

**Electronic Supplementary Information**

**Induced Cytotoxicity of Peptides by  
Intracellular Native Chemical Ligation**

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### ***Experimental Section***

**Cell lines and culture conditions.** A549 human lung adenocarcinoma epithelial cells, HTC116 human colorectal cancer cells and HeLa human cervical cancer cells were obtained from American Type Culture Collection (ATCC) and cultured in DMEM medium. Cells were incubated at 37 °C in a 5% CO<sub>2</sub>-containing humidified incubator.

**ATP-Glo cell viability assay.** Cells (1,000 cells/well) were seeded in 96-well white microplates and incubated at 37 °C in a 5% CO<sub>2</sub>-containing humidified incubator. After 16 h of incubation, the cells were treated with or without various concentrations of peptide. After 24 h of treatment, the cell viability was analyzed using ATP-Glo cell viability assay kit (Promega, Madison, WI, USA). Measurements were performed according to the manufacturer's protocol using a Luminometer (Perkin Elmer, Meriden, CT, USA).

**Statistical Analysis.** All grouped data are presented as mean ± S.E.M. Differences between groups were analyzed by Student's t-test or ANOVA using GraphPad Prism software (GraphPad Software, Inc, La Jolla, CA, USA). All experiments were repeated in at least duplicate with triplicate technical replicates. P values less than 0.05 was considered statically significant.

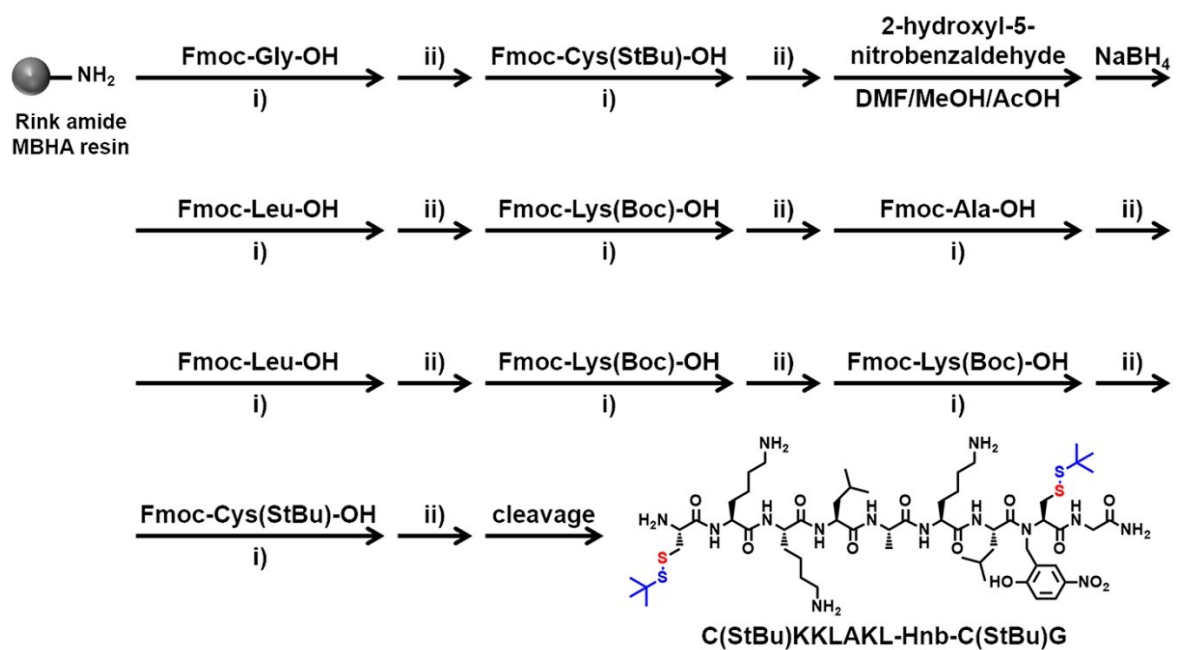


Fig. S1 Synthetic route to NCL-Pep-1 peptide.

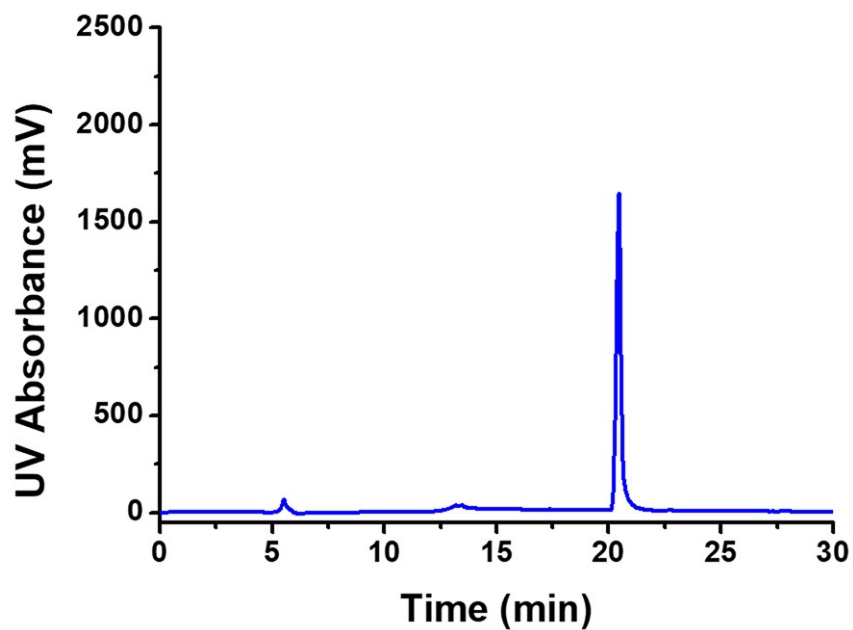


Fig. S2 HPLC chromatogram of NCL-Pep-1.

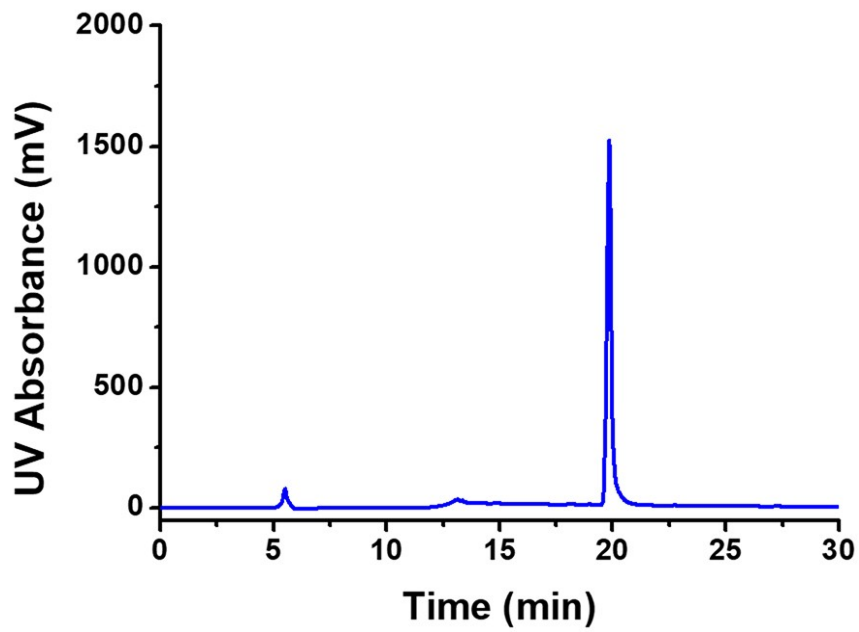


Fig. S3 HPLC chromatogram of NCL-Pep-2.

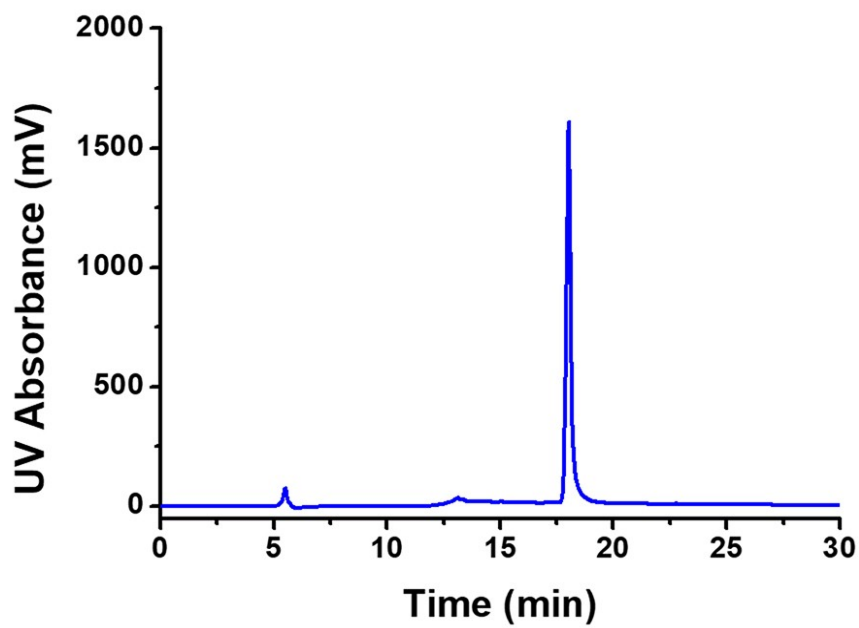


Fig. S4 HPLC chromatogram of NCL-Pep-3.

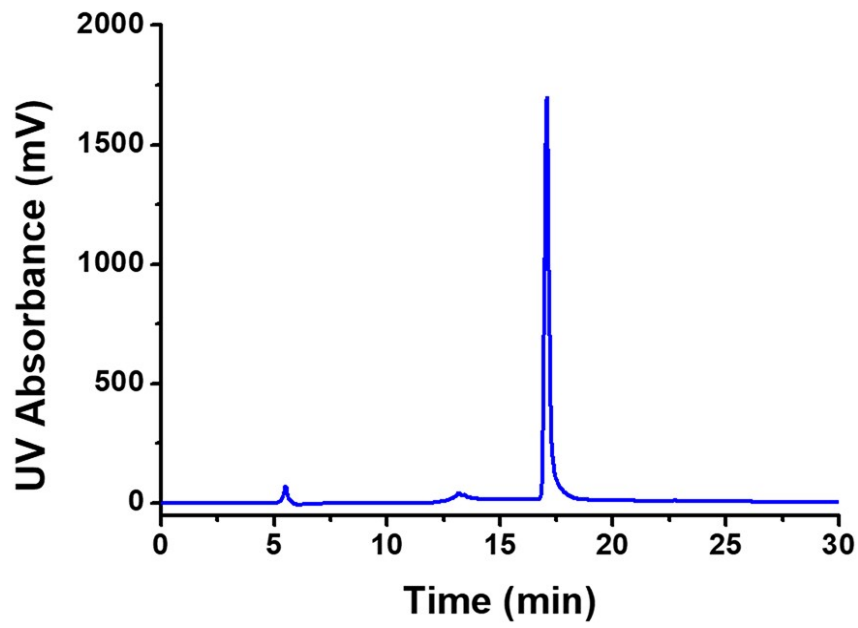


Fig. S5 HPLC chromatogram of NCL-Pep-4.

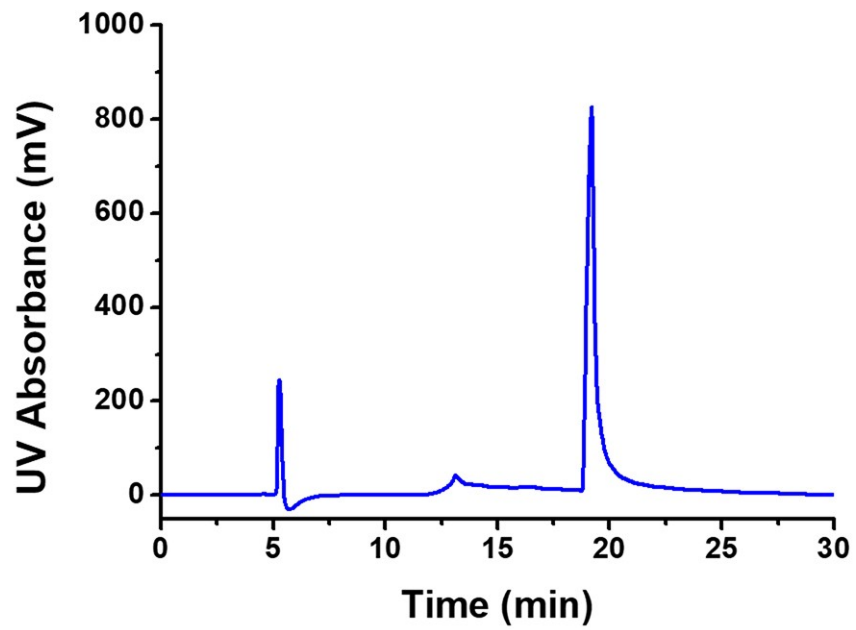
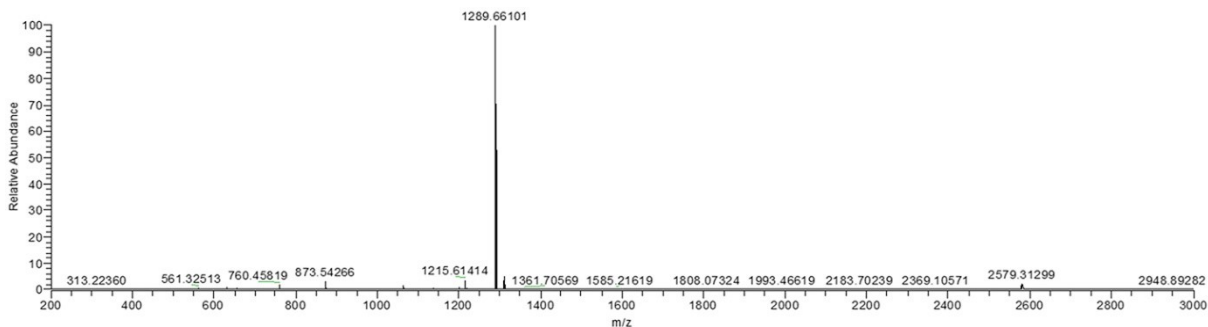
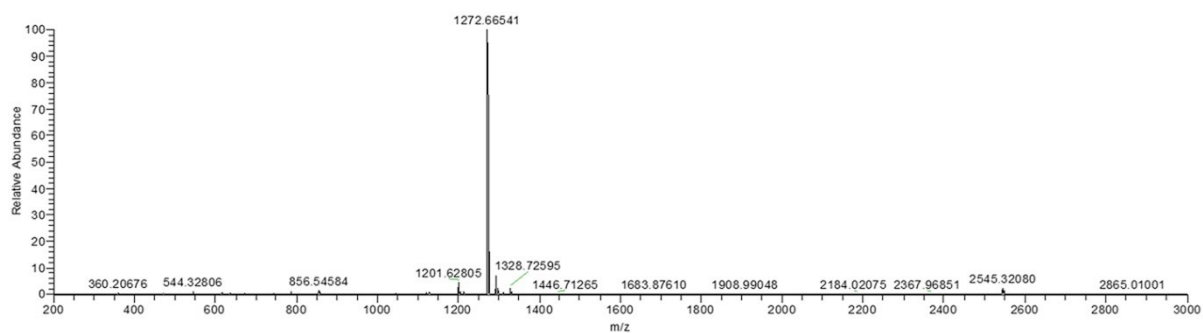


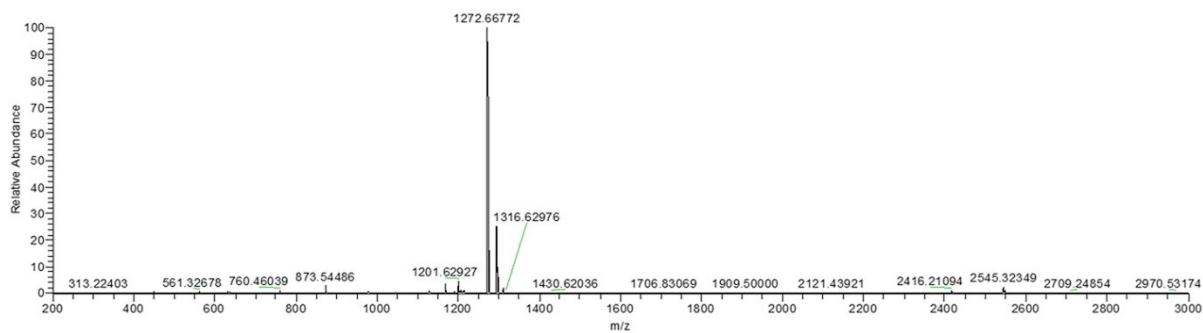
Fig. S6 HPLC chromatogram of linear (CKKLAKL)<sub>2</sub> peptide.



**Fig. S7** LC-Mass spectrum of NCL-Pep-1.



**Fig. S8** LC-Mass spectrum of NCL-Pep-2.



**Fig. S9** LC-Mass spectrum of NCL-Pep-3.

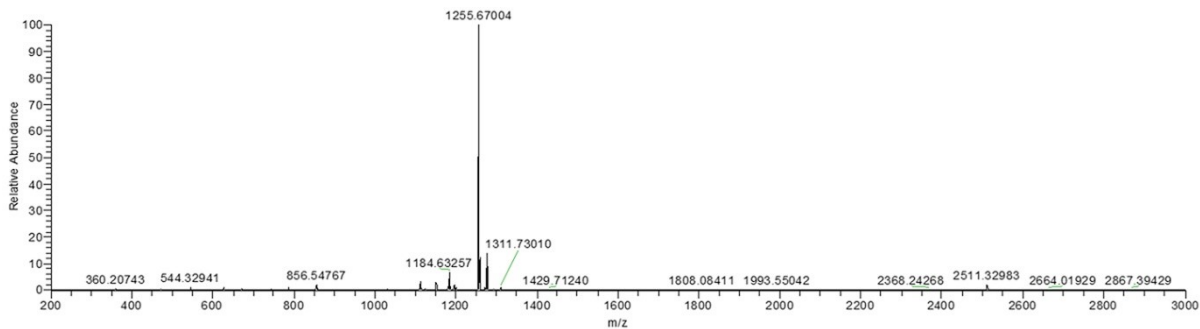


Fig. S10 LC-Mass spectrum of NCL-Pep-4.

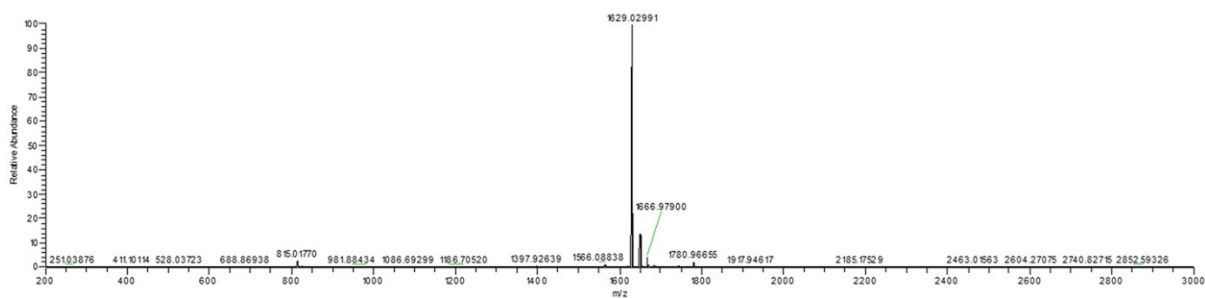


Fig. S11 LC-Mass spectrum of linear (CKKLAKL)<sub>2</sub> peptide.

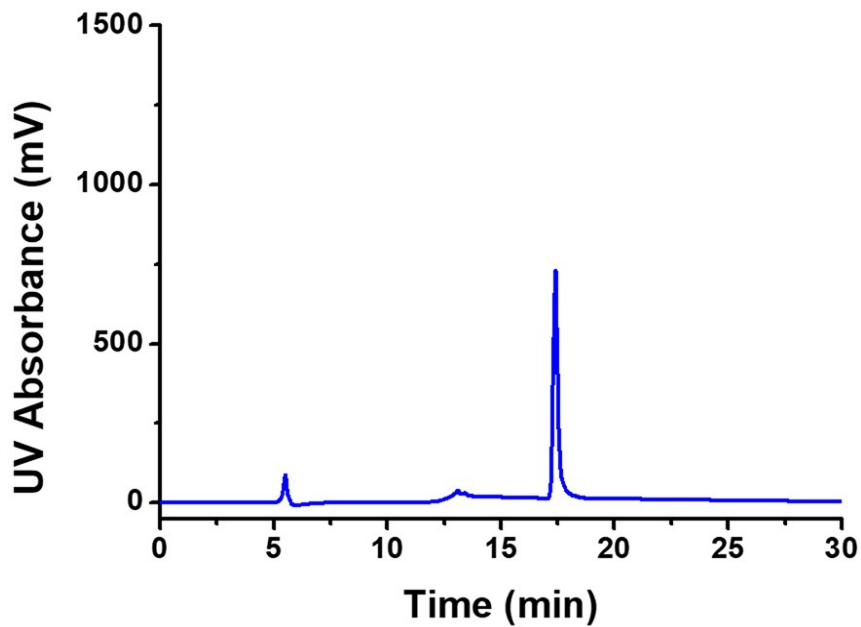


Fig. S12 HPLC chromatogram of NCL-Pep-1 after NCL reaction.

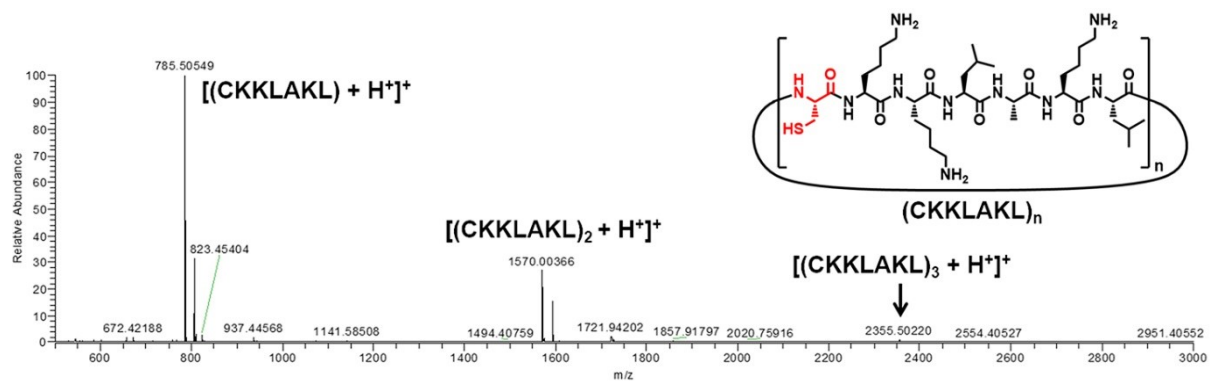


Fig. S13 LC-Mass spectrum of NCL-Pep-1 after NCL reaction.