

*Supplementary materials*

## **Chemical and Pharmaceutical Bulletin**

Hydrazide-Mediated Solubilizing Strategy for Poorly Soluble Peptides Using  
a Dialkoxybenzaldehyde Linker

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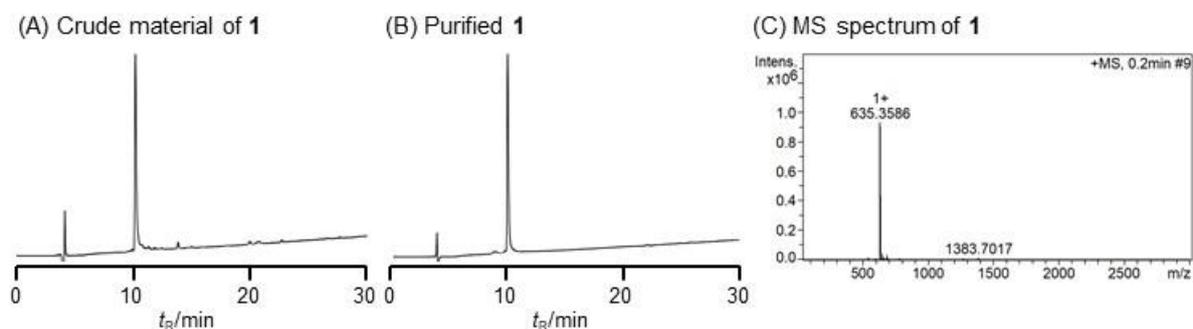
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## General information

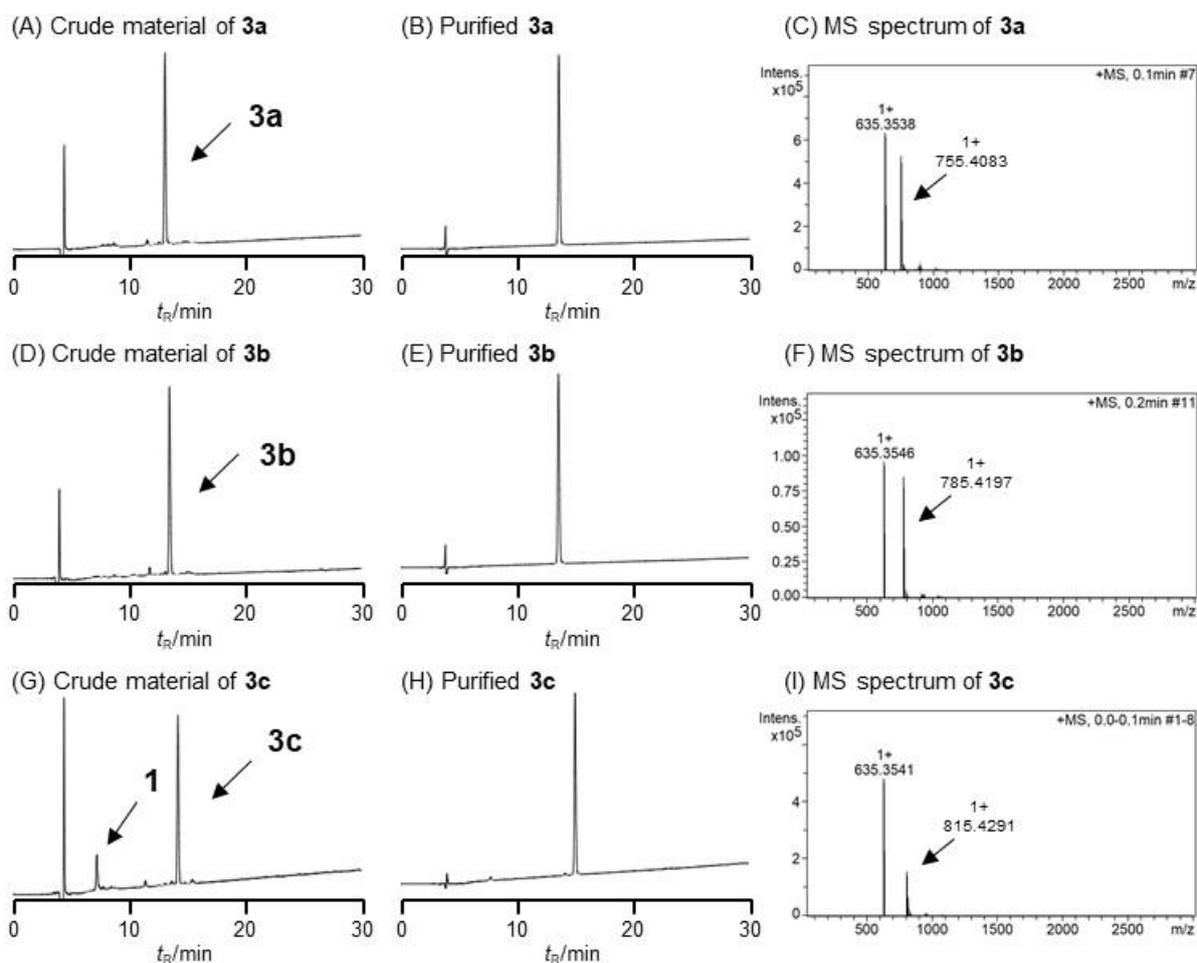
HPLC separations used a Cosmosil 5C<sub>8</sub>-AR-300 analytical column (Nacalai Tesque, 4.6 × 250 mm, flow rate 1.0 mL/min), a Cosmosil 5C<sub>18</sub>-AR-II analytical column (Nacalai Tesque, 4.6 × 250 mm, flow rate 1.0 mL/min), a Cosmosil Protein-R analytical column (Nacalai Tesque, 4.6 × 250 mm, flow rate 1.0 mL/min), a Cosmosil 5C<sub>8</sub>-AR-300 preparative column (Nacalai Tesque, 20 × 250 mm, flow rate 8.0 mL/min), a Cosmosil 5C<sub>18</sub>-AR-II preparative column (Nacalai Tesque, 20 × 250 mm, flow rate 8.0 mL/min), or a Cosmosil Protein-R semi-preparative column (Nacalai Tesque, 10 × 250 mm, flow rate 3.0 mL/min), and the eluted products were detected by UV at 220 nm.

Trityl-OH ChemMatrix resin was purchased from Biotage Japan. Dry dichloromethane (DCM), dry *N,N*-dimethylformamide (DMF), methanol (MeOH), *n*-hexane, ethyl acetate (EtOAc), acetic acid (AcOH), tetrahydrofuran (THF), dimethyl sulfoxide (DMSO), 4-anisaldehyde, sodium nitrite (NaNO<sub>2</sub>), disodium hydrogen phosphate (Na<sub>2</sub>HPO<sub>4</sub>), *N,N*-diisopropylethylamine (DIPEA) and sodium *N,N*-diethyldithiocarbamate trihydrate were purchased from Kanto Chemical. 9-Fluorenylmethyl carbazate, trifluoroacetic acid (TFA), triisopropylsilane (TIS), *m*-cresol, 2-picoline-borane complex (pic-BH<sub>3</sub>), sodium 2-mercaptoethanesulfonate (MESNa), piperidine, tetrakis(triphenylphosphine)palladium(0) (Pd(PPh<sub>3</sub>)<sub>4</sub>), phenylsilane (PhSiH<sub>3</sub>), 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), ethyl 4-bromobutyrate, 2,4-dimethoxybenzaldehyde and 2,4,6-trimethoxybenzaldehyde were purchased from Tokyo Chemical Industry. DMF, diethyl ether (Et<sub>2</sub>O), acetonitrile (CH<sub>3</sub>CN), tris(2-carboxyethyl)phosphine hydrochloride (TCEP·HCl), 2,2'-azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride (VA-044), 4-formyl-3-methoxyphenol, cesium carbonate (Cs<sub>2</sub>CO<sub>3</sub>), sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>), 6 mol/L hydrochloric acid, (+)-biotin and benzamide were purchased from Fuji Film Wako Pure Chemical Industries. Thioanisole, guanidine hydrochloride (Gn·HCl) and *O*-methylhydroxylamine hydrochloride (MeONH<sub>2</sub>·HCl) were purchased from Nacalai Tesque. *N,N'*-Diisopropylcarbodiimide (DIPCI) and Boc-Cys(Trt)-OH were purchased from Watanabe Chemical Industries. 4-Mercaptophenylacetic acid (MPAA) and methyl thioglycolate were purchased from Sigma-Aldrich. Ethyl cyanohydroxyiminoacetate (Oxyma), Fmoc-Leu-Thr(Ψ<sup>Me,Me</sup>pro)-OH, Fmoc-Ile-Thr(Ψ<sup>Me,Me</sup>pro)-OH, Fmoc-Leu-Ser(Ψ<sup>Me,Me</sup>pro)-OH, Fmoc-Ser-Thr(Ψ<sup>Me,Me</sup>pro)-OH and Rink Amide AM resin (100-200 mesh) were purchased from Merck. Sodium hydroxide (NaOH) and thionyl chloride were purchased from Kishida Chemical. Fmoc-Ala-OH·H<sub>2</sub>O and Fmoc-Met-OH were purchased from Bachem. Fmoc-Cys(Trt)-OH, Fmoc-Asp(*O**t*-Bu)-OH, Fmoc-Glu(*O**t*-Bu)-OH, Fmoc-Phe-OH, Fmoc-Gly-OH, Fmoc-His(Boc)-OH, Fmoc-Ile-OH, Fmoc-Lys(Boc)-OH, Fmoc-Leu-OH, Fmoc-Asn(Trt)-OH, Fmoc-Pro-OH, Fmoc-Gln(Trt)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Ser(*t*-Bu)-OH, Fmoc-Thr(*t*-Bu)-OH, Fmoc-Val-OH, Fmoc-Tyr(*t*-Bu)-OH and Fmoc-Lys(Alloc)-OH were purchased from CEM. 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate (HATU) was purchased from Funakoshi.

## HPLC and MS analyses of model compounds

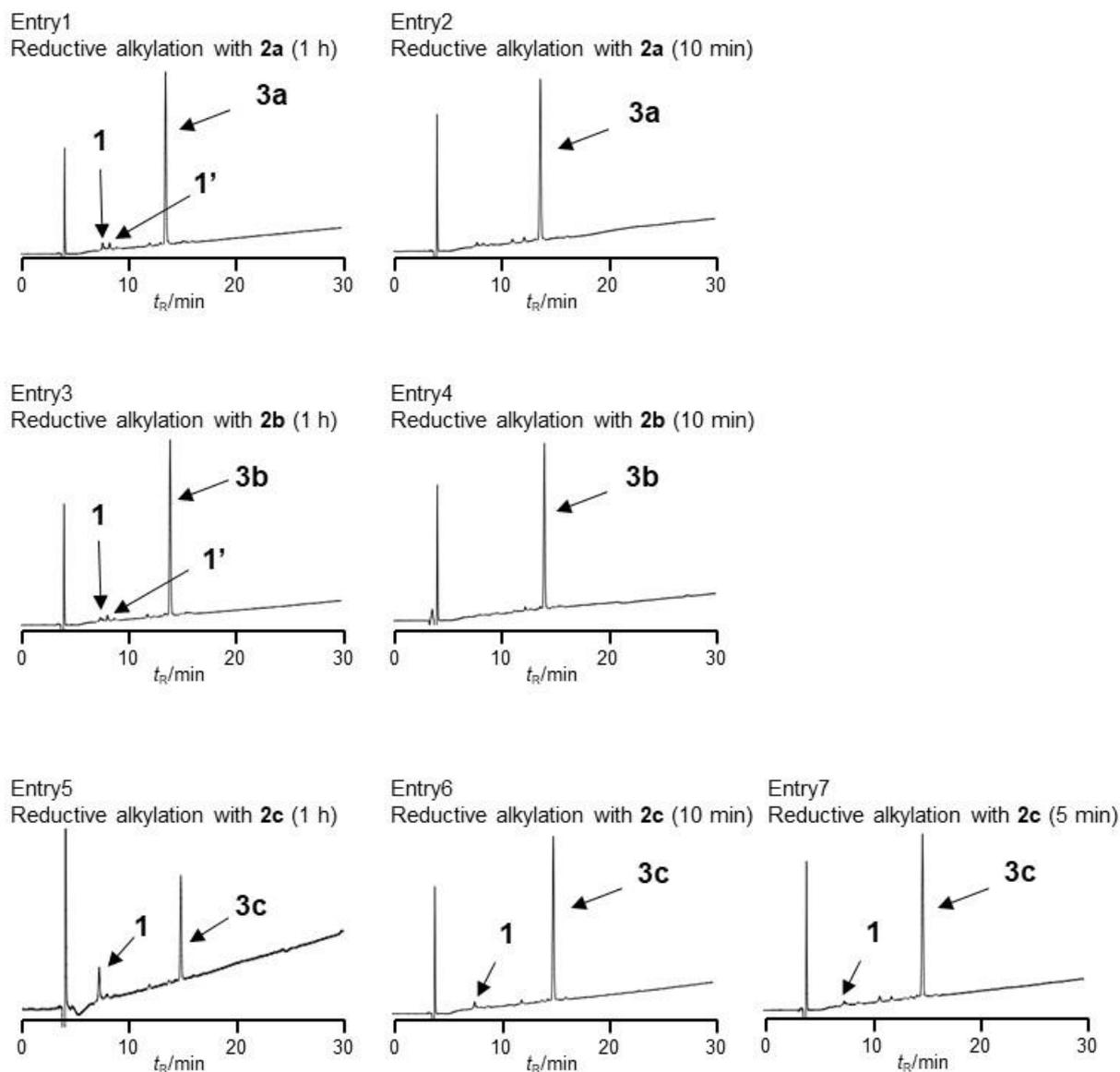


**Figure S1.** Analytical HPLC of peptide hydrazide **1** using a Cosmosil 5C<sub>8</sub>-AR-300 analytical column with the linear gradient of solvent B in solvent A, 10% to 60% over 30 min. (A) crude material of **1**, (B) purified **1** and (C) MS spectrum of **1**.

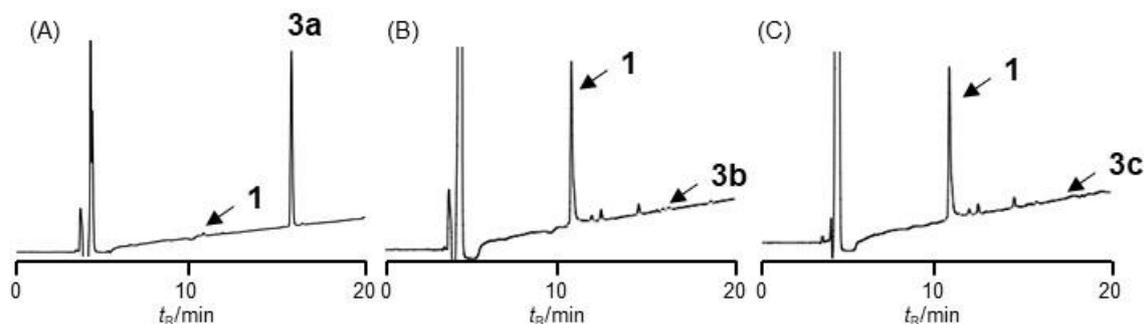


**Figure S2.** Analytical HPLC of reductive alkylation using a Cosmosil 5C<sub>8</sub>-AR-300 analytical column with the linear gradient of solvent B in solvent A, 15% to 50% over 30 min. (A) crude **3a**; (B) purified **3a**; (C) MS spectrum of **3a**; (D) crude material of **3b**; (E) purified **3b**; (F) MS spectrum of **3b**; (G) crude material of **3c**; (H) purified **3c**; (I) mass spectrum of **3c**.

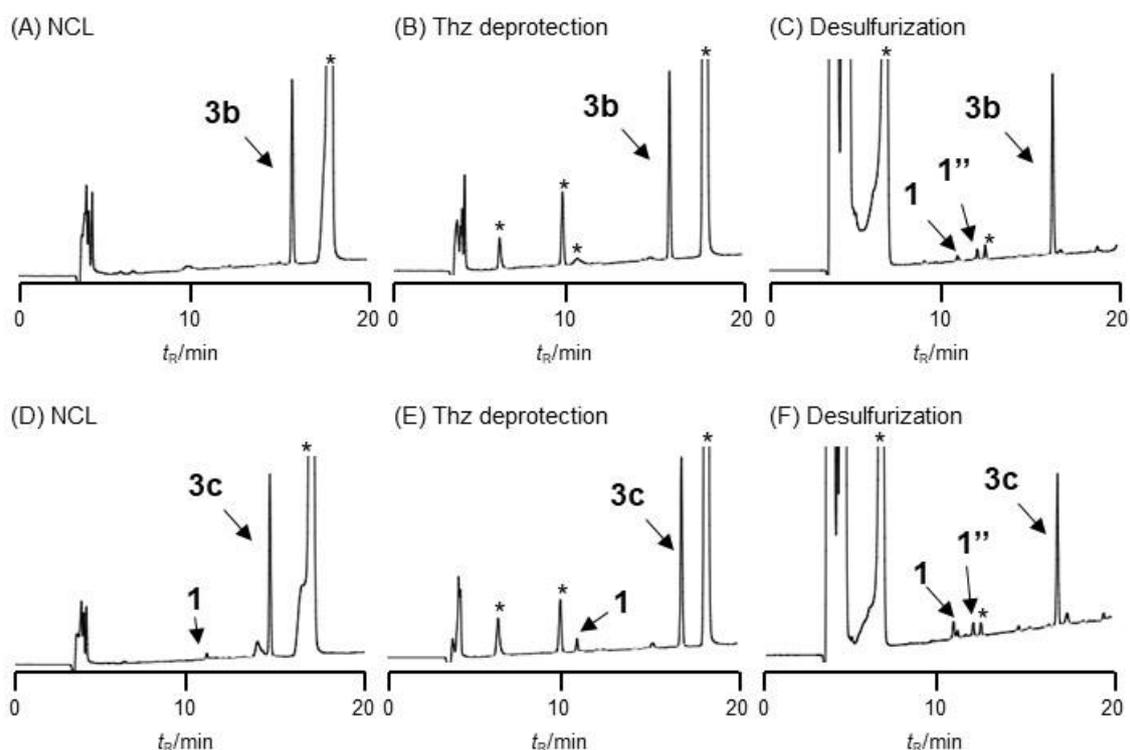
## Analytical HPLC of model reactions



**Figure S3.** Analytical HPLC of reductive alkylation between model peptide **1** and aldehyde **2** using a Cosmosil 5C<sub>8</sub>-AR-300 analytical column with the linear gradient of solvent B in solvent A, 15% to 50% over 30 min. **1'**: Ac-LYRAG-NH<sub>2</sub> (retention time: 8.4 min; obs [M+H]<sup>+</sup> = 620.3 (calc [M+H]<sup>+</sup> = 620.4)).



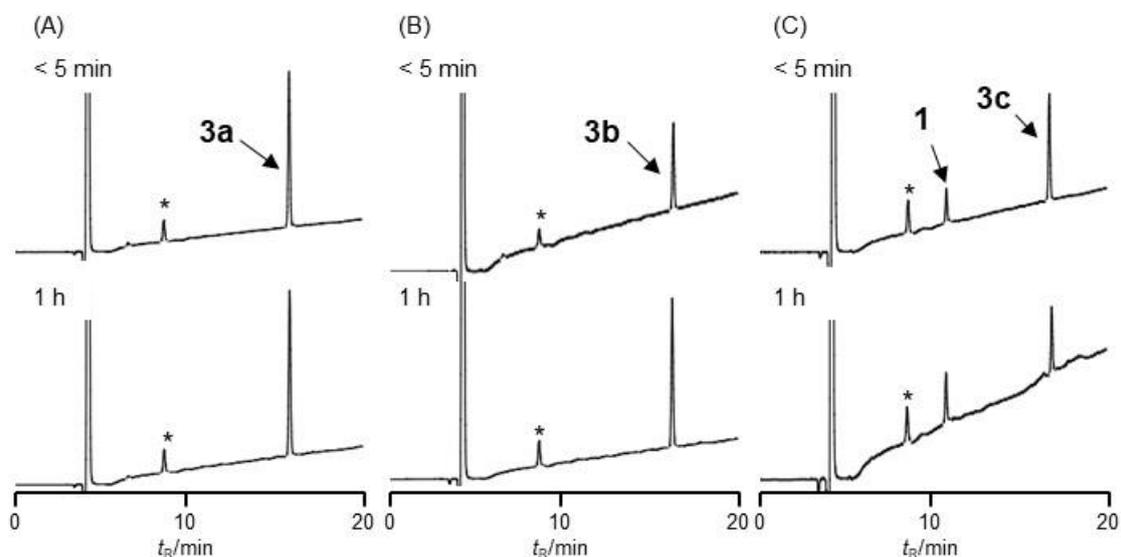
**Figure S4.** Analytical HPLC of tag removal using a Cosmosil 5C<sub>8</sub>-AR-300 analytical column with the linear gradient of solvent B in solvent A, 10% to 40% over 20 min. (A) After 1 h using **3a**; (B) After 1 h using **3b**; (C) After 1 h using **3c**.



**Figure S5.** Analytical HPLC of stability check of N-alkyl hydrazide peptide using a Cosmosil 5C<sub>8</sub>-AR-300 analytical column with the linear gradient of solvent B in solvent A, 10% to 40% over 20 min. (A) NCL 12 h, (B) Thz deprotection 12 h, (C) desulfurization 12 h using **3b**; (D) NCL 12 h, (E) Thz deprotection 12 h, (F) desulfurization 12 h using **3c**. **1''**: Ac-LYRAG-OH (retention time: 12.0 min; obs [M+H]<sup>+</sup> = 621.3 (calc [M+H]<sup>+</sup> = 621.3)). Asterisk indicates non-peptidic compounds derived from buffer components.

### Stability of N-alkyl hydrazide peptide in 50% (v/v) AcOH-HFIP

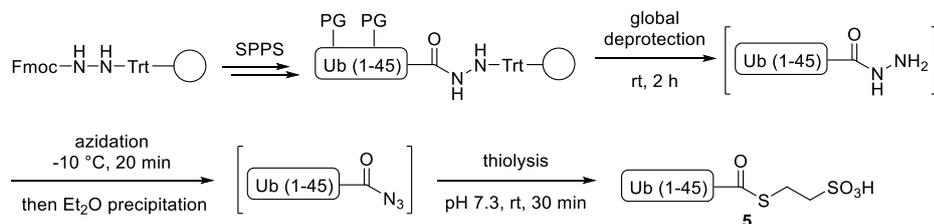
N-alkyl hydrazide peptide **3** (0.10  $\mu\text{mol}$ ) was dissolved in 50% (v/v) AcOH-HFIP (100  $\mu\text{L}$ ) and incubated at 37  $^{\circ}\text{C}$ . An aliquot of the reaction mixture was analyzed by HPLC.



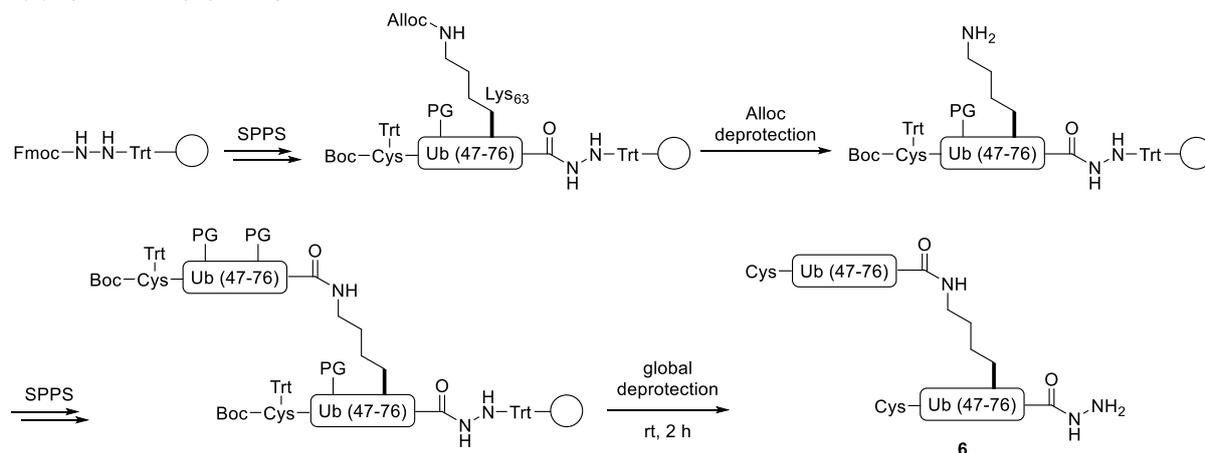
**Figure S6.** Analytical HPLC of stability check of N-alkyl hydrazide to AcOH-HFIP solution using a Cosmosil 5C<sub>8</sub>-AR-300 analytical column with the linear gradient of solvent B in solvent A, 10% to 40% over 20 min. (A) Stability of **3a**; (B) Stability of **3b**; (C) Stability of **3c**. Asterisk indicates benzamide used as an internal standard.

## HPLC and MS analyses for synthesis of Lys<sub>63</sub>-linked di-ubiquitin (Ub) derivative

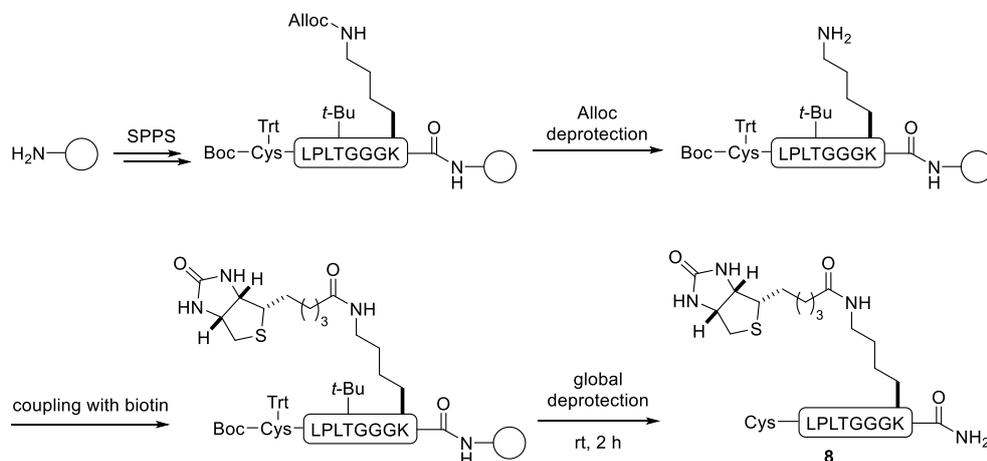
### (A) Synthesis of peptide thioester **5**



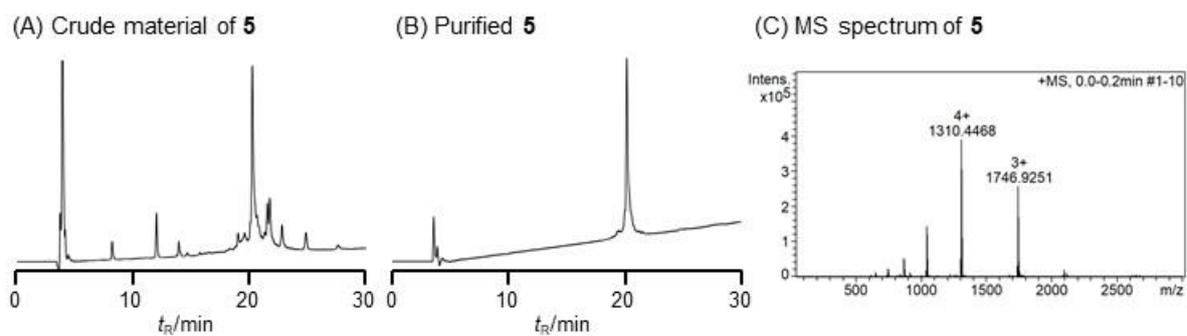
### (B) Synthesis of peptide hydrazide **6**



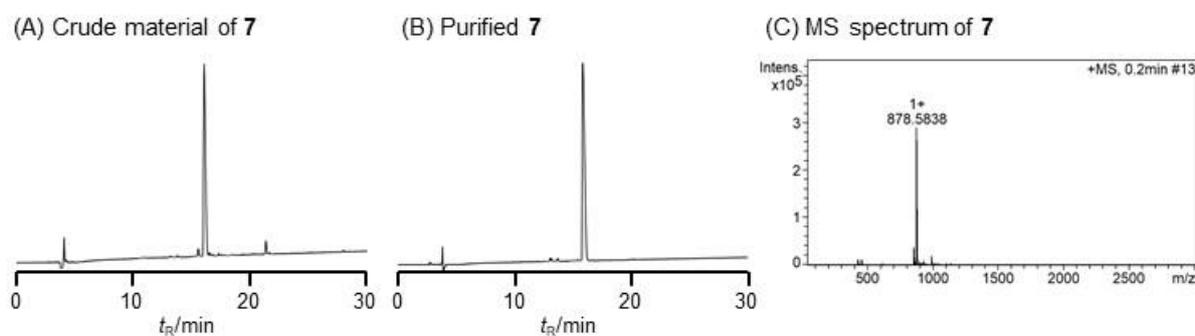
### (C) Synthesis of biotin peptide **8**



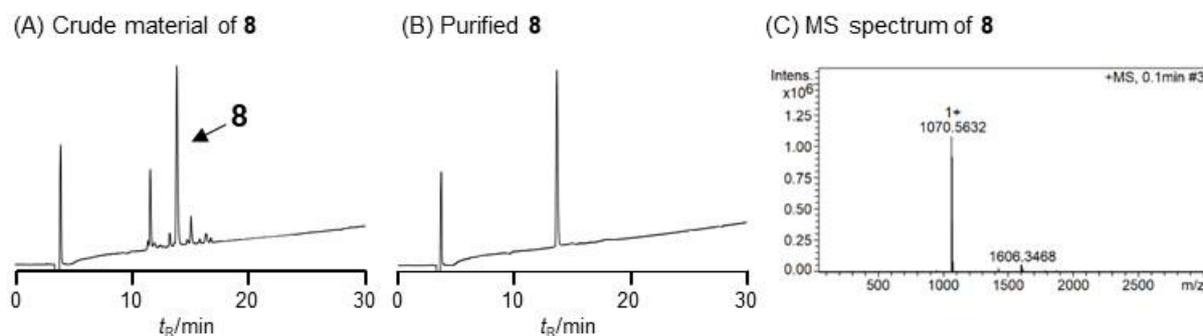
**Chart S1.** Synthesis of peptide fragments for Lys<sub>63</sub>-branched di-ubiquitin derivative. PG: protecting groups.



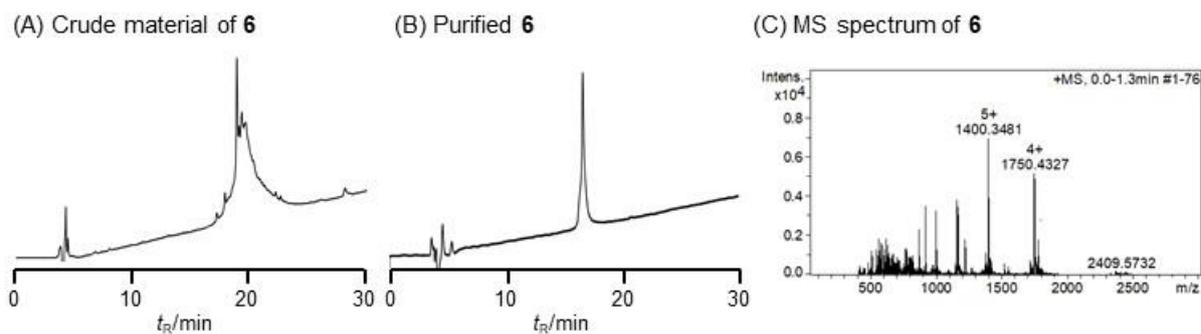
**Figure S7.** Analytical HPLC of peptide thioester **5** using a Cosmosil Protein-R analytical column with the linear gradient of solvent B in solvent A, 10% to 60% over 30 min. (A) crude **5**, (B) purified **5** and (C) MS spectrum of **5**.



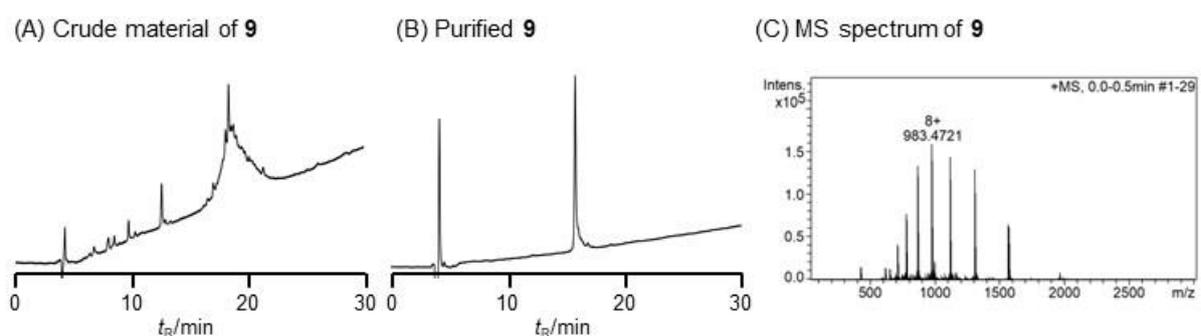
**Figure S8.** Analytical HPLC of solubilizing tag **7** using a Cosmosil 5C<sub>18</sub>-AR-II analytical column with the linear gradient of solvent B in solvent A, 5% to 35% over 30 min. (A) crude **7**, (B) purified **7** and (C) MS spectrum of **7**.



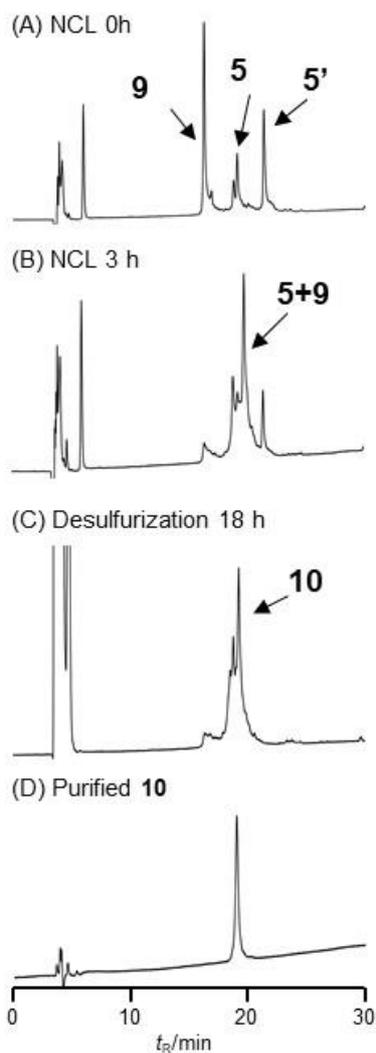
**Figure S9.** Analytical HPLC of peptide **8** using a Cosmosil 5C<sub>18</sub>-AR-II analytical column with the linear gradient of solvent B in solvent A, 10% to 60% over 30 min. (A) crude **8**, (B) purified **8** and (C) MS spectrum of **8**.



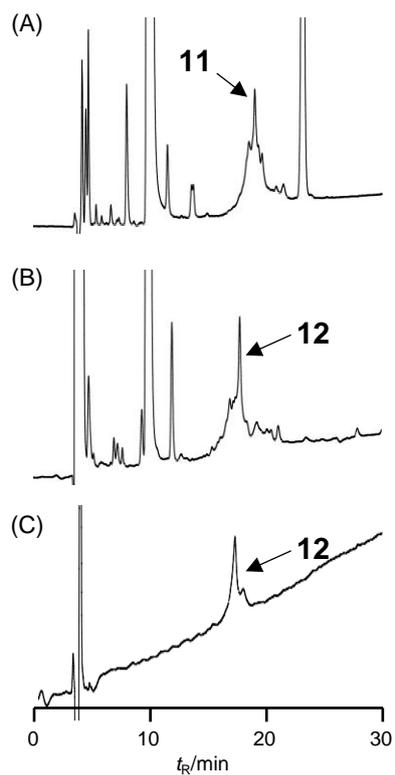
**Figure S10.** Analytical HPLC of peptide **6** using a Cosmosil Protein-R analytical column with the linear gradient of solvent B in solvent A, 10% to 60% over 30 min. (A) crude **6**, (B) purified **6** and (C) MS spectrum of **6**.



**Figure S11.** Analytical HPLC of peptide **9** using a Cosmosil Protein-R analytical column with the linear gradient of solvent B in solvent A, 10% to 60% over 30 min. (A) Reductive *N*-alkylation ( $t = 10$  min), (B) purified **9** and (C) MS spectrum of **9**.

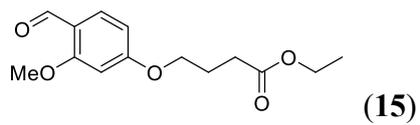


**Figure S12.** Analytical HPLC of NCL of **5** and **9** followed by desulfurization using a Cosmosil Protein-R analytical column with the linear gradient of solvent B in solvent A, 20% to 50% over 30 min. (A) NCL ( $t = <5$  min). (B) NCL ( $t = 3$  h), ligated **5+9** (retention time: 19.8 min; obs  $[M+15H]^{15+} = 1204.0$  (calc  $[M+15H]^{15+} = 1203.7$ )). (C) desulfurization ( $t = 18$  h). (D) Peptide **10** after purification. **5'**: exchanged thioester to methyl thioglycolate (retention time: 21.4 min; obs  $[M+4H]^{4+} = 1300.7$  (calc  $[M+4H]^{4+} = 1300.7$ )).

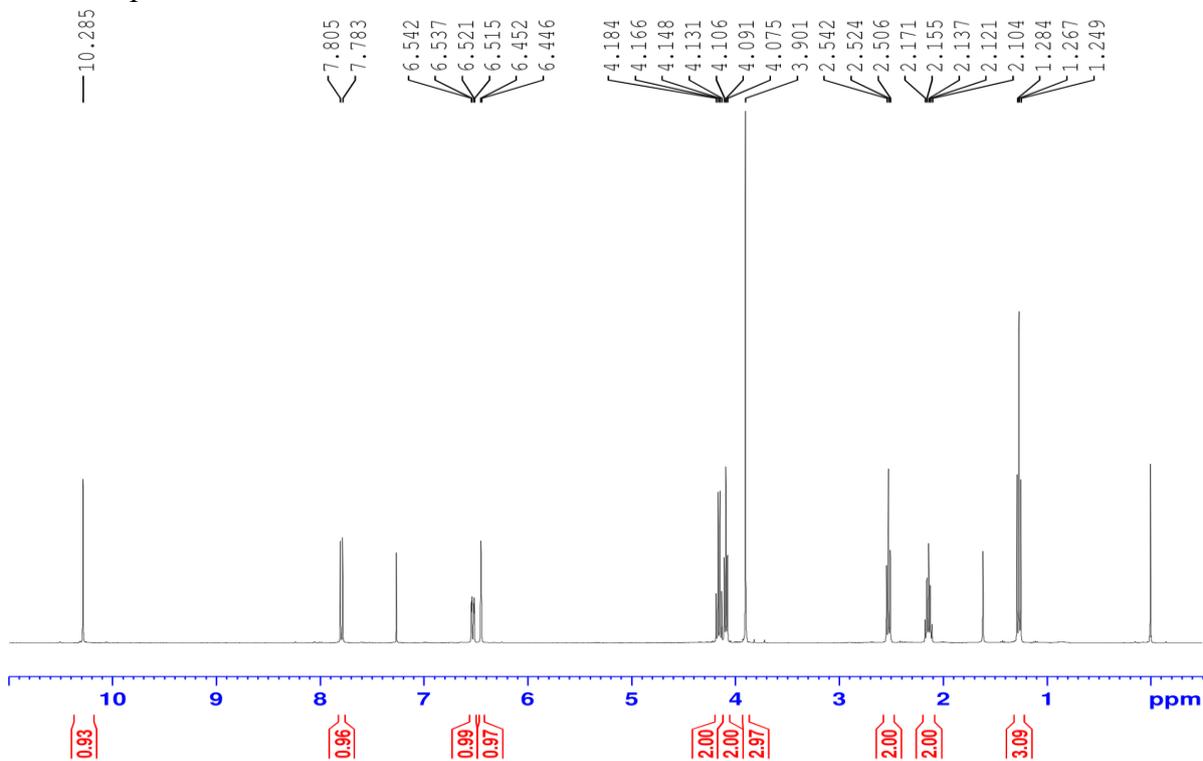


**Figure S13.** Analytical HPLC of tag removal of **10** followed by NCL with **8** using a Cosmosil Protein-R analytical column with the linear gradient of solvent B in solvent A, 25% to 45% over 30 min). (A) Tag removal ( $t = 1$  h). (B) NCL ( $t = 5$  h). (C) purified **12**.

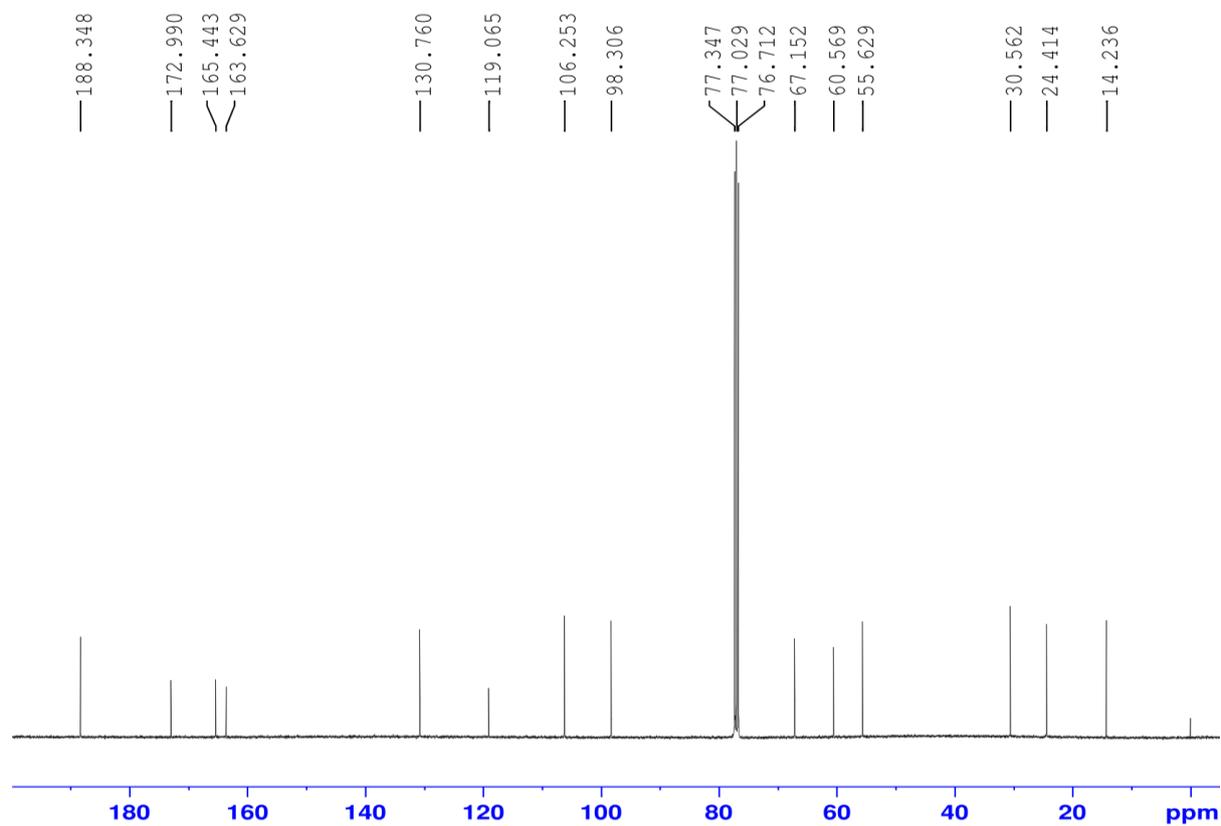
# <sup>1</sup>H and <sup>13</sup>C NMR spectra

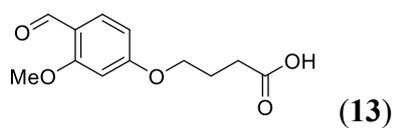


## <sup>1</sup>H NMR spectrum of 15

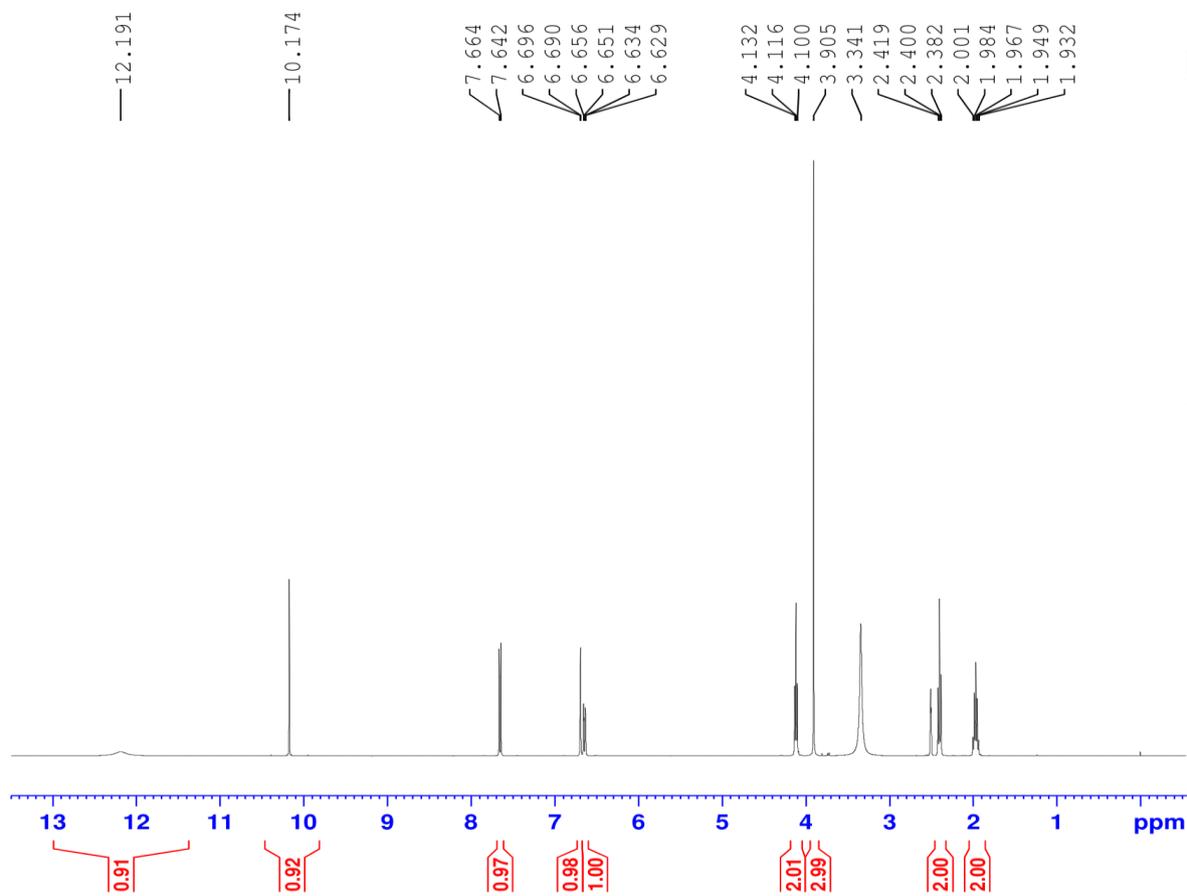


## <sup>13</sup>C NMR spectrum of 15





<sup>1</sup>H NMR spectrum of **13**



<sup>13</sup>C NMR spectrum of **13**

