

## Design, Synthesis and Evaluation of *N*-Aryl-Glyoxamide Derivatives as Structurally Novel Bacterial Quorum Sensing Inhibitors

Shashidhar Nizalapur,<sup>a</sup> Önder Kimyon,<sup>b</sup> Nripendra Nath Biswas,<sup>a</sup> Christopher R. Gardner,<sup>a</sup> Renate Griffith,<sup>c</sup> Scott A. Rice,<sup>d, e</sup> Mike Manefield,<sup>b</sup> Mark Willcox,<sup>f</sup> David StC. Black<sup>a</sup> and Naresh Kumar<sup>a\*</sup>

- a. School of Chemistry, UNSW Australia, Sydney, NSW 2052, Australia.
- b. School of Biotechnology and Biomolecular Sciences (BABS), UNSW Australia, Sydney, NSW 2052, Australia.
- c. School of Medical Sciences, UNSW Australia, Sydney, NSW 2052, Australia.
- d. Centre for Marine Biology, School of Biological, Earth and Environmental Sciences, UNSW Australia, Sydney, NSW 2052, Australia.
- e. The Singapore Centre on Environmental Life Sciences Engineering and the School of Biological Sciences, Nanyang Technological University, Singapore.
- f. School of Optometry and Vision Science, UNSW Australia, Sydney, NSW 2052, Australia

\* Corresponding author

E-mail: n.kumar@unsw.edu.au\* Tel: +61 29385 4698; Fax: +61 29385 6141.

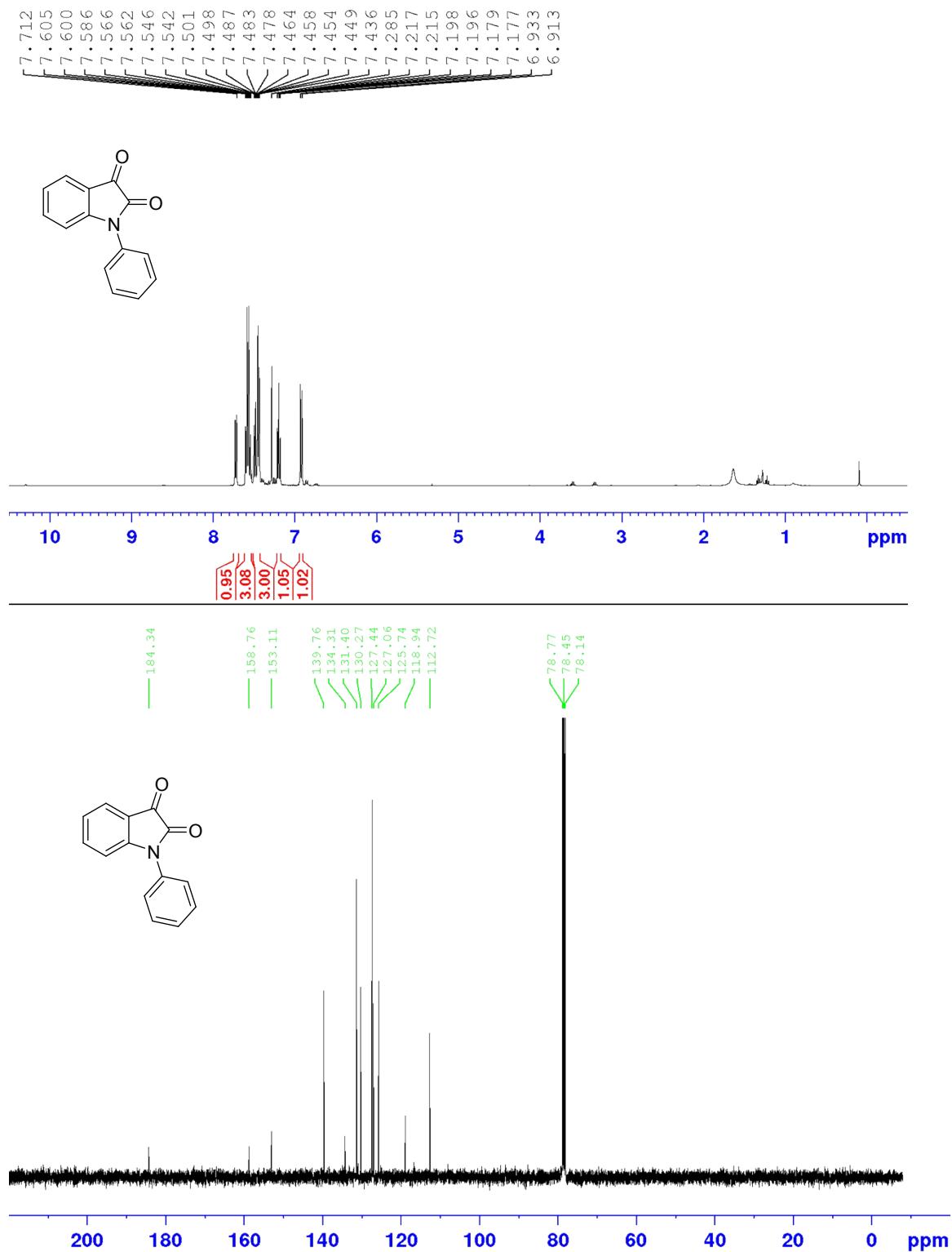
## Table of contents

• $^1\text{H}$ NMR and $^{13}\text{C}$ NMR spectra of 1-phenylindoline-2,3-dione ( <b>10</b> ) .....	4
• $^1\text{H}$ NMR and $^{13}\text{C}$ NMR spectra of 1-(4-methoxyphenyl) indoline-2,3-dione ( <b>11</b> ) .....	5
• $^1\text{H}$ NMR and $^{13}\text{C}$ NMR spectra of 1-(3-fluorophenyl) indoline-2, 3-dione ( <b>12</b> ) .....	6
• $^1\text{H}$ NMR and $^{13}\text{C}$ NMR spectra of 1-(4-fluorophenyl) indoline-2,3-dione ( <b>13</b> ) .....	7
• $^1\text{H}$ NMR and $^{13}\text{C}$ NMR spectra of 1-(4-nitrophenyl) indoline-2, 3-dione ( <b>14</b> ) .....	8
• $^1\text{H}$ NMR and $^{13}\text{C}$ NMR spectra of 1-(2,4-dinitrophenyl)indoline-2,3-dione ( <b>15</b> ) .....	9
• $^1\text{H}$ NMR and $^{13}\text{C}$ NMR spectra of 1-(2-(phenylamino) phenyl)-2-(pyrrolidin-1-yl) ethane-1,2-dione ( <b>16</b> ) .....	10
• $^1\text{H}$ NMR and $^{13}\text{C}$ NMR spectra of 1-(2-(phenylamino)phenyl)-2-(piperidin-1-yl)ethane-1,2-dione ( <b>17</b> ) .....	11
• $^1\text{H}$ NMR and $^{13}\text{C}$ NMR spectra of 1-(2-((3-fluorophenyl)amino)phenyl)-2-(pyrrolidin-1-yl)ethane-1,2-dione ( <b>18</b> ) .....	13
• $^1\text{H}$ NMR and $^{13}\text{C}$ NMR spectra of 1-(2-((4-fluorophenyl)amino)phenyl)-2-(pyrrolidin-1-yl)ethane-1,2-dione ( <b>19</b> ) .....	15
• $^1\text{H}$ NMR and $^{13}\text{C}$ NMR spectra of 1-(2-((4-fluorophenyl)amino)phenyl)-2-(piperidin-1-yl)ethane-1,2-dione ( <b>20</b> ) .....	17
• $^1\text{H}$ NMR and $^{13}\text{C}$ NMR spectra of 1-(2-((4-nitrophenyl)amino)phenyl)-2-(pyrrolidin-1-yl)ethane-1,2-dione ( <b>21</b> ) .....	19
• $^1\text{H}$ NMR and $^{13}\text{C}$ NMR spectra of 1-(2-((4-methoxyphenyl)amino)phenyl)-2-(pyrrolidin-1-yl)ethane-1,2-dione ( <b>22</b> ) .....	20
• $^1\text{H}$ NMR and $^{13}\text{C}$ NMR spectra of 1-(2-((2,4-dinitrophenyl)amino)phenyl)-2-(pyrrolidin-1-yl)ethane-1,2-dione ( <b>23</b> ) .....	21
• $^1\text{H}$ NMR and $^{13}\text{C}$ NMR spectra of 1-(2-((2,4-dinitrophenyl)amino)phenyl)-2-(piperidin-1-yl)ethane-1,2-dione ( <b>24</b> ) .....	23
• $^1\text{H}$ NMR and $^{13}\text{C}$ NMR spectra of <i>N</i> -butyl-2-oxo-2-(2-(phenylamino)phenyl)acetamide ( <b>25</b> ) .....	24
• $^1\text{H}$ NMR and $^{13}\text{C}$ NMR spectra of <i>N</i> -octyl-2-oxo-2-(2-(phenylamino)phenyl)acetamide ( <b>26</b> ) .....	25
• $^1\text{H}$ NMR and $^{13}\text{C}$ NMR spectra of <i>N</i> -butyl-2-(2-((3-fluorophenyl)amino)phenyl)-2-oxoacetamide ( <b>27</b> ) .....	26
• $^1\text{H}$ NMR and $^{13}\text{C}$ NMR spectra of <i>N</i> -butyl-2-(2-((4-methoxyphenyl)amino)phenyl)-2-oxoacetamide ( <b>28</b> ) .....	28
• $^1\text{H}$ NMR and $^{13}\text{C}$ NMR spectra of <i>N</i> -butyl-2-(2-((2,4-dinitrophenyl)amino)phenyl)-2-oxoacetamide ( <b>29</b> ) .....	29

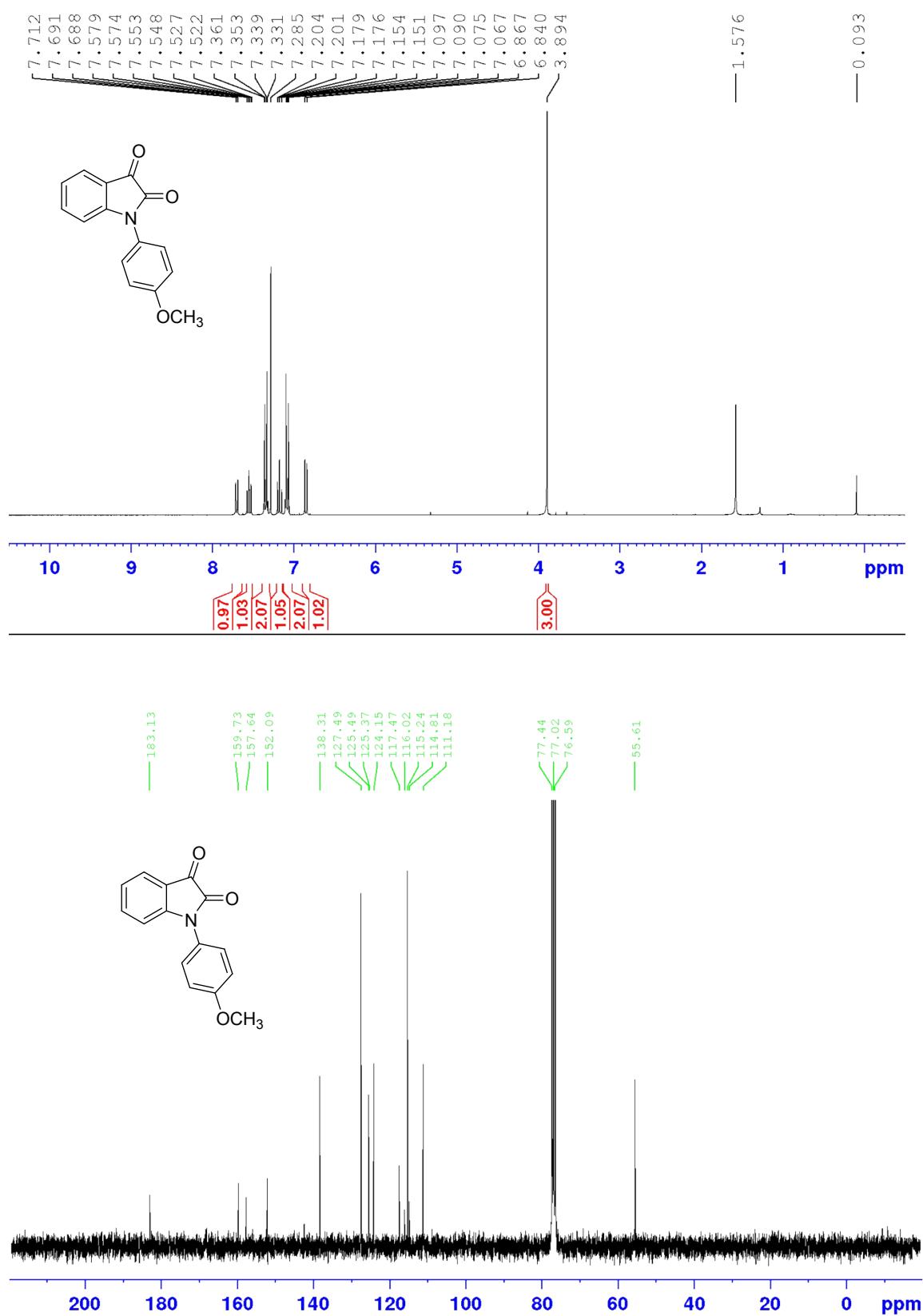
• <sup>1</sup> HNMR and <sup>13</sup> CNMR spectra of 2-(2-((2,4-dinitrophenyl)amino)phenyl)-2-oxo- <i>N</i> -pentylacetamide ( <b>30</b> ).....	31
• <sup>1</sup> HNMR and <sup>13</sup> CNMR spectra of Ethyl (2-(2-((2,4-dinitrophenyl)amino)phenyl)-2-oxoacetyl)glycinate( <b>31</b> ) .....	32
• <sup>1</sup> HNMR and <sup>13</sup> CNMR spectra of Methyl (2-(2-((2,4-dinitrophenyl)amino)phenyl)-2-oxoacetyl)-L-alaninate ( <b>32</b> ) .....	33
• <sup>1</sup> HNMR and <sup>13</sup> CNMR spectra of Methyl (2-(2-((2,4-dinitrophenyl)amino)phenyl)-2-oxoacetyl)-L-valinate ( <b>33</b> ) .....	34
• <sup>1</sup> HNMR and <sup>13</sup> CNMR spectra of Methyl (2-(2-((2,4-dinitrophenyl)amino)phenyl)-2-oxoacetyl)-L-leucinate ( <b>34</b> ) .....	35
• <sup>1</sup> HNMR and <sup>13</sup> CNMR spectra of Methyl (2-(2-((2,4-dinitrophenyl)amino)phenyl)-2-oxoacetyl)-L-phenylalaninate ( <b>35</b> ) .....	37
• <sup>1</sup> HNMR and <sup>13</sup> CNMR spectra of Methyl (2-(2-((2,4-dinitrophenyl)amino)phenyl)-2-oxoacetyl)-L-tryptophanate ( <b>36</b> ).....	38
• Table 1: Growth inhibition by the synthesized compounds against the PAMH602 and <i>E. coli</i> MT102 strains at three different concentrations .....	39

## NMR Spectra

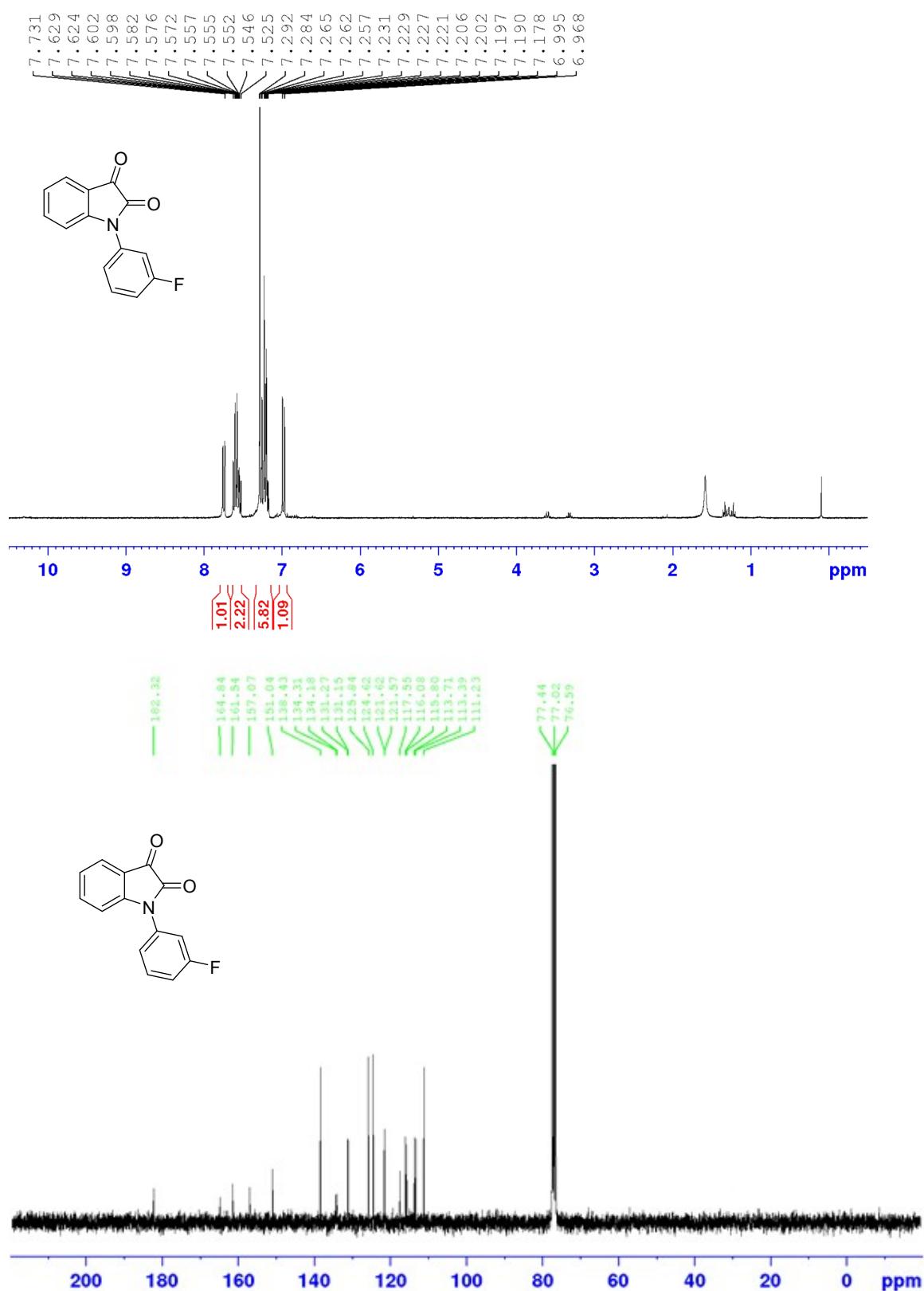
### 1-phenylindoline-2,3-dione (**10**)



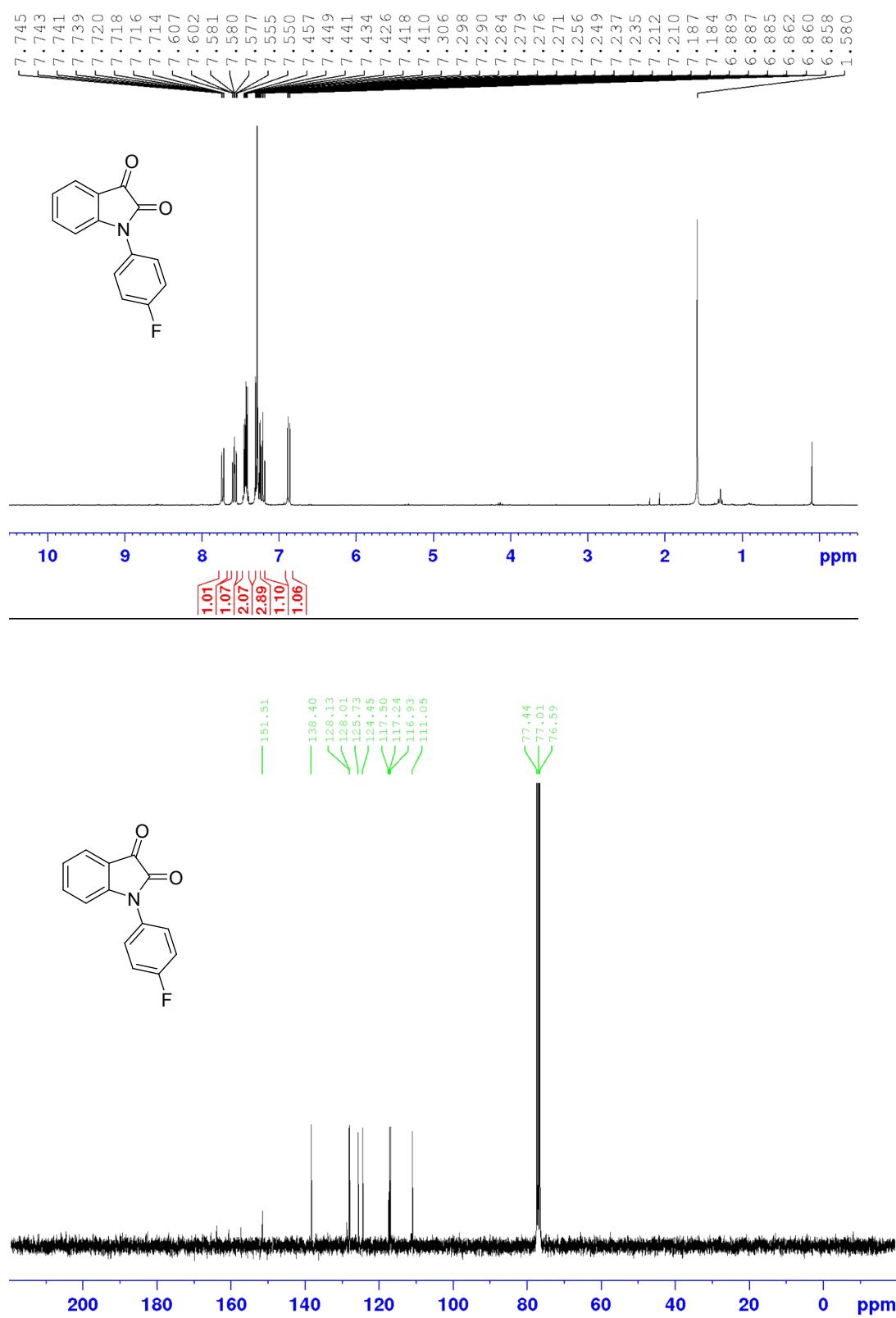
<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of 1-(4-methoxyphenyl) indoline-2,3-dione (**11**)



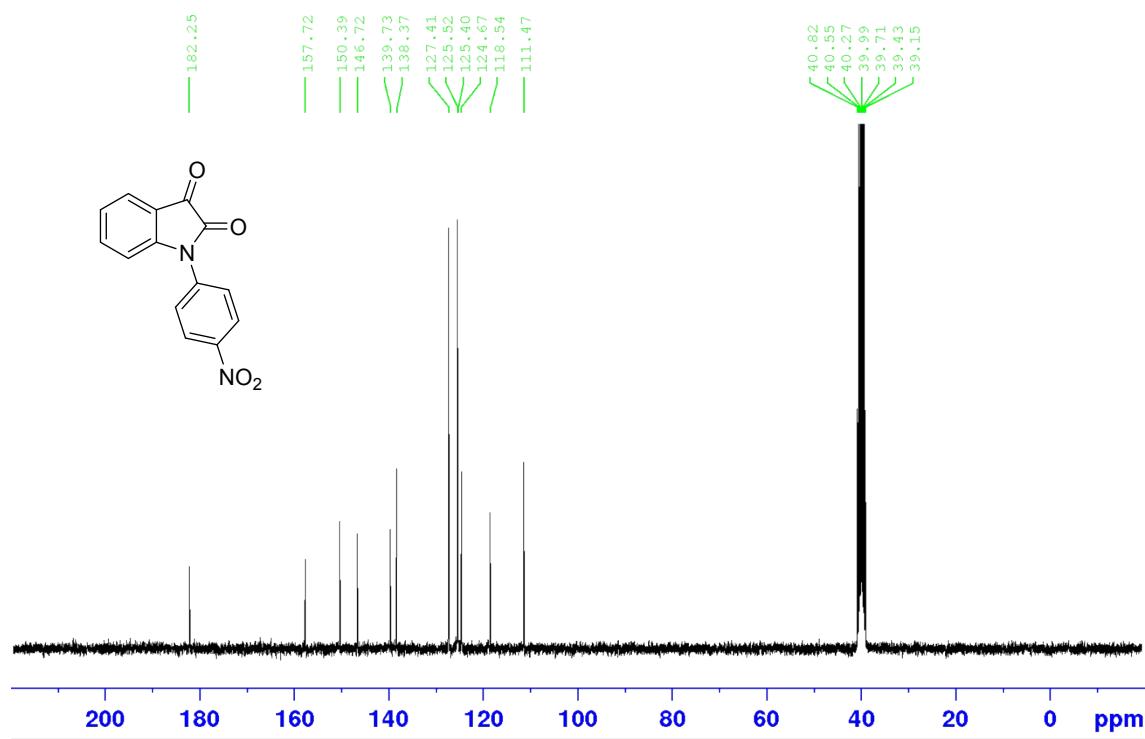
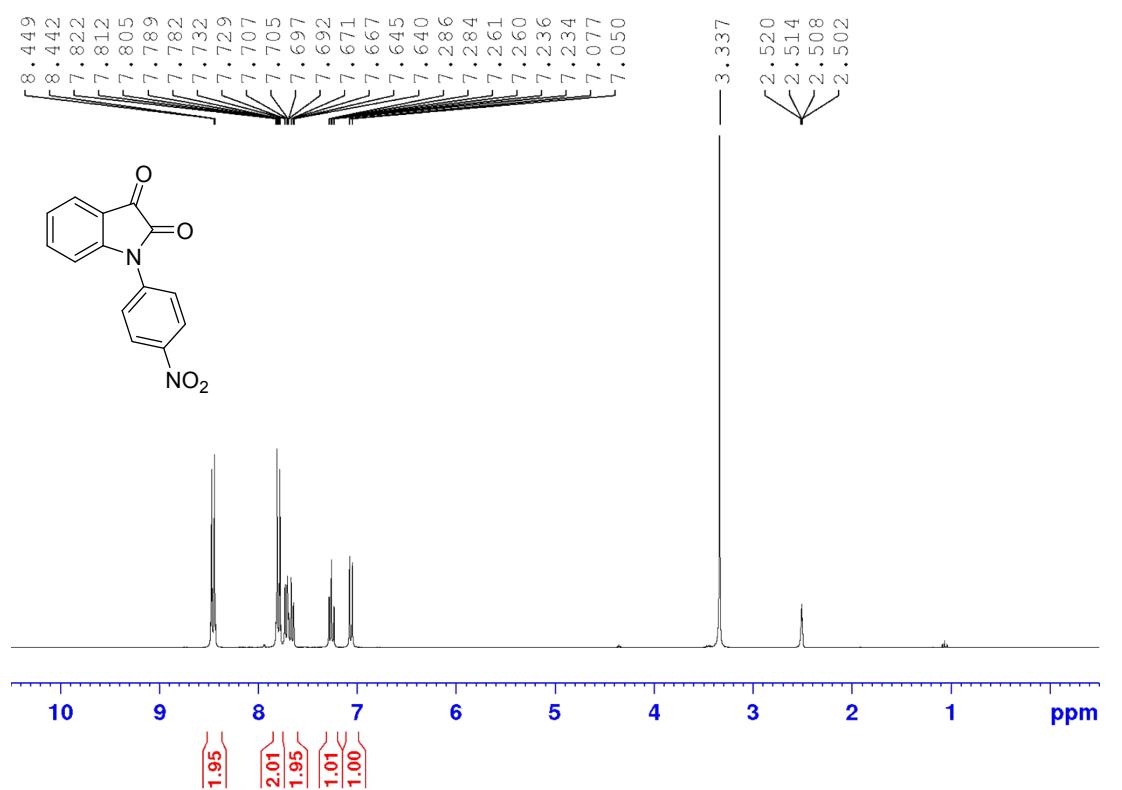
<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of 1-(3-fluorophenyl) indoline-2, 3-dione (**12**)



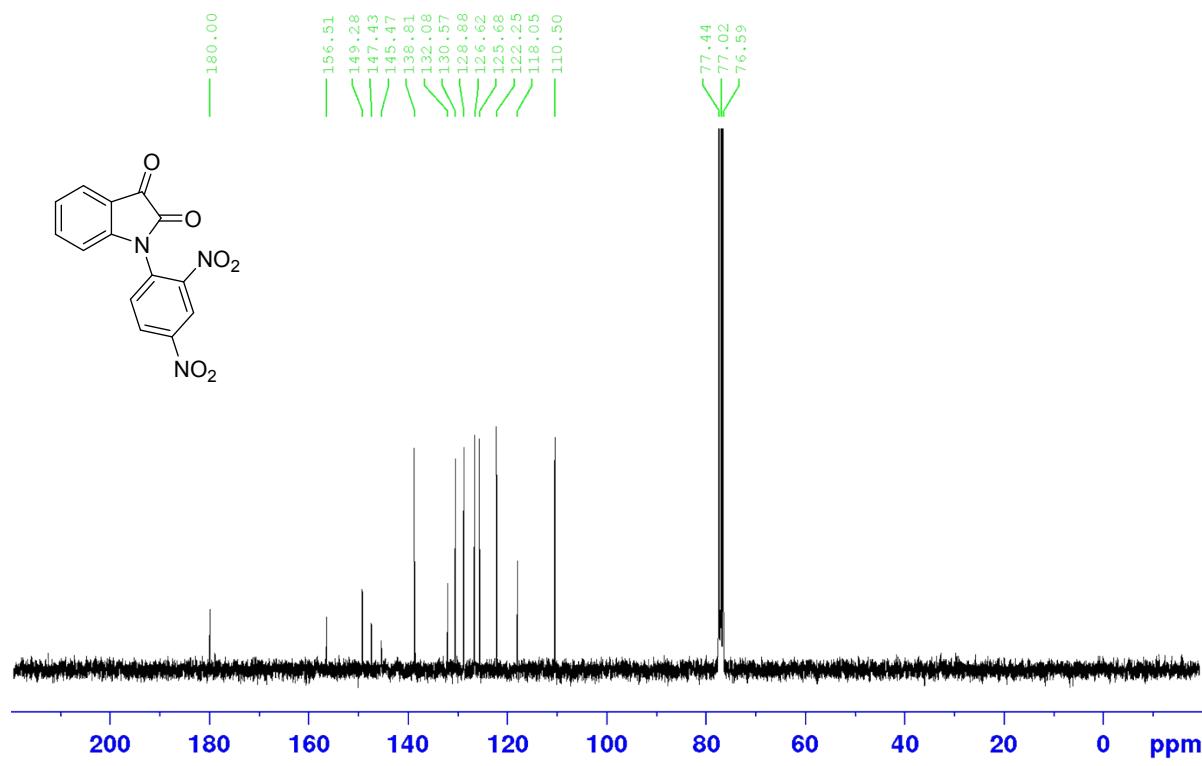
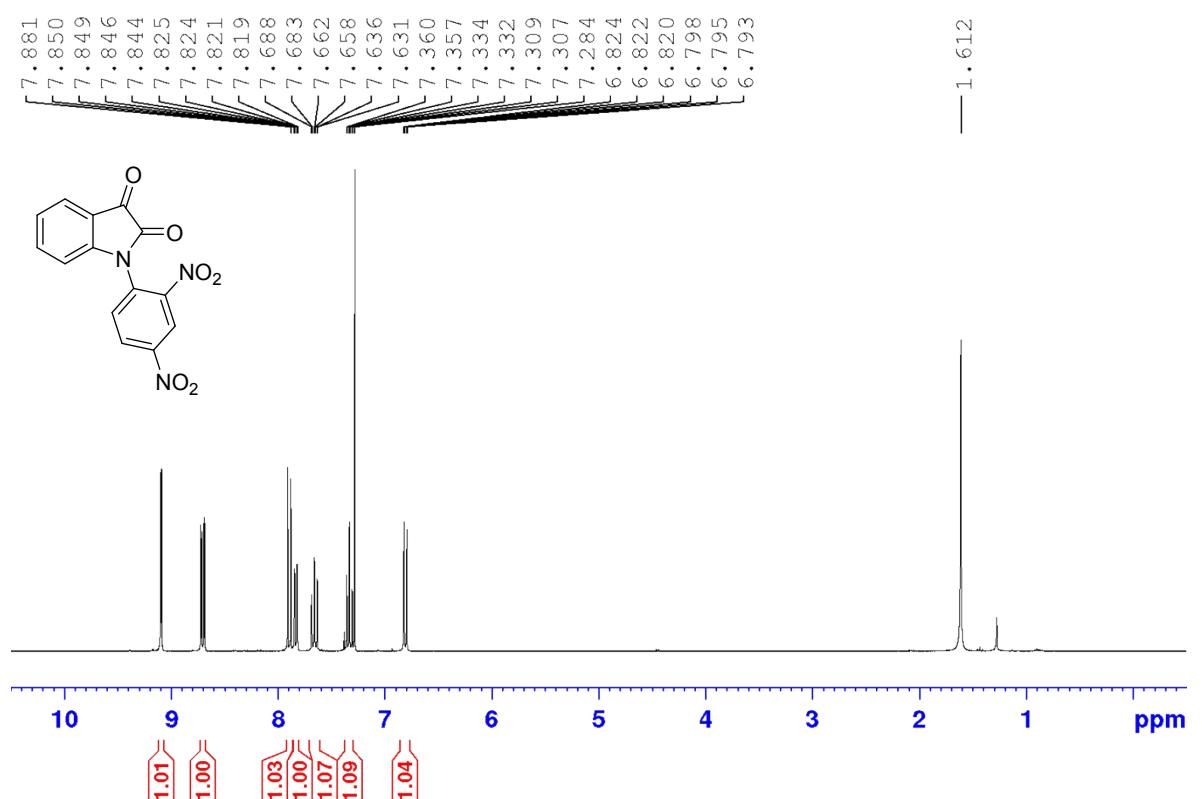
<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of 1-(4-fluorophenyl) indoline-2,3-dione (**13**)



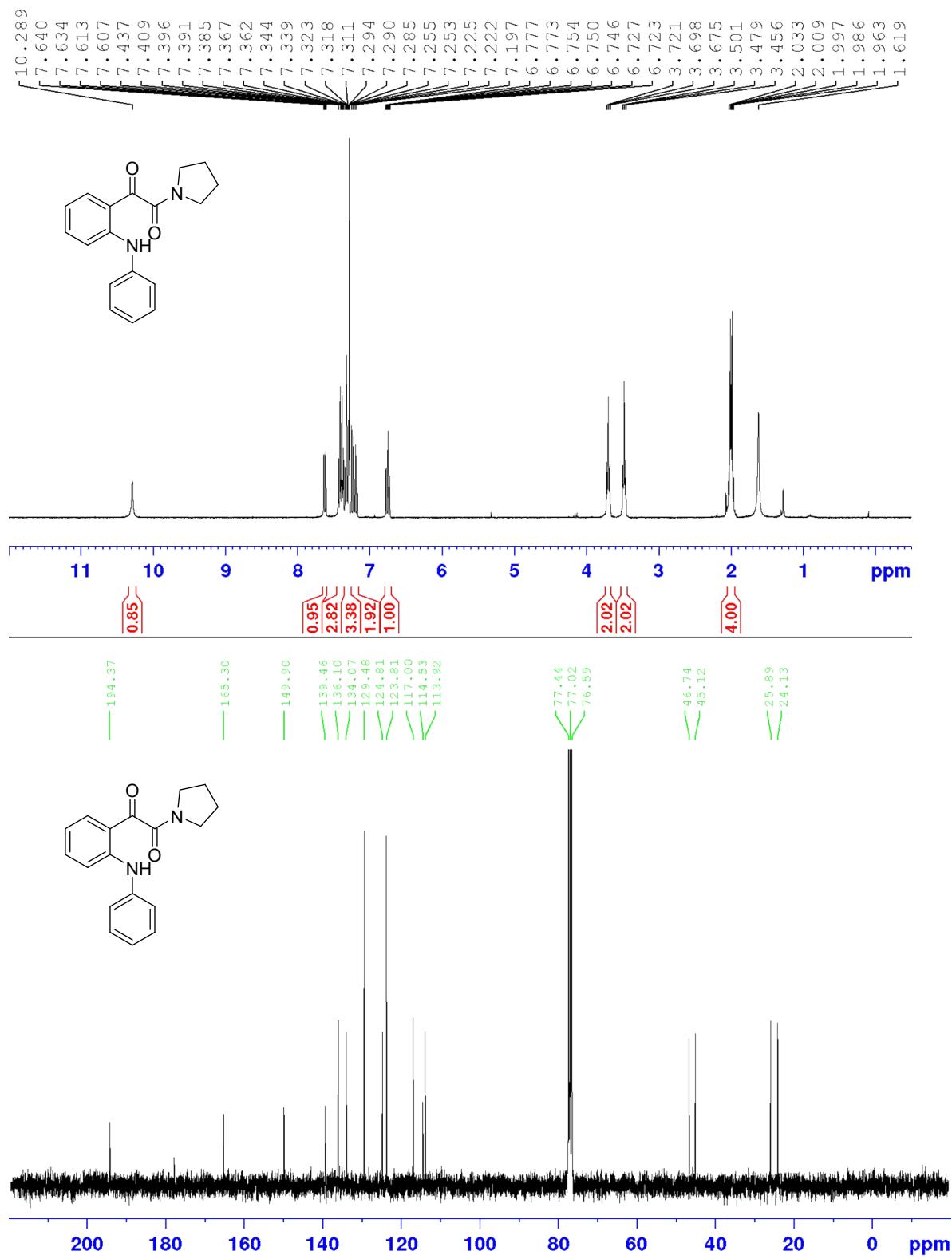
<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of 1-(4-nitrophenyl) indoline-2, 3-dione (**14**)



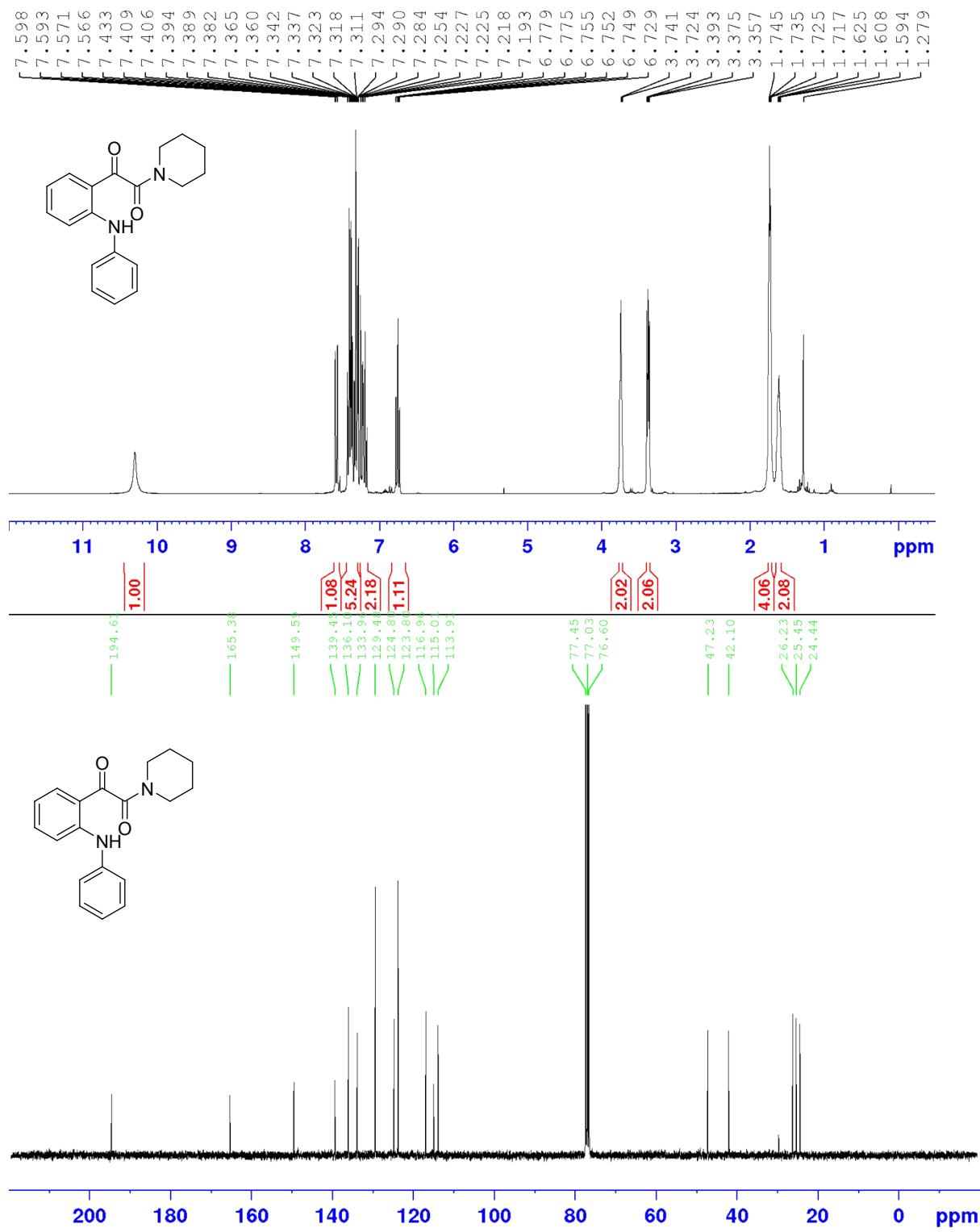
<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of 1-(2,4-dinitrophenyl)indoline-2,3-dione (**15**)



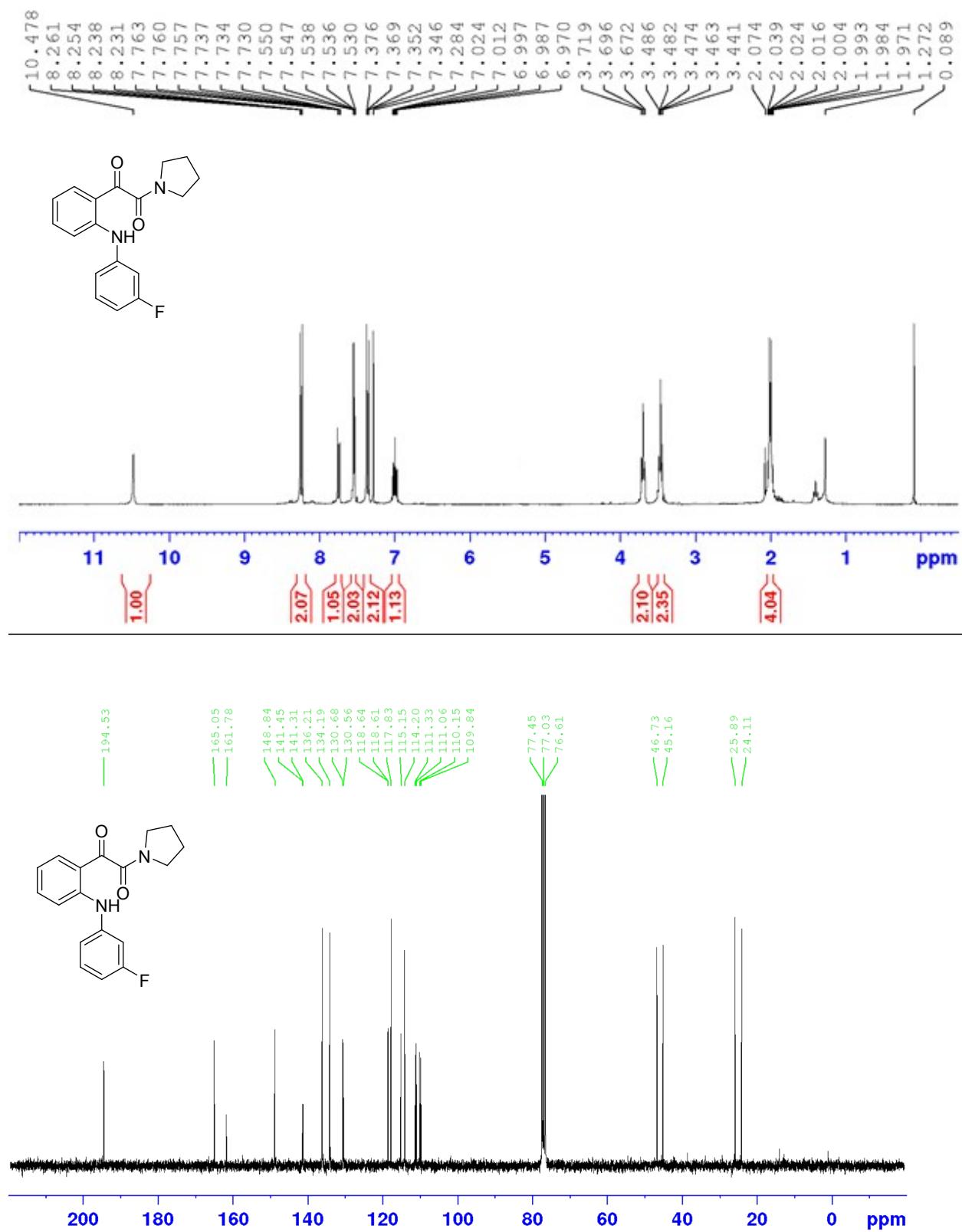
<sup>1</sup>HNMR and <sup>13</sup>CNMR spectra of 1-(2-(phenylamino) phenyl)-2-(pyrrolidin-1-yl) ethane-1,2-dione (**16**)



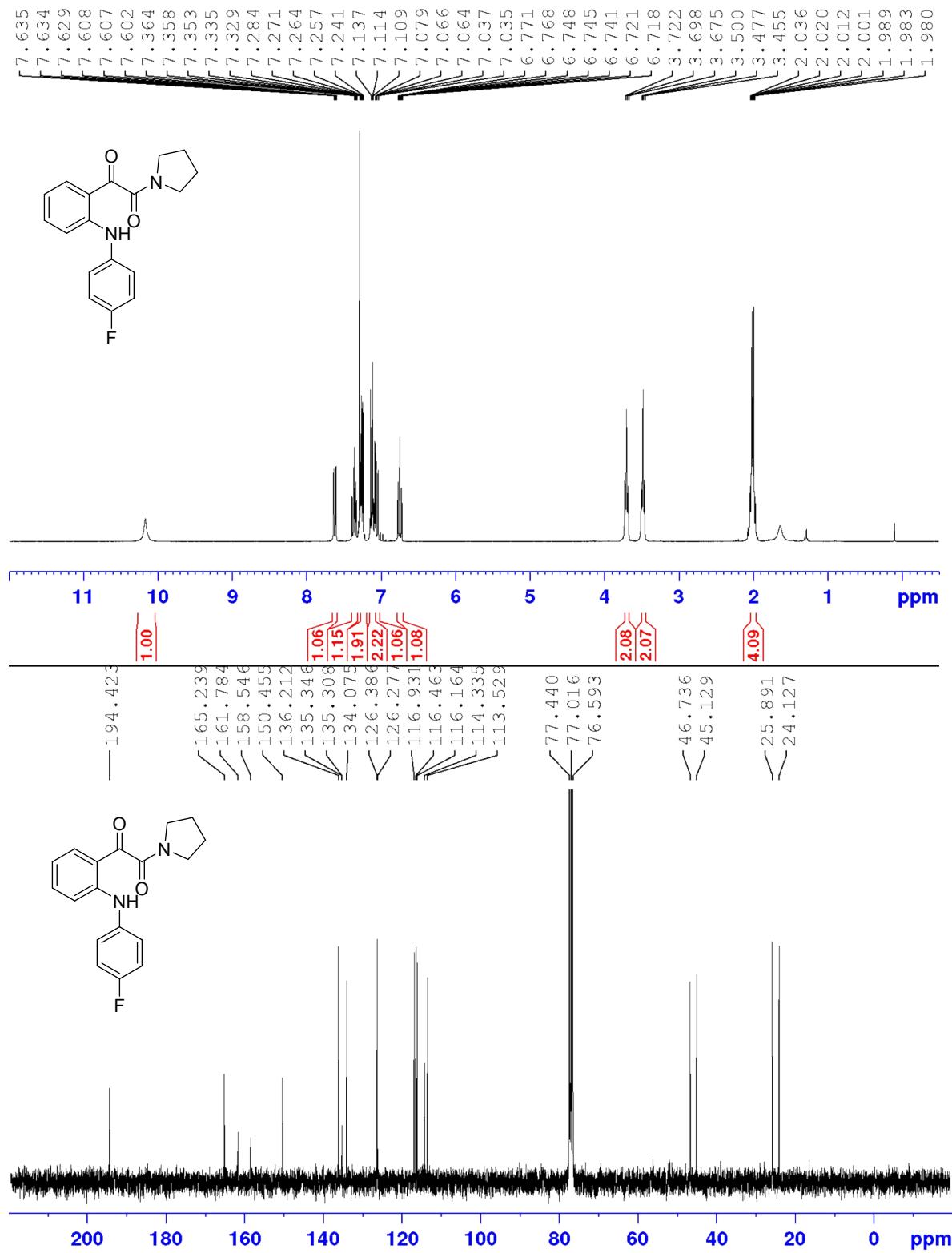
<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of 1-(2-(phenylamino)phenyl)-2-(piperidin-1-yl)ethane-1,2-dione (**17**)



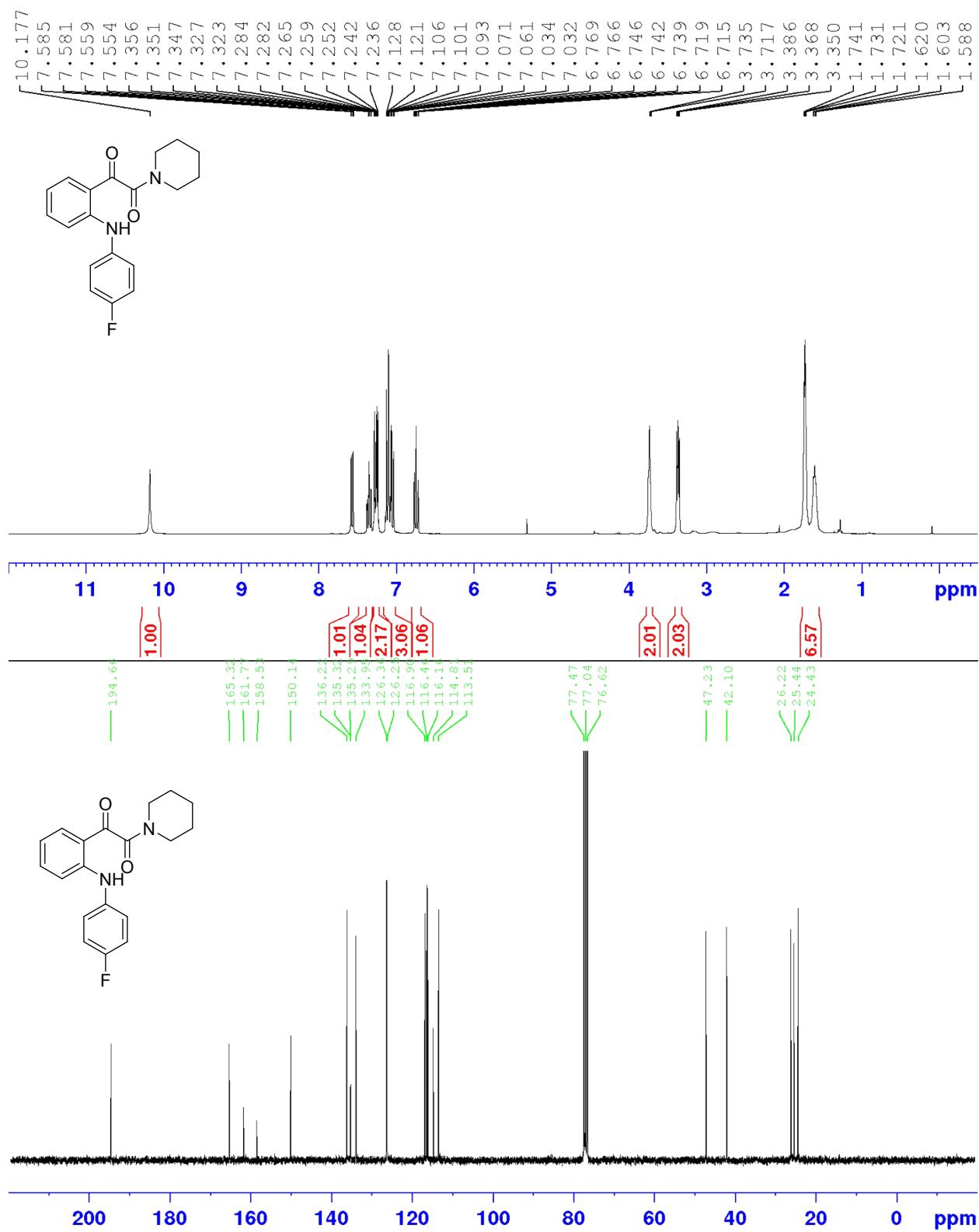
<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of 1-(2-((3-fluorophenyl)amino)phenyl)-2-(pyrrolidin-1-yl)ethane-1,2-dione (**18**)



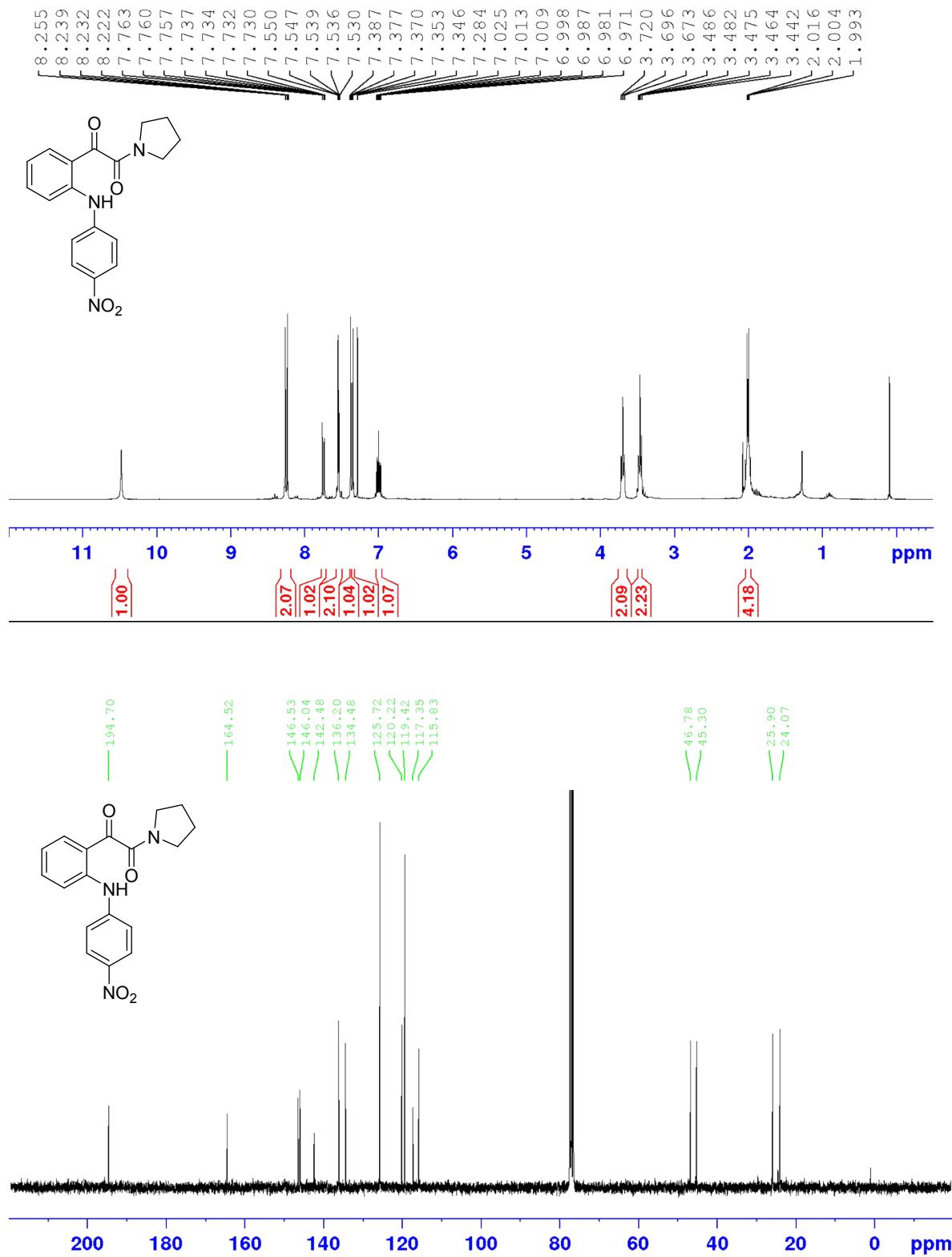
<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of 1-(2-((4-fluorophenyl)amino)phenyl)-2-(pyrrolidin-1-yl)ethane-1,2-dione (**19**)



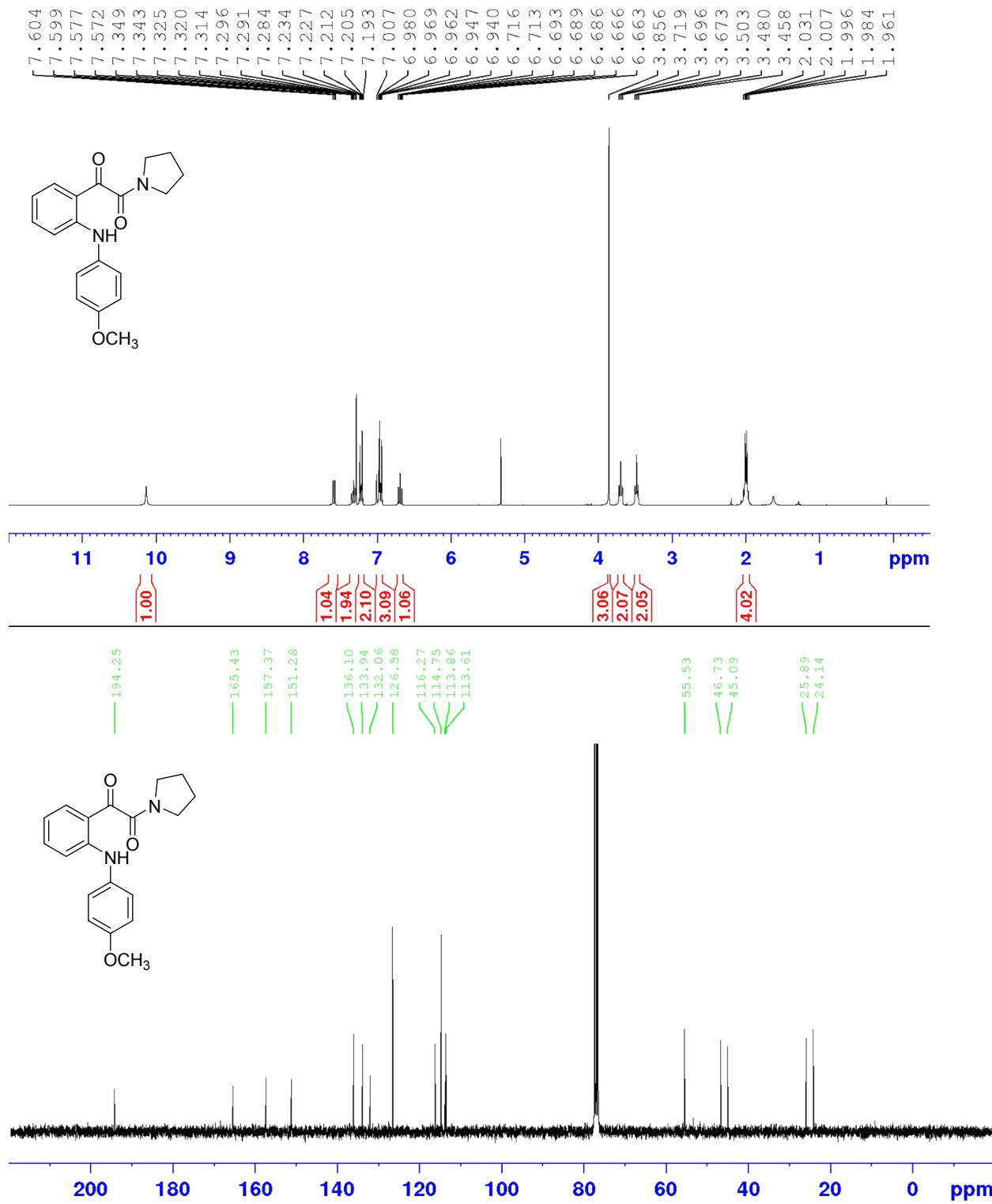
<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of 1-(2-((4-fluorophenyl)amino)phenyl)-2-(piperidin-1-yl)ethane-1,2-dione (**20**)



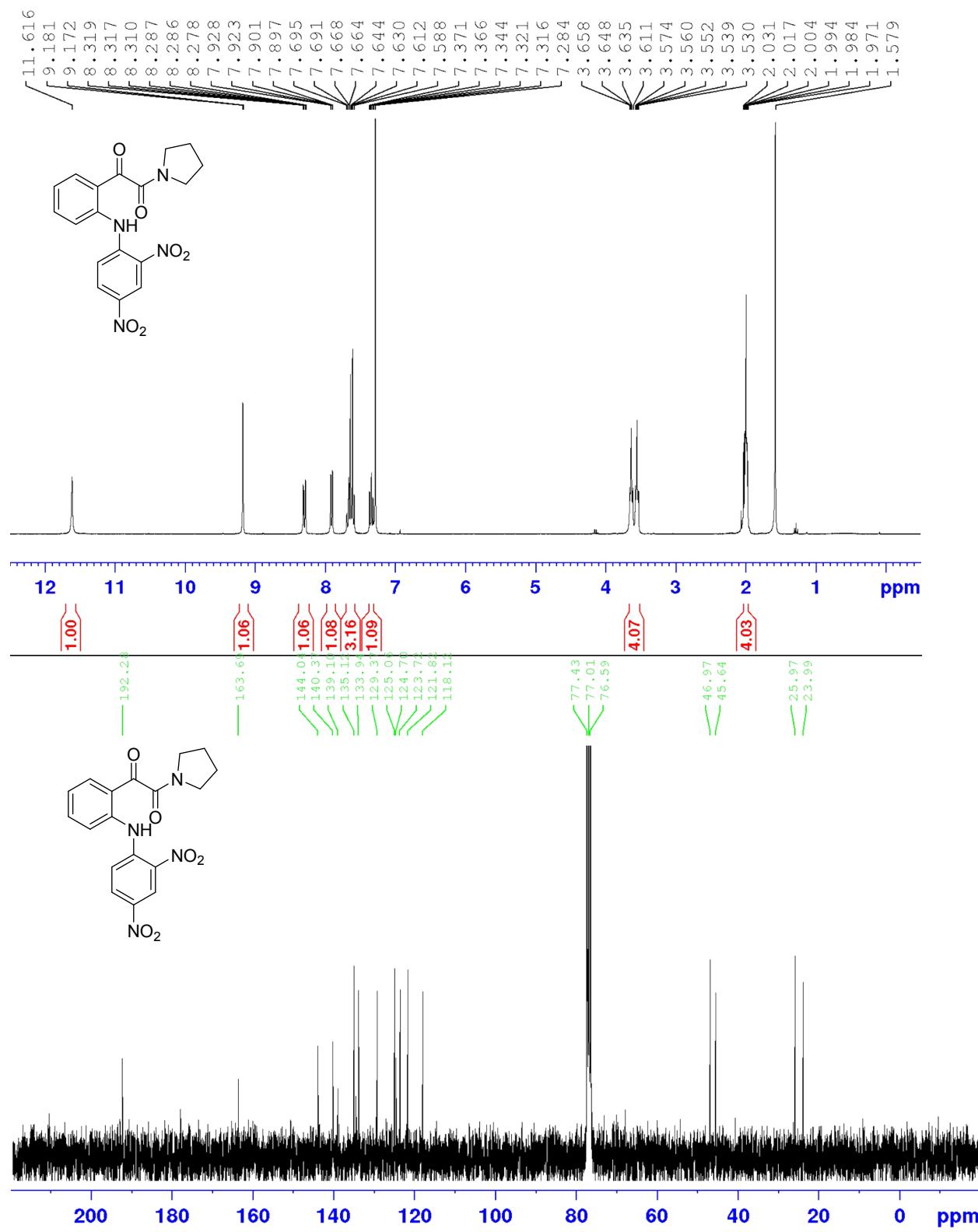
<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of 1-(2-((4-nitrophenyl)amino)phenyl)-2-(pyrrolidin-1-yl)ethane-1,2-dione (**21**)



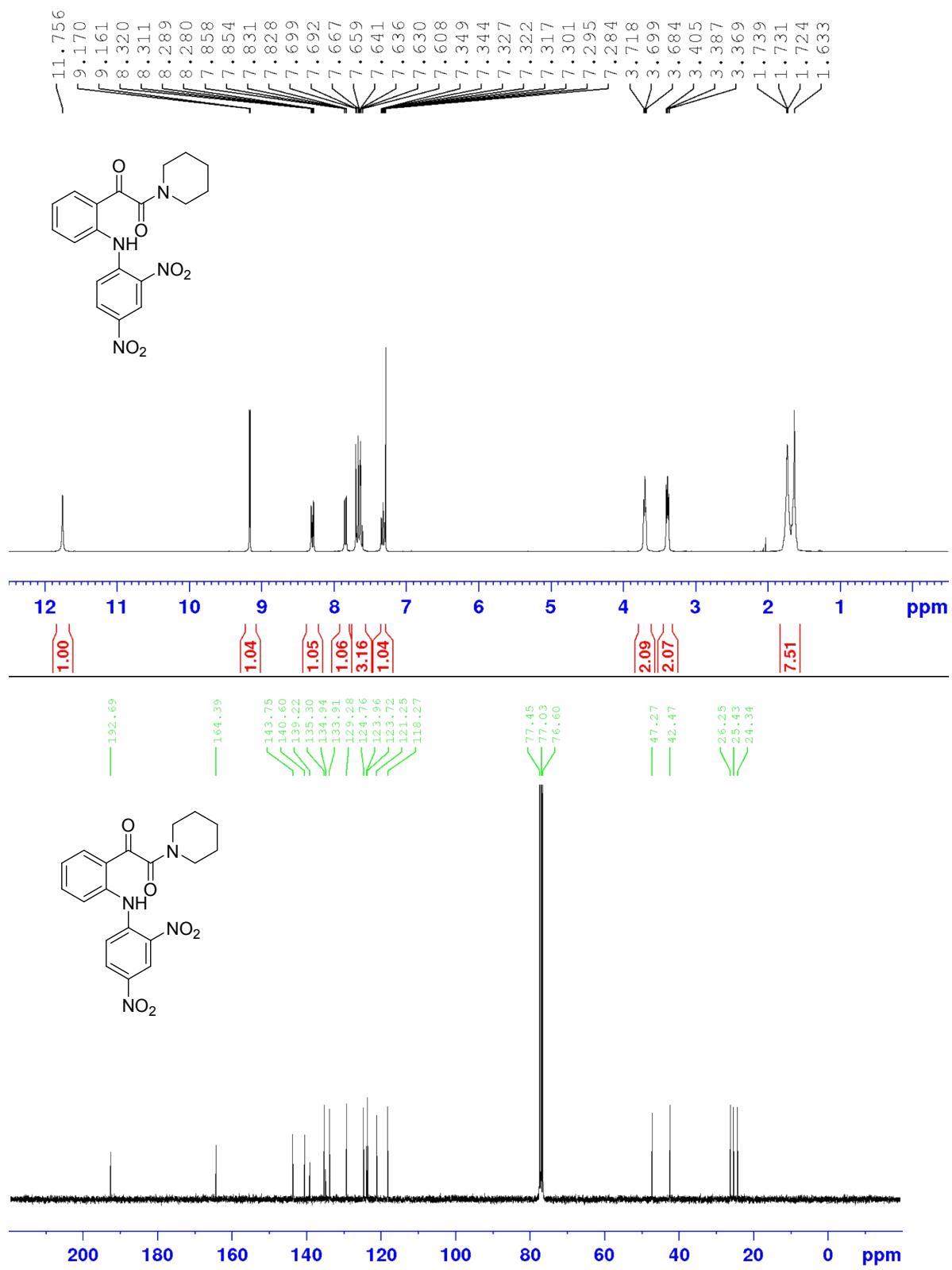
<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of 1-(2-((4-methoxyphenyl)amino)phenyl)-2-(pyrrolidin-1-yl)ethane-1,2-dione (**22**)



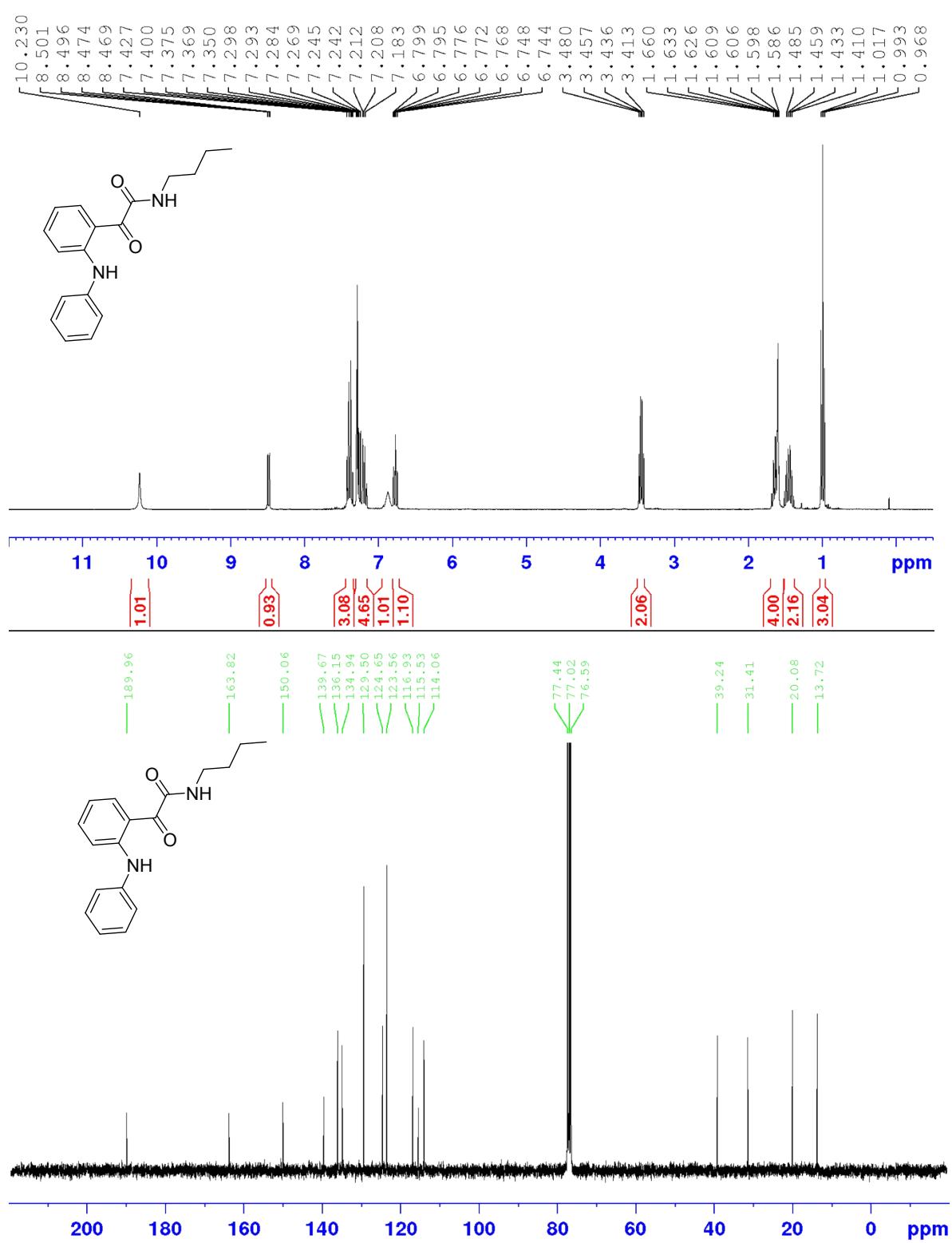
<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of 1-(2-((2,4-dinitrophenyl)amino)phenyl)-2-(pyrrolidin-1-yl)ethane-1,2-dione (**23**)



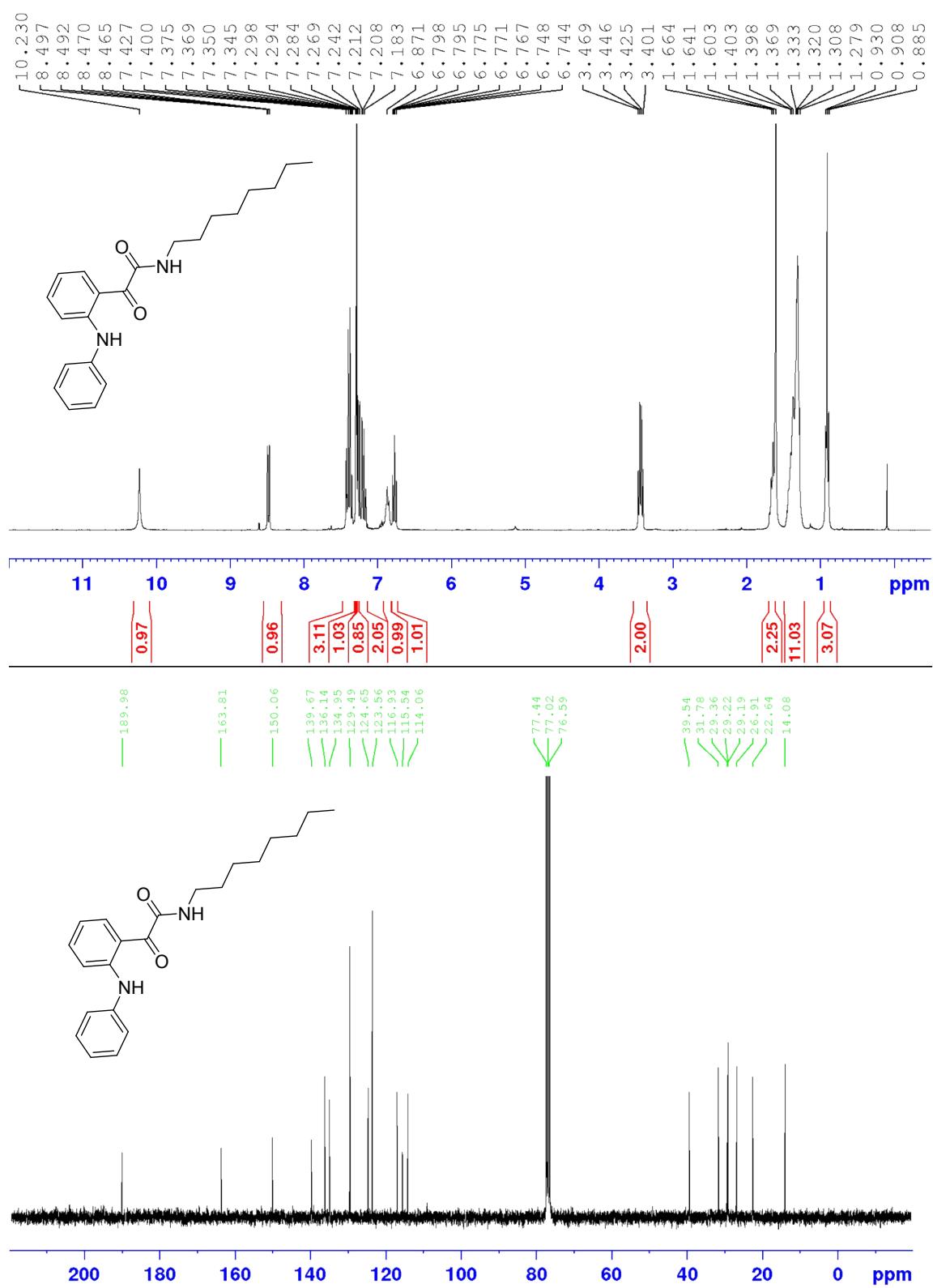
<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of 1-(2-((2,4-dinitrophenyl)amino)phenyl)-2-(piperidin-1-yl)ethane-1,2-dione (**24**)



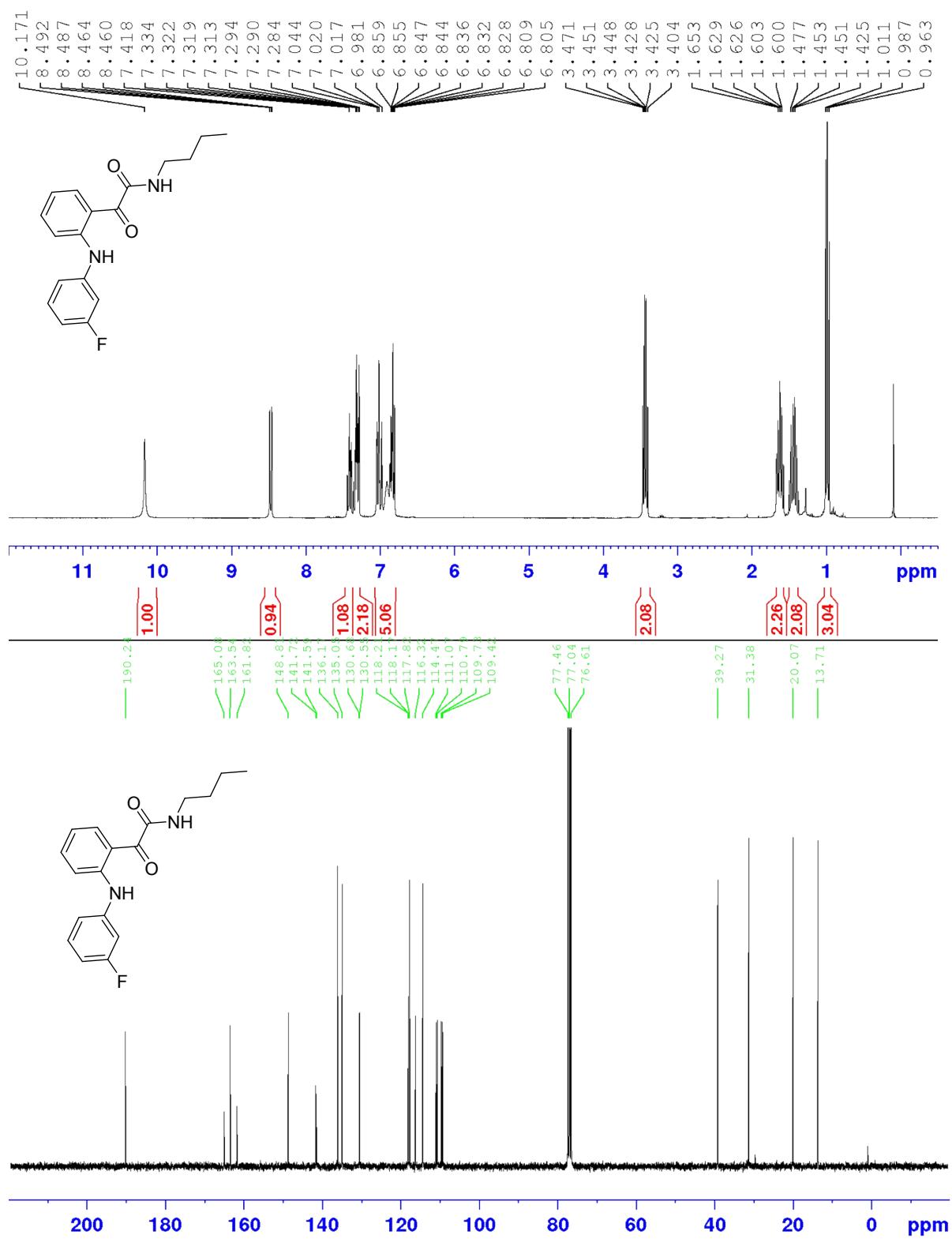
<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of *N*-butyl-2-oxo-2-(2-(phenylamino)phenyl)acetamide (**25**)



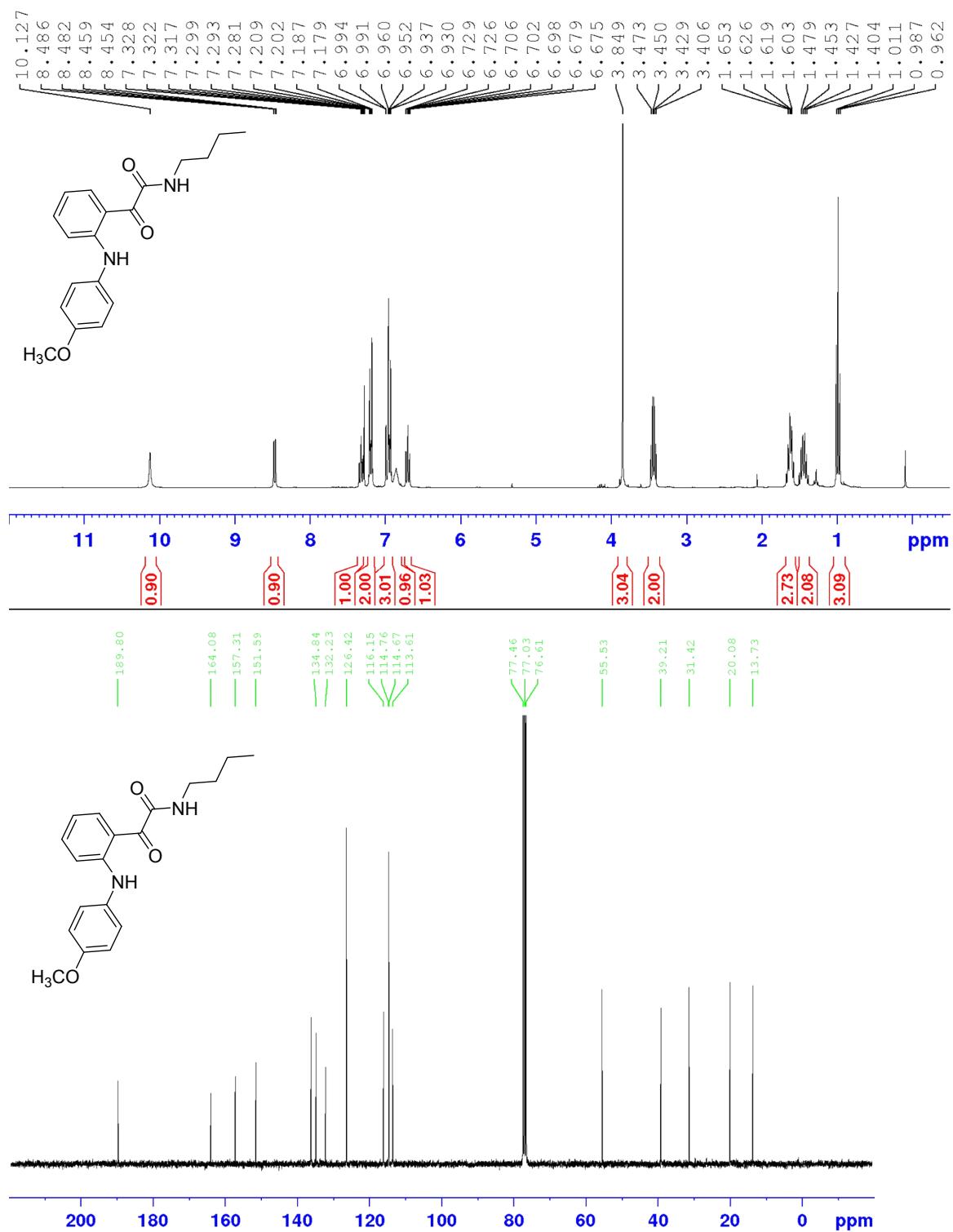
<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of *N*-octyl-2-oxo-2-(2-(phenylamino)phenyl)acetamide (**26**)



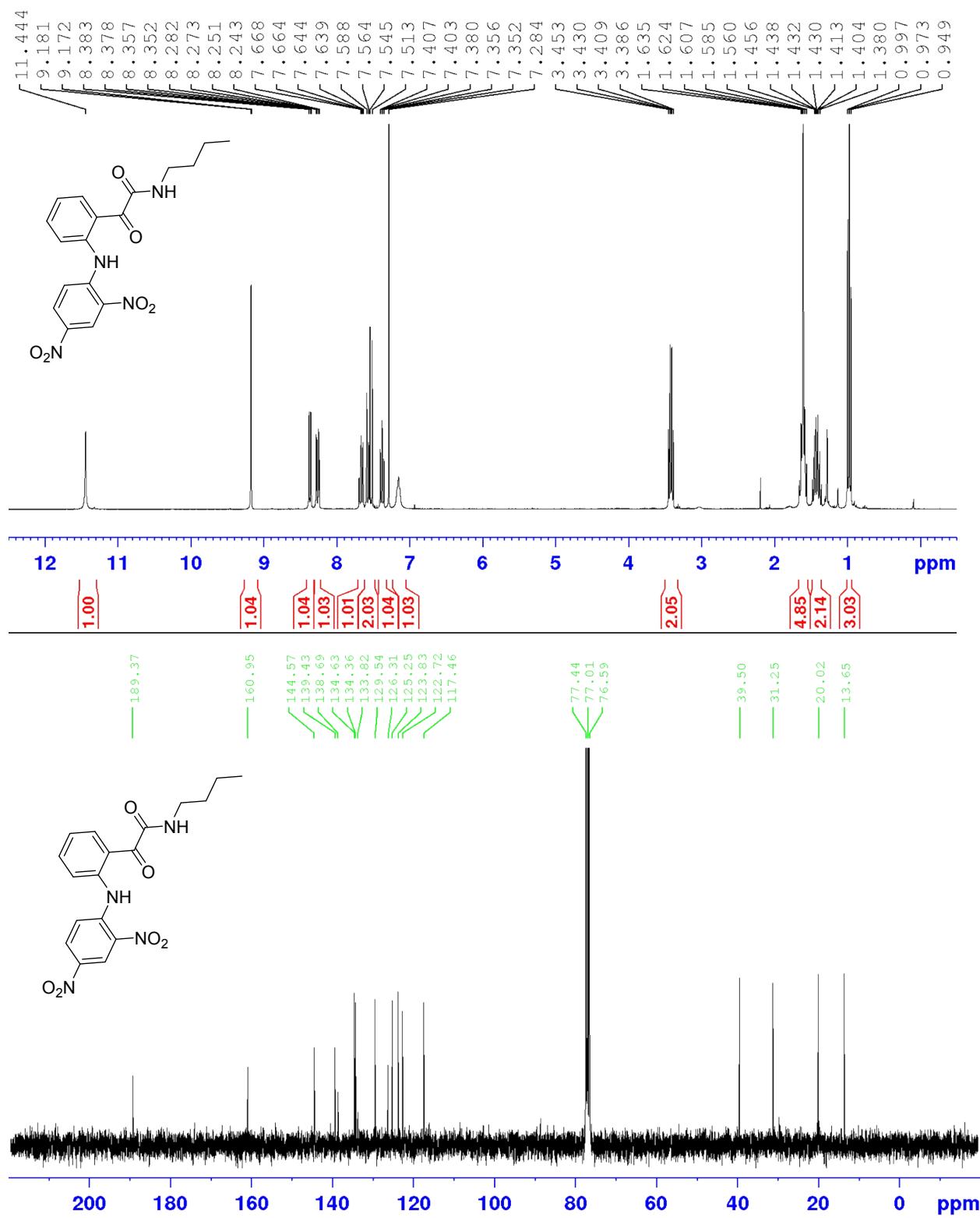
<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of *N*-butyl-2-(2-((3-fluorophenyl)amino)phenyl)-2-oxoacetamide  
**(27)**



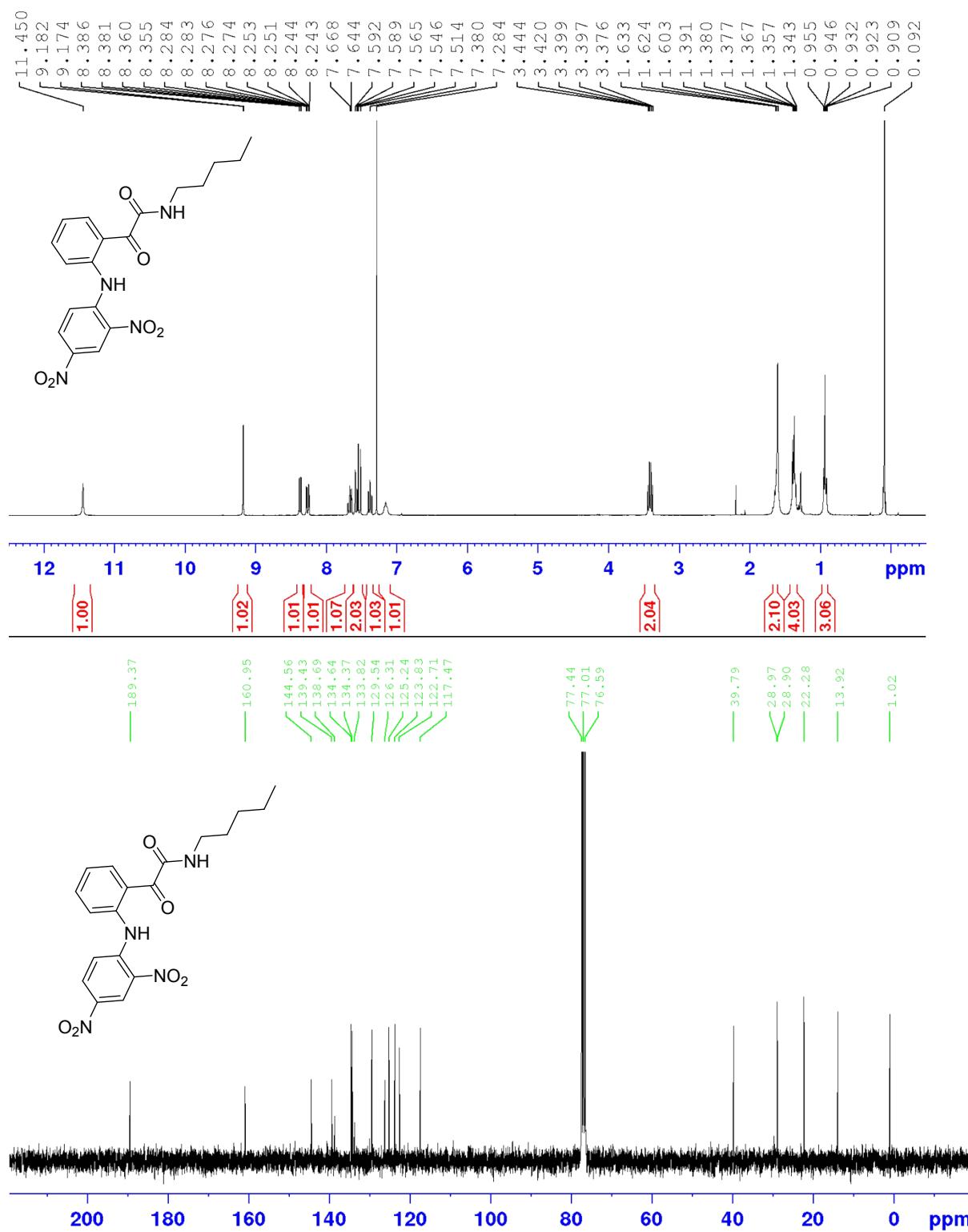
**<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of *N*-butyl-2-(2-((4-methoxyphenyl)amino)phenyl)-2-oxoacetamide (**28**)**



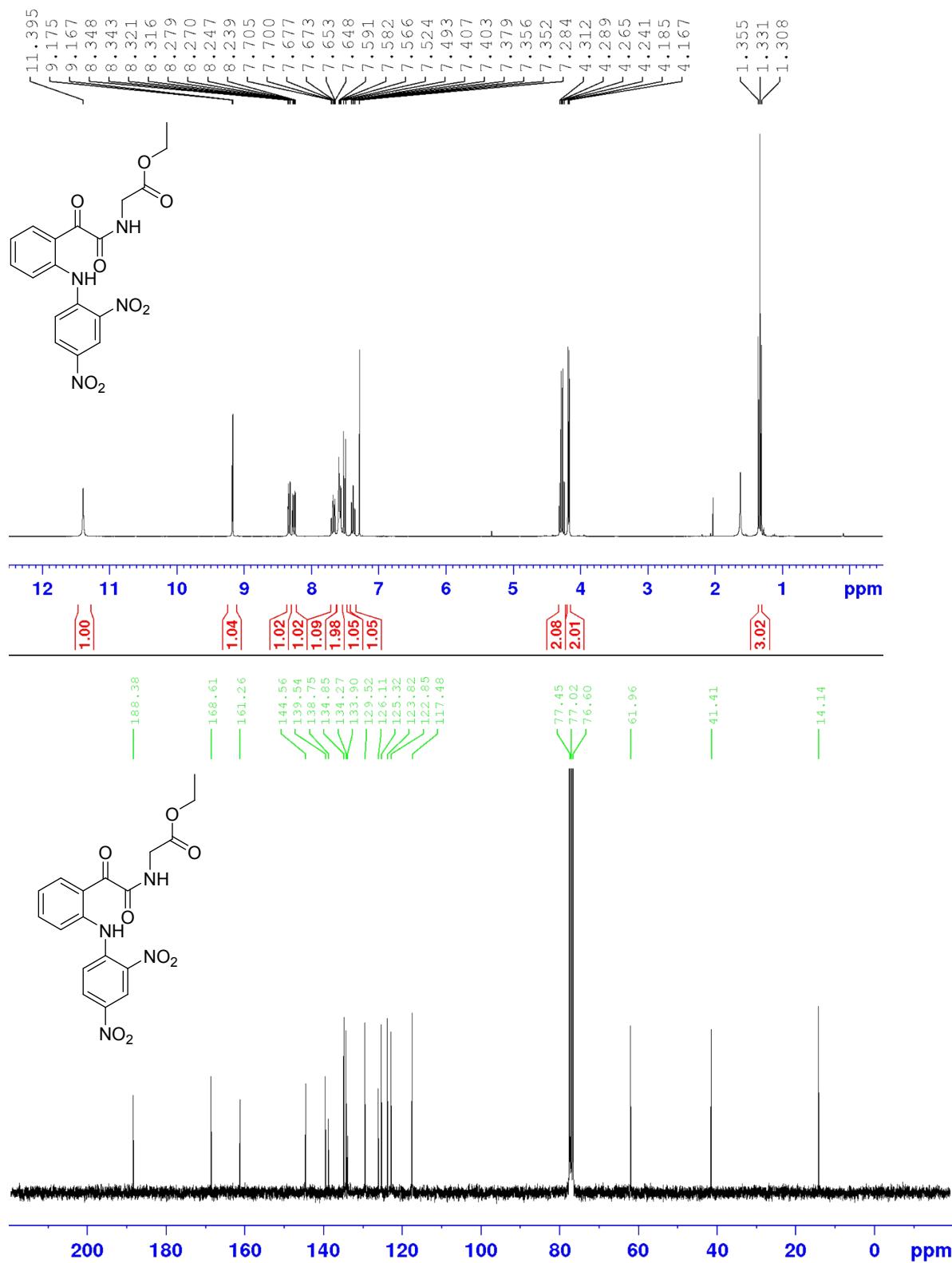
<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of *N*-butyl-2-(2-((2,4-dinitrophenyl)amino)phenyl)-2-oxoacetamide (**29**)



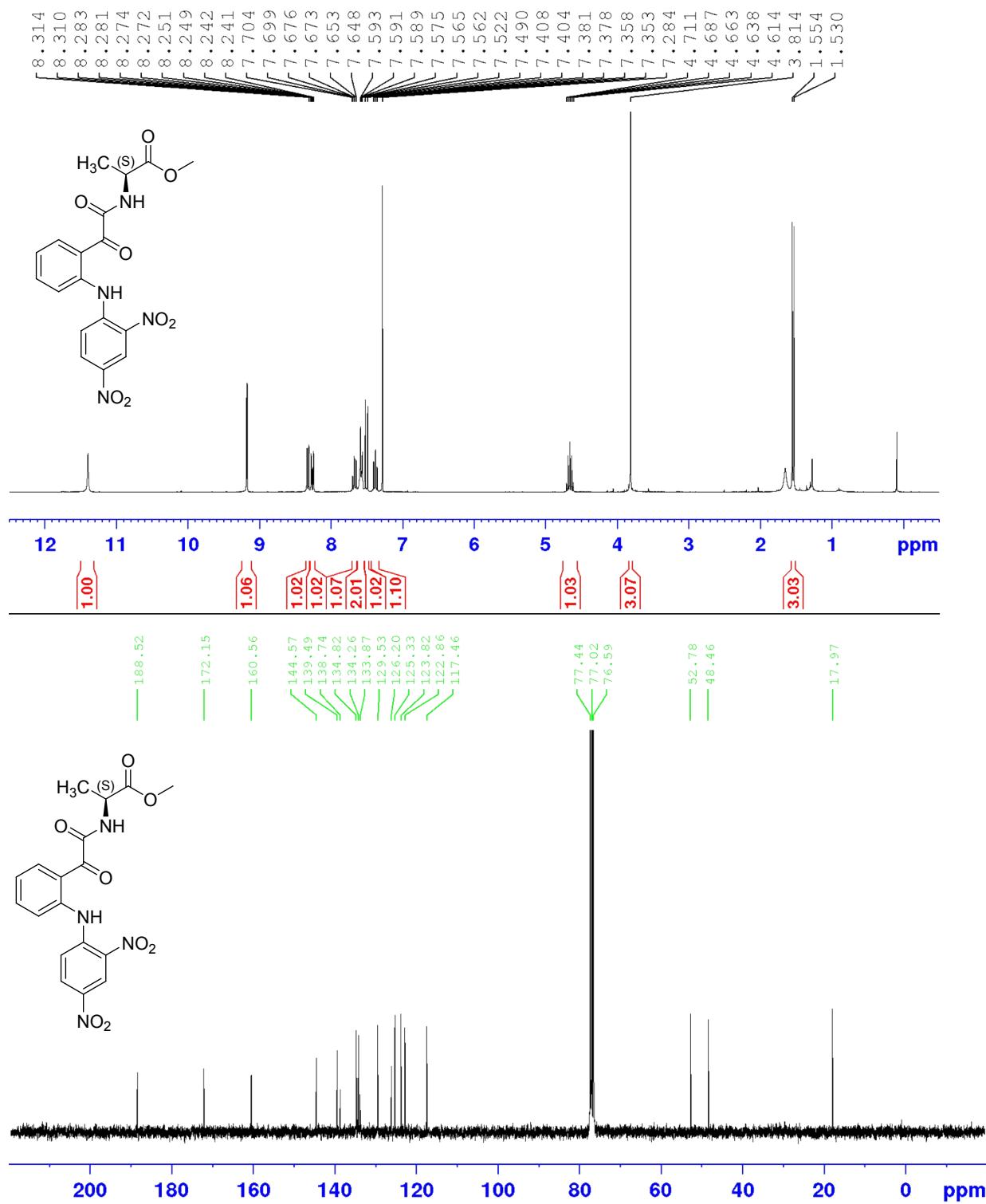
<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of 2-(2-((2,4-dinitrophenyl)amino)phenyl)-2-oxo-N-pentylacetamide (**30**)



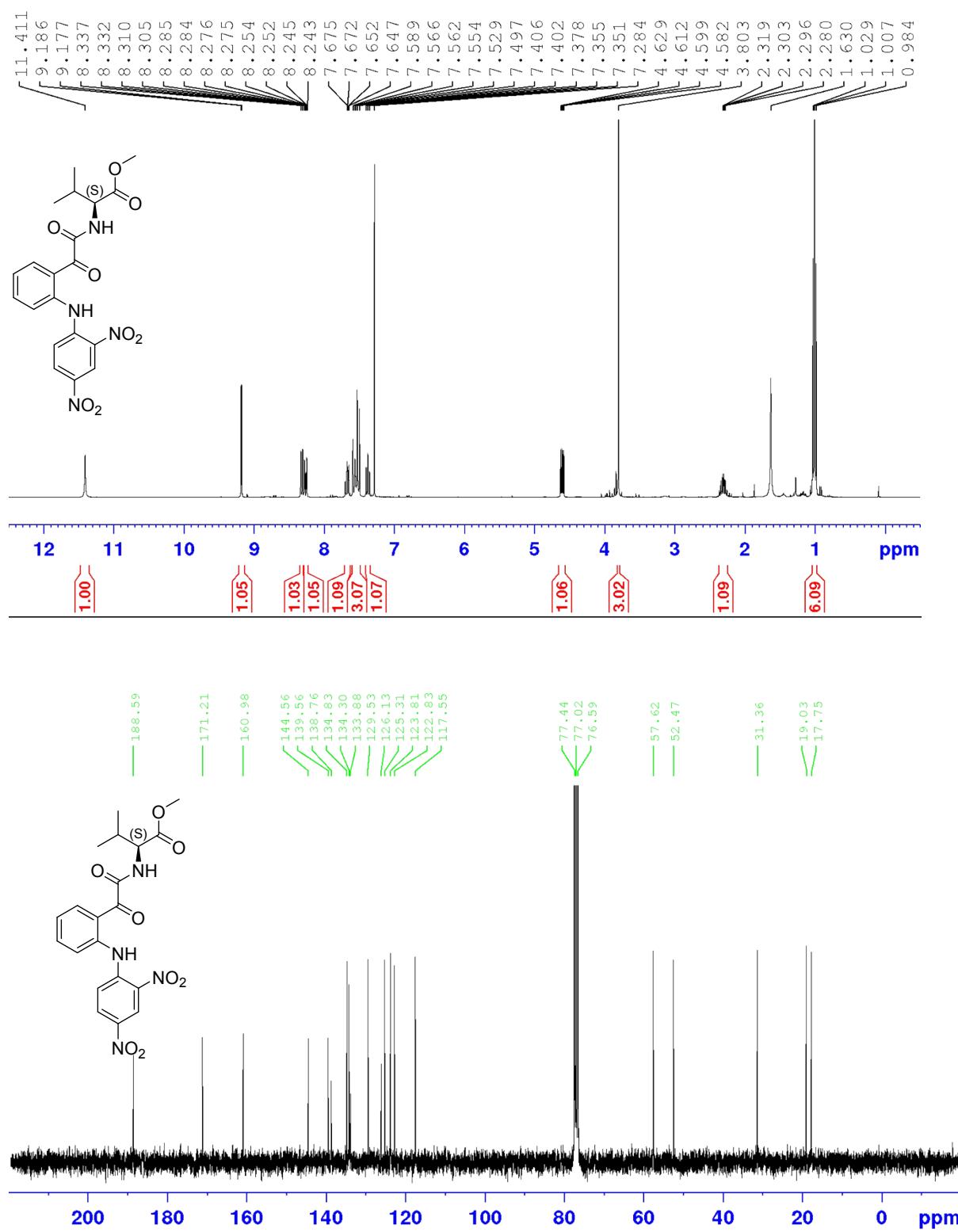
**<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of Ethyl (2-(2-((2,4-dinitrophenyl)amino)phenyl)-2-oxoacetyl)glycinate(31)**



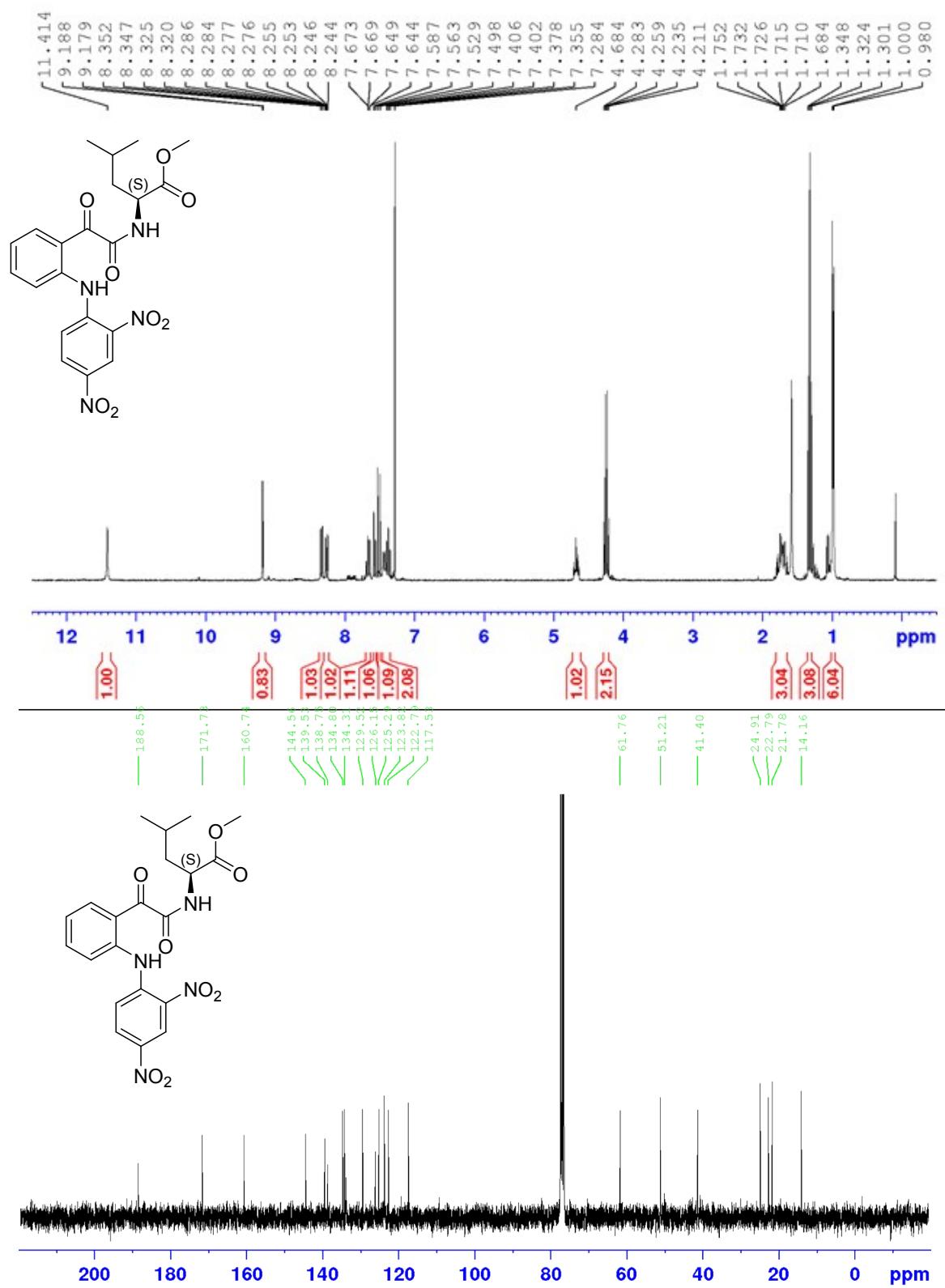
**<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of Methyl (2-((2,4-dinitrophenyl)amino)phenyl)-2-oxoacetyl)-L-alaninate (**32**)**



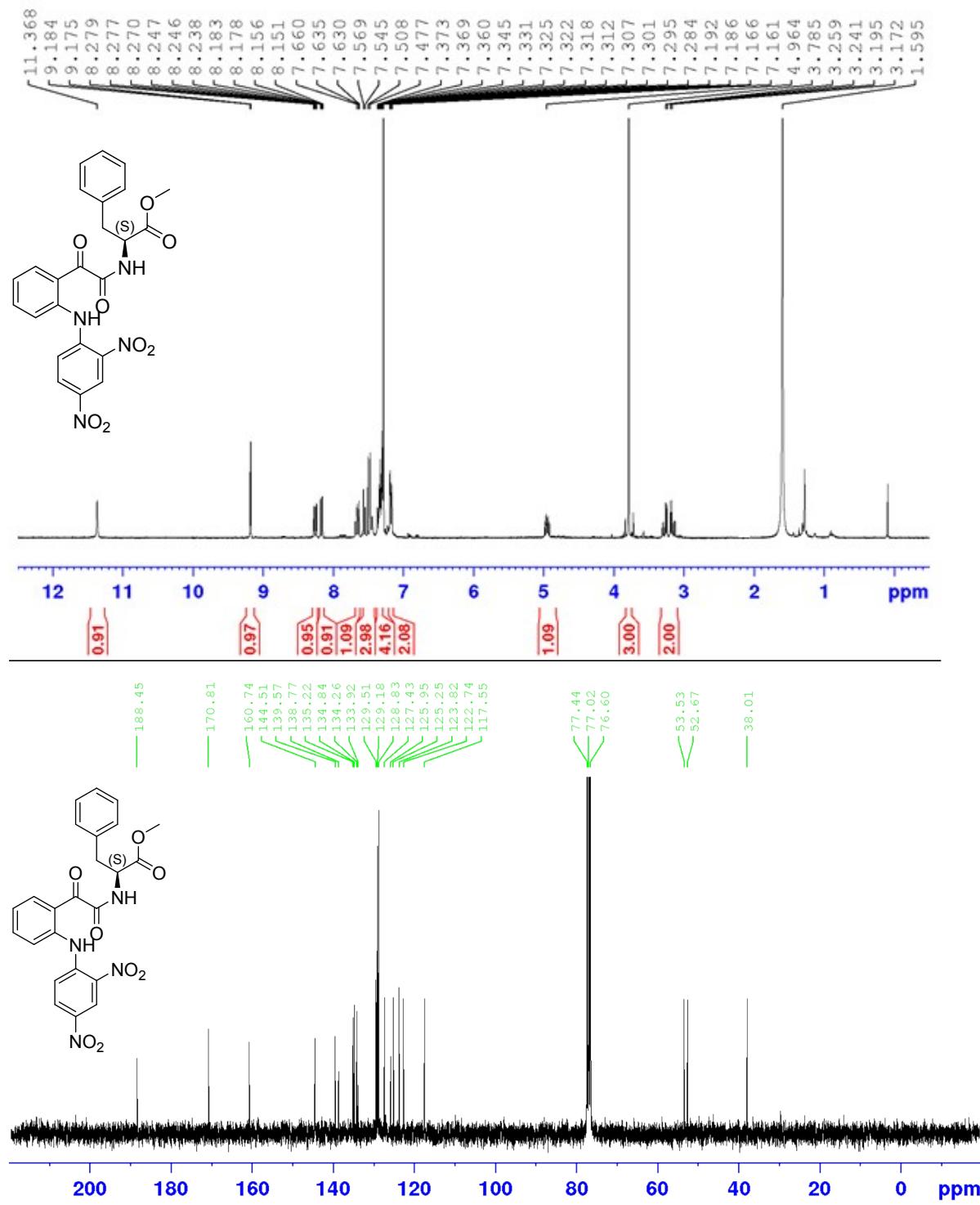
**<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of Methyl (2-((2,4-dinitrophenyl)amino)phenyl)-2-oxoacetyl)-L-valinate (**33**)**



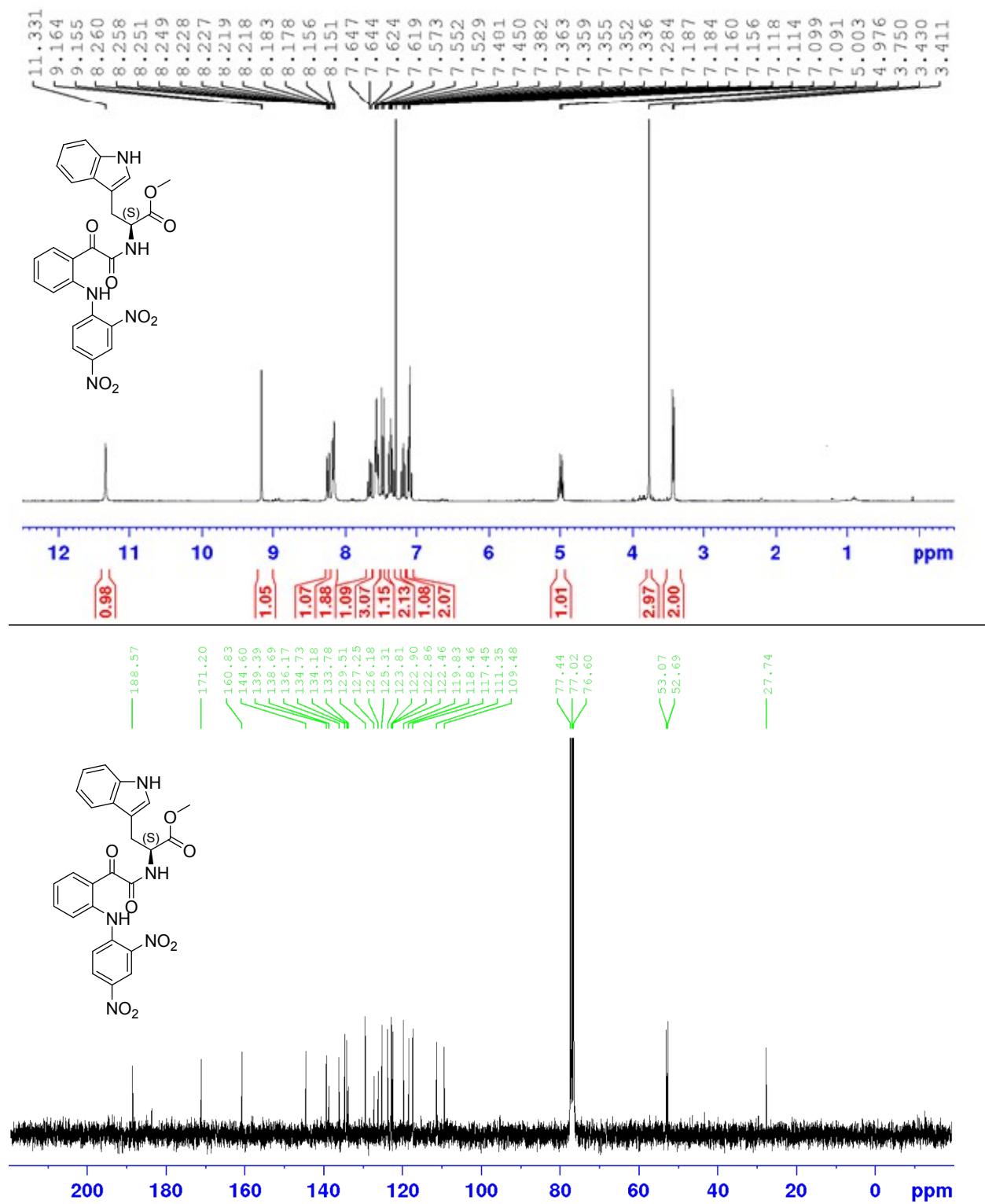
<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of Methyl (2-((2,4-dinitrophenyl)amino)phenyl)-2-oxoacetyl)-L-leucinate (**34**)



<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of Methyl (2-((2,4-dinitrophenyl)amino)phenyl)-2-oxoacetyl)-L-phenylalaninate (35)



<sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of Methyl (2-((2,4-dinitrophenyl)amino)phenyl)-2-oxoacetyl)-L-tryptophanate (**36**)



### **Optical Density (OD) Measurements**

**Table 1:** Growth inhibition by the synthesized compounds against the PAMH602 and *E. coli* MT102 strains at three different concentrations.

Compound	PAMH602			<i>E. coli</i> MT102		
	Concentration ( $\mu\text{M}$ )					
	250	125	62.5	250	125	62.5
<b>16</b>	0	0	0	4.6 $\pm$ 8.2	2.8 $\pm$ 1.1	0
<b>17</b>	0	0	0	14.9 $\pm$ 1.3	4.2 $\pm$ 0.3	1.7 $\pm$ 0.2
<b>18</b>	1.3 $\pm$ 1.2	0	0	17.2 $\pm$ 5.2	4 $\pm$ 0.7	0
<b>19</b>	0	0	0	14.8 $\pm$ 6.8	4 $\pm$ 0.6	0
<b>20</b>	0	0	0	17.8 $\pm$ 6.0	8.1 $\pm$ 1.8	0
<b>21</b>	0	0	0	20.6 $\pm$ 3.7	18.6 $\pm$ 2.5	12.1 $\pm$ 1.2
<b>22</b>	0	0	0	15.4 $\pm$ 5.8	0	5.6 $\pm$ 1.0
<b>23</b>	0	0	0	20.6 $\pm$ 3.7	18.6 $\pm$ 2.5	12.1 $\pm$ 1.2
<b>24</b>	0	0	0	8.9 $\pm$ 2.1	0	0
<b>25</b>	0	0	0	3.6 $\pm$ 5.7	0	0
<b>26</b>	0.1 $\pm$ 0.9	0	0	12.7 $\pm$ 3.4	0	0
<b>27</b>	8.3 $\pm$ 3.0	5.2 $\pm$ 2.5	0	17.2 $\pm$ 5.2	4.0 $\pm$ 0.7	0
<b>28</b>	0	0	0	5.1 $\pm$ 2.1	0	0
<b>29</b>	0	0	0	19.3 $\pm$ 3.5	19.5 $\pm$ 1.1	11 $\pm$ 0.9
<b>30</b>	0	0	0	17.9 $\pm$ 2.1	13.2 $\pm$ 1.5	9.8 $\pm$ 0.8
<b>31</b>	0	0	0	18.1 $\pm$ 2.3	1.6 $\pm$ 0.7	-1.6 $\pm$ 0.6
<b>32</b>	0	0	0	22.5 $\pm$ 1.4	0.6 $\pm$ 0.4	0
<b>33</b>	0	0	0	37 $\pm$ 2.4	9.9 $\pm$ 2.4	6.1 $\pm$ 0.8
<b>34</b>	3.3 $\pm$ 2.6	0.8 $\pm$ 1.6	0	26.1 $\pm$ 6.7	5.2 $\pm$ 0.8	0
<b>35</b>	0	0	0	12.2 $\pm$ 4.4	0	0
<b>36</b>	1.2 $\pm$ 2.2	0	0	26.4 $\pm$ 1.1	2.3 $\pm$ 1.5	0
<b>Fu-30</b>	51.9 $\pm$ 0.2	36.9 $\pm$ 1.6	4.3 $\pm$ 0.6	98.8 $\pm$ 11.7	99.7 $\pm$ 0.3	75.6 $\pm$ 0.5

Growth inhibition  $\pm$  standard deviation of mean from at least two independent experiments.  
 Compounds tested thrice in triplicate. 0 = No growth inhibition.