

Supporting Information

Investigating the Kinetic Mechanism of Inhibition of Elongation Factor 2 Kinase (eEF-2K) by NH125: Evidence for a Common *in* *vitro* Artifact

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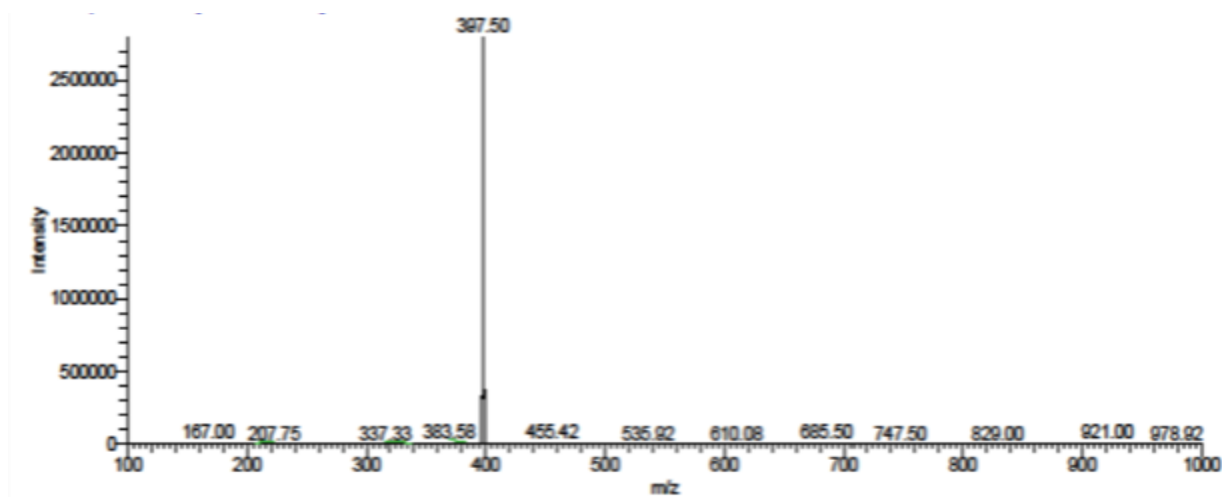


Figure S1: MS data of NH125. ESI-MS $[M+H]^+$ calculated, 397.4 ; observed, 397.5.

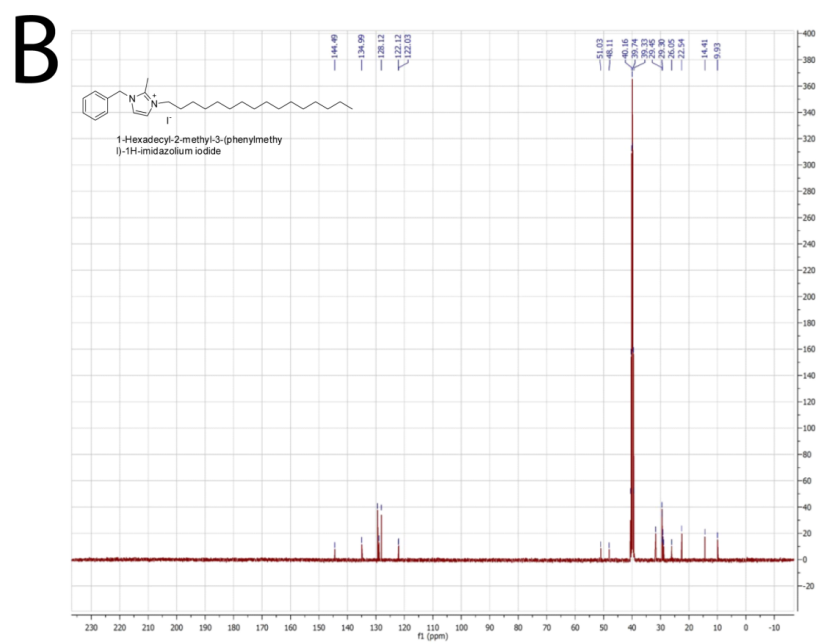
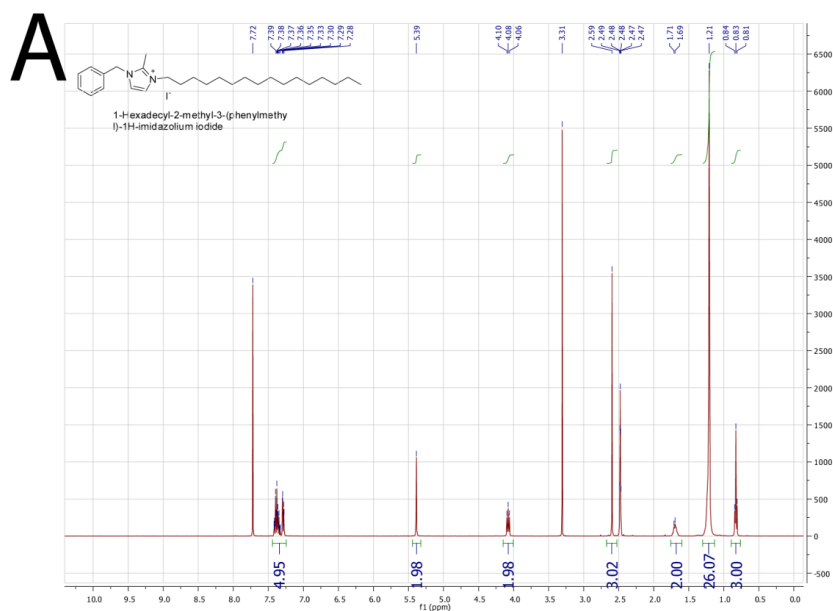


Figure S2: NMR spectra of NH125. White solid (1.74 g, 52.4%). A) ^1H NMR (400 MHz DMSO- d_6) δ 7.72 (s, 2H), 7.43-7.33 (m, 3H), 7.3 (d, 2H, J = 6.4 Hz), 5.39 (s, 2H), 4.1 (t, 2H, J = 7.2 Hz), 2.60 (s, 3H), 1.71 (br, 2H), 1.21 (br, 26H), 0.83 (t, 3H, J = 6.8 Hz); and B) ^{13}C NMR (100.6 MHz DMSO- d_6) δ 144.76, 135.0, 129.44, 128.92, 128.13, 122.13, 122.03, 51.03, 48.11, 31.73, 29.49, 29.45, 29.39, 29.36, 29.31, 29.15, 26.06, 22.54, 14.41, 9.94.

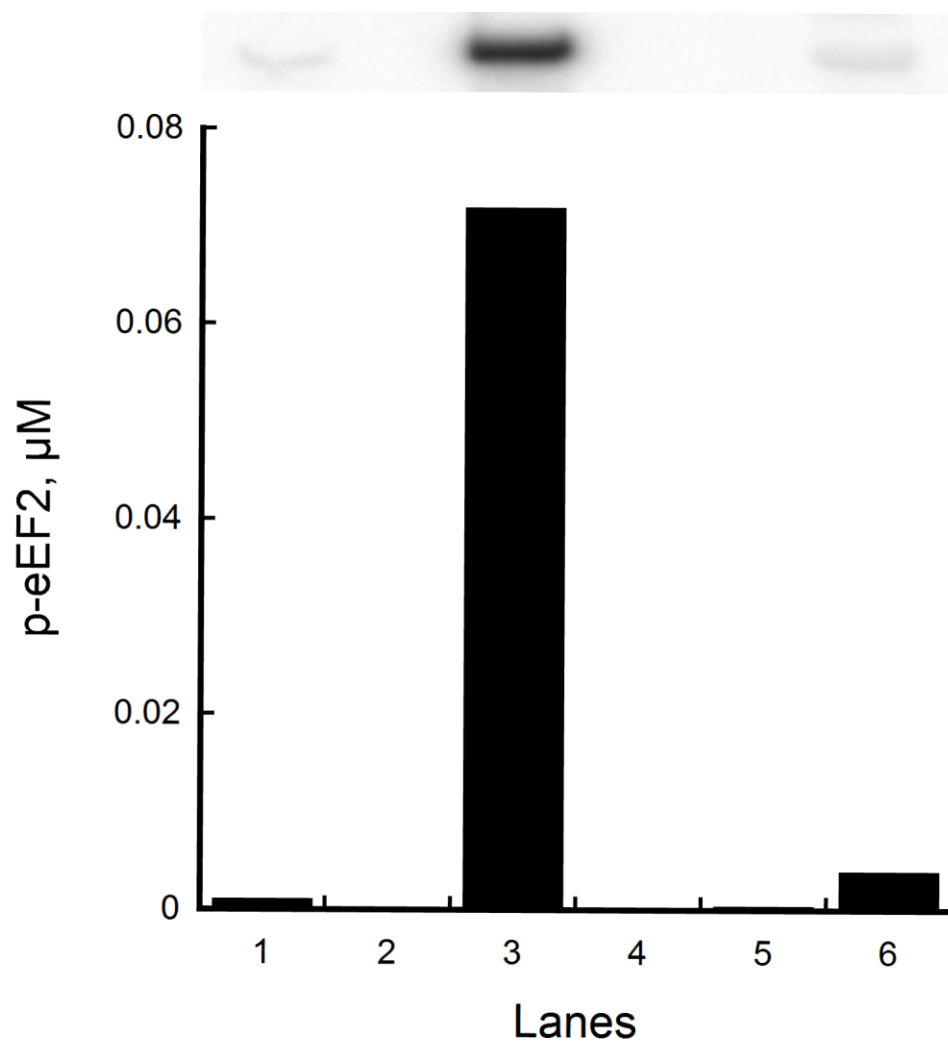


Figure S3. Phosphorylation assays of wheat germ EF2 by eEF-2K performed at higher concentrations of NH125 (lane 3, 0 μM ; lane 4, 50 μM ; lane 5, 25 μM ; lane 6, 12.5 μM). Control reactions with no eEF2 (lane 1) or no eEF-2K (lane 2) are also shown. Phosphorylation reactions, quenched after 5 minutes and resolved by SDS-PAGE, were visualized by autoradiography (top) and also quantified using the scintillation counter (bottom).