Electronic Supplementary Material (ESI) for Chemical Communications

SUPPORTING INFORMATION

Fluorescence Amplified Detection of Proteases by the Catalytic Activation of A Semisynthetic Sensor

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General considerations and materials: Chemicals and peptide coupling reagents were purchased from Sigma-Aldrich, Alfa Aesar and Advanced Chemtech and used without further purification. Solvents (DMF, DCM, hexane, ethyl acetate and methanol) from Sigma-Aldrich were used without further treatment and distillation. Thin layer chromatography (TLC) was performed on TLC-aluminum sheets (Silica gel 60 F_{254}). Flash column chromatography was performed with Merck silica gel (230-400 mesh). ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Varian Mercury-400 with chemical shifts (δ) reported in ppm relative to the solvent residual signals of CD_3OD (3.30 ppm), $CDCl_3$ (7.24 ppm), DMSO-D_6 (2.49 ppm) and coupling constants reported in Hz. UV absorption and fluorescence emission spectra were recorded using TECAN Infinite M200Pro. High resolution mass spectra (HRMS) were measured on a Finnigan/Thermo Quest MAT 95XL.

Protein expression and purification: Recombinant protein SNAP_DEVD_HCA was expressed with N-terminal Strep-tag and C-terminal 10XHis-tag in the *E. coli* strain BL21. Bacterial cultures in LB medium were grown at 37 ˚C to an OD_{600nm} of 1.2. Expression of the recombinant protein was then induced by the addition of 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG). The bacteria were grown for an additional 16 hours at 18 ˚C and harvested by centrifugation. They were lysed by sonication and insoluble protein and cell debris were removed by centrifugation. We used a two-step purification procedure starting with Ni-NTA (Qiagen) followed by *Strep*-Tactin superflow (IBA) according to the instructions of the suppliers. The purified fusion proteins were stored in 50 mM HEPES pH 7.2, 50 mM NaCl at 4 ˚C until further use, and were stable for several weeks when stored under these conditions. The protein concentration was determined using NanoDrop 2000 (Thermo Scientific) with absorption

wavelength (280 nm) using BSA as a standard. About 10 mg SNAP_DEVD_HCA was obtained after Histag and Streptag purification from 1 L culture medium.

Sensor labeling: The compound BG-$(C6)_3$-*para*SA **1** was added to a final concentration of 10 μM to a solution of 2 μM SNAP_DEVD _HCA fusion protein in HEPES buffer (50 mM HEPES, 50 mM NaCl, pH 7.2), 1 mM dithiothreitol (DTT) and 10 μM $ZnCl_2$. The solutions were incubated for 2 hour at 37°C. The labeled SNAP-tag fusion proteins were concentrated by using centrifugal filter device (Amicon Ultra-30K Centrifugal Filters, Millipore) without further purification. Three washing cycles using HEPES buffer were performed. The labeled fusion proteins were stored at 4 °C until further use.

Detection of caspase-3 activity: 5 μM labeled semisynthetic sensor and various concentrations of caspase-3 in 10 μL HEPES buffer (50 mM HEPES, 50 mM NaCl, pH 7.2) were incubated at 37°C for one hour. The sensor/caspase-3 mixture was diluted 10-fold by HEPES buffer and 1 μL FLDA (from 500 μM FLDA ACN stock solution). Final concentration for fluorescence amplification: 0.5 μM sensor, 5 μM FLDA and 1% ACN. The fluorescence intensity was measured on TECAN Infinite M200Pro every 5 minutes. λ_{ex} = 460 nm, λ_{em} =513 nm.

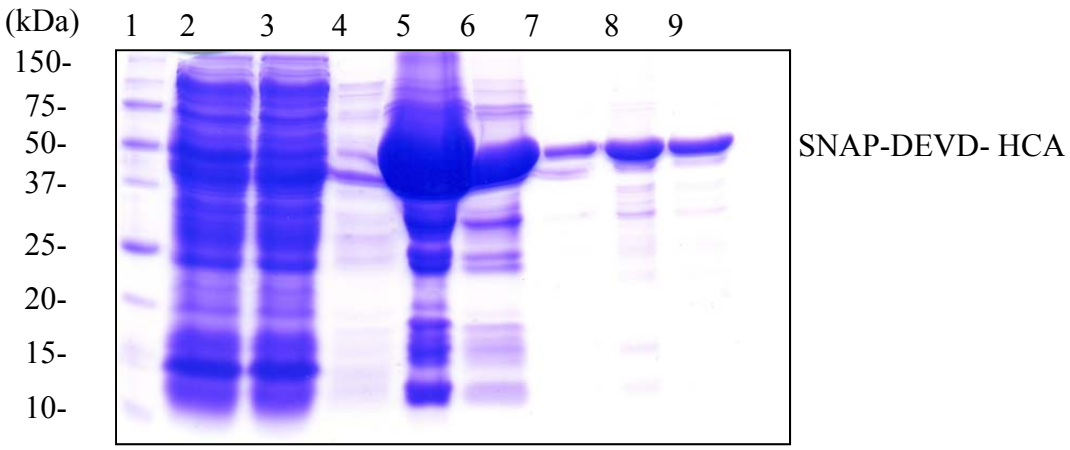
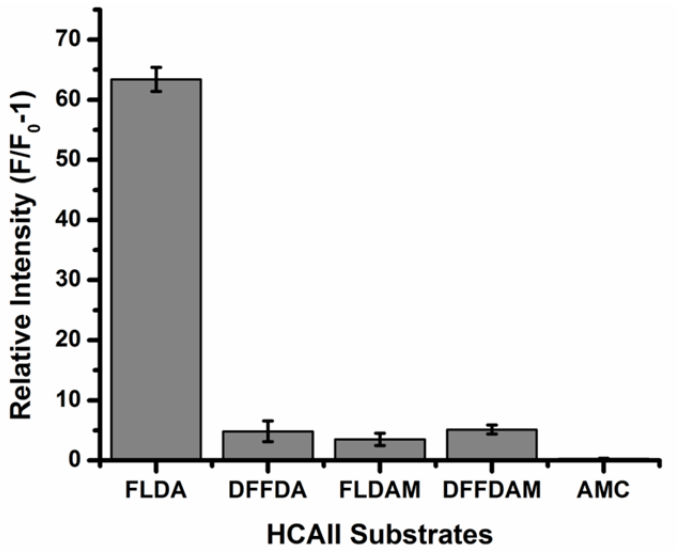


Fig. S1. Expression and purification of SNAP_DEVD_HCAII protein. Lane 1：Marker, Lane 2：post-sonication, Lane 3：His-tag loading buffer, Lane 4：His-tag wash buffer, Lane 5：His-tag elution buffer, Lane 6：Strep-tag loading buffer, Lane 7：Strep-tag wash buffer, Lane 8：Strep-tag elution buffer, Lane 9：Strep-tag elution buffer.

a)



b)

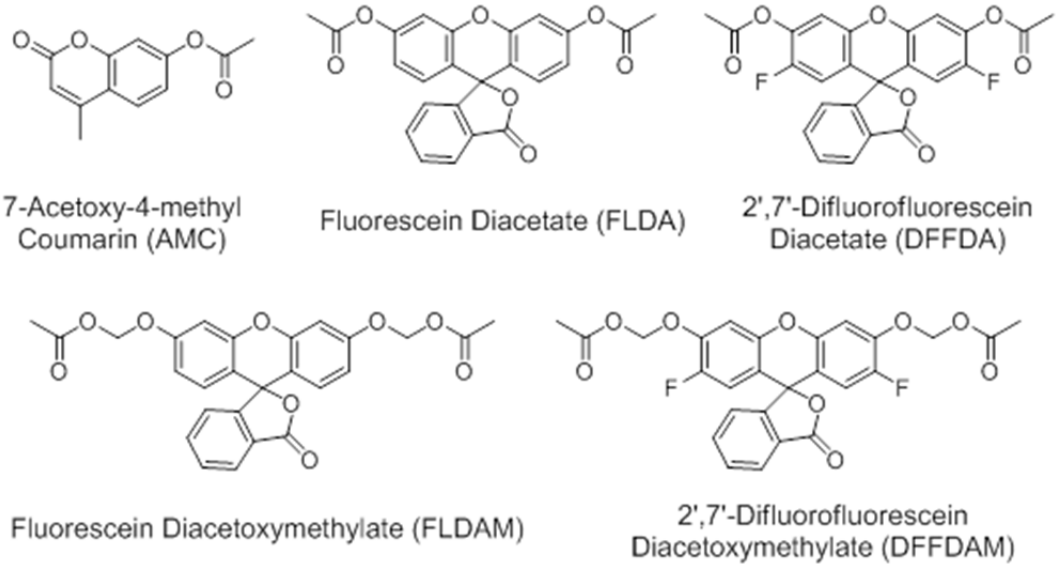


Fig. S2. a) Relative fluorescence intensity of different acetate-protected fluorophores were hydrolyzed by SNAP_DEVD_HCA after 45 minutes of incubation. The results show that FLDA is the most suitable HCAII substrate. b) Molecular structures of HCAII fluorophore substrates.

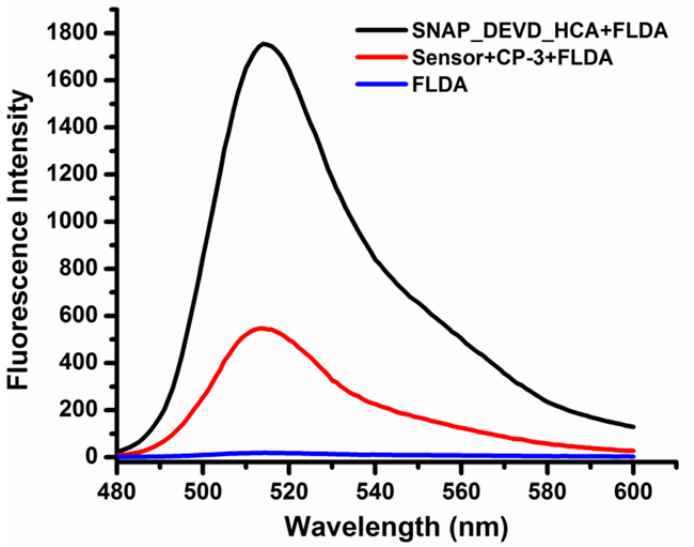


Fig. S3. Fluorescence spectra of FLDA hydrolysis to generate fluorophore FL by HCAII after 45 minutes of incubation. In general, the hydrolysis rate of FLDA by SNAP_DEVD_HCA alone (black line) is 3 times faster than with sensor and caspase-3 (red line). FLDA in HEPES buffer shows only very weak fluorescence (blue line).

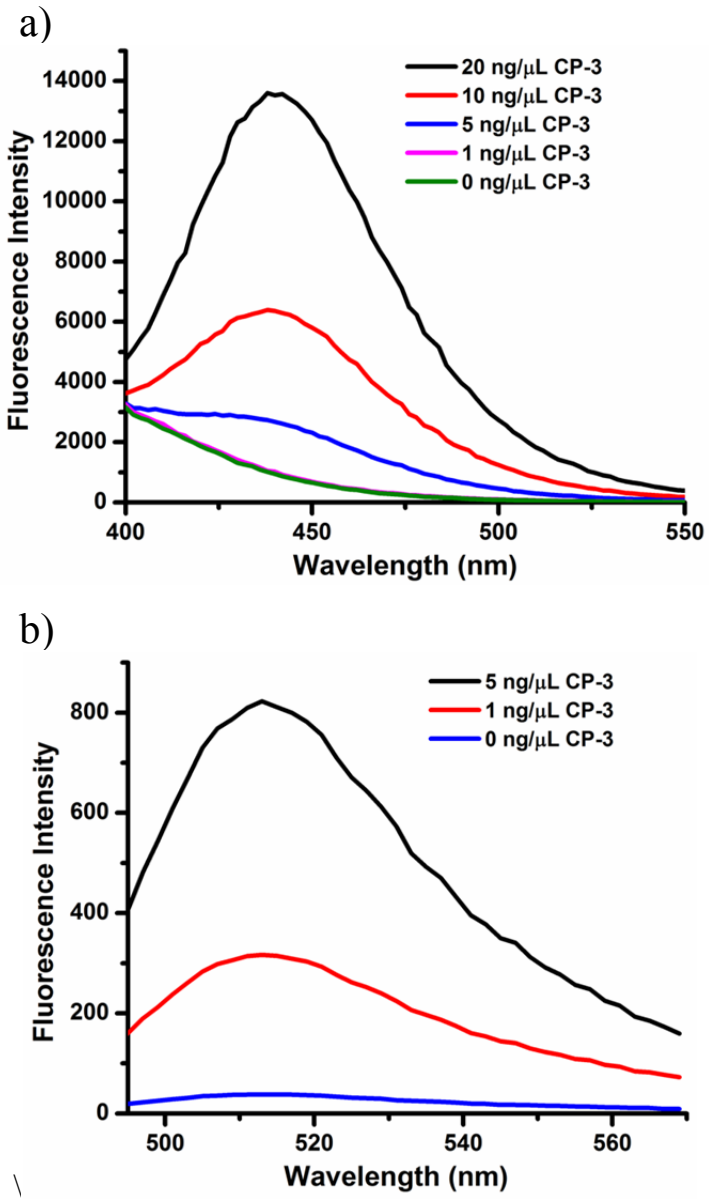
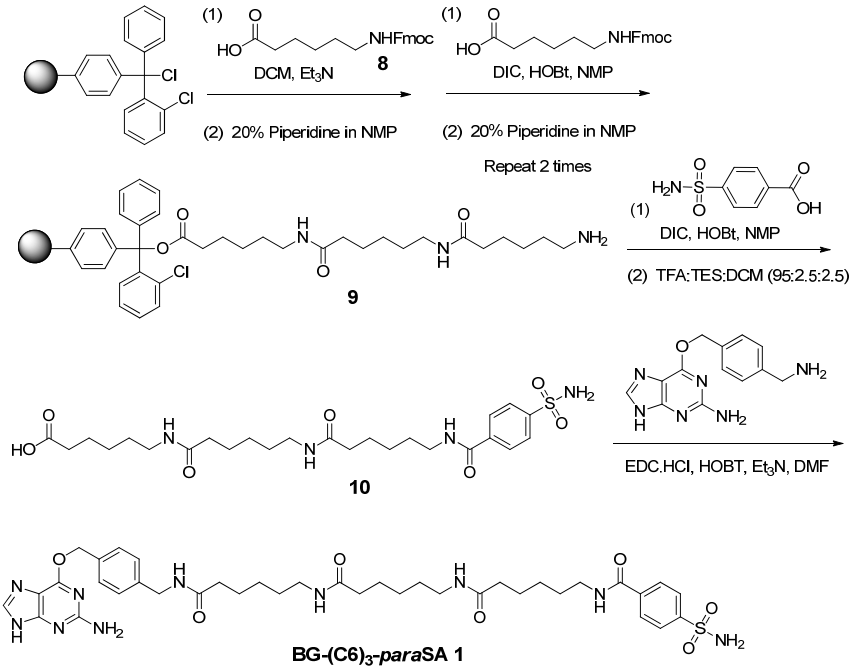


Fig. S4. Comparing the sensitivities of synthetic substrate Ac_DMQD_AMC and semisynthetic sensor in detecting caspase-3. a) 50 μM of Ac_DMQD_AMC was incubated with various concentrations of caspase-3 at 37°C for one hour. λ_{ex} = 365 nm The synthetic substrate cannot detect 1ng/μL of caspase-3. b) Sensing condition of the semisynthetic sensor: 5 μM sensor and various concentrations of caspase-3 in 10 μL HEPES buffer, pH 7.2, 1 hour at 37°C. Signal amplification was initiated by addition of 5 μM FLDA. The spectrum was recorded after 45 minutes incubation. λ_{ex} = 460 nm.

Synthesis of BG-(C6)$_3$-*para*SA 1

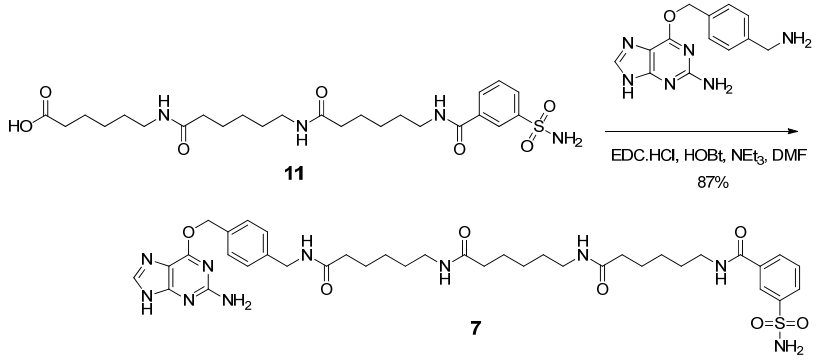


Scheme S1. Synthetic Scheme of BG-(C6)$_3$-*para*SA **1**.

Synthesis of Compound 10: Compound **10** was constructed using standard solid-phase Fmoc methodology. 100 mg of 2-chlorotrityl chloride (1.5 mmol/g) resin was dried in vacuo overnight. The resin was reacted with 1.2 equavalent of **8** and 3 equivalent of Et_3N in DCM for 2 hours. The Fmoc group was deprotected using 20% piperidine in NMP for 5 minutes (repeated 3 times). The amide bond coupling reactions were performed with 3 equivalents of DIC, HOBt and amino acid (or 4-Sulfamoylbenzoic acid) in NMP for 2 hours. Compound **10** was cleaved from the resin with 3 mL TFA:TES:DCM (95:2.5:2.5) for 2 hours to afford 86 mg of product. Compound **10** was used for next step without further purification.

Synthesis of BG-(C6)$_3$-*para*SA 1: Compound **10** (10 mg, 18 μmol), EDC.HCl (10 mg, 64 μmol), HOBt (8 mg, 59 μmol) and Et_3N (12 μL) in DMF (1 mL) were added to BG-NH_2 (6 mg, 22 μmol) at room temperature and stirred overnight. The crude product was purified by preparative HPLC to give the desired product **1** as a white powder after lyophilization. BG-NH_2 was prepared as previously reported.[1] **Yield** = 63%, 11 mg; Purity > 95% (HPLC); **^{1}H NMR** (400 MHz, CD_3OD) δ 8.33 (s, 1H), 7.94 (q, *J* = 8.8 Hz, 4H), 7.50 (d, *J* = 8.0 Hz, 2H), 7.32 (d, *J* = 8.0 Hz, 2H), 5.63 (s, 2H), 4.36 (s, 2H), 3.37 (d, *J* = 7.0 Hz, 2H), 3.13 (dd, *J* = 8.8, 5.0 Hz, 4H), 2.25 – 2.12 (m, 6H), 1.69 – 1.19 (m, 18H) ppm; **^{13}C NMR** (100 MHz, CD_3OD) δ 165.99, 130.12, 129.19, 126.93, 119.68, 118.93, 118.66, 117.31, 58.66, 34.44, 33.82, 30.95, 30.19, 26.97, 20.09, 17.55, 16.70 ppm; **HRMS** (ESI): m/z calc. for $C_{38}H_{52}N_{10}O_7S$ 792.3741, found 793.3828 $[M+H]^+$.

Synthesis of BG-(C6)$_3$-*meta*SA 7



Scheme S2. Synthetic Scheme of BG-(C6)$_3$-*meta*SA **7**.

Synthesis of BG-(C6)$_3$-*meta*SA 7: Compound **11** was prepared by the same protocol as compound **10**. Compound **11** (10 mg, 18 μmol), EDC.HCl (10 mg, 64 μmol), HOBt (8 mg, 59 μmol) and Et_3N (12 μL) in DMF (1 mL) were added to BG-NH_2 (6 mg, 22 μmol) at room temperature and stirred overnight. The crude product was purified by preparative HPLC to give the desired product **7** as a white powder after lyophilization. yield: 87%, 15 mg; Purity > 95%

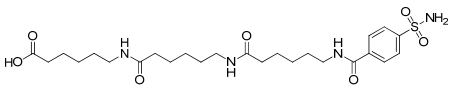
(HPLC); **^{1}H NMR** (400 MHz, CD_3OD) δ 8.32 (s, 1H), 8.11 – 7.89 (m, 4H), 7.62 (dd, *J* = 9.8, 6.0 Hz, 1H), 7.50 (d, *J* = 7.5 Hz, 1H), 7.32 (d, *J* = 8.1 Hz, 1H), 5.63 (s, 2H), 4.36 (s, 2H), 3.37 (d, *J* = 7.2 Hz, 2H), 3.16 – 3.08 (m, 2H), 2.28 – 2.10 (m, 4H), 2.25 – 2.12 (m, 6H), 1.69 – 1.19 (m, 18H) ppm; **^{13}C NMR** (100 MHz, CD_3OD) δ 166.01, 158.54, 151.06, 135.64, 133.95, 131.66, 131.09, 129.08, 128.03, 126.82, 125.12, 121.53, 120.39, 120.23, 119.83, 119.63, 119.19, 118.77, 118.56, 118.18, 116.12, 61.07, 54.92, 33.77, 30.95, 30.18, 26.94, 20.08, 17.54, 16.70 ppm; HRMS (ESI): m/z calc. for $C_{38}H_{52}N_{10}O_7S$ 792.3741, found 793.3808 $[M+H]^+$.

Synthesis of BG-$(EG)_{11}$-*para*SA 6: BG-$(EG)_{11}$-NH_2 (5 mg, 5.7 μmol), 4-carboxy-benzenesulfonamide (2 mg, 11 μmol) in DMF (1 mL) were added to EDC.HCl (1 mg, 5.7 μmol), HOBt (1 mg, 5.7 μmol) and Et_3N (5 μL) at room temperature and stirred overnight. The crude product was purified by preparative HPLC to give the desired product **6** as a white powder after lyophilization in 40% yield. BG-$(EG)_{11}$-NH_2 was prepared as previously reported.[2] **Yield** = 40%; Purity > 95% (HPLC); **^{1}H NMR** (400 MHz, MeOD) δ 8.41 (s, 1H), 7.98 (m, 5H), 7.52 (d, *J* = 8.0, 2H), 7.36 (d, *J* = 8.0, 2H), 5.66 (s, 2H), 4.41 (s, 2H), 3.75 (t, *J* = 5.9, 4H), 3.70 – 3.51 (m, 44H), 2.49 (t, *J* = 5.9, 4H) ppm; **HRMS** (ESI) *m/z* calc. for $C_{47}H_{72}N_8O_{17}S^+$ 1053.4816, found 1053.4827 $[M+H]^+$.

References:

1. A. Keppler, S. Gendreizig, T. Gronemeyer, H. Pick, H. Vogel, K. Johnsson, *Nat. Biotechnol.* **2003**, *21*, 86-89.

2. M. A. Brun, K.-T. Tan, E. Nakata, M. J. Hinner, K. Johnsson, *J. Am. Chem. Soc.* **2009**, *131*, 5873-5884.



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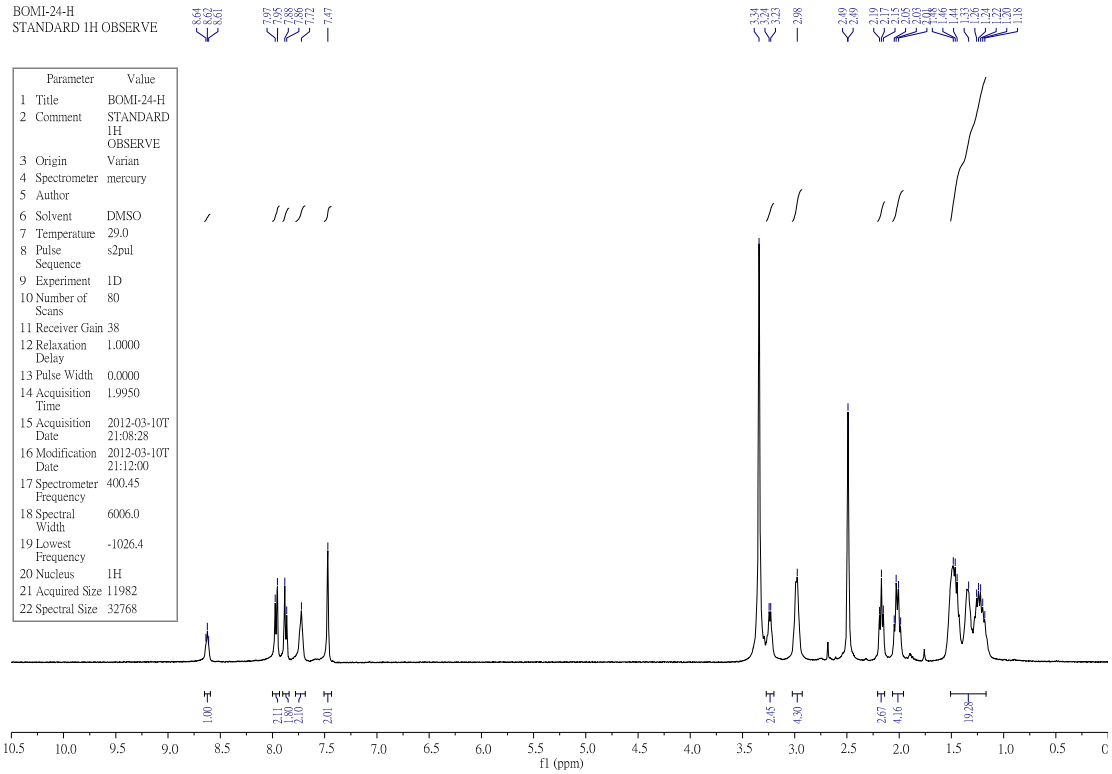

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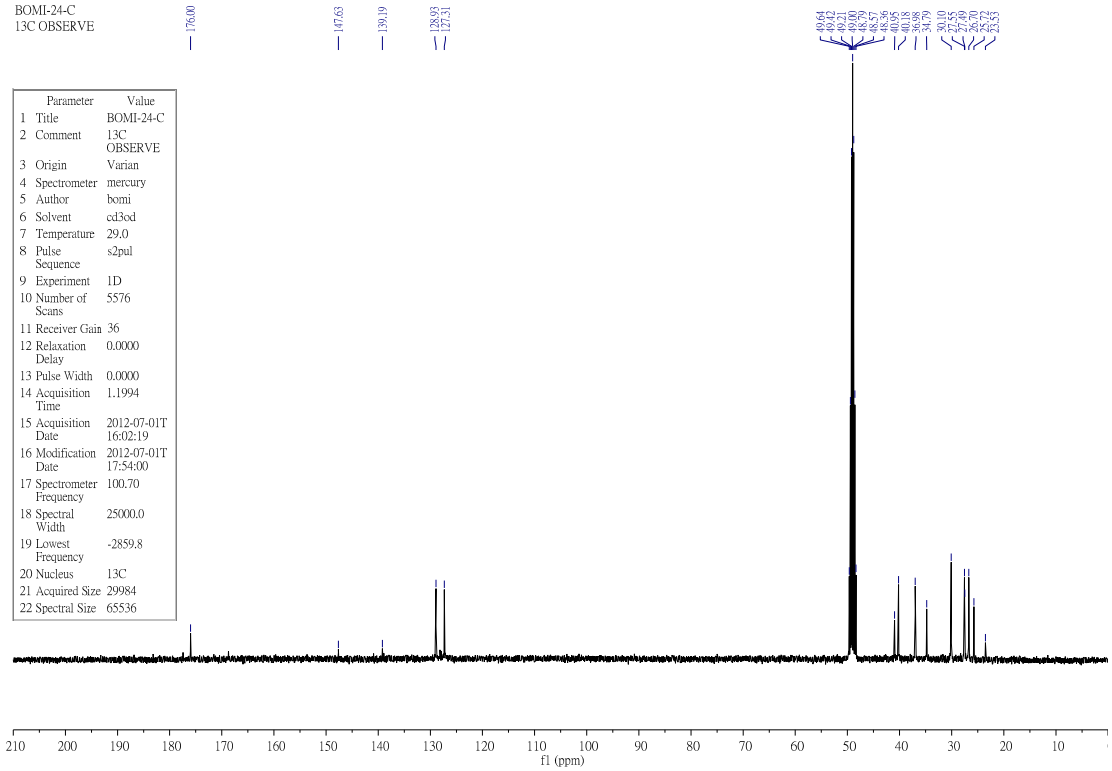

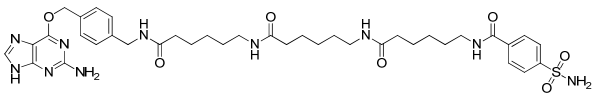

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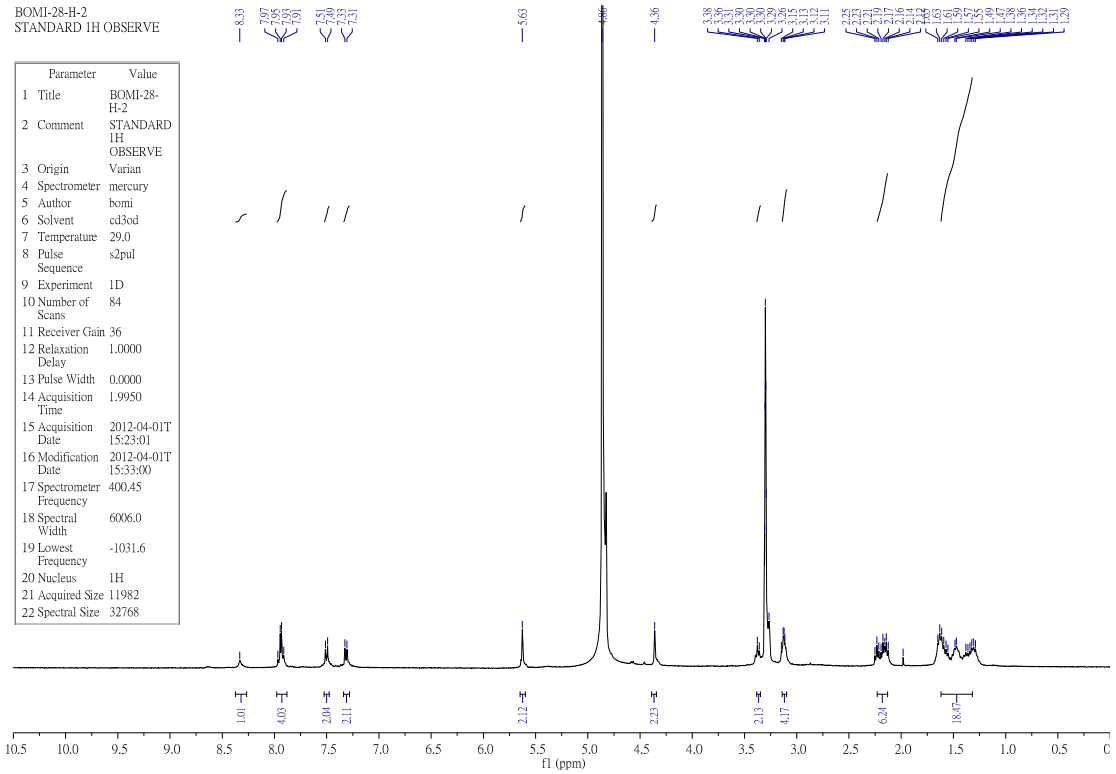

BG-_C6_3-para_SA
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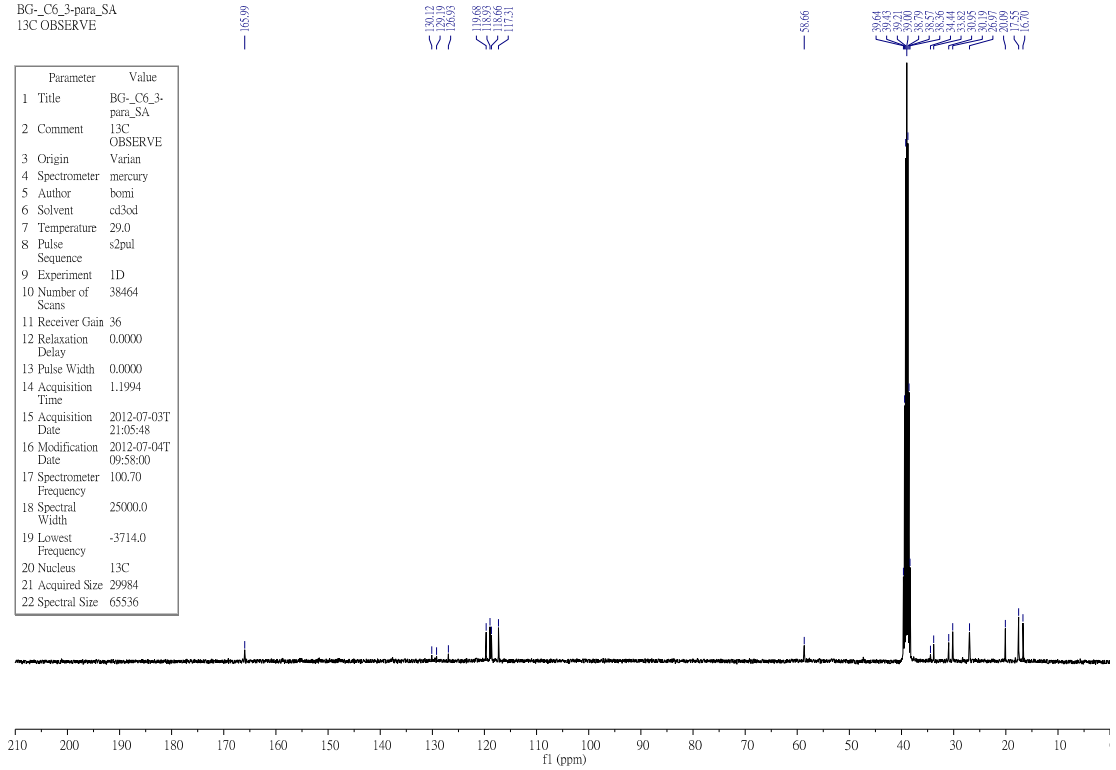

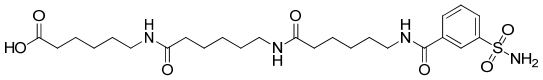

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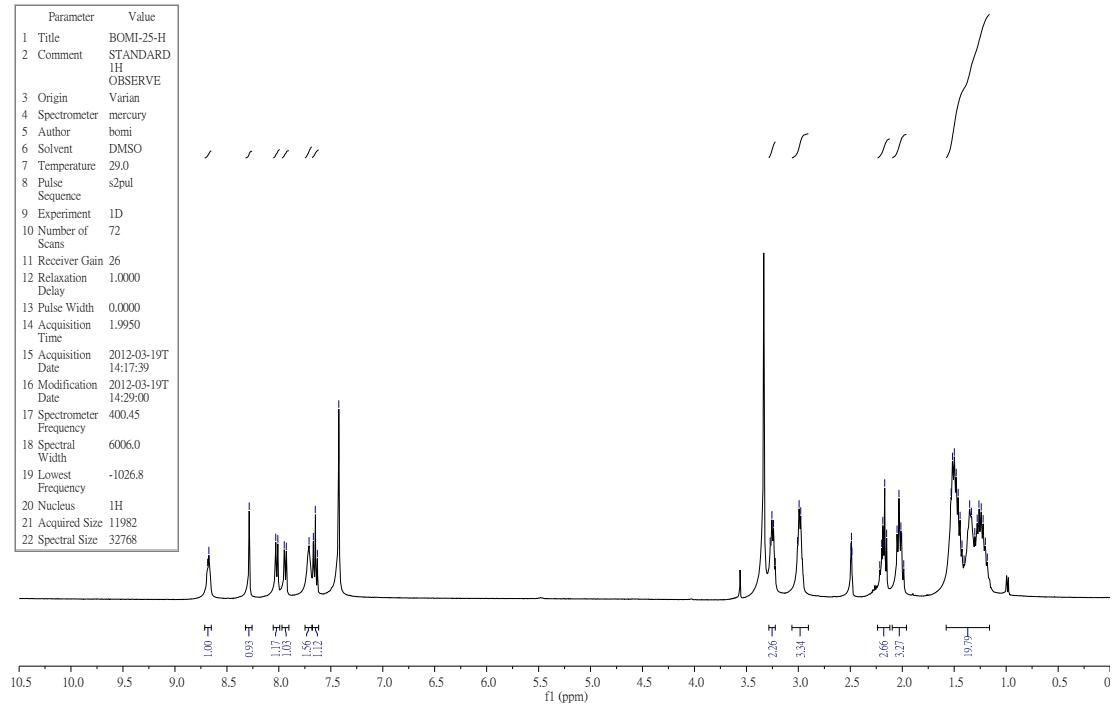

Linker6_3_Meta_SA
13C OBSERVE

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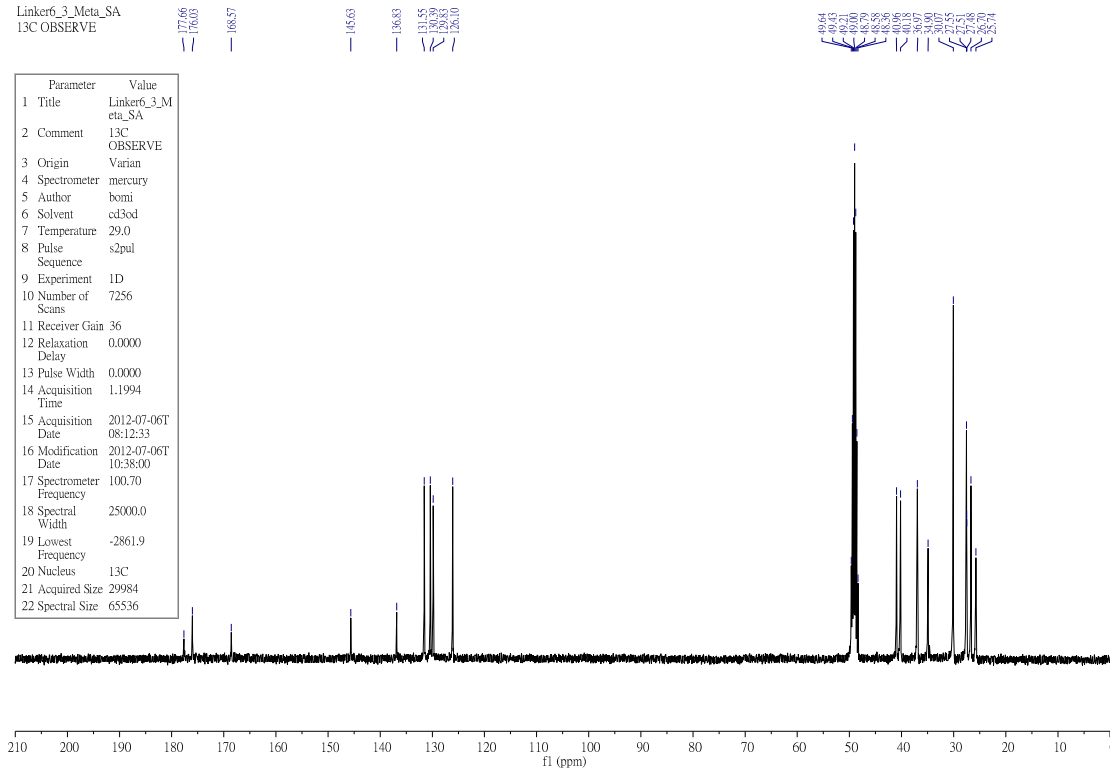

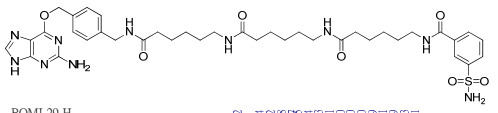

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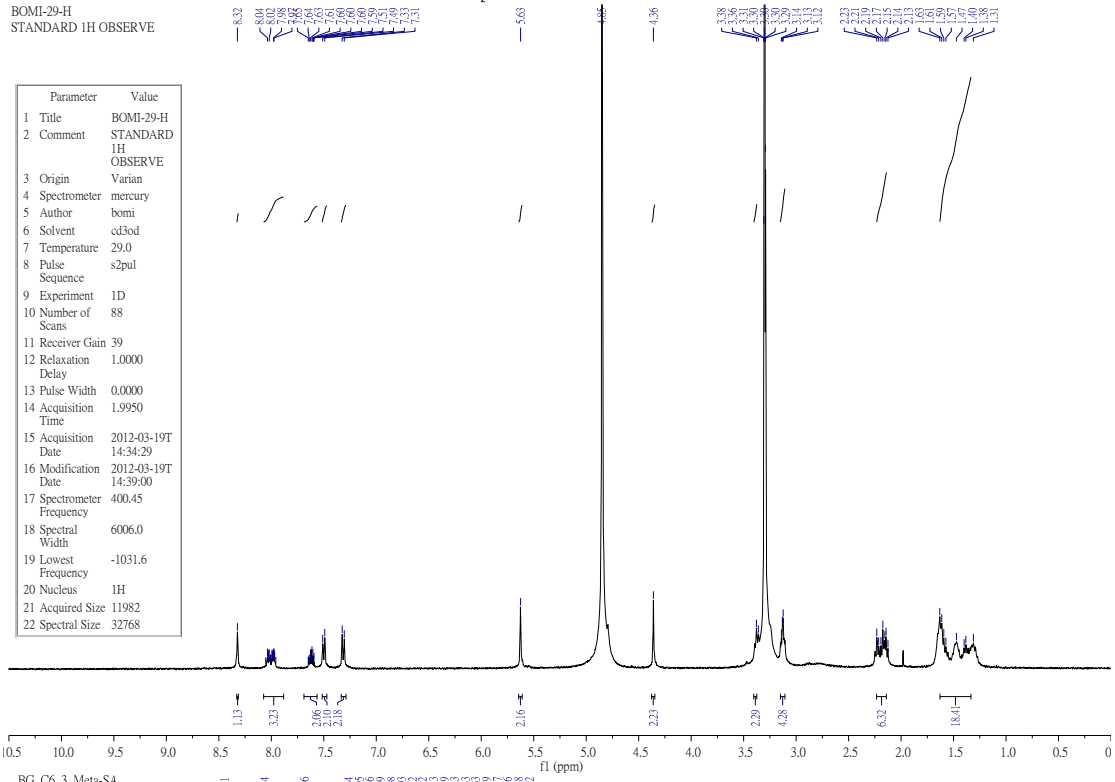

BG_C6_3_Meta-SA
13C OBSERVE

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