

Supporting Information for

Bis-Michael Acceptors as Novel Probes to Study the  
Keap1/Nrf2/ARE Pathway

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Canada

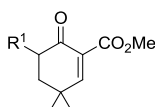
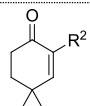
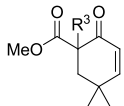
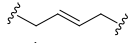

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**Table S1.** Fold-induction of NQO1 by qPCR

Compound	Fold-induction at: <sup>α</sup>			
	2 μM	5 μM	10 μM	
Sulforaphane	0.81 ± 0.08	1.38 ± 0.15	2.02 ± 0.08	
<b>7</b>	 R <sup>1</sup> = H	2.02 ± 0.15	2.25 ± 0.20	4.29 ± 0.20
<b>8</b>	R <sup>1</sup> = Hex	1.62 ± 0.45	5.92 ± 1.38	10.99 ± 2.82
<b>11</b>	R <sup>1</sup> = Bn	1.96 ± 0.72	3.41 ± 0.37	5.99 ± 0.75
<b>15</b>	R <sup>1</sup> = CH <sub>2</sub> CN	0.63 ± 0.19	2.35 ± 0.57	1.98 ± 0.56
<b>17</b>	R <sup>1</sup> = (CH <sub>2</sub> ) <sub>2</sub> CO <sub>2</sub> Me	0.57 ± 0.18	1.94 ± 0.35	1.30 ± 0.75
<b>18</b>	 R <sup>2</sup> = CO <sub>2</sub> All	3.59 ± 0.11	14.89 ± 2.04	30.22 ± 4.87
<b>19</b>	R <sup>2</sup> = CO <sub>2</sub> <i>t</i> -Bu	5.56 ± 1.64	8.76 ± 0.76	19.71 ± 2.79
<b>23</b>	R <sup>2</sup> = Cl	2.78 ± 0.77	1.99 ± 0.52	2.05 ± 0.23
<b>27</b>		2.48 ± 0.09	5.42 ± 1.58	8.08 ± 0.80
<b>30</b>	R <sup>4</sup> = CH <sub>2</sub>	5.97 ± 0.73	10.17 ± 1.03	22.41 ± 3.04
<b>31</b>	R <sup>4</sup> = (CH <sub>2</sub> ) <sub>3</sub>	3.18 ± 0.17	8.96 ± 0.65	18.63 ± 5.98
<b>32</b>	R <sup>4</sup> = (CH <sub>2</sub> ) <sub>4</sub>	8.38 ± 0.62	12.92 ± 1.45	13.96 ± 1.47
<b>33</b>	R <sup>4</sup> = 	14.97 ± 4.77	15.60 ± 3.56	13.92 ± 1.88
<b>34</b>	R <sup>4</sup> = 	14.38 ± 2.12	19.47 ± 2.64	25.62 ± 1.62

<sup>α</sup> no toxicity was observed at these concentrations

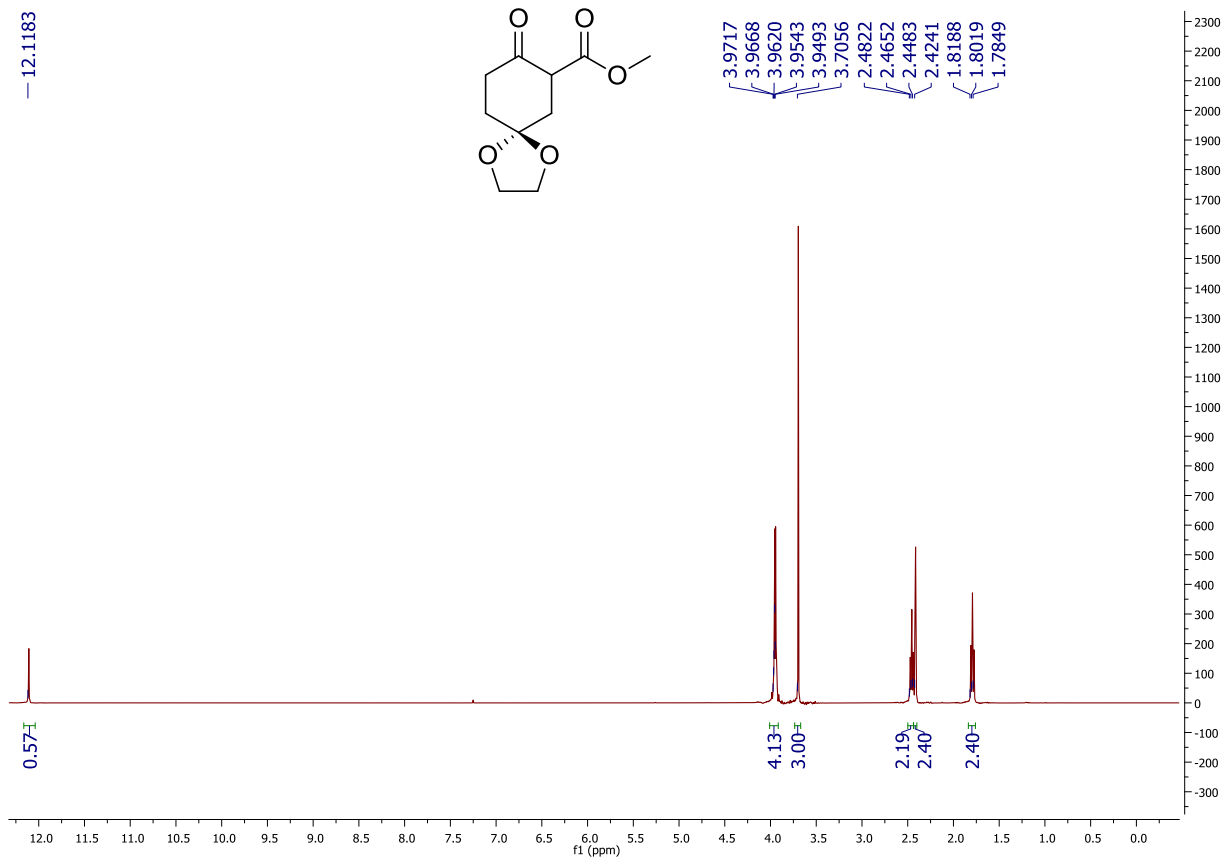
**Table S2.** % Mortality of all compounds evaluated in the LDH assay <sup>a</sup>

Compound		% Mortality at:			
		1 $\mu$ M	20 $\mu$ M	100 $\mu$ M	
3		R <sup>1</sup> = Hex	Not tested	2.39 $\pm$ 0.06	4.23 $\pm$ 0.11
4		R <sup>1</sup> = Me	"	4.04 $\pm$ 0.29	Not tested
7		R <sup>1</sup> = H	"	0.85 $\pm$ 0.26	1.91 $\pm$ 0.43
8		R <sup>1</sup> = Hex	"	0.49 $\pm$ 0.85	1.93 $\pm$ 0.47
9		R <sup>1</sup> = Me	"	1.33 $\pm$ 0.35	3.23 $\pm$ 1.49
10		R <sup>1</sup> = allyl	"	2.00 $\pm$ 0.25	3.54 $\pm$ 0.70
11		R <sup>1</sup> = Bn	"	1.25 $\pm$ 0.23	1.04 $\pm$ 0.80
12		R <sup>1</sup> = propargyl	"	1.33 $\pm$ 0.35	7.26 $\pm$ 3.12
13		R <sup>1</sup> = CH <sub>2</sub> CF <sub>3</sub>	"	3.25 $\pm$ 0.93	11.43 $\pm$ 0.03
14		R <sup>1</sup> = CH <sub>2</sub> CO <sub>2</sub> <i>t</i> -Bu	"	1.68 $\pm$ 0.32	0.86 $\pm$ 0.74
15		R <sup>1</sup> = CH <sub>2</sub> CN	"	- 1.09 $\pm$ 0.20	- 0.94 $\pm$ 0.02
16		R <sup>1</sup> = CH <sub>2</sub> C(O)Me	"	- 0.68 $\pm$ 0.04	- 0.26 $\pm$ 0.27
17		R <sup>1</sup> = (CH <sub>2</sub> ) <sub>2</sub> CO <sub>2</sub> Me	"	0.44 $\pm$ 0.06	- 0.31 $\pm$ 0.01
18		R <sup>2</sup> = CO <sub>2</sub> All	"	0.49 $\pm$ 0.85	16.80 $\pm$ 0.09
19		R <sup>2</sup> = CO <sub>2</sub> <i>t</i> -Bu	"	- 1.25 $\pm$ 0.23	15.63 $\pm$ 0.43
20		R <sup>2</sup> = CO <sub>2</sub> H	"	1.19 $\pm$ 0.29	1.12 $\pm$ 0.21
23		R <sup>2</sup> = Cl	"	1.19 $\pm$ 0.29	1.12 $\pm$ 0.21
24		R <sup>2</sup> = I	"	1.19 $\pm$ 0.29	1.12 $\pm$ 0.21
25		R <sup>2</sup> = CH <sub>2</sub> OH	"	0.85 $\pm$ 0.26	1.91 $\pm$ 0.43
26		R <sup>2</sup> =	"	2.28 $\pm$ 0.29	4.37 $\pm$ 1.07
21			"	1.07 $\pm$ 0.35	2.63 $\pm$ 0.60
27		R <sup>3</sup> = H	"	1.07 $\pm$ 0.35	4.31 $\pm$ 2.28
28		R <sup>3</sup> = Me	"	1.19 $\pm$ 0.29	1.12 $\pm$ 0.21
29			"	2.28 $\pm$ 0.29	3.20 $\pm$ 0.45
30		R <sup>4</sup> = CH <sub>2</sub>	"	2.28 $\pm$ 0.29	24.39 $\pm$ 1.16
31		R <sup>4</sup> = (CH <sub>2</sub> ) <sub>3</sub>	"	2.62 $\pm$ 0.01	35.61 $\pm$ 12.02
32		R <sup>4</sup> = (CH <sub>2</sub> ) <sub>4</sub>	2.68 $\pm$ 0.05	Not tested	Not tested
33		R <sup>4</sup> =	1.36 $\pm$ 0.27	Not tested	Not tested
34		R <sup>4</sup> =	0.80 $\pm$ 0.04	Not tested	Not tested
35			Not tested	3.48 $\pm$ 0.27	11.78 $\pm$ 1.65
36		36 n = 1 37 n = 3	"	3.91 $\pm$ 0.06	9.43 $\pm$ 0.61
37			"	3.38 $\pm$ 2.01	15.24 $\pm$ 2.21

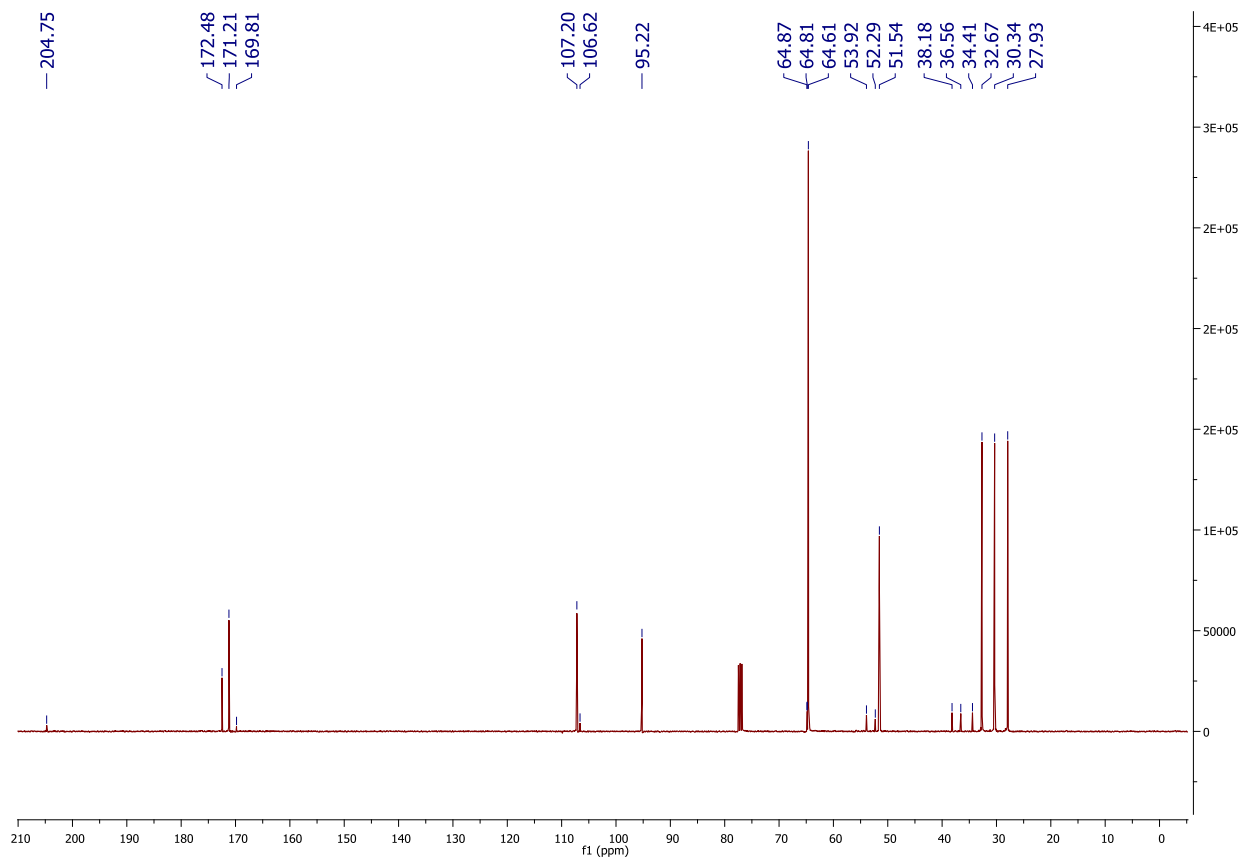
<sup>a</sup> The data are reported as Mean  $\pm$  SEM based on two separated experiments

# Methyl 8-oxo-1,4-dioxaspiro[4.5]decane-7-carboxylate (2)

## <sup>1</sup>H NMR spectrum

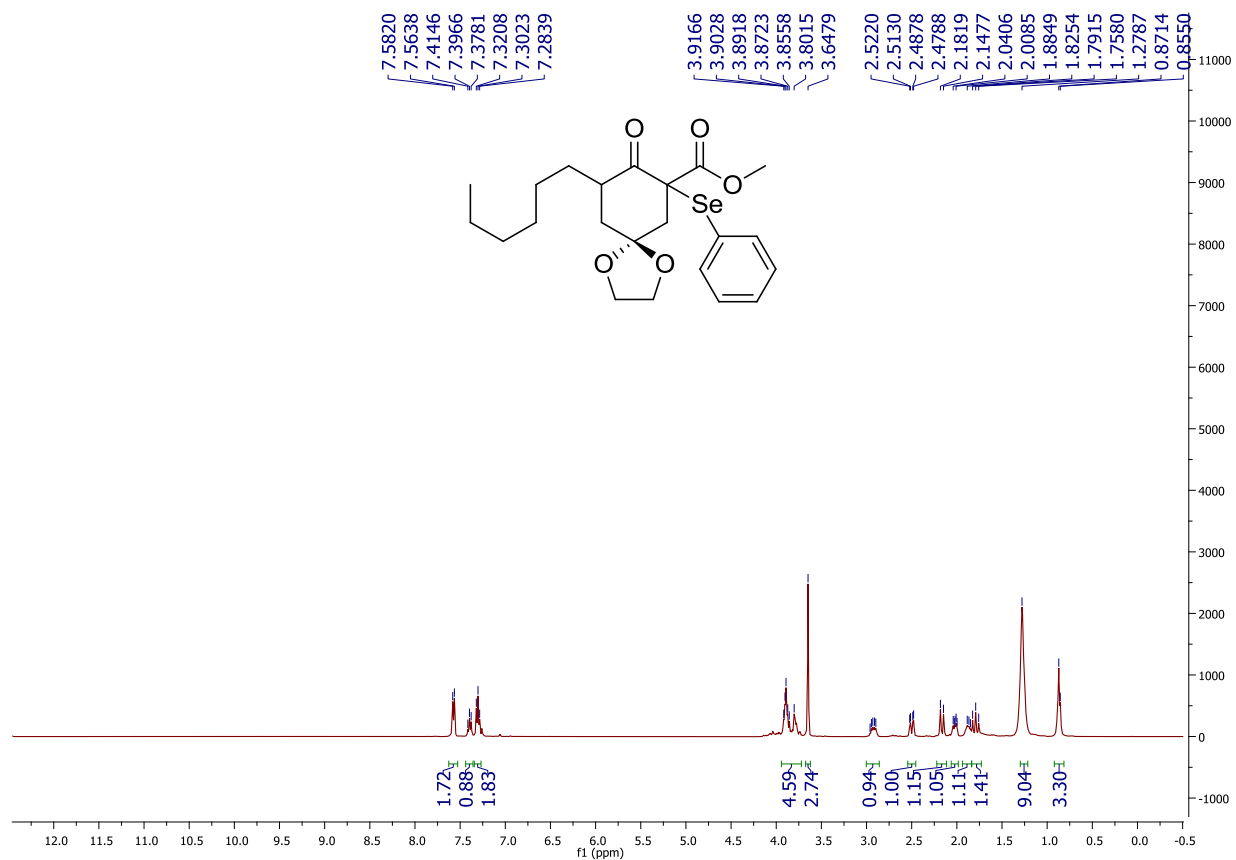


## <sup>13</sup>C NMR spectrum

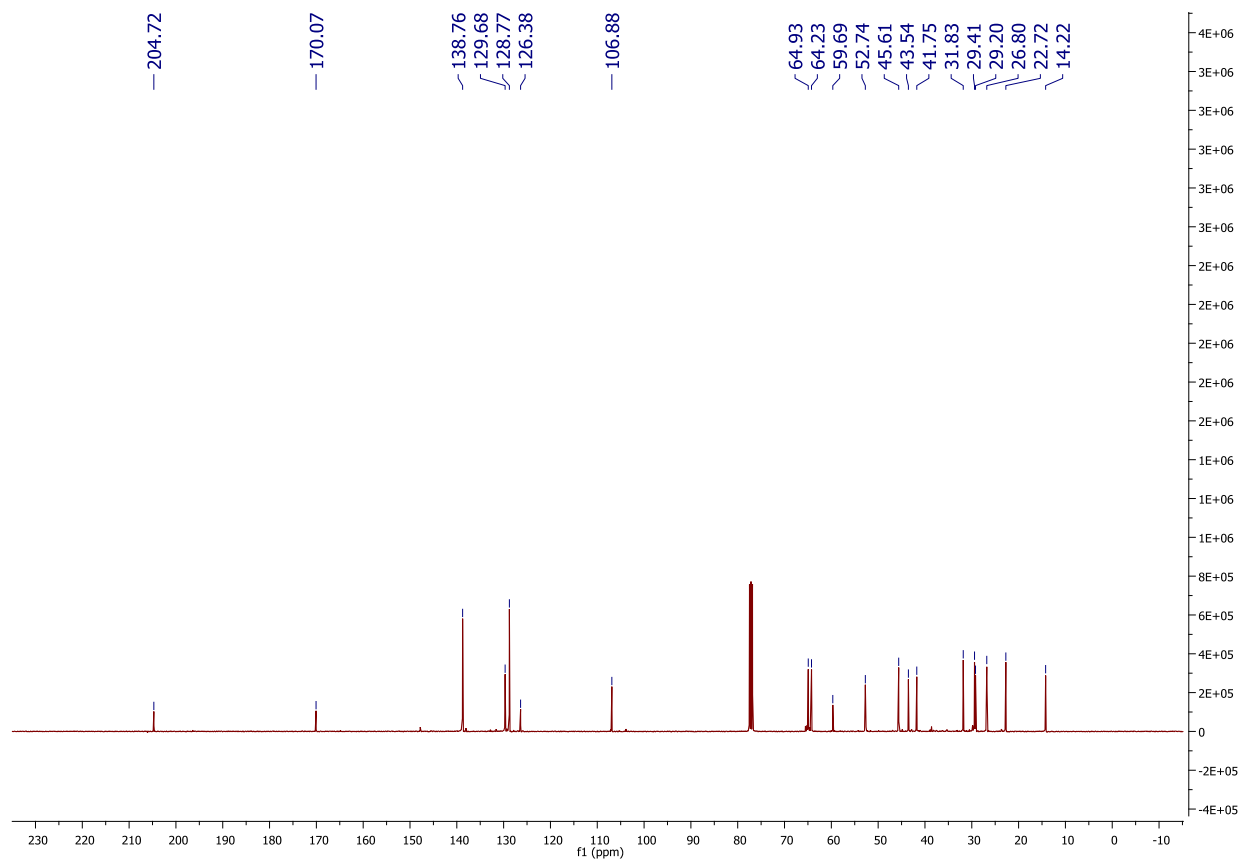


# Methyl 9-methyl-8-oxo-1,4-dioxaspiro[4.5]decane-7-carboxylate

## <sup>1</sup>H NMR spectrum

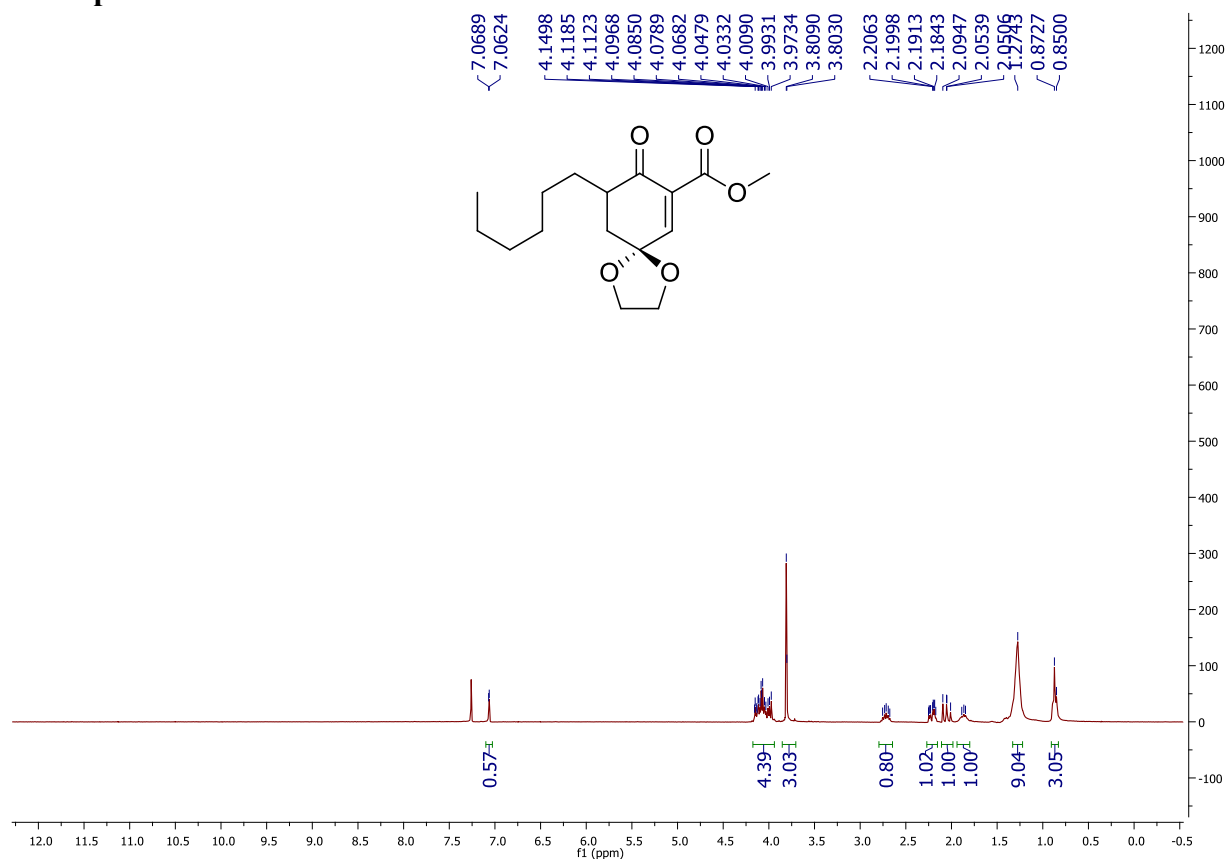


## <sup>13</sup>C NMR spectrum

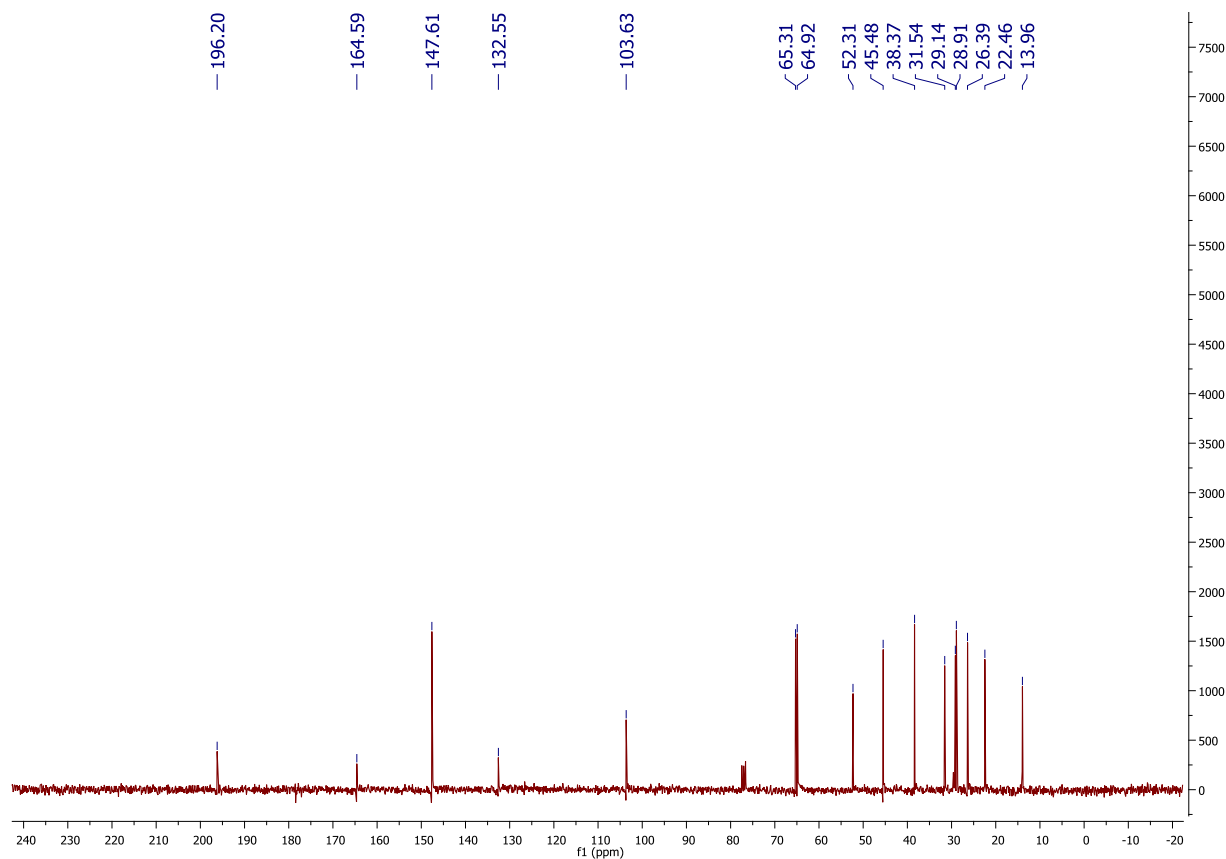


# Methyl 9-hexyl-8-oxo-1,4-dioxaspiro[4.5]dec-6-ene-7-carboxylate (3)

## <sup>1</sup>H NMR spectrum

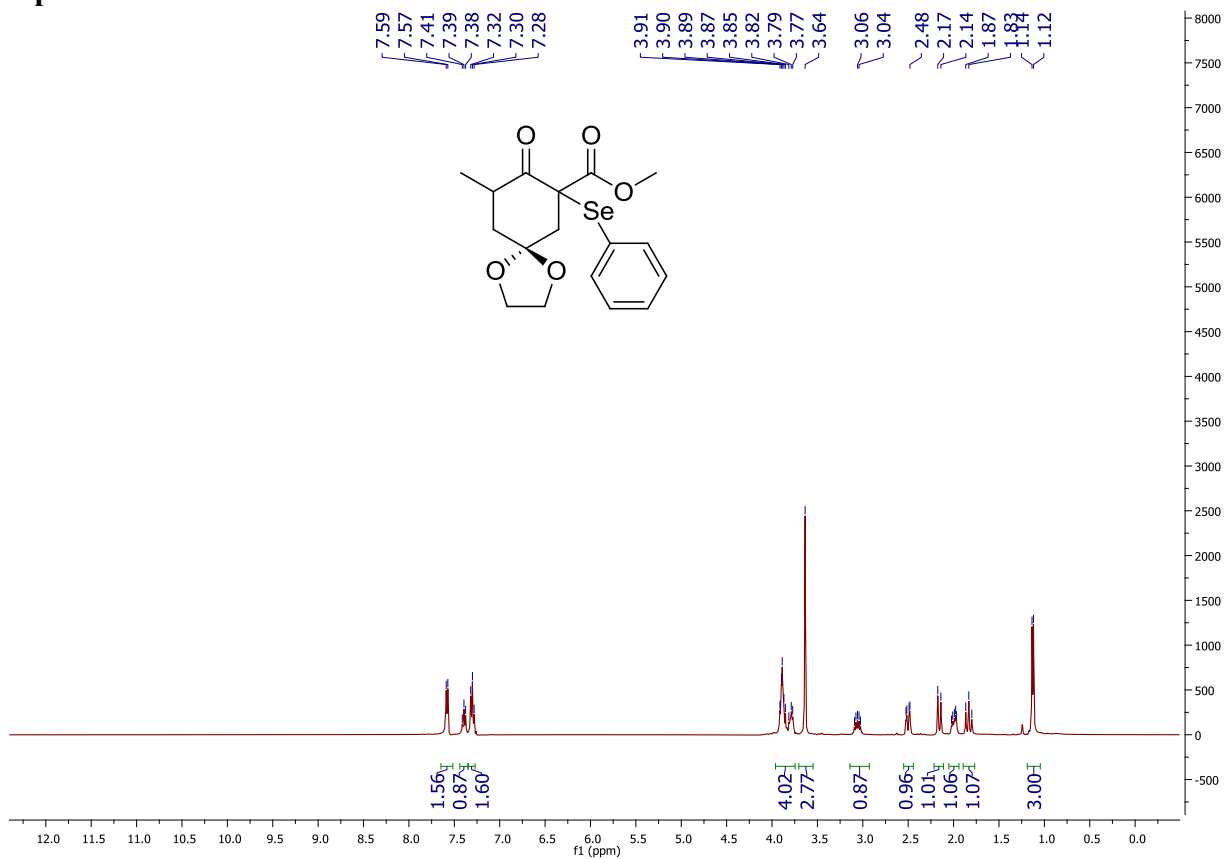


## <sup>13</sup>C NMR spectrum

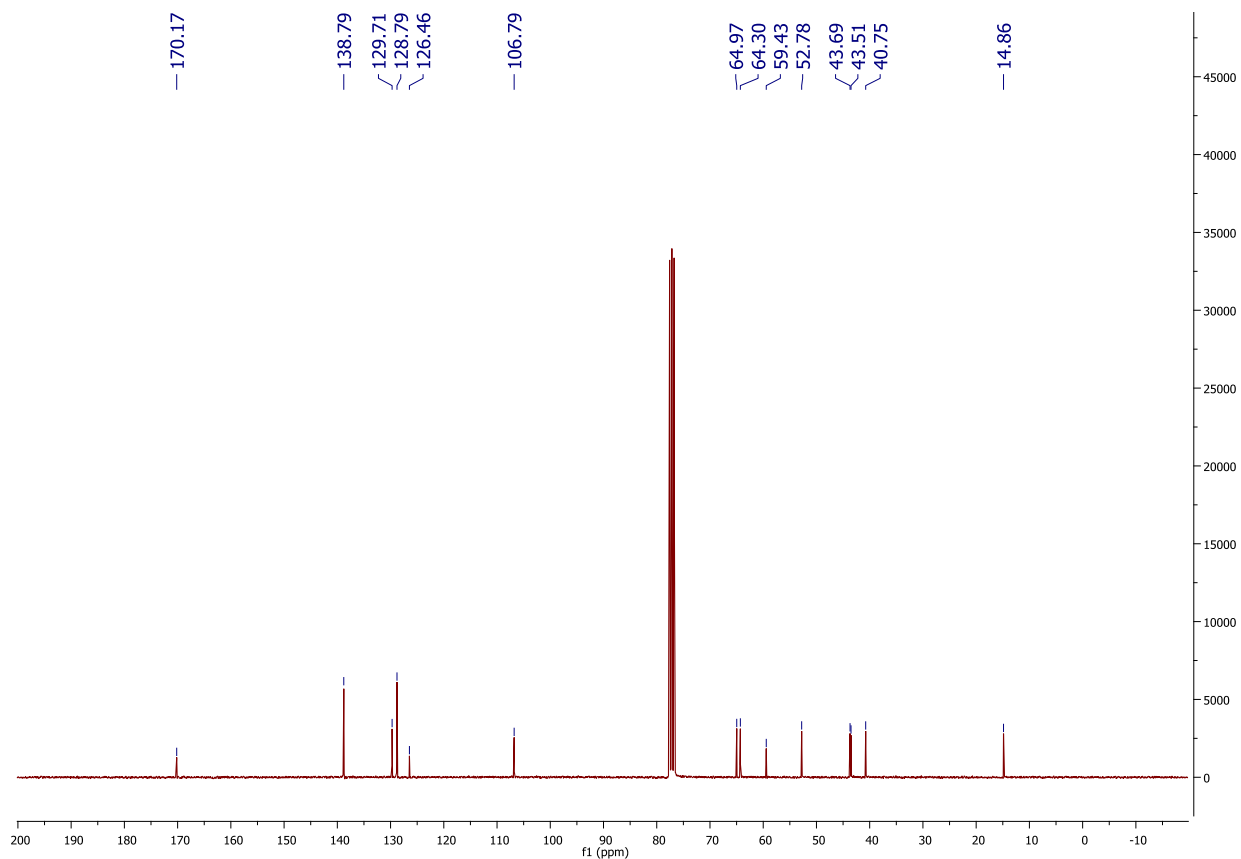


# Methyl 9-methyl-8-oxo-1,4-dioxaspiro[4.5]decane-7-carboxylate

## <sup>1</sup>H NMR spectrum

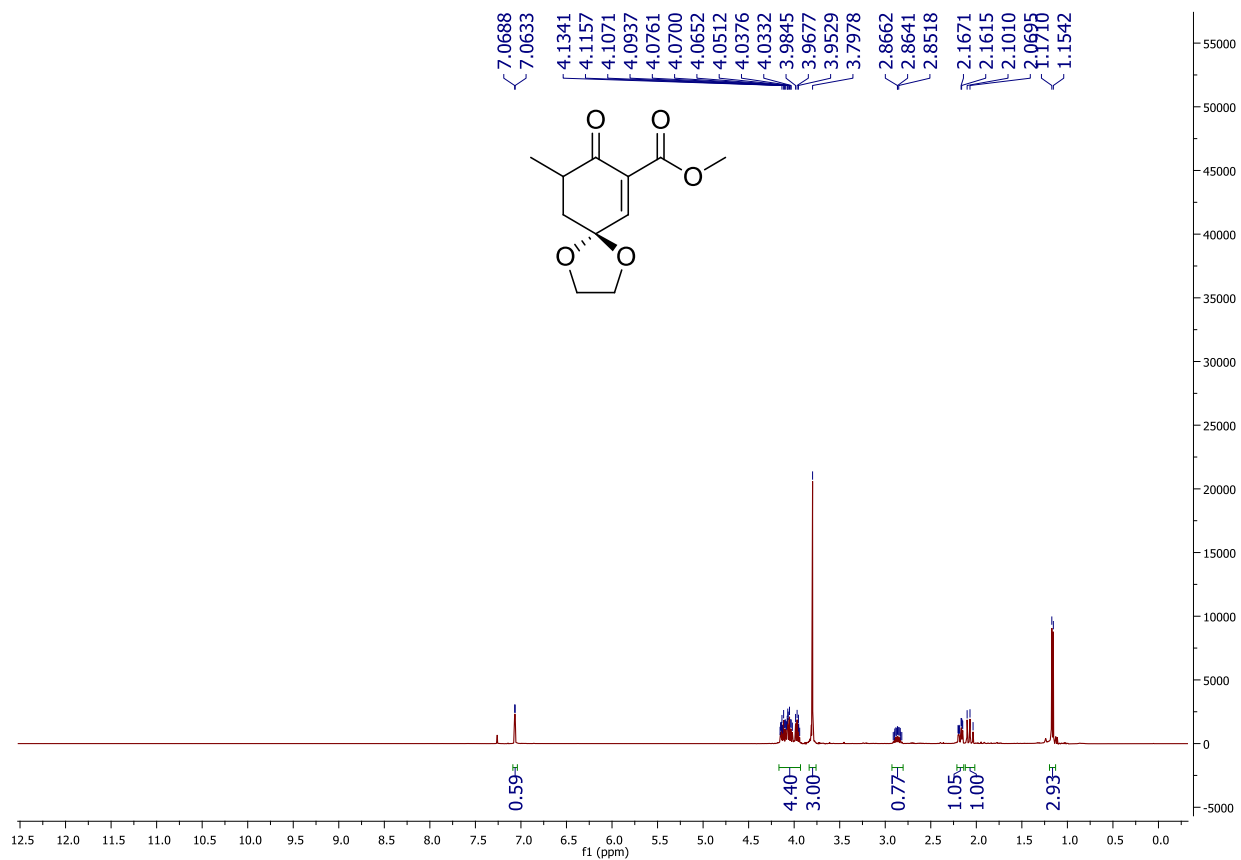


## <sup>13</sup>C NMR spectrum

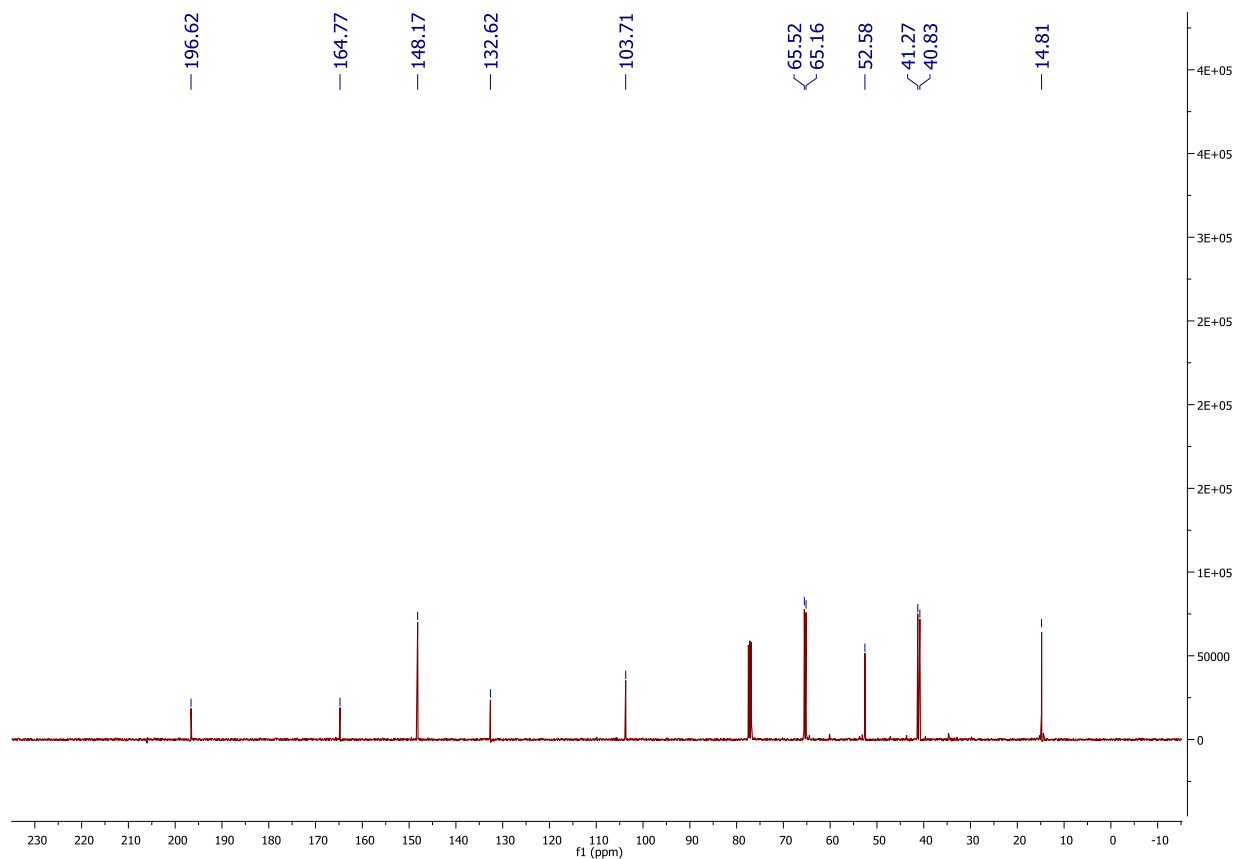


# Methyl 9-methyl-8-oxo-1,4-dioxaspiro[4.5]dec-6-ene-7-carboxylate (4)

## <sup>1</sup>H NMR spectrum



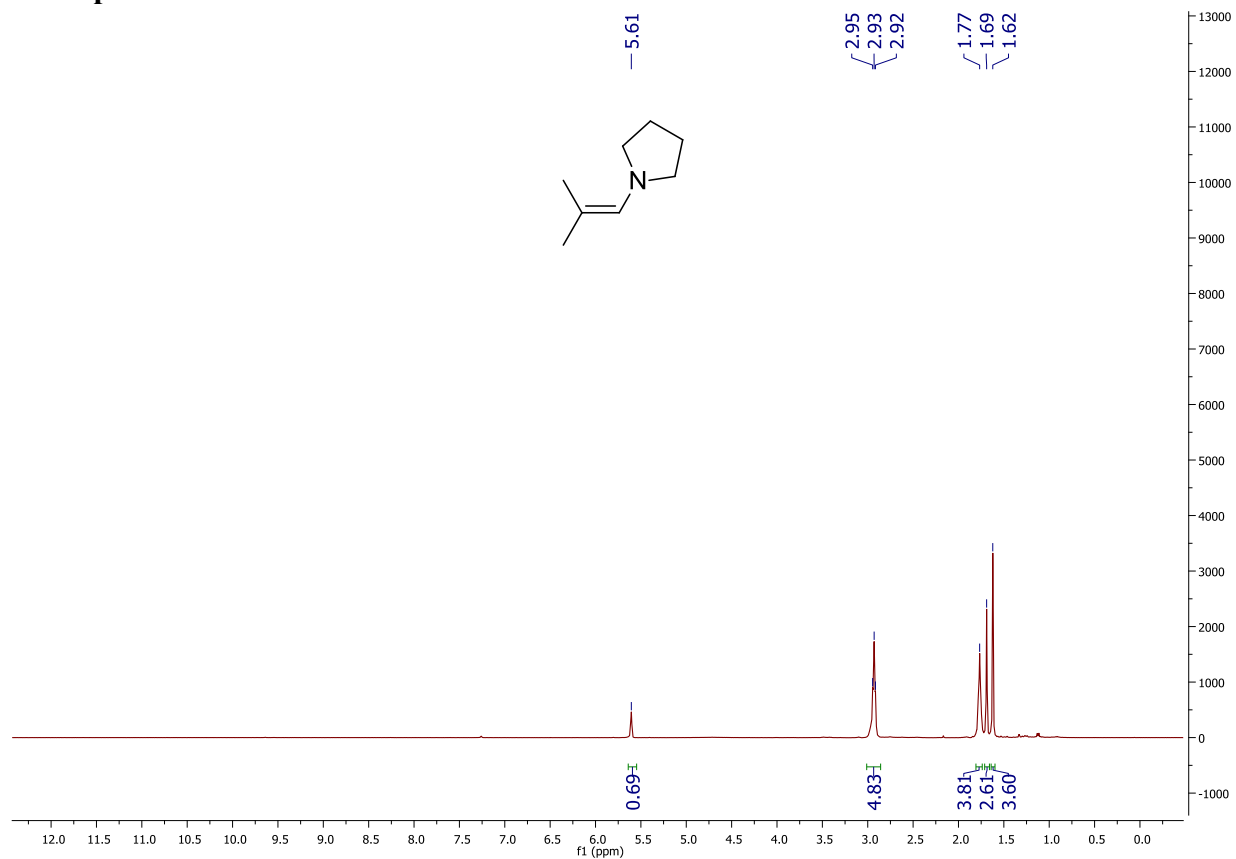
## <sup>13</sup>C NMR spectrum





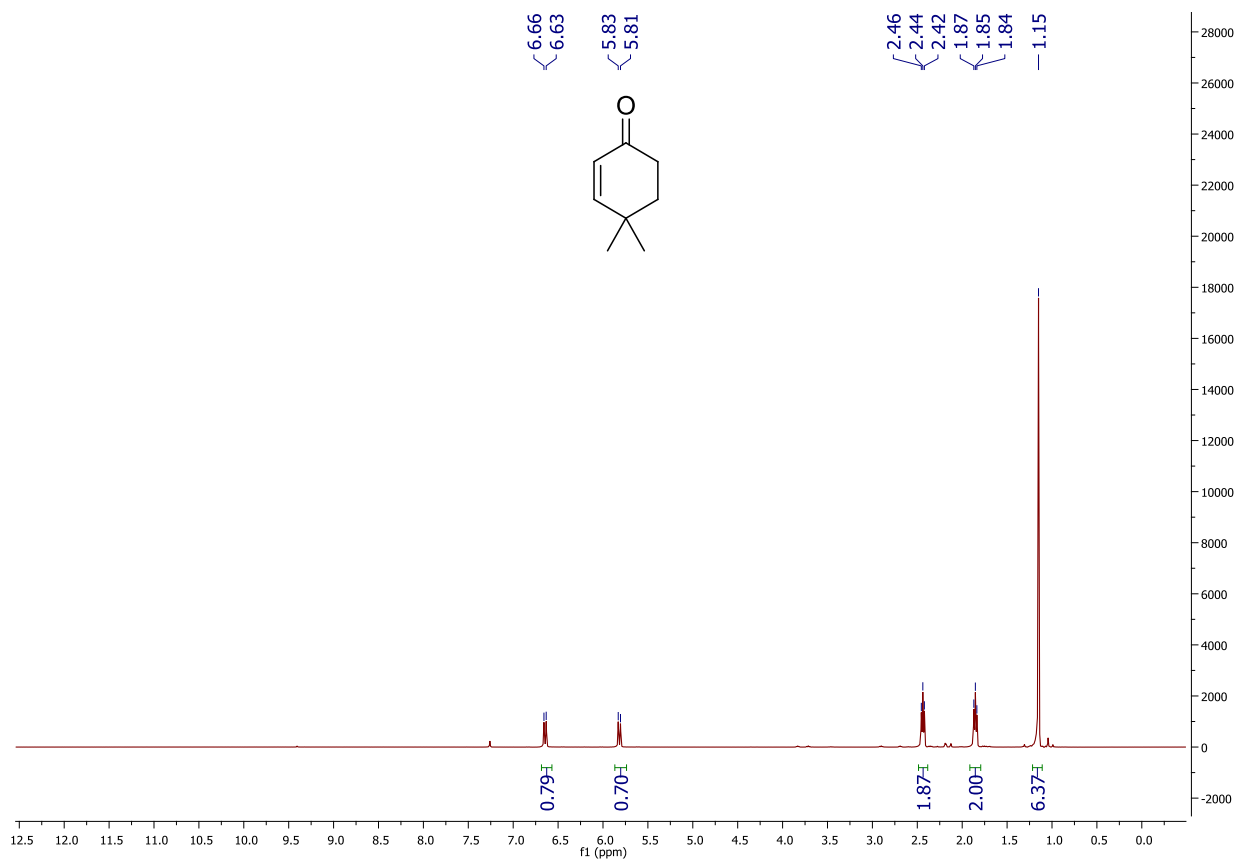
# 1-(2-methylprop-1-en-1-yl)pyrrolidine

## <sup>1</sup>H NMR spectrum



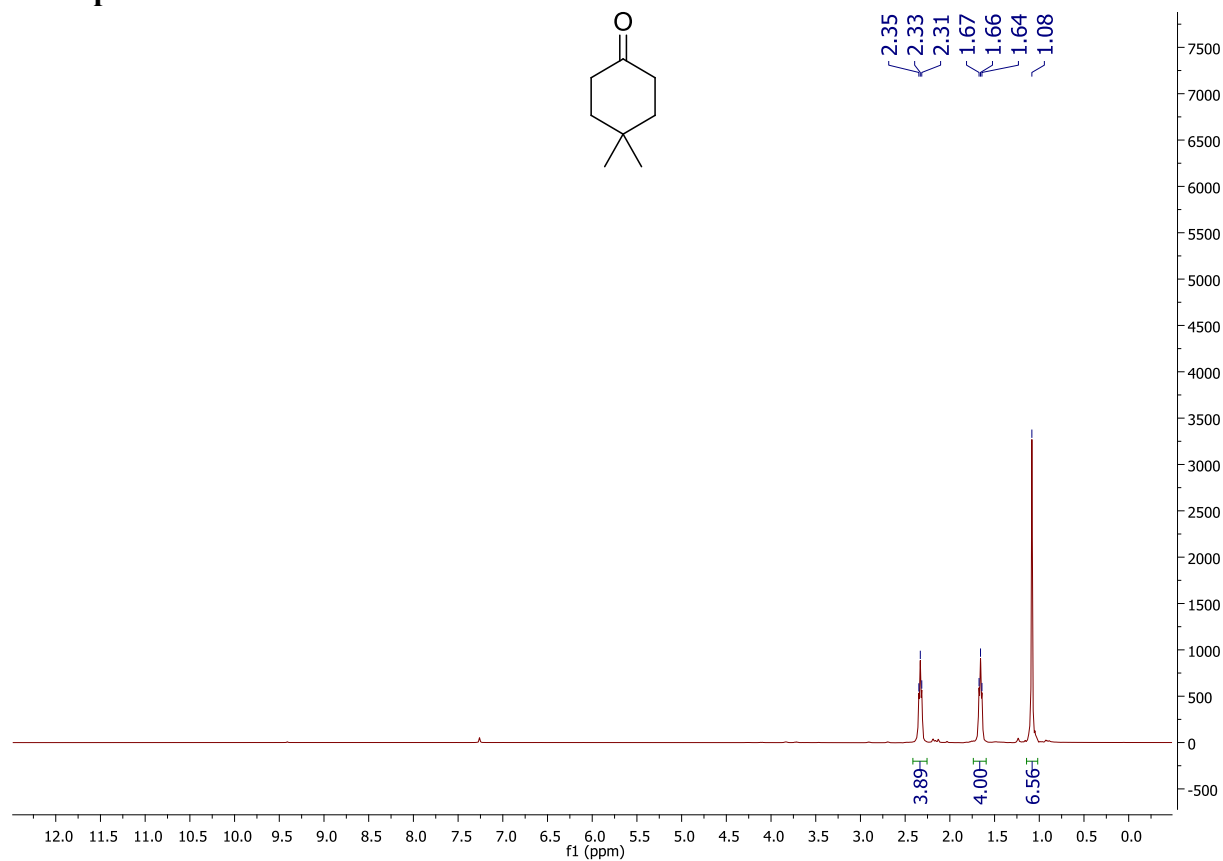
# 4,4-dimethylcyclohex-2-enone (22)

## <sup>1</sup>H NMR spectrum

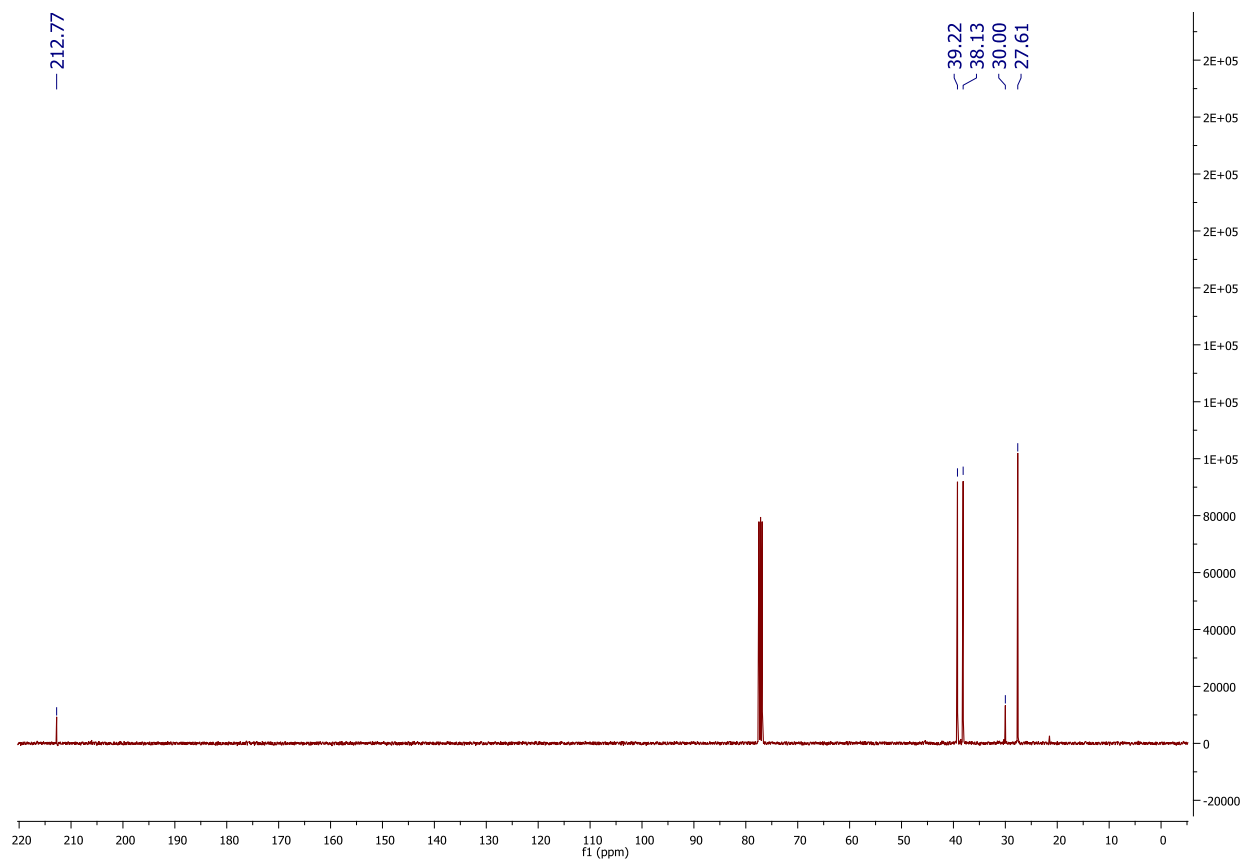


# 4,4-dimethylcyclohexanone (5)

## <sup>1</sup>H NMR spectrum

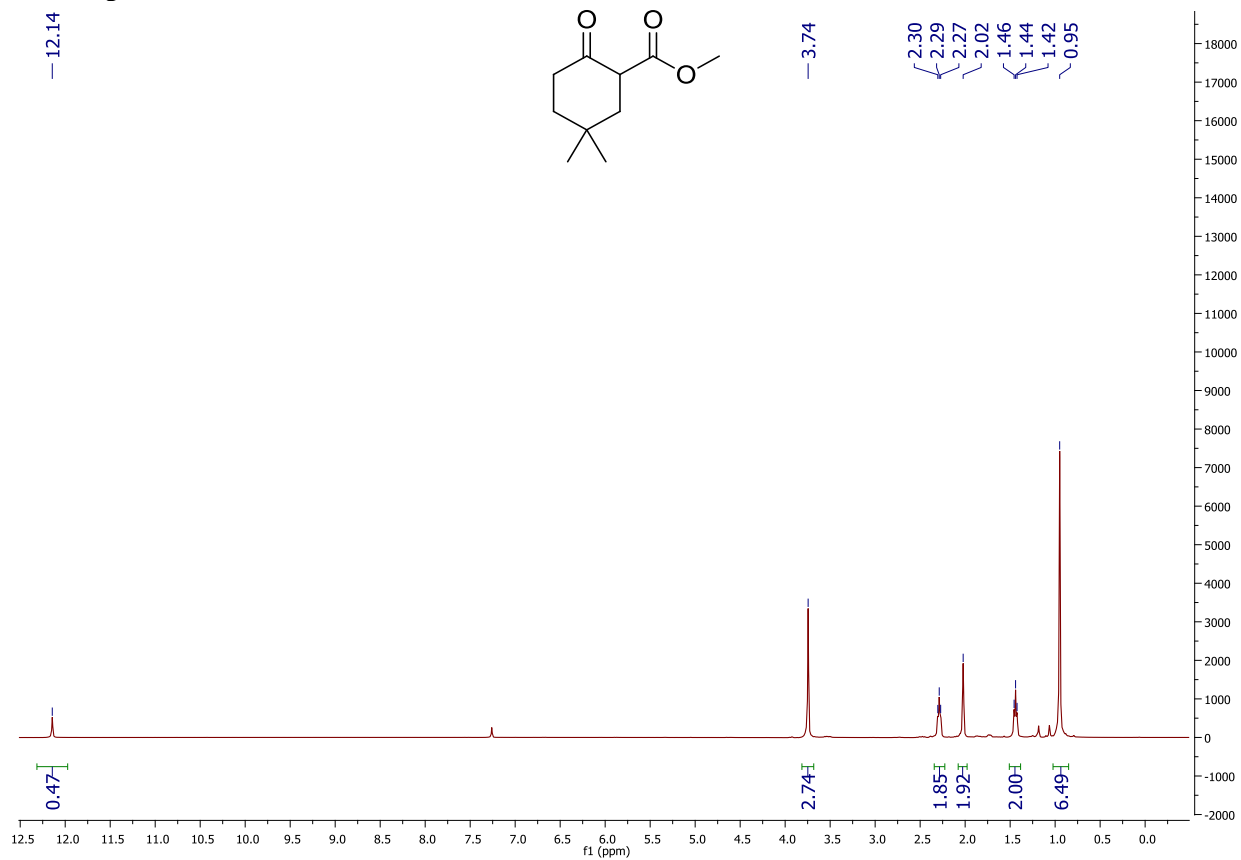


## <sup>13</sup>C NMR spectrum



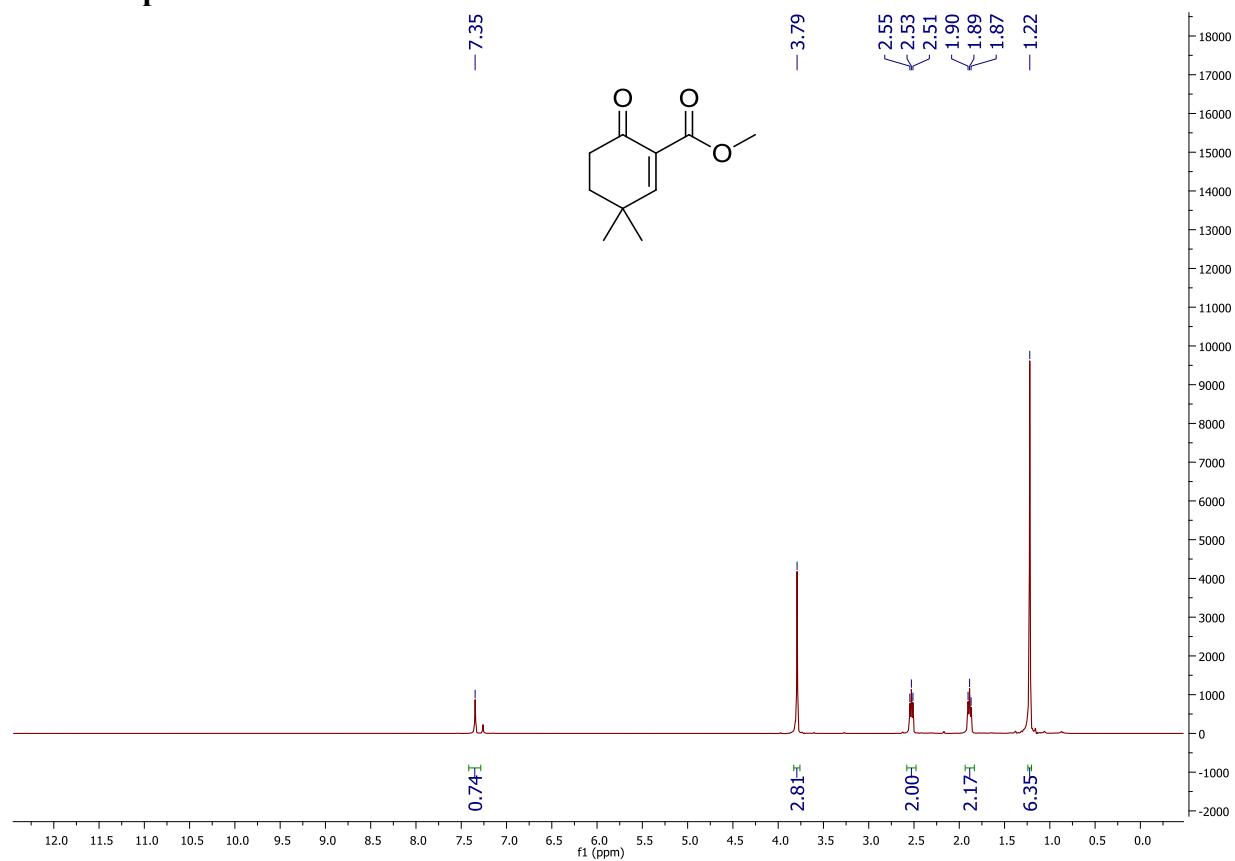
# Methyl 5,5-dimethyl-2-oxocyclohexanecarboxylate (6)

## <sup>1</sup>H NMR spectrum

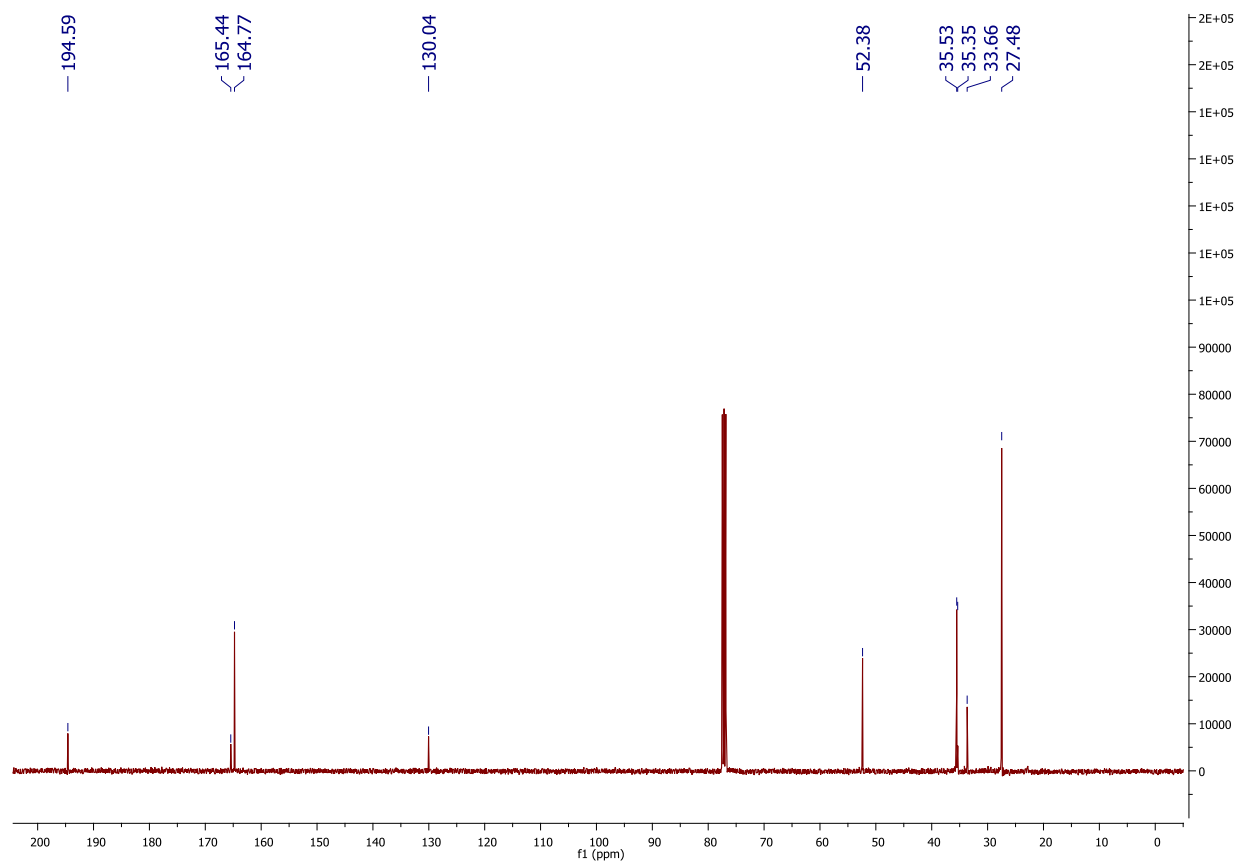


# Methyl 3,3-dimethyl-6-oxocyclohex-1-enecarboxylate (7)

## <sup>1</sup>H NMR spectrum

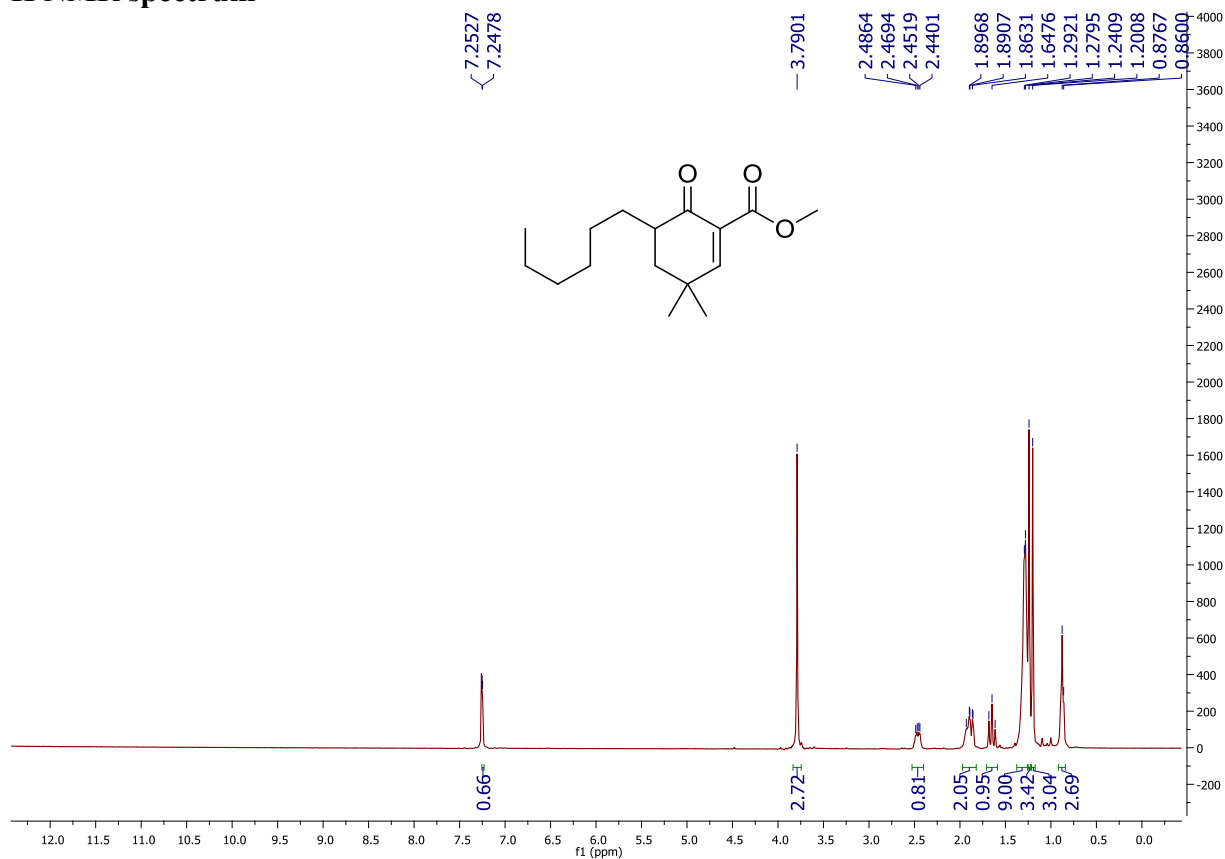


## <sup>13</sup>C NMR spectrum

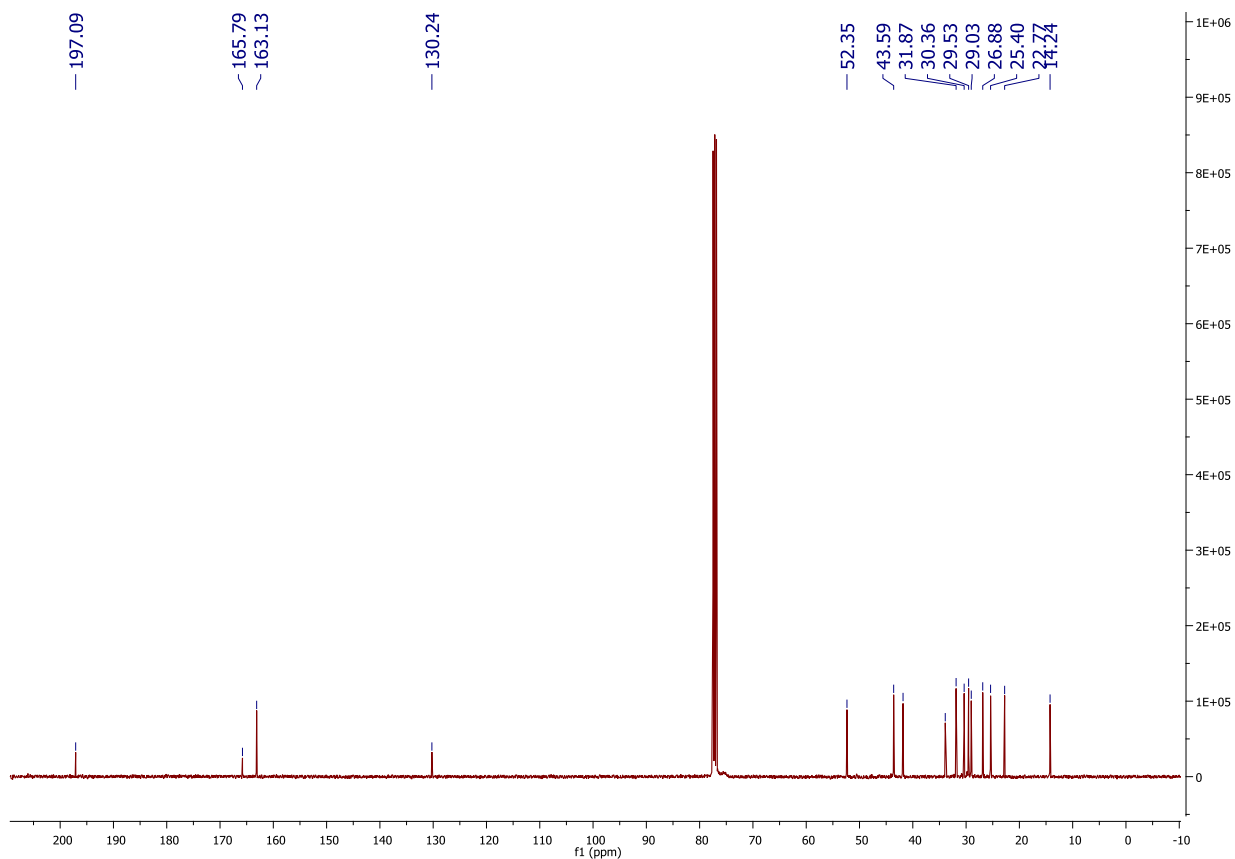


# Methyl 5-hexyl-3,3-dimethyl-6-oxocyclohex-1-enecarboxylate (8)

## <sup>1</sup>H NMR spectrum

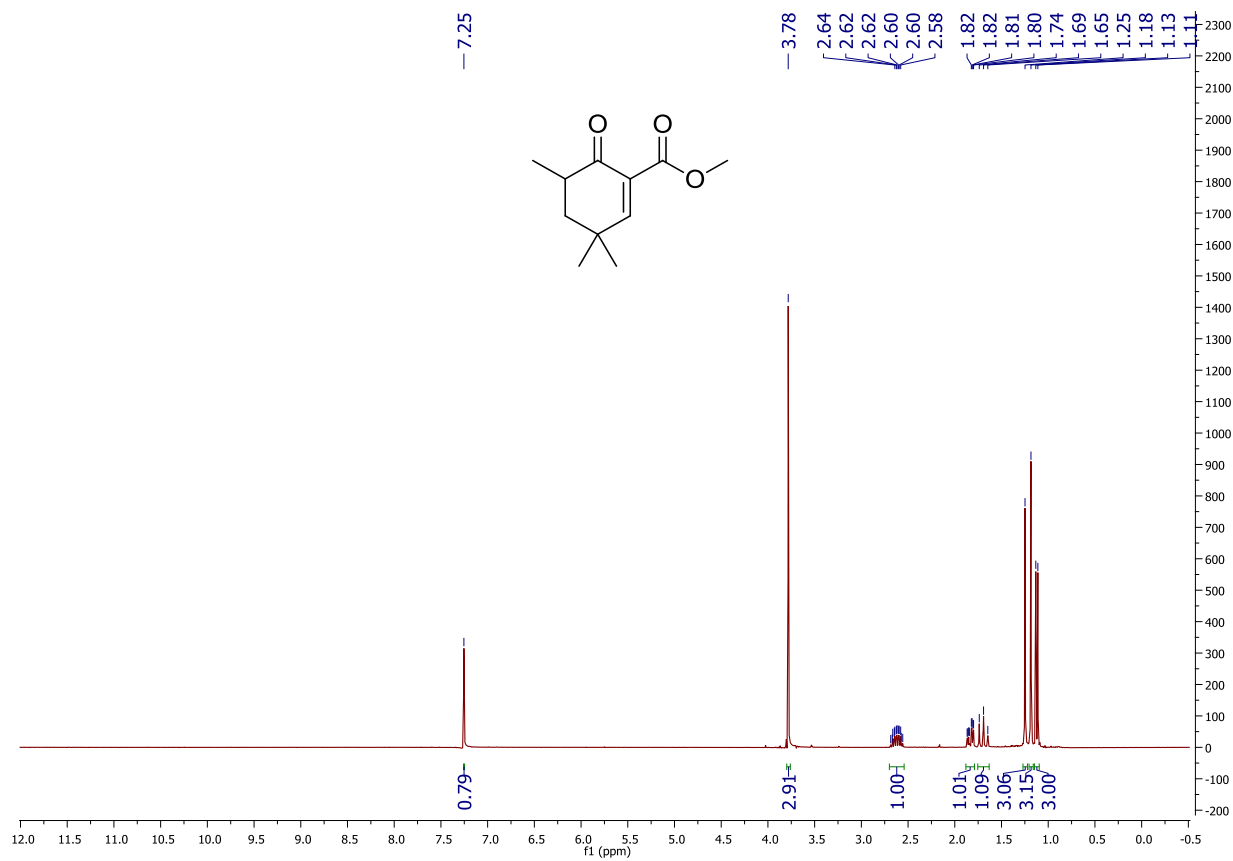


## <sup>13</sup>C NMR spectrum

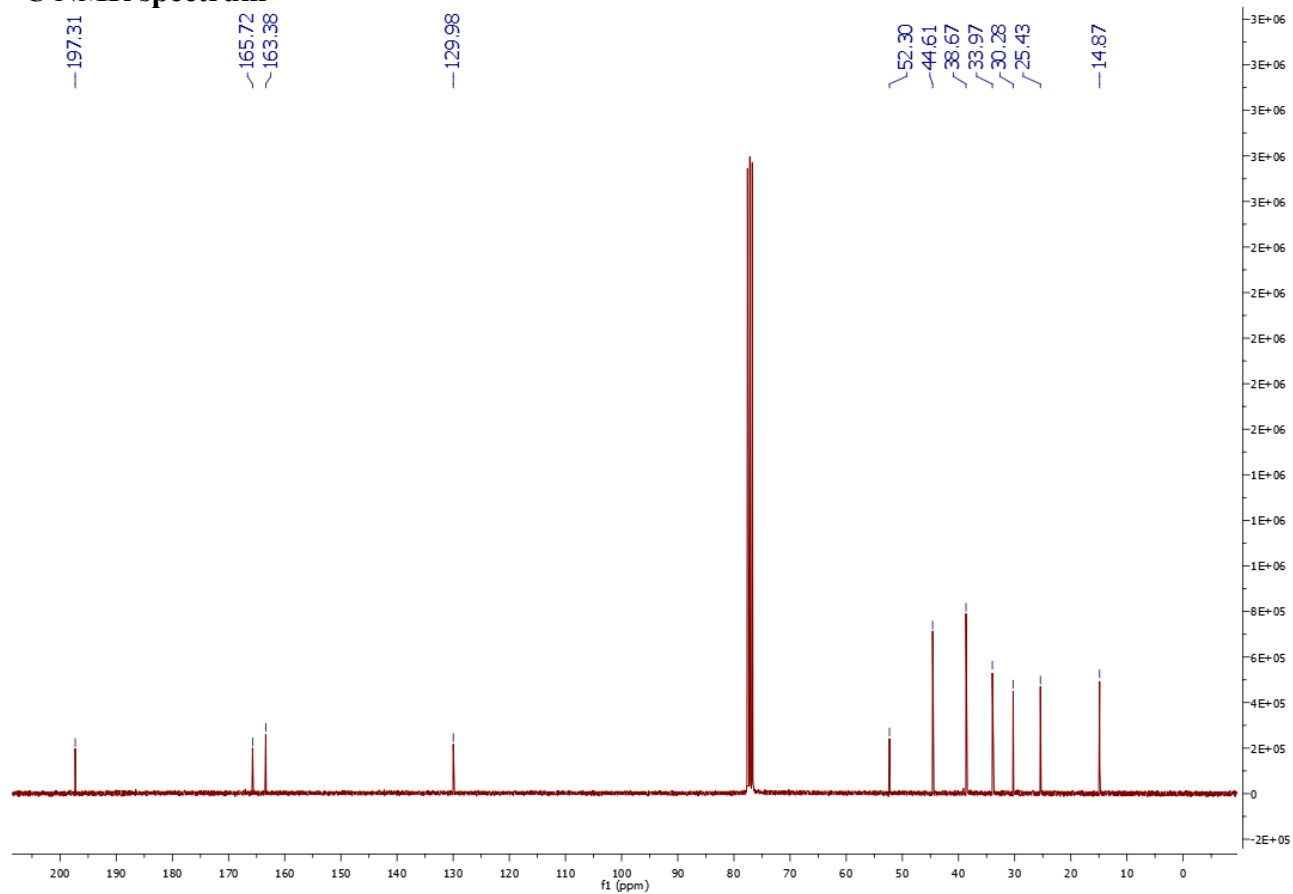


# Methyl 3,3,5-trimethyl-6-oxocyclohex-1-enecarboxylate (9)

## <sup>1</sup>H NMR spectrum

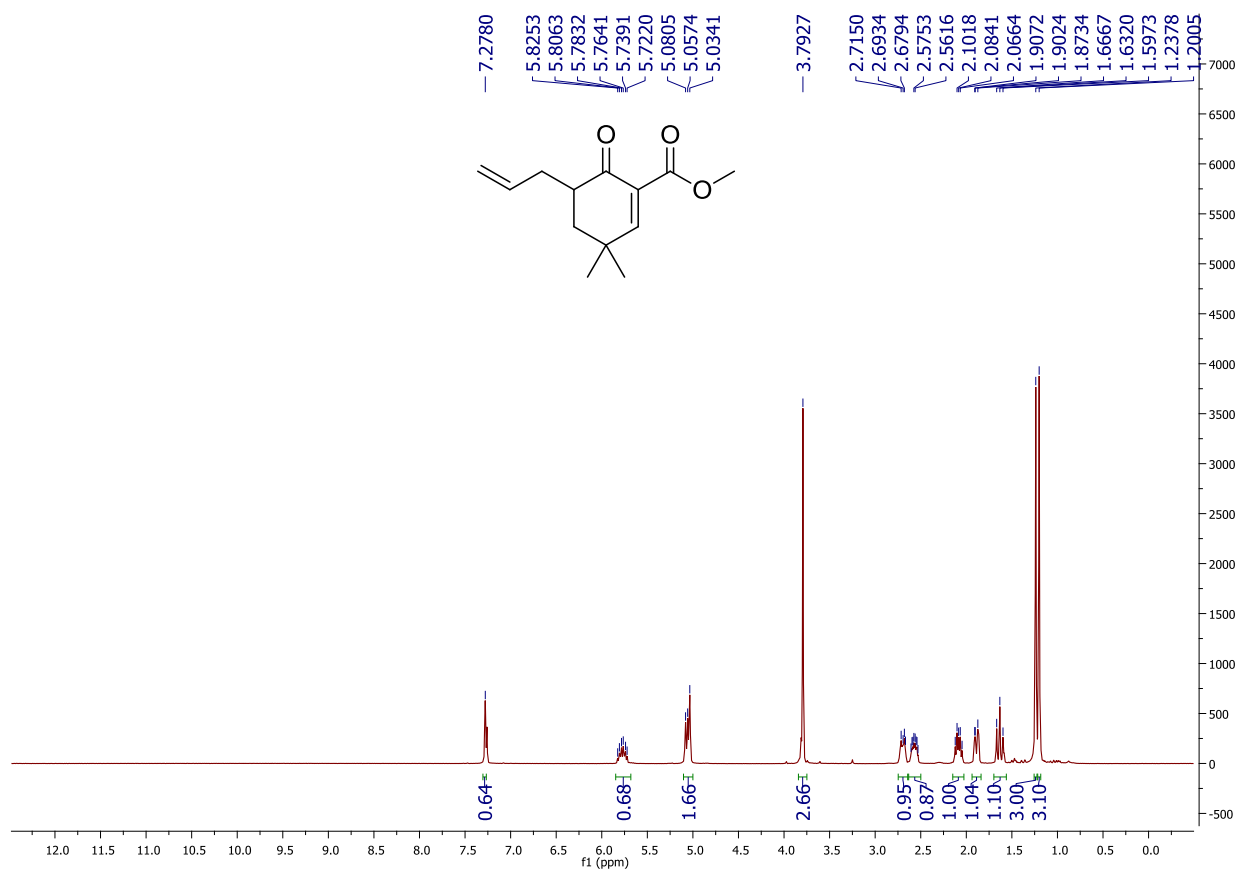


## <sup>13</sup>C NMR spectrum



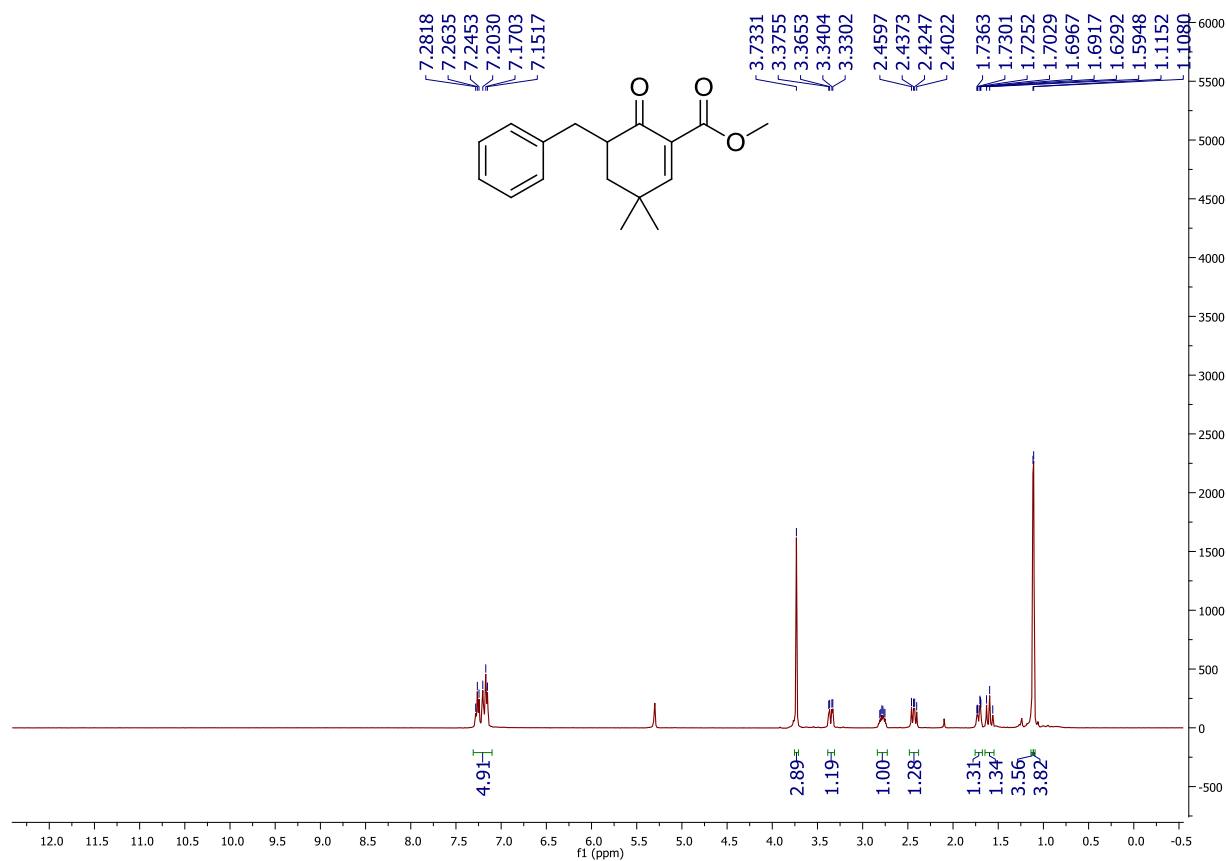
# Methyl 5-allyl-3,3-dimethyl-6-oxocyclohex-1-enecarboxylate (10)

## <sup>1</sup>H NMR spectrum

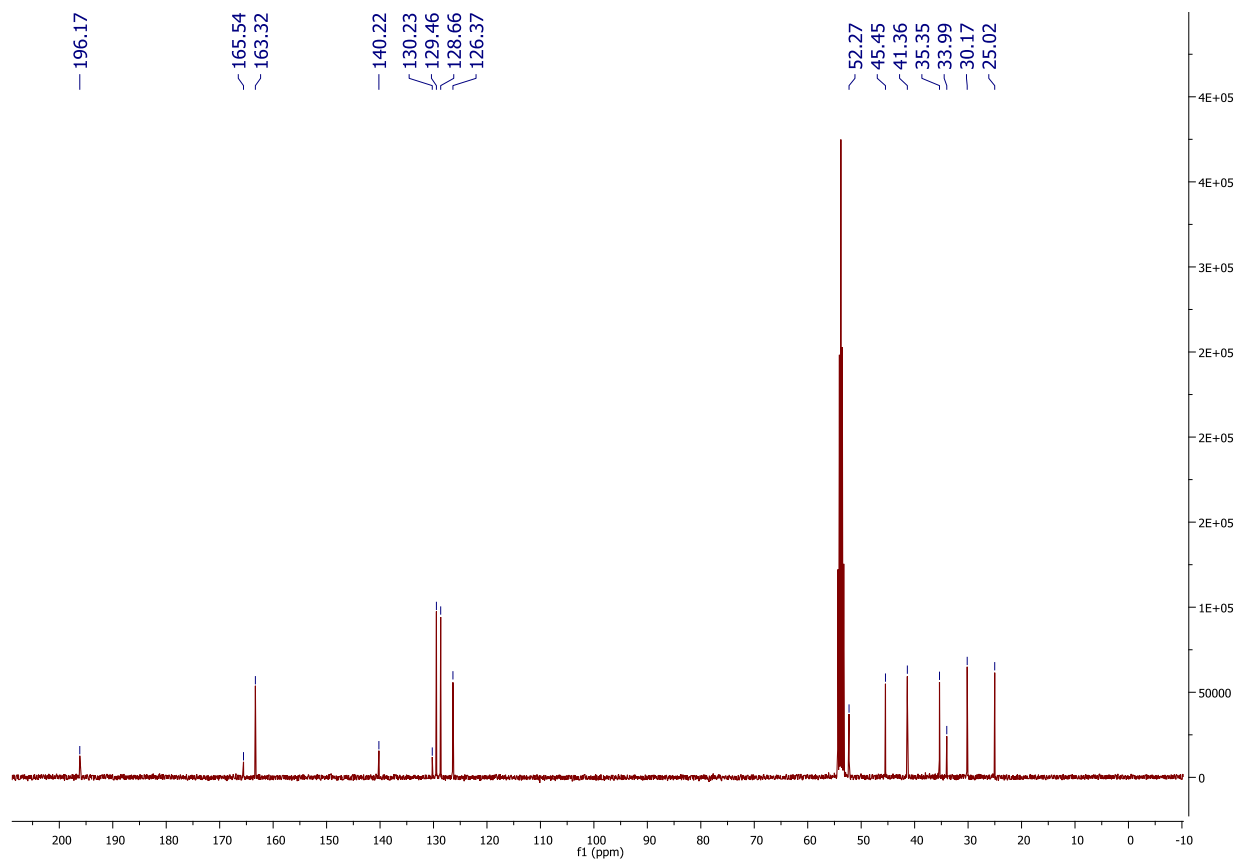


# Methyl 5-benzyl-3,3-dimethyl-6-oxocyclohex-1-enecarboxylate (11)

## <sup>1</sup>H NMR spectrum



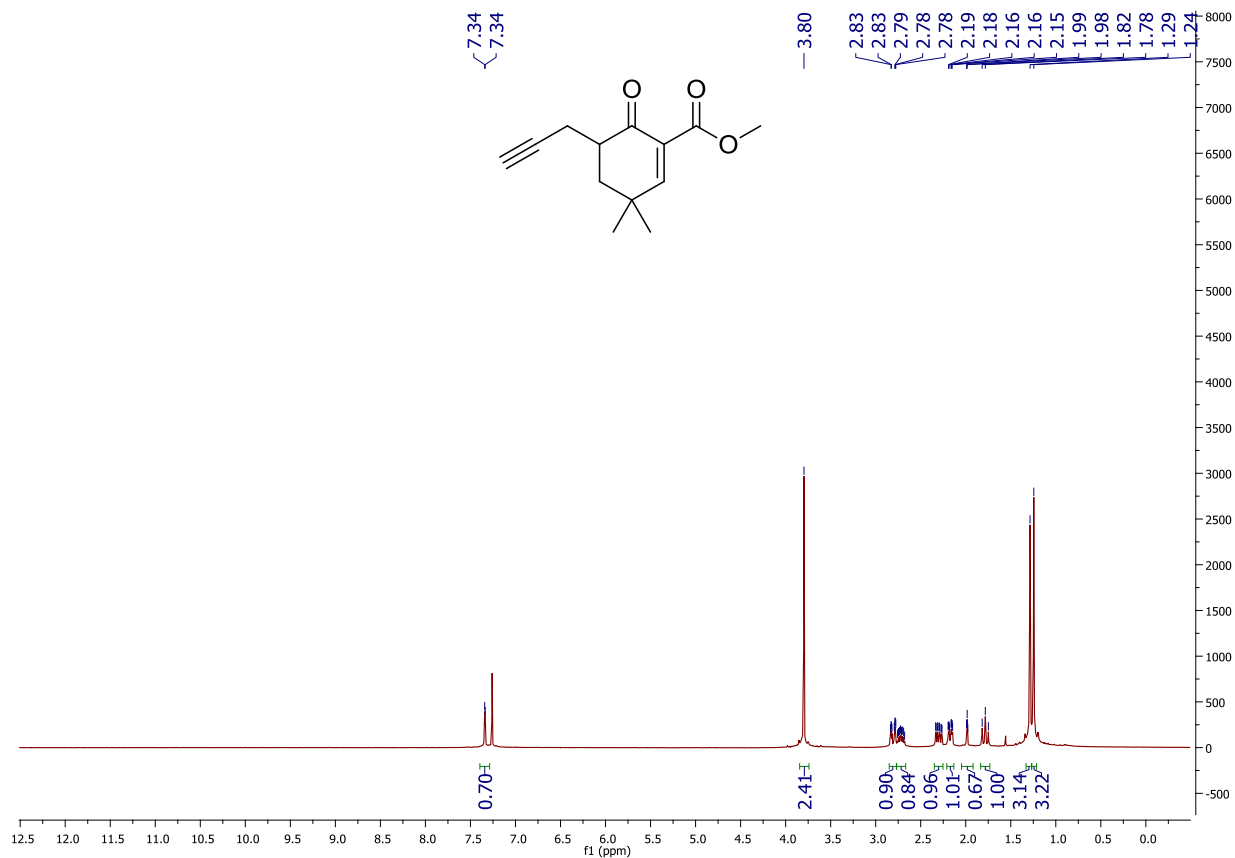
## <sup>13</sup>C NMR spectrum



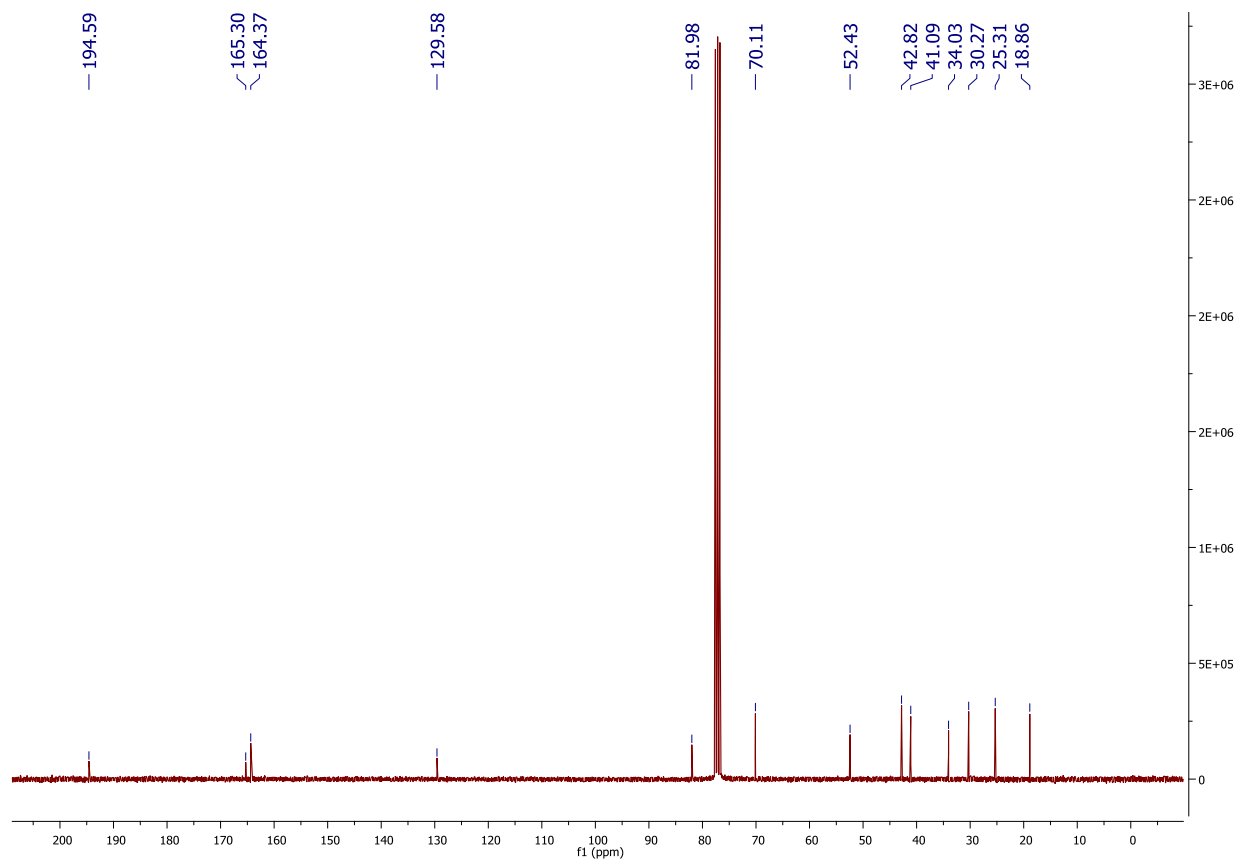


# Methyl 3,3-dimethyl-6-oxo-5-(prop-2-yn-1-yl)cyclohex-1-enecarboxylate (12)

## <sup>1</sup>H NMR spectrum

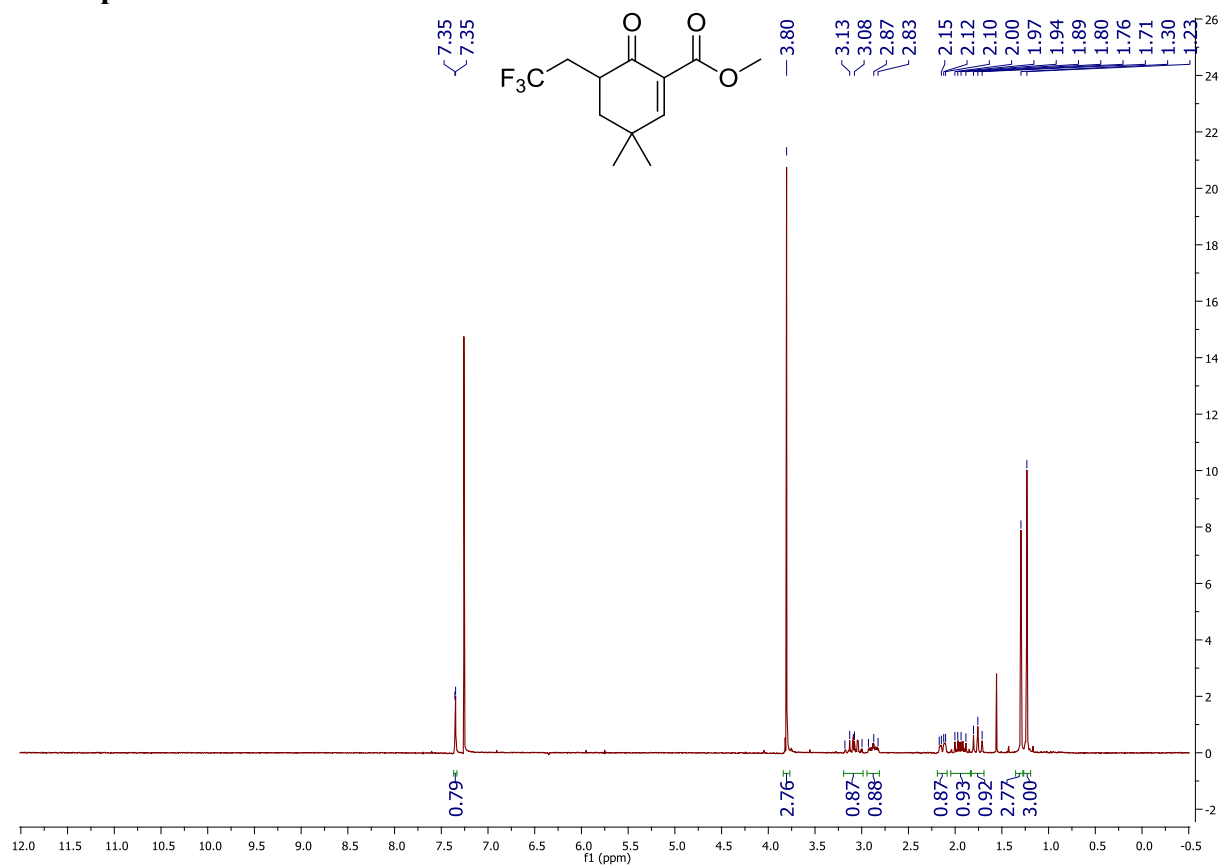


## <sup>13</sup>C NMR spectrum

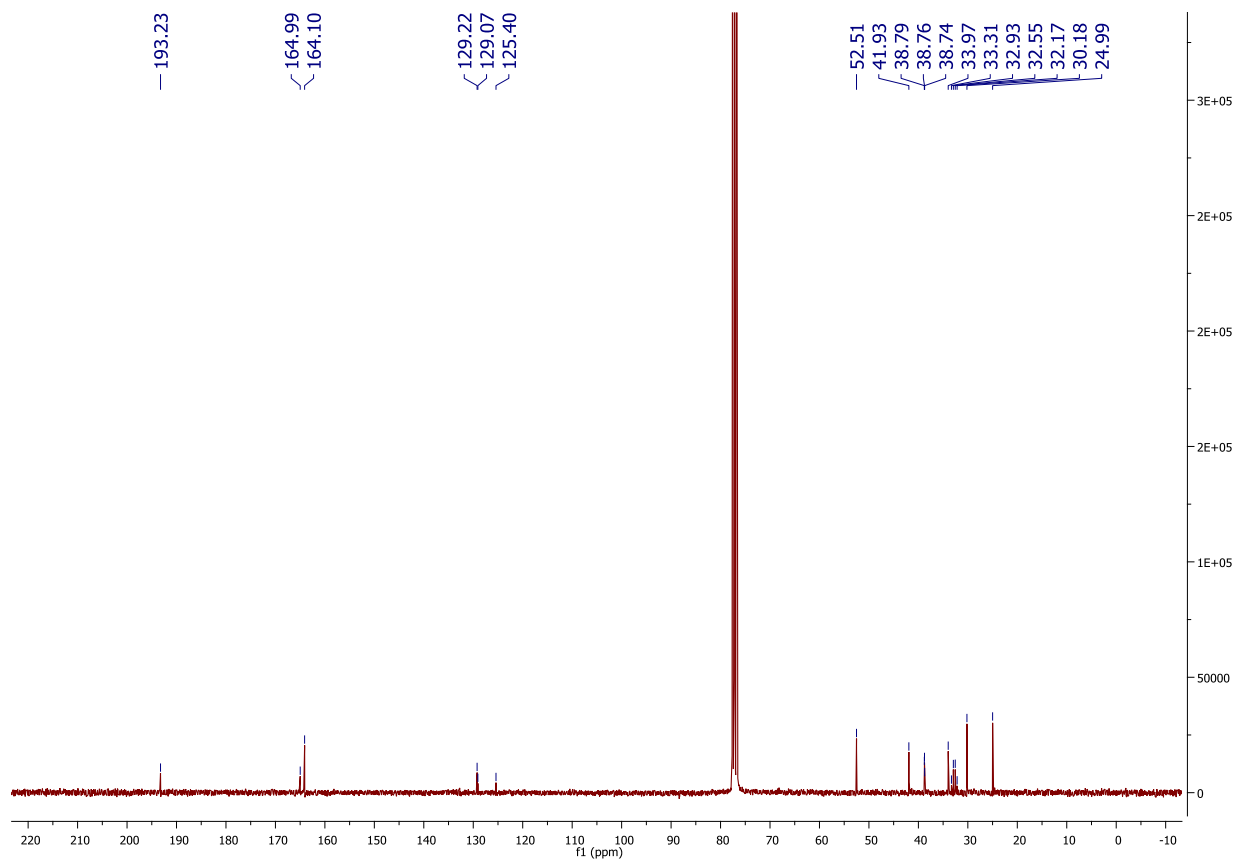


# Methyl 3,3-dimethyl-6-oxo-5-(2,2,2-trifluoroethyl)cyclohex-1-enecarboxylate (13)

## <sup>1</sup>H NMR spectrum

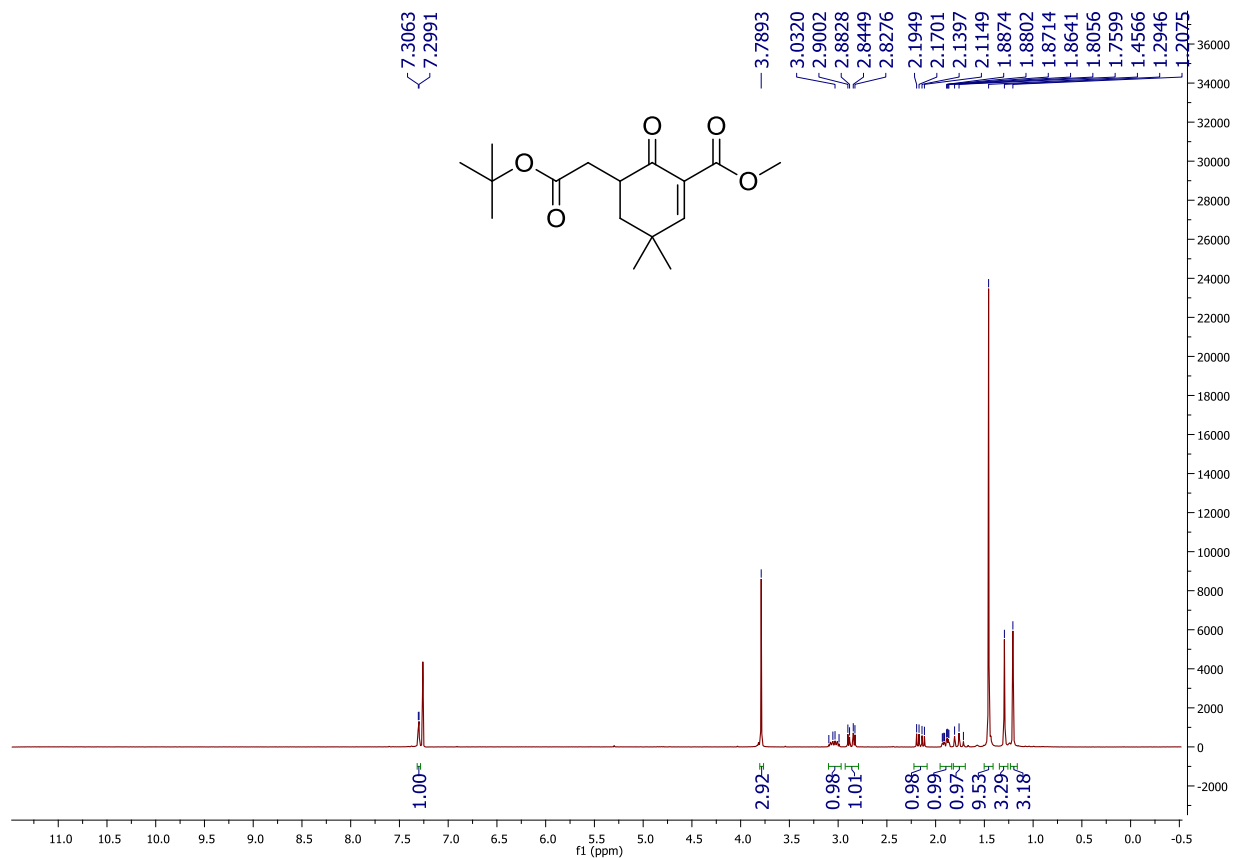


## <sup>13</sup>C NMR spectrum

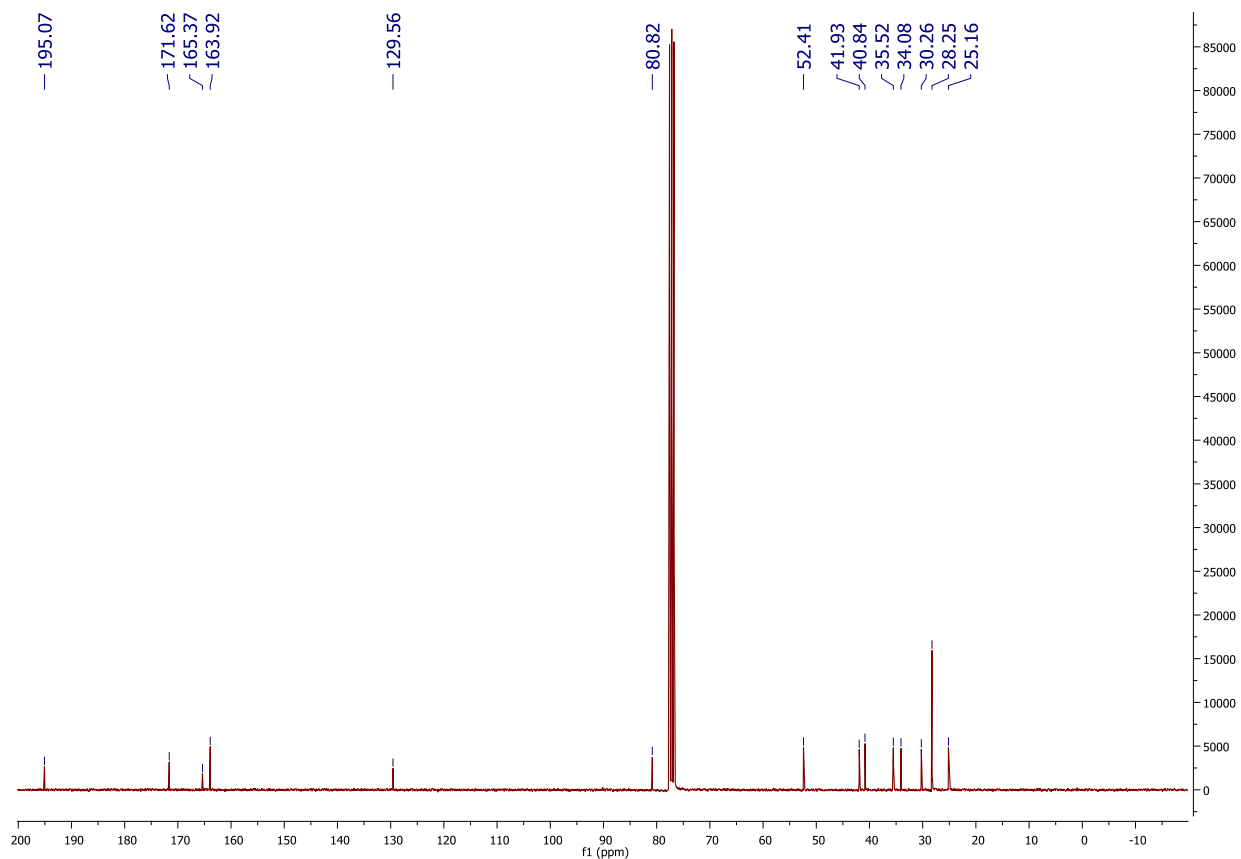


# Methyl 5-(2-(tert-butoxy)-2-oxoethyl)-3,3-dimethyl-6-oxocyclohex-1-enecarboxylate (14)

## <sup>1</sup>H NMR spectrum

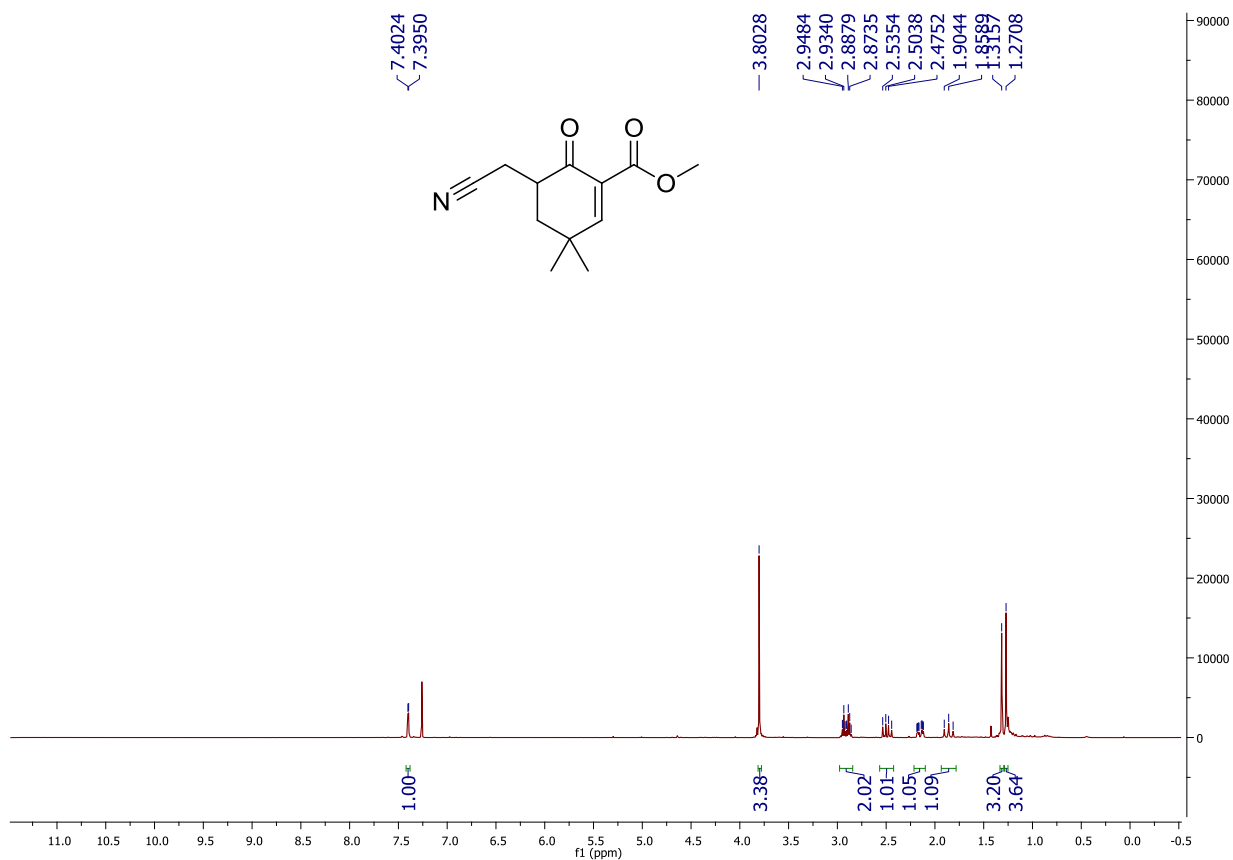


## <sup>13</sup>C NMR spectrum

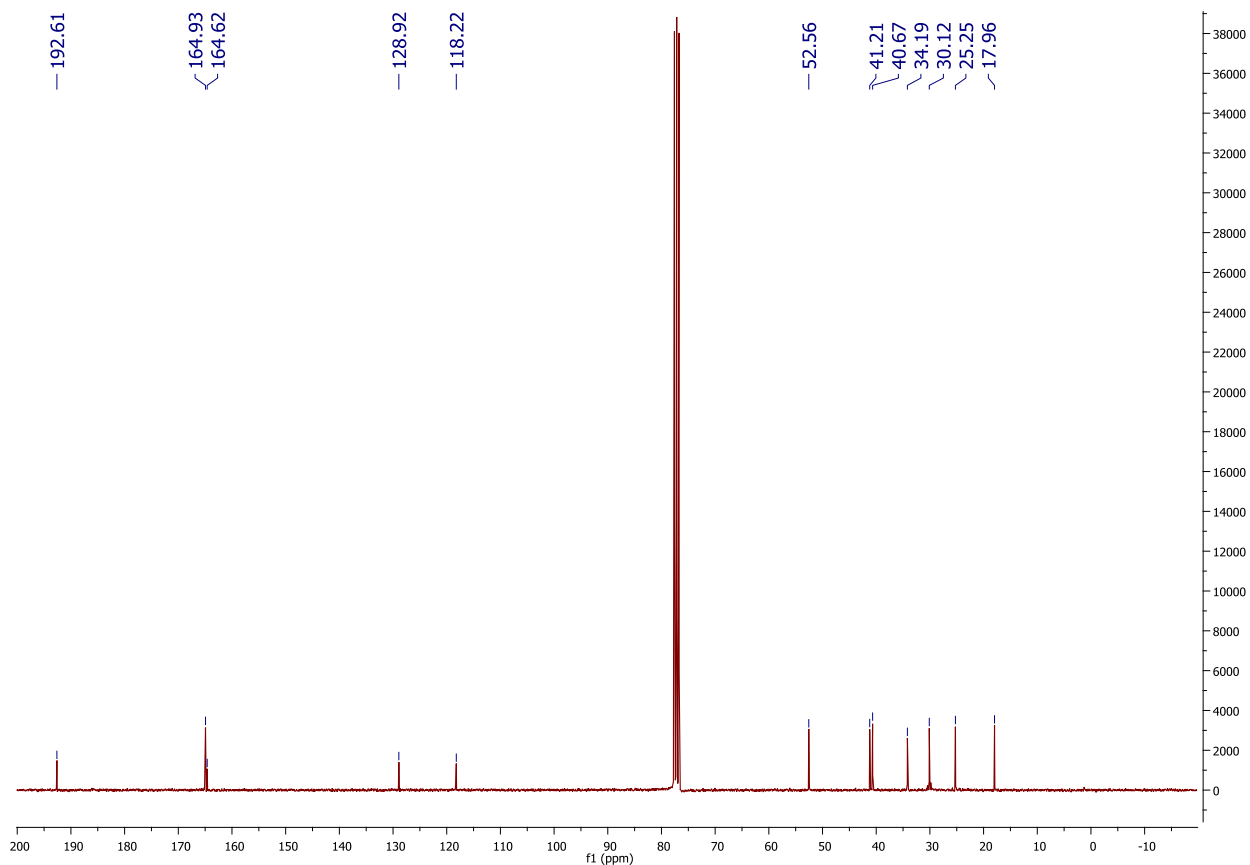


# Methyl 5-(cyanomethyl)-3,3-dimethyl-6-oxocyclohex-1-enecarboxylate (15)

## <sup>1</sup>H NMR spectrum

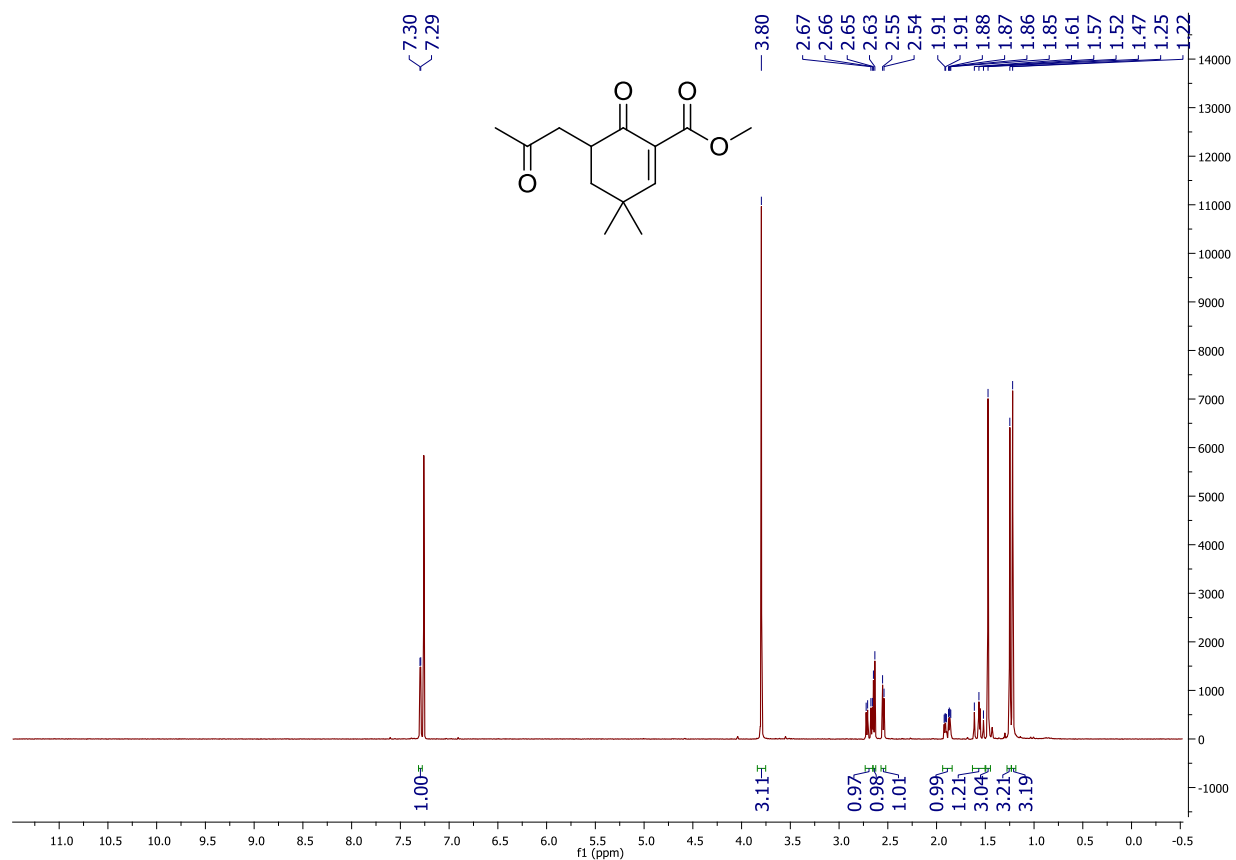


## <sup>13</sup>C NMR spectrum

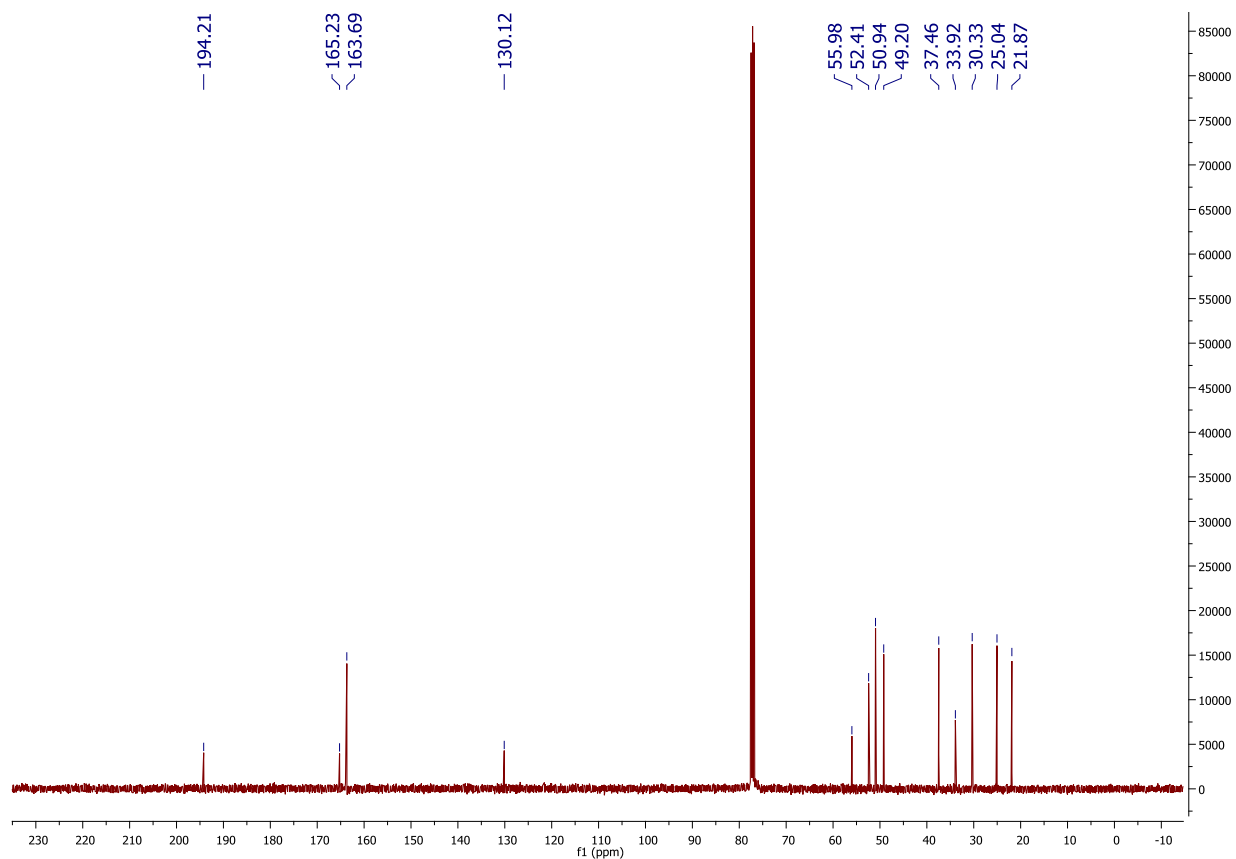


# Methyl 3,3-dimethyl-6-oxo-5-(2-oxopropyl)cyclohex-1-enecarboxylate (16)

## <sup>1</sup>H NMR spectrum

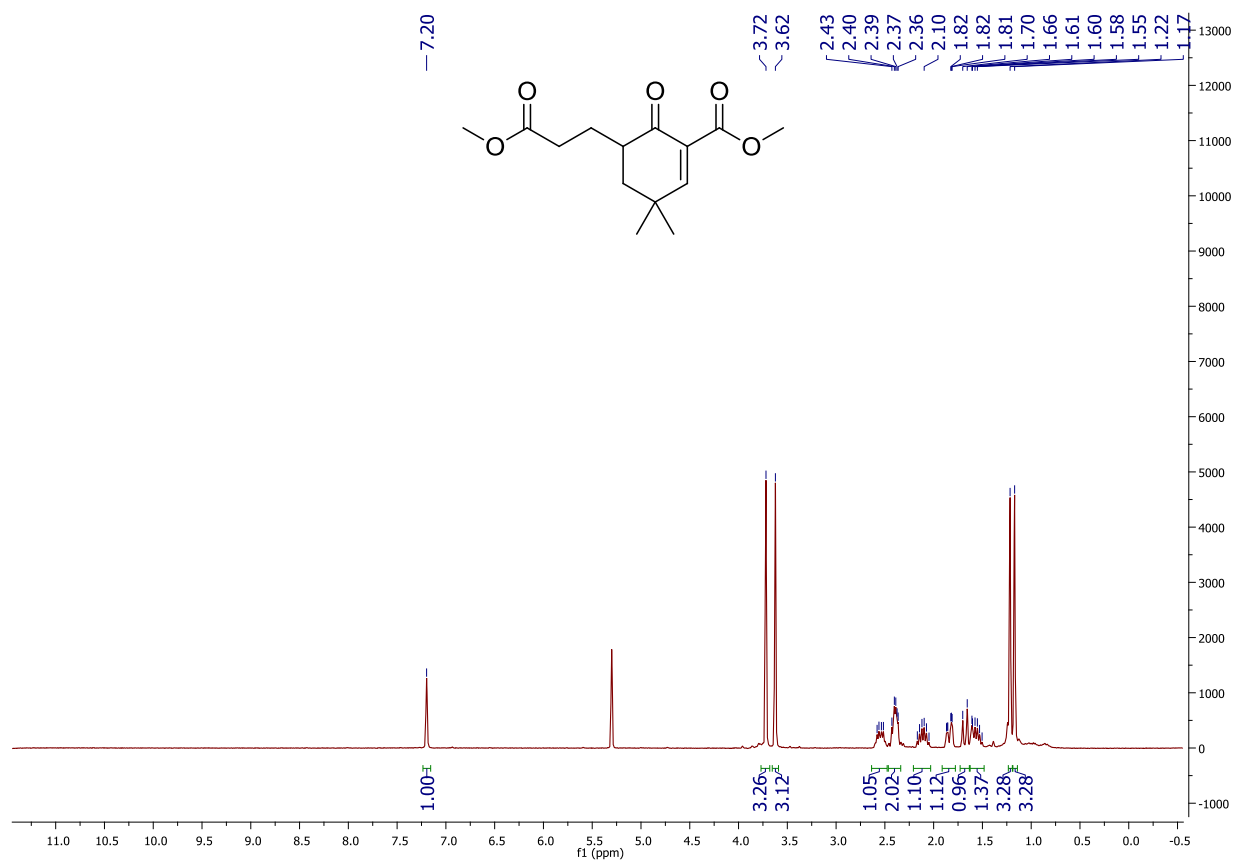


## <sup>13</sup>C NMR spectrum

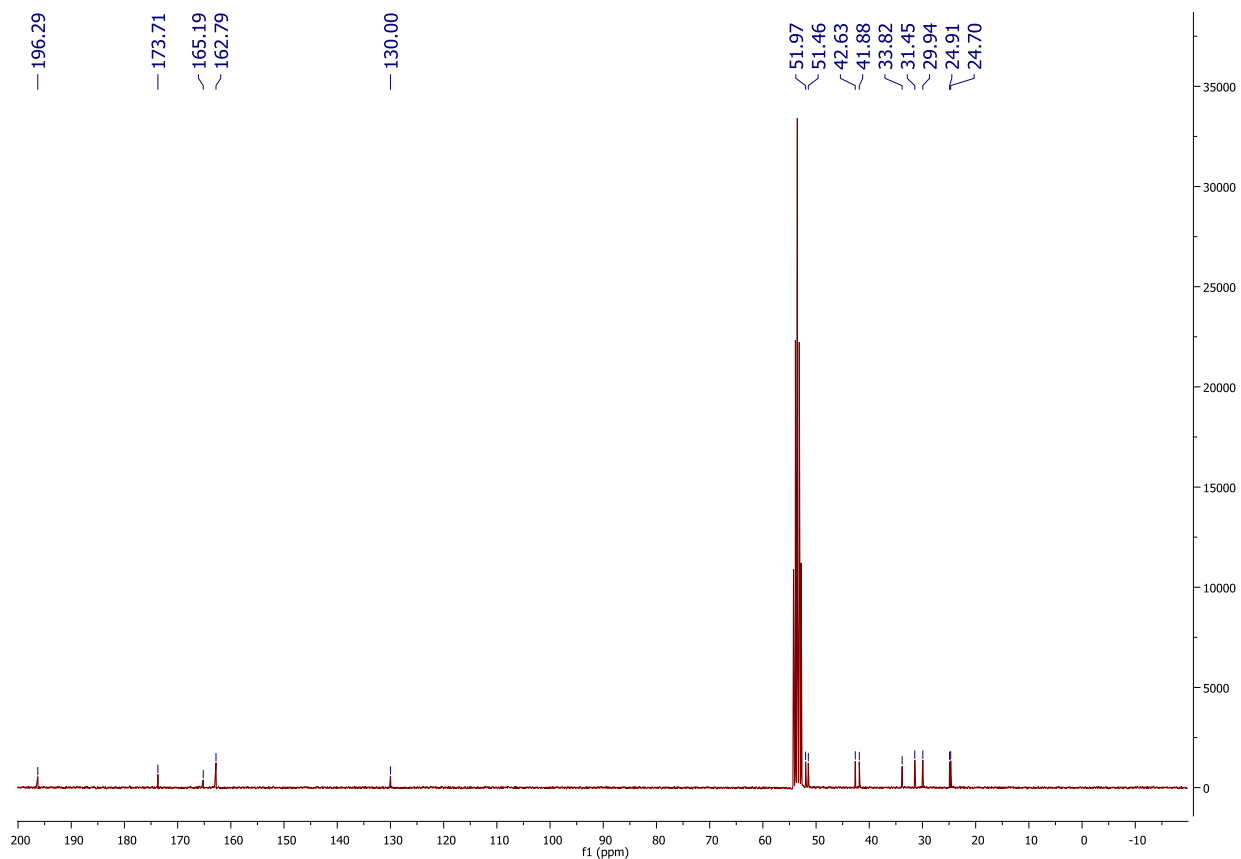


# Methyl 5-(3-methoxy-3-oxopropyl)-3,3-dimethyl-6-oxocyclohex-1-enecarboxylate (17)

## <sup>1</sup>H NMR spectrum

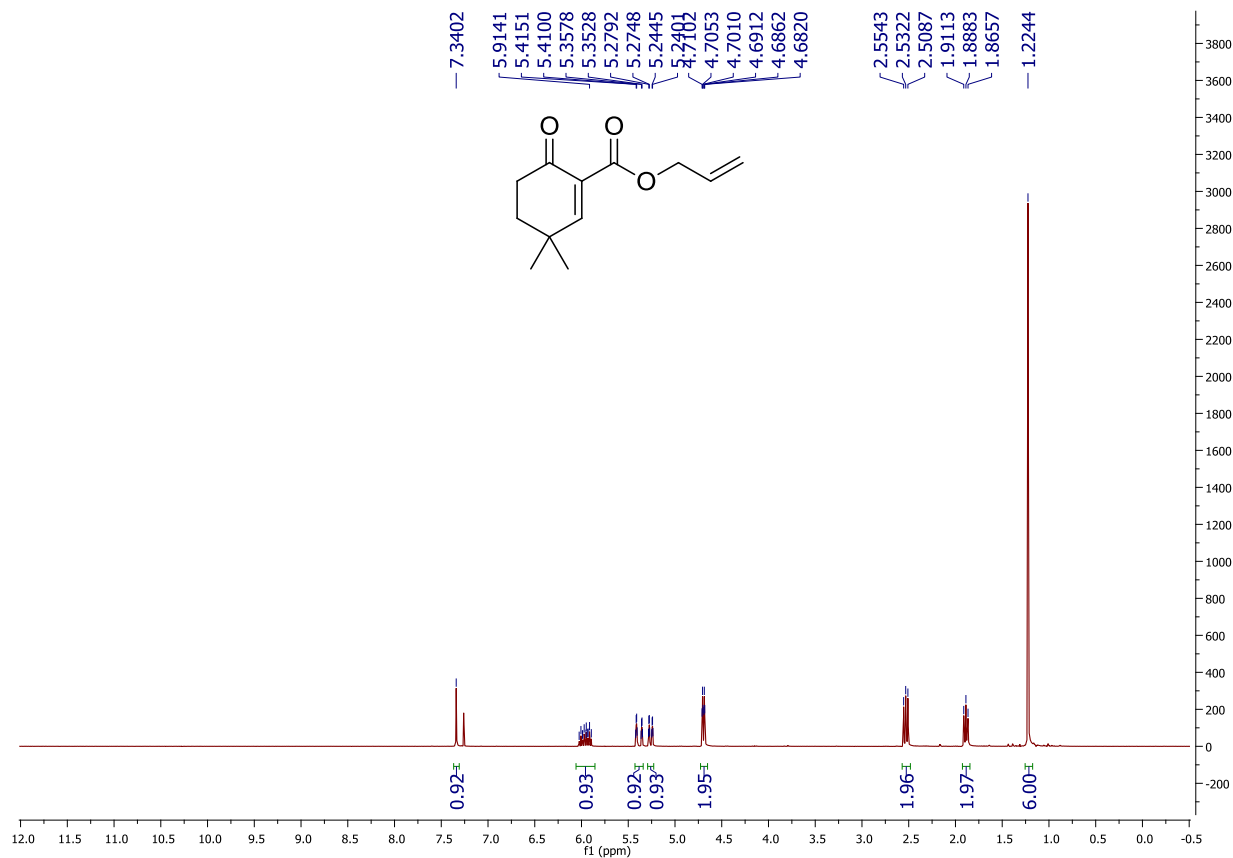


## <sup>13</sup>C NMR spectrum

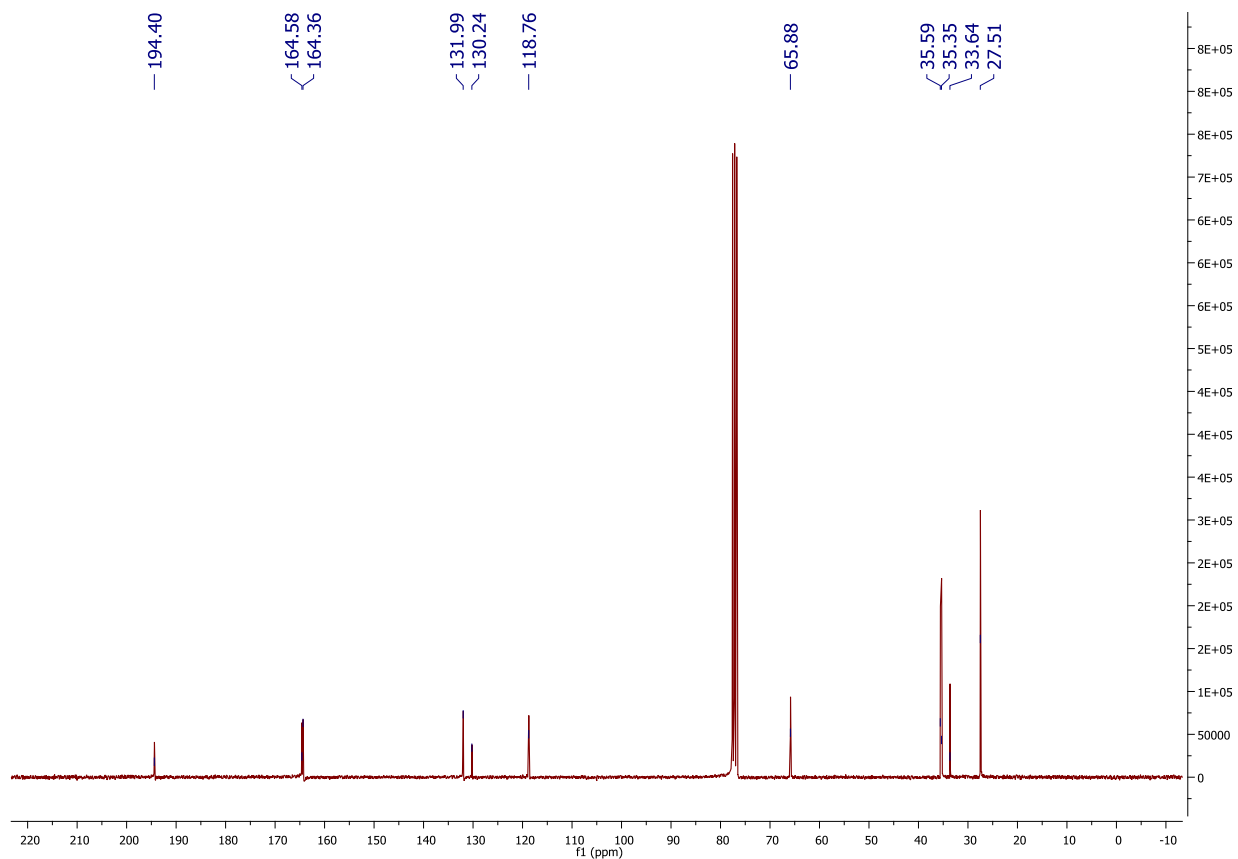


# Allyl 3,3-dimethyl-6-oxocyclohex-1-enecarboxylate (18)

## <sup>1</sup>H NMR spectrum

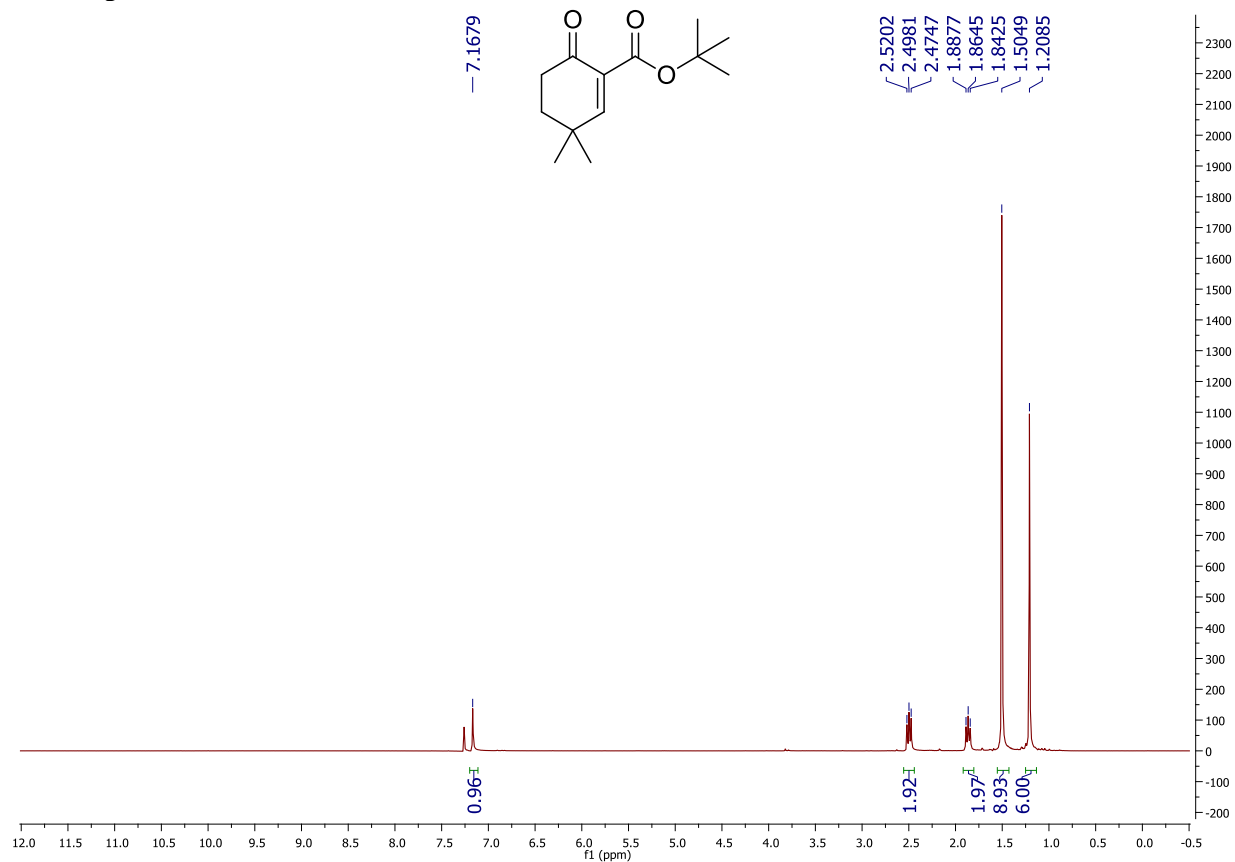


## <sup>13</sup>C NMR spectrum

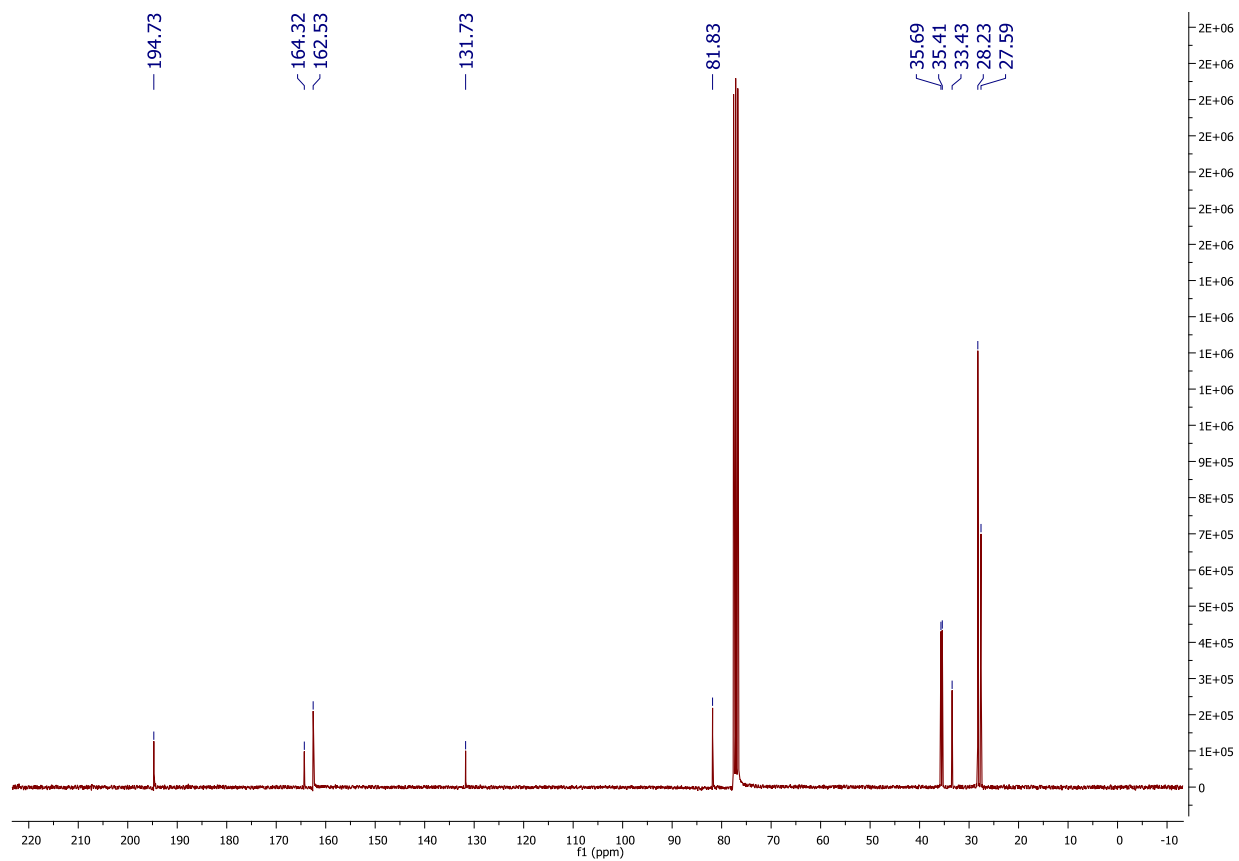


# *tert*-Butyl 3,3-dimethyl-6-oxocyclohex-1-enecarboxylate (19)

## <sup>1</sup>H NMR spectrum



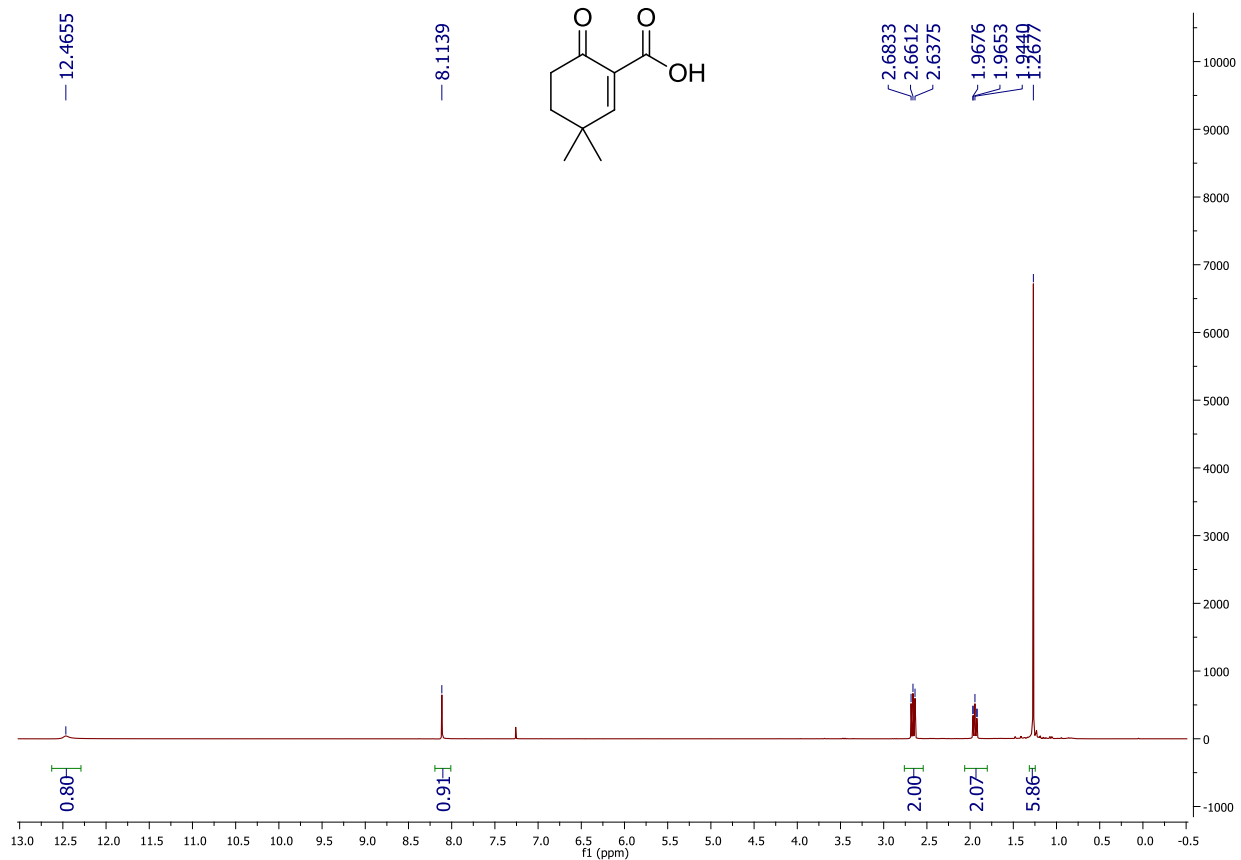
## <sup>13</sup>C NMR spectrum



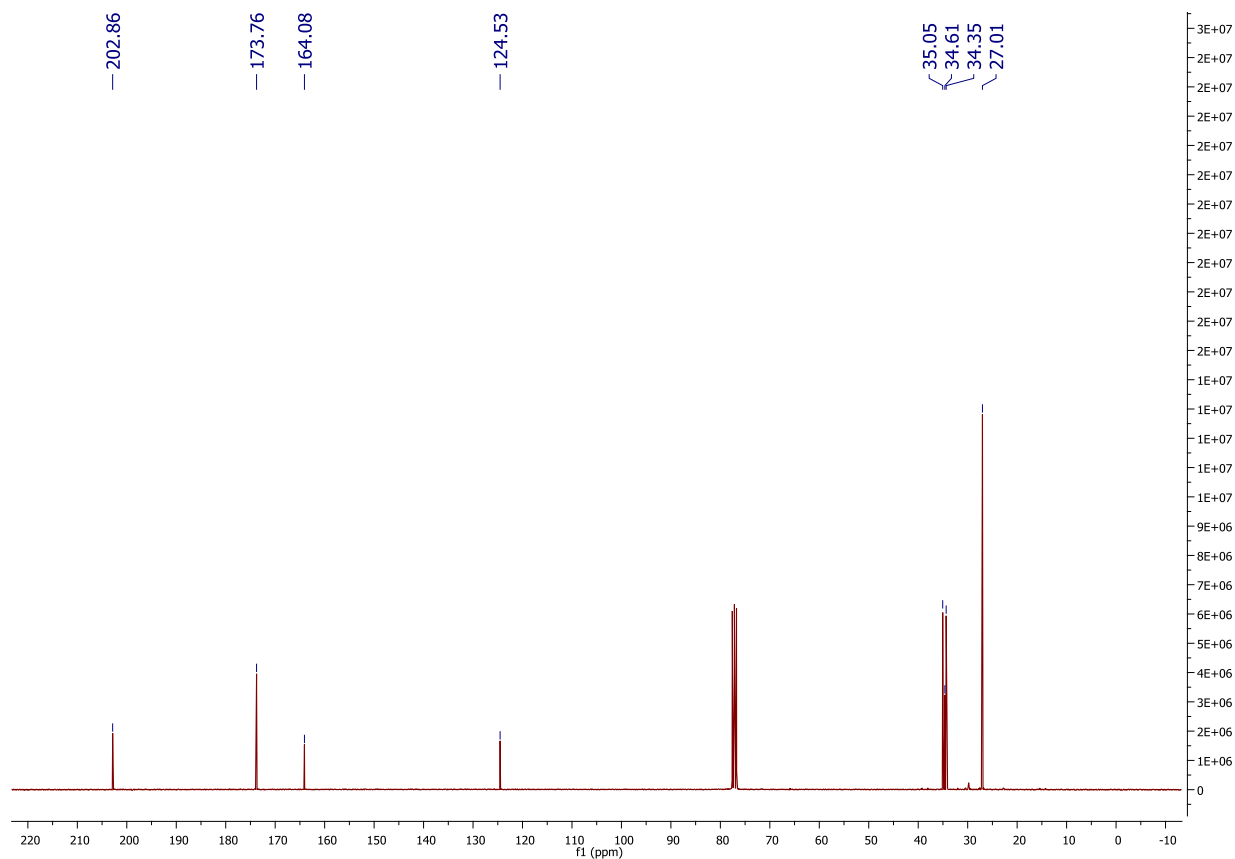


# 3,3-dimethyl-6-oxocyclohex-1-enecarboxylic acid (20)

## <sup>1</sup>H NMR spectrum

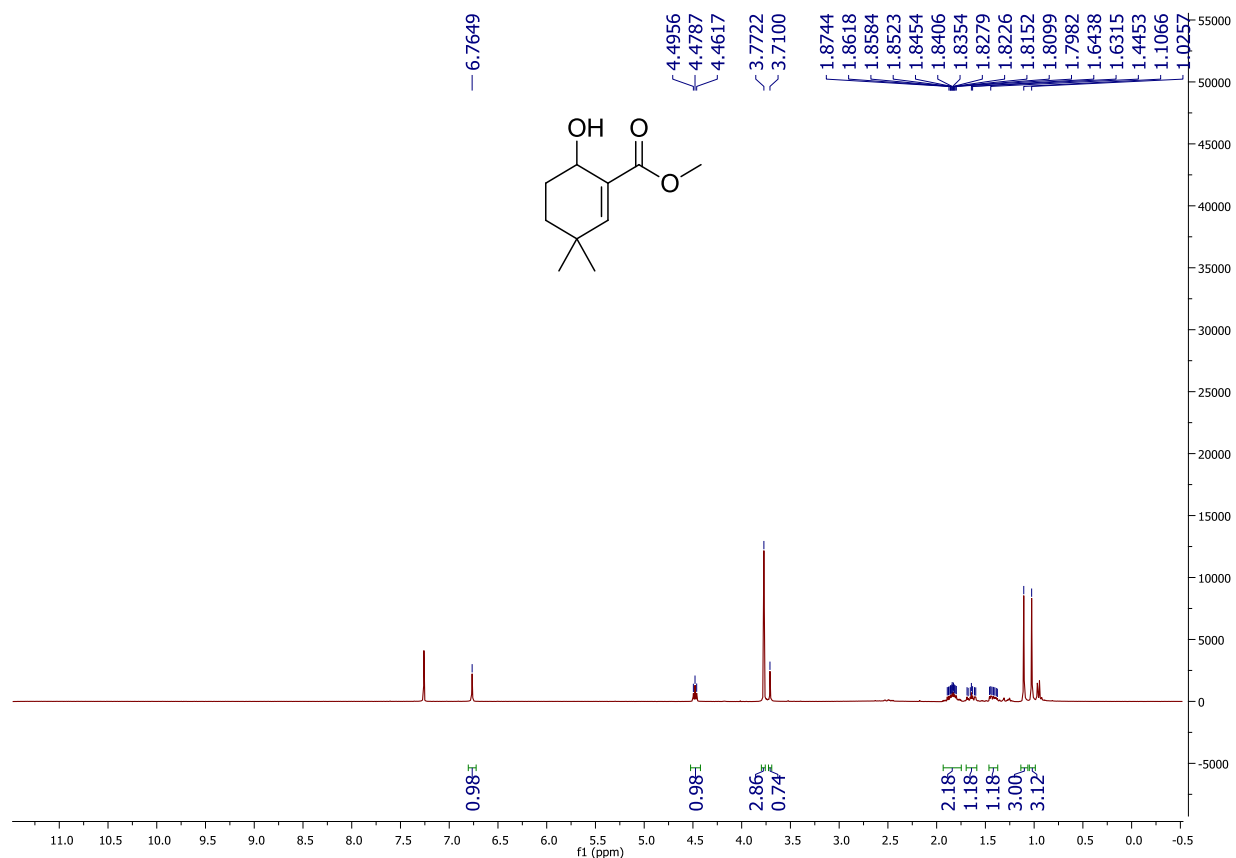


## <sup>13</sup>C NMR spectrum

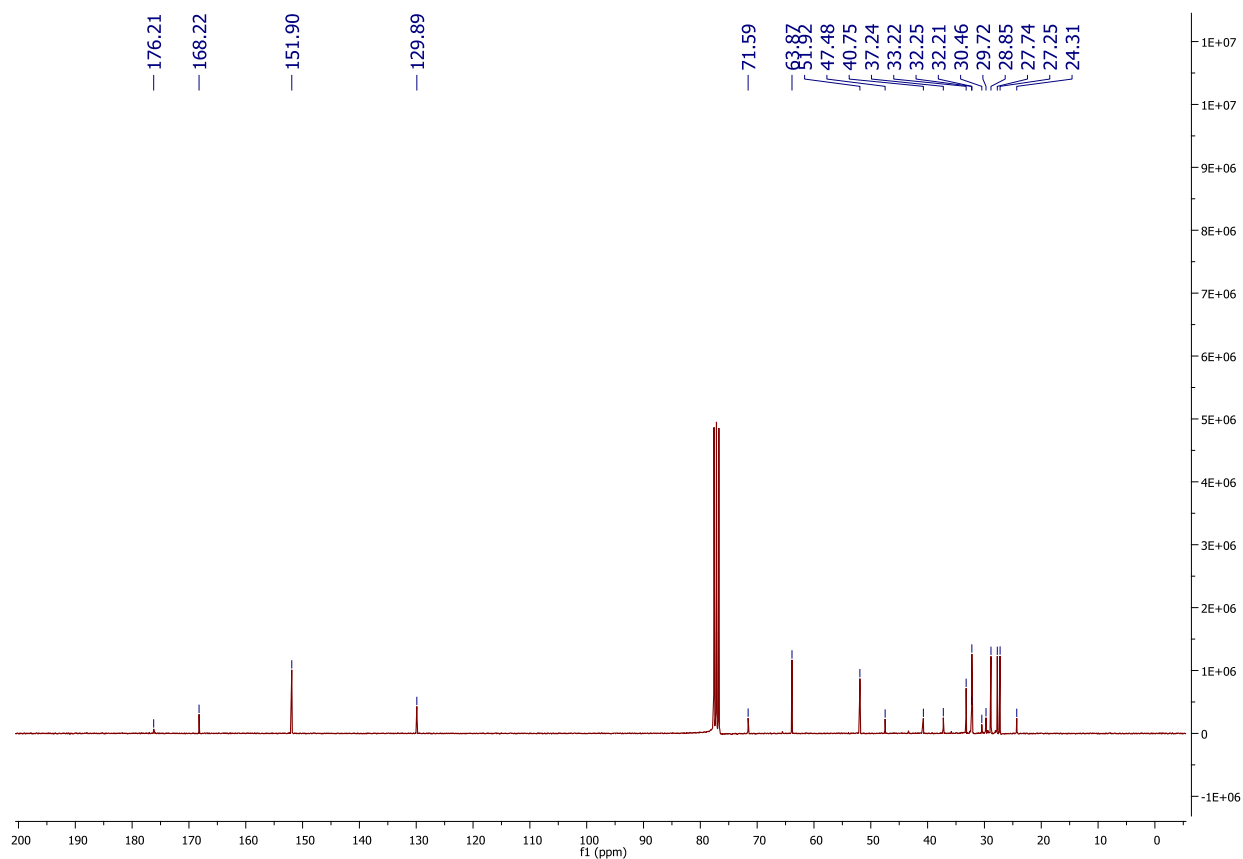


# Methyl 6-hydroxy-3,3-dimethylcyclohex-1-enecarboxylate (21)

## <sup>1</sup>H NMR spectrum

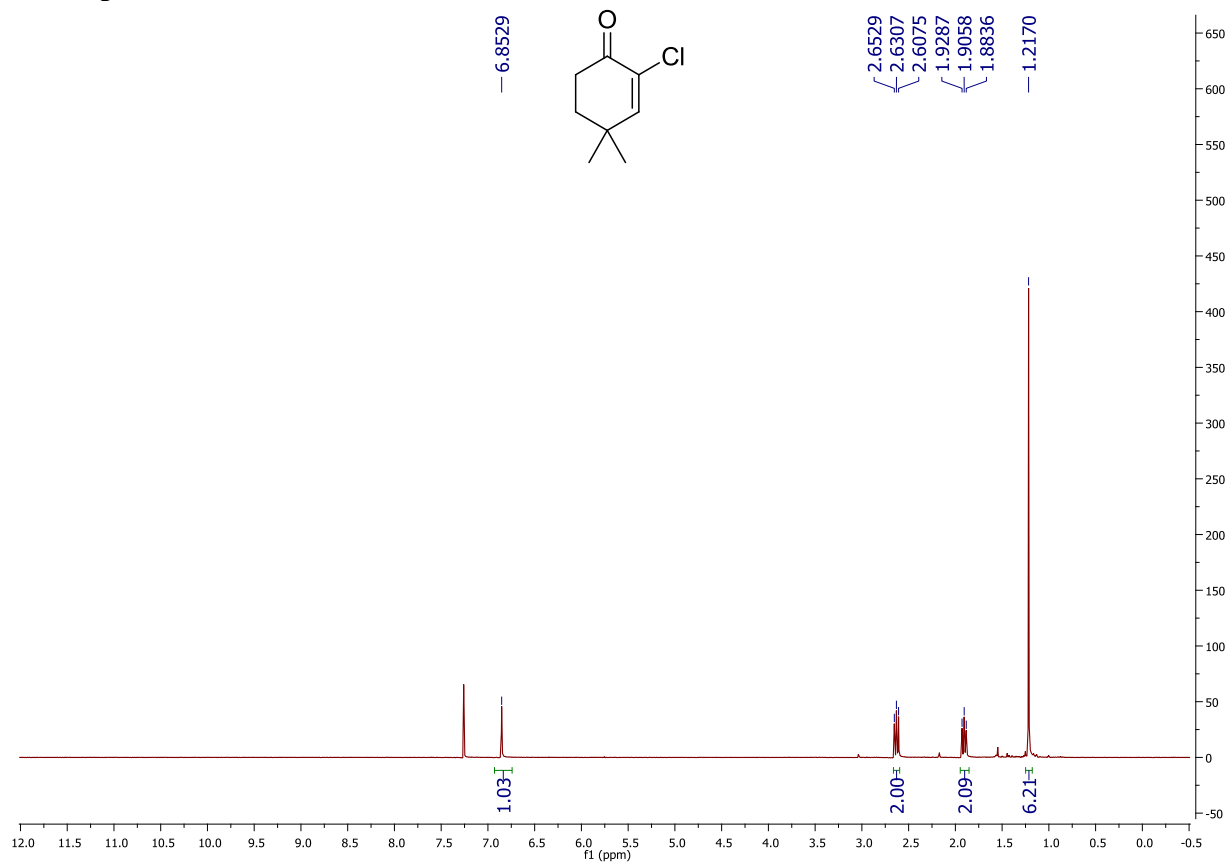


## <sup>13</sup>C NMR spectrum

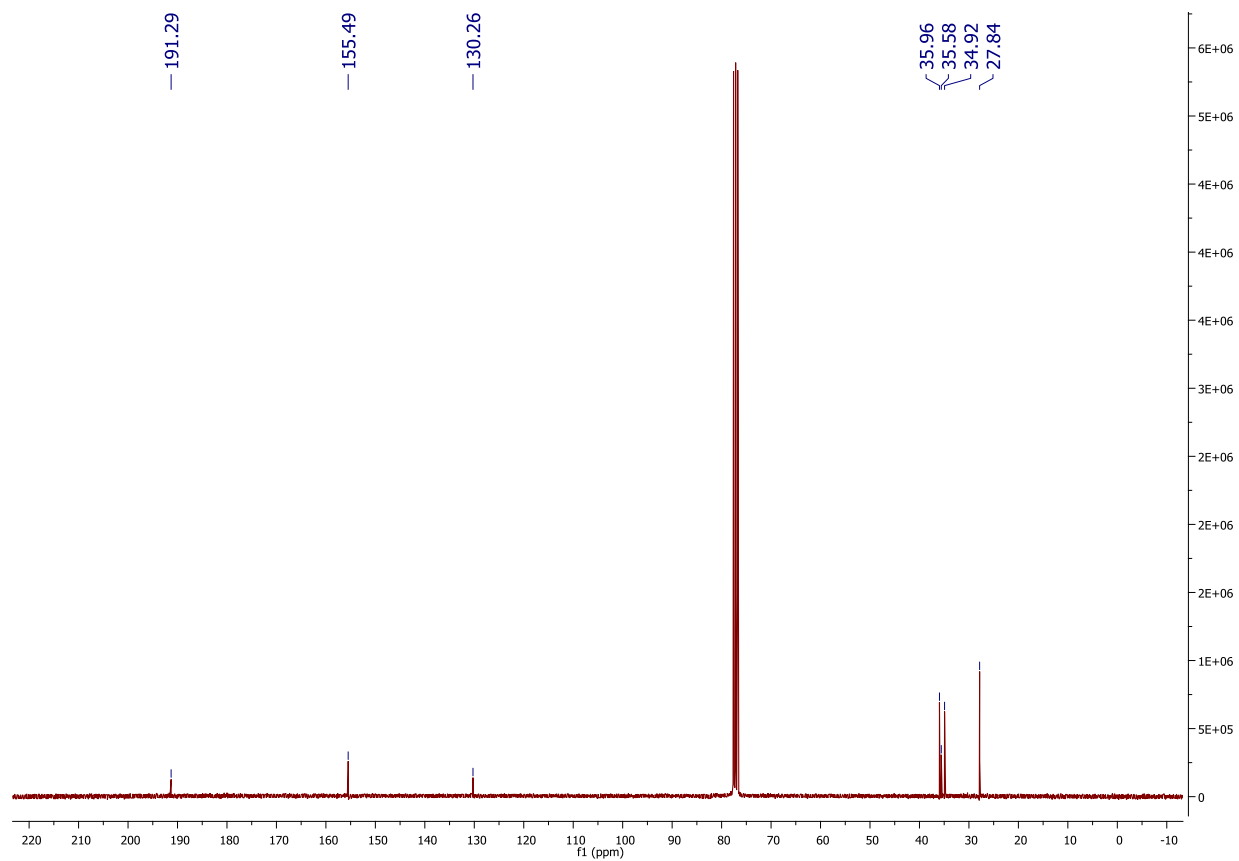


# 2-chloro-4,4-dimethylcyclohex-2-enone (23)

## <sup>1</sup>H NMR spectrum

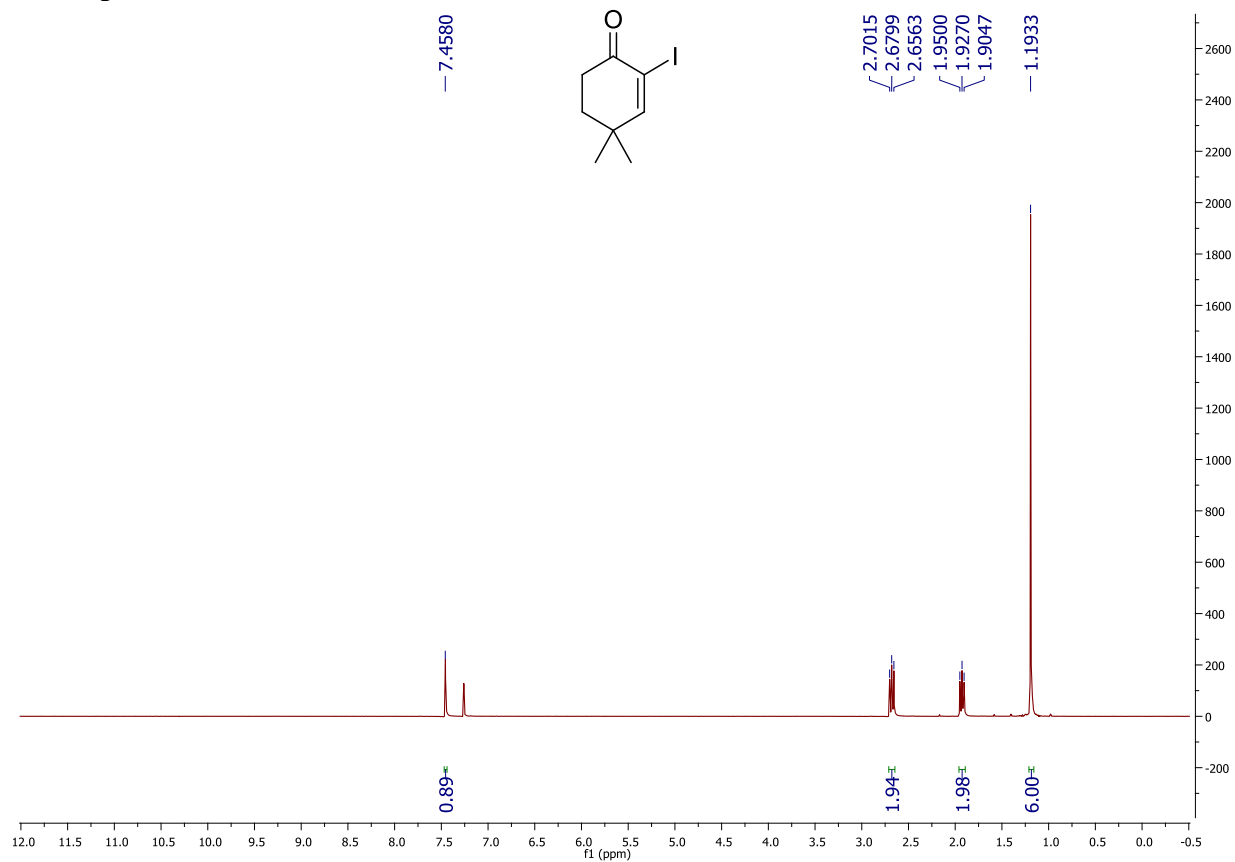


## <sup>13</sup>C NMR spectrum



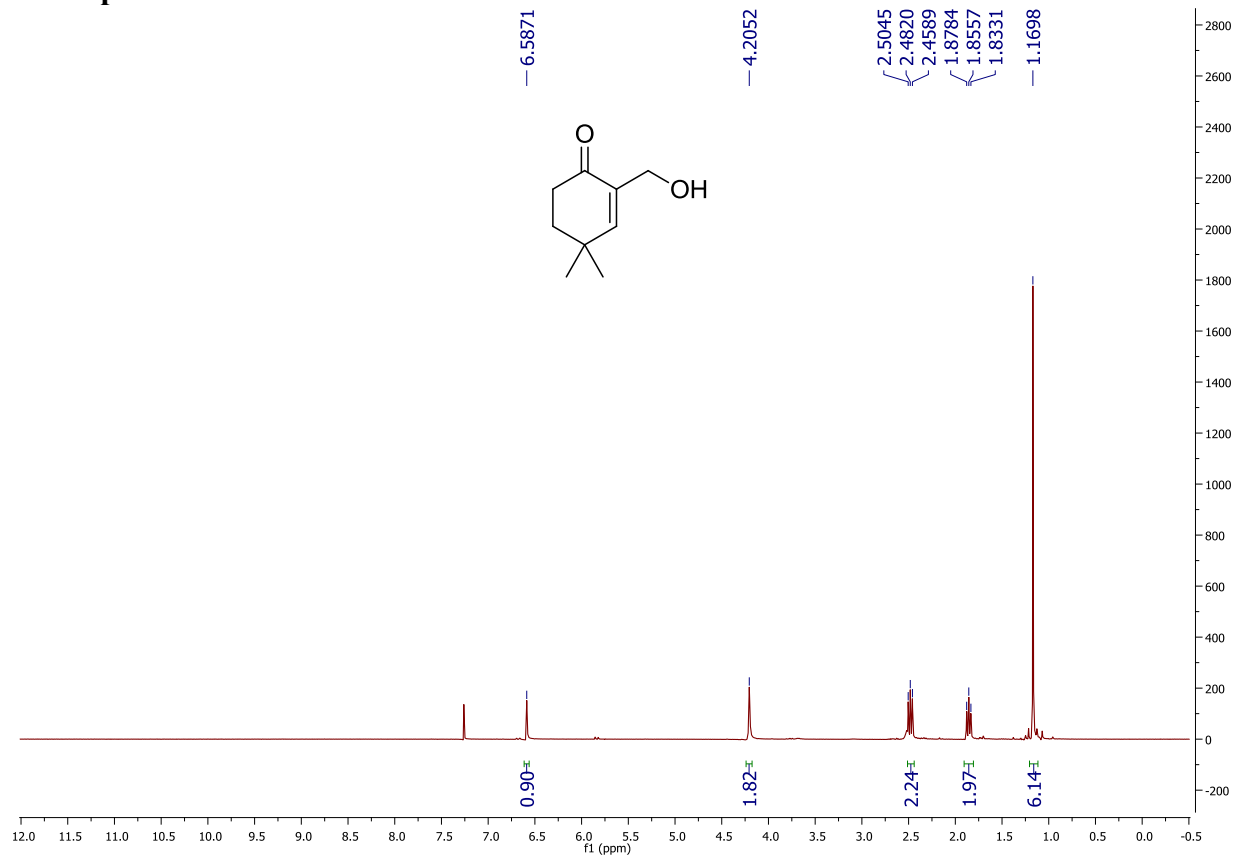
## 2-iodo-4,4-dimethylcyclohex-2-enone (24)

$^1\text{H}$  NMR spectrum

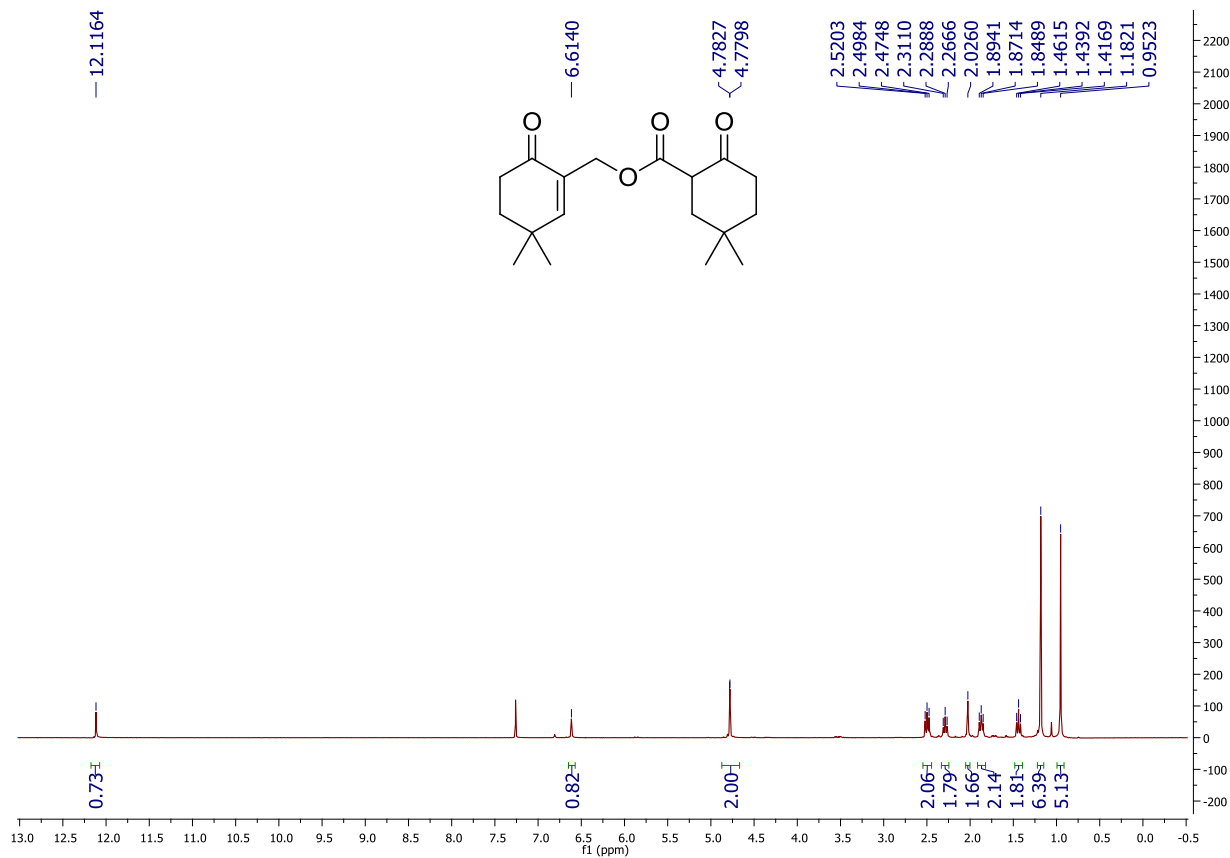


## 2-(hydroxymethyl)-4,4-dimethylcyclohex-2-enone (25)

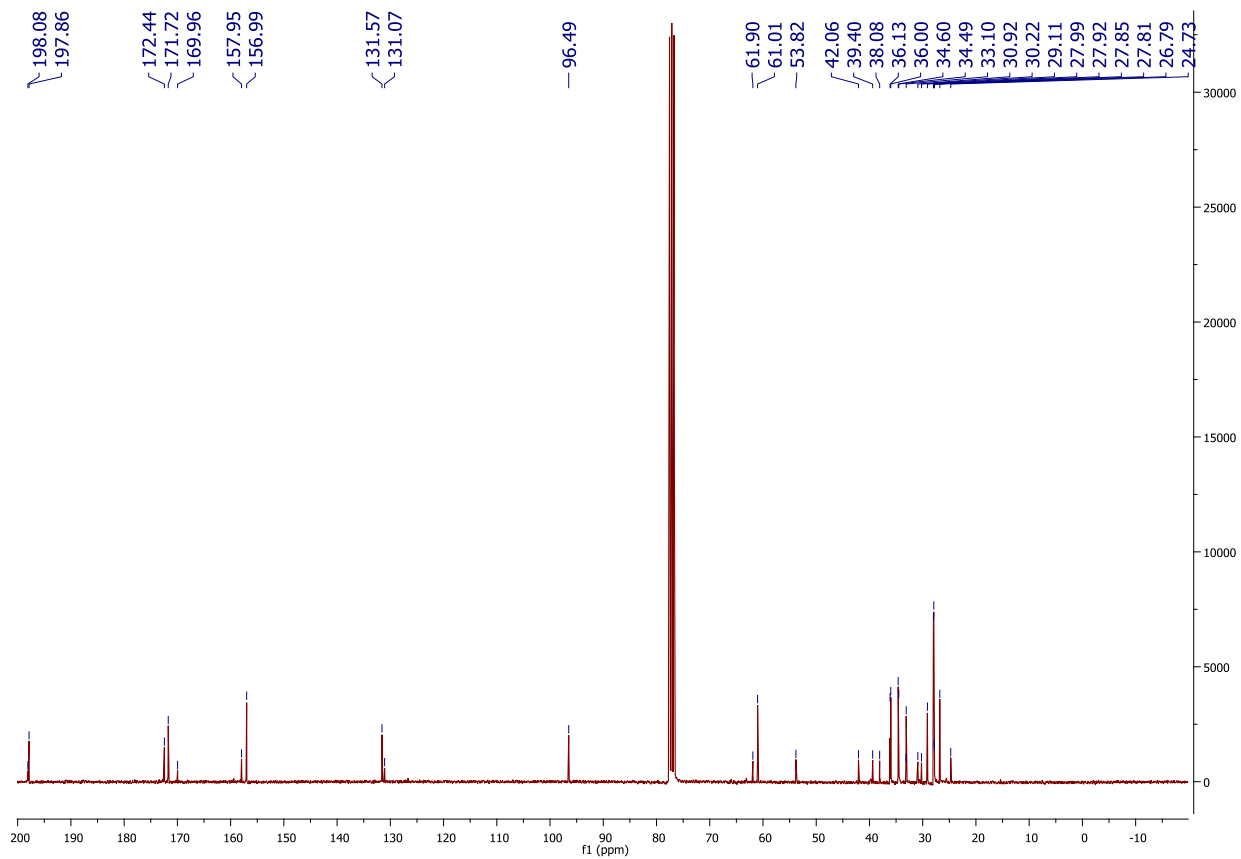
$^1\text{H}$  NMR spectrum



**(3,3-dimethyl-6-oxocyclohex-1-en-1-yl)methyl 5,5-dimethyl-2-oxocyclohexanecarboxylate (26)**  
**<sup>1</sup>H NMR spectrum**

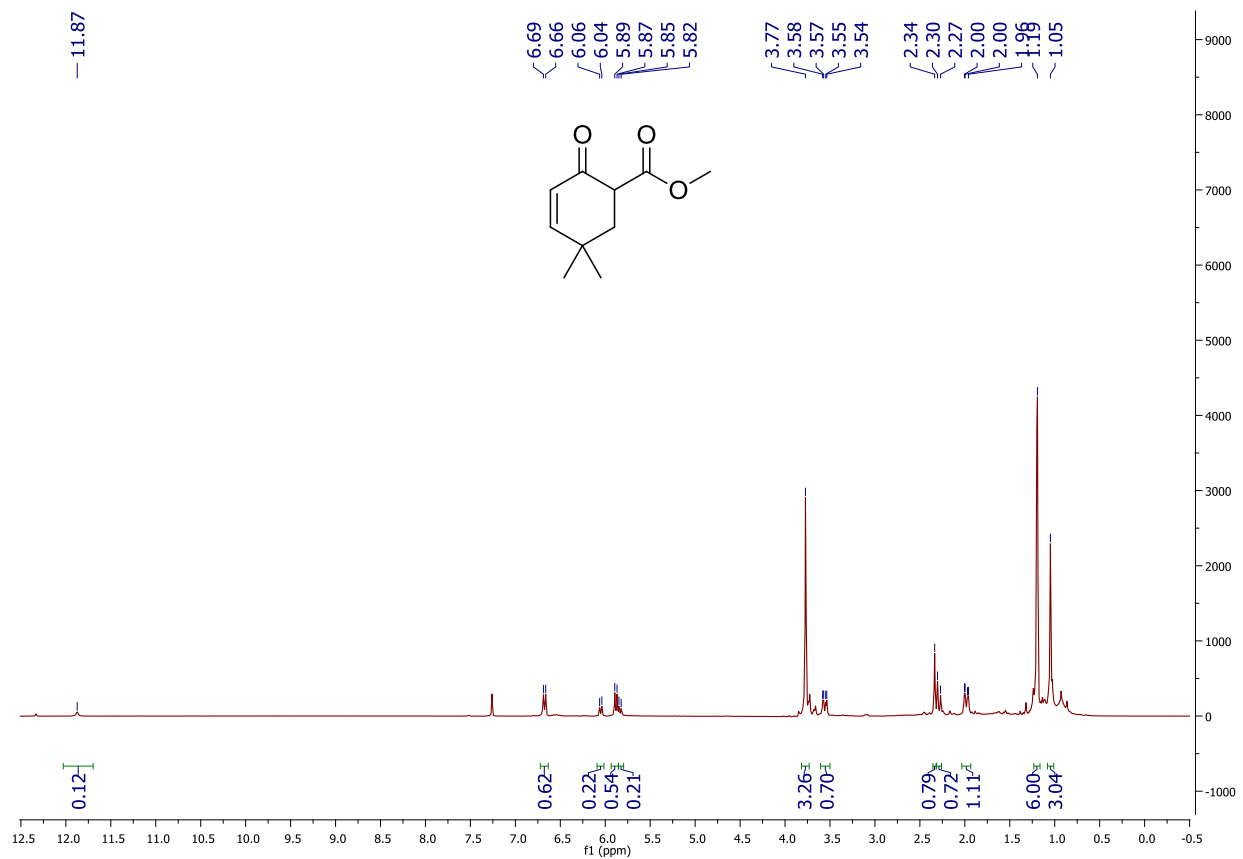


**<sup>13</sup>C NMR spectrum**



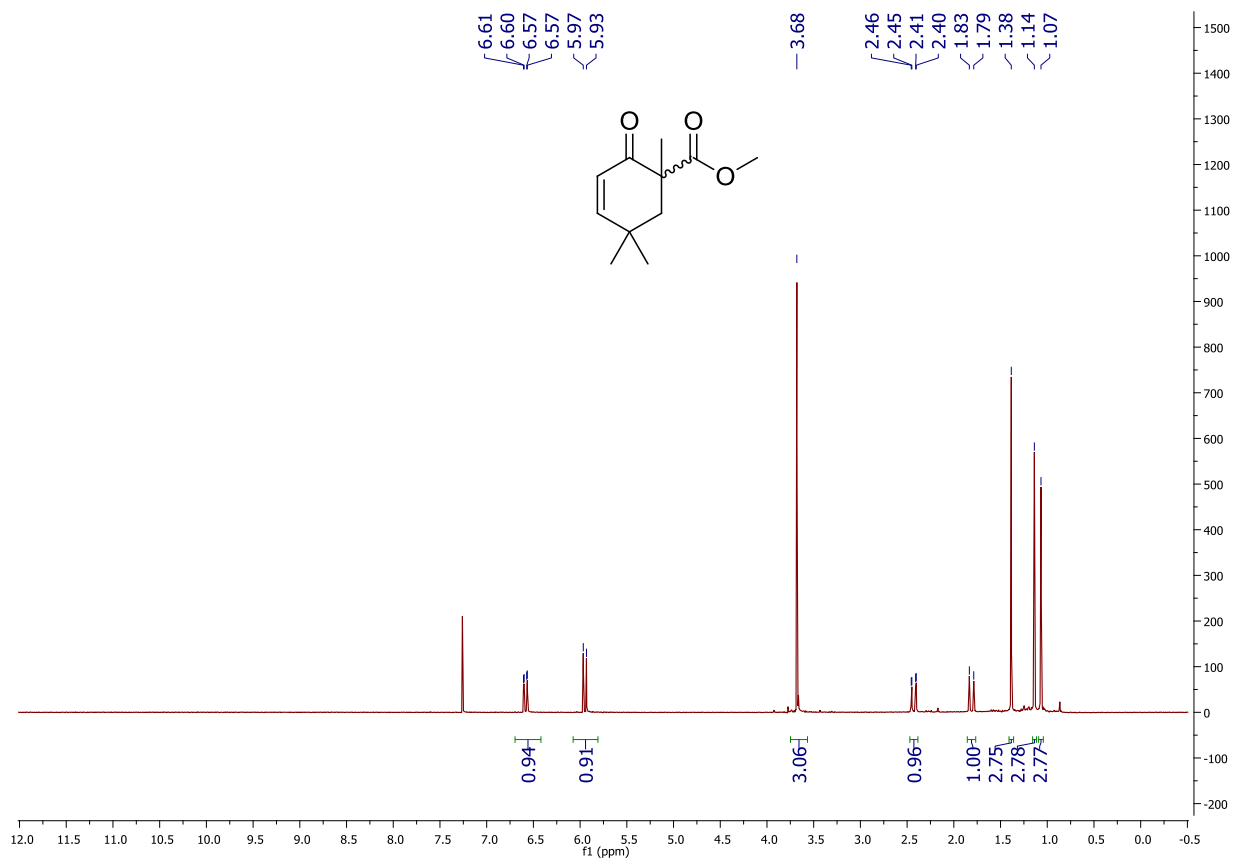
# Methyl 5,5-dimethyl-2-oxocyclohex-3-enecarboxylate (27)

## <sup>1</sup>H NMR spectrum

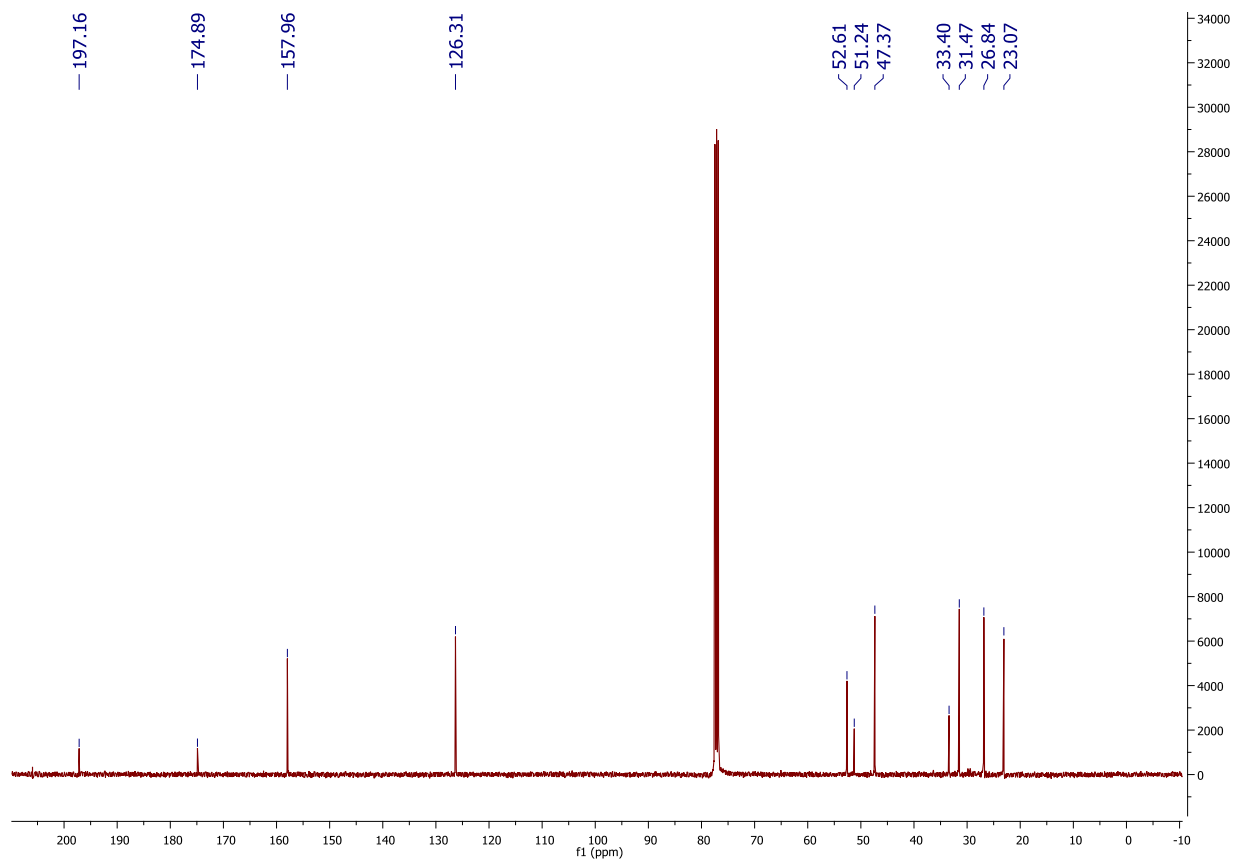


# Methyl 1,5,5-trimethyl-2-oxocyclohex-3-enecarboxylate (28)

## <sup>1</sup>H NMR spectrum

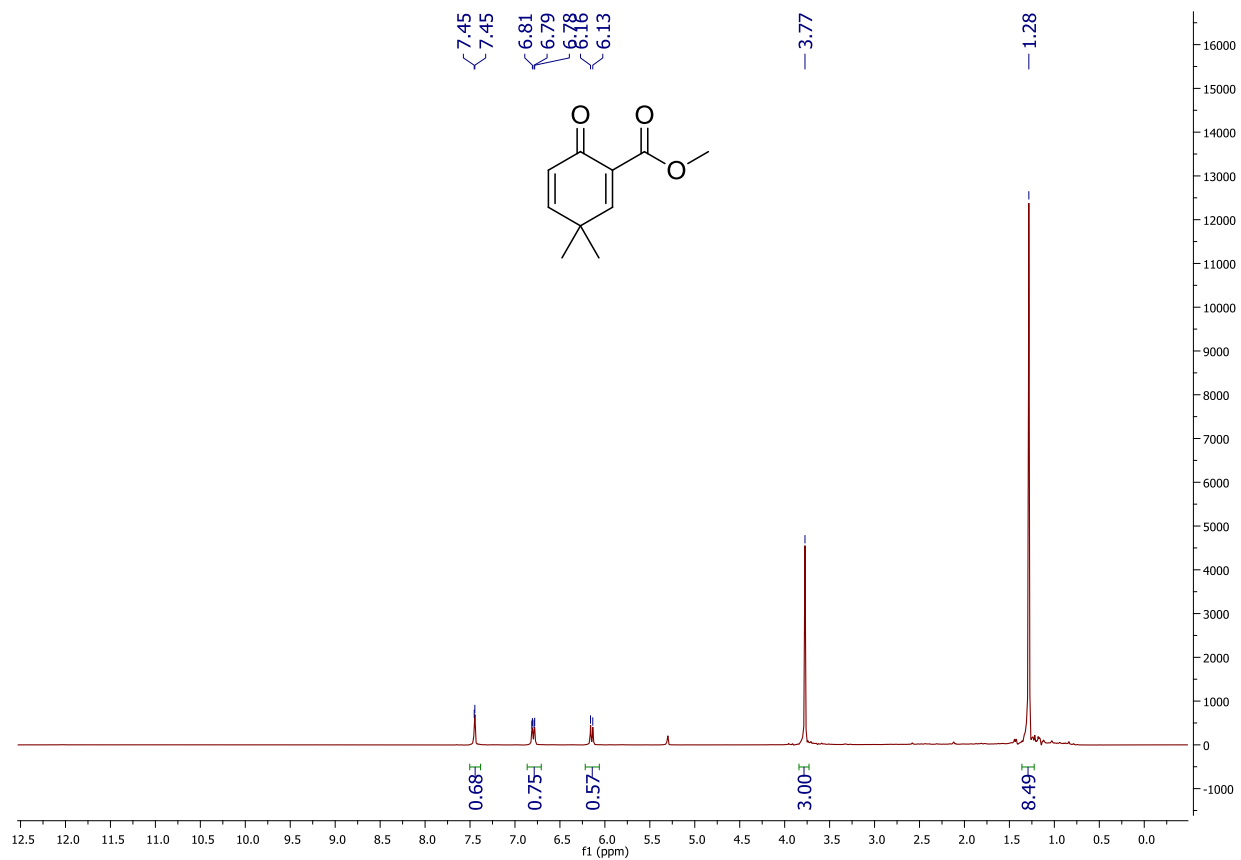


## <sup>13</sup>C NMR spectrum

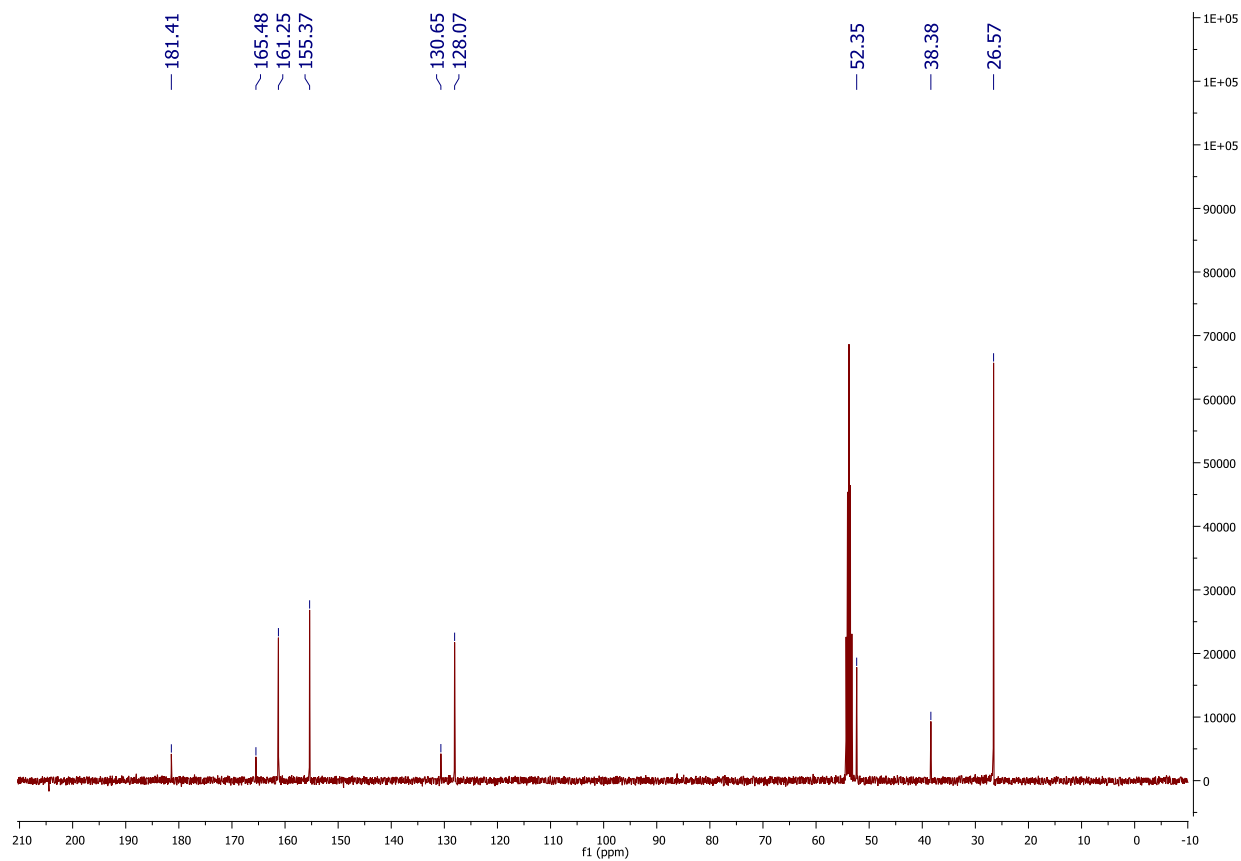


# Methyl 3,3-dimethyl-6-oxocyclohexa-1,4-dienecarboxylate (29)

## <sup>1</sup>H NMR spectrum



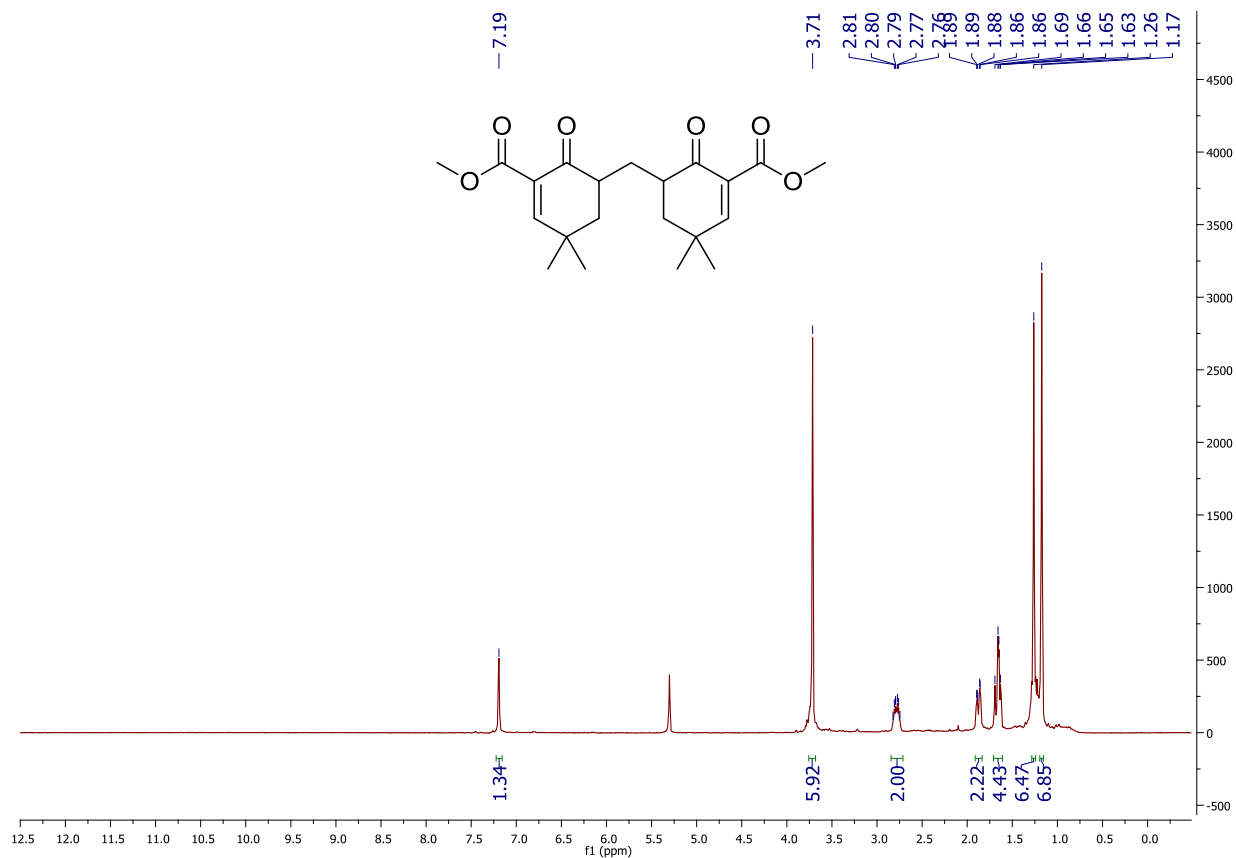
## <sup>13</sup>C NMR spectrum



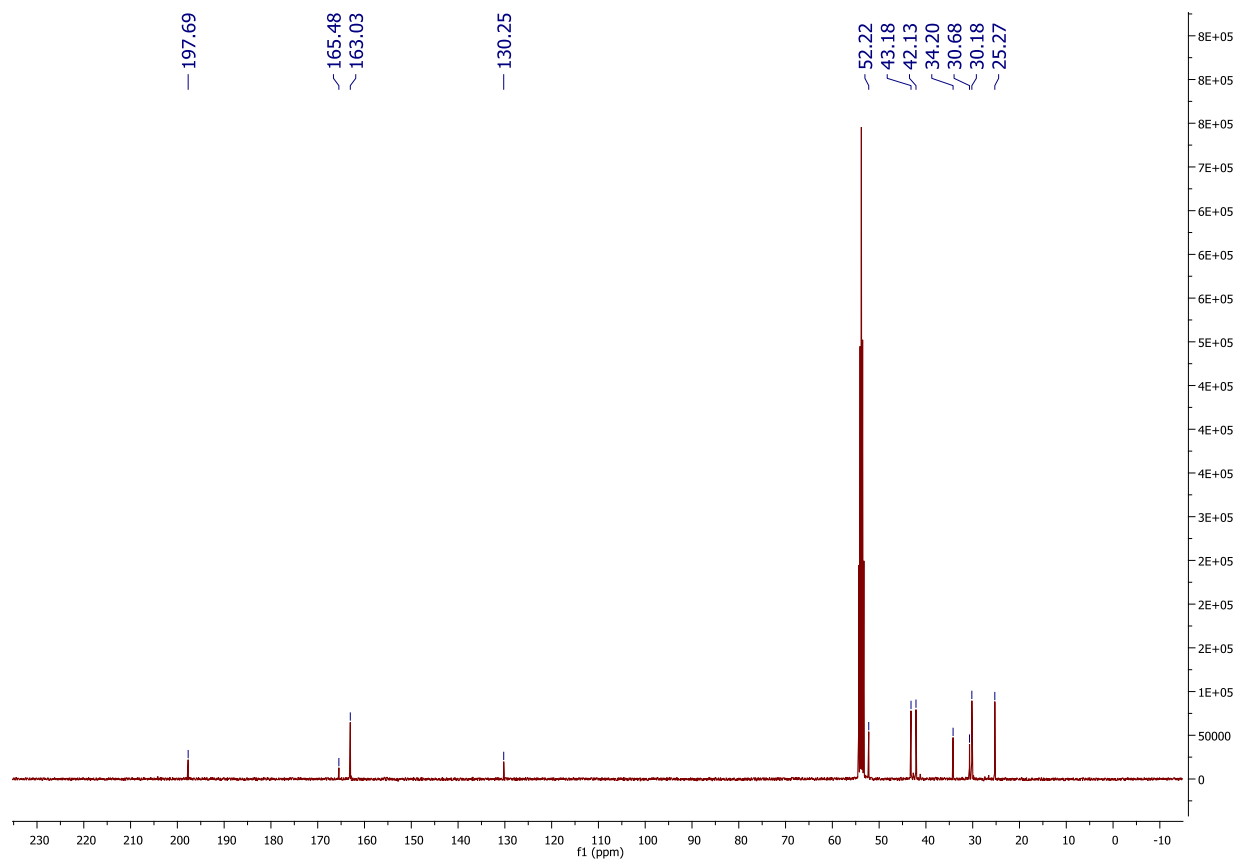


# Dimethyl 5,5'-methylenebis(3,3-dimethyl-6-oxocyclohex-1-enecarboxylate) (30)

## <sup>1</sup>H NMR spectrum

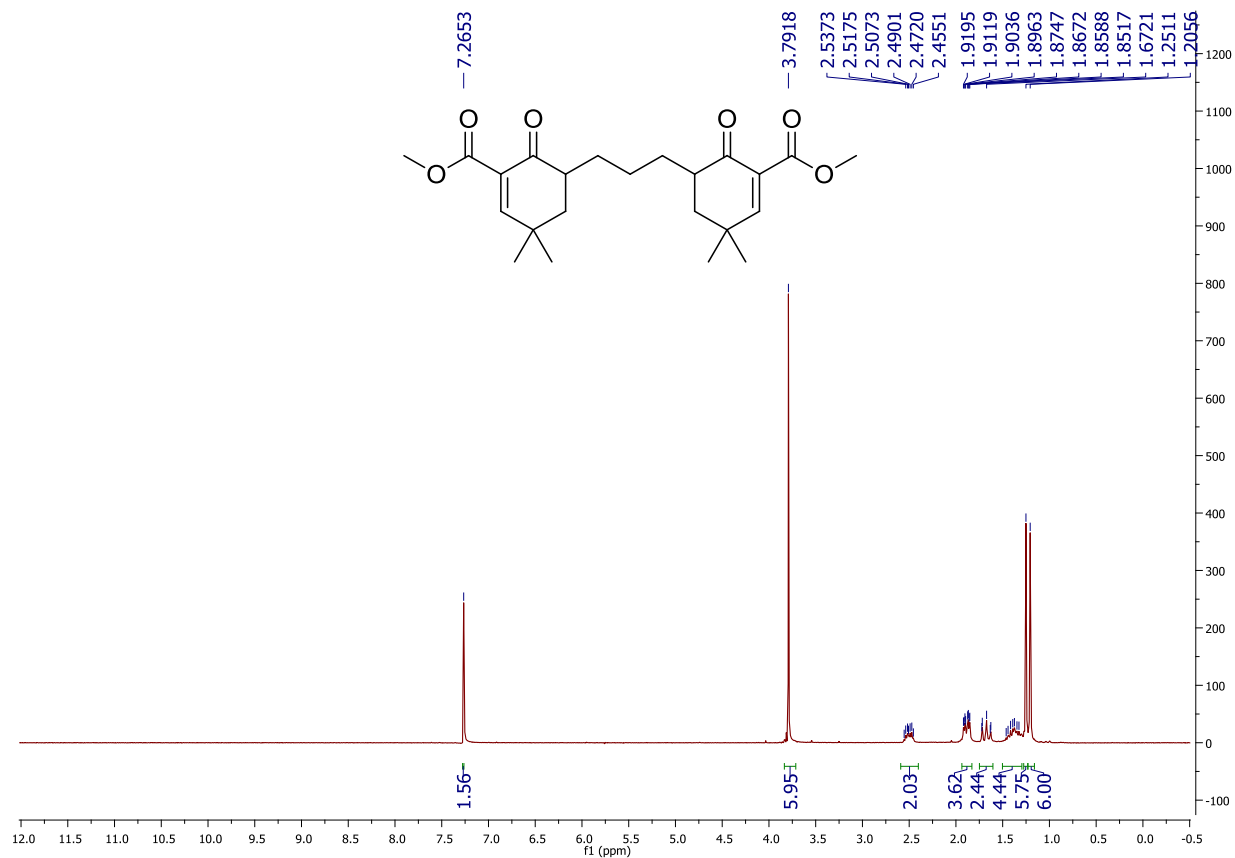


## <sup>13</sup>C NMR spectrum

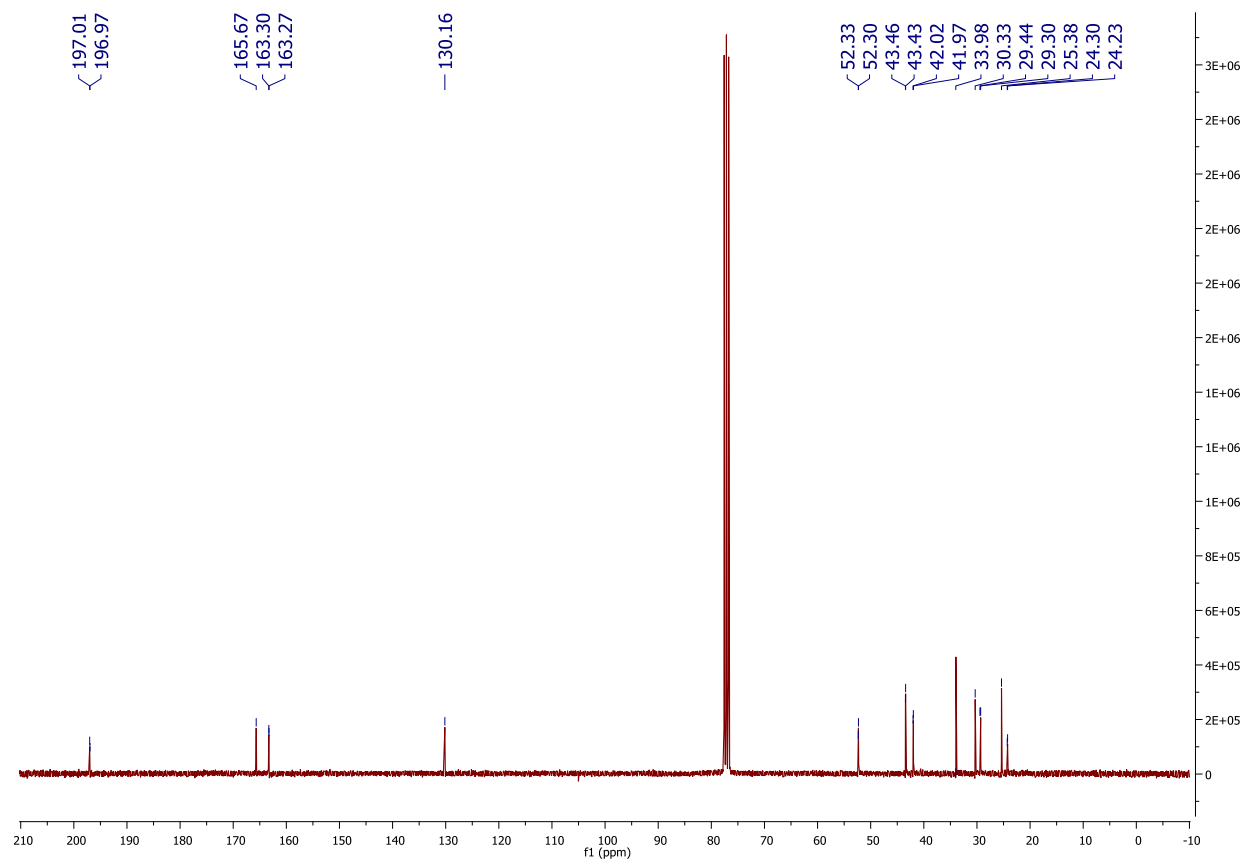


# Dimethyl 5,5'-(propane-1,3-diyl)bis(3,3-dimethyl-6-oxocyclohex-1-enecarboxylate) (31)

## <sup>1</sup>H NMR spectrum

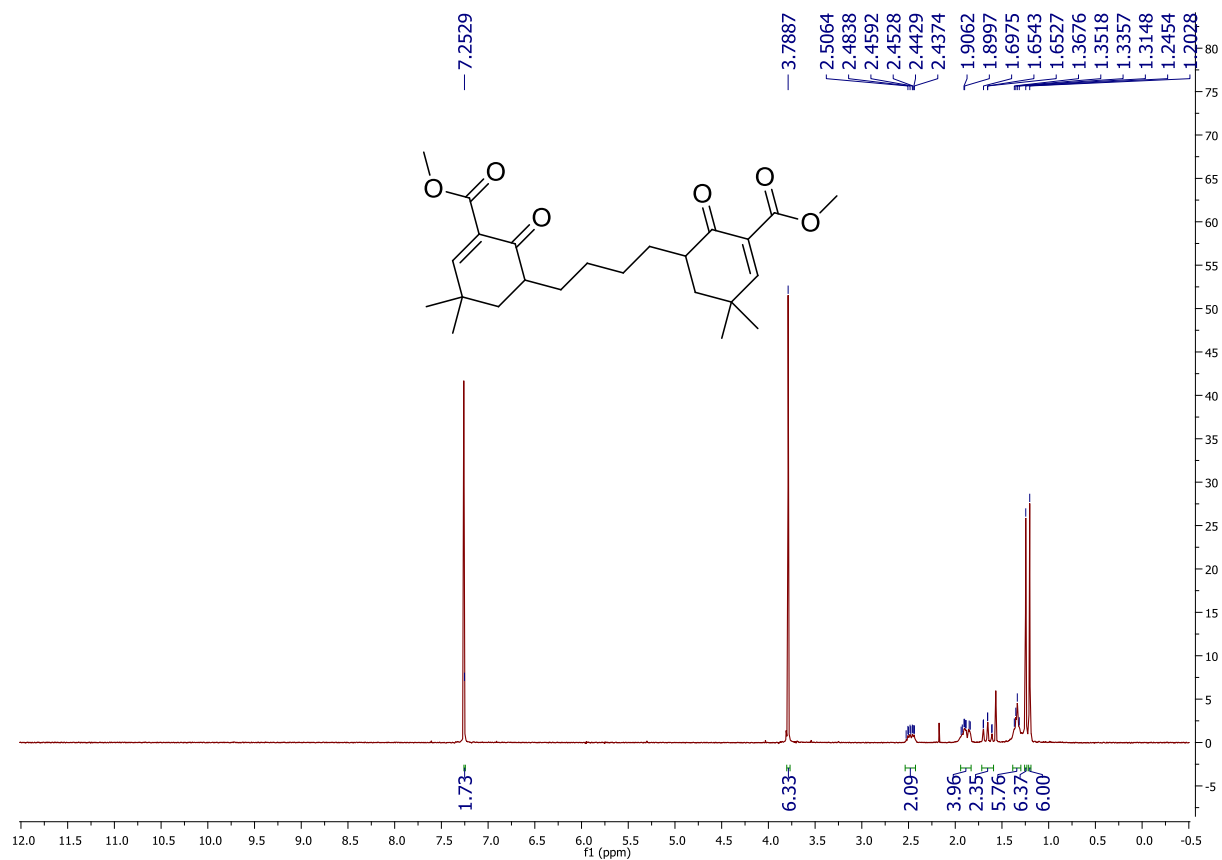


## <sup>13</sup>C NMR spectrum

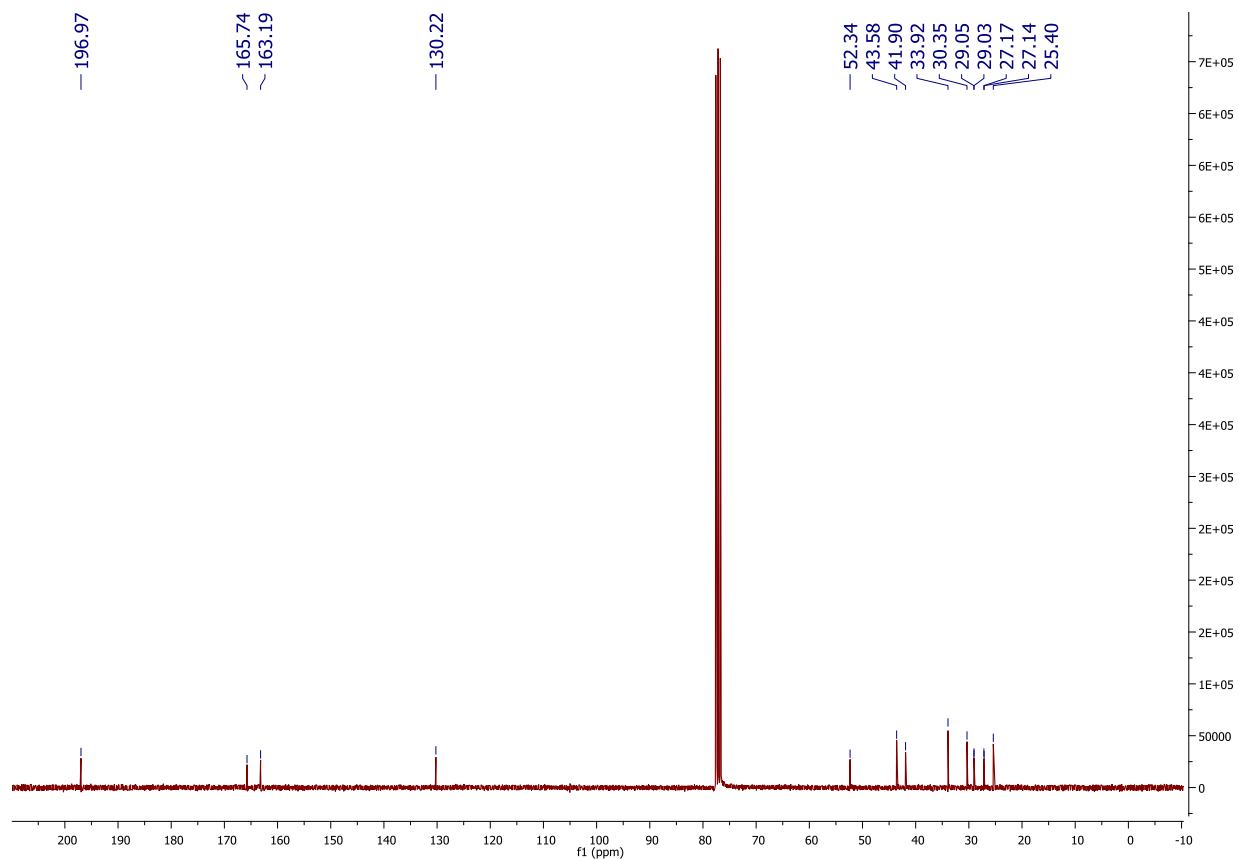


# Dimethyl 5,5'-(butane-1,4-diyl)bis(3,3-dimethyl-6-oxocyclohex-1-enecarboxylate) (32)

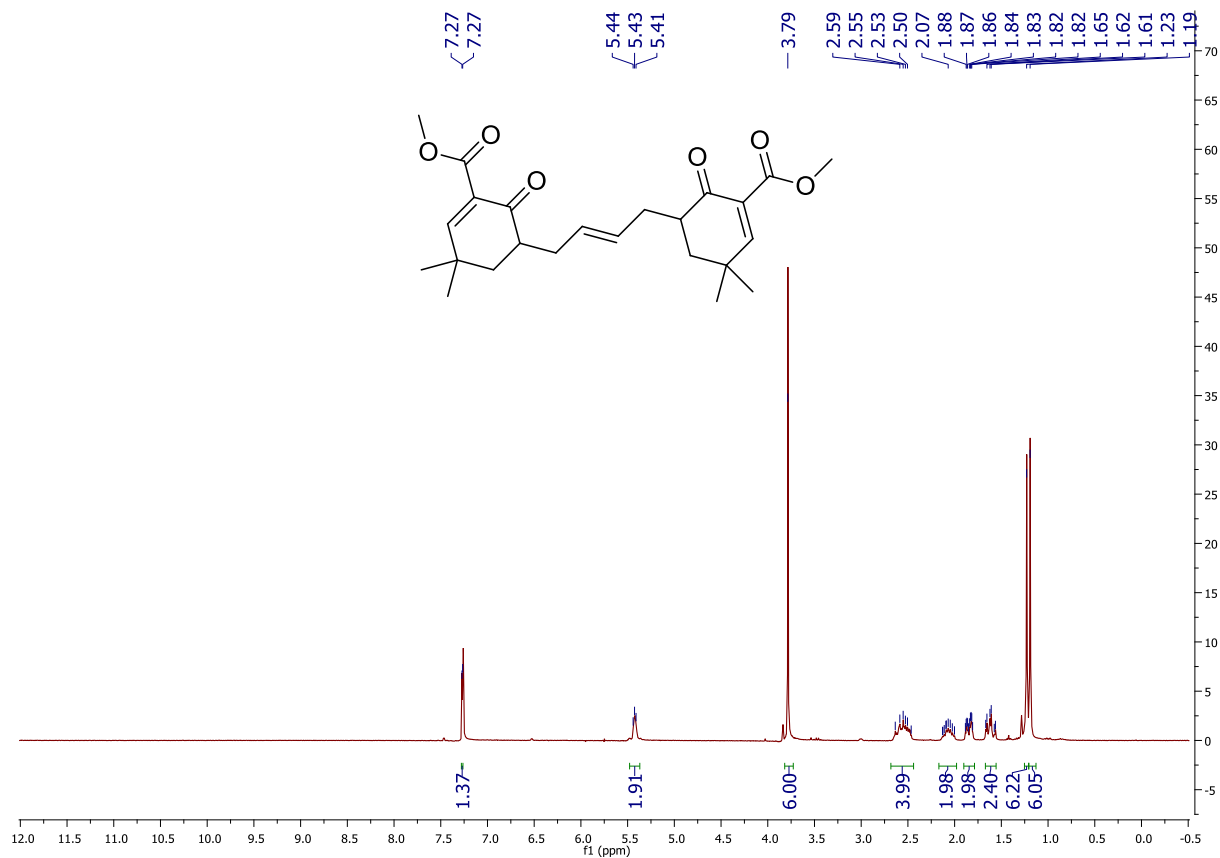
## <sup>1</sup>H NMR spectrum



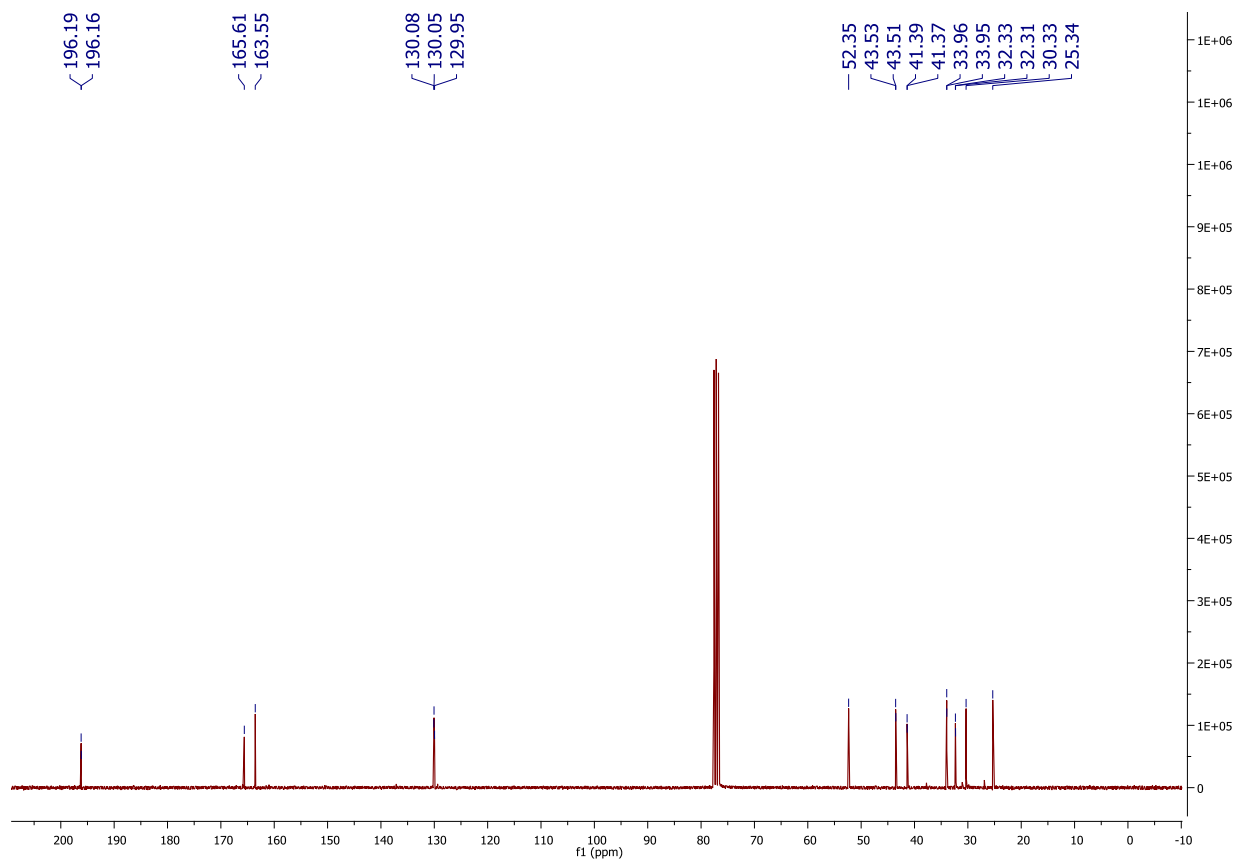
## <sup>13</sup>C NMR spectrum



**(E)-dimethyl 5,5'-(but-2-ene-1,4-diyl)bis(3,3-dimethyl-6-oxocyclohex-1-enecarboxylate) (33)**  
**<sup>1</sup>H NMR spectrum**



**<sup>13</sup>C NMR spectrum**

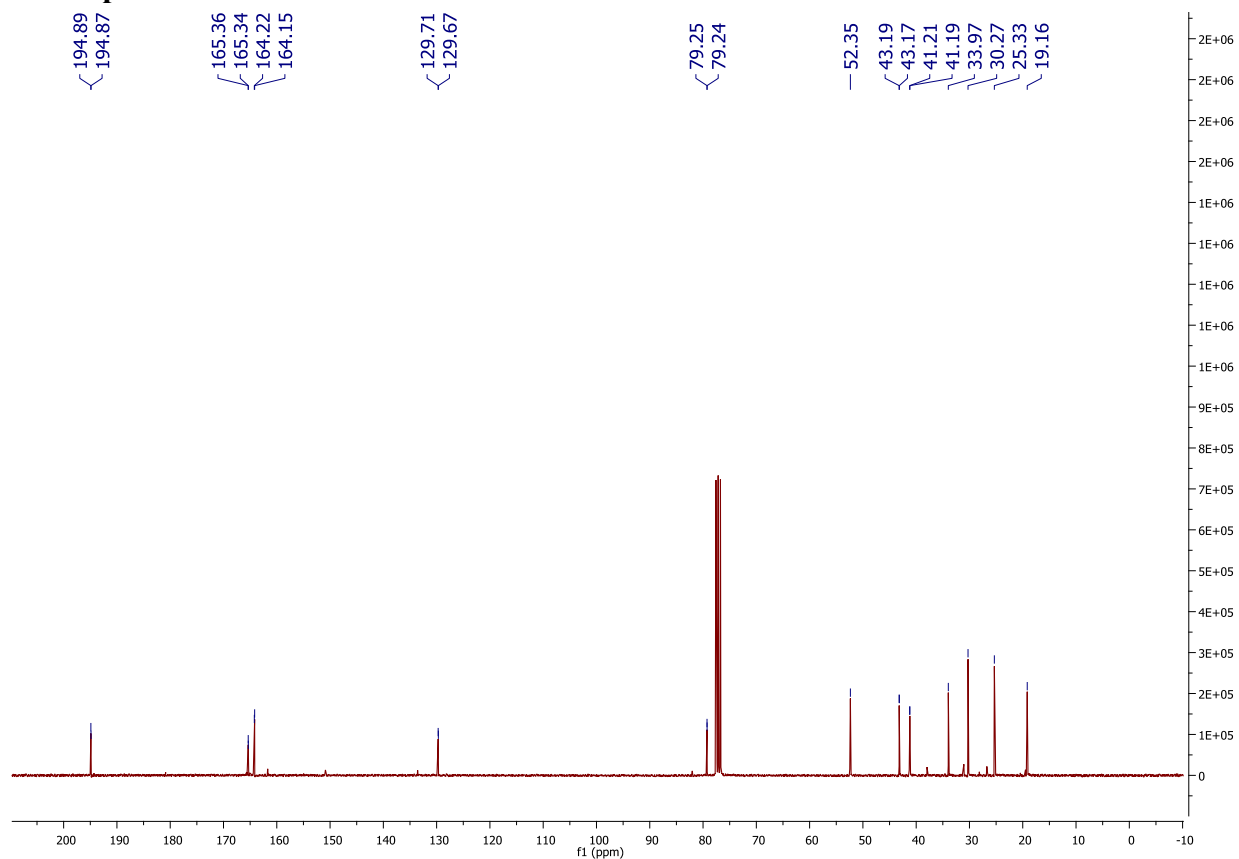


# Dimethyl 5,5'-(but-2-yne-1,4-diyl)bis(3,3-dimethyl-6-oxocyclohex-1-enecarboxylate) (34)

## <sup>1</sup>H NMR spectrum

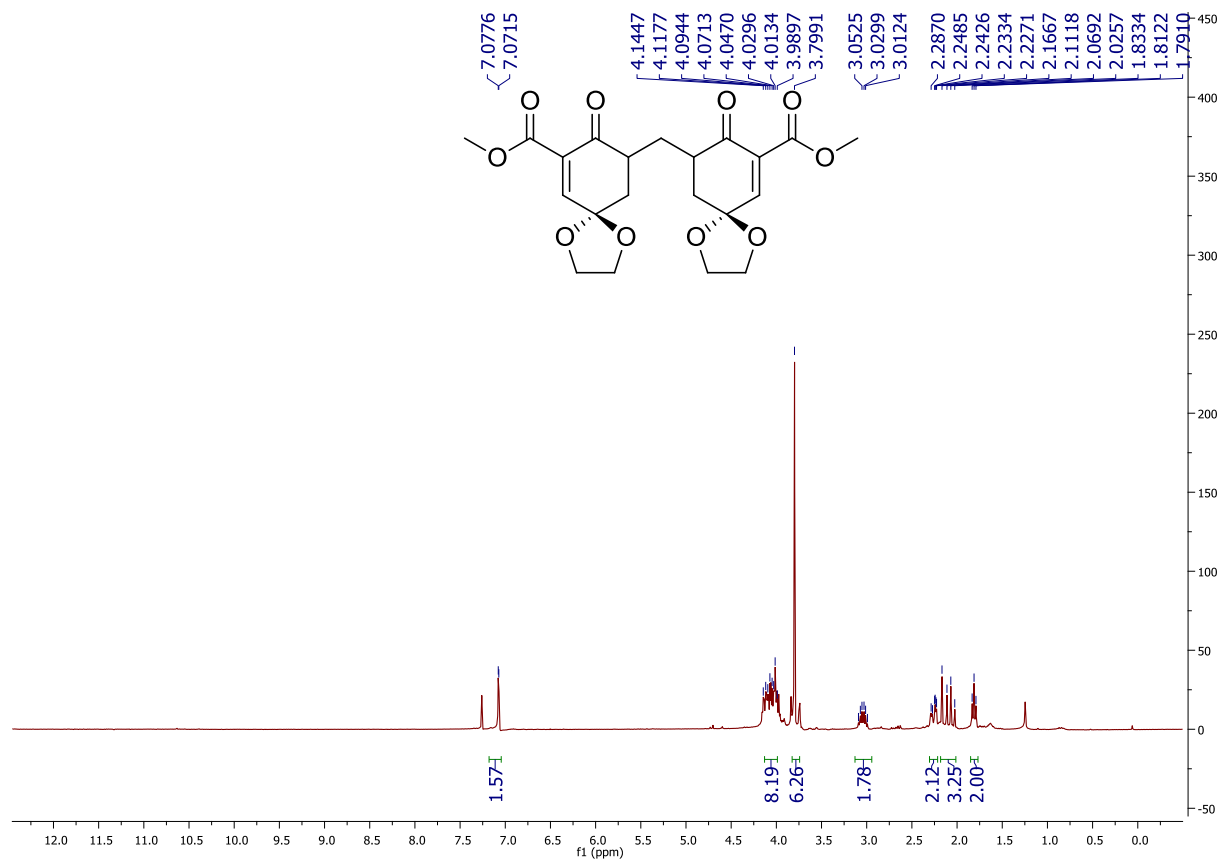


## <sup>13</sup>C NMR spectrum

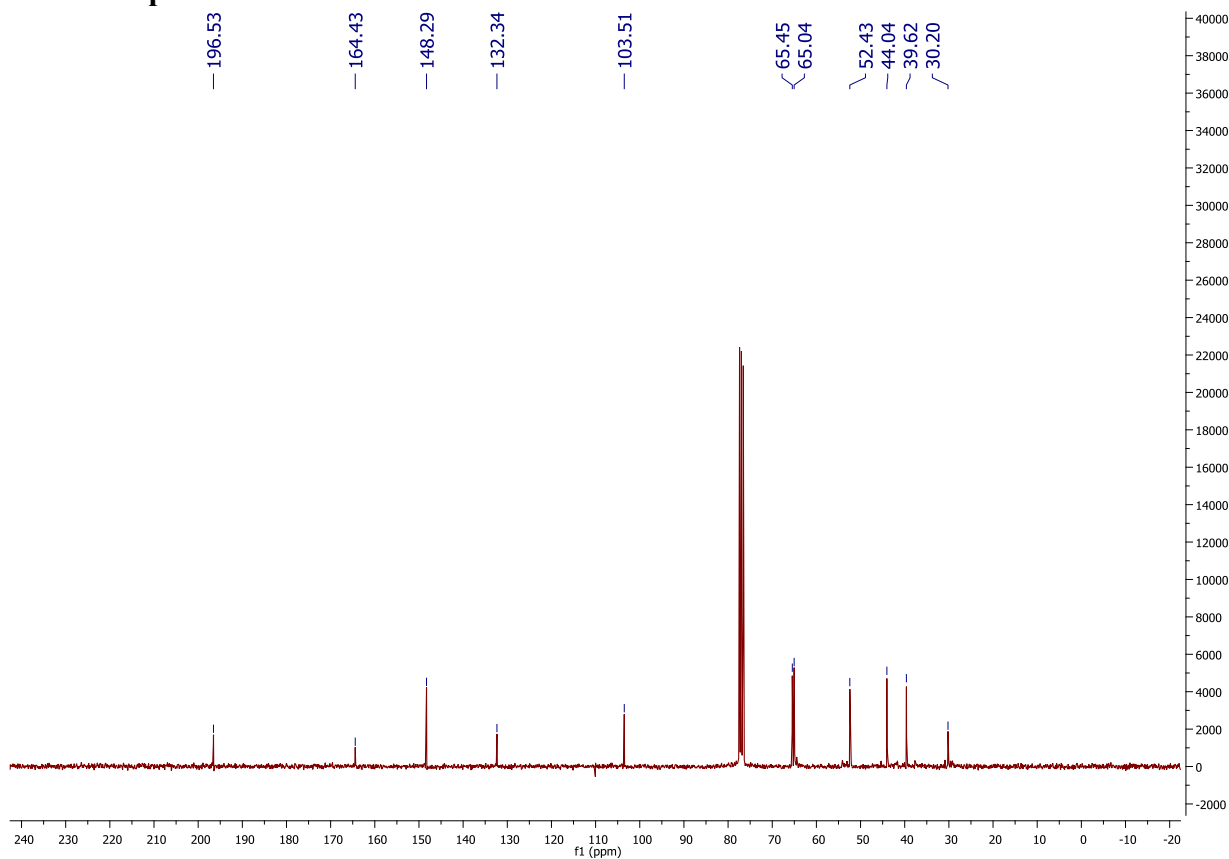


# Bis(methyl 8-oxo-1,4-dioxaspiro[4.5]dec-6-ene-7-carboxylate) methylene (35)

## <sup>1</sup>H NMR spectrum

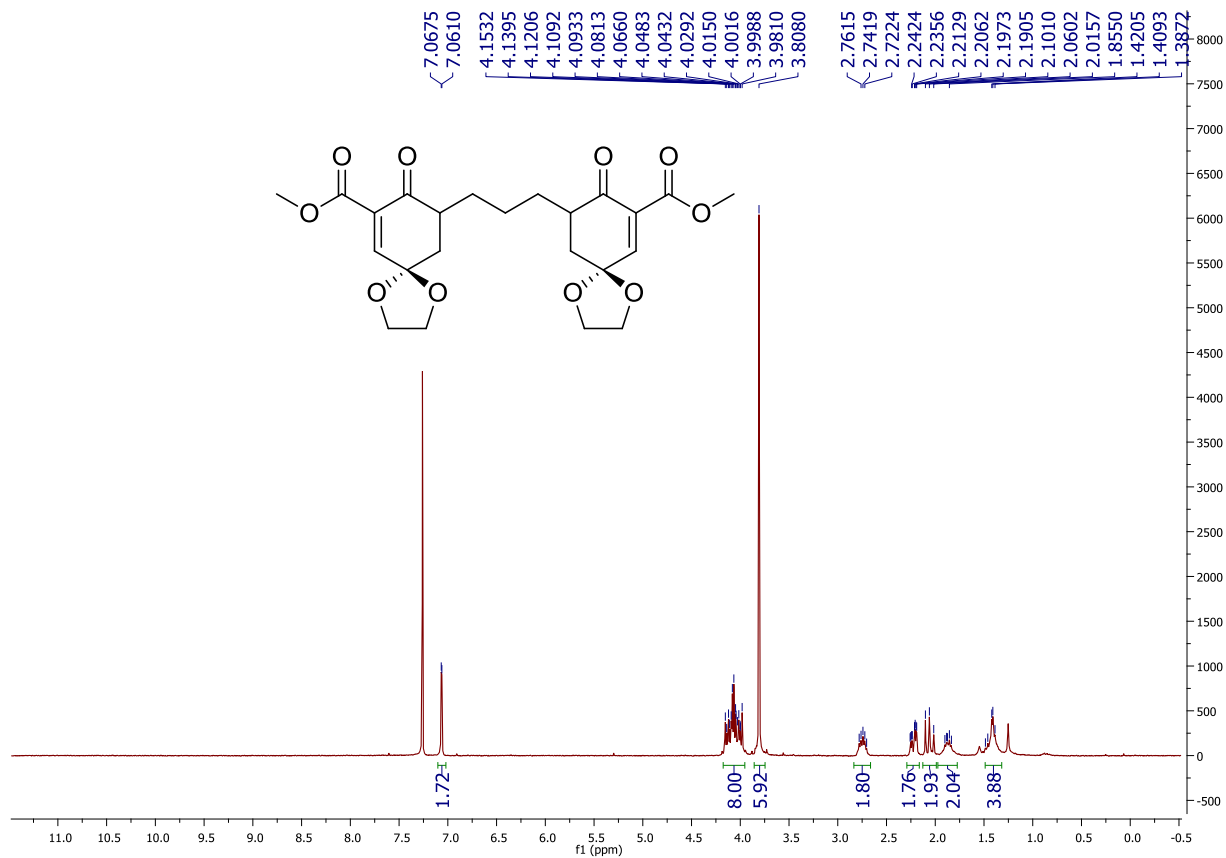


## <sup>13</sup>C NMR spectrum

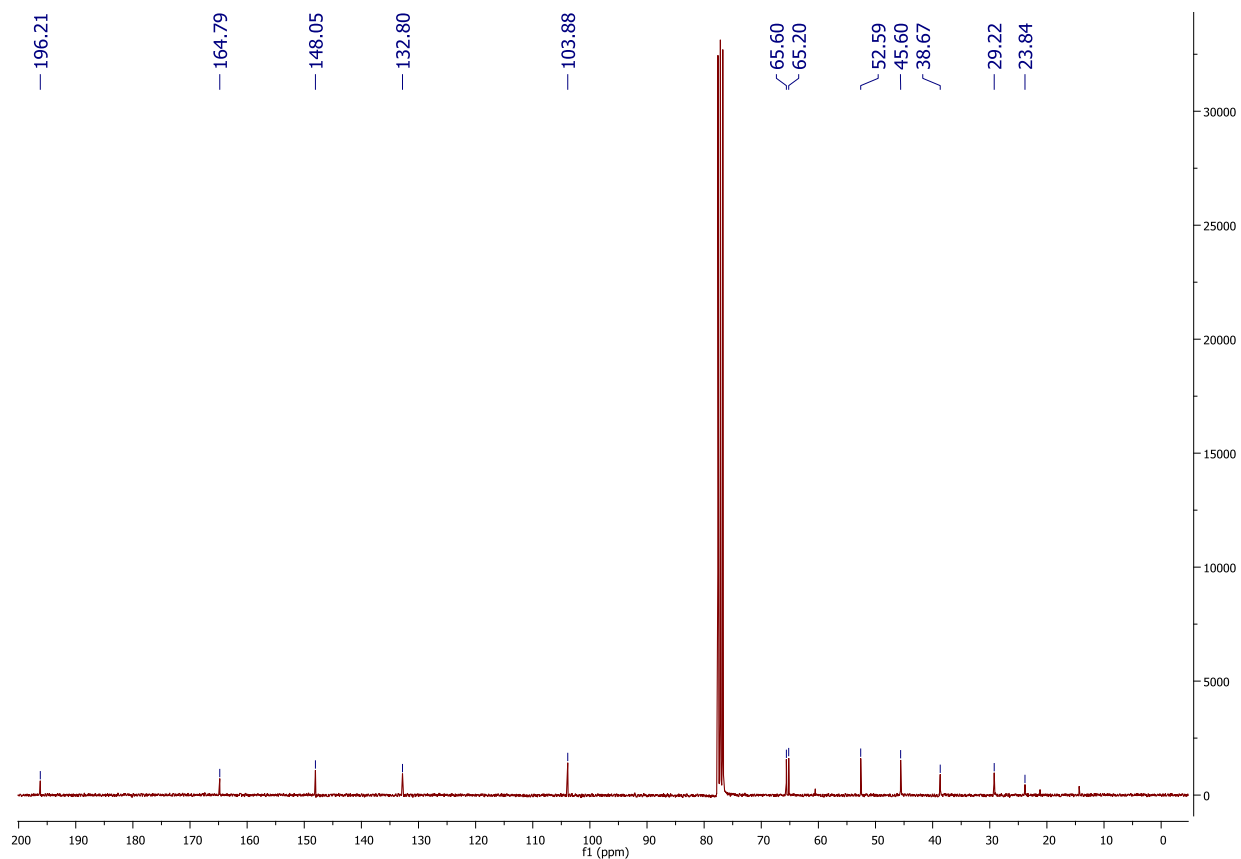


# Bis(methyl 8-oxo-1,4-dioxaspiro[4.5]dec-6-ene-7-carboxylate) propylene (36)

## <sup>1</sup>H NMR spectrum

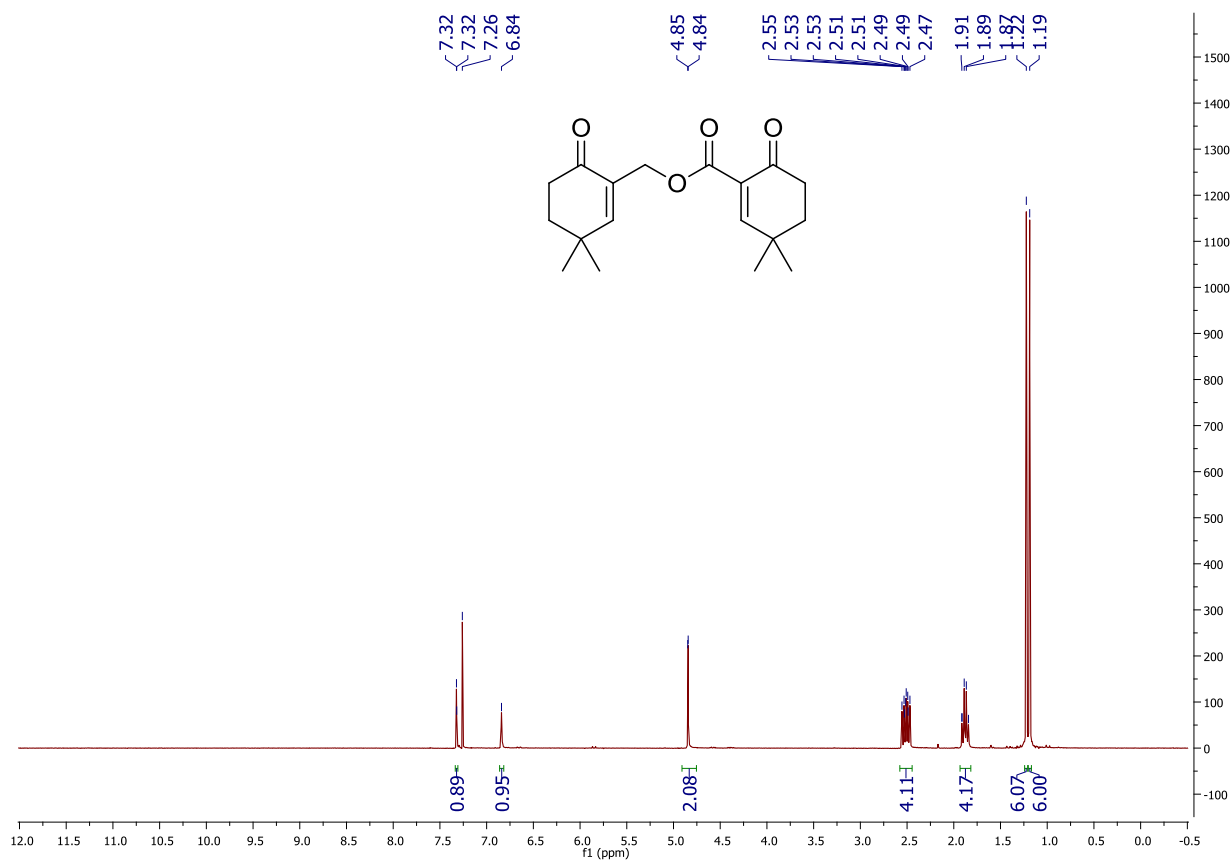


## <sup>13</sup>C NMR spectrum



# (3,3-dimethyl-6-oxocyclohex-1-en-1-yl)methyl 3,3-dimethyl-6-oxocyclohex-1-enecarboxylate (37)

## <sup>1</sup>H NMR spectrum



## <sup>13</sup>C NMR spectrum

