

## Supplemental information for

### Pipecolic Esters as Minimized Templates for Proteasome Inhibition

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Page 2.....Docking studies rationale & results

Page 3.....<sup>1</sup>H and <sup>13</sup>C NMR spectra's of compounds B1, 2-15

## Docking studies rationale & results

The large size of the human proteasome can make docking studies complicated due to the large search space and numerous possible binding pockets. Additionally, a completely exhaustive search is infeasible with the hardware available in most labs as the large number of rotatable bonds in the peptidic ester structures increases the computational expense. To overcome these limitations, docking was undertaken in three parts:

### 1) The whole 20S proteasome

The entirety of the proteasome was searched with a low exhaustiveness (30-40) in multiple individual runs (on average ~5 times). This sampling revealed more binding modes within the alpha ring than the beta rings.

### 2) Alpha and Beta Rings

After (1), the search space was reduced, and exhaustiveness increased (~60). In two separate runs, each compound was docked against the beta rings, and then the alpha rings. The average binding affinity difference was ~1.2 kcal/mol suggesting a large preference for the alpha ring system.

### 3) Alpha Ring Site refinement

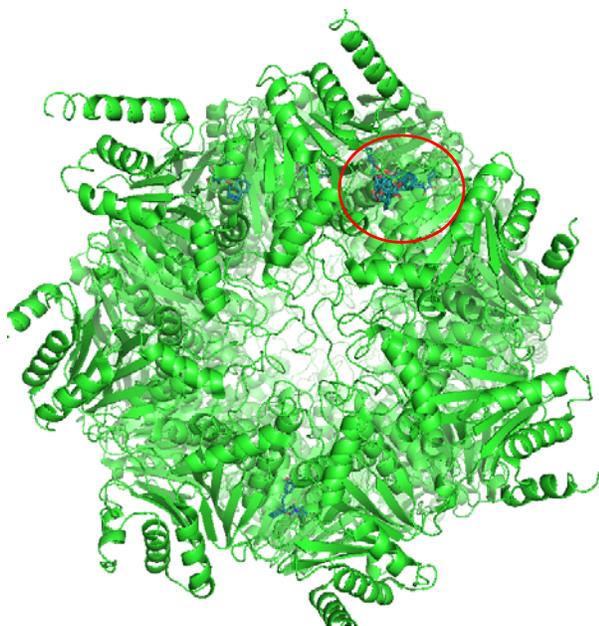
Finally, the search space was limited to the minimum space necessary to encompass just the alpha ring and as high an exhaustiveness as possible on our hardware:

Center: (135.9681, -38.0296, 65.3754)

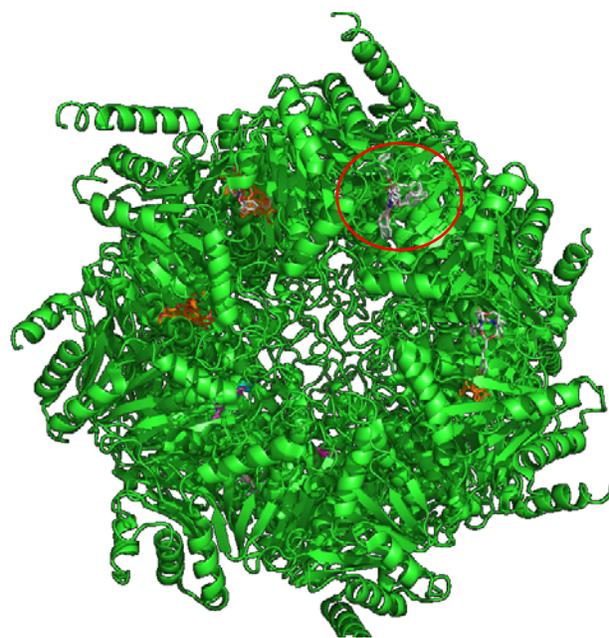
Dimensions (Angstrom): X: 122.9455 Y: 138.030 Z: 53.1835 Exhaustiveness: 80

Remarkably, the most potent compounds gathered in only a few of the intersubunit pockets (red circles).

While, the less active compounds preferred either a different binding pocket or no preference.

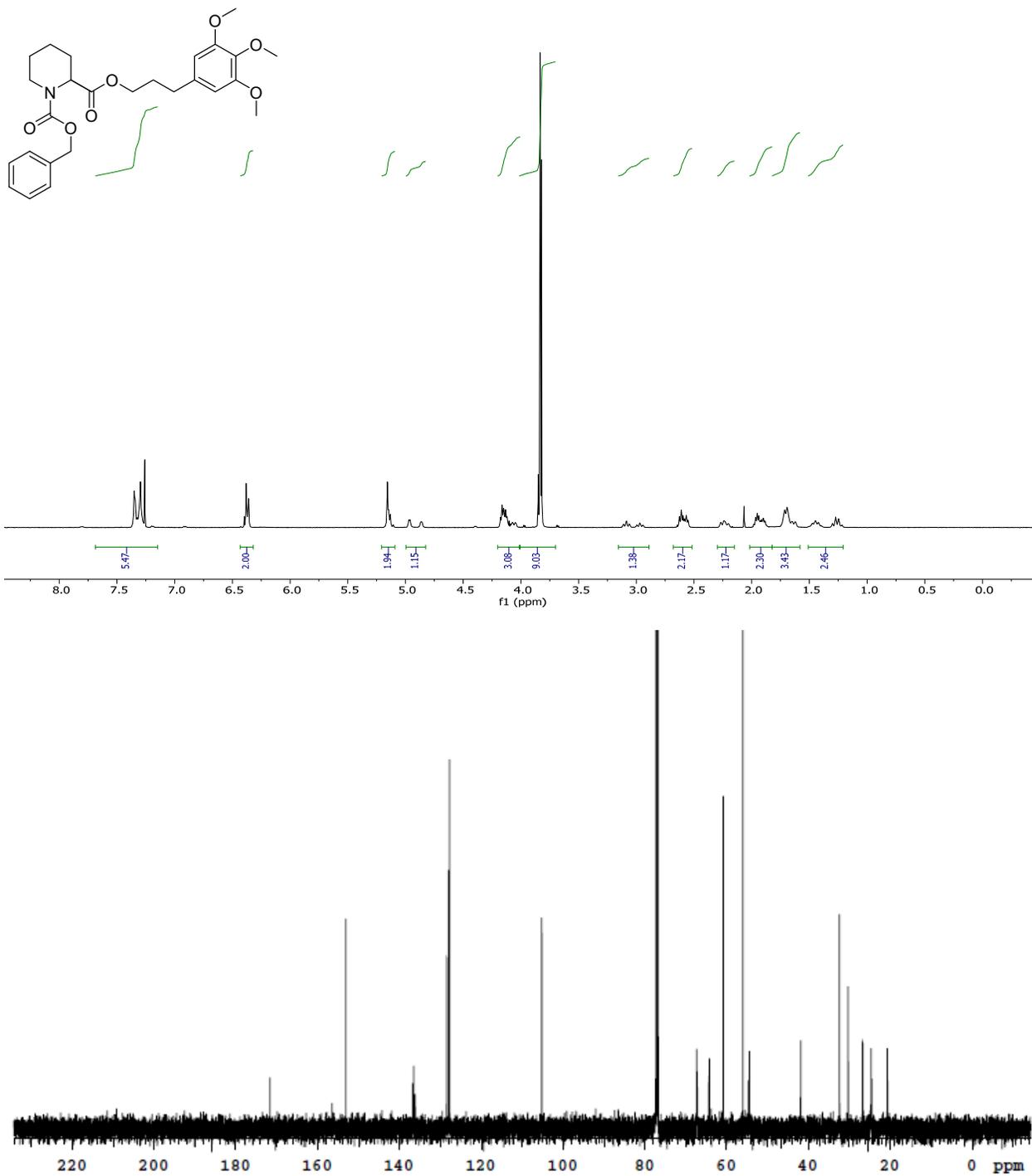


Compound B1 binding poses

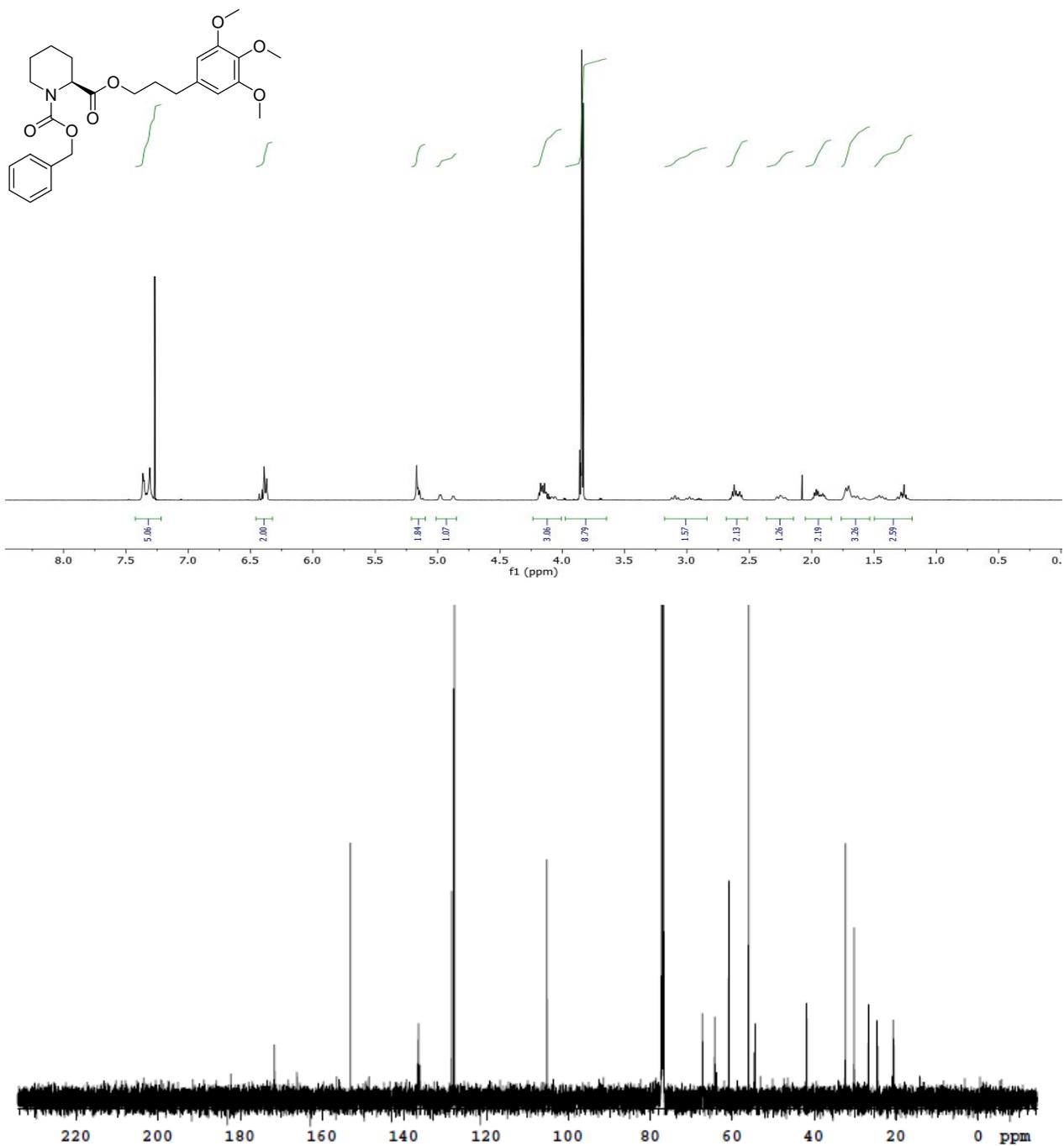


Multiple binding modes

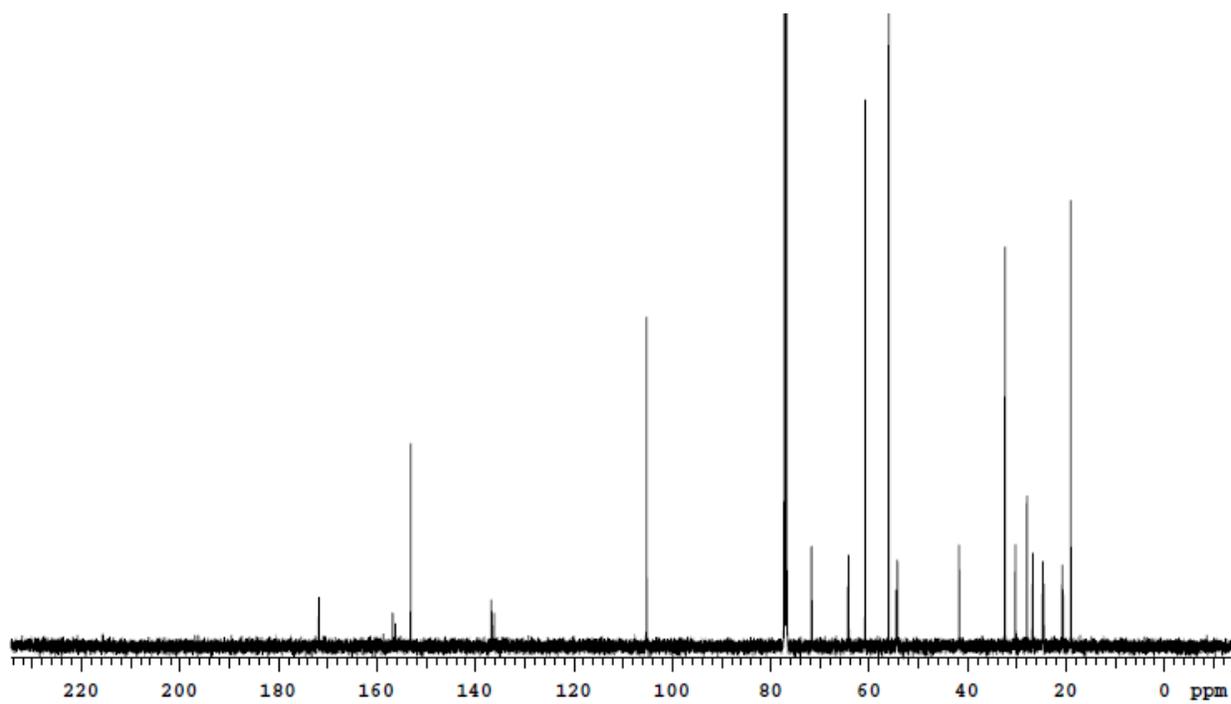
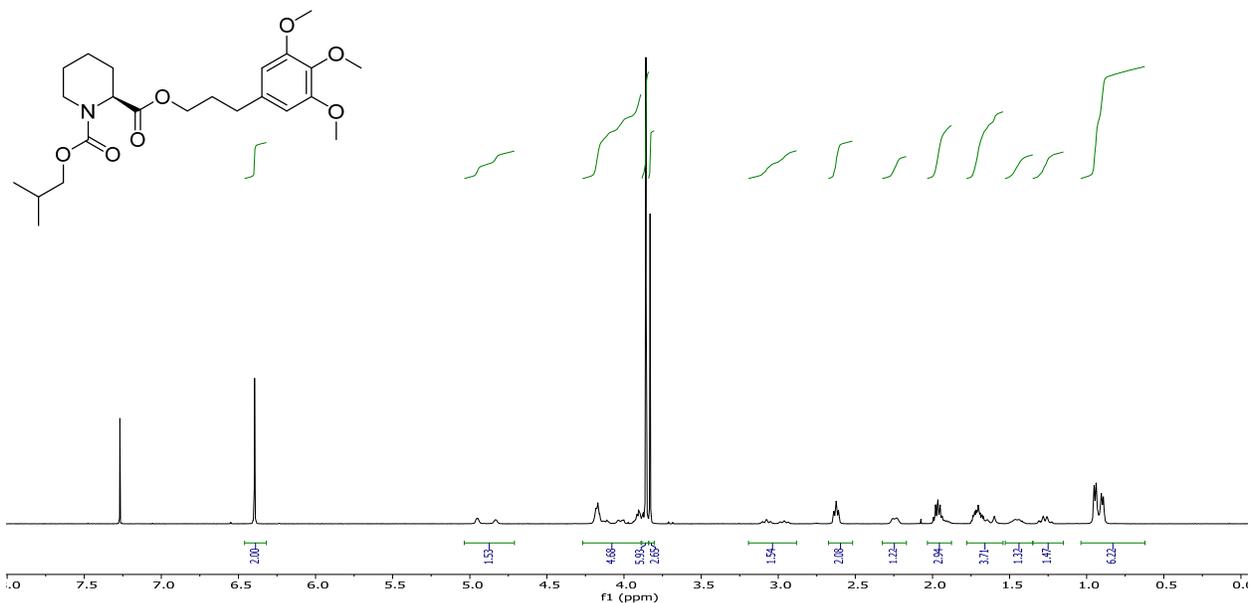
**Compound 2** (in CDCl<sub>3</sub>, standardized to 7.26 signal)



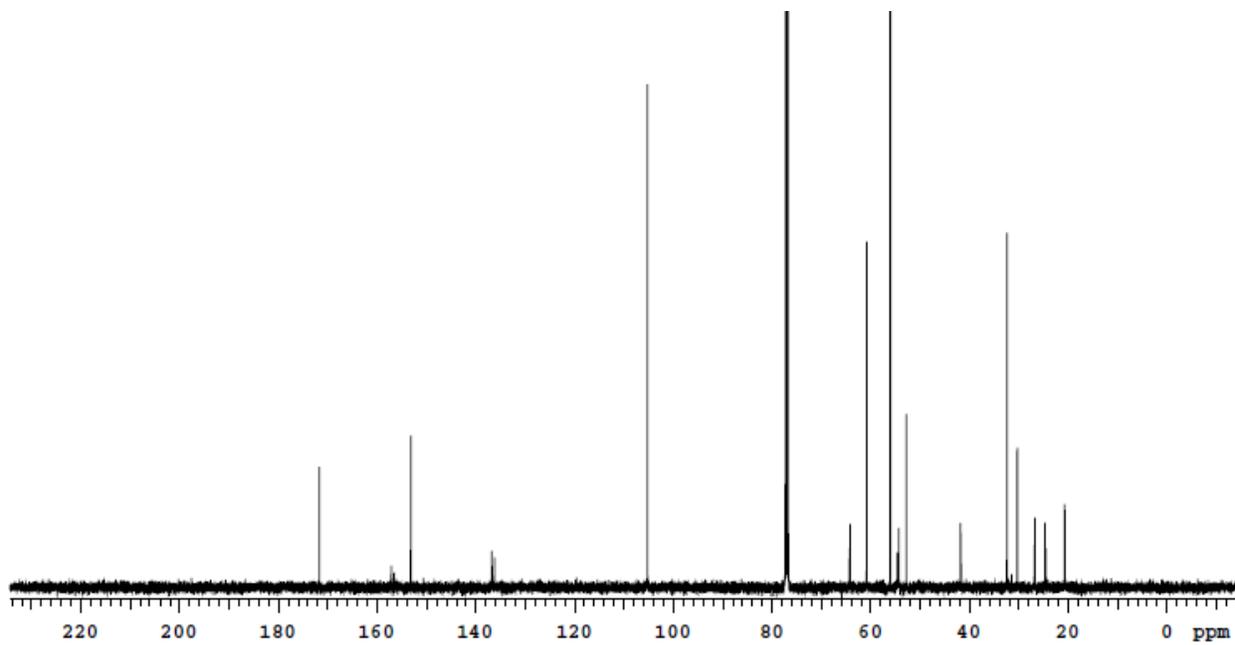
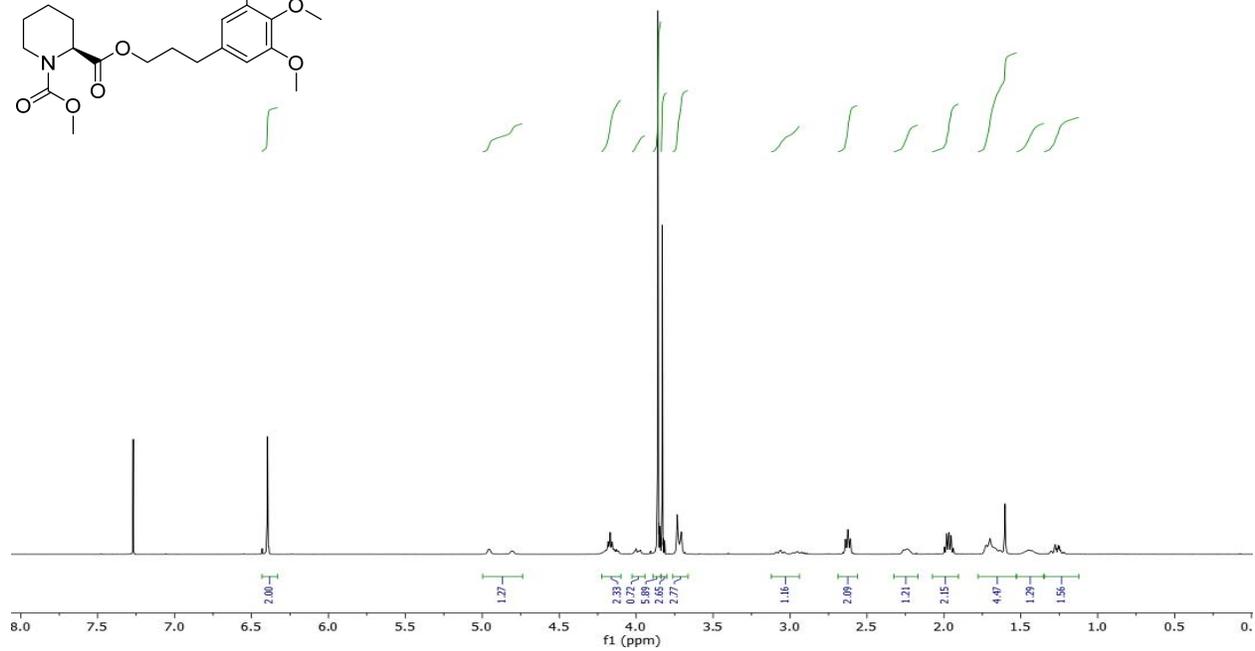
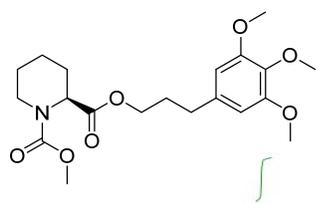
Compound 3 (in CDCl<sub>3</sub>, standardized to 7.26 signal)



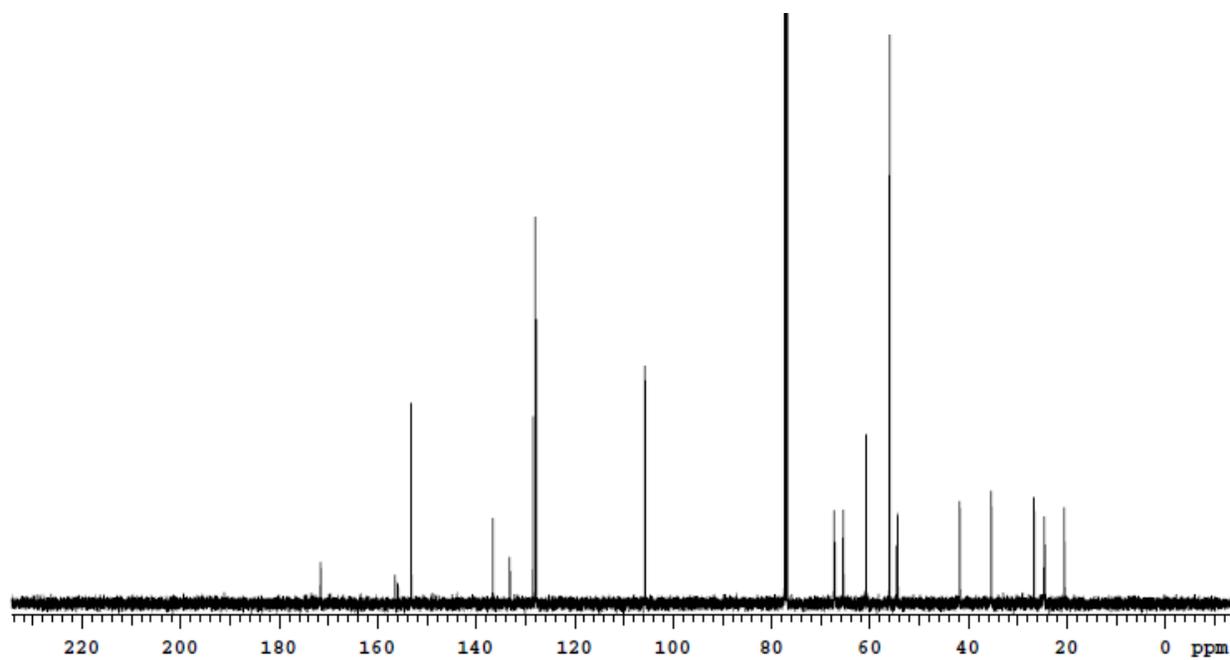
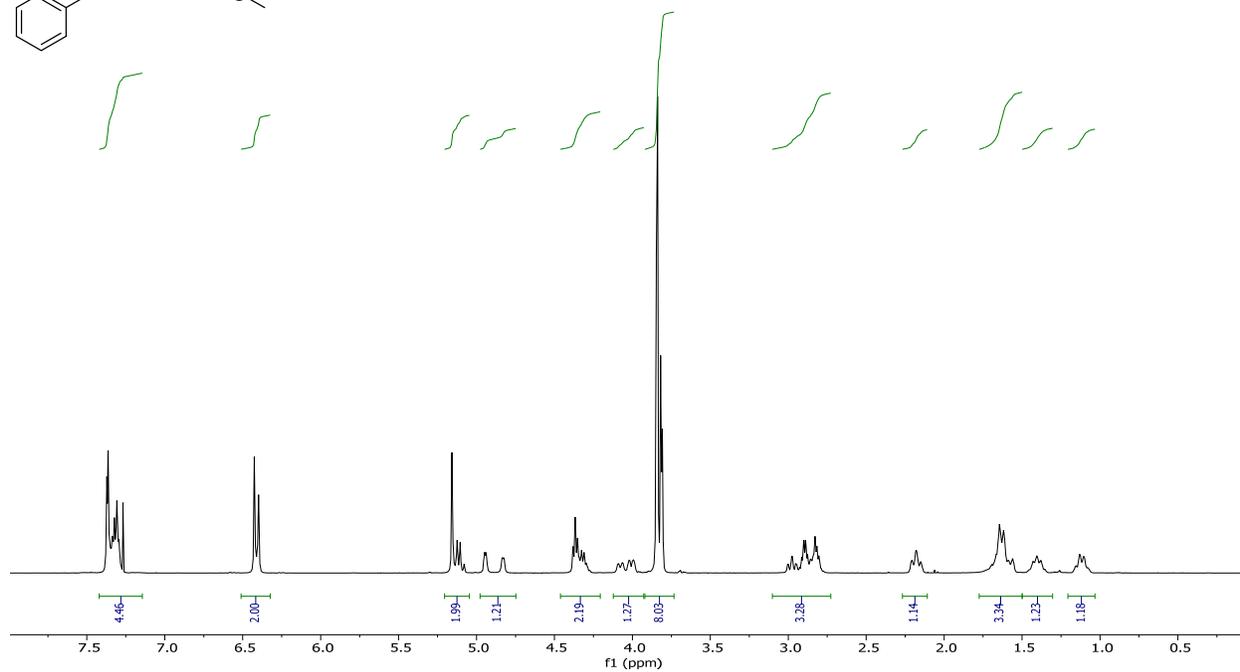
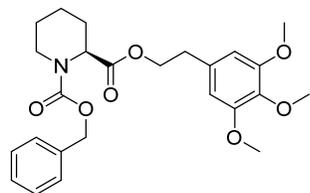
**Compound 4** (in CDCl<sub>3</sub>, standardized to 7.26 signal)



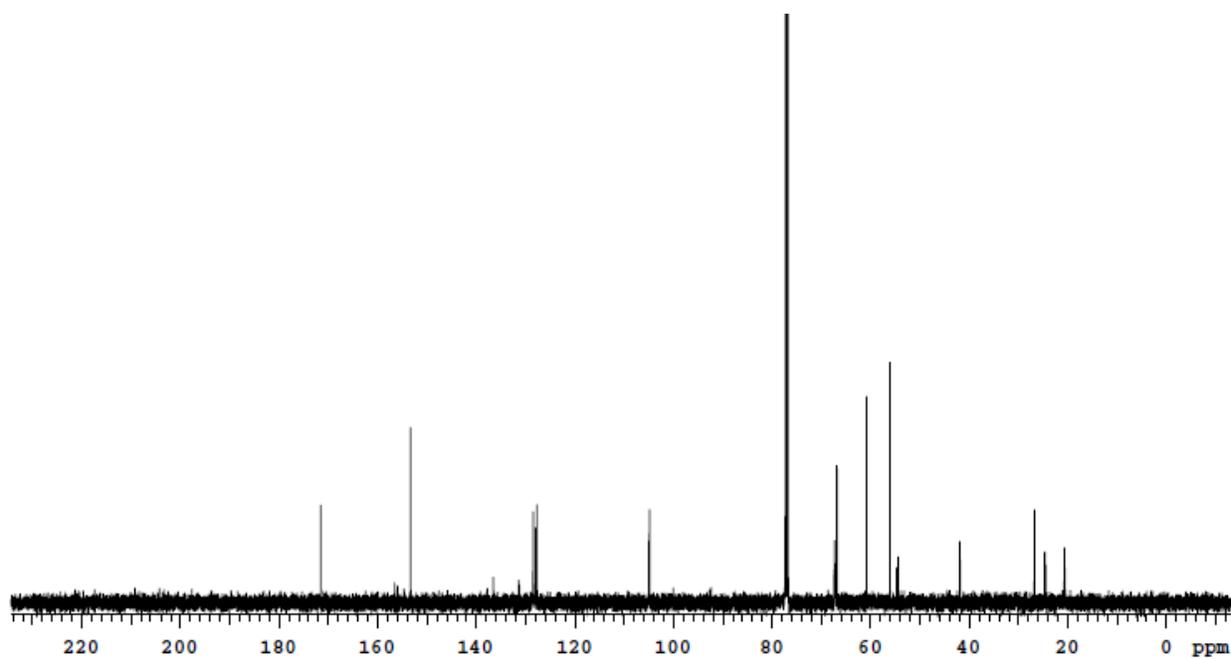
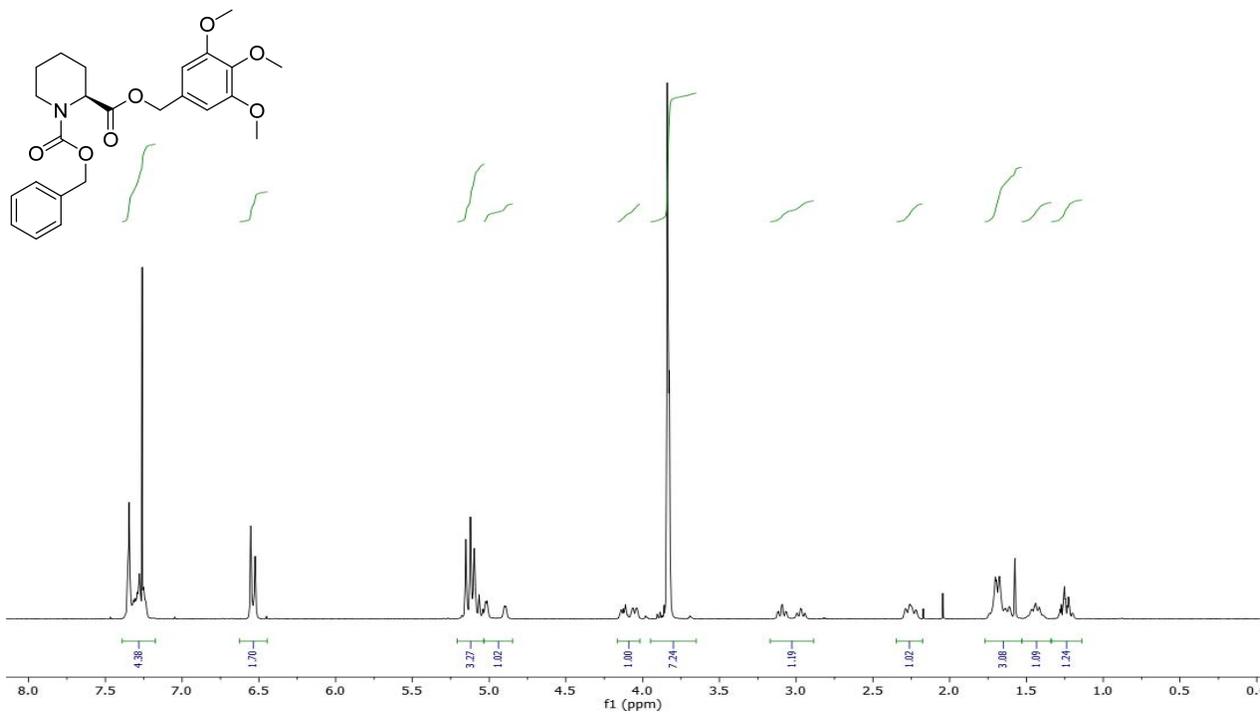
**Compound 5** (in CDCl<sub>3</sub>, standardized to 7.26 signal)



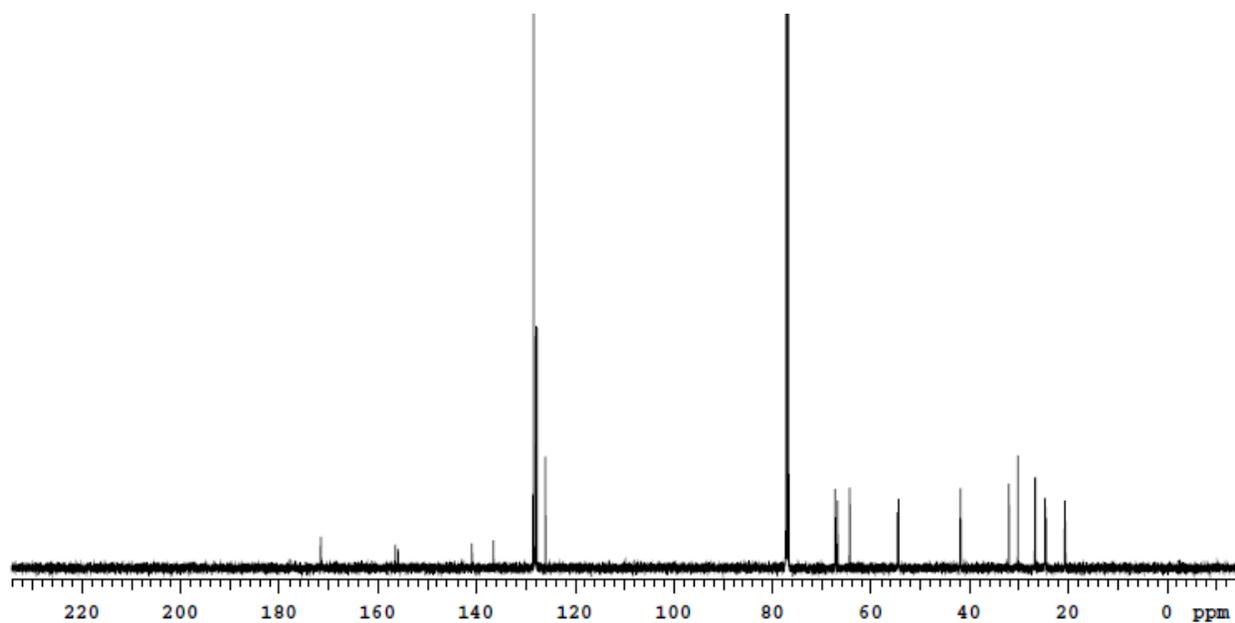
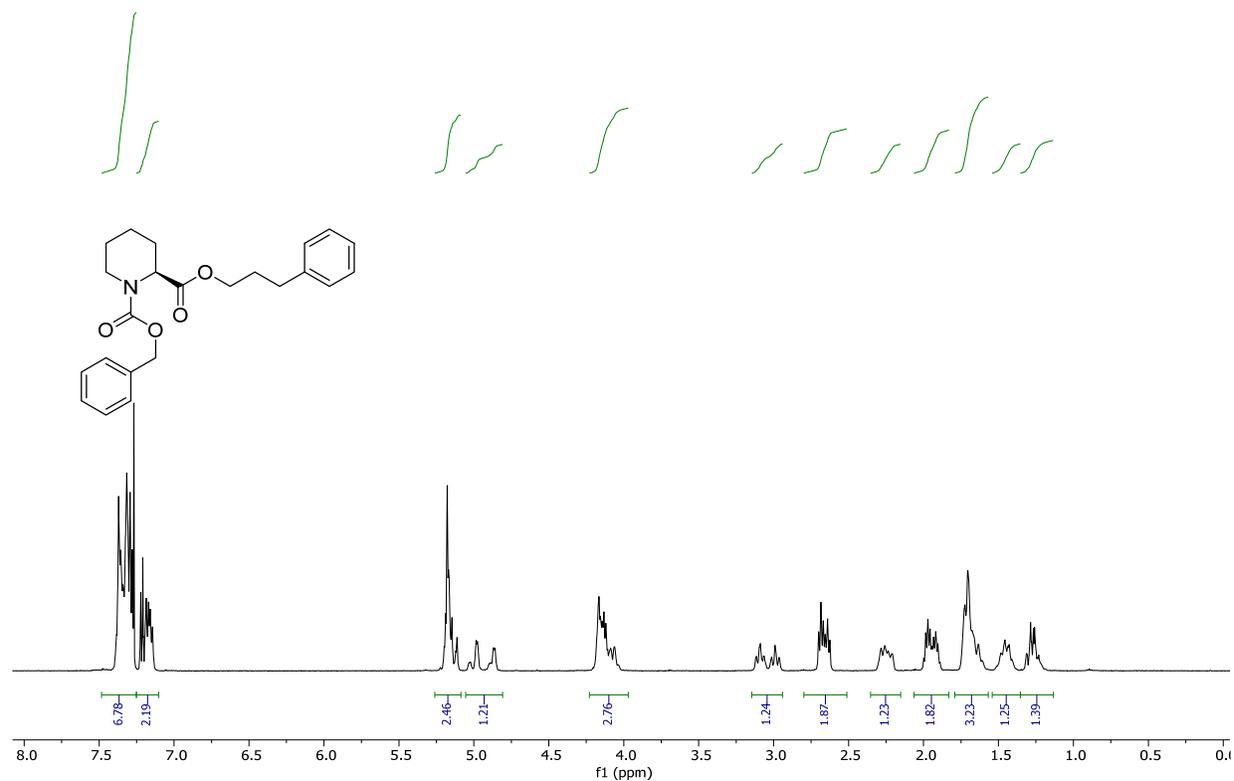
**Compound 6** (in CDCl<sub>3</sub>, standardized to 7.26 signal)



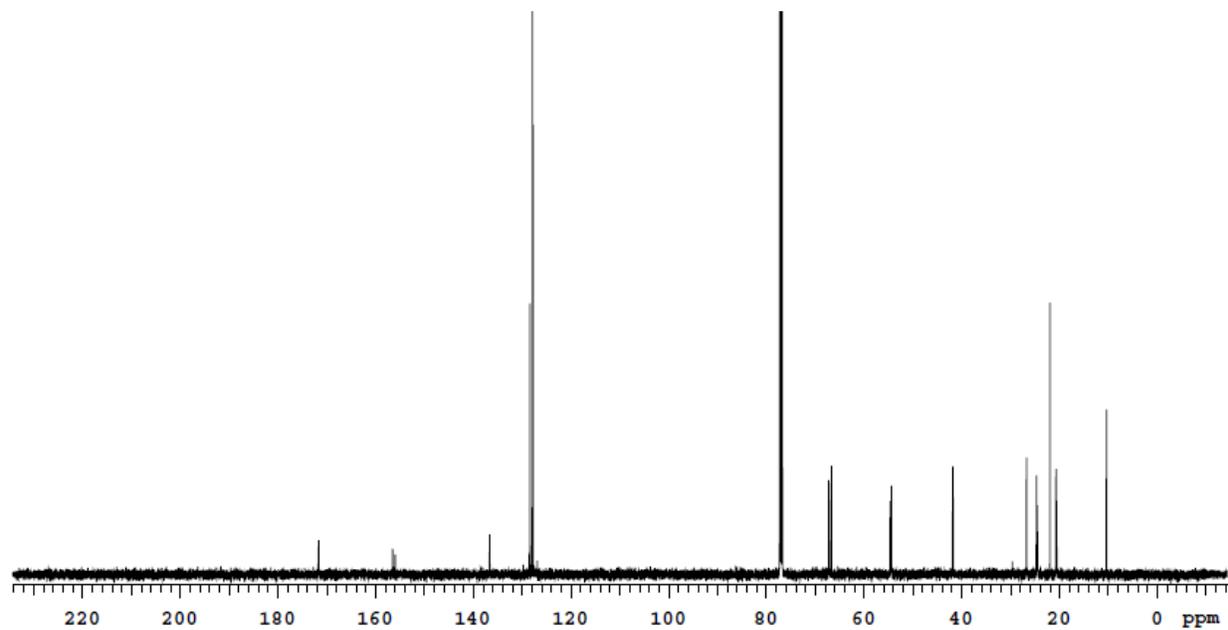
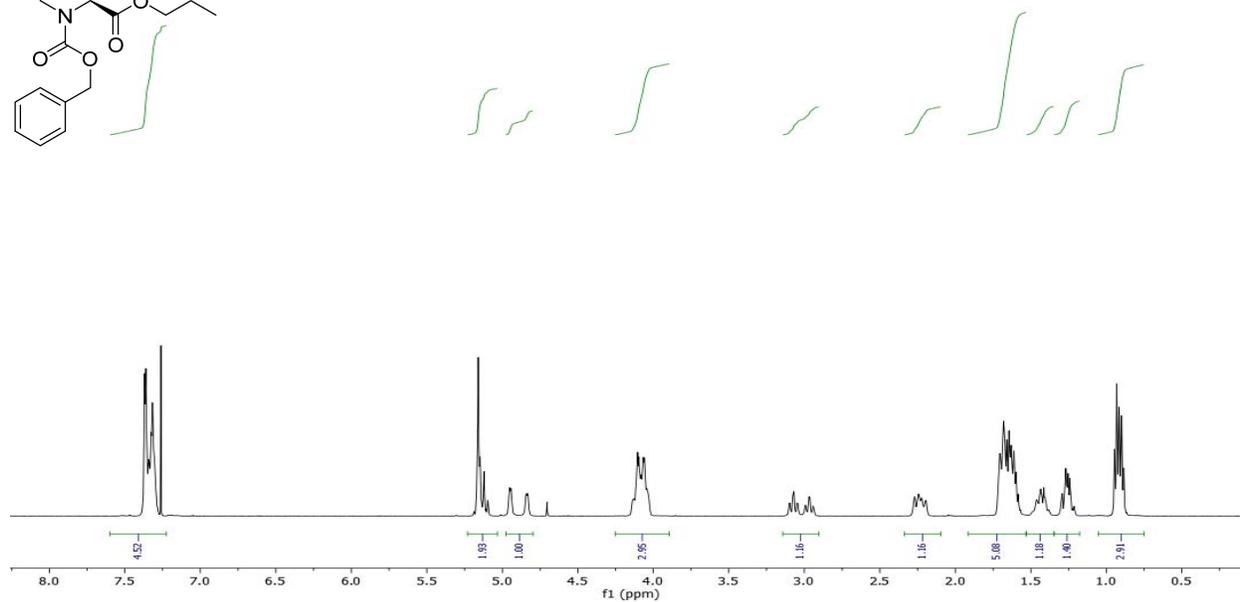
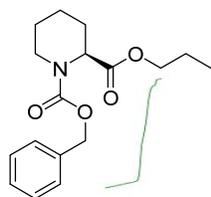
**Compound 7** (in CDCl<sub>3</sub>, standardized to 7.26 signal)



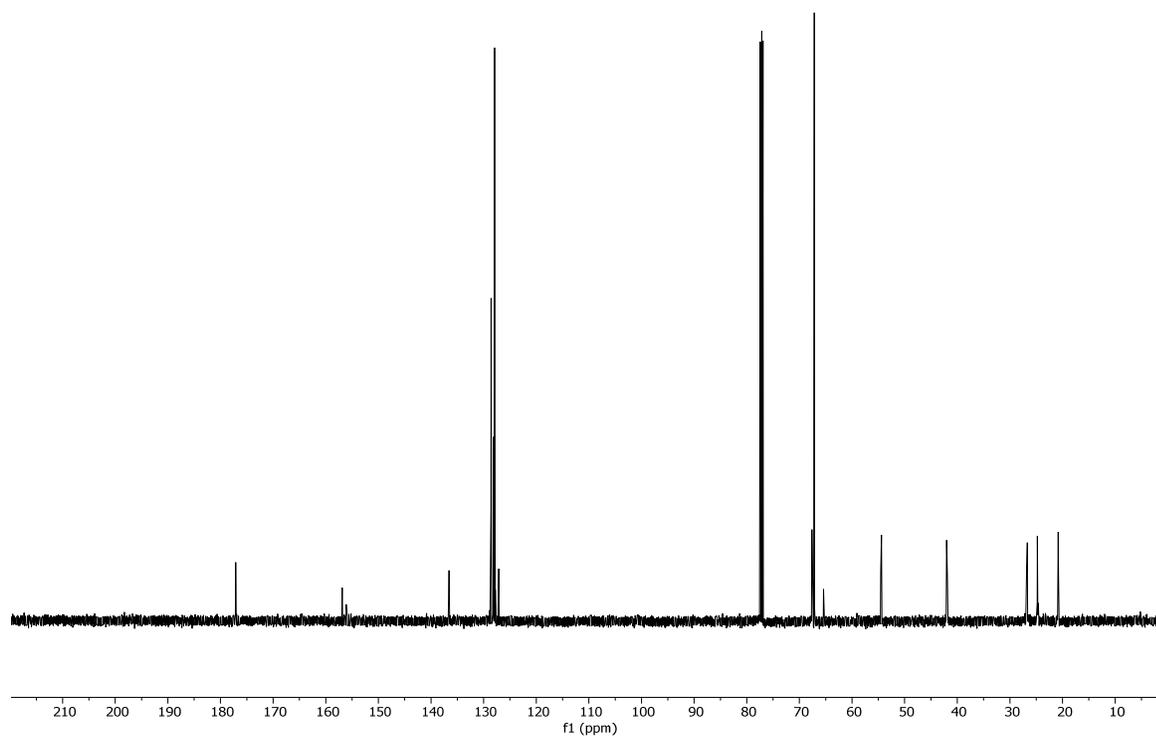
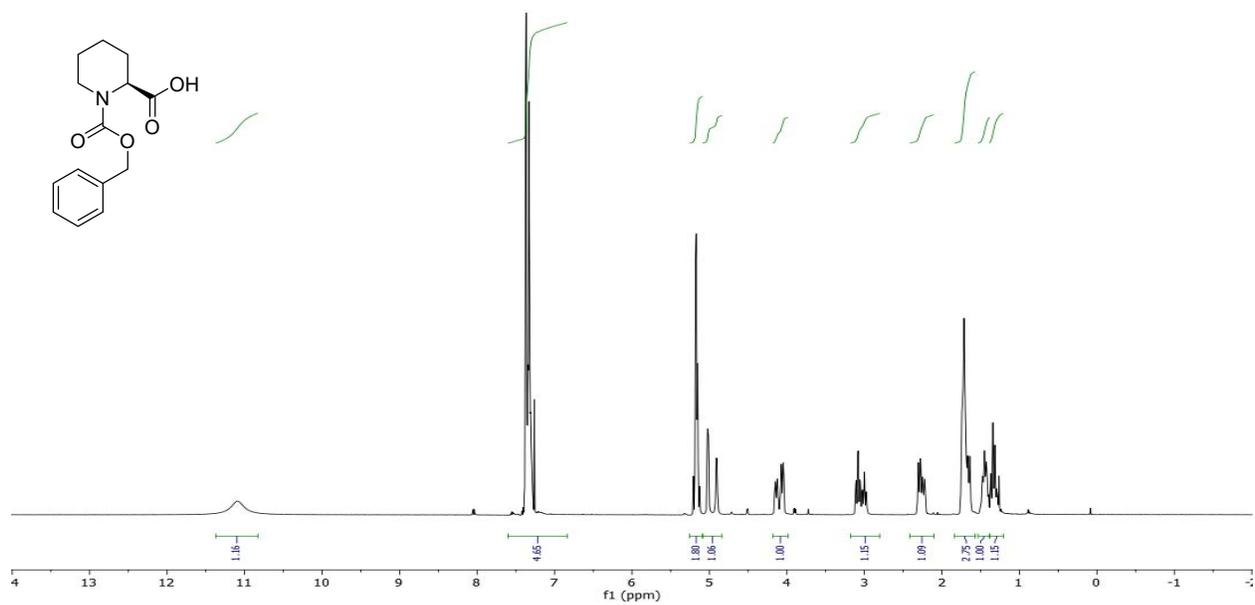
**Compound 8** (in CDCl<sub>3</sub>, standardized to 7.26 signal)



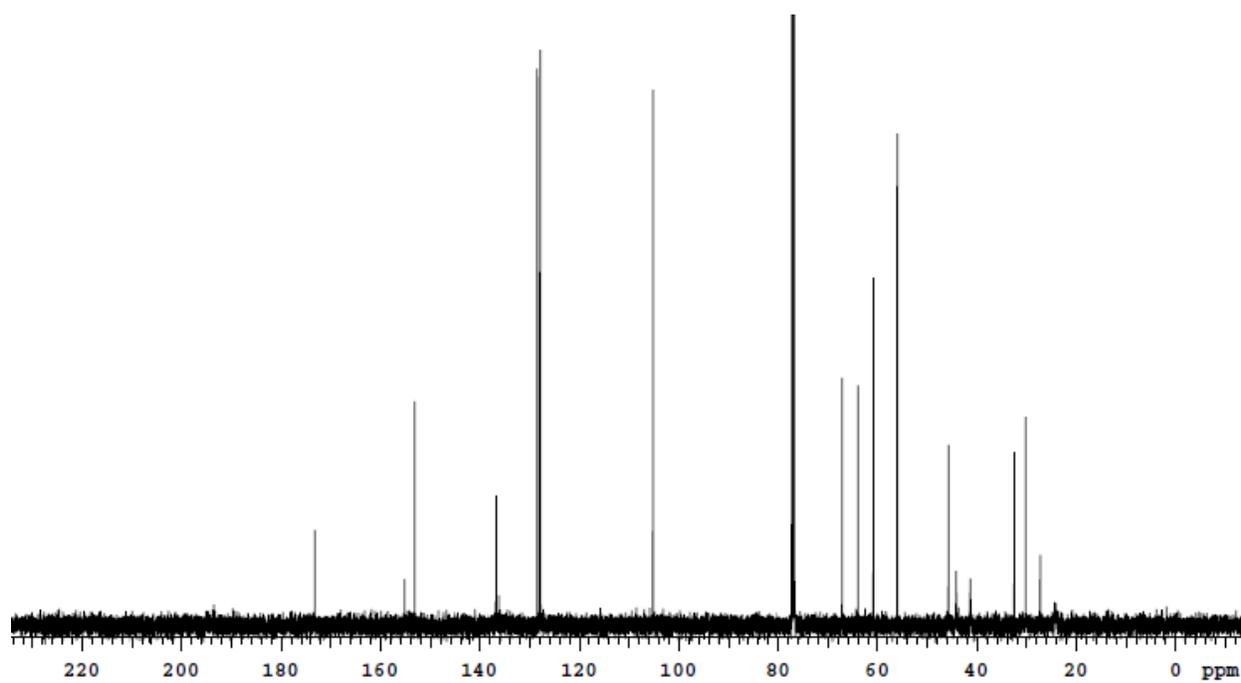
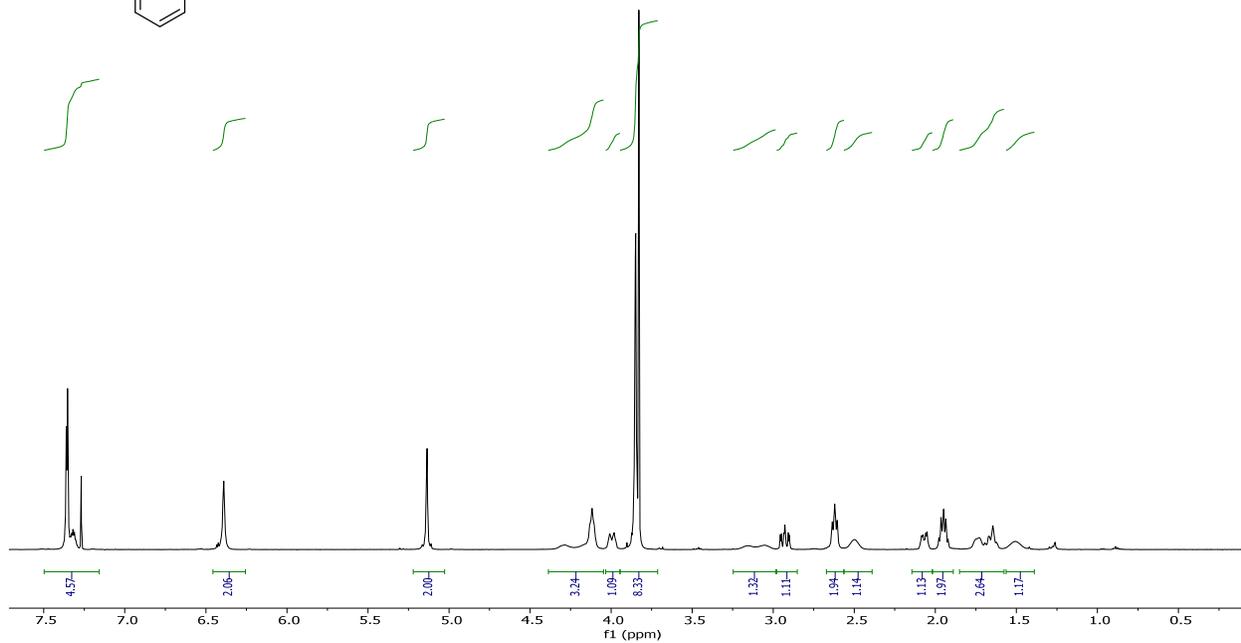
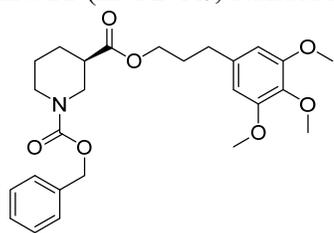
**Compound 9** (in CDCl<sub>3</sub>, standardized to 7.26 signal)



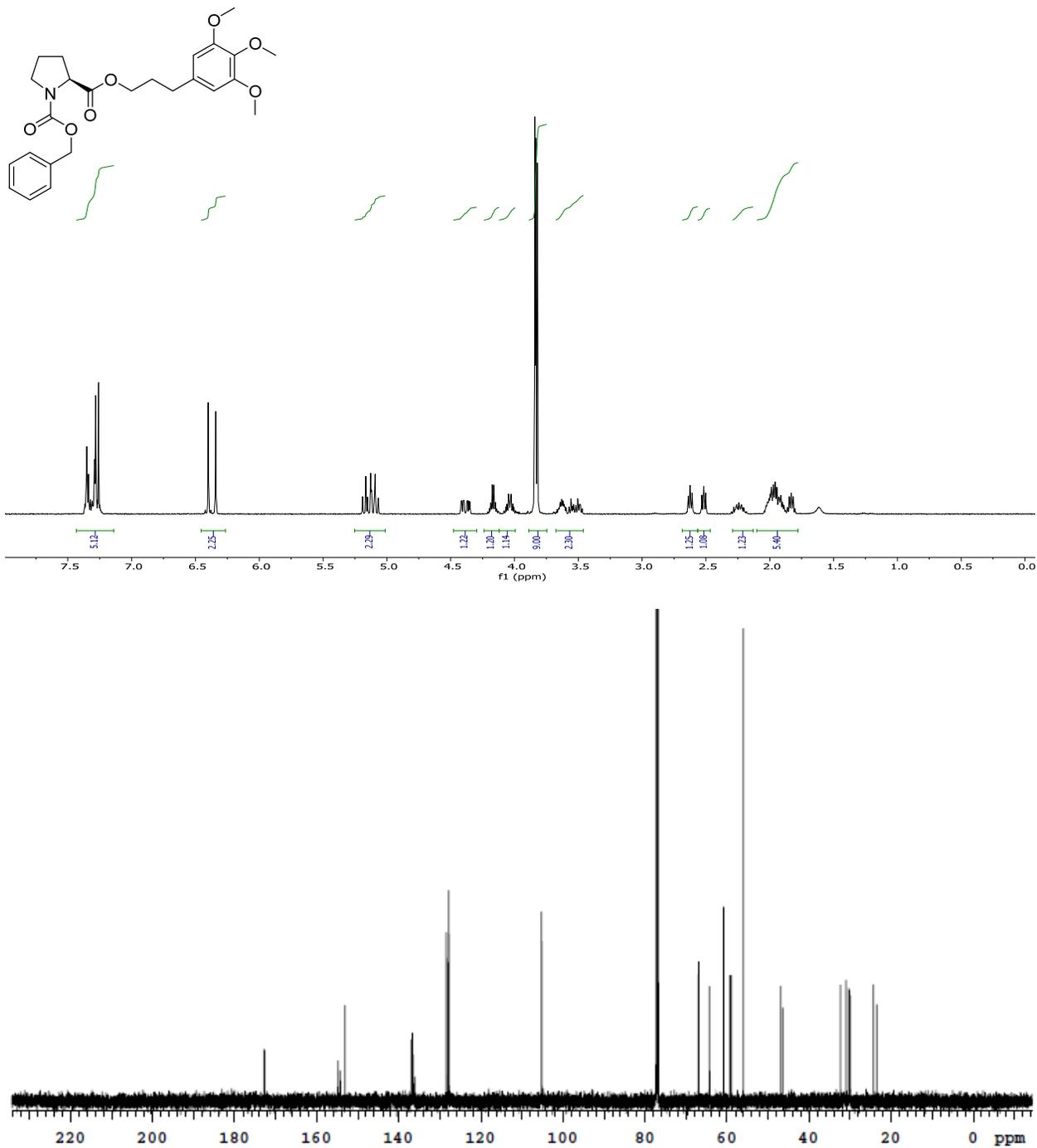
**Compound 10** (in CDCl<sub>3</sub>, standardized to 7.26 signal)



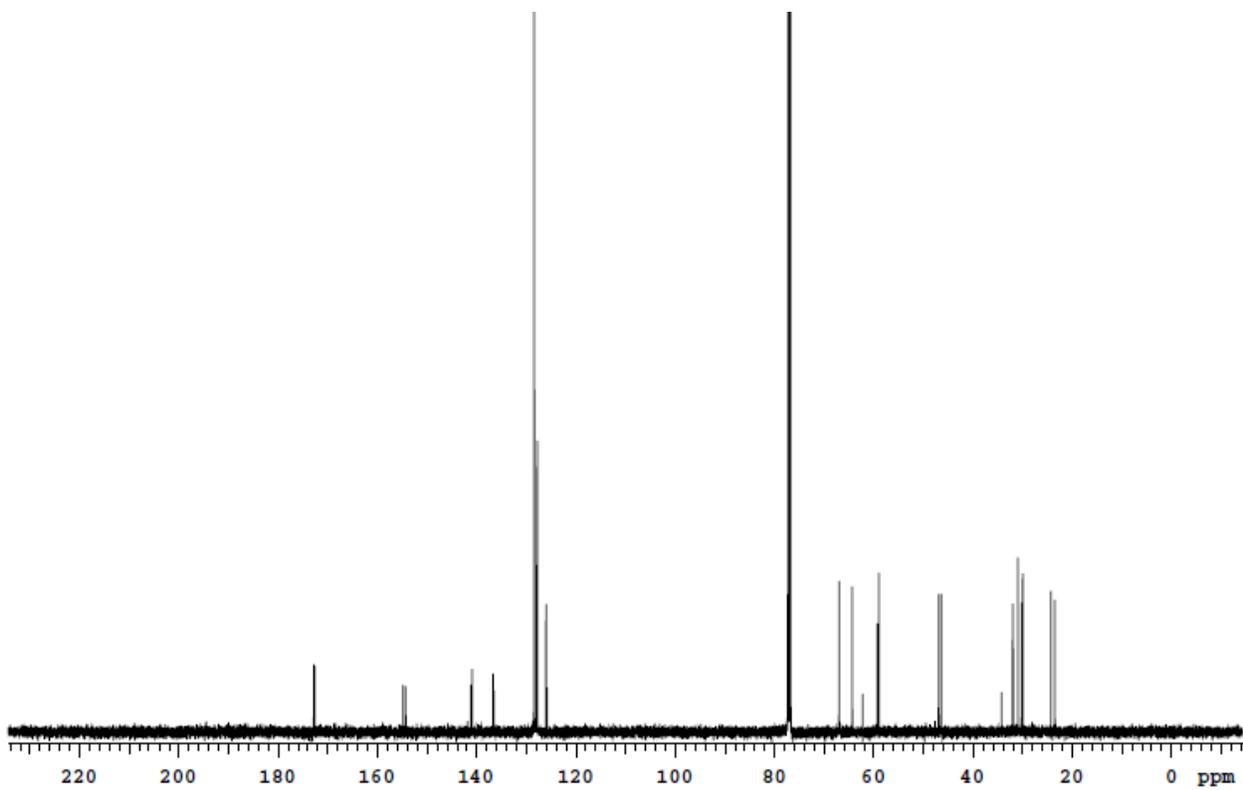
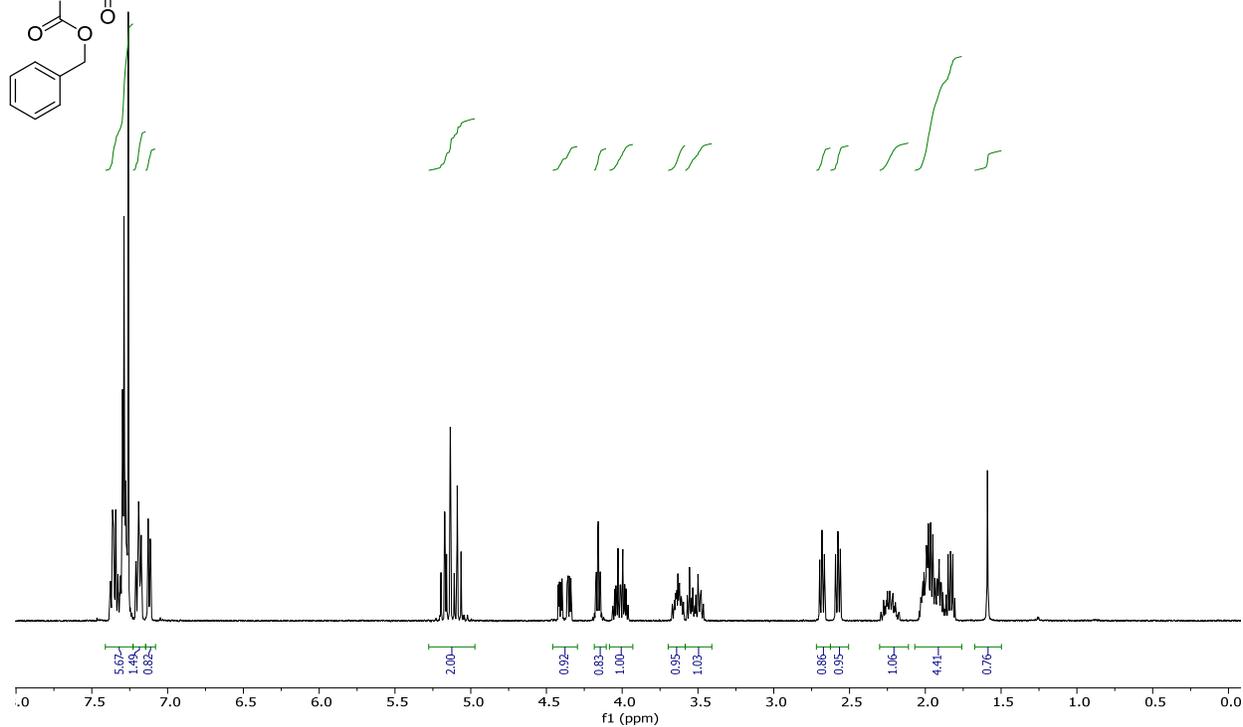
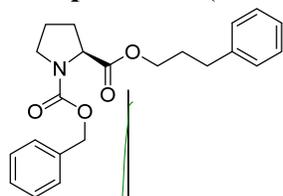
**Compound 11** (in CDCl<sub>3</sub>, standardized to 7.26 signal)



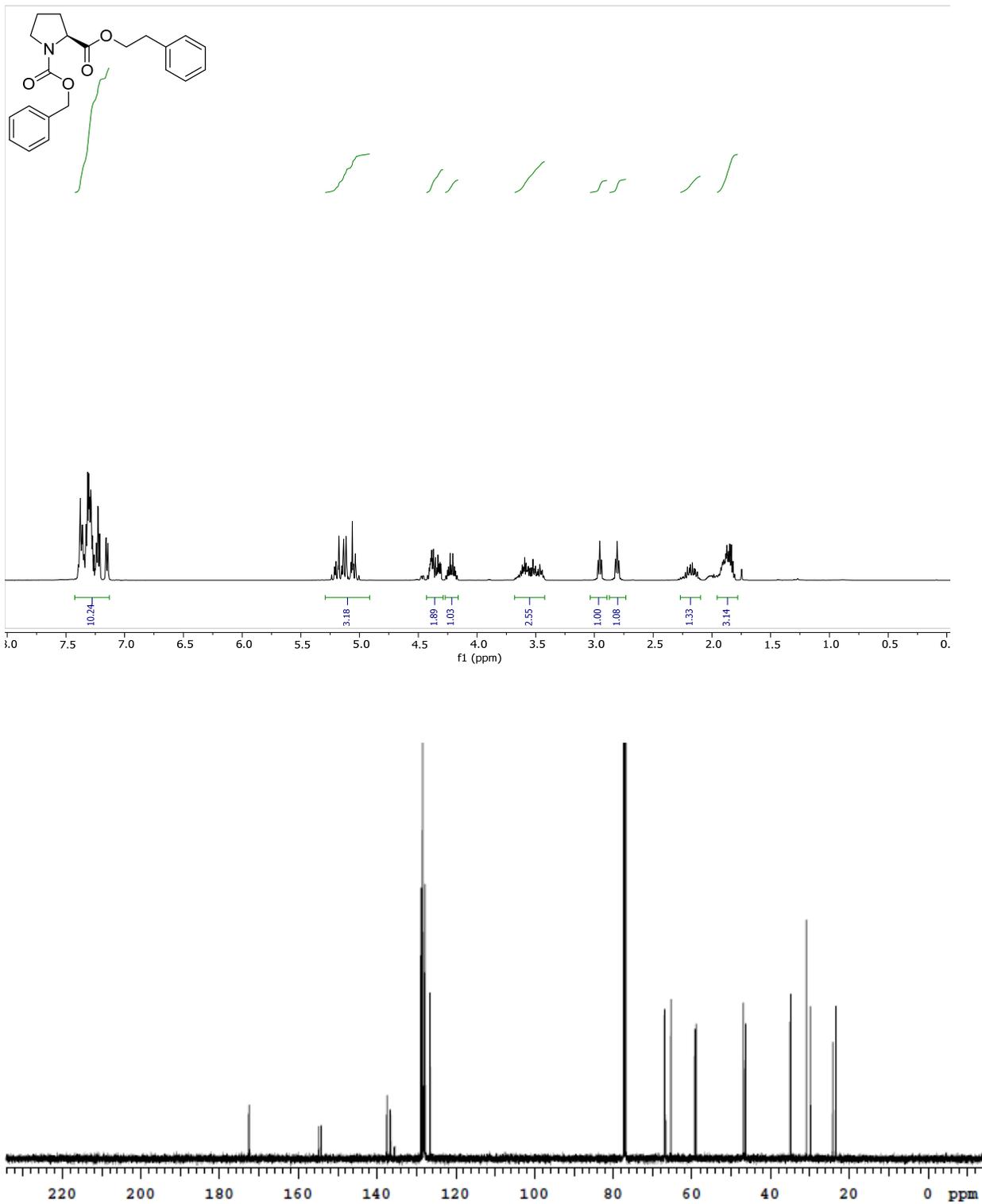
**Compound 12** (in CDCl<sub>3</sub>, standardized to 7.26 signal)



**Compound 13** (in CDCl<sub>3</sub>, standardized to 7.26 signal)



**Compound 14** (in CDCl<sub>3</sub>, standardized to 7.26 signal)



**Compound 15** (in CDCl<sub>3</sub>, standardized to 7.26 signal)

