

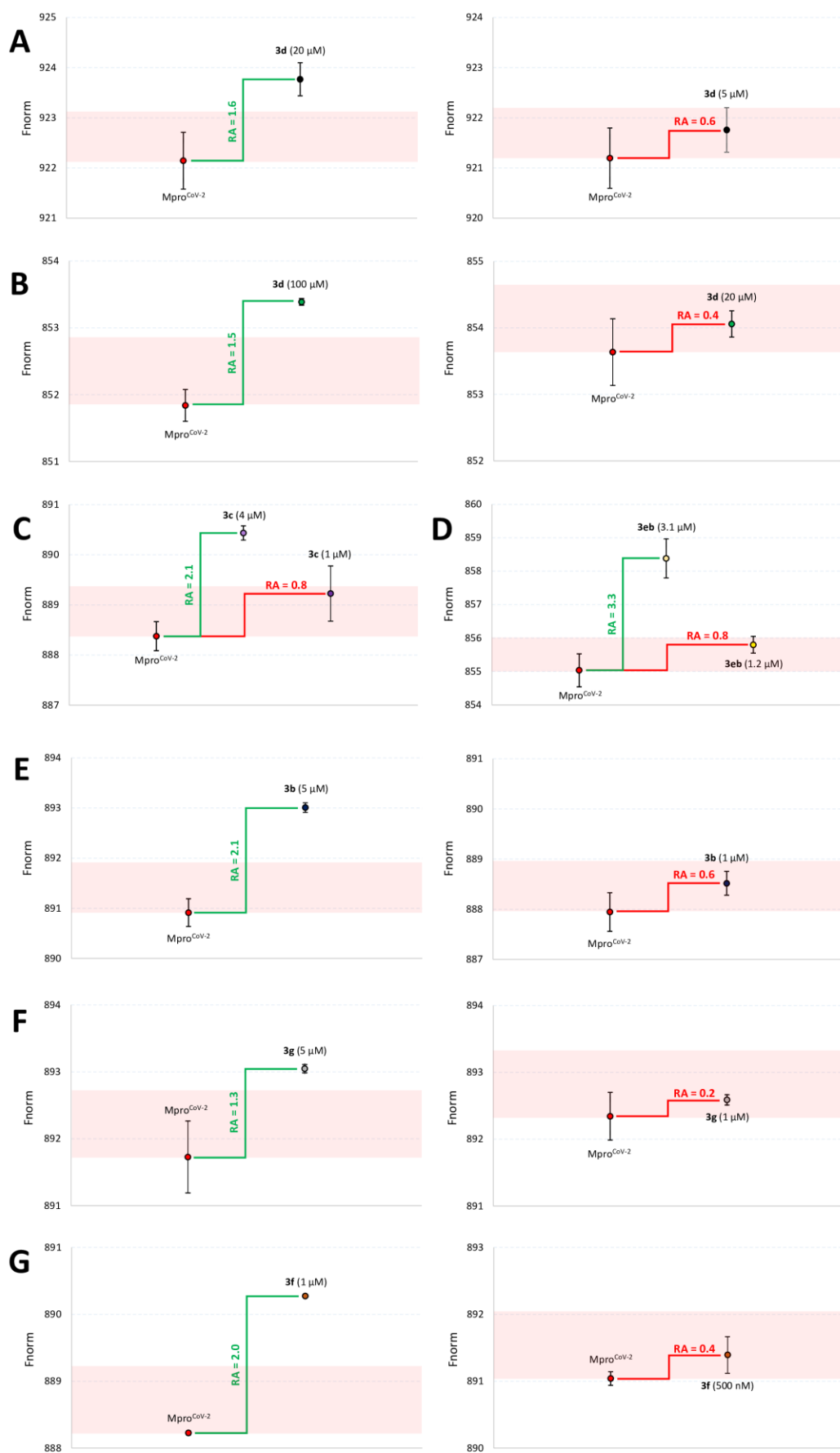
# **Electronic supporting information *for***

## **Computational Design, Synthesis, and Biophysical Evaluation of $\beta$ -Amido Boronic Acids as SARS-CoV-2 M<sup>pro</sup> Inhibitors**

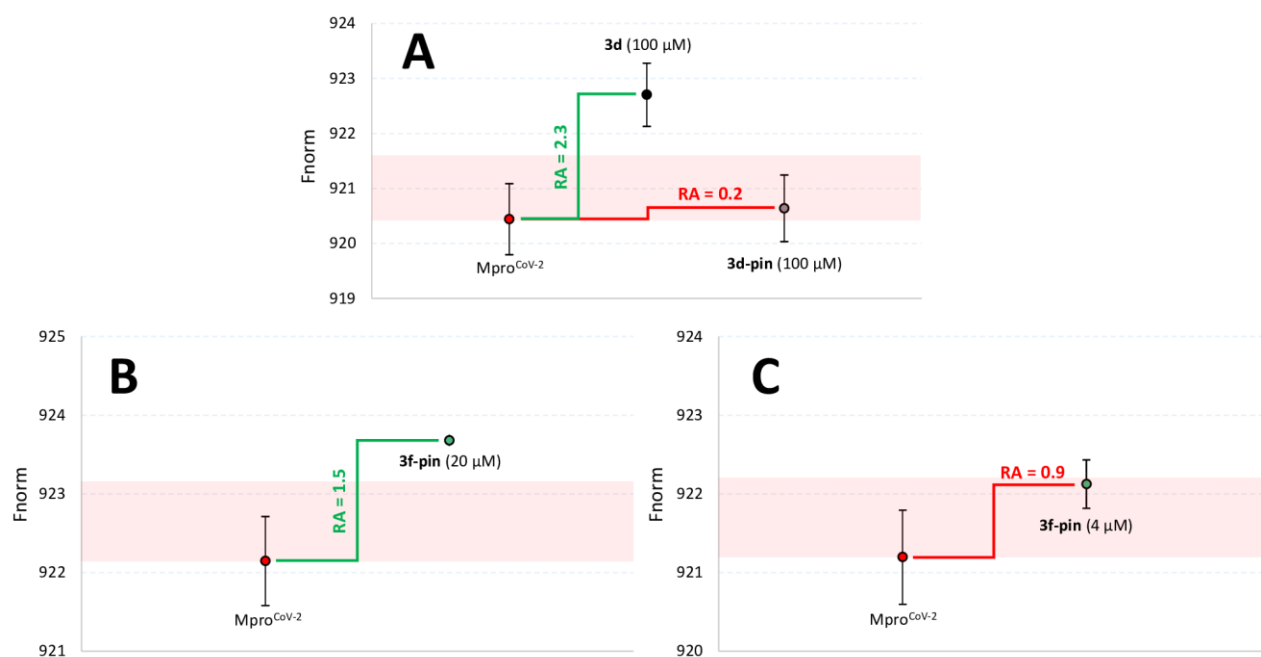
Enrico M. A. Fassi, Marco Manenti, Andrea Citarella, Michele dei Cas, Sara Casati, Nicola Micale, Tanja Schirmeister, Gabriella Roda, Alessandra Silvani, and Giovanni Grazioso

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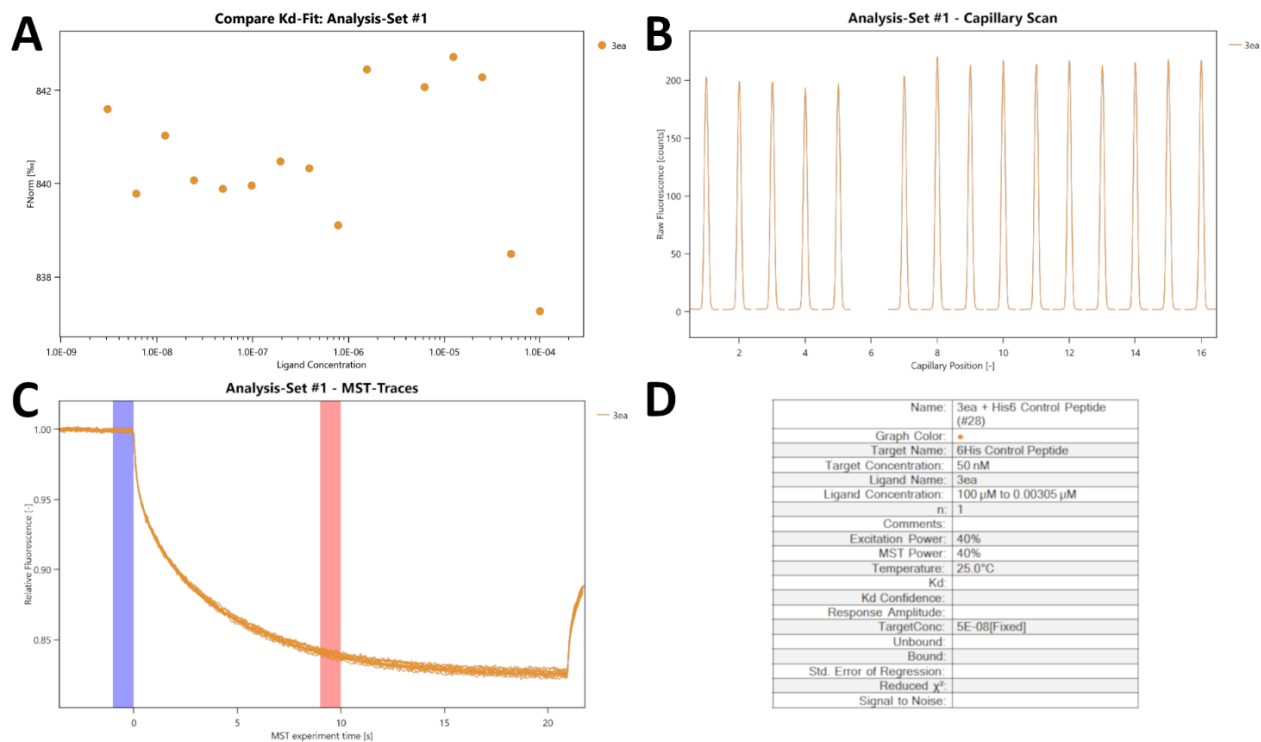
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- Copies of  $^1\text{H}$ ,  $^{13}\text{C}$  and  $^{11}\text{B}$  NMR spectra



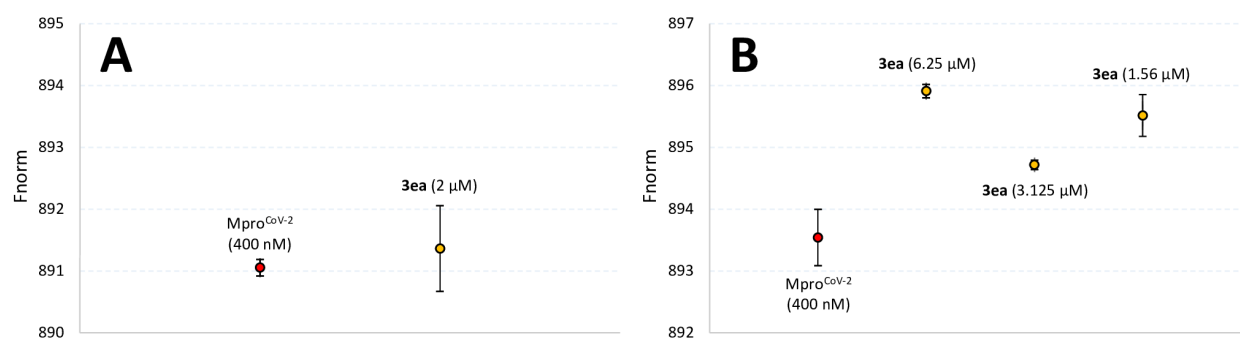
**Figure S1.** MST binding check experiments of  $\beta$ -amido boronic compounds reported in Table 2 on the manuscript. In red is highlighted the “no binding” Fnorm area due to an insufficient Response Amplitude (RA).



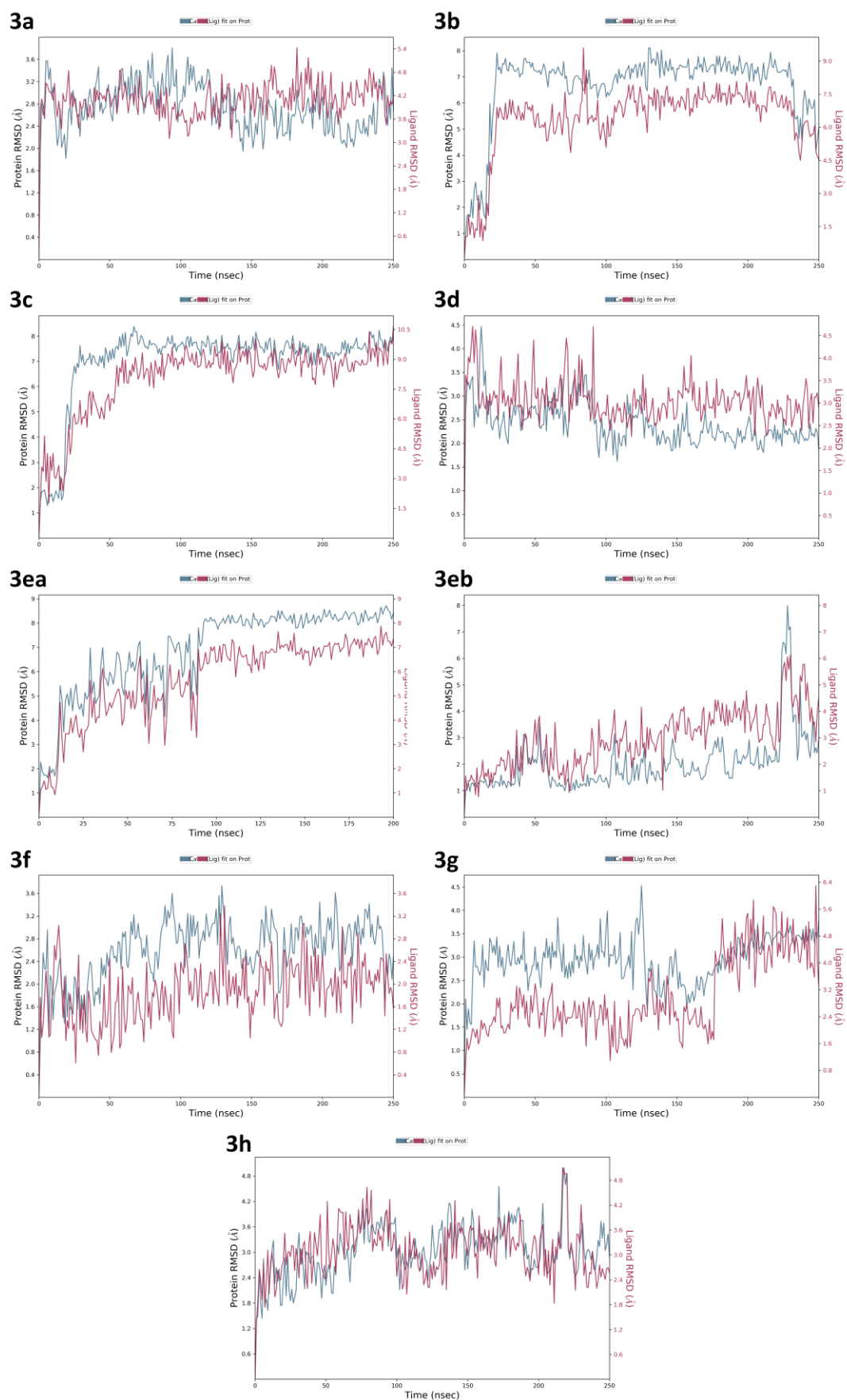
**Figure S2.** MST binding check experiment of  $\beta$ -amido boronic compounds protected by the pinacol group. (A) Comparison between **3d** and **3d-pin** at a concentration of 100  $\mu\text{M}$ . (B-C) **3f-pin** at a concentration of 20  $\mu\text{M}$  and 4  $\mu\text{M}$ , respectively. In red is highlighted the “no binding”  $F_{\text{norm}}$  area due to an insufficient Response Amplitude (RA).



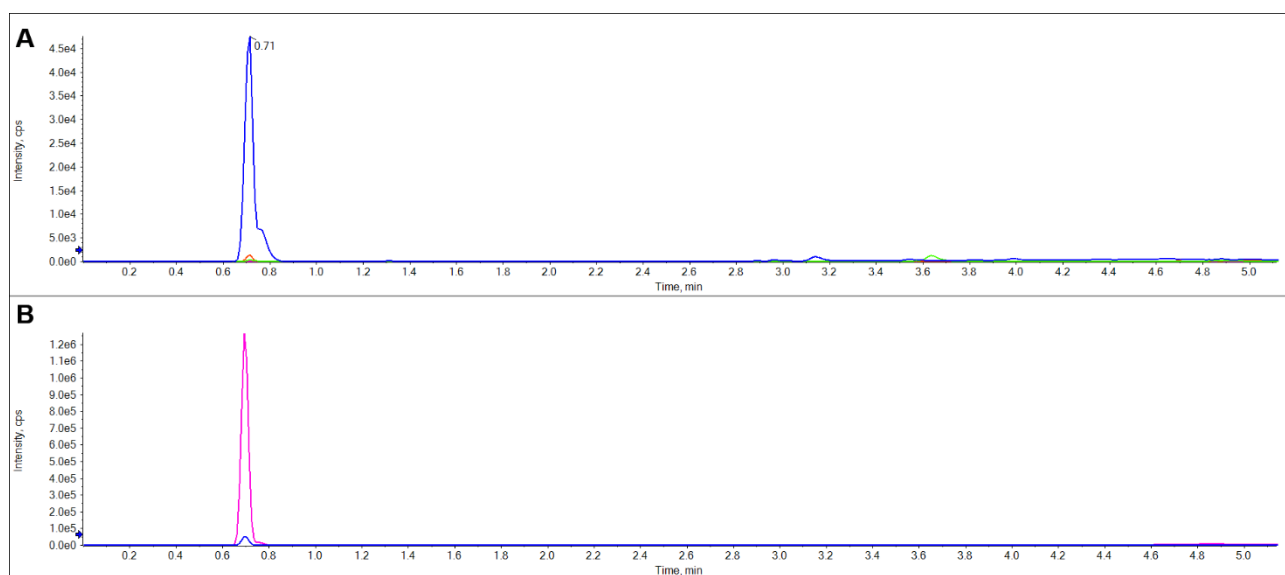
**Figure S3.** MST analysis reporting the binding affinity assay of **3ea** on 6His Control Peptide. (A)  $F_{\text{norm}}$  values, (B) raw fluorescence values and (C) MST traces observed for each capillary. (D) Dataset overview. The 6<sup>th</sup> point, corresponding to a concentration of 3.1  $\mu$ M, was discarded since it appears to be a clear outlier.



**Figure S4.** MST binding check experiments to determine whether if **3ea** compound binds reversibly or irreversibly to the Mpro<sup>Cov-2</sup>. (A) Fnorm values of protein alone compared to **3ea**(2 μM)/Mpro<sup>Cov-2</sup> complex. (B) Fnorm values of protein alone compared to **3ea**(6.25 μM)/protein complex and its dilutions.

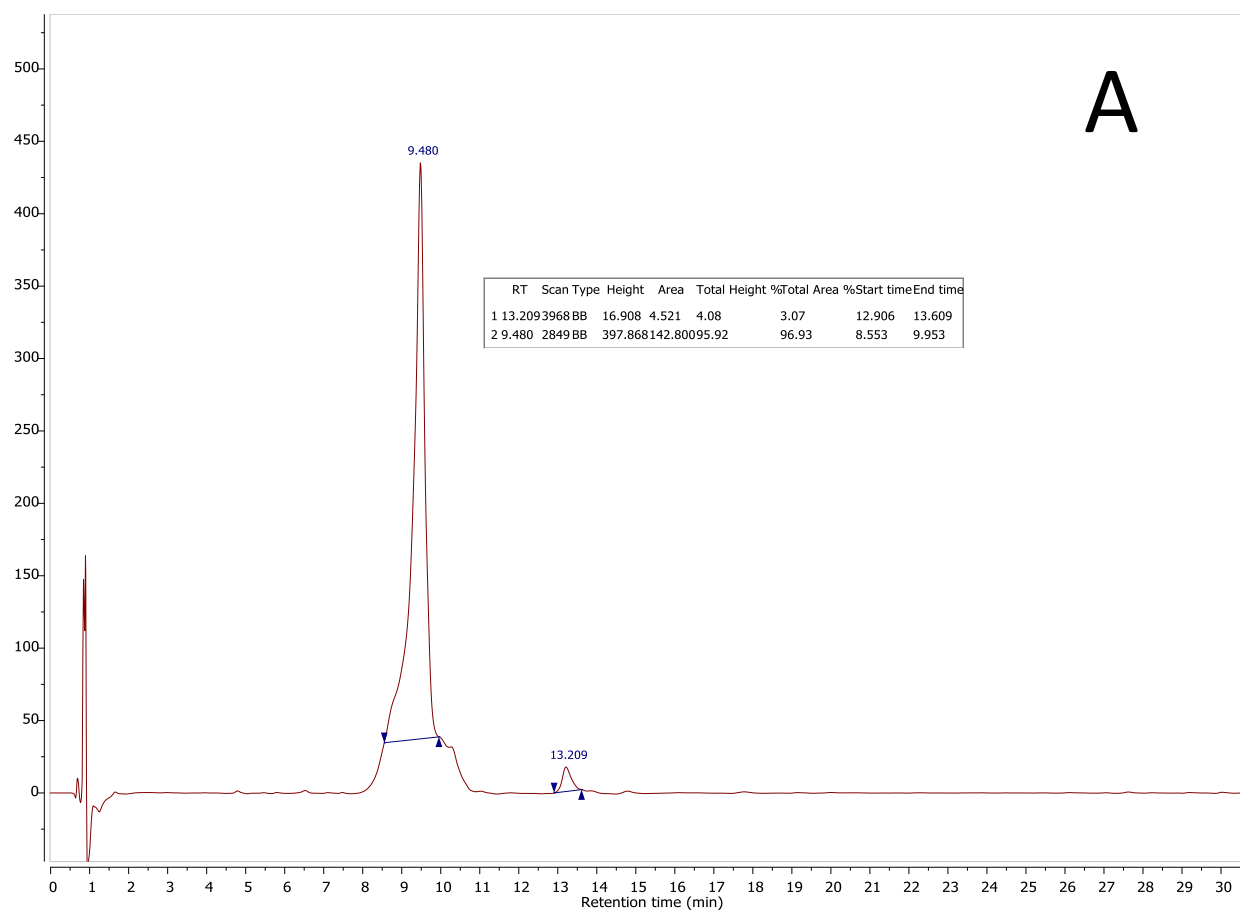


**Figure S5.** RMSD versus time plots of the synthesized  $\beta$ -amido boronic compounds reported in Table 1 in the main text. All the compounds were subjected to a 250 ns-long MD simulations.

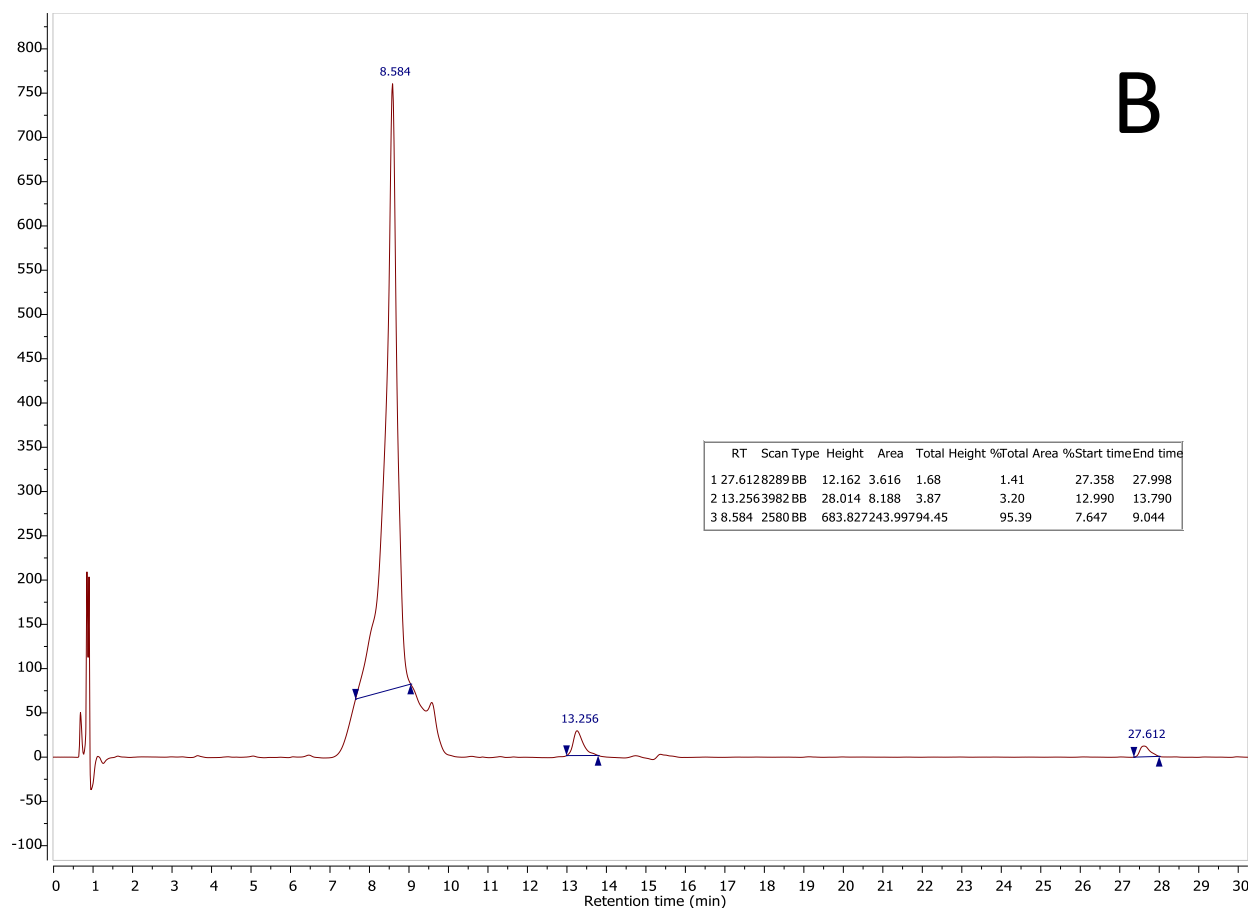


**Figure S6.** LC-MS/MS experiment on a  $\beta$ -amido boronic acid. (A) Extracted ion chromatograms of **3ea**  $[M+H]^+$  and (B) **3ea**  $[M+2H]^{2+}$ .

A

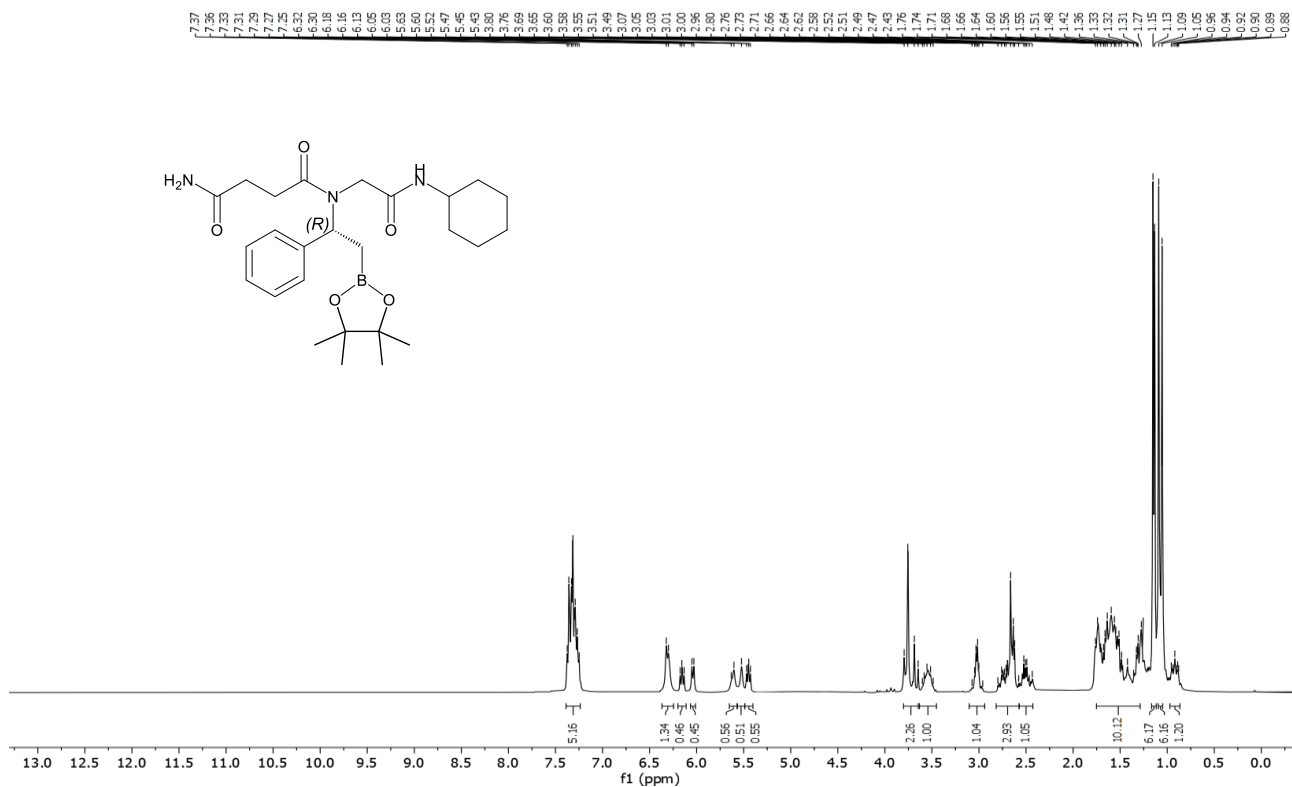




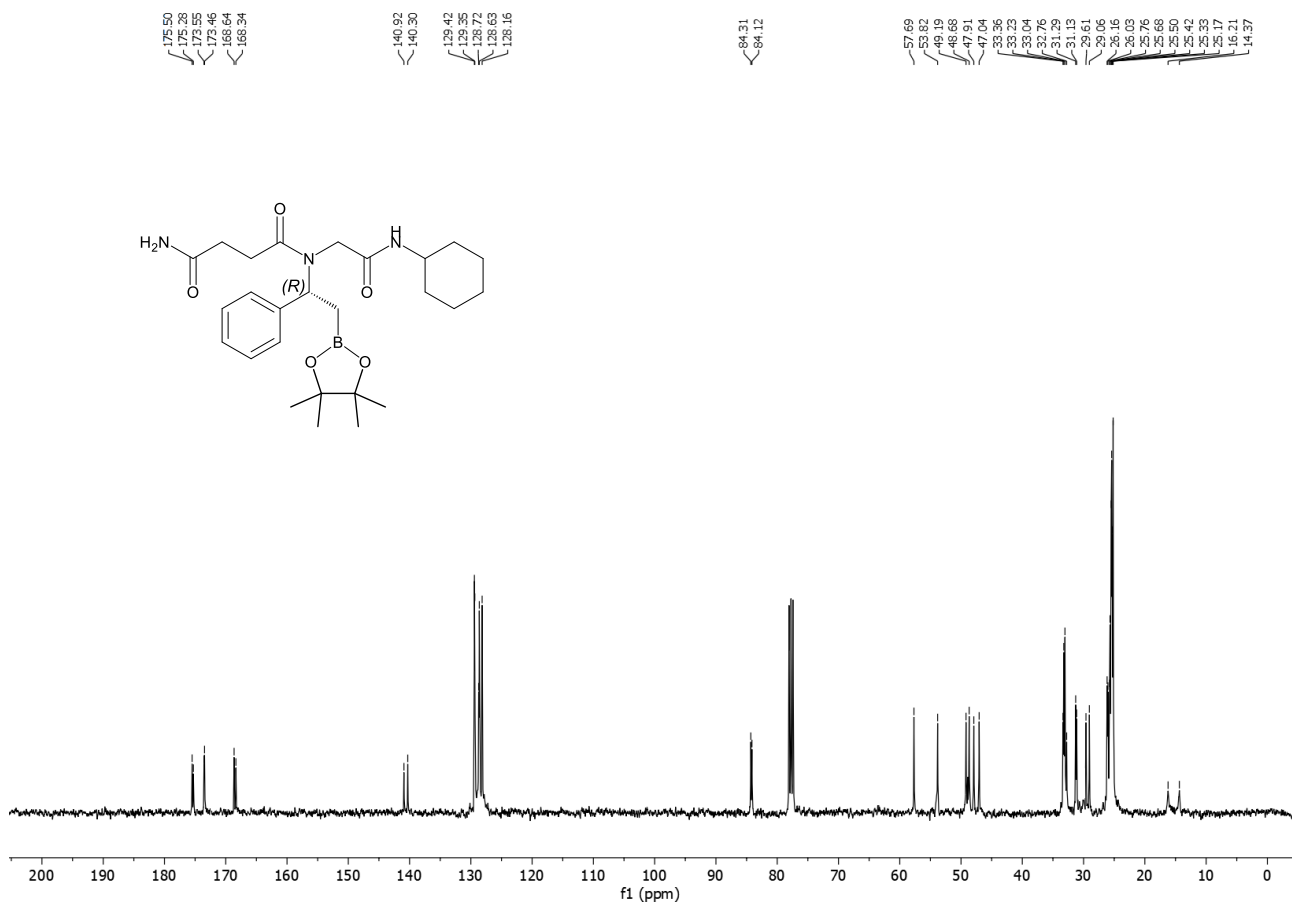


**Figure S7. HPLC chromatograms for compounds 3a (panel A) and 3ea (panel B).** HPLC was performed on Agilent 1100 Series System using a Gemini 5  $\mu$ M C18 110 Å LC Column 150  $\times$  3 mm and a gradient of H<sub>2</sub>O/ACN from 5% ACN to 100% ACN in 30 min, flux of 1.0 mL/min and sample injection of 20  $\mu$ L. The wavelength used for detection was 220 nm.

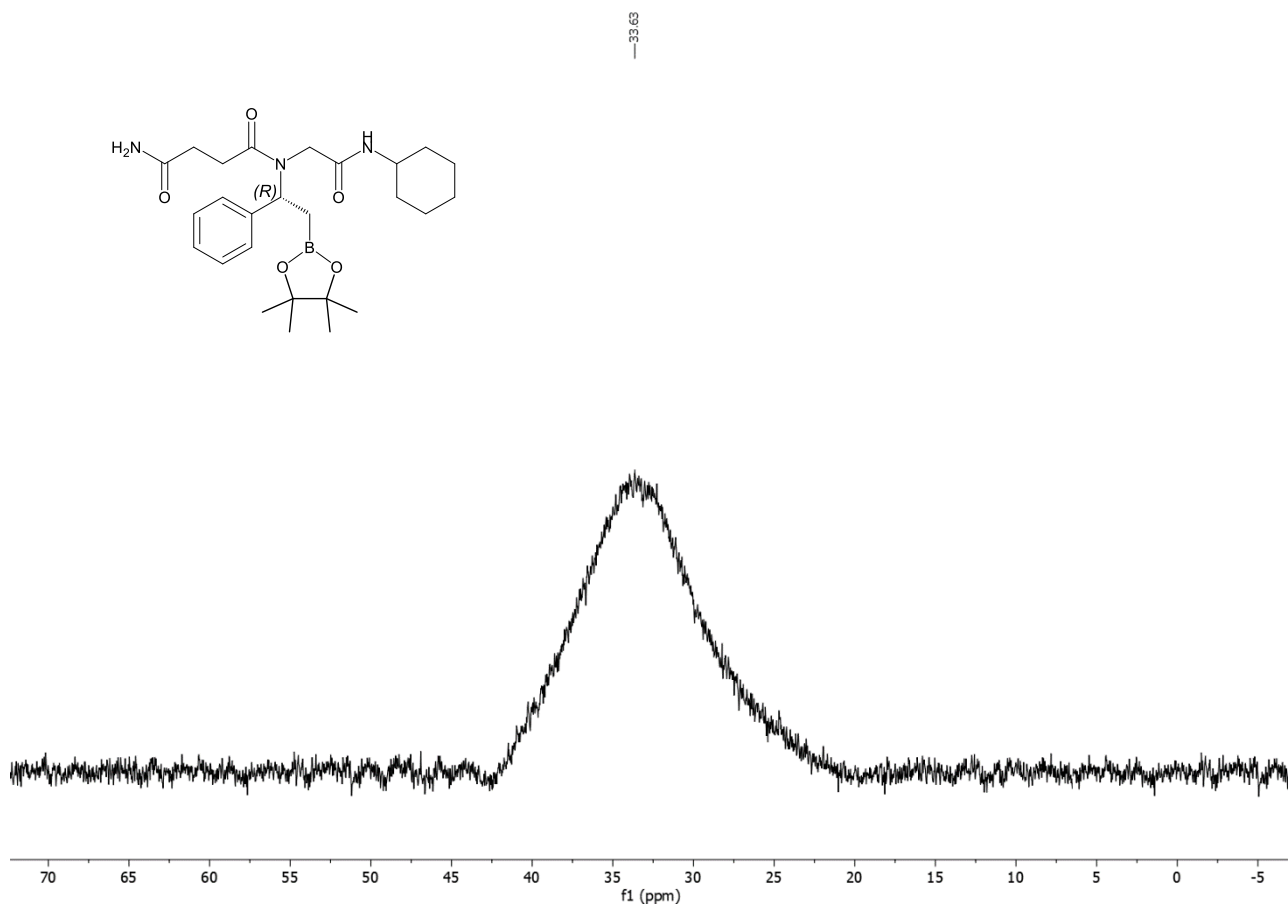
$^1\text{H}$  NMR of compound **2a** (400 MHz,  $\text{CDCl}_3$  55:45 rotameric mixture)



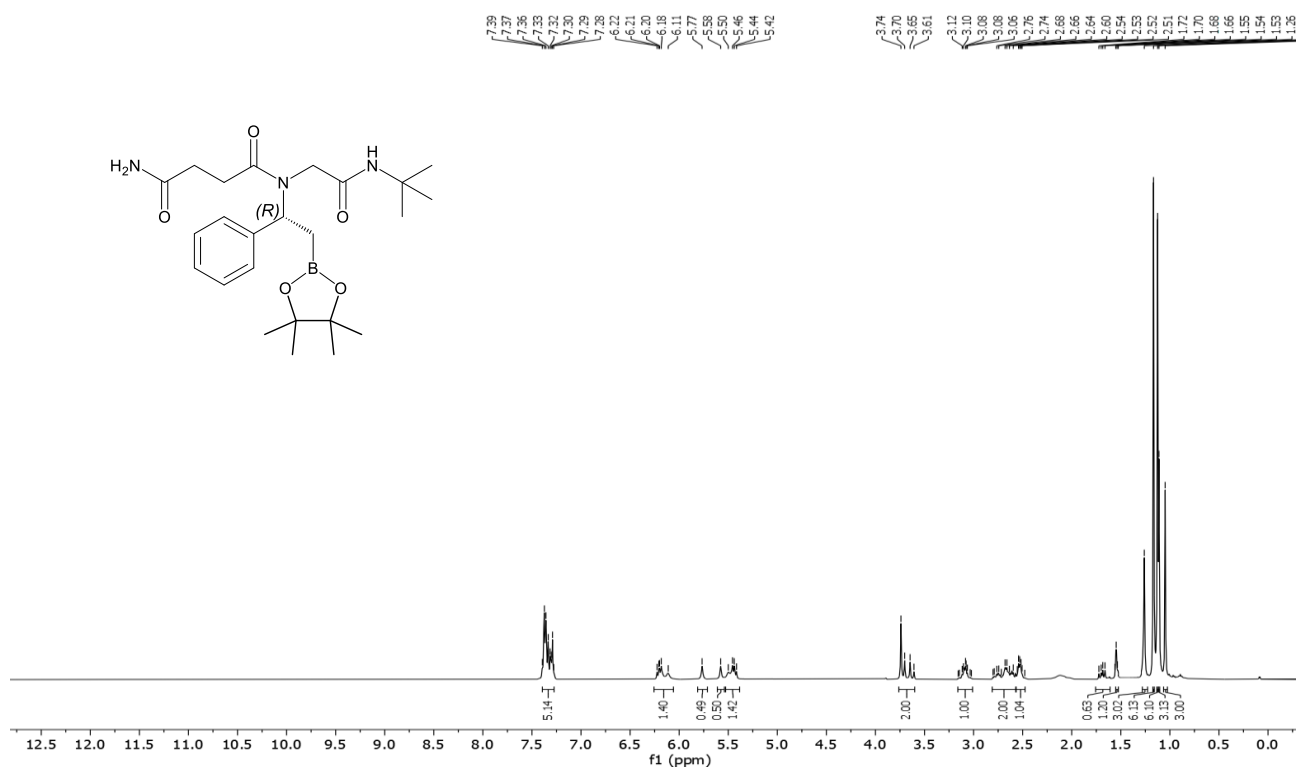
$^{13}\text{C}$  NMR of compound **2a** (101 MHz,  $\text{CDCl}_3$  55:45 rotameric mixture)



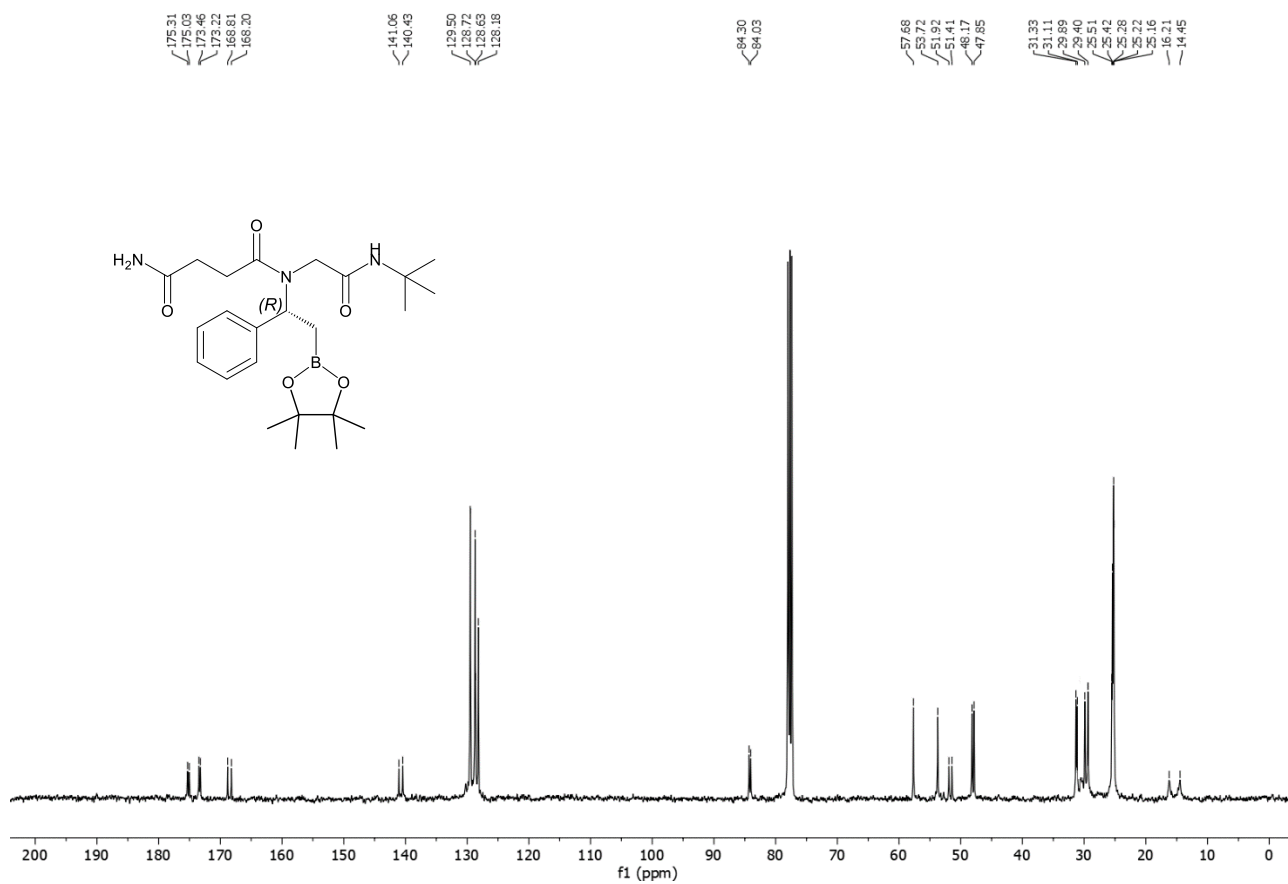
$^{11}\text{B}$  NMR of compound **2a** (128 MHz,  $\text{CDCl}_3$ )



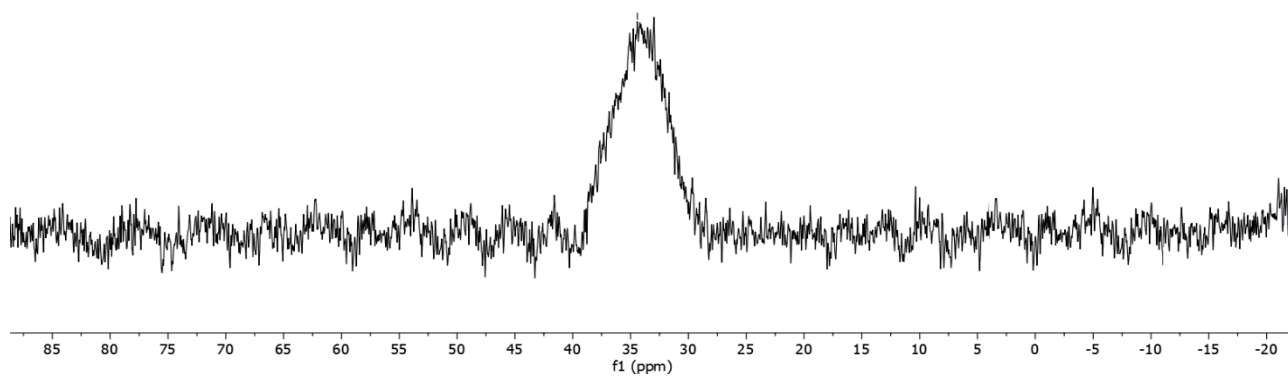
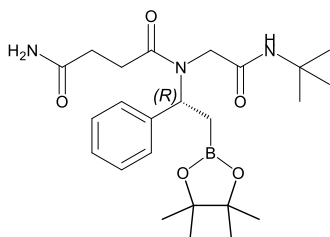
$^1\text{H}$  NMR of compound **2b** (400 MHz,  $\text{CDCl}_3$ , 50:50 rotameric mixture)



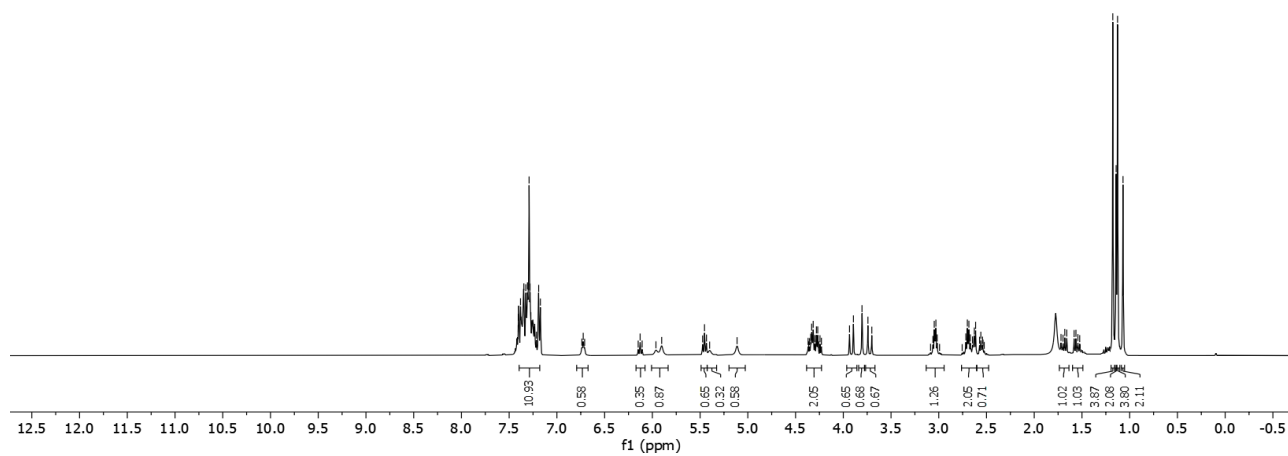
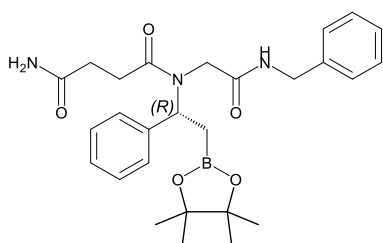
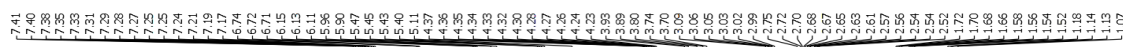
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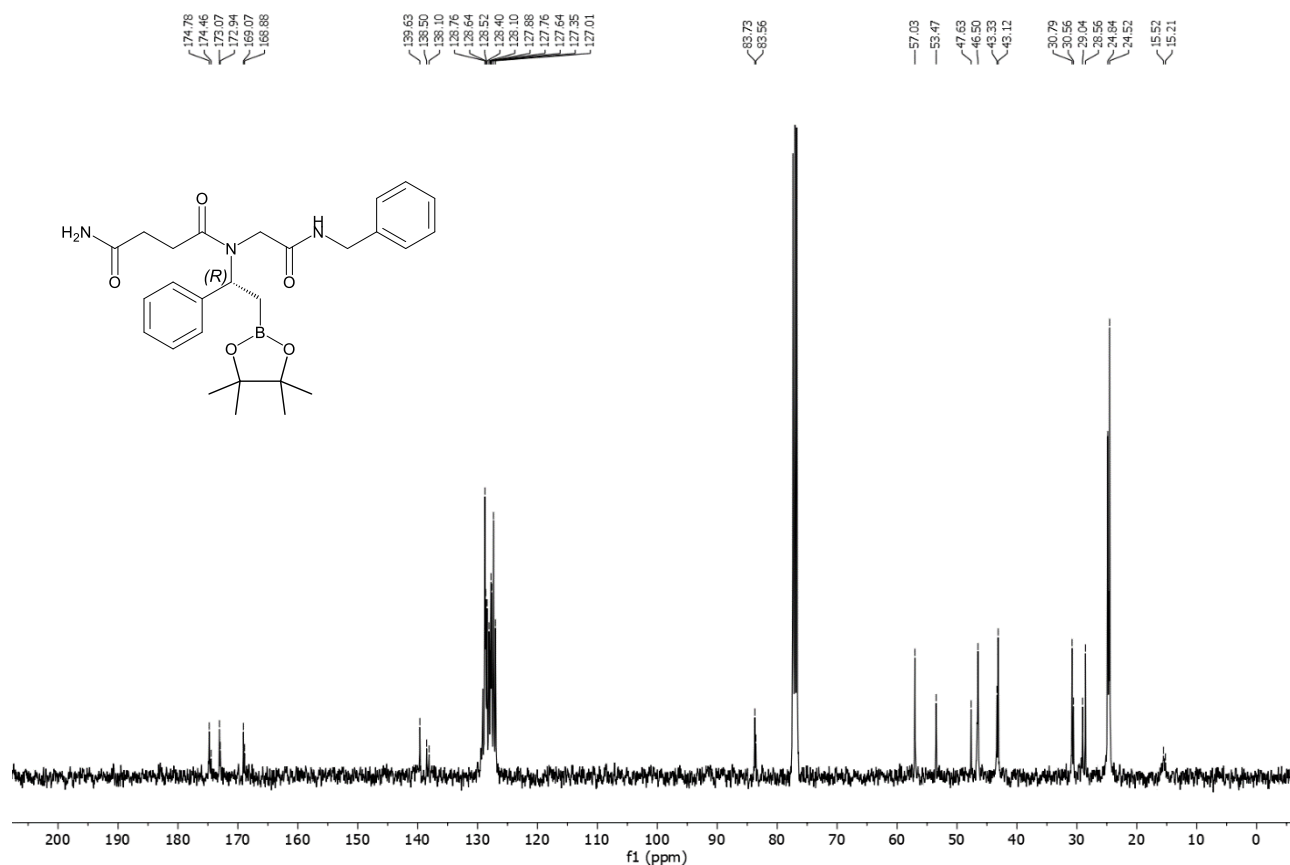
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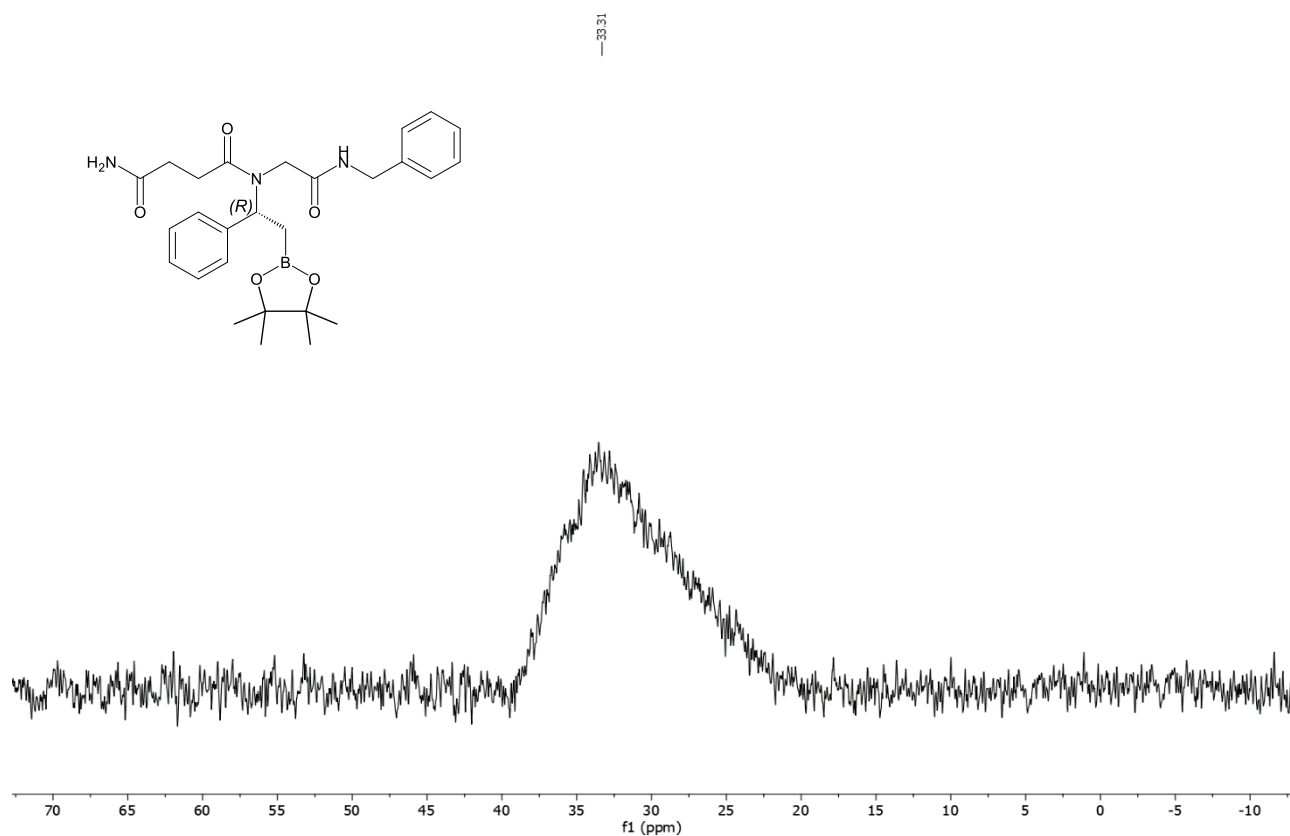
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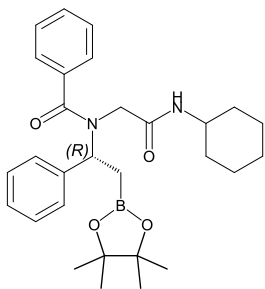
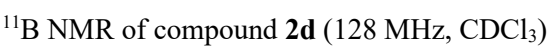
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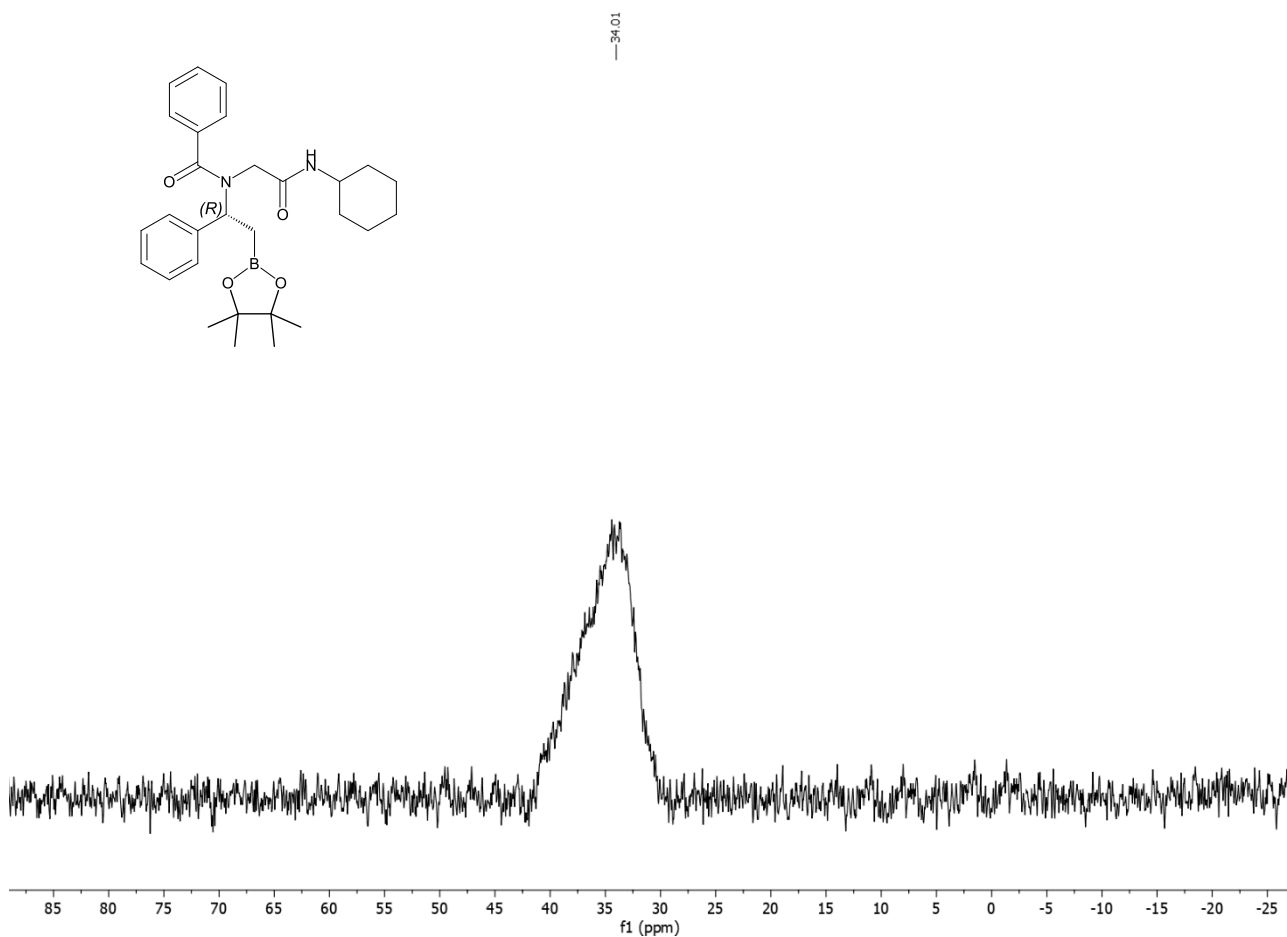


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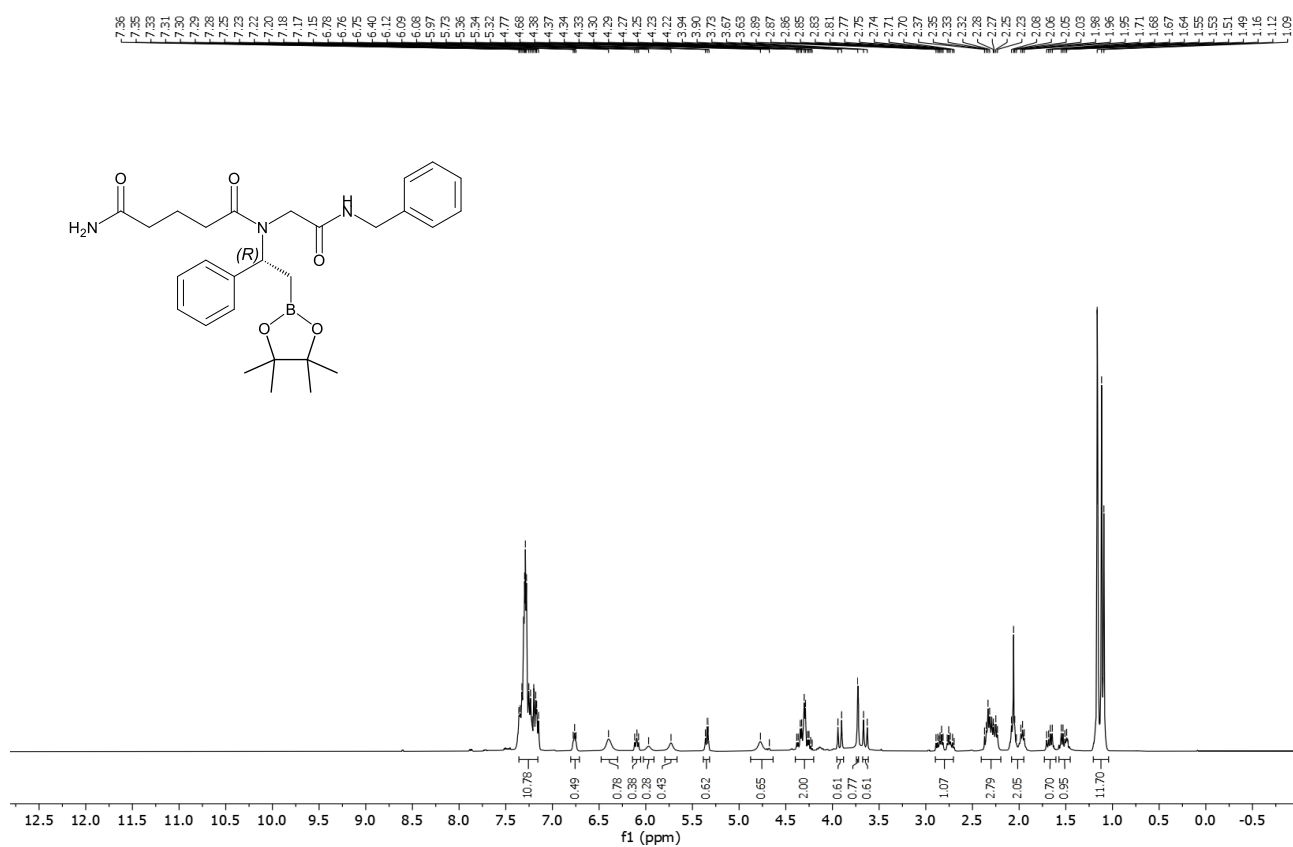


$^1\text{H}$  NMR of compound **2d** (400 MHz,  $\text{CDCl}_3$ , complex rotameric mixture: the section by section integration proves the overall number of protons)

[illegible]

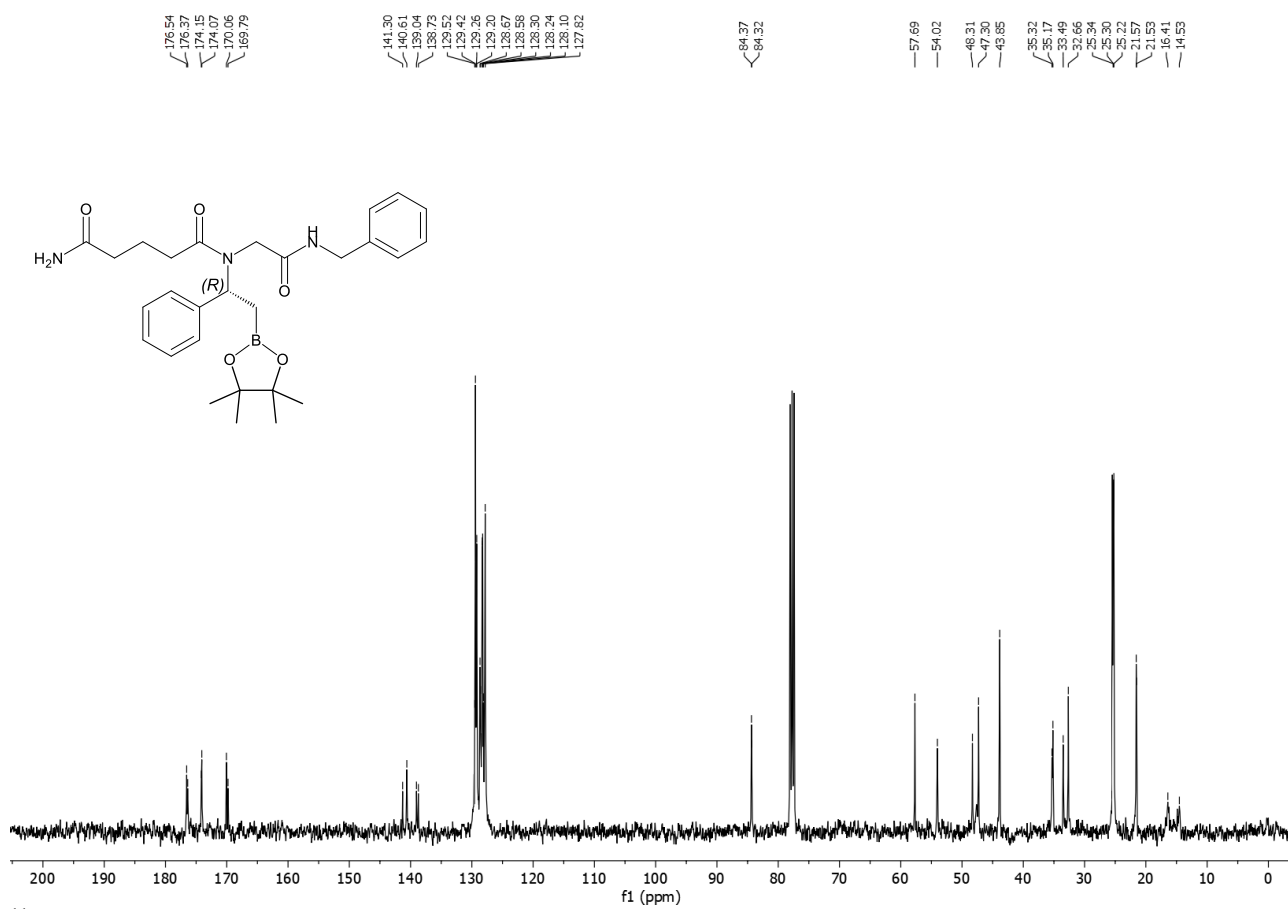


<sup>1</sup>H NMR of compound **2ea** (400 MHz, CDCl<sub>3</sub> 60:40 rotameric mixture)

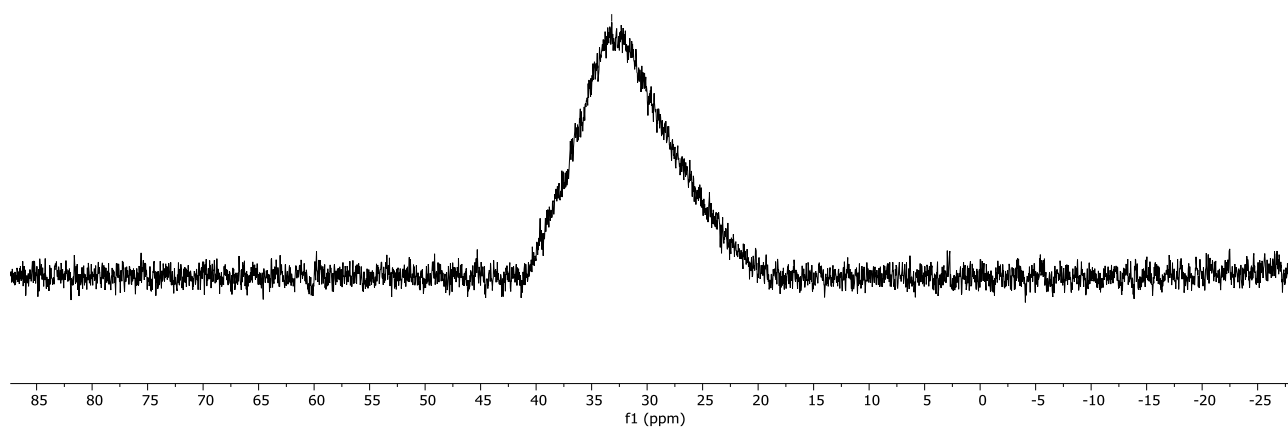
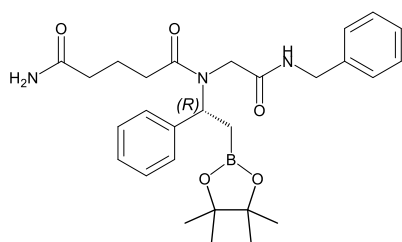




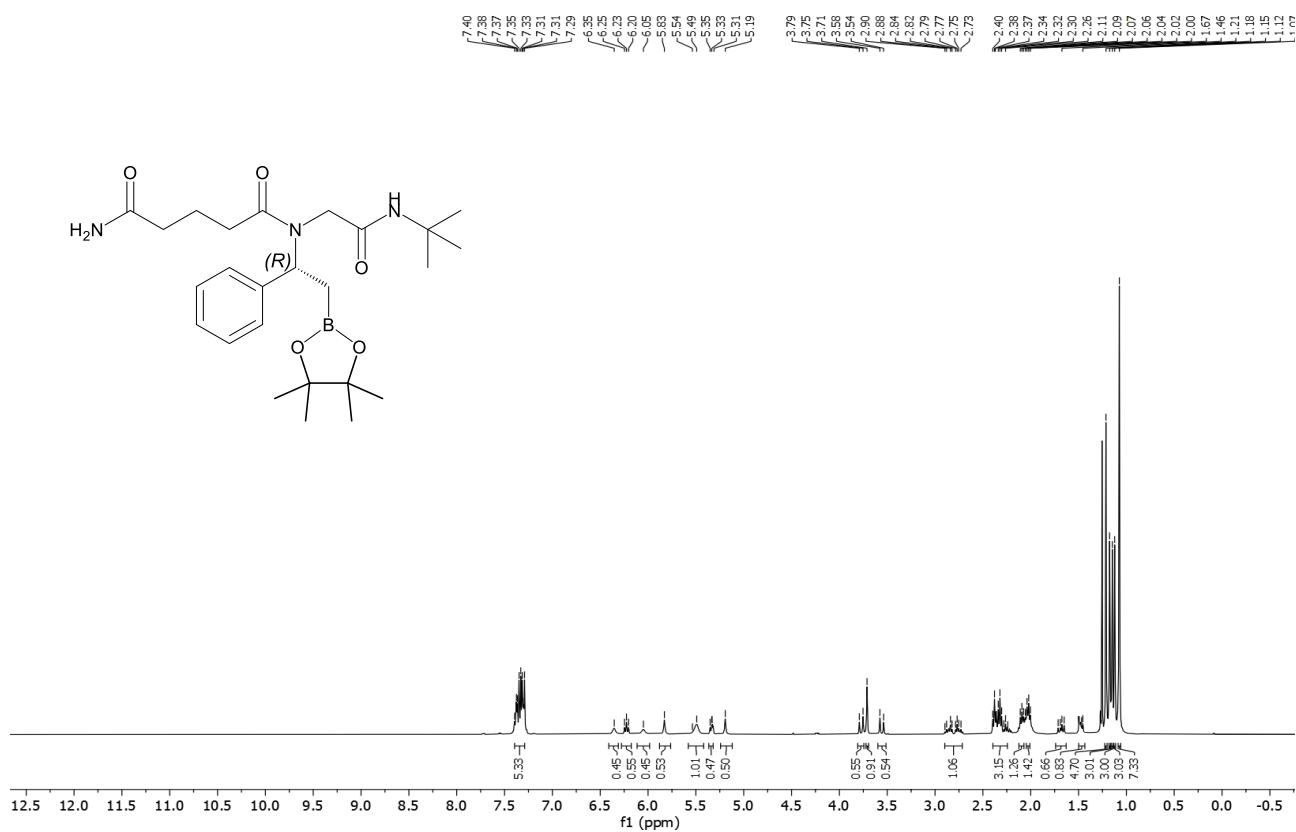
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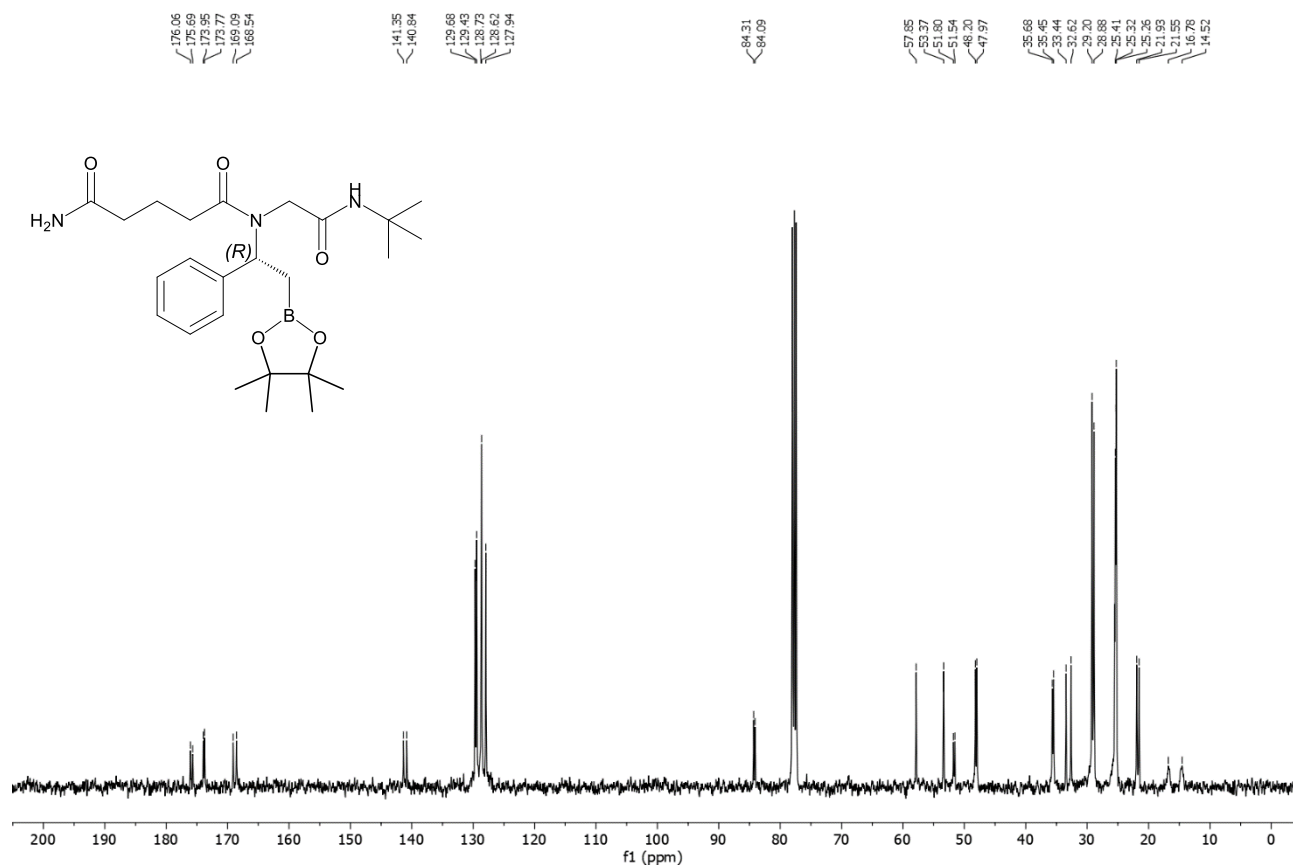
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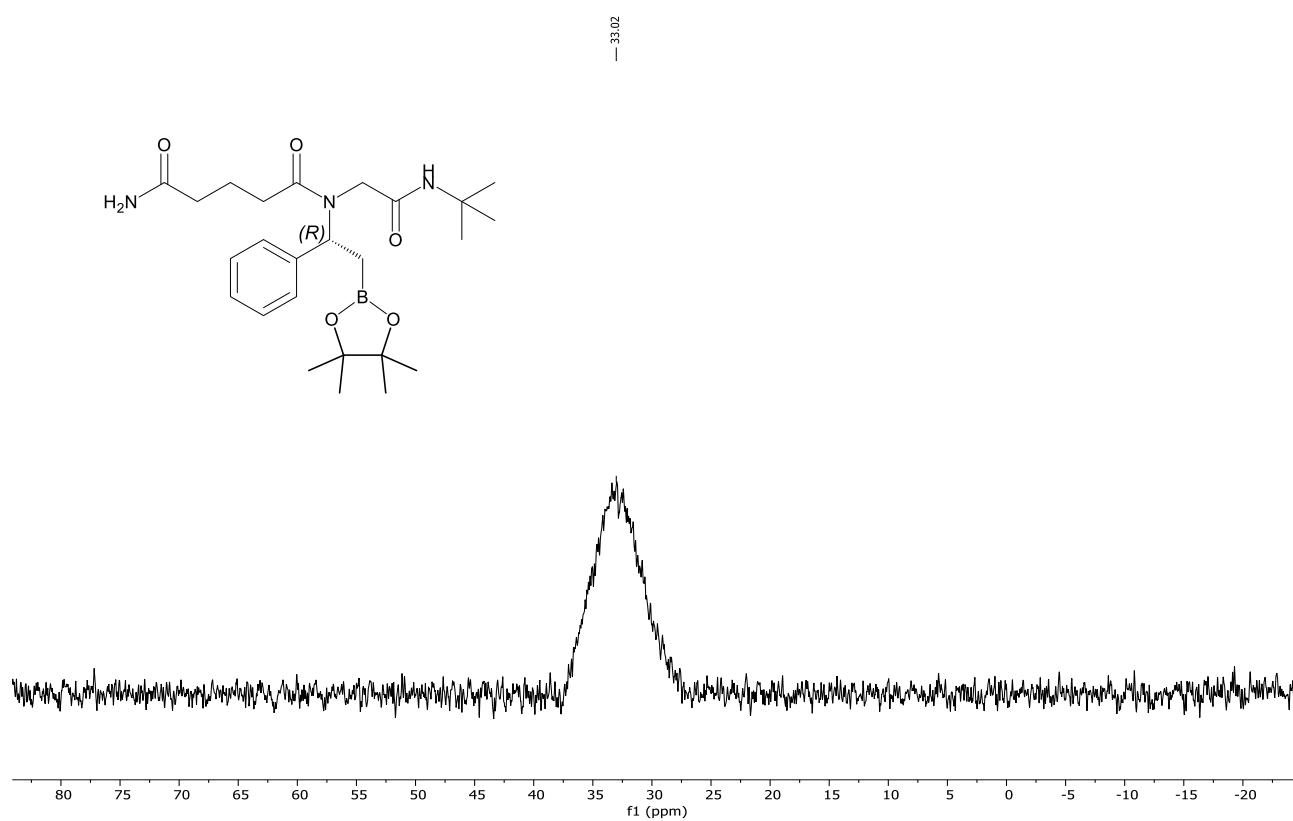
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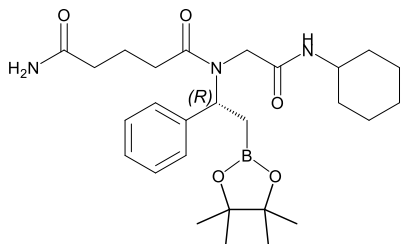
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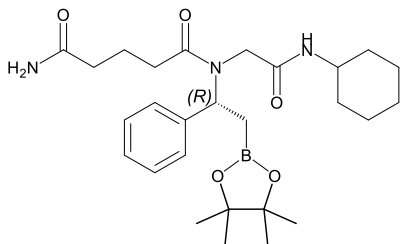
<sup>11</sup>B NMR of compound **2f** (128 MHz, CDCl<sub>3</sub>)

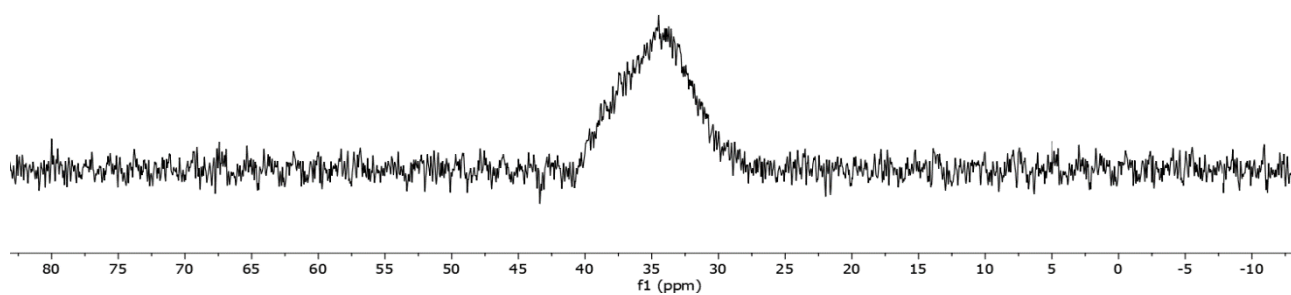
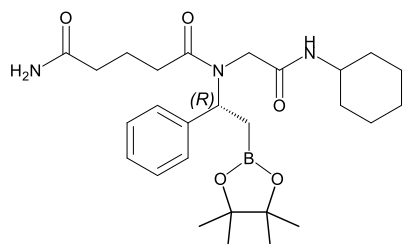


<sup>1</sup>H NMR of compound **2g** (400 MHz, CDCl<sub>3</sub> 65:35 rotameric mixture)

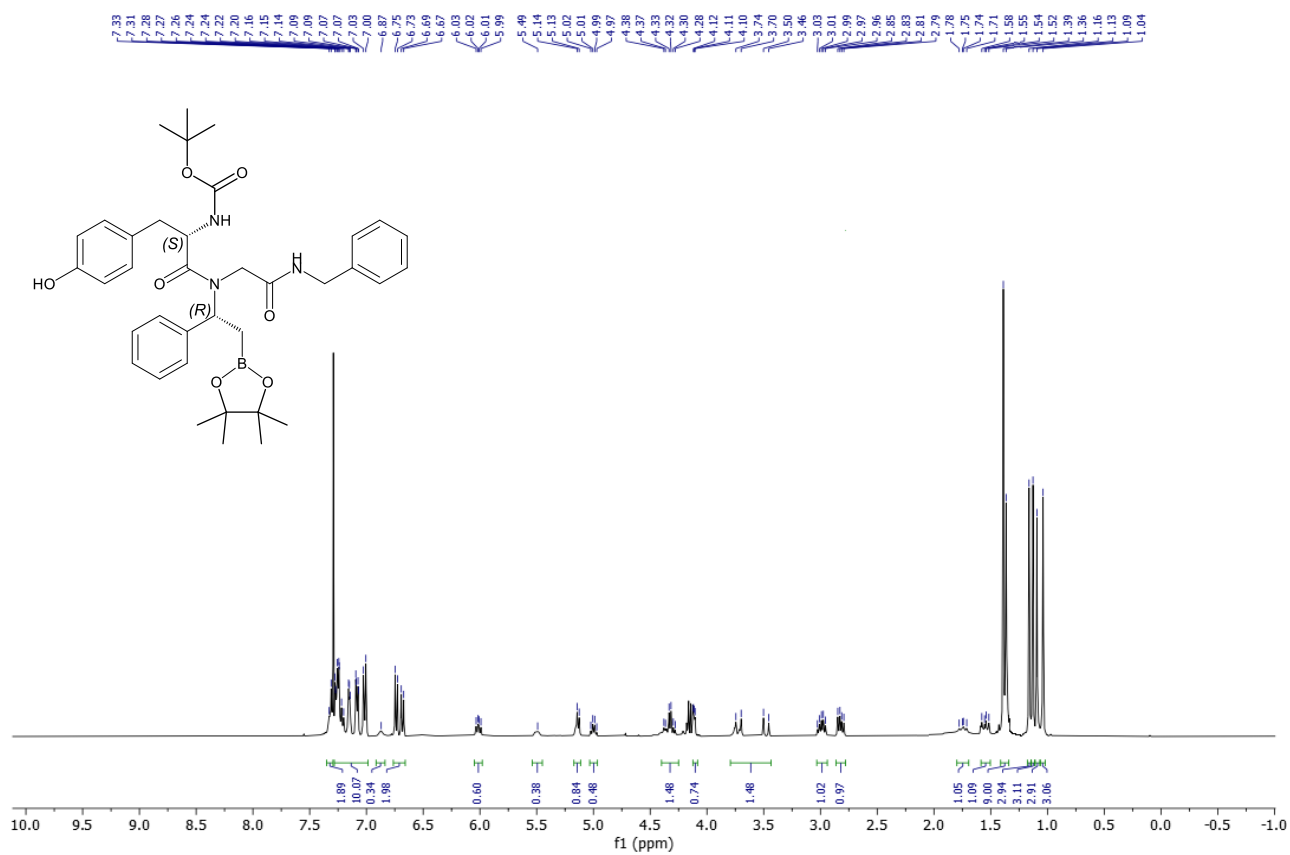


175.25	174.85	173.22	166.33	167.90	140.74	140.14	128.91	128.73	128.01	127.96	127.80	127.60	127.25	83.65	83.59	57.19	52.81	48.24	47.98	47.39	46.76	35.06	35.06	32.60	32.72	32.68	32.51	32.13	31.96	25.52	25.36	24.90	24.83	24.68	24.58	24.30	21.30	20.91	15.94	13.72
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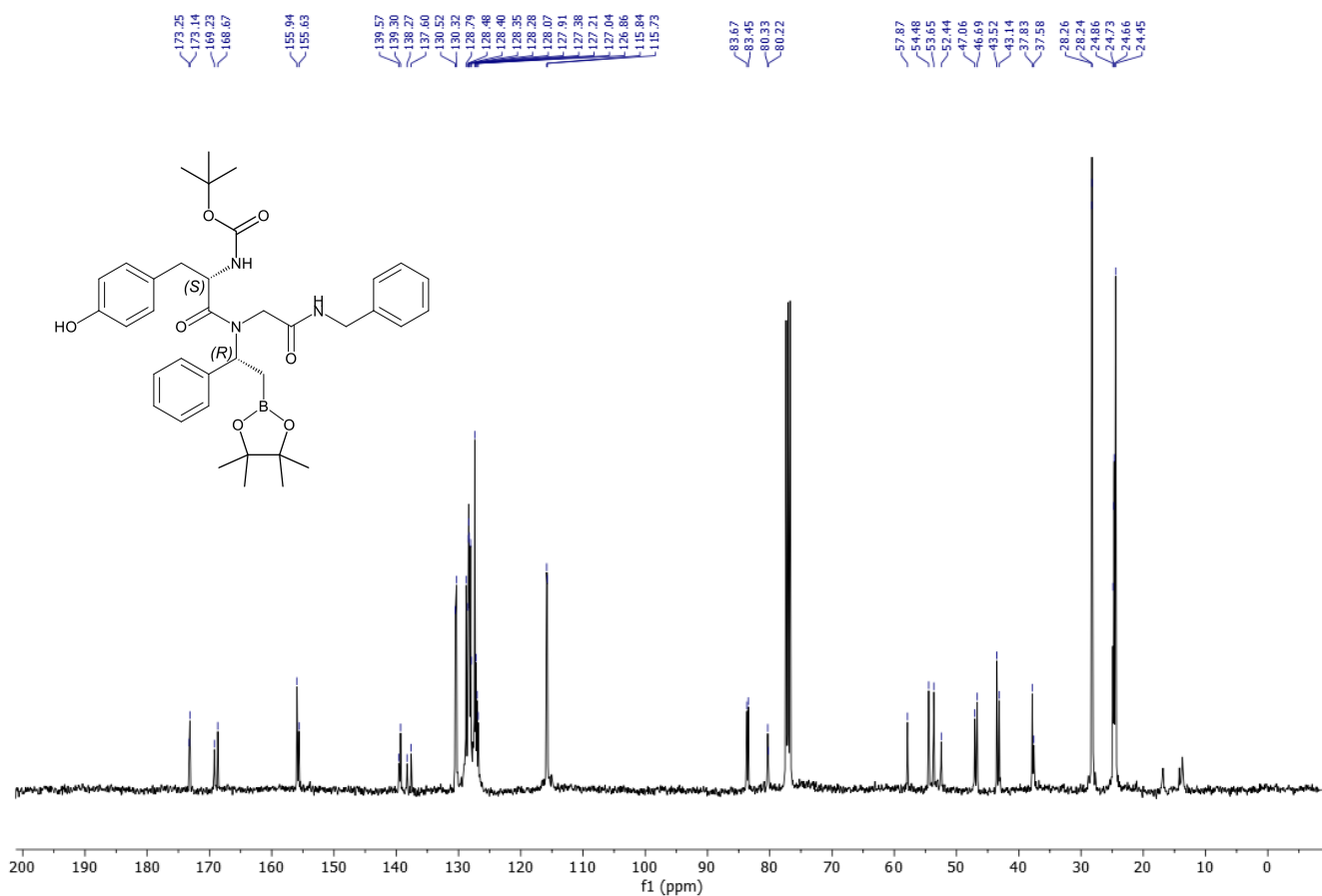
<sup>11</sup>B NMR of compound **2g** (128 MHz, CDCl<sub>3</sub>)



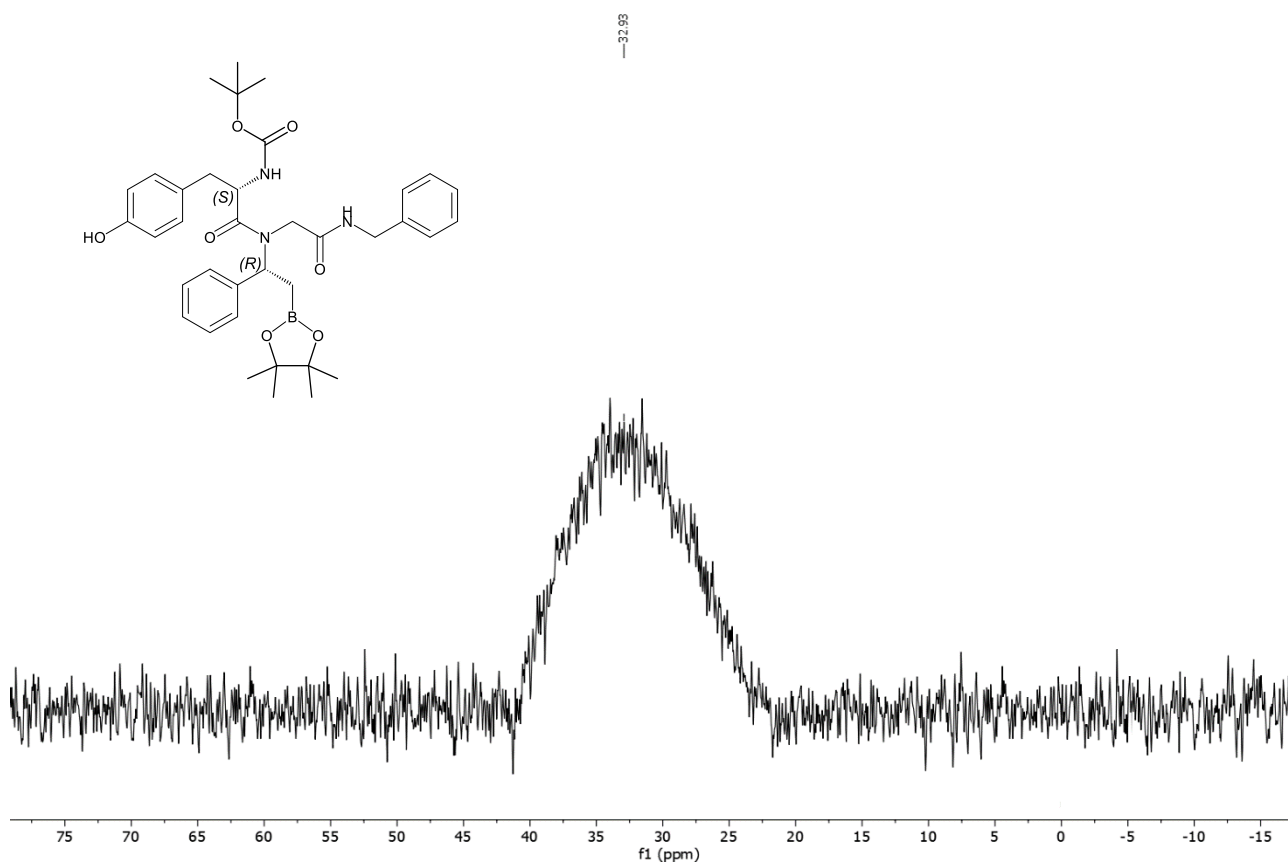
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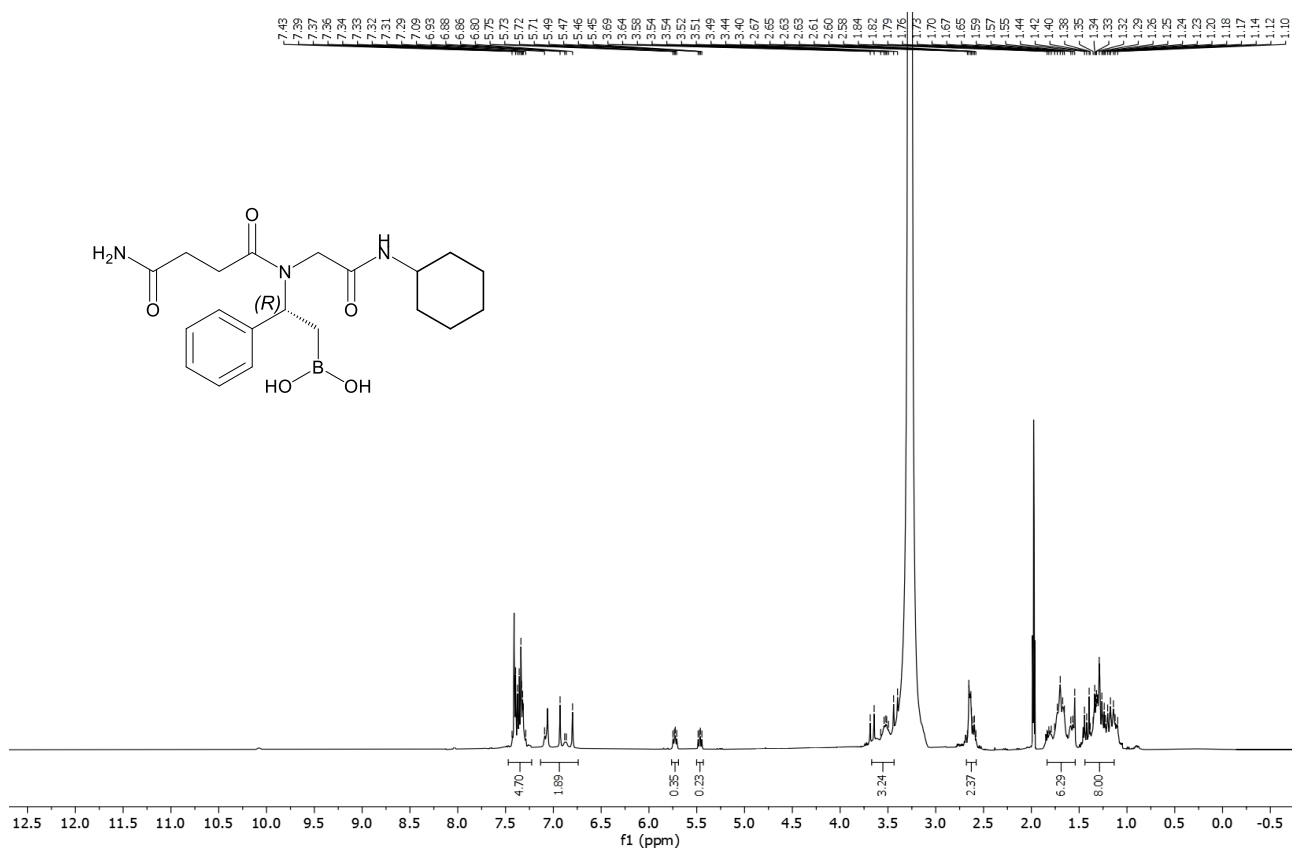
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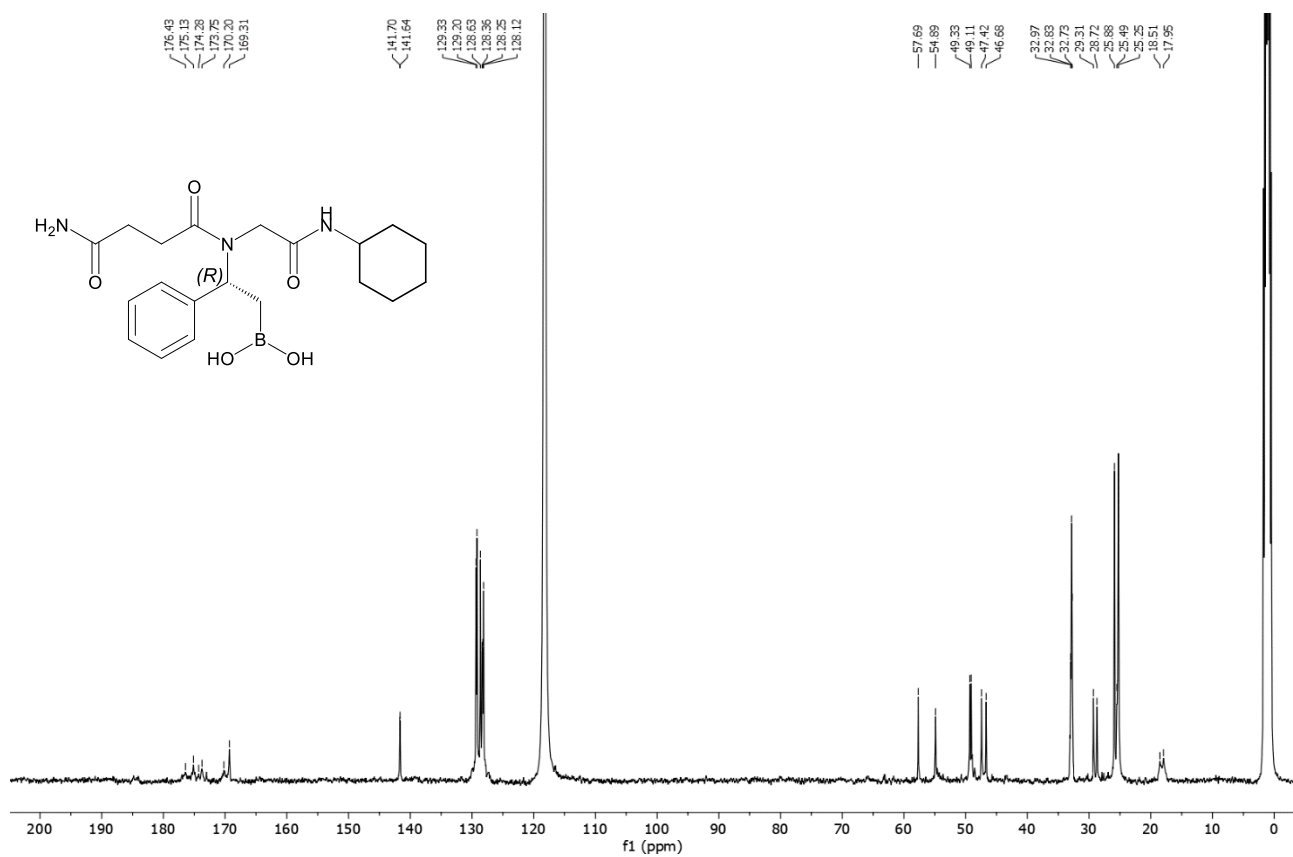
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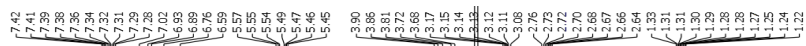
<sup>1</sup>H NMR of compound **3a** (400 MHz, CD<sub>3</sub>CN + 1 drop of H<sub>2</sub>O, 60:40 rotameric mixture)



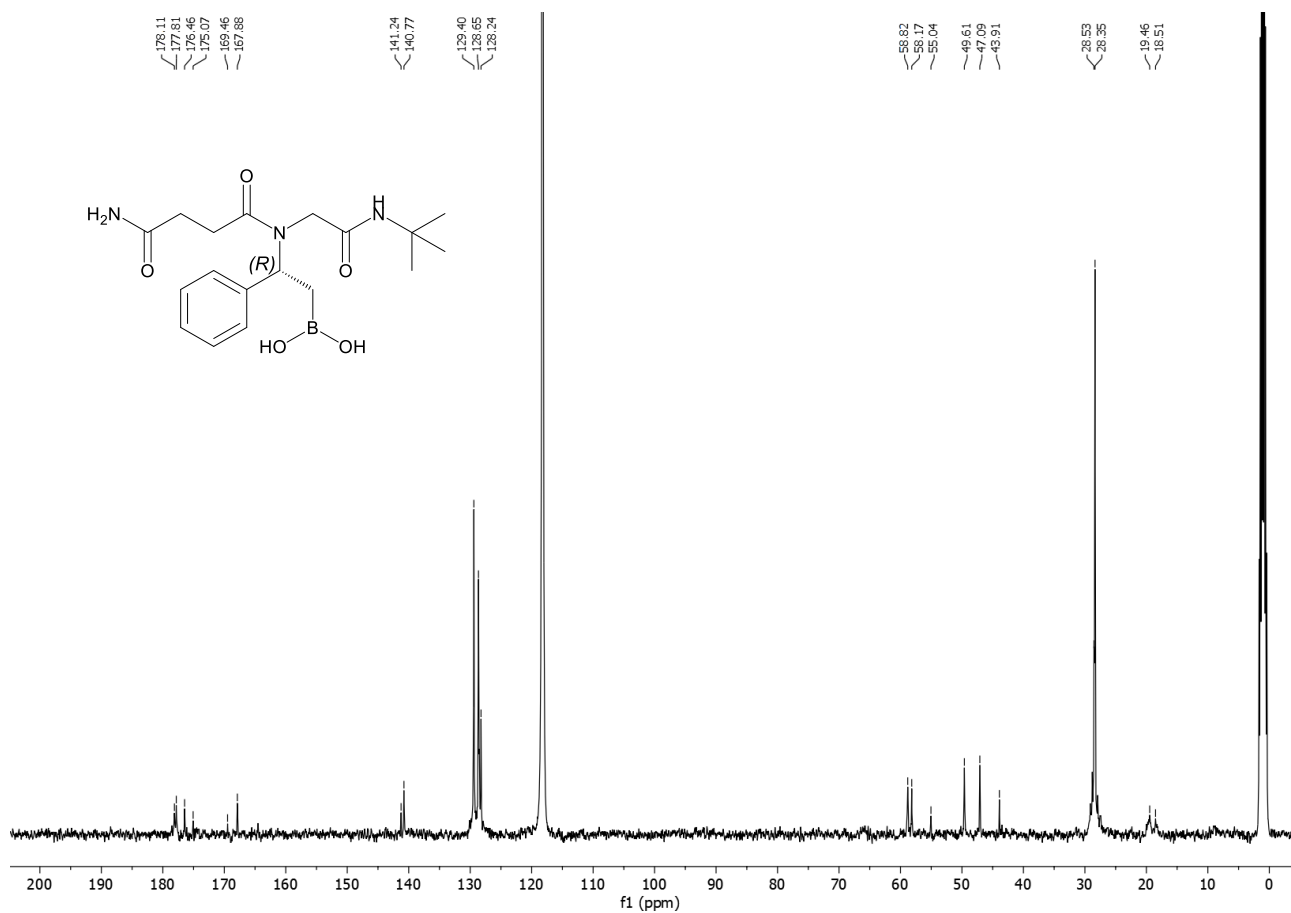
<sup>13</sup>C NMR of compound **3a** (101 MHz, CD<sub>3</sub>CN + 1 drop of H<sub>2</sub>O, 60:40 rotameric mixture)



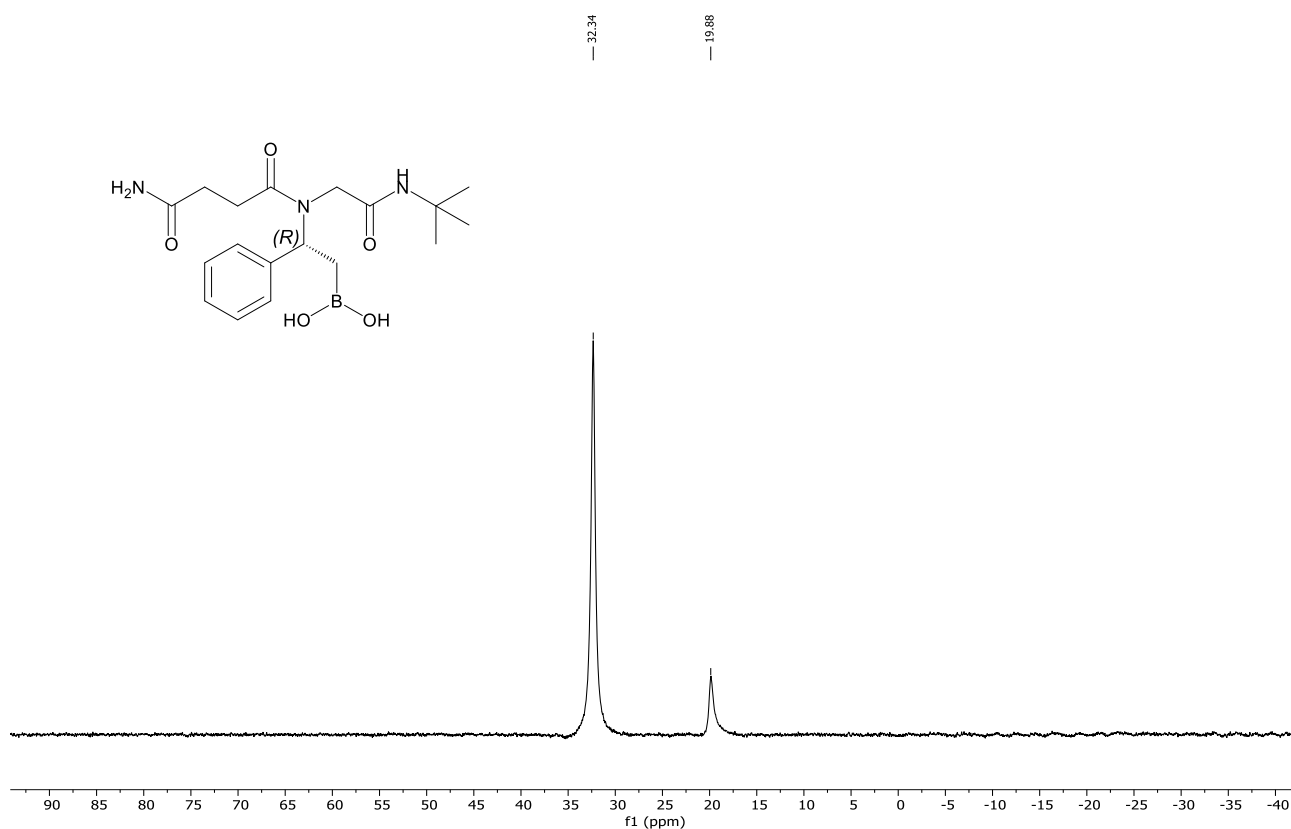
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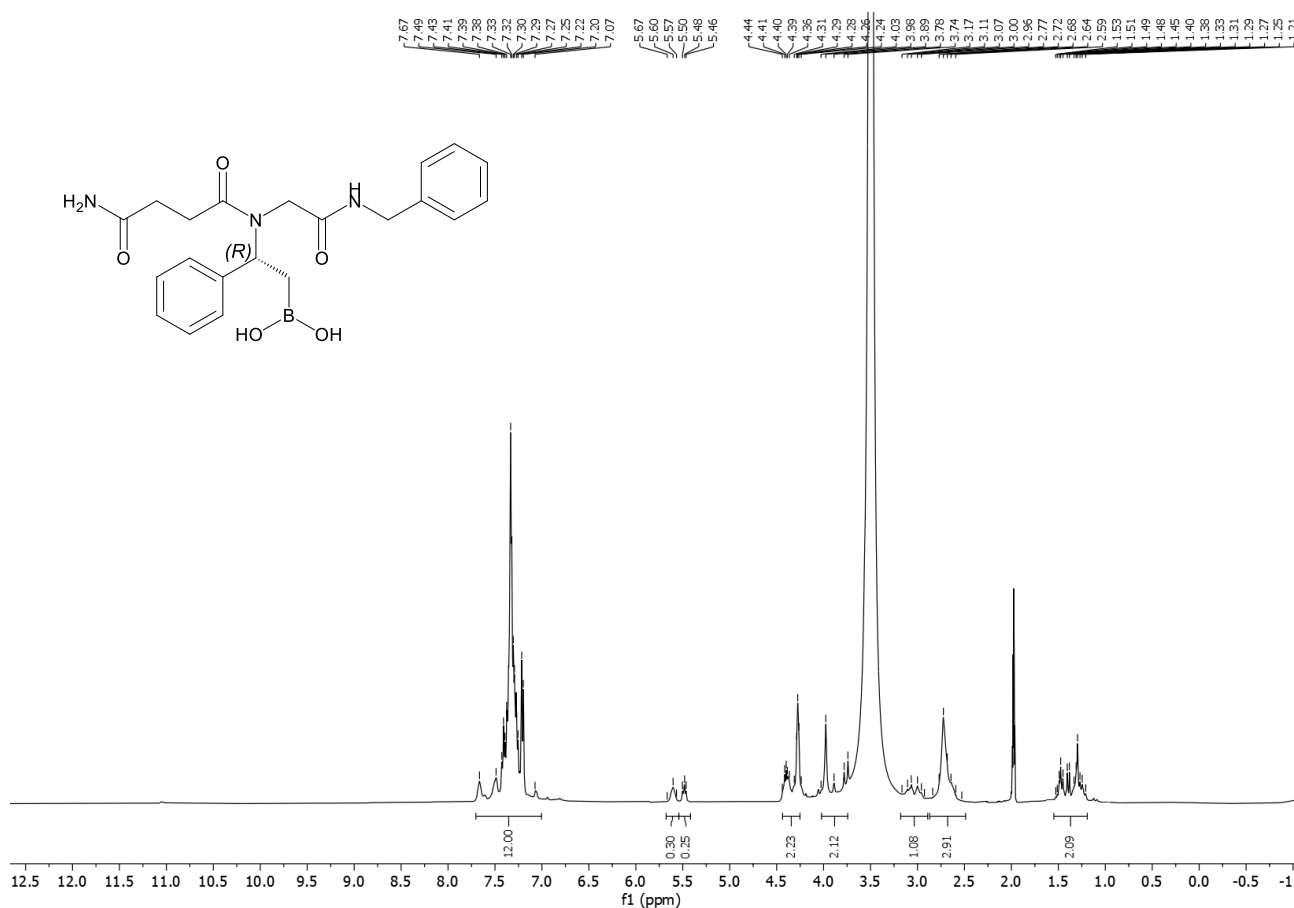




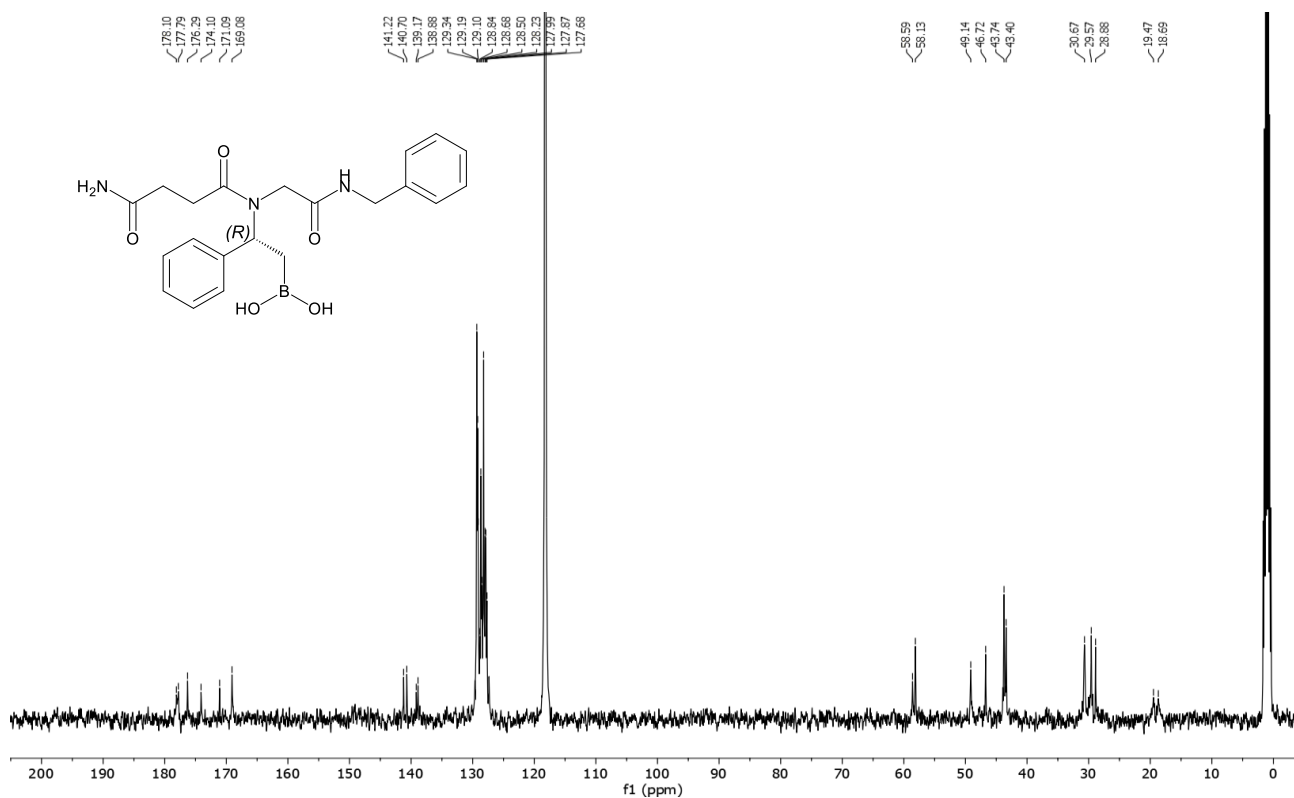
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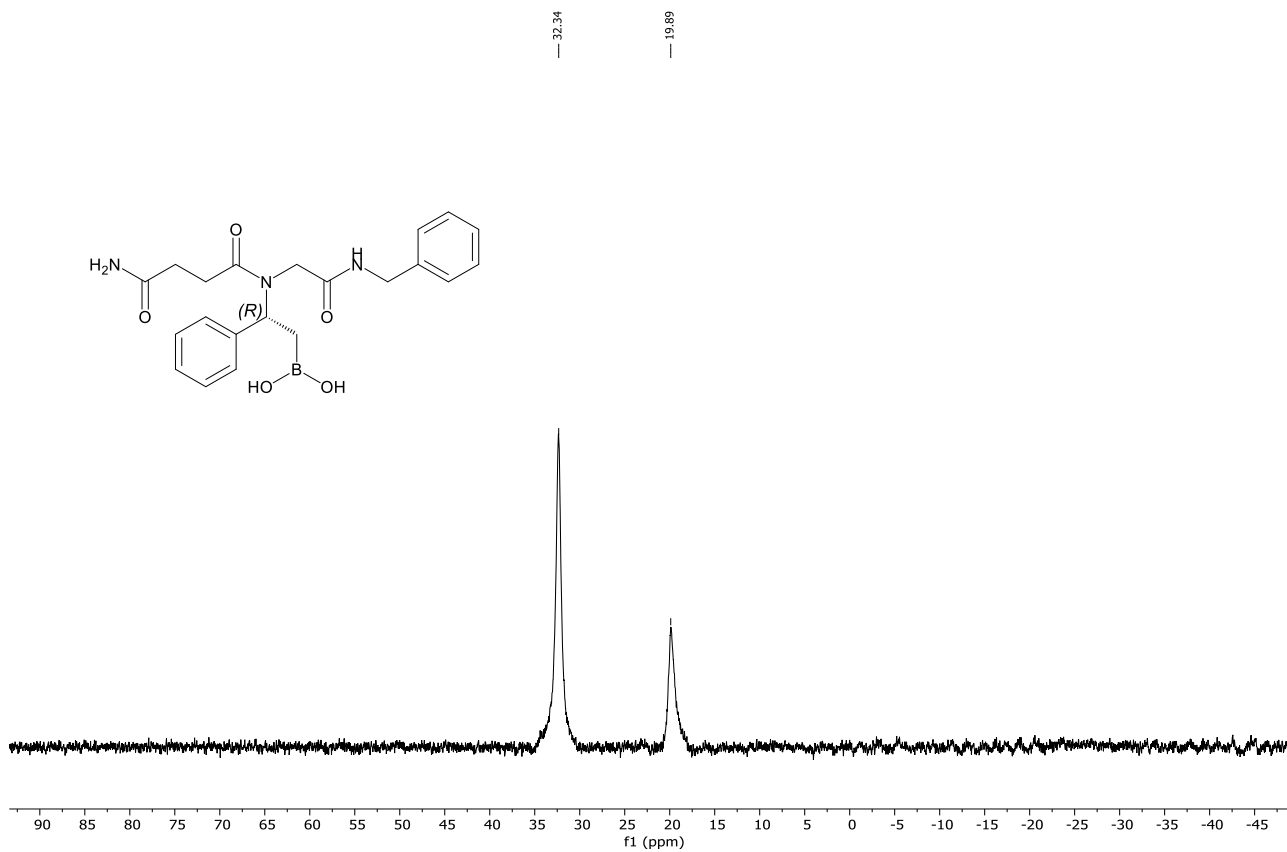
<sup>1</sup>H NMR of compound **3c** (400 MHz, CD<sub>3</sub>CN + 1 drop of H<sub>2</sub>O, 55:45 rotameric mixture)



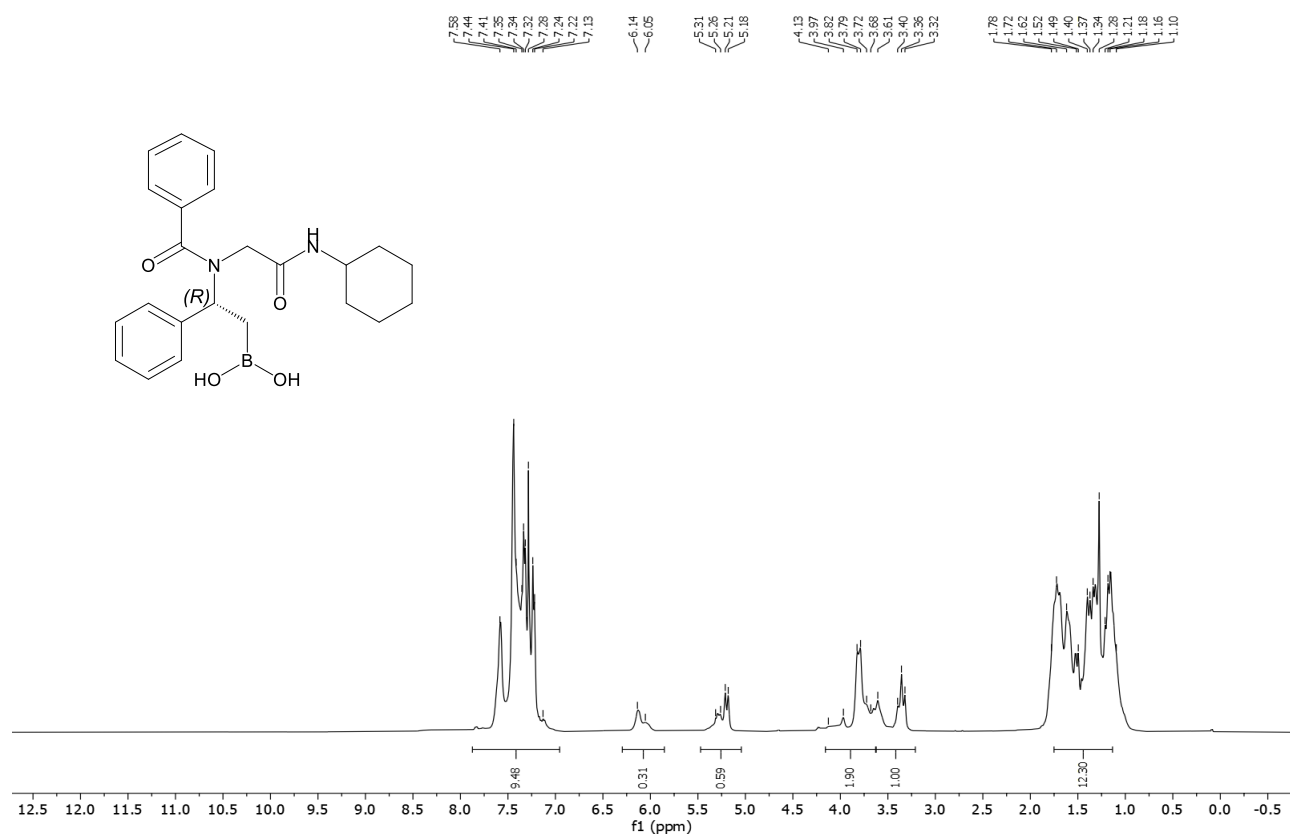
**<sup>13</sup>C NMR of compound 3c** (101 MHz, CD<sub>3</sub>CN + 1 drop of H<sub>2</sub>O, 55:45 rotameric mixture)



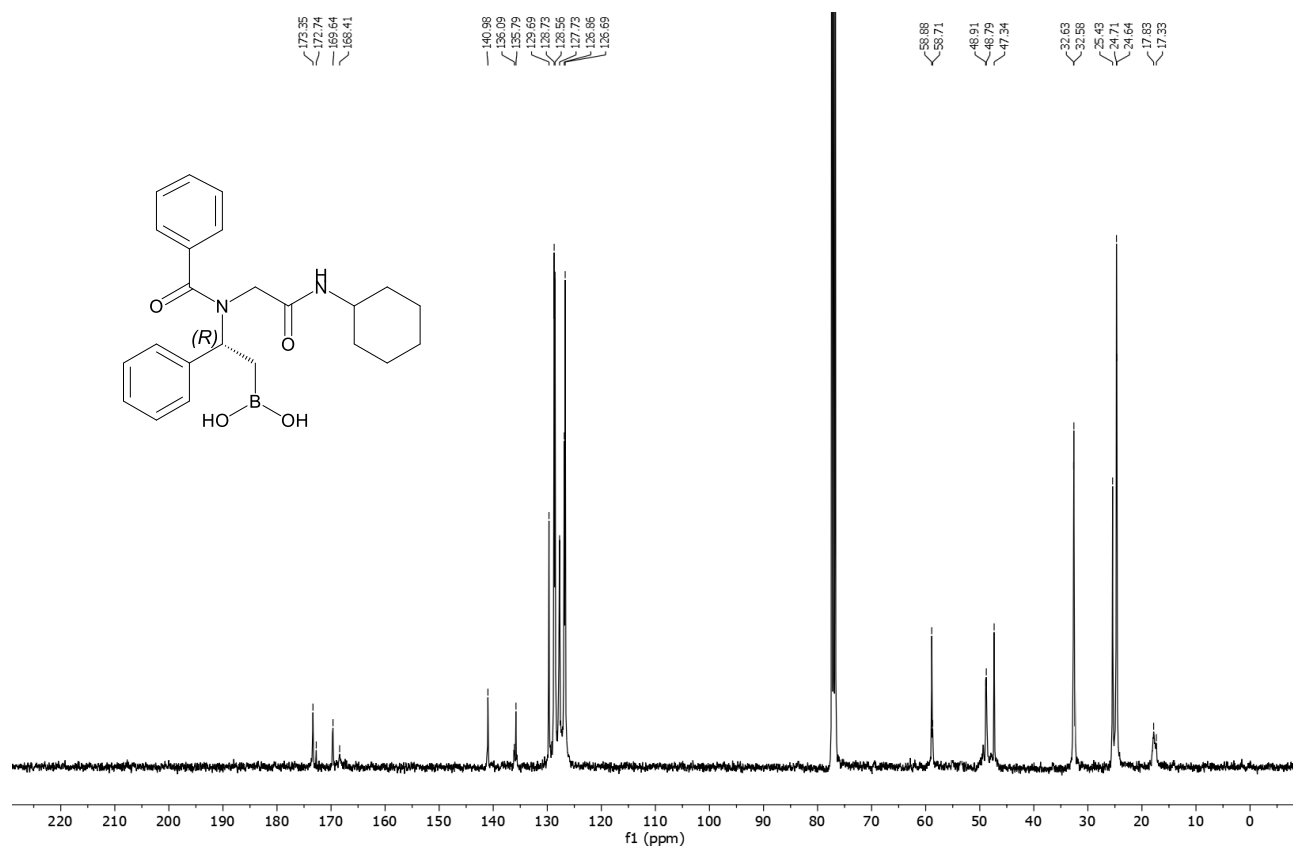
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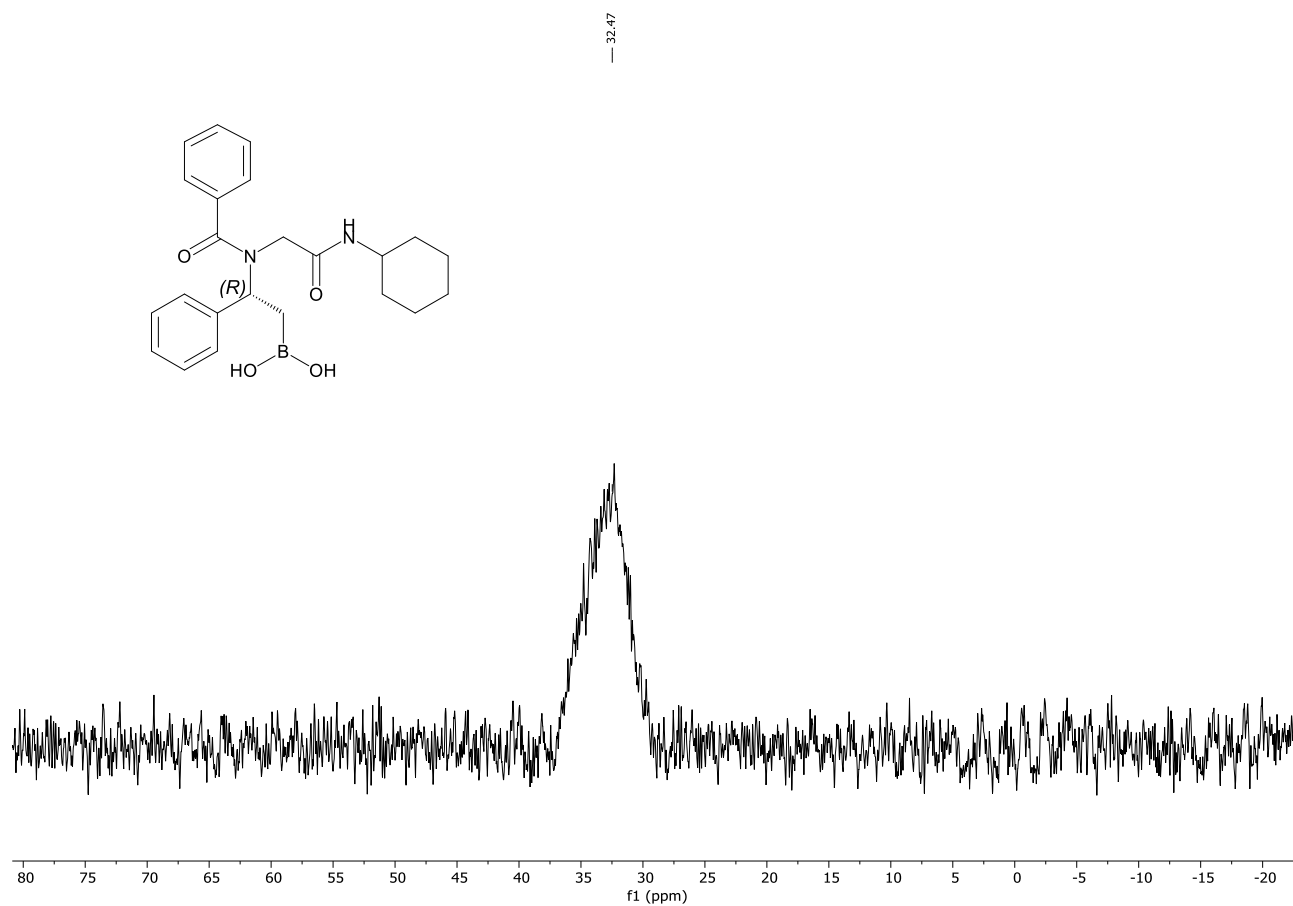
$^1\text{H}$  NMR of compound **3d** (400 MHz,  $\text{CDCl}_3$ , complex rotameric mixture: the section by section integration proves the overall number of protons)



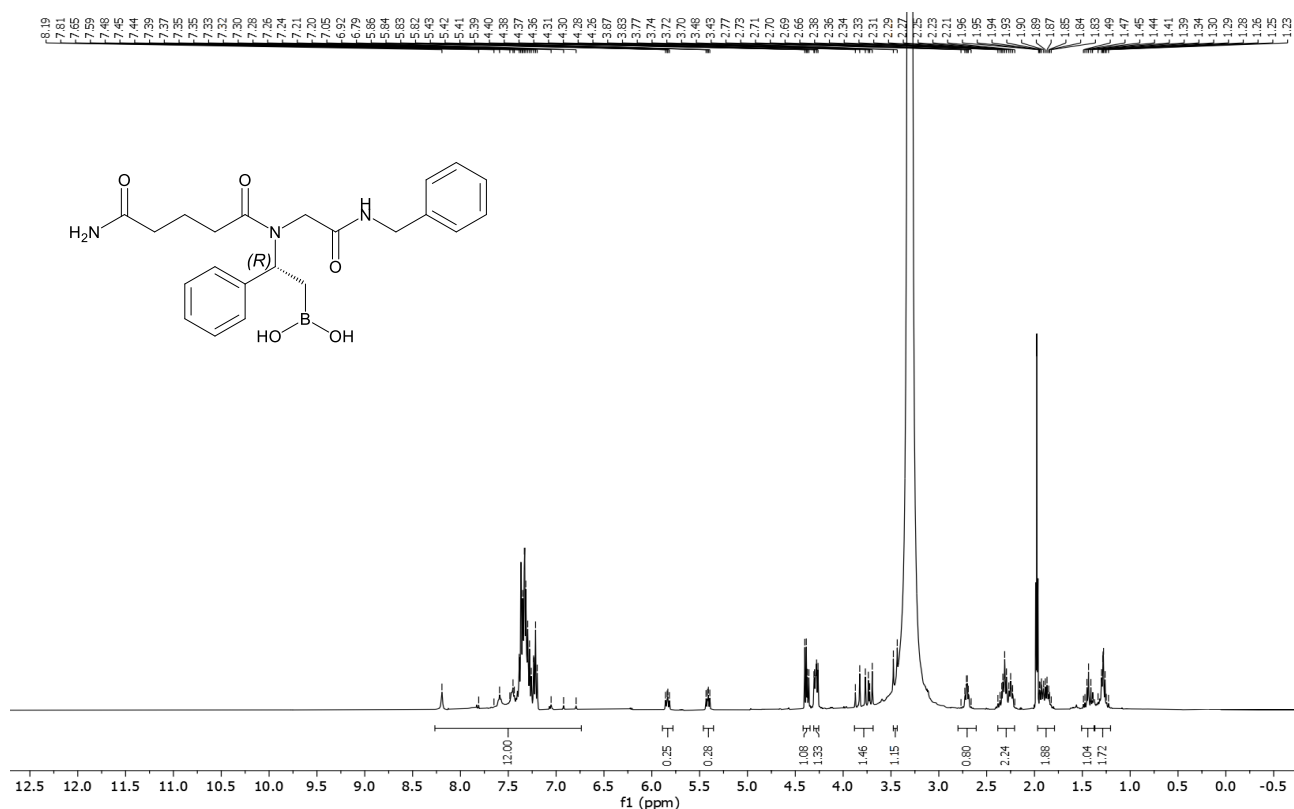
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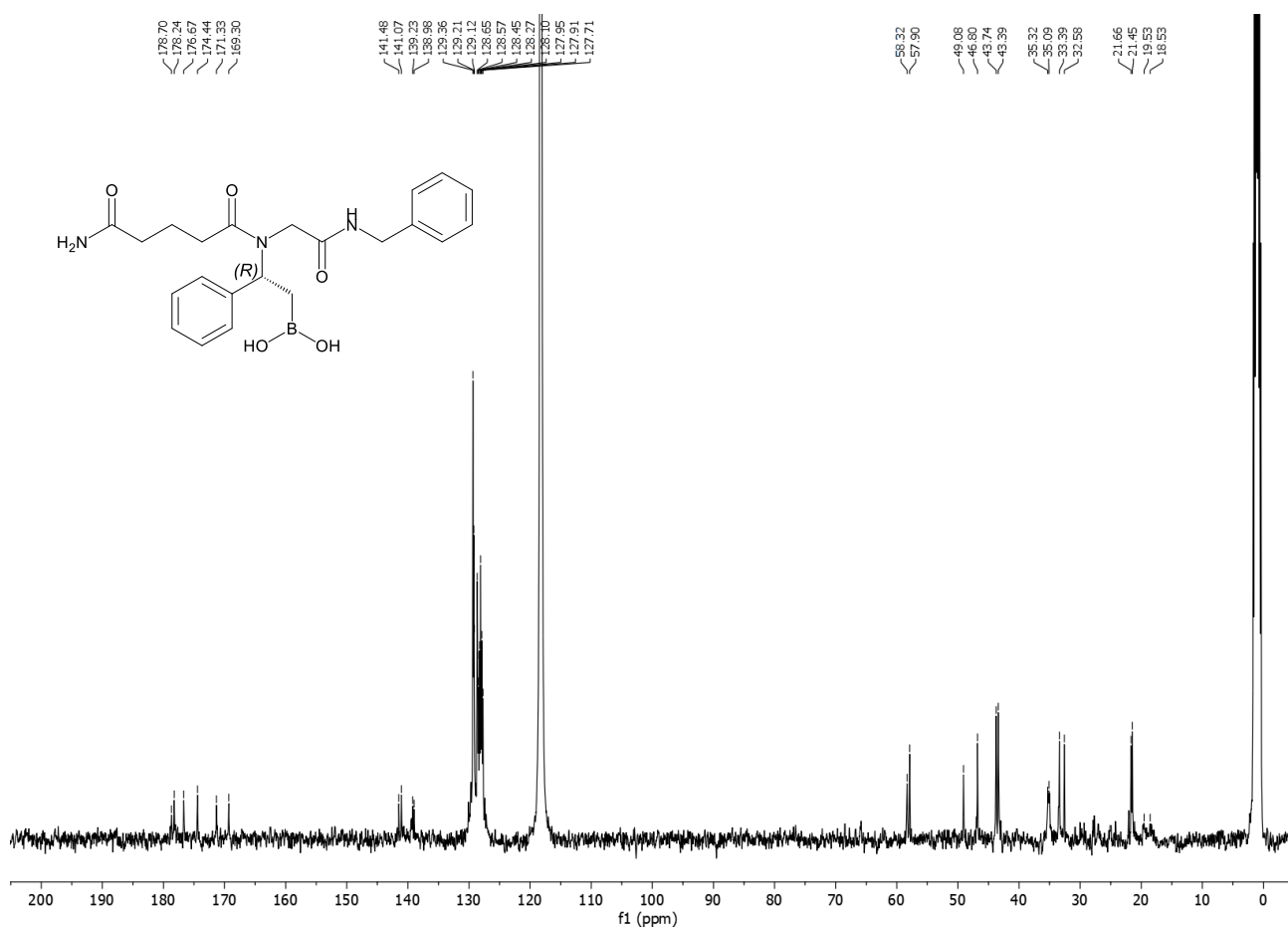
<sup>11</sup>B NMR of compound **3d** (128 MHz, CDCl<sub>3</sub>)



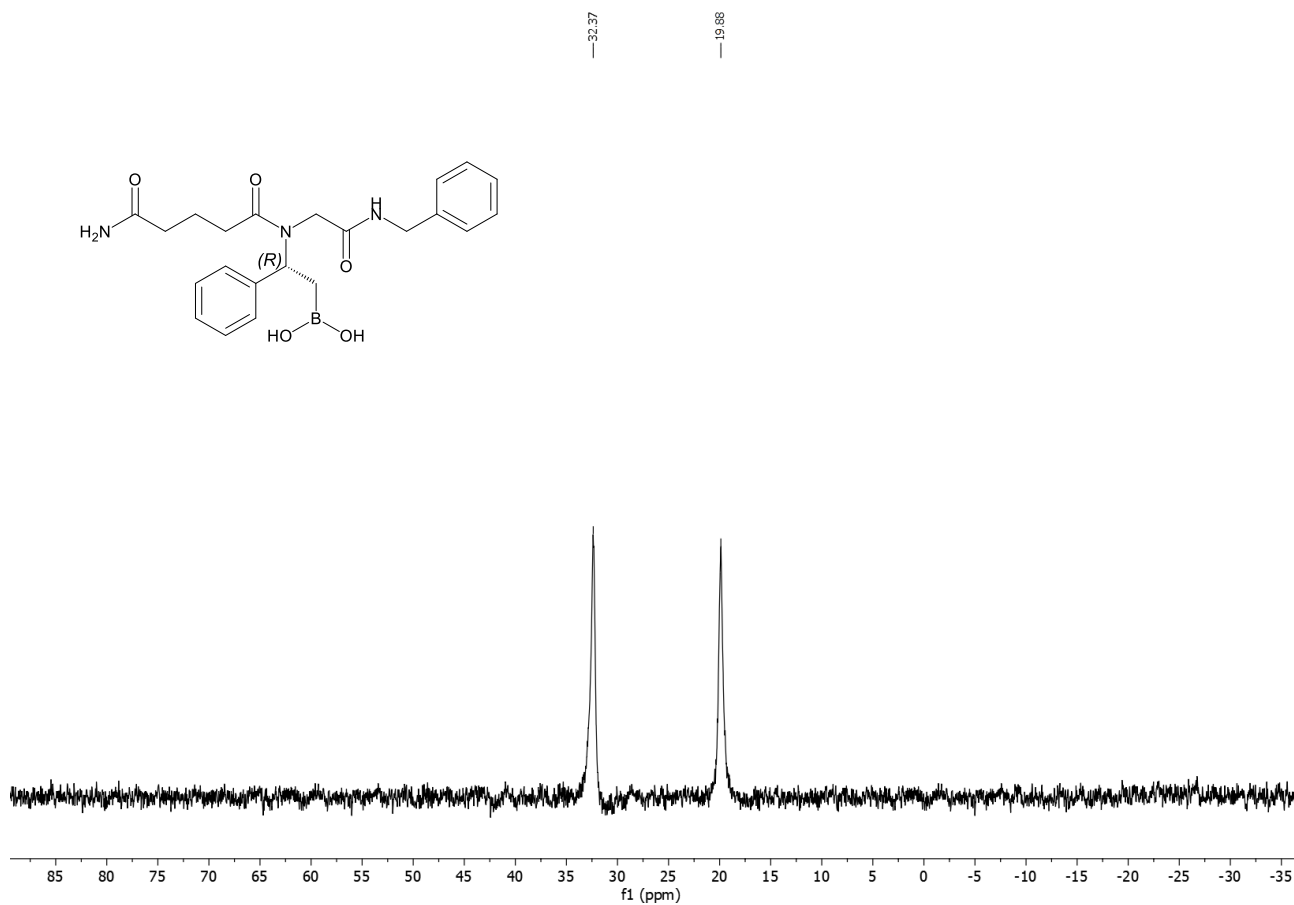
<sup>1</sup>H NMR of compound **3ea** (400 MHz, CD<sub>3</sub>CN + 1 drop of H<sub>2</sub>O, 55:45 rotameric mixture)



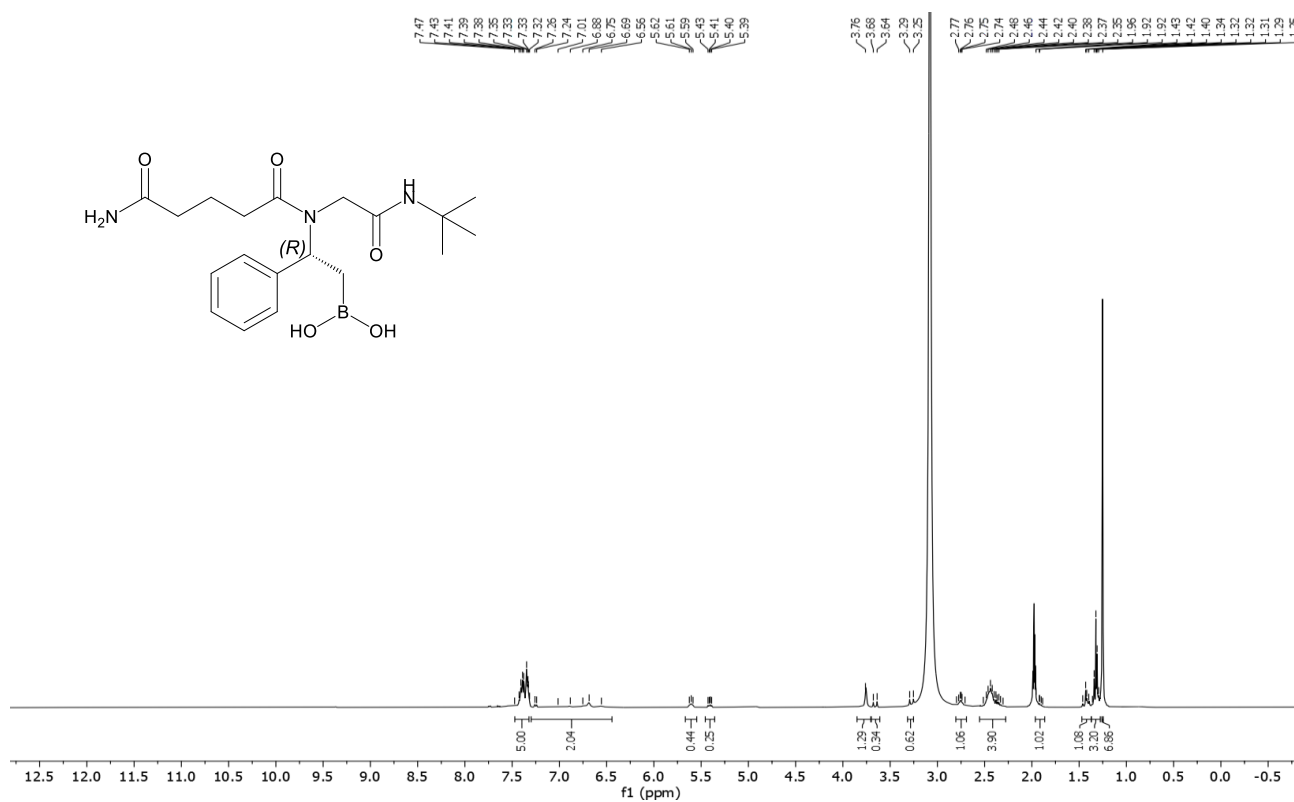
**<sup>13</sup>C NMR of compound **3ea** (101 MHz, CD<sub>3</sub>CN + 1 drop of H<sub>2</sub>O, 55:45 rotameric mixture)**



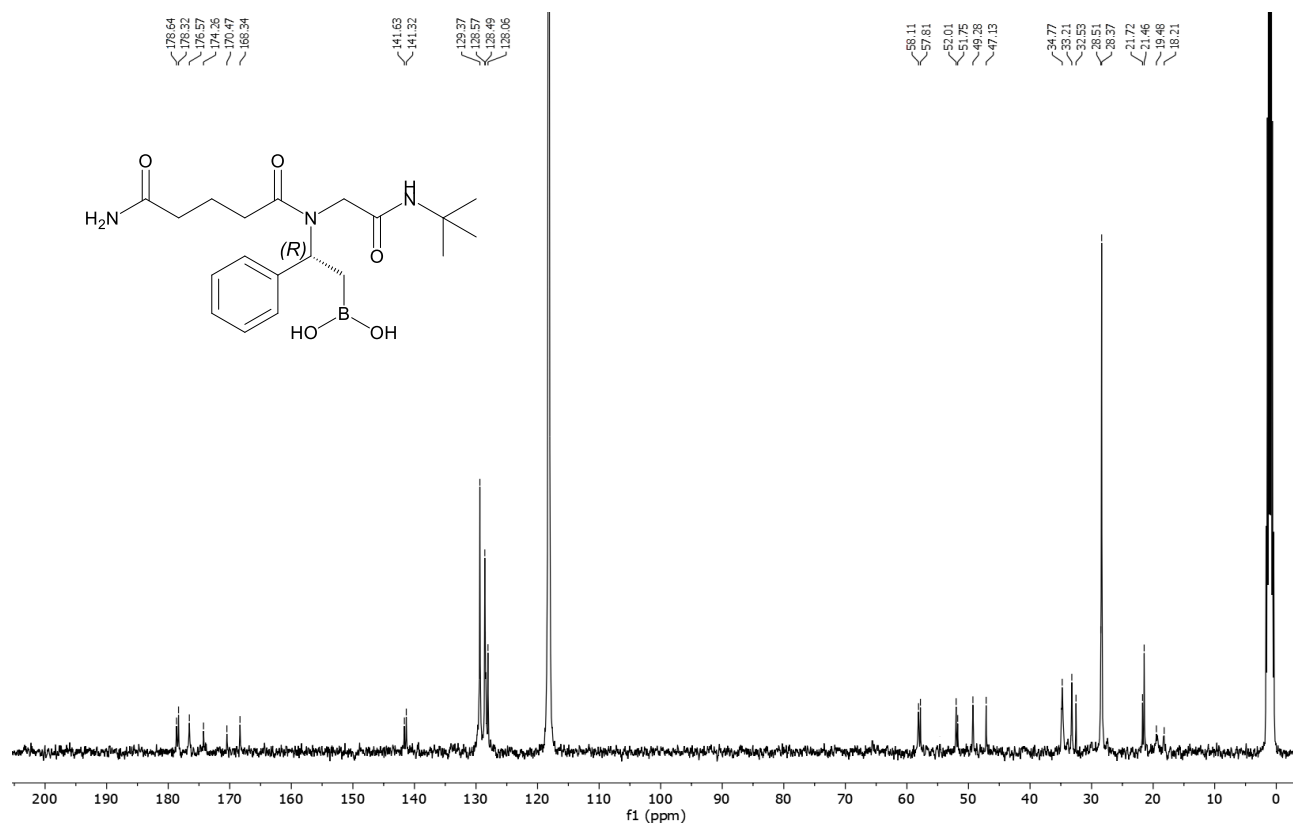
**<sup>11</sup>B NMR of compound **3ea** (128 MHz, CD<sub>3</sub>CN + 1 drop of H<sub>2</sub>O)**



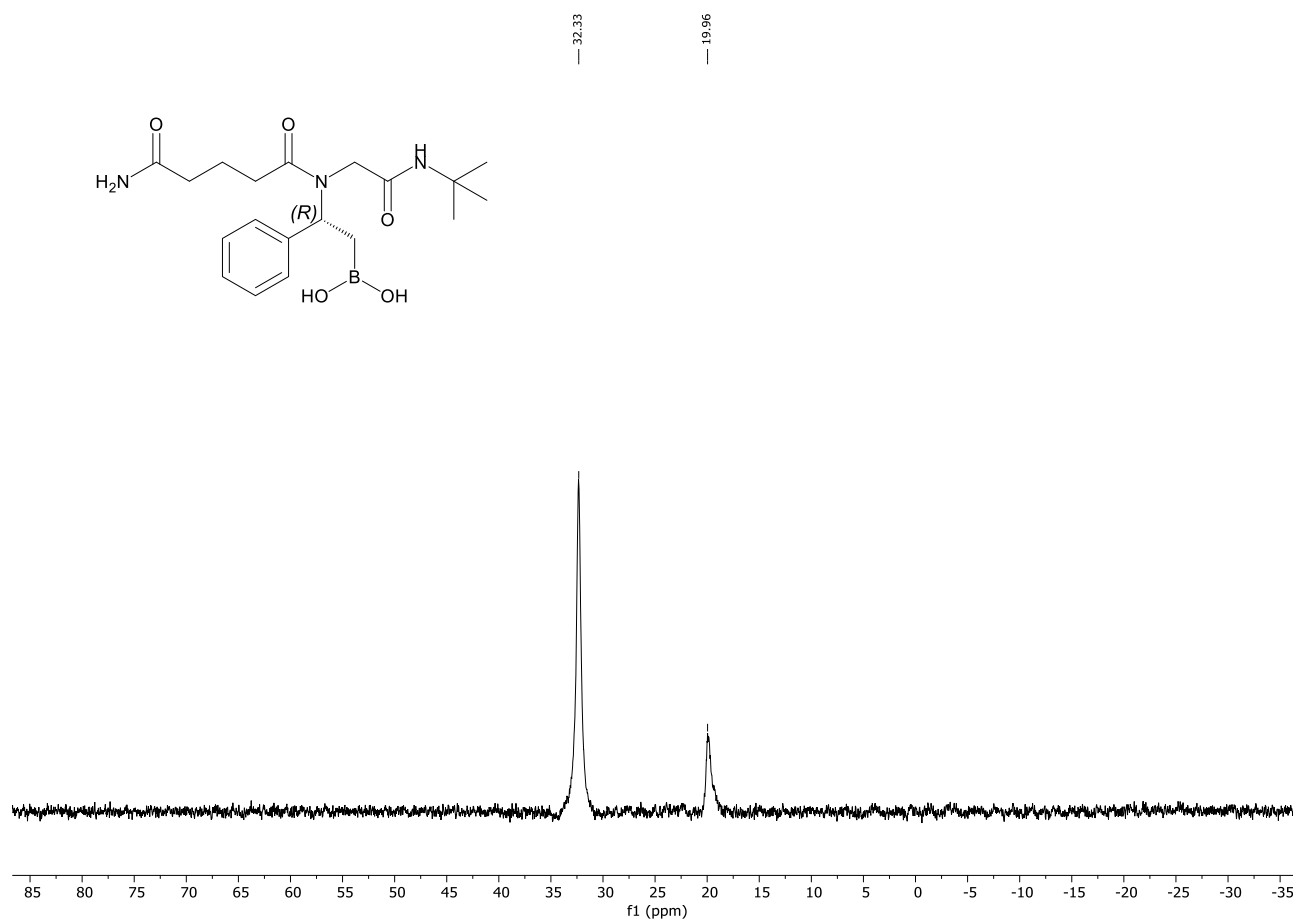
$^1\text{H}$  NMR of compound **3f** (400 MHz,  $\text{CD}_3\text{CN}$  + 1 drop of  $\text{H}_2\text{O}$ , 65:35 rotameric mixture)



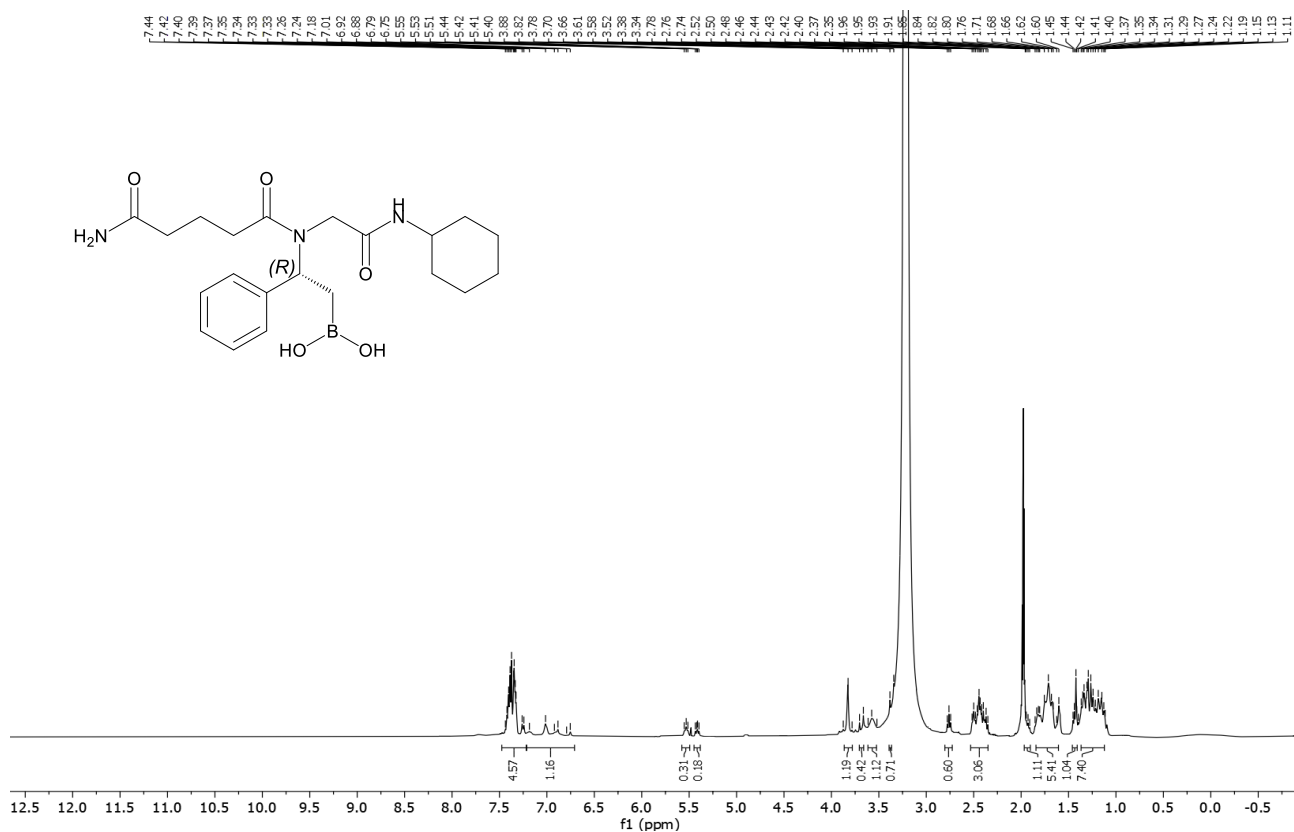
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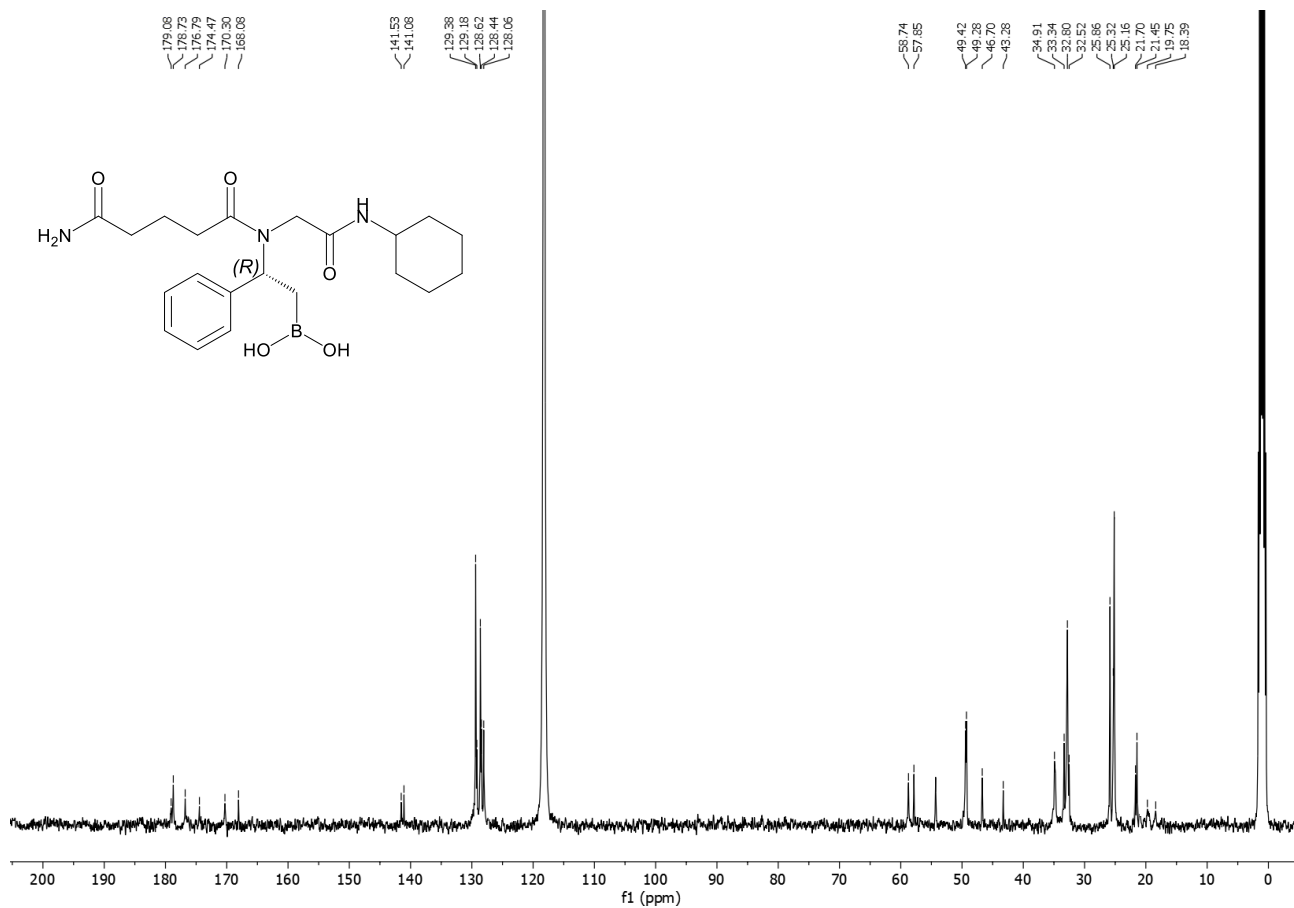
$^{11}\text{B}$  NMR of compound **3f** (128 MHz,  $\text{CD}_3\text{CN}$  + 1 drop of  $\text{H}_2\text{O}$ )



$^1\text{H}$  NMR of compound **3g** (400 MHz,  $\text{CD}_3\text{CN}$  + 1 drop of  $\text{H}_2\text{O}$ , 60:40 rotameric mixture)



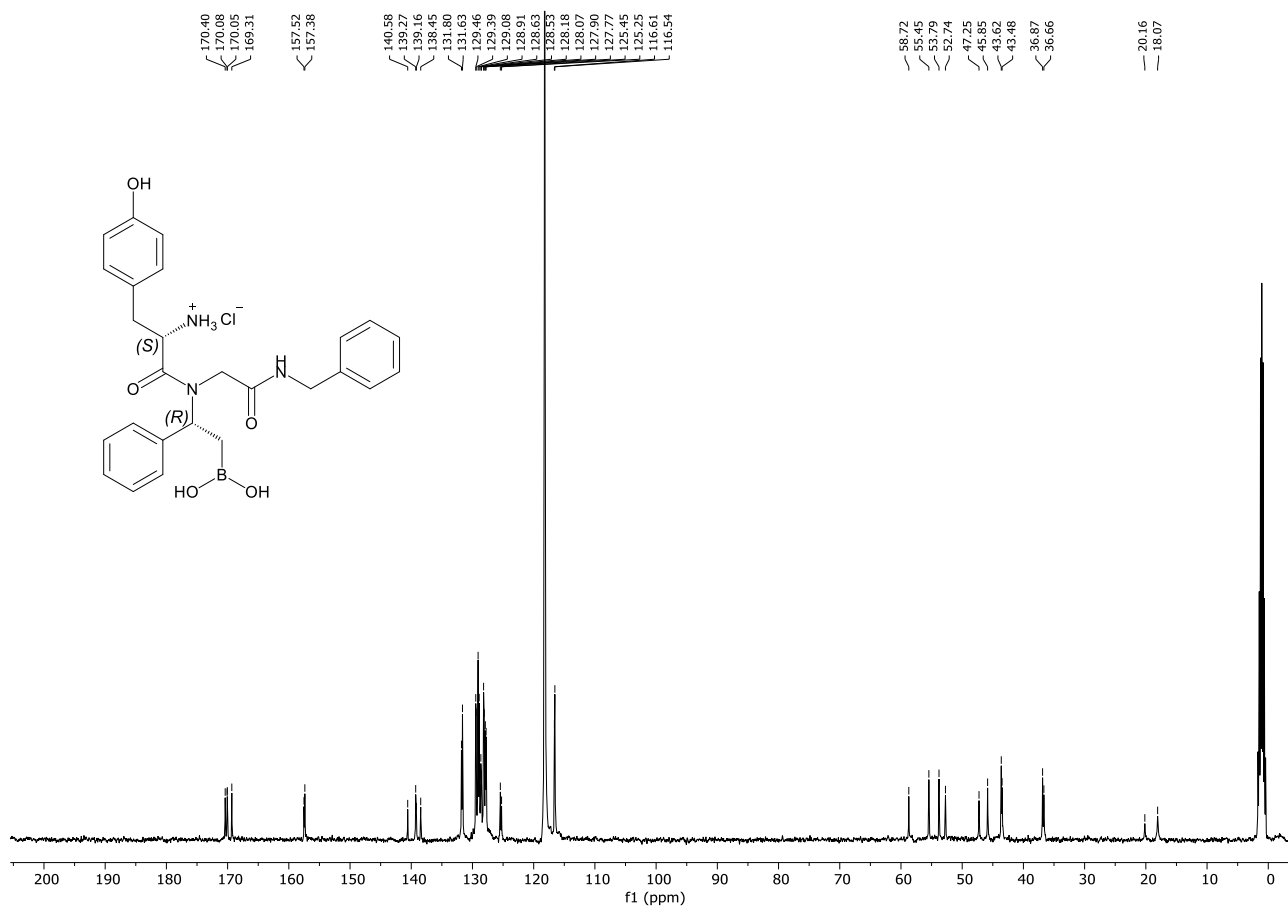
**<sup>13</sup>C NMR of compound 3g** (101 MHz, CD<sub>3</sub>CN + 1 drop of H<sub>2</sub>O, 60:40 rotameric mixture)



**<sup>11</sup>B NMR of compound 3g** (128 MHz, CD<sub>3</sub>CN + 1 drop of H<sub>2</sub>O)







$^{11}\text{B}$  NMR of compound **3h** (128 MHz,  $\text{CD}_3\text{CN}$  + 1 drop of  $\text{H}_2\text{O}$ )

