz), 1.50-1.69 (m, 3H), 2.05 (dd, 1H, *J* = 7.9, 4.1 Hz), 2.73-2.80 (m, 1H), 2.80-2.91 (m, 1H), 3.06-3.17 (m, 1H), 4.20 (d, 1H, *J* = 9.5 Hz), 4.34 (dd, 1H, *J* = 9.5, 4.8 Hz). **^{13}C NMR (100 MHz, $CDCl_3$) δ [ppm]:** 21.2, 22.4, 22.5, 23.8, 27.8, 29.6, 36.2, 38.2, 41.8, 67.2, 172.8, 202.8. **MS (EI, 70eV): *m/z* (%):** 210 (M^+, 1), 167 (12), 153 (40), 140 (94), 125 (45), 122 (100), 95 (28), 69 (39), 53 (36), 43 (47). **IR (solid, ν_{max} [cm^{-1}]):** 766, 1089, 1390, 1698, 1769, 2872, 2956. ***I* (HP5-MS):** 1629.

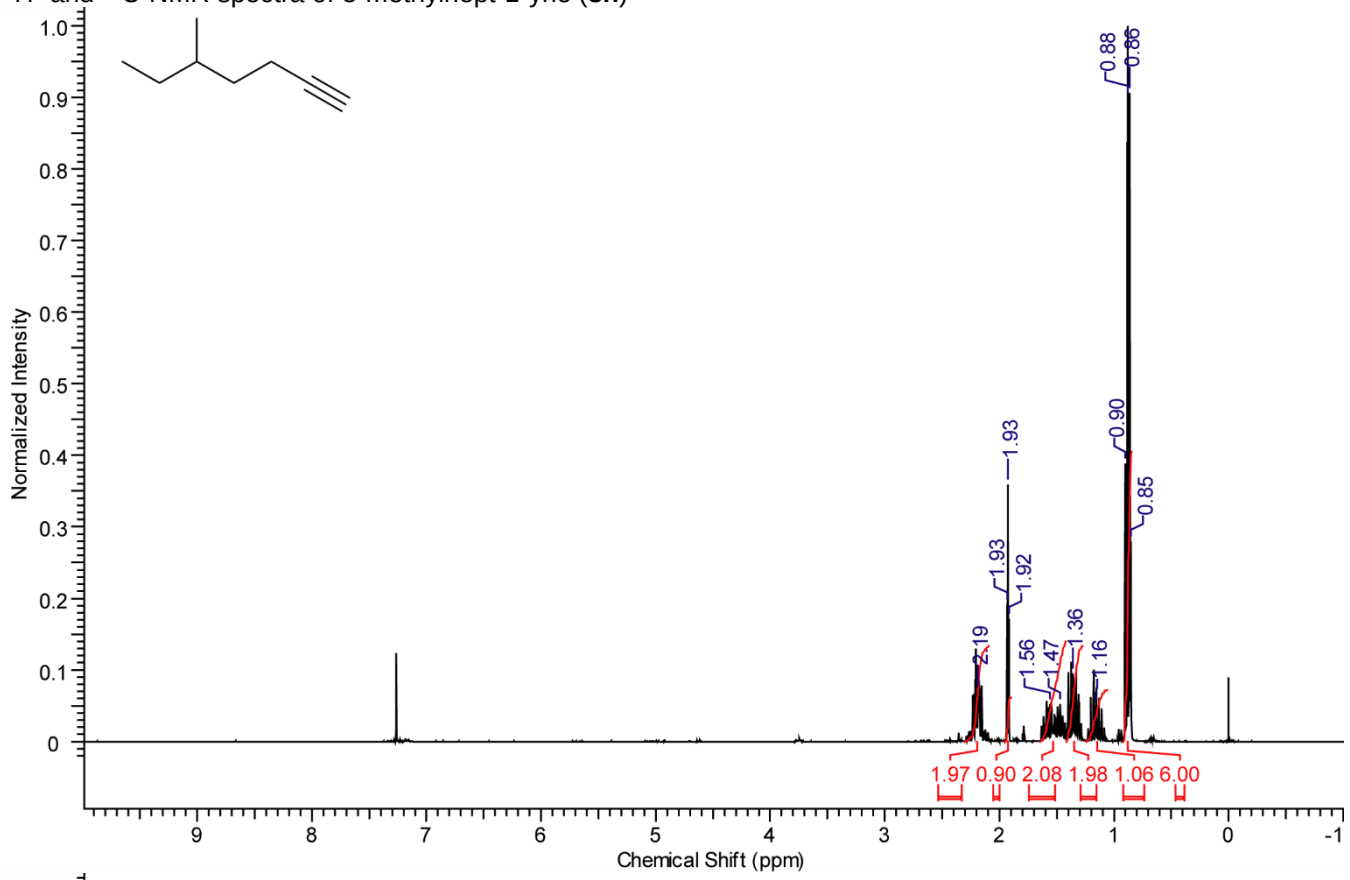
Part 4: NMR Spectra

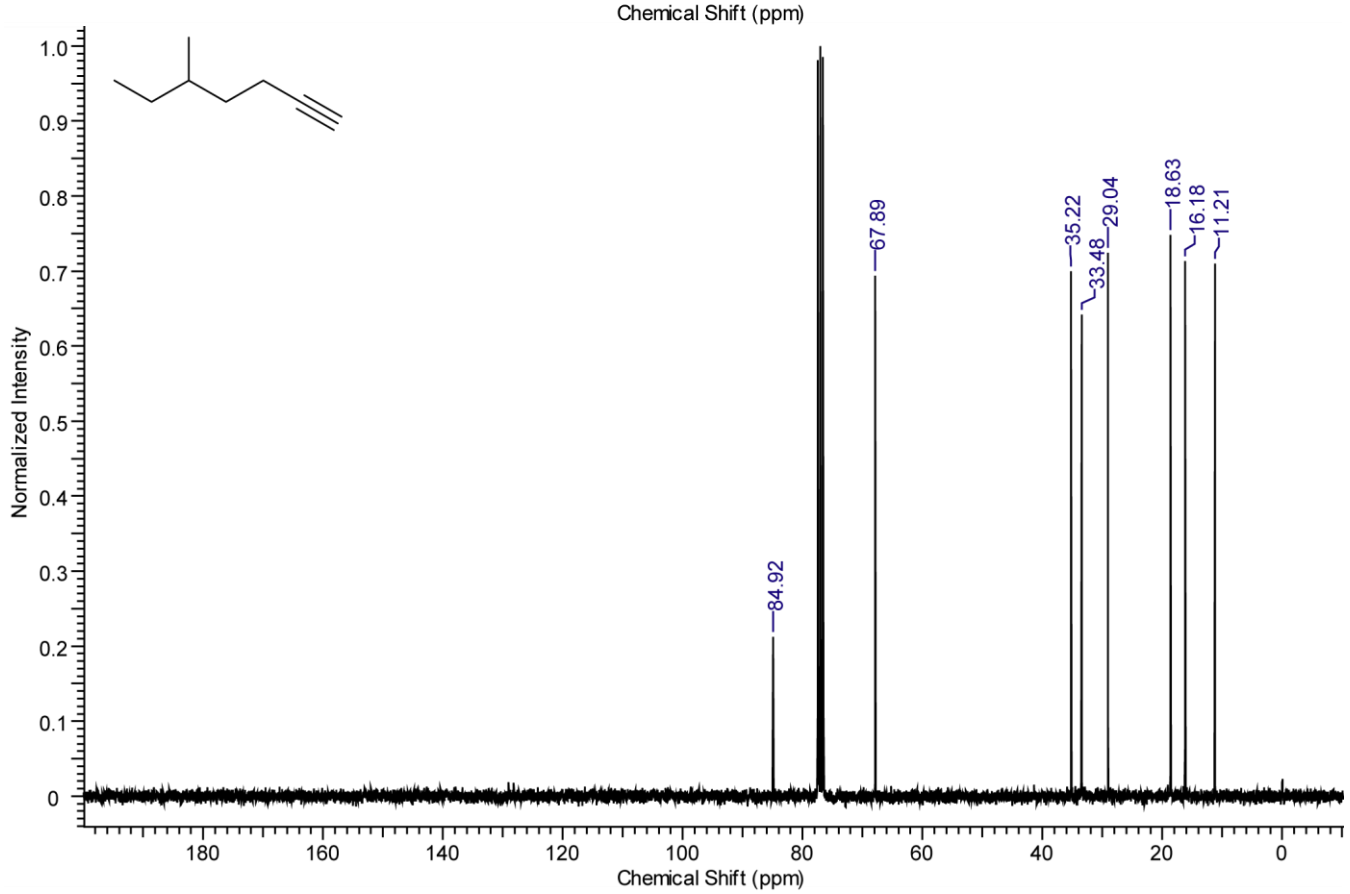
^{1}H- and ^{13}C-NMR spectra of 3-methyl-1-bromopentane (**2h**)



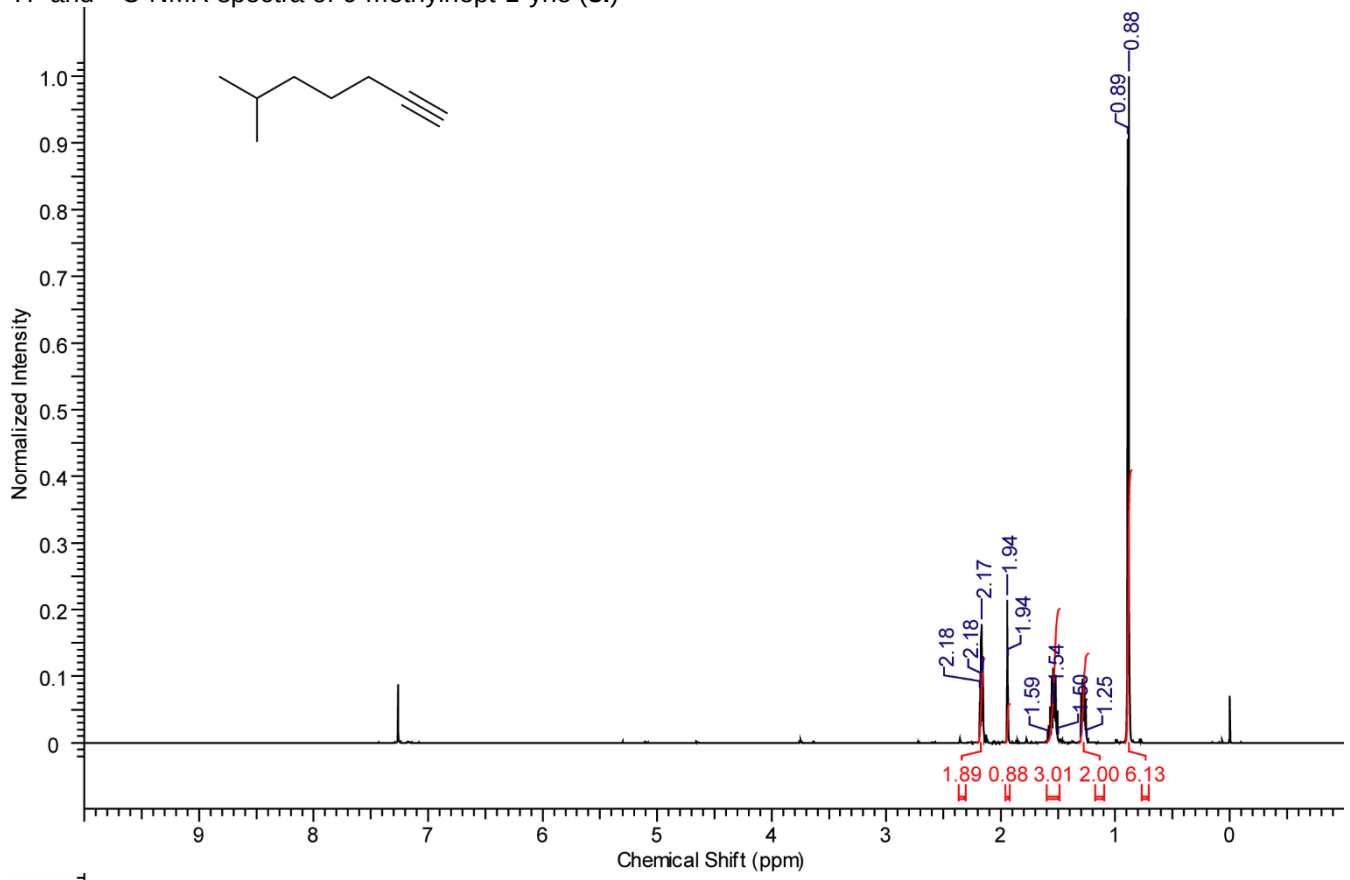


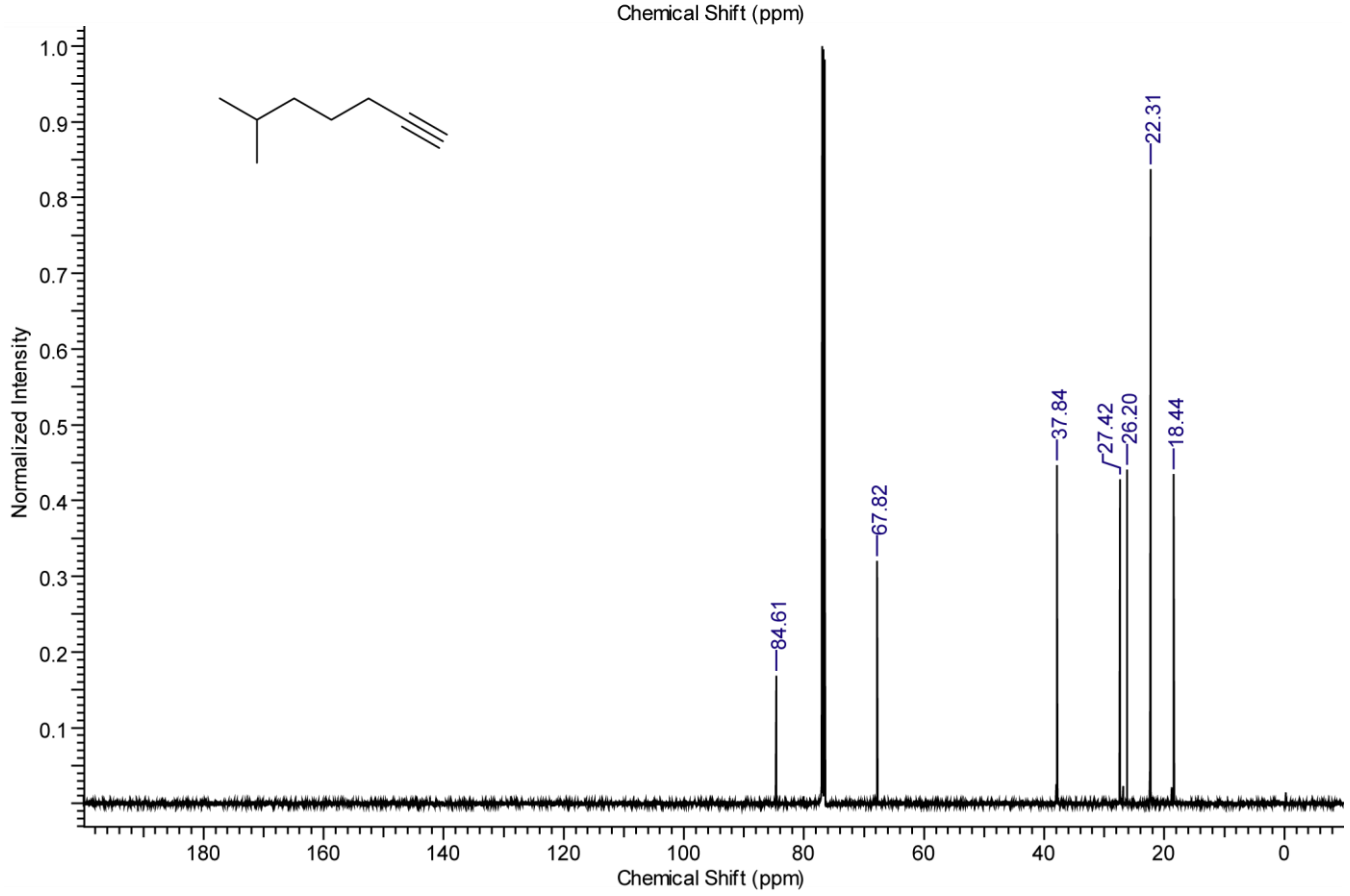
^{1}H- and ^{13}C-NMR spectra of 5-methylhept-1-yne (**3h**)



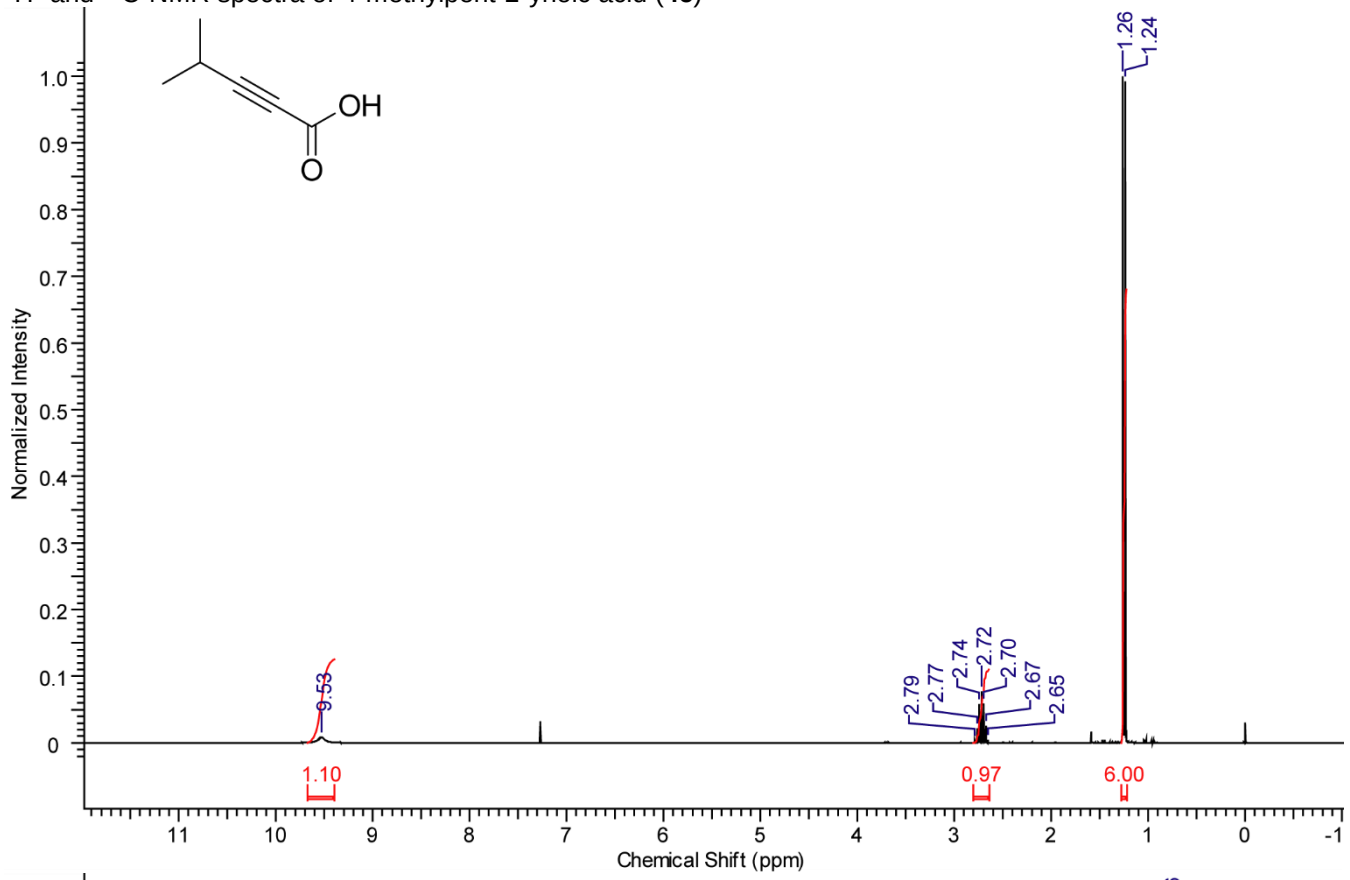


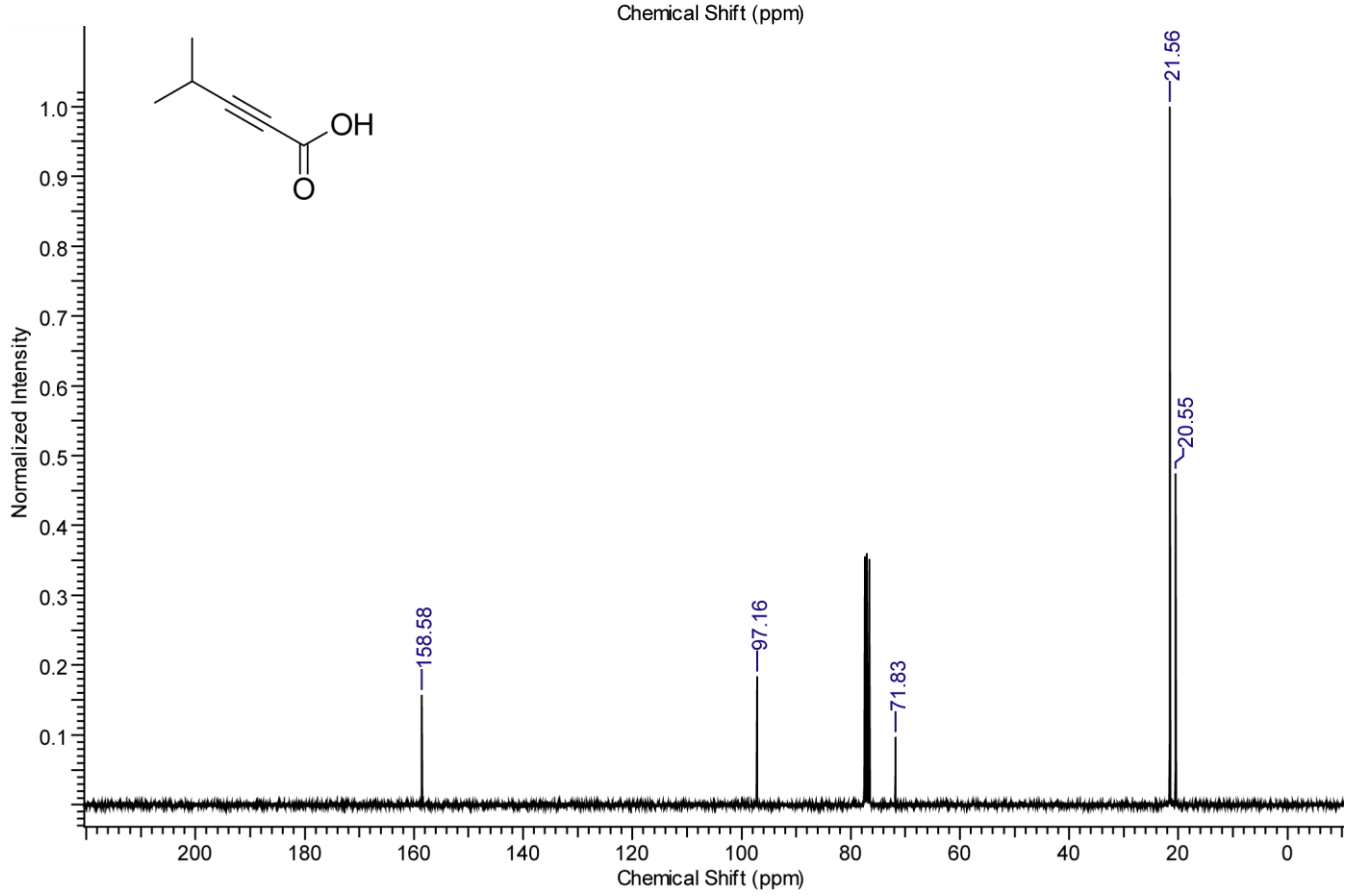
^{1}H- and ^{13}C-NMR spectra of 6-methylhept-1-yne (**3i**)



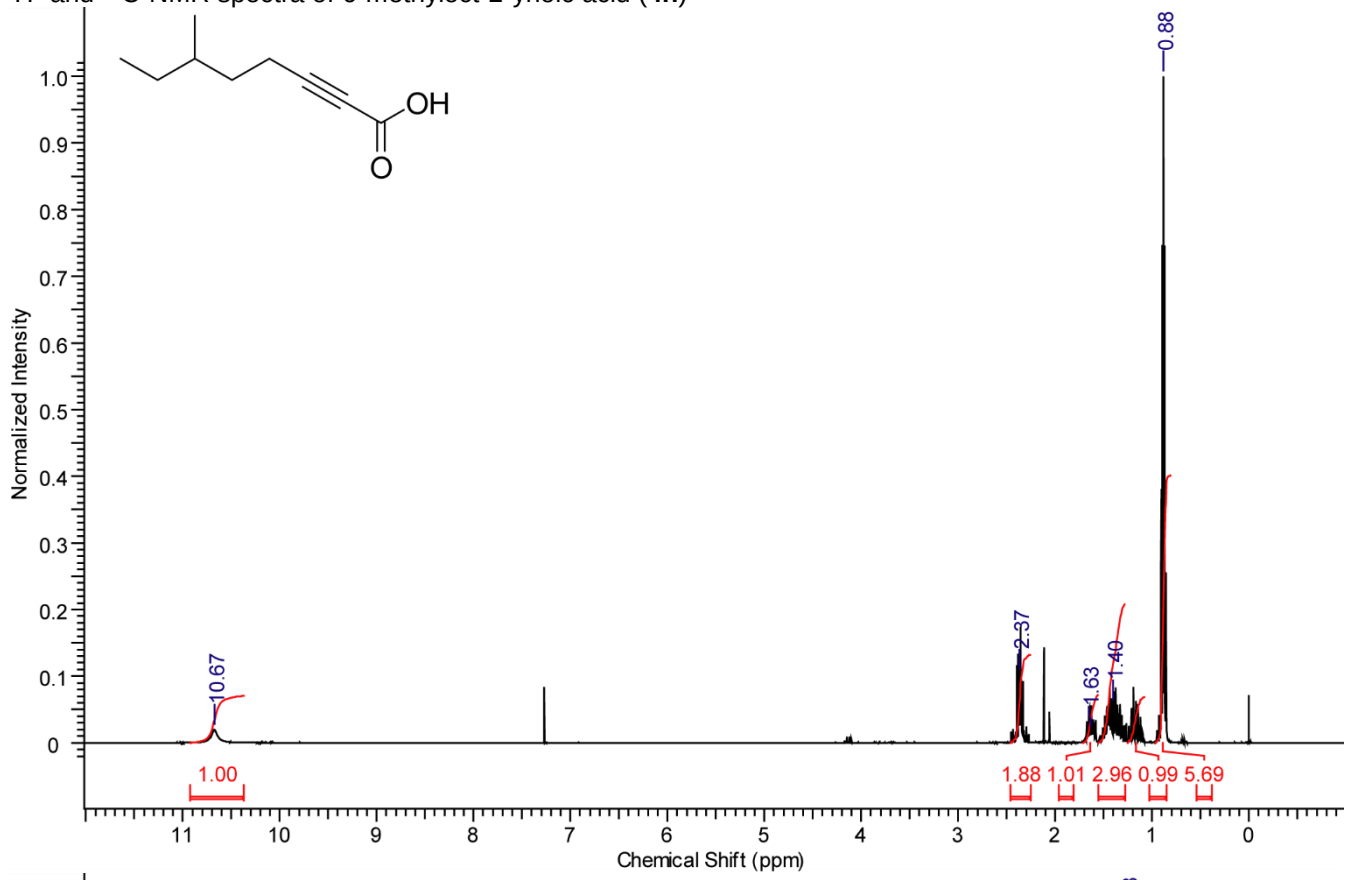


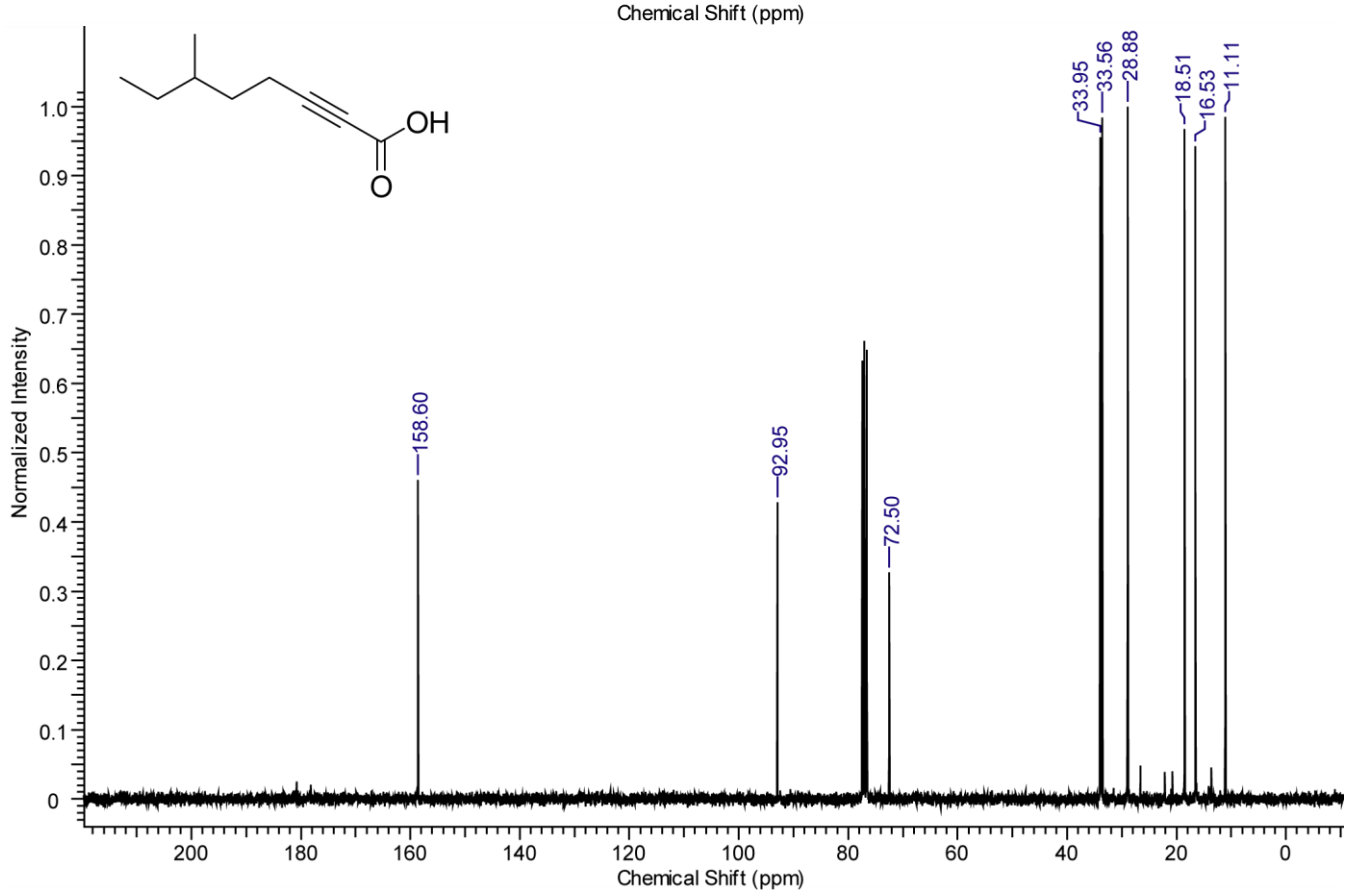
^{1}H- and ^{13}C-NMR spectra of 4-methylpent-2-ynoic acid (**4e**)





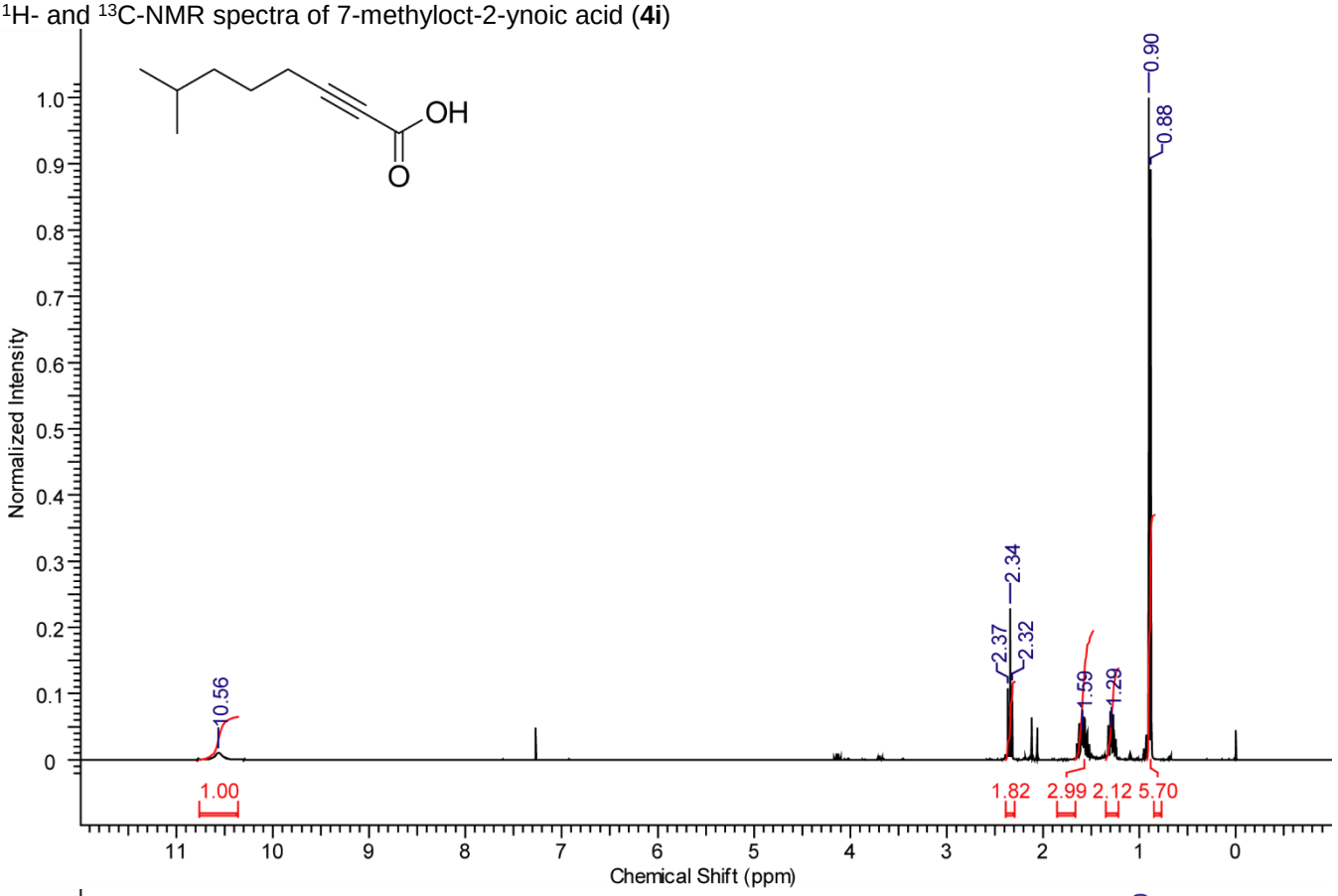
^{1}H- and ^{13}C-NMR spectra of 6-methyloct-2-ynoic acid (**4h**)

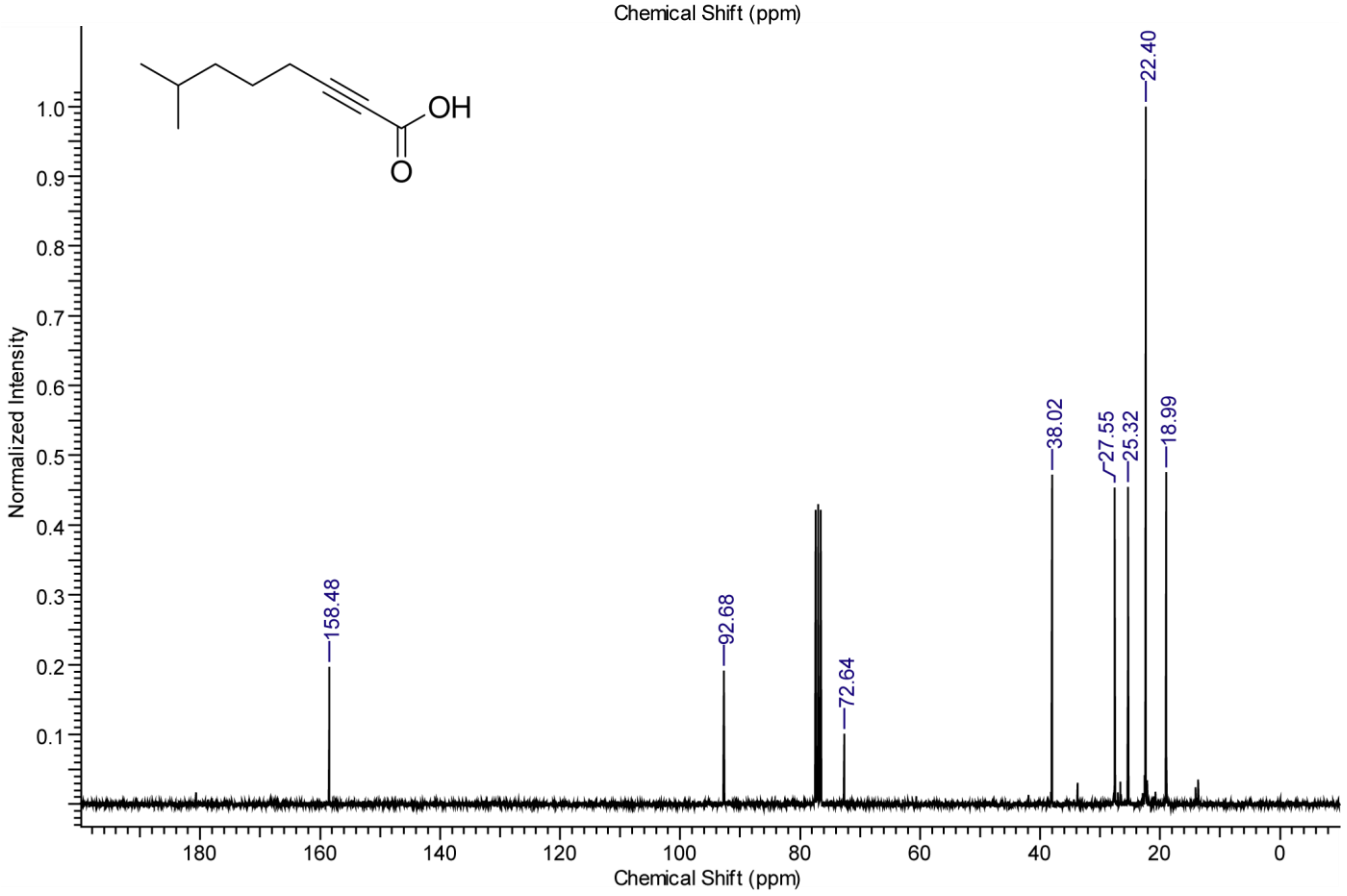




SUPPORTING INFORMATION

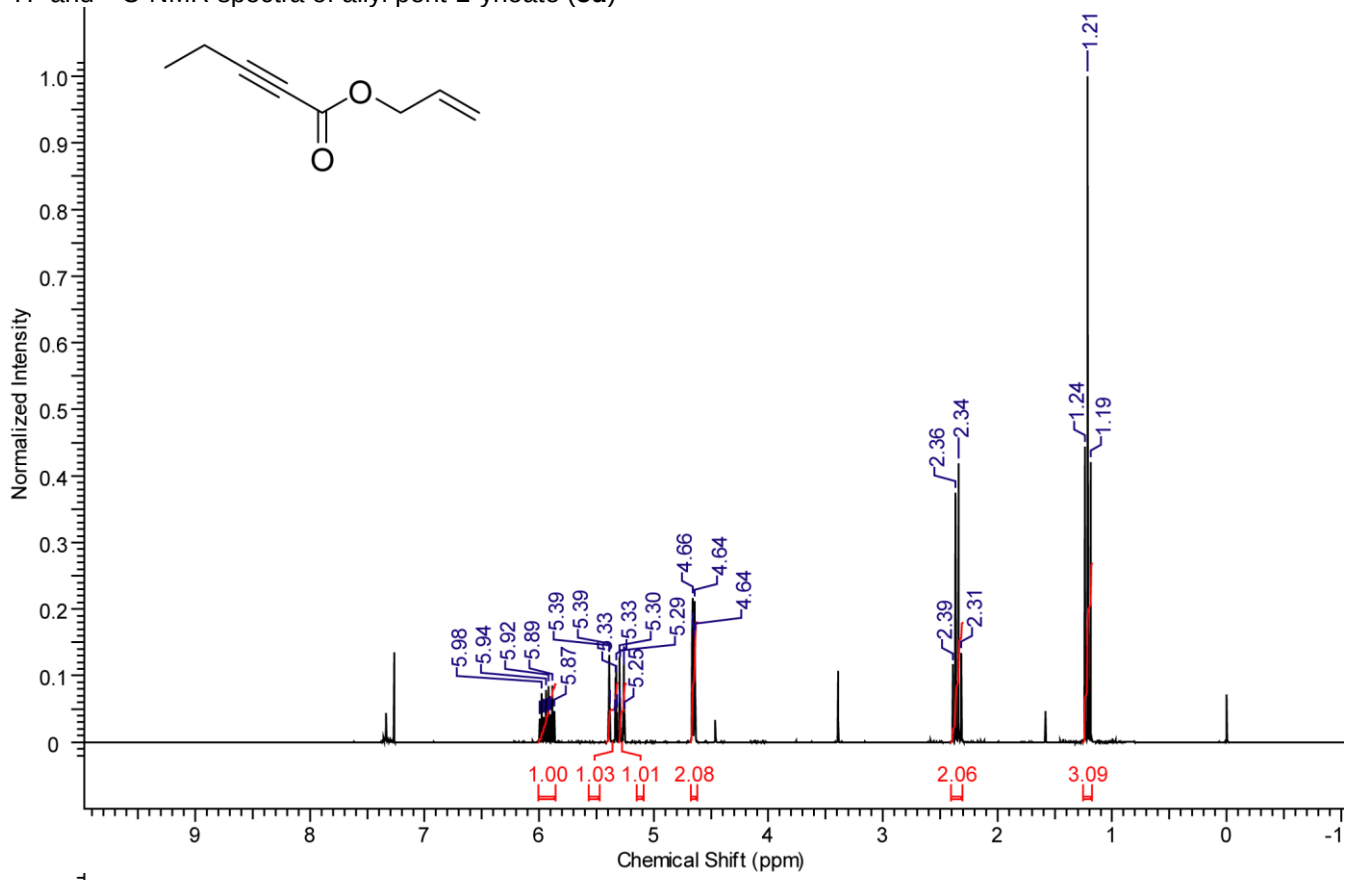
^{1}H- and ^{13}C-NMR spectra of 7-methyloct-2-ynoic acid (**4i**)

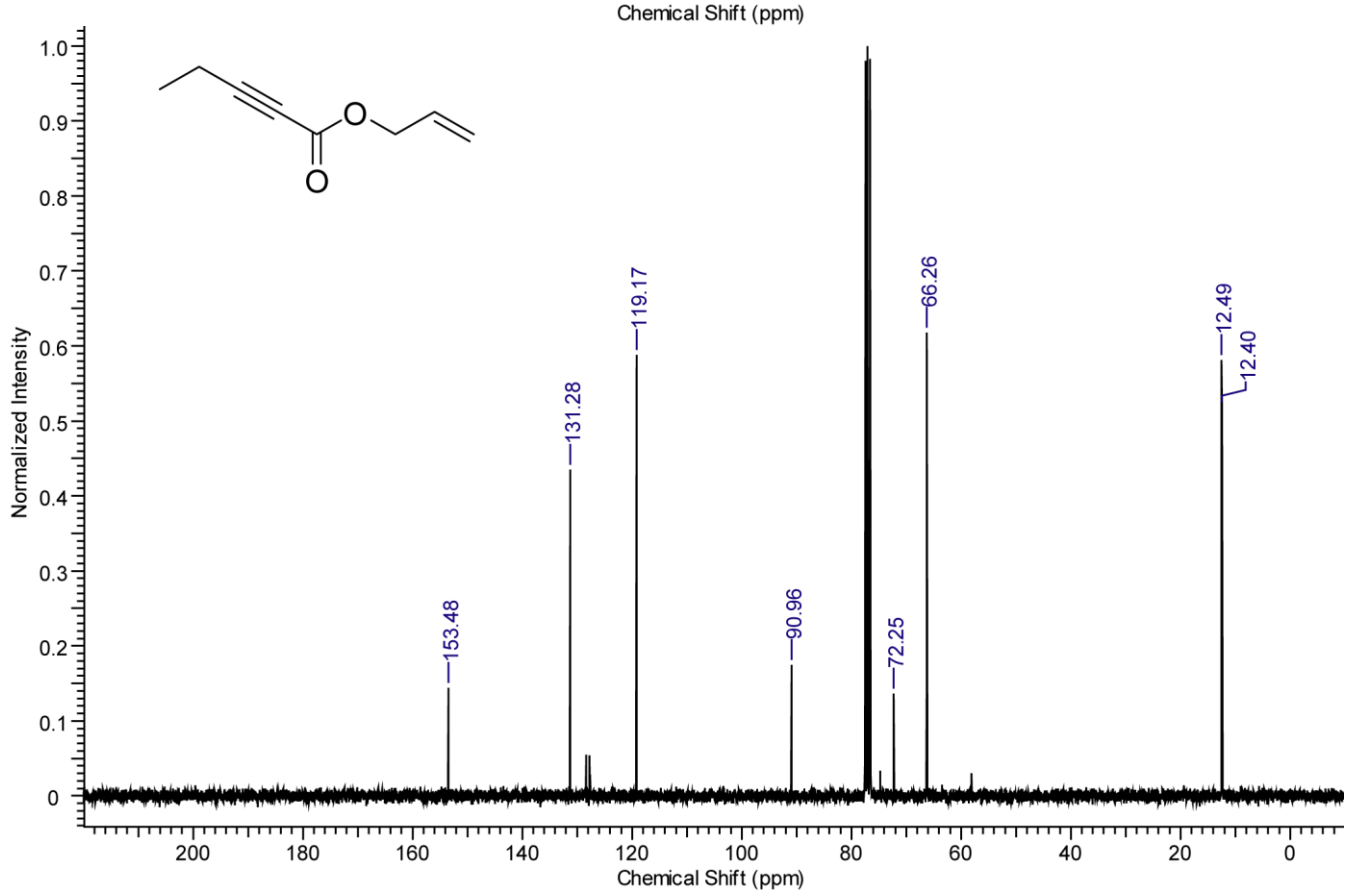




SUPPORTING INFORMATION

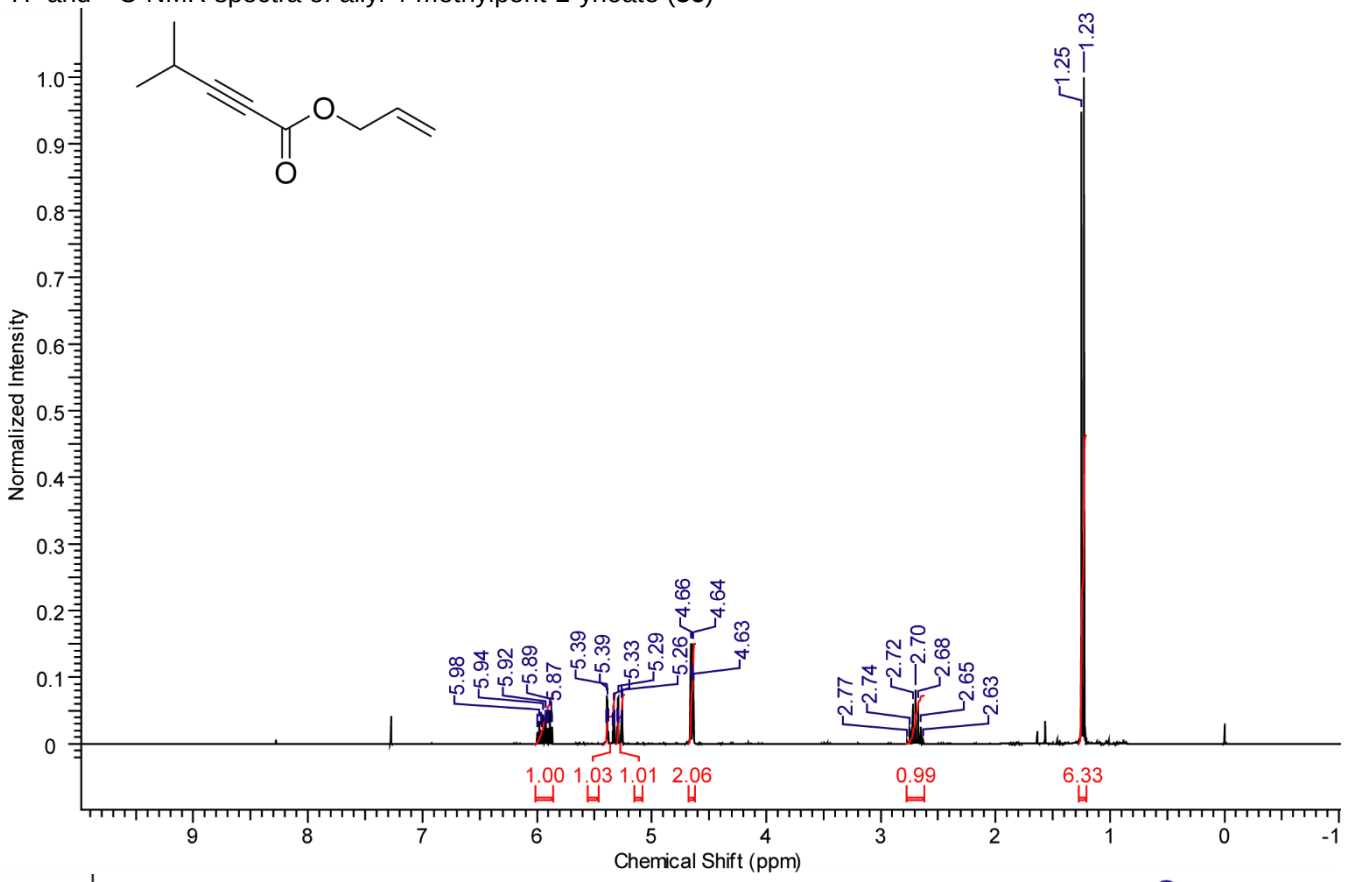
^{1}H- and ^{13}C-NMR spectra of allyl pent-2-ynoate (**5d**)

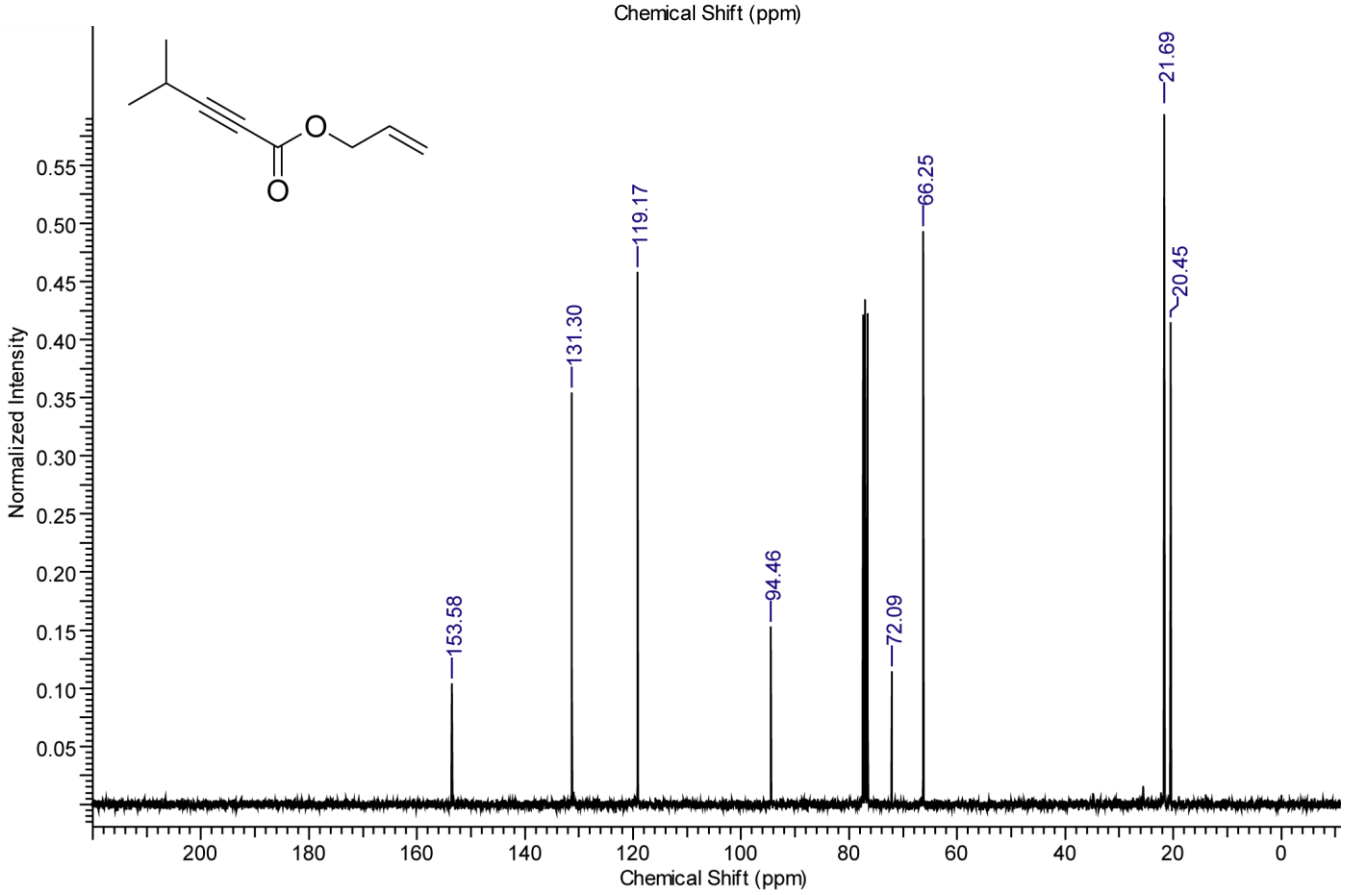




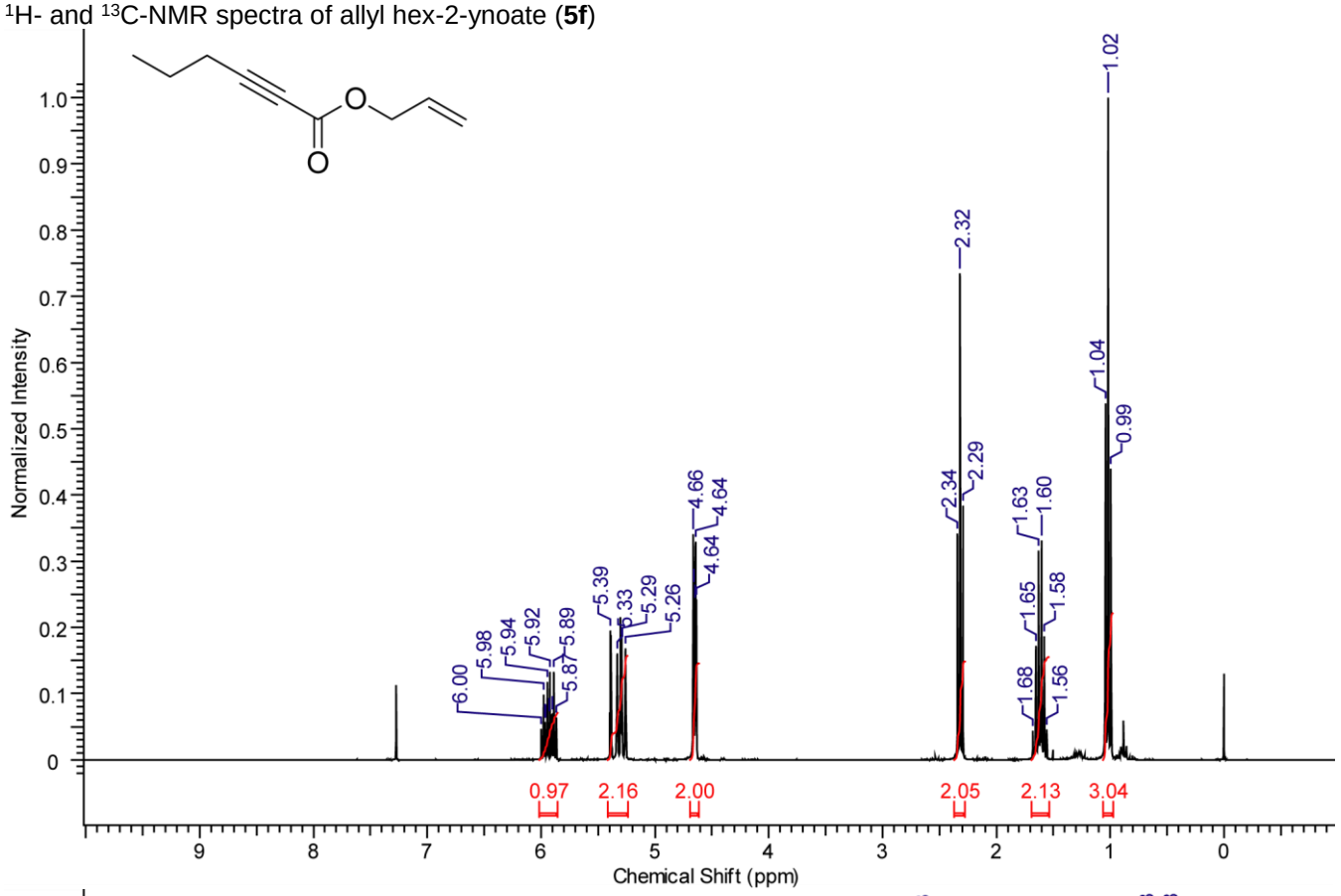
SUPPORTING INFORMATION

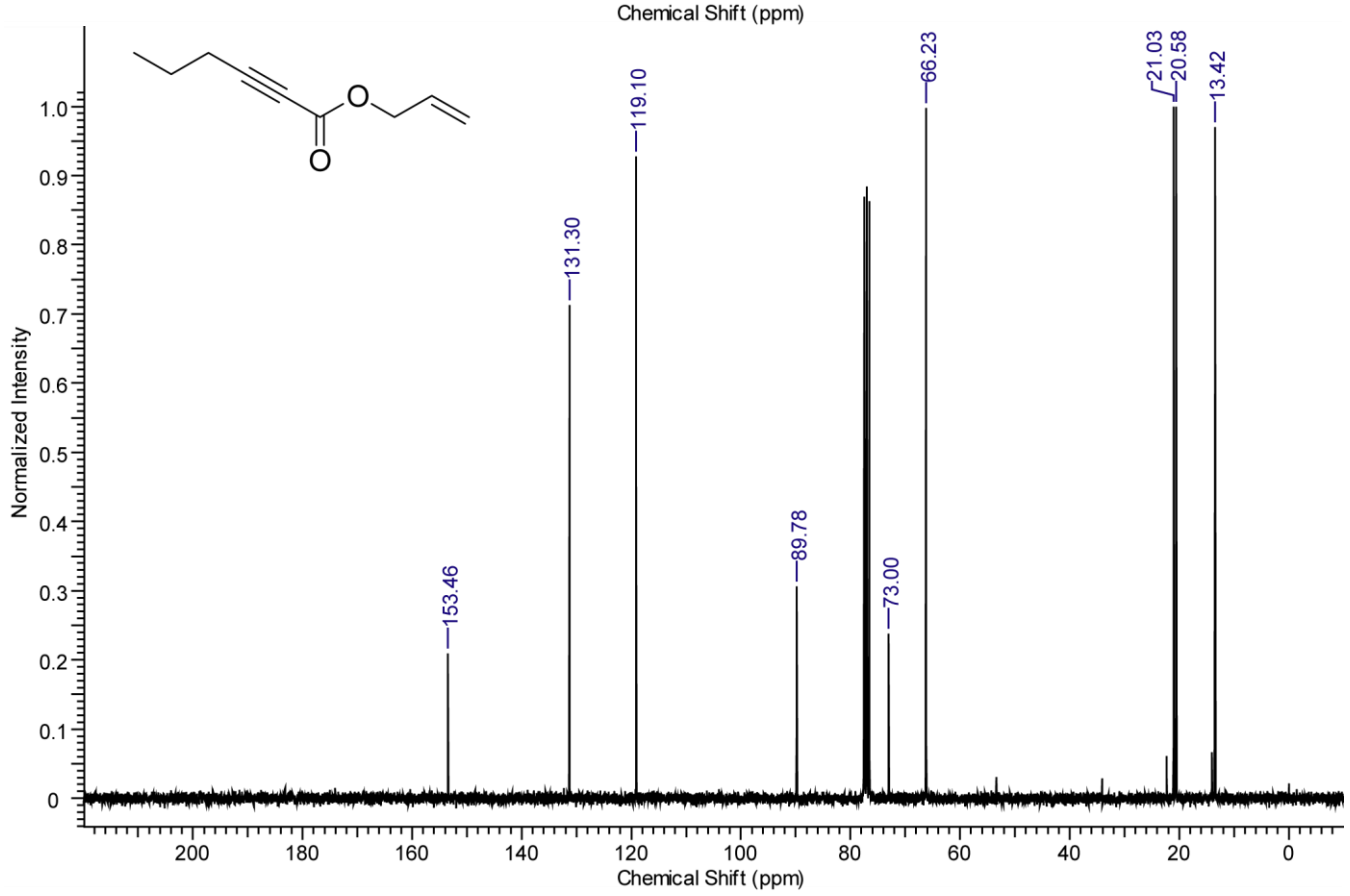
^{1}H- and ^{13}C-NMR spectra of allyl 4-methylpent-2-ynoate (**5e**)



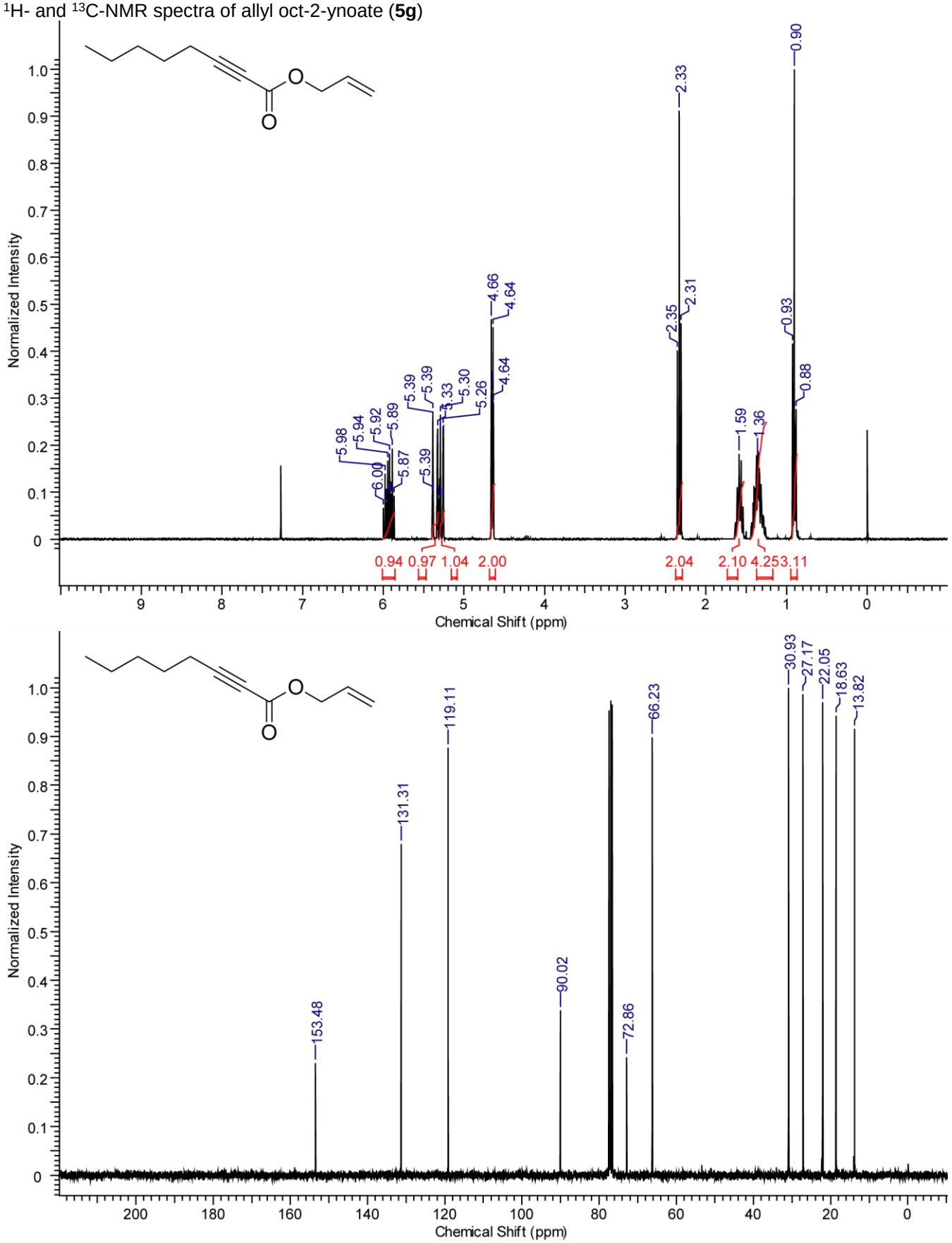


^{1}H- and ^{13}C-NMR spectra of allyl hex-2-ynoate (**5f**)

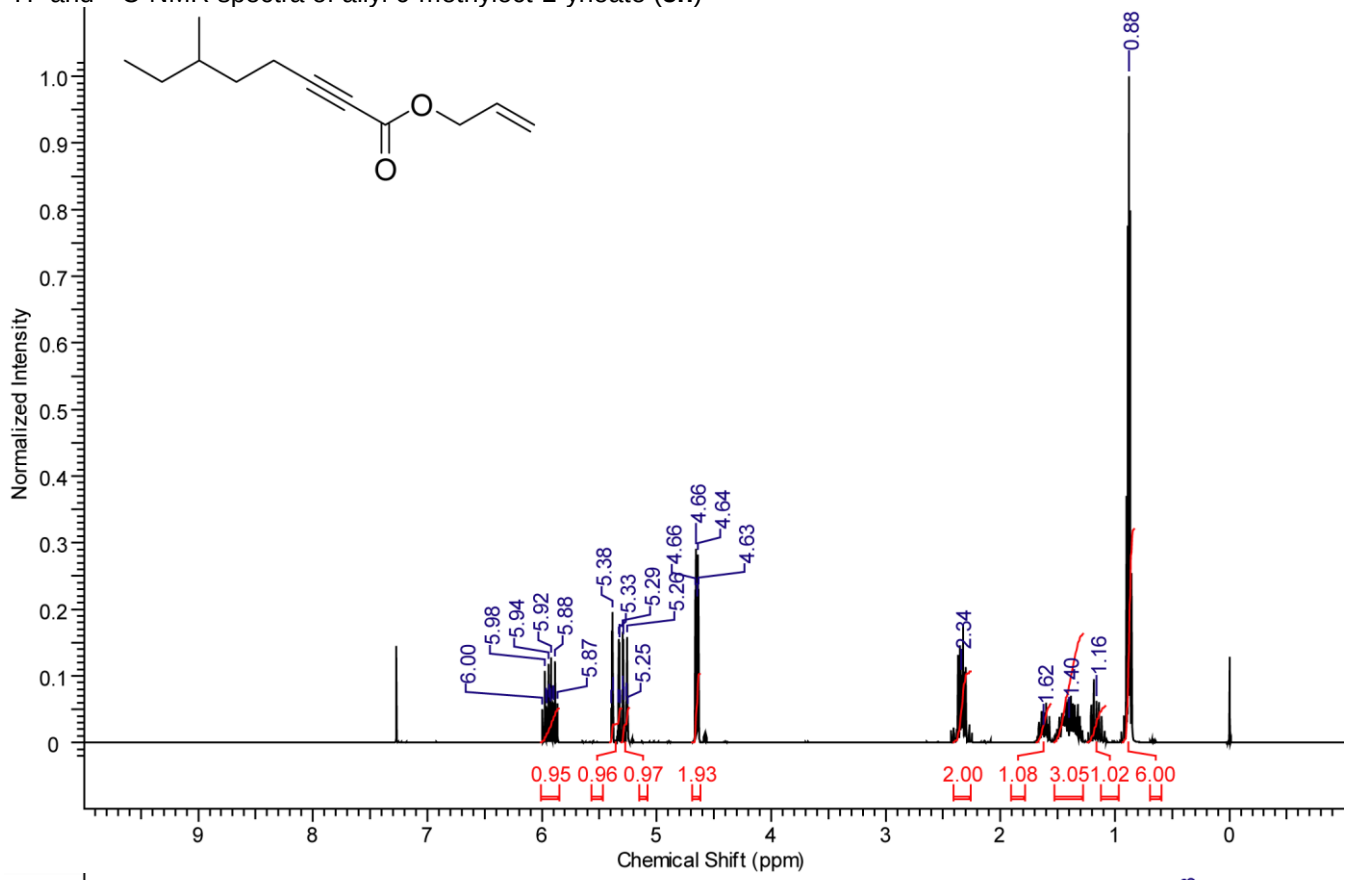


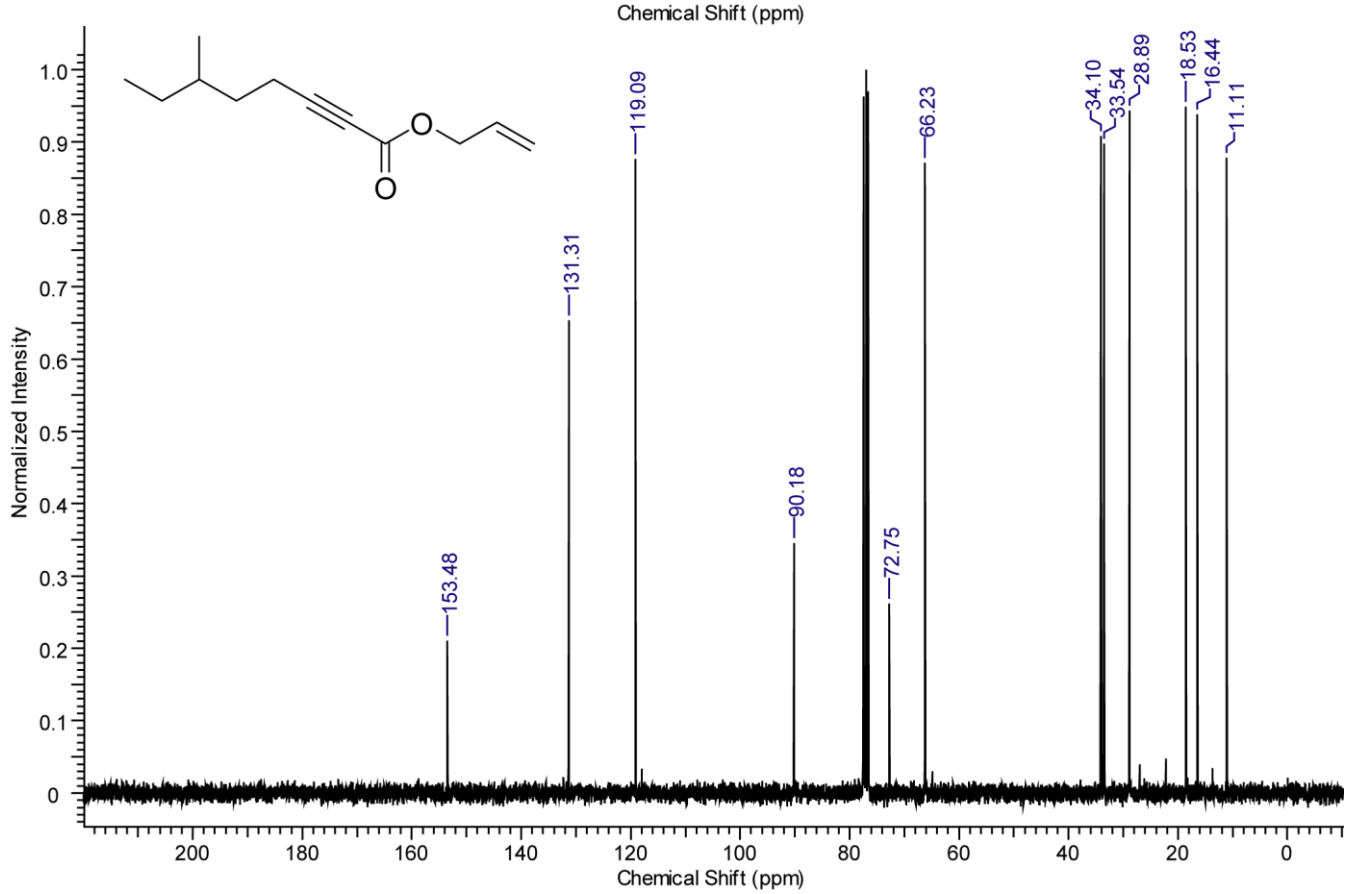


^{1}H- and ^{13}C-NMR spectra of allyl oct-2-ynoate (**5g**)

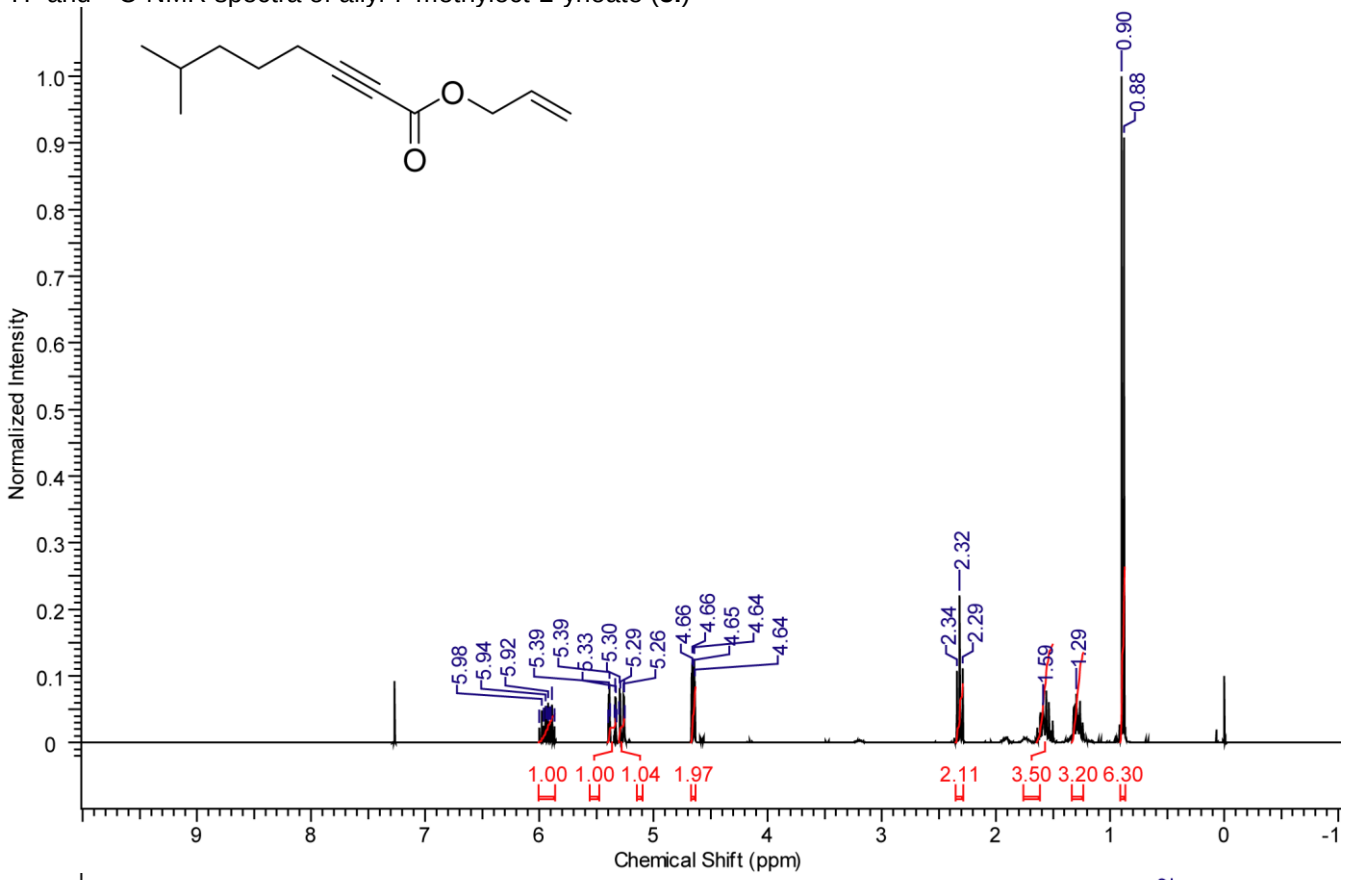


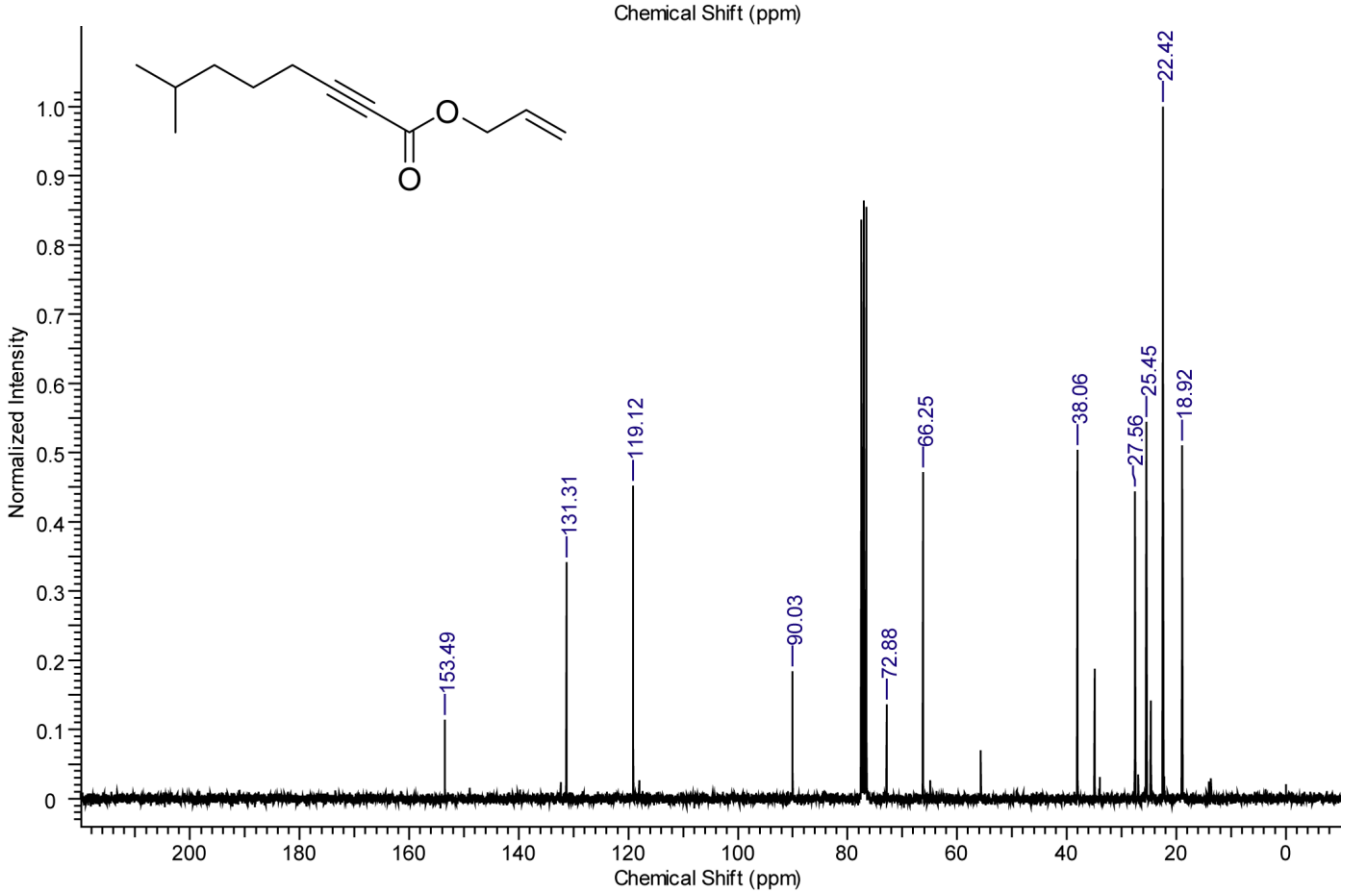
^{1}H- and ^{13}C-NMR spectra of allyl 6-methyloct-2-ynoate (**5h**)



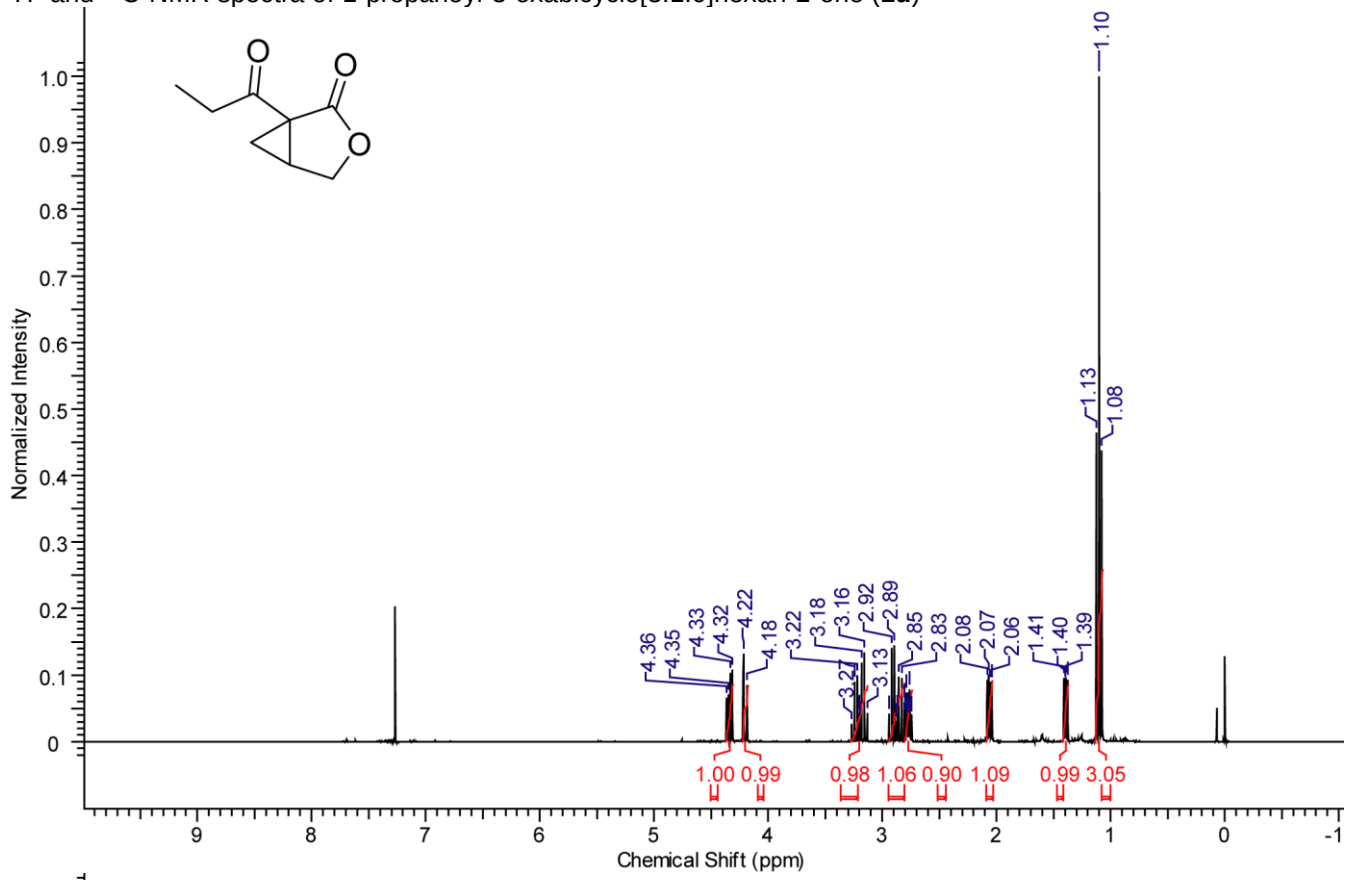


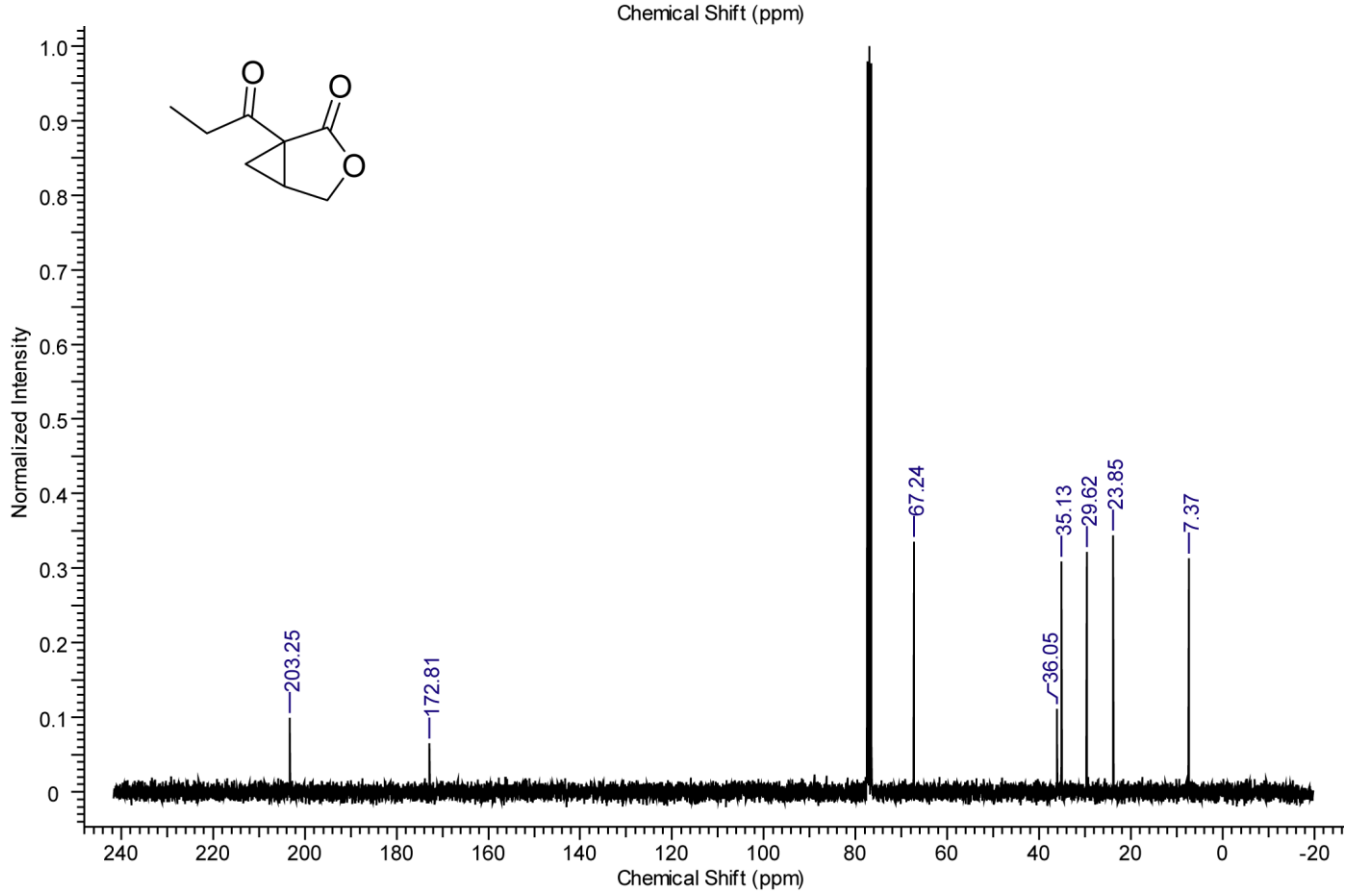
^{1}H- and ^{13}C-NMR spectra of allyl 7-methyloct-2-ynoate (**5i**)



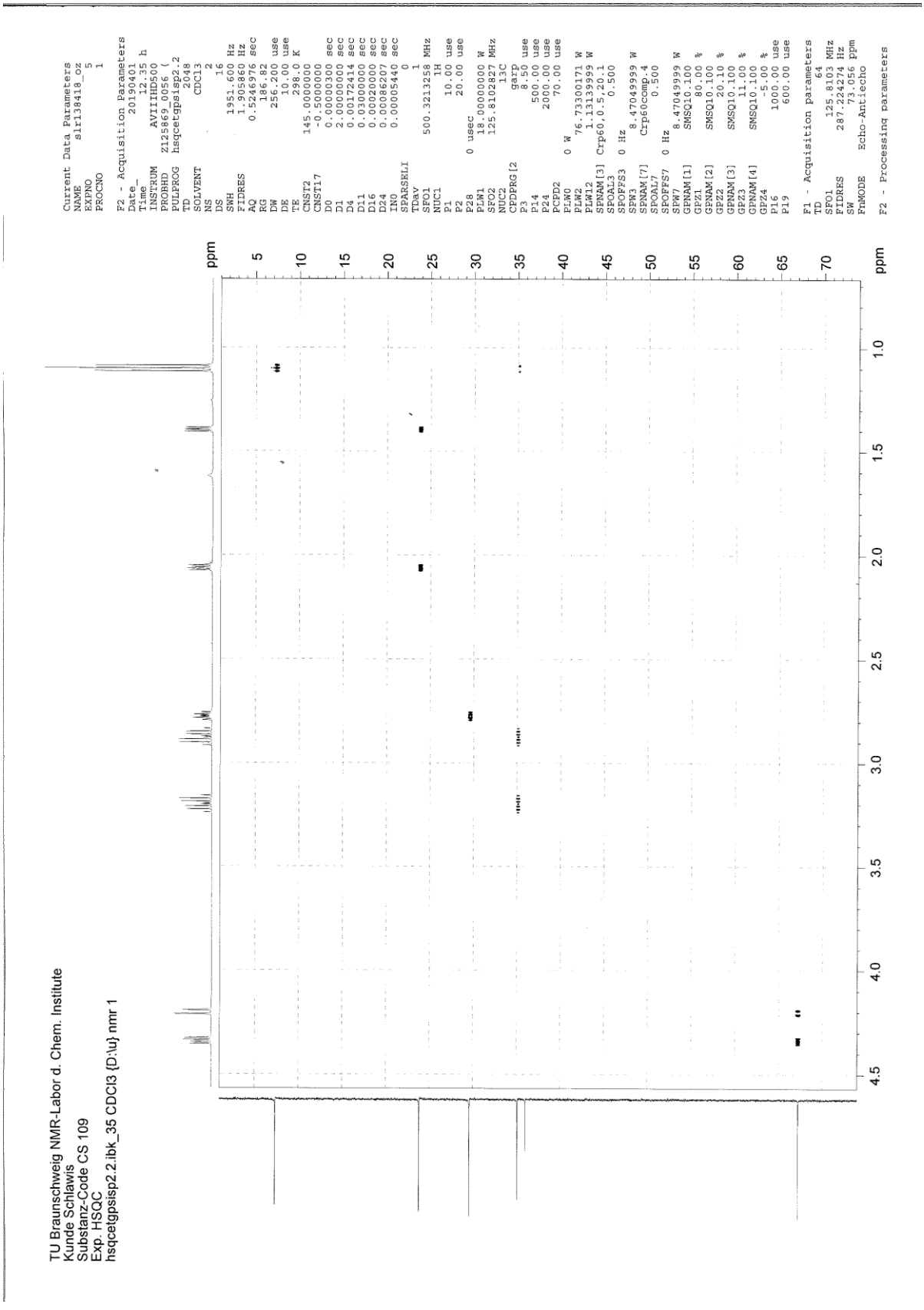


^{1}H- and ^{13}C-NMR spectra of 1-propanoyl-3-oxabicyclo[3.1.0]hexan-2-one (**1d**)



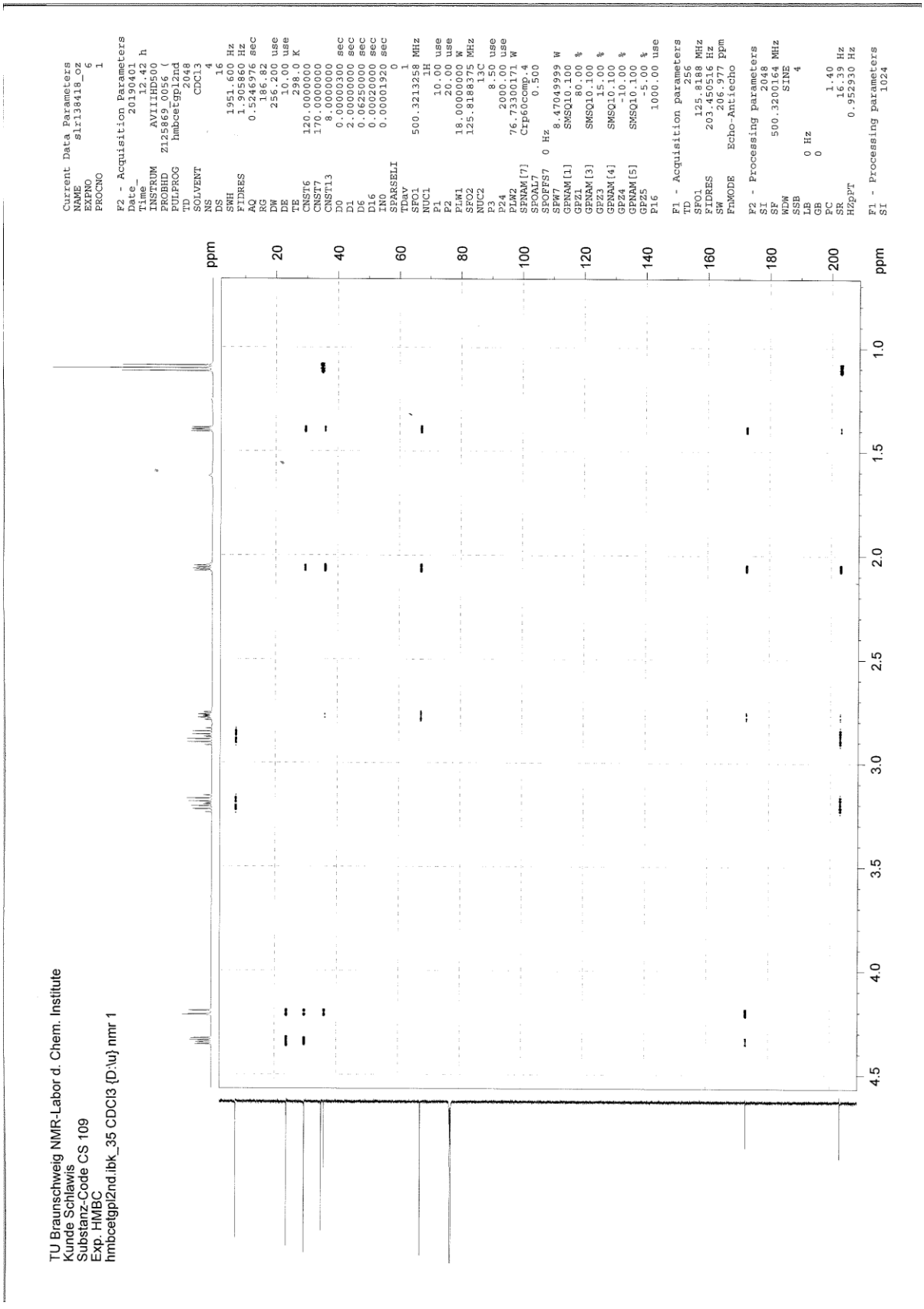


HC-HSQC-NMR spectrum of 1-propanoyl-3-oxabicyclo[3.1.0]hexan-2-one (**1d**)

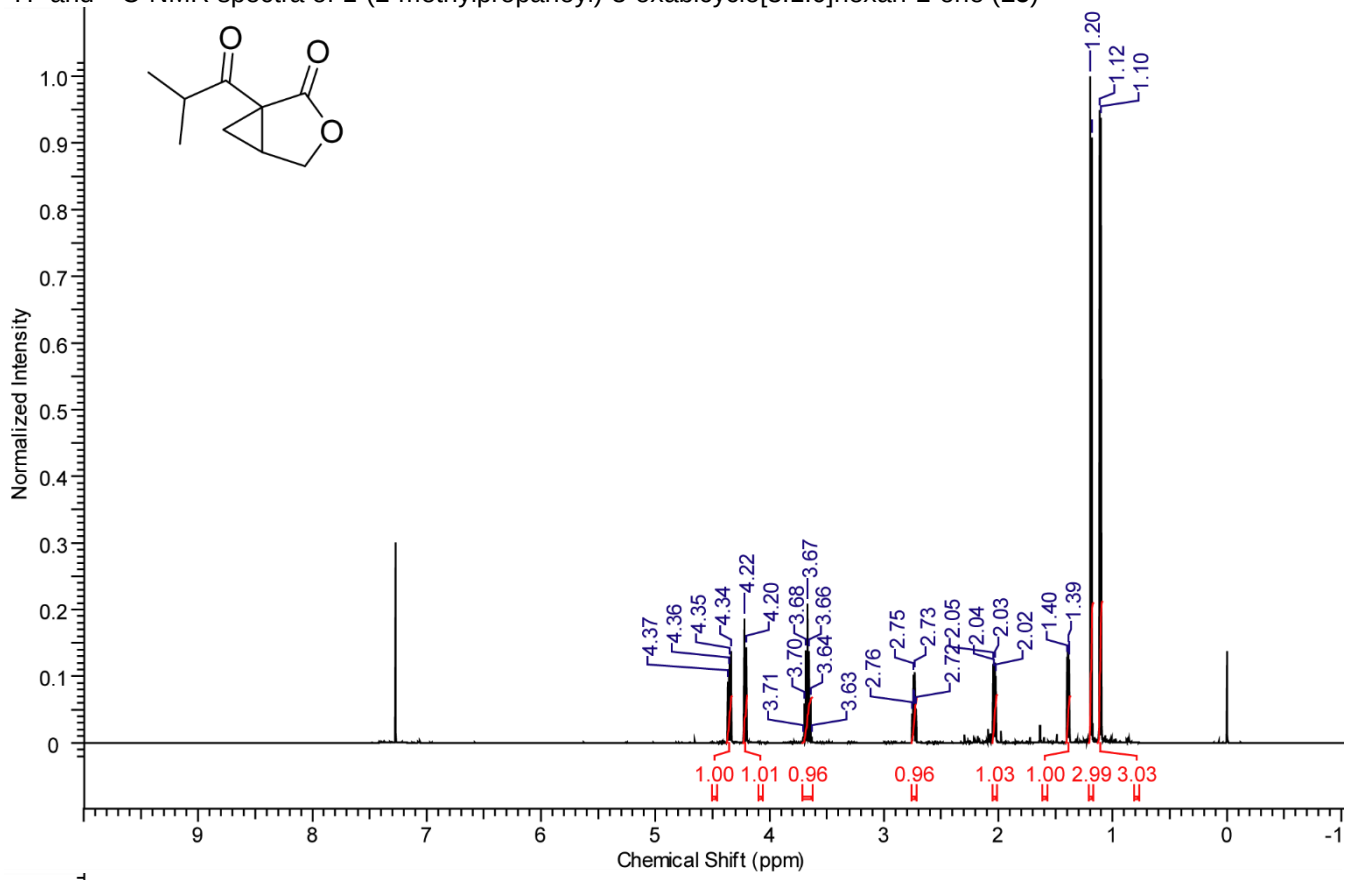


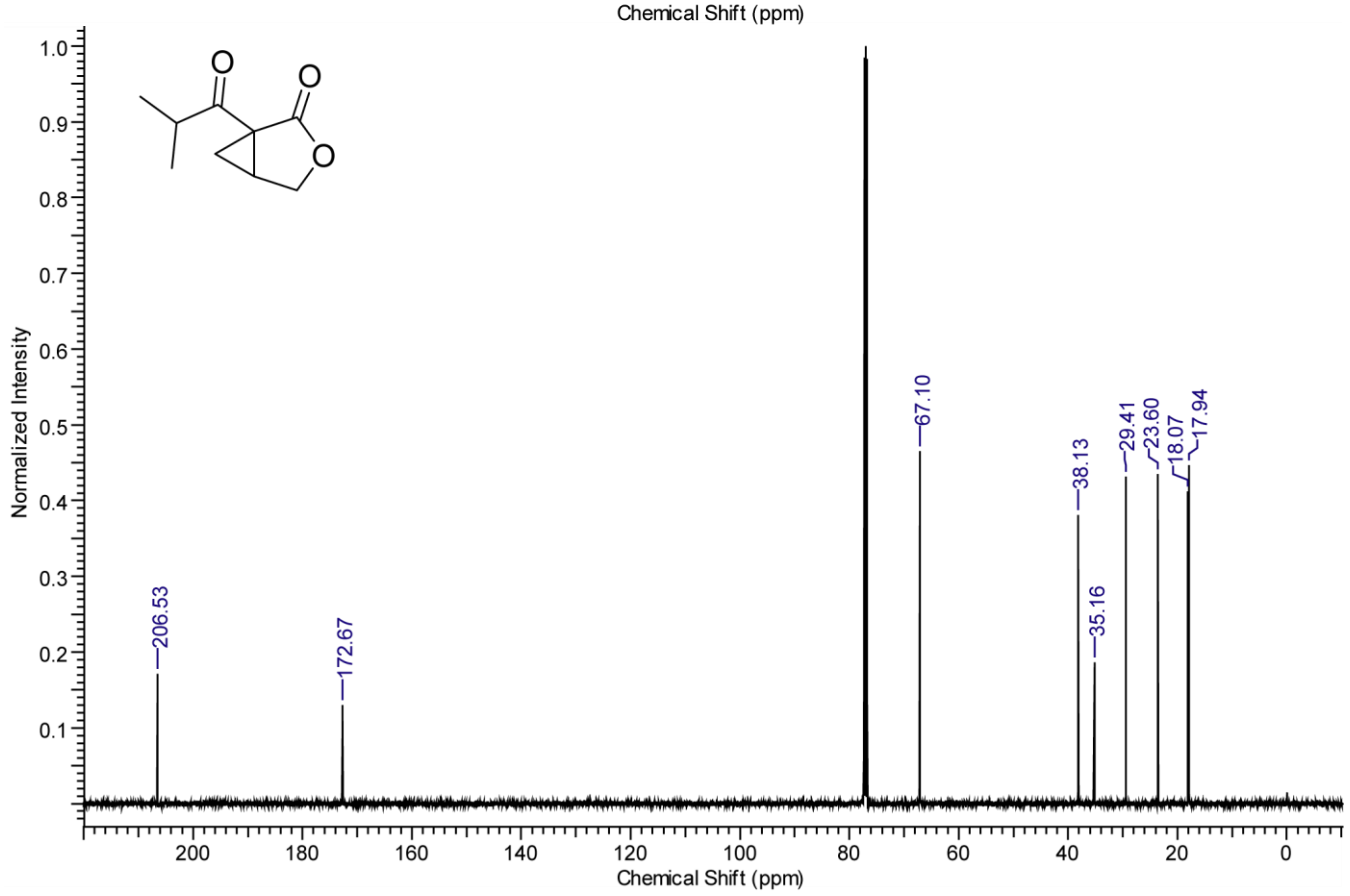
SUPPORTING INFORMATION

HC-HMBC-NMR spectrum of 1-propanoyl-3-oxabicyclo[3.1.0]hexan-2-one (**1d**)



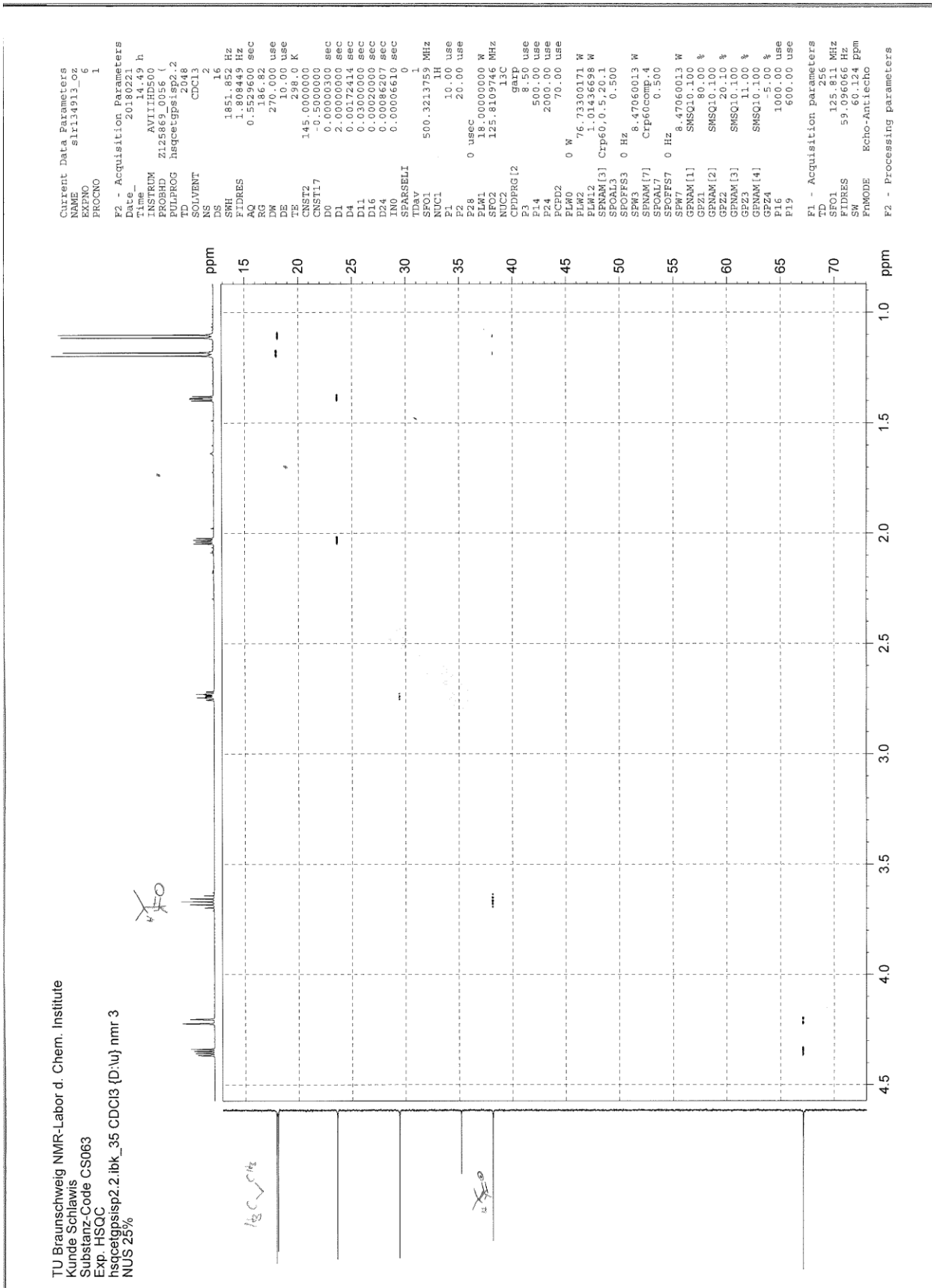
^{1}H- and ^{13}C-NMR spectra of 1-(2-methylpropanoyl)-3-oxabicyclo[3.1.0]hexan-2-one (**1e**)





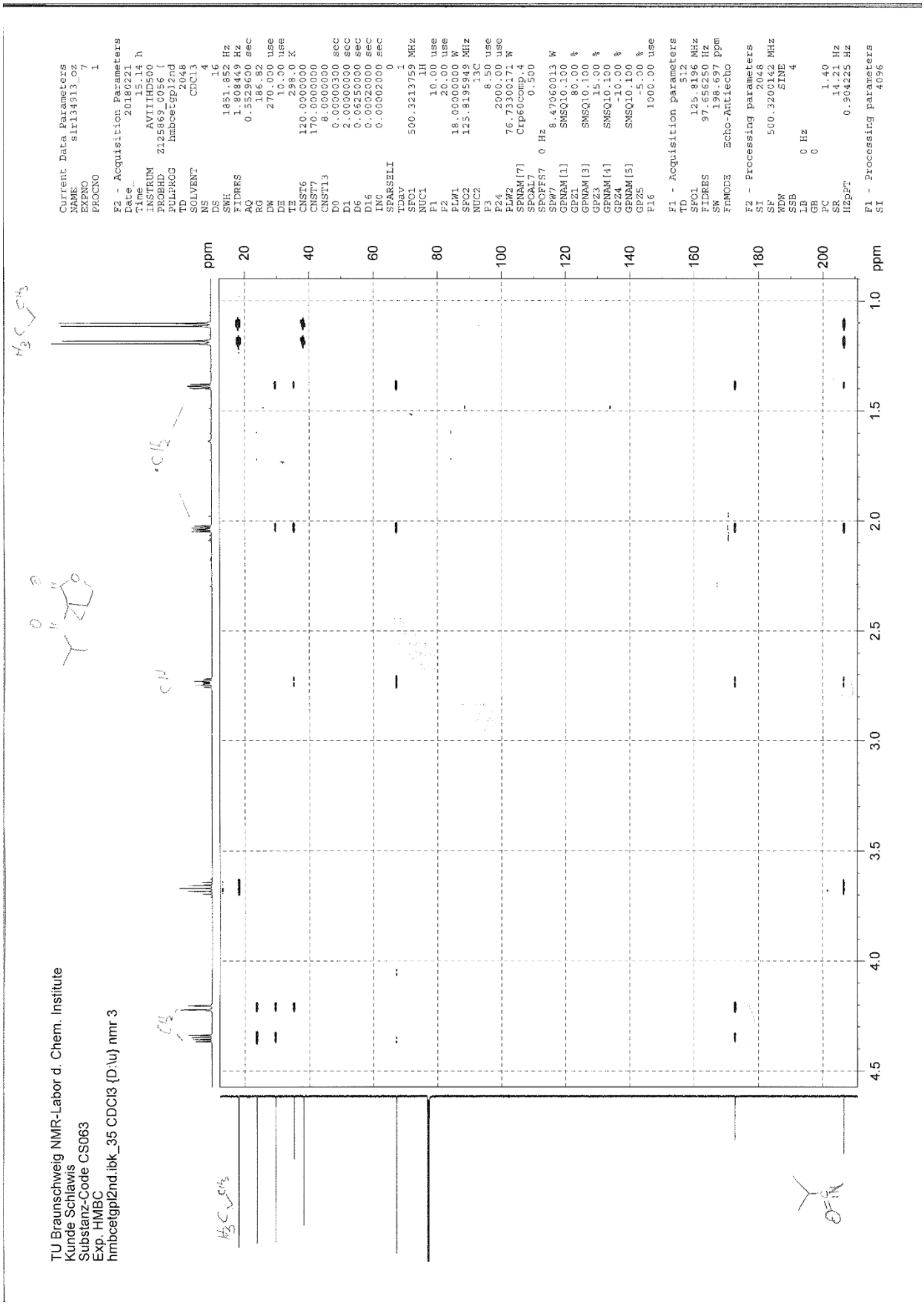
SUPPORTING INFORMATION

HC-HSQC-NMR spectrum of 1-(2-Methylpropanoyl)-3-oxabicyclo[3.1.0]hexan-2-one (**1e**)

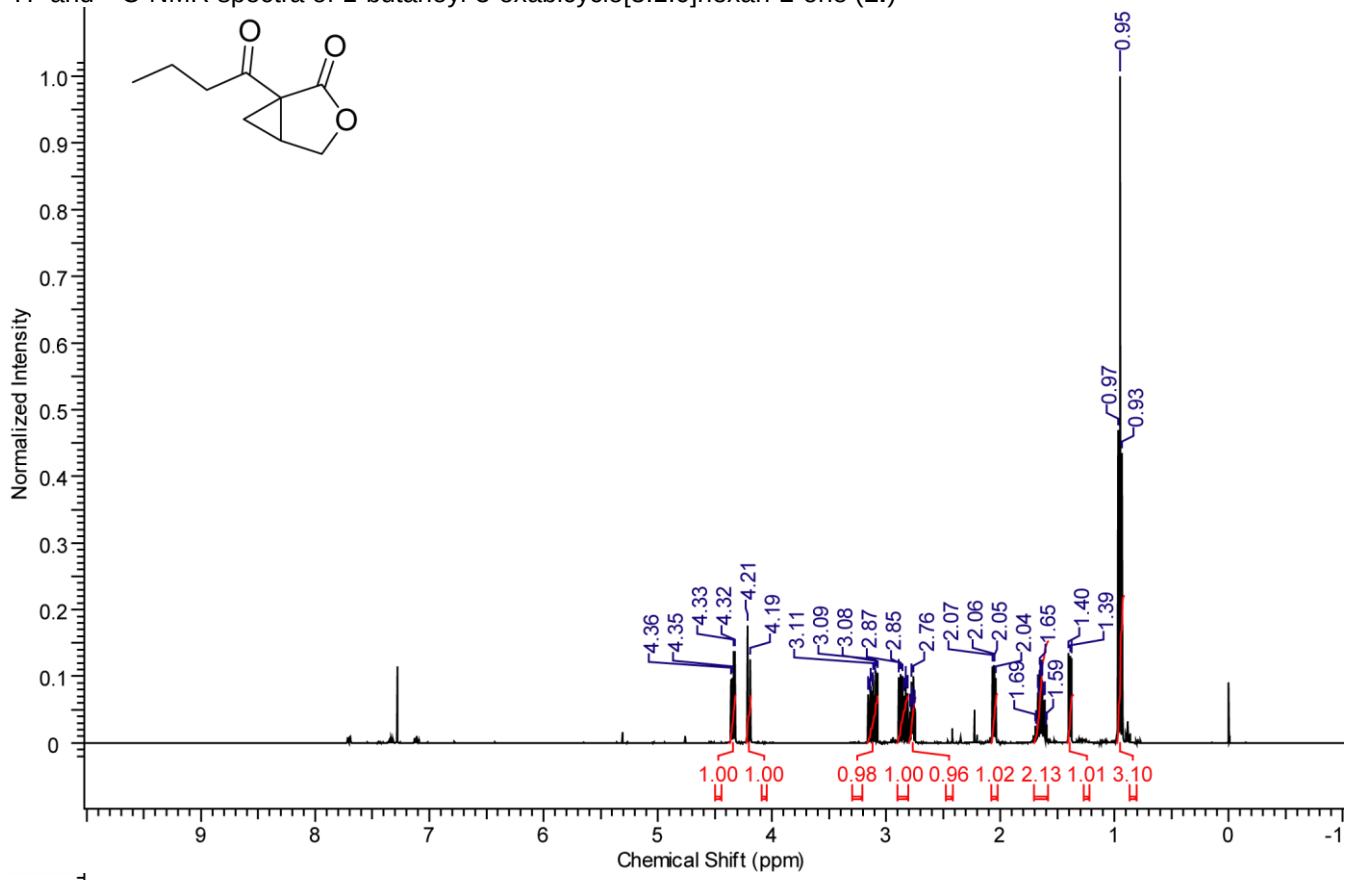


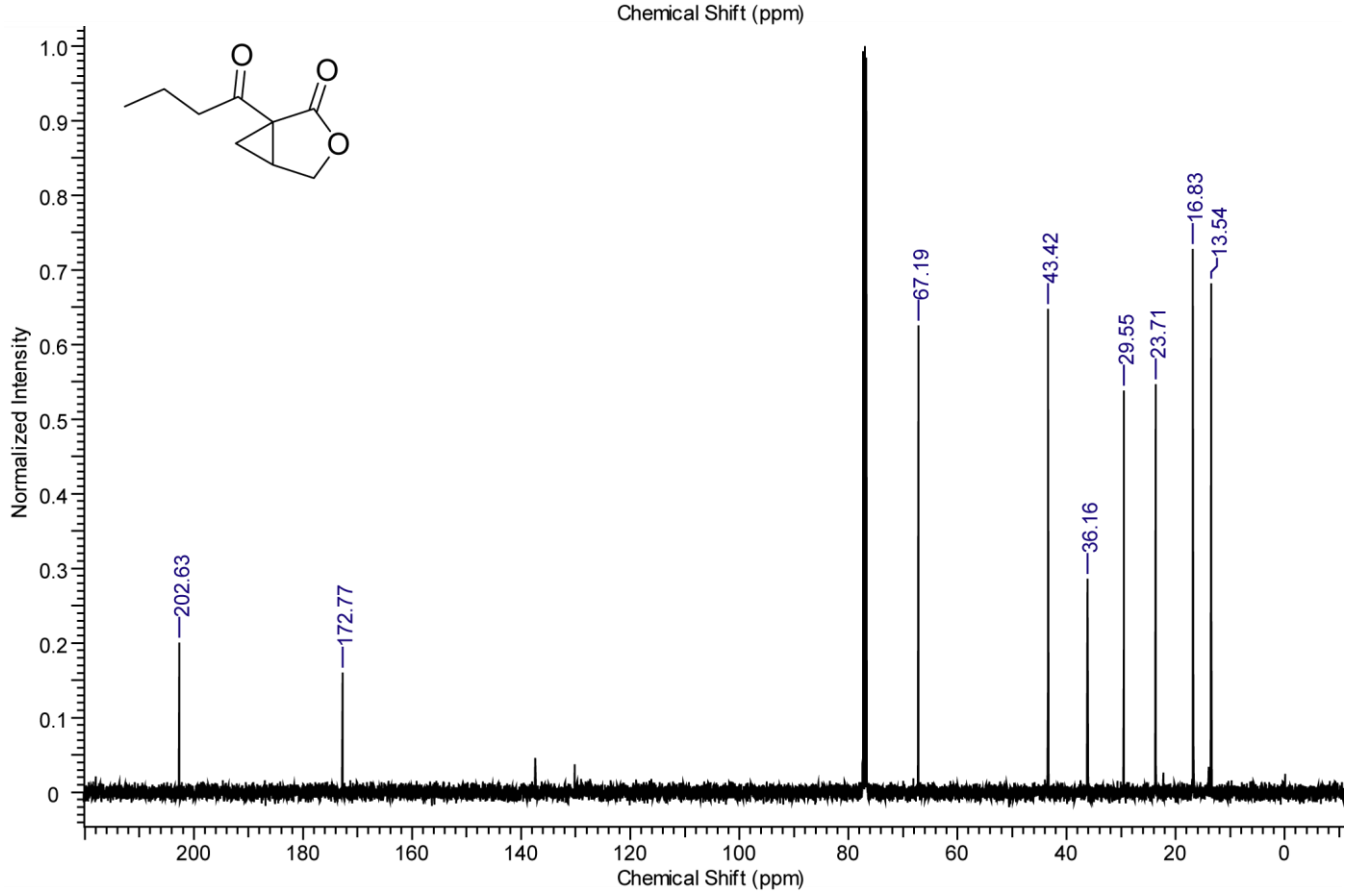
SUPPORTING INFORMATION

HC-HMBC-NMR spectrum of 1-(2-methylpropanoyl)-3-oxabicyclo[3.1.0]hexan-2-one (**1e**)



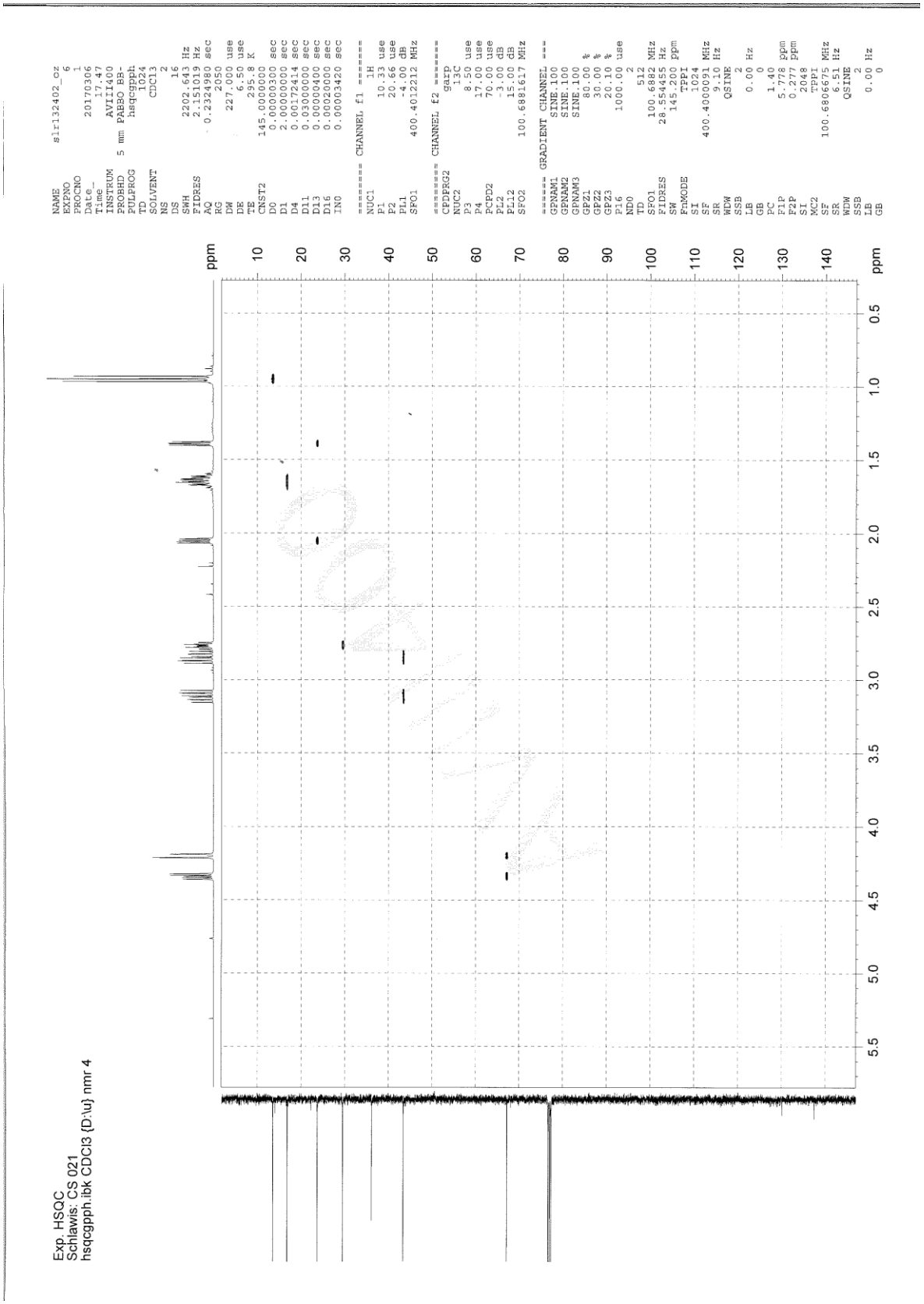
^{1}H- and ^{13}C-NMR spectra of 1-butanoyl-3-oxabicyclo[3.1.0]hexan-2-one (**1f**)





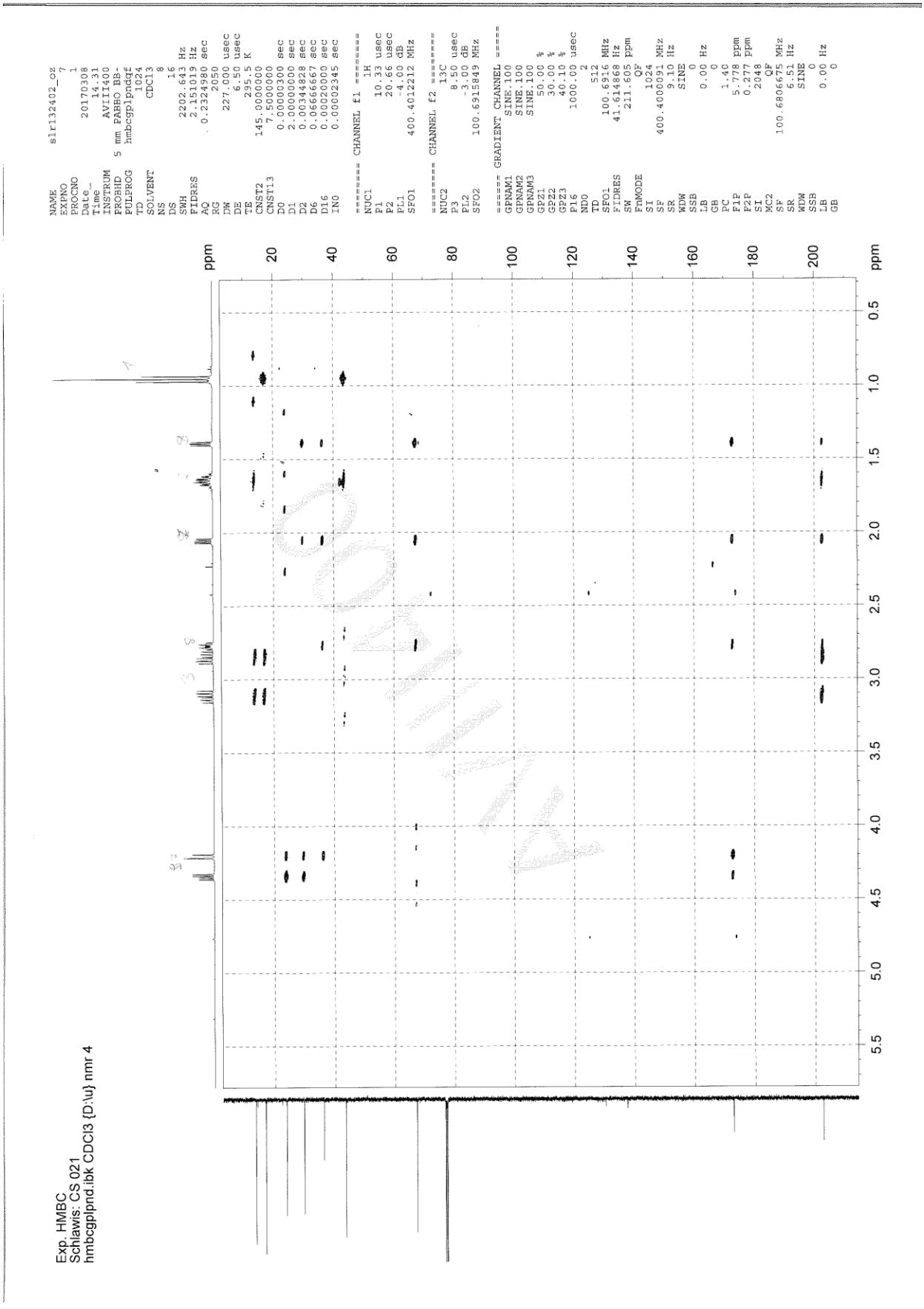
SUPPORTING INFORMATION

HC-HSQC-NMR spectrum of 1-butanoyl-3-oxabicyclo[3.1.0]hexan-2-one (**1f**)



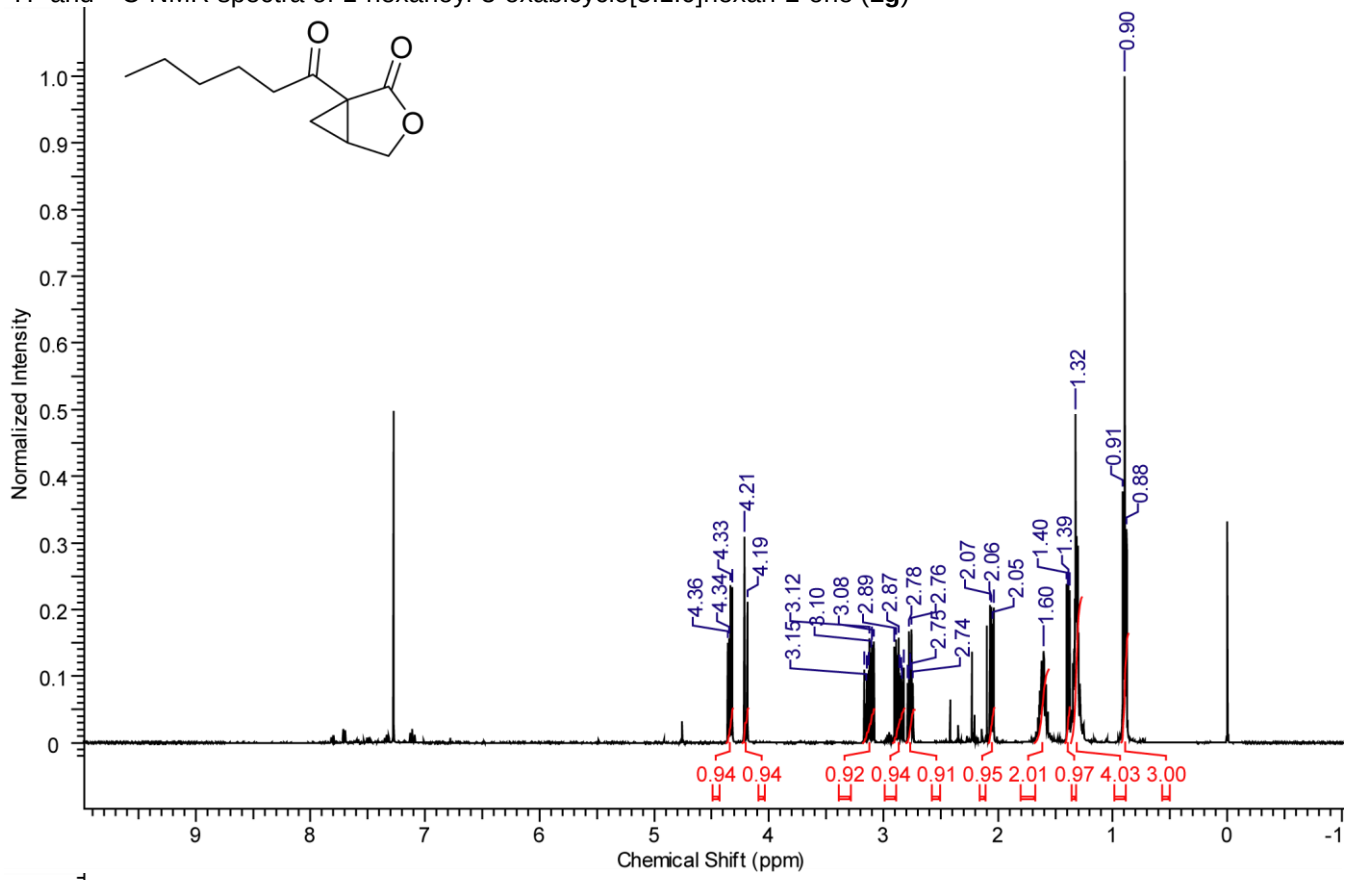
SUPPORTING INFORMATION

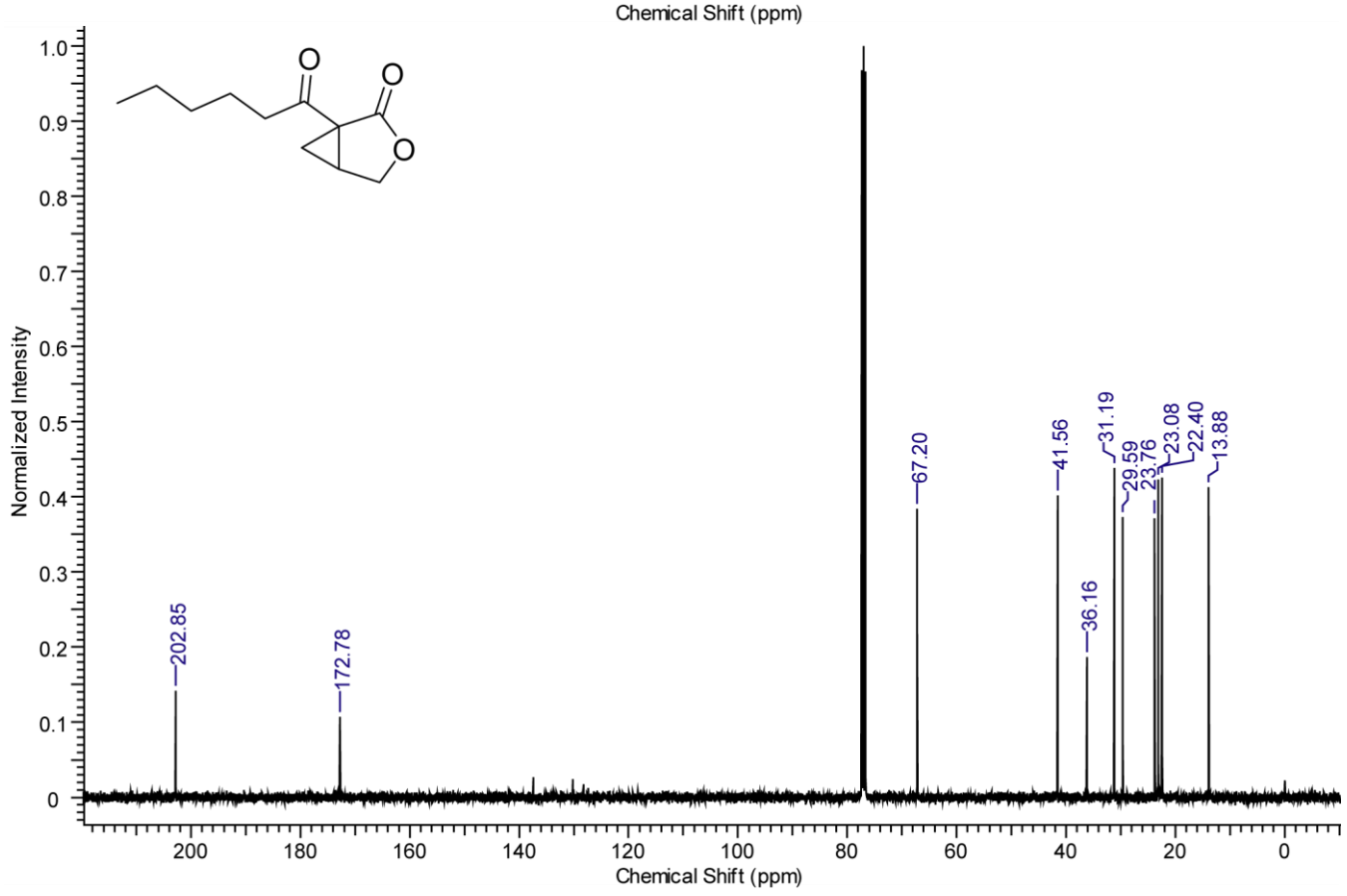
HC-HMBC-NMR spectrum of 1-butanoyl-3-oxabicyclo[3.1.0]hexan-2-one (**1f**)



SUPPORTING INFORMATION

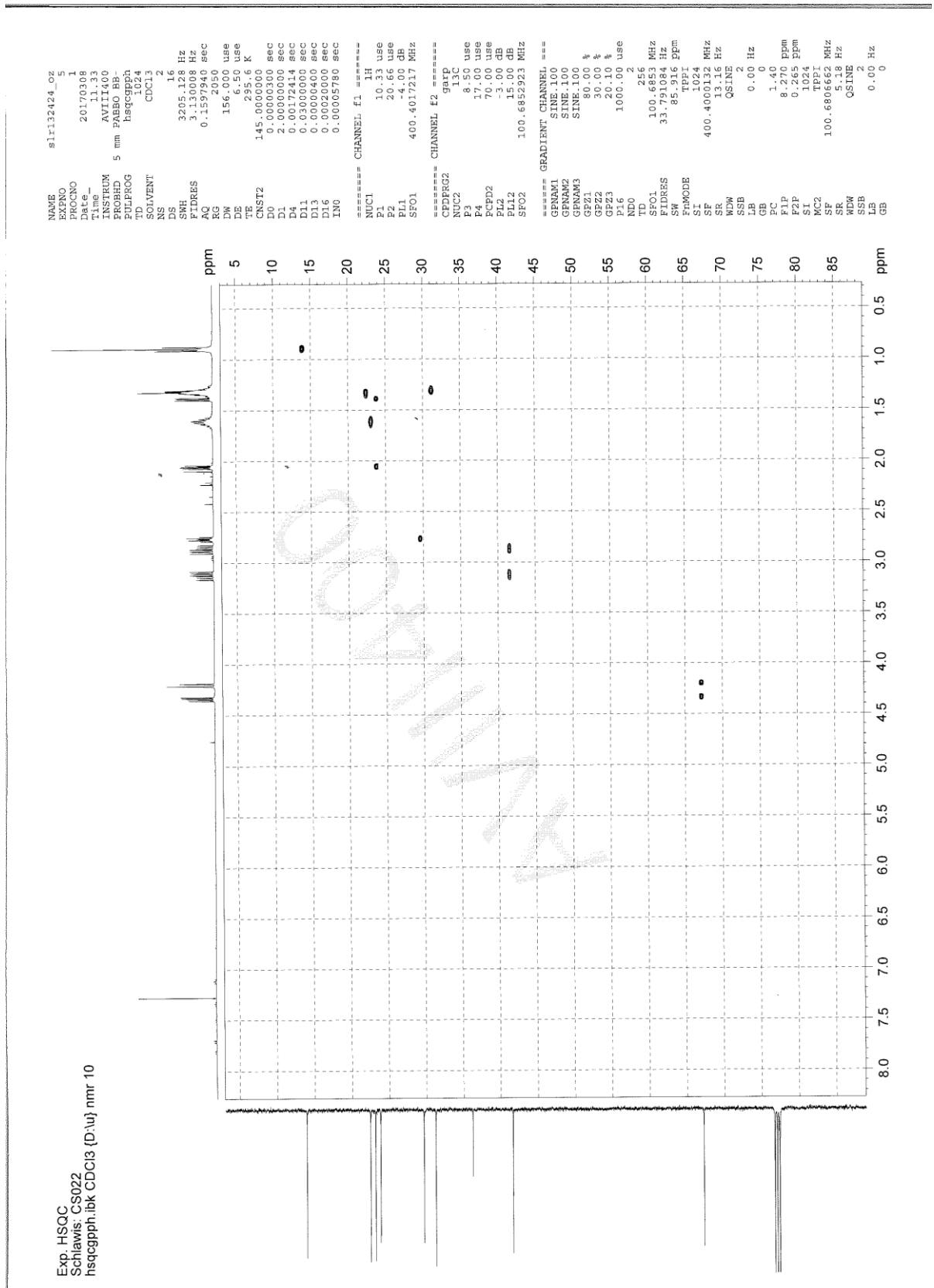
^{1}H- and ^{13}C-NMR spectra of 1-hexanoyl-3-oxabicyclo[3.1.0]hexan-2-one (**1g**)





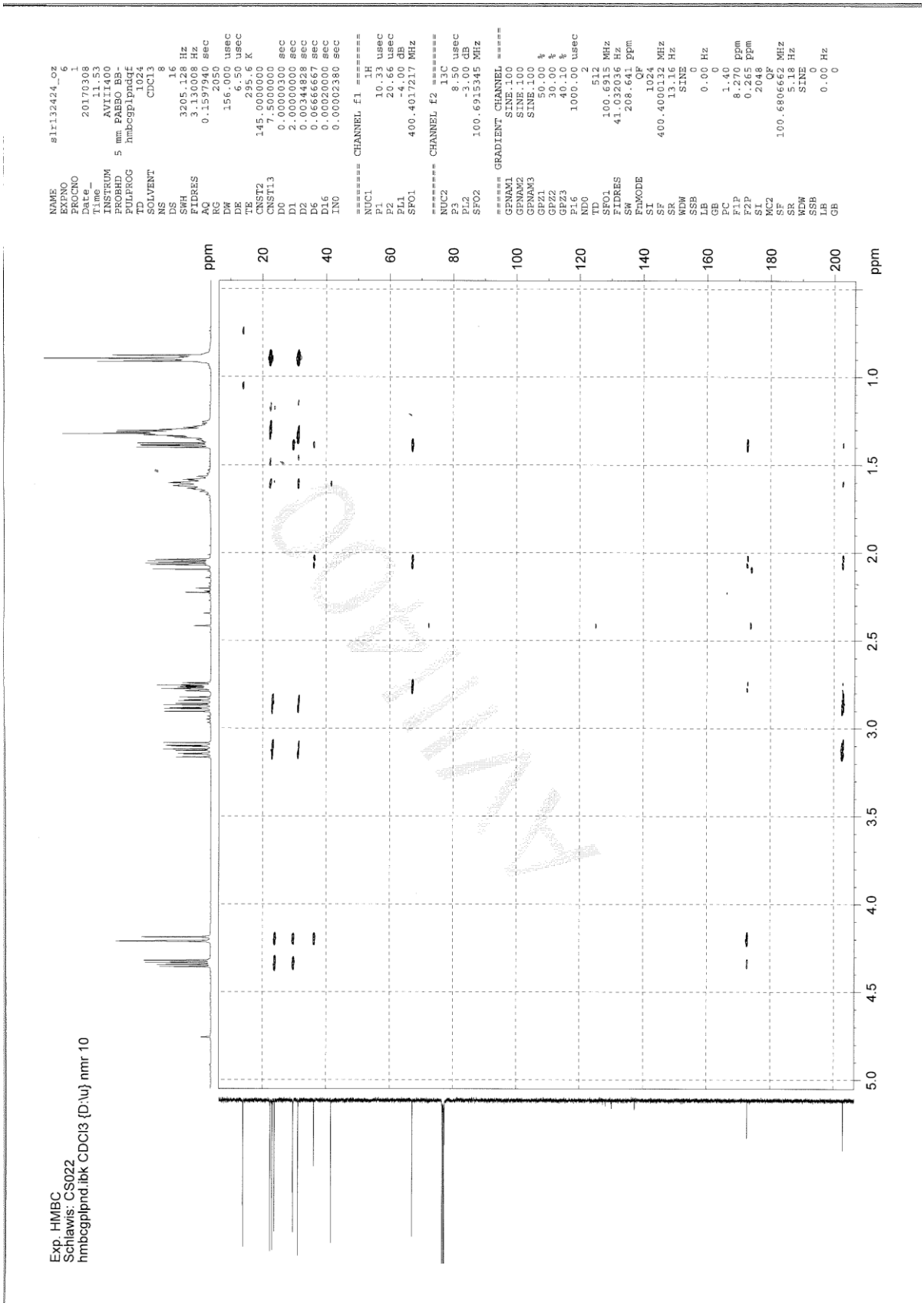
SUPPORTING INFORMATION

HC-HSQC-NMR spectrum of 1-hexanoyl-3-oxabicyclo[3.1.0]hexan-2-one (**1g**)

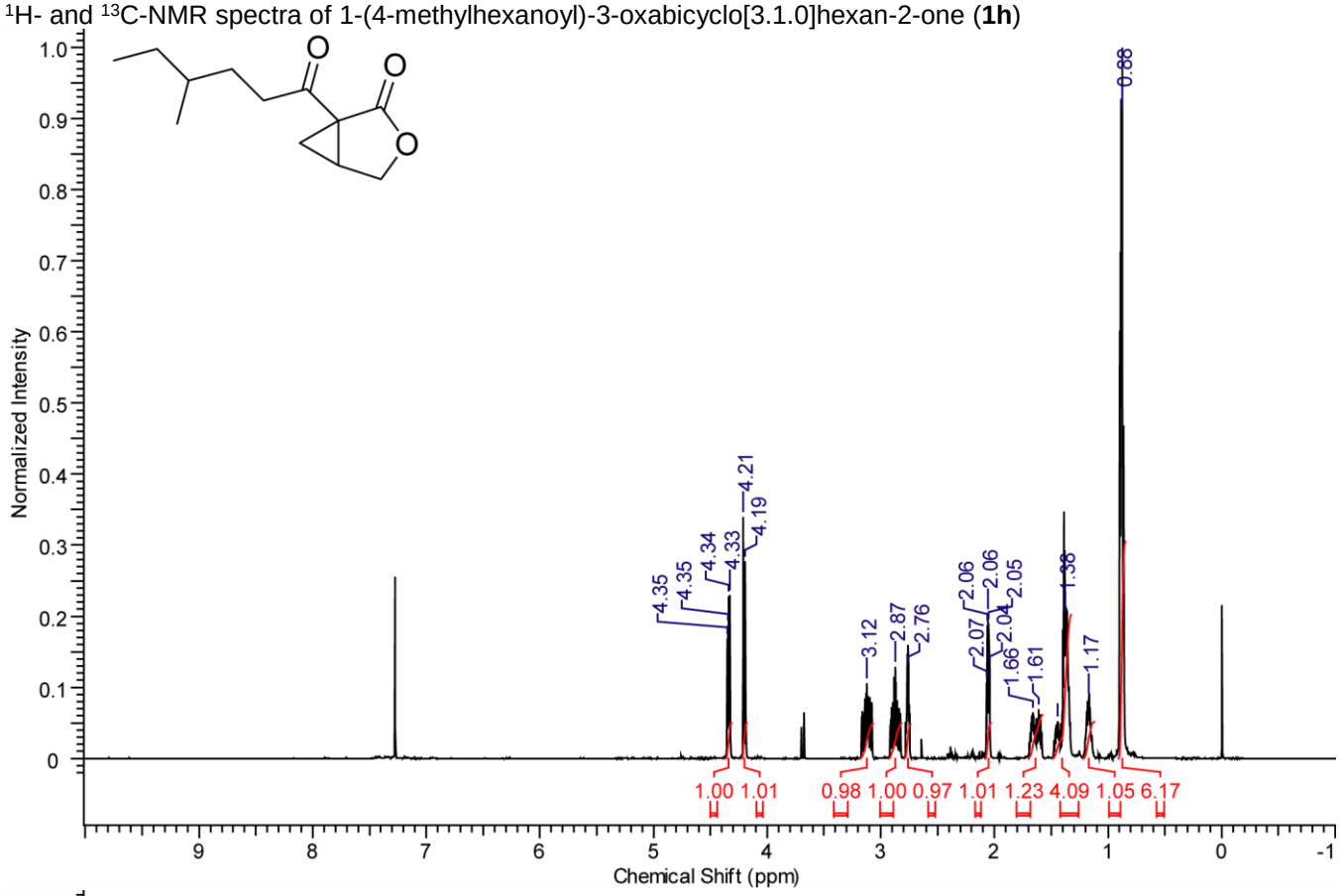


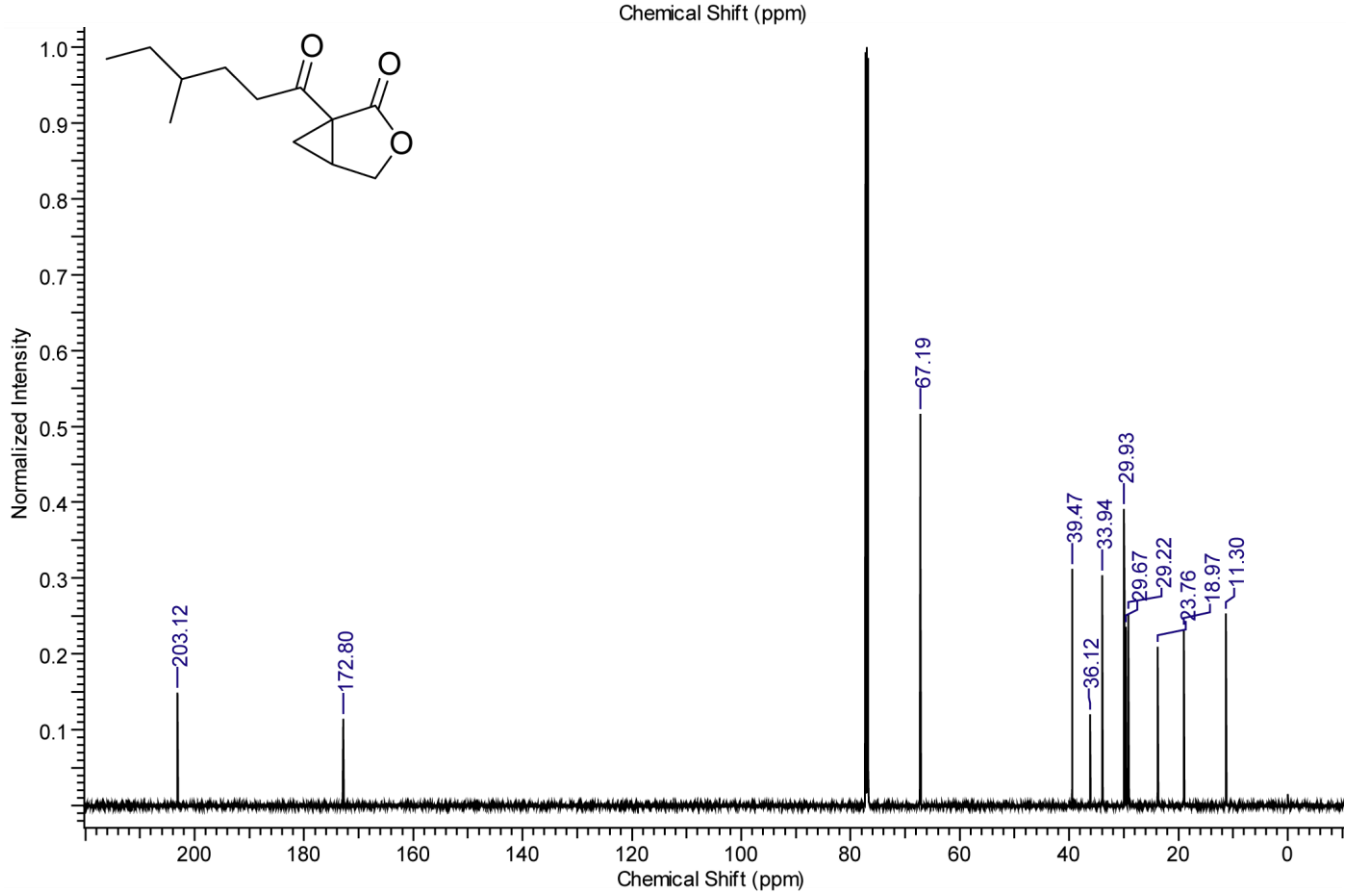
SUPPORTING INFORMATION

HC-HMBC-NMR spectrum of 1-hexanoyl-3-oxabicyclo[3.1.0]hexan-2-one (**1g**)



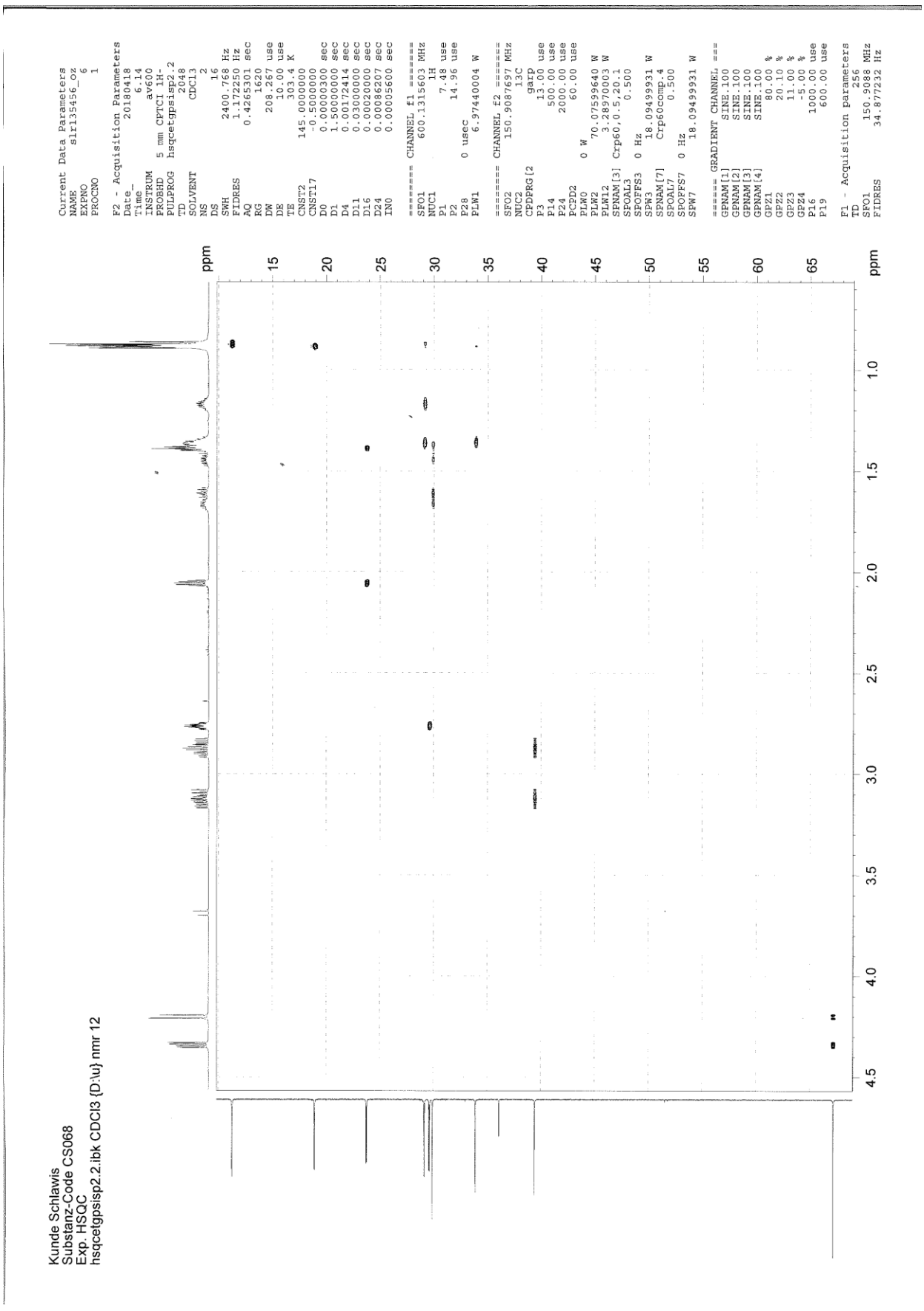
^{1}H- and ^{13}C-NMR spectra of 1-(4-methylhexanoyl)-3-oxabicyclo[3.1.0]hexan-2-one (**1h**)





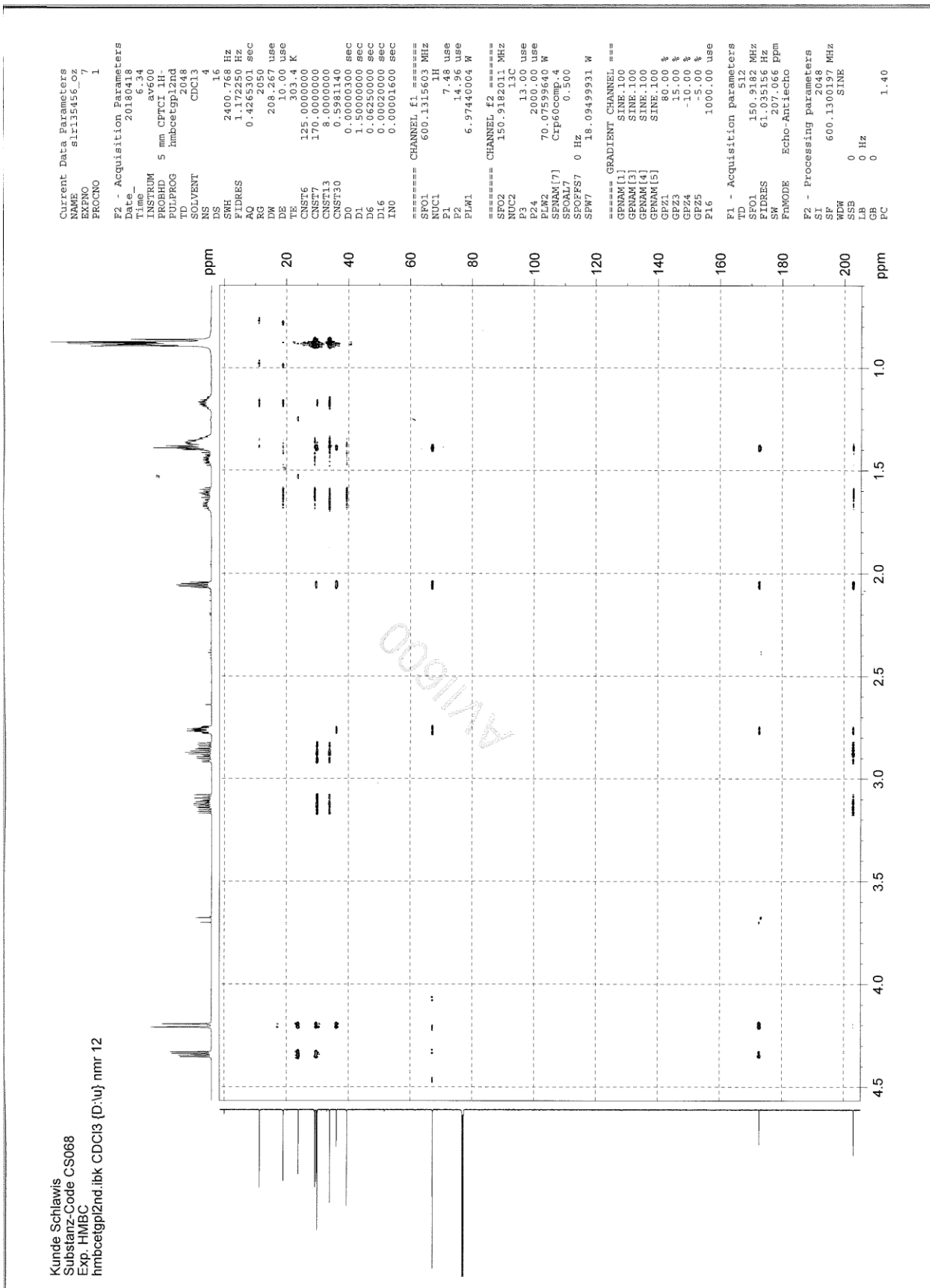
SUPPORTING INFORMATION

HC-HSQC-NMR spectrum of 1-(4-methylhexanoyl)-3-oxabicyclo[3.1.0]hexan-2-one (**1h**)

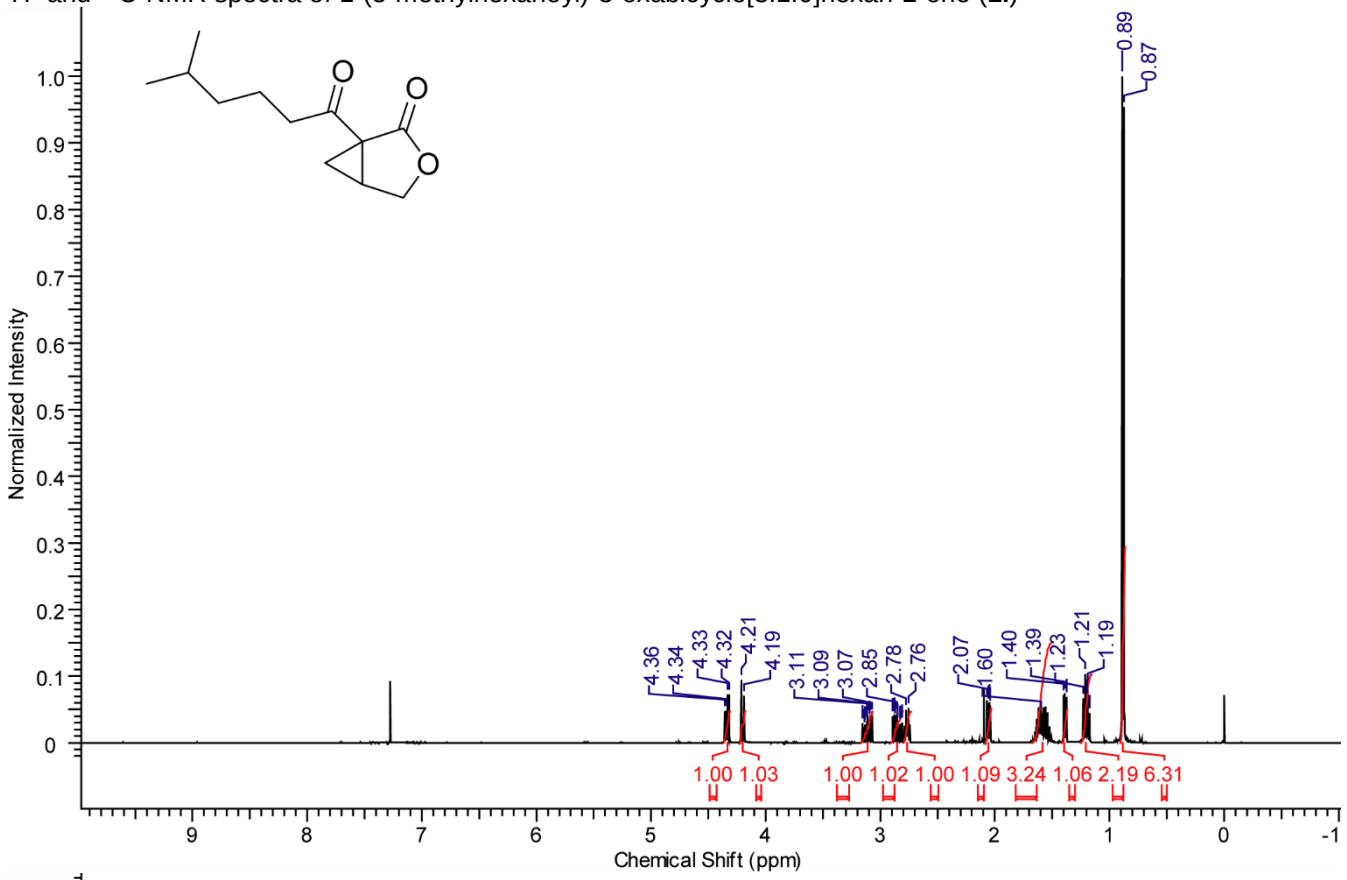


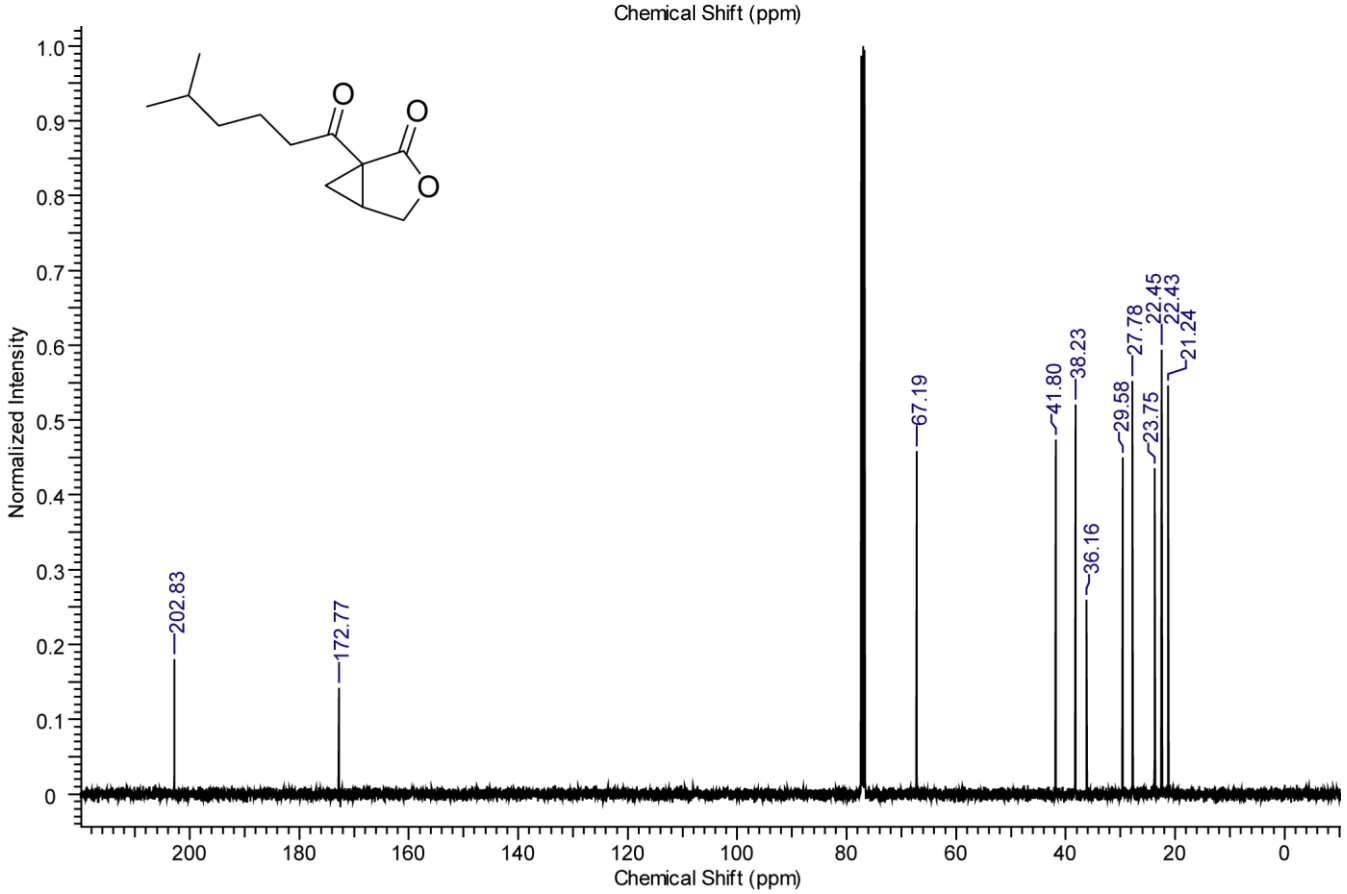
SUPPORTING INFORMATION

HC-HMBC-NMR spectrum of 1-(4-methylhexanoyl)-3-oxabicyclo[3.1.0]hexan-2-one (**1h**)



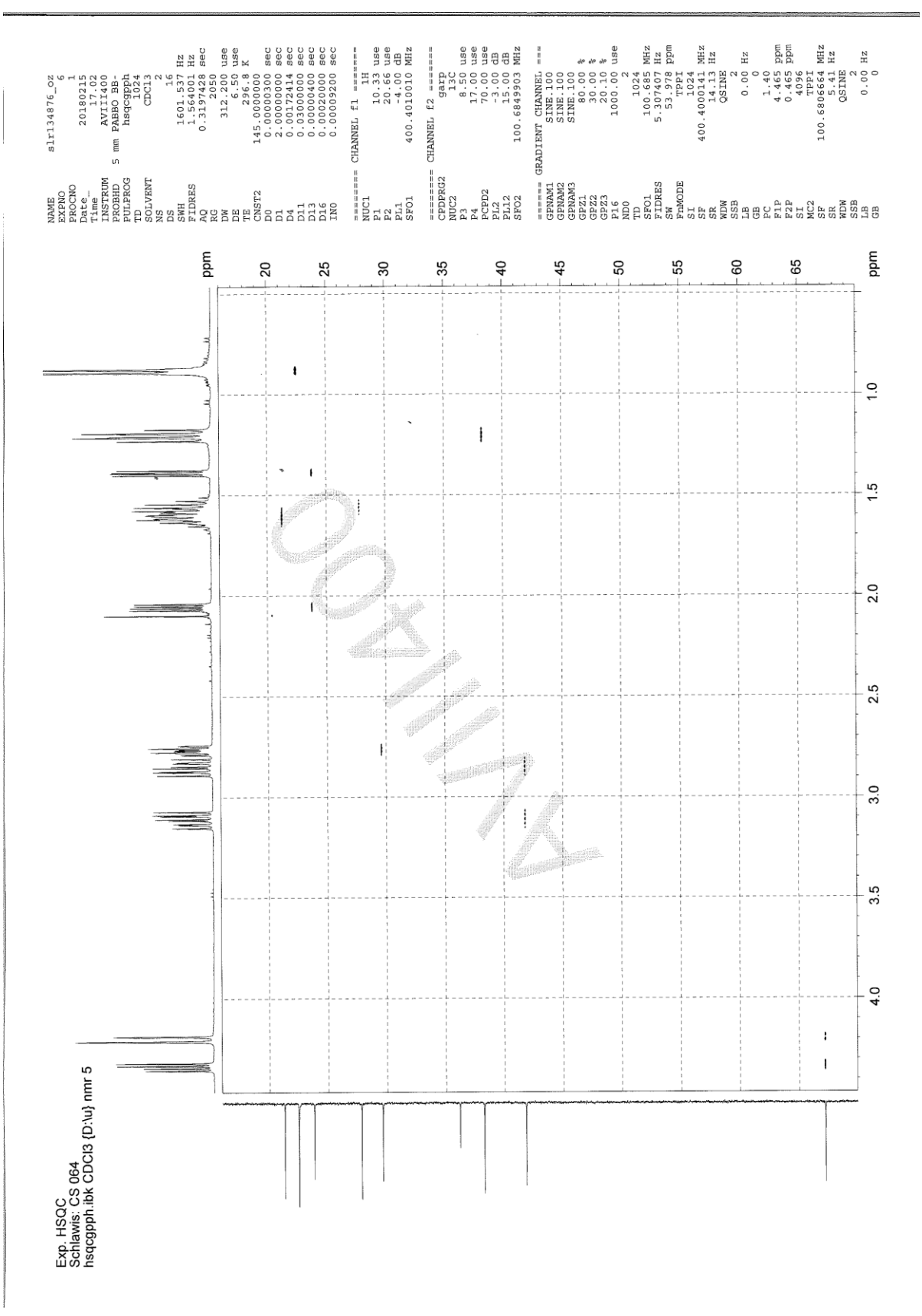
^{1}H- and ^{13}C-NMR spectra of 1-(5-methylhexanoyl)-3-oxabicyclo[3.1.0]hexan-2-one (**1i**)





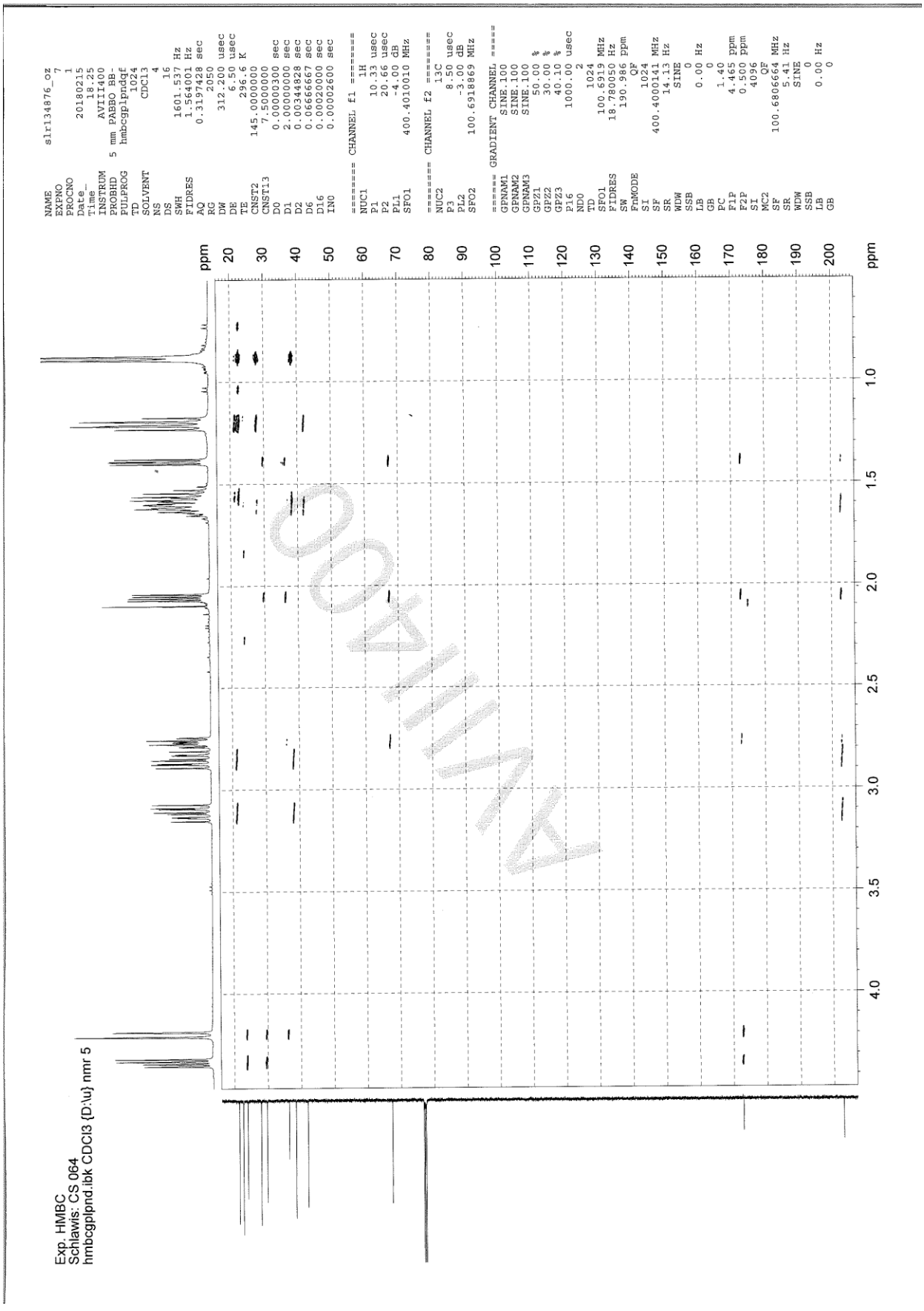
SUPPORTING INFORMATION

HC-HSQC-NMR spectrum of 1-(5-methylhexanoyl)-3-oxabicyclo[3.1.0]hexan-2-one (**1i**)



SUPPORTING INFORMATION

HC-HMBC-NMR spectrum of 1-(5-methylhexanoyl)-3-oxabicyclo[3.1.0]hexan-2-one (**1i**)



SUPPORTING INFORMATION

Part 5: References

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