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< Supplementary Information >

Nonspecific Protein Labeling of Photoaffinity Linkers Correlates with Their Molecular Shapes in Living Cells

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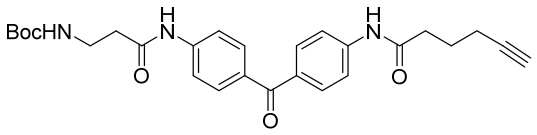
1. General Information for Synthesis

The ^{1}H and ^{13}C NMR spectra were recorded on a Varian DD2MR400 and a Varian Inova-500 [Varian Assoc., USA], and chemical shifts were measured in ppm downfield from internal tetramethylsilane (TMS) standard or specific solvent signal. Multiplicity was indicated as follows: s (singlet); d (doublet); t (triplet); q (quartet); m (multiplet); dd (doublet of doublet); dt (doublet of triplet); td (triplet of doublet); qd (quartet of doublet); quind (quintet of doublet); brs (broad singlet), etc. Coupling constants were reported in Hz. Low resolution mass spectrometry (LRMS) analyses were performed with Finnigan MSQ Plus Surveyer HPLC/MS system [Thermo Electron Corp., USA] using electron spray ionization (ESI). Analytical thin-layer chromatography (TLC) was performed using 0.25-mm silica-gel-coated Kiselgel 60 F254 plates, and the components were visualized by observation under UV light (254 and 365 nm) or by treating TLC plates with anisaldehyde, potassium permanganate or phosphomolybdic acid followed by thermal visualization. Silica gel 60 (0.040–0.063 mm) used in flash column chromatography was purchased from Merck. All reactions were conducted in oven-dried glassware under dry argon atmosphere, unless otherwise specified. All solvents and organic reagents were purchased from commercial venders [Sigma-Aldrich, TCI, and Alfa Aesar] and used without further purification unless otherwise mentioned.

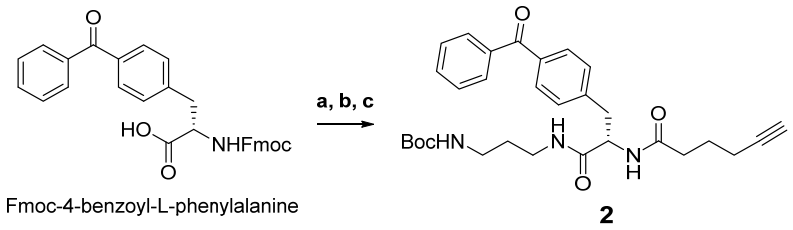
2. Preparation of Benzophenone Linkers – compound **1, 2**

■ *tert*-Butyl (3-((4-(4-(hex-5-ynamido)benzoyl)phenyl)amino)-3-oxopropyl)carbamate (**1**)



The synthesis of **1** was performed using previously reported procedure.[1]

Scheme S1. Synthesis of Benzophenone Linker **2**



Reagents and conditions: (a) *N*-Boc-1,3-propanediamine, HATU, DIPEA, DMF, 0 °C, 1 h; (b) Piperidine, DMF, r.t., 30 min; (c) 5-Hexynoic acid, HATU, DIPEA, DMF, r.t., 1 h, overall yield: 31%.

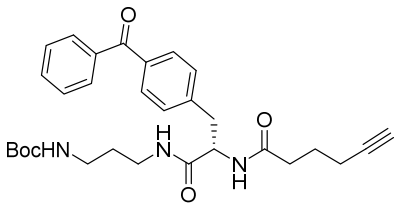
(Step **a)** To a 3 mL anhydrous DMF solution of Fmoc-4-benzoyl-L-phenylalanine (0.41 mmol, 1 equiv.) and HATU (1.2 equiv.), *N*-Boc-1,3-propanediamine (1.1 equiv.) and DIPEA (1.5 equiv.) were added at 0 °C. The reaction mixture was stirred at 0 °C for 1 h. Then, the reaction solution was diluted with ethyl acetate and water. The aqueous layer was extracted three times with ethyl acetate. The organic layer was evaporated under reduced pressure to provide the crude amine product. The combined organic layer was dried over anhydrous $MgSO_4$(s) and filtered through a celite-packed glass filter. The filtrate was concentrated *in vacuo* and was directly for next reaction without further purification.

(Step **b)** To a solution of the resulting crude product in DCM (5 mL), excess amount of piperidine (1 mL) was added. The solution was stirred at room temperature for 1 h. After completion of the reaction, the reaction mixture was concentrated *in vacuo* and purified with silica-gel flash column chromatography

(DCM:methanol = 7:1) to provide the desired Fmoc-deprotected product. (R_f=0.44 at DCM:methanol = 8:1)

(Step **c)** To a resulting amine product of step **b** and 5-hexynoic acid (2 equiv.) in DMF (5 mL), HATU (1.5 equiv.) and diisopropylethylamine (3 equiv.) were added. The reaction mixture was stirred for 1 h at room temperature. Then, the reaction solution was diluted with ethyl acetate and water. The aqueous layer was extracted three times with ethyl acetate. The combined organic layer was dried over anhydrous $MgSO_4$(s) and filtered through a celite-packed glass filter. The filtrate was concentrated *in vacuo* and purified with silica-gel flash column chromatography to provide the desired product **2**. (3-step yield: 31%)

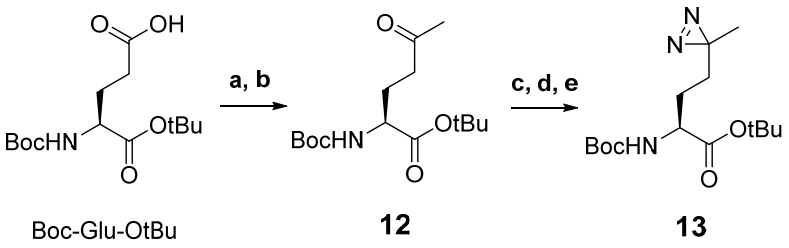
■ *tert*-Butyl (*S*)-(3-(3-(4-benzoylphenyl)-2-(hex-5-ynamido)propanamido)propyl)carbamate (**2**)



R_f=0.68 (DCM:methanol = 8:1, v/v); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.69–7.75 (m, 4H), 7.56 (t, *J* = 7.5 Hz, 1H), 7.45 (t, *J* = 7.8 Hz, 1H), 7.31 (d, *J* = 8.5 Hz, 1H), 7.01 (brs, 1H), 6.59 (d, *J* = 7.5 Hz, 1H), 4.91 (brs, 1H), 4.75 (d, *J* = 7.3 Hz, 1H), 3.09–3.28 (m, 4H), 2.91–2.99 (m, 2H), 2.32 (t, *J* = 7.3 Hz, 1H), 2.12–2.18 (m, 2H), 1.93 (t, *J* = 2.5 Hz, 1H), 1.74–1.81 (m, 2H), 1.51 (brs, 2H), 1.38 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 196.5, 172.5, 171.0, 156.8, 142.0, 137.7, 136.4, 132.6, 130.6, 130.2, 129.5, 128.5, 83.5, 79.6, 69.6, 54.4, 38.8, 37.2, 36.2, 35.0, 30.1, 28.6, 24.2, 18.0; LRMS(ESI$^+$) m/z calcd for $C_{30}H_{37}N_3O_5$ $[M+H]^+$ 520.28; Found 520.3.

3. Preparation of Diazirine Linker Precursors – compounds **12** & **13**

Scheme S2. Synthesis of Diazirine Linker Precursor **13**



Boc-Glu-OtBu **12** **13**

Reagents and conditions: (a) *N*,*O*-Dimethylhydroxylamine hydrochloride, NMM, EDC·HCl, DCM, –15 °C → r.t., 12 h; (b) Methylmagnesium bromide solution, THF, –78 °C → 0 °C, 12 h, 74%; (c) Liquid ammonia, –78 °C → r.t., 12 h; (d) Hydroxylamine-*O*-sulfonic acid, methanol, r.t., 12 h; (e) I_2, TEA, 0 °C, 2 h, 49%.

(Step **a)** To a solution of Boc-Glu-OtBu (2.0 g, 1 equiv.) in anhydrous DCM (20 mL) at –15 °C, *N*,*O*-dimethylhydroxylamine hydrochloride (1.05 equiv.) and *N*-methylmorpholine (1.05 equiv.) were added. After stirring for 5 min, EDC hydrochloride (1.05 equiv.) in DCM (10 mL) was added over 15 min. Then the reaction mixture was stirred for 12 h at room temperature. Then the reaction mixture was diluted with DCM and ddH_2O. The aqueous layer was extracted three times with DCM. The combined organic layer was dried over anhydrous $MgSO_4$(s) and filtered through a celite-packed glass filter. The filtrate was concentrated *in vacuo* and the resulting clear oil was directly for the subsequent substitution reaction without further purification.

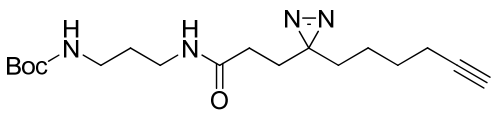
(Step **b)** To a solution of a resulting crude mixture (from step **a**) in anhydrous THF (50 mL), 3.0 M solution of methylmagnesium bromide in diethyl ether (3 equiv.) was added at –78 °C. The resulting mixture was then stirred for 3 h with the temperature warming up to 0 °C. Subsequently, the reaction was quenched by addition of water (30 mL) followed by extraction with three times with DCM. The organic layer was washed with brine. The combined organic layer was dried over anhydrous $MgSO_4$(s) and filtered through a celite-packed glass filter. The filtrate was concentrated *in vacuo* and purified with

silica-gel flash column chromatography to provide the desired product **12** (yield: 74%). NMR spectra of compound **12** was in agreement with that reported in previous literature.[2]

(Step **c, d, e)** The synthesis of **13** from **12** was performed using previously reported procedure.[2]

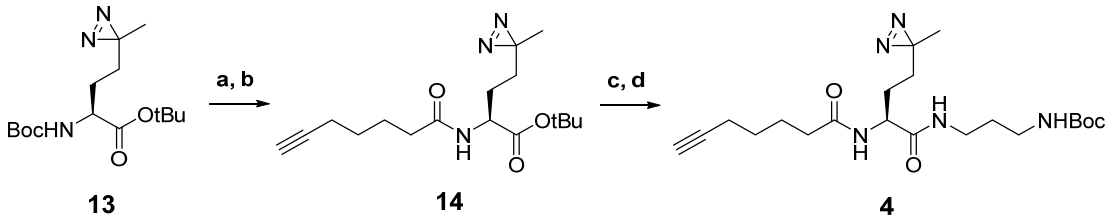
4. Preparation of Diazirine Linkers – compounds **3**, **4**, **5**, **14**, and **15**

■ *tert*-Butyl (3-(3-(3-(hex-5-yn-1-yl)-3*H*-diazirin-3-yl)propanamido)propyl)carbamate (**3**)



The synthesis of **3** was performed using the previously reported procedure.[1]

Scheme S3. Synthesis of diazirine linker **4**



Reagents and conditions: (a) HCl, dioxane, r.t., 15 min; (b) 6-Heptynoic acid, EDC·hydrochloride, HOBt, TEA, r.t., 12 h, 42%; (c) TFA, DCM, r.t., 14 h; (d) *N*-Boc-1,3-propanediamine, EDC·hydrochloride, HOBt, TEA, r.t., 12 h, 63%.

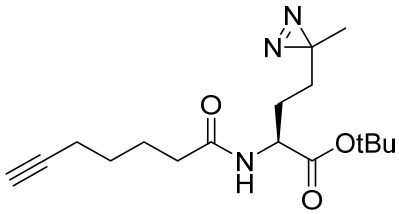
(Step **a)** To a solution of **13** (0.75 mmol, 1 equiv.) in anhydrous 1,4-dioxane (3 mL), 4 N HCl solution in 1,4-dioxane (3 mL) was added and the reaction mixture was stirred at room temperature for 15 min. Saturated aqueous bicarbonate solution was added to the solution and the mixture was extracted with DCM. The organic layer was evaporated under reduced pressure to provide crude amine product.

(Step **b)** To a resulting crude product of step **a** and 6-heptynoic acid (1.5 equiv.) in 1,4-dioxane (3 mL), EDC·hydrochloride (1.5 equiv.), hydroxybenzotriazole (1.5 equiv.), and triethylamine (6 equiv.) were added. The reaction mixture was stirred for 12 h at room temperature. Then, the reaction solution was diluted with DCM and water. The aqueous layer was extracted three times with DCM. The combined organic layer was dried over anhydrous $MgSO_4$(s) and filtered through a celite-packed glass filter. The filtrate was concentrated *in vacuo* and purified with silica-gel flash column chromatography to provide the desired product **14**. (2-step yield: 42%)

(Step **c)** Compound **14** (1 equiv.) was dissolved in 15% (v/v) trifluoroacetic acid in DCM (2 mL). The mixture was stirred at room temperature for 14 h and condensed under reduced pressure to provide substituted amine TFA salt. The resulting mixture was used directly for the subsequent amide coupling reaction without further purification.

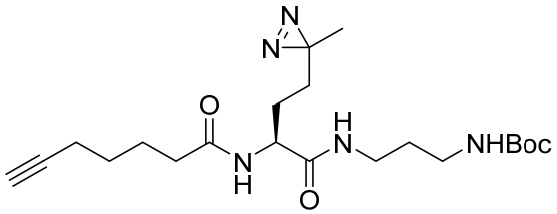
(Step **d)** To a resulting crude salt of step **c** and *N*-Boc-1,3-propanediamine (1.5 equiv.) in 1,4-dioxane (3 mL), EDC·hydrochloride (1.5 equiv.), hydroxybenzotriazole (1.5 equiv.), and triethylamine (6 equiv.) were added. The reaction mixture was stirred for 12 h at room temperature. Then, the reaction solution was diluted with DCM and water. The aqueous layer was extracted three times with DCM. The combined organic layer was dried over anhydrous $MgSO_4$(s) and filtered through a celite-packed glass filter. The filtrate was concentrated *in vacuo* and purified with silica-gel flash column chromatography to provide the desired product **4**. (2-step yield: 63%)

■ *tert*-Butyl (*S*)-2-(hept-6-ynamido)-4-(3-methyl-3*H*-diazirin-3-yl)butanoate (**14**)



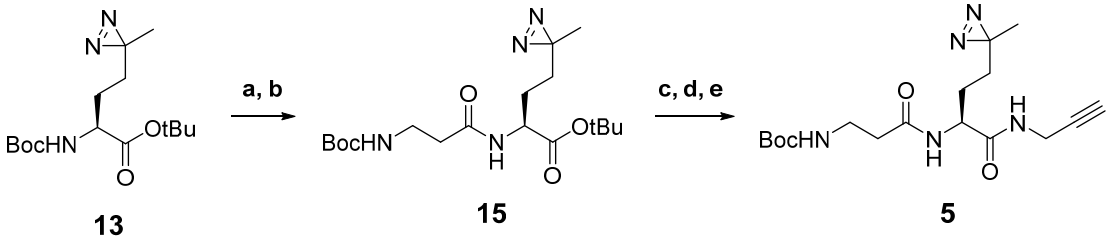
R_f = 0.30 (ethyl acetate:n-hexane = 1:3, v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.00 (d, *J* = 7.2 Hz, 1H), 4.46 (q, *J* = 6.5 Hz, 1H), 2.18–2.25 (m, 4H), 1.95 (t, *J* = 2.6 Hz, 1H), 1.66–1.80 (m, 2H), 1.38–1.60 (m, 4H), 1.44 (s, 9H), 1.21–1.31 (m, 2H), 1.00 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4, 171.3, 84.1, 82.7, 68.8, 52.0, 36.1, 30.5, 28.1, 28.0, 27.4, 25.4, 24.7, 19.7, 18.3.

■ *tert*-Butyl (*S*)-(3-(2-(hept-6-ynamido)-4-(3-methyl-3*H*-diazirin-3-yl)butanamido)propyl)carbamate (**4**)



R_f = 0.75 (DCM:methanol = 10:1, v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.07 (brs, 1H), 6.35–6.38 (m, 1H), 4.91 (brs, 1H), 4.41 (q, *J* = 6.5 Hz, 1H), 3.26 (q, *J* = 6.3 Hz, 2H), 3.10–3.14 (m, 2H), 2.17–2.26 (m, 4H), 1.95 (t, *J* = 2.4 Hz, 1H), 1.68–1.78 (m, 2H), 1.39–1.64 (m, 6H), 1.43 (s, 9H), 1.23–1.34 (m, 2H), 1.00 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.9, 171.4, 156.8, 84.1, 79.6, 68.9, 52.5, 37.2, 36.1, 36.0, 30.6, 30.1, 28.5, 28.0, 27.5, 25.5, 24.7, 19.7, 18.3; LRMS(ESI$^+$) m/z calcd for $C_{21}H_{35}N_5O_4$ $[M+H]^+$ 422.28; Found 422.28.

Scheme S4. Synthesis of diazirine linker **5**



Reagents and conditions: (a) HCl, dioxane, r.t., 15 min; (b) N-Boc-β-alanine, EDC hydrochloride, HOBt, TEA, 54%; (c) TFA, DCM, r.t., 12 h; (d) Di-*tert*-butyl dicarbonate, NaOH, water, THF, 20 h; (e) propargylamine, EDC·hydrochloride, HOBt, TEA, 1,4-dioxane, r.t., 12 h, 9%.

(Step **a)** To a solution of **13** (0.75 mmol, 1 equiv.) in anhydrous 1,4-dioxane (3 mL), 4 N HCl solution in 1,4-dioxane (3 mL) was added and the reaction mixture was stirred at room temperature for 15 min. Saturated aqueous bicarbonate solution was added to the solution and the mixture was extracted with DCM. The organic layer was evaporated under reduced pressure to provide the crude amine product.

(Step **b)** To a resulting crude product of step **a** and N-Boc-β-alanine (1.5 equiv.) in 1,4-dioxane (3 mL), EDC·hydrochloride (1.5 equiv.), hydroxybenzotriazole (1.5 equiv.), and triethylamine (6 equiv.) were added. The reaction mixture was stirred for 12 h at room temperature. Then, the reaction solution was diluted with DCM and water. The aqueous layer was extracted three times with DCM. The combined organic layer was dried over anhydrous $MgSO_4$(s) and filtered through a celite-packed glass filter. The filtrate was concentrated *in vacuo* and purified with silica-gel flash column chromatography to provide the desired product **15**. (2-step yield: 54%)

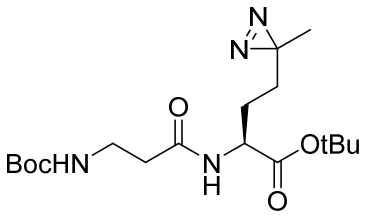
(Step **c)** Compound **15** (1 equiv.) was dissolved in 15% (v/v) trifluoroacetic acid in DCM (2 mL). The mixture was stirred at room temperature for 14 h and condensed under reduced pressure to provide substituted amine TFA salt. The resulting mixture was directly for the subsequent Boc-protection reaction without further purification.

(Step **d)** To a solution of the resulting crude salt of step **c** in water (3 mL), 2 N sodium hydroxide aqueous solution (3 mL) was added at room temperature followed by addition of di-*tert*-butyl dicarbonate (1.1 equiv.) in THF (6 mL). The reaction mixture was stirred for 20 h at room temperature and concentrated

under reduced pressure. Excess equivalent of saturated aqueous ammonium chloride solution was added to the solution and the mixture was extracted three times with DCM. The combined organic layer was dried over anhydrous $MgSO_4$(s) and filtered through a celite-packed glass filter. The filtrate was concentrated *in vacuo* to provide crude Boc-protected product.

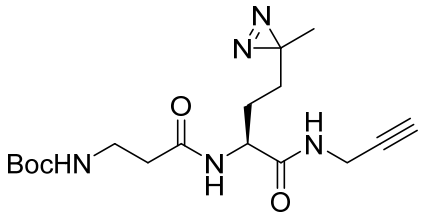
(Step **e)** To a resulting crude product of step **d** and propargylamine (1.5 equiv.) in 1,4-dioxane (3 mL), EDC·hydrochloride (1.5 equiv.), hydroxybenzotriazole (1.5 equiv.), and triethylamine (6 equiv.) were added. The reaction mixture was stirred for 12 h at room temperature. Then, the reaction solution was diluted with DCM and water. The aqueous layer was extracted three times with DCM. The combined organic layer was dried over anhydrous $MgSO_4$(s) and filtered through a celite-packed glass filter. The filtrate was concentrated *in vacuo* and purified with silica-gel flash column chromatography to provide the desired product **8**. (3-step yield: 9%)

■ *tert*-Butyl (*S*)-2-(3-((tert-butoxycarbonyl)amino)propanamido)-4-(3-methyl-3*H*-diazirin-3-yl)butanoate (**15**)



R_f = 0.56 (ethyl acetate:n-hexane = 1:1, v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.14 (d, J = 7.6 Hz, 1H), 5.12 (brs, 1H), 4.40–4.46 (m, 1H), 3.39 (brs, 2H), 2.38–2.44 (m, 2H), 1.68–1.75 (m, 1H), 1.35–1.55 (m, 20H), 1.23–1.32 (m, 1H), 1.00 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 171.1, 156.1, 82.8, 79.5, 52.1, 36.8, 36.3, 30.5, 28.5, 28.1, 27.2, 25.4, 19.7.

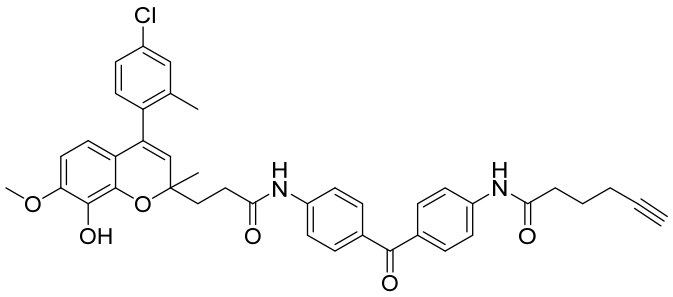
■ *tert*-Butyl (*S*)-(3-((4-(3-methyl-3*H*-diazirin-3-yl)-1-oxo-1-(prop-2-yn-1-ylamino)butan-2-yl)amino)-3-oxopropyl)carbamate (**5**)



R_f = 0.07 (ethyl acetate:n-hexane = 1:1, v/v); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.62–6.66 (m, 1H), 6.41 (brs, 1H), 5.08 (brs, 1H), 4.41 (q, *J* = 6.9 Hz, 1H), 4.02 (q, *J* = 2.7 Hz, 2H), 3.40 (q, *J* = 6.1 Hz, 2H), 2.44 (q, *J* = 5.3 Hz, 1H), 2.24 (t, *J* = 2.6 Hz, 1H), 1.43–1.52 (m, 11H), 1.24–1.37 (m, 2H), 1.02 (s, 3H); LRMS(ESI$^+$) m/z calcd for $C_{17}H_{27}N_5O_4$ $[M+H]^+$ 366.21; Found 366.17.

5. Synthesis of Photoaffinity Probes of **6** – compounds **7**, **8**, **9**, **10**, and **11**

■ *N*-(4-(4-(3-(4-(4-Chloro-2-methylphenyl)-8-hydroxy-7-methoxy-2-methyl-2*H*-chromen-2-yl)propanamido)benzoyl)phenyl)hex-5-ynamide (**7**)



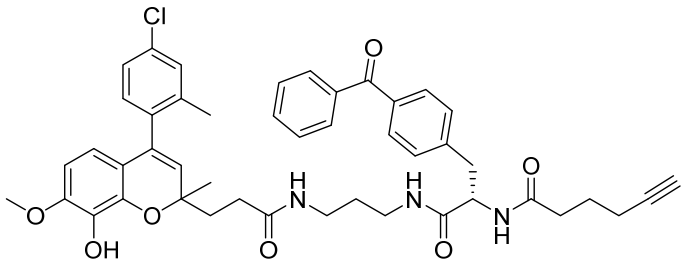
The synthesis of **7** was performed using the previously reported procedure. [3]

[General synthetic procedure for compounds **8**, **9**, **10**, and **11**]

First, compound **2**, **3**, **4**, or **5** was dissolved in 15% (v/v) trifluoroacetic acid in DCM (2 mL). The mixture was stirred at room temperature for 12 h and condensed under reduced pressure to provide crude amine TFA salt. The resulting mixture was directly for the subsequent amide coupling reaction without further purification.

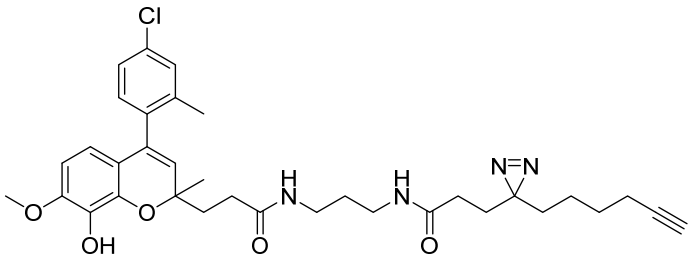
To a resulting crude amine salt (1.5 equiv.) of previous step and 3-(4-(4-chloro-2-methylphenyl)-8-hydroxy-7-methoxy-2-methyl-2*H*-chromen-2-yl)propanoic acid (1 equiv.)[3] in 1,4-dioxane (3 mL), EDC·hydrochloride (1.5 equiv.), hydroxybenzotriazole (1.5 equiv.), and triethylamine (6 equiv.) were added. The reaction mixture was stirred for 12 h at room temperature. Then, the reaction solution was diluted with DCM and water. The aqueous layer was extracted three times with DCM. The combined organic layer was dried over anhydrous $MgSO_4$(s) and filtered through a celite-packed glass filter. The filtrate was concentrated *in vacuo* and purified with silica-gel flash column chromatography to provide the desired product **8** (yield: 78%), **9** (yield: 59%), **10** (yield: 57%), or **11** (yield: 66%). In case of **10** and **11**, one single diastereomer was purified by normal-phase HPLC for NMR spectrum.

■ *N*-((2*S*)-3-(4-Benzoylphenyl)-1-((3-(3-(4-(4-chloro-2-methylphenyl)-8-hydroxy-7-methoxy-2-methyl-2*H*-chromen-2-yl)propanamido)propyl)amino)-1-oxopropan-2-yl)hex-5-ynamide (**8**)



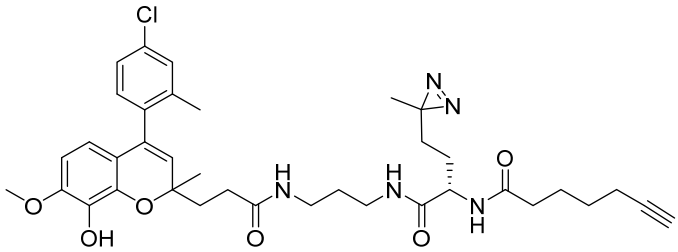
R_f = 0.41 (DCM:methanol = 10:1, v/v); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68–7.75 (m, 4H), 7.56 (t, *J* = 7.5 Hz, 1H), 7.44 (td, *J* = 7.6, 1.7 Hz, 2H), 7.32 (d, *J* = 8.5 Hz, 2H), 7.12–7.23 (m, 2H), 6.98–7.07 (m, 2H), 6.38–6.66 (m, 2H), 6.30–6.32 (m, 1H), 6.05–6.08 (m,1H), 5.29 (t, *J* = 6.5 Hz, 1H), 4.74–4.79 (m, 1H), 3.81 (s, 3H), 2.96–3.21 (m, 6H), 2.31–2.49 (m, 4H), 1.99–2.19 (m, 7H), 1.91–1.94 (m, 1H), 1.74–1.82 (m, 2H), 1.41–1.51 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 196.6, 173.9, 173.8 172.6 171.5, 171.3, 148.5, 141.9, 140.2, 140.0, 138.6, 138.4, 137.5, 136.4, 136.2, 134.8, 134.4, 134.3, 134.2, 133.5, 133.4, 132.6, 131.1, 130.9, 130.4, 130.1, 130.0, 129.9, 129.4, 128.4, 126.0, 126.0, 116.1, 116.0, 103.8, 103.8, 83.4, 79.0, 78.9, 69.5, 56.2, 54.5, 54.4, 38.6, 36.8, 36.6, 36.5, 36.2, 34.9, 31.8, 31.7, 29.8, 29.4, 26.3, 26.2, 26.0, 25.8, 24.2, 19.9, 19.6, 17.9 (peaks of two diastereomers); LRMS(ESI$^+$) m/z calcd for $C_{46}H_{48}ClN_3O_7$ $[M+H]^+$ 790.33; Found 790.3.

■ 3-(4-(4-Chloro-2-methylphenyl)-8-hydroxy-7-methoxy-2-methyl-2*H*-chromen-2-yl)-*N*-(3-(3-(3-(hex-5-yn-1-yl)-3*H*-diazirin-3-yl)propanamido)propyl)propanamide (**9**)



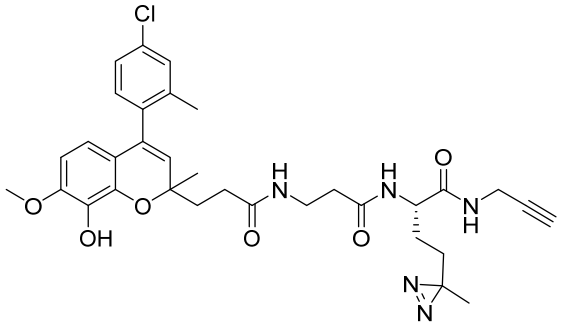
The synthesis of **9** was performed using the previously reported procedure.[1]

■ *N*-((2*S*)-1-((3-(3-(4-(4-chloro-2-methylphenyl)-8-hydroxy-7-methoxy-2-methyl-2*H*-chromen-2-yl)propanamido)propyl)amino)-4-(3-methyl-3*H*-diazirin-3-yl)-1-oxobutan-2-yl)hept-6-ynamide (**10**)



R_f = 0.36 (DCM:methanol = 10:1, v/v); ^{1}H NMR (400 MHz, CD_3OD) δ 7.26 (d, *J* = 5.2 Hz, 1H), 7.22 (d, *J* = 8.4 Hz, 1H), 7.08–7.13 (m, 1H), 6.40 (dd, *J* = 8.8, 2.0 Hz, 1H), 6.00 (dd, *J* = 8.2, 2.2 Hz, 1H), 5.38 (d, *J* = 12.0 Hz, 1H), 4.19 (dd, *J* = 9.0, 5.4 Hz, 1H), 3.81 (s, 1H), 3.05–3.25 (m, 4H), 2.38–2.46 (m, 2H), 1.98–2.29 (m, 11H), 1.26–1.74 (m, 15H), 0.99 (s, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 176.2, 176.0, 174.0, 132.2, 130.7, 127.1, 127.0, 126.9, 117.5, 116.6, 116.5, 105.0, 99.8, 84.6, 79.6, 71.5, 69.8, 56.5, 54.4, 37.8, 37.6, 36.1, 32.4, 32.3, 32.0, 30.1, 29.2, 27.5, 26.5, 26.2, 26.1, 25.9, 20.1, 19.7, 18.9, 18.8; LRMS(ESI$^+$) m/z calcd for $C_{37}H_{46}ClN_4O_6$ $[M+H]^+$ 692.32; Found 692.28.

■ (2*S*)-2-(3-(3-(4-(4-chloro-2-methylphenyl)-8-hydroxy-7-methoxy-2-methyl-2*H*-chromen-2-yl)propanamido)propanamido)-4-(3-methyl-3*H*-diazirin-3-yl)-*N*-(prop-2-yn-1-yl)butanamide (**11**)



R_f = 0.62 (DCM:methanol = 10:1, v/v); ^{1}H NMR (400 MHz, CD_3OD) δ 7.26 (d, *J* = 5.6 Hz, 1H), 7.22 (d, *J* = 8.0 Hz, 1H), 7.10 (d, *J* = 7.6 Hz, 1H), 6.40 (d, *J* = 6.8 Hz, 1H), 6.00 (d, *J* = 7.2 Hz, 1H), 5.38 (d, *J* = 13.2 Hz, 1H), 4.23 (dd, *J* = 8.8, 5.6 Hz, 1H), 3.85–3.98 (m, 2H), 3.81 (s, 3H), 3.36–3.50 (m, 2H), 2.57 (dd, *J* = 4.2, 2.6 Hz, 1H), 1.98–2.50 (m, 10H), 1.23–1.74 (m, 9H), 0.98 (s, 3H); LRMS(ESI$^+$) m/z calcd for $C_{33}H_{38}ClN_5O_6$ $[M+H]^+$ 636.26; Found 636.28.

6. Cell Culture Procedure

HeLa (human cervical adenocarcinoma cell line) was obtained from American Type Culture Collection [ATCC, USA]. HeLa cells were cultured in RPMI 1640 [GIBCO, USA] supplemented with 10% (v/v) fetal bovine serum [GIBCO, USA] and 1 % (v/v) antibiotic-antimycotic solution [GIBCO, USA]. The cells were maintained in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C, and cultured in T75 Flask [Nalgene Nunc International, USA] according to manufacturers' instruction. The growth medium was changed every two to three days. Cells were grown to confluence prior to the experiment.

7. Cell Viability Assay of Photoaffinity Probes

Antiproliferative activity assay – Cell viability was measured to determine the cytotoxicity of our probe using the Cell Counting Kit (CCK)-8 assay [Dojindo, Japan] and the experimental procedure is based on the manufacturer's manual. HeLa cells were cultured into 96-well plates at a density of 2×10^3 cells/well for 24 h, followed by the treatment of **6**, **7**, **8**, **9**, **10**, and **11**. After 72 h incubation with each compound, 10 μL of WST-8 solution [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium, monosodium salt] was added to each well, and plates were incubated for additional 1 h at 37 °C. The absorbance of each well at 450 nm was measured using Synergy HT [Bio-Tek, USA]. The percentage of cell viability was calculated by following formula: % cell viability = (mean absorbance in test wells) / (mean absorbance in control well) × 100. Each experiment was performed in triplicate.

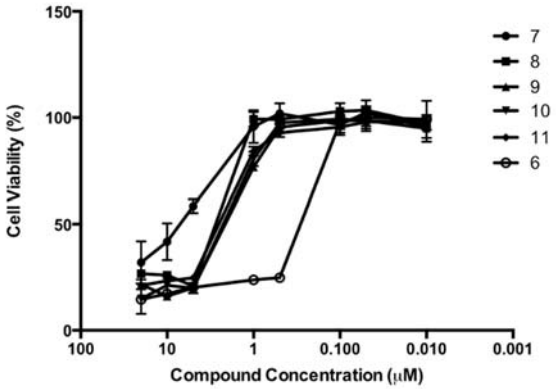


Figure S1. Cell viability against tubulin inhibitor **6** and its photoaffinity probes **7**, **8**, **9**, **10**, and **11**. All the photoaffinity probes have similar range of anticancer activity.

8. 1D Gel Fluorescence Imaging

Protein profiling of photoaffinity linkers 1, 2, 3, 4 and 5, and photoaffinity probes

Cell lysate labeling – HeLa cell (150ϕ dish × 3) was scrapped with cold Ca^{2+}- and Mg^{2+}-free phosphate buffered saline (PBS) [WelGENE, S. Korea] and centrifuged. The supernatant was discarded, and cell pellet was kept at –80 °C until use. RIPA buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 1% NP-40, 0.5% deoxycholate, protease inhibitor [Roche, Switzerland]) was added to the cells for lysis. The cells were incubated for 15 min on ice. The cell lysate was centrifuged at 4 °C, 13000 rpm for 15 min. Supernatant was taken, and the protein concentration was measured with BCA assay kit [Thermo, USA], and protein concentration was adjusted to 1 mg/mL. The protein and compounds was mixed. The mixture was incubated at RT for 30 min. 365-nm UV light from BLAK-Ray (B-100AP) UV lamp [UVP, USA] was irradiated to the mixture for 30 min on ice. The mixture was under click chemistry with Cy5-azide [Lumiprobe, USA] (40 μM), tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA) [Sigma, USA] (100 μM), $CuSO_4$ [Sigma, USA] (1 mM), tris(2-carboxyethyl)phosphine (TCEP) [TCI, Japan] (1 mM) and *t*-BuOH [Sigma, USA] (5%) for 1 h. 5 × Laemmli buffer was added and incubated at 95 °C for 5 min. The protein samples were separated by 1DGE and scanned with Typhoon Trio.

Live cell labeling – HeLa cells were seeded on 6 well plates. Compounds were treated for 3 h. 356-nm UV light was irradiated to the mixture for 30 min on ice. The cells were wash with PBS and kept at –80 °C until use. RIPA buffer was added to the cells for lysis. The cells were incubated for 15 min on ice. The cell lysate was scrapped and centrifuged at 4 °C, 13000 rpm for 15 min. Supernatant was taken and the protein concentration was measured with BCA assay, and protein concentration was adjusted to 1 mg/mL. The mixture was under click chemistry with Cy5–azide (40 μM), TBTA (100 μM), $CuSO_4$ (1 mM), TCEP (1 mM) and tBuOH (5%) for 1 h. 5 × Laemmli sample buffer was added and incubated at 95 °C for 5 min. The protein samples were separated by 1DGE and scanned with Typhoon Trio.

Fluorescence detection and quantification

The *in-gel* fluorescence signal was visualized at the Cy3 (532 nm excitation) or Cy5 (633 nm excitation) channel by Typhoon Trio [Amersham Bioscience, USA] and analyzed by ImageQuant TL program [Amersham Bioscience, USA].

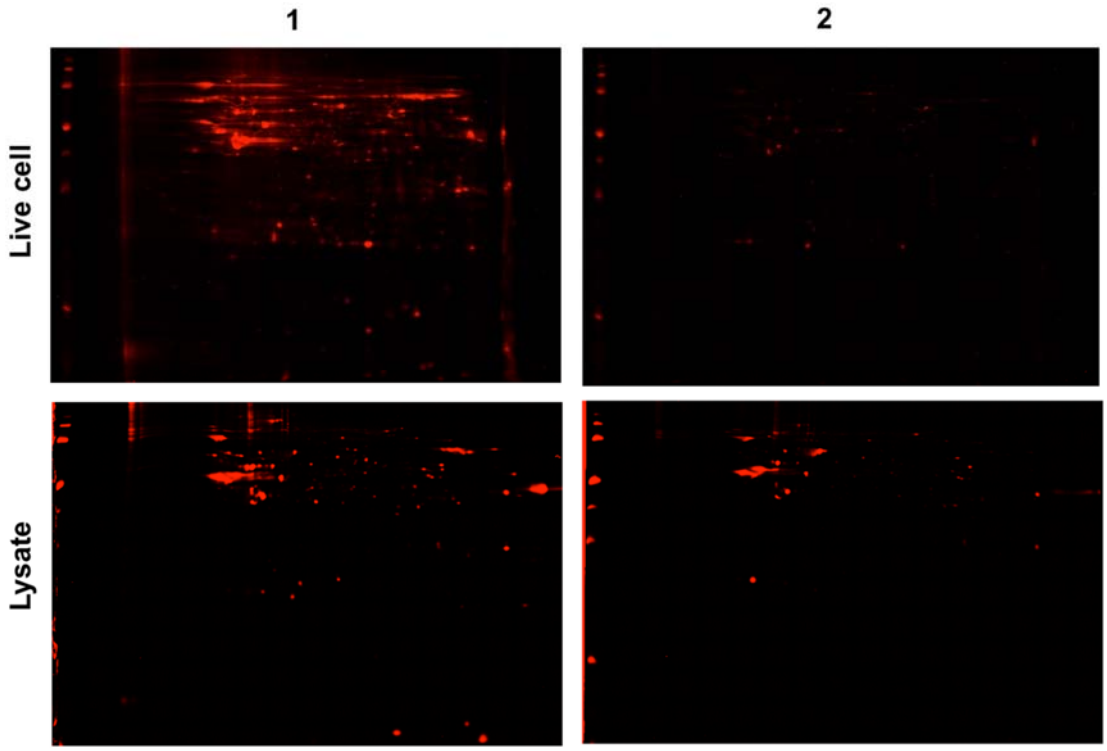


Figure S2. The proteome labeled by PLs **1** and **2** in living cells and cell lysates was subjected to 2D-gel electrophoresis. (**1** and **2**: 10 μM, HeLa cell).

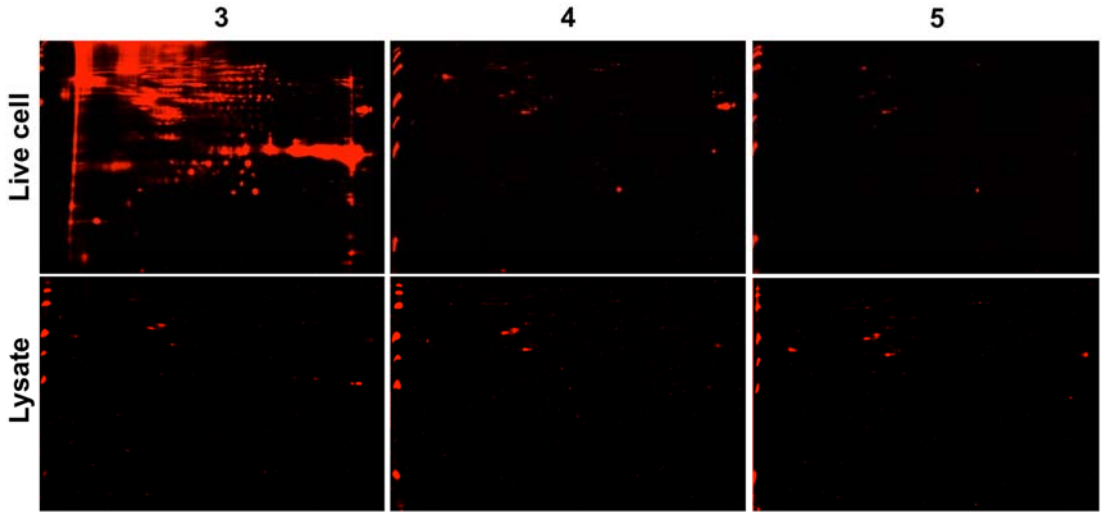


Figure S3. The proteome labeled by PLs **3, 4**, and **5** in living cells and cell lysates was subjected to 2D-gel electrophoresis. (**3**, **4**, and **5**: 10 μM, HeLa cell).

9. 2D Gel Fluorescence Imaging

Cell lysate labeling – HeLa cell (150ϕ dish × 3) was scrapped in cold PBS and centrifuged. The supernatant was discarded, and cell pellet was kept at –80 °C until use. RIPA buffer was added to the cells for lysis. The cells were incubated for 15min on ice. The cell lysate was centrifuged at 4 °C, 13000 rpm for 15 min. Supernatant was taken, the protein concentration was measured with BCA assay, and protein concentration was adjusted to 1 mg/mL. The protein and compounds were mixed. The mixture was incubated at RT for 30 min. 356-nm UV light was irradiated to the mixture for 30 min on ice. The mixture was under click chemistry with Cy5-azide or Cy3-azide [Lumiprobe, USA] (40 μM), TBTA (100 μM), $CuSO_4$ (1 mM), TCEP (1 mM) and *t*-BuOH (5%) for 1 h. Acetone was added to the mixture for precipitation, and the mixture was kept at –20 °C for 20 min. The mixture was centrifuged at 4 °C, 14000 rpm for 10 min. Supernatant was discarded, and the pellet was washed with cold acetone two times. The pellet was resolved with rehydration buffer – 7 M Urea, 2 M Thiourea, 2% CHAPS(w/v), 40 mM DTT, IPG buffer(5 μl/ml) in ddwater. The proteome labeled with photoaffinity probe in the absence (labeled with Cy5-azide) and presence of **6** (labeled with Cy3-azide) were mixed (1:1 ratio). The proteins were separated on 2DGE and scanned with Typhoon Trio.

Live cell labeling – HeLa cells were seeded on 6 well plate. The cells were treated with compounds for 3 h. 356-nm UV light was irradiated to the mixture for 30 min on ice. The cells were wash with PBS and kept at –80°C until use. RIPA buffer was added to the cells for lysis. The cells were incubated for 15 min on ice. The cell lysate was scrapped and centrifuged at 4 °C, 13000 rpm for 15 min. Supernatant was taken and the protein concentration was measured with BCA assay and protein concentration was adjusted to 1 mg/mL. The mixture was under click chemistry with Cy5-azide or Cy3-azide (40 μM), TBTA (100 μM), $CuSO_4$ (1 mM), TCEP (1 mM) and *t*BuOH (5%) for 1 h. Acetone was added to the mixture for precipitation, and the mixture was kept at –20 °C for 20 min. The mixture was centrifuged at 4 °C, 14000 rpm for 10 min. Supernatant was discarded, and the pellet was washed with cold acetone two times. The pellet was resolved with rehydration buffer. The proteome labeled with photoaffinity probe in the absence (labeled with Cy5-azide) and presence of **6** (labeled with Cy3-azide) were mixed (1:1 ratio). The proteins were separated on 2DGE and scanned with Typhoon Trio.

2 dimensional gel electrophoresis (2DGE) – Isoelectric focusing (IEF) was performed with 24 cm pH 3–10 Immobiline^{TM} Drystrip gel [GE healthcare, USA] using Ettan IPGphor3 IEF system [GE healthcare, USA]. The Drystrip was loaded to 12% SDS-PAGE gel, and proteins were separated by Ettan Daltsix [GE healthcare, USA].

10. MS Analysis for Protein Identification

Protein spots were excised from 2D gel and dehydrated in acetonitrile for 10 min. Acetonitrile was removed and dried under reduced pressure. For mass analysis, the resulting gel pieces were re-swelled at 4 °C for 45 min in buffer containing trypsin and 50 mM $(NH_4)_2CO_3$ and incubated overnight at 37 °C for tryptic digestion. The samples with gel pieces were centrifuged, and the supernatant was collected for mass analysis. The residual peptides in gel pieces were further extracted with 50% acetonitrile containing 20 mM $(NH_4)_2CO_3$ and 5% formic acid three times at room temperature. The combined peptide samples were condensed down in SpeedVac until the desired sample concentration was reached. MS analysis was performed with the instruments in NICEM at Seoul National University. LC-MS/MS experiments were performed using an integrated system consisting of HPLC [Dionex, Ultimate 3000 RSLCnano system, USA] interfaced to Q Exactive [Thermo Scientific, USA] equipped with a nano-electrospray ionization source. Peptides were reconstituted in solvent A [water/acetonitrile (98:2, v/v), 0.1% formic acid] and then injected into LC-nano ESI-MS/MS system. Samples were first trapped on a trap column [Acclaim pepmap100, 100 μm i.d. × 20 mm, 5 μm, 10 nm, Thermo Scientific, USA] and washed for 10 min with 98% solvent A and 2% solvent B [water/acetonitrile (2:98, v/v), 0.1% Formic acid] at a flow rate of 4 μL/min, and then separated on a Zorbax 300SB-C18 capillary column (75 μm i.d. × 150 mm, 3.5 μm, 10 nm) at a flow rate of 300 nL/min. The LC gradient was run at 2% to 45% solvent B over 30 min, then from 45% to 70% over 10 min, followed by 70% solvent B for 5 min, and finally 5% solvent B for 15 min. Resulting peptides were electrosprayed through a coated silica tip [FS360-20-10-N20-C12, PicoTip emitter, New Objective] at an ion spray voltage of 2,000 eV. The raw data from Q Exactive was processed with Proteome Discoverer 1.3 software [Thermo Scientific, USA]. To process an MS/MS spectrum, a thorough search was performed against a NCBI database. All the protein MS data can be found in excel file of supporting information.

11. Whole Proteome Staining of 1D and 2D Gel Images

Cellular proteomes labeled by photoaffinity linker or target ID probes were visualized with Coomassie staining or silver staining. Cellular protein expression pattern was not affected by the treatment of photoaffinity linker and photoaffinity probes. Among whole cellular proteins, only few proteins were labeled by probes in a structure dependent manner.

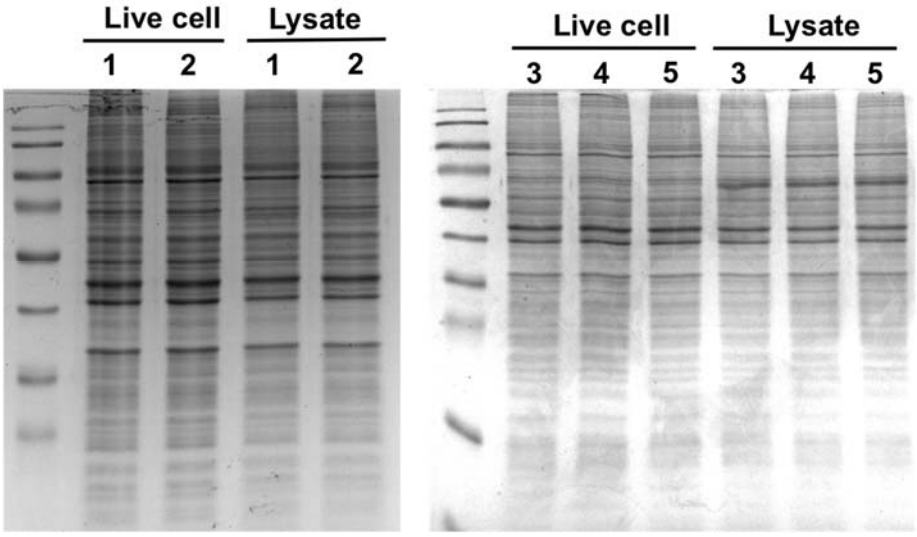


Figure S4. Coomassie staining of 1D gel image in Figure 1d. Expression patterns of proteomes were not changed by proteome labeling by photoaffinity linkers, but the proteome-labeling pattern was affected by the molecular shape of photoaffinity linker in a structure-dependent manner.

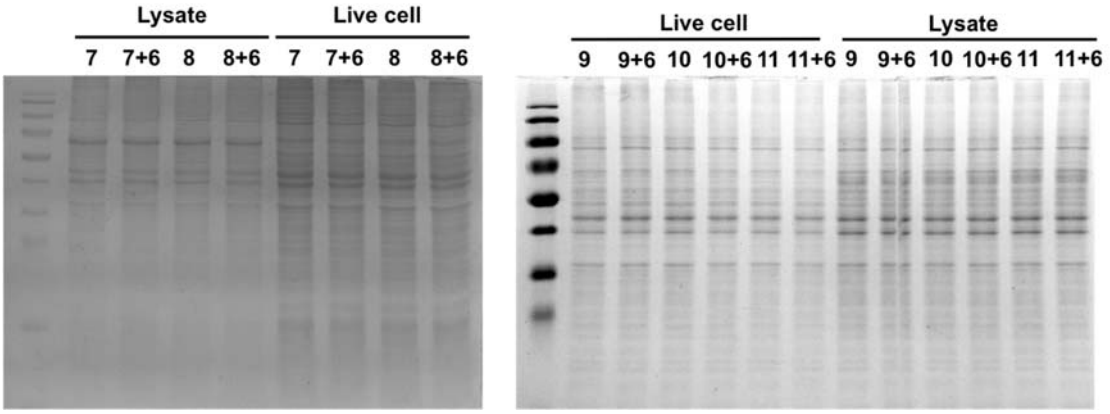


Figure S5. Coomassie staining of 1D gel image in Figure 2b and 3b. Expression pattern of proteomes was not changed by proteome labeling upon treatment with probes.

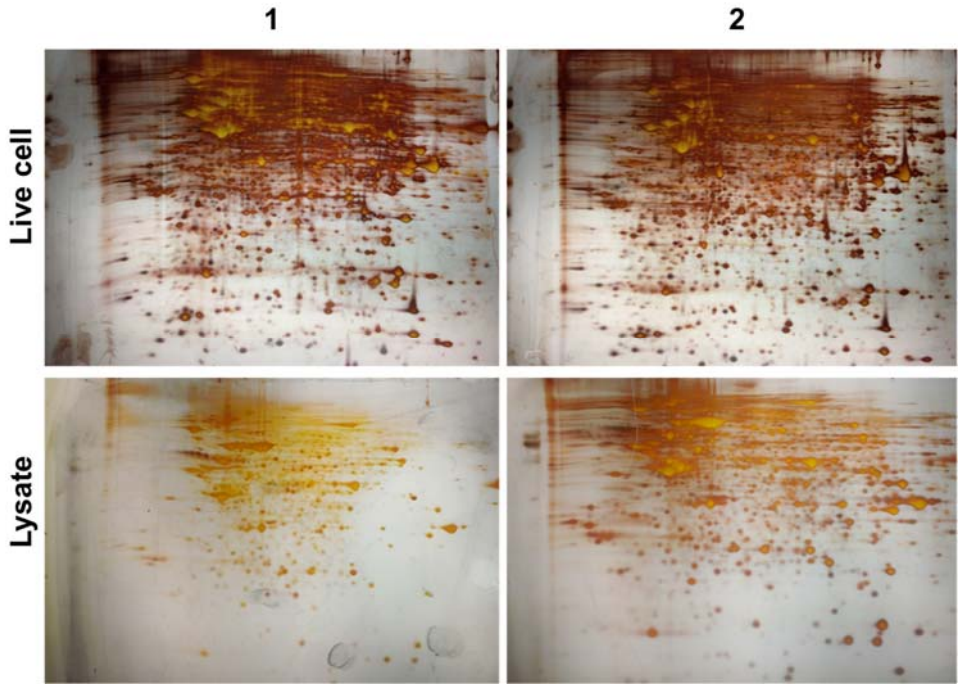


Figure S6. Silver staining of 2D gel image in Figure S2. Whole proteome expression pattern was not affected by proteome labeling by photoaffinity linkers. Only few proteins out of whole proteome are labeled by each photoaffinity linkers.

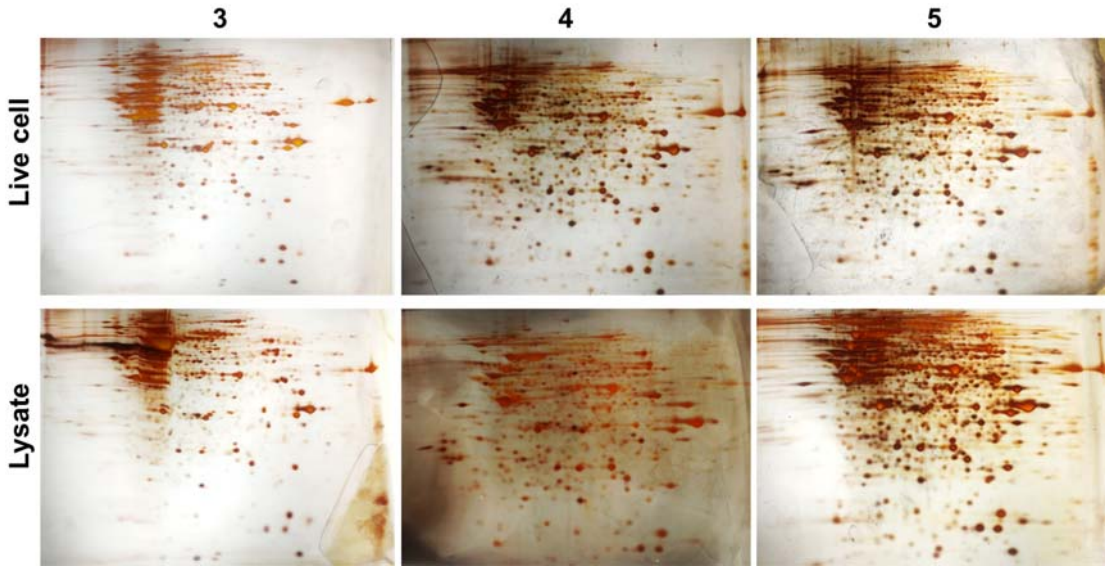


Figure S7. Silver staining of 2D gel image in Figure S3. Whole proteome expression pattern was not affected by proteome labeling by photoaffinity linkers. Only few proteins out of whole proteome are labeled by each photoaffinity linkers.

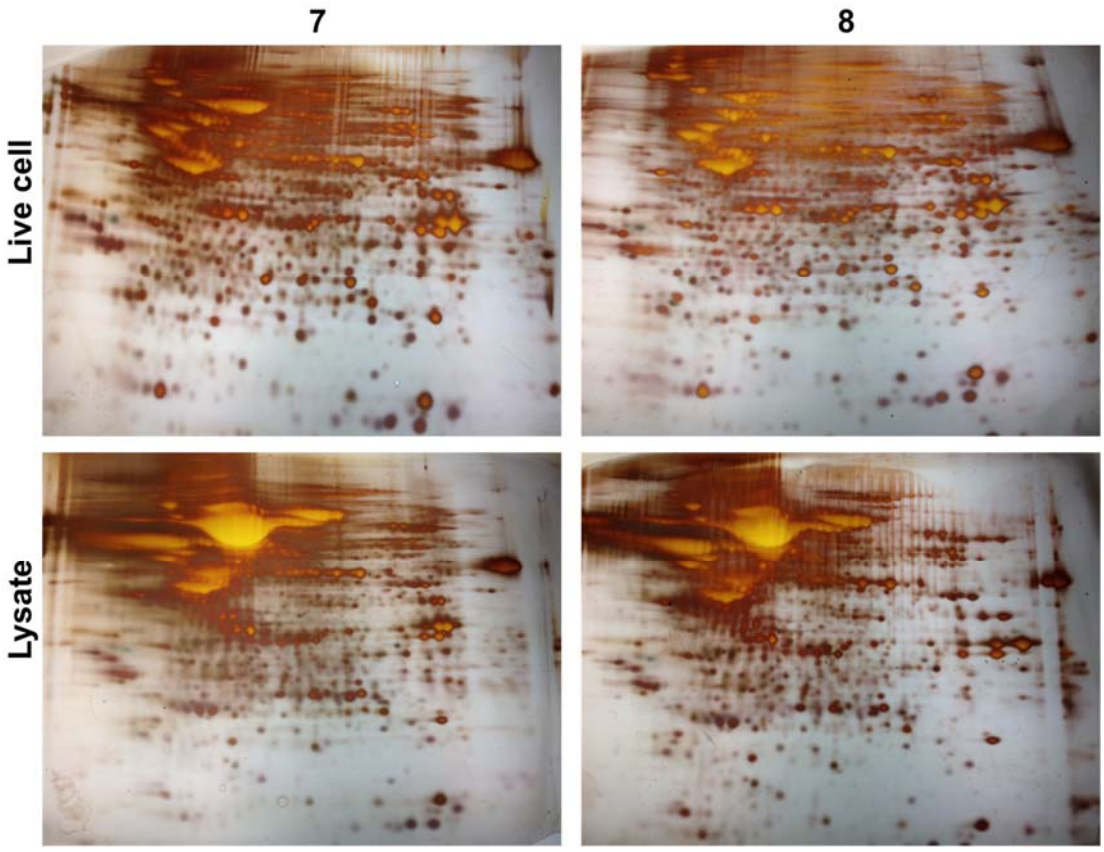


Figure S8. Silver staining of 2D gel image in Figure 2c. Whole proteome expression pattern was not affected by proteome labeling by photoaffinity linkers. Only few proteins out of whole proteome are labeled by each photoaffinity linkers.

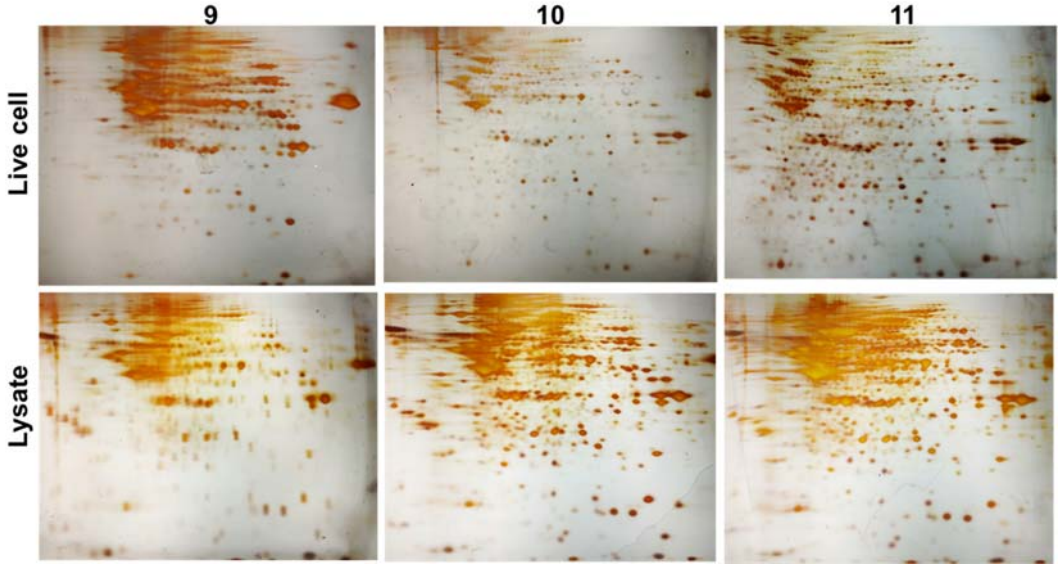


Figure S9. Silver staining of 2D gel image in Figure 3c. Whole proteome expression pattern was not affected by proteome labeling by photoaffinity linkers. Only few proteins out of whole proteome are labeled by each photoaffinity linkers.

12. Immunofluorescent Staining of Cellular Vimentin Structure

Cells were treated with compounds for 4 h. Cells were fixed with 3.7% formaldehyde in PBS for 15 min. The cells were washed with PBS twice. To the fixed cells, a solution of 0.5 % Triton X-100 in PBS was added. The cells were incubated at 4 °C for 15 min and washed with ice-cold PBS three times. The cells were incubated with 1% BSA in TBST for 60 min. Vimentin antibody [#5741, Cell signaling, USA] in TBST with 1% BSA was added to cells under different experimental conditions overnight at 4 °C. The cells were washed with TBST three times. Anti-rabbit IgG Alexa Flour 647-linked antibody [ab150075, Abcam, UK] in TBST with 1% BSA was added to the cells at room temperature for 1 h. The cells were washed with TBST three times and observed with fluorescent microscope [IX71, Olympus, Japan].

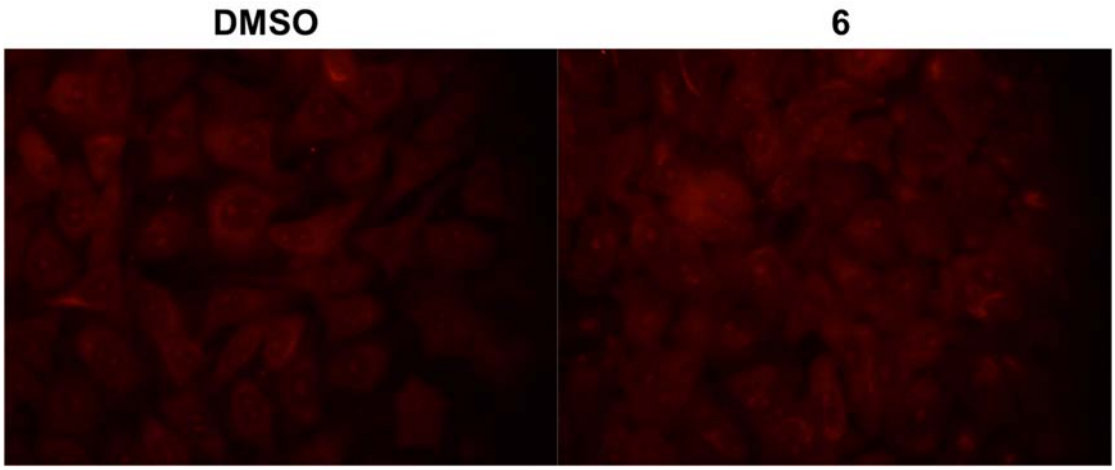


Figure S10. Immunostaining of vimentin structure. Treatment of **6** does not affect the vimentin cellular structures.

13. Protein Spot MS Analysis Data

MS data of Protein a

Description	Score	Coverage	# Proteins	# Unique Peptides	# Peptides	# PSMs	# AAs	MW [kDa]	calc. pI
vimentin variant 3	41.27	34.57	89	14	14	17	431	49.6	5.25

A2	Sequence	# PSMs	# Proteins	# Protein Groups	Protein Group	Modifications	ΔCn	XCorr	Probability	Charge	MH+ [Da]	ΔM [ppm]	RT [min]
High	KVESLQEEIAFLK	1	13	1	167887751		0.0000	3.34	0.00	2	1533.85796	3.65	45.22
High	ISLPLPNFSSLNLR	1	10	1	167887751		0.0000	2.81	0.00	2	1570.90129	3.86	45.21
High	LGDLYEEEMR	1	12	1	167887751		0.0000	2.70	0.00	2	1254.56999	2.27	43.69
High	EEAENTLQSFR	3	12	1	167887751		0.0000	2.68	0.00	2	1323.61882	0.93	43.17
High	ILLAELEQLKGQGK	1	12	1	167887751		0.0000	2.60	0.00	3	1539.91495	2.86	45.33
High	SLYASSPGGVYATR	1	8	1	167887751		0.0000	2.45	0.00	2	1428.71330	1.05	43.64
High	QDVDNASLAR	2	14	1	167887751		0.0000	2.44	0.00	2	1088.53581	2.46	33.66
High	FADLSEAANR	1	14	1	167887751		0.0000	2.42	0.00	2	1093.52800	0.62	43.58
High	VELQELNDR	1	34	1	167887751		0.0000	2.37	0.00	2	1115.57036	1.06	43.36
High	DNLAEDIMR	1	14	1	167887751		0.0000	2.26	0.00	2	1076.50713	2.76	43.74
High	LQDEIQNMK	1	13	1	167887751		0.0000	2.15	0.00	2	1118.55254	1.29	41.48
High	GTNESLER	1	24	1	167887751		0.0000	2.10	0.00	2	905.43437	2.24	33.34
Medium	LLEGEESR	1	67	1	167887751		0.0000	2.07	0.00	2	932.46910	0.75	41.45
Medium	NLQEAEEWYK	1	18	1	167887751		0.0000	2.04	0.00	2	1309.60771	1.35	43.68

MS data of Protein b

Description	Score	Coverage	# Proteins	# Unique Peptides	# Peptides	# PSMs	# AAs	MW [kDa]	calc. pI
Tubulin, beta	145.26	47.52	35	5	15	50	444	49.6	4.86

A2	Sequence	# PSMs	# Proteins	# Protein Groups	Protein Group	Modifications	ΔCn	XCorr	Probability	Charge	MH+ [Da]	ΔM [ppm]	RT [min]
High	SGPFGQIFRPDNFVFG	1	31	3	19437378 5;1808871		0.0000	4.36	0.00	3	2798.34903	1.97	45.99
High	MAVTFIGNSTAIQELFK	3	18	1	18088719		0.0000	4.23	0.00	2	1869.98113	1.68	45.85
High	GHYTEGAELVDSVLDV	6	32	2	18088719; 11960877		0.0000	4.09	0.00	2	1958.98430	1.23	46.68
High	FWEVISDEHGIDPTGT	1	9	1	18088719		0.0000	3.91	0.00	4	3102.41274	1.63	45.53
High	LHFFMPGFAPLTSR	5	51	3	19437378 5;1808871		0.0000	3.58	0.00	3	1620.83878	1.95	46.73
High	FPGQLNADLR	2	68	3	19437378 5;1808871		0.0000	3.26	0.00	2	1130.59868	2.95	43.74
High	ISVYYNEATGGK	11	9	1	18088719		0.0000	3.19	0.00	2	1301.63945	1.70	41.55
High	AILVDLEPGTMDSVR	1	25	1	18088719		0.0000	3.12	0.00	2	1615.84282	4.17	44.43
High	EVDEQMLNVQNK	12	36	2	18088719; 11960877		0.0000	3.04	0.00	2	1446.69280	2.35	37.65
High	NSSYFVEWIPNNVK	1	54	3	19437378 5;1808871		0.0000	2.68	0.00	2	1696.83989	4.06	44.36
High	ALTVPELTQQVFDAK	2	13	1	18088719		0.0000	2.62	0.00	2	1659.90129	3.62	44.59
High	KLAVNMVPFPR	1	64	3	19437378 5;1808871		0.0000	2.47	0.00	2	1271.73406	3.70	45.15
High	LAVNMVPFPR	1	64	3	19437378 5;1808871		0.0000	2.46	0.00	2	1143.63994	4.86	44.38
High	IREEYPDR	1	43	2	18088719; 11960877		0.0000	2.44	0.00	2	1077.53655	3.86	44.40
High	IMNTFSVVPSPK	2	30	3	19437378 5;1808871		0.0000	2.35	0.00	2	1319.70708	3.20	43.96

MS data of Protein c

Description	Score	Coverage	# Proteins	# Unique Peptides	# Peptides	# PSMs	# AAs	MW [kDa]	calc. pI
60 kDa heat shock	158.48	39.97	18	17	18	47	573	61.0	5.87

A2	Sequence	# PSMs	# Proteins	# Protein Groups	Protein Group	Modifications	ΔCn	XCorr	Probability	Charge	MH+ [Da]	ΔM [ppm]	RT [min]
High	LVQDVANNTNEEAG	4	8	1	31542947		0.0000	6.62	0.00	2	2560.25298	1.69	22.12
High	VGEVIVTKDDAMLL	4	10	1	31542947		0.0000	4.75	0.00	3	1630.91269	2.56	25.67
High	KPLVIIAEDVDGEAL	2	12	1	31542947		0.0000	4.57	0.00	3	2365.33829	1.89	36.35
High	ALMLQGVDLLADAV	4	10	1	31542947		0.0000	4.42	0.00	2	2113.14238	1.27	46.00
High	ISSIQSIVPALEIANA	3	10	1	31542947		0.0000	3.88	0.00	3	1919.07474	1.96	32.32
High	IQEIIEQLDVTTSEY	1	8	1	31542947		0.0000	3.86	0.00	2	2038.02532	1.27	30.60
High	IQEIIEQLDVTTSEY	4	7	1	31542947		0.0000	3.65	0.00	3	2295.16422	1.71	30.72
High	TVIIEQSWGSPK	3	9	1	31542947		0.0000	3.63	0.00	2	1344.71868	2.10	23.80
High	GVMLAVDAVIAELKK	2	10	1	31542947		0.0000	3.51	0.00	2	1556.90935	0.78	39.51
High	RIQEIIEQLDVTTSE	1	7	1	31542947		0.0000	3.49	0.00	3	2451.26731	2.40	32.26
High	TLNDELEIIEGMK	2	12	1	31542947		0.0000	3.46	0.00	2	1504.75859	1.44	32.70
High	KISSIQSIVPALEIAN	3	10	1	31542947		0.0000	3.44	0.00	3	2047.17020	2.08	30.73
High	NAGVEGSLIVEK	3	9	1	31542947		0.0000	3.28	0.00	2	1215.66008	1.70	20.60
High	VGLQVVAVK	4	10	1	31542947		0.0000	3.21	0.00	2	912.58934	1.77	21.39
High	IMQSSSEVGYDAM	1	9	1	31542947		0.0000	3.16	0.00	2	2508.11577	2.60	34.63
High	GYISPYFINTSK	3	13	1	31542947		0.0000	2.92	0.00	2	1389.70842	2.52	26.74
Low	TLNDELEIIEGMKFD	1	10	1	31542947		0.0000	2.46	0.00	3	1922.95584	1.53	34.06
High	IGIEIIKR	2	9	1	31542947		0.0000	2.41	0.00	2	941.61577	1.58	21.33

14. Computational Analysis and Calculation

1) Surface modeling of 5 PLs:

The molecular surface charge of PLs was calculated by Marvin Sketch 15.6.29.0.

2) The conformer number of 5 PLs near the energy minimum:

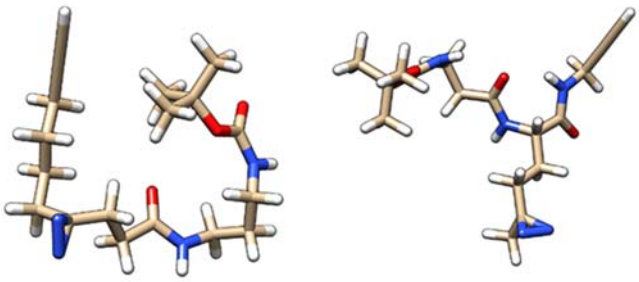


Figure S11. Lowest-energy conformation of PL **3** (linear) and **5** (branched)

Cpd.	ClogP	P.E.	< 1	< 2
1	4.0	42.8	26	40
2	3.8	25.4	9	23
3	2.4	-3.6	36	68
4	2.1	-4.4	5	20
5	1.0	-4.3	5	13

Figure S12. cLogP, the minimum energy of the most stable conformer, the number of conformers of **1**, **2**, **3**, **4**, and **5** within a range of 1 or 2 kcal/mol. cLogP value was calculated by ChemBioDraw Ultra 14.0. Energy minimization and structural optimization were performed by Gaussian W09, potential energies of compounds were calculated by Vconf2.0 from previous optimized structures. Number of conformers within 1 or 2 kcal mol^{-1} from energy minimum was calculated by $V_{conf}2.0$ based on Tork conformational analysis method.[4]

3) Docking simulation and Graphical analyses

The binding pose of **6** at the combretastatin-binding site was predicted by docking simulation with co-crystal structure (PDB: 1SA0) of tubulin with colchicine, which shares the binding site of combretastatin, using the Discovery Studio 1.7 program. The colchicine-binding site of β-tubulin from tubulin-colchicine co-crystal structure was defined from receptor cavities, and the LigandFit module implemented in the Receptor-Ligand Interaction protocol was used for detailed calculations. Image of 3 different tubulin binding sites were reproduced based 1Z2B, 1JFF, 1SA0 pdb files. Graphical analyses were performed with UCSF Chimera package version 1.8.

15. Reference

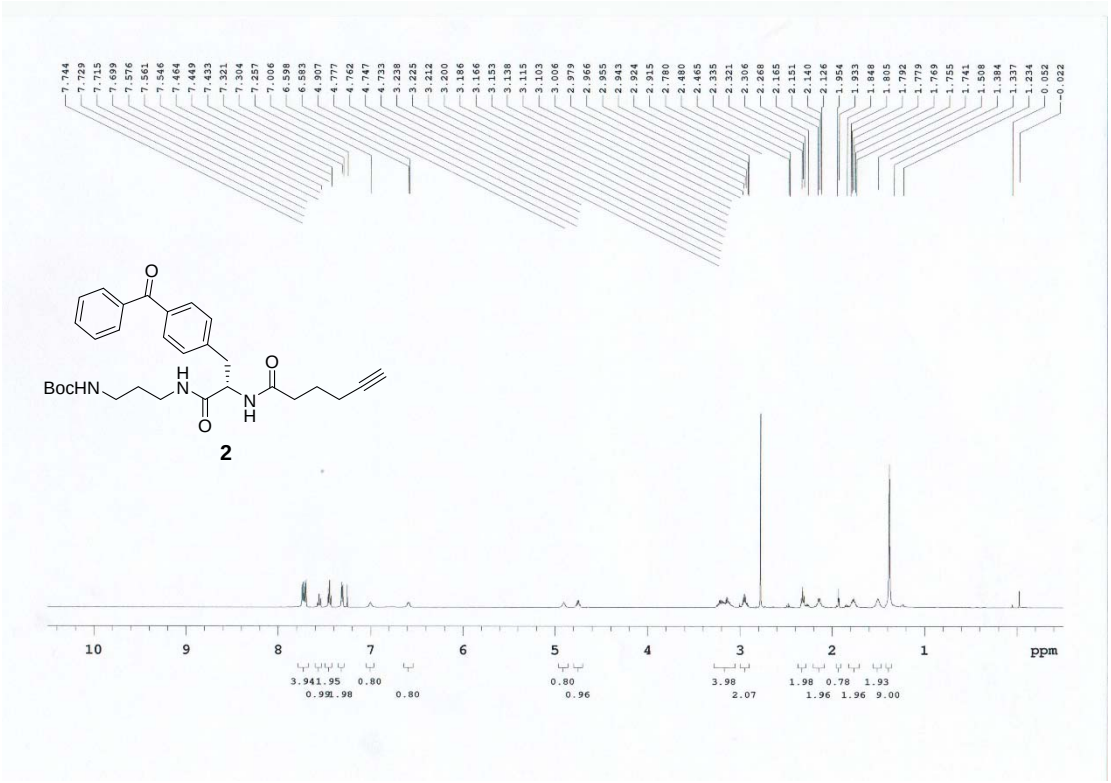
[1] J. Park, M. Koh, J. Y. Koo, S. Lee, S. B. Park, *ACS Chem. Biol.* **2015**, DOI: 10.1021/acschembio.5b00671.

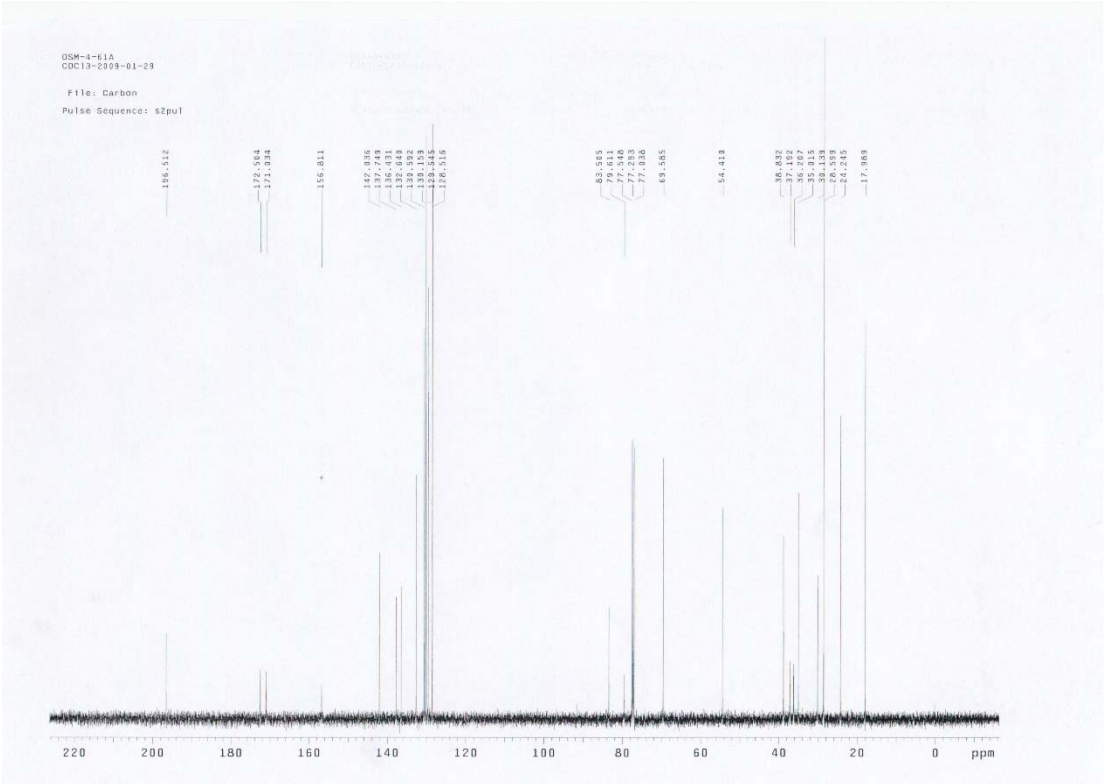
[2] H. Shi, C.–J. Zhang, G. Y. J. Chen, S. Q. Yao, *J. Am. Chem. Soc.* **2012**, *134*, 3001-3014.

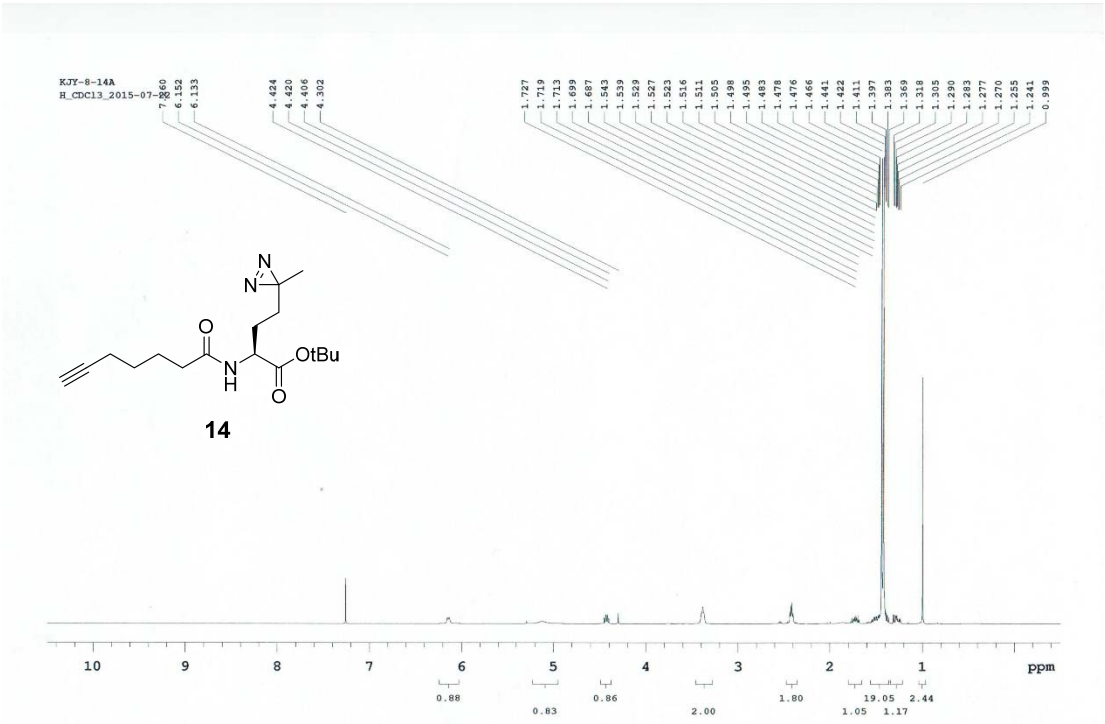
[3] J. Park, S. Oh, S. B. Park, *Angew. Chem.* **2012**, *124*, 5543–5547; *Angew. Chem. Int. Ed.* **2012**, *51*, 5447–5451.

[4] C.–E. Chang, M. K. Gilson, *J. Comput. Chem.* **2003**, *24*, 1987–1998.

16. NMR Spectra of All New Compounds



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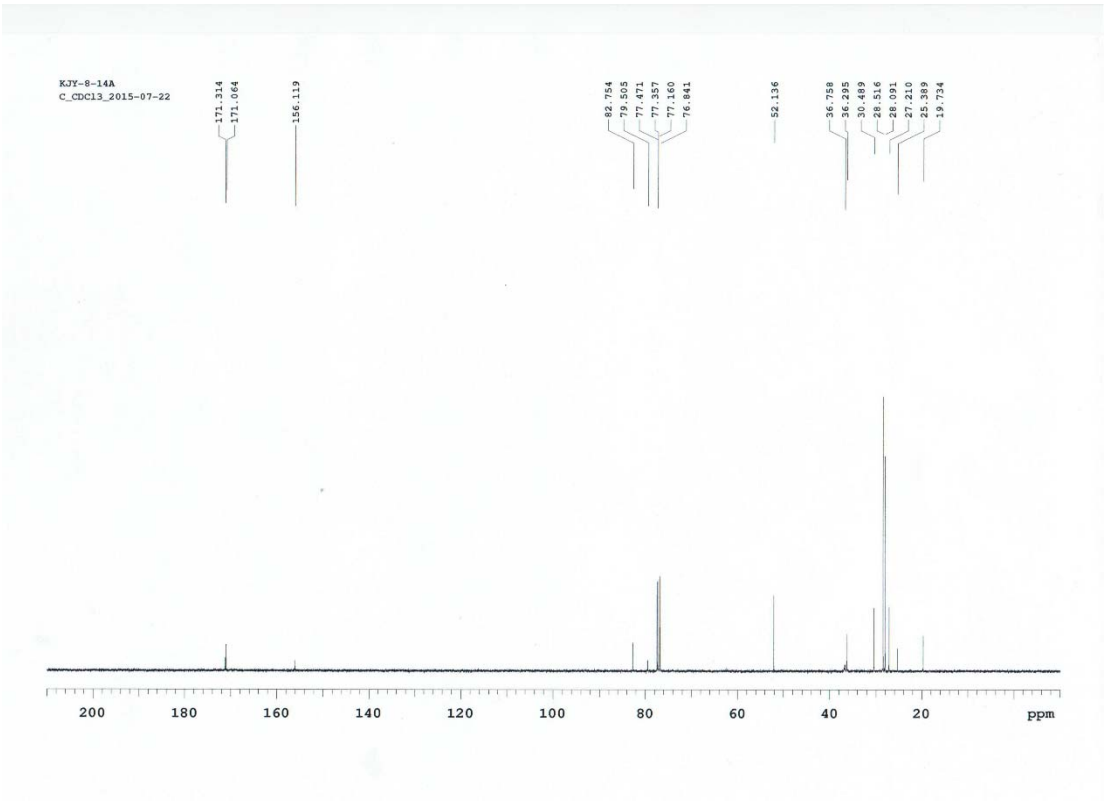
ppm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KJY-8-14A
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128 increments
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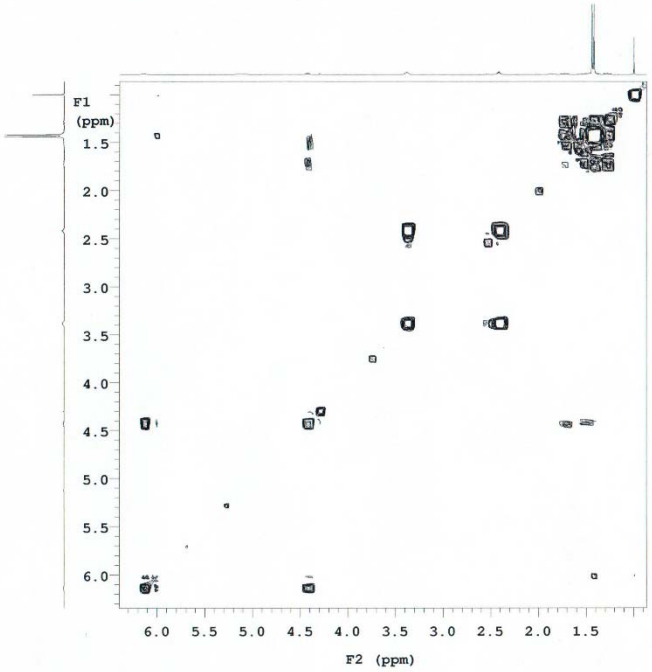
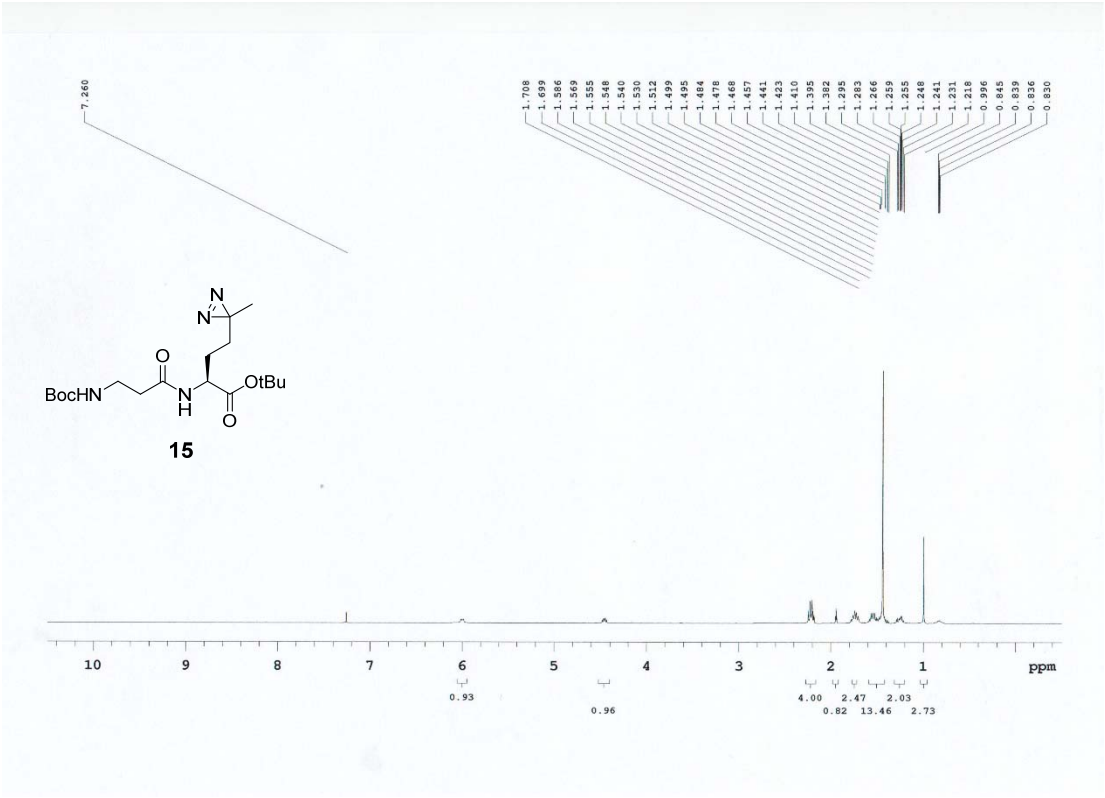
Total time 3 min 10 sec

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1.266
1.259
1.255
1.248
1.241
1.231
1.218
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0.839
0.836
0.830
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O
BocHN
N
H
OtBu
O
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10
9
8
7
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2
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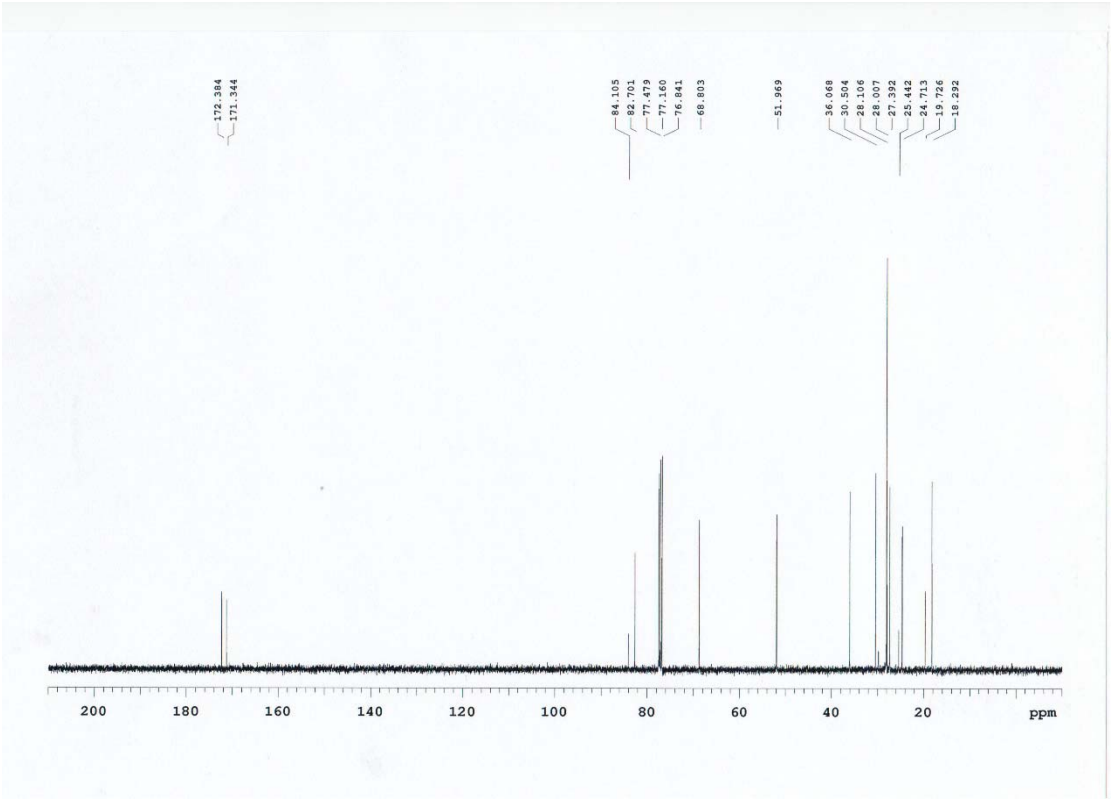
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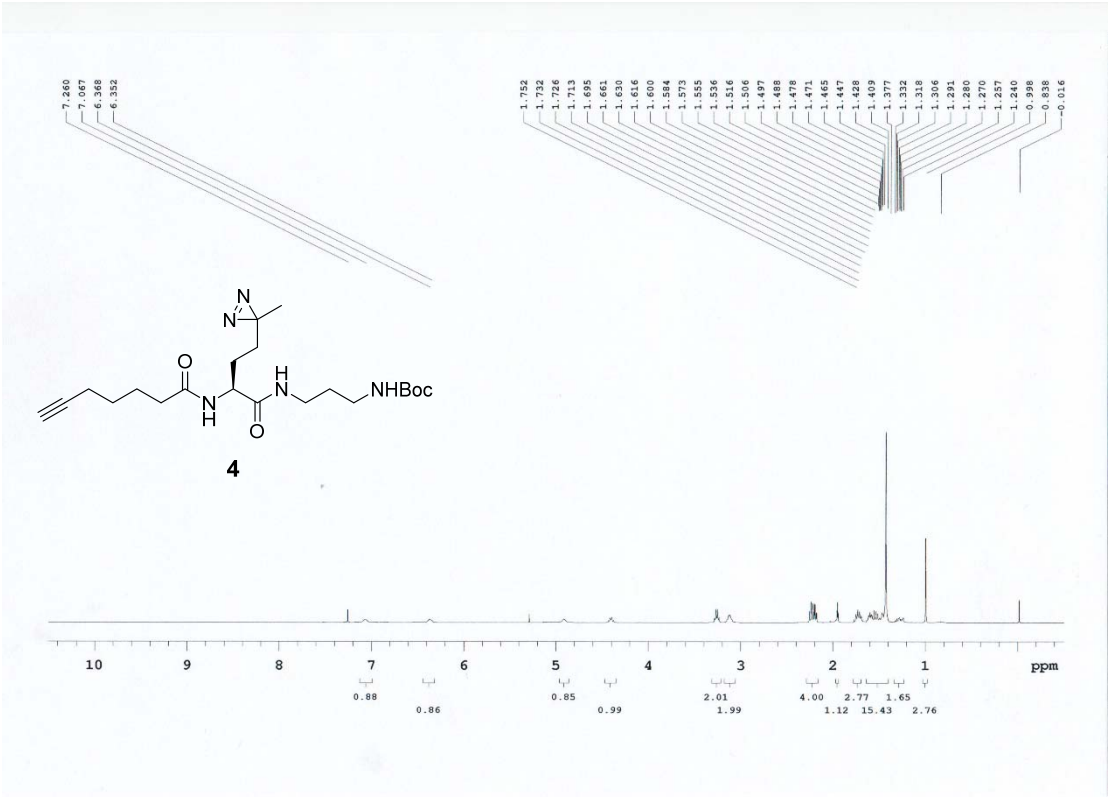
140
120
100
80
60
40
20
ppm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1.584
1.573
1.555
1.536
1.516
1.506
1.497
1.488
1.478
1.471
1.465
1.447
1.428
1.409
1.377
1.332
1.318
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1.291
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1.270
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0.016
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N
H
H
N
O
NHBoc
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10
9
8
7
6
5
4
3
2
1
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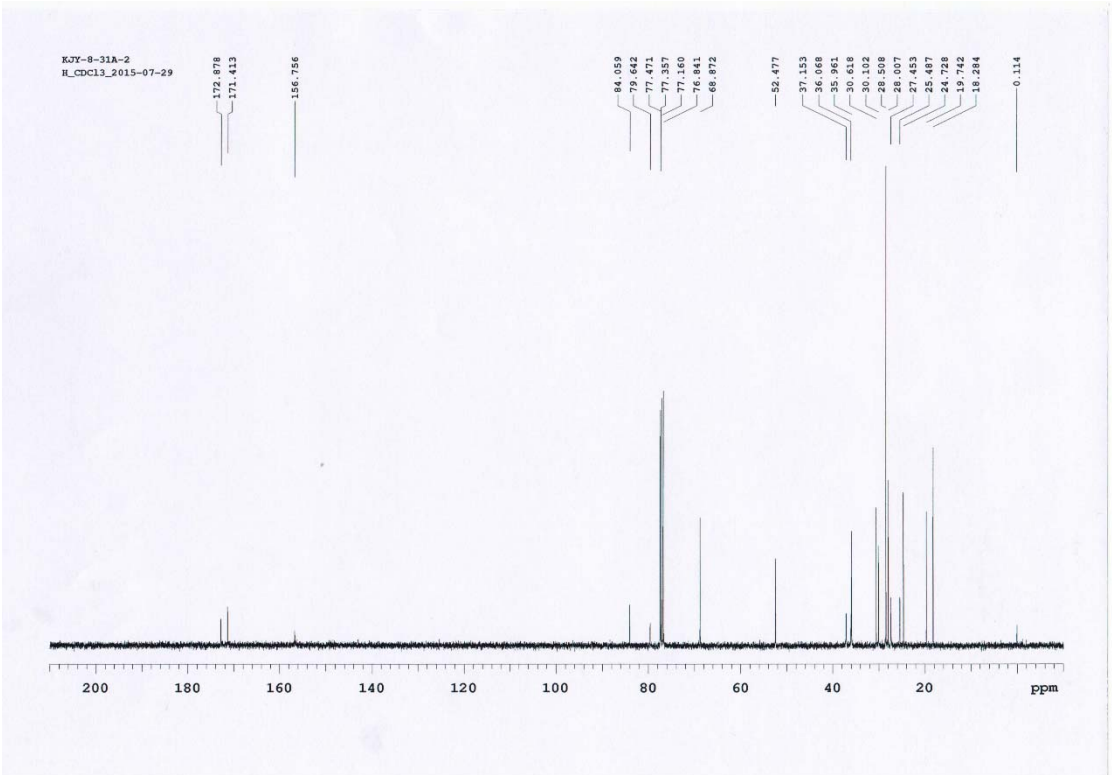
1.65
2.76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200
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60
40
20
ppm

KJY-8-31A-2
H_CDCl3_2015-07-29

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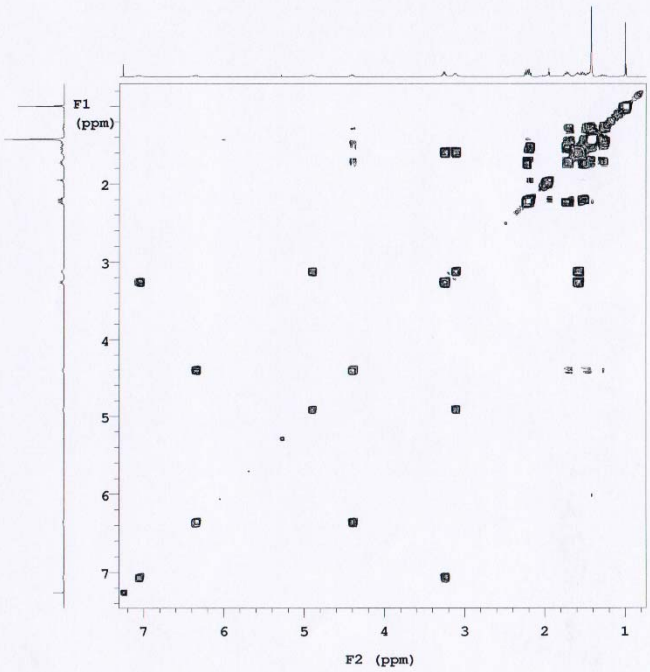
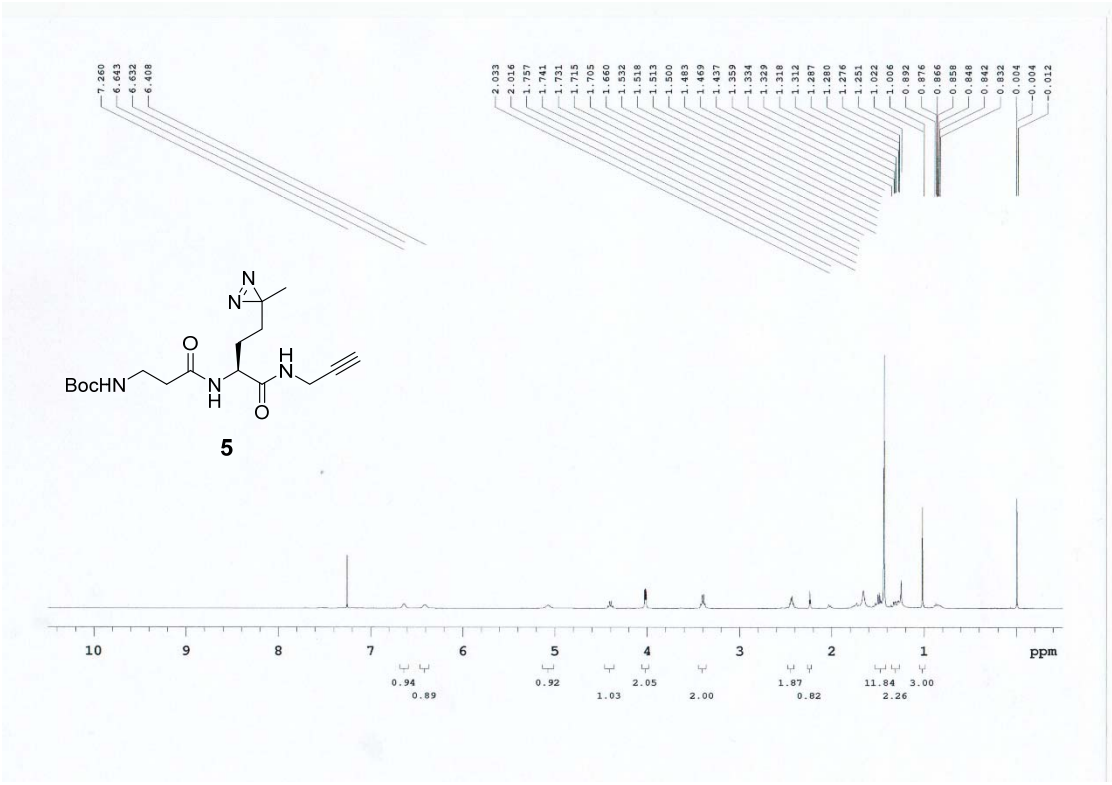
FT size 2048 x 2048
Total time 3 min 10 sec

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8
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6
5
4
3
2
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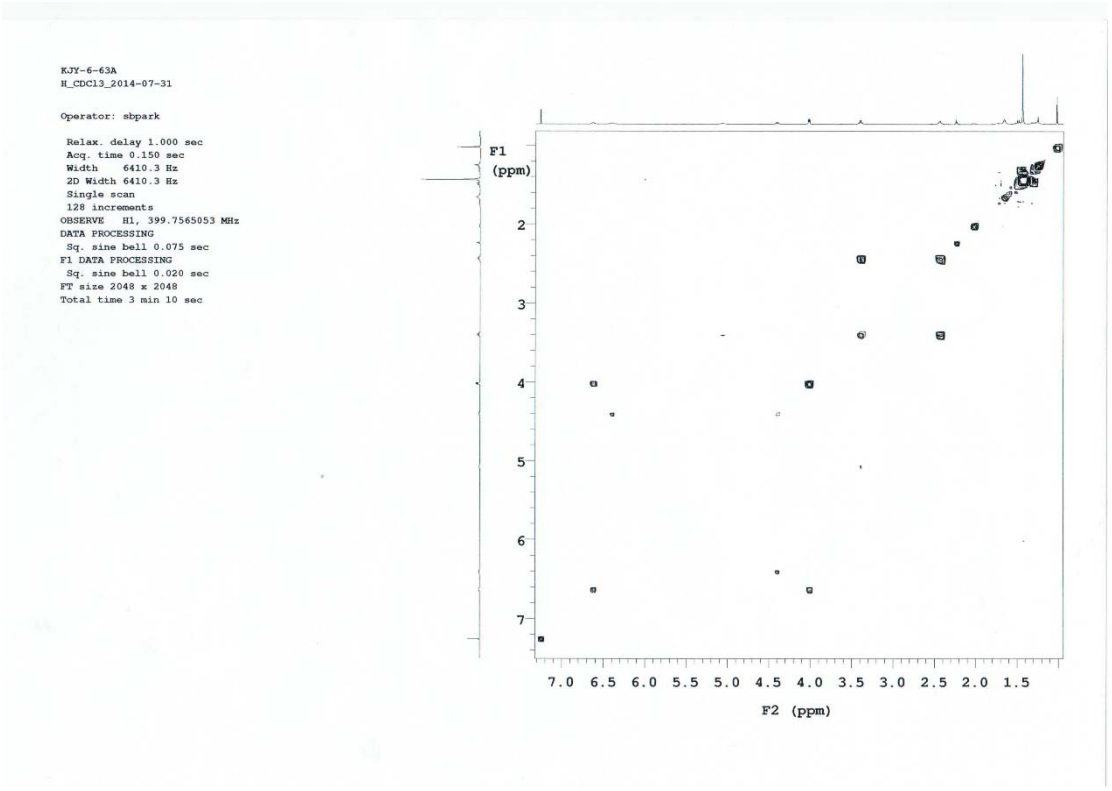
3.00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F1 DATA PROCESSING
Sq. sine bell 0.020 sec
FT size 2048 x 2048
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5
6
7
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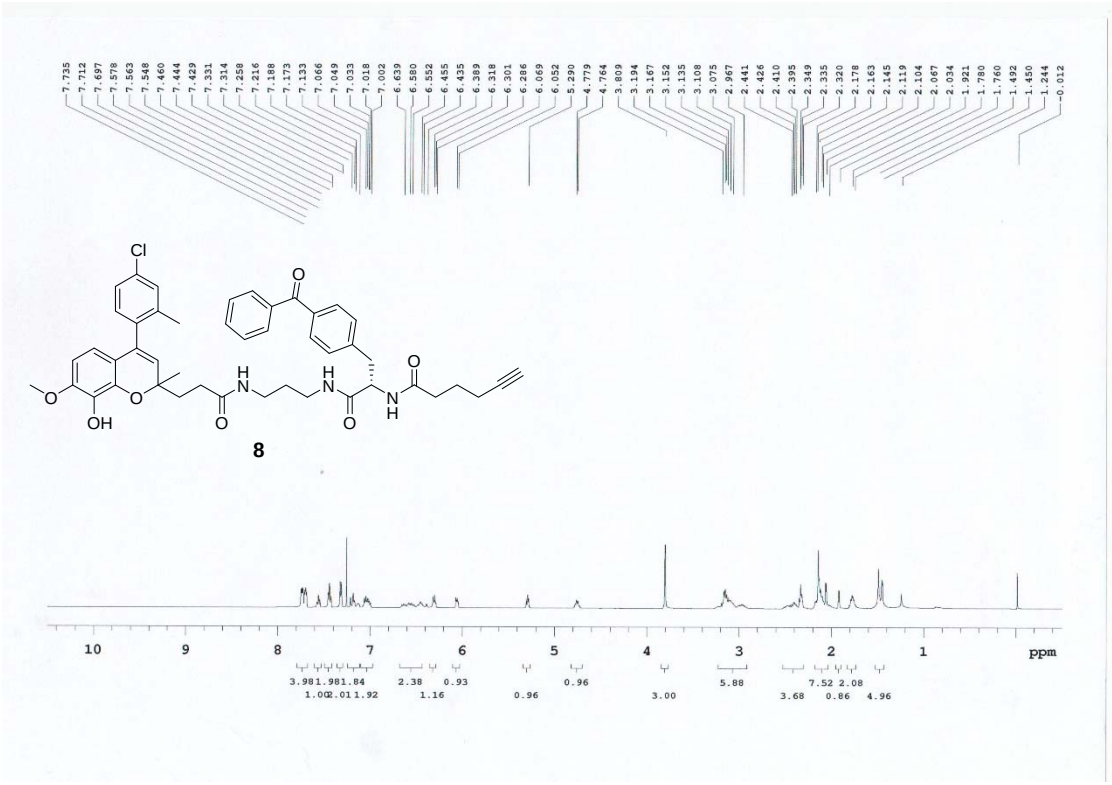
1.5
F2 (ppm)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H
N
O
H
N
O
O
OH
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10
9
8
7
6
5
4
3
2
1
ppm
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1.00 2.01 1.92
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3.00
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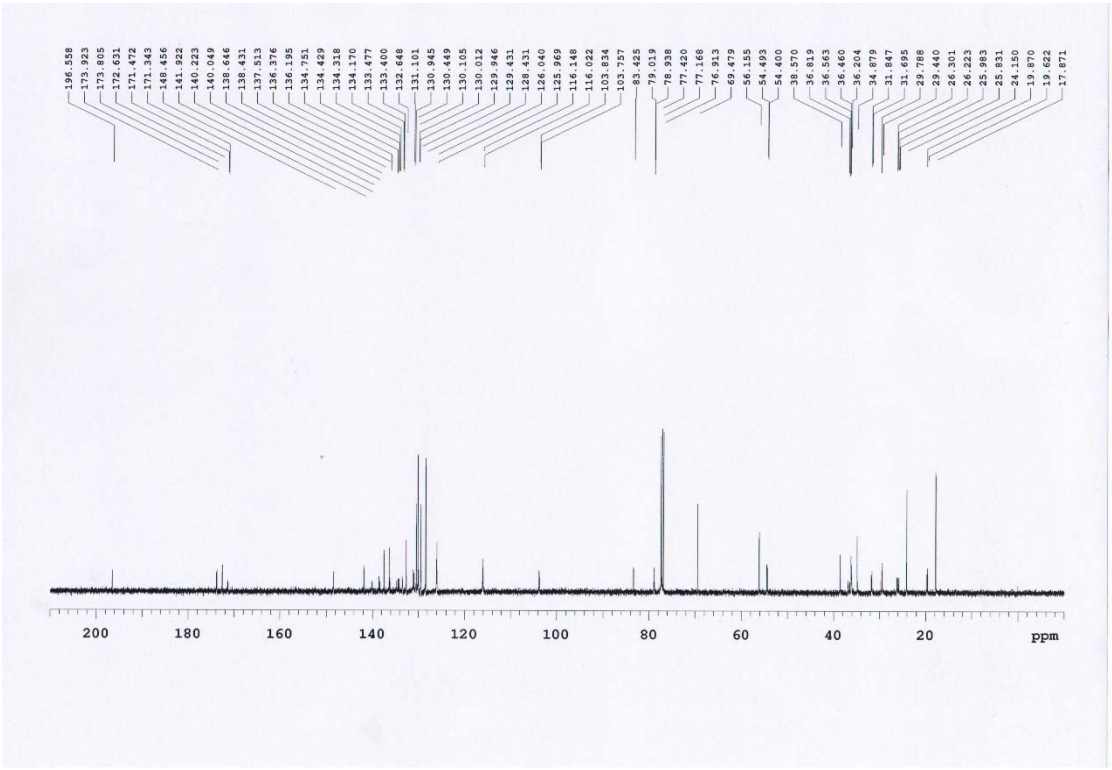
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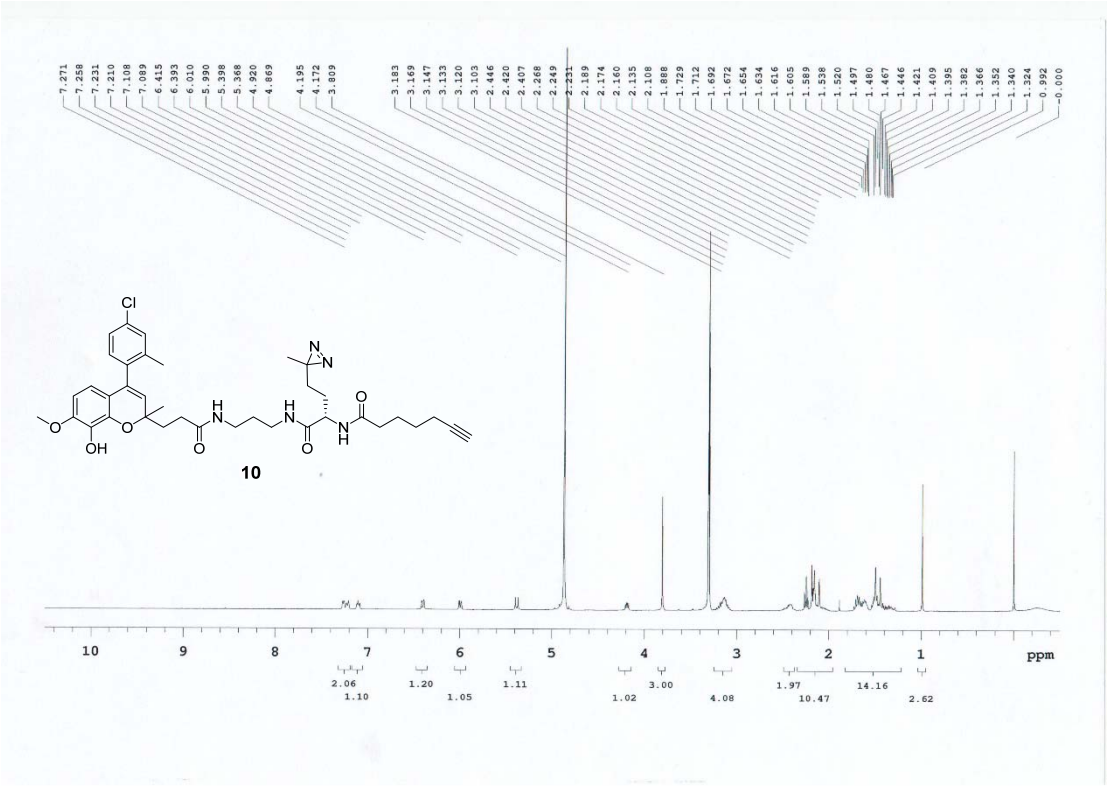
20
ppm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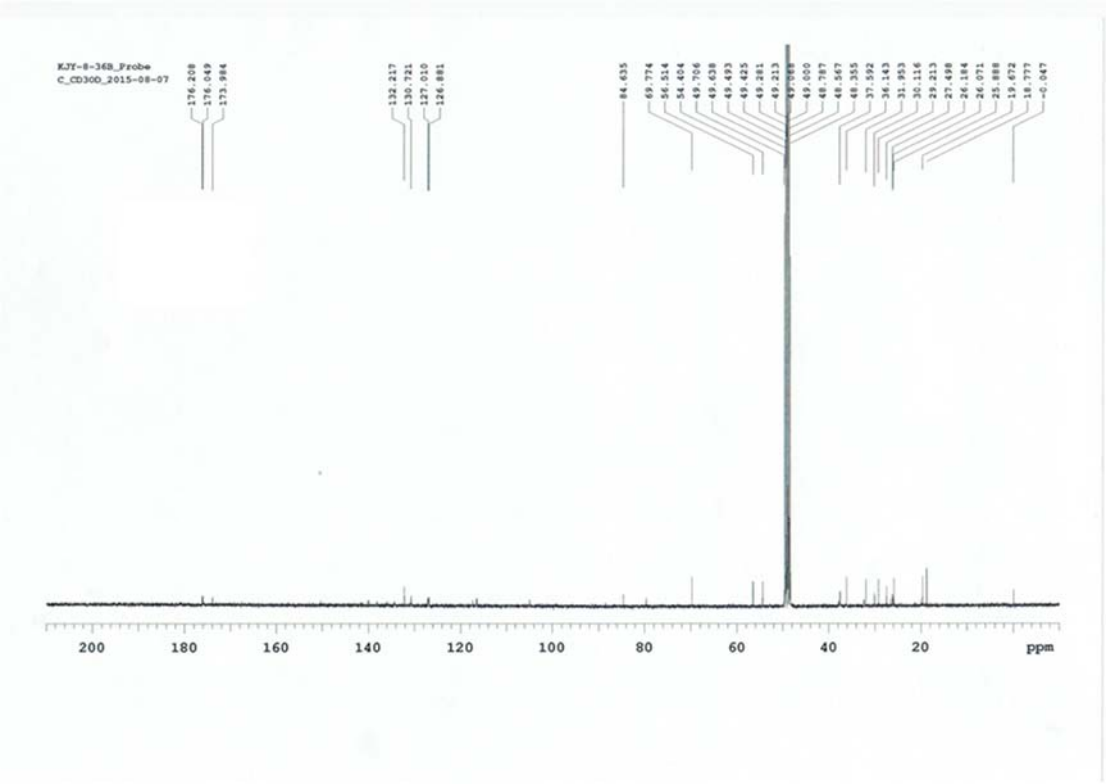
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ppm

KJY-8-36B_Probe
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128 increments
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F1 DATA PROCESSING
Sq. sine bell 0.020 sec
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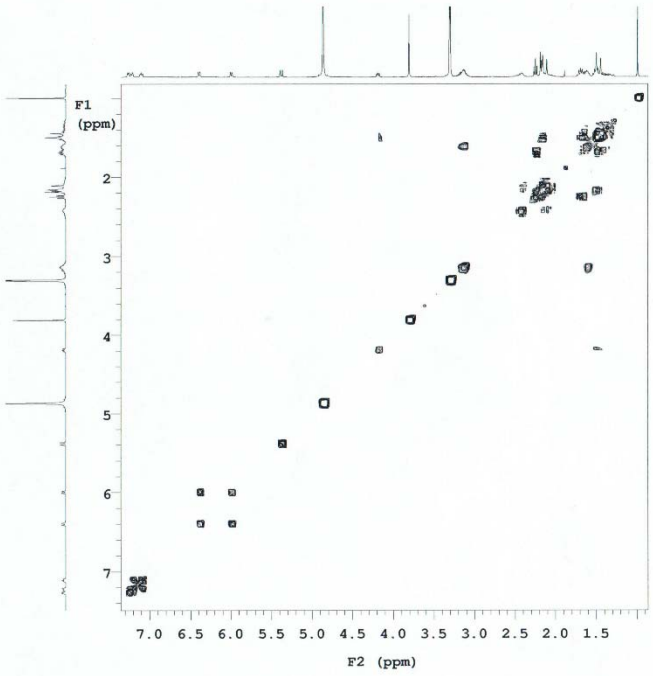

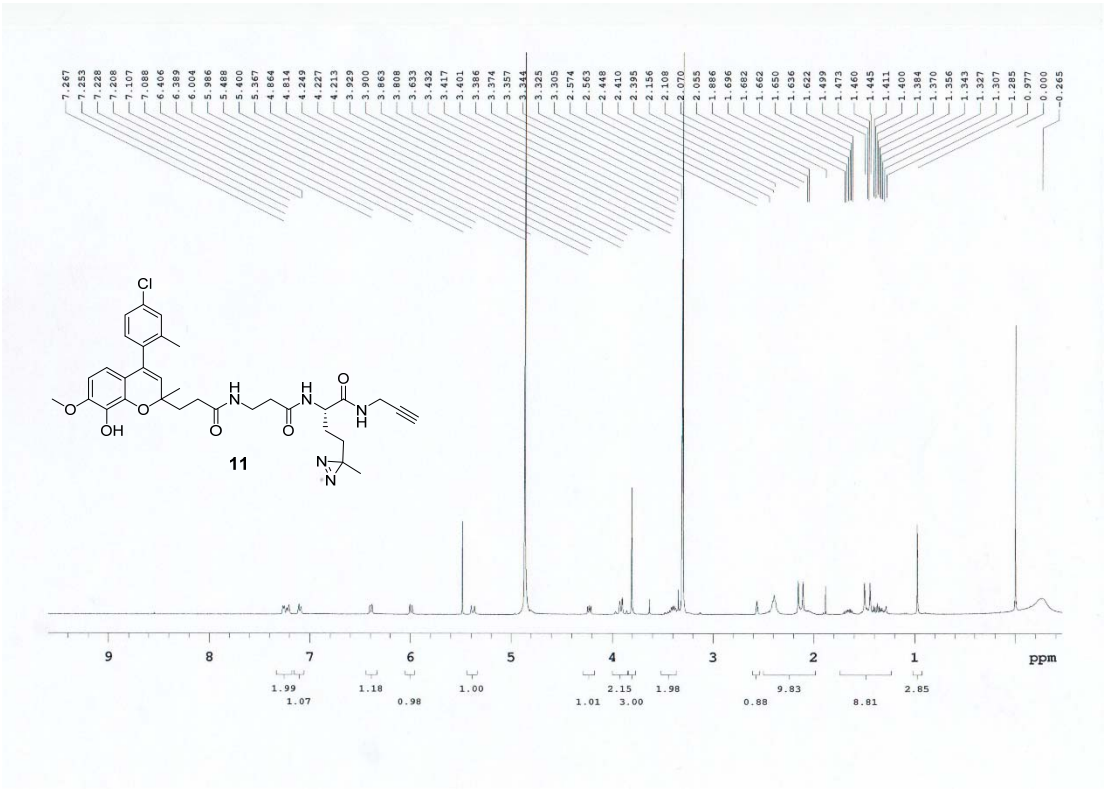
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3
2
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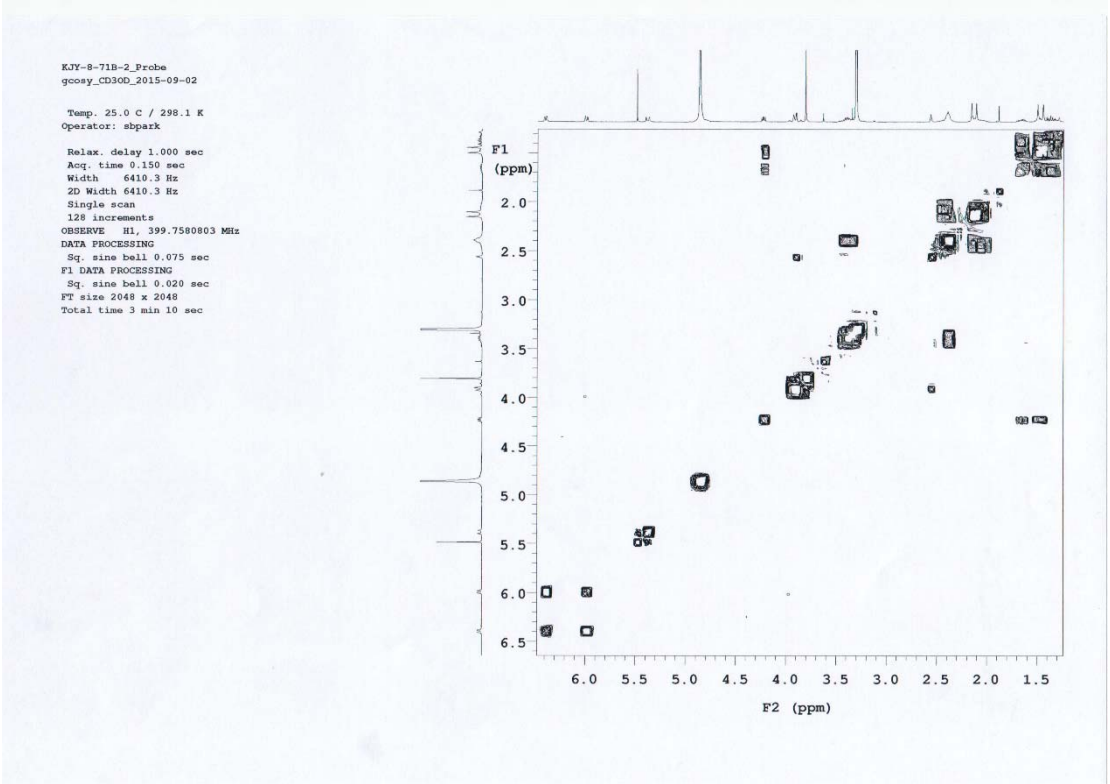
1.01
2.15
3.00
1.98
0.88
9.83
8.81
2.85


KJY-8-71B-2_Probe
gcosy_CD3OD_2015-09-02
Temp. 25.0 C / 298.1 K
Operator: sbpark
Relax. delay 1.000 sec
Acq. time 0.150 sec
Width 6410.3 Hz
2D Width 6410.3 Hz
Single scan
128 increments
OBSERVE H1, 399.7580803 MHz
DATA PROCESSING
Sq. sine bell 0.075 sec
F1 DATA PROCESSING
Sq. sine bell 0.020 sec
FT size 2048 x 2048
Total time 3 min 10 sec
F1
(ppm)
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
F2 (ppm)