

## Electronic Supplementary Material

### Simultaneous Quantification of Four Major Metabolites of Embryotoxic *N*-Methyl- and *N*-Ethyl-2-Pyrrolidone in Human Urine by Cooled-Injection Gas Chromatography and Isotope Dilution Mass Spectrometry

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NMR and mass-spectra of 5-hydroxy-*N*-ethyl-2-pyrrolidone (5-HNEP) and 2-hydroxy-*N*-ethylsuccinimide (2-HESI), two newly identified presumed metabolites of NEP, and their deuterium-labeled analogues with N-C<sub>2</sub>D<sub>5</sub> groups (5-HNEP-d<sub>5</sub> and 2-HESI-d<sub>5</sub>)

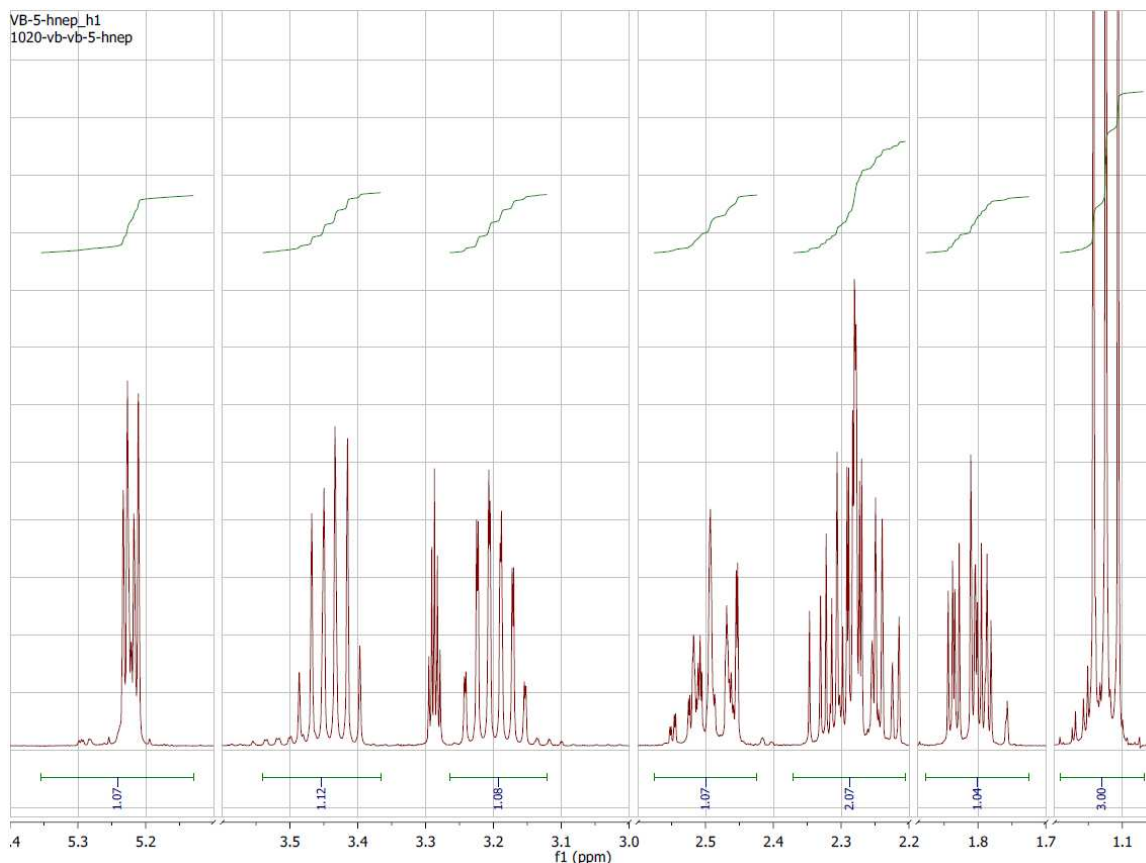


Figure S-1. <sup>1</sup>H-NMR spectrum of 5-HNEP in MeOH-d<sub>4</sub> (400 MHz). Assignments of the signals and coupling constants are given in the experimental part of the main text.

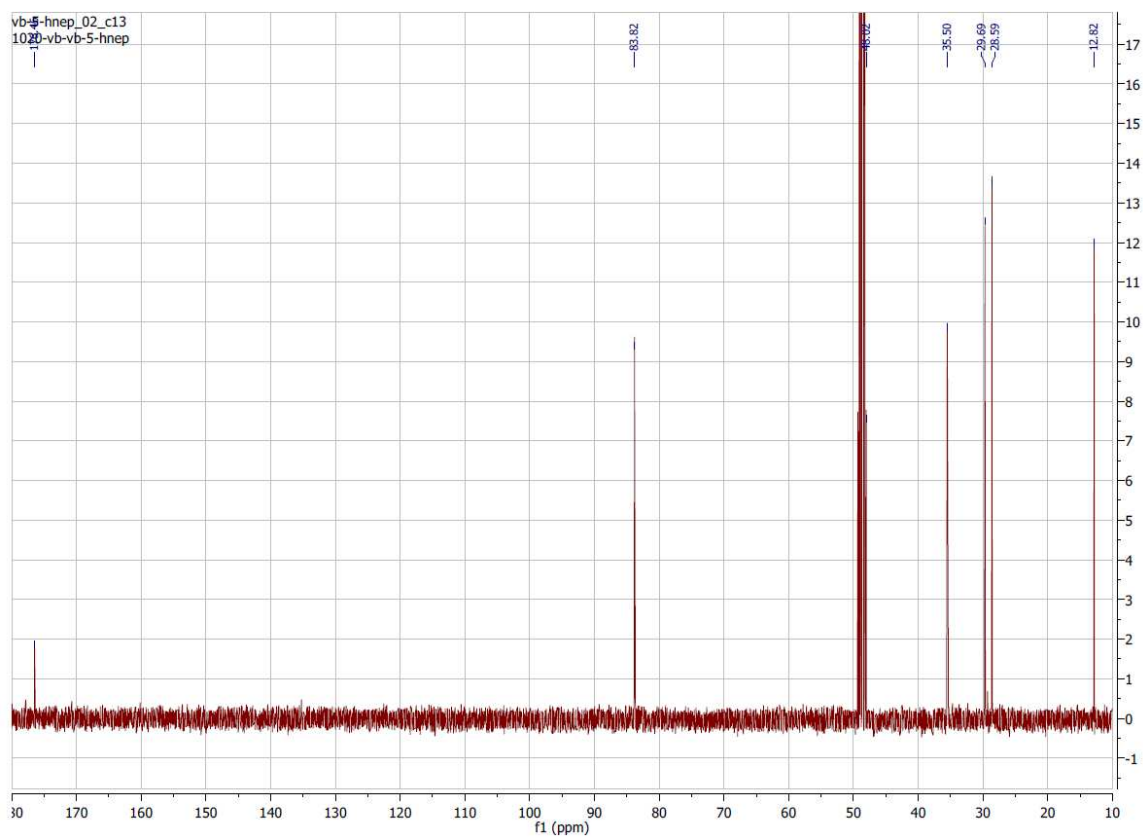
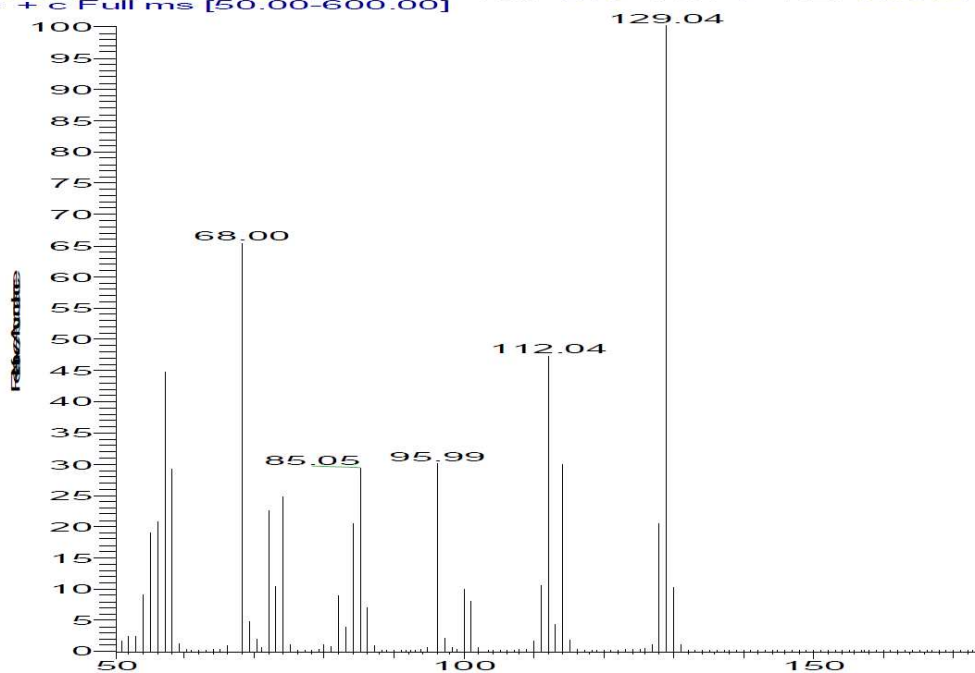


Figure S-2.  $^{13}\text{C}$ -NMR spectrum of 5-HNEP in  $\text{MeOH-d}_4$  (400 MHz). Assignments of the signals are given in the experimental part of the main text.

ms\_direkt\_ei\_vb\_5hnep #60 RT: 1.15 AV: 1 NL: 3.59E7  
T: + c Full ms [50.00-600.00]



ms\_direkt\_ei\_vb\_5hnep+5 #11-102 RT: 0.23-1.93 AV: 92  
T: + c Full ms [50.00-600.00]

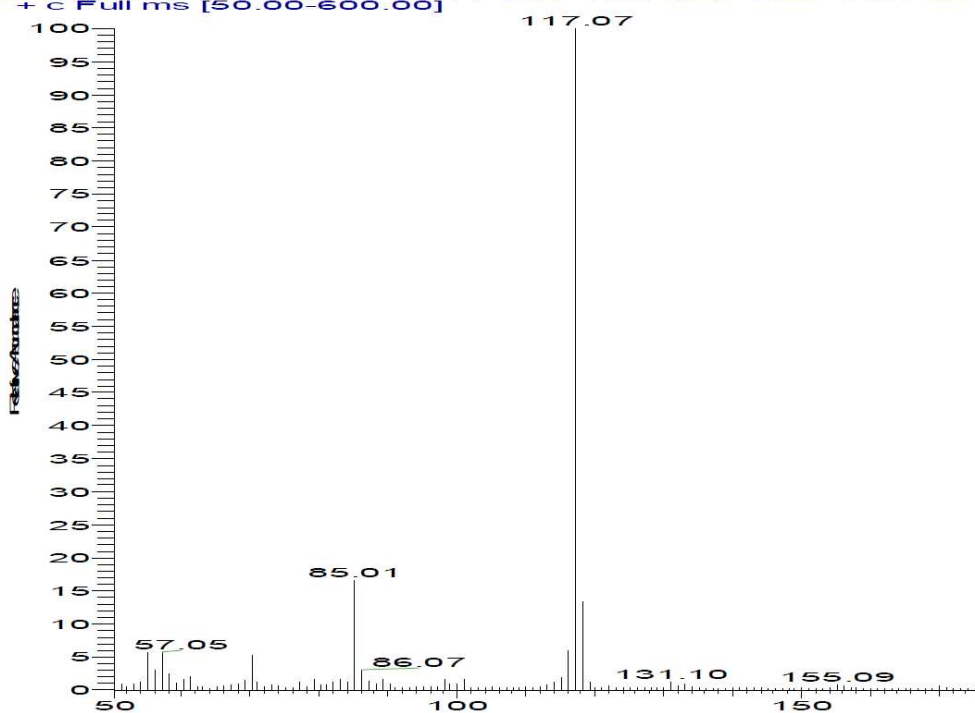


Figure S-3. EI-MS of compounds 5-HNEP and 5-HNEP-d<sub>5</sub>. Fragmentation patterns are given in the experimental part of the main text.

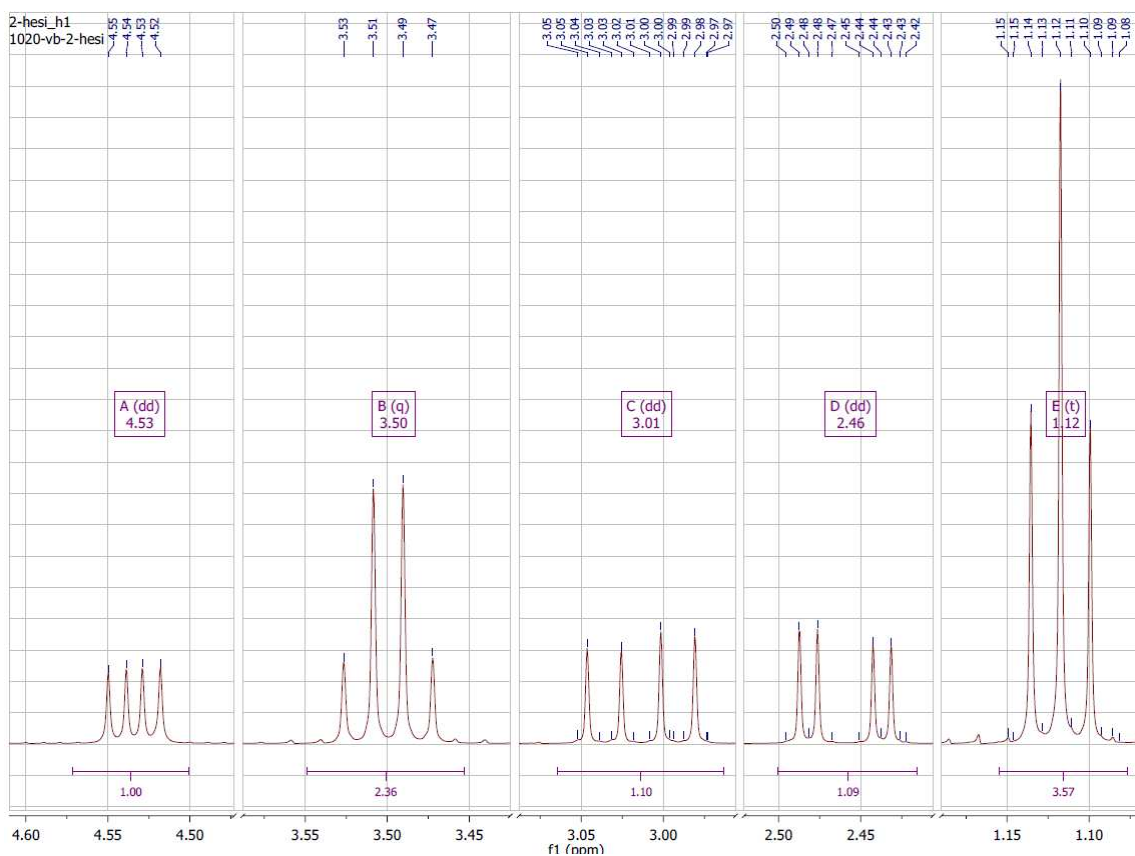


Figure S-4.  $^1\text{H}$ -NMR spectrum of 2-HESI in  $\text{MeOH-d}_4$  (400 MHz). Assignments of the signals and coupling constants are given in the experimental part of the main text.

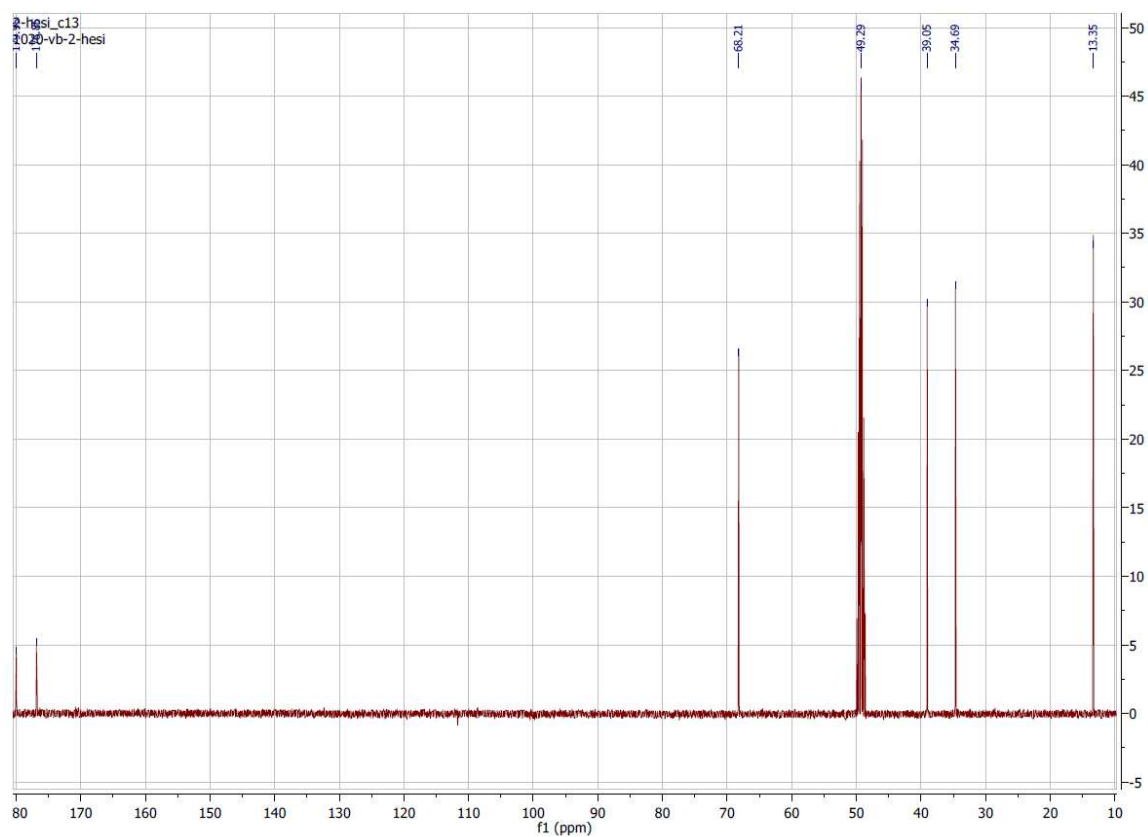


Figure S-5.  $^{13}\text{C}$ -NMR spectrum of 2-HESI in MeOH-d<sub>4</sub> (400 MHz). Assignments of the signals and coupling constants are given in the experimental part of the main text.

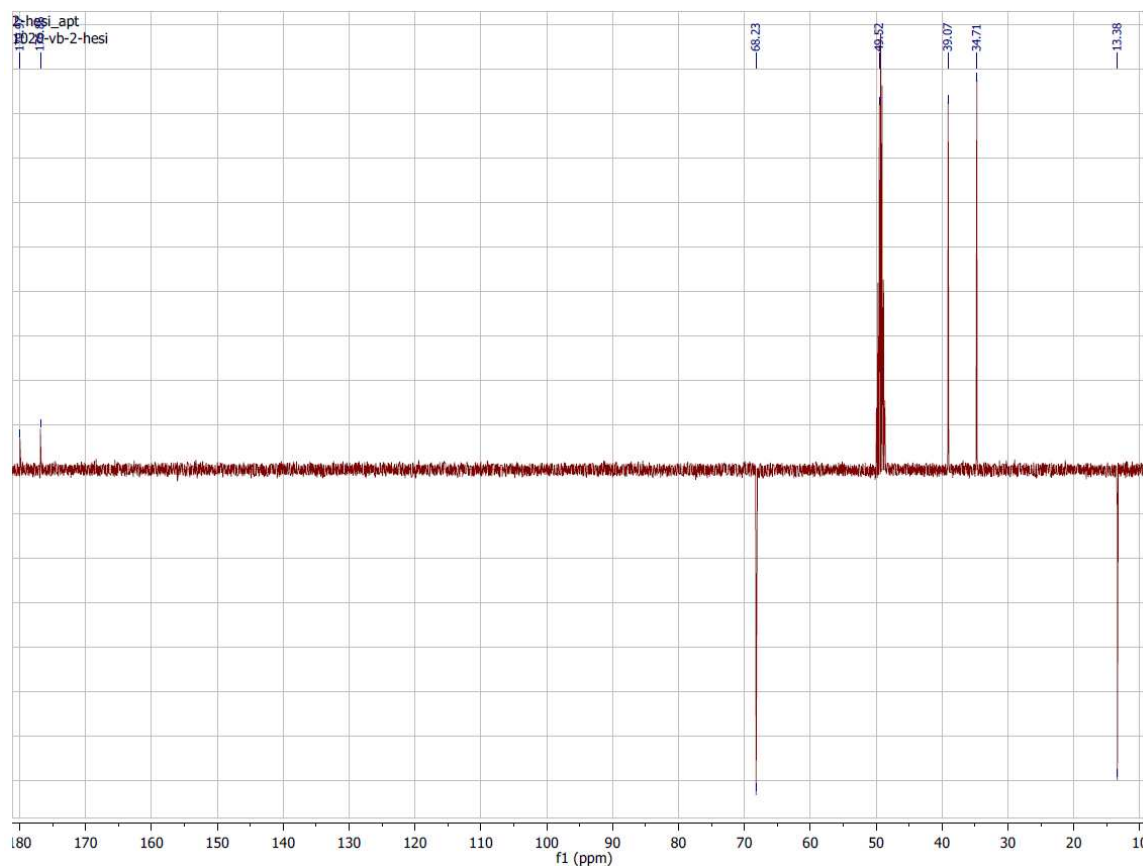
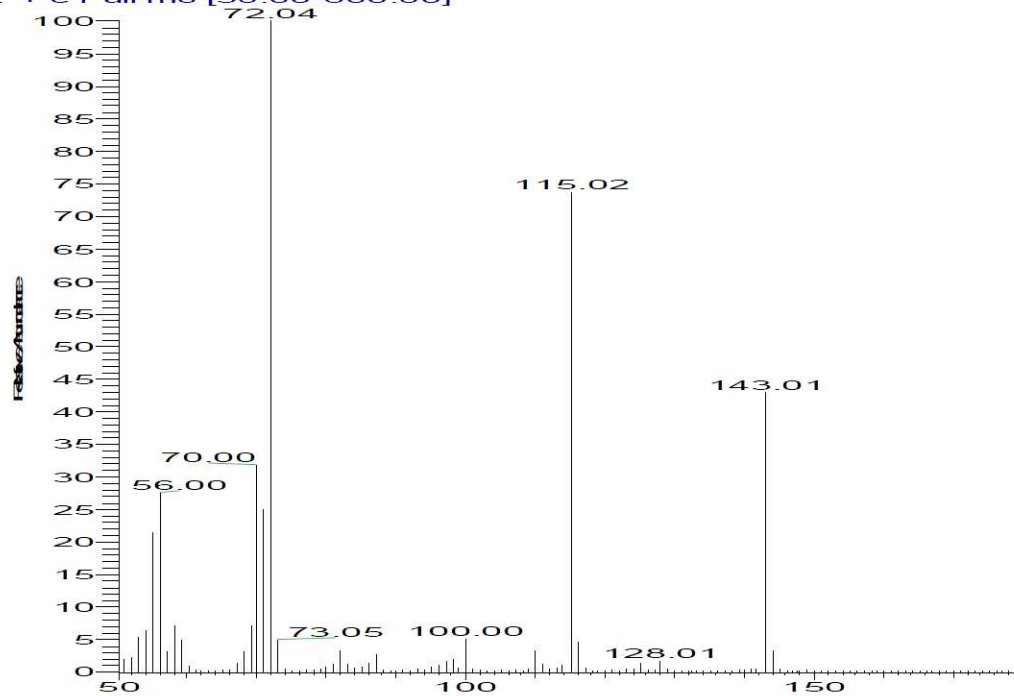


Figure S-6. APT mode of  $^{13}\text{C}$ -NMR spectrum of 2-HESI in  $\text{MeOH-d}_4$  (400 MHz). (signals of  $\text{CH}_3$  and  $\text{CH}$  groups have negative intensity, and signals of  $\text{CH}_2$  groups, as well as quaternary carbons – positive intensity). Assignments of the signals are given in the experimental part of the main text.

ms\_direkt\_el\_vb\_2\_hesi #3-74 RT: 0.08-1.41 AV: 72 NL: 3  
T: + c Full ms [50.00-600.00]



ms\_direkt\_el\_vb\_2hesi+5 #3-93 RT: 0.08-1.77 AV: 91 NL: 3  
T: + c Full ms [50.00-600.00]

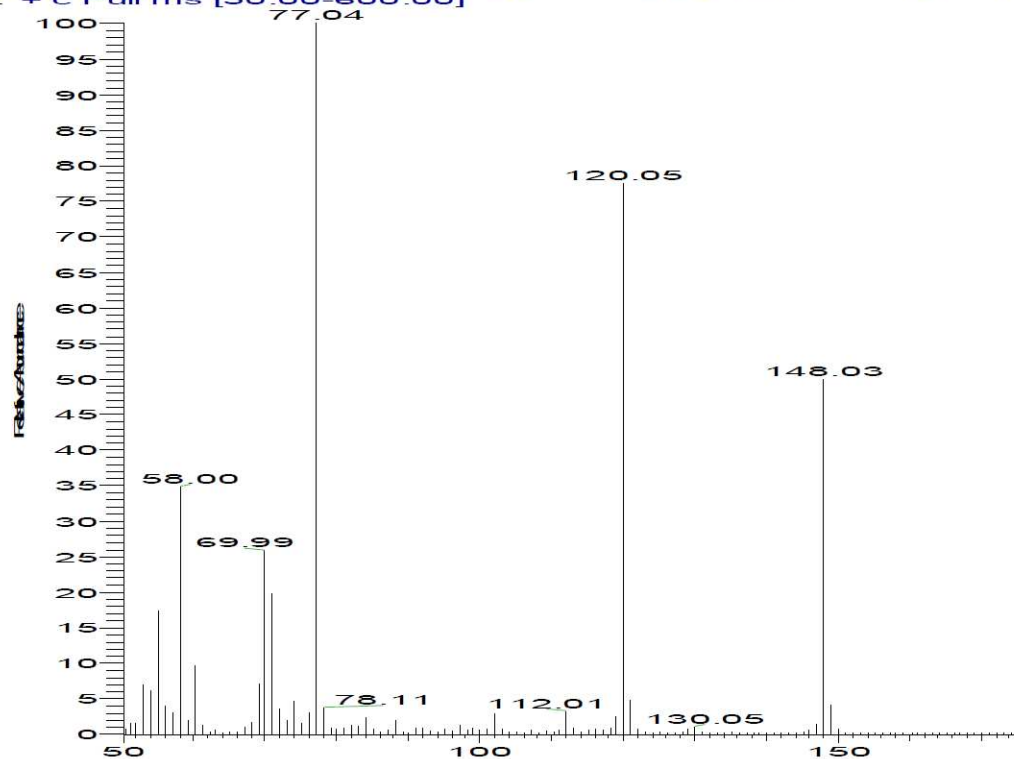


Figure S-7. EI-MS of compounds 5-HESI and 5-HESI-d<sub>5</sub>. Fragmentation patterns are given in the experimental part of the main text.



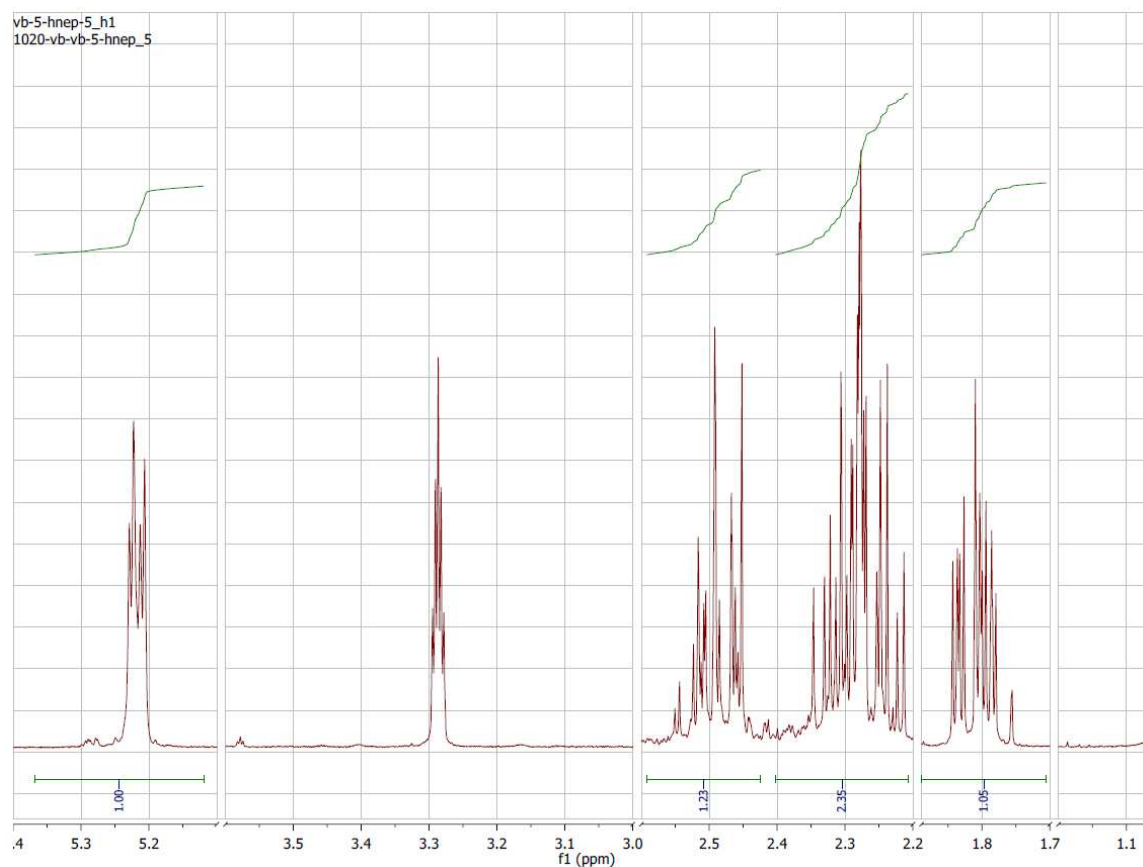


Figure S-8.  $^1\text{H}$ -NMR spectrum of 5-HNEP- $\text{d}_5$  in  $\text{MeOH-d}_4$  (400 MHz). Note the complete absence of the signals attributed to *N*-ethyl group (*cf.* Fig. 1). Assignments of the other signals and coupling constants are given in the experimental part of the main text.

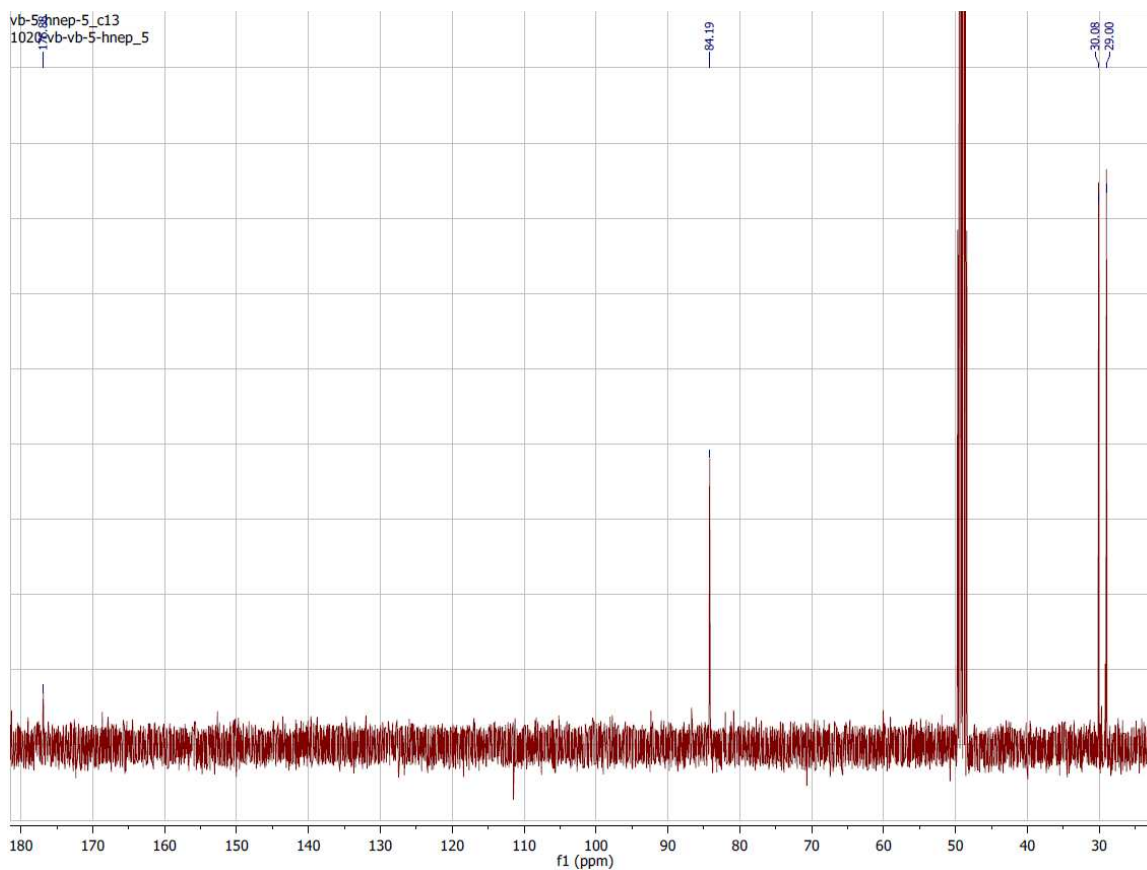


Figure S-9.  $^{13}\text{C}$ -NMR spectrum of 5-HNEP- $\text{d}_5$  in  $\text{MeOH-d}_4$  (400 MHz). Note the complete absence of the signals attributed to *N*-ethyl group (*cf.* Fig. 2). Assignments of the other signals are given in the experimental part of the main text.

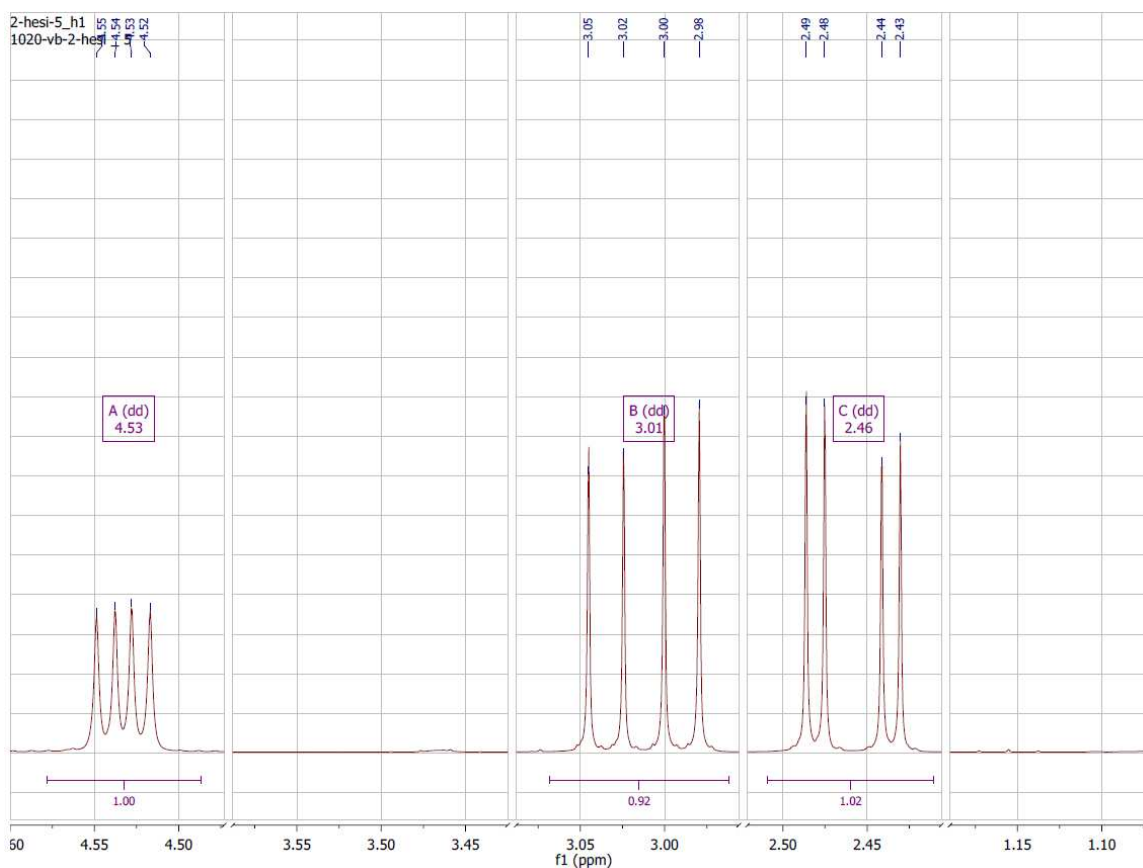


Figure S-10.  $^1\text{H}$ -NMR spectrum of 2-HESI- $\text{d}_5$  in  $\text{MeOH-d}_4$  (400 MHz). Note that the signals of N-ethyl group are absent (*cf.* Fig. 5). Assignments of the other signals and coupling constants are given in the experimental part of the main text.

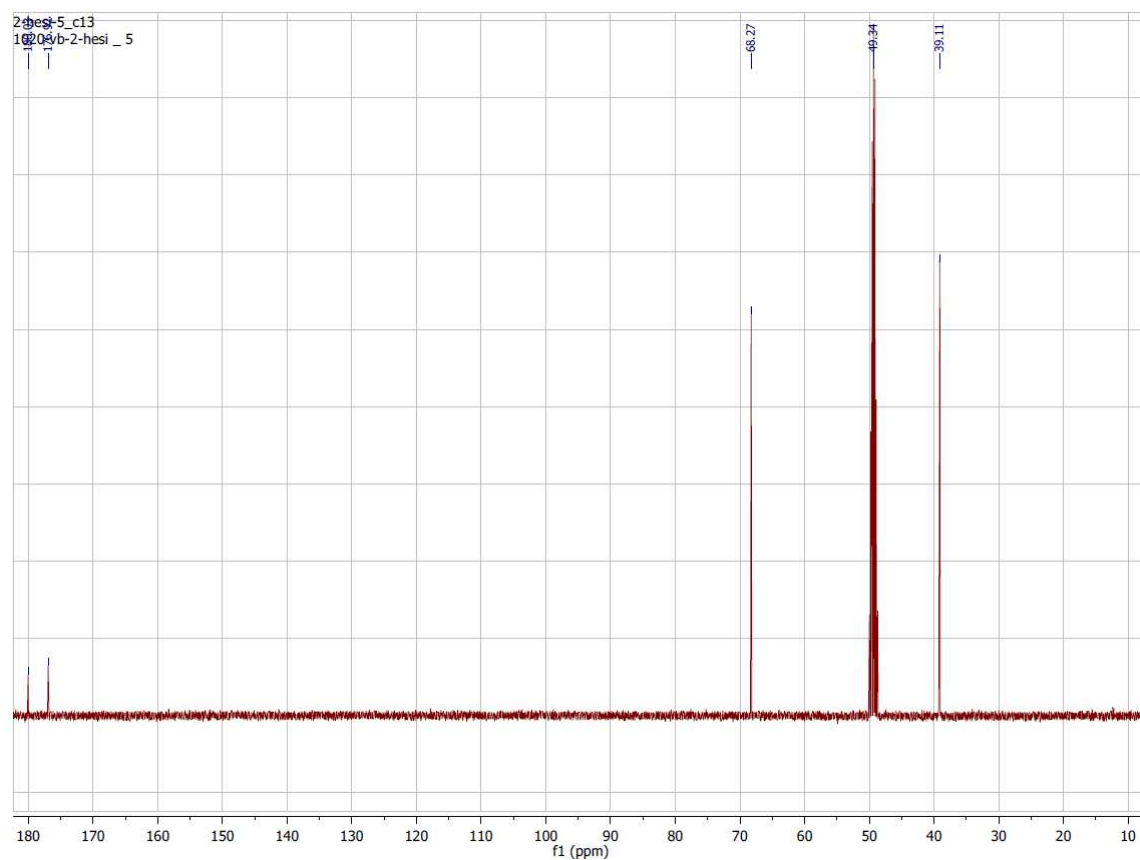


Figure S-11.  $^{13}\text{C}$ -NMR spectrum of 2-HESI- $\text{d}_5$  in  $\text{MeOH-d}_4$  (400 MHz). Note that the signals of N-ethyl group are absent (*cf.* Fig. 6). Assignments of the signals are given in the experimental part of the main text.

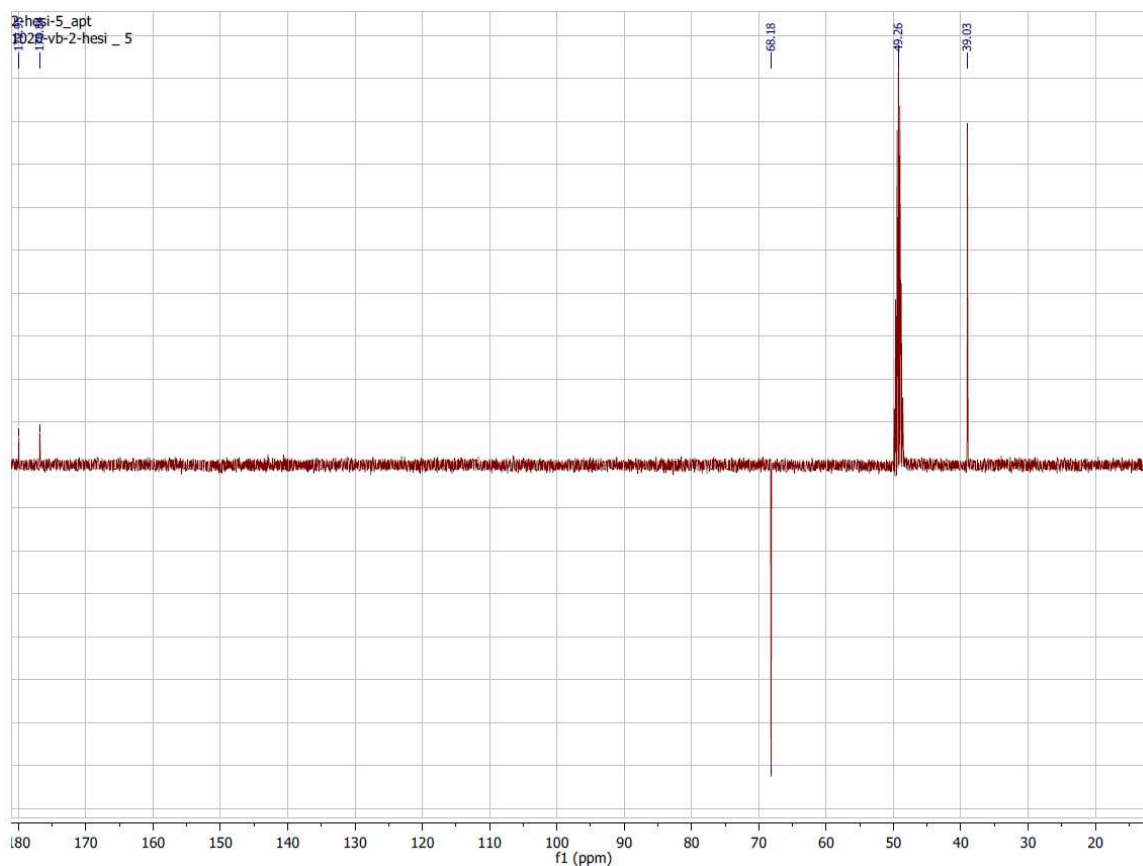


Figure S-12. APT mode of  $^{13}\text{C}$ -NMR spectrum of 2-HESI- $\text{d}_5$  in  $\text{MeOH-d}_4$  (400 MHz) (signals of  $\text{CH}_3$  and  $\text{CH}$  groups have negative intensity, and signals of  $\text{CH}_2$  groups, as well as quaternary carbons – negative intensity). Note that the signals of N-ethyl group are absent (*cf.* Figures 6 and 9). Assignments of the signals are given in the experimental part of the main text.