

## A novel route for preparing 5' cap mimics and capped RNAs: phosphate-modified cap analogues obtained *via* click chemistry

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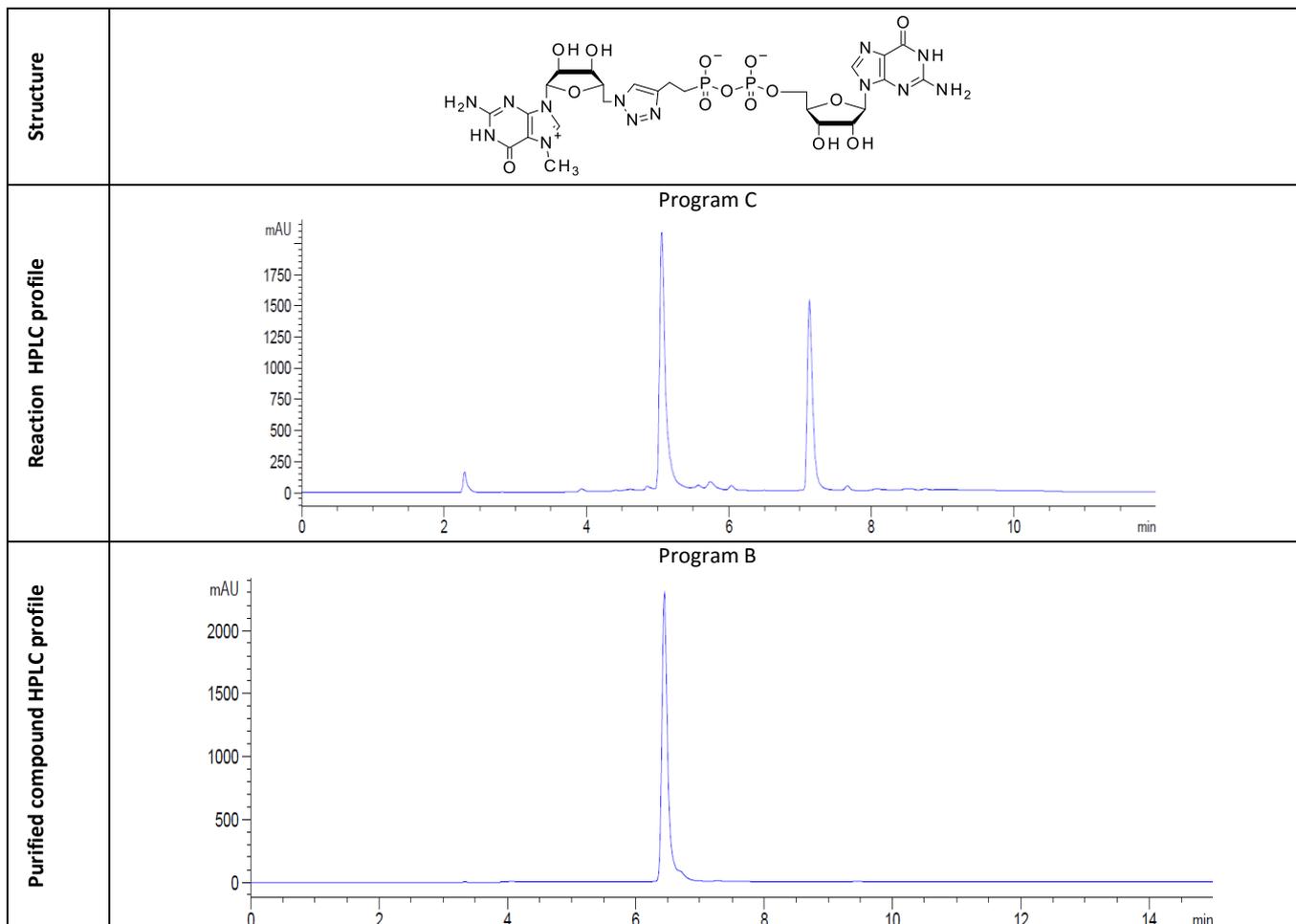
## HPLC gradients

Program A - linear gradient 0–25% of methanol in 0.05 M ammonium acetate buffer (pH 5.9) in 15 min

Program B - linear gradient 0–50% of methanol in 0.05 M ammonium acetate buffer (pH 5.9) in 15 min

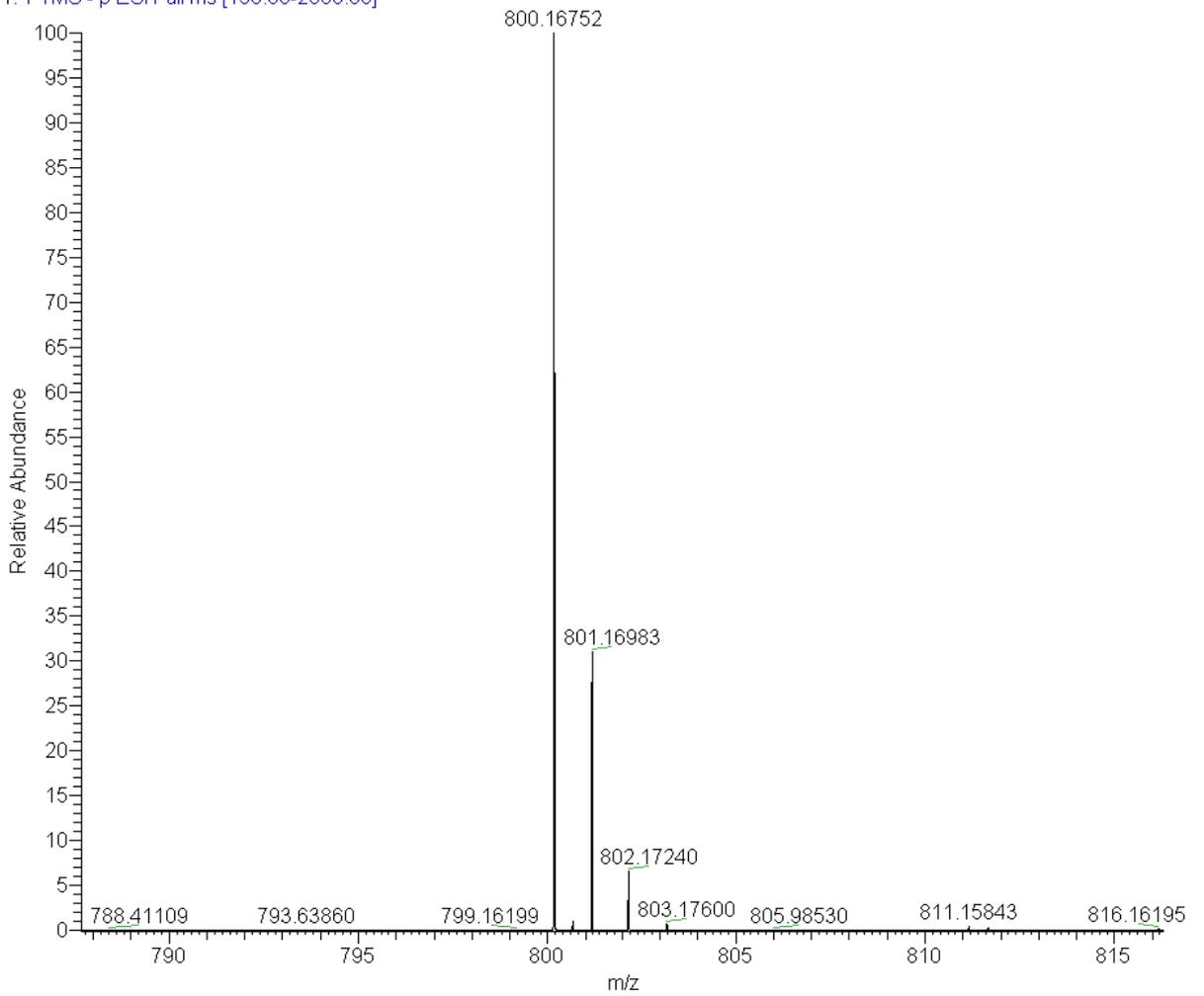
Program C – linear gradient 0–50% of methanol in 0.05 M ammonium acetate buffer (pH 5.9) in 7.5 min and then isocratic elution (50% of methanol) until 15 min

### (1a) m<sup>7</sup>G-triazole-C<sub>2</sub>H<sub>4</sub>ppG

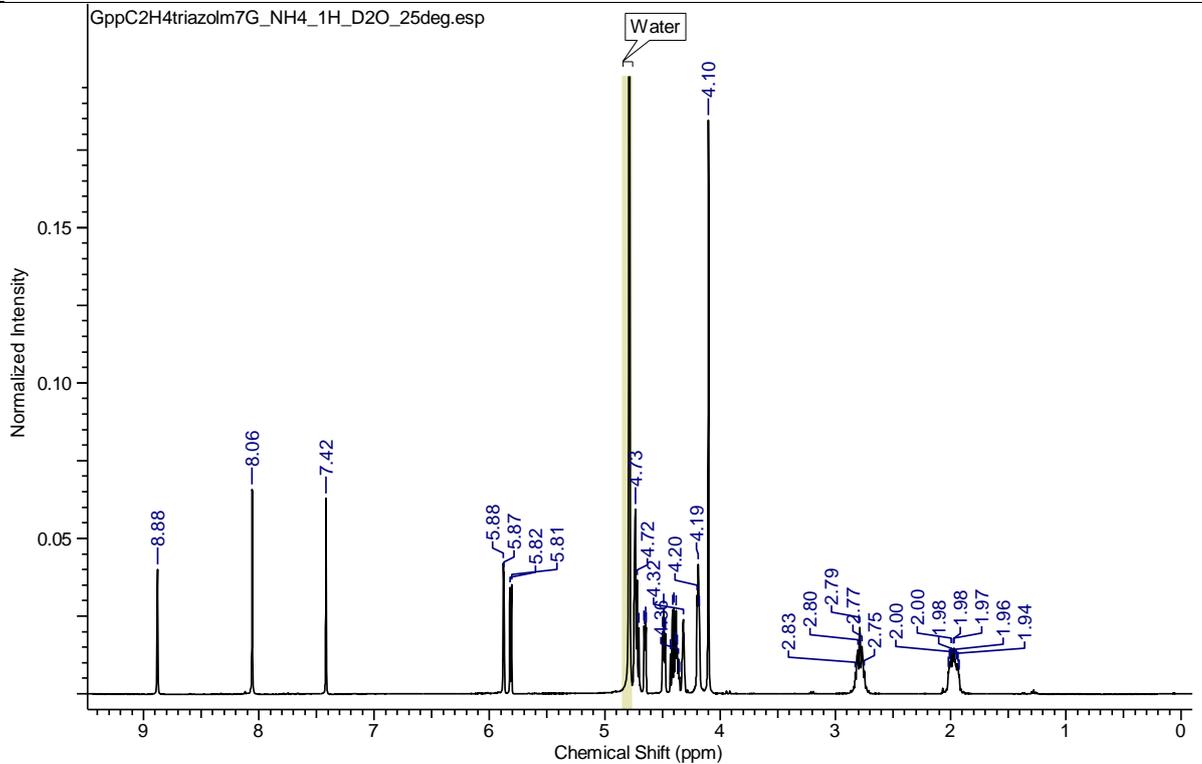


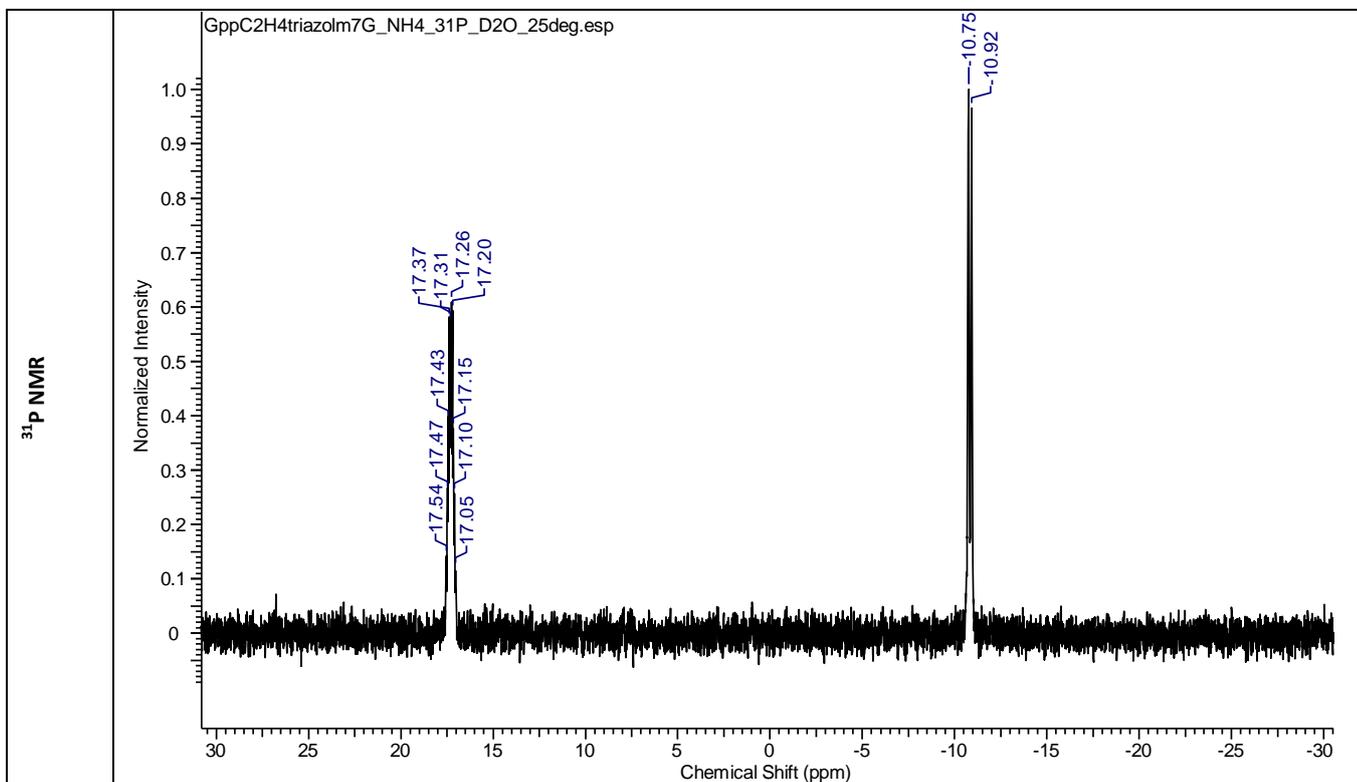
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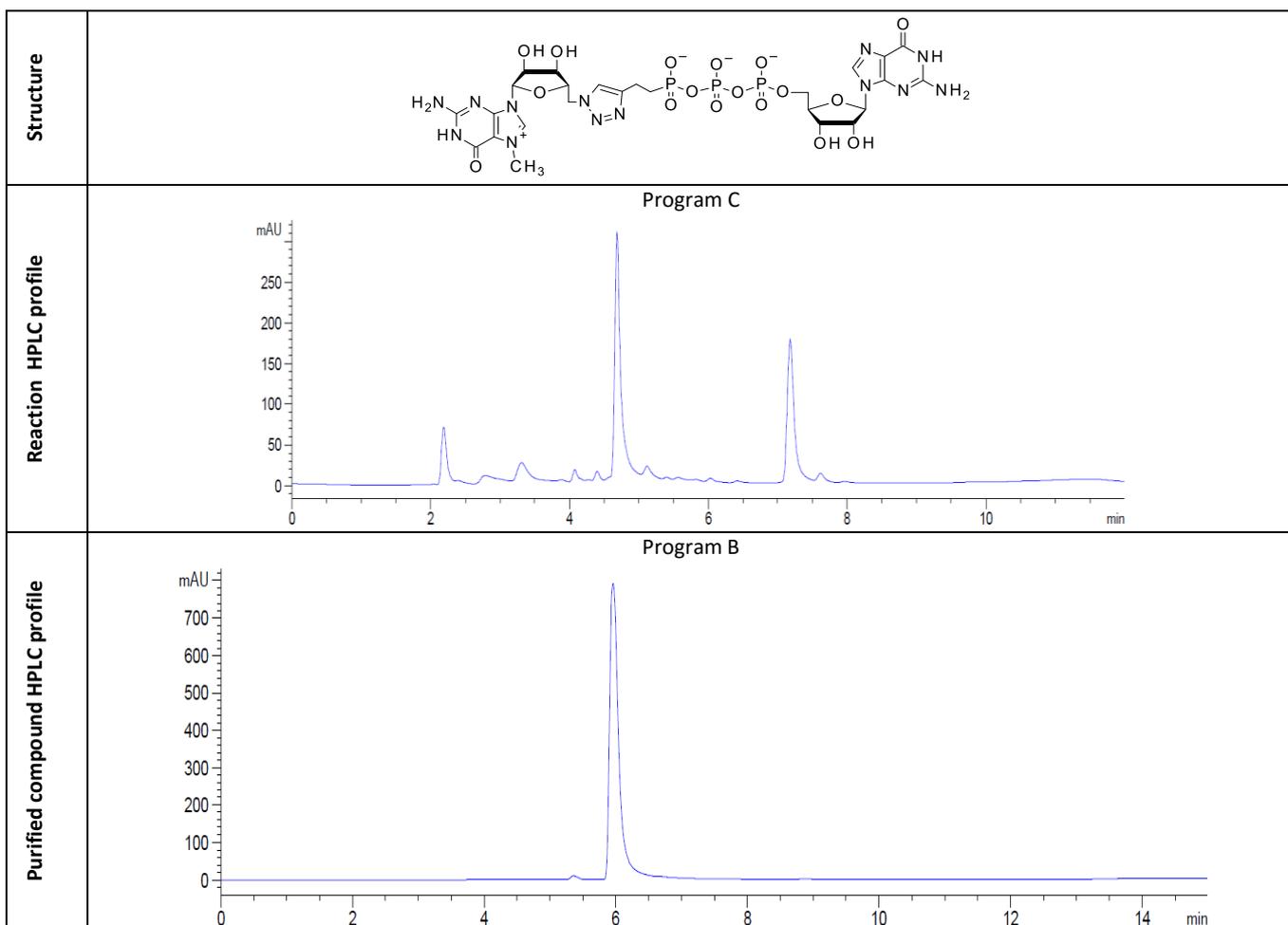


<sup>1</sup>H NMR



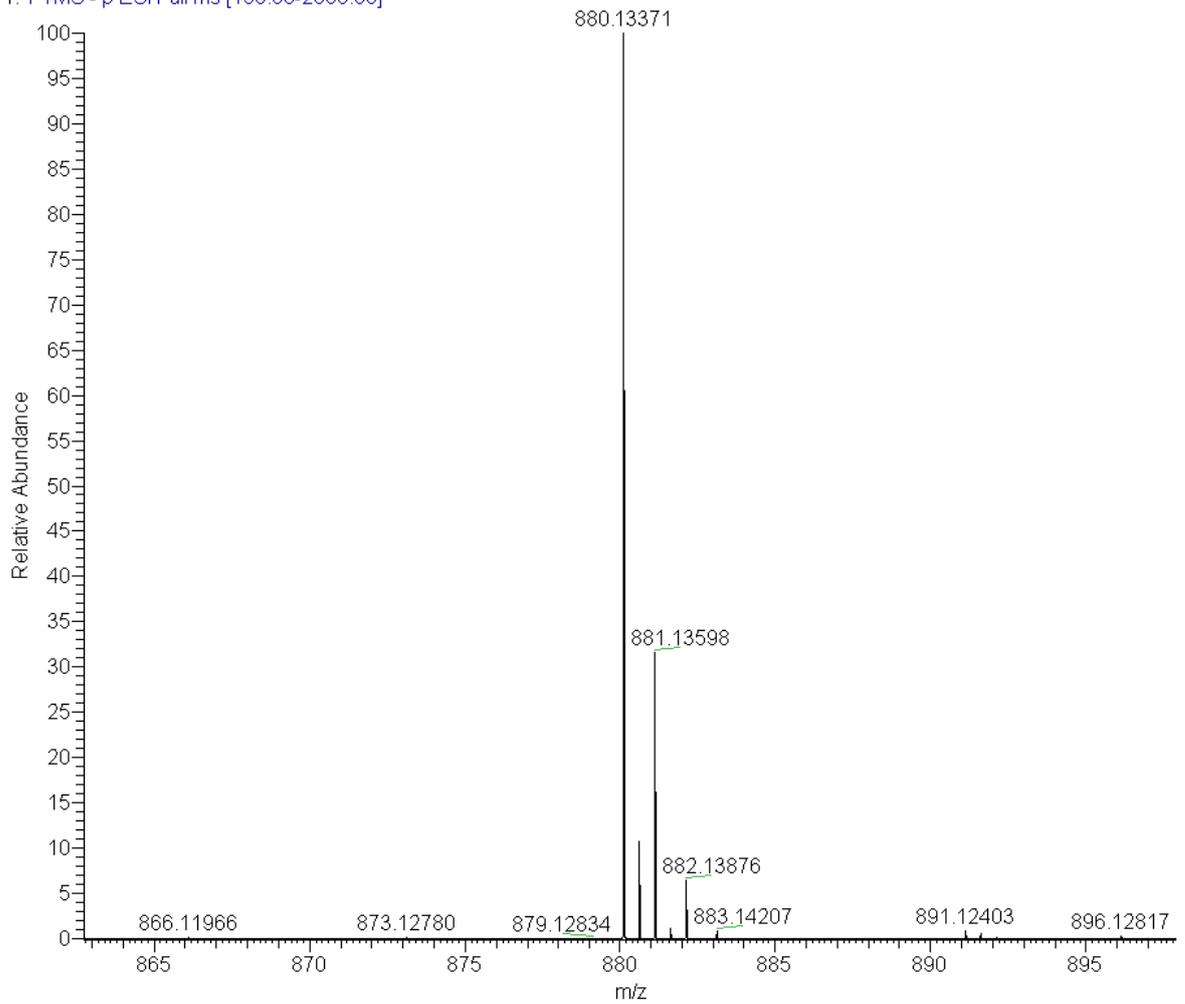


(1b)  $m^7G$ -triazole- $C_2H_4pppG$

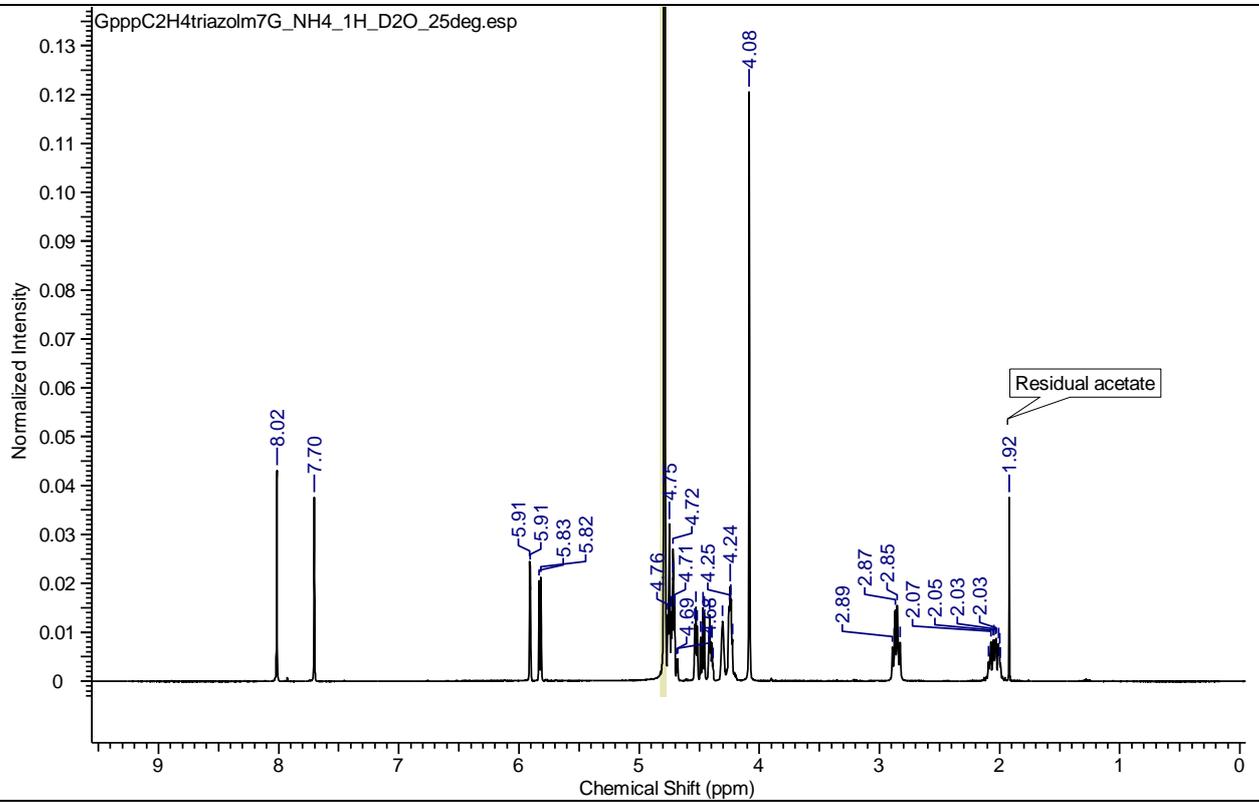


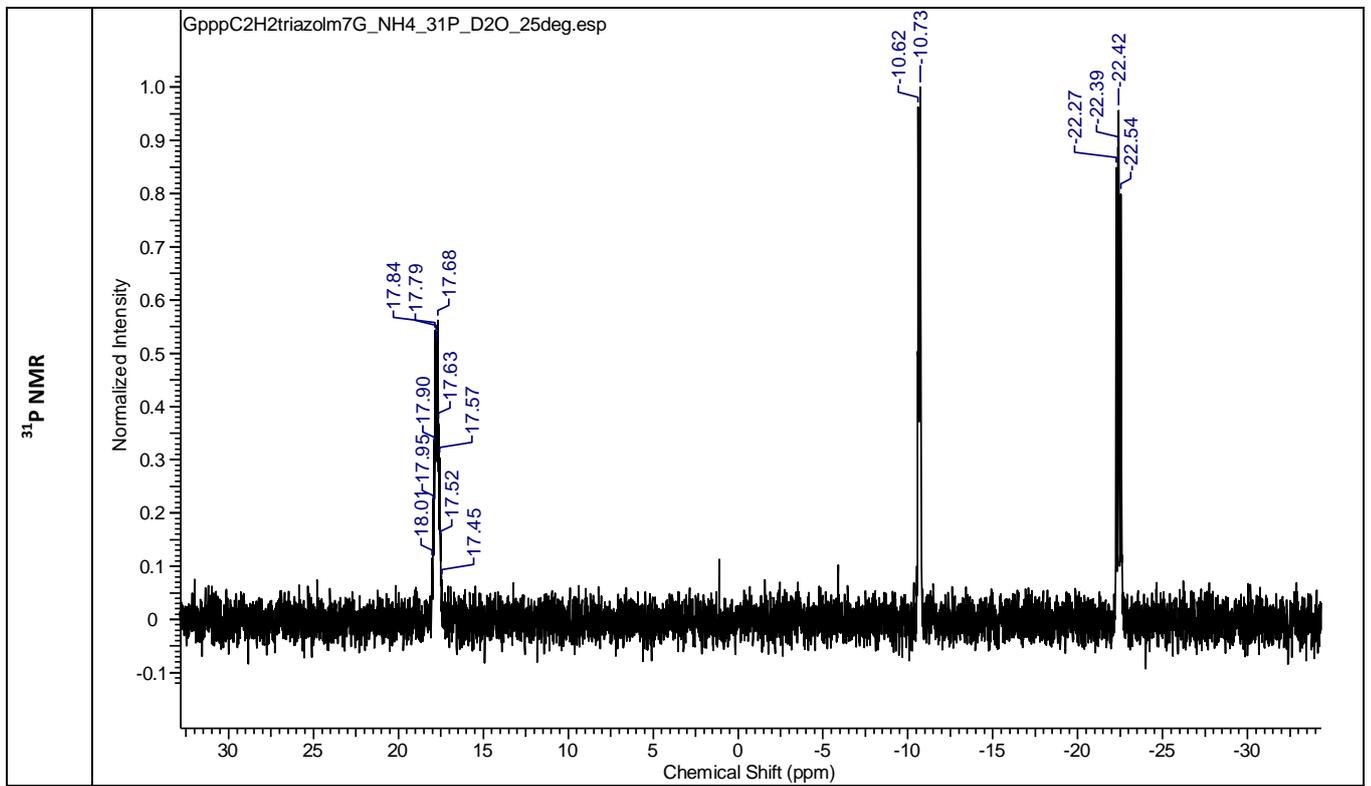
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HRMS

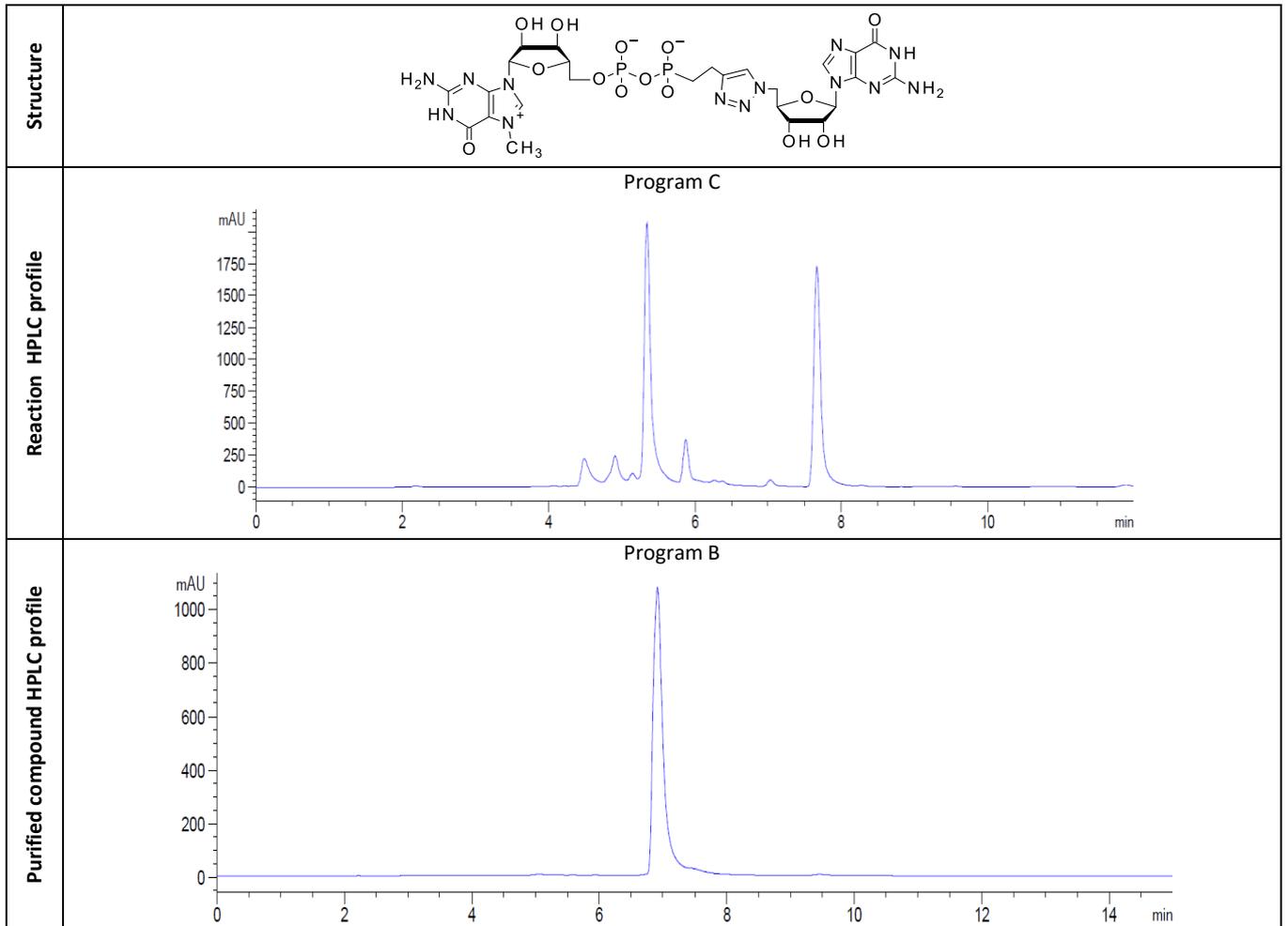


<sup>1</sup>H NMR



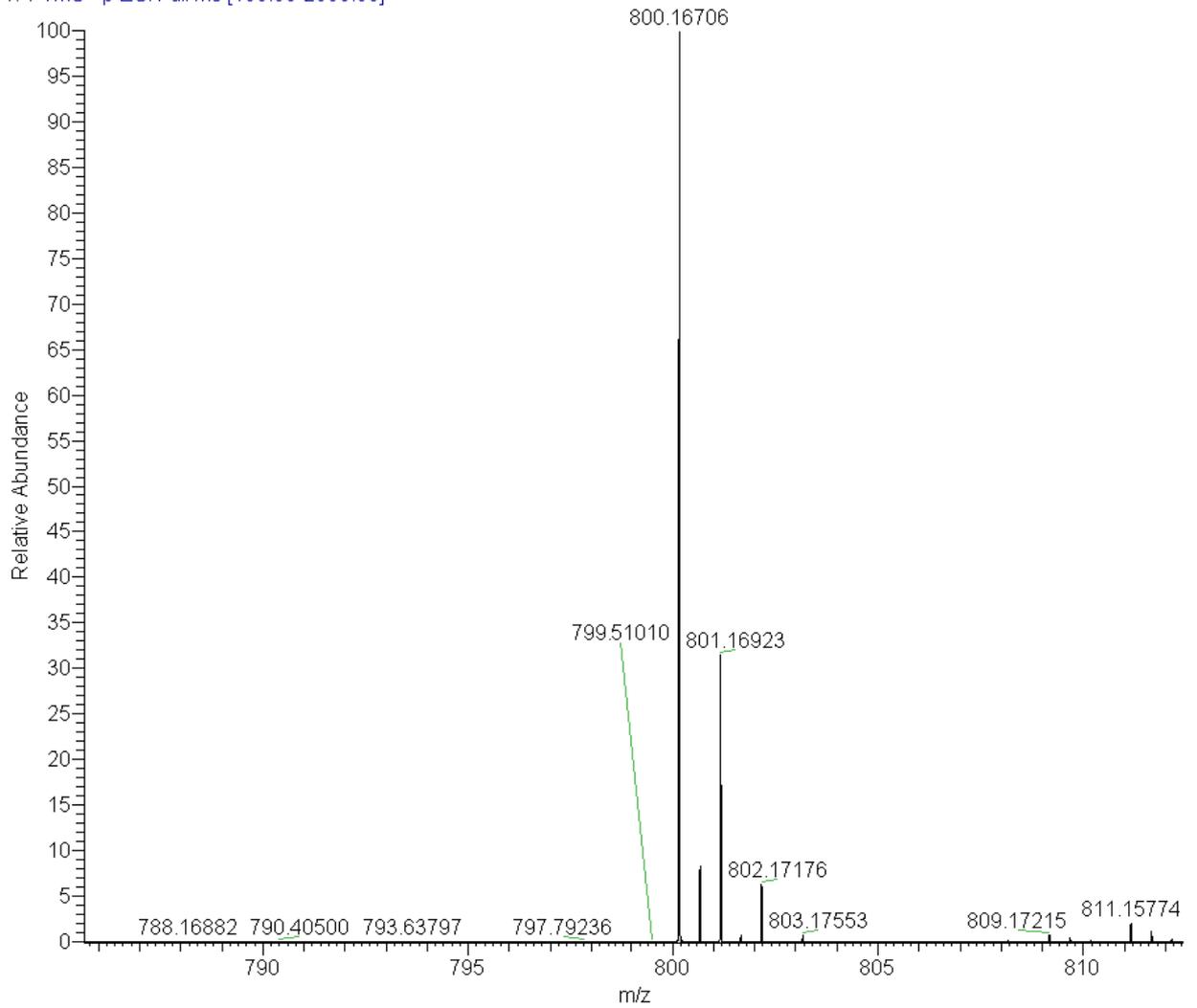


**(1c) m<sup>7</sup>GppC<sub>2</sub>H<sub>4</sub>-triazole-G**

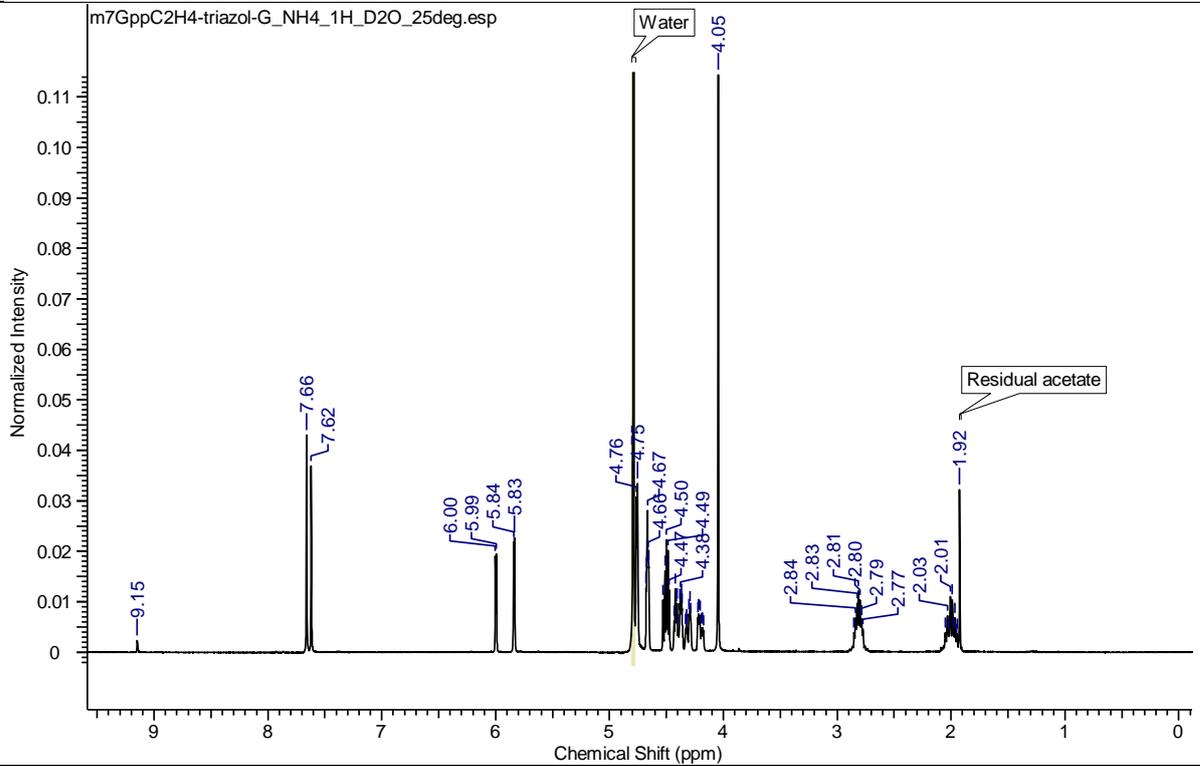


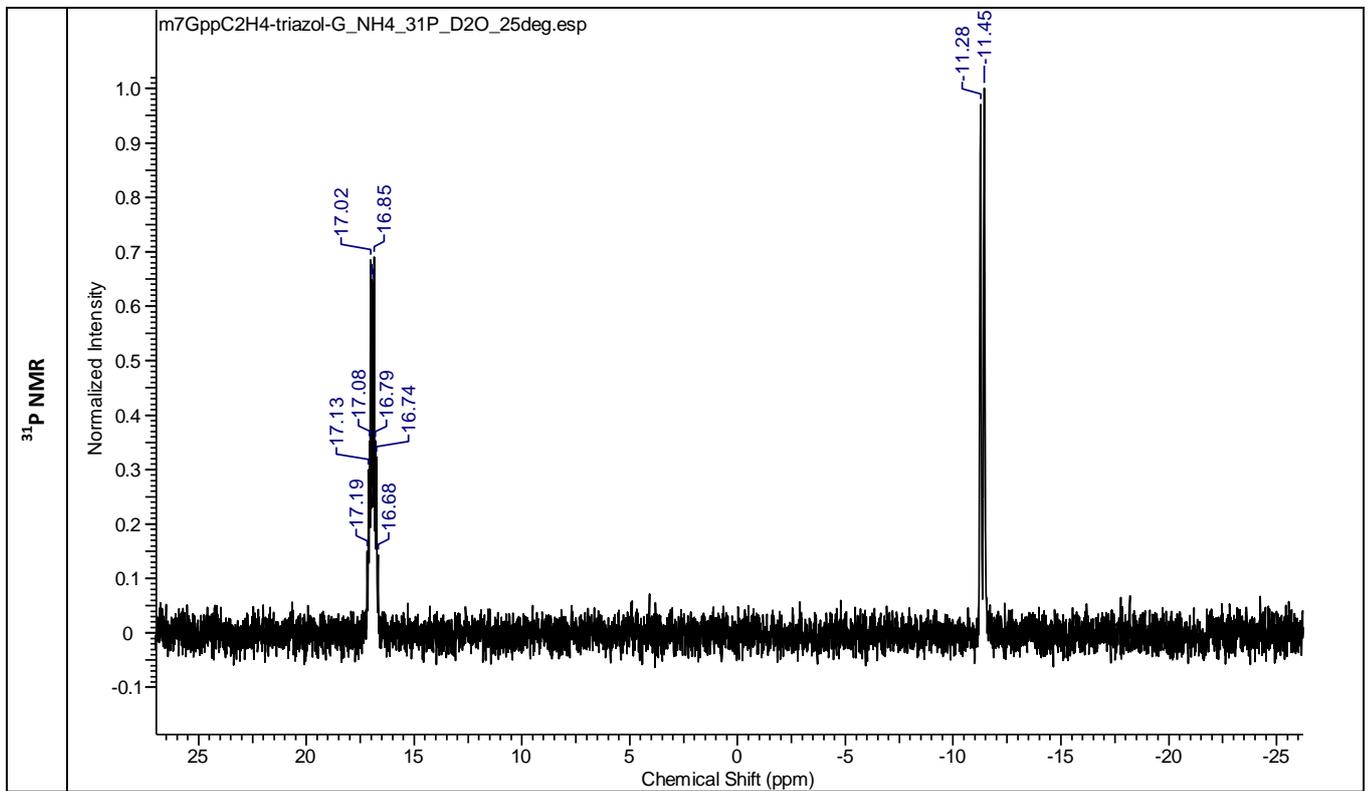
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HRMS

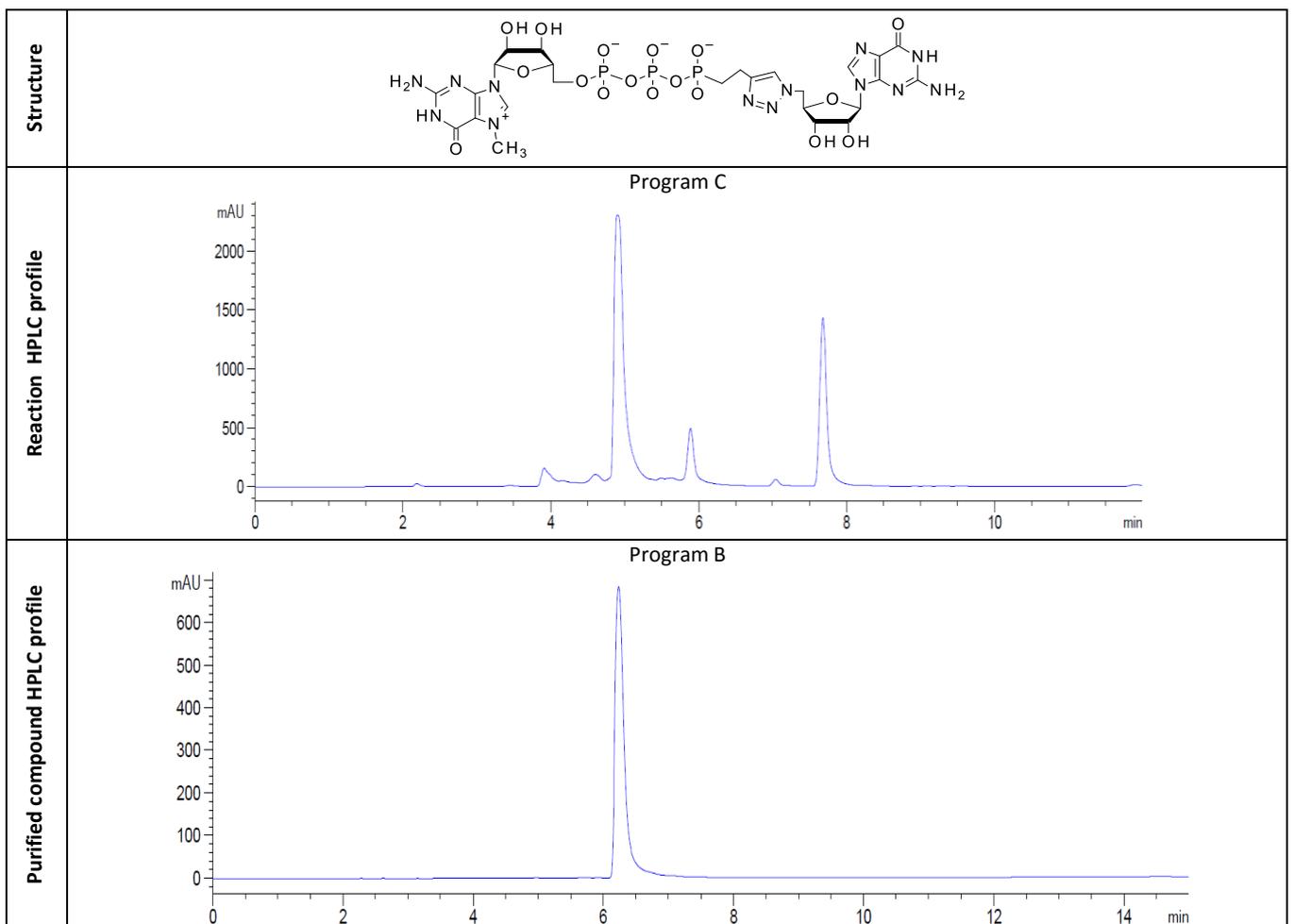


<sup>1</sup>H NMR



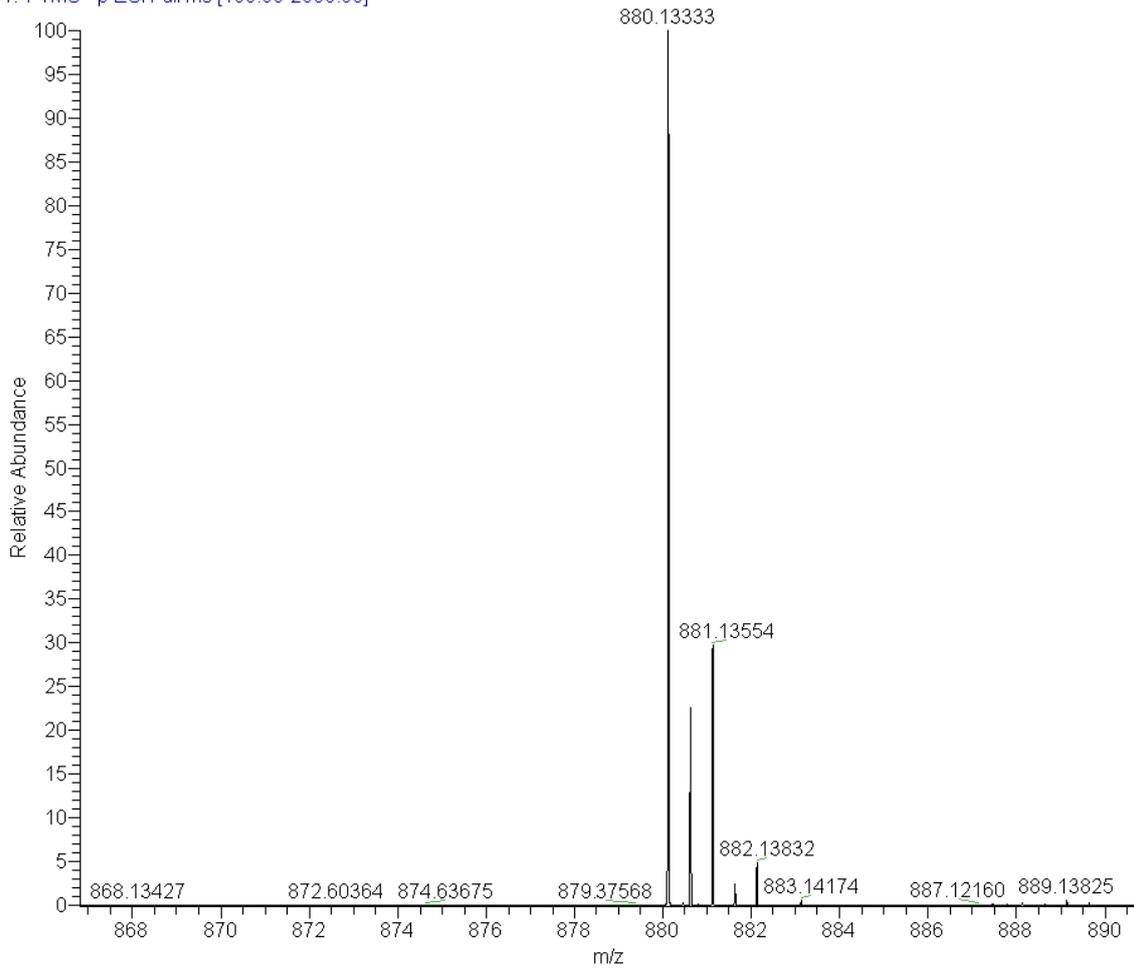


(1d) m<sup>7</sup>GpppC<sub>2</sub>H<sub>4</sub>-triazole-G

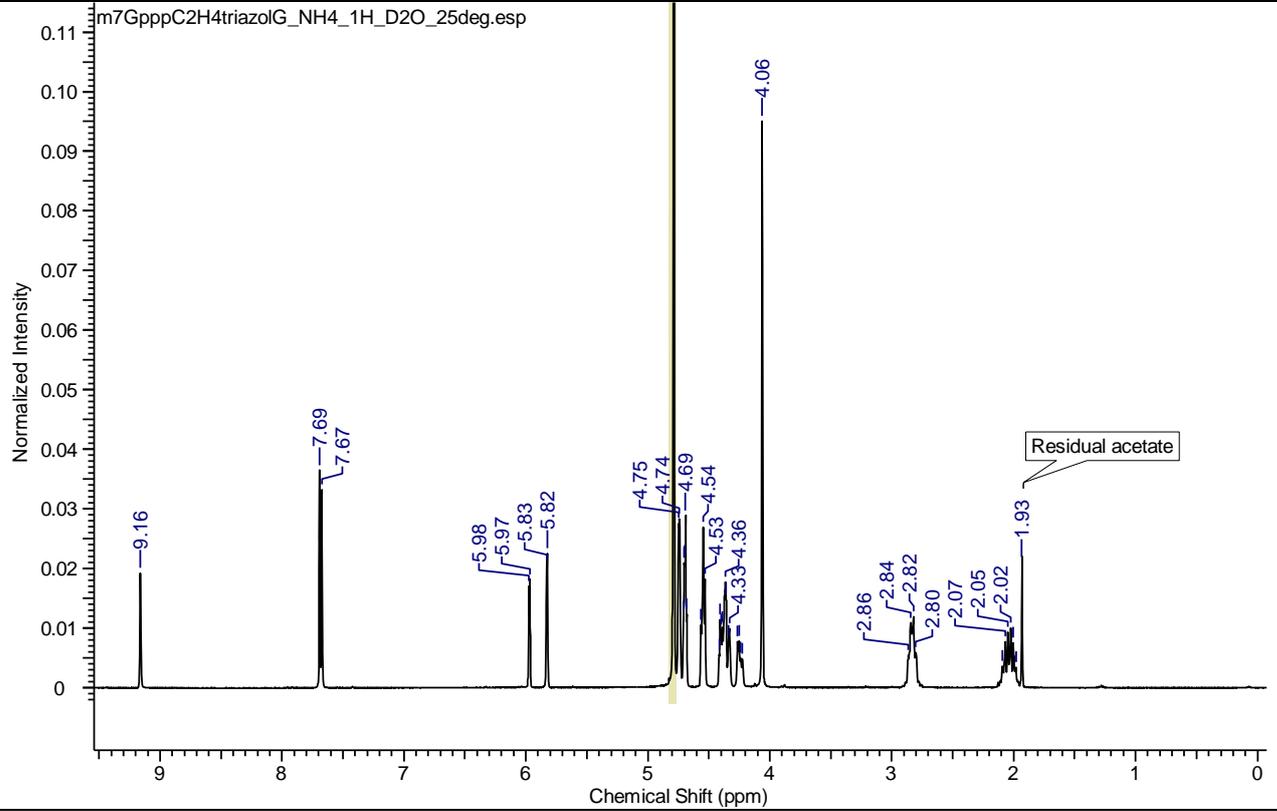


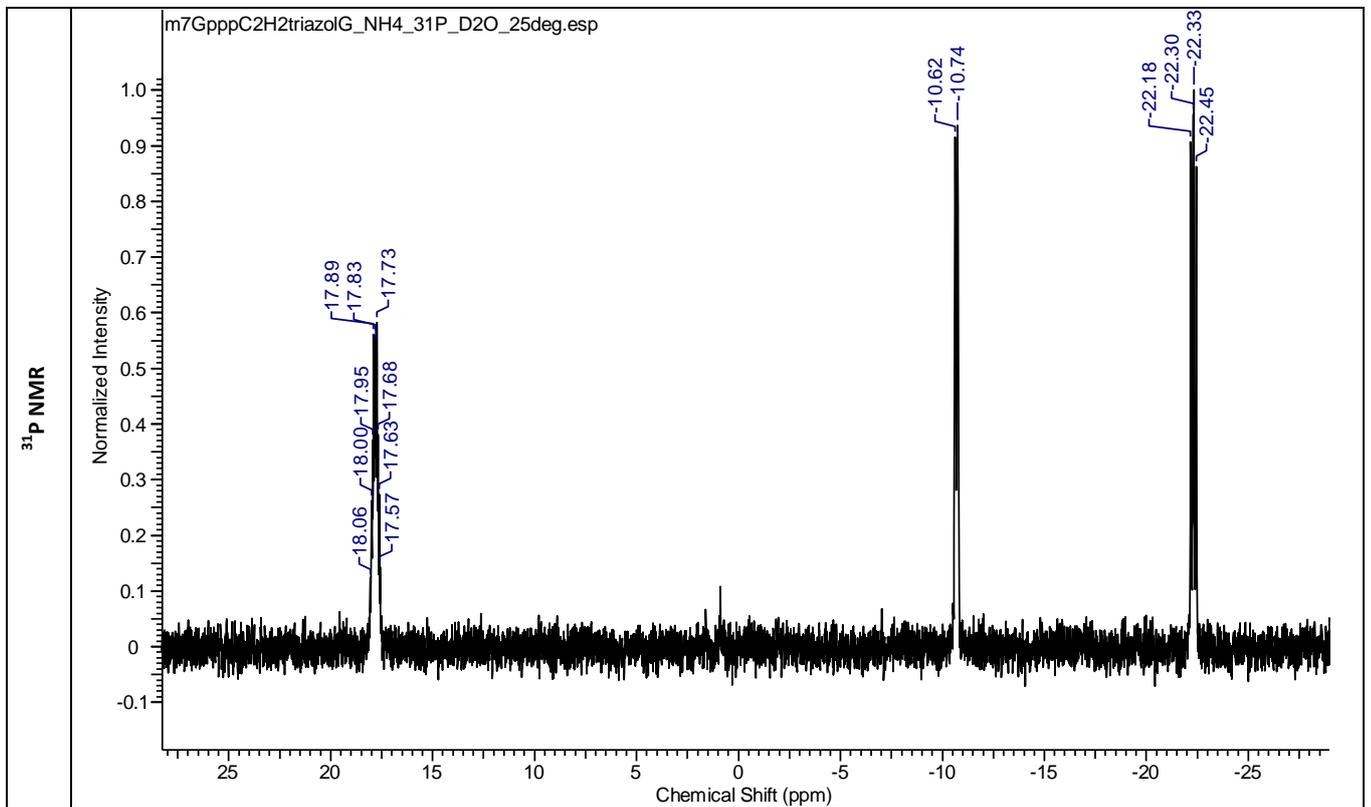
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HRMS

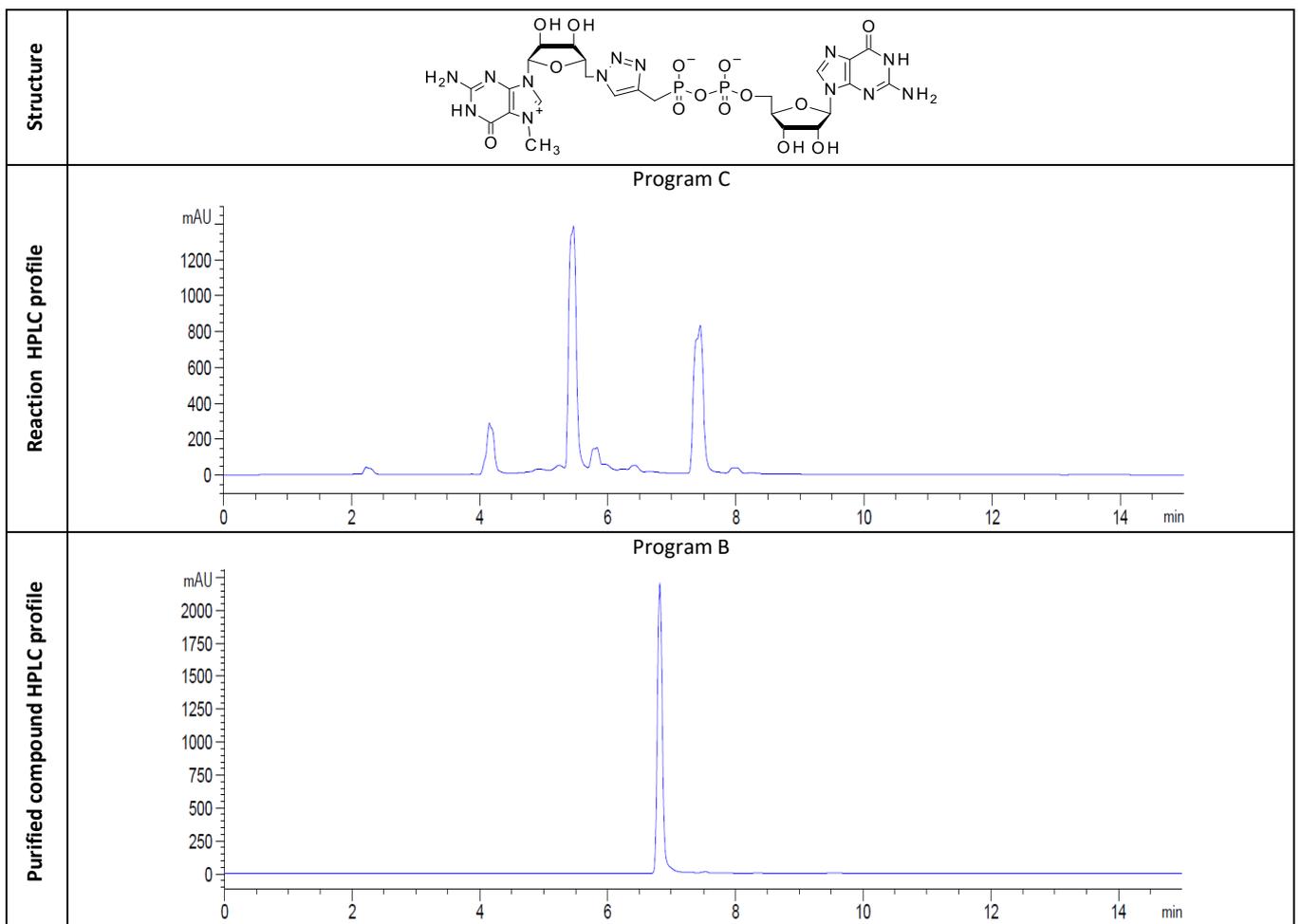


<sup>1</sup>H NMR



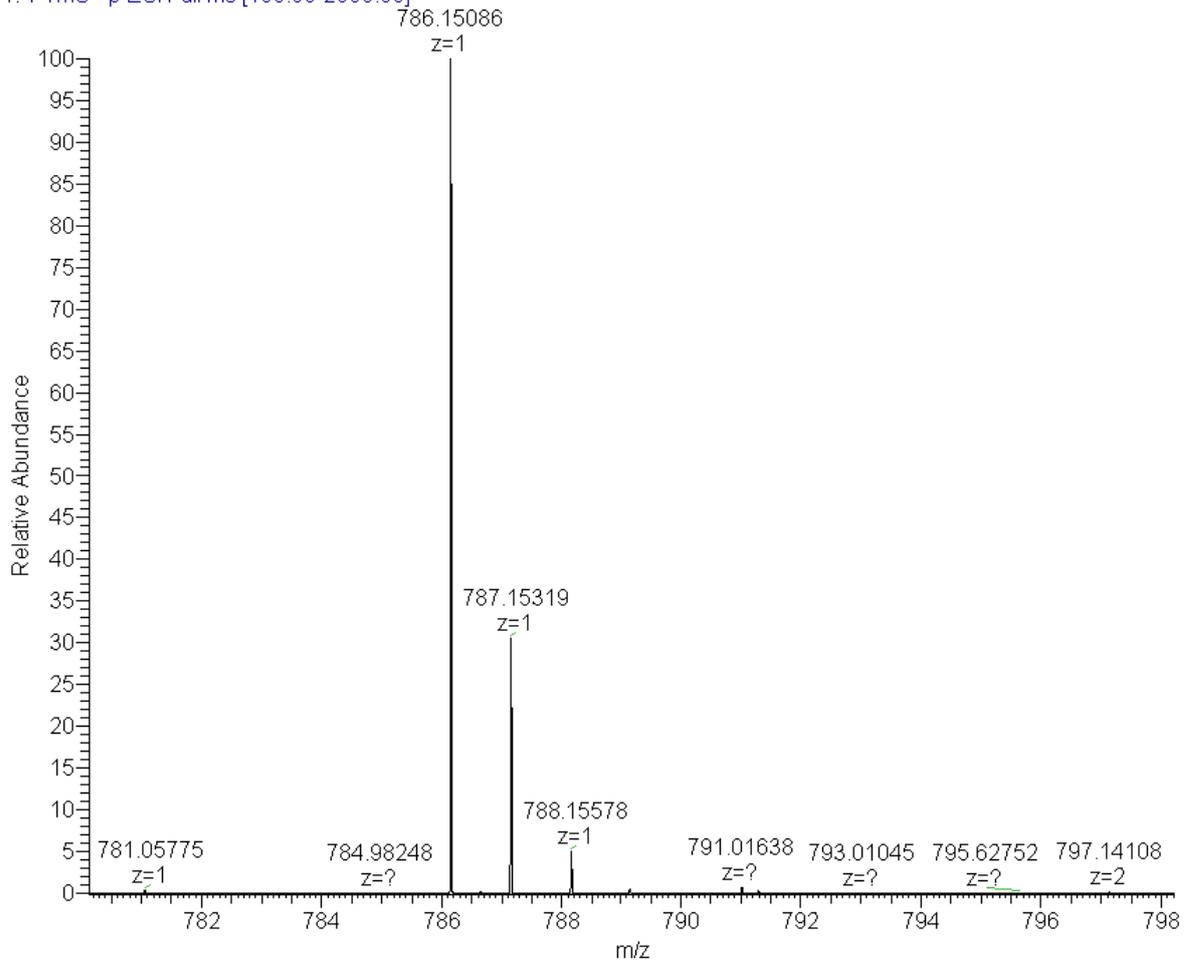


**(2a) m<sup>7</sup>G-triazole-CH<sub>2</sub>ppG**

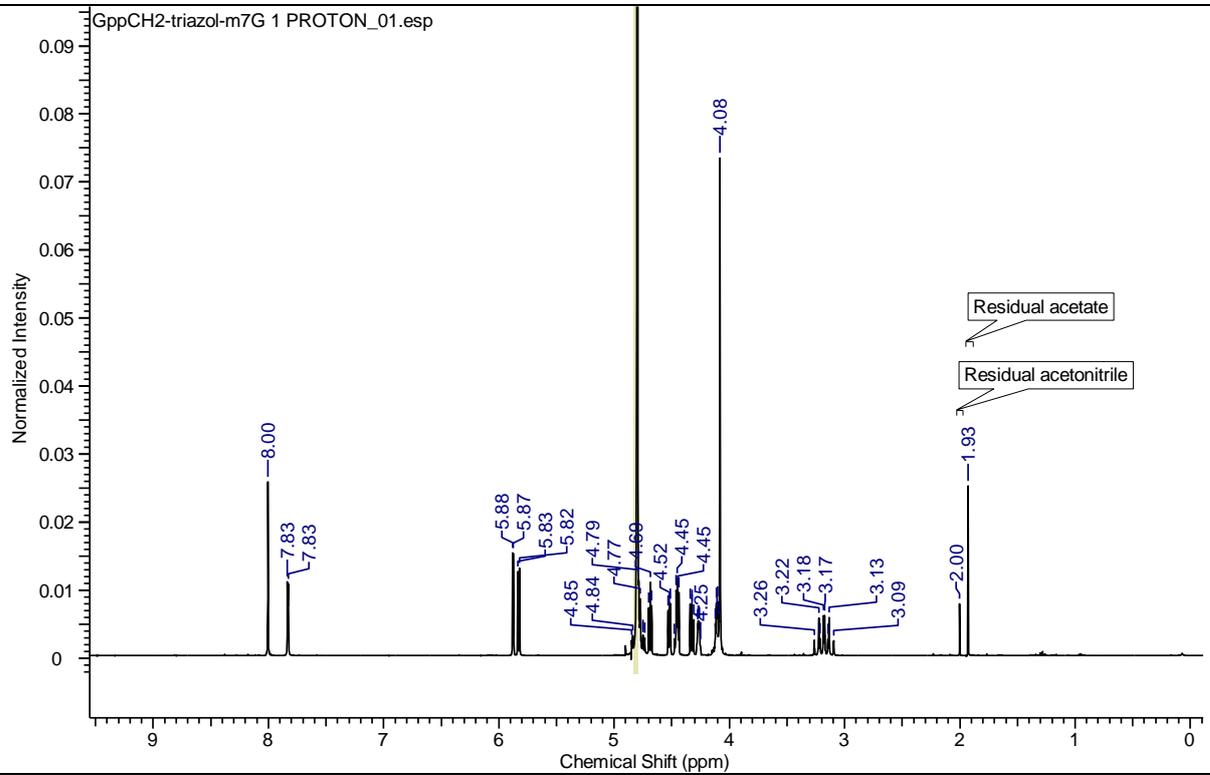


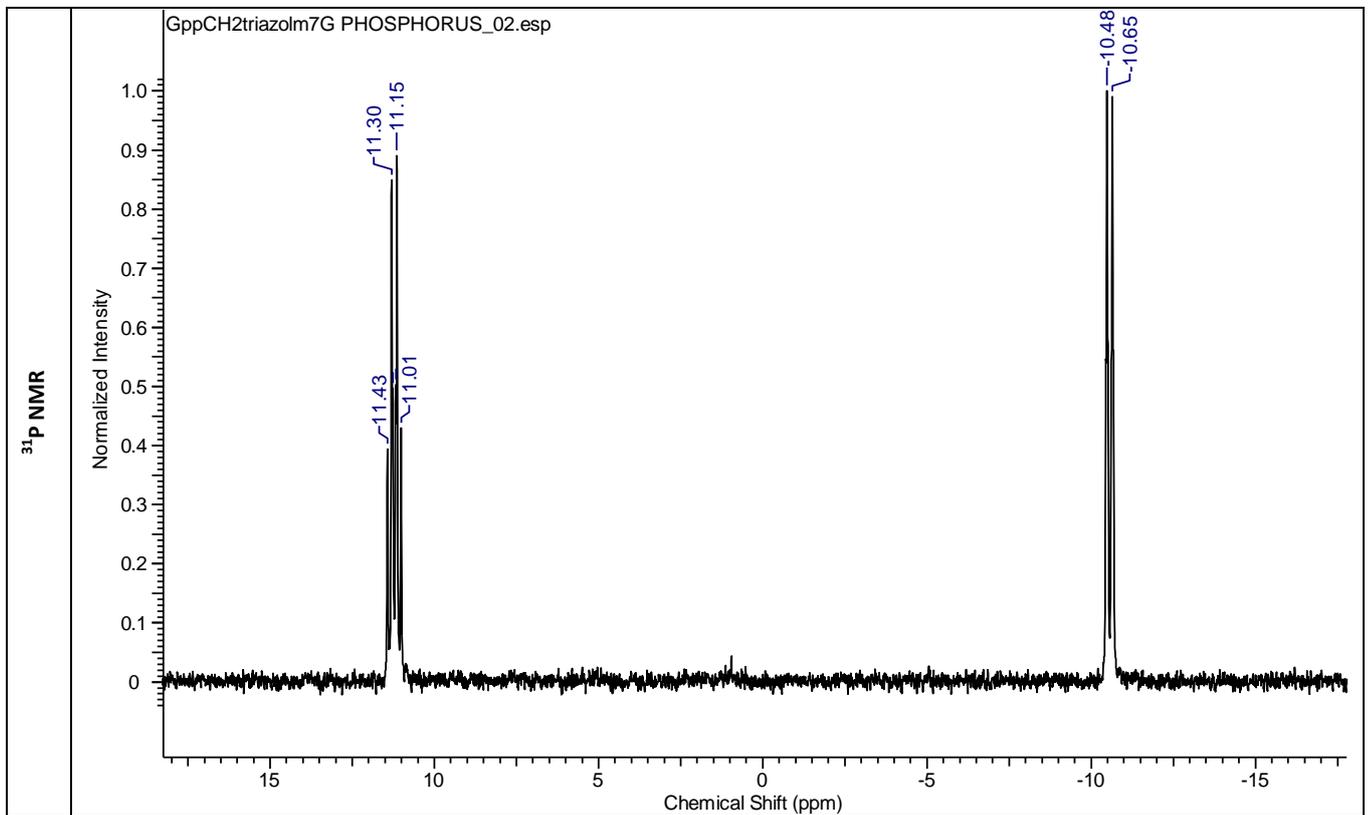
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HRMS

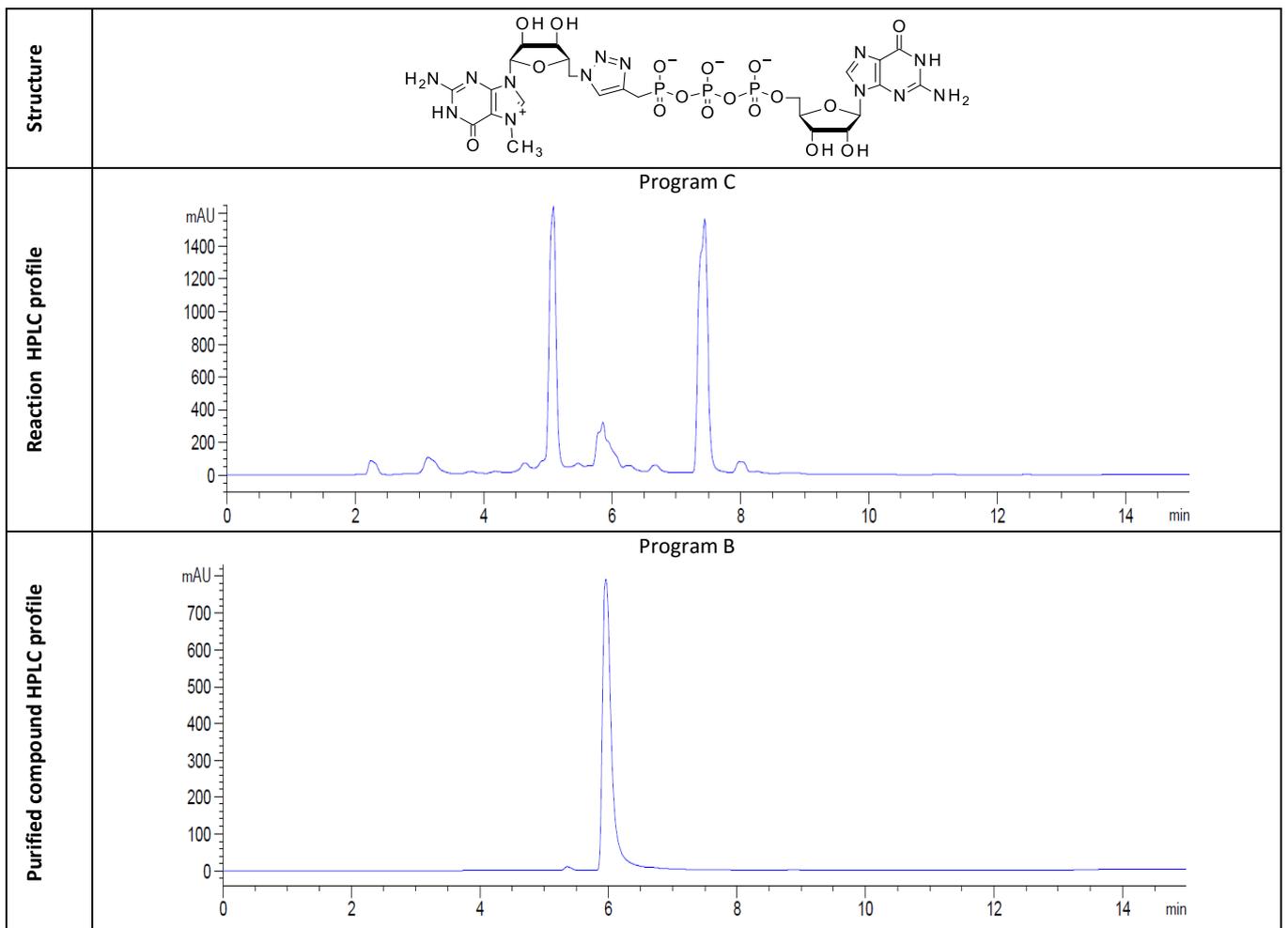


<sup>1</sup>H NMR



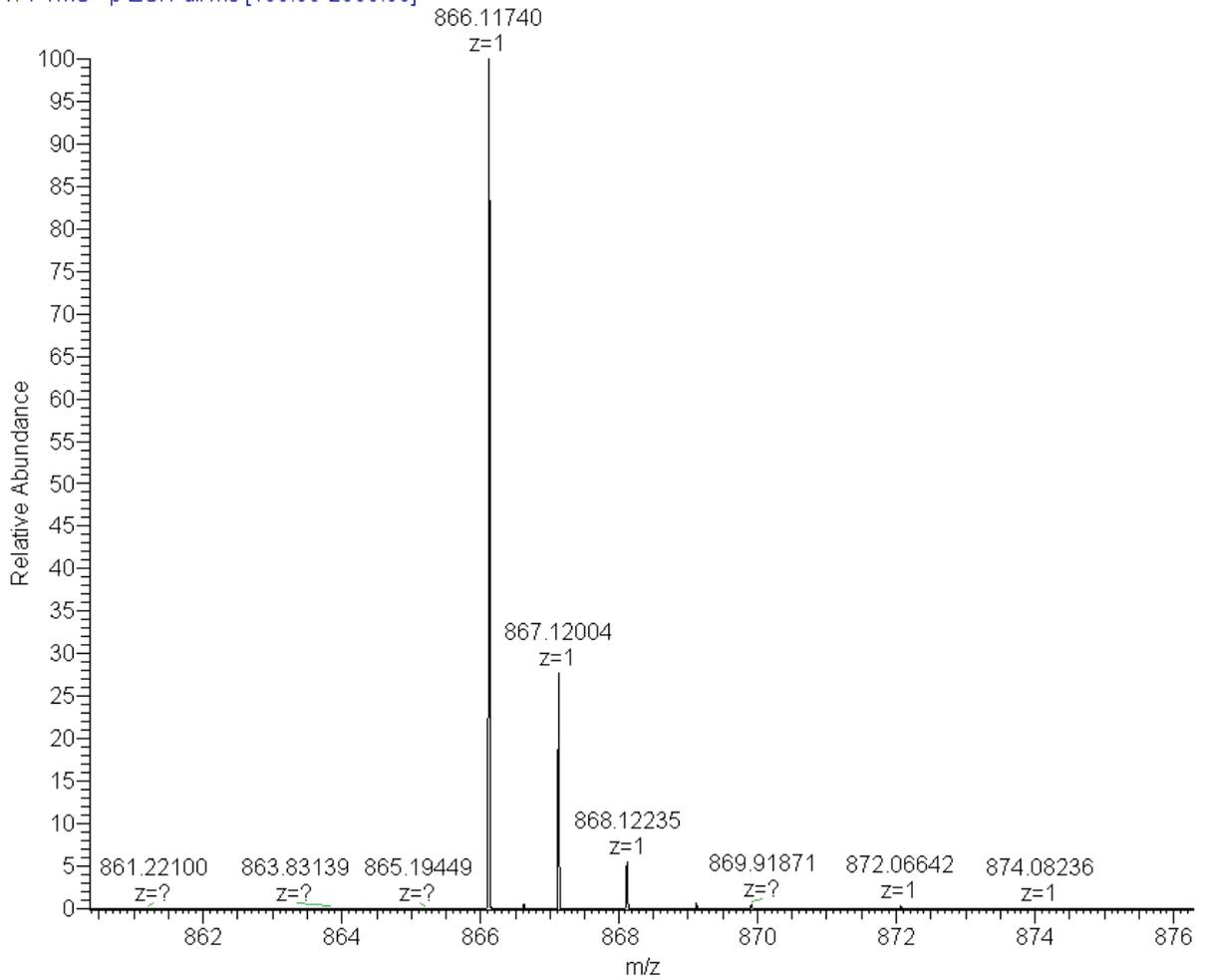


**(2b) m<sup>7</sup>G-triazole-CH<sub>2</sub>pppG**

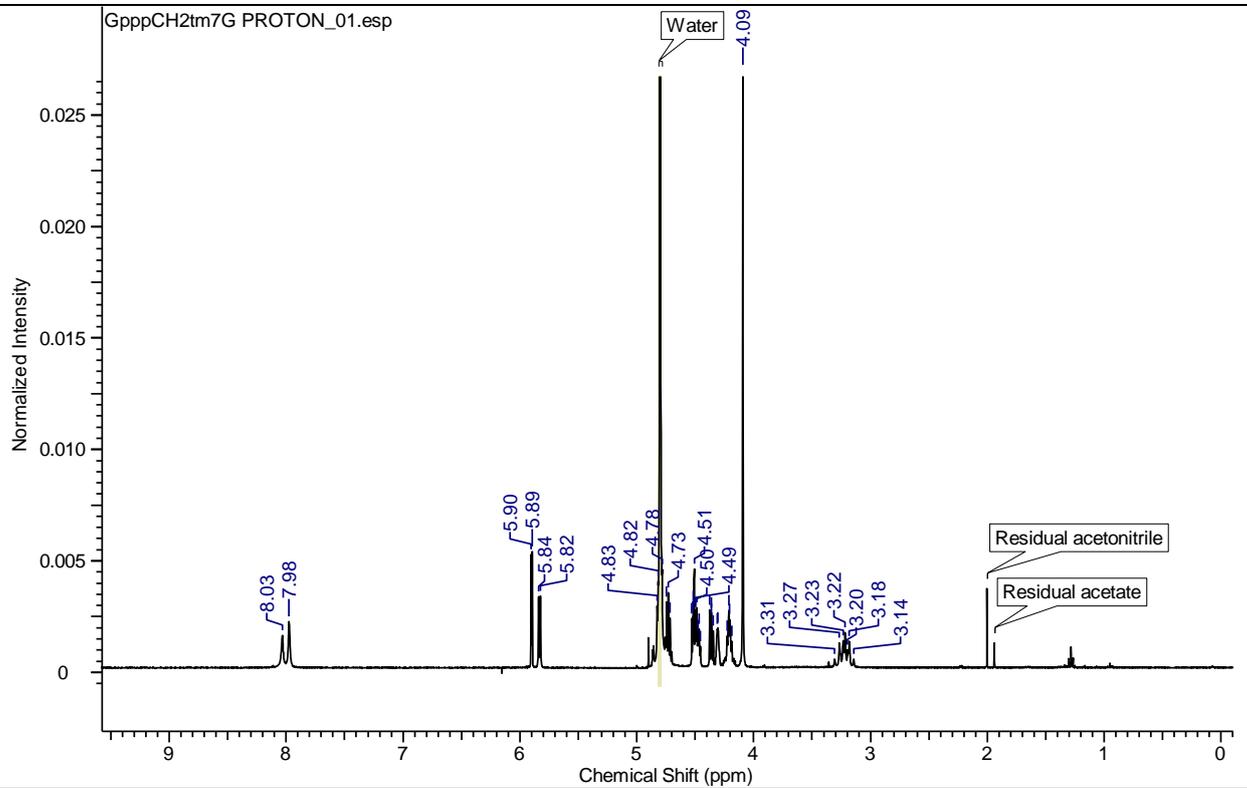


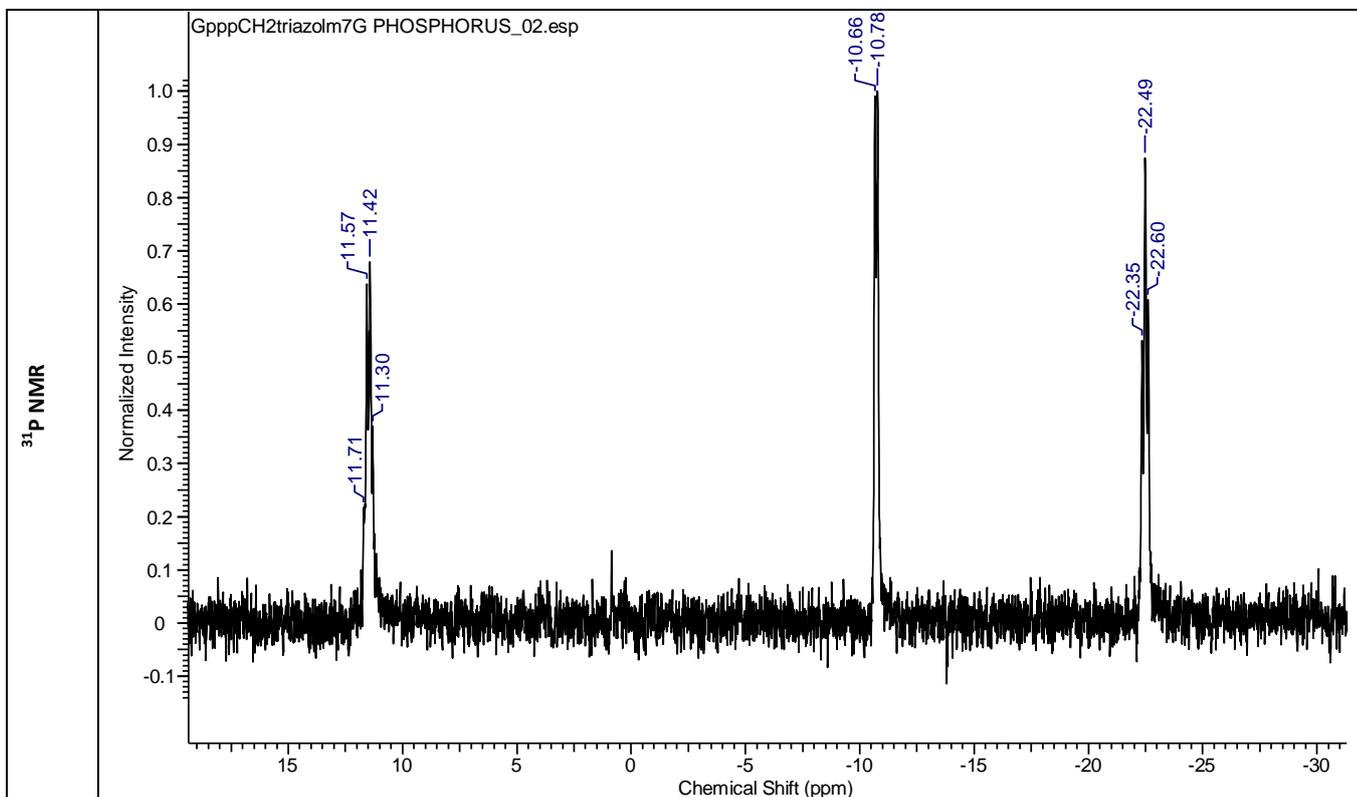
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HRMS

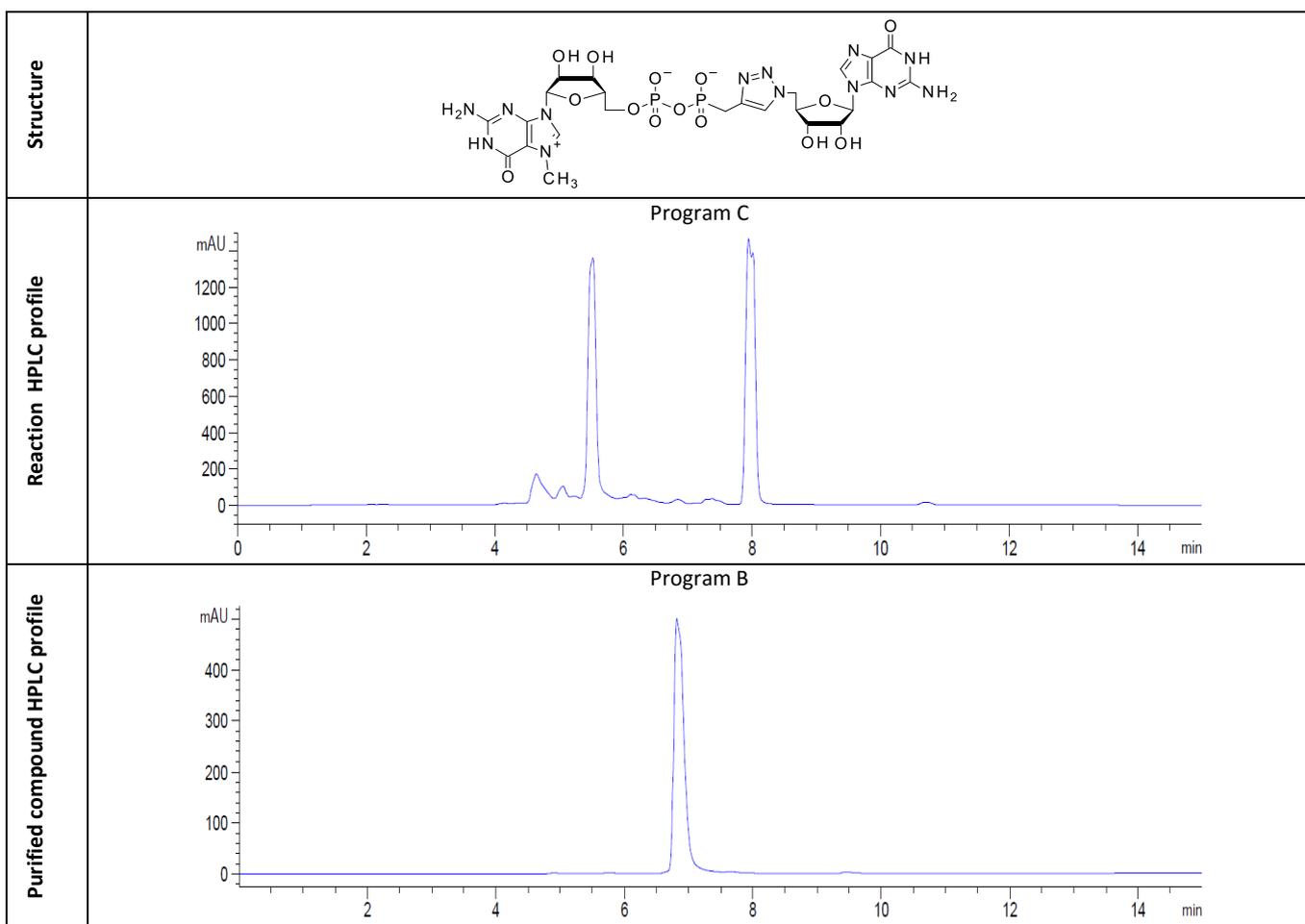


<sup>1</sup>H NMR



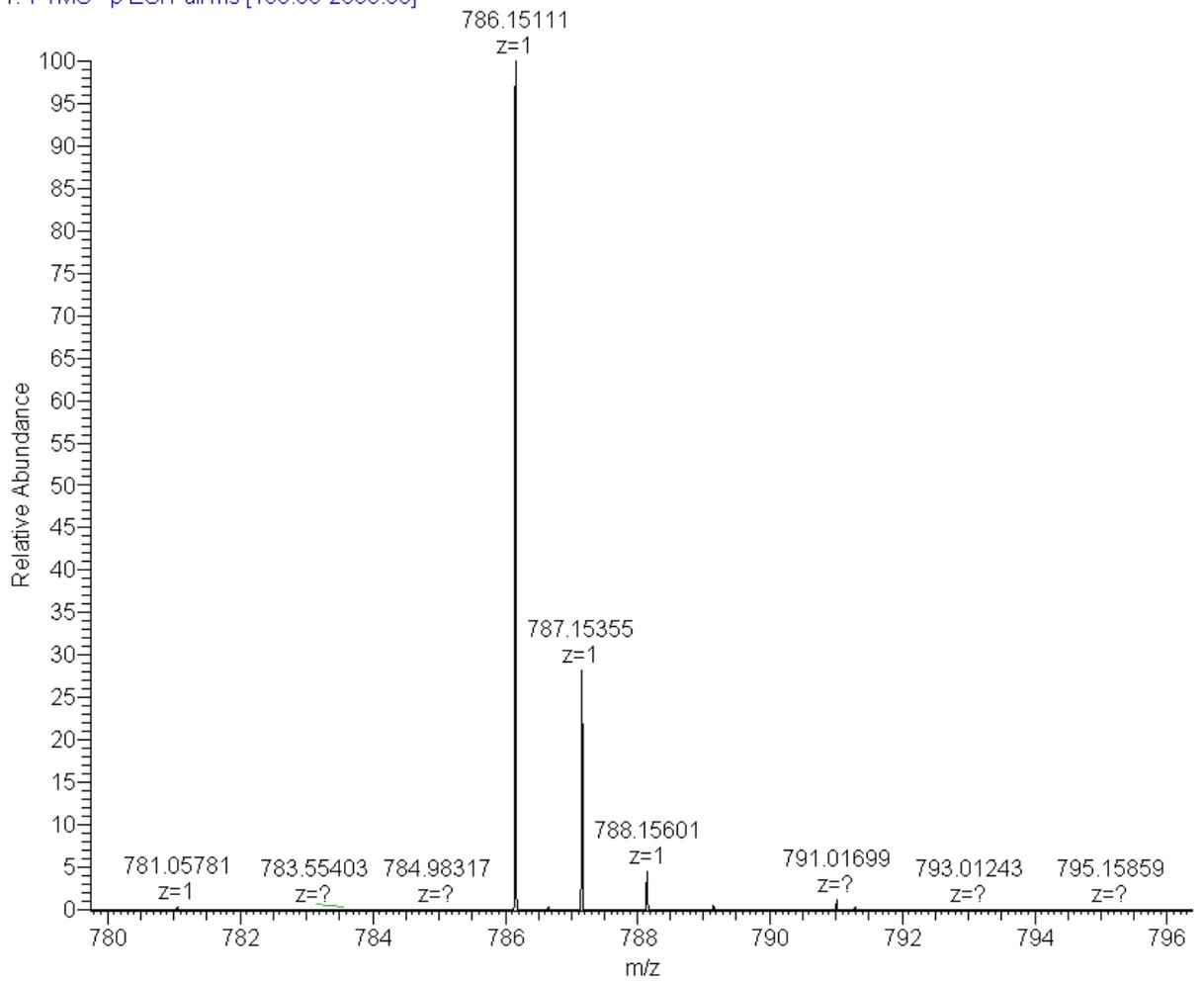


(2c) m<sup>7</sup>GppCH<sub>2</sub>-triazole-G

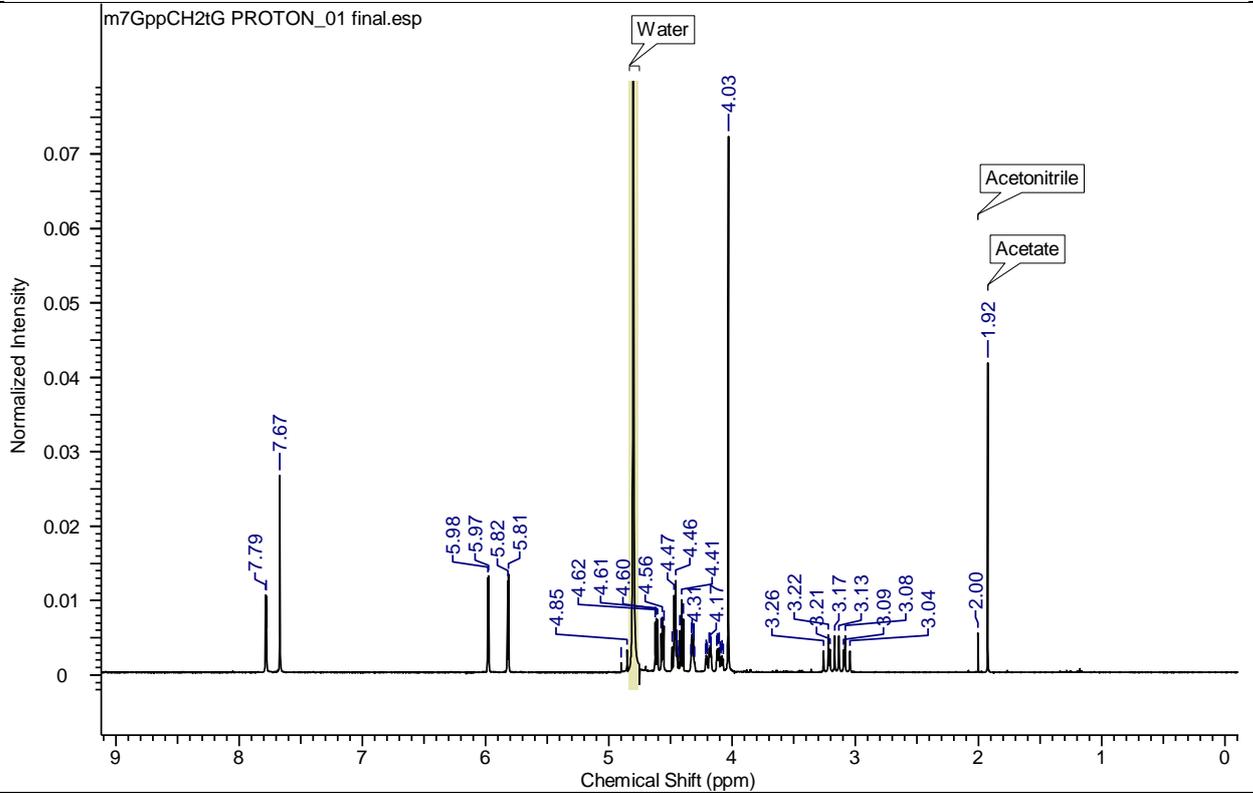


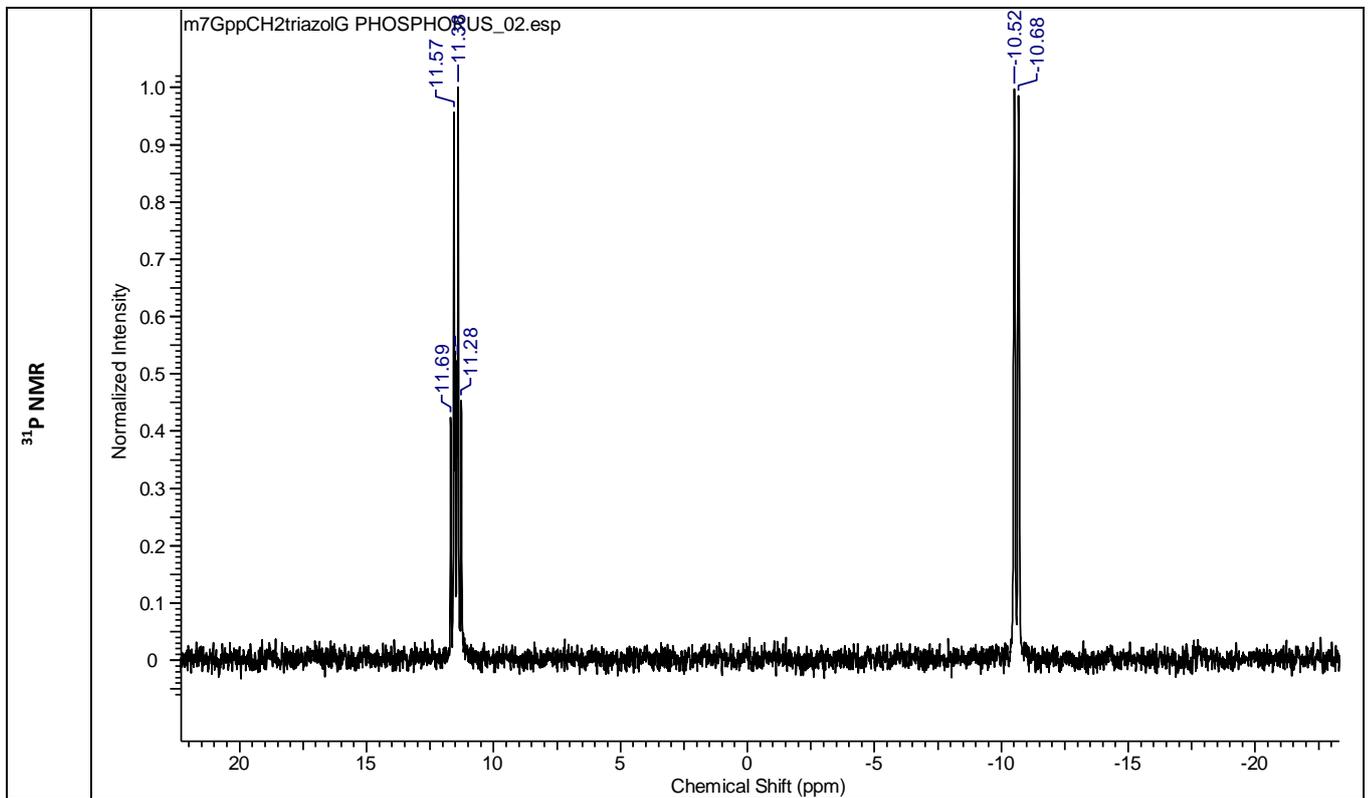
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HRMS

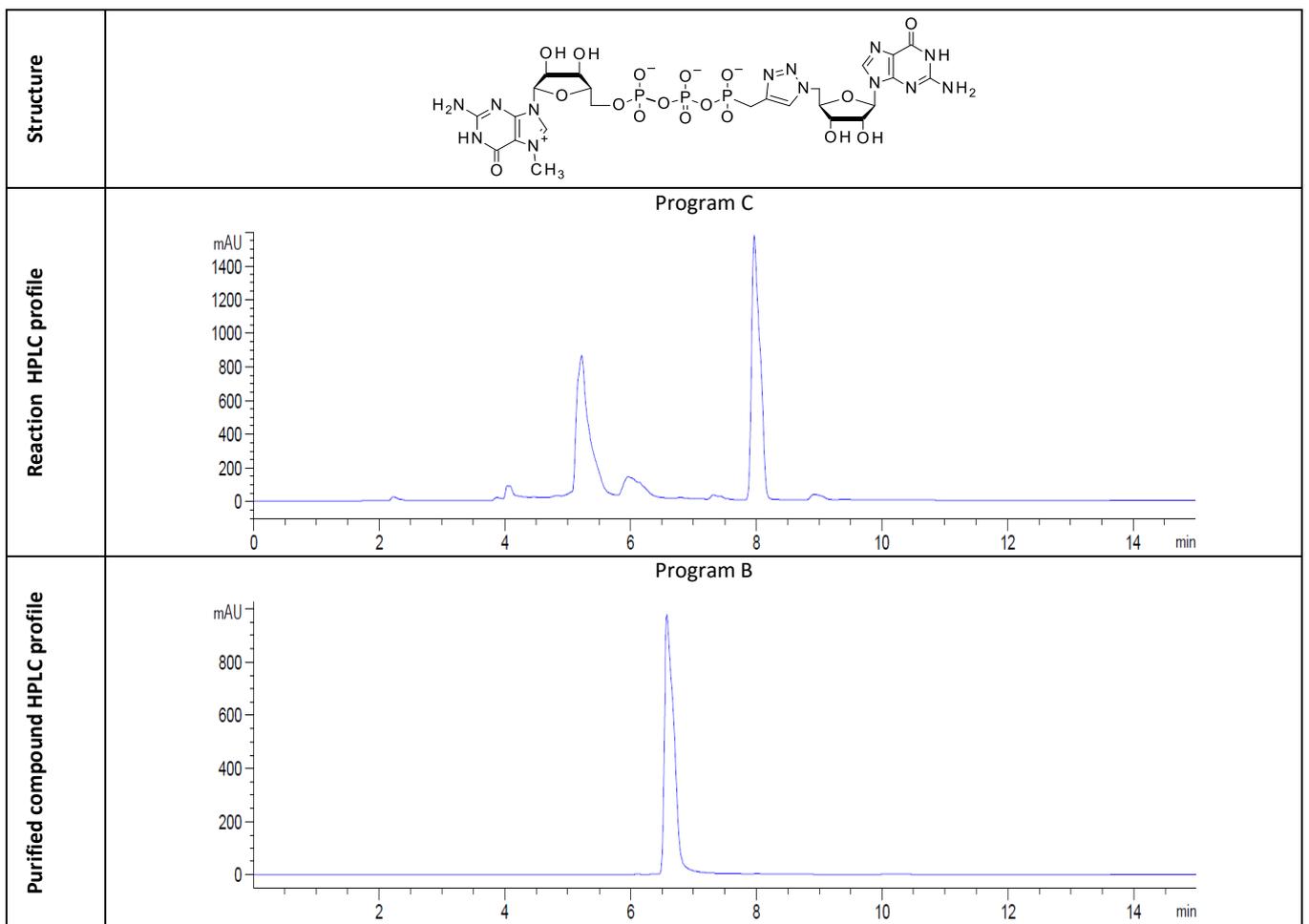


<sup>1</sup>H NMR



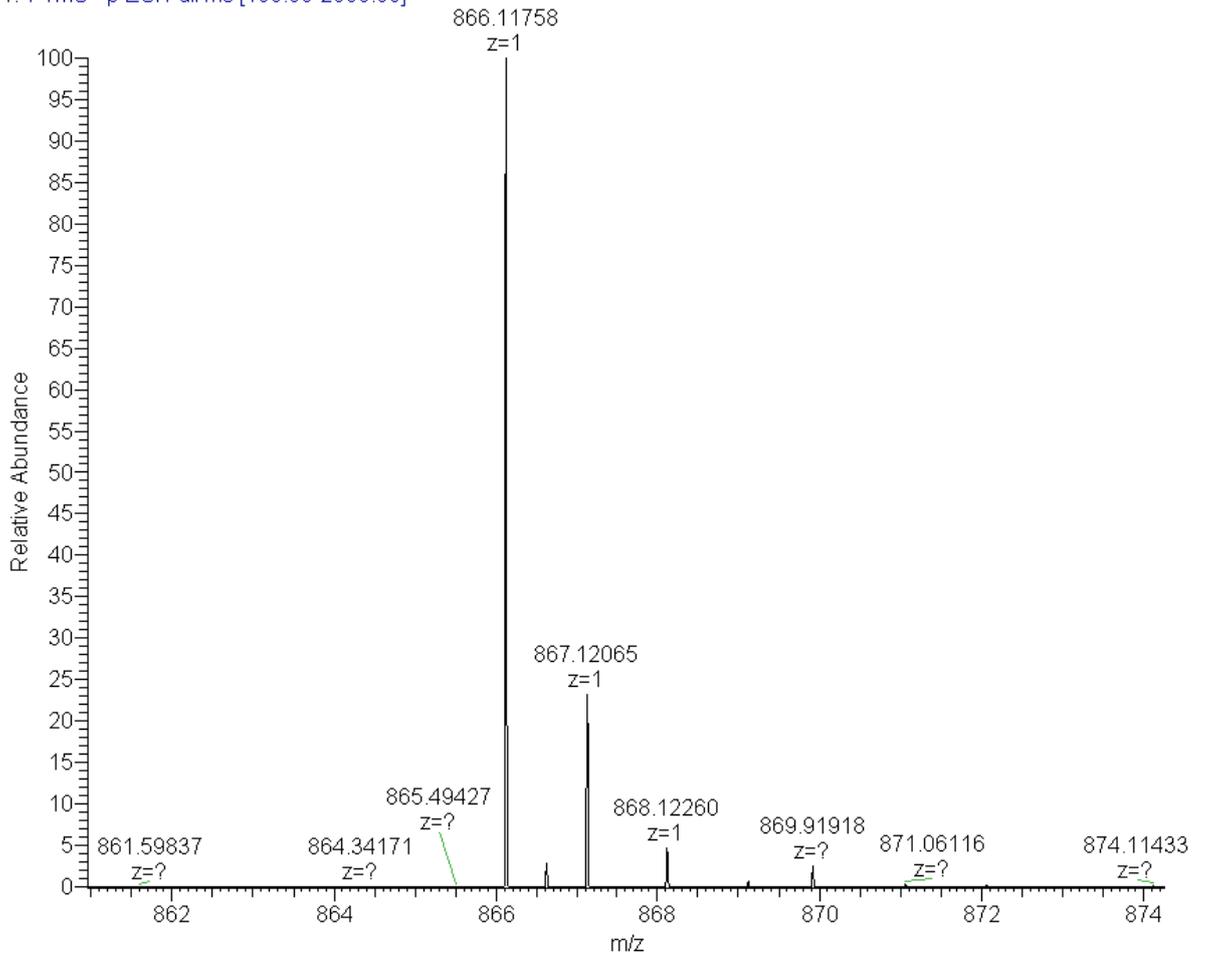


**(2d) m<sup>7</sup>GpppCH<sub>2</sub>-triazole-G**

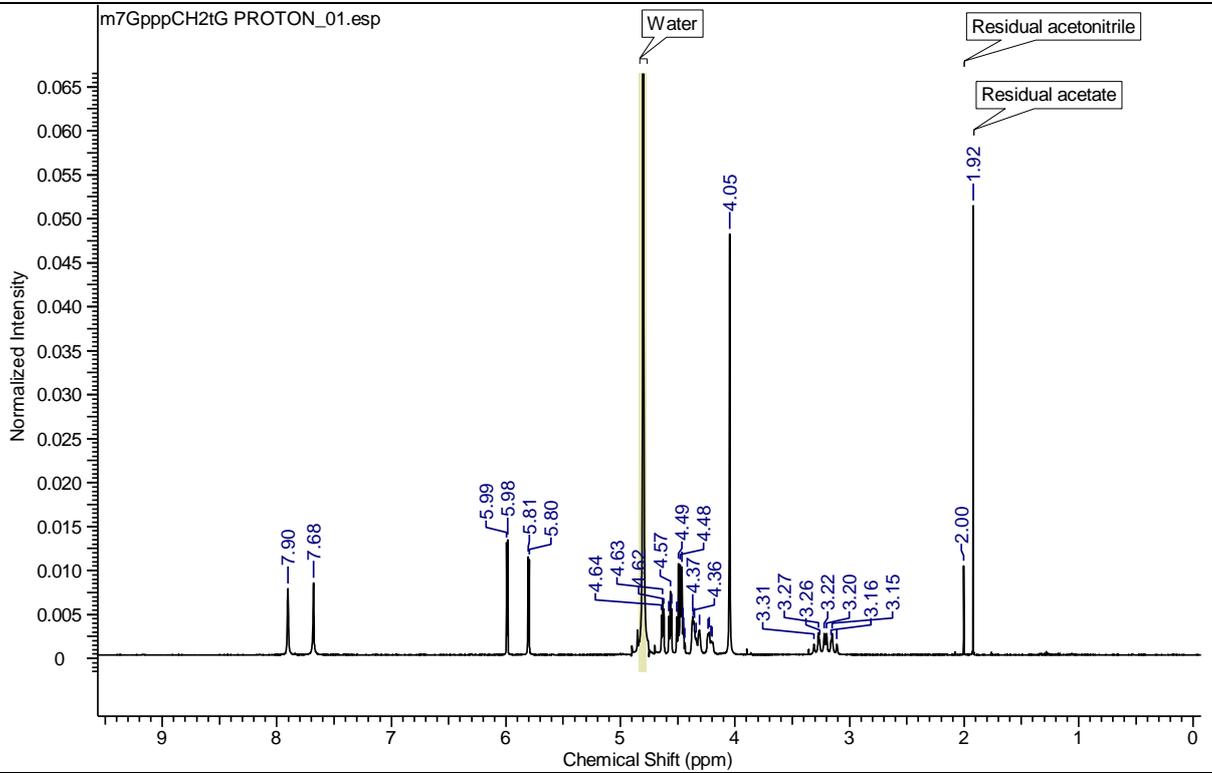


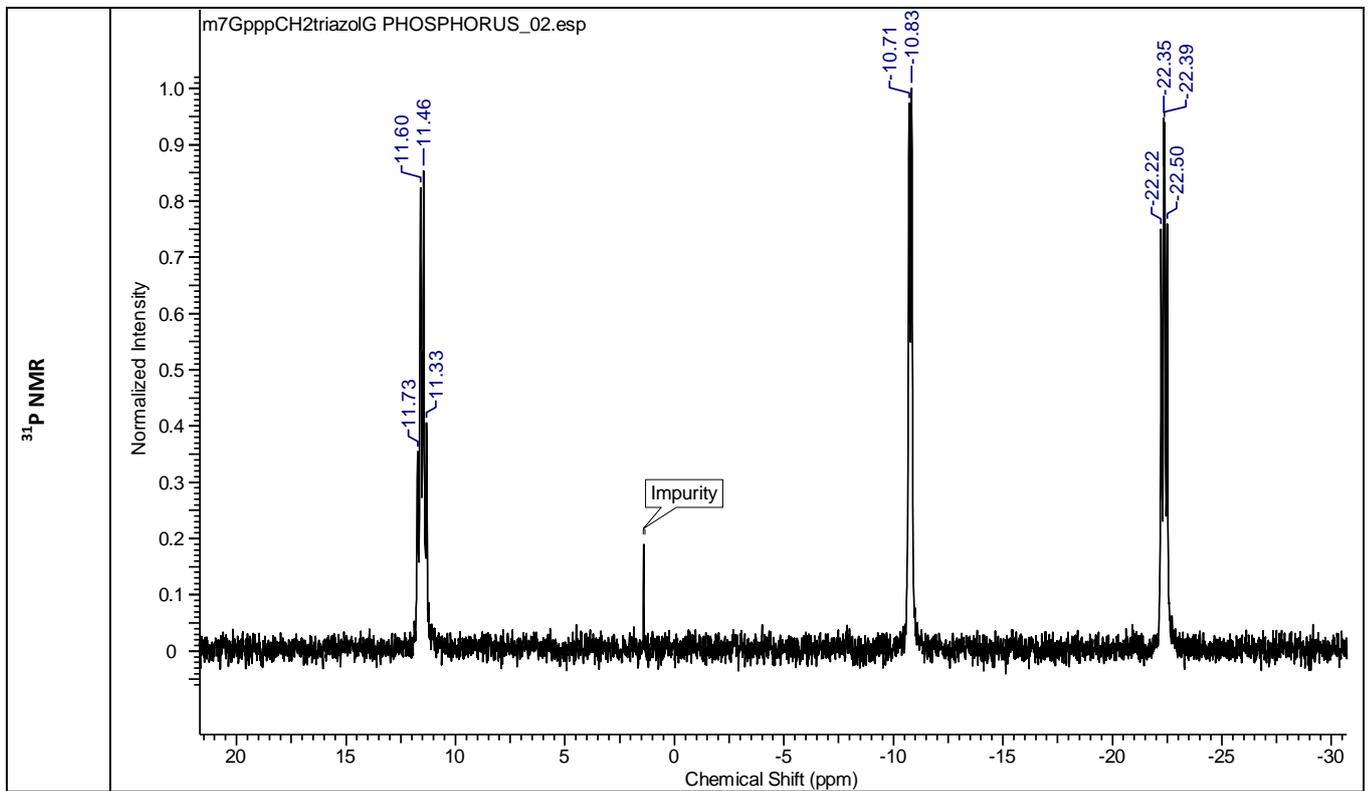
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HRMS

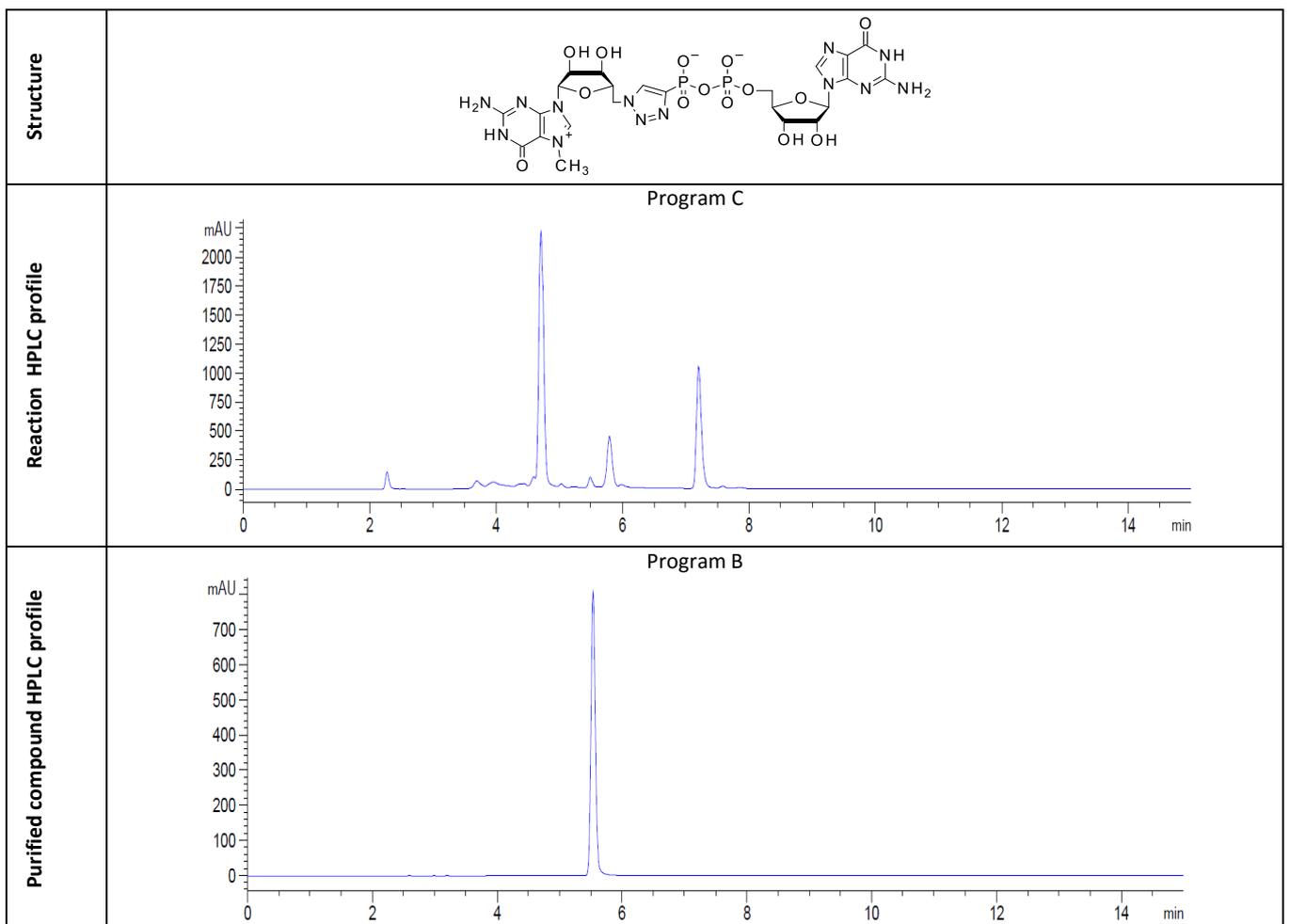


<sup>1</sup>H NMR



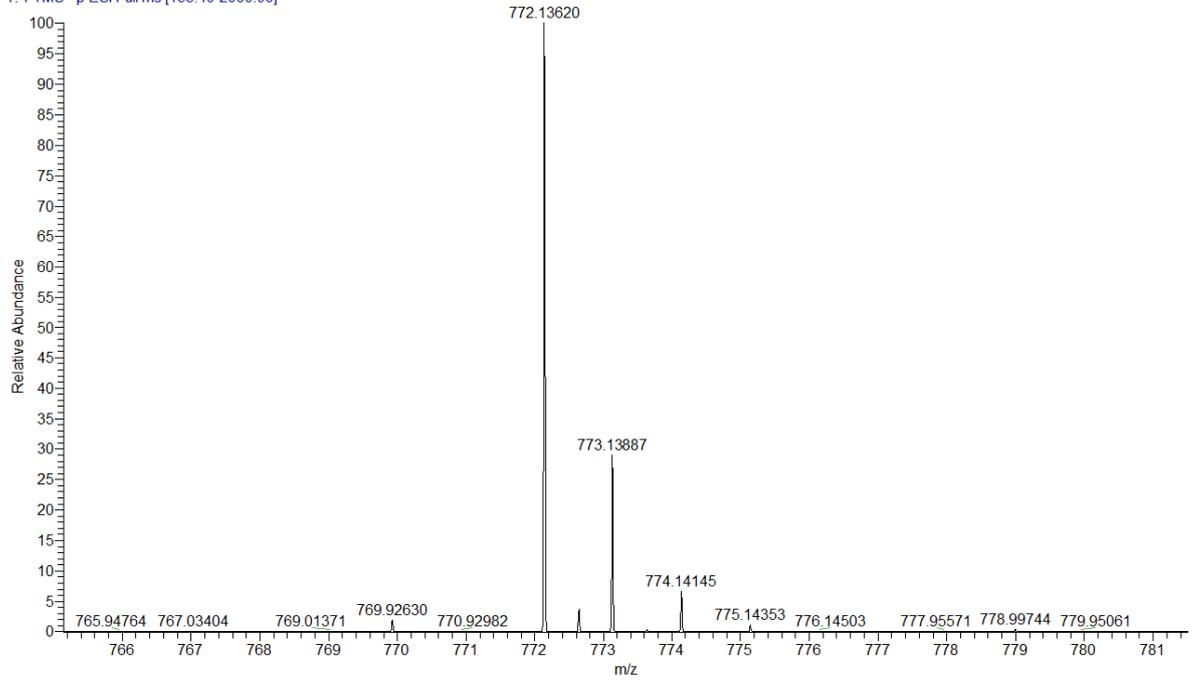


(3a) m<sup>7</sup>G-triazole-ppG

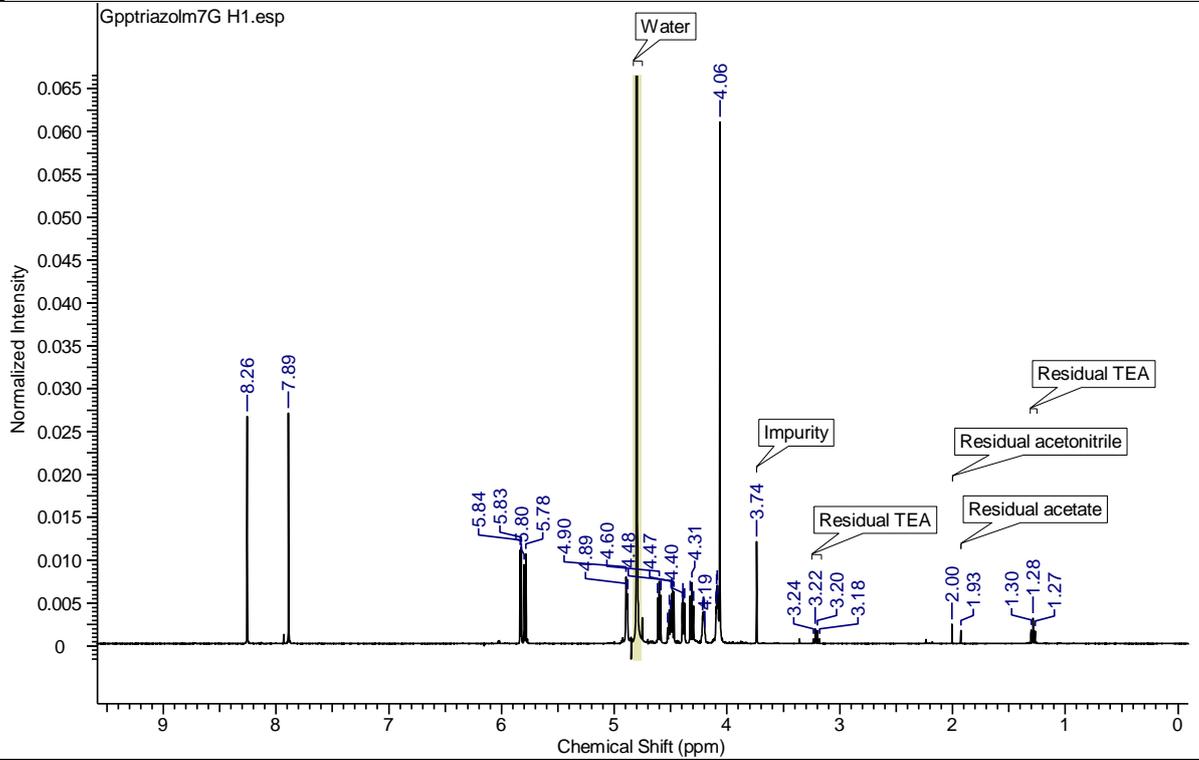


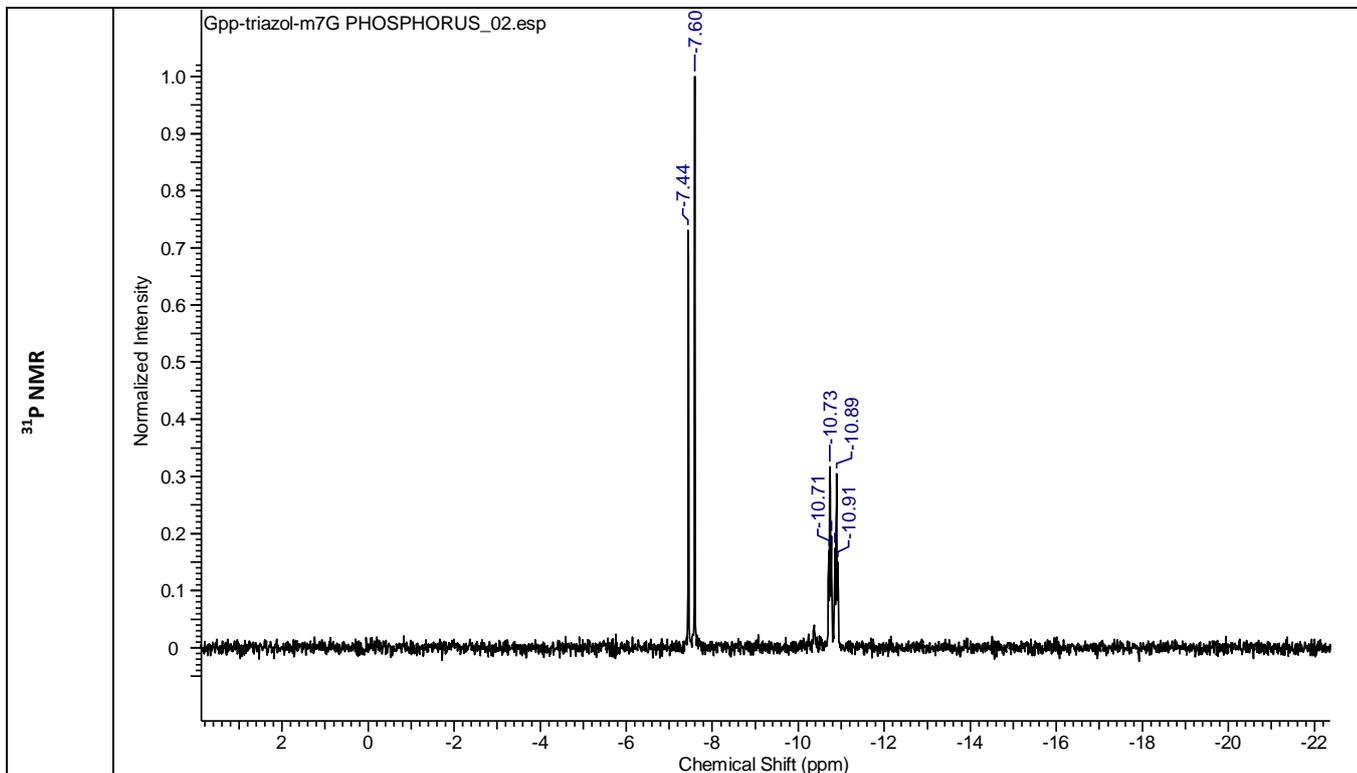
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HRMS

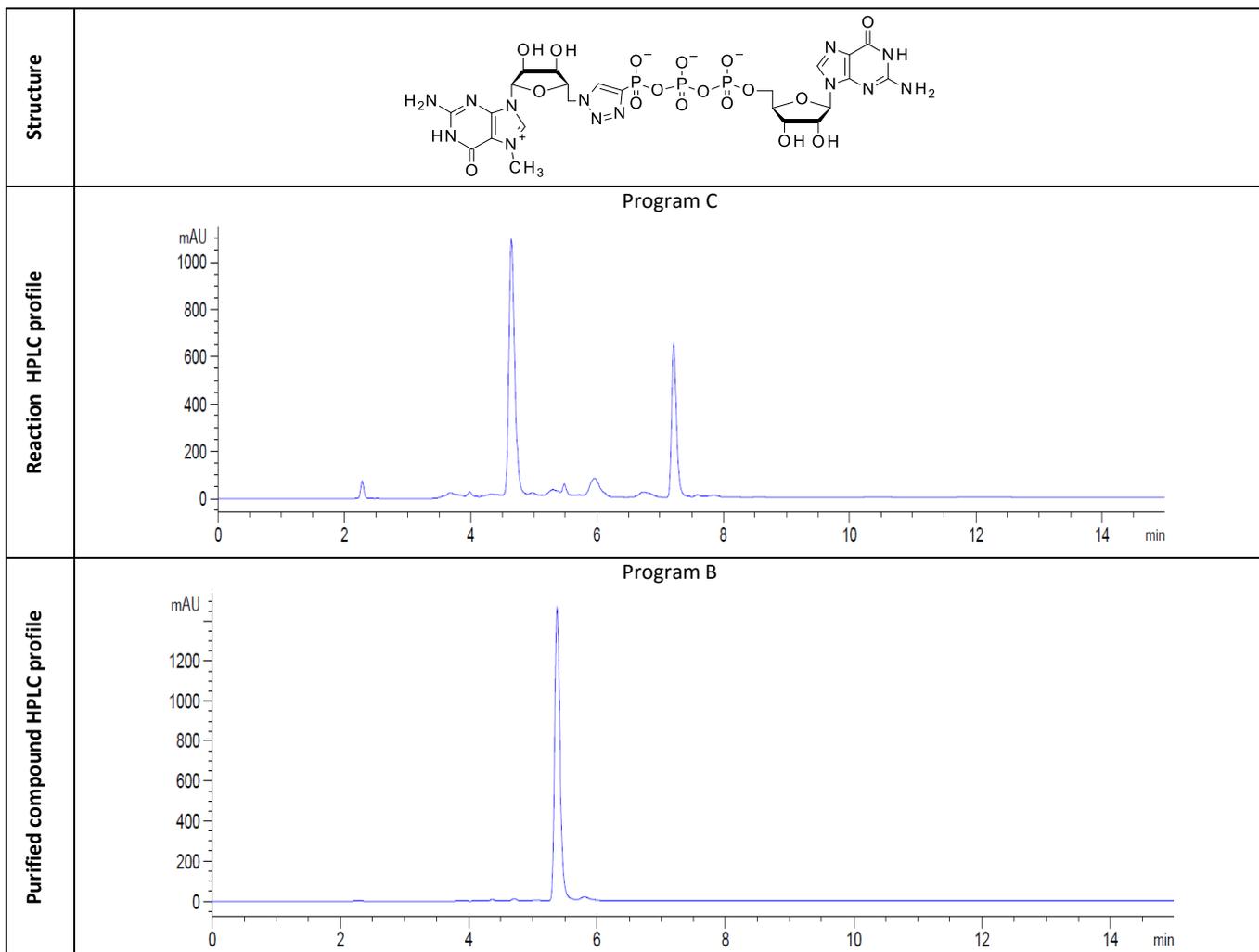


<sup>1</sup>H NMR



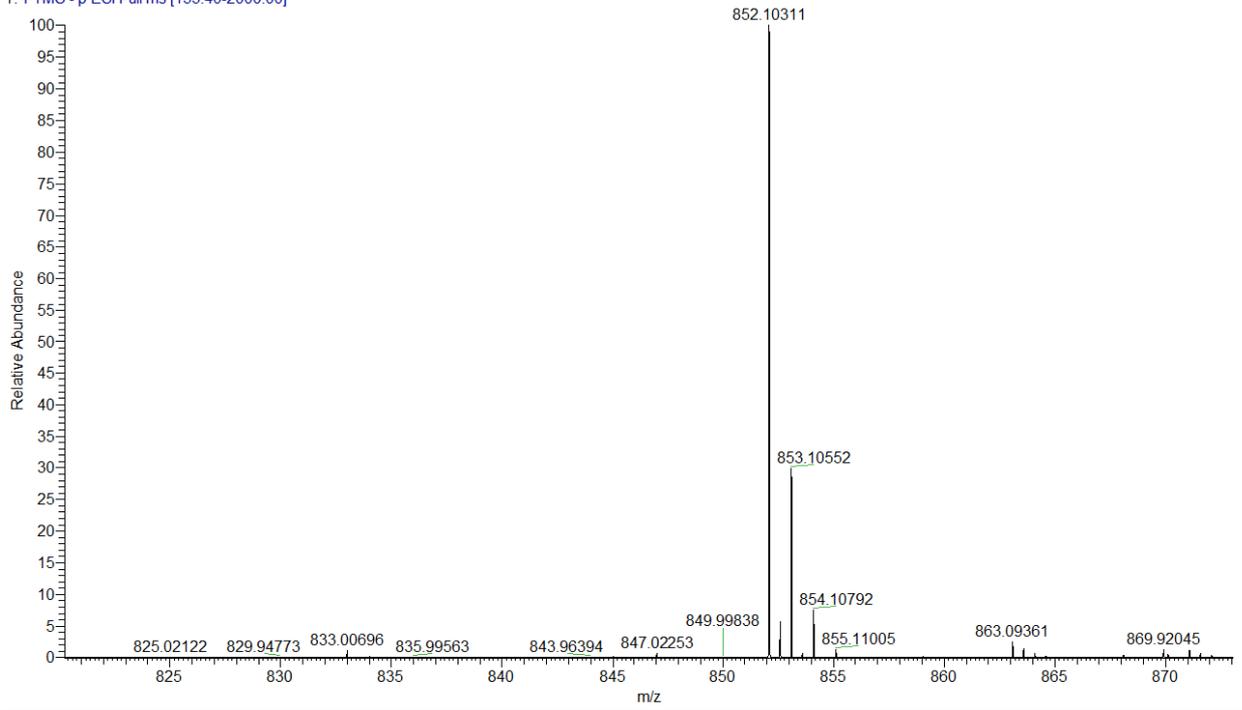


**(3b) m<sup>7</sup>G-triazole-pppG**

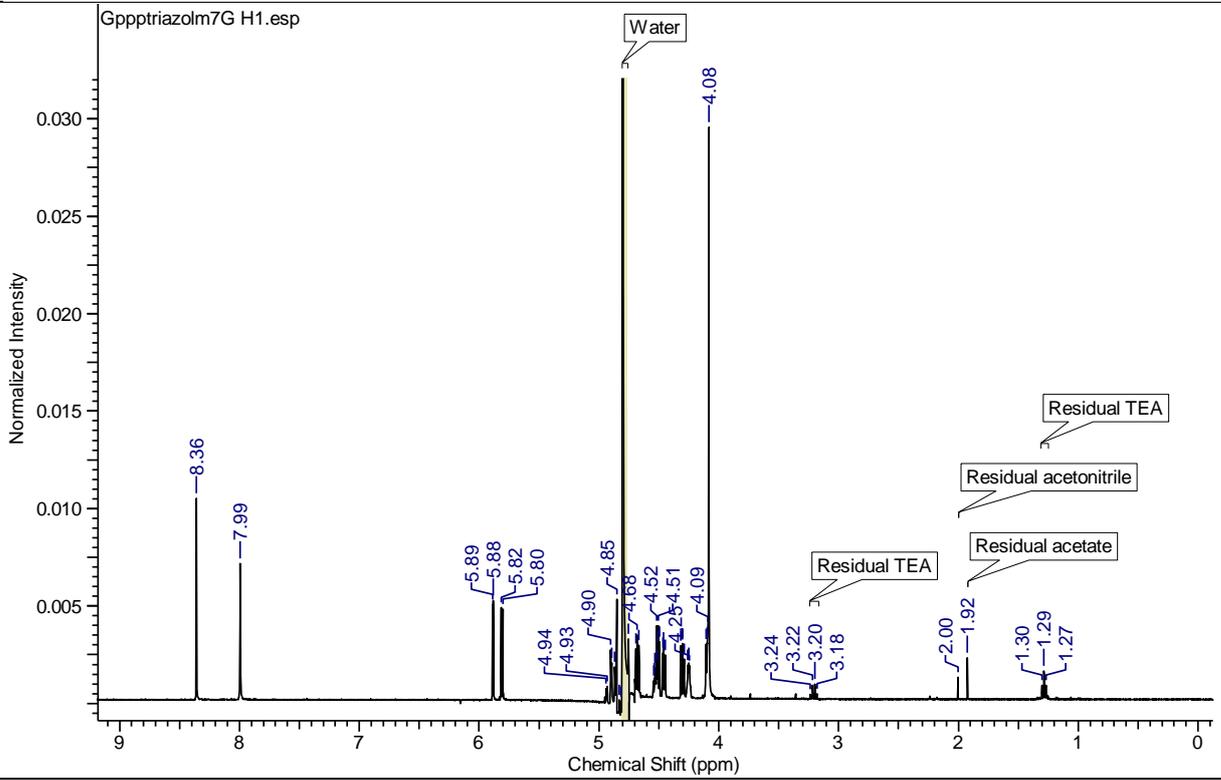


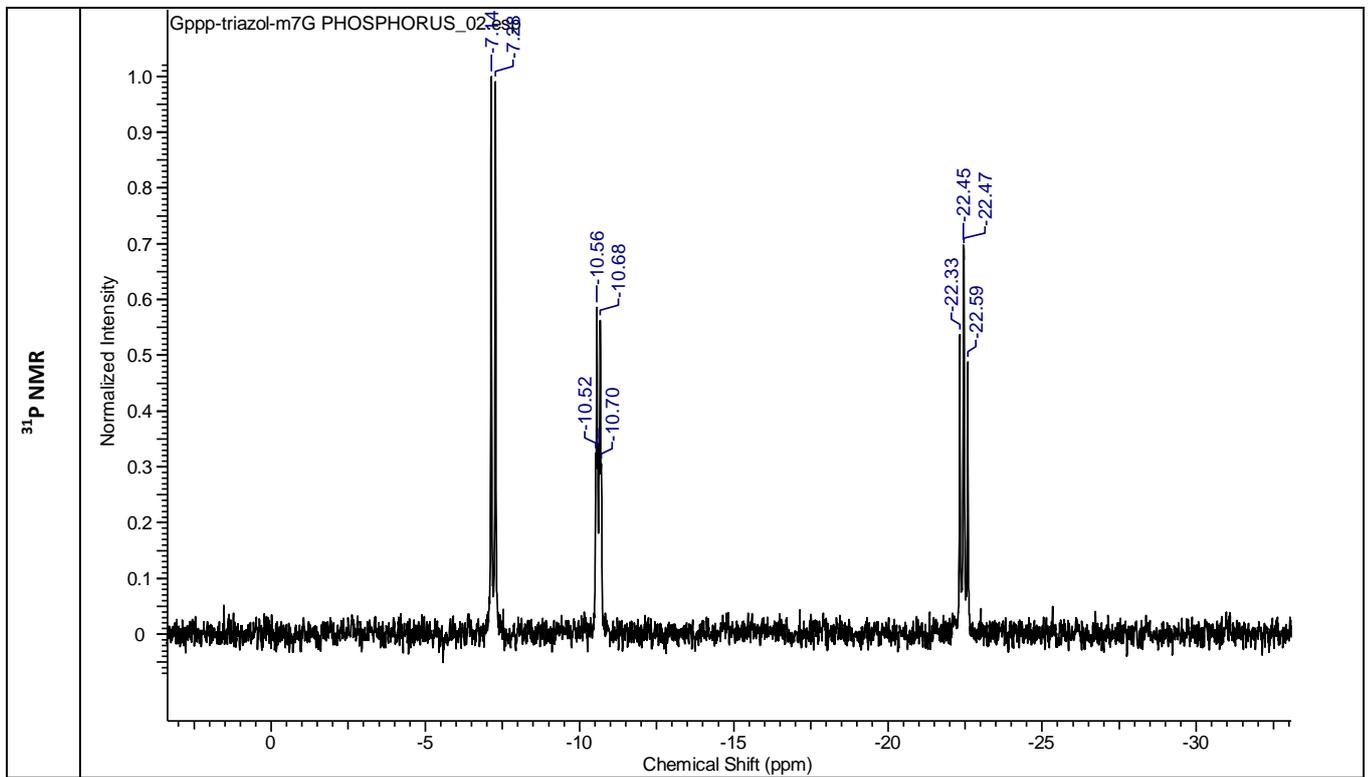
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HRMS

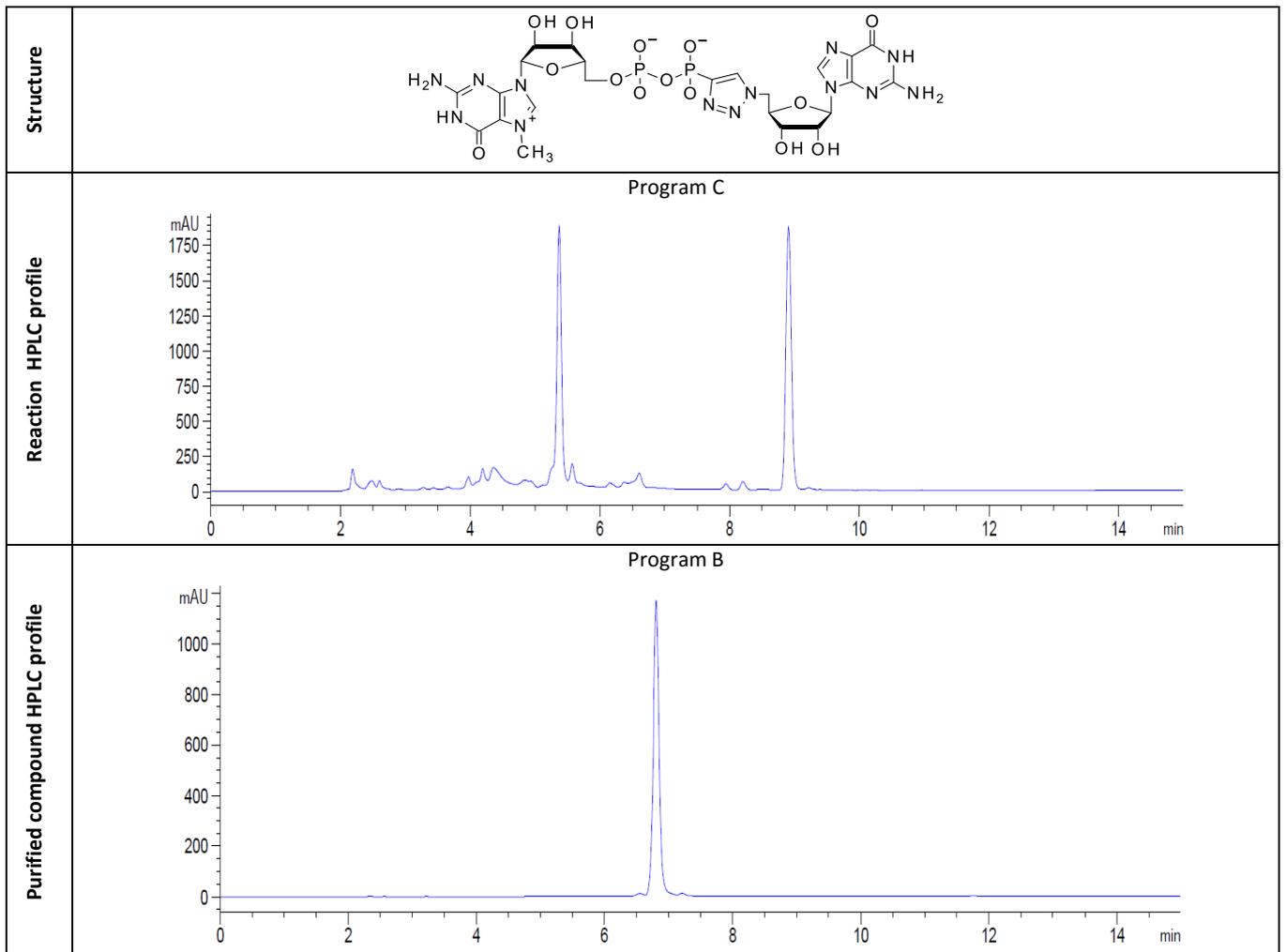


<sup>1</sup>H NMR



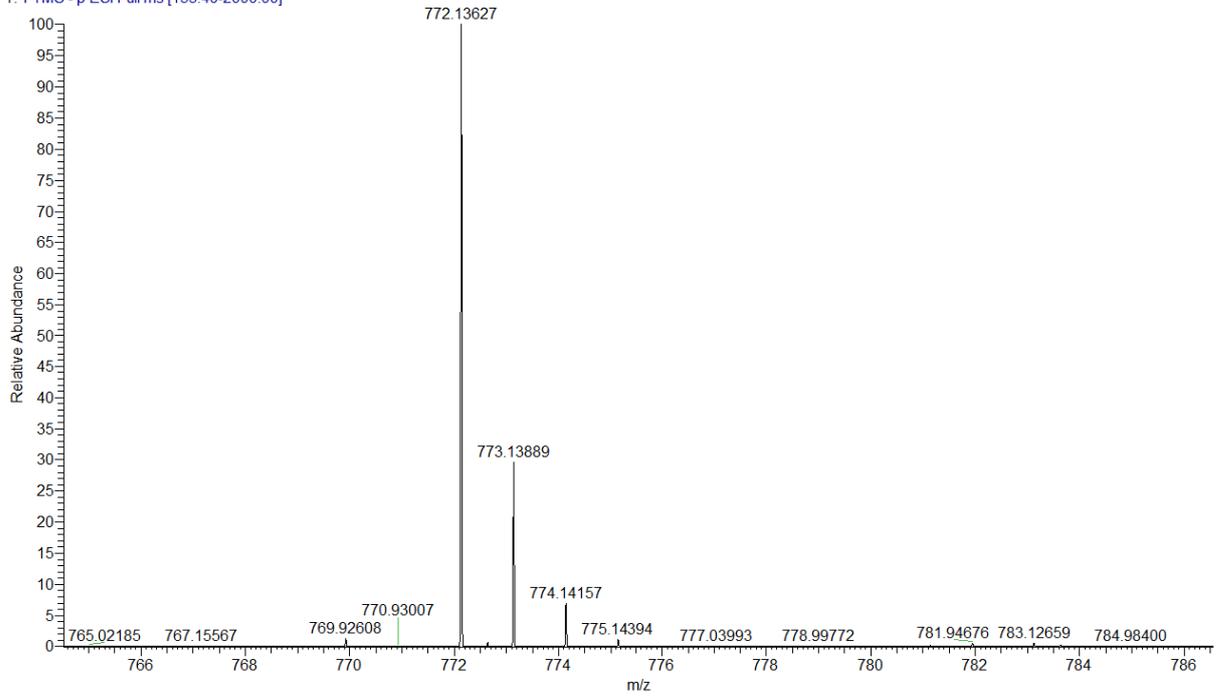


**(3c) m<sup>7</sup>Gpp-triazole-G**

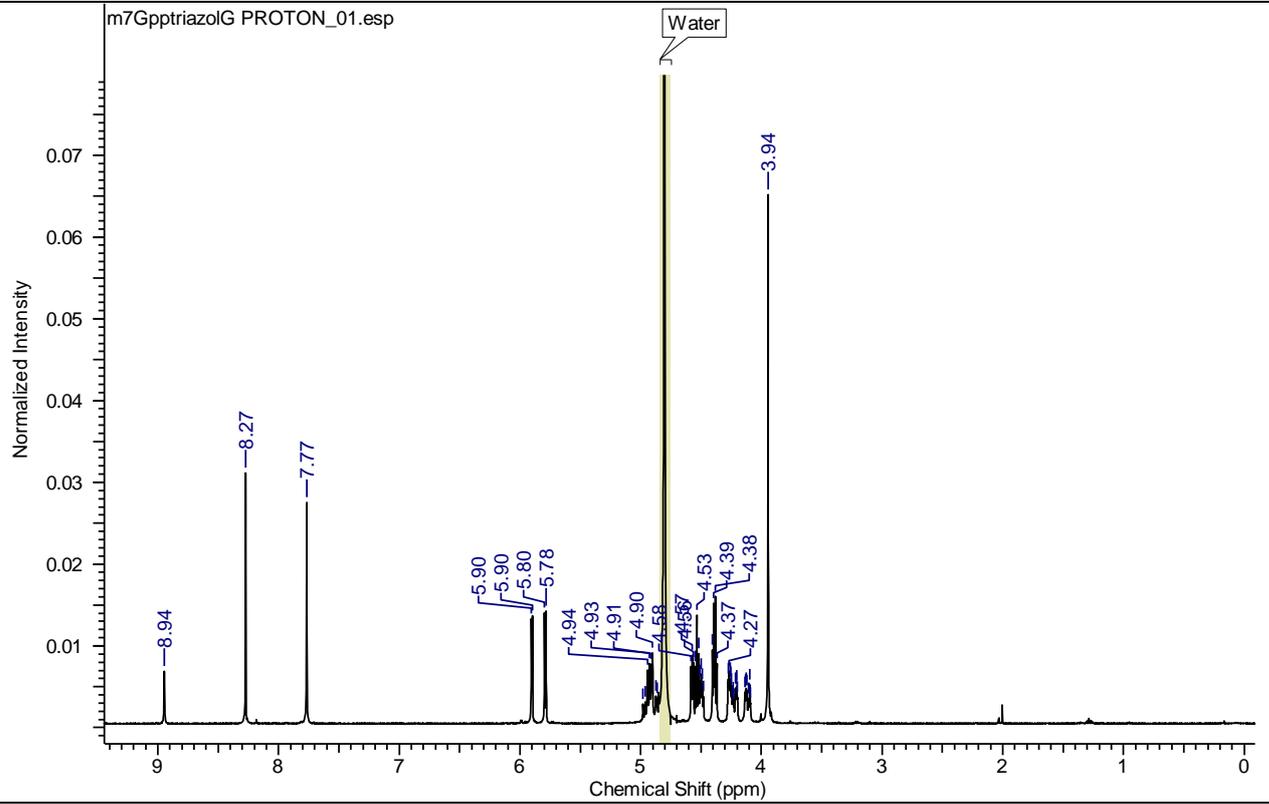


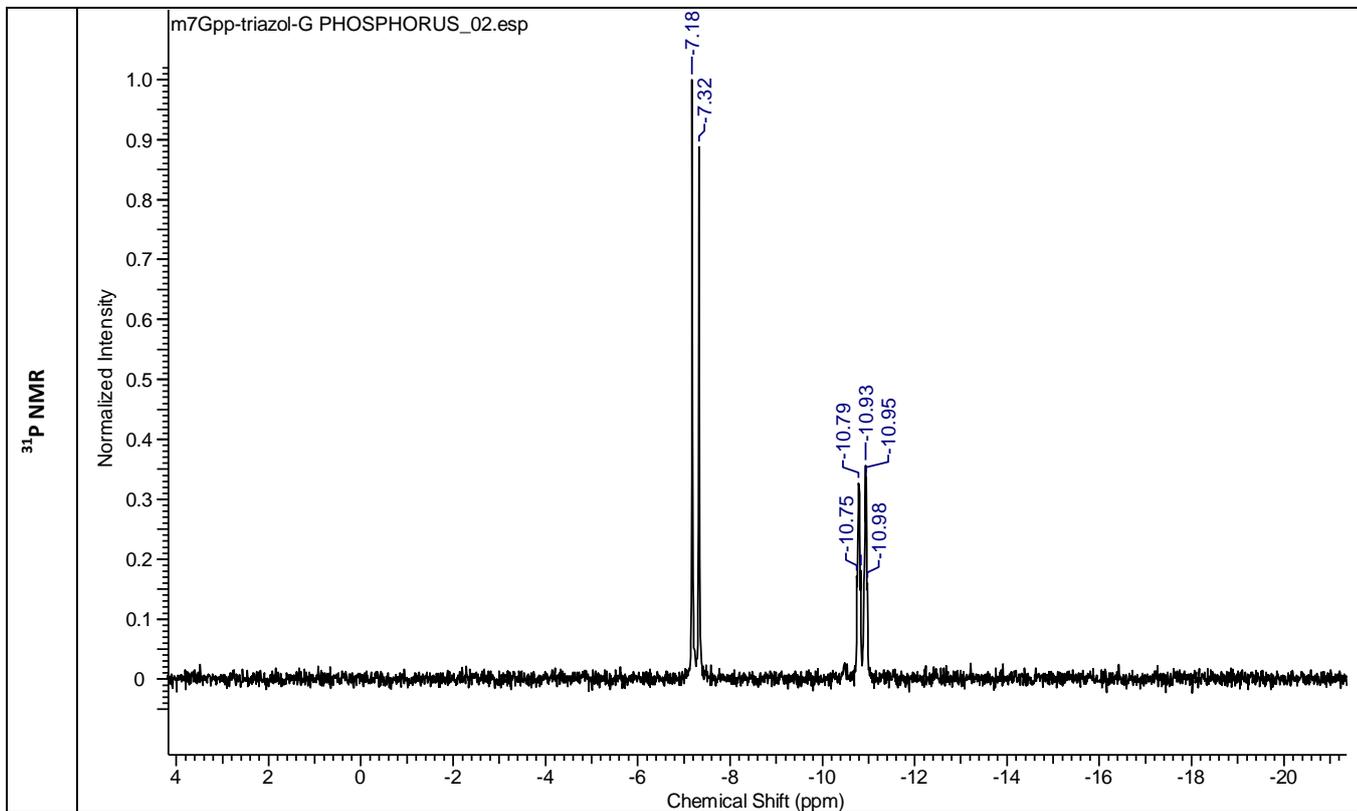
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HRMS

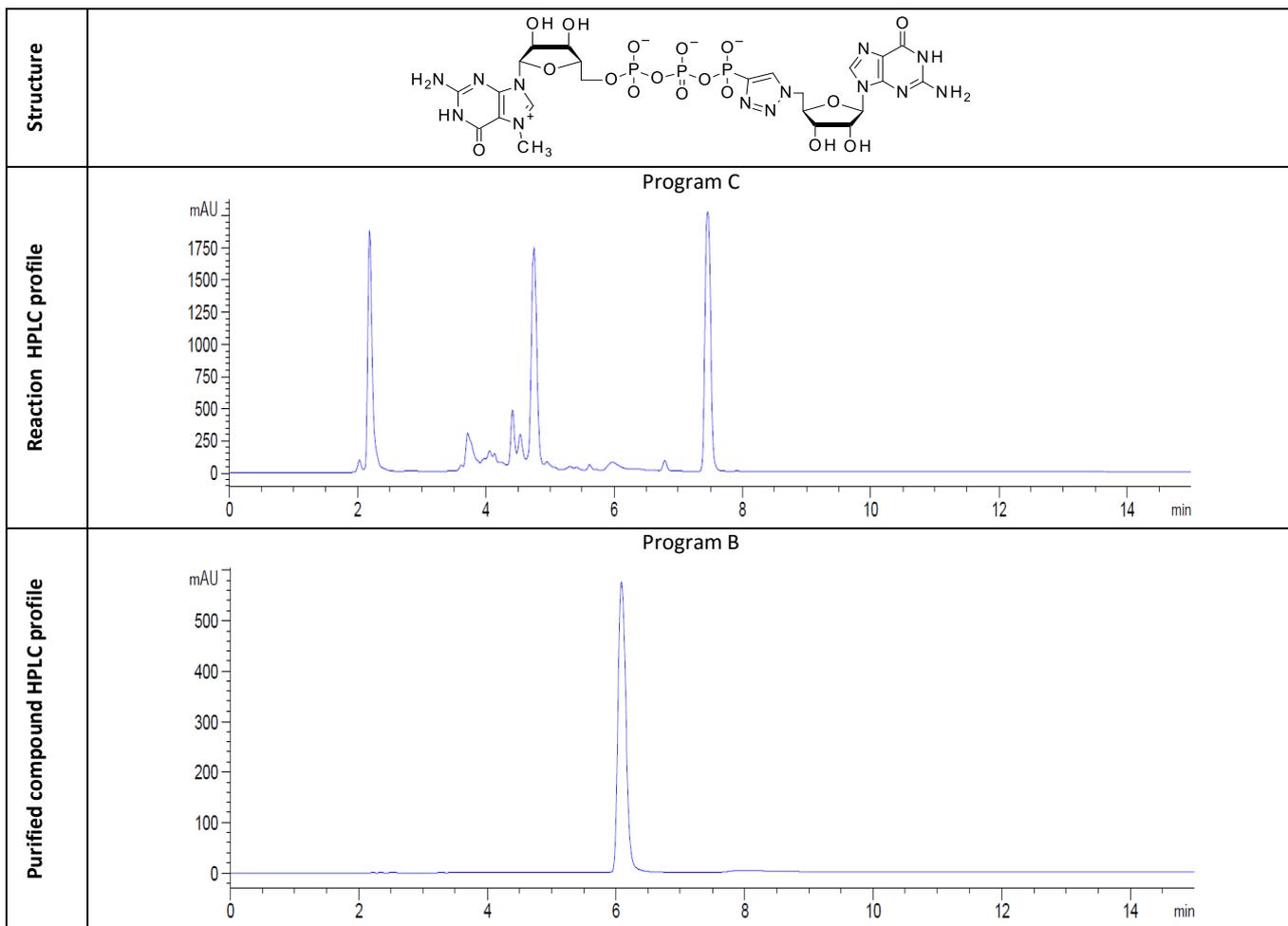


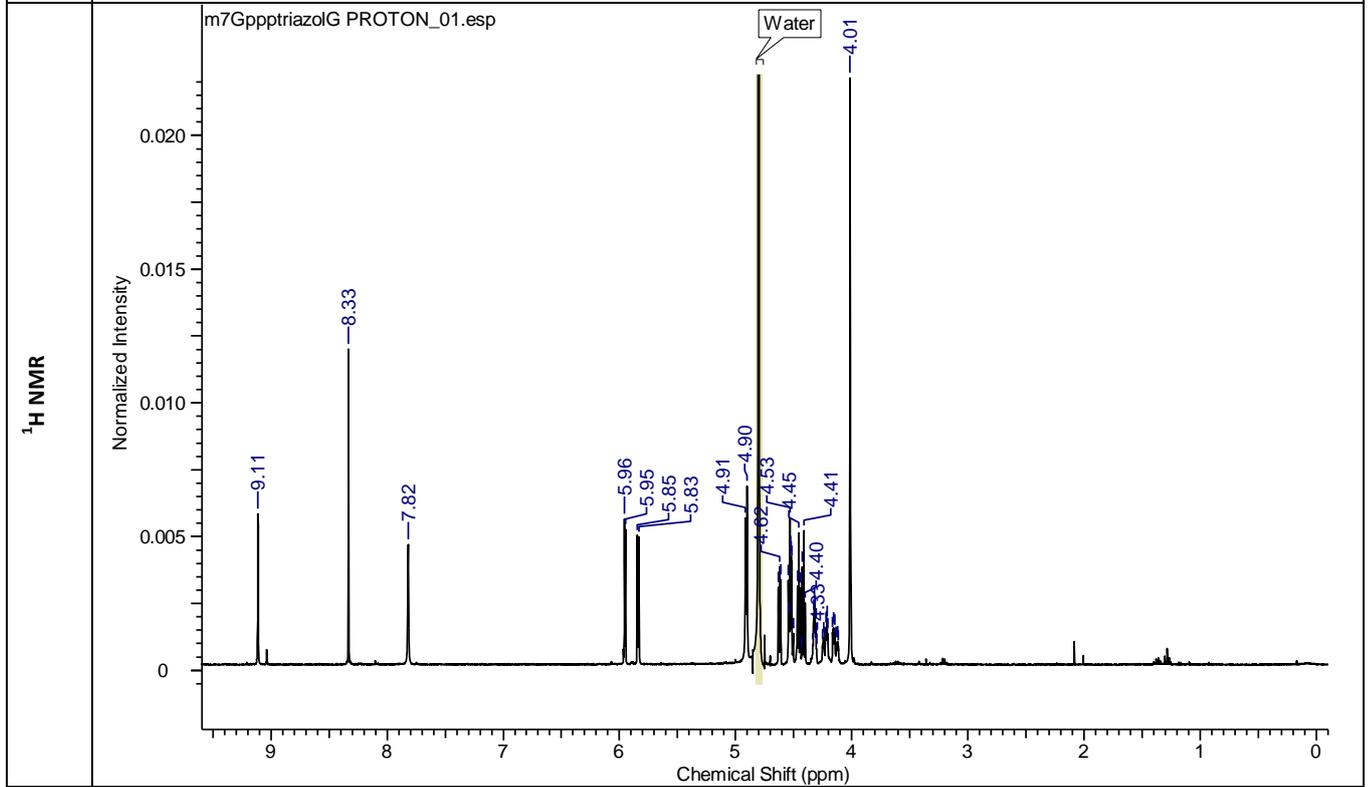
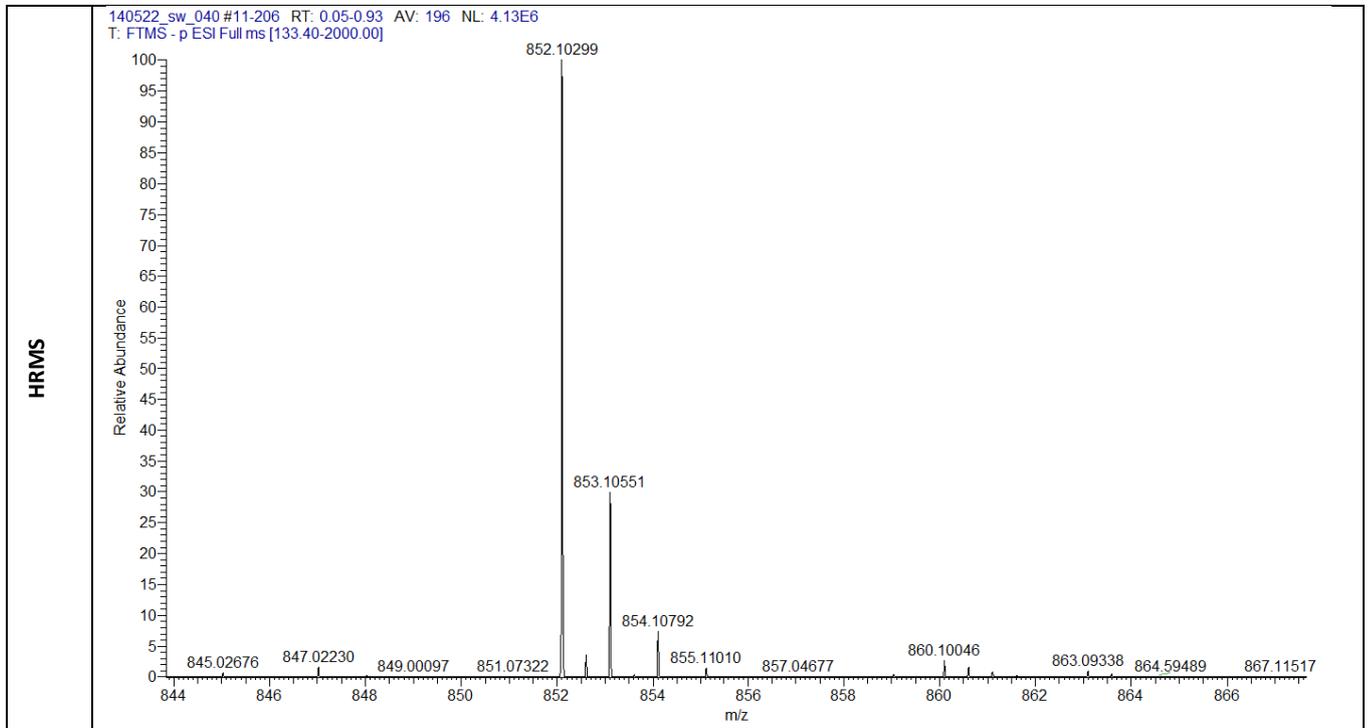
<sup>1</sup>H NMR

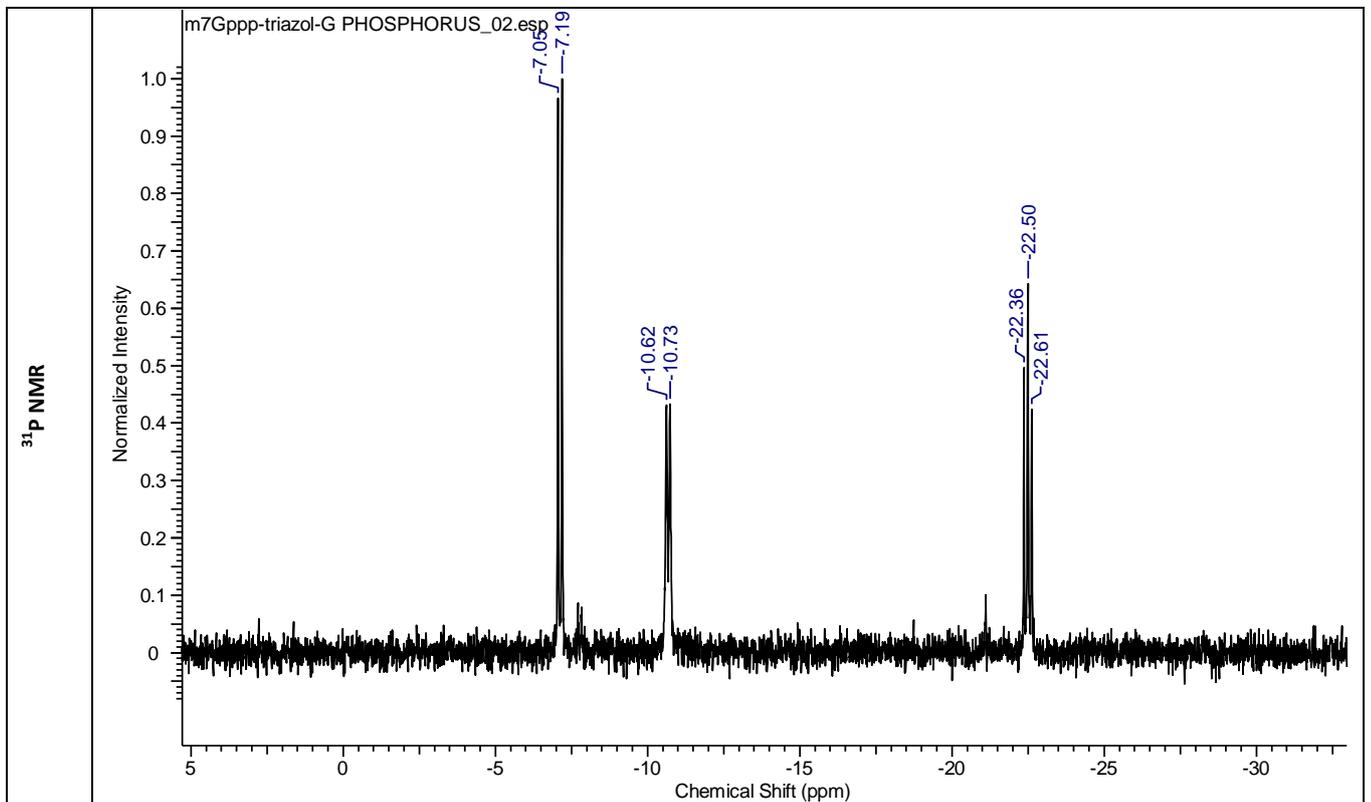




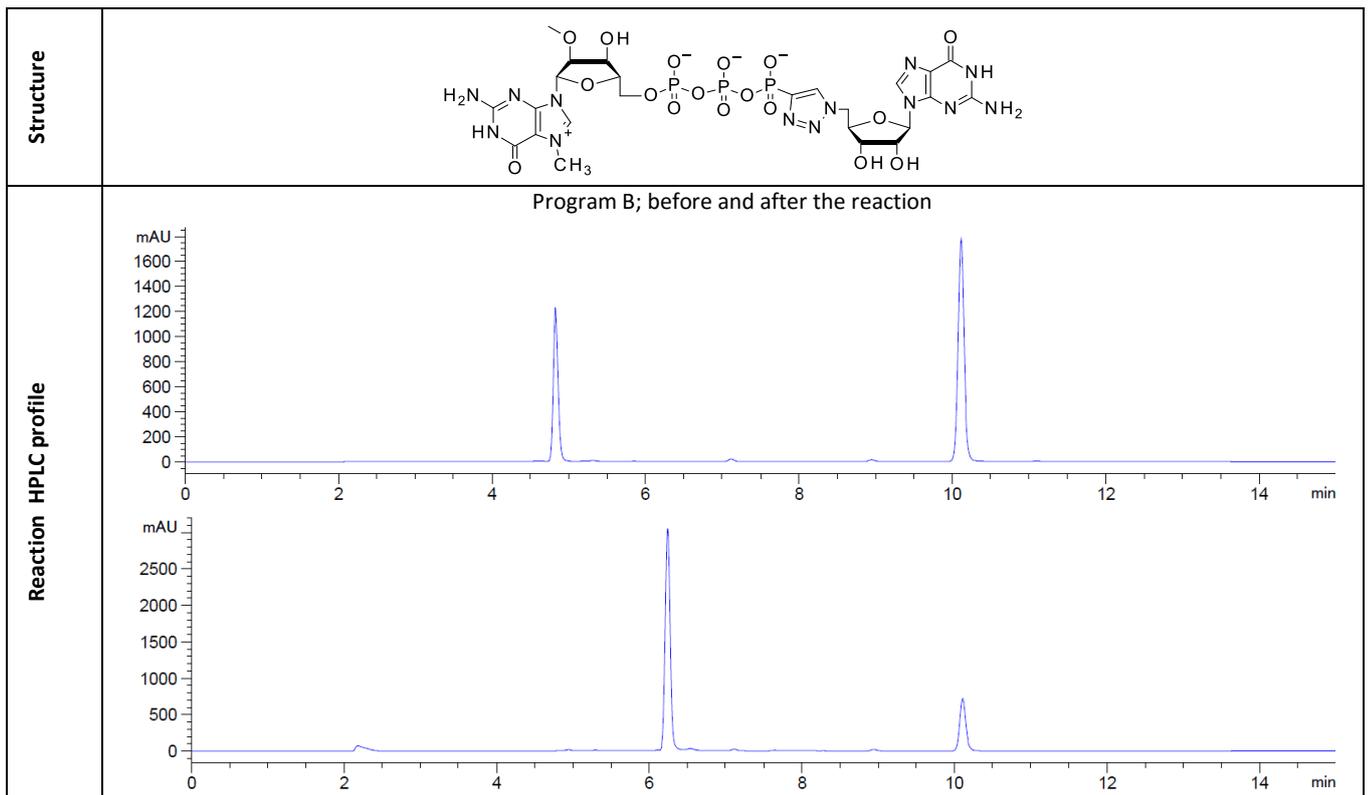
**(3d) m<sup>7</sup>Gppp-triazole-G**

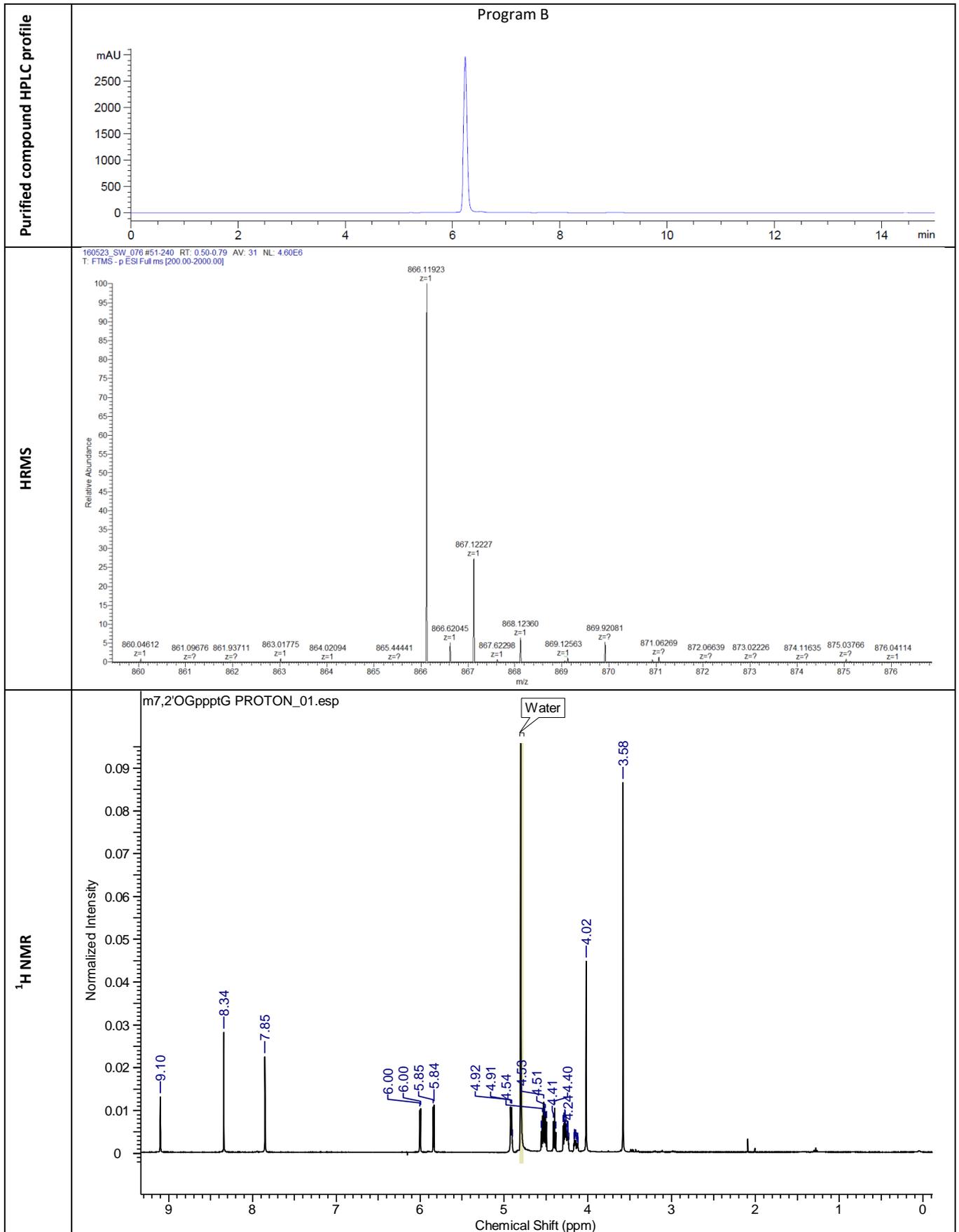


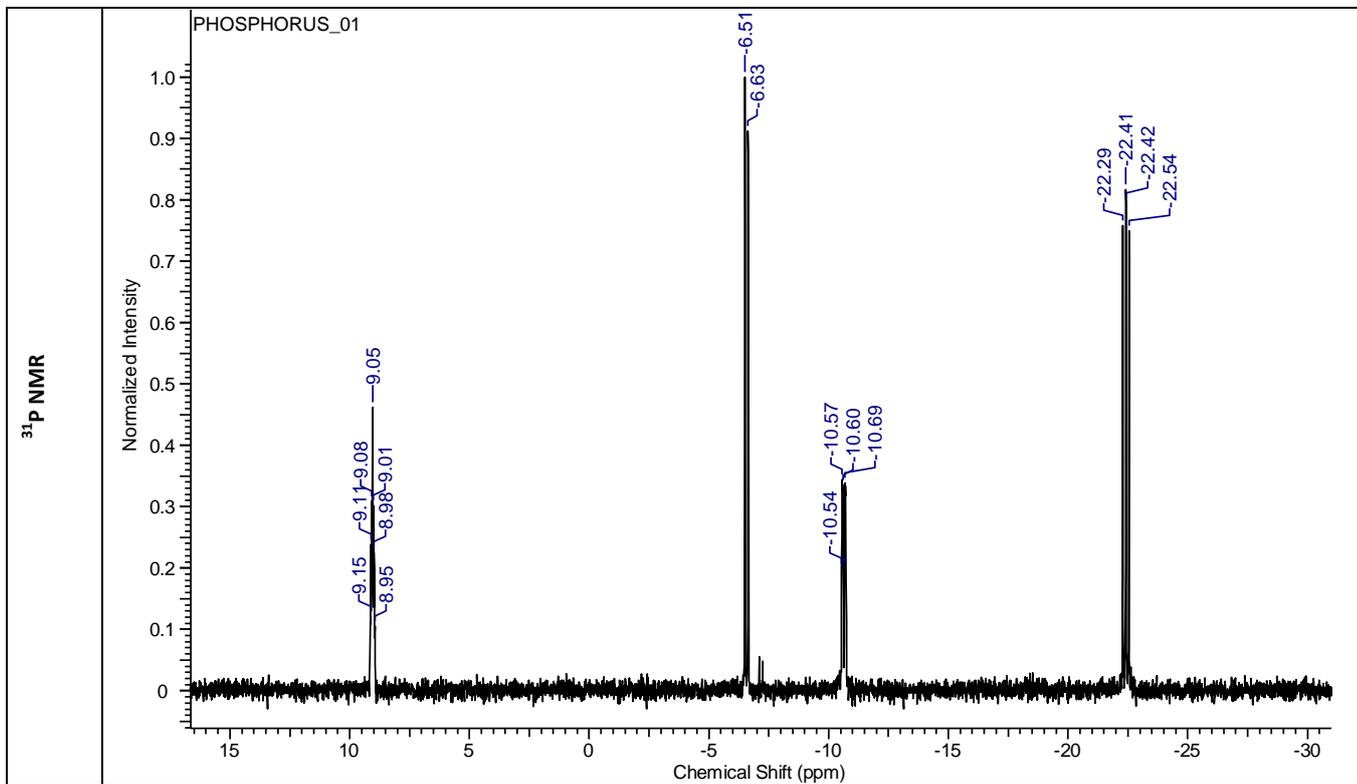




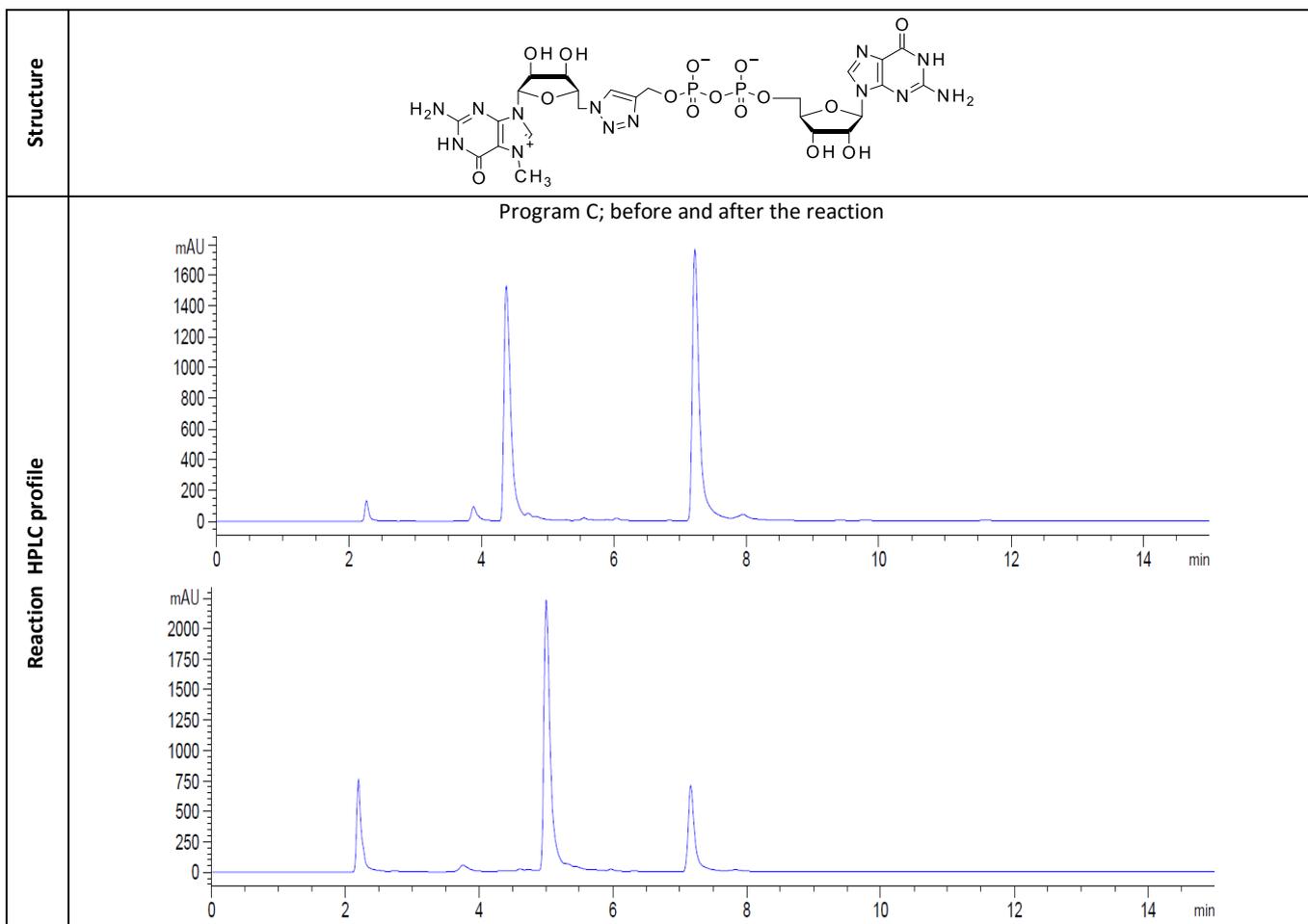
**(3e) m<sub>2</sub><sup>7,2'-O</sup>Gppp-triazole-G**



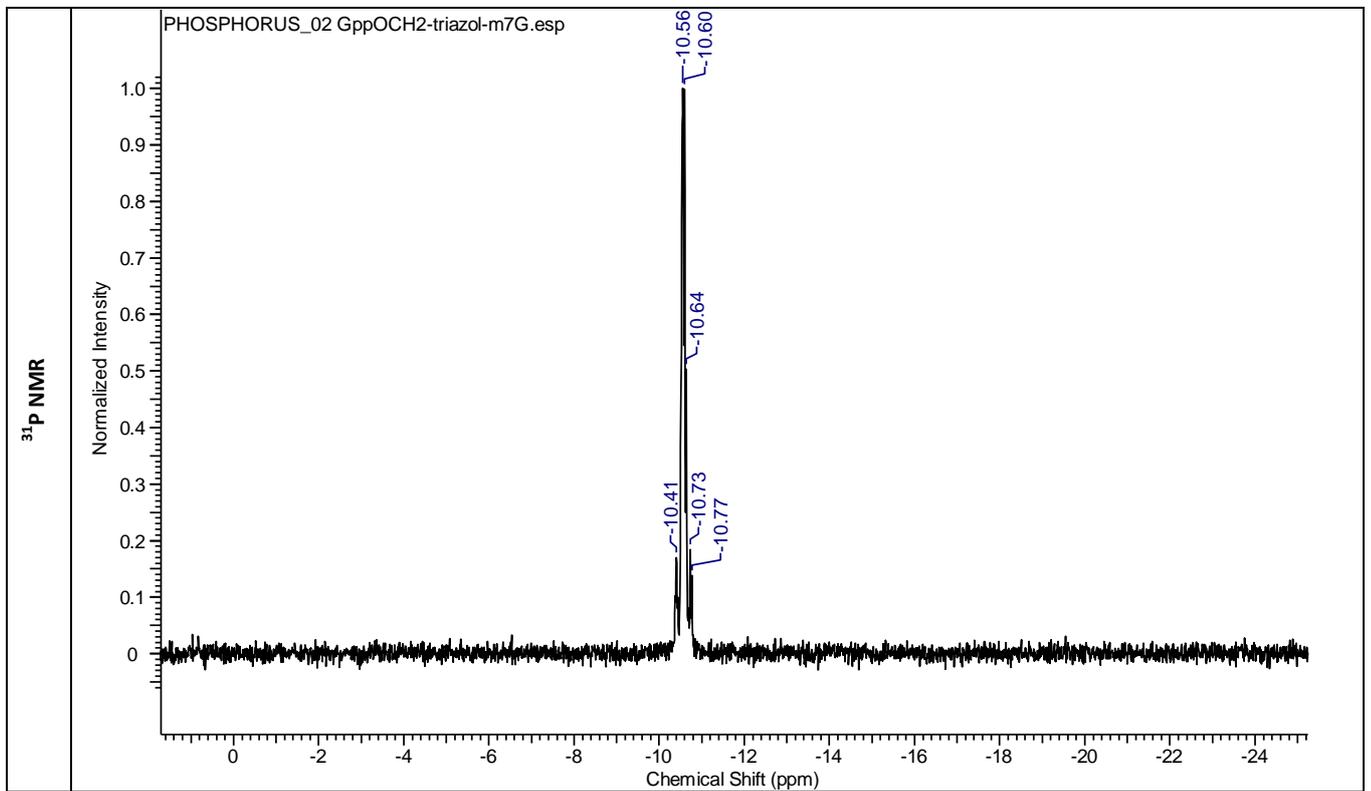




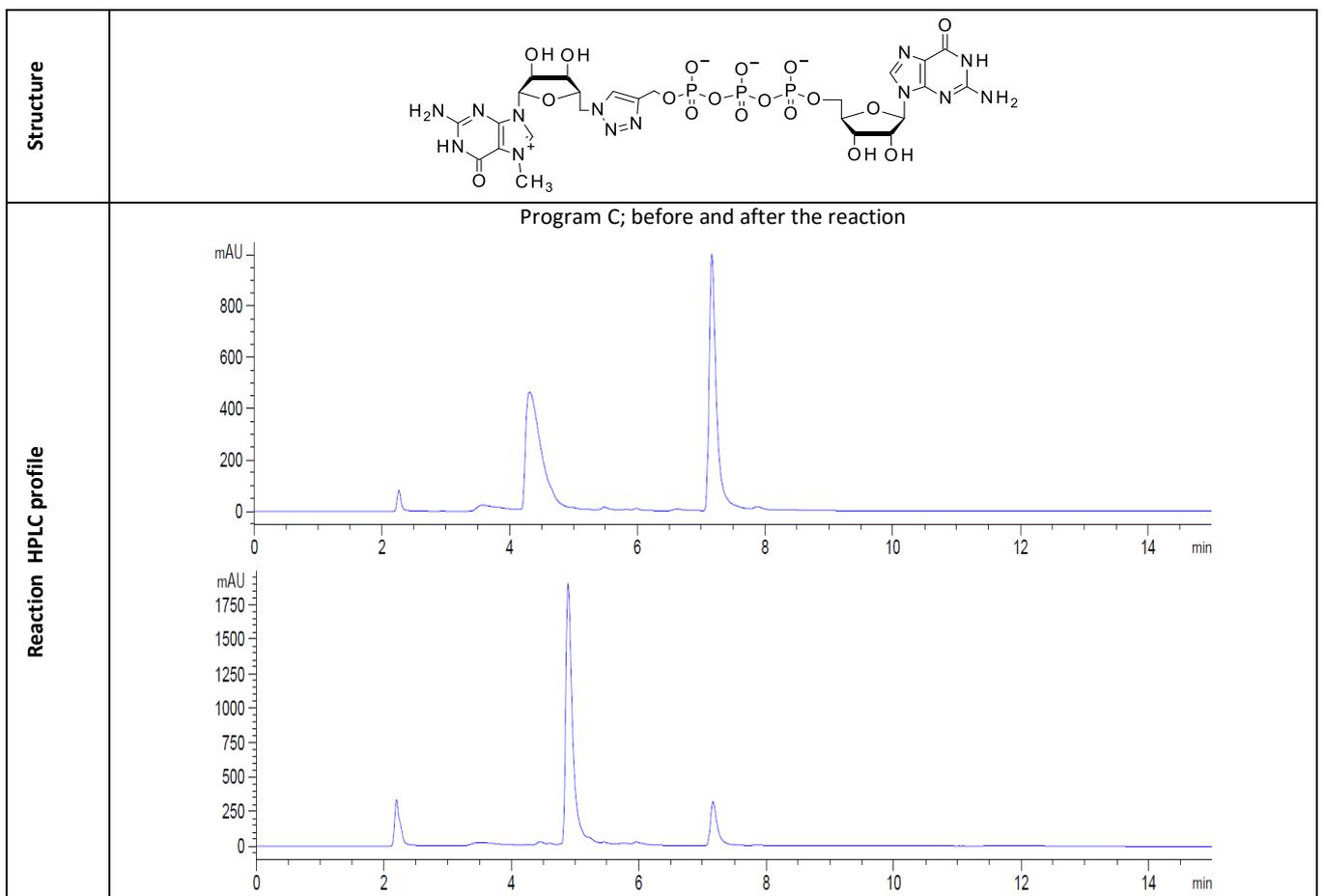
**(4a) m<sup>7</sup>G-triazole-CH<sub>2</sub>OppG**

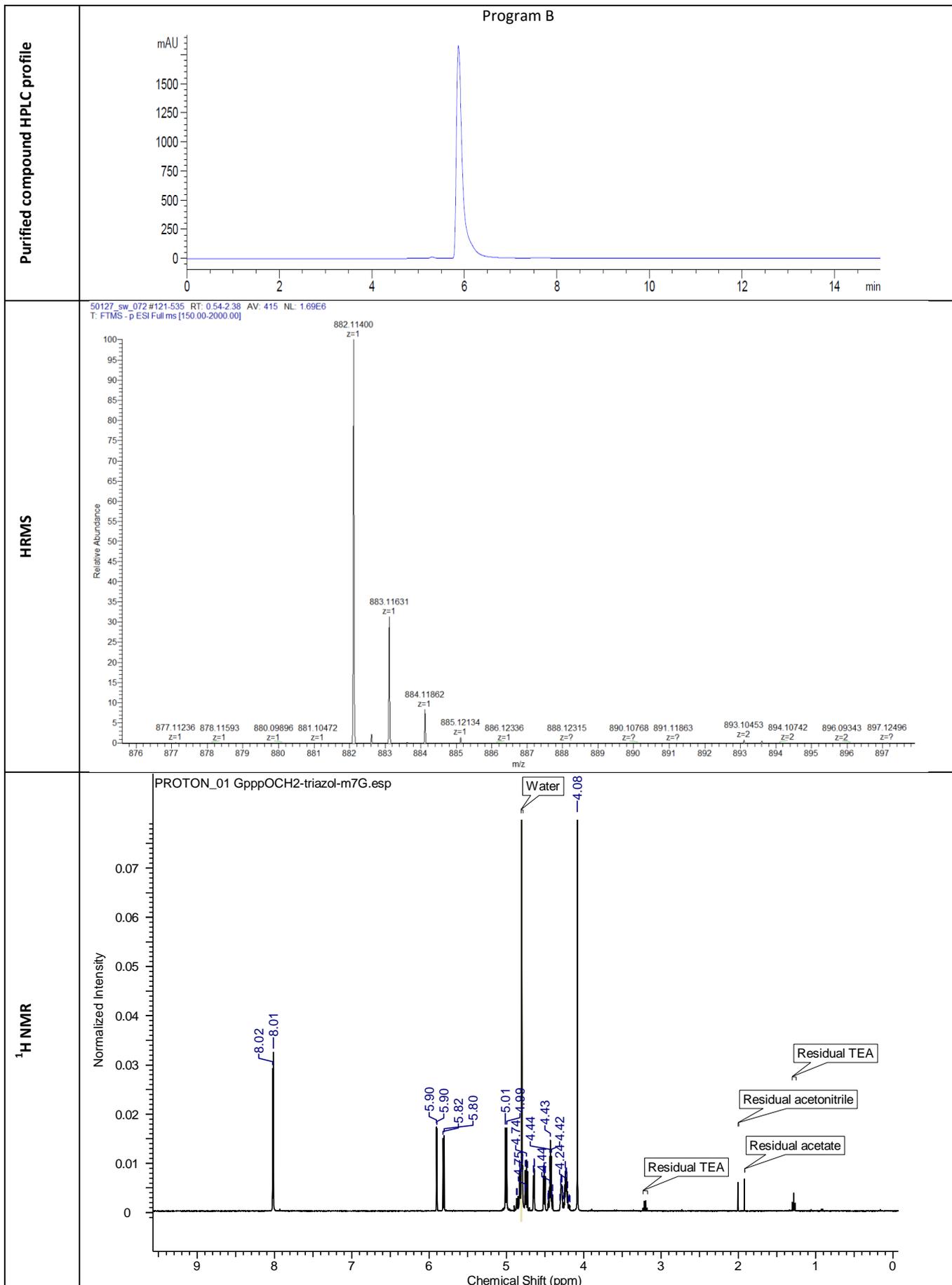


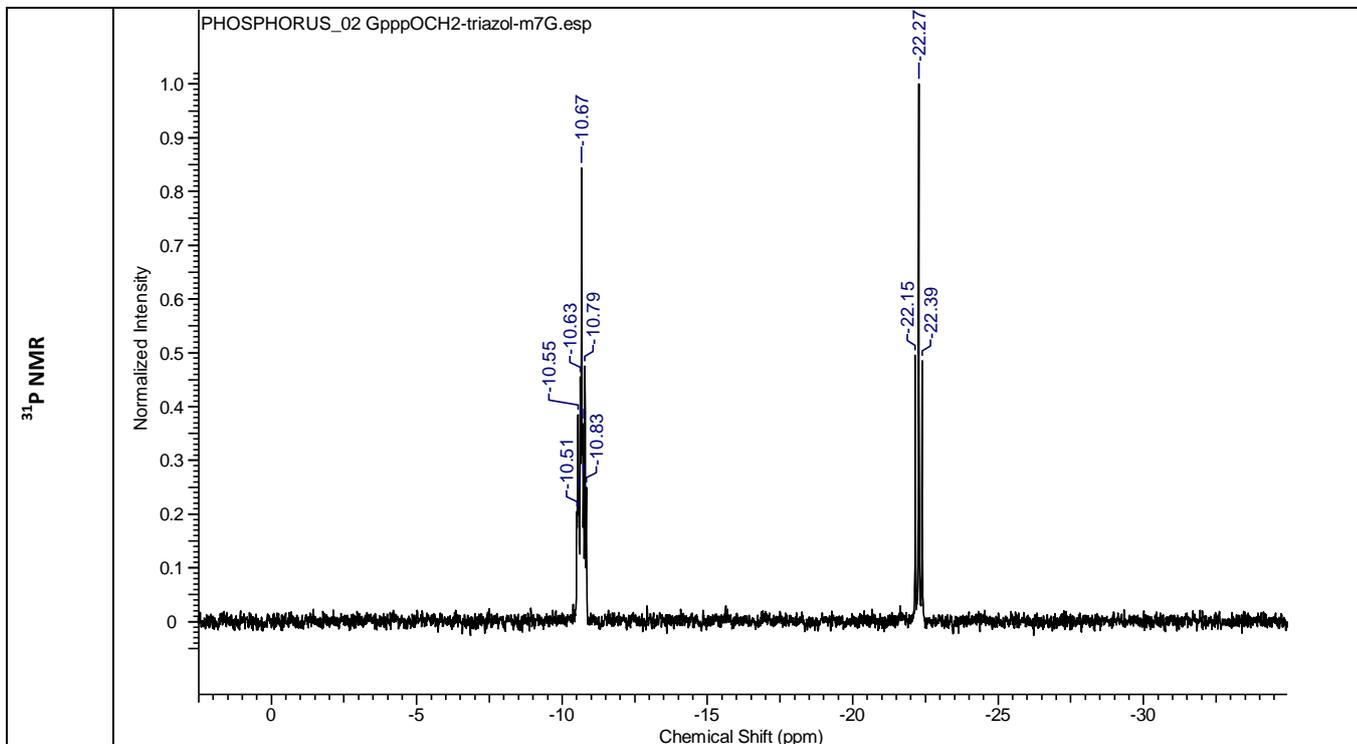




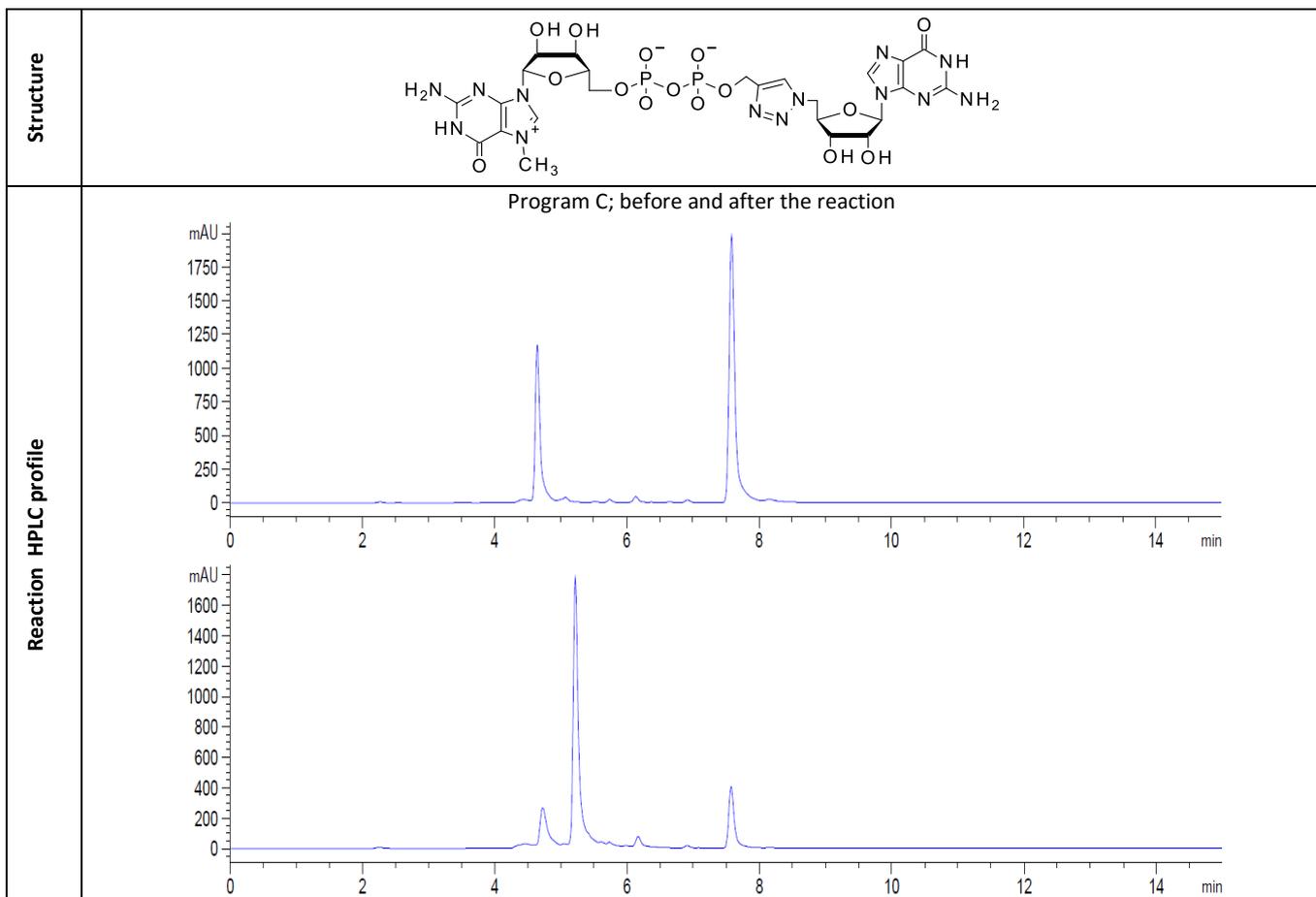
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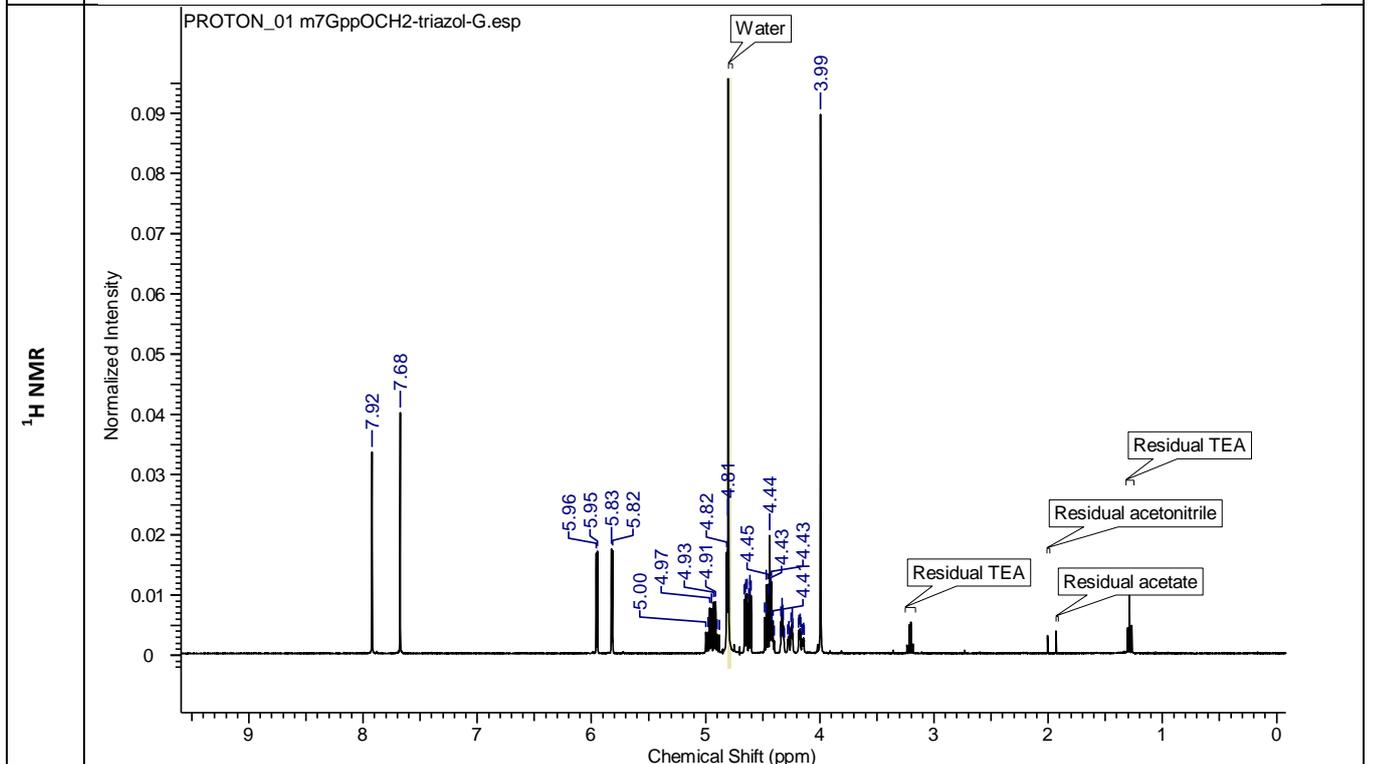
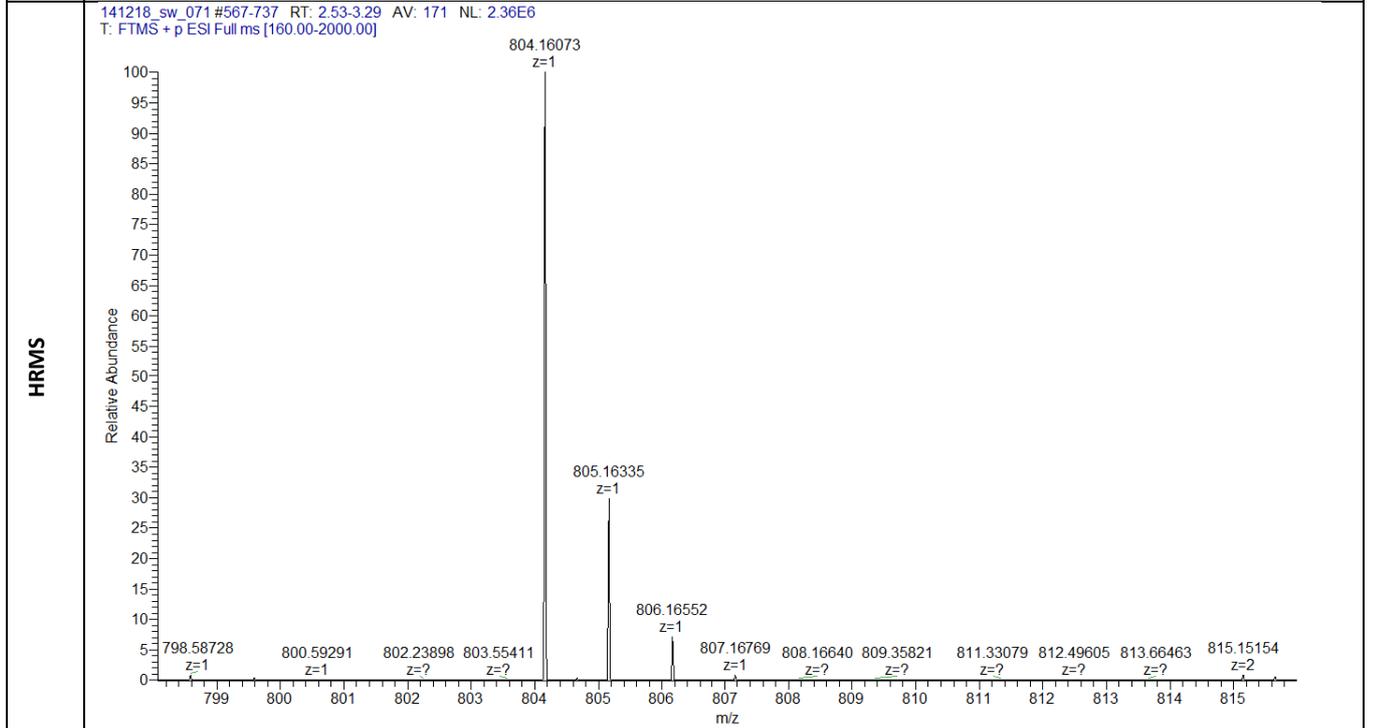
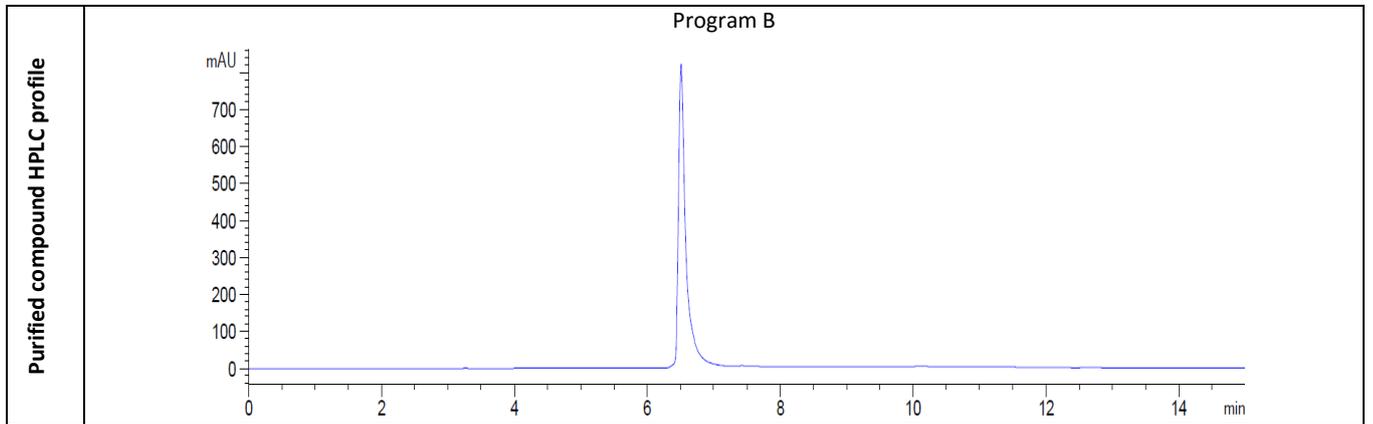


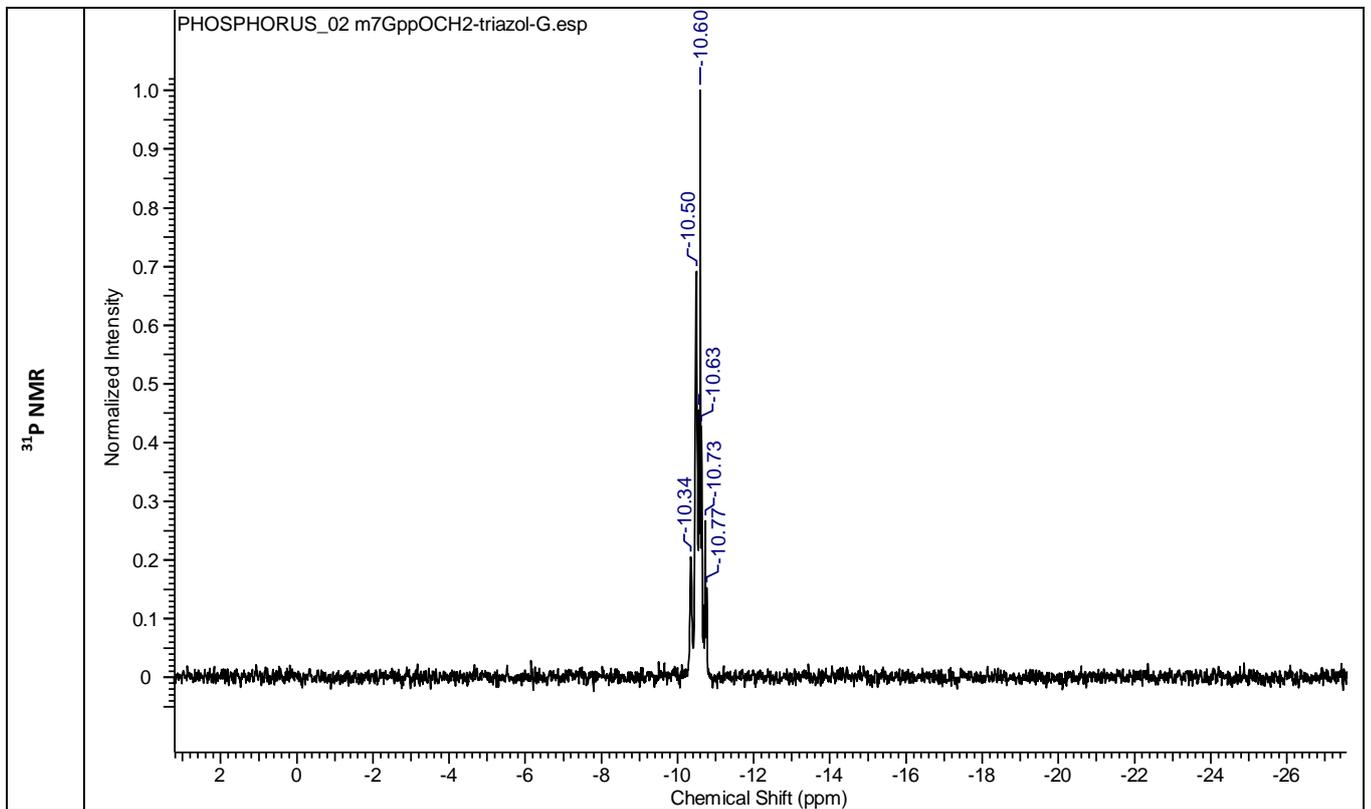




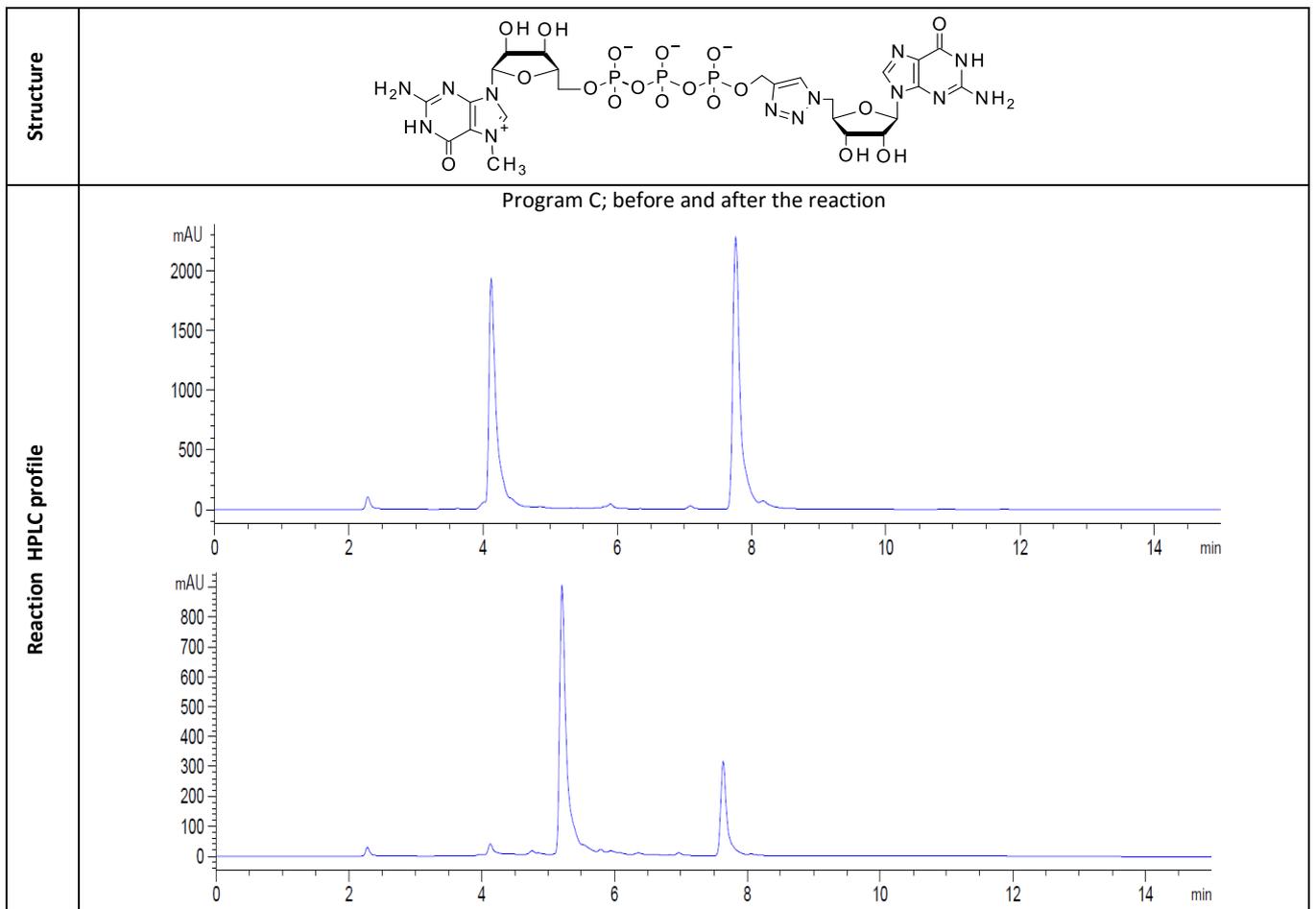
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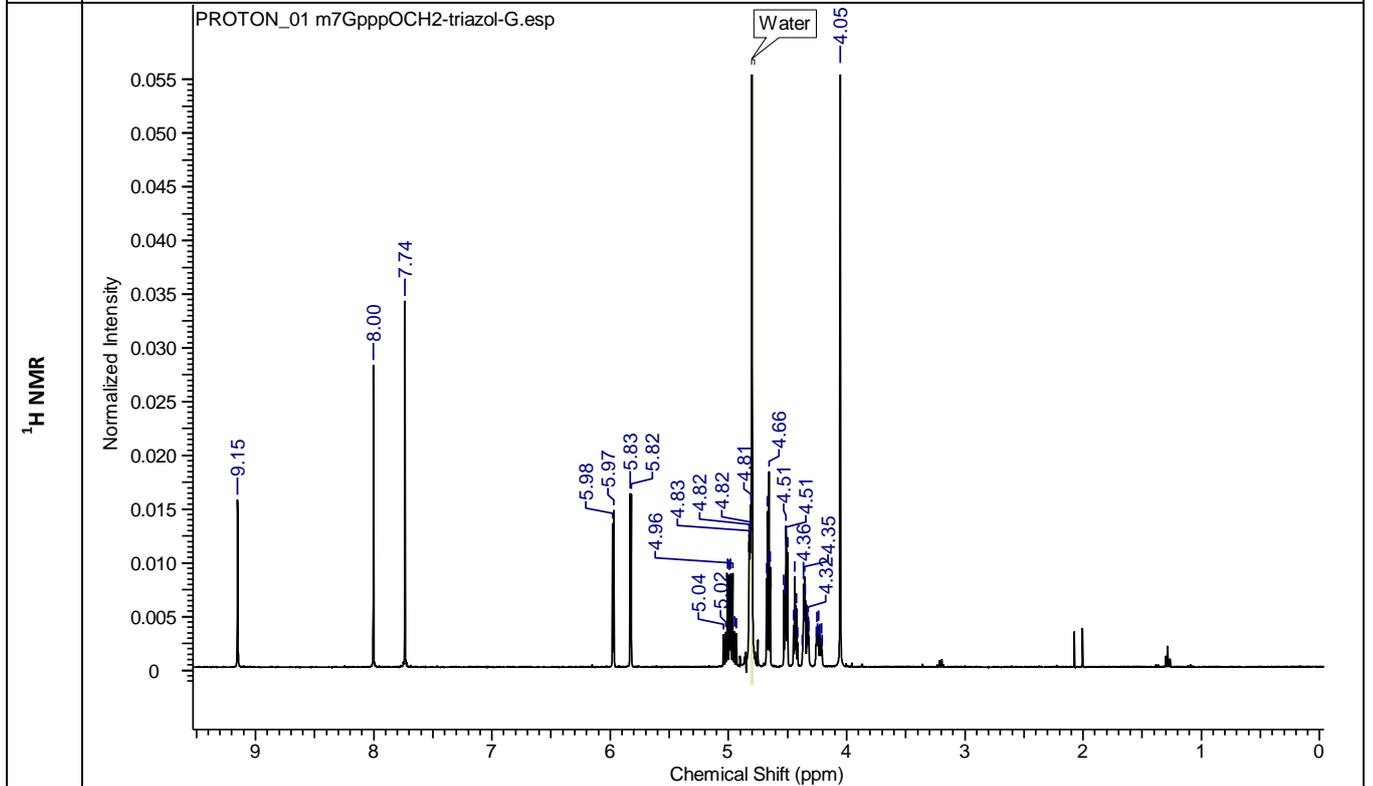
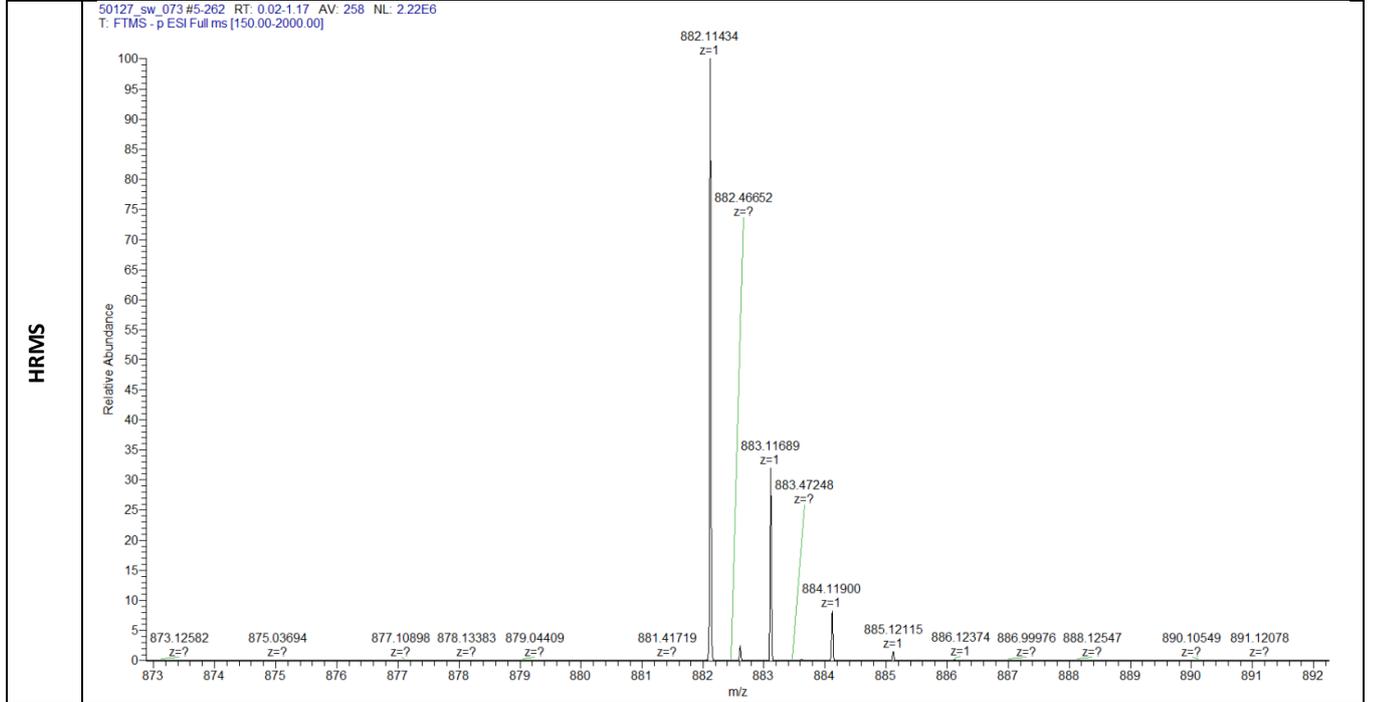
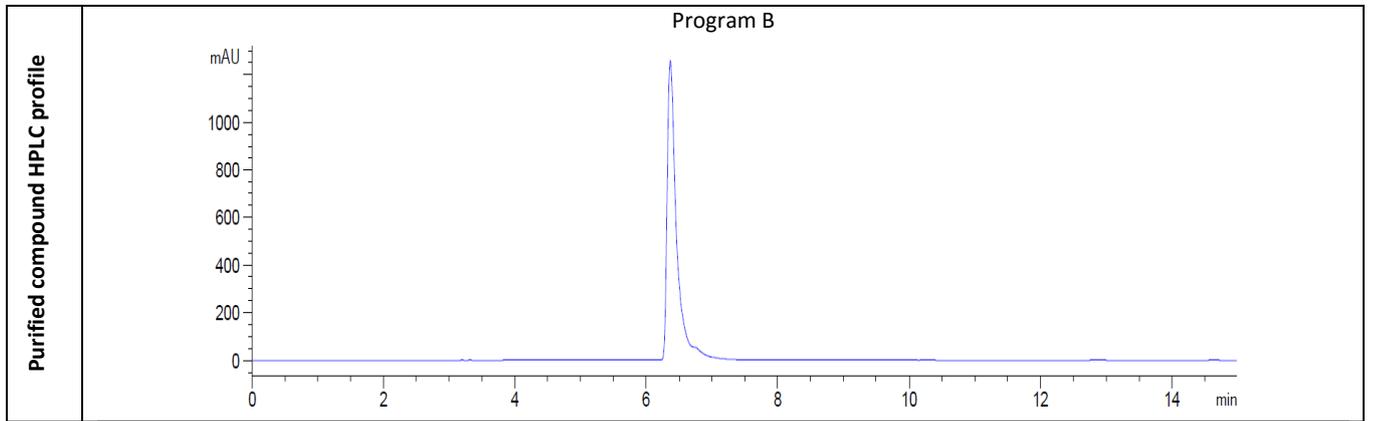


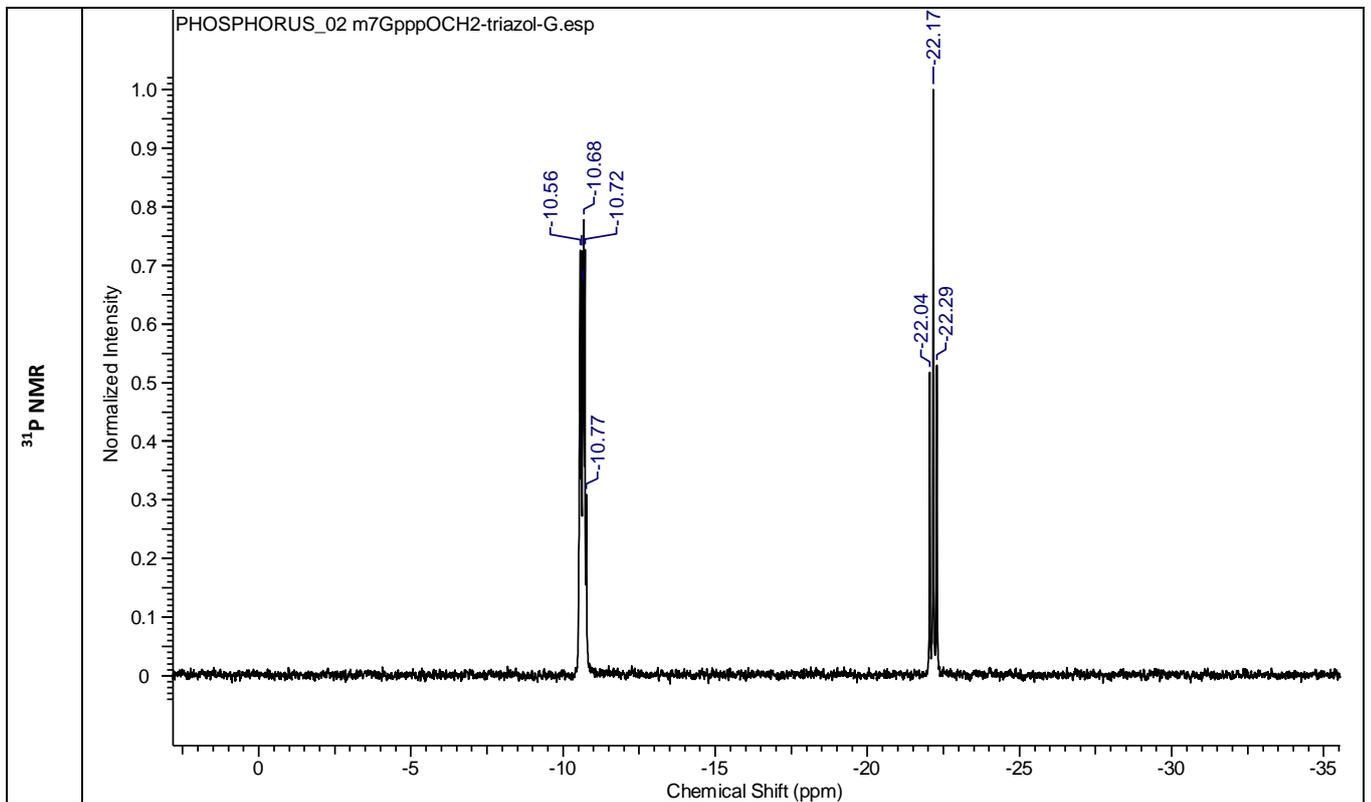




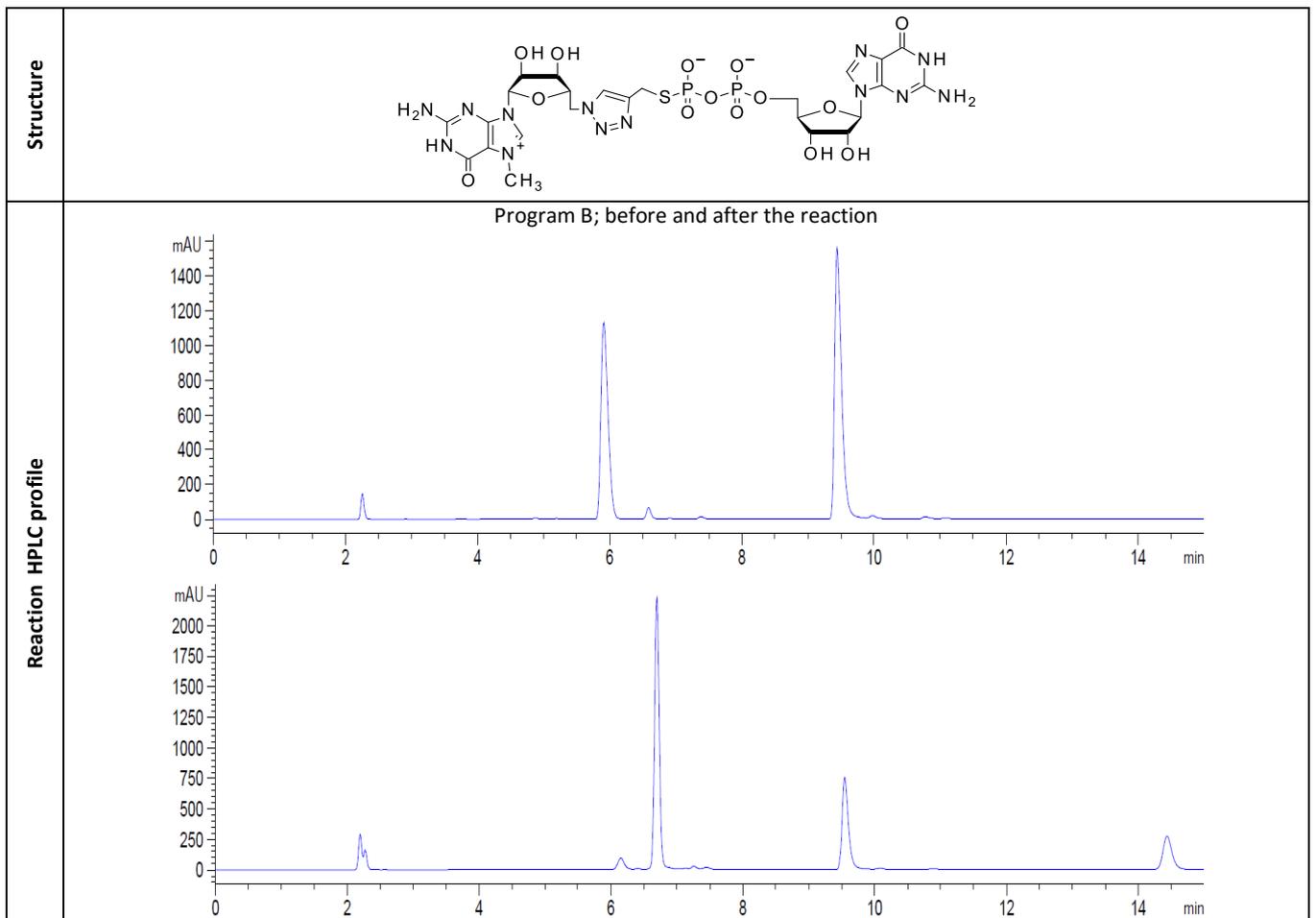
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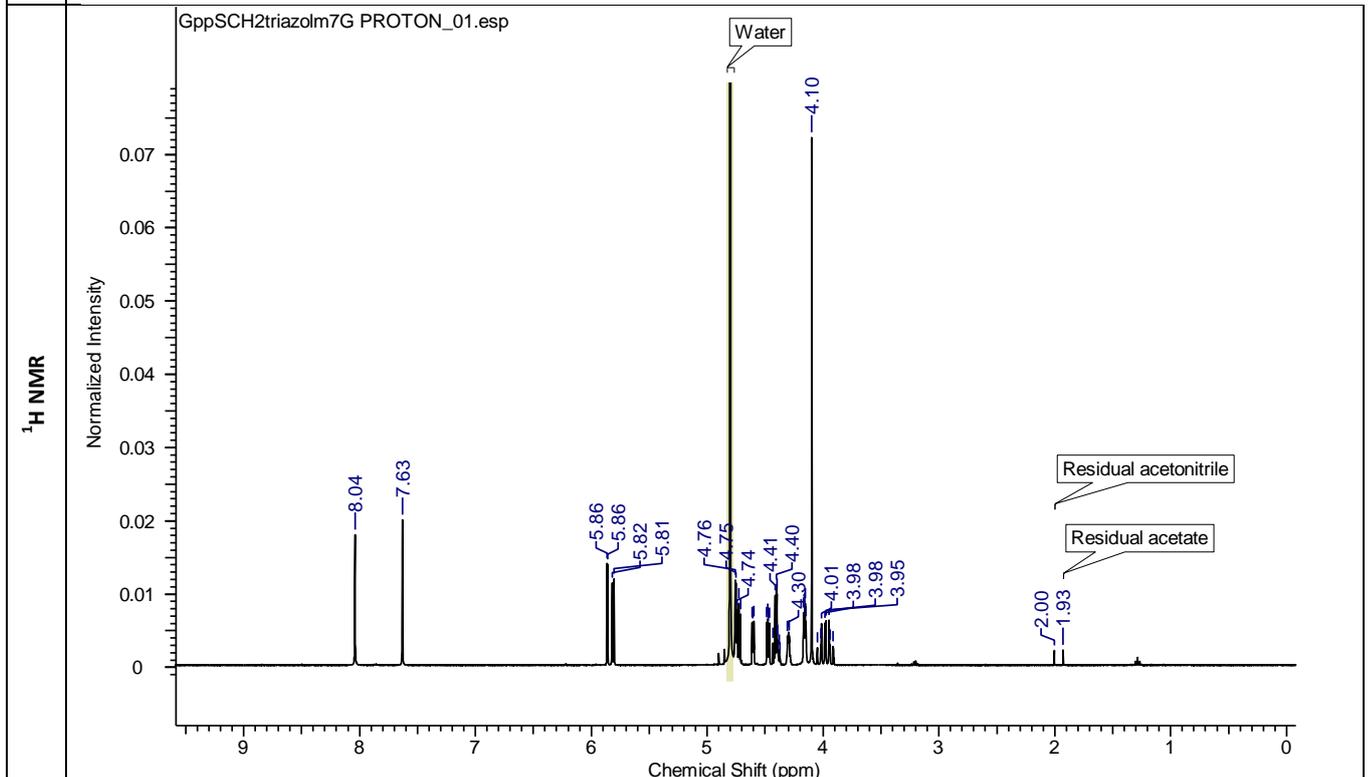
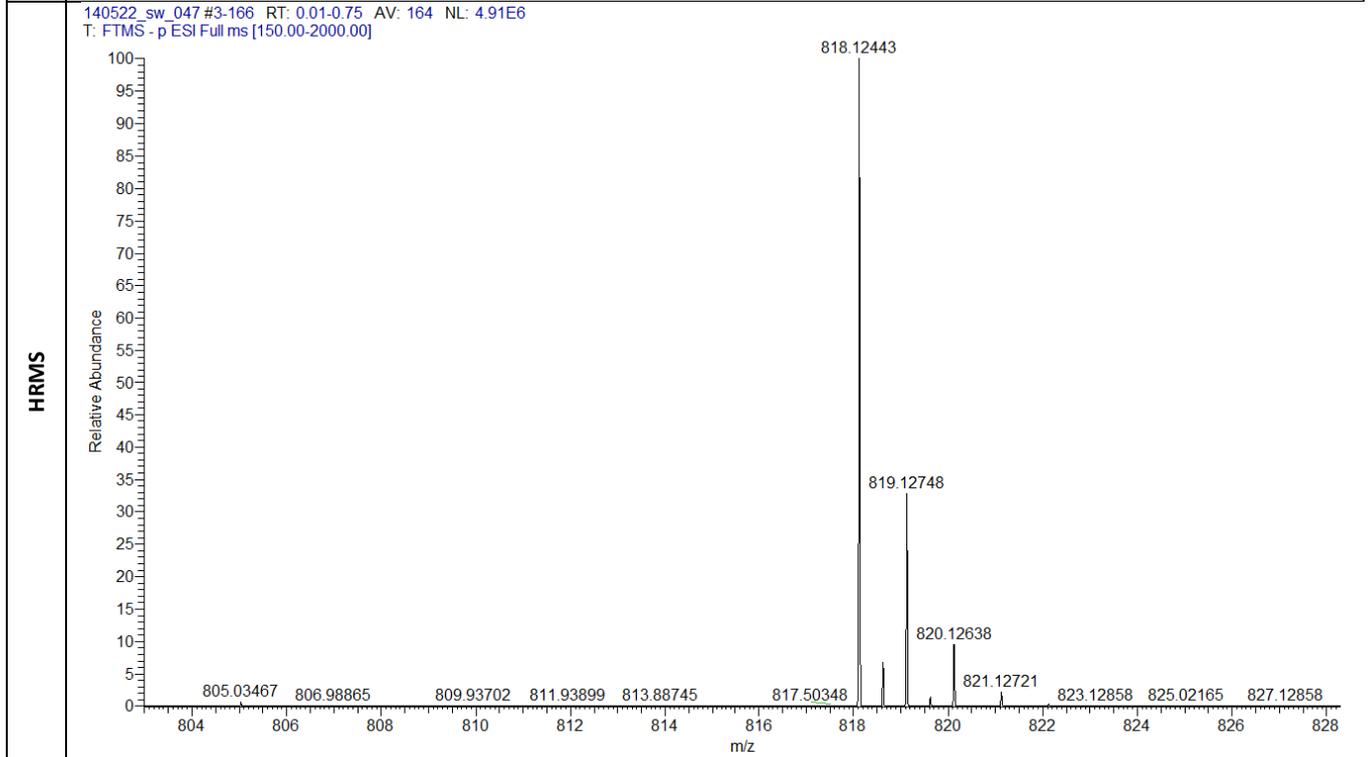
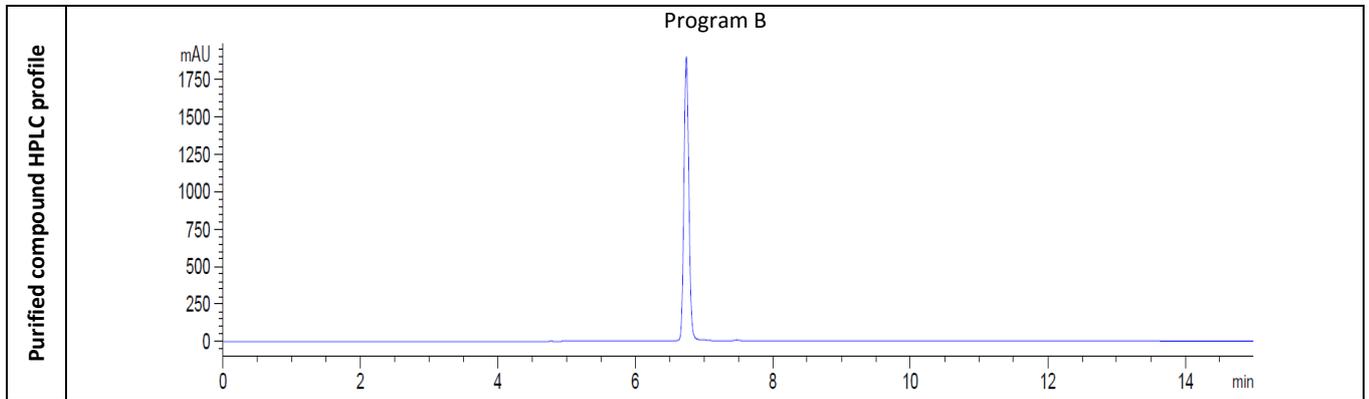


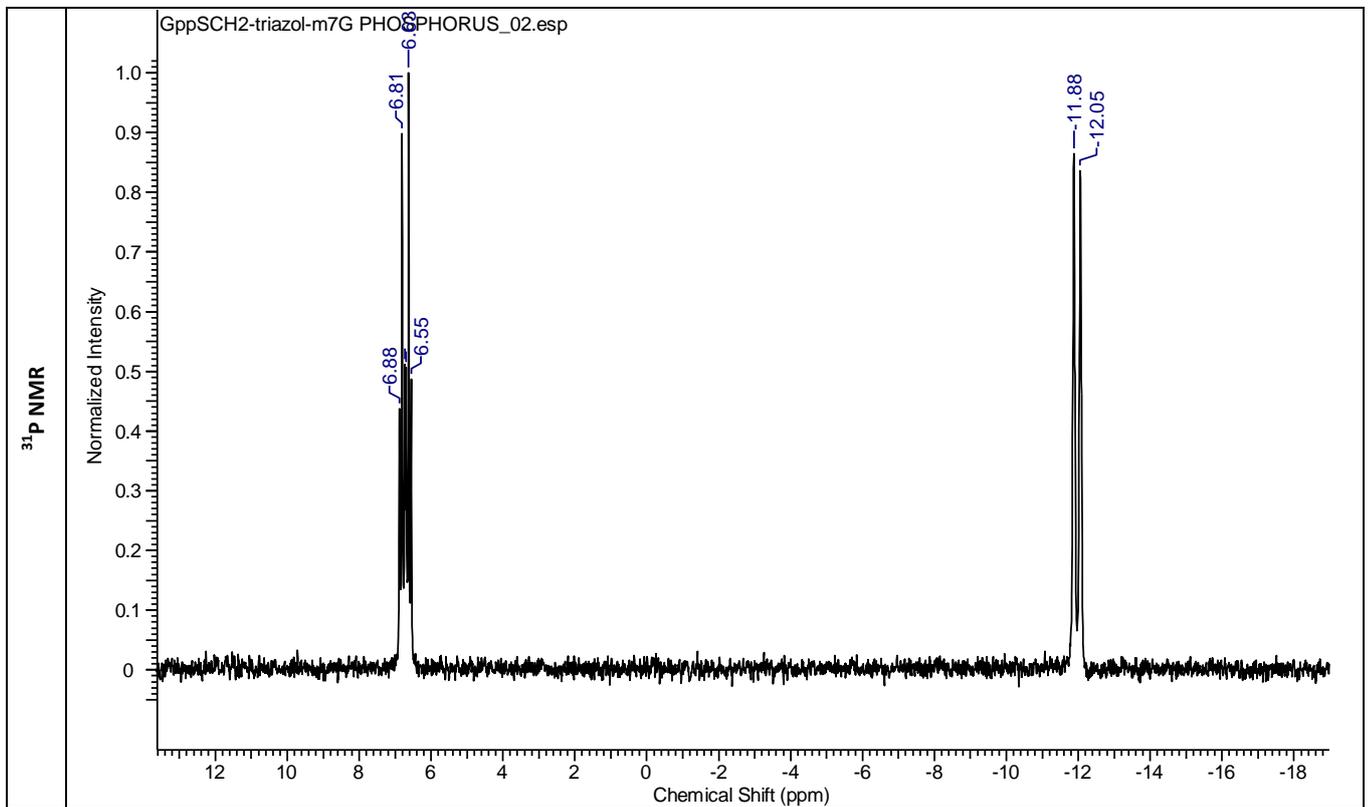




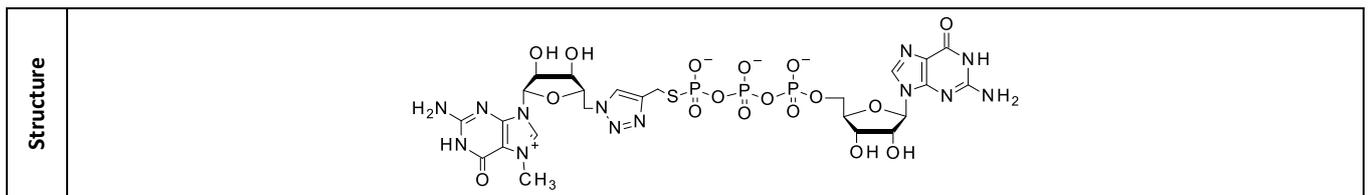
**(5a) m<sup>7</sup>G-triazole-CH<sub>2</sub>SppG**

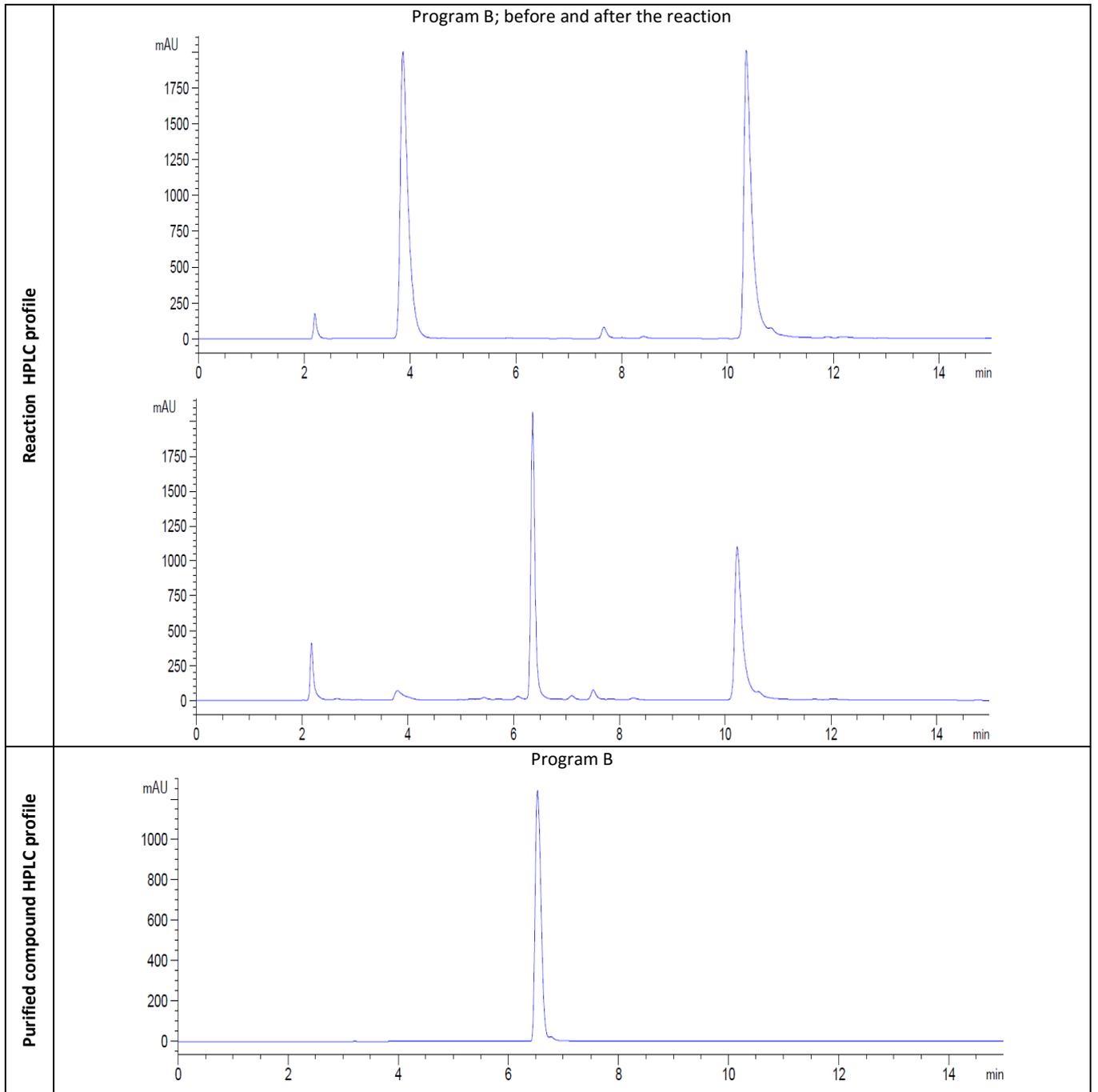






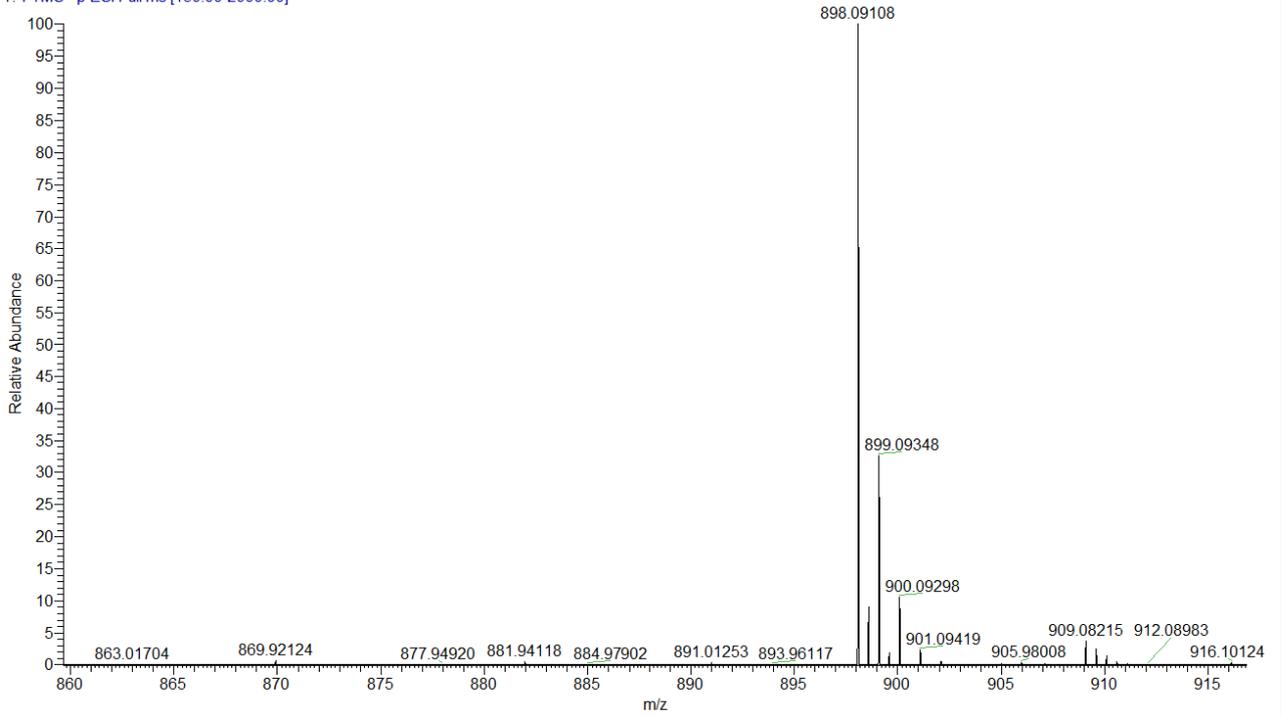
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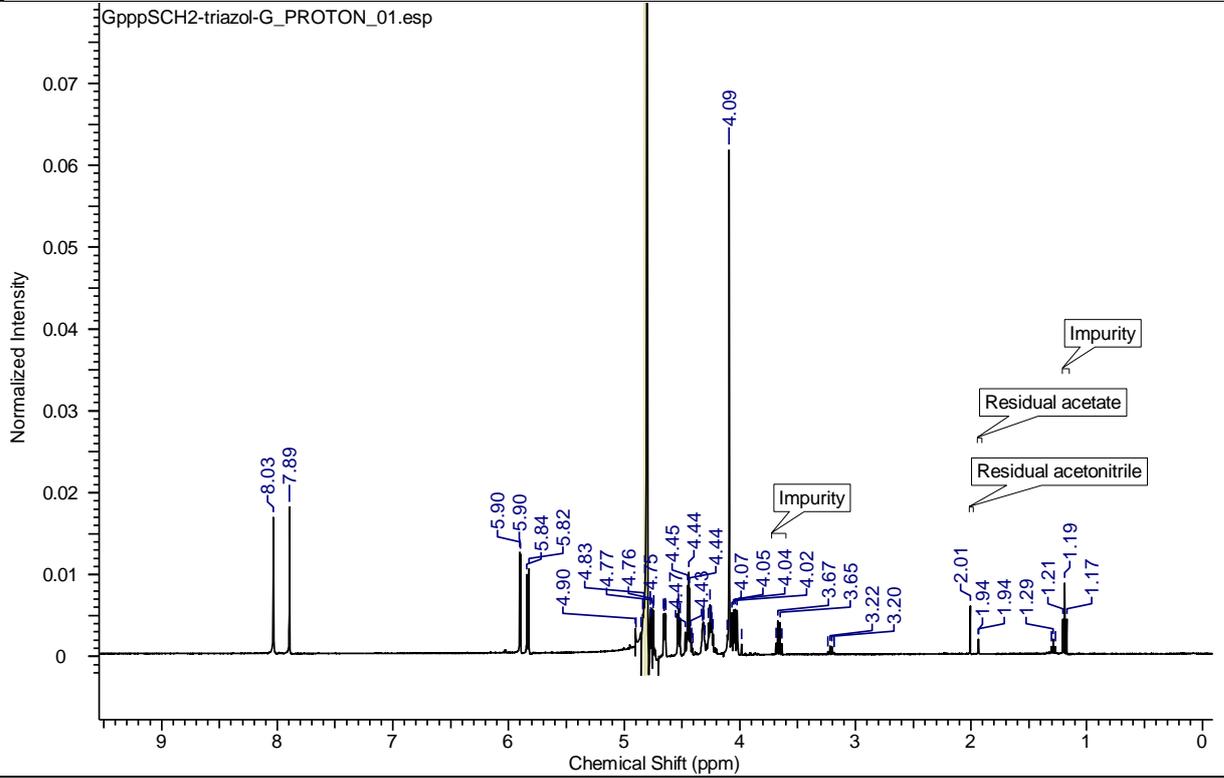


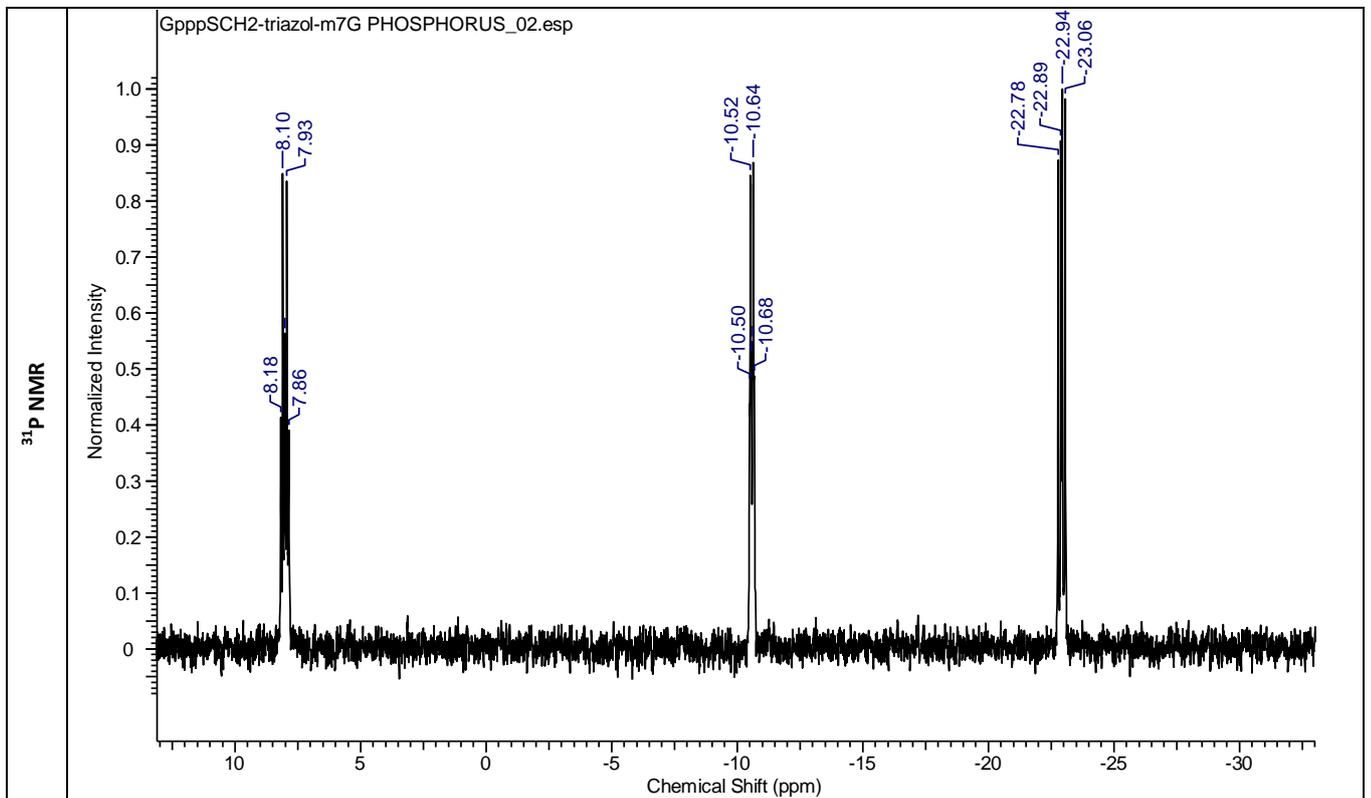
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HRMS

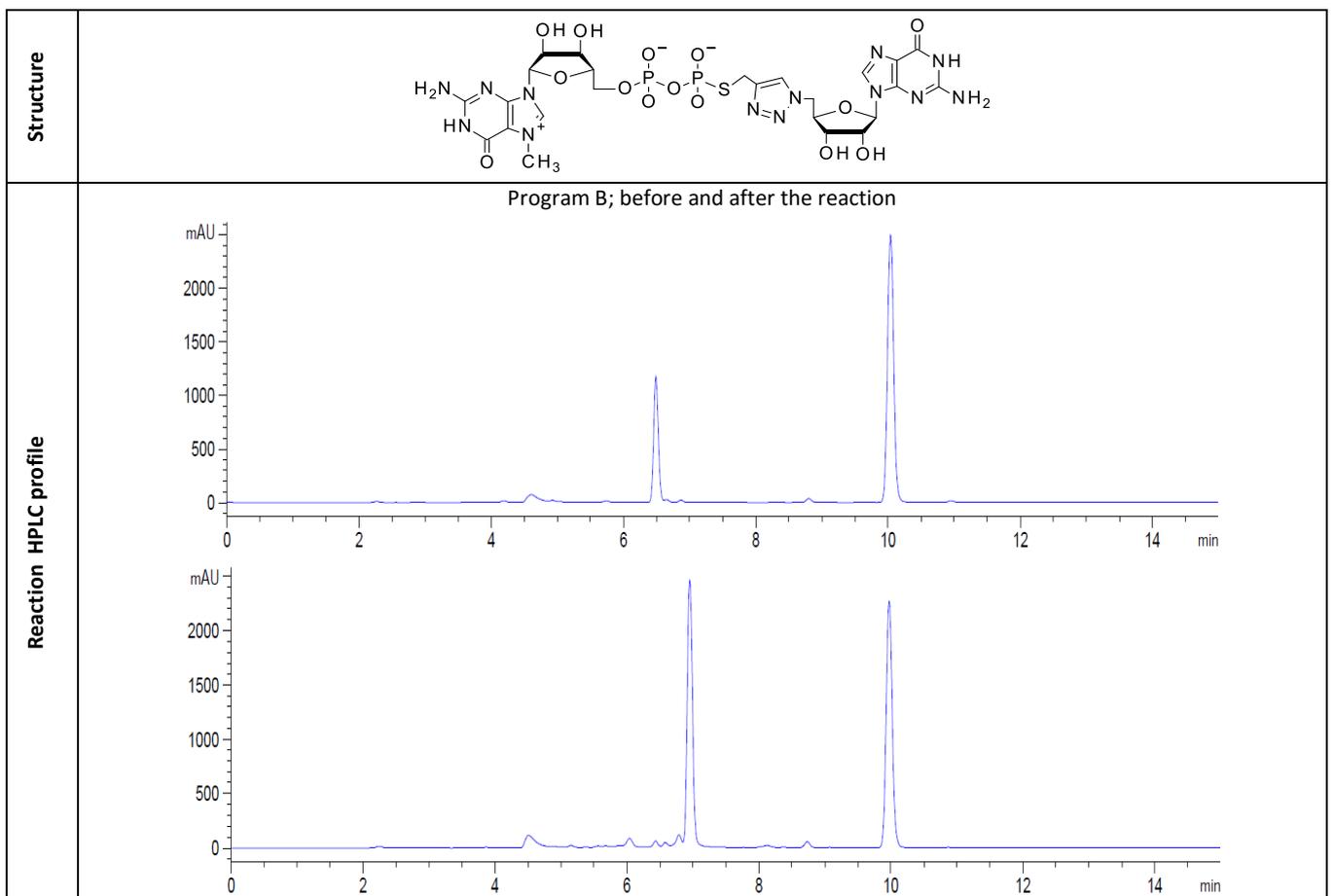


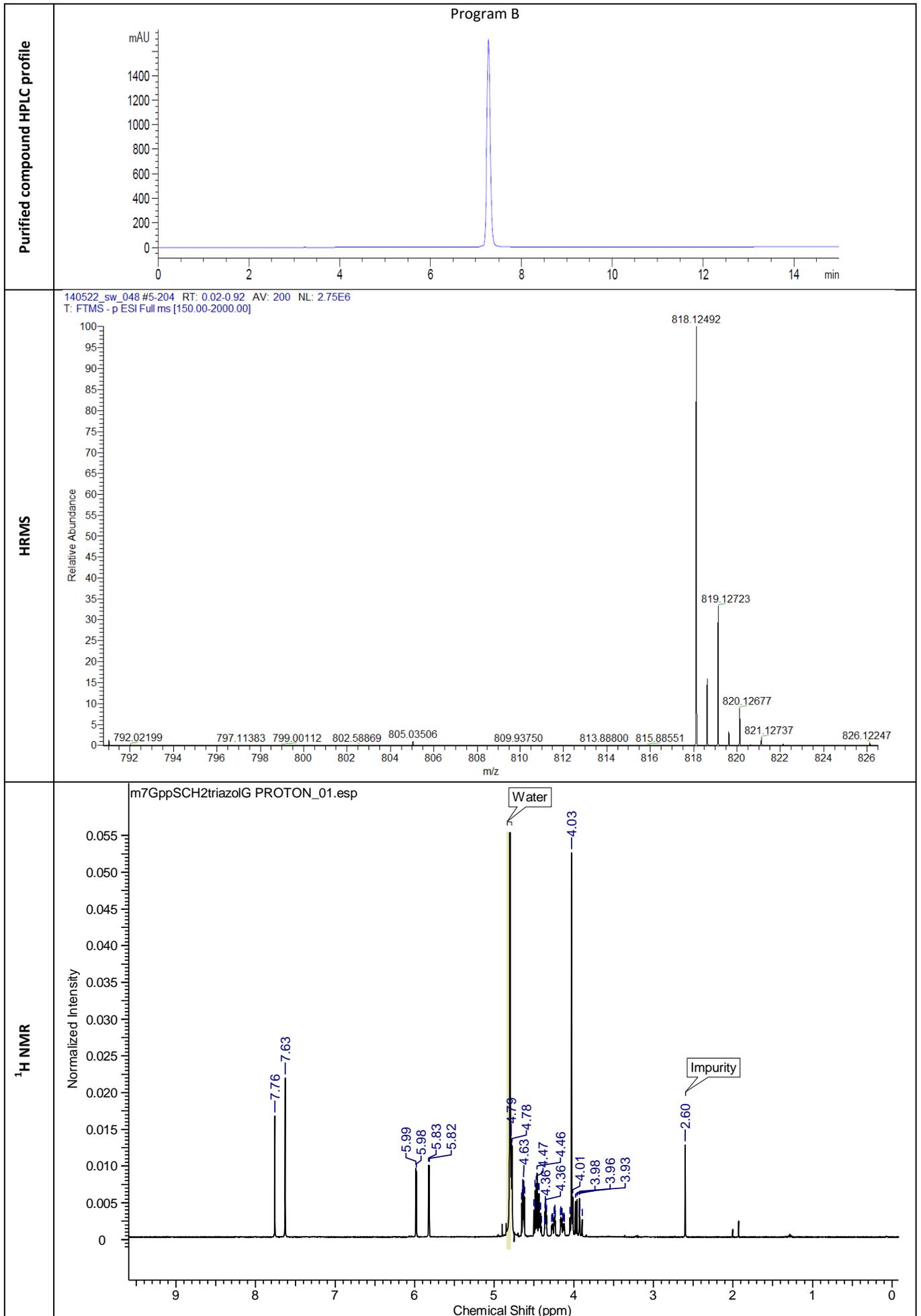
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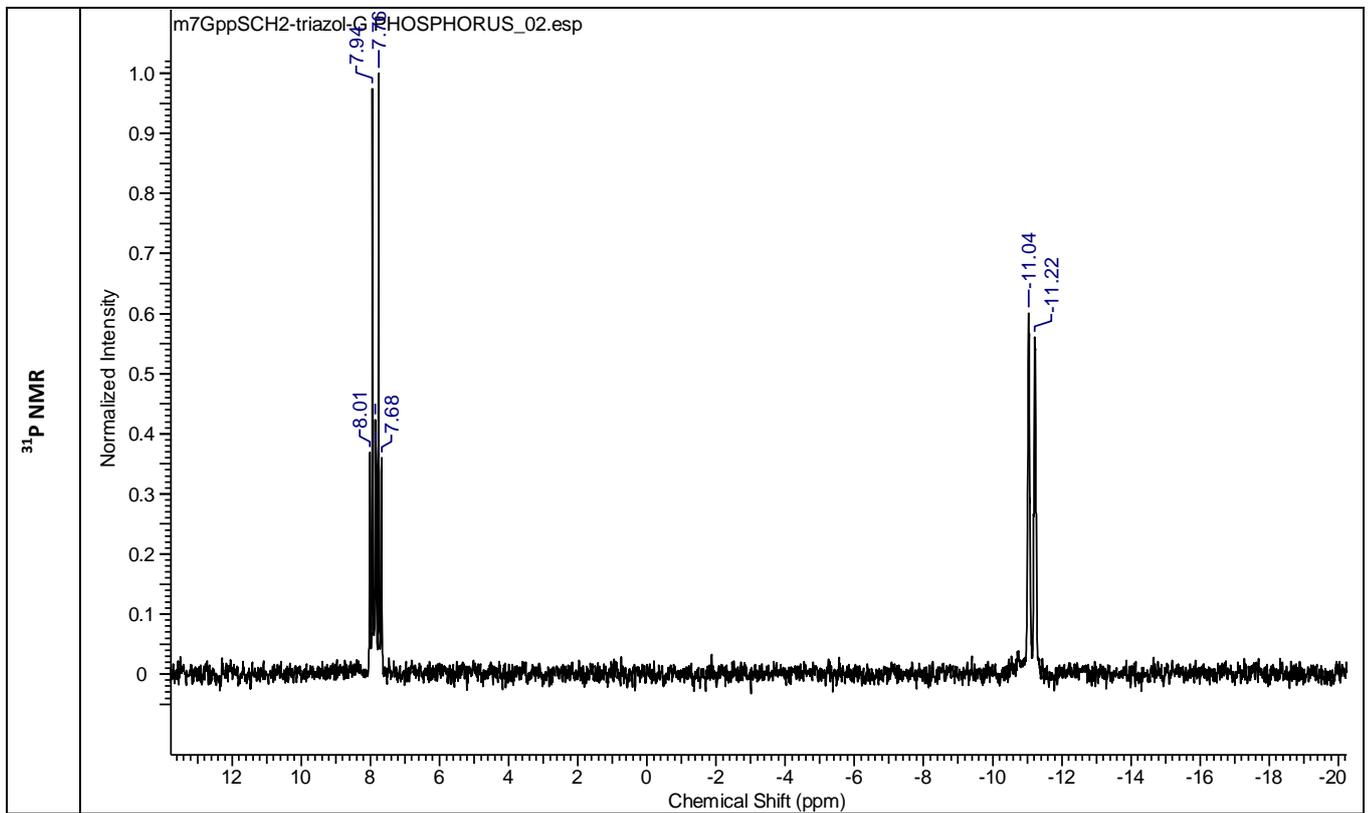




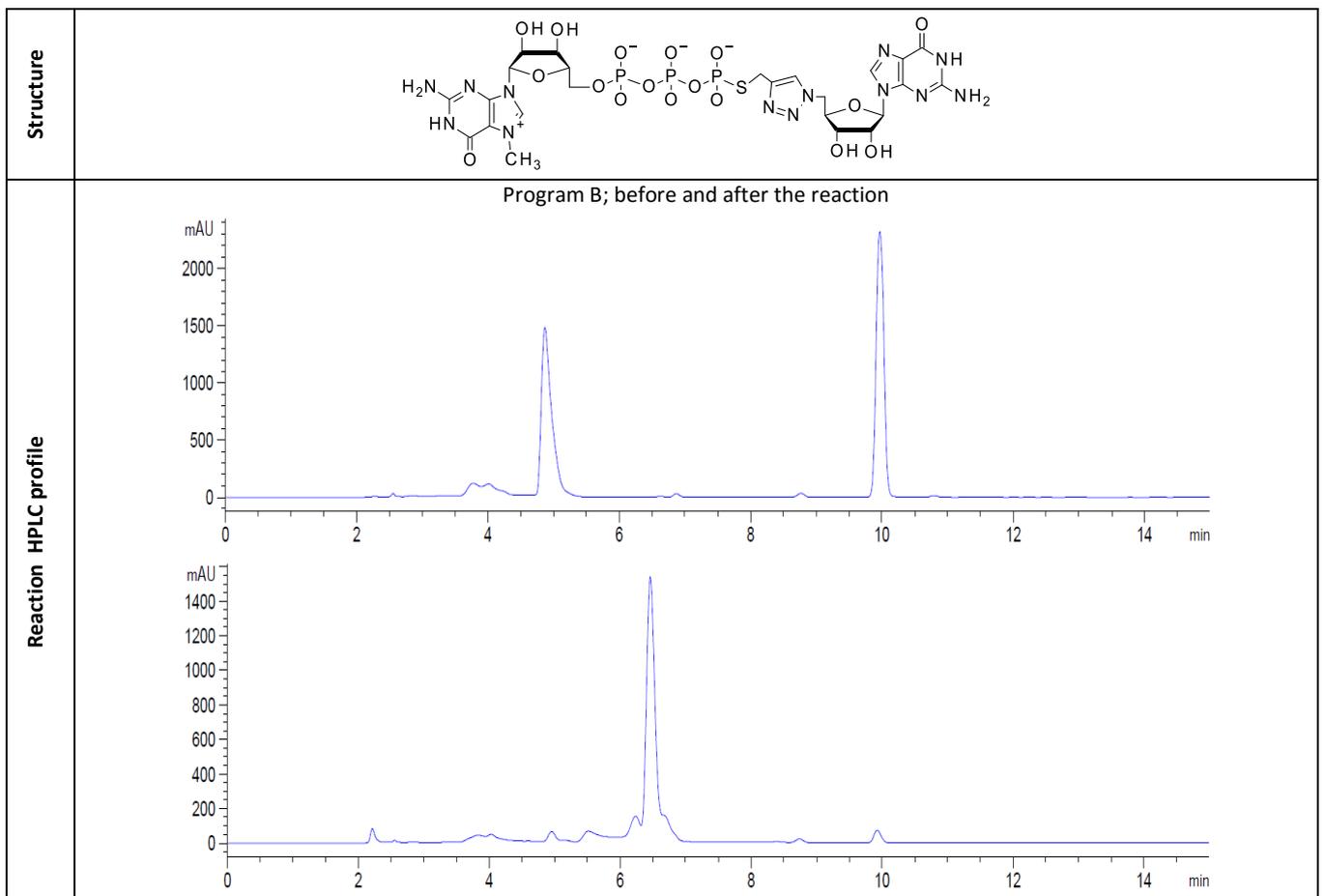
**(5c) m<sup>7</sup>GppSCH<sub>2</sub>-triazole-G**

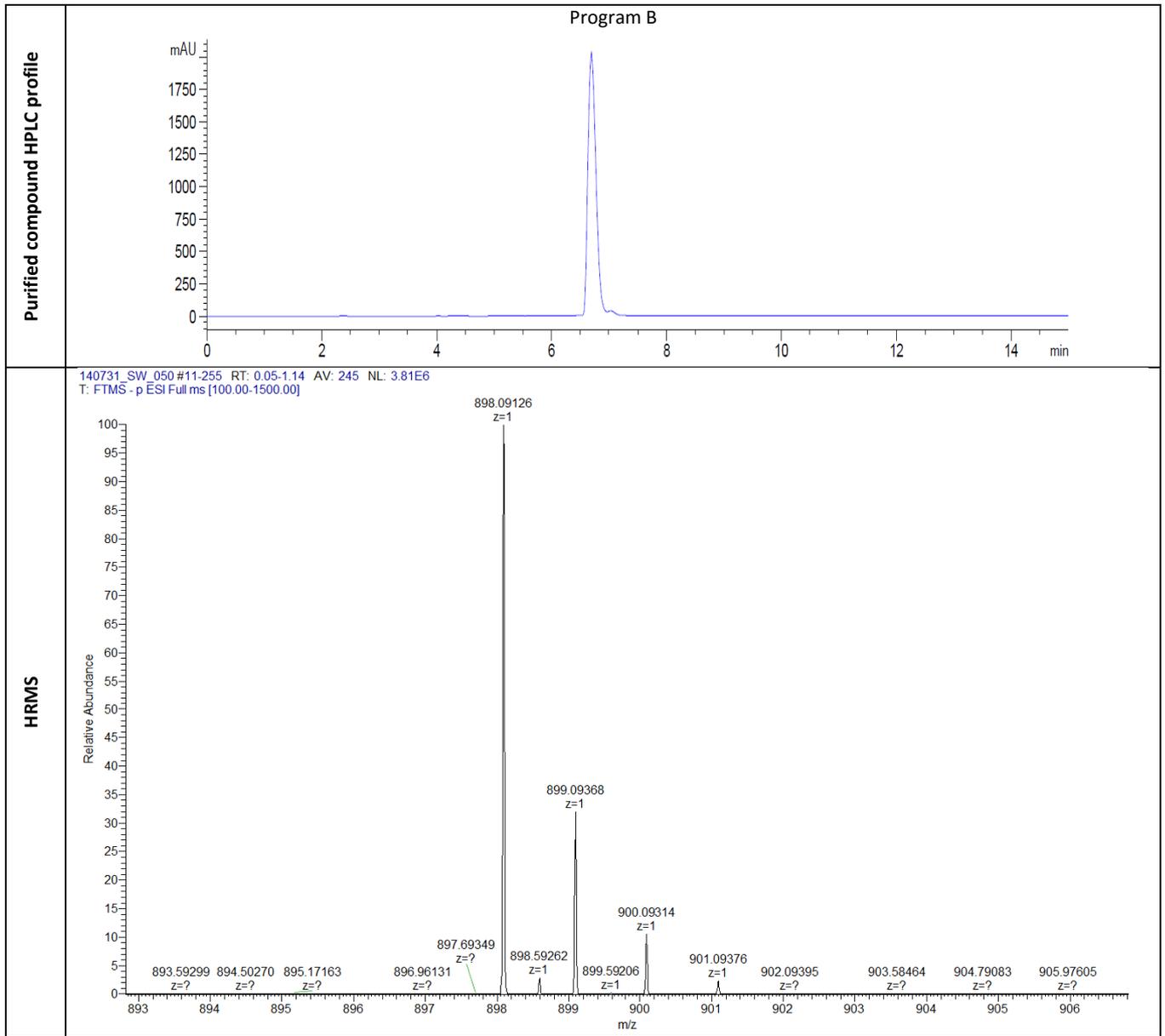


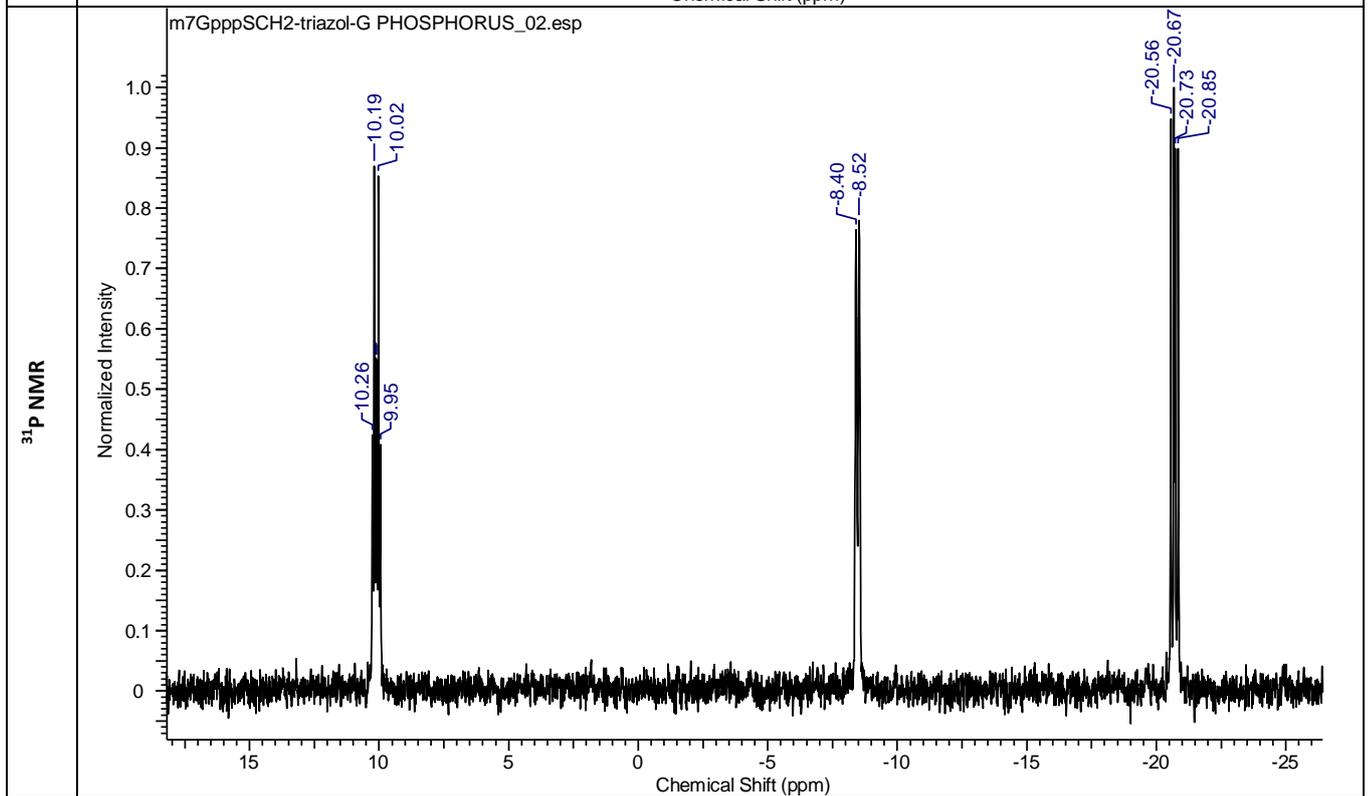
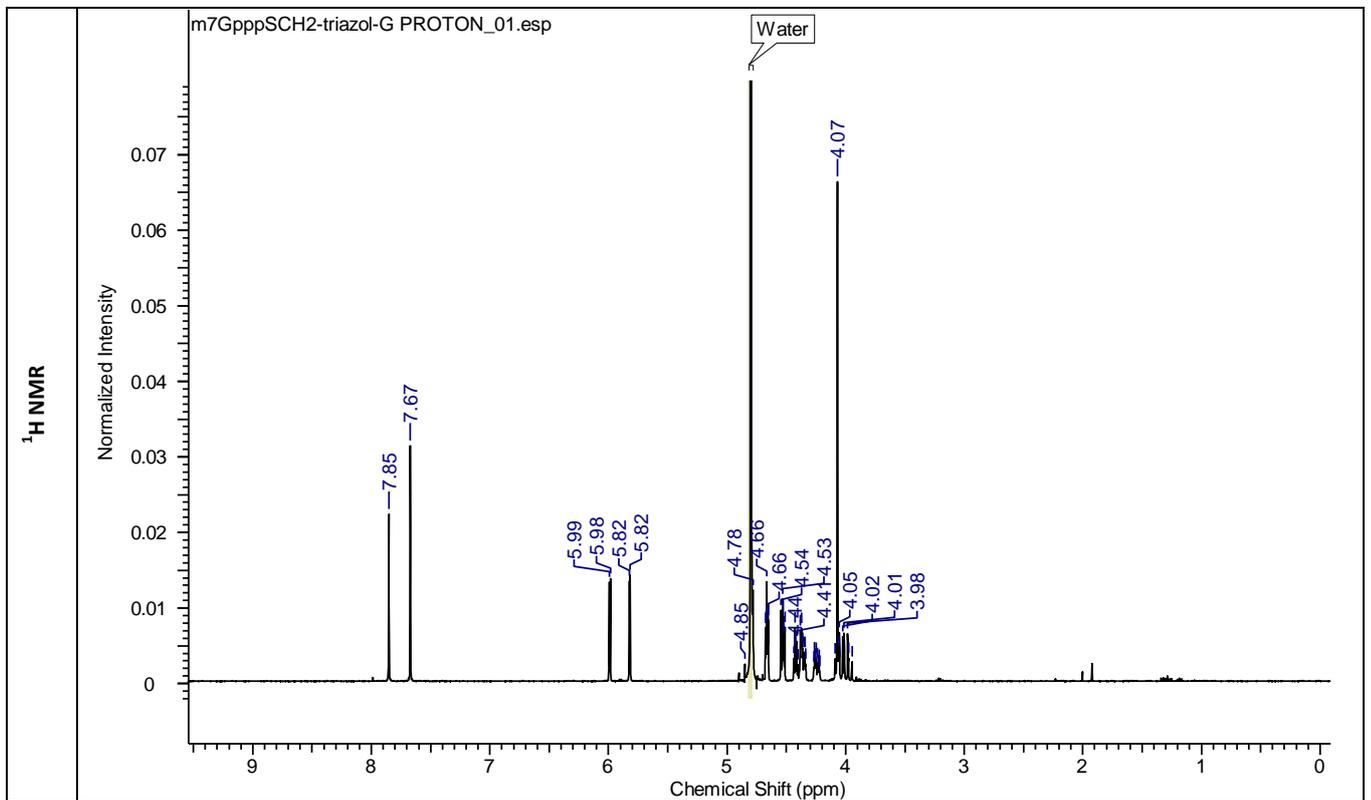




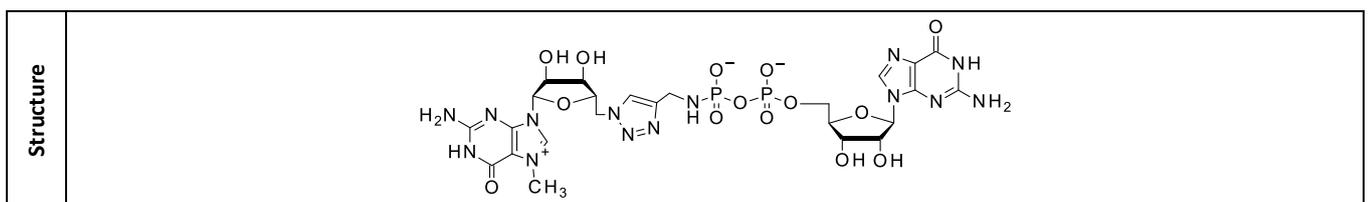
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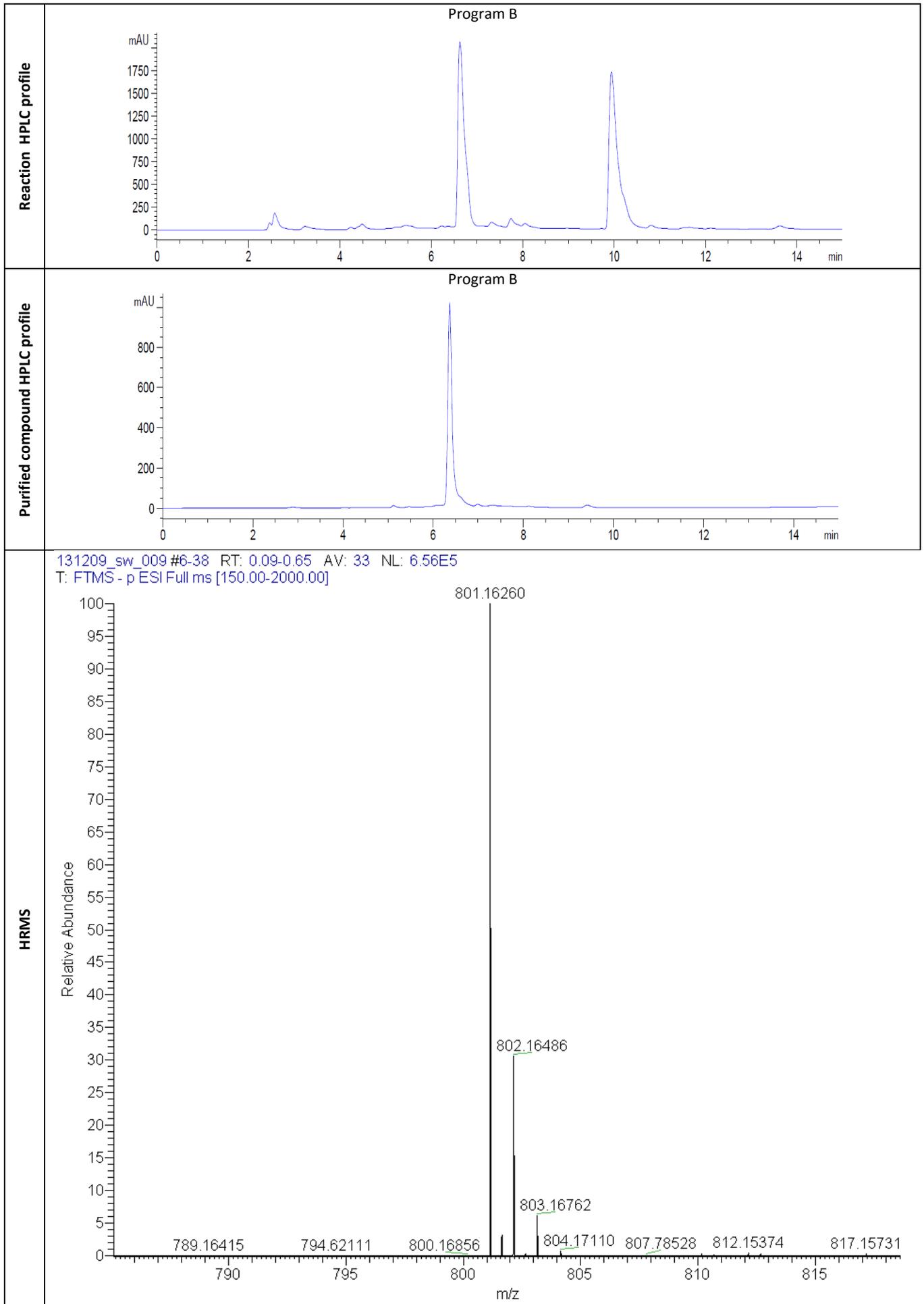


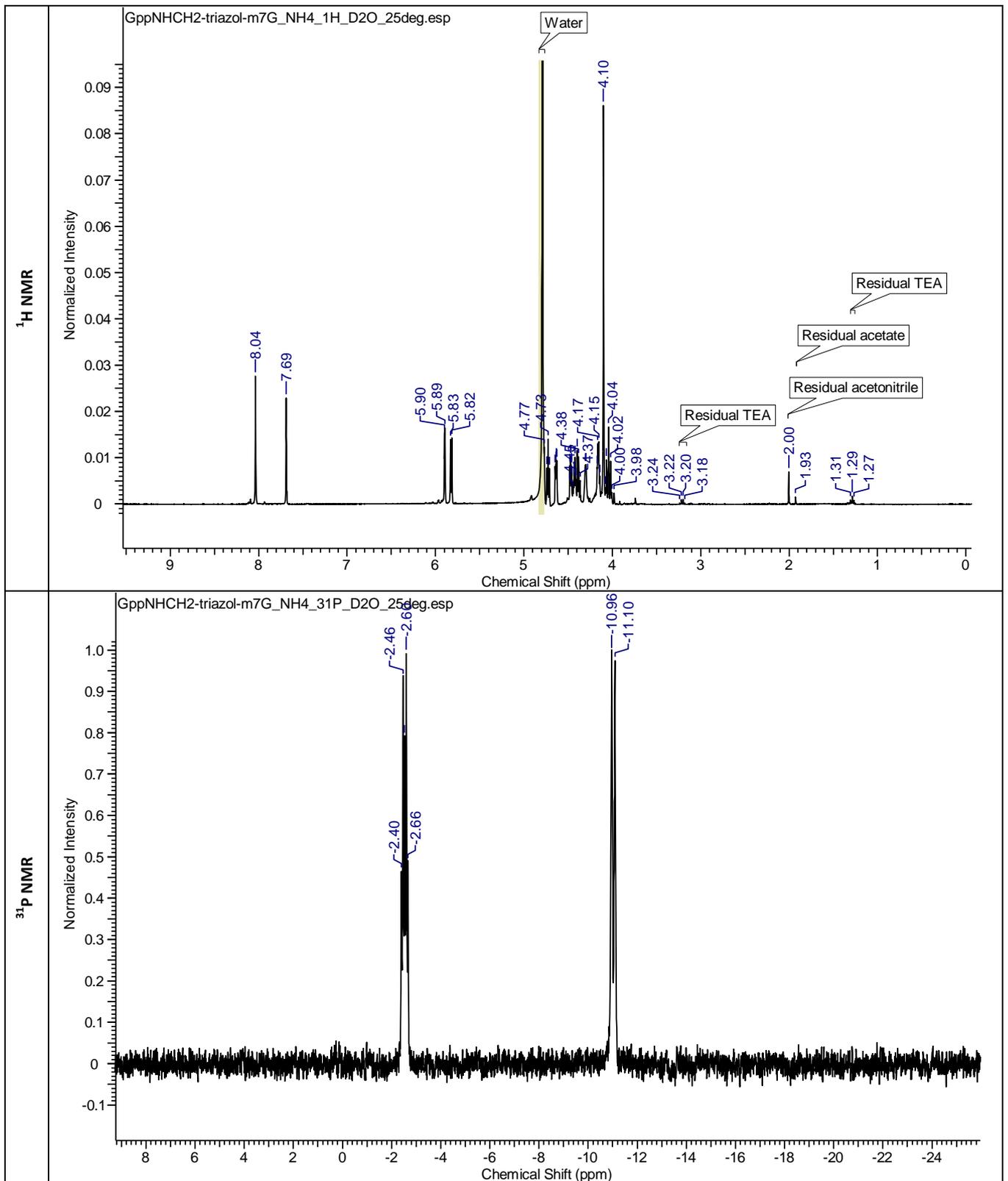




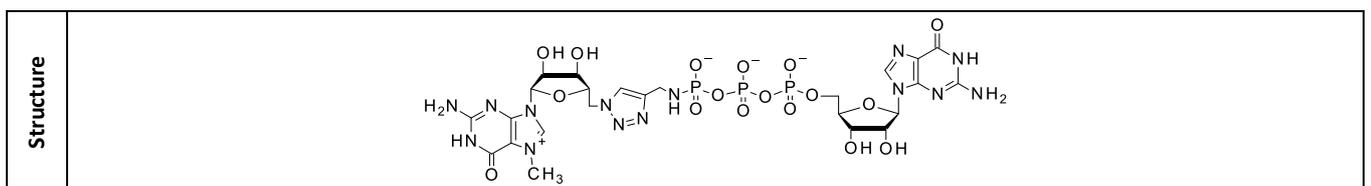
**(6a) m<sup>7</sup>G-triazole-CH<sub>2</sub>NHppG**

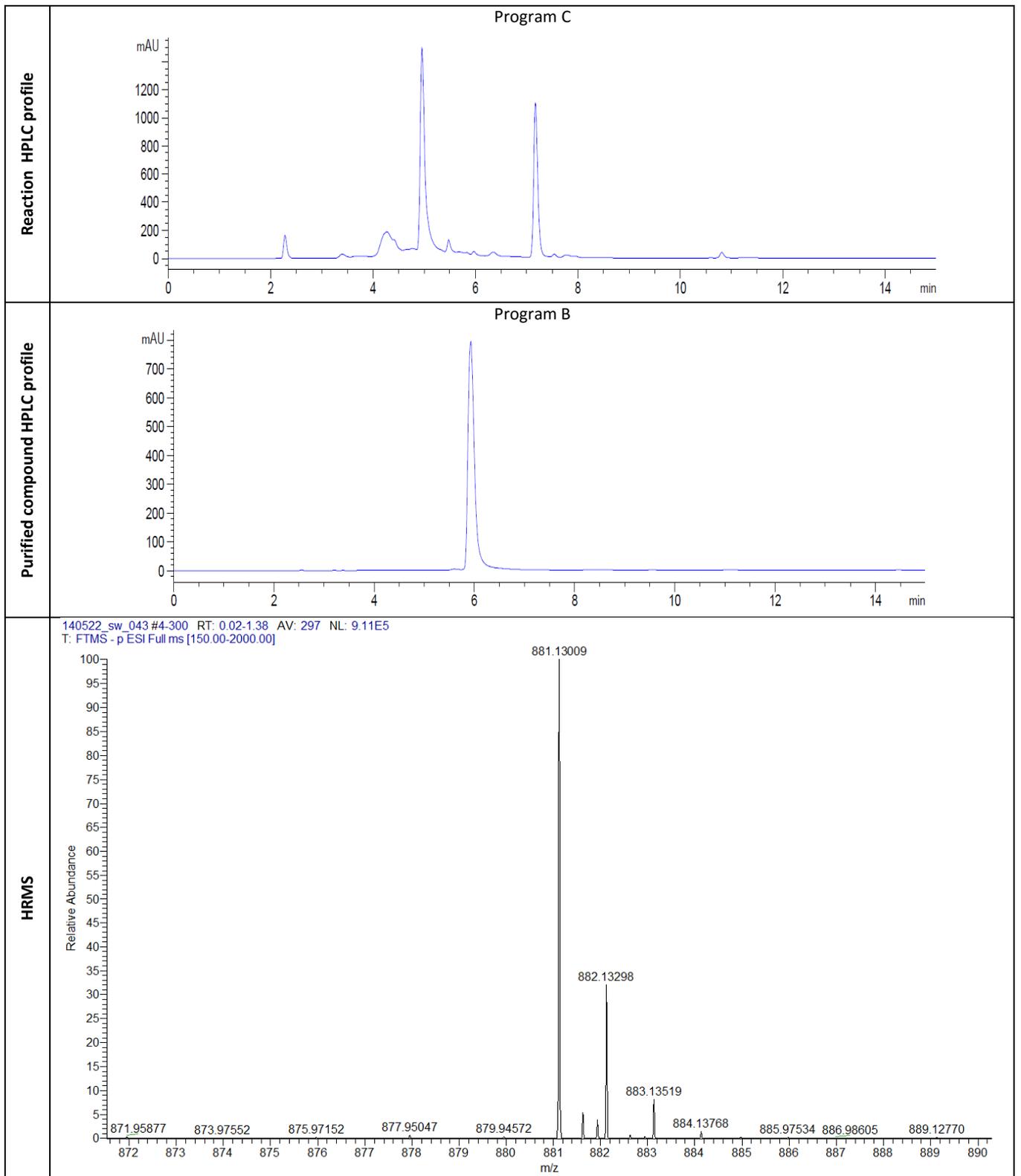


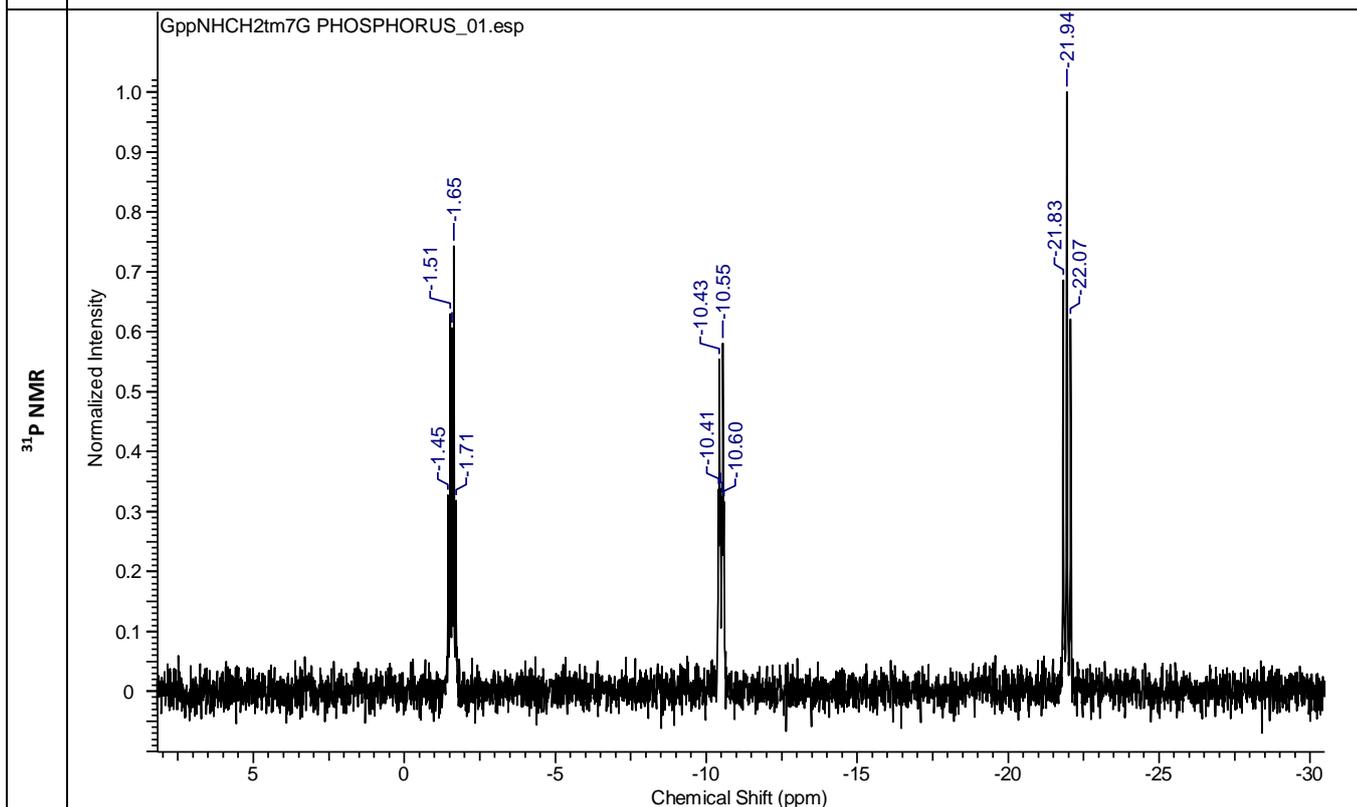
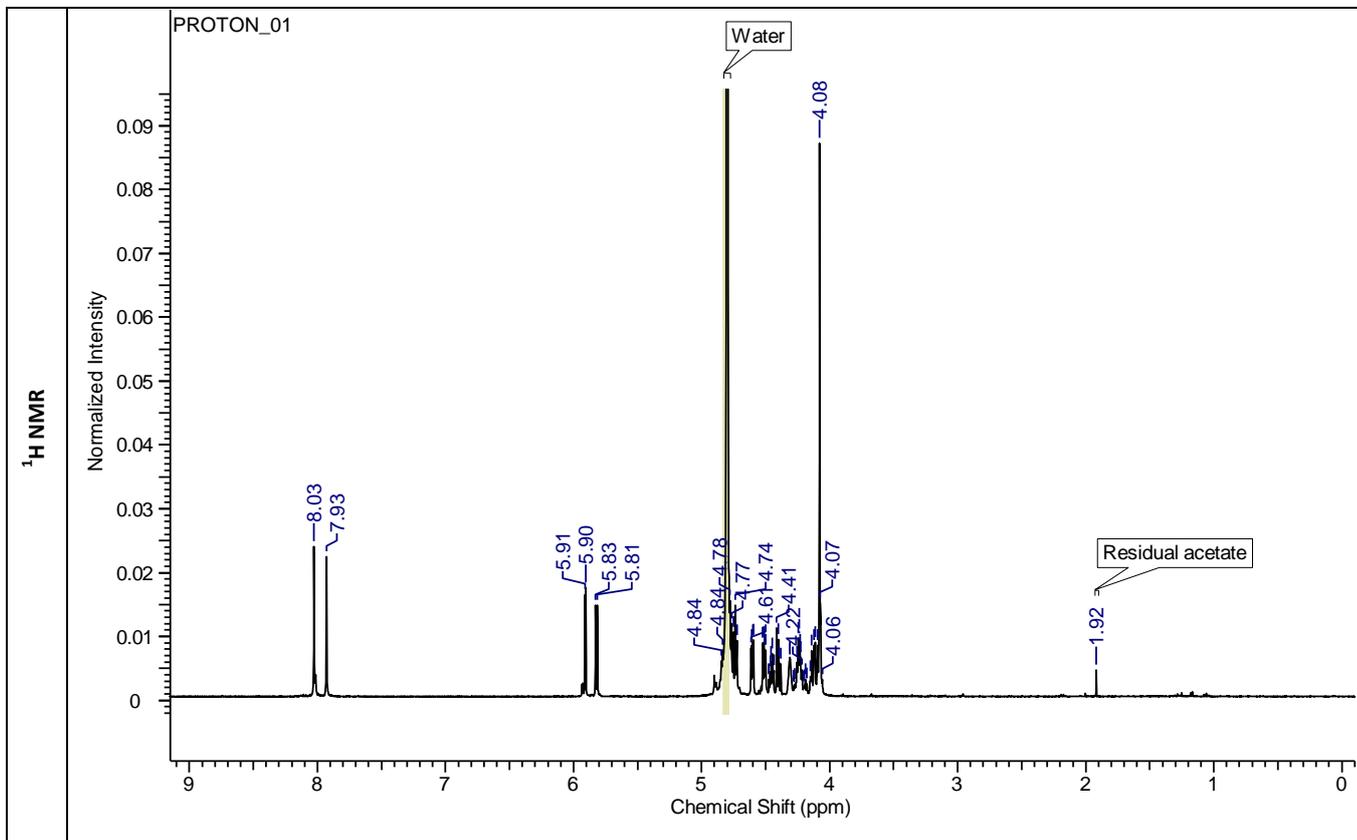




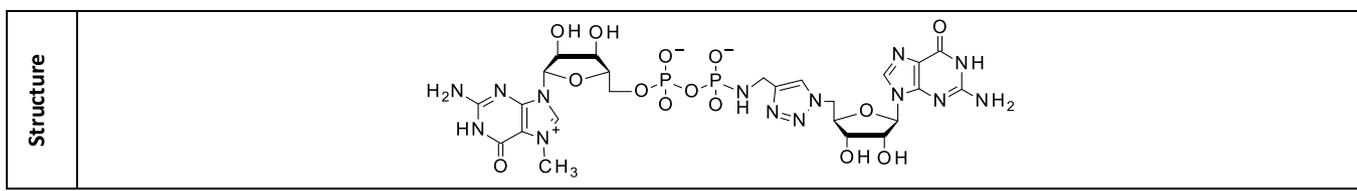
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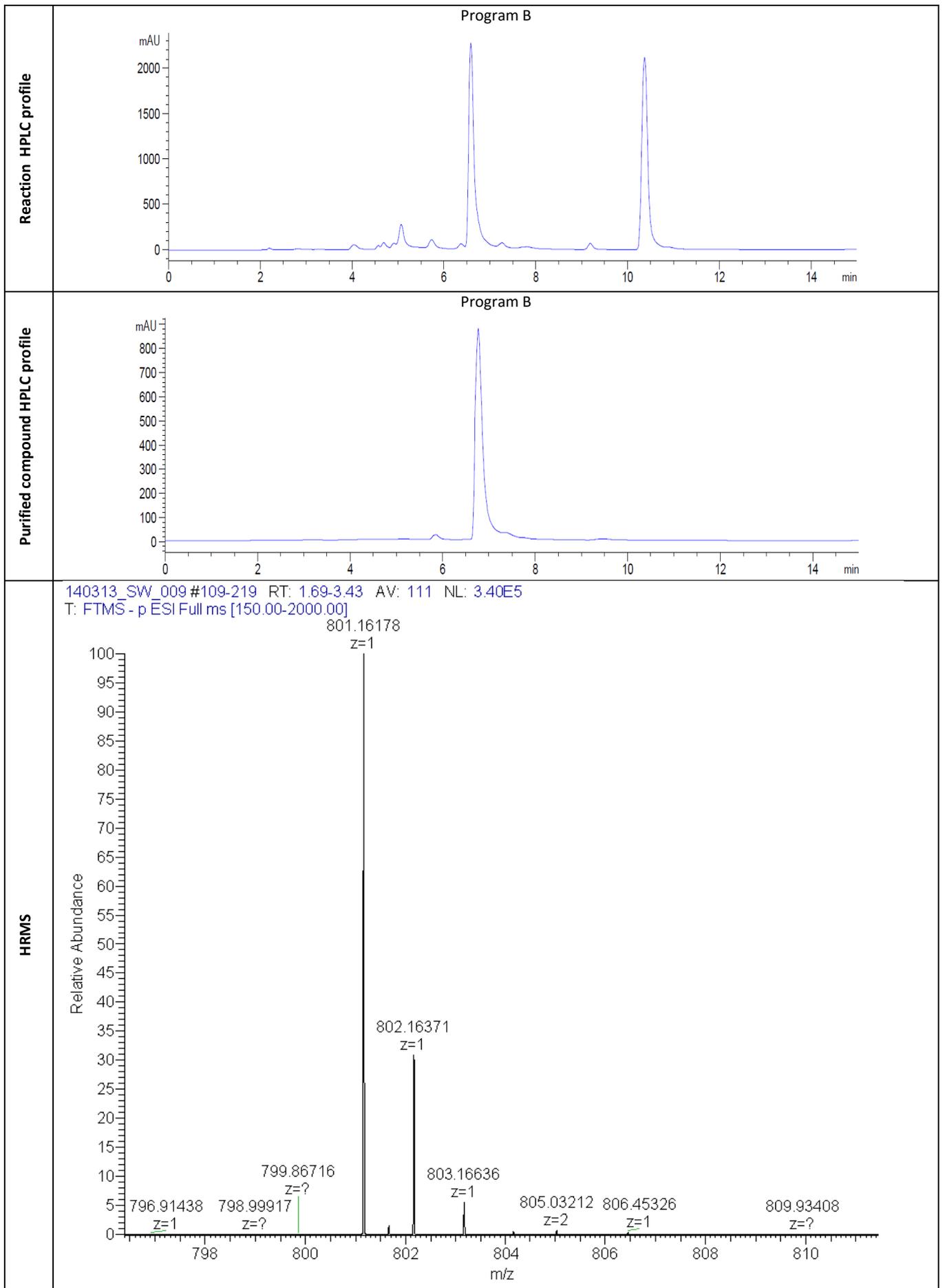


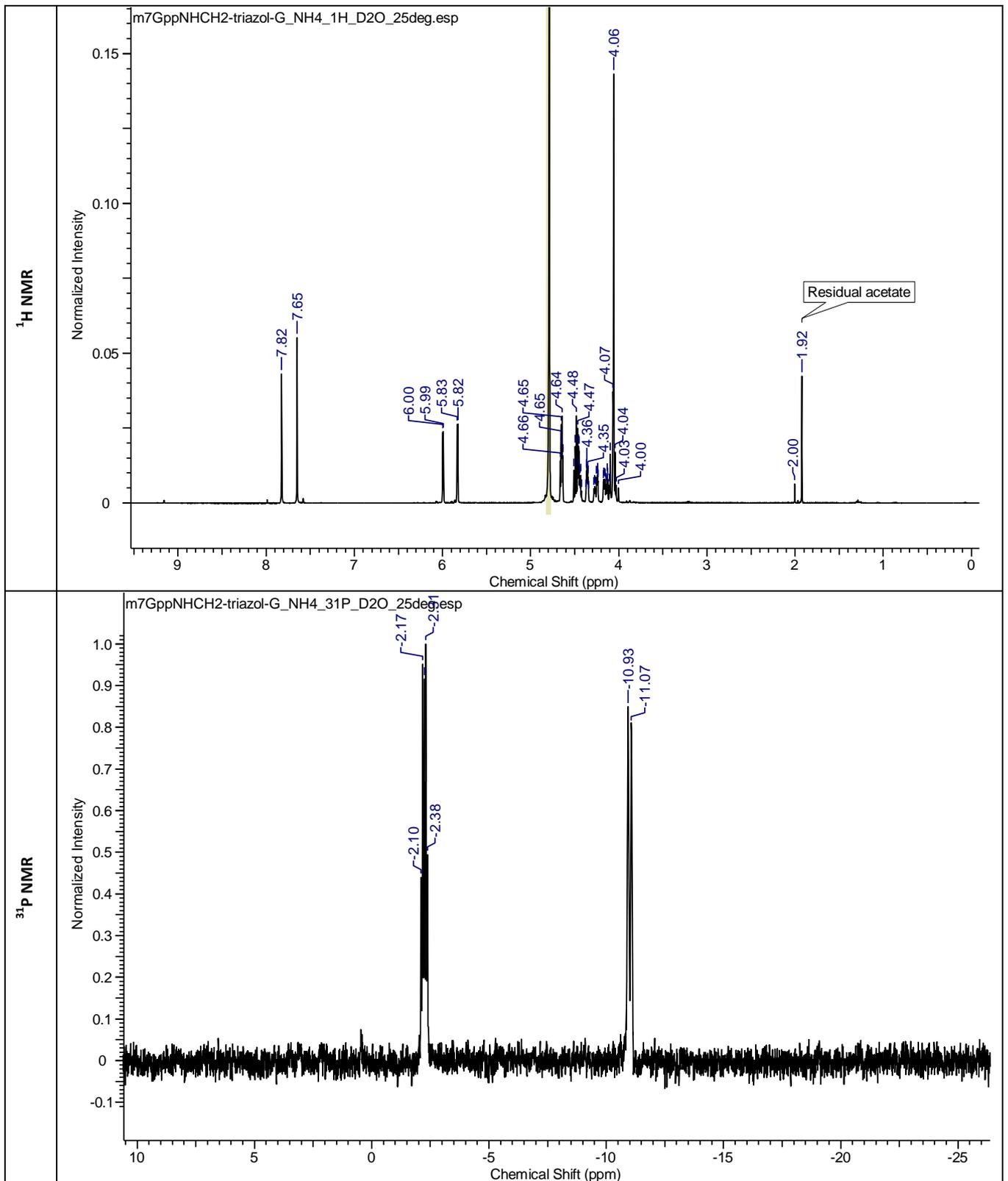




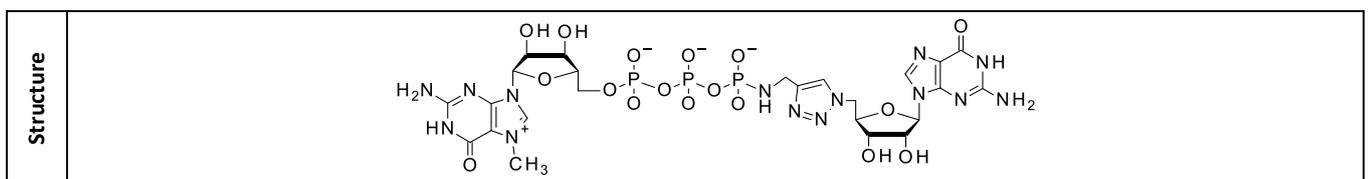
**(6c) m<sup>7</sup>GppNHCH<sub>2</sub>-trazole-G**

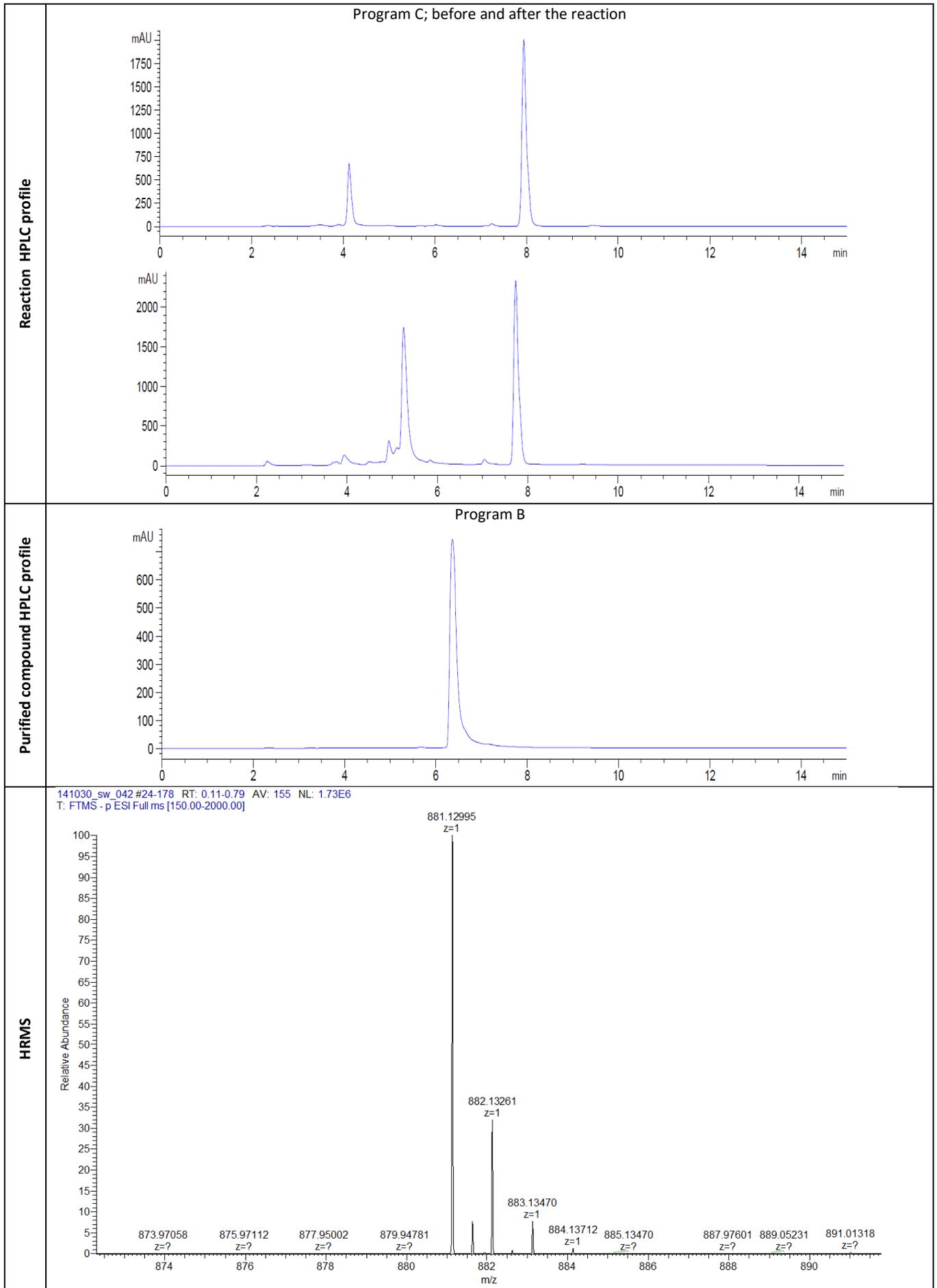


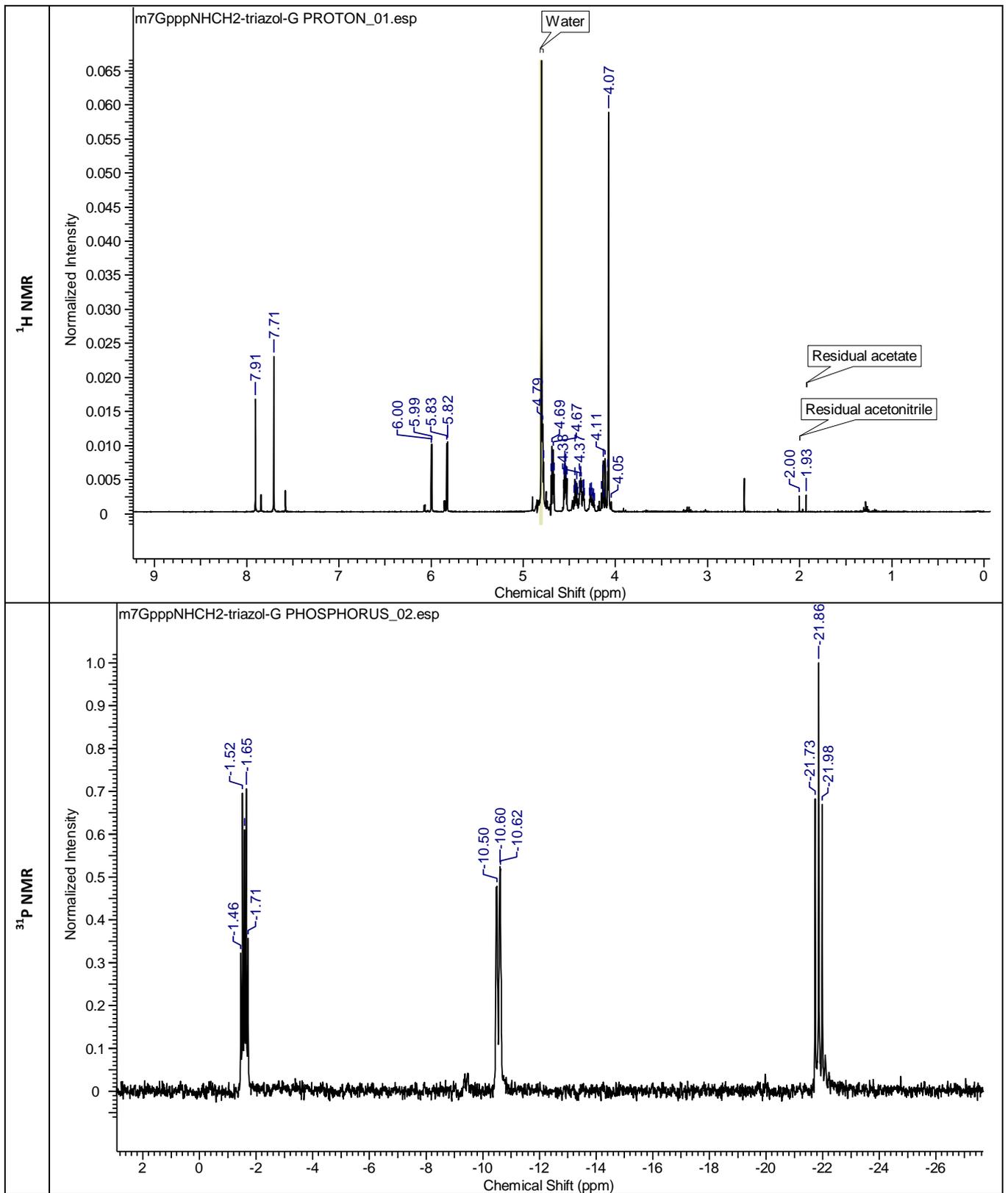




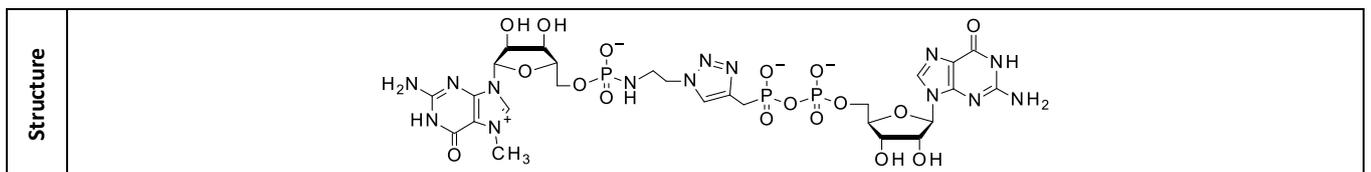
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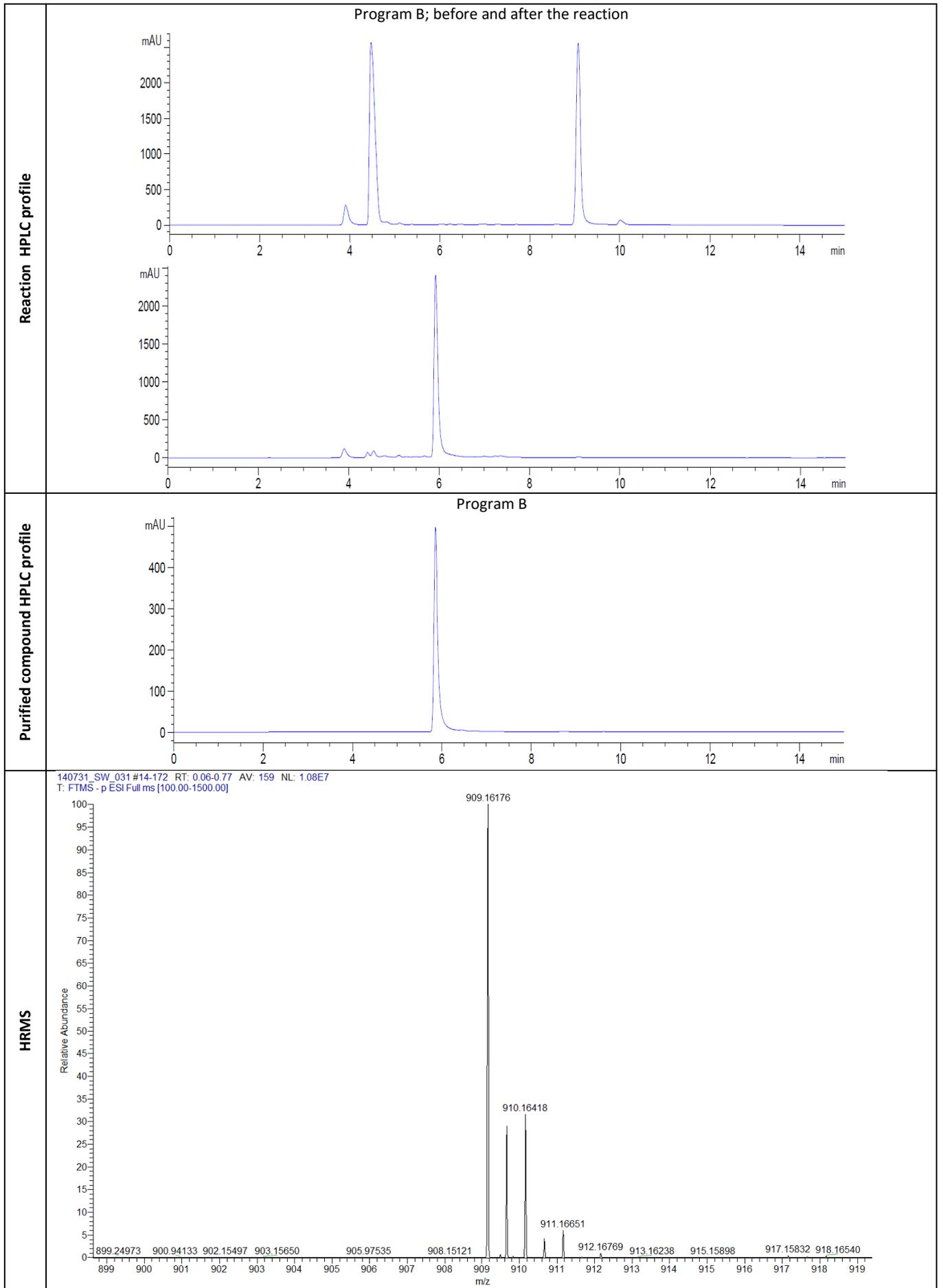


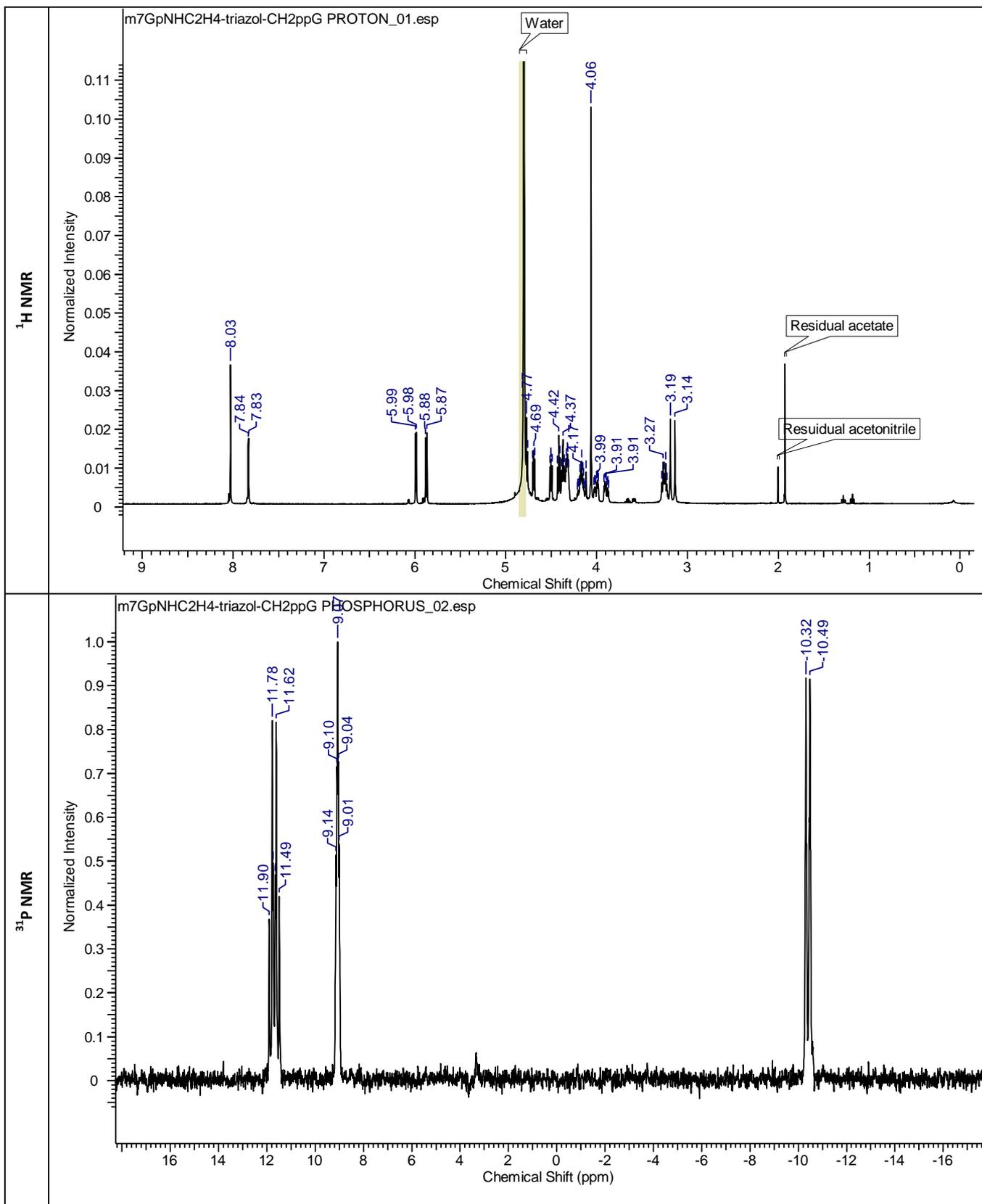




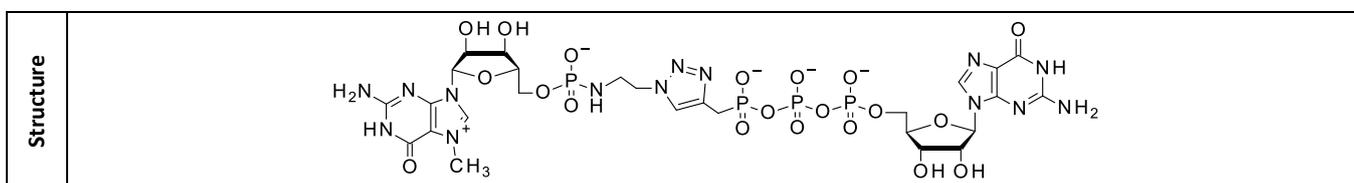
**(7a) m<sup>7</sup>GpNHC<sub>2</sub>H<sub>4</sub>-triazole-CH<sub>2</sub>ppG**

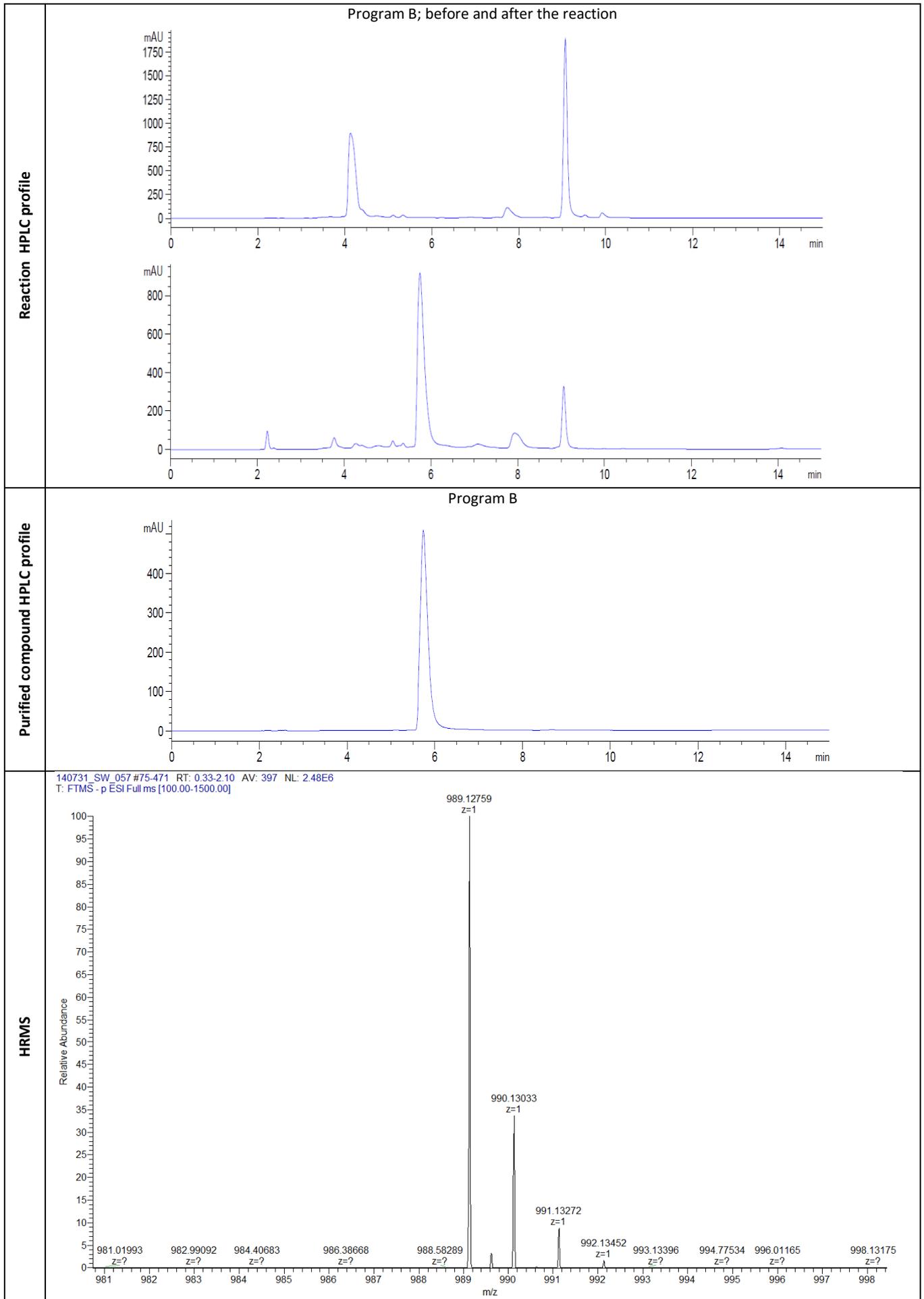


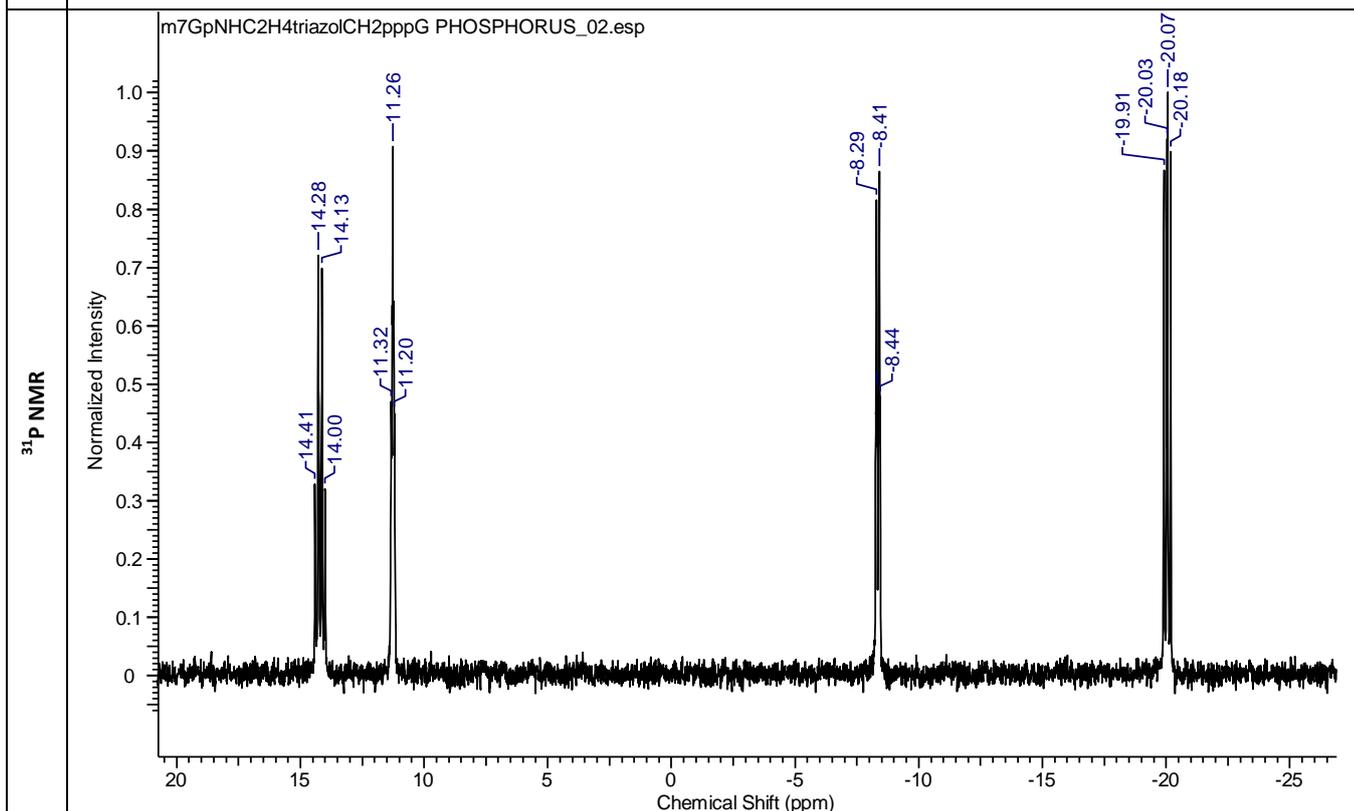
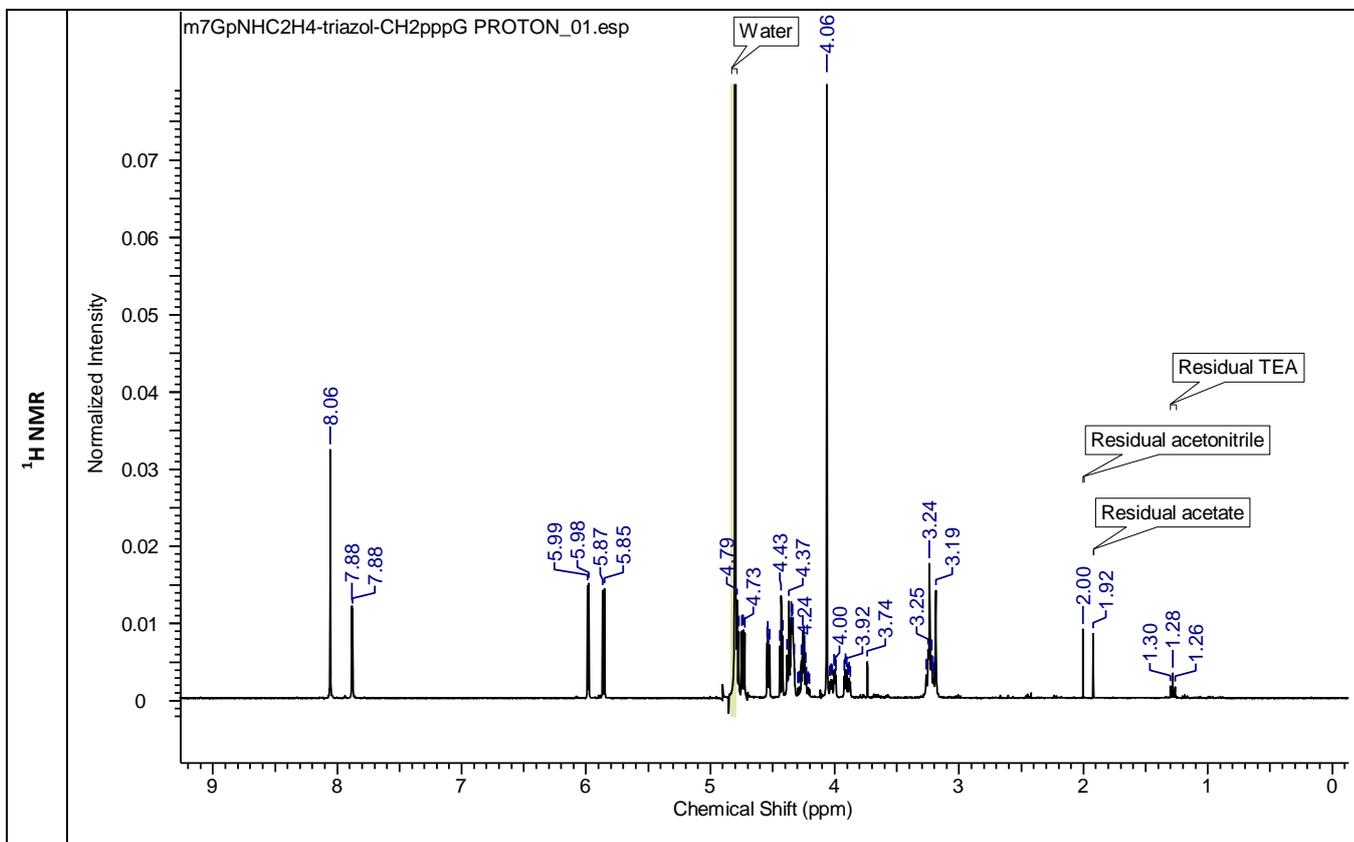




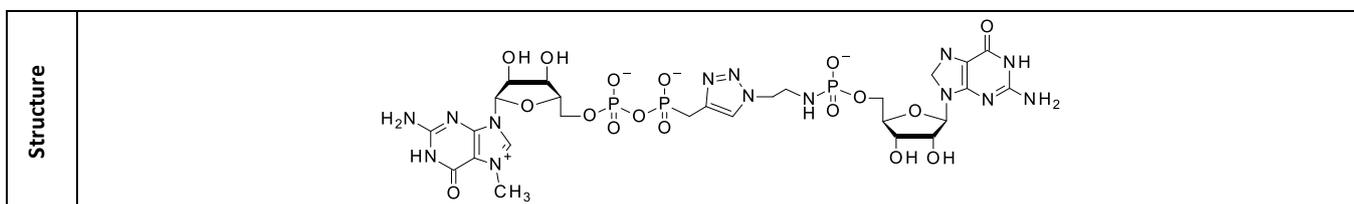
**(7b) m<sup>7</sup>GpNHC<sub>2</sub>H<sub>4</sub>-triazole-CH<sub>2</sub>pppG**

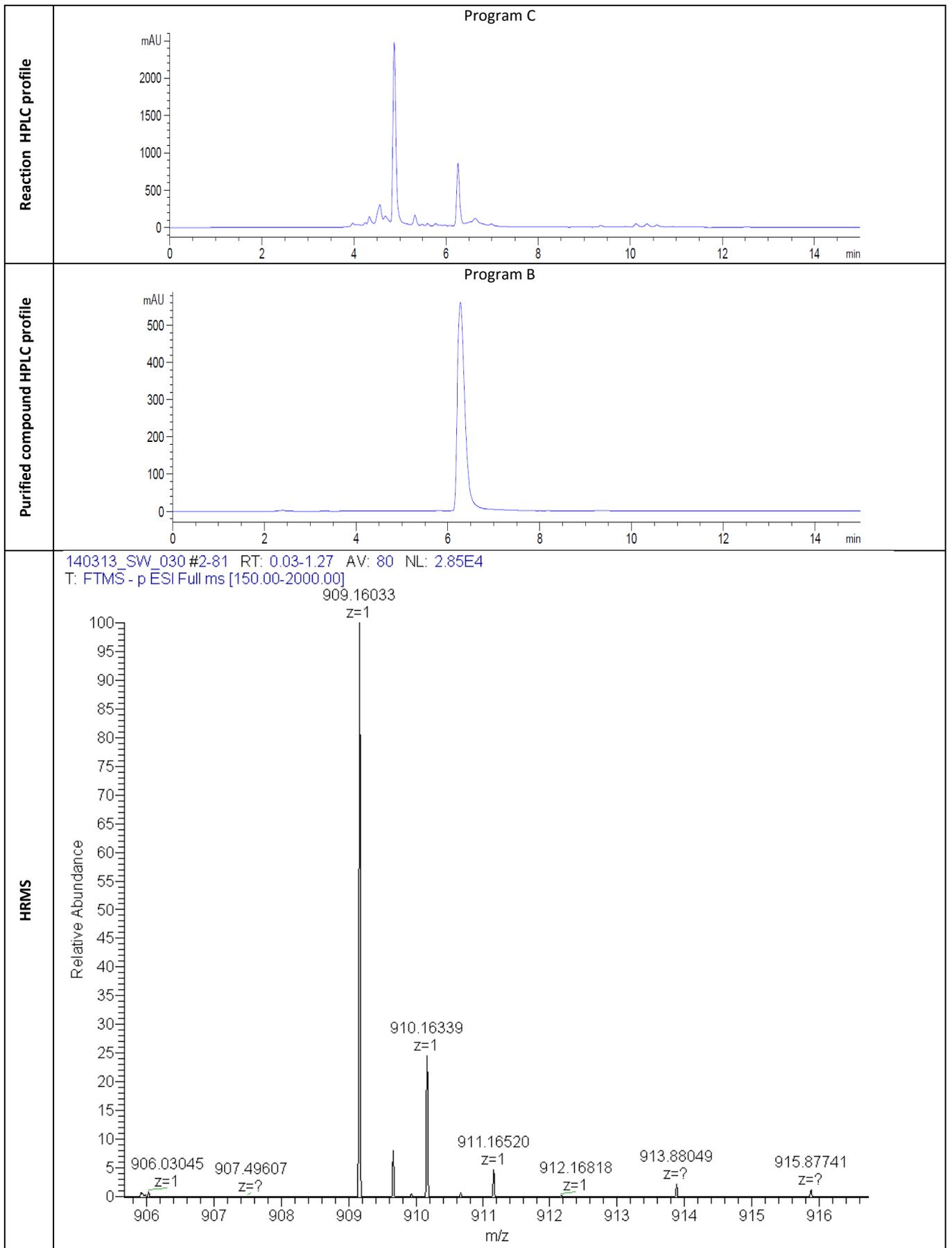


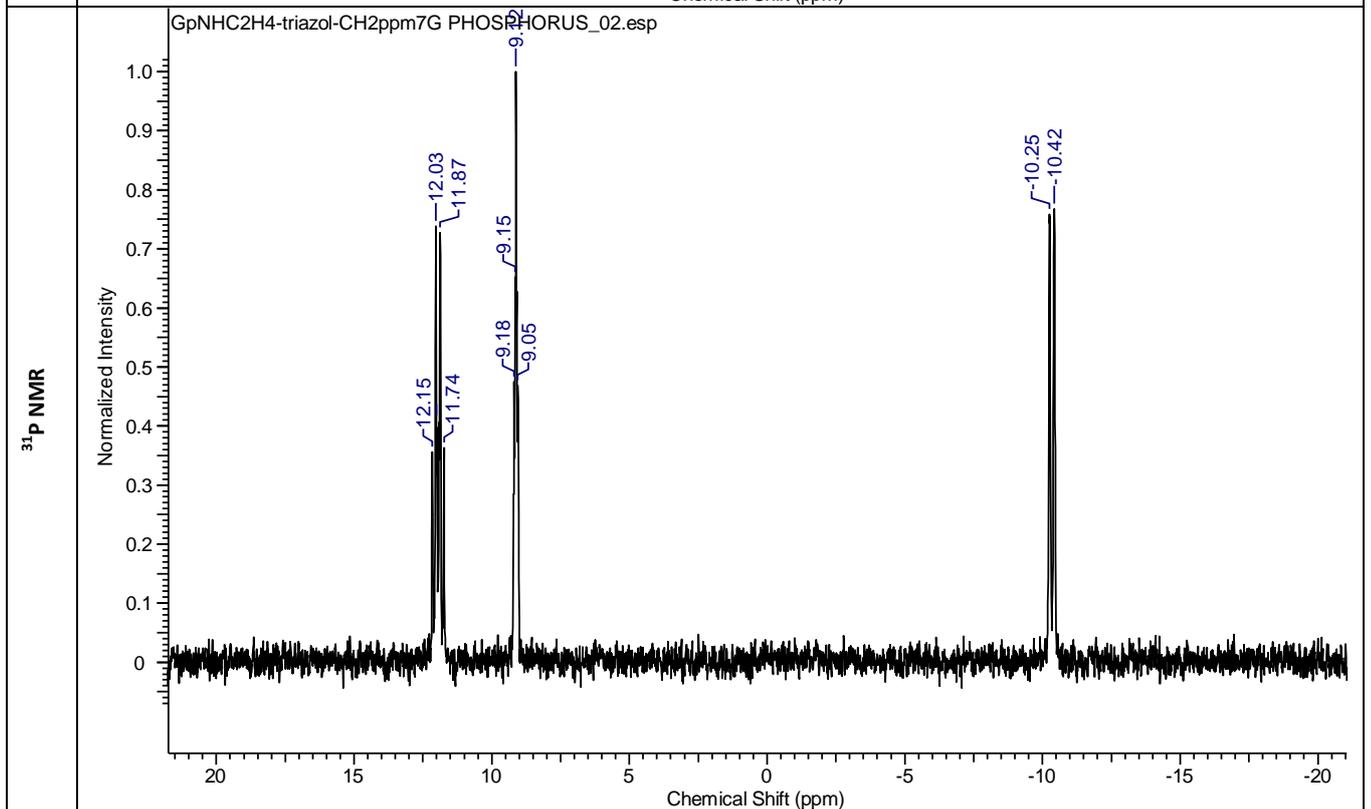
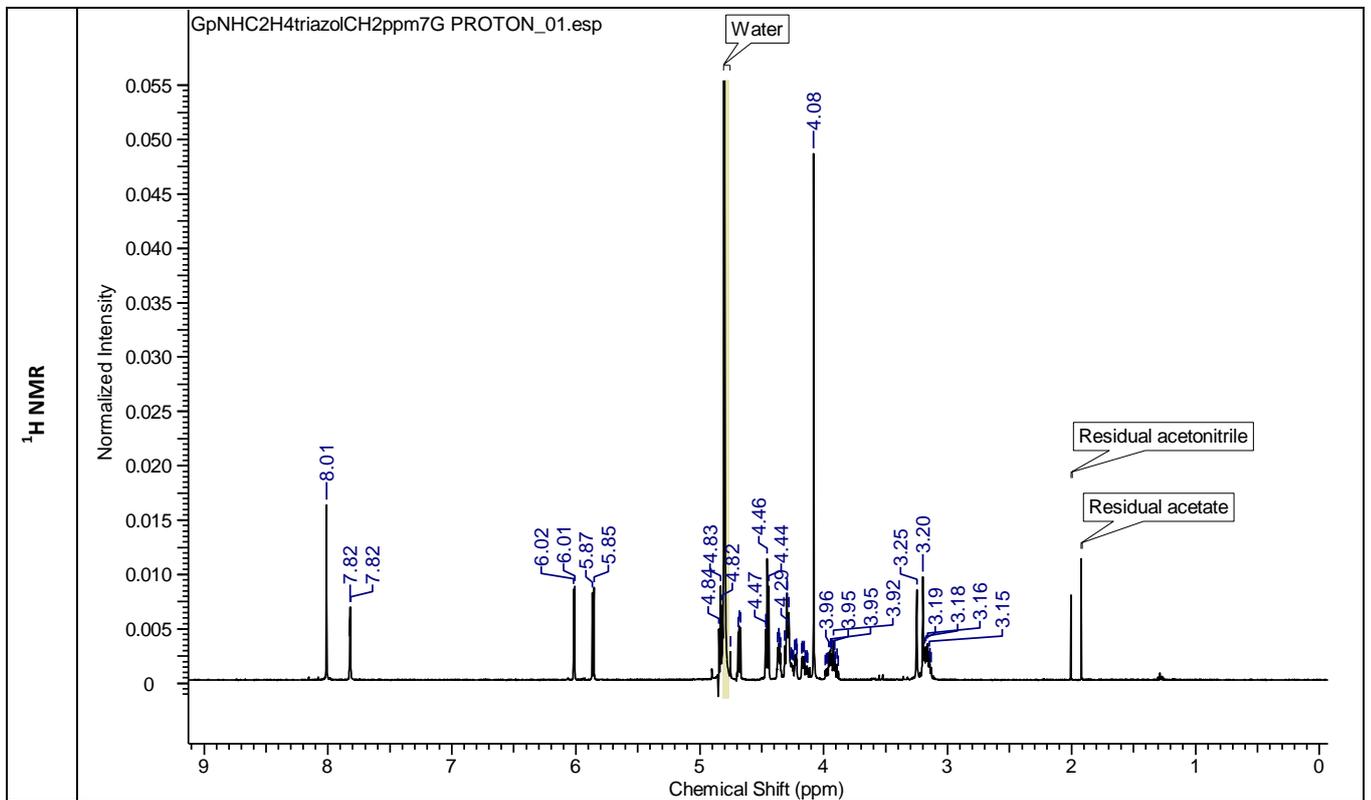




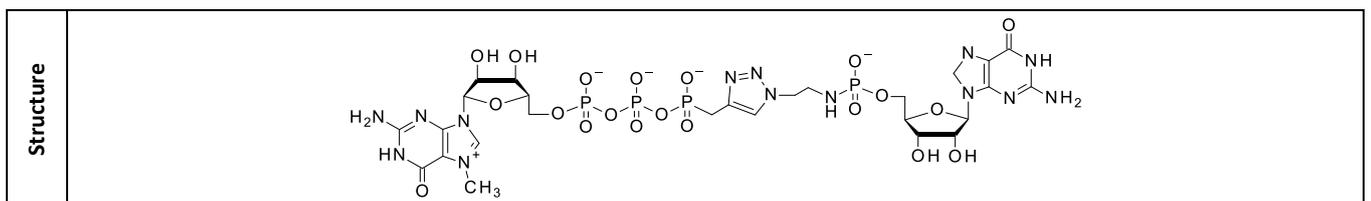
**(7c) m<sup>7</sup>GppCH<sub>2</sub>-triazole-C<sub>2</sub>H<sub>4</sub>NHpG**

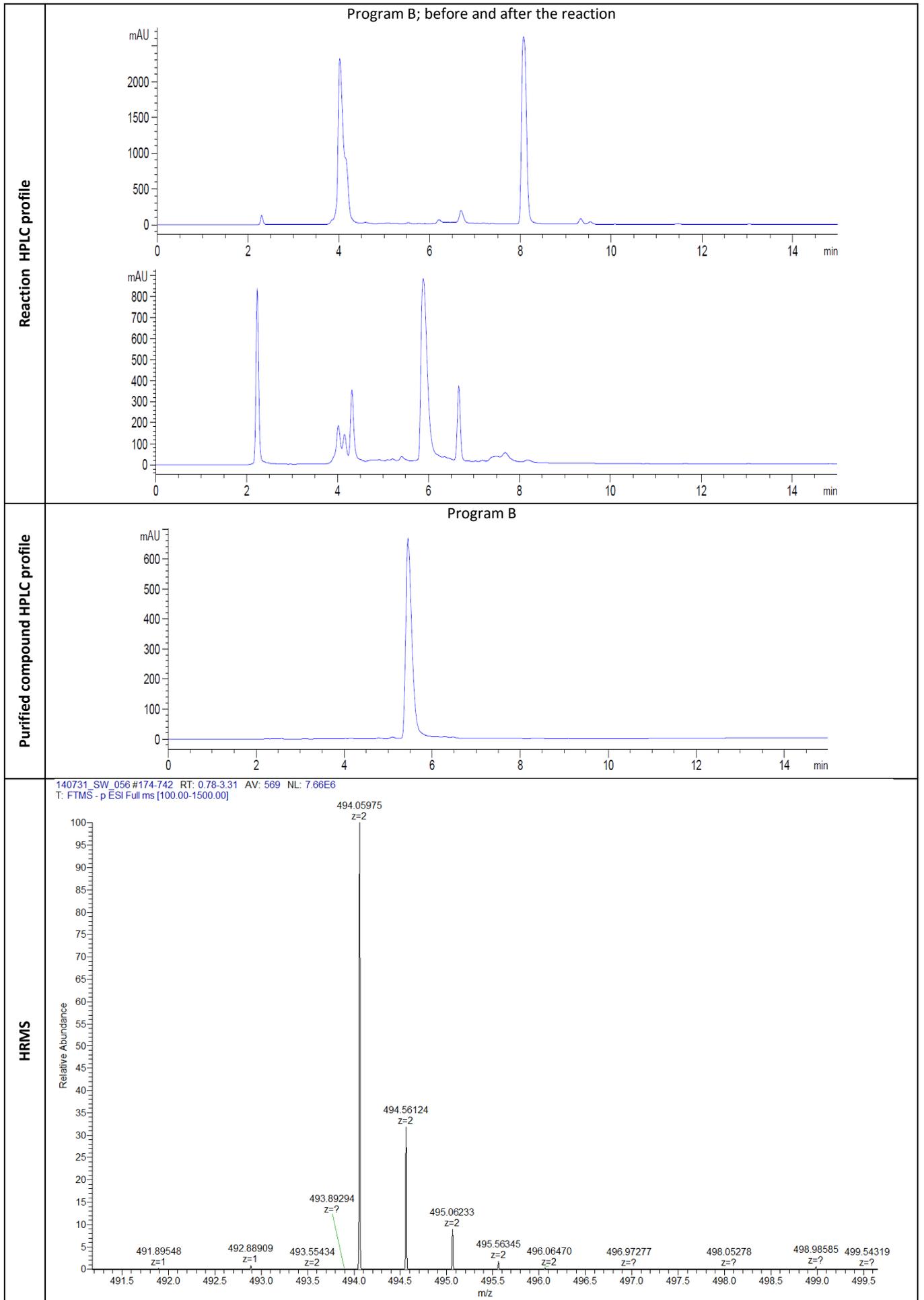


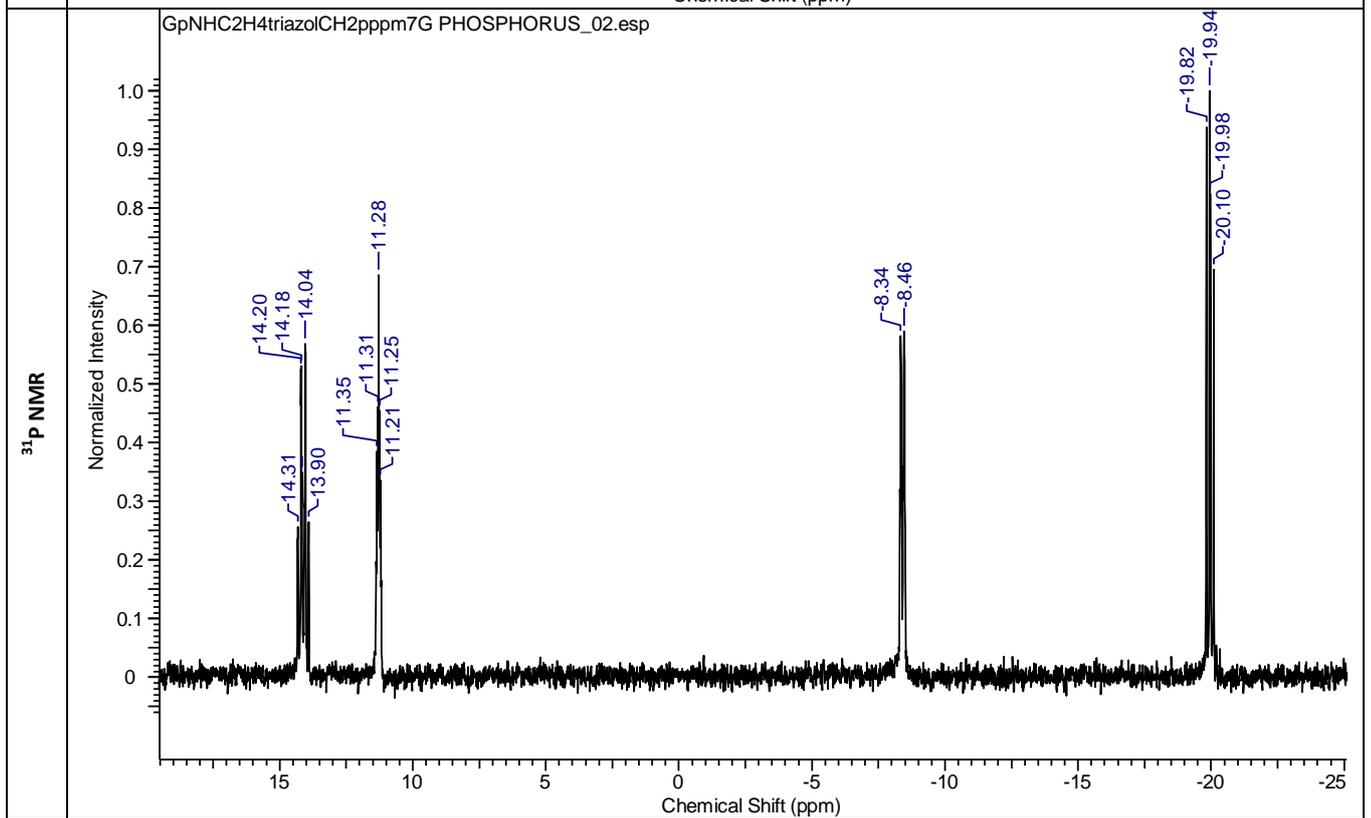
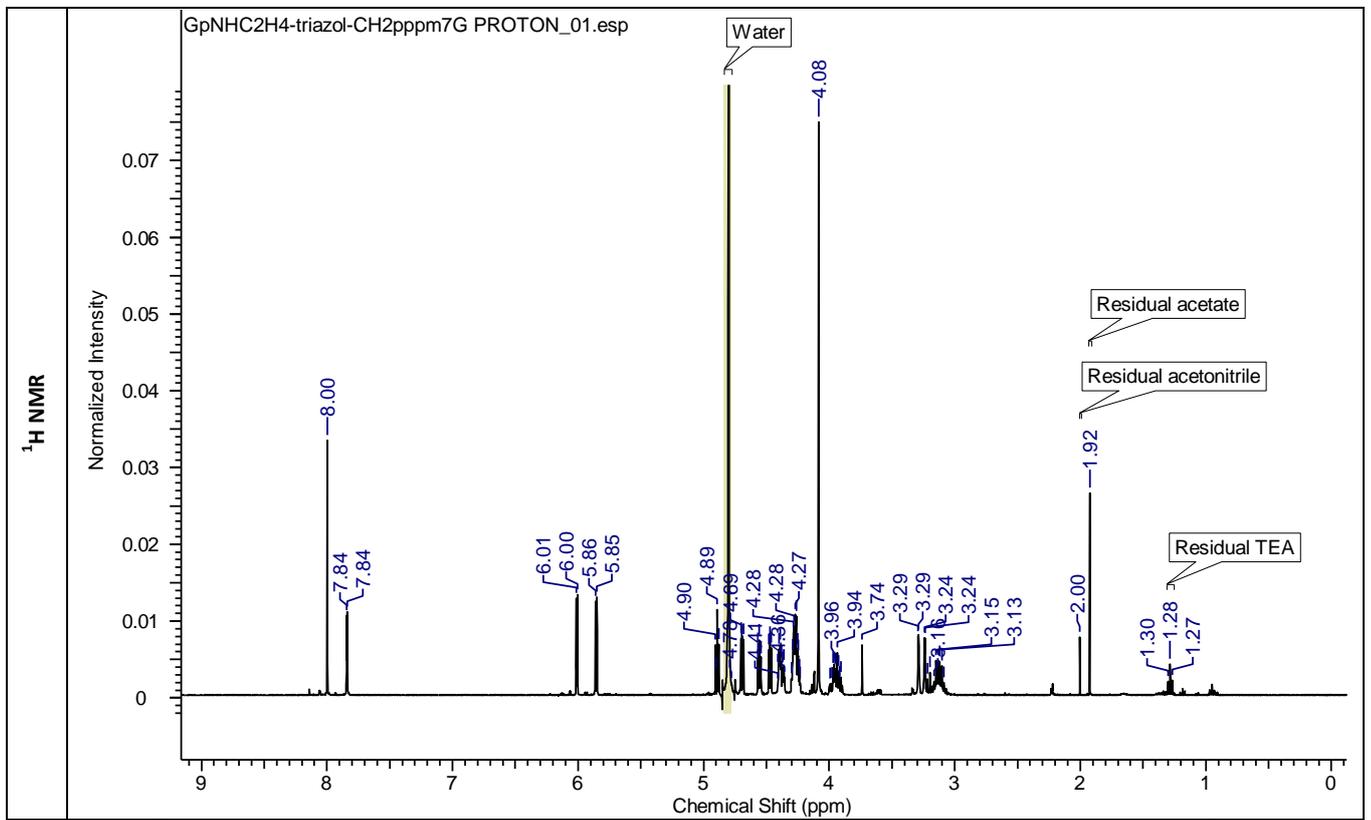




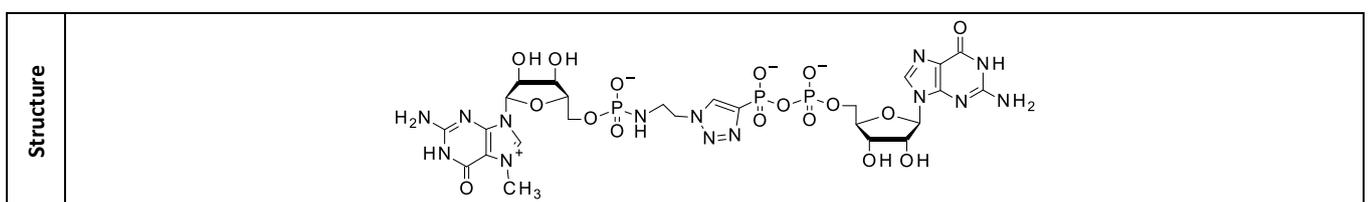
**(7d) m<sup>7</sup>GpppCH<sub>2</sub>-triazole-C<sub>2</sub>H<sub>4</sub>NHpG**

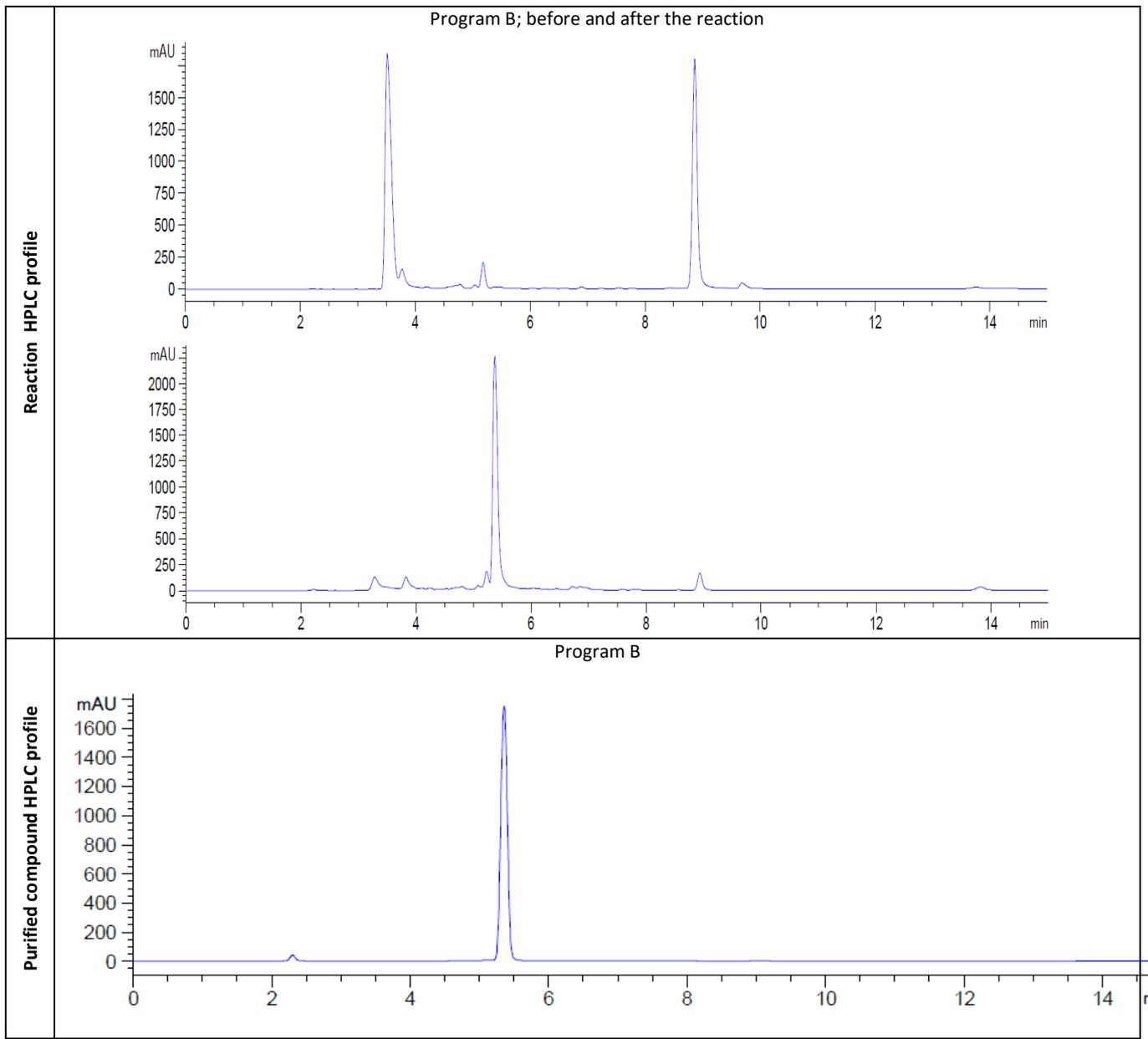






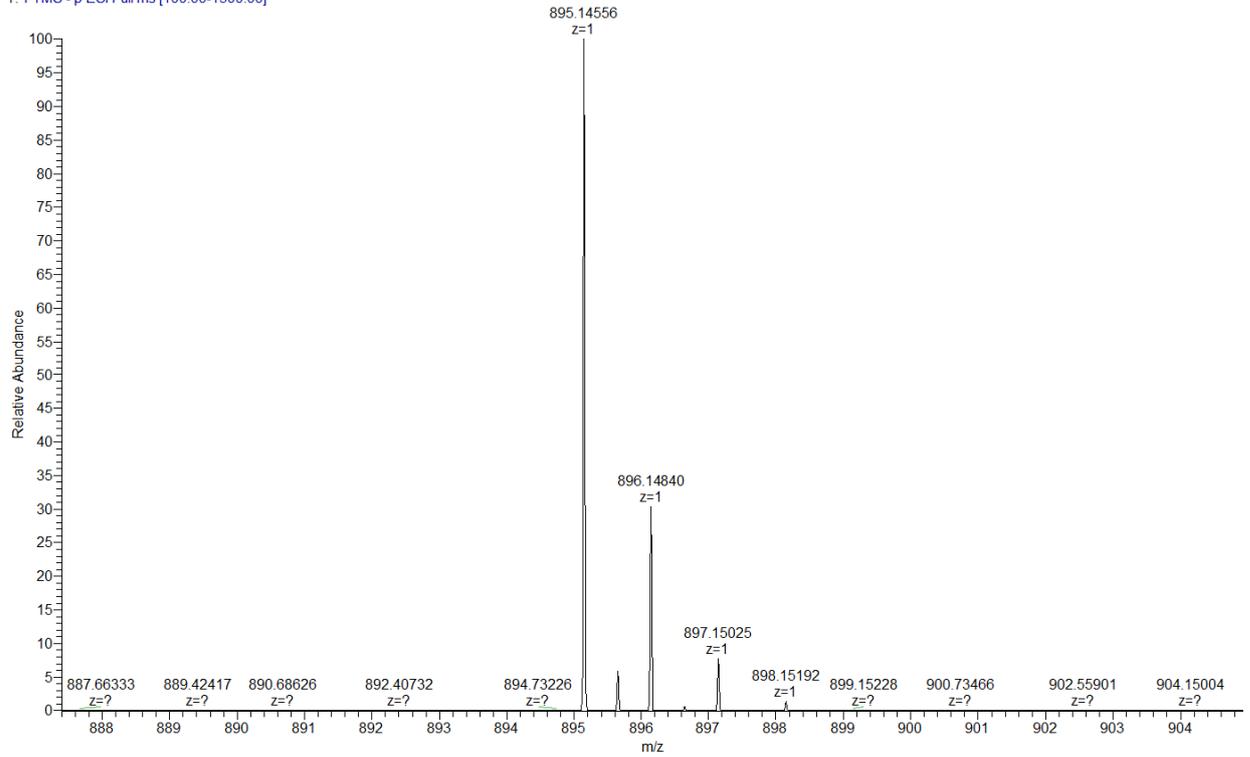
**(8a) m<sup>7</sup>GpNHC<sub>2</sub>H<sub>4</sub>-triazole-ppG**



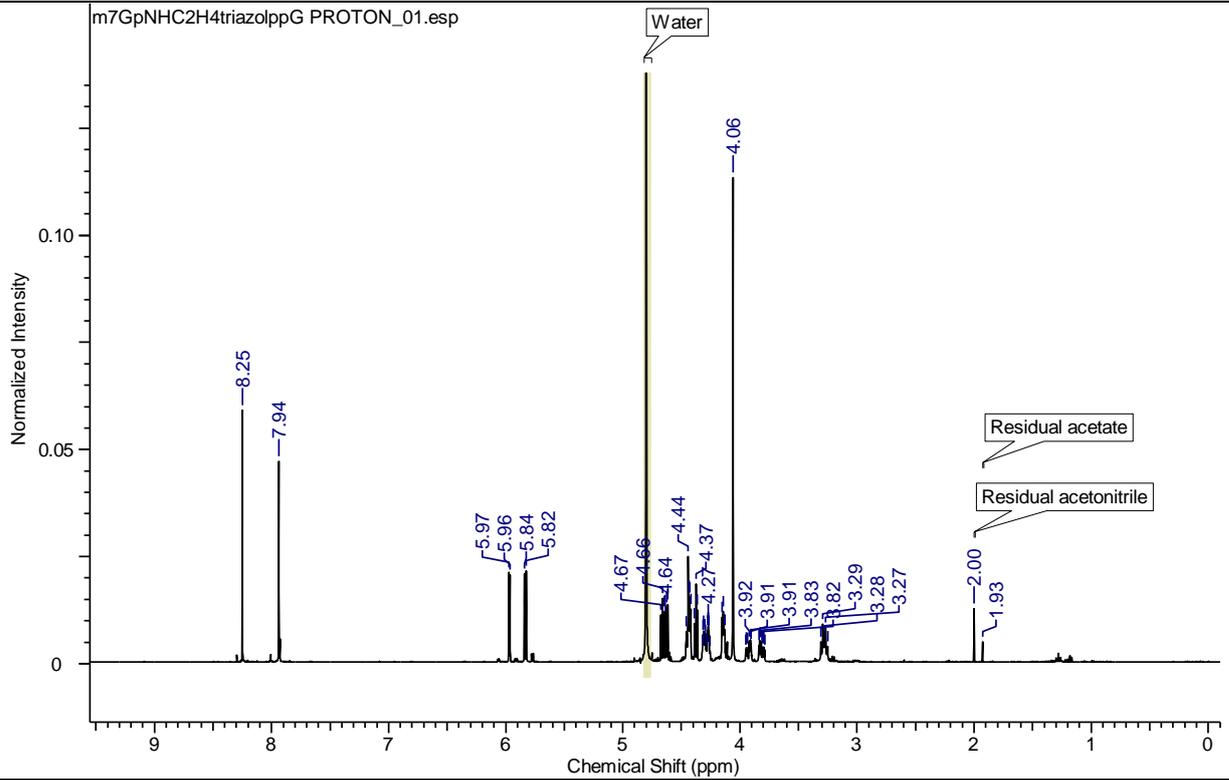


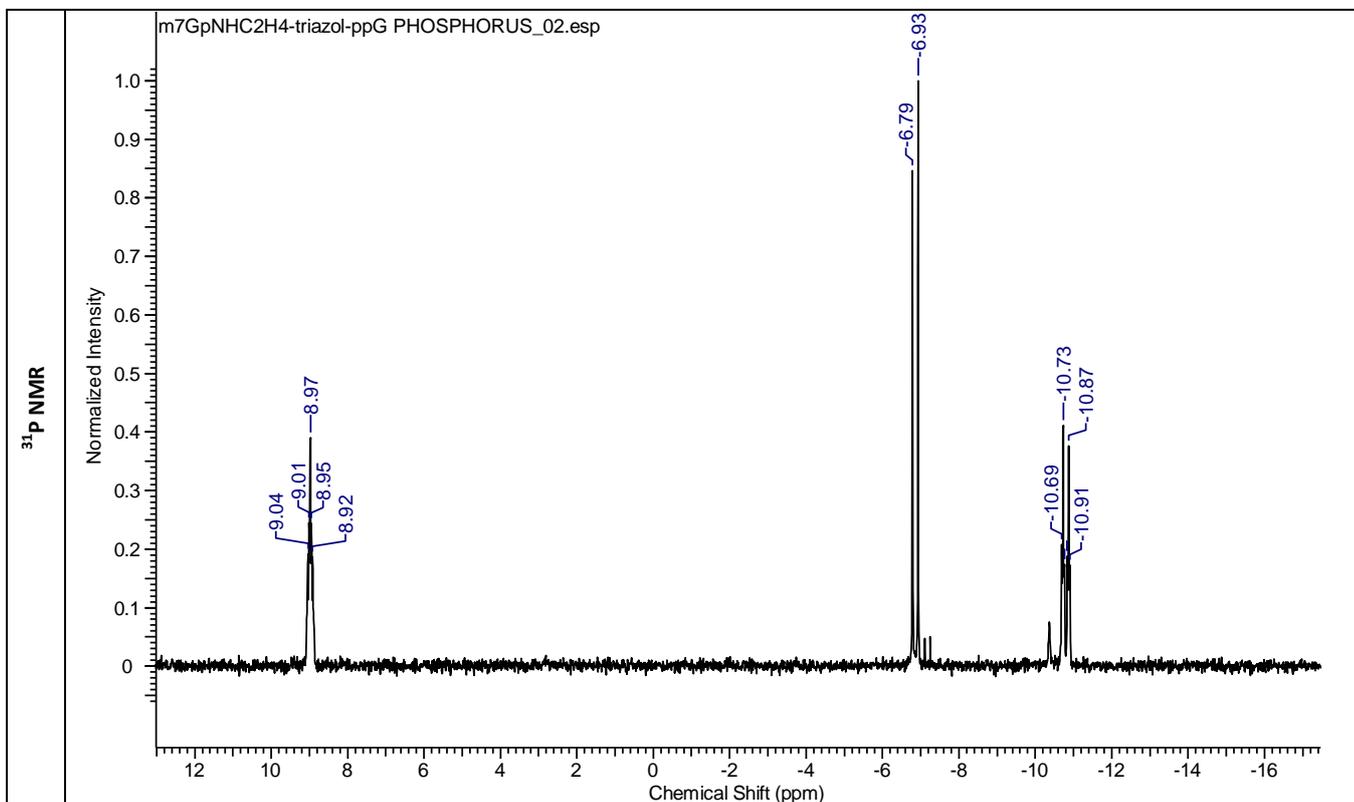
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HRMS

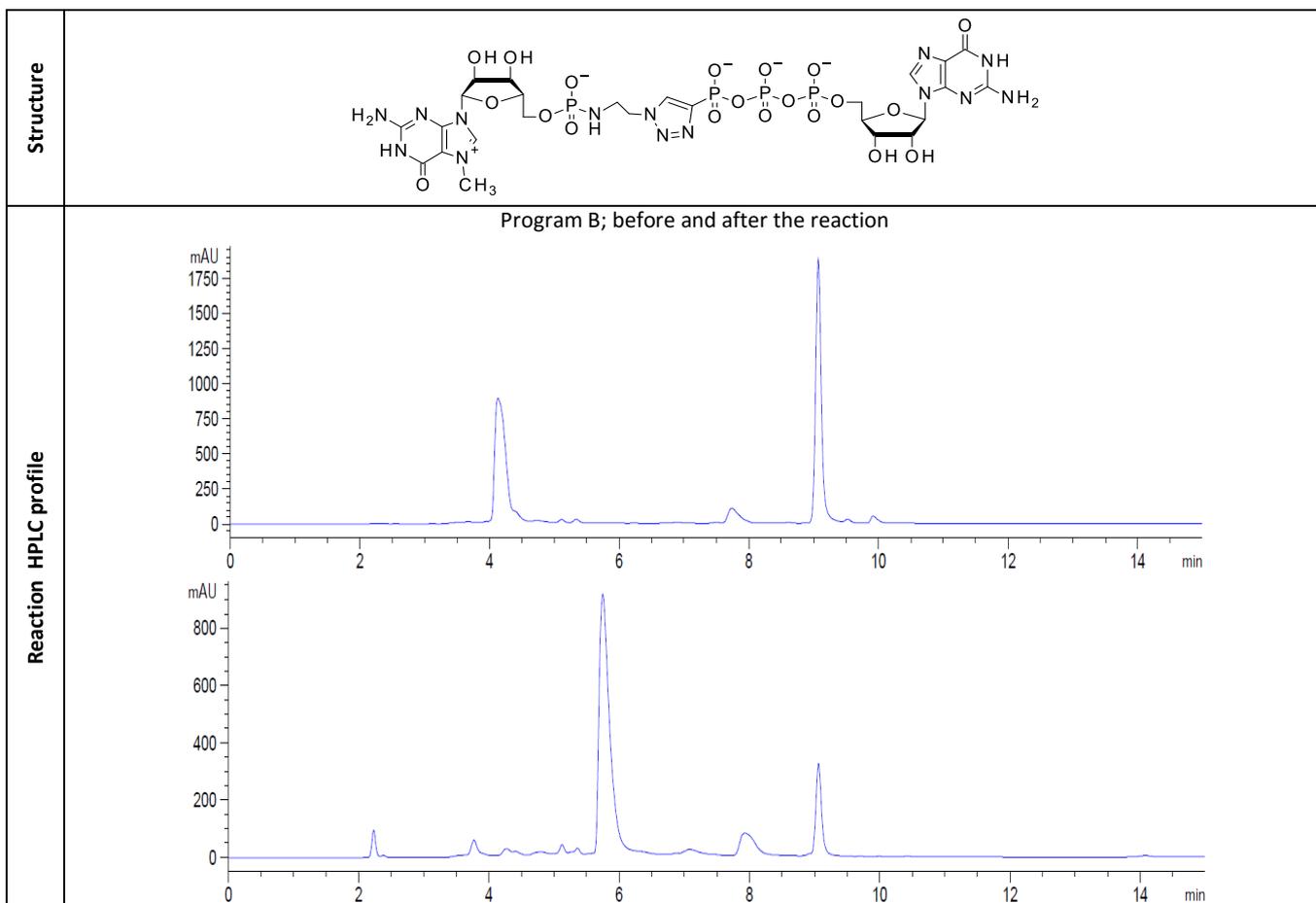


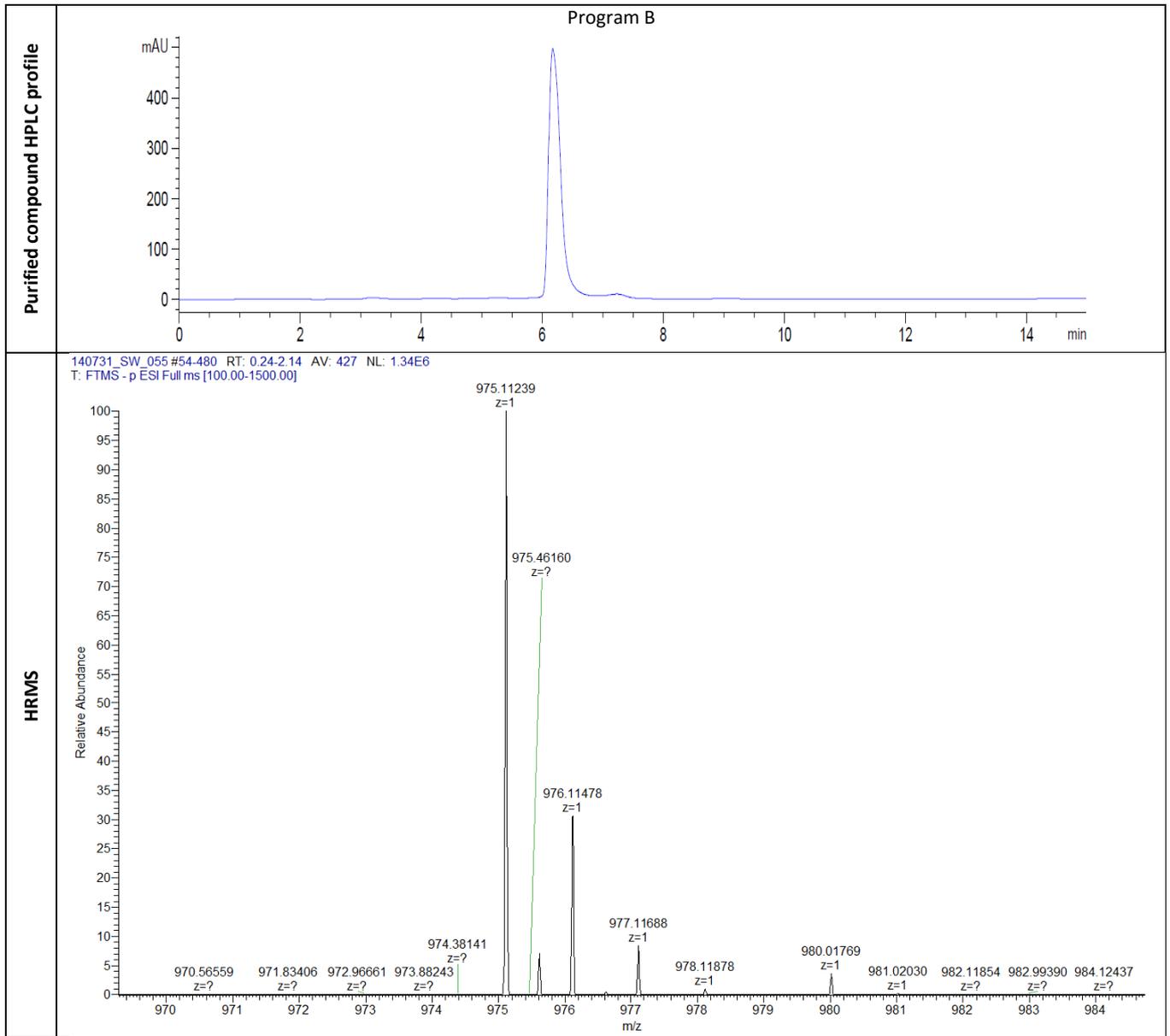
<sup>1</sup>H NMR

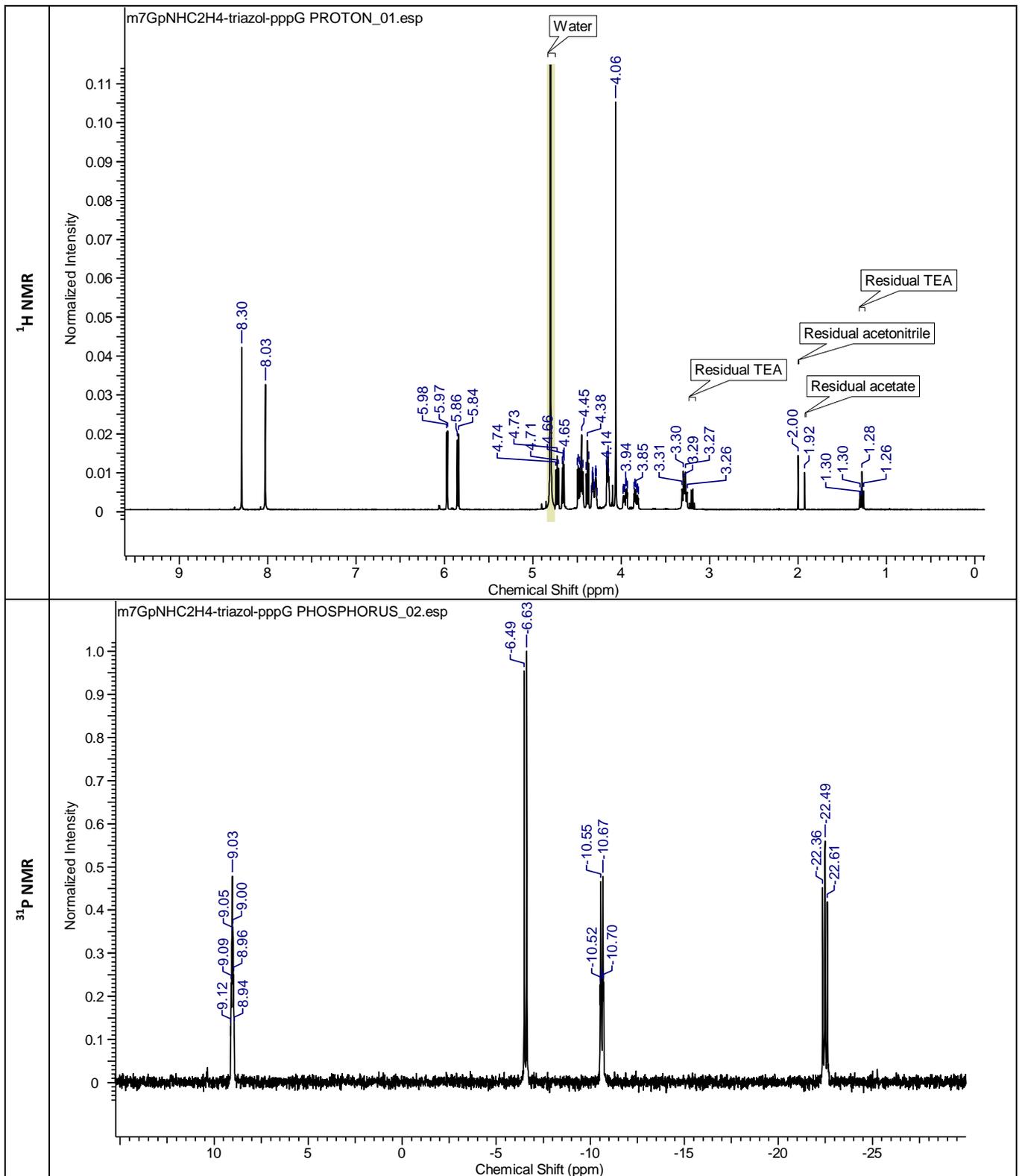




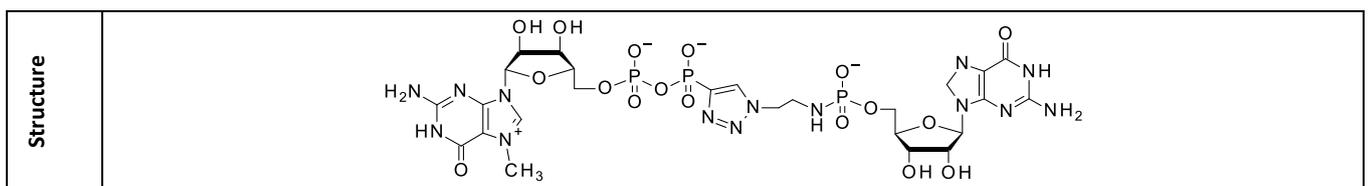
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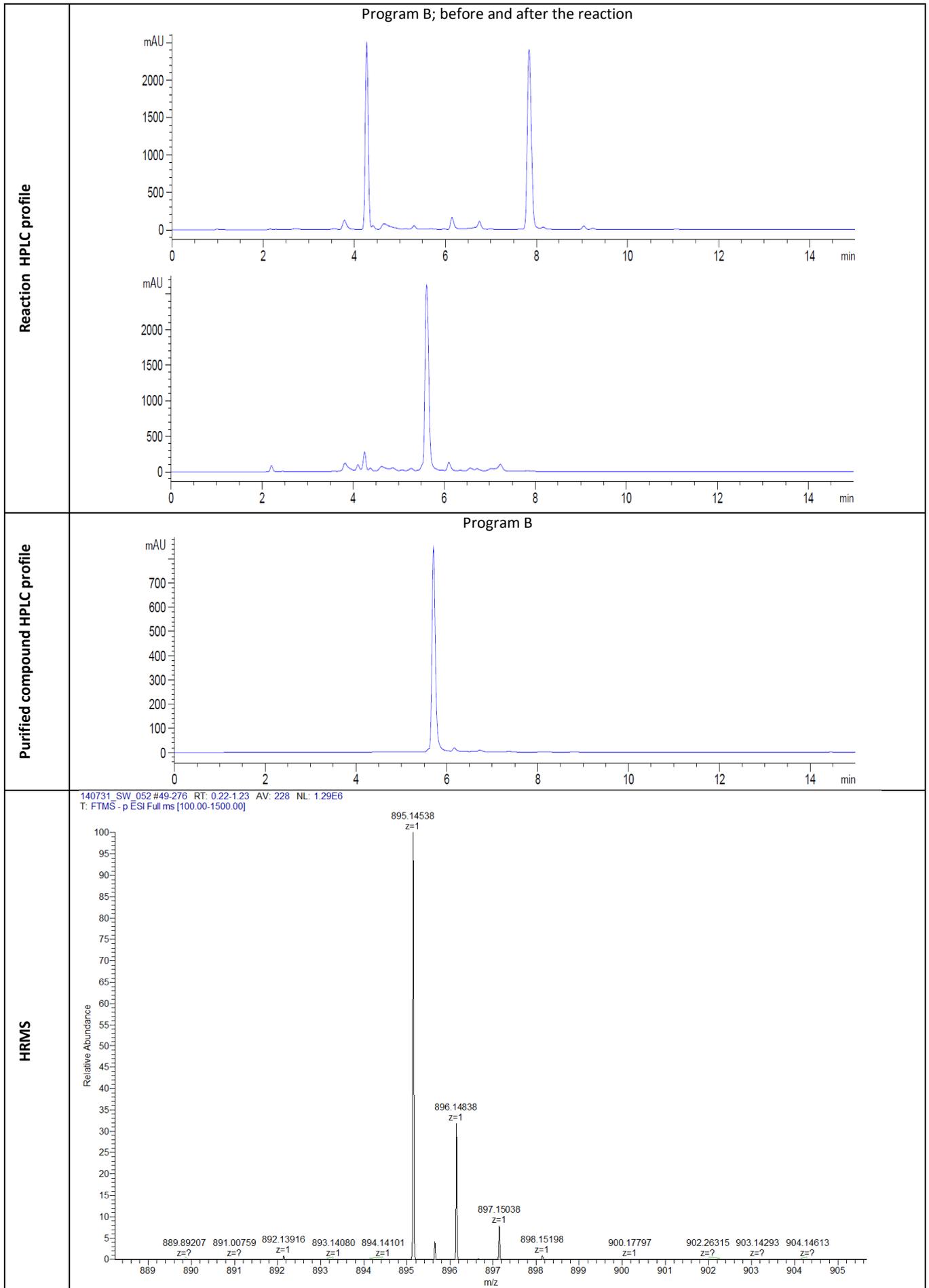




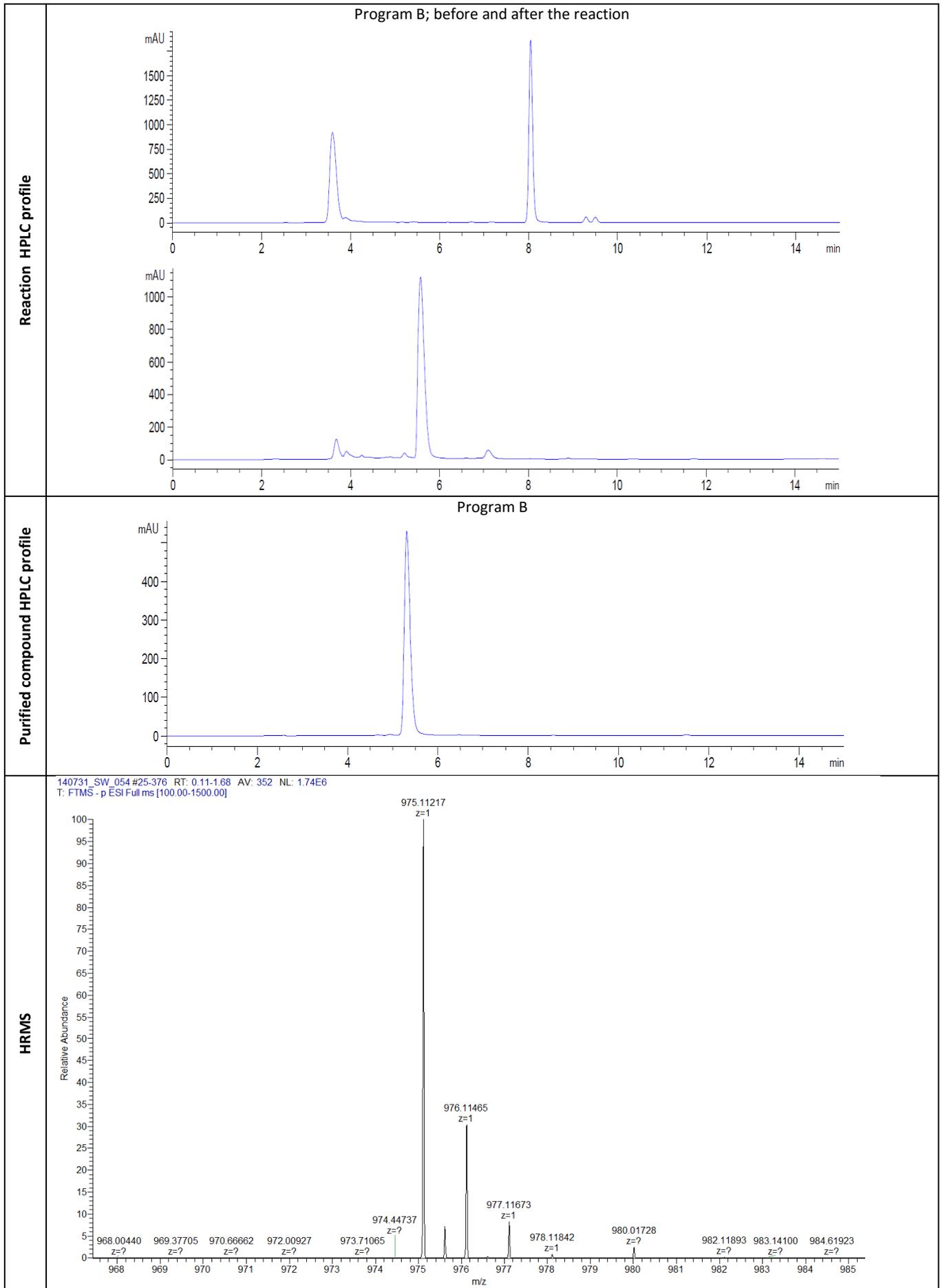


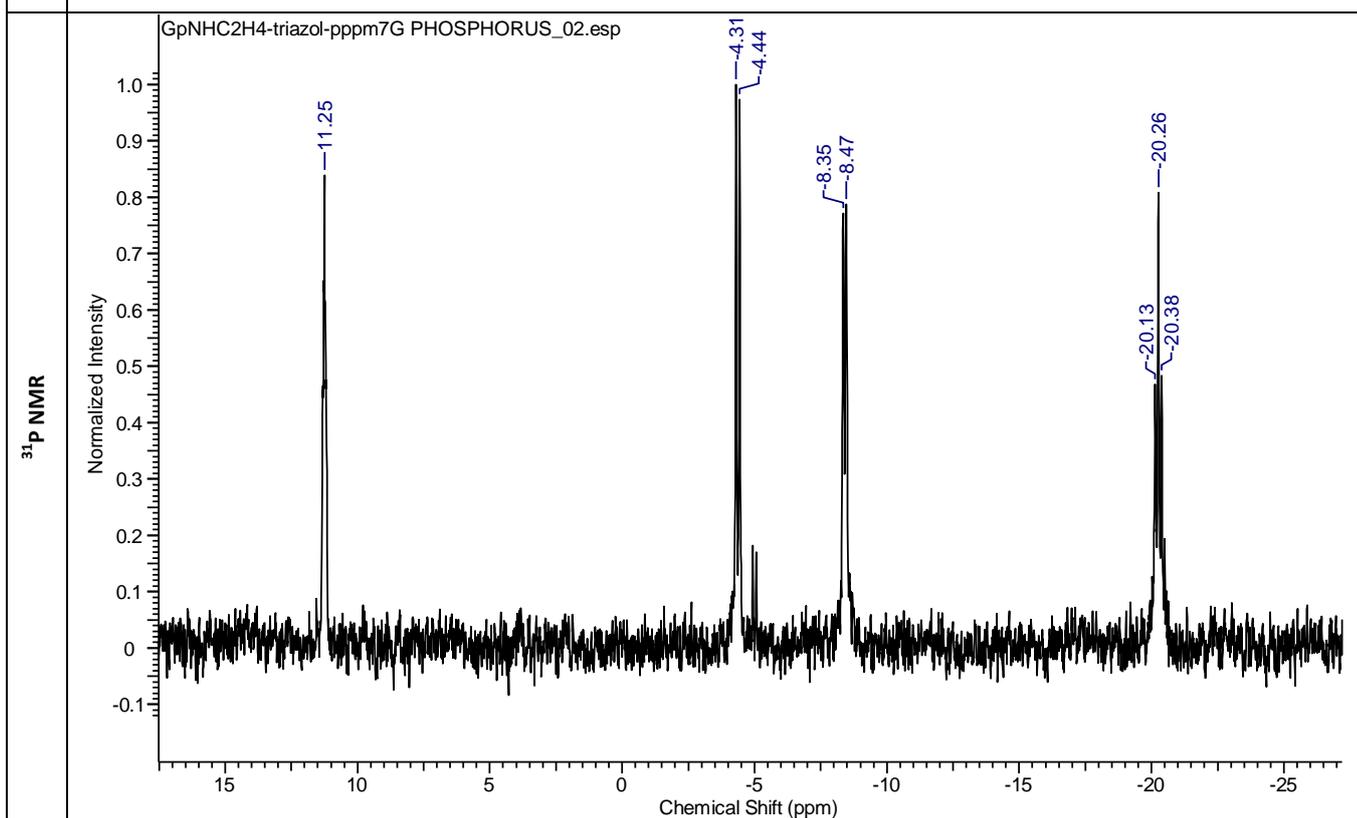
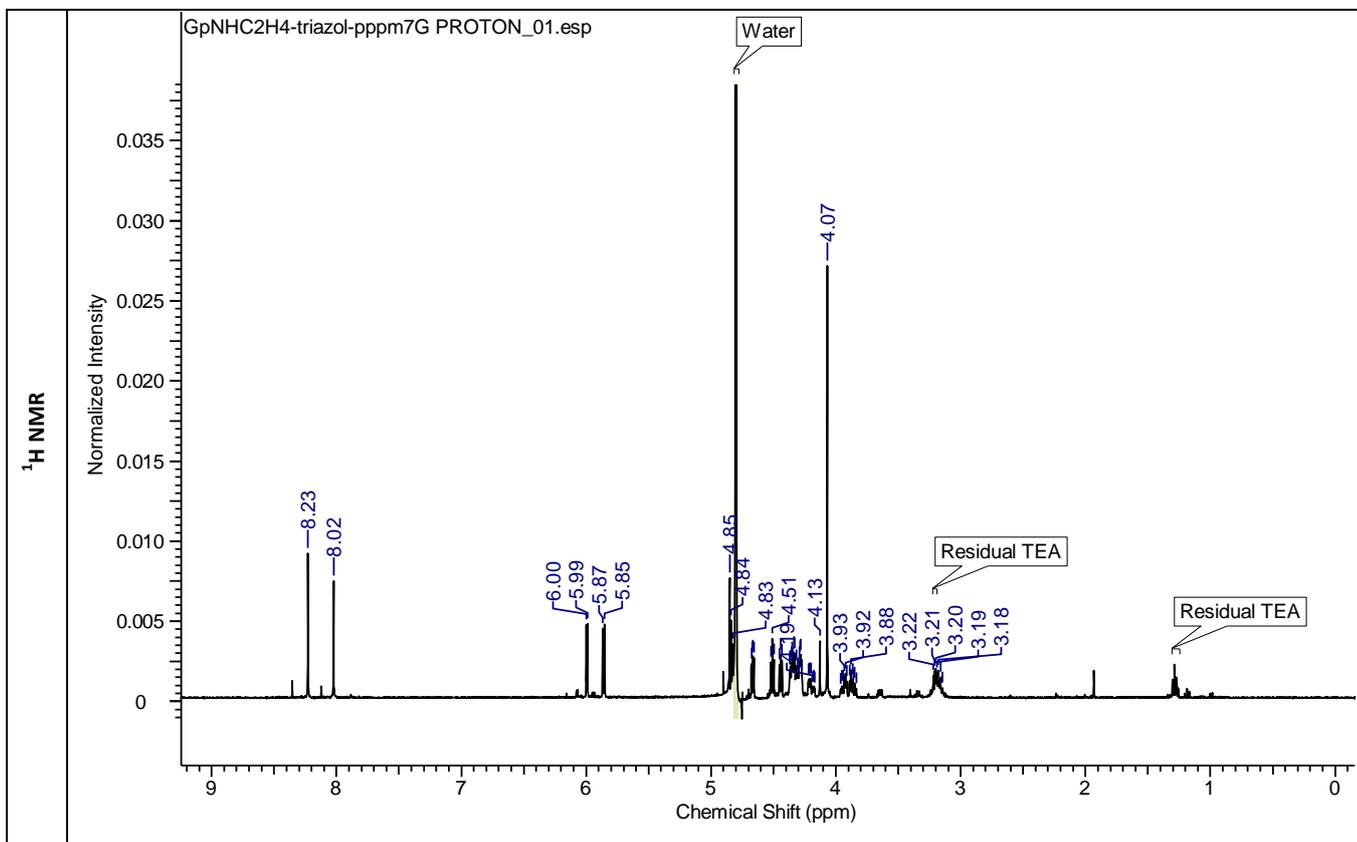
**(8c) m<sup>7</sup>Gpp-triazole-C<sub>2</sub>H<sub>4</sub>NHpG**



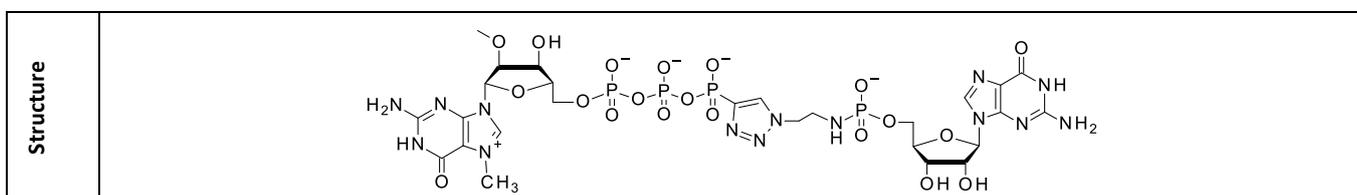


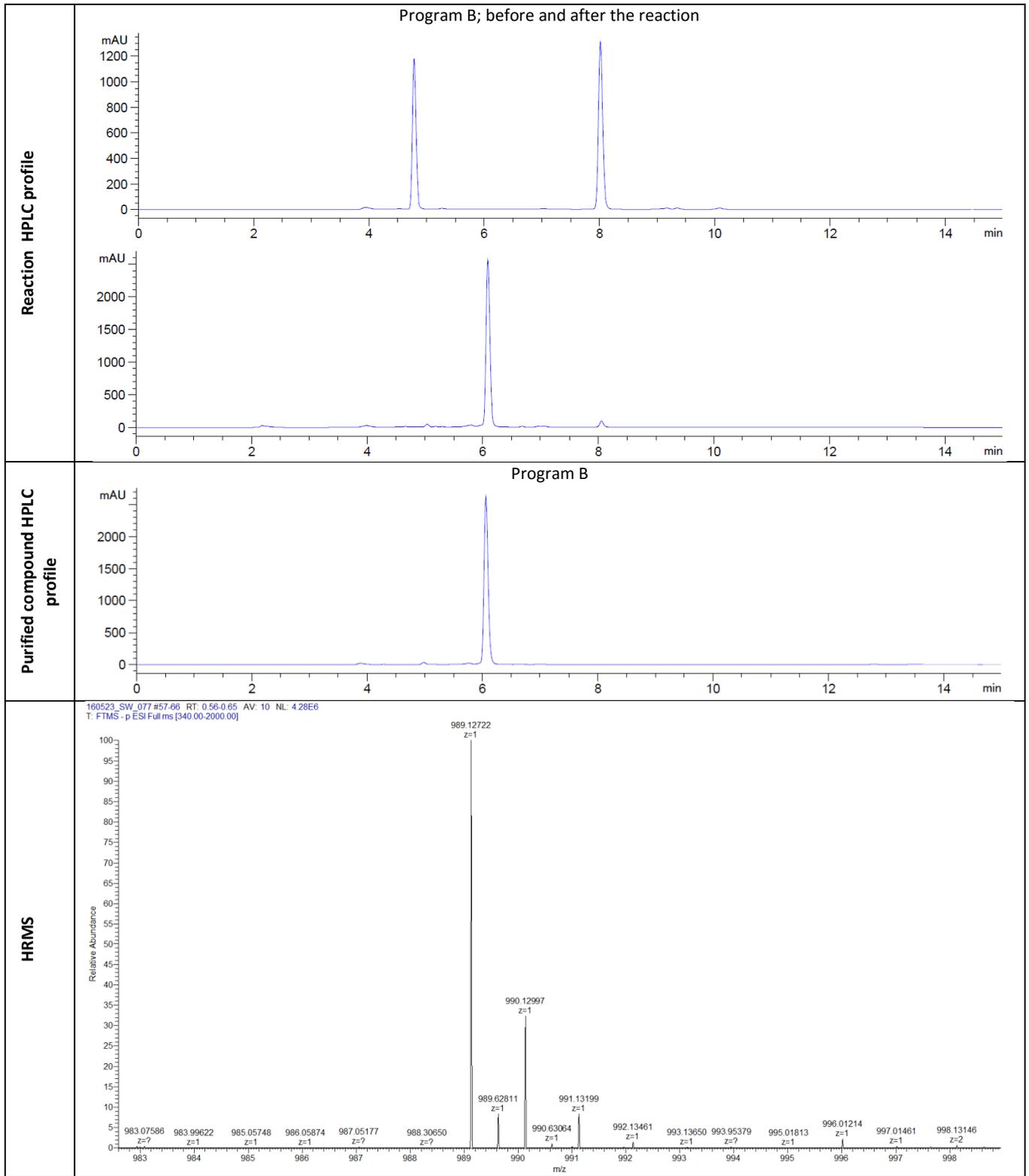


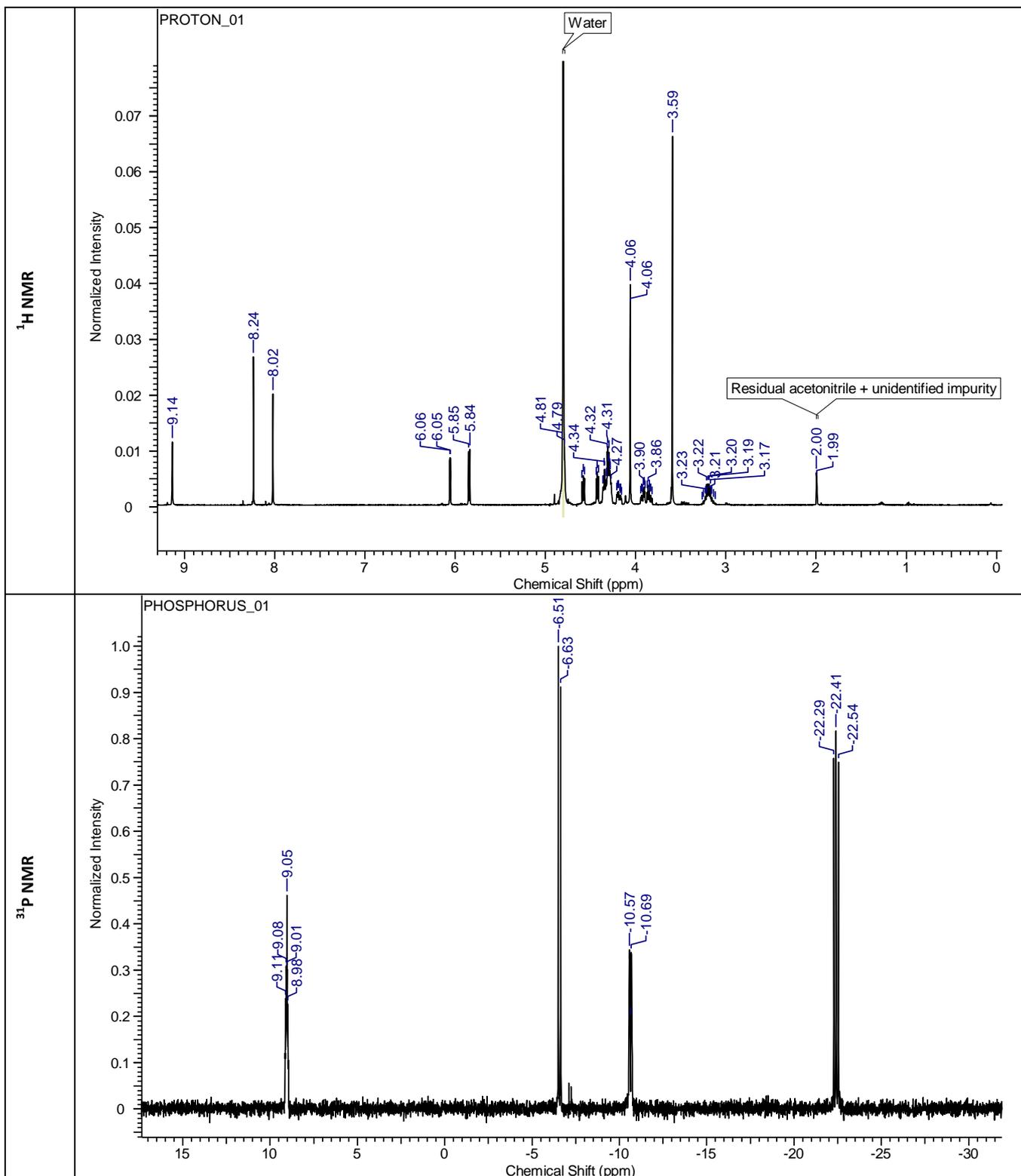




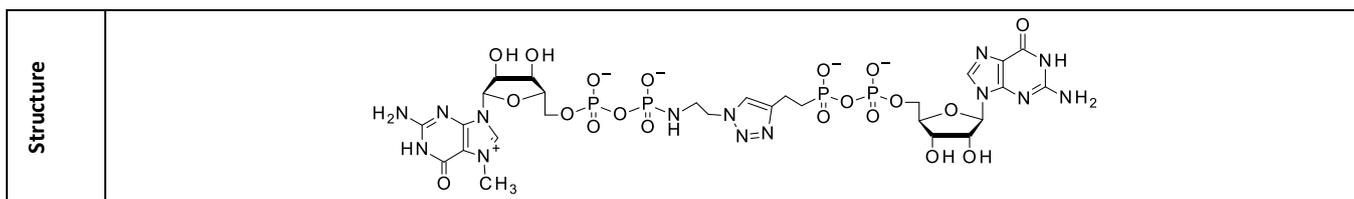
**(8e) m<sub>2</sub><sup>7,2'-O</sup>Gppp-triazole-C<sub>2</sub>H<sub>4</sub>NHppG**

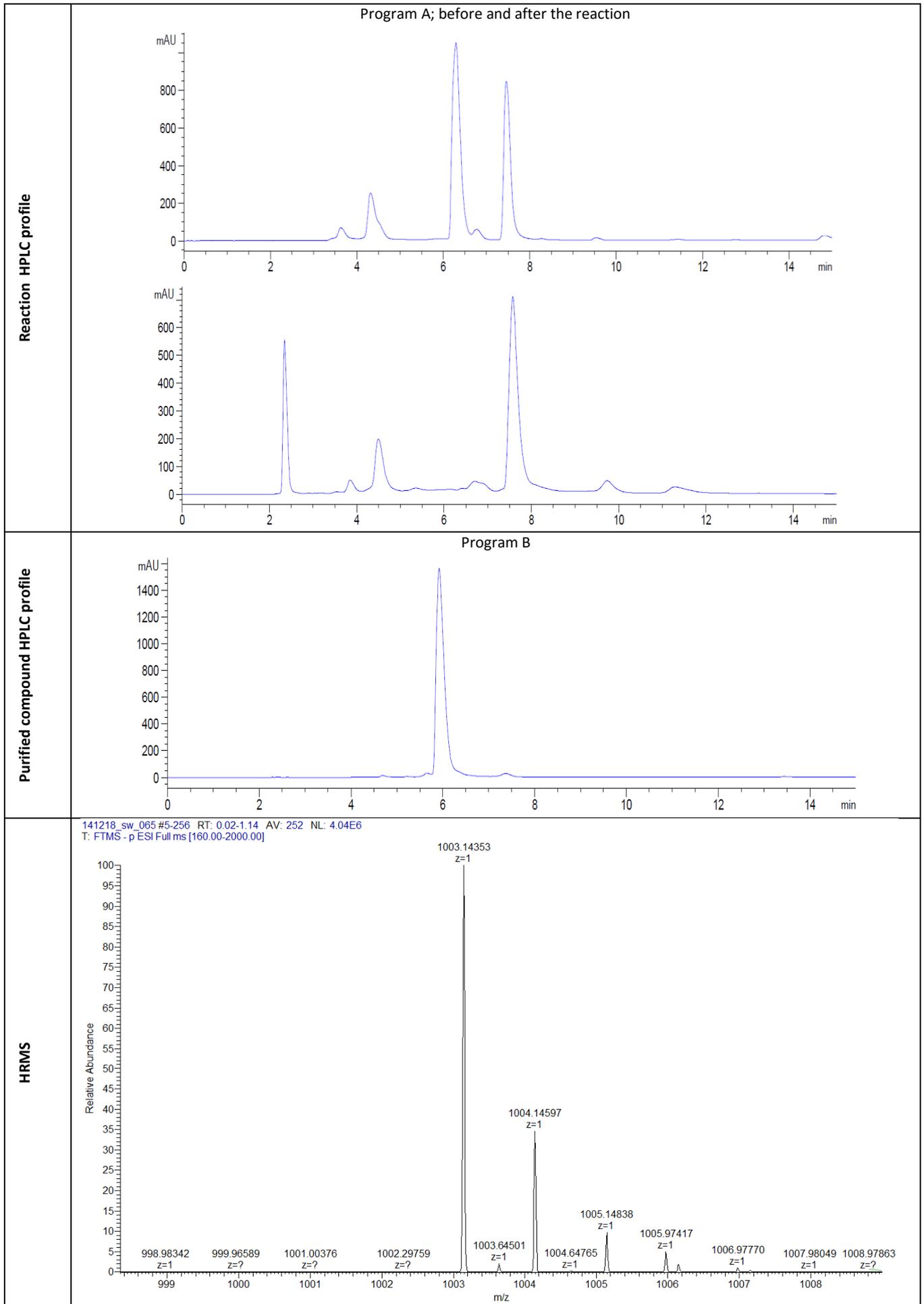


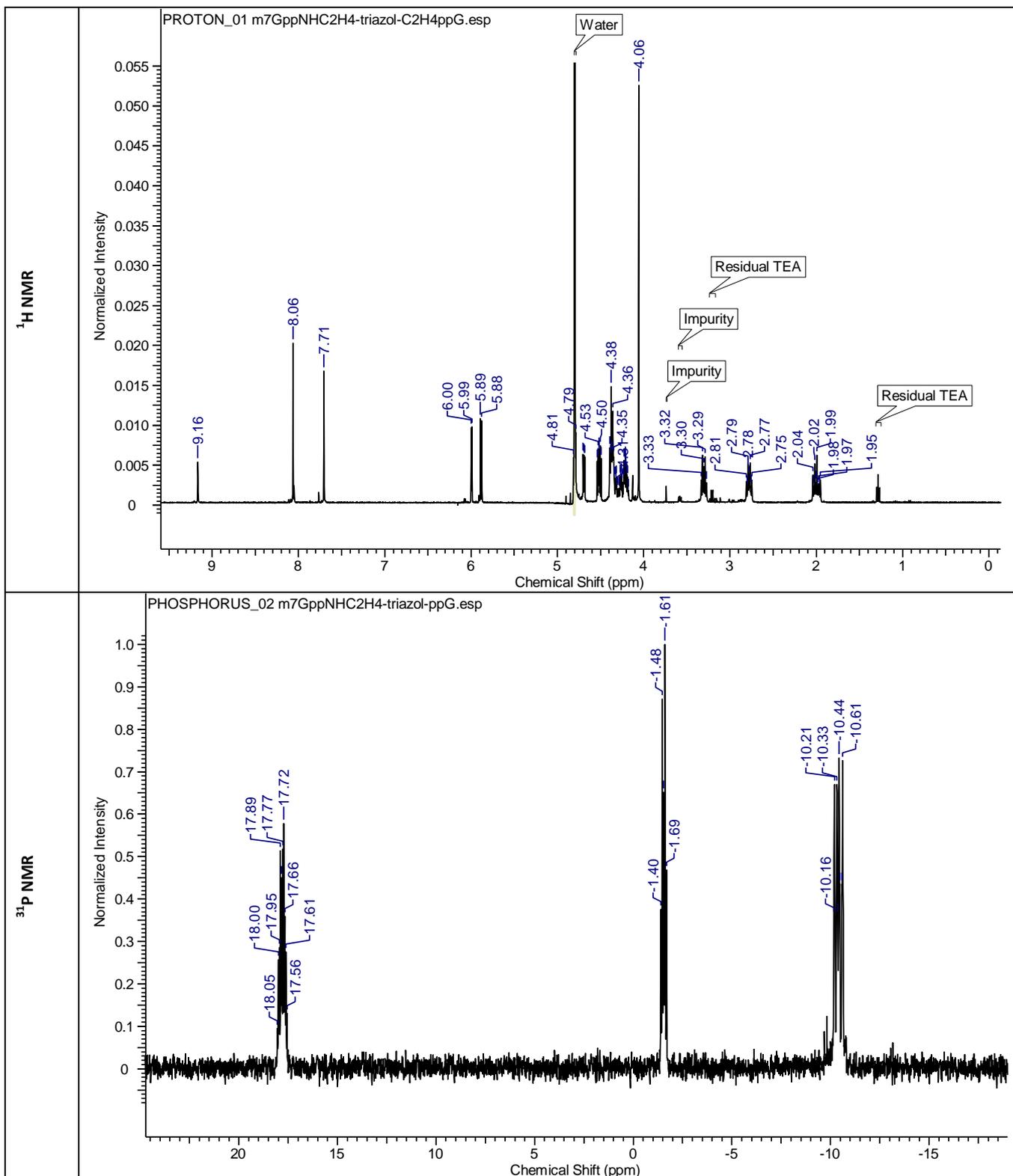




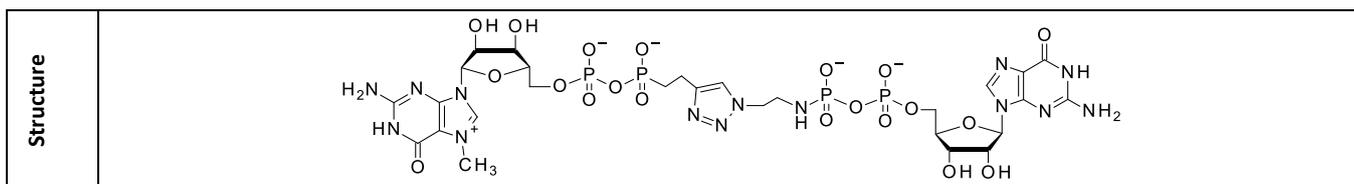
**(9a) m<sup>7</sup>GppNHC<sub>2</sub>H<sub>4</sub>-triazole-C<sub>2</sub>H<sub>4</sub>ppG**

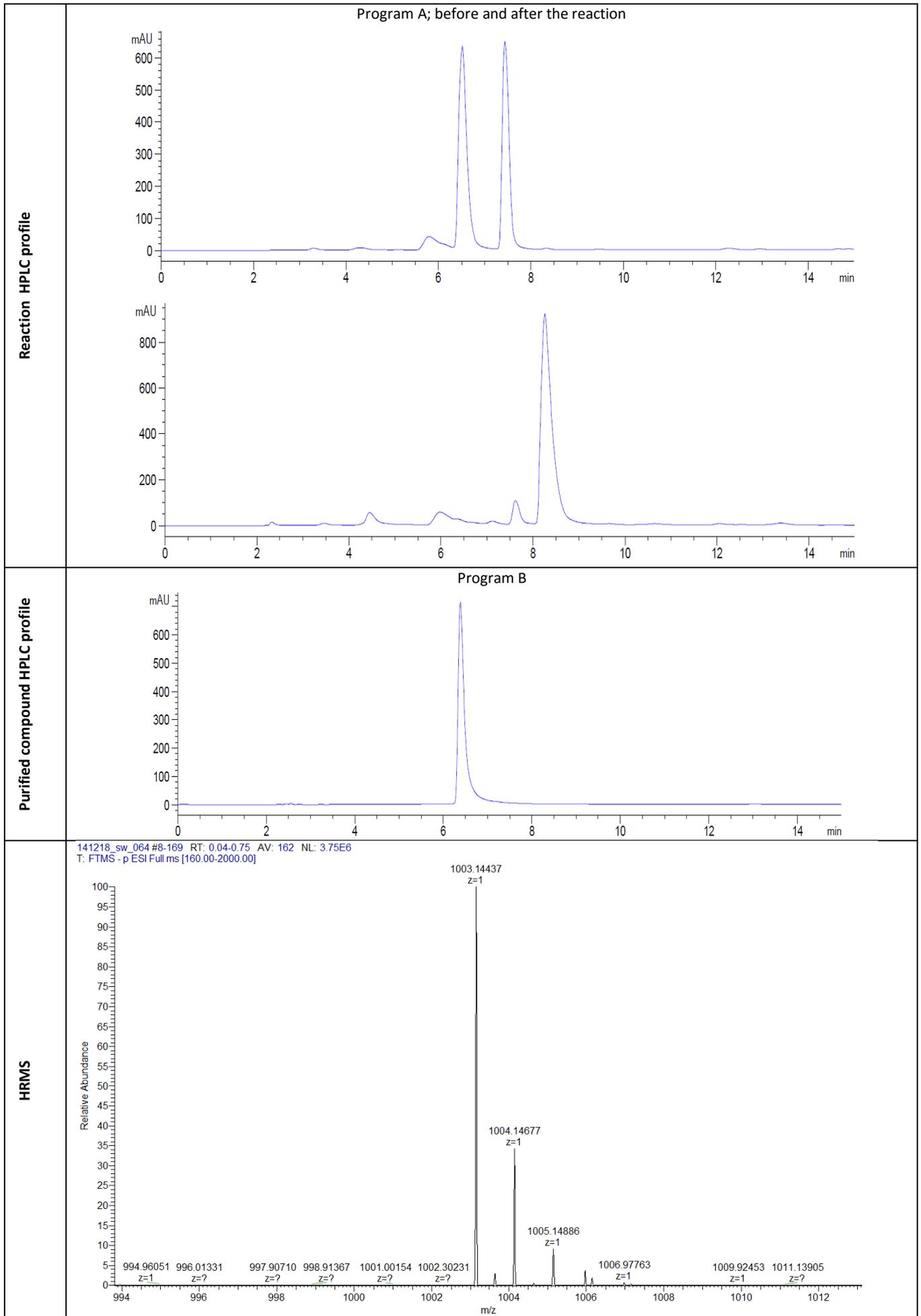


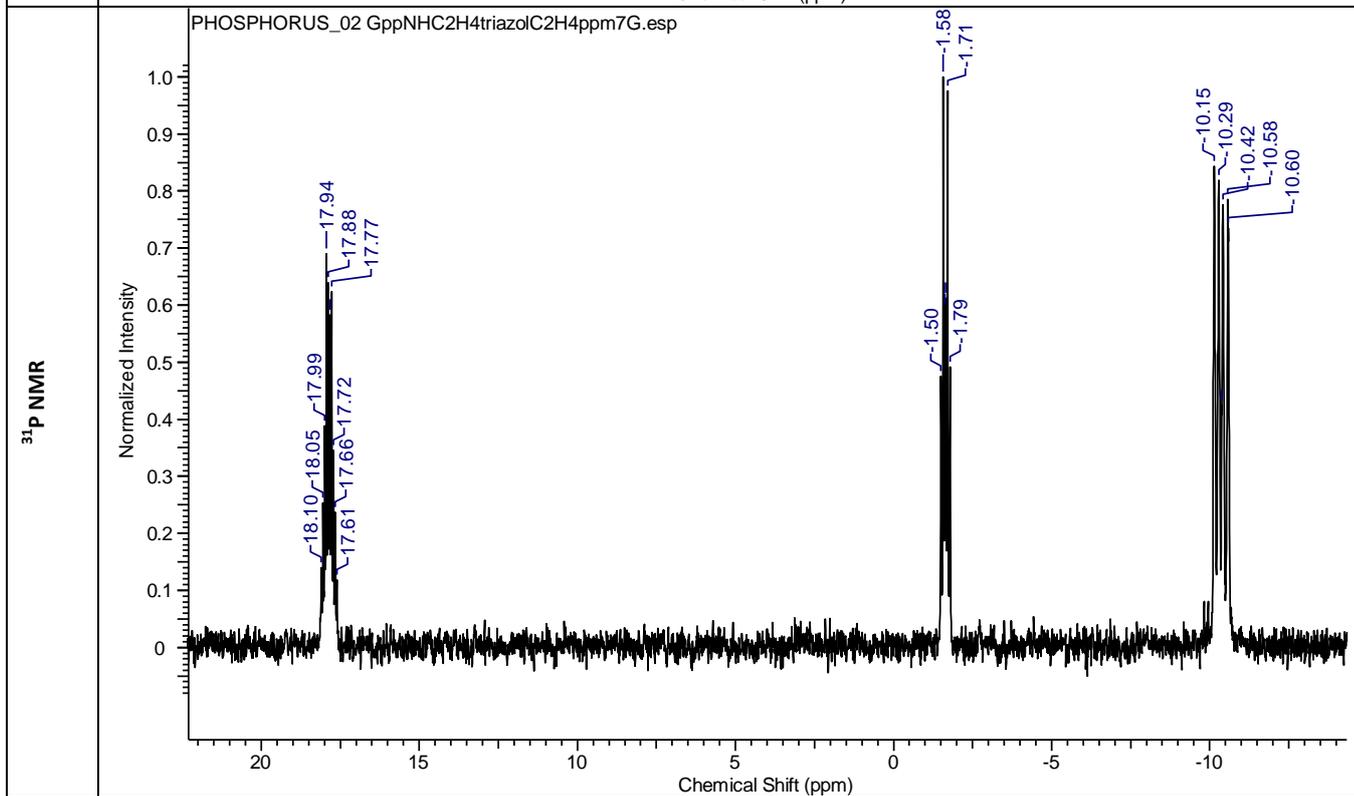
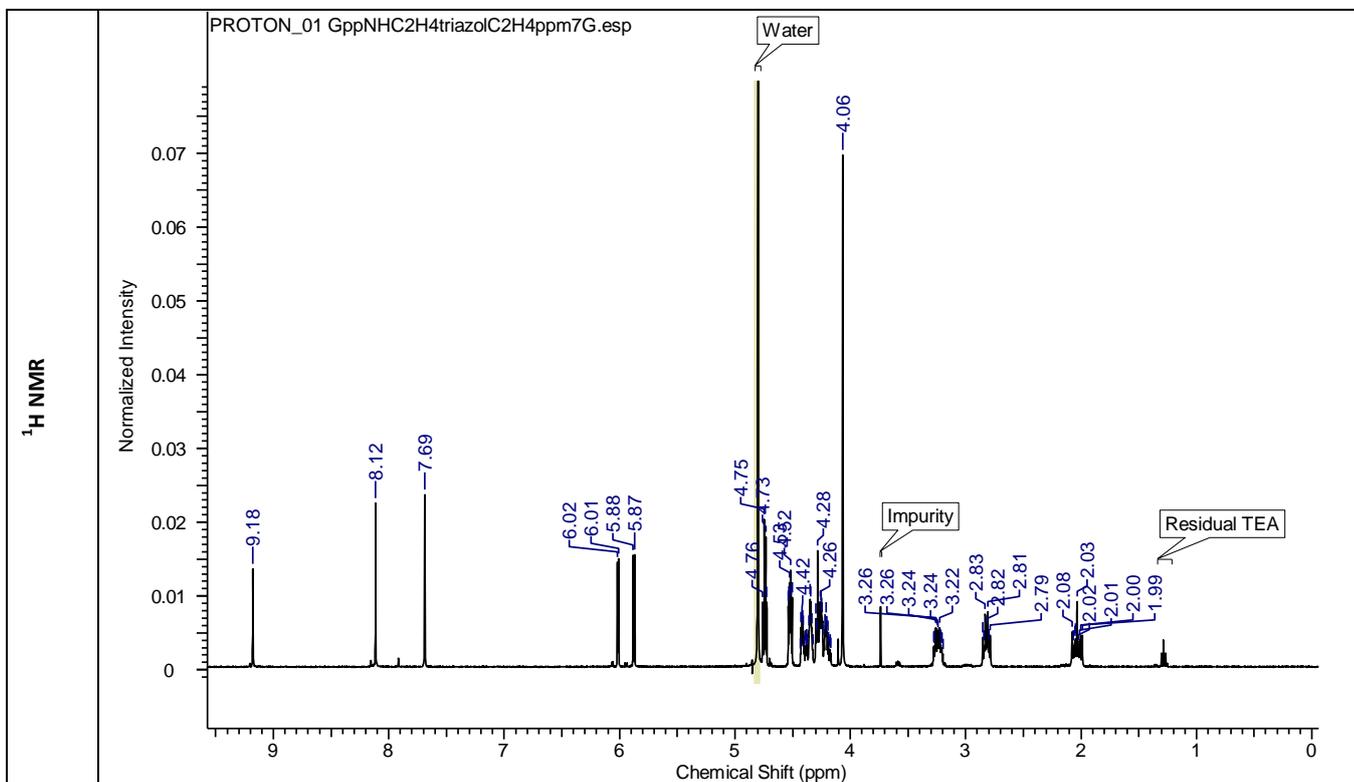




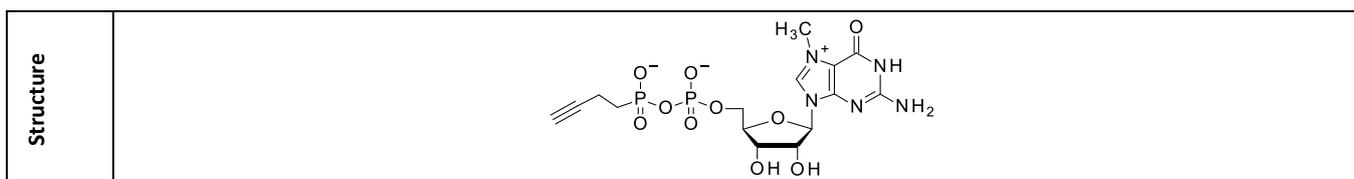
**(9b) m<sup>7</sup>GppC<sub>2</sub>H<sub>4</sub>-triazole-C<sub>2</sub>H<sub>4</sub>NHppG**

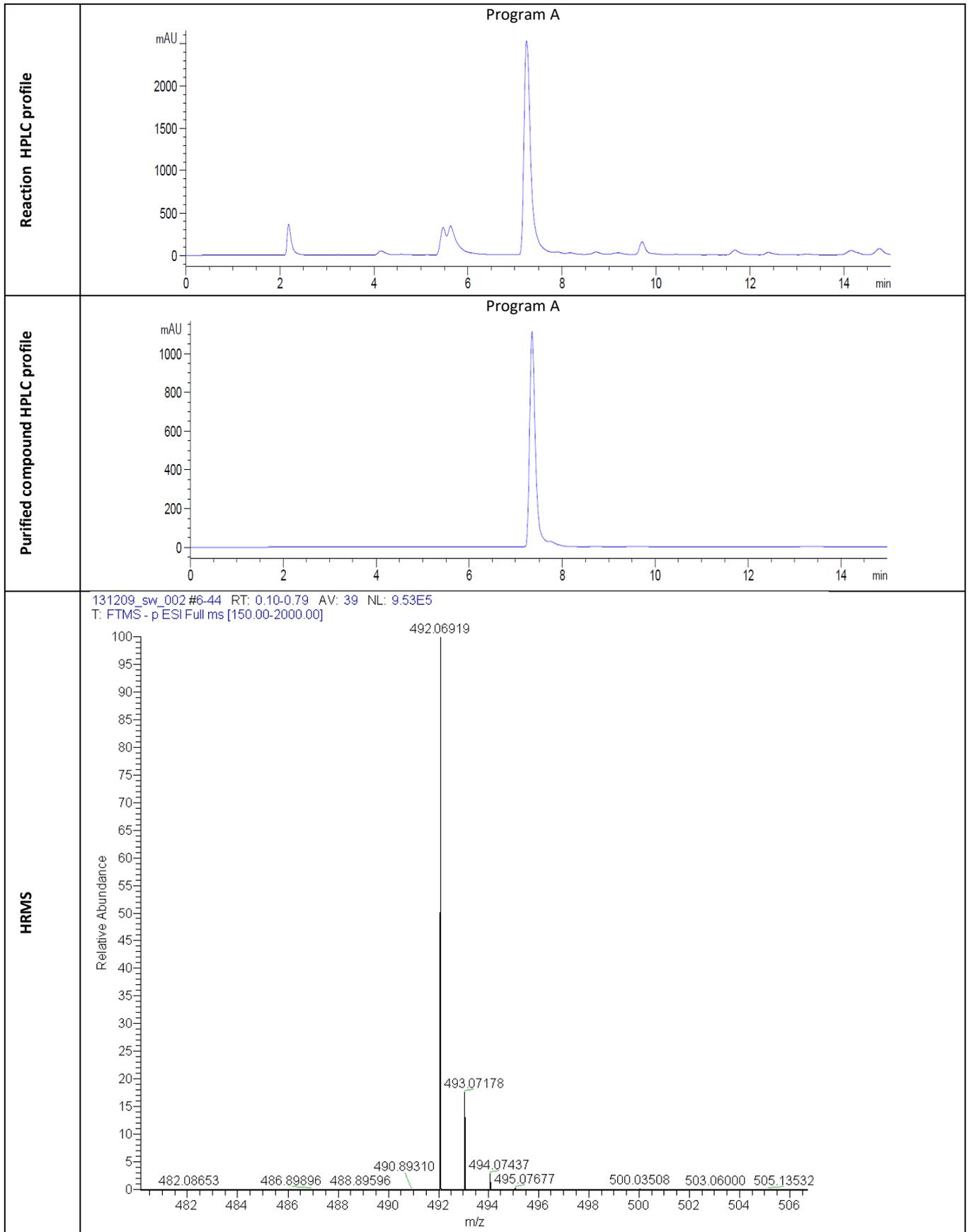


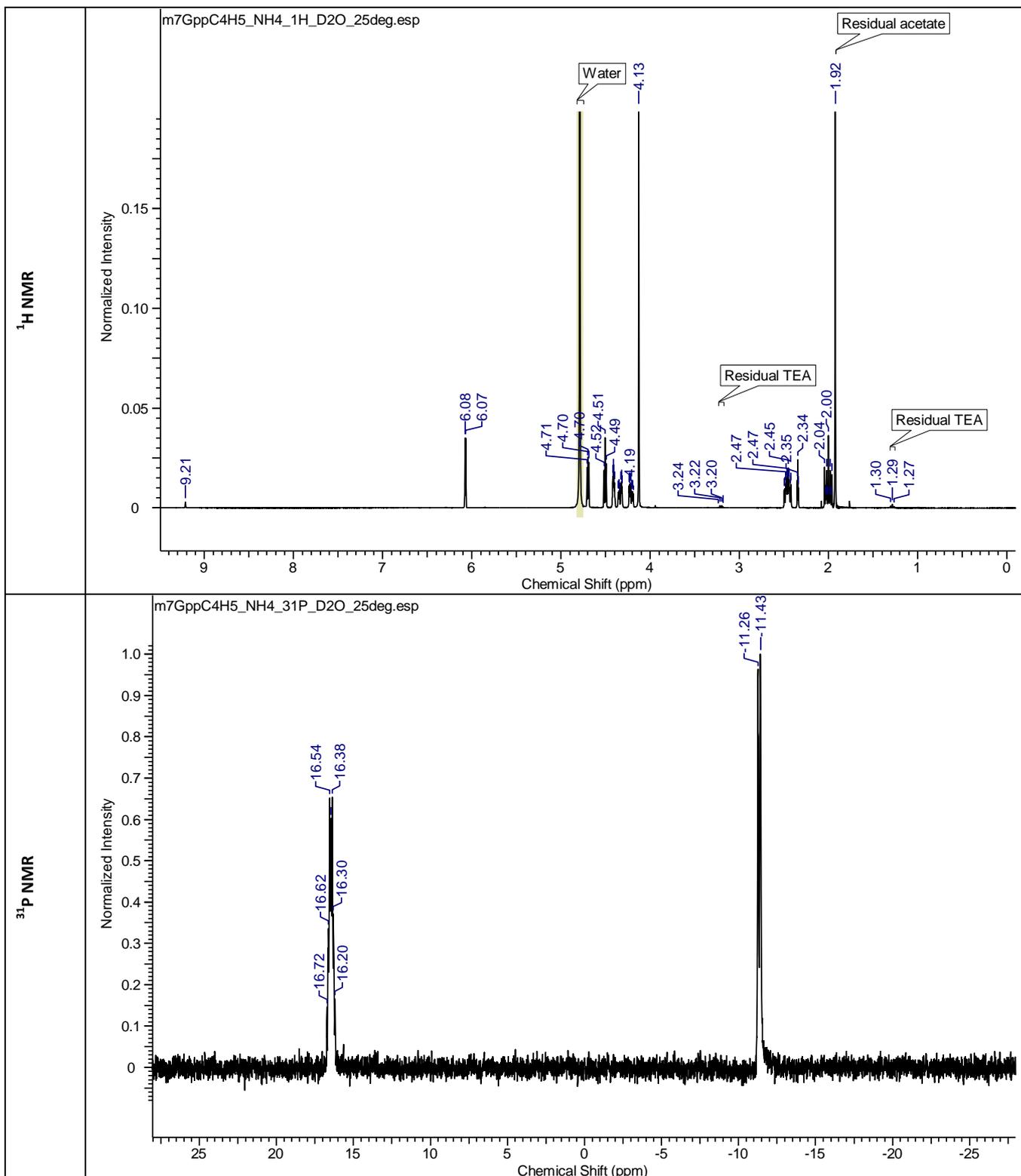




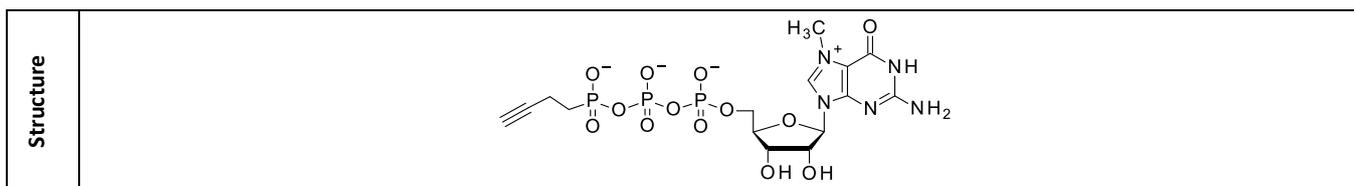
(10c) m<sup>7</sup>GppC<sub>4</sub>H<sub>5</sub>

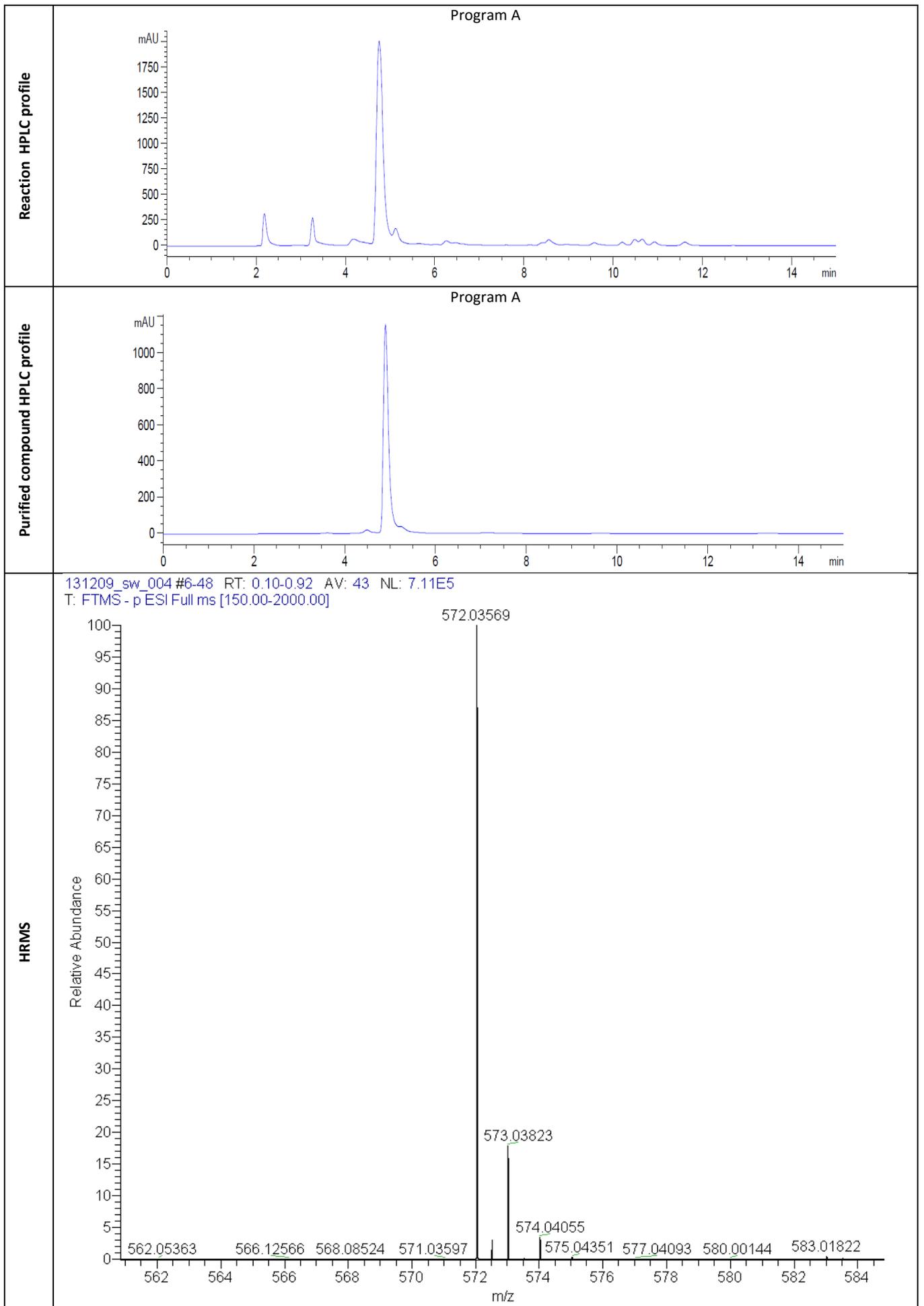


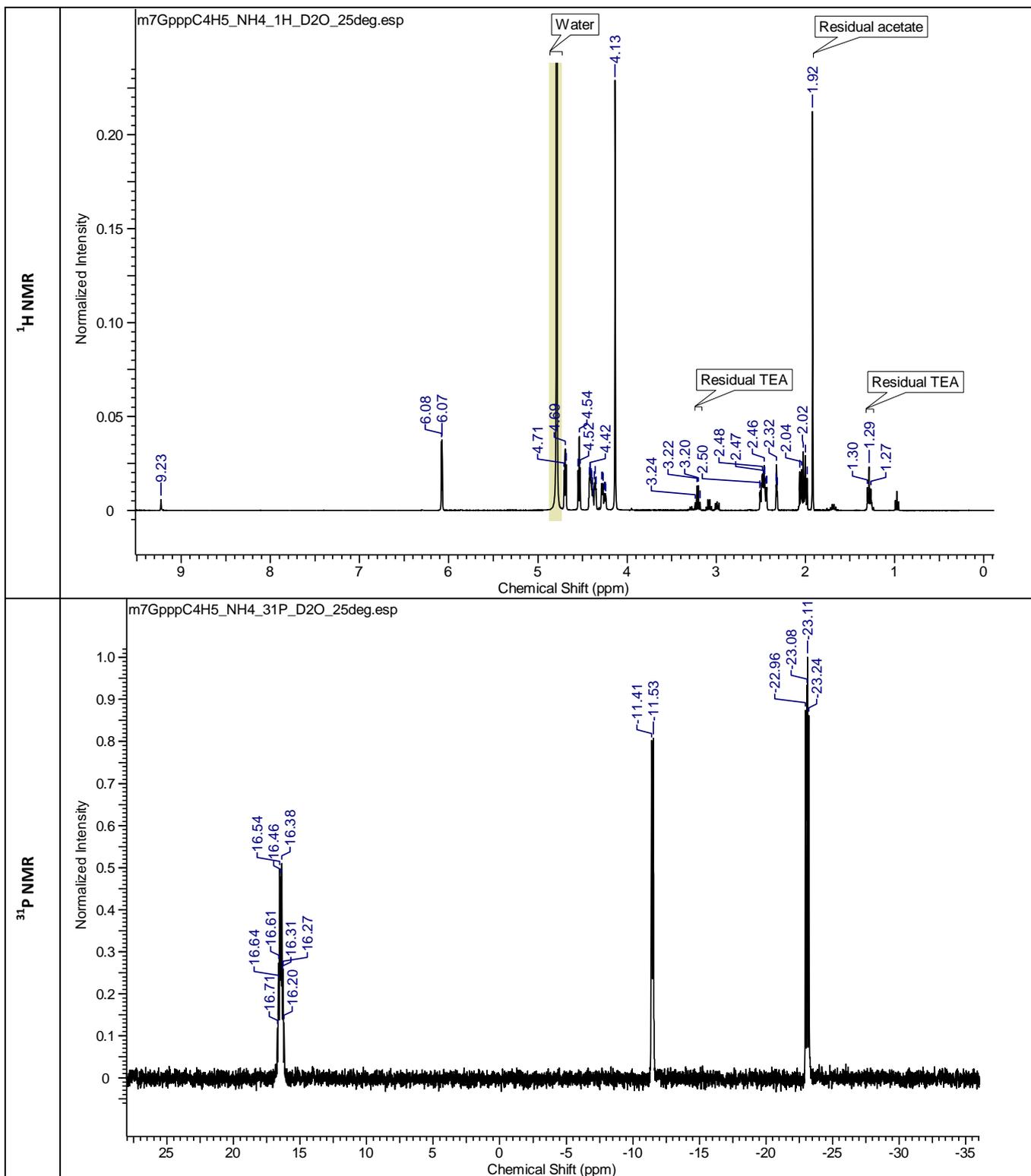




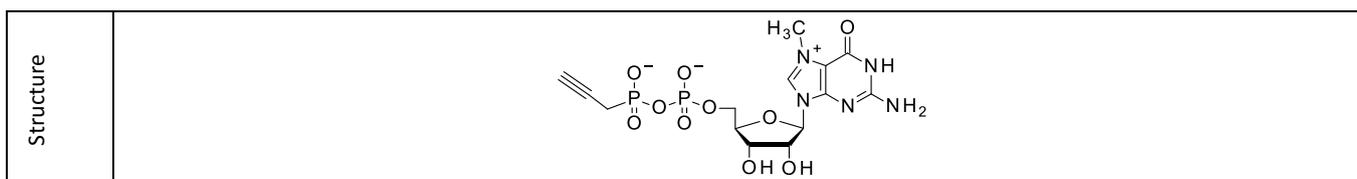
(10d) m<sup>7</sup>GpppC<sub>4</sub>H<sub>5</sub>

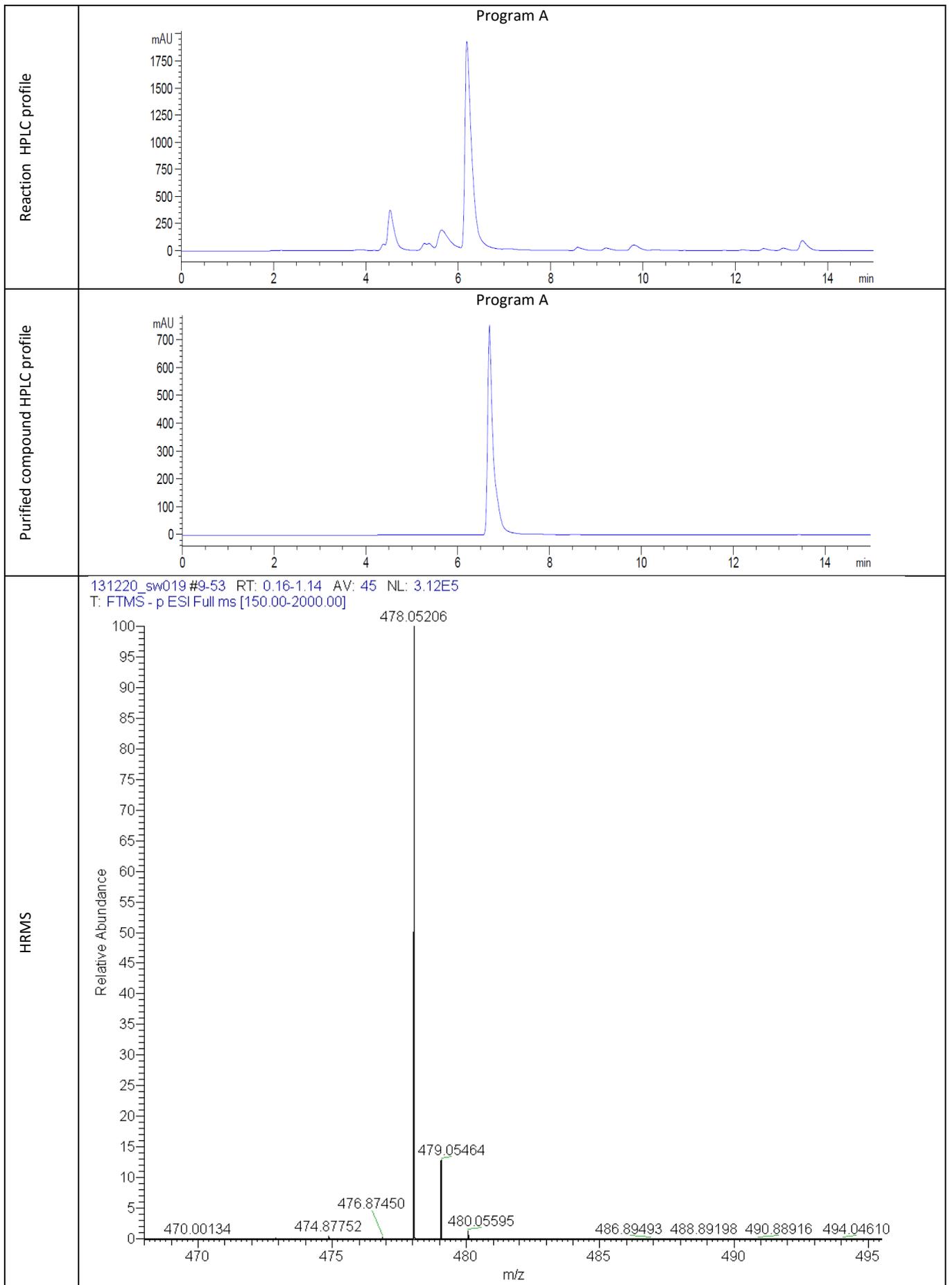


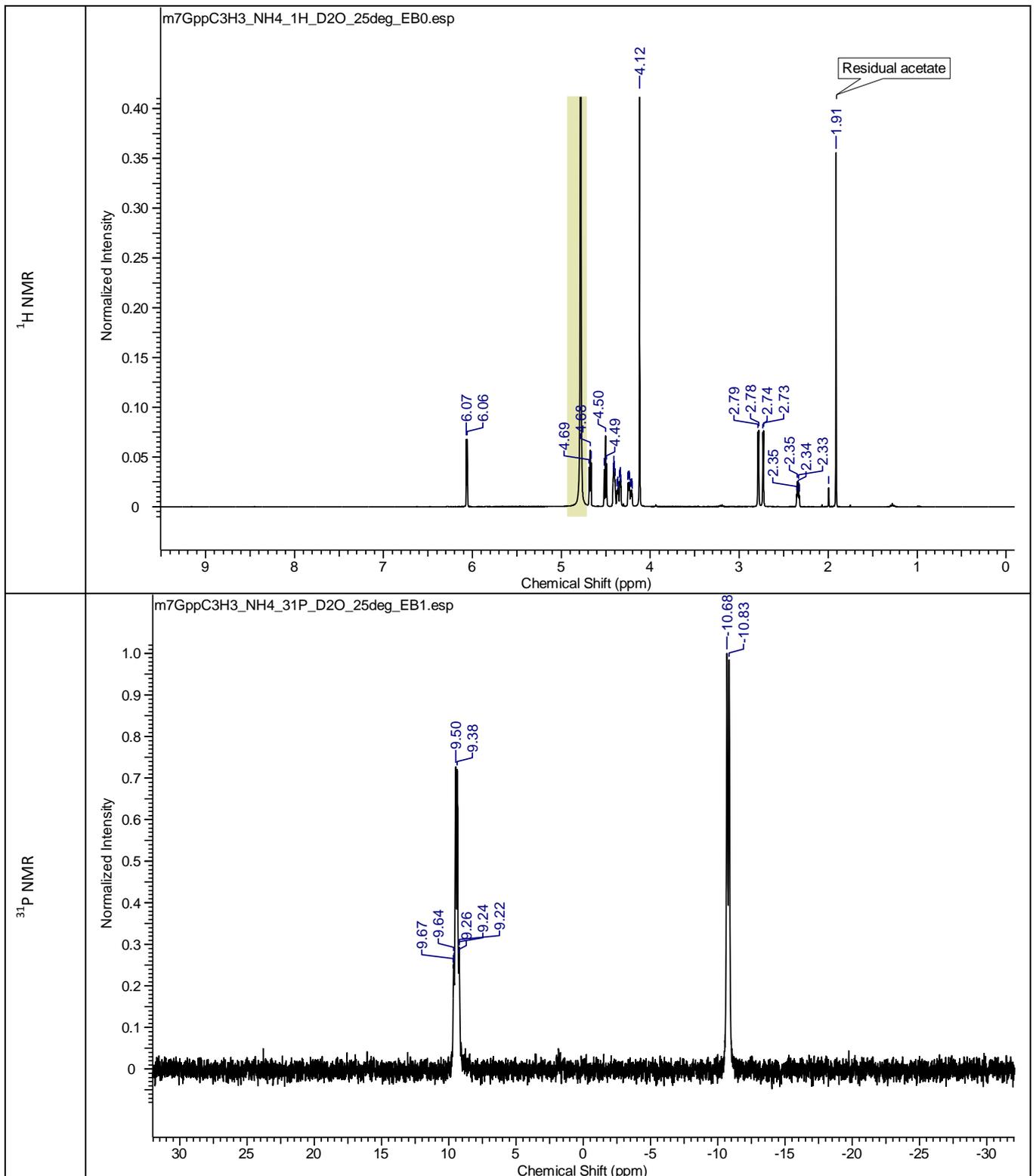




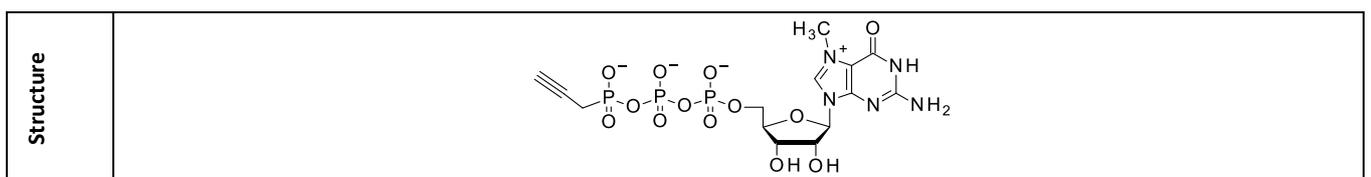
**(11c) m<sup>7</sup>GppC<sub>3</sub>H<sub>3</sub>**

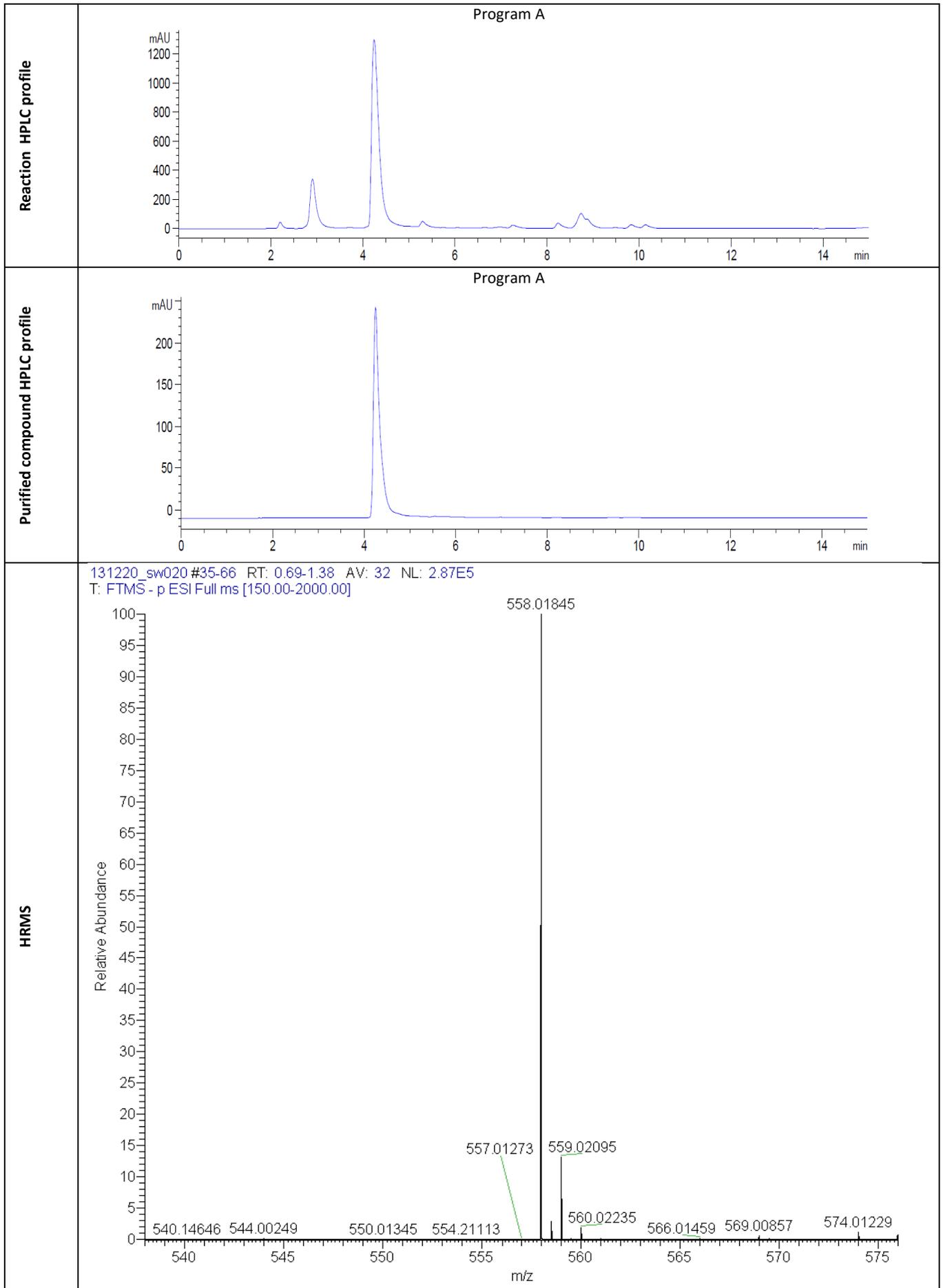


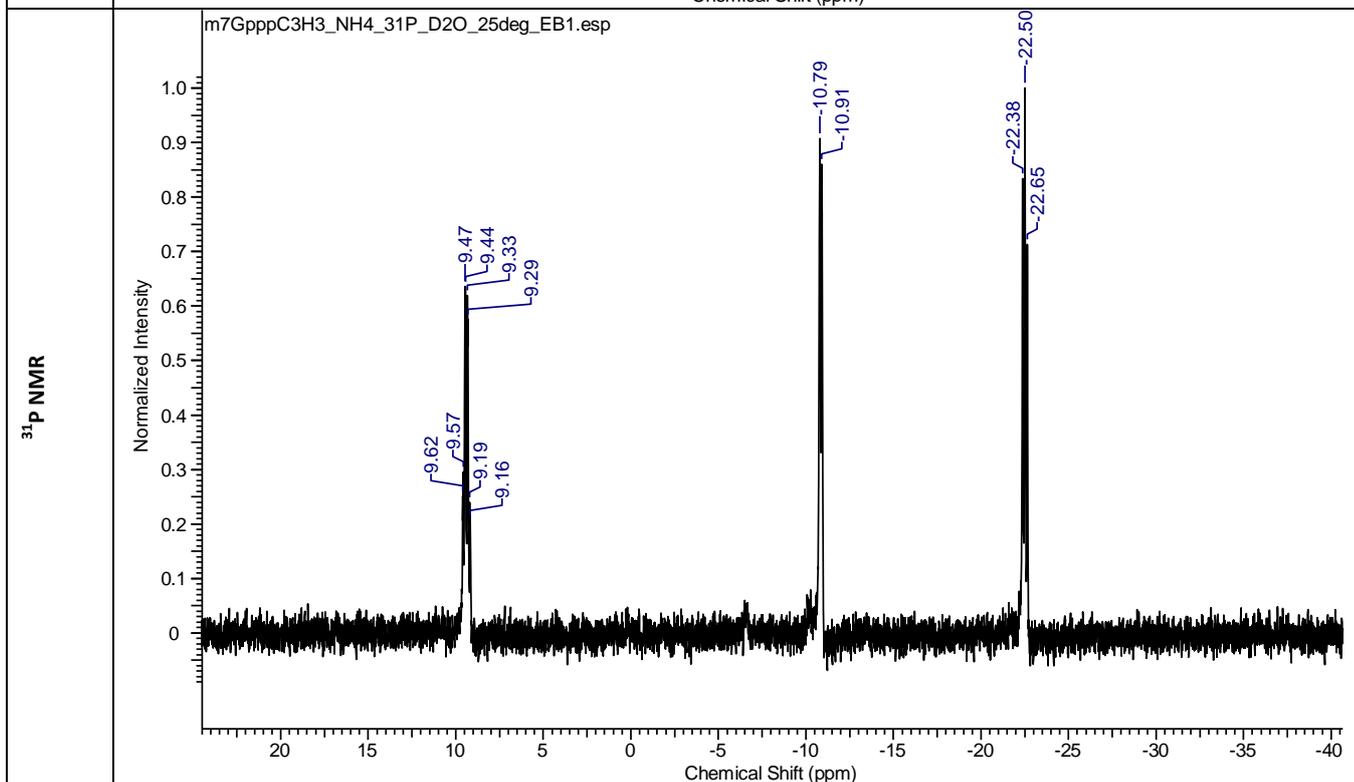
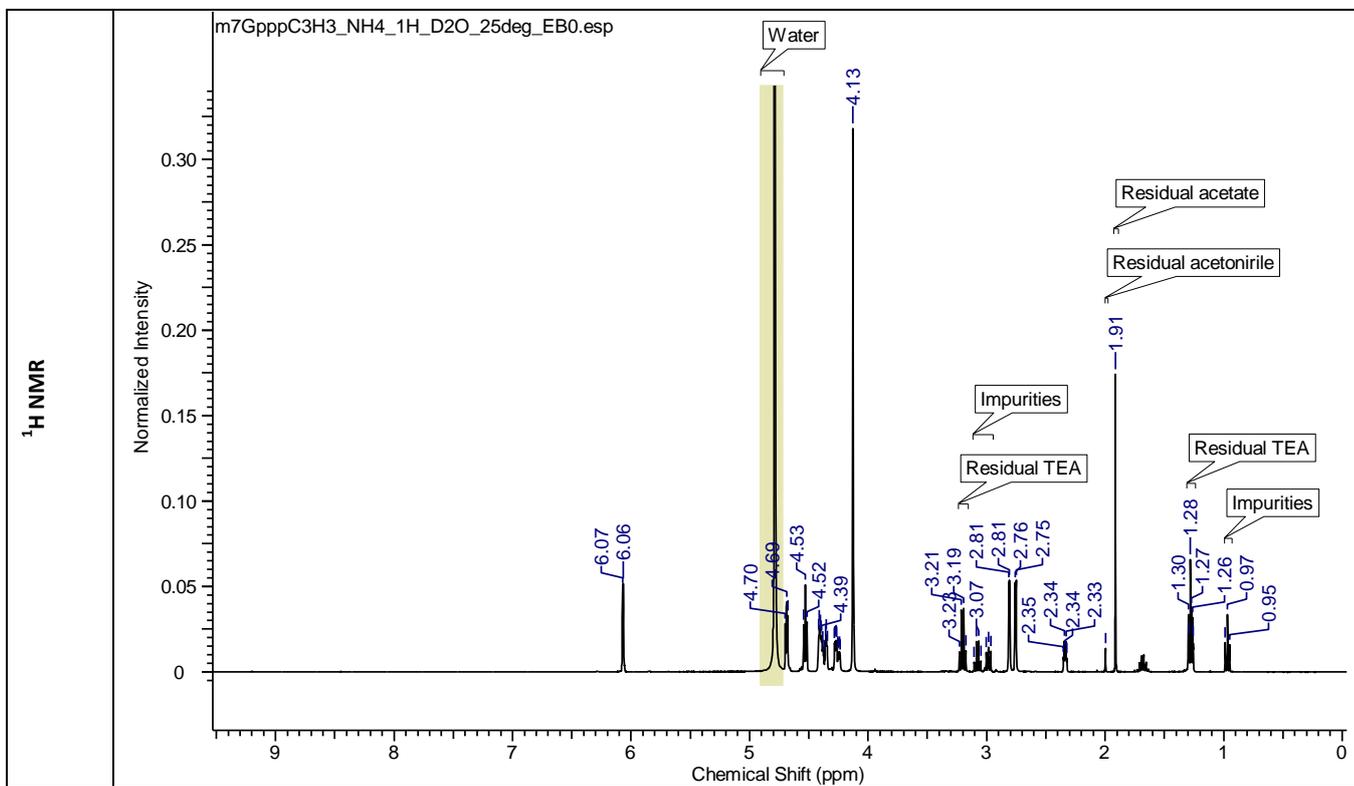




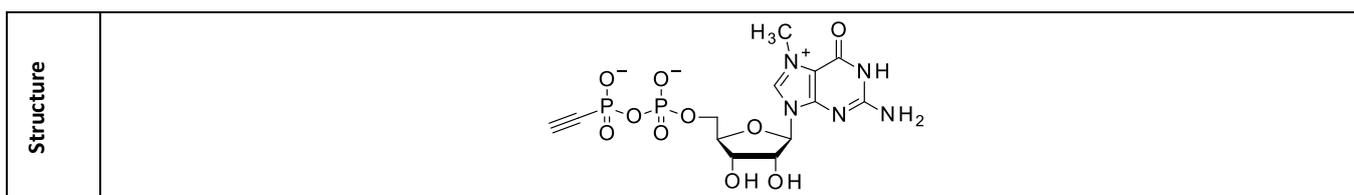
**(11d) m<sup>7</sup>GpppC<sub>3</sub>H<sub>3</sub>**

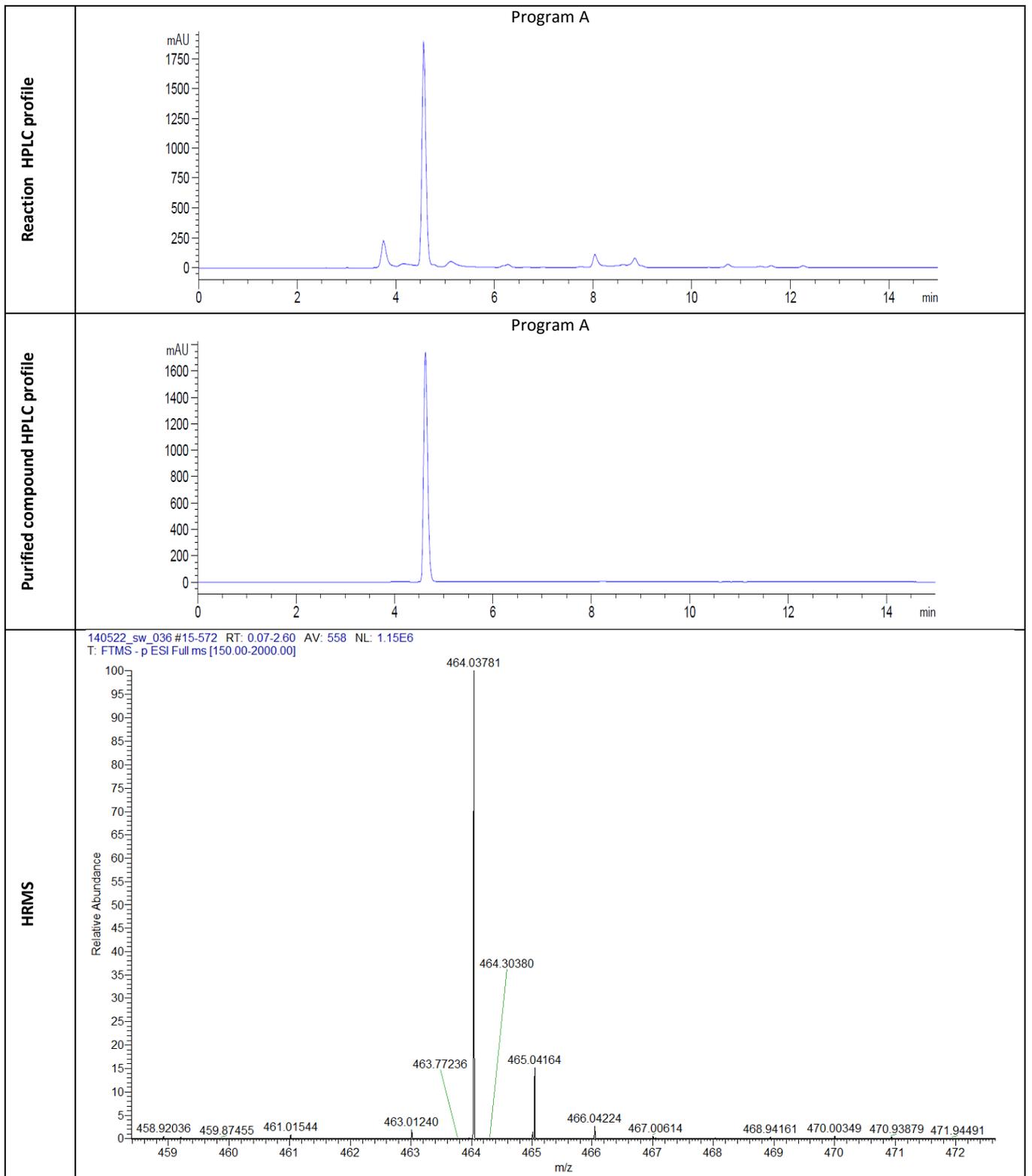


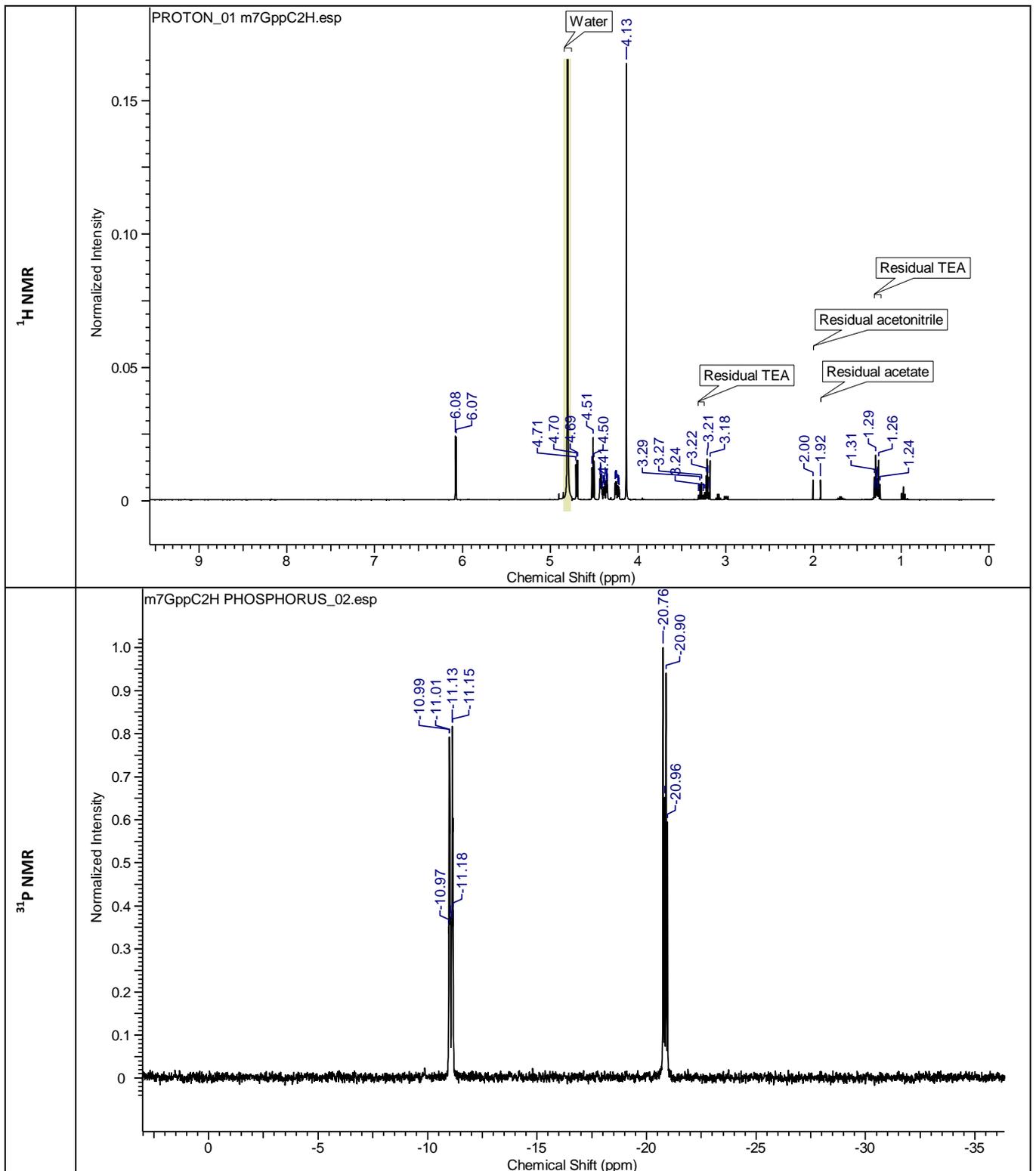




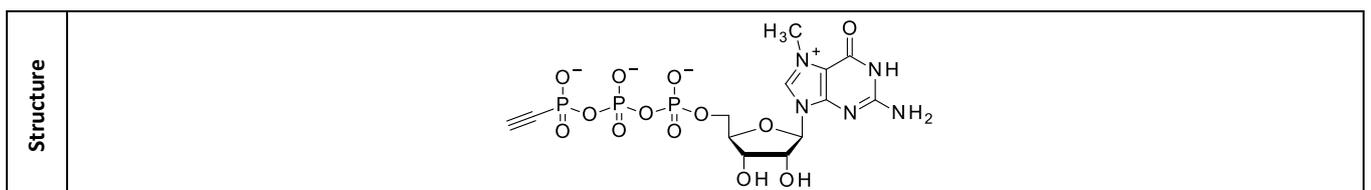
**(12c) m<sup>7</sup>GppC<sub>2</sub>H**

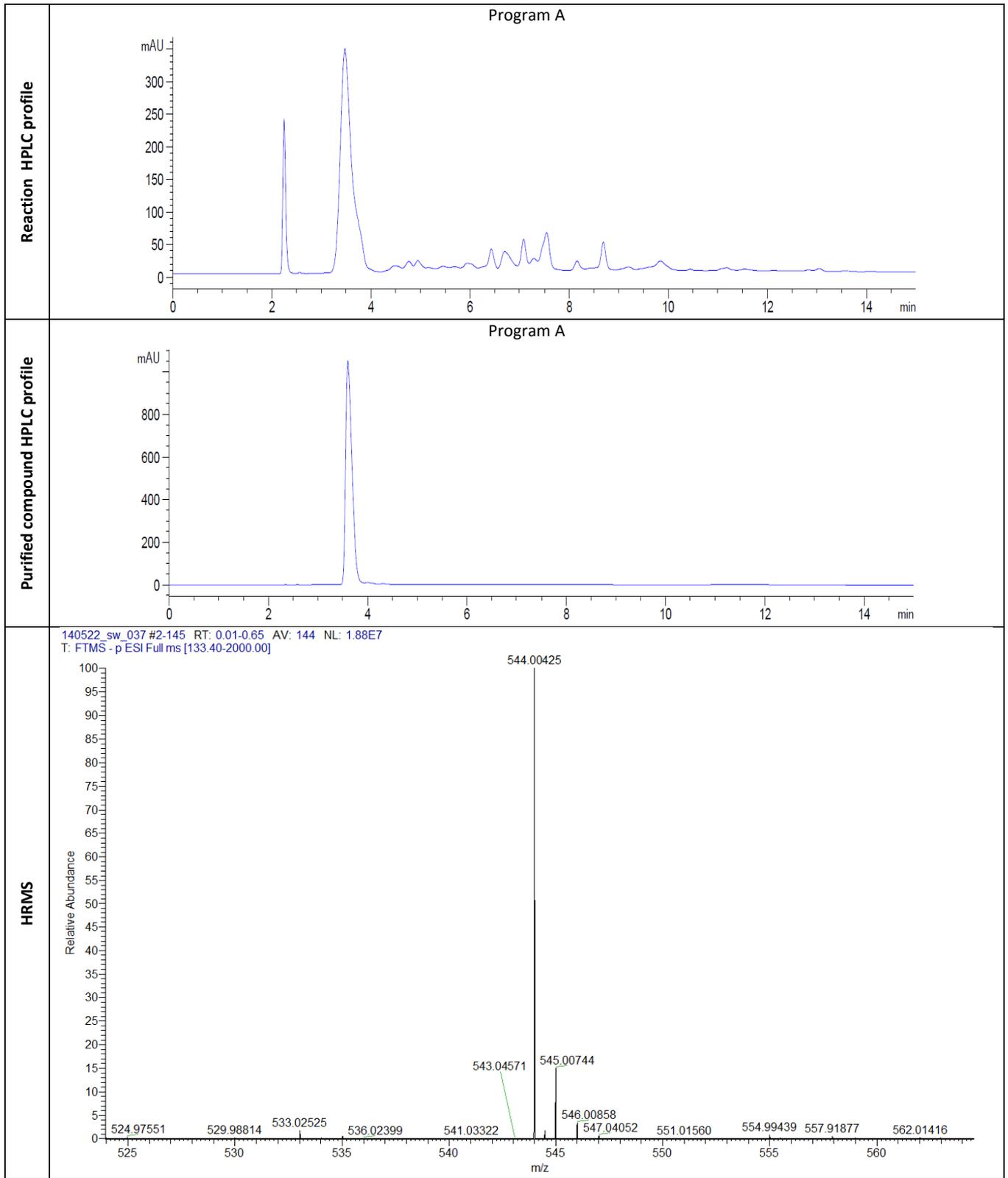


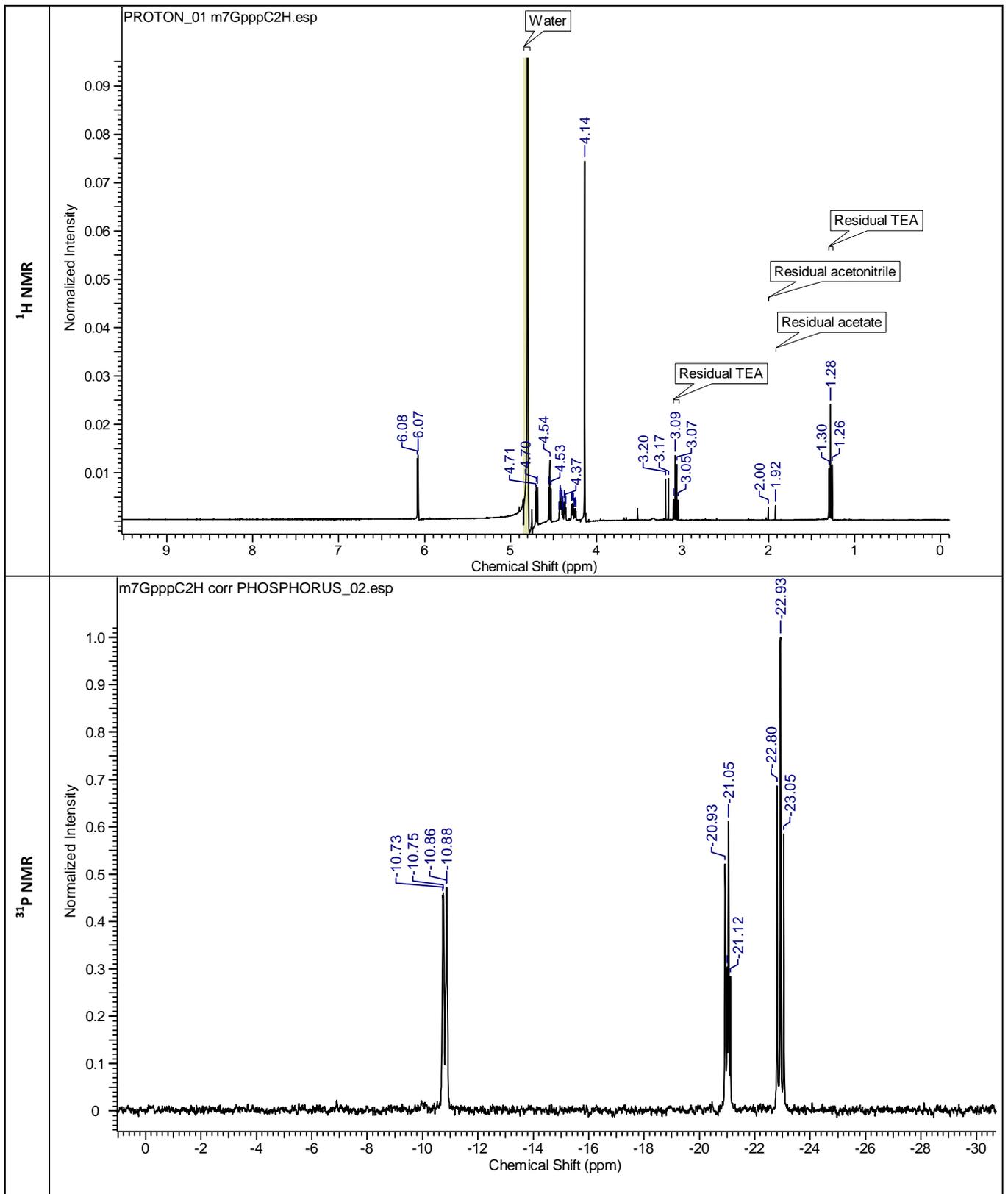




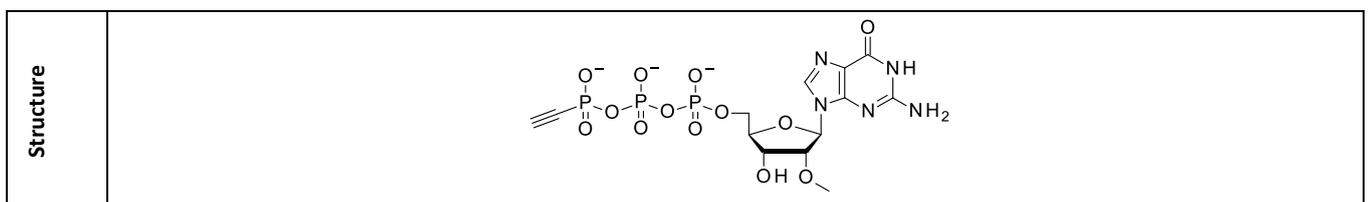
**(12d) m<sup>7</sup>GpppC<sub>2</sub>H**

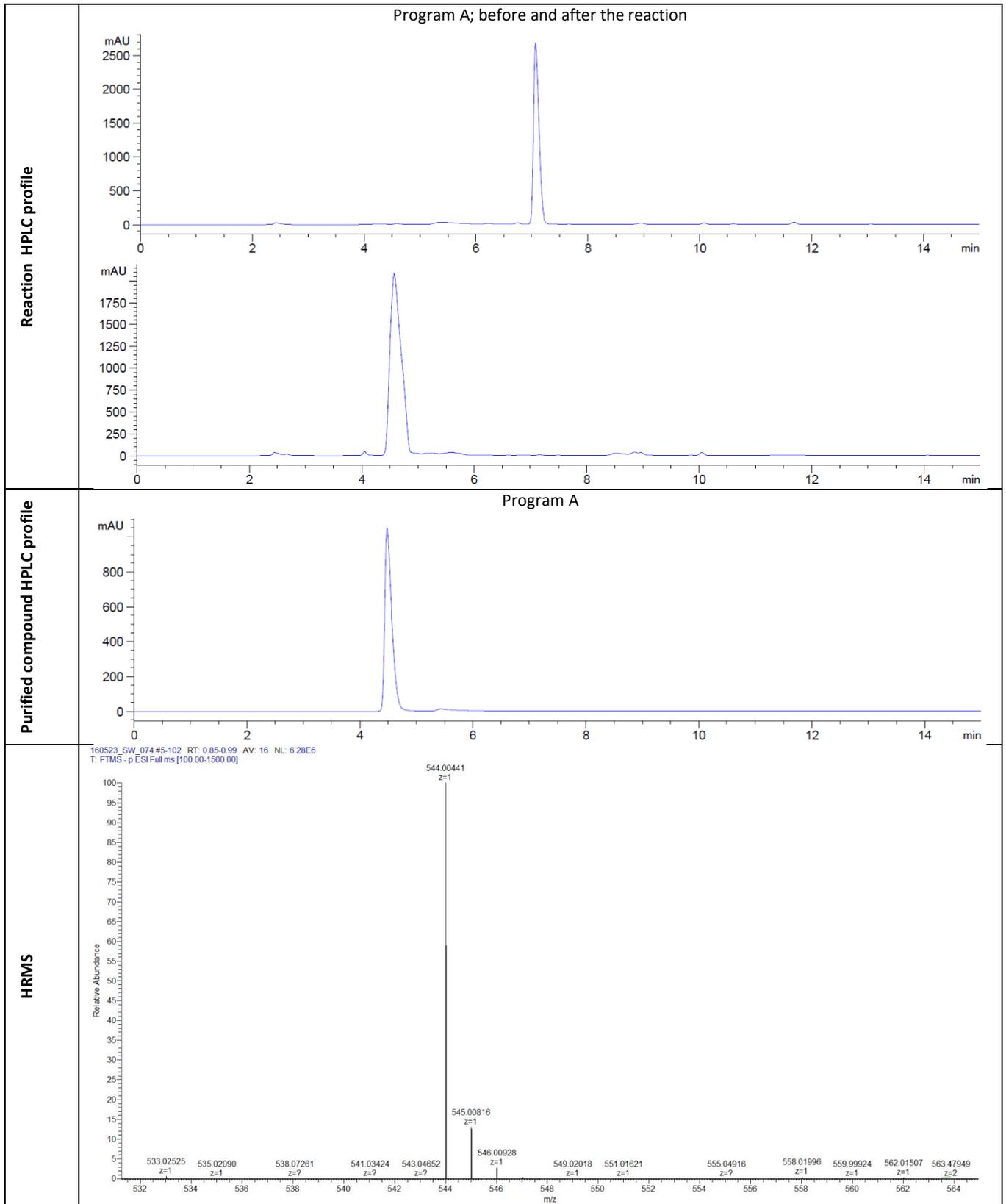


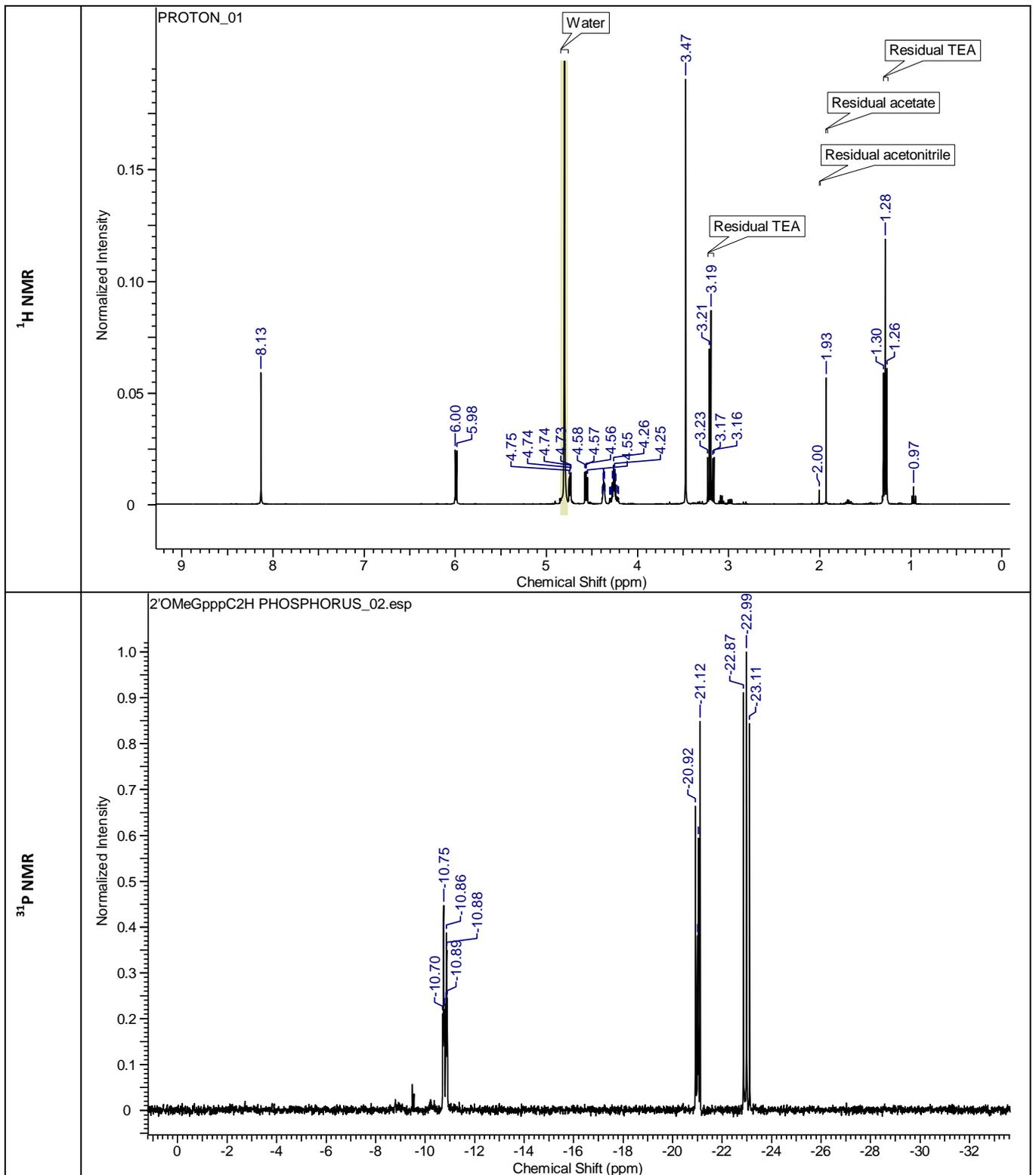




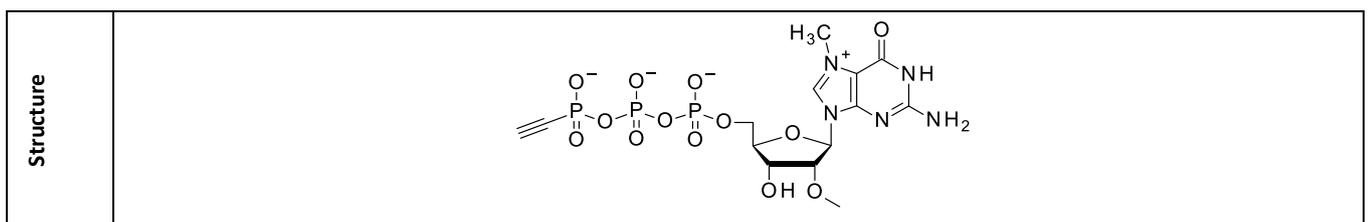
(12e) m<sup>2'-O</sup>GpppC<sub>2</sub>H

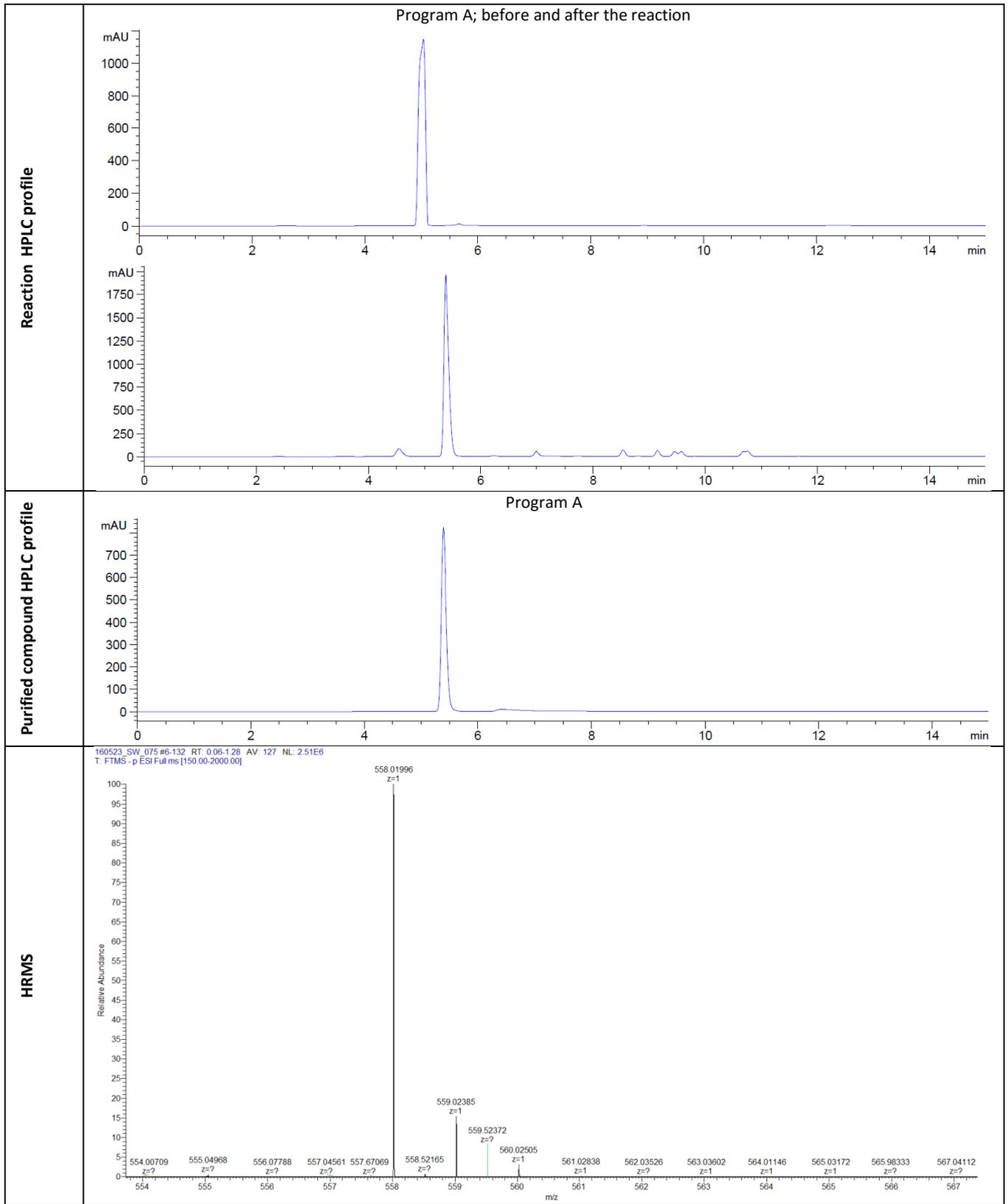


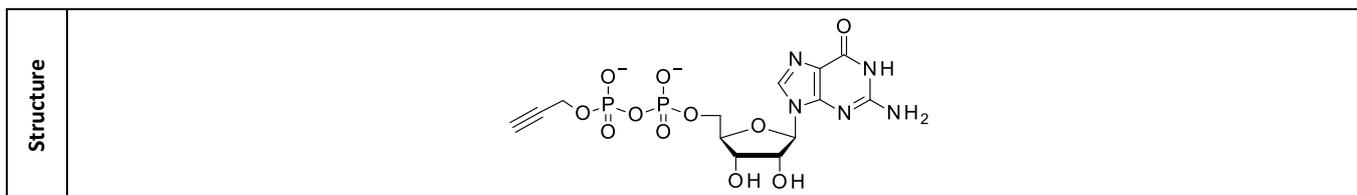
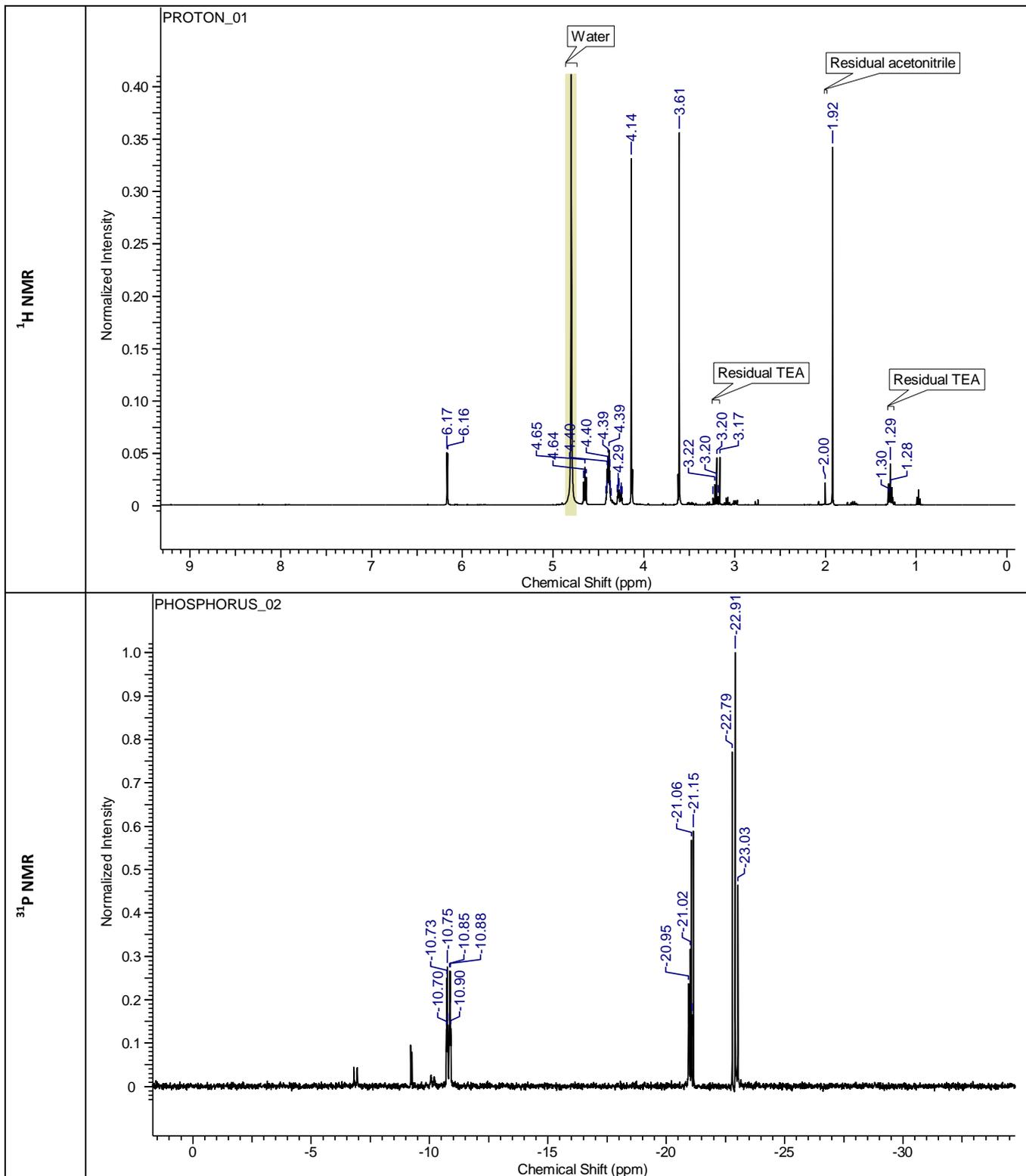


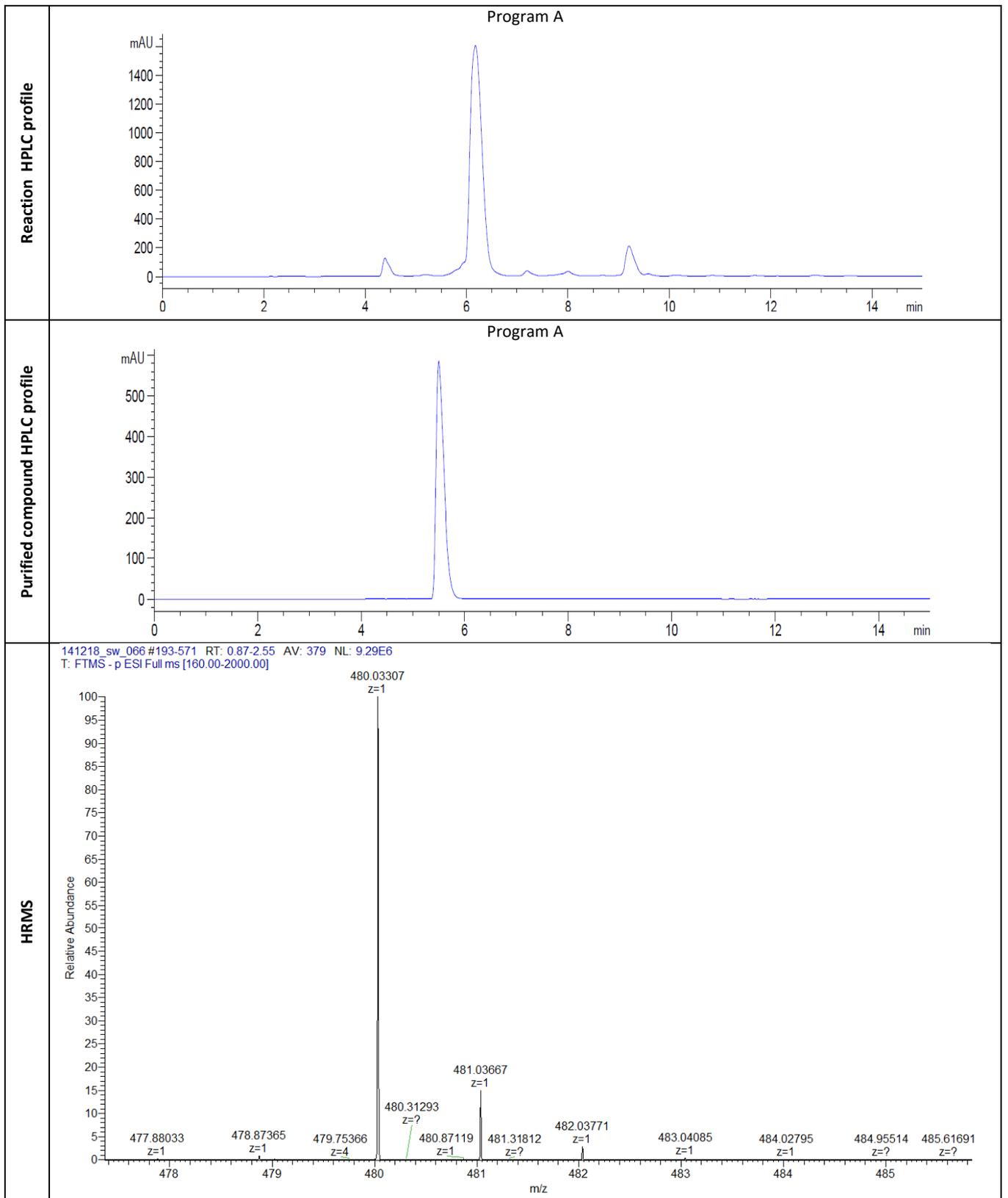


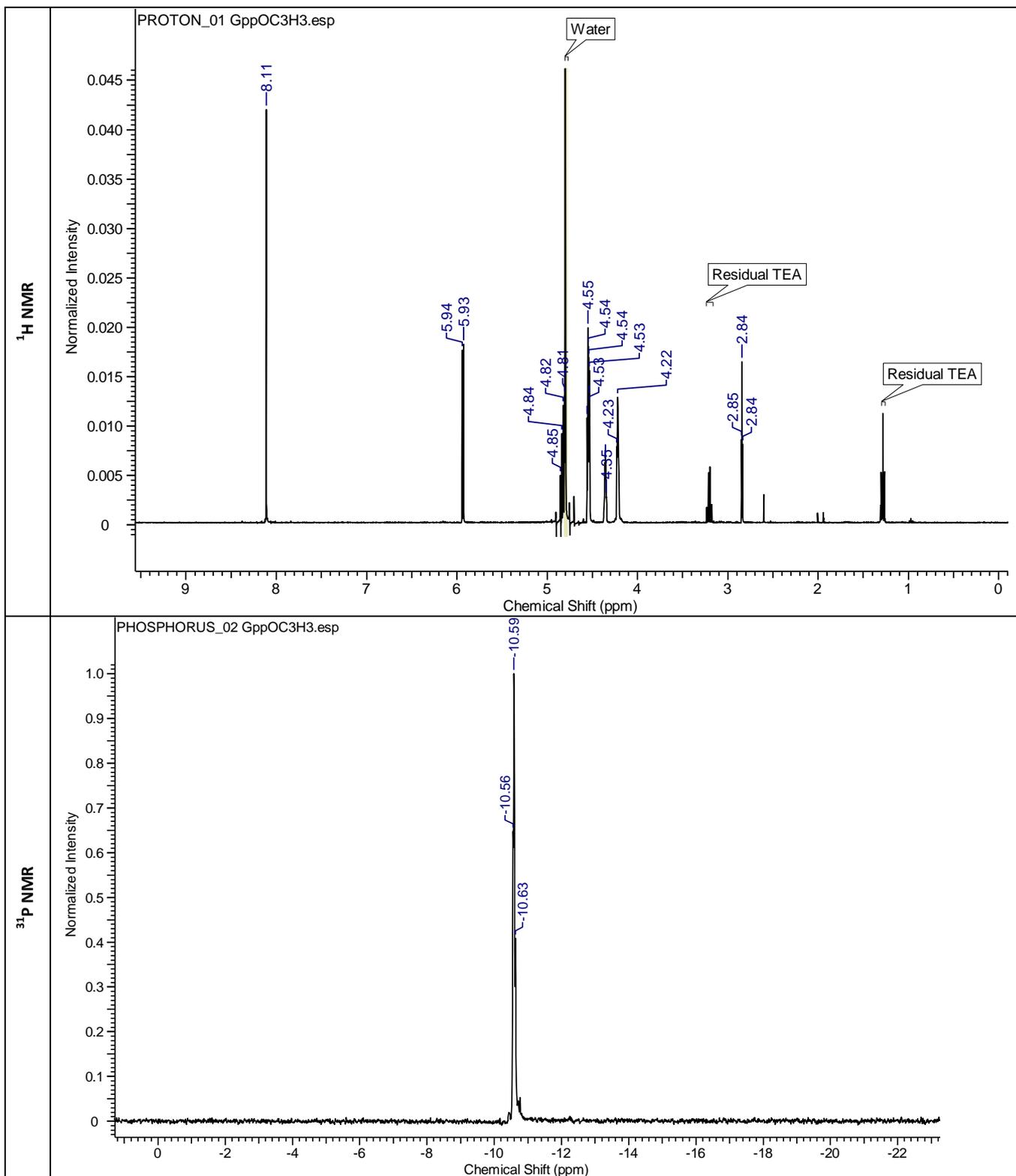
(12f)  $m_2^{7,2'-O}$ GpppC<sub>2</sub>H



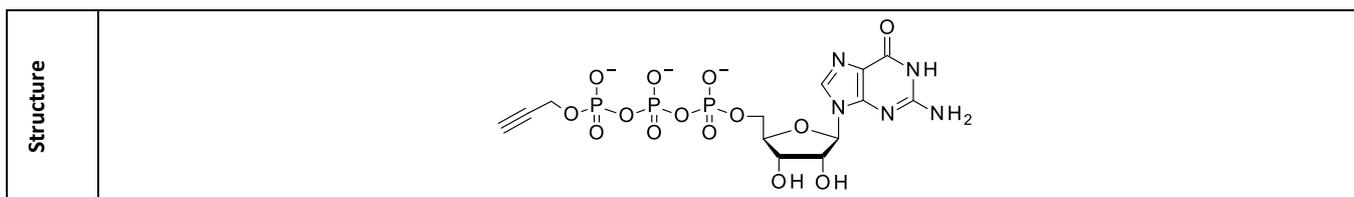


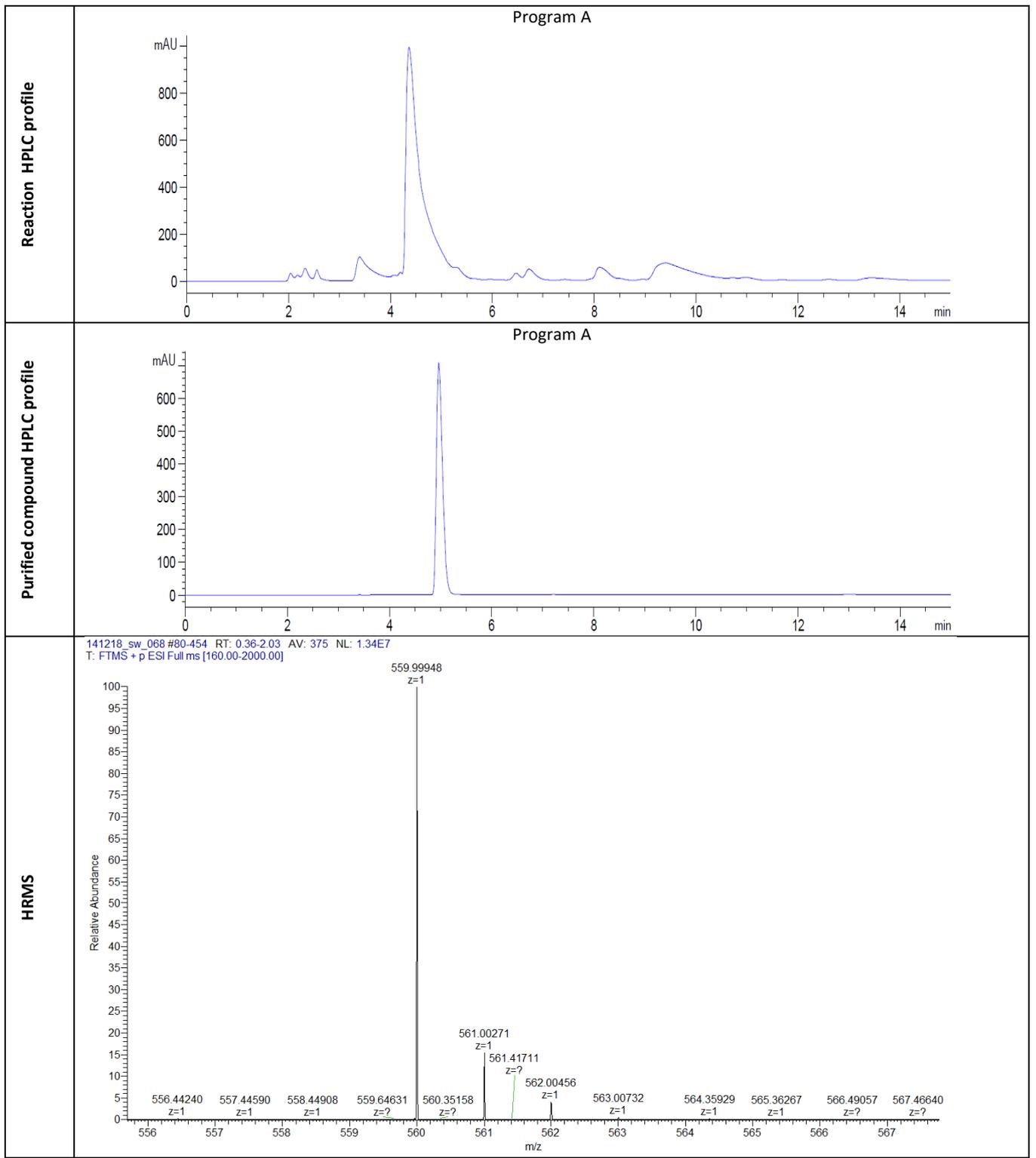


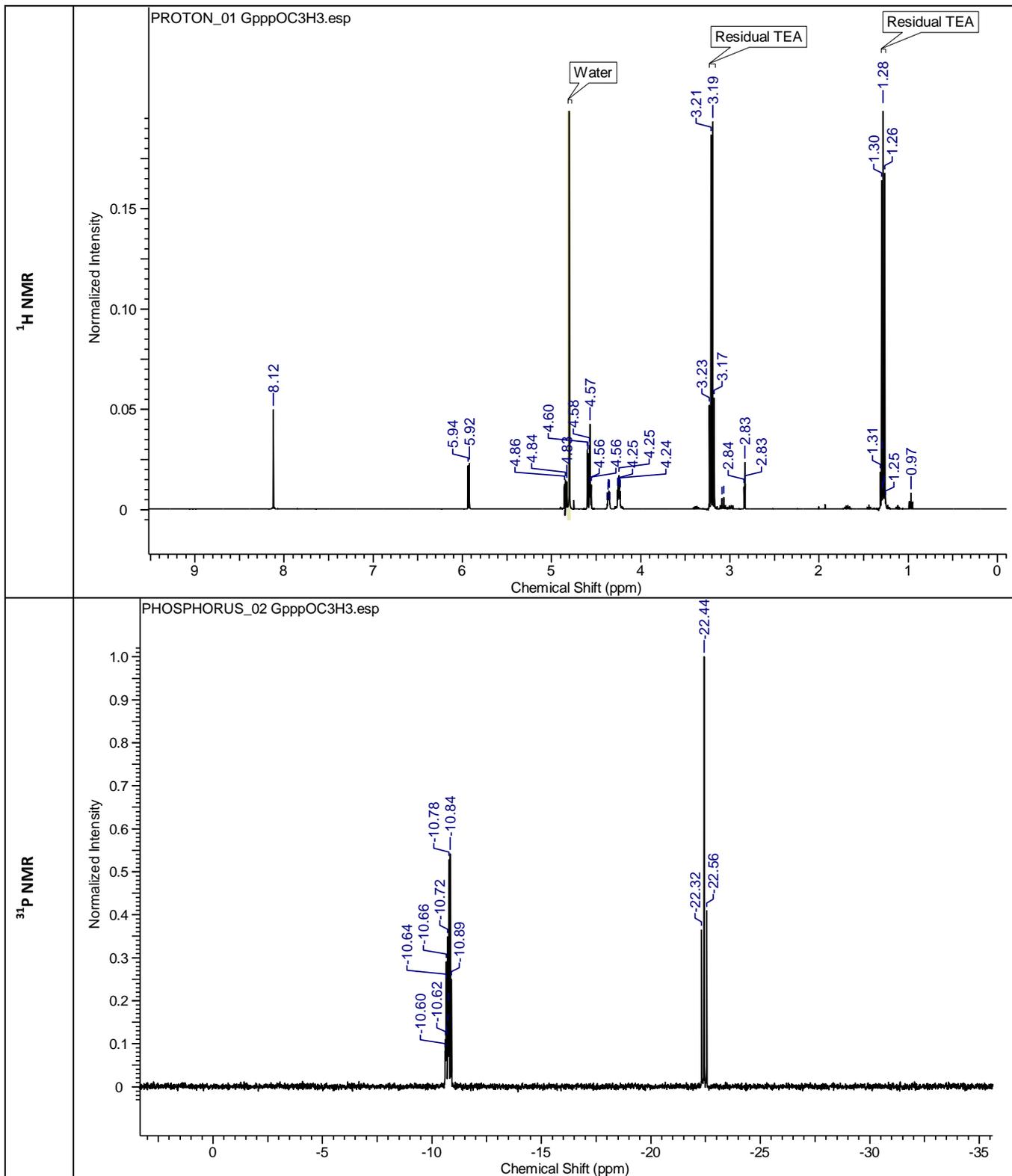




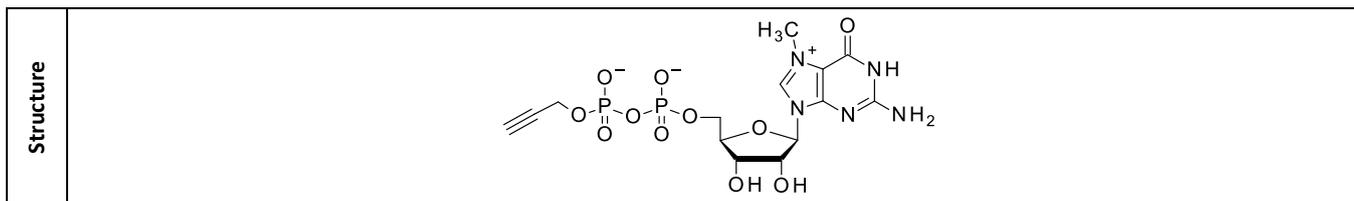
**(13b) GpppOC<sub>3</sub>H<sub>3</sub>**

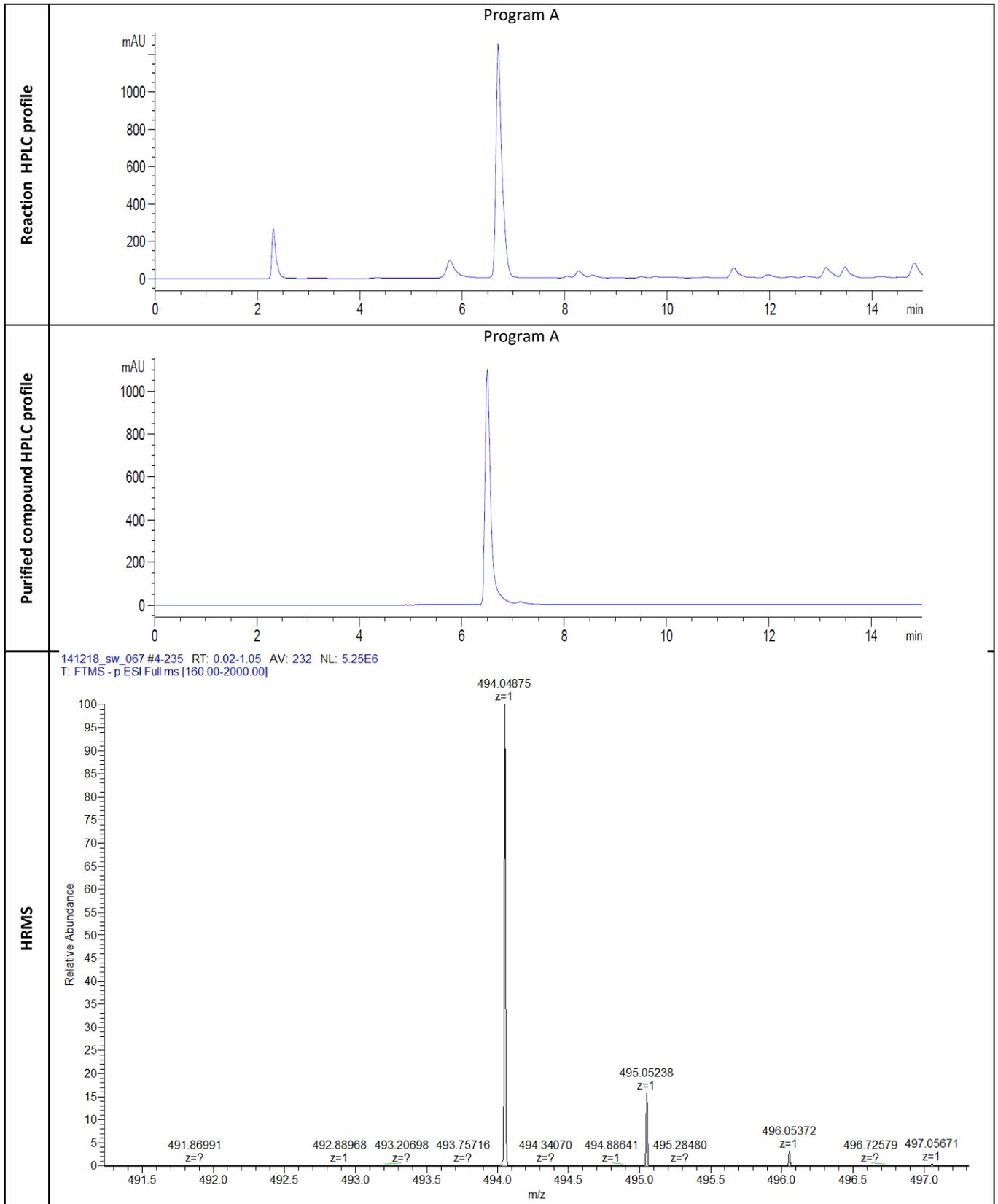


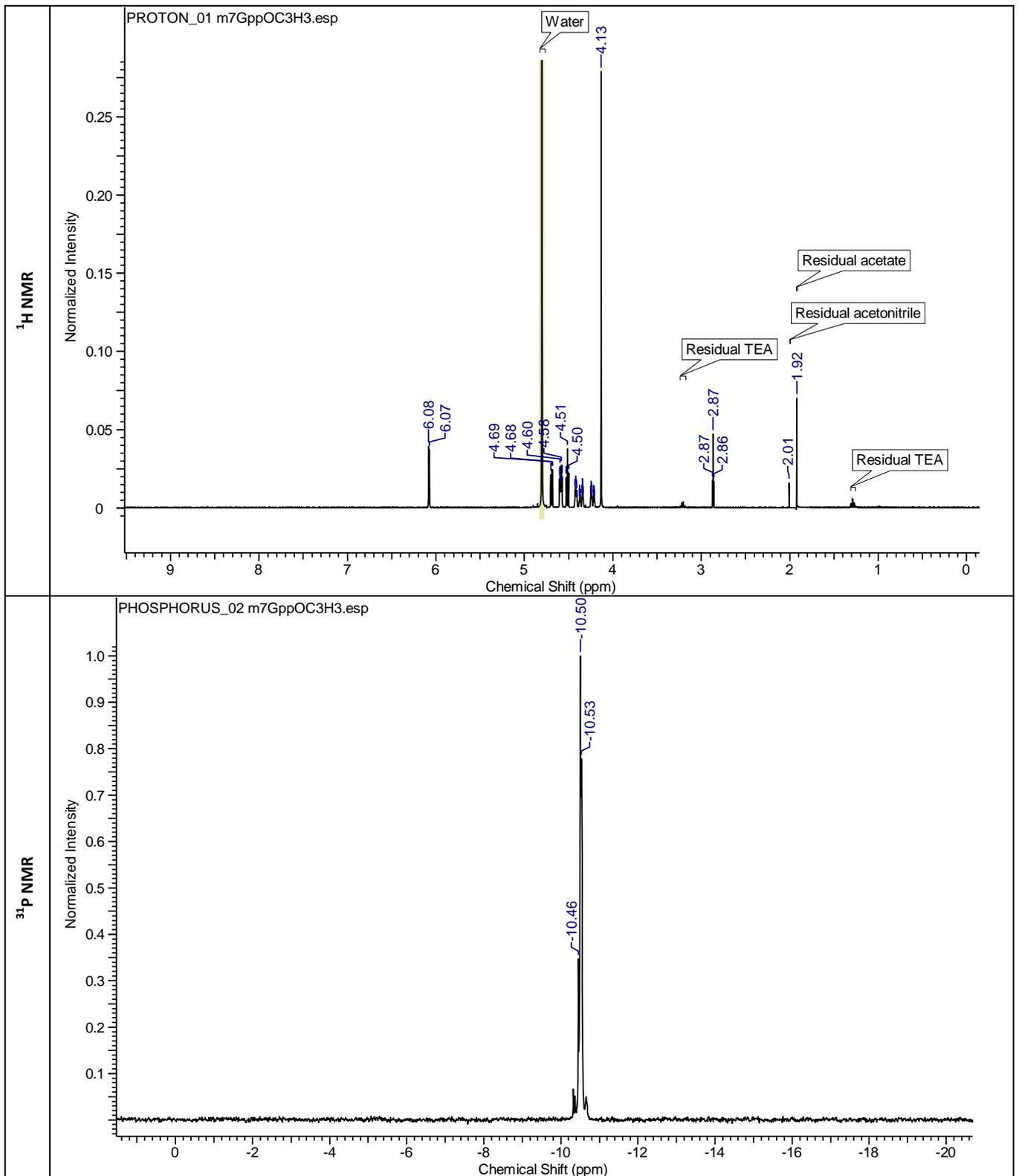




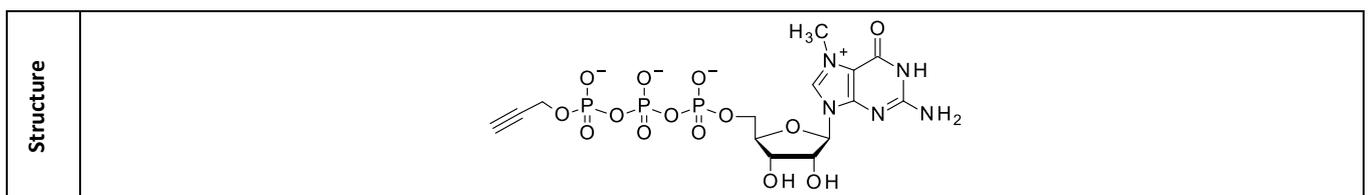
(13c) m<sup>7</sup>GppOC<sub>3</sub>H<sub>3</sub>

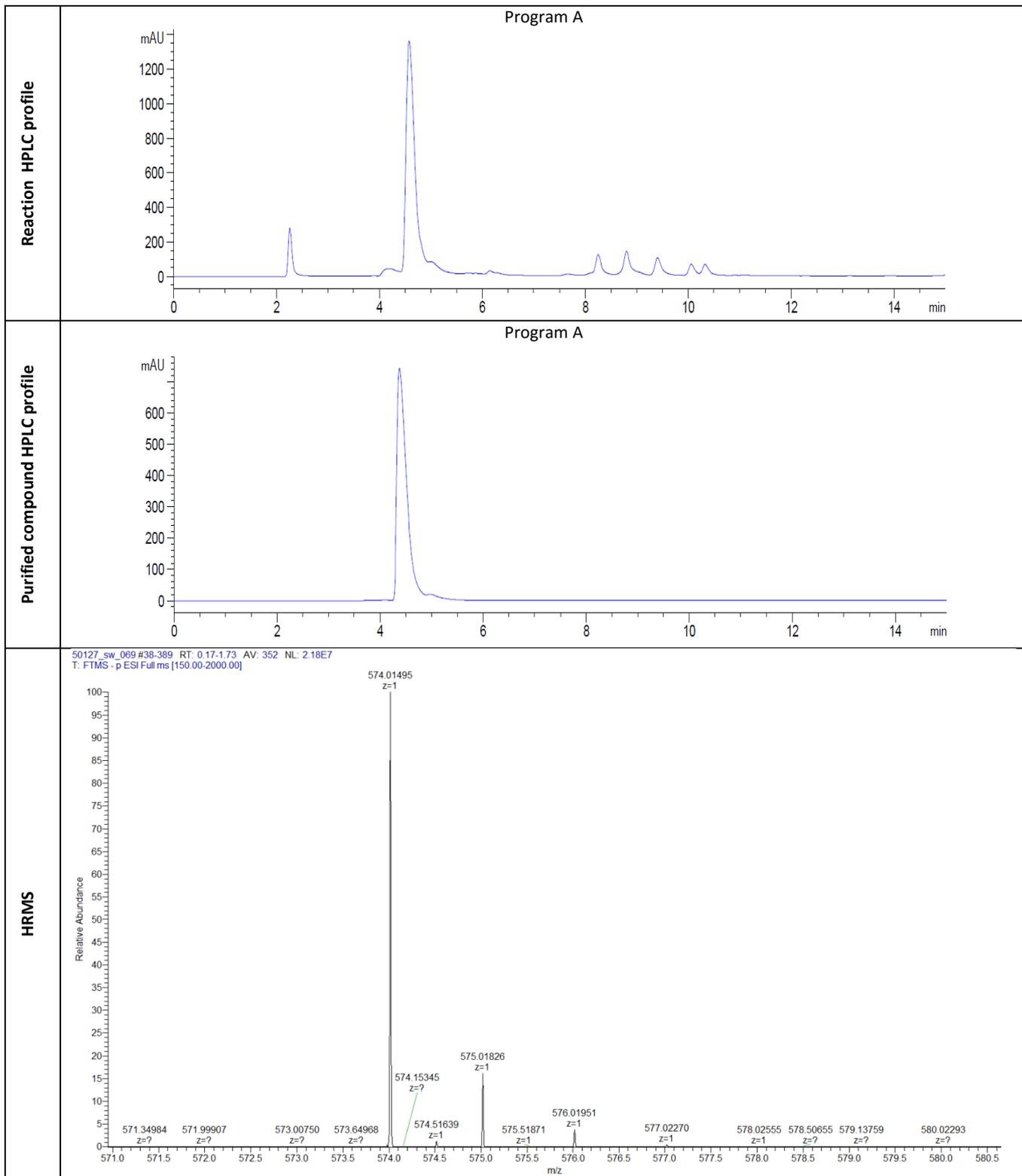


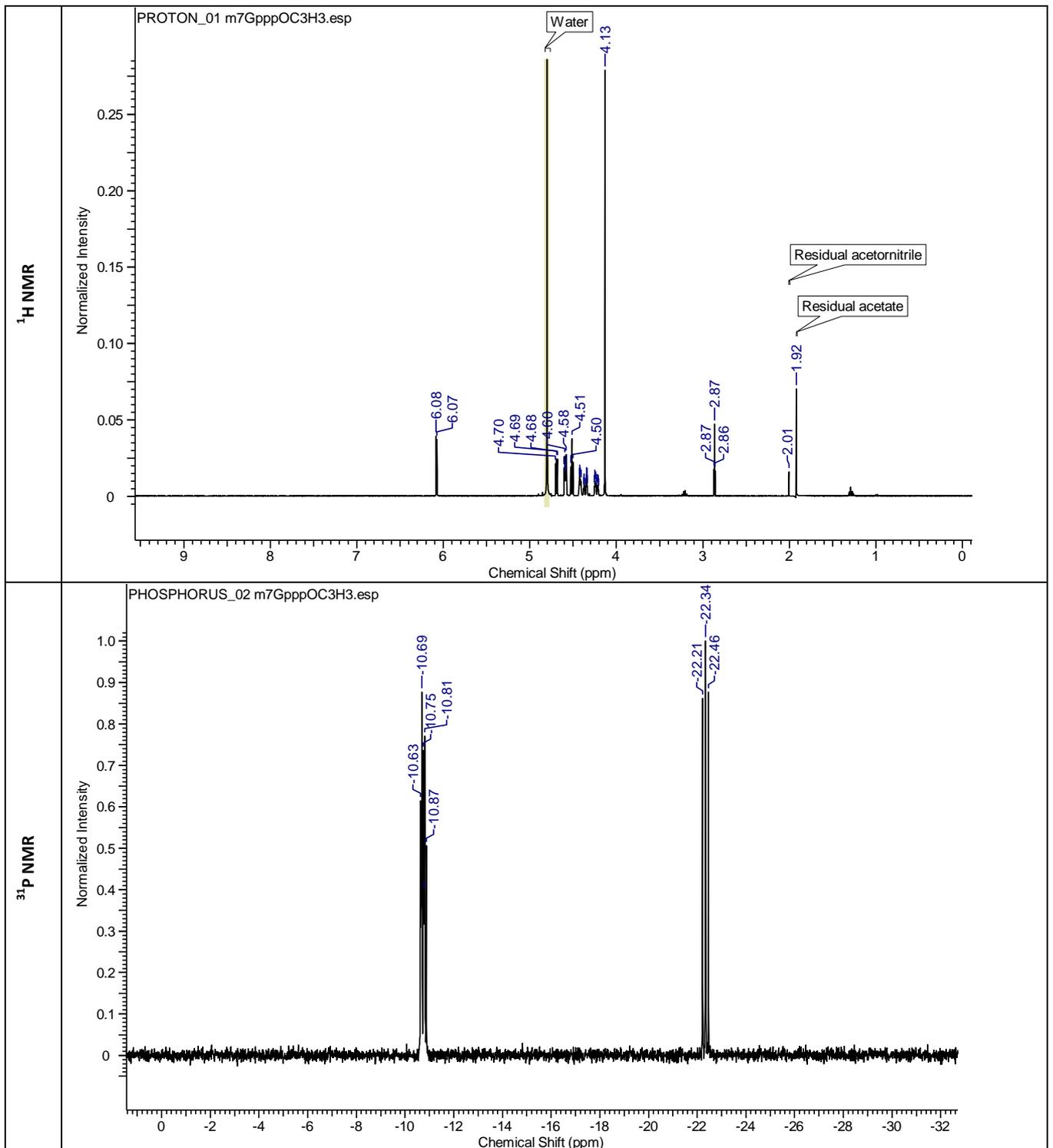




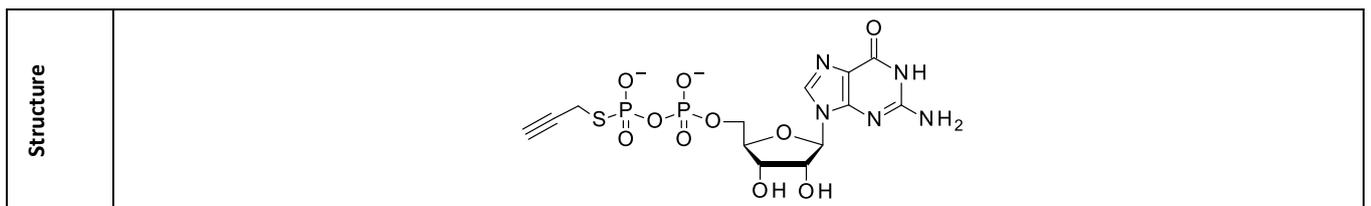
(13d) m<sup>7</sup>GppOC<sub>3</sub>H<sub>3</sub>

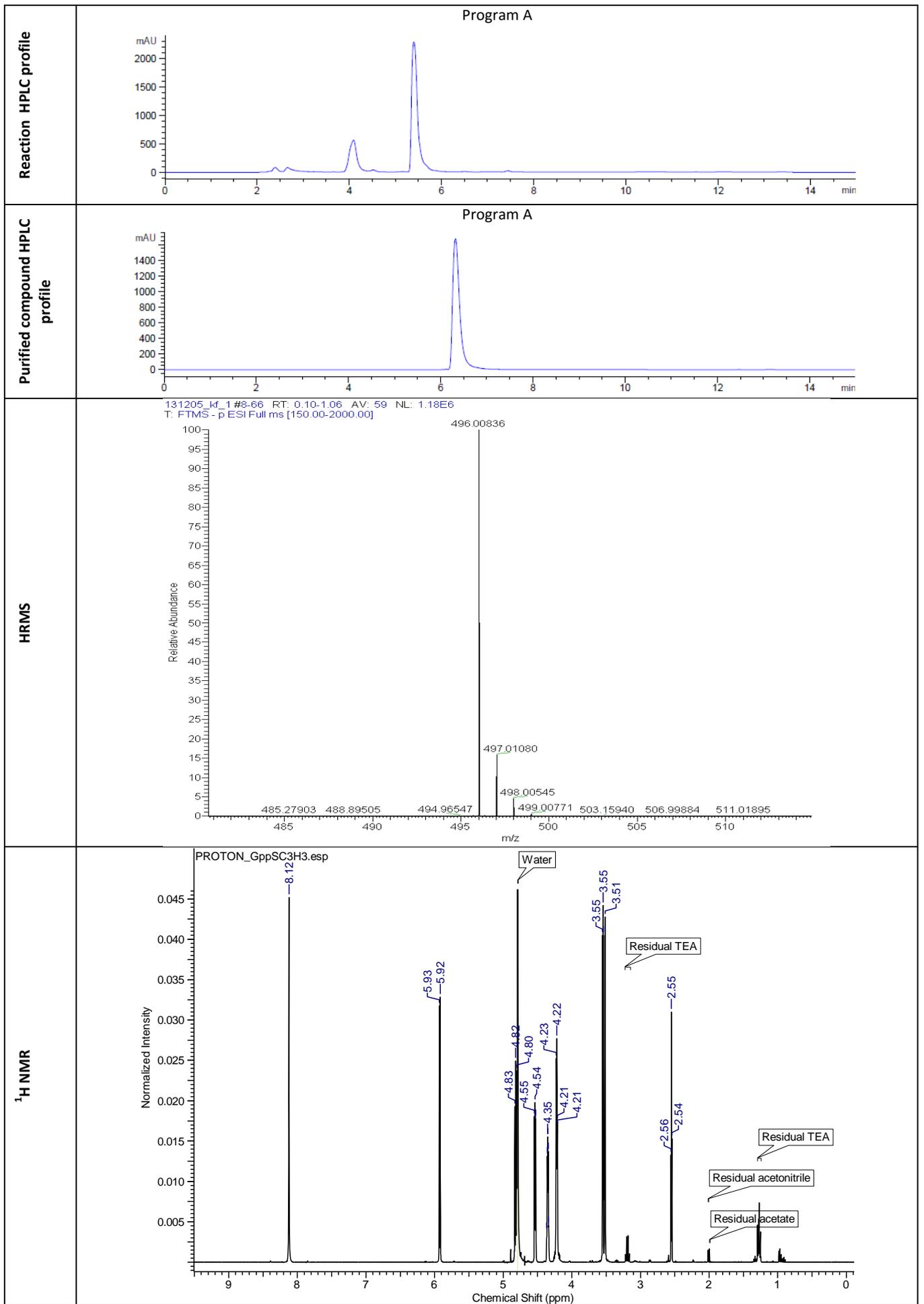


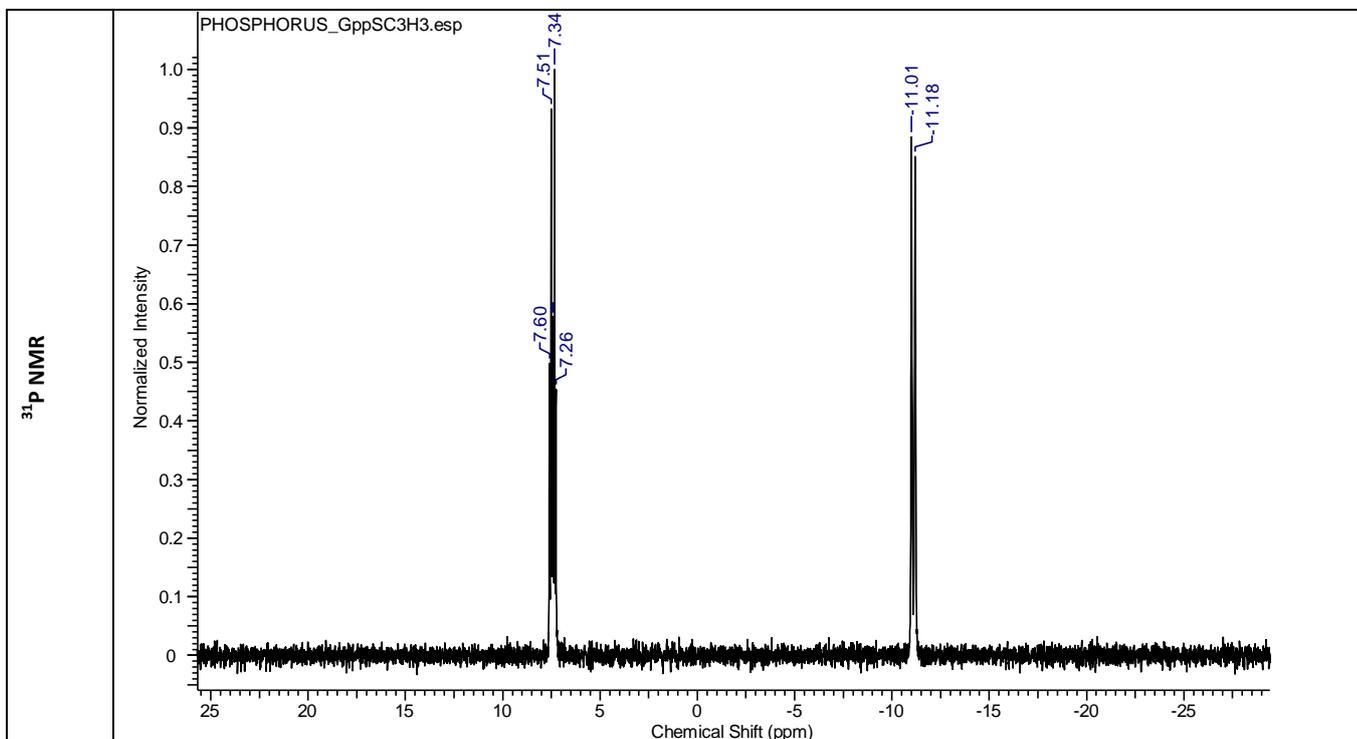




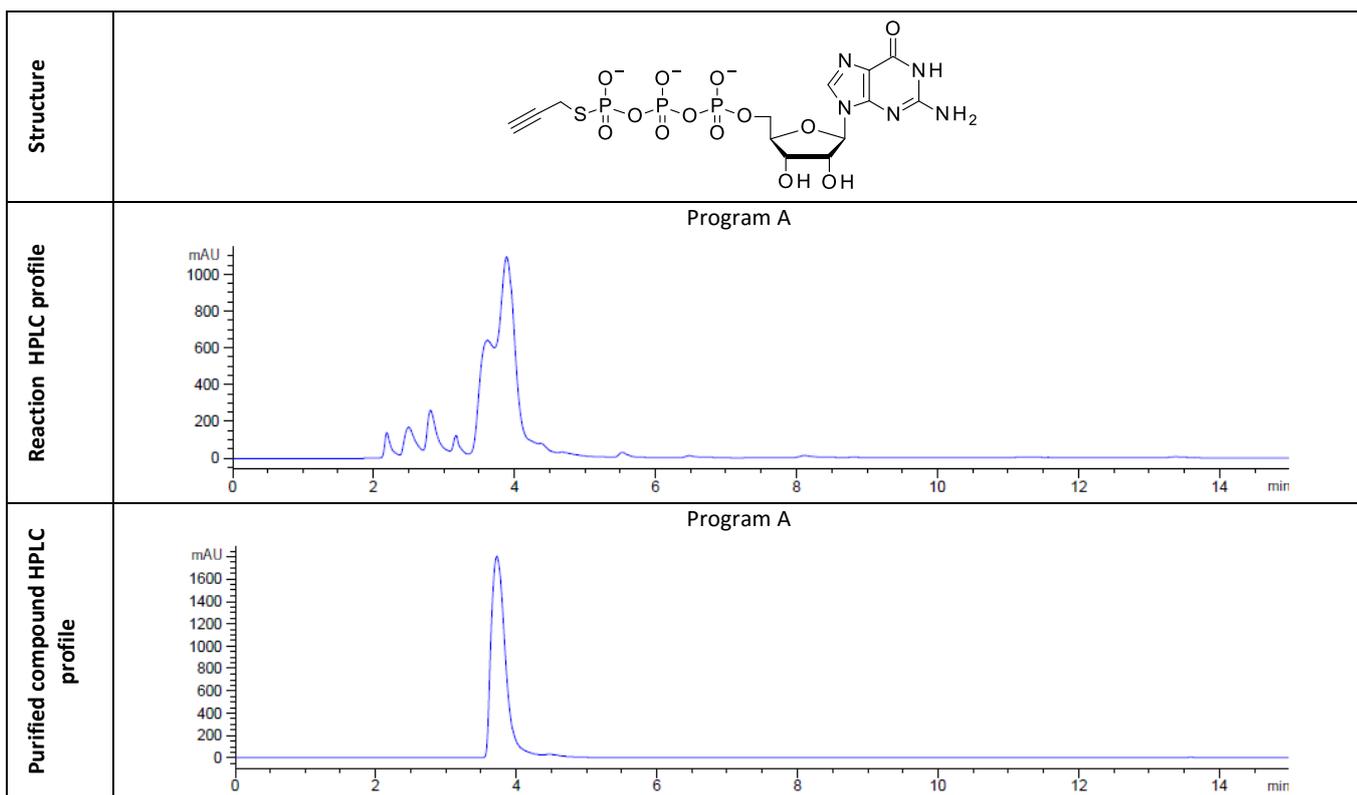
**(14a) GppSC<sub>3</sub>H<sub>3</sub>**

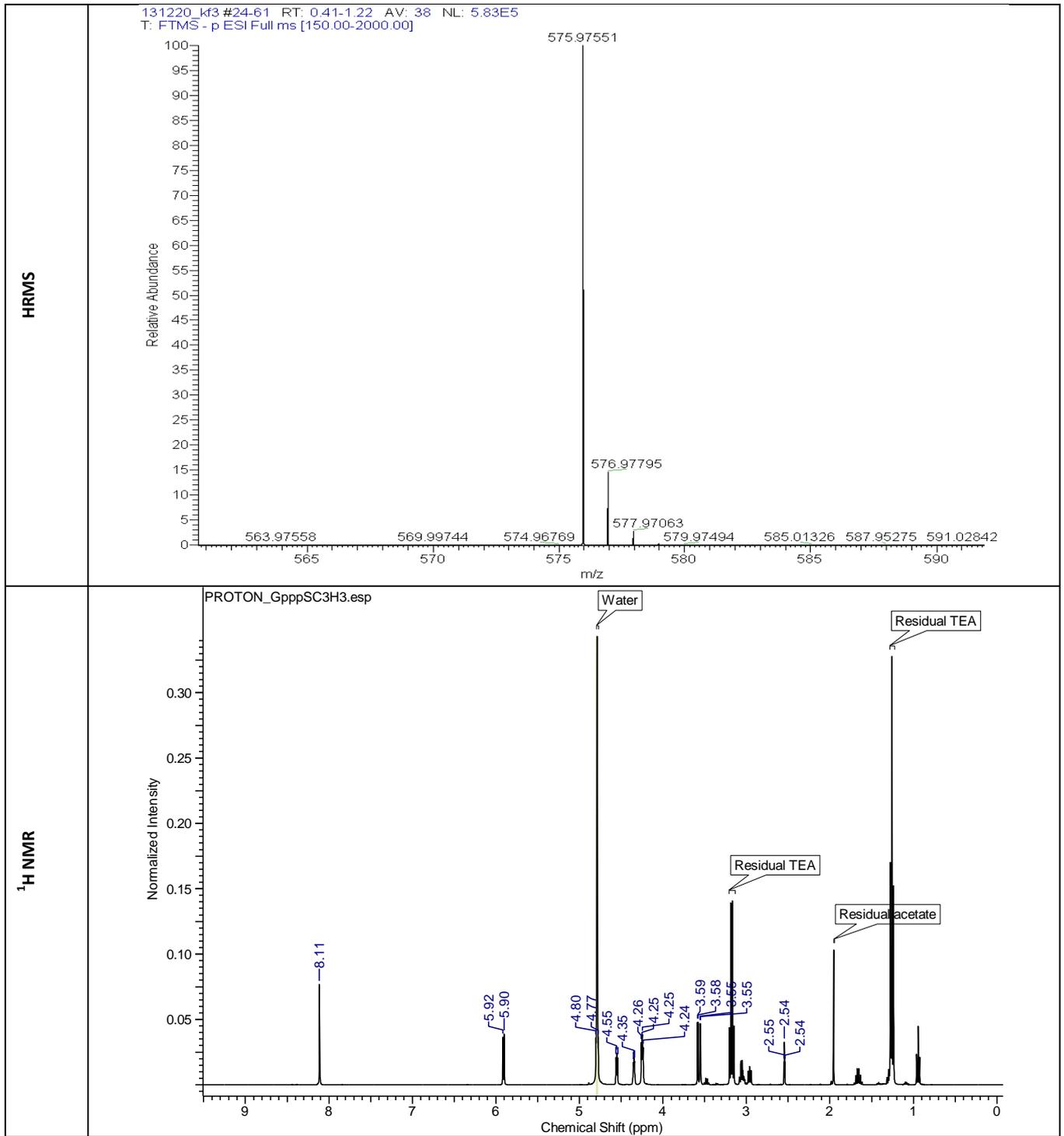


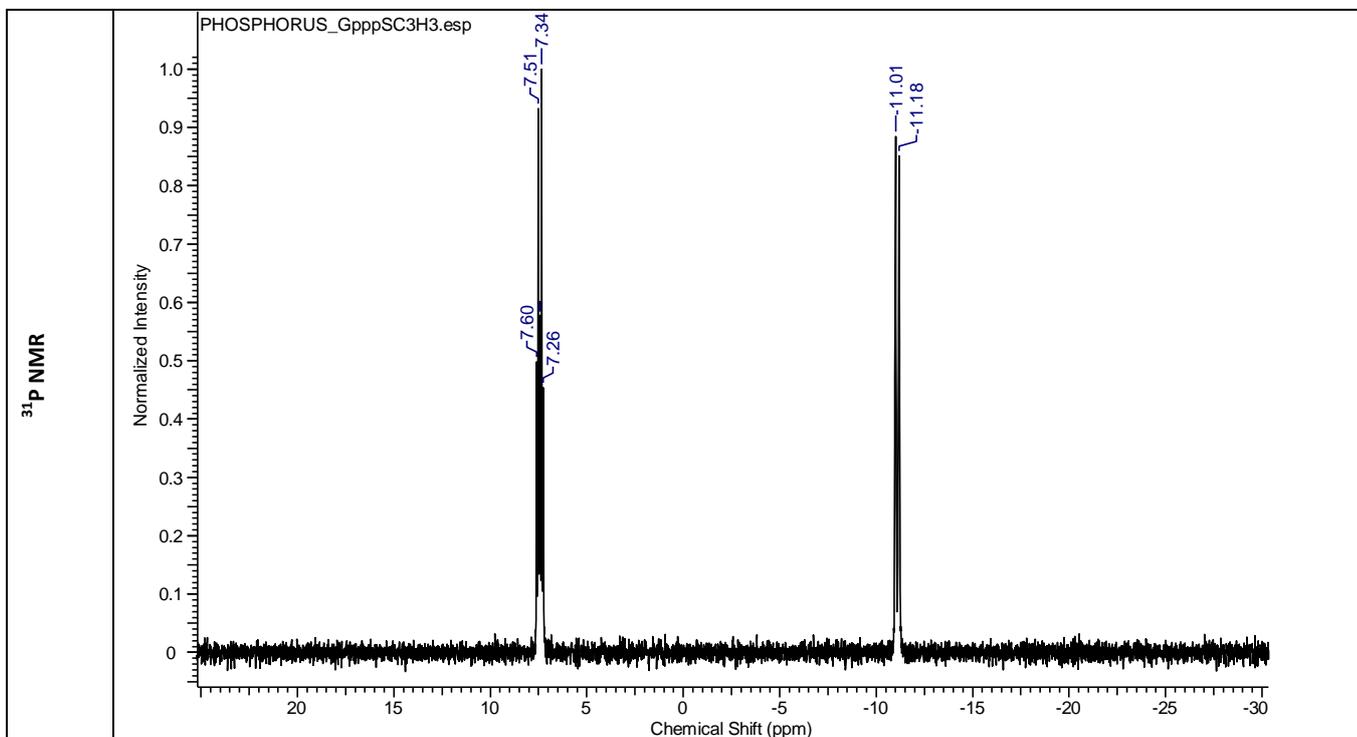




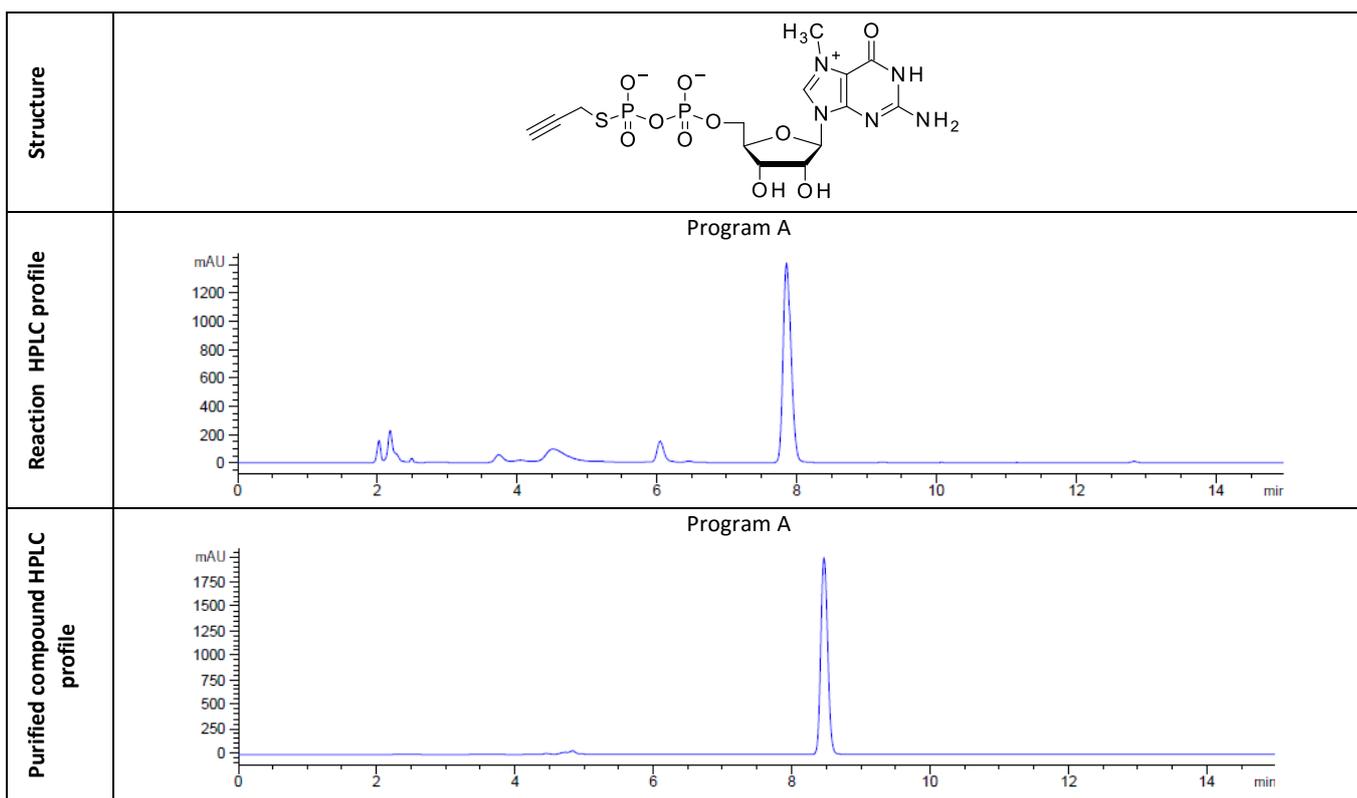
(14b) GpppSC<sub>3</sub>H<sub>3</sub>

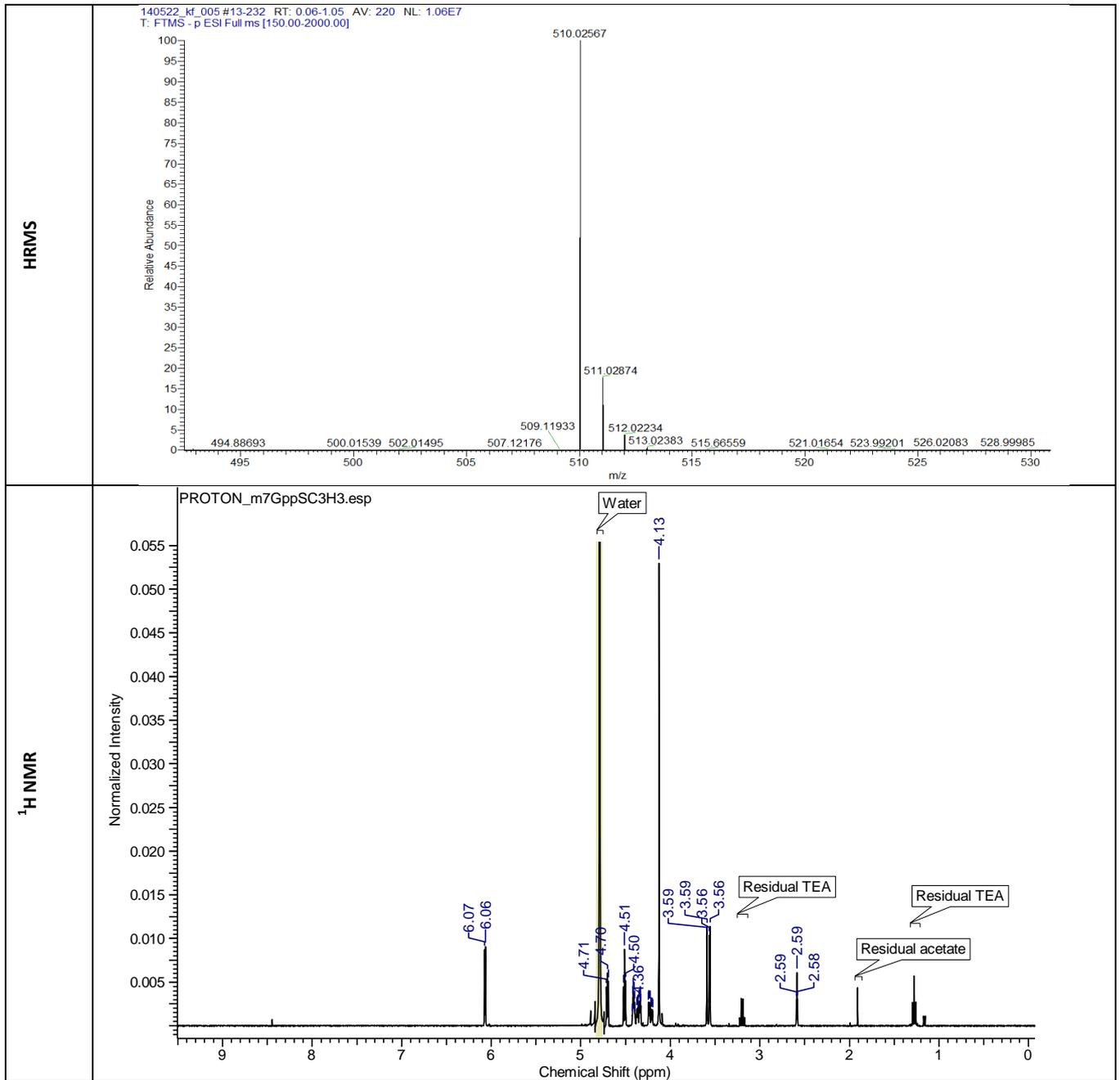


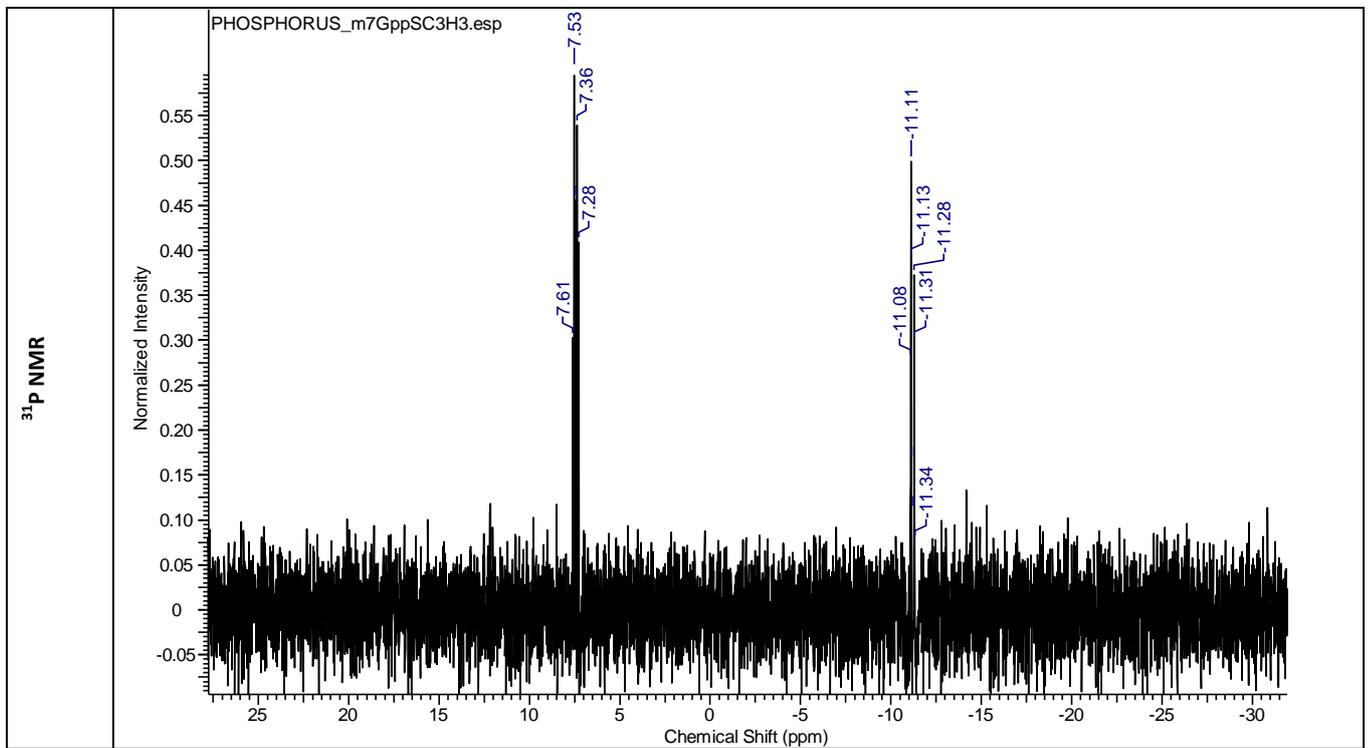




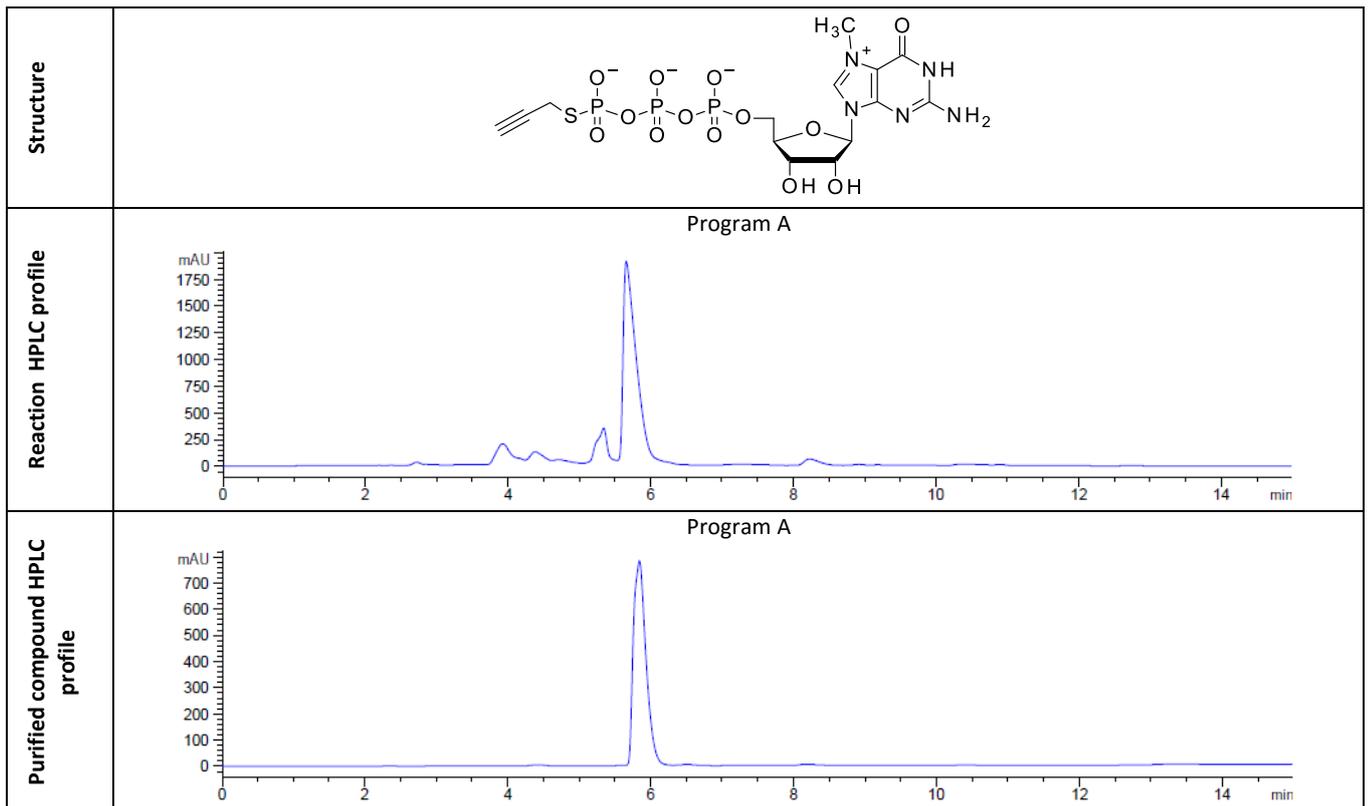
(14c) m<sup>7</sup>GppC<sub>3</sub>H<sub>3</sub>

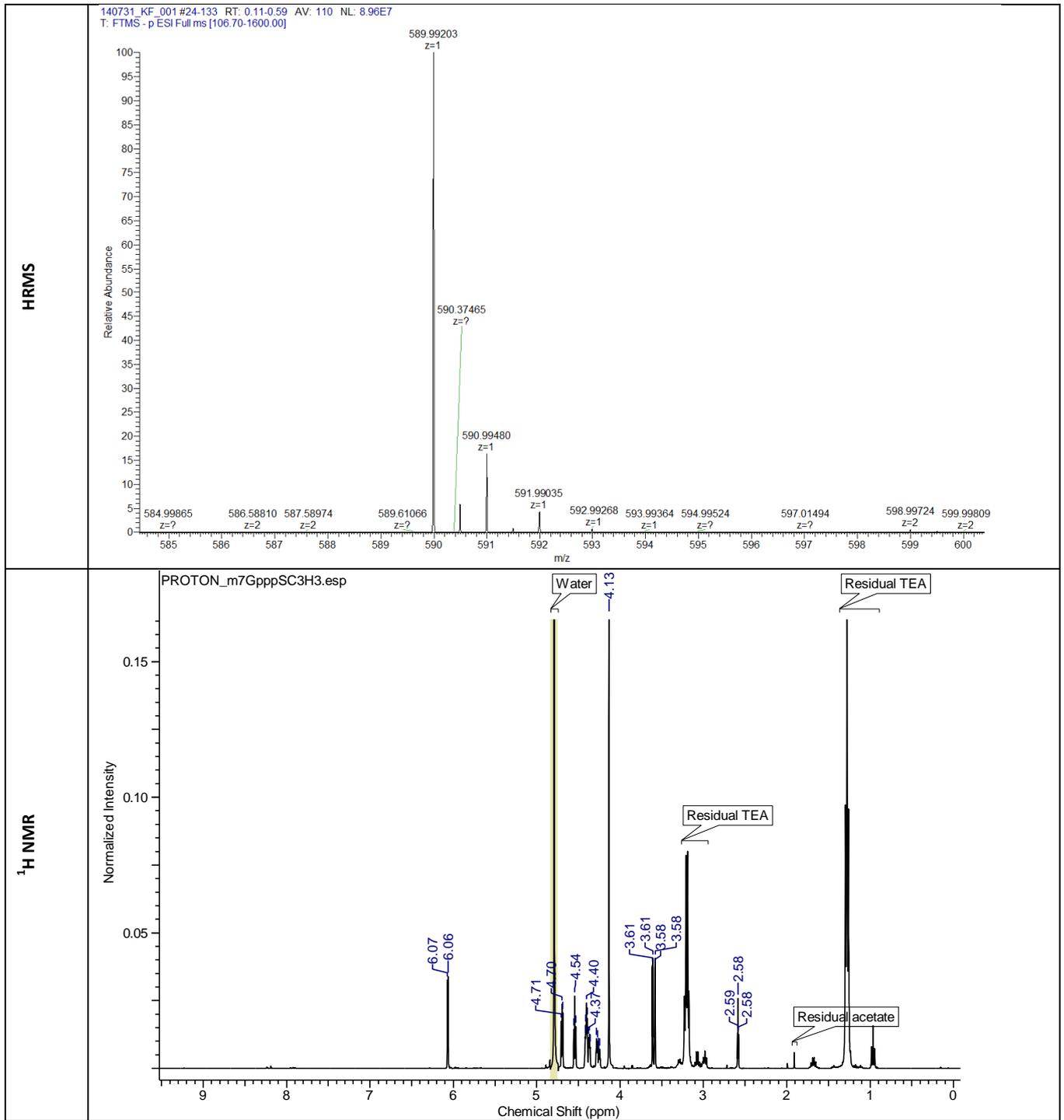


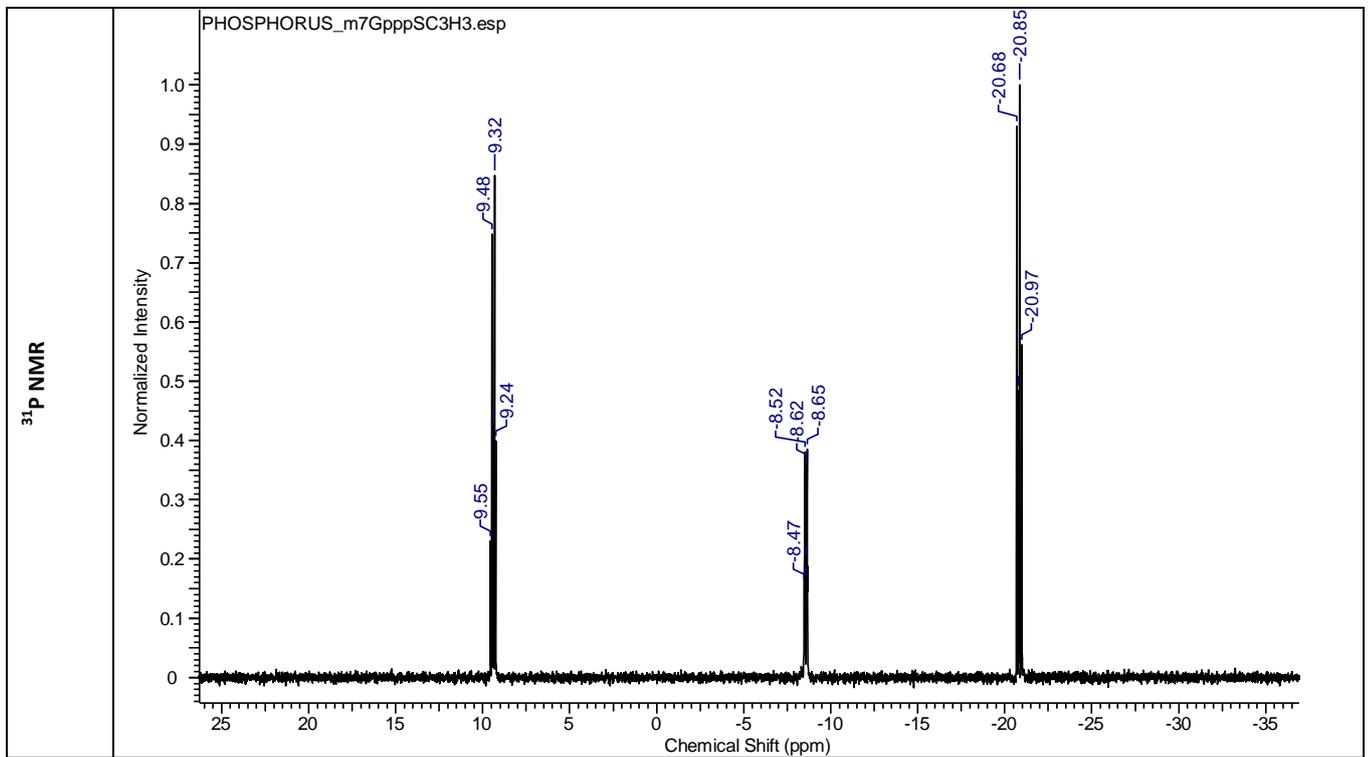




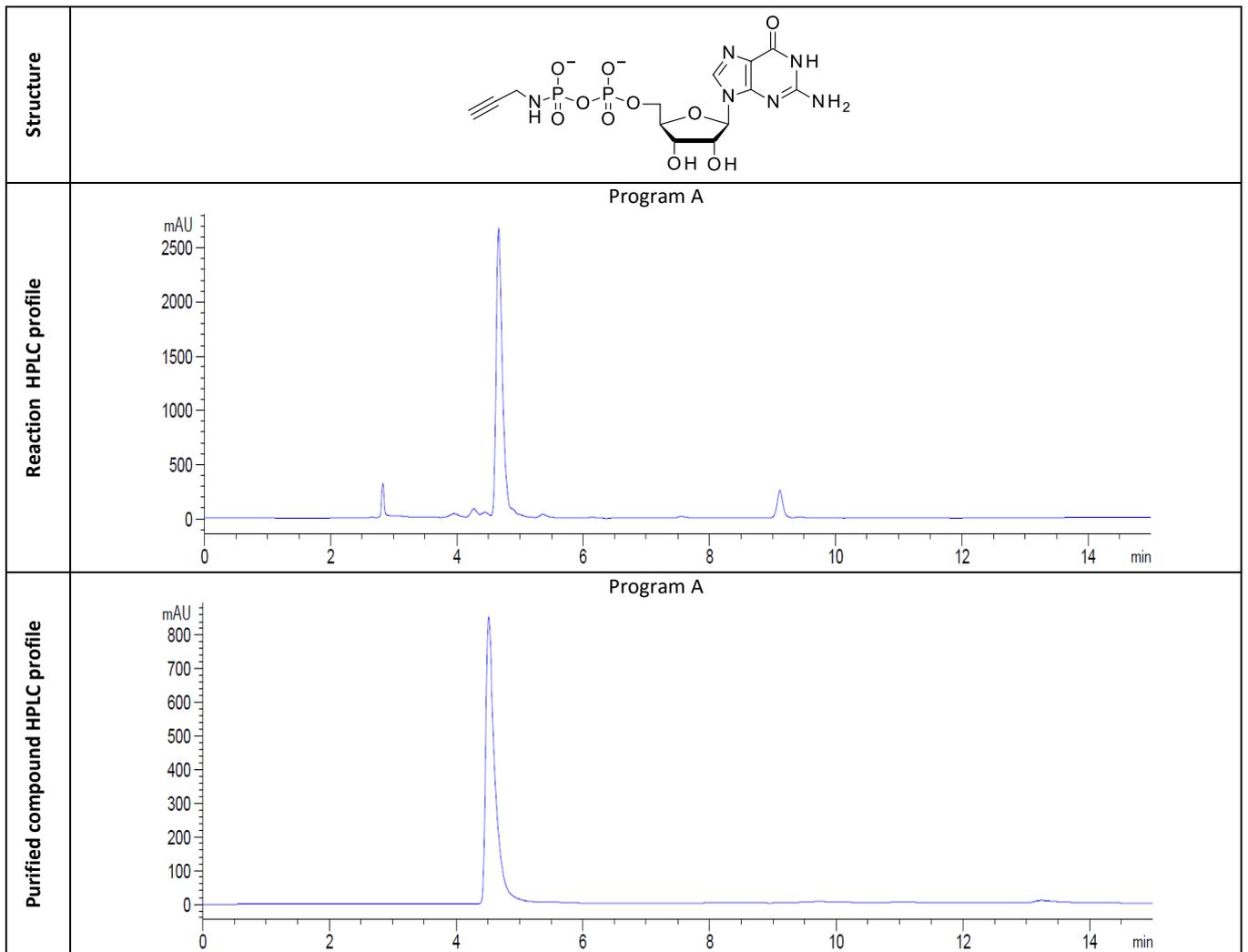
(14d) m<sup>7</sup>GpppSC<sub>3</sub>H<sub>3</sub>





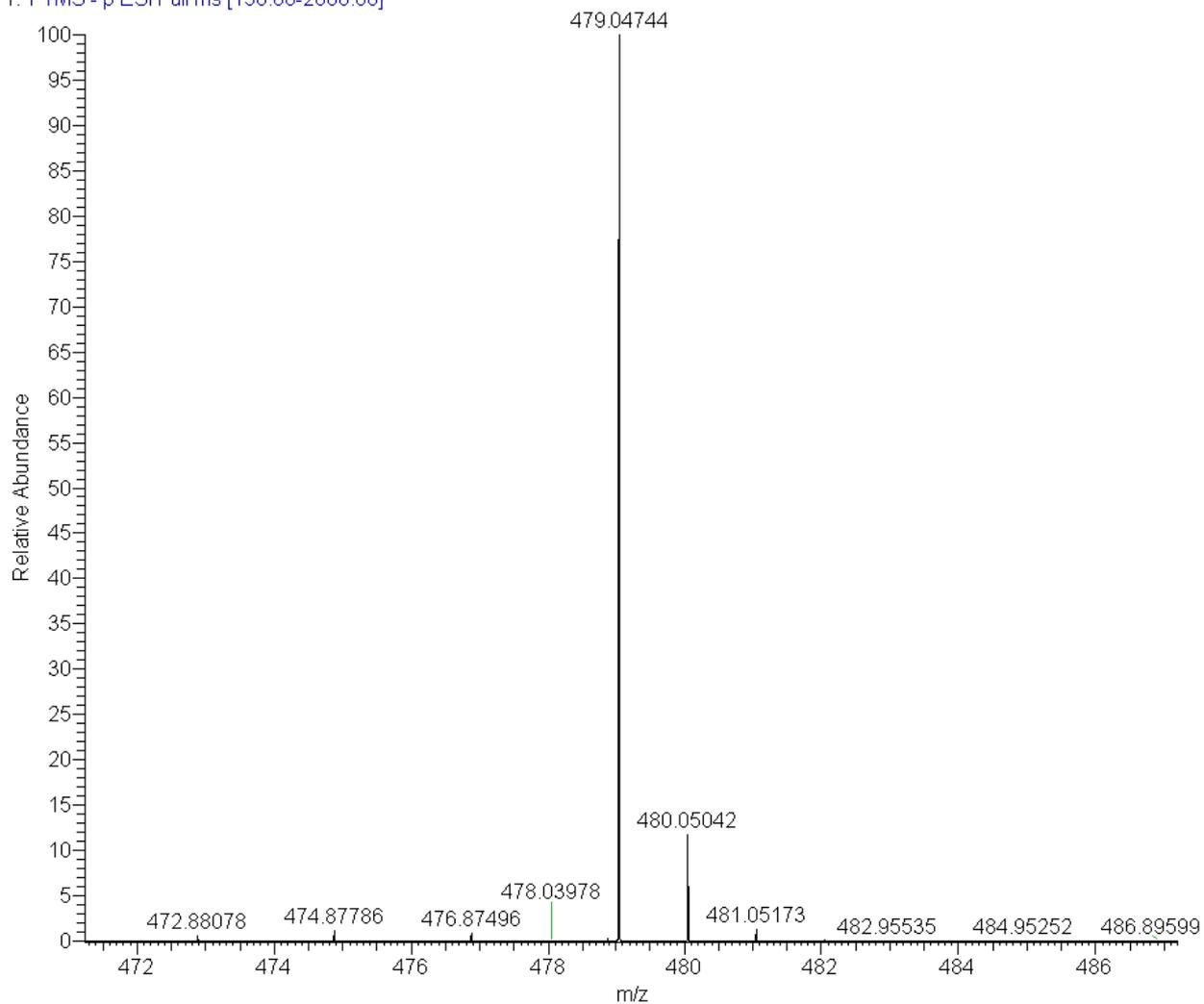


**(15a) GppNHC<sub>3</sub>H<sub>3</sub>**

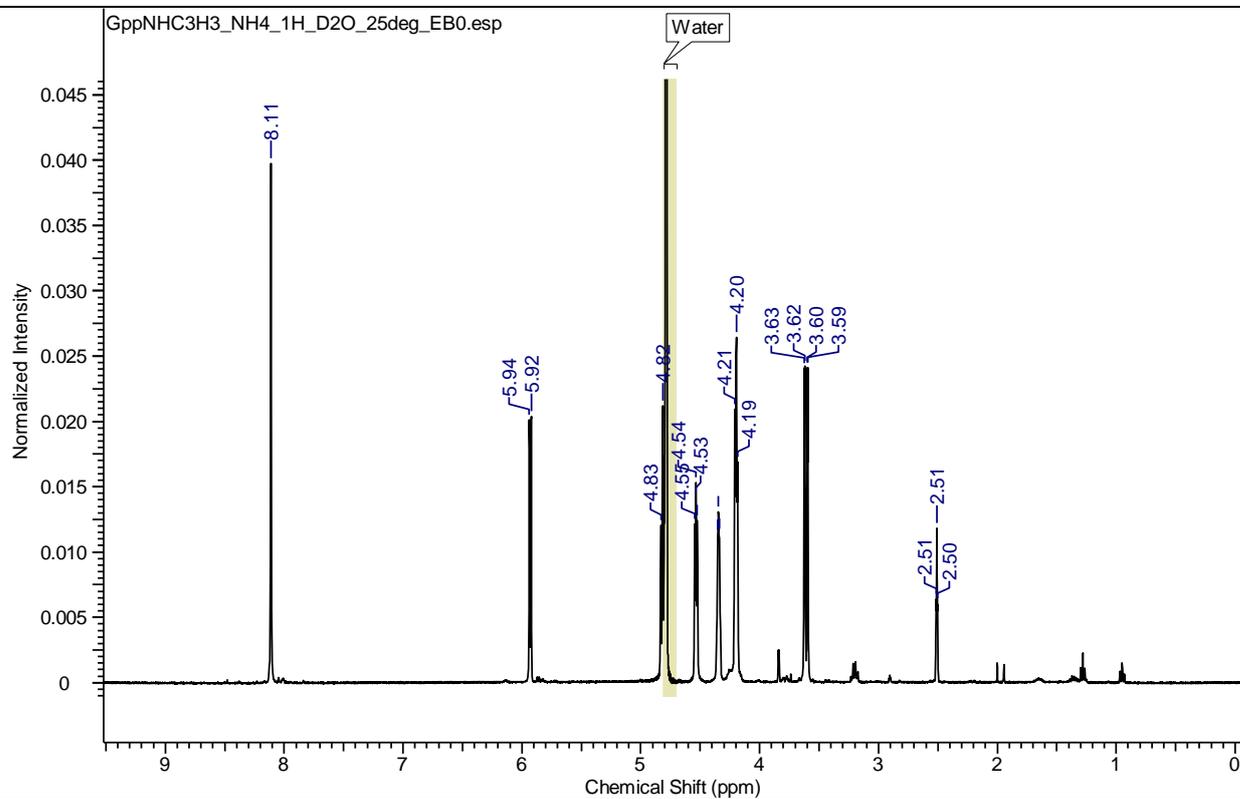


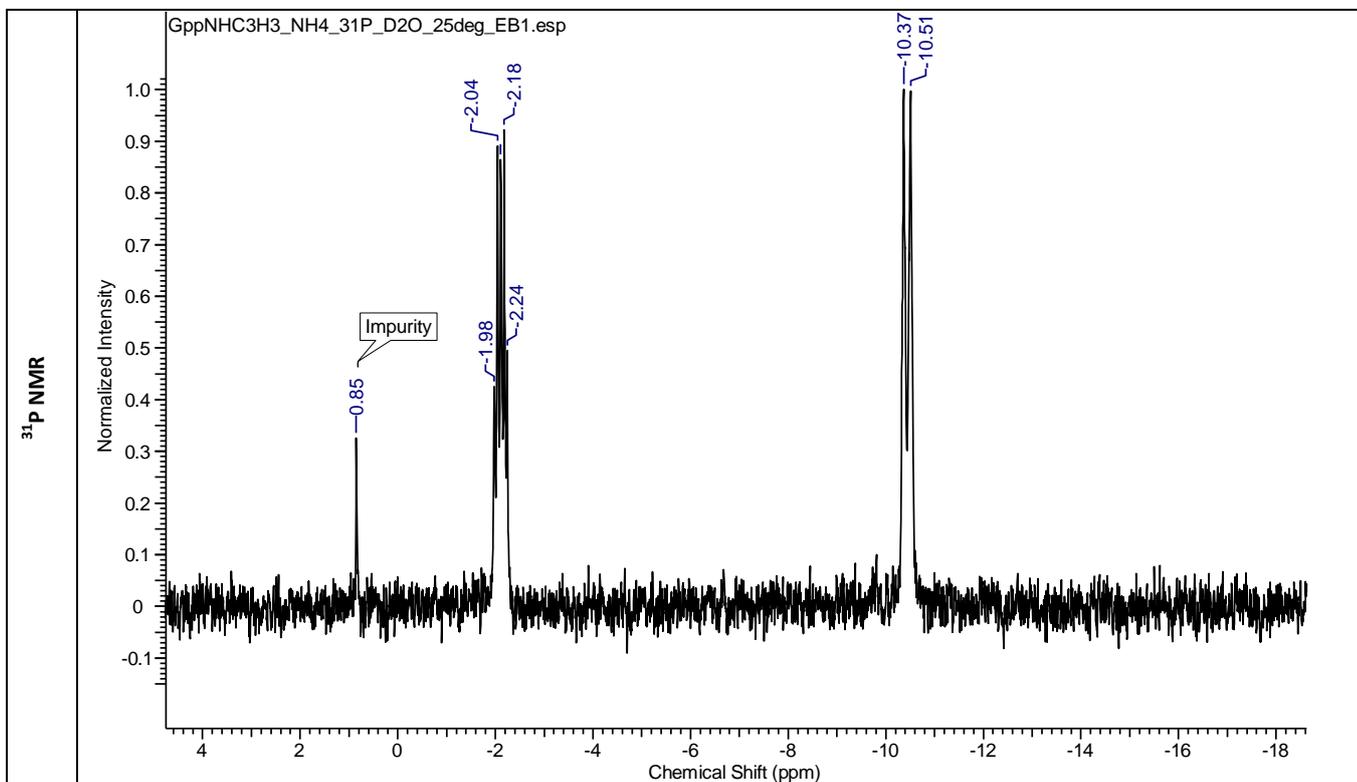
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T: FTMS - p ESI Full ms [150.00-2000.00]

HRMS

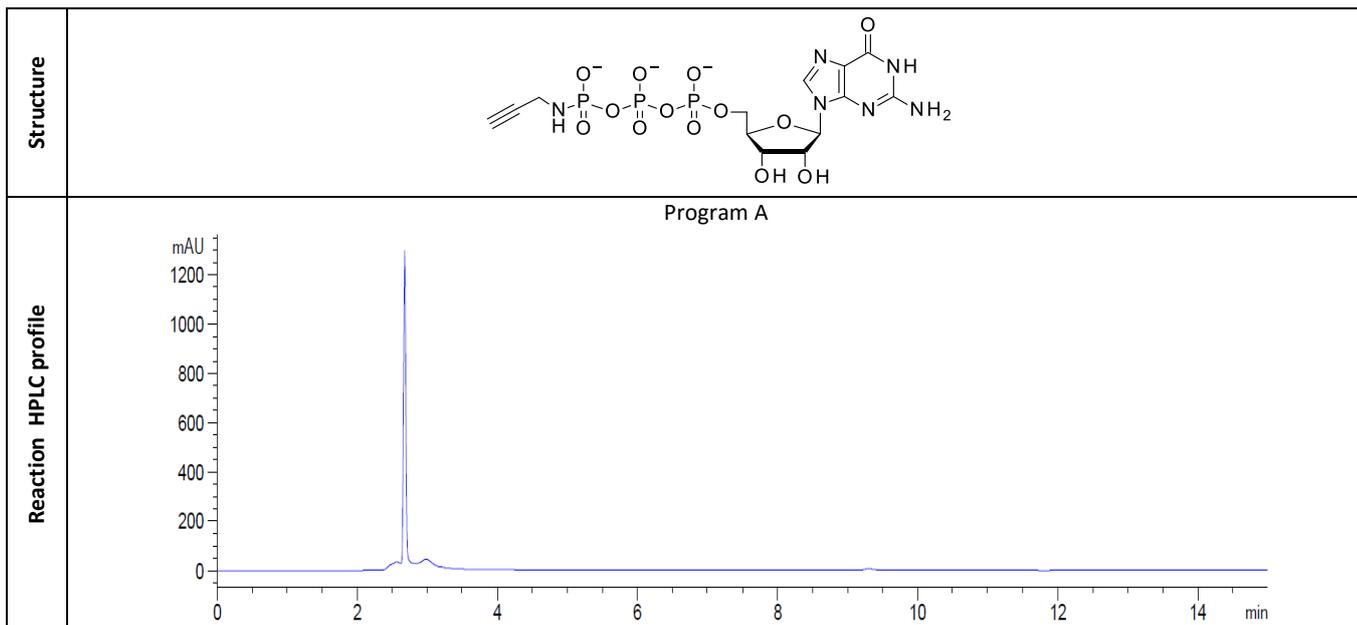


<sup>1</sup>H NMR

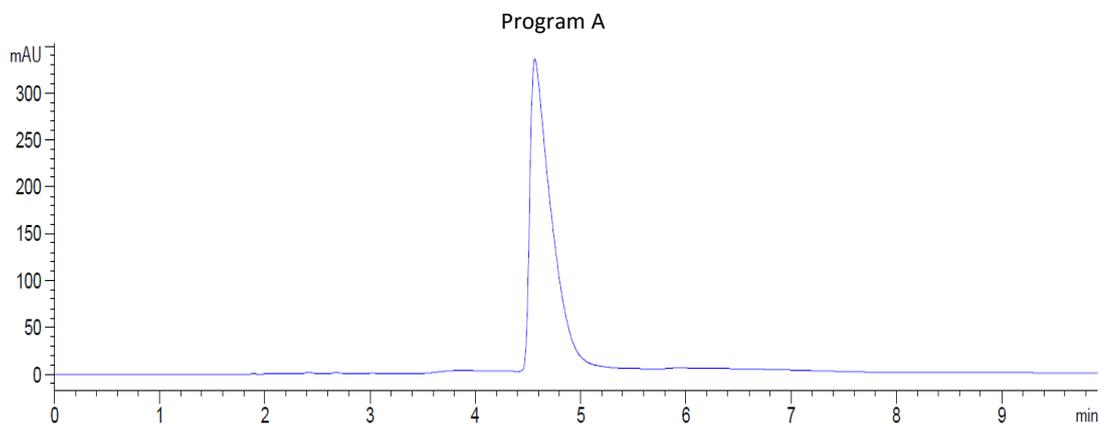




**(15b) GpppNHC<sub>3</sub>H<sub>3</sub>**

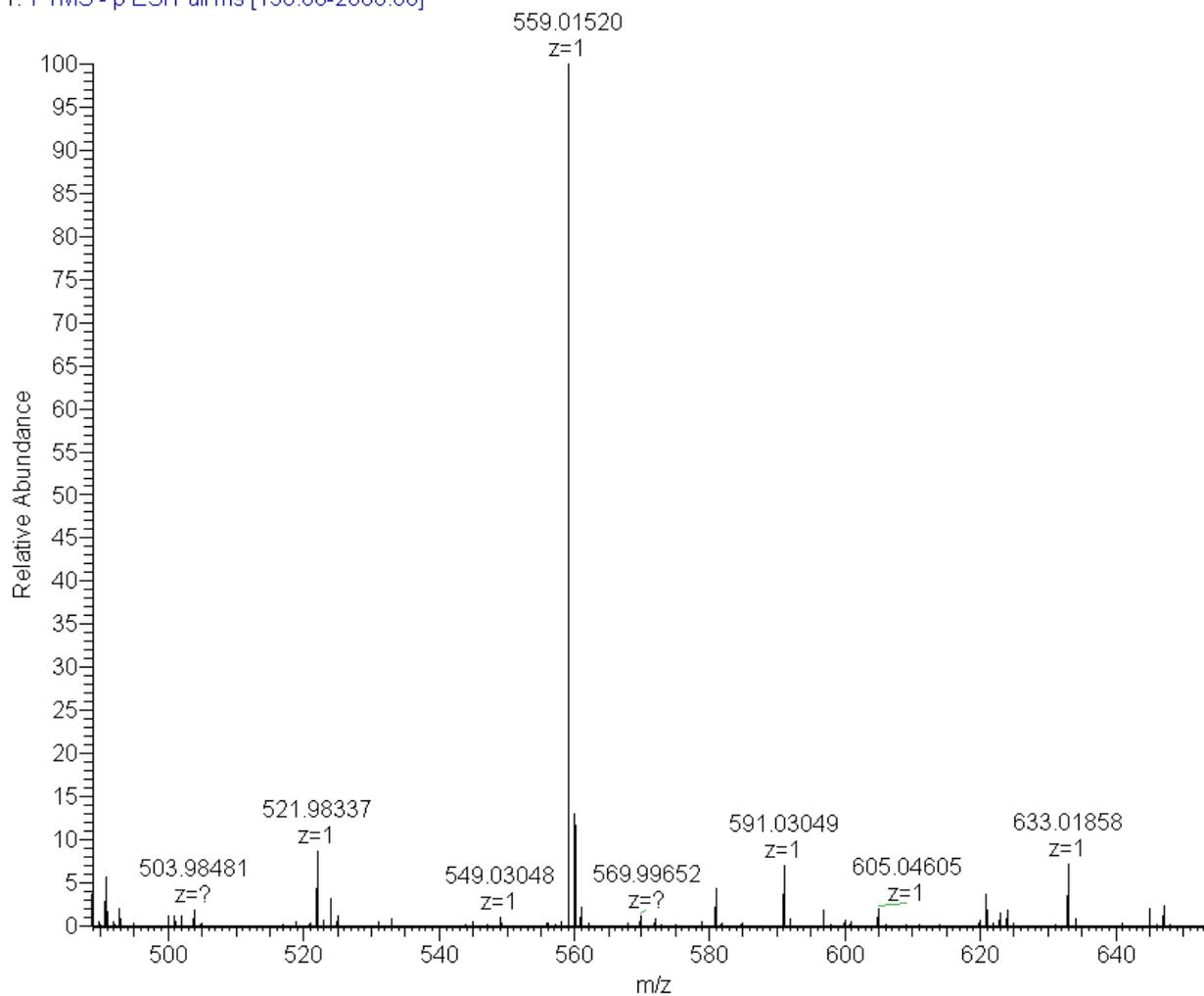


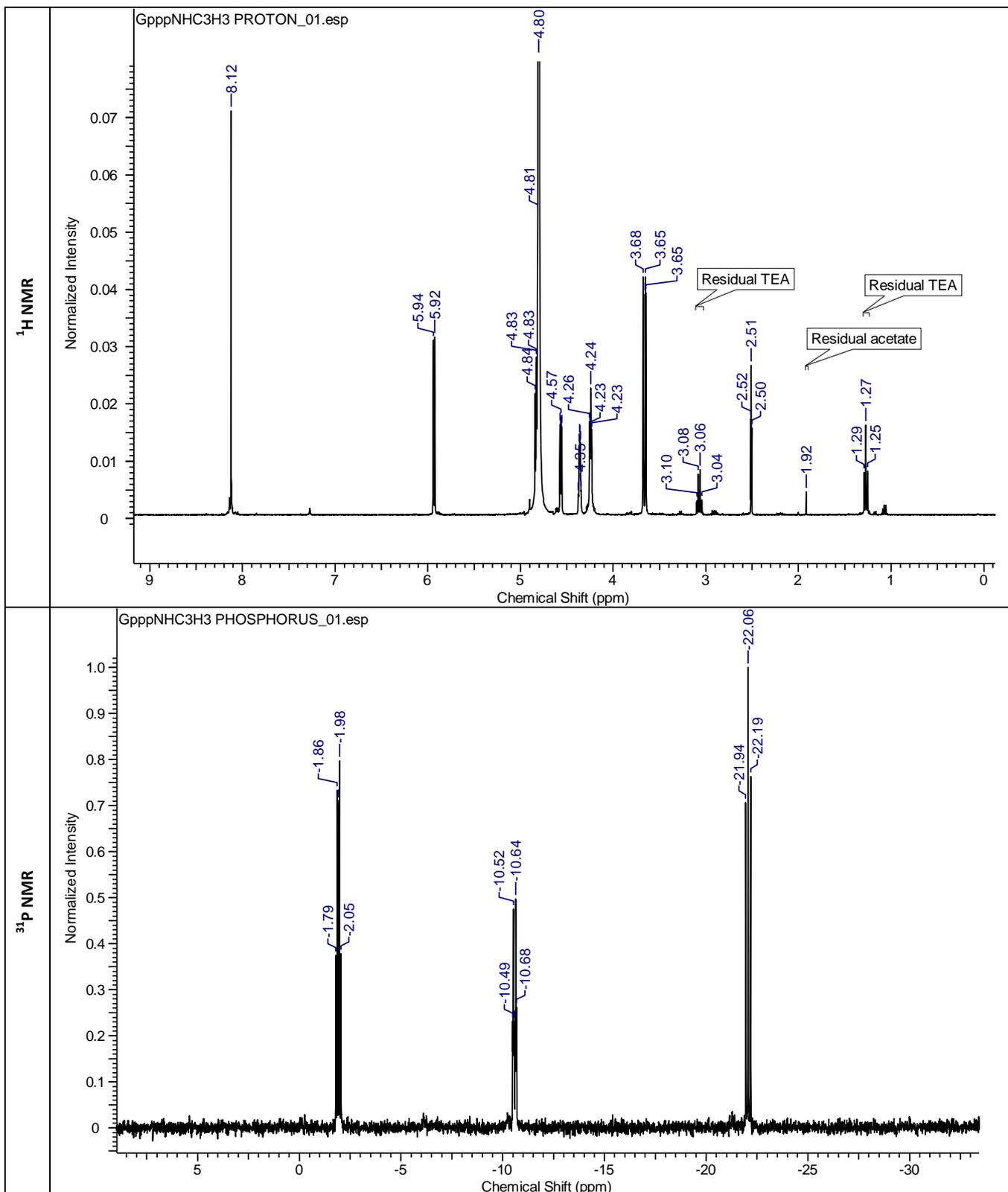
Purified compound HPLC profile



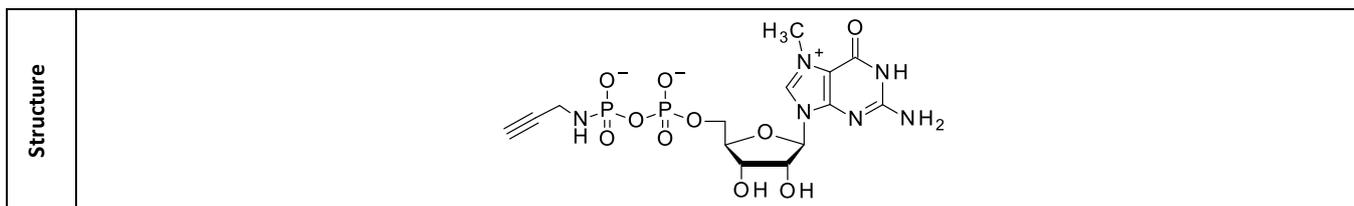
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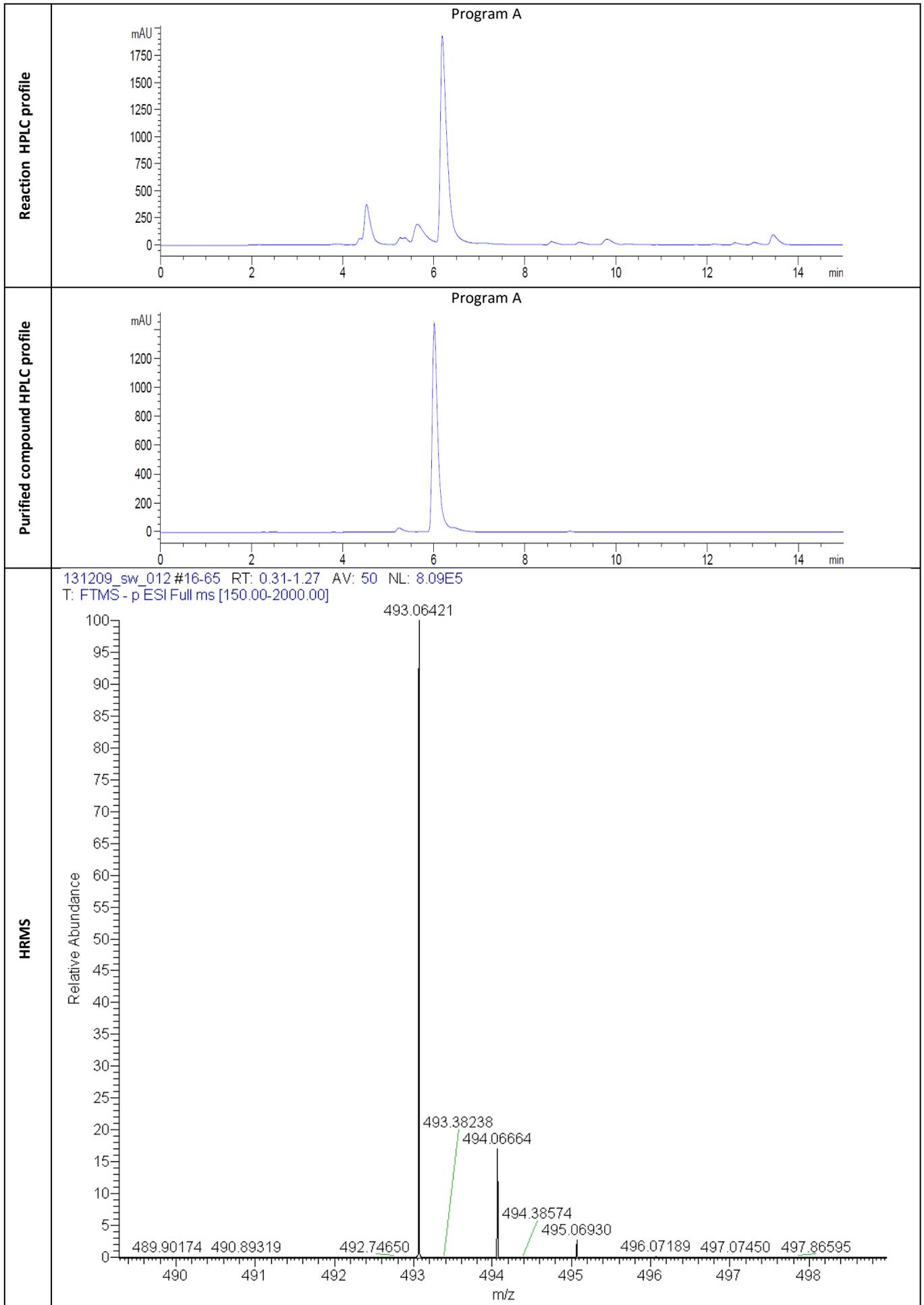
HRMS

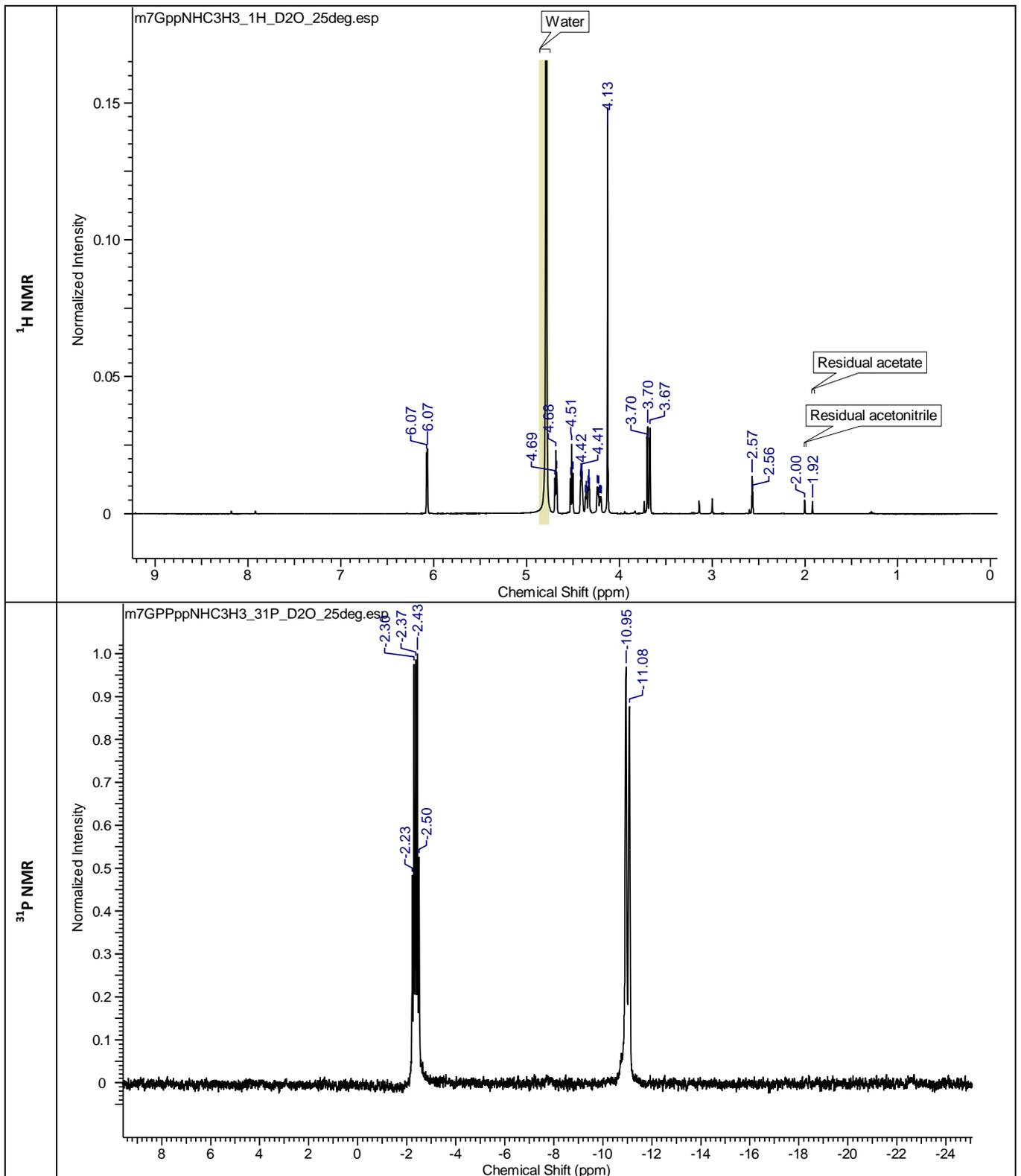




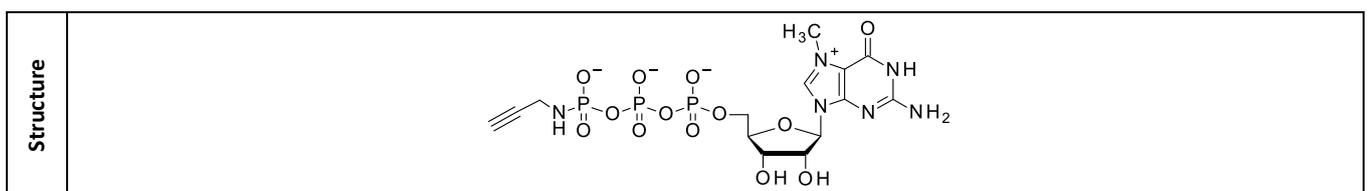
(15c) m<sup>7</sup>GppNHC<sub>3</sub>H<sub>3</sub>

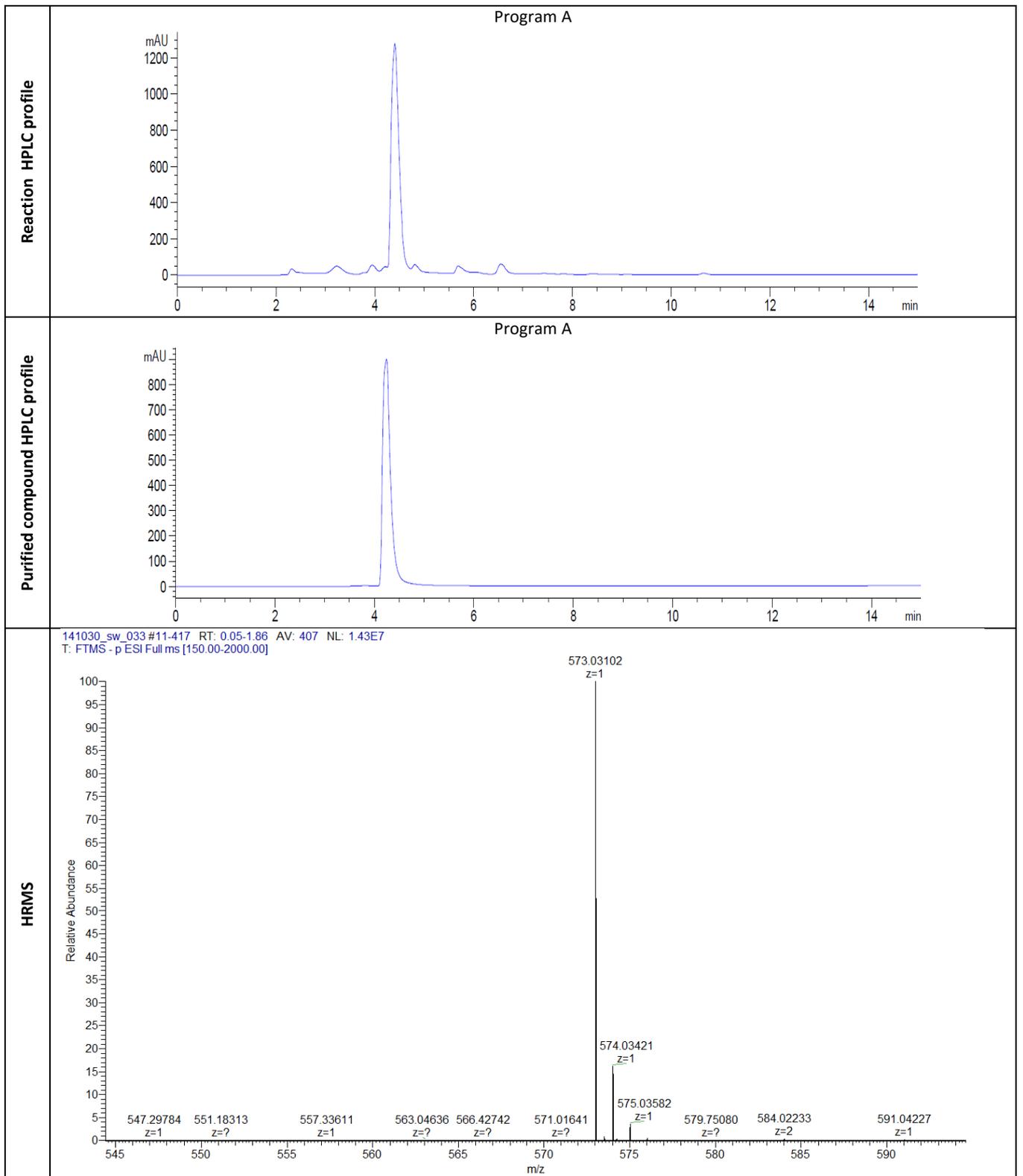


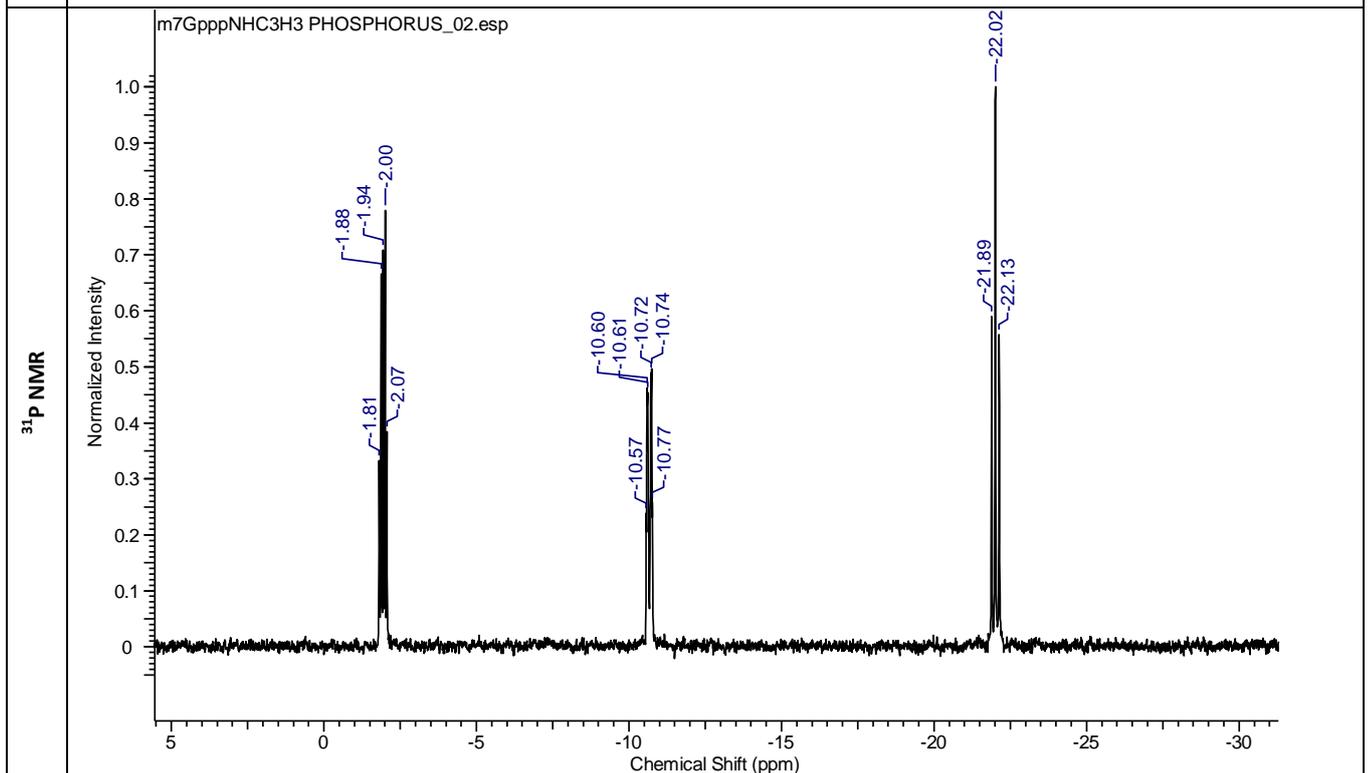
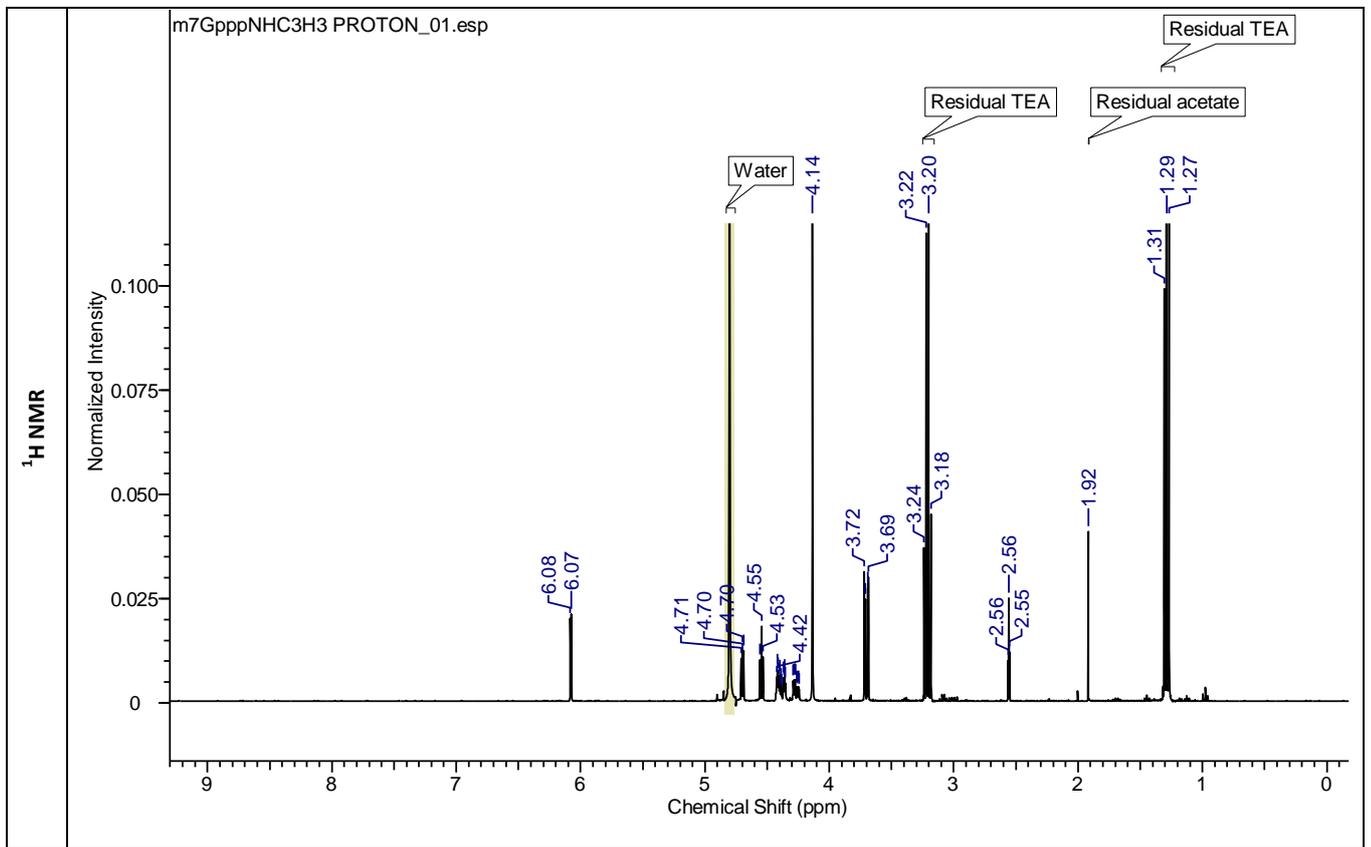




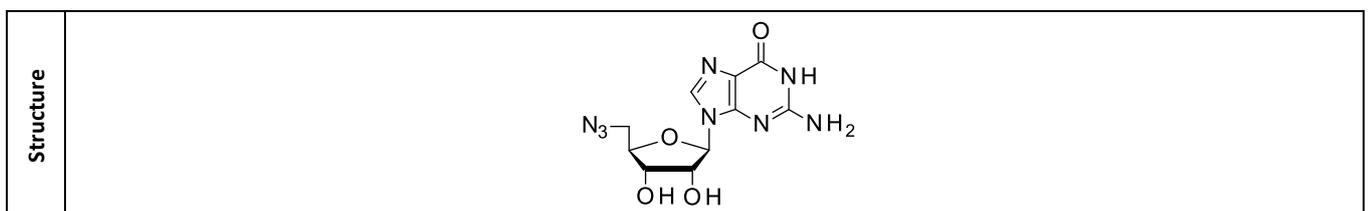
(15d) m<sup>7</sup>GpppNHC<sub>3</sub>H<sub>3</sub>

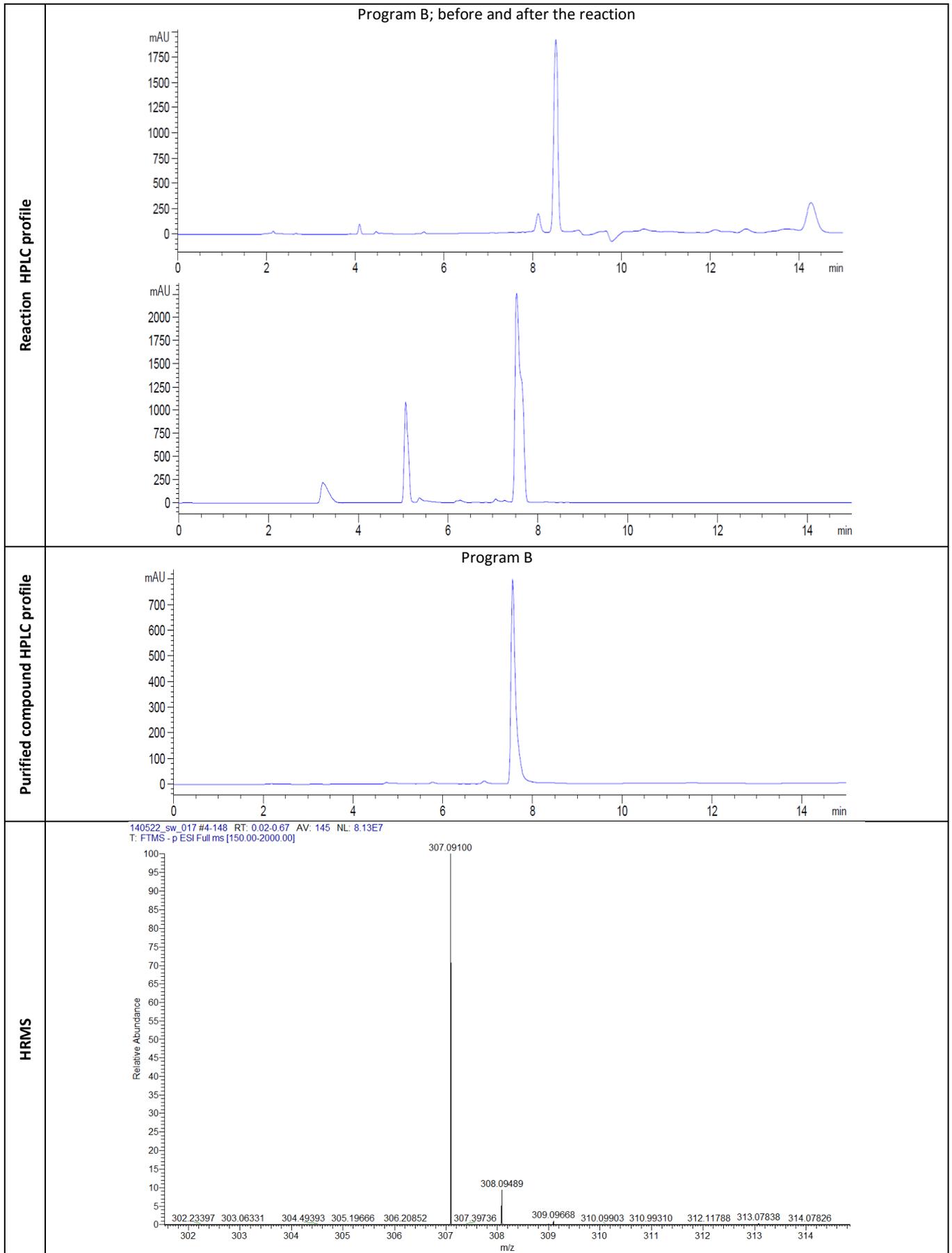


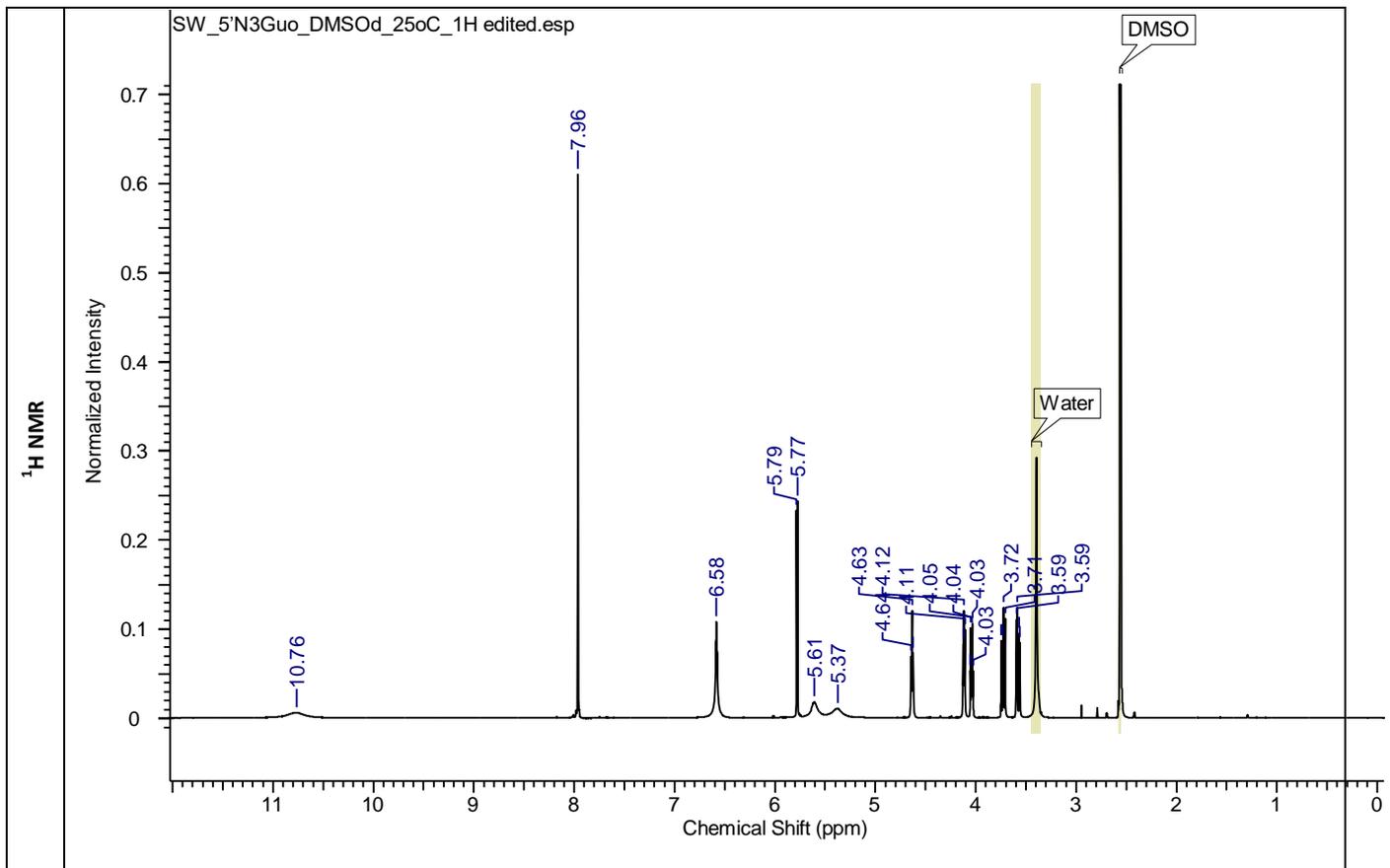




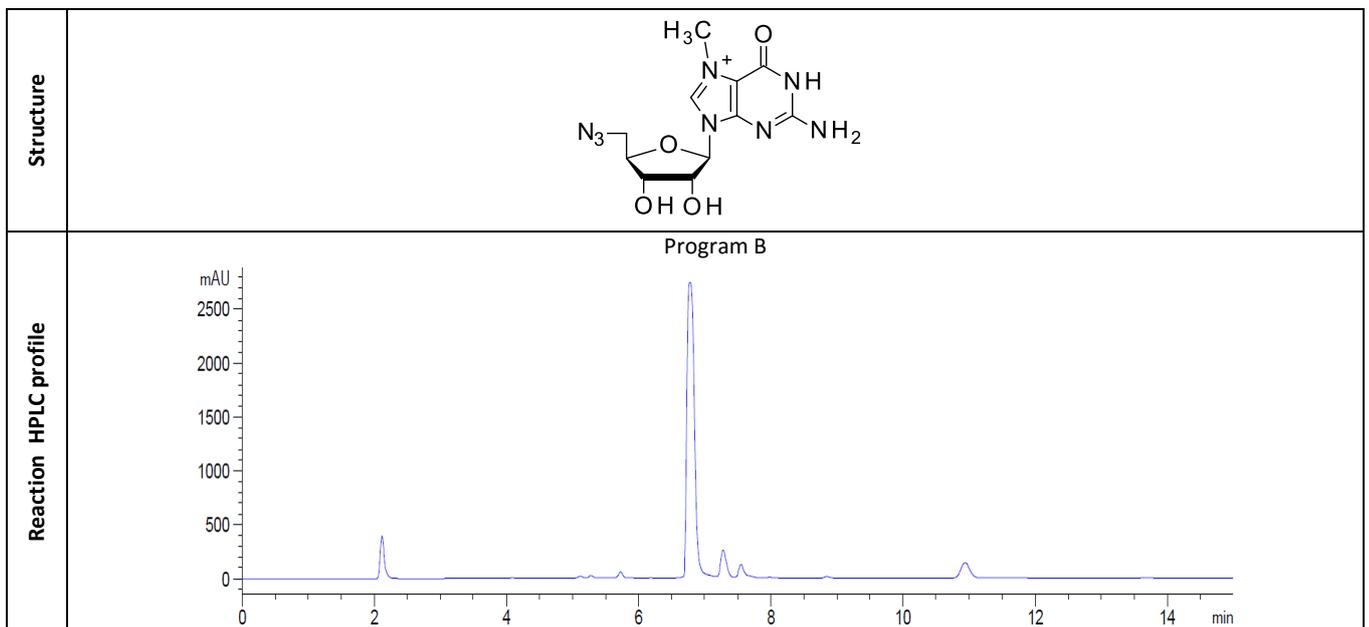
**(16a) 5'-N<sub>3</sub>-Guo**

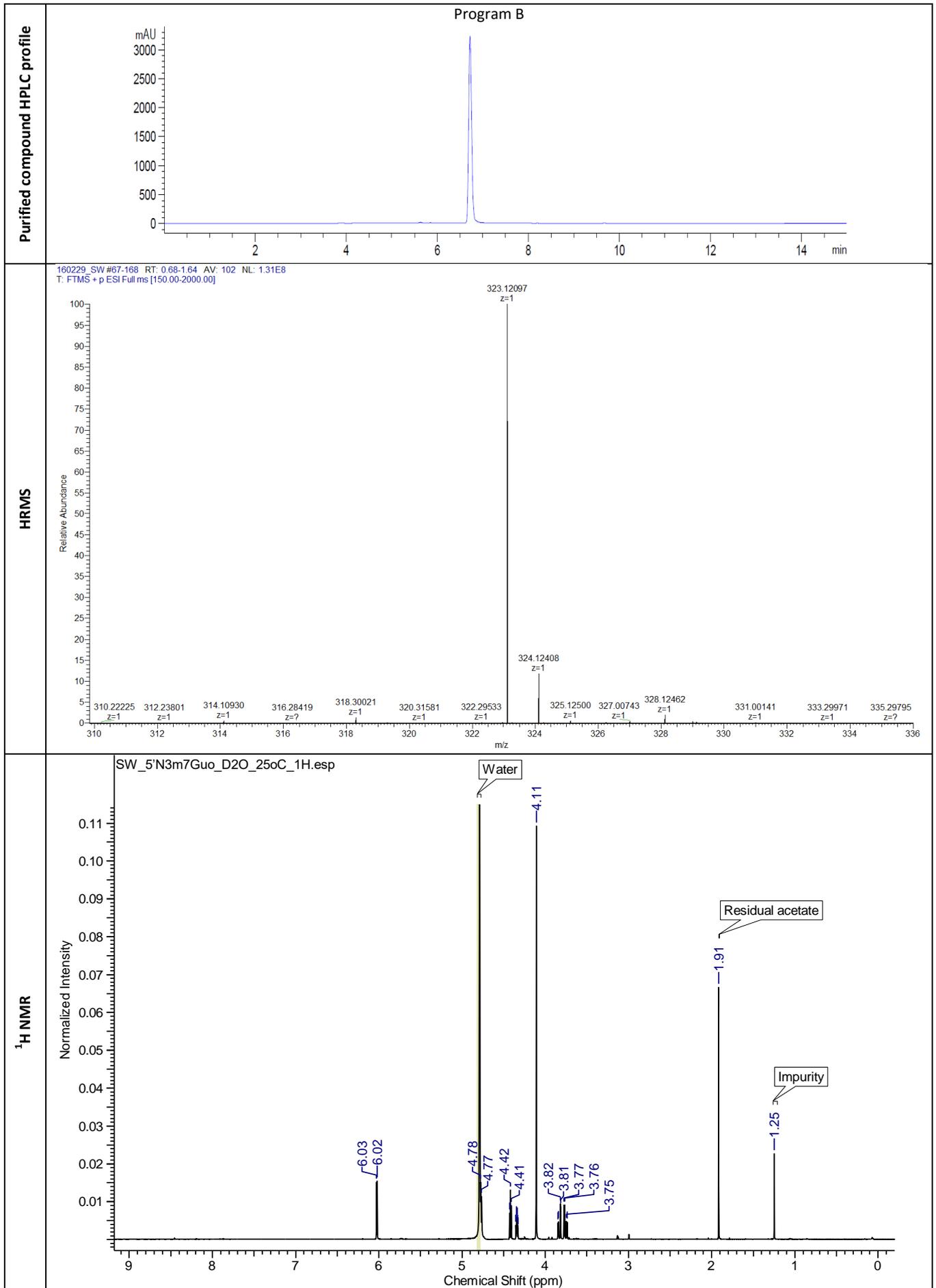




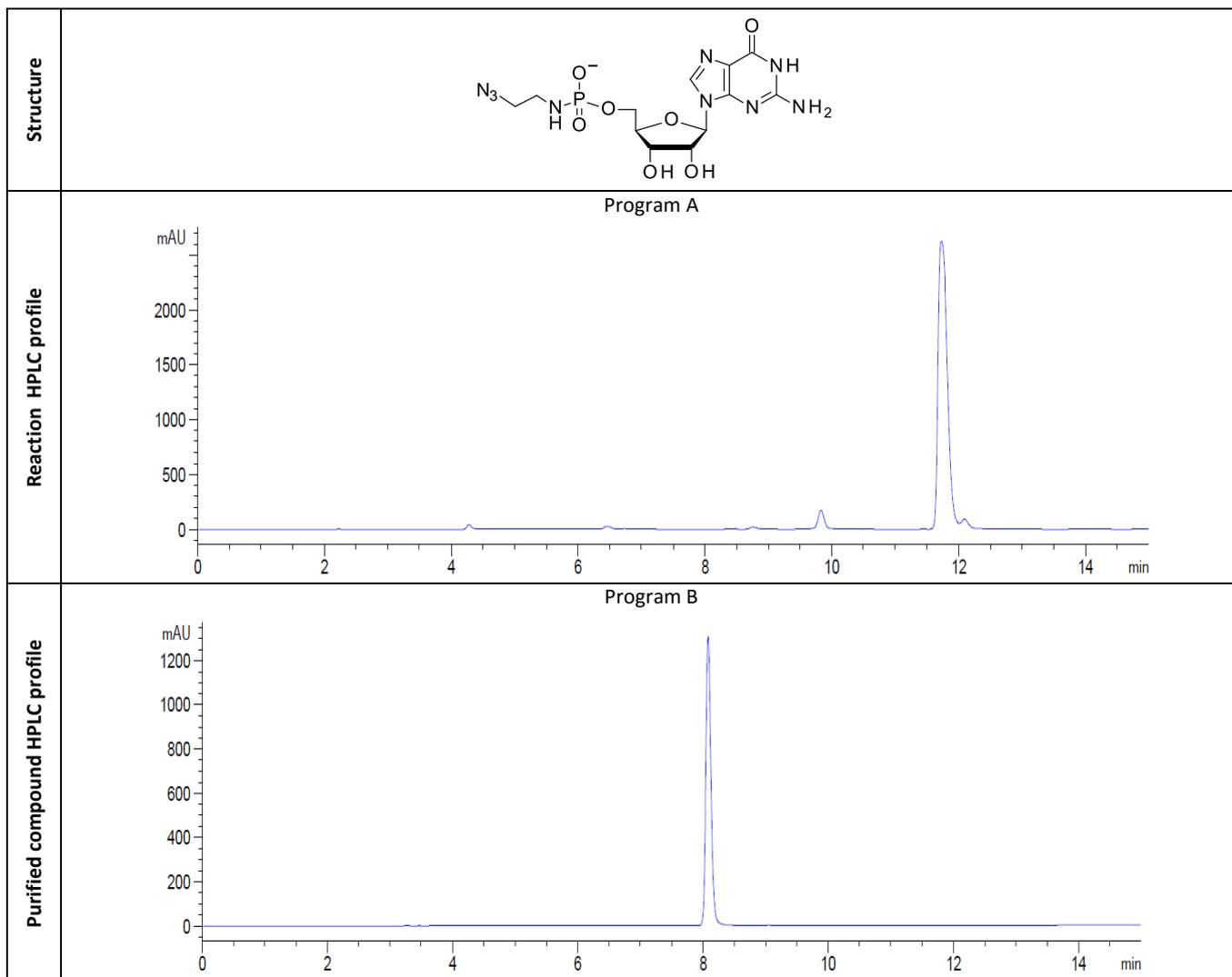


**(16b) 5'-N<sub>3</sub>-m<sup>7</sup>Guo**



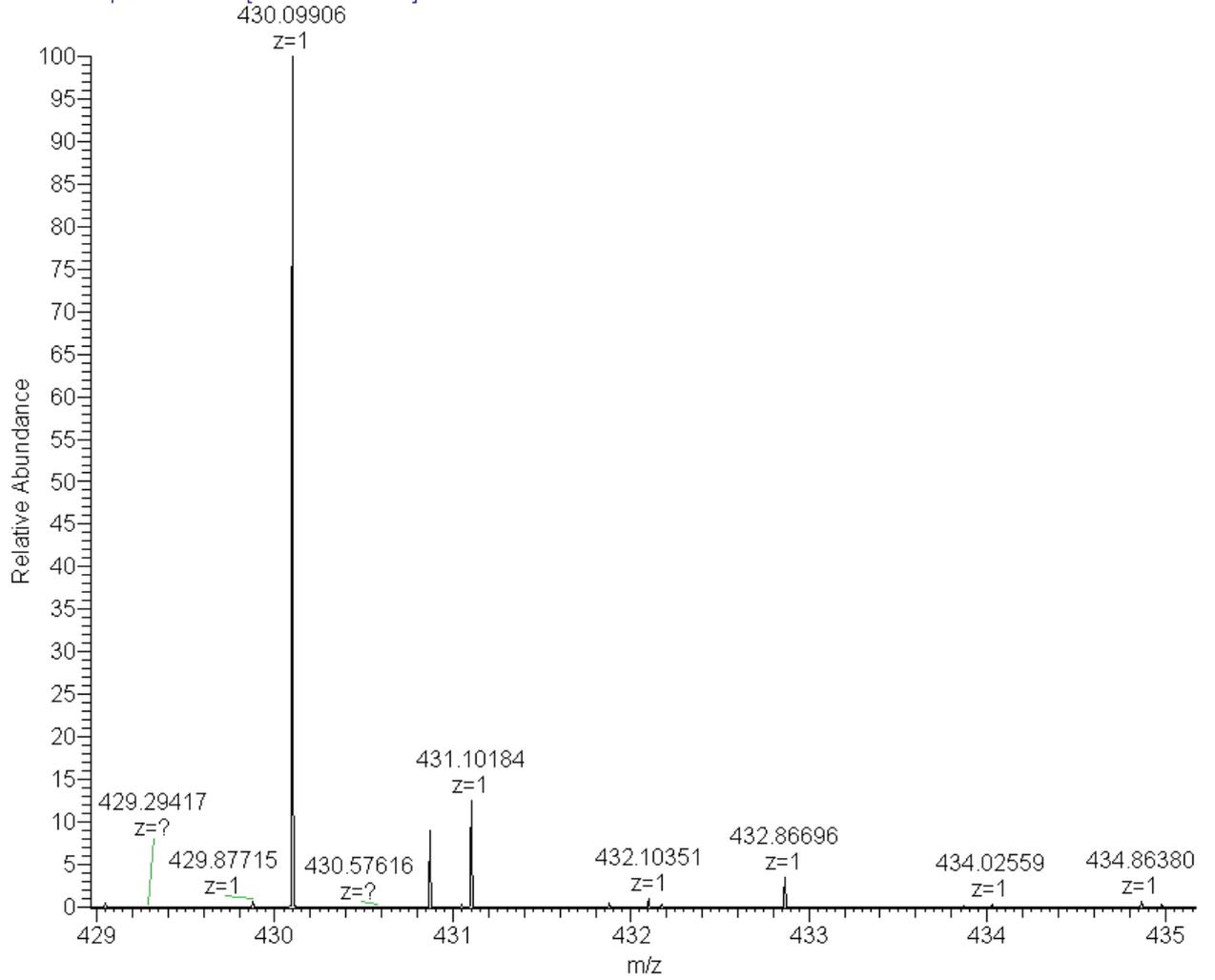


(17a) GpNHC<sub>2</sub>H<sub>4</sub>N<sub>3</sub>

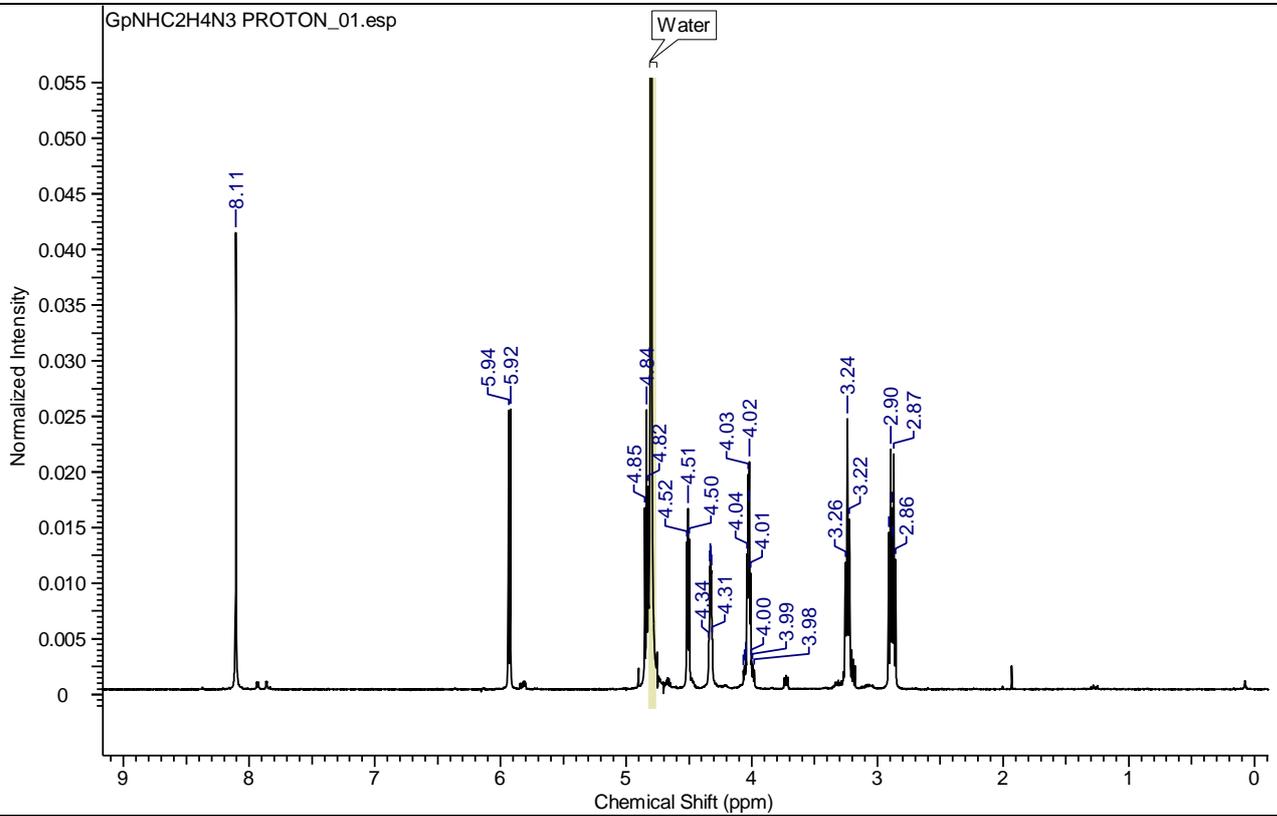


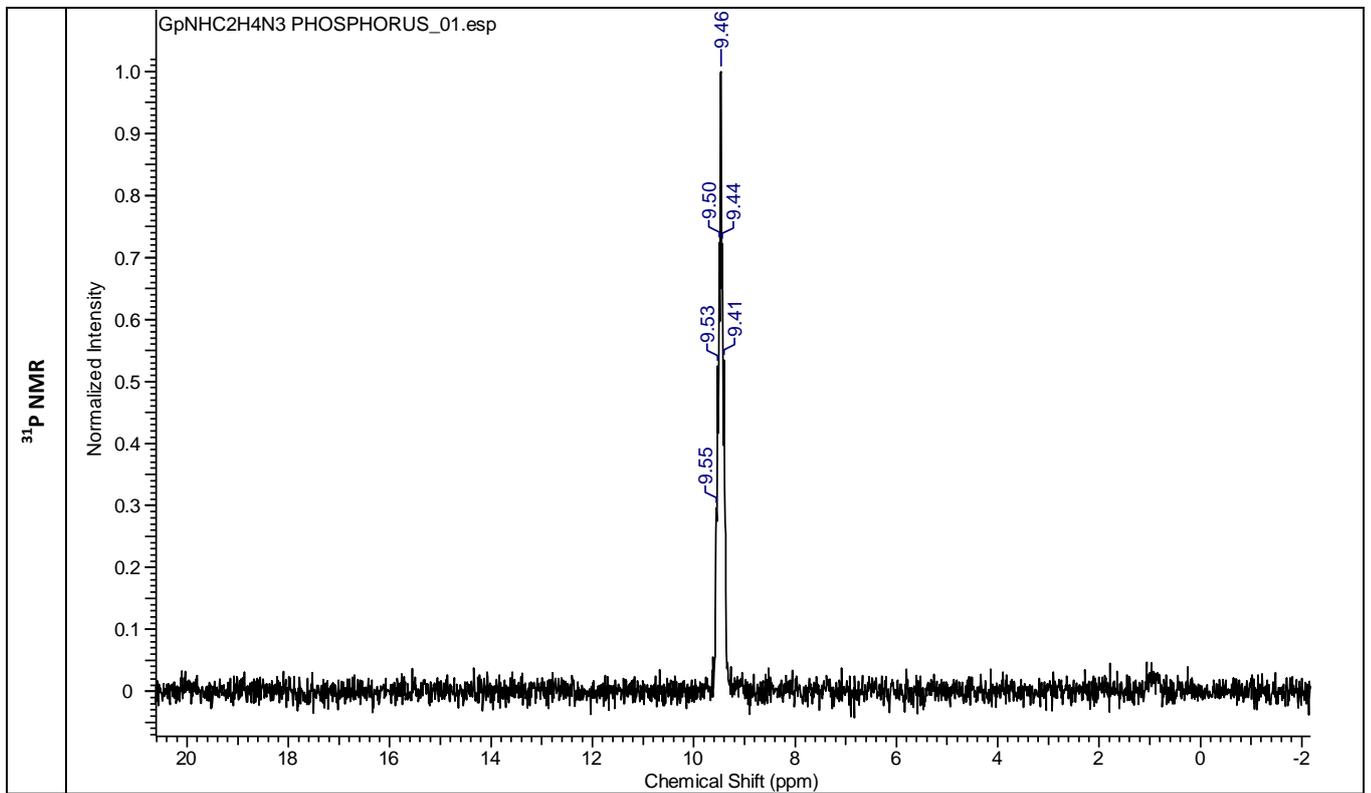
140313\_SW\_028 #60-125 RT: 0.92-1.98 AV: 66 NL: 2.87E5  
T: FTMS - p ESI Full ms [150.00-2000.00]

HRMS

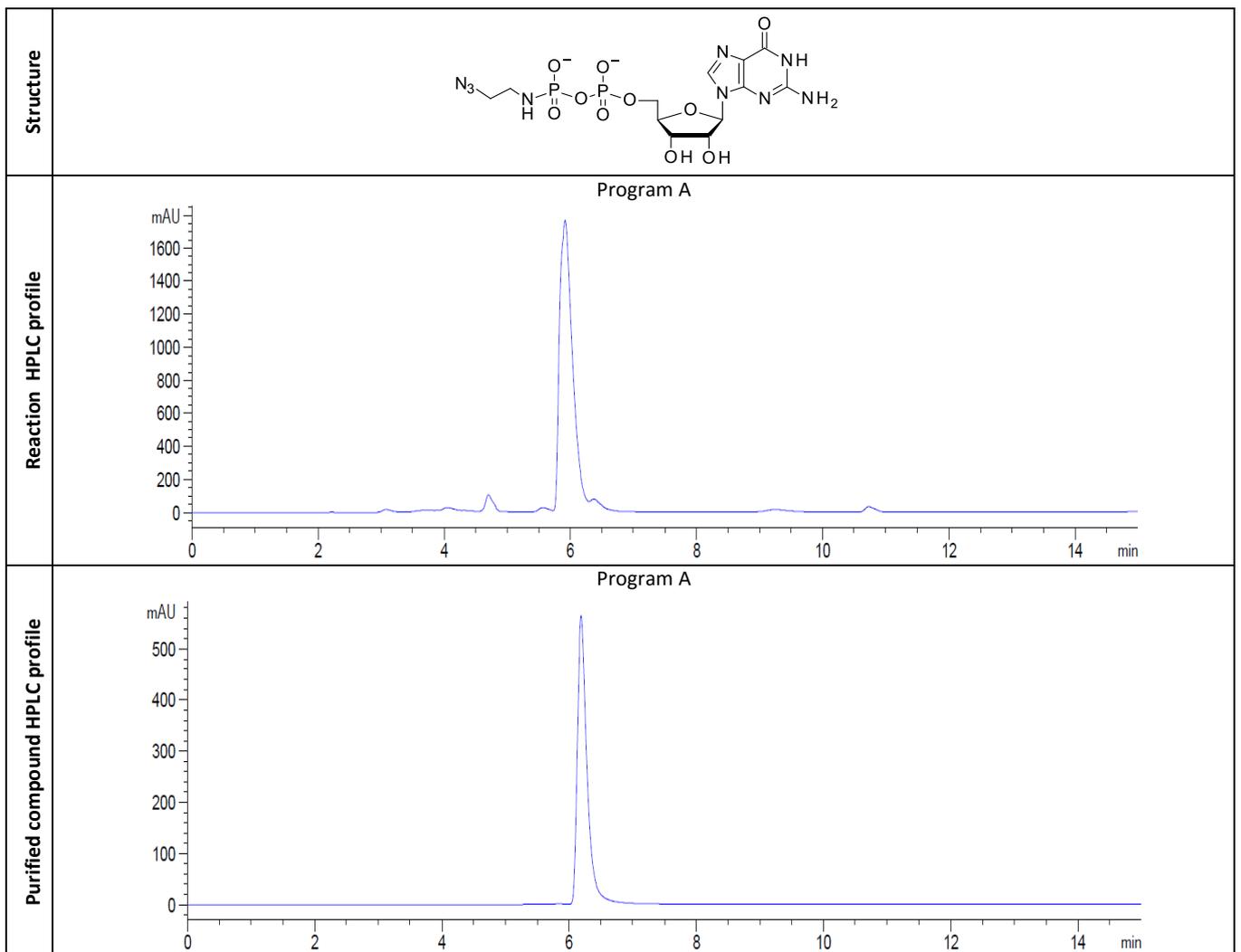


<sup>1</sup>H NMR



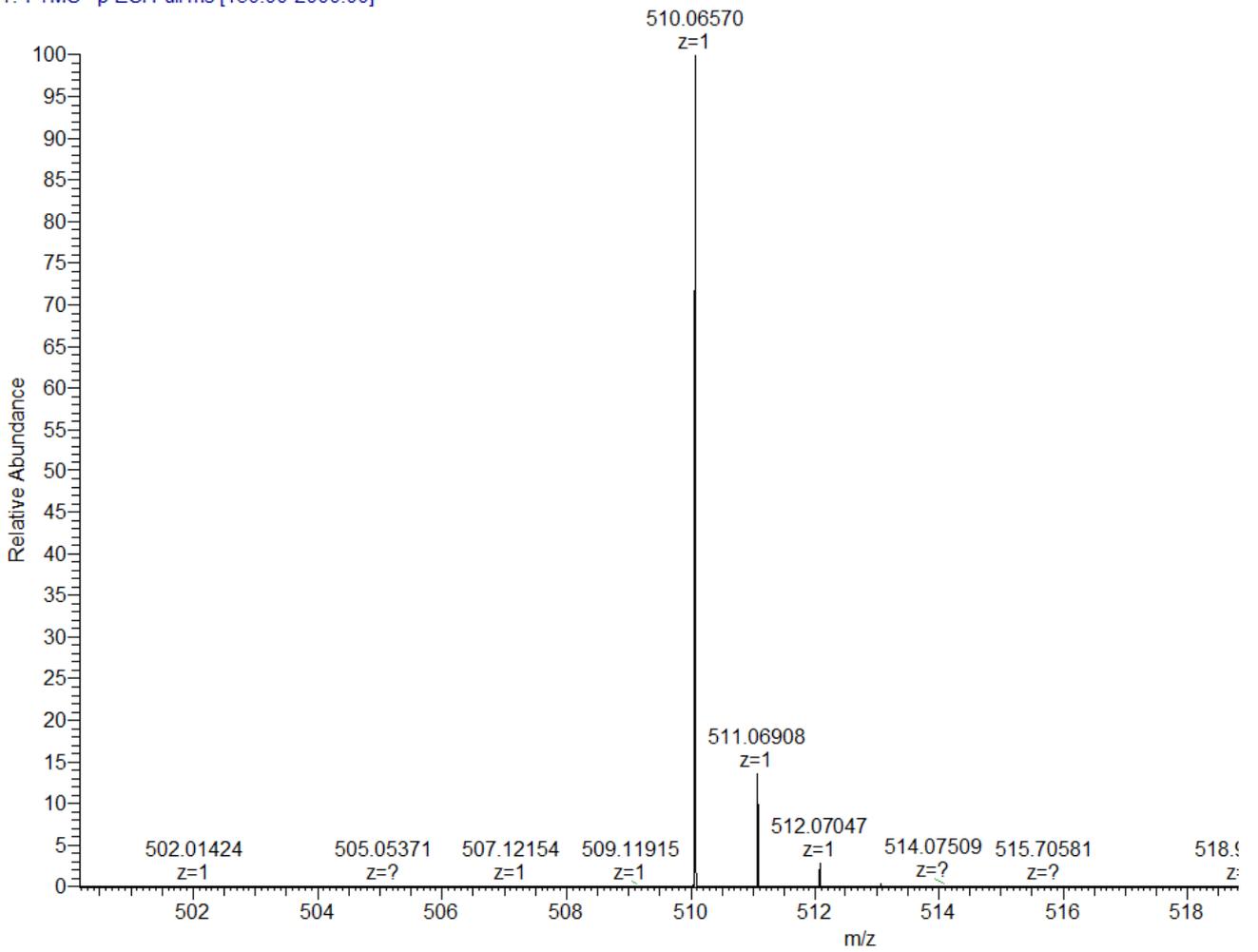


**(17b) GppNHC<sub>2</sub>H<sub>4</sub>N<sub>3</sub>**

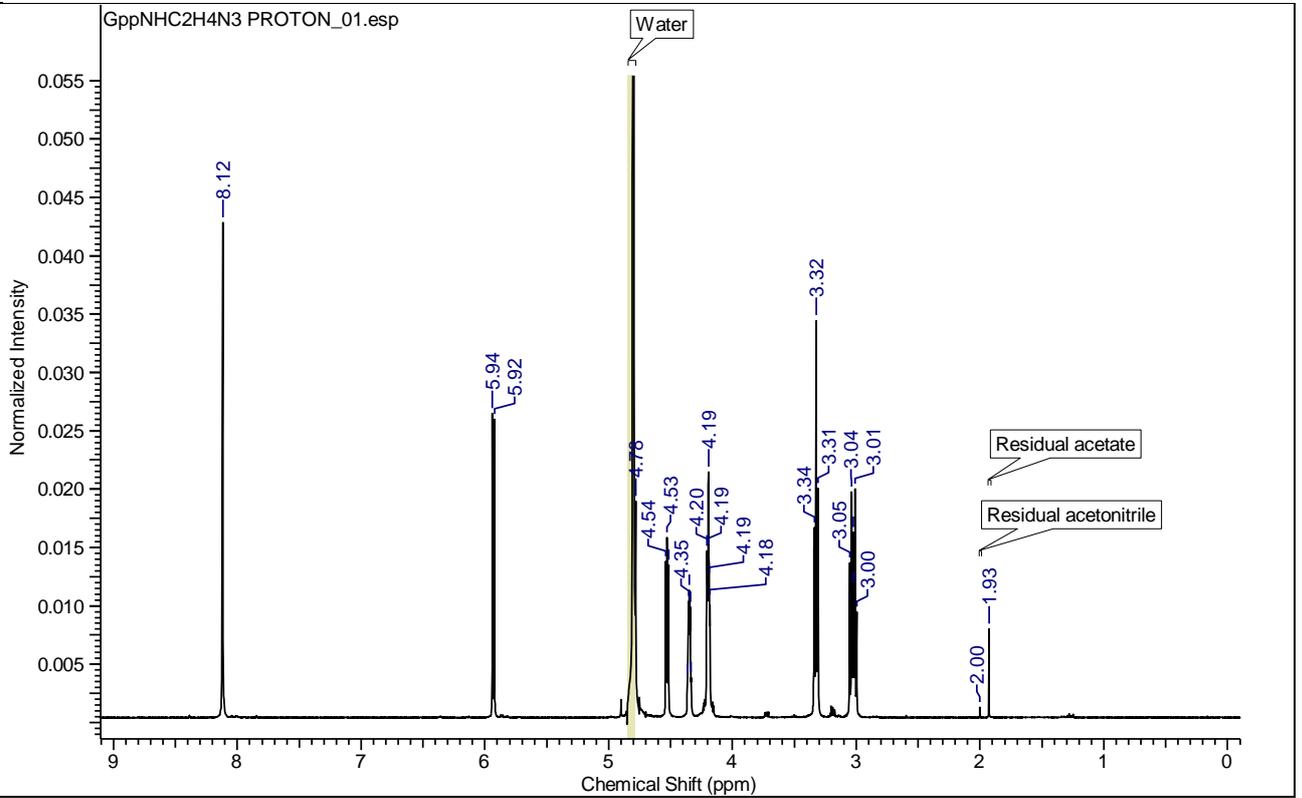


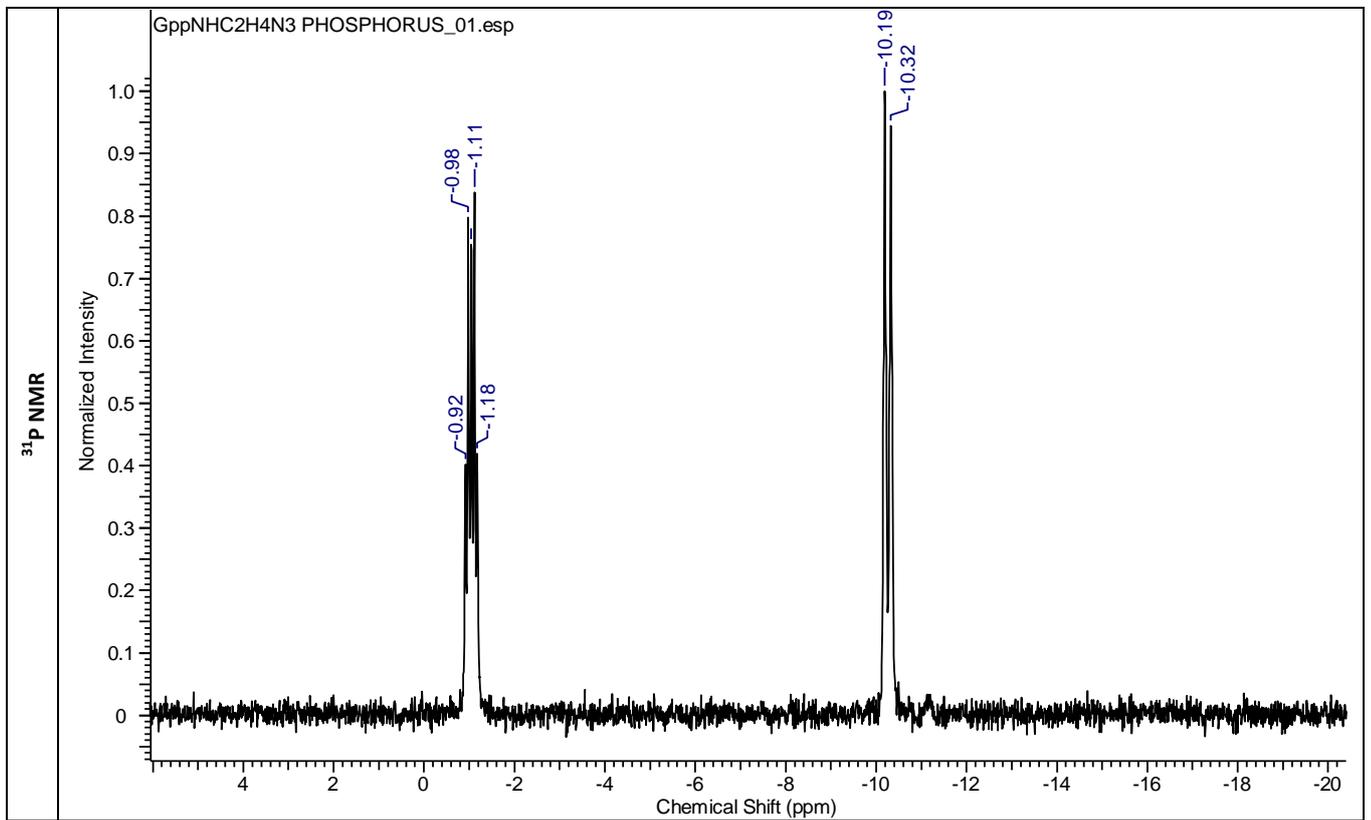
141030\_sw\_060 #6-204 RT: 0.03-0.91 AV: 199 NL: 2.69E7  
T: FTMS - p ESI Full ms [150.00-2000.00]

HRMS

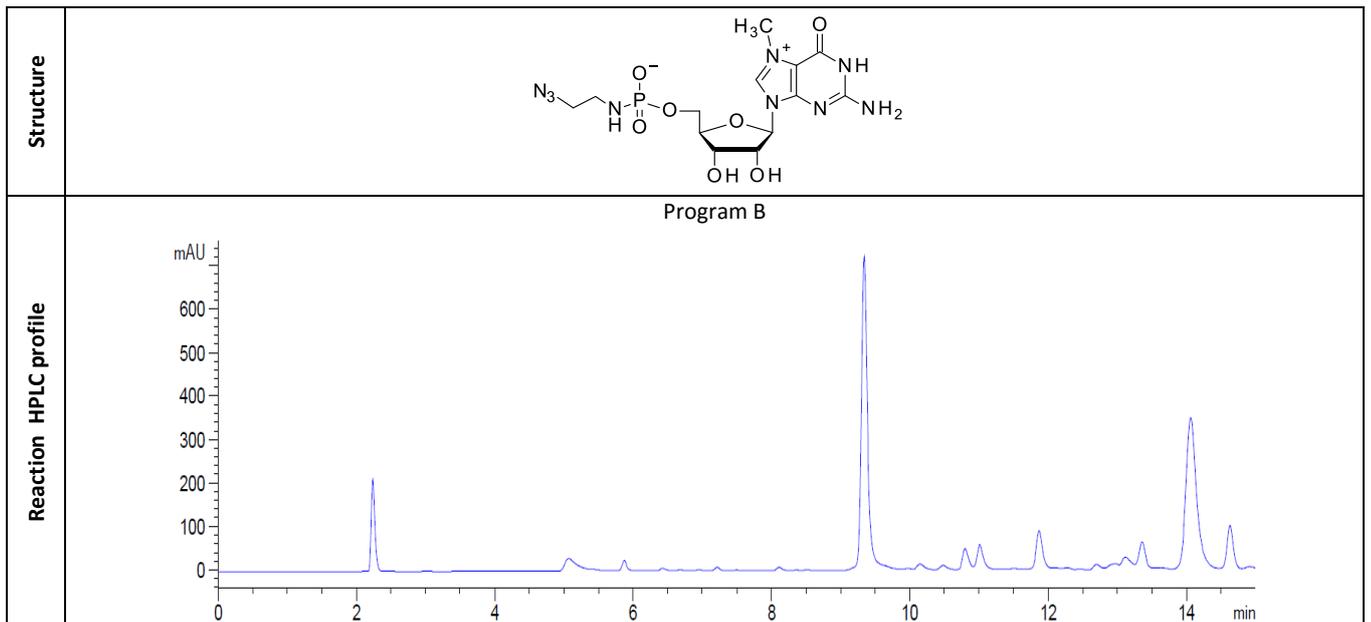


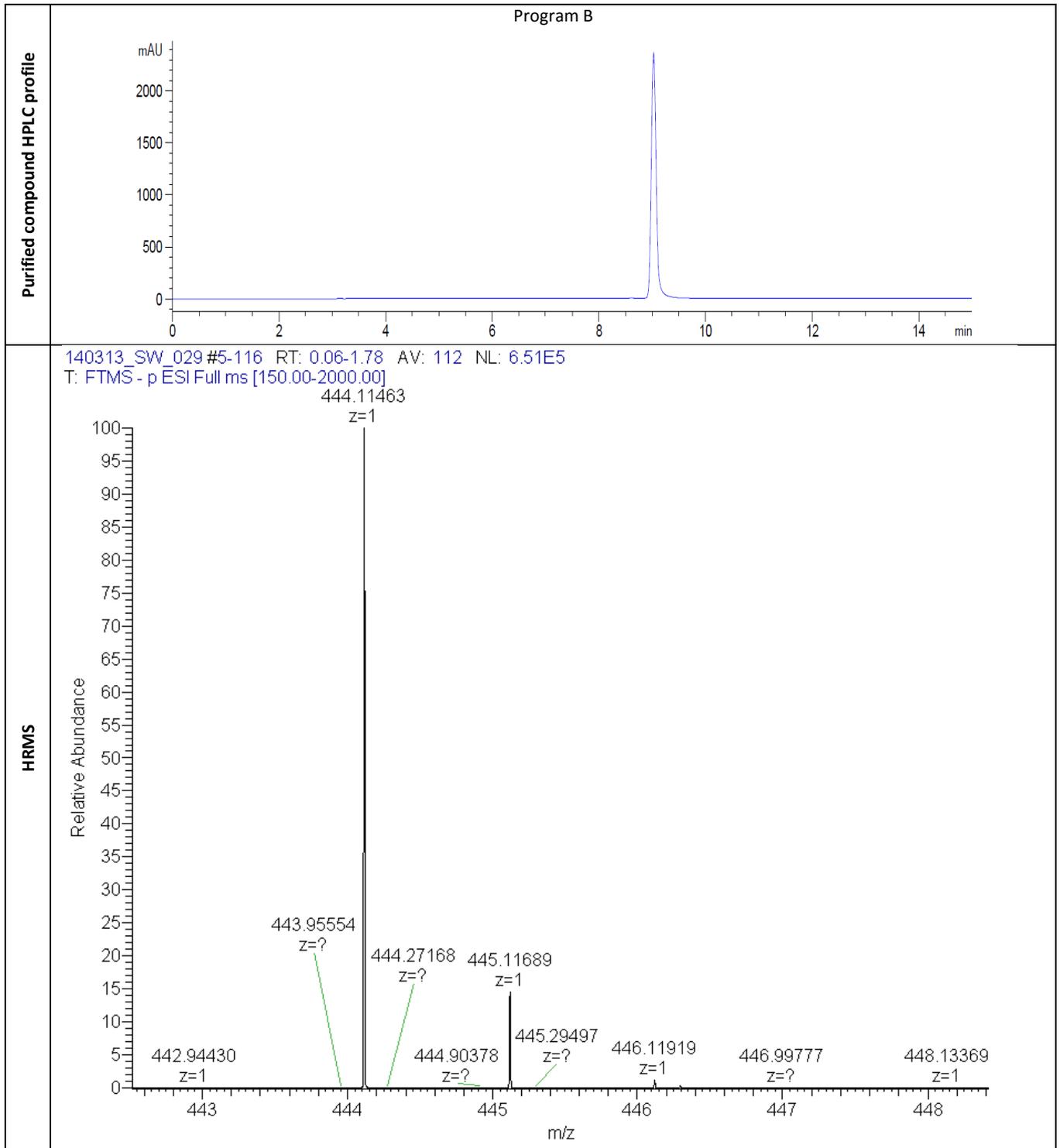
<sup>1</sup>H NMR

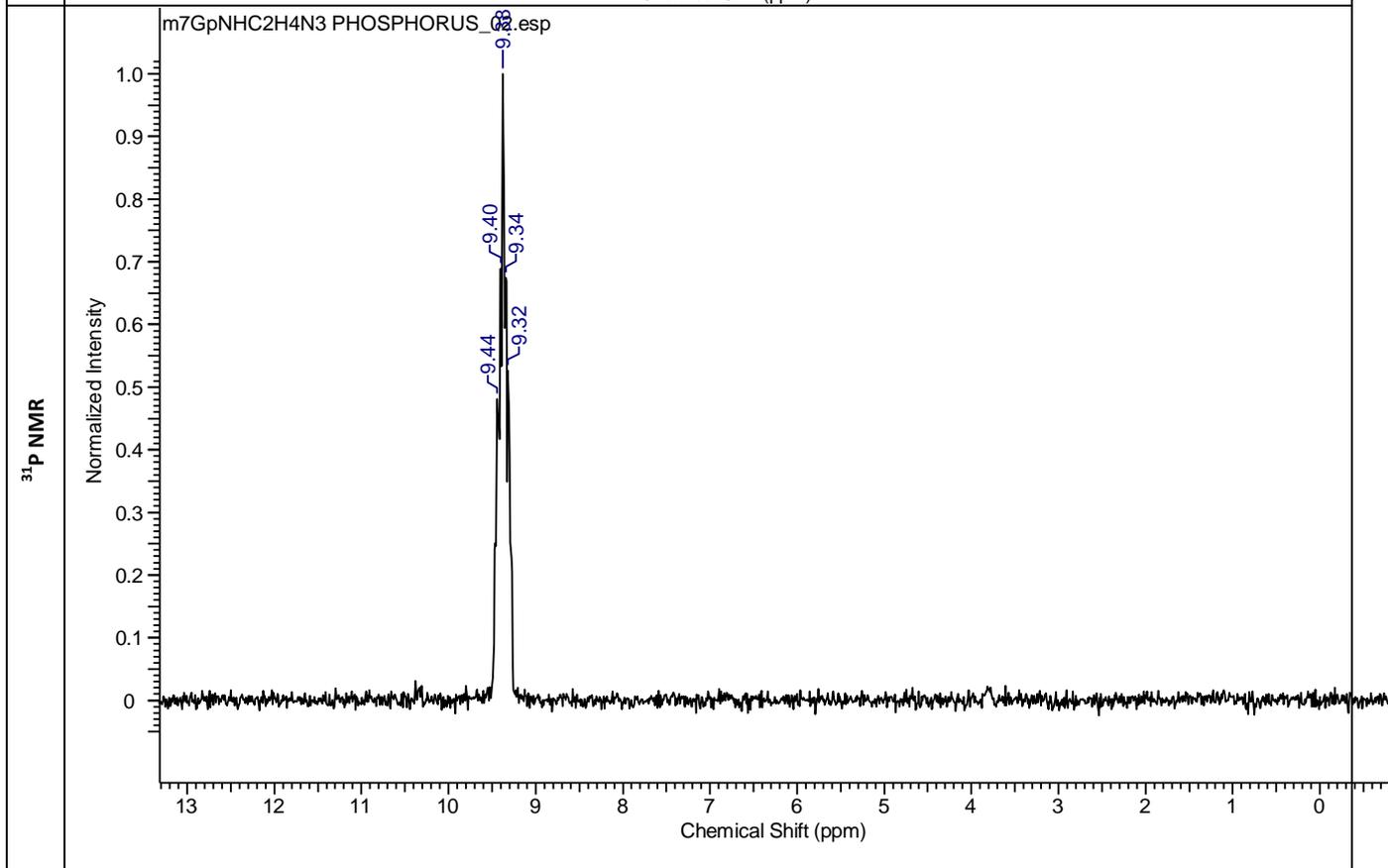
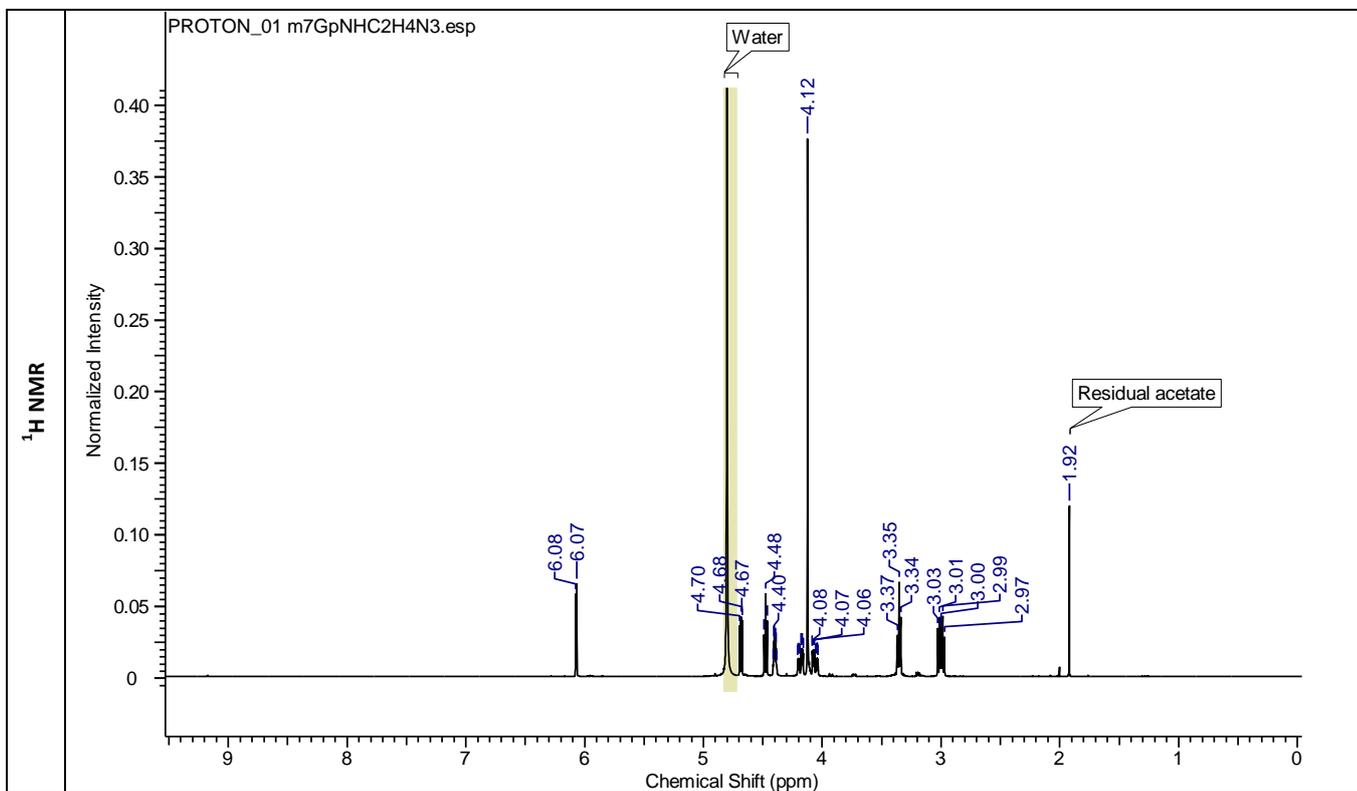




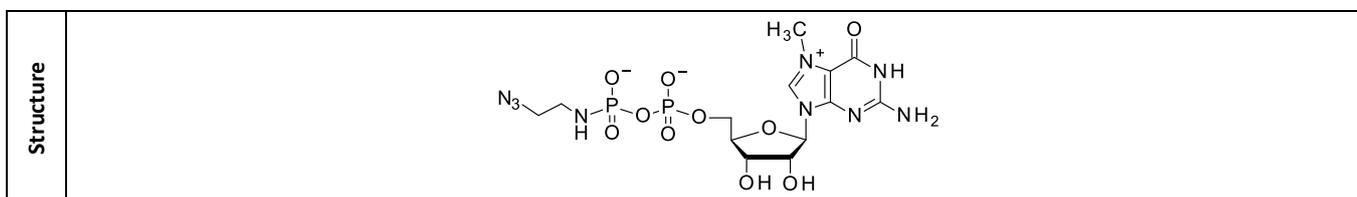
(17c) m<sup>7</sup>GpNHC<sub>2</sub>H<sub>4</sub>N<sub>3</sub>

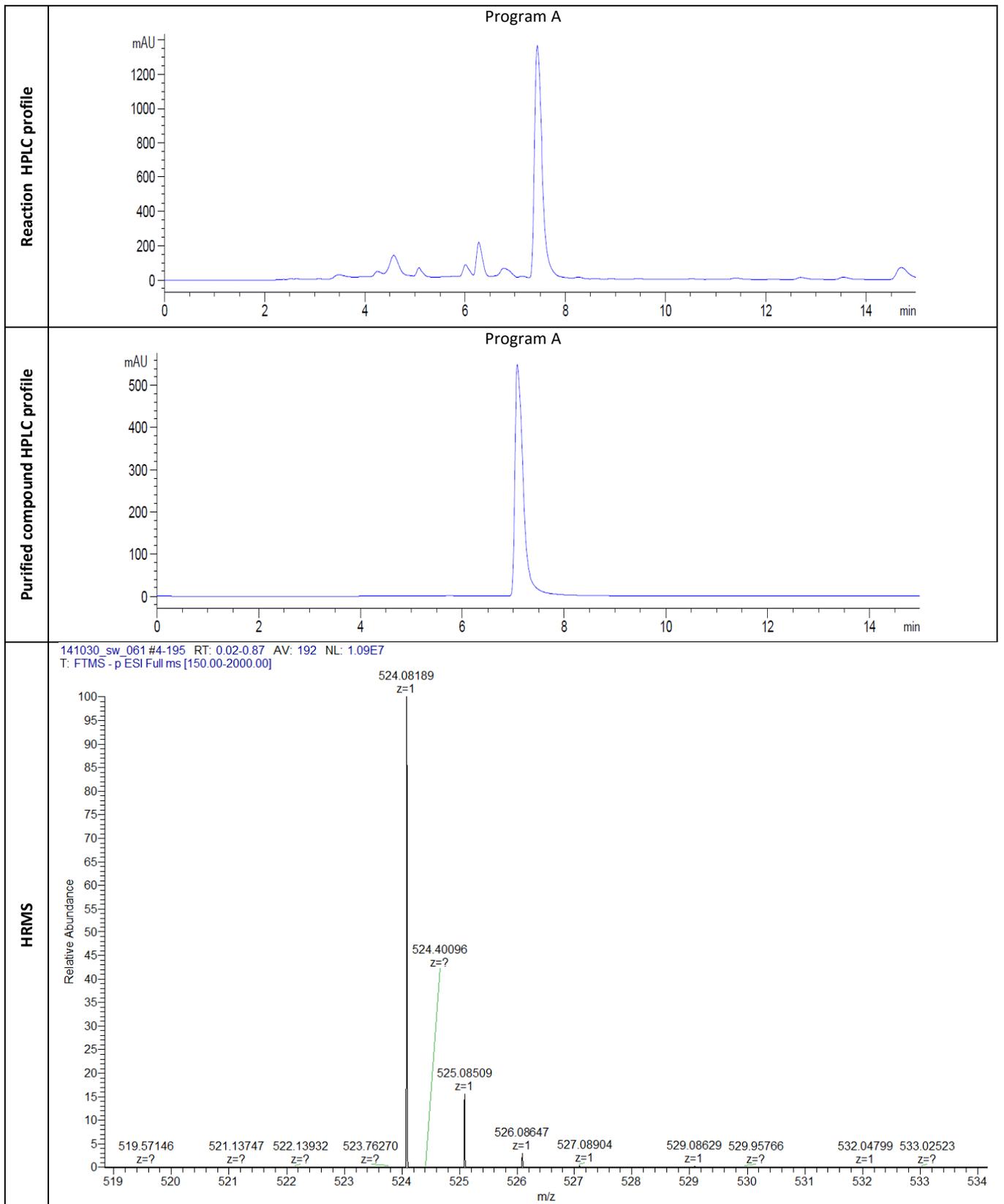


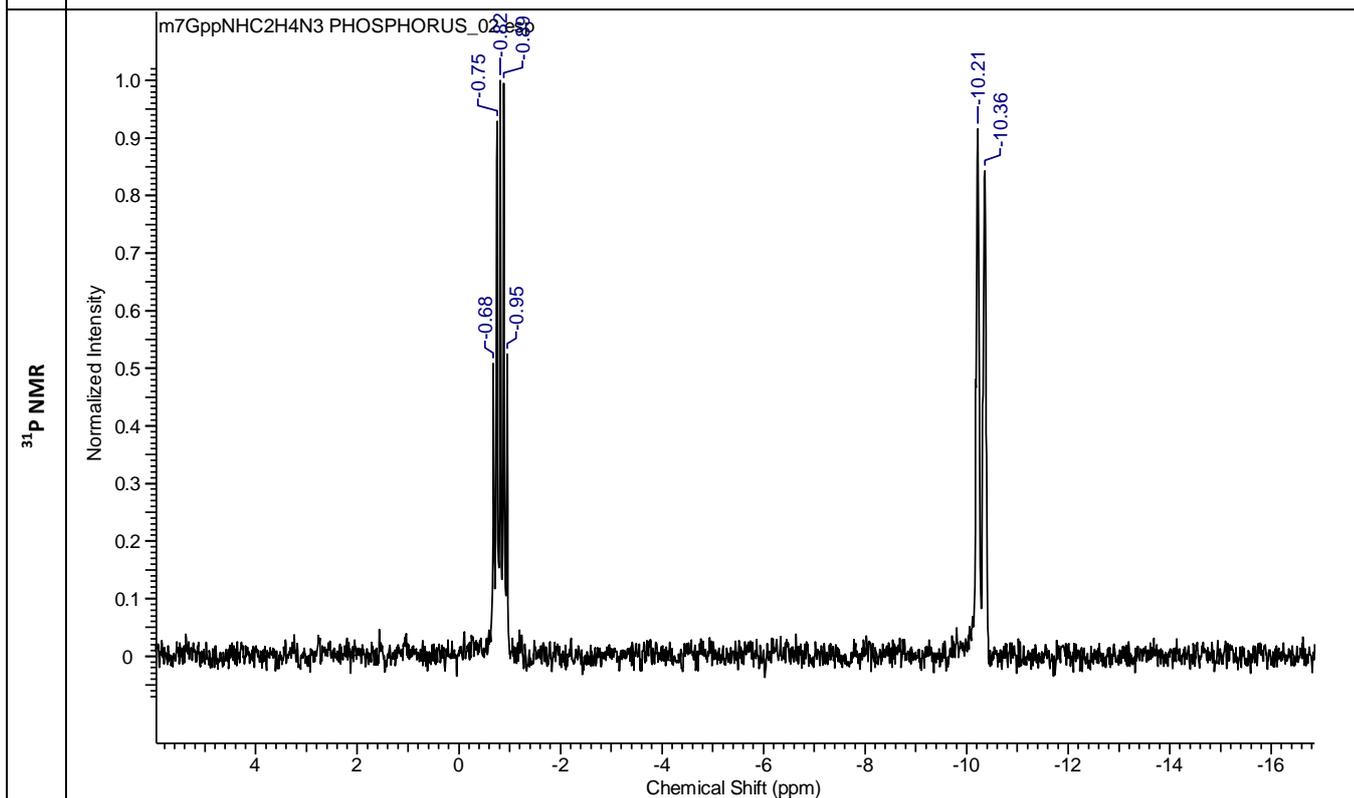
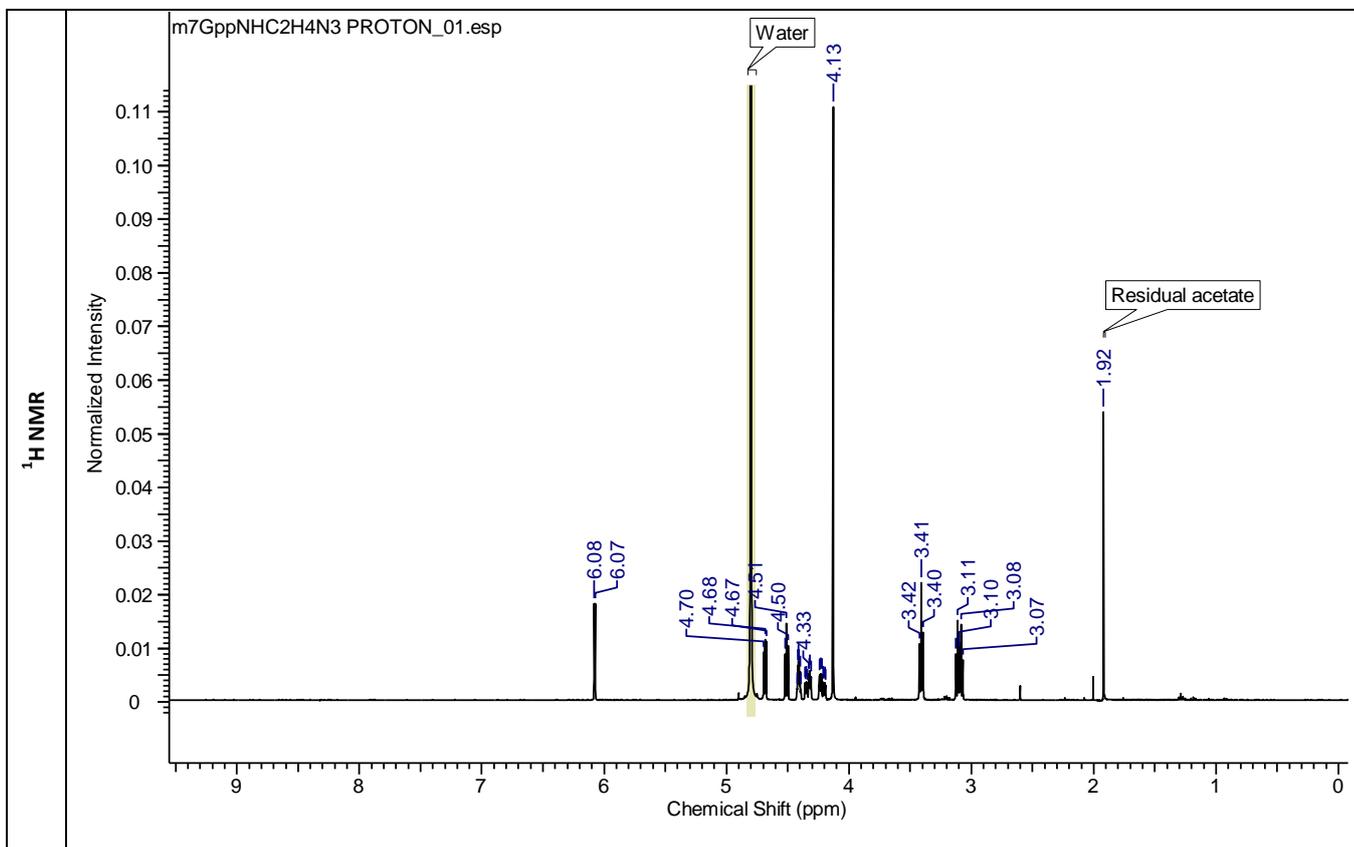




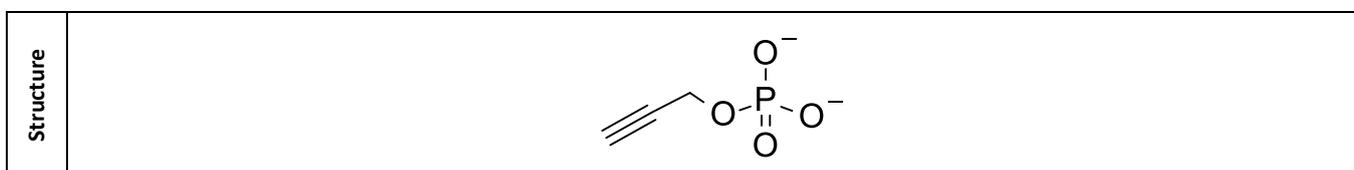
**(17d) m<sup>7</sup>GppNHC<sub>2</sub>H<sub>4</sub>N<sub>3</sub>**





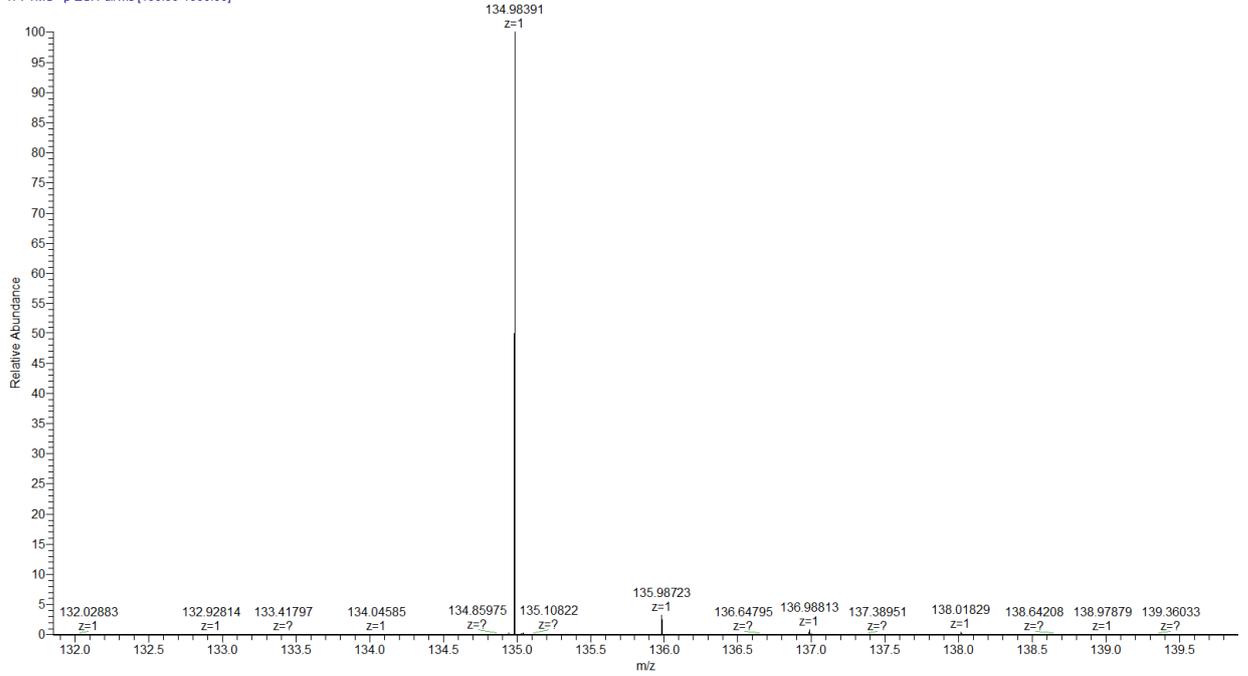


**(18d) O-(2-propynyl) phosphate ester**



160317\_PW\_007 #18-116 RT: 0.17-1.10 AV: 99 NL: 2.15E8  
T: FTMS - p ESI Full ms [100.00-1500.00]

HRMS



<sup>1</sup>H NMR

