

## Supporting Information- File 2

for

### **Total synthesis of leopolic acid A, a natural 2,3-pyrrolidinedione with antimicrobial activity**

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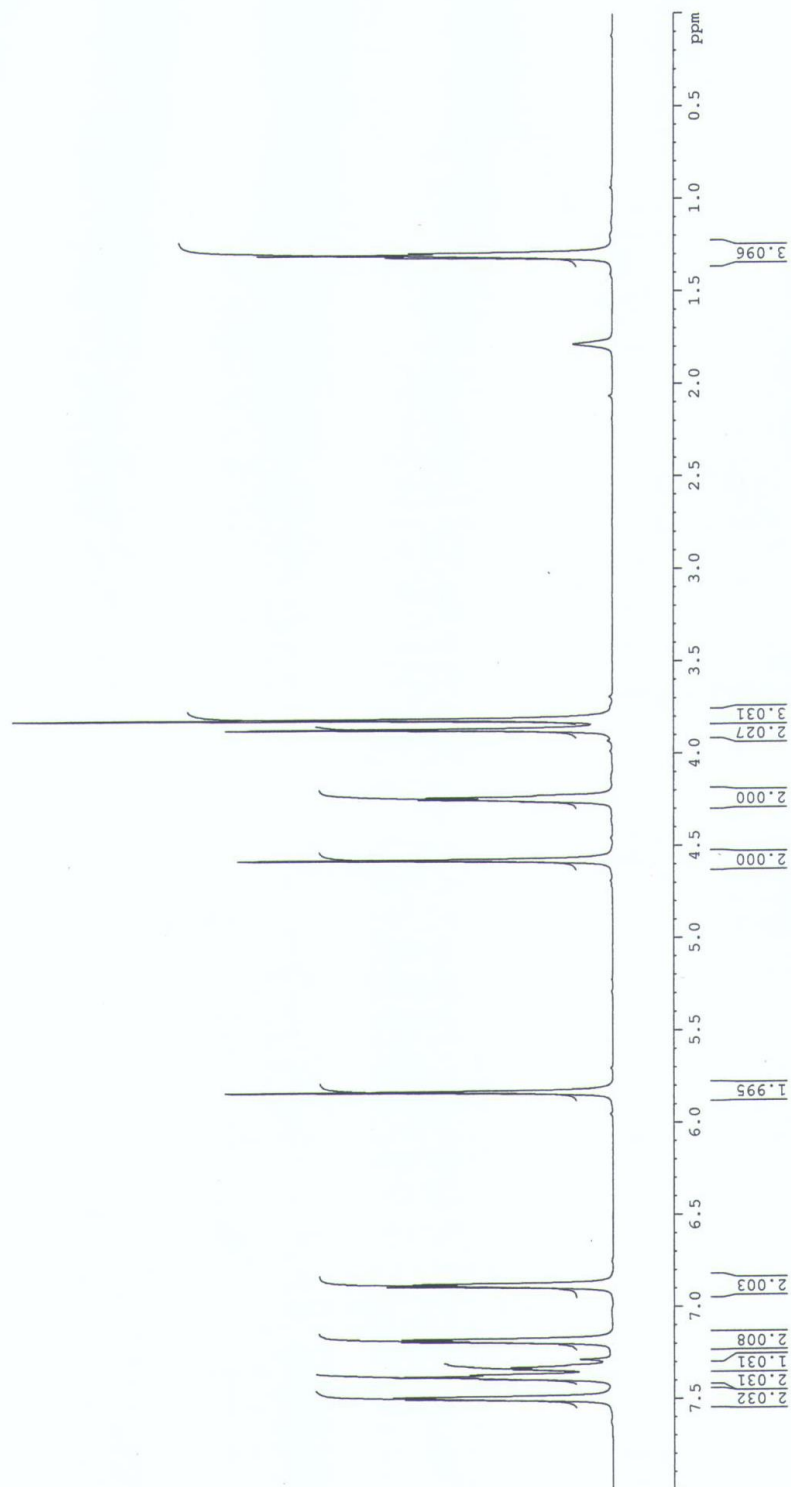
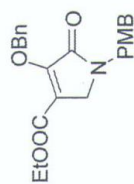
**<sup>1</sup>H and <sup>13</sup>C NMR spectra of all the new compounds; 2D HMBC, HSQC spectra of compounds 1, and 17, COSY spectrum of compound 1, MIC of compounds 1 and 17 against *Staphylococcus pseudintermedius* and *Escherichia coli* strains**

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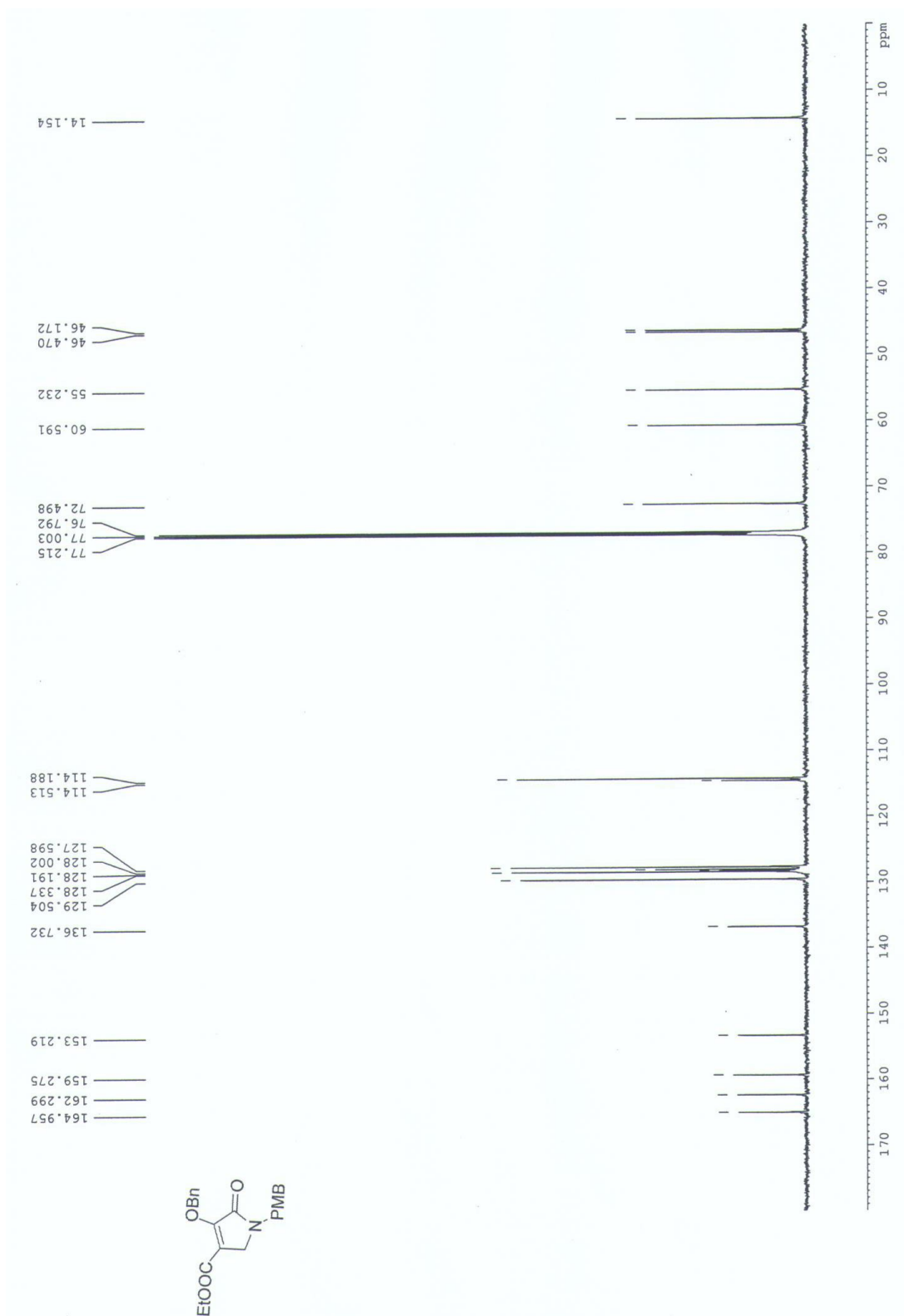
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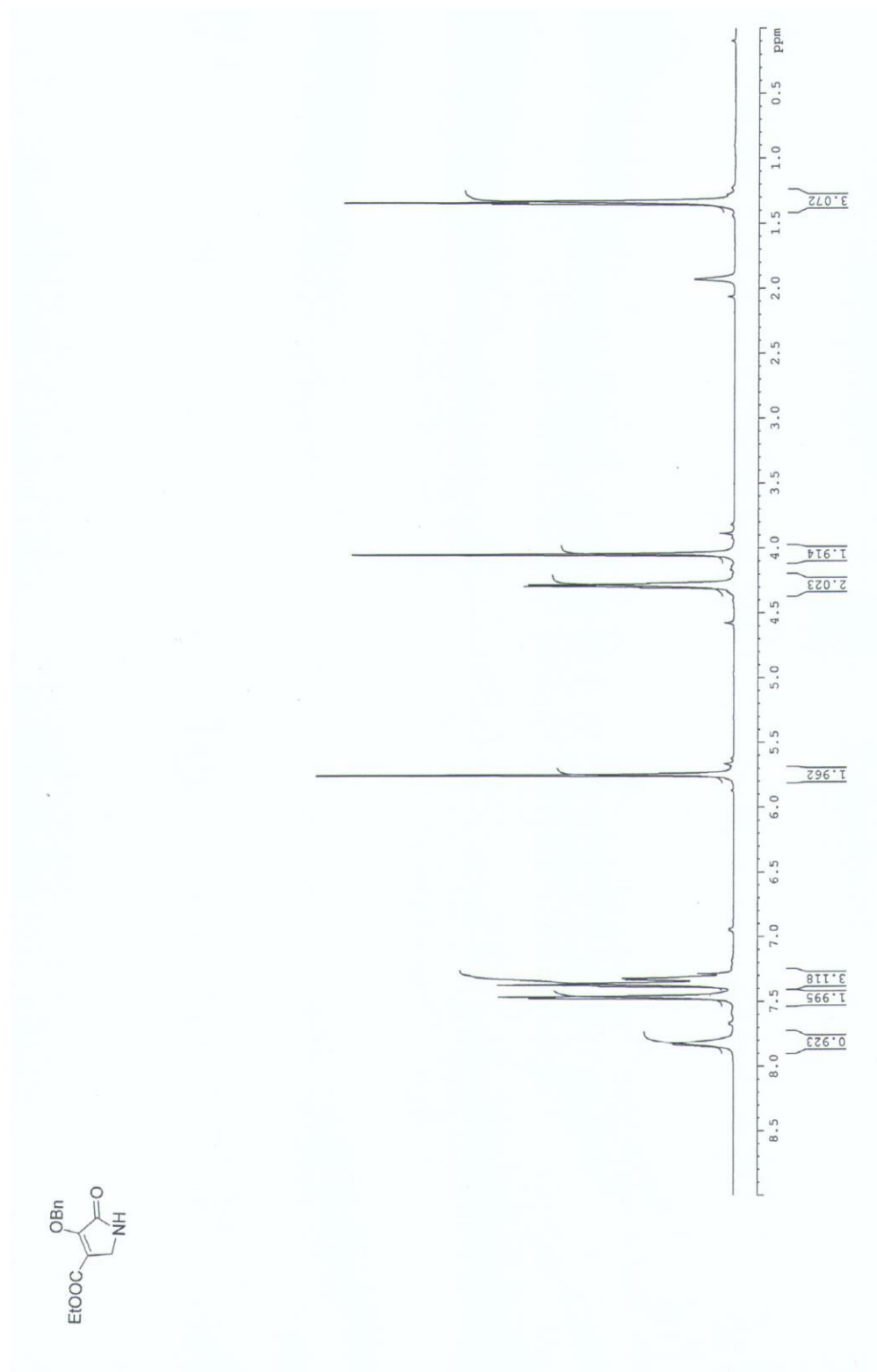
$^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ ) spectrum of compound **4**



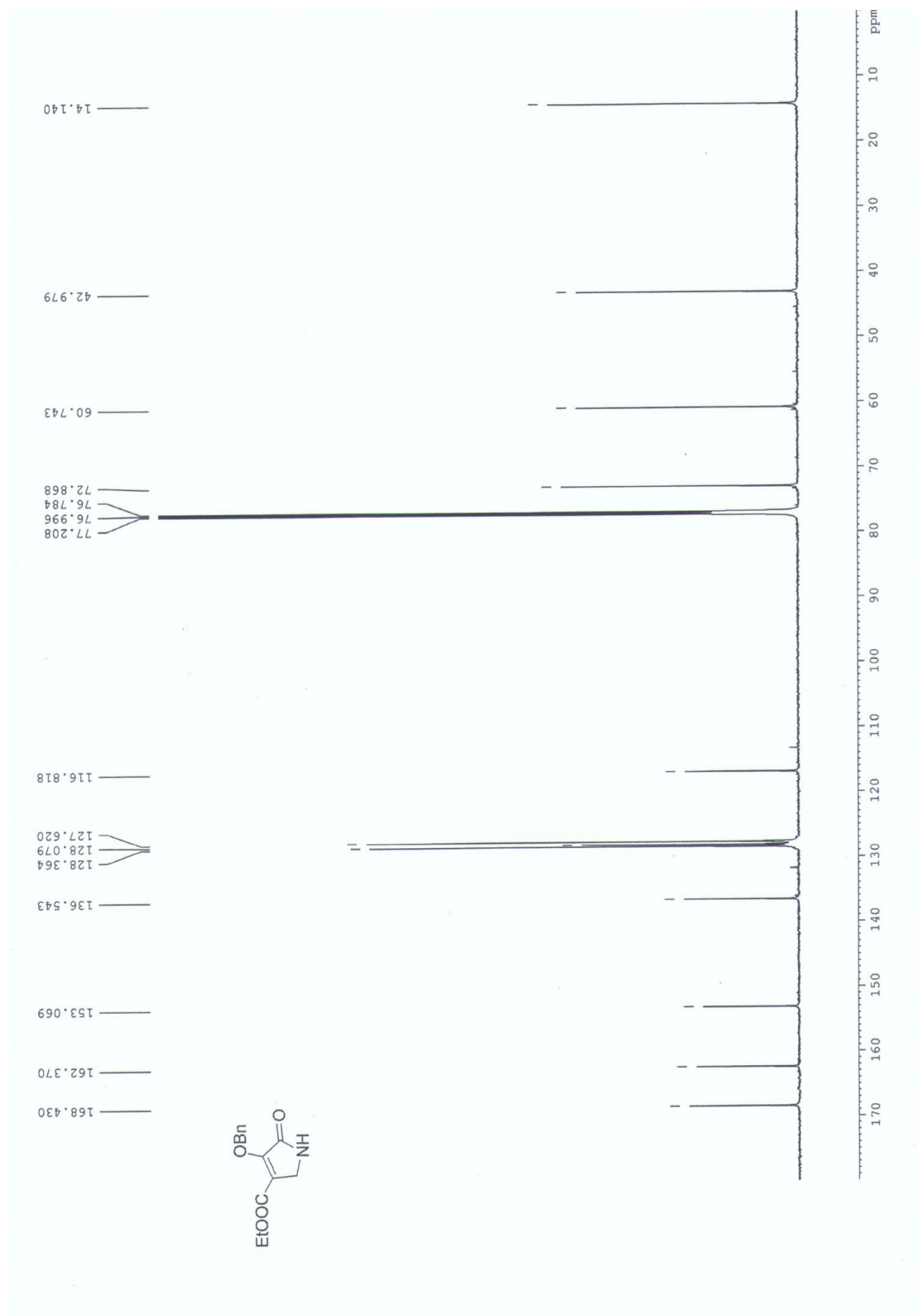
$^{13}\text{C}$  NMR (600 MHz,  $\text{CDCl}_3$ ) spectrum of compound **4**



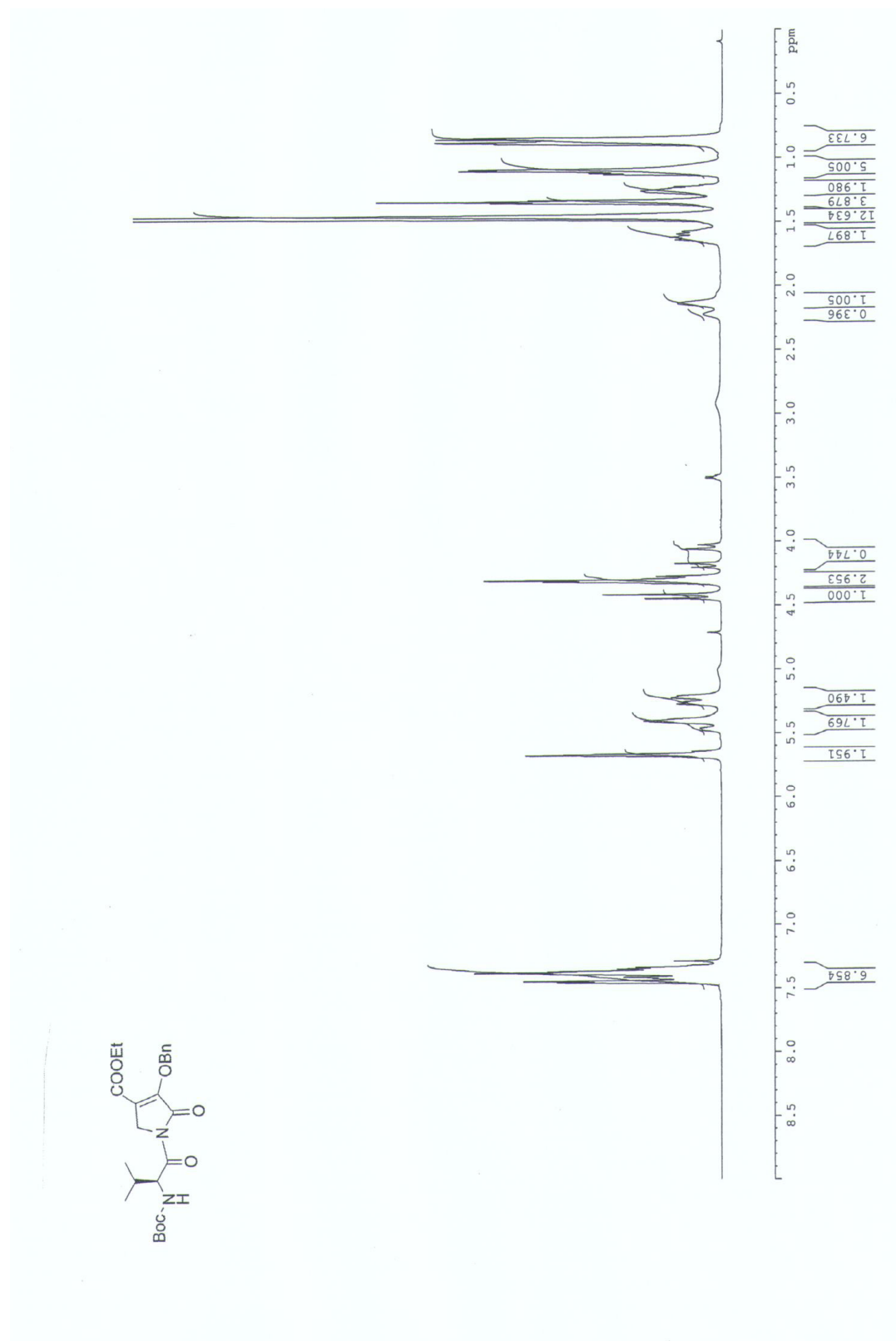
$^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ ) spectrum of compound 7



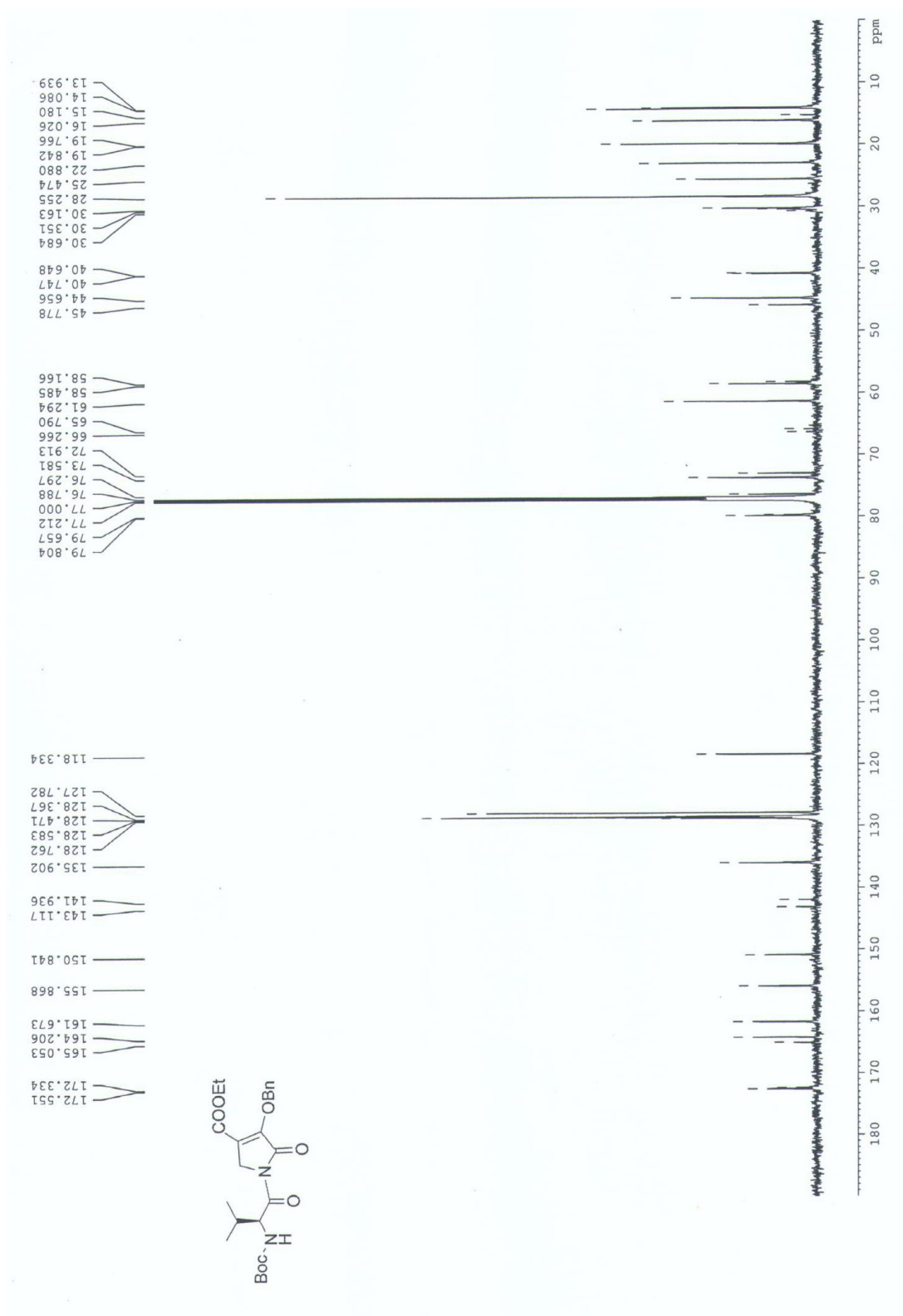
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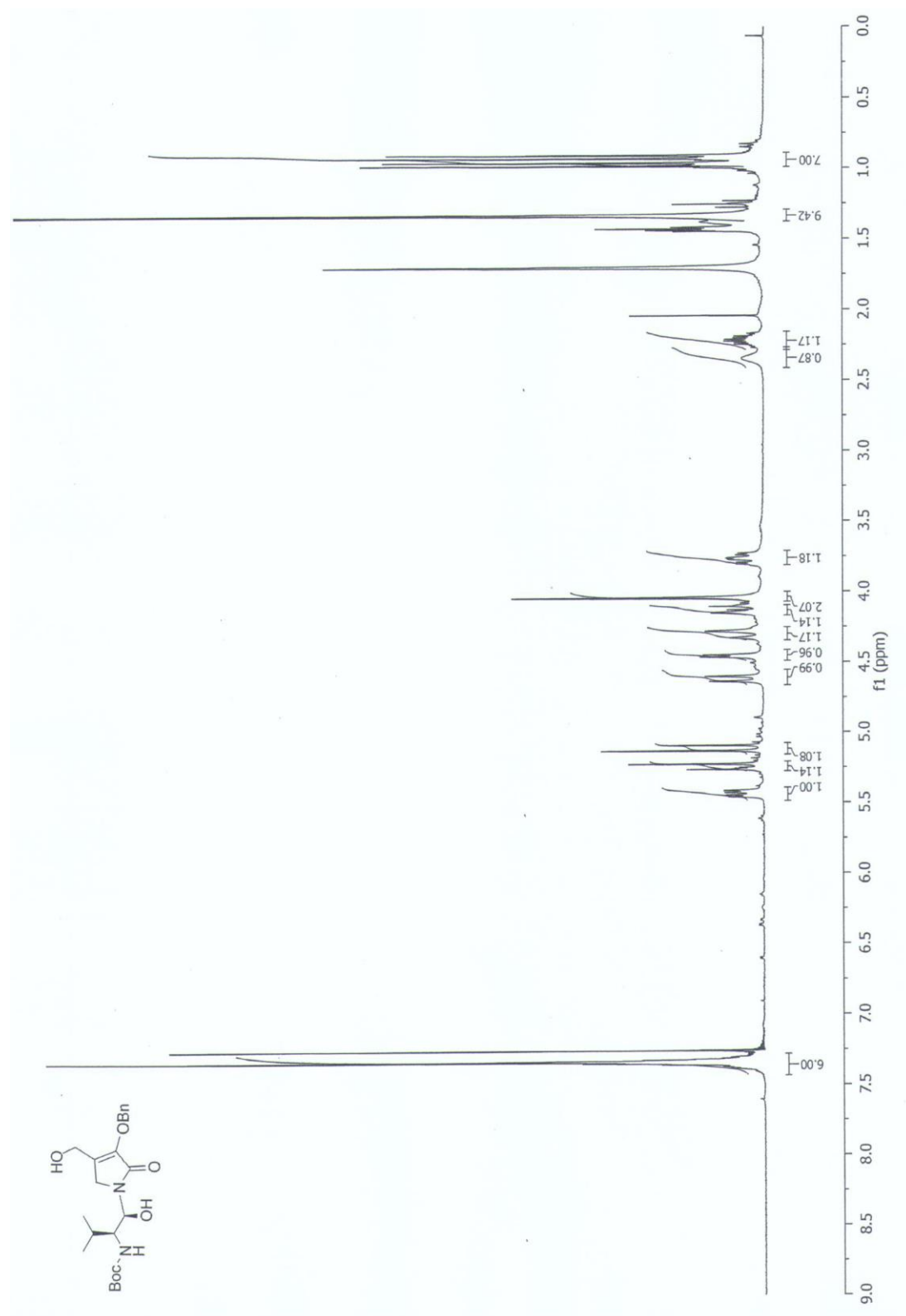
<sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) spectrum of compound **8** (mixture of rotamers)



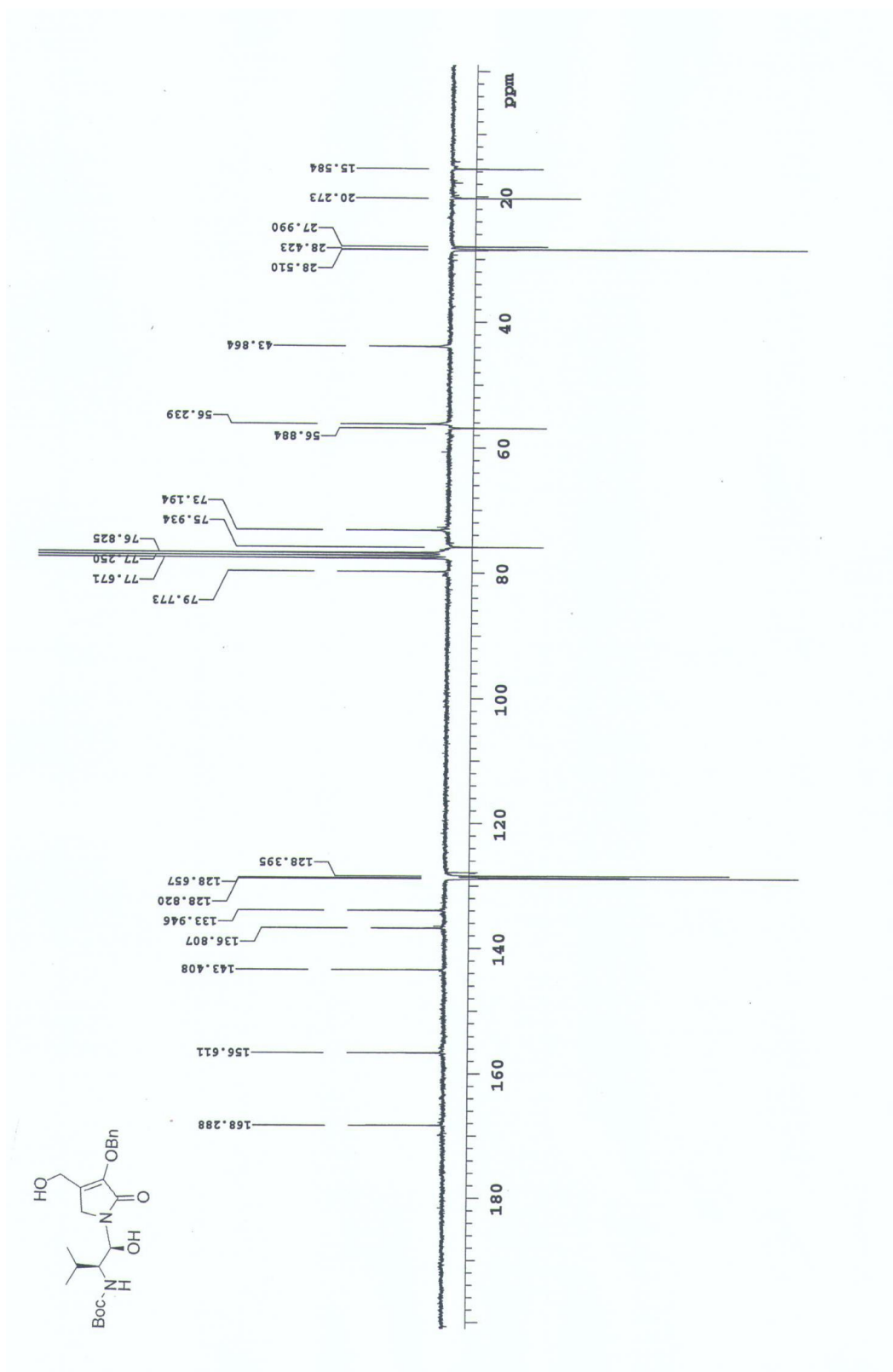
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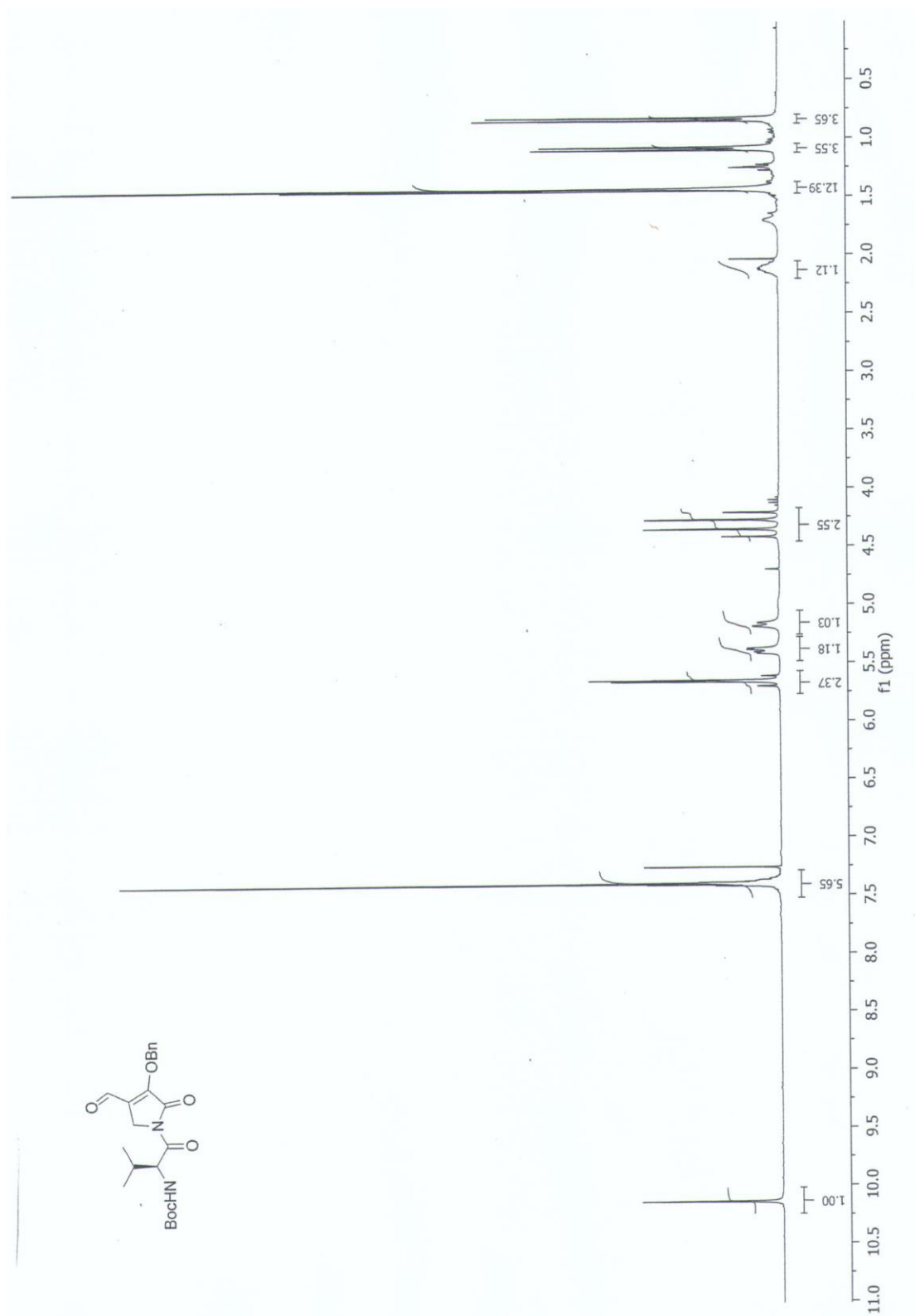
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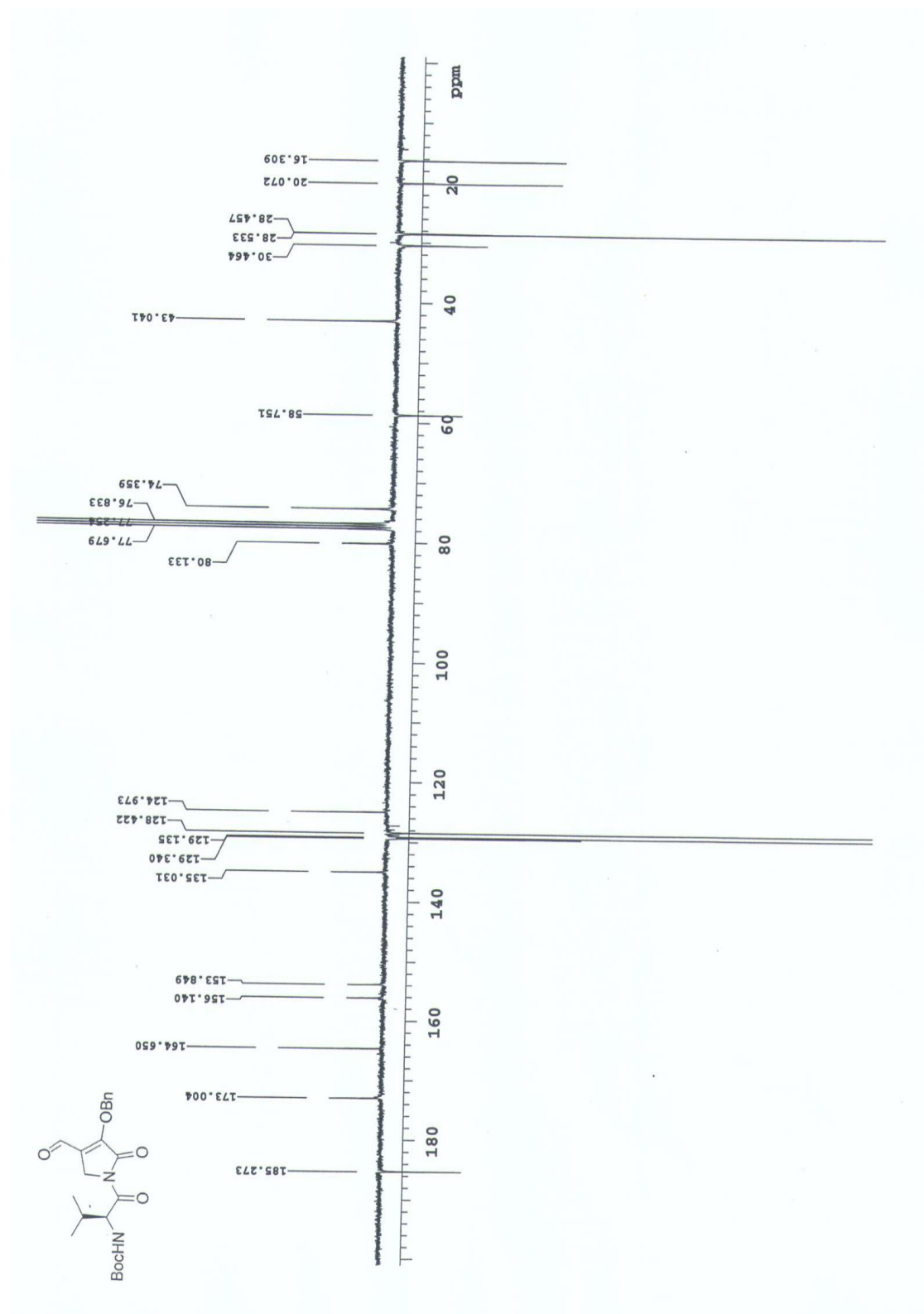
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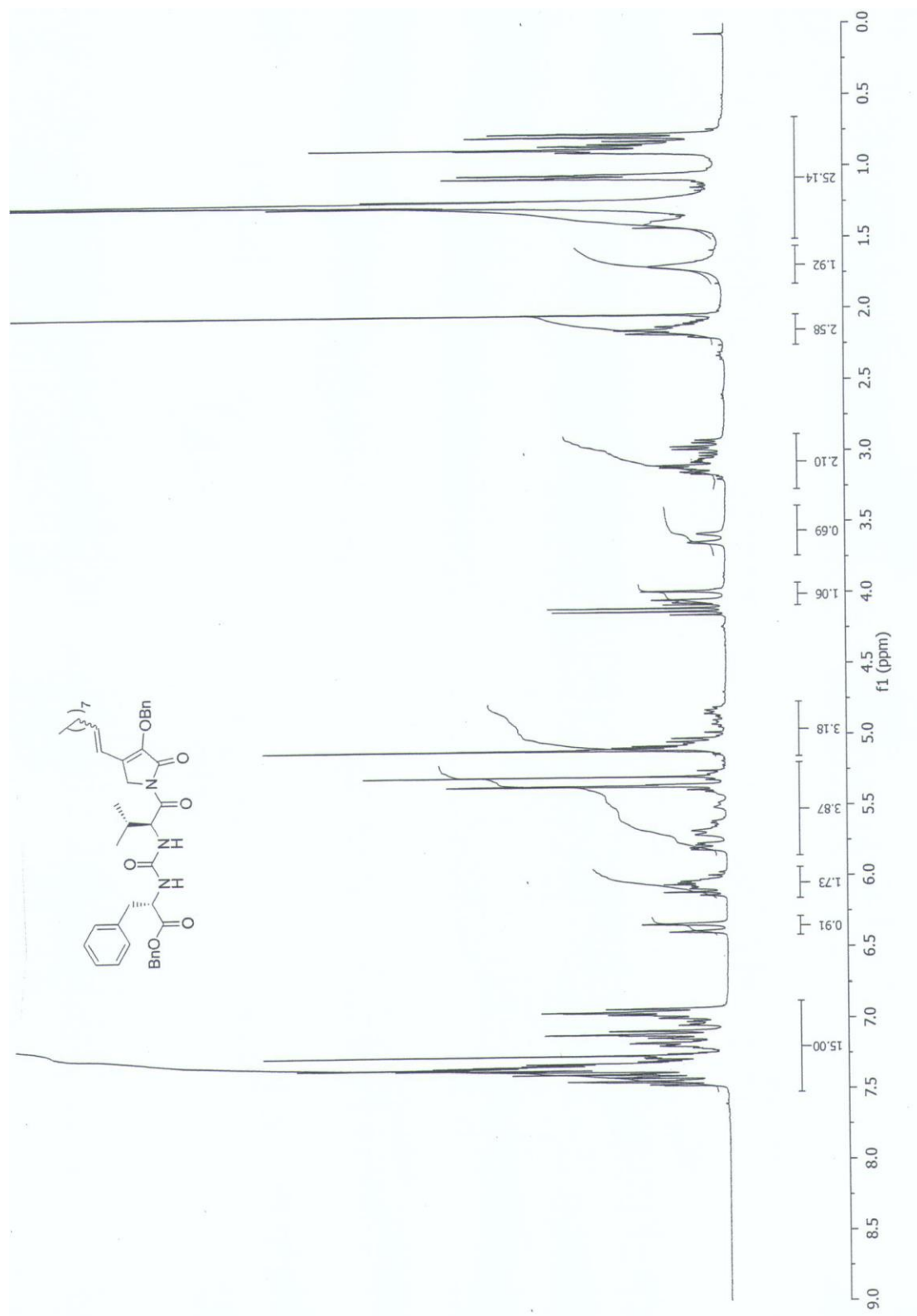
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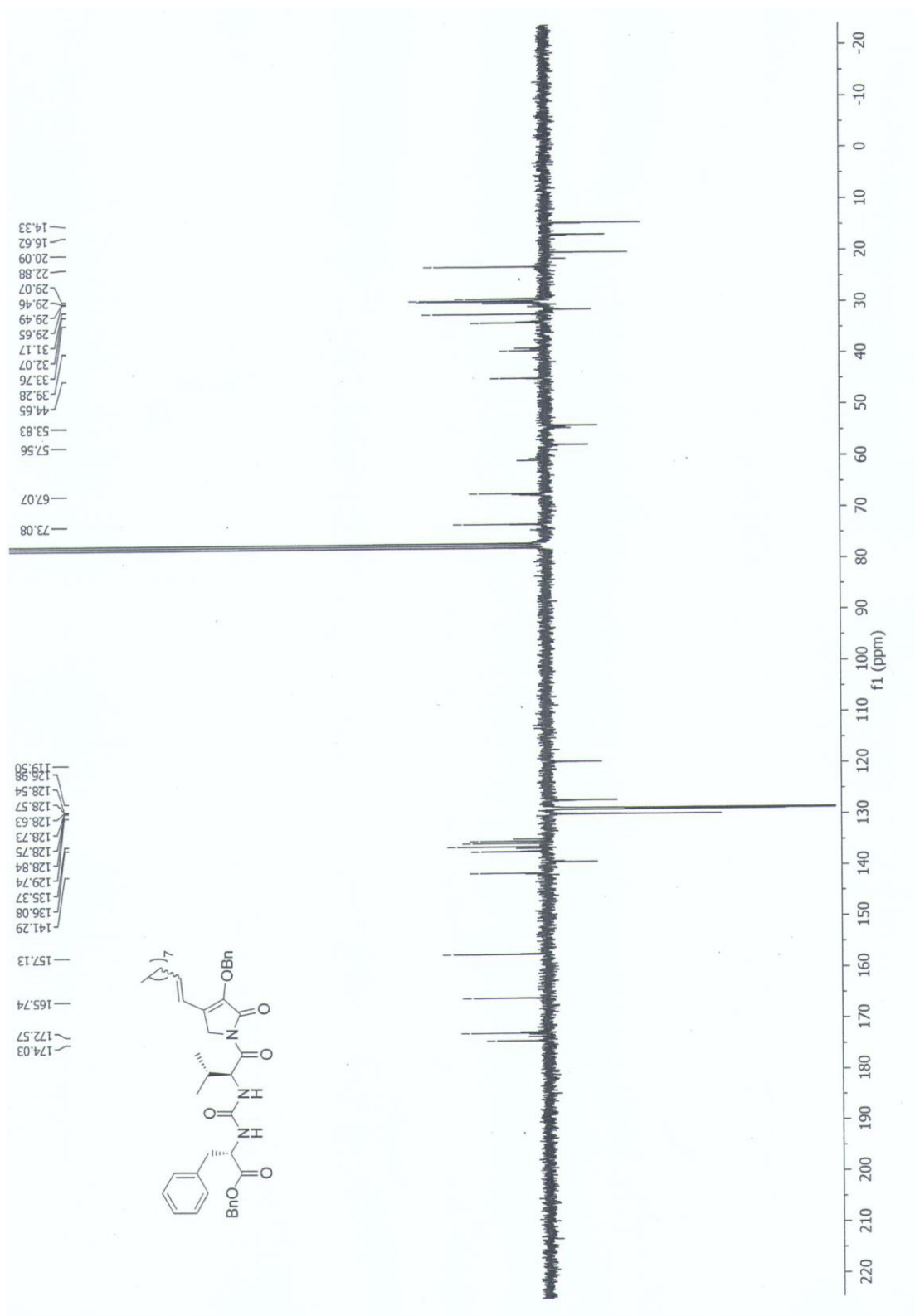
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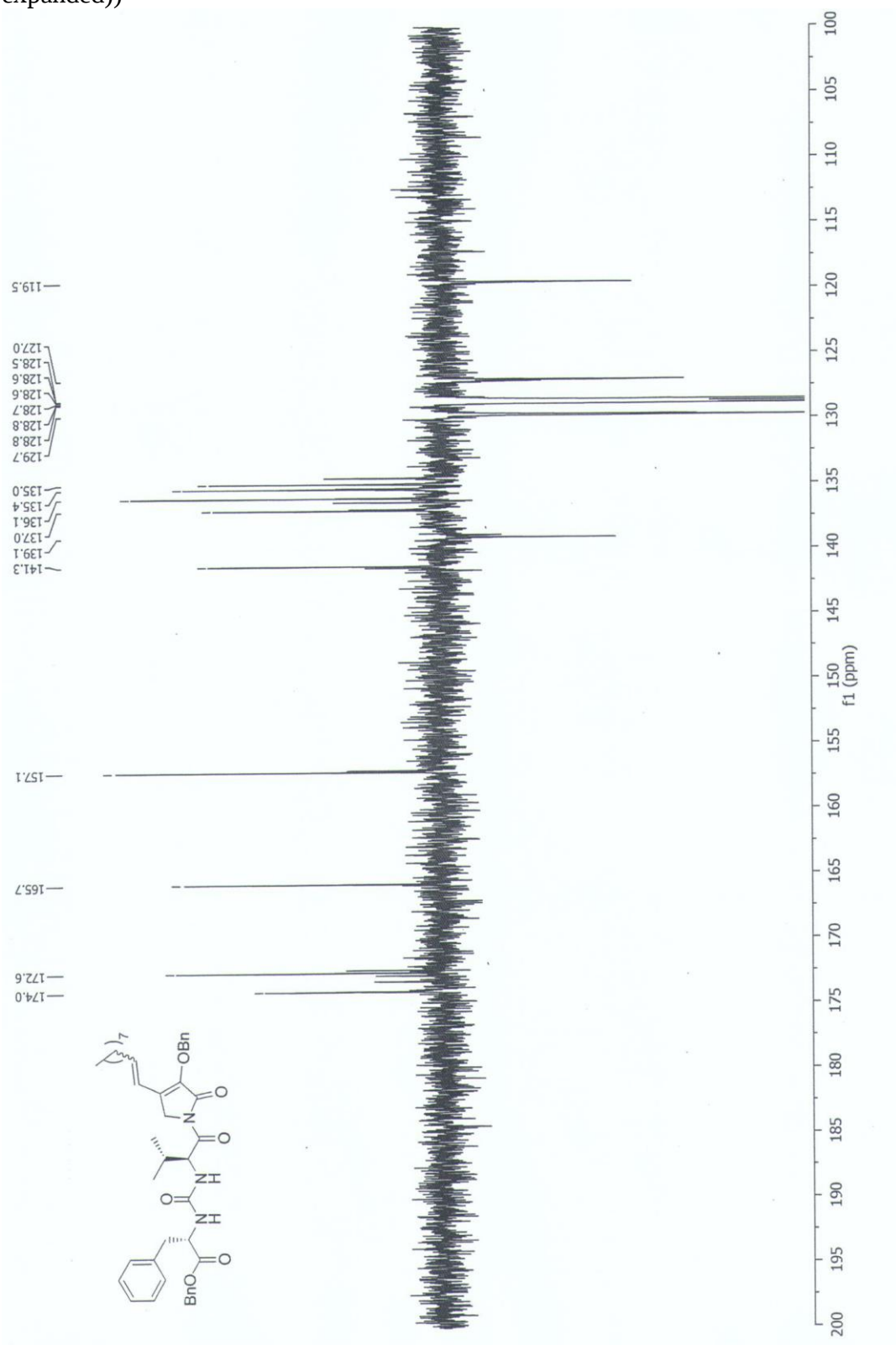
$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ) spectrum of compound **13** (mixture of stereoisomers)



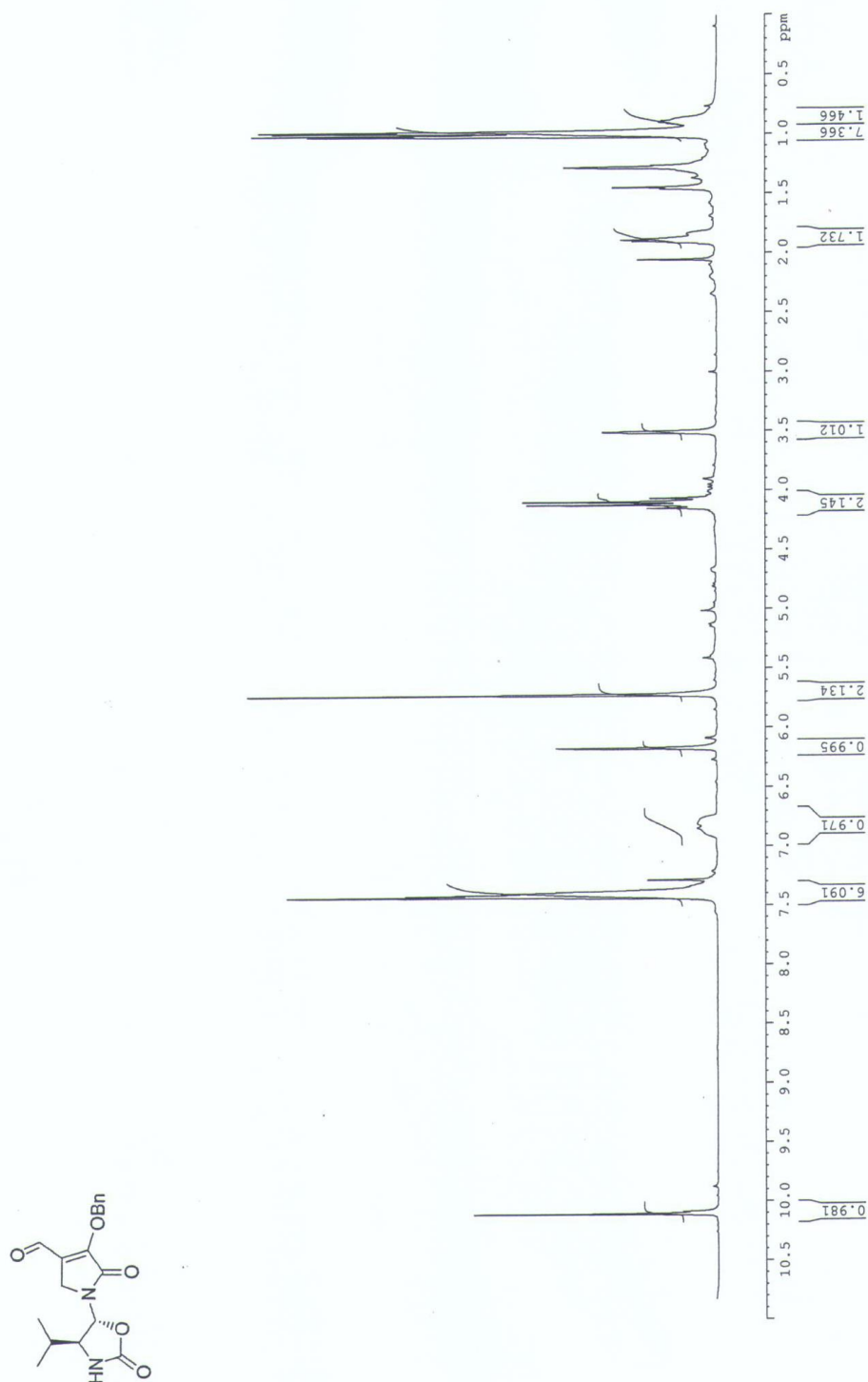
$^{13}\text{C}$  NMR (300 MHz,  $\text{CDCl}_3$ ) spectrum of compound **13** (mixture of stereoisomers) (APT)



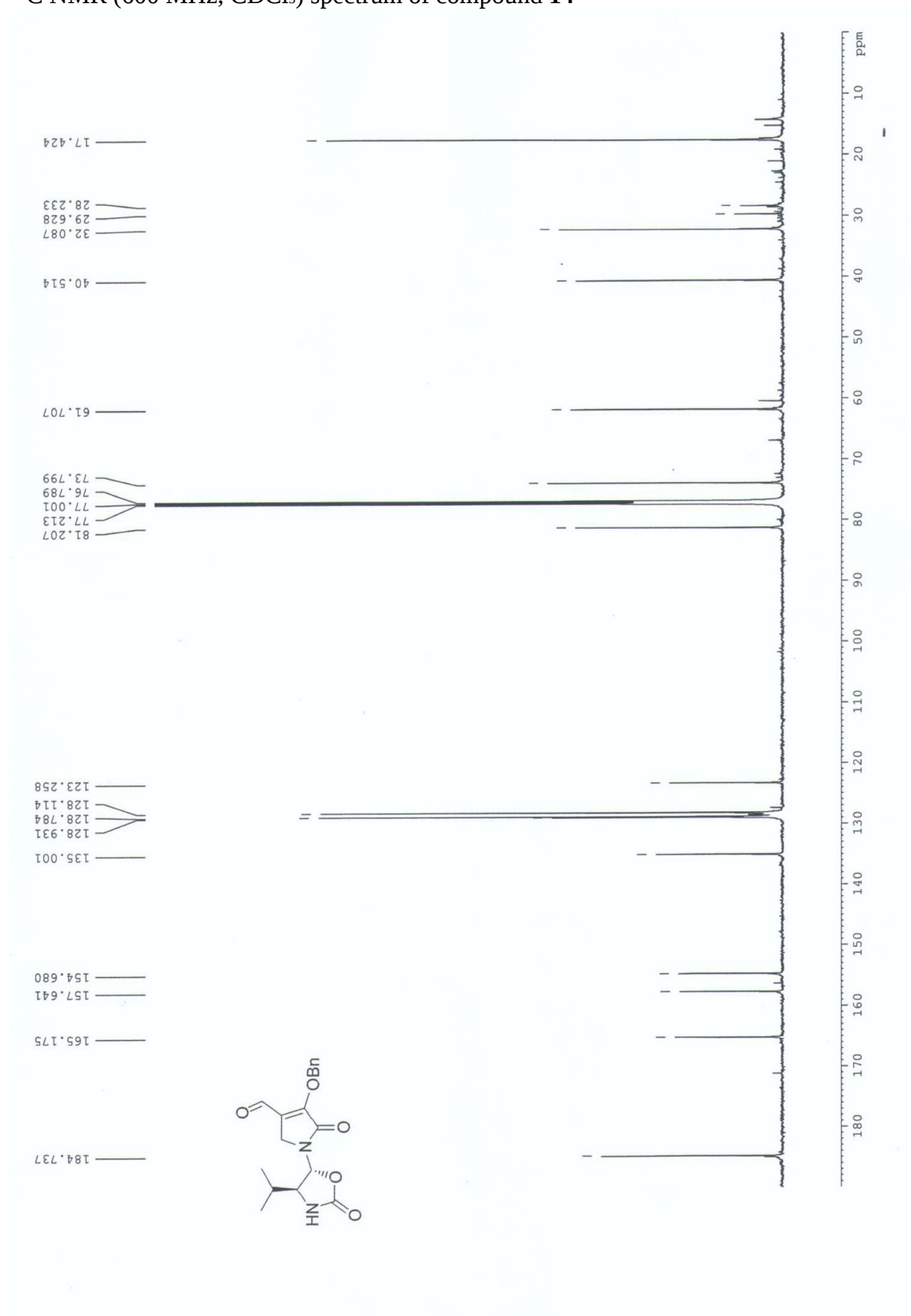
$^{13}\text{C}$  NMR (300 MHz,  $\text{CDCl}_3$ ) spectrum of compound **13** (mixture of stereoisomers) (APT, expanded))



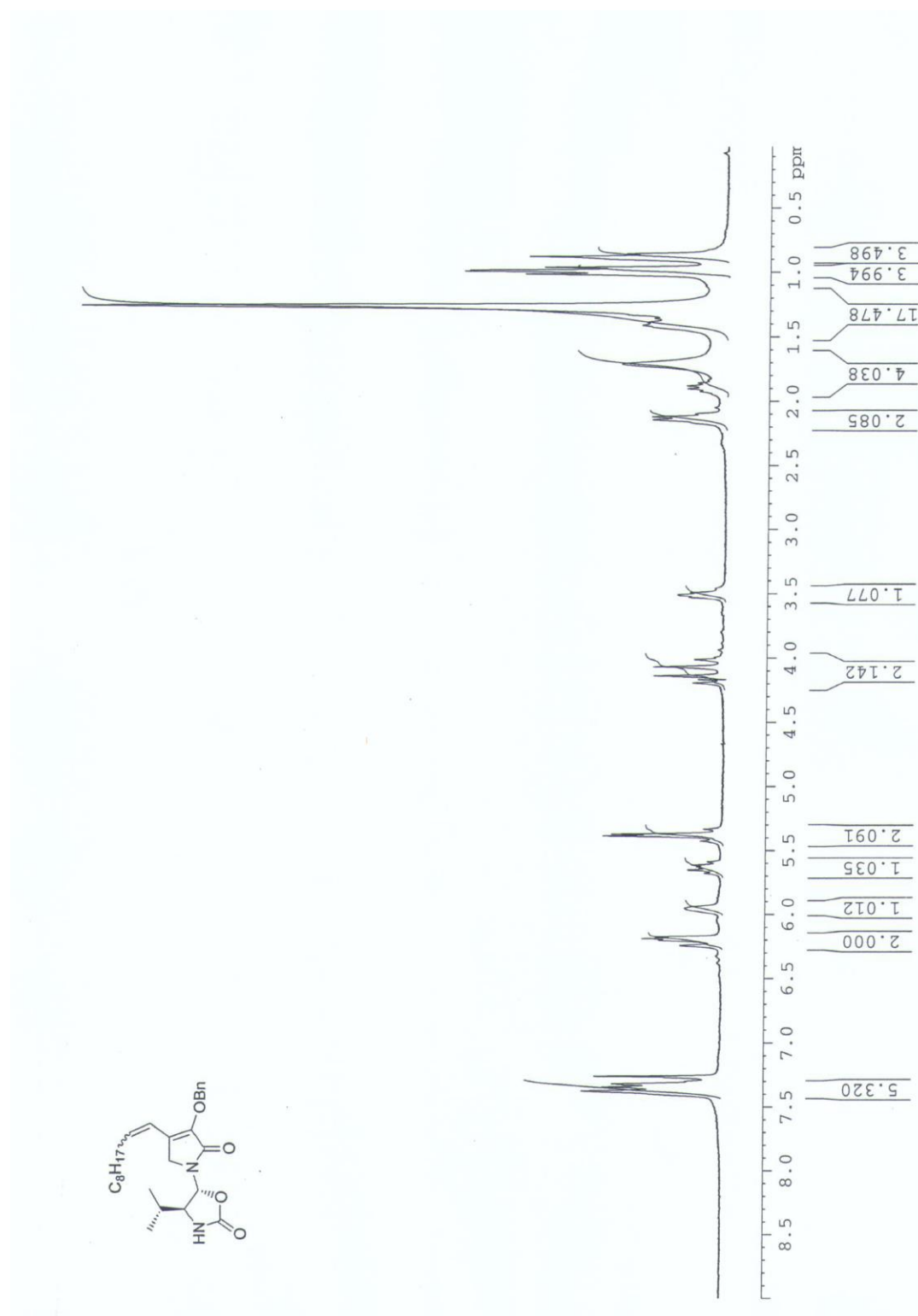
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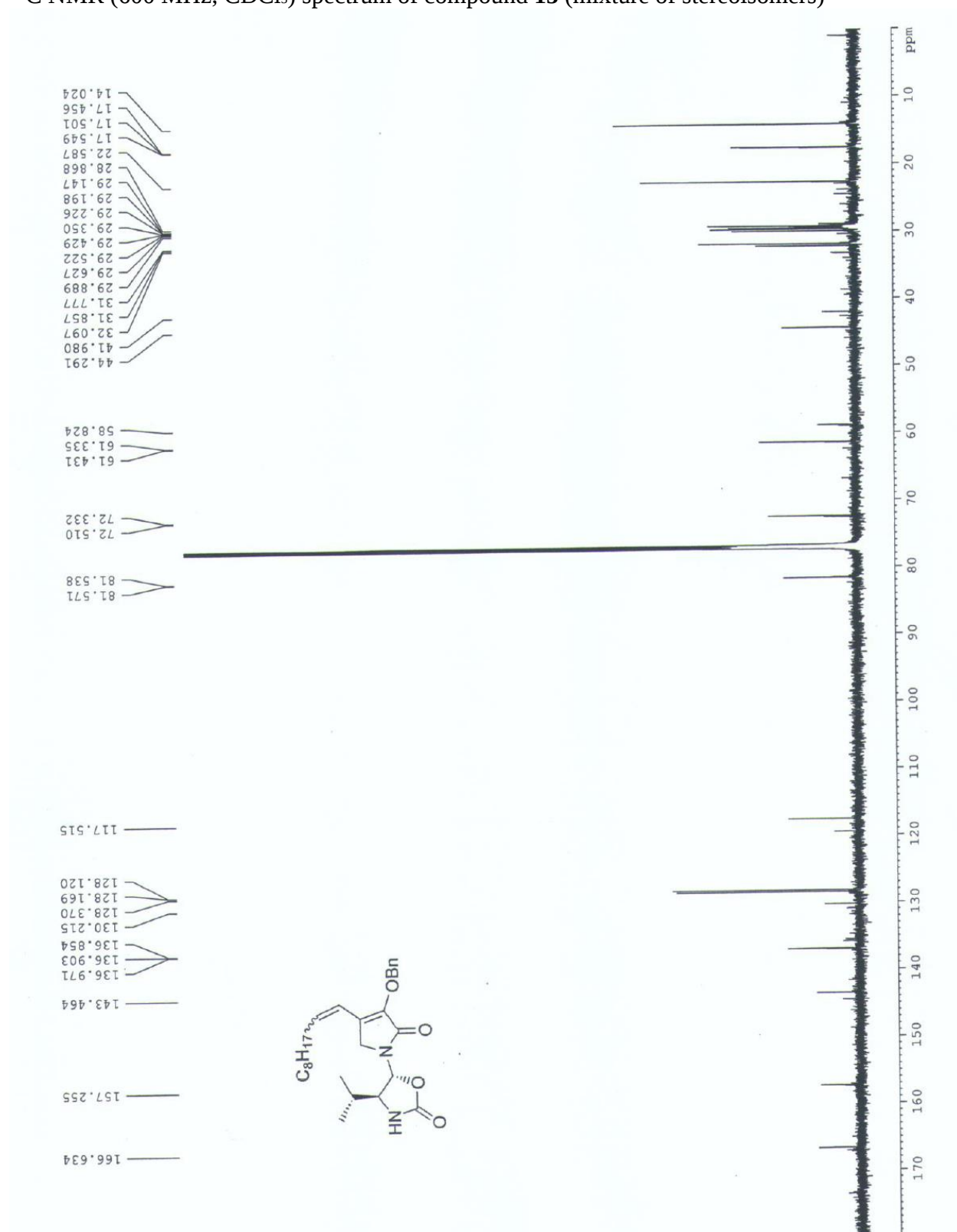
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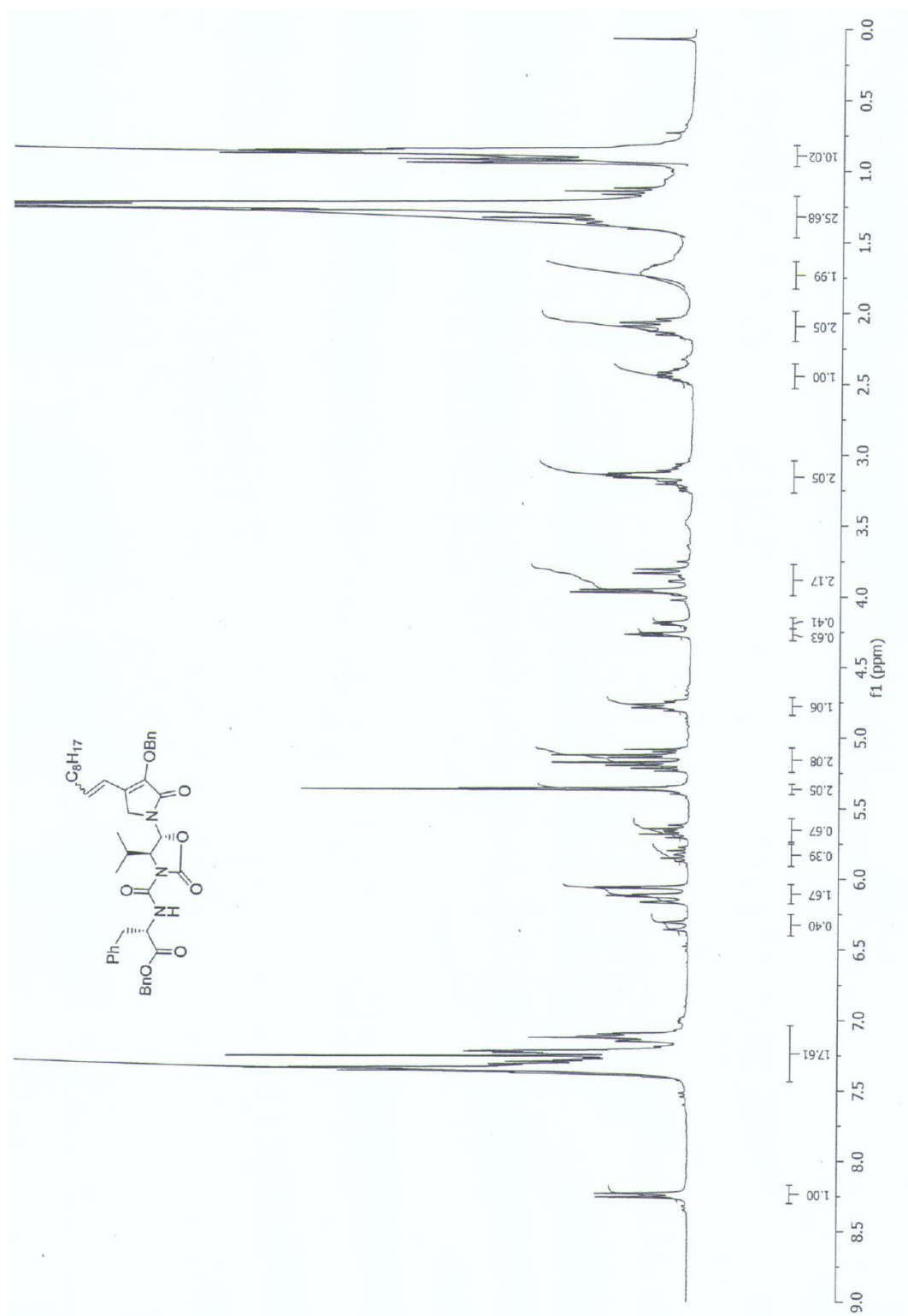
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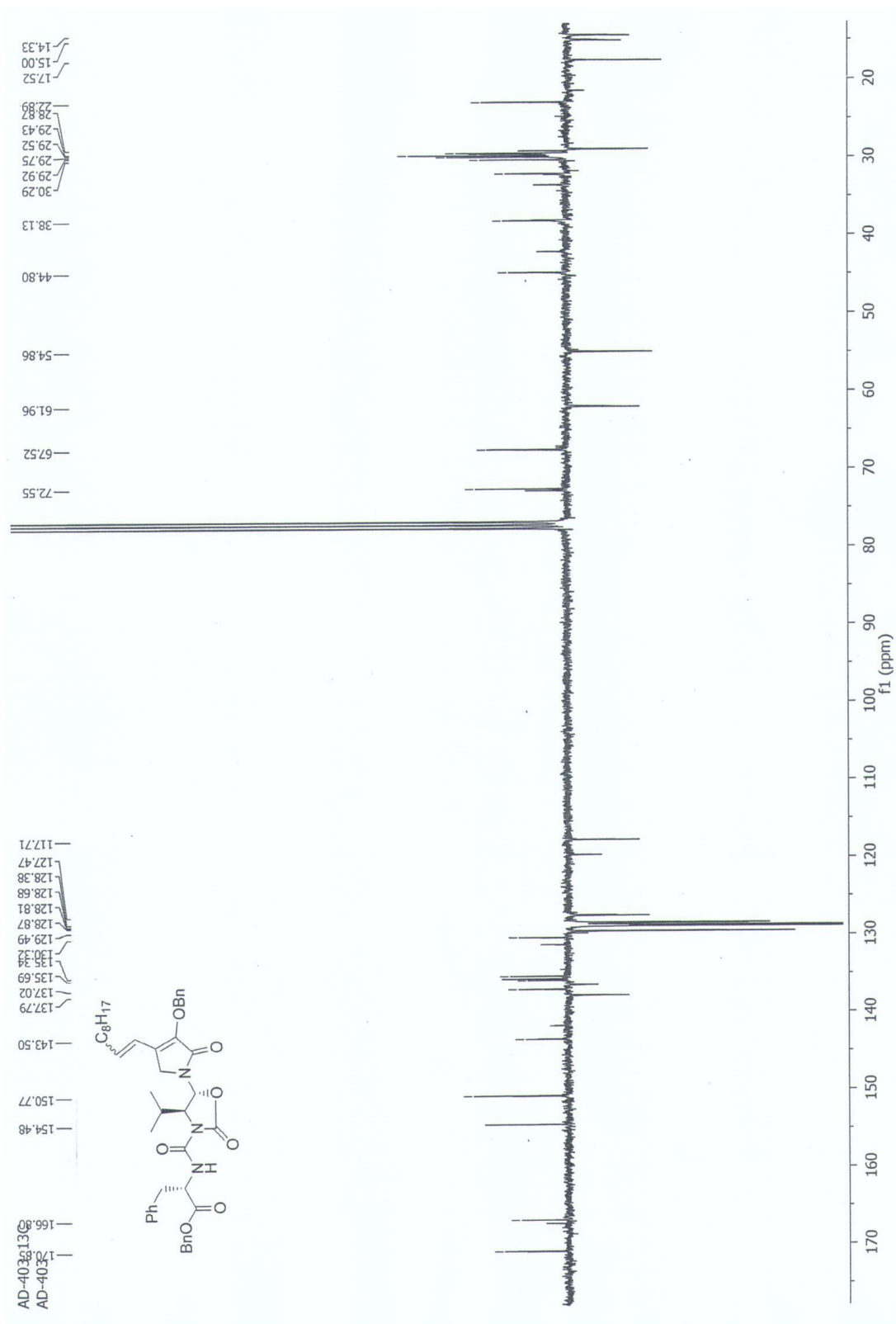
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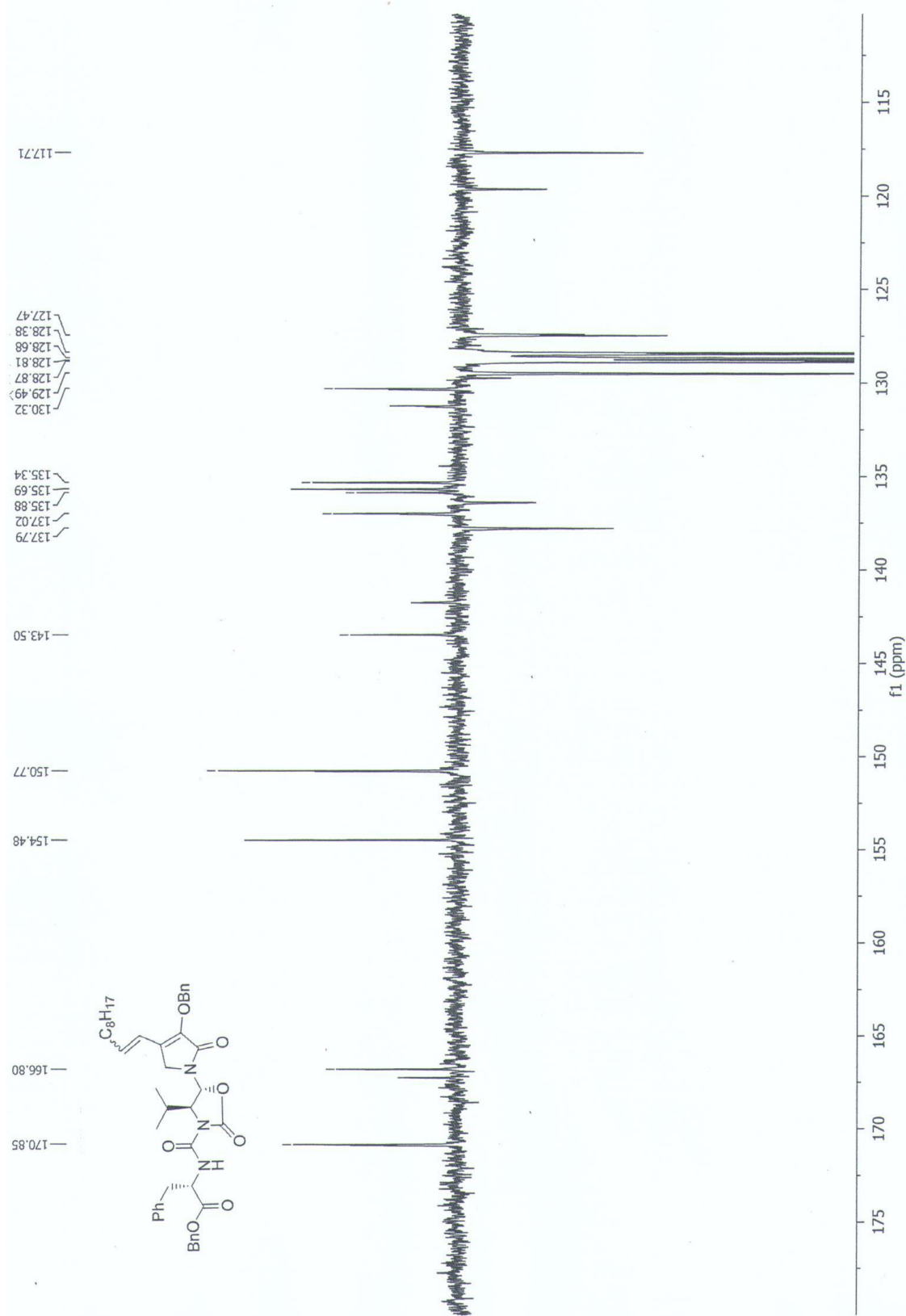
$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ) spectrum of compound **16** (mixture of stereoisomers)



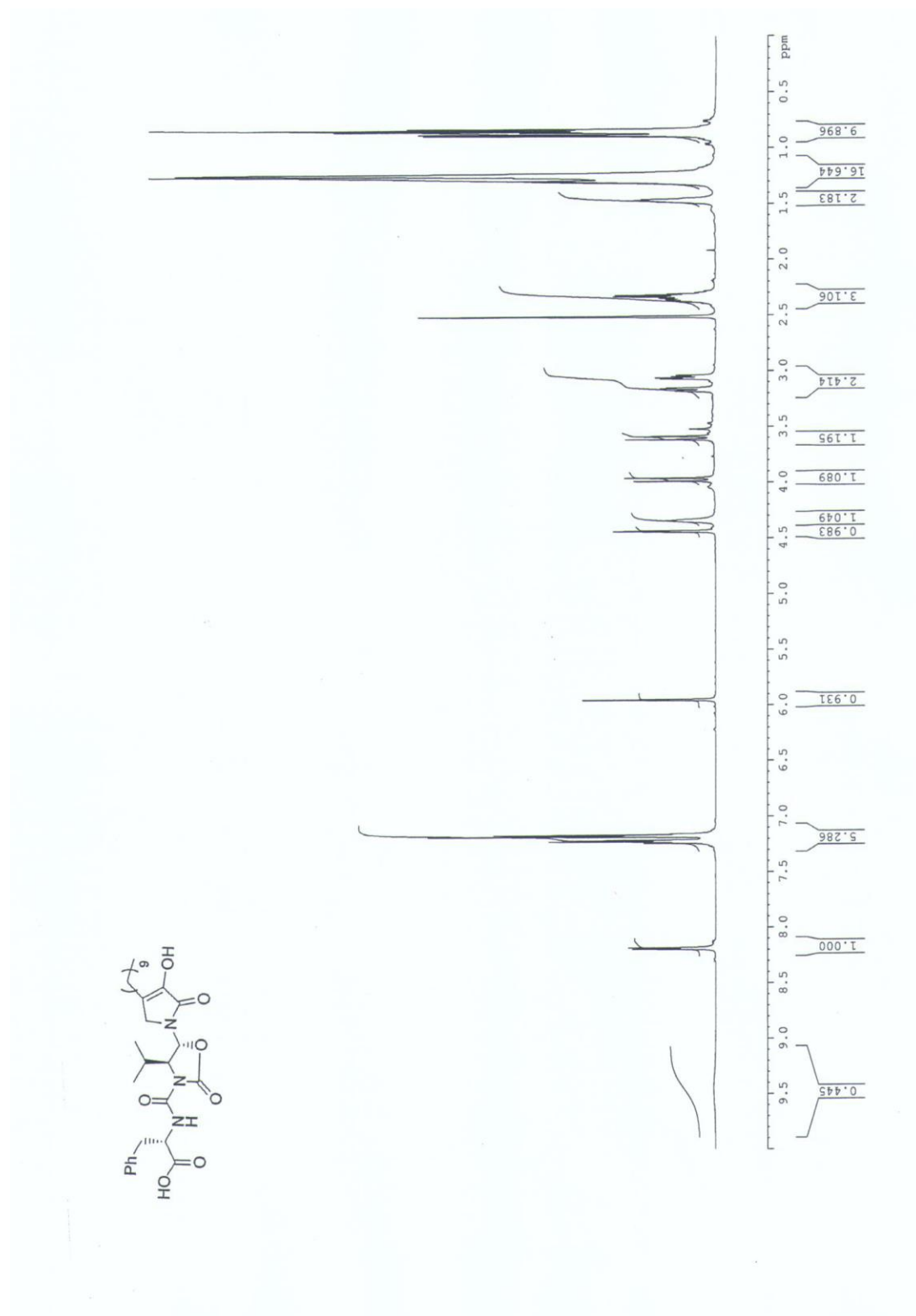
$^{13}\text{C}$  NMR (300 MHz,  $\text{CDCl}_3$ ) spectrum of compound **16** (mixture of stereoisomers)



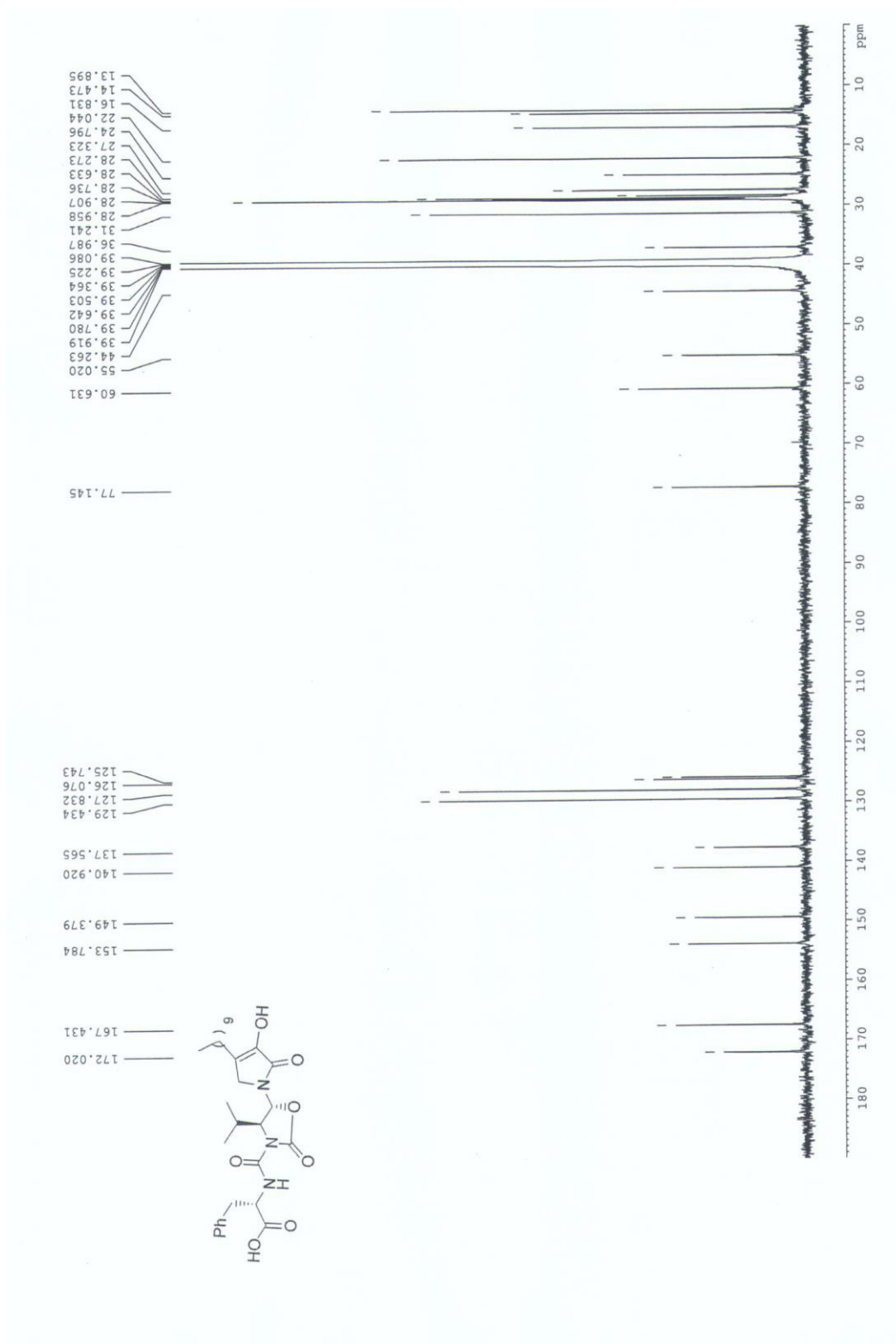
$^{13}\text{C}$  NMR (300 MHz,  $\text{CDCl}_3$ ) spectrum of compound **16** (mixture of stereoisomers, expanded)



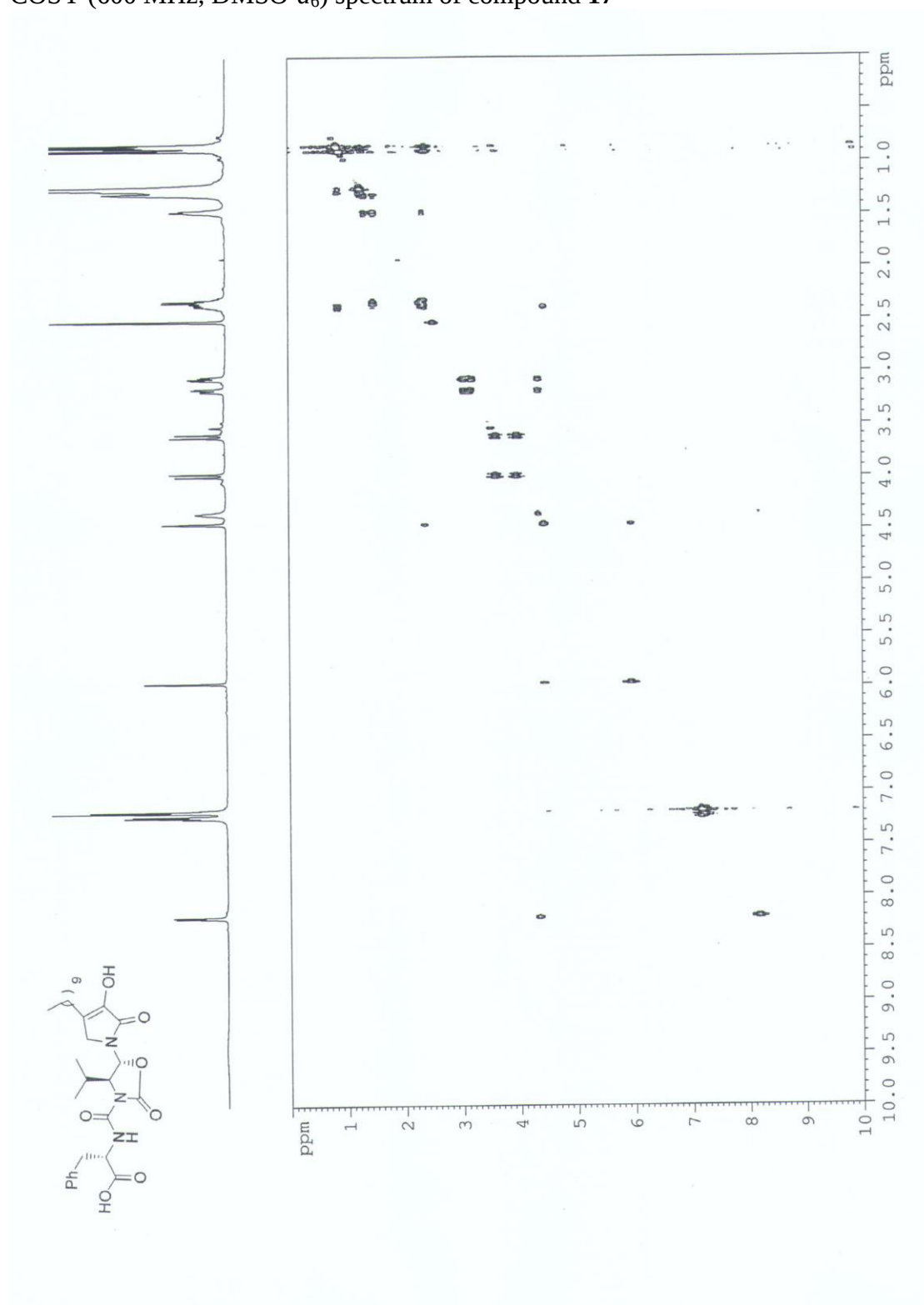
$^1\text{H}$  NMR (600 MHz,  $\text{DMSO-}d_6$ ) spectrum of compound **17**



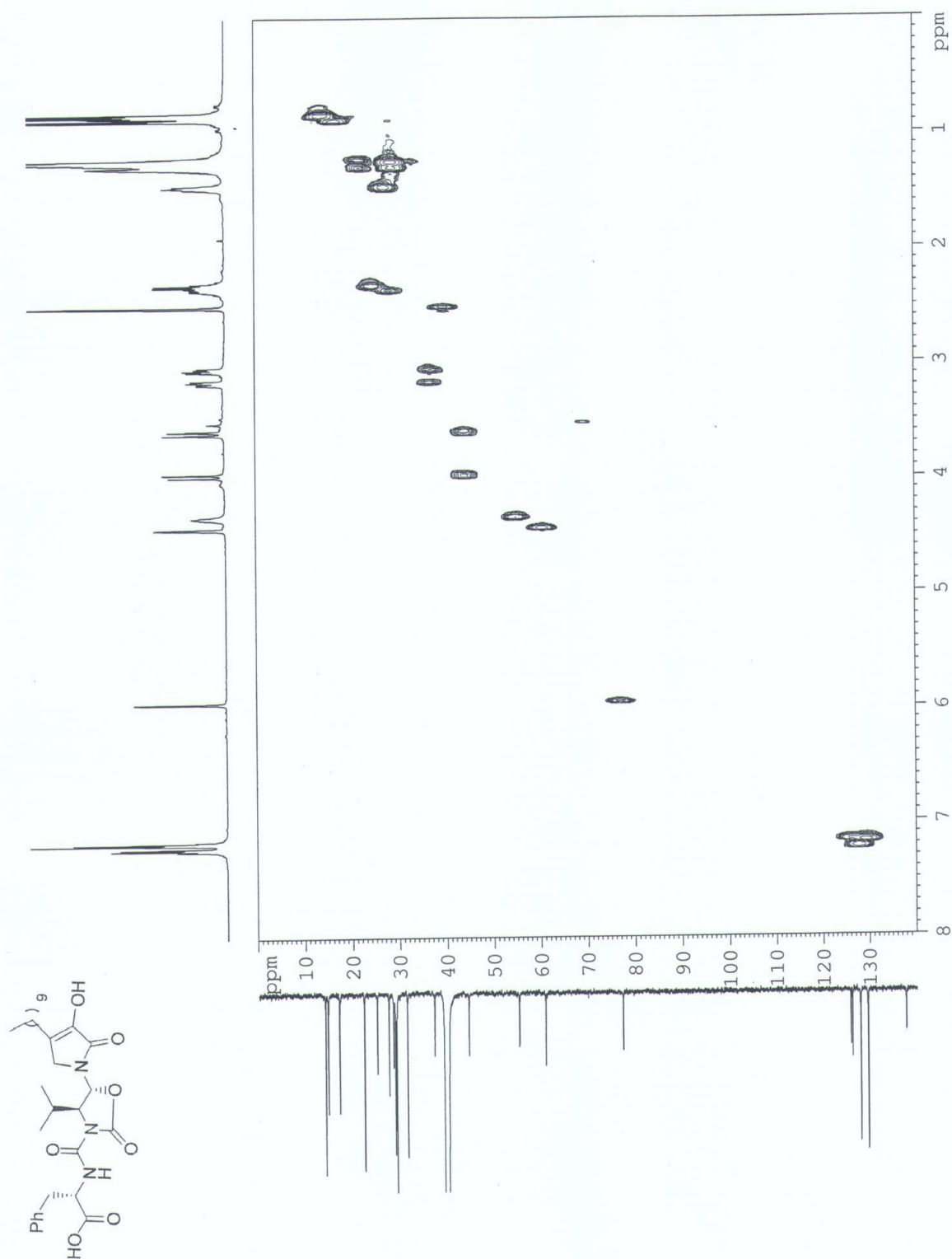
$^{13}\text{C}$  NMR (600 MHz,  $\text{DMSO-}d_6$ ) spectrum of compound **17**



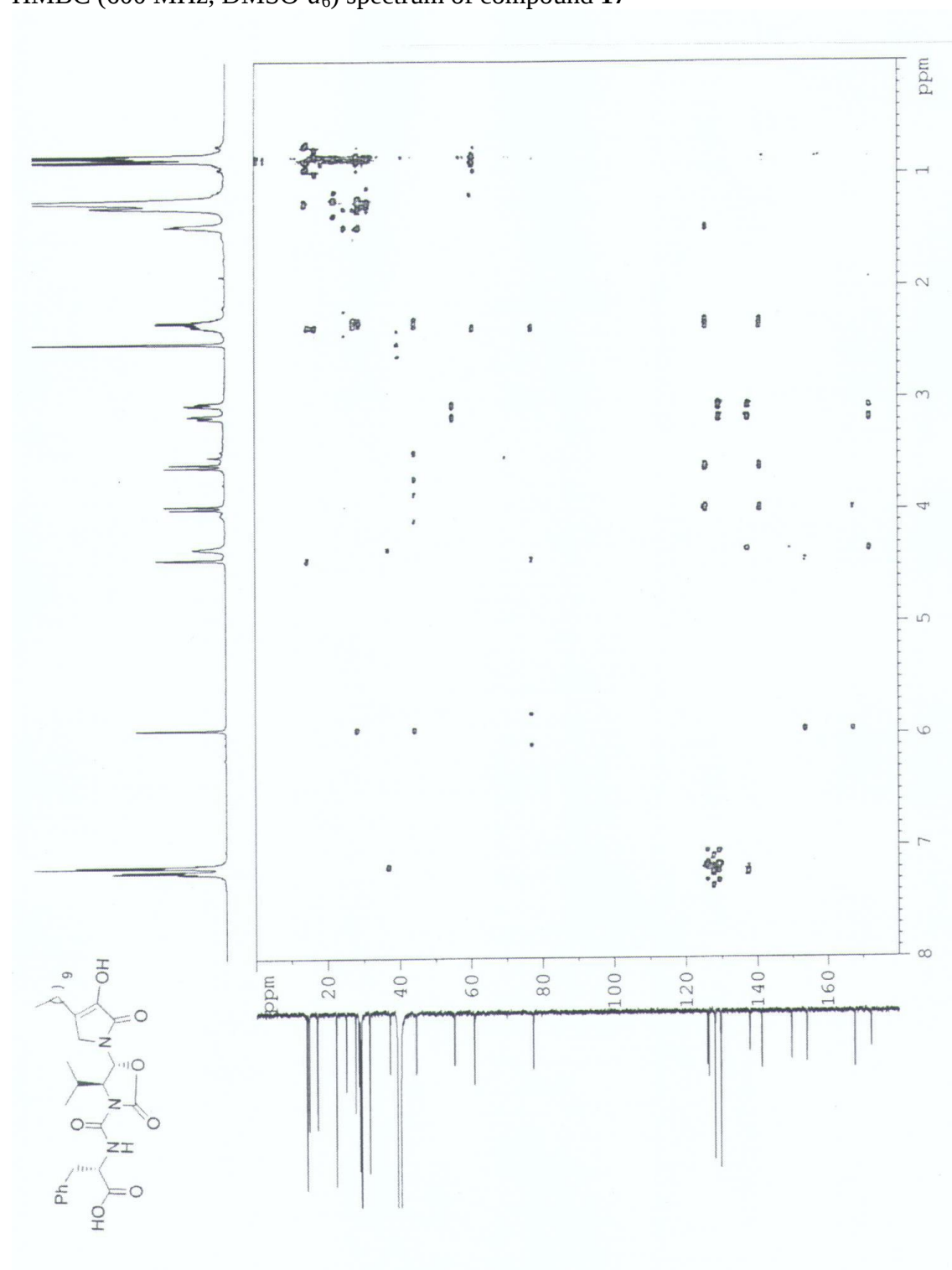
COSY (600 MHz, DMSO- $d_6$ ) spectrum of compound **17**



HSQC (600 MHz, DMSO- $d_6$ ) spectrum of compound **17**



HMBC (600 MHz, DMSO- $d_6$ ) spectrum of compound **17**

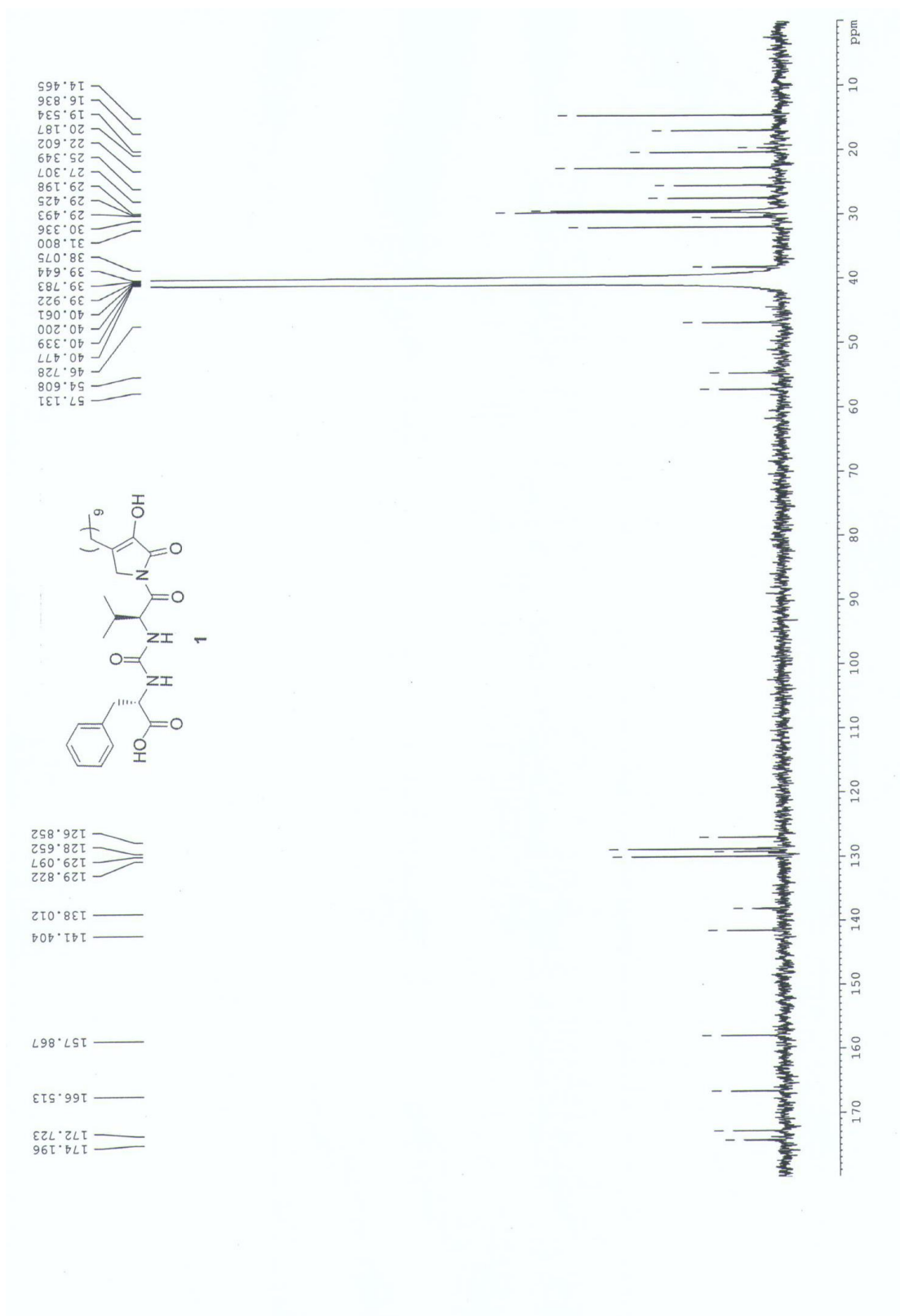


CC(C)[C@@H](NC(=O)C(=O)O[C@H](c1ccccc1)C(=O)N)C(=O)Nc1cc(O)c(C2=CC=CC=C2)nc12

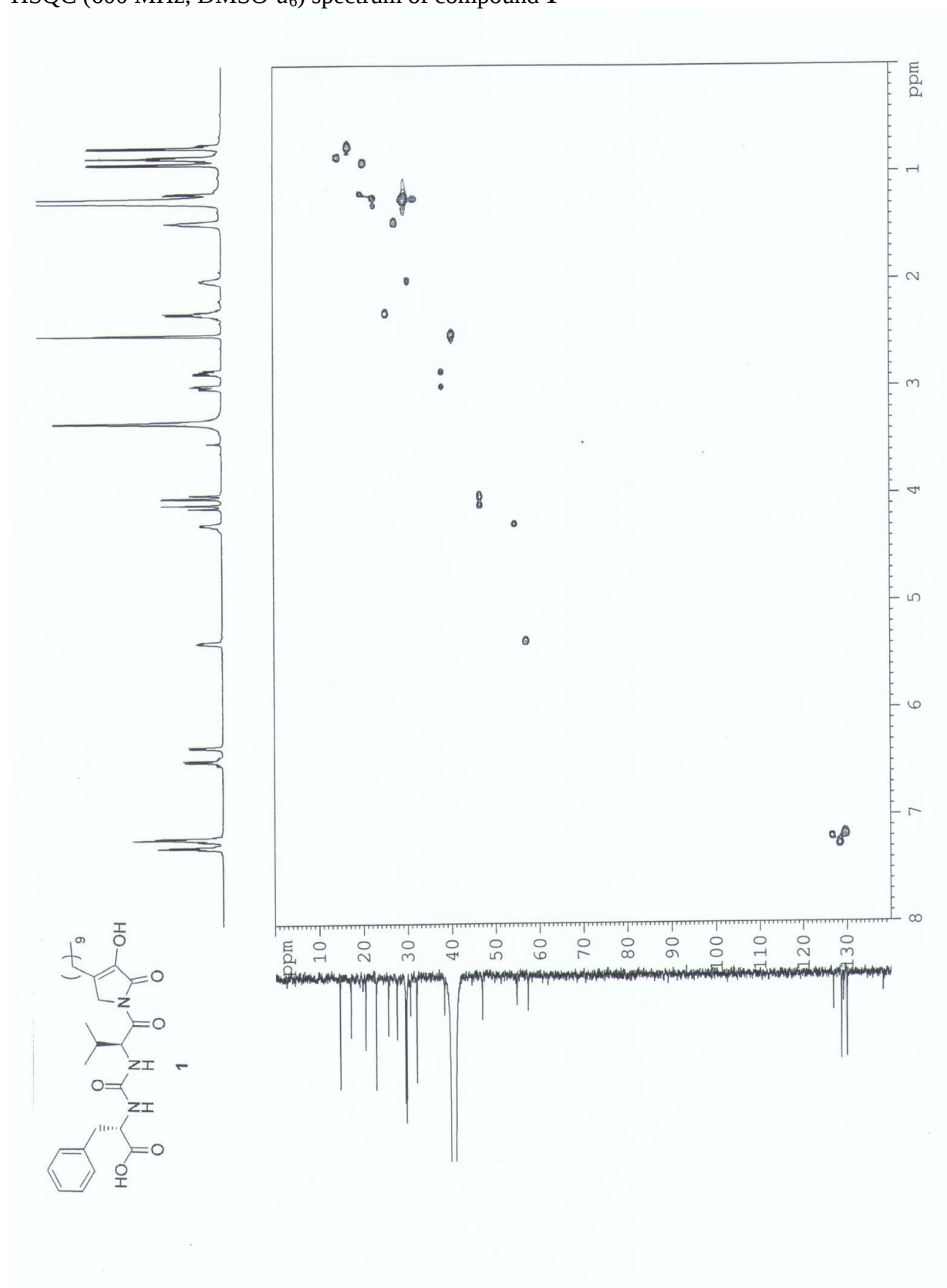
<sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) spectrum of compound 1

| Chemical Shift (ppm) | Integration |
|----------------------|-------------|
| 0.752                | 3H          |
| 0.975                | 3H          |
| 1.054                | 1H          |
| 1.012                | 1H          |
| 1.126                | 1H          |
| 1.7429               | 1H          |
| 2.327                | 1H          |
| 2.032                | 1H          |
| 2.138                | 1H          |
| 2.324                | 1H          |
| 3.135                | 1H          |
| 4.367                | 2H          |
| 2.001                | 2H          |
| 0.926                | 1H          |

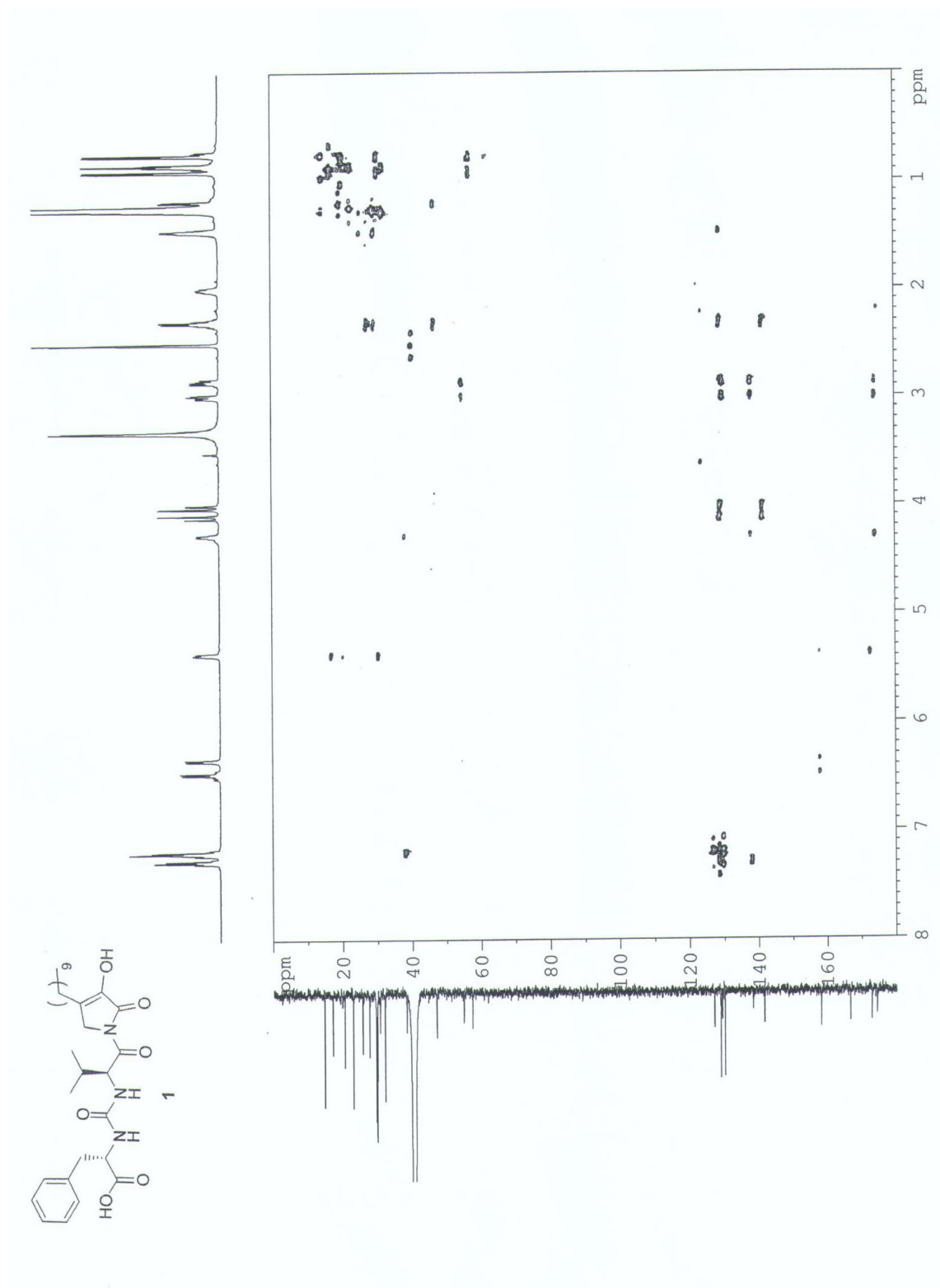
$^{13}\text{C}$  NMR (600 MHz,  $\text{DMSO-}d_6$ ) spectrum of compound **1**



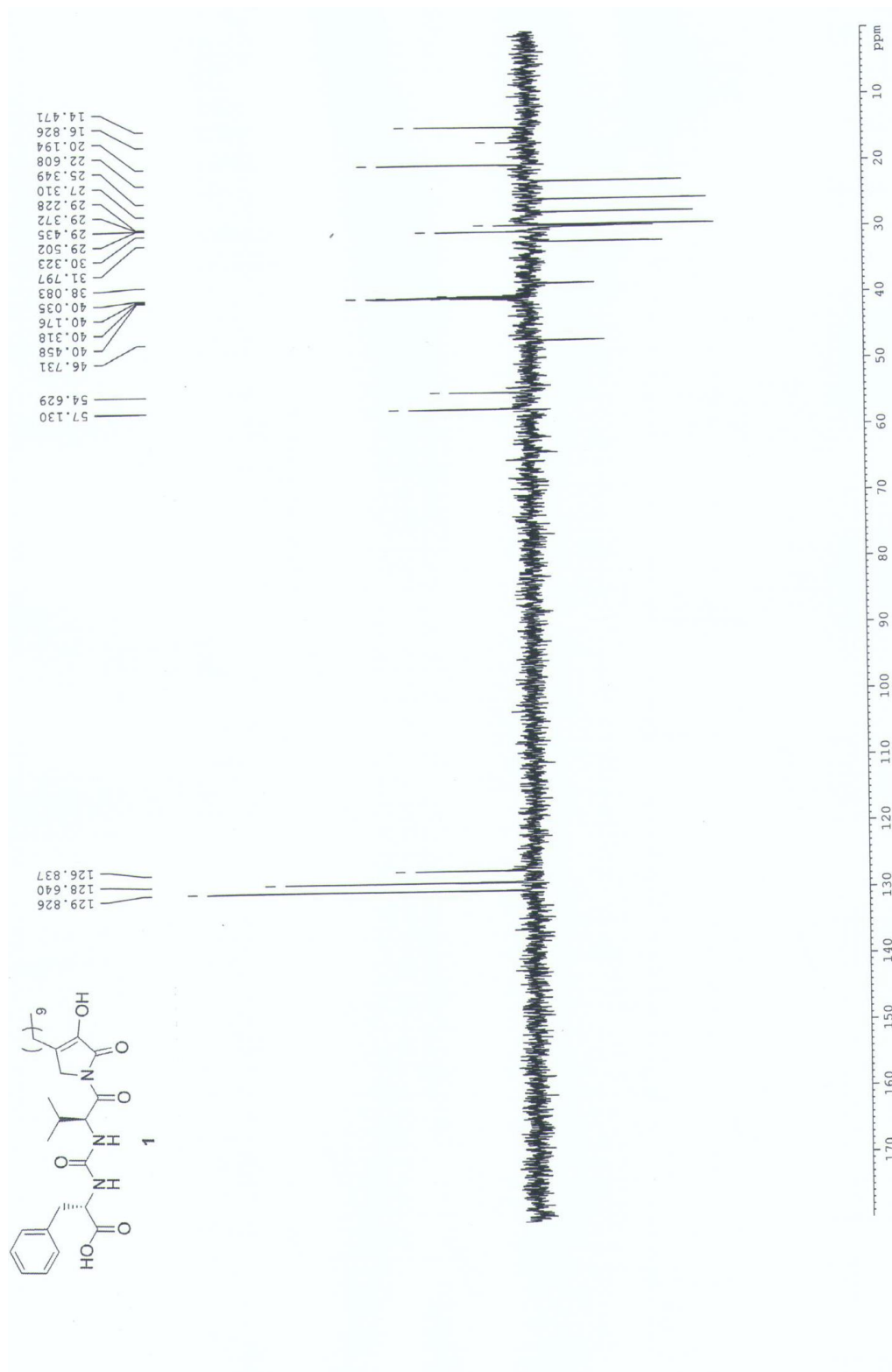
HSQC (600 MHz, DMSO- $d_6$ ) spectrum of compound **1**



HMBC(600 MHz, DMSO- $d_6$ ) spectrum of compound **1**



DEPT (600 MHz, DMSO-*d*<sub>6</sub>) spectrum of compound **1**



**MIC of 1 against *Staphylococcus pseudintermedius* strains<sup>a</sup>**

| Strain     | MIC (µg/mL) |
|------------|-------------|
| 1          | 16          |
| 2          | 16          |
| 3          | 16          |
| 4          | 8           |
| 5          | 16          |
| 6          | 16          |
| 7          | 16          |
| 8          | 16          |
| 9          | 16          |
| 10         | 16          |
| 11         | 16          |
| 12         | 16          |
| 13         | 16          |
| 14         | 16          |
| 15         | 16          |
| 16         | 16          |
| 17         | 16          |
| 18         | 16          |
| 19         | 16          |
| 20         | 16          |
| 21         | 16          |
| 22         | 16          |
| 23         | 16          |
| 24         | 16          |
| 25         | 16          |
| ATCC 25923 | 16          |

<sup>a</sup>Sampled in Milano (Italy) in 2015

# **MIC of 17 against *Staphylococcus pseudintermedius* strains<sup>a</sup>**

| Strain     | MIC (µg/mL) |
|------------|-------------|
| 1          | 16          |
| 2          | 32          |
| 3          | 64          |
| 4          | 64          |
| 5          | 32          |
| 6          | 32          |
| 7          | 32          |
| 8          | 64          |
| 9          | 64          |
| 10         | 64          |
| 11         | 32          |
| 12         | 32          |
| 13         | 32          |
| 14         | 32          |
| 15         | 32          |
| 16         | 32          |
| 17         | 64          |
| 18         | 64          |
| 19         | 64          |
| 20         | 32          |
| 21         | 32          |
| 22         | 32          |
| 23         | 32          |
| 24         | 32          |
| 25         | 64          |
| ATCC 25923 | 32          |

<sup>a</sup>Sampled in Milano (Italy) in 2015

**MIC of 1 against *Escherichia coli* strains**

| Strain     | MIC (µg/mL) |
|------------|-------------|
| 1          | 128         |
| 2          | 128         |
| 3          | 128         |
| 4          | 128         |
| 5          | 128         |
| 6          | 128         |
| 7          | 128         |
| 8          | 128         |
| 9          | 128         |
| 10         | 128         |
| 11         | 128         |
| 12         | 128         |
| 13         | 128         |
| 14         | 128         |
| 15         | 128         |
| 16         | 128         |
| 17         | 128         |
| 18         | 128         |
| 19         | 128         |
| 20         | 128         |
| ATCC 25923 | 128         |

### MIC of 17 against *E. coli* strains

| Strain     | MIC (µg/mL) |
|------------|-------------|
| 1          | 128         |
| 2          | 128         |
| 3          | 128         |
| 4          | 128         |
| 5          | 128         |
| 6          | 128         |
| 7          | 128         |
| 8          | 128         |
| 9          | 128         |
| 10         | 128         |
| 11         | 128         |
| 12         | 128         |
| 13         | 128         |
| 14         | 128         |
| 15         | 128         |
| 16         | 128         |
| 17         | 128         |
| 18         | 128         |
| 19         | 128         |
| 20         | 128         |
| ATCC 25923 | 128         |